ORGANIC CHEMISTRY REVIEW

At Med-Pathway, we love Organic Chemistry (O Chem) and so does the MCAT. OChem provides a very good foundation for understanding various aspects of medicine including biochemistry, drug development, and drug design. Med-Pathway’s very own Dr. Phil Carpenter studied O Chem under the world famous Dr. Bruice at UCSB and was a Master Trainer and Teacher for MCAT© Ochem for years.

We present a comprehensive subject review. The website has nearly 100 challenging assessment questions/passages to prepare you for O Chem mastery on the MCAT. Med-Pathway O Chem Review has been derived from a careful consideration of the AAMC MCAT© Content Guideline. Stereochemistry and Amino Acids and Proteins are discussed in detail in other subject modules available on the website. This module is divided into three sections. The following topics are discussed and assessed in each chapter of this module.

Chapter I

- Carbon: Bonding, Hybridization, and Saturation
- Induction and Resonance
- General Nomenclature
- Alkanes (Free radical halogenation, Conformation of cycloalkanes)
- Alkenes
- Alcohols (Nomenclature, Physical Properties, Acidity, Synthesis, Oxidation, S_N1 and S_N2 Substitution Reactions, Preparation of Mesylates and Tosylates, Protection of Alcohols)
- Carbonyl Chemistry: Aldehydes & Ketones (Physical Preparation & Synthesis, Oxidation-Reduction, Keto-enol tautomerization, Aldol condensation reactions, kinetic & thermodynamic enolates, formation of acetals & hemiketals)
- Cyanohydrins

CHAPTER I

Carbon

Carbon is central to organic chemistry. The stability of carbon-carbon bonds is an essential reason carbon is a major ingredient in important biopolymers such as proteins, nucleic acids, and polysaccharides. Because carbon is an electro
neutral atom, its bonding to more electronegative atoms generates polar bonds (dipoles) that formulate potential sites of chemistry. We will see this point over and over.

With an atomic number of six, carbon has four electrons that participate in the generation of covalent bonds as shown below. Recall that the orbitals of valence electrons combine to form new hybrid orbitals. Be familiar with how hybridization of orbitals and molecular shapes are related.

![molecular structures](image)

Single bonds are known as sigma bonds (σ) and double bonds are also called pi bonds (π) bonds. A double bond contains one σ and one π bond. A triple bond contains one σ and two π bonds.

There are a few points regarding hybridization that are important to know:

1) The greater the s character, the greater the bond dissociation energy.
2) The greater the s character, the shorter and stronger the bond.
3) Therefore, carbon-carbon triple bonds are shorter than double bonds, but require more energy to dissociate as they have higher bond dissociation energies.
4) Acidity is inversely proportional to s character. Thus, the hydrogen atoms in acetylene (C₂H₂), a molecule that has a triple bond, are more acidic than the hydrogen atoms in ethylene (C₂H₄), a molecule that has a single double bond.

Degrees of unsaturation

The number of double bonds and/or rings in a compound is referred to as its degrees (or units) of unsaturation. This can be figured out given the molecular
formula of the compound. Remember that all hydrocarbons have an even number of hydrogen atoms, otherwise they have charged carbon atoms (+ or -) or free radicals.

Saturated compounds are those that contain only sigma bonds and have all carbons with a maximal number of bonded hydrogen atoms (2N +2 where N = the number of carbons atoms). A general formula for the degrees of unsaturation of a compound can be described as:

**Degrees of unsaturation = 2N + 2 –X/2**

where

C = # of carbon atoms

N = # of hydrogen atoms

X = # Halogens

H = # Hydrogen atoms

# Oxygen atoms = 0

And nitrogen atoms  n = n +1, x = x +1

Using this formula, the number of units (degrees) of unsaturation of benzene (C₆H₆) can be calculated as:

Degrees of unsaturation = 2N + 2 –X/2

2(6) + 2 -6/2 = 4 units of unsaturation. This should make sense as benzene has one ring and 3 double bonds.

**Chemical Induction**

In chemistry, induction refers to the uneven distribution of electrons within a sigma bond. Although bonds are usually depicted in Lewis dot structures as well as stick structures as equally sharing electrons, the transmission of charge through dipoles often unequally distributes electrons. Such polarization of electrons (i.e. induction) within bonds is largely determined by the electronegativity of the participating atoms. The strength of inductive groups depends on distance from the group and the atom that it is acting on.
The image below shows some important examples of induction. **Panel A** shows water and the carbonyl unit. Note the presence of the electronegative oxygen atoms bound to either hydrogen (water) or carbon (carbonyl). As oxygen is more electronegative than either hydrogen or carbon, the electrons in the bond are not shared equally, resulting in dipole moments. This is reflected in the partial positive and negative charges.

**Panel B** shows three carbocations (tertiary, secondary, and primary). In contrast to oxygen, alkyl groups donate their electrons through induction. As a consequence, tertiary carbocations are more stable than secondary and primary carbocations as the relative induction of electrons reduces the overall positive charge on the carbon. Recall that carbon is electro neutral and more stable carbocations have charges more towards neutrality.

**Panel C** shows chlorinated derivatives of acetic acid. As chlorine is an electronegative halogen, it will draw electrons towards itself, even over distances. Notice that the pKₐ of acetic acid is normally 4.86, but decreases as a function of the number of chlorine atoms added to the molecule increases. The pKₐ of trichloroacetic acid is 0.7. This is due to the inductive effects of chlorine. As more electron withdrawing groups are added to acetic acid, the O-H bond becomes more acidic and more capable of releasing the proton.
Resonance

The structure of molecules is commonly described through Lewis structures. However, some molecules can be drawn with more than one Lewis structure. In some cases, delocalized pi electrons exist as resonance hybrid structures and can be described through multiple Lewis structures. Importantly, each form contributes in proportion to the overall stability of the molecule. Resonance structures are not transient states, but rather the true molecular structure is the resonance hybrid and this structure represents the overall lowest total energy.

Benzene and Resonance

![Benzene Resonance Structures](image)

<table>
<thead>
<tr>
<th>BOND</th>
<th>LENGTH (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td>1.54</td>
</tr>
<tr>
<td>C=O</td>
<td>1.34</td>
</tr>
<tr>
<td>C-C (Benzene)</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Therefore, resonance is important for molecular stability. The resonance structures for benzene ($C_6H_6$) are shown. Note that two resonance structures of benzene can be written in the Lewis dot format. As written, benzene has both single and double carbon-carbon bonds, but the average bond length for the carbon-carbon bonds in benzene falls in between what is expected for single and double bonds (see image). That is, the bond lengths in benzene are in between single and double bonds due to resonance delocalization of the pi electrons.
electrons. Note that the pi electrons can move from one space to another, but the atoms themselves do not move. This is shown for aniline.

![Resonance Structures of Aniline](image)

Notice that in the resonance structures of aniline, the negative charges are in the ortho and para positions and this explains why addition of groups to aniline occur at these two positions.

**The basic rules for determining the most optimal resonance structures are as follows:**

1) Maintain the octet rule for atoms
2) Minimize formal charges, but electronegative atoms prefer negative charges
3) Maintain aromaticity
4) Minimize formal charges
5) Minimize separation of charges

**Nomenclature**

Common and IUPAC names will be used on the MCAT. The general rules are as follows:

A) Count the longest continuous carbon chain.
B) Assign numbers to carbon atoms in the chain such that the sum of the attached substituent groups is the lowest.
C) Name the substituent groups.
D) Name the compound using substituent groups in alphabetical order.

In some cases, the functional groups can be named as the parental compound in terms of the substituents.
Let’s examine some examples. More will be presented as the chemistry of each functional group is discussed.

Organic compounds contain a large array of functional groups. Alkanes, alkenes, and alkynes differ in the number of double bonds between carbon-carbon covalent bonds. The following functional groups will appear on the MCAT and will be discussed here. Other groups such as alkenes and ethers will also be examined. Alkynes are rare in biological systems and do not appear to be naturally present in humans.
ALKANES

Saturated hydrocarbons are known as alkanes and have the formula of:

\[ C_nH_{2n+2} \]

They are relatively unreactive, but undergo two significant reactions:

A) **Combustion reactions.** In the presence of heat and oxygen, alkanes are oxidized to \( CO_2 \) and \( H_2O \). These exothermic reactions release a lot of heat. Think of burning gas in your car.

B) **Free radical substitution**

Alkanes can be halogenated in free radical reactions. The resulting alkyl halides are often used further as substrates in additional reactions. Free radicals are highly reactive molecules. The reaction occurs in the presence of light or heat.
to induce homolytic dissociation of the diatomic halogen. All free radical reactions occur with three major steps:

1) Initiation 2) Propagation 3) Termination. Shown in the figure is the free radical chlorination of methane, the simplest alkane.

**Free Radical Halogenation**

<table>
<thead>
<tr>
<th>Initiation</th>
<th>Propagation (2 Steps)</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Initiation: Diatomic Chlorine Molecule Split" /></td>
<td><img src="image" alt="Propagation: Methane and Chlorine Interaction" /></td>
<td><img src="image" alt="Termination: Chlorine atoms colliding" /></td>
</tr>
</tbody>
</table>

**Initiation**: The diatomic chlorine molecule is split in a homolytic fashion by light or heat. Homolytic cleavage generates free radical chlorine atoms that contain one unpaired electron. Note that initiation increases the number of free radicals in the system.

**Propagation**: This occurs in two steps. The methane alkane interacts with the free radical chlorine atom. A hydrogen atom is taken from the alkane, generating a primary, free radical intermediate and another chlorine free radical. This initiates a chain reaction. In the second step, the alkyl free radical collides with a diatomic chloride molecule forming the alkyl halide and another chloride radical.

**Termination**: Any reaction that decreases the number of free radicals in the system is a termination event. Two termination reactions are shown above.
Stereochemistry in Free Radical Halogenation

Free radical halogenation reactions often produce stereoisomers. Before we examine a specific example, let’s review some basic tenants of stereoisomers.

Isomers that differ in their 3D-spatial arrangement of atoms are termed stereoisomers. Any molecule that cannot be superimposed on its mirror image exhibits chirality. The simplest way to envision chirality is to note that your left hand cannot be superimposed on your right hand: they are mirror images of each other. Chirality refers to “handedness” and exists throughout nature. Helices in nucleic acids and proteins have handedness. Thus, chirality is a term referring to handedness or symmetry, and is an intrinsic property of stereoisomers.

