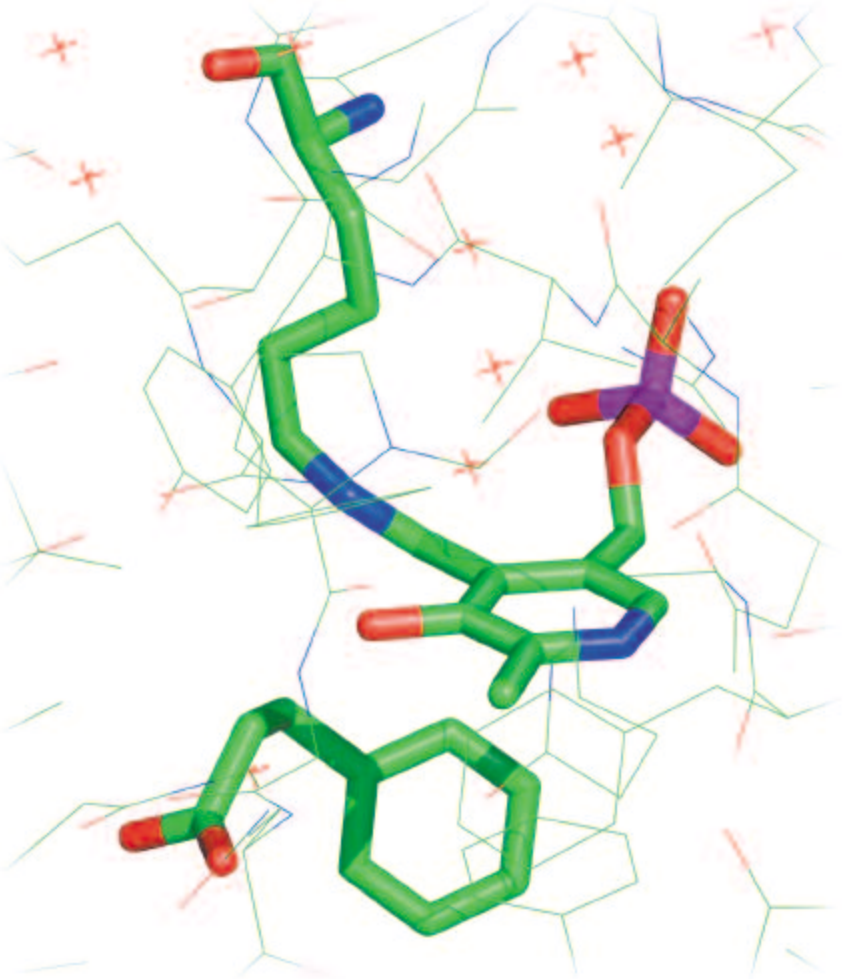
1 Common Mechanisms in Biological Chemistry



This scheme shows the active site of the enzyme that catalyzes the transamination reaction in the biosynthesis of phenylalanine from chorismate. A mechanistic understanding of biosynthetic pathways is a powerful tool for elucidating the chemical logic of living systems.

The final decades of the 20th century saw the beginning of a scientific revolution. Based on our newly acquired ability to manipulate, sequence, and synthesize deoxyribonucleic acid (DNA), the way is now open to isolate, study, and eventually modify each of the approximately 30,000 genes in our bodies. Medicines will become safer, more effective, and more specific; terrible genetic diseases such as sickle-cell anemia and cystic fibrosis will be cured; life spans will increase and the quality of life will improve as heart disease and cancer are brought under control.

None of these changes could occur without a detailed knowledge of chemistry, for it is our understanding of life processes at the molecular level that has made this revolution possible and that will sustain it. Biochemical processes are not mysterious. It's true that the many proteins, enzymes, nucleic acids, polysaccharides, and other substances in living organisms are enormously complex, but despite their

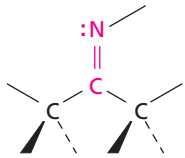
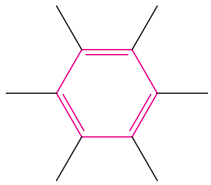
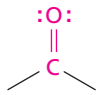
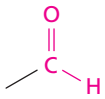
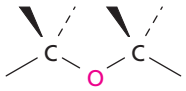
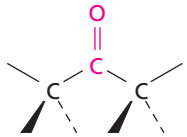
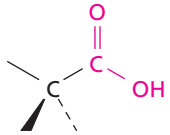
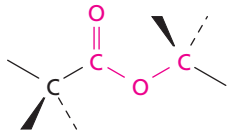
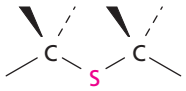
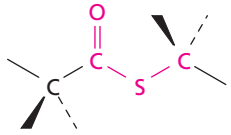
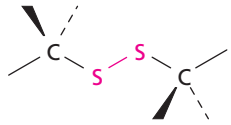
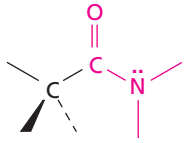
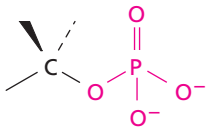
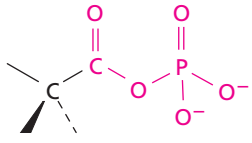
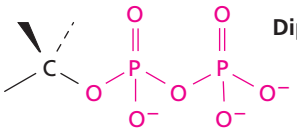
complexity, they are still molecules. They are subject to the same chemical laws as all other molecules, and their reactions follow the same rules of reactivity and take place by the same mechanisms as those of far simpler molecules.

The focus of this book is on examining biochemical processes from a chemical perspective. We'll begin with a brief review of organic chemistry, looking first at the common functional groups found in biological molecules and then at some fundamental mechanisms by which organic molecules react. Following this general review of organic reactivity, we'll look at the structures and chemical characteristics of the main classes of biomolecules: carbohydrates, lipids, proteins, enzymes, and nucleic acids. Finally, we'll come to the heart of the matter: the organic chemistry of biological transformations. We'll dissect the details of important biochemical pathways to see both *how* and *why* these pathways occur. The result will be both a deeper understanding of biochemistry and a deeper appreciation for the remarkable subtleties by which living organisms function.

1.1 Functional Groups in Biological Chemistry

Chemists have learned through experience that organic compounds can be classified into families according to their structural features and that members of a given family have similar chemical reactivity. The structural features that make such classifications possible are called *functional groups*. A **functional group** is a group of atoms within a molecule that has a characteristic chemical behavior. Chemically, a functional group behaves in pretty much the same way in every molecule where it occurs. An ester (RCO_2R), for instance, usually undergoes a hydrolysis reaction with water to yield a carboxylic acid (RCO_2H) and an alcohol (ROH); a thiol (RSH) usually undergoes an oxidation reaction to yield a disulfide (RSSR); and so on. Table 1.1 lists some common functional groups found in biological molecules.

Table 1.1 Common Functional Groups in Biological Molecules

Structure*	Name	Structure*	Name
	Alkene (double bond)		Imine (Schiff base)
	Arene (aromatic ring)		Carbonyl group
	Alcohol		Aldehyde
	Ether		Ketone
	Amine		Carboxylic acid
	Thiol		Ester
	Sulfide		Thioester
	Disulfide		Amide
	Monophosphate		Acyl phosphate
	Diphosphate		

* The bonds whose connections aren't specified are assumed to be attached to carbon or hydrogen atoms in the rest of the molecule.

Alcohols, ethers, amines, thiols, sulfides, disulfides, and phosphates all have a carbon atom singly bonded to an electronegative atom. Alcohols, ethers, and phosphates have a carbon atom bonded to oxygen; amines have a carbon atom bonded to nitrogen; and thiols, sulfides, and disulfides have a carbon atom bonded to sulfur. In all cases, the bonds are polar, with the carbon atom being electron-poor and thus bearing a partial positive charge ($\delta+$), while the electronegative atom is electron-rich and thus bears a partial negative charge ($\delta-$). These polarity patterns are shown in Figure 1.1.

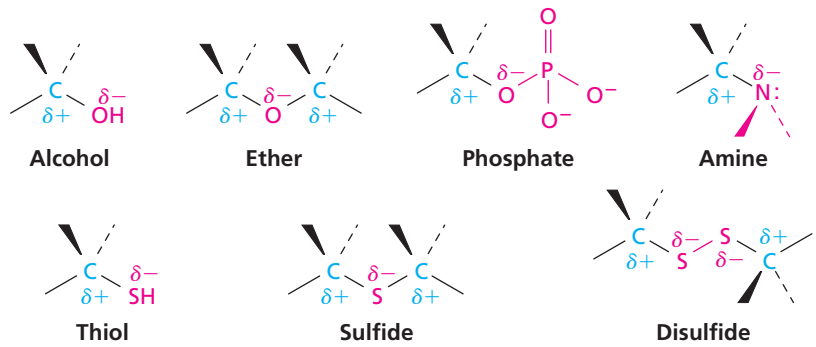


FIGURE 1.1 Polarity patterns of some common functional groups. The electronegative atom bears a partial negative charge ($\delta-$), and the carbon atom bears a partial positive charge ($\delta+$).

Note particularly in Table 1.1 the different families of compounds that contain the **carbonyl group, C═O**. Carbonyl groups are present in the vast majority of biological molecules. These compounds behave similarly in many respects but differ depending on the identity of the atoms bonded to the carbonyl-group carbon. Aldehydes have at least one hydrogen bonded to the C═O; ketones have two carbons bonded to the C═O; carboxylic acids have an —OH group bonded to the C═O; esters have an ether-like oxygen (−OR) bonded to the C═O; thioesters have a sulfide-like sulfur (−SR) bonded to the C═O; amides have an amine-like nitrogen ($-NH_2$, −NHR, or $-NR_2$) bonded to the C═O; and acyl phosphates have a phosphate group ($-OPO_3^{2-}$) bonded to the C═O. You might note that an acyl phosphate is structurally (and chemically) similar to a carboxylic acid anhydride.

As shown in Figure 1.2, carbonyl compounds are polar, with the electron-poor C═O carbon atom bearing a partial positive charge and the electron-rich oxygen atom bearing a partial negative charge.

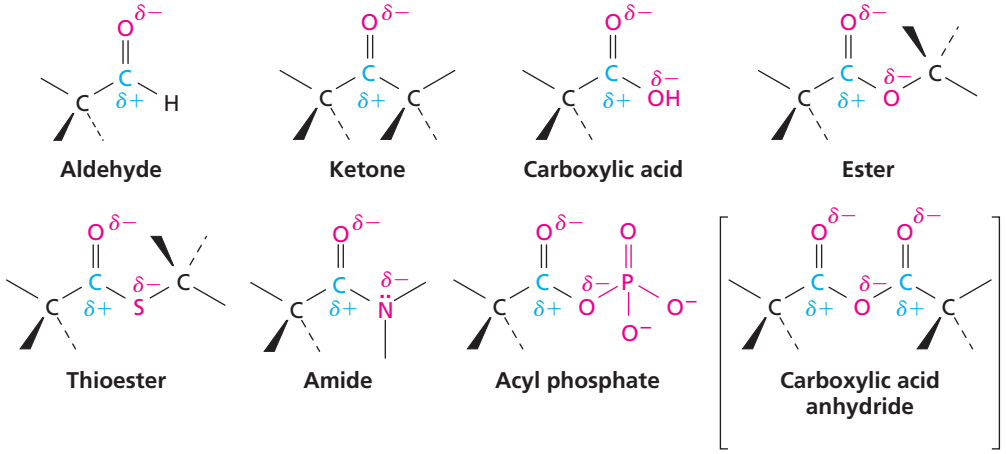


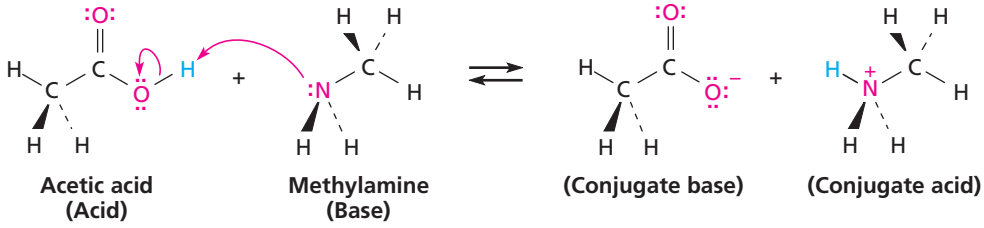
FIGURE 1.2 Polarity patterns in some carbonyl-containing functional groups. The carbonyl carbon atom is electron-poor ($\delta+$) and the oxygen atom is electron-rich ($\delta-$).

1.2 Acids and Bases; Electrophiles and Nucleophiles

Brønsted–Lowry Acids and Bases

Acids and bases are enormously important in biochemistry. The vast majority of biological transformations are catalyzed by acids or bases, and a thorough knowledge of acid–base chemistry is crucial for understanding how reactions occur.

According to the Brønsted–Lowry definition, an **acid** is a substance that donates a proton (hydrogen ion, H^+), and a **base** is a substance that accepts a proton. A carboxylic acid such as acetic acid, for example, can donate its —OH proton to a base such as methylamine in a reversible, **proton-transfer reaction**. The product that results by loss of H^+ from an acid is the **conjugate base** of the acid, and the product that results from addition of H^+ to a base is the **conjugate acid** of the base.



