Organic Mechanisms

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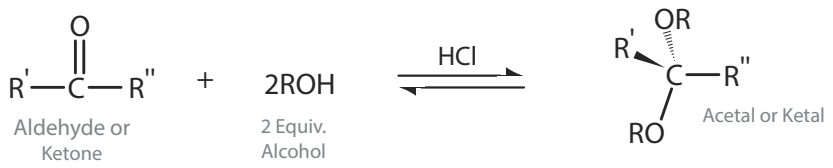
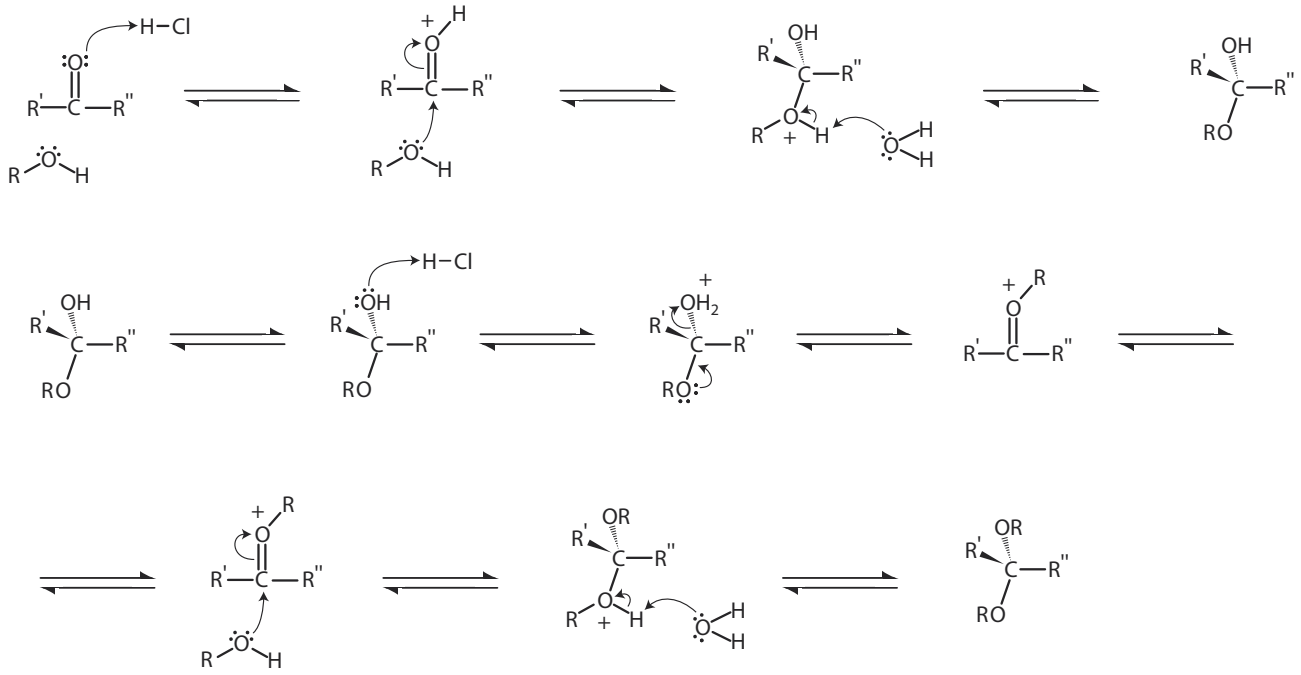
Acetal Formation

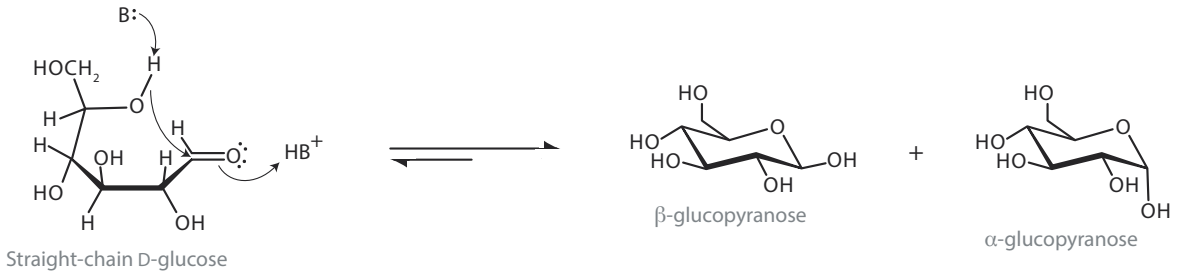




Acetal formation is the quintessential representative of an important class of reactions, nucleophilic additions, in which a nucleophile attaches to a carbonyl carbon. The greater electronegativity of oxygen over carbon in the carbonyl group makes carbonyl carbon somewhat electron deficient. This is why carbonyl compounds are subject to nucleophilic addition. A nucleophile is a reagent that can supply an electron pair to form a new bond, allowing the carbonyl carbon needs to resolve itself in a thermodynamically favorable direction. In acetal formation, nucleophilic addition happens twice. The first addition forms the simple addition product, a hemiacetal. Following dehydration of the hemiacetal, a second addition of alcohol forms the acetal.

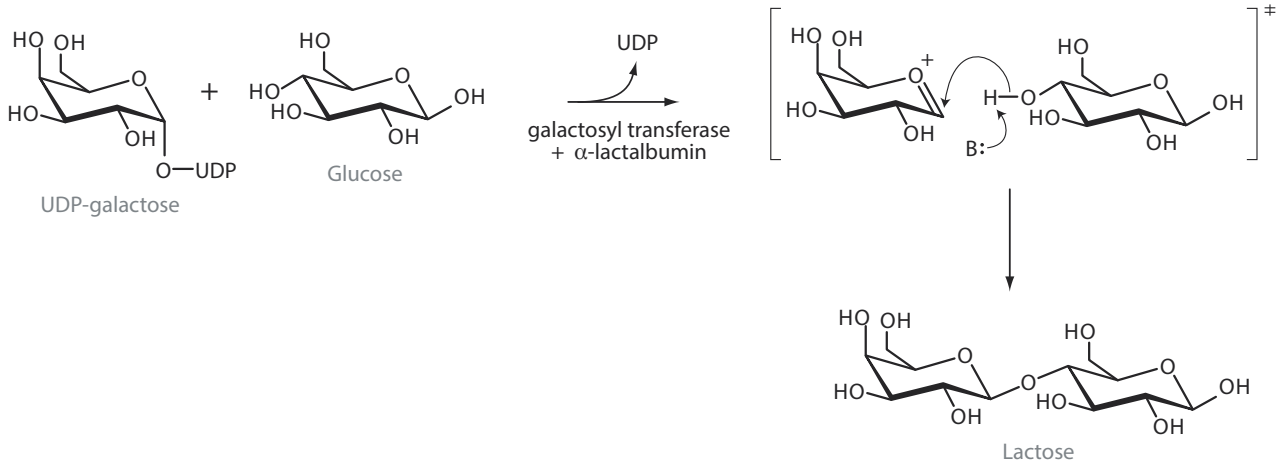
The reaction mechanism in acetal formation begins with protonation of the ketone or aldehyde carbonyl oxygen by an acid catalyst. Protonation of oxygen increases the attractiveness of the carbonyl group carbon, making it even more electropositive. Next, dehydration of the tetrahedral intermediate forms an oxonium cation which is then approached by another alcohol nucleophile. This forms the acetal. On the benchtop, acetal formation is often employed as a strategy for protecting carbonyl groups from hostile reaction conditions.

Glucose Ring Formation



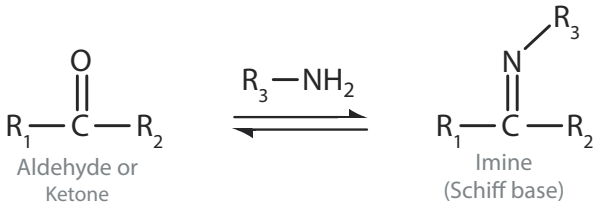
The open form of D-glucose, like many sugars, can cyclize in aqueous solution to form a hemiacetal. This reaction is an example of the hemiacetal phase of acetal formation in which an equivalent of alcohol forms a tetrahedral intermediate with an aldehyde. In glucose ring formation the nucleophilic hydroxyl group approaches the anomeric carbonyl from either below or above the plane of the forming ring, so two stereoisomers are possible, either β-D-glucopyranose or α-D-glucopyranose. β-D-glucopyranose is somewhat more stable. The α and β forms of D-glucose interconvert in aqueous solution, by a process called mutarotation. The equilibrium mixture consists of about one-third α-D-glucose and two-thirds β-D-glucose, along with very small amounts of the linear form.

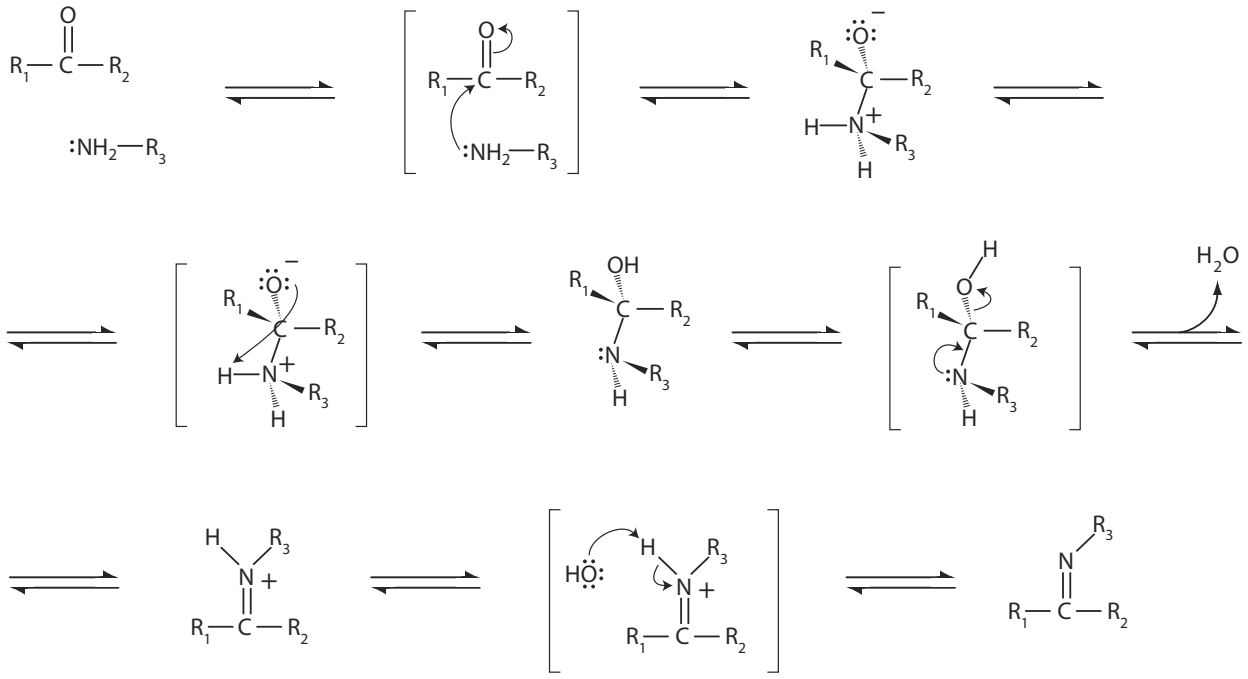
Glycosidic Bond Formation



Formation of the glycosidic bond in lactose is an example of the second phase of the general acetal formation reaction. In the general mechanism, after dehydration of the hemiacetal under acid catalysis, a second alcohol attacks the carbon of the resonant oxonium cation and forms the acetal. In lactose formation, playing the role of the hemiacetal is UDP-galactose (UDP leaving group promotes oxoniuim cation formation like acid catalysis does in the general mechanism). The alcohol in the reaction is the C-4 hydroxyl of the second sugar, glucose. This hydroxyl attacks the galactose C1 (anomeric) carbon from above, producing a β orientation in the resulting disaccharide, a β-1-4 glycosidic linkage.

Imine Formation

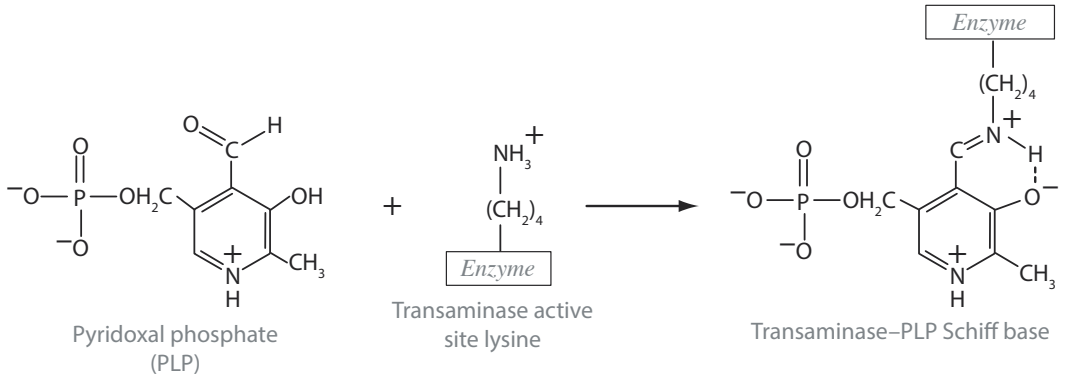




Imine formation involves the addition of a nucleophile to the carbonyl carbon of an aldehyde or ketone just like acetal formation, another important reaction in the category of nucleophilic additions to aldehydes and ketones. In acetal formation the nucleophile is an alcohol while in imine formation the nucleophile is a primary amine. The mechanism with imine formation is somewhat different concerning what happens after the addition. The overall process of imine formation produces a compound in which the C=O double bond has been replaced by a C=N double bond. This product is called an imine by organic chemists, but biochemists call this type of compound a Schiff base.

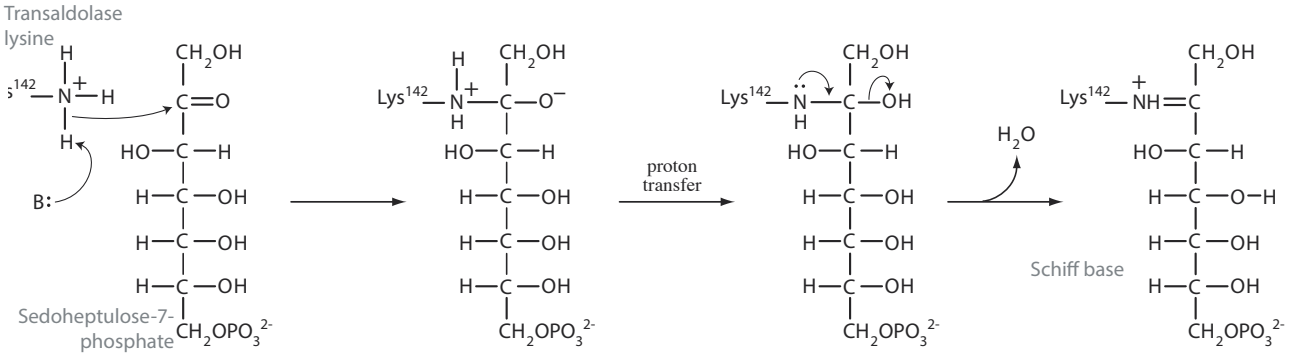
Comparing the mechanism of imine formation to acetal formation helps the understanding of both. In both reactions, after addition of the nucleophile, dehydration resolves the tetrahedral intermediate to a doublebonded, resonant cation. The oxonium cation in acetal formation then becomes a target for an additional alcohol addition. With imine formation, however, the nitrogen still possesses a bond to hydrogen which serves as an electron source to resolve its electron deficiency with the simple loss of the proton, so there is no necessity for another nucleophilic attack. Because it resolves itself with an internal shift, imine formation is like acetal formation except that it stops a step earlier.

Formation of Schiff Base - Transaminase with PLP



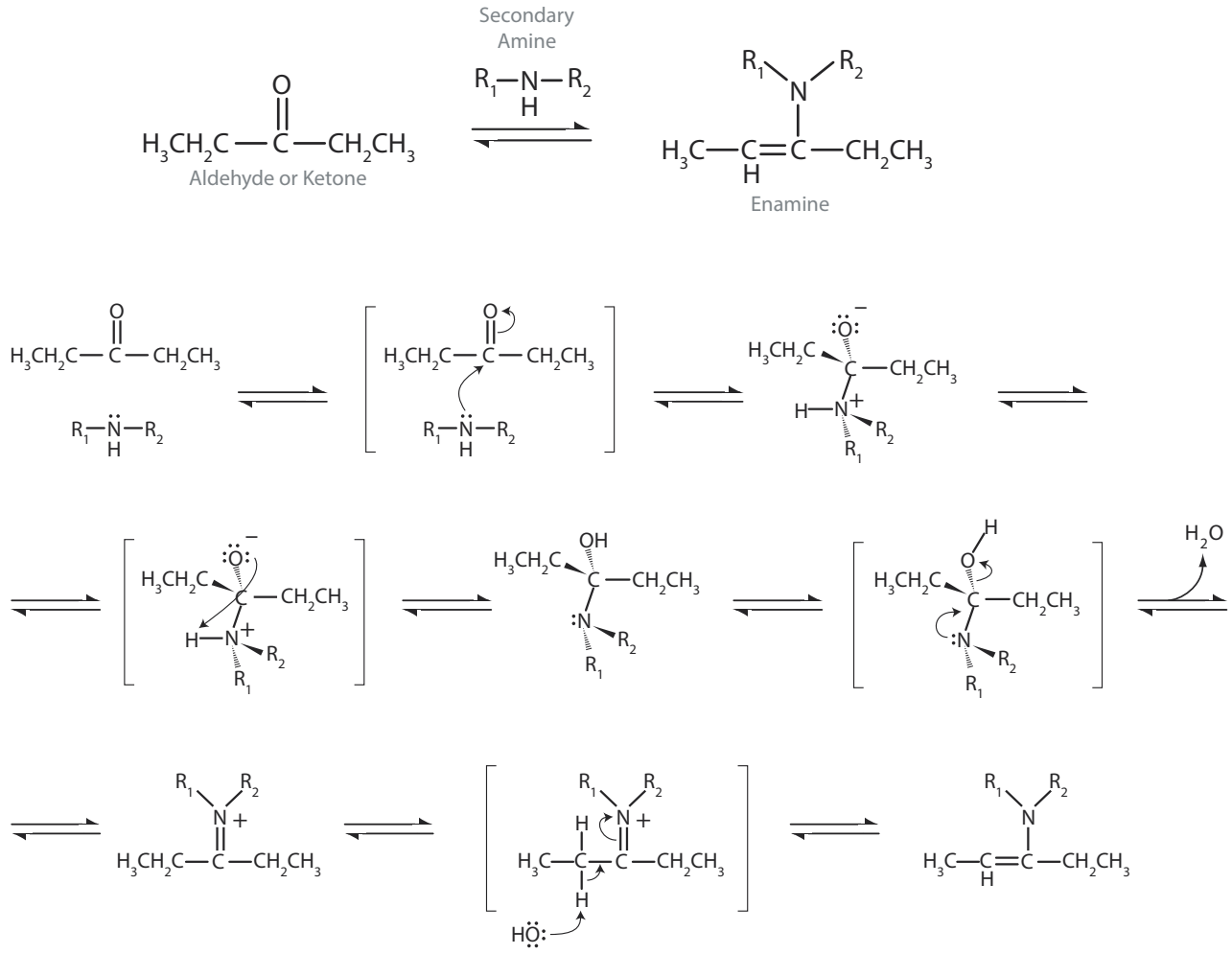
Imine formation is often seen in biochemistry. The example above depicts the formation of a Schiff base between a lysine residue of the enzyme transaminase and the coenzyme PLP. (In the biochemical context we should be in the habit of calling imines Schiff bases.) Transaminase is an important enzyme in the context of metabolic integration as well as nitrogen waste removal. Transaminase catalyzes the interconversion of α-amino amino acids with their corresponding α-keto-acids such as the conversion of glutamate to α-ketoglutarate. In the transaminase mechanism the lysine residue demonstrates an important aspects of its personality, the ability to covalently bind substrates (or in this case a coenzyme) through Schiff base formation.