A molecule and its non-superimposable mirror image are called enantiomers. The two enantiomers of the amino acid alanine are shown below. The red asterisk designates the chiral carbon stereocenter. The two molecules are non-superimposable mirror images. Enantiomers possess identical physical properties (i.e. boiling and melting points) with the exception of how they interact with light. This is further elaborated on in the Med-Pathway Stereochemistry and Isomers Testing Module. A 1:1 mixture of enantiomers is called a racemic mixture. Any ratio of enantiomers deviating from 1:1 is called a scalemic mixture.

Stereoisomers possess one or more stereogenic atoms or stereocenters. According to Mislow and Siegel, a stereocenter represents a position in a molecule where the exchange of two groups generates a stereoisomer. Stereocenters are often referred to as chiral or asymmetric centers. Although many atoms can exhibit chirality, the MCAT will largely, if not exclusively, focus on carbon.
The free radical chlorination of butane generates a racemic mixture of 2-chlorobutane. Note that butane can generate both primary and secondary free radicals. Because secondary free radicals are more stable than primary free radicals, the predominant products will substituted at position 2. For a free radical halogenation reactions, the image below shows the generation of the free radical intermediate after abstraction of the secondary hydrogen. Importantly, this intermediate is planar, meaning that in the second step, the chlorine free radical can react from either the top or the bottom of the plane. As a result, two products are created with equal probability. Note that the product is chiral as the carbon at the reaction center has four different substituents bound to it. As discussed in the Stereochemistry Test Module, one is termed R and the other is termed S.

Selectivity in free radical halogenation

In the example of free radical chlorination of butane (\(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3\)) shown above, there are two types of hydrogen atoms that can participate in the reaction: primary (1°) and secondary (2°). Although chlorination will occur at all unique hydrogen positions, the major products (racemic mixture) are those
substitutions that take place with the $2^\circ$ hydrogen atoms. This is because $2^\circ$ free radical intermediates are more stable than $1^\circ$ hydrogen atoms.

The relative stability of free radicals is shown below. Note that the benzyl radical is the most stable one because of resonance stabilization, and the relative stabilities of the alkyl radicals are governed by induction. Recall that alkyl groups (represented by R) are electron donating. Therefore, the more R groups on a carbon atom with one unpaired electron, the more that carbon atom is effectively closer to its satisfied octet structure.

![Free Radical Stability Diagram]

**Conformations of cyclohexanes**

Ring structures often exist in multiple conformations. In the example of cyclohexane, the six-membered ring structure is often presented as a flat, planar structure. However, if this depiction were accurate, the bond angles would be $120^\circ$. As the bonds in cyclohexane are sp$^3$ hybridized, the most stable structure would have angles of $109.5^\circ$. Thus, being planar is not favorable for cyclohexane. To accommodate optimal bond angles, cyclohexane exists in the chair and boat conformational isomers (conformers) as shown below.

When ring structures such as cyclohexane have substituted groups (i.e. 1, 3, dimethylocyclohexane), both cis and trans configurations result. The most stable forms are those that have the groups in the equatorial positions due to steric effects.
Cyclohexane and its derivatives are often drawn from a top down view as well as the chair view. One common MCAT line of questioning regarding cyclohexane and its derivatives tests the ability to convert from one form to the other.

To interconvert between the top down view and the chair structure, we draw cyclohexane with equatorial and axial groups as shown (“The Wheel”). Note how the equatorial and axial groups alternate as you go around the wheel. As you observe the two views of cis-1, 2 dichlorocyclohexane, convince yourself that the two chlorine atoms both lie above the plane in the chair structure. Thus, they are cis.
**ALKENES**

Alkenes are hydrocarbons that contain double bonds (sp\(^2\) carbons). Alkanes are more stable than alkenes due to differences in bond energies between \(\sigma\) and \(\pi\) bonds. Alkanes only contain C-C and C-H \(\sigma\) bonds, but alkenes contain C-C bonds that contain both a \(\sigma\) and a \(\pi\) bond. Compare the approximate bond energies of an average C-C single bond (~ 350 kJ/mol), an average C-H bond (~360 kJ/mol), and the average C-C \(\pi\) bond (260 kJ/mol). When considering these values, it is apparent that the C-C bond in alkanes takes considerably more energy to break relative to the C-C \(\pi\) bond in alkenes. Thus, the alkane is more stable, but the alkene is more reactive.

The presence of the double bond in an alkene is indicated by the “ene” suffix. Those alkenes with two double bonds are designated as “dienes”. The molecules are numbered such that the position of the double bond has the lowest number (see 2-butene above). Cyclical alkenes such as cyclopentane are not numbered as it is assumed that the double bond is between the 1 and 2 carbon atoms.
As for alkanes, the longest chain of carbon atoms that contains the functional group is numbered such that the functional group (double bond) is given the lowest number. Take the case of 2-ethylbutene for example. Although the longest continuous chain contains 5 carbons (shown in yellow), the longest chain containing the double bond functional group is four. Thus, the parental name is butane, and the proper name is 2-ethyl-1-butene.

The energy barrier for rotation around double bonds is high (~260 kJ/mol). This is over 20 fold higher than the rotation around C-C sigma bonds. As a consequence, atoms attached to the sp2 carbons in alkenes are virtually "locked" in position. However, in response to sufficient levels of light, isomerization of cis-retinal to trans-retinal is important in vision and this is further discussed in the context of Vitamin A.

Alkenes such as 2-butene can exist in either the cis or trans form as shown in the image. The cis form has the “R” groups on the same side of the double bond and the trans form has the “R” groups on opposite sides of the double bond. Such geometric isomers have different physical properties including dipole moments as well as boiling points. The boiling points of cis-2-butene and trans-2-butene are 3.7 °C and 0.9 °C. Unlike cis-2-butene, trans-2-butene has no net dipole moment.
In some cases, using the cis and trans system to designate alkenes does not work. This occurs when four different atoms are bonded to the sp² carbons of the alkene. In this case, the Z and E system is used. For this, priorities are assigned to each of the atoms attached to the sp² carbons. The highest priority goes to the atom with the highest atomic number. If the highest priorities are on the same side, then the alkene is designated as “Z”, and if the highest priorities are on opposite sides, then the alkene is designated as “E”.

Alkene Reactivity

Alkenes are electron rich compounds (i.e. nucleophiles) because of the pi electrons that lie above and below the plane. The pi bond is reactive and far easier to break than sigma bonds. The electron rich pi bond is attracted to positively charged atomic centers (i.e. electrophile). This reaction with alkenes underscores a central theme in organic chemistry: the attraction between electron-rich species (nucleophiles) and electron-poor species (i.e. electrophiles).

Electrophilic addition reactions
Electrophilic addition across a double bond generates two new sigma bonds from a pi bond. The mechanism of the reaction between 1-propene and HCl is shown above. The pi electrons attack the electrophile and this generates a carbocation intermediate. Notice that two different carbocation species can be formed: a primary and a secondary. As the secondary carbocation is the most stable of the two, this species will be the most stable intermediate and will react with the chlorine nucleophile to form the product 2-chloroethane. Therefore, because the reaction that is favored is the one that creates the most stable carbocation intermediate, electrophilic addition reactions are considered “regioselective”.

**Carbocation rearrangements**

Some molecules with carbocation intermediates will rearrange to form more stable carbocation intermediates (i.e. 2° to 3°). An example of this is shown below with 3-methyl-1-butene. Note that during the rearrangement, a hydride anion (H:) is moved from one carbon to an adjacent carbon. This is known as the 1, 2 hydride shift. The nomenclature of using 1, 2 refers to the fact that the shift occurs on adjacent carbon atoms.

Additionally, methyl groups can also shift to form more stable carbocations in a process known as a 1, 2 methyl shift. It is really a methide shift. This is shown below with 3, 3 dimethyl-1-hexene. Note that a secondary carbocation is initially formed, but through displacement of a methyl group via a shift, a tertiary carbocation will be formed. The major products are derived from this rearrangement. As the tertiary carbocation intermediate is sp² planar, the chloride nucleophile can attack from the top or the bottom of the plane. Addition of chloride at this position generates a new chiral center. Therefore, the major products constitute a racemic mixture.
Formation of alkenes from elimination reactions

Alkenes can be formed via elimination reactions that use alkyl halides as substrates. Alcohols can also be used and this is examined below. The mechanism of this concerted reaction is shown below. A strong base abstracts a proton that is in the Beta position relative to the leaving group. This means that the proton is attached to a carbon atom that is adjacent to the C-Cl bond. Afterwards, the electrons “collapse” to form a new alkene. During this process, a good leaving group (i.e. halogen) is eliminated provided that it is in the anti-conformation (180 degrees apart as shown). Therefore, elimination reactions involving alkenes are sensitive to the conformation of the substrate. The most stable products formed follow Zaistev’s rule. This states that the alkene with the most R groups (alkyl) attached to the sp² carbons are the most stable. Further, the trans isomer is more stable than the cis isomer. As we will see below, alcohols are also good substrates for the formation of alkenes. This occurs through dehydration reactions.
Alcohols

Nomenclature. Alcohols are molecules that contain an OH functional group (R-OH), a polar non-covalent bond. Monoalcohols have the generic formula of:
\[ C_nH_{2n+1}OH \]

Alcohols are named in a similar manner to alkanes, but molecules containing the -OH group have the IUPAC suffix of -ol provided that the alcohol functional group has the highest priority. In those molecules where the OH functional group does not have the highest priority (i.e. ketone, aldehyde, carboxylic acid), then the prefix “hydroxy” is used.

Alcohols are classified as 1°, 2°, and 3°. The image below shows several of such alcohols.

Alcohol Nomenclature

- n-butanol
- 2-butanol
- cyclohexanol
- 2-methyl 2 butanol
However, the names of some alcohols do not conform to systematic nomenclature. Some examples of these are shown below.

**Alcohols**

\[ \text{Glycerol} \quad \text{Allyl alcohol} \quad \text{t-butyl alcohol} \quad \text{Benzyl alcohol} \quad \text{Glucose} \]

**Physical Properties of alcohols**

**Boiling Point.** Alcohols have relatively higher boiling points due to their ability to form hydrogen bonds. Appreciate that hydrogen bonds between donors and acceptors are composed of electronegative atoms (i.e. F, O, N), hydrogen, and a second electronegative atom. In the case of alcohols, the hydrogen in the –O-H group, by virtue of being covalently linked to the electronegative O atom, is a donor. Acceptor atoms could be the oxygen in \( \text{H}_2\text{O} \) as well as the carbonyl oxygen in a ketone (\( \text{R}_2\text{C}=\text{O} \)).

The amino acid side chains of Tyrosine, Serine and Threonine have alcohol functional groups. These side chains are involved in hydrogen bonding as well as other reactions such as phosphorylation and glycosylation.

