CHEM 322 LECTURE CONTENT



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Chem 322 Lecture Content

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Glossary

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CHAPTER OVERVIEW

1: Phosphate Transfer Reactions

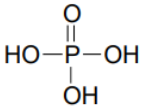
This chapter is about the chemistry of phosphates, a ubiquitous functional group in biomolecules that is based on phosphoric acid.

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1.1: Prelude to Phosphate Transfer Reactions

This chapter is about the chemistry of phosphates, a ubiquitous functional group in biomolecules that is based on phosphoric acid:



phosphoric acid

In late 2010, people around the world found themselves getting a crash course in phosphate chemistry as they watched the evening news. Those who paid close attention to the developing story also got an interesting glimpse into the world of scientific research and debate.

It all started when the American National Aeronautics and Space Administration (NASA) released the following statement to the news media:

> ***"NASA will hold a news conference at 2 p.m. EST on Thursday, Dec. 2, to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life."***

The wording of the statement attracted widespread media attention, and had some people holding their breath in anticipation that NASA would be introducing a newly discovered alien life form to the world. When December 2nd came, however, those hoping to meet ET were disappointed – the life form being introduced was a bacterium, and it was from our own planet. To biologists and chemists, though, the announcement was nothing less than astounding.

The NASA scientists worked hard to emphasize the significance of their discovery during the news conference. Dr. Felicia Wolfe-Simon, a young postdoctoral researcher who had spearheaded the project, stated that they had "cracked open the door to what's possible for life elsewhere in the universe - and that's profound". A senior NASA scientist claimed that their results would "fundamentally change how we define life", and, in attempting to convey the importance of the discovery to a reporter from the newspaper USA Today, referred to an episode from the original Star Trek television series in which the crew of the Starship Enterprise encounters a race of beings whose biochemistry is based on silica rather than carbon.

The new strain of bacteria, dubbed 'GFAJ-1', had been isolated from the arsenic-rich mud surrounding salty, alkaline Mono Lake in central California. What made the strain so unique, according to the NASA team, was that it had evolved the ability to substitute arsenate for phosphate in its DNA. Students of biology and chemistry know that phosphorus is one of the six elements that are absolutely required for life as we know it, and that DNA is a polymer linked by phosphate groups. Arsenic, which is directly below phosphorus on the periodic table, is able to assume a bonding arrangement like that of phosphate, so it might seem reasonable to wonder whether arsenate could replace phosphate in DNA and other biological molecules. Actually finding a living thing with arsenate-linked DNA would indeed be a momentous achievement in biology, as this would represent a whole new chemistry for the most fundamental molecule of life, and would change our understanding of the chemical requirements for life to exist on earth - and potentially other planets.

In 1987, Professor F.H. Westheimer of Harvard University published what would become a widely read commentary in Science Magazine entitled "Why Nature Chose Phosphates". In it, he discussed the chemical properties that make the phosphate group so ideal for the many roles that it plays in biochemistry, chief among them the role of a linker group for DNA polymers. One of the critical characteristics of phosphate that Westheimer pointed out was that the bonds linking phosphate to organic molecules are stable in water. Clearly, if you are selecting a functional group to link your DNA, you don't want to choose one that will rapidly break apart in water. Among the functional groups that Westheimer compared to phosphate in terms of its suitability as a potential DNA linker was arsenate –but he very quickly dismissed the idea of arsenate-linked DNA because it would be far too unstable in water.



Mono Lake, California. (photo credit https://www.flickr.com/photos/slolane/)

Given this background, it is not hard to imagine that many scientists were puzzled, to say the least, by the NASA results. While the popular media took the announcement at face value and excitedly reported the results as a monumental discovery – NASA is, after all, a highly respected scientific body and the study was being published in Science Magazine, one of the most prestigious scientific journals in the world – many scientists quickly voiced their skepticism, mainly in the relatively new and unconstrained venue of the blogosphere. Microbiologist Rosie Redfield of the University of British Columbia, writing in her blog devoted to 'open science', wrote a detailed and highly critical analysis of the study. She pointed out, among other things, that the experimenters had failed to perform the critical purification and mass spectrometry analyses needed to demonstrate that arsenate was indeed being incorporated into the DNA backbone, and that the broth in which the bacteria were being grown actually contained enough phosphate for them to live and replicate using normal phosphate-linked DNA. Science journalist Carl Zimmer, in a column in the online magazine Slate, contacted twelve experts to get their opinions, and they were overwhelmingly negative. One of the experts said bluntly, “This paper should not have been published". Basically, the NASA researchers were making an astounding claim that, if true, would refute decades of established knowledge about the chemistry of DNA – but the evidence they presented was far from convincing. Carl Sagan's widely quoted dictum - “extraordinary claims require extraordinary evidence” - seemed to apply remarkably well to the situation.

What followed was a very public, very lively, and not always completely collegial debate among scientists about the proper way to discuss science: the NASA researchers appeared to dismiss the criticism amassed against their study because it came from blogs, websites, and Twitter feeds. The proper venue for such discussion, they claimed, was in the peer-reviewed literature. Critics countered that their refusal to respond to anything outside of the traditional peer-review system was disingenuous, because they had made full use of the publicity-generating power of the internet and mainstream media in the first place when they announced their results with such fanfare.

The traditional venue for debate, while quite a bit slower than the blogosphere, did eventually come through. When the full paper was published in Science a few months later, it was accompanied by eight 'technical comments' from other researchers pointing out deficiencies in the study, an 'editors note', and a broader news article about the controversy. In July of 2012, a paper was published in Science under the title “GFAJ-1 Is an Arsenate-Resistant, Phosphate-Dependent Organism”. The paper reported definitive evidence that DNA from GFAJ-1, under the conditions described in the NASA paper, did not have arsenate incorporated into its structure. Just like professor Westheimer discussed in the 1980s, it appears that nature really did choose phosphate – and only phosphate – after all . . . at least on this planet.

Background reading and viewing:

- Youtube video of the NASA press conference: http://www.youtube.com/watch?v=WVuhBt03z8g.
- Wolfe-Simon, F. et al. Science Express, Dec 2, 2010. The first preview article on the proposed 'arsenic bacteria'.
- Wolfe-Simon, F. et al., Science 2011, 332, 1163. The full research paper in Science Magazine.
- Westheimer, F.H. Science 1987, 235, 1173. The article by Westheimer titled 'Why Nature Chose Phosphates'.

- Zimmer, Carl, Slate, Dec 7, 2010: Blog post by Carl Zimmer titled 'This Paper Should Not Have Been Published'. http://www.slate.com/articles/health...published.html
- Redfield, R. Blog post Dec 4, 2010: http://rrresearch.fieldofscience.com...ria-nasas.html
- Science 2012, 337, 467. The paper in Science Magazine refuting the validity of the arsenic bacteria claim.

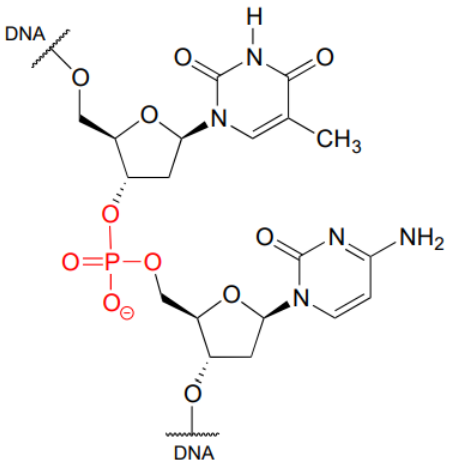
Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

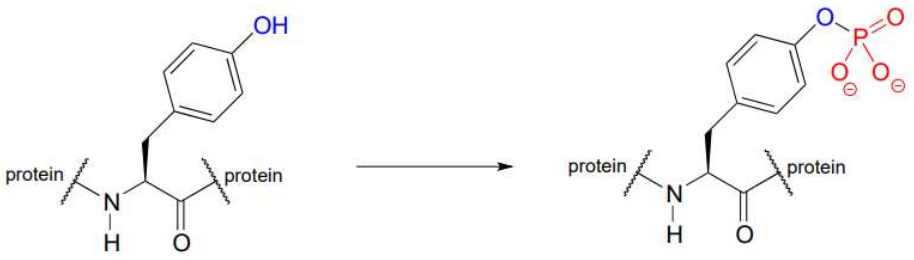
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1.2: Overview of Phosphate Groups

Phosphate is everywhere in biochemistry. As we were reminded in the introduction to this chapter, our DNA is linked by phosphate:



The function of many proteins is regulated - switched on and off - by enzymes which attach or remove a phosphate group from the side chains of serine, threonine, or tyrosine residues.



tyrosine residue phosphotyrosine residue

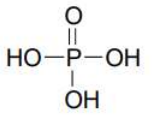
Countless diseases are caused by defects in phosphate transferring enzymes. As just one example, achondroplasia, a common cause of dwarfism, is caused by a defect in an enzyme whose function is to transfer a phosphate to a tyrosine residue in a growth-related signaling protein.

Finally, phosphates are excellent leaving groups in biological organic reactions, as we will see many times throughout the remainder of this book.

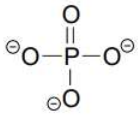
Clearly, an understanding of phosphate chemistry is central to the study of biological organic chemistry. We'll begin with an overview of terms used when talking about phosphates.

Terms and abbreviations

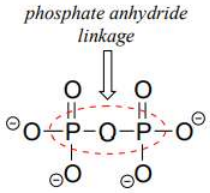
The fully deprotonated conjugate base of phosphoric acid is called a phosphate ion, or inorganic phosphate (often abbreviated 'P_i'). When two phosphate groups are linked to each other, the linkage itself is referred to as a 'phosphate anhydride', and the compound is called 'inorganic pyrophosphate' (often abbreviated PP_i).



phosphoric acid

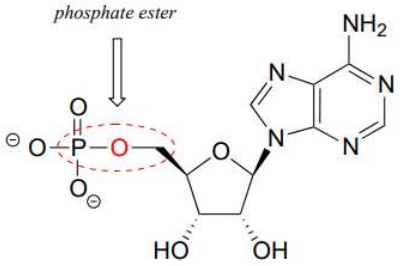


inorganic phosphate (P_i)



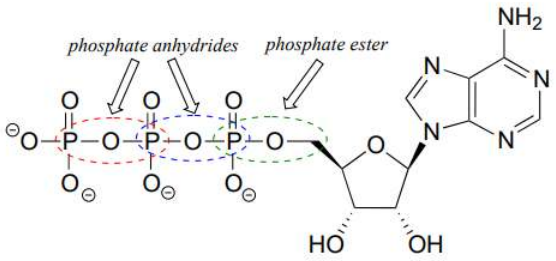
inorganic pyrophosphate (PP_i)

The chemical linkage between phosphate and a carbon atom is a phosphate ester. Adenosine monophosphate (AMP) has a single phosphate ester linkage.



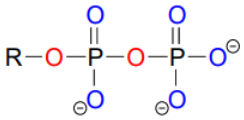
adenosine monophosphate (AMP)

Adenosine triphosphate has one phosphate ester linkage and two phosphate anhydride linkages.



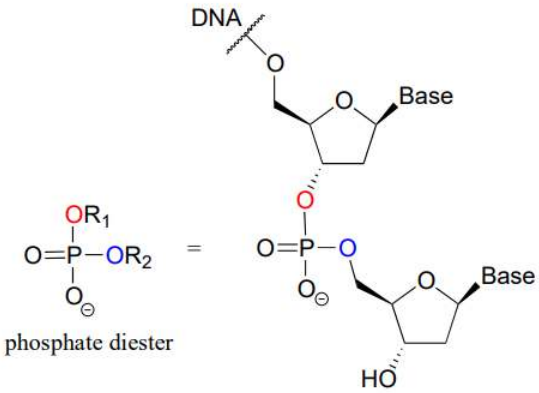
adenosine triphosphate (ATP)

Oxygen atoms in phosphate groups are referred to either as 'bridging' or 'non-bridging', depending on their position. An organic diphosphate has two bridging oxygens (one in the phosphate ester linkage and one in the phosphate anhydride linkage) and five non-bridging oxygens:



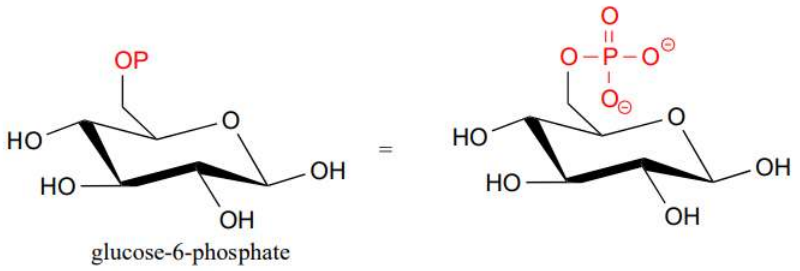
red = bridging oxygen
blue = non-bridging oxygen

A single phosphate is linked to two organic groups is called phosphate diester. The backbone of DNA is linked by phosphate diesters.



phosphate diester

Organic phosphates are often abbreviated using '*OP*' and '*OPP*' for mono- and diphosphates, respectively. For example, glucose-6-phosphate and isopentenyl diphosphate are often depicted as shown below. Notice that the 'P' abbreviation includes the associated oxygen atoms and negative charges.



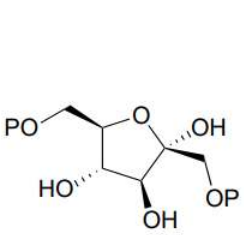
glucose-6-phosphate



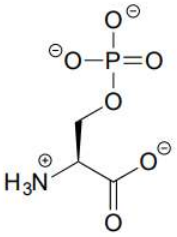
isopentenyl diphosphate

Exercise 1.2.1

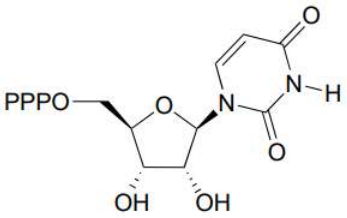
Consider the biological compounds below, some of which are shown with abbreviated structures:



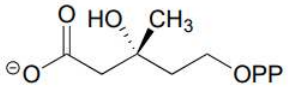
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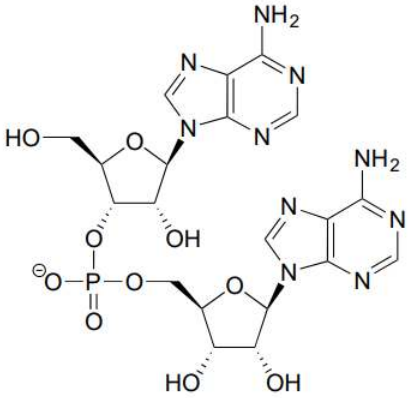
II



III



IV

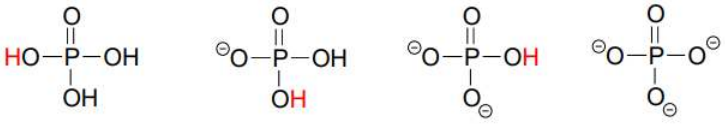


V

a. Which contain one or more phosphate anhydride linkages? Specify the number of phosphate anhydride linkages in your answers.
b. Which contain one or more phosphate monoesters? Again, specify the number for each answer.
c. Which contain a phosphate diester?
d. Which could be described as an organic diphosphate?
e. For each compound, specify the number of bridging and non-bridging oxygens in the phosphate group.

Acid constants and protonation states

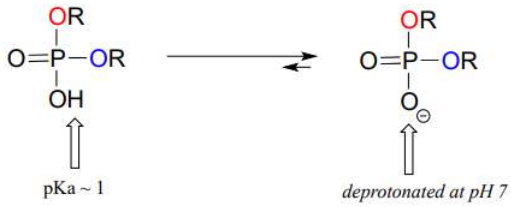
Phosphoric acid is triprotic, meaning that it has three acidic protons available to donate, with pK_a values of 1.0, 6.5, and 13.0, respectively. (da Silva and Williams)



H_3PO_4 pKa = 1.0 | $H_2PO_4^{-1}$ pKa = 6.5 | HPO_4^{-2} pKa = 13.0 | PO_4^{-3}

These acid constant values, along with the Henderson-Hasselbalch equation (section 7.2) tell us that, at the physiological pH of approximately 7, somewhat more than half of the phosphate species will be in the HPO_4^{-2} state, and slightly less than half will be in the $H_2PO_4^{-1}$ state, meaning that the average net charge is between -1.5 and -2.0.

Phosphate diesters have a pK_a of about 1, meaning that they carry a full negative charge at physiological pH.



Organic monophosphates, diphosphates, and triphosphates all have net negative charges and are partially protonated at physiological pH, but by convention are usually drawn in the fully deprotonated state.

Exercise 1.2.2

Explain why the second pK_a of phosphoric acid is so much higher than the first pK_a.

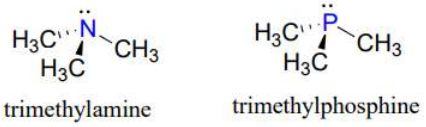
Exercise 1.2.3

What is the approximate net charge of inorganic phosphate in a solution buffered to $pH1$?

Recall from section 8.4 that good leaving groups in organic reactions are, as a rule, weak bases. In laboratory organic reactions, leaving groups are often halides or toluenesulfonates (section 8.4), both of which are weak bases. In biological organic reactions, phosphates are very common leaving groups. These could be inorganic phosphate, inorganic pyrophosphate, or organic monophosphates, all of which are weakly basic, especially when coordinated to metal cations such as Mg^{+2} in the active site of an enzyme. We will see many examples of phosphate leave groups in this and subsequent chapters.

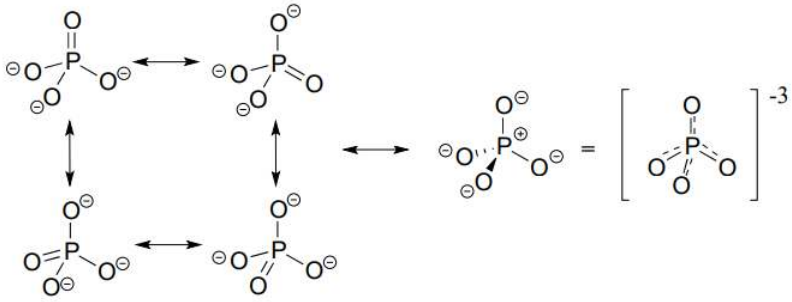
Bonding in phosphates

Looking at the location of phosphorus on the periodic table, you might expect it to bond and react in a fashion similar to nitrogen, which is located just above it in the same column. Indeed, phosphines - phosphorus analogs of amines - are commonly used in the organic laboratory.



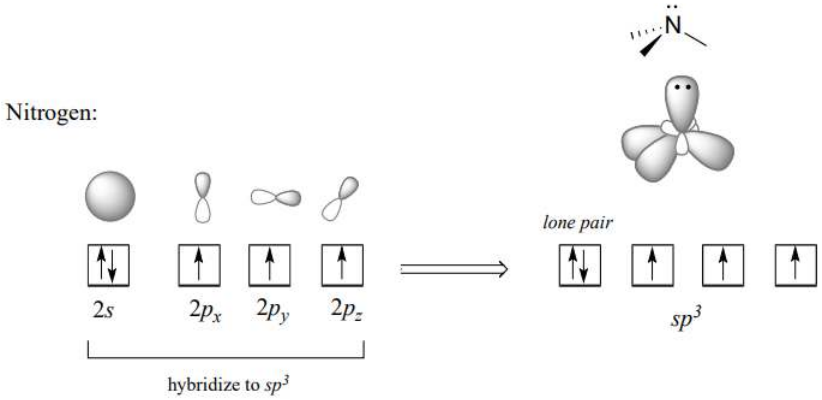
However it is in the form of phosphate, rather than phosphine, that phosphorus plays its main role in biology.

The four oxygen substituents in phosphate groups are arranged about the central phosphorus atom with tetrahedral geometry, however there are a total of five bonds to phosphorus - four s bonds and one delocalized π bond.

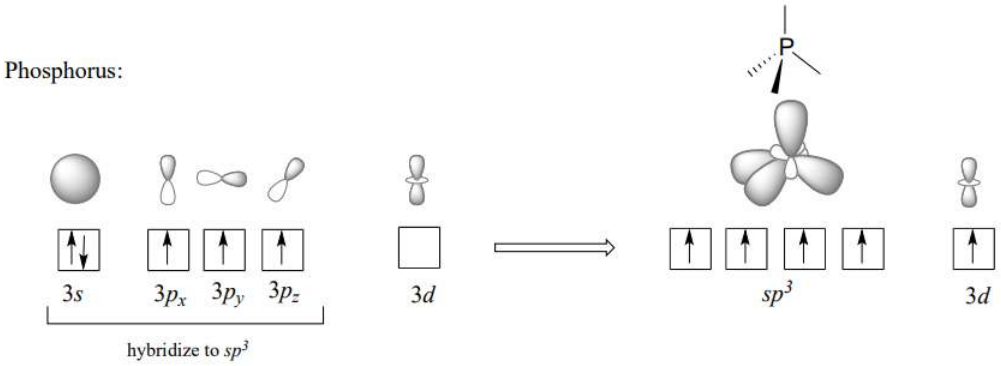


Phosphorus can break the 'octet rule' because it is on the third row of the periodic table, and thus has d orbitals available for bonding. The minus 3 charge on a fully deprotonated phosphate ion is spread evenly over the four oxygen atoms, and each phosphorus-oxygen bond can be considered to have 25% double bond character: in other words, the bond order is 1.25.

Recall from section 2.1 the hybrid bonding picture for the tetrahedral nitrogen in an amine group: a single **2*s*** and three **2*p*** orbitals combine to form four sp^3 hybrid orbitals, three of which form s bonds and one of which holds a lone pair of electrons.

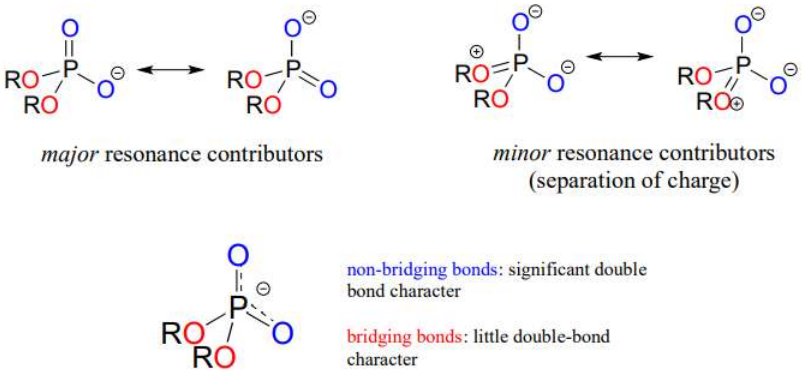


In the hybrid orbital picture for phosphate ion, a single **3*s*** and three **3*p*** orbitals also combine to form four sp^3 hybrid orbitals with tetrahedral geometry. In contrast to an amine, however, four of the five valance electrons on phosphorus occupy sp^3 orbitals, and the fifth occupies an unhybridized **3*d*** orbital.



This orbital arrangement allows for four s bonds with tetrahedral geometry in addition to a fifth, delocalized p bond formed by p overlap between the half-filled **3*d*** orbital on phosphorus and **2*p*** orbitals on the oxygen atoms.

In phosphate esters, diesters, and anhydrides the π bonding is delocalized primarily over the non-bridging bonds, while the bridging bonds have mainly single-bond character. In a phosphate diester, for example, the two non-bridging oxygens share a -1 charge, as illustrated by the two major resonance contributors below. The bonding order for the bridging $P-O$ bonds in a phosphate diester group is about 1, and for the non-bridging $P-O$ bonds about 1.5. In the resonance contributors in which the bridging oxygens are shown as double bonds (to the right in the figure below), there is an additional separation of charge - thus these contributors are minor and make a relatively unimportant contribution to the overall bonding picture.



Exercise 1.2.4

Draw all of the resonance structures showing the delocalization of charge on a (fully deprotonated) organic monophosphate. If a 'bond order' of 1.0 is a single bond, and a bond order of 2.0 is a double bond, what is the approximate bond order of bridging and non-bridging $P-O$ bonds?

Throughout this book, phosphate groups will often be drawn without attempting to show tetrahedral geometry, and π bonds and negative charges will usually be shown localized to a single oxygen. This is done for the sake of simplification - however it is important always to remember that the phosphate group is really tetrahedral, the negative charges are delocalized over the non-bridging oxygens, and that there is some degree of protonation at physiological pH (with the exception of the phosphate diester group).

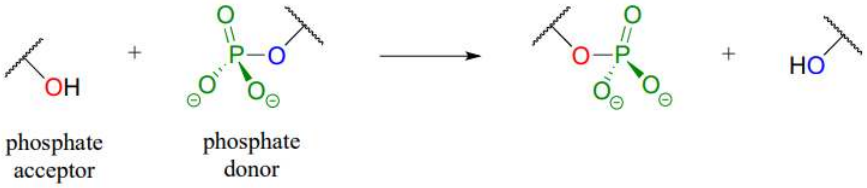
Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

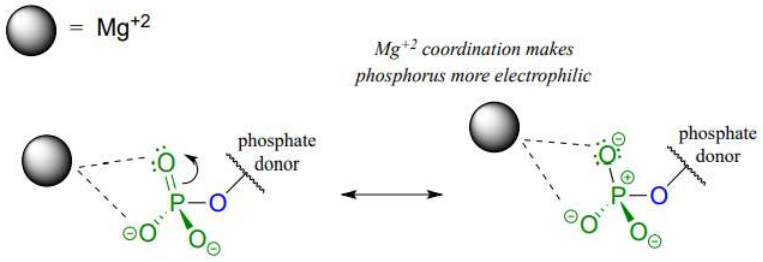
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1.3: Phosphate Transfer Reactions - An Overview

In a phosphate transfer reaction, a phosphate group is transferred from a **phosphate group** donor molecule to a phosphate group acceptor molecule:



A very important aspect of biological phosphate transfer reactions is that the electrophilicity of the phosphorus atom is usually enhanced by the Lewis acid (electron-accepting) effect of one or more magnesium ions. Phosphate transfer enzymes generally contain a Mg^{2+} ion bound in the active site in a position where it can interact with non-bridging phosphate oxygens on the substrate. The magnesium ion pulls electron density away from the phosphorus atom, making it more electrophilic.

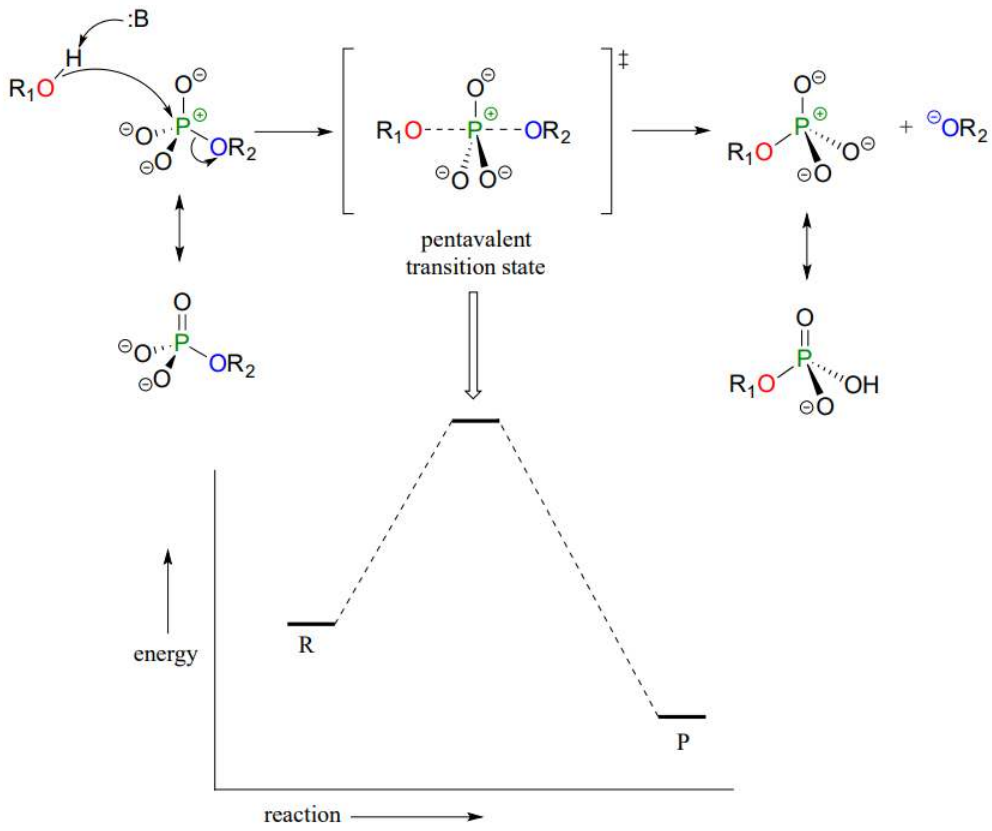


Without this metal ion interaction, a phosphate is actually a poor electrophile, as the negatively-charged oxygens shield the phosphorus center from attack by a nucleophile.

> **Note**
>
> For the sake of simplicity and clarity, we will not draw the magnesium ion or other active site groups interacting with phosphate oxygens in most figures in this chapter - but it is important to keep in mind that these interactions play an integral role in phosphate transfer reactions.

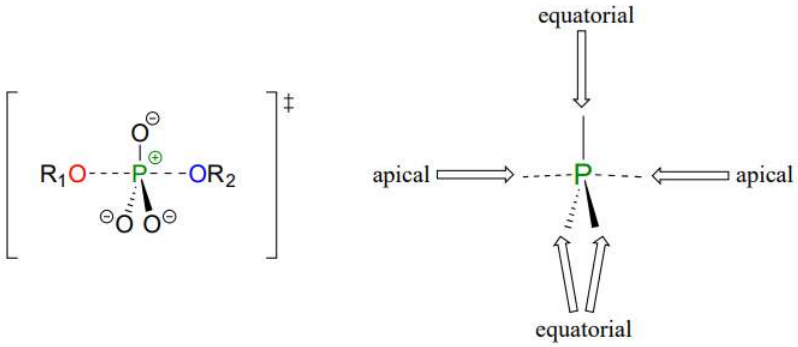
Mechanistically speaking, a phosphate transfer reaction at a phosphorus center can be though of as much like a S_N2 reaction at a carbon center. Just like in an S_N2 reaction, the nucleophile in a phosphoryl transfer approaches the electrophilic center from the backside, opposite the leaving group:

Concerted model:

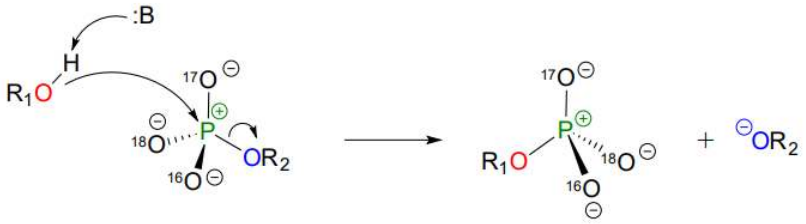


As the nucleophile gets closer and the leaving group begins its departure, the bonding geometry at the phosphorus atom changes from tetrahedral to trigonal bipyramidal at the pentavalent (5-bond) transition state. As the phosphorus-nucleophile bond gets shorter and the phosphorus-leaving group bond grows longer, the bonding picture around the phosphorus atom returns to its original tetrahedral state, but the stereochemical configuration has been 'flipped', or inverted.

In the trigonal bipyramidal transition state, the five substituents are not equivalent: the three non-bridging oxygens are said to be equatorial (forming the base of a trigonal bipyramid), while the nucleophile and the leaving group are said to be apical (occupying the tips of the two pyramids).



Although stereochemical inversion in phosphoryl transfer reactions is predicted by theory, the fact that phosphoryl groups are achiral made it impossible to observe the phenomenon directly until 1978, when a group of researchers was able to synthesize organic phosphate esters in which stable oxygen isotopes ^{17}O and ^{18}O were specifically incorporated. This created a chiral phosphate center.



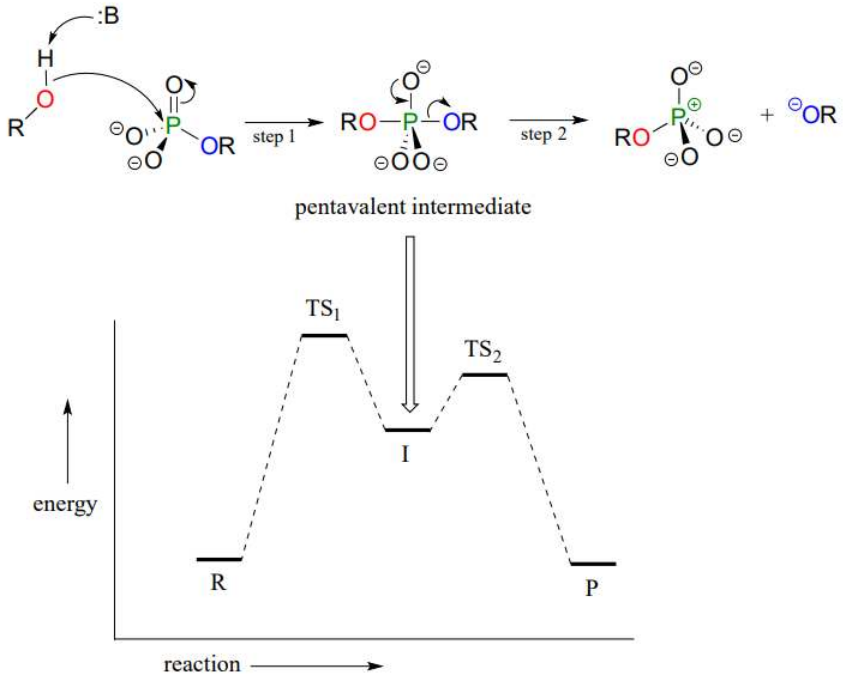
phosphate has *S* configuration phosphate has *R* configuration

Subsequent experiments with phosphoryl transfer-catalyzing enzymes confirmed that these reactions proceed with stereochemical inversion. (Nature 1978 275, 564; Ann Rev Biochem 1980 49, 877).

The concerted (S_N2-like) is not the only mechanism that has been proposed for these reactions - in fact, two other possible mechanisms have been suggested. In an alternative two-step mechanistic model, the nucleophile could attack first, forming

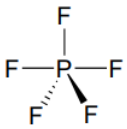
a pentavalent, trigonal bipyramidal intermediate (as apposed to a pentavalent transition state). The reaction is completed when the leaving group is expelled. The intermediate species would occupy an energy valley between the two transition states.

Addition-elimination model:

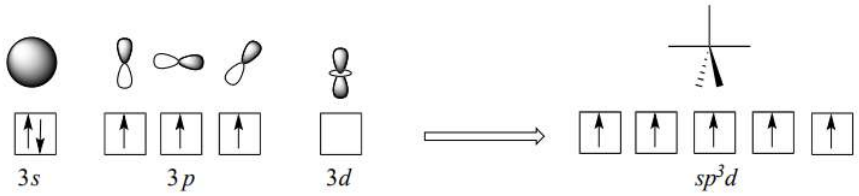


This is often referred to as an 'addition-elimination' mechanism - the nucleophile adds to the phosphate first, forming a pentavalent intermediate, and then the leaving group is eliminated.

An addition-elimination mechanism with a pentavalent intermediate is not possible for an S_N2 reaction at a carbon center, because carbon, as a second-row element, does not have any d orbitals and cannot form five bonds. Phosphorus, on the other hand, is a third-row element and is quite capable of forming more than four bonds. Phosphorus pentachloride, after all, is a stable compound that has five bonds to chlorine arranged in trigonal bipyramidal geometry around the central phosphorus.

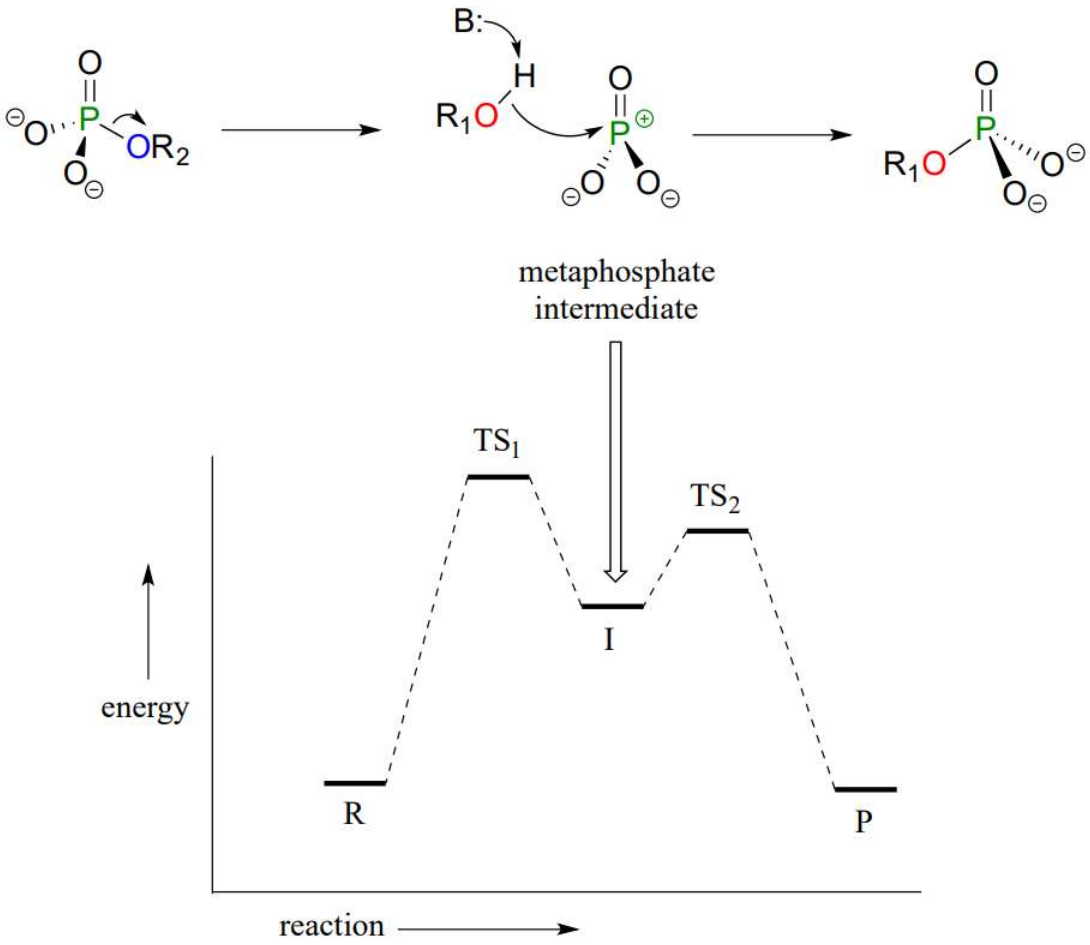


The phosphorus atom in PCl_5 (and in the hypothetical pentavalent intermediate pictured above) is considered to be sp^3d hybridized:



There is a third possibility: the reaction could proceed in an S_N1-like manner: in other words, elimination-addition. In this model, the phosphorus-leaving group bond breaks first, resulting in a metaphosphate intermediate. This intermediate, which corresponds to the carbocation intermediate in an S_N1 reaction and like a carbocation has trigonal planar geometry, is then attacked by the nucleophile to form the reaction product.

Elimination-addition model:



So which mechanistic model - concerted (SN_2-like), addition-elimination, or elimination-addition - best describes enzymatic phosphate transfer reactions? Chemists love to investigate and debate questions like this! Just like with the S_N1/S_N2 argument discussed in chapter 8, it really boils down to one question: which happens first, bond-forming or bond-breaking - or do these two events occur at the same time? From the evidence accumulated to date, it appears that many enzymatic phosphate transfer reactions can best be described by the concerted model, although there is still argument about this, and still many unanswered questions about other details of how these reactions are catalyzed in active sites. Considering the importance of phosphate transfer reactions in metabolic pathways, this area is clearly a very promising one for further investigation. If you are interesting in learning more

about this research, a great place to start is a review article written by Professor Daniel Herschlag at Stanford University (Annu. Rev. Biochem. 2011, 80, 669).

For the sake of simplicity and clarity, phosphoryl transfers in this text will be depicted using the concerted model.

Exercise 1.3.1

Predict the approximate angles between the two bonds indicated in a phosphate transfer transition state. O_a refers to an oxygen at the apical position, and O_e to an oxygen in the equatorial position.

a. $O_a - P - O_a$

b. $O_a - P - O_e$

c. $O_e - P - O_e$

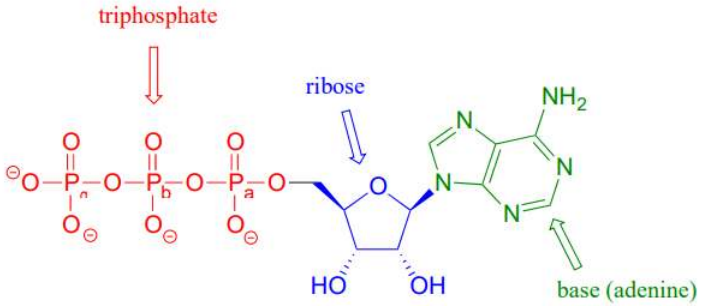
Contributors

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1.4: ATP, The Principal Phosphate Group Donor

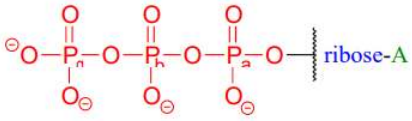
Thus far we have been very general in our discussion of phosphate transfer reactions, referring only to generic 'donor' and 'acceptor' species. It's time to get more specific. The most important donor of phosphate groups in the cell is a molecule called adenosine triphosphate, commonly known by its abbreviation ATP.

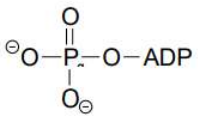


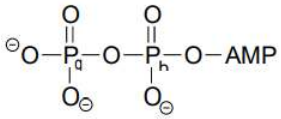
adenosine triphosphate (ATP)

that there are essentially three parts to the ATP molecule: an adenine nucleoside 'base', a five-carbon sugar (ribose), and triphosphate. The three phosphates are designated by Greek letters a, b, and g, with the a phosphate being the one closest to the ribose. Adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are also important players in the reactions of this chapter.

ATP is a big molecule, but the bond-breaking and bond-forming events we will be studying in this chapter all happen in the phosphate part of the molecule. You will see structural drawings of ATP, ADP, and AMP abbreviated in many different ways in this text and throughout the biochemical literature, depending on what is being illustrated. For example, the three structures below are all abbreviated depictions of ATP:



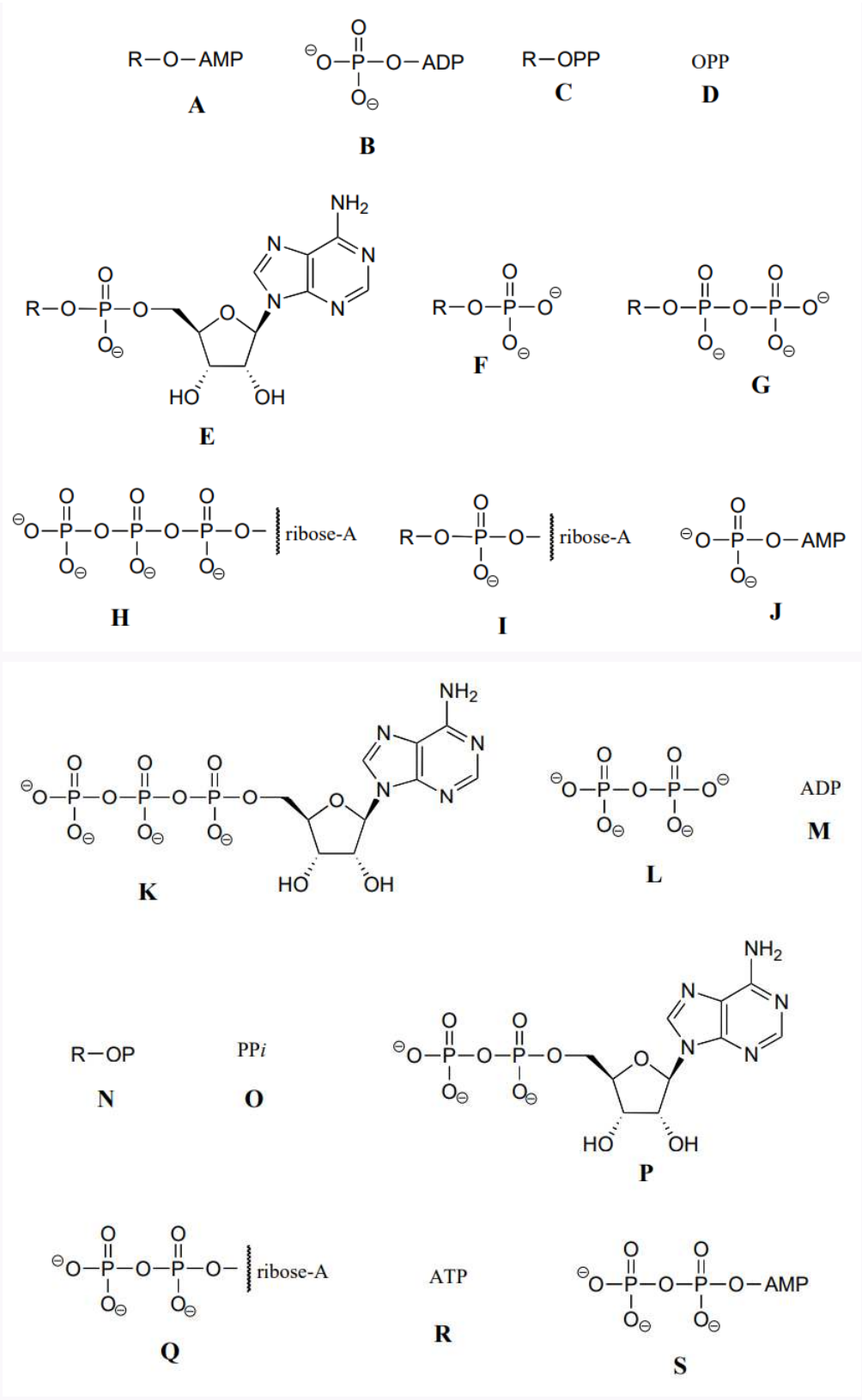




The following exercise will give you some practice in recognizing different abbreviations for ATP and other biological molecules that contain phosphate groups.

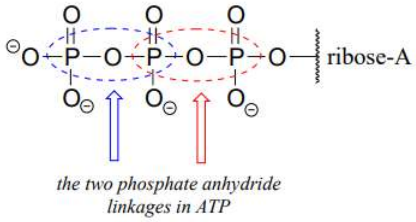
Exercise 1.4.1

Below are a number of representations, labeled A-S, of molecules that contain phosphate groups. Different abbreviations are used. Arrange A-S into groups of drawings that depict the same species (for example, group together all of the abbreviations which depict ATP).

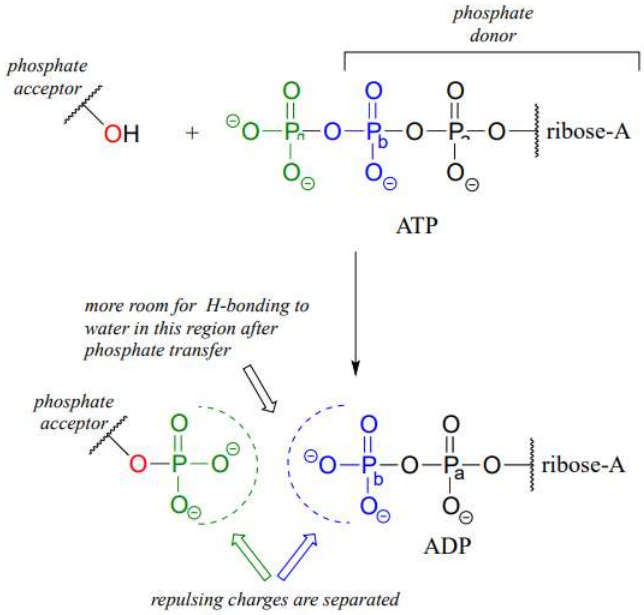


You are probably familiar with the physiological role of ATP from your biology classes - it is commonly called 'the energy currency of the cell'. What this means is that ATP stores energy we get from the oxidation of fuel molecules such as carbohydrates or fats.

The energy in ATP is stored in the two high-energy phosphate anhydride linkages.



When one or both of these phosphate anhydride links are broken as a phosphate group is transferred to an acceptor, a substantial amount of energy is released. The negative charges on the phosphate groups are separated, eliminating some of electrostatic repulsion that existed in ATP. One way to picture this is as a coil springing open, releasing potential energy.

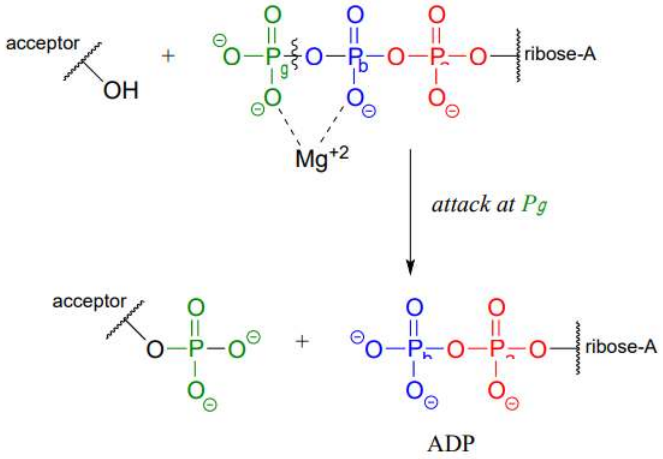


In addition, cleavage of a phosphate anhydride bond means that surrounding water molecules are able to form more stabilizing hydrogen-bonding interactions with the products than was possible with the starting materials, again making the reaction more 'downhill', or exergonic.

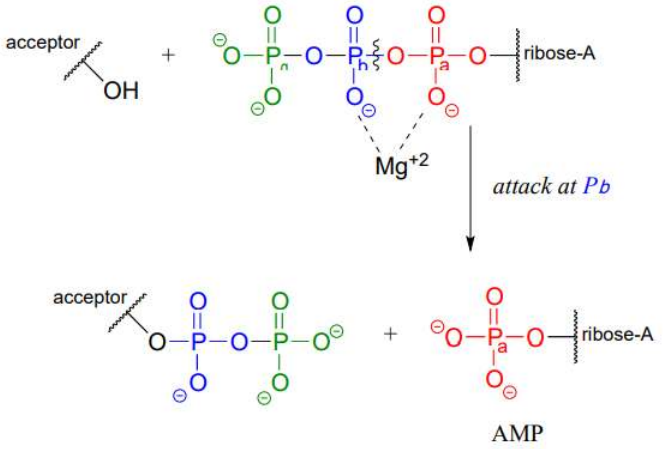
It is important to understand that while the phosphate anhydride bonds in ATP are thermodynamically unstable (they contain a great deal of chemical energy), they are at the same time kinetically stable: ATP-cleaving reactions are exothermic, but also have a high energy barrier, making them very slow unless catalyzed by an enzyme. In other words, the release of the energy contained in ATP is highly energetic but also subject to tight control by the interaction of highly evolved enzymes in our metabolic pathways.

ATP is a versatile phosphate group donor: depending on the site of nucleophilic attack (at the α, β, or γ phosphorus), different phosphate transfer outcomes are possible. Below are the three most common patterns seen in the central metabolic pathways. A 'squigly' line in each figure indicates the $P-O$ bond being broken. We will study specific examples of each of these in the coming sections.

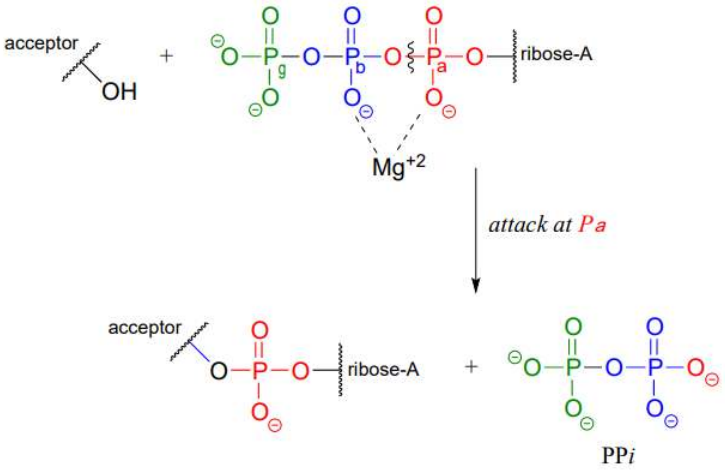
Attack at the γ-phosphate:



Attack at the β-phosphate:



Attack at the α-phosphate:



Note

The common thread running through all of the ATP-dependent reactions we will see in this section is the idea that the phosphate acceptor molecule is undergoing a thermodynamically 'uphill' transformation to become a more reactive species. The energy for this uphill

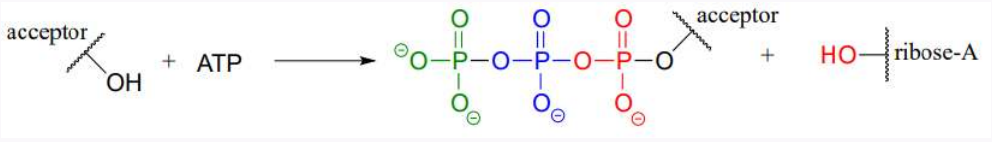
transformation comes from breaking a high-energy phosphate anhydride bond in ATP. That is why ATP is often referred to as 'energy currency': the energy in its anhydride bonds is used to 'pay for' a thermodynamically uphill chemical step.

Exercise 1.4.2

Propose a fourth hypothetical phosphate transfer reaction between ATP and the generic acceptor molecule in the figure above, in which inorganic phosphate (Pi) is a by-product.

Exercise 1.4.3

Why is this hypothetical phosphate transfer reaction less energetically favorable compared to all of the possible ATP-cleaving reactions shown in the figure above?



Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

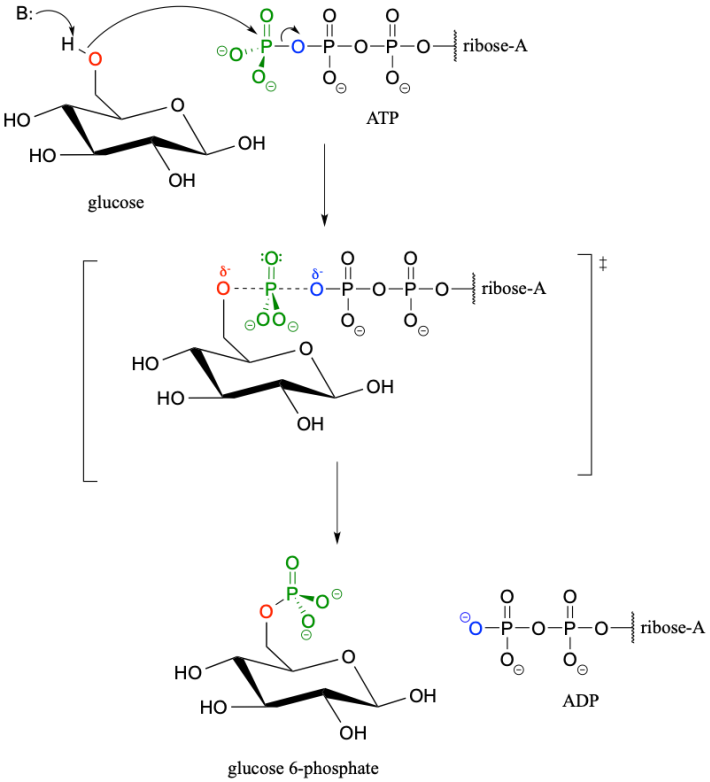
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1.5: Phosphorylation of Alcohols

A broad family of enzymes called kinases catalyze transfer of a phosphate group from ATP to an alcohol acceptor. Mechanistically, the alcohol oxygen acts as a nucleophile, attacking the electrophilic γ-phosphorus of ATP and expelling ADP.

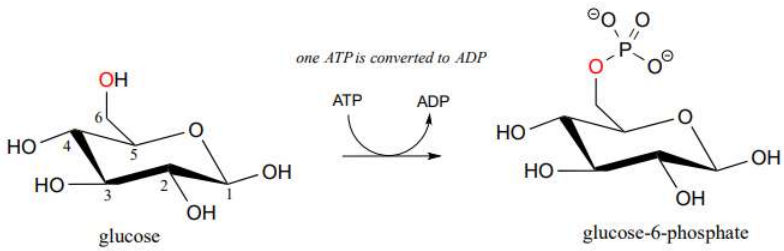
Glucose is phosphorylated in the first step of the glycolysis pathway by the enzyme hexose kinase (EC 2.7.1.1), forming glucose-6-phosphate.

Hexose kinase mechanism

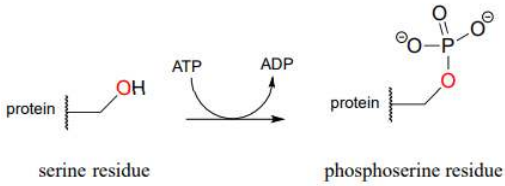


Video Tutorial

Here is a shorthand way to depict this reaction. Notice the "ATP in, ADP out" notation used below, indicating that ATP is one of the reactants and ADP is one of the products. From here on, we will frequently use this common convention to indicate reaction participants whose structures are not drawn out in a figure.



The biological activity of many proteins is regulated by protein kinases. In a protein kinase reaction, the side chain hydroxyl groups on serine, threonine, or tyrosine residues of certain proteins are phosphorylated by ATP:



The conversion of a neutral hydroxyl group to a charged phosphate represents a very dramatic change in the local architecture of the protein, potentially altering its folding pattern and ability to bind to small molecules or other proteins. A protein's biological function can be 'switched on' by phosphorylation of a single residue, and switched off again by removal of the phosphate group. The latter reaction we will examine later in this chapter.

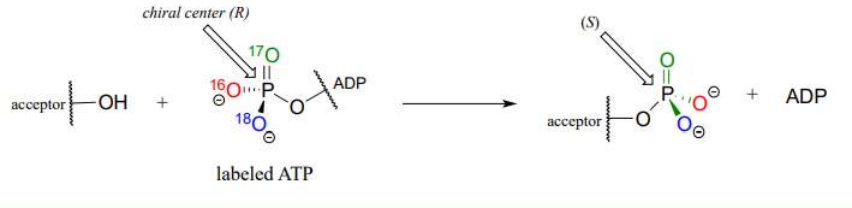
Exercise 1.5.1

a. Draw a curved-arrow mechanism, using abbreviations as appropriate, for the serine kinase reaction.

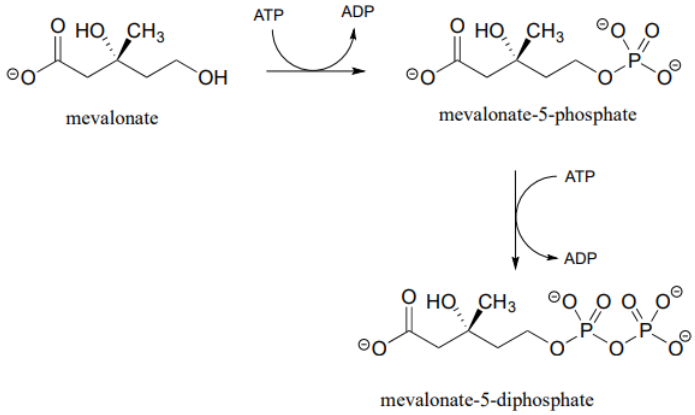
b. Threonine kinase catalyzes the phosphorylation of the side chain hydroxyl group of threonine residues in proteins. Draw the structure, including the configuration of all stereocenters, of a phosphothreonine residue. Explain how you can predict the stereochemistry of the side chain.

Note

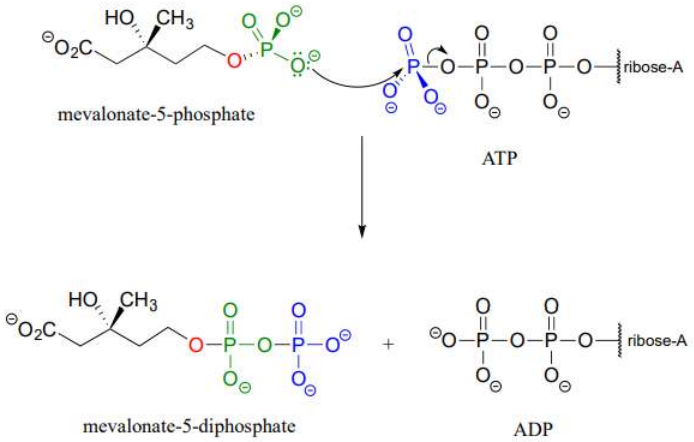
Although stereochemical inversion in phosphate transfer is predicted by theory, the fact that phosphate groups are achiral made it impossible for a long time to verify the phenomenon directly. This was finally accomplished in the late 1970's, when a group of researchers demonstrated phosphate inversion in kinase enzymes using chemically synthesized ATP in which three different isotopes of oxygen were incorporated into the γ-phosphate, thus creating a chiral phosphorus center. (Ann. Rev. Biochem. 1980 49, 877).



Alcohols can be converted into organic diphosphates in two different ways. A two-step process simply involves successive transfers of the γ-phosphate groups of two ATP donors, such as in these sequential steps in isoprenoid biosynthesis. (EC 2.7.1.36; EC 2.7.4.2). A compound called mevalonate is diphosphorylated in this way in the early phase of the biosynthetic pathway for cholesterol, steroid hormones, and other isoprenoid molecules.



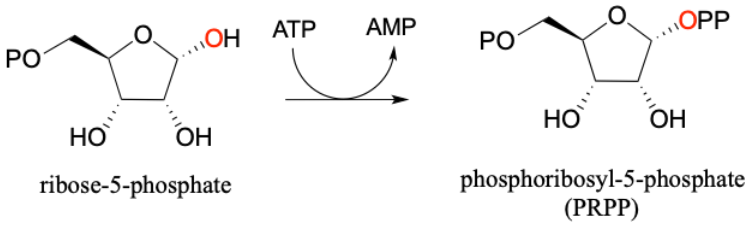
The mechanism for the first phosphorylation step is analogous to that for an alcohol kinase reaction, which we have just seen. In the second phosphate transfer step, catalyzed by a separate enzyme, one of the phosphate oxygens on the organic monophosphate acts as a nucleophilic phosphate acceptor, attacking the γ-phosphate of a second ATP.



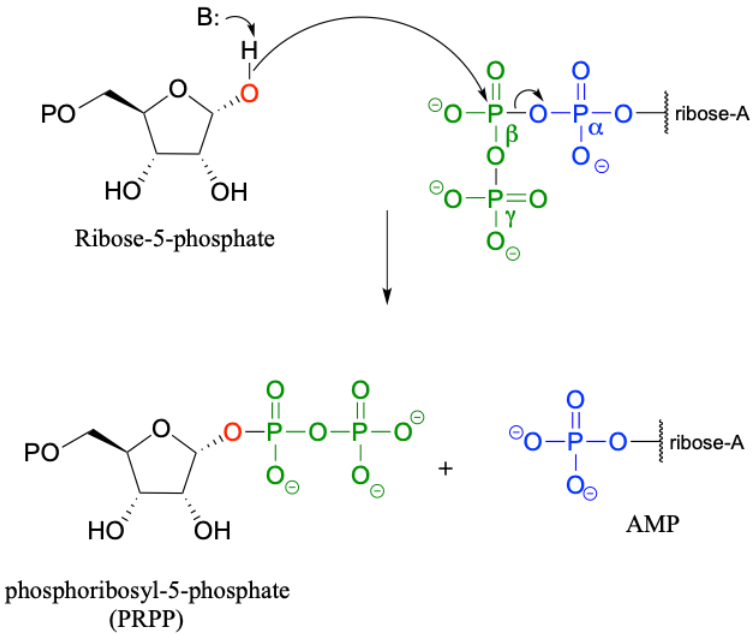
In some metabolic pathways, diphosphorylation occurs by a different mechanism from the one above. stead of sequentially transferring two phosphates from two ATP donors, the alternate mechanism occurs in a single step: the nucleophilic acceptor molecule attacks the β-phosphate of ATP, rather than the γ-phosphate. After formation of the trigonal bipyramidal intermediate, it is AMP (not ADP) which is expelled, and what started out as the β and γ phosphates of ATP both remain with the acceptor.

In the biosynthesis of DNA and RNA nucleotides, one of the hydroxyl groups on ribose-5-phosphate is diphosphorylated (EC 2.7.6.1) in a one-step mechanism:

A one-step alcohol diphosphorylation reaction (PRPP synthase):



Mechanism



The metabolic role of both of the diphosphorylation processes we just saw is to convert a hydroxyl group into a good leaving group (recall that hydroxide ions are strong bases and poor leaving groups, while phosphates/diphosphates, especially when stabilized in an enzyme active site, are weak bases and very good leaving groups). In nucleoside biosynthesis pathways, the diphosphate group of PRPP acts as a leaving group in the very next metabolic step, which is an S_N1 reaction: we have already seen this reaction in section 8.7).

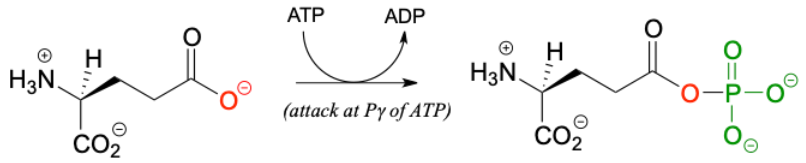
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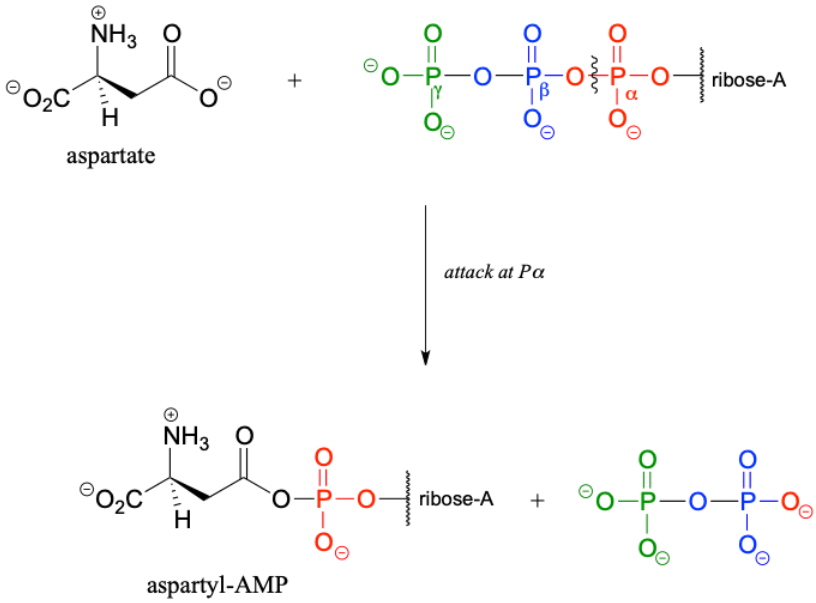
1.6: Phosphorylation of Carboxylates

Thus far we have seen hydroxyl oxygens and phosphate oxygens acting as nucleophilic accepting groups in ATP-dependent phosphate transfer reactions. Carboxylate oxygens can also accept phosphate groups from ATP. This typically happens in two different ways. First, the carboxylate can attack the γ-phosphate of ATP to accept phosphate, generates a species known as an 'acyl phosphate'. An example is the first part of the reaction catalyzed by glutamine synthase (EC 6.3.1.2):



glutamate glutamyl phosphate

Alternatively, carboxylate groups are often converted into a species referred to as an 'acyl-AMP' . Here, the carboxylate oxygen attacks the α-phosphate of ATP leading to release of inorganic pyrophosphate. An example is the first part of the reaction catalyzed by the enzyme asparagine synthetase: (EC 6.3.5.4):

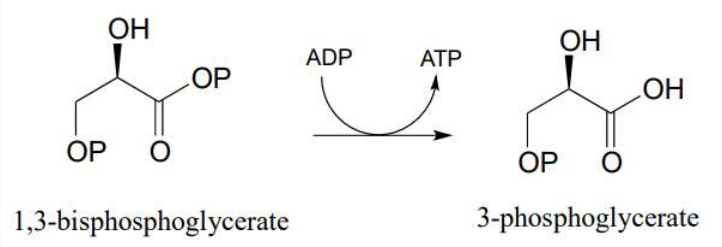


aspartate

aspartyl-AMP

Exercise 1.6.1

Draw a curved-arrow mechanism for the phosphate transfer reaction shown below (EC 2.7.2.3), which is from the glycolysis pathway. Note that ADP is on the reactant side and ATP is a product (the opposite of what we have seen so far). Hint: What functional group is the nucleophile? What functional group is the leaving group?



1,3-bisphosphoglycerate 3-phosphoglycerate

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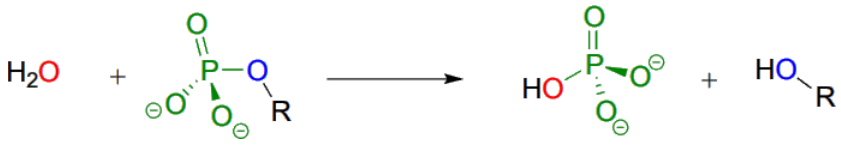
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1.7: Hydrolysis of Organic Phosphates

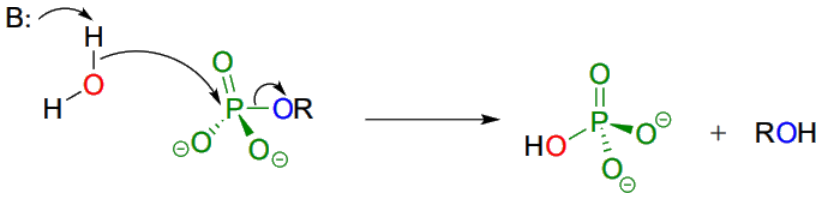
While kinase enzymes catalyze the phosphorylation of organic compounds, enzymes called phosphatases catalyze dephosphorylation reactions. The reactions catalyzed by kinases and phosphatases are not the reverse of one another: kinases irreversibly transfer phosphate groups from ATP (or sometimes other nucleoside triphosphates) to various organic acceptor compounds, while phosphatases transfer phosphate groups from organic compounds to water: these are hydrolysis reactions. Kinase reactions involve an inherently 'uphill' step (phosphorylation of an alcohol, for example) being paid for with an inherently 'downhill' step (cleavage of an anhydride bond in ATP). Phosphatase reactions, on the other hand, are thermodynamically 'downhill', and while they require an enzyme to speed them up, they do not involve 'spending' energy currency the way kinase reactions do.

Phosphatase reaction:

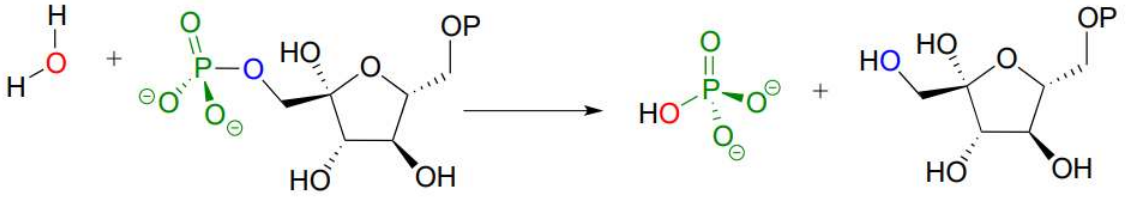


There are two possible general mechanisms for a phosphatase reaction. Some enzymes catalyze direct hydrolysis reactions, in which the phosphate group is removed by direct attack of a water molecule at the phosphate center:

Phosphatase mechanism (direct hydrolysis):



One of the two phosphate groups on fructose 1,6-bisphosphate is hydrolyzed in such a way late in the gluconeogenesis pathway. (Biochemistry 2000, 39, 8565; EC 3.1.3.11)



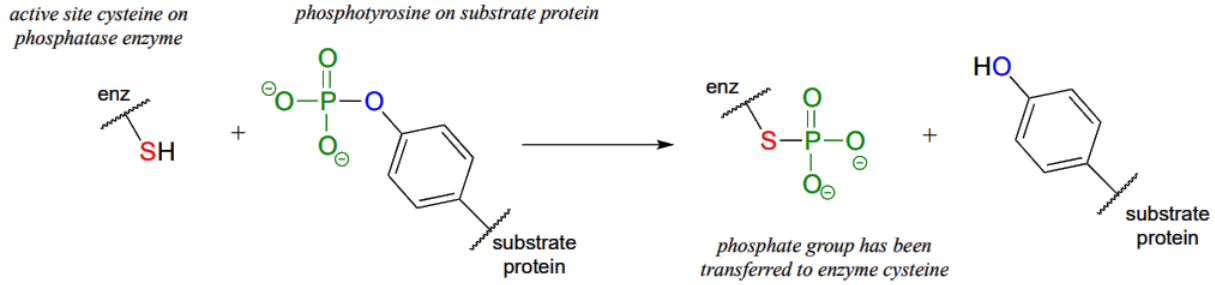
fructose 1,6-bisphosphate

fructose-6-phosphate

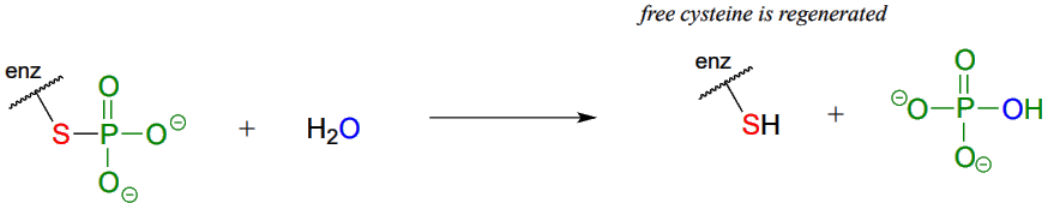
Many phosphatase reactions, however, operate by a slightly more complicated mechanism than what is shown above. In the first phase, a nucleophilic enzyme group (typically a cysteine, aspartate, glutamate, or histidine side chain–cysteine is used in the figure below) attacks the phosphate group. In the second phase, the phosphorylated residue is hydrolyzed. For example, protein tyrosine phosphatase catalyzes the dephosphorylation of phosphotyrosine residues in some proteins - this is the other half of the regulatory 'on-off switch' that we discussed earlier in the context of protein kinases. In the first step, the phosphate group is directly donated to a cysteine side chain in the phosphatase enzyme's active site. In the second step, the phosphocysteine intermediate is cleaved by water to form inorganic phosphate and regenerate the free cysteine in the active site.

Indirect phosphatase reaction:

Step 1:



Step 2:



Notice that in the end, the phosphate group has still been transferred to a water molecule, albeit indirectly. How would you know, just by looking at the substrate and product of the protein tyrosine phosphatase reaction, that the phosphate is not transferred directly to a water molecule? Simply put, you wouldn't know this information without the benefit of knowledge gained from biochemical experimentation.

Exercise 1.7.1

If you were to look just at the substrates and products of a phosphatase reaction without knowing anything about the mechanism, it is apparent that a *nucleophilic substitution* mechanism could theoretically account for the products formed. Draw out a hypothetical nucleophilic substitution mechanism for the hydrolysis of a phosphoserine residue and show how researchers, by running the reaction in H218O, (isotopically labeled water), could potentially distinguish between a nucleophilic substitution and phosphate group transfer mechanism by looking at where the 18O atom ends up in the products.

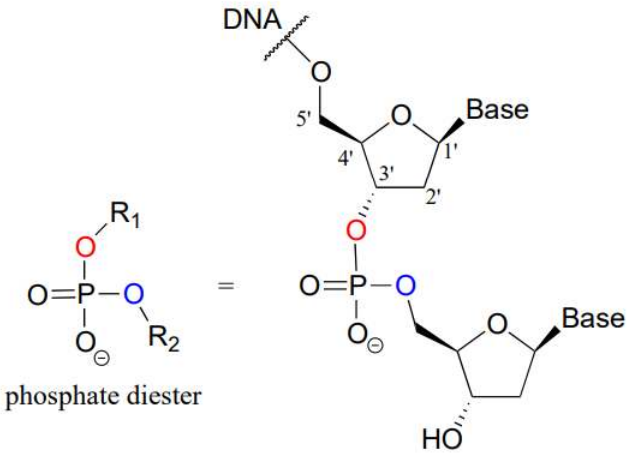
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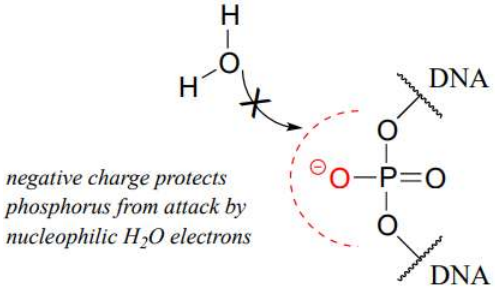
1.8: Phosphate Diesters in DNA and RNA

Phosphate diesters play an absolutely critical role in nature - they are the molecular 'tape' that connect the individual nucleotides in DNA and RNA via a sugar-phosphate backbone. Take note of the 1' - 5' carbon numbering shown below for the ribose sugar - these numbers will be used frequently in the coming discussion. The 'prime' symbol (') is used to distinguish the ribose carbon numbers from the nucleotide base carbon numbers (which are not shown here).



The introduction to this chapter referenced a widely-read 1987 commentary in Science Magazine, in which F.H. Westheimer of Harvard University addressed the question of why phosphates were 'chosen' by nature for critical biochemical job of linking DNA (Science 1987, 235, 1173). He emphasizes how critical it is for the phosphate diester linkage in DNA to be stable in water – in other words, it must be resistant to spontaneous (nonenzymatic) hydrolysis. Even very infrequent occurrence of such an undesired hydrolysis event could be disastrous for an organism, given that an intact DNA strand is a long-term storage mechanism for genetic information.

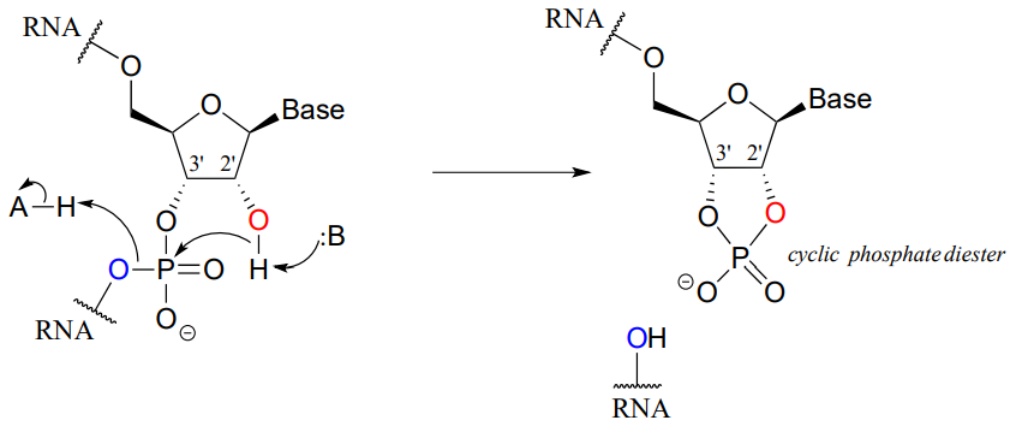
Westheimer pointed out that the inherent stability of DNA is a due in large part to the negative charge on the non-bridging oxygen of the phosphate diester linker, which effectively repels nucleophilic water molecules and shields the electrophilic phosphorus atom from attack.



While DNA is quite stable with regard to spontaneous hydrolysis, it of course can be degraded by specific enzymatic hydrolysis, where the phosphate electrophile is activated for attack through noncovalent interactions (e.g., with Mg^{2+}) in the active site. Enzymes that hydrolyze the phosphate diester bonds in DNA are called nucleases, and we will learn more about them in section 9.8.

Unlike DNA, RNA is quite vulnerable to spontaneous hydrolysis in aqueous solution. This does not present a physiological dilemma, because the function of RNA is to encode genetic information on a temporary rather than long-term basis. Why does hydrolysis occur so much more rapidly in RNA than in DNA? The answer has everything to do with the lowered entropic barrier to the reaction (you might want to quickly review the concept of entropy at this point). RNA nucleotides, unlike the deoxynucleotides of DNA, have a hydroxyl group at the neighboring 2' carbon. The 2' hydroxyl group is right next to the electrophilic phosphorus

atom, poised in a good position to make a nucleophilic attack, breaking the RNA chain and forming a cyclic phosphate diester intermediate (see figure below).



Researchers working with RNA have to be careful to store their samples at very cold temperatures, preferably freeze-dried or precipitated in ethanol, to avoid hydrolysis. The problem of RNA decomposition is compounded by the fact that RNAase enzymes, which catalyze RNA hydrolysis, are present on the surface of human skin and are very stable, long-lived, and difficult to destroy.

In contrast, DNA samples can be safely stored in aqueous buffer in a refrigerator, or in a freezer for longer-term storage.

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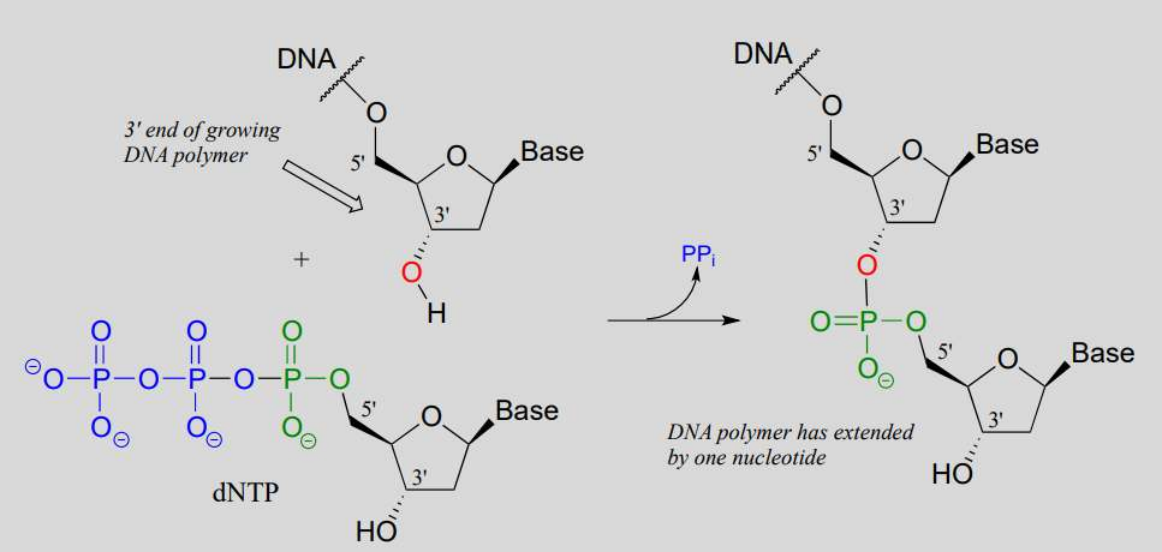
1.9: The Organic Chemistry of Genetic Engineering

Many enzymes that catalyze reactions involving the phosphate diester bonds of DNA have been harnessed for use in genetic engineering - techniques in which we copy, snip, and splice DNA in order to create custom versions of genes. The tools of genetic engineering have become indispensable and commonplace in the past decade, and most researchers working on the biological side of chemistry use them extensively. The days of painstakingly purifying an enzyme from bacterial cultures or ground-up cow livers are pretty much gone. Now scientists clone the gene that encodes the enzyme, make any desired changes (by site-directed mutagenesis, for example), and use a host such as *E. coli* or yeast to produce 'recombinant' enzyme from the cloned gene. You will learn the details of many of these procedures in a biochemistry or molecular biology course. What we will focus on now is applying what we have learned about phosphate group transfer reactions so that we can recognize some of the organic chemistry that is happening in a cloning experiment.

The first thing you have to do in a gene cloning procedure is to copy a DNA strand. This is accomplished by an enzyme called DNA polymerase (EC 2.7.7.7), which uses a single strand of DNA as a template to synthesize a second, complementary strand (the full picture of this complex process is well beyond the scope of this book, but recall that we talked about the discovery of thermostable DNA polymerase in the introduction to chapter 6).

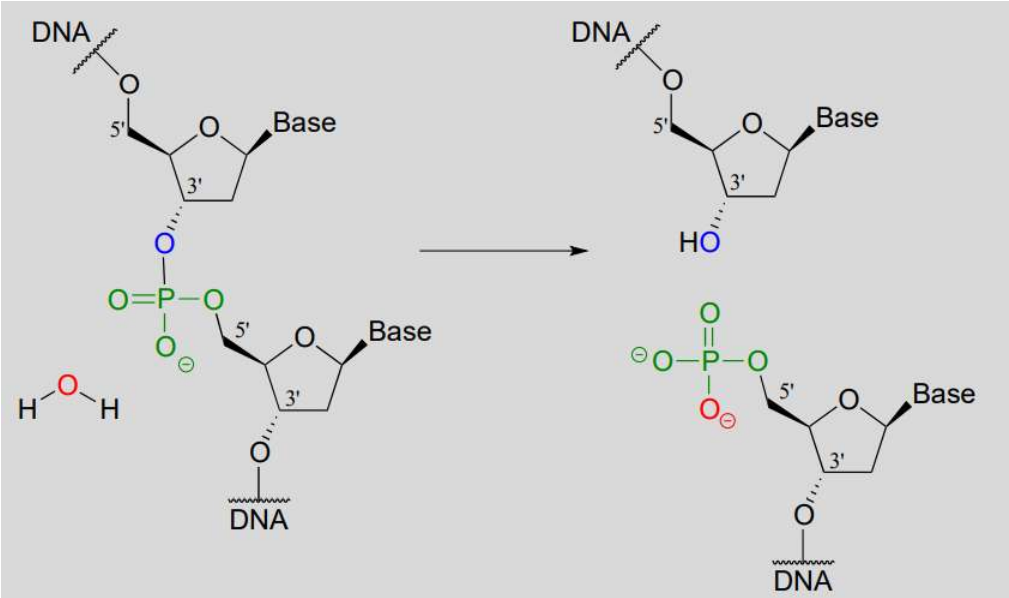
may have learned in a biology class that DNA is synthesized in the 3' to 5' direction. Notice below that the 3' hydroxyl group on the end of the growing DNA strand attacks the α-phosphate of a 2'-deoxynucleoside triphosphate (dNTP), expelling inorganic pyrophosphate.

DNA polymerase reaction:



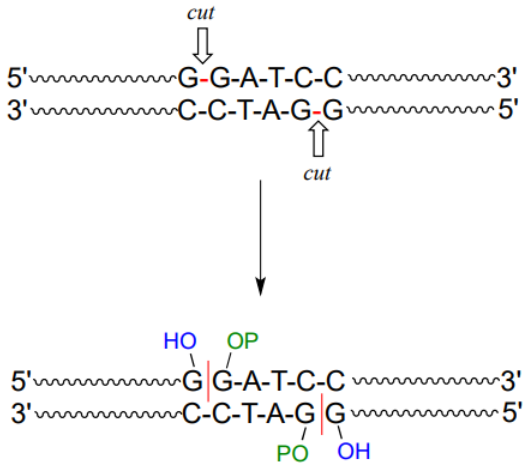
Scientists are able to cut DNA using 'molecular scissor' enzymes called restriction endonucleases that cleave double-stranded DNA by hydrolysis at specific base sequences.

DNA hydrolysis by restriction endonucleases:



Notice that the result of this hydrolytic cleavage reaction is one segment of DNA with a hydroxyl group at the 3' position, and a second segment with a phosphate group at the 5' position.

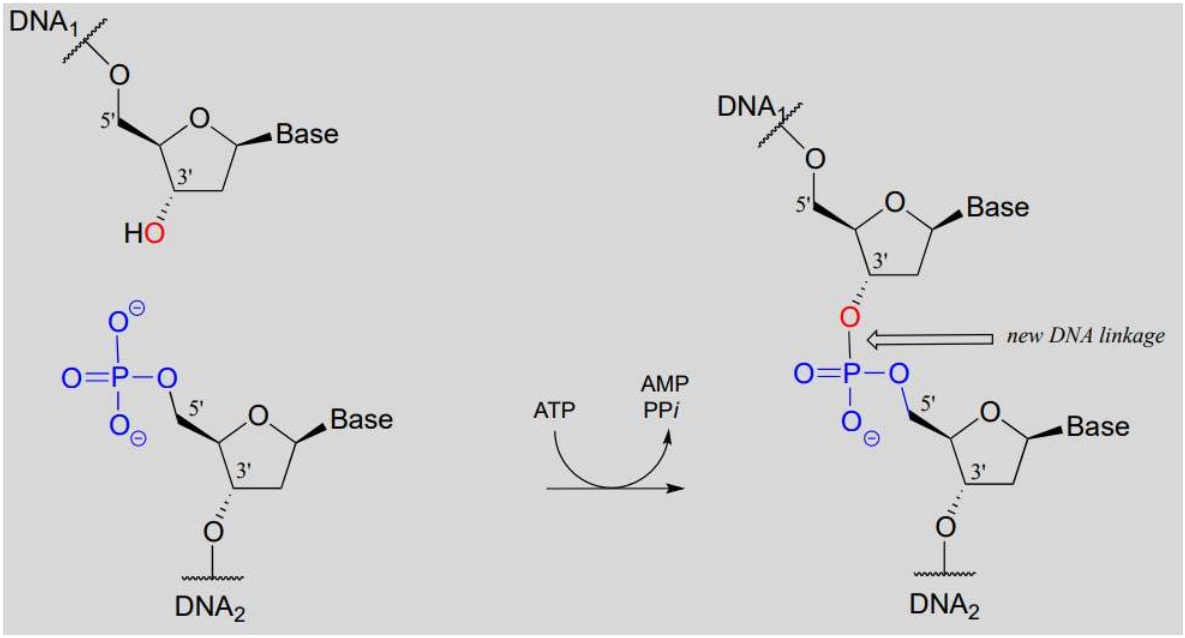
A commonly used restriction endonuclease called 'BamHI' cleaves double-stranded DNA specifically at the following 6-base sequence:



Notice that a 'staggered' cut is made: this is a common (and useful) property of many endonucleases, although some make 'blunt-ended' cuts.

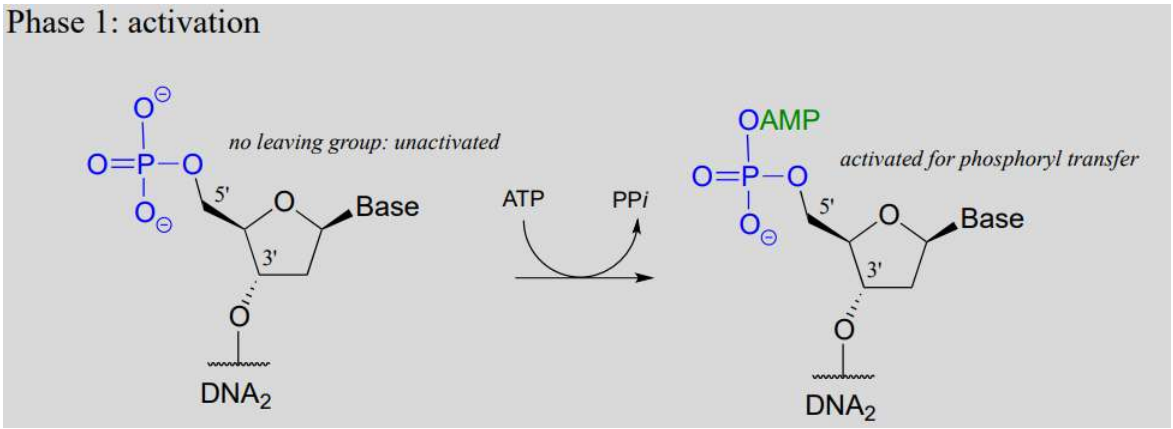
While an endonuclease cleaves a phosphodiester linkage in a DNA strand, DNA ligase (EC 6.5.1.1) accomplishes the reverse process: it catalyzes the formation of a new 3'-5' link between two strands:

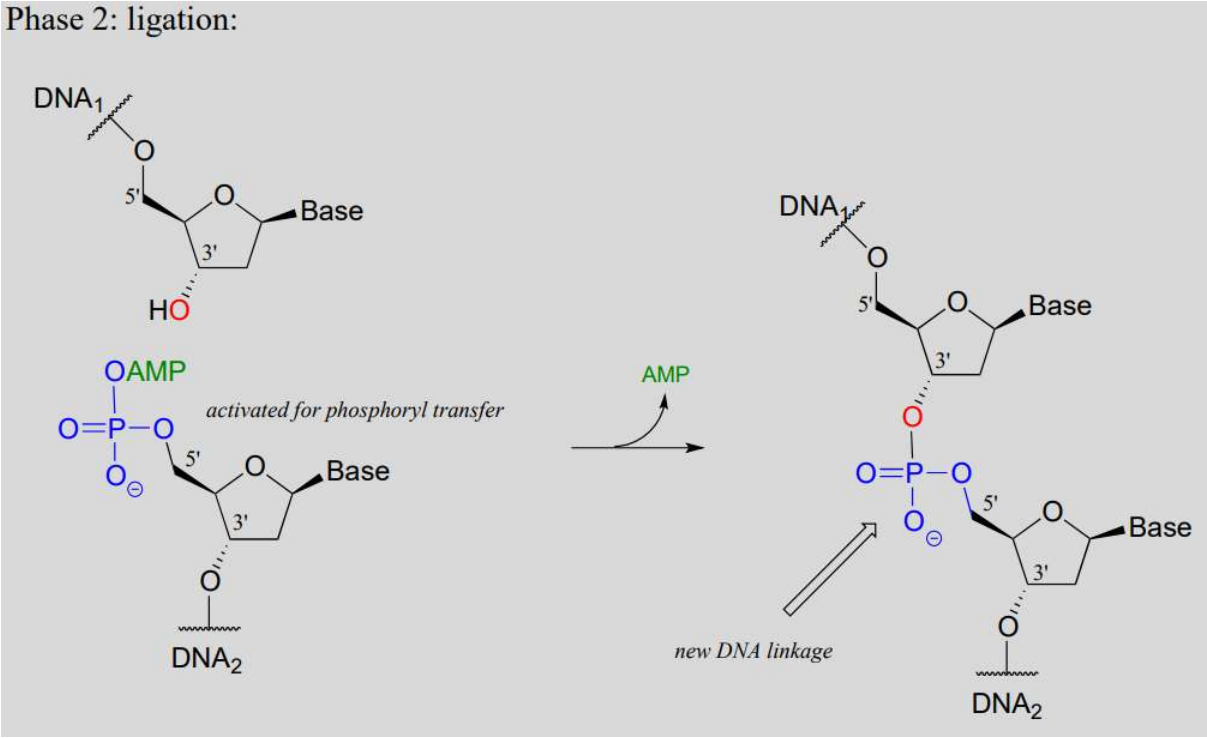
DNA ligase reaction:



Note that there is initially no leaving group on the 5' phosphate of DNA_2, which makes a direct phosphate transfer reaction impossible. The strategy employed by the DNA ligase enzyme is to first activate the 5' phosphate of DNA_2 using ATP (phase 1 below), then the ligation reaction can proceed (phase 2)

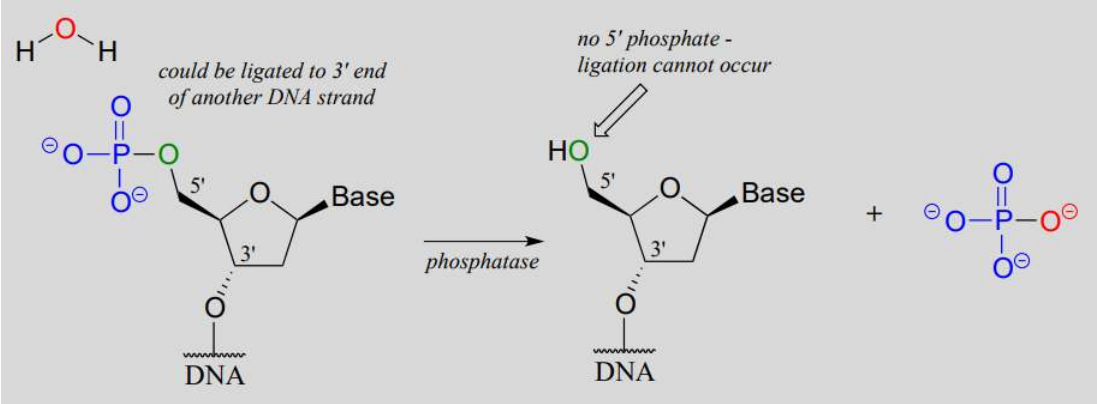
DNA ligation





One more enzymatic tool in the genetic engineering arsenal bears mention. In some cloning procedures, a researcher may want to prevent unwanted ligation of DNA. This can be accomplished by using the enzyme alkaline phosphatase (EC 3.1.3.1), which catalyzes the dephosphorylation of many different organic phosphates, including 5'-phosphorylated DNA (recall that we discussed phosphatases in section 9.6).

Alkaline phosphatase reaction:



With the phosphate group removed, ligation is impossible - there is no way to make a new phosphodiester bond without a 5' phosphate group!

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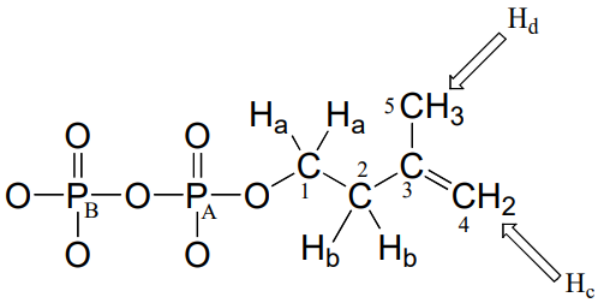
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1.10: NMR of phosphorylated compounds

Because so many biological molecules contain phosphoryl groups, it is worthwhile to look at how scientists use NMR to determine the structure of these molecules. Recall from section 5.1 that ^{31}P, the most abundant isotope of phosphorus, is NMR active: it can be directly observed by $^{31}P-NMR$, and indirectly observed in $^{1}H-NMR$ and $^{13}C-NMR$ through its spin-coupling interactions with neighboring protons and carbons, respectively.

Consider the case of isopentenyl diphosphate, the building block molecule used by cells to make 'isoprenoid' compounds such as cholesterol (in many animals), or β-carotene (in some plants). NMR spectra of this molecule were taken in a D_2O solvent, buffered with ND_4OD (the deuterium equivalent of aqueous ammonium hydroxide, NH_4OH) (J. Org. Chem. 1986, 51, 4768). In our discussion, carbon atoms are specified with numbers, protons with lower case letters, and phosphorus atoms with upper case letters.



First, let's look at the proton spectrum:

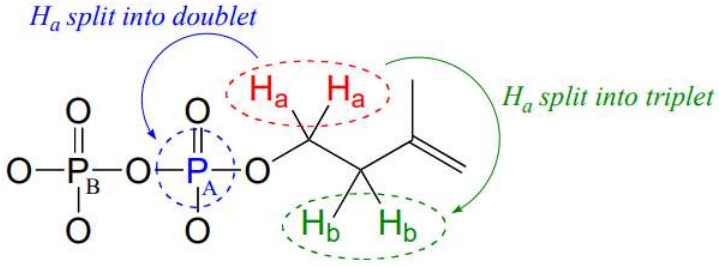
^{1}H-NMR

H_a: 4.05 ppm (td); $^{3}J_{Ha\text{-}Hb}$ = 6.6 Hz; $^{3}J_{Ha\text{-}PA}$ = 3.3 Hz.

H_b: 2.39 ppm (t) $^{3}J_{Ha\text{-}Hb}$ = 6.6 Hz

H_c: 4.86 ppm (s)

H_d: 1.77 ppm (s)



The signals for H_b, H_c, and H_d look like we would expect from our discussion in chapter 5, with the exception of H_c which you will be invited to discuss in the exercise below. Why, though, is the signal for H_a split into a triplet of doublets (td)? First of all, as, expected, the two neighboring H_b protons split the Ha signal into a triplet, with $^{3}J_{H\text{-}H}$ = 6.6 Hz. Then, **the signal is further split into doublets ($^{3}J_{H\text{-}P}$ = 3.3 Hz) by P_A, the closer of the two phosphorus atoms**. A phosphorus atom will spin-couple with protons up to three bonds away.

Exercise 1.10.1

The signal for the two H_c protons in isopentenyl diphosphate is reported above as a singlet integrating to $2H$. Are these two protons really chemically equivalent, and, according to what you know about proton NMR, should this signal really be a singlet? If not, what kind of signal(s) would you

expect to see? Explain any discrepancies between what you would expect to see and the actual reported data.

Now, let's look at the ^{13}C spectrum of IPP:

$^{13}C-NMR$ (proton-decoupled)

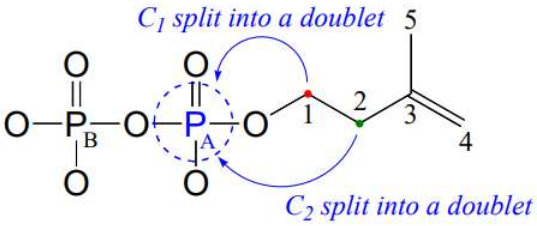
C_1: 63.0 ppm (d); $^2J_{C1\text{-}PA}$ = 7.2 Hz

C_2: 37.4 ppm (d); $^3J_{C2\text{-}PA}$ = 4.0 Hz

C_3: 142.9 ppm

C_4: 111.2 ppm

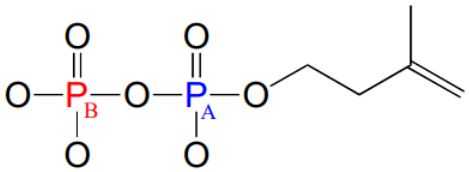
C_5: 21.6 ppm



Notice that the signals for both C_1 and C_2 are split into doublets by the magnetic field of P_A. Phosphorus atoms will spin-couple with ^{13}C nuclei up to three bonds away. Notice also that the 2-bond coupling between C_1 and P_A is larger than the 3-bond coupling between C_2 and P_A (7.2 Hz vs. 4.0 Hz). Finally, notice that we do not observe 4-bond $C-P$ coupling: C_3 is not spin-coupled to P_A, and P_B is not coupled to any of the ^{13}C or ^1H nuclei on the molecule.

Remember that when processing a typical $^{13}C-NMR$ spectrum, we electronically 'turn off' spin coupling between carbons and neighboring protons in order to simplify the spectrum (this is referred to as 'proton decoupling'). Proton decoupling does not turn off $C-P$ spin coupling.

Because ^{31}P is NMR-active, we can also, with an NMR spectrophotometer equipped with a phosphorus probe, directly observe the phosphorus NMR signals, just as we can directly observe the signals from protons and ^{13}C nuclei. On an NMR instrument where protons resonate at 300 MHz and ^{13}C nuclei resonate at 75 MHz, phosphorus resonates at 32 MHz. In $^{31}P-NMR$ experiments, the reference standard used to determine the 0 ppm point is usually phosphoric acid (tetramethylsilane, the standard 0 ppm point for ^1H - and $^{13}C-NMR$, doesn't have a phosphorus atom!). The $^{31}P-NMR$ spectrum of isopentenyl diphosphate has, as expected, two peaks, each of which is upfield of the phosphoric acid standard (negative chemical shifts!) and split into a doublet ($^2J_{P-P} = 20$ Hz) due to 2-bond coupling between the two phosphorus nuclei.



P_A: (-)11.03 ppm (d, $^2J_{P\text{-}P}$ = 20 Hz)
P_B: (-)7.23 ppm (d, $^2J_{P\text{-}P}$ = 20 Hz)

Notice that although the C_1 and C_2 signals were split by P_A in our $^{13}C-NMR$ spectrum, in the $^{31}P-NMR$ spectrum the converse is not true: the P_A signal is not split by C_1 or C_2. Both of these carbons are NMR-inactive ^{12}C isotope in 99 out of 100 molecules. In addition, $P-H$ splitting is not observed in this ^{31}P spectrum, because proton decoupling is in effect.

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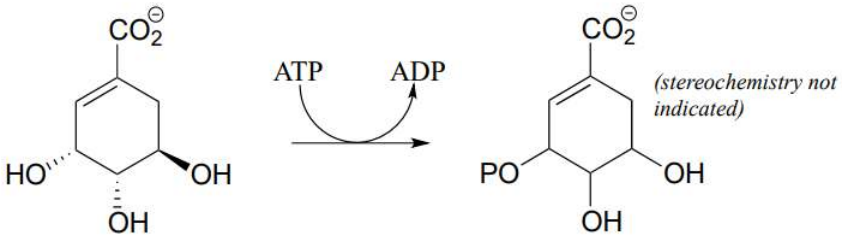
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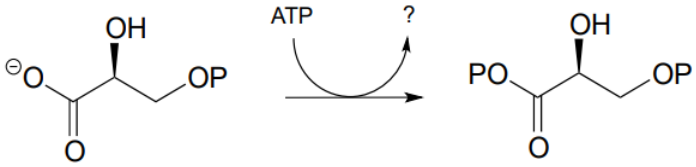
1.P: Phosphate Transfer Reactions (Problems)

In all of the problems that follow, feel free to use appropriate abbreviations when drawing structures. However, always be sure not to abbreviate regions of a structure which are directly involved in bond-breaking or bond-forming events.

P1.1: Draw a likely mechanism for reaction catalyzed by shikimate kinase (EC 2.7.1.71) in the aromatic amino acid biosynthesis pathway). Stereochemistry of the product is not indicated in the figure below - in your mechanism, show the stereochemistry of the product, and explain how you are able to predict it from your knowledge of kinase reactions.

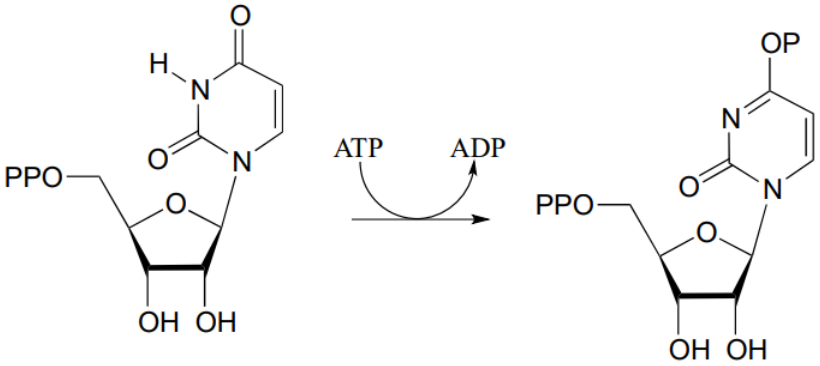


P1.2: Draw a likely mechanism for the following reaction (EC 2.7.2.3) in the gluconeogenesis pathway, and predict what compound is indicated by the question mark.

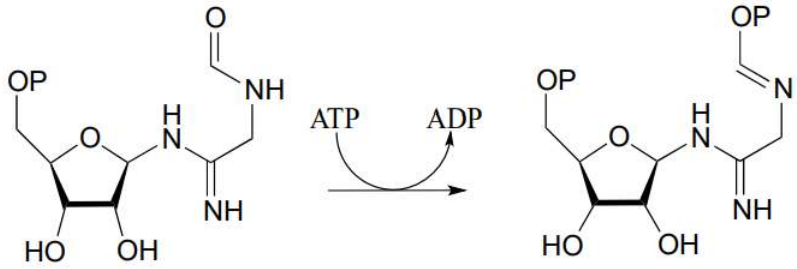


P1.3:

a. Draw a likely mechanism for the following reaction (EC 6.3.4.2) from ribonucleotide biosynthesis. Hint: what is the nucleophilic group? How could the enzyme increase it's nucleophilicity?

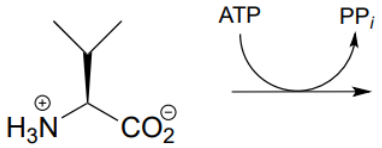


b. Draw a mechanism for the following reaction, also from ribonucleotide biosynthesis (EC 6.3.3.1):



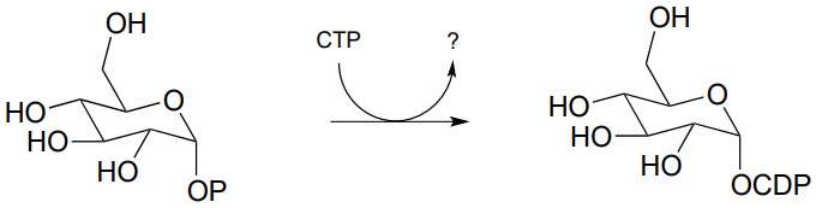
P1.4:

a. The carboxylate group on the amino acid valine is activated in an early step in the biosynthesis of the antibiotic penicillin. Predict the product of this reaction, and draw the likely intermediate of the phosphate group transfer reaction.



valine

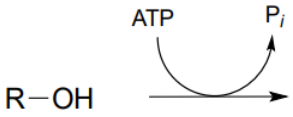
P1.5: The reaction below is an early step in the synthesis of tyvelose, a sugar found on the surface of some pathogenic bacteria. Notice that CTP plays the role of the phosphate group donor in this case, rather than ATP.



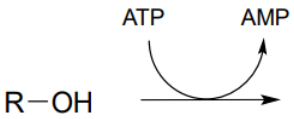
Draw a mechanism for the reaction, and indicate the second product that is released by the enzyme. (J. Biol. Chem. 2005, 280, 10774)

P1.6: Draw the likely product of the following hypothetical phosphate group transfer reactions. Specify which phosphate group of ATP is the electrophile in each case.

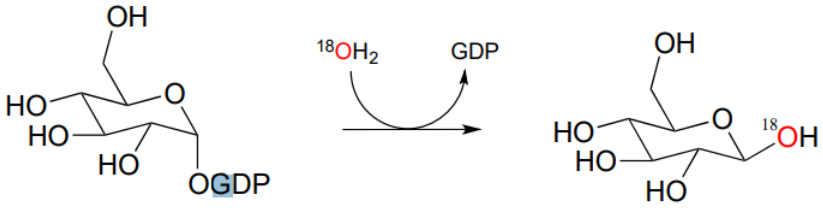
a.



b.



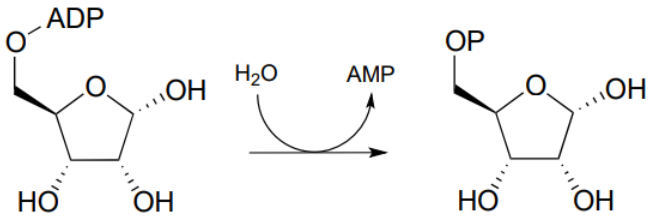
P1.7: The figure below illustrates an experiment in which a reaction catalyzed by an E. coli enzyme was run in isotopically labeled water.



a. The researchers concluded that the reaction was not a phosphate group transfer. Explain their reasoning.
b. Draw the products that would be expected if the reaction actually did proceed by a phosphate transfer mechanism (be sure to show stereochemistry and the location of the ^{18}O atom).

P1.8: The reaction below proceeds with a direct attack by a water molecule on the substrate, but the hydrolysis could be expected to proceed through two possible mechanisms. Draw two possible mechanisms for the reaction run in $H_2^{18}O$. Trace the progress of the

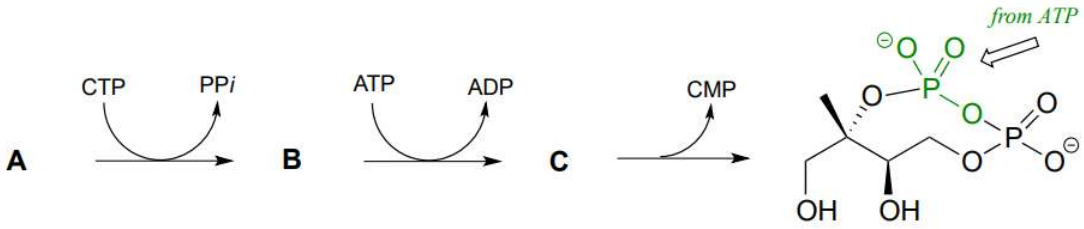
^{18}O 'label' throughout each mechanism to see where it ends up: this should indicate to you how the two mechanisms could be (and in fact were!) distinguished experimentally.



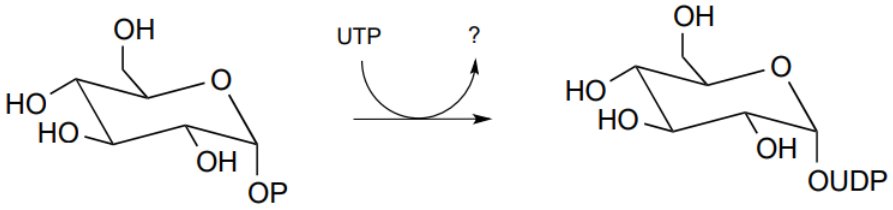
P1.9: Glucose-6-phosphate is dephosphorylated to glucose in the last step of the gluconeogenesis pathway (EC 5.3.1.9). The reaction is not a direct hydrolysis: like the phosphotyrosine phosphatase reaction we saw in this chapter it involves formation of a phosphoenzyme intermediate, but in this case the enzyme residue acting as the initial phosphate acceptor is an active site histidine rather than an asparate. Given this information, propose a likely mechanism for the reaction.

P1.10: (This question assumes a basic knowledge of DNA structure and the idea of supercoiling). DNA topoisomerase enzymes catalyze the temporary 'nicking' of one strand of double-stranded DNA, which allows supercoiled DNA to 'unwind' before the nicked strand is re-ligated. During the unwinding process, the 5' end of the nicked strand is transferred to a tyrosine in the enzyme's active site, effectively holding it in place while the 3' end rotates. Overall, the stereochemical configuration of the bridging phosphate is retained. Propose a likely mechanism for this nicking and re-ligating process. (Biochemistry 2005, 44, 11476.)

P1.11: Pictured below is a series of phosphate group transfer steps in the early part of isoprenoid biosynthesis in bacteria. With the knowledge that the atoms in green are derived from ATP, predict the structures of compounds A, B and C.

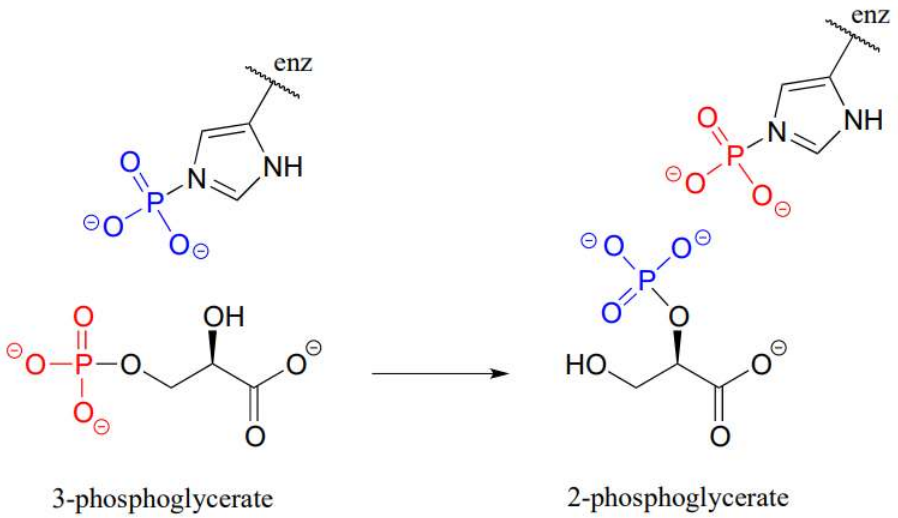


P1.12: The reaction below shows the synthesis of glucose-UDP, an important intermediate in carbohydrate biosynthesis. Notice that UTP (instead of ATP) is the phosphate donor. Identify the by-product denoted below by a question mark.

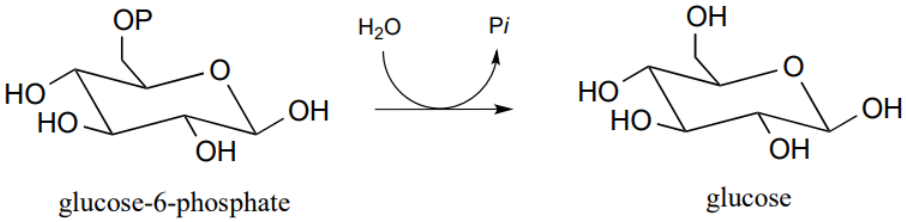


P1.13: Isomerization of 3-phosphoglycerate to 2-phosphoglycerate (EC 5.4.2.1, a reaction in glycolysis) has been shown to occur with the participation of a phosphohistidine residue in the enzyme's active site. The two phosphate groups are distinguished in the figure below by color. With this information, propose a mechanism for the reaction.

https://chem.libretexts.org/@go/page/291122



P1.14: The gluconeogenesis (sugar-building) pathway enzyme glucose-6-phosphatase catalyzes an indirect phosphate hydrolysis reaction with a phosphohistidine intermediate ('indirect hydrolysis' in this context means that a water molecule does not directly attack glucose-6-phosphate).



Researchers wanted to confirm that the hydrolysis in this reaction is indirect, rather than direct. It turns out that the same enzyme is also capable of catalyzing the transfer of the phosphate group from glucose-6-phosphate to the hydroxyl group on carbon #6 of another glucose molecule (instead of to water, which is the natural reaction). The enzyme-catalyzed transfer of phosphate between two glucose substrates is reversible.

The researchers incubated the enzyme with labeled glucose-6-phosphate, in which in the phosphate center was chiral (with the R configuration) due to the incorporation of ^{17}O and ^{18}O isotopes. They also included a high concentration of glucose in the reaction mixture, which ensured that the glucose-to-glucose transfer reaction predominated and hydrolysis (the 'natural' reaction) did not take place. After allowing the reaction to reach equilibrium, they isolated the glucose-6-phosphate and looked at the configuration of the phosphate group.

Given what you have just learned about the enzyme mechanism, predict what the researchers found in this experiment, explain your prediction, and draw the appropriate structure(s), including stereochemistry. Assume that the glucose-to-glucose mechanism is identical to the hydrolysis mechanism, aside from the identity of the ultimate phosphate acceptor.

Contributors

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1.S: Phosphate Transfer Reactions (Summary)

All of the reactions detailed in this chapter involved the transfer of a phosphate group - usually a phosphate, diphosphate, or AMP group - from one molecule (the donor) to another (the acceptor). Your learning goal for this chapter is to recognize and understand what is happening in these phosphate group transfer reactions, and to gain a basic understanding of the chemistry of phosphate and other phosphate groups.

Be sure that you can identify and provide examples of the following terms:

inorganic phosphate	organic triphosphate
inorganic phosphate	phosphate (mono)ester
inorganic pyrophosphate	phosphate diester
organic (mono)phosphate	phosphate anhydride
organic diphosphate	bridging/non-bridging oxygen

- Also, make sure that you can recognize and use appropriately the various abbreviations introduced in this chapter:

P_i PP_i $R-OP$

$R-OPP$ $R-OAMP$ ATP

ADP AMP

. . . in addition to the various structural abbreviations for adenosine mono-, di-, and triphosphate.

- You should know the approximate pK_a values for phosphoric acid and an organic monophosphate, and be able to state the approximate net charge (to the nearest 0.5 charge unit) of these species in buffers of different pH levels.
- You should be able to describe, in words and pictures, the tetrahedral sp3d bonding picture for the phosphorus atom of a phosphate group. Even though the geometry is not always shown in every drawing, always keep in mind that the phosphate group is tetrahedral.
- You should be able to draw resonance contributors for different phosphate groups, identify major versus minor contributors, and explain why some are major and some are minor. Remember - charges are shared between non-bridging oxygens, even if they are not drawn that way!
- ***Absolutely critical to your success with this chapter is being able to picture and illustrate the mechanistic pattern which we refer to as a phosphate group transfer.***
- Though not usually included in reaction illustrations, always remember that charge-charge interactions with magnesium ions and hydrogen bonds to active site amino acids both serve to increase the electrophilicity of a phosphorus atom in donor compounds such as ATP.
- You should understand the distinctions between the three mechanistic models for phosphate transfer reactions - concerted, addition-elimination, and elimination-addition - and know that the concerted model probably most closely describes biochemical reactions.
- You should be able to identify the apical and equatorial positions in the pentavalent transition state of a phosphate transfer reaction, and recognize that the reaction results in inversion at the phosphorus center.
- You should be able to identify the a, b, and g phosphate groups of ATP, as well as the ribose and adenosine parts of the molecule.
- You should be able to explain how ATP acts as a phosphate group donor, and why such reactions are thermodynamically favorable.
- You should be able to draw a curved-arrow mechanism for reactions in which ATP acts as a phosphate group donor in the phosphorylation and/or diphosphorylation of an alcohol. You should be able to predict the result of nucleophilic attack at the α, β, or γ phosphates of ATP.
- In general, you should be able to propose a likely mechanism for any phosphate transfer reaction, given the starting compounds and products.

- Given information about the existence of a covalently linked enzyme-substrate complex in an enzyme mechanism, you should be able to propose a likely mechanism that accounts for such an intermediate. For example, after being told that the phosphotyrosine phosphatase reaction involves a phosphocysteine intermediate, you should be able to propose a mechanism.

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CHAPTER OVERVIEW

2: Nucleophilic Carbonyl Addition Reactions

2.1: Prelude to Nucleophilic Carbonyl Addition Reactions
2.2: Nucleophilic Additions to Aldehydes and Ketones - An Overview
2.3: Hemiacetals, Hemiketals, and Hydrates
2.4: Acetals and Ketals
2.5: N-glycosidic Bonds
2.6: Imines
2.7: A Look Ahead - Addition of Carbon and Hydride Nucleophiles to Carbonyls
2.P: Nucleophilic Carbonyl Addition Reactions (Problems)
2.S: Nucleophilic Carbonyl Addition Reactions (Summary)

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2.1: Prelude to Nucleophilic Carbonyl Addition Reactions

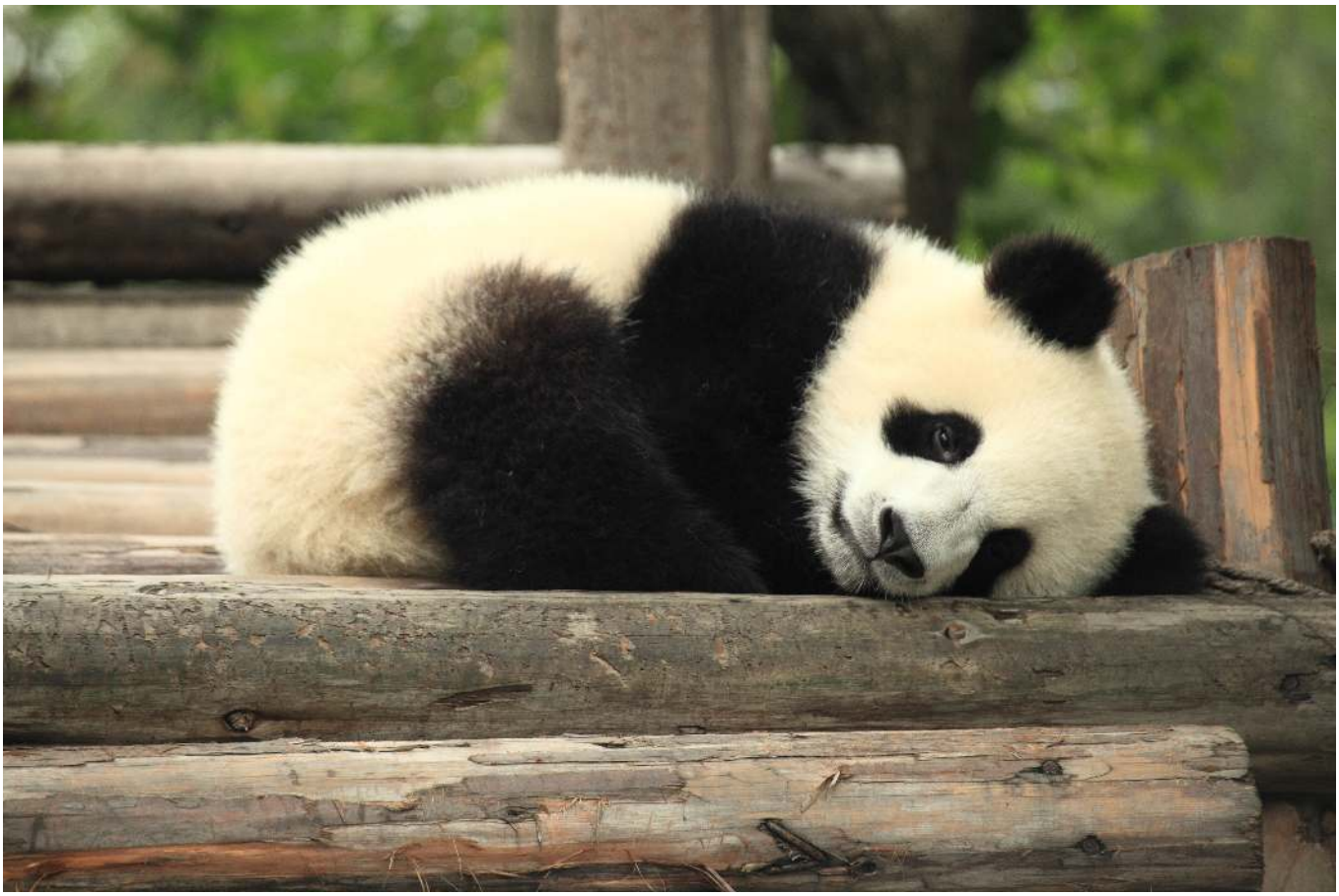


Figure* 2.1.1*: Panda (photo credit: https://www.flickr.com/photos/gzlu/)

Introduction

It's possible that the fuel for the car you drive thirty years from now will come from the back end of a panda. Not literally, of course – but it just might turn out that future biofuel technology will be derived in part from the stuff that workers have to clean out of the enclosure housing Ya Ya and Le Le, the two resident pandas at the Memphis Zoo. At least, that's the hope of Dr. Ashli Brown, a biochemistry professor at Tennessee State University.

First, a little background. If you are like most people in the United States, you are already burning ethanol every time you drive: in 2012, the U.S. Department of Energy reports that over 13 million gallons of ethanol were sold at gas stations nationwide, most often as a 10% mixture along with 90% conventional gasoline. The ethanol we burn today is made by fermenting the sugars present in edible corn. The use of corn ethanol, while a significant step forward in the effort to move away from petroleum fuels and towards carbon-neutral, renewable energy sources, is far from a permanent, sustainable solution to the world's ever-increasing energy needs. Growing corn crops requires a lot of energy and expense, from running the large equipment used to plow and harvest the fields, to manufacturing and applying pesticides and fertilizers, all the way to trucking the corn to the ethanol plant. In fact, some calculation methods suggest that more energy goes into producing a gallon of corn-based ethanol than is released when the ethanol is burned.

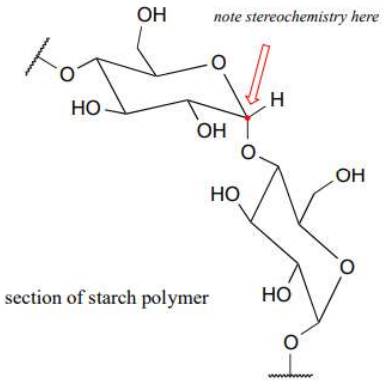
Moreover, growing corn requires a lot of water, and takes up land which otherwise could be used for growing food, or preserved as a natural habitat. A recent study by scientists in South Dakota reported that between 2006 and 2011, a full 1.3 million acres of wetland and prairie were plowed over and converted to biofuel crop production in five Midwestern states.

What would be much better in the long run is if we could produce ethanol or other biofuels not from resource-intensive food crops like corn, but from non-edible plant materials: grasses, trees, and agricultural byproducts such as the cobs and stalks from corn

plants. Switchgrass, for example, is a native North American prairie grass that is thought to have high potential for biofuel production.

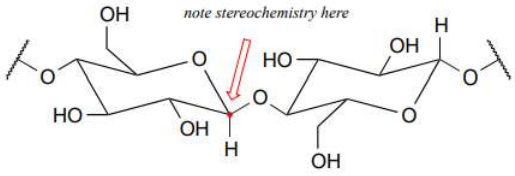
So if we can make ethanol from corn, couldn't we just change over to switchgrass using the same technology?