Note the standard convention used to show how this proton-transfer reaction occurs: A curved arrow (red) indicates that a pair of electrons moves *from* the atom at the tail of the arrow (the nitrogen in methylamine) *to* the atom at the head of the arrow (the acidic hydrogen in acetic acid). That is, the electrons used to form the new N—H bond flow from the base to the acid. As the N—H bond forms, the O—H bond breaks and its electrons remain with oxygen, as shown by a second curved arrow. *A curved arrow always represents the movement of electrons, not atoms.*

Acids differ in their ability to donate protons. Recall from general chemistry that the strength of an acid HA in water solution is expressed by its **pK_a**, the negative common logarithm of its **acidity constant, K_a**. A stronger acid has a smaller pK_a (or larger K_a); a weaker acid has a larger pK_a (or smaller K_a).

For the reaction: $HA + H_2O \rightleftharpoons A^- + H_3O^+$

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} \text{ and } pK_a = -\log K_a$$

Stronger acid—smaller pK_a
Weaker acid—larger pK_a

Table 1.2 lists the pK_a's of some typical acids encountered in biochemistry. Note that the pK_a of water is 15.74, the value that results when K_w, the ion-product constant for water, is divided by the molar concentration of pure water, 55.5 M:

$$HA + H_2O \rightleftharpoons A^- + H_3O^+ \qquad \text{where HA} = H_2O, A^- = OH^-$$

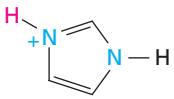
$$K_a = \frac{[H_3O^+][A^-]}{[HA]} = \frac{[H_3O^+][OH^-]}{[H_2O]} = \frac{K_w}{55.5}$$

$$= \frac{1.00 \times 10^{-14}}{55.5} = 1.80 \times 10^{-16}$$

$$pK_a = -\log 1.80 \times 10^{-16} = 15.74$$

Note also in Table 1.2 that carbonyl compounds are weakly acidic, a point we'll discuss in more detail in Section 1.7.

Table 1.2 Relative Strengths of Some Acids

Functional group	Example	pK_a	
Carboxylic acid	CH_3COH (O)	4.76	Stronger acid
Imidazolium ion		6.95	
Ammonia	NH_4^+	9.26	
Thiol	CH_3SH	10.3	
Alkylammonium ion	$CH_3NH_3^+$	10.66	
β-Keto ester	$CH_3CCH_2COCH_3$ (O, O)	10.6	
Water	H_2O	15.74	
Alcohol	CH_3CH_2OH	16.00	
Ketone	CH_3CCH_3 (O)	19.3	
Thioester	CH_3CSCH_3 (O)	21	
Ester	CH_3COCH_3 (O)	25	Weaker acid

Just as acids differ in their ability to donate a proton, bases differ in their ability to accept a proton. The strength of a base B in water solution is normally expressed using the *acidity* of its conjugate acid, BH^+.

For the reaction: $BH^+ + H_2O \rightleftharpoons B + H_3O^+$

$$K_a = \frac{[B][H_3O^+]}{[BH^+]}$$

so

$$K_a \times K_b = \left(\frac{[B][H_3O^+]}{[BH^+]}\right)\left(\frac{[BH^+][OH^-]}{[B]}\right)$$

$$= [H_3O^+]\,[OH^-] = K_w = 1.00 \times 10^{-14}$$

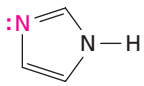
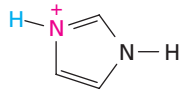
$$\text{Thus} \qquad K_a = \frac{K_w}{K_b} \qquad \text{and} \qquad K_b = \frac{K_w}{K_a}$$

$$\text{so} \qquad pK_a + pK_b = 14 \qquad \text{and} \qquad pK_b = 14 - pK_a$$

Stronger base—larger pK_a for BH^+
Weaker base—smaller pK_a for BH^+

These equations say that we can determine the basicity of a base B by knowing the K_a of its conjugate acid BH^+. A stronger base holds H^+ more tightly, so it has a weaker conjugate acid (larger pK_a); a weaker base holds H^+ less tightly, so it has a stronger conjugate acid (smaller pK_a). Table 1.3 lists some typical bases found in biochemistry. Note that water can act as either a weak acid or a weak base, depending on whether it donates a proton to give OH^- or accepts a proton to give H_3O^+. Similarly with imidazole, alcohols, and carbonyl compounds, which can either donate or accept protons depending on the circumstances.

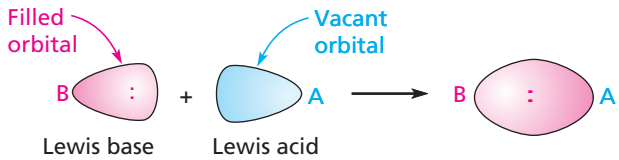
Table 1.3 Relative Strengths of Some Bases

Functional group	Example		pK_a of BH^+	
Hydroxide ion	$:\ddot{O}H^-$	H_2O	15.74	Stronger base
Guanidino	$:NH \parallel H_2NCNHCH_2CH_3$	$^+NH_2 \parallel H_2NCNHCH_2CH_3$	12.5	
Amine	$CH_3\ddot{N}H_2$	$CH_3\overset{+}{N}H_3$	10.66	
Ammonia	$:NH_3$	$^+NH_4$	9.26	
Imidazole			6.95	
Water	$H_2\ddot{O}:$	H_3O^+	−1.74	
Alcohol	$CH_3\ddot{O}H$	$CH_3\overset{+}{O}H_2$	−2.05	
Ketone	$:O: \parallel CH_3CCH_3$	$^+OH \parallel CH_3CCH_3$	−7.5	Weaker base

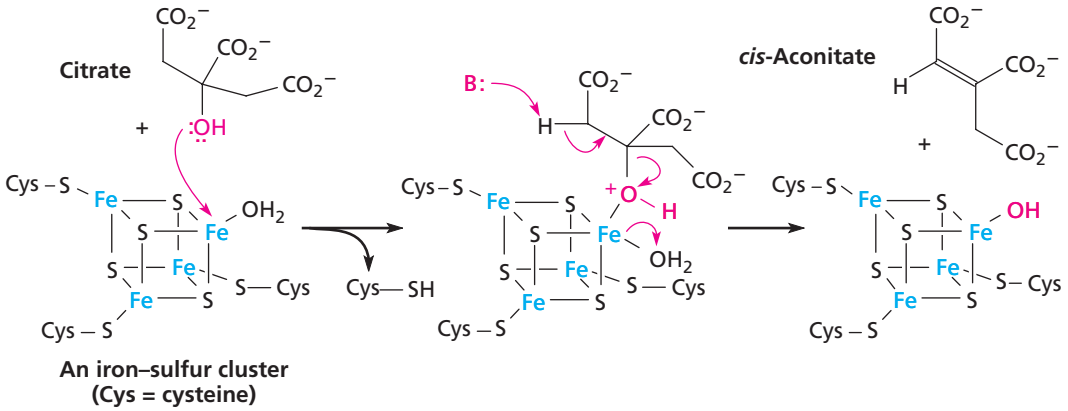
Lewis Acids and Bases

The Brønsted–Lowry definition of acids and bases covers only compounds that donate or accept H^+. Of more general use, however, is the Lewis definition. A **Lewis acid** is a substance that accepts an electron pair from a base, and a **Lewis base** is a substance that donates an electron pair to an acid. For all practical purposes, Lewis and Brønsted–Lowry bases are the same: Both have lone pairs of electrons that they donate to acids. Lewis and Brønsted–Lowry acids, however, are *not* necessarily the same.

The fact that a Lewis acid must be able to accept an electron pair means that it must have a vacant, low-energy orbital. Thus, the Lewis definition of acidity is much broader than the Brønsted–Lowry definition and includes many species in addition to H^+. For example, various metal cations and transition-metal compounds, such as Mg^{2+}, Zn^{2+}, and Fe^{3+} are Lewis acids.



Lewis acids are involved in a great many biological reactions, often as cofactors in enzyme-catalyzed processes. Metal cations such as Mg^{2+} and Zn^{2+} are particularly common, but complex compounds such as iron–sulfur clusters are also found. We'll see an example in Section 4.4 where citrate undergoes acid-catalyzed dehydration to yield *cis*-aconitate, a reaction in the citric acid cycle.



Electrophiles and Nucleophiles

Closely related to acids and bases are *electrophiles* and *nucleophiles*. An **electrophile** is a substance that is "electron-loving." It has a positively polarized, electron-poor atom and can form a bond by accepting a pair of electrons from an electron-rich atom. A **nucleophile**, by contrast, is "nucleus-loving." It has a negatively polarized, electron-rich atom and can form a bond by donating a pair of electrons to an electron-poor atom. Thus, electrophiles are essentially the same as Lewis acids and nucleophiles are the same as Lewis bases. In practice, however, the words "acid" and "base" are generally used when electrons are donated to H^+ or a metal ion, and the words "electrophile" and "nucleophile" are used when electrons are donated to a carbon atom.

Electrophiles are either positively charged or neutral and have a positively polarized, electron-poor atom that can accept an electron pair from a nucleophile/base. Acids (H^+ donors), trialkylsulfonium compounds (R_3S^+), and carbonyl compounds are examples (Figure 1.3).

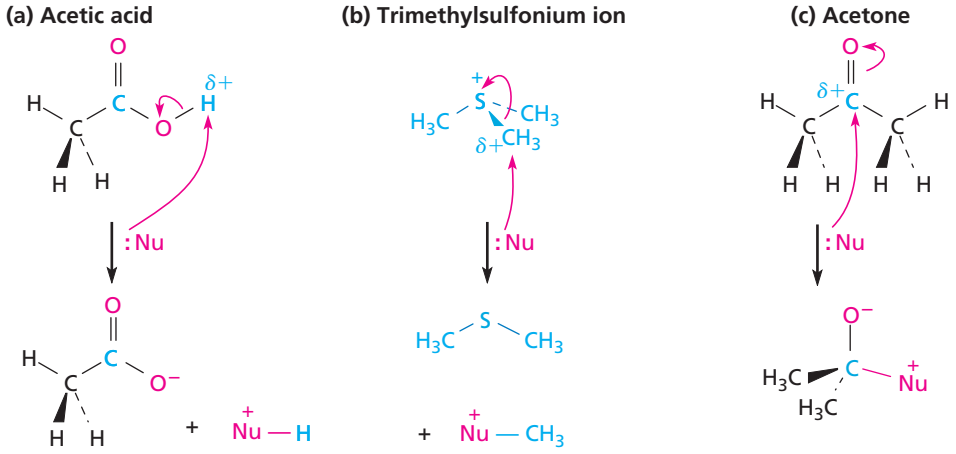


FIGURE 1.3 Some electrophiles and their reactions with nucleophiles (:Nu).

Nucleophiles are either negatively charged or neutral and have a lone pair of electrons they can donate to an electrophile/acid. Amines, water, hydroxide ion, alkoxide ions (RO^-), and thiolate ions (RS^-) are examples.

1.3 Mechanisms: Electrophilic Addition Reactions

Chemical reactions carried out in living organisms follow the same rules of reactivity as reactions carried out in the laboratory. The "solvent" is often different, the temperature is often different, and the catalyst is certainly different, but the reactions occur by the same fundamental mechanisms. That's not to say that *all* bioorganic reactions have obvious laboratory counterparts—some of the most chemically interesting biotransformations cannot be duplicated in the laboratory without an enzyme because too many side reactions would occur. Nevertheless, the chemical mechanisms of biotransformations can be understood and accounted for by organic chemistry. In this and the remaining sections of Chapter 1, we'll look at some fundamental organic reaction mechanisms, beginning with the electrophilic addition reactions of C═C bonds.

An **electrophilic addition reaction** is initiated by addition of an electrophile to an unsaturated (electron-rich) partner, usually an alkene, and leads to formation of a saturated product. In the laboratory, for example, water undergoes an acid-catalyzed electrophilic addition to 2-methylpropene to yield 2-methyl-2-propanol. The reaction takes place in three steps and proceeds through a positively charged, carbocation intermediate (Figure 1.4). In the first step, electrons from the nucleophilic C═C bond attack an electrophilic hydrogen atom of H_3O^+, forming a C—H bond. The intermediate carbocation then reacts with water as nucleophile, giving first a protonated alcohol and then the neutral alcohol after a proton-transfer step that regenerates H_3O^+. Note that the initial protonation takes place on the less highly substituted carbon of the double bond, leading to the more highly substituted, more stable, carbocation.