**SERINE**

**THREONINE**
Acidity of alcohols

Alcohols are weak Bronsted-Lowry acids:

\[ R-\text{OH} = R-\text{O}^- + \text{H}^+ \]

The conjugate base of an alcohol is termed an alkoxide and the conjugate acid of an alcohol is called an oxonium ion \((\text{ROH}^+)\). This is derived from the protonation of an alcohol as follows:

\[ R-\text{OH} + \text{H}_2\text{O} = R-\text{OH}_2^+ + \text{HO}^- \]

Any factor that generates a more stable conjugate base will increase the acidity of an alcohol (or any other molecule for that matter). Common factors contributing to this include induction, resonance, and solvation.

Aliphatic alcohols have \(pK_a\) values in the range of 15-20. They are therefore very weak acids, especially when compared to carboxylic acids that have \(pK_a\) values of ~ 2.0. Remember that the \(pK_a\) is defined as:

\[ pK_a = -\log [K_a] \]

where \(K_a\) describes the generic acid dissociation reaction:

\[ \text{HA} = \text{H}^+ + \text{A}^- \]

\[ K_a = [\text{H}^+][\text{A}^-]/[\text{HA}] \]

\(\text{HA} = \) conjugate acid; \(\text{A}^- = \) conjugate base

Larger \(K_a\) values mean that more products are formed at equilibrium. Therefore, relatively smaller \(pK_a\) values are indicative of relatively stronger acids (and corresponding weaker conjugate bases).

In contrast, aryl alcohols (i.e. those containing a benzene ring) have lower \(pK_a\) values relative to their aliphatic counterparts. This is due to resonance stabilization of the conjugate base. Additionally, the presence of additional functional groups can drastically alter the \(pK_a\) as shown below. Note that the electron withdrawing groups attached to the benzene ring reduce the \(pK_a\) values, meaning that the presence of such groups stabilizes the conjugate base. This is both an inductive effect and a resonance effect.
The immediate steric environment surrounding the oxygen atom of an alcohol influences its physical properties. This includes boiling points and acidic strength. A significant physical principle behind acidic strength is the solvation effect, the ability of water to interact and stabilize the alkoxide anion upon dissociation of the proton from the OH group of the alcohol. Note that due to steric effects, a primary alcohol is more solvated than tertiary alcohols. Deduce from this that methanol is more acidic than 2-methylpropanol.

**Synthesis of alcohols**

**Biological synthesis**

Alcohols are synthesized as intermediates in fatty acid metabolism and are synthesized as end products in some forms of fermentation. Alcohols are generated as intermediates in the context of the catabolism of fatty acids (Step 3) through a hydration reaction. In this reaction, water is added across the double bond of an alkene:

\[
\text{H}_2\text{O} + \text{alkene} = \text{alcohol}
\]

The reverse reaction occurs during fatty acid synthesis and is an example of a dehydration event. These reactions are common in biology and increase the
bond order as a compound with a double bond (alkene) is generated from a reactant with a single bond.

Fatty acid metabolism is an important topic on the MCAT as well as in medicine. Because fatty acid synthesis is essentially the reverse pathway of catabolism, alcohols are also generated in this pathway (not shown). Fatty acid catabolism occurs in the mitochondria and requires oxygen. Fatty acids derived from adipose tissue are catabolized in various tissues such as the liver. The process is summarized:

Step 1. The fatty acid is initially activated to generate a fatty acyl CoA.
Step 2. The β carbon is oxidized to the level of the alkene.
Step 3. Addition of water (hydration) to the alkene creates 3-hydroxyacyl CoA, an alcohol intermediate.

Step 4. Oxidation of the alcohol generates a ketone.

Step 5. A β ketothiolase enzyme uses CoASH to generate Acetyl CoA as well as a fatty acid of N-2 carbons.

Fermentation

Fermentation is an anaerobic process that occurs in both bacteria and yeast. Ethanol is the end product of fermentation in yeast and its formation occurs through the partial oxidation of glucose as shown below. One mole of glucose is converted into 2 moles of pyruvate via glycolysis. Pyruvate decarboxylase releases CO₂ to generate acetaldehyde. Alcohol dehydrogenase then reduces the aldehyde to ethanol.

In humans, alcohol is metabolized to acetaldehyde by the action of alcohol dehydrogenase. The expression of alcohol dehydrogenase allows for the consumption and metabolism of alcohol. Many people of Asian descent have a variant of this enzyme that causes the build up of potentially toxic levels of acetaldehyde that is causal for the “alcohol flush reaction”. Therefore, alcohols undergo oxidation reactions. Primary alcohols such as ethanol are metabolically oxidized into aldehydes that can be further oxidized into carboxylic acids. Secondary alcohols are oxidized into ketones and tertiary alcohols cannot be oxidized as shown below.

In the laboratory, 1° and 2° alcohols can be oxidized to the aldehyde state or ketone state of oxidation via mild oxidizing agents such as PCC (Pyridinium chlorochromate). However, strong oxidizing agents such as potassium permanganate (KMnO₄) and potassium dichromate (K₂Cr₂O₇) can oxidize the 1° alcohol to the level of the carboxylic acid.

Alcohols and substitution reactions (Sₙ1 and Sₙ2)

Alcohols undergo substitution (and elimination) reactions. We will examine both Sₙ1 and Sₙ2 mechanisms as they are specifically listed in the AAMC Content Guideline. Elimination reactions are not specifically listed in the
Content Guideline, so they will be deemphasized here. However, they were discussed above in the context of alkenes. In many cases, substitution reactions compete with elimination reactions. However, in biological enzymes one is preferred over the other given that enzymes catalyze the reactions. Although other compounds besides alcohols (i.e. alkyl halides) undergo $S_N1$ and $S_N2$ reactions, we will introduce the concept in terms of alcohols. You should be able to apply these principles to additional reactions outside of alcohols. $S_N1$ reactions (Substitution nucleophilic first order reactions) occur through the formation of a carbocation intermediate. The rate law can be written as $r = k[\text{electrophile}]$ as the formation of the carbocation electrophile is the rate limiting step. Therefore, these reactions are first order.

Recall that electrophiles usually have a positive charge (full or partial) and love electrons. As they can accept electrons from nucleophiles, electrophiles can also be thought of as Lewis acids.

The mechanism of a generic $S_N1$ reaction is shown below with a $3^\circ$ alcohol bonded to three different alkyl groups ($R_1$, $R_2$, and $R_3$). Note that the carbon bonded to the alcoholic group is asymmetric (or chiral). This will have implications for the stereochemistry of the product.
Alcohols are notoriously poor leaving groups as HO is a strong base. However, when the reaction is performed at low pH, the alcohol becomes protonated. This will generate the H₂O leaving group that is a better leaving group than OH. Recall that the weakest base is always the best leaving group. This is because weak bases hold electrons weaker than strong bases. Therefore the bond will be broken easier.

Sₙ₁ reactions are performed with polar protic solvents. Appreciate that protic solvents are capable of forming hydrogen bonds as they have a hydrogen atom bonded to a nitrogen or an oxygen atom. The polar protic solvent stabilizes the carbocation and provides the energy to dissociate the leaving group from the tertiary carbon. In many cases for Sₙ₁ reactions, the nucleophile is the same as the solvent (solvolysis).

The carbocation has an empty p orbital and is therefore planar in structure (i.e. sp² hybridized). As shown, the nucleophile (i.e. negatively charged halogen) can attack the planar carbocation intermediate from either the top or the bottom of the plane (i.e. wedged or hatched line structure). As a result two different bonds are formed with the carbon atom (i.e. enantiomers = racemic mixture).

Although Sₙ₁ reactions proceed through carbocation intermediates, primary, vinyl, and methyl carbocations rarely, if ever, undergo Sₙ₁ reactions due to their instability. The relative stability of carbocations is shown below. Note that carbocation stability is enhanced by the presence of alkyl substituents as they tend to donate electrons. This decreases the positive charge on carbon, effectively making it more electro neutral. Further, resonance contributes to the stability of carbocations as seen for the primary benzyl and primary allylic carbocations.
Carbocation rearrangements in Alcohol reactions

We have seen that some molecules with carbocation intermediates will rearrange to form more stable carbocation intermediates (i.e. 2° to 3°). An example of this with alcohols is shown below. Note that during the rearrangement, a hydride anion (H⁻) is moved from one carbon to an adjacent carbon. This is known as the 1, 2 hydride shift. The nomenclature of using 1, 2 refers to the fact that the shift occurs on adjacent carbon atoms. Additionally, methyl groups can also shift to form more stable carbocations in a process known as a 1, 2 methyl shift and was shown above with alkene reactivity. The bottom line is that when you have a reaction with a carbocation intermediate, you must examine its potential to rearrange to a more stable intermediate.
Substitution nucleophilic second order ($S_{N2}$) reactions occur with alcohols (as well as other molecules such as alkyl halides). The rate law is written as:

$$r = k[\text{electrophile}][\text{nucleophile}]$$

The reaction is considered a “concerted” reaction because the nucleophile attacks the electrophilic carbon at the same time that the leaving group is ejected from the molecule. Therefore, a pentavalent intermediate exists for a brief time (not shown).

$S_{N2}$ reactions occur in polar aprotic solvents. In this case, the acidic pH allows for protonation of the alcohol. As we saw with the $S_{N1}$ reaction, this will generate a better leaving group than the HO$^-$ leaving group. A protic solvent would be expected to solvate the nucleophile and hinder its ability to attack the electrophilic center. As shown in the figure, the negatively charged nucleophile attacks from the opposite side of the leaving group (“backside attack”). As a result, the $S_{N2}$ reaction works best when the path to the electrophilic carbon is least hindered. That is, steric hindrance with alkyl groups impedes $S_{N2}$ reactions. Therefore, in contrast to $S_{N1}$ reactions, which are favored in polar
protic solvents, $S_N2$ reactions proceed in polar aprotic solvents and rarely, if ever, occur with $3^\circ$ carbon atoms.

**SN2 mechanism**

As is the case for $S_N1$ reactions, there are stereochemical considerations for $S_N2$ reactions. Due to the backside attack of the nucleophile, a new bond is formed that is of the opposite configuration of the leaving group (see figure above). Therefore, $S_N2$ reactions proceed through “inversion of configuration”. In the example shown here, the electrophilic carbon is chiral as there are four different substituent groups bound to it (Ethyl, Methyl, Hydrogen, and OH). The resulting $S_N2$ product will be of opposite configuration of the starting reactant molecule.

A comparison of $S_N1$ and $S_N2$ mechanisms is shown below.