Unfortunately, it's not nearly that simple. Ethanol is made by 'feeding' glucose to living yeast cells, allowing them to break down the sugar into ethanol – a metabolic process called fermentation. Corn kernels contain sugar in the form of starch, a polysaccharide of linked glucose molecules. Enzymes called 'amylases' are used to break up the starch polymer into individual glucose molecules (as well as two-glucose units called cellobiose), which are then fermented by the yeast.



section of starch polymer

The rest of the corn plant – the stalks, leaves, and cobs – is composed in large part of another glucose polymer called cellulose.



section of cellulose polymer

Cellulose is a major component of plant cell walls, and is the most abundant organic compound on the planet - an enormous source of glucose for fermentation! The problem, from a renewable energy perspective, is how to get at the glucose monomers that make up the polymer. Look closely at the bond connecting two glucose monomers in starch, and then compare it to the same bond in cellulose. They both link the same two carbons of glucose, but with opposite stereochemistry. Recall that enzymes are very sensitive to the stereochemical configuration of their substrate molecules. It should come as no surprise, then, that the amylase enzymes which are so efficient at breaking apart starch are completely ineffective at breaking apart cellulose. Other enzymes, known as cellulases, are needed for this job. These enzymes do exist in nature: just think about what happens to tree branches, leaves, and other cellulose-rich plant matter that lies on the forest floor. These slowly rot away, the cellulose broken apart by cellulase enzymes in microscopic fungi.

The key word here, though, is 'slowly'. Fungi living on the forest floor are not in any great hurry to degrade the leaves and wood around them – the cellulose is not going anywhere. Fungal cellulases are, comparatively speaking, very slow, inefficient enzymes. Herein lies the biggest challenge to the development of economically viable production of ethanol from cellulosic sources such as switchgrass or wood. Breaking the glucose-glucose bonds in cellulose is the main bottleneck in the whole process.

This is where the pandas come in.

Pandas live primarily on a diet of bamboo, obtaining their energy from the cellulose in the plant. Like other plant-eaters such as cows, horses, and sheep, pandas do not make their own cellulase enzymes. Rather, they rely on a diverse population of symbiotic microbes inhabiting their digestive tracts to do the job of cellulose digestion for them. Unlike the microbes living the slow-paced

lifestyle of the forest floor, though, the panda's microbes don't have a lot of time to spare - the food is moving through the system pretty quickly. In theory, evolutionary pressure should have resulted in panda-gut microbes with speedy cellulase enzymes, and that is what Dr. Ashli Brown at Tennessee State was hoping to find as she and her research students analyzed panda feces from the Memphis Zoo. They have had some success: at the fall, 2013 meeting of the American Chemical Society, Dr. Brown announced that her group, working in cooperation with colleagues at the University of Wisconsin, had found over forty cellulose-digesting bacteria, courtesy of Ya Ya and Le Le. The next step is to clone the cellulase-encoding genes, use the DNA to produce recombinant enzyme, and see just how fast they are.

Other less cuddly and photogenic animals are also being studied with similar goals in mind. Dr. Falk Warnecke, working at the U.S. Department of Energy Joint Genome Institute in Northern California, has been investigating the microbes that live in the guts of wood-eating termites, and many other researchers around the world are interested in the symbiotic bugs which inhabit the rumen of cows and sheep.

The problematic chemical reaction catalyzed by cellulase enzymes is, in organic chemistry terminology, an 'acetal hydrolysis'. Acetals are derived from aldehydes. The reactions that occur at the carbonyl carbon of aldehydes and ketones is absolutely central to the chemistry of carbohydrates such as starch and cellulose, and it is this chemistry that is the subject of the chapter we are about to begin.

Contributors

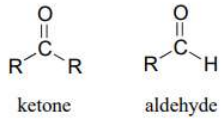
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2.2: Nucleophilic Additions to Aldehydes and Ketones - An Overview

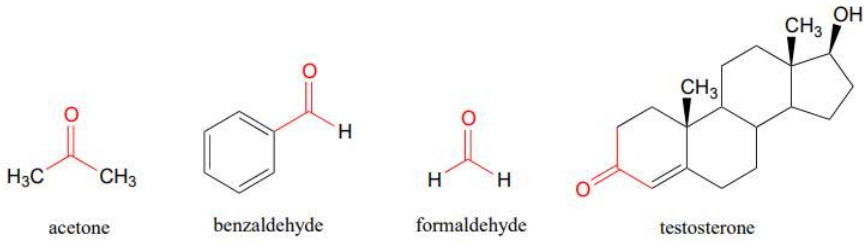
The aldehyde and ketone functional groups

Recall from before that the ketone functional group is made up of a carbonyl bonded to two carbons, while in an aldehyde one (or both) of the neighboring atoms is a hydrogen.

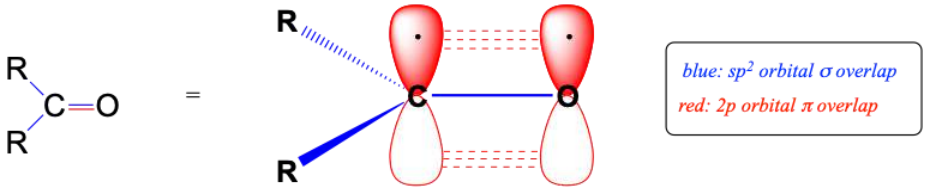


Organic Chemistry With a Biological Emphasis
Tim Soderberg

You probably are familiar with the examples shown below: acetone, the simplest ketone compound, is a common solvent in nail polish remover, benzaldehyde is the flavoring in maraschino cherries, and formaldehyde (a special case in which the carbonyl carbon is bonded to hydrogens on both sides) is the nasty-smelling stuff that was used to preserve the unlucky frog that you dissected in high school biology class. The male sex hormone testosterone contains a ketone group in addition to alcohol and alkene groups.

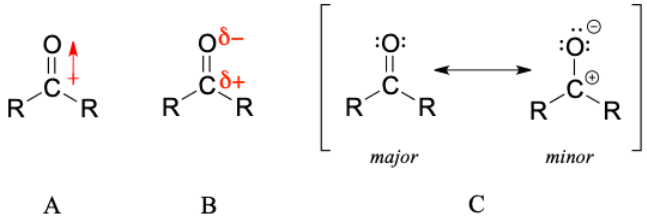


Recall from chapter 2 (last semester) the bonding picture in a ketone or aldehyde: the carbonyl carbon is sp^2-hybridized, with its three trigonal planar sp^2 orbitals forming σ bonds with orbitals on the oxygen and on the two carbon or hydrogen atoms. The remaining unhybridized 2p orbital is perpendicular to the plane formed by the sp^2 orbitals, and forms a π bond through a side-by-side overlap with a 2p orbital on the oxygen. The σ and π bonds between the carbon and oxygen combine to make the C=O double bond that defines the carbonyl functionality.



Nucleophilic addition

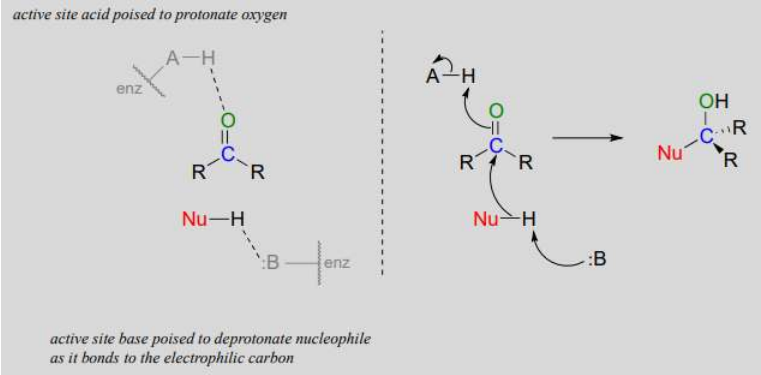
The carbon-oxygen double bond is polar: oxygen is more electronegative than carbon, so electron density is higher on the oxygen end of the bond and lower on the carbon end. Recall that bond polarity can be depicted with a dipole arrow (A in the figure below), or by showing the oxygen as bearing a partial negative charge and the carbonyl carbon a partial positive charge (B).



A third way to illustrate the carbon-oxygen dipole (C in the figure above) is to consider the two main resonance contributors: the major form, which is what you typically see drawn in Lewis structures, and a minor but very important contributor in which both electrons in the p bond are localized on the oxygen, giving it a full negative charge. The latter depiction shows the carbon with an empty 2p orbital and a full positive charge.

However the bond polarity is depicted, the end result is that the carbonyl carbon is electron-poor – in other words, it is an electrophile. In addition, the trigonal planar geometry means that the carbonyl group is unhindered). Thus, it is an excellent target for attack by an electron-rich nucleophilic group, a mechanistic step called nucleophilic addition:

Nucleophilic addition to an aldehyde or ketone (enzymatic)

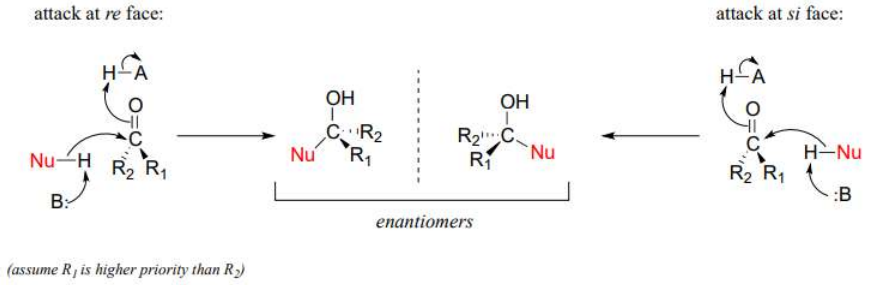


Notice the acid-base catalysis in this generalized mechanism: in the enzyme active site, a basic group is poised to deprotonate the nucleophile (thus enhancing its nucleophilicity) as begins to attack the carbonyl carbon, while at the same time an acidic proton on another active site group is poised just above the carbonyl oxygen (thus enhancing the electrophilicity of the carbon), ready to protonate the oxygen and neutralize any negative charge that builds up.

Stereochemistry of nucleophilic addition to a carbonyl

Recall from last semester that when the two groups adjacent to a carbonyl are not the same, we can distinguish between the re and si 'faces' of the planar structure.

The concept of a trigonal planar group having two distinct faces comes into play when we consider the stereochemical outcome of a nucleophilic addition reaction. Notice that in the course of a carbonyl addition reaction, the hybridization of the carbonyl carbon changes from sp^2 to sp^3, meaning that the bond geometry changes from trigonal planar to tetrahedral. If the two R groups are not equivalent, then a chiral center is created upon addition of the nucleophile. The configuration of the new chiral center depends upon which side of the carbonyl plane the nucleophile attacks from.



If the reaction is catalyzed by an enzyme, the stereochemistry of addition is (as you would expect) tightly controlled, and leads to one stereoisomer exclusively–the nucleophilic and electrophilic substrates are bound in specific positions within the active site, so that attack must occur specifically from one side and not the other. Nonenzymatic reactions of this type often result in a 50:50 mixture of stereoisomers, but it is also possible that one stereoisomer may be more abundant, depending on the structure of the reactants and the conditions under which the reaction takes place. We'll see some examples of this phenomenon soon when we look at cyclic forms of sugar molecules.

Contributors

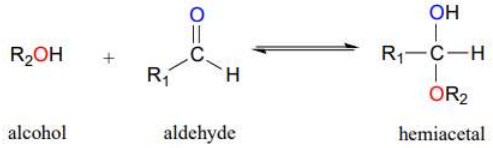
- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

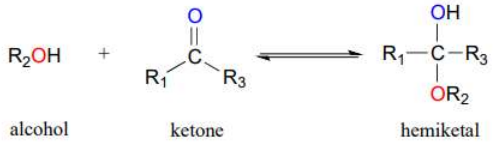
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2.3: Hemiacetals, Hemiketals, and Hydrates

Overview

One of the most important examples of a nucleophilic addition reaction in biochemistry, and in carbohydrate chemistry in particular, is the addition of an alcohol to a ketone or aldehyde. When an alcohol adds to an aldehyde, the result is called a hemiacetal; when an alcohol adds to a ketone the resulting product is a hemiketal.

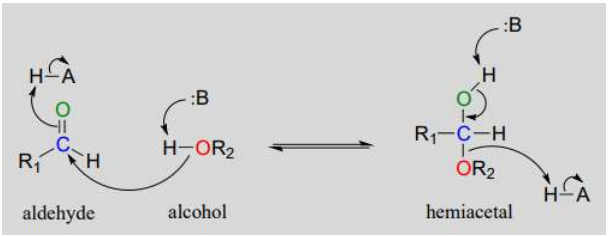




(The prefix ‘hemi’ (half) is used in each term because, as we shall soon see, addition of a second alcohol nucleophile can occur, resulting in species called acetals and ketals.)

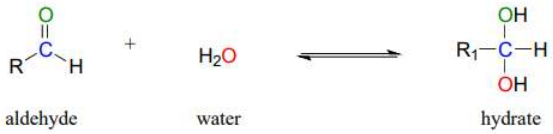
The conversion of an alcohol and aldehyde (or ketone) to a hemiacetal (or hemiketal) is a reversible process. The generalized mechanism for the process at physiological pH is shown below.

Biochemical mechanism of hemiacetal formation:



In general, hemiacetals (and hemiketals) are higher in energy than their aldehyde-alcohol components, so the equilibrium for the reaction lies to the left. As we will soon see in the context of glucose and other sugars, however, five- and six-membered cyclic hemiacetals are considerably lower in energy, and are favored at equilibrium: recall from last semester the inherent stability of five- and six-membered rings.

Aldehydes and ketones, when in aqueous solution, exist in equilibrium with their hydrate form. A hydrate forms as the result of a water molecule adding to the carbonyl carbon of the aldehyde or ketone.

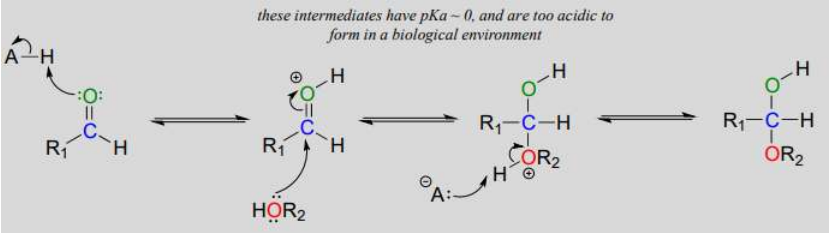


Although you should be aware that aldehyde and ketone groups may exist to a considerable extent in their hydrated forms when in aqueous solution (depending upon their structure), they are usually drawn in their non-hydrated form for the sake of simplicity.

The mechanism we just saw for hemiacetal formation applies to biochemical reactions occurring at physiological ***pH***. In the organic laboratory, however, hemiacetal and hemiketal formation usually takes place in the presence of a strong acid. The acid catalyzes the reaction by protonating the carbonyl oxygen, thus increasing the electrophilicity of the carbonyl carbon. Notice in the

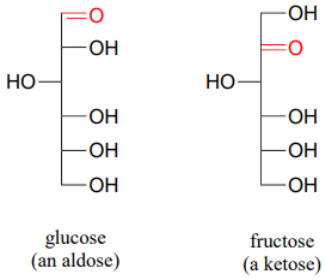
mechanism below that highly acidic intermediates are drawn which would be unreasonable to propose for the corresponding biochemical mechanisms occurring at physiological pH.

Acid-catalyzed hemiacetal formation (non-biological):



Sugars as intramolecular hemiacetals and hemiketals

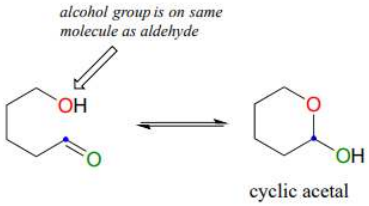
As stated above, the reactions of hemiacetals and hemiketals are central to the chemistry of carbohydrates. Recall that sugar molecules generally contain either an aldehyde or a ketone functional group, in addition to multiple alcohol groups. Aldehyde sugars are often referred to as aldoses; ketone sugars as ketoses. For example, glucose is an aldose, and fructose is a ketose - their structures are drawn below in Fischer projection:



Exercise 2.3.1

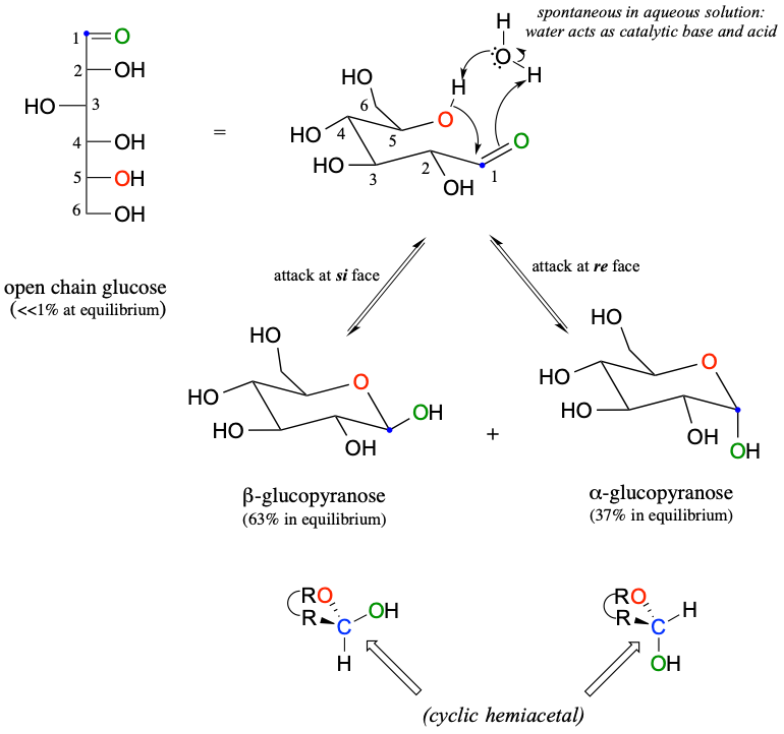
What term describes the relationship between glucose and fructose (in other words, what kind of isomers are they)?

Glucose and fructose are shown above in their open-chain form. However, in aqueous solution, glucose, fructose, and other sugars of five or six carbons rapidly interconvert between straight-chain and cyclic forms. This occurs through the formation of intramolecular hemiacetals and hemiketals. This simply means that the 'R' group of the alcohol is already covalently attached to the 'R 'group of the aldehyde (R1 in our general mechanism).



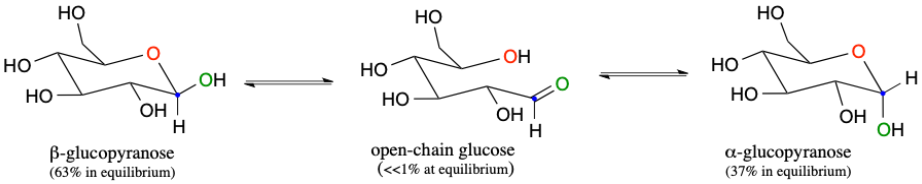
Unlike most of the biochemical reactions you will see in this text, sugar cyclization reactions are not catalyzed by enzymes: they occur spontaneously and reversibly in aqueous solution. For most five- and six-carbon sugars, the cyclic forms predominate in equilibrium.

The cyclic form of glucose is a six-membered ring, with an intramolecular hemiacetal formed by attack of the hydroxl on carbon #5 to the aldehyde carbon (carbon #1, also called the anomeric carbon in carbohydrate terminology).



The cyclic form of glucose is called glucopyranose. As was discussed above, nucleophilic attack on a planar carbonyl group can occur at either face of the plane, leading to two different stereochemical outcomes - in this case, to two different diastereomers. In carbohydrate nomenclature, these two diastereomers are referred to as the α and β anomers of glucopyranose.

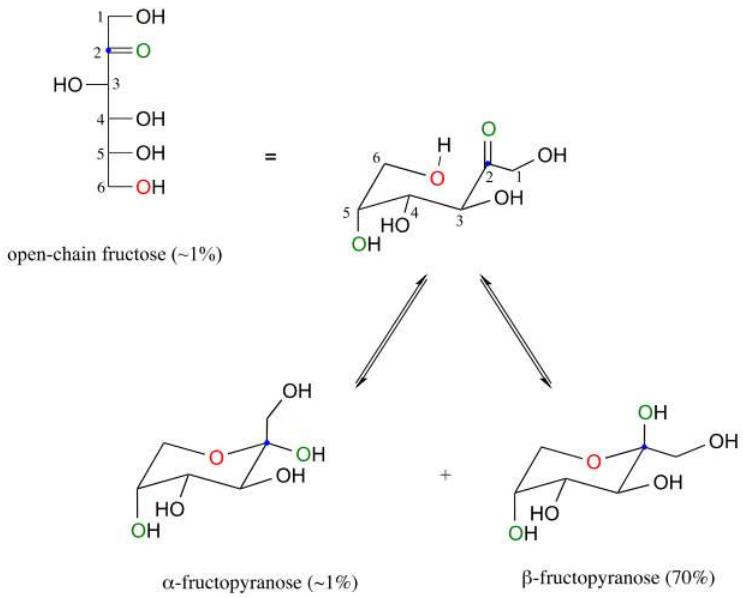
Because the formation of glucopyranose occurs spontaneously without enzyme catalysis, shouldn't equal amounts of these two anomers form? In fact, this does not happen: there is almost twice as much of one anomer than the other at equilibrium. Why is this? Remember (section 3.2) that six-membered rings exist predominantly in the chair conformation, and that the lower energy chair conformation is that in which unfavorable interactions between substituents are minimized – in most cases, this is the conformation in which larger substituents are in the equatorial position. In the lower-energy chair conformation of the major β anomer of glucopyranose, all of the hydroxyl groups are in the equatorial position, but in the minor a anomer one hydroxyl group is forced into the axial position. As a result, the α anomer is higher in energy, and less abundant at equilibrium.



Exercise 2.3.2

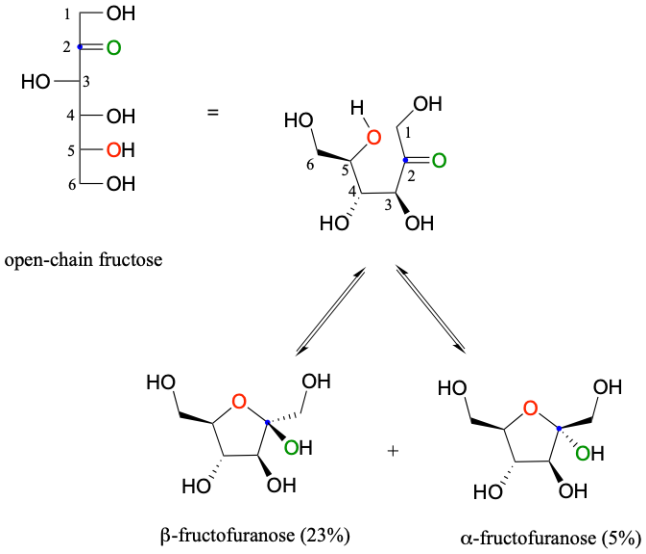
Draw a mechanism for the conversion of α-glucopyranose to open-chain glucose.

Fructose in aqueous solution forms a six-membered cyclic hemiketal called fructopyranose when the hydroxyl oxygen on carbon #6 attacks the ketone carbon (carbon #2, the anomeric carbon in fructose).



In this case, the β anomer is heavily favored in equilibrium by a ratio of 70:1, because in the minor a anomer the bulkier CH_2OH group occupies an axial position.

Notice in the above figure that the percentages of α and β anomers present at equilibrium do not add up to 100%. Fructose also exists in solution as a five-membered cyclic hemiketal, referred to in carbohydrate nomenclature as fructofuranose. In the formation of fructofuranose from open-chain fructose, the hydroxyl group on the fifth carbon attacks the ketone.



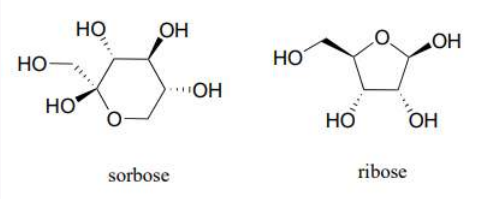
In aqueous solution, then, fructose exists as an equilibrium mixture of 70% β-fructopyranose, 23% β-fructofuranose, and smaller percentages of the open chain and cyclic α-anomers. The β-pyranose form of fructose is one of the sweetest compounds known, and is the main component of high-fructose corn syrup. The β-furanose form is much less sweet.

Although we have been looking at specific examples for glucose and fructose, other five- and six-carbon monosaccharides also exist in solution as equilibrium mixtures of open chains and cyclic hemiacetals and hemiketals. Shorter monosaccharides are unlikely to undergo analogous ring-forming reactions, however, due to the inherent instability of three and four-membered rings.

Exercise 2.3.3

a. Identify the anomeric carbon of each of the sugars shown below, and specify whether the structure shown is a hemiacetal or hemiketal.

b. Draw mechanisms for cyclization of the open-chain forms to the cyclic forms shown.



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2.4: Acetals and Ketals

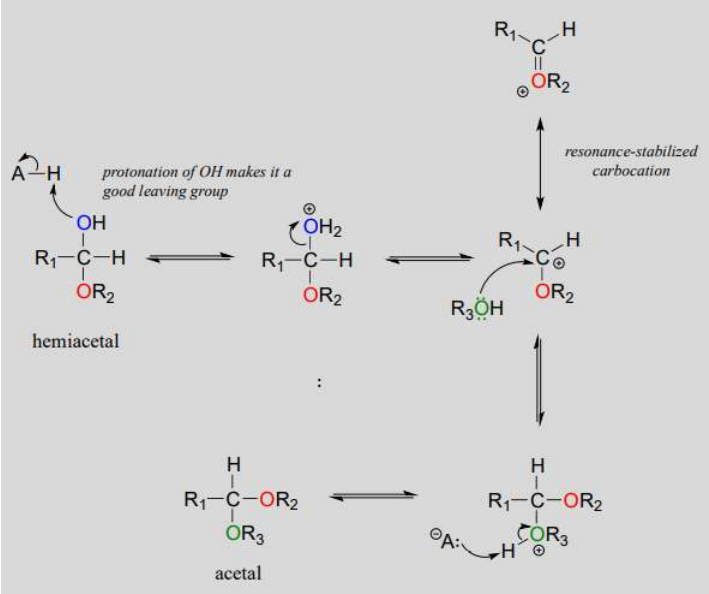
Overview

Hemiacetals and hemiketals can react with a second alcohol nucleophile to form an acetal or ketal. The second alcohol may be the same as the first (ie. if $R_2 = R_3$ in the scheme below), or different.



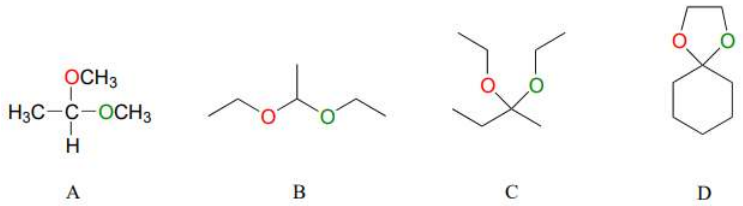
Although we focus here on biological reactions, it is instructive in this case to consider non-biological acetal-forming reactions before we look at their biochemical counterparts. In a non-enzymatic context, acetal/ketal formation - just like hemiacetal/hemiketal formation - is generally catalyzed by a strong acid.

Acid-catalyzed acetal formation (non-biological)



The role of the acid catalyst is to protonate the OH group of the acetal, thus making it a good leaving group (water). Notice something important here: the conversion of a hemiacetal to an acetal is simply an S_N1 reaction, with an alcohol nucleophile and water leaving group. The carbocation intermediate in this S_N1 mechanism is stabilized by resonance due to the oxygen atom already bound to the electrophilic carbon.

Below are some examples of simple, non-biological acetal and ketals.

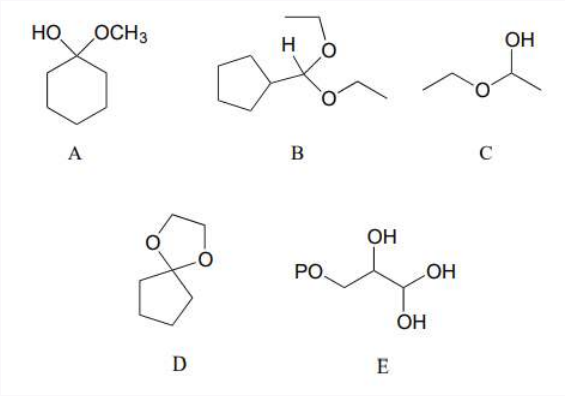


Exercise 2.4.1

For each acetal/ketal A-D in the figure above, specify the required aldehyde/ketone and alcohol starting materials.

Exercise 2.4.2

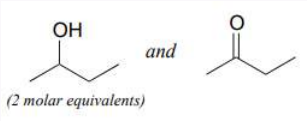
Categorize each of the following molecules as a hemiacetal, hemiketal, acetal, ketal, hydrate of an aldehyde, or hydrate of a ketone.



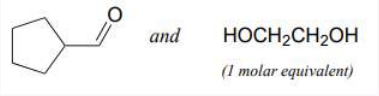
Exercise 2.4.3

Specify the acetal/ketal that would form from a reaction between the given starting compounds.

a.



b.

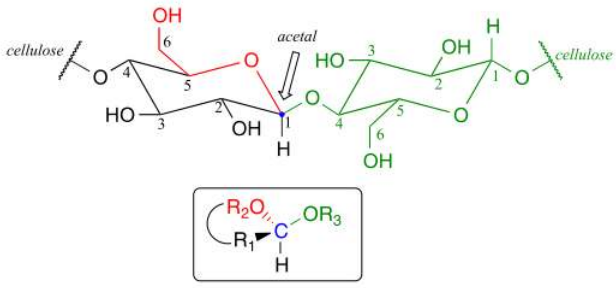


Exercise 2.4.4

Specify the aldehyde/ketone and alcohol combination that would be required to form the compounds in exercise 10.5.

Glycosidic bond formation

Now, let's consider acetal formation in a biochemical context. A very important example of the acetal/ketal group in biochemistry is the glycosidic bonds which link individual sugar monomers to form polysaccharides (see section 1.3 for a quick review). Look at the glycosidic bond between two glucose monomers in a cellulose chain:

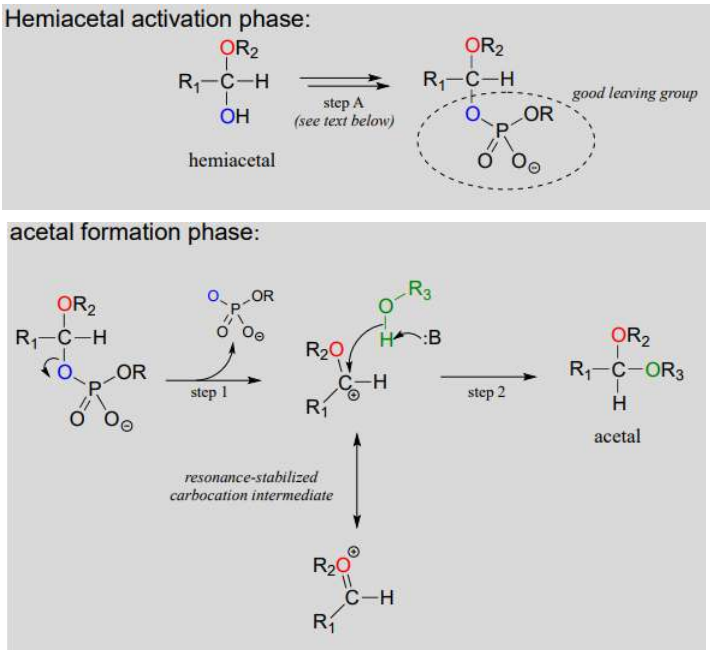


If you look carefully, you should recognize that carbon #1, the anomeric carbon on the left-side glucose monomer, is the central carbon of an acetal group. Biochemists refer to this as a β-1,4 linkage, because the stereochemistry at carbon #1 is β in the specialized carbohydrate nomenclature system, and it is linked to carbon #4 of the next glucose on the chain. The vast structural diversity of carbohydrates stems in large part from the different linkages that are possible - both in terms of which two carbons are

linked, and also the stereochemistry of the linkage. You will see many more variations of glycosidic bond linkage patterns if you study carbohydrate biochemistry in greater depth.

Reactions in which new glycosidic bonds are formed are catalyzed by enzymes called glycosyltransferases, and in organic chemistry terms these reactions represent the conversion of a hemiacetal to an acetal (remember that sugar monomers in their cyclic form are hemiacetals and hemiketals). The mechanism for glycosidic bond formation in a living cell parallels the acid-catalyzed (non-biological) acetal-forming mechanism, with an important difference: rather than being protonated, the OH group of the hemiacetal is converted to a good leaving group by phosphorylation (this is a pattern that we are familiar with from chapter 9). The specific identity of the activating phosphate group varies for different reactions, so it is generalized in the figure below.

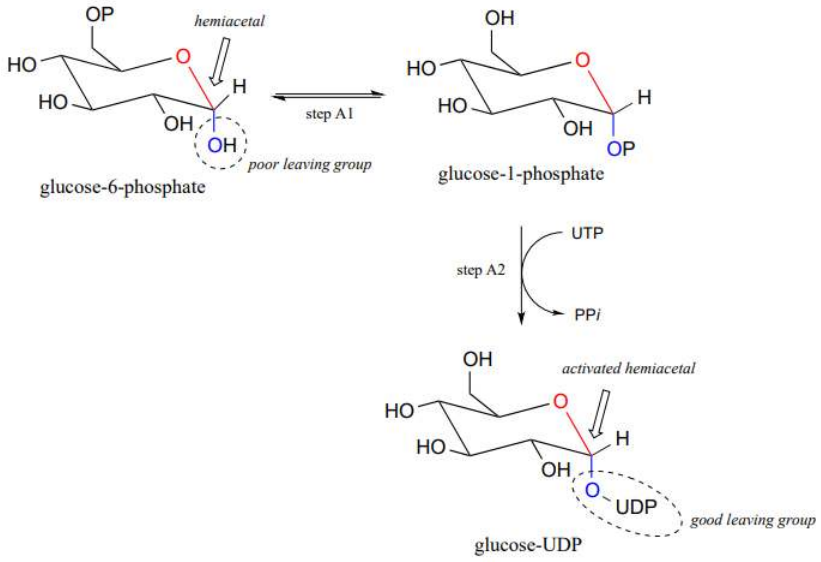
Mechanism for (biochemical) acetal formation:



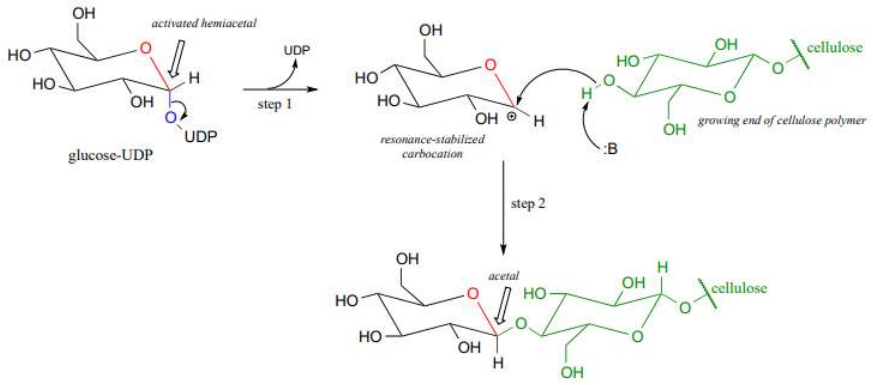
- Step A (Activation phase): This phase of the reaction varies according to the particular case, but always involves phosphate group transfer steps that are familiar from chapter 1. What is most important for our present discussion, however, is simply that the hydroxyl group on the hemiacetal has been activated (i.e., made into a good leaving group) by phosphorylation.
- Step 1: Now that the hydroxyl group has been activated by converting it to a good leaving group, it does its job and leaves, resulting in a resonance-stabilized carbocation.
- Step 2: A nucleophilic alcohol on the growing cellulose chain attacks the highly electrophilic carbocation to form an acetal. Here is where the stereochemistry of the new glycosidic bond is determined: depending on the reaction, the alcohol nucleophile could approach from either side of the planar carbocation.

To reiterate: it is important to recognize the familiar S_N1 mechanistic pattern in play here: in step A, a poor leaving group is converted into a good leaving group, in step 1 the leaving group leaves and a stabilized carbocation is left behind, and in step 2 a nucleophile attacks to form a new bond and complete the substitution process. Look back at the S_N1 reactions we saw last semester if you are having trouble making this mechanistic connection.

Now, let's look specifically at the glycosyl transferase reaction mechanism in which a new glycosidic bond is formed on a growing cellulose chain. Glucose (a hemiacetal) is first activated through two enzymatic phosphate transfer steps: step A1, a phosphate isomerization reaction with a mechanism similar to the reaction in problem P1.13, followed by a UTP-dependent step A2, for which you were invited to propose a mechanism in problem P1.12.

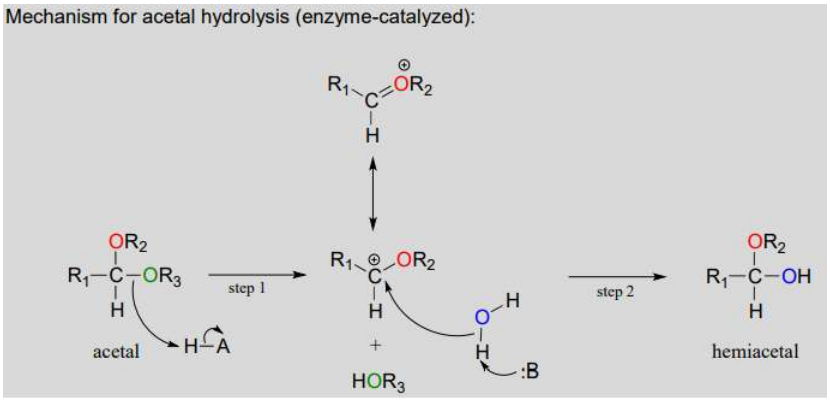


The UDP group on glucose-UDP then leaves (step 1 below), forming a resonance-stabilized carbocation intermediate. Attack by the alcohol group on the growing cellulose chain in step 2 forms the glycosidic (acetal) bond. Note the inversion of stereochemistry.



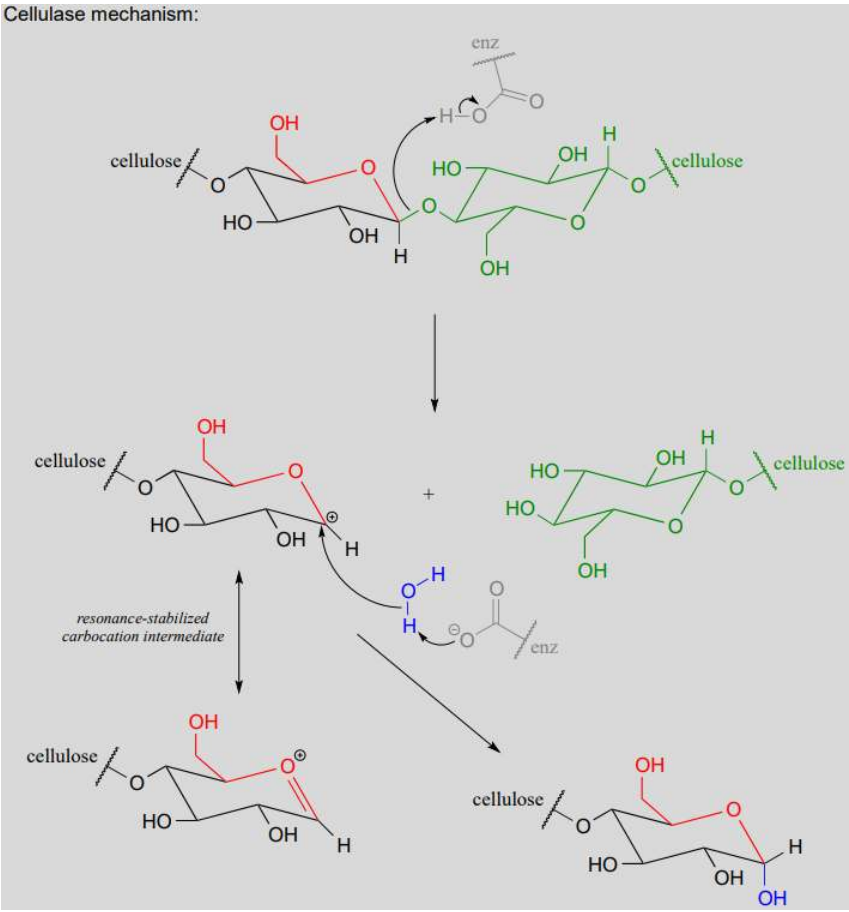
Glycosidic bond hydrolysis

Acetals can be hydrolyzed back to hemiacetals. Notice that an acetal to hemiacetal conversion is an S_N1-type reaction with a water nucleophile and an alcohol leaving group.



In step 1, an alcohol is protonated by a nearby acid group as it breaks away to form a resonance-stabilized carbocation intermediate. The carbocation is attacked by a nucleophilic water molecule in step 2 to form a hemiacetal.

The general mechanism above applies to reactions catalyzed by glycosidase enzymes, which catalyze the cleavage of glycosidic bonds in carbohydrates. In the introduction to this chapter, we learned about ongoing research in the field of cellulosic ethanol. Recall that the main bottleneck in the production of ethanol from sources such as switchgrass or wood is the cellulase-catalyzed step in which the glycosidic bonds in cellulose are cleaved. Cellulose-digesting microbes have several different but closely related forms of cellulase enzymes, all working in concert to cleave cellulose into smaller and smaller pieces until individual glucose molecules are free to be converted to ethanol by the fermentation process. Below is a representative mechanism for a cellulase reaction.



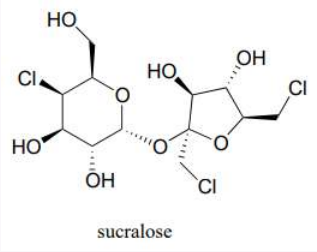
The starch-digesting amylase enzymes used in the corn ethanol production process catalyze similar glycoside hydrolysis reactions, the main difference being the opposite stereochemistry at the anomeric carbon of the substrate.

Exercise 2.4.5

Notice that the cellulose glycososide bond-forming reaction requires the cell to 'spend' a high-energy UTP molecule, but the cellulase glycoside bond-breaking reaction does not. Use your knowledge of chemical thermodynamics to explain this observation.

Exercise 2.4.6

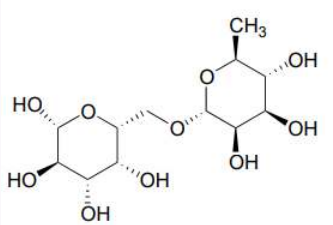
Below is the structure of the artificial sweetener sucralose. Identify the two anomeric carbons in the disaccharide.



sucralose

Exercise 2.4.7

Robinose is a disaccharide found in 'Chenille Plant', a flowering shrub native to the Pacific Islands.



robinose

a. Identify the two anomeric carbons and the glycosidic bond in robinose.
b. Using the same carbon numbering system as for glucose in the earlier figure, fill in in the carbon numbers (#1 through #6) for each of the monosaccharides that make up robinose.
c. Based on what you know of glycosidic bond-forming reactions in nature, propose a reasonable mechanism for the linking of the two monosaccharides, starting with the activated hemiacetal species, assuming that it is a UDP species as in the cellulose gycosidic bond-forming reaction.
d. Draw the open chain form of each of the monosaccharides

Exercise 2.4.8

Look again at the structures of the two-glucose fragments of cellulose and amylose shown the introduction to this chapter. A structural feature of the cellulose polymer makes it inherently more resistant to enzymatic hydrolysis compared to starch. Explain.

Hint

Think about intermolecular interactions.

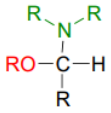
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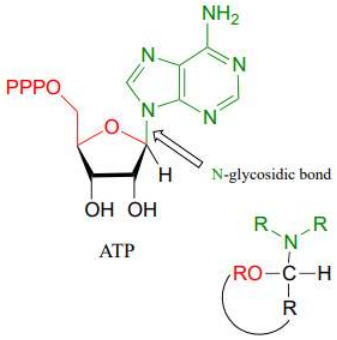
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2.5: N-glycosidic Bonds

We have just seen that when a second alcohol attacks a hemiacetal or hemiketal, the result is an acetal or ketal, with the glycosidic bonds in carbohydrates providing a biochemical example. But if a hemiacetal is attacked not by a second alcohol but by an amine, what results is a kind of 'mixed acetal' in which the anomeric carbon is bonded to one oxygen and one nitrogen.

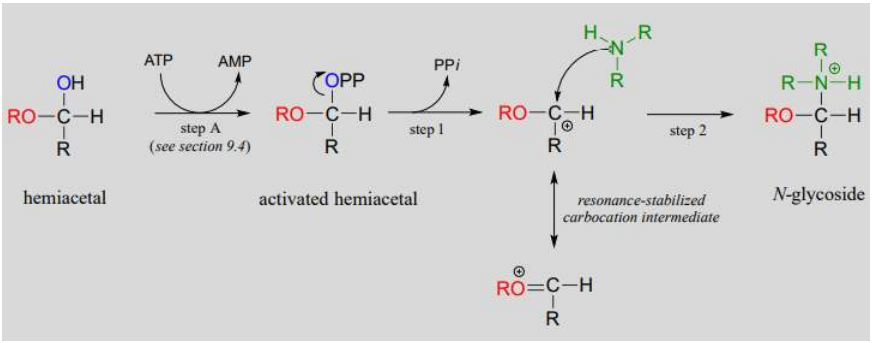


This arrangement is referred to by biochemists as an N-glycosidic bond. You may recognize these as the bonds in nucleosides and nucleotides that link the G, C, A, T, or U base to the sugar.



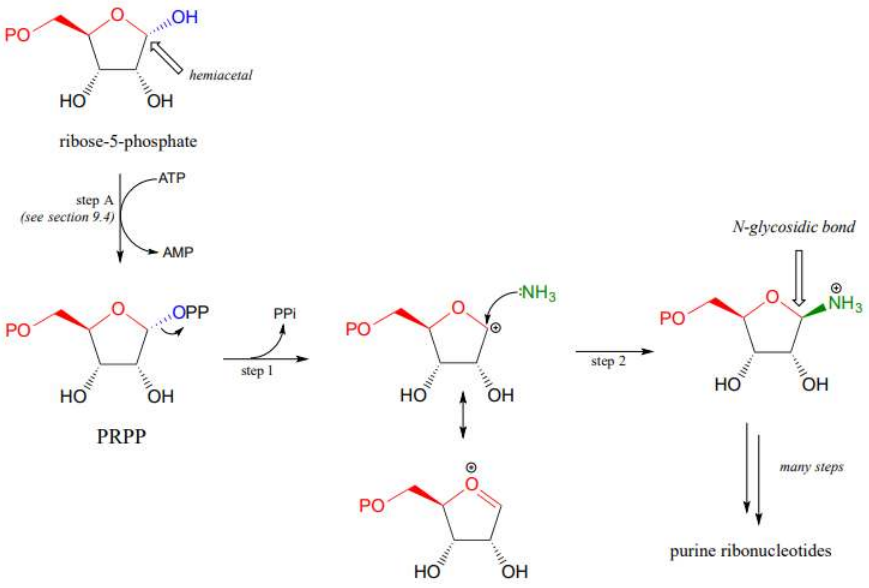
The formation of ***N***-glycosidic bonds in ribonucleotides is closely analogous to the formation of glycosidic bonds in carbohydrates – again, it is an S_N1-like process with an activated water leaving group. Typically, the hemiacetal is activated by diphosphorylation, as illustrated in step A of the general mechanism below.

Mechanism for formation of an ***N***-glycosidic bond:



The starting point for the biosynthesis of purine (G and A) ribonucleotides is a five-carbon sugar called ribose-5-phosphate, which in solution takes the form of a cyclic hemiacetal. The critical ***N***-glycosidic bond is established through substitution of NH_3 for OH at the anomeric carbon of the ribose. The anomeric OH group is first activated (step A below) to form an activated intermediate called phosphoribosylpyrophosphate (PRPP). The inorganic pyrophosphate then leaves to generate a resonance-stabilized carbocation (step 1) which is attacked by a nucleophilic ammonia in step 2 to establish the ***N***-glycosidic bond.

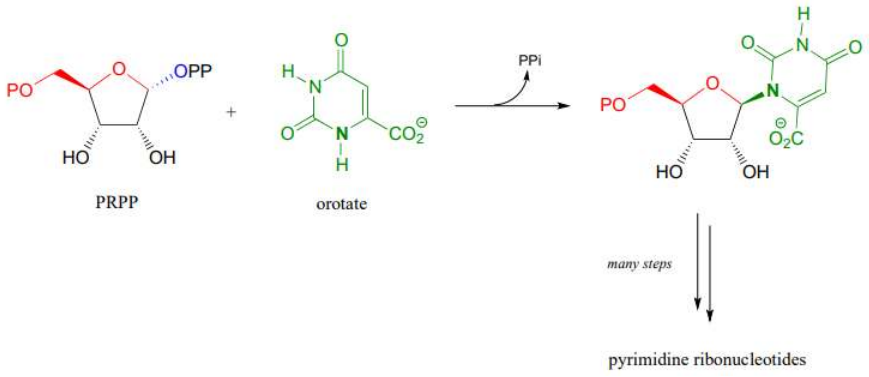
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With the ***N***-glycosidic bond in place, the rest of the purine base is assembled piece by piece by other biosynthetic enzymes.

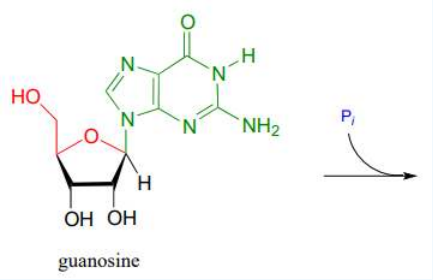
(The mechanism above should look familiar - we saw step A in chapter 9 as an example of alcohol diphosphorylation , and steps 1 and 2 in chapter 8 as an example of a biochemical S_N1 reaction).

Establishment of the ***N***-glycosidic bond in biosynthesis of the pyrimidine ribonucleotides and (U, C and T) also begins with PRPP, but here the ring structure of the nucleotide base part of the biomolecule has already been 'pre-fabricated' in the form of orotate:



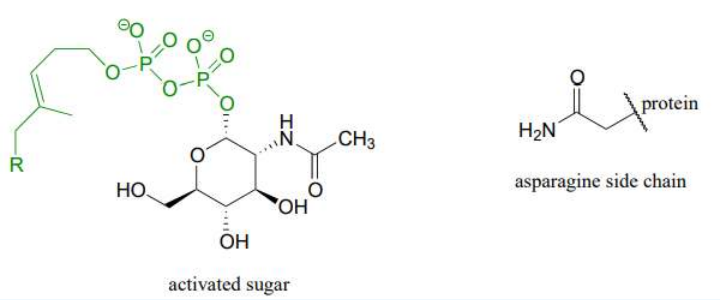
? Exercise 10.5.1

We have just seen an illustration of the formation of an N-glycosidic bond in a biosynthetic pathway. In the catabolic (degradative) direction, an N-glycosidic bond must be broken, in a process which is analogous to the hydrolysis of a glycosidic bond (illustrated earlier). In the catabolism of guanosine nucleoside, the N-glycosidic bond is broken by inorganic phosphate (not water!) apparently in a concerted (SN2-like) displacement reaction (Biochemistry 2011, 50, 9158). Predict the products of this reaction, and draw a likely mechanism.



Exercise 10.5.2

Glycoproteins are proteins that are linked, by glycosidic or *N*-glycosidic bonds, to sugars or carbohydrates through an asparagine, serine, or threonine side chain on the protein. As in other glycosylation and *N*-glycosylation reactions, the hemiacetal of the sugar must be activated prior to glycosidic bond formation. Below is the structure of the *activated* sugar hemiacetal substrate in an asparagine glycosylation reaction



Draw the product of the asparagine glycosylation reaction, assuming inversion of configuration of the anomeric carbon.

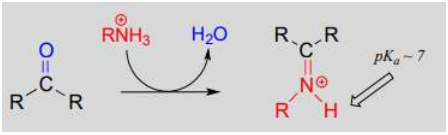
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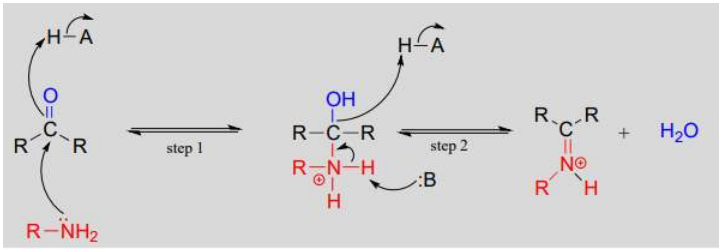
2.6: Imines

The electrophilic carbon atom of aldehydes and ketones can be the target of nucleophilic attack by amines as well as alcohols. The end result of attack by an amine nucleophile is a functional group in which the C=O double bond is replaced by a C=N double bond, and is known as an imine. (An equivalent term is 'Schiff base', but we will use 'imine' throughout this book). Recall from section 7.5B that imines have a pKa of approximately 7, so at physiological pH they can be accurately drawn as either protonated (iminium ion form) or neutral (imine).

Iminium ion formation:



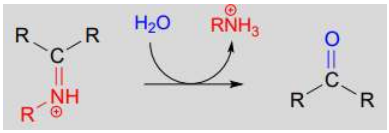
Mechanism (enzymatic):



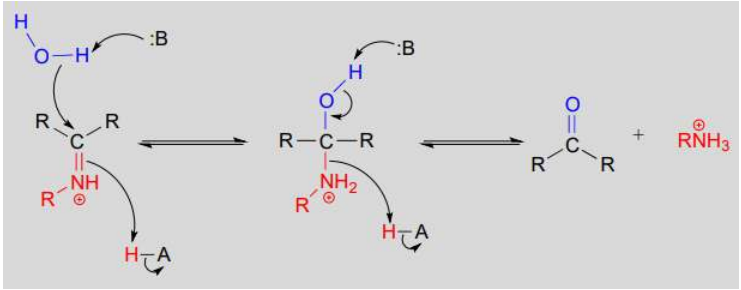
Mechanistically, the formation of an imine involves two steps. First, the amine nitrogen attacks the carbonyl carbon in a nucleophilic addition step (step 1) which is closely analogous to hemiacetal and hemiketal formation. Based on your knowledge of the mechanism of acetal and ketal formation, you might expect that the next step would be attack by a second amine to form a compound with a carbon bound to two amine groups – the nitrogen version of a ketal or acetal. Instead, what happens next (step 2 above) is that the nitrogen lone pair electrons 'push' the oxygen off of the carbon, forming a $C = N$ double bond (an iminium) and a displaced water molecule.

The conversion of an iminium back to an aldehyde or ketone is a hydrolytic process (bonds are broken by a water molecule), and mechanistically is simply the reverse of iminiom formation:

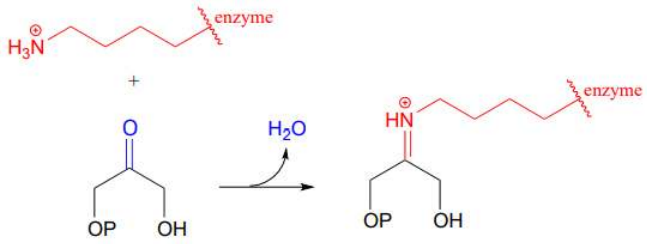
Hydrolysis of an iminium ion:



Mechanism (enzymatic):

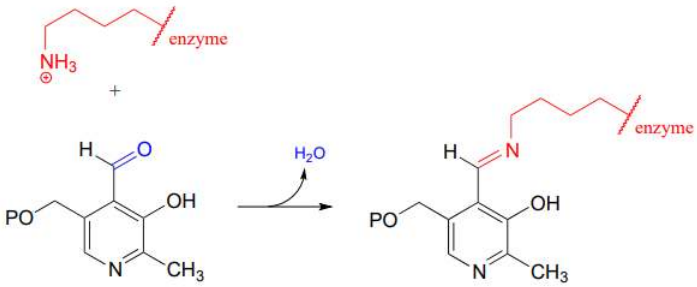


Carbon-carbon bond forming enzymes called aldolases (which we'll cover in detail in a later chapter) often form iminium links between a carbonyl carbon on a substrate and a lysine residue from the active site of the enzyme, as in this aldolase reaction from the Calvin Cycle:

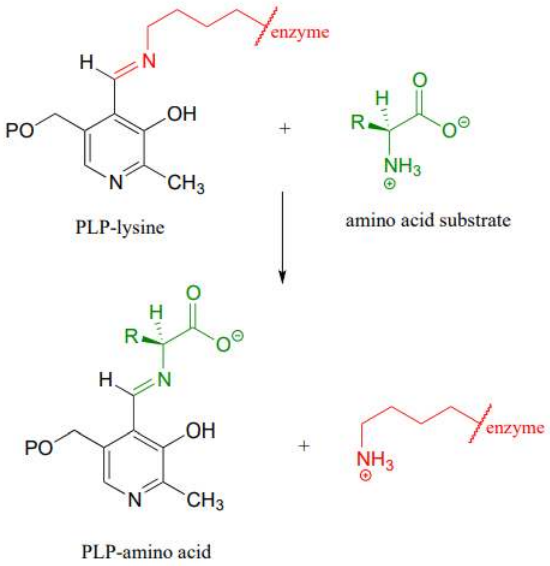


After the carbon-carbon bond forming part of an aldolase reaction is completed, the iminium linkage is hydrolyzed, freeing the product so that it can diffuse out of the active site and allow another catalytic cycle to begin.

In a later chapter, we will learn about reactions that are dependent upon a coenzyme called pyridoxal phosphate (***PLP***), also known as vitamin ***B6***. In these reactions, the aldehyde carbon of ***PLP*** links to an enzymatic lysine in the active site:

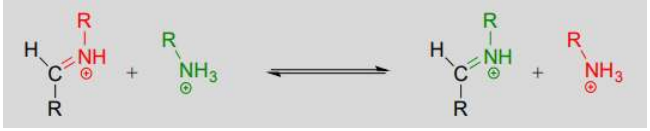


Then, the ***PLP***-lysine imine linkage is traded for an imine linkage between ***PLP*** and the amino group on the substrate, in what can be referred to as a transimination.

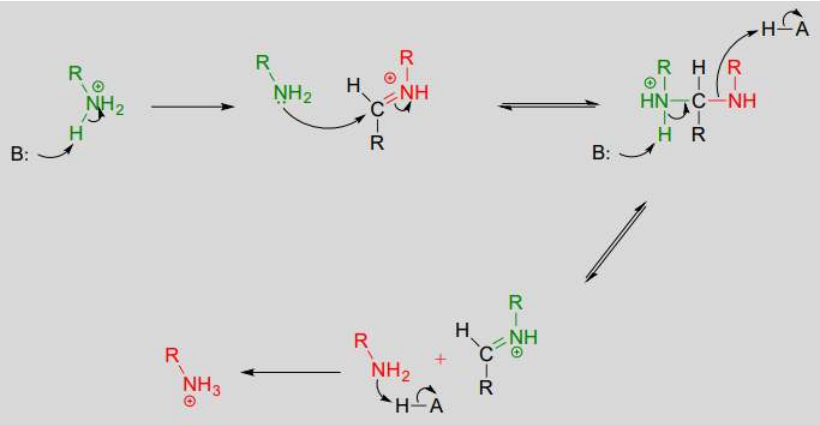


The mechanism for a transimination is very similar to that of imine formation:

Transimination reaction:



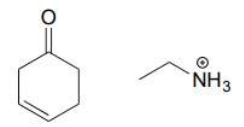
Mechanism:



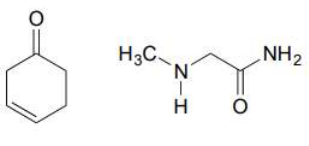
Exercise 2.6.1

Draw an imine that could be formed between each pair of compounds.

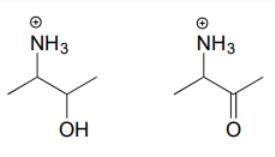
a.



b.

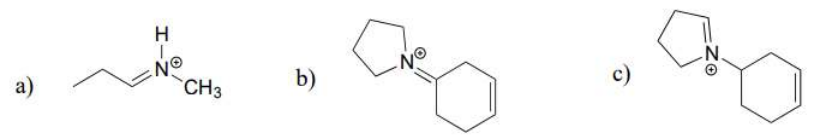


c.



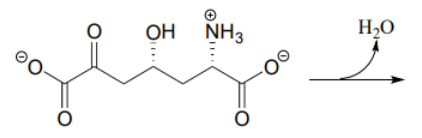
Exercise 2.6.2

Draw the iminium hydrolysis product for each of the following compounds.

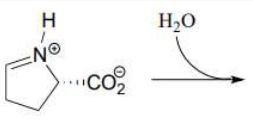


Exercise 2.6.3

a. The metabolic intermediate shown below undergoes an intramolecular imine formation as a step in the biosynthesis of lysine (EC 4.3.3.7). Draw the product of this intramolecular imine formation step.



b. Predict the product of this iminium hydrolysis step (EC 2.3.1.117) from the proline degradation pathway.



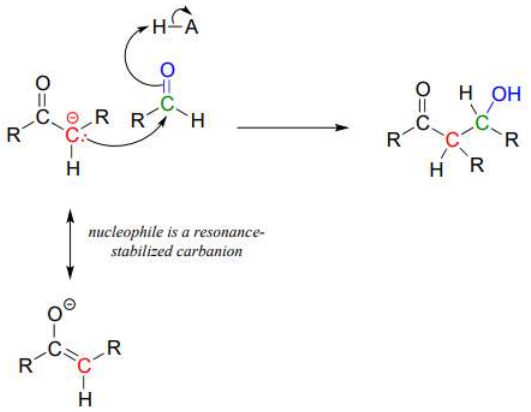
Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

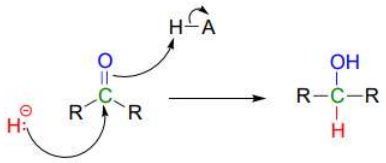
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2.7: A Look Ahead - Addition of Carbon and Hydride Nucleophiles to Carbonyls

We have seen in this chapter a number of reactions in which oxygen and nitrogen nucleophiles add to carbonyl groups. Other nucleophiles are possible in carbonyl addition mechanisms: in chapters 7 and 8, for example, we will examine in detail some enzyme-catalyzed reactions where the attacking nucleophile is a resonance stabilized carbanion (usually an enolate ion):



Then in chapter 6, we will see how the carbonyl groups on aldehydes and ketones can be converted to alcohols through the nucleophilic addition of what is essentially a hydride (H^-) ion.



Contributors

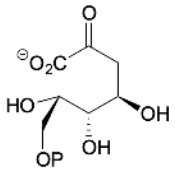
- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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2.P: Nucleophilic Carbonyl Addition Reactions (Problems)

P2.1: Draw a mechanism showing the formation of an imine linkage between a lysine side chain and α-ketobutyrate (this is the first step in the degradation of lysine, EC 1.5.1.8).

P2.2: Draw four possible cyclic hemiketal isomers of the compound below.



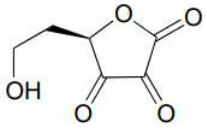
P2.3: A downstream intermediate in the lysine degradation pathway undergoes imine hydrolysis to release two amino acid products (EC 1.5.1.1). Draw a mechanism for this hydrolysis reaction, and show the structures of the two products formed.

P2.4: Below is the structure of lactose, the sugar found in dairy products.

Lactose is a disaccharide of galactose and glucose. People who are lactose intolerant do not produce enough lactase - the enzyme that hydrolyzes the glycosidic bond linking the two monosaccharides - to be able to fully digest dairy products.

a. Draw a likely stabilized carbocation intermediate in the hydrolysis reaction catalyzed by lactase.
b. Draw, in the chair conformation, the structure of what you predict would be the most abundant form of the galactose monosaccharide in aqueous solution.
c. Is galactose an aldose or a ketose?
d. Draw, showing sterochemistry, the open-chain form of galactose.

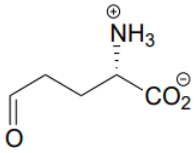
P2.5: You probably know that ascorbic acid (vitamin C) acts as an antioxidant in the body. When vitamin C does its job, it ends up being oxidized to dehydroascobate, which is usually drawn as shown below, in the so-called tricarbonyl form.



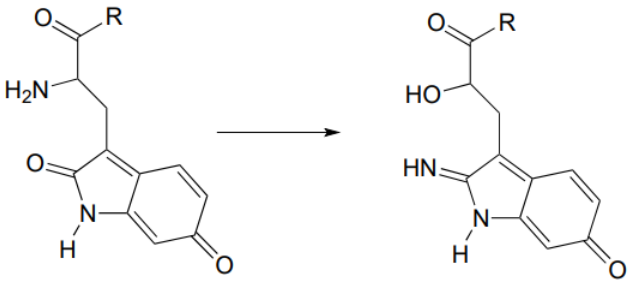
dehydroascorbate
(tricarbonyl form)

Evidence suggests, however, that the most important form of dehydroascorbate in a physiological context is one in which one of the ketone groups is in its hydrated form, and the other is an intramolecular hemiketal (see Chemical and Engineering News, Aug. 25, 2008, p. 36). Show the structure of this form of dehydroascorbic acid.

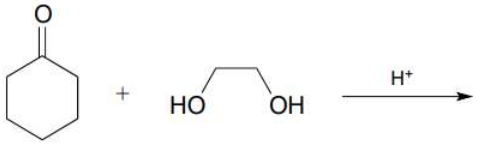
P2.6: The compound below is the product of a ring-opening imine hydrolysis step in the degradation pathway for proline, one of the amino acids. Draw the structure of the starting compound.



P2.7: The rearrangement below was proposed to proceed via imine formation followed by nucleophilic substitution. Propose a mechanism that fits this description. (J. Biol. Chem. 280, 12858, scheme 2 part 2) .

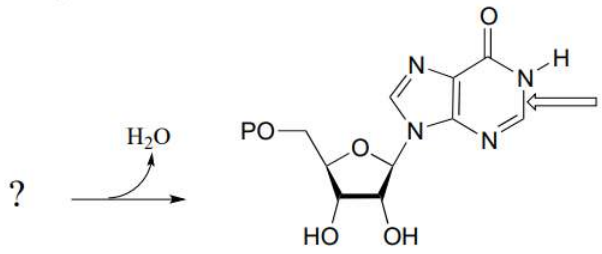


P2.8: The biochemical acetal-forming reactions we learned about in this chapter all require activation of the hemiacetal through phosphorylation. In the organic synthesis lab, non-enzymatic acetal-forming reactions are carried out with a catalytic amount of strong acid, which serves to activate the hemiacetal. Predict the product of the following acetal forming reaction, and propose a reasonable mechanism for the reaction. Remember that the reaction is carried out under acidic conditions, which means that the protonation state of intermediates will be different than biochemical reactions occurring at neutral ***pH***.



Problems 9-15 all involve variations on, and combinations of, the nucleophilic addition steps that we studied in this chapter. Although the reactants and/or products may look somewhat different from the simpler aldehydes, acetals, imines, etc. that we used as examples in the chapter, the key steps still involve essentially the same mechanistic patterns. Before attempting these problems, you may want to review tautomerization reactions in section 7.6.

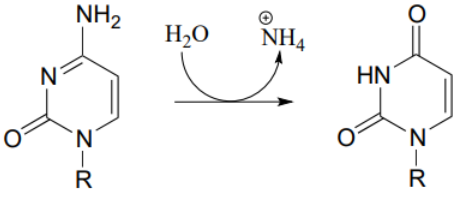
P2.9: The final step in the biosynthesis of inosine monophosphate (IMP, a precursor to both AMP and GMP), is a ring-closing reaction in which a new nitrogen-carbon bond (indicated by an arrow in the structure below) is formed. Predict the starting substrate for this reaction, and propose a mechanism that involves a slight variation on typical imine formation. (EC 3.5.4.10)



inosine monophosphate

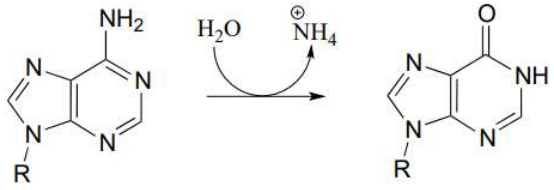
P2.10: Propose a mechanism for these steps in nucleotide metabolism:

a. (EC 3.5.4.5)



Cytidine Uridine

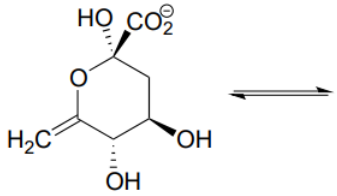
b. (EC 3.5.4.4)



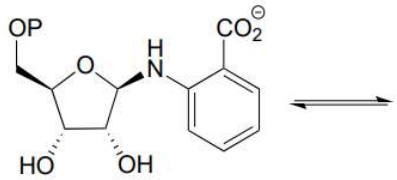
Adenosine Inosine

P2.11

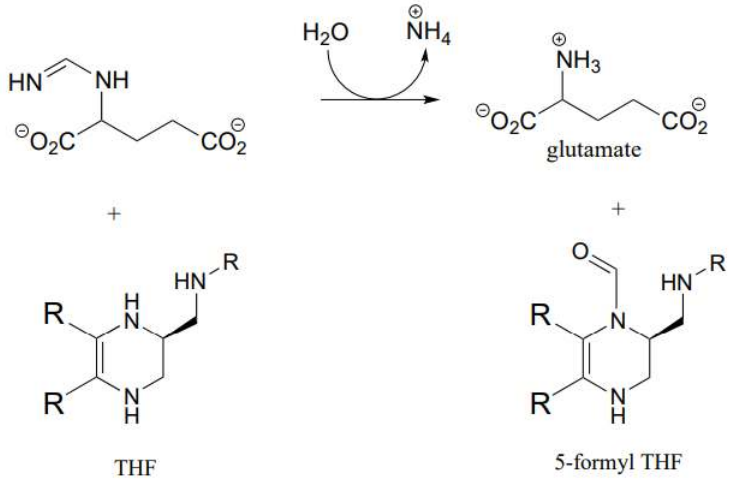
a. Draw the structure (including stereochemistry) of the compound that results when the cyclic hemiketal shown below coverts to an open-chain compound with two ketone groups.



b. The compound shown below undergoes a ring-opening reaction to form a species that can be described as both an enol and an enamine. Draw the structure (including stereochemistry) of this product, and a likely mechanism for its formation. (EC 5.3.1.24)



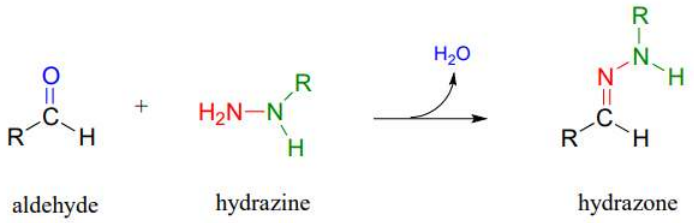
P2.12: Tetrahydrofolate (***THF***) is a coenzyme that serves as a single-carbon donor in many biochemical reactions. Unlike ***S***-adenosylmethionine (SAM, see section 8.8), the carbon being transferred in a ***THF***-dependent reaction is often part of a carbonyl. Below is a reaction in the histidine degradation pathway (EC 3.5.3.8). The mechanism involved is thought to be an transimination, followed by a imine-to-imine tautomerization, followed by an imine hydrolysis. Propose a reasonable mechanism that fits this description. Hint: first identify the carbon atom being transferred.

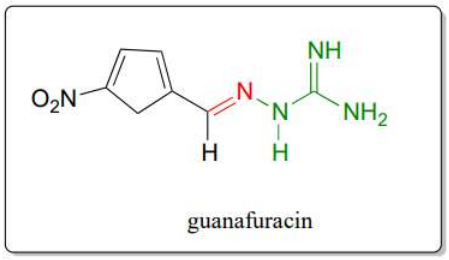


P2.13: Hydrazones are close relatives of imines, formed in reactions between aldehydes/ketones and hydrazines, a functional group containing a nitrogen-nitrogen bond. The mechanism for hydrazone formation is analogous to that of imine formation.

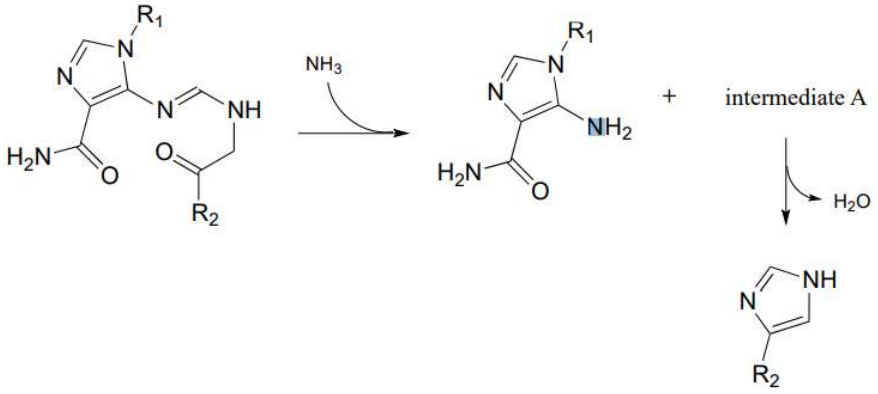
Guanafuracin, a known antibiotic compound, is a hydrazone, and can be prepared easily in the laboratory by combining equimolar amounts of the appropriate aldehyde and hydrazine in water (no heat or acid catalyst is required, and the reaction is complete in seconds).

Determine the starting materials required for the synthesis of guanafuracin, and propose a likely mechanism for the reaction.





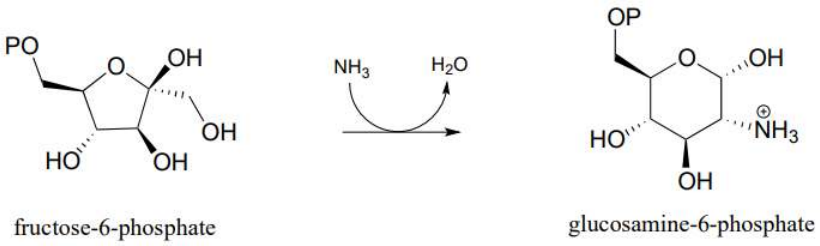
P2.14: Propose reasonable mechanisms for the following steps from the histidine biosynthesis pathway, and predict the structure of intermediate A (which is open-chain, not cyclic).



The last several problems are quite challenging!

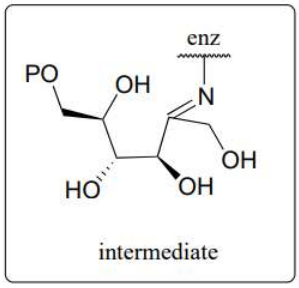
P2.15: Propose a likely mechanism for the synthesis of glucosamine 6-phosphate from fructose-6-phosphate. One of several intermediates is shown. (EC 2.6.1.16.)

https://chem.libretexts.org/@go/page/291133



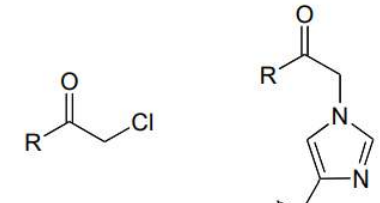
fructose-6-phosphate

glucosamine-6-phosphate



intermediate

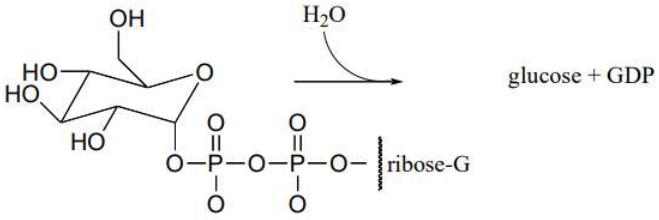
P2.16: α-chloromethyl ketones (structure below) are effective irreversible inhibitors of proteolytic (peptide-bond breaking) enzymes such as chymotrypsin. In these enzymes, a nucleophilic serine plays a key role in the reaction. The mechanism for inactivation of a-chymotrypsin is thought to involve, as a first step, nucleophilic attack by the active site serine on the carbonyl of the inhibitor. However, when the inactivated enzyme is analyzed, an active site histidine rather than the serine, is found to be covalently modified by the inhibitor. The structure of the modified histidine is shown below. The mechanism of inactivation is thought to involve an epoxide intermediate - with this in mind, propose a reasonable mechanism of inactivation.



-chloromethyl
ketone inhibitor

modified histidine

P2.17: An enzyme in E. coli bacteria catalyzes the hydrolysis of a-glucose-GDP to glucose.



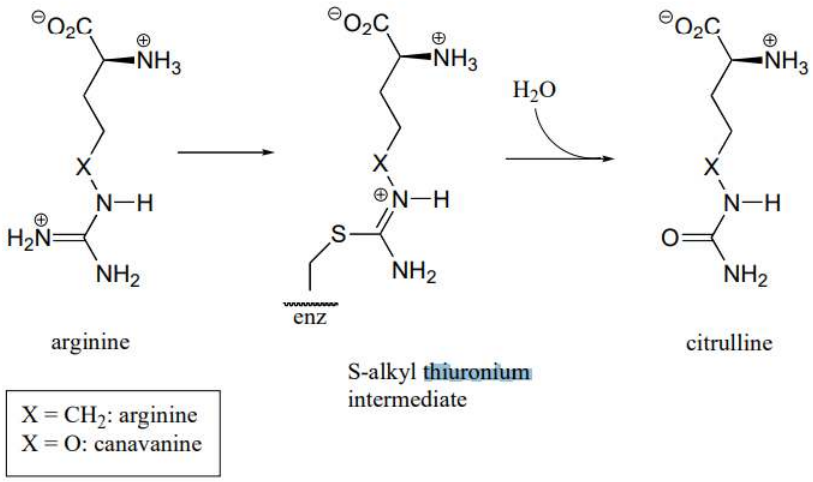
glucose + GDP

^1H-NMR analysis of the reaction in progress showed the initial appearance of a doublet at 4.64 ppm with J = 7.9 Hz (the spectrum contained other signals as well, of course). After 20 minutes (at which point the hydrolysis reaction has been complete for some time), another doublet began to appear slightly downfield, this one with J = 4.0 Hz. Over time, the strength of the downfield signal gradually increased and that of the upfield signal gradually decreased, until they stabilized at constant levels.

Draw a mechanism for the enzymatic hydrolysis reaction, and correlate your mechanism to the NMR data (including the appearance of the second doublet).

https://chem.libretexts.org/@go/page/291133

P2.18: Arginine deaminase, an enzyme in the arginine degradation pathway, catalyzes the transformation of (***L***)-arginine to (***L***)-citrulline via a covalent substrate-cysteine intermediate.



This enzyme is the target for the development of drugs for cancer and immunological diseases such as arthritis. However, rather than completely and permanently shutting down the enzyme (eg. with an irreversible inhibitor), researchers are looking for a way to temporarily 'turn down' the activity of the enzyme. One strategy that has recently been reported involves the use of an oxygen-containing arginine analog, called canavanine, which reacts in the same way as arginine except that the second (hydrolysis) step is very slow. While the enzyme is covalently attached to the inhibitor (in the ***S***-alkyl thiuronium stage), it is inactivated.

a. Show a mechanism for the reaction catalyzed by arginine deaminase.
b. Explain how the electronic effect of the oxygen substituent would slow down the hydrolysis step of the reaction, and why the rate of the hydrolysis step is more affected by the oxygen substitution than the ***S***-alkylthiuronium-forming step.

Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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2.S: Nucleophilic Carbonyl Addition Reactions (Summary)

Before moving on to the next chapter, you should be confident in your ability to:

- Recognize aldehyde and ketone groups in organic biomolecules
- Draw/explain the bonding picture for aldehyde and ketone groups
- Explain why the carbonyl carbon in an aldehyde or ketone is electrophilic
- Draw complete curved arrow mechanisms for the following reaction types:
  - formation of a hemiacetal/hemiketal
  - collapse of a hemiacetal/hemiketal to revert to an aldehyde/ketone
  - formation and hydrolysis of an acetal/ketal
  - formation and hydrolysis of an N-glycosidic bond
  - formation and hydrolysis of an imine
  - transimination
- Explain how the carbocation intermediates in glycosidic bond formation and hydrolysis reactions are stabilized by resonance
- Explain the stereochemical considerations of a nucleophilic addition to an aldehyde/ketone, especially in the context of glycosidic bond formation. Be able to identify the re and si faces of an aldehyde, ketone, or imine.
- In addition to these fundamental skills, you should develop your confidence in working with end-of-chapter problems involving more challenging, multi-step biochemical reactions.

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CHAPTER OVERVIEW

3: Oxidation and Reduction Reactions

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3.3: Oxidation and Reduction in the Context of Metabolism
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3.1: Prelude to Oxidation and Reduction Reactions



Introduction

Theo Ross was not doing very well at his summer job, and he was frustrated. His boss had given him specific instructions, and yet Theo kept botching the job, over and over again. Theo was not used to failure – he had achieved almost perfect scores on both the ACT and SAT college entrance exams, and was headed to Stanford University in the fall. Why couldn't he get it right? It wasn't brain surgery, after all.

Well, actually – it was brain surgery.

An April 17, 2014 article in Sports Illustrated tells Theo's story. Wanting to do something interesting over the summer of 2010 before he started college, Theo had applied for a research internship at the National Institutes of Health in Bethesda, Maryland. This was an extremely competitive program normally reserved for outstanding college students, but somehow Theo had managed to win a coveted spot in the program, working with Dr. Dorian McGavern, a neurologist studying how meningitis effects the brain. Dr. McGavern assigned Theo the task of performing 'skull-thinning' surgery on mice, part of which involved using a special saw to shave down a small section of the bone in order to gain access to the brain. It was a delicate procedure, something that even some experienced neurosurgeons who had tried it had found challenging. Any small slip resulted in a concussion to the mouse's brain, rendering it useless for the study. Theo just couldn't get the hang of it, and ended up concussing one mouse after another.

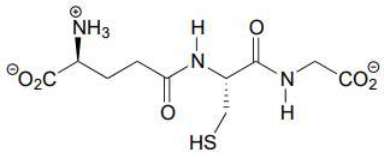
You have probably heard the old expression: “when life gives you lemons, make lemonade”. Theo made a lot of lemonade that summer.

Theo and Dr. McGavern eventually realized that his failure at the procedure actually presented an opportunity to observe what happens to a brain right after a concussive injury. Theo started doing more skull-thinning surgeries, but now the goal was to cause concussion, rather than to avoid it. The concussed mice (who had been anaesthetized prior to the surgery) were immediately strapped under a microscope so that Theo could observe how their brains responded to the injury. This was new, and very exciting stuff: most of what neurologists knew about concussions up to that point had come from MRI (magnetic resonance imaging – see chapter 5) or autopsies. Nobody knew very much about what happens at the cellular level in a brain in the minutes and hours after a concussion has occurred. In addition, the problem of traumatic head injuries and the long-lasting effects they cause was becoming an increasingly hot topic in the news, critically relevant to thousands of veterans returning from Iraq and Afghanistan as well as to football players and other athletes in contact sports– including Theo, who had been a competitive wrestler in high school. (You might have been wondering why Theo's story appeared in Sports Illustrated – now you know.)

Theo spent the rest of that summer, and every spring break and summer vacation over the next few years, working in McGavern's lab on the new project. He and McGavern found evidence that the 'hidden' damage to a concussed brain – that which went

undetected in MRI scans but could come back to haunt the victim years later in the form of recurring headaches, memory loss, and depression – may be caused by a type of molecule referred to as 'reactive oxygen species', or ROS, leaking from damaged tissues into the brain. ROS are potentially harmful byproducts of respiration such as hydrogen peroxide (H_2O_2) that are constantly being produced in our cells. Although ROS can cause serious oxidative damage if they are allowed to build up, our bodies have evolved ways to deal with them, using so-called 'ROS scavengers' to convert them to something innocuous like water.

With this new understanding, Theo and his mentor had another idea: what if they could prevent the ROS from causing further damage to a recently concussed brain by applying an scavenger to the injury? After some trial and error, they found that an ROS scavenger compound called glutathione, when applied directly to the skull of a concussed mouse within a few minutes to three hours after the injury, could permeate the bone and react with the ROS. Brain cells from these glutathione-treated mice appeared normal, with none of the signs of ROS damage Theo was used to seeing.



glutathione

The road from an initial scientific discovery to a safe and effective medical treatment is often a very long one, but Theo Roth and Dorian McGavern appear to have made a discovery that could eventually help prevent some of the most devastating and long-term damage caused by traumatic head injuries. In the end, it's a very good thing that Theo's hands were not cut out for brain surgery.

The chemistry of oxidation and reduction - often called 'redox' chemistry - is central to Theo Roth's discovery about what happens to a concussed brain at the molecular level. This chapter is dedicated to redox chemistry. We'll begin with a reminder of what you learned in General Chemistry about the fundamentals of redox reactions in the context of inorganic elements such as iron, copper and zinc: reduction is a gain of electrons, and oxidation is a loss of electrons. Then, we'll expand our understanding to include bioorganic redox reactivity, examining among other things how alcohols are converted to ketones and aldehydes, aldehydes are converted to carboxylic acids, and amines are converted to imines. We will also talk about redox reactions in the broader context of metabolism in living things.

A central player in some of the biochemical redox reactions we will see is the coenzyme called glutathione, Theo Roth's 'magic bullet' molecule that was able to rescue mouse brain cells from death by oxidation. We'll see how glutathione acts as a mediator in the formation and cleavage of disulfide bonds in proteins, and how it acts as an 'ROS scavenger' to turn hydrogen peroxide into water.

Contributors

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3.2: Oxidation and Reduction of Organic Compounds - An Overview

You are undoubtedly already familiar with the general idea of oxidation and reduction: you learned in general chemistry that when a compound or element is oxidized it loses electrons, and when it is reduced it gains electrons. You also know that oxidation and reduction reactions occur in tandem: if one species is oxidized, another must be reduced at the same time - thus the term 'redox reaction'.

Most of the redox reactions you have seen previously in general chemistry probably involved the flow of electrons from one metal to another, such as the reaction between copper ion in solution and metallic zinc:

$$Cu^{+2}_{(aq)} + Zn_{(s)} \rightarrow Cu_{(s)} + Zn^{+2}_{(aq)} \tag{3.2.1}$$

Exercise 3.2.1

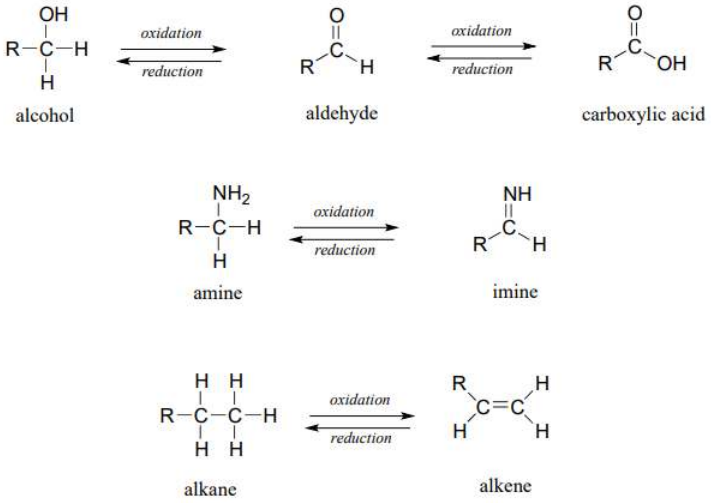
Reading the reaction above from left to right, which chemical species is being oxidized? Which is being reduced?

When we talk about the oxidation and reduction of organic compounds, what we are mainly concerned with is the number of carbon-heteroatom bonds in the compound compared to the number of carbon-hydrogen bonds. (Remember that the term 'heteroatom' in organic chemistry generally refers to oxygen, nitrogen, sulfur, or a halogen).

Note

- Oxidation of an organic compound results in an increase in the number of carbon-heteroatom bonds, and/or a decrease in the number of carbon-hydrogen bonds.
- Reduction of an organic compound results in a decrease in the number of carbon-heteroatom bonds, and/or an increase in the number of carbon-hydrogen bonds.

Below are a number of common functional group transformations that are classified as redox.



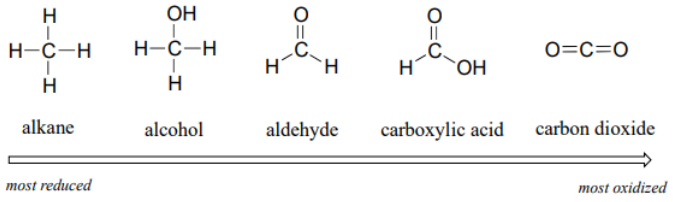
Heteroatoms such as oxygen and nitrogen are more electronegative than carbon, so when a carbon atom gains a bond to a heteroatom, it loses electron density and is thus being oxidized. Conversely, hydrogen is less electronegative than carbon, so when a carbon gains a bond to a hydrogen, it is gaining electron density, and thus being reduced.

Exercise 3.2.2

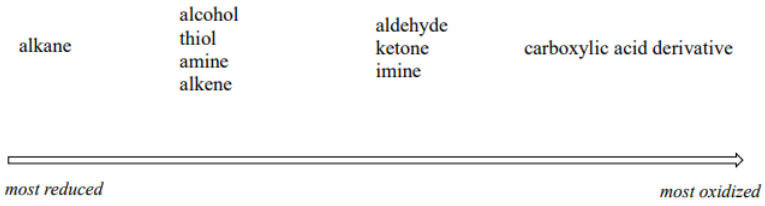
The hydration of an alkene to an alcohol is not classified as a redox reaction. Explain.

For the most part, when talking about redox reactions in organic chemistry we are dealing with a small set of very recognizable functional group transformations. The concept of oxidation state can be useful in this context. When a compound has lots of carbon-hydrogen bonds, it is said to be in a lower oxidation state, or a more reduced state. Conversely, if it contains a lot of carbon-heteroatom bonds, it is said to be in a higher oxidation state.

We'll start with a series of single carbon compounds as an example. Methane, in which the carbon has four bonds to hydrogen, is the most reduced member of the group. The compounds become increasingly oxidized as we move from left to right, with each step gaining a bond to oxygen and losing a bond to hydrogen. Carbon dioxide, in which all four bonds on the carbon are to oxygen, is in the highest oxidation state.

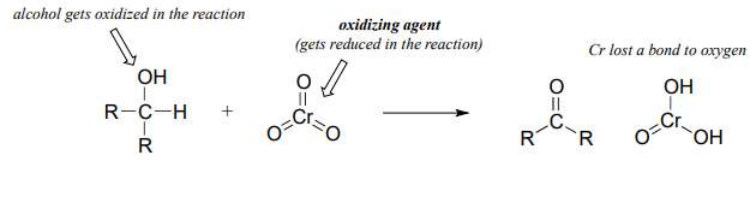


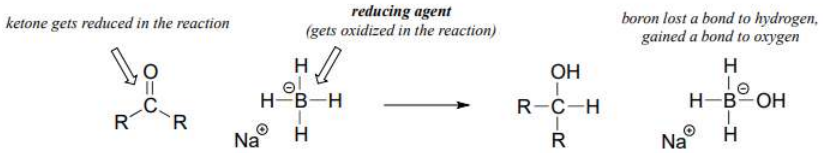
More generally, we can rank the oxidation state of common functional groups:



The alkane oxidation state is the most reduced. Alcohols, thiols, amines, and alkenes are all at the same oxidation state: therefore, a reaction converting one of these groups to another - an alcohol to alkene conversion, for example - is not a redox reaction. Aldehydes, however, are at a higher oxidation state than alcohols, so an alcohol to aldehyde conversion is an oxidation. Likewise, an imine to amine conversion is a reduction, but an imine to ketone conversion is not a redox reaction.

It is important to keep in mind that oxidation and reduction always occurs in tandem: when one compound is oxidized, another compound must be reduced. Often, organic chemists will use the terms oxidizing agent and reducing agent to refer to species that are commonly used, by human chemists or by nature, to achieve the oxidation or reduction of a variety of compounds. For example, chromium trioxide (CrO_3) is a laboratory oxidizing agent used by organic chemists to oxidize a secondary alcohol to a ketone, in the process being reduced to H_2CrO_3. Sodium borohydride ($NaBH_4$) is a laboratory reducing agent used to reduce ketones (or aldehydes) to alcohols, in the process being oxidized to $NaBH_3OH$.





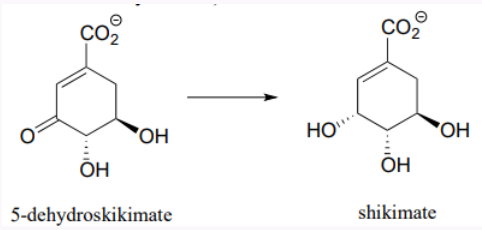
There is a wide selection of oxidizing and reducing agents available for use in the organic chemistry laboratory, each with its own particular properties and uses. For example, while sodium borohydride is very useful for reducing aldehyde and ketone groups to alcohols, it will not reduce esters and other carboxylic acid derivatives. If you take a course in synthetic organic chemistry, you will learn about the use of many of these agents.

In this book, of course, we are concerned primarily with the organic chemistry that occurs within a living cell. A large part of this chapter will be spent looking at the action of two very important classes of coenzymes - the nicotinamides and the flavins - that serve as biochemical oxidizing and reducing agents. We also consider the oxidation and reduction of sulfur atoms in thiol groups, especially the thiol group on the side chain of cysteine residues in proteins.

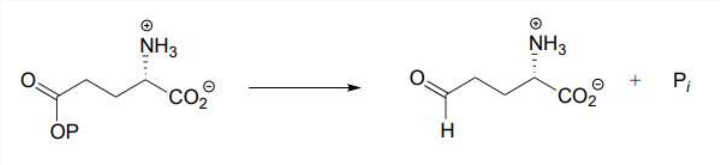
Exercise 3.2.3

Each of the biochemical transformations shown below is a step in amino acid metabolism. For each, state whether the substrate is being oxidized, reduced, or neither oxidized nor reduced.

a. (from aromatic amino acid biosynthesis)



b. (from the biosynthesis of arginine and proline)



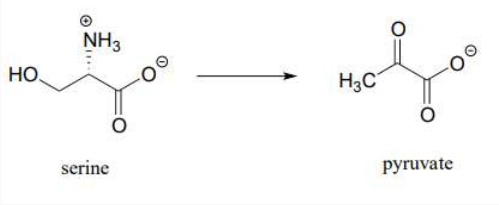
c. (from the catabolism of lysine)



d. (from the catabolism of tryptophan)



e. (from the catabolism of serine)



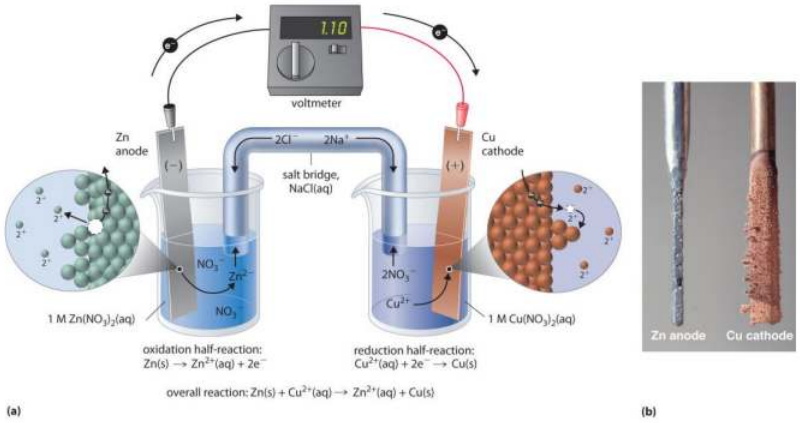
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3.3: Oxidation and Reduction in the Context of Metabolism

Think back again to the redox chemistry that you learned in your general chemistry course. A common experiment in a general chemistry lab is to set up a galvanic cell consisting of a copper electrode immersed in an aqueous copper nitrate solution, connected by a wire to a zinc electrode immersed in an aqueous zinc nitrate solution.



When the cell is completed with a salt bridge, an electrical current begins to flow - what we have is a simple battery (figure a above). Over time, the copper electrode gets heavier as metallic copper is deposited on the copper cathode, while the zinc anode slowly dissolves into solution (figure b above). The redox reaction occurring here is:

$$\mathrm{Cu^{+2}\,(aq) + Zn\,(s) \;\rightarrow Cu\,(s) + Zn^{+2}\,(aq) + energy} \tag{3.3.1}$$

Electrons flow from zinc metal to copper cations, creating zinc cations and copper metal: in other words, zinc metal is being oxidized to zinc cation and copper cation is being reduced to copper metal, as expressed by the two relevant half-cell reactions:

$$\mathrm{Cu^{+2}\,(aq) + 2\,e^{-} \;\rightarrow Cu^{0}\,(s)} \tag{3.3.2}$$

$$\mathrm{Zn^{0}\,(s) \;\rightarrow Zn^{+2}\,(aq) + 2\,e^{-}} \tag{3.3.3}$$

We can predict before we set up the cell that the spontaneous flow of electrons will go in the zinc to copper direction, just by looking at a table of standard reduction potentials (such a table was no doubt in your general chemistry text).

Table **3.3.1**: Standard reduction potentials at **25°**

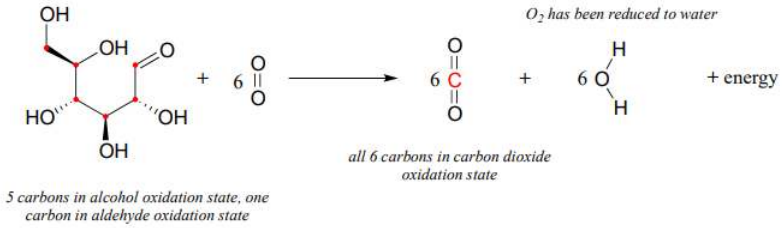
Reduction half-reaction	Reduction potential (volts)
$\mathrm{Ag^{+1}\,(aq) + e^{-} \;\rightarrow Ag^{0}\,(s)}$	0.800
$\mathrm{Cu^{+2}\,(aq) + 2\,e^{-} \;\rightarrow Cu^{0}\,(s)}$	0.337
$\mathrm{H^{+1}\,(aq) + 2\,e^{-} \;\rightarrow H_2\,(g)}$	0 (Standard)
$\mathrm{Pb^{+2}\,(aq) + 2\,e^{-} \;\rightarrow Pb^{0}\,(s)}$	-0.126
$\mathrm{Fe^{+2}\,(aq) + 2\,e^{-} \;\rightarrow Fe^{0}\,(s)}$	-0.441
$\mathrm{Zn^{+2}\,(aq) + 2\,e^{-} \;\rightarrow Zn^{0}\,(s)}$	-0.763

Copper ion (Cu^{+2}) has a higher standard reduction potential than zinc ion (Zn^{+2}), meaning that, under identical conditions, more energy is released by reducing one mole of Cu^{+2} ion to Cu^{0} metal than is released by reducing one mole of Zn^{+2} ion to Zn^{0} metal.. Another way to think about this is to imagine that the copper ion 'wants' to gain electrons more than the zinc ion does. Conversely, zinc metal 'wants' to lose electrons more than the copper metal does. Therefore, transfer of two electrons from zinc metal to Cu^{2+} is a thermodynamically downhill process, whereas the reverse process - transfer of two electrons from copper metal to Zn^{2+} - is thermodynamically uphill.

$$Cu^{+2}(aq) + Zn(s) \rightarrow Cu(s) + Zn^{+2}(aq) + energy \quad (3.3.4)$$

$$Cu(s) + Zn^{+2}(aq) + energy \rightarrow Cu^{+2}(aq) + Zn(s) \quad (3.3.5)$$

Let's now extend the idea of redox reactions to the context of metabolism in living things. When we 'burn' glucose for energy, we transfer (by a series of enzyme-catalyzed reactions) electrons from glucose to molecular oxygen (O_2), oxidizing the six carbon molecules in glucose to carbon dioxide and at the same time reducing the oxygen atoms in O_2 to water. The overall chemical equation is:



The transfer of electrons from glucose to O_2 is a thermodynamically downhill, energy-releasing process, just like the transfer of electrons from zinc metal to copper ion. And while you could have used the energy released by the zinc/copper redox reaction to light a small light bulb, your cells use the energy released by the glucose/oxygen redox process to carry out a wide variety of energy-requiring activities, such as walking to your organic chemistry lecture.

In your general chemistry copper/zinc experiment, was it possible to reverse the reaction so that it runs in the uphill direction - in other words, to oxidize copper and reduce zinc?

$$Zn^{+2}(aq) + Cu(s) + energy \rightarrow Zn(s) + Cu^{+2}(aq) \quad (3.3.6)$$

Just ask yourself the question: is it possible to get water to flow uphill? Of course it is - but only if you supply a pump and some energy!

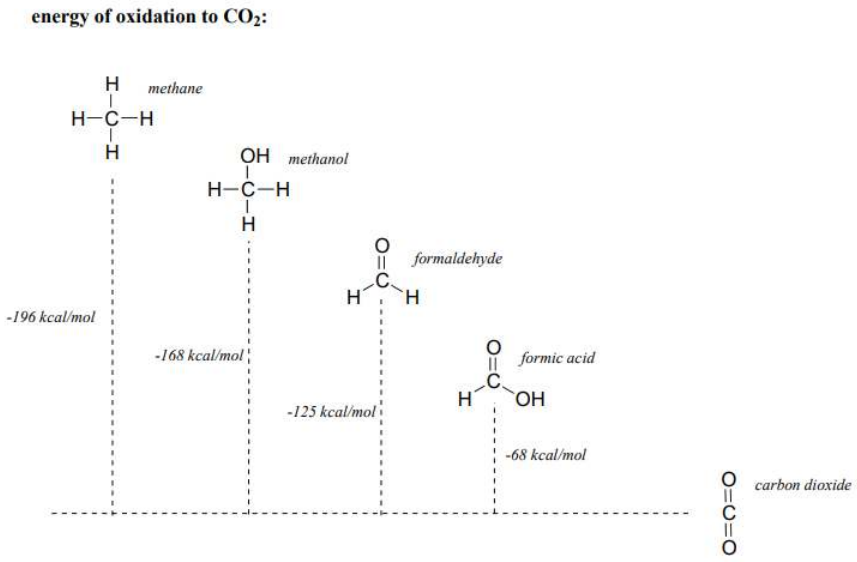
The same idea applies to 'pumping' electrons uphill in your copper-zinc electrochemical cell: all you need to do is to provide some energy in the form of an external electrical current in order to pump the electron flow in the uphill direction. You are recharging your battery.

Thinking again in a biochemical context: plants are able, by a process called photosynthesis, to reduce carbon dioxide and oxidize water to form glucose and molecular oxygen: essentially recharging the ecosystem's biochemical battery using energy from the sun.

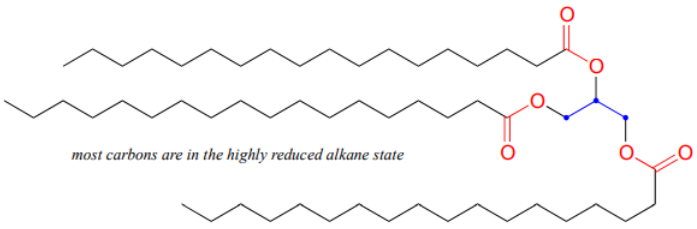
$$6\,CO_2 + 6\,H_2O + energy \rightarrow C_6H_{12}O_6 + 6\,O_2 \quad (3.3.7)$$

On a global scale, oxidation of the carbons in glucose to CO_2 by non-photosynthetic organisms (like people) and the subsequent reductive synthesis of glucose from CO_2 by plants is what ecologists refer to as the 'carbon cycle'.

In general the more reduced an organic molecule is, the more energy is released when it is oxidized to CO_2. Going back to our single-carbon examples, we see that methane, the most reduced compound, releases the most energy when oxidized to carbon dioxide, while formic acid releases the least:

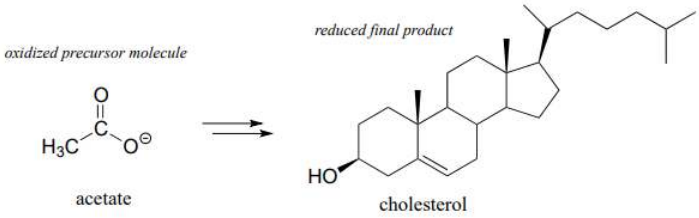


A lipid (fat) molecule, where most of the carbons are in the highly reduced alkane state, contains more energy per gram than glucose, where five of the six carbons are in the more oxidized alcohol state (look again at the glucose structure we saw just a couple of pages back).



a saturated fat molecule

After we break down and oxidize sugar and fat molecules to obtain energy, we use that energy to build large, complex molecules (like cholesterol, or DNA) out of small, simple precursors. Many biosynthetic pathways are reductive: the carbons in the large biomolecule products are in a reduced state compared to the small precursors. Look at the structure of cholesterol compared to that of acetate, the precursor molecule from which all of its carbon atoms are derived - you can see that cholesterol is overall a more reduced molecule.



acetate

cholesterol

While we are focusing here on the mechanistic details of the individual organic redox reactions involved in metabolism, if you take a course in biochemistry you will learn much more about the bigger picture of how all of these reactions fit together in living systems.

Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

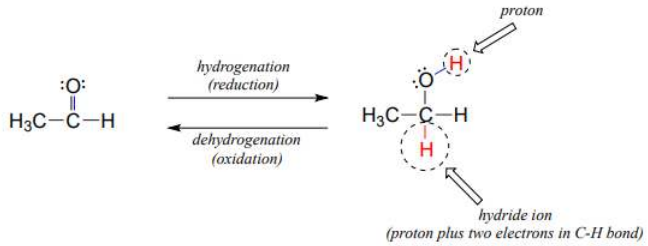
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3.4: Hydrogenation of Carbonyl and Imine Groups

Next, we'll go on to look at the actual chemical mechanisms involved in the enzyme-catalyzed oxidation and reduction of biomolecules.

An overview of hydrogenation and dehydrogenation

Many of the redox reactions that you will encounter when studying the central metabolic pathways are classified as hydrogenation or dehydrogenation reactions. Hydrogenation is simply the net addition of a hydrogen (H_2) molecule to a compound, in the form of a hydride ion (H^-, a proton plus a pair of electrons) and a proton. Hydrogenation corresponds to reduction. Dehydrogenation reactions are the reverse process: loss of a hydride and a proton. Dehydrogenation corresponds to oxidation.



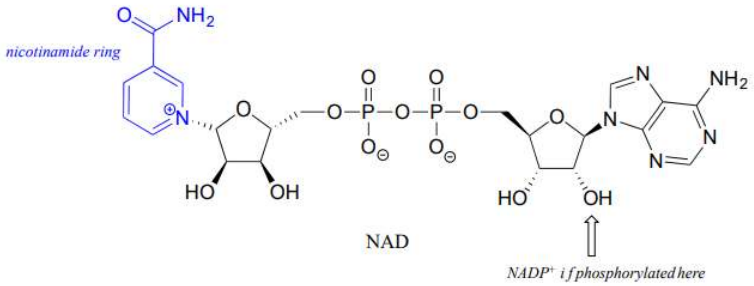
Hydrogenation and dehydrogenation reactions can also be called hydride transfer reactions, because a hydride ion is transferred from the molecule being oxidized to the one being reduced. In the next few sections, we will learn about two important classes of coenzyme molecules that serve hydride ion acceptors (oxidizing agents) and hydride ion donors (reducing agents) in biochemical redox reactions.

> **Note**
>
> Be careful not to confuse the terms *hydrogenation* and *dehydrogenation* with *hydration* and *dehydration* - the latter terms refer to the gain or loss of water, while the former terms refer to the gain or loss of hydrogen.

Many mechanistic patterns that we have already learned about in previous chapters will come into play again in this discussion, with the only variation being that here, a hydride ion will act as a nucleophile (in the hydrogenation direction) or as a leaving group (in the dehydrogenation direction). The key to understanding these reactions will be to understand how a hydride can act as a nucleophile or leaving group.

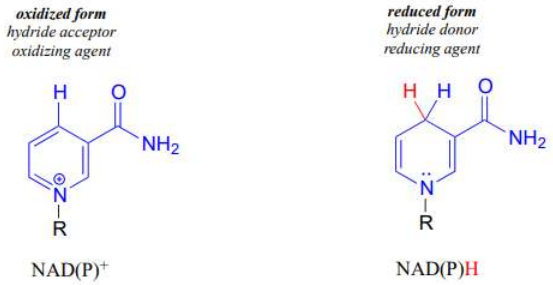
Nicotinamide adenine dinucleotide - a hydride transfer coenzyme

Although we are talking here about hydrides acting as nucleophiles and leaving groups, you already know that literal hydride ions are far too unstable to exist as discreet intermediates in the organic reactions of living cells (the pK_a of H_2, the conjugate acid of hydride, is about 35: a very weak acid, meaning hydride is a very strong base and not a reasonable species to propose for a biochemical reaction). As was alluded to earlier, biochemical hydrogenation/dehydrogenation steps require the participation of a specialized hydride transfer coenzyme. The most important of these is a molecule called **nicotinamide adenine dinucleotide**. The full structure of the oxidized form of this coenzyme, abbreviated NAD^+, is shown below, with the active nicotinamide group colored blue. Because the redox chemistry occurs specifically at the nicotinamide ring (in blue in the figure below), typically the rest of the molecule is simply designated as an 'R' group.



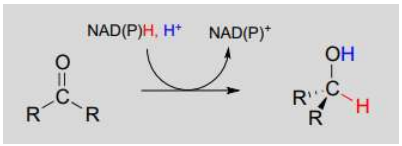
If the hydroxyl group indicated by the arrow is phosphorylated, the coenzyme is called $NADP^+$. The phosphate is located far from the nicotinamide ring and does not participate directly in the hydride transfer function of the cofactor. It is, however, important in a larger metabolic context: as a general rule, redox enzymes involved in catabolism (the breakdown of large molecules) typically use the non-phosphorylated coenzyme, while those involved in anabolism (biosynthesis of large molecules from small precursors) use the phosphorylated coenzyme.

NAD^+ and $NADP^+$ both function in biochemical redox reactions as hydride acceptors: that is, as oxidizing agents. The reduced forms of the coenzyme, abbreviated NADH and NADPH, serve as hydride donors: that is, as reducing agents.

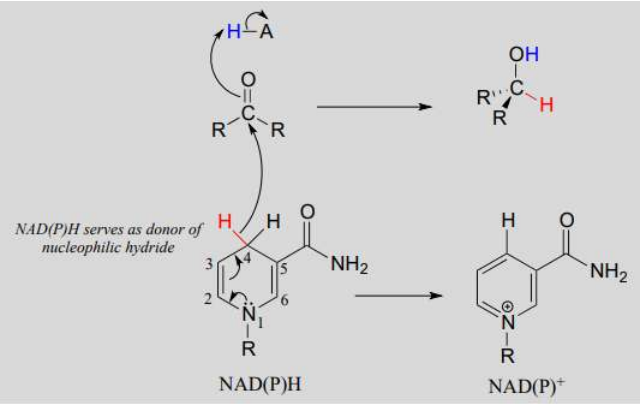


To understand how the nicotinamide coenzymes function in hydride transfer, let's look at a general picture of a reversible, redox conversion from a ketone to a secondary alcohol. Mechanistically, the reaction we are about to see can be described as a nucleophilic addition to a carbonyl - a mechanism type we studied in chapter 10 - with the twist that the nucleophilic species is a hydride ion. At the beginning of the reaction cycle, both the ketone substrate and the NADH cofactor are bound in the enzyme's active site, and carbon #4 of the nicotinamide ring is positioned very close to the carbonyl carbon of the ketone.

NAD(P)H-dependent hydrogenation (reduction) of a ketone



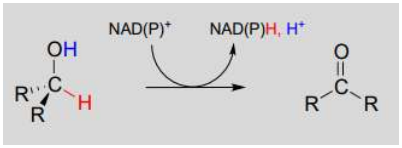
Mechanism:



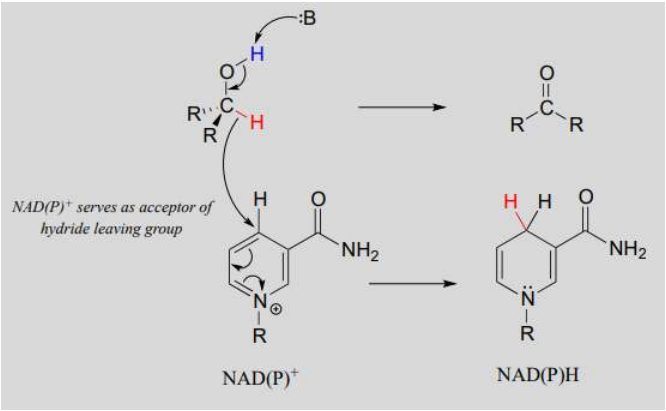
As an enzymatic group transfers a proton to the ketone oxygen, the carbonyl carbon loses electron density and becomes more electrophilic, and is attacked by a hydride from NADH. Because carbon #4 of NADH is bound in such close proximity to the electrophile, this step can occur without generating a free hydride ion intermediate – the two hydride electrons can be pictured as shifting from one carbon to another. Note the products of this reaction: the ketone (which accepted a hydride and a proton) has been reduced to an alcohol, and the NADH cofactor (which donated a hydride) has been oxidized to NAD^+.

The dehydrogenation of an alcohol by NAD^+ is simply the reverse of a ketone hydrogenation:

$NAD(P)^+$-dependent dehydrogenation (oxidation) of an alcohol



Mechanism:



An enzymatic base positioned above the carbonyl removes a proton, and the electrons in the $O-H$ bond shift down and push out the hydride, which shifts over to carbon #4 of NAD^+. Note that the same process with a primary alcohol would yield an aldehyde instead of a ketone.

Exercise 3.4.1

Draw general mechanisms for:

a. hydrogenation of an imine

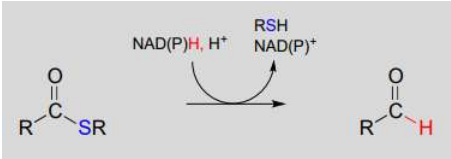
b. dehydrogenation of an amine

Exercise 3.4.2

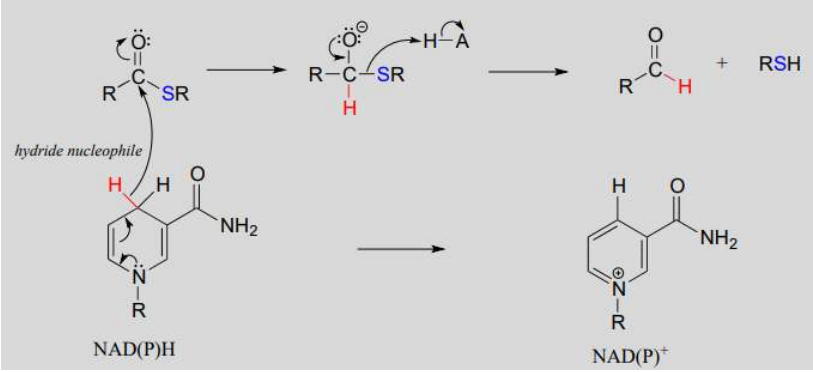
We just saw that when the nucleophile in a nucleophilic carbonyl addition step is a hydride ion from NADH, the result is a ketone/aldehyde hydrogenation reaction. As a review: what kind of reaction step results when the nucleophile in this process is not a hydride ion but a) an alcohol, or b) an enolate carbon?

The nicotinamide coenzymes also serve as hydride donors/acceptors in the redox reactions interconverting carboxylic acid derivatives and aldehydes. Notice that these reactions can be thought of as nucleophilic acyl substitution reactions (Chapter 6) in which the nucleophile or leaving group is a hydride ion.

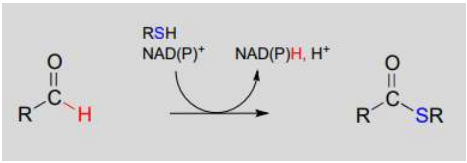
NAD(P)H-dependent hydrogenation (reduction) of a thioester to an aldehyde:



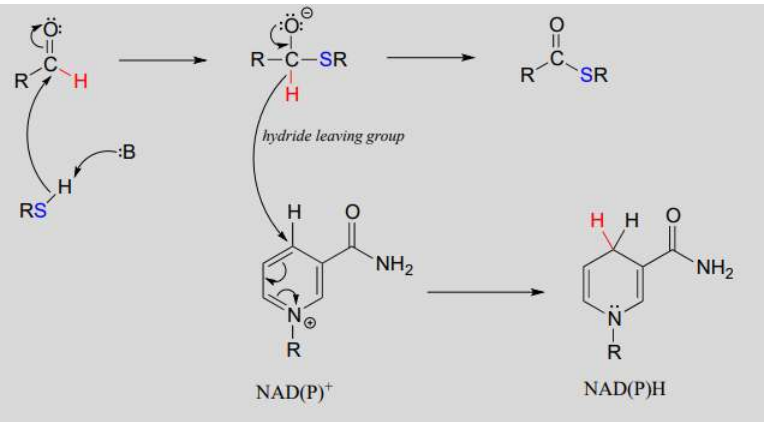
Mechanism:



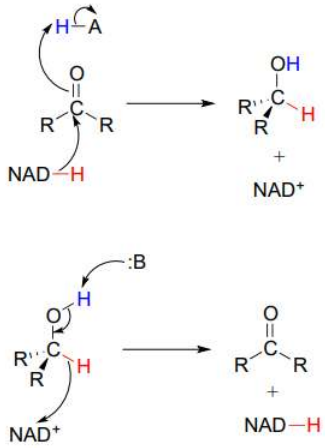
$NAD(P)^+$-dependent dehydrogenation (oxidation) of an aldehyde to a thioester:



Mechanism:



To simplify figures, hydrogenation and dehydrogenation reactions are often drawn with the role of the coenzyme abbreviated:



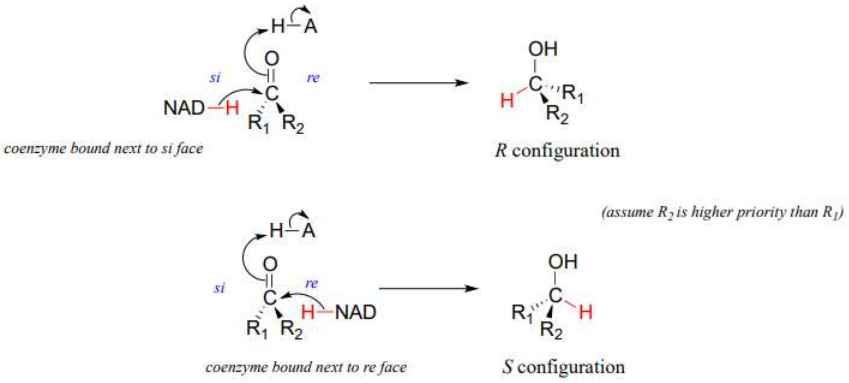
However, it is very important to make sure that you can remember and draw out the full mechanism, including the role of the coenzyme, in these types of reactions.

Caution

A very common error made by students learning how to draw biochemical redox mechanisms is to incorrectly show nicotinamide coenzymes acting as acids or bases. Remember: NADH and NADPH are hydride donors, **NOT** proton donors. NAD^+ and $NADP^+$ are hydride acceptors, **NOT** proton acceptors.

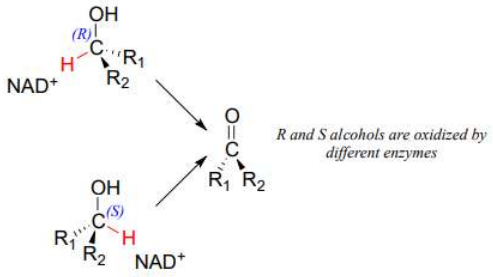
Stereochemistry of ketone hydrogenation

It should not surprise you that the stereochemical outcomes of enzymatic hydrogenation / dehydrogenation steps are very specific. Consider the hydrogenation of an asymmetric ketone: In the hydrogenation direction, attack by the hydride can occur from either the *re* or the *si* face of an asymmetric ketone, leading specifically to the S or R alcohol.



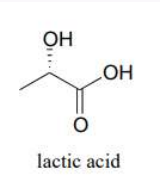
The stereochemical configuration of the product depends on which side of the ketone substrate the NAD(P)H coenzyme is bound in the active site. Any given enzyme will catalyze its reaction with one of these two stereochemical outcomes, not both.

Stereochemical considerations apply in the dehydrogenase direction as well: in general, enzymes specifically catalyze the oxidation of either an R or S alcohol, but not both.



Exercise 3.4.3

During an intense workout, lactic acid forms in muscle tissue as the result of enzymatic reduction of a ketone group in the precursor molecule (EC 1.1.1.27). It is the lactate that you can blame for the sore muscles you feel the day after a workout.



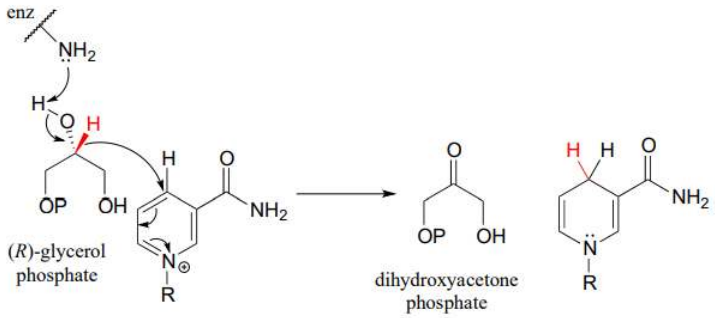
a. Draw the structure of the starting ketone in this reaction.

b. Which face of the ketone is the coenzyme positioned next to in the active site of the enzyme?

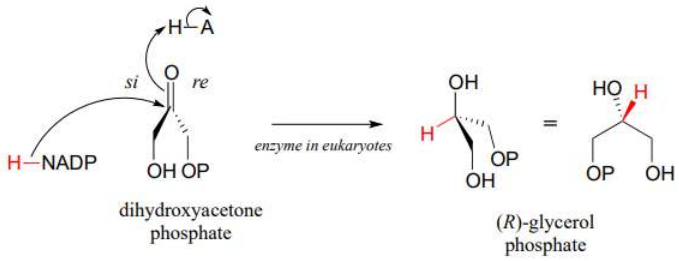
Examples of biochemical carbonyl/imine hydrogenation

Now that we have covered the basics, let's look at some real examples of hydrogenation and dehydrogenation reactions.

Glycerol phosphate dehydrogenase (EC 1.1.1.8) catalyzes one of the final chemical steps in the breakdown of fat molecules. The enzyme specifically oxidizes (R)-glycerol phosphate to dihydroxyacetone phosphate. (S)-glycerol phosphate is not a substrate for this enzyme. J. Mol. Biol. 200



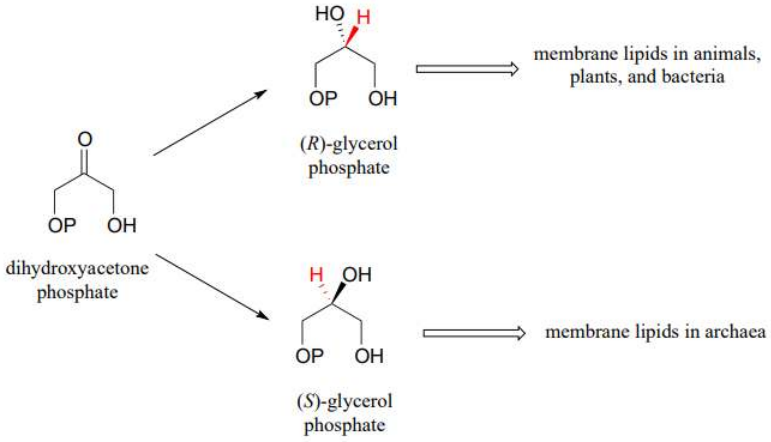
The reverse reaction (catalyzed by the same enzyme) converts dihydroxyacetone phosphate to (R)-glycerol phosphate, which serves as a starting point for the biosynthesis of membrane lipid molecules.



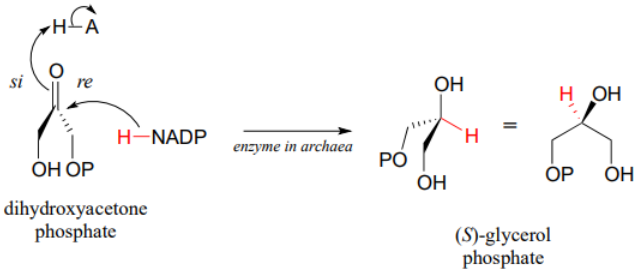
Exercise 3.4.4

X-ray crystallography experiments reveal that in the active site of glycerol phosphate dehydrogenase, a zinc cation (Zn^{+2}) is coordinated to the oxygen atom of the carbonyl/alcohol group. How does this contribute to catalysis of the reaction?

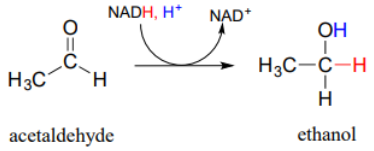
While the cell membranes of animals, plants, and bacteria are made from lipids with the R stereochemistry exclusively, archaeal microbes (the so-called 'third kingdom of life) are distinguished in part by the S stereochemistry of their membranes.



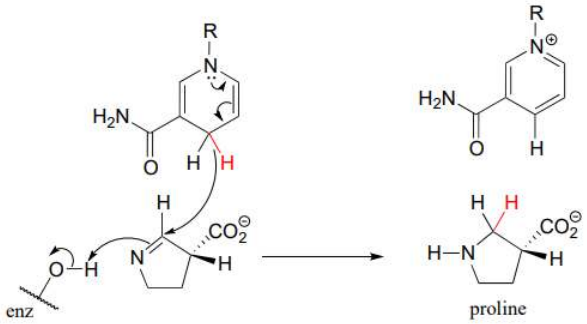
Archaea have an enzyme that catalyzes hydrogenation of dihydroxyacetone with the opposite stereochemistry compared to the analogous enzyme in bacteria and eukaryotes. This archaeal enzyme was identified and isolated in 1997.



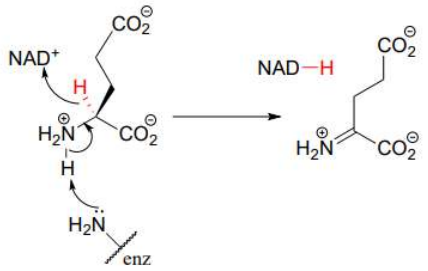
In a reaction that is relevant to people who enjoy the occasional 'adult beverage', an NADH-dependent enzyme (EC 1.1.1.1) in brewer's yeast produces ethanol by reducing acetaldehyde. This is the final step in the process by which yeast ferment glucose to ethanol.



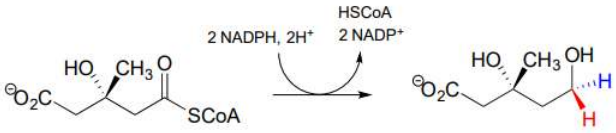
The reaction below, which is the final step in the biosynthesis of proline (EC 1.5.1.2), is an example of an enzymatic reduction of an imine to an amine. J. Mol. Biol. 2005, 354, 91.



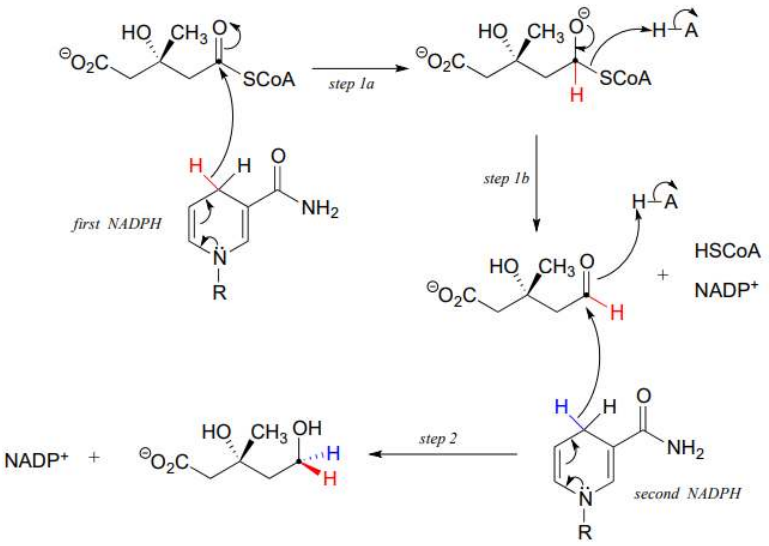
This step in the breakdown of the amino acids glutamate (EC 1.4.1.2) provides an example of the oxidation of an amine to an imine: Structure 1999, 7, 769.