Biological examples of electrophilic addition reactions occur frequently in the biosynthetic routes leading to steroids and other terpenoids, although they are less common elsewhere. The electrophile in such reactions is a positively charged or positively polarized carbon atom, which often adds to a C═C bond within the same molecule. As an example, α-terpineol, a substance found in pine oil and used in perfumery, is derived biosynthetically from linalyl diphosphate by an internal electrophilic addition reaction. Following formation of an allylic carbocation by dissociation of the diphosphate (here abbreviated PPO),

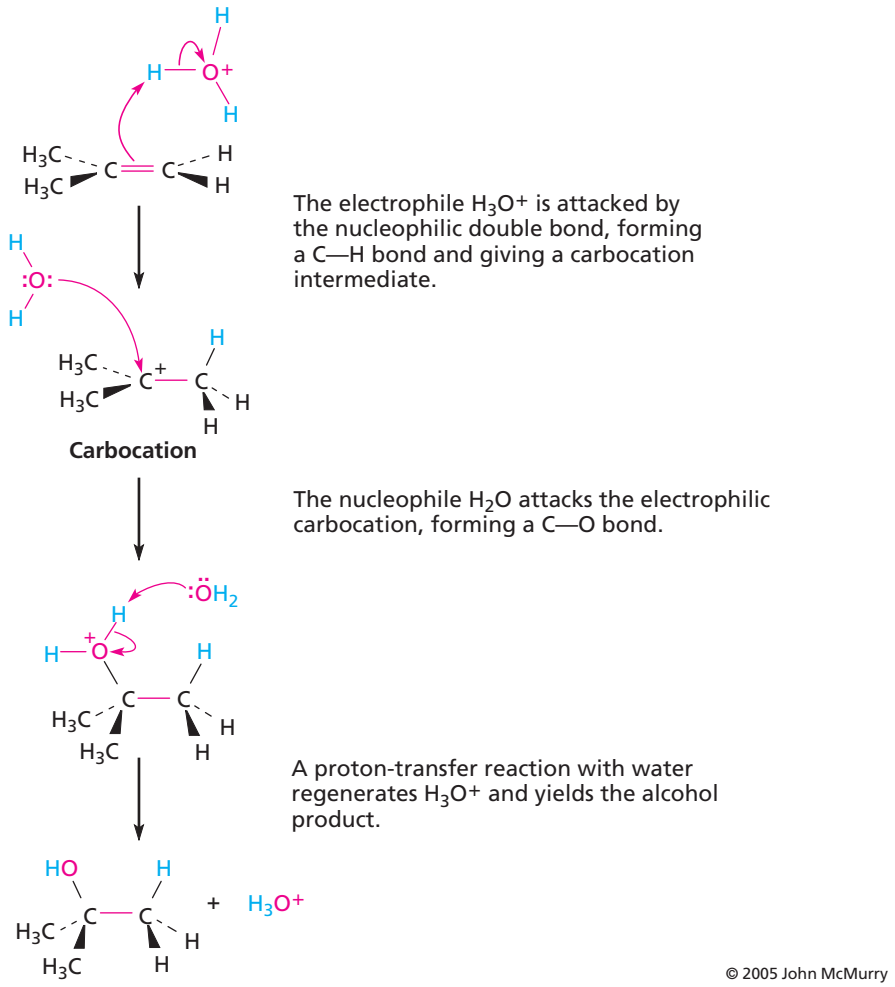


FIGURE 1.4 The mechanism of the acid-catalyzed electrophilic addition of water to 2-methylpropene. The reaction involves a carbocation intermediate.

electrophilic addition to the nucleophilic C═C bond at the other end of the molecule occurs, giving a second carbocation that then reacts with nucleophilic water. A proton transfer from the protonated alcohol to water yields α-terpineol (Figure 1.5). We'll see more such examples when we look at steroid biosynthesis in Section 3.5.

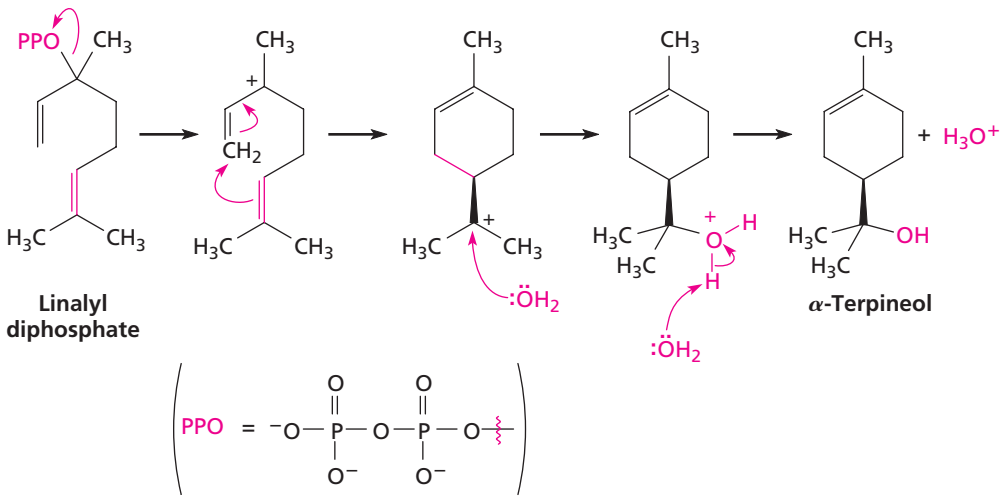


FIGURE 1.5 The biosynthesis of α-terpineol from linalyl diphosphate occurs by an electrophilic addition reaction.

1.4 Mechanisms: Nucleophilic Substitution Reactions

A **nucleophilic substitution reaction** is the substitution of one nucleophile (the *leaving group*) by another on a saturated, sp^3-hybridized carbon atom: Br^- by OH^-, for example. Nucleophilic substitution reactions in the laboratory generally proceed by either an **S_N1 mechanism** or an **S_N2 mechanism** depending on the reactants, the solvent, the pH, and other variables. S_N1 reactions usually take place with tertiary or allylic substrates and occur in two steps through a carbocation intermediate. S_N2 reactions usually take place with primary substrates and take place in a single step without an intermediate.

The mechanism of a typical S_N1 reaction is shown in Figure 1.6. As indicated, the substrate undergoes a spontaneous dissociation to generate a carbocation intermediate, which reacts with the substituting nucleophile to give product.

The mechanism of a typical S_N2 process is shown in Figure 1.7 for the reaction of hydroxide ion with (*S*)-2-bromobutane. The reaction takes place in a single step when the incoming nucleophile uses a lone pair of electrons to attack the

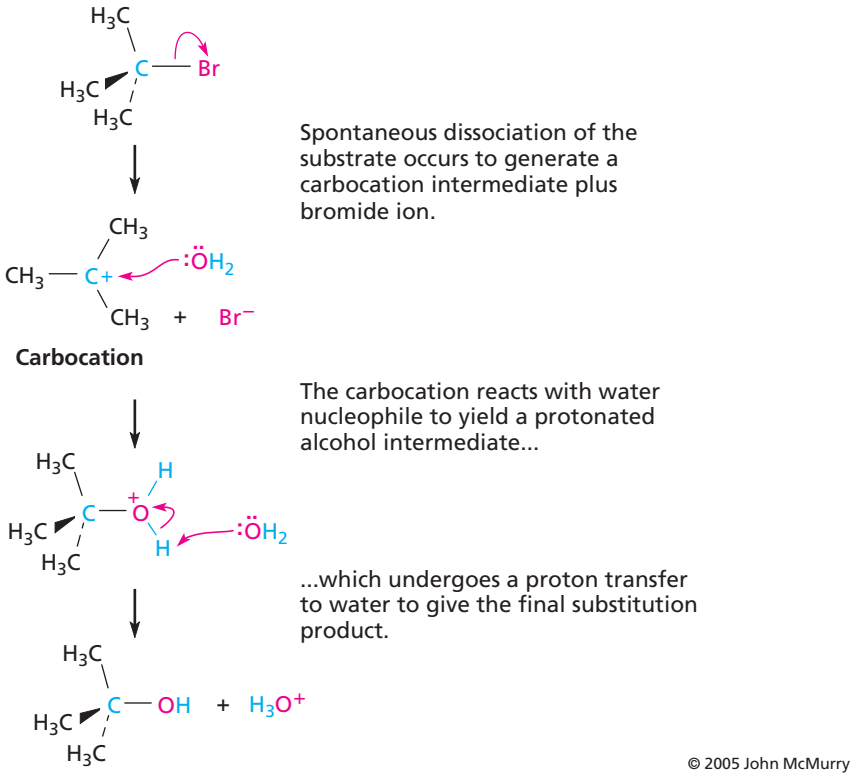


FIGURE 1.6 Mechanism of the S_N1 reaction of 2-bromo-2-methylpropane with water to yield 2-methyl-2-propanol. The reaction occurs by a spontaneous dissociation to give a carbocation intermediate, which reacts with water.

electrophilic carbon atom of the alkyl halide from a direction 180° opposite the C—Br bond. As the OH^- comes in and a new O—C bond begins to form, the old C—Br bond begins to break and the Br^- leaves. Because the incoming and outgoing nucleophiles are on opposite sides of the molecule, the stereochemistry at the reacting center inverts during an S_N2 reaction. (*S*)-2-Bromobutane yields (*R*)-2-butanol, for example. (There is no guarantee that inversion will change the assignment of a stereocenter from *R* to *S* or vice versa, because the relative priorities of the four groups attached to the stereocenter may also change.)

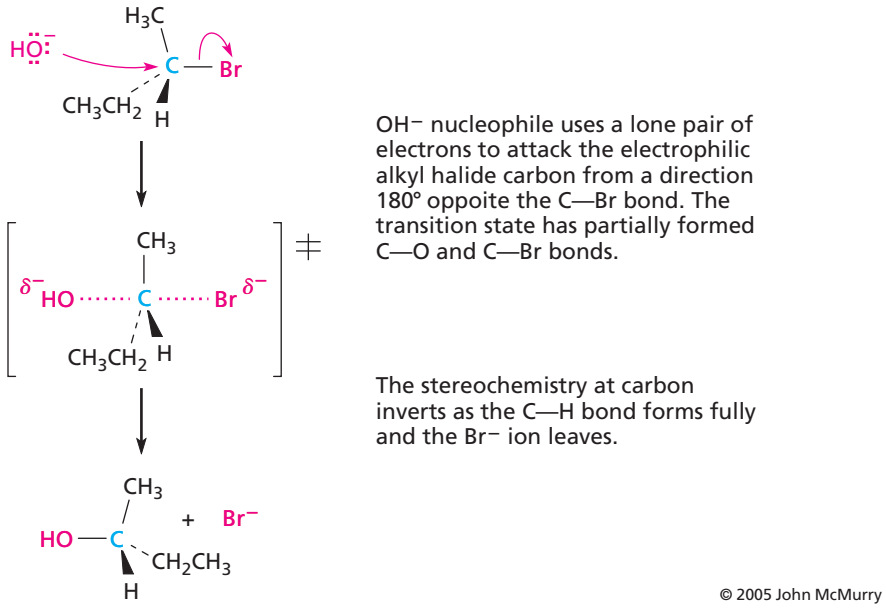
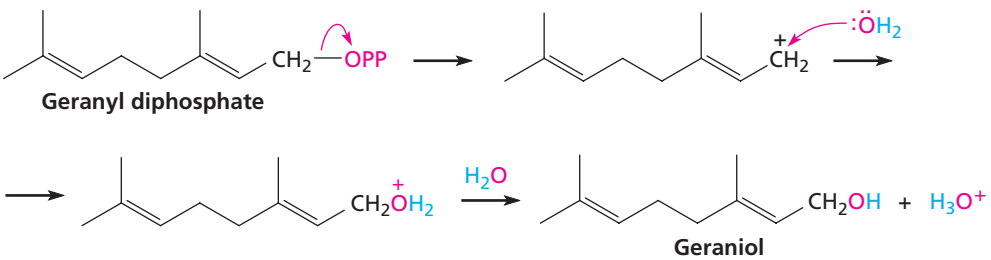


FIGURE 1.7 Mechanism of the S_N2 reaction of (*S*)-2-bromobutane with hydroxide ion to yield (*R*)-2-butanol. The reaction occurs in a single step with inversion of stereochemistry at the reacting carbon atom.

Biochemical examples of both S_N1 and S_N2 reactions occur in numerous pathways. An S_N1 reaction, for instance, takes place during the biological conversion of geranyl diphosphate to geraniol, a fragrant alcohol found in roses and used in perfumery. Initial dissociation of the diphosphate gives a stable allylic carbocation, which reacts with water nucleophile and transfers a proton to yield geraniol.

An S_N1 reaction



An S_N2 reaction is involved in biological methylation reactions whereby a —CH_3 group is transferred from *S*-adenosylmethionine to various nucleophiles.

In the biosynthetic transformation of norepinephrine to epinephrine (adrenaline), for instance, the nucleophilic amine nitrogen atom of norepinephrine attacks the electrophilic methyl carbon atom of *S*-adenosylmethionine in an S_N2 reaction, displacing *S*-adenosylhomocysteine as the leaving group (Figure 1.8).