**$S_N1$ vs $S_N2$ Reactions**

<table>
<thead>
<tr>
<th>$S_N1$</th>
<th>$S_N2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rate = $k$[electrophile]</td>
<td>Rate = $k$[electrophile][nucleophile]</td>
</tr>
<tr>
<td>2. Polar Protic solvent</td>
<td>Polar Aprotic solvent</td>
</tr>
<tr>
<td>3. Order of reactivity: $3^\circ &gt; 2^\circ &gt; 1^\circ$</td>
<td>Order of reactivity: $1^\circ &gt; 2^\circ &gt; 3^\circ$</td>
</tr>
<tr>
<td>4. sp$^2$ carbocation Intermediate</td>
<td>Pentavalent intermediate</td>
</tr>
<tr>
<td>5. Possible stereoisomer products</td>
<td>Inversion of configuration</td>
</tr>
<tr>
<td>6. Carbocation rearrangements possible</td>
<td>No Carbocation rearrangements</td>
</tr>
</tbody>
</table>
Additional alcohol reactions

1) Synthesis of Alkyl Halides

Alcohols can be converted into alkyl halides through either of two reactions:

A) The reaction between an alcohol and thionyl chloride produces an alkyl halide.

\[ R-OH + SOCl_2 = R-Cl + SO_2 \]

B) This can also be achieved with phosphorous trihalides as shown with phosphorous trichloride:

\[ R-OH + PCl_3 = R-Cl + HOPCl_2 \]

2) Preparation of Mesylates and Tosylates

Mesolytes and Tosylates are specifically listed in the AAMC MCAT Content Outline. They are intimately linked to the chemistry of alcohols.

In general, alcohols are poor leaving groups because the hydroxyl anion is a strong base. Besides protonation, another way to convert alcohols into good leaving groups is to generate organosulfonates. In contrast to the \( S_{N1} \) and \( S_{N2} \) reactions, these reactions do not change any stereochemistry.

Starting with an alcohol and either Mesyl chloride (MsCl) or Tosyl chloride (TsCl) as shown in the image, an activated alcohol is generated with the OH nucleophile and the sulfur electrophile. The reaction is usually performed with pyridine and the products are sulfonate esters that are derivatives of sulfonic acid, a strong acid with a \( pK_a \approx -3.0 \). This is 100,000 times more acidic than a carboxylic acid with a \( pK_a \approx 2.0 \! \) Addition of nucleophile to mesylates (OMs) or tosylates (OTs) readily creates a substitution product. This is because the sulfonate is a great leaving group as it is the weak conjugate base of a strong acid.
Protection of alcohols

Protection of alcohols is specifically listed in the AAMC MCAT Content Guide (Download a free copy from our site). Addition of a protecting group to an alcohol, or any other functional group for that matter, is a temporary step in a synthesis. The protecting group is removed later in the synthesis. Protecting groups are added to a functional group when it is incompatible with a set of reaction conditions. Therefore, protection, as its name suggests, can be used to mask a functional group such as an alcohol from performing an undesired side reaction. Protecting groups mask functional groups and ensure chemo selectivity in organic synthesis.

Alcohols are versatile molecules. In addition to participating in redox reaction, alcohols can act as nucleophiles when deprotonated and as leaving groups when protonated. Therefore, protection of alcohols is important in organic chemistry reactions.
There are multiple ways to protect alcohols. One example is shown in the figure above. Panel A shows the desired reaction between an alkyne carbanion and a molecule with two functional groups: a halogen (chlorine) and a primary alcohol. The desired product is formed through an $S_N^2$ mechanism as shown by the arrow. However, the major product (Panel B) is a closed heterocyclic structure that results from an intramolecular nucleophilic attack.

So, how can the desired product be made? The answer is to protect the alcohol and this is shown in Panel C. Note how the protecting group is a tertiary alcohol that forms a carbocation that engages in a $S_N^1$ reaction with the primary alcohol of the reactant molecule. The product is a trimethyl ether linkage (R-O-R), a stable bulky group that masks the alcohol. Now, this protected molecule can be reacted with the alkyne carbanion under $S_N^2$ conditions. After release of the protected group via acid and heat, the desired product is formed.

Carbonyl Chemistry
The carbonyl functional group is commonly seen in biomolecules. Due to the strong electronegativity of oxygen, the generic C=O functional group is a polar covalent bond. This dipole moment generates a potentially electrophilic center at the carbon atom.

Aldehydes and Ketones

The general formula for aldehydes and ketones is shown below. Aldehydes have one R group and ketones have two R groups attached to their carbonyl carbons as shown.

Nomenclature

Aldehydes are named with the suffix “al” and ketones are named with the “one” suffix. For ketones, the carbonyl carbon should be specified unless it is in a cyclical structure. In this case, the carbonyl carbon is assumed to be in position 1. Some examples are shown below. Note that the ubiquinone molecule has been truncated for simplicity.
Both aldehydes and ketones are hydrogen bond acceptors, often with amines and alcohols. This is shown below. Note the optimal 180° bond angles between the donor and the acceptor. Aldehydes and ketones have lower boiling points relative to alcohols as the R-OH group can participate as both.
donors and acceptors.

**Reactivity**

**Oxidation-Reduction**

As we saw earlier with alcohols, aldehydes and ketones participate in oxidation-reduction reactions. Reducing agents such as LiAlH₄ take ketones to aldehydes and even alkanes.

One important redox reaction concerns ubiquinone, a ketone in the electron transport chain. Also known as coenzyme Q, recall that this important molecule is central to the electron transport chain and accepts and donates electrons. Specifically, ubiquinone forms part of electron transport chain center II. Med-Pathway covers electron transport in physical and chemical detail in both the Biochemistry and Thermodynamics and Kinetics diagnostic modules.

![Ubiquinone to Ubiquinol Reaction](image)

**Keto-enol tautomers**

Hydrogen atoms bonded to alpha carbons in ketones and aldehydes are acidic and participate in important biological reactions, including keto-enol tautomerizations and aldol condensation reactions. Note that the hydrogen atom immediately bonded to the carbonyl carbon of aldehydes is not acidic.
As shown for acetone, the acidic alpha hydrogen is abstracted from the ketone molecule by a strong base (i.e. hydroxide or ethoxide anion). This generates a carbanion that is stabilized by resonance with the carbonyl group as shown. The resulting enolate anion can pick up a proton from the media to generate an enol. Therefore, molecules such as acetone can exist in a keto-enol equilibrium as shown.

Note that the keto and enol forms are constitutional isomers and are called tautomers. In most cases, the equilibrium is heavily shifted towards the keto form, but there are some exceptions. Importantly, phenol mostly exists in the enol form as this form is most stabilized by resonance. The keto tautomer does not enjoy the resonance stabilization of the benzene ring. In addition to resonance, hydrogen bonding can also stabilize the enol form over the keto form.
Aldol condensation reaction

The aldol condensation reaction exploits the chemistry of the acidity of the alpha hydrogen atom of either an aldehyde or ketone. In this reaction, the enolate anion reacts with a carbonyl compound (aldehyde or ketone) to generate either a β-hydroxyaldehyde or β-hydroxyketone. After dehydration by heat (Step 3), an α, β unsaturated product (i.e. conjugated eneone) is generated. The reaction scheme is shown below for the aldol condensation reaction of acetaldehyde.

**Step 1** is performed in the presence of a strong base at 5 °C. The base pulls off the acidic alpha hydrogen and generates a resonance-stabilized carbanion that is a strong nucleophile. This targets the electrophilic carbonyl of another molecule of acetaldehyde in solution.

**Step 2** generates a β-hydroxyaldehyde. Upon heating, the molecule loses water (hence condensation) to form the α, β unsaturated product. Note that in the aldol condensation, the total number of carbons in the product is the sum of the number of carbons that participate in the reaction. This will come in handy when you are trying to figure out the products of an aldol condensation.
Retro aldol condensation

The aldol condensation reaction is reversible. Such a reaction, the retro aldol condensation, is specifically mentioned in The AAMC Content Guide. A classic example of this reaction (shown below) occurs in the glycolytic pathway where the enzyme aldolase uses the substrate Fructose 1, 6 biphosphate (F 1, 6-P₂) and converts it into two small carbohydrates: 3-phosphoglyceraldehyde (G-3P) and dihydroxyacetone phosphate (DHAP). Recall that these intermediates are further oxidized and are precursors to pyruvate. As enzymes such as aldolase also catalyze the reverse reaction during the process of gluconeogenesis, the aldol condensation generates F 1, 6-P₂ from G-3P and DHAP.
Kinetic and thermodynamic enolates.

This topic frequently appears on the MCAT. We present it here as well as in the Thermodynamics/Kinetics module.

Reactions that produce multiple products can have both kinetically and thermodynamically controlled products. The most rapidly formed product is the kinetic product and the most stably formed product is the thermodynamic product. Through controlling various parameters (i.e. temperature, ionic strength, buffer, etc.), the reaction can be either kinetically or thermodynamically controlled.

Shown above is a typical MCAT style problem with a cyclical ketone molecule. There are two possible products. Can you tell which one is the thermodynamically controlled product and which one is the kinetically controlled product? Note that the products produced from each reaction are enolates, and as the name implies, they are molecules that have both an alcohol
group and an alkene group. Therefore, arriving at the correct answer requires that you bring in some knowledge of alkene chemistry.

As shown in the image, the most substituted alkene is the most stable product as it forms the most stable enolate. In contrast, the kinetic enolate is the molecule that has the most easily accessible alpha hydrogen atoms. That is, the base can pull off a proton fastest from the least sterically hindered molecule. Note the presence of the additional methyl group sterically hinders accessibility to the alpha proton.

**Formation of acetals and hemiketals**

As shown below, aldehydes and ketones undergo nucleophilic attack as the partial positive charge on the carbonyl carbon is an electrophilic center. This generates a tetrahedral intermediate (Panel A) that forms a central theme in the chemistry of the carbonyl functional group.
As shown, when the nucleophile attacking the aldehyde or the ketone is an alcohol, hemiacetals and hemiketals are produced. In general, these are unstable compounds, but further addition of alcohol generates the stable compounds, acetals and ketals.

Hemiacetals and acetals are commonly scene in the context of carbohydrate chemistry. As shown, glucose is in a hemiacetal form, but maltose, a disaccharide of glucose, consists of acetal linkages in the glycosidic bond. This will be elaborated on in the carbohydrate section.
The formation of gemdiols and cyanohydrins are two additional nucleophilic reactions with aldehydes and ketones that are listed on the AAMC checklist. Both of these are shown below.
Chapter II

☐ Imines & Enamines
☐ Carboxylic acids & Their derivatives
☐ Decarboxylation & Carboxylation
☐ Acyl Halides
☐ Acid Anhydrides
☐ Esters
☐ Claissen Condensation
☐ Amides
☐ Amides and Protein Structure (Hydrolysis of Peptide Bonds)
☐ Relative Reactivity of Carbonyl Compounds

Formation of imines and enamines

Imines are important biological molecules that are formed from the addition of an aldehyde or ketone to a primary amine. An enamine is formed in the presence of a secondary amine. This is a condensation reaction (loss of water) that occurs in a weakly acidic buffer. An example is shown below with lysine (1° amine) and acetaldehyde.