The 'double reduction' reaction below (EC 1.1.1.34) is part of the isoprenoid biosynthetic pathway, which eventually leads to cholesterol in humans.

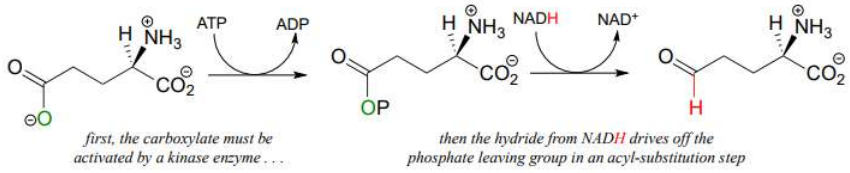


In this reaction, a thioester is first reduced to an aldehyde in steps 1a and 1b:

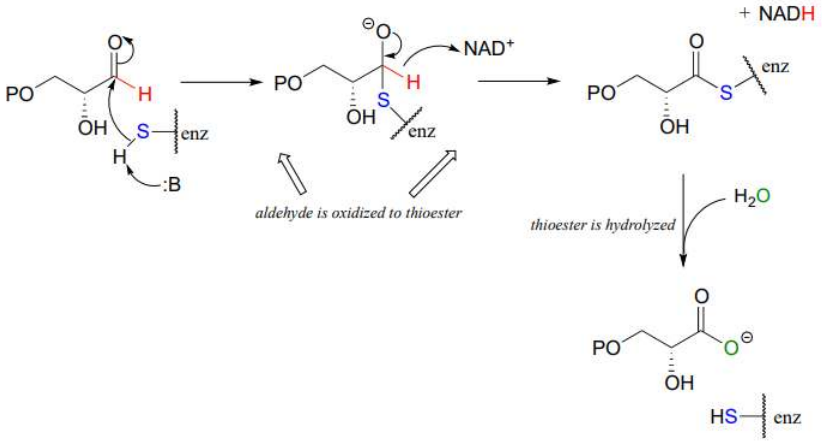


Then in step 2, the aldehyde is in turn reduced by the same enzyme (and a second NADPH that enters the active site) to a primary alcohol. This enzyme is inhibited by atorvastatin and other members of the statin family of cholesterol-lowering drugs. Atorvastatin, marketed under the trade name Lipitor by Pfizer, is one of the all-time best-selling prescription medications.

Carboxylates are not reactive in acyl substitution steps, so it follows that they cannot be directly reduced to aldehydes by an enzyme in the same way that thioesters can. However, a carboxylate can be converted to its 'activated' acyl phosphate form, which can then be hydrogenated. An example of this is found in a two-reaction sequence found in amino acid metabolism (EC 2.7.2.11; EC1.2.1.41).



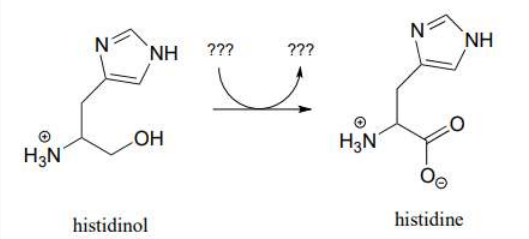
Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) , a key enzyme in the glycolysis pathway, provides an example of the oxidation of an aldehyde to a thioester, in this case a thioester linkage between the substrate and a cysteine residue in the enzyme's active site. In the second phase of the reaction, the thioester intermediate is hydrolyzed to free the carboxylate product.



https://chem.libretexts.org/@go/page/291170

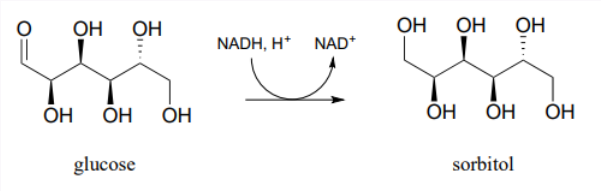
Exercise 3.4.5

Below is the final step in the biosynthesis of the amino acid histidine (EC 1.1.1.23). Fill in the species that are indicated with question marks. Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 1859



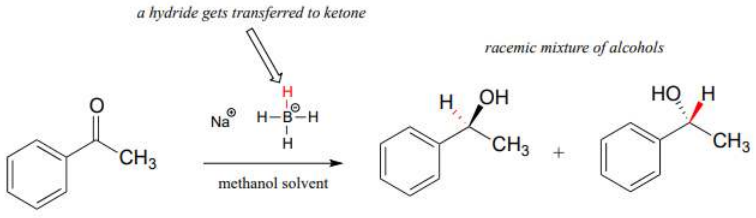
Exercise 3.4.6

Draw a likely mechanism for the conversion of glucose to sorbitol, a process that occurs in the liver. Do not abbreviate the nicotinamide ring structure.



Reduction of ketones and aldehydes in the laboratory

Although our focus in this book is biological organic reactions, it is interesting to note that synthetic organic chemists frequently perform hydrogenation reactions in the lab that are similar in many respects to the NAD(P)H-dependent reactions that we have just finished studying. A reagent called sodium borohydride ($NaBH_4$) is very commonly used, often in methanol solvent, to reduce ketones and aldehydes to alcohols. The reagent is essentially a laboratory equivalent of NADH (or NADPH: it serves as a source of nucleophilic hydride ions. Sodium borohydride is a selective reagent in the sense that it will reduce ketones and aldehydes but not carboxylic acid derivatives such as esters (carbonyl carbons of carboxylic acid derivatives are less potent electrophiles than the carbonyl carbons of ketones and aldehydes). Unlike the enzymatic hydrogenation reactions we saw earlier, the reduction of asymmetric ketones with sodium borohydride usually results in a 50:50 racemic mixture of the R and S enantiomers of the alcohol product.



Synthetic organic chemists have at their disposal a wide range of other reducing and oxidizing reagents with varying specificities and properties, many of which you will learn about if you take a course in laboratory synthesis.

Exercise 3.4.7

Camphor can be easily reduced by sodium borohydride. However, the mixture of stereoisomeric alcohols that results is not 50:50.



camphor

a. Draw the two stereoisomers of the alcohol products of this reaction, and explain why they are not formed in a 50:50 ratio.

b. Which analytical technique - ^1H-NMR, IR, UV, or MS - could best be used to determine the ratio of the stereoisomers in the product mix? Describe how this analysis could be accomplished.

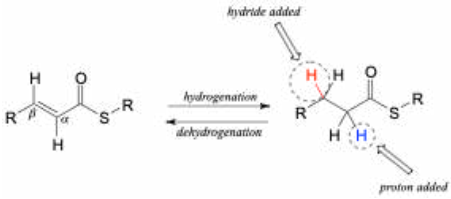
Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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3.5: Hydrogenation of Alkenes and Dehydrogenation of Alkanes

We turn next to reactions in which a hydrogen molecule is added to the double bond of an alkene, forming an alkane - and the reverse, in which H_2 is eliminated from an alkane to form an alkene. Many biochemical reactions of this type involve α, β-unsaturated thioesters.



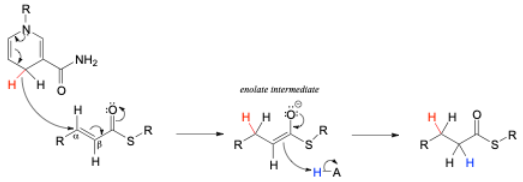
Alkene hydrogenation

In the cell, alkene hydrogenation most often occurs at the α and β position relative to a carbonyl. This type of alkene hydrogenation is essentially a conjugate addition (section 11.4) of hydrogen, with a hydride ion (often from NAD(P)H acting as the nucleophile in the first step.

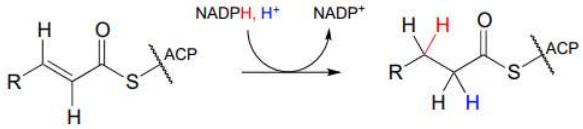
NAD(P)H-dependent hydrogenation (reduction) of an α, β-conjugated alkene:



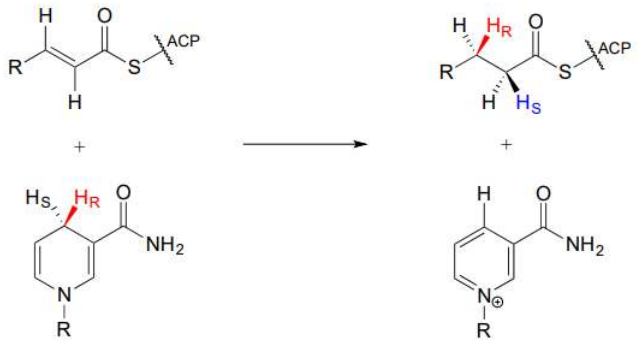
Mechanism:



As part of the fatty acid synthesis pathway, a double bond between the α and β carbons of a fatty acid is reduced to a single bond by hydrogenation (EC 1.3.1.10). The fatty acid is attached to an acyl-carrier protein via a thioester linkage (section 11.5).



It can be easy to forget but important to remember that there is a lot of stereospecificity inherent in biochemical reactions, including this one - even though no chiral centers are involved. First, notice that the substrate contains a trans (E) alkene. Next, let's add some new information about prochirality:

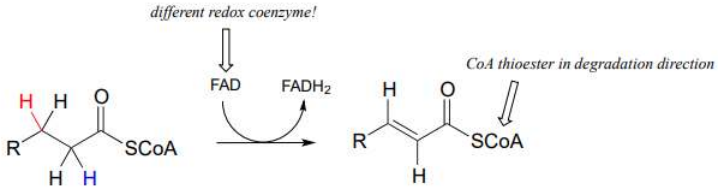


Notice that in this particular reaction it is specifically the pro-R hydride on NADPH that is delivered to the substrate. Also notice that the hydride and proton are added to the same side of the alkene, and become the pro-R and pro-S hydrogen atoms, respectively, on the substrate. This level of stereospecificity, you should recall from previous discussions, stems from the highly precise positioning of substrate and cofactor within the active site of the enzyme.

Other hydrogenase enzymes are known to deliver the pro-S hydride of NADH or NADPH to their substrate, and there are many examples of biochemical conjugate addition reactions in which the nucleophile and proton are added from opposite sides. Always keep in mind that stereochemistry is a key element in the amazing diversity of biological organic reactions.

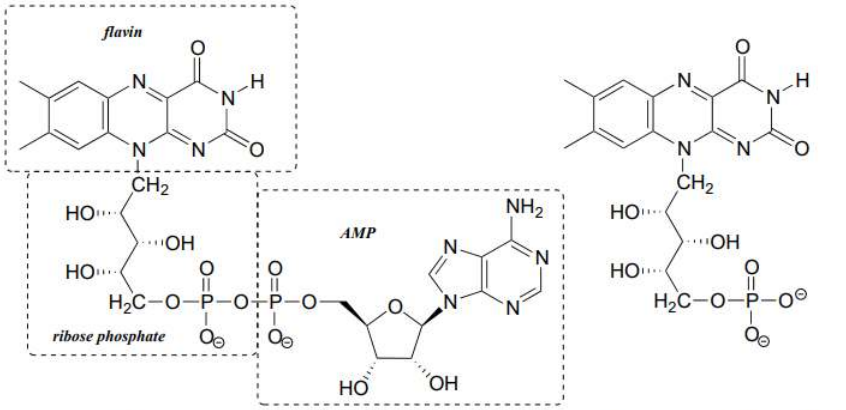
Flavin-dependent alkane dehydrogenation

Next let's consider an alkane dehydrogenation reaction (EC 1.3.99.3) in the fatty acid degradation pathway. Here, a double bond is introduced between the α and β carbons, with concurrent loss of a hydride ion and a proton.



This reaction is clearly not the reverse of the hydrogenation reaction we just saw from fatty acid biosynthesis. First of all, you should notice that the thioester linkage is to coenzyme A rather than acyl carrier protein ACP. More importantly to this discussion, while the hydride donor in the biosynthetic hydrogenation reaction is NADPH, the relevant coenzyme in the catabolic direction is not NAD^+ or $NADP^+$ - rather, it is a **flavin coenzyme**.

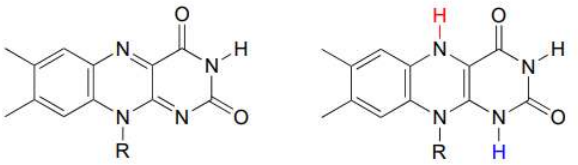
Flavin adenine dinucleotide (FAD) is composed of three components: the three-ring flavin system, ribose phosphate, and AMP. An alternate form, which is missing the AMP component, is called **flavin mononucleotide** (FMN).



flavin adenine dinucleotide (FAD) flavin mononucleotide (FMN)

The reactive part of the coenzyme is the flavin group, so usually the rest of the molecule is abbreviated with 'R'.

FAD and FMN are the oxidized form of flavin. The reduced (hydrogenated) forms of these cofactors are abbreviated $FADH_2$ and $FMNH_2$.

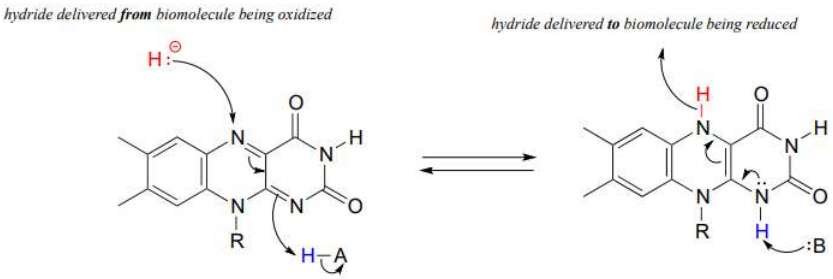


FAD or FMN
oxidized flavin
hydride acceptor
oxidizing agent

$FADH_2$ or $FMNH_2$
reduced flavin
hydride donor
reducing agent

The flavin coenzymes are synthesized in humans from riboflavin (vitamin B_2, which we obtain from our diet (the structure of riboflavin is the same as that of FMN, except that riboflavin lacks the phosphate group). Notice the extended conjugated π system in the three fused rings: the flavin system absorbs light in the visible wavelengths and has a distinctive deep yellow color - it is riboflavin, and to some extent FAD and FMN, that give urine its color.

Like the nicotinamide coenzymes, flavin serves as a hydride donor or acceptor. FAD and FMN are able to accept a hydride ion (and a proton), and $FADH_2$ and $FMNH_2$ in turn can serve as hydride donors in hydrogenation reactions.

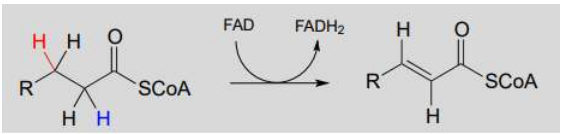


oxidized flavin
hydride acceptor
oxidizing agent

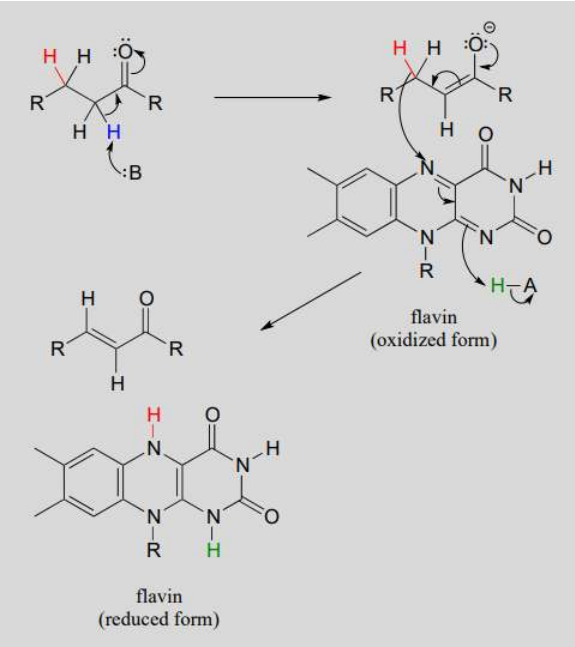
reduced flavin
hydride donor
reducing agent

Below is a general mechanism for the dehydration of an alkene at the α, β position - notice that it is mechanistically an E1cb elimination of H_2.

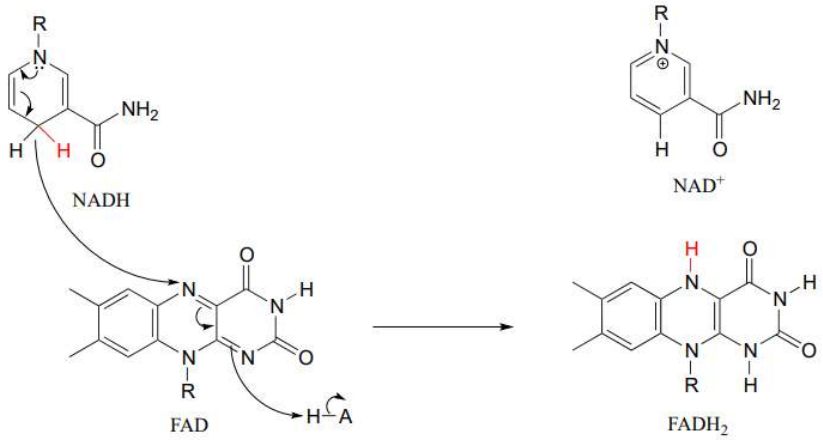
α, β dehydrogenation (oxidation) of an alkane:



Mechanism:



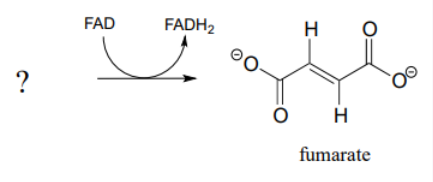
In many enzymatic reactions in which $FADH_2$ acts as the reducing agent, the reaction cycle is completed when FAD, rather than being released from the active site, is recycled back to $FADH_2$ with the concomitant oxidation of NADH.



Hydride ion transfer with flavin or nicotinamide coenzymes is a two electron redox process. However, unlike the nicotinamide cofactors, flavins are also able to function in single electron transfer (radical) mechanisms. We will come back to this idea briefly in the chapter 16.

Exercise 3.5.1

Fumarate is formed in an alkane dehydrogenation reaction (EC 1.3.5.1) which is part of the citric acid cycle:



a. Predict the structure of the starting substrate in this reaction

b. Draw the structure of the enolate intermediate

Exercise 3.5.2

Reduced flavin can serve as the hydride donor in some hydrogenation reactions. Degradation of the RNA base uracil begins with hydrogenation of a conjugated alkene group by a flavin-dependent hydrogenase enzyme (EC 1.3.1.2). Predict the product of this step, and draw curved arrows for the first mechanistic step.

Contributors

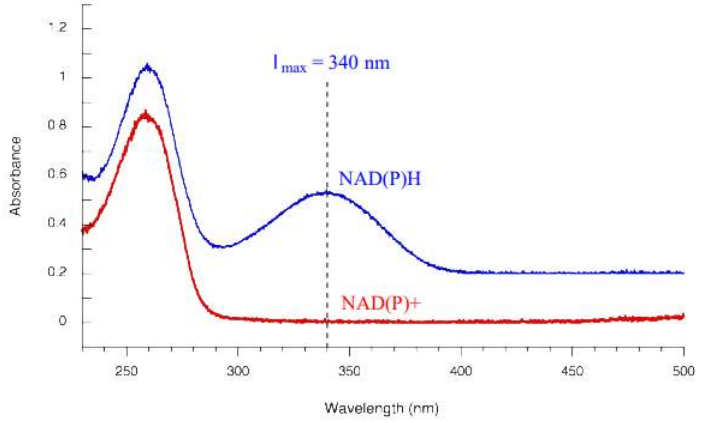
- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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3.6: Monitoring Hydrogenation and Dehydrogenation Reactions by UV Spectroscopy

In order to study any enzyme-catalyzed reaction, a researcher must have available some sort of test, or assay, in order to observe and measure the reaction's progress and measure its rate. In many cases, an assay simply involves running the reaction for a specified length of time, then isolating and quantifying the product using a separation technique such as high performance liquid chromatography (HPLC) or gas chromatography (GC). This type of assay can be extremely time-consuming, however, so it is to the researcher's great advantage if a more convenient assay can be found.

Redox reactions in which a nicotinamide coenzyme participates as a hydride donor or acceptor are generally quite convenient to assay. In fact, the progress of these reactions can usually be observed in real time, meaning that the researcher doesn't need to stop the reaction in order to see how far it has progressed. ***NADPH*** and ***NADH*** have distinctive $n - \pi^*$ UV absorbance bands centered at 340 nm, with a molar absorptivity of 6290 M^{-1} cm^{-1} (section 4.4). The oxidized coenzymes ***NADP*****+** and ***NAD*****+** do not absorb at this wavelength.



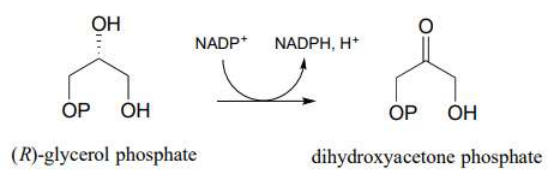
Therefore, the course of a hydrogenation reaction, in which ***NAD(P)H*** is converted to ***NAD(P)*****+**, can be observed in real time if it is run in a quartz cuvette in a UV spectrometer. By observing the decrease in absorbance at 340 nm, the researcher can calculate how much ***NAD(P)H*** has been oxidized to ***NAD(P)*****+** at any given time point, and this number is the molar equivalent of the amount of organic substrate that has been reduced:



Likewise, a ***NAD*****+**-dependent dehydrogenase reaction can be followed in real time by monitoring the increase in absorbance at 340 nm as ***NAD*****+** is converted to ***NADH***.

Exercise 3.6.1

You are observing the progress of the (R)-glycerol phosphate dehydrogenase reaction shown in the figure below.



You run the reaction in a quartz cuvette (path length 1 cm) in a total solution volume of 1 mL. start with 200 μM substrate and 100 μM ***NADP*****+** in solution, zero the UV spectrophotometer, then add the

enzyme to start the reaction. After 5 minutes, the A340 reading has climbed from 0.000 to 0.096. At this time point:

a. How many moles of substrate have been oxidized?
b. What is the solution concentration of $NADP^+$?
c. The enzyme has a mass of 25 kilodaltons (25,000 g/mol).You added 5 mL of a 2 ng/mL solution of pure enzyme to start the reaction. How many reactions does each enzyme molecule catalyze, on average, per second? (This number is referred to by biochemists as the 'turnover number').

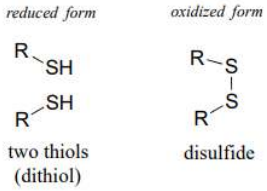
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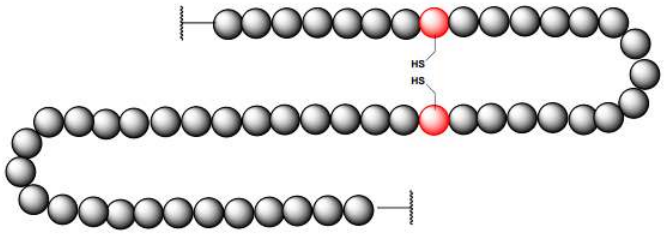
3.7: Redox Reactions of Thiols and Disulfides

A **disulfide bond** is a sulfur-sulfur bond, usually formed from two free thiol groups.

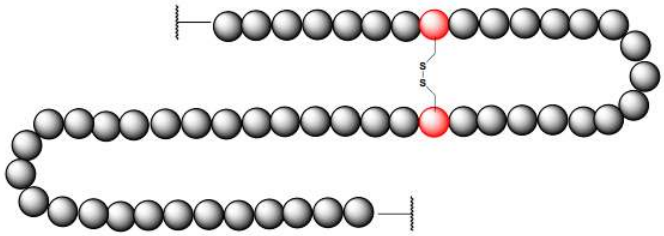


The interconversion between dithiol and disulfide groups is a redox reaction: the free dithiol form is in the reduced state, and the disulfide form is in the oxidized state. Notice that in the oxidized (disulfide) state, each sulfur atom has lost a bond to hydrogen and gained a bond to sulfur.

As you may recall from your biology courses, disulfide bonds between cysteine residues are an integral component of the three-dimensional structure of many extracellular proteins and signaling peptides.

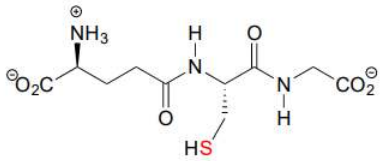


reduced protein (free cysteines)

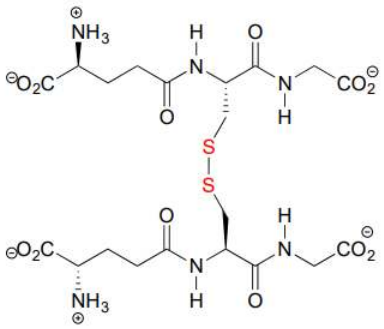


oxidized protein (disulfide)

A thiol-containing coenzyme called glutathione is integrally involved in many thiol-disulfide redox processes (recall that glutathione was a main player in this chapter's introductory story about concussion research). In its reduced (thiol) form, glutathione is abbreviated 'GSH'. In its oxidized form, glutathione exists as a dimer of two molecules linked by a disulfide group, and is abbreviated 'GSSG'.



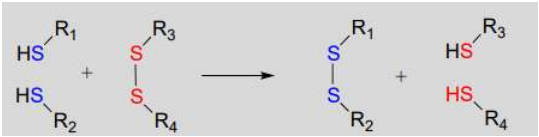
glutathione (GSH)
reduced state



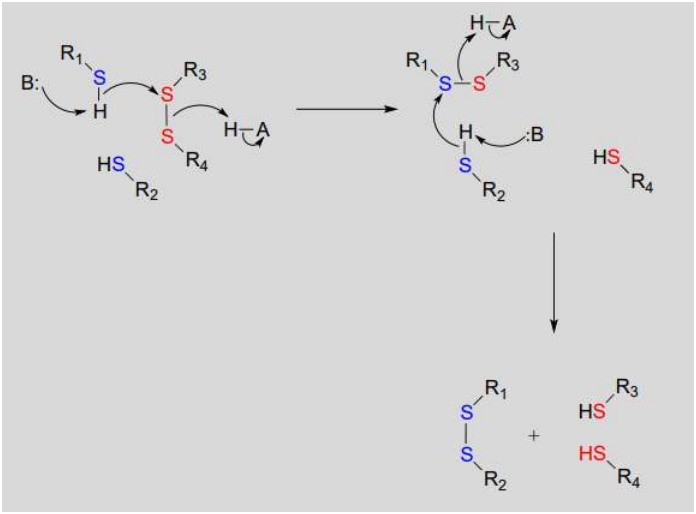
glutathione dimer (GSSG)
oxidized state

Disulfide bonds and free thiol groups in both proteins and smaller organic molecules like glutathione can 'trade places' through a disulfide exchange reaction. This process is essentially a combination of two direct displacement (S_N2-like) events, with sulfur atoms acting as nucleophile, electrophile and leaving group.

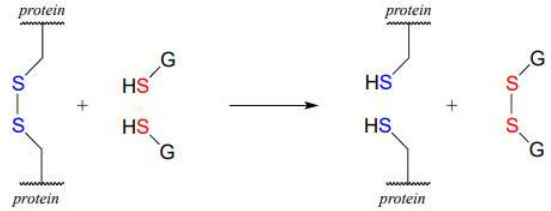
Disulfide exchange reaction



Mechanism:

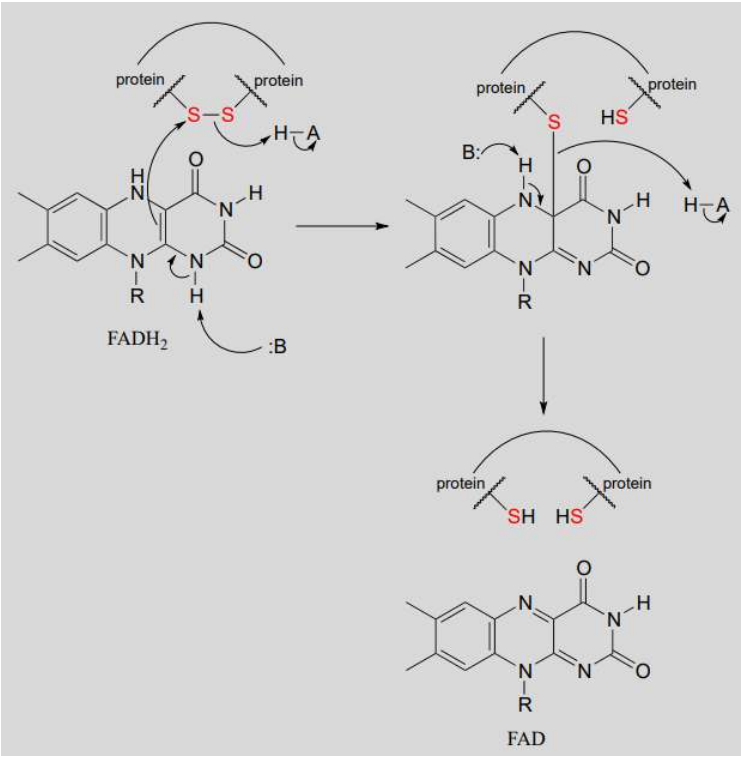


In eukaryotes, the cysteine side chains of intracellular (inside the cell) proteins are almost always in the free thiol (reduced) state due to the high concentration of reduced glutathione (GSH) in the intracellular environment. A disulfide bond in an intracellular protein will be rapidly reduced in a disulfide exchange reaction with excess glutathione.

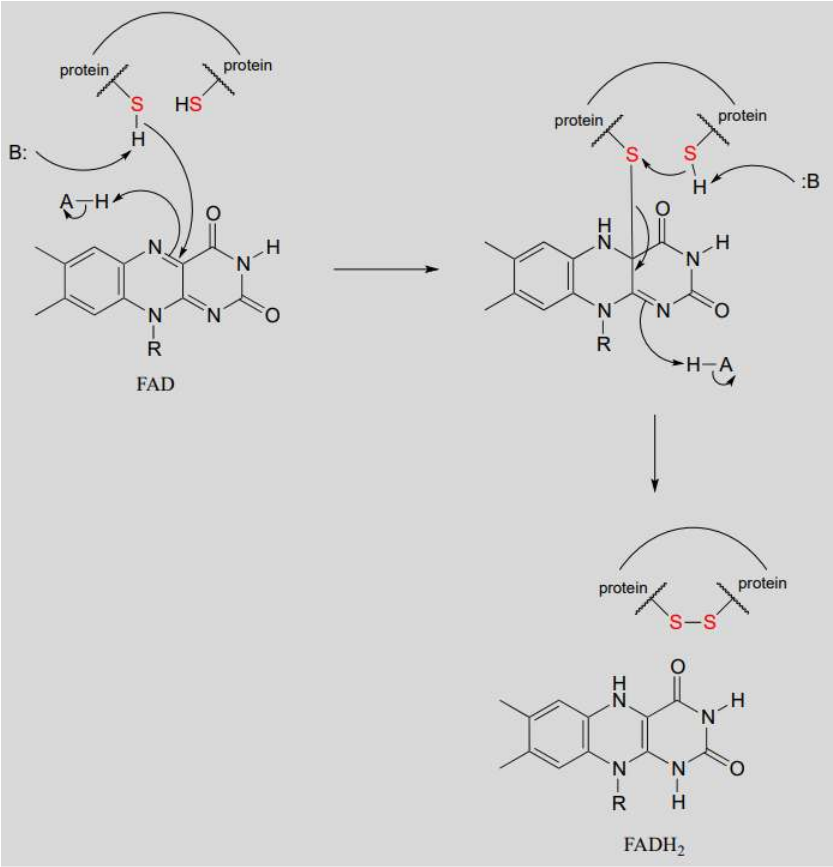


The interconversion of free thiols and disulfides is also mediated by flavin in some enzymes.

Flavin-mediated reduction of a protein disulfide bond

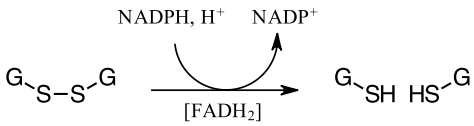


Flavin-mediated oxidation of a protein disulfide bond



As was stated earlier, a high intracellular concentration of reduced glutathione (GSH) serves to maintain proteins in the free thiol (reduced) state. An enzyme called glutathione reductase catalyzes the reduction of GSSG in a flavin-mediated process, with ***NADH*** acting as the ultimate hydride donor.

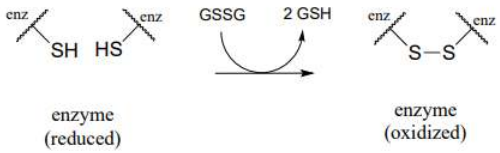
Gluthione reductase reaction:



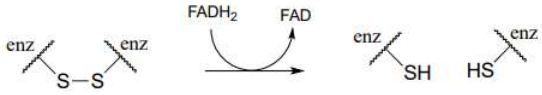
This figure shows oxidized glutathione converted to reduced glutathione by NADPH.

The mechanism for this and other similar reactions is not yet completely understood, but evidence points to an initial thiol-disulfide exchange reaction with a pair of cysteines from the enzyme, (phase 1 below) followed by flavin-dependent reduction of the cysteine-cysteine disulfide (phase 2). Finally, (phase 3) ***FAD*** is reduced back to ***$FADH_2$*** by ***NADH***. Frey and Hegeman, Enzymatic Reaction Mechanisms, p. 699

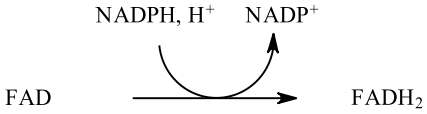
Phase 1: thiol-disulfide exchange (see earlier figure for mechanism):



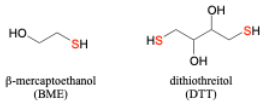
Phase 2: Reduction of protein disulfide by ***$FADH_2$*** (see earlier figure for mechanism)



Phase 3: regeneration of ***$FADH_2$*** by ***NADH*** (see section 15.4B for mechanism)



In the biochemistry lab, proteins are often maintained in their reduced (free thiol) state by incubation in buffer containing an excess concentration of β-mercaptoethanol (BME) or dithiothreitol (DTT). These reducing agents function in a manner similar to that of GSH, except that DTT, because it has two thiol groups, can form an intramolecular disulfide in its oxidized form.



Exercise 3.7.1

Draw structures of the oxidized (disulfide) forms of BME and DTT.

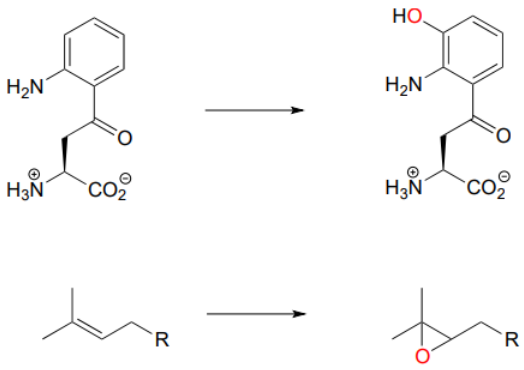
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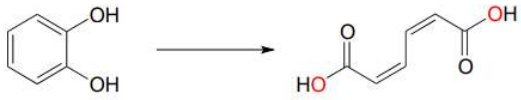
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3.8: Flavin-Dependent Monooxygenase Reactions - Hydroxylation, Epoxidation, and the Baeyer-Villiger Oxidation

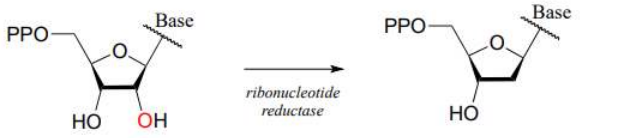
Up to now, the redox reaction examples we have seen have all been either hydrogenation/dehydrogenation transformations or interconversions between free thiols and disulfides. However, there are many important redox reactions in biological chemistry which do not fall under either of these descriptions. Oxygenase enzymes catalyze the insertion of one or two oxygen atoms from molecular oxygen (O_2) into an organic substrate molecule. Enzymes which insert a single oxygen atom are called monooxygenases. Below are two examples of biochemical transformations catalyzed by monooxygenase enzymes: one is a hydroxylation, the other is an epoxidation (an epoxide functional group is composed of a three-membered carbon-carbon-oxygen ring - epoxides are somewhat rare in biological organic chemistry but are very common and useful intermediates in laboratory organic synthesis).



Dioxygenase enzymes insert both oxygen atoms from O_2 into the substrate, and usually involve cleavage of an aromatic ring. Below is an example of a dioxygenase reaction, catalyzed by catechol dioxygenase:



In the reduction direction, reductases *remove* oxygen atoms, or sometimes other electronegative heteroatoms such as nitrogen or halides. For example, DNA **deoxy**ribonucleosides are converted from their corresponding RNA ribonucleosides by the action of reductase enzymes:

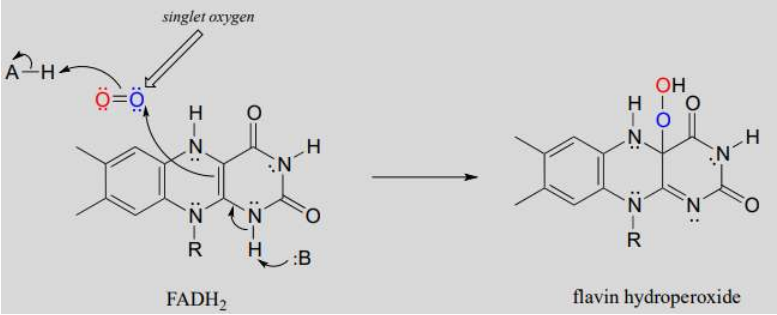


Many oxygenase and reductase reactions involve the participation of enzyme-bound transition metals - such as iron or copper - and the mechanistic details of these reactions are outside the scope of our discussion. A variety of biochemical monooxygenase reactions, however, involve flavin as a redox cofactor, and we do have sufficient background knowledge at this point to understand these mechanisms. In flavin-dependent monooxygenase reactions, the key intermediate species is flavin hydroperoxide.

The term 'peroxide' refers to a functional group characterized by an oxygen-oxygen single bond. The simplest peroxide is hydrogen peroxide ($HOOH$) about which we will have more to say below. In flavin hydroperoxide, the peroxide group is linked to one of the carbons of the reactive triple-ring system of the coenzyme. A possible mechanism for the formation of flavin peroxide from $FADH_2$ and molecular oxygen is shown below.

Silverman, R.B. The Organic Chemistry of Enzyme-Catalyzed Reactions, p. 121-122, Scheme 3.33. 2000, Academic Press, San Diego.

Mechanism for the formation of flavin hydroperoxide:



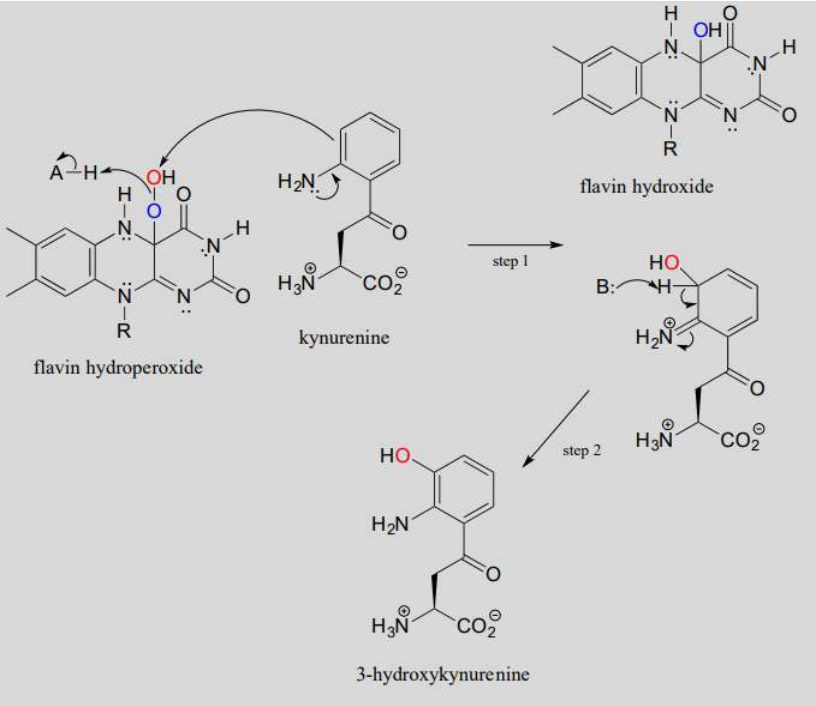
(Note: Implicit in this mechanism is that the molecular oxygen first undergoes spin inversion from the triplet state to the higher energy 'singlet' state. You may recall from your general chemistry course that molecular oxygen exists in two states: 'singlet' oxygen has a double bond and no unpaired electrons, while 'triplet' oxygen has a single $O-O$ bond and two unpaired electrons - a kind of 'double radical'. Molecular orbital theory - and experimental evidence - show that the triplet state is lower in energy.

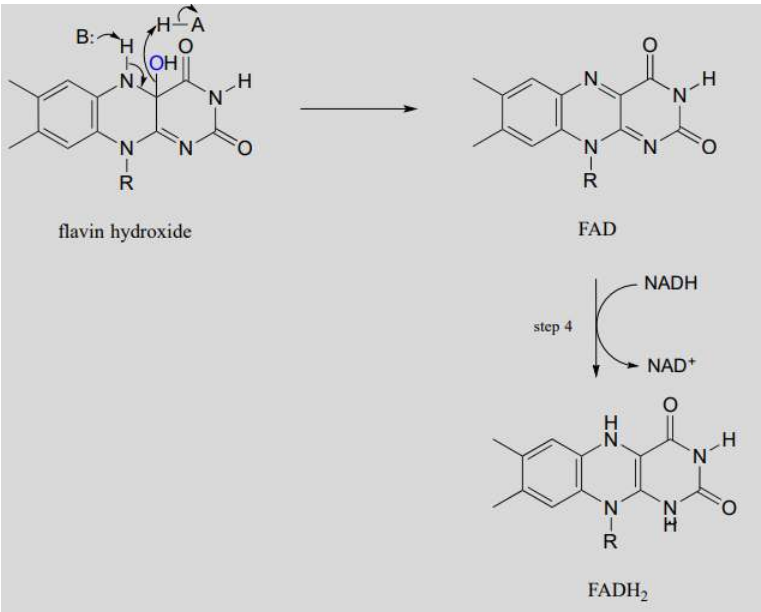


The mechanism shown above is one proposed mechanism, another proposal involves triplet oxygen reacting with flavin in a series of radical-intermediate, single-electron steps.)

Flavin hydroperoxide can be thought of as an activated form of molecular oxygen. Peroxides in general are potent oxidizing agents, because the oxygen-oxygen single bond is quite weak: only 138 kJ/mole, compared to 339 kJ/mol for a carbon-carbon bond, and 351 kJ/mol for a carbon-oxygen bond. When the 'outer' oxygen of flavin hydroperoxide (red in our figure above) comes into close proximity to the π-bonded electrons of an alkene or aromatic group, the $O-O$ bond will break, leaving an empty orbital on the outer oxygen to be filled by the π electrons - thus, a new carbon-oxygen bond is formed. This is what is happening in step 1 of a reaction in the tryptophan degradation pathway catalyzed by kynurenine 3-monooxygenase. Step 2 completes what is, mechanistically speaking, an electrophilic aromatic substitution reaction with an peroxide oxygen electrophile.

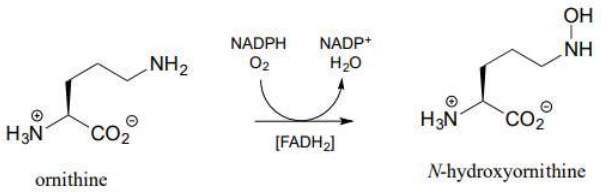
Mechanism for the flavin hydroperoxide-dependent hydroxylation of kynurenine:





Elimination of water from the hydroxyflavin intermediate then leads to formation of ***FAD*** (step 3), which is subsequently reduced back to $FADH_2$ by ***NADH*** (step 4).

The ***N***-hydroxylation reaction below, which is part of the of the biosynthetic pathway of an iron-binding molecule in the pathogenic bacterium *Pseudomonas aeruginosa*, is mechanistically similar to the ***C***-hydroxylation reaction we just saw, except that the nucleophile is an amine nitrogen. Note that $FADH_2$ is shown in brackets below the reaction arrow, indicating that reduced flavin participates in the reaction but is not used up - rather it is regenerated in the active site at the end of the reaction cycle.



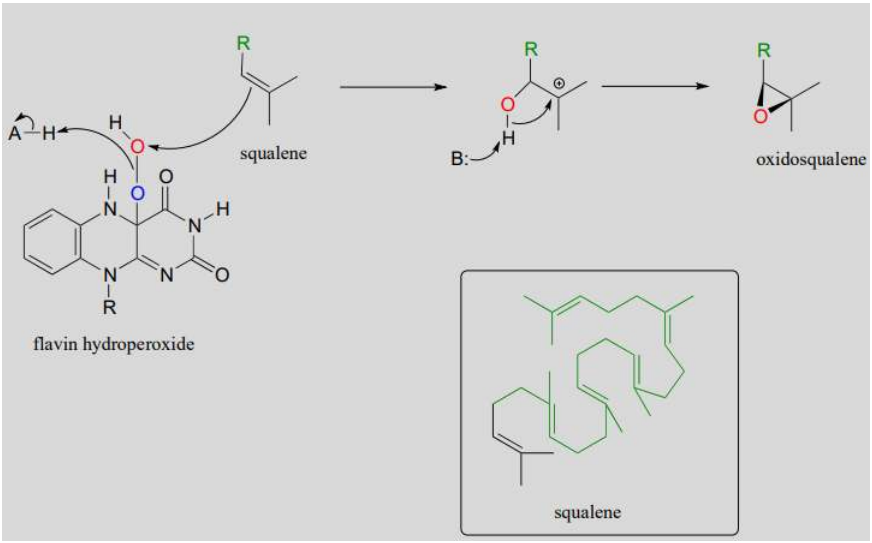
Exercise 3.8.1

Draw arrows for the $N-O$ bond-forming step in the ornithine hydroxylation reaction above.

Epoxides, characterized by a three-membered ring composed of two carbons and one oxygen, are a very common and useful functional group employed in synthetic organic chemistry. Although rare, there are some interesting epoxide-forming reactions in biochemical pathways, catalyzed by flavin-dependent monooxygenase enzymes.

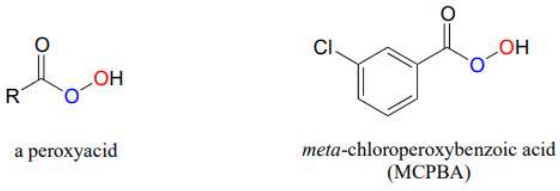
In a key step in the biosynthesis of cholesterol and other steroid compounds, an alkene is converted to an epoxide in a precursor molecule called squalene. Flavin hydroperoxide also serves as the direct oxidizing agent in this step:

Mechanism for the flavin-hydroperoxide-dependent epoxidation of squalene:



Oxidosqualene goes on to cyclize to lanosterol in a complex and fascinating electrophilic reaction.

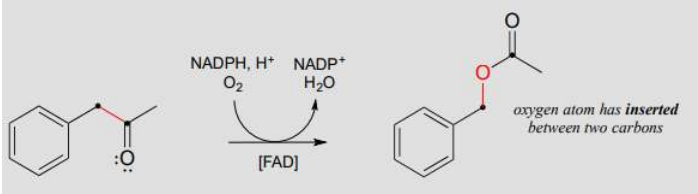
Epoxidation reactions have a parallel in the synthetic organic laboratory, and in fact are very important tools in organic synthesis. In laboratory epoxidations, peroxyacids are the counterpart to flavin hydroperoxide in biochemical epoxidations. meta-Chloroperoxybenxoic acid (MCPBA) is a commonly used peroxyacid.



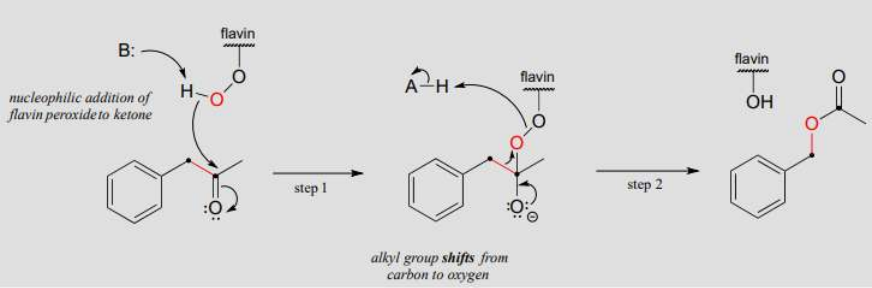
The Baeyer-Villiger oxidation, in which a ketone is converted to an ester through treatment with a peroxide reagent, is an extremely useful laboratory organic synthesis reaction discovered in the late 19th century. Recently, many biochemical examples of Baeyer-Villiger oxidations have been discovered: the reaction below, for example, is catalyzed by a monooxygenase in a thermophilic bacterium:

(Proc. Natl. Acad. Sci. U.S.A. 2004, 101, 13157)

A Baeyer-Villiger oxidation:



Mechanism:



The Baeyer-Villiger mechanism is differs significantly from the hydroxylation reactions we saw earlier, although flavin hydroperoxide (abbreviated in the above figure) still plays a key role. Here, the peroxide oxygen is a nucleophile, rather than an electrophile, attacking the ketone carbonyl in step 1. Step 2 is a rearrangement, similar in many ways to the hydride and alkyl shifts we learned about in section 14.5. The electrons in the red bond in the figure shift over one atom: from the carbonyl carbon to the outer peroxide oxygen. The end result is that an oxygen atom, from O_2 via flavin hydroperoxide, has been inserted between the carbonyl carbon and a neighboring methylene (CH_2) carbon, forming an ester.

Note that in the reaction mechanism above, the ketone substrate is asymmetric: on one side of the carbonyl there is a benzyl group (CH_2-phenyl), and on the other side a methyl group. Note also that it is the benzyl group, not the methyl, that shifts in step 2 of the mechanism. For reasons that are not yet well understood, in Baeyer-Villiger reactions the alkyl group with higher carbocation stability has a higher migratory aptitude: in other words, it has a lower energy barrier for the shifting step.

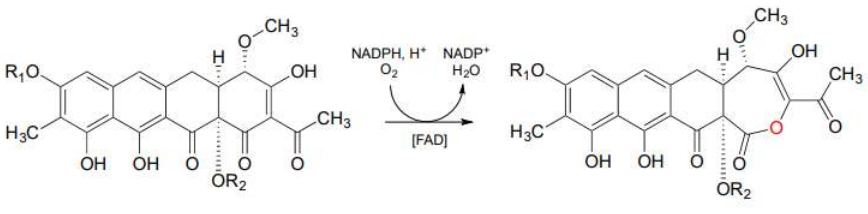
Exercise 3.8.2

Draw the product of a hypothetical Baeyer-Villiger reaction involving the same substrate as the above figure, in which the methyl rather than the benzyl group shifts.

Exercise 3.8.3

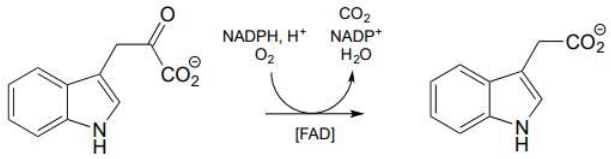
Draw the likely major product of a hypothetical Baeyer Villiger reaction starting with 2-methylcyclopentanone as the substrate. Take into account the idea of migratory aptitude.

Below is another example of a Baeyer-Villiger reaction in which a cyclic ketone is oxidized to a lactone (cyclic ester). Notice that oxygen insertion expands the ring from 6 to 7 atoms. This is the third-to-last step in the biosynthesis of the anti-cancer agent mithromycin in some bacterial species (ACS Chem. Biol. 2013, 8, 2466).



(R_1 and R_2 are triglycerides)

Yet another variety of flavin-dependent monooxygenase, which bears some mechanistic similarity to the Baeyer-Villiger oxidation, is the decarboxylative reaction below from biosynthesis of the plant hormone auxin: (J. Biol. Chem. 2013, 288, 1448)



auxin

Exercise 3.8.4

Propose a mechanism for the above reaction, starting with flavin hydroperoxide.

Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

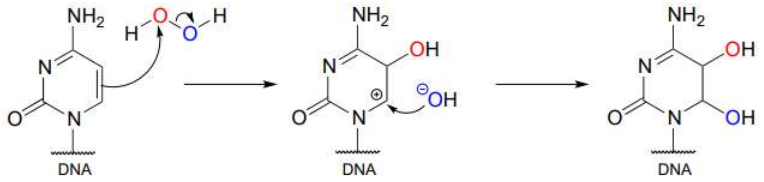
This page titled 3.8: Flavin-Dependent Monooxygenase Reactions - Hydroxylation, Epoxidation, and the Baeyer-Villiger Oxidation is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Tim Soderberg.

3.9: Hydrogen Peroxide is a Harmful Reactive Oxygen Species

We get our energy from the oxidation of organic molecules such as fat and carbohydrates, as electrons from these reduced compounds are transferred to molecular oxygen, thereby reducing it to water. Reducing O_2, however, turns out to be a hazardous activity: harmful side products called reactive oxygen species (ROS) are inevitably formed in the process. Recall from the story introducing this chapter that ROS appear to play an important role in the damage that occurs to the brain immediately after a concussion.

Hydrogen peroxide, $HOOH$, is an ROS. Recall peroxides are potent oxidizing agents due to the weakness of the $O-O$ single bond. It is this same weak bond that causes hydrogen peroxide to be dangerous when produced in our bodies, as it can react spontaneously with oxygen or nitrogen nucleophiles and π bonds.

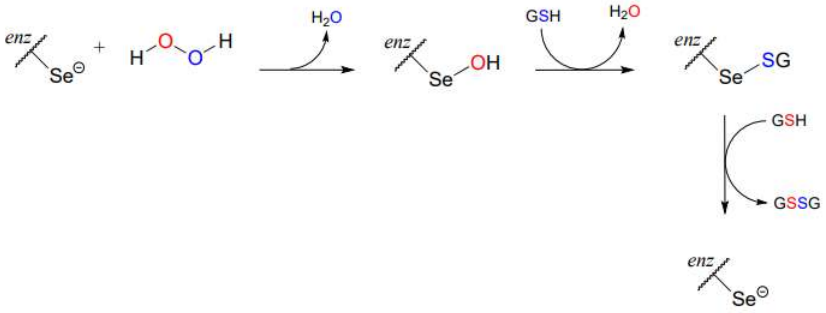
Peroxide formed as a by-product of our metabolism is particularly harmful when it oxidizes DNA bases. In just one of many known examples of oxidative damage, the DNA base cytosine is oxidized to thymine glycol in the presence of hydrogen peroxide. Although mechanistic details for reactions such as these are not yet well understood, one possibility is electrophilic addition:



cytosine thymine glycol

Our bodies have evolved ways to dispose of the harmful reactive oxygen species that are continuously being formed (the only way to stop the production of ROS is to stop breathing oxygen!). Glutathione peroxidase is a remarkable enzyme in that its active site contains selanocysteine, a modified cysteine residue in which the side chain sulfur is replaced by selenium (selenium is very toxic, but we do need a very small amount of it in our diet). Look at a periodic table: selenium is below oxygen and sulfur in the same column. If you think back to the vertical periodic trends in nucleophilicity, you'll recall that just as a thiol is a better nucleophile than an alcohol, a selanol (RSeH) is even more nucleophilic than a thiol. Moreover, the vertical periodic trend in acidity tells us that a selenol should be more acidic than a thiol - in fact, the pKa of a selenocysteine is about 5.5, meaning that it is mostly in its deprotonated state at physiological pH, making it even more nucleophilic.

Glutathione peroxidase very efficiently catalyzes the reduction of hydrogen peroxide to water and the oxidation of glutathione (GSH) to GSSG, beginning with nucleophilic attack by the enzymatic selanocysteine on a peroxide oxygen. The intermediates in this process are shown below: each step can be thought of as a concerted nucleophilic displacement similar to those that take place in a disulfide exchange reaction.



Contributors

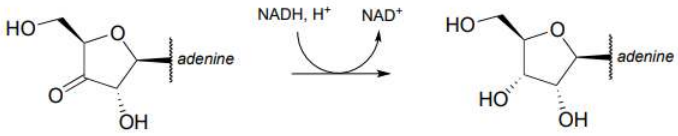
- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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3.P: Oxidation and Reduction Reactions (Problems)

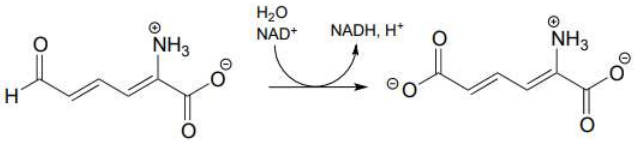
P3.1: Show a mechanism for each of the redox reactions below. Do not abbreviate the reactive parts of the redox coenzyme.

a)

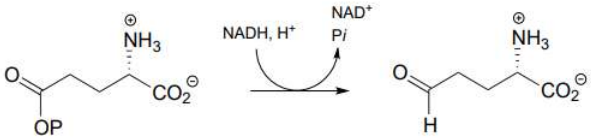


adenosine

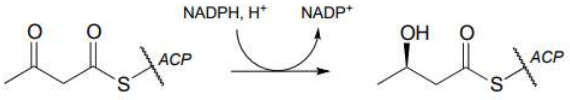
b)



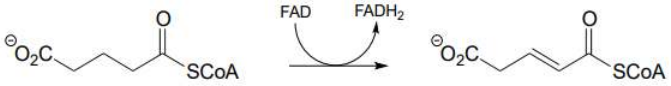
c)



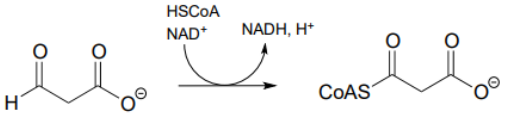
d)



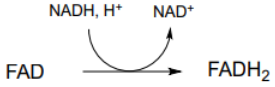
e)



f)



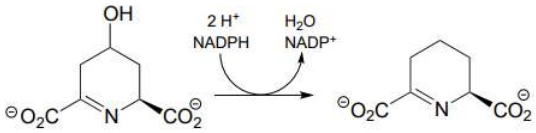
g) regeneration of reduced flavin by $NADH$):



h) In reaction (a), near which face of the substrate is the cofactor bound in the active site?

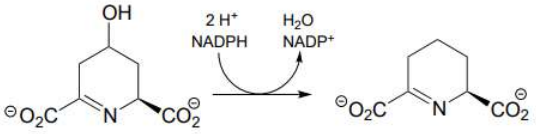
i) In reaction (d), near which face of the product is the cofactor bound in the active site?

P3.2: In the reactions below ((EC 2.7.2.4; EC 1.2.1.11) , the side chain of aspartate is altered, but the main peptide chain is not affected. Show the most probable structure of species A and B.

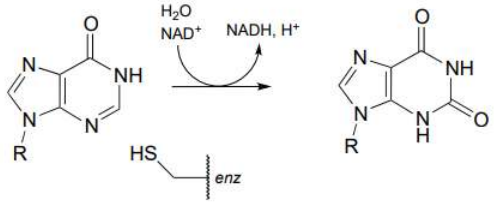


P3.3: Propose complete mechanisms for the following reactions.

a. (EC 1.3.1.26, from lysine biosynthesis)

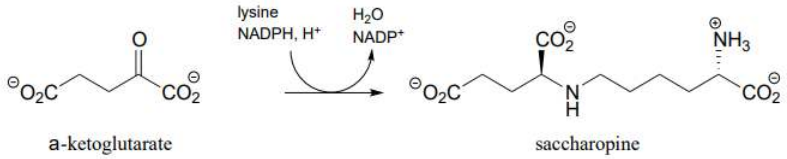


b. (EC 1.1.1.205, from guanosine ribonucleotide biosynthesis) The mechanism involves a covalent cysteine-linked enzyme-substrate intermediate.



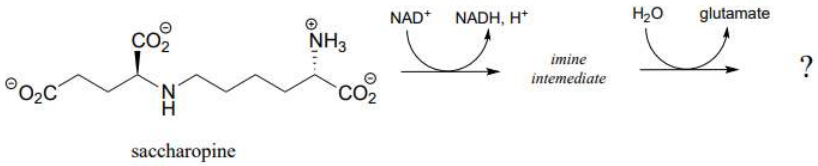
P3.4: The first step in the lysine degradation pathway is a reductive condensation with a-ketoglutarate to form an intermediate called saccharopine. (EC 1.5.1.8)

a. Propose a mechanism for this transformation.



a-ketoglutarate saccharopine

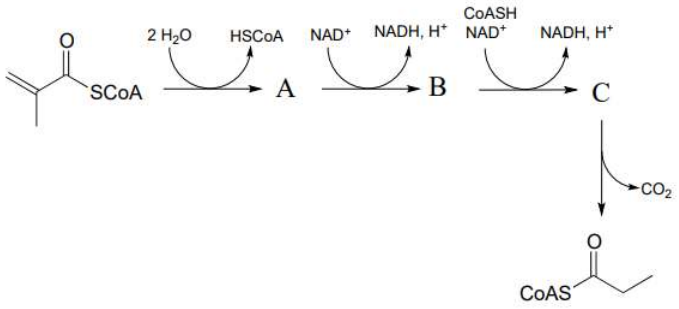
b. Saccharopine (see part (a) above) is then broken up to yield glutamate and a second product that contains an aldehyde group. Predict the structure of this second product, and propose a likely mechanism for the reaction which involves and imine intermediate. (EC 1.5.1.10)



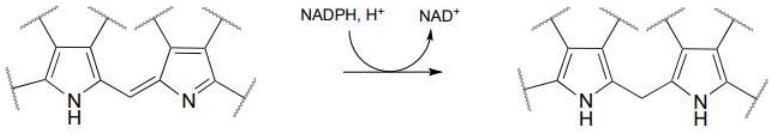
saccharopine

P5.5: Predict the structures of species A and B in the pathway below.

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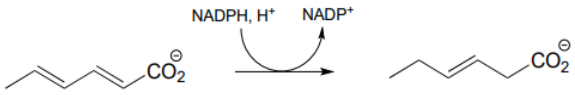
P3.6: Bilirubin, the molecule responsible for the yellowish color of bruises, is formed from the ***NADPH***-dependent hydrogenation of a double bond in biliverdin (EC 1.3.1.24), which is a product of heme degradation (heme is an iron-containing coenzyme in the oxygen-carrying blood protein hemoglobin). Draw a likely mechanism for this reaction.



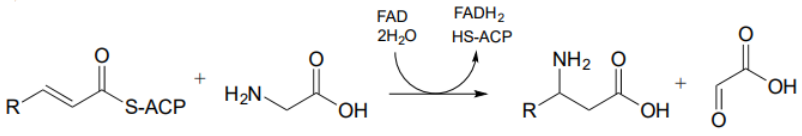
biliverdin bilirubin

P3.7: Draw a likely mechanism for each of the reactions below.

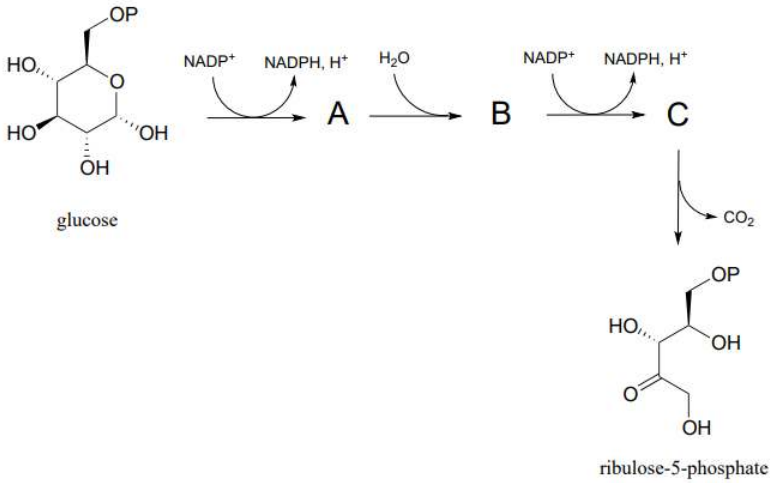
a. From the oxidation of polyunsaturated fatty acids:



b.

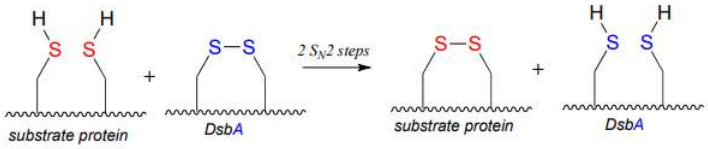


P3.8: Predict the structures of pathway intermediates A, B, and C:

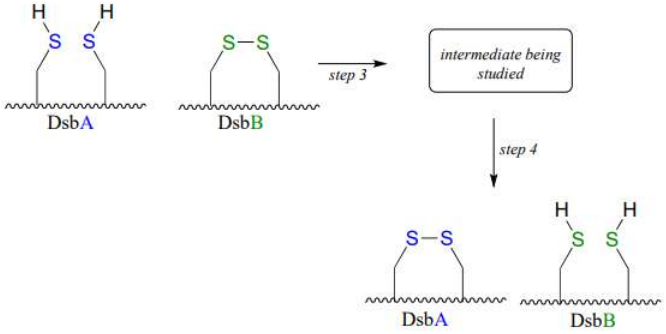


glucose ribulose-5-phosphate

P3.9: An enzyme called DsbA (EC1.8.4.2) is responsible for the formation of disulfide bonds in bacterial proteins. The process - which can be thought of as a 'disulfide exchange', involves the cleavage of a disulfide bond between two active site cysteines in DsbA. It is accomplished through two successive S_N2 displacements.



DsbA is then returned to it's starting (disulfide) state through a second disulfide exchange reaction with another protein called DsbB:

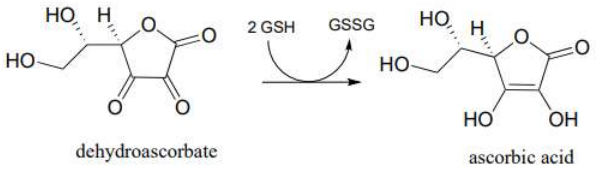


Scientists were interested in studying the intermediate species formed in step 3, but found that it is very short-lived and difficult to isolate. In order to address this problem, they ran the reaction with a synthetic analog of DsbB that contained an unnatural bromoalanine amino acid in place of one of the active site cysteines.

a. Draw a complete mechanism for the disulfide exchange reaction between DsbA and DsbB.
b. Show how the bromoalanine-containing DsbB analog allowed for the isolation of an intermediate that resembles the true, short-lived intermediate. (J. Am. Chem. Soc. 2004, 126, 15060; J. Nat. Prod. 2001, 64, 597).

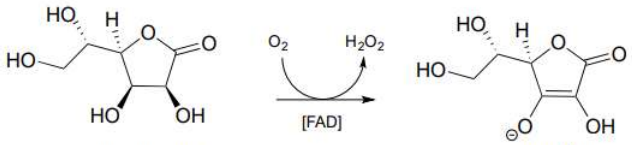
P3.10:

a. In chapter 16 we will learn how ascorbate (vitamin C) acts as a 'radical scavenger' antioxidant to protect our cells from damage by free radical species. When ascorbate scavenges a radical, it ends up being converted to dehydroascorbate . One possible metabolic fate of dehydroascorbate is to be recycled back to ascorbate through an enzyme-free reaction with glutathione. Biocemistry 1999, 38, 268.



Suggest a likely mechanism for the enzyme-free reaction.

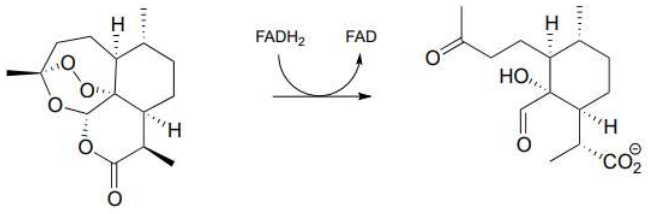
b. In the introduction to chapter 16, we will learn that most animals - but not humans - are able to synthesize their own ascorbate. Humans cannot synthesize vitamin C because we lack the enzyme for the final step in the biosynthetic pathway, gulonolactone oxidase:



gulonolactone ascorbate

This enzyme uses ***FAD*** as an oxidizing agent, and ***$FADH_2$*** is oxidized back to ***FAD*** at the end of the catalytic cycle by molecular oxygen, with hydrogen peroxide as a side product. Draw out a likely mechanism showing how gulonolactone is converted to ascorbate, and how ***FAD*** is regenerated.

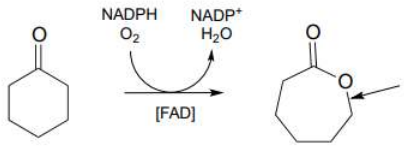
c. Artemisinin is a naturally occurring compound with demonstrated antimalarial properties. It is thought to act by depleting the malaria-causing microbe's store of reduced flavin, thus disrupting the redox balance. The relevant reaction is shown below:



artemisinin

Draw mechanistic arrows for the step as shown above. (Molecules 2010, 15, 1705)

d. Draw the mechanistic step in the reaction below in which the C-O bond indicated by the arrow in the figure below is formed.



P3.11: Methanogens are a class of microorganisms in the domain archaea which inhabit a diversity of anaerobic (oxygen-lacking) environments, from the intestines of humans, to swamp mud, to the base of deep sea hot water vents. They obtain energy by reducing carbon dioxide to methane:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O + \text{energy} \qquad (3.P.1)$$

Methanogenesis', like the oxidation of glucose in animals, is not accomplished in a single reaction - it requires a long series of enzymatic steps, and involves the participation of several unique coenzymes (if you are interested in learning more, see FEMS Microbiol. Rev. 1999, 23, 13 for a detailed review of the enzymatic reactions of methanogenesis).

The oxidation of methane to carbon dioxide when you burn natural gas for heating your house is obviously an exergonic process. How then is it possible that reducing carbon dioxide to methane could also be exergonic? Explain.

Multistep pathway prediction problems

In chapters 12 and 13, we were introduced to the challenge of mapping out potential multi-step transformations using the retrosynthesis approach, where we start with the more complex molecule and take it apart step be step. These problems have necessarily involved hypothetical, generalized transformations, because many of the reaction types involved in actual biochemical transformations were as-yet unfamiliar to us.

We have now reached a point in our study of organic reactivity where we can look at an actual metabolic pathway and probably recognize most of the reactions taking place - so were are ready to try our hand at mapping out real metabolic pathways.

Before we dive in, you may want to go back to the retrosynthesis interchapter and review the key elements of the retrosynthetic approach. You may (or may not) also need a little more practice with some simpler, shorter, hypothetical problems, similar to what

we worked on in the last three chapters but incorporating some of the redox reactions that we have just finished learning about. These are provided in problems 15.12 and 15.13 below.

As before, your job is to draw out a 'pathway diagram' for each transformation, using the 'arrow in - arrow out' convention to indicate the role of other necessary participants in each reaction, such as ATP, ***NADH***, water, or another organic molecule. An example for a simple two-step pathway is provided in problem 15.12 below. A three-step pathway would of course show two intermediate compounds.

Remember, it is most important that your proposed pathway be chemically reasonable - in other words, each hypothetical reaction that you propose should be very similar to a reaction pattern that we have seen in chapters 8-15. You should be able to put a name on each step: for example, 'step 1 is a Claisen condensation; step 2 is a ketone reduction', and so forth.

Also remember that there is usually no one correct way to approach problems like this - they are puzzles to solve, and success will be dependent in large part on having a solid grasp on the chemical 'tools' available to us: in other words, the biochemical reaction types that we have been studying, starting with nucleophilic substitutions in chapter 8.

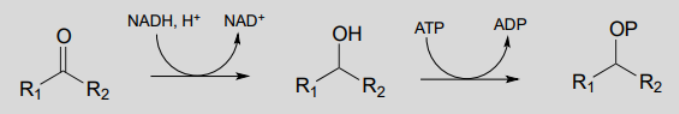
P3.12: Hypothetical 2-step transformations:

Each of the generalized transformations below would be expected to require two enzymatic steps. Draw a reasonable pathway diagram for each transformation.

Example:

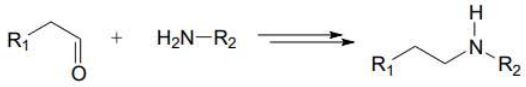


Diagram:

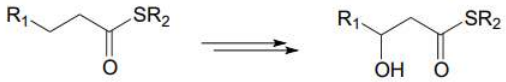


The first step is a nicotinamide-dependent ketone reduction/hydrogenation, and step 2 is ATP-dependent phosphorylation (ie. a kinase reaction). Note that the reducing agent in the first step could also be ***NADPH***)

a)



b)

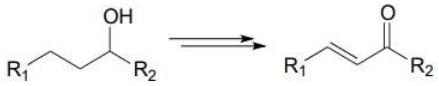


c)

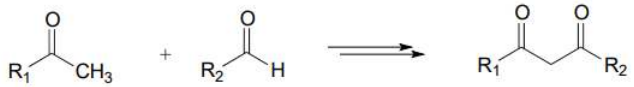


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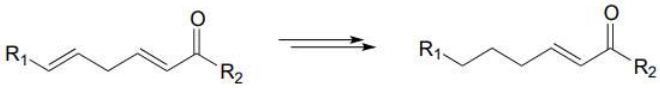
d)



e)



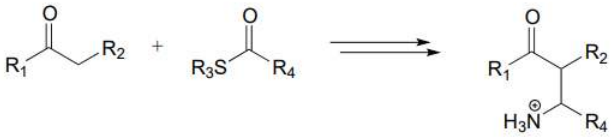
f)



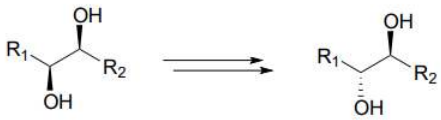
P3.13: Hypothetical 3-step transformations:

Each of the generalized transformations below would be expected to require three enzymatic steps. Draw a reasonable pathway diagram for each transformation.

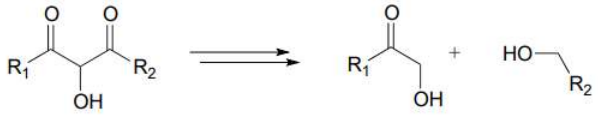
a)



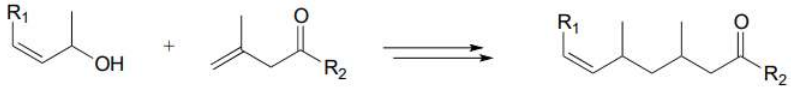
b)



c)



d)

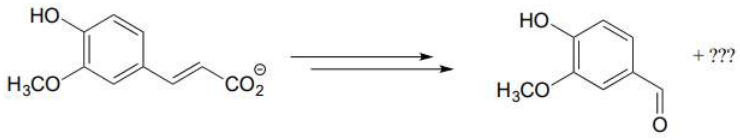


P3.14: Now, let's try our hand at predicting the steps of some actual metabolic pathways. For each, draw a complete pathway diagram. As needed, use any of the coenzymes we have studied, water, and ammonia.

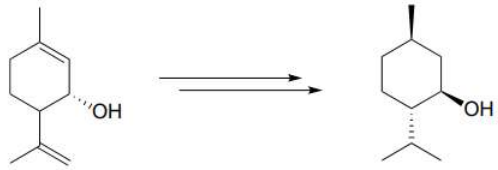
Note: you will probably find these quite challenging! Do not expect to be able to figure them out in a few minutes - rather, think of them as puzzles to work on over a period of time, sharing ideas and strategies with classmates. Remember, if one side of the transformation is larger or more complex, start there and work towards the simpler molecules. It is also a good idea, when applicable, to start the process by a) counting carbons on each side of the transformation, and b) identifying the key bond being formed (or broken) in the transformation.

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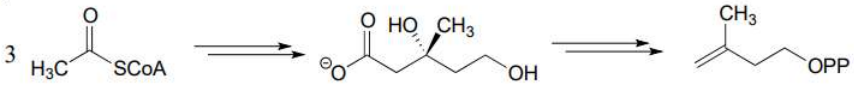
a)



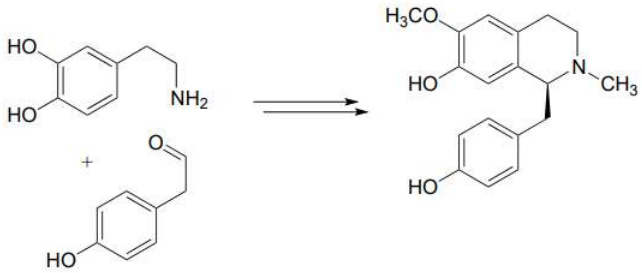
b)



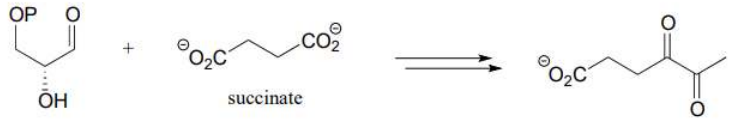
c)



e)



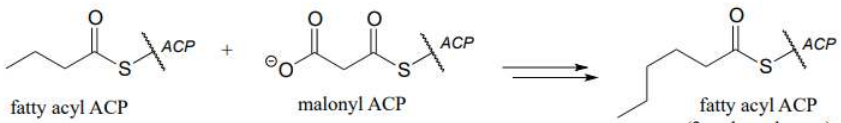
f)



glyceraldehyde-3-phosphate

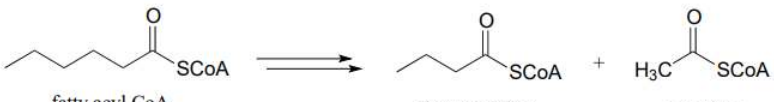
P3.15: Propose a pathway diagram for each of the metabolic pathways below. Note that some pathways contain steps that will be unfamiliar to you, and are therefore provided already.

a. one cycle of fatty acid biosynthesis:



fatty acyl ACP | malonyl ACP | fatty acyl ACP (2 carbons longer)

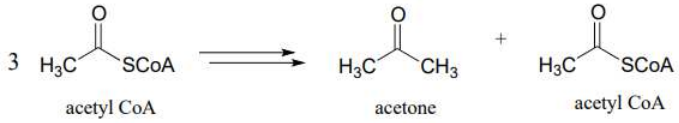
b. one cycle of fatty acid degradation:



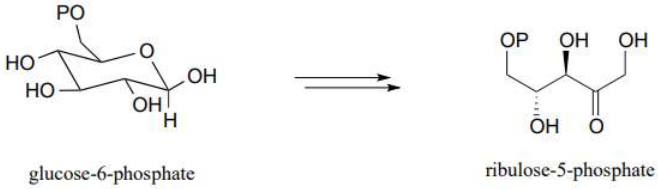
fatty acyl CoA | fatty acyl CoA (2 carbons shorter) | acetyl CoA

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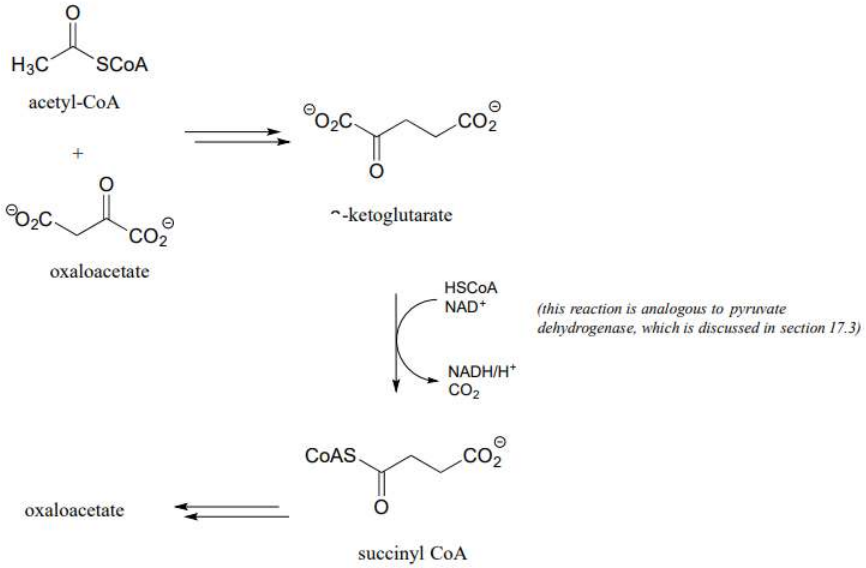
c. Diabetics and people who adopt an extreme low-carbohydrate diet sometimes have breath that smells like acetone, due to 'ketone body' formation that occurs when acetyl-***CoA*** from fatty acid oxidation (see part (b) above) is not able to enter into the citric acid cycle. Draw a pathway diagram showing how three molecules of excess acetyl ***CoA*** combine to form acetone (all three acetyl-***CoA*** molecules first link together, but one is left over at the end of the process).



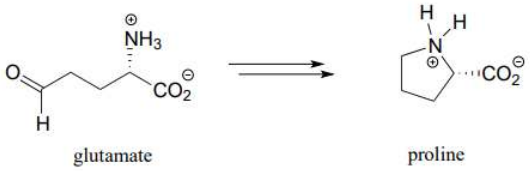
d. Pentose phosphate pathway (oxidative branch):



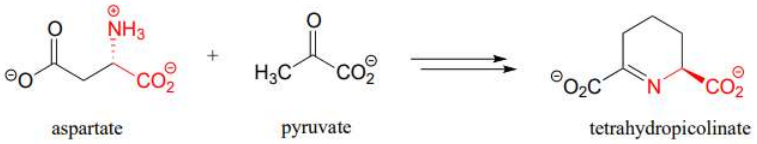
e. Citric acid (Krebs) cycle:



f. proline biosynthesis:

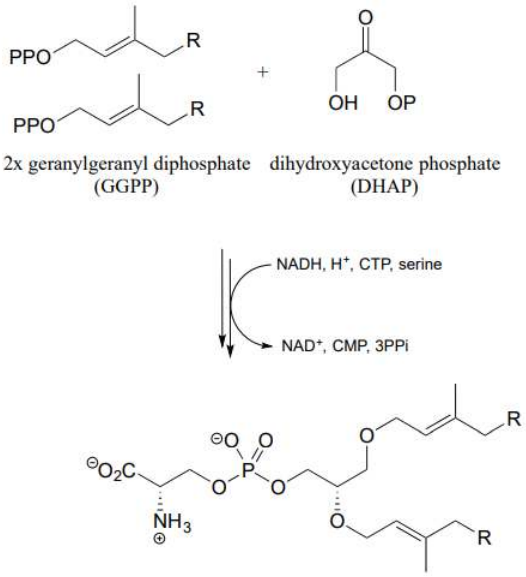


g. First half of lysine biosynthesis:



h. From the biosynthesis of membrane lipid in archaea: J. Bacteriol. 2003, 185, 1181

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Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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3.S: Oxidation and Reduction Reactions (Summary)

Before moving on to the next chapter, you should be able to:

- Recognize when an organic molecule is being oxidized or reduced, and distinguish between redox and non-redox organic reactions.
- Draw complete mechanisms for the following reaction types, including the structure of the reactive part of the redox coenzyme (it is strongly recommended that you commit to memory the structures of the reactive parts of the nicotinamide and flavin coenzymes).
  - oxidation of an alcohol to an aldehyde or ketone
  - oxidation of an amine to an imine
  - oxidation of an aldehyde to a carboxylic acid derivative (usually a thioester or carboxylate)
  - oxidation of an alkane to an alkene at the a,b position relative to a carbonyl or imine
  - reduction of an aldehyde or ketone to an alcohol
  - reduction of an imine to an amine
  - reduction of a carboxylic acid derivative to an aldehyde
  - reduction of an α,β - conjugated alkene to an alkane
  - oxidation of two thiol groups to a disulfide in a disulfide-exchange type reaction
  - reduction of a disulfide group by flavin
  - flavin hydroperoxide-dependent hydroxylation, epoxidation, and Baeyer-Villiger reactions
  - reduction of FAD (or FMN) to $FADH_2$ (or $FMNH_2$) by $NAD(P)H$.
  - spontaneous oxidation of an alkene group in a biomolecule by hydrogen peroxide
  - reduction of hydrogen peroxide by glutathione peroxidase
- In addition, you should be able to draw complete mechanisms for hydrogenation-dehydrogenation and disulfide exchange reactions that we have not yet seen specific examples of, based on your understanding of the chemistry involved in these reaction types and organic reaction patterns in general. Several exercises and end-of-chapter problems provide opportunities practice with inferring and drawing mechanisms of less familiar redox reactions.
- Given a multistep pathway diagram, you should be able to recognize the transformations taking place and fill in missing intermediate compounds or reagents (problem 15.5 is an example of this type).
- You should be working on gaining proficiency at solving multi-step pathway elucidation problems, such as those at the end of this chapter's problem section.

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CHAPTER OVERVIEW

4: Arenes, Electrophilic Aromatic Substitution

Benzene and other aromatic hydrocarbons usually have such strikingly different properties from typical open-chain conjugated polyenes, such as 1,3,5-hexatriene, that it is convenient to consider them as a separate class of compounds called **arenes**. In this chapter we shall outline the essential features of the chemistry of arenes, particularly their reactions with electrophilic reagents which result in the substitution of a ring hydrogen with other functional groups. Some of the important properties of benzene were discussed in Chapter 21 in connection with the valence-bond and molecular-orbital theories, which rationalize the bonding in benzene and account for the remarkable stability and low reactivity of benzene (Section 21-3A). This chapter is especially concerned with chemical properties of benzene and its derivatives as well as related ring systems.

4.1: Nomenclature of Arenes
4.2: Physical Properties of Arenes
4.3: Spectroscopic Properties of Arenes
4.4: Molecular orbital theory- conjugation and aromaticity
4.5: Electrophilic Aromatic Substitution
4.6: Effect of Substituents on Reactivity and Orientation in Electrophilic Aromatic Substitution
4.7: Orientation in Disubstituted Benzenes
4.8: Addition Reactions of Arenes
4.9: Oxidation Reactions
4.10: Sources and Uses of Aromatic Hydrocarbons
4.11: Some Conjugated Cyclic Polyenes
4.12: Fluxional Compounds
4.P: Arenes, Electrophilic Aromatic Substitution (Problems)

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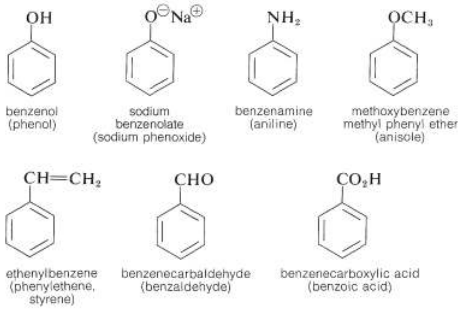
John D. Robert and Marjorie C. Caserio (1977) *Basic Principles of Organic Chemistry, second edition.* W. A. Benjamin, Inc. , Menlo Park, CA. ISBN 0-8053-8329-8.

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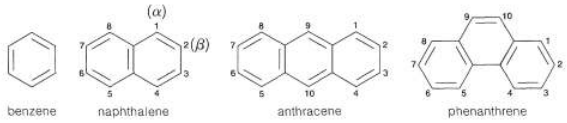
4: Arenes, Electrophilic Aromatic Substitution is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

4.1: Nomenclature of Arenes

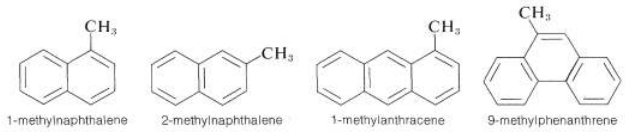
The naming of benzene derivatives was considered previously and is relatively straightforward. However, many benzene derivatives have acquired trivial names, and we draw your attention to a few of these below. The accepted name for the C_6H_5- group as a substituent is **phenyl**.



The more complex ring systems having two or more fused benzene rings have nonsystematic names and illogical numbering systems. They are described as polynuclear aromatic hydrocarbons (PAHs), the three most important examples being naphthalene, anthracene, and phenanthrene. In anthracene the rings are connected in a *linear* manner, whereas in phenanthrene they are connected *angularly*:



The accepted numbering system for these hydrocarbons is as shown in the structures. The 1- and 2-positions of the naphthalene ring sometimes are designated as α and β, but we prefer not to use these designations. Some illustrative substitution products are:



The names that have been given to these and other more elaborate types of polynuclear aromatic hydrocarbons are for the most part distressingly uninformative with respect to their structures.[1]

[1]A thorough summary of names and numbering systems has been published by A. M. Patterson, L. T. Capell, and D. F. Walker, *Ring Index*, 2nd ed., American Chemical Society, 1960. Less complete but useful summaries are given in various handbooks of chemistry.

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4.2: Physical Properties of Arenes

The pleasant odors of the derivatives of many arenes is the origin of the name **aromatic** hydrocarbons. The arenes themselves generally are quite toxic; some are carcinogenic and inhalation of their vapors should be avoided. The volatile arenes are highly flammable and burn with a luminous sooty flame, in contrast to alkanes and alkenes, which usually burn with a bluish flame leaving little carbon residue.

The more common arenes and their physical properties are given in Table 4-1. They are less dense than water and are highly insoluble. Boiling points increase regularly with increasing molecular weight, but there is little correlation between melting point and molecular weight. The melting point is highly dependent on the symmetry of the compound; thus benzene melts **100°** higher than methylbenzene, and the more symmetrical 1,4-dimethylbenzene (*para*-xylene) has a higher boiling point than either the 1,2- or the 1,3-isomer. This latter fact is utilized in the separation by fractional crystallization of 1,4-dimethylbenzene from mixtures of isomers produced from petroleum.

Table 4-1: *Physical Properties of Arenes*

Compound	Mp, °C	Bp, °C	Density, d_4^{20}
benzene	5.5	80	0.8790
methylbenzene (toluene)	−95	111	0.866
ethylbenzene	−94	136	0.8669
propylbenzene	−99	159	0.8617
isopropylbenzene [(1-methylethyl)benzene]	−96	152	0.8620
tert-butylbenzene [(2,2-dimethylethyl)benzene]	−58	168	0.8658
1,2-dimethylbenzene (*ortho*-xylene)	−25	144	0.8968
1,3-dimethylbenzene (*meta*-xylene)	−47	139	0.8811
1,4-dimethylbenzene (*para*-xylene)	13	138	0.8541
1,3,5-trimethylbenzene (mesitylene)	−45	165	0.8634
1,2,4,5-tetramethylbenzene (durene)	80	197	
naphthalene	80	218	
anthracene	216	340	
phenanthrene	101	340	

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4.3: Spectroscopic Properties of Arenes

Infrared Spectra

The presence of a phenyl group in a compound can be ascertained with a fair degree of certainty from its infrared spectrum. For example, in Figure 4-1 we see the infrared spectra of methylbenzene, and of 1,2-, 1,3-, and 1,4-dimethylbenzene. That each spectrum is of a benzene derivative is apparent from certain common features. The two bands near $1600\ \text{cm}^{-1}$ and $1500\ \text{cm}^{-1}$, although of variable intensity, have been correlated with the stretching vibrations of the carbon-carbon bonds of the aromatic ring; also, the sharp bands near $3030\ \text{cm}^{-1}$ are characteristic of aromatic C—H bonds. Other bands in the spectra, especially those between $1650\ \text{cm}^{-1}$ and $2000\ \text{cm}^{-1}$, between $1225\ \text{cm}^{-1}$ and $950\ \text{cm}^{-1}$, and below $900\ \text{cm}^{-1}$, have been correlated with the number and position of ring substituents. Although we shall not document all these various bands in detail, each of the spectra in Figure 4-1 is marked to show some of the correlations that have been made.

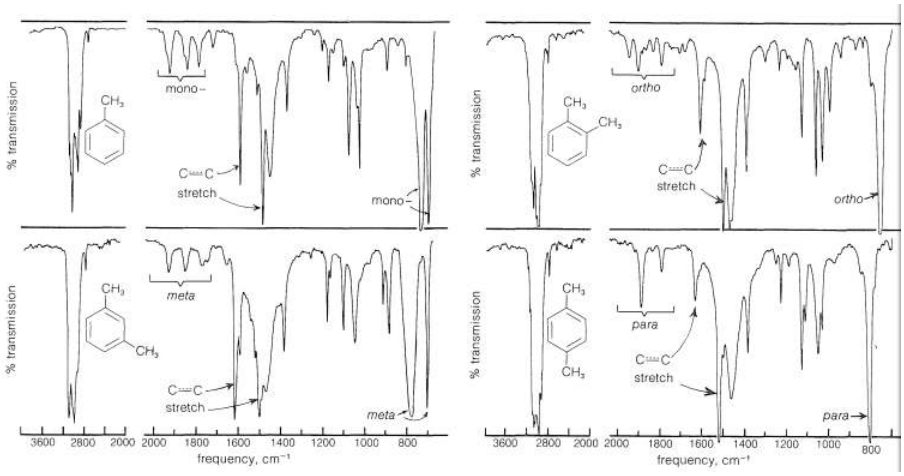
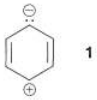


Figure 4-1: *Infrared spectra of methylbenzene and the 1,2-, 1,3-, and 1,4-dimethylbenzenes. The number and positions of ring substituents determine the pattern of the low-intensity bands in the region* $2000\ \text{cm}^{-1}$ *to* $1650\ \text{cm}^{-1}$ *and the positions of the stronger bands in the region* $800\ \text{cm}^{-1}$ *to* $690\ \text{cm}^{-1}$. *The sharp bands near* $3030\ \text{cm}^{-1}$ *arise from* C—H *stretching vibrations.*

Electronic Absorption Spectra

Compared to straight-chain conjugated polyenes, aromatic compounds have relatively complex absorption spectra with several bands in the ultraviolet region. Benzene and the alkylbenzenes show two bands in which we shall be primarily interested, one near $200\ \text{nm}$ and the other near $260\ \text{nm}$. The 200-nm band is of fairly high intensity and corresponds to excitation of a π electron of the conjugated system to a π^* orbital (i.e., a $\pi \rightarrow \pi^*$ transition). The excited state has significant contributions from dipolar structures such as **1**:



1

This is analogous to the absorption bands of conjugated dienes except that the wavelength of absorption of benzenes is shorter. In fact, the 200-nm absorptions of benzene and the alkylbenzenes are just beyond the range of most commercial quartz spectrometers. However, these absorptions (which we say arise from the benzene **chromophore**[2]) are intensified and shifted to longer wavelengths when the conjugated system is extended by replacement of the ring hydrogens by unsaturated groups (e.g., $-\text{CH=CH}_2$, $-\text{C} \equiv \text{CH}$, $-\text{CH=O}$, and $-\text{C} \equiv \text{N}$; see Table 4-2). The delocalized π-electron system of the absorbing chromophore now includes the electrons of the unsaturated substituent as well as those of the ring. In the specific case of ethenylbenzene the excited state is a hybrid structure composite of ***2a*** and ***2b*** and other related dipolar structures:

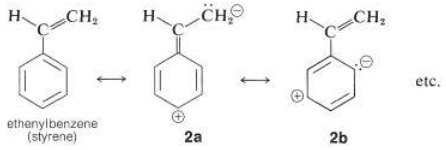
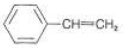
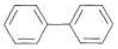
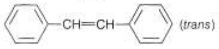
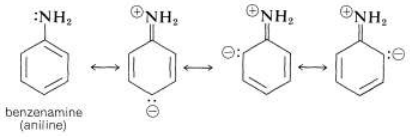


Table 4-2: *Effect of Conjugation on Electronic Absorption by the Benzene Chromophore*

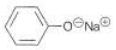
Compound	λ_{max}, nm[a]	ϵ_{max}
	203	8,000
	244	12,000
	244	15,000
	250	18,000
	295	27,000

Similar effects are observed for benzene derivatives in which the substituent has unshared electron pairs that can be conjugated with the benzene ring (e.g., $-NH_2$, $-OH$, $-Cl$). An unshared electron pair is to some extent delocalized to become a part of the aromatic π-electron system in both the ground and excited states, but more importantly in the excited state. This is illustrated for benzenamine (aniline) by the following structures, which contribute to the hybrid structure:



The data of Table 4-3 show the effect on the benzene chromophore of this type of substituent - the substituent often being called an **auxochrome**.[2] This term means that, although the substituent itself is not responsible for the absorption band, it shifts the absorption of the chromophoric group, in this case the benzene ring, toward *longer* wavelengths. The auxochromic groups usually increase the intensity of the absorption also.

Table 4-3: *Effect of Auxochromic Substituents on Electronic Absorption by the Benzene Chromophore*

Compound	λ_{max}, nm[a]	ϵ_{max}
	203	8,000
	210	6,200
	235	9,400
	226	13,000
	230	8,600

[a]In ethanol or water

The benzene chromophore itself gives rise to a second band at longer wavelengths. This band, shown for benzene in Figure 4-3, is of relatively low intensity and is found under high resolution to be a composite of several narrow peaks. It appears to be characteristic of aromatic hydrocarbons because no analogous band is found in the spectra of conjugated acyclic polyenes. For this reason it often is called the **benzenoid band**. The position and intensity of this band, like the one at shorter wavelengths, is affected by the nature of the ring substituents, particularly by those that extend the conjugated system, as may be seen from the date in Table 4-4.

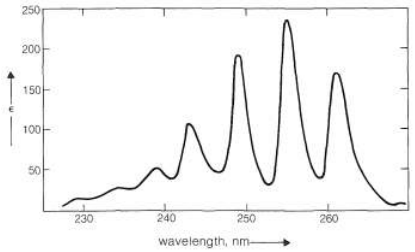
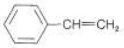
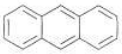
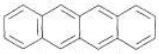
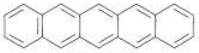


Figure 4-3: *Ultraviolet absorption spectrum of benzene (in cyclohexane) showing the "benzenoid" band.*

Table 4-4: *Effects of Structure on Electronic Absorption Corresponding to the Benzenoid Band*

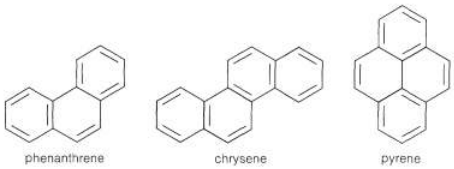
Compound	λ_{max}, nm*	ϵ_{max}
	255	230
	261	300
	282	450
	256	800
	280	1,430
	314	316
	380	7,900
 (naphthacene)	480	11,000
 (pentacene)	580	12,600

*Mostly in ethanol solution

The benzenoid band corresponds to a low-energy $\pi \rightarrow \pi^*$ transition of the benzene molecules. The absorption intensity is weak because the π^* state involved has the same electronic symmetry as the ground state of benzene, and transitions between symmetrical states usually are forbidden. The transitions are observed in this case only because the vibrations of the ring cause it to be slightly distorted at given instants. In the valence-bond treatment this excited state of benzene is an antibonding state of the π electrons.

The electronic spectra of polynuclear aromatic hydrocarbons such as naphthalene and anthracene, in which aromatic rings are fused together in a linear manner, resemble the spectrum of benzene except that the bands are shifted to longer wavelengths. In fact, with the four linearly connected rings of naphthacene, the benzenoid band is shifted far enough to longer wavelengths to be in the visible region of the spectrum (see Table 4-4). Accordingly, naphthacene is yellow. The next higher member, pentacene, is blue.

Compounds such as phenanthrene, chrysene, and pyrene, in which the aromatic rings are fused in an angular manner, have complex electronic spectra with considerable fine structure. The λ_{max} values normally are at shorter wavelengths than those of their linear isomers.



Nuclear Magnetic Resonance Spectra

The chemical shifts of arene protons (**6.5 ppm** to **8.0 ppm**) characteristically are toward lower magnetic fields than those of protons attached to ordinary double bonds (**4.6 ppm** to **6.9 ppm**). The difference of about **2 ppm** cannot be easily explained because the

hydrogens in both types of systems are bonded to carbon through sp^2-σ bonds.

At least part of the chemical-shift difference between arene protons and alkene protons is the result of the special property of π electrons in aromatic systems of circulating freely above and below the plane of the carbon nuclei, as shown in Figure 4-4. When a molecule such as benzene is subjected to a magnetic field that has a component *perpendicular* to the plane of the ring, the electrons circulate around the ring in such a way as to produce a local magnetic dipole in the direction *opposite* to the applied field. This *diamagnetic shielding* effect acts to reduce the applied field in the center of the ring. Therefore, if a proton could be located in the center of the ring, the applied field would have to be higher than normal to counteract the local diamagnetic field and bring the proton into resonance. A proton outside the ring is affected in the opposite way (*paramagnetic deshielding* effect) because, as can be seen from the diagram, such protons are located in the return path of the lines of force associated with the local field and thus are in a field greater than that arising from the external magnet alone. When the plane of the molecule is oriented parallel to the field, the diamagnetic circulation is cut off. As a result, as the molecules tumble over and over in the liquid the component of magnetization perpendicular to the plane of the ring varies rapidly. Nonetheless, a substantial *net* paramagnetic effect is experienced by the ring hydrogens. The resonance line positions therefore are shifted to lower magnetic fields.

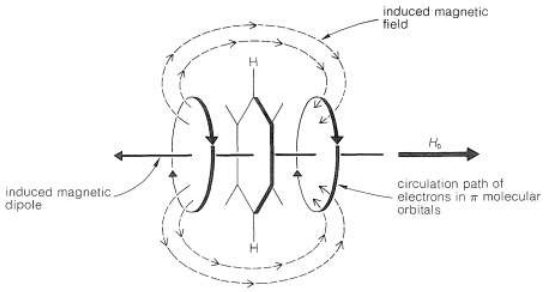
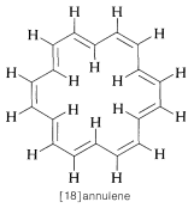


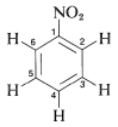
Figure 4-4: *Diagram representing the circulation of the π electrons of an aromatic ring under the influence of an applied magnetic field, H_0. This diagram corresponds to the same kind of effect as that shown in Figure 9-26. The strength of the induced magnetic field, or dipole, is proportional to the applied field.*

Strong evidence in confirmation of the above explanation of the chemical shifts of aromatic hydrogens is provided by a study of the cyclic conjugated polyene [18]annulene, which has hydrogens both "inside" and "outside" the ring:



The *inside* hydrogens are strongly deshielded, coming at 1.9 ppm *upfield* from tetramethylsilane, while the *outside* hydrogens are deshielded and come at 8.8 ppm *downfield* from TMS. As we shall see, the ring current effect is quite general and constitutes a widely used test for aromatic character in conjugated polyene ring systems.

In general, the spin-spin splittings observed between the five protons of a phenyl group can be extremely complex. An example is afforded by nitrobenzene (Figure 4-5), which has different chemical shifts for its ortho, meta, and para hydrogens and six different spin-spin interaction constants: J_{23}, J_{24}, J_{25}, J_{26}, J_{34}, J_{35}, (the subscripts correspond to position numbers of the protons):



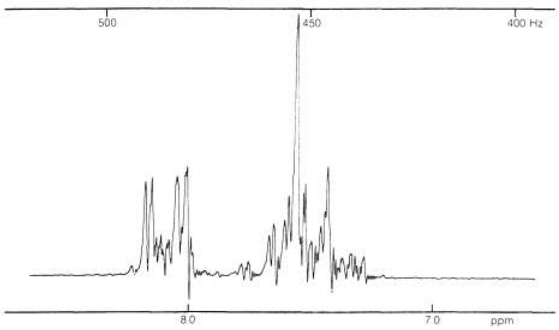
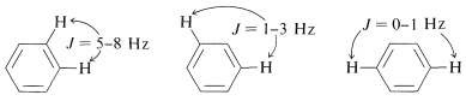


Figure 4-5: *Nmr spectrum of nitrobenzene at* **60 MHz** *with reference to TMS at* **0.00 ppm**.

Such a spectrum is much too complex to be analyzed by any simple procedure. Nonetheless, nuclear magnetic resonance can be useful in assigning structures to aromatic derivatives, particularly in conjunction with integrated line intensities and approximate values of the coupling constants between the ring hydrogens, as shown below:



[2]A chromophore is a grouping of atoms in an organic molecule that gives rise to color, or has the potential of doing so when other groups called **auxochromes** are present.

Contributors and Attributions

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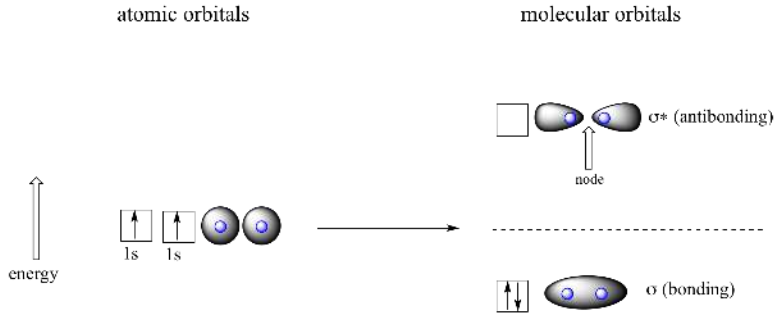
4.3: Spectroscopic Properties of Arenes is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

4.4: Molecular orbital theory- conjugation and aromaticity

Valence bond theory does a remarkably good job at explaining the bonding geometry of many of the functional groups in organic compounds. There are some areas, however, where the valence bond theory falls short. It fails to adequately account, for example, for some interesting properties of compounds that contain alternating double and single bonds. In order to understand these properties, we need to think about chemical bonding in a new way, using the ideas of molecular orbital (MO) theory.

Let's go back and consider again the simplest possible covalent bond: the one in molecular hydrogen (H_2). When we described the hydrogen molecule using valence bond theory, we said that the two 1*s* orbitals from each atom overlap, allowing the two electrons to be shared and thus forming a covalent bond. In molecular orbital theory, we make a further statement: we say that the two atomic 1*s* orbitals mathematically combine to form two new orbitals. Recall that an atomic orbital (such as the 1s orbital of a hydrogen atom) describes a region of space around a single atom inside which electrons are likely to be found. *A molecular orbital describes a region of space around two or more atoms inside which electrons are likely to be found.*

Mathematical principles tell us that when orbitals combine, the number of orbitals before the combination takes place must equal the number of new orbitals that result from the combination – orbitals don't just disappear! We saw this previously when we discussed hybrid orbitals: one *s* and three *p* orbitals make four sp^3 hybrids. When two atomic 1*s* orbitals combine in the formation of H_2, the result is *two* **sigma (σ) orbitals**.



Molecular orbitals for H_2

According to MO theory, one sigma orbital is lower in energy than either of the two isolated atomic 1*s* orbitals –this lower sigma orbital is referred to as a **bonding molecular orbital**. The second, **'sigma star' (sigma*, σ*) orbital** is higher in energy than the two atomic 1*s* orbitals, and is referred to as an **antibonding molecular orbital**.

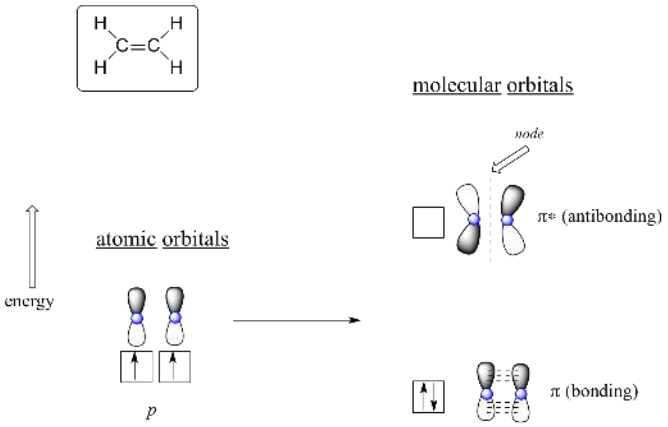
The bonding sigma orbital, which holds both electrons in the ground state of the molecule, is egg-shaped, encompassing the two nuclei, and with the highest likelihood of electrons being in the area between the two nuclei. The high-energy, antibonding sigma* orbital can be visualized as a pair of droplets, with areas of higher electron density near each nucleus and a 'node', (area of zero electron density) midway between the two nuclei.

Remember that we are thinking here about electron behavior as *wave behavior*. When two separate waves combine, they can do so with **constructive interference**, where the two amplitudes build up and reinforce one another, or **destructive interference**, where the two amplitudes cancel one another out. Bonding MOs are the consequence of constructive interference between two atomic orbitals, which results in an attractive interaction and an increase in electron density between the nuclei. Antibonding MO's are the consequence of destructive interference which results in a repulsive interaction and a region of zero electron density between the nuclei (in other words, a node).

Following the same *aufbau* ('building up') principle you learned in General Chemistry for writing out electron configurations, we place the two electrons in the H_2 molecule in the lowest energy molecular orbital, which is the (bonding) sigma orbital. The bonding (attracting) MO is full, and the antibonding (repulsing) MO is empty.

MO theory and conjugated pi bonds

The advantage of using MO theory to understand bonding in organic molecules becomes more apparent when we think about pi bonds. Let's first consider the pi bond in ethene from an MO theory standpoint (in this example we will be disregarding the sigma bonds in the molecule, and thinking *only* about the pi bond). We start with two atomic orbitals: one unhybridized 2p orbital from each carbon. Each contains a single electron. In MO theory, the two atomic combine mathematically to form two **pi (π) molecular orbitals**, one a low-energy pi bonding orbital and one a high-energy pi* (π*) antibonding orbital.



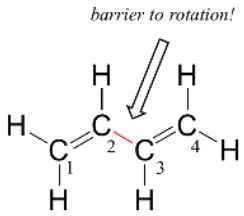
Molecular orbitals for ethene (ethylene)

In the bonding pi orbital, the two shaded lobes of the *p* orbitals interact *constructively* with each other, as do the two unshaded lobes (remember, the arbitrary shading choice represents mathematical (+) and (-) signs for the mathematical wavefunction describing the orbital). There is increased electron density between the two carbon nuclei in the molecular orbital - it is a bonding interaction.

In the higher-energy antibonding pi* orbital, the shaded lobe of one *p* orbital interacts *destructively* with the unshaded lobe of the second *p* orbital, leading to a node between the two nuclei and overall repulsion between the carbon nuclei.

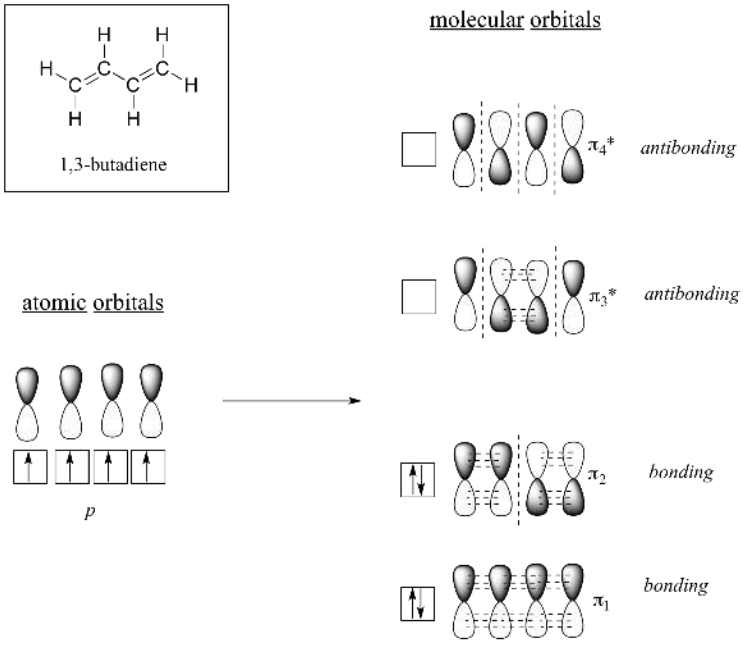
Again using the 'building up' principle, we place the two electrons in the lower-energy, bonding pi molecular orbital. The antibonding pi* orbital remains empty.

Next, we'll consider the 1,3-butadiene molecule. From valence orbital theory alone we might expect that the C_2-C_3 bond in this molecule, because it is a sigma bond, would be able to rotate freely.



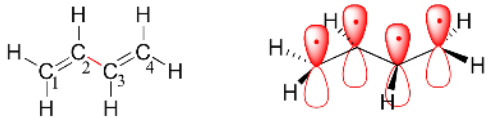
Experimentally, however, it is observed that there is a significant barrier to rotation about the C_2-C_3 bond, and that the entire molecule is planar. In addition, the C_2-C_3 bond is 148 pm long, shorter than a typical carbon-carbon single bond (about 154 pm), though longer than a typical double bond (about 134 pm).

Molecular orbital theory accounts for these observations with the concept of **delocalized pi bonds**. In this picture, the four *2p* atomic orbitals combine mathematically to form four pi molecular orbitals of increasing energy. Two of these - the bonding pi orbitals - are lower in energy than the *p* atomic orbitals from which they are formed, while two - the antibonding pi* orbitals - are higher in energy.



The lowest energy molecular orbital, pi_1, has only constructive interaction and zero nodes. Higher in energy, but still lower than the isolated *p* orbitals, the pi_2 orbital has one node but two constructive interactions - thus it is still a bonding orbital overall. Looking at the two antibonding orbitals, pi_3* has two nodes and one constructive interaction, while pi_4* has three nodes and zero constructive interactions.

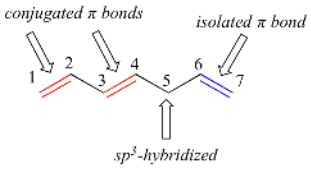
By the *aufbau* principle, the four electrons from the isolated $2p_z$ atomic orbitals are placed in the bonding pi_1 and pi_2 MO's. Because pi_1 includes constructive interaction between C_2 and C_3, there is a degree, in the 1,3-butadiene molecule, of pi-bonding interaction between these two carbons, which accounts for its shorter length and the barrier to rotation. The valence bond picture of 1,3-butadiene shows the two pi bonds as being isolated from one another, with each pair of pi electrons 'stuck' in its own pi bond. However, molecular orbital theory predicts (accurately) that the four pi electrons are to some extent delocalized, or 'spread out', over the whole pi system.



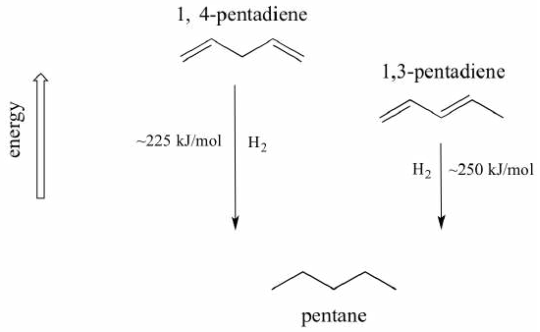
1,3-butadiene

space-filling view

1,3-butadiene is the simplest example of a system of **conjugated pi bonds**. To be considered conjugated, two or more pi bonds must be separated by only one single bond – in other words, there cannot be an intervening sp^3-hybridized carbon, because this would break up the overlapping system of parallel *p* orbitals. In the compound below, for example, the C_1-C_2 and C_3-C_4 double bonds are conjugated, while the C_6-C_7 double bond is **isolated** from the other two pi bonds by sp^3-hybridized C_5.

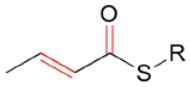


A very important concept to keep in mind is that *there is an inherent thermodynamic stability associated with conjugation.* This stability can be measured experimentally by comparing the **heat of hydrogenation** of two different dienes. (Hydrogenation is a reaction type that we will learn much more about in chapter 15: essentially, it is the process of adding a hydrogen molecule - two protons and two electrons - to a pi bond). When the two *conjugated* double bonds of 1,3-pentadiene are 'hydrogenated' to produce pentane, about 225 kJ is released per mole of pentane formed. Compare that to the approximately 250 kJ/mol released when the two *isolated* double bonds in 1,4-pentadiene are hydrogenated, also forming pentane.

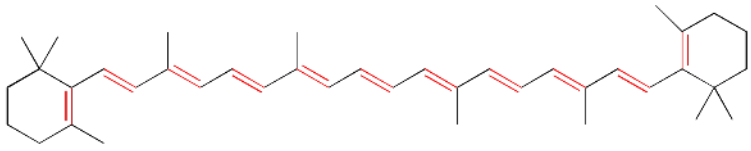


The conjugated diene is lower in energy: in other words, it is more stable. In general, conjugated pi bonds are more stable than isolated pi bonds.

Conjugated pi systems can involve oxygen and nitrogen atoms as well as carbon. In the metabolism of fat molecules, some of the key reactions involve alkenes that are conjugated to carbonyl groups.



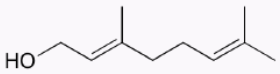
In chapter 4, we will see that MO theory is very useful in explaining why organic molecules that contain extended systems of conjugated pi bonds often have distinctive colors. beta-carotene, the compound responsible for the orange color of carrots, has an extended system of 11 conjugated pi bonds.

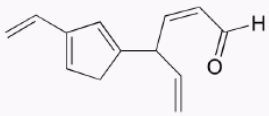


beta-carotene

Exercise 4.1

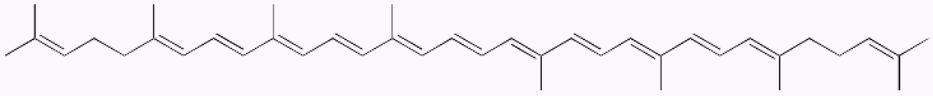
Identify all conjugated and isolated double bonds in the structures below. For each conjugated pi system, specify the number of overlapping *p* orbitals, and how many pi electrons are shared among them.





Exercise 4.2

Identify all isolated and conjugated double bonds in lycopene, the red-colored compound in tomatoes. How many pi electrons are contained in the conjugated pi system?

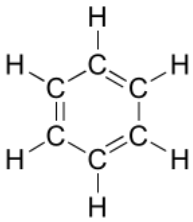


lycopene

Solutions to exercises

Aromaticity

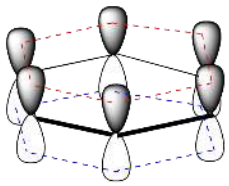
Molecular orbital theory is especially helpful in explaining the unique properties of **aromatic** compounds such as benzene:



3D interactive model of benzene

Although benzene is most often drawn with three double bonds and three single bonds, in fact all of the carbon-carbon bonds are exactly the same length (138 pm). In addition, the pi bonds in benzene are significantly less reactive than 'normal' pi bonds, either isolated or conjugated. Something about the structure of benzene makes its pi bonding arrangement especially stable. This 'something' has a name: it is called 'aromaticity'.

What exactly is this 'aromatic' property that makes the pi bonds in benzene so stable? In large part, the answer to this question lies in the fact that benzene is a *cyclic* molecule in which all of the ring atoms are sp^2-hybridized. This allows the pi electrons to be delocalized in molecular orbitals that extend all the way around the ring, above and below the plane. For this to happen, of course, the ring must be planar – otherwise the *p* orbitals couldn't overlap properly. Benzene is indeed known to be a flat molecule.



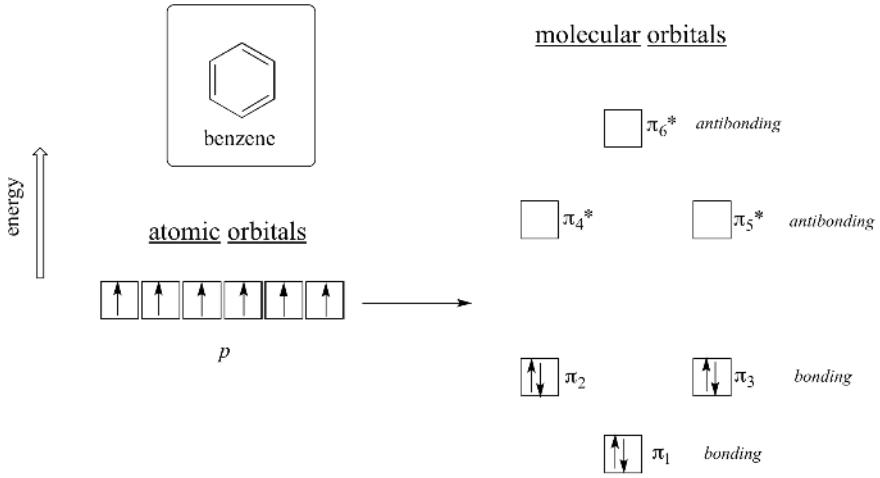
π electrons delocalized around the ring, above and below the plane

Do all cyclic molecules with alternating single and double bonds have this same aromatic stability? The answer, in fact, is 'no'. The eight-membered cyclooctatetraene ring shown below is *not* flat, and its pi bonds react like 'normal' alkenes. π



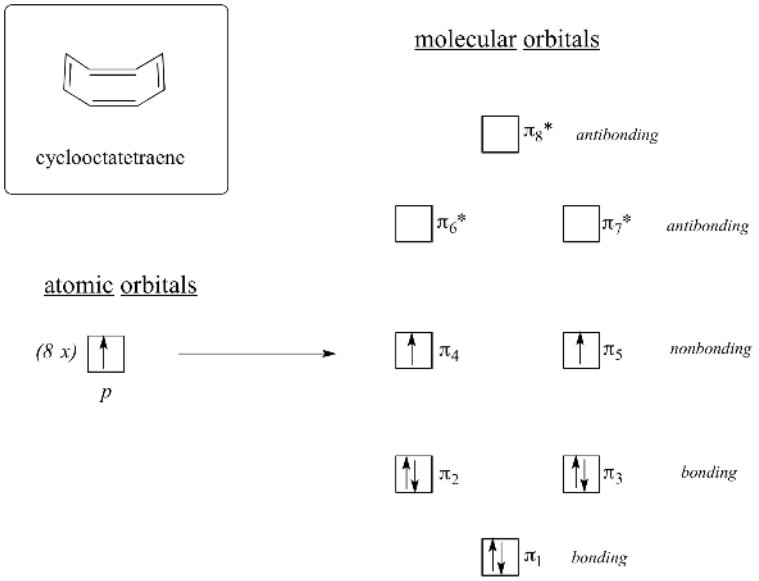
cyclooctatetraene
(not aromatic)

Clearly it takes something more to be aromatic, and this can best be explained with molecular orbital theory. Let's look at an energy diagram of the pi molecular orbitals in benzene.



Quantum mechanical calculations tell us that the six pi molecular orbitals in benzene, formed from six atomic *p* orbitals, occupy four separate energy levels. pi_1 and pi_6* have unique energy levels, while the pi_2 - pi_3 and pi_4*- pi_5* pairs are **degenerate**, meaning they are at the same energy level. When we use the *aufbau* principle to fill up these orbitals with the six pi electrons in benzene, we see that the bonding orbitals are completely filled, and the antibonding orbitals are empty. This gives us a good clue to the source of the special stability of benzene: a full set of bonding MO's is similar in many ways to the 'full shell' of electrons in the atomic orbitals of the stable noble gases helium, neon, and argon.

Now, let's do the same thing for cyclooctatetraene, which we have already learned is *not* aromatic.



The result of molecular orbital calculations tells us that the lowest and highest energy MOs (pi_1 and pi_8*) have unique energy levels, while the other six form degenerate pairs. Notice that pi_4 and pi_5 are at the same energy level as the isolated $2p_z$ atomic orbitals: these are therefore neither bonding nor antibonding, rather they are referred to as **nonbonding MOs**. Filling up the MOs with the eight pi electrons in the molecule, we find that the last two electrons are unpaired and fall into the two degenerate nonbonding orbitals. Because we don't have a perfect filled shell of bonding MOs, our molecule is not aromatic. As a consequence, each of the double bonds in cyclooctatetraene acts more like an *isolated* double bond.

Here, then, are the conditions that must be satisfied for a molecule or group to be considered aromatic:

Criteria for aromaticity:

The molecule or group must be cyclic.

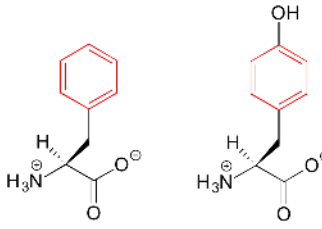
The ring must be planar.

Each atom in the ring must be sp^2-hybridized.

The number of pi electrons in the ring must equal $4n+2$, where n is any positive integer including zero.

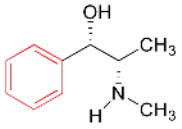
Rule #4 is known as the **Hückel rule**, named after Erich Hückel, a German scientist who studied aromatic compounds in the 1930's. If n = 0, the Hückel number is **2**. If n = 1, the Hückel number is **6** (the Hückel number for benzene). The series continues with **10, 14, 18, 22,** and so on. Cyclooctatetraene has eight pi electrons, which is *not* a Hückel number. Because six is such a common Hückel number, chemists often use the term '**aromatic sextet**'.

Benzene rings are ubiquitous in biomolecules and drugs - below are just a few examples.

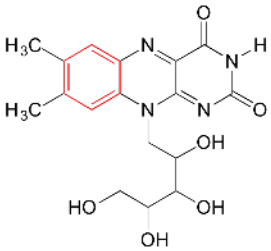


phenylalanine
(an amino acid)

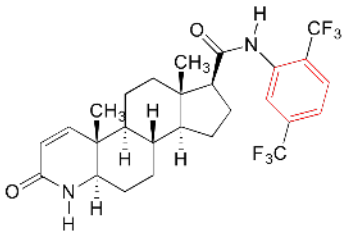
tyrosine
(an amino acid)



pseudoephidrine
(decongestant)



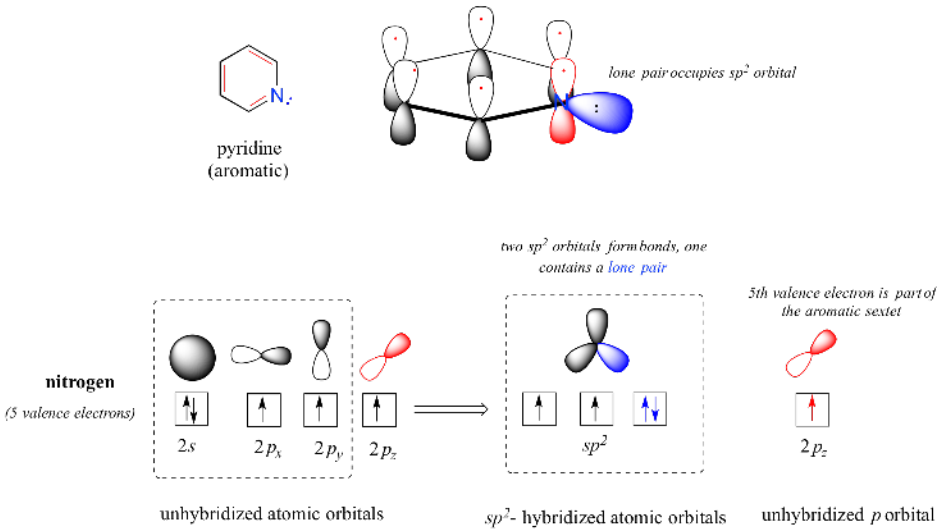
riboflavin
(a vitamin)



dutasteride
(GlaxoSmithKline drug for urinary condition in men)

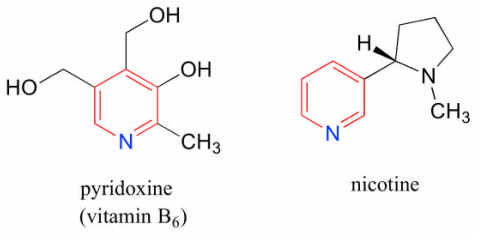
Recall that a benzene ring with a hydoxyl substituent -such as seen in the tyrosine structure above - is called a **phenol**.

Heterocycles - cyclic structures in which the ring atoms may include oxygen or nitrogen - can also be aromatic. Pyridine, for example, is an aromatic heterocycle. In the bonding picture for pyridine, the nitrogen is sp^2-hybridized, with two of the three sp^2 orbitals forming sigma overlaps with the sp^2 orbitals of neighboring carbon atoms, and the third nitrogen sp^2 orbital containing the lone pair. The unhybridized p orbital contains a single electron, which is part of the 6 pi-electron system delocalized around the ring.