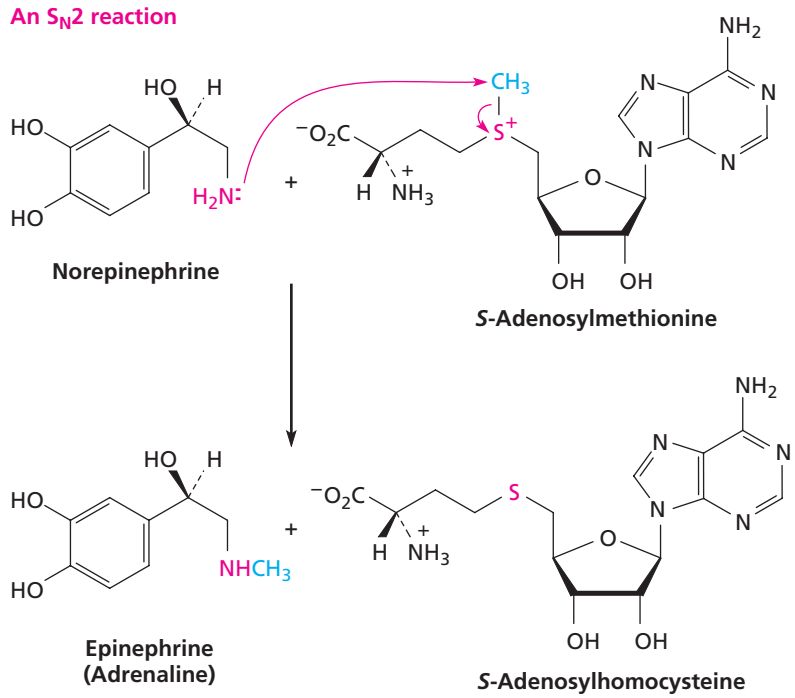
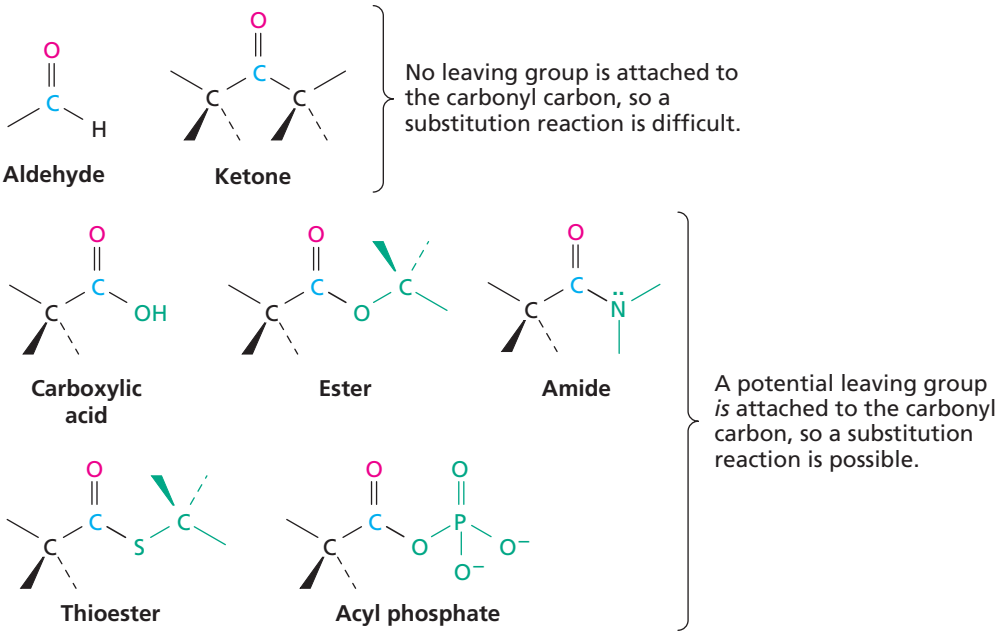


FIGURE 1.8 The biosynthesis of epinephrine from norepinephrine occurs by an S_N2 reaction with *S*-adenosylmethionine.

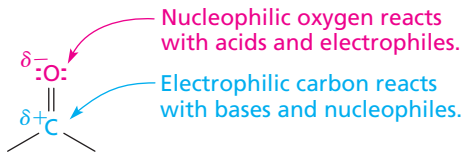
1.5 Mechanisms: Nucleophilic Carbonyl Addition Reactions

Carbonyl groups are present in the vast majority of biological molecules, and carbonyl reactions are thus encountered in almost all biochemical pathways. In discussing carbonyl-group chemistry, it's useful to make a distinction between two general classes of compounds. In one class are aldehydes and ketones, which have their carbonyl carbon bonded to atoms (C and H) that can't stabilize a negative charge and therefore don't typically act as leaving groups in substitution reactions. In the second class are carboxylic acids and their derivatives, which have

their carbonyl carbon bonded to an electronegative atom (O, N, or S) that *can* stabilize a negative charge and thus *can* act as a leaving group in a substitution reaction.



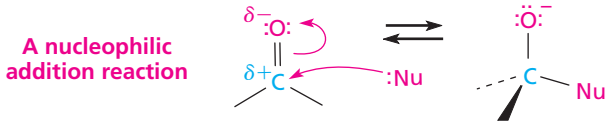
The C═O bond in every carbonyl compound, regardless of structure, is polarized with the oxygen negative and the carbon positive. As a result, the carbonyl oxygen is *nucleophilic* and reacts with acids/electrophiles, while the carbon is *electrophilic* and reacts with bases/nucleophiles. These simple reactivity patterns show up in almost all biological pathways.



Nucleophilic Addition Reactions

A **nucleophilic addition reaction** is the addition of a nucleophile (:Nu) to the electrophilic carbon of an aldehyde or ketone. As an electron pair from the nucleophile forms a bond to the carbon, an electron pair from the C═O bond moves toward

oxygen, giving an alkoxide ion, RO^-. The carbonyl carbon rehybridizes from sp^2 to sp^3 during the process, so the alkoxide product has tetrahedral geometry.



Once formed, the tetrahedral alkoxide ion can do any of several things, as shown in Figure 1.9. When a nucleophile such as hydride ion ($H:^-$) or a carbanion ($R_3C:^-$) adds, the alkoxide ion undergoes protonation to yield a stable alcohol.

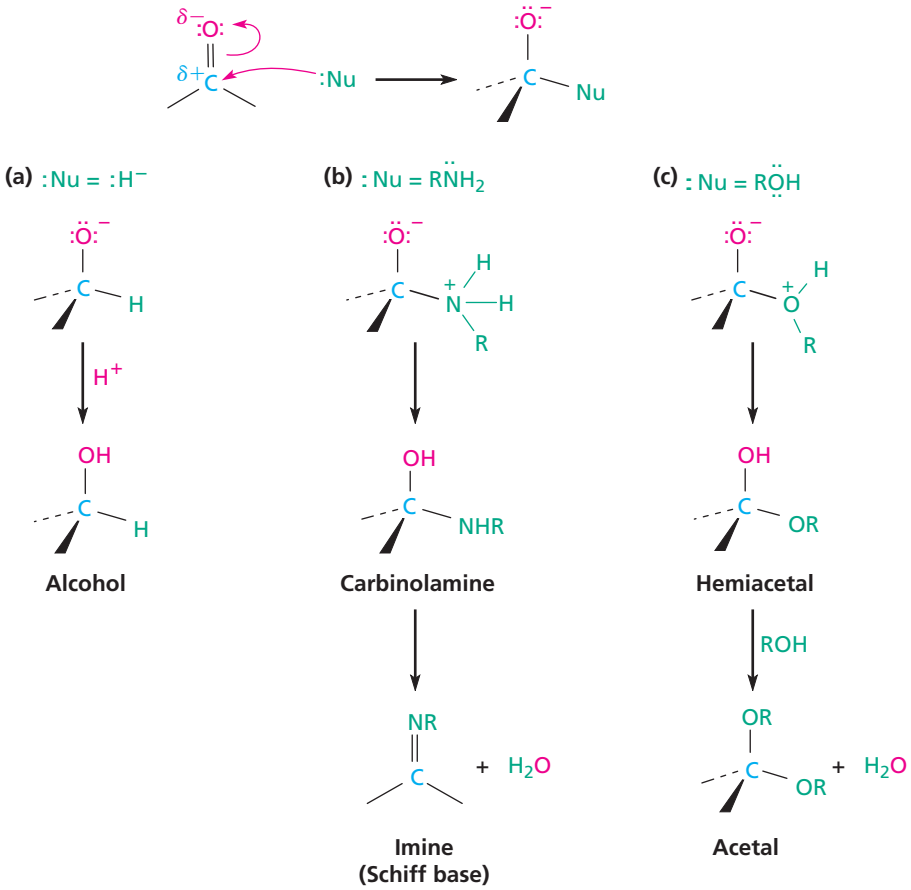
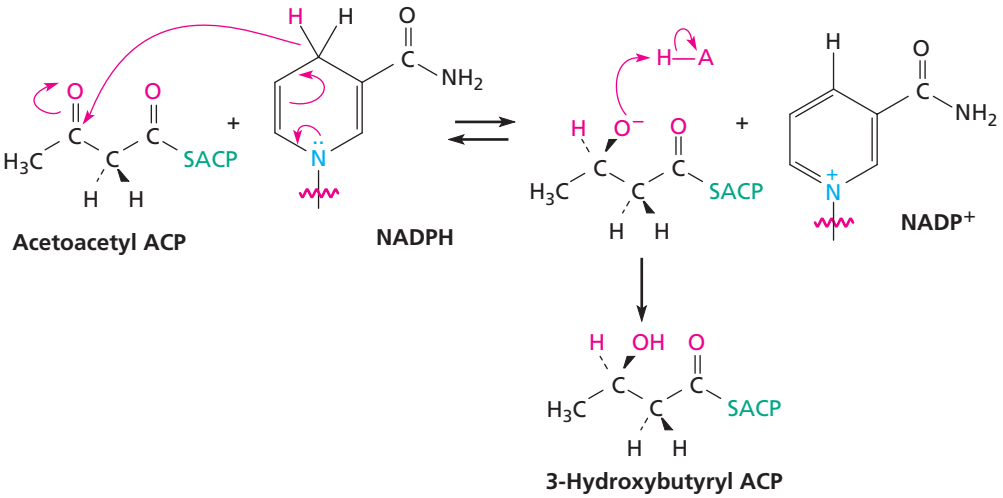


FIGURE 1.9 Some typical nucleophilic addition reactions of aldehydes and ketones. (a) With a hydride ion as nucleophile, protonation of the alkoxide intermediate leads to an alcohol. (b) With an amine as nucleophile, proton transfer and loss of water leads to an imine. (c) With an alcohol as nucleophile, proton transfer leads to a hemiacetal, and further reaction with a second equivalent of alcohol leads to an acetal.

When a primary amine nucleophile (RNH_2) adds, the alkoxide ion undergoes a proton transfer to yield a **carbinolamine**, which loses water to form an **imine** ($R_2C{=}NR'$), often called a **Schiff base** in biochemistry. When an alcohol nucleophile (ROH) adds, the alkoxide undergoes proton transfer to yield a **hemiacetal**, which can react with a second equivalent of alcohol and lose water to give an **acetal** [$R_2C(OR')_2$]. In all the reactions that follow, note the role of acid and base catalysts.

Alcohol Formation

In the laboratory, the conversion of an aldehyde or ketone to an alcohol is generally carried out using $NaBH_4$ as the nucleophilic hydride-ion donor. In biological pathways, however, NADH (reduced nicotinamide adenine dinucleotide) or the closely related NADPH (reduced nicotinamide adenine dinucleotide phosphate) is the most frequently used hydride-ion donor. An example that occurs in the pathway by which organisms synthesize fatty acids is the conversion of acetoacetyl ACP (acyl carrier protein) to 3-hydroxybutyryl ACP. We'll look at the details of the process in Section 3.4.



Imine (Schiff Base) Formation

Imines are formed in a reversible, acid-catalyzed process that begins with nucleophilic addition of a primary amine to the carbonyl group of an aldehyde or ketone. The initial dipolar addition product undergoes a rapid proton transfer that removes an H^+ from N and places another H^+ on O to give a carbinolamine, which is

protonated on the oxygen atom by an acid catalyst. The effect of this protonation is to convert —OH into a much better leaving group ($—OH_2^+$) so that it can be expelled by the electrons on nitrogen. Deprotonation of the resultant iminium ion then gives the imine product. The mechanism is shown in Figure 1.10.