![IMINE FORMATION](image)

The mechanism of imine formation is shown below. Note that all steps are reversible. For simplicity, the lysine side chain is denoted as R-NH₂. Note that under normal conditions at pH = 5.0, the primary amine side chain of lysine would exist largely in the protonated form as the pKₐ of a primary amine is ~11.0. However, as lysine is part of the primary structure of proteins, the
pKₐ values can vary widely, meaning that more of the unprotonated amine would be available for the nucleophilic attack that is essential to the mechanism of imine formation.

**Mechanism of Imine Formation**

**Step 1:** The carbonyl group of the aldehyde is protonated, generating a more electrophilic carbonyl carbon that is attacked by the unprotonated lysine. A tetrahedral intermediate is formed.

**Step 2:** The tetrahedral intermediate has its alcohol group protonated, turning the weak OH leaving group into the strong leaving group water. In addition, a conjugate base pulls off a proton from the quaternary amine.

**Step 3:** The tetrahedral intermediate collapses, kicking out water (i.e. condensation).

**Steps 4, 5:** The protonated imine (Schiff base) that forms upon collapse of the tetrahedral intermediate is in equilibrium with the unprotonated imine, the final product. Note the R-C=N-R characteristic of imines.

**Enamine formation and reactivity**

Enamines are formed from aldehydes/ketones and secondary amines. When the lone pair of electrons on nitrogen is donated, a resonance-stabilized carbanion is formed. Therefore, enamines are reactive at the α carbon. Think of enamines as you do for enolates: resonance stabilized carbanions. These
Carbanions can attack various electrophiles to perform a variety of reactions including acylation and alkylation.

**ENAMINE REACTIVITY AT α CARBON**

ENAMINE

**Carboxylic Acids & Their Derivatives**

Carboxylic acids have the general formula \((R-COOH)\) and they are usually named with the suffix “oic”. When ionized, the suffix “ate” is often used. Thus, pyruvate is the conjugate base of pyruvic acid. Some examples are shown below.

Due to resonance stabilization after ionization, carboxylic acids have higher boiling points to comparable alcohols. Further, carboxylic acids participate in hydrogen bonding.

**CARBOXYLIC ACIDS**

- Methanoic Acid (Formic Acid)
- Pyruvic acid
- 4-methylhexanoic acid
- Benzoic acid
- Levulinic acid
- 6-chlorohexanoic acid
Decarboxylation and carboxylation reactions

Decarboxylation and carboxylation reactions occur at multiple places throughout metabolism. In most cases, these reactions are catalyzed by enzymes. Thiamine pyrophosphate (TPP: Vitamin B1) and pyridoxal phosphate (Vitamin B_6) are co-factors that participate in decarboxylation whereas biotin (Vitamin B_7) performs carboxylation reactions.

One important carboxylation reaction occurs during fatty acid synthesis. In this pathway, acetyl CoA is converted into malonyl CoA by the enzyme acetyl CoA carboxylase. This reaction is the committed step in fatty acid synthesis and occurs in an ATP-dependent manner. Note how acetyl CoA carboxylase uses bicarbonate as the source of the CO_2 is positively regulated by insulin and negatively regulated by glucagon.

Two decarboxylation reactions are shown below. In Panel A, the spontaneous decarboxylation of acetoacetate, a ketone body produced in the liver, to acetone is shown. Acetone is a volatile substance that is often detected in the breath of people who have type I diabetes mellitus. Note that acetoacetate is a β-ketoacid. Decarboxylation of β-ketoacids often occurs spontaneously because the carbanion that results after decarboxylation is stabilized by resonance.

Panel B shows elements of the pyruvate dehydrogenase complex, a group of mitochondrial enzymes that oxidizes pyruvate into acetyl CoA, a two-carbon carrier of activated carbon atoms. Note that the reaction utilizes an E1 enzyme that is bound to thiamine pyrophosphate (TPP), also known as vitamin B_1.
Carboxylic acids & Their Derivatives

There are numerous carboxylic acid derivatives. The important ones for the MCAT are shown below.

### CARBOXYLC ACID DERIVATIVES

<p>| | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
</table>
| **A** | Acyl Halide | Synthesis: **Alcohol + PBr$_3$ or SOBr$_2$**  
Reactions: Conversion to Esters & Amides |
| ![Acyl Halide](image) | Acetyl Bromide  
Ethanoyl Bromide | ![Acyl Halide](image) |
| **B** | Acid Anhydride | Synthesis: 1) **From two carboxylic acids**  
2) **Acyl Halide + Carboxylic acid**  
Reactions: Conversion to Acids, Esters, and Amides |
| ![Acid Anhydride](image) | Acetic Anhydride  
Ethanoic Anhydride | ![Acid Anhydride](image) |
| **C** | Ester | Synthesis: 1) **Alcohol + Acid, Acyl Halide, or Acid Anhydride**  
Reactions: Conversion to Acids + Alcohol, Transesterification, Amides, Claissen Rxn |
| ![Ester](image) | Ethyl Acetate  
ethylethanolate | ![Ester](image) |
| **D** | Amide | Synthesis: 1°, 2° Amines + Acyl Halides, Esters, Acid anhydrides  
Reactions: Hydrolysis to acid + amine, Transamination, Reduction to amines |
| ![Amide](image) | N-methylacetamide | ![Amide](image) |
**Acyl Halides**

Acyl halides are very reactive as the covalently linked halogen is a strong leaving group. Four reactions with a model acyl halide are shown below in the image. The mechanism for the formation of each carboxylic acid derivative is analogous and proceeds through a nucleophilic substitution-elimination reaction. In this reaction scheme, a nucleophile (i.e. hydroxide) attacks the electrophilic carbon in the carbonyl group. This forms a tetrahedral intermediate that collapses. As bromide is the weakest base, it is the best leaving group. As a result a carboxylic acid is formed. The only mechanism shown in the image is for the conversion of acyl halides into carboxylic acids. The formation of anhydrides, esters, and amides occurs through a similar mechanism.

**B. Acid Anhydrides**

The synthesis of acetic anhydride is shown in the image below along with the reaction of acetic anhydride with methanol. Note that for the nomenclature of anhydrides, the name of the acid is replaced with anhydride for symmetrical anhydrides (i.e. acetic anhydride). For asymmetrical anhydrides, the acid names are used in alphabetical order followed by anhydride.
As seen above for acyl halides, anhydride synthesis as well as degradation occurs through a nucleophilic substitution-elimination reaction. The image also shows the synthesis of acetic anhydride from two carboxylic acids through such a mechanism. Likewise, for the reaction of acetic anhydride with methanol, a nucleophilic substitution-elimination reaction occurs to form an acid and an ester. Amides and esters can also be formed from anhydrides by reacting with amines (not shown).

C. Esters

For naming esters, the “ate” suffix is used. The group attached to the carbonyl oxygen is named first and then the group attached to the carbonyl carbon is then named with the “ate” suffix.

Esters are important biological molecules especially in the context of fatty acid and triglyceride metabolism. Although a fatty acid plus an alcohol can form an ester, biological systems activate fatty acids with ATP to form Coenzyme A (CoA or CoASH) derivatives that are actually thioesters. You are likely to encounter esters on the MCAT in the context of fatty acid metabolism.
The synthesis of an ester from a fat and an alcohol is shown. This step is the initial one in the synthesis of triglycerides. Note that the fatty acid is palmitic acid (C16:0). The fatty acid synthase enzyme synthesizes this metabolite. However, in biological systems fatty acids are activated by thiokinases. These enzymes use ATP and Coenzyme A (CoA or CoASH) to generate thioesters, in this case palmitoyl CoA. Next, glycerol-3 phosphate, an activated form of the glycerol alcohol, performs a nucleophilic-substitution reaction that generates the ester. The reaction is actually a “transesterification” reaction as a new ester is generated from another one. After two more rounds of esterification a triglyceride is made. Triglyceride synthesis occurs in the liver, intestines, and adipose tissue.

Esters undergo substitution-elimination reactions with a number of molecules including alcohols, amines, and water. The reaction mechanisms are similar in that a nucleophile attacks the electrophilic carbonyl of the ester, generating a tetrahedral intermediate that collapses and kicks out a leaving group to restore the carbonyl group.

The most common reaction with esters that you see will be hydrolysis, especially of triglycerides. This and two other reactions are shown below. Observe that water is a good nucleophile with its lone pair of electrons. These
electrons attack the electrophilic carbonyl of the ester linkage. After formation and collapse of the tetrahedral intermediate, an alcohol leaves. Therefore, the generic description of the hydrolysis of an ester that you will not forget is:

**ESTER + H₂O = ACID + ALCOHOL**

Importantly, some esters occur in a cyclical format. Such structures are known as lactones. Their hydrolysis also produces an acid and an alcohol, but both functional groups are on the same molecule as shown. The third reaction shows the hydrolysis of a triglyceride. This occurs in adipose tissue in response to low blood sugar (i.e. running, fasting, sleeping). Under these conditions, epinephrine stimulates the activity of hormone sensitive lipase, an enzyme that hydrolyzes triglycerides into fatty acids (R-COOH and alcohols such as glycerol).

**Claissen Condensation Reaction**

In addition to nucleophilic-substitution elimination reactions observed for hydrolysis of esters, esters also undergo the Claissen condensation reaction. As observed above for ketones and aldehydes in the Aldol condensation reaction, the Claissen condensation exploits the acidic α hydrogen atoms present in esters. After a strong base pulls off this acidic proton, a resonance-stabilized carbanion acts as a nucleophile that attacks a second ester molecule. A
tetrahedral intermediate is formed and upon collapse kicks out an alcohol in the protonated form (R-OH).

**Amides**
Amides can be linear or cyclical structures. Cyclical amides are known as lactams. Amide nomenclature replaces the “oic” in acid with the suffix -amide. The carbonyl carbon is assumed to be in the #1 position. Secondary amides are designated with an upper case “N” to show that the alkyl group is bonded to the nitrogen. The alkyl group is named. Some examples are shown below in the Formation of Amides schematic.
As we have seen for the formation of other molecules, amides can be formed by nucleophilic substitution-elimination reactions. Amides can be synthesized from primary and secondary amines when combined with esters, acyl halides, acid anhydrides, and esters. Amides are not normally synthesized from carboxylic acids as an acid plus a base (amine) will generate a salt.