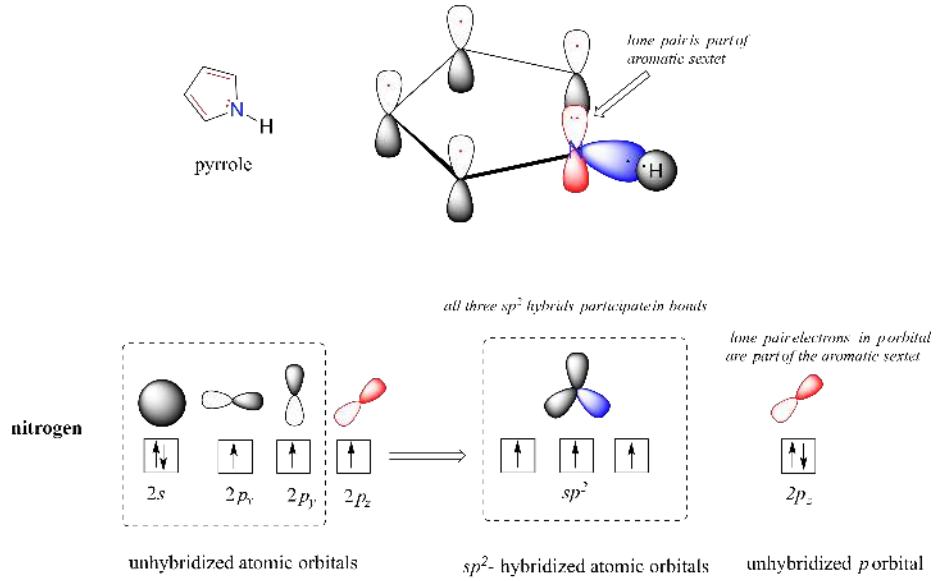


another image of orbitals in pyridine

Pyridoxine, commonly known as vitamin B_6, and nicotine are both substituted pyridines.

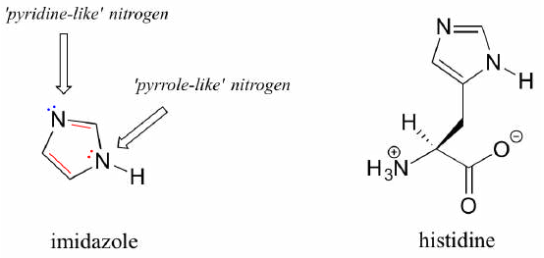


Pyrrole is a five-membered aromatic heterocycle. In pyrrole, the lone pair electrons on the sp^2-hybridized nitrogen are part of the aromatic sextet (contrast this to pyridine, where the lone pair occupies one of the sp^2 hybrid orbitals).



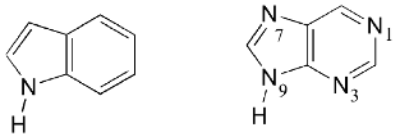
Why don't we assume that the nitrogen in pyrrole is sp^3-hybridized, like a normal secondary amine? The answer is simple: if it were, then pyrrole could not be aromatic, and thus it would not have the stability associated with aromaticity. In general, *if a molecule or group can be aromatic, it will be*, just as water will always flow downhill if there is a downhill pathway available.

Imidazole is another important example of an aromatic heterocycle found in biomolecules - the side chain of the amino acid histidine contains an imidazole ring.

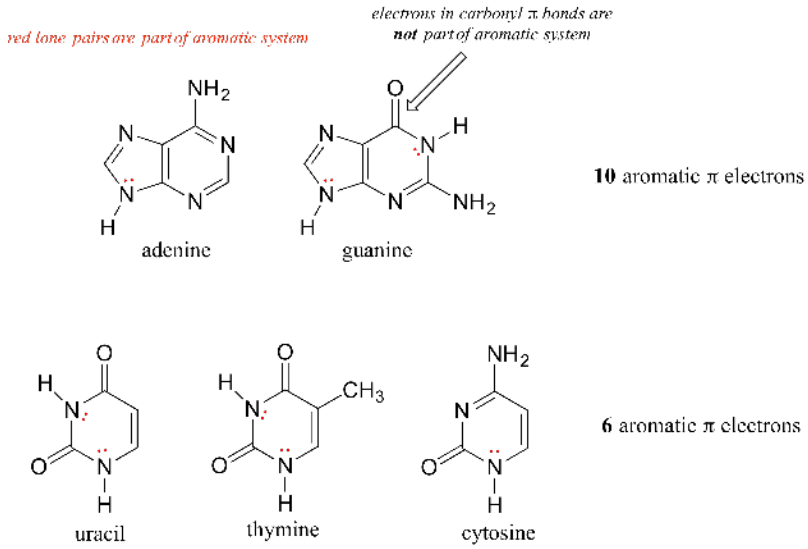


In imidazole, one nitrogen is 'pyrrole-like' (the lone pair contributes to the aromatic sextet) and one is 'pyridine-like' (the lone pair is located in an sp^2 orbital, and is *not* part of the aromatic sextet).

Fused-ring structures can also fulfill the Hückel criteria, and often have many of the same properties as monocyclic aromatic compounds, including a planar structure. Indole (a functional group on the side chain of the amino acid tryptophan) and purine (found in guanine and adenine nucleotide bases) both have a total of ten pi electrons delocalized around two rings.



The nucleic acid bases of DNA and RNA - guanine, adenine, cytosine, thymine, and uracil - are all aromatic systems, with the characteristic aromatic properties of planarity and delocalized pi electron density. When you study the structure and function of DNA and RNA in a biochemistry or molecular biology course, you will see that the planar shape of the bases plays a critically important role.

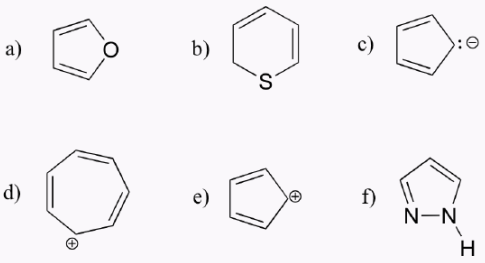


Exercise 4.3

Classify the nitrogen atoms of indole and purine as either 'pyrrole-like' or 'pyridine-like', in terms of where the lone pair electrons are located.

Exercise 4.4

Are the following molecules/ions aromatic? Explain, using criteria you learned in this section.



Solutions to exercises

Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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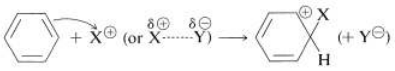
4.5: Electrophilic Aromatic Substitution

4-5A Scope and Mechanism

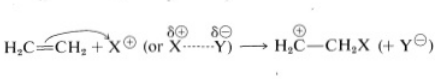
In this section we shall be mainly interested in the reactions of arenes that involve attack on the carbon atoms of the aromatic ring. We shall not elaborate now on the reactions of substituent groups around the ring.

The principal types of reactions involving aromatic rings are substitution, addition, and oxidation. Of these, the most common type is electrophilic substitution. A summary of the more important substitution reactions of benzene is given in Figure 4-7. Many of the reagents used to achieve these substitutions will be familiar to you in connection with electrophilic addition reactions to alkenes (e.g., Cl_2, Br_2, H_2SO_4, and HOCl). Electrophilic addition to alkenes and electrophilic aromatic substitution are both polar, stepwise processes, and the key step for each is attack of an electrophile at carbon to form a cationic intermediate. We may represent this type of reaction by the following general equations, in which the attacking reagent is represented either formally as a cation, $X^{\oplus}$, or as a neutral but polarized molecule, $\overset{\delta\oplus}{X}\text{---}\overset{\delta\ominus}{Y}$:

electrophilic aromatic substitution (first step)



electrophilic addition to alkenes (first step)



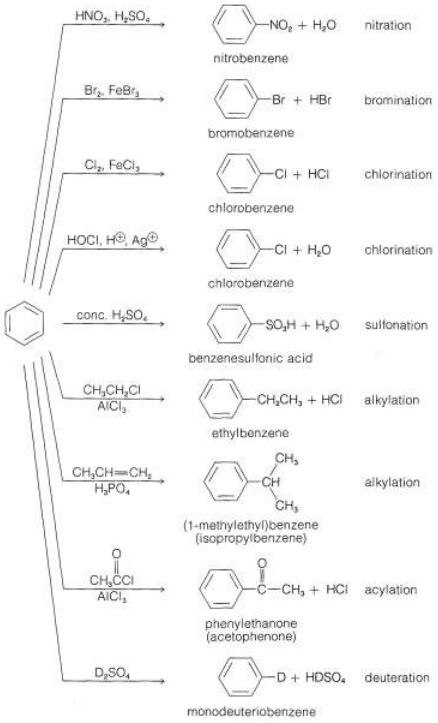
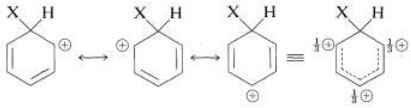


Figure 4-7: *Typical benzene substitution reactions.*

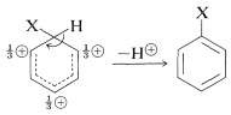
The intermediate shown for aromatic substitution no longer has an aromatic structure; rather, it is a cation with four π electrons delocalized over five carbon nuclei, the sixth carbon being saturated with sp^3-hybrid bonds. It may be formulated in terms of the following contributing structures, which are assumed to contribute essentially equally:



The importance of writing the hybrid structure with the partial charges at these three positions will become evident later. This kind of ion is referred to as a σ *complex* or a *benzenium ion*.

The aromatic ring is regenerated from this cationic intermediate by loss of a proton from the sp^3-hybridized carbon. The electron pair of this $\mathbf{C{-}H}$ bond then becomes part of the aromatic π-electron system and a substitution product of benzene, $\mathbf{C_6H_5X}$, is formed.

electrophilic aromatic substitution (second step)



The gain in stabilization attendant on regeneration of the aromatic ring is sufficiently advantageous that this, rather than combination of the cation with $\mathbf{Y^{\ominus}}$, normally is the favored course of reaction. Herein lies the difference between aromatic substitution and alkene addition. In the case of alkenes there usually is no substantial resonance energy to be gained by loss of a proton from the intermediate, which tends therefore to react by combination with a nucleophilic reagent.

electrophilic addition to alkenes (second step)

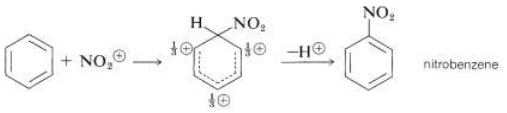
$$\mathbf{\overset{\oplus}{C}H_2{-}CH_2X + Y^{\ominus} \rightarrow YCH_2{-}CH_2X} \tag{4.5.1}$$

4-5B Nature of the Substituting Agent

It is important to realize that in aromatic substitution the actual electrophilic substituting agent, $\mathbf{\overset{\oplus}{X}}$ or $\mathbf{\overset{\delta\oplus}{X}-\overset{\delta\ominus}{Y}}$, is not necessarily the reagent that is added to the reaction mixture. For example, nitration in mixtures of nitric and sulfuric acids is not brought about by attack of the nitric acid molecule on the aromatic compound, but by attack of a more electrophilic species, the nitronium ion, $\mathbf{NO_2^+}$. This ion is formed from nitric acid and sulfuric acid according to the following equation:

$$\mathbf{HNO_3 + 2H_2SO_4 \rightleftharpoons NO_2^+ + H_3O^+ + 2HSO_4^-} \tag{4.5.2}$$

The nitronium ion attacks the aromatic ring to give first a nitrobenzenium ion and then an aromatic nitro compound:

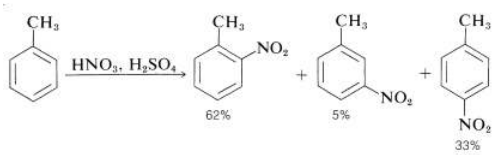


In general, the function of a catalyst (which is so often necessary to promote aromatic substitution) is to generate an electrophilic substituting agent from the given reagents. Thus it is necessary to consider carefully for each substitution reaction what the actual substituting agent may be. This problem does not arise to the same degree in electrophilic additions to alkenes, because alkenes are so much more reactive than arenes that the reagents employed (e.g., $\mathbf{Br_2}$, $\mathbf{Cl_2}$, $\mathbf{HCl}$, $\mathbf{HOCl}$, $\mathbf{HOBr}$, $\mathbf{H_3O^{\oplus}}$) themselves are sufficiently electrophilic to react with alkenes without the aid of a catalyst. In fact, conditions that lead to substitution of arenes, such as nitration in mixtures of nitric and sulfuric acid, often will degrade the carbon skeleton of alkenes.

Now we shall consider the individual substitution reactions listed in Figure 4-1 with regard to the nature of the substituting agent and the utility for synthesis of various classes of aromatic compounds.

4-5C Nitration

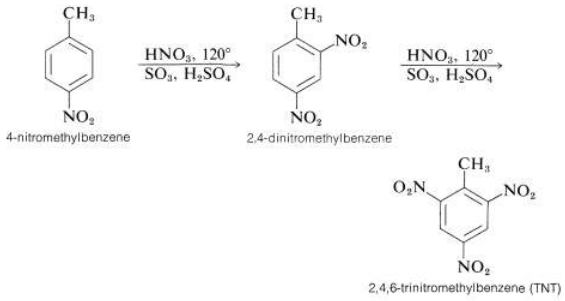
The nitronium ion, $\mathbf{NO_2^+}$, is the active nitrating agent in nitric acid-sulfuric acid mixtures. The nitration of methylbenzene (toluene) is a typical example of a nitration that proceeds well using nitric acid in a 1:2 mixture with sulfuric acid. The nitration product is a mixture of 2-, 3-, and 4-nitromethylbenzenes:



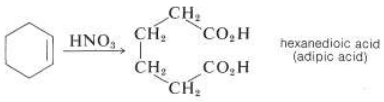
The presence of appreciable amounts of water in the reaction mixture is deleterious because water tends to reverse the reaction by which nitronium ion is formed:

$$NO_2^+ + H_2O \overset{HSO_4^-}{\rightleftharpoons} HNO_3 + H_2SO_4 \qquad (4.5.3)$$

For this reason the potency of a nitric-sulfuric acid mixture can be considerably increased by using fuming nitric and fuming sulfuric acids. With such mixtures nitration of relatively unreactive compounds can be achieved. For example, 4-nitromethylbenzene is far less reactive than methylbenzene, but when heated with an excess of nitric acid in fuming sulfuric acid, it can be converted successively to 2,4-dinitromethylbenzene and to 2,4,6-trinitromethylbenzene (TNT):

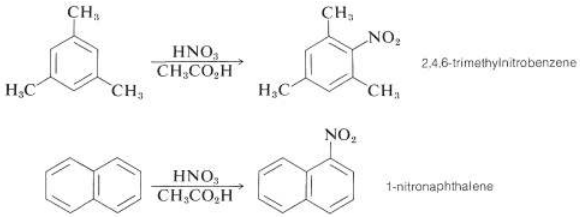


There are several interesting features about the nitration reactions thus far discussed. For instance, the conditions required for nitration of 4-nitromethylbenzene would rapidly oxidize an alkene by cleavage of the double bond:

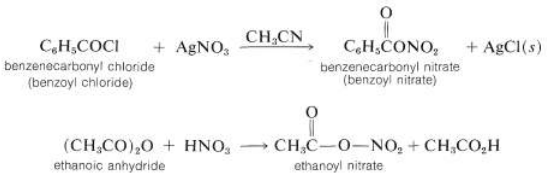


Also the mononitration of methylbenzene does not lead to equal amounts of the three possible products. The methyl substituent apparently orients the entering substituent preferentially to the 2 and 4 positions. This aspect of aromatic substitution will be discussed in Section 4-5 in conjunction with the effect of substituents on the reactivity of aromatic compounds.

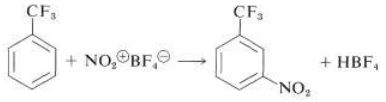
Some compounds are sufficiently reactive that they can be nitrated with nitric acid in ethanoic acid. Pertinent examples are 1,3,5-trimethylbenzene and naphthalene:



Other convenient nitrating reagents are benzoyl nitrate, $C_6H_5COONO_2$, and ethanoyl nitrate, CH_3COONO_2. These reagents provide a source of NO_2^+ and have some advantage over $HNO_3 \cdot H_2SO_4$ mixtures in that they are soluble in organic solvents such as ethanenitrile or nitromethane. Having homogeneous solutions is especially important for kinetic studies of nitration. The reagents usually are prepared in solution as required from the corresponding acyl chloride and silver nitrate or from the acid anhydride and nitric acid. Such reagents are hazardous materials and must be handles with care.

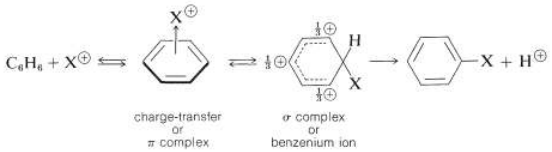


Nitronium salts of the type $NO_2^+X^-$ are very powerful nitrating agents. The counterion, X^-, must be non-nucleophilic and usually is fluoroborate, BF_4^- or SbF_4^-:



4-5D Halogenation

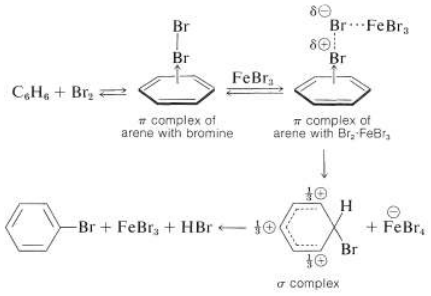
To some degree we have oversimplified electrophilic substitution by neglecting the possible role of the 1:1 charge-transfer complexes that most electrophiles form with arenes (see Section 10-3C for discussion of analogous complexes of alkenes):



With halogens, especially iodine, complex formation is visually evident from the color of solutions of the halogen in arenes. Although complex formation may assist substitution by bringing the halogen and arene in close proximity, substitution does not necessarily occur. A catalyst usually is required, and the catalysts most frequently used are metal halides that are capable of accepting electrons (i.e., Lewis acids such as $FeBr_3$, $AlCl_3$, and $ZnCl_2$). Their catalytic activity may be attributed to their ability to polarize the halogen-halogen bond in the following way:

$$\overset{\delta\oplus}{Br}\cdots\overset{\delta\ominus}{Br}\cdots FeBr_3 \tag{4.5.4}$$

The positive end of the dipole attacks the aromatic compound while the negative end is complexed with the catalyst. We can represent the reaction sequence as follows, with the slow step being formation of a σ bond between $Br^{\oplus}$ and the aromatic ring:



The order of reactivity of the halogens is $F_2 > Cl_2 > Br_2 > I_2$. Fluorine is too reactive to be of practical use for the preparation of aromatic fluorine compounds and indirect methods are necessary (see Section 23-10B). Iodine usually is unreactive. It has been stated that iodination fails because the reaction is reversed as the result of the reducing properties of the hydrogen iodide that is formed:

$$C_6H_6 + I_2 \rightleftarrows C_6H_5I + HI \tag{4.5.5}$$

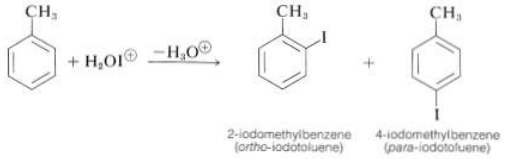
This view is not correct because, as Kekule himself showed, iodobenzene is not reduced by hydroiodic acid except at rather high temperatures.

The way to achieve direct iodination in the absence of powerful activating substituent groups is to convert molecular iodine to some more active species (perhaps $H_2OI^{\oplus}$ or $I^{\oplus}$) with an oxidizing agent such as nitric acid or hydrogen peroxide:

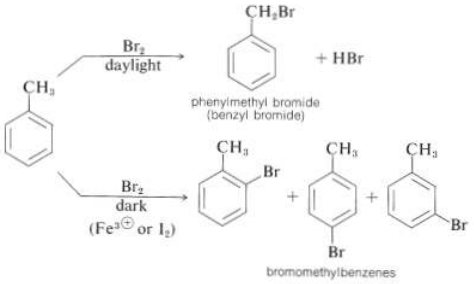
$$I_2 + 4HNO_3 \rightarrow 2H_2O{-}I^+ + 2NO_2 + 2NO_3^- \quad (4.5.6)$$

$$I_2 + H_2O_2 + 2H^+ \rightarrow 2H_2OI^+ \quad (4.5.7)$$

With combinations of this kind good yields of iodination products are obtained:



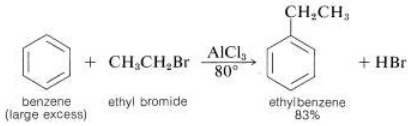
Halogen substitution reactions with chlorine or bromine must be carried out with adequate protection from strong light. If such precautions are not taken, an *alkyl*benzene will react rapidly with halogen by a photochemical process to substitute a hydrogen of the alkyl group rather than of the aromatic ring. The reaction has a light-induced, radical-chain mechanism of the kind discussed for the chlorination of propene. Thus methylbenzene reacts with bromine when illuminated to give phenylmethyl bromide; but when light is excluded and a Lewis acid catalyst is present, substitution occurs to give principally the 2- and 4-bromomethylbenzenes. Much less of the 3-bromomethylbenzene is formed:



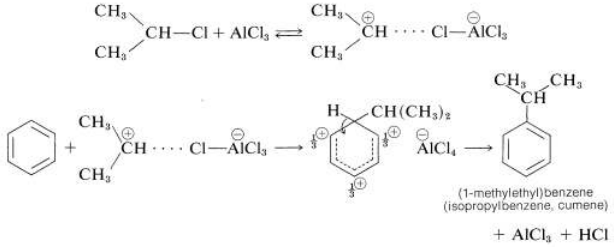
Benzene itself can be induced to *add* halogens on strong irradiation to give polyhalocyclohexanes (see Sections 21-3A and 4-9C):

4-5E Alkylation

An important method of synthesis of alkylbenzenes utilizes an alkyl halide as the alkylating agent and a metal halide, usually aluminum chloride, as catalyst:

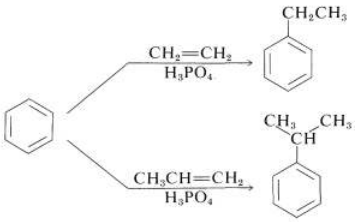


This class of reaction is called **Friedel-Crafts alkylation** in honor of its discoverers, C. Friedel (a French chemist) and J. M. Crafts (an American chemist). The metal-halide catalyst functions much as it does in halogenation reactions to provide a source of a positive substituting agent, which in this case is a carbocation:



Alkylation is not restricted to alkyl halides; alcohols and alkenes may be used to advantage in the presence of acidic catalysts such as H_3PO_4, H_2SO_4, HF, BF_3, or $HF{-}BF_3$. Ethylbenzene is made commercially from benzene and ethene using phosphoric acid as the

catalyst. Isopropylbenzene is made similarly from benzene and propene:

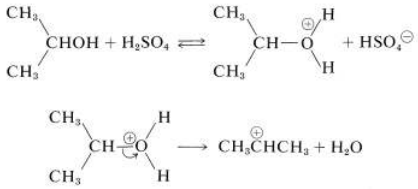


Under these conditions the carbocation, which is the active substituting agent, is generated by protonation of the alkene:

$$CH_2{=}CH_2 + H^+ \rightleftharpoons CH_3CH_2^+ \tag{4.5.8}$$

$$CH_3CH{=}CH_2 + H^+ \rightleftharpoons CH_3\overset{+}{C}HCH_3 \tag{4.5.9}$$

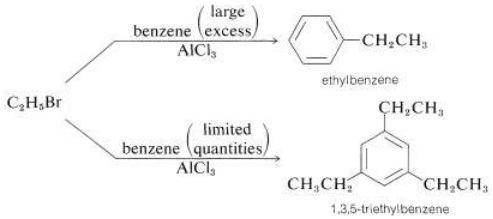
With alcohols the electrophile can be formed by initial protonation by the acid catalyst and subsequent cleavage to a carbocation:



Limitations of Alkylation Reactions

Polysubstitution

There are several factors that limit the usefulness of alkylation reactions. First, it may be difficult to limit reaction to monosubstitution because introduction of one alkyl substituent tends to activate the ring towards a second substitution. Therefore, to obtain reasonable yields of a monoalkylbenzene, it usually is necessary to use a large excess relative to the alkylating agent:



Rearrangement of the alkylating agent

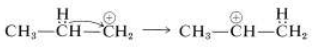
A second limitation is the penchant for the alkylating reagent to give rearrangement products. As an example, the alkylation of benzene with 1-chloropropane leads to a mixture of propylbenzene and isopropylbenzene. We may write the reaction as first involving formation of a propyl cation, which is a *primary* carbocation:

$$CH_3CH_2CH_2Cl + AlCl_3 \rightarrow CH_3CH_2CH_2^+ + \bar{A}lCl_4 \tag{4.5.10}$$

This ion either can alkylate benzene to give propylbenzene,

$$C_6H_6 + CH_3CH_2CH_2^+ \rightarrow C_6H_5CH_2CH_2CH_3 + H^+ \tag{4.5.11}$$

or it can rearrange to a more stable secondary ion by the transfer of a hydrogen from a neighboring carbon together with its bonding electron pair (i.e., 1,2-hydride shift). The positive charge is thereby transferred from C_1 to C_2:



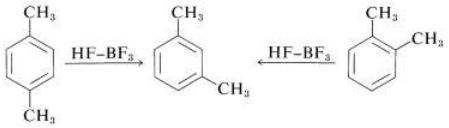
Alkylation of benzene with the isopropyl cation then produces isopropylbenzene:

$$C_6H_6 + CH_3\overset{\oplus}{C}HCH_3 \rightarrow C_6H_5CH(CH_3)_2 + H^{\oplus} \quad (4.5.12)$$

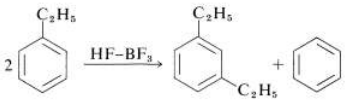
Rearrangements of this type involving carbocation intermediates often occur in Friedel-Crafts alkylations with primary and secondary alkyl groups larger than C_2 and C_3. Related carbocation rearrangements were discussed in the alkene chapter last semester.

Rearrangement of products

Further complications arise from the fact that the alkylation reactions sometimes are under equilibrium control rather than kinetic control. Products often isomerize and disproportionate, particularly in the presence of large amounts of catalyst. Thus 1,2- and 1,4-dimethylbenzenes (*ortho*- and *para*-xylenes) are converted by large amounts of Friedel-Crafts catalysts into 1,3-dimethylbenzene (*meta*-xylene):

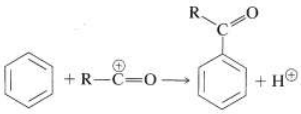


Ethylbenzene disproportionates under the influence of excess $HF-BF_3$ to benzene and 1,3-diethylbenzene:

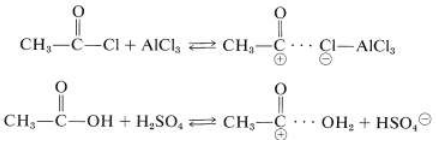


4-5F Acylation

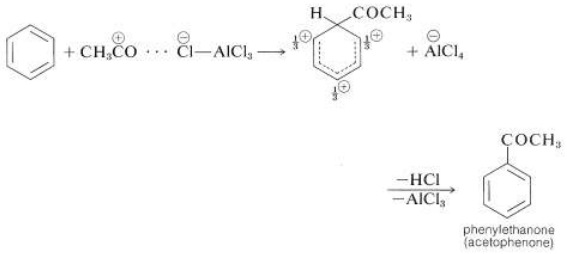
Acylation and alkylation of arenes are closely related. Both reactions were developed as the result of the collaboration between Friedel and Crafts, in 1877. The acylation reaction introduces an acyl group, RCO, into an aromatic ring and the product is an aryl ketone:



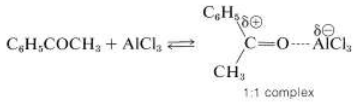
The acylating reagents commonly used are carboxylic acid halides, RCOCl, anhydrides, $(RCO)_2O$, or the acid itself, RCO_2H. A strong proton or other Lewis-acid catalyst is essential. The catalyst functions to generate the acyl cation:



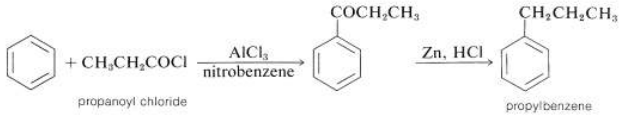
The catalyst most commonly used with acyl halides and anhydrides is aluminum chloride:



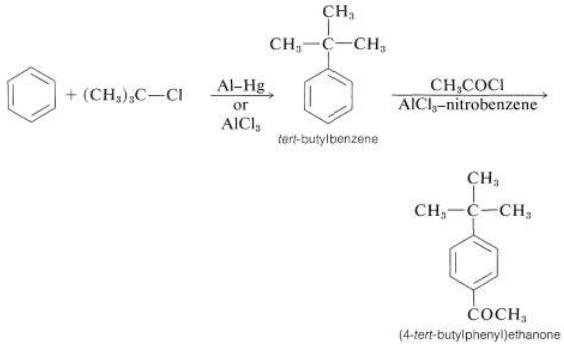
Acylation differs from alkylation in that the reaction usually is carried out in a solvent, commonly carbon disulfide, CS_2, or nitrobenzene. Furthermore, acylation requires more catalyst than alkylation, because much of the catalyst is tied up and inactivated by complex formation with the product ketone:



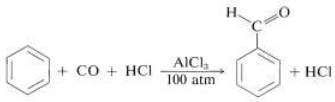
Unlike alkylation, acylation is controlled easily to give monosubstitution, because once an acyl group is attached to a benzene ring, it is not possible to introduce a second acyl group into the same ring. Because of this, a convenient synthesis of alkylbenzenes starts with acylation, followed by reduction of the carbonyl group with zinc and hydrochloric acid. For example, propylbenzene is prepared best by this two-step route because, as we have noted, the direct alkylation of benzene with propyl chloride produces considerable amounts of isopropylbenzene and polysubstitution products:



In the acylation of alkylbenzene the product almost always is the *para* isomer. The synthesis of (4-*tert*-butylphenyl)ethanone illustrates this as well as the sequential use of alkylation and acylation reactions:



Chemists are inclined to give names to reactions that associate them either with their discoverers or with the products they give. This practice can be confusing because many named reactions (or "name reactions") which once were thought to be quite unrelated, have turned out to have very similar mechanisms. Thus we have two closely related acylation reactions: one is the Friedel-Crafts ketone synthesis, in which the electrophile is $\mathbf{R-\overset{\oplus}{C}=O}$; and the other is the **Gattermann-Koch aldehyde synthesis**, in which the electrophile is $\mathbf{H-\overset{\oplus}{C}=O}$:

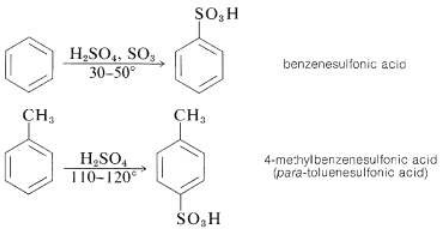


The latter reaction utilizes carbon monoxide and **HCl** under pressure in the presence of aluminum chloride. The electrophile may be considered to be formed as follows:

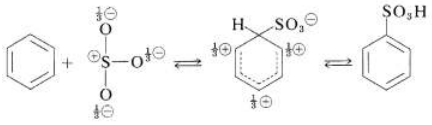
$$\mathbf{C=O + HCl + AlCl_3 \rightleftharpoons H-\overset{\oplus}{C}=O + \overset{\ominus}{AlCl_4}} \qquad (4.5.13)$$

4-5G Sulfonation

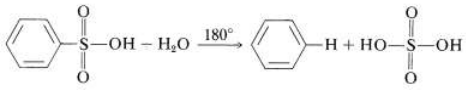
Substitution of the sulfonic acid ($\mathbf{-SO_3H}$) group for a hydrogen of an aromatic hydrocarbon can be carried out by heating the hydrocarbon with a slight excess of concentrated or fuming sulfuric acid:



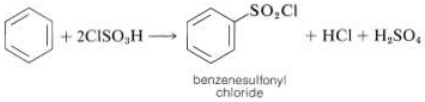
The actual sulfonating agent normally is the SO_3 molecule, which, although neutral, has a powerfully electrophilic sulfur atom:



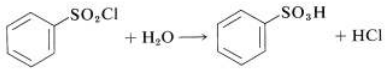
Sulfonation is reversible and the $-SO_3H$ group can be removed by hydrolysis at 180°:



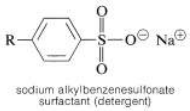
A useful alternative preparation of sulfonyl derivatives is possible with chlorosulfonic acid:



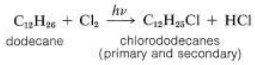
This procedure has an advantage over direct sulfonation in that sulfonyl chlorides usually are soluble in organic solvents and may be easily separated from the reaction mixture. Also, the sulfonyl chloride is a more useful intermediate than the sulfonic acid, but can be converted to the acid by hydrolysis if desired:



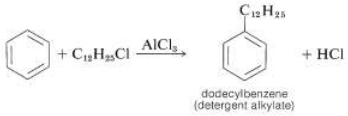
Sulfonation is important in the commercial production of an important class of detergents - the sodium alkylbenzenesulfonates:



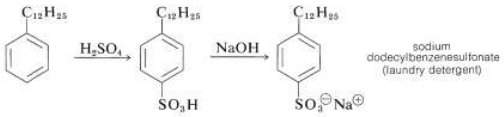
The synthesis illustrates several important types of reactions that we have discussed in this and previous chapters. First, the alkyl group R usually is a C_{12} group derived from the straight-chain hydrocarbon, dodecane, which on photochlorination gives a mixture of chlorododecanes:



This mixture of chlorododecanes is used to alkylate benzene, thereby giving a mixture of isomeric dodecylbenzenes, called *detergent alkylate*:



Sulfonation of the detergent alkylate gives exclusively the 4-dodecylbenzenesulfonic acids, which with sodium hydroxide form water-soluble dodecylbenzenesulfonates:

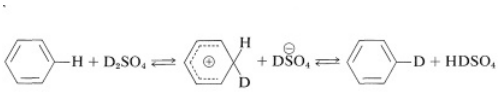


In many countries it is prohibited by law to market detergents of this type, which have highly branched alkyl groups. The reason is that quaternary carbons and, to a lesser extent, tertiary carbons are not degraded readily by bacteria in sewage treatment plants:



4-5H Hydrogen Exchange

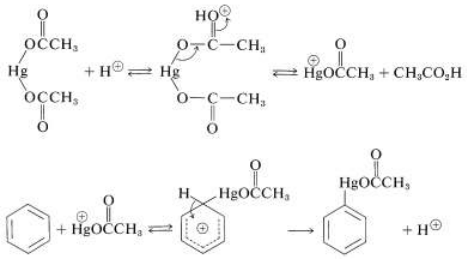
It is possible to replace the ring hydrogens of many aromatic compounds by exchange with strong acids. When an isotopically labeled acid such as $\mathbf{D_2SO_4}$ is used, this reaction is an easy way to introduce deuterium. The mechanism is analogous to other electrophilic substitutions:



Perdeuteriobenzene[3] can be made from benzene in good yield if a sufficiently large excess of deuteriosulfuric acid is used. Deuteration might appear to be competitive with sulfonation, but deuteration actually occurs under much milder conditions.

4-5I Aromatic Substitution by Electrophilic Metalation

Because metals are electropositive elements they can be considered potential electrophiles. Their reactions with arenes have been investigated most thoroughly for mercury. Benzene can be substituted with $\mathbf{HgX^{\oplus}}$ derived from a mercuric salt, $\mathbf{HgX_2}$, in the presence of an acid catalyst. The salt most commonly used is mercuric ethanoate, $\mathbf{Hg(OOCCH_3)_2}$. The catalyst is considered to function by assisting the generation of the active electrophile, $\mathbf{HgX^{\oplus}}$. Other metals that may be introduced directly into an aromatic ring in this manner include thallium and lead.



[3]The prefix *per*, as in perdeuterio- or perfluoro-, means that *all* the hydrogens have been replaced with the named substituent, **D** or **F**. Perhydro means saturated or fully hydrogenated.

Contributors and Attributions

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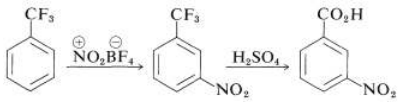
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4.6: Effect of Substituents on Reactivity and Orientation in Electrophilic Aromatic Substitution

In planning syntheses based on substitution reactions of substituted benzenes, it is imperative to be able to predict in advance which of the available positions of the ring are likely to be most reactive. This is now possible with a rather high degree of certainty, thanks to the work of many chemists during the past 100 years. Few, if any, other problems in organic chemistry have received so much attention over so many years, and there are now sufficient data on the orientation and reactivity effects of ring substituents in electrophilic substitution to permit the formation of some very valuable generalizations.

Basically, three experimental problems are involved in the substitution reactions of aromatic compounds: (1) proof of structure of the isomers that are formed; (2) determination of the percentage of each isomer formed, if the product is a mixture; and (3) measurement of the reactivity of the compound being substituted relative to some standard substance, usually benzene.

For benzenoid compounds, structures can be established by the historically important substitution method (Section 1-1F) or with the aid of correlations between spectroscopic properties and positions of substitution, as we indicated in Section 4-3. Also, it is often possible to identify the isomers by converting them to compounds of known structure. For example, trifluoromethylbenzene on nitration gives only one product, which has been shown to be the 3-nitro derivative by conversion to the known 3-nitrobenzoic acid by concentrated sulfuric acid:

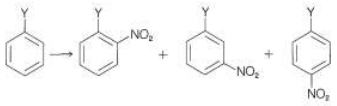


The ratios of isomers formed in substitution reactions can be determined by spectroscopic means or by the analytical separation methods discussed in Section 9-2. We mainly are concerned in this chapter with the reactivity and orientation observed in aromatic substitution.

4-6A The Pattern of Orientation in Aromatic Substitution

The reaction most studied in connection with the orientation problem is nitration, but the principles established also apply for the most part to the related reactions of halogenation, sulfonation, alkylation, and acylation. Some illustrative data for the nitration of a number of mono-substituted benzene derivatives are given in Table 4-6. The table includes the percentage of ortho, meta, and para isomers formed, along with their reactivities relative to benzene. We see that there is a wide range of reactivity according to the nature of the substituent, and that the ortho, meta, and para positions are *not* equally reactive. Although these substituent effects may appear complex, they are related closely to substituted alkenes (Section 10-4), as will be explained in the following section.

Table 4-6: *Orientation and Rate Data for Nitration of Some Monosubstituted Benzene Derivatives*[a]



	Orientation			
Substituent, Y	% ortho	% meta	% para	Relative reactivity
—H	–	–	–	1
—CH_3	56.5	3.5	40	24
—$C(CH_3)_3$	12.0	8.5	79.5	15.7
—CH_2Cl	32.0	15.5	52.5	0.302
—Cl	29.6	0.9	68.5	0.033
—Br	36.5	1.2	62.4	0.030
—NO_2	6.4	93.2	0.3	$\sim 10^{-7}$
—$CO_2C_2H_5$	28.3	68.4	3.3	0.003
—CF_3		100		
—$\overset{\oplus}{N}(CH_3)_3$		89	11	

[a]The data are representative but will vary to some extent with the reaction conditions and nature of the substituting agent.

4-6B Electronic Effects

It is helpful to construct an energy diagram for substitution by an electrophilic $\mathbf{X^{\oplus}}$ of a benzene derivative, $\mathbf{C_6H_5Y}$, in which **Y** is a substituent group (Figure 4-8). The rate of substitution at any one position (we have arbitrarily chosen in Figure 4-8 to compare the 3 and 4 positions) will depend on the height of the energy barrier between the reactants and the transition state. Effects that act to lower the heights of the barriers increase the rates of substitution. Because the transition state and the positively charged intermediate for aromatic substitution have much the same energy, any effect that stabilizes this intermediate is likely to lower the energy of the transition state and increase the rate of substitution. Thus under conditions of *kinetic control* the preferred arene substitution product, as in alkene addition, will be that derived from the most stable of the possible intermediates. Therefore the problem of predicting relative rates and orientation in aromatic substitution becomes one of deciding what factors are likely to stabilize or destabilize the various possible intermediates relative to one another and to the ground state.

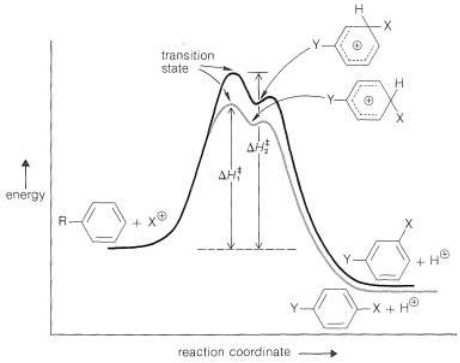
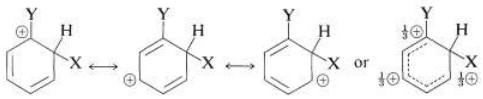


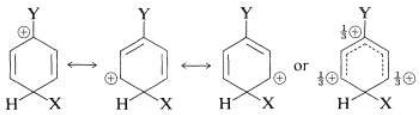
Figure 4-8: *Energy diagram for the substitution of a compound* $\mathbf{C_6H_5Y}$ *in the 3 and 4 positions. It is assumed here that the relative rates are determined by differences in* $\Delta H^{\ddagger}$ *and not in* $\Delta S^{\ddagger}$*. Because* $\Delta H_1^{\ddagger}$ *is less than* $\Delta H_2^{\ddagger}$*, substitution to give the 4-isomer is "kinetically preferred".*

We now can examine the structures of the three substitution intermediates with a view to deciding how the substituent might affect their stability. According to the valence-bond method, the positive charge in the ring is dispersed mainly on alternate carbons, as shown below.

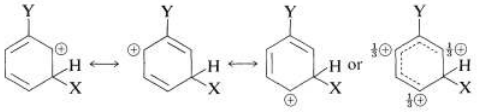
ortho substitution



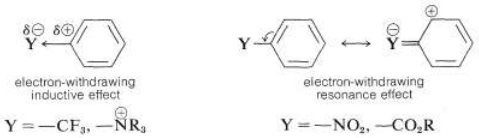
para substitution



meta substitution



The substituent **Y** should (and does) exert its electronic influence more strongly from the ortho and para positions than from the meta position because **Y** in the ortho and the para positions is close to a positively charged ring carbon. This electronic influence will be stabilizing if **Y** has a net electron-donating effect, and destabilizing if **Y** is electron withdrawing. A group can withdraw electrons relative to hydrogen if it is more electronegative than hydrogen and this is called the electron-withdrawing inductive effect (also see Section 18-2B). A group also can withdraw electrons by the **resonance effect**:



Accordingly, substituents fall into one of the following categories.

Meta-directing substituents

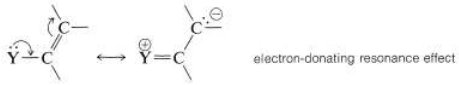
A ring substituent **Y** that is electron withdrawing relative to hydrogen and has no capacity to donate electrons by a resonance effect will *decrease* the reactivity of C_6H_5Y, especially at the ortho and para positions. The result is a sluggish reaction (**deactivation**) with substitution occurring preferentially at the meta position. Substituents in this category are $-NO_2$, $-CF_3$, $-CO_2R$, $-\overset{\oplus}{N}R_3$, and so on (also see Tables 4-5 and 4-6). No groups are known that direct the electrophile to the meta position and, at the same time, make the phenyl derivative *more* reactive relative to benzene.

Table 4-6: *Orientation and Reactivity Effects of Ring Substituents*

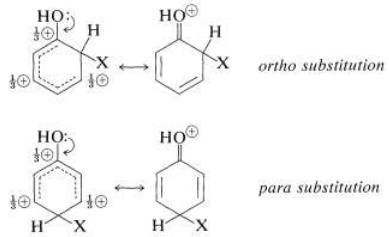
ortho-para-Orientation, with activation	ortho-para-Orientation, with deactivation	meta-Orientation, with deactivation	
—OH	$-CH_2Cl$	$-NO_2$	$-SO_3H$
$-O^{\ominus}$	—F	$-\overset{\oplus}{N}H_3$	$-SO_3R$
—OR	—Cl	$-\overset{\oplus}{N}R_3$	$-CO_2H$
$-OC_6H_5$	—Br	$-\overset{\oplus}{P}R_3$	$-CO_2R$
$-NH_2$	—I	$-\overset{\oplus}{S}R_2$	$-CONH_2$
$-NR_2$	$-CH{=}CHNO_2$	$-\overset{\oplus}{I}C_6H_5$	—CHO
$-NHCOCH_3$		$-CF_3$	—COR
—alkyl (e.g., CH_3)		$-CCl_3$	—C≡N
—aryl (e.g., C_6H_5)			

Ortho-para directing substituents

1. A ring substituent, $-Y$, that has an electron pair on the atom adjacent to the ring gives ortho-para substitution in preference to meta substitution. The reason is that the intermediate can be stabilized by an electron-donating resonance effect from **Y** that is effective from the ortho and para positions only:



This effect is made clear in the valence-bond structures for the ortho-para substitution intermediates from benzenol (phenol):



Substituents of the type $-Y$ include $-OH$, $-OR$, $-SR$, $-NH_2$, and halogens. Most of these groups also are electron withdrawing by an inductive effect that opposes their resonance effect. However, as we saw in the case of alkene additions (Section 10-4C), even when $-Y$ is an electronegative group, stabilization of the intermediate cation by donation of unshared electrons of **Y** to the adjacent positive carbon more than compensates for the polar electron-withdrawing properties of **Y**. Electron donation thus controls the orientation. If, however, the group is *strongly* electron withdrawing (e.g., $-Y = -F$, $-Cl$, $-Br$, $-I$), the reactivity of the compound C_6H_5Y may be reduced. Groups of this kind are ortho-para directing with deactivation.

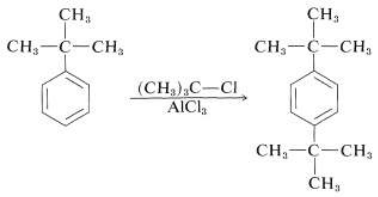
But if the polar effect is not pronounced, then substitution can be powerfully assisted by the substituent. This is ortho-para direction with activation and is provided by groups such as $-OH$, $-OR$, $-SR$, and $-NH_2$. A more comprehensive list of substituents and their orientation effects is provided in Table 4-6.

2. When no important π-electron effect is possible, as with alkyl groups, the orientation effect of a substituent is controlled by its polar effect and the degree to which it polarizes the bonding electrons of the ring. Alkyl groups actually are electron donating and therefore are ortho-para directing with activation.



4-6C Steric Effects

Thus far we have made no distinction between the reactivities of the ortho and the para positions, yet they clearly are not equal. If they were equal, the ortho:para ratio would be 2:1, thereby reflecting the fact that there are two ortho positions but only one para position in monosubstituted benzenes. Most substitution reactions favor the para product, sometimes by a considerable amount (see Table 4-6). A reasonable explanation is that ortho substitution is subject to steric hindrance between the substituent and the entering group. *tert*-Butylbenzene, for example, gives much less ortho nitration than methylbenzene (Table 4-6), thereby suggesting that the size of the substituent is important. Also, *tert*-butylbenzene gives no ortho alkylation with *tert*-butyl chloride, suggesting that the size of the entering group is also important:



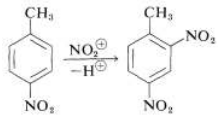
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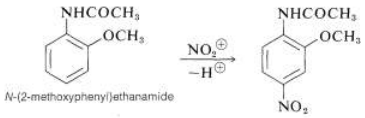
4.6: Effect of Substituents on Reactivity and Orientation in Electrophilic Aromatic Substitution is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

4.7: Orientation in Disubstituted Benzenes

The orientation and reactivity effects of substituents discussed for the substitution of monosubstituted benzenes also hold for disubstituted benzenes, except that the directing influences now come from two groups. Qualitatively, the effects of the two substituents are additive on the reactivity. We therefore would expect 4-nitromethylbenzene to be less reactive than methylbenzene because of the deactivating effect of a nitro group. Also, the most likely position of substitution should be, and is, ortho to the methyl group and meta to the nitro group:



When the two substituents have opposed orientation effects, it is not always easy to predict what products will be obtained. For example, N-(2-methoxyphenyl)ethanamide has two powerful *o*,*p*-directing substituents, $-OCH_3$ and $-NHCOCH_3$. Nitration of this compound gives mainly the 4-nitro derivative, which indicates that the $-NHCOCH_3$ exerts a stronger influence than $-OCH_3$:



Seemingly anomalous effects of substituents are known, but such effects may be due to equilibrium control. One example is the aluminum chloride-catalyzed alkylation of benzene, which leads to the formation of a 1,3,5-trialkylbenzene in preference to the expected 1,2,4-isomer (see Section 22-4E). The preferred reaction occurs particularly readily because alkylation is reversible and because alkylation is one of the least selective of the electrophilic aromatic substitutions (considerable meta isomer is formed even under conditions where kinetic control is dominant). Equilibrium control, which favors the 1,3,5-product rather than the less stable 1,2,4-product, becomes most evident when the reaction time, the reaction temperature, and aluminum chloride concentration are increased. Another source of anomalous substituent effects is discussed in the next section.

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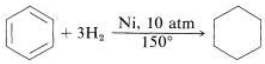
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4.7: Orientation in Disubstituted Benzenes is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

4.8: Addition Reactions of Arenes

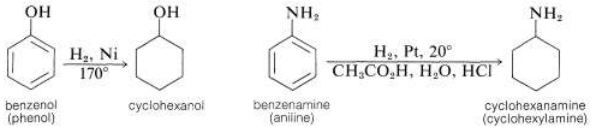
4-8A Catalytic Hydrogenation

Benzenoid compounds are not readily converted to cyclohexane derivatives. Nevertheless, several addition reactions are carried out on an industrial scale. Mention was made previously of the hydrogenation of benzene to cyclohexane in the presence of a nickel catalyst:

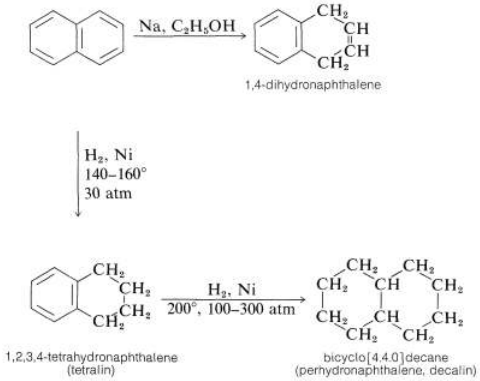


The reaction is very important because cyclohexane is used widely as a solvent and also is oxidized to cyclohexanone, and important intermediate in the synthesis of hexanedioic (adipic) acid and azacycloheptan-2-one (caprolactam), which are used in the preparation of nylon (Section 24-3C).

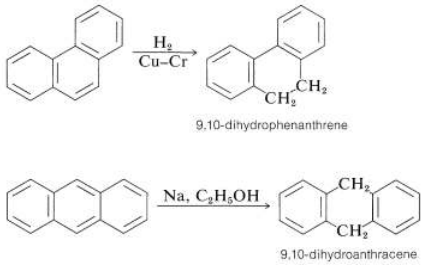
Other cyclohexyl compounds are obtained by catalytic hydrogenation of the corresponding benzene derivatives. Thus cyclohexanol is obtained from benzenol, and cyclohexanamine is obtained from benzenamine (aniline):



Naphthalene can be reduced more easily than benzene. With sodium in alcohol, 1,4-dihydronaphthalene is formed. Catalytic hydrogenation gives tetralin (1,2,3,4-tetrahydronaphthalene). Further reduction to give perhydronaphthalene (decalin) can be achieved on prolonged catalytic hydrogenation at relatively high temperatures and pressures:

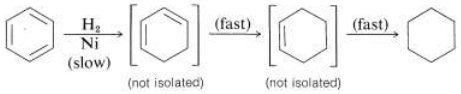


Phenanthrene and anthracene are reduced readily to the dihydro level by addition to the 9,10 positions. Further reduction of the terminal benzene rings is relatively difficult:

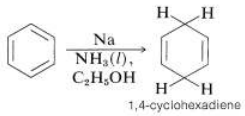


4-8B Reduction of Arenes with Metals

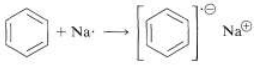
Catalytic hydrogenation of benzene cannot be stopped at cyclohexane or cyclohexadiene; it proceeds to cyclohexane. This is because the rate of the first addition step is much slower than of the subsequent steps:



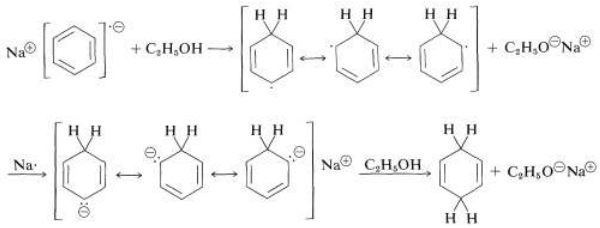
However, benzene and its derivatives can be reduced to cyclohexadienes by solutions of metals such as **Li**, **Na**, **K**, **Zn**, and **Hg** in a weakly acidic solvent, such as liquid ammonia, amines, or ether-alcohol mixtures. This general type of reaction is known as the **Birch reduction** after the Australian chemist, A. J. Birch. With benzene, reduction with metals leads to 1,4-cyclohexadiene:



The initial step of the Birch reduction is an electron transfer to the lowest unoccupied molecular orbital of the benzene π system (see Figure 21-5) to form a radical anion:

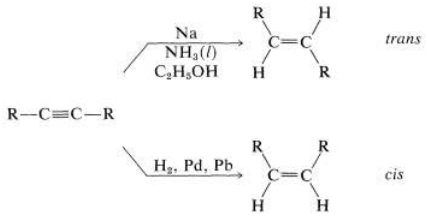


Subsequent steps include a sequence of proton- and electron-transfer steps as follows:



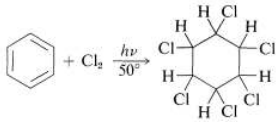
Substituent effects observed for this reaction are entirely consisent with those described for electrophilic substitution and addition - only reversed. That is, the reactivity of an arene in metal reductions is increased by electron-withdrawing groups and decreased by electron-donating groups. Substituents that can stabilize the anion-radical intermediate facilitate the reduction.

Reduction with metals in weakly acidic solvents is not restricted to arenes. A useful related reaction reduces alkynes to *trans*-alkenes, and provides a useful alternative to catalytic hydrogenation, which favors formation of *cis*-alkenes (Section 11-2A):

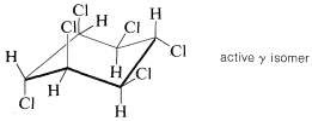


4-8C Halogen Addition

Benzene will add chlorine on irradiation with light to give the fully saturated hexachlorocyclohexane as a mixture of stereoisomers:

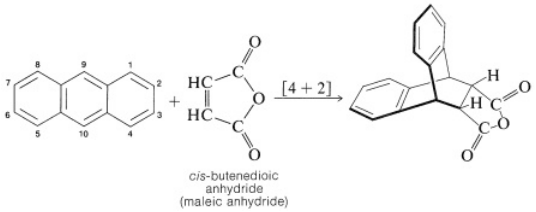


The reaction is commercially important because one of the isomers is a potent insecticide. The product is marketed as a mixture of isomers in which the active isomer (γ) is optimally about **40%** by weight. It has a variety of trade names: Fortified, BHC, Lindane, Gammexane, Hexachlor.

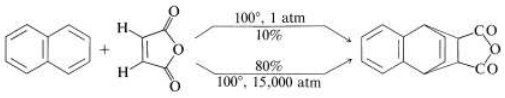


4-8D Cycloaddition

In Chapter 13 we encountered the Diels-Aler reaction, which involves addition of a reactive alkene (dienophile) to the 1,4 positions of a conjugated diene. Neither benzene nor naphthalene reacts significantly with dienophiles on simple heating, but anthracene does react. Cycloaddition occurs between the 9,10 positions:



Reactions in which the transition state has a *smaller* volume than the reactants are speeded up by an increase in pressure. This is the case with naphthalene and *cis*-butenedioic anhydride. An **80%** yield of adduct is obtained at **100°** at 15,000 atmospheres pressure, whereas at one atmosphere and **100°**, the yield is only **10%**.



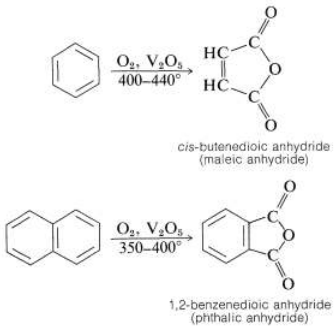
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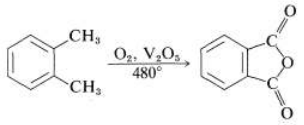
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4.9: Oxidation Reactions

The reagents usually employed for the oxidation of alkenes (e.g., CrO_3, $KMnO_4$, H_2O_2, OsO_4) normally do not attack benzene. At high temperatures, benzene can be oxidized to *cis*-butenedioic (maleic) anhydride by air with a vanadium pentoxide catalyst. Naphthalene can be similarly oxidized to 1,2-benzenedioic (phthalic) anhydride:

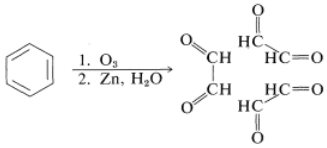


Both anhydrides are prepared in this manner on a large scale for use in the production of ester polymers (Section 29-5A). Phthalic anhydride also is prepared by the oxidation of 1,2-dimethylbenzene:

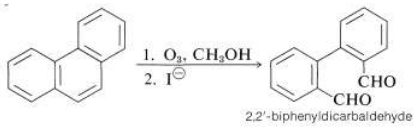


Phthalic anhydride is used to make anthraquinone and to make esters of phthalic acid, which are used widely to plasticize polymers.

Ozonization of aromatic hydrocarbons is possible. Benzene itself gives ethanedial (glyoxal):



The double-bond character of the 9,10 bond in phenanthrene is particularly evident in ozonization. This bond is attacked preferentially, which leads to the formation of a dialdehyde when the ozonide is reduced with iodide ion:



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4.10: Sources and Uses of Aromatic Hydrocarbons

Benzene and many of its derivatives are manufactured on a large scale for use in high-octane gasolines and in the production of polymers, insecticides, detergents, dyes, and many miscellaneous chemicals. Prior to World War II, coal was the only important source of aromatic hydrocarbons, but during the war and thereafter, the demand for benzene, methylbenzene, and the dimethylbenzenes rose so sharply that other sources had to be found. Today, most of the benzene and almost all of the methylbenzene and the dimethylbenzenes produced in the United States are derived from petroleum.

Coal tar, which is a distillate obtained in the coking of coal (Section 4-2), is a source of an amazing number of aromatic compounds. Some of these are listed in Table 4-7, which includes nitrogen, oxygen, and sulfur compounds, as well as hydrocarbons. Although petroleum from some locations contains fairly substantial amounts of aromatic hydrocarbons, it is not a principal source for such compounds. Rather, aromatic compounds are synthesized from the C_6-C_{10} gasoline fraction from petroleum refining by a process referred to in the petroleum industry as **catalytic re-forming** or **hydroforming**. This involves heating a C_6-C_{10} fraction with hydrogen in the presence of a catalyst to modify the molecular structure of its components. Some amazing transformations take place, and the C_6-C_7 alkanes can be converted to cycloalkanes, which, in turn, are converted to arenes. Benzene, methylbenzene (toluene), and the dimethylbenzenes (xylenes) are produced primarily in this way:

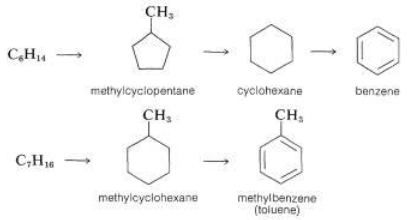
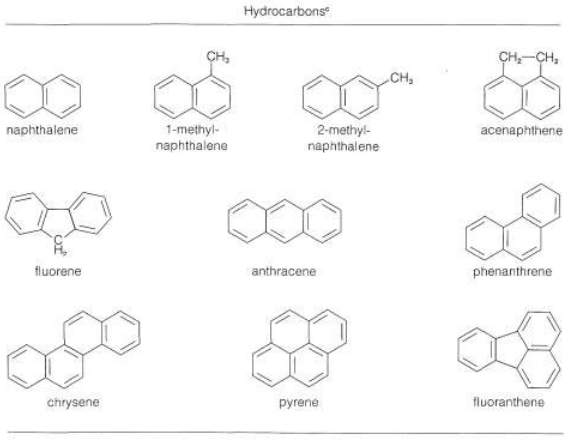
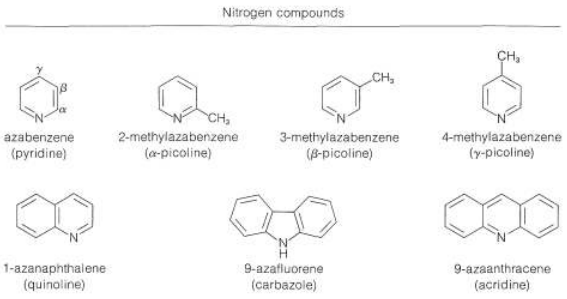
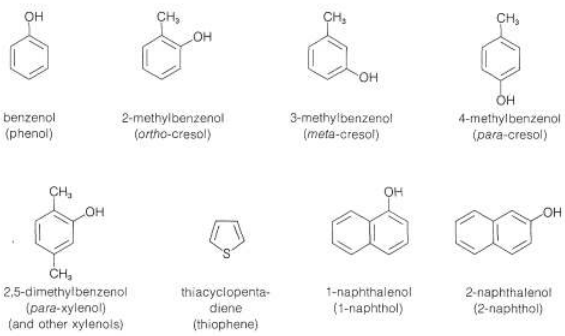


Table 4-7: *Principal Compounds Obtained from Coal Tar*[a,b]





Oxygen and sulfur compounds



[a]Compiled from *Chemistry of Coal Utilization*, National Research Council Committee, H. H. Lowry (Ed.), John Wiley and Sons, Inc., 1945.

[b]None of the compounds listed are very abundant in coal tar. Even naphthalene, which is present in the largest amount, constitutes only 10–11% of coal tar.

[c]Benzene and the mono- and dimethylbenzenes are present in very small amount (0.3% total) in coal tar.

Much of the aromatic product obtained by catalytic re-forming is blended with other fractions from petroleum refining to give high-octane gasoline. The rest is separated into its component hydrocarbons, which then are utilized by the chemical industry for the production of chemicals derived from benzene, methylbenzene, and the dimethylbenzenes, as summarized in Figures 4-9 and 4-10.

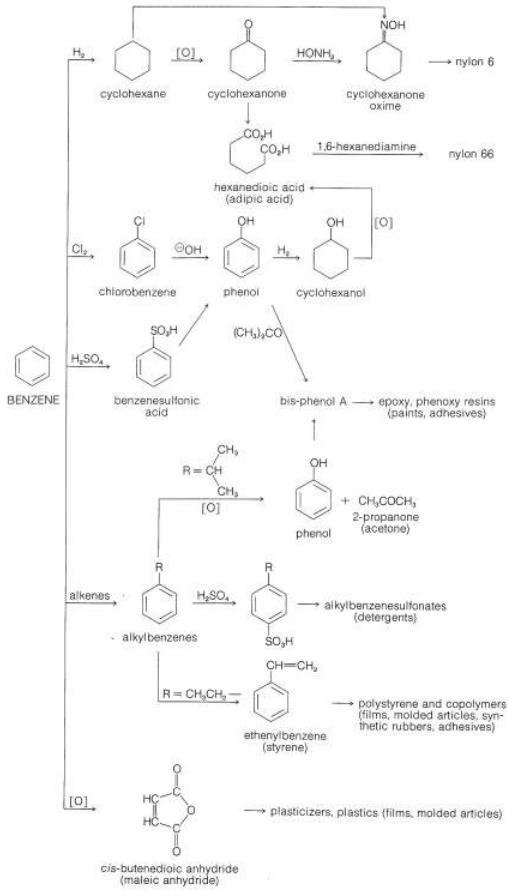


Figure 4-9: *Important chemicals derived from benzene.*

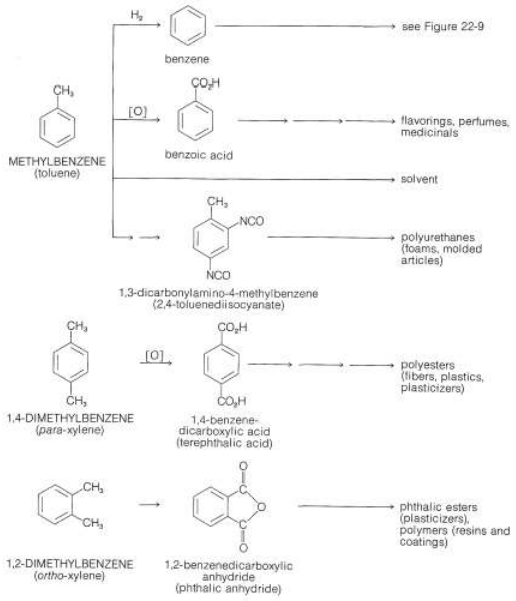


Figure 4-10: *Important chemicals derived from methyl-substituted benzenes.*

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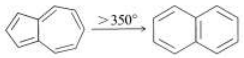
4.11: Some Conjugated Cyclic Polyenes

4-11A Azulene

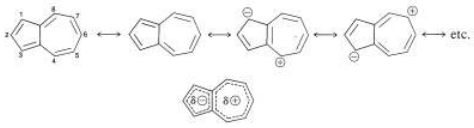
There are several compounds that possess some measure of aromatic character typical of benzene, but do not possess a benzenoid ring. Appropriately, they have $(4n + 2)$ π electrons and are classified as nonbenzenoid aromatic compounds (Section 21-9). An example is azulene, which is isomeric with naphthalene and has a five- and a seven-membered ring fused through adjacent carbons:



As the name implies, it is deep blue. It is less stable than naphthalene, to which it isomerizes quantitatively on heating above $350°$ in the absence of air:

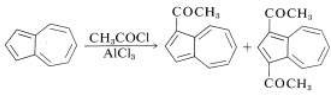


Azulene has a significant polarity, with the five-membered ring negative and the seven-membered ring positive. The structure can be represented as a hybrid of neutral and ionic structures:

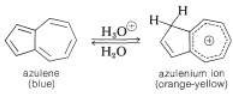


The polarization that has the five-membered ring negative and the seven-membered ring positive corresponds to ionic structures that have *six* (i.e., $4n + 2$) electrons in both the five- and seven-membered rings (Section 21-9B).

In keeping with its aromatic character and unsymmetrical charge distribution, azulene undergoes certain typical electrophilic substitution reactions at the 1 and 3 positions. Thus Friedel-Crafts acylation leads to a mixture of 1-ethanoylazulene and 1,3-diethanoylazulene:

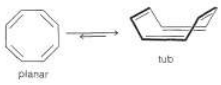


Furthermore, in the presence of strong acids the 1 position is protonated to give a derivative of the relatively stable cycloheptatrienyl (tropylium) ion (Section 21-9B):



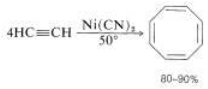
4-11B Cyclooctatetraene

1,3,5,7-Cyclooctatetraene (or simply cyclooctatetraene) is a bright-yellow, nonplanar, nonaromatic compound (Section 21-9A). Apparently the resonance energy of a planar structure is insufficient to overcome the unfavorable angle strain of a planar structure would have with its C—C—C bond angles of $135°$. Cyclooctatetraene normally assumes a "tub" structure with alternating single and double bonds:



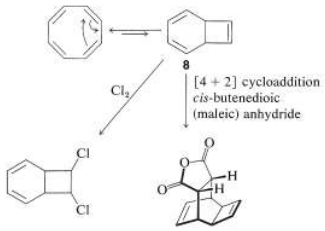
There is, however, nmr evidence that indicates that the tub form is in rapid equilibrium with a very small amount of the planar form at room temperature. There is about a 15-kcal mol^{-1} energy difference between the two forms. The dication, $C_8H_8^{2+}$, and the dianion of cyclooctatetraene, $C_8H_8^{2-}$, which have $(4n + 2)$ π electrons, appear to exist in planar conformation.

Cyclooctatetraene can be prepared readily by polymerization of ethyne in the presence of nickel cyanide:

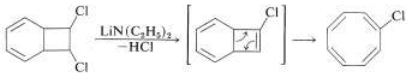


It could be manufactured on a large scale, but no large-scale commercial uses of the substance have yet been developed.

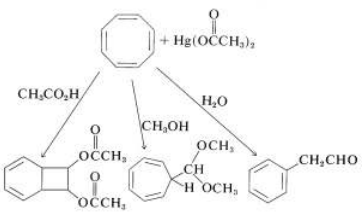
The chemistry of cyclooctatetraene is interesting and unusual. Particularly noteworthy is the way in which it undergoes addition reactions to form products that appear to be derived from the bicyclic isomer, bicyclo[4.2.0]2,4,7-octatriene, **8**. In fact, there is an electrocyclic equilibrium between cyclooctatetraene and **8** (Section 21-10D) and, although the position of equilibrium lies far on the side of cyclooctatetraene, **8** is more reactive and leads to the observed addition products:



Treatment of the bridged dichloride with strong bases causes elimination of hydrogen chloride and formation of chlorocyclooctatetraene:



The diverse ways in which cyclooctatetraene can react with a give reagent under different conditions is well illustrated by the variety of products obtained with mercuric ethanoate in ethanoic acid, methanol, and water:



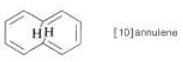
Efforts to prepare "pentalene", a bridged analog of cyclooctatetraene, have not been very successful so far. A substance that appears to be a methylpentalene has been characterized at **−180°** by its spectral properties. On warming to **−105°** it forms a dimer.



4-11C Annulenes

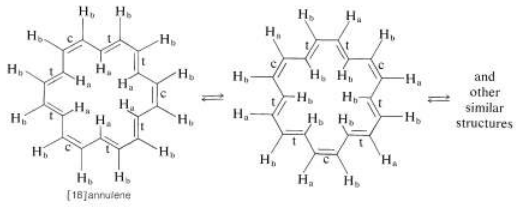
Cyclooctatetraene is nonplanar. One reason is that the angle strain is severe in the planar form. Is it possible that larger-ring conjugated polyalkenes may have strainless planar structures? Models show that a strainless structure can be achieved with two or more of the double bonds only in *trans* configurations, and then only with a large enough ring that the "inside" hydrogens do not interfere with one another.

In discussing compounds of this type, it will be convenient to use the name *[n]annulene* to designate the simple conjugated cyclic polyalkenes, with *n* referring to the number of carbons in the ring - benzene being [6]annulene. The simplest conjugated cyclic polyolefin that could have a strainless planar ring containing trans double bonds, except for interferences between the inside hydrogens, is [10]annulene:



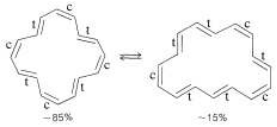
Inside-hydrogen interferences are likely to be of some importancee in all annulenes up to [30]annulene. Many annulenes have been synthesized by F. Sondheimer.

We have mentioned already (Section 22-3C) the large differences in nmr chemical shifts between the inside and outside hydrogens of [18]annulene - a substance which with 18 π electrons should be aromatic by the $4n + 2$ rule. These differences are observed only at low temperatures. The proton nmr spectrum of [18]annulene at room temperature is a single resonance, which indicates that the inside (H_a) and outside (H_b) hydrogens are equilibrating rapidly. This can take place only if cis-trans interconversion occurs about the double bonds (marked *c* and *t*):



At low temperatures, this equilibrium is slow enough that separate groups of resonances for the inside and outside hydrogens can be discerned in an nmr experiment (see Sections 9-10C and 27-2).

A theoretical prediction that has been borne out by experiment is that an annulene with $4n$ π electrons should have a *paramagnetic* circulation of electrons - that is, opposite in direction to that shown in Figure 22-4 for benzene. For example, [16]annulene, which has $4n$ electrons, is not very stable and exists as a very rapidly interconverting mixture of two configurational isomers:



At very low temperatures $(-155°)$, the proton nmr spectrum shows the *inner* hydrogens at δ12.9-10.5 and the outer hydrogens at δ5.7 -6.4, which is in exactly the opposite order to the shifts with [18]annulene and the other known $[4n + 2]$ π-electron annulenes.

The annulenes generally are not stable compounds, but the $[4n + 2]$annulenes clearly show typical aromatic reactions. For instance [18]annulene has been converted to the nitro, ethanoyl, bromo, and carbaldehyde derivatives by electrophilic substitution reactions.

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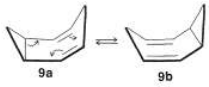
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4.12: Fluxional Compounds

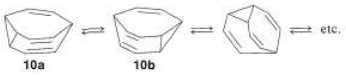
A number of compounds are known to rearrange from one structure to an entirely equivalent structure, sometimes with extraordinary facility. Such compounds are said to be **fluxional** to distinguish from tautomers (which usually involve rearrangements between nonequivalent structures). Simple examples are the Cope rearrangement of 1,5-hexadiene,



and the electrocyclic rearrangement of bicyclo[5.1.0]-2,5-octadiene,



If the two methylenes of **9** are bridged with two double-bonded carbons, we get the remarkable structure **10**, called "bullvalene", which rapidly interconverts amongst equivalent structures:



Equilibration of fluxional molecules must not be confused with resonance. In each electrocyclic reaction, the nuclei alter their positions as bond lengths and angles change. Interconversion of fluxional molecules also must not be confused with conformational changes (as in the interconversion of two equivalent chair forms of cyclohexane). In the interconversion of fluxional molecules, chemical bonds are broken and made; in conformational changes, no bonds are broken and no bonds are made.

> *Equilibration of fluxional molecules must not be confused with resonance.*

A theoretical prediction is that the very-large-ring annulenes, even those with $[4n+2]$ π electrons, may not have sufficient resonance energy to maintain equal bond lengths between the carbons and hence would be most stable with alternating double and single bonds. These substances then would be as Kekule thought benzene to be - a fluxional, equilibrating mixture of cyclohexatrienes! This does *not* mean that there would be no π-electron delocalization in fluxional cyclic polyenes; it means only that the VB structures would *not* be exactly equivalent and the MO model would have a σ-bond framework with alternating short and long C–C bonds. The theory suggests that if alternation in bond lengths occurs, then neither diamagnetic nor paramagnetic circulation of the π electrons should be important. The synthesis and study of [26]- and [30]annulenes seems to bear out this prediction, in that with these substances there appears to be no ring-current effect on the proton chemical shifts.

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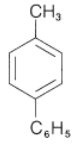
4.P: Arenes, Electrophilic Aromatic Substitution (Problems)

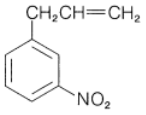
Problem 4-1 How many structurally different monomethyl derivatives are possible for each of the following compounds? Name each.

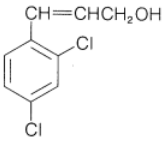
a. naphthalene
b. anthracene
c. phenanthrene

Problem 4-2 How many isomeric products could each of the dimethylbenzenes give on introduction of a third substituent? Name each isomer, using chlorine as the third substituent.

Problem 4-3 Name each of the following compounds by the IUPAC system:

a. $(C_6H_5)_2CHCl$
b. $C_6H_5CHCl_2$
c. $C_6H_5CCl_3$
d. 

e. 

f. 

Problem 4-4 Identify the two compounds with molecular formula C_7H_7Cl from the infrared spectra shown in Figure 4-2.

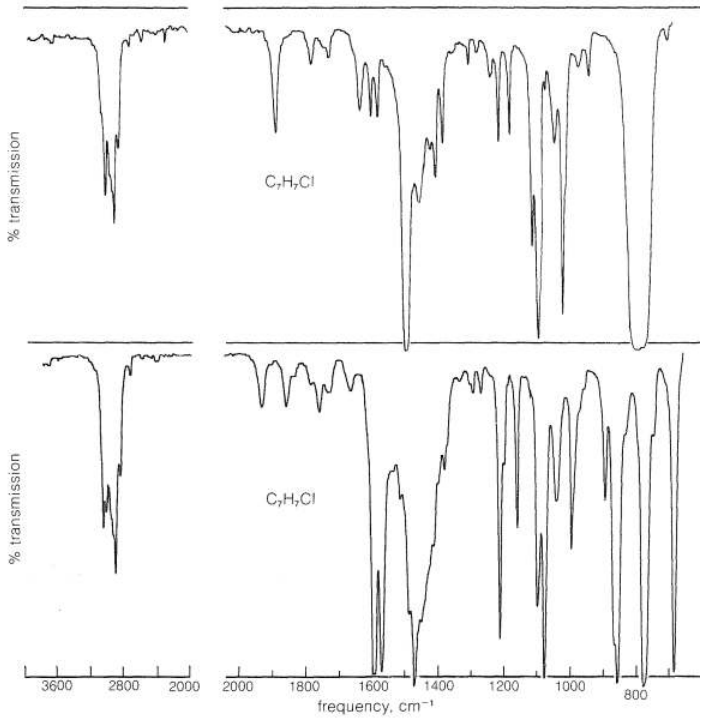
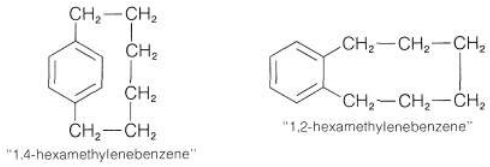


Figure 4-2: Infrared spectra of two isomeric compounds of formula C_7H_7Cl (see Problem 4-4)

Problem 4-5 Predict the effect on the ultraviolet spectrum of a water solution of benzenamine when hydrochloric acid is added. Explain why a solution of sodium benzenoxide absorbs at longer wavelengths than a solution of benzenol (see Table 4-3).

Problem 4-6* Estimate the chemical shifts of the protons of (a) the separate CH_2 of "1,4-hexamethylenebenzene" as compared with "1,2-hexamethylenebenzene"; and (b) cyclooctatetraene (see Section 21-9A).



Problem 4-7 Establish the structures of the following benzene derivatives on the basis of their empirical formulas and NMR spectra shown in Figure 4-6. Remember that equivalent protons normally do not split each other's resonances.

a. C_8H_{10}
b. C_8H_7OCl
c. $C_9H_{10}O_2$
d. C_9H_{12}

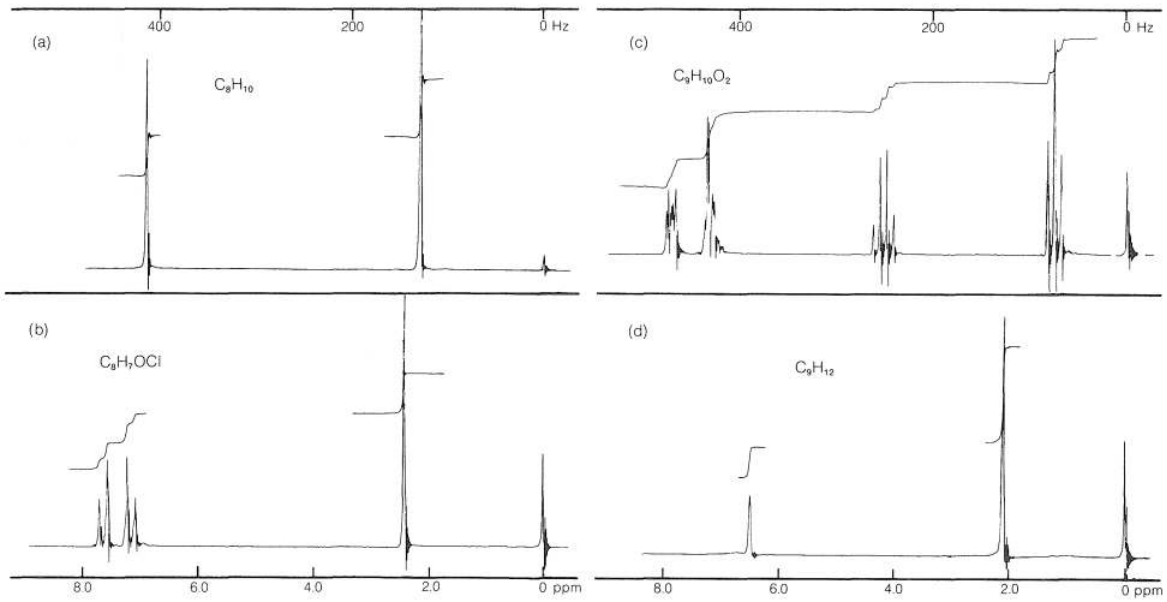
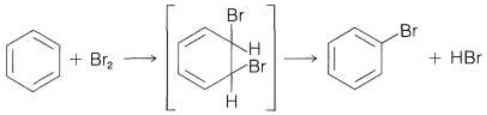


Figure 4-6: Proton NMR spectra of some benzene derivatives at 60 MHz with reference to TMS at 0 ppm (see Problem 4-7).

Problem 4-8 Calculate from appropriate bond-energy and stabilization-energy tables (4-3 and 21-1) the heats of chlorine with benzene to give (a) chlorobenzene and (b) 5,6-dichloro-1,3-cyclohexadiene. Your answer should indicate that substitution is energetically more favorable than addition.

Problem 4-9 Devise an *experimental* test to determine whether the following addition-elimination mechanism for bromination of benzene actually takes place.



Problem 4-10 Why is nitration with ethanoyl nitrate accelerated by added fluoroboric acid, HBF_4, but retarded by added hydrochloric acid?

Problem 4-11 Why do fairly reactive arenes, such as benzene, methylbenzene, and ethylbenzene, react with excess nitric acid in nitromethane solution at a rate that is *independent of the concentration of the arene* (i.e., zero order in arene concentration)? Does this lack of dependencies on arene concentration mean that nitration of an equimolar mixture of benzene and methylbenzene would necessarily give an equimolar mixture of nitrobenzene and nitromethylbenzenes? Why or why not?

Problem 4-12 Reagents, besides the molecular halogens, that effect halogen substitution include hypochlorous and hypobromous acids. They are most effective when a strong acid is present and care is taken to exclude formation of halide ions. Account for the catalytic effect of acid and the anticatalytic effect of halide ions.

Problem 4-13 Arrange the following bromine-containing species in order of their expected reactivity in achieving electrophilic aromatic bromination: $HOBr$, Br_2, $Br^{\oplus}$, $Br^{\ominus}$, HBr, $H_2OBr^{\oplus}$, $BrCl$.

Problem 4-14 Aluminum chloride is a much more powerful catalyst than ferric bromide for bromination of benzene. Would you expect the combination of aluminum chloride and bromine to give much chlorobenzene in reaction with benzene? Explain.

Problem 4-15

a. The bromination of benzene is catalyzed by small amounts of iodine. Devise a possible explanation for this catalytic effect.

b. The kinetic expression for the bromination of naphthalene in ethanoic acid involves a term that is first order in naphthalene and second order in bromine. How can two molecules of bromine and one of naphthalene be involved in the rate-determining step of bromination? Explain why the kinetic expression simplifies to first order in naphthalene and first order in bromine in 50% aqueous ethanoic acid.

Problem 4-16 Write a mechanism for the alkylation of benzene with 2-propanol catalyzed by boron trifluoride.

Problem 4-17 Explain how it is possible that the ratio of products isolated from equilibration of 1,2-, 1,3-, and 1,4-dimethylbenzenes is 18:58:24 if the presence of a small amount of $HF{-}BF_3$, but is essentially 0:100:0 in the presence of excess $HF{-}BF_3$. Notice that HBF_4 is an extremely strong acid.

Problem 4-18 Account for the following observations:

a. 3-Methyl-2-butanol alkylates benzene in HF to give (1,1-dimethylpropyl)benzene.

b. 1-Chloronorbornane will not alkylate in the presence of $AlCl_3$.



1-chloronorbornane

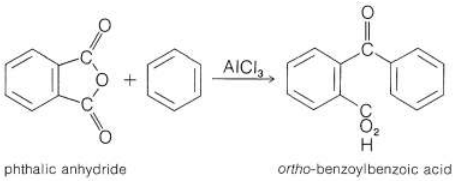
c. 1-Methylcyclopentyl cation is formed from each of the compounds shown below under the indicated conditions at low temperatures (-70°).

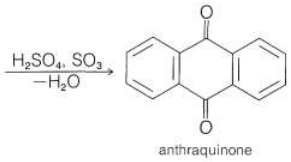
chlorocyclohexane $\xrightarrow{SbF_5{-}SO_2}$

cyclohexene $\xrightarrow{HF{-}SbF_5{-}SO_2}$

cyclohexanol $\xrightarrow{FSO_3H{-}SbF_5}$

Problem 4-19 Anthraquinone can be synthesized from phthalic anhydride and benzene in two steps. The first step is catalyzed by $AlCl_3$, the second by fuming sulfuric acid. Write mechanisms for both reactions and suggest why fuming sulfuric is required in the second step but not in the first.





Problem 4-20 Suggest possible routes for the synthesis of the following compounds:

a. diphenylmethane from benzoic acid and benzene
b. 1-ethyl-4-methylbenzene from methylbenzene

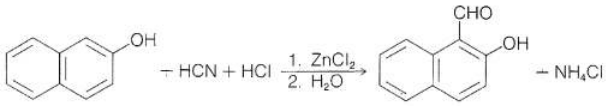
Problem 4-21

a. Substitution of a chloromethyl group, $-CH_2Cl$, on an aromatic ring is **chloromethylation** and is accomplished using methanal, HCl, and a metal-halide catalyst ($ZnCl_2$). Write reasonable mechanistic steps that could be involved in this reaction:

$$C_6H_6 + CH_2O + HCl \xrightarrow{ZnCl_2} C_6H_5CH_2Cl + CH_2O \qquad (4.P.1)$$

b. Phenylmethyl chloride can be formed from benzene and chloromethyl methyl ether, $ClCH_2OCH_3$, in the presence of stannic chloride, $SnCl_4$. Write reasonable mechanistic steps, again supported by analogy, for this reaction. Notice that $SnCl_4$ is a Lewis acid.

Problem 4-22 The **Gattermann reaction** (not to be confused with the Gattermann-Koch aldehyde synthesis) introduces the $H-C=O$ function into reactive aromatic compounds such as 2-naphthalenol. The necessary reagents are HCN, HCl, and a metal-halide catalyst ($ZnCl_2$ or $AlCl_3$), and the initial product must be treated with water. Write a mechanism for this reaction that is supported by analogy to other reactions discussed in this chapter.



Problem 4-23 Show explicitly how an alkyl side chain of alkylbenzenesulfonates could be formed with a quaternary carbon, if the C_{12} alkane used at the start of the synthesis contained any branched-chain C_{12} isomers.

Problem 4-24 Draw the structures of the intermediate cations for nitration of nitrobenzene in the 2, 3, and 4 positions. Use the structures to explain why the nitro group is meta-orienting with deactivation. Use the same kind of arguments to explain the orientation observed with $-CF_3$, $-CHO$, $-CH_2Cl$, and $-NH_2$ groups in electrophilic aromatic substitution (Table 4-6).

Problem 4-25* The product distribution in the bromination of methylbenzene (toluene) depends on the nature of the brominating agent. Pertinent information follows:

reagent	% of isomeric bromomethylbenzenes		
	2-	3-	4-
Br_2	33	<1	67
$H_2OBr^{\oplus}$	58	19	23

Explain why the distribution varies with the nature of the substituting agent. Predict the product distribution of isomeric ions if $Br^{\oplus}$ were to add to methylbenzene in the *gas phase*.

Problem 4-26 Construct an energy diagram, similar to Figure 4-8, for nitration of phenyltrimethylammonium ion in the meta and para positions.

Problem 4-27 Using the rationale developed in Section 4-5, predict the major products of nitration of the following compounds. It will help to work out the Lewis structures of the substituent groups.

a. phenylnitromethane, $C_6H_5CH_2NO_2$
b. methylthiobenzene, $C_6H_5SCH_3$
c. nitrosobenzene, C_6H_5NO
d. phenyldimethylphosphine oxide, $C_6H_5PO(CH_3)_2$

Problem 4-28 The energy diagram in Figure 4-8 represents a two-step reaction in which the first step is slower than the second. This circumstance is found in nitration and halogenation reactions. Show how this diagram would change when (a) the rate-determining step is loss of a proton from the intermediate ion, (b) the reactants rapidly form a π complex prior to the slow step of the electrophilic attack at carbon, and (c) the rate-determining step is π-complex formation.

Problem 4-29 Predict the favored position(s) of substitution in the nitration of the following compounds:

a. 4-nitro-1-phenylbenzene
b. 4-methylbenzenecarboxylic acid
c. 3-methylbenzenecarboxylic acid
d. 1,3-dibromobenzene
e. 1-fluoro-3-methoxybenzene
f. 1,3-dimethylbenzene

Problem 4-30*

a. In the nitration of *para*-cymene by ethanoyl nitrate in ethanoic anhydride, the observed product composition at 0° is 41% 5 and 6, 41% 3, 8% 4, and 10% of 4-nitromethylbenzene. Use these results to determine the relative reactivities of the *para*-cymene ring carbons towards NO_2^+. Give your answer relative to C_3 as unity (C_3 is the carbon next to the isopropyl group). determine the relative reactivities based on the data obtained in Equation 4-1. How does neglect of *ipso* substitution affect calculation of relative reactivities of the ring carbons?

b. Write a mechanism for the solvolytic conversion of **5** and **6** to **3**.

Problem 4-31 Draw the Kekulé-type valence-bond structures for napthalene, anthracene, and phenanthrene. Estimate the percentage of double-bond character for the 9,10 bond of phenanthrene, assuming that each of the valence-bond structures contributes equally to the hybrid structure.

Problem 4-32 Devise an experiment that would establish whether the acylation of naphthalene in the 2 position in nitrobenzene solution is the result of *thermodynamic control* of the orientation.

Problem 4-33 Predict the orientation in the following reactions:

a. 1-methylnaphthalene $+Br_2$
b. 2-methylnaphthalene $+HNO_3$
c. 2-napthalenecarboxylic acid $+HNO_3$

Problem 4-34 Show how one can predict qualitatively the character of the 1,2 bond in acenapthylene.



Problem 4-35* Explain why sodium in liquid ammonia reduces methoxybenzene (anisole) to 1-methoxy-1,4-cyclohexadiene, whereas it reduces sodium benzoate to sodium 2,5-cyclohexadienecarboxylate:



Problem 4-36 Predict the Birch reduction products of the following reactions:

a. anthracene $\xrightarrow[C_2H_5OH]{Na}$

b. naphthalene $\xrightarrow[NH_3(l)]{Na}$

c.* methylbenzene $\xrightarrow[NH_3(l)]{Na,\ C_2H_5OH}$

Problem 4-37* A side reaction when reducing benzene derivatives to 1,4-cyclohexadienes with lithium or sodium in liquid ammonia is over-reduction to give cyclohexenes. Addition of ethanol greatly reduces the importance of this side reaction. Explain what role ethanol plays in preventing over-reduction.

Problem 4-38 Neglecting steric-hindrance effects use the stabilization energies in Table 21-1 to explain why *cis*-butenedioic anhydride adds more readily to anthracene than to benzene and adds across the 9,10 positions but not the 1,4 positions of anthracene.

Problem 4-39 What products would you expect to be formed in the ozonization of the following substances? Consider carefully which bonds are likely to be most reactive.

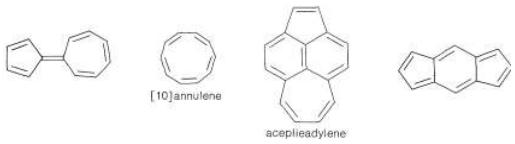
a. 1,2-dimethylbenzene
b. naphthalene
c. acenaphthylene (see Problem 4-34)

Problem 4-40* The rate of the Diels-Alder addition between cyclooctatetraene and tetracyanoethene is proportional to the tetracyanoethene concentration, $[C_2(CN)_4]$, at low concentrations of the addends but becomes independent of $[C_2(CN)_4]$ at high concentrations. Write a mechanism that accounts for this behavior.

Problem 4-41* Write reasonable mechanisms for the different oxidation reactions of cyclooctatetraene with mercuric ethanoate in ethanoic acid, methanol, and water solutions. Notice that compounds of the type $Hg(OR)_2$ appear to act in some cases as $^{\oplus}OR$-donating agents and also that the oxide produced from cyclooctatetraene and peroxyacids (Section 15-11C) rearranges readily in the presence of acids to phenylethanal.

Problem 4-42* The dianion $C_8H_6^{2-}$, which corresponds to pentalene, has been prepared and appears to be reasonably stable. Why may the dianon be more stable than pentalene itself? (See Section 21-9B.)

Problem 4-43* Predict which of the following compounds may have some aromatic character. Give your reasons.



Problem 4-44 Write structural formulas for all of the possible isomers of C_8H_{10} that contain one benzene ring. Show how many different mononitration products each could give if no carbon skeleton rearrangements occur but nitration is possible either in the ring or side chain. Name all of the mononitration products by an accepted system.

Problem 4-45 Write structural formulas (more than one may be possible) for aromatic substances that fit the following descriptions:

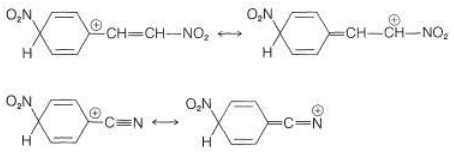
a. C_8H_{10}, which can give only one theoretically possible ring nitration product
b. $C_6H_3Br_3$, which can give three theoretically possible nitration products.
c. $C_6H_3Br_2Cl$, which can give two theoretically possible nitration products.
d. $C_8H_8(NO_2)_2$, which can give only two theoretically possible different ring monobromosubstitution products.

Problem 4-46 Predict the most favorable position for mononitration for each of the following substances. Indicate whether the rate is greater, or less, than for the nitration of benzene. Give your reasoning in each case.

a. fluorobenzene
b. trifluoromethylbenzene
c. phenylethanone
d. phenylmethyldimethylamine oxide, $C_6H_5CH_2\overset{\oplus}{N}(CH_3)_2\overset{\ominus}{O}$
e. diphenylmethane
f. 4-bromo-1-methoxybenzene
g. phenylsulfinylbenzene, $C_6H_5SOC_6H_5$
h. 1-*tert*-butyl-4-methylbenzene
i. diphenyliodonium nitrate, $(C_6H_5)_2\overset{\oplus}{I}\overset{\ominus}{N}$ \ce{O_3}\)
j. 1,3-diphenylbenzene (*meta*-terphenyl)
k. N-(4-phenylphenyl)ethanamide

Problem 4-47 Explain why the bromination of benzenamine (aniline) gives 2,4,6-tribromobenzenamine (2,4,6-tribromoaniline), whereas the nitration with mixed acids gives 3-nitrobenzenamine (*meta*-nitroaniline).

Problem 4-48 Explain how comparison of the following resonance structures for para substitution with the corresponding ones for meta substitution may (or may not) lead to the expectation that ortho-para orientation would be favored for the nitro, cyano, and $-CH{=}CHNO_2$ groups.



Problem 4-49 Starting with benzene, show how the following compounds could be prepared. Specify the required reagents and catalysts.

a. 1-bromo-4-nitrobenzene
b. 4-isopropyl-3-nitrobenzenesulfonic acid
c. 4-*tert*-butylbenzenecarbaldehyde
d. $C_6H_5COCH_2CH_2CO_2H$
e. 1,2,4,5-tetrachlorocyclohexane

Problem 4-50 Offer a suitable explanation of each of the following facts:

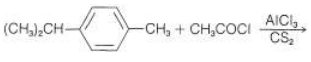
a. Nitration of arenes in concentrated nitric acid is retarded by added nitrate ions and strongly accelerated by small amounts of sulfuric acid.

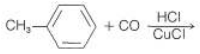
b. Nitrobenzene is a suitable solvent to use in Friedel-Crafts acylation of benzene derivatives.

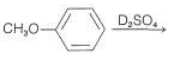
c. Benzene and other arenes usually do not react with *nucleophiles* by either addition or substitution.

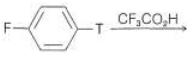
d. Pyridine is almost inert to nitration with mixed nitric and sulfuric acids, a reaction the proceeds readily with benzene.

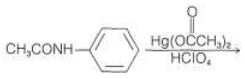
Problem 4-51 Indicate the structures of the major product(s) expected in the following reactions:

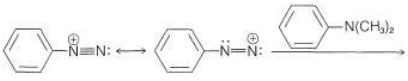
a. 

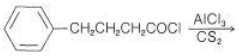
b. 

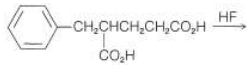
c. 

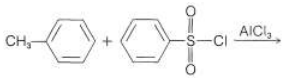
d.  (**T** is ^{3}**H**, or tritium)

e. 

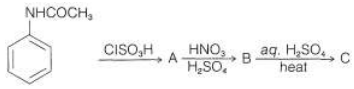
f. 

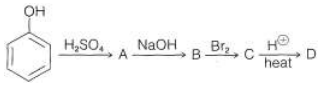
g. 

h. 

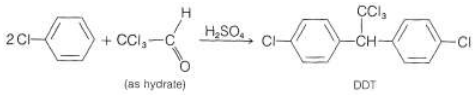
i. 

Problem 4-52 Draw the structures of the products A, B, C, and D in the stepwise reaction sequences shown.

a. 

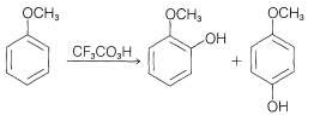
b. 

Problem 4-53 The pesticide DDT is made commercially by the reaction of chlorobenzene with trichloroethanal (chloral) in the presence of an acid catalyst (H_2SO_4). Show the steps that are likely to be involved in this reaction:



Problem 4-54 Hexachlorophene, the controversial germicide, is prepared from 2,4,5-trichlorobenzenol (2 moles) and methanal (1 mole) in the presence of concentrated sulfuric acid. Show the steps involved and the expected orientation of the substituents in the final product.

Problem 4-55 Trifluoroperoxyethanoic acid, $CF_3C(O)O{-}OH$ reacts with methoxybenzene to give 2- and 4-methoxybenzenols:



Explain the nature of this reaction. What is likely to be the substituting agent? What products would you expect from trifluoroperoxyethanoic acid and fluorobenzene? Would fluorobenzene be more, or less, reactive than methoxybenzene?

Problem 4-56 Ethanoic anhydride reacts with concentrated nitric acid to yield the rather unstable ethanoyl nitrate (acetyl nitrate), which is a useful nitrating agent. With mixtures of benzene and methylbenzene, ethanoyl nitrate products a mixture of nitrobenzene and 2- and 4-nitromethylbenzenes. When nitrated separately, each compound reacts at the **same** overall rate, but when mixed together, 25 times more nitromethylbenzene is formed than nitrobenzene.

a. Write equations for the formation of ethanoyl nitrate and its use in nitration of benzene derivatives.

b. Consider possible mechanisms for nitrations with ethanoyl nitrate and show how the above observations with benzene and methylbenzene alone or in mixtures can be rationalized by proper choice of the rate-determining step.

Problem 4-57 4-Nitromethylbenzene-2,6-D_2 is nitrated by a mixture of nitric and sulfuric acids at the same rate as ordinary 4-nitromethylbenzene under conditions in which the rate of nitration v is given by $v = k\,[\text{nitromethylbenzene}]\,[NO_2^+]$. (Review Section 15-6B.)

a. Explain what conclusion may be drawn from this result as to the mechanism of nitration under these conditions.

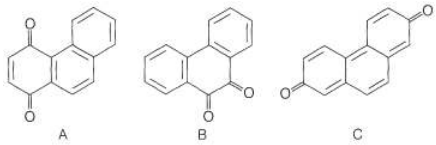
b. What would you expect the nitration rate of C_6D_6 to be as compared with C_6H_6 in the ethanoyl nitrate nitration in Problem 4-56?

Problem 4-58

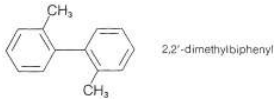
a. From the data of Table 21-1 estimate the overall loss in stabilization energy for the addition of chlorine to the 1,4-positions of naphthalene and to the 9,10 positions of phenanthrene. Which is likely to be the more favorable reaction?

b. Predict whether anthracene is more likely to undergo electrophilic substitution at the 1,2 or 9 position. Show your reasoning.

Problem 4-59 Phenanthrene is oxidized more easily than benzene or naphthalene. Chromic acid oxidation of phenanthrene forms a substance known as phenanthraquinone. Which structure, A, B, or C, would you expect to be formed most readily by oxidation of phenanthrene? Explain.



Problem 4-60 Explain why the nitration and halogenation of biphenyl (phenylbenzene) goes with activation at the ortho and para positions but with deactivation at the meta position. Suggest a reason why biphenyl is more reactive than 2,2'-dimethylbiphenyl in nitration.



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4.P: Arenes, Electrophilic Aromatic Substitution (Problems) is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

CHAPTER OVERVIEW

5: Organohalogen and Organometallic Compounds

The general term of "organohalogen" refers to compounds with covalent carbon-halogen bonds. Substances such a bromomethane (CH_3Br) and chloroethene ($CH_2=CHCl$), are examples of organohalogen compounds, whereas others such as the methylammonium chloride salt, which have no carbon-halogen bonds, are not. This chapter is only concerned with compounds that have covalent carbon-halogen bonds.

5.1: Prelude to Organohalogen and Organometallic Compounds
5.2: Physical Properties of Organohalogen and Organometallic Compounds
5.3: Spectroscopic Properties of Alkyl Halides
5.4: Alkyl Halides
5.5: Alkenyl and Alkynyl Halides
5.6: Cycloalkyl Halides
5.7: Aryl Halides
5.8: Polyhalogenated Alkanes and Alkenes
5.9: Organometallic Compounds from Organohalogen Compounds
5.10: Properties of Organometallic Compounds
5.11: Preparation of Organometallic Compounds
5.12: Organomagnesium Compounds
5.13: Organomagnesium and Organolithium Compounds in Synthesis
5.P: Organohalogen and Organometallic Compounds (Problems)

Thumbnail: Structure of 1-Chlorobenzene.

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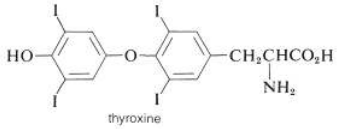
John D. Robert and Marjorie C. Caserio (1977) *Basic Principles of Organic Chemistry, second edition.* W. A. Benjamin, Inc. , Menlo Park, CA. ISBN 0-8053-8329-8. This content is copyrighted under the following conditions, "You are granted permission for individual, educational, research and non-commercial reproduction, distribution, display and performance of this work in any format."

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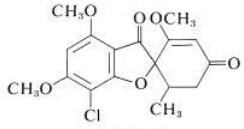
5.1: Prelude to Organohalogen and Organometallic Compounds

There is wide diversity in the nature of organohalogen compounds but, of necessity, we have restricted this chapter to alkyl, cycloalkyl, alkenyl, alkynyl, and aryl halides. Some of the chemistry of the carbon-halogen bonds already will be familiar to you because it involves the addition, substitution, and elimination reactions discussed in previous chapters. To some extent, we will amplify these reactions and consider nucleophilic substitution by what are called the *addition-elimination* and *elimination-addition* mechanisms. Subsequently, we will discuss the formation of carbon-metal bonds from carbon-halogen bonds. The latter type of reaction is of special value because compounds that have carbon-metal bonds are potent reagents for the formation of carbon-carbon bonds, as we will show later in this chapter.

Although large numbers of organohalogens are known, very few of them occur naturally. Thyroid hormones (e.g., thyroxine) that contain iodine are exceptions; other organohalogens are found as mold metabolites (such as griseofulvin) and in marine organisms:

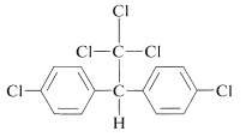


thyroxine

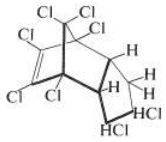


griseofulvin

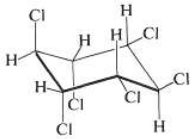
Almost all of the organohalogen compounds in use today are synthetic in origin. You may wonder why, if nature doesn't choose to make them, man elects to do so. The main interest to us here is that they are very useful intermediates for the synthesis of a wide range of other compounds. However, vast quantities of synthetic halogen compounds, particularly polyhalogen compounds, are used as pesticides, cleaning solvents, anaesthetics, aerosol propellants, refrigerants, polymers, and so on. The wisdom of this massive use of materials that are foreign to our natural environment gradually is being reevaluated as the long-term detrimental effects of many of these chemicals become known. For example, many of the chlorinated hydrocarbons such as DDT, Chlordane, and Lindane, which have been used very widely as insecticides, now are at least partially banned because of concern for their long-term effects on nontarget species, including man.



1,1,1-trichloro-2-bis(4-chlorophenyl)ethane (DDT)



(Chlordane)



1,2,3,4,5,6-hexachlorocyclohexane (Lindane)

Sometimes the long-term effects are quite unexpected and difficult to predict. For example, millions of kilograms of CF_2Cl_2, which is used as a propellant, have been released into the atmosphere from aerosol cans. This compound appears to be wholly free of direct adverse physiological effects. However, as the substance diffuses into the upper atmosphere, it is slowly decomposed by sunlight to produce chlorine atoms. Serious danger then is possible because chlorine atoms are known to catalyze the decomposition of ozone, and it is the ozone layer in the upper atmosphere that absorbs most of the sun's ultraviolet radiation that is strongly harmful to life.

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5.1: Prelude to Organohalogen and Organometallic Compounds is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

5.2: Physical Properties of Organohalogen and Organometallic Compounds

The physical properties of haloalkanes are much as one might expect. Volatility decreases: (a) with increasing molecular weight along a homologous series, (b) with increasing atomic number of the halogen, and (c) with the structure of the alkyl group in the order such that *tertiary* < *secondary* < *primary* for isomeric halides. These trends are apparent from the physical properties listed in Table 5-1, which includes data for simple halogen derivatives of alkanes, alkenes, alkynes, and arenes.

Table 5-1: *Physical Properties of Organic Halides*

Name	Formula	MW	Mp, °C	Bp, °C	Density, d_4^{20}, g ml^{-1}
Alkyl halides					
fluoromethane	CH_3F	34	−142	−78	–
chloromethane	CH_3Cl	50.5	−97	−24	–
bromomethane	CH_3Br	95	−94	4.5	$1.730^{0/4}$
iodomethane	CH_3I	142	−67	42.8	2.28
tetrafluoromethane	CF_4	88	−184	−129	–
tetrachloromethane	CCl_4	154	−23	76.7	1.594
chloroethane	CH_3CH_2Cl	64.5	−138	12.3	$0.9214^{0/4}$
1-chloropropane	$CH_3CH_2CH_2Cl$	78.5	−122	47	$0.890^{20/20}$
1-chlorobutane	$CH_3CH_2CH_2CH_2Cl$	92.5	−123	78.5	0.886
2-chlorobutane	$CH_3CH_2CH(Cl)CH_3$	92.5	−131	68	0.871
2-chloro-2-methyl-propane	$(CH_3)_3C—Cl$	92.5	−27	51	$0.847^{15/4}$
Alkenyl halides					
fluoroethene	$CH_2=CHF$	46	−161	−72	–
chloroethene	$CH_2=CHCl$	62.5	−160	−14	0.911
1,1-dichloroethene	$CH_2=CCl_2$	97	−123	31.7	1.213
tetrafluoroethene	$CF_2=CF_2$	100	−131	−76	1.519
tetrachloroethene	$CCl_2=CCl_2$	165.8	−22	121	1.623
Alkynyl halides					
fluoroethyne	$HC\equiv CF$	44	>−196	−104	–
chloroethyne	$HC\equiv CCl$	60.5	−126	−32	–
bromoethyne	$HC\equiv CBr$	105	–	4.7	–
iodoethyne	$HC\equiv CI$	152	–	32	–
Aryl halides					
fluorobenzene	C_6H_5F	96.0	−42	85	1.024
chlorobenzene	C_6H_5Cl	112.5	−45	132	1.107
bromobenzene	C_6H_5Br	157	−31	156	1.495
iodobenzene	C_6H_5I	204	−31	189	1.832
2-chloromethylbenzene		126.5	−36	159	1.0817
3-chloromethylbenzene		126.5	−49	162	1.0732
4-chloromethylbenzene		126.5	7.5	162	1.0697

The boiling points of many halogen compounds are similar to hydrocarbons of the same molecular weight, but there are some conspicuous exceptions. Iodomethane, for example, has about the same molecular weight as decane (MW 142), but the boiling point of iodomethane is **132°** *lower* than that of decane. Likewise, fluorocarbons (e.g., tetrafluoromethane, CF_4, MW 88, bp **−129°**) are far more volatile than hydrocarbons of similar weights (e.g., hexane, C_6H_{14}, MW 86, bp **69°**).

In general, halogen compounds are insoluble in water but are readily soluble in organic solvents and, with the exception of some fluoro and monochloro compounds, they are more dense than water. Aryl halides are fairly pleasant smelling liquids, but arylmethyl (benzylic) halides of structure $ArCH_2X$ are irritating to the eyes, skin, and nasal passages. Toxicity varies, but the chlorinated hydrocarbons such as CCl_4 ("carbon tet") and $CHCl_2—CHCl_2$ are quite toxic and should be used with care.

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5.3: Spectroscopic Properties of Alkyl Halides

Organohalogen compounds give rise to strong absorptions in the *infrared* arising from stretching vibrations of the carbon-halogen bond. The frequency of absorption decreases as the mass of the halogen increases. For monohaloalkanes the absorptions useful for identification are those of C—F at 1100-1000 cm^{-1} and C—Cl at 850-550 cm^{-1}. The C—Br and C—I absorptions are below 690 cm^{-1} and therefore are out of range of most commercial spectrophotometers. Because these bands are in the fingerprint region or far infrared, it is difficult to infer the presence of halogen in a molecule solely from its infrared spectrum.

Apart from fluorine, the magnetic properties of halogen nuclei do not complicate proton or ^{13}C *nuclear magnetic resonance spectra* of organohalogen compounds. But fluorine (^{19}F) has a spin of 1/2 and causes spin-spin splitting of the resonances of neighboring magnetic nuclei (^{13}C, ^{1}H, and other ^{19}F nuclei). Proton chemical shifts are influenced strongly by the presence of halogen, which serves to deshield neighboring protons by electronegativity effects (see Section 9-10E).

The *mass spectra* of chlorine- and bromine-containing compounds clearly show the abundance ratios of the stable isotopes ^{35}Cl:^{37}Cl = 3:1 and ^{79}Br:^{81}Br = 1:1 in the molecular ions and those ionic fragments which contain halogens (Section 9-11).

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5.3: Spectroscopic Properties of Alkyl Halides is shared under a not declared license and was authored, remixed, and/or curated by LibreTexts.

5.4: Alkyl Halides

The important chemistry of alkyl halides, **RX**, includes the nucleophilic (S_N) displacement and elimination (E) reactions discussed last semester. Recall that *tertiary* alkyl halides normally are reactive in ionization (S_N1) reactions, whereas *primary* halides, and to a lesser extent *secondary* halides, are reactive in S_N2 reactions, which occur by a concerted mechanism with inversion of configuration.

Elimination competes with substitution in many S_N reactions and can become the major pathway at high temperatures or in the presence of strong base. Elimination (E_2), unlike displacement (S_N2), is insensitive to steric hindrance in the alkyl halide. In fact, the E_2 reactivity of alkyl halides is *tert* **RX** > *sec* **RX** > *prim* **RX**, which is opposite to their S_N2 reactivity.

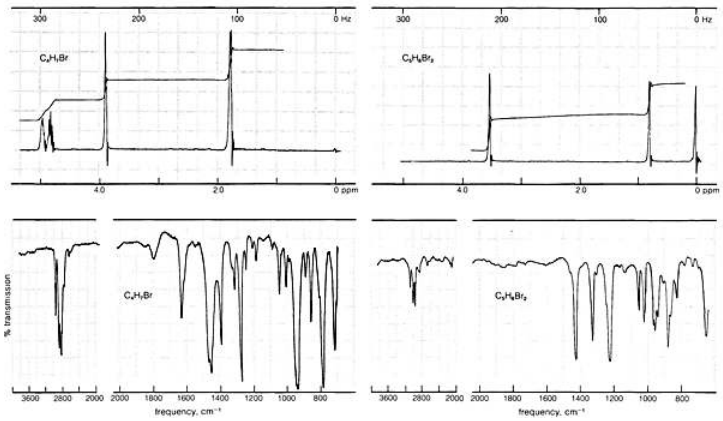


Figure 5-1: *Infrared and proton nmr spectra of substance* **C_4H_7Br** *and substance* **$C_5H_8Br_2$**. *NMR spectra are at* **60 MHz** *with reference to TMS at 0.0.*

Several useful reactions for the synthesis of alkyl halides that we already have encountered are summarized below with references to the sections that supply more detail:

$$RH + Cl_2 \xrightarrow{h\nu} RCl + HCl \qquad \text{(Section 4-5)}$$

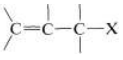
$$RCH{=}CH_2 + HCl \longrightarrow RCHCH_3 \quad (Cl) \qquad \text{(Section 10-4)}$$

$$RCH{=}CH_2 + HBr \xrightarrow[\text{or peroxides}]{h\nu} RCH_2CH_2Br \qquad \text{(Section 10-7)}$$

$$ROH + HBr \longrightarrow RBr + H_2O \qquad \text{(Section 8-7D)}$$

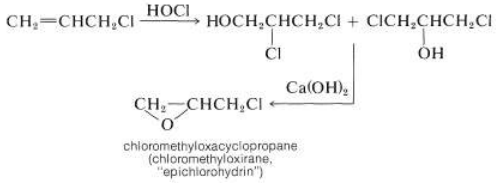
A summary of these and some other reactions for the synthesis of alkyl halides or organohalogen compounds is given in Table 5-5.

5-4A Allylic (2-Propenyl) Halides

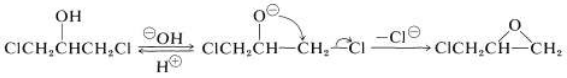
Halogen compounds in which the carbon-halogen bond is adjacent to a double bon, as in  are known as **allylic halides**. The simplest example is 3-chloropropene, **$CH_2{=}CHCH_2Cl$**, which is made on a large scale by the radical chlorination of propene at **400°**:

$$CH_2{=}CH{-}CH_3 + Cl_2 \xrightarrow{400°} CH_2{=}CH{-}CH_2{-}Cl + HCl$$

Most of the 3-chloropropene prepared in this manner is converted to other important compounds. For example, addition of hypochlorous acid gives a mixture of dichloropropanols, which on treatment with base gives a substance known commercially as "epichlorohydrin":



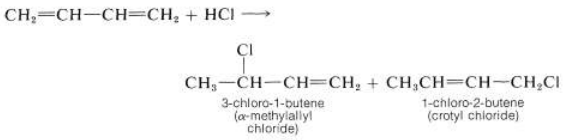
The ring closure reaction with $Ca(OH)_2$ is an internal S_N2 reaction. Hydroxide ion converts the alcohol to an alkoxide ion that acts as a nucleophile in displacing the neighboring chlorine:



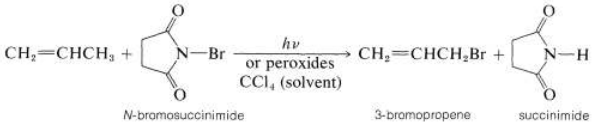
The epichlorohydrice so produced is used primarily to make epoxy resins, although some of it is hydrolyzed to glycerol:



A general method for preparing allylic halides is by addition of hydrogen halides to conjugated dienes. This reaction usually produces a mixture of 1,2- and 1,4-addition products:

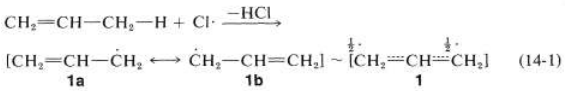


A second general method involves the bromination of alkene with *N*-bromosuccinimide (the **Wohl-Ziegler** reaction). A radical-chain reaction takes place between *N*-bromosuccinimide (NBS) and alkenes, which commonly is initiated by light, peroxides, or other catalysts, and yields allylic bromides:

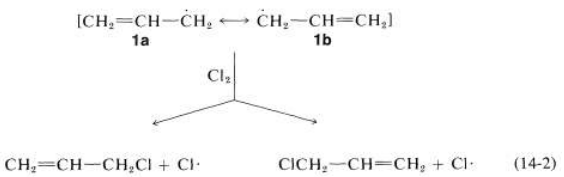


This reaction, like the chlorination of propene, is highly selective in that the so-called allylic **C—H** is attacked preferentially.

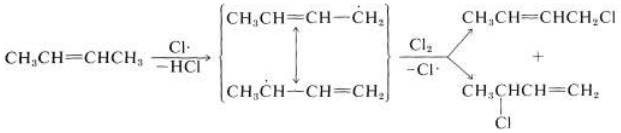
From bond energies (Table 4-6) we know that the weakest **C—H** bonds of propene are to the allylic hydrogens, $H_2C{=}CHCH_2{-}H$. Therefore, in the first step of radical-chain chlorination of propene, an allylic hydrogen is removed by a chlorine atom (Equation 5-1). The allylic **C—H** bonds are weaker than the alkenic **C—H** bonds because of the extra stabilization of the radical obtained on hydrogen abstraction (Equation 14-1). Two equivalent valence-bond structures (**1*a*** and **1*b***) can be written for the 2-propenyl radical; the electron delocalization enhances the stability of the radical:



In the second step of the chain reaction (Equation 14-2) the propenyl radical can form a carbon-halogen bond at either end by abstracting a halogen atom from the halogenating agent:

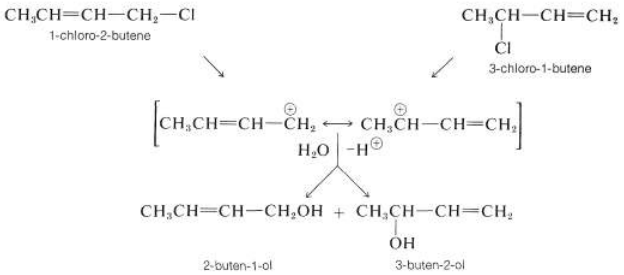


The $Cl\cdot$ atom produced now can participate in Reaction 5-1, thereby continuing the chain. With propene the intermediate radical gives the same product, 2-propenyl chloride, irrespective of whether a chlorine atom is transferred to the 1- or 3-carbon. However, the radical formed by removal of an allylic hydrogen from 2-butene gives a mixture of products:



5-4B S_N Reactions of Allylic Halides

The carbon-halogen bonds of allylic halides are especially reactive in both S_N1 and S_N2 reactions. The reasons for the enhanced S_N1 reactivity have been discussed previously. For example, the ease with which 1-chloro-2-butene ionizes compared to 1-chlorobutane is attributed to the stability of the 2-butenyl cation, which is distributed between C_1 and C_3, and the nucleophile (water) attacks at both positions to give mixtures of products. The same results are obtained if one starts with 3-chloro-1-butene because the same cation is formed:



The high reactivity of allylic halides in S_N2 reactions indicates some special stabilization of the transition state ascribable to resonance involving the adjacent π bond. We can express this in terms of the valence-bond structures, **2*a*-2*c***, for the transition state of the reaction of iodide with 3-chloropropene. The extra stabilization over the corresponding transition state for the reaction of iodide with a saturated chloride (e.g., $CH_3CH_2CH_2Cl + I^{\ominus} \rightarrow CH_3CH_2CH_2I + Cl^{\ominus}$) arises from the contribution of **2*c***. An alternative atomic-orbital picture of the transition state is shown in Figure 5-2.

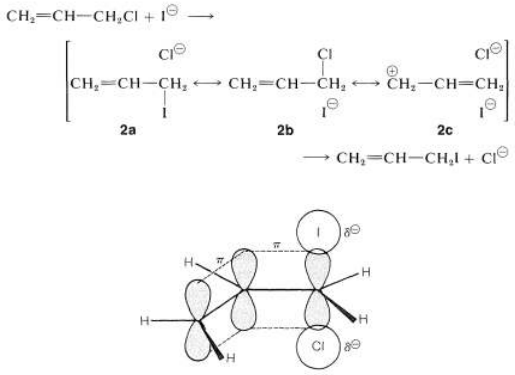
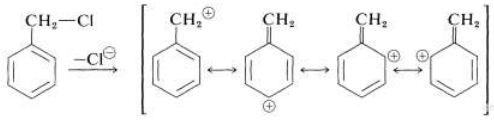


Figure 5-2: *Atomic-orbital representation of the transition state for S_N2 displacement of 3-chloropropene (allyl chloride) with iodide ion. The halide orbitals are represented as spherical for simplicity. The purpose of this figure is to show the π bonding, which can stabilize the transition state.*

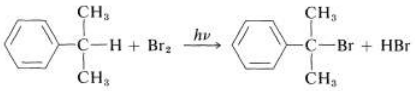
5-4C Benzylic (Phenylmethyl) Halides

Reactivities comparable to allylic halides are found in the nucleophile displacement reactions of benzylic halides by S_N1 and S_N2 mechanisms. The ability of the benzylic halides to undergo S_N1 reactions clearly is related to the stability of the resulting benzylic cations, the electrons of which are extensively delocalized. Thus, for phenylmethyl chloride,



When the halogen substituent is located two or more carbons from the aryl group as in 2-phenylethyl bromide, $C_6H_5CH_2CH_2Br$, the pronounced activating effect evident in benzylic halides disappears, and the reactivity of the halides is essentially that of a primary alkyl halide (e.g., $CH_3CH_2CH_2Br$).

Benzylic halides can be prepared by the same radical-halogenating agents that give allylic halides from alkenes. These include Cl_2, Br_2, *N*-bromosuccinimide, SO_2Cl_2, and *tert*-butyl hypochlorite:



The benzylic C—H bond is weaker and more restrictive then primary alkane C—H bonds because of the stabilization of benzylic radicals.

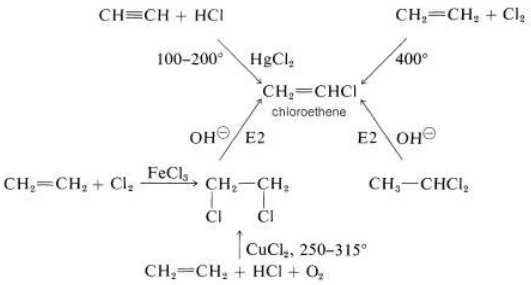
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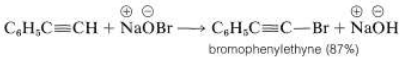
5.5: Alkenyl and Alkynyl Halides

The most readily available alkenyl halide is chloroethene (vinyl chloride), which can be prepared by a number of routes:



The most economical commercial preparation is high-temperature chlorination of ethene. A useful modification of this process uses hydrogen chloride in place of chlorine. An oxidizing agent is required to raise the oxidation state of chlorine in $\mathbf{HCl}$ to that of $\mathbf{Cl_2}$; molecular oxygen is used for this purpose along with cupric salts as catalysts.

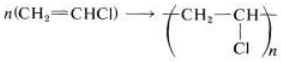
General methods of preparation for alkenyl and alkynyl halides are listed in Table 5-5. By the alkynyl halides we mean 1-halo-alkynes. One interesting method by which they may be prepared employs 1-alkynes with hypohalites:



This kind of reaction does not proceed with either alkanes or alkenes.

Uses of Alkenyl Halide

Chloroethene is produced in vast quantities for the production of polymers (polyvinyl chloride) and copolymers:



These polymers commonly are described as PVC plastics or less specifically as “vinyl.” They are materials that may be either flexible or rigid according to what they are mixed with, and they are used in the manufacture of many familiar articles such as plastic curtains, rainwear, floor tile, synthetic leather goods, upholstery, table mats, phonograph records, insulation, plastic pipes, tubing, and packaging materials.

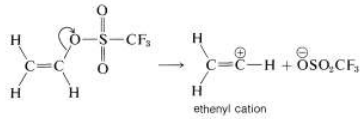
Recently, it has been found that persons working in plants that manufacture and use chloroethene have an unusually high incidence of an unusual type of liver cancer. As a result, strict safety regulations and pollution standards have been set for plants where chloroethene is made or used. The once widespread use of chloroethene as a propellant for aerosol cans has been curtailed. Polyvinyl chloride itself seems to be quite safe, but there are possible problems with its incorporation into interior building materials, clothing, and upholstery because heat, such as fire, causes polyvinyl chloride to decompose, thereby producing hydrogen chloride as one decomposition product. In closed areas the toxicity of hydrogen chloride gas may be as serious a hazard as the fire itself. Other polymers may give off similarly toxic products on strong heating.

5-5B Chemical Properties

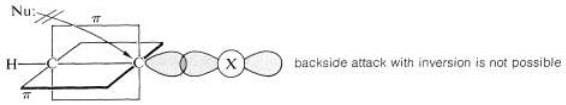
The outstanding chemical characteristic of alkenyl halides is their general inertness in S_N1 and S_N2 reactions. Thus chloroethene fails to react with silver nitrate in ethanol (i.e., low S_N1 reactivity), fails to react with potassium iodide in acetone (i.e., low S_N2 reactivity), and only reacts slowly with sodium hydroxide to give ethyne (low $E2$ reactivity). The haloalkynes, such as $\mathbf{RC \equiv C{-}Cl}$, are similarly unreactive.

It is not surprising that $\mathbf{=C{-}X}$ and $\mathbf{\equiv C{-}X}$ bonds are hard to break heterolytically. In general, $\mathbf{C{-}X}$ bonds are strong in alkenyl halides (cf. Table 4-6) and this property tends to make them less reactive than alkyl halides. Furthermore, double- and triple-bonded carbons are more strongly electron-attracting than saturated sp^3 carbons, which is the reason why 1-alkynes and alkenes are stronger acids (Section 11-8) than alkanes. Consequently it is easier to break a $\mathbf{\equiv C{-}H}$ bond in the sense $\mathbf{C^{\ominus}H^{\oplus}}$ than as $\mathbf{\equiv C^{\oplus}H^{\ominus}}$. It

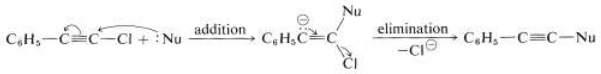
also will be more difficult to ionize a carbon-halogen bond to $C^{\oplus}X^{\ominus}$ if the carbon is unsaturated. Therefore ethenyl and ethynyl cations, such as $CH_2{=}CH^{\oplus}$ and $HC{\equiv}C^{\oplus}$, are difficult to generate from the corresponding halides. Superior leaving groups are required, such as trifluoromethanesulfonate, $-OSO_3CF_3$ (Section 8-7C):



The reason for the lack of S_N2 reactivity in ethenyl or ethynyl halides may be that the attacking nucleophile is unable to react by the concerted inversion mechanism that invariably is observed with alkyl halides:



Nevertheless, substitution of the halogen does occur under some circumstances. In such cases, the nucleophile first *adds* to the multiple bond, and in a subsequent step the halogen leaves as halide ion. This is an “addition-elimination” mechanism, of which we will have more examples later:



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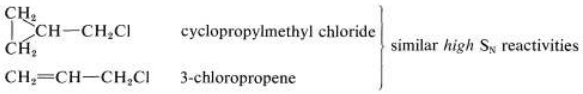
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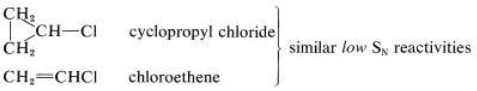
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5.6: Cycloalkyl Halides

The cycloalkyl halides, except for cyclopropyl halides, have physical and chemical properties that are similar to those of the open-chain secondary halides and can be prepared by the same types of reactions (Table 5-5). All the cycloalkyl halides undergo S_N2 reactions rather slowly and, with nucleophiles that are reasonably basic ($^{\ominus}OH$, $^{\ominus}OC_2H_5$, $^{\ominus}C \equiv N$, etc.), $E2$ reactions can be expected to predominate (Table 5-6). The rate of carbocation formation leading to S_N1 and $E1$ reactions is sensitive to ring size but, except for the small-ring halides, the carbocation reactions are normal in most other respects.

The cyclopropyl halides are exceptional in that their behavior is much more like alkenyl halides than like secondary alkyl halides. Thus cyclopropyl chloride undergoes S_N1 and S_N2 reactions much less rapidly than isopropyl or cyclohexyl chlorides. A relationship between the reactivity of cyclopropyl chloride and chloroethene is not surprising in view of the general similarity between cyclopropane rings and double bonds (Section 12-5). This similarity extends to cyclopropylmethyl derivatives as well. Cyclopropylmethyl chloride is reactive in both S_N1 and S_N2 reactions in much the same way as 3-chloropropene:





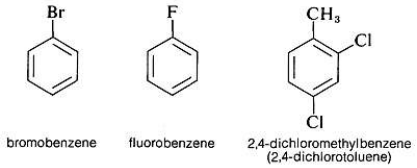
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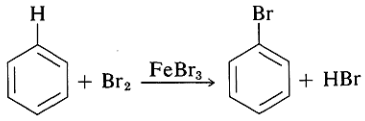
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5.7: Aryl Halides

Aryl halides have a halogen directly bonded to a carbon of an aromatic ring. Examples are bromobenzene, fluorobenzene, and 2,4-dichloromethylbenzene:



Some of the methods by which alkyl halides are prepared do not work for aryl halides because it is difficult to form C-halogen bonds at aromatic ring carbons by nucleophilic displacement reactions. The most common ways of forming C_{aryl}-halogen bonds are by substitution of $C_{aryl}–H$ by electrophilic halogenating agents (e.g., Br_2 or Cl_2),



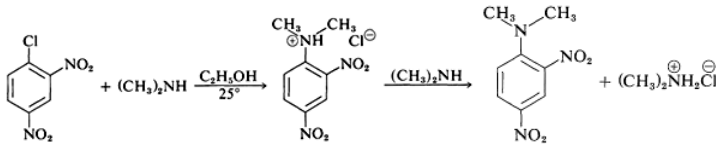
and by replacement of $C–NH_2$ by C–halogen. These reactions are listed in Table 5-5 and will be discussed in more detail in Chapters 22 and 23.