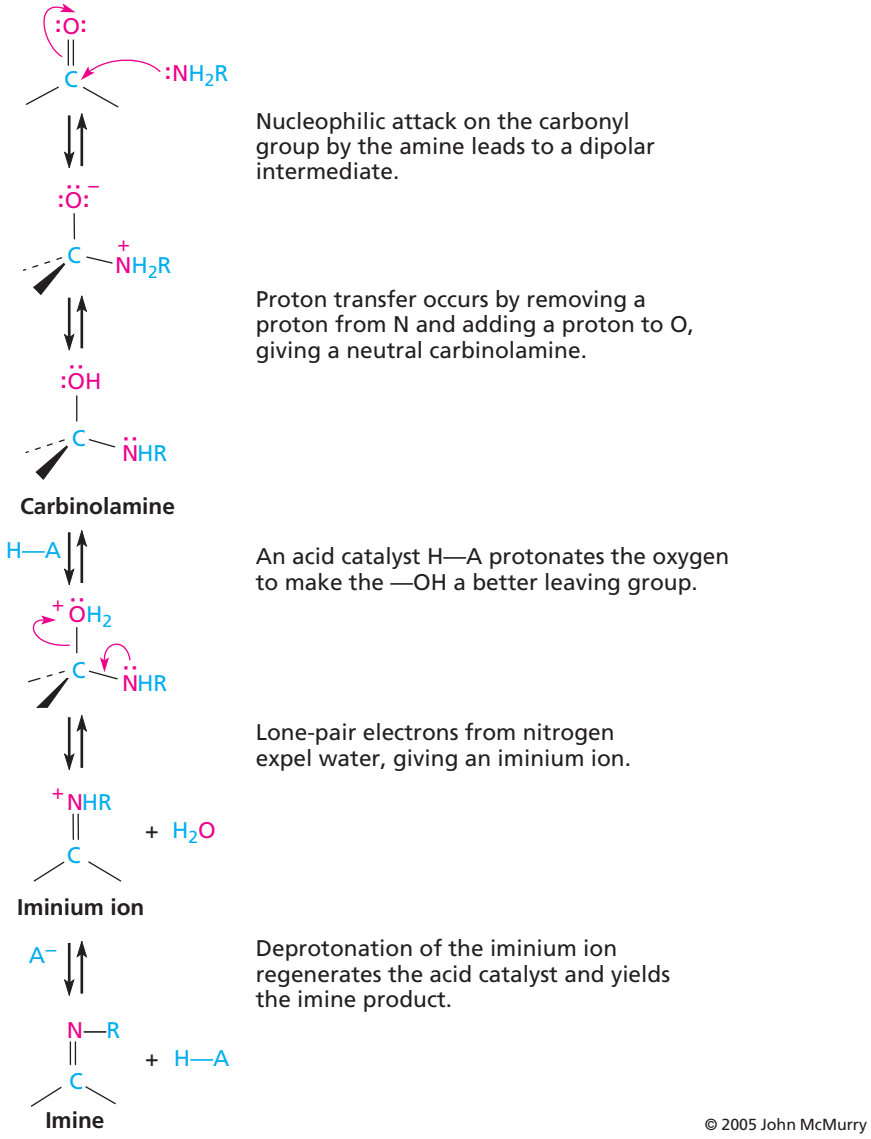
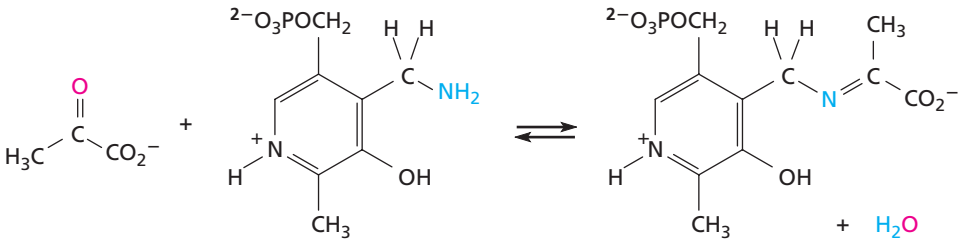


FIGURE 1.10 Mechanism of acid-catalyzed imine (Schiff base) formation by reaction of an aldehyde or ketone with a primary amine, RNH_2.

The conversion of a ketone to an imine is a step in numerous biological pathways, including the route by which many amino acids are synthesized in the body. For instance, the ketone pyruvate and the amine pyridoxamine phosphate, a derivative of vitamin B_6, form an imine that is converted to the amino acid alanine. We'll look at the details in Section 5.1.



Pyruvate **Pyridoxamine phosphate** **An imine**

Acetal Formation

Acetals, like imines, are formed in a reversible, acid-catalyzed process. The reaction begins with protonation of the carbonyl oxygen to increase its reactivity, followed by nucleophilic addition of an alcohol. Deprotonation then gives a neutral hemiacetal, and reprotonation on the hydroxyl oxygen converts the —OH into a better leaving group so that it can be expelled by electrons on the neighboring —OR to produce an oxonium ion. This oxonium ion behaves much like the protonated carbonyl group in the first step, undergoing a second nucleophilic addition with alcohol. A final deprotonation then gives the acetal product. The mechanism is shown in Figure 1.11.

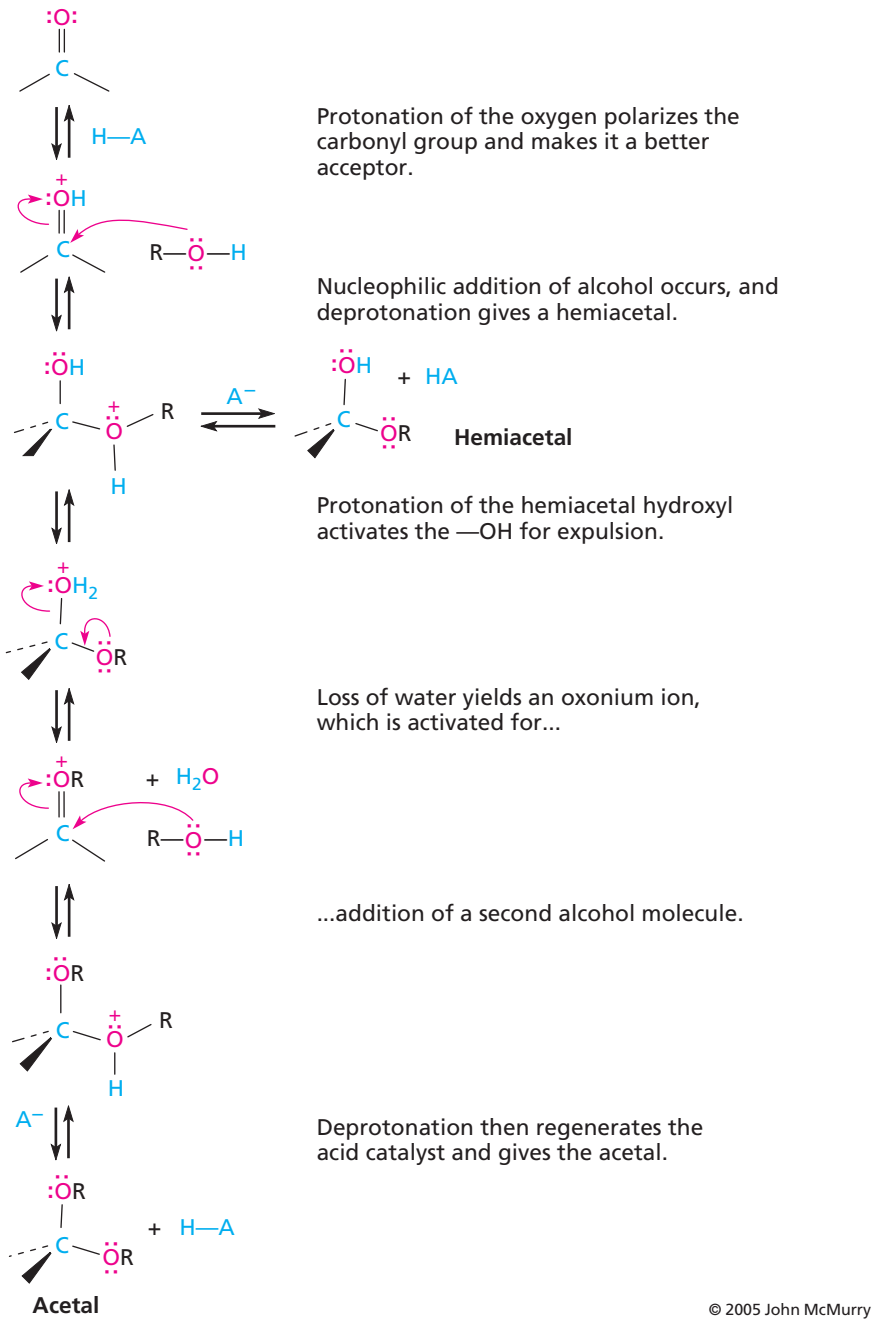
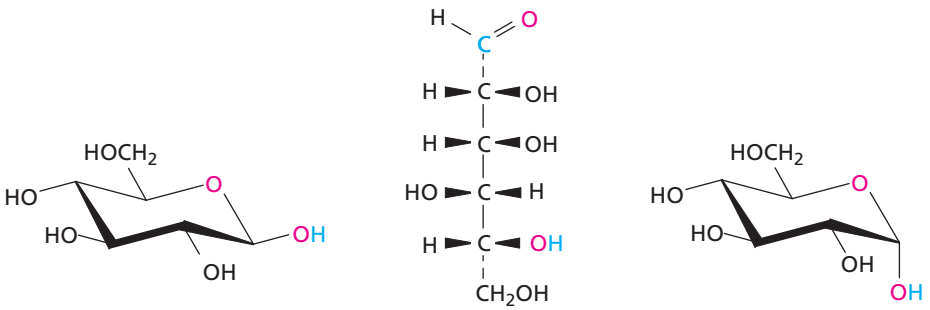


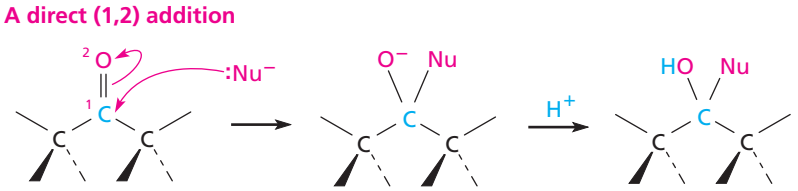
FIGURE 1.11 Mechanism of acid-catalyzed hemiacetal and acetal formation by reaction of an aldehyde or ketone with an alcohol.

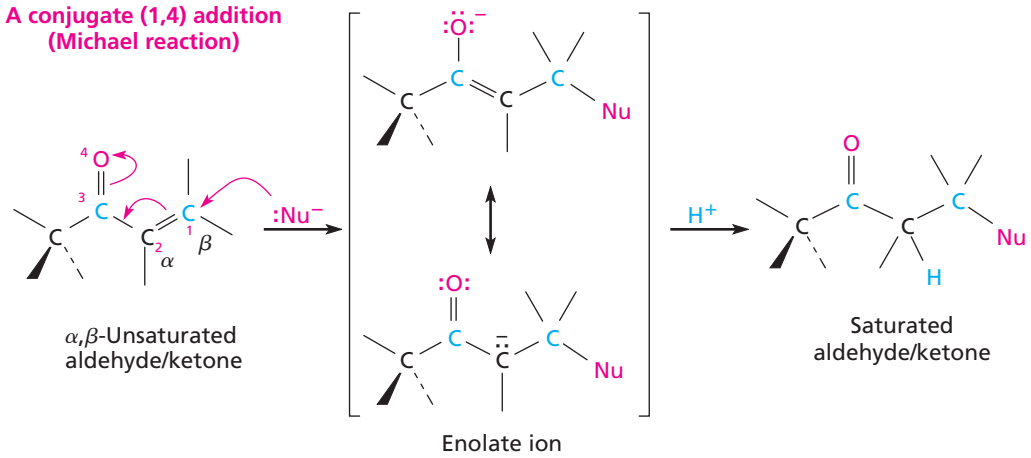
The formation of hemiacetals and acetals is a central part of carbohydrate chemistry. Glucose, for instance, is in a readily reversible equilibrium between open (aldehyde + alcohol) and closed (cyclic hemiacetal) forms. Many glucose molecules can then join together by acetal links to form starch and cellulose (Section 4.1).



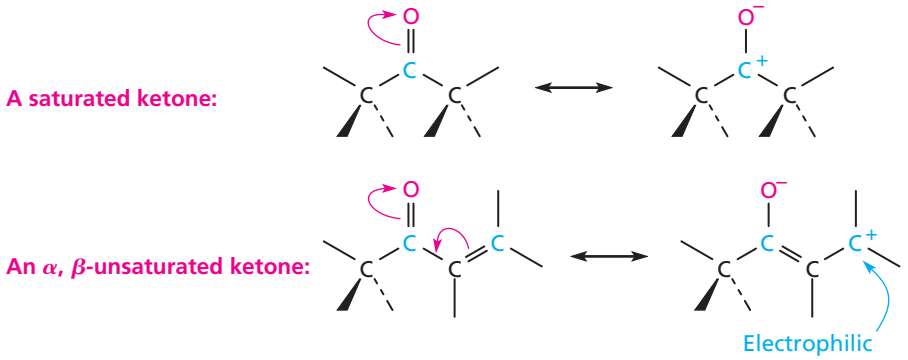
Conjugate (1,4) Nucleophilic Additions

Closely related to the direct (1,2) addition of a nucleophile to the C═O bond of an aldehyde or ketone is the **conjugate (1,4) addition**, or **Michael reaction**, of a nucleophile to the C═C bond of an α,β-unsaturated aldehyde or ketone (or thioester). The initial product, a resonance-stabilized **enolate ion**, typically undergoes protonation on the α carbon (the carbon *next to* the C═O) to give a saturated aldehyde or ketone (or thioester) product.

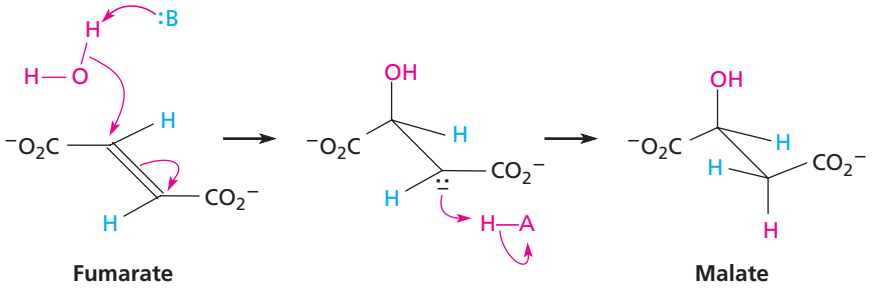




Note that this conjugate addition reaction gives an overall result similar to that of the electrophilic alkene addition discussed in Section 1.3, but the mechanisms of the two processes are entirely different. Isolated alkenes react with *electrophiles* and form carbocation intermediates; α,β-unsaturated carbonyl compounds react with *nucleophiles* and form enolate-ion intermediates. Conjugate addition occurs because the electronegative oxygen atom of the α,β-unsaturated carbonyl compound withdraws electrons from the β carbon, thereby making it more electron-poor and more electrophilic than a typical alkene C═C bond.