Panel A in “Formation of Amides” shows the reaction mechanism between a primary amine (Ethylamine) and an ester (Methyl acetate). Note that the acid catalysis protonates the carbonyl carbon as well as the oxygen in the leaving group. Protonation of the carbonyl oxygen generates a stronger electrophilic carbon. The lone pair of electrons residing on the ethylamine nucleophile attacks this carbon atom and forms a tetrahedral intermediate. Upon collapse of the tetrahedral intermediate, methanol leaves and the amide linkage is formed. Note that protonation of the oxygen in the ester linkage, which generates methanol (CH₃OH), is the leaving group rather than the methoxide anion (CH₃O⁻). As methanol is a weaker base than methoxide, it is a much better leaving group. Thus, acid catalysis generates both a better electrophile for the amine as well as a better leaving group during the formation of the amide.
Panels B and C illustrate the formation of esters from primary and secondary amines with acetyl chloride and acetic anhydride, respectively. The mechanisms of these reactions are not shown but are analogous to the reaction shown in Panel A.

Amides and Protein structure

Amides are very important biological molecules, especially in the context of protein structure and function. This is because the peptide bond that forms the backbone of protein structure is an amide linkage.

Peptide bonds are synthesized during ribosomal translation. Due to the formation of resonance structures (see image), peptide bonds have partial double bond character. As rotation is limited around double bonds, peptide bonds adopt a planar structure that usually favors the trans position as shown with the arrows pointing in opposite directions.
Hydrolysis of Peptide Bonds

Protease enzymes catalyze the hydrolysis of amide (peptide) bonds. Below is a hypothetical scheme for acid/base catalyzed hydrolysis of a peptide bond using two key active site residues: lysine (Lys) and serine (Ser). In this scenario, an uncharged lysine side chain acts as a base by accepting a proton from water, generating the stronger, negatively charged hydroxyl nucleophile. As shown, the HO\(^-\) nucleophile attacks the electrophilic carbon at the carbonyl group of the peptide bond, forming a tetrahedral intermediate.

Note that the serine residue forms a hydrogen bond with the carbonyl oxygen and helps keep the peptide bond situated in the active site of the enzyme. During the collapse of the tetrahedral intermediate, the newly protonated lysine residue behaves as a Bronsted-Lowry acid through donating its proton to the nitrogen atom in the peptide bond. This generates a stronger leaving group (NH\(_2\) vs NH\(^-\)). Recall that the best leaving group is always the weakest base. (Weak bases do not share electrons very well, making the bond easier to break.) Without receiving the proton from lysine, the leaving group would be R\(_1\)NH\(^-\), but with the addition of the proton, the leaving group becomes R\(_1\)NH\(_2\), a neutral species and weaker base than R\(_1\)NH\(^-\).
Relative reactivity of carbonyl compounds

We have examined four types of carbonyl compounds: acyl halides, anhydrides, esters, and amides. Each class of compound undergoes nucleophilic-substitution elimination reactions as described above. The relative reactivity of these derivatives is shown below. As discussed previously, the weakest conjugate base is always the best leaving group. Weak bases such as chloride are stable leaving groups and have strong conjugate acids (HCl is a strong acid). In contrast, strong bases such as R-NH are reactive and are poor leaving groups. Therefore, amides are the most stable of the carbonyl derivative compounds.

Relative reactivity of carbonyl derivatives

Acyl Halide | Anhydride | Ester | Amide
---|---|---|---

Cl⁻ | O⁻ | O⁻ | H⁻

Weakest Base (Most stable leaving group) | Strongest Base (Least stable leaving group)

Chapter III

Carbohydrates, Lipids, Steroids, and Vitamins

☐ Carbohydrates (Nomenclature, Formation of hemiacetals & acetals, mutarotation and anomers, glycogen & cellulose, reducing sugars and the Benedict’s test)
☐ Fatty acids & Lipids (saturated vs unsaturated, formation of micelles, triglycerides, saponification, phospholipids, phosphatids, sphingolipids)
☐ Cholesterol
☐ Bile Acids

MCAT Prep Med-Pathway.com Organic Chem Review The MCAT Experts
☐ Steroids
☐ Prostaglandins
☐ Fat-soluble vitamins (Vitamin D, Vitamin A, Vitamin K, Vitamin E and lipid peroxides)

Carbohydrates

Carbohydrates are molecules that usually have the general formula \(\text{C}_n\text{H}_{2n}\text{O}_n\). They are obviously important in metabolism. This section will focus on their structures and reactivity. Common carbohydrates are sugars, starches, and cellulose. They usually contain aldehyde or ketone groups and exist as monomers (monosaccharides) or polymers (polysaccharides). Monosaccharides cannot be hydrolyzed into smaller units and are therefore the smallest of the carbohydrates. Polysaccharides are monomers linked together through glycosidic bonds. Carbohydrates are classified by three major criteria:

1) The type of carbonyl group. If the carbohydrate has an aldehyde or ketone group, it is called an aldose or ketose, respectively. Some carbohydrates also contain additional functional groups (acids, amides).
2) The number of carbon atoms in the molecule. Carbohydrates with five and six carbons are called pentoses and hexoses, respectively. A pentose is often referred to as a furanose and a hexose is often referred to as a pyranose.
3) Chiral handedness. Carbon atoms in carbohydrates are often asymmetric in that they are bound to four distinct substituents. This means that they are stereocenters that can exist in either an R or an S configuration. We will not go into depth with respect to absolute configuration here. However, chirality is extensively covered in the Stereochemistry and Isomers module of Med-Pathway.
The system for naming carbohydrates is based upon chirality and uses the D and L nomenclature. The assignment of D and L refers to the stereochemical assignment of the asymmetric carbon furthest from the carbonyl group (carbon #1) in the Fischer projection. This is shown below for the two isomers of glyceraldehyde, the simplest carbohydrate. If the OH group attached to the penultimate carbon is on the right side, then the carbohydrate is designated as “D”. If it is on the left side, then it is designated as “L”. D and L glyceraldehyde are mirror images of each other and are therefore enantiomers.

Relationship between R/S and, + and −, and d/l system

The nomenclature of carbohydrates can be tricky. Recall that chiral molecules rotate the plane of polarized light in either a clockwise (+) or counterclockwise (-) manner, which is linked to the d (dextrorotatory) and l (levorotatory) nomenclature. This is used to distinguish how a chiral molecule rotates the plane of polarized light. The (+)-d and (-)-l system describes how an entire molecule interacts with polarized light. In contrast, the R and S system defines the absolute configuration of individual chiral centers within a molecule. There is no relationship between the two nomenclature systems; meaning that a chiral molecule with a single stereocenter assigned R does not indicate how this molecule will rotate the plane of polarized light (+ or -). This must be determined experimentally.
Further, when considering the d and l system, it is necessary to distinguish the meaning of the lower case nomenclature (direction of rotation in response to polarized light) to the uppercase D and L nomenclature. Uppercase D and L nomenclature refers to an alternative system for designating absolute configuration for amino acids and sugars. Therefore, D and L isomers can also be assigned through the R and S system by application of the Cahn-Ingold-Prelog rules.

Historically, when glyceraldehyde is drawn in a Fischer projection, if the OH group is drawn on the right then the molecule is the D isomer. If the OH is drawn on the left, then this represents the L isomer. As stated, D and L isomers are also known as enantiomers. If the chiral carbons in D and L glyceraldehyde were assigned an absolute configuration based upon the Cahn-Ingold-Prelog rules, then it is usually the case that the D isomer represents the R configuration and the L enantiomer represents the L configuration. However, you cannot always assume that D and L configurations will equate to R and S assignments.

The convention for amino acids such as serine is slightly different. If the $\alpha$-NH$_3^+$ group is on the right in the Fischer projection, then the D configuration is assigned. The conversion into the R and S system is accomplished through the priority assignments.

Biological significance

For enantiomers, it is common for one only one of the isomers to be biologically active. This is naturally seen with the L-amino acids as they are incorporated into proteins, but the D isomers are not. This is further seen with carbohydrates as they naturally exist in the D configuration in the human body. Thus, stereochemistry is highly relevant to pharmaceutical drug design and synthesis. Understanding stereochemistry is a prerequisite for the development of pharmaceuticals....and the MCAT!

**Common Carbohydrates**

The image below shows some common carbohydrates and their chemical properties. In **Panel A**, the ketose fructose is show in both its linear Fischer projection and its circularized Haworth projection. Fructose and glucose are structural isomers; their interconversion is facilitated by basic conditions. Note that fructose is a ketose and the carbonyl carbon is carbon #2 in the closed circular structure. Further, this carbon is in a hemiacetal linkage and the significance of this will be discussed below.
Panel B shows the structure of sucrose, a disaccharide composed of glucose and fructose. This is common table sugar. Note that carbon atoms 1 and 2 from glucose and fructose form a glycosidic bond. This is an acetal linkage and is very stable. Further note that the orientation of the oxygen in the linkage is pointing down below the plane. This is said to be in the $\alpha$ configuration. Sucrose is also called Glu-$\alpha$-1, 2-Fru.

Panel C shows galactose. Note that the HO group attached to carbon #4 in galactose is pointing up. In contrast, the HO group attached to carbon #4 in glucose points down (see Panel D). Notice that the other groups are in identical orientations. Therefore, the groups attached to carbon #4 are in opposite chiral configurations, making galactose and glucose epimers; they have multiple chiral carbon centers but differ in configuration at only one of them. Appreciate that epimers are a type of diastereomer.

Panel D shows the structure of lactose, a disaccharide composed of galactose and glucose. Note that the oxygen atom in the glycosidic bond (between carbon atoms 1 and 4) is pointing up and is therefore in the $\beta$ configuration. As for sucrose, the glycosidic bond in lactose is in an acetal linkage. Lactose is also written as $\beta$-D-galactopyranosyl-(1→4)-D-glucose. Humans express the enzyme
lactase, but there are some people with mutations. Such lactose malabsorbers often experience cramps and diarrhea after consuming food sources with lactose (i.e. dairy products).

Acetals and hemiacetals were first mentioned in the carbonyl chemistry of aldehydes and ketones section. Recall that they are formed from a carbonyl and an alcohol. Hemiacetals are labile bonds, but acetals are stable and must be hydrolyzed by an enzyme. Thus, formation of a hemiacetal is a reversible reaction, but the formation of an acetal is not. Therefore, glycosidic linkages, which are acetal linkages, are very stable bonds.