5-7A Nucleophilic Aromatic Displacement Reactions

The carbon-halogen bonds of aryl halides are like those of alkenyl halides in being much stronger than those of alkyl halides (see Table 4-6). *The simple aryl halides generally are resistant to attack by nucleophiles in either* S_N1 *or* S_N2 *reactions* (Table 5-7). However, this low reactivity can be changed dramatically by changes in the reaction conditions and the structure of the aryl halide. In fact, nucleophilic displacement becomes quite rapid (a) when the aryl halide is activated by substitution with strongly electron-attracting groups such as NO_2, and (b) when very strongly basic nucleophilic reagents are used.

5-7B Addition-Elimination Mechanism of Nucleophilic Substitution

Although the simple aryl halides are inert to the usual nucleophilic reagents, *considerable activation is produced by strongly electron-attracting substituents provided these are located in either the* ortho *or* para *positions, or both*. For example, the displacement of chloride ion from 1-chloro-2,4-dinitrobenzene by dimethylamine occurs readily in ethanol solution at room temperature. Under the same conditions chlorobenzene completely fails to react; thus the activating influence of the two nitro groups amounts to a factor of at least 10^8:

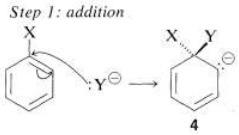


A related reaction is that of 2,4-dinitrofluorobenzene with the amino groups of peptides and proteins, and this reaction provides a means for analysis of the *N*-terminal amino acids in polypeptide chains. (See Section 25-7B.)

In general, the reactions of activated aryl halides closely resemble the S_N2-displacement reactions of aliphatic halides. The same nucleophilic reagents are effective (e.g., $CH_3O^{\ominus}$, $HO^{\ominus}$, and RNH_2); the reactions are second order overall (first order in halide and first order in nucleophile); and for a given halide the more nucleophilic the attacking reagent, the faster the reaction. However, there must be more than a subtle difference in mechanism because an aryl halide is unable to pass through the same type of transition state as an alkyl halide in S_N2 displacements.

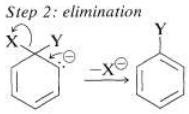
The generally accepted mechanism of nucleophilic aromatic substitution of aryl halides carrying activating groups involves two steps that are closely analogous to those briefly described in Section 5-4 for alkenyl and alkynyl halides. The first step involves attack of the nucleophile $Y^{\ominus}$ at the carbon bearing the halogen substituent to form an intermediate carbanion **4** (Equation 5-3). The

aromatic system is destroyed on forming the anion, and the carbon at the reaction site changes from planar (sp^2 bonds) to tetrahedral (sp^3 bonds).



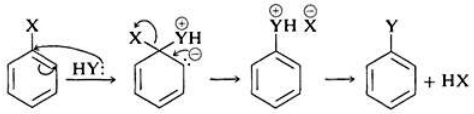
(14-3)

In the second step, loss of an anion, $X^{\ominus}$ or $Y^{\ominus}$, regenerates an aromatic system, and, if $X^{\ominus}$ is lost, the overall reaction is nucleophilic displacement of **X** by **Y** (Equation 5-4).

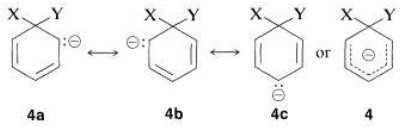


(14-4)

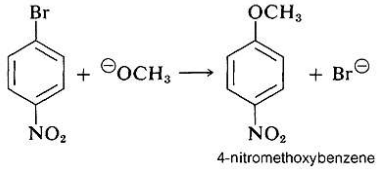
In the case of a neutral nucleophilic reagent, **Y** or **HY**, the reaction sequence would be the same except for the necessary adjustments in the charge of the intermediate:



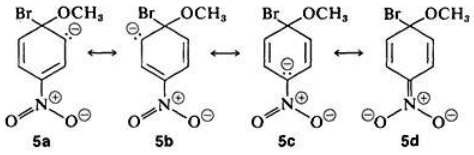
Why is this reaction pathway generally unfavorable for the simple aryl halides? The answer is that the intermediate **4**, which we can express as a hybrid of the valence-bond structures **4*a*-4*c***, is too high in energy to be formed at any practical rate. Not only has **4** lost the aromatic stabilization of the benzene ring, but its formation results in transfer of negative charge to the ring carbons, which themselves are not very electronegative:



However, when s*trongly electron-attracting groups are located on the ring at the* ortho-para *positions, the intermediate anion is stabilized by delocalization of electrons from the ring carbons to more favorable locations on the substituent groups*. As an example, consider the displacement of bromine by OCH_3 in the reaction of 4-bromonitrobenzene and methoxide ion:



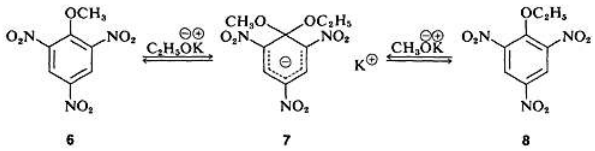
The anionic intermediate formed by *addition* of methoxide ion to the aryl halide can be described by the valence-bond structures **5*a*-5*d***. Of these structures **5*d*** is especially important because in it the charge is transferred from the ring carbons to the oxygen of the nitro substituent:



Substituents in the meta positions have much less effect on the reactivity of an aryl halide because delocalization of electrons to the substituent is not possible. No formulas can be written analogous to **5*c*** and **5*d*** in which the negative charges are both on atoms next to positive nitrogen, $\ddot{C}-\overset{\oplus}{N}-\ddot{O}$ and $\ddot{O}-\overset{\oplus}{N}-\ddot{O}$,

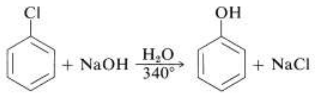


In a few instances, stable compounds resembling the postulated reaction intermediate have been isolated. One classic example is the complex **7** (isolated by J. Meisenheimer), which is the product of the reaction of either the methyl aryl ether **6** with potassium ethoxide, or the ethyl aryl ether **8** and potassium methoxide:

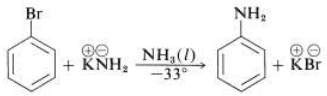


5-7C Elimination-Addition Mechanism of Nucleophilic Aromatic Substitution. Arynes

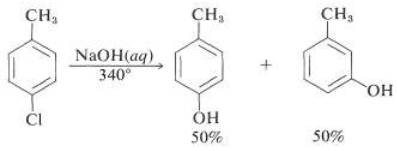
The reactivities of aryl halides, such as the halobenzenes, are exceedingly low toward nucleophilic reagents that normally effect displacements with alkyl halides and activated aryl halides. Substitutions do occur under forcing conditions of either high temperatures or very strong bases. For example, chlorobenzene reacts with sodium hydroxide solution at temperatures around **340°** and this reaction was once an important commercial process for the production of benzenol (phenol):



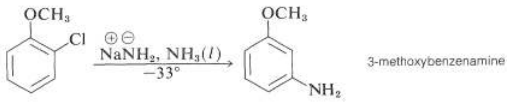
In addition, aryl chlorides, bromides, and iodides can be converted to areneamines **$ArNH_2$** by the conjugate bases of amines. In fact, the reaction of potassium amide with bromobenzene is extremely rapid, even at temperatures as low as **−33°** with liquid ammonia as solvent:



However, displacement reactions of this type differ from the previously discussed displacements of activated aryl halides in that rearrangement often occurs. That is, *the entering group does not always occupy the same position on the ring as that vacated by the halogen substituent*. For example, the hydrolysis of 4-chloromethylbenzene at **340°** gives an equimolar mixture of 3- and 4-methylbenzenols:

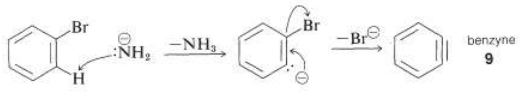


Even more striking is the exclusive formation of 3-methoxybenzenamine in the amination of 2-chloromethoxybenzene. Notice that this result is a violation of the principle of least structural change (Section 1-1H):



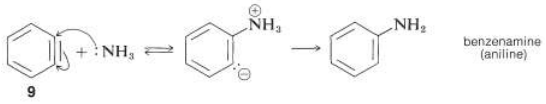
The mechanism of this type of reaction has been studied extensively, and much evidence has accumulated in support of a stepwise process, which proceeds first by base-catalyzed *elimination* of hydrogen halide (**HX**) from the aryl halide - as illustrated below for the amination of bromobenzene:

Elimination

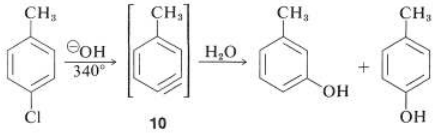


The product of the elimination reaction is a highly reactive intermediate **9** called **benzyne**, or **dehydrobenzene**, which differs from benzene in having two less hydrogen and an extra bond between two ortho carbons. Benzyne reacts rapidly with any available nucleophile, in this case the solvent, ammonia, to give an addition product:

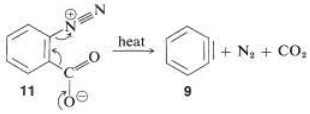
Addition



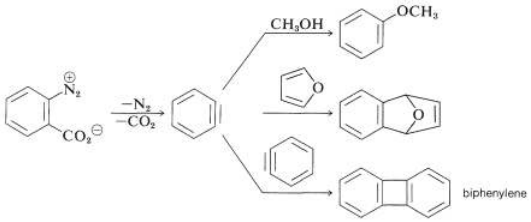
The rearrangements in these reactions result from the attack of the nucleophile at one or the other of the carbons of the extra bond in the intermediate. With benzyne the symmetry is such that no rearrangement would be detected. With substituted benzynes isomeric products may result. Thus 4-methylbenzyne, **10**, from the reaction of hydroxide ion with 4-chloro-1-methylbenzene gives both 3- and 4-methylbenzenols:



In the foregoing benzyne reactions the base that produces the benzyne in the elimination step is derived from the nucleophile that adds in the addition step. This need not always be so, depending on the reaction conditions. In fact, the synthetic utility of aryne reactions depends in large part of the success with which the aryne can be generated by one reagent but captured by another. One such method will be discussed in Section 5-10C and involves organometallic compounds derived from aryl halides. Another method is to generate the aryne by thermal decomposition of a 1,2-disubstituted arene compound such as **11**, in which both substituents are leaving groups - one leaving with an electron pair, the other leaving without:



When **11** decomposes in the presence of an added nucleophile, the benzyne intermediate is trapped by the nucleophile as it is formed. Or, if a conjugated diene is present, benzyne will react with it by a [4 + 2] cycloaddition. In the absence of other compounds with which it can react, benzyne will undergo [2 + 2] cycloaddition to itself:

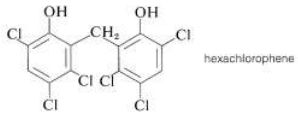


5-7D Uses for Aryl Halogen Compounds

As with most organic halides, aryl halides most often are synthetic intermediates for the production of other useful substances. For example, chlorobenzene is the starting aryl halide for the synthesis of DDT; it also is a source of benzenol (phenol, Section 5-7C) which, in turn, has many uses (Section 26-1).

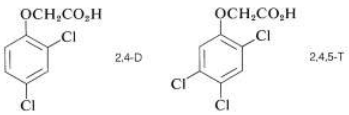
Several aromatic chloro compounds are used extensively as insecticides, herbicides, fungicides, and bactericides. They also have acquired much notoriety because in some instances their indiscriminate usage has led to serious problems. For example, hexachlorophene is an external bactericide that until recently was used in cosmetic preparations such as soaps, deodorants, and so

on. Its use has been discontinued because of compelling evidence that it can be absorbed through the skin in amounts that are dangerous, if not lethal, for infants and small children.



hexachlorophene

Other pesticides, notably DDT and the herbicides 2,4-D and 2,4,5-T have been partially banned for different reasons.



Contributors and Attributions

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5.8: Polyhalogenated Alkanes and Alkenes

5-8A Useful Compounds

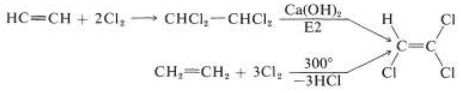
Polychlorination of methane yields the di-, tri-, and tetrachloromethanes cheaply and efficiently:



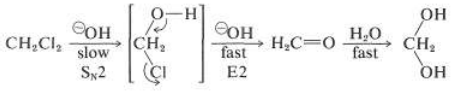
These substances have excellent solvent properties for nonpolar and slightly polar substances. Chloroform once was used widely as an inhalation anesthetic. However, it has a deleterious effect on the heart and is oxidized slowly by atmospheric oxygen to highly toxic carbonyl dichloride (phosgene, $\mathbf{COCl_2}$). Commercial chloroform contains about **1%** ethanol, which destroys any $\mathbf{COCl_2}$ formed by oxidation.

Carbon tetrachloride commonly was employed as a cleaning solvent, although its considerable toxicity entails considerable hazard when used indiscriminately. It has been used as a fire-extinguishing fluid for petroleum fires, but its toxicity and tendency to form still more toxic carbonyl dichloride makes it undesirable for confined areas. The common laboratory practice of removing traces of water from solvents with metallic sodium should *not* be applied to halogenated compounds; carbon tetrachloride-sodium mixtures are shock sensitive and can detonate.

Trichloroethene ("Tri-Clene", bp **87°**) is a widely used dry-cleaning solvent. It can be prepared from either ethene or ethyne:



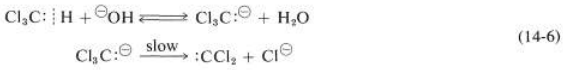
Compared with monohaloalkanes, polyhalogen compounds have quite different reactivities and behavior toward nucleophiles and bases. Thus dichloromethane reacts with hydroxide ion by an $\boldsymbol{S_N2}$ mechanism much less readily than methyl chloride. The chloromethanol formed then undergoes a rapid $\boldsymbol{E2}$ elimination to give methanal (formaldehyde), a substance that exists in water largely as dihydroxymethane:



Trichloromethane (chloroform) reacts quite differently with base than does chloromethane or dichloromethane - as will be described in the following section.

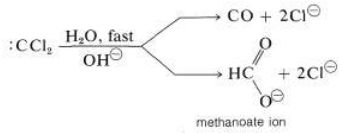
5-8B α Elimination. Carbenes

Trihalomethanes, such as trichloromethane (chloroform), are quite reactive toward strong base. The base, such as hydroxide, removes the hydrogen of $\mathbf{HCCl_3}$ as a proton much more rapidly than it attacks the carbon in the $\boldsymbol{S_N2}$ manner. The carbanion so formed, $\mathbf{Cl_3C^{\ominus}}$, is unstable and loses chloride ion to form a highly reactive neutral intermediate, $\mathbf{:CCl_2}$, called **dichlorocarbene**:



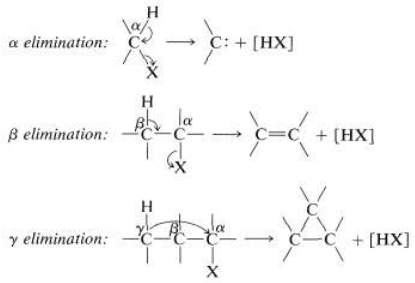
(14-6)

This intermediate has only six valence electrons around carbon and therefore is strongly electrophilic. In aqueous solution it reacts rapidly to form carbon monoxide and methanoate (formate) ion:



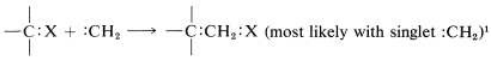
The formation of $\mathbf{:CCl_2}$ from $\mathbf{HCCl_3}$ by the reactions of Equation 5-6 results in the elimination of **HCl** - the leaving groups, **H** and **Cl**, both originating from the *same* carbon atom. Such reactions are not uncommon and are called $\boldsymbol{\alpha}$ eliminations or 1,1 eliminations

to distinguish them from $E1$ and $E2$ reactions, which are β eliminations or 1,2 eliminations. Still other possibilities are reactions such as γ or 1,3 eliminations, but these take on the character of internal S_N2 reactions and will not be considered in detail here.

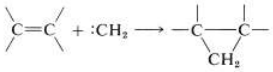


The product of α elimination is a neutral species that resembles a carbocation in having only six carbon valence electrons. The simplest carbene is $:CH_2$, methylene. *Carbenes are highly reactive*, so much so that they cannot be isolated. Their involvement in reactions usually has to be inferred from the nature of the products or the reaction kinetics. The characteristic carbene reactions involve forming an electron-pair bond to the carbene carbon by reacting with σ bonds, π bonds, or unshared pairs (n). Some of these reactions are illustrated here for methylene $:CH_2$.[1]

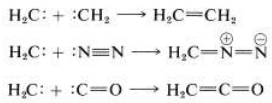
with σ bonds (insertion):



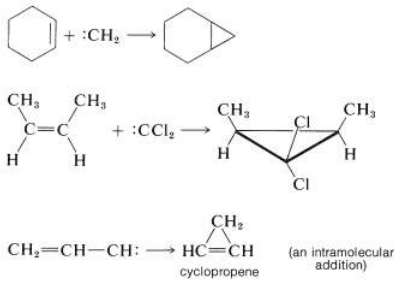
with π bonds ([2 + 1] cycloaddition):



with unshared pairs (dimerization, addition):

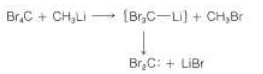
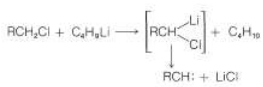
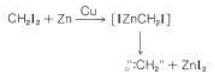
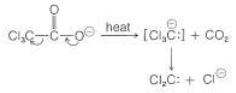
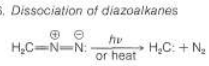
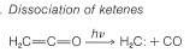


Carbenes are much more reactive toward carbon-carbon double bonds than toward single bonds. Without doubt the most useful feature of α elimination is that it provides a practical route to cyclopropanes and cyclopropenes by [2 + 1] cycloaddition of carbenes to double or triple bonds. These additions are stereospecific *suprafacial* additions if they involve singlet carbenes, but can give mixtures with triplet carbenes:



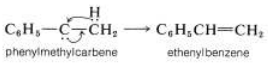
Carbene precursors are compounds that have or acquire good leaving groups (e.g., halide ions). Thus, halogen compounds frequently are carbene sources. Trihalomethanes are the oldest known sources of dihalocarbenes; but there are other methods for generating carbenes, and some of these are listed for reference in Table 5-2 (see also Section 5-10C). There is a question as to whether a "free" carbene actually is formed in some of these reactions, particularly those involving metals, but for our purposes we will classify them as routes to carbenes or carbenelike species.

***Table 5-2:** α-Elimination Reactions Producing Carbene Intermediates*[a]

Reaction	Comments
1. *Trihalomethanes and strong base*	Gives dihalocarbenes; to trap the carbene with an alkene, an aprotic solvent is preferred.
2. *Haloalkanes and alkyllithiums* 	Tetrahalomethanes give dihalocarbenes. (See Section 14-10C.)
	Suitable for generation of alkyl or aryl carbenes.
3. *Diiodomethane and zinc* (Simmons–Smith Reaction) 	Appears to generate a singlet $:CH_2$ metal complex; zinc must be activated with copper.
4. *Dissociation of trihaloacetates* 	Salt is heated in aprotic solvent (e.g., 1,2-dimethoxyethane).
5. *Dissociation of trihalomethylmercury compounds*	
6. *Dissociation of diazoalkanes* 	Widely applicable to formation of alkyl, aryl, keto, and carboalkoxy carbenes.
7. *Dissociation of ketenes* 	

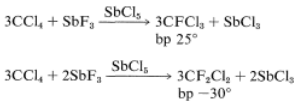
[a]These reactions normally produce singlet carbenes. However, the singlet CH_2: formed in Reactions 6 and 7 may undergo some interconversion to the more stable triplet form,[1] especially if an otherwise inert material is present that can facilitate the singlet–triplet transformation.

Many carbenes, like carbocations, rearrange to more stable structures by the migration of a neighboring group to the electron-deficient carbon. Thus phenylmethylcarbene rearranges to ethenylbenzene (styrene):



5-8C Fluorochloromethanes

Replacement of either one or two of the chlorines of carbon tetrachloride by fluorine can be achieved readily with antimony trifluoride containing some antimony pentachloride. The reaction stops after two chlorines have been replaced. The antimony trifluoride can be regenerated continuously from the antimony chloride by addition of anhydrous hydrogen fluoride:



Both products are useful as refrigerants, particularly for household refrigerators and air-conditioning units, under the trade name Freon. Difluorodichloromethane (Freon 12) also is employed as a propellant in aerosol bombs, shaving-cream dispensers, and other such containers. It is nontoxic, odorless, nonflammable, and will not react with hot concentrated mineral acids or metallic sodium. This lack of reactivity is generally characteristic of the difluoromethylene group, provided the fluorines are not located on an unsaturated carbon. Attachment of a fluorine atom to a carbon atom bonded to one or more chlorine atoms tends greatly to reduce the reactivity of the chlorines toward almost all types of reagents. Possible environmental problems associated with these substances were discussed in the introduction to this chapter.

5-8D Fluorocarbons

During World War II, plastics and lubricating compounds of unusual chemical and thermal stability were required for many applications, in particular for pumping apparatus used to separate ^{235}U from ^{238}U by diffusion of corrosive uranium hexafluoride

through porous barriers. It was natural to consider the use of substances made only of carbon and fluorine (fluorocarbons) for such purposes, and considerable effort was spent on methods of preparing compounds such as $-(CF_2)-_n$. Today, many such substances are in common use. These often are called "perfluoro-" compounds, which indicates that all available hydrogens of the parent compound are replaced by fluorine. Thus perfluorocyclohexane is $(CF_2)_6$. A widely used perfluorocarbon is the plastic material $-(CF_2)-_n$, which is produced in quantity by radical polymerization of tetrafluoroethene:

$$n(CF_2{=}CF_2) \xrightarrow{R\cdot} +CF_2{-}CF_2+_n$$

The product ("Teflon") is a solid, chemically inert substance that is stable to around 300°. It makes excellent electrical insulation and gasket materials. It also has self-lubricating properties, which are exploited in the preparation of low-adhesion surfaces (such as "nonstick" fry pans) and light-duty bearings.

Tetrafluoroethene can be made on a commercial scale by the following method:

$$3CHCl_3 + 2SbF_3 \xrightarrow{SbCl_5} 3CHClF_2 + 2SbCl_3$$
$$2CHClF_2 \xrightarrow[90\%\ \text{yield}]{700\text{–}900^\circ} CF_2{=}CF_2 + 2HCl$$

The latter reaction involves difluorocarbene ($:CF_2$):

$$CHClF_2 \longrightarrow :CF_2 + HCl$$
$$2\ :CF_2 \longrightarrow CF_2{=}CF_2$$

In the presence of peroxides, tetrafluoroethene polymerizes to the long-chain polymer. If peroxides are excluded, [2 + 2] cycloaddition occurs in high yield to give octafluorocyclobutane (see Section 13-3D):

$$2CF_2{=}CF_2 \longrightarrow \begin{matrix} CF_2{-}CF_2 \\ | \quad\quad | \\ CF_2{-}CF_2 \end{matrix}$$

Similar cycloaddition reactions occur with chlorotrifluoroethene and 1,1-dichloro-2,2-difluoroethene.

Radical polymerization of chlorotrifluoroethene gives a useful polymer (Kel-F) that is similar to Teflon.

An excellent elastomer of high chemical resistance (Viton) can be made by copolymerizing hexafluoropropene with 1,1-difluoroethene. The product is stable to 300° and is not attacked by hot concentrated nitric acid. Although expensive, it is unrivaled among elastomers for chemical durability under extreme conditions.

5-8E Properties of Fluorocarbons

The fluorocarbons have extraordinarily low boiling points relative to the hydrocarbons of comparable molecular weight. As seen in Figure 5-3, their boiling points are nearly the same or even lower than those of the alkanes or cycloalkanes with the same number of carbons. Thus octafluorocyclobutane or boils 17° lower than cyclobutane, despite an almost fourfold greater molecular weight!

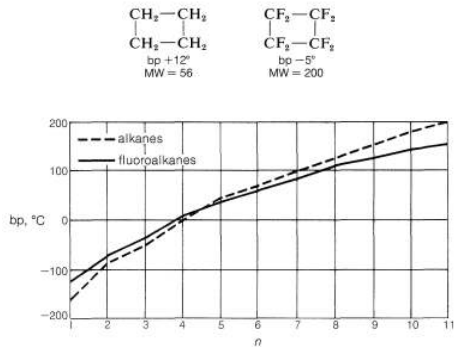


Figure 5-3: Boiling points of straight-chain fluorocarbons ($C_nF_{2n_2}$) and hydrocarbons ($C_nH_{2n_2}$).

Fluorocarbons are very insoluble in most polar solvents and are only slightly soluble in alkanes in the kerosene range. The higher-molecular-weight fluorocarbons are not even miscible in all proportions with their lower-molecular-weight homologs.

The physiological properties of organofluorine compounds vary widely. Dichlorodifluoromethane and the saturated fluorocarbons appear to be completely nontoxic. In contrast, perfluoro-2-methylpropene is exceedingly toxic, more so than the war gas, carbonyl dichloride ($COCl_2$). Sodium fluoroethanoate (CH_2FCO_2Na) and 2-fluoroethanol are toxic fluorine derivatives of oxygen-containing organic substances. The fluoroethanoate salt is sold commercially as a rodenticide. Interestingly, sodium trifluoroethanoate is nontoxic.

Fluorocarbon derivatives have another interesting and potentially useful property. They dissolve large quantities of oxygen. This fact, combined with their nontoxicity, has led to their use as blood replacements in heart surgery on experimental animals. Mice can live totally immersed in oxygen-saturated liquid fluorocarbons.

[1]Life with carbenes is substantially complicated by the fact that there are two different forms (singlet and triplet) of $:CH_2$ and presumably of all other carbenes. The two forms of $:CH_2$ differ considerably in their reactivity. One is the **singlet**, which has its unshared electrons paired, while the other is the **triplet** with the same electrons unpaired. For $:CH_2$, the singlet form is the less stable and more reactive, whereas with $:CCl_2$, the triplet is the less stable and more reactive.

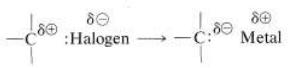
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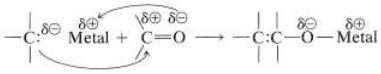
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5.9: Organometallic Compounds from Organohalogen Compounds

One of the more important reactions of organohalogen compounds is the formation of **organometallic** compounds by replacement of the halogen by a metal atom. Carbon is positive in carbon-halogen bonds and becomes negative in carbon-metal bonds, and therefore carbon is considered to be *reduced* in formation of an organometallic compound (Section 11-1):



This transformation is of value because it makes an electrophilic carbon into a nucleophilic carbon. Organometallic compounds are a convenient source of nucleophilic carbon. A typical example of their utility is the way the achieve addition of nucleophilic carbon to carbonyl groups with formation of carbon-carbon bonds:



In this chapter we will restrict our discussion of organometallic compounds to the alkyl and aryl compounds of magnesium and lithium, and the sodium and potassium salts of 1-alkynes. These substances normally are derived directly or indirectly from organohalogen compounds and are used very widely in organic synthesis. Organometallic compounds of transition metals and of boron are discussed in Chapters 11 and 31.

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5.10: Properties of Organometallic Compounds

How carbon-metal bonds are formed depends on the metal that is used. Conditions that are suitable for one metal may be wholly unsuited for another. Some organometallic compounds react very sluggishly even toward acids, whereas others react avidly with water, oxygen, carbon dioxide, and almost all solvents but the alkanes themselves. Reactivity increases with increasing polarity of the carbon-metal bond, which is determined by the electropositivity of the metal. Strongly electropositive metals, such as sodium and potassium, form largely ionic bonds to carbon, as we have mentioned in the case of alkynide salts, $RC \equiv C^{\ominus}Na^{\oplus}$. Estimates of the ionic character of various carbon-metal bonds are given in Table 5-3, and it will be seen that *organosodium and organopotassium compounds have the most ionic bonds and they are, in fact, among the most reactive organometallic compounds known*. Many organosodium and organopotassium compounds burn spontaneously when exposed to air and react violently with water and carbon dioxide. As might be expected from their saltlike character, they are nonvolatile and do not dissolve readily in nonpolar solvents. In contrast, the more covalent, less ionic, organometallic compounds, such as $(CH_3)_2Hg$, are far less reactive; they are stable in air, quite volatile, and dissolve in nonpolar solvents.

Table 5-3: Percent Ionic Character of Carbon-Metal Bonds[a]

Bond	Percent Ionic Character	Bond	Percent Ionic Character	Bond	Percent Ionic Character
C-K	51%	**C-Mg**	35%	**C-Sn**	12%
C-Na	47%	**C-Al**	22%	**C-Pb**	12%
C-Li	43%	**C-Zn**	18%	**C-Hg**	9%
C-Ca	43%	**C-Cd**	15%		

Source: L. Pauling, The Nature of the Chemical Bond, Cornell University Press, Ithaca, N.Y. 3rd, 1960, Chap. 3

All of these compounds must be handled with great care because some are dangerously reactive and others are very toxic. They seldom are isolated from the solutions in which they are prepared, but are used immediately in other reactions.

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5.11: Preparation of Organometallic Compounds

5-11A Metals with Organic Halides

The reaction of a metal with an organic halide is a convenient method for preparation of organometallic compounds of reasonably active metals such as lithium, magnesium, and zinc. Ethers, particularly diethyl ether and oxacyclopentane (tetrahydrofuran), provide inert, slightly polar media in which organometallic compounds usually are soluble. Care is necessary to exclude moisture, oxygen, and carbon dioxide, which would react with the organometallic compound. This can be accomplished by using an inert atmosphere of nitrogen or helium.

$$CH_3Br + 2Li \xrightarrow{(CH_3CH_2)_2O} CH_3Li + LiBr \quad \text{methyllithium}$$

$$CH_3CH_2Br + Mg \xrightarrow{(CH_3CH_2)_2O} CH_3CH_2MgBr \quad \text{ethylmagnesium bromide}$$

The reactivity order of the halides is $\mathbf{I > Br > Cl \gg F}$. Whereas magnesium and lithium react well with chlorides, bromides, and iodides, zinc is satisfactory only with bromides and iodides. Mercury only reacts when amalgamated with sodium. Sodium and potassium present special problems because of the high reactivity of alkylsodium and alkylpotassium compounds toward ether and organic halides. Alkane solvents usually are necessary.

Alkenyl, alkynyl, and aryl halides, like alkyl halides, can be converted to the corresponding magnesium and lithium compounds. However, the reaction conditions, such as choice of solvent, can be critical. Bromoethene, for instance, can be converted to ethenylmagnesium bromide in good yield if the solvent is oxacyclopentane [tetrahydrofuran, $(CH_2)_4O$]:

$$CH_2{=}CH{-}Br + Mg \xrightarrow[CH_2\text{-}CH_2]{O} CH_2{=}CH{-}MgBr$$

The more reactive allylic and benzylic halides present a problem - not so much in forming the organometallic derivative as in keeping it from reacting further with the starting halide. An often unwanted side reaction in the preparation of organometallic compounds is a displacement reaction, probably of the S_N2 type:

$$CH_2{=}CH{-}CH_2{-}Cl: + R^{\delta\ominus}\cdots{}^{\delta\oplus}MgX \longrightarrow CH_2{=}CH{-}CH_2{-}R + MgXCl$$

This problem can be lessened greatly by using a large excess of magnesium and dilute solutions of the allylic halide to minimize the coupling reaction.

The same difficulty also occurs in the preparation of alkylsodium compounds. The starting halide **RX** couples with **RNa** (to give **R–R** and **NaX**) or is converted to an alkene. These reactions appear to involve radical intermediates undergoing combination and disproportionation (Section 10-8C):

$$CH_3CH_2{-}Br + Na\cdot \longrightarrow CH_3CH_2\cdot + Br^{\ominus} + Na^{\oplus}$$

$$CH_3CH_2\cdot + Na\cdot \longrightarrow CH_3CH_2Na$$

$$2CH_3CH_2\cdot \begin{cases} \longrightarrow CH_3CH_2CH_2CH_3 & \text{coupling (combination)} \\ \longrightarrow CH_2{=}CH_2 + CH_3CH_3 & \text{disproportionation} \end{cases}$$

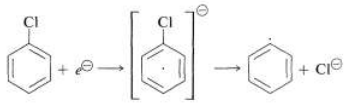
In the absence of metallic sodium, ethylsodium probably still reacts with ethyl bromide by a radical reaction rather than S_N2 or $E2$. This happens because $CH_3CH_2^-$ tends to lose an electron easily and can act like metallic sodium to donate an electron to CH_3CH_2Br to form an ethyl radical and itself become an ethyl radical:

$$CH_3CH_2{:}^{\ominus} + CH_3CH_2{-}Br \longrightarrow 2CH_3CH_2\cdot + Br^{\ominus}$$

Reactions between the resulting radicals then produce butane, ethane, and ethene.

The point at which one can expect S_N2 and $E2$ reactions to go faster than radical formation as the structures of the halides and the nature of the metal are changed is not yet clearly defined. However, it is becoming increasingly evident that there are substitution

reactions of "unactivated" aryl halides that proceed without rearrangement by way of radical intermediates. The key step in these reactions is donation of an electron to one of the unfilled π orbitals of the ring and subsequent ejection of a halide ion:



Such a mechanism probably is involved in the formation of organometallic compounds from aryl halides and metals.

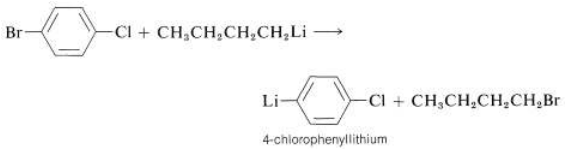
5-11B Some Other Preparations of Organometallic Compounds

Brief descriptions follow of less general but very useful methods of forming organometallic compounds (also see Table 5-7). In each of these preparations the solvent must be inert to all of the organometallic compounds involved.

Halogen-Metal Exchange

$$\mathbf{RBr + R'Li \rightleftharpoons RLi + R'Br} \tag{5.11.1}$$

The equilibrium in these reactions favors formation of the organometallic compound with the metal attached to the more electronegative **R** group. The method is mainly used in the preparation of organolithium compounds derived from unreactive halides such as aryl, ethenyl, or ethynyl halides. These halides do not always react readily with lithium metal, but may react well with butyllithium:



Displacement of One Metal by Another

$$\mathbf{R_2Hg + 2Na \rightleftharpoons 2RNa + Hg} \tag{5.11.2}$$

Here the equilibrium is such that the **R** group favors attachment to the more electropositive metal.

Organometallic Compounds with Metal Halides

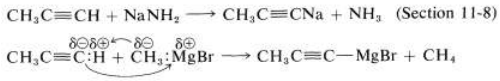
$$\mathbf{RMgCl + HgCl_2 \rightleftharpoons RHgCl + MgCl_2} \tag{5.11.3}$$

$$\mathbf{RLi + CuI \rightleftharpoons RCu + LiI} \tag{5.11.4}$$

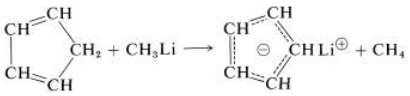
The equilibrium favors the products with **R** connected to the less electropositive metal so the reaction tends to form a less reactive organometallic compound from a more reactive one.

Organometallic Compounds from Acidic Hydrocarbons

Some organometallic compounds are prepared best by the reaction of a strong base or an alkyl metal derivative with an acidic hydrocarbon, such as an alkyne:

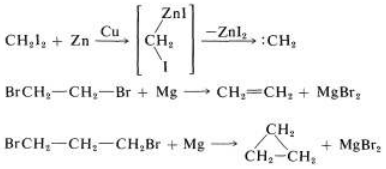


An especially important example is that of 1,3-cyclopentadiene, which is acidic because its conjugate base (cyclopentadienide anion) is greatly stabilized by electron delocalization. The anion is formed easily from the hydrocarbon and methyllithium:



5-11C Organometallic Compounds from Polyhalogen Compounds

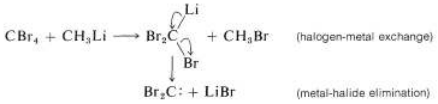
Diorganometallic compounds cannot be prepared from dihalides if the halogens are separated by three C—C bonds or less because elimination or other reactions usually predominate. With active metals and 1,1-, 1,2-, or 1,3-dihalides, the following reactions normally occur:



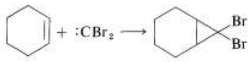
When the halogens are at least four carbons apart a diorganometallic compound can be formed:



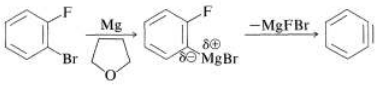
Carbenes, $R_2C:$ (Section 5-7B) are produced by α eliminations from polyhalogen compounds with organometallic reagents. The first step is halogen-metal exchange and this is followed by elimination of metal halide:



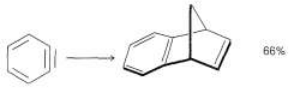
Elimination reactions of this type can be useful in synthesis for the formation of carbon-carbon bonds. For example, if dibromocarbene is generated in the presence of an alkene, it will react by cycloaddition to give a cyclopropane derivative:



A related example is the generation of benzyne from 1-bromo-2-fluorobenzene with magnesium in oxacyclopentane (tetrahydrofuran). If the temperature is kept around 0°, 2-fluorophenylmagnesium bromide is formed. At higher temperatures, magnesium halide is eliminated and benzyne results:



If a diene is present, the benzyne will react with it by a [4 + 2] cycloaddition as in the following example:



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5.12: Organomagnesium Compounds

For many years the most important organometallic compounds for synthetic purposes have been the organomagnesium halides, or **Grignard reagents**. They are named after Victor Grignard, who discovered them and developed their use as synthetic reagents, for which he received a Nobel Prize in 1912. As already mentioned, these substances customarily are prepared in dry ether solution from magnesium turnings and an organic halide:

$$CH_3I + Mg \xrightarrow{\text{ether}} CH_3MgI \qquad 95\%\ \text{yield}$$

Chlorides often react sluggishly and, in addition, may give an unwelcome precipitate of magnesium chloride, which, unlike magnesium bromide and iodide, is only very slightly soluble in ether. Organomagnesium fluorides eluded preparation until quite recently.

Although we usually write the structure of a Grignard reagent as $\mathbf{RMgX}$, in which $\mathbf{X}$ is a halogen, the structure of the reagent in ether solution is more complex. There is a rapidly established equilibrium between the organomagnesium halide ($\mathbf{RMgX}$) and the corresponding dialkylmagnesium ($\mathbf{RMgR}$):

$$\mathbf{2RMgX \rightleftharpoons R_2Mg + MgX_2} \tag{5.12.1}$$

Both of these species, $\mathbf{RMgX}$ and $\mathbf{R_2Mg}$, are reactive, and in ether solvents are solvated by coordination of the ether oxygen to magnesium. They further associate as dimers or higher polymers in solution. Although it is an oversimplification to regard a Grignard reagent as $\mathbf{RMgX}$, most of the reactions can be rationalized easily by this simple structure.

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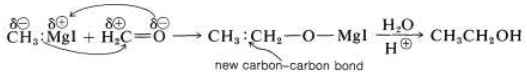
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5.13: Organomagnesium and Organolithium Compounds in Synthesis

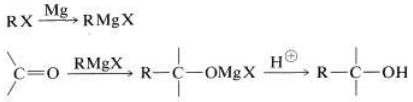
5-13A Additions to Carbonyl Groups. Synthesis of Alcohols

The most important synthetic use of Grignard reagents and organolithium reagents is to form new carbon-carbon bonds by addition to *polar* multiple bonds, particularly carbonyl bonds. An example is the addition of methyl-magnesium iodide to methanal:

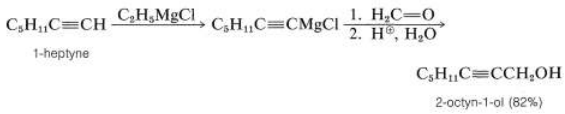


The yields of addition products in reactions of this kind are generally high. The adducts have metal-oxygen bonds that can be broken readily by acid hydrolysis to give the organic product. Grignard reagents seldom add to carbon-carbon multiple bonds (however, see Section 5-13D).

With suitable variations of the carbonyl compound, a wide range of compounds can be built up from substances containing fewer carbon atoms per molecule. The products formed when several types of carbonyl compounds react with Grignard reagents are listed in Table 5-4. The sequence of reactions starting with an organic halide, **RX**, amounts to the addition of **R–H** across a carbonyl bond.[2]



Primary alcohols can be prepared by the addition of **RMgX** or **RLi** to methanal, **CH_2=O**,



Alcohols of formula **RCH_2CH_2OH** can be prepared by addition of **RMgX** to oxacyclopropane (oxirane):

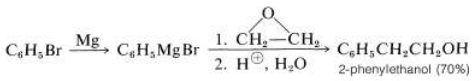
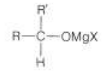
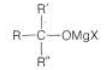
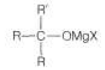
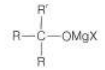
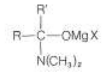
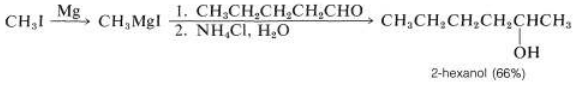
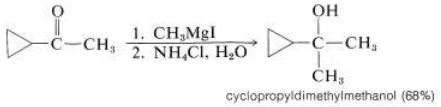


Table 5-4: *Products from the Reaction of Grignard Reagents* (**RMgX**) *with Carbonyl Compounds*

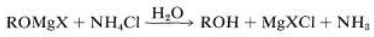
Reactant		Product	Hydrolysis product	Customary yield
methanal (formaldehyde)		RCH_2OMgX	*prim*-alcohol, RCH_2OH	good
other aldehydes			*sec*-alcohol, 	good
ketone			*tert*-alcohol, 	good to poor
carbon dioxide	CO_2	RCO_2MgX	carboxylic acid, RCO_2H	good
carboxylic ester			*tert*-alcohol, 	good to poor
acid chloride			*tert*-alcohol, 	good to poor
N,N-dimethylcarboxamide			ketone, 	good to poor

Secondary alcohols are obtained from aldehydes, whereas ketones give *tertiary alcohols*:

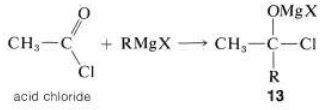


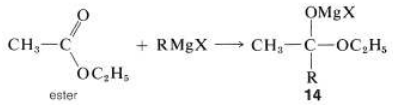


Hydrolysis of the intermediate **R–OMgX** compound is achieved best with aqueous ammonium chloride solution. Addition of water gives an unpleasant mess of $Mg(OH)_2$ whereas addition of strong acids such as **HCl** or H_2SO_4 can lead to side reactions of dehydration and so on, especially with tertiary alcohols (Section 8-9C):

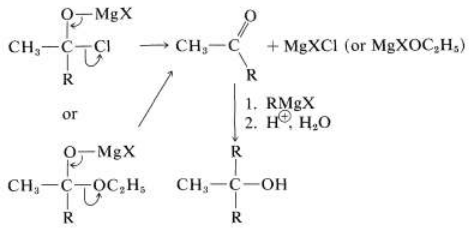


Tertiary alcohols also are obtained from both acyl halides, **RCOCl**, and esters, RCO_2R, by the addition of *two* moles of Grignard reagent. The first mole of **RMgX** adds to the carbonyl bond to give the adducts **13** or **14**:

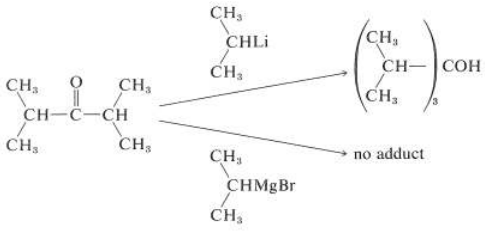




However, these first-formed adducts are unstable and decompose to a ketone, CH_3COR, and magnesium salts, **MgXCl** or $MgXOC_2H_5$. The ketone usually cannot be isolated, but reacts rapidly with more **RMgX** ultimately to give a tertiary alcohol:

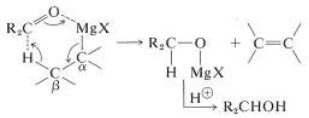


Organolithium compounds behave very much like Grignard reagents, but with increased reactivity. They offer advantages over the magnesium compounds when the **R** group or the carbonyl compound is highly branched. For instance, isopropyllithium adds in good yield to 2,4-dimethyl-3-pentanone, whereas isopropylmagnesium bromide fails completely to give the normal addition product:



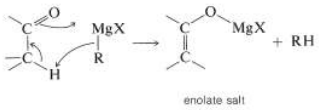
Failure of Grignard reagents to add in the normal way generally is because reactions by alternative paths occur more rapidly. If the Grignard reagent has a hydrogen on the carbon adjacent to the point of attachment of **–MgX** (i.e., a β hydrogen), then reduction can occur, with the effect of adding H_2 to the carbonyl group.

Side reactions - reduction



Furthermore, if the carbonyl compound has a hydrogen located on the carbon next to the carbonyl group, the Grignard reagent can behave as a base and remove this hydrogen as a proton. The result is that the compound becomes an enolate salt and **RMgX** becomes **RH**.

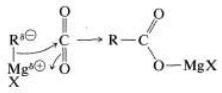
Side reactions - enolization



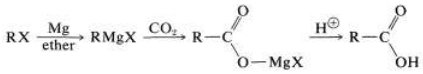
Apparently, the complicating side reactions observed with **RMgX** are not nearly as important with **RLi**. The reasons for this difference are not well understood.

5-13B Synthesis of Carboxylic Acids

The reaction of carbon dioxide with Grignard reagents initially gives a magnesium salt of a carboxylic acid, RCO_2MgX:

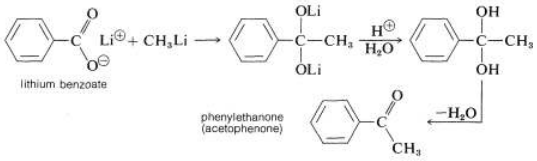


This salt, which has a carbonyl group, in principle could add a second **RMgX**. However, further addition is usually slow, and for most practical purposes the reaction stops at this stage. If the reaction can go further, the *worst* way to run it is by bubbling CO_2 into the Grignard solution. This exposes the first-formed RCO_2MgX to excess **RMgX** and may lead to further addition reactions. The easy way to avoid this problem is to pour the **RMgX** solution onto powdered dry ice (solid CO_2). Hydrolysis of the product (here a stronger acid than NH_4Cl is required) generates the carboxylic acid, RCO_2H:

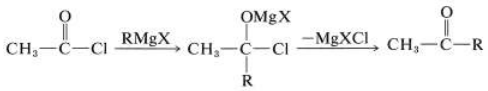


5-13C Synthesis of Ketones

Although organomagnesium compounds are not sufficiently reactive to add to carboxylate anions, alkyllithium compounds add quite well. A useful synthesis of methyl ketones involves the addition of methyllithium to the lithium salt of a carboxylic acid:



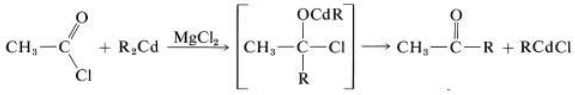
Other methods begin with acid chlorides or esters and attempt to add only *one* mole of **RMgX**:



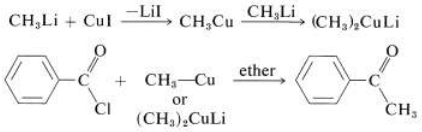
The disadvantage of using Grignard reagents for this purpose is that they add very rapidly to the ketone as it is formed. There are two ways in which this disadvantage can be minimized. First, one can add the Grignard solution to an excess of acid chloride solution (the so-called “inverse addition” procedure) to keep the concentration of **RMgX** in the reaction mixture low, and hope that the reaction will stop at the ketone stage. However, this device seldom works very well with acid chlorides. Better results can be obtained with **RMgX** and $R'CON(CH_3)_2$. The second method is to use a less reactive organometallic compound - one that will react with **RCOCl** but not with $R_2C{=}O$. One easy way to do this is to add cadmium chloride to the Grignard solution, whereby an

organocadmium compound, R_2Cd, is formed (cf. Section 5-10B, Method 3). In the presence of magnesium halides, R_2Cd reacts moderately rapidly with acid chlorides, but only slowly with ketones. The addition therefore can be arrested at the ketone stage:

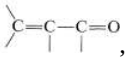
$$2RMgCl + CdCl_2 \rightarrow R_2Cd + 2MgCl_2 \qquad (5.13.1)$$



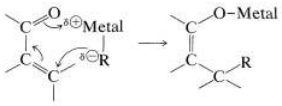
Alkylcopper compounds, R—Cu, also are selective reagents that react with acid chlorides to give ketones, but do not add to esters, acids, aldehydes, or ketones. The R—Cu compounds can be prepared from CuI and the alkyllithium. With an excess of the alkyllithium, the alkylcopper is converted to R_2CuLi:



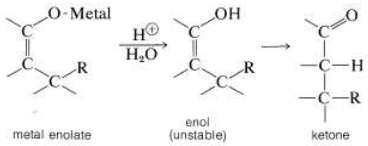
5-13D 1,4 Additions to Unsaturated Carbonyl Compounds

A conjugated alkenone,  , can react with an organometallic reagent by a normal 1,2 addition across the carbonyl group, or by 1,4 addition to the conjugated system.

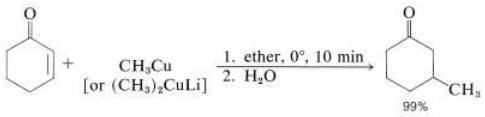
1,4 addition



On hydrolysis, the 1,4 adduct first yields the corresponding enol, but this is normally unstable and rearranges rapidly to the ketone. The final product therefore corresponds to addition of R—H across the carbon-carbon double bond:

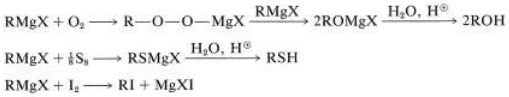


Organomagnesium and organolithium compounds can add both 1,2 and 1,4 to alkenones, and the relative importance of each mode of addition depends on the structure of the reactants. This sort of dual behavior can be a nuisance in synthetic work because it leads to separation problems and low yields. Organocopper compounds are a great help in this situation because they show a very high selectivity for 1,4 addition and add to unsaturated ketones in excellent yield:



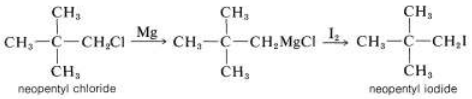
5-13E Oxygen, Sulfur, and Halogens

Grignard reagents react with oxygen, sulfur, and halogens to form substances containing C—O, C—S, and C—X bonds, respectively:



These reactions are not often important for synthesis because the products, ROH, RSH, and RX, can be obtained more conveniently and directly from alkyl halides by S_N1 and S_N2 displacement reactions, as described in Chapter 8. However, when both S_N1 and

S_N2 reactions are slow or otherwise impractical, as for neopentyl derivatives, the Grignard reactions can be very useful:



Also, oxygenation of a Grignard reagent at *low* temperatures provides an excellent method for the synthesis of hydroperoxides:

$$RMgX + O_2 \xrightarrow{-70^\circ} ROOMgX \xrightarrow{H^\oplus} ROOH$$

To prevent formation of excessive amounts of the alcohol, **inverse addition** is desirable (i.e., a solution of Grignard reagent is added to ether through which oxygen is bubbled rather than bubbling oxygen through a solution of the Grignard reagent).

Table 5-5: *Methods of Preparation of Organic Halides*

Reaction	Comments
Alkyl halides	
1. *Alcohols and hydrogen halides* $ROH + HBr \longrightarrow RBr + H_2O$ $ROH + HCl \xrightarrow{ZnCl_2} RCl + H_2O$	Reactivity: *tertiary* > *secondary* > *primary*; chlorides require $ZnCl_2$ as catalyst (Section 15-5A); rearrangement is common when the displacement is S_N1 type.
2. *Alcohols and phosphorus halides* $3ROH + PBr_3 \xrightarrow{\text{pyridine}} 3RBr + H_3PO_3$	Best with *primary* ROH; pyridine (azabenzene, a weak nitrogen base) acts to keep the acidity low, which may reduce side reactions such as rearrangement.
3. *Alcohols and thionyl chloride* $ROH + SOCl_2 \xrightarrow{\text{pyridine}} RCl + SO_2 + HCl$	As in Method 2. (See Section 15-5A.)
4. *Halogenation of hydrocarbons* $RH + X_2 \xrightarrow{h\nu} RX + HX$	Radical-chain reaction (Sections 4-4 and 4-5); Br_2 is more selective than Cl_2; used for preparation of 2-propenyl and arylmethyl halides; other reagents include SO_2Cl_2 (Section 4-5B), *tert*-butyl hypochlorite and *N*-bromosuccinimide (Section 14-3A).
5. *Addition of hydrogen halides to alkenes* $RCH{=}CH_2 \xrightarrow{HX} RCH(X){-}CH_3$ $RCH{=}CH_2 \xrightarrow{HBr,\ peroxides} RCH_2CH_2Br$	See Sections 10-4 and 10-7.
6. *Addition of halogens to alkenes* $RCH{=}CH_2 + X_2 \longrightarrow RCHXCH_2X$	Gives 1,2-dihalides (Section 10-3A).
7. *Inorganic halides and carbonyl compounds* $R_2C{=}O \xrightarrow{PCl_5} R_2CCl_2$ $R_2C{=}O \xrightarrow{MoF_6} R_2CF_2$	Gives 1,1-dihalides (Section 16-4D).
8. *Organometallic compounds and halogens* $RMgX + X_2 \longrightarrow RX + MgX_2$ $RHgX + X_2 \longrightarrow RX + HgX_2$	Of limited use because the RX compound is usually the source of the organometallic reagent (however, see Section 14-12E).
9. *Chloromethylation of arenes* $ArH + CH_2O + HCl \xrightarrow[(-H_2O)]{} ArCH_2Cl$	Electrophilic substitution of ArH; gives benzylic (arylmethyl) chlorides (see Section 26-4A, Exercise 22-21).
Alkenyl halides	
10. *Addition of halogens and hydrogen halides to alkynes* $RC{\equiv}CH + HX \longrightarrow RCX{=}CH_2 + RCH{=}CHX$ $RC{\equiv}CH + X_2 \longrightarrow RCX{=}CHX$	May require mercury catalyst (Section 10-5).
11. *Elimination of hydrogen halides from dihalides* $RCHXCH_2X \xrightarrow{\text{base}} RCH{=}CHX + RCX{=}CH_2$ $RCH_2CHX_2 \xrightarrow{\text{base}} RCH{=}CHX$	E2 (see Section 8-8).
1-Alkynyl halides	
12. *Halogenation of alkynide salts* $RC{\equiv}CNa + X_2 \longrightarrow RC{\equiv}CX$	As in Method 8.
13 *Halogenation of 1-alkynes with hypohalites* $RC{\equiv}CH + HOX \longrightarrow RC{\equiv}CX$	Very useful (Section 14-4).

14. *Elimination of hydrogen halides* $RCH{=}CX_2 \xrightarrow{\text{base}} RC{\equiv}CX$	As in Method 11.
15. *Chloroethynylation of electrophilic carbon* $ClC{\equiv}CH \xrightarrow{NaNH_2} ClC{\equiv}CNa \xrightarrow{RX} ClC{\equiv}CR$	See Sections 14-10B and 11-8C.
Aryl halides	
16. *Halogenation of arenes* $ArH + X_2 \longrightarrow ArX + HX$	Electrophilic substitution of ArH; may require a catalyst [Fe(II) or I_2]; used for Cl_2 and Br_2 (see Section 22-4D).
17. *From aromatic amines* $ArNH_2 \xrightarrow{HONO} Ar\overset{\oplus}{N_2} \xrightarrow[Cu(I)/Cu(II)]{X^{\ominus}} ArX$	Amine is converted to diazonium salt ($ArN_2^{\oplus}$), which is replaced by halide ion; a copper catalyst is required (see Section 23-10B).

Table 5-6: *Reactivities of Organohalogen Compounds in Displacement and Elimination Reactions*

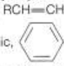
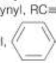
Organic halide	S_N2	Reactivity E2	S_N1, E1
prim-alkyl, RCH_2X	good	fair	very poor
sec-alkyl, R_2CHX	fair	fair	fair
tert-alkyl, R_3CX	poor	good	good
allylic, $RCH{=}CHCH_2X$	good	–	good
benzylic,  $-CH_2X$	good	–	good
alkenyl, $RCH{=}CHX$	poor	fair	poor
alkynyl, $RC{\equiv}CX$	poor	–	poor
aryl,  $-X$	very poor	–	poor

Table 5-7: *Methods of Preparation of Organometallic Compounds*

Method	Reaction
1. *Organohalogen compounds with metals:* solvent is diethyl ether or oxacyclopentane (tetrahydrofuran); inert atmosphere desirable to keep out moisture and air; mostly used for magnesium and lithium	$RX + Mg \xrightarrow{\text{ether}} RMgX$
2. *Halogen–metal exchange:* used when Method 1 fails because the halide is unreactive	$RX + R'Li \longrightarrow RLi + R'X$
3. *Metal–metal exchange:* replacement of one metal by a less electropositive metal	$RLi + CuI \longrightarrow RCu + LiI$
4. *Metal–metal exchange:* replacement of one metal by a more electropositive metal	$R_2Mg + 2Li \longrightarrow 2RLi + Mg$
5. *Replacement of acidic hydrogen*	$RC{\equiv}CH \xrightarrow[NH_3]{NaNH_2} RC{\equiv}CNa$ $RC{\equiv}CH + RMgX \longrightarrow RC{\equiv}CMgX + RH$

[2]It is not possible to add RH to $>C{=}O$ directly because ΔG^0 generally is somewhat unfavorable [$+5$ kcal for $CH_4 + (CH_3)_2C{=}O \rightarrow (CH_3)_3COH$]. How we get around this unfavorable equilibrium in practice provides an interesting example of how energy can be (and is) squandered to achieve some particular desired result; for example, the reaction $CH_3CH_3 + CH_3CHO \rightarrow CH_3CH_3CH(CH_3)OH$ has $\Delta H^0 = -12$ kcal but $\Delta G^0 = +0.5$ kcal. A possible sequence is

$$CH_3CH_3 + Br_2 \longrightarrow CH_3CH_2Br + HBr \qquad \Delta H^0 = -10 \text{ kcal}$$
$$CH_3CH_2Br + 2Li \longrightarrow CH_3CH_2Li + LiBr \qquad \Delta H^0 = -76 \text{ kcal}$$
$$\left.\begin{array}{l} CH_3CH_2Li + CH_3CH{=}O \longrightarrow CH_3CH_2CH(CH_3)OLi \\ CH_3CH_2CH(CH_3)OLi + H_2O \longrightarrow \\ \qquad CH_3CH_2CH(CH_3)OH + LiOH\ (aq) \end{array}\right\} \quad \Delta H^0 = -71 \text{ kcal}$$

The overall result is the expenditure of $10 + 76 + 71 = 157$ kcal to achieve a reaction that itself has $\Delta H^0 = -12$ kcal, but an unfavorable ΔG^0. (Li is used in this example rather than Mg because the heat of formation of C_2H_5MgBr is not available.)

Contributors

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5.P: Organohalogen and Organometallic Compounds (Problems)

Problem 5-1 Deduce the structures of the two compounds whose NMR and infrared spectra are shown in Figure 5-1. Assign as many of the infrared bands as you can and analyze the NMR spectra in terms of chemical shifts and spin-spin splittings.

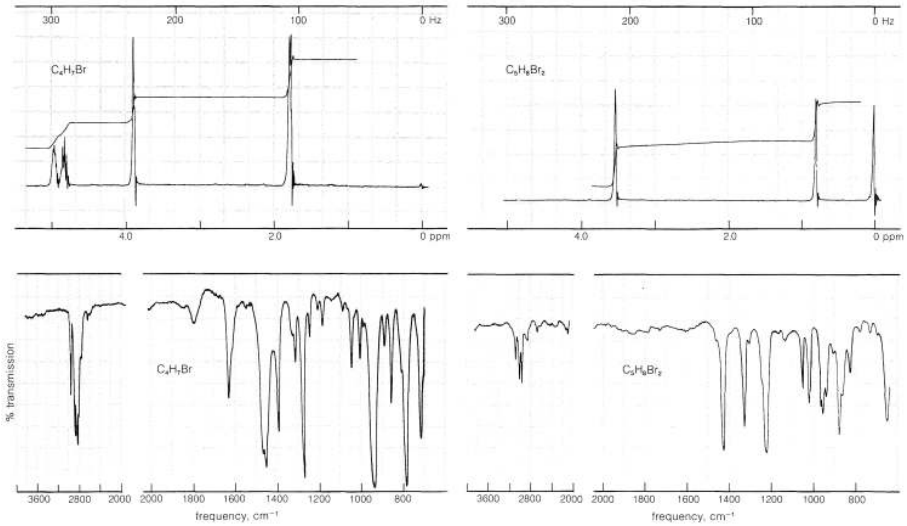


Figure 5-1: *Infrared and proton NMR spectra of substance* $\mathbf{C_4H_7Br}$ *and substance* $\mathbf{C_5H_8Br_2}$ *(see Problem 5-1). NMR spectra are at* $\mathbf{60\ MHz}$ *with reference to TMS at* $\mathbf{0.0}$.

Problem 5-2

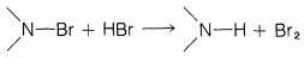
a. Methyl iodide can be prepared from potassium iodide and dimethyl sulfate. Why is dimethyl sulfate preferable to methanol in reaction with potassium iodide?

b. 1-Bromobutane can be prepared from 1-butanol and sodium bromide in concentrated sulfuric acid. What is the function of the sulfuric acid?

c. Some people like to put salt in their beer. Assess the possibility of $\mathbf{CH_3CH_2Cl}$ poisoning from the reaction of $\mathbf{NaCl}$ with the ethanol in beer. Give your reasoning.

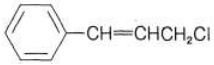
d. Both isopropyl bromide and *tert*-butyl bromide react with sodium ethoxide in ethanol. Which bromide would give the most alkene? Which bromide would give the most alkene on solvolysis in $\mathbf{60\%}$ aqueous ethanol? Of the two reagents, sodium ethoxide in ethanol or $\mathbf{60\%}$ aqueous ethanol, which would give the most alkene with each bromide? Give your reasoning.

Problem 5-3 The reaction of **N**-bromosuccinimide (NBS) with alkenes to produce allylic bromides is though to involve molecular bromine produced by the reaction



Show the propagation steps that probably are involved in the radical-chain bromination of cyclohexane with NBS, assuming that bromine atoms are produced from NBS in the initiation steps. What by-products would you anticipate?

Problem 5-4 Give a reasonable method for the synthesis of the following compounds from organic compounds not containing a halogen. Indicate the structures of any major by-products expected.

a. 

b. $\mathbf{HC \equiv CCH_2Br}$

c. 

d. $CH_3-CH(Cl)-CH=CH-CH_2CH_3$

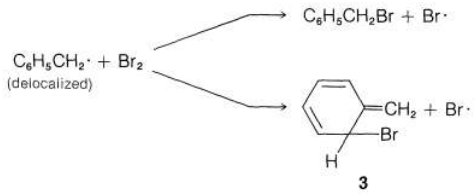
e. $(CH_2=CH)_2CHCl$

Problem 5-5 In the presence of only traces of ionizing agents, either pure 1-chloro-2-butene or 3-chloro-1-butene is converted slowly to a 50-50 equilibrium mixture of the two chlorides. Explain.

Problem 5-6

a. Write the initiation and propagation steps involved in the radical bromination of methylbenzene (toluene) with bromine. Write the low-energy valence-bond structures of the intermediate phenylmethyl radical.

b. Calculate ΔH^0 for the following reactions of the radical, using the C—Br bond strength of $C_6H_5CH_2Br$ (55 kcal), and any other necessary bond energies. Assume that stabilization arising from electron delocalization is 38 kcal for a phenyl group (Section 6-5A) and 5 kcal for the triene structure **3**.



3

What can you conclude from these calculations about the stability of **3** and the likelihood of its formation in this kind of bromination?

Problem 5-7 Explain the following observations.

a. 1,2-Propadiene gives 3-chloropropyne on radical chlorination with either Cl_2 or *tert*-butyl hypochlorite.

b. 1-Chloro-2-propanone could be regarded as a kind of allylic chloride, but it is very unreactive under S_N1 conditions although it is highly reactive in S_N2 reactions.

c. The enantiomers of 3-chloro-1-butene racemize somewhat more rapidly than they give solvolysis products, under conditions that favor S_N1 reactions when a good ionizing but *weakly nucleophilic* solvent is used.

Problem 5-8 Would you expect the behavior of 3-chloropropyne to more nearly resemble 1-chloropropane or 3-chloropropane in nucleophilic displacement reactions? Give your reasoning.

Problem 5-9 Arrange the following halides in order of expected *increasing reactivity* towards (a) sodium iodide in acetone and (b) silver nitrate in ethanol. Indicate your reasoning.

$$C_6H_5CH_2CH_2Cl,\ C_5H_5C\equiv CCl,\ C_6H_5C\equiv CCH_2Cl,\ C_6H_5CH=CHCl \tag{5.P.1}$$

Problem 5-10 Write a reasonable mechanism for the formation of phenylethanoic acid on heating phenylbromoethyne with potassium hydroxide in aqueous alcohol:

$$C_6H_5C\equiv CBr \xrightarrow[KOH]{H_2O-C_2H_5OH} \xrightarrow{H^\oplus} C_6H_5CH_2CO_2H \tag{5.P.2}$$

Problem 5-11

a. Write resonance structures analogous to structures 5*a* through 5*d* to show the activating effect of $-C\equiv N$ and $-SO_2R$ groups in nucleophilic substitution of the corresponding 4-substituted chlorobenzenes.

b. How would you expect the introduction of methyl groups *ortho* to the activating group to affect the reactivity of 4-bromonitrobenzene and 4-bromocyanobenzene toward ethoxide ion? (Investigate the geometry of the anion intermediate.)

Problem 5-12 Would you expect 4-bromonitrobenzene or (4-bromophenyl)-trimethylammonium chloride to be more reactive in bimolecular replacement of bromine by ethoxide ion?

Problem 5-13* Would you expect 4-chloromethoxybenzene and 4-chlorotrifluoromethylbenzene to be more, or less, reactive than chlorobenzene toward methoxide ion? Explain.

Problem 5-14* Whereas the order of reactivity of alkyl halides toward a given nucleophile is $\mathbf{I > Br > Cl \gg F}$, the reverse order of reactivity frequently is observed with aryl halides ($\mathbf{F \gg Cl \cong Br \cong I}$). What does this signify regarding the relative rates of the addition and elimination steps (Equations 5-3 and 5-4) in this kind of aromatic substitution?

Problem 5-15 The reactions of several 1-substituted 2,4-dinitrobenzenes with piperidine (azacyclohexane), Equation 5-5, proceed at nearly the same rate, *independent of the nature of* **X**. Rationalize this observation in terms of a mechanism of nucleophilic aromatic substitution.

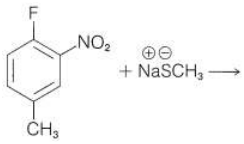


X = C_6H_5S, Br, Cl, $C_6H_5SO_2$, *p*-$NO_2C_6H_4O$, I

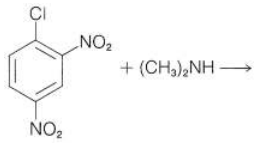
Problem 5-16* The reaction of 1-fluoro-2,4-dinitrobenzene with dimethylamine is catalyzed by weak bases. How may this observation be explained? (Consider possible intermediates, rate-determining steps, etc.)

Problem 5-17 Write the products of the following reactions:

a.



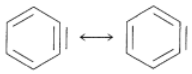
b.



c.



Problem 5-18 Two valence-bond structures are possible for benzyne:



How do these differ from the Kekulé structures usually written for benzene? Devise an atomic-orbital model for benzyne.

Problem 5-19 The intervention of benzyne in the amination of chlorobenzene, bromobenzene, and iodobenzene with sodium amide in liquid ammonia originally was demonstrated by J. D. Roberts using ^{14}C-labeled halobenzenes. Show explicitly how the use of a chlorobenzene-^{14}C label could differentiated between amination by *addition-elimination* (Section 5-6B) versus amination by *elimination-addition (benzyne mechanism)*.

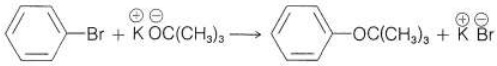
Problem 5-20 In the hydrolysis of chlorobenzene-1-^{14}C with **4 M** aqueous sodium hydroxide at **340°**, the products are **58%** benzenol-1-^{14}C and **42%** benzenol-2-^{14}C. Calculate the percentage of reaction proceeding (a) by an elimination-addition mechanism, and (b) by direct nucleophilic displacement. Would you expect the amount of direct displacement to increase, or decrease, if the reaction were carried out (a) at **240°** and (b) with lower concentrations of sodium hydroxide? Give you reasoning.

Problem 5-21 Explain the following observations:

a. 2,6-Dimethylchlorobenzene does not react with potassium amide in liquid ammonia at **−33°**.

b. Fluorobenzene, labeled with deuterium in the 2- and 6-positions, undergoes rapid exchange of deuterium for hydrogen in the presence of potassium amide in liquid ammonia, but does not form benzenamine (aniline).

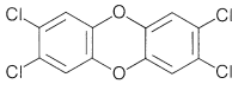
Problem 5-22* Bromobenzene reacts rapidly with potassium *tert*-butoxide in $(CH_3)_2SO$ (methylsulfinylmethane, dimethyl sulfoxide, DMSO) to give *tert*-butyl phenyl ether:



A comparable reaction does not take place in *tert*-butyl alcohol as solvent (see Section 8-7F). Suggest a mechanism for the reaction and explain why DMSO is a better solvent for the reaction than *tert*-butyl alcohol. What products would you expect to be formed using 4-bromo-1-methylbenzene in place of bromobenzene?

Problem 5-23* Both 2,4-D and 2,4,5-T are herbicides that have been used for weed control and as defoliating agents in jungle warfare. Apart from the arguments for or against the use of chemicals for such purposes, there have been reports of serious dermatitis among the industrial workers who produce these substances.

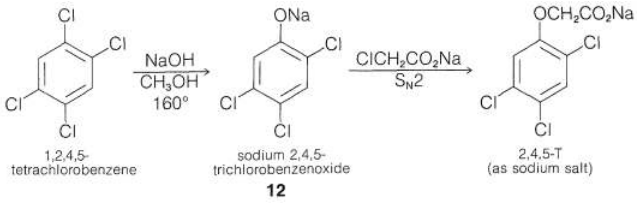
The cause finally was traced to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is produced as an impurity in the manufacture of 2,4,5-T.



TCDD

This substance (TCDD) is very toxic. In addition to the dermatitis in produces, it is a potent teratogen (induces birth abnormalities). The lethal does is less than 10^{-6} g for guinea pigs. Its presence in 2,4,5-T can be eliminated, but the conditions by which it is formed are pertinent to our present discussion.

The production of 2,4,5-T involves the substitution of one chlorine of 1,2,4,5-tetrachlorobenzene with hydroxide ion to give **12**. This is followed by a second displacement reaction, this time on chloroethanoate by the sodium salt of **12**:



12

If the temperature of the first step exceeds **160°**, then two molecules of **12** react in a double nucleophilic displacement to give TCDD.

a. Write reasonable mechanisms for the steps by which two molecules of **12** are converted to TCDD.

b. Would you expect TCDD to be formed in the preparation of 2,4-D from 1,2,4-trichlorobenzene? Explain.

Problem 5-24 What products would you expect from the reaction of bromoform, $CHBr_3$, with potassium *tert*-butyl alcohol in the presence of (a) *trans*-2-butene and (b) *cis*-2-butene?

Problem 5-25* Devise atomic-orbital models of the singlet and triplet forms of $:CH_2$. Of these one has a much greater H–C–H angle than the other. Deduce whether the triplet or the singlet form should have the wider H–C–H angle. (Remember the Pauli principle, Section 6-1.)

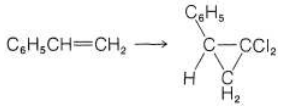
Problem 5-26 Write the structures of the products of the following equations:

a. $C_6H_5CH_2CH_2MgBr + (CH_3)_2SO_4 \rightarrow$
b. $C_2H_5MgBr + CH_3C \equiv C{-}CH_2Br \rightarrow$
c. $CH_2{=}CH{-}CH_2Li + CH_2{=}CH{-}CH_2Cl \rightarrow$
d. $CH_3CH_2CH_2MgBr + ClCH_2OCH_3 \rightarrow$
e. $C_4H_9Na + C_4H_9Br \rightarrow$

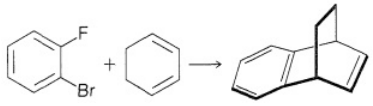
Problem 5-27* Show the steps involved in the formation of 2,3-pentadiene from *cis*-2-butene (1 mole), carbon tetrabromide (1 mole), and methyllithium (2 moles). (See Section 5-7B for pertinent reactions.)

Problem 5-28* Show how the following transformations might be achieved. Define the reaction conditions as closely as possible. More than one step may be required.

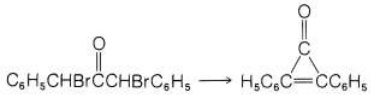
a.



b.



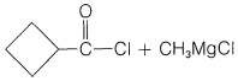
c.



Problem 5-29 Addition of Grignard reagents, $RMgX$, to diethyl carbonate, $O{=}C(OC_2H_5)_2$, gives tertiary alcohols, R_3COH, on hydrolysis. Write the steps involved in this reaction.

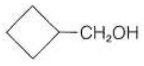
Problem 5-30 Write structures for the products of the following reactions involving Grignard reagents. Show the structures of both the intermediate substances and the substances obtained after hydrolysis with NH_4Cl solution. Unless otherwise specified, assume that sufficient Grignard reagent is used to effect those addition reactions that occur readily at room temperature.

a. $C_6H_5MgBr + C_6H_5CHO$
b. $CH_3MgI + CH_3CH_2CO_2C_2H_5$
c. $CH_3CH_2MgBr + ClCO_2C_2H_5$
d. $C_6H_5MgBr + (CH_3O)_2C{=}O$
e.



Problem 5-31 Show how each of the following substances could be prepared from the indicated organic halide and any other appropriate organic compounds:

a.


from


b. $CH_2{=}CHC(CH_3)_2OH$ from CH_3I

c. $C_6H_5C \equiv CCH_2OH$ from C_2H_5Cl

d. $CH_3CH_2CH(OH)CH_3$ from C_2H_5Cl

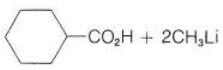
Problem 5-32 Write structures for the addition, enolization, and reduction products possible for the following reactions:

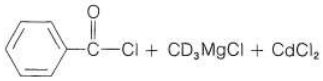
a. $CH_3COCH_3 + (CH_3)_3CMgX$
b. $C_6H_5COC_6H_5 + CH_3CH_2MgX$
c. $C_6H_5CH{=}CHCO_2C_2H_5 + C_6H_5MgX$

Problem 5-33 Grignard reagents, such as CH_3MgI, often add to the triple bond of nitriles, $RC \equiv N$, to give adducts that, on hydrolysis, yield ketones, $RCOCH_3$. Show the possible steps involved.

Problem 5-34 Show the products expected from the following combinations of reagents. Write the structures of the initial adducts and also the products they give on acid hydrolysis:

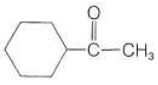
a. $(CH_3)_3CMgCl + CO_2$
b.



c. 

d. $CH_3{-}COCl + CD_3Li + CuI$

Problem 5-35 Show how the following compounds could be synthesized by reasonable reactions from the indicated compound and any other needed reagents:

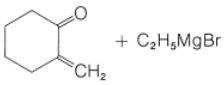
a.  from chlorocyclohexane

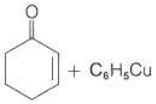
b. $C_6H_5C \equiv CCO_2H$ from phenylethyne

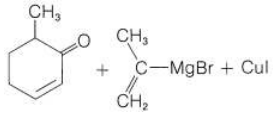
c. $ClC \equiv CCO_2H$ from ethyne

Problem 5-36 Predict the products expected from the reactions of the following compounds:

a. $CH_2{=}CH{-}CO{-}CH_3 + C_2H_5MgBr$

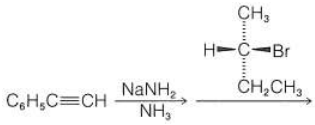
b. 

c. 

d. 

Problem 5-37 Would you expect the overall energy change (*after hydrolysis*) to be more favorable for 1,2 or 1,4 addition of a Grignard reagent to $CH_2{=}CH{-}COCH_3$? Give your reasoning.

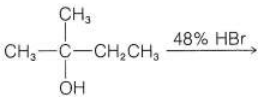
Problem 5-38 What products do you expect from the following reactions? Give your reasoning. Show the structures of the intermediate compounds in sequences of the type $\rightarrow\rightarrow$.

a. 

b. $CH_2{=}CH_2 \xrightarrow{ICl} \xrightarrow[heat]{KOH}$

c. 

d. $CH_3CH_2OH + KI + H_3PO_4 \xrightarrow{reflux}$

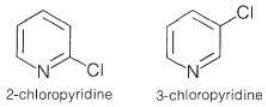
e. 

Problem 5-39 In the reaction of 5-38e, when the aqueous acid is mixed with 2-methyl-2-butanol, the mixture is initially homogenous, but it soon separates into two phases. Explain why two phases appear. On separation of the phases using a separatory funnel, which layer (upper or lower) would contain the organic product? If you were unsure, how could you quickly find out?

Problem 5-40 Suppose one could hydrolyze pure *cis*-1-chloro-2-butene exclusively by (a) the S_N1 mechanism or (b) the S_N2 mechanism. Would you expect the 2-butenol formed in each case to be the cis isomer, the trans isomer, or a mixture? Explain.

Problem 5-41 The intermediate carbocation formed by S_N1 reactions of either 3-chloro-3-methyl-1-butene or 4-chloro-2-methyl-2-butene reacts with water to give a mixture of 2-methyl-3-buten-2-ol and 3-methyl-2-buten-1-ol. Which alcohol would you expect to predominate under conditions of *equilibrium* control? On the bases of steric hindrance, charge distribution in the cation, and so on, which alcohol should be favored under conditions of *kinetic* control? (Review Sections 6-5C, 10-4A, and 11-3.) Give your reasoning.

Problem 5-42 Explain why 2-chloropyridine is more reactive than 3-chloropyridine in nucleophilic substitution reactions.

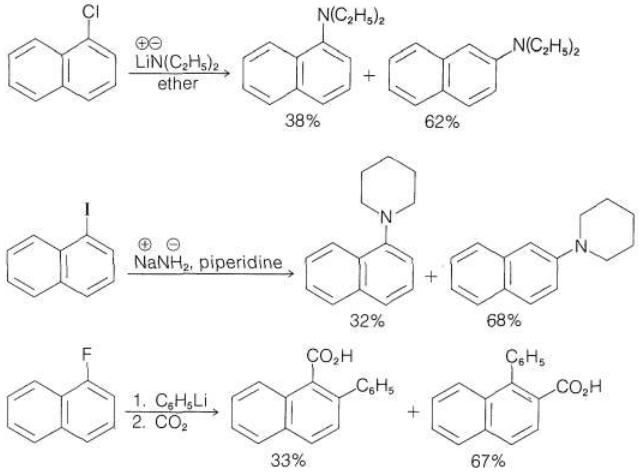


Problem 5-43 Explain why 2-chloropyridine reacts with potassium amide (KNH_2) in liquid ammonia solution at $-33°$ to give 2-aminopyridine, whereas 3-chloropyridine under the same conditions gives a mixture of 65% 4-amino- and 35% 3-aminopyridine.

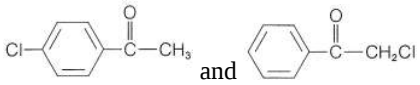
Problem 5-44 Write a structural formula for a compound that fits each of the following descriptions:

a. an aryl halogen compound that reacts with sodium iodide in acetone but not with aqueous silver nitrate solution
b. an organic fluoro compound that is more reactive in displacement reactions than the corresponding iodo compound
c. an aryl bromide that cannot undergo substitution by the elimination-addition (benzyne) mechanism
d. the monobromonitronaphthalene expected to be *least* reactive toward ethoxide ion in ethanol

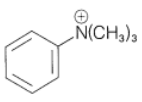
Problem 5-45 Explain why the substitution reactions of the following halonaphthalenes give about the same ratio of 1- and 2-naphthyl products independently of the halogen substituent and the nucleophilic reagent. Show the steps involved.



Problem 5-46 For each of the following pairs of compounds describe a chemical test, preferably a test-tube reaction, that will distinguish between the two compounds. Write a structural formula for each compound and equations for the reactions involved.

a. chlorobenzene and phenylmethyl chloride
b. 4-nitrochlorobenzene and 3-nitrochlorobenzene
c.  and
d. $CH_3C \equiv C{-}Br$ and $BrCH_2C \equiv CH$

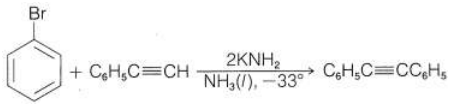
Problem 5-47 Show how benzyne can be formed from the following reagents:

a. fluorobenzene and phenyllithium
b.  $+KNH_2$

c. 1,2-dibromobenzene + butyllithium

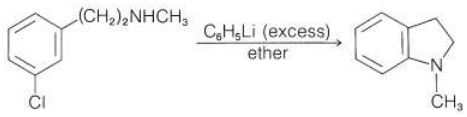
Problem 5-48 Indicate the steps involved in each of the following transformations:

a.

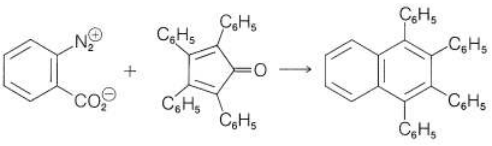


The reaction does not occur unless more than one mole of KNH_2 per mole of C_6H_5Br is used.

b.

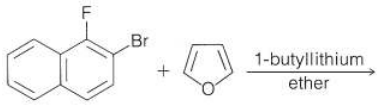


c.

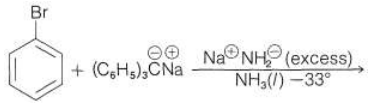


Problem 5-49 Predict the products of the following reactions:

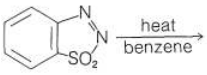
a.



b.



c.



Problem 5-50 Nucleophilic displacement of the halogen of 3,5-dimethyl-4-nitrobromobenzene is much slower than with the corresponding compound lacking the methyl groups. Give a reasonable explanation of this observation. (Construction of molecular models will help.)

Problem 5-51 Methylmagnesium halides have been employed as analytical reagents for the determination of the number of acidic hydrogens in a molecule (the *Zerewitinoff determination*). The method involves measuring the amount of methane produced from a given weight of compound (such as **RH**, with an acidic hydrogen) by the following reaction:

$$CH_3MgI + RH \rightarrow CH_4 + RMgI \qquad (5.P.3)$$

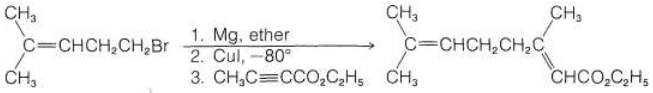
Excess methylmagnesium iodide and $0.1776\ g$ of Compound **A** (formula $C_4H_{10}O_3$) react to give $84.1\ mL$ of methane collected over mercury at $740\ mm$ and $25°$. How many acidic hydrogens does Compound **A** possess per molecule? Suggest a possible structure on the basis that spectral data indicate (a) there is no C=O group in the molecule and (b) **A** is achiral.

Problem 5-52 From the nature of the carbon-metal bonds in organometallic compounds, predict the products of the following reactions. Give your reasoning.

a. $CH_3MgCl + ICl$
b. $C_6H_5Li + CH_3OH$
c. $CH_3Li + HC \equiv CH$
d. $C_6H_5Li + CuI$

Problem 5-53

a. Show the steps and reaction intermediates by which the product is formed in the following reaction sequence:



b. Draw structures for the products in each step of the following sequence:

$$C_2H_5Cl \xrightarrow{Mg,\ ether} \xrightarrow{C_6H_5C\equiv CH} \xrightarrow{CO_2} \xrightarrow{CH_3Li} \xrightarrow{H^{\oplus},\ H_2O} \tag{5.P.4}$$

Problem 5-54 The following experimental observations have been reported:

1. *tert*-Butyl chloride was added to lithium metal in dry ether at **35°**. A vigorous reaction ensued with evolution of hydrocarbon gases. After all the lithium metal was consumed, the mixture was poured onto dry ice. The only acidic product that could be isolated (small yield) was 4,4-dimethylpentanoic acid.

2. *tert*-Butyl chloride was added to lithium metal in dry ether at **−40°**. After all the lithium had reacted, the mixture was carbonated and gave a good yield of 2,2-dimethylpropanoic acid.

3. *tert*-Butyl chloride was added to lithium metal in dry ether at **−40°**. After all the lithium was consumed, ethene was bubbled through the mixture at **−40°** until no further reaction occurred. Carbonation of this mixture gave a good yield of 4,4-dimethylpentanoic acid.

a. Give a reasonably detailed analysis of the results obtained and show as best you can the mechanisms involved in each reaction.

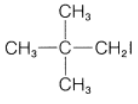
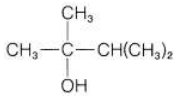
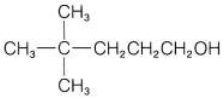
b. Would similar behavior be expected with methyl chloride? Explain.

c. Would you expect that a substantial amount of 6,6-dimethylheptanoic acid would be found in Observation 3? Explain.

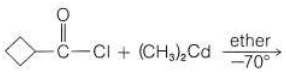
Problem 5-55 Predict the products of each of the following Grignard reactions before and after hydrolysis. Give reasoning or analogies for each.

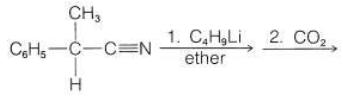
a. $CH_3MgI + HCO_2C_2H_5 \rightarrow$
b. $CH_3CH_2CH(MgBr)CH_3 +$ 2,4-dimethyl-3-pentanone $\rightarrow$
c. $CH_3CH_2MgBr + CS_2 \rightarrow$
d. $CH_3CH_2MgBr + NH_3 \rightarrow$

Problem 5-56 Show how each of the following substances can be synthesized from the indicated starting materials by a route that involves organometallic substances in at least one step.

a. $(CH_3)_3C{-}D$ from $(CH_3)_3CCl$
b. $CH_3C\equiv C{-}CO_2H$ from $CH\equiv CH$
c.  from $(CH_3)_4C$
d.  (three ways)
e.  from 
f.  from $(CH_3)_3CCH_2Cl$
g. 1,5-hexadiene from propene

Problem 5-57 Predict the products of each of the following reactions both before and after hydrolysis:

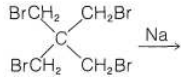
a. 



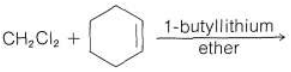
b.

c. $HC \equiv CCH_2Br \xrightarrow{Mg\text{–ether}} \xrightarrow{HC\equiv CCH_2Br}$

d. $(CH_3)_3CBr \xrightarrow{Mg\text{–ether}} \xrightarrow{O_2,\ -70^\circ}$



e.



f.

Problem 5-58 Each of the following equations represents a "possible" but not actually feasible Grignard synthesis. Consider each equation and determine why it will not proceed satisfactorily as written. *Give your reasoning* and show what the actual product will be.

a. $(CH_3)_3CMgBr + [(CH_3)_2CH]_2C{=}O \rightarrow\rightarrow (CH_3)_3C[(CH_3)_2CH]_2COH$

b. $(CH_3)_3CCH_2MgBr + [(CH_3)_3C]_2C{=}O \rightarrow\rightarrow (CH_3)_3CCH_2[(CH_3)_3C]_2COH$

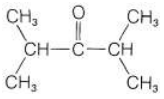
c. $CH_3MgI + CH_3(CH_3)_2COCl \rightarrow\rightarrow CH_3(CH_3)_2COCH_3$

d. $CH_3MgI + CH_3CCH{=}N{-}CH_3 \rightarrow\rightarrow CH_3CH_2N(CH_3)_2$

e. $BrCH_2CH_2O_2CCH_3 \xrightarrow[(CH_2H_5)_2O]{Mg}$ Grignard reagent $\xrightarrow{CH_2O} \rightarrow HOCH_2CH_2CH_2O_2CCH_3$

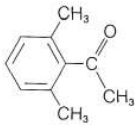
f. $CH_2{=}CHCH_2MgCl + C_2H_5Br \rightarrow CH_2{=}CH(CH_2)_2CH_3$

Problem 5-59 What products would you expect to predominate in the following reactions:



a. $C_6H_5MgBr +$

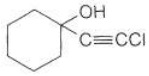
b. $CH_3MgBr + (CH_3)_3CCOC(CH_3)_3$



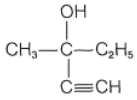
c. $CH_3MgBr +$

Problem 5-60 Suggest possible reactions by which the following compounds could be prepared from ethyne and any other necessary compounds:

a. $ClC \equiv C(CH_2)_4C \equiv CCl$



b.

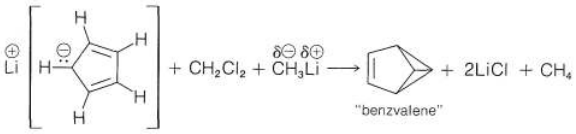


c.

d. $CH_2{=}CHCH_2C \equiv CCH_3$

Problem 5-61* The rate of addition of dimethylmagnesium to excess diphenylmethanone (benzophenone) in diethyl ether initially is cleanly second order, that is, first order in ketone and first order in $(CH_3)_2Mg$. As the reaction proceeds, the rate no longer follows a strictly second-order rate overall. Suggest how the apparent specific rate could change as the reaction proceeds.

Problem 5-62* An interesting isomer of benzene is "benzvalene" (tricyclo[2.1.1.$0^{5,6}$]-2-hexene). This substance, which like prismane (Section 12-10) can decompose with explosive violence, has been synthesized by T. Katz from lithium cyclopentadienide, dichloromethane, and methyllithium:



Write a mechanism for this reaction that you can support by analogy with other reactions discussed in this chapter or by reasonable mechanistic arguments.

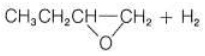
Problem 5-63 Table 5-4 shows that the addition of $\mathbf{RMgX}$ to $\mathbf{R'CON(CH_3)_2}$ gives $\mathbf{RCOR'}$, although addition of $\mathbf{RMgX}$ to $\mathbf{R'CO_2CH_3}$ and $\mathbf{R'COCl}$ lead to $\mathbf{R'R_2COH}$. Assuming that similar mechanistic steps are involved throughout, why might $\mathbf{R'CON(CH_3)_2}$ give a different product than $\mathbf{R'CO_2CH_3}$ or $\mathbf{R'COCl}$? Show your reasoning.

Problem 5-64 The formation of alcohols with organometallic compounds by the Grignard synthesis can be used to achieve the reaction $\mathbf{RH + -C{=}O}$ to give $\mathbf{R{-}C{-}OH}$, which generally has an *unfavorable* equilibrium constant. If you were looking for an industrial synthesis of 2-butanol by a reaction that would have a *favorable* equilibrium constant, which of the following might be *better* candidates than $\mathbf{CH_3CH_3 + CH_3CH{=}O \rightarrow CH_3CH_2CH(CH_3)OH}$. Give your reasoning.

a. $\mathbf{CH_2{=}CH_2 + CH_3CH_2OH}$

b. $\mathbf{CH_3CH_3 + CH_3CH_2OH}$

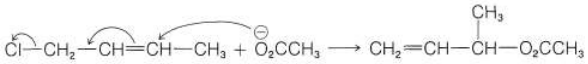
c. $\mathbf{CH_3CH_2CH_2CH_3 + \frac{1}{2}O_2}$

d. 

e. $\mathbf{CH_3CH_2CH{=}CH_2 + H_2O}$

Problem 5-65 Compound X, of formula $\mathbf{C_3H_5Br_3}$, with methyllithium formed bromocyclopropane and 3-bromopropene. The NMR spectrum of X showed a one-proton triplet at $\mathbf{5.9\ ppm}$, a two-proton triplet at $\mathbf{3.55\ ppm}$, and a complex resonance centered at $\mathbf{2.5\ ppm}$ downfield from TMS. What is the structure of X? Account for the products observed in its reaction with methyllithium.

Problem 5-66* There have been many proposals for the occurrence of a so-called $\mathbf{S_N2'}$ mechanism that would produce a concerted substitution with rearrangement for allylic halides. One possible example is



Consider whether this mechanism is operating for the particular example from the following experimental results. Give your reasoning.

1. Silver ethanoate in ethanoic acid with 1-chloro-2-butene gives $\mathbf{65\%}$ 2-butenyl ethanoate and $\mathbf{35\%}$ 1-methyl-2-propenyl ethanoate.

2. Ethanoate ion $\mathbf{(1\ M)}$ in ethanoic acid with 1-chloro-2-butene gives a mixture of about $\mathbf{85\%}$ 2-butenyl ethanoate and $\mathbf{15\%}$ 1-methyl-2-propenyl ethanoate. About $\mathbf{36\%}$ of the overall reaction product results from a process that is *zero order* in ethanoate ion, whereas $\mathbf{64\%}$ comes from a process that is *first order* in ethanoate ion.

3. Ethanoate ion in 2-propanone reacts with 1-chloro-2-butene to give only 2-butenyl ethanoate, and with 3-chloro-1-butene to give only 1-methyl-2-propenyl ethanoate

(This Problem involves many of the ideas developed in Chapter 8 and you may wish to review Sections 8-4A, 8-4B, and 8-7, especially 8-7F.)

Contributors

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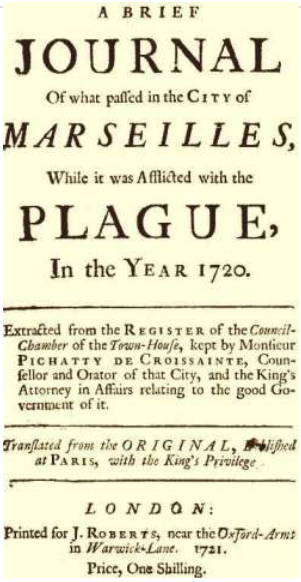
CHAPTER OVERVIEW

6: Nucleophilic Acyl Substitution Reactions

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6.3: The Nucleophilic Acyl Substitution Mechanism
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6.6: Synthesis of Carboxylic Acid Derivatives
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6.8: Protein Synthesis on the Ribosome
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6.P: Nucleophilic Acyl Substitution Reactions (Problems)
6.S: Nucleophilic Acyl Substitution Reactions (Summary)

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6.1: Prelude to Nucleophilic Acyl Substitution Reactions



A BRIEF

JOURNAL

Of what paſſed in the CITY of

MARSEILLES,

While it was Afflicted with the

PLAGUE,

In the YEAR 1720.

Extracted from the REGISTER of the *Council-Chamber* of the *Town-Houſe*, kept by Monſieur PICHATTY DE CROISSAINTE, Counſellor and Orator of that City, and the King's Attorney in Affairs relating to the good Government of it.

Tranſlated from the ORIGINAL, Publiſhed at PARIS, *with the King's Privilege.*

LONDON:

Printed for J. ROBERTS, near the *Oxford-Arms* in *Warwick-Lane.* 1721.

Price, One Shilling.

Introduction

The 26th of July, Notice is given to the Sheriffs, that in the Street of Lescalle, a Part of the old Town inhabited only by poor People, Fifteen Persons are suddenly fallen sick: They dispatch thither Physicians and Surgeons; they examine into the Distemper, and make Report; some, that 'tis a Malignant Fever; others, a contagious or pestilential Fever, occasioned by bad Food, which Want had long forced those poor Creatures to live upon . . .

The 27th, Eight of those Sick dye; the Sheriffs themselves go to their Houses to cause them to be searched; Buboes [swelling of the lymph nodes] are found on Two of them: The Physicians and Surgeons still hold the same Language, and impute the Cause of the Distemper to unwholsome Food. Notwithstanding which, as soon as Night comes, M. Moustier repairs to the Place, sends for Servants from the Infirmaries, makes them willingly or by Force, take up the Bodies, with all due Precautions; they are carried to the Infirmaries, where they are buried with Lime; and all the rest of the Night he causes the remaining Sick, and all those of their Houses, to be removed to the Infirmaries.

The 28th, very early in the Morning, Search is made every where for those who had Communication with them, in order to confine them: Other Persons in the same Street fall sick, and some of those who first sicken'd dye. ..

The People who love to deceive themselves, and will have it absolutely not to be the Plague, urge a Hundred false Reasons on that Side. Would the Plague, say they, attack none but such poor People? Would it operate so slowly?

Let them have but a few Days Patience, and they will see all attacked without Distinction, with the swiftest Rage, and the most dreadful Havock, that ever was heard of.

(source: Gutenberg Project http://www.gutenberg.org/files/45673...-h/45673-h.htm)

In late May of 1720, a ship arrived in the Mediterranean port city of Marseille, having recently departed from Cyprus and Tripoli. Although several crew members had fallen ill and died during the journey, the ship was allowed to unload after only a very brief quarantine, the result of political pressure on port authorities from local businessmen who wanted quick access to the valuable silk and cotton waiting in the ship's hold.

Along with silk and cotton, the hold carried rats. The rats, in turn, carried fleas. The fleas carried a microscopic mass murderer: Yersinia pestis, the species of bacteria that causes bubonic plague.

It is next to impossible to estimate how many people have died from bubonic plague over the course of human history. In the time of the 'Black Death' in the 14th century, it wiped out more than half the population of Europe. In the Great Plague of Marseille in 1720, over 100,000 people succumbed to Y. pestis infection in the city and surrounding provinces. At the height of the outbreak,

corpses piled up in city streets, and a fortified wall, the 'mur de la peste' was constructed in an attempt to prevent people from traveling north to the neighboring city of Aix.

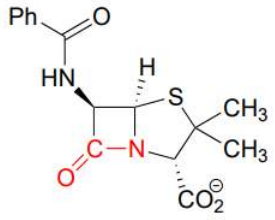
Throughout history, bacteria have been the cause of untold human death and suffering, making the threat posed by more obviously frightening species - lions and bears, spiders and snakes – seem inconsequential by comparison. As recently as the mid-1940s, a minor cut or cold could become a life-threatening event if a bacterial infection were to set in, and even in developed countries, one in twenty infants did not survive to celebrate their first birthday.

Since then, the infant mortality rate in developed countries has declined by a factor of ten. You probably don't worry very much when a small cut on your hand becomes infected. The idea of half of the population of the United States dying in a plague is, in most people's minds, the stuff of zombie movies, not reality. Bacteria are, for now at least, no longer public enemy #1.

How did this happen?

For an answer, we move to a September morning in 1928, in the laboratory of Alexander Fleming, a Scottish bacteriologist working at St. Mary's Hospital in London. As a young man serving in the British Medical Corps during World War I, Fleming saw first-hand how deadly bacteria could be, as he watched countless soldiers in his battlefield hospital die from infected wounds. After returning to civilian life, he began to study Staphylococci bacteria, a common source of life-threatening infections in humans, hoping to discover new antibacterial agents that were more effective than those he had used in the war. He spent a lot of his time growing Staphylococcus cultures in petri dishes for his experiments, and, notoriously untidy, he tended to leave piles of culture dishes lying around his lab. One morning, he returned from a short vacation to find that one of the cultures he had left out had some mold growing on it. He was about to throw it away, but happened to notice something curious: surrounding the small spot of mold was a circle of clear medium, where no bacteria were growing. He realized that the mold must be secreting something that killed bacteria.

As it turned out, the mold was a of strain called Penicillium notatum, and the 'something' killing the bacteria was an organic compound that came to be known as penicillin.



penicillin

Fleming published his findings in the British Journal of Experimental Pathology, but made only passing reference to the potential therapeutic value of penicillin. The paper received little attention.

Fast-forward now to early February 1941, with the world once again at war. One morning, a policeman named Albert Alexander living in Oxford, England, had an unfortunate gardening accident. While he was trimming some roses on his day off, his shears slipped and gave him a nasty cut on the side of his mouth. The cut became infected, and after a few days it appeared as if the infection would kill him. Then, he got a visit in his hospital room from some chemists at nearby Oxford University.

For the last few years, the chemists had been hard at work isolating pure penicillin from mold cultures, a tricky job because the compound tends to degrade during purification. It is a feat that Alexander Fleming -who, after all, was a bacteriologist, not a chemist - had never been able to accomplish, but the Oxford researchers had realized how valuable penicillin might be to the war effort, and had finally met with some success. They needed a human subject on whom to test the ability of their compound to treat infected wounds, and Albert was their man for the job. They injected him with penicillin, and within a day his infection cleared up. It was a new day in the history of medicine.

At the heart of a penicillin molecule is an amide functional group - more specifically, a cyclic amide, or 'lactam'. To understand how penicillin works at the molecular level as it prevents bacteria from multiplying, we first need to know more about the chemistry of amides and other carboxylic acid derivative functional groups, and a type of organic reaction mechanism called 'nucleophilic acyl substitution'. Understanding the reactivity of carboxylic acid derivative groups will also allow us to appreciate

why penicillin is so prone to degradation, and why - very significantly for all of us - the era of not having to worry about bacterial infections may be near an end, as common toxic bacterial species such as Staphylococcus develop increasingly robust resistance to antibiotics.

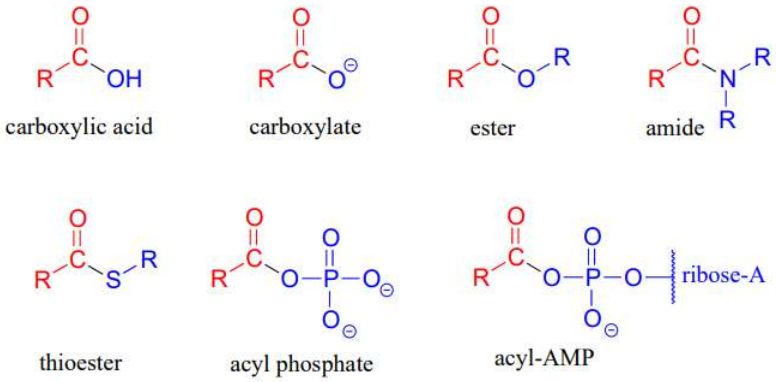
Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

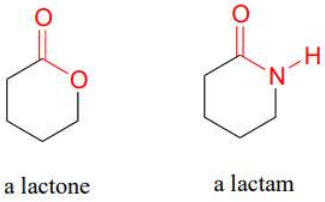
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6.2: Carboxylic Acid Derivatives

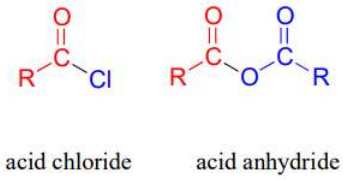
The functional groups at the heart of this chapter are called **carboxylic acid derivatives**: they include carboxylic acids themselves, carboxylates (deprotonated carboxylic acids), amides, esters, thioesters, and acyl phosphates.



Cyclic esters and amides are referred to as **lactones** and **lactams**, respectively.



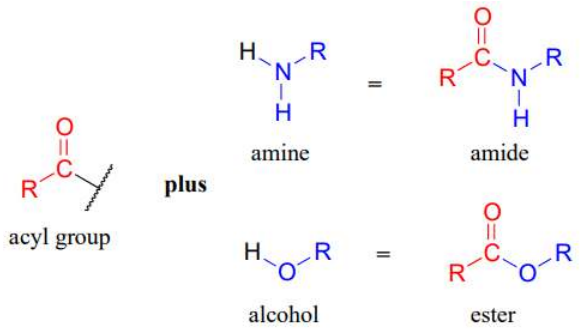
Carboxylic acid anyhydrides and acid chlorides, which also fall under the carboxylic acid derivative category, are not generally found in biomolecules but are useful intermediates in laboratory synthesis. They are discussed in a section on laboratory reactions at the end of this chapter.



Carboxylic acid derivatives can be distinguished from aldehydes and ketones by the presence of a group containing an electronegative heteroatom - usually oxygen, nitrogen, or sulfur – bonded directly to the carbonyl carbon. You can think of a carboxylic acid derivative as having two sides. One side is the acyl group, which is the carbonyl plus the attached alkyl (R) group. In the specific cases where R is a hydrogen or methyl, chemists use the terms formyl and acetyl group, respectively. On the other side is the heteroatom-linked group: in this text, we will sometimes refer to this component as the ‘acyl X' group (this, however, is not a standard term in organic chemistry).



Notice that the acyl X groups are simply deprotonated forms of other functional groups linked to the acyl group: in an amide, for example, the acyl X group is an amine, while in an ester the acyl X group is an alcohol.



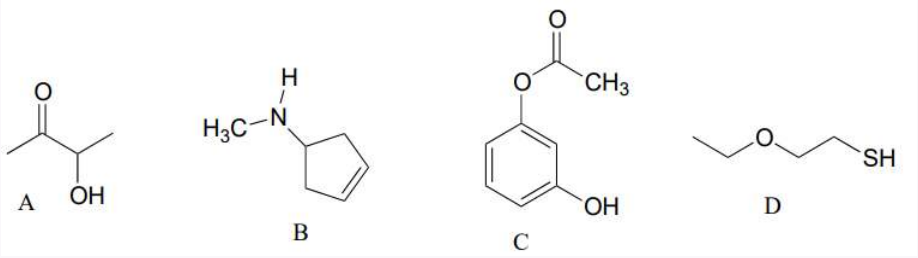
Exercise 6.2.1

What is the ‘acyl X’ group in:

a. an acid anhydride?
b. a thioester?
c. a carboxylic acid?
d. an acyl phosphate?

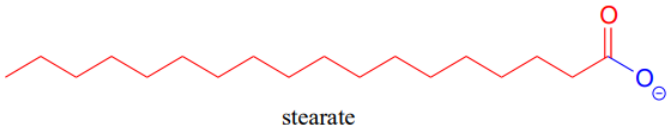
Exercise 6.2.2

Draw the structures indicated:

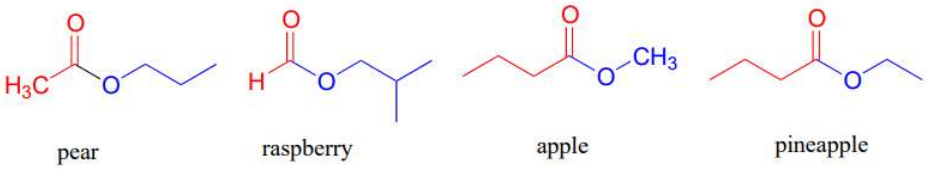


a. compound A after is has been acetylated (ie. an acetyl group added)
b. compound B after it has been formylated
c. compound C after it has been formylated
d. Compound D after it has been acetylated

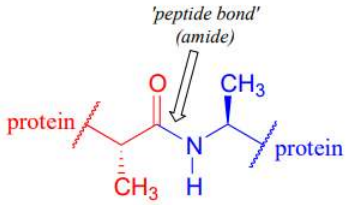
'Fatty acid' molecules such as stearate are carboxylates with long carbon chains for acyl groups.



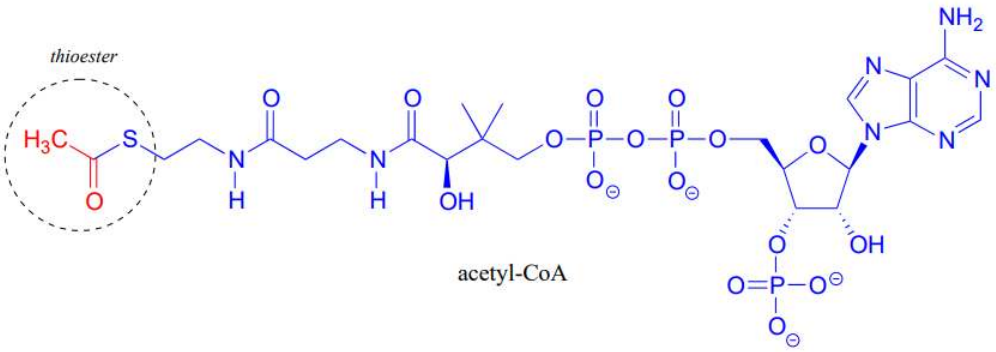
The aromas of many fruits come from small ester-containing molecules:



The 'peptide bonds' that link amino acids together in proteins are amides.



Acetyl-Coenzyme A, a very important two carbon (acetyl group) 'building block' molecule in metabolism, is characterized by reactions at its thioester functional group:

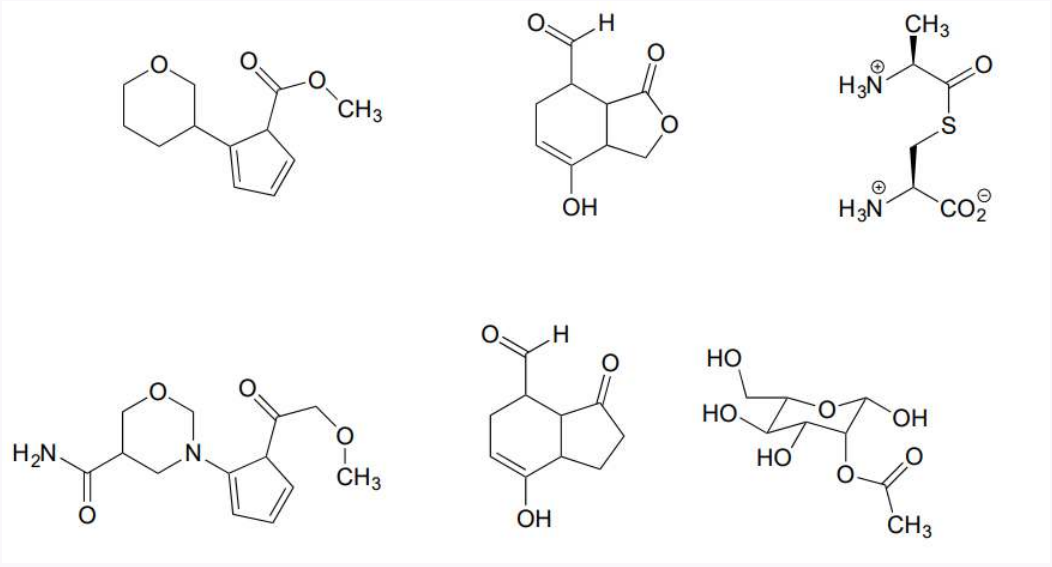


Exercise 6.2.3

There are two amide groups in acetyl-CoA: identify them.

Exercise 6.2.4

Name all carboxylic acid derivative groups in the molecules below.



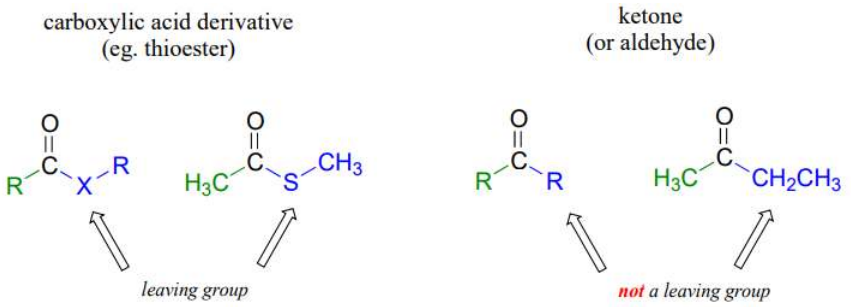
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6.3: The Nucleophilic Acyl Substitution Mechanism

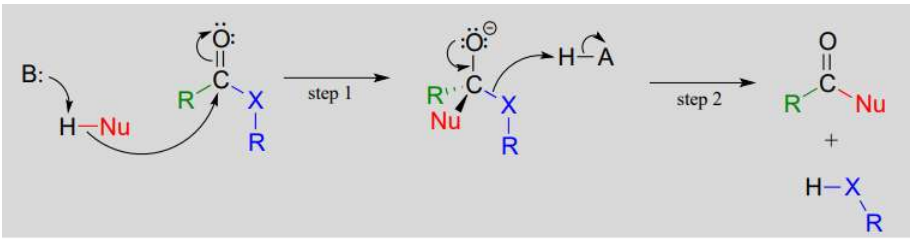
The fact that one of the atoms adjacent to the carbonyl carbon in carboxylic acid derivatives is an electronegative heteroatom – rather than a carbon like in ketones or a hydrogen like in aldehydes - is critical to understanding the reactivity of carboxylic acid derivatives. The most significant difference between a ketone/aldehyde and a carboxylic acid derivative is that the latter has a potential leaving group - what we are calling the 'acyl X group' - bonded to the carbonyl carbon.



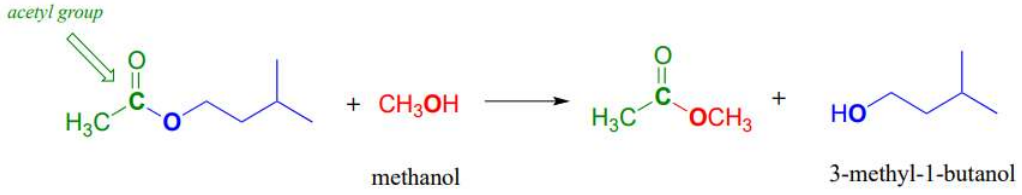
As a result, carboxylic acid derivatives undergo nucleophilic acyl substitution reactions, rather than nucleophilic additions like ketones and aldehydes.

A nucleophilic acyl substitution reaction starts with nucleophilic attack at the carbonyl, leading to a tetrahedral intermediate (step 1 below). In step 2, the tetrahedral intermediate collapses and the acyl X group is expelled, usually accepting a proton from an enzymatic acid in the process.

Mechanism for a nucleophilic acyl substitution reaction:



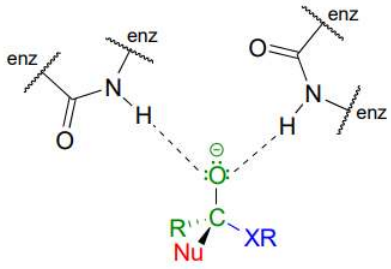
Notice that in the product, the nucleophile becomes the new acyl X group. This is why this reaction type is called a nucleophilic acyl substitution: one acyl X group is substituted for another. For example, in the reaction below, one alcohol 'X group' (methanol), substitutes for by another alcohol 'X group' (3-methyl-1-butanol) as one ester is converted to another.



Another way of looking at this reaction is to picture the acyl group being transferred from one acyl X group to another: in the example above, the acetyl group (in green) is transferred from 3-methyl-1-butanol (blue) to methanol (red). For this reason, nucleophilic acyl substitutions are also commonly referred to as acyl transfer reactions.

Enzymes catalyzing nucleophilic acyl substitution reactions have evolved ways to stabilize the negatively charged, tetrahedral intermediate, thus lowering the activation energy of the first, rate-determining step (nucleophilic attack). The late transition state of the first step resembles the tetrahedral intermediate that results: recall from chapter 6 that the Hammond postulate tells us that anything that stabilizes the tetrahedral intermediate will also stabilize the transition state. In many cases, for example, enzymatic amino acid residues are positioned in the active site so as to provide stabilizing hydrogen bond donating interactions with the

negatively-charged oxygen. This arrangement is sometimes referred to in the biochemistry literature as an oxanion hole. The figure below shows a tetrahedral intermediate stabilized by hydrogen bond donation from two main chain (amide) nitrogen atoms.



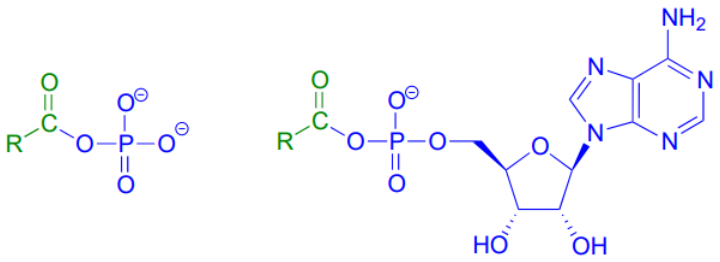
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6.4: Acyl Phosphates

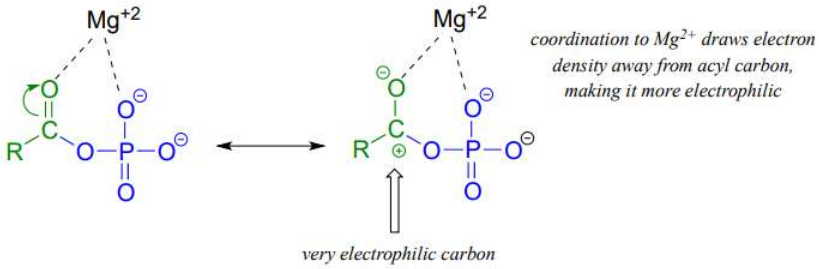
Acyl phosphates, because they are so reactive towards acyl substitutions, are generally seen as reaction intermediates rather than stable metabolites in biochemical pathways. Acyl phosphates usually take one of two forms: a simple acyl monophosphate, or acyl-adenosine monophosphate.



acyl monophosphate

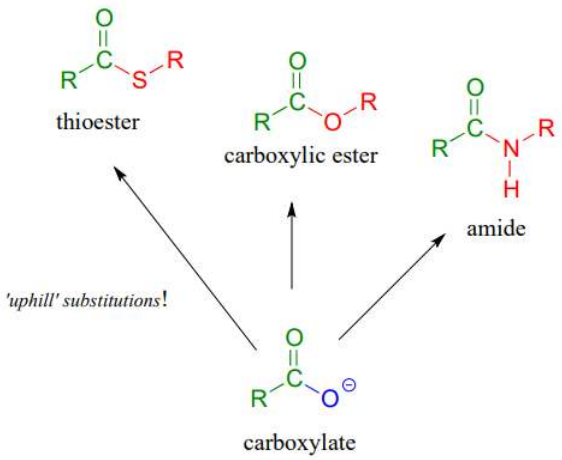
acyl adenosine monophosphate
(acyl AMP)

Both forms are highly reactive to acyl substitution reactions, and are often referred to as ‘activated acyl groups’ or ‘activated carboxylic acids’ for reasons that will become clear soon. The tendency of phosphates to form stabilizing complexes with one or more magnesium ions in an enzyme's active site contributes in a large way to the reactivity of acyl phosphates.



A magnesium ion acts as a Lewis acid, accepting electron density from the oxygen end of the acyl carbonyl bond, which greatly increases the degree of partial positive charge - and thus the electrophilicity - of the carbonyl carbon. The magnesium ion also balances negative charge on the phosphate, making it a weak base and excellent leaving group.

We have already learned that the carboxylate functional group is the least reactive substrate for an enzyme-catalyzed acyl substitution reactions. In biology, though, carboxylates are frequently transformed into thioesters, carboxylic esters, and amides, all of which are higher in energy, meaning that these transformations are thermodynamically 'uphill'.



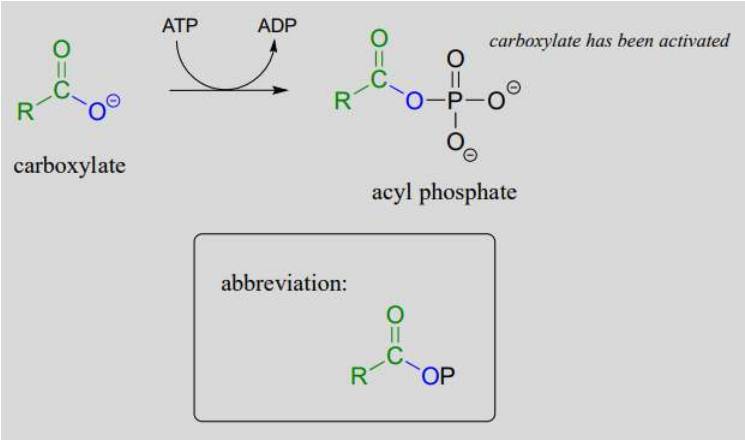
How are these uphill substitutions accomplished? They are not carried out directly:

like all thermodynamically unfavorable reactions in biochemistry, they are linked to an energy-releasing, 'downhill' reaction. In this case, (and many others), the linked reaction that 'pays for' the uphill reaction is hydrolysis of ATP.

In order to undergo an acyl substitution reaction, a carboxylate must first be activated by phosphorylation. You are already familiar with this phosphoryl group transfer process from a previous chapter.

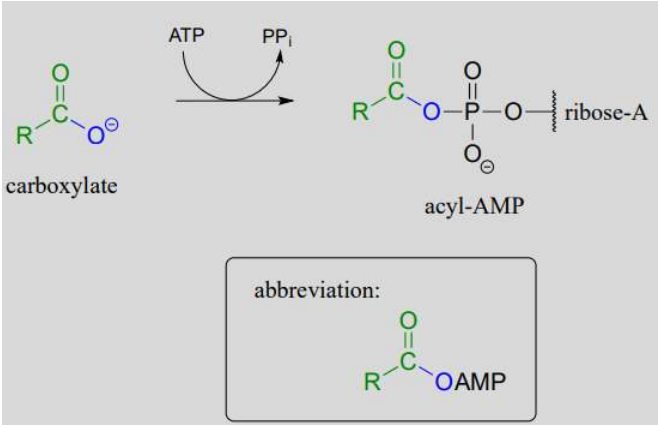
In many cases, enzymes activate a carboxylate group by converting it to an acyl phosphate (the most reactive of the carboxylic acid derivatives), at the expense of an ATP: the mechanism for this type of transformation is shown in Chapter 1.

Formation of an acyl phosphate (see Chapter 1 for the complete mechanism):

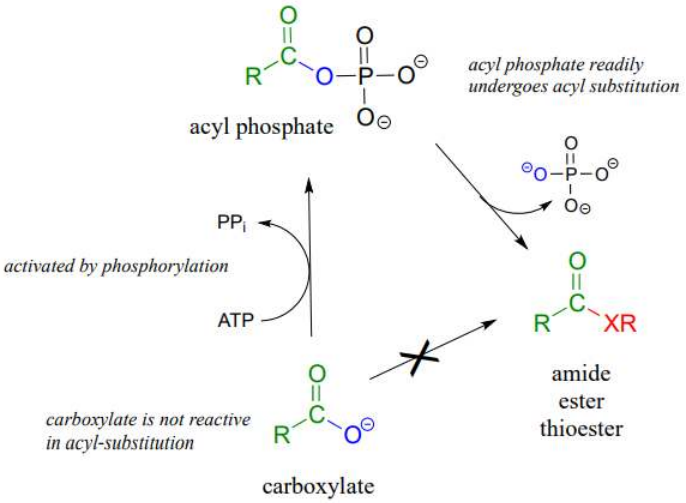


As a common alternative, some enzymatic reactions begin with the conversion of a carboxylate to an acyl-AMP intermediate:

Formation of an acyl -AMP (see Chapter 1 for the complete mechanism):



In either case, once the carboxylate group has been activated, the reactive acyl phosphate/acyl-AMP intermediate can go on to act as the electrophile in an energetically favorable nucleophilic acyl substitution reaction.



You have probably heard ATP referred to as the 'energy currency' molecule. The reactions in this section provide a more concrete illustration of that concept. A lower-energy group (a carboxylate) is converted to a higher-energy group (a thioester, for example) by 'spending' a high-energy ATP.

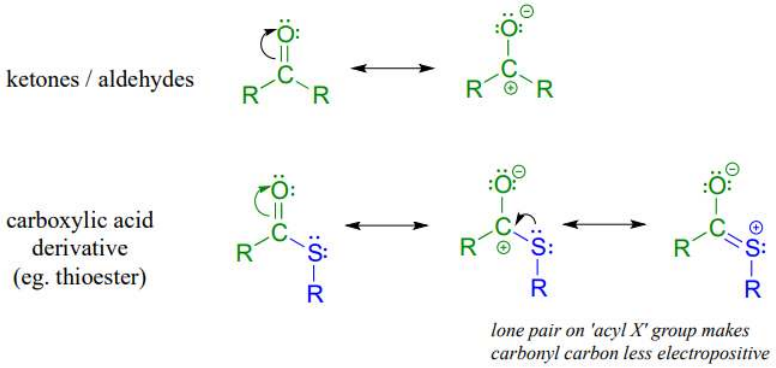
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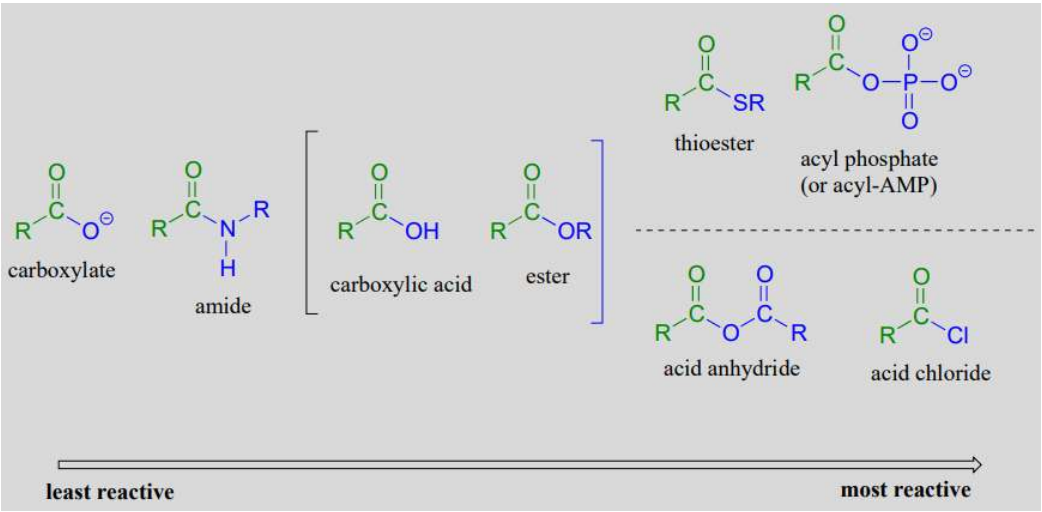
6.5: The Relative Reactivity of Carboxylic Acid Derivatives

In carboxylic acid derivatives, the partial positive charge on the carbonyl carbon is stabilized by electron donation from nonbonding electrons on the adjacent heteroatom, which has the effect of decreasing electrophilicity.



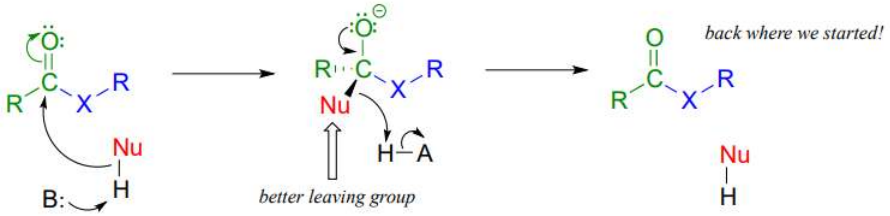
Among the carboxylic acid derivatives, carboxylate groups are the least reactive towards nucleophilic acyl substitution, followed by amides, then carboxylic esters and carboxylic acids, thioesters, and finally acyl phosphates, which are the most reactive among the biologically relevant acyl groups. Acid anhydrides and acid chlorides are laboratory reagents that are analogous to thioesters and acyl phosphates, in the sense that they too are highly reactive carboxylic acid derivatives. A section near the end of this chapter includes information about the chemistry of these two reagents.

Relative reactivity of carboxylic acid derivatives:



The reactivity trend of the carboxylic acid derivatives can be understood by evaluating the basicity of the leaving group (acyl X group) - remember that weaker bases are better leaving groups. A thioester is more reactive than an ester, for example, because a thiolate (RS^-) is a weaker base and better leaving group than an alkoxide (RO-). Recall that the pK_a of a thiol is about 10, while the pK_a of an alcohol is 15 or higher: a stronger conjugate acid means a weaker conjugate base.