Formation of Schiff Base - Transaldolase with Sedoheptulose-7-Phosphate



Transaldolase is an enzyme from the nonoxidative portion of the pentose phosphate pathway (pp 110-111). Transaldolase moves a 3-carbon segment of sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate forming erythrose-4-phosphate and fructose-6-phosphate. In this portion of the transaldolase mechanism, an enzyme lysine residue forms a Schiff base with the carbonyl of the sedoheptulose-7-phosphate substrate. Formation of the Schiff base with lysine readies the substrate for cleavage because the 3-carbon segment formed next will be stabilized by imine-enamine tautormerism. (See pg 35 for the complete transaldolase mechanism.)

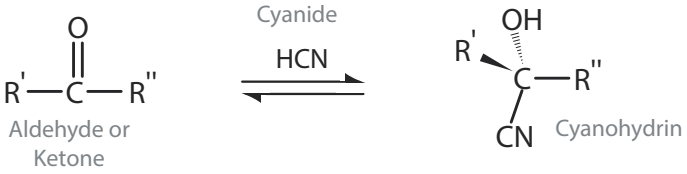
Enamine Formation

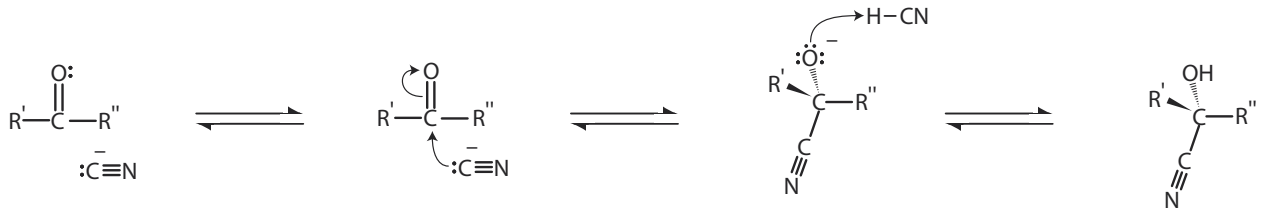


Secondary amines react with aldehydes and ketones to form enamines. In the case of a primary amine nucleophile an imine is formed instead (pg 6). In imine formation an original hydrogen remains from the primary amine on the iminium cation after addition and hydrolysis, and this nitrogen-hydrogen bond can be broken heterolytically to satisfy the iminium cation's electron deficiency. With enamine formation, however, where the nucleophile is a secondary amine not a primary amine, there is no such nitrogen-hydrogen bond at the iminium cation stage. The iminium cation intermediate must look elsewhere for an electron source to satisfy its thermodynamic drive to reduce internal energy.

The carbon-hydrogen bond at a position alpha to carbon-nitrogen double bond is an acceptable electron source. After that bond is broken, electron density can flow by conjugation to satisfy the iminium cation. This occurs by formation of a carbon-carbon double bond interior to the alpha carbon which causes the carbon-nitrogen double bond on the other side to become a single bond, releasing an electron pair to become the sole property of nitrogen. Between carbon and nitrogen there is now a single bond and the nitrogen atom is neutral. These electron movements are very similar to the electron movements that occur in keto-enol tautomerism (pg 23). It can be productive to think of enamines as the nitrogen-carbon cousins of enols. (See imine-enamine tautomerism, pg 31.)

Cyanohydrin Formation

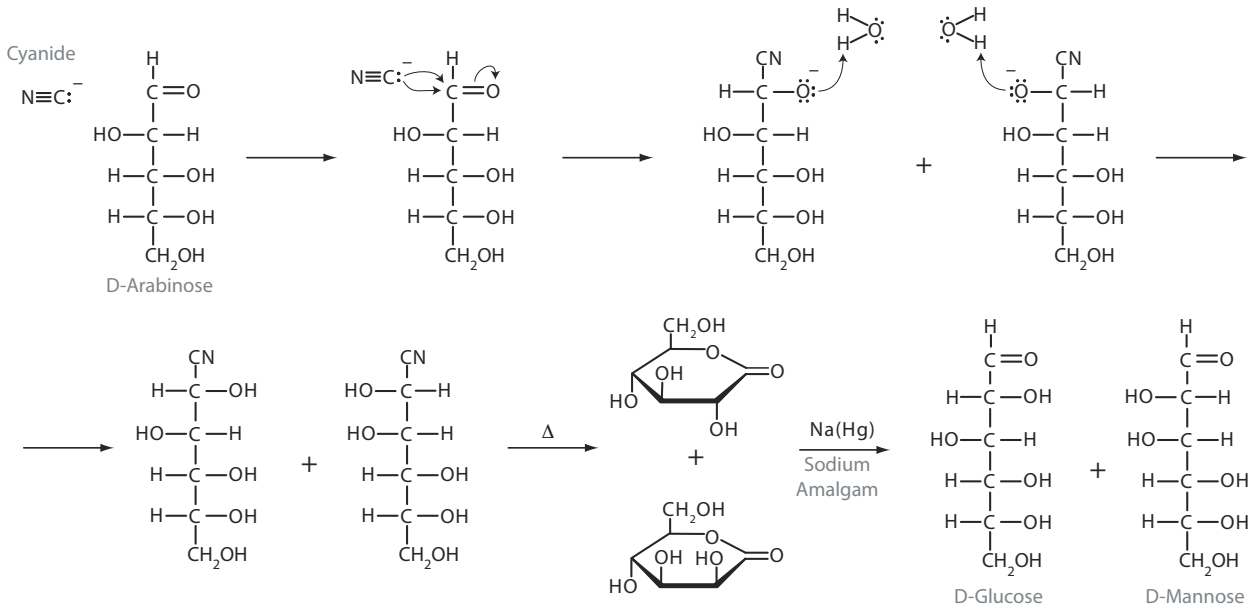




The cyanohydrin functional group possesses both a hydroxyl and a cyano group bonded to the same carbon atom. Cyanohydrins are produced through the reversible nucleophilic addition of cyanide anion (CN^-) to an aldehyde or ketone. The nucleophilic cyanide anion attacks the carbonyl carbon on the ketone to form the stable, tetrahedral cyanohydrin.

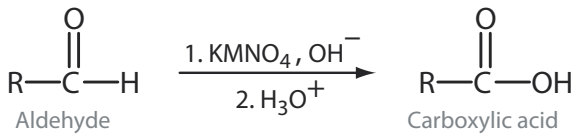
* A cyanide nucleophile is similarly employed in the Strecker amino acid synthesis (pg 117), but to attack an imine.

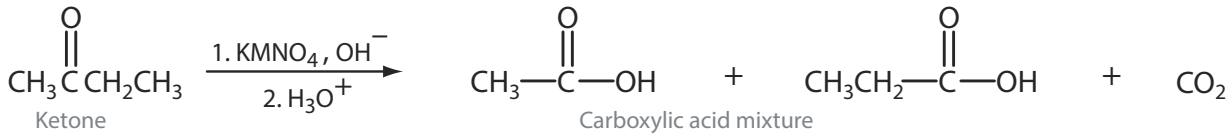
Kilani-Fischer Synthesis of D-Glucose and D-Mannose from D-Arabinose

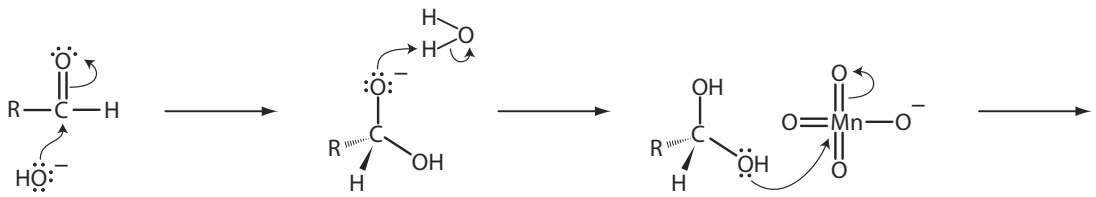


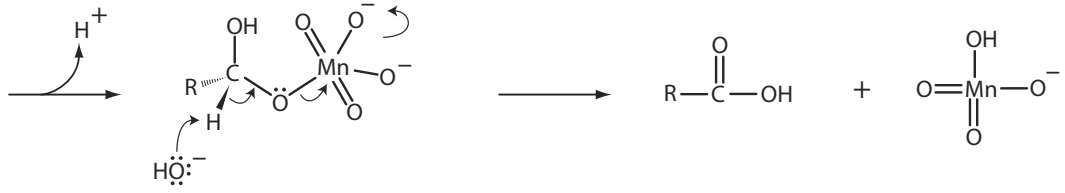
Cyanohydrins are intermediates in the Kilani-Fischer synthesis of D-glucose and D-mannose from D-arabinose. While not an MCAT required reaction, Kilani-Fischer synthesis is useful to review because it emphasizes the usefulness of cyanohydrin formation for synthesis. Cyanohydrin formation creates new carbon-carbon bonds.

Oxidation of Aldehydes & Ketones







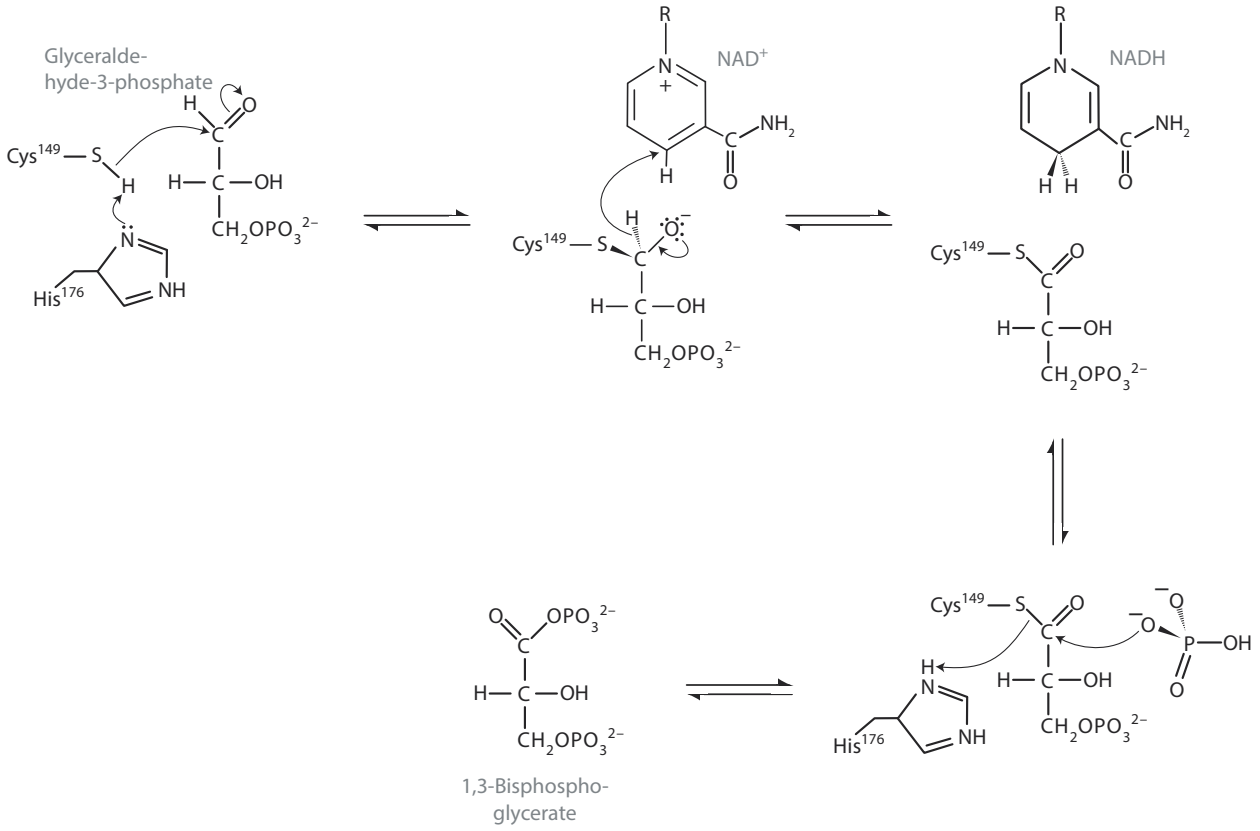


Many of the stronger oxidizing agents such as $KMnO_4$ will transform aldehydes into carboxylic acids. Tollens' reagent $[Ag(NH_3)_2]^+$ is one such oxidant (pg 17). A shiny mirror of metallic silver is deposited through oxidation of aldehydes by Tollens' reagent, so it is a frequently used test for aldehydes in qualitative analysis. Aldehydes are themselves oxidation products of alcohols.

Be cognizant of the spectrum of oxidation states for organic carbon-oxygen functional groups, beginning with alcohols, which are more highly reduced than aldehydes or ketones. Aldehydes and ketones are in turn more reduced than carboxylic acids and carboxylic acid derivatives.

A strong oxidizing agent like $KMnO_4$ will oxidize a primary alcohol past the aldehyde and up to the carboxylic acid oxidation state, while other, weaker oxidizing agents, like PCC, can be used to form aldehydes from alcohols, not proceeding to oxidize the aldehyde further. In general, normal ketones are not oxidized except under extreme conditions. At high temperature, ketones are cleavage oxidized by a strong oxidizing agent like $KMnO_4$. An exception is a benzylic carbonyl group, which $KMnO_4$ oxidizes easily.

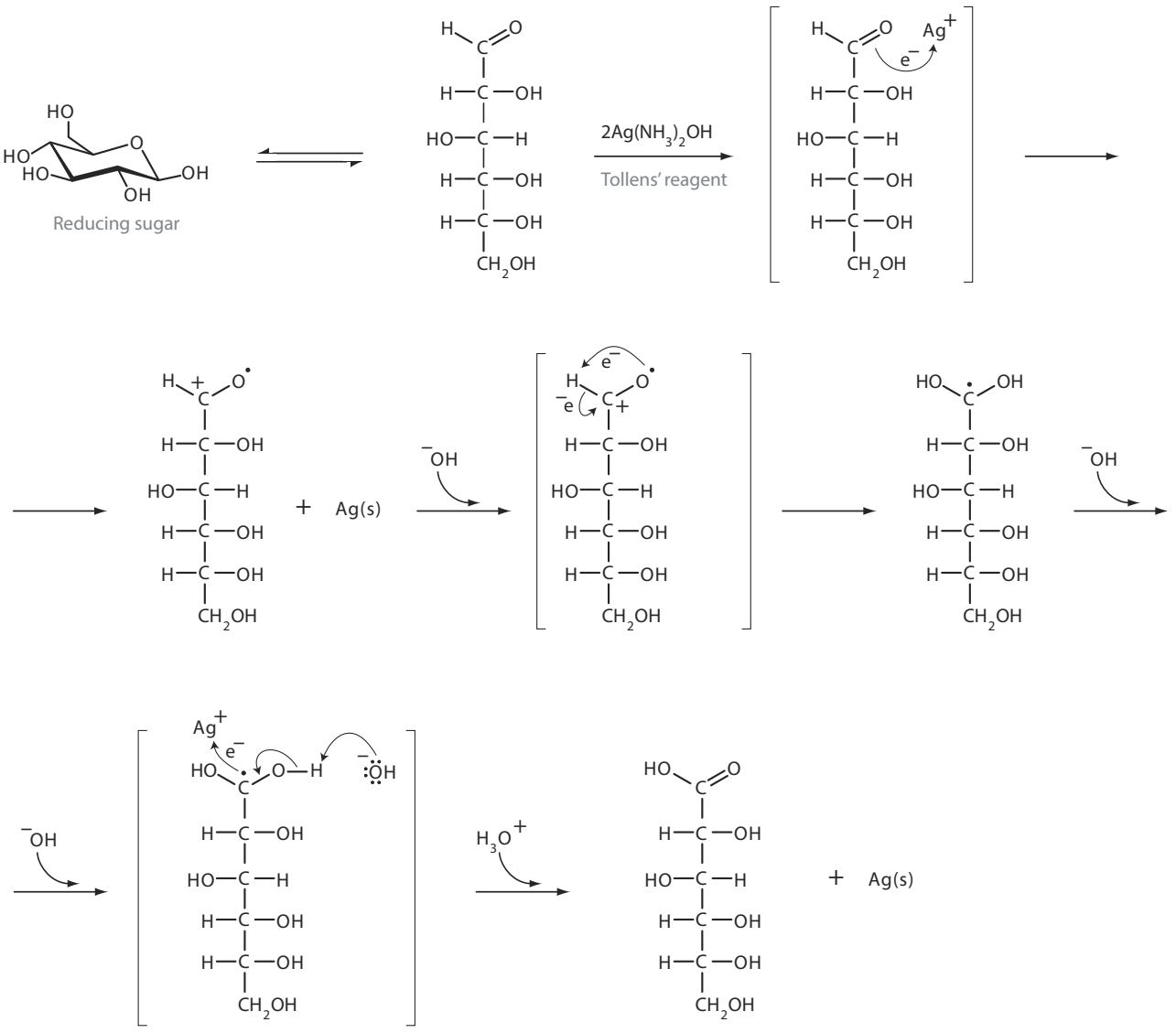
Glyceraldehyde-3-phosphate Dehydrogenase



Glyceraldehyde 3-phosphate dehydrogenase, which catalyzes the sixth step of glycolysis (pg 105), is a fascinating enzyme. The GAPDH mechanism demonstrates a central motif of energy metabolism, which is the coupling of an exergonic oxidation process with the establishment of phosphoryl transfer potential. In transitioning from an aldehyde to a carboxylic acid in a normal redox process, the system would be expected to lose free energy in heat flow to the environment, but here, the transition produces a phosphate anhydride, 1,3-bisphosphoglycerate, which has preserved the redox energy in the form of a compound with a very high phosporyl transfer potential.

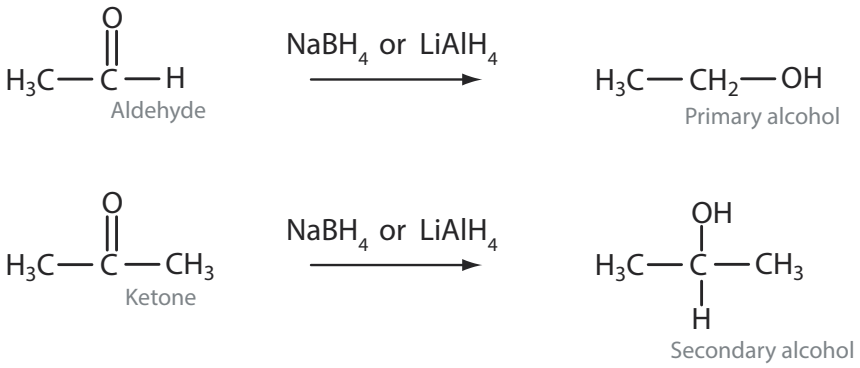
The mechanism starts with a cysteine residue in the active site of GAPDH attacking the carbonyl group of GAP, creating a hemithioacetal intermediate. Next, the coenzyme NAD+ accepts a hydride ion (H^-) becoming NADH while oxidizing GAP to a thioester intermediate. Like phosphate anydrides, thioesters are activated carboxylic acid derivatives (pg 58). This is how the redox energy is preserved. Thioesters are much higher in energy than the carboxylic acid species that would have resulted from the simple oxidation of an aldehyde. Finally, a molecule of inorganic phosphate attacks the thioester forming a tetrahedral intermediate, which then collapses through the acyl substitution addition-elimination pathway (pg 58) becoming 1,3-bisphosphoglycerate while releasing the thiol group of the enzyme's cysteine residue.