**Mutarotation and anomers**

Glucose is an aldose (aldehyde sugar) and exists in multiple forms as shown below in the figure. Analogous to glyceraldehyde, in the Fischer projection for glucose, the most oxidized carbon is designated as #1 and if the last chiral OH group (#5) is on the right then the glucose molecule is in the D configuration (left = L configuration).
Very little glucose exists in the free, linear form. Rather the hydroxyl group at position 5 (shown in red in image) acts as a nucleophile and attacks the aldehyde carbon at position #1, or the anomeric carbon. Because the carbonyl carbon is in a planar, sp²-hybridized form, the OH attacks from either above or from below the plane. This creates a new hemiacetal linkage that is sp³ hybridized and chiral. Convince yourself that this is a hemiacetal linkage. The anomeric carbon is a newly created stereocenter as it is a carbon bonded to four different substituent atoms. Therefore, there are two possible structures resulting from the circularization of linear glucose: one with a hydroxyl group pointing up (β) and with one pointing down (α). The term “anomers” is used to describe the relationship between these two structures.

As the new linkage at the anomeric carbon is in a hemiacetal linkage, it is labile, as opposed to the stable acetal linkage found in the glycosidic bond of a disaccharide. Thus, there is rapid conversion between the a and b forms in solution through the linear intermediate. As the b form can be converted into the a form through a linear intermediate, monosaccharides such as fructose and glucose undergo mutarotation. Note that at equilibrium, the β form (65%) is more stable than the α form (35%). The reason for this can be seen by comparing the Haworth and chair structures of glucose as shown in the right panel of the image above. This is because the β form in a Haworth represents the equatorial form in a chair conformation. Groups that occupy the equatorial position in a chair conformation are more stable than those that occupy an axial position due to less steric hindrance.

Anomers have opposite chiral configurations (i.e. R vs S) at carbon #1, yet the same configuration at the other chiral centers in glucose, the a and b forms of glucose are also related to each other as diastereomers. Further, diastereomers that differ in configuration about one carbon are also referred to as epimers. Thus, some anomers are epimers, but not all epimers are anomers! But they are all diastereomers, meaning that they have at least two chiral centers.

**Glycogen and Cellulose**

Glycogen is an important polymer of glucose that is stored in tissues such as the liver and spleen. Glycogen is to animals as starch is to plants: they are both storage forms of glucose. Glycogen is an important source of energy for tissues, and its synthesis and degradation are heavily regulated processes (see below). Glycogen synthase adds glucose units to a growing glycogen chain under conditions of energy excess (i.e. high insulin). UDP-glucose, an activated sugar, is used as a substrate for glycogen synthase. In contrast, during times of
energy need (i.e., high epinephrine and glucagon), the glycogen polymer is broken down by phosphorylase, an enzyme that releases a glucose-1 phosphate molecule from the glycogen polymer. After conversion into glucose 6-phosphate, the activated glucose monomer can enter into the glycolytic pathway for oxidation.

Note that the glycogen polymer as shown in the figure has α 1, 4 glycosidic bonds. Glycogen also contains α 1, 6 linkages that assist in making the polymer more soluble (not shown).

In contrast to glycogen, cellulose is a polymer of glucose, but contains β 1, 4 linkages. Humans do not express an enzyme capable of cleaving this particular bond. Therefore, cellulose is a type of “fiber”, a carbohydrate polymer that cannot be digested. Cellulose is a widely present in nature and is a component of the cell wall of plants and algae and is therefore often consumed in the diet.

Reducing sugars

Some sugars act as reducing agents because they have free aldehyde groups (Note keto groups can be converted into aldehydes, especially in the presence of base). The part of the molecule where this occurs is known as the reducing end. One test for the presence of reducing groups on sugars is known as the Benedict’s test. In the presence of an aldehyde (i.e. glucose) and copper in a
basic solution (hydroxide), the aldehyde can be oxidized to an acid (See below). Therefore, the aldehyde is a reducing agent. Initially, the copper solution is blue, but in the presence of the aldehyde reducing agent, Cu$_2$O is formed and the solution turns red. Note that Benedict’s reagent is not specific for sugars per se, but rather is specific for aldehyde groups.

**Benedict’s Reaction**

\[
\text{Glucose} + 2 \text{Cu(OH)}_2 \rightarrow \text{Gluconic Acid} + 2 \text{Cu}_2\text{O} + 2 \text{H}_2\text{O}
\]

**Fatty acids & Lipids: Descriptions and Types**

Fatty acids and lipids are important biomolecules that are certain to be seen on the MCAT. We have already seen these with respect to esters. Although the terms fatty acid and lipid are often used interchangeably, a lipid is a molecule that is either a fatty acid or its derivative and is insoluble in water but soluble in an organic reagent. Many of these molecules have polar head groups and a hydrophobic core and are therefore classified as amphiphiles.

Fatty acid metabolism (i.e. storage and utilization) is paramount to numerous aspects of biology and medicine. Fatty acids are molecules with long aliphatic chains and a terminal carboxyl group. The aliphatic chains can be branched and may or may not be saturated. In biological systems, most fatty acids have an even number of carbons.

Fatty acids rarely circulate freely in serum. Rather, they are often found esterified in various forms: triglycerides, cholesterol esters, and phospholipids.

Shown below are three important fatty acids. Palmitic acid is the most common fatty acid. Designated as C16:0 due to the 16 carbons and 0 degrees of saturation, palmitic acid is the terminal fatty acid synthesized by cytosolic fatty acid synthase. Longer chain fatty acids are synthesized in the endoplasmic reticulum. Note that the carbon adjacent to the carboxyl carbon is designated as the α carbon or the #1 carbon. The last carbon, regardless of the number of
carbons in the chain is designated as the $\omega$ carbon. During fatty acid oxidation in the mitochondria, the $\beta$ carbon of palmitic acid is oxidized.

Phytanic acid is a 16 carbon branched chain fatty acid. Also known as 3, 7, 11, 15 tetramethylhexadecanoic acid, phytanic acid is not produced in the human body. Rather, humans consume this in their diets by eating ruminant animals and some forms of fish. Phytanic acid cannot be degraded by the $\beta$ oxidation machinery in the mitochondria as the presence of the methyl group at the $\beta$ position sterically interferes with the enzymes involved in this process. Rather, phytanic acid is degraded in the peroxisome by the process of $\alpha$ oxidation.

Linoleic acid is not produced by the human body and is considered an essential fatty acid. It is often taken as a supplement. With two double bonds in the cis position, linoleic acid is known as an omega-6 fatty acid that is often found in flax seed oil. Linoleic acid is often represented as $18:2^{\Delta 9,12}$ where the 2 and $\Delta$ represents the number and positions of the double bonds, respectively. Linoleic acid is a precursor to arachidonic acid which itself is a precursor to important inflammatory regulators such as prostaglandins, leukotrienes, and thromboxanes. As prostaglandins are important in chemistry and medicine, they are specifically listed on the AAMC MCAT content outline. We will examine this in more detail below.
Saturated vs unsaturated fatty acids

Most animal fats are saturated, but notable exceptions like linoleic acid exist. The introduction of a double bond in unsaturated fatty acids alters their properties. Saturated fatty acids are usually solid at room temperature, while unsaturated are liquids. However, at body temperature, both are liquids. The presence of the double bond in unsaturated fats causes the molecule to kink or bend. This causes a reduction in the melting temperature as the van der Waals interactions between the chains of fatty acids are reduced.

Structural formation of fatty acids

Fatty acids and bile salts (derived from cholesterol) form micelles at a critical micellar concentration (CMC). This is shown in the image. Below the CMC value, the lipids exist as a solution of monomers. Above the CMC, the lipid forms micelles, or colloidal suspensions of surfactants. The concentration of lipids required to form micelles can be determined through multiple methods including conductivity and absorbance. Cholesterol, bile salts, and lipids such as phosphatidylethanolamine, all form micelles that incorporate dietary fatty acids for absorption into the GI tract.
Triglycerides

Triglycerides are esters and the storage form of fat and are synthesized primarily in the liver, intestines, and adipose tissue. They are generated from excess carbon (i.e. sugar, proteins) and stored in the adipose. We have seen triglycerides before as they are esters and are synthesized from fatty acids and glycerol.

The image shows the mechanism of esterification of glycerol from palmitic acid. Fatty acids such as palmitic acid are initially activated by ATP and Coenzyme A. This generates derivatives of coenzyme A. These high energy, activated thioesters are esterified with glycerol. As glycerol is a tri-alcohol, it can accommodate three fatty acid chains.

Fatty acids and triglycerides are very hydrophobic. Therefore, their transport through the body poses a challenge with respect to solubility. To circumvent this, fatty acids and triglycerides are transported with various partners that solubilize them. For example, fatty acids derived from the hydrolysis of triglycerides in times of energy need are bound to serum albumin.
Triglycerides synthesized from caloric excess are packaged into either chylomicrons or very low density lipoproteins (VLDLs). See the image below for elaboration. These important lipoprotein particles are of high clinical value. Chylomicrons are formed in the intestines from consumption of dietary fatty acids (i.e. meat and dairy products). VLDL particles are generated in the liver from consumption of excess sugars and amino acids. Both chylomicrons and VLDL particles arrive at the adipose tissue where they encounter lipoprotein lipase (LPL), an enzyme that hydrolyzes the esters into carboxylic acids and glycerol. The free fatty acids are imported into the adipose tissue where they are reconstituted into triglycerides for storage. Note that triglycerides cannot freely cross cell membranes.

Saponification

Saponification is the process of producing soap from triglycerides such as stearin, a molecule formed from three molecules of stearic acid and glycerol. The major source of triglycerides is animal fats and vegetable oils.
When stearin is treated with a strong hydroxide base (i.e. lye), the ester is hydrolyzed into three molecules of stearic acid and glycerol. Saponification is also a major method for producing industrial glycerol.

The mechanism of saponification is similar to the hydrolysis of esters: nucleophilic substitution-elimination reactions. The hydroxide anion is a strong nucleophile that attacks the electrophilic carbonyl carbon in the ester. The tetrahedral intermediate is formed and upon collapse an acid and an alcohol are created.

**Phospholipids and phosphatids**

Phospholipids are major components of cell membranes and form membrane bilayers. This is unlike fatty acids that form micelles as described above. Phospholipids are important for maintaining structural integrity of the cell and also perform important signaling functions. Phospholipids contain a polar phosphate head and a tail consisting of two hydrophobic chains (generically designated as R1 and R2). Two examples are shown below. Note that both phosphatidic acid and phosphatidylserine are both synthesized from a glycerol backbone that contains three alcohols. R1 and R2 are esters and the charged phosphate head forms the third phosphoester. In the case of...
phosphatidylserine (PS), the amino acid serine is linked to the phosphate head group. PS is a key membrane component and is important in apoptotic signaling.