In general, if the incoming nucleophile is a weaker base than the ‘acyl X’ group that is already there, it will also be the better leaving group, and thus the first nucleophilic step will simply reverse itself and we’ll get the starting materials back:



In general, acyl substitution reactions convert higher energy carboxylic acid derivatives into derivatives of lower energy. Thioesters, for example, are often converted directly into carboxylic esters in biochemical reactions, but not the other way around. To go 'uphill' - from a carboxylate to a thioester, for example, requires the 'coupling' of the uphill reaction to an energetically favorable reaction. We will see how this works in the next section.

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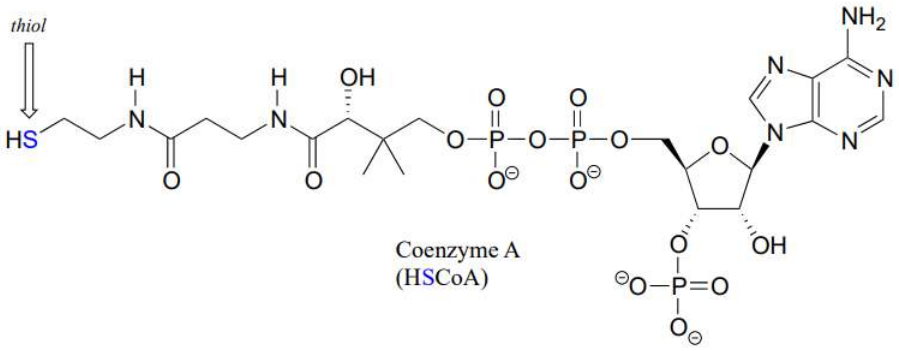
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6.6: Synthesis of Carboxylic Acid Derivatives

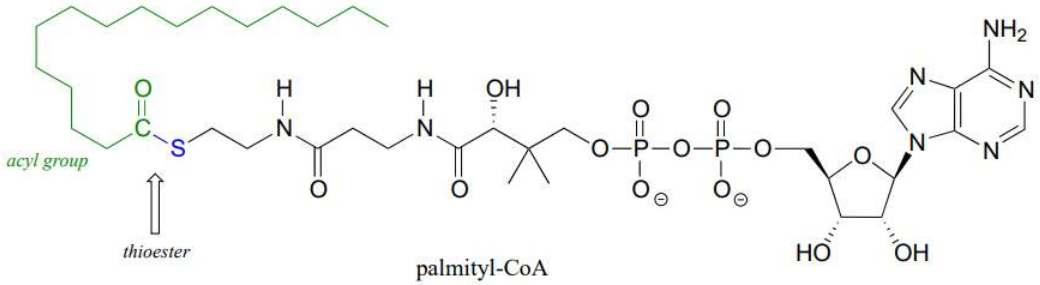
Thioester formation

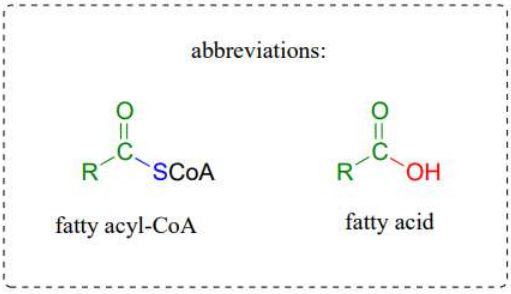
Thioesters, which are themselves quite reactive in acyl substitution reactions (but less so than acyl phosphates), play a crucial role in the metabolism of fatty acids The ‘acyl X group’ in a thioester is a thiol.

Coenzyme A is a thiol-containing coenzyme that plays a key role in metabolism. Coenzyme A is often abbreviated 'HSCoA' in order to emphasize the importance of the thiol functionality.

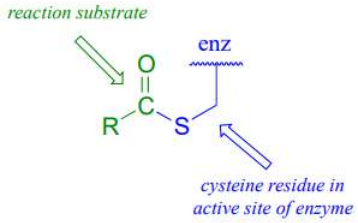


Coenzyme A serves as a 'carrier' group in lipid biosynthesis, and is attached by a thioester linkage to growing fatty acid chains. Palmityl is shown below as an example of a typical fatty acyl-CoA thioester.



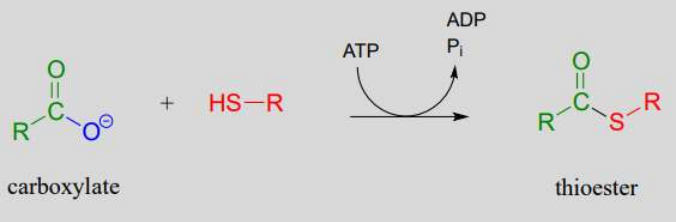


As we look at reactions involving thioesters in this and future sections, we will frequently see Coenzyme A playing a key role. We will also see the formation and breaking of thioester linkages between an acyl group and other thiol-containing species, such as a cysteine residue on the enzyme:



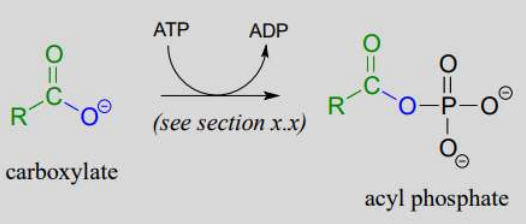
The term 'thioesterification' refers to the formation of a thioester functional group. In a typical biochemical thioesterification reaction, a carboxylate is first converted into an acyl phosphate (in other words, it is activated), then the acyl phosphate undergoes an acyl substitution reaction with a thiol nucleophile.

Thioesterification reaction:

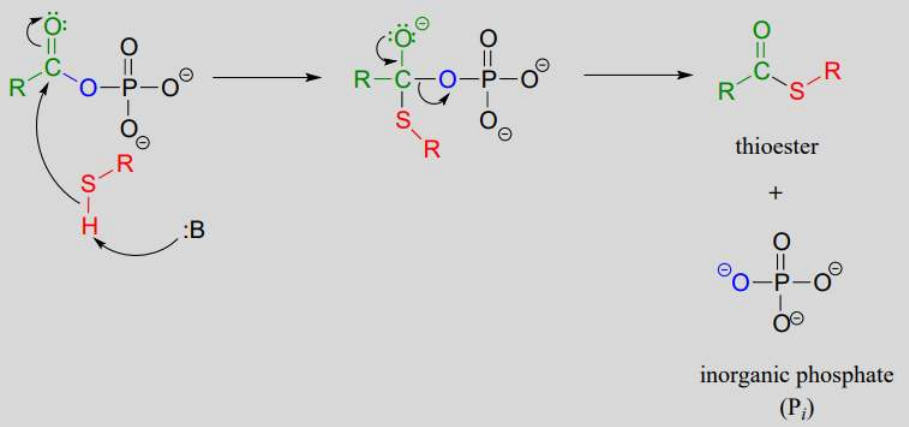


Mechanism:

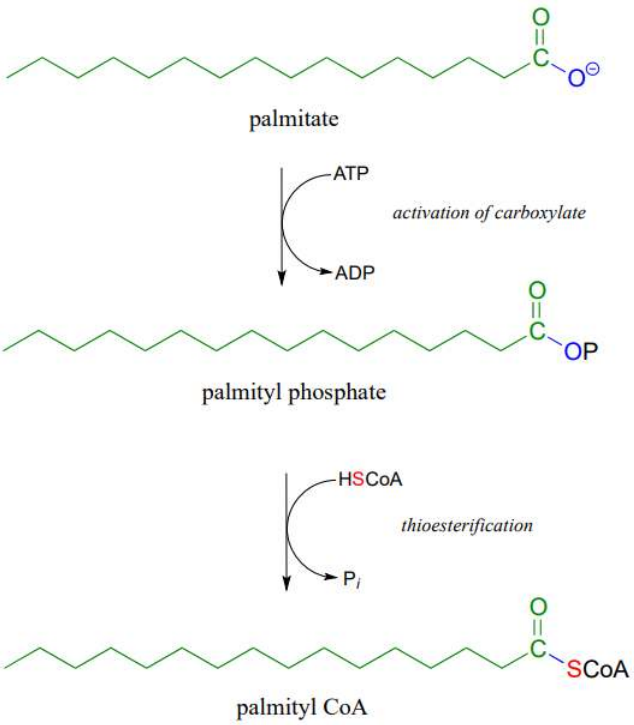
1. activation phase:



2. acyl substitution phase:

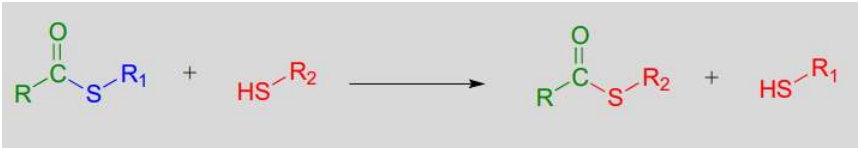


Fatty acids such as palmitate , from fats and oils in your food, are converted to a coenzyme A thioester prior to being broken down by the fatty acid degradation pathway.

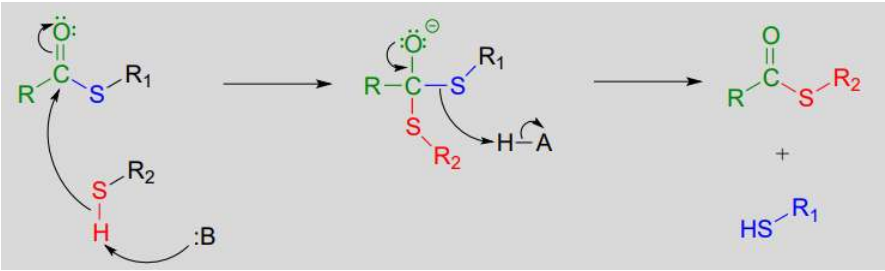


A transthioesterification reaction is a thioester to thioester conversion - in other words, an acyl group is transferred from one thiol to another.

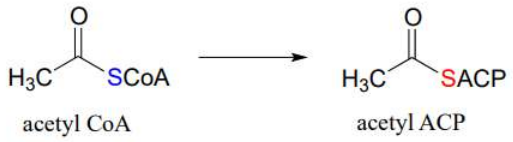
Transthioesterification:

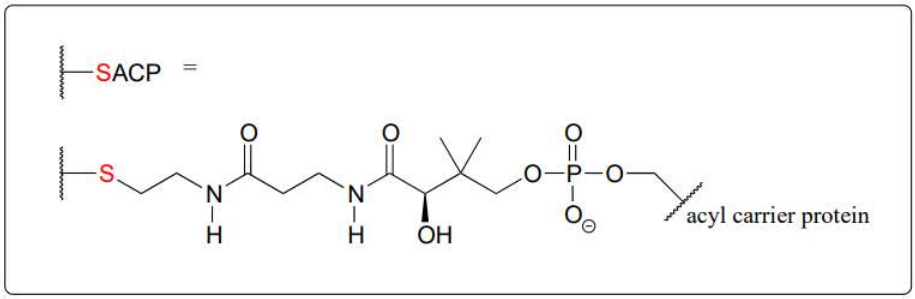


Mechanism:



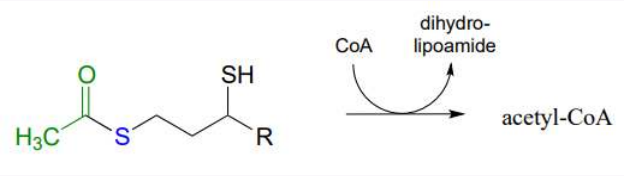
For example, when your body synthesizes fatty acids, the two-carbon fatty acid 'building block' acetyl CoA is first converted to acetyl ACP (EC 2.3.1.38). ACP is an abbreviation for 'Acyl Carrier Protein', a modified protein with a thiol-containing prosthetic group attached to one of its serine side chains. Throughout the fatty acid chain elongation process, the growing hydrocarbon chain remains linked to ACP.





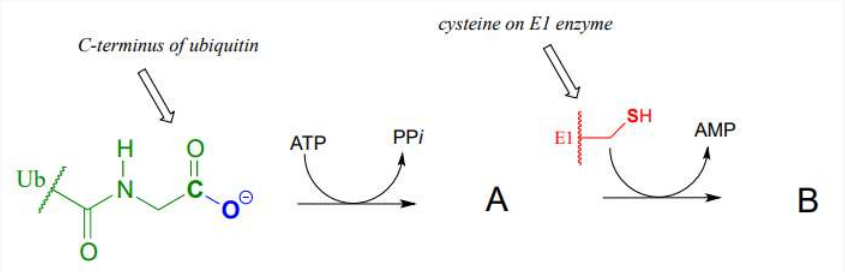
Exercise 6.6.1

The pyruvate dehydrogenase complex (EC 1.2.4.1) catalyzes one of the most central of all central metabolism reactions, the conversion of pyruvate to acetyl-CoA, which links the gycolytic pathway to the citric acid (Krebs) cycle. The reaction is quite complex, and we are not yet equipped to follow it through from start to finish (we will finally be ready to do this in section 17.3). The final step, however, we can understand: it is a transthioesterification, involving a dithiol coenzyme called dihydrolipoamide and coenzyme A. Given the information below, draw out a reasonable mechanism for the reaction.



Exercise 6.6.2

Ubiquitin is a protein which plays a key role in many cellular processes by reversibly attaching to other proteins, thus altering or regulating their function. Recently, a team of researchers uncovered details of the mechanism by which ubiquitin (abbreviated Ub) is transferred by the ubiquitin activating enzyme (abbreviated E1) to target proteins. In the first part of this process, the carboxy terminus of ubiquitin is linked to a cysteine side chain on E1, as shown in the incomplete reaction sequence below. Complete the figure by drawing the structures of species A and B.



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Formation of esters

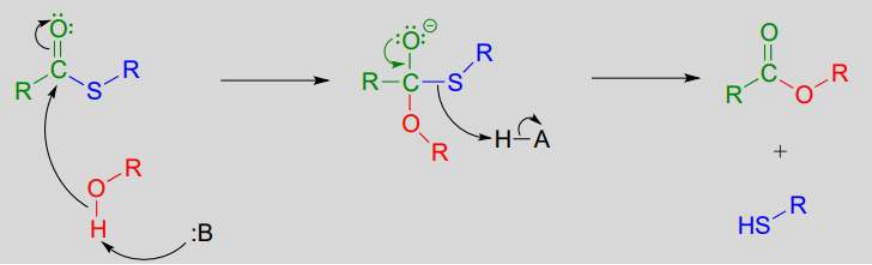
Esterification refers to the formation of a new ester functional group.

In a typical biochemical esterification, a thioester is subjected to nucleophilic attack from an alcohol, leading to the formation of an ester and a thiol.

Esterification reaction (from thioester):

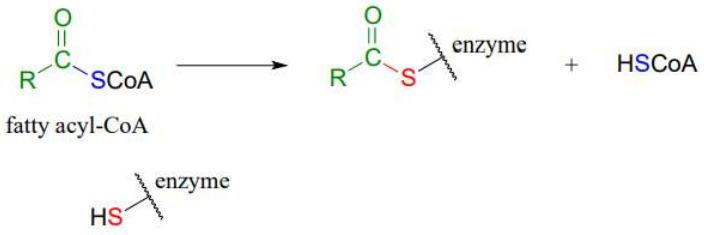


Mechanism:

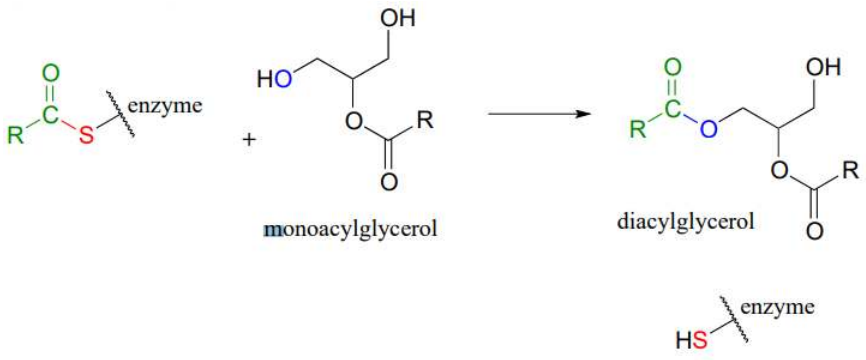


The reaction below is from the synthesis of triacylglycerol, the form in which fat is stored in our bodies.

Phase 1 (transthioesterification):



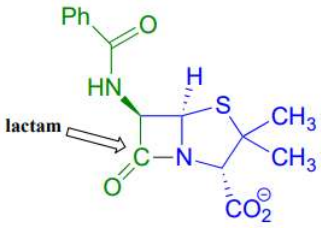
Phase 2 (esterification):



The reaction, catalyzed by monoacylglycerol acyltransferase (EC 2.3.1.22), begins (phase 1 above) with a preliminary transthioesterification step in which the fatty acyl group is transferred from coenzyme A to a cysteine residue in the active site of the enzyme. Recall that it is a common strategy for enzymes to first form a covalent link to one substrate before catalyzing the 'main' chemical reaction.

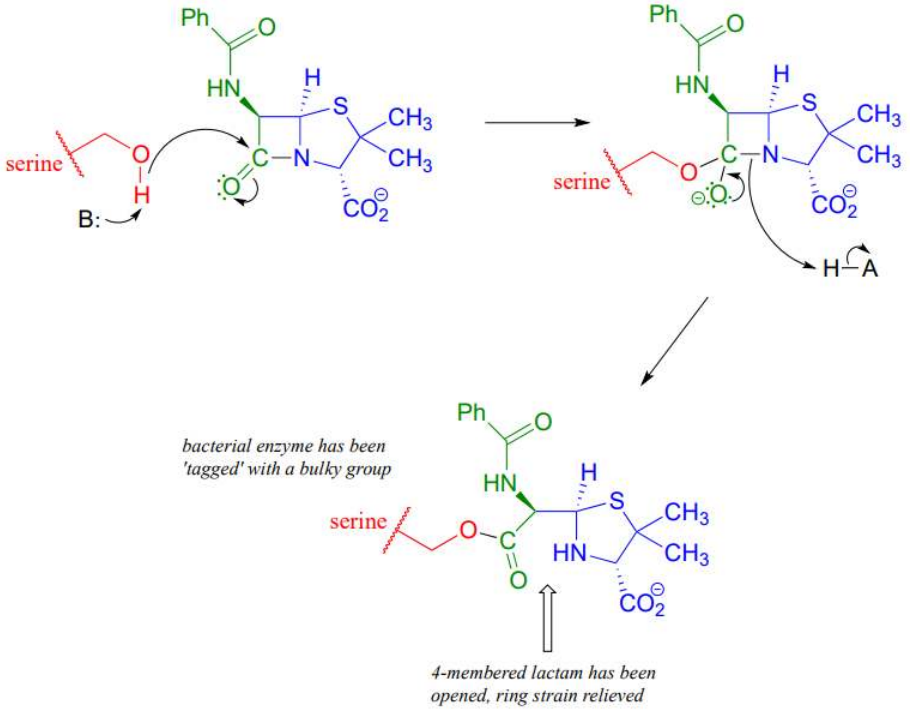
In phase 2 of the reaction, the fatty acyl group is now ready to be transferred to glycerol, trading its thioester linkage to the cysteine for a new ester linkage to one of the alcohol groups on glycerol.

An esterification reaction has tremendous importance in the history of drug development, a story that we heard in the introduction to this chapter. The discovery of penicillin was arguably one of the most important events in the history of modern medicine. The key functional group in penicillin is the four-membered lactam (recall that a lactam is a cyclic amide).



benzyl penicillin

Penicillin, and later generations of antibiotic drugs, have saved countless lives from once-deadly bacterial infections. The elucidation of the chemical mechanism of penicillin action was also a milestone in our developing understanding of how drugs function on a molecular level. We now know that penicillin, and closely related drugs such as ampicillin and amoxycillin, work by inhibiting an enzyme that is involved in the construction of the peptide component bacterial cell walls. The details of the wall-building reaction itself are outside the scope of this discussion, but it is enough to know that the process involves the participation of a nucleophilic serine residue in the active site of the enzyme. The penicillin molecule is able to enter the active site, and once inside, the lactam group serves as an electrophilic 'bait' for the nucleophilic serine:



Although you might expect that an amide-to-ester conversion such as what is shown above would be energetically unfavorable based on the reactivity trends we have learned, this lactam is in fact much more reactive than an ordinary amide group due to the effect of ring strain: recall from section 3.2 that four-membered rings are highly strained, and considerable energy is released when they are opened.

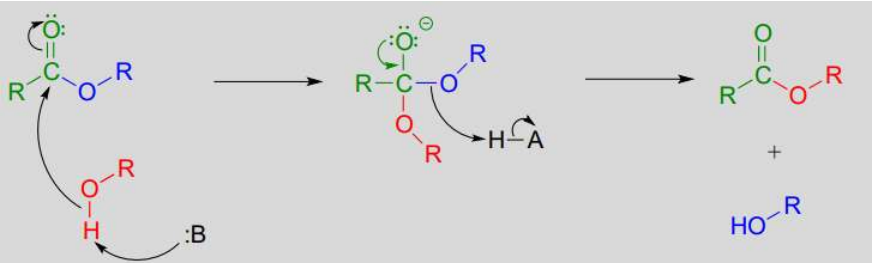
Ring strain also accounts for why penicillin has a tendency to degrade: when in contact with water, the lactam will spontaneously hydrolyze over time, which opens the ring and forms a carboxylate group.

Unfortunately, many strains of bacteria have acquired an enzyme called β-lactamase (EC 3.5.2.6), that catalyzes rapid hydrolysis of the lactam ring in penicillin-based drugs, rendering them inactive. These bacteria are consequently resistant to penicillin and related

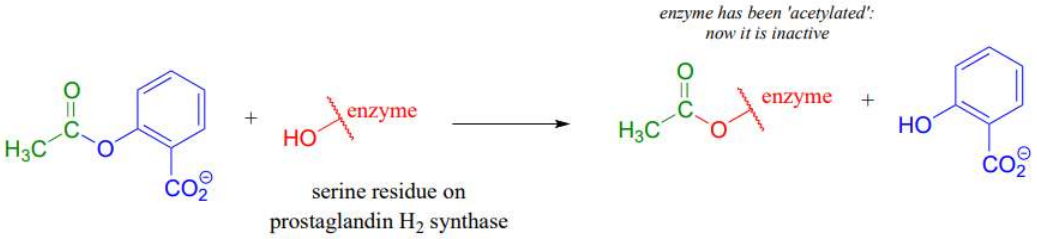
antibiotics. As you are probably aware, the evolution of drug resistance in bacteria is a major, world-wide health problem, and scientists are engaged in a constant battle to develop new antibiotics as the older ones become less and less effective.

In a transesterification reaction, one ester is converted into another by an acyl substitution reaction.

Mechanism for a transesterification reaction:



If studying organic chemistry sometimes gives you a headache, you might want to turn to a transesterification reaction for help. Prostaglandins are a family of molecules that promote a wide range of biological processes, including inflammation. Acetylsalicylic acid, commonly known as aspirin, acts by transferring - through a transesterification reaction - an acetyl group to a serine residue on the enzyme responsible for the biosynthesis of prostaglandin H2 (one member of the prostaglandin family).



Acetylation of this serine blocks a channel leading to the active site, effectively shutting down the enzyme, impeding prostaglandin production, and inhibiting the inflammation process that causes headaches.

In section 6.9, we will see two laboratory acyl substitution reactions that lead to the formation of aspirin and ibuprofen.

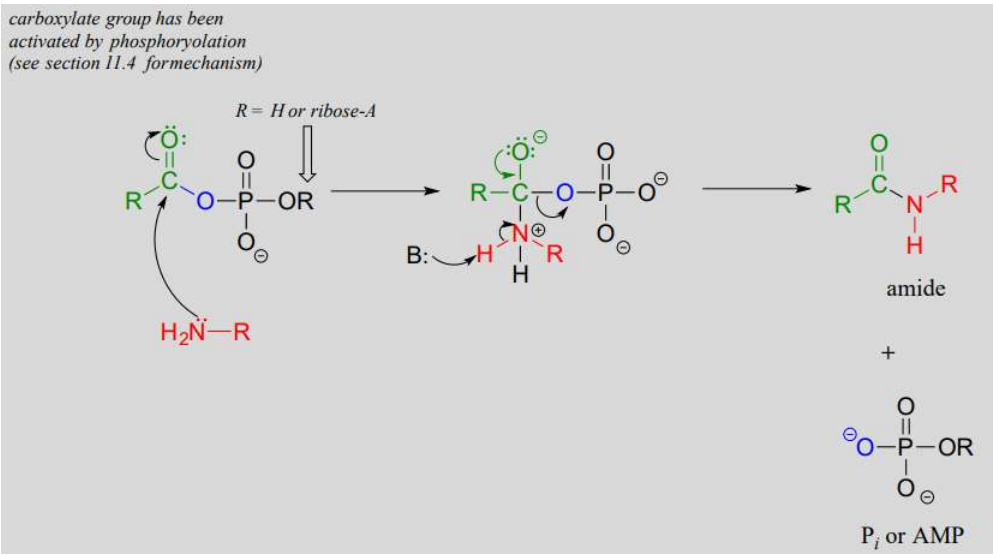
Exercise 6.6.3

Discuss the key structural feature of aspirin that makes it so effective at transferring its acetyl group - in other words, why is the ester group in aspirin more reactive than a typical ester?

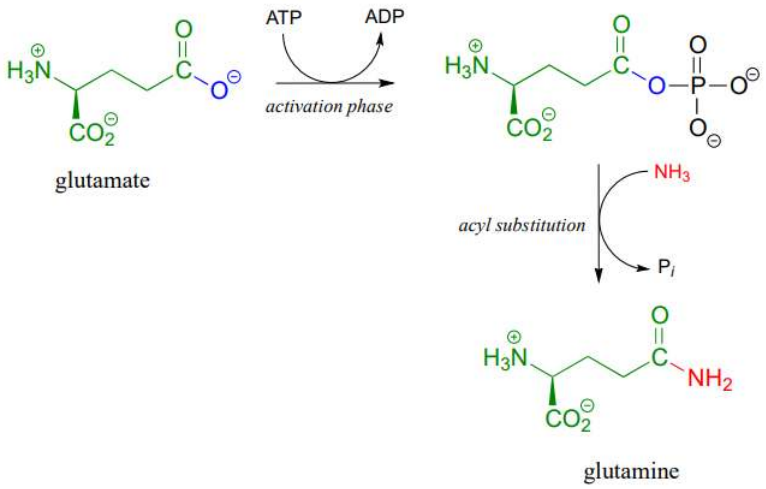
Amide formation

An activated carboxylate group (in other words, acyl phosphate or acyl-AMP) can be converted to an amide through nucleophilic attack by an amine.

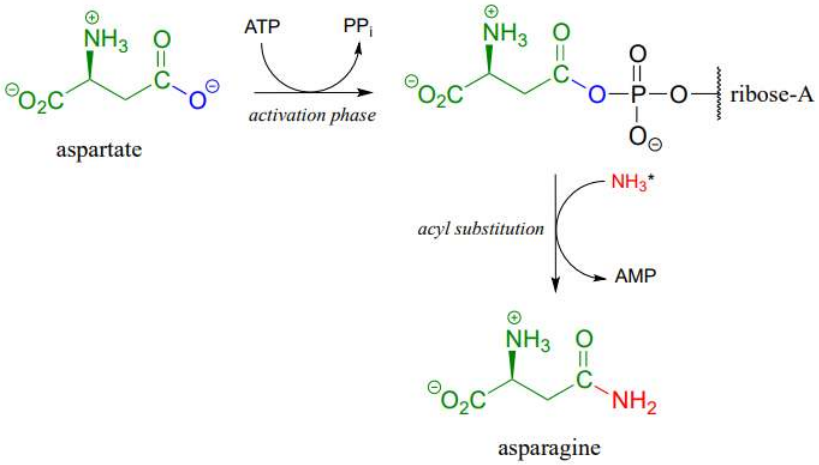
Mechanism for amide formation:



The amino acid biosynthesis pathways provide examples of amide formation in biology. The amino acid glutamine is synthesized in most species by converting the carboxylate side chain of glutamate (another amino acid) to an amide, after first activating the carboxylate by monophosphorylation: (EC 6.3.1.2)



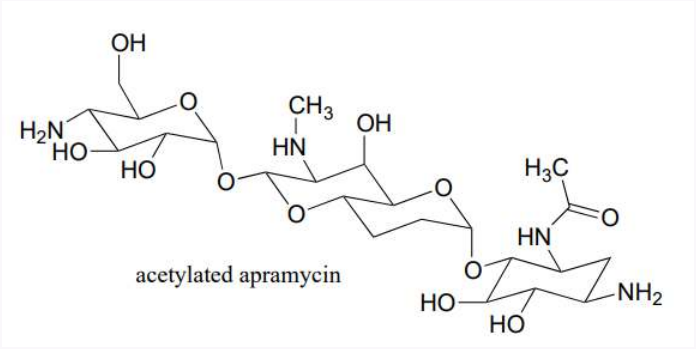
A similar process takes place in the synthesis of asparagine from aspartate, except that the activated carboxylate in this case is an acyl-AMP:



*In the asparagine synthesis reaction, the ammonia nucleophile actually comes from hydrolysis of a glutamine molecule.

Exercise 6.6.4

A enzyme in bacteria is thought to be responsible for resistance to a class of antibiotics that includes apramycin, ribostamycin and paromomycin. The enzyme catalyzes acetylation of the antibiotic compound with acetyl-CoA as an additional substrate. The structure of acetylated apramycin is shown below.



a. a) Identify the acetyl group that has been transferred to apramycin, (and thus inactivating it).
b. b) What functional group acts as an acetyl group donor? What functional group acts as an acetyl group?
c. c) What is the coproduct of the reaction?

Contributors

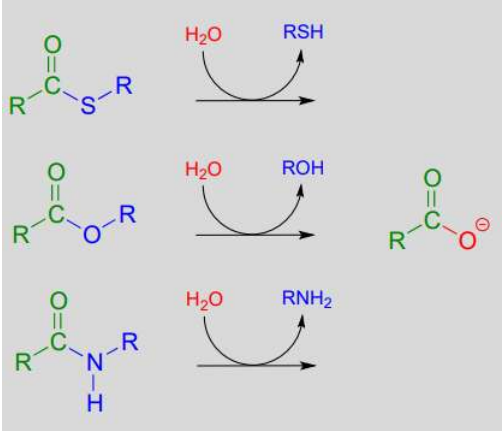
- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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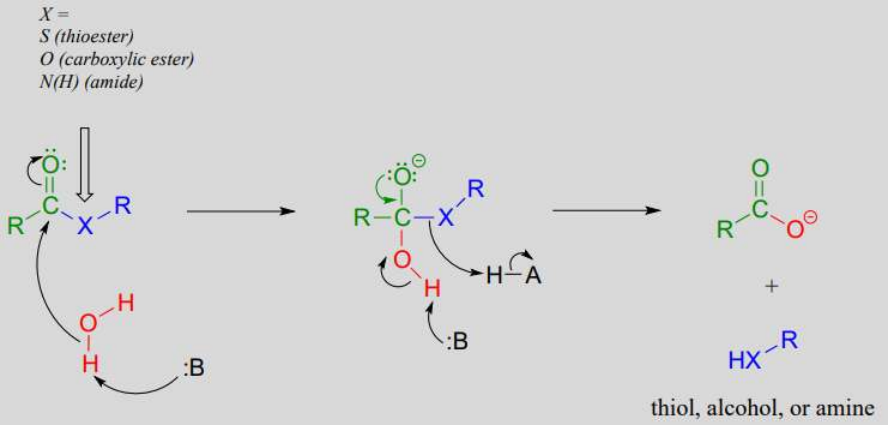
6.7: Hydrolysis of Thioesters, Esters, and Amides

So far we have been looking at the formation of thioesters, carboxylic esters, and amides, starting from carboxylates. In hydrolytic acyl substitution reactions, nucleophilic water is the incoming nucleophile and a carboxylate group is the final product. Because carboxylates are the least reactive among the carboxylic acid derivatives, these hydrolysis reactions are thermodynamically favorable, with thioester hydrolysis the most favorable of the three.

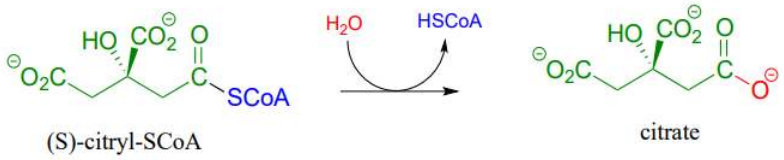
Thioester, carboxylic ester, and amide hydrolysis:



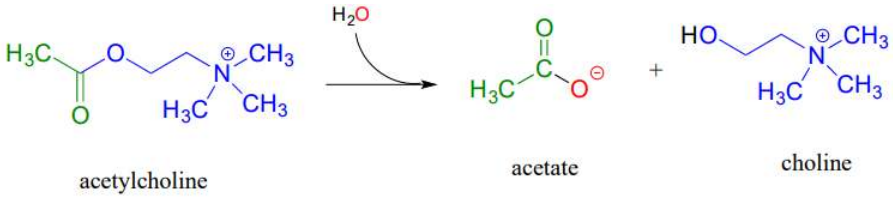
Mechanism:



In the citric acid (Krebs) cycle, (S)-citryl CoA is hydrolyzed to citrate (EC 2.3.3.8):



Acetylcholinesterase (EC 3.1.1.7), an enzyme present in the synapse, catalyzes hydrolysis of the ester group in acetylcholine, a neurotransmitter that triggers muscle contraction.



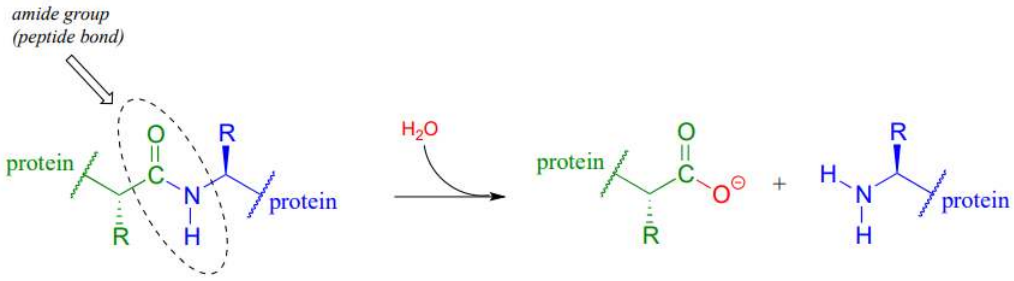
Like many other hydrolytic enzymes, the acetylcholinesterase reaction proceeds in two phases: first, a covalent enzyme-substrate intermediate is formed when the acyl group of acetylcholine is transferred to an active-site serine on the enzyme (a transesterification reaction). A water nucleophile then attacks this ester, driving off acetate and completing the hydrolysis.

Exercise 6.7.1

Based on the above description, draw the structure of the covalent enzyme-substrate intermediate in the acetylcholinesterase reaction.

If the action of acetylcholinesterase is inhibited, acetylcholine in the synapse does not get hydrolyzed and thus accumulates, resulting in paralysis and death in severe cases. Sarin nerve gas is a potent inhibitor of acetylcholinasterase action. Some victims of the Tokyo subway sarin attack in 1995 who were exposed to low levels of the gas reported that they initially realized that something was wrong when they noticed how dark everything seemed around them. This was due to uncontrolled contraction of their pupils. You will be invited to consider the mechanism of inhibition by sarin in problem 11.6.4.

Peptide (amide) bonds in proteins and polypeptides are subject to spontaneous (nonenzymatic) hydrolysis in water.

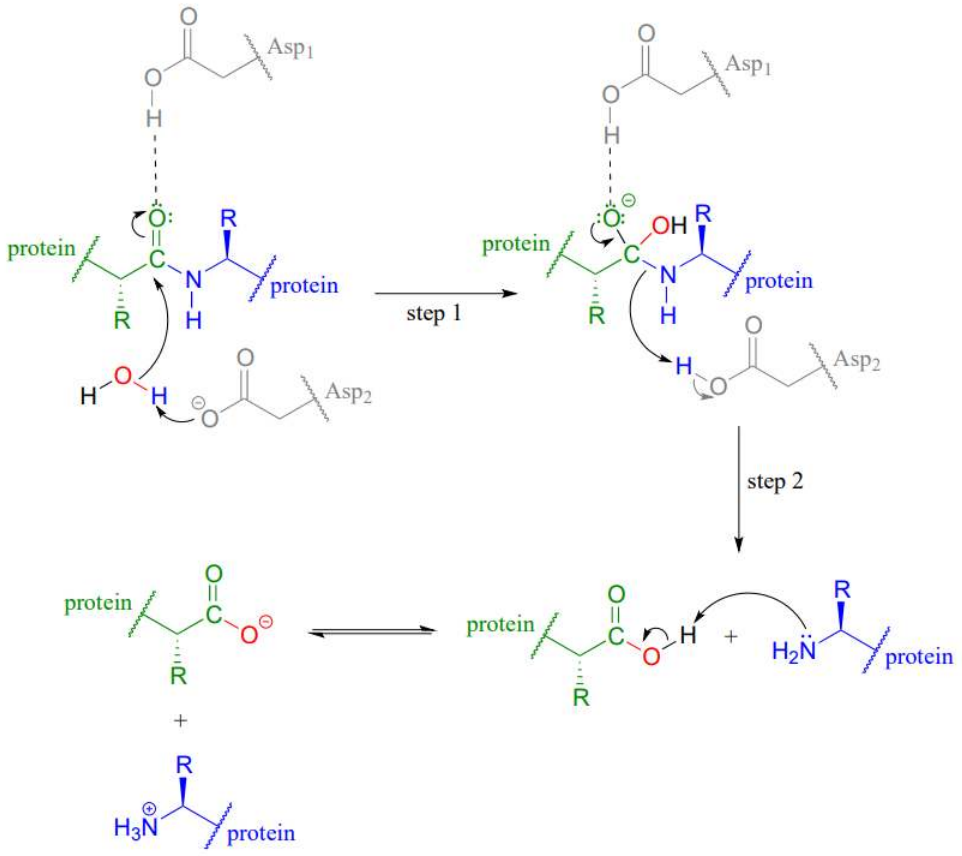


Although this amide to carboxylate conversion is thermodynamically a downhill reaction, peptide bonds are kinetically very stable (they react slowly) at neutral pH. In fact, the half-life for uncatalyzed hydrolysis of a peptide bond in pH 7 water is by some estimates as high as 1000 years. (Ann. Rev. Biochem. 2011, 80, 645.)

The stability of peptides bonds makes good physiological sense: we would all be in trouble if our enzymes, receptors, and structural proteins were hydrolyzing away while we slept! That being said, it is also true that controlled, specific hydrolysis of peptide bonds, catalyzed by a large, diverse class of enzymes called proteases, is a critical biochemical reaction type that can occur very rapidly, in many different biological contexts. For example, many proteins only become active after they have been ‘processed' - in other words, hydrolyzed at a specific amino acid location by a specific protease.

Although all proteases catalyze essentially the same reaction – amide hydrolysis - different protease subfamilies have evolved different catalytic strategies to accomplish the same result. HIV protease is the target of some the most recently-developed anti-HIV drugs. It plays a critical role in the life cycle if the HIV virus, hydrolyzing specific peptide bonds of essential viral proteins in order to convert them to their active forms. HIV protease is a member of the aspartyl protease subfamily, so-named because of the two aspartate residues located in the active sites of these enzymes. HIV protease is also, as you are probably aware, the target of HIV protease inhibitor drugs, which are a component of the most effective treatment currently available for HIV infection.

In HIV protease and other aspartyl proteases, the two enzymatic aspartates residues (shaded grey and abbreviated 'Asp1' and 'Asp2 'in the figure below) work in concert to activate the electrophile, nucleophile, and leaving group in the reaction.

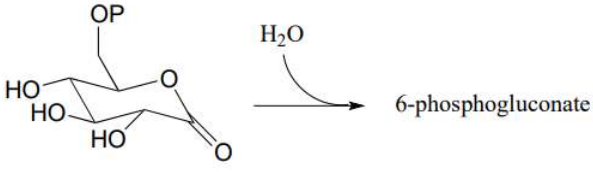


Exactly how this works is a subject of some debate and the details may well vary according to the enzyme in question, but one likely mechanism is illustrated in the figure above, where Asp1, which is initially in its protonated form, contributes a hydrogen bond to draw electron density away from the carbonyl carbon, making it more electrophilic. At the same time, Asp2, which begins the reaction cycle in its anionic form, deprotonates the water molecule to make it more nucelophilic. In step 2, Asp2 donates a proton back to the nitrogen, making it a better leaving group.

HIV protease inhibitors shut down this reaction, which prevents the virus from processing the proteins that it uses to bond to host cells.

Exercise 6.7.2

Lactonase (EC 3.1.1.17), the second enzyme in the oxidative branch of the pentose phosphate pathway, catalyzes hydrolysis of the lactone (cyclic ester) group in 6-phosphogluconolactone. Draw the structure of 6-phosphogluconate, the product of this reaction.



6-phosphogluconolactonate

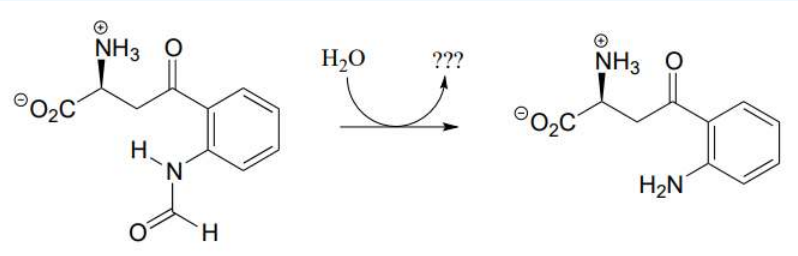
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Exercise 6.7.3

Draw the product of the β-lactamase-catalyzed hydrolysis of penicillin as described in section 11.6.

Exercise 6.7.4

What is the missing product (designated below by question marks) in the reaction below, which is part of degradation pathway for the amino acid tryptophan? How could you describe this reaction in organic chemistry terminology?



Answer

Add answer text here and it will automatically be hidden if you have a "AutoNum" template active on the page.

Contributors and Attributions

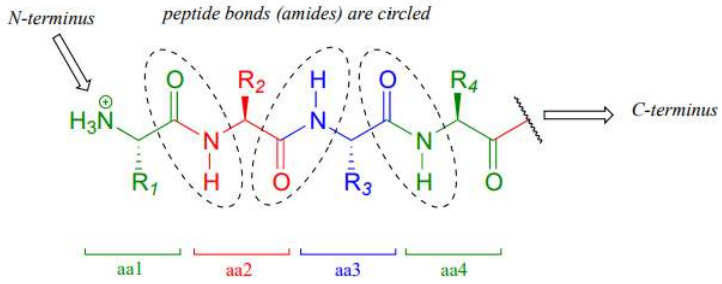
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6.8: Protein Synthesis on the Ribosome

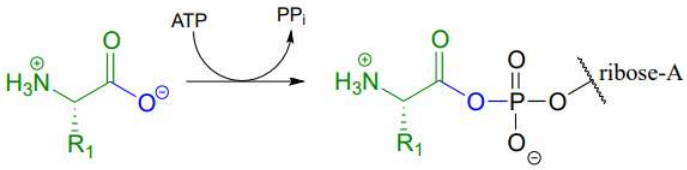
Recall from section 1.3D that the 'peptide bonds' which link amino acids to form polypeptides and proteins are in fact amide functional groups. The figure below shows the first four amino acid residues in a protein, starting at the amino terminus.



Let's take a look at the chemistry behind the formation of a new peptide bond between the first two amino acids - which we will call $aa-1$ and $aa-2$ - in a growing protein molecule. This process takes place on the ribosome, which is essentially a large biochemical 'factory' in the cell, composed up of many enzymes and RNA molecules, and dedicated to the assembly of proteins. You will learn more in a biochemistry or cell biology course about the complex but fascinating process of ribosomal protein synthesis. For now, we will concentrate on the enzyme-catalyzed organic transformation that is taking place: the formation of an amide from a carboxylate and an amine.

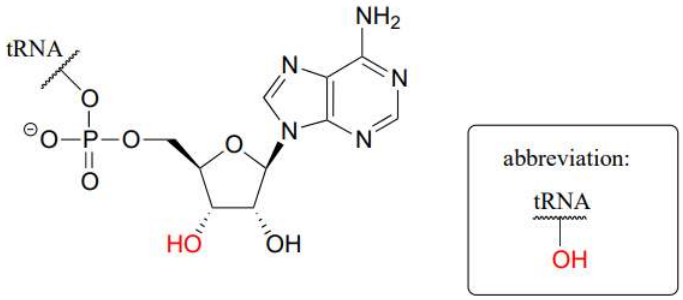
We have seen amide-forming reactions before – think back to the glutamine and asparagine synthetase reactions (section 11.5). The same ideas that we learned for those reactions hold true for peptide bond formation: the carboxylate group on a substrate amino acid must first be activated, and the energy for this activation comes from ATP.

The carboxylate group of aa-1 is first transformed to an acyl-AMP intermediate through a nucleophilic substitution reaction at the α -phosphate of ATP.

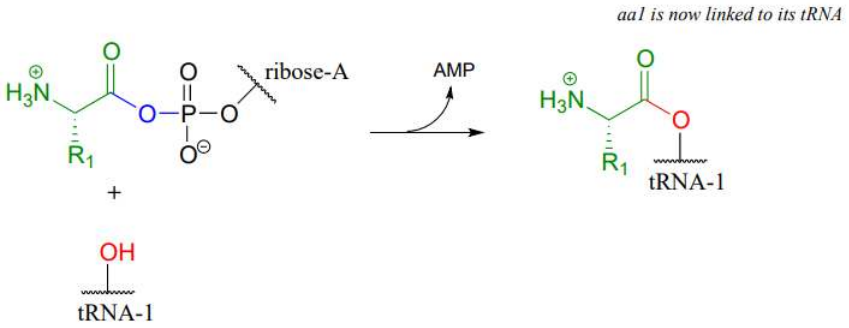


activated acyl-AMP intermediate

In the next step, the amino acid is transferred to a special kind of RNA polymer called transfer RNA, or $tRNA$ for short. We need not concern ourselves here with the structure of $tRNA$ molecules- all we need to know for now is that the nucleophile in this reaction is a hydroxyl group on the terminal adenosine of a $tRNA$ molecule. Because this $tRNA$ molecule is specific to $aa-1$, we will call it $tRNA-1$

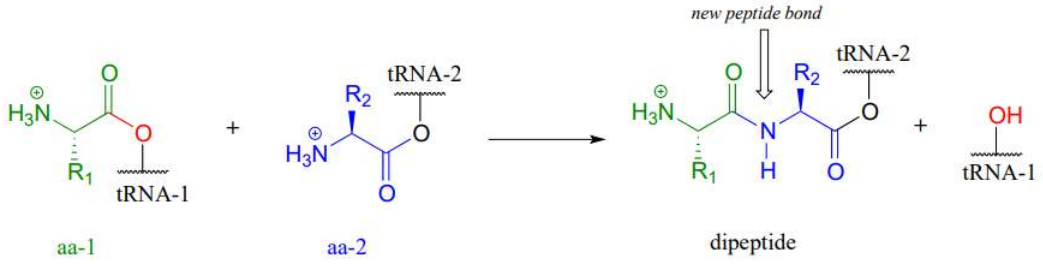


The incoming nucleophile is an alcohol, thus what we are seeing is an esterification: an acyl substitution reaction between the activated carboxylate of $aa-1$ and an alcohol on $tRNA-1$ to form an ester.

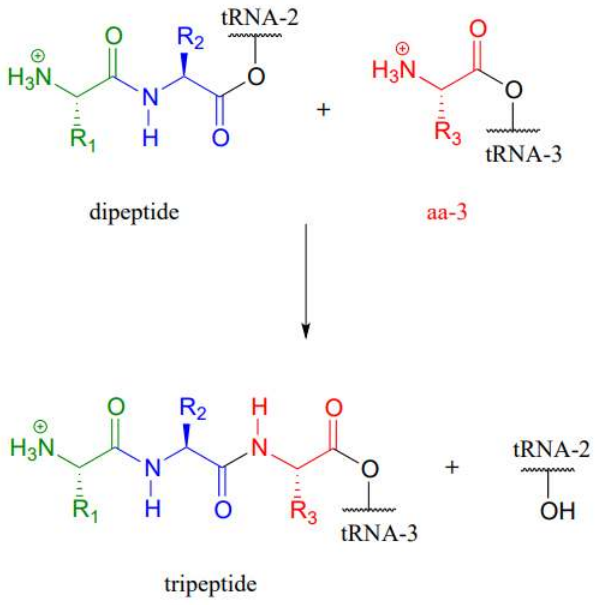


This reaction, starting with activation of the amino acid, is catalyzed by a class of enzymes called aminoacyl-$tRNA$ synthetases (there are many such enzymes in the cell, each one recognizing its own amino acid - $tRNA$ pair).

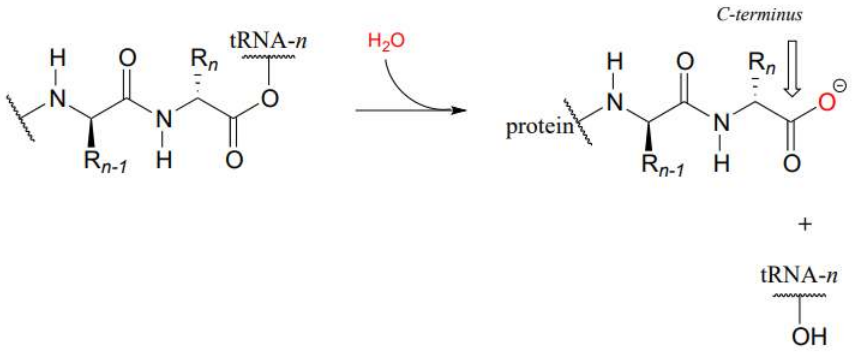
The first amino acid is now linked via an ester group to $tRNA-1$. The actual peptide bond-forming reaction occurs when a second amino acid (aa-2) also linked to its own $tRNA-2$ molecule, is positioned next to the first amino acid on the ribosome. In another acyl substitution reaction, catalyzed by an enzymatic component of the ribosome called peptidyl transferase (EC 2.3.2.12), the amino group on $aa-2$ displaces $tRNA1$: thus, an ester has been converted to an amide (thermodynamically downhill, so ATP is not required).



This process continues on the ribosome, as one amino acid after another is added to the growing protein chain:



When a genetically-coded signal indicates that the chain is complete, an ester hydrolysis reaction – as opposed to another amide formation - occurs on the last amino acid, which we will call $aa-n$. This reaction is catalyzed by proteins called release factors (RFs).



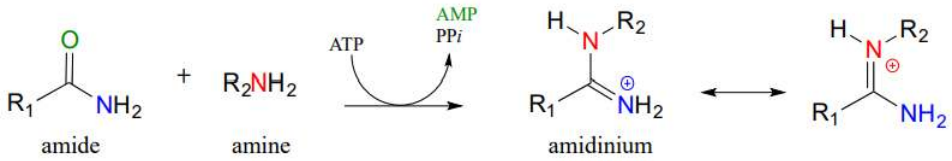
This hydrolysis event frees the mature protein from the ribosome, and results in the formation of a free carboxylate group at the end of the protein (this is called the carboxy-terminus, or *C*-terminus of the protein, while the other end – the 'starting' end – is called the *N*-terminus).

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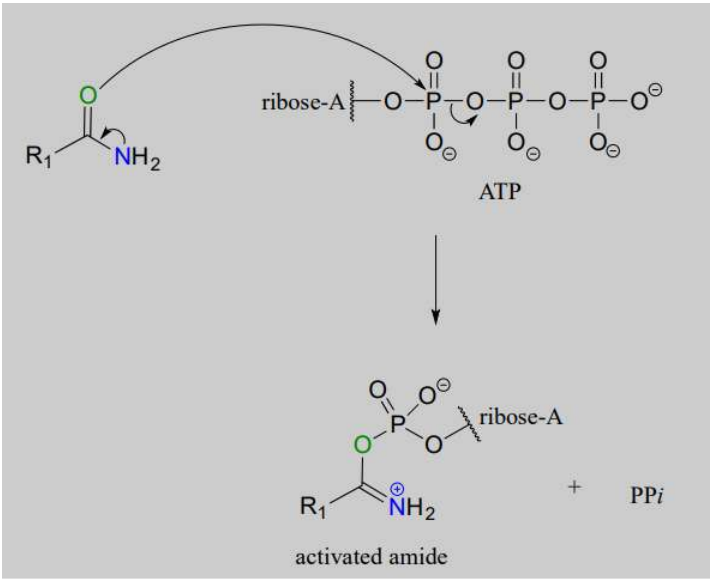
6.9: Nucleophilic Substitution at Activated Amides and Carbamides

In discussing the nucleophilic acyl substitution reactions of acyl phosphates, thioesters, esters, and amides, we have seen many slight variations on one overarching mechanistic theme. Let's now look at a reaction that can be thought of as a 'cousin' of the nucleophilic acyl substitution, one that follows the same general pattern but differs in several details. Below is a generic illustration of this reaction type:



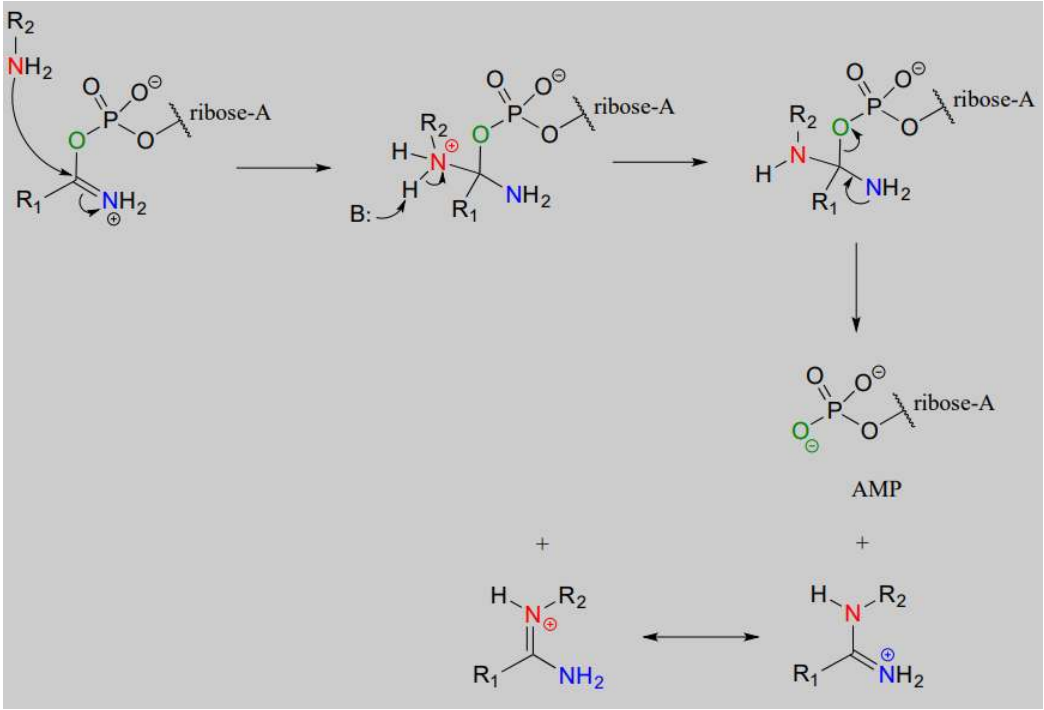
Looking at this reaction, you can see that substitution is occurring at an amide group, but the atom that gets expelled is the amide oxygen rather than the amide nitrogen. Also, we see that the substituting nucleophile is an amine, and the product is a functional group referred to as an amidinium ion (the uncharged conjugate base is called an amidine, and the pKa of the group is close enough to 7 that it can be shown in either protonated or deprotonated form in a biological context). As we learned previously in this chapter, amides are comparatively stable to nucleophilic substitution, and thus it stands to reason that the starting amide must be activated before the reaction can take place. This occurs, as you might predict, through the formation of an acyl phosphate intermediate at the expense of one ATP molecule. The amide oxygen acts as a nucleophile, attacking the α-phosphate of ATP to form the activated acyl-AMP intermediate:

Substitution at an activated amide, phase 1 (activation):

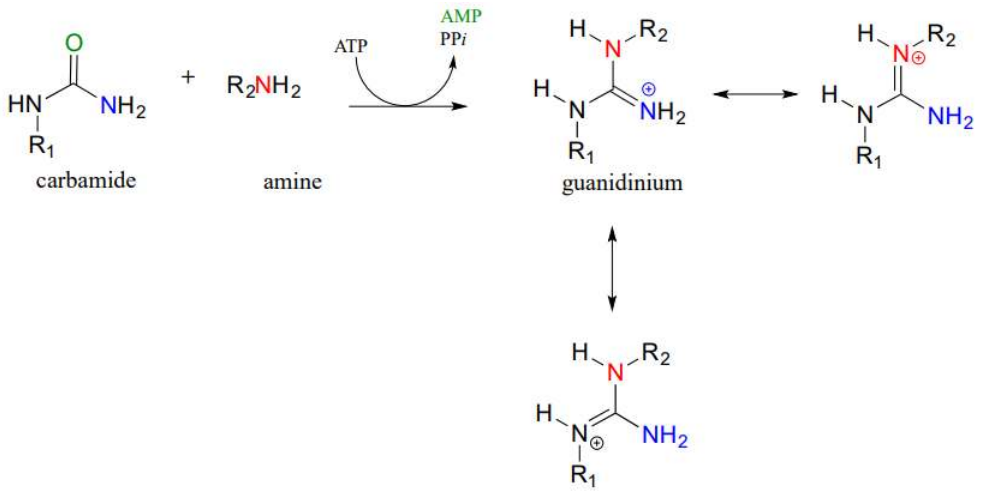


Next, a kind of acyl substitution occurs that we have not yet seen: an amine nitrogen attacks the electrophilic carbon of a carbon-nitrogen double bond, and the reaction proceeds through a tetrahedral intermediate before the AMP group is expelled, taking with it what was originally the carbonyl oxygen of the starting amide.

Substitution at an activated amide, phase 2:

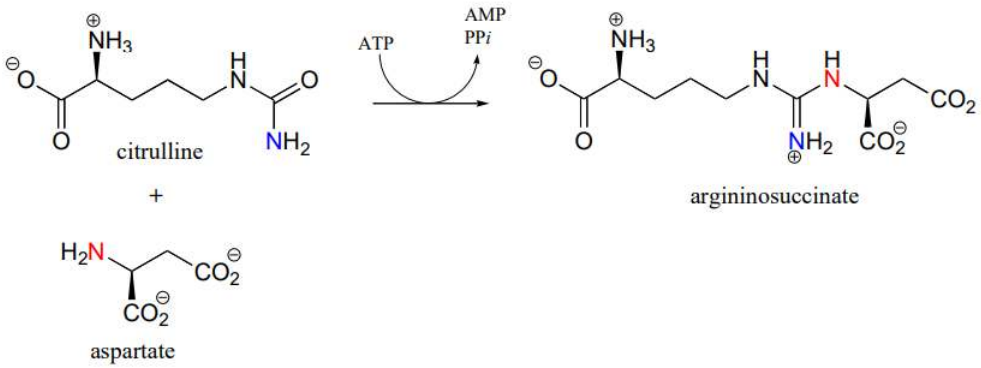


Carbamides, a kind of 'double amide' functional group in which a carbonyl oxygen is bonded to two nitrogens, also undergo this type of reaction, leading to the formation of a guanidinium/gaunidine functional group (look again at the structure of the amino acid arginine and you will see that it contains a guanidinium group on the side chain).



The reaction catalyzed by argininosuccinate synthetase (EC 6.3.4.5) is the second step in the urea cycle, a four-step series of reactions in which ammonia is converted into urea for elimination in urine. Note that the substitution takes place at the carbamide group of citrulline, the nucleophilic amine is on aspartate, and the product has a guanidinium functional group.

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Exercise 6.9.1

a. Draw the structure of the 'activated carbamide' intermediate in the reaction above.

b. Draw the structure of the cyclic product of a hypothetical intramolecular substitution reaction of citrulline (ie. aspartate is not involved).

Contributors and Attributions

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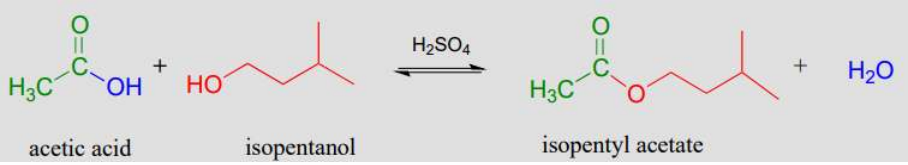
6.10: Nucleophilic Acyl Substitution Reactions in the Laboratory

All of the biological nucleophilic acyl substitution reactions we have seen so far have counterparts in laboratory organic synthesis. Mechanistically, one of the biggest differences between the biological and the lab versions is that the lab reactions usually are run with a strong acid or base as a catalyst, whereas biological reactions are of course taking place at physiological pH. When proposing mechanisms, then, care must be taken to draw intermediates in their reasonable protonation states: for example, a hydronium ion (H_3O^+) intermediate is reasonable to propose in an acidic reaction, but a hydroxide (OH^-) intermediate is not.

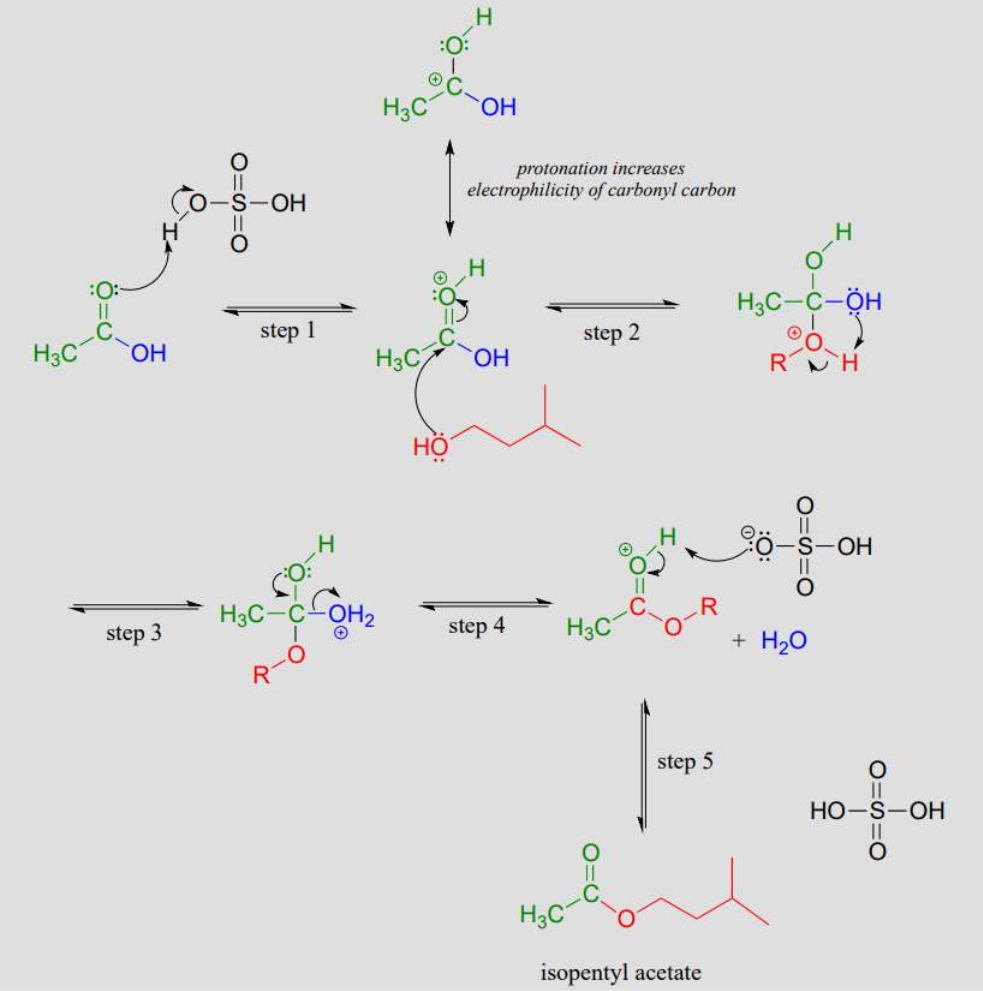
Ester reactions - bananas, soap and biodiesel

Acid-catalyzed synthesis of flavor compounds such as isopentyl acetate (an ester with the flavor of banana) is simple to carry out in the lab. In this esterification reaction, acetic acid is combined with isopentyl alcohol along with a catalytic amount of sulfuric acid.

Acid-catalyzed esterification (laboratory reaction):



Mechanism:



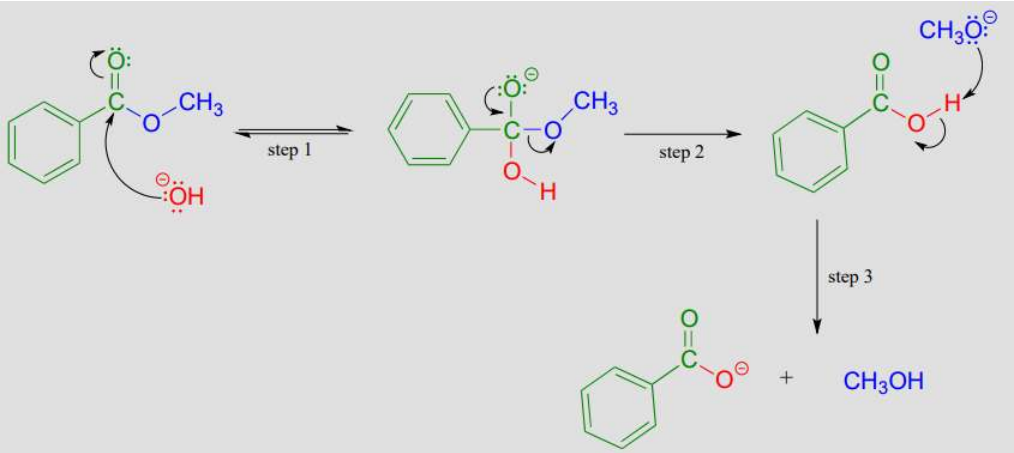
The carbonyl oxygen of acetic acid is first protonated (step 1), which draws electron density away from the carbon and increases its electrophilicity. In step 2, the alcohol nucleophile attacks: notice that under acidic conditions, the nucleophile is not deprotonated

simultaneously as it attacks (as we would show in a biochemical mechanism), and the tetrahedral intermediate is a cation rather than an anion. In step 3, a proton is transferred from one oxygen atom to another, creating a good leaving group (water) which is expelled in step 4. Finally (step 5), the carbonyl oxygen on the ester is deprotonated, regenerating the catalytic acid.

This reaction is highly reversible, because carboxylic acids are approximately as reactive as esters. In order to obtain good yields of the ester, an excess of acetic acid can be used, which by Le Chatelier's principle (see your General Chemistry textbook for a review) shifts the equilibrium toward the ester product.

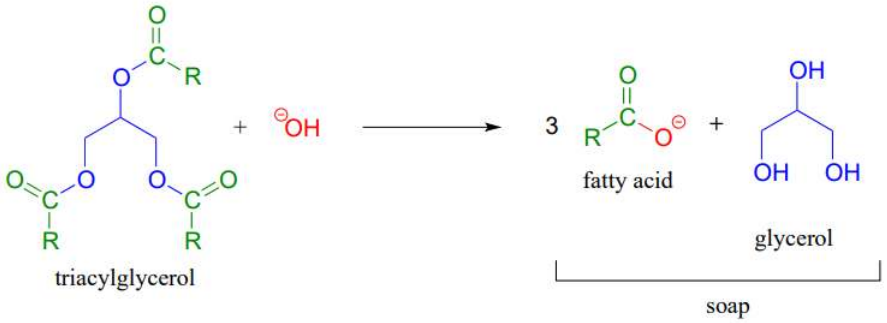
Saponification is a common term for base-induced hydrolysis of an ester. For example, methyl benzoate will hydrolyze to benzoate and methanol when added to water with a catalytic amount of sodium hydroxide.

Mechanism of base-catalyzed ester hydrolysis (saponification):

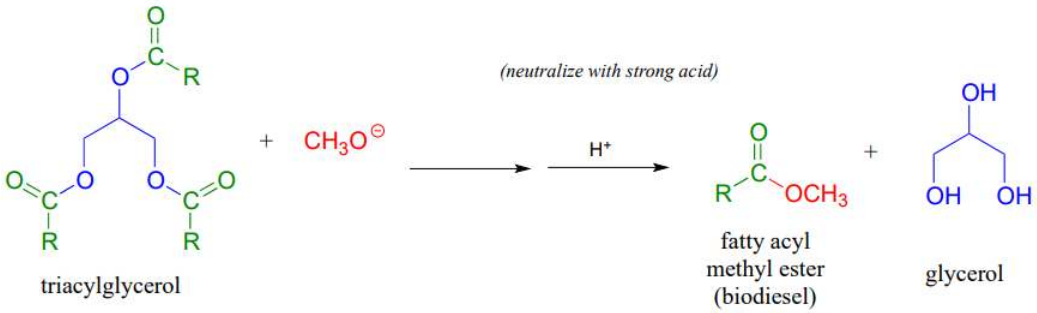


Addition of the base provides hydroxide ion to act as a nucleophile (hydroxide is of course a better nucleophile than water) in step 1. The tetrahedral intermediate (anionic in this case, because the reaction conditions are basic) then collapses in step 2, and the alkoxide (CH_3O^-) leaves. We are not used to seeing alkoxides or hydroxides as leaving groups in biochemical reactions, because they are strong bases - but in a basic solution, this is a reasonable chemical step. Step 3 is simply an acid-base reaction between the carboxylic acid and the alkoxide. Note that this is referred to as base-induced rather than base-catalyzed because hydroxide is not regenerated, and thus a full molar equivalent of base must be used.

The saponification process derives its name from the ancient craft of soap-making, in which the ester groups of triacylglycerols in animal fats are hydrolized under basic conditions to glycerol and fatty acyl anions (see section 2.5A for a reminder of how fatty acyl anions work as soap).



We learned earlier about transesterification reactions in the context of the chemical mechanism of aspirin. Transesterification also plays a key role in a technology that is already an important component in the overall effort to develop environmentally friendly, renewable energy sources: biodeisel. You may have heard stories about people running their cars on biodeisel from used french fry oil. To make biodeisel, triacylglycerols in fats and oils can be transesterified with methanol or ethanol under basic conditions. The fatty acyl methyl and ethyl ester products are viable motor fuels.



Exercise 6.10.1

Draw structures of the carboxylic acid and alcohol starting materials that could be used to synthesize the fragrant fruit esters shown in section 6.2.

Exercise 6.10.2

What would happen if you tried to synthesize isopentyl acetate (banana oil) with basic rather than acidic conditions? Would this work?

Exercise 6.10.3

Consider the reverse direction of the acid-catalyzed esterification reaction. What would you call this reaction in organic chemistry terms?

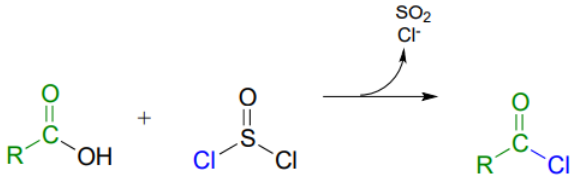
Exercise 6.10.4

An alternative way to synthesize esters is to start with a carboxylate and an alkyl halide. Draw a mechanism for such a synthesis of methyl benzoate - what type of reaction mechanism is this?

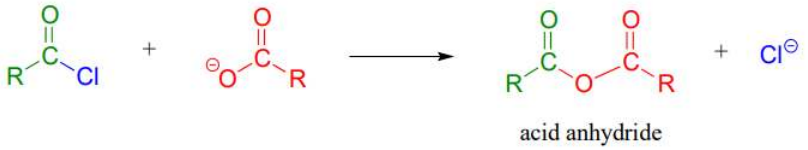
Acid chlorides and acid anhydrides

In the cell, acyl phosphates and thioesters are the most reactive of the carboxylic acid derivatives. In the organic synthesis lab, their counterparts are acid chlorides and acid anhydrides, respectively. Of the two, acid chlorides are the more reactive, because the chloride ion is a weaker base and better leaving group than the carboxylate ion (the pK_a of HCl is -7, while that of carboxylic acids is about 4.5: remember, a stronger conjugate acid means a weaker conjugate base).

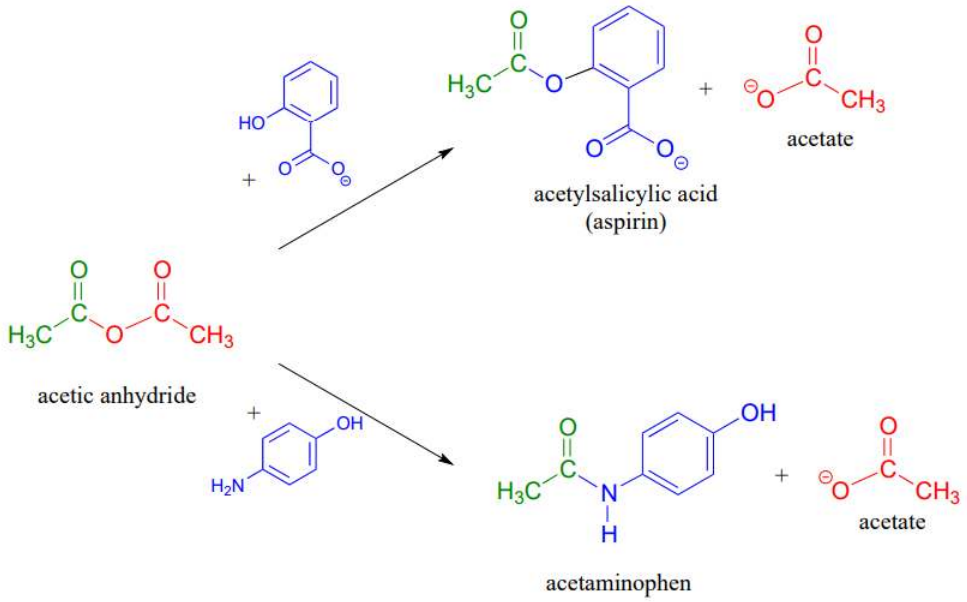
Acid chlorides can be prepared from carboxylic acids using $SOCl_2$:



Acid anhydrides can be prepared from carboxylic acids and an acid chloride under basic conditions:



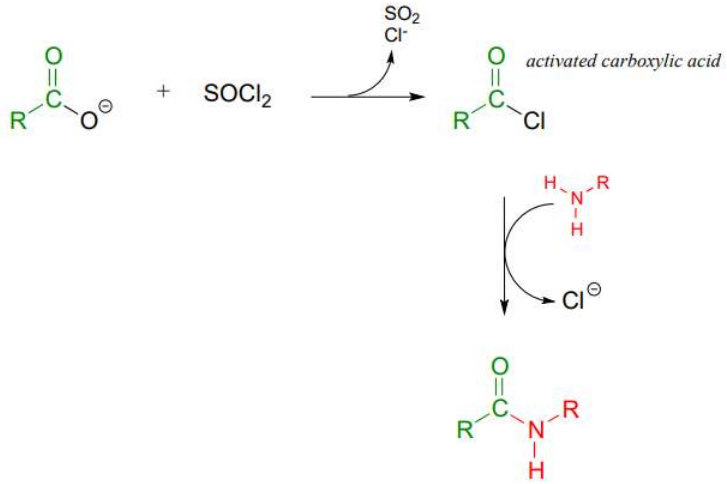
Acetic anhydride is often used to prepare acetate esters and amides from alcohols and amines, respectively. The synthesis of aspirin and acetaminophen are examples:



A carboxylic acid cannot be directly converted into an amide because the amine nucleophile would simply act as a base and deprotonate the carboxylic acid:



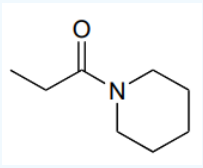
Instead, the carboxylic acid is first converted to an acid chloride (in other words, the carboxylic acid is activated), then the acid chloride is combined with an amine to make the amide.



This sequence of reactions is a direct parallel to the biochemical glutamine and asparagine synthase reactions we saw earlier in the chapter (section 11.5), except that the activated form of carboxylic acid is an acid chloride instead of an acyl phosphate or acyl-AMP.

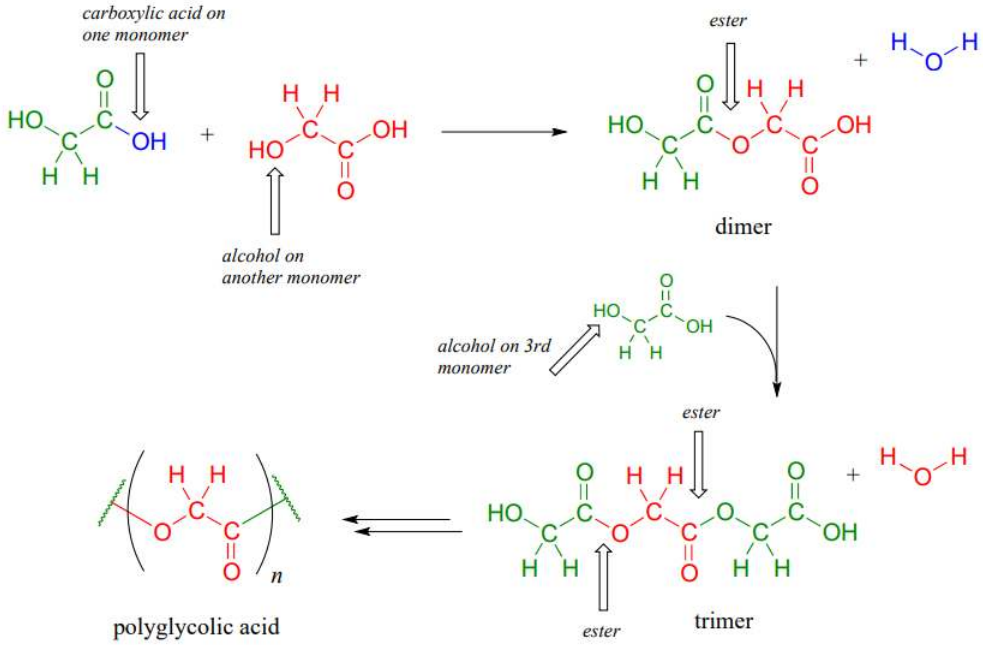
Exercise 6.10.5

For the preparation of the amide below, show a starting carboxylate and amine and the intermediate acid chloride species.



Polyesters and polyamides

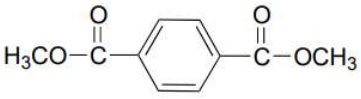
If you have ever had the misfortune of undergoing surgery or having to be stitched up after a bad cut, it is likely that you benefited from our increasing understanding of polymers and carboxylic ester chemistry. Polyglycolic acid is a material commonly used to make dissolving sutures. It is a polyester - a polymer linked together by ester groups - and is formed from successive acyl substitution reactions between the alcohol group on one end of a glycolic acid monomer and the carboxylic acid group on a second:



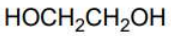
The resulting polymer - in which each strand is generally several hundred to a few thousand monomers long - is strong, flexible, and not irritating to body tissues. It is not, however, permanent: the ester groups are reactive to gradual, spontaneous hydrolysis at physiological ***pH***, which means that the threads will dissolve naturally over several weeks, eliminating the need for them to be cut out by a doctor.

Exercise 6.10.6

Dacron, a polyester used in clothing fiber, is made of alternating dimethyl terephthalate and ethylene glycol monomers.



dimethyl terephthalate

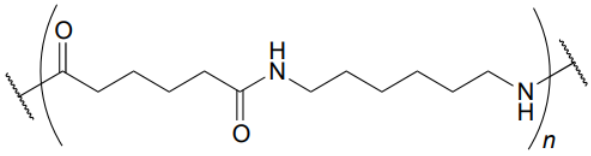


ethylene glycol

a. Draw the structure of a Dacron tetramer (in other words, four monomers linked together).
b. Water is a side product of glycolic acid polymerization. What is the equivalent side product in Dacron production?

Exercise 6.10.7

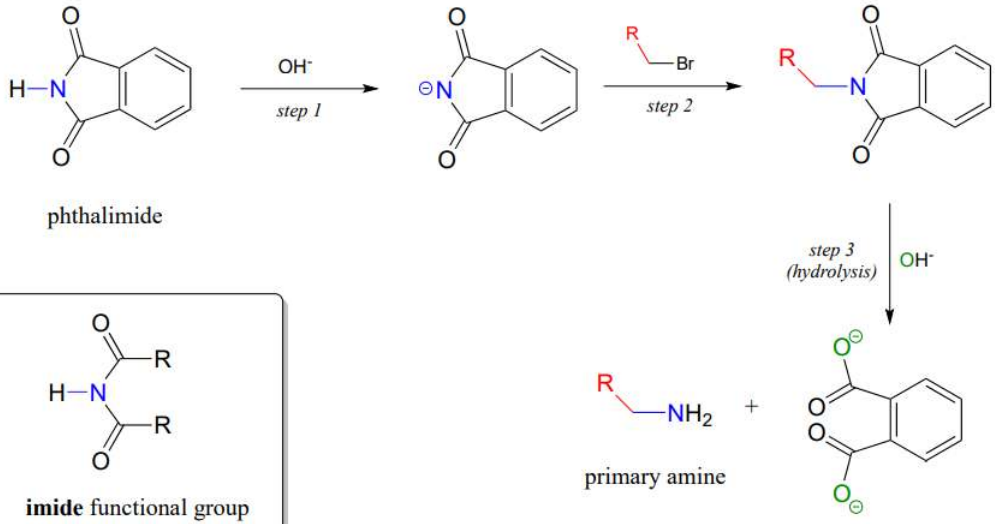
Nylon 6,6 is a widely used polyamide composed of alternating monomers. Nylon 6,6 has the structure shown below -the region within the parentheses is the repeating unit, with 'n' indicating a large number of repeats. Identify the two monomeric compounds used to make the polymer.



Nylon 66

The Gabriel synthesis of primary amines

The Gabriel synthesis, named after the 19th-century German chemist Siegmund Gabriel, is a useful way to convert alkyl halides to amines and another example of S_N2 and acyl substitution steps in the laboratory. The nitrogen in the newly introduced amine group comes from phthalimide. In the first step of the reaction, phthalimide is deprotonated by hydroxide, then in step 2 it acts as a nucleophile to displace a halide in an S_N2 reaction (phthalimide is not a very powerful nucleophile, so this reaction works only with unhindered primary or methyl halides).



phthalimide

primary amine

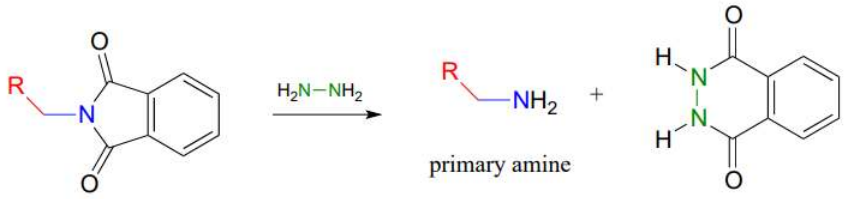
imide functional group

Step 3 is simply a pair of hydrolytic acyl substitution steps to release the primary amine, with an aromatic dicarboxylate by-product.

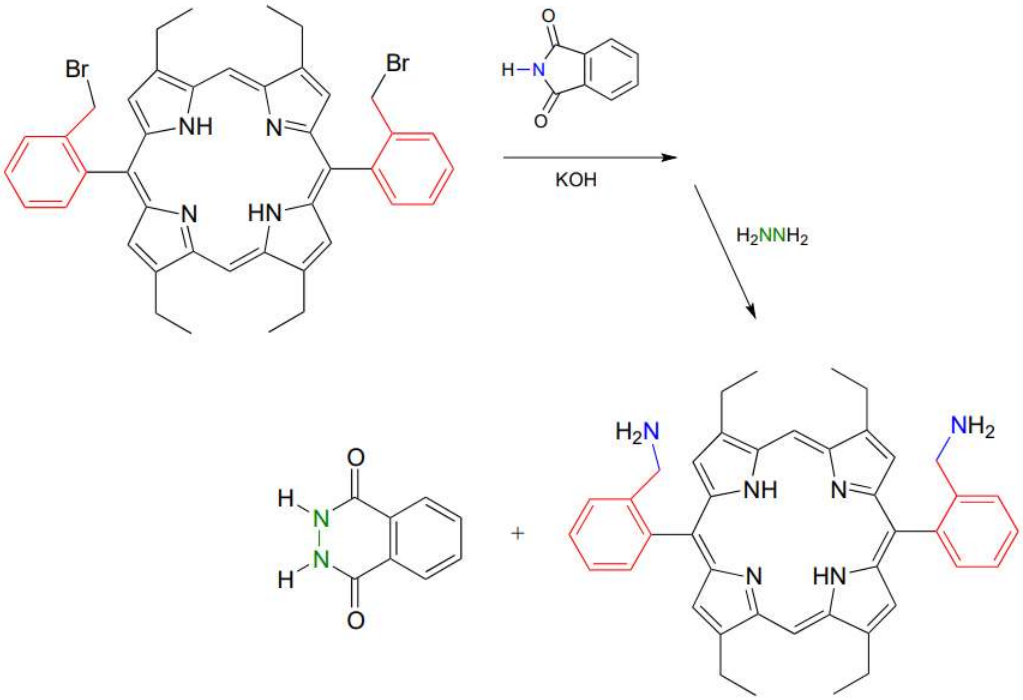
Exercise 6.10.8

Phthalimide contains an 'imide' functional group, and has a pK_a of approximately 10. What makes the imide group so much more acidic than an amide, which has a pK_a of approximately 17?

As an alternative procedure, release of the amine in step 3 can be carried out with hydrazine (H_2NNH_2) instead of hydroxide. Again, this occurs through two nucleophilic acyl substitution reactions.



In 2000, chemists at MIT synthesizing a porphyrin-containing molecule introduced two amine groups using the Gabriel synthesis with hydrazine. Porphyrins, which include the 'heme' in our red blood cells, are an important family of biomolecules with a variety of biochemical function (J. Org. Chem. 2000, 65, 5298).



Contributors and Attributions

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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6.11: A Look Ahead - Acyl Substitution Reactions with a Carbanion or Hydride Ion Nucleophile

Although we have seen many different types of nucleophilic acyl substitutions in this chapter, we have not yet encountered a reaction in which the incoming nucleophile is a carbanion or a hydride. Recall that in the previous chapter on aldehydes and ketones, we also postponed discussion of nucleophilic carbonyl addition reactions in which a carbanion or a hydride is the nucleophile. The reason for putting off these discussions is that these topics are both important and diverse enough to warrant their own dedicated chapters.

In the next chapter, we will see many carbonyl addition and acyl substitution reactions where the nucleophilic species is a resonance-stabilized carbanion such as an enolate (section 7.6). Then in chapter 14, we will encounter nucleophilic addition and acyl substitution reactions in which a hydride ion (H^-) essentially plays the part of a nucleophile. In these chapters we will see how nucleophilic carbanion and hydride species are generated in a biochemical context. For now, see if you can predict the result of the following biochemical reactions.

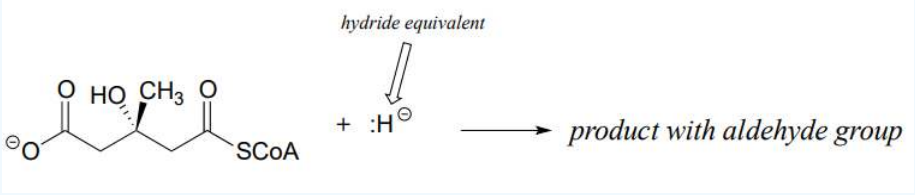
Exercise 6.11.1

Predict the products of the following nucleophilic acyl substitution reactions, both of which are part of the biosynthesis of isoprenoid compounds such as cholesterol and lycopene:

a. acetoacetyl CoA acetyltransferase reaction (enolate nucleophile)



b. HMG-CoA reductase reaction (to repeat, the nucleophile here is not literally an isolated hydride ion, which would be a very unlikely species in a physiological environment. We will learn in chapter 16 what is actually going on, but for the time being, just predict the result of an acyl substitution reaction with a "hydride ion" nucleophile.)



Contributors and Attributions

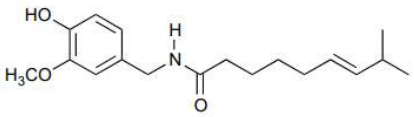
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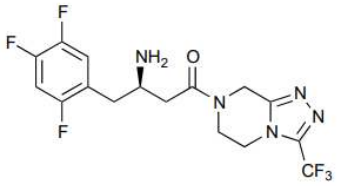
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6.P: Nucleophilic Acyl Substitution Reactions (Problems)

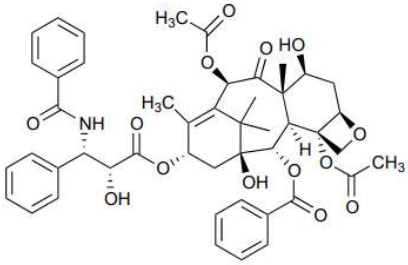
P6.1: Here is some practice in recognizing carboxylic acid derivative functional groups in large, complex biological molecules. There are seven amide and four ester groups in the molecules below - see if you can find them all.



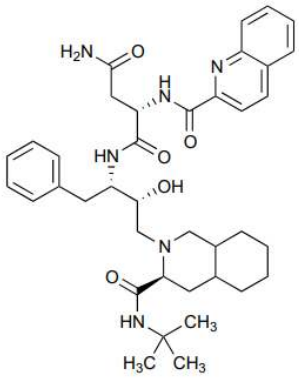
capsaicin
(the 'hot' molecule in hot peppers)



sitagliptin
(component of Janumet, a diabetes drug from Merck Pharmaceuticals)



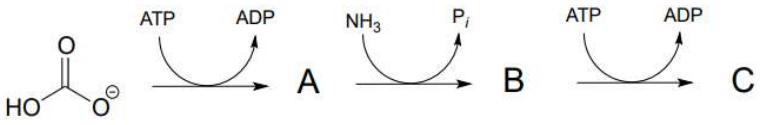
paclitaxel
(cancer drug Taxol from Bristol-Myers Squibb)



Saquinavir
(first HIV protease inhibitor to be approved by FDA)

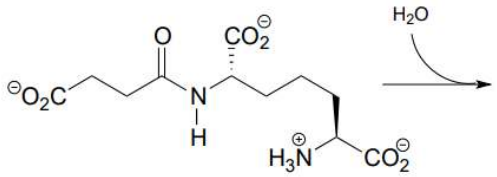
P6.2

a. a) Predict the structures of intermediate compounds A, B, and C in the reaction below (EC 6.3.4.16). Compound C contains an activated carboxylate functionality. Use abbreviations as appropriate.
b. Draw a reasonable mechanism for the A to B step



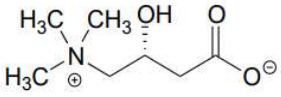
P6.3: Imagine that acetylcholine is combined with acetylcholinesterase (section 11.6) in a buffer made from ^{18}O-labeled water. Where would you expect to find the ^{18}O label in the products?

P6.4: Predict the products of this hydrolysis reaction (EC 3.5.1.18):



P6.5: The breakdown of fat in our bodies begins with the action of lipase enzymes, which catalyze the cleavage of fatty acids from t glycerol backbone of triacylglycerol (see section 1.3 for a reminder of the structure of triacylglycerol). A serine residue in the lipase acti site plays a key nucleophilic role in the reaction. Draw the single mechanistic step in which the covalent link between a fatty acid and t glyceryl backbone is broken, using curved arrow notation and appropriate abbreviation.

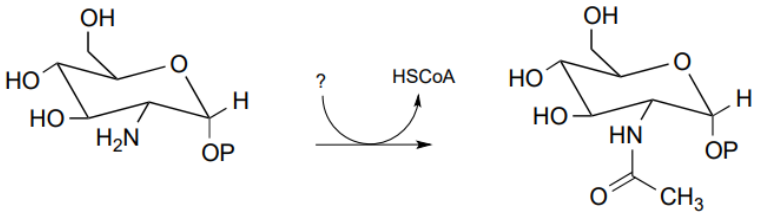
P6.6: Before long-chain fatty acids are transported across the inner mitochondrial membrane, they are temporarily transferred from Coenzyme A to a transport molecule called carnitine, to which they are linked by an ester group (EC 2.3.1.21).



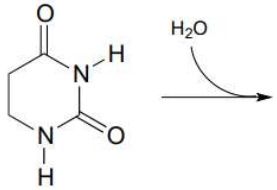
carnitine

Draw the structure of fatty acyl carnitine (use R to denote the hydrocarbon chain of the fatty acid)

P6.7: Below is a reaction from carbohydrate metabolism (EC 2.3.1.157). Identify the compound designated with a question mark.



P6.8: Propose the most likely enzymatic hydrolysis product of the substrate below (hint - think about electrophilicity when consideri regiochemical outcomes!) (EC 3.5.2.2) J. Biol. Chem. 2006, 281, 13762 scheme 2)

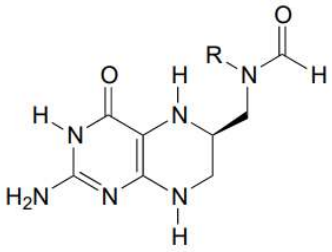


P6.9: The coenzyme tetrahydrofolate (***THF***) participates in single-carbon transfer reactions. One derivative of ***THF***, called 10-form ***THF*** (abbreviated structure shown below), transfers a formyl group early in purine ribonucleotide biosynthesis to glycinami ribonucleotide.

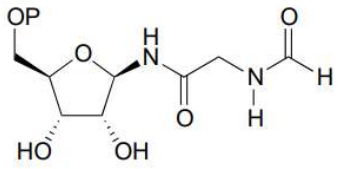
10-formyl-THF + glycinamide ribonucleotide

↓

THF + formylglycinamide ribonucleotide



10-formyl-THF

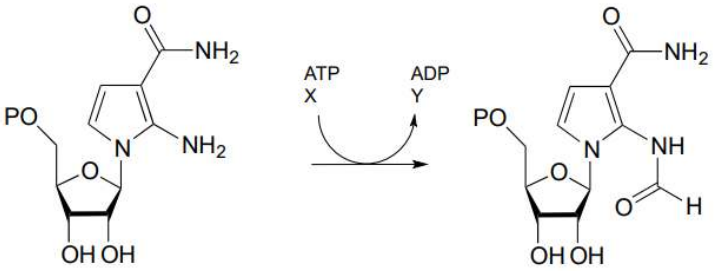


formylglycinamide ribonucleotide

Draw a nucleophilic attack step for this reaction (assume that acyl transfer between the two substrates is direct, without any covalent enzyme-substrate intermediates being formed).

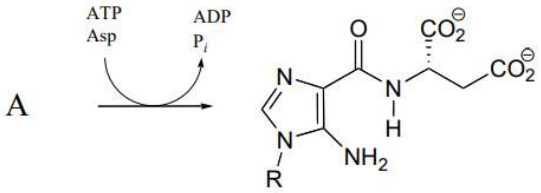
P6.10: One of the key steps in the biosynthesis of purine nucleotides (guanosine and adenosine) in archaea is shown below.

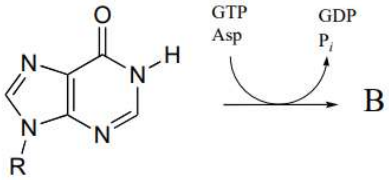
https://chem.libretexts.org/@go/page/291145



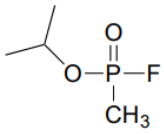
Identify the missing compounds X and Y in the figure above, and draw the structure of an acyl phosphate intermediate.

P6.11: The reactions below are part of nucleotide biosynthesis. Predict the structures of compounds A and B. Compound A contains a carboxylate group, and the reaction that forms compound B is of the type discussed in section 11.8, in which an amine group substitutes at an activated amide to form an amidine/amidinium group.



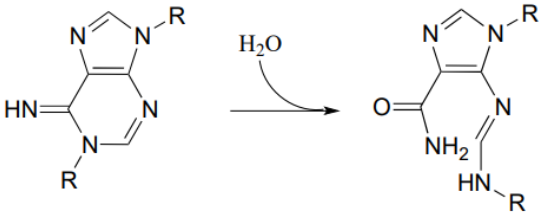


P6.12: Recall from section 11.6 that acetylcholinesterase catalyzes the hydrolysis of the ester group in acetylcholine, going through intermediate in which the acetyl group on the substrate is transferred to a serine on the enzyme by a transesterification reaction. The ner gas sarin acts by blocking this initial transesterification step: the drug enters the active site and attaches to the active site serine. Given t structure of sarin below, propose a mechanism for how this happens.



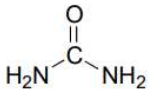
sarin

P6.13: Propose a mechanism for the following reaction from histidine biosynthesis (EC 3.5.4.19).

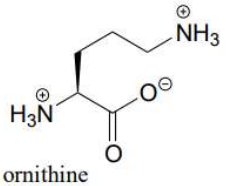


P6.14 (Challenging!) In the final step of the urea cycle (a phase of amino acid degradation pathways), the amino acid arginine hydrolyzed to urea and ornithine (EC 3.5.3.1). Propose a reasonable mechanism.

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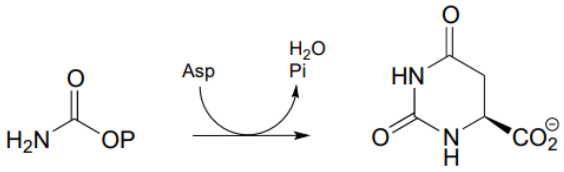


urea



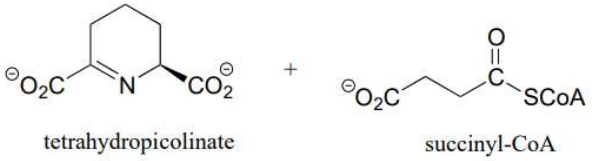
ornithine

P6.15: In the biosynthetic pathway for the DNA/RNA bases uridine and cytidine, a reaction occurs in which carbamoyl phospha condenses with aspartate, and the resulting intermediate cyclizes to form dihydroorotate. Propose a mechanism for this transformatic Hint: in a very unusual step, a carboxylate group is at one point in the process directly subjected to an acyl transfer reaction, without pr activation by phosphorylation. The enzyme accomplishes this with the help of two bo.und zinc ions, which serve to stabilize the negati charge on a hydroxide leaving group. (EC 3.5.2.3) (Biochemistry 2001, 40, 6989, Scheme 2)



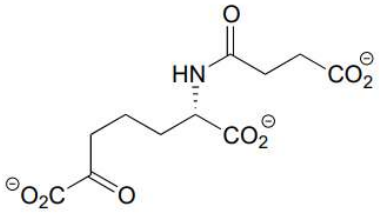
dihydroorotate

P6.16: Propose a reasonable mechanism for the reaction below (from lysine biosynthesis), and fill in the missing species indicated by question marks.



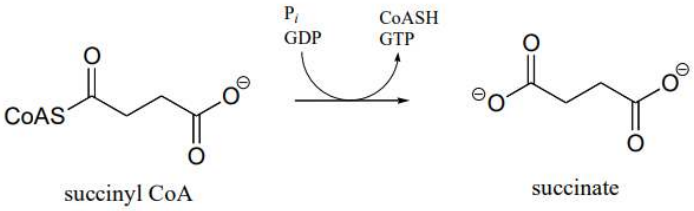
tetrahydropicolinate

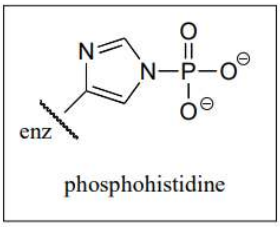
succinyl-CoA



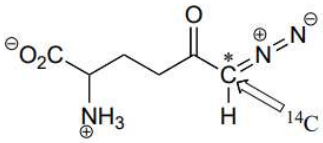
P6.17: In a step in the citric acid cycle, hydrolysis of succinyl-***CoA*** is coupled to phosphorylation of GDP. The mechanism involves t transient phosphorylation of an active site histidine. Suggest a (multi-step) mechanism for this process (EC 6.2.1.4).

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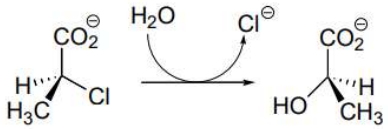


P6.18: A ^{14}C-labeled diazoketone compound (structure below) was used to irreversibly inactivate an enzyme called glutaminase Inactivation was shown to occur with ^{14}C labeling of an active site cysteine.
a)Propose a mechanism of inactivation and cysteine labeling.



b) To a lesser extent, inactivation of the enzyme and labeling of the cysteine was found to occur with release of a radioactive compou from the active site. Propose a mechanism for the mode of inactivation.

P6.19: Dehelogenase enzymes catalyze the cleavage of carbon-halogen bonds, and are of interest to scientists looking for new ways detoxify organohalogen pollutants that make their way into the environment. One such dehalogenase catalyzes the following reaction:



An active site aspartate is thought to carry out the initial nucleophilic attack that expels the chloride.

a. Draw a likely mechanism for the complete reaction shown above. Look carefully at the stereochemical progress!
b. When the active site aspartate was mutated to asparagine, the enzyme still maintained activity. Mass spectrometry analysis indicated that, at one point in the catalytic cycle of the mutant enzyme, the asparagine exists as a cyanoalanine. Draw a likely mechanism for the reaction as catalyzed by the mutant enzyme, including formation of the transient cyanoalanine residue and subsequent regeneration of the asparagine.

Contributors

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6.S: Nucleophilic Acyl Substitution Reactions (Summary)

Before moving forward you should be able to:

- Recognize and draw examples of carboxylic acid derivative functional groups:
  - carboxylic acids/carboxylates
  - acyl phosphates (both acyl monophosphate and acyl-AMP)
  - thioesters
  - esters
  - amides
  - acid chlorides
  - carboxylic acid anhydrides
- Know the meaning of the terms 'acyl', 'acetyl', 'formyl', 'lactone', and 'lactam'.
- You need not memorize the structure of coenzyme A, but you should recognize that it contains a key thiol group and often forms thioester linkages, particularly in fatty acid metabolism.
- Understand what happens in a nucleophilic acyl substitution (also called acyl transfer reaction), and be able to draw mechanistic arrows for a generalized example.
- Know the trends in relative reactivity for the carboxylic acid derivatives:
  - in a biological context (acyl phosphates and thioesters as activated acyl groups)
  - in a laboratory context (acid chlorides and carboxylic acid anhydrides as activated acyl groups)
- Recognize and understand the most important types of nucleophilic acyl substitution reactions in biology:
  - How a carboxylate group, which is unreactive to nucleophilic acyl substitution reactions, is activated in the cell by ATP-dependent phosphorylation to either acyl monophosphate or acyl-AMP.
  - Conversion of an acyl phosphate to a thioester, a (carboxylic) ester, or an amide.
  - Transthioesterification, esterification, and transesterification reactions.
  - Conversion of a thioester or ester to an amide
  - Hydrolysis of a thioester, a (carboxylic) ester, or an amide to a carboxylate.
- Understand the energetics of the above reactions:
  - Carboxylate to acyl phosphate is 'uphill' energetically, paid for by coupling to hydrolysis of one ATP
  - Other conversions above are 'downhill': it is unlikely, for example, to see a direct conversion of an amide to an ester. (Notable exception: the lactam (cyclic amide) group in penicillin is very reactive due to ring strain, and forms an ester with an active site serine residue in the target protein)
- You need not memorize all of the details of peptide bond formation on the ribosome, but you should be able to follow the description in section 7 and recognize the nucleophilic acyl substitution reactions that are occuring.
- Be able to draw complete mechanisms for the following lab reactions:
  - acid-catalyzed esterification of a carboxylic acid
  - saponification (base-catalyzed hydrolysis of an ester), application to soap-making
  - base-catalyzed transesterification, application to biodiesel production
- Understand how acid chlorides and carboxylic acid anhydrides serve as activated acyl groups in laboratory synthesis. Be able to describe how an amide to ester conversion could be carried out in the laboratory.
- Understand how polyesters and polyamides are formed. Given the structure of a polymer be able to identify monomer(s), and vice-versa.
- Be able to recognize, predict products of, and draw mechanisms for the Gabriel synthesis of primary amines, using either hydroxide ion or hydrazine to release the amine product.

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CHAPTER OVERVIEW

7: Reactions at the α-Carbon, Part I

7.1: Prelude to Reactions at the α-carbon, part I

7.2: Review of Acidity at the α-Carbon

7.3: Isomerization at the α-Carbon

7.4: Aldol Addition

7.5: α-Carbon Reactions in the Synthesis Lab - Kinetic vs. Thermodynamic Alkylation Products

7.P: Reactions at the α-Carbon, Part I (Problems)

7.S: Reactions at the α-Carbon, Part I (Summary)

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7.1: Prelude to Reactions at the α-carbon, part I



Introduction

There are lots of things that can kill you in northern Australia. On land, there is the death adder, the tiger snake and the redback spider; in the water, you'd be well advised to give wide berth to the salt water crocodile, the stonefish, the great white shark, and of course, the duck-billed platypus.

The duck-billed platypus?

Consider this: in 1991, a man fishing a river in northern Queensland, Australia happened across a platypus sitting on a log. Thinking it was injured, he picked it up. For his trouble, he spent the next six days in a nearby hospital, suffering from two puncture wounds in his right hand that resulted in "immediate, sustained, and devastating" pain, against which the usual analgesic drugs were almost completely useless. His hand "remained painful, swollen and with little movement for three weeks. Significant functional impairment . . . persisted for three months".

Meanwhile, on the other side of the planet, deep in the rain forests that straddle the border between eastern Peru and Brazil, a young man of the Matses tribe prepares himself to receive the 'hunting magic'. He holds the end of a short wooden stick in a fire for a few minutes, then removes it and presses the red-hot end into the skin of his chest, holding it there for long enough for the skin to be burned. Then he scrapes the burned skin away, and rubs into the wound a paste made from saliva mixed with secretions taken from the skin of a giant leaf frog.

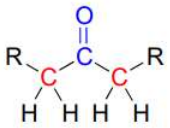
An American journalist named Peter Gorman, who reports having had the frog-skin paste administered in the same manner during a visit to a Matses village, describes what happens next:

> *Instantly my body began to heat up. In seconds I was burning from the inside . . . I began to sweat. My blood began to race. My heart pounded. I became acutely aware of every vein and artery in my body and could feel them opening to allow for the fantastic pulse of my blood. My stomach cramped and I vomited violently. I lost control of my bodily functions. . . (and) fell to the ground. Then, unexpectedly, I found myself growling and moving about on all fours. I felt as though animals were passing through me, trying to express themselves through my body.*

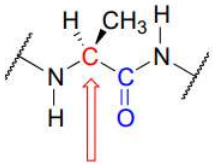
After the immediate violent effects pass, the Matses hunter is carried by his friends to a hammock to recover. After sleeping for a day, he awakens to find himself with what his people call the 'hunting magic': a state of heightened awareness, possessed of

tremendous energy and an abnormally keen sense of vision, hearing and smell. In the words of Mr. Gorman, "everything about me felt larger than life, and my body felt immensely strong... [I was] beginning to feel quite godlike".

There is a connection between the killer platypus in Australia and the 'hunting magic' in the Amazon, and it has to do with the structure and reactivity of what organic chemists refer to as the α-carbon: the carbon atom positioned adjacent to a carbonyl or imine group in an organic molecule:



α-carbons on a ketone



the α-carbon of an alanine amino acid residue in a protein

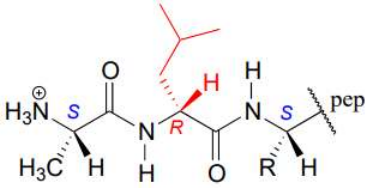
It is this chemistry that we are going to be studying for the next two chapters. But first, let's go back to that river in northern Australia and the fisherman who apparently didn't pay enough attention in his high school wildlife biology class.

The platypus, along with a few species of shrews and moles, is an example of a very rare phenomenon in nature: a venomous mammal. The male platypus possesses a pair of sharp spurs on each of his hind legs near the ankle. These spurs are hollow, and connected by a duct to a venom-producing gland in the thigh. The consensus among scientists who study the platypus is that males use their venomous barbs mainly when fighting each other over territory during mating season. Because healthy animals are often found with multiple scars from spur wounds, a platypus who gets spurred during a fight with a rival will not always die, but the experience is unpleasant enough that he will start looking for real estate a healthy distance down the river.

It is not easy to milk the venom from an angry, thrashing platypus, but there are scientists out there who have done it. It turns out that, like snake and spider venom, the venom from a platypus spur consists of a mixture of neuroactive peptides (peptides are very short proteins - less than 50 amino acids long). Recently, a team of biochemists from the Universities of Sydney, Queensland, and Adelaide reported that they were able to isolate from platypus venom two forms of a 39-amino acid peptide. Further analysis using NMR and mass spectrometry revealed that the two forms of the peptide differed in structure only at a single amino acid: the leucine at the #2 position. In one form, the leucine had the L configuration (or S if using the R/S system), just like the amino acids in virtually all other peptides and proteins found in nature. In the other form, this leucine had the unusual D, or R configuration.



peptide with L-Leu at position 2



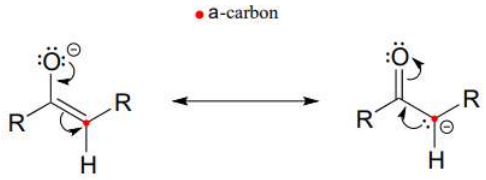
peptide with D-Leu at position 2

Peptides or proteins incorporating D-amino acids are not unheard of in nature, but this was the first time that one had been found in a mammal. Interestingly, the venom from certain marine cone snails and spiders - and, yes, the skin of the giant leaf frog in the Amazon rain forest - also contain neuroactive peptides with D-amino acids.

What is the advantage - to a platypus, cone snail, spider, or frog - of making a venomous peptide with D stereochemistry on one or more of its amino acids? It all has to do with generating diversity of shape and function. These are neuroactive peptides: each one interacts in a very specific way with a specific neural protein, thus exerting a specific neurological effect on the person or animal exposed to the venom. The different spatial arrangement of atoms about the α-carbon of D- and L-amino acids will cause a peptide with a D-leucine at position #2, for example, to fold into a different shape than its counterpart with an L-leucine at the same position. Thus, the two peptides may bind differently to one or more proteins in the nervous system, and ultimately may exert different neurological effects - such as intense pain in the case of playpus venom, or the 'hunting magic' effect in the case of the peptide from frog skin. The ability to incorporate D-amino acids greatly expands the potential structural and functional diversity of these short peptides.

The two stereoisomeric platypus venom peptides are encoded by the same gene. The peptides are initially synthesized using all L-amino acids, and then the leucine at position #2 undergoes a 'post-translational modification': in other words, a specific enzyme binds the all-L peptide after it has been synthesized on the ribosome and changes the leucine residue to the D configuration.

It is this reaction - a stereoisomerization reaction that takes place at the α-carbon of an amino acid - that brings us to the central topic of this chapter and the next: chemistry at the α-carbon. The key concept to recall from what we have learned about acidity and basicity in organic chemistry, and to keep in mind throughout this discussion, is that α-protons (in other words, protons on α-carbons) are weakly acidic. Loss of an α-proton forms an enolate - a species in which a negative formal charge is delocalized between a carbon and an oxygen. The 'enolate' term will be very important in the next two chapters, because most of the reactions we see will go through an enolate intermediate.



an enolate

In this chapter, we will first see several examples of isomerization reactions, in which an enzyme acts at the α-carbon of a substrate to catalyze the interconversion of two constitutional isomers or stereoisomers. Then, we will be introduced to a reaction type known as the 'aldol addition' and its reverse counterpart, the 'retro-aldol' cleavage reaction. Up to now, we have seen plenty of reactions where bonds were formed and broken between carbon and oxygen, nitrogen, or sulfur. Here, for the first time, we will see how enzymes can catalyze the formation or cleavage - again, at the a position - of *carbon-carbon* bonds: in other words, we will learn how an α-carbon can be either a nucleophile or a leaving group in an enzymatic reaction. This has clear importance for an understanding of metabolism in living things: the molecules of life, after all, are built upon a framework of carbon-carbon bonds, and metabolism is the process by which living cells build up and break down complex biomolecules.

It all starts with the α-carbon - and as both the Australian fisherman and the Amazonian hunter could attest, what happens at the α-carbon can have some rather dramatic consequences.

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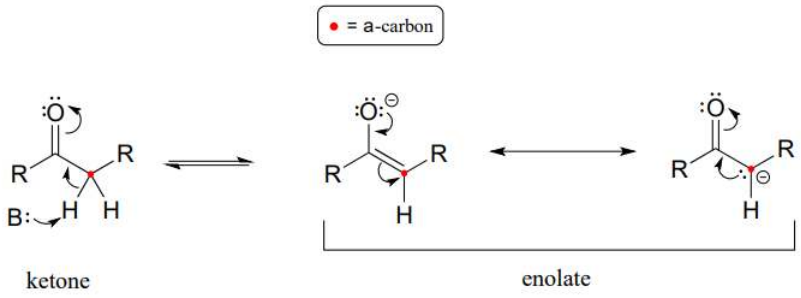
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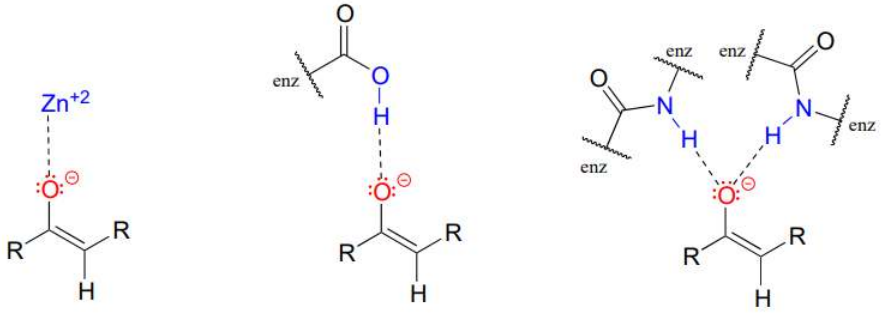
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7.2: Review of Acidity at the α-Carbon

Let's review what we learned in section 7.6 about the acidity of a proton on an α-carbon and the structure of the relevant conjugate base, the enolate ion. Remember that this acidity can be explained by the fact that the negative charge on the enolate conjugate base is delocalized by resonance to both the α-carbon and the carbonyl oxygen.



The α-carbon on the enolate is sp^2-hybridized with trigonal planar geometry, as are the carbonyl carbon and oxygen atoms (now would be a good time to go back to section 2.1, section 2.2, and section 2.3 to review, if necessary, the geometry of π-bonding in conjugated systems). pK_a of a typical a-proton in aqueous solution is approximately 18-20: acidic, but only weakly so. Recall from section 7.8, however, that the effective pK_a of a functional group on an enzyme-bound molecule can be altered dramatically by the 'microenvironment' of the active site. In order to lower the pK_a of an α-proton, an enzyme catalyzing a reaction that begins with an a-proton abstraction step must further stabilize the negative charge that develops on the oxygen atom of the (enolate) conjugate base. Different enzymes have evolved different strategies for accomplishing this task: in some cases, a metal cation (often Zn^{+2}) is bound in the active site to provide a stabilizing ion-ion interaction. In other cases, stabilization is provided by a proton-donating group positioned near the oxygen. As a third possibility, the active site architecture sometimes provides one or more stabilizing hydrogen bond donor groups.



In most of the mechanism illustrations in this chapter where an enolate intermediate is depicted, stabilizing metal ions or hydrogen bond interactions will not be explicitly drawn, for the sake of clarity. However, whenever you see an enolate intermediate in an enzyme-catalyzed reaction, you should remember that there are stabilizing interactions in play inside the active site.

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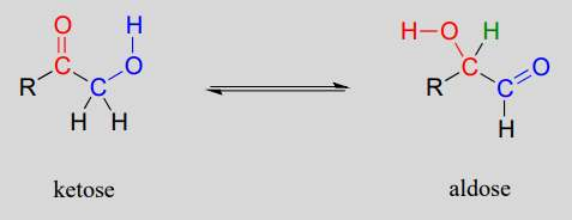
7.3: Isomerization at the α-Carbon

Enolate ions, as well as enols and enamines (section 7.6) are the key reactive intermediates in many biochemical isomerization reactions. Isomerizations can involve either the interconversion of constitutional isomers, in which bond connectivity is altered, or of stereoisomers, where the stereochemical configuration is changed. Enzymes that interconvert constitutional isomers are usually called isomerases, while those that interconvert the configuration of a chiral carbon are usually referred to as racemases or epimerases.

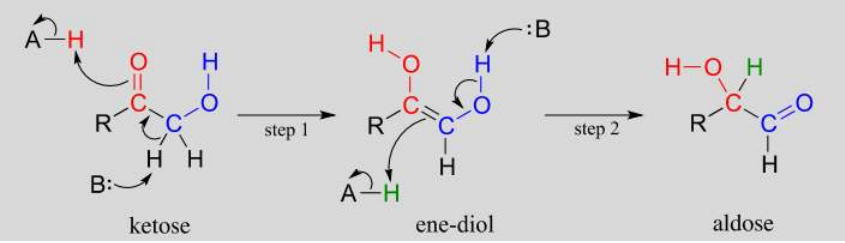
Carbonyl isomerization

One very important family of isomerase enzymes catalyzes the shifting of a carbonyl group in sugar molecules, often converting between a ketose and an aldose (recall that the terms ketose and aldolse refer to sugar molecules containing ketone and aldehyde groups, respectively).

Carbonyl isomerization:

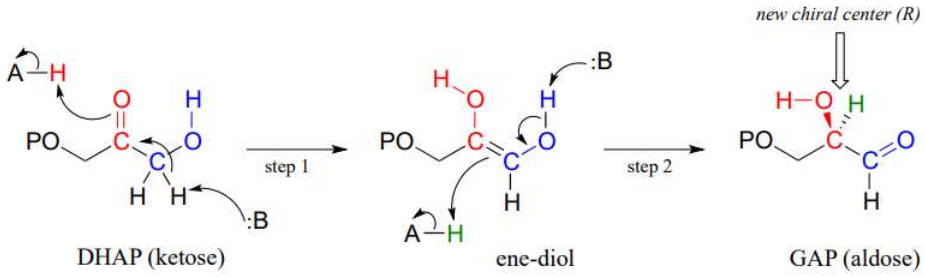


Mechanism:



The ketose species is first converted to its enol tautomer in step 1 (actually, this particular intermediate is known as an 'ene-diol' rather than an enol, because there are hydroxyl groups on both sides of the carbon-carbon double bond). Step 2 leads to the aldose, and is simply another tautomerization step. However, because there is a hydroxyl group on the adjacent (blue) carbon, a carbonyl can form there as well as at the red carbon.

An example is the glycolysis pathway reaction catalyzed by the enzyme triose phosphate isomerase (EC 5.3.1.1). Here, dihydroxyacetone phosphate (DHAP) is reversibly converted to glyceraldehyde phosphate (GAP).



Notice that DHAP is achiral while GAP is chiral, and that a new chiral center is introduced at the middle (red) carbon of GAP. As you should expect, the enzyme is stereoselective: in step 2 a proton is delivered to the red carbon, from behind the plane of the page, to yield the R enantiomer.

Also in the glycolysis pathway, glucose-6-phosphate (an aldose) and fructose-6-phosphate (a ketose) are interconverted through an ene-diol intermediate (EC 5.3.1.9) by an enzyme that is closely related to triose-phosphate isomerase.



glucose-6-phosphate fructose-6-phosphate

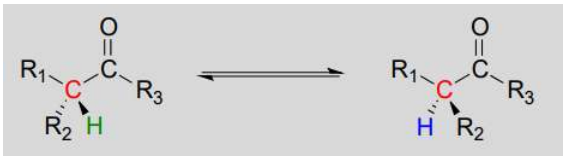
Exercise 7.3.1

Draw the ene-diol intermediate in the phosphoglucose isomerase reaction.

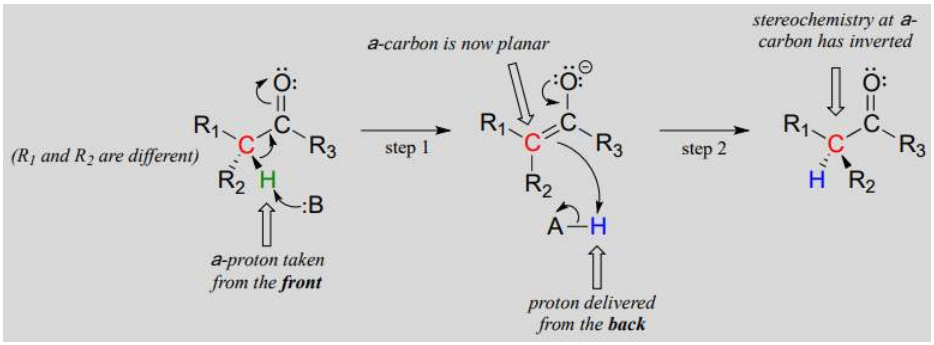
Stereoisomerization at the α-carbon

Enolates are a common intermediate in reactions where the stereochemical configuration of a chiral α-carbon is interconverted. These are commonly referred to as racemization or epimerization reactions, depending on whether the interconverted isomers are enantiomers or epimers (recall that the term 'epimer' refers to a pair of diastereomers that differ by a single chiral center).

Racemization/ epimerization:

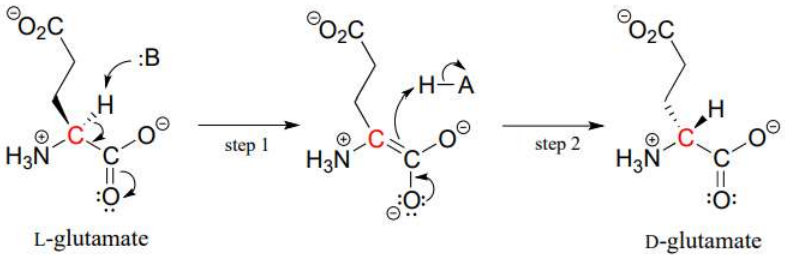


Mechanism:

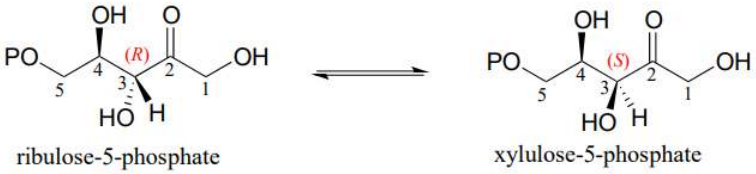


These reactions proceed though a deprotonation-reprotonation mechanism, illustrated above. In step 1, the chiral α-carbon is deprotonated, leading to a planar, achiral enolate. In step 2, a proton is delivered back to the α-carbon, but from the opposite side from which the proton was taken in step 1, resulting in the opposite stereochemistry at this carbon. Two acid-base groups, positioned at opposing sides of the enzyme's active site, work in tandem to accomplish this feat.

The proteins and peptides in all known living things are constructed almost exclusively of L-amino acids, but in rare cases scientists have identified peptides which incorporate D-amino acids, which have the opposite stereochemistry at the α-carbon. Amino acid racemase enzymes catalyze the interconversion of L and D amino acids. As you may recall from the introductory section to this chapter, the venom of the male platypus contains a neurotoxic peptide in which an L-leucine amino acid has been converted by a racemase enzyme to D-leucine. In another example, the cell walls of bacteria are constructed in part of peptides containing D-glutamate, converted from L-glutamate by the enzyme glutamate racemase. (EC 5.1.1.3) (Biochemistry 2001, 40, 6199).



A reaction (EC 5.1.3.1) in sugar metabolism involves the interconversion of the epimers ribulose-5-phosphate and xylulose-5-phosphate. The enzyme that catalyzes this reaction is called an 'epimerase'. (J. Mol. Biol. 2003, 326, 127).

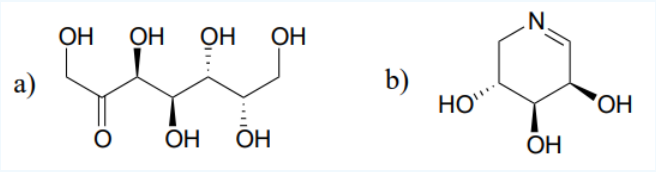


Exercise 7.3.2

Draw a reasonable mechanism for the ribulose-5-phosphate epimerization reaction above. Your mechanism should show an enolate intermediate and specify stereochemistry throughout.

Exercise 7.3.3

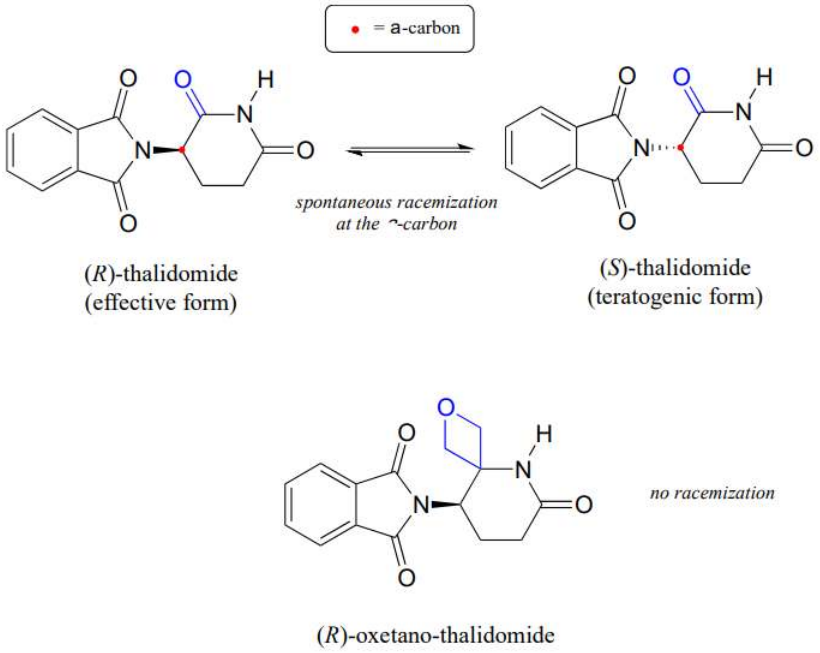
Predict the products of epimerization reactions starting with each of the substrates shown.



Hint

Carbons next to imine groups can also be considered α-carbons!

Recall from chapter 3 that a major issue with the drug thalidomide is the fact that the R enantiomer, which is an effective sedative, rapidly isomerizes in the body to the terotogenic (mutation-causing) S enantiomer. Note that the chiral center in thalidomide is an α -carbon!

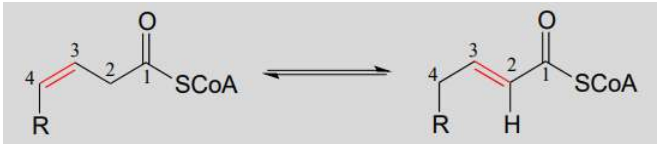


Recently chemists reported the synthesis of a thalidomide derivative in which the carbonyl group is replaced by an 'oxetane' ring, with the aim of making an isotopically stable form of the drug (because the carbonyl group has been removed, racemization is no longer possible - there is no α-carbon!) (Org. Lett. 2013, 15, 4312.)

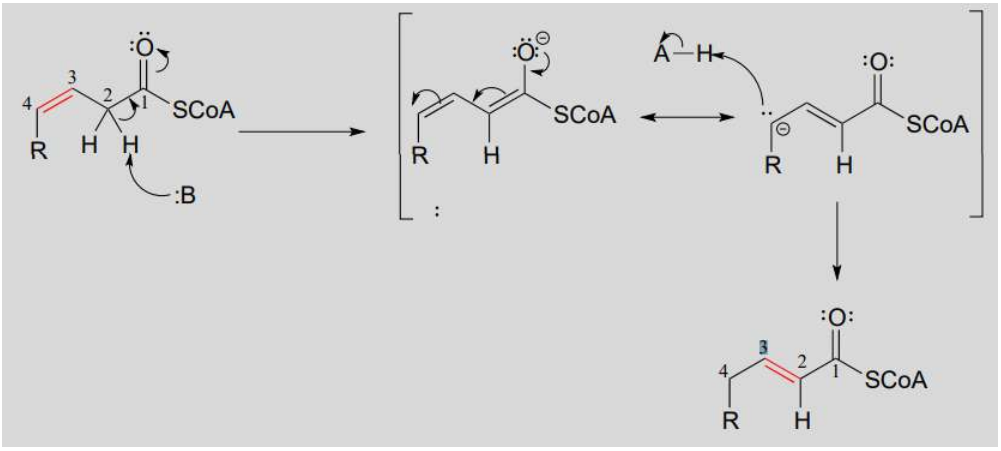
Alkene regioisomerization

The position of an alkene group can also be changed through a reaction in which the first step is abstraction of an α-proton and formation of an enolate intermediate. degradation pathway for unsaturated fatty acids (fatty acids whose hydrocarbon chains contain one or more double bonds) involves the 'shuffling' of the position of a carbon-carbon double bond, from a cis bond between carbon #3 and carbon #4 to a trans bond between carbon #2 and carbon #3. This is accomplished by the enzyme enoyl ***CoA*** isomerase (EC 5.3.3.8). (J. Biol Chem 2001, 276, 13622).