Among the many biological examples of conjugate additions is the conversion of fumarate to malate by reaction with water, a step in the citric acid cycle by which acetate is metabolized to CO_2.

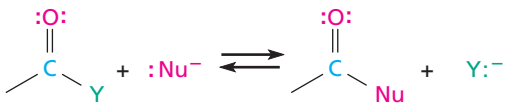


Fumarate Malate

1.6 Mechanisms: Nucleophilic Acyl Substitution Reactions

Carboxylic acids and their derivatives are characterized by the presence of an electronegative atom (O, N, S) bonded to the carbonyl carbon. As a result, these compounds can undergo **nucleophilic acyl substitution reactions**—the substitution of the leaving group bonded to the carbonyl carbon (:Y) by an attacking nucleophile ($:Nu^-$).

A nucleophilic acyl substitution reaction



As shown in Figure 1.12 for the reaction of OH^- with methyl acetate, a nucleophilic acyl substitution reaction is initiated by addition of the nucleophile to the carbonyl carbon in the usual way. But the tetrahedrally hybridized alkoxide intermediate is not isolated; instead, it reacts further by expelling the leaving group and forming a new carbonyl compound. The overall result of nucleophilic acyl substitution is the replacement of the leaving group by the attacking nucleophile, just as occurs in the nucleophilic *alkyl* substitutions discussed in Section 1.4. Only the *mechanisms* of the two substitutions are different.

Both the addition step and the elimination step can affect the overall rate of a nucleophilic acyl substitution reaction, but the first step is generally rate-limiting. Thus, the greater the stability of the carbonyl compound, the less reactive it is. We therefore find that, of the carbonyl-containing functional groups commonly

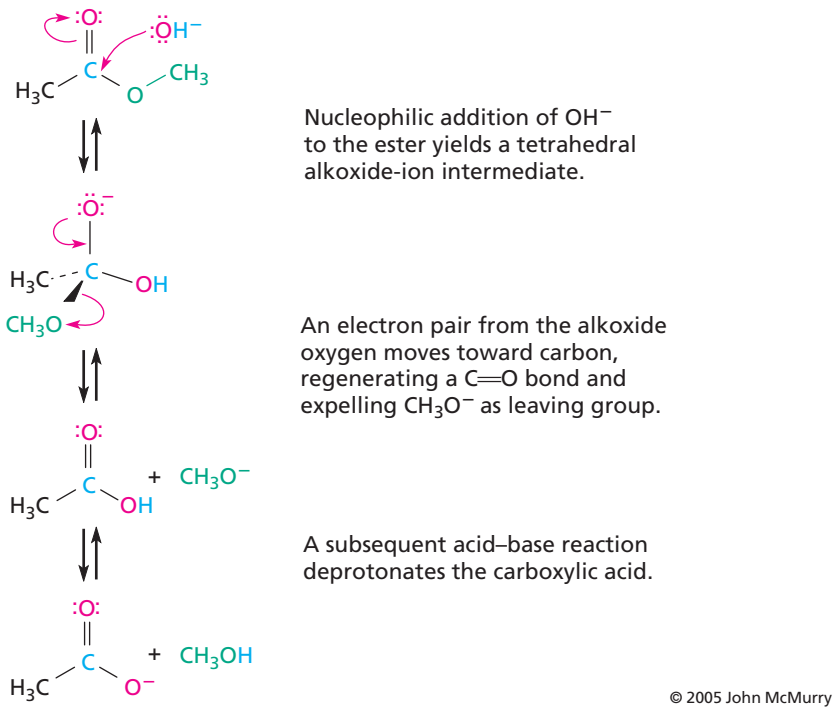
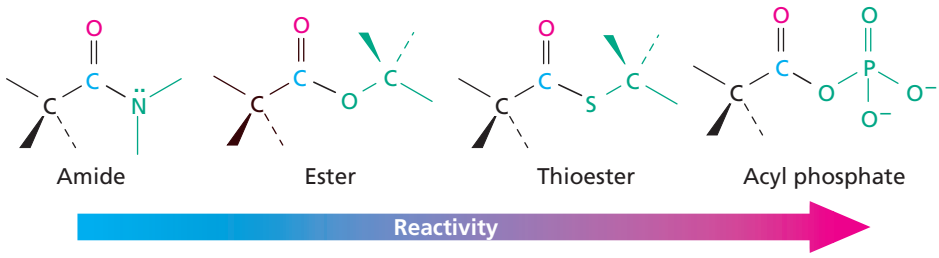
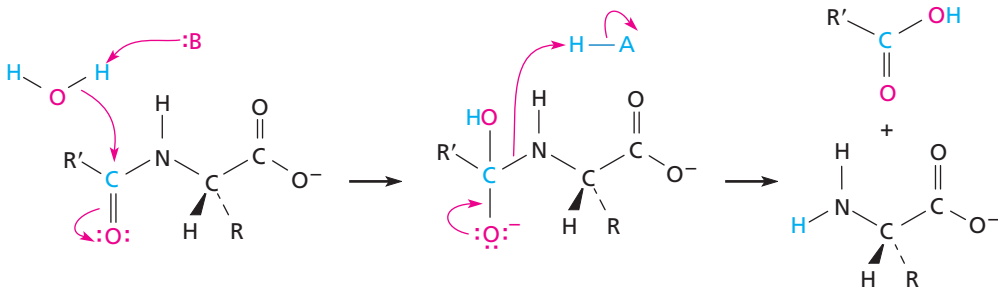


FIGURE 1.12 Mechanism of the nucleophilic acyl substitution reaction of OH^- with methyl acetate to give acetate. The reaction occurs by a nucleophilic addition to the carbonyl group, followed by expulsion of the leaving group in a second step.

found in living organisms, amides are the least reactive because of resonance stabilization; esters are somewhat more reactive; and thioesters and acyl phosphates are the most reactive toward substitution. (Acyl phosphates are generally further activated for substitution by complexation with a Lewis acidic metal cation such as Mg^{2+}.)

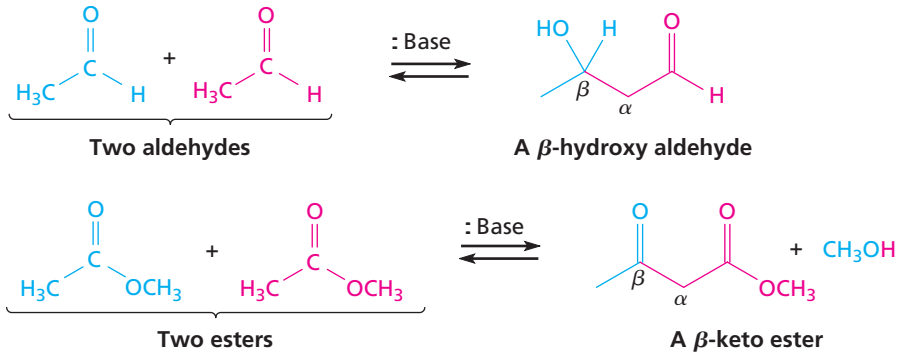


Nucleophilic acyl substitution reactions occur frequently in biochemistry. For example, the carboxypeptidase-catalyzed hydrolysis of the C-terminal amide bond in proteins is one such process.

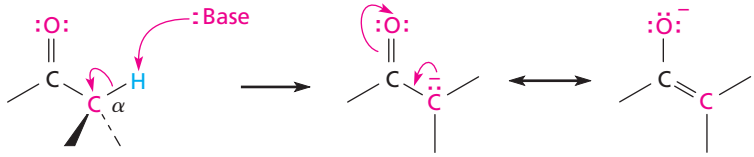


1.7 Mechanisms: Carbonyl Condensation Reactions

The third major reaction of carbonyl compounds, **carbonyl condensation**, occurs when two carbonyl compounds join to give a single product. When an aldehyde is treated with base, for example, two molecules combine to yield a β-hydroxy aldehyde product. Similarly, when an ester is treated with base, two molecules combine to yield a β-keto ester product.



Carbonyl condensation results in bond formation between the carbonyl carbon of one partner and the α carbon of the other partner. The reactions occur because the α hydrogen of a carbonyl compound is weakly acidic and can therefore be removed by reaction with a base to yield an enolate ion. Like other anions, enolate ions are nucleophiles.



A carbonyl compound **An enolate ion**

The acidity of carbonyl compounds is due to resonance stabilization of the enolate ion, which allows the negative charge to be shared by the α carbon and the electronegative carbonyl oxygen. As shown in Table 1.4, aldehydes and ketones are the most acidic monocarbonyl compounds, with thioesters, esters,

Table 1.4 Acidity Constants of Some Carbonyl Compounds

Carbonyl compound	Example	pK_a	
Carboxylic acid	CH_3COH	4.7	Stronger acid
1,3-Diketone	$CH_3CCH_2CCH_3$	9.0	
β-Keto ester	$CH_3CCH_2COCH_3$	10.6	
1,3-Diester	$CH_3OCCH_2COCH_3$	12.9	
Aldehyde	CH_3CH	17	
Ketone	CH_3CCH_3	19.3	
Thioester	CH_3CSCH_3	21	
Ester	CH_3COCH_3	25	
Amide	$CH_3CN(CH_3)_2$	30	Weaker acid

and amides less so. Most acidic of all, however, are 1,3-*dicarbonyl* compounds (β-dicarbonyl compounds), which have an α position flanked by *two* adjacent carbonyl groups.

The condensation of an aldehyde or ketone is called the **aldol reaction** and takes place by the mechanism shown in Figure 1.13. One molecule reacts with base to give a nucleophilic enolate ion, which then adds to the second molecule in a nucleophilic addition reaction. Protonation of the initially formed alkoxide ion yields the neutral β-hydroxy aldehyde or ketone product. Note that the condensation is reversible: A β-hydroxy aldehyde or ketone can fragment on treatment with base to yield two molecules of aldehyde or ketone.

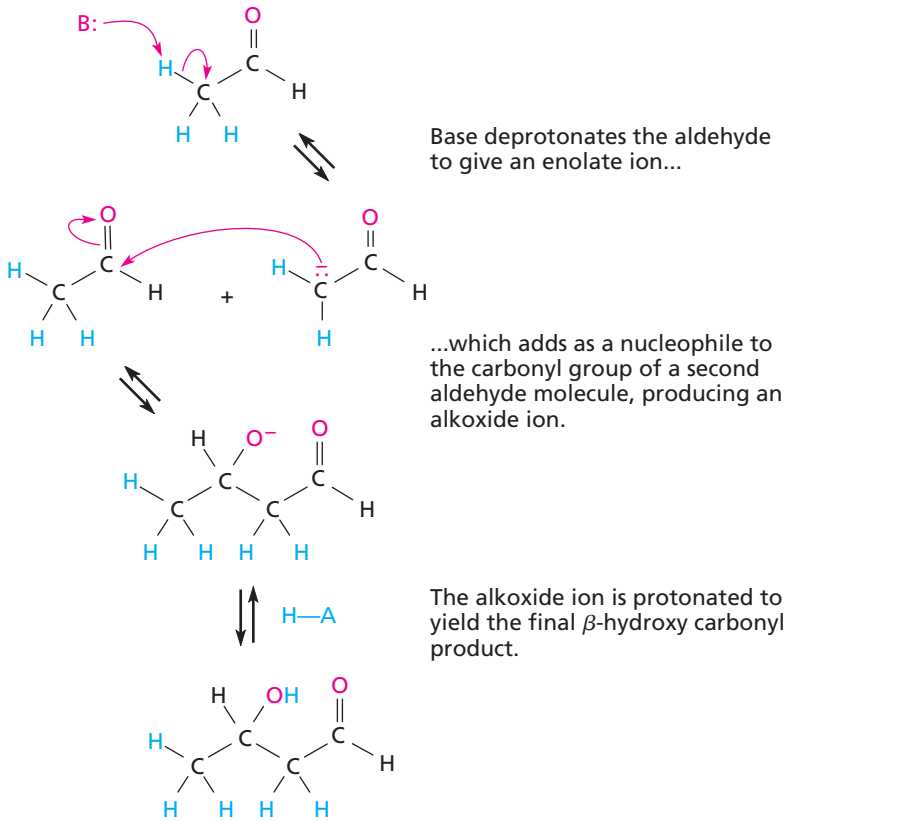


FIGURE 1.13 Mechanism of the aldol reaction, a reversible, base-catalyzed condensation reaction between two molecules of aldehyde or ketone to yield a β-hydroxy carbonyl compound. The key step is nucleophilic addition of an enolate ion to a C═O bond.