**Phospholipids**

**Lipid Bilayer**

**Sphingolipids**

Sphingolipids are a type of lipid containing the sphingosine backbone. Sphingosine is a lipid derived from serine and palmitoyl CoA. This is shown below. Ceramides are sphingolipids, waxy phospholipids synthesized from sphingosine and any of several fatty acids (i.e. C16:0). As we have described, C16:0 is shorthand nomenclature for palmitic acid, a common saturated fatty acid. These lipids normally reside in cell membranes where they serve important structural and cell signaling roles. Sphingolipid catabolism occurs in the lysosome, an acidic organelle often referred to as the “recycling” center of the cell. Dysfunctional sphingolipid catabolism formulates a class of diseases known as “sphingolipidoses” where sphingolipid accumulation leads to its aberrant deposition in organs. Med-Pathway examines experimental treatment of the Niemann Pick sphingolipid disease in the “Cell Biology & Genetics” Testing module.
The image below shows several types of ceramides, lipids that are synthesized from sphingosine, a lipid derived from palmitoyl CoA and serine. Addition of a fatty acyl moiety generates a ceramide. Note that because various fatty acids can be added to sphingosine that there can be different ceramides. Hence, the term: “A ceramide”. Addition of phosphocholine generates a sphingomyelin, but addition of sugar(s) (i.e. glucose or lactose) generates a cerebroside and addition of sialic acid produces a ganglioside.
Cholesterol, Bile, and Steroids

Cholesterol is an important component of animal cell membranes that provides membrane fluidity. Thus, it is no surprise that organisms (i.e. shellfish) that live at relatively cold temperatures have abundant levels of cholesterol in their membranes. As the structure of cholesterol shows, it is highly nonpolar and composed of 27 carbon atoms with a hydroxyl group. The solubility of cholesterol is very low, about $10^{-12}$ M. Thus, cholesterol is an alcohol. It is also
an alkene. Esterification of cholesterol by enzymes such as LCAT generates more hydrophobic forms of the lipid.

The synthesis of cholesterol is a complex pathway consisting of numerous steps. As shown in the figure below, the mevalonate pathway is key in understanding the synthesis of cholesterol. Further, it is an important point in the application of cholesterol lowering drugs known as statins. Some of the key features of the pathway are described.

First, observe that cholesterol is synthesized from acetyl CoA. From here, mevalonate pyrophosphate is generated and converted into the isoprenoids: isopentyl pyrophosphate and dimethylallyl-pyrophosphate. Note that these five carbon species (i.e. terpenoids) are fundamental building blocks of nature. Cholesterol is built by combining these activated five carbon species by generating geranyl pyrophosphate (10 carbons) and eventually squalene, a 30-carbon derivative of the isoprenoids. Squalene is modified into the 27-carbon species cholesterol. Cholesterol is a precursor for steroid hormones (i.e. testosterone and estrogen). Further, isoprenoids such as farnesyl pyrophosphate can be added to proteins. Such posttranslational modifications are generically referred to as “prenylation” and serve to keep proteins anchored into the cell membrane.
Synthesis of Bile Salts

Bile is made in the liver and secreted into the duodenum during digestion. It is important for emulsifying fats, a process that makes them better substrates for pancreatic lipases. Cholesterol is the precursor in the synthesis of bile salts. The pathways for synthesizing bile salts are shown below. Note that each one has a distinct pKₐ value. Appreciate that lower pKₐ values allow for the acid to be charged at a lower pH. Charged species are more soluble than uncharged ones.

STEROIDS

In addition to bile acids, steroid hormones are derived from cholesterol. Cholesterol is initially converted into pregnenalone by the cholesterol side chain cleavage enzyme. Anterior pituitary hormones control expression of this cytochrome P450 enzyme. Pregnenalone is the common precursor to the steroid hormones as shown. Appreciate the similarities between cholesterol (30 carbons) and the other hormones, especially with respect to the four-ring structure. The synthesis of the various steroid hormones occurs in many steps through various chemical reactions that you have already seen, including oxidation and reduction. Don’t memorize the structures, but rather, expect the MCAT to test your organic chemistry knowledge by asking you to figure out which type of reaction/enzyme would perform a given step. Significantly,
notice that testosterone and estradiol are related to each other as keto-enol tautomers.

**Prostaglandins**

Prostaglandins are important mediators in the inflammatory response and their synthesis is the target of numerous drugs, including aspirin and ibuprofen. Prostaglandins are derived from signal transduction pathways at the surface of the cell. Signaling pathways that activate phospholipase A2 release fatty acids from the C-2 carbon of the glycerol backbone. This generates arachidonic acid (C20:4 Δ5, 8, 11, 14), a signaling molecule that is converted into various prostaglandin mediators (PGG2, PGH2, and PGD2) by the action of two cyclooxygenase (COX) enzymes, COX1 and COX2 (see below). These enzymes are similar in structure and function, but have different modes of expression.
COX enzymes are inhibited by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). COX1 is constitutively expressed, but COX2 is inducible in response to various signals, including those that activate inflammatory pathways such as tumor necrosis factor (TNF).

COX1 and COX2 are redox enzymes that generate the prostanoids, including prostaglandins and prostacyclin as well as leukotrienes. In platelets, only COX1 is present and it generates thromboxane A2, a mediator of platelet aggregation and blood clotting. In addition, COX enzymes regulate angiogenesis, production of the mucus lining, and blood pressure in renal arterioles. Because
of their pivotal role in the critical physiological processes, inhibition of COX enzymes is a highly active area of study.

**Fat Soluble Vitamins**
The fat-soluble vitamins are vitamins D, A, K, and E. The bioavailability of fat-soluble vitamins is regulated by their cognate binding proteins. For example, vitamin D binding protein (DBP), a member of the albumin family, is synthesized in the liver and binds tightly to the major circulating form of the sterol vitamin D₃. Although required for normal homeostasis, the fat-soluble vitamins can be toxic in high doses (hypervitaminosis).

**Vitamin D**
The synthesis of vitamin D occurs in multiple places as shown below in the image. Cholecalciferol, the inactive precursor to vitamin D₃, is generated in the skin via the action of UV light on 7-dehydrocholesterol. Cholecalciferol is then transported to the liver where it is modified through hydroxylation to create 25, hydroxycholecalciferol [25(OH)D₃]. This compound is transported to the kidney and activated via a PTH-induced renal hydroxylase to generate the active form of vitamin D₃ that is commonly abbreviated as 1, 25 (OH)₂D₃.

A major function of vitamin D₃ and its vitamers is to regulate the intestinal absorption of ions such as calcium and phosphate. Indeed, deficiencies in vitamin D₃ are well known to result in poorly calcified and improperly structured bone (i.e. rickets and osteomalacia).
The active form of vitamin D activates transcriptional programs in target cells as shown in the image. Vitamin D circulates with its binding protein (VDB) and diffuses through cell membranes where it interacts with the vitamin D receptor (VDR). In conjunction with RXR, vitamin D regulates gene expression as shown.

Vitamin A

Vitamin A is composed of various compounds as shown below that participate in many cellular processes. Although most notorious for its role in vision, the most common function of vitamin A is to regulate gene expression in the form of retinoic acid. Such regulation is important in processes such as development and immunity.
β-carotenes and other carotenoids (i.e. lycopene) are pro-vitamins consumed from various plants and are converted into vitamin A compounds in the intestine as shown below. These are converted into retinyl esters and transported with chylomicrons in the lymph and blood to the liver and adipose tissue. Retinols secreted from the liver and adipose circulate with the retinol binding protein (RBP) and arrive at target tissues. Once across the membrane, free retinol is converted into retinoic acid where it participates in gene expression programs.
Various forms of vitamin A are critical in the visual cycle. Indeed, deficiencies in vitamin A are notorious for night blindness. A simplified version of the visual cycle in the retina is shown below. Key to this is the visual pigment rhodopsin that consists of the G-protein opsin covalently linked to 11-cis retinal, a chromophore. Upon interacting with light, 11-cis retinal is photo isomerized into all trans retinal. This cis to trans isomerization induces a conformational change in opsin that generates a signal cascade that hyperpolarizes the photoreceptor cell. This is the molecular basis for vision.

During the visual cycle, 11-cis retinal, bound through a Schiff base to opsin, is converted into all trans retinal (Panel A), an isomerization event that releases the retinoid from opsin and transduces a signal to the brain. The system is recharged by converting all trans retinal back into 11 cis-retinal (Panel B). Although shown in the figure with a straight arrow, there are multiple intermediate steps in this conversion.
Vitamin K

The principle function of vitamin K is to participate in blood clotting and bone metabolism through the carboxylation of various vitamin-K dependent proteins. Deficiencies in vitamin K lead to osteoporosis and easy bruising. Inhibition of the vitamin K biochemical pathway formulates a class of drugs known as “blood thinners”. This will be elaborated on below.

The vitamin K family is comprised of various derivatives of 1, 4 Naphthoquinone as shown. We have seen biological quinones before in electron transport. Therefore, vitamin K might be expected to participate in redox reactions. Vitamin K1 is made by plants and is plentiful in green leafy vegetables. The gut micro biota can convert Vitamin K1 into vitamin K2 and can extend the length of the isoprenoid chain. Thus, vitamin K2 has several forms. Vitamin K3 is a synthetic form.
Vitamin K is a cofactor for enzymes that carboxylate glutamate (GLU) residues in vitamin-K dependent proteins. This forms γ-glutamyl carboxylated (GLA) side chains. This redox reaction is accompanied by the consumption of oxygen and the formation of water as shown. γ-glutamyl carboxylase concomitantly forms an epoxide intermediate in the vitamin K cycle. Conversion of the epoxide back into the active hydroquinone form is conducted by vitamin K epoxide reductase (VKOR). This enzyme performs two separate steps in the process and a second enzyme is capable of reducing vitamin K to the dihydroquinone form. Notably, VKOR is inhibited by warfarin (i.e. Coumadin), a well-studied pharmacological agent that is used as an anticoagulant (“blood thinner”). Clotting factors for II, VII, IX, and X are affected by warfarin.
Vitamin E comprises a group of compounds (i.e. tocopherols and tocotrienols) and is an antioxidant found in membranes. Vitamin E has been shown to have numerous health benefits. Vitamin E deficiency leads to abnormalities in fat absorption. This can lead to neurological problems due to perturbations in nerve membrane and conduction. As shown in the image, all forms of vitamin E contain a chromane double ring equipped with a hydroxyl function that reduces free radicals. The hydrophobic side chain allows for the positioning of vitamin E into the membrane.
The chief role of vitamin E is to prevent free radical chain reactions for lipid peroxidation events that occur in the membrane (see image). Tocopheroxyl radicals (α-TO) are generated from lipid peroxide radicals (ROO) and participate in initiation and propagation events. Importantly, the reaction between α-TO and ROO generates non-radical termination products.