Alkene isomerization:



Mechanism:

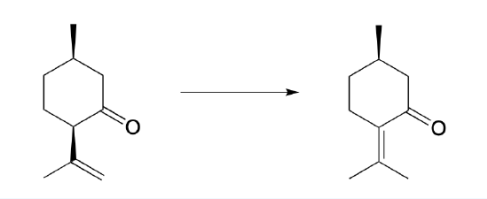


Exercise 7.3.4

Consider the structures of the substrate and product of the isomerization reaction above. What two factors contribute to the thermodynamic 'driving force' for the transformation?

Exercise 7.3.5

The reaction below is part of the biosynthetic pathway for menthol. Suggest a mechanism that includes an enolate intermediate.



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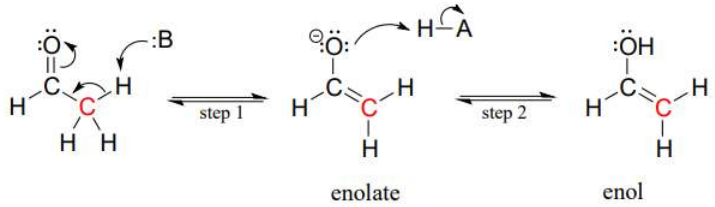
7.4: Aldol Addition

We arrive now at one of the most important mechanisms in metabolism: the aldol addition.

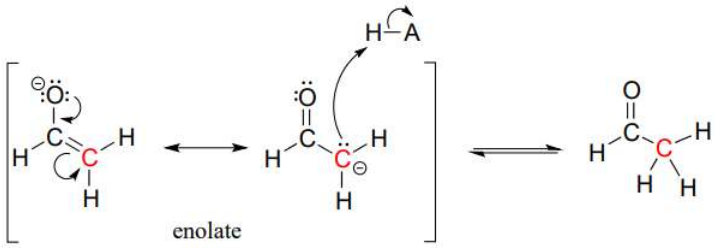
Along with Claisen condensation reactions, which we will study in the next chapter, aldol additions are responsible for most of the carbon-carbon bond forming events that occur in a living cell. Because biomolecules are built upon a framework of carbon-carbon bonds, it is difficult to overstate the importance of aldol addition and Claisen condensation reactions in the chemistry of living things!

Overview of the aldol addition reaction

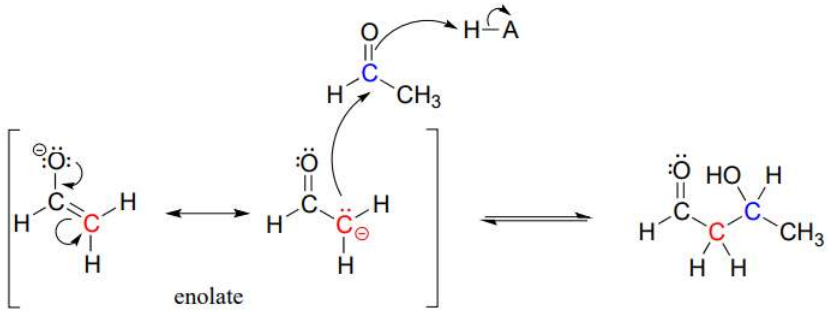
Consider the potential pathways available to a reactive enolate intermediate once the a-proton has been abstracted. We'll use acetaldehyde as a simple example. The oxygen, which bears most of the negative charge, could act as a base, (step 2 below) and the result would be an enol.



Alternatively, the enolate carbon, which bears a degree of negative charge, could act as a base, which is simply the reverse of the initial deprotonation step that formed the enolate in the first place. This of course just takes us right back to the starting aldehyde.

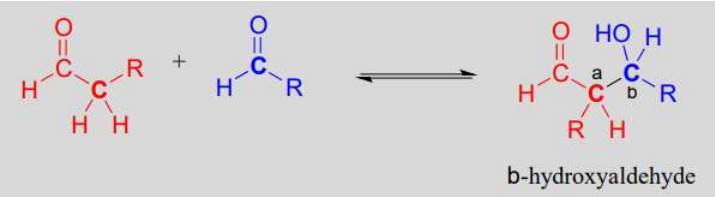


In both of these cases, the electron-poor species attacked by the enolate is an acidic proton. What if the electron-poor species - the electrophile - is not a proton but a carbonyl carbon? In other words, what if the enolate acts not as a base but rather as a nucleophile in a carbonyl addition reaction? For example, the enolate of acetaldehyde could attack the carbonyl group of a second acetaldehyde molecule. The result is the formation of a new carbon-carbon bond:

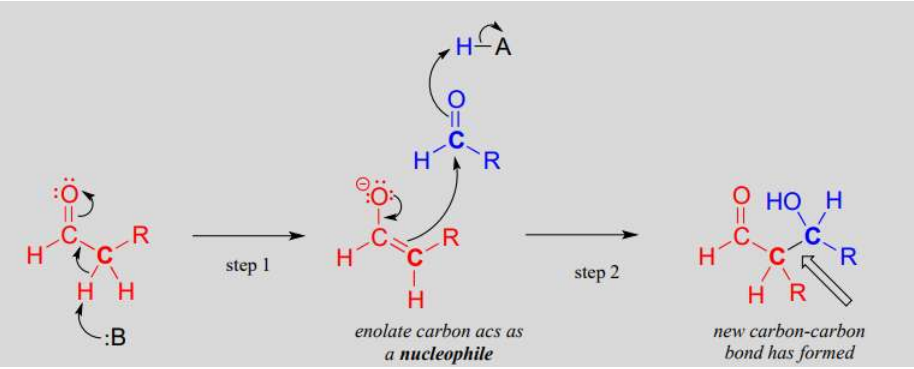


This type of reaction is called an aldol addition. It can be very helpful to think of an aldol addition reaction as simply a nucleophilic carbonyl addition (Chapter 10) reaction with an enolate α-carbon (rather than an alcohol oxygen or amine nitrogen) as the nucleophile.

An aldol addition reaction:



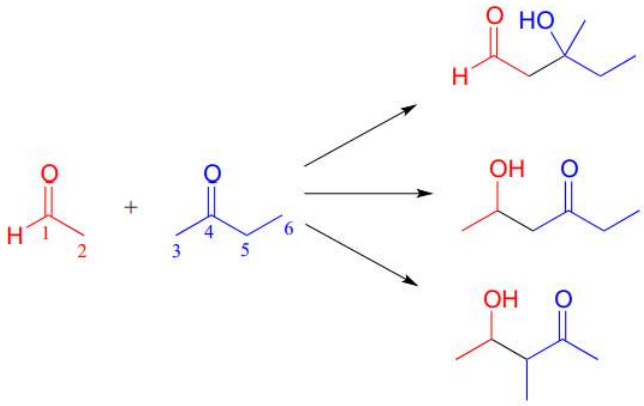
Mechanism:



Historically, the first examples of this mechanism type to be studied involved reactions very similar to what is shown above: an aldehyde reacting with itself. Because the resulting product contained both an aldehyde and an alcohol functional group, the reaction was referred to as an 'aldol' addition, a terminology that has become standard for reactions of this type, whether or not an aldehyde is involved. More generally, an aldol addition is characterized as a nucleophilic addition to an aldehyde, ketone, or imine electrophile where the nucleophile is the α-carbon in an aldehyde, ketone, imine, ester, or thioester. The enzymes that catalyze aldol reactions are called, not surprisingly, **aldolases**.

Note that the aldol reaction results in a product in which a hydroxide group is two carbons away from the carbonyl, in the β position. You can think of the β-hydroxy group as a kind of 'signature' for an aldol addition product.

Depending on the starting reactants, *nonenzymatic* aldol reactions can take more than one route to form different products. For example, a reaction between acetaldehyde and 2-butanone could potentially result in in three different aldol addition products, depending on which of the three α-carbons (carbons 2, 3, and 5 below) becomes the attacking nucleophile.



Exercise 7.4.1

a. Fill in the appropriate carbon numbers for each of the three possible aldol addition products shown above.

b. Draw arrows for the carbon-carbon bond forming step that leads to each of the three products.

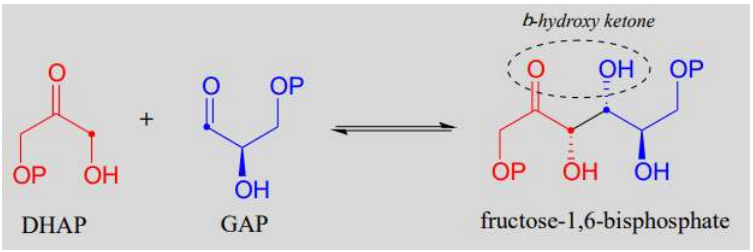
Hint

For each reaction, first identify the nucleophilic and electrophilic carbon atoms on the starting compounds!

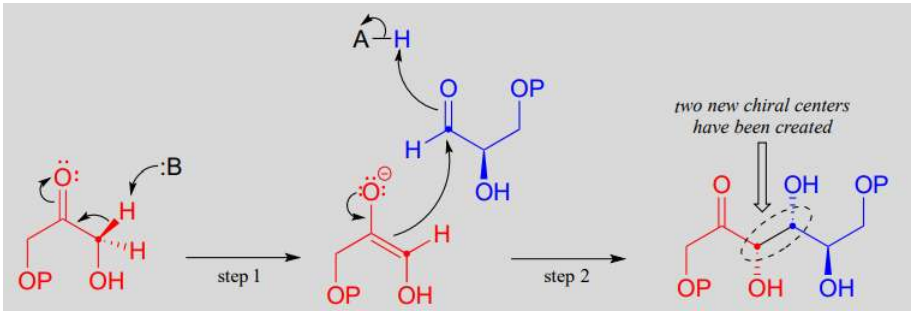
Biochemical aldol addition reactions

Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13) is an enzyme that participates in both the glycolytic (sugar catabolism) and gluconeogenesis (sugar synthesis) biochemical pathways. The reaction catalyzed by fructose 1,6-bisphosphate aldolase links two 3-carbon sugars, glyceraldehyde-3-phosphate (GAP, the electrophile in the reaction) and dihydroxyacetone phosphate (DHAP, the nucleophile), forming a 6-carbon product. In the figures below, the nucleophilic and electrophilic carbons are identified with dots.

The fructose 1,6-bisphosphate aldolase reaction



Mechanism:



In step 1 of the reaction, an α-carbon on DHAP is deprotonated, leading to an enolate intermediate. this and many other aldolase reactions, a zinc cation (Zn^{+2}) is positioned in the enzyme's active site so as to interact closely with - and stabilize - the negatively charged oxygen of the enolate intermediate. This is one important way in which the enzyme lowers the energy barrier to the reaction.

Next, (step 2), the deprotonated α-carbon attacks the carbonyl carbon of GAP in a nucleophilic addition reaction, leading to the fructose 1,6-bisphosphate product.

Notice that two new chiral centers are created in this reaction. This reaction, being enzyme-catalyzed, is highly stereoselective due to the precise position of the two substrates in the active site: only one of the four possible stereoisomeric products is observed. The enzyme also exhibits tight control of regiochemistry: GAP and DHAP could potentially form two other aldol products which are constitutional isomers of fructose 1,6-bisposphate.

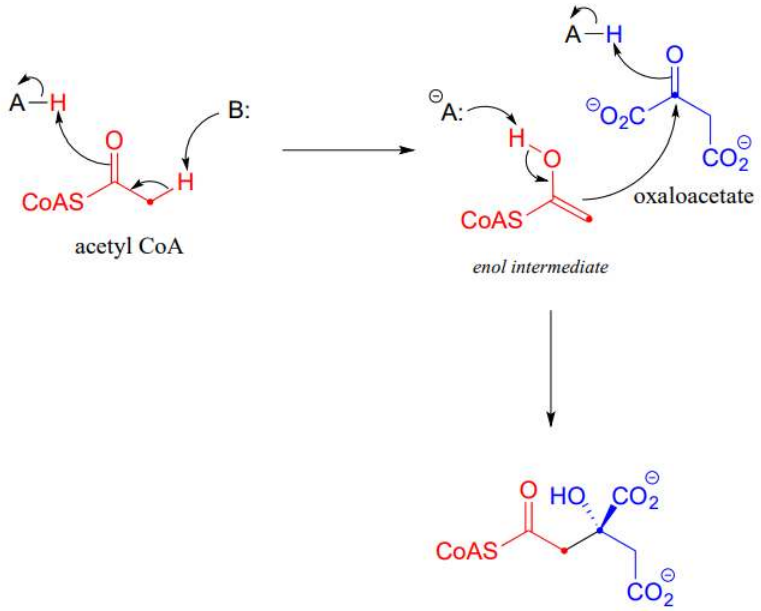
Exercise 7.4.2

a. Fill in the blanks with the correct term: (pro-R, pro-S, re, si). You may want to review the terminology in section 3.11.

In the fructose 1,6-bisphosphate aldolase reaction, the _____ proton on the α-carbon of DHAP is abstracted, then the _____ face of the resulting enolate α-carbon attacks the _____ face of the aldehyde carbon of GAP.

b. Draw structures of the two other constitutional isomers that could hypothetically form in aldol addition reactions between GAP and DHAP. How many stereoisomers exist for these two alternative products?

Along with aldehydes and ketones, esters and thioesters can also act as the nucleophilic partners in aldol reactions. In the first step of the citric acid (Krebs) cycle, acetyl ***CoA*** (a thioester nucleophile) adds to oxaloacetate (a ketone electrophile) (EC 2.3.3.8).

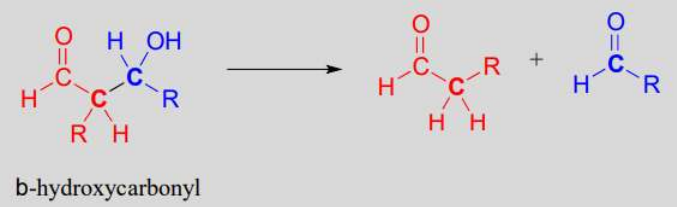


Notice that the nucleophilic intermediate is an enol, rather than a zinc-stabilized enolate as was the case with the fructose 1,6-bisphosphate aldolase reaction. An enol intermediate is often observed when the nucleophilic substrate is a thioester rather than a ketone or aldehyde.

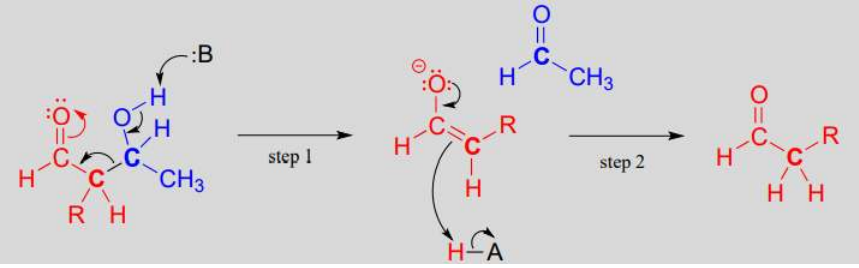
Going backwards: the retro-aldol cleavage reaction

Although aldol reactions play a very important role in the formation of new carbon-carbon bonds in metabolic pathways, it is important to emphasize that they can also be reversible: in most cases, the energy level of starting compounds and products are very close. This means that, depending on metabolic conditions, aldolases can also catalyze retro-aldol reactions: the reverse of aldol reactions, in which carbon-carbon bonds are broken.

A retro-aldol cleavage reaction:



Mechanism:

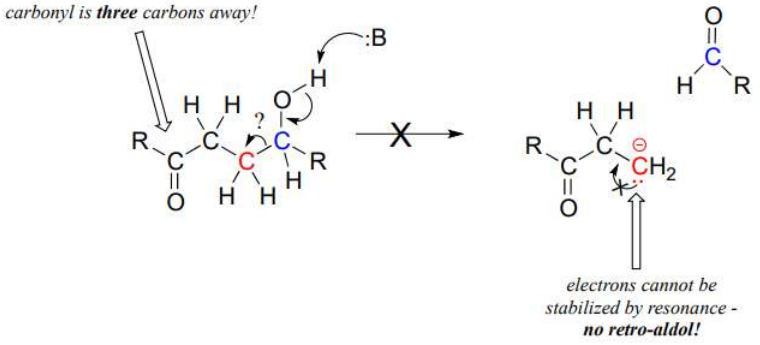


In the retro-aldol cleavage reaction the β-hydroxy group is deprotonated (step 1 above), to form a carbonyl, at the same time pushing off the enolate carbon, which is now a leaving group rather than a nucleophile.

Is an enolate a good enough leaving group for this step to be chemically reasonable? Sure it is: the same stabilizing factors that explain why it can form as an intermediate in the forward direction (resonance delocalization of the negative charge to the oxygen, interaction with a zinc cation) also explain why it is a relatively weak base, and therefore a relatively good leaving group (remember, weak base = good leaving group!). All we need to do to finish the reaction off is reprotonate the enolate (step 2) to yield the starting aldehyde, and we are back where we started.

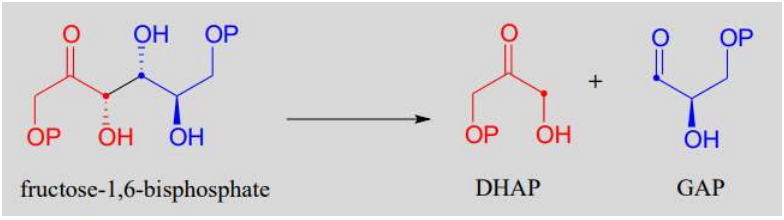
The key thing to keep in mind when looking at a retro-aldol mechanism is that, when the carbon-carbon bond breaks, the electrons must have 'some place to go' where they will be stabilized by resonance. Most often, the substrate for a retro-aldol reaction is a β-hydroxy aldehyde, ketone, ester, or thioester.

If the leaving electrons cannot be stabilized, a retro-aldol cleavage step is highly unlikely.

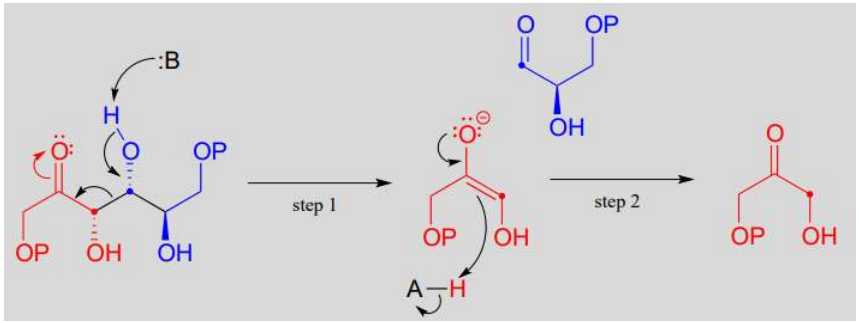


The fructose 1,6-bisphosphate aldolase reaction we saw in the previous section is an excellent example of an enzyme whose metabolic role is to catalyze both the forward and reverse (retro) directions of an aldol reaction. The same enzyme participates both as an aldolase in the sugar-building gluconeogenesis pathway, and as a retro-aldolase in the sugar breaking glycolysis pathway. We have already seen it in action as an aldolase in the gluconeogenesis pathway. Here it is in the glycolytic direction, catalyzing the retro-aldol cleavage of fructose bisphosphate into DHAP and GAP:

The fructose 1,6-bisphosphate aldolase reaction (retro-aldol direction)

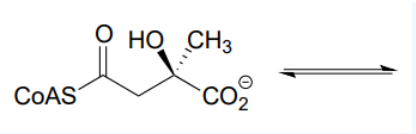


Mechanism:



Exercise 7.4.3

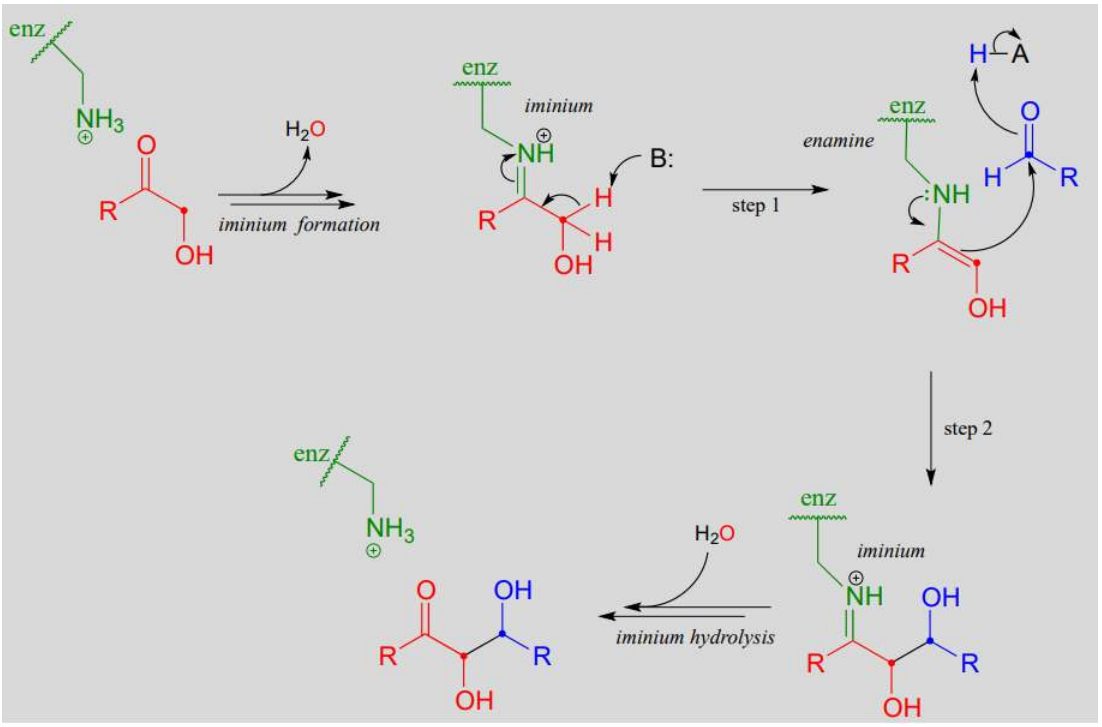
Predict the products of a retro-aldol reaction with the given substrate.



Aldol addition reactions with enzyme-linked enamine intermediates

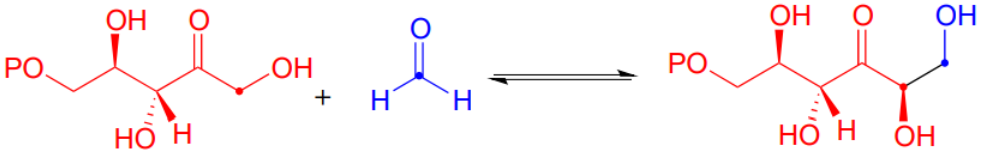
Earlier we looked at the mechanism for the fructose 1,6-bisphosphate aldolase reaction in bacteria. Interestingly, it appears that the enzyme catalyzing the exact same reaction in plants and animals evolved differently: instead of going through a zinc-stabilized enolate intermediate, in plants and animals the key intermediate is an enamine. The nucleophilic substrate is first linked to the enzyme through the formation of an iminium with a lysine residue in the enzyme's active site (refer to section 10.5 for the mechanism of iminium formation). This effectively forms an 'electron sink', in which the positively-charged iminium nitrogen plays the same role as the Zn^{+2} ion in the bacterial enzyme.

Mechanism for an aldol addition reaction with an enzyme-linked enamine intermediate



The α-proton, made more acidic by the electron-withdrawing effect of the imminium nitrogen, is then abstracted by an active site base to form an enamine (step 1). In step 2 , the α-carbon attacks the carbonyl carbon of an aldehyde, and the new carbon-carbon bond is formed. In order to release the product from the enzyme active site and free the enzyme to catalyze another reaction, the iminium is hydrolyzed back to a ketone group (see section 10.5 to review the imine/imminium hydrolysis mechanism).

There are many more examples of aldol/retroaldol reactions in which the key intermediate is a lysine-linked imine. Many bacteria are able to incorporate formaldehyde, a toxic compound, into carbohydrate metabolism by linking it to ribulose monophosphate. The reaction (EC 4.1.2.43) proceeds through imine and enamine intermediates.

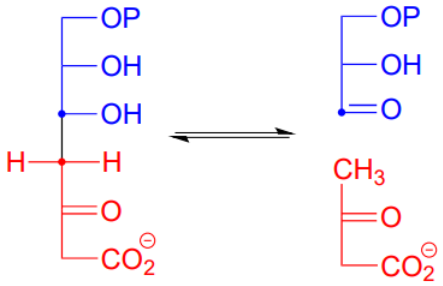


ribulose-5-phosphate formaldehyde hexulose-6-phosphate

Exercise 7.4.4

Draw the carbon-carbon bond-forming step for the hexulose-6-phosphate aldolase reaction shown above.

Here is an example of an enamine intermediate retro-aldol reaction from bacterial carbohydrate metabolism (EC 4.1.2.14). Notice that the structures are drawn here in the Fischer projection notation - it is important to practice working with this drawing convention, as biologists and biochemists use it extensively to illustrate carbohydrate chemistry. Proc. Natl. Acad. Sci. 2001, 98, 3679



Exercise 7.4.5

Draw the carbon-carbon bond breaking step in the reaction above. Use the Fischer projection notation.

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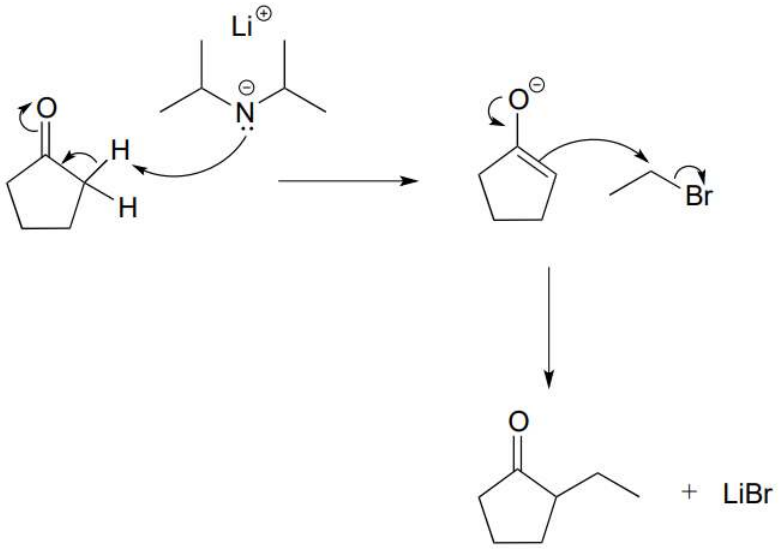
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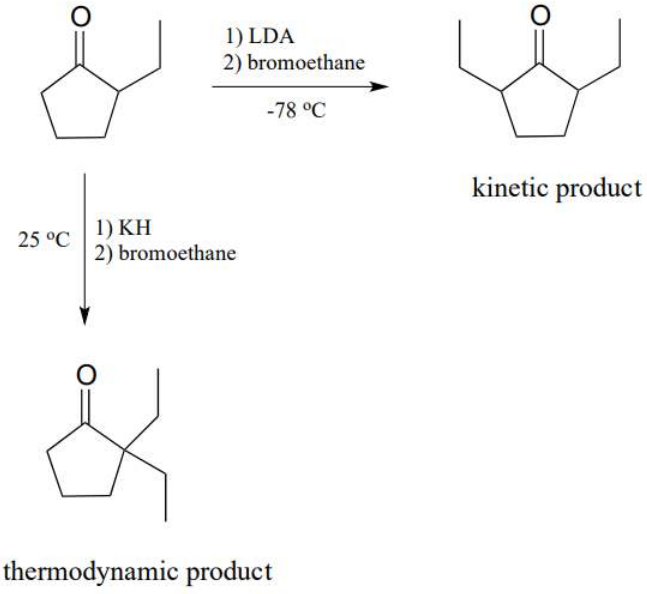
7.5: α-Carbon Reactions in the Synthesis Lab - Kinetic vs. Thermodynamic Alkylation Products

While aldol addition reactions are widespread in biochemical pathways as a way of forming carbon-carbon bonds, synthetic organic chemists working the lab also make use of aldol-like reactions for the same purpose. Consider this reaction:



Here, cyclopentanone is deprotonated at an α-carbon by lithium diisopropylamide (LDA), a very strong base commonly used in the synthesis lab. Addition to the reaction mixture of an electrophile in the form of a primary alkyl bromide results in formation of a new carbon-carbon bond. Notice that this is a kind of 'S_N2 variation' on the aldol addition reactions we saw above, because the enolate nucleophile is attacking in S_N2 fashion rather than in a carbonyl addition fashion.

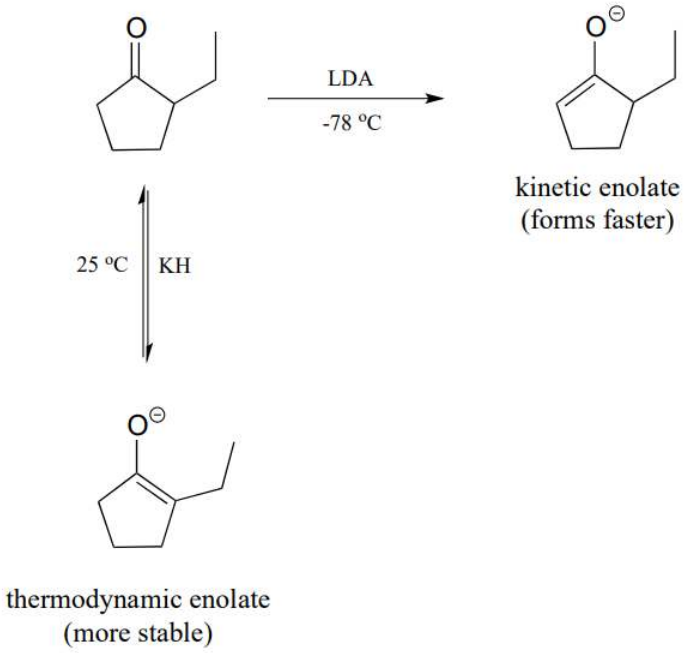
What would happen, though, if we started with 2-ethylcyclopentantone? Because the starting ketone is no longer symmetrical, we could hypothetically obtain two different products:



It turns out that we can control which product we get by selecting the base used in the reaction, and the reaction temperature. If we use LDA and immerse the reaction flask in a dry ice-acetone bath ($-78°C$), we get mainly 2,5-diethyl cyclopentanone. If we use

potassium hydride (***KH***) and run the reaction at room temperature, we get mainly 2,2-diethylcyclopentanone.

LDA is a very *hindered* base: the basic nitrogen atom is surrounded by two bulky isopropyl groups, and thus it is more difficult for it to come into contact with an ***α***-proton. The ***α***-protons on the less substituted side of 2-ethylcyclopentanone are less hindered and more accessible to the base. In addition, the cold reaction temperature means that the deprotonation step is irreversible: the system does not have enough energy to overcome the energy barrier for the reverse (reprotonation) reaction. The less substituted enolate forms faster, and once it forms it goes on to attack the bromoethane rather then reversing back to the ketone form. Because it is the rate of enolate formation that determines the major product under these conditions, we say that this reaction is under kinetic control, and the less substituted enolate intermediate is called the kinetic enolate.



If, on the other hand, we use ***KH*** as a base, hindrance is no longer an issue because the base is a hydride ion. We run this reaction at room temperature, so the system has enough energy to overcome the energy barrier for re-protonation, and enolate formation is reversible. The enolate in most abundance at equilibrium is therefore not the one that forms fastest, but the one that is more stable. The more substituted enolate is more stable (recall that alkenes are more stable when they are more substituted - the same idea applies here). The more substituted enolate leads to the 2,2-diethyl cyclopentanone product. Because it is the stability of the enolate intermediate that determines the major product under these conditions, we say that this reaction is under thermodynamic control, and the more substituted enolate intermediate is the thermodynamic enolate.

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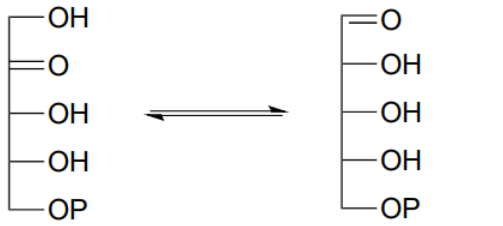
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7.P: Reactions at the α-Carbon, Part I (Problems)

P12.1: The enzyme ribulose-5-phosphate isomerase (EC 5.3.1.6), which is active in both the Calvin cycle and the pentose phosphate pathway, catalyzes an aldehyde-to-ketone isomerization between two five-carbon sugars.

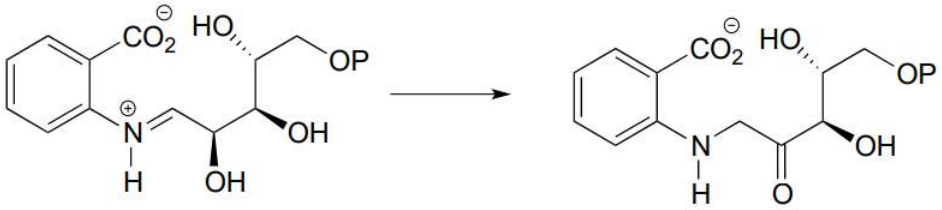
a. Draw a mechanism for this step.



ribulose 5-phosphate ribose 5-phosphate

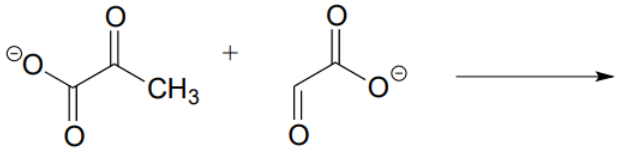
b. What ^1H-NMR signal would most clearly differentiate the aldose from the ketose in this reaction?

P12.2: Provide a likely mechanism for the reaction below, from tryptophan biosynthesis (EC 5.3.1.24) Hint: this is mechanistically very similar to a carbonyl isomerization reaction.



P12.3:

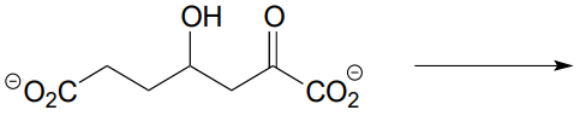
a. Draw the product of an aldol addition reaction between pyruvate and glyoxylate (EC 4.1.3.16):



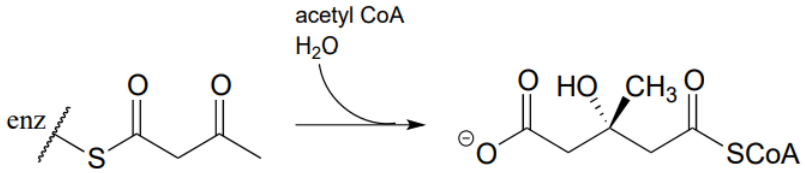
pyruvate glyoxylate

b. Draw the product of an aldol addition reaction between two molecules of pyruvate (EC 4.1.3.17).

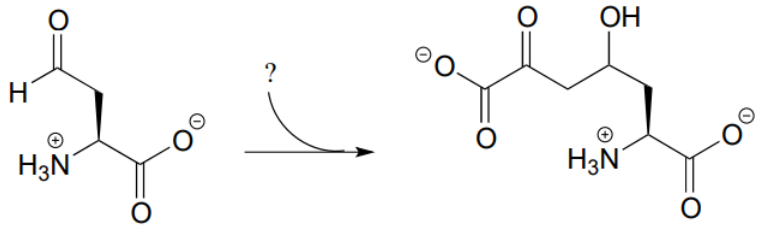
c. The molecule below undergoes a retroaldol cleavage reaction in E. coli (J. Biol. Chem. 2012, 287, 36208). Draw the structure of the products.



d. Propose a mechanism for this early reaction in the biosynthesis of isoprenoids (EC 2.3.3.10). Hint: this is an aldol reaction, followed by thioester hydrolysis.

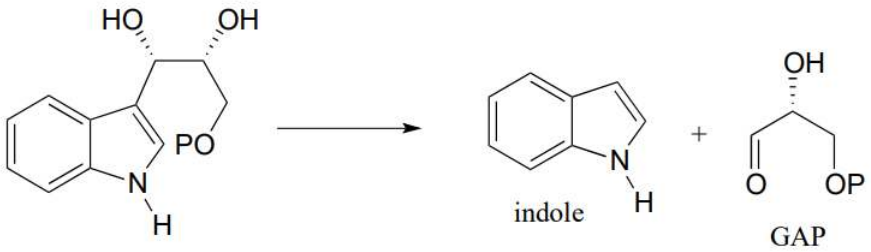


e. The carbon-carbon bond cleaving reaction below was reported to take place in many species of bacteria. Predict the structure of product X, and draw a mechanism for the reaction. Assume that an imine linkage to an enzymatic lysine residue does not play a part in the mechanism. (J. Bacteriol. 2009, 191, 4158).

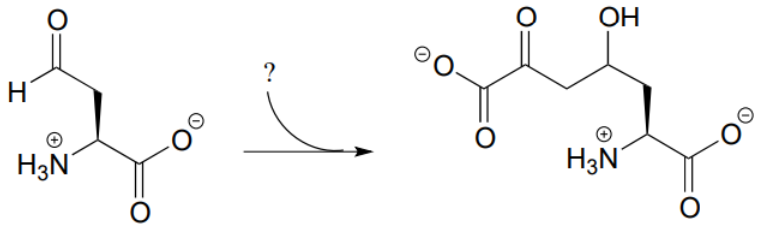


aspartate semialdehyde

P12.4: Below is a step in the biosynthesis of tryptophan. Draw a likely mechanism. Hint: you will need to show an enamine to imine tautomerization step first, then the carbon-carbon bond breaking step will become possible.



P12.5: The following step in the biosynthesis of lysine makes a connection between aspartate semialdehyde and a common metabolic intermediate. Identify the intermediate, and propose a mechanism for the reaction.



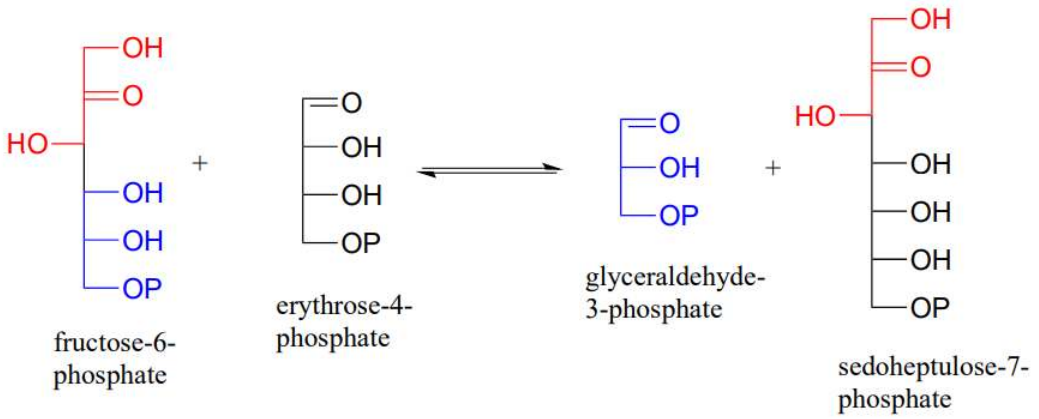
aspartate semialdehyde

P12.6: Sugar-interconverting transaldol reactions play an important role in sugar metabolism. In a transaldolase reaction, a ketose (e.g. fructose-6-phosphate) first breaks apart in a retro-aldol step to release an aldose (e.g. glyceraldehyde-3-phosphate) from the active site. Then, in a forward aldol step, a second aldose (e.g. erythrose-4-phosphate) enters the active site and connects to what remains from the original ketose (the red part in the figure below) to form a new ketose (e.g. sedoheptulose-7-phosphate). Transaldolase enzymes generally have a lysine in the active site that is covalently bound to the substrate throughout the reaction cycle.

Draw curved-arrow diagrams showing

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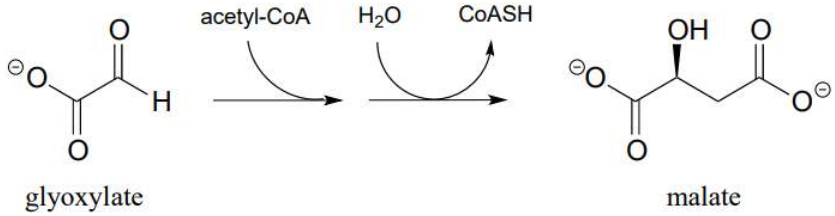
a. the carbon-carbon bond breaking step of the reaction cycle
b. he carbon-carbon bond forming step.



P12.7: Scientists are investigating the enzymatic reaction below, which is part of the biosynthesis of the outer membrane of gram-negative bacteria, as a potential target for new antibiotic drugs. Draw a likely mechanism for the reaction. (J. Biol. Chem. 2008, 283, 2835).

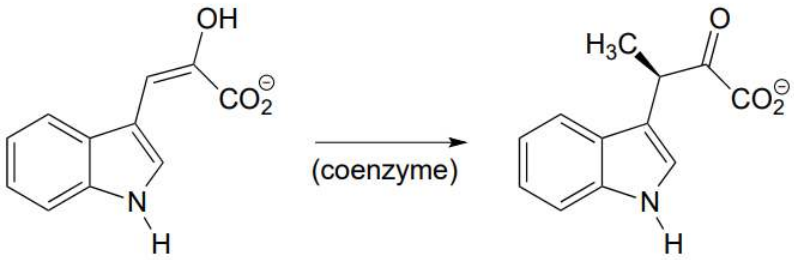


P12.8: The reaction below, catalyzed by the enzyme malate synthase, is part of the glyoxylate cycle of plants and some bacteria. It is the glyoxylate cycle that allows these organisms to convert acetyl CoA, derived from the metabolism of oils, into glucose.

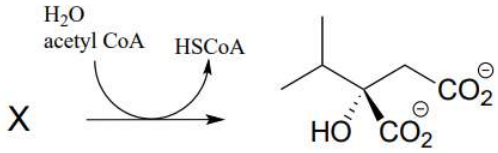


a. Propose a mechanism.
b. Predict the signals you would expect to see in a ^1H-NMR spectrum of malate.

P12.9: The reaction below, from the biosynthetic pathway for the amino acid tryptophan, is dependent upon a coenzyme that we learned about in an earlier chapter. Based on the reaction, identify this coenzyme and propose a mechanism.

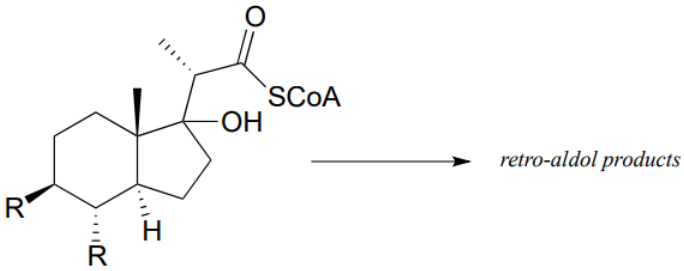


P12.10: In the biosynthesis of leucine, acetyl CoA condenses with another metabolic intermediate ‘X” to form 1-isopropylmalate (EC 2.3.3.13). Give the structure for substrate X, and provide a mechanism for the reaction.

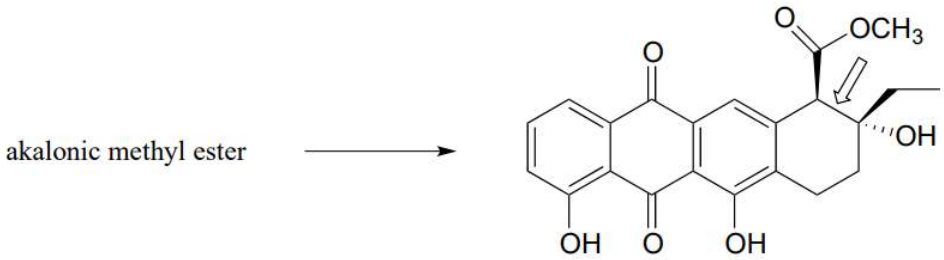


P12.11:

a. Mycobacterium tuberculosis, the microbe that causes tuberculosis, derives energy from the metabolism of cholesterol from infected patients. The compound below is predicted to be an intermediate in that metabolic pathway, and to undergo a retro-aldol cleavage reaction. Predict the retro-aldol products and show the mechanism involved. (Crit. Rev. Biochem. Mol. Biol. 2014, 49, 269, fig 5).

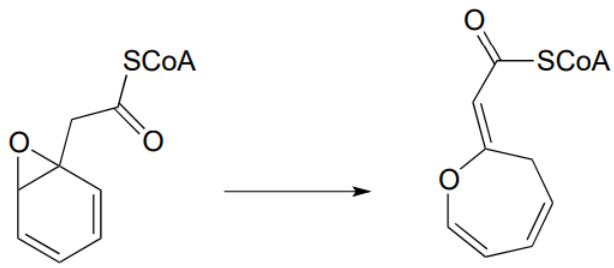


b. Polyketides are a structurally diverse class of biomolecules produced by almost all living things. Many drugs are derived from polyketide precursors. The cancer drug doxorubicin (trade name Adriamycin) is derived from a bacterial polyketide called rhodomycinone. Aklaviketone, an intermediate in the biosynthesis of rhodomycinone, is derived in a single enzymatic step from akalonic methyl ester, in a reaction in which the carbon-carbon bond indicated by an arrow is formed. Predict the structure of akalonic methyl ester.



aklaviketone

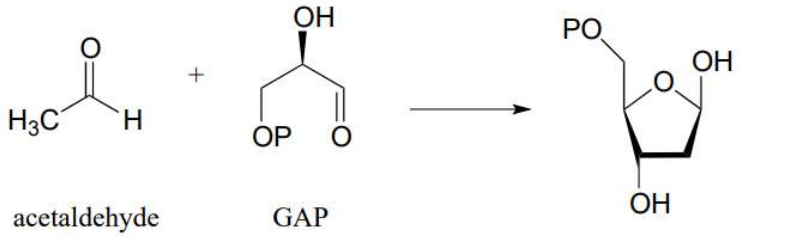
P12.12: The unusual isomerization reaction shown below has been reported recently to occur in some bacteria. Propose a mechanism that begins with formation of an enolate intermediate. (J. Biol. Chem. 2012, 287, 37986).



P12.13: The reactions in parts a) and b) below both proceed through lysine-linked enamine intermediates. Show the carbon-carbon bond forming step for each reaction. Hint: you will want to consider the straight-chain (ie. aldose/ketose) form of the sugars in both

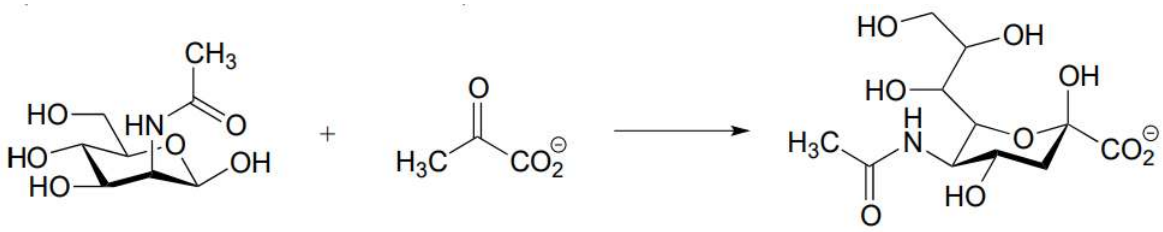
cases.

a. (J. Bacteriol. 2004, 186, 4185)

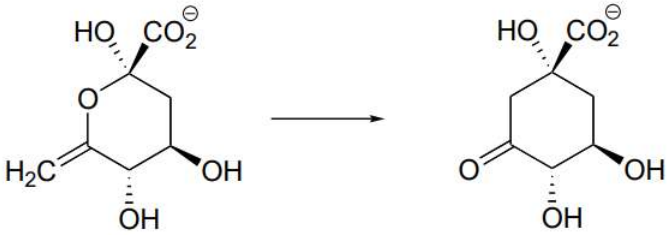


acetaldehyde GAP deoxyribose 5-phosphate

b. (J. Biol. Chem. 2011, 286, 14057)



P12.14: Suggest a mechanism for the following transformation from aromatic amino acid biosynthesis (EC 4.2.3.4). Hint – only two mechanistic steps are required.



Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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7.S: Reactions at the α-Carbon, Part I (Summary)

Before moving on to the next chapter, you should:

- Understand what is meant by 'a and b positions' relative to a carbonyl group.
- Understand how an enzyme can increase the acidity of an a-proton through the active site microenvironment
- Understand the 3D bonding arrangement of an enolate ion
- Be able to recognize and draw reasonable mechanisms for the following reaction types:
  - tautomerizations: keto-enol, imine-enamine
  - racemization/epimerization
  - carbonyl isomerization (changing position of a carbonyl group)
  - alkene isomerization (changing position of an alkene relative to carbonyl)
  - aldol addition, retro-aldol cleavage (both enolate intermediate and enamine intermediate mechanisms)
- Be able to draw a mechanism for a laboratory alkylation reaction at the a-carbon of a ketone or aldehyde. Understand the difference between kinetic and thermodynamic control of this reaction type, and be able to predict the regiochemical outcome of the reaction based on reaction conditions.

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CHAPTER OVERVIEW

8: Reactions at the α-Carbon, Part II

8.1: Prelude to Reactions at the α-Carbon, Part II
8.2: Decarboxylation
8.3: An Overview of Fatty Acid Metabolism
8.4: Claisen Condensation
8.5: Conjugate Addition and Elimination
8.6: Carboxylation
8.P: Reactions at the α-Carbon, Part II (Problems)
8.S: Reactions at the α-Carbon, Part II (Summary)

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8.1: Prelude to Reactions at the α-Carbon, Part II



Introduction

We begin this chapter with the story of two men, and two chemical reactions.

The two men couldn't be more different. One was an acclaimed scientist who lived and continued to work productively into his eighties. The other was struck down as a young boy by what was assumed at the time to be a fatal disease. With the heroic support of his parents and caregivers, though, he lived to his thirtieth birthday and provided the inspiration for development of a medical treatment that could potentially save thousands of lives.

The two chemical reactions in this story are closely related, and both involve the metabolism of fats in the human body. One serves to build up fatty acid chains by repeatedly linking together two-carbon units, while the other does the reverse, progressively breaking off two-carbon pieces from a long chain fatty acid molecule. The life and work of the two men are inextricably linked to the two reactions, and while we will be learning all about the reactions in the main part of this chapter, we'll begin with the stories of the two men.

On a Saturday in January of 2007, Dr. Hugo Moser passed away in the Johns Hopkins Hospital in Baltimore, succumbing to pancreatic cancer. He was 82 years old. A neurologist who had taught and researched for much of his career at Johns Hopkins, he was well known for his workaholic nature: he had signed off on his last grant application while on the way to the hospital for major surgery just a few months previously. Two days after his death, his wife and colleague Ann Moser was back in their lab, because, she said, “He gave us all a mandate to continue with the work”. Dr. Moser was a highly esteemed scientist who had devoted his life to understanding and eventually curing a class of devastating neurodegenerative diseases, most notably adrenoleukodystrophy, or ALD. In his work he was careful, rational, painstaking, and relentless – a classic scientist. But in the minds of many movie fans, he became a Hollywood villain.

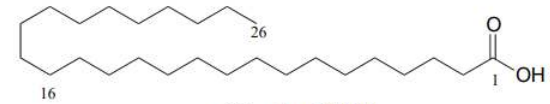
Only 17 months after the death of Dr. Moser, newspapers around the world published moving obituaries marking the passing, at age 30, of Lorenzo Odone. In one, written by his older sister and published in the British newspaper The Guardian, Lorenzo as a young boy is described as “lively and charming . . .he displayed a precocious gift for languages as he mastered English, Italian and French. He was funny, articulate and favored opera over nursery rhymes.” But for more than 20 years leading up to his death, he had been confined to a wheelchair, blind, paralyzed, and unable to communicate except by blinking his eyes. Because he was unable to swallow, he needed an attendant to be with him around the clock to suction saliva from his mouth so he wouldn't choke.

When he was he six years old, Lorenzo started to show changes in behavior: a shortening attention span, moodiness. More disturbing to his parents, Augusto and Michaela Odone, was their suspicion that he was having trouble hearing. They took him in to be examined, and although his hearing was fine, the doctors noticed other behavioral symptoms that concerned them, and so ordered more neurological tests. The results were a kick to the stomach: Lorenzo had a fatal neurodegenerative disease called adrenoleukodystrophy. There was no cure; his nervous system would continue to degenerate, and he would probably be dead within two years.

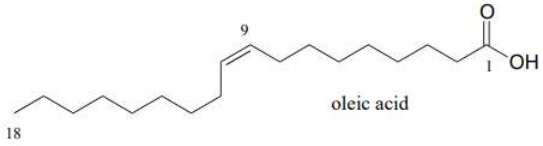
What happened next became such a compelling story that it was eventually retold by director George Miller in the 1992 movie Lorenzo's Oil, starring Nick Nolte and Susan Sarandon as Augusto and Michaela Odone and Peter Ustinov as a character based on Dr. Hugo Moser. The Odones were unwilling to accept the death sentence for their son and, despite having no scientific or medical training, set about to learn everything they could about ALD.

They found out that the cause of ALD is a mutation in a gene that plays an important role in the process by which saturated fatty acids of 26 or more carbons are broken down in the body. When these 'very long chain fatty acids' (VLCFAs) accumulate to excessive levels, they begin to disrupt the structure of the myelin sheath, a protective fatty coating around nerve axons, leading eventually to degradation of the nervous system.

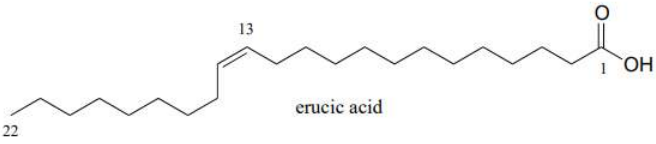
Researchers had found that restricting dietary intake of VLCFAs did not help – apparently much of the damage is done by the fats that are naturally synthesized by the body from shorter precursors. The Odones realized that the key to preventing destruction of the myelin sheath might be to somehow disrupt the synthesis of VLCFAs in Lorenzo's cells. The breakthrough came when they came across studies showing that the carbon chain-elongating enzyme responsible for producing VLCFAs is inhibited by oleic and erucic acids, which are monounsaturated fatty acids of 18 and 22 carbons, respectively and are found in vegetable oils.



a 26-carbon VLCFA



oleic acid



erucic acid

Administration of a mixture of these two oils, which eventually came to be known as 'Lorenzo's Oil' , was shown to lead to a marked decrease in levels of VLCFAs in ALD patients.

This was, however, a therapy rather than a miracle cure – and tragically for the Odones and the families of other children afflicted with ALD, the oil did not do anything to reverse the neurological damage that had already taken place in Lorenzo's brain. Although he was profoundly disabled, with round-the-clock care and a daily dosage of the oil Lorenzo was able to live until a day after his 30th birthday, 22 years longer than his doctors had predicted.

The story does not end there. Although the discovery of the treatment that bears his name came too late for Lorenzo Odone, might daily consumption of the oil by young children who are at a high genetic risk for ALD possibly prevent onset of the disease in the first place, allowing them to live normal lives? This proposal was not without a lot of controversy. Many ALD experts were very skeptical of the Lorenzo's oil treatment as there was no rigorous scientific evidence for its therapeutic effectiveness, and indeed erucic oil was thought to be potentially toxic in the quantities ingested by Lorenzo. Most doctors declined to prescribe the oil for their ALD patients until more studies could be carried out. The Hollywood version of Lorenzo's story cast the medical and scientific establishment, and Dr. Hugo Moser in particular, in a strikingly negative light – they were portrayed as rigid, callous technocrats who cared more about money and academic prestige than the lives of real people. Dr. Moser was not mentioned by name in the movie, but the character played by Peter Ustinov was based closely on him: as his obituary in the Washington Post recounts, Dr. Moser once told an interviewer "The good guys were given real names. The bad guys were given pseudonyms."

What Hugo Moser in fact did was what a good scientist should always do: he kept an open mind, set up and performed careful, rigorous experiments, and looked at what the evidence told him. In a 2005 paper, Moser was finally able to confidently report his

results: when young children at risk of developing ALD were given a daily dose of Lorenzo's oil, they had significantly better chance of avoiding the disease later on.

When he died, Dr. Moser was tantalizingly close to demonstrating conclusively that a simple and rapid blood test that he and his team had developed could reliably identify newborns at high risk of developing ALD – but it was not until after his death that his colleagues, including his wife, Ann Moser, were able to publish results showing that the test worked. The hope is that many lives might be saved by routinely screening newborns for ALD and responding with appropriate preventive treatments - possibly including Lorenzo's oil.

The biochemical reactions at the heart of the Lorenzo's oil story – the carbon-carbon bond forming and bond breaking steps in the synthesis and degradation of fatty acids - both involve chemistry at the α-carbon and proceed through enolate intermediates, much like the aldol and isomerization reactions we studied in chapter 11. They are known as 'Claisen condensation' and 'retro-Claisen cleavage' reactions, respectively, and represent another basic mechanistic pattern - in addition to the aldol reaction - that is ubiquitous in metabolism as a means of forming or breaking carbon-carbon bonds.

To begin this chapter, we will first learn about 'carboxylation' and 'decarboxylation' reactions, in which organic molecules gain or lose a bond to carbon dioxide, respectively, in a mechanism that is really just an extension of the aldol/retro-aldol reactions we learned about in the previous chapter. As part of this discussion, we will work through the mechanism of the carbon-fixing enzyme in plants commonly known as 'Rubisco', which is thought to be the most abundant enzyme on the planet. Then, we will move to the Claisen reactions that are so central to lipid metabolism and the story of Lorenzo Odone. Finally, we will study 'conjugate additions' and 'β-eliminations', common reaction patterns that involve double bonds in the $\alpha - \beta$ position relative to a carbonyl group, and which, again, proceed via enolate intermediates.

Contributors and Attributions

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8.2: Decarboxylation

Many carbon-carbon bond-forming and bond-breaking processes in biological chemistry involve the gain or loss, by an organic molecule, of a single carbon atom in the form of CO_2. You undoubtedly have seen this chemical equation before in an introductory biology or chemistry class:

$$CO_2\,(g) + 6\,H_2O\,(l) + \text{energy} \rightarrow C_6H_{12}O_6\,(aq) + 6\,O_2\,(g) \tag{8.2.1}$$

This of course represents the photosynthetic process, by which plants (and some bacteria) harness energy from the sun to build glucose from individual carbon dioxide molecules. The key chemical step in plants in which a carbon dioxide molecule is 'fixed' (linked to a larger organic molecule) is a carboxylation reaction, and is catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase, commonly known as Rubisco.

The reverse chemical equation is also probably familiar to you:

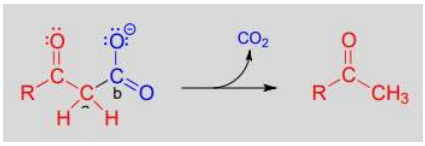
$$C_6H_{12}O_6\,(aq) + 6\,O_2\,(g) \rightarrow 6\,CO_2\,(g) + 6\,H_2O\,(l) + \text{energy} \tag{8.2.2}$$

This equation expresses what happens in the process known as respiration: the oxidative breakdown of glucose to form carbon dioxide, water, and energy (in a non-biological setting, it is also the equation for the uncatalyzed combustion of glucose). In respiration, each of the carbon atoms of glucose is eventually converted to a CO_2 molecule. The process by which a carbon atom - in the form of carbon dioxide - breaks off from a larger organic molecule is called decarboxylation.

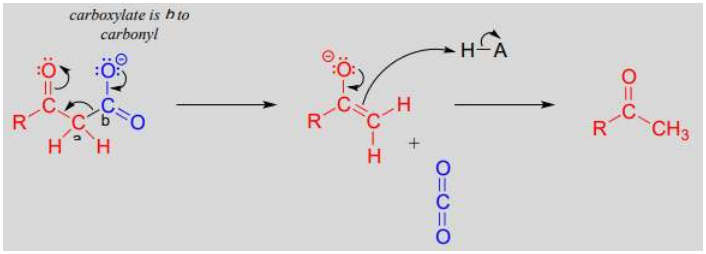
We will look now at the biochemical mechanism of decarboxylation reactions. Later in the chapter, we will look at the carboxylation reaction catalyzed by the Rubisco enzyme.

Decarboxylation steps occur at many points throughout central metabolism. Most often, the substrate for a decarboxylation step is a β-carboxy ketone or aldehyde.

Decarboxylation of a β-carboxy ketone or aldehyde:

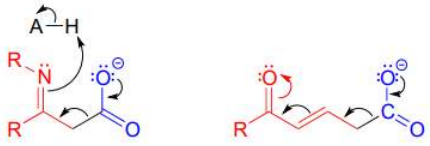


Mechanism:



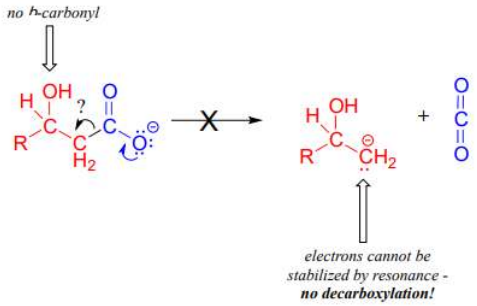
Just as in a retro-aldol reaction, a carbon-carbon bond is broken, and the electrons from the broken bond must be stabilized for the step to take place. Quite often, the electrons are stabilized by the formation of an enolate, as is the case in the general mechanism pictured above.

The electrons from the breaking carbon-carbon bond can also be stabilized by a conjugated imine group and/or by a more extensively conjugated carbonyl.

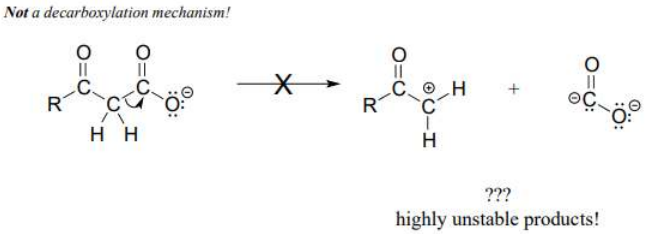


The key in understanding decarboxylation reactions is to first mentally 'push' the electrons away from the carboxylate group, then ask yourself: where do these electrons go? If the electrons cannot 'land' in a position where they are stabilized, usually by resonance with an oxygen or nitrogen, then a decarboxylation is very unlikely.

The compound below is not likely to undergo decarboxylation:



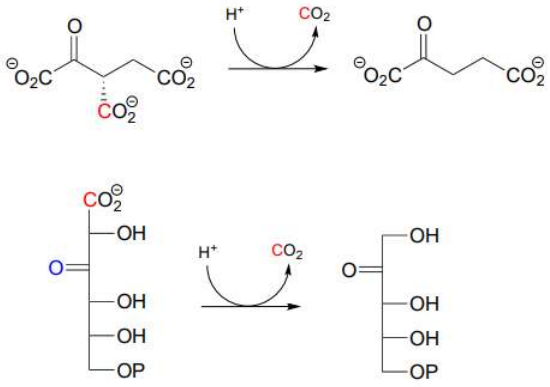
Be especially careful, when drawing decarboxylation mechanisms, to resist the temptation to treat the CO_2 molecule as the leaving group in a mechanistic sense:



The above is not what a decarboxylation looks like! (Many a point has been deducted from an organic chemistry exam for precisely this mistake!) Remember that in a decarboxylation step, it is the rest of the molecule that is, in fact, the leaving group, 'pushed off' by the electrons on the carboxylate.

Decarboxylation reactions are generally thermodynamically favorable due to the entropic factor: one molecule is converted into two, one of which is a gas - this represents an increase in disorder (entropy). Enzymatic decarboxylation steps in metabolic pathways are also generally irreversible.

Below are two decarboxylation steps (EC 1.1.1.42; EC 1.1.1.43) in central catabolic metabolism (specifically the citric acid cycle and pentose phosphate pathway catabolism, respectively). Each step representing a point at which a carbon atom derived from the food we eat is released as carbon dioxide:

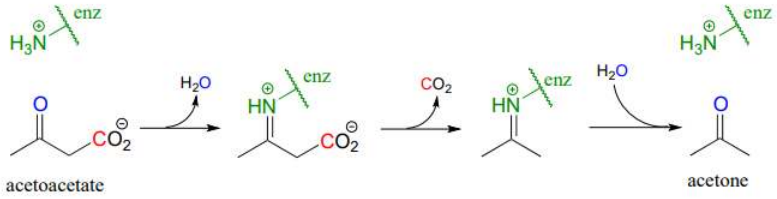


Exercise 8.2.1

Draw mechanistic arrows showing the carbon-carbon bond breaking step in each of the reactions

shown above.

The reaction catalyzed by acetoacetate decarboxylase (EC 4.1.1.4) relies on an iminium (protonated imine) link that forms temporarily between the substrate and a lysine residue in the active site, in a strategy that is similar to that of the enamine-intermediate aldolase reactions we saw in chapter 12. (Recall from section 7.5 that the pK_a of an iminium cation is approximately 7, so it is generally accurate to draw either the protonated iminium or the neutral imine in a biological organic mechanism).

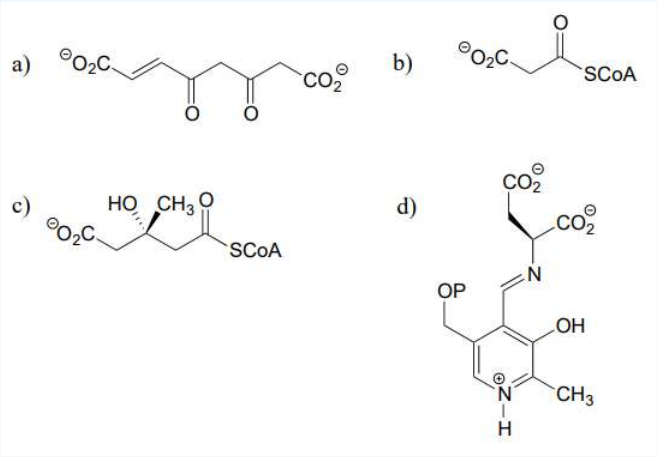


Exercise 8.2.2

Draw a mechanism for the carbon-carbon bond breaking step in the acetoacetate decarboxylase reaction.

Exercise 8.2.3

Which of the following compounds could be expected to potentially undergo decarboxylation? Draw the mechanistic arrows for the decarboxylation step of each one you choose, showing how the electrons from the breaking carbon-carbon bond can be stabilized by resonance.



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8.3: An Overview of Fatty Acid Metabolism

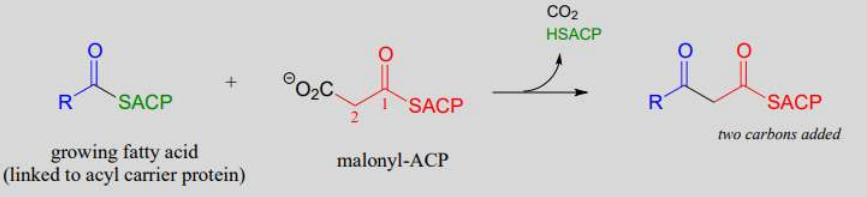
In the introduction to this chapter, we learned about a patient suffering from a rare disease affecting fatty acid metabolism. The reaction mechanisms that we are about to learn about in the next two sections are central to the process by which fatty acids are assembled (synthesis) and taken apart (degradation), so it is worth our time to go through a brief overview before diving into the chemical details.

Fatty acid metabolism is a two-carbon process: in the synthetic directions, two carbons are added at a time to a growing fatty acid chain, and in the degradative direction, two carbons are removed at a time. In each case, there is a four-step reaction cycle that gets repeated over and over. We will learn in this chapter about steps I and III in the synthesis direction, and steps II and IV in the degradative direction. The remaining reactions, and the roles played by the coenzymes involved, are the main topic of chapter 15.

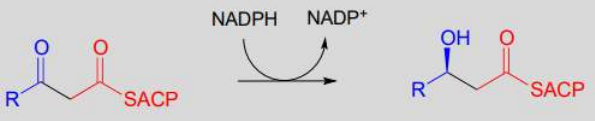
Fatty acid synthesis

'ACP' stands for 'acyl carrier protein', which is a protein that links to growing fatty acid chains through a thioester group (see section 11.5)

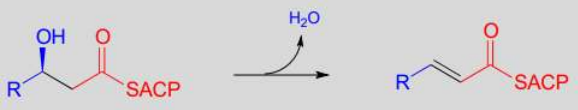
Step I: Condensation (covered in section 13.3)



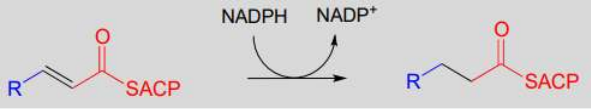
Step II: Ketone hydrogenation (covered in section 15.3)



Step III: Elimination (covered in section 13.4)



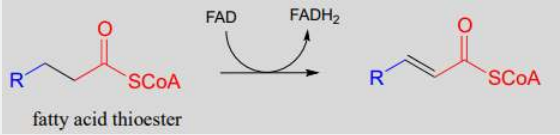
Step IV: Alkene hydrogenation (covered in section 15.4)



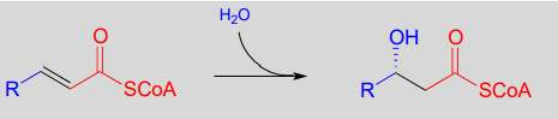
. . . back to step I, add another malonyl-ACP, repeat.

Fatty acid degradation

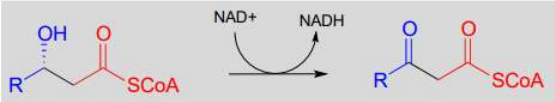
Step I: Alkane oxidation (covered in section 15.4)



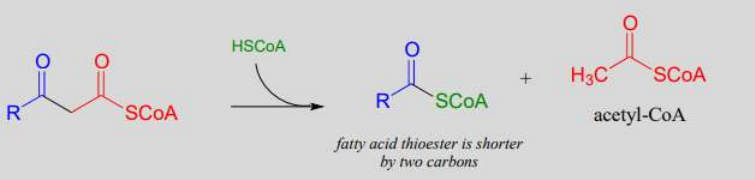
Step II: Addition of water (covered in section 13.4)



Step III: Oxidation of alcohol (covered in section 15.3)



Step IV: Cleavage (covered in section 13.3)



. . . back to step I

When looking at these two pathways, it is important to recognize that they are not the reverse of each other. Different coenzymes are in play, different thioesters are involved (coenzyme A in the degradative direction, acyl carrier protein in the synthetic direction), and even the stereochemistry is different (compare the alcohols in steps II/III of both pathways). As you will learn in more detail in a biochemistry course, metabolic pathways that work in opposite directions are generally not the exact reverse of each other. In some, like fatty acid biosynthesis, all of the steps are catalyzed by different enzymes in the synthetic and degradative directions. Other 'opposite direction' pathways, such as glycolysis/gluconeogenesis, contain mostly reversible reactions (each catalyzed by one enzyme working in both directions), and a few irreversible 'check points' where the reaction steps are different in the two directions. As you will learn when you study metabolism in biochemistry course, this has important implications in how two 'opposite direction' metabolic pathways can be regulated independently of one another.

Recall that in Chapter 12 we emphasized the importance of two reaction types - the aldol addition and the Claisen condensation - in their role in forming (and breaking) most of the carbon-carbon bonds in a living cell. We have already learned about the aldol addition, and its reverse, the retro-aldol cleavage. Now, we will study the Claisen condensation reaction, and its reverse, the retro-Claisen cleavage. Step I in fatty acid synthesis is a Claisen condensation, and step IV in fatty acid degradation is a retro-Claisen cleavage.

In section 13.4, we will look more closely at the reactions taking place in step III of fatty acid synthesis (an elimination) and step II of fatty acid degradation (a conjugate addition).

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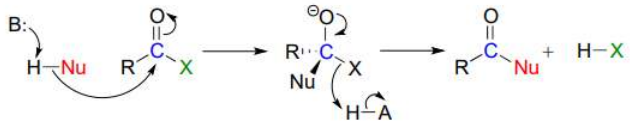
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8.4: Claisen Condensation

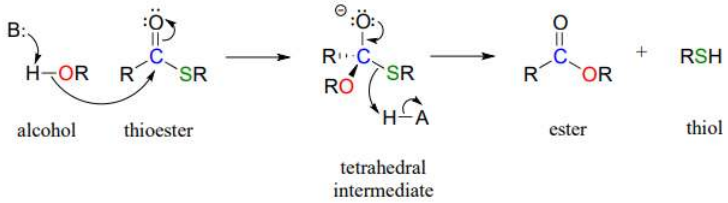
Claisen condensation - an overview

Recall the general mechanism for a nucleophilic acyl substitution mechanism, which we studied in chapter 10:



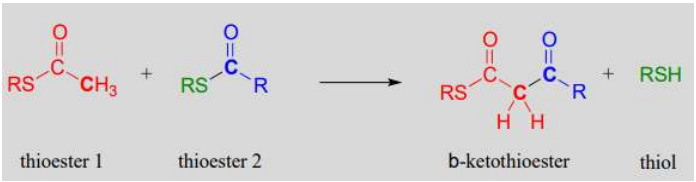
The major points to recall are that a nucleophile attacks a carboxylic acid derivative, leading to a tetrahedral intermediate, which then collapses to expel the leaving group (X). The whole process results in the formation of a different carboxylic acid derivative.

A typical nucleophilic acyl substitution reaction might have an alcohol nucleophile attacking a thioester, driving off a thiol and producing an ester.

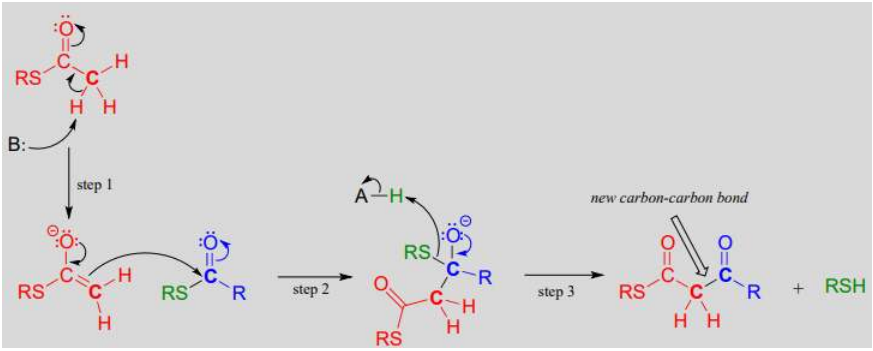


If, however, the attacking nucleophile in an acyl substitution reaction is the α-carbon of an enolate, a new carbon-carbon bond is formed. This type of reaction is called a **Claisen condensation**, after the German chemist Ludwig Claisen (1851-1930).

A Claisen condensation reaction



Mechanism:



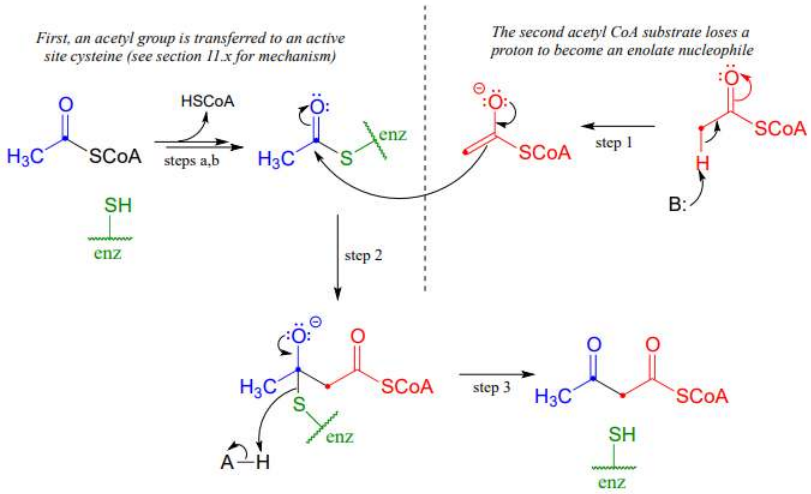
In step 1, the αcarbon of one thioester substrate is deprotonated to form an enolate, which then goes on to attack the second thioester substrate (step 2). Then the resulting tetrahedral intermediate collapses (step 3), expelling the thiol leaving group and leaving us with a β-keto thioester product (a thioester with a ketone group two carbons away).

To reiterate: *A Claisen condensation reaction is simply a nucleophilic acyl substitution (Chapter 11) reaction with an enolate carbon nucleophile.*

Biochemical Claisen condensation examples

A Claisen condensation between two acetyl ***CoA*** molecules (EC 2.3.1.9) serves as the first step in the biosynthesis of cholesterol and other isoprenoid compounds in humans (see section 1.3 for a reminder of what isoprenoids are). First, a transthioesterase reaction transfers the acetyl group of the first acetyl ***CoA*** to a cysteine side chain in the enzyme's active site (steps a, b). (This preliminary event is typical of many enzyme-catalyzed Claisen condensation reactions, and serves to link the electrophilic substrate covalently to the active site of the enzyme).

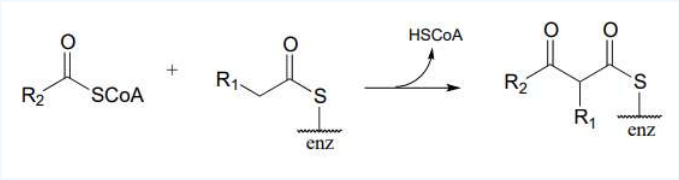
In the 'main' part of the Claisen condensation mechanism, the α-carbon of a second acetyl ***CoA*** is deprotonated (step 1), forming a nucleophilic enolate.



The enolate carbon attacks the electrophilic thioester carbon, forming a tetrahedral intermediate (step 2) which collapses to expel the cysteine thiol (step 3).

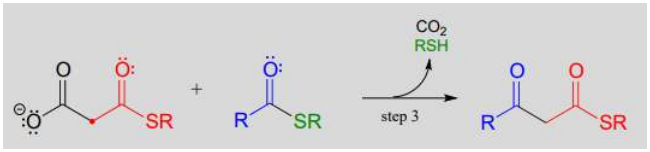
Exercise 8.4.1

Draw curved arrows for the carbon-carbon bond-forming step in mechanism for this condensation reaction between two fatty acyl-thioester substrates. R1 and R2 can be hydrocarbon chains of various lengths. (J. Biol. Chem. 2011, 286, 10930.)

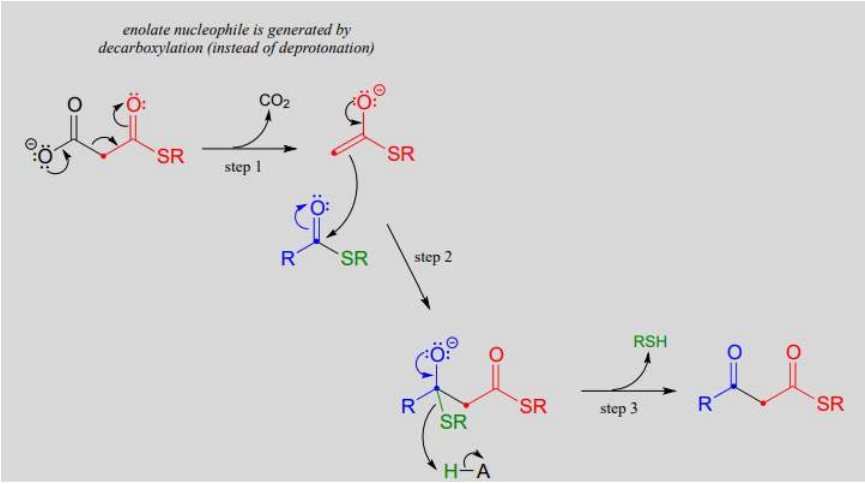


In an alternative mechanism, Claisen condensations in biology are often initiated by decarboxylation at the α-carbon of a thioester, rather than by deprotonation:

Decarboxylation/Claisen condensation:



Mechanism:

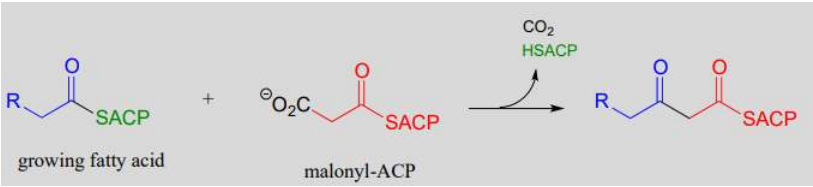


The thing to notice here is that the nucleophilic enolate (in red) is formed in the first step by decarboxylation, rather than by deprotonation of an α-carbon. Other than that, the reaction looks just like the Claisen condensation reactions we saw earlier.

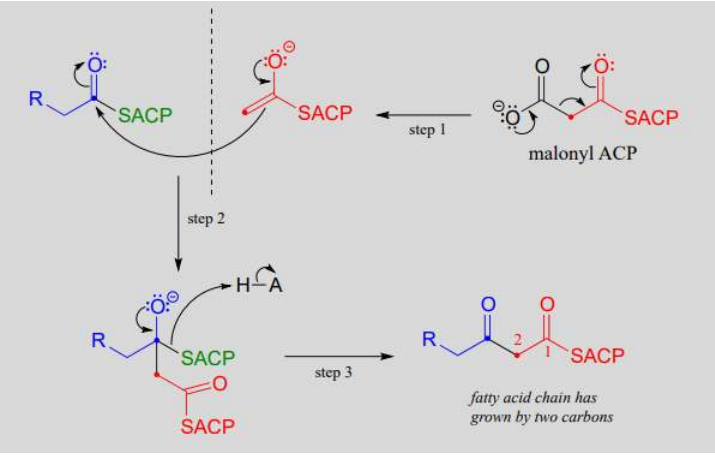
Now, we can finally understand the fatty acid chain-elongation step that we heard about in the chapter introduction in the context of the Lorenzo's oil story, which is a decarboxylation/Claisen condensation between malonyl-ACP (the donor of a two-carbon unit) and a growing fatty acyl CoA molecule. Notice that, again, the electrophilic acyl group is first transferred to an active site cysteine, which then serves as the leaving group in the carbon-carbon bond forming process.

Chain elongating (Claisen condensation) reaction in fatty acid biosynthesis

(step 1 in fatty acid synthesis cycle)

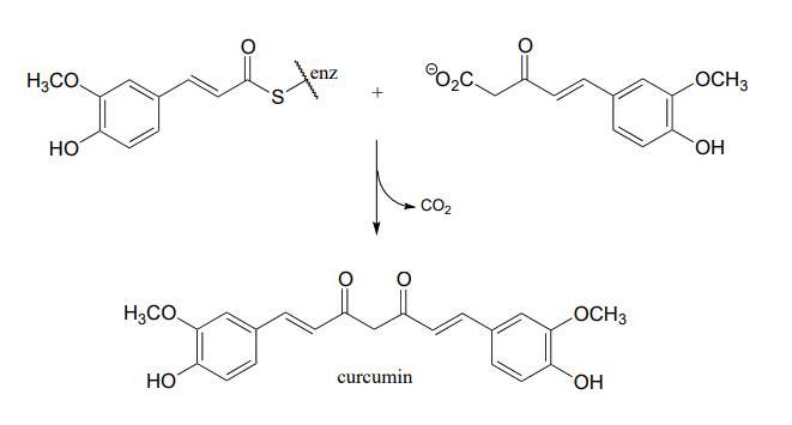


Mechanism:



Exercise 8.4.2

Curcumin is the compound that is primarily responsible for the distinctive yellow color of turmeric, a spice used widely in Indian cuisine. The figure below shows the final step in the biosynthesis of curcumin. Draw a mechanism for this step.



Retro-Claisen cleavage

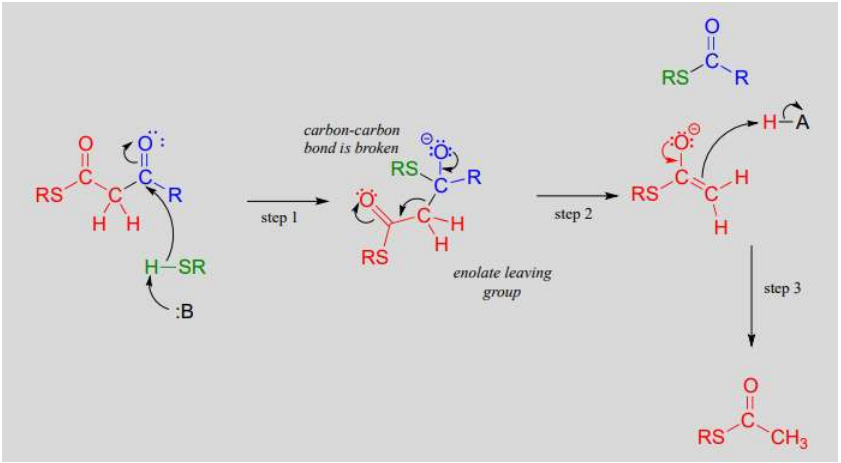
Just like the aldol mechanism, Claisen condensation reactions often proceed in the 'retro', bond-breaking direction in metabolic pathways.

A typical Retro-Claisen cleavage reaction

(thiol nucleophile)



Mechanism:



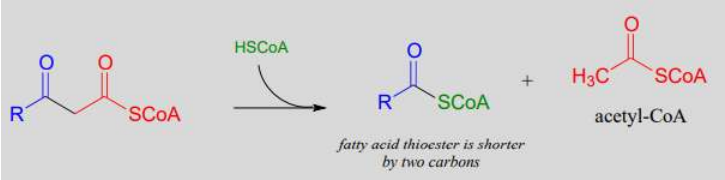
In a typical retro-Claisen reaction, a thiol (or other nucleophile such as water) attacks the carbonyl group of a β-thioester substrate (step 1), and then the resulting tetrahedral intermediate collapses to expel an enolate leaving group (step 2) - this is the key carbon-carbon bond-breaking step. The leaving enolate reprotonates (step 3) to bring us back to where we started, with two separate thioesters. You should look back at the general mechanism for a forward Claisen condensation and convince yourself that the retro-Claisen mechanism illustrated aboveis a step-by-step reverse process.

Exercise 8.4.3

Is a decarboxylation/Claisen condensation step also likely to be metabolically relevant in the 'retro' direction? Explain.

When your body 'burns' fat to get energy, it is a retro-Claisen cleavage reaction (EC 2.3.1.16) that is responsible for breaking the carbon-carbon bonds in step IV of the fatty acid degradative pathway. A cysteine thiol on the enzyme serves as the incoming nucleophile (step 1 in the mechanism below), driving off the enolate leaving group as the tetrahedral intermediate collapses (step 2). The enolate is then protonated to become acetyl CoA (step 3), which goes on to enter the citric acid (Krebs) cycle. Meanwhile, a transthioesterification reaction occurs (steps a and b) to free the enzyme's cysteine residue, regenerating a fatty acyl CoA molecule which is two carbons shorter than the starting substrate.

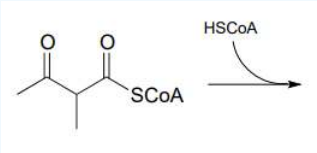
The retro-Claisen reaction (step IV) in fatty acid degradation



Mechanism:

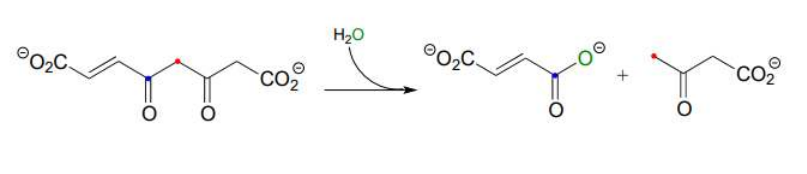
Exercise 8.4.4

In a step in the degradation if the amino acid isoleucine, the intermediate compound 2-methyl-3-keto-butyryl *CoA* undergoes a retro-Claisen cleavage. Predict the products.



Exercise 8.4.5

Many biochemical retro-Claisen steps are hydrolytic, meaning that water, rather than a thiol as in the example above, is the incoming nucleophile that cleaves the carbon-carbon bond. One example (EC 3.7.1.2) occurs in the degradation pathway for tyrosine and phenylalanine:



a. Propose a likely mechanism for the reaction shown.
b. The b-diketone substrate in the reaction above could hypothetically undergo a different retro-Claisen cleavage reaction in which the nucleophilic water attacks the other ketone group. Predict the products of this hypothetical reaction.

Contributors and Attributions

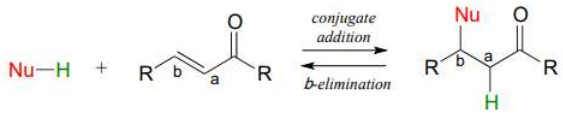
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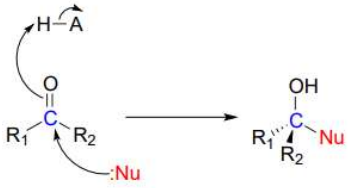
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8.5: Conjugate Addition and Elimination

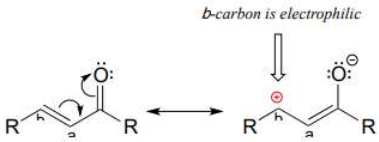
In this section, we will look at two more common biochemical reactions that proceed through enolate intermediates. In a typical **conjugate addition**, a nucleophile and a proton are 'added' to the two carbons of an alkene which is conjugated to a carbonyl (i.e. in the $\alpha - \beta$ position). a β-elimination step, the reverse process occurs:



Earlier we learned about nucleophilic carbonyl addition reactions, including the formation of hemiacetals, hemiketals, and imines. In all of these reactions, a nucleophile directly attacks a carbonyl carbon.

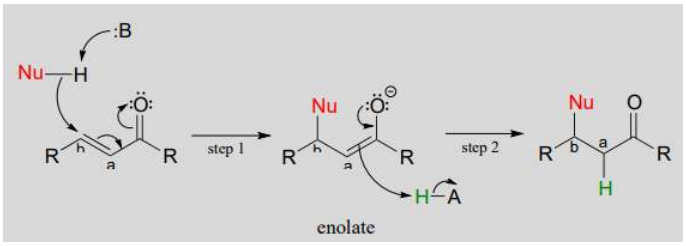


If, however, the electrophilic carbonyl is $\alpha - \beta$-unsaturated - if, in other words, it contains a double bond conjugated to the carbonyl - a different reaction pathway is possible. A resonance structure can be drawn in which the β-carbon has a positive charge, meaning that the β-carbon also has the potential to be an electrophilic target.



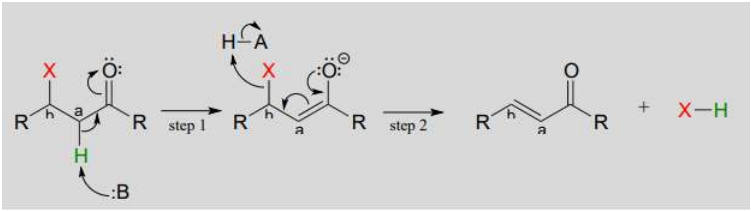
If a nucleophile attacks at the β-carbon, an enol or enolate intermediate results (step 1 below). In many cases this intermediate collapses and the α-carbon is protonated (step 2). This type of reaction is known as a **conjugate addition**.

Mechanism of a conjugate addition reaction



The reverse of a conjugate addition is a **β-elimination**, and is referred to mechanistically the abbreviation $E1cb$.

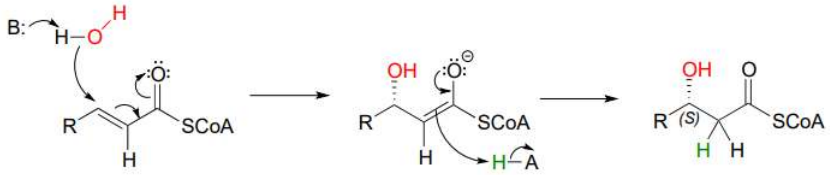
Mechanism of an E1cB elimination



The E stands for 'elimination'; the numeral 1 refers to the fact that, like the S_N1 mechanism, it is a stepwise reaction with first order kinetics. 'cB' designation refers to the intermediate, which is the **c**onjugate **b**ase of the starting compound. In step 1, an α-carbon is

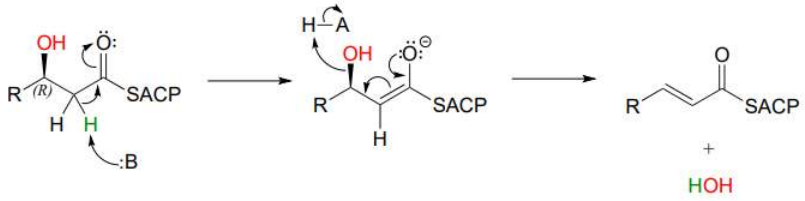
deprotonated to produce an enolate, just like in aldol and Claisen reactions we have already seen. In step 2, the excess electron density on the enolate expels a leaving group at the β position (designated 'X' in the figure above). Notice that the α and β carbons change from sp^3 to sp^2 hybridization with the formation of a conjugated double bond.

Step II of fatty acid degradation is a conjugate addition of water, or hydration.



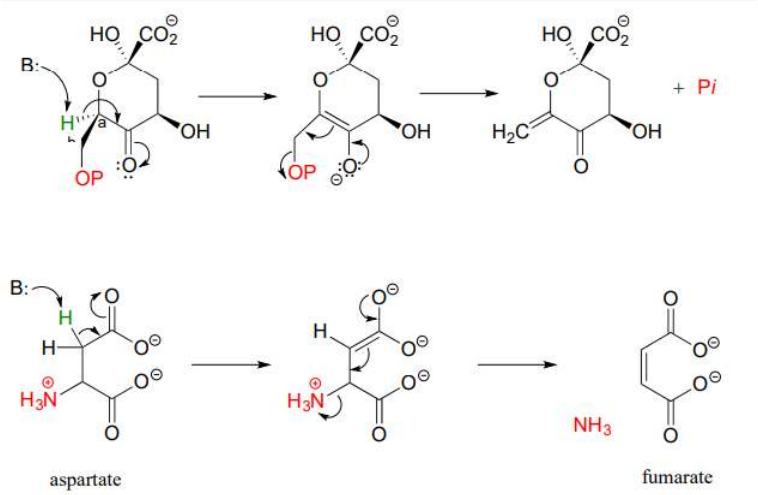
Note the specific stereochemical outcome: in the active site, the nucleophilic water is bound behind the plane of the conjugated system (as drawn in the figure above), and the result is S configuration in the β-hydroxy thioester product.

In step III of the fatty acid synthesis cycle we saw an $E1cb$ β-elimination of water (dehydration):



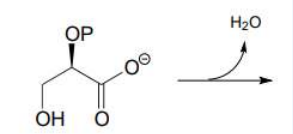
Notice that the stereochemistry at the β-carbon of the starting alcohol is R, whereas the hydration pathway (step II) reaction in the fatty acid degradation cycle pathway results in the S stereoisomer. These two reactions are not the reverse of one another!

Here are two more examples of β-elimination reactions, with phosphate and ammonium respectively, as leaving groups. The first, 3-dehydroquinate synthase (EC 4.2.3.4) is part of the biosynthesis of aromatic amino acids, the second, aspartate ammonia lyase (EC 4.3.1.1) is part of amino acid catabolism.



Exercise 8.5.1

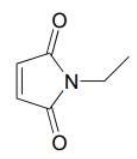
In the glycolysis pathway, the enzyme 'enolase' (EC 4.2.1.11) catalyzes the $E1cb$ dehydration of 2-phosphoglycerate. Predict the product of this enzymatic step.



2-phosphoglycerate

Exercise 8.5.2

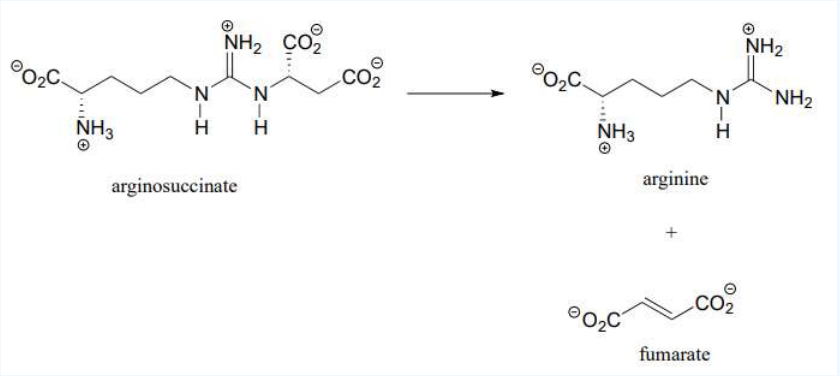
N-ethylmaleimide (NEM) is an irreversible inhibitor of many enzymes that contain active site cysteine residues. Inactivation occurs through conjugate addition of cysteine to NEM: show the structure of the labeled residue. (Michael addition)



NEM

Exercise 8.5.3

Argininosuccinate lyase (4.3.2.1), an enzyme in the metabolic pathway that serves to eliminate nitrogen from your body in the form of urea in urine, catalyzes this β-elimination step:



Propose a complete mechanism.

Hint

Don't be intimidated by the size or complexity of the substrate - review the β-elimination mechanism, then identify the leaving group and breaking bond, the α-carbon which loses a proton, the carbonyl that serves to stabilize the negatively-charged (enolate) intermediate, and the double bond that forms as a result of the elimination. You may want to designate an appropriate 'R' group to reduce the amount of drawing.

Contributors and Attributions

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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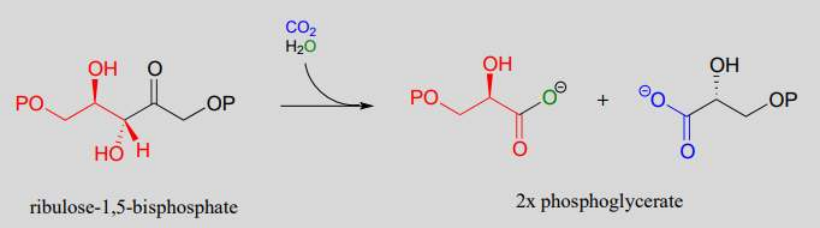
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8.6: Carboxylation

It is difficult to overstate the importance to biology and ecology of the enzymatic reaction we are going to see next: ribulose 1,5-bisphosphate carboxylase (Rubisco) plays a key role in closing the 'carbon cycle' in our biosphere, catalyzing the incorporation of a carbon atom - in the form of carbon dioxide from the atmosphere - into organic metabolites and eventually into carbohydrates, lipids, nucleic acids, and all of the other organic molecules in living things. Rubisco is probably the most abundant enzyme on the planet.

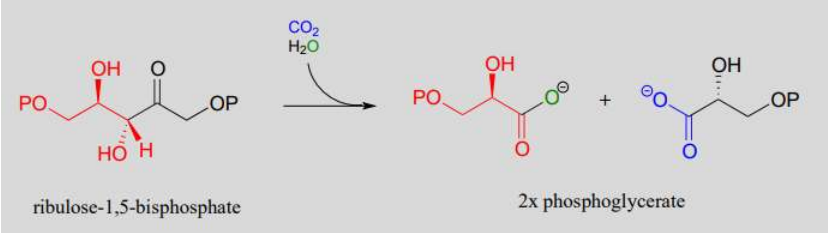
You can think of a carboxylation reaction as essentially a special kind of aldol reaction, except that the carbonyl electrophile being attacked by the enolate is CO_2 rather than a ketone or aldehyde:

Mechanism for carboxylation of an enolate

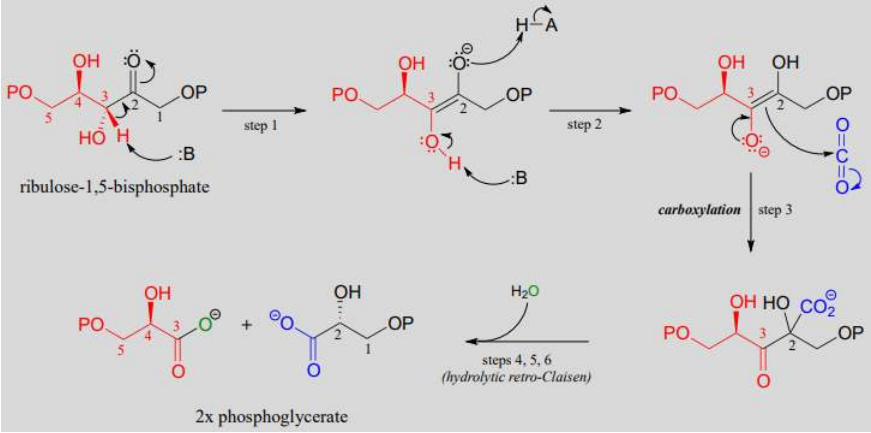


Here is the full Rubisco reaction. Notice that the carbon dioxide (in blue) becomes incorporated into one of the two phosphoglycerate products.

The Rubisco reaction:



Mechanism:



The mechanism for the Rubisco reaction is somewhat involved, but if we break it down into its individual steps, it is not terribly difficult to follow. In step 1, an α-carbon on ribulose 1,5-bisphosphate is deprotonated to form an enolate. In step 2, the oxygen at carbon #3 is deprotonated while the oxygen at carbon #2 is protonated: combined, these two steps have the effect of creating a different enolate intermediate and making carbon #2, rather than carbon #3, into the nucleophile for an aldol-like addition to CO_2 (step 3). Carbon dioxide has now been 'fixed' into organic form - it has become a carboxylate group on a six-carbon sugar derivative. Steps 4, 5, and 6 make up a hydrolytic retro-Claisen cleavage reaction (in other words, water is the bond-breaking

nucleophile) producing two molecules of 3-phosphoglycerate. Phosphoglycerate is channeled into the gluconeogenesis pathway to eventually become glucose.

Exercise 8.6.1

Draw out the full mechanism for steps 4-6 in the Rubisco reaction.

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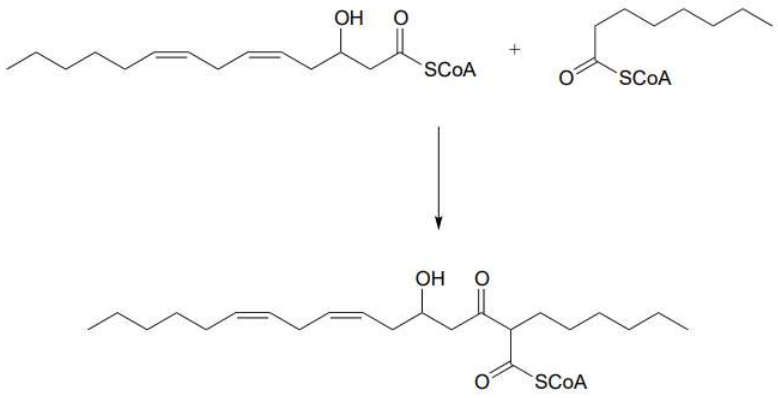
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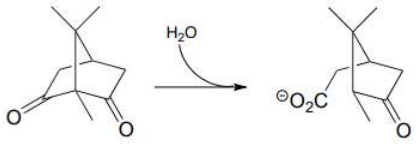
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8.P: Reactions at the α-Carbon, Part II (Problems)

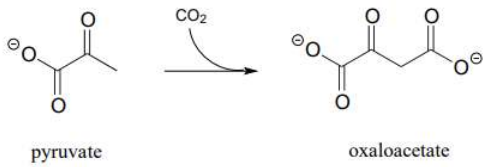
P13.1: Tetrahydrolipastatin, a potent inhibitor of lipase enzymes (see section 11.6) is being tested as a possible anti-obesity drug. Lipastatin, a close derivative, is synthesized by the bacterium Streptomyces toxytricini. The biosynthetic pathway involves the following step shown below - draw a likely mechanism. (J. Biol. Chem. 1997, 272, 867).



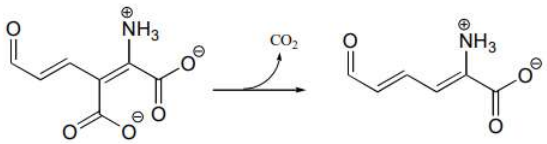
P13.2: The metabolism of camphor by some bacteria involves the step below. Draw a likely mechanism. (J. Biol. Chem 2004, 279, 31312)



P13.3: The glucogeogenesis pathway, by which glucose is synthesized from pyruvate, begins with a reaction catalyzed by pyruvate carboxylase. The enzyme requires the CO_2-carrying biotin to function, but the final step is thought to be the simple carboxylation of pyruvate by free carbon dioxide (Biochem. J. 2008, 413, 369). Draw a mechanism for this step.

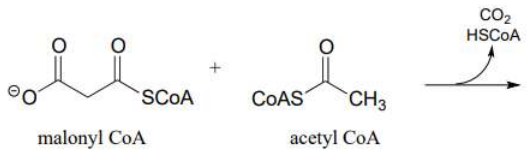


P13.4: Draw a reasonable mechanism for this decarboxylation step in tryptophan biosynthesis (EC 4.1.1.45). Hint: a tautomerization step precedes the decarboxylation.

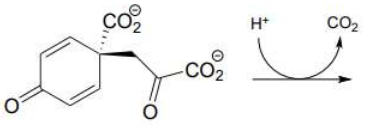


P13.5: The biosynthetic pathway for the antibiotic compound rabelomycin begins with the condensation of malonyl ***CoA*** and acetyl ***CoA***. Predict the product of this reaction, and propose a likely mechanism. (Org. Lett. 2010 12, 2814.)

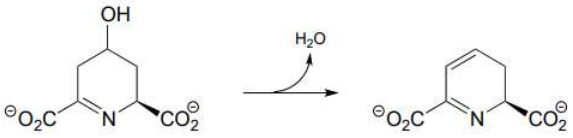
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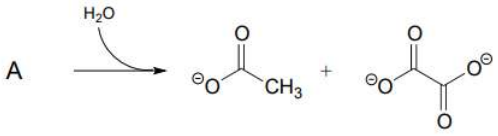
P13.6: Predict the product of this decarboxylation step in the biosynthesis of the amino acid tyrosine. Hint: think about comparative stability when you are considering where protonation will occur!



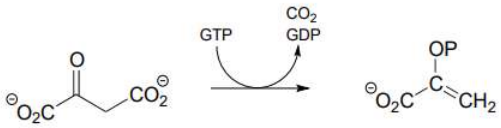
P13.7: Show a likely mechanism for this reaction from lysine biosynthesis:



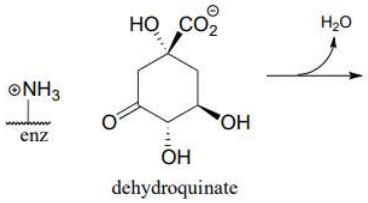
P13.8: Compound A undergoes hydrolytic cleavage in some fungi to form the products shown. Predict the structure of A. (J. Biol. Chem. 2007, 282, 9581)



P13.9: Propose a mechanism for the following reaction from the gluconeogenesis pathway (EC 4.1.1.32):

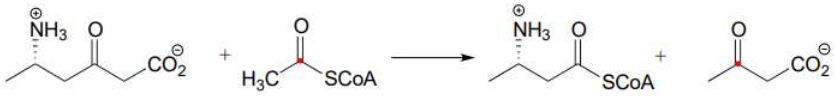


P13.10: Dehydroquinate undergoes dehydration (EC 4.2.1.10) in aromatic amino acid biosynthesis. Experimental and genomic evidence points to a lysine-linked iminium intermediate. More than one dehydration product is possible for dehydroquinate, but in this case the most stable product is the one that forms. Predict the structure of the product, explain why it is the more stable of the possible dehydration products, and draw a mechanism for its formation.

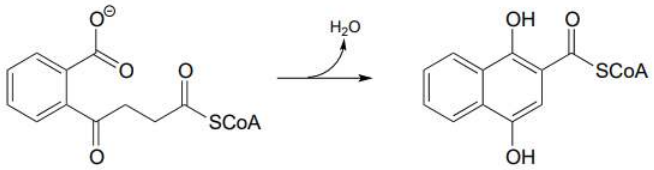


P13.11: The enzyme catalyzing the reaction below, thought to participate in the fermentation of lysine in bacteria, was recently identified and characterized (J. Biol. Chem. 2011, 286, 27399). Propose a likely mechanism. Hint: the mechanism involves two separate carbon-carbon bond forming and bond breaking steps. C_1 of acetyl ***CoA*** is identified with a red dot to help you trace it through to the product.

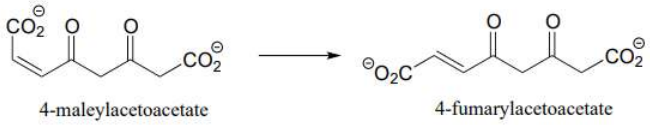
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P13.12: Menaquinone (Vitamin K) biosynthesis in bacteria includes the following step:

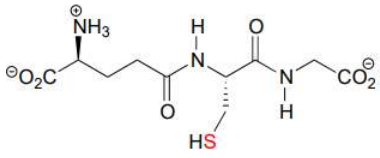


Propose a likely mechanism. Hint: the mechanism involves a Claisen condensation step which is unusual in that the electrophile is a carboxylic acid group rather than a thioester. What is the driving force that allows this unusual step to occur? (J. Biol. Chem. 2010, 285, 30159)



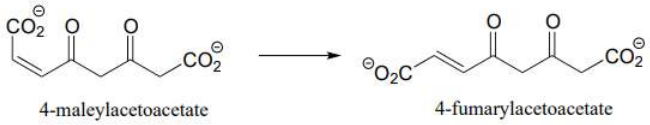
4-maleylacetoacetate

4-fumarylacetoacetate



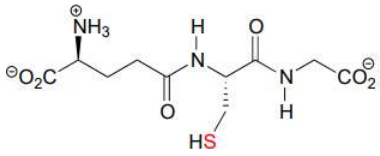
Glutathione
(GSH)

P13.13: 4-maleylacetoacetate isomerase (EC 5.2.1.2) catalyzes the following cis to trans alkene isomerization as part of the degradation of the aromatic amino acids phenylalanine and tyrosine.



4-maleylacetoacetate

4-fumarylacetoacetate



Glutathione
(GSH)

The enzyme uses the thiol-containing coenzyme glutathione, which is also involved in the formation of disulfide bonds in proteins, but in this case glutathione serves as a 'thiol group for hire'. The mechanism for the reaction is essentially a reversible conjugate addition of glutathione. Draw out the steps for this mechanism, showing how the cis-trans isomerization could be accomplished. Also, explain why the equilibrium for this reaction favors 4-fumarylacetoacetate. The structure of glutathione is shown, but you can use the abbreviation GSH in your mechanism.

P13.14: Based on the mechanistic patterns you have studied in this chapter, propose a likely mechanism for this final reaction in the degradation of the amino acid cysteine in mammals.

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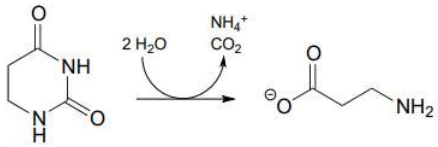


pyruvate

P13.15: Propose a mechanism for the following carboxylation reaction (EC 6.4.1.4) in the leucine degradation pathway. The complete reaction is dependent on the CO_2-carrying coenzyme biotin as well as ATP, but assume in your mechanism that the actual carboxylation step occurs with free CO_2 (you don't need to account for the role played by biotin or ATP).

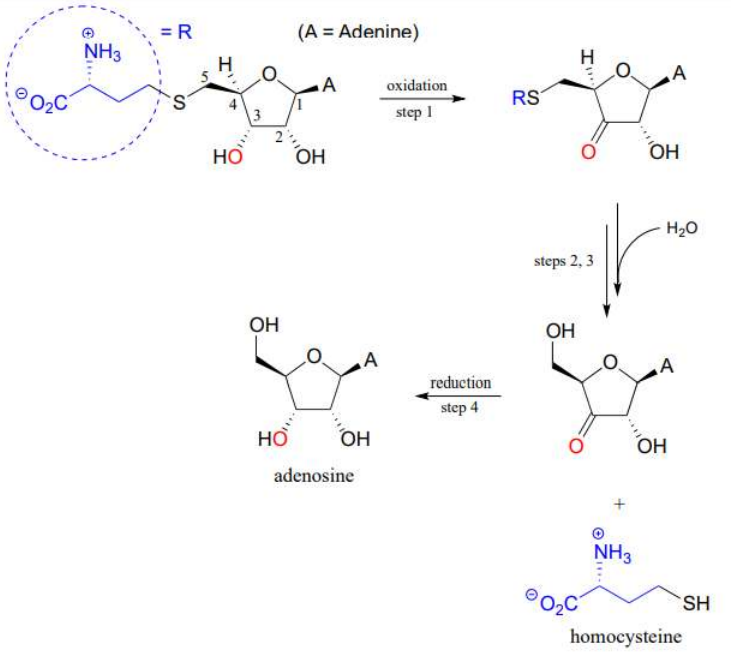


P13.16: Propose a mechanism for the following reaction, which is part of the degradation pathway for the nucleotide uridine.



P13.17: Illustrated below is a series of reactions in the degradation pathway for the amino acid methionine. In step 1, an alcohol group on C_3 is oxidized to a ketone, and in step 4 the ketone is reduced back to an alcohol - we will study these reactions in chapter X. In steps 2 and 3, the thiol (homocysteine) is replaced by water - but this does NOT involve a nucleophilic substitution process.

a. Draw a likely mechanism for step 2
b. Draw a likely mechanism for step 3
c. How does the involvement of the redox steps (steps 1 and 4) provide evidence that overall substitution of water for homocysteine is not a nucleophilic substitution?



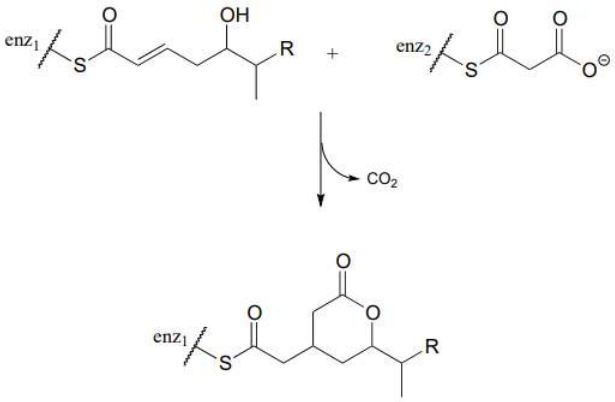
adenosine

homocysteine

P13.18: (Challenging!) A recently discovered reaction in the biosynthesis of rhizoxin, a potent virulence factor in the rice-seedling blight fungus Rhizopus microsporus, is illustrated below (Angewandte Chemie 2009, 48, 5001). The reaction takes place at the intersection of two 'modules' of a multi-enzyme complex, and provides an example of a biochemical conjugate addition step that

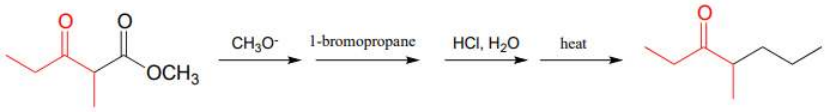
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results in the formation of a new carbon-carbon bond (conjugate addition of a carbon nucleophile is referred to as a Michael addition). Draw a likely mechanism.



P13.19:

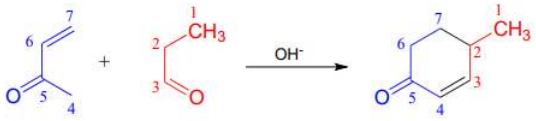
a. The 'acetoacetic ester synthesis' is a useful carbon-carbon bond-forming reaction in the laboratory. The reaction mechanism is described as a-carbon deprotonation to form an enolate, followed by SN2 alkylation, ester hydrolysis, and decarboxylation. Below is an example:



Draw out a reasonable mechanism, taking care to propose reactive intermediates that are appropriate given the basic or acidic conditions present (note that the reaction starts under basic conditions, then is later acidified).

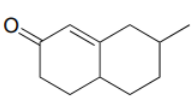
b. Suggest starting compounds for the synthesis of 4-phenyl-2-butanone by the acetoacetic ester reaction.

c. A very useful ring-forming reaction in laboratory synthesis is called 'Robinson annulation' (Sir Robert Robinson was an English chemist who won the 1947 Nobel Prize in Chemistry, and the term ‘annulation’ comes from the Latin annulus, meaning ‘ring’.) The reaction, which takes place in basic conditions, consists of a conjugate (Michael) addition step, followed by aldol addition and finally dehydration (*β*-elimination of water). A typical example is shown below, with carbons numbered to help you to follow the course of the reaction.

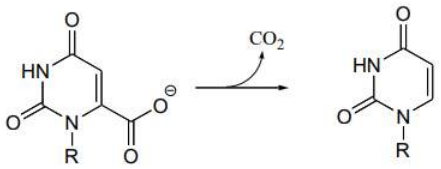


Draw a mechanism for this reaction (when proposing intermediate species, keep in mind that the reaction is occurring in a basic environment, and choose protonation states accordingly).

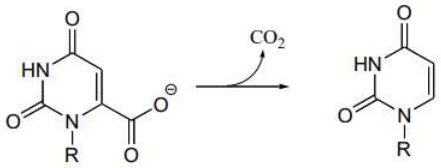
d. Propose starting compounds for the Robinson annulation synthesis of the following product:



P13.20: The reaction shown below, catalyzed by orotidine monophosphate decarboxylase (EC 4.1.1.23), is one of the most extensively studied enzymatic transformations. It is known to occur without the participation of any coenzymes.

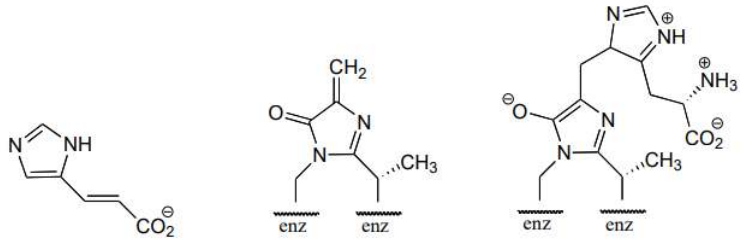


a. Look at the reaction closely: what is unique about it?
b. In 1997, a paper was published in which the authors predicted, based on theoretical calculations, that this reaction proceeded through a carbene intermediate (carbenes are not covered in this text – you may need to look them up). This was prior to the publication of an x-ray crystal structure. What kind of active site environment does this imply?
c. When the crystal structure was published a few years later, we learned that an aspartate residue (predicted to be negatively charged) is positioned very near the substrate carboxylate group, and a lysine residue (predicted to be positively charged) is positioned nearby on the opposite side (see figure below). What roles do you think were predicted for these two active site residues?



P13.21: In the histidine degradation pathway, histidine undergoes elimination of ammonia to form trans-urocanate. The enzyme catalyzing this reaction (E.C. 4.3.1.3) has been shown to use an unusual 'coenzyme', 4-methylideneimidazole-5-one (MIO), which is formed from the cyclization of an alanine-serine-glycine stretch of the enzyme itself.

A mechanism has been proposed in which the MIO coenzyme plays the role of electron sink, and the intermediate shown below forms.



Propose a full mechanism for this reaction according to this information.

P13.22: The product that forms in the reaction between benzaldehyde and acetophenone (along with a catalytic amount of sodium hydroxide) has a 1H-NMR spectrum in which all of the signals are between 7-8 ppm. Give the structure of product.

Contributors

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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8.S: Reactions at the α-Carbon, Part II (Summary)

Before moving on to the next chapter, you should:

- Be able to draw reasonable mechanisms for reactions of the following type:
  - Decarboxylation of a β-carboxy ketone or aldehyde
  - Claisen condensation and retro-Claisen cleavage
  - Hybrid decarboxylation-Claisen condensation
  - Conjugate addition
  - $E1cb$ elimination
- Understand (though not necessarily memorize) the fatty acid synthesis and degradation cycles, and how the Claisen. retro-Claisen, conjugate addition, and $E1cb$ elimination steps fit in.
- Be able to draw a complete mechanism for the Rubisco reaction.

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CHAPTER OVERVIEW

9: Radical Reactions

9.1: Prelude to Radical Reactions
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9.1: Prelude to Radical Reactions



Introduction

Imagine that you are an 18th century British sailor about set out with Commodore George Anson to raid Spanish shipping fleets in the Pacific. You know full well that you are signing up for a long and arduous ordeal, with months of constant seasickness, bad food, cramped, unsanitary conditions, and brutal warfare. You are mentally ready for these hardships, but what you are not prepared for is to watch your own body rot away – to literally fall apart.

Below is a description of the suffering endured by many sailors of the time:

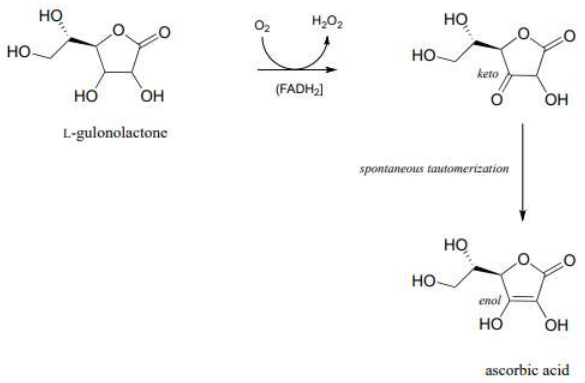
> *Some lost their very substance and their legs became swollen and puffed up while the sinews contracted and turned coal-black and, in some cases, all blotched with drops of purplish blood. Then the disease would creep up to the hips, thighs and shoulders, arms and neck. And all the sick had their mouths so tainted and their gums so decayed that the flesh peeled off down to the roots of their teeth, which almost all fell out. . .*

There were devastating neurological as well as physiological effects. Scurvy had the ability to inhibit a person's normal restraints on emotion: they became intensely homesick and nostalgic, wept at the slightest disappointment, and screamed in agony upon smelling the scent of flower blossoms drifting across the water from a nearby shore.

The disease afflicting the sailors was scurvy, which we now know is caused by a deficiency of vitamin C in the diet. European sea voyagers in the 18th century and earlier subsisted mainly on a diet of salted meat, hard biscuits, pea soup, oatmeal, and beer. After the first couple of weeks at sea, fresh fruits and vegetables - and the nutrients they contained – were all consumed or spoiled. The salted meat and hardtack diet provided salt and calories, but little else of nutritional value.

Although it is rare now, scurvy has plagued sailors for centuries, with records of its occurrence on ships going back as far as the 15th century voyages of Magellan and Vasco de Gama, both of whom lost up to three quarters of their crew to the disease on long ocean crossings. Various cultures made the connection between scurvy and diet, and learned effective preventative measures: sailors with the 16th century French explorer Jacques Cartier, for example, were cured of their scurvy upon arriving in Canada and taking the advice of native people to eat the leaves and bark of pine trees. These were lessons, unfortunately, that often had to be relearned time and again, as the knowledge gained by one culture was not effectively recorded and passed along to others.

Vitamin C, or ascorbic acid as it is known to chemists, plays an essential helping role in a variety of essential biochemical reactions. Most living things are able to synthesize ascorbic acid – the exceptions include humans and other higher primates, several species of bats, and some rodents such as guinea pigs and capybaras. Humans lack the last enzyme in the ascorbic acid biosynthetic pathway, L-gulonolactone oxidase. (EC 1.1.3.8) (You were invited to propose the mechanism for this redox reaction in problem 15.10).



Because we cannot make our own ascorbic acid, we need to get it in our diet. It is abundant in many plant-based foods, citrus fruits in particular. The traditional diet of the Inuit people of the arctic region contains virtually no plant products, but vitamin C is obtained from foods such as kelp, caribou livers, and whale skin. For a time in the 18th century, the observation that citrus fruits quickly cured scurvy led to the practice of including in a ship's stores a paste prepared from boiled lemon juice. Unfortunately, ascorbic acid did not survive the boiling process, rendering the paste ineffective against scurvy. Captain James Cook, the legendary explorer and the first European to make it to the east coast of Australia and the Hawaiian islands, brought along sourkraut (fermented cabbage), a somewhat more effective vitamin C supplement. According to his own account, Cook's sailors at first refused to eat the pungent preparation, so the captain engaged in a little psychological trickery: he declared that it would only be served to officers. The enlisted sailors quickly took offense, and demanded their own sourkraut ration. Later, the British navy famously adopted the practice of adding lemon or lime juice to their ships' rum rations, leading to the birth of the slang term 'Limey' used by European and American sailors to refer to their British counterparts.

The biochemical role of ascorbic acid is to facilitate the transfer of single electrons in a variety of redox reactions - note here the emphasis on single electrons, as opposed to the redox reactions we studied in chapter 15 in which electrons were transferred in pairs. The subject of this chapter is single-electron chemistry, and the free radical intermediates that are involved in single electron reaction steps.

Later in this chapter we will learn the chemical details of why ascorbic acid deficiency causes scurvy, how the act of breathing makes you get old, how polystyrene packing foam is made, and other interesting applications of single-electron chemistry. But first we need to cover some basics ideas about single electron chemical steps, and the free radical intermediates that result from them.

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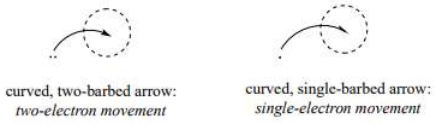
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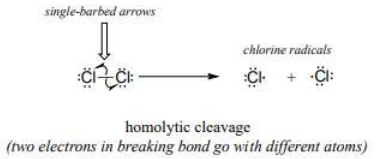
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9.2: Overview of Single-Electron Reactions and Free Radicals

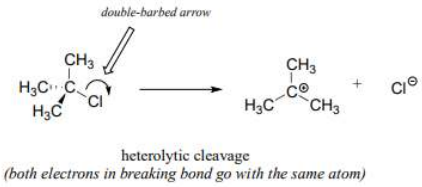
Beginning with acid-base reactions in chapter x and continuing though the chapters on nucleophilic substitution, carbonyl addition, acyl substitution, α-carbon chemistry, and electrophilic reactions , we have been studying reaction mechanisms in which both electrons in a covalent bond or lone pair move in the same direction. In this chapter, we learn about reactions in which the key steps involve the movement of single electrons. Single electron movement is depicted by single-barbed 'fish-hook' arrows (as opposed to the familiar double-barbed arrows that we have been using throughout the book to show two-electron movement).



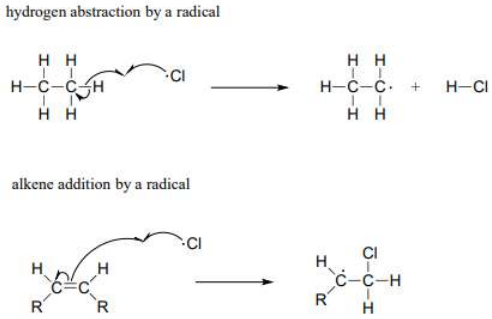
Single-electron mechanisms involve the formation and subsequent reaction of free radical species, highly unstable intermediates that contain an unpaired electron. Free radicals are often formed from homolytic cleavage, an event in which the two electrons in a breaking covalent bond move in opposite directions. The bond in molecular chlorine, for example, is subject to homolytic cleavage when chlorine is subjected to heat or light. The result is two chlorine radicals. Note that each radical has a formal charge of zero.



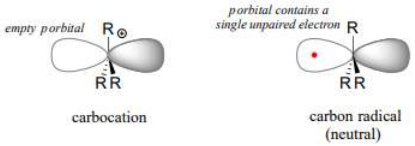
In contrast, essentially all of the reactions we have studied up to now involve bond-breaking events in which both electrons move in the same direction: this is called heterolytic cleavage.