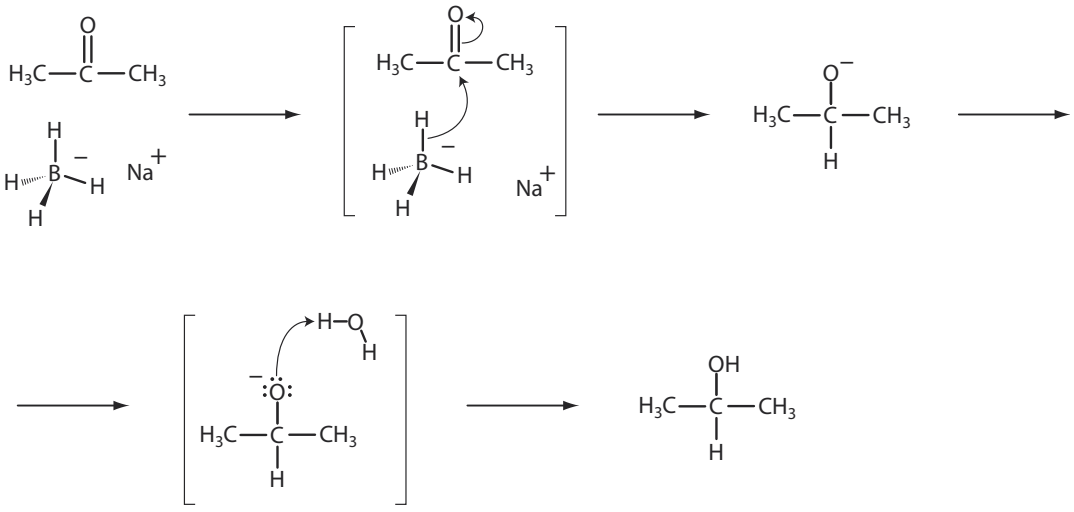
Tollens' Test



Tollens' test employs a silver ammonia complex $[Ag(NH_3)_2]^+$ to diagnose the presence of aldehyde. A positive test is indicated by the precipitation of elemental silver producing a characteristic "silver mirror" on the inner surface of the reaction vessel. Biochemists use Tollens' test to determine the presence of a reducing sugar, a sugar capable of acting as a reducing agent, in the case of Tollens' test, to reduce silver ions. Reducing sugars possess a free aldehyde group. The cyclic hemiacetal forms of aldoses can open to reveal an aldehyde and certain ketoses can undergo tautomerization to become aldoses. Nonreducing disaccharides like sucrose have glycosidic bonds between their anomeric carbons and thus can't convert to an open-chain form. Reducing disaccharides like lactose and maltose have only one of their two anomeric carbons involved in the glycosidic bond, so through the reverse of hemiacetal formation, they can convert to an open-chain form having an aldehyde group.

Reduction of Aldehydes and Ketones

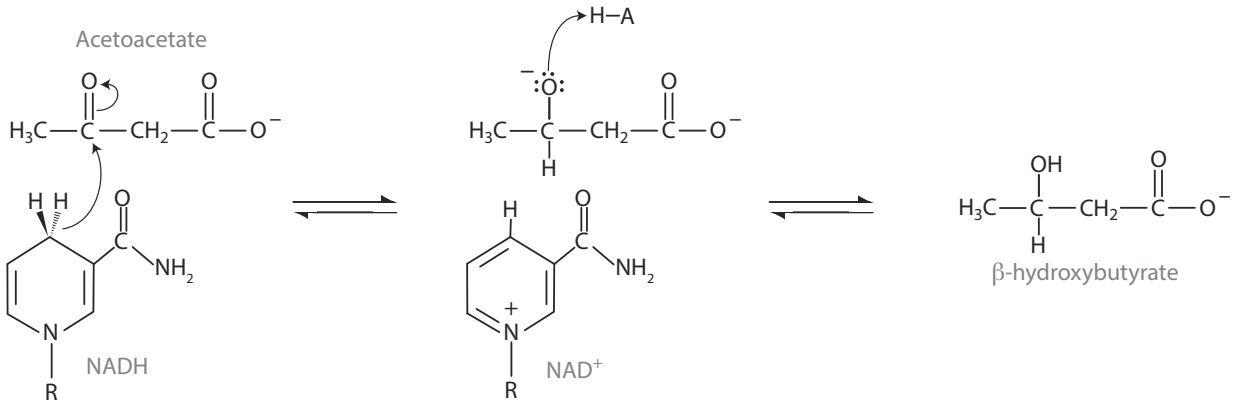




The reducing agents $NaBH_4$ or $LiAlH_4$ transform an aldehyde or a ketone into an alcohol. The reduced form of an aldehyde is a primary alcohol. For a ketone the reduced form is a secondary alcohol. Some reductions, such as Wolff-Kishner or Clemmensen reduction, will reduce aldehydes and ketones all the way to alkanes. Catalytic hydrogenation will reduce a benzylic carbonyl group to an aliphatic carbon as well.

Be sure of the sequence of oxidation states of carbon among the various functional groups. This is extremely important in biochemistry. The progression from more reduced to more highly oxidized forms goes as follows: alkyl, alcohol, aldehyde, ketone, carboxylic acid, carbon dioxide. A very useful exercise is to build the habit of assigning changes in oxidation state in terms of oxidation numbers when you see movement between these forms through oxidation-reduction. Remember that in assigning oxidation numbers, electron control over shared electrons is attributed to the more electronegative atom. Then you decide whether a particular element has gained or lost electrons in forming the bond. For example, in the reduction of an aldehyde to a primary alcohol shown at left, the oxidation number of the relevant carbon has changed from +1 to –1. For the reduction of the ketone, the oxidation number of the carbon has changed from from +2 to 0.

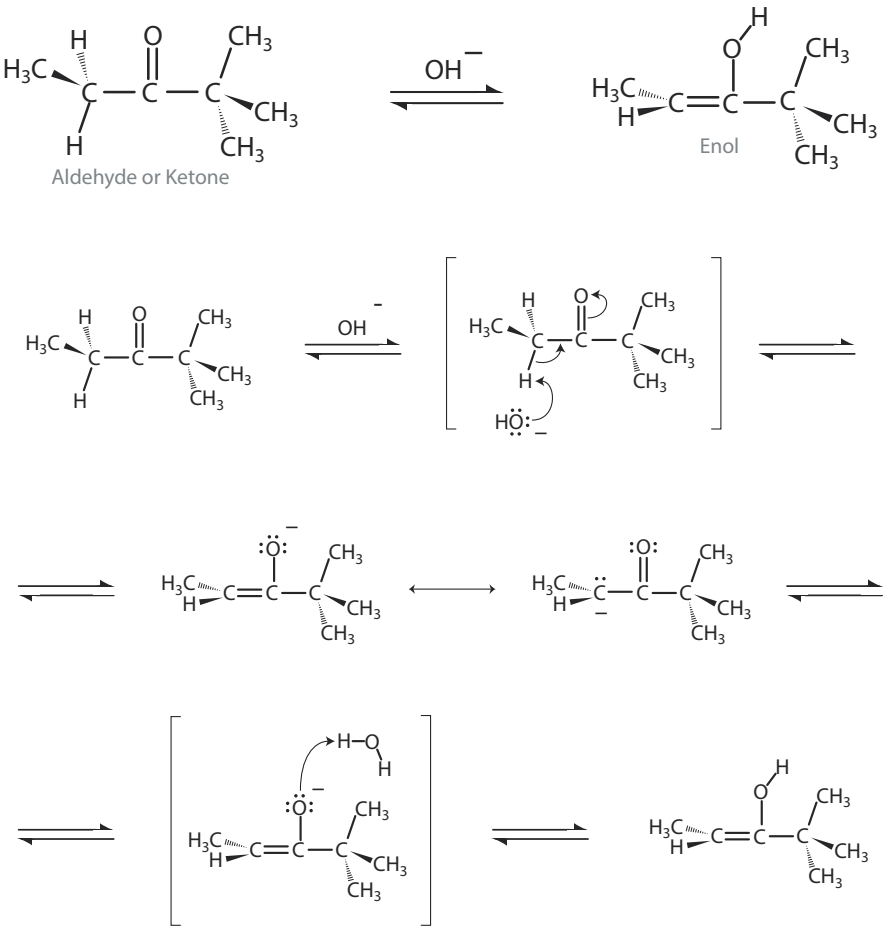
β-Hydroxybutyrate Dehydrogenase



When the body is in the fasted state, oxaloacetate becomes in short supply in the liver because it is being used to form glucose through the process of gluconeogenesis (pg 108). Oxaloacetate is thus unavailable to bind with acetyl CoA. Additionally, the high level of acetyl CoA present in the cell inhibits the pyruvate dehydrogenase complex (pg 106), whereas pyruvate carboxylase (pg 29) becomes activated for gluconeogenesis. High levels of ATP and NADH inhibit the enzymes citrate synthase and isocitrate dehydrogenase in the TCA cycle (pg 107). Under these conditions acetyl CoA is diverted into the formation of ketone bodies, which are water-soluble transportable forms of acetyl units and which serve as a perfectly acceptable fuel for most of the needs of peripheral tissues. The three ketone bodies are acetoacetate, β-hydroxybutyrate, and acetone.

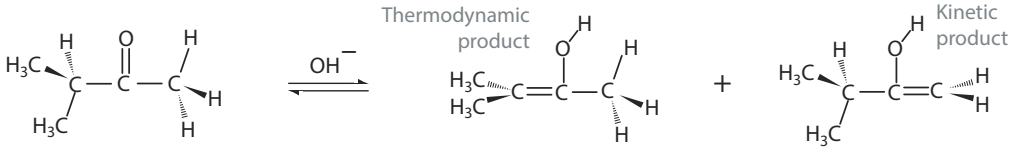
β-hydroxybutyrate is formed by the reduction of acetoacetate by the enzyme β-hydroxybutyrate dehydrogenase. This activity allows the liver to dispose of some of the surplus hydrogen that accumulates during β-oxidation of fats. β-hydroxybutyrate dehydrogenase employs NADH in its typical role as a hydride donor, reducing the carbonyl group of acetoacetate to a secondary alcohol. The activity of β-hydroxybutyrate dehydrogenase depends on the NADH/NAD^+ ratio within hepatocyte mitochondria.

Keto-Enol Tautomerism

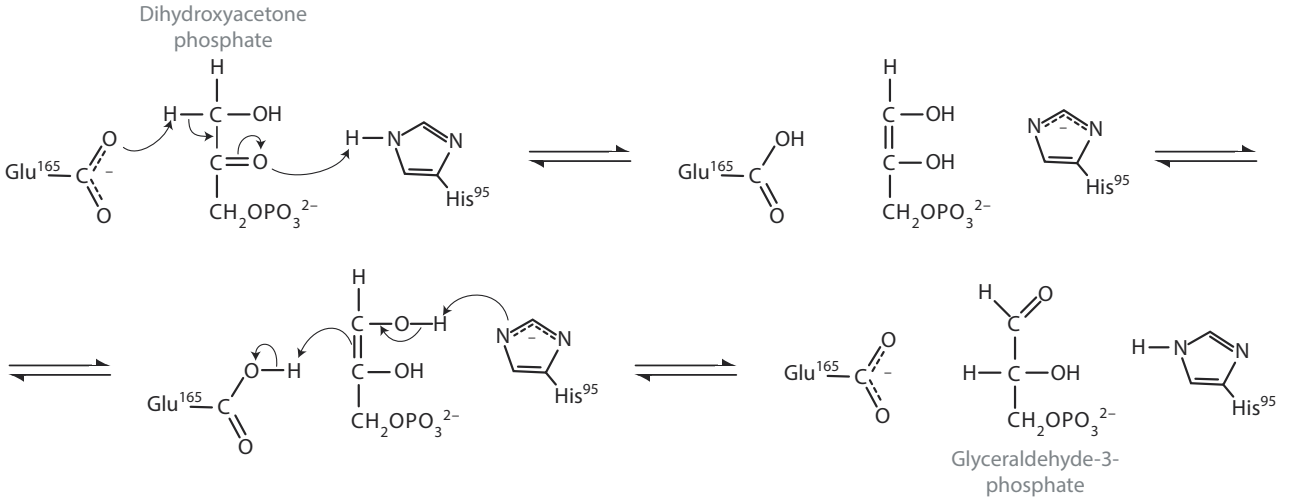


Keto-enol tautomerism is an important factor in the reactivity of aldehydes and ketones. Keto-enol tautomerism can occur with carbonyl compounds if the carbon adjacent to the carbonyl, the α carbon, possesses a hydrogen. Such α hydrogens are acidic. The conjugate base formed is resonance stabilized. The proton departs and electrons shift to form a carbon-carbon double bond with another electon pair moving by conjugation up onto the oxygen. The carbonyl group becomes an alkoxide anion and then captures a proton to become a hydroxyl group. The net process is the transfer of a proton from the α carbon to the carbonyl oxygen accompanied by the shifting of electrons up toward oxygen. The vinylic alcohol formed is referred to as an enol.

A ketone with α hydrogens at two positions has two possible tautomers. Enolization toward the less substituted position will generally be advantaged kinetically (lower activation energy) while enolization towards an interior position will be advantaged thermodynamically (lower free energy). At low temperature, kinetic control prevails and the less substituted double bond is the main reaction product. At high temperature after long reaction times, chemical equilibrium can assert itself and the thermodynamically more stable interior double bond is formed.

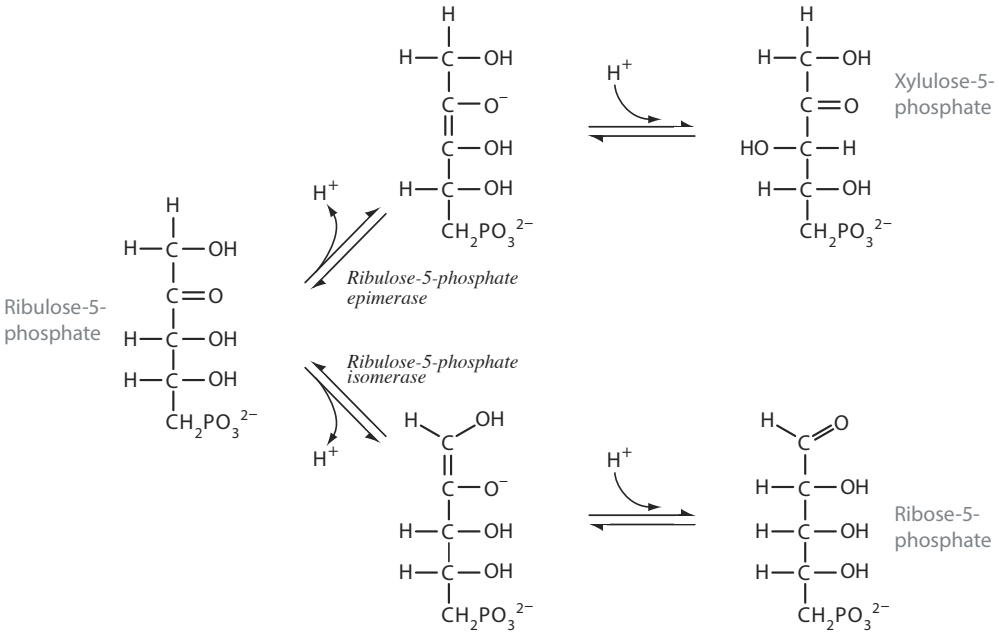


Triose Phosphate Isomerase



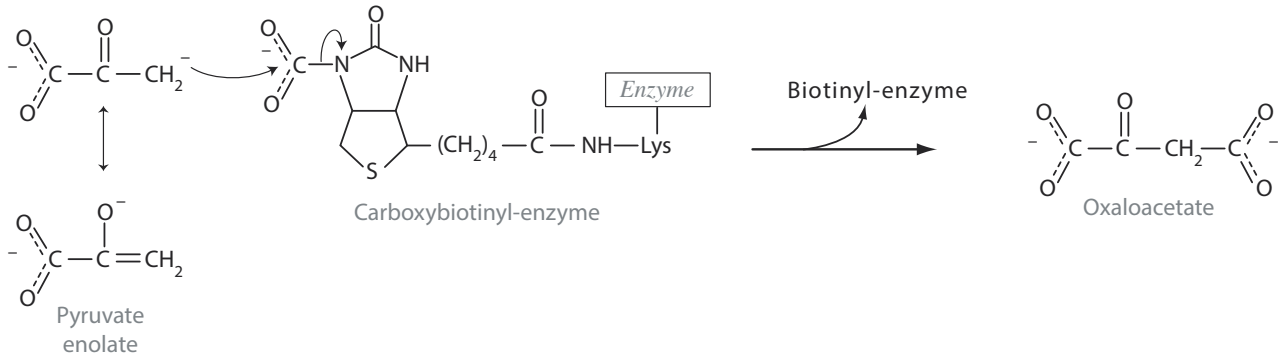
Triose phosphate isomerase (TPI) plays an important role in glycolysis (pg 105) and gluconeogenesis (pg 108) catalyzing the reversible interconversion of the ketose dihydroxyacetone phosphate (DHAP) with its aldose isomer glyceraldehyde 3-phosphate (GAP). The mechanism provides an interesting example of keto-enol tautomerism. The interconversion of DHAP and GAP proceeds through an intermediate that is an enol tautomer of either triose. Because there are two hydroxyl groups to choose from in returning to the carbonyl form, keto-enol tautomerism can lead back and forth between the two sugars.

Kilani-Fischer Synthesis of D-Glucose and D-Mannose from D-Arabinose



The non-oxidative phase of the pentose phosphate pathway (pg 109) begins with keto-enol tautomerism. Ribulose-5-phosphate 3-epimerase produces an epimer of ribulose-5-phosphate. The other branch, ribulose-5-phosphate isomerase, transforms the aldose ribulose-5-phosphate into the ketose ribose-5-phosphate. The ribulose-5-phosphate epimerase and isomerase reactions are very similar to the triose phosphate isomerase mechanism, employing a common enol tautomer to move between ketose and aldose forms.

Pyruvate Carboxylase



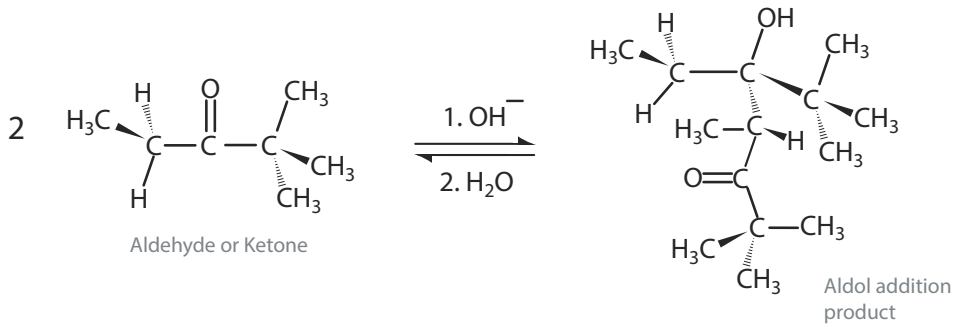
Gluconeogenesis (pg 108) begins in the mitochondria with the pyruvate carboxylase reaction, which carries out the formation of oxaloacetate by the carboxylation of pyruvate. In the next step of gluconeogenesis, oxaloacetate will be decarboxylated and phosphorylated by PEP carboxykinase to form phosphoenolpyruvate. Utilizing ATP and GTP, respectively, these two reactions push the substrate up the energy hill, part of the particular reactions, along with fructose bisphosphatase and glucose 6-phosphatase that sidestep the exergonic reactions of glycolysis on the reverse glucogenic path.

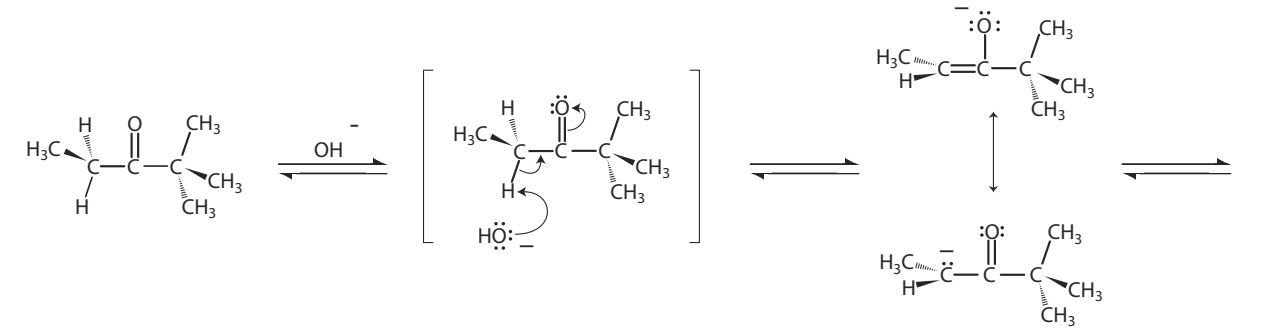
Pyruvate carboxylase is an important control point for the gluconeogenic pathway. The enzyme is stimulated by high levels of acetyl-CoA (the signal of β-oxidation of fatty acids) and inhibited by high levels of ADP and glucose.

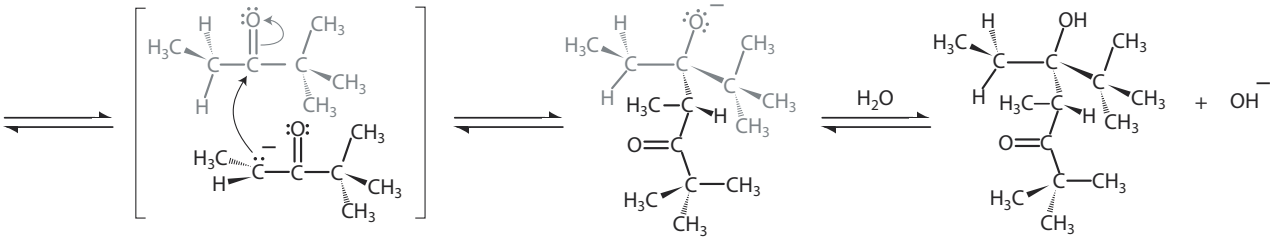
Pyruvate carboxylase relies on a covalently attached prosthetic group, biotin. Biotin serves as a carrier of activated (high energy) CO_2, which it obtains from another active site on the enzyme, the ATP-bicarbonate site. Carboxylation of biotin is the process requiring ATP. An interesting feature of this prosthetic group is that the long chain holding biotin to its enzyme allows it to swing between active sites. In this way biotin is like lipoamide in the pyruvate dehydrogenase complex (pg 106).

After activation, the carboxy-biotin swings over to the pyruvate site of the enzyme, and the enol form of pyruvate attacks it. This mechanism illustrates a key feature of the reactivity of aldehydes and ketones in biochemistry, an aspect you will see over and over again. The α carbon of an enol formed through keto-enol tautomerism is nucleophilic. The nucleophilicity of α carbons is a fundamentally important aspect of the reactivity of aldehydes and ketones. We

Aldol Addition

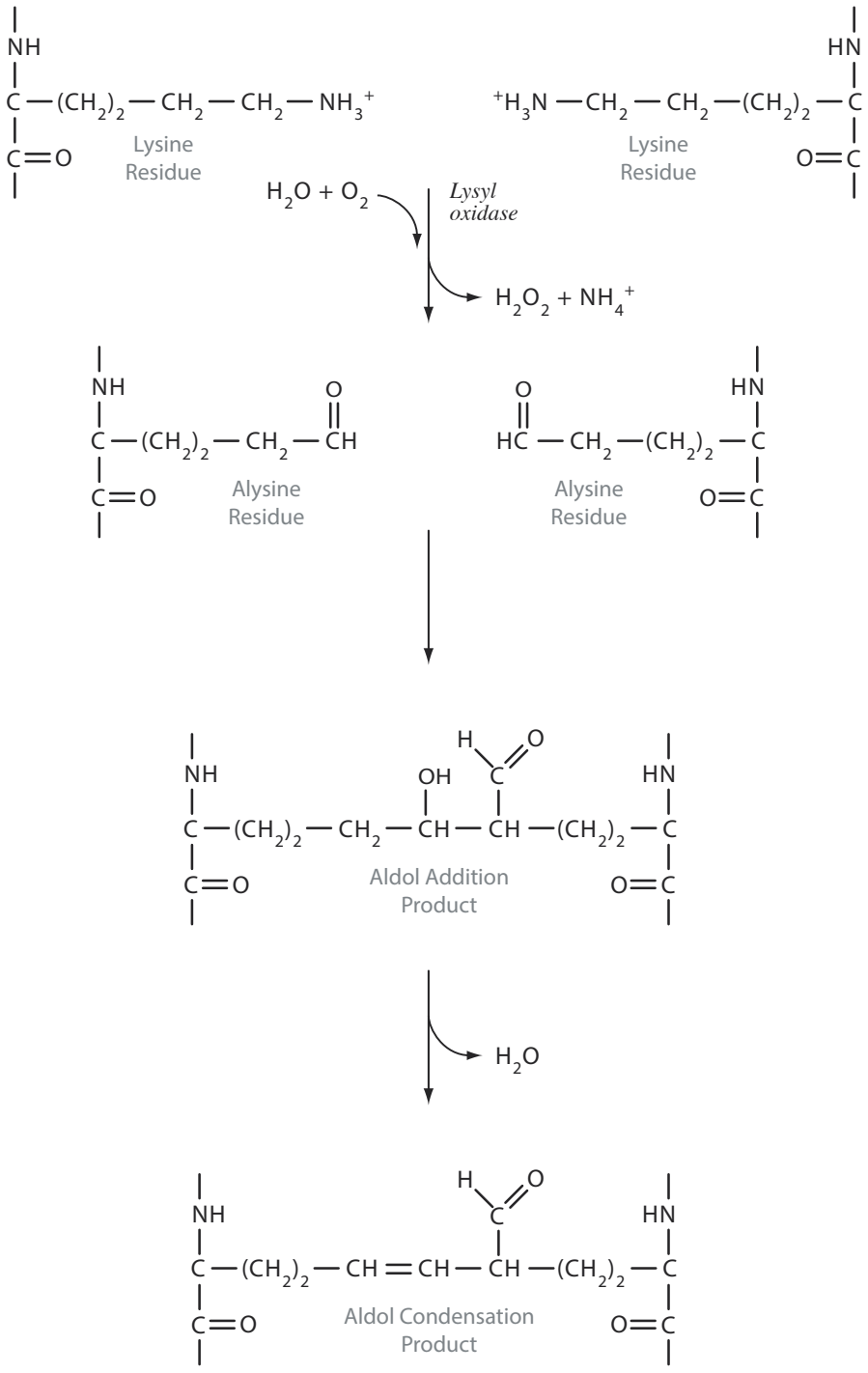






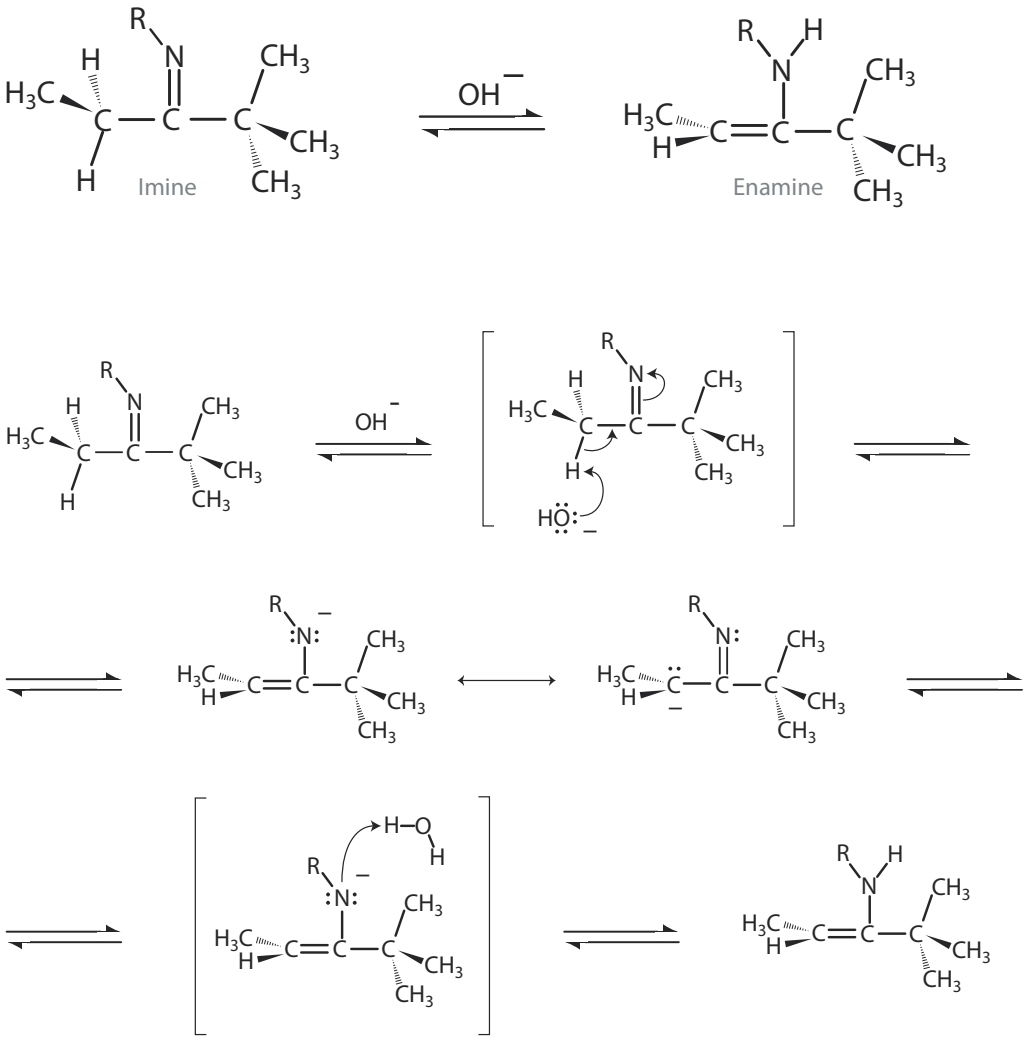
It can be helpful to see that most of the reactions of aldehydes and ketones fit into either one of two categories. For one group the mechanism relies on the partial positive charge of the carbonyl carbon, which makes it subject to attack by nucleophiles. Examples include acetal formation (pg 1) and imine formation (pg 5). In the other group the reaction depends upon the nucleophilicity of the enolate α carbon. In other words, most reactions involving aldehydes or ketones are either involving nucleophilic attack upon the carbonyl carbon or they involve keto-enol tautomerism with electrophilic attack upon the α carbon. Aldol addition is a reaction that can't be said to belong solely to either category because it belongs to both. In aldol addition the enolate form of one aldehyde or ketone acts as a nucleophile to form a bond with the carbonyl carbon of another aldehyde or ketone (or another equivalent of the same aldehyde or ketone.)

Crosslinking in Collagen



Lysyl oxidase is an extracellular enzyme catalyzing formation of aldehydes, allysine residues, from lysine residues in tropocollagen. Allysine residues are highly reactive, and undergo spontaneous aldol condensation (aldol addition followed by dehydration) with other lysyl oxidase-derived allysine residues. An allysine residue may also form a cross-link through imine formation with an unmodified lysine residue. Cross-linking in collagen and elastin is essential for stabilization of collagen fibrils.

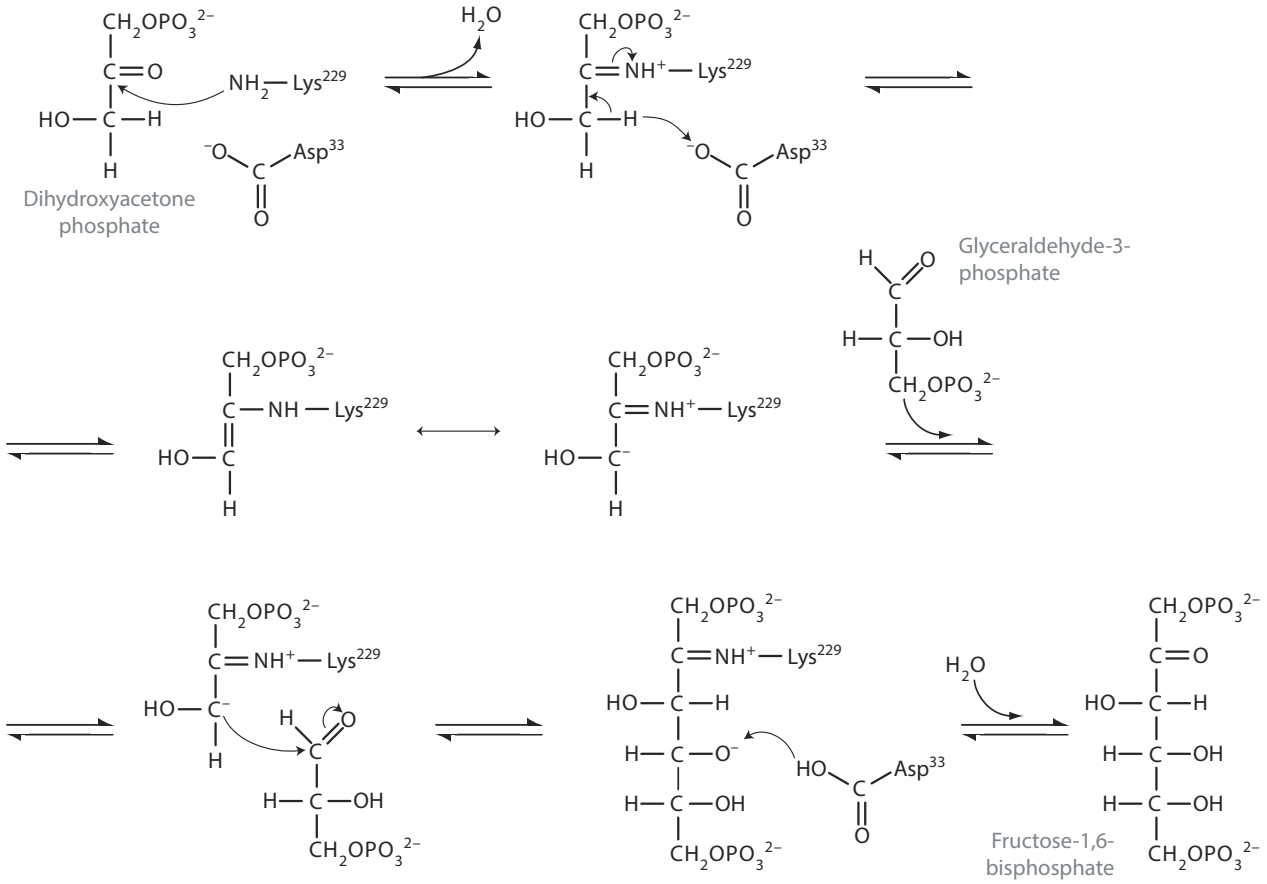
Imine-Enamine Tautomerism



In aqueous solution an equilibrium will be established between between an imine (or Schiff base) and its enamine tautomer, if the imine possesses an α hydrogen, in much the same way that we see an equilibrium established between and aldehyde or ketone and its enol tautomer. Imine-enamine tautomerism is the nitrogen analog to keto-enol tautomerism. In both cases, a hydrogen atom exchanges between the heteroatom (oxygen or nitrogen) and the second carbon atom.

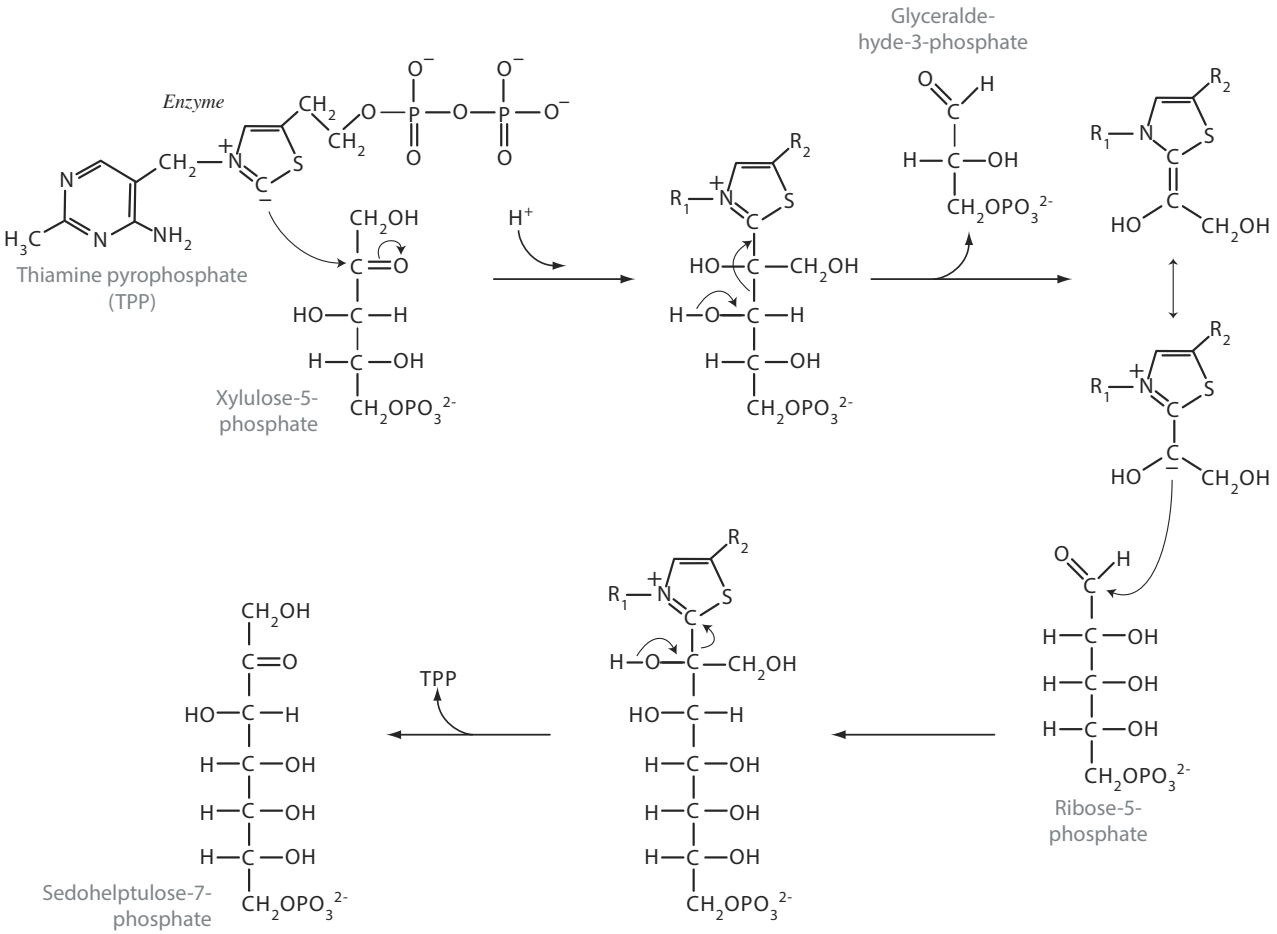
An enamine tautomer behaves a lot like an enol, and like enols, enamines are nucleophilic at the α carbon. When you see an imine in solution, you should also recognize that the enamine tautomer will also be present in some percentage. Imine-enamine tautomerism gives imines a whole set of possible reaction pathways arising from the nucleophilicity of the α carbon.

Aldolase



Aldolase reversibly splits fructose 1,6-bisphosphate into the triose phosphates dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. The reaction appears in both glycolysis and gluconeogenesis. Glycolysis (pg 105) is a catabolic pathway and uses the forward reaction. Gluconeogenesis (pg 108) is an anabolic pathways and uses the reverse reaction. The mechanism shown above represents the reverse direction. It's useful to study aldolase in the reverse mechanism because this makes it easier to see the reaction as a variant of aldol addition. In the aldolase mechanism, however, unlike aldol addition, the carbanion nucleophile is the resonance stabilized imine-enamine intermediate of an enzyme-lysine linked Schiff base instead of appearing through keto-enol tautomerism. Hydrolysis resolves the adduct so that the same net process occurs in either case.

Transketolase

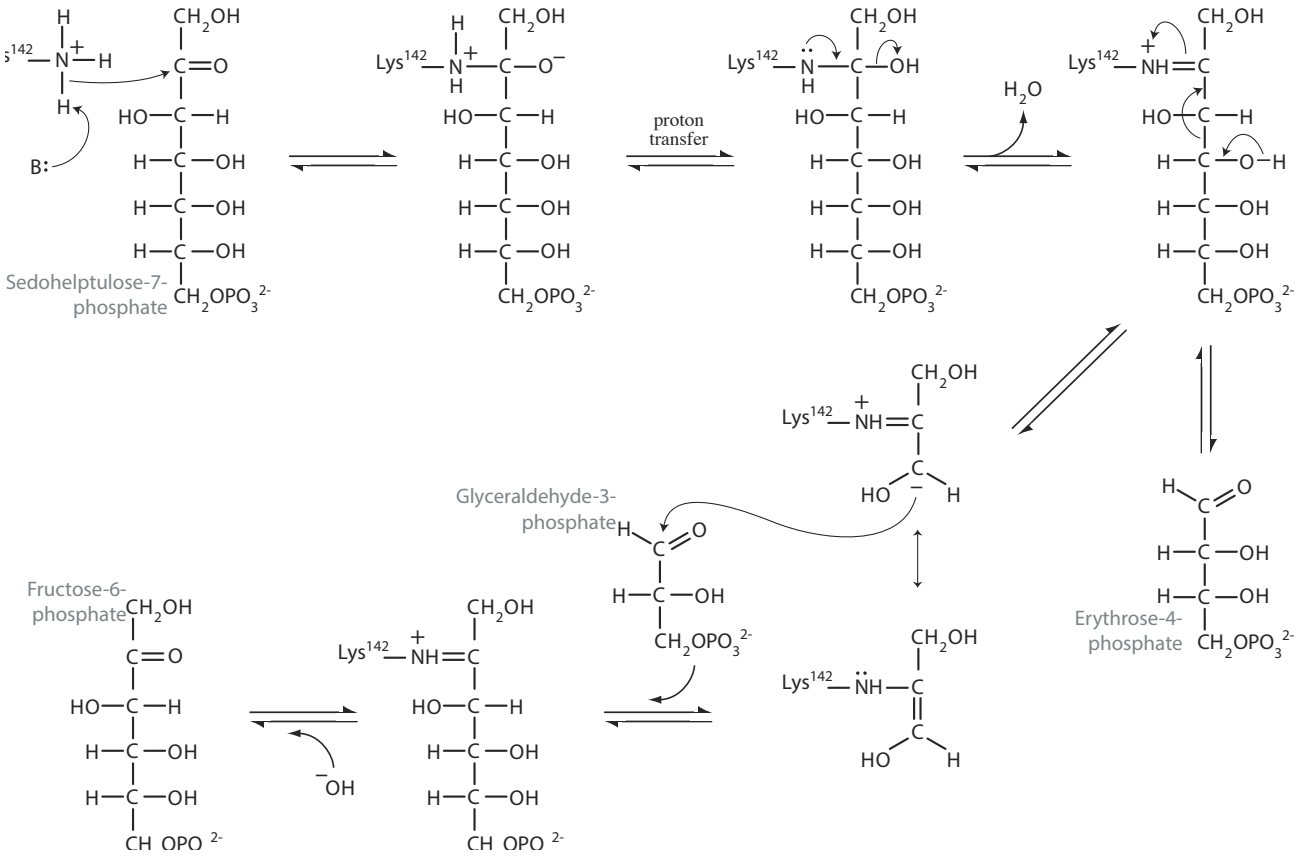


The overall process of the non-oxidative phase of the pentose phosphate pathway (pg 109) is to convert three C_5 sugars into two C_6 sugars and one C_3 sugar. Transketolase carries out the first of these reactions with transaldolase carrying out the second reaction and transketolase appearing again to carry out the third. In the first transketolase reaction, shown above, the cofactor thiamine diphosphate accepts a 2-carbon fragment from the 5-carbon ketose, xylose-5-phosphate, leaving the 3-carbon glyceraldehyde-3-phosphate. Transketolase then transfers this fragment to the 5-carbon aldose, ribose-5-phosphate, to form a 7-carbon ketose, sedoheptulose-7-phosphate.

A recurrent motif in biochemistry is that enzymatic reactions in which carbon-carbon bonds are broken or formed will often involve a stabilized carbanion intermediate. Such a carbanion can serve as a nucleophile to add to the electropositive carbonyl carbon of a substrate. In the transketolase mechanism, the ylide* form of the cofactor thiamine pyrophosphate is used to form such an intermediate in joining with the two-carbon fragment removed from xylulose-5-phosphate, a resonance stabilized imine-enamine carbanion intermediate, which can next perform nucleophilic addition upon the aldehyde group of ribose-5-phosphate forming sedoheptulose-7-phosphate.

* An ylide is a compound with positive and negative charges on adjacent atoms.

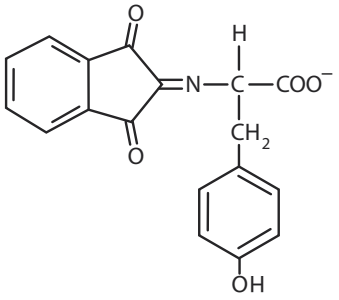
Transaldolase



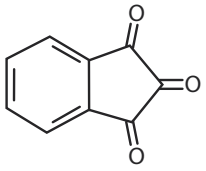
Transaldolase is an enzyme of the non-oxidative phase of the pentose phosphate pathway. Transaldolase catalyzes the transfer of a 3-carbon unit from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate. This yields erythrose-4-phosphate and fructose-6-phosphate. The transaldolase mechanism begins with a lysine residue forming a Schiff base with sedoheptulose-7-phosphate. Formation of this Schiff base creates the conditions for a variation of retro-aldol cleavage to occur (pg 37) in which a 3-carbon portion is broken from sedoheptulose-7-phosphate as an enzyme bound resonance stabilized imine-enamine intermediate with the remainder released as erythrose-4-phosphate. Formation of this Schiff-base stabilized intermediate is the vehicle for the enzyme to move the 3-carbon portion from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate. The intermediate is resonance stabilized and in possession of a nucleophilic carbon that can undergo addition to the glyceraldehyde-3-phosphate carbonyl group. Nucleophilic addition to glyceraldehyde-3-phosphate forms enzyme bound fructose-6-phosphate. The Schiff base link between the enzyme and fructose-6-phosphate is next broken by hydrolysis, regenerating transaldolase and releasing fructose-6-phosphate.

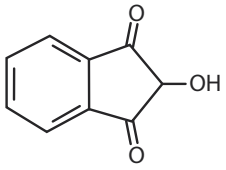
Aldehydes & Ketones Practice Items

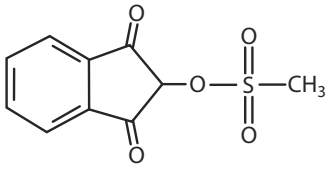
1. The substance ninhydrin is a tool of quantitative analysis. The reaction of two equivalents of ninhydrin with an α-amino acid produces both CO_2 and the intensely colored Ruheman's purple. Reaction with the first equivalent of ninhydrin produces a ketamine such as is depicted in the figure below.

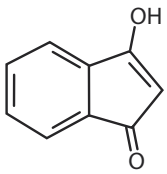


Which figure below represents the structure of ninhydrin?

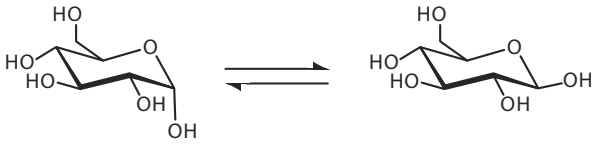
A.


B.


C.


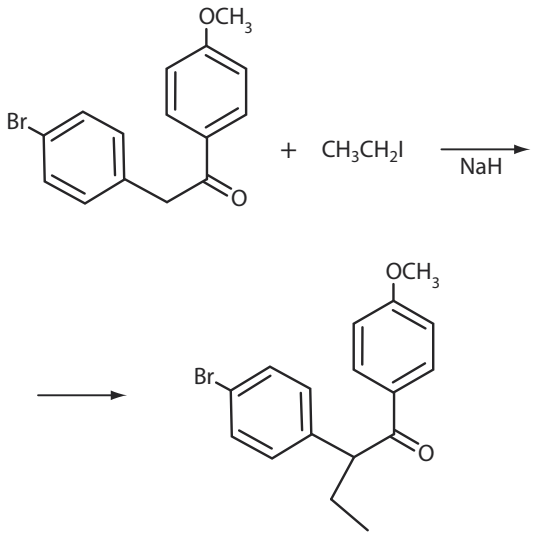
D.


2. Which of the following choices best describes the pathway of interconversion of α-glucopyranose and β-glucopyranose?



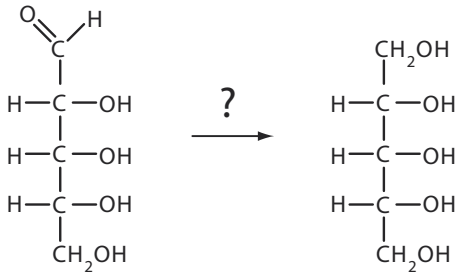
A. tautomerization
B. ring flipping
C. hemiacetal formation
D. aldol cleavage

3. The figure below shows a step in the benchtop synthesis of tamoxifen, a medication used to treat hormone-receptor positive early and metastatic breast cancers. What is the mechanism of this reaction?



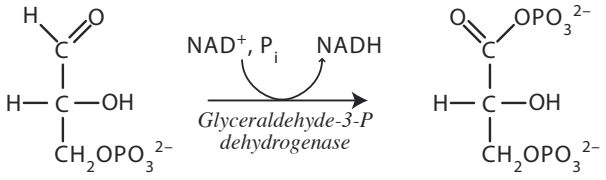
A. SN2 substitution
B. cyanohydrin formation
C. aldol addition
D. nucleophilic acyl substitution

4. Which reagent could be used to carry out the conversion of D-ribose to D-ribitol?



A. FAD
B. $NaBH_4$
C. KOH
D. PCC

5. In one of the reactions of the glycolytic pathway, glyceraldeyde-3-phosphate is oxidized by NAD^+ to form 1,3-bisphosphoglycerate in a reaction catalyzed by the enzyme glyceraldehyde-3-phosphate dehydrogenase.

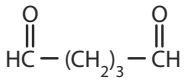


What change has occurred to the oxidation state of the aldehyde carbon of glyceraldeyde-3-phosphate as a result of this reaction?

A. +1 → +2
B. +1 → +3
C. +2 → +3
D. +2 → +4

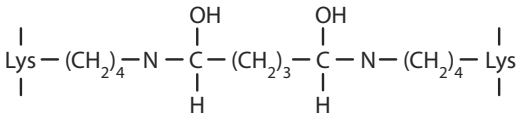
6. A method for quaternary structure analysis, which is especially useful for oligomeric proteins that decompose easily, employs cross-linking agents.

Glutaraldehyde is a bifunctional reactent that reacts to covalently crosslink two Lys residues.

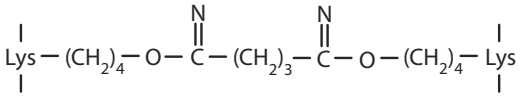


Which of the following structures below represents the cross-links formed by treatment of a multi-subunit protein with glutaraldehyde?

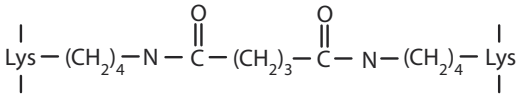
A.



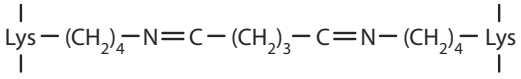
B.



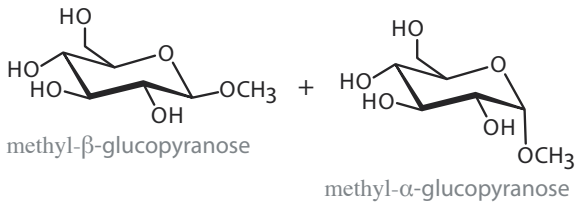
C.



D.



7. Under conditions of acid catalysis, glucose reacts with methanol to form a mixture of glucoside anomers.



methyl-β-glucopyranose

methyl-α-glucopyranose

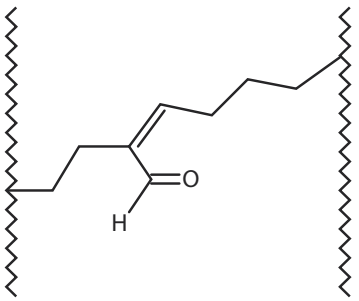
Because normal glucose crystalizes as the α form, a solution of the pure α anomer of glucose can be obtained upon dissolving crystalized glucose in water. Excess methanol was introduced under acidic conditions to a pure solution of the α anomer of glucose and the reaction completed prior to to any signficant mutarotation having occurred. In other words, only the α form and not the β form of the glucose reagent was available to react. The rate of glucaside formation at normal temperatures is several orders of magnitude faster than mutarotation of glucose. The optical activity of the solution was measured upon completion of synthesis of the methyl glucoside. The optical activity measurement obtained was most likely consistent with which of the following solutions?

A. pure methyl-α-glucopyranose
B. pure methyl-β-glucopyranose
C. a mixture of the α and β forms with a greater concentration of the β form
D. a racemic mixture

8. To elongate the carbon chain of an aldose, the Kilani-Fisher synthesis utilizes a particular reagent to form a new carbon-carbon bond. What is that reagent?

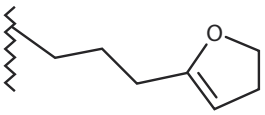
A. sodium cyanide
B. dihydroxyacetone
C. sodium amalgum
D. methyl amine

9. Subsequent to its export to the extracellular compartment by a fibroblast, tropocollagen is assembled into collagen fibrils via cross-linking. The figure below depicts a crosslink between two modified amino acid side chains in side-by-side tropocollagen helices.

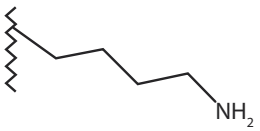


Which of the following depictions represents one of the modified side chains prior to the formation of the cross-link?

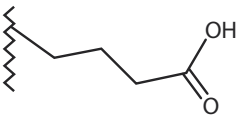
A.



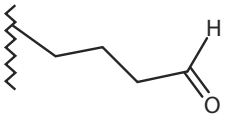
B.



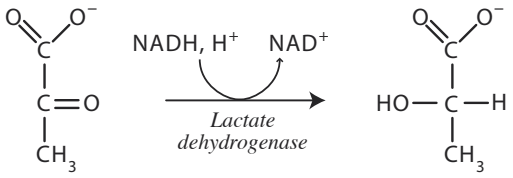
C.



D.



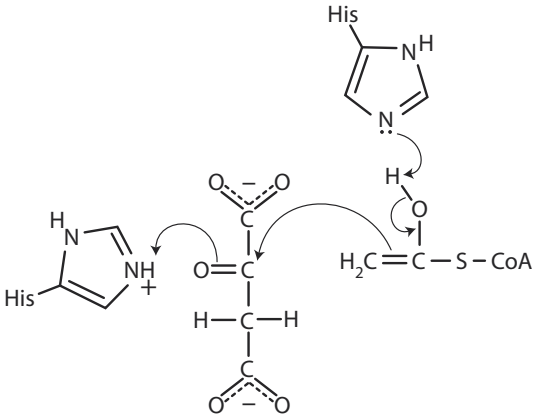
10. In the process of fermentation, pyruvate is reduced by NADH to form lactate in a reaction catalyzed by the enzyme lactate dehydrogenase.



What change has occurreed to the oxidation state of the carbonyl carbon of pyruvate as a result of this reaction?

A. –2 → 0
B. –2 → –1
C. +2 → +1
D. +2 → 0

11. What reaction is occuring in this step of the citrate synthase mechanism?



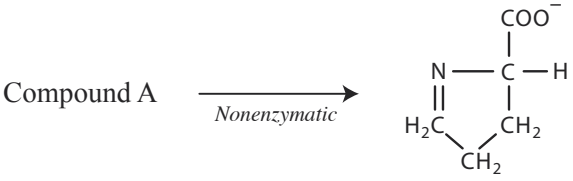
A. Michael addition
B. Claisen condensation
C. Aldol addition
D. Hydrolysis of a thioester

12. Fill in the blank to complete the following analogy:

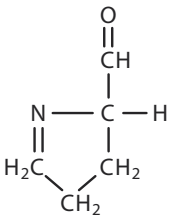
An enol is to a ketone as an enamine is to a(n) _____.

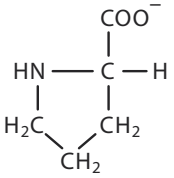
A. amine
B. amide
C. nitrile
D. Schiff base

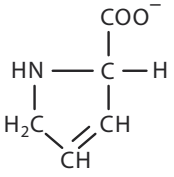
13. A step in the biosynthesis of proline involves the non-enzymatic comversion of Compound A into 1-pyrroline-5-carboxylic acid.

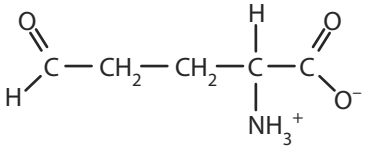


Which of the following is Compound A?

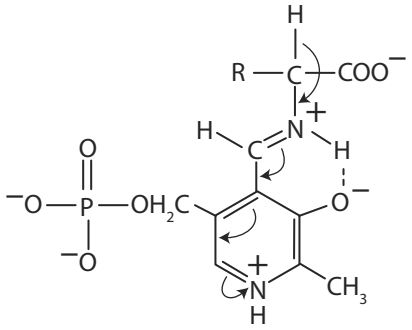
A.


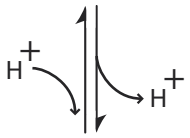
B.


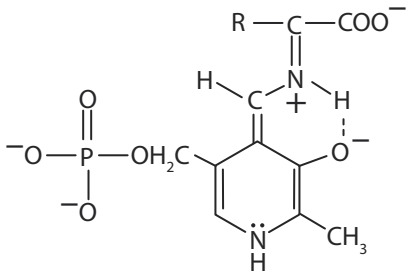
C.


D.


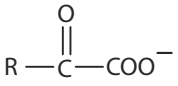
14. The figure below depicts a step in the transminase mechanism. Loss of a proton occurs in this step from the PLP-amino acid Schiff base, leading to formation of a resonance stabilized ketimine intermediate.

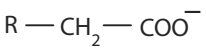


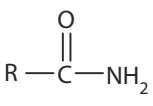


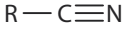


The mechanism then proceeds with subsequent hydrolysis of the ketamine intermediate. Which of the following is a product of the subsequent hydrolysis of the ketamine intermediate?

A. 

B. 

C. 

D. 

15. Which of the following reaction mechanisms involves an enolate or enol intermediate?

I. decarboxylation of acetoacetate
II. pyruvate kinase
III. enoyl CoA hydratase
IV. triose phosphate isomerase

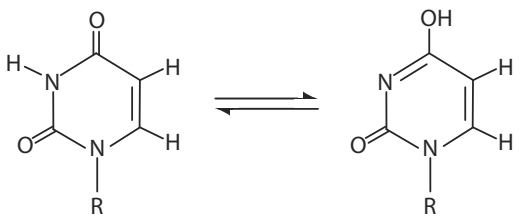
A. I and II
B. II and IV
C. I, II, and III
D. I, II, III and IV

16. The liberation of cyanide serves as a defense mechanism against herbivores and microbial attack in plants. The activity of which of the following enzymes corresponds to this phenotype?

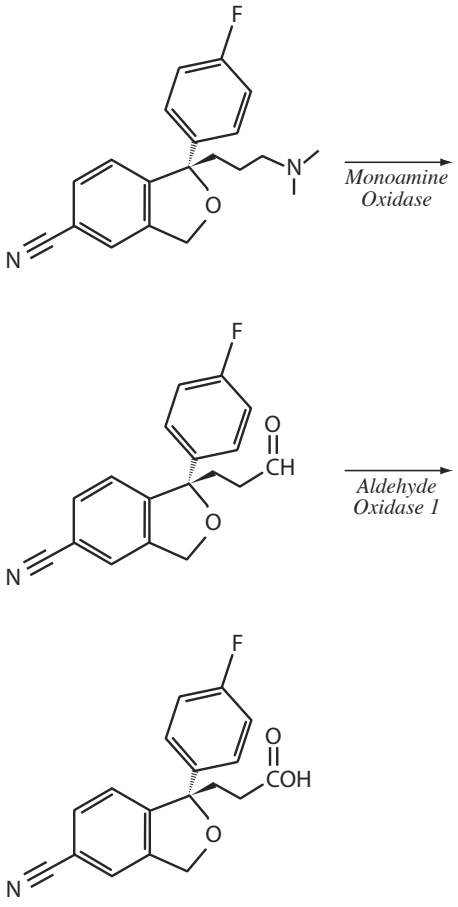
A. hydroxynitrile lyase
B. transaminase
C. hydroxylamine oxidoreductase
D. fatty acylamidase

17. The interconversion shown below between the lactam and lactim forms of uracil is a type of

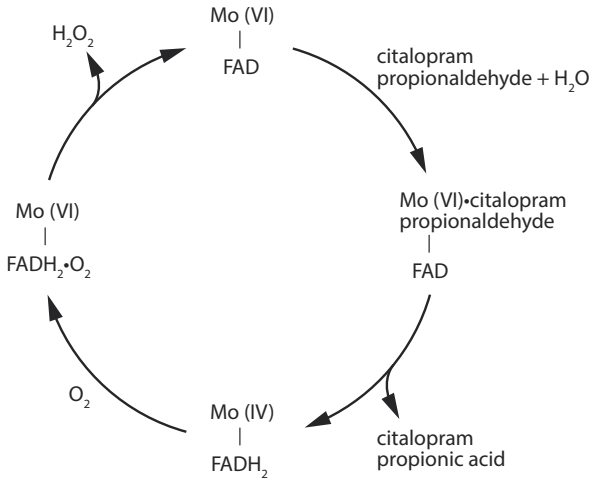
A. mutarotation
B. epimerization
C. tautomerization
D. resonance



18. One of the pathways in the liver for the metabolism of the drug citalopram involves the activity of the enzymes monoamine oxidase and aldehyde oxidase.



The figure below shows the catalytic cycle of aldehyde oxidase.



Which of the following is a true description of the net aldehyde oxidase reaction in the metabolism of citalopram?

A. a transfer of two electrons from molecular oxygen to citalopram propionaldehyde

B. a transfer of four electrons from citalopram propionaldehyde to molybdenum and FAD

C. a transfer of two electrons from citalopram propionate to molecular oxygen

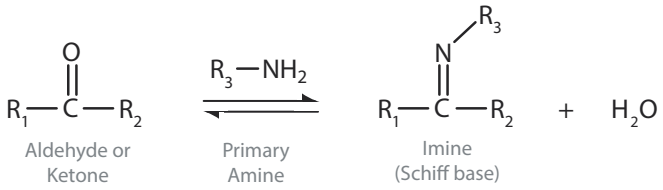
D. a transfer of two electrons from citalopram propionaldehyde to molecular oxygen

Aldehydes & Ketones

Answers and Explanations

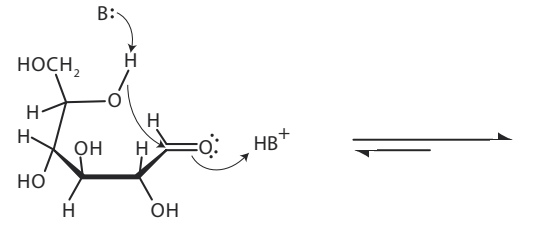
1. A

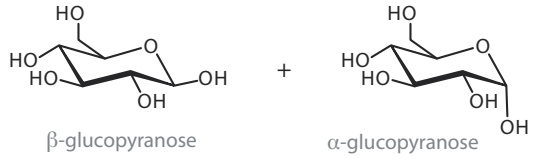
The reaction which has occurred is imine formation. The amino acid tyrosine has reacted with the ninhydrin reagent through nucleophilic addition of its α-amine group of the to the carbonyl group of the ninhydrin. Imine formation proceeds first with nucleophlic addition to form a tetrahedral intermediate followed by dehydration to form the imine.



2. C

Glucose ring formation is an example of the hemiacetal phase of acetal formation in which an equivalent of alcohol forms a tetrahedral intermediate with an aldehyde.

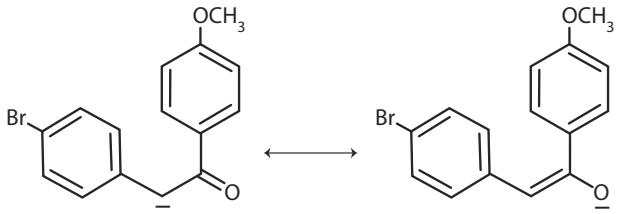




The intercoversion of the α and β anomeric forms of glucopyranose occurs through the straight chain, ring opening by hemiacetal formation in reverse, and followed by closure to form the hemiacetal now inverted at C1.

3. A

Sodium hydride base converts the reagent into a resonance stabilized enolate anion.



The negatively charged carbon depicted in one of the resonance forms of the enolate anion is nucleophilic. The reaction proceeds via SN2 substitution.

4. B

The reducing agents $NaBH_4$ or $LiAlH_4$ transform an aldehyde or a ketone into an alcohol. The reduced form of an aldehyde is a primary alcohol.

5. B

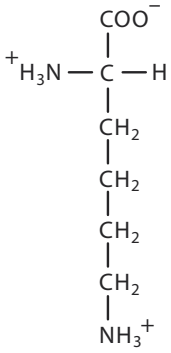
When you have the structural formula of an organic compound, assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

Each of the electrons carbon brought to the double bond in the carbonyl bond to oxygen in G3P is assigned to oxygen. Those are under oxygen's control. They are oxygen's property in the oxidation-reduction accountig system. However, that carbon has also 'gained' the one electron that hydrogen brought to its bond to the carbon. The carbon in the aldehyde at the start, in other words, is in the position of having lost two and having gained one, so the oxidation state of the aldehyde carbon in glyceraldehyde-3-phosphate is +1. Remember henceforth that the oxidation state of a carbonyl carbon in an aldeyde is +1.

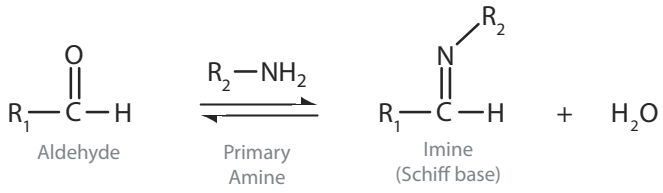
After the reaction, the carbon will now have three electrons invested in bonds to oxygen (a double bond and a single bond), so its oxidation state in 1,3-bisphosphoglycerate has become +3. It has been oxidized by NAD^+ in a two electron transfer. These two electrons are now the property of NADH. Remember that the oxidation state of a carbon in a carboxylic acid (or carboxylic acid derivative, here a phosphate anhydride) is +3.

6. D

The amino acid lysine possesses a primary amine side-chain.

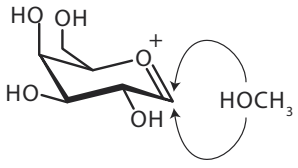


A primary amine reacts with an aldehyde to form an amine.



7. C

The ring form of glucose is a hemiacetal (See question 2 explanation). The methyl glucoside formed in this reaction is an acetal. Formation of an acetal from a hemiacetal proceeds through nucleophlic attack of an alcohol upon an oxonium cation intermediate. Because the attack can occur from either plane of the ring, a mixture of α and β forms is obtained.

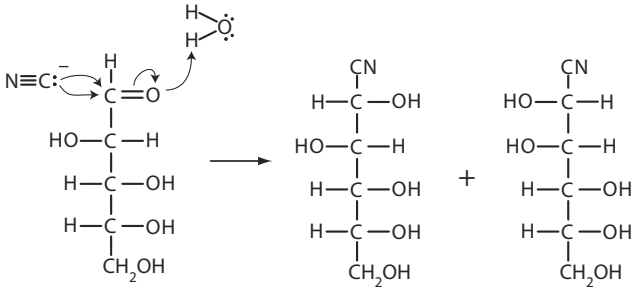


Because the β form possesses lower free energy, it will form predominatly. Even if the two forms occurred at equal concentration, however, the choice could not be 'D' because the solution would not be racemic. The α and β forms are not enantiomers. They are diasteriomers and a solution with equal concentrations of the two would still show optical activity.

8. A

The first step in Kilani-Fischer synthesis is to react the starting sugar with aqueous cyanide. The cyanide undergoes nucleophilic addition to the carbonyl group of the sugar forming a cyanohydrin. Cyanohydrin formation is an important tool of organic synthesis because it can form a new carbon-carbon bond.

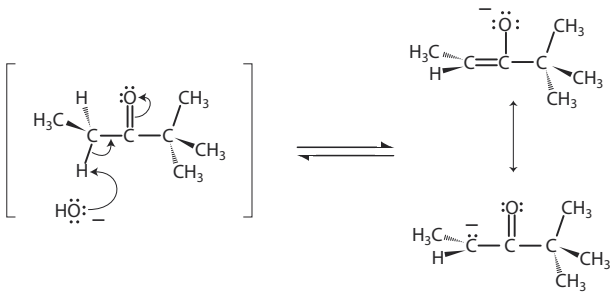
While sugars tend to exist mainly as cyclic hemiacetal, they are always in chemical equilibrium with their open-chain aldehyde or ketone forms, and in the case of these aldoses it is that aldehyde form that reacts in this synthesis.



Note that because the cyanide can add to either plane of the trigonal planar carbonyl group, the cyanohydrin resulting from this addition consists of a mixture of two diastereomers. The stereochemistry of all the previously present chiral carbons is preserved.

9. D

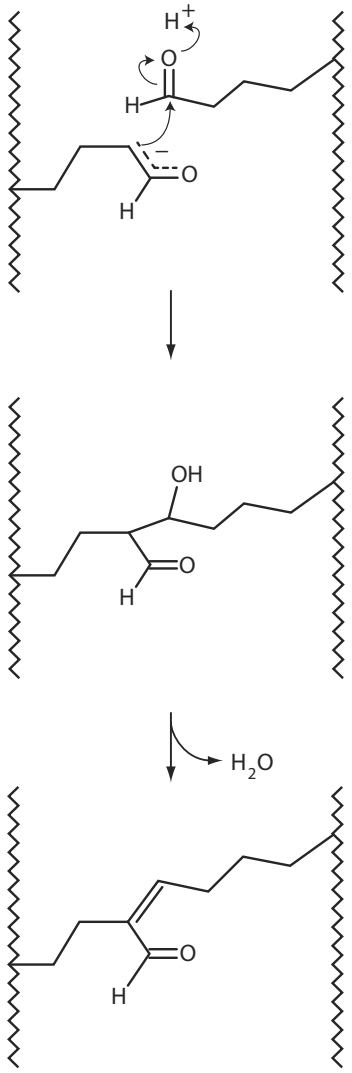
Keto-enol tautomerism can occur with carbonyl compounds if the carbon adjacent to the carbonyl, the α carbon, possesses a hydrogen. Such α hydrogens are acidic. The conjugate base formed is resonance stabilized. Keto-enol tautomerism is an important factor in the reactivity of aldehydes and ketones.



One of the MCAT's favorite reactions is aldol addition, in which the enolate form of one aldehyde or ketone acts as a nucleophile to attack the carbonyl carbon of another

(or another equivalent of the same aldehyde or ketone). The cross-link in collagen depicted in the problem has formed between two *alllysine* residues. Allysine is an aldehyde derivative of lysine produced by the action of the enzyme lysyl oxidase.

The reaction below would be termed aldol condensation. Aldol condensation is aldol addition followed by a dehydration step. An aldol condensation product is an α,β unsaturated carbonyl compound.



To be able to work backwards from an aldol addition product or an aldol condensation product to determine the original carbonyl compounds involved in forming the product is an important figure of merit for the MCAT. The key is locating the original α carbon in the product (it will be adjacent to a carbonyl group) and the original carbonyl carbon, which will either possess a hydroxyl group, in the case of aldol addition, or be on the far side of a double bond in the case of the full aldol condensation.

10. D

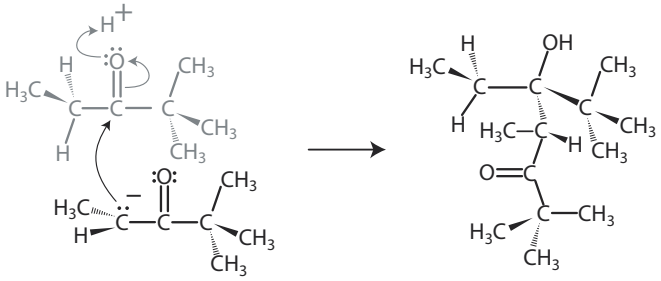
When you have the structural formula of an organic compound, assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

Each of the electrons carbon brought to the double bond in the carbonyl bond to oxygen in pyruvate is assigned to oxygen. Those are under oxygen's control. They are oxygen's property in the oxidation-reduction accountig system. The C2 carbon of pyruvate at the start, in other words, is in the position of having lost two electrons, so the oxidation state at the start is +2. Remember henceforth that the oxidation state of a carbonyl carbon in an ketone is +2.

After the reaction, the carbon will now have one electron invested in a bond to oxygen, so it loses that one, but it also has a bond to hydrogen, so it gains an electron there, the electron hydrogen brought to that bond. The carbon's oxidation state in lactate is therefore 0. It has been reduced by NADH in a two electron transfer.

11. C

In aldol addition, the enolate of one carbonyl compound nucleophilically attacks the carbonyl of another.

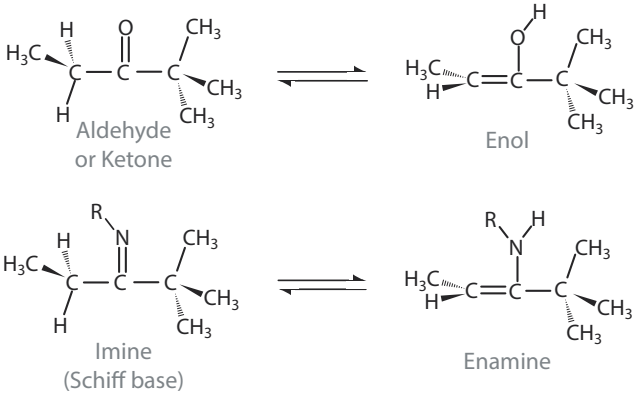


In the step of the citrate synthase mechanism shown in the problem, the enolate ester of acetyl CoA is acting as a nucleophile to attach to the carbonyl group of oxaloacetate.

12. D

Two substances with the same molecular formula but different connectivities in their structural formulas are called constitutional isomers. Tautomers are constitutional isomers which readily interconvert.

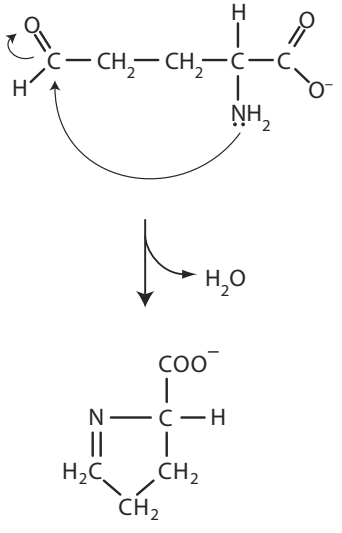
As long as it possesses an α hydrogen, a ketone will interconvert with its enol tautomer. This means that some of the enol form will be present in aqueous solution, as well as the enolate intermediate in the conversion (in neutral to basic conditions). Keto-enol tautomerism opens aldehydes and ketones up to a whole dimension of reactivity. Similarly, an imine possessing an α hydrogen will be interconverting with its enamine tautomer, which opens up imines to a dimension of reactivity.



In biochemistry imines (the subset possessing an 'R' group on the nitrogen) are referred to as Schiff bases. In other words, what an organic chemist would call it an imine (or a ketimine), a biochemist calls a Shiff base. Imine-enamine tautomerism is at least as important to biochemistry as keto-enol tautomerism, and keto-enol tautomerism is important!

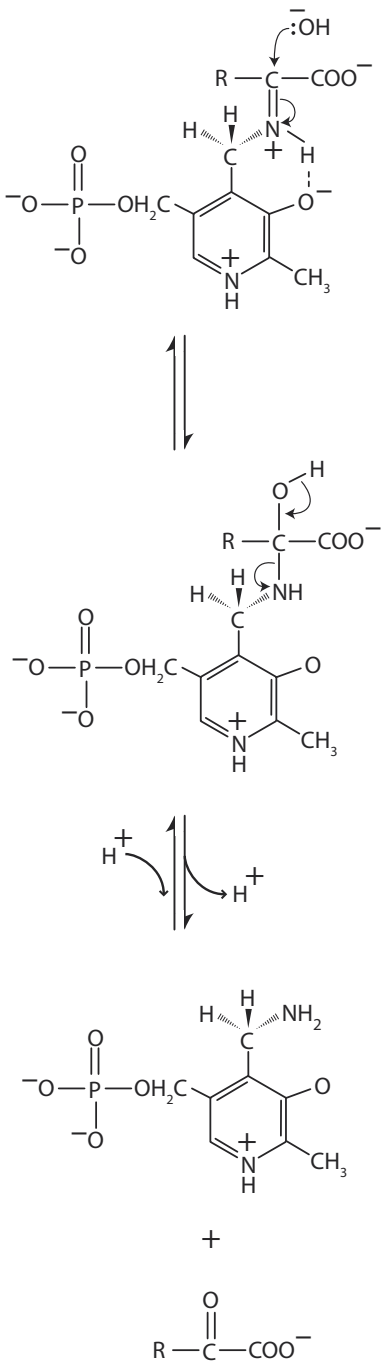
13. D

The intermediate which converts nonenzymatically into 1-pyrroline-5-carboxylic acid in proline biosynthesis is glutamate-5-semialdehyde. The reaction that occurs is an intramolecular imine formation.



14. A

Hydrolysis of an imine is simply imine formation in reverse.



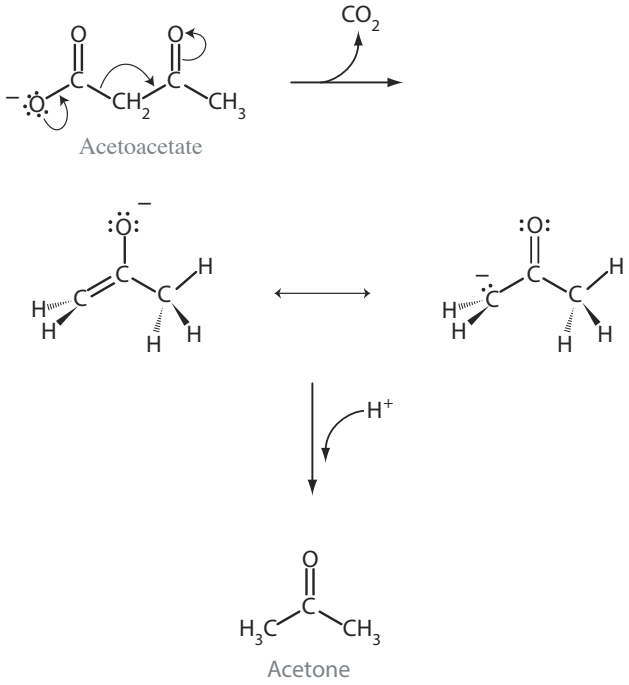
Transaminase interconverts α-amino acids and α-keto acids. Examples the transaminase reaction include the interconversion of alanine and pyruvate, aspartate and oxaloacetate and glutamate and α-ketoglutarate. Transaminase is an key enzyme in amino acid breakdown and synthesis, the malate aspartate shuttle, and the urea cycle.

15. D

It is an important point to emphasize in biochemistry that many biochemical processes are facilitated because their occurance coincides with formation of a resonance stabilized enolate anion.

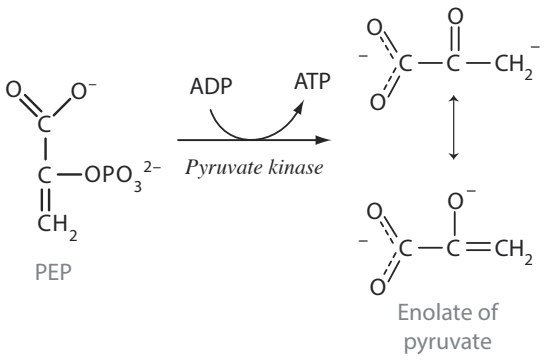
Decarboxylation of acetoacetate:

The ketone body acetoacetate undergoes a steady rate of nonenzymatic decarboxylation forming acetone in the blood. Acetoacetate is a β-keto acid. β-keto acids lose CO_2 very easily because the reaction passes through a resonance stabilized enolate anion.



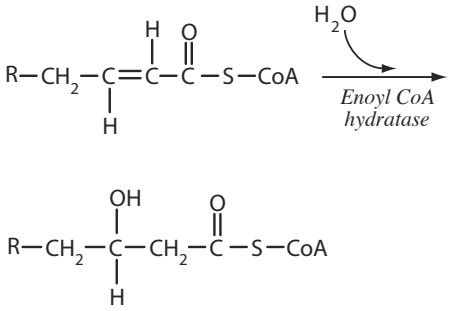
Pyruvate kinase:

One of the driving factors behind the phosphoryl transfer potential of phosphoenolpyruvate (PEP) is that transfer of the phosphoryl group onto ADP leaves behind an enolate anion.

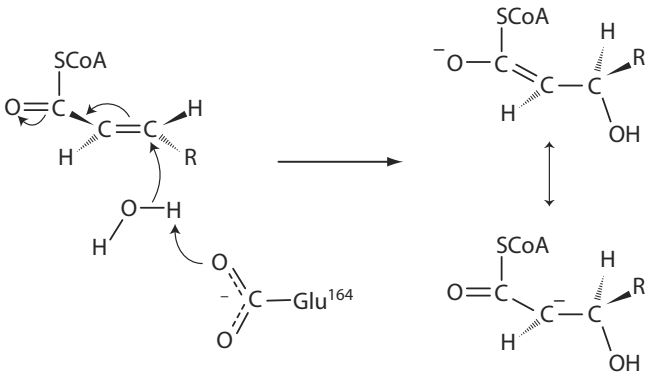


Enoyl CoA hydratase:

Enoyl CoA hydratase is a step in the pathway for the oxidation of fatty acids.

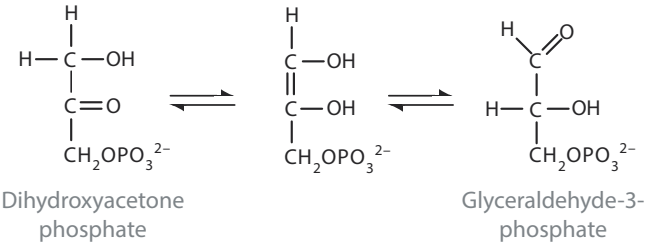


The reaction is a Michael addition. Although Michael addition is not on the list of MCAT reactions, from the AAMC section bank, knowledge of Michael addition does appear to be figure of merit for the exam. In Michael addition, a nucleophile attacks the β carbon of an α,β unsaturated carbonyl compound. Nucleophiles attack the β carbon because the addition leads to an enolate intermediate as in the enoyl CoA hydratase mechanism:



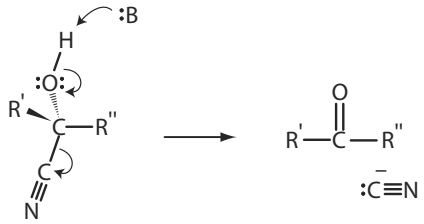
Triose phosphate isomerase:

In glycolysis (and gluconeogenesis) triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The enol of both is an intermediate in the interconversion.



16. A

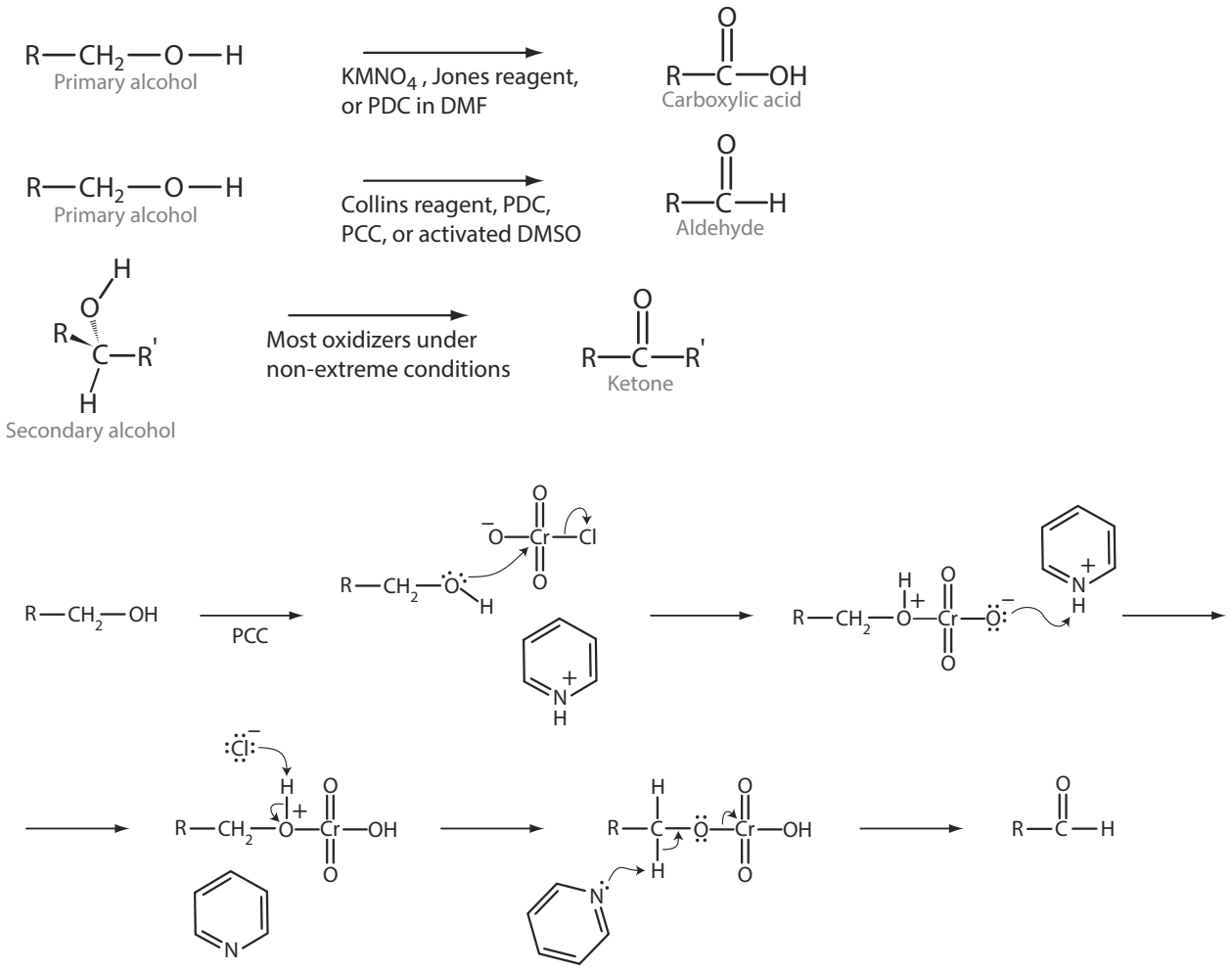
Hydroxynitriles are more often referred to as cyanohydrins. In cyanohydrin formation, cyanohydrins are produced through the reversible nucleophilic addition of cyanide anion (CN–) to an aldehyde or ketone. Reasoning function from its name, hydroxynitrile lyase is the only plausible choice among those given which could liberate cyanide. The enzyme catalyzes the reverse reaction.



17. C

Constitutional isomers are two molecules with the same molecular formula but different connectivity. Constitutional isomers that can interconvert in a rapid equilibrium are tautomers. The lactam and lactim forms of uracil are tautomers. The interconversion would be a tautomerization.

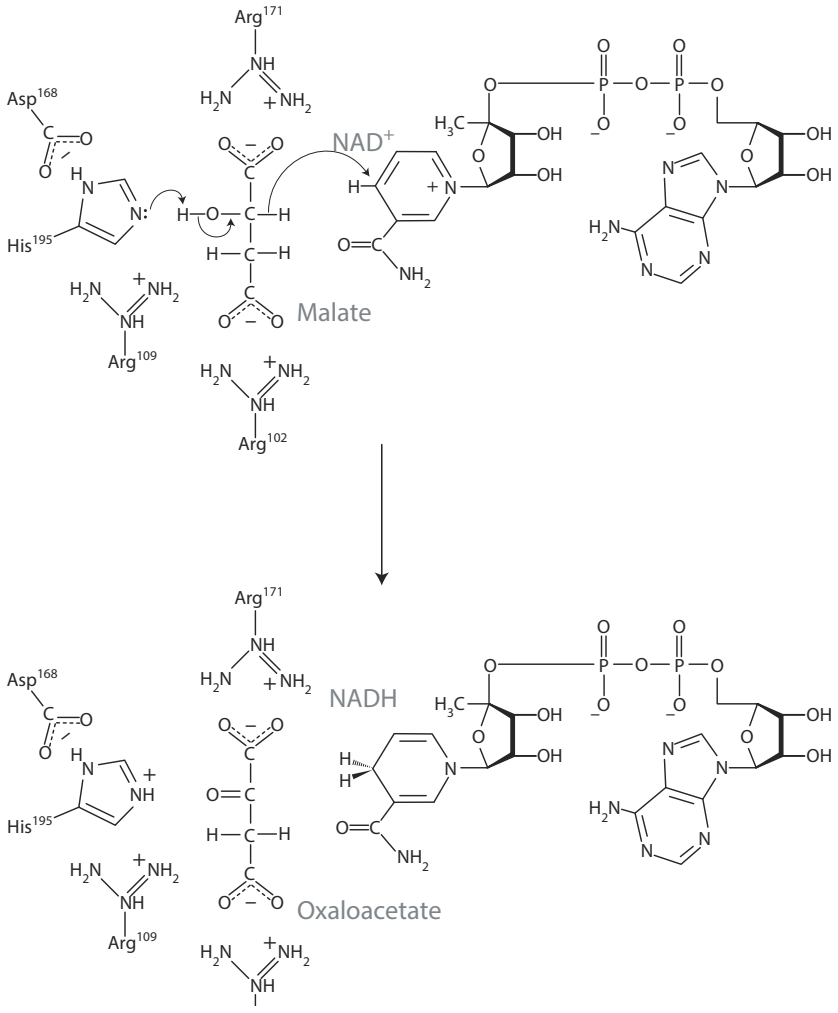
Oxidation of Alcohols



Primary alcohols can be oxidized to aldehydes or to carboxylic acids. In aqueous media, the carboxylic acid is usually the major product. The oxidation of secondary alcohols normally terminates at the ketone stage.

The figure above depicts the mechanism of the oxidation of a primary alcohol by pyridinium chlorochromate (PCC). In the first step the alcohol forms a chromate ester. This places electron withdrawing pressure on oxygen. Oxygen is the second most electronegative element after fluorine. Here oxygen now finds itself in a tug-of-war across chromium with three other oxygen atoms. When pyridinium abstracts a proton from the carbon bonded to oxygen, this solves the problem, because electrons that had been under the control of carbon are free now to shift to oxygen. Oxygen would rather own these electrons than be in a tug-of-war over the electrons it owns in redox terms in its bond with the chromium atom that is in a +6 oxidation state, so it leaves those electrons to become the property chromium. Carbon loses and chromium gains. Oxidation occurs as the carbonyl group forms and the Cr species leaves with an electron pair. In this reaction carbon has been oxidized from a –1 to +1 oxidation state and chromium has been reduced from a +6 to a +4 oxidation state.

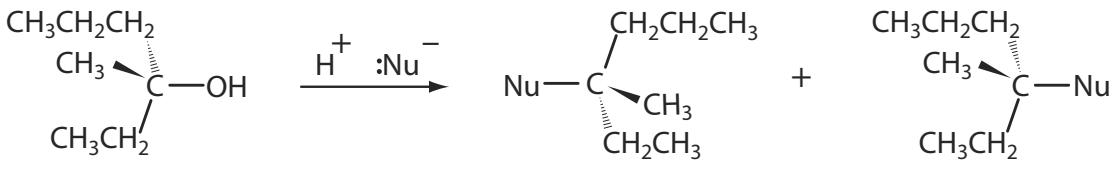
Malate Dehydrogenase

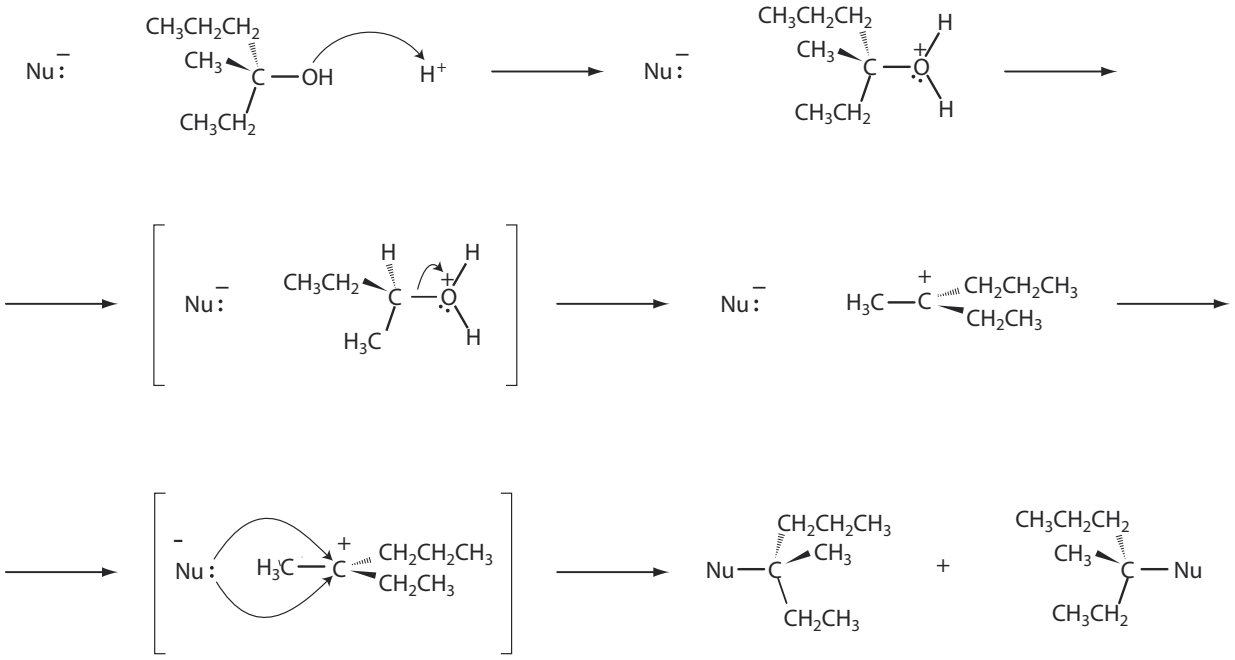


In the citric acid cycle (pg 107), malate dehydrogenase reversibly catalyzes the oxidation of malate to oxaloacetate with reduction of NAD^+ to NADH. Malate dehydrogenase also plays a role in gluconeogenesis (pg 108), although it is not in the net pathway. In gluconeogenesis, pyruvate carboxylase (pg 29) forms oxaloacetate from pyruvate in the mitochondria. Malate dehydrogenase reduces the oxaloacetate to malate so that it can traverse the inner mitochondrial membrane. Once in the cytosol, the malate is oxidized back to oxaloacetate by a cytosolic form of malate dehydrogenase so that gluconeogenesis can resume with the PEP carboxykinase step.

The malate dehydrogenase mechanism illustrates a recurrent theme in energy metabolism, the oxidation of an alcohol to a carbonyl group by NAD^+. Such reactions involve the removal of two hydrogen atoms from the reactant in the form of a hydride ion (H^-), and a proton (H^+). The proton is received by a histidine residue (ultimately released to the solution) while NAD^+ is reduced to NADH by transfer of the hydride to the nicotinamide ring. Note that when the NAD^+ is reduced, the nicotinamide ring loses its aromaticity. This is why NADH such a powerful reducing agent. When it donates these electrons to NADH:ubiquinone oxidoreductase (Complex I) later, the nicotinamide ring will regain its aromaticity.

SN1 Substitution with Alcohols



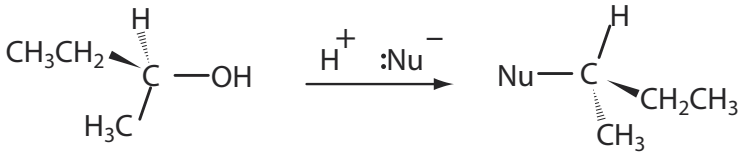


Substitution reactions upon an alcohol substrate involve replacement of the hydroxyl group with a new functional group. However, hydroxide is a strong base, making hydroxyl groups poor leaving groups. For this reason substitution reactions of alcohols are generally acid catalyzed. Acid catalysis makes it possible for the hydroxyl group to leave as water.

Upon acid catalysis, the SN1 variation of alcohol substitution begins with departure of water and formation of a carbocation. The "1" in SN1 refers to the fact that only the substrate appears in the rate determining step in the SN1 mechanism, carbocation formation, and thus the rate expression is first order. Because the reaction passes through a carbocation intermediate, the SN1 mechanism is much more likely for tertiary alcohols than primary alcohols, where SN2 substitution predominates (pg 47). Tertiary carbocations are significantly more stable than primary carbocations. Also, remember that rearrangement might occur within a carbocation intermediate if a methyl or hydride shift transforming a secondary into a tertiary carbocation is possible.

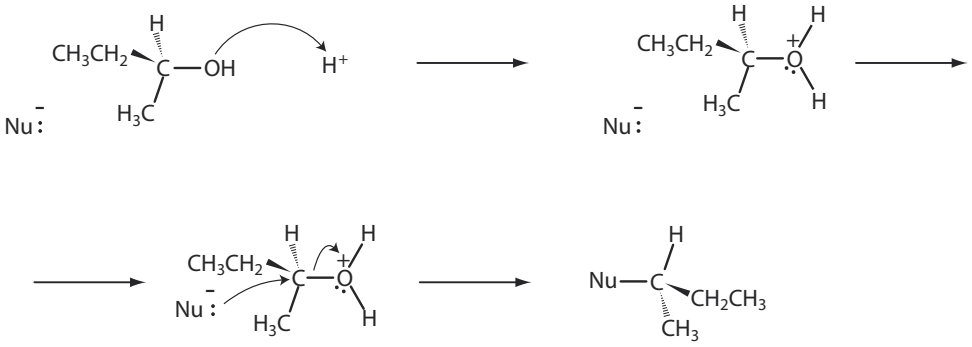
The carbocation intermediate is trigonal planar and attack by the nucleophile can occur on either face of the carbocation. This means that even if the reagent is a pure enantiomer, the SN1 mechanism leads at least to partial racemization.

SN2 Substitution with Alcohols



1° or 2° Alcohol

Substitution product

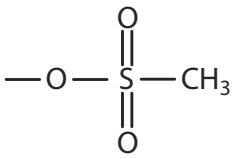


As with SN1 substitution (pg 46), SN2 substitution of alcohols is generally acid catalyzed, so that hydroxyl group can leave as water.

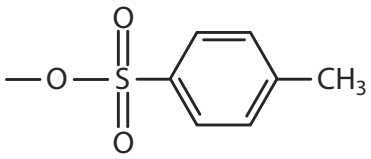
Tertiary alcohols are more likely to react with the SN1 mechanism. SN2 is the favored process for primary alcohols. In the SN2 mechanism, the nucleophile donates an electron pair from one side while the water departs with an electron pair on the other side of the carbon. Because attack of the nucleophile occurs in concert with departure of the leaving group, the SN2 mechanism always occurs with inversion of configuration. If the reagent alcohol is pure enantiomer, the product will also be a pure enantiomer, though with inverted configuration.

The "2" in SN2 substitution refers to the fact that both the nucleophile and the substrate appear in the rate determining step in the SN2 mechanism, and thus the rate expression is second order.

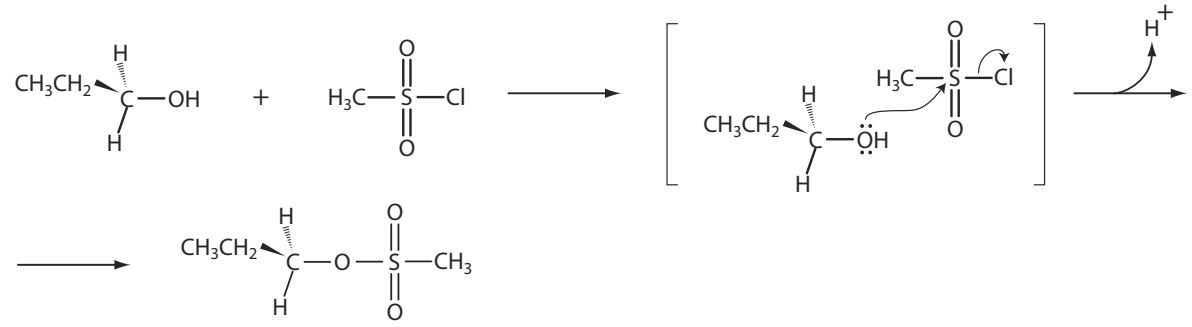
Mesylate & Tosylate Leaving Groups

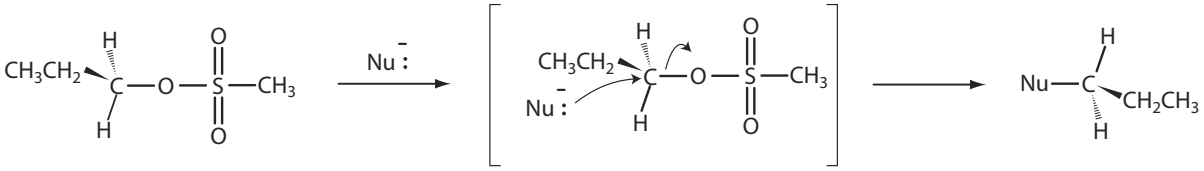


Mesylate



Tosylate

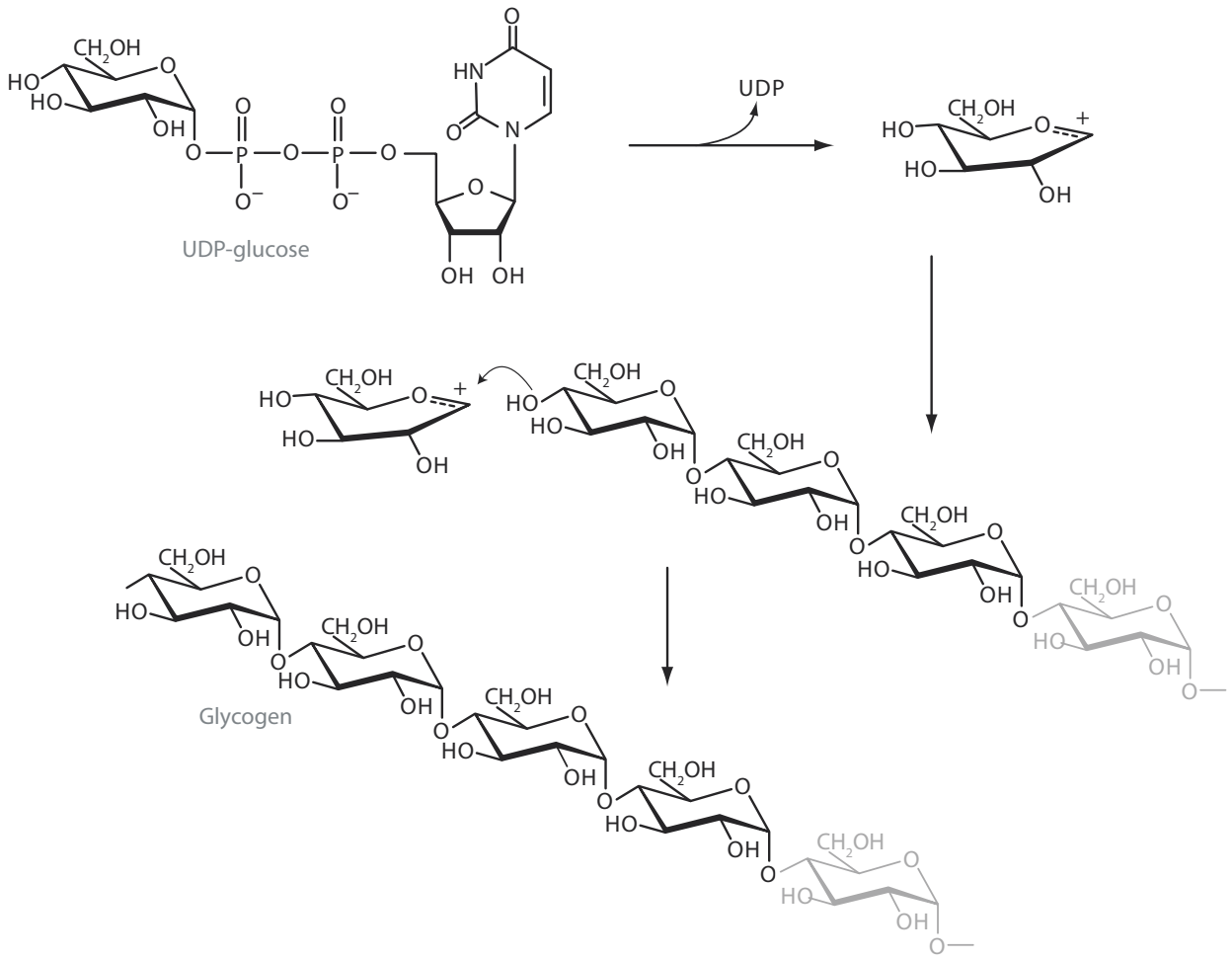




What makes a good leaving group? When a leaving group departs from a substrate in a substitution reaction, it takes an electron pair with it. How stable will the leaving group be after it departs? Can the negative charge spread out or does it remain compressed within a small region? This type of discussion of internal energy is similar to the one informing the determination of the position of equilibrium for an acid-base couple. Will the conjugate base be very stable? If so, the acid will be strong. In fact, as a general rule of thumb the best leaving groups are also very weak bases. Strong bases are poor leaving groups. Weak bases are substances that can manage being negatively charged in solution without taking on high energy.

This presents a problem for substitution reactions involving alcohols. Because hydroxide is a strong base it makes a poor leaving group. This problem can be circumvented with acid catalysis, but when that isn't practical, another solution to the problem is by turning the alcohol into a sulfonic ester. A commonly employed method being either to form an organic mesylate or an organic tosylate by treatment of the alcohol with either methanesulfonyl chloride or para-toluene sulfonyl chloride. Mesylate and tosylate groups are excellent leaving groups in nucleophilic substitution reactions because the negative charge on the leaving group is stabilized by resonance.

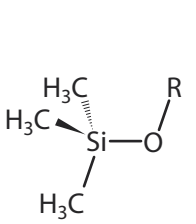
Glycogen Synthase



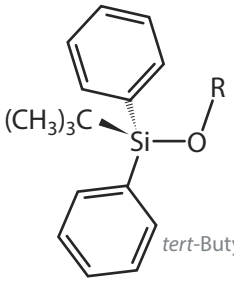
The formation of the glycosidic linkage between two carbohydrate moeities is an instance of the second part of acetal formation (pg 1) in biochemistry in which an alcohol adds to a hemiacetal.With reference to acetal formation, the second sugar's anomeric carbon begins the process at the hemiacetal stage (pp 3-4). The cyclic form of a sugar is a hemiacetal. In glycosidic bond formation, the hydroxyl group of one sugar carries out nucleophilic addition upon the hemiacetal anomeric carbon of another. Before this carbon can receive the nucleophile, this anomeric carbon must be transformed into an oxonium cation through departure of its hydroxyl group. However, hydroxide is a poor leaving group. In benchtop acetal formation, oxonium cation formation occurs through acid catalyzed dehydration. In the biochemical context, however, such significantly acidic conditions are not feasible, so instead, the hydroxyl group must first be transformed into a good leaving group.

Glycogen synthesis includes such a strategy. In this pathway, the UDP moeity is positioned by UDP-glucose pyrophosphorylase (pg 103), extending from the phosphate of glucose-1-phosphate, to form an excellent leaving group at the C-1 position. Activating glucose in this way enables oxonium cation formation through UDP departure in glycogen synthesis. The substrate is then prepared for nucleophilic attack and the formation of the glycosidic linkage between the anomeric carbon and the non-reducing end of the growing glycogen chain.

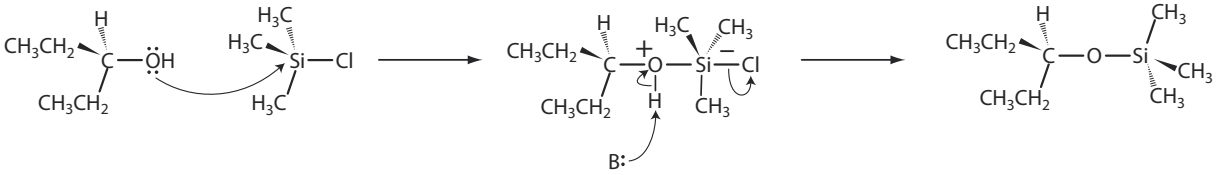
Silyl Protecting Groups

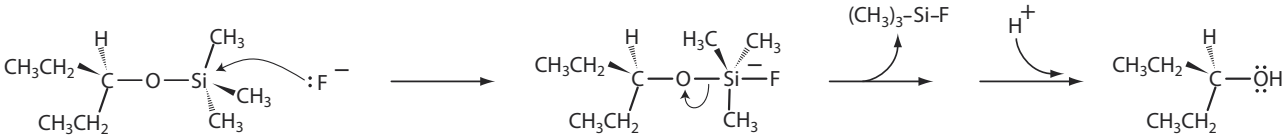


Trimethylsilyl ether



tert-Butyldiphenylsilyl ether

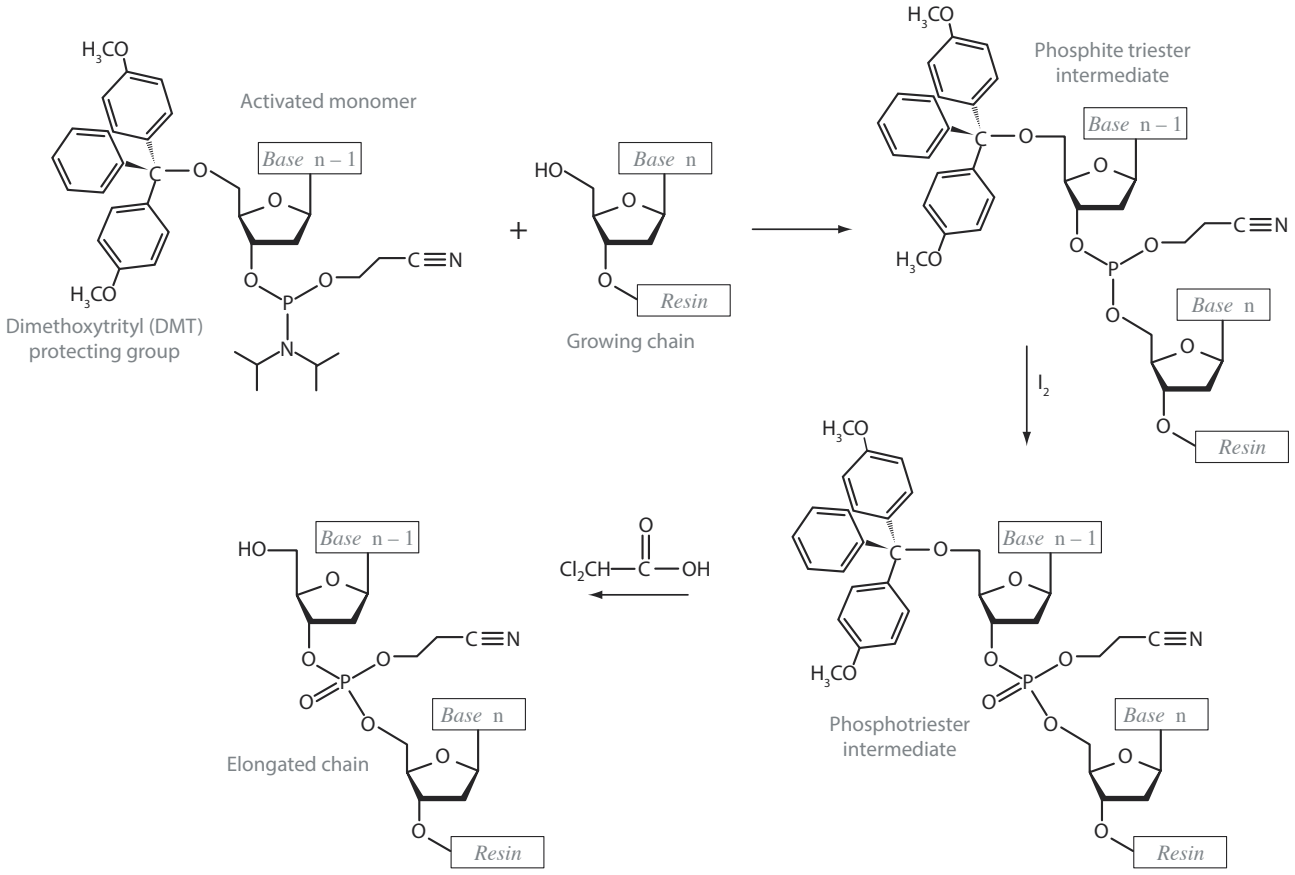




Silylating agents are often used in synthetic organic chemistry to protect hydroxyl groups from adverse reaction conditions. In the role of protection, they group forms a silyl ether with the substrate. Silyl groups are particularly useful for this purpose because they can be installed and removed very selectively under mild conditions. Common silyl protecting groups include trimethylsilyl (TMS) and tert-butyldiphenylsilyl (TBDPS).

There are some interesting aspects regarding the use of silyl protecting groups. For example, the bulklier silyl protecting groups, such as TBDPS, will selectively protect primary alcohols in the presence of secondary and tertiary alcohols. Additionally, silyl groups are generally created with at least two of the R groups on silicon being the same. Otherwise, the silicon of the silyl group would be a chiral center. Protection using a racemic mixture of such a silylating agent could create a new stereogenic center and formation of a mixture of diastereomers which might complicate downstream handling.

Solid Phase DNA Synthesis



The dimethoxytrityl (DMT) protecting group is widely used for protection of 5'-hydroxy group in nucleosides, particularly in oligonucleotide synthesis, such as in the phosphite triester method of solid phase DNA synthesis shown above. The 5'-hydroxy group of the monomer to be added has been rendered unreactive (protected) by attaching DMT, preventing undesirable side reactions. Upon the addition of the monomer, the DMT will be removed with weak acid.

The gist of the phosphite triester method is that naturally occurring nucleotide phosphate esters are insufficiently reactive to afford convenient synthetic preparation of oligonucleotides in high yields. Using a phosphoramidite derivative dramatically improves the selectivity and the rate of the formation of internucleosidic linkages.

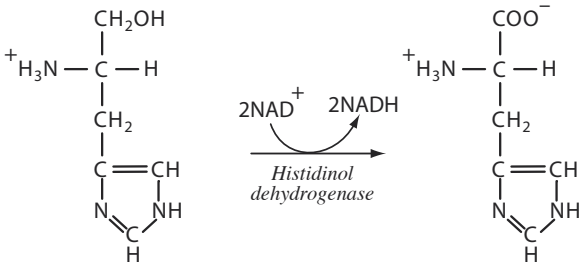
While not specific MCAT content, solid phase DNA synthesis is indicative of the type of procedure the test-writers are apt to choose as the subject matter of a passage. Difficult passages are almost never about whether you have seen the particulars before but about whether you can break things down in terms of the fundamental principles you already know.

Reactions of Alcohols Practice Items

1. Ethanol is metabolized to acetaldehyde in the cytosol of liver cells by alcohol dehydrogenase. Consumption of ethanol in excess can disregulate cellular metabolism by inhibiting gluconeogenesis and promoting fatty acid synthesis by making conditions in the cytosol too . . .

A. reducing
B. oxidizing
C. acidic
D. basic

2. Histidinol dehydrogenase is the final step in histidine biosynthesis.



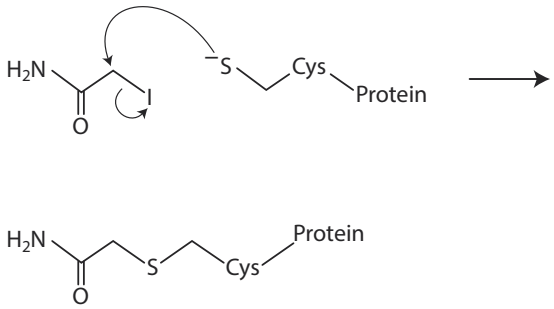
L-Histidinol Histidine

What change has occurred to the oxidation state of the hydroxyl bearing carbon of histidinol as a result of this reaction?

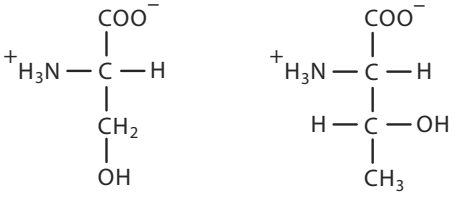
A. –1 → +3
B. 0 → +3
C. 0 → +4
D. +1 → –3

3. 2-Iodoacetamide is an alkylating agent used for peptide mapping purposes. It binds covalently with the thiol groups of cysteine residues preventing the formation of disulfide bonds. 2-Iodoacetamide may also be utilized as an irreversible inhibitor of enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, that employ a reactive cysteine in their mechanism.

The figure below depicts the alkylating mechanism of 2-iodoacetamide.



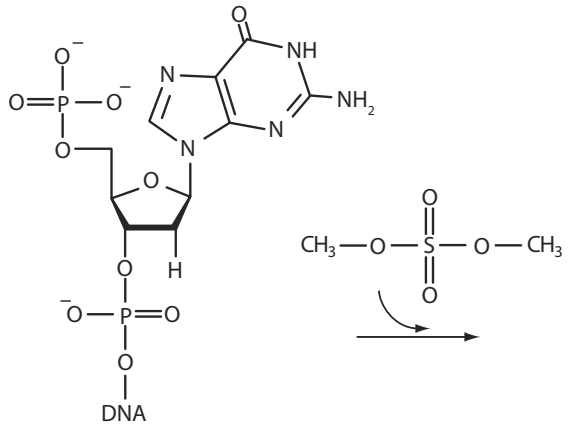
The alkylation may also occur at other locations in undesireable side reactions including upon N-terminal serines or threonines with the former more apt to occur. Which of the following describes one of the reasons that serine residues are more likely to undergo alkylation than threonine residues?



Serine Threonine

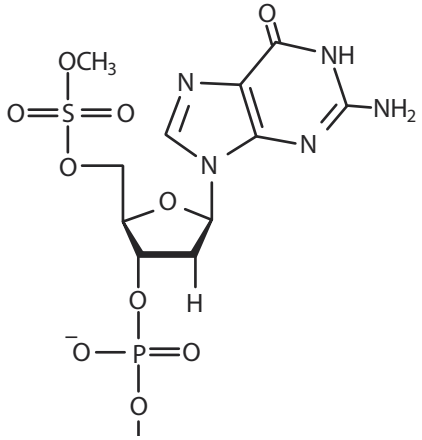
A. The hydroxyl group is a poorer leaving group from threonine than serine.
B. Carbocation formation is more likely to occur with threonine than with serine.
C. The serine hydroxyl group is less hindered than the threonine hydroxyl group.
D. The hydroxyl group of serine has a higher pK_a than that of threonine.

4. Below is the first step in the chemical cleavage method of DNA sequencing developed by Maxam and Gilbert.

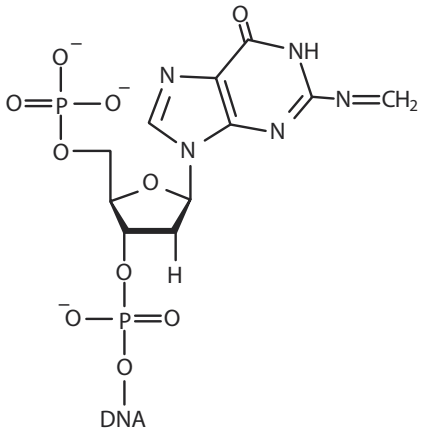


Which of the following structures represents the product of this reaction?

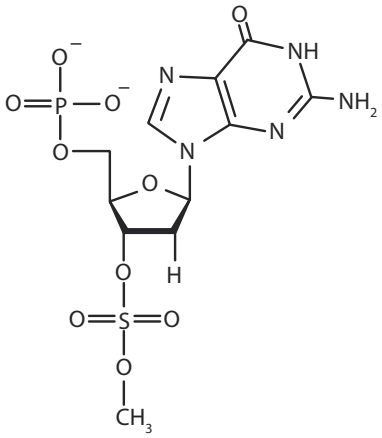
A.



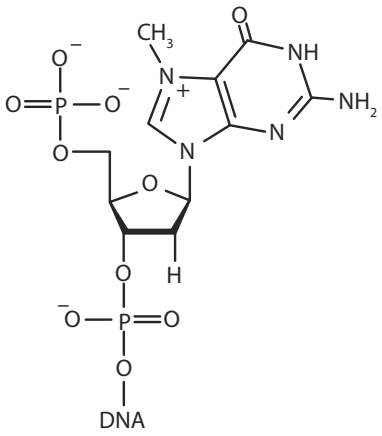
B.



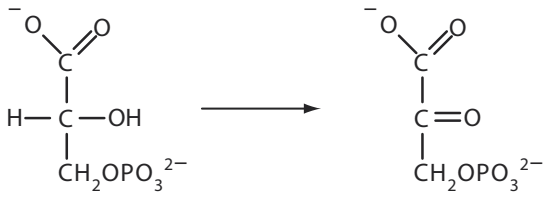
C.



D.



5. 3-phosphoglycerate dehydrogenase is a step in serine biosynthesis.