The condensation of an ester is called the **Claisen condensation reaction** and takes place by the mechanism shown in Figure 1.14. One molecule reacts with base to give a nucleophilic enolate ion, which adds to the second molecule in a nucleophilic acyl substitution reaction. The initially formed alkoxide ion

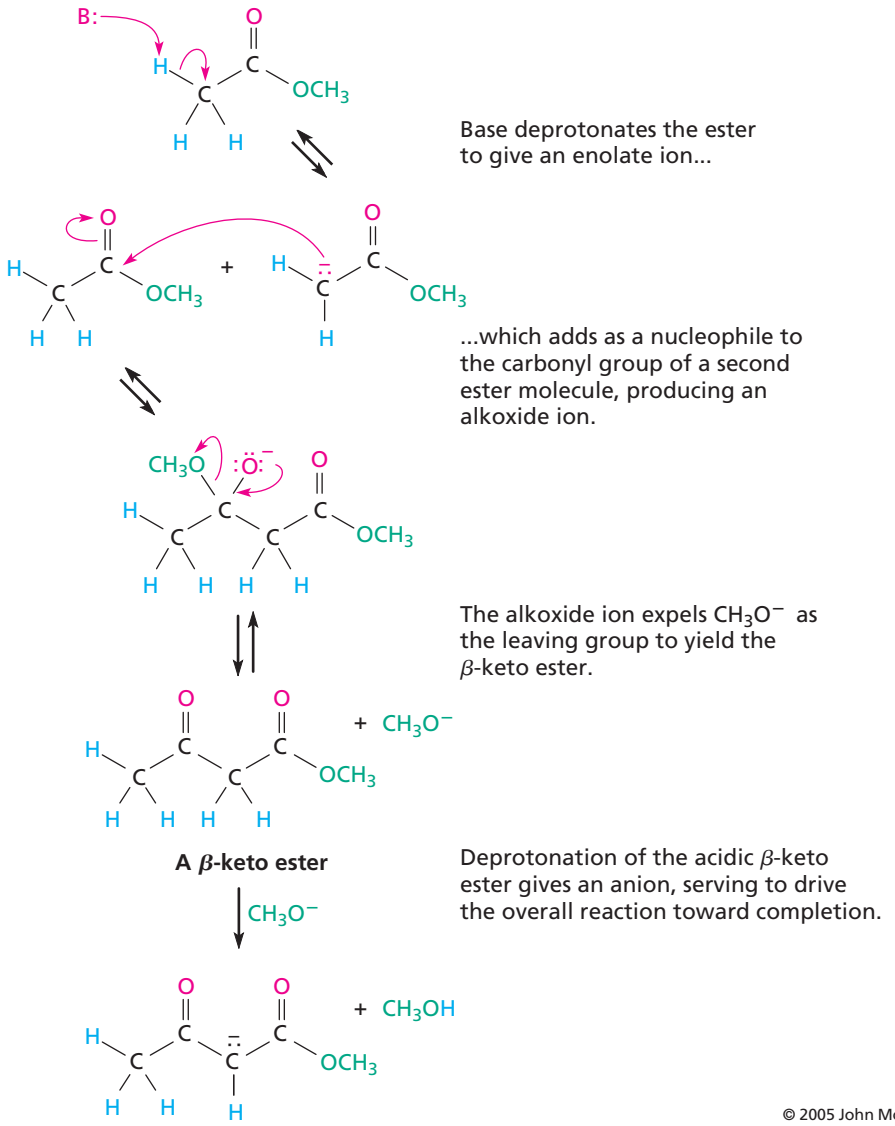
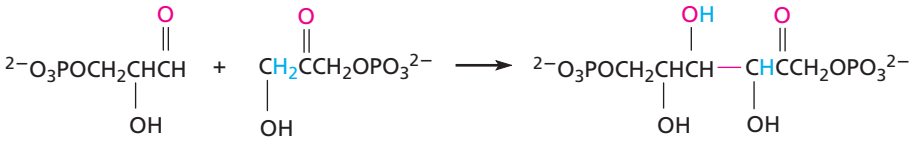


FIGURE 1.14 Mechanism of the Claisen condensation reaction, a reversible, base-catalyzed condensation reaction between two molecules of ester to yield a β-keto ester. The key step is nucleophilic acyl substitution by an enolate ion, with expulsion of an alkoxide leaving group.

expels CH_3O^- as the leaving group to regenerate a C═O bond and form the β-keto ester product. As with the aldol reaction, the Claisen condensation is reversible: A β-keto ester can fragment on treatment with base to yield two molecules of ester.

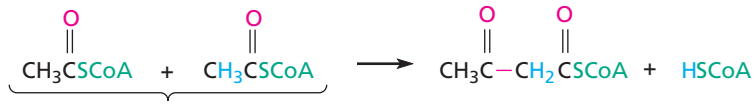
Carbonyl condensation reactions are involved in nearly all biochemical pathways and serve as the primary biological method for forming and breaking carbon–carbon bonds. For instance, one step in the biosynthesis of glucose from pyruvate is the aldol reaction of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate. As another example, the biological pathway for terpenoid and steroid biosynthesis begins with a Claisen condensation of the thioester acetyl CoA (coenzyme A) to give acetoacetyl CoA. We'll look at the details of glucose biosynthesis in Section 4.5 and the details of steroid biosynthesis in Section 3.6.

Aldol reaction



Glyceraldehyde 3-phosphate **Dihydroxyacetone phosphate** **Fructose 1,6-bisphosphate**

Claisen condensation reaction



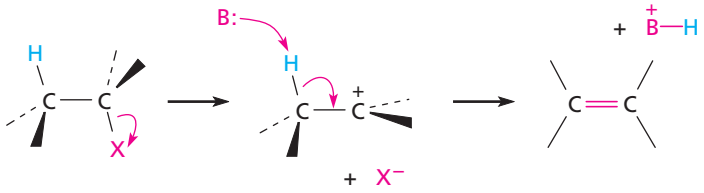
Acetyl CoA **Acetoacetyl CoA**

1.8 Mechanisms: Elimination Reactions

The elimination of HX to yield an alkene appears to be the simple reverse of an electrophilic addition of HX. In fact, though, **elimination reactions** are a good deal more complex than additions and can occur by any of several mechanisms. In the laboratory, the three most common processes are the E1, E2, and E1cB reactions, which differ in the timing of C—H and C—X bond-breaking. In the E1 reaction, the C—X bond breaks first to give a carbocation intermediate, which then undergoes base abstraction of H^+ to yield the alkene (the exact reverse of the electrophilic addition reaction described in Section 1.3). In the E2

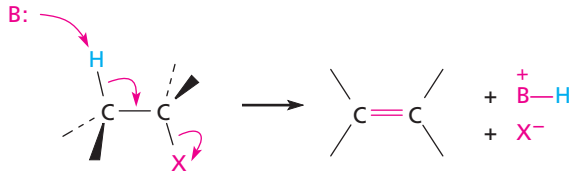
reaction, base-induced C—H bond cleavage is simultaneous with C—X bond cleavage, giving the alkene in a single step. In the E1cB reaction (cB for "conjugate base"), base abstraction of the proton occurs first, giving a carbanion intermediate that undergoes loss of X^- in a subsequent step to give the alkene.

E1 Reaction: C–X bond breaks first to give a carbocation intermediate, followed by base removal of a proton to yield the alkene.

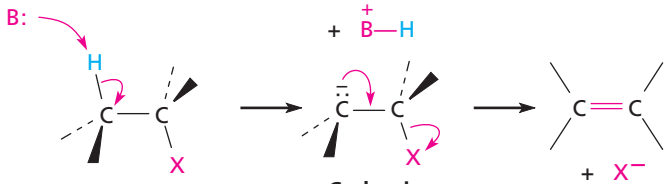


Carbocation

E2 Reaction: C–H and C–X bonds break simultaneously, giving the alkene in a single step without intermediates.



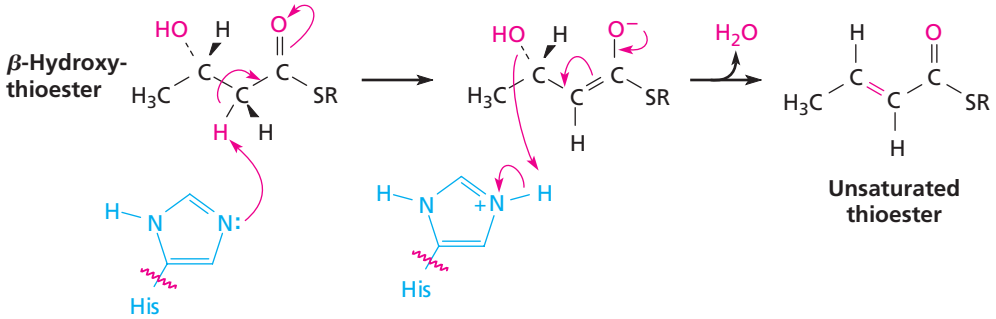
E1cB Reaction: C–H bond breaks first, giving a carbanion intermediate that loses X^- to form the alkene.



Carbanion

Examples of all three mechanisms occur in different biological pathways, but the E1cB mechanism is particularly common. The substrate is usually an alcohol (X = OH) or protonated alcohol (X = OH_2^+), and the H atom that is removed is usually made acidic, particularly in E1cB reactions, by being adjacent to a carbonyl group. Thus, β-hydroxy carbonyl compounds (aldol reaction products) are frequently converted to α,β-unsaturated carbonyl compounds by elimination

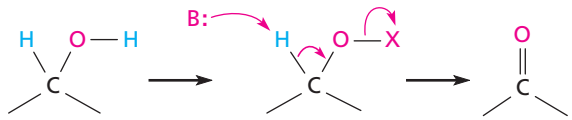
reactions. An example is the dehydration of a β-hydroxy thioester to the corresponding unsaturated thioester, a reaction that occurs in fatty-acid biosynthesis (Section 3.4). The base in this reaction is a histidine residue in the enzyme, and the elimination is assisted by complexation of the —OH group to the protonated histidine as a Lewis acid. Note that the reaction occurs with *syn* stereochemistry, meaning that the —H and —OH groups in this example are eliminated from the same side of the molecule.



1.9 Oxidations and Reductions

Oxidation–reduction, or **redox**, chemistry is a large and complicated, but extremely important, topic. Rather than attempt a complete catalog of the subject at this point, however, let's focus on the mechanisms of two of the more commonly occurring biological redox processes: the oxidation of an alcohol and the reduction of a carbonyl compound. We'll look at the mechanisms of other kinds of biological oxidations in later chapters as the need arises when we discuss specific pathways.

In the laboratory, alcohol oxidations generally occur through a mechanism that involves attachment to oxygen of a leaving group X, usually a metal in a high oxidation state such as Cr(VI) or Mn(VII). An E2-like elimination reaction then forms the C═O bond and expels the metal in a lower oxidation state. Note that the C—H hydrogen is removed by base as H^+ during the elimination step.



Although a similar mechanism does occasionally occur in biological pathways, many biological oxidations of an alcohol occur by a reversible hydride-transfer

mechanism involving one of the coenzymes NAD^+ (oxidized nicotinamide adenine dinucleotide) or $NADP^+$ (oxidized nicotinamide adenine dinucleotide phosphate). As shown in Figure 1.15, the reaction occurs in a single step without intermediates when a base B: abstracts the acidic O—H proton, the electrons from the O—H bond move to form a C═O bond, and the hydrogen attached to carbon is transferred to NAD^+. Note that the C—H hydrogen is transferred as H^-, in contrast to the typical laboratory oxidation where it is removed as H^+. Note also that the hydride ion adds to the C═C—C═N^+ part of NAD^+ in a conjugate nucleophilic addition reaction, much as water might add to the C═C—C═O part of an α,β-unsaturated ketone (Section 1.5).

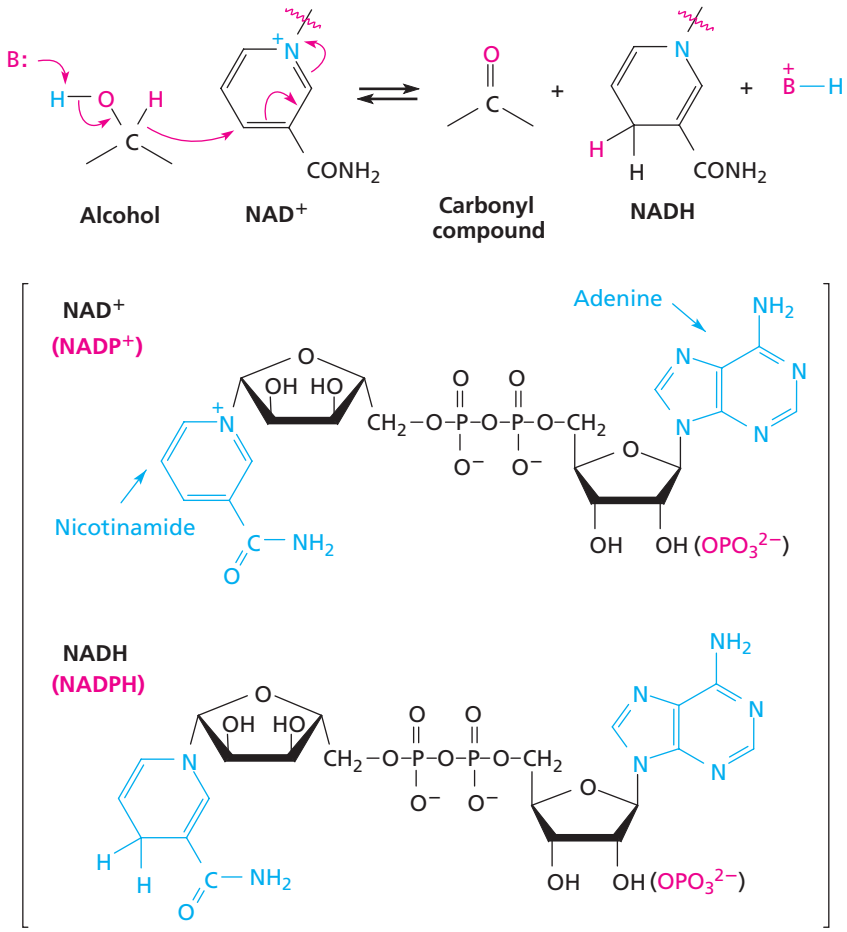
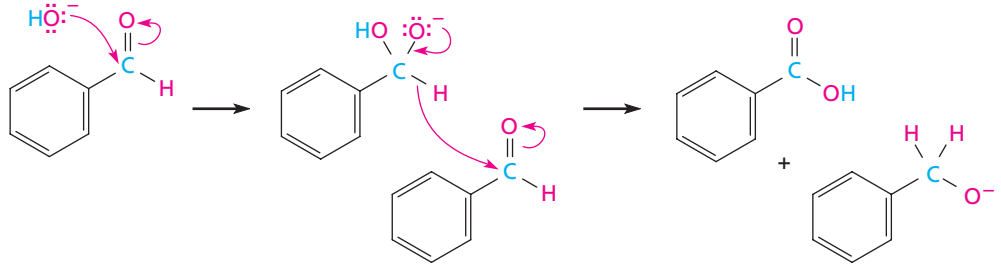


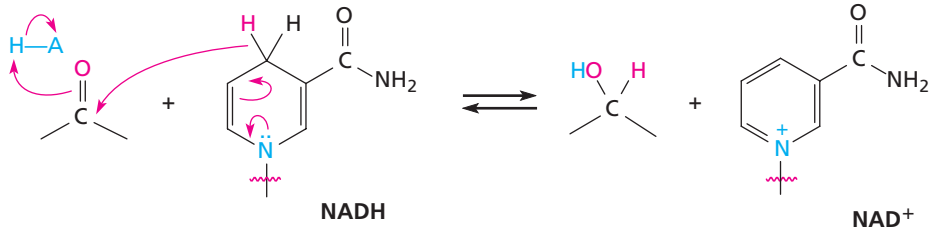
FIGURE 1.15 Mechanism of alcohol oxidation by NAD^+.

Expulsion of hydride ion is not often seen in laboratory chemistry because, as noted at the beginning of Section 1.5, H^- is a poor leaving group. One analogous process that does occur in laboratory chemistry, however, is the Cannizzaro reaction, which involves the disproportionation of an aromatic aldehyde on treatment with base. Benzaldehyde, for example, is converted to a mixture of benzyl alcohol and benzoic acid by reaction with NaOH. Hydroxide ion first adds to the aldehyde carbonyl group to give an alkoxide intermediate, which transfers a hydride ion to a second molecule of aldehyde. The first aldehyde is thereby oxidized, and the second aldehyde is reduced.

Cannizzaro reaction



Biological reductions are the reverse of oxidations. As noted in Section 1.5, NADH transfers a hydride ion to the carbonyl group in a nucleophilic addition reaction, and the alkoxide intermediate is protonated.



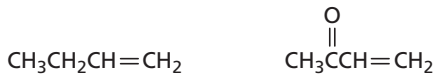
Problems

1.1 Which of the following substances can behave as either an acid or a base, depending on the circumstances?

(a) CH_3SH **(b)** Ca^{2+} **(c)** NH_3 **(d)** CH_3SCH_3

(e) NH_4^+ **(f)** $H_2C{=}CH_2$ **(g)** $CH_3CO_2^-$ **(h)** $^+H_3NCH_2CO_2^-$

1.2 Which C=C bond do you think is more nucleophilic, that in 1-butene or that in 3-buten-2-one? Explain.



1-Butene **3-Buten-2-one**

1.3 Rank the following compounds in order of increasing acidity:

(a) Acetone, $pK_a = 19.3$ **(b)** Phenol, $pK_a = 9.9$

(c) Methanethiol, $pK_a = 10.3$ **(d)** Formic acid, $K_a = 1.99 \times 10^{-4}$

(e) Ethyl acetoacetate, $K_a = 2.51 \times 10^{-11}$

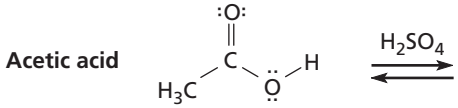
1.4 Rank the following compounds in order of increasing basicity:

(a) Aniline, $pK_{a(BH^+)} = 4.63$ **(b)** Pyrrole, $pK_{a(BH^+)} = 0.4$

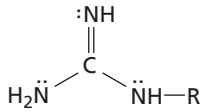
(c) Dimethylamine, $pK_{a(BH^+)} = 10.5$

(d) Ammonia, $K_{a(BH^+)} = 5.5 \times 10^{-10}$

1.5 Protonation of acetic acid by H_2SO_4 might occur on either of two oxygen atoms. Draw resonance structures of both possible products, and explain why protonation occurs preferentially on the double-bond oxygen.



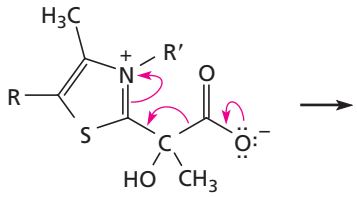
1.6 Protonation of a guanidino compound occurs on the double-bond nitrogen rather than on either of the single-bond nitrogens. Draw resonance structures of the three possible protonation products, and explain the observed result.



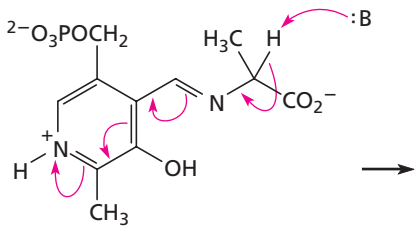
A guanidino compound

1.7 Predict the product(s) of the following biological reactions by interpreting the flow of electrons as indicated by the curved arrows:

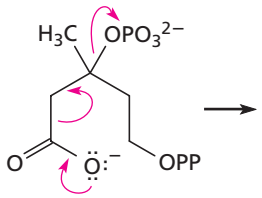
(a)



(b)

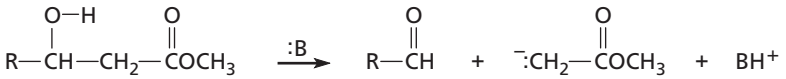


(c)

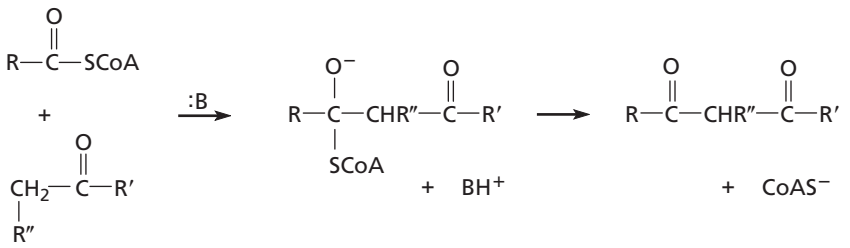


1.8 Complete the following biological mechanisms by adding curved arrows to indicate electron flow:

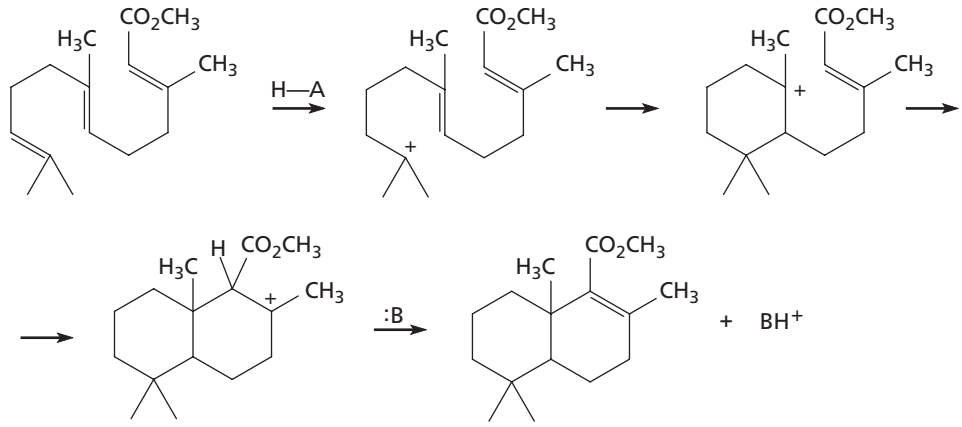
(a)



(b)

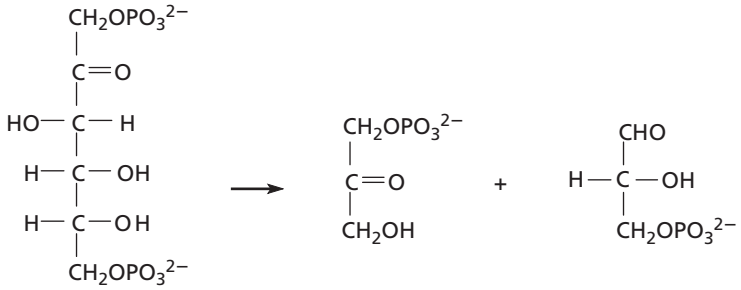


(c)



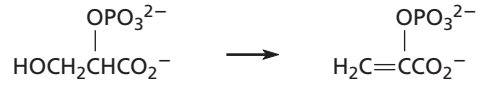
1.9 Propose a mechanism for the following step in the β-oxidation pathway for degradation of fatty acids. HSCoA is the abbreviation for coenzyme A, a thiol.

1.10 The conversion of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate is a step in the glycolysis pathway for degrading carbohydrates. Propose a mechanism.



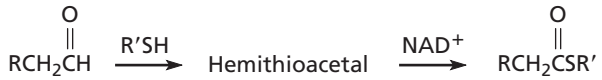
Fructose 1,6-bisphosphate | **Dihydroxyacetone phosphate** | **Glyceraldehyde 3-phosphate**

1.11 Propose a mechanism for the conversion of 3-phosphoglycerate to phosphoenolpyruvate (PEP), a step in the glycolysis pathway.

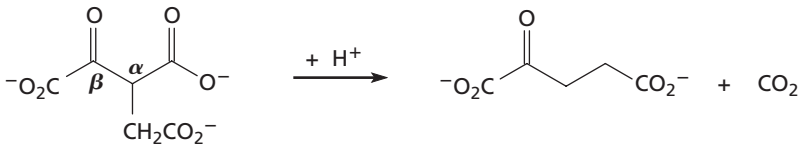


2-Phosphoglycerate | **Phosphoenolpyruvate**

1.12 The biological conversion of an aldehyde to a thioester occurs in two steps: (1) nucleophilic addition of a thiol to give a hemithioacetal, and (2) oxidation of the hemithioacetal by NAD^+. Show the structure of the intermediate hemithioacetal, and propose mechanisms for both steps.

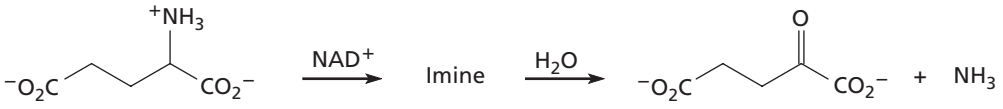


1.13 The loss of CO_2 (decarboxylation) from a β-keto acid happens frequently in biological chemistry and takes place by a mechanism that is closely related to a retroaldol reaction. Propose a mechanism for the following reaction that occurs in the citric acid cycle.



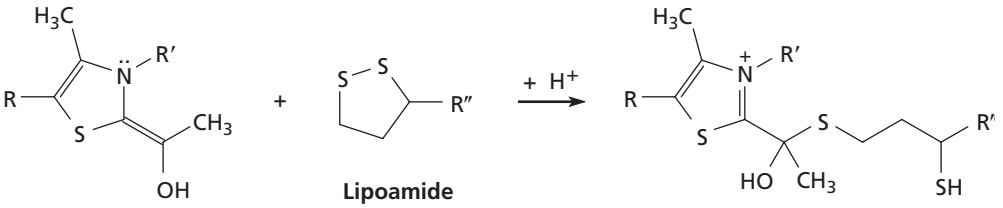
A β-keto acid **α-Ketoglutarate**

1.14 One of the biological pathways by which an amine is converted to a ketone involves two steps: (1) oxidation of the amine by NAD^+ to give an imine, and (2) hydrolysis of the imine to give a ketone plus ammonia. Glutamate, for instance, is converted by this process into α-ketoglutarate. Show the structure of the imine intermediate, and propose mechanisms for both steps.



Glutamate **α-Ketoglutarate**

1.15 The following reaction is part of the sequence by which pyruvate is converted to acetyl CoA. Propose a mechanism, and tell what kind of reaction is occurring.



Lipoamide

1.16 The amino acid methionine is formed by a methylation reaction of homocysteine with *N*-methyltetrahydrofolate. The stereochemistry of the reaction has been probed by carrying out the transformation using a donor with a "chiral methyl group" in which deuterium (D) and tritium (T) isotopes of hydrogen are present. Does the methylation reaction occur with inversion or retention of configuration? What mechanistic inferences can you draw?