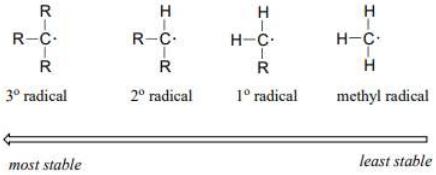
Two other homolytic cleavage reactions that we will see in this chapter can be described as 'radical hydrogen atom abstraction' and 'radical alkene addition':



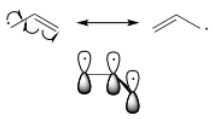
Single-electron reaction mechanisms involve the formation of radical species, and in organic reactions these are often carbon radicals. A carbon radical is sp^2 hybridized, with three s bonds arranged in trigonal planar geometry and the single unpaired electron occupying an unhybridized p orbital. Contrast this picture with a carbocation reactive intermediate, which is also sp^2 hybridized with trigonal planar geometry but with an empty p orbital.



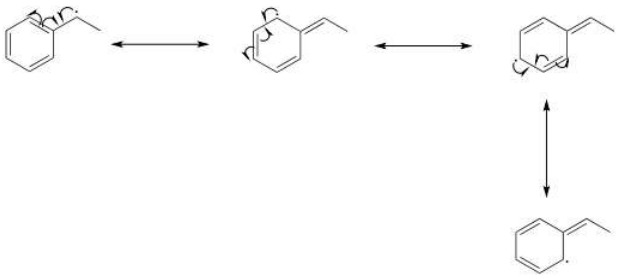
When we studied electrophilic reactions in chapter 14, a major concern when evaluating possible mechanisms was the stability of any carbocation intermediate(s). Likewise, the stability of proposed radical intermediates is of great importance when evaluating the likelihood of possible single-electron mechanisms. Fortunately, the trend in the stability of carbon radicals parallels that of carbocations (section 8.5): tertiary radicals, for example, are more stable than secondary radicals, followed by primary and methyl radicals. This should make intuitive sense, because radicals, like carbocations, are electron deficient, and thus are stabilized by the electron-donating effects of nearby alkyl groups.



Benzylic and allylic radicals are more stable than alkyl radicals due to resonance effects - an unpaired electron (just like a positive or negative charge) can be delocalized over a system of conjugated π bonds. An allylic radical, for example, can be pictured as a system of three parallel p orbitals sharing three electrons.

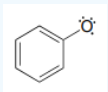


The drawing below shows how a benzylic radical is delocalized to three additional carbons around the aromatic ring:

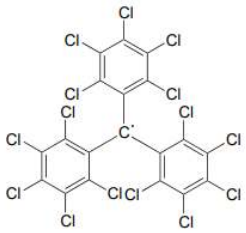


Exercise 9.2.1

Just as phenolate ions are less reactive (less basic) than alkoxide ions, phenolic radicals are less reactive than alkoxide radicals. Draw one resonance contributor of a phenolic radical showing how the radical electron is delocalized to a ring carbon. Include electron-movement arrows.



While radical species are almost always very reactive and short-lived, in some extreme cases they can be unreactive. One example of an inert organic radical structure is shown below.



The already extensive benzylic resonance stabilization is further enhanced by the fact that the large electron clouds on the chlorine atoms shield the radical center from external reagents. The radical is, in some sense, inside a protective 'cage'.

Exercise 9.2.2

Draw a resonance contributor of the structure above in which the unpaired electron is formally located on a chlorine atom (include electron movement arrows)

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9.3: Radical Chain Reactions

Because of their high reactivity, free radicals have the potential to be extremely powerful chemical tools - but as we will see in this chapter, they can also be extremely harmful in a biological/environmental context. Key to understanding many types of radical reactions is the idea of a **radical chain reaction**.

Radical chain reactions have three distinct phases: initiation, propagation, and termination. We'll use a well-known example, the halogenation of an alkane such as ethane, to illustrate. The overall reaction is:

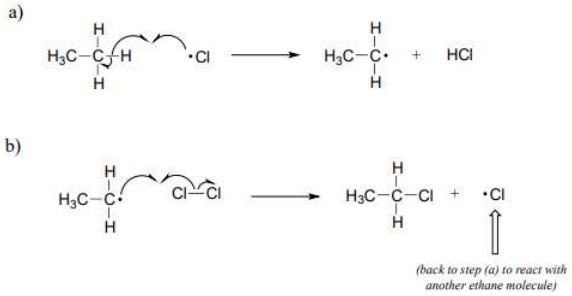
$$CH_3CH_3 + Cl_2 \underset{h\nu \text{ or } \Delta}{\rightarrow} CH_3CH_2Cl + HCl \cdots h\nu \text{ means light } \Delta \text{ means heat} \tag{9.3.1}$$

The initiation phase in a radical chain reaction involves the homolytic cleavage of a weak single bond in a non-radical compound, resulting in two radical species as products.

Often, heat or light provides the energy necessary to overcome an energy barrier for this type of event. The initiation step in alkane halogenation is homolysis of molecular chlorine (Cl_2) into two chlorine radicals. Keep in mind that that virtually all radical species, chlorine radicals included, are highly reactive.

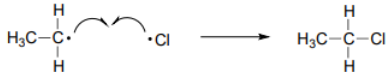
$$Cl \curvearrowright\!\curvearrowright Cl \rightarrow 2Cl \tag{9.3.2}$$

The **propagation** phase is the 'chain' part of chain reactions.



Once a reactive free radical (chlorine radical in our example) is generated in the initiation phase, it will react with relatively stable, non-radical compounds to form a new radical species. In ethane halogenation, a chlorine radical generated in the initiation step first reacts with ethane in a hydrogen abstraction step, generating HCl and an ethyl radical (part a above). Then, the ethyl radical reacts with another (non-radical) Cl_2 molecule, forming the chloroethane product and regenerating a chlorine radical (part b above). This process repeats itself again and again, as chlorine radicals formed in part (b) react with additional ethane molecules as in part (a).

The termination phase is a radical combination step, where two radical species happen to collide and react with each other to form a non-radical product and 'break the chain'. In our ethane chlorination example, one possible termination event is the reaction of a chlorine radical with an ethyl radical to form chloroethane.

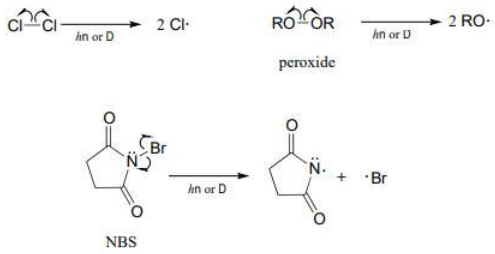


Exercise 9.3.1

Draw two alternative chain termination steps in the ethane chlorination chain reaction. Which one leads to an undesired product?

Because radical species are so reactive and short-lived, their concentration in the reaction mixture at any given time is very low compared to the non-radical components such as ethane and Cl_2. Thus, many cycles of the chain typically occur before a termination event takes place. In other words, a single initiation event leads to the formation of many product molecules.

Compounds which readily undergo homolytic cleavage to generate radicals are called radical initiators. As we have just seen, molecular chlorine and bromine will readily undergo homolytic cleavage to form radicals when subjected to heat or light. Other commonly used as radical initiators are peroxides and ***N***-bromosuccinimide (NBS).



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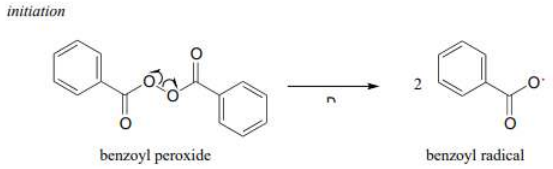
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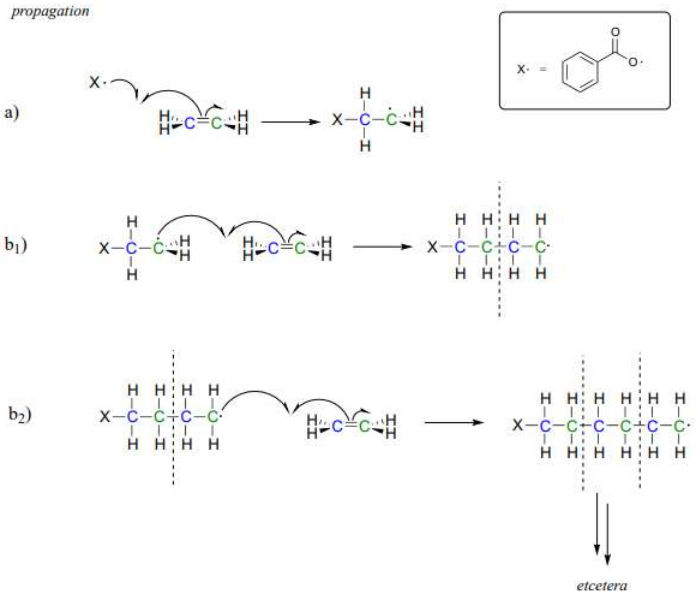
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9.4: Useful Polymers formed by Radical Chain Reactions

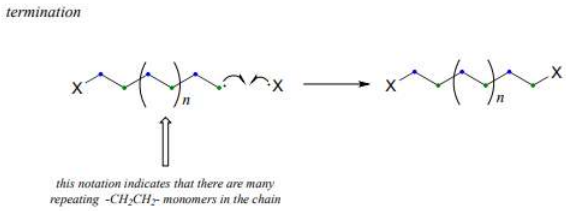
Many familiar household materials polymers made from radical chain reaction processes. Polyethylene (PET), the plastic material used to make soft drink bottles and many other kinds of packaging, is produced by the radical polymerization of ethylene (ethylene is a common name for what we call 'ethene' in IUPAC nomenclature). The process begins when a radical initiator such as benzoyl peroxide undergoes homolytic cleavage at high temperature:



In the propagation phase, the benzoyl radical (***X***· in the figure below) adds to the double bond of ethylene, generating a new organic radical.

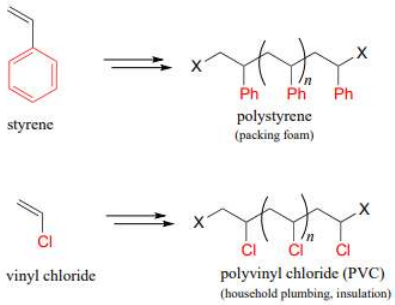


Successive ethylene molecules add to the growing polymer, until termination occurs when two radicals happen to collide.



The length of the polymer is governed by how long the propagation phase continues before termination, and can usually be controlled by adjusting reaction conditions.

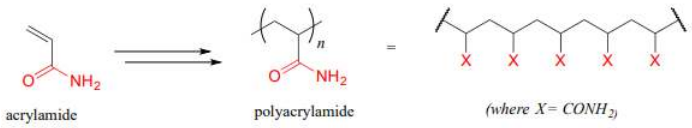
Other small substituted alkene monomers polymerize in a similar fashion to form familiar polymer materials. Two examples are given below.

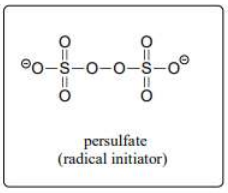


Exercise 9.4.1

Show a mechanism for the formation of a 2-unit long section of polystyrene, starting with the monomer and benzoyl peroxide initiator. Keep in mind the relative stability of different radical intermediates.

A common way to separate proteins in the biochemistry lab is through a technique called polyacrylamide gel electrophoresis (**PAGE**). The polyacrylamide gel is formed through radical polymerization of acrylamide monomer, with the ammonium salt of persulfate used as the radical initiator.





In the end of chapter problems, you will be invited to propose a mechanism showing how a molecule called 'bis-acrylamide' serves as a 'crosslinker' between linear polyacrylamide chains to allow for formation of a net-like structure for the PAGE gel.

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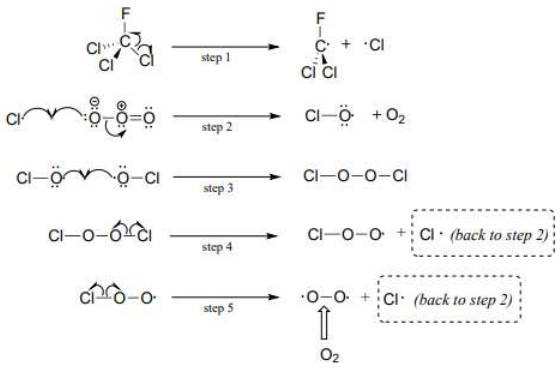
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9.5: Destruction of the Ozone Layer by a Radical Chain Reaction

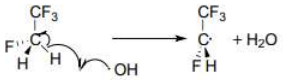
The high reactivity of free radicals and the multiplicative nature of radical chain reactions can be useful in the synthesis of materials such as polyethylene plastic - but these same factors can also result in dangerous consequences in a biological or ecological context. You are probably aware of the danger posed to the earth's protective stratospheric ozone layer by the use of chlorofluorocarbons (*CFCs*) as refrigerants and propellants in aerosol spray cans. Freon-11, or $CFCl_3$, is a typical *CFC* that was widely used until late in the 20th century. It can take months or years for a *CFC* molecule to drift up into the stratosphere from the surface of the earth, and of course the concentration of *CFCs* at this altitude is very low. Ozone, on the other hand, is continually being formed in the stratosphere. Why all the concern, then, about destruction of the ozone layer - how could such a small amount of *CFCs* possibly do significant damage? The problem lies in the fact that the process by which ozone is destroyed is a chain reaction, so that a single *CFC* molecule can initiate the destruction of many ozone molecules before a chain termination event occurs.

Although there are several different processes by which the ozone destruction process might occur, the most important is believed to be the chain reaction shown below.



First, a *CFC* molecule undergoes homolytic cleavage upon exposure to UV radiation, resulting in the formation of two radicals (step 1). The chlorine radical rapidly reacts with ozone (step 2) to form molecular oxygen and a chlorine monoxide radical. Step 3 appears to be a chain termination step, as two chlorine monoxide radicals combine. The Cl_2O_2 condensation product, however, is highly reactive and undergoes two successive homolytic cleavage events (steps 4 and 5) to form O_2 and two chlorine radicals, which propagates the chain.

To address the problem of ozone destruction, materials chemists have developed new hydrofluorocarbon refrigerant compounds. The newer compounds contain carbon-hydrogen bonds, which are weaker than the carbon-halogen bonds in *CFCs*, and thus are susceptible to homolytic cleavage caused by small amounts of hydroxide radical present in the lower atmosphere:



This degradation occurs before the refrigerant molecules have a chance to drift higher up to the stratosphere where the ozone plays its important protective role. The degradation products are quite unstable and quickly degrade further into relatively harmless by-products. The hydroxide radical is sometimes referred to as an atmospheric 'detergent' due to its ability to degrade escaped refrigerants and other volatile organic pollutants.

Hydrofluorocarbons do, however, act as greenhouse gases, and are thought to contribute to climate change.

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9.6: Oxidative Damage to cells, Vitamin C, and Scurvy

While the hydroxide radical can be a beneficial 'detergent' in the atmosphere, it is harmful when present in a living cell. Hydroxide radical is one of the reactive oxygen species (ROS) that we learned about in chapter 15. Recall that ROS are continuously produced as minor but harmful side-products in the reduction of O_2 to H_2O in respiration.

You may recall from your general chemistry course that molecular oxygen exists in two states: 'singlet' oxygen has a double bond and no unpaired electrons, while 'triplet' oxygen has a single O-O bond and two unpaired electrons. Molecular orbital theory - and experimental evidence - show that the triplet state is lower in energy.



***Figure* 9.6.1**

ROS are highly reactive oxidizing agents, capable of inflicting damage to DNA, proteins, and the lipids of cell membranes - they are thought to play a major role in the aging process. Hydroxide radical, for example, can initiate a radical chain reaction with the hydrocarbon chain of an unsaturated membrane lipid molecule, resulting in the formation of lipid hydroperoxide.

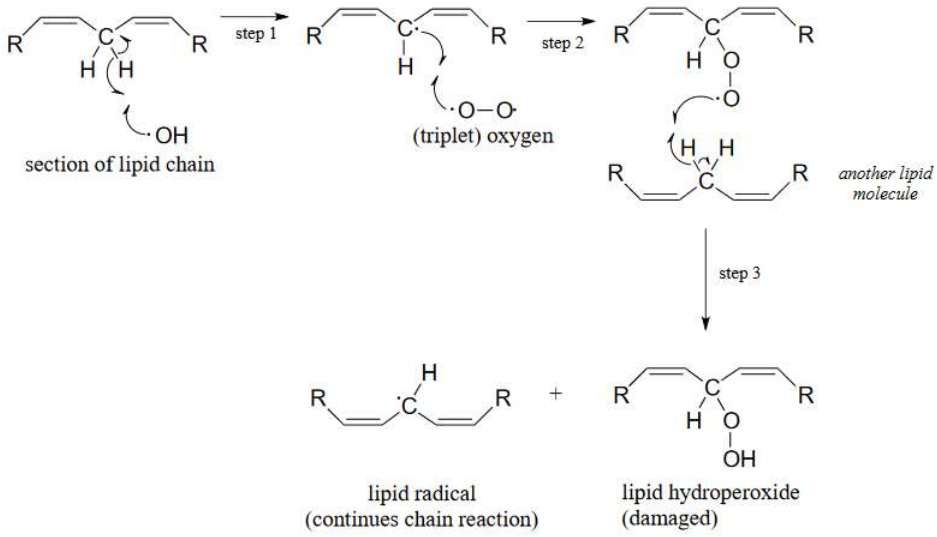


***Figure* 9.6.2**

The allylic lipid radical formed as the result of homolytic hydrogen abstraction by hydroxide radical (step 1 above) reacts with one of the unpaired electrons in triplet oxygen (step 2) forming a peroxy radical. This radical species in turn homolytically abstracts a hydrogen from another lipid molecule (step 3), thus propagating the chain.

Many edible plants contain various antioxidant compounds, also known as 'free radical scavengers', which serve to protect cells from the oxidative effects of hydroxide radical and other harmful radical intermediates. Simply put, a free radical scavenger is a molecule that reacts with a potentially damaging free radical species, forming a more stable radical species which can be metabolized by the body before any damage is done to cell constituents.

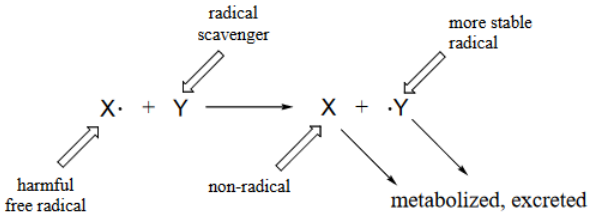


***Figure* 9.6.3**

In the introduction to this chapter, we learned about scurvy, the disease long dreaded by sailors, and how it is caused by a deficiency of ascorbic acid (vitamin C) in the diet. We will soon get to the connection between ascorbic acid and scurvy, but first, let's look at how ascorbic acid functions as a free radical scavenger in your body.

The pK_a of ascorbic acid is about 4.1, so in a physiological environment it exists mainly as ascorbate anion, the conjugate base. When ascorbate encounters a hydroxide radical (or any other potentially damaging radical species), it donates a single electron, thus reducing the hydroxide radical to hydroxide ion and becoming itself an ascorbyl radical.

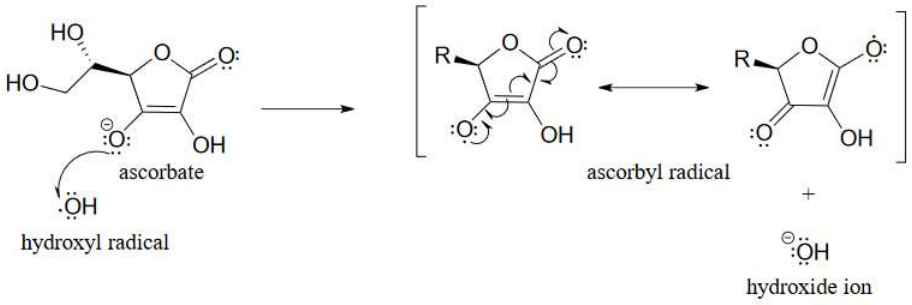


***Figure* 9.6.4**

The ascorbyl radical is stabilized by resonance. The end result of this first step is that a very reactive, potentially harmful hydroxide radical has been 'quenched' to hydroxide ion and replaced by a much less reactive (and thus less harmful) ascorbyl radical.

The ascorbyl radical can then donate a second electron to quench a second hydroxide radical, resulting in the formation of dehydroascorbate, the oxidized form of ascorbate.

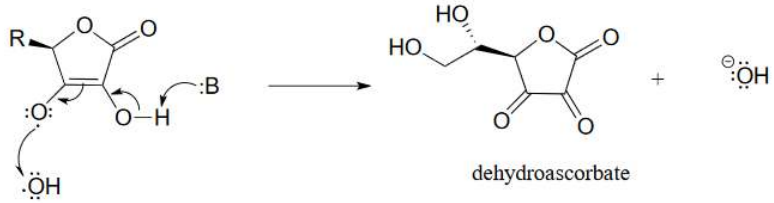


***Figure* 9.6.5**

One ascorbate molecule is thus potentially able to scavenge two harmful radical species.

Dehydroascorbate is subsequently either broken down and excreted, or else enzymatically recycled (reduced) back to ascorbic acid. You were invited to propose a mechanism for the latter (redox) step in problem 15.10. J. Am. Coll. Nutr., 2003, 22, 18

We learned in the introduction to this chapter about the gruesome effects of long-term ascorbic acid deficiency. What, then, is the chemical connection between ascorbic acid and scurvy?

The symptoms association with scurvy are caused by the body's failure to properly synthesize collagen, the primary structural protein in our connective tissues. Essential to the stability of collagen is its ability to form a unique triple-helical structure, in which three protein strands coil around each other like a woven rope. Collagen strands are not able to pack together properly into their

triple helix structure unless certain of their proline amino acid residues are hydroxylated: the electronegative OH group on hydroxyproline causes the five-membered ring in the amino acid to favor a particular 'envelope' conformation (section 3.2) as well as the 'trans' peptide conformation, both of which are necessary for stable triple-helix formation.

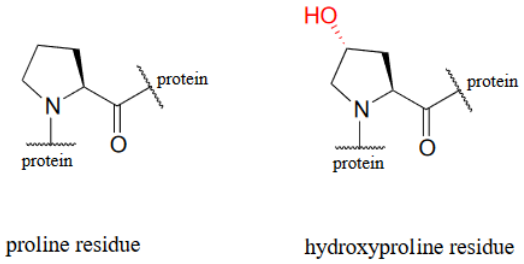


***Figure* 9.6.6**

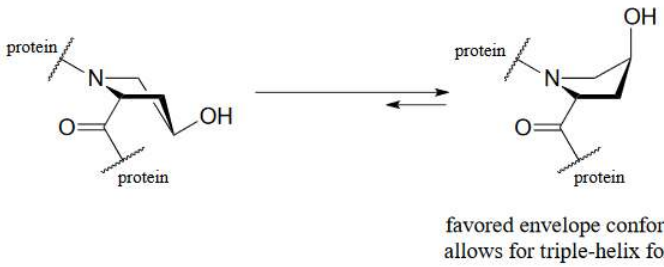


***Figure* 9.6.7**

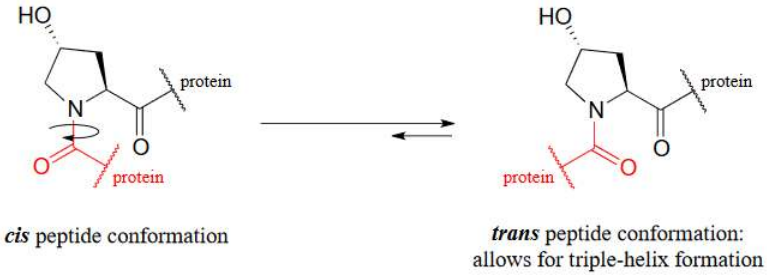


***Figure* 9.6.8**

Proline hydroxylase, the enzyme responsible for this key modification reaction, depends in turn upon the presence of ascorbate. The hydroxylating reaction is complex, and involves electron-transfer steps with enzyme-bound iron - mechanistic details that are well outside of our scope here, but which you may learn about in a bioinorganic chemistry course. It is enough for us to know that iron starts out in the Fe^{+2} state, and during the course of the reaction it loses an electron to assume the Fe^{+3} state. In order for the enzyme to catalyze another reaction, the iron must be reduced back to its active Fe^{+2} state - it must accept a single electron. The donor of this single electron is ascorbate.

(For more information, see Crit. Rev. Biochem. Mol. Biol. 2010, 405, 106.)

So, to sum up: If we fail to get enough ascorbic acid in our diet (in other words, if we don't eat our fruits and vegetables!) the iron in our proline hydroxylase enzymes won't be returned to the active Fe^{+2} state, so the catalytic cycle is broken and we can't turn prolines into hydroxyprolines. Without the hydroxy group, the proline residues of our collagen proteins won't assume the proper conformation, and as a consequence the collagen triple helix structures will be unstable. At physiological temperature, our collagen will literally melt apart - and with it, our gums, our capillaries, and anything else held together by connective tissue. This is scurvy.

You have probably heard that many fruits and vegetables contain natural 'polyphenol' antioxidant compounds that are thought to be beneficial to our health. Apigenin, for example, is found in parsley and celery, while the skins of grapes used to produce red wine

are particularly rich in resveratrol, as well as many other polyphenols. Curcumin is the compound responsible for the distinctive yellow color of turmeric, a ubiquitous spice in Indian cuisine.

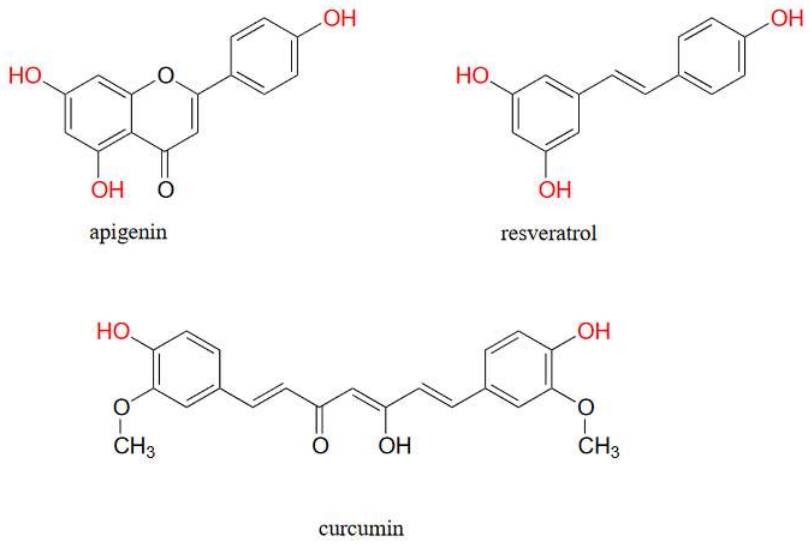


***Figure* 9.6.9**

While much remains to be learned about exactly how these polyphenols exert their antioxidant effects, it is likely that they, like ascorbic acid, act as radical scavengers. For example, resveratrol could donate a single electron (and a proton) to hydroxide radical to reduce it to water. The phenolic radical that results is stabilized by resonance, and is much less likely than hydroxide radical to cause damage to important biomolecules in the cell.

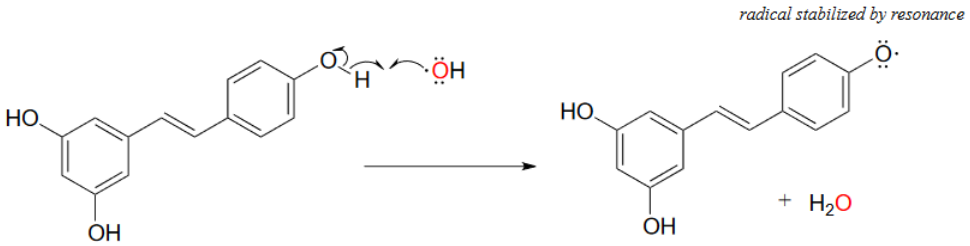


***Figure* 9.6.10**

Contributors and Attributions

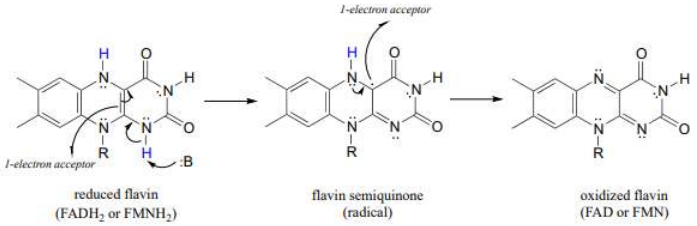
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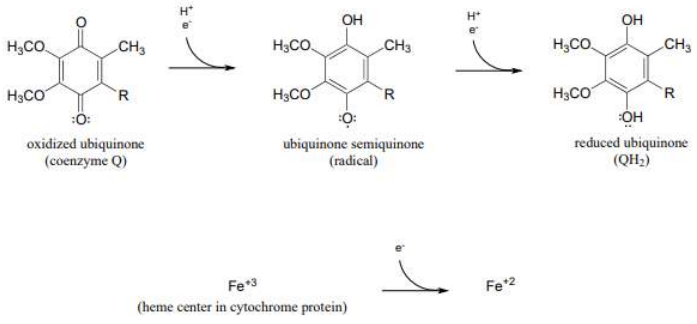
9.7: Flavin as a One-Electron Carrier

In chapter 15 we saw how a nicotinamide and flavin coenzymes can act as acceptors or donors of two electrons in hydride-transfer redox steps. Recall that it was mentioned that flavin, (but not nicotinamide) can also participate in single-electron transfer steps through a stabilized radical intermediate called a semiquinone.. Frey p. 162 fig 3-30; Silverman p. 122 sch. 3.34; J Phys Chem A. 2013, 117, 11136 fig 2)



Note in this reaction that overall, flavin loses or gains two electrons and two protons, just like in the flavin-dependent redox reactions we saw in chapter 15. The difference here is that the electrons are transferred one at a time, rather than paired in the form of a hydride ion.

Two important examples single-electron acceptor species in human metabolism are ubiquinone (coenzyme Q) and the oxidized form of cytochrome. Ubiquinone is a coenzyme that can transfer single electrons via a semiquinone state analogous to that of flavin, and cytochrome is a protein containing a 'heme' iron center which shuttles between the Fe^{+3} (oxidized) and Fe^{+2} (reduced) state.



Further discussion of the mechanisms of single-electron flavin reactions is beyond our scope here, but when you study the 'respiratory chain' in a biochemistry course you will gain a deeper appreciation for the importance of flavin in single-electron transfer processes.

Contributors and Attributions

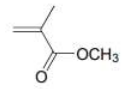
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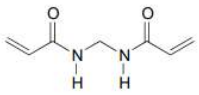
9.P: Radical Reactions (Problems)

P16.1: Plexiglass is a polymer of methyl methacrylate. Show a mechanism for the first two propagation steps of polymerization (use $X\cdot$ to denote the radical initiator), and show a structure for the plexiglass polymer. Assume an alkene addition process similar to that shown in the text for polyethylene.



methyl methacrylate

P16.2: In section 16.3 we saw how acrylamide polymerizes to form the polyacrylamide used in PAGE protein gels. Polyacrylamide by itself is not sufficient by itself to form the gel - the long polyacrylamide chains simply slip against each other, like boiled spaghetti. To make a PAGE gel, with pores for the proteins to slip through, we need a crosslinker - something to tie the chains together, forming a three-dimensional web-like structure. Usually, a small amount of bis-acrylamide is added to the acrylamide in the polymerization mixture for this purpose.



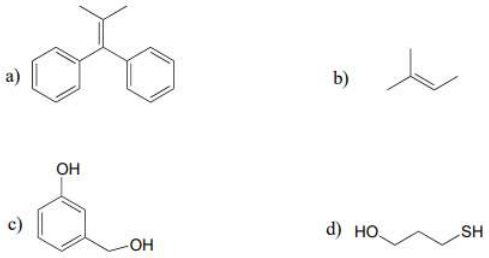
bis-acrylamide

Propose a radical mechanism showing how bis- acrylamide might form crosslinks between two polyacrylamide chains.

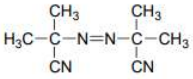
P16.3: Resveratrol is a natural antioxidant found in red wine (see section 16.5 for the structure).

a. Draw one resonance structure to illustrate how the resveratrol radical is delocalized by resonance.
b. Indicate all of the carbons on your structure to which the radical can be delocalized.
c. Draw an alternate resveratrol radical (one in which a hydrogen atom from one of the other two phenolic groups has been abstracted). To how many carbons can this radical be delocalized?
d. The curcumin structure is shown in the same figure as that of resveratrol, in section16.5. Draw two resonance contributors of a curcumin radical, one in which the unpaired electron is on a phenolic oxygen, and one in which the unpaired electron is on a ketone oxygen.

P16.4: Draw the radical intermediate species that you would expect to form when each of the compounds below reacts with a radical initiator.



P16.5: Azobis(isobutyronitrile) is a widely used radical initiator which rapidly undergoes homolytic decomposition when heated.



Azobis(isobutyronitrile)

Predict the products of this decomposition reaction, and show a likely mechanism. What is the thermodynamic driving force for homolytic cleavage?

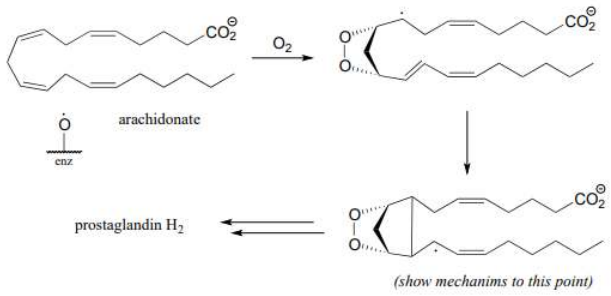
P16.6:

a. When 2-methylbutane is subjected to chlorine gas and heat, a number of isomeric chloroalkanes with the formula $C_5H_{11}Cl$ can form. Draw structures for these isomers, and for each draw the alkyl radical intermediate that led to its formation.
b. In part a), which is the most stable radical intermediate?
c. In the reaction in part a), the relative abundance of different isomers in the product is not exclusively a reflection of the relative stability of radical intermediates. Explain.

P16.7: We learned in chapter 14 that HBr will react with alkenes in electrophilic addition reactions with 'Markovnikov' regioselectivity. However, when the starting alkene contains even a small amount of contaminating peroxide (which happens when it is allowed to come into contact with air), a significant amount of 'anti-Markovnikov' product is often observed.

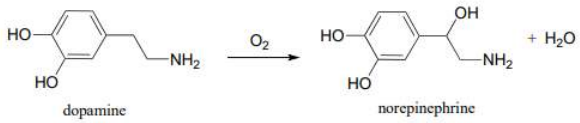
a. Propose a mechanism for formation of the anti-Markovnikov addition product when 1-butene reacts with HBr containing a small amount of benzoyl peroxide
b. Predict the product and propose a mechanism for the addition of ethanethiol to 1-butene in the presence of peroxide.

P16.8: In section 11.5 we learned that aspirin works by blocking the action of an enzyme that catalyzes a key step in the biosynthesis of prostaglandins, a class of biochemical signaling molecules. The enzyme in question, prostaglandin H synthase (EC 1.14.99.1) catalyzes the reaction via several single-electron steps. First, an iron-bound oxygen radical in the enzyme abstracts a hydrogen atom from arachidonate. The arachidonate radical intermediate then reacts with molecular oxygen to form a five-membered oxygen-containing ring, followed by closure of a cyclopentane ring to yield yet another radical intermediate. (Biochemistry 2002, 41, 15451.)



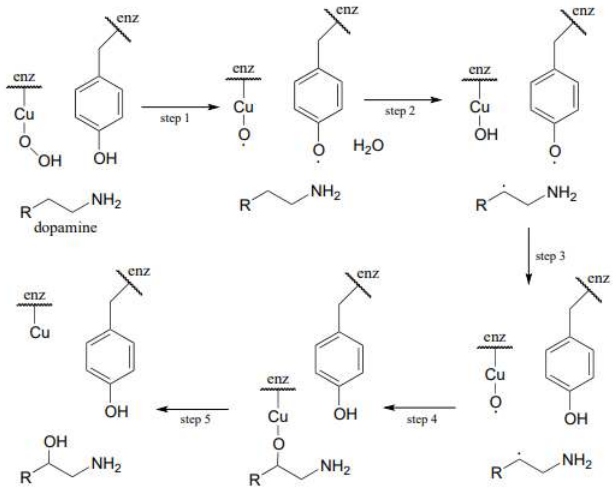
Propose a mechanism for the steps of the reaction that are shown in this figure.

P16.9: Some redox enzymes use copper to assist in electron transfer steps. One important example is dopamine b-monooxygenase (EC 1.14.1.1), which catalyzes the following reaction:



The following intermediates have been proposed: (see Biochemistry 1994, 33, 226) Silverman p. 222

https://chem.libretexts.org/@go/page/291186



Draw mechanistic arrows for steps 1-4.

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CHAPTER OVERVIEW

10: The Organic Chemistry of Vitamins

10.1: Prelude to the Organic Chemistry of Vitamins

10.2: Pyridoxal Phosphate (Vitamin B6)

10.3: Thiamine Diphosphate (Vitamin B1)

10.4: Thiamine Diphosphate, Lipoamide and the Pyruvate Dehydrogenase Reaction

10.5: Folate

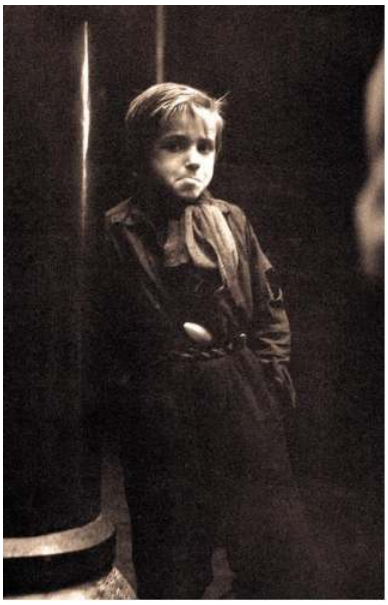
10.E: The Oganic Chemistry of Vitamins (Exercises)

10.S: The Oganic Chemistry of Vitamins (Summary)

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10.1: Prelude to the Organic Chemistry of Vitamins

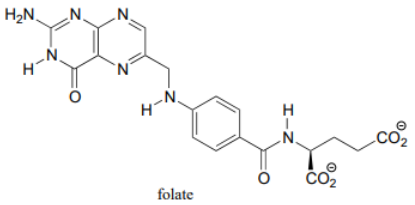
The old black and white photograph is haunting. A young boy, perhaps 10 or 11 years old, huddles against a wall outside a soup kitchen, his mouth in an odd twisted shape that could be expressing either pain or defiance, his eyes staring straight into those of the viewer. Tucked into his pants, almost like a pistol in a holster, is a metal spoon.



The photograph was taken in the Netherlands in 1945, at the height of what the Dutch people still refer to simply as "The Hunger Winter". With the western part of the country still occupied by the Nazis, the Dutch resistance government, based in London, had called for a railway strike with the aim of stopping German troop movements before a planned airborne invasion by Allied forces. In retaliation, the Germans cut off all food shipments to cities in the western Netherlands. The Allied invasion failed to liberate the country, and the winter of 1944-1945 turned out to be bitterly cold. With food supplies dwindling, rations were cut first to 1000 calories per day, then to 500. People resorted to eating grass and tulip bulbs just to stay alive. Over 20,000 people died of starvation before food shipments were restored in the spring.

As tragic as the Hunger Winter was for the Dutch people, some good did come from it. For medical scientists, the event became a unique 'social experiment': unlike most episodes of famine throughout human history, the Hunger Winter had a clearly defined beginning, end, and geographic boundary, and it occurred in a technologically advanced society that continued to keep thorough records before, during, and after the ordeal. Scientists knew exactly who suffered from famine and for precisely how long, and in the years that followed they were able to look at the long-term effects of famine, particularly on developing embryos. Researchers found that babies who had been conceived during the famine were born with a significantly higher incidence of neurological birth defects such as spina bifida, a condition in which a portion of the neural tube protrudes from between vertebrae which did not properly fuse together during fetal development. Later in life, members of this same cohort of 'famine babies' were more likely to be obese, and to suffer from schizophrenia.

These initial findings spurred interest in further research into the consequences of prenatal deprivation. In particular, carefully controlled studies later led to the recognition of the importance of the vitamin folate in ensuring proper neurological development in early-term fetuses.



folate

Folate - the conjugate base of folic acid - is an organic coenzyme: a helper molecule that binds in the active site of certain enzymes and plays a critical role in the biochemical reaction being catalyzed. Recall that we have seen coenzymes at work before: ***SAM***, ***ATP***, $NAD(P)^+$ and $NAD(P)H$, flavin and glutathione are all important coenzymes with which we are already familiar.

Because prenatal folate deficiency was found to be directly related to the incidence of spina bifida and other neural tube defects, health officials in the United States and many other countries changed their official guidelines to include a specific recommendation that women begin taking folate supplements as soon as they knew that they are pregnant, or better yet as soon as they begin trying to become pregnant. A number of studies conducted during the 1980s and early 1990s consistently showed that folate supplementation correlated with a 50-70% reduction in neural tube defects.

The molecular role of folate in prenatal neurological development is not understood in detail, but most researchers agree that it probably has a lot to do with DNA biosynthesis. Like ***S***-adenosyl methionine (***SAM***), folate functions in 1-carbon transfer reactions, including several critical steps in the nucleic acid biosynthesis pathways. The rapidly dividing cells of the developing brain of an early term fetus appear to be especially sensitive to folate deficiency in the mother's diet: insufficient folate leads to impaired DNA biosynthesis, which in turn leads to defects in brain development.

Folate also serves as a 1-carbon donor in the pathway by which ***SAM*** is regenerated after it donates a methyl group. You may recall from the introduction to chapter 8 that methylation of cytosine bases in DNA by ***SAM*** results in permanent changes to a individual's genome - this was the reason why the two 'identical' twin sisters in that introductory story turned out to be, as they grew older, not so identical after all. It is likely that the folate deprivation that afflicted expectant mothers during the Dutch Hunger Winter also caused epigenetic changes (in other words, changes in the extent of DNA methylation) in their developing fetuses, which decades later manifested in the form of an increased incidence of conditions such as obesity and schizophrenia. All the more reason, we now know, to make sure that women get plenty of folate in their diet early in the first trimester of pregnancy.

In this final chapter, we focus on the organic chemistry of folate, along with three other coenzymes: pyridoxal phosphate, thiamine diphosphate, and lipoamide. Humans can synthesize lipoamide, but we depend on dietary sources for the other three: pyridoxal phosphate is a form of vitamin B6, and thiamine diphosphate is a form of vitamin B1. In a mechanistic sense, there is really nothing new in this chapter. All of the reaction mechanism types that we will see are already familiar to us, ranging from nucleophilic substitutions (chapter 8) to disulfide exchanges (chapter 15). We will soon see, however, how each coenzyme plays its own specific and crucial role in assisting enzymes with the catalysis of key reactions of metabolism. We will begin with pyridoxal phosphate and its various roles in amino acid metabolism.

Additional reading:

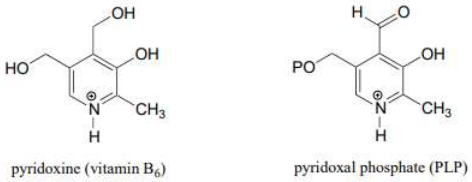
http://www.naturalhistorymag.com/fea...na-epigenetics

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10.2: Pyridoxal Phosphate (Vitamin B6)

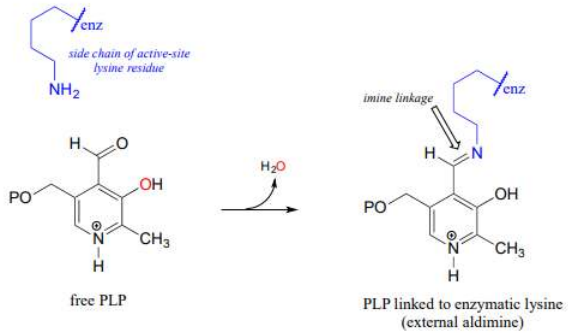
The coenzyme pyridoxal phosphate (commonly abbreviated PLP) is the active form of vitamin B_6, or pyridoxine.



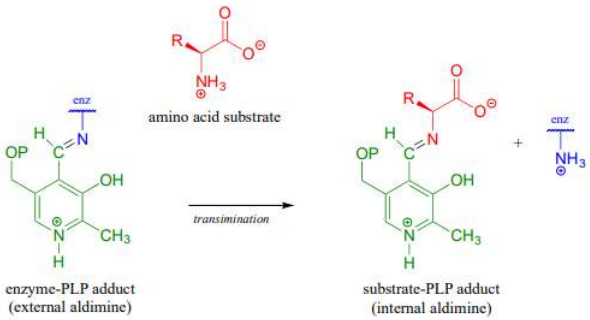
PLP is required for over 100 different reactions in human metabolism, primarily in the various amino acid biosynthetic and degradation pathways. The essential function of PLP is to act as an 'electron sink', stabilizing a negative formal charge that develops on key reaction intermediates. Some of reactions will be familiar to you from chapter 12 and 13 we will see examples, for instance, of -dependent α-carbon racemization, as well as aldol- and Claisen-type reactions. Other reactions will be less familiar: for example, the participation of allows for decarboxylation of amino acids, a chemical step which would be highly unlikely without the coenzyme, and PLP is also required for a very important class of biochemical transformation called 'transamination', in which the amino group of an amino acid is transferred to an acceptor molecule. Before we dive into the reactions themselves, though, we need to begin by looking at a key preliminary step that is common to all of the PLP reactions we will see in this section.

PLP in the active site: the imine linkage

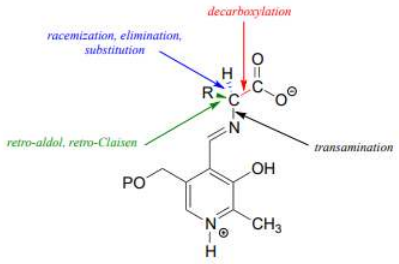
The common catalytic cycle of a PLP-dependent enzyme begins and ends with the coenzyme covalently linked to the enzyme's active site through an imine linkage between the aldehyde carbon of PLP and the amine group of a lysine residue (see section 10.5 to review the mechanism for imine formation). For a PLP-dependent enzyme to become active, a PLP molecule must first enter the active site of an enzyme and form an imine link to the lysine. This state is often referred to as an **internal aldimine**.



The first step of virtually all PLP-dependent reactions is transimination (section 10.5), as the amino group on the amino acid substrate displaces the amino group of the enzymatic lysine. This state - where the coenzyme is covalently linked to the substrate or product of the reaction - is often referred to as an **external aldimine**.



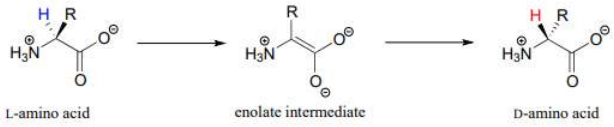
With the preliminary transimination accomplished, the real PLP chemistry is ready to start. The versatility of PLP in terms of its ability to assist with a wide variety of reaction types is illustrated by the figure below, showing how, depending upon the reaction/enzyme in question, PLP can assist in the cleavage of any one of the four bonds to the α-carbon of the amino acid substrate.



Let's look first at the reaction catalyzed by PLP-dependent alanine racemase. (EC 5.1.1.1).

PLP-dependent amino acid racemization

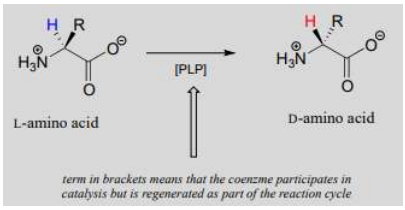
In section 12.2 we saw an example of a PLP-independent amino acid racemization reaction, in which the negatively-charged intermediate was simply the enolate form of a carboxylaten:



Many other amino acid racemase reactions, however, require the participation of PLP.

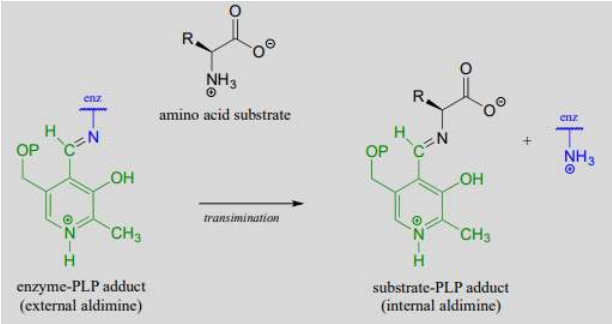
Like all other PLP-dependent reactions that we will see in this section, PLP-dependent amino acid racemization begins with a preliminary step in which the substrate becomes attached to the coenzyme through a transimination. Once it is linked to PLP in the active site, the a-proton of an amino acid substrate is abstracted by an active site base (step 1 below). The negative charge on the carbanion intermediate can, of course, be delocalized to the carboxylate group. The PLP coenzyme, however, provides an expanded network of conjugated π-bonds over which the electron density can be delocalized all the way down to the PLP nitrogen. This is what we mean when we say that the job of PLP is to act as an 'electron sink': the coenzyme is very efficient at absorbing, or delocalizing, the excess electron density on the deprotonated α-carbon of the reaction intermediate. PLP is helping the enzyme to increase the acidity of the α-hydrogen by stabilizing the conjugate base. A PLP-stabilized carbanion intermediate is commonly referred to as a quinonoid intermediate. Note that in the overall reaction equation below, PLP appears below the reaction arrow in brackets, indicating that it participates in the mechanism but is regenerated as part of the reaction cycle.

PLP-dependent amino acid racemization:

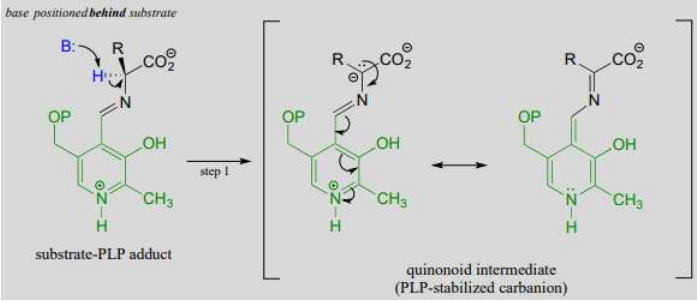


Mechanism:

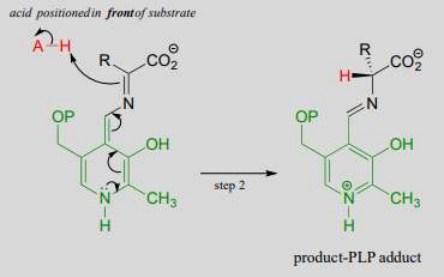
Preliminary step - transimination



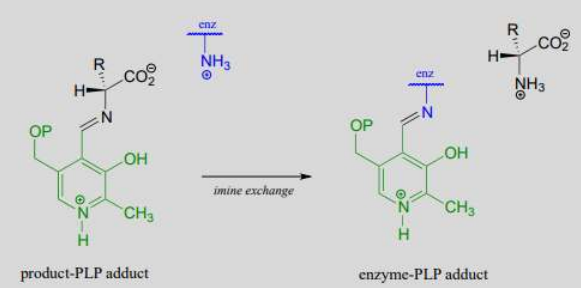
First step - deprotonation:



Second step - reprotonation from the opposite side:



Final step - transimination:



Just as in the PLP-independent racemase reactions, reprotonation occurs on the opposite side of the substrate (step 2), leading to the ***D***-amino acid product.

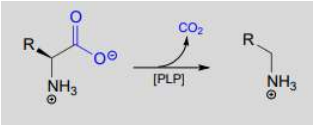
All that remains is the final imine exchange which frees the ***D***-amino acid product and re-attaches the coenzyme to the enzymatic lysine side-chain, ready to begin another catalytic cycle.

To simplify matters, from here on we will not include the preliminary and final transimination steps in our PLP reaction figures - we will only show mechanistic steps that occur while the substrate is attached to the coenzyme (the internal aldimine forms).

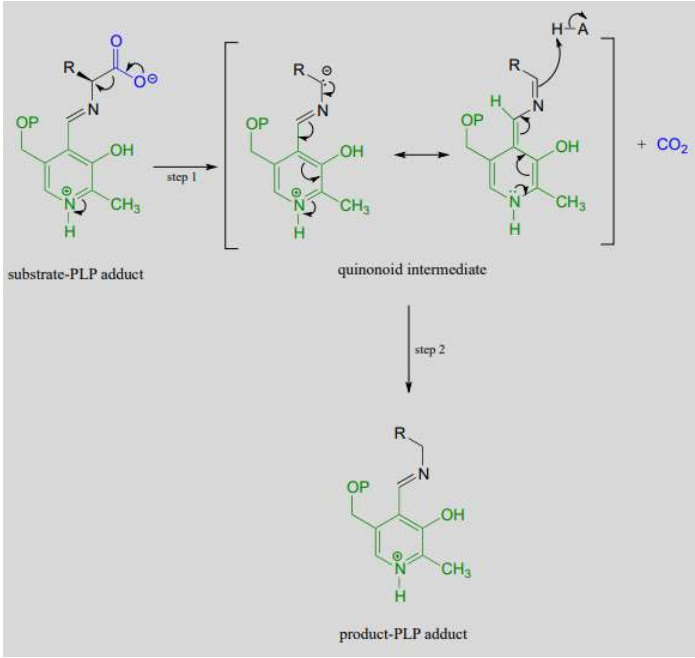
PLP-dependent decarboxylation

In the amino acid racemase reaction above, PLP assisted in breaking the α-carbon to α-proton bond of the amino acid. Other PLP-dependent enzymes can catalyze the breaking of the bond between the α-carbon and the carboxylate carbon by stabilizing the resulting carbanion intermediate: these are simply decarboxylation reactions.

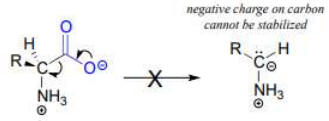
PLP-depended amino acid decarboxylation:



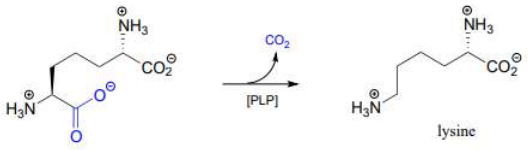
Mechanism:



Notice something very important here: while in racemization reactions the assistance of PLP can be seen as 'optional' (in the sense that some racemase enzyme use PLP and others do not), the coenzyme is essential for amino acid decarboxylation steps. Without PLP, there is no way to stabilize the carbanion intermediate, and decarboxylation is not a chemically reasonable step.



One example of a PLP-facilitated decarboxylation reaction is the final step in the lysine biosynthesis pathway: (EC 4.1.1.20).



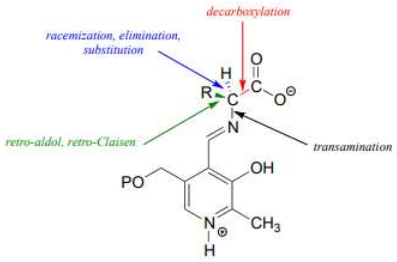
? Exercise 17.2.1

Draw mechanistic arrows for the carbon-carbon bond-breaking step of the PLP-dependent decarboxylation reaction above.

PLP-dependent retroaldol and retro-Claisen cleavage

(*It would be a good idea before reading this section to review aldol/retro-aldol and Claisen/retro-Claisen reaction mechanisms in sections 12.3 and 13.3, respectively*)

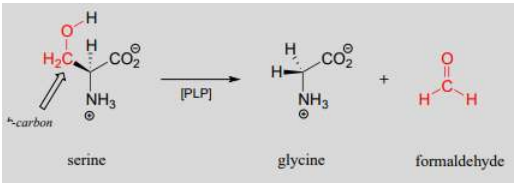
So far we have seen PLP playing a role in breaking the bond between the α-carbon and its α-proton (in the racemization reaction), and the bond between the α-carbon and carboxylate carbon (in the decarboxylation reaction).



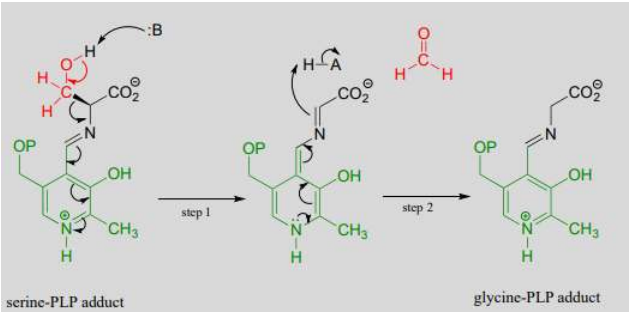
Other PLP-dependant enzymes catalyze cleavage of the bond between the α-carbon and the first carbon on the amino acid side chain, otherwise known as the β-carbon. In the serine degradation pathway, serine is first converted to glycine by a retro-aldol cleavage reaction. (). Although a reasonable mechanism could be proposed without the participation of PLP, this reaction in fact requires the coenzyme to assist in stabilization of the negative charge on the carbanion intermediate.

A PLP-dependent retro-aldol cleavage reaction

(serine hydroxymethyl transferase, EC 2.1.2.1)



Mechanism:

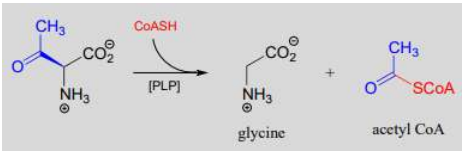


Note that, in this reaction just as in the racemase reaction described previously, the key intermediate is a PLP-stabilized carbanion, or quinonoid.

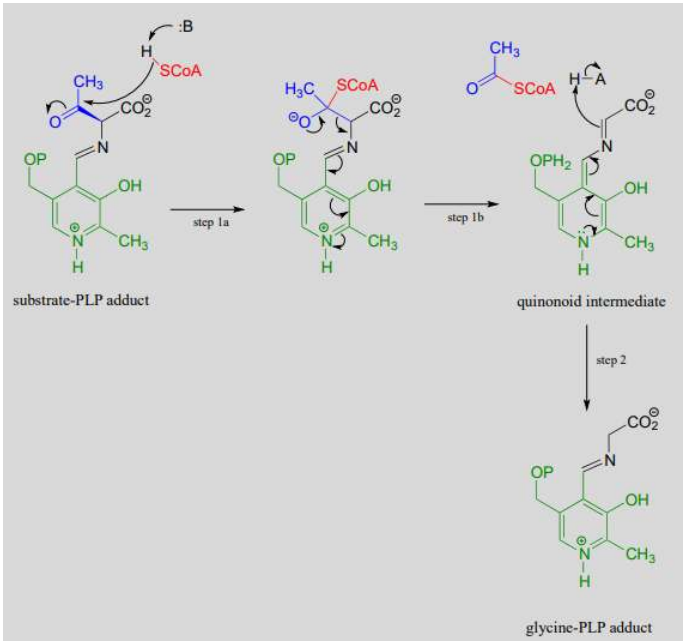
What happens to the (toxic!) formaldehyde produced in this reaction? We will see later in this chapter how the serine hydroxymethyltransferase enzyme goes on to use another coenzyme called tetrahydrofolate to prevent the formaldehyde from leaving the active site and causing damage to the cell.

PLP also assists in retro-Claisen cleavage reactions (section 13.3), such as this step in the degradation of threonine. (EC 2.3.1.29)

A PLP-dependent retro-Claisen reaction:



Mechanism:

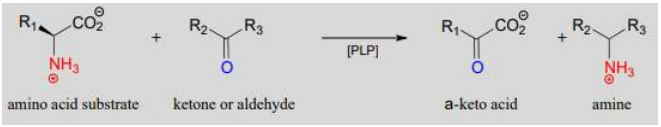


Notice how, like the retro-aldol reaction, the bond between the α-carbon and the β-carbon of the amino acid substrate is broken (in step 1b).

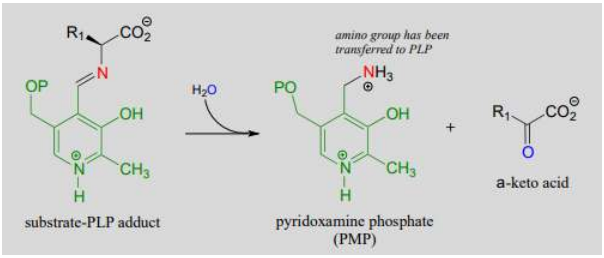
PLP-dependent transamination

One of the most important reaction types in amino acid metabolism is transamination, in which an amino group on a donor molecule (often an amino acid) is transferred to a ketone or aldehyde acceptor molecule.

A transamination reaction:

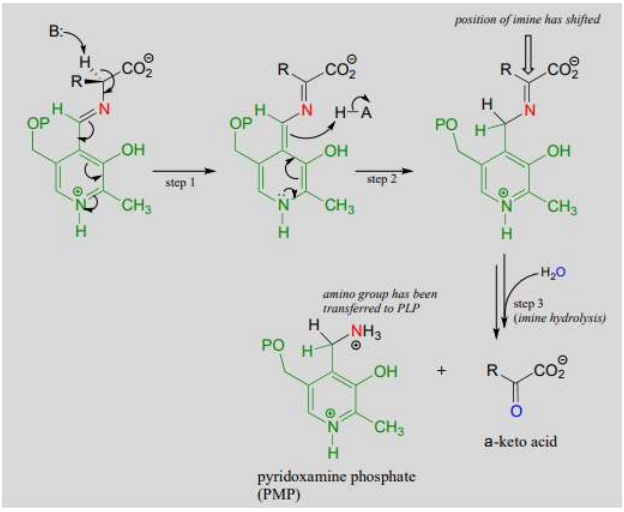


Transamination phase 1 (transfer of amino group from amino acid substrate to coenzyme)



Mechanism:

https://chem.libretexts.org/@go/page/291189

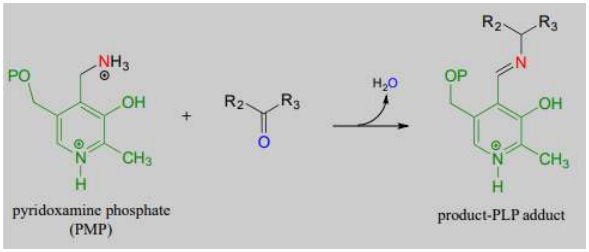


Once again, step 1 is abstraction of the α-proton from the PLP-substrate adduct. However, in a transaminase reaction this initial deprotonation step is immediately followed by reprotonation at what was originally the aldehyde carbon of PLP (step 2 above), which results in a new carbon-nitrogen double bond (in other words, an imine) between the α-carbon and the nitrogen atom of the original amino acid. The repositioned imine group is then hydrolyzed (step 3 above), breaking the carbon-nitrogen bond, transferring the amino group to the coenzyme, and releasing an α-keto acid.

The coenzyme, which now carries an amine group and is called pyridoxamine phospate (***PMP***), next transfers the amine group to α-ketoglutarate (to form glutamate) through a reversal of the whole process depicted above.

Transamination reaction, phase 2

(transfer of amino group from coenzyme to acceptor molecule)



Mechanism:

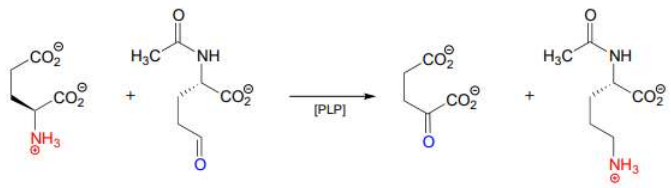
see exercise below

In a transamination reaction, the PLP coenzyme not only provides an electron sink, it also serves as a temporary 'parking place' for an amino group as it is transferred from donor to acceptor.

? Exercise 17.2.2

Show a complete, step-by-step mechanism for 'phase 2' of the transamination reaction above.

Here is an example of a transamination reaction in the arginine biosynthesis pathway: EC 2.6.1.11

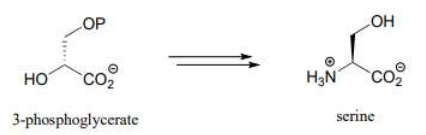


Exercise 17.2.3

a. Draw arrows for the first mechanistic step of 'phase 2' of the above transaminase reaction.
b. Which carbon on the substrate side of the reaction will eventually become the α-carbon of arginine?

Exercise 17.2.4

Propose a pathway, with three enzymatic steps, for the biosynthesis of serine from 3-phosphoglycerate. Include a generalized ('-ase') enzyme name for each step. Glutamate plays a role in the process as an amino group donor.



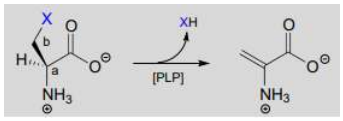
PLP-dependent *beta*-elimination and β-substitution

(*Before starting this section, it would be a good idea to review* ***E1cb*** *β-elimination and conjugate addition reaction mechanisms in section 13.4*)

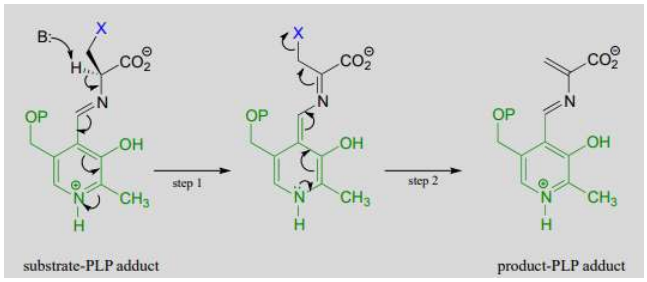
By now it should be pretty apparent that PLP is a pretty versatile coenzyme! Two more reaction types in the PLP toolbox are β-elimination and β-substitution on amino acid substrates.

In a PLP-dependent β-elimination reaction, the coenzyme simply helps to stabilize the carbanion intermediate of the ***E1cb*** mechanism:

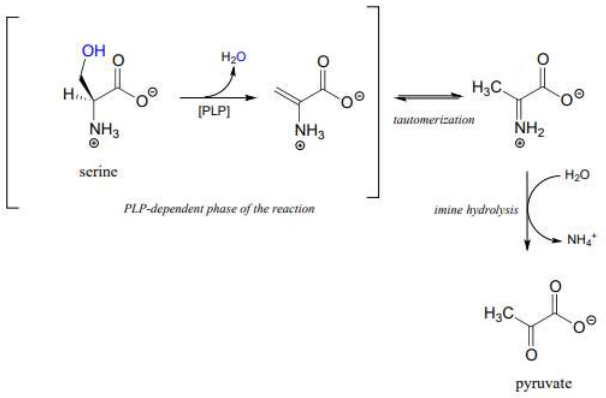
PLP-dependent β-elimination reaction



Mechanism:

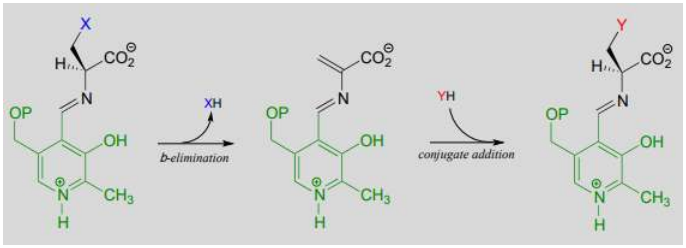


Serine dehydratase (EC 4.2.1.13) catalyzes a PLP-dependent β-elimination in the first step of the serine degradation pathway:

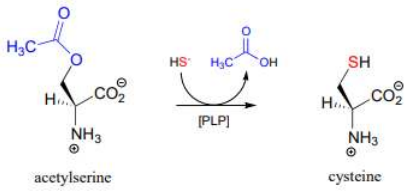


A β-substitution reaction is simply ***E1cb*** elimination followed directly by the reverse reaction (conjugate addition) with a different nucleophile (Y in the figure below):

A β-substitution reaction:



In many bacteria, the synthesis of cysteine from serine includes a PLP-dependent β-substitution step (EC 2.5.1.47).



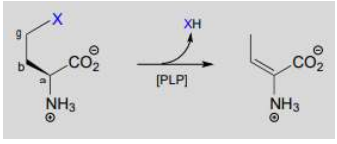
? Exercise 17.2.5

Draw a mechanism for the conjugate addition phase of the reaction above (end with the cysteine-PLP adduct).

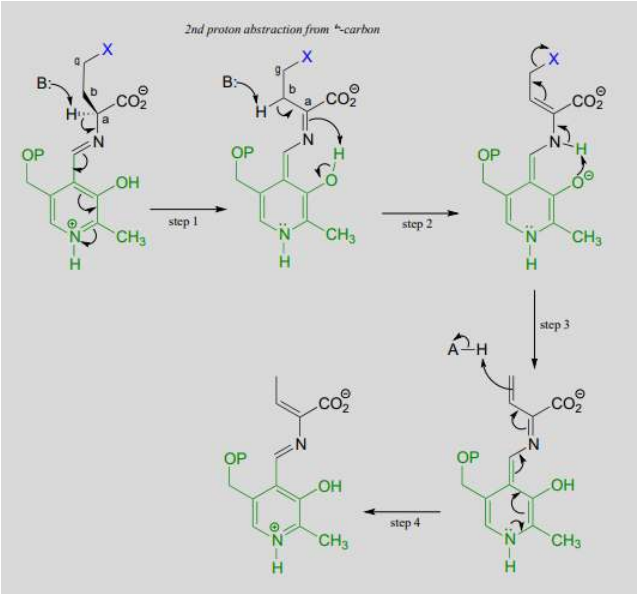
PLP-dependent γ-elimination and γ-substitution reactions

The electron sink capability of PLP allows some enzymes to catalyze eliminations at the γ-carbon of some amino acid side chains, rather than at the β-carbon. secret to understanding the mechanism of a γ-elimination is that PLP essentially acts as an electron sink twice - it absorbs the excess electron density from not one but two proton abstractions.

γ-elimination

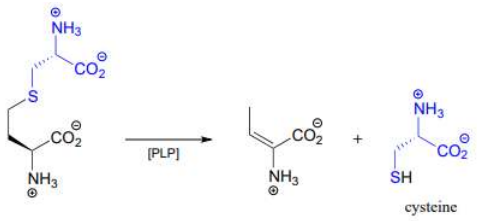


Mechanism:



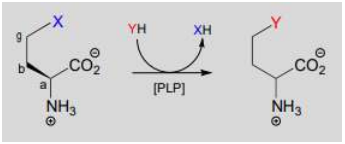
In a familiar first step, the α-proton of the amino acid is abstracted by an enzymatic base, and the electron density is absorbed by PLP. Next comes the new part - before anything happens to the electron density from the first proton abstraction, a second proton, this time from the β-carbon on the side chain, is abstracted, forming an enamine intermediate (step 2). The phenolic proton on the pyridoxal ring of PLP donates a proton to the nitrogen. step 3, the leaving is expelled and a new π-bond forms between the β and γ carbons (step 3). This π-bond is short-lived, however, as the electron density from the first proton abstraction, which has been 'stored' in PLP all this time, flows back up to protonate the α-carbon (step 4), leaving the γ-elimination product linked to PLP via the usual imine connection.

An example is the cystathionine γ-lyase reaction in the methionine degradation pathway (EC 4.4.1.1):

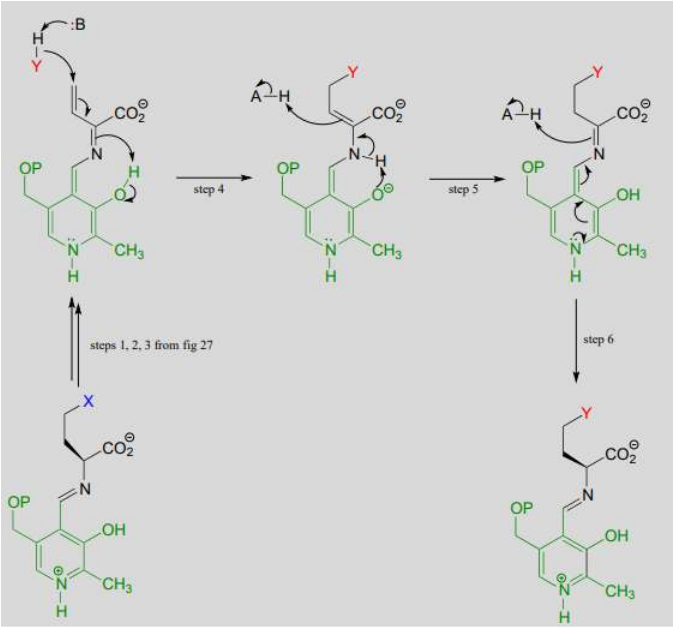


A related reaction is PLP-dependent γ-substitution, which again is simply γ-elimination of a leaving group (X in the figure below) followed directly by the reverse process (a γ-addition) with a different nucleophile ('Nu' in the figure below).

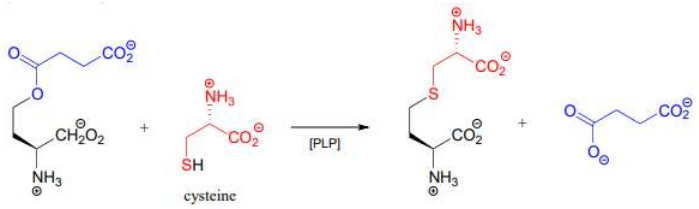
PLP-dependent γ-substitution:



Mechanism:



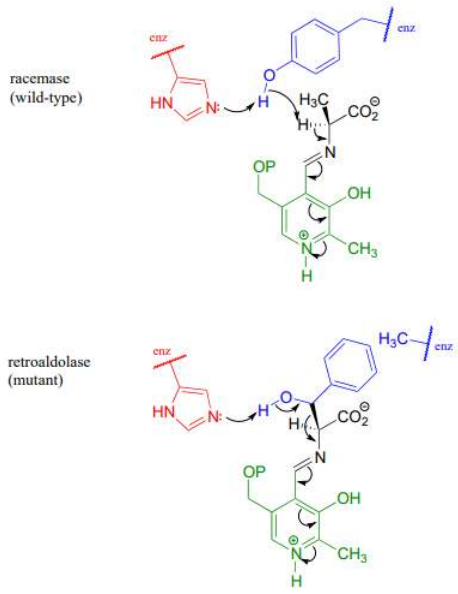
Below is a PLP-dependent γ-substitution reaction in the methionine degradation pathway (EC 4.2.1.22):



Racemase to aldolase: altering the course of a PLP reaction

We have seen how PLP-dependent enzymes catalyze a variety of reaction types - racemization, retroaldol/retro-Claisen cleavage, transaminination, elimination, and substitution - which, despite their apparent diversity, are all characterized by formation of a critical carbanion intermediate which is stabilized by the 'electron sink' property of the PLP coenzyme. Given this common mechanistic feature, it would be reasonable to propose that the active site architecture of these enzymes might also be quite close. This idea was nicely illustrated by an experiment in which researchers found that changing a single active site amino acid of PLP-dependent alanine racemase was sufficient to turn it into a retro-aldolase (J. Am. Chem. Soc. 2003, 125, 10158).

In the 'wild-type' (natural) alanine racemase reaction, an active site histidine (red in figure below) deprotonates a neighboring tyrosine residue (blue), which in turn acts as the catalytic base abstracting the α-proton of the substrate. When researchers changed the tyrosine to an alanine (using a technique called 'site-directed mutagenesis'), and substituted β-hydroxytyrosine for the alanine substrate, the new 'mutant' enzyme catalyzed a retro-aldol reaction.

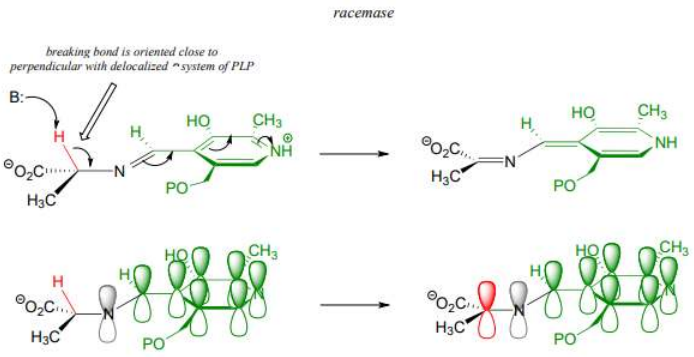


Notice what has happened here: the basic histidine, with no tyrosine to deprotonate because of the mutation, is instead positioned to abstract a proton from the β-hydroxyl group of the new substrate, setting up a retroaldol cleavage. That was all it took to change a racemase into a retroaldolase, because the necessary PLP electron sink system was all left in place. The researchers predicted correctly that the phenyl ring of β-hydroxy tyrosine would fit nicely in the space left empty due to the tyrosine to alanine change in the mutant enzyme's structure

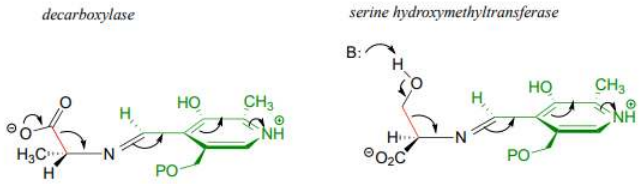
These results underline the close mechanistic relationship between two PLP-dependent reactions which, at first glance, appear to be quite different - and suggest that PLP-dependent racemases and aldolases may have evolved from a common 'ancestor' enzyme.

Stereoelectronic considerations of PLP-dependent reactions

Recall that all PLP-dependent reactions involve the cleavage of one of the bonds coming from the α-carbon of an amino acid substrate, with the coenzyme serving as an 'electron sink' to stabilize the intermediate that results. PLP-dependent enzymes accelerate this bond-breaking step by binding the substrate-PLP adduct in a conformation such that the bond being broken is close to perpendicular to the plane formed by the conjugated π system of PLP: this way, as the α-carbon transitions from sp^3 to sp^2 hybridization, the unhybridized π orbital is already oriented to overlap with the rest of the conjugated system. For example, in alanine racemase the first step is cleavage of the $C\alpha - H$ bond, so it must be that bond which is positioned near-perpendicular to the PLP plane:



Likewise, in an amino acid decarboxylase, the $C\alpha$-carboxylate bond is held near-perpendicular to the PLP plane, and in hydroxymethyltransferase, the $C\alpha - C\beta$ bond is in the perpendicular orientation:



These are all good examples of how enzymatic catalysis is achieved, in part, by the ability of the active site to bind the substrate molecule in a specific conformation which contributes to the lowering of the activation energy of a key reaction step.

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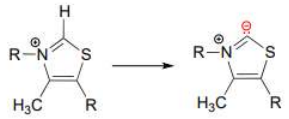
10.3: Thiamine Diphosphate (Vitamin B1)

Thiamine diphosphate (***ThDP***, sometimes also abbreviated ***TPP*** or ***ThPP***) is a coenzyme which, like PLP, acts as an electron sink to stabilize key carbanion intermediates. The most important part of the ***ThDP*** molecule from a catalytic standpoint is its thiazole ring.



thiamine diphosphate (ThDP)

The proton on the carbon between nitrogen and sulfur on the thiazole ring is weakly acidic, with a pK_a of about 18.

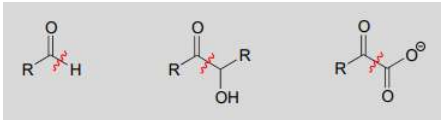


The reason for its acidity lies partly in the ability of the neighboring sulfur atom to accept, in its open ***d***-orbitals, some of the excess electron density of the conjugate base. Another reason is that the positive charge on the nitrogen helps to stabilize the negative charge on the conjugate base. The deprotonated thiazole is called an ylide, which is a general term for a species with adjacent positively and negatively charged atoms.

The negatively charged carbon on the ylide form of ***ThDP*** is nucleophilic, and as we shall soon see, the first step of most ***TPP***-dependent reactions is nucleophilic attack of the ylide carbon on a carbonyl group of the reaction substrate.

ThDP plays a key role in a variety of reaction types, but the common theme in all ***ThDP***-dependent reactions is cleavage of a bond adjacent to the carbonyl carbon of a ketone or aldehyde.

Thiamine diphosphate assists in breaking bonds next to a ketone or aldehyde:



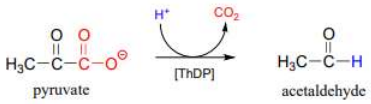
Consider this hypothetical decarboxylation step:



Hopefully, you can quickly recognize that this is not a chemically reasonable step, because the intermediate species which results from decarboxylation has a negative charge localized on the ketone carbon - a very unstable, unlikely intermediate indeed.

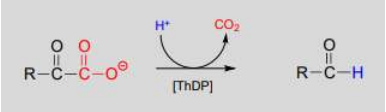
(Recall from section 13.1 that decarboxylation steps usually result in intermediates in which the negative formal charge is delocalized to an oxygen or nitrogen - in other words, enolates or enamines.)

Now consider, however, a reaction going on in your cells right now, catalyzed by the enzyme pyruvate decarboxylase (EC 4.1.1.1):

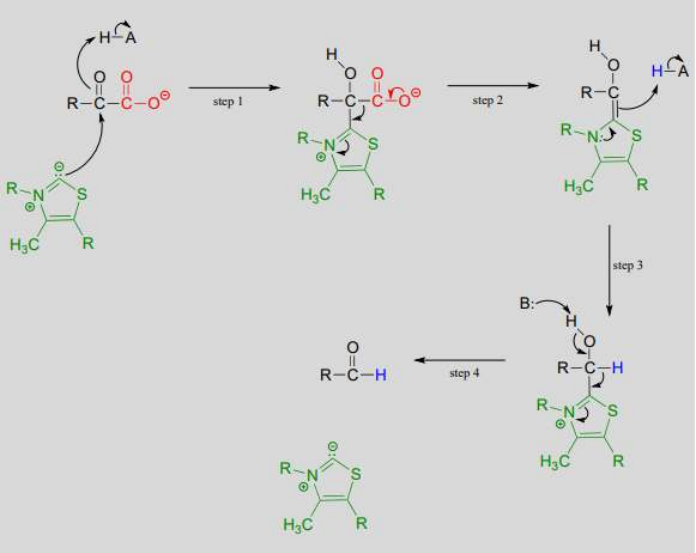


Somehow, the enzyme manages to accomplish this 'impossible' decarboxylation. How does this happen? Here is where the thiamine diphosphate coenzyme comes in.

A ***ThDP***-dependent decarboxylation reaction (pyruvate decarboxylase):



Mechanism:



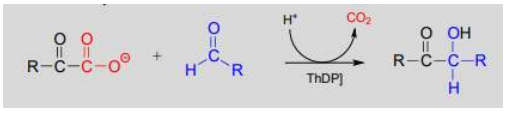
Upon binding to the enzyme's active site, ***ThDP*** quickly loses a proton. The nucleophilic ylide carbon then adds to the carbonyl carbon of pyruvate.

Look carefully at the intermediate that results from step 1 in the mechanism above. The thiazole ring of ***ThDP***, once it has added to the carbonyl of pyruvate, provides an 'electron sink' to absorb the electrons from decarboxylation (step 2). Note which bond is breaking in step 2 - as was mentioned earlier, the common function of ***ThDP*** is to make possible the cleavage of a bond to a carbonyl carbon.

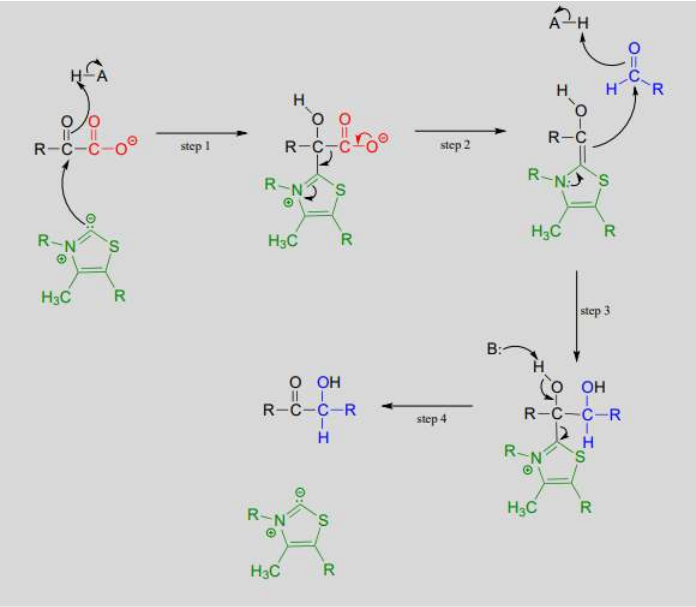
In step 3, the electrons from decarboxylation flow back to abstract a proton from an acidic group in the active site. All that remains is for the product to break free of thiamine in step 4.

Thiamine can also assist in decarboxylation-addition reactions:

ThDP-dependent decarboxylation-addition:

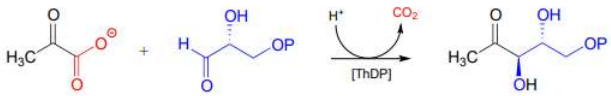


Mechanism:



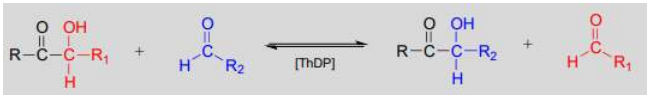
Here, the electron-rich intermediate formed from the decarboxylation step (step 2) simply goes on to act as a nucleophile rather than as a base, adding to the carbonyl group of an aldehyde or ketone (step 3). As before, the product breaks free of ***ThDP*** in step 4.

An example is the first step in the biosynthetic pathway for isoprenoid compounds in bacteria:

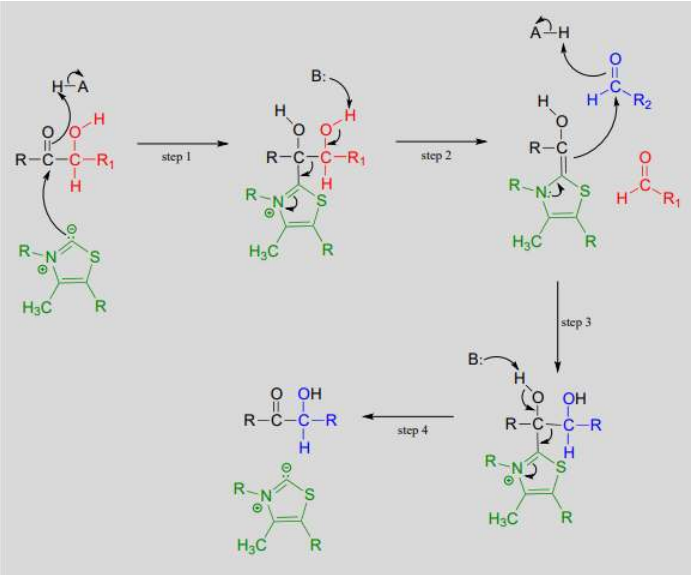


Transketolase, a ***ThDP***-dependent enzyme in the pentose phosphate pathway of sugar metabolism, catalyzes a carbon-carbon bond break step, followed by a carbon-carbon bond forming step. The substrates and products are at similar energy levels, so the reaction is completely reversible.

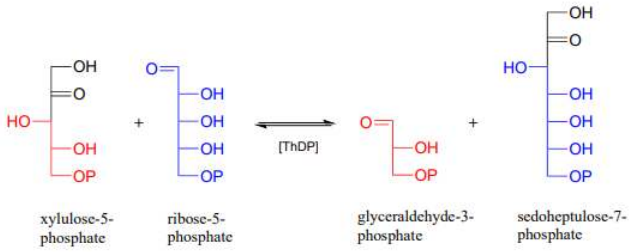
Transketolase reaction:



Mechanism:



Below is an actual example of a transketolase-catalyzed transformation from the pentose phosphate pathway (shown in Fischer projections, as is common for sugar structures).

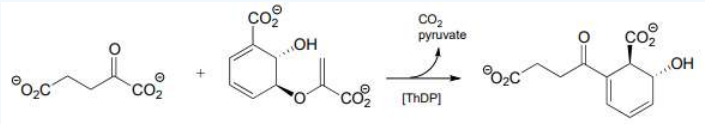


? Exercise 17.3.1

As was mentioned above, the transketolase reaction is highly reversible. Do you think the same can be said for the decarboxylation and decarboxylation-addition reactions we saw in this section? Why or why not?

? Exercise 17.3.2

(Challenging!) Propose a mechanism for the reaction below.

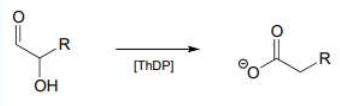


Hint

This is a *ThDP*-facilitated decarboxylation/ Michael addition, followed by \(E1cb\) elimination of pyruvate. A Michael addition is the name for a conjugate addition with a carbon nucleophile. (J. Mol. Biol. 2010, 401, 253).

Exercise 17.3.3

Propose a mechanism for the reaction below.



Hint

The mechanism can be described as a $ThDP$-facilitated dehydration step, followed by a tautomerization step, followed by a hydrolytic expulsion of $ThDP$ (a different kind of $ThDP$ expulsion from what we have seen so far!)

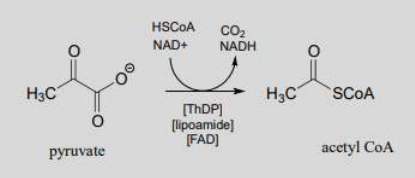
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10.4: Thiamine Diphosphate, Lipoamide and the Pyruvate Dehydrogenase Reaction

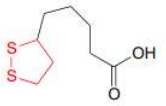
The enzyme pyruvate dehydrogenase is one of the most central of all the enzymes of central metabolism: by converting pyruvate to acetyl-***CoA***, it links glycolysis (where glucose is broken down into pyruvate) to the citric acid cycle, into which carbons enter in the form of acetyl-***CoA***. Five ceonzymes are involved: coenzyme A, nicotinamide, thiamine diphosphate, ***FAD***, and finally lipoamide, one which is new to us at this point.

Reaction catalyzed by pyruvate dehydrogenase:

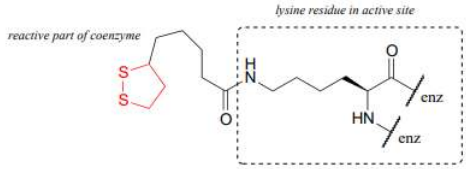


You will learn more about the structure and metabolic role of this complex and remarkable enzyme in a biochemistry course. Here, we will focus on the multi-step organic reaction it catalyzes, which we are at long last equipped to understand.

Looking at the reaction, you should recognize that, first of all, the pyruvate substrate is being oxidized - it starts out as a ketone, and ends up as a thioester, losing carbon dioxide in the process. Ultimately, the oxidizing agent in this reaction is ***NAD***$^+$, but the reduction of ***NAD***$^+$ is linked to the oxidative decarboxylation of pyruvate by ***FAD*** and a disulfide-containing coenzyme called lipoamide, which is lipoic acid attached by an amide linkage to a lysine residue on the enzyme.



lipoic acid

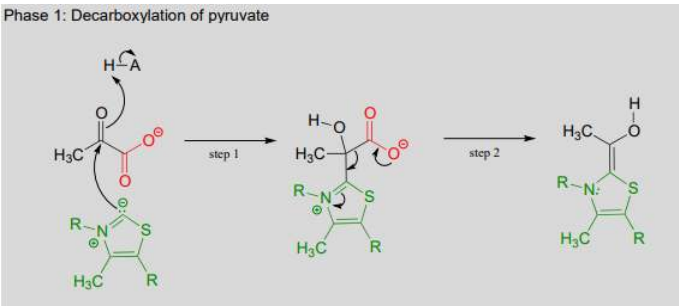


lipoamide
(linked to lysine residue in enzyme)

The second thing to notice is that, because the reaction involves breaking the bond between the ketone carbon and an adjacent carbon, thiamine diphosphate (***ThDP***) coenzyme is required. In fact, the first phase of the reaction (steps 1 and 2 below) is identical to that of pyruvate decarboxylase, an enzyme we discussed a few pages ago.

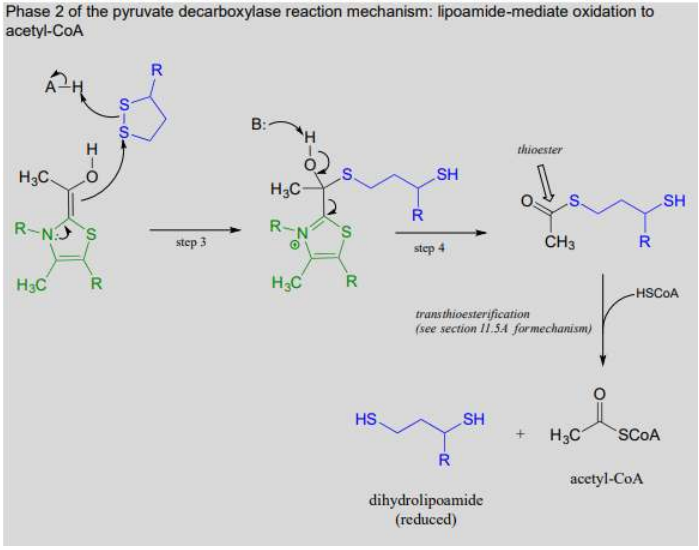
The pyruvate decarboxylase reaction mechanism

Phase 1: Decarboxylation of pyruvate



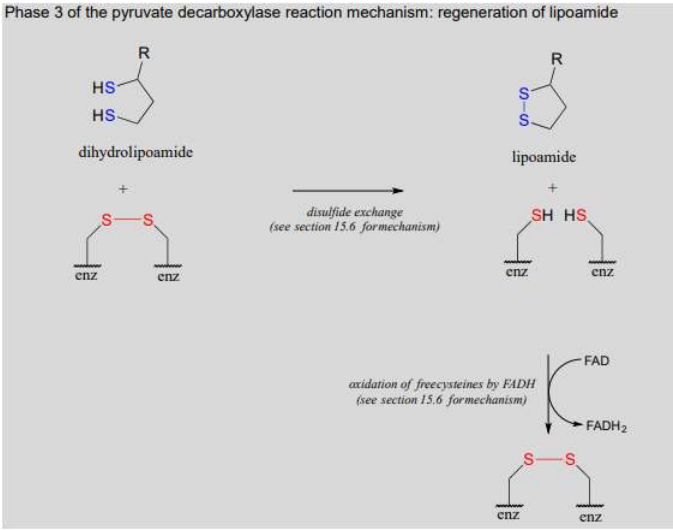
The ***ThDP***-stabilized carbanion then acts as a nucleophile, cleaving the disulfide bridge of lipoamide (step 3 below). It is in this step that oxidation of the substrate is actually occurring. After the resulting thioester product is released from ***ThDP*** (step 4 below), it undergoes transesterification form acetyl-***CoA***, the product of the reaction.

Phase 2 of the pyruvate decarboxylase reaction mechanism: lipoamide-mediate oxidation to acetyl-***CoA***



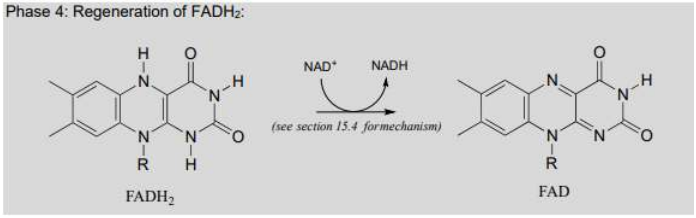
We are not done yet! In order for the catalytic cycle to be complete, the reduced dihydrolipoamide must be regenerated back to its oxidized state through disulfide exchange with a disulfide bond on the enzyme. The pair of enzymatic cysteines is then oxidized back to disulfide form by an ***FAD***-dependent reaction.

Phase 3 of the pyruvate decarboxylase reaction mechanism: regeneration of lipoamide



Finally, ***FAD*** is regenerated with concurrent reduction of NAD^+:

Phase 4: Regeneration of $FADH_2$:

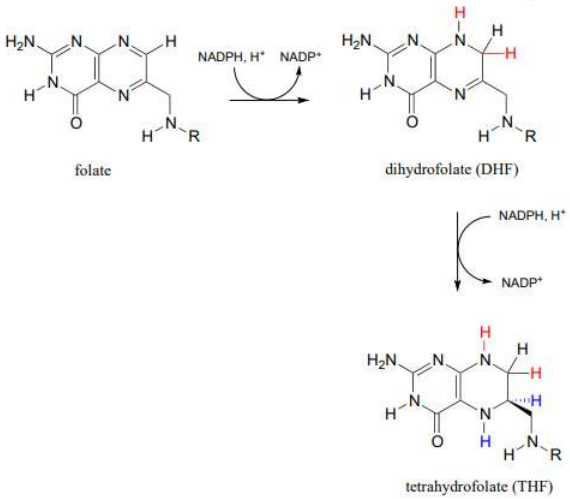


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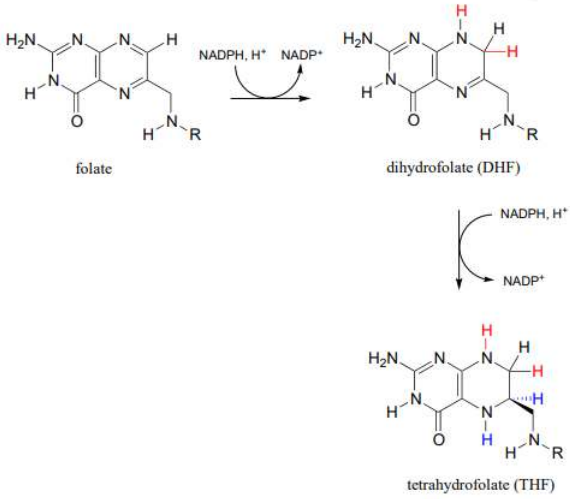
10.5: Folate

Folate, or vitamin B_9, is essential for a variety of important reactions in nucleotide and amino acid metabolism. The reactive part of folate is the **pterin** ring system, shown in red below. The conventional atom numbering system for folate is also indicated.

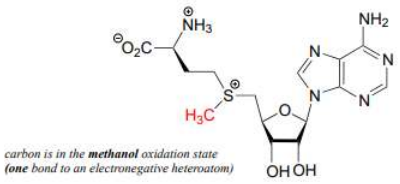


Active forms of folate

Folate is active as a coenzyme in its reduced forms, dihydrofolate and tetrahydrofolate, which are formed by $NADPH$–dependent imine hydrogenation steps (section 15.3).

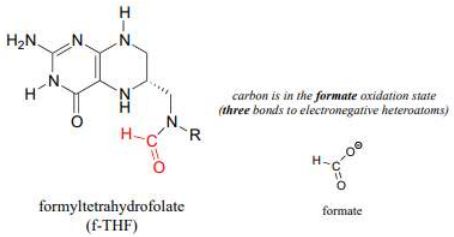


The metabolic role of folate is to serve as a donor or acceptor in single-carbon transfer reactions. How is folate different from S-adenosylmethionine (SAM, section 8.8) which also serves as a single-carbon donor? Recall that SAM donates a single carbon in the form of a methyl group: essentially, the single carbon of SAM is in the methanol (CH_3OH) oxidation state, because it has only one bond to a heteroatom (specifically, to sulfur). (Refer to section 15.1 for a review of oxidation states).



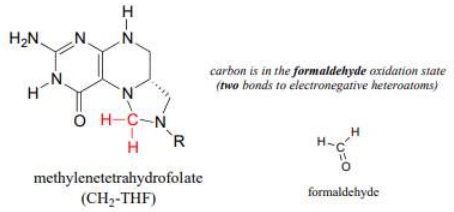
S-adenosylmethionine
(SAM)

Folate coenzymes, on the other hand, can carry a single carbon in the formaldehyde and formate oxidation states, in addition to the methanol oxidation state. By 'formaldehyde' and 'formate' oxidation state, we mean that the carbon has two and three bonds to heteroatoms, respectively.



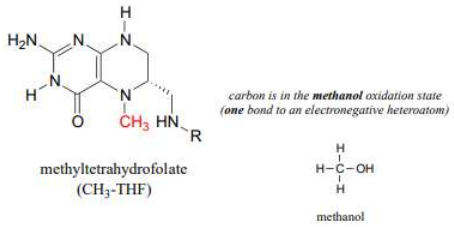
formyltetrahydrofolate
(f-THF)

formate



methylenetetrahydrofolate
(CH_2-THF)

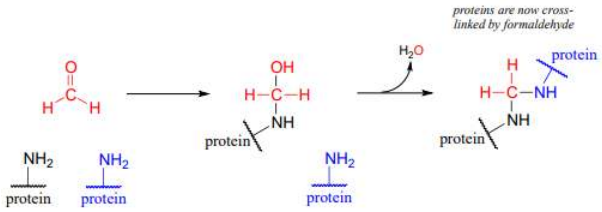
formaldehyde



methyltetrahydrofolate
(CH_3-THF)

methanol

Some key reactions in nucleic acid and amino acid metabolic pathways involve transfer of a single carbon in the formaldehyde or formate states. However, this could present problems. Formaldehyde by itself is very toxic: in particular, it tends to spontaneously form unwanted crosslinks between amine groups (eg. lysine side chains) in proteins.



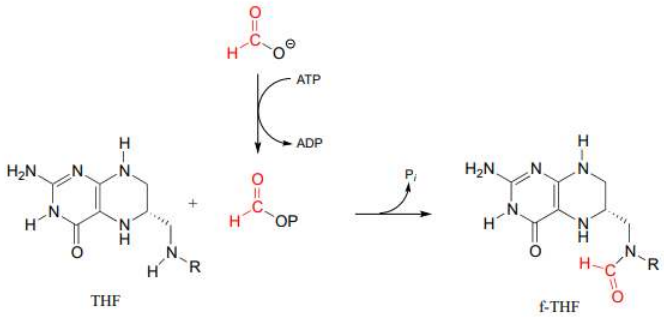
Free formaldehyde is too reactive, and would cause damage to a cell. The CH_2-THF coenzyme is stable in solution, but in the active site of certain enzymes it is reactive enough to serve as a formaldehyde donor, as we will see shortly.

Free formate, on the other hand, is a carboxylate, and we know from chapter 11 that carboxylates are not reactive in acyl substitution steps. Formate could be activated by phosphorylation, of course, but the resulting formyl phosphate would be too

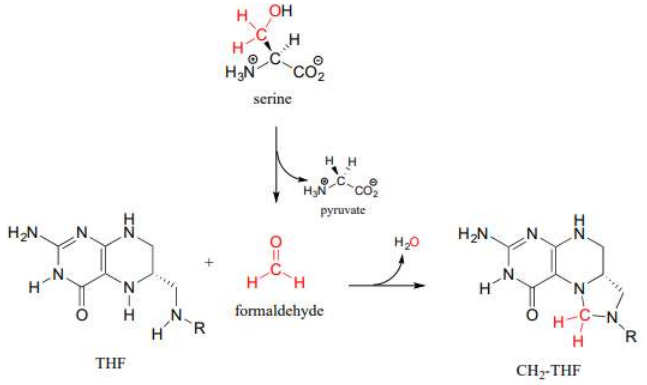
reactive in many enzyme active sites. A 'happy medium' has been found in which carbons in the formate oxidation state are carried by folate in the form of $f-THF$: once again, the carbon donor is stable in solution, but sufficiently reactive in certain enzyme active sites to accomplish controlled transfer of a formate group.

Formation of formyl-THF and methylene-THF

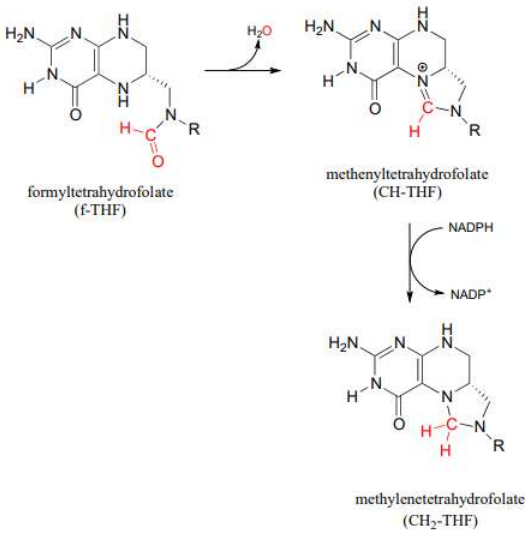
Formyltetrahydrofolate ($f-THF$) is formed from THF and a formate molecule which has been activated by phosphorylation (formyl phosphate, as stated in the paragraph above, is a high reactive intermediate, but is held inside the enzyme's active site for immediate reaction with the incoming amine group of THF).



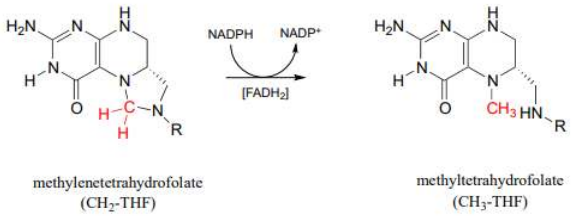
There are two main metabolic routes to CH_2-THF. One route is just the last step of the serine hydroxymethyltransferase reaction we have already seen in section 17.1: the formaldehyde formed in the PLP-dependent phase of the reaction stays in the active site, and the oxygen is displaced by successive attacks from the amine nucleophiles at the 5 and 10 positions of THF. (Notice the similarity to the formaldehyde-protein crosslinking reaction shown earlier in this section.)



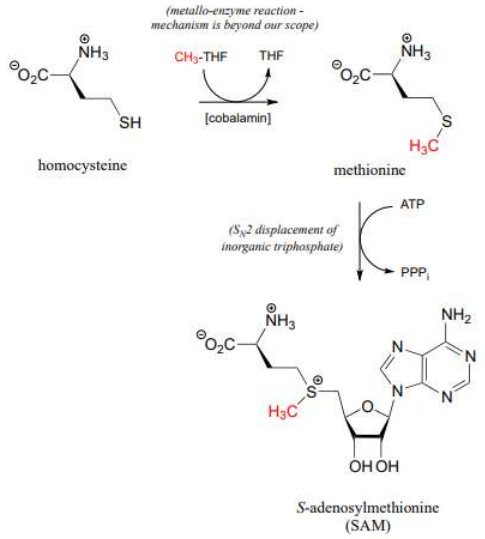
In the second route, $f-THF$ is dehydrated, then the resulting methenyl-THF intermediate is reduced by $NADPH$.



Methylene-THF (CH_2-THF) is reduced to methyl-THF (CH_3-THF) in a flavin-dependent reaction. *Biochemistry 2001, 40, 6216*



Recall from the introduction to this chapter that babies who were in the womb during the Dutch Hunger Winter faced a higher risk, when they reached adulthood, of conditions such as obesity and schizophrenia, most likely caused by disruptions in the S-adenosylmethionine-dependent methylation of their DNA, which was in turn caused by their mothers not getting enough folate. CH_3-THF is the source of the methyl group in methionine, which ultimately becomes the methyl group in SAM. The methylation of homocysteine to methionine (below) involves a cobalt-containing coenzyme called cobalamin, but the mechanism for this reaction is beyond the scope of our discussion. The second reaction below (formation of SAM) is simply an SN_2 displacement of the inorganic triphosphate leaving group on ATP by the nucleophilic sulfur in methionine.

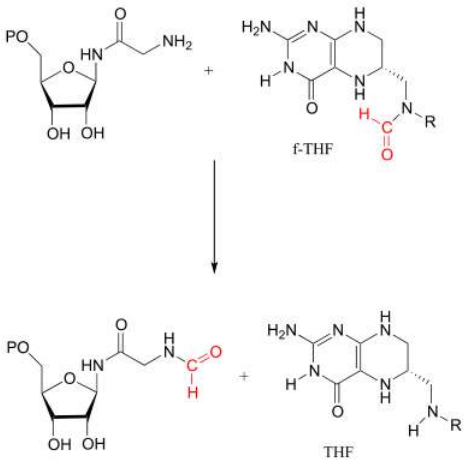


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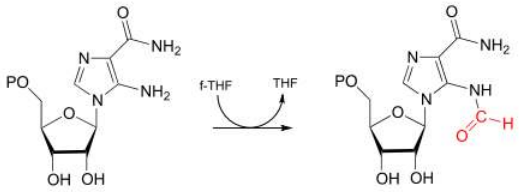
Single-carbon transfer with formyl-THF

There are two important $f-THF$-dependent formylation steps in the biosynthetic pathways for purine nucleophiles. Both are simply transamidation reactions: in other words, conversion by the nucleophilic acyl substitution mechanism (chapter 11) of one amide to another.

Glycinamide ribonucleotide transformylase reaction:



Aminoimidazole carboxamide transformylase reaction:

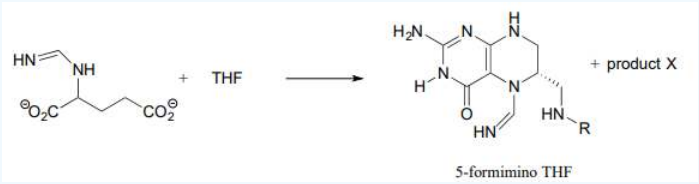


? Exercise 17.5.1

Which of the two transformylase reactions above would you expect to be more kinetically favorable? Explain your reasoning.

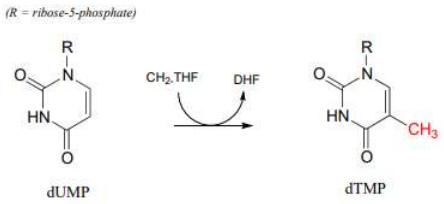
? Exercise 17.5.2

Predict the structure of 'product X' in the reaction below, from the histidine degradation pathway.

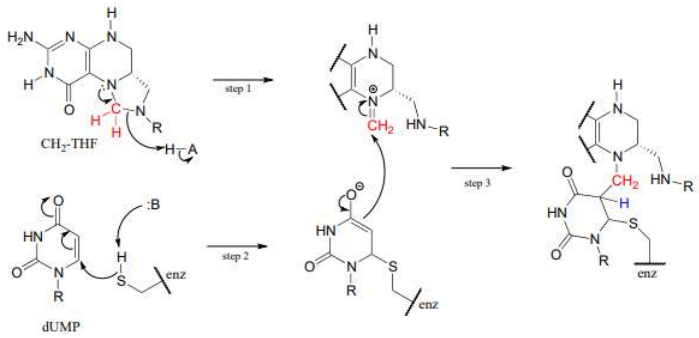


Single-carbon transfer with methylene-THF

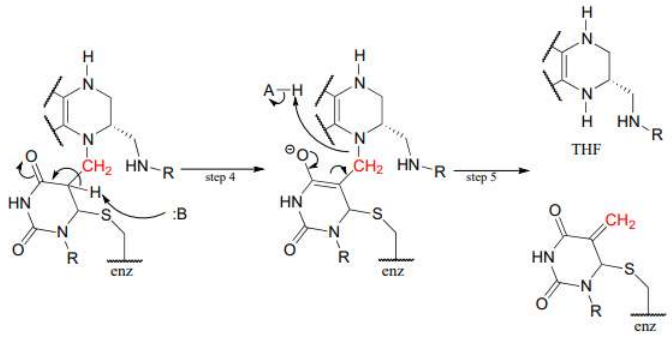
CH_2-THF serves as a single-carbon donor in a somewhat complicated reaction in the biosynthesis of the DNA monomer doexythymidine monophosphate ($dTMP$).



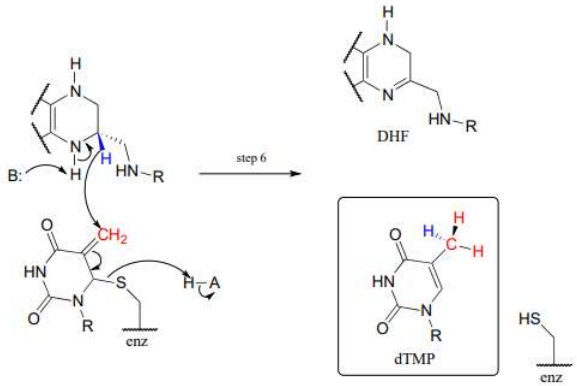
The reactions begins with the five-membered ring of $CH_2 - THF$ breaking apart to create an imine intermediate (step 1 below). The imine becomes an electrophile in a conjugate addition step (section 13.4, steps 2-3 below). Note that after step 1, the second ring in the pterin system of the coenzyme is abbreviated for clarity.



Next, tetrahydrofolate is eliminated in an $E1cb$ elimination mechanism (steps 4 and 5). Notice that this is where the single carbon is transferred from methyl-THF to dUMP.



The final step in the mechanism is where it gets really interesting: a hydride ion is transferred from the tetrahydrofolate coenzyme to the methylene (CH_2) group on the deoxynucleotide substrate. Essentially, this is a conjugated SN_2 step with hydride as the nucleophile and the active site cysteine as the leaving group.



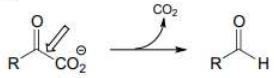
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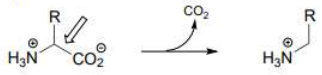
10.E: The Oganic Chemistry of Vitamins (Exercises)

P17.1: Here is a chance to test your ability to recognize reactions catalyzed by enzymes using three coenzymes - thiamine diphosphate, pyridoxal phosphate, and folate - that we have studied in this chapter. For each generalized reaction, look carefully at the transformation that is taking place, and decide which of the three coenzymes is likely to be required. Then, draw the single mechanistic step by which the bond identified by an arrow is broken or formed. In the cases where a double bond is indicated, show the mechanistic step in which the s bond is formed. In each case, your drawing should include the structure of the reactive part of the coenzyme, and should clearly show the role it plays in catalyzing the mechanistic step you are drawing.

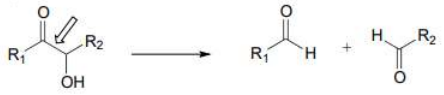
a)



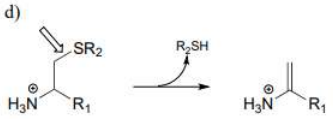
b)



c)



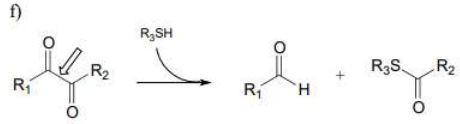
d)



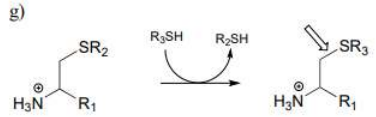
e)



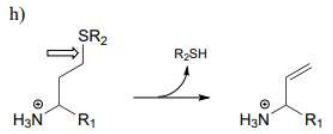
f)



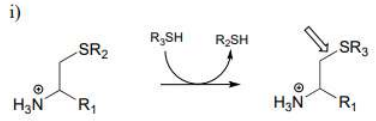
g)



h)

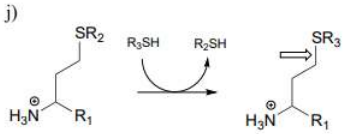


i)

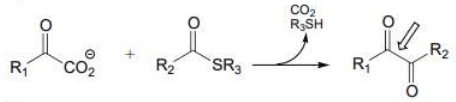


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j)



k)



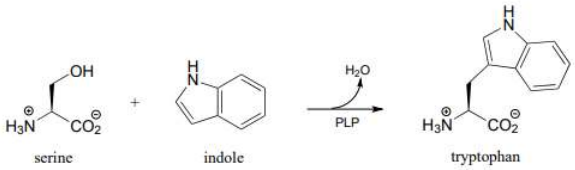
l)



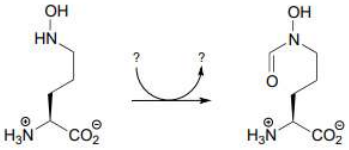
m)



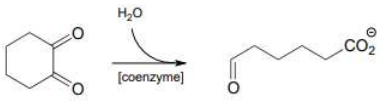
P17.2: The final step in the biosynthesis of the amino acid tryptophan is a PLP-dependent condensation between serine and indole, shown below (EC 4.2.1.20). The reaction mechanism involves steps that are familiar from this chapter, but also incorporates a reaction type we studied in chapter 14. Propose a mechanism.



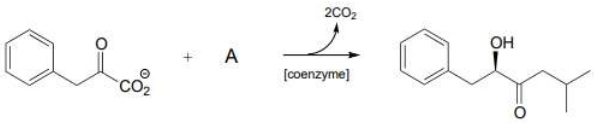
P17.3: Draw a reasonable mechanism for the following reaction, identifying the species denoted by questions marks. (Biochemistry 2012, 51, 3059)



P17.4: Propose a mechanism for the reaction below, which is part of the anaerobic catabolism of alcohols in some species of bacteria. (ChemBioChem 2014, 15, 389)

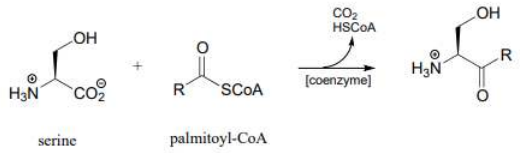


P17.5: Identify cosubstrate A and propose a mechanism for the reaction shown below, which was reported to occur in the thermophilic bacterium *Thermosporothrix hazakensis*. (ChemBioChem 2014 15, 527).



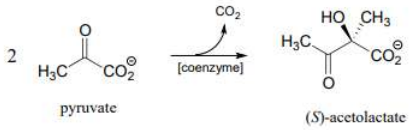
P17.6: Propose a mechanism for each of the reactions below, being sure to show the role played by the coenzyme (you need to determine which coenzyme is needed in each case).

a)



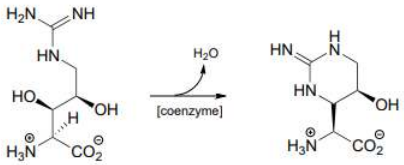
serine palmitoyl-CoA

b) (E.C. 2.2.1.6)



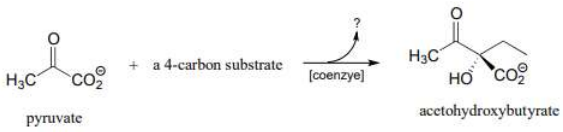
pyruvate (*S*)-acetolactate

c) *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 1943.



P17.7: Acetohydroxybutyrate is formed in a coenzyme-dependent reaction between pyruvate and a 4-carbon compound. What is a likely second substrate, coenzyme, and by-product (indicated below with a question mark)?

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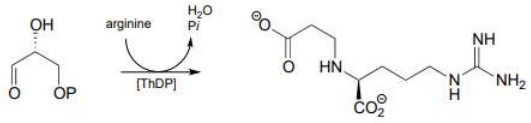
pyruvate acetohydroxybutyrate

P17.8: The 'benzoin condensation' reaction was discovered in the 19th century, and led eventually to a better understanding of ***ThDP***-dependent reactions in the cell. In a traditional benzoin condensation reaction, cyanide ion (instead of ThDP) plays the role of electron acceptor. Enzyme-catalyzed benzoin condensation reactions are also known to occur in some bacteria: Pseudomonas fluorescens, for example, contains an enzyme that catalyzes the synthesis of (R)-benzoin.



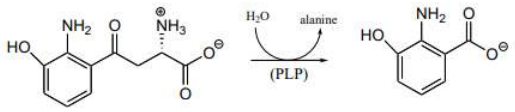
benzaldehyde benzoin

a. Draw a mechanism for the enzyme-catalyzed (***ThDP***-dependent) benzoin condensation reaction.
b. Draw a mechanism for the cyanide-catalyzed benzoin condensation reaction (non-enzymatic, basic conditions).
c. The following ***ThDP***-reaction was recently reported to be part of the biosynthetic pathway for clavulanic acid, a compound that inhibits the action of b-lactamases (b-lactamases are bacterial enzymes that hydrolyze penicillin-based antibiotic drugs, rendering them ineffective). As is typical for ***ThDP***-dependent reactions, the first step is addition of the ylide form of the coenzyme to the substrate carbonyl. The next steps are (in order): dehydration, tautomerization, elimination of phosphate, conjugate addition of arginine, and finally hydrolytic cleavage of the coenzyme-product bond. Draw out a complete mechanism that corresponds to this description.

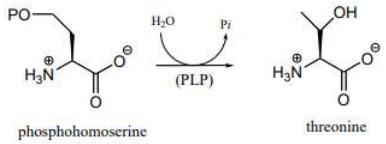


P17.9: Practice with PLP-dependent reactions:

a. Propose a mechanism for this reaction, which is part of the tryptophan degradation pathway (EC 3.7.1.3).



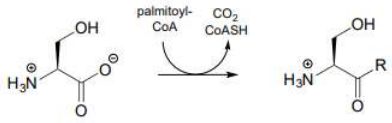
b. Propose a mechanism for the final step of the threonine biosynthesis pathway (EC 4.2.3.1).



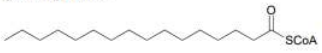
phosphohomoserine threonine

c. Propose a mechanism for the reaction catalyzed by aspartate β-decarboxylase (EC 4.1.1.12), which converts aspartate to alanine in a PLP-dependent reaction.

d. Sphingolipids are a type of membrane lipid found in the membranes of all eukaryotic cells, and are most abundant in the cells of central the central nervous system. Synthesis of sphingolipids involves the PLP-dependent reaction below, catalyzed by serine palmitoyl transferase (EC 2.3.1.50). Propose a mechanism.



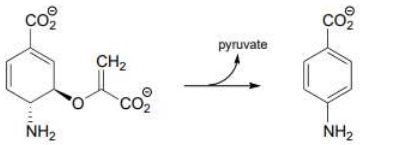
palmitoyl-CoA:



abbreviated:

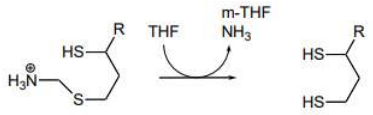


P17.10: As we saw in this chapter, PLP-dependent enzymes usually catalyze reactions involving amino acid substrates. Here is an exception, a PLP-dependent β-elimination reaction in the folate biosynthetic pathway (EC 4.1.3.38). Propose a mechanism for this reaction.

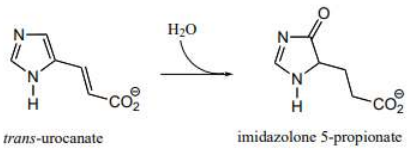


chorismate *para*-aminobenzoate

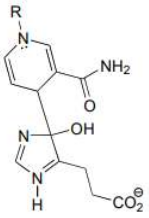
P17.11: The final step in the degradation pathway for the amino acid glycine (also known as the 'glycine cleavage system') is shown below. Propose a likely mechanism, given that evidence suggests that $CH_2NH_2^+$ is an intermediate.



P17.12: As we saw in chapter 15, the usual biochemical role of NAD^+ is to act as a hydride acceptor in dehydrogenation reactions. An exception is the reaction catalyzed by the histidine degradation pathway enzyme urocanase (EC 4.2.1.49).



In this reaction, NAD^+ acts as a catalytic, electron-sink coenzyme - it temporarily accepts electrons from a pi bond in the substrate, resulting in a covalent substrate-NAD adduct. This allows a key isomerization step to occur on the substrate through a protonation-deprotonation mechanism, followed by addition of water, cleavage of the substrate-NAD adduct to regenerate NAD^+, and finally tautomerization to the product. Propose a mechanism that fits this description, and involves the intermediate below.



reaction intermediate

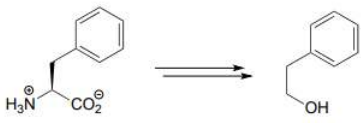
Pathway prediction problems

P17.13: Propose a multistep pathway for each of the following transformations. All involve at least one step requiring PLP, $ThDP$, or folate.

a. Below is portion of the biosynthesis of a modified membrane lipid in Salmonella and other pathogenic bacteria. The modified membrane confers antibiotic resistance to the bacterium.

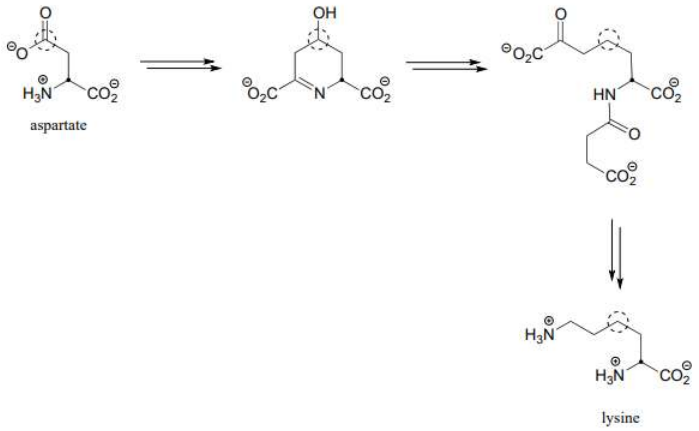


b. Below is the biosynthetic pathway for phenethanol in yeast. Phenethanol, which has a rose scent, is commonly used as a fragrance - this pathway has been proposed as a potential 'green' enzymatic synthesis to replace the traditional industrial synthesis, which uses toxic reagents.

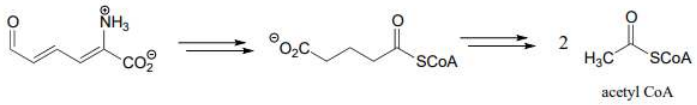


phenylalanine phenethanol

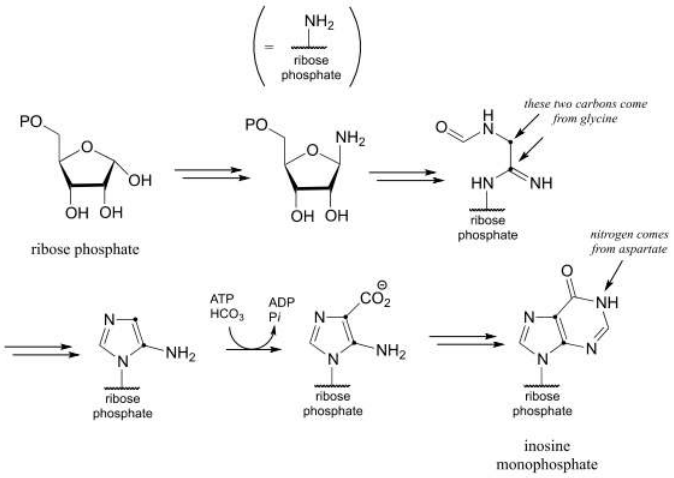
c. Below is an incomplete pathway diagram for the biosynthesis of the amino acid lysine, starting from aspartate. Fill in the missing steps and reactants/coenzymes to complete the diagram. The solid dot and dashed circle are provided to help you to trace two of the carbons from substrate to product.



d. Below is the second half of the tryptophan degradation pathway. Fill in the missing steps and reactants/coenzymes to complete the diagram.

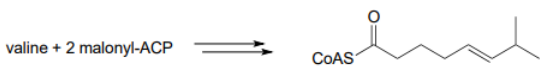


e. Below is an incomplete pathway diagram for the biosynthesis of inosine monophosphate, a precursor to the nucleotides adenosine monophosphate (***AMP***) and guanosine monophosphate (***GMP***). Fill in missing steps and reactants/coenzymes to complete the diagram. Note that one enzymatic step is provided (this is a carboxylation reaction of a type that we have not studied).

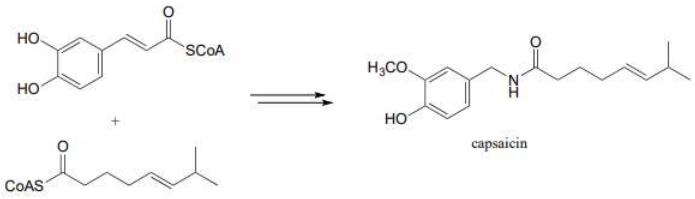


f. We begin our study of organic chemistry with a story about a hot pepper eating contest in Wisconsin (see the introduction the Chapter 1), and a compound called capsaicin which causes the 'hot' in hot peppers. As our last problem, let's try to predict some of the key steps in the biosynthesis of capsaicin.

Phase 1:



Phase 2:



Contributors and Attributions

- Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

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10.S: The Oganic Chemistry of Vitamins (Summary)

After completing this chapter, you should be able to:

- Understand how pyridoxal phosphate (PLP) acts as an 'electron sink' in a variety of reactions in amino acid metabolism.
- Recognize and draw mechanisms for PLP-dependent transformations of the following types:
  - racemization
  - decarboxylation
  - transamination
  - retroaldol cleavage
  - retro-Claisen cleavage
  - β-elimination
  - β-substitution
  - γ-elimination
  - γ-substitution
- Recognize transformations - amino acid decarboxylation and transamination, for example - in which chemical steps occur that simply don't 'make sense' unless the electron sink role of PLP is taken into account.
- Understand how the orientation of the substrate in relation to the plane formed by the conjugated π system of PLP is a major factor in catalysis of PLP-dependent reactions.
- Understand how thiamine diphosphate ($ThDP$) acts as an 'electron sink' in a variety of reactions in which a bond to a carbonyl carbon is broken, and how these steps do not 'make sense' unless the electron sink role of $ThDP$ is taken into account.
- Recognize transformations for which $ThDP$ is likely required, and be able to draw reasonable mechanisms for them.
- Understand how $ThDP$ acts in tandem with lipoamide, flavin, and nicotinamide in the reaction catalyzed by pyruvate dehydrogenase.
- Recognize folate in its various forms - DHF, THF, $f-THF$, CH_2-THF, and CH_3-THF - functions in a variety of one-carbon transfer steps. Be able to recognize the oxidation state of the carbon being transferred in a folate-depenent step.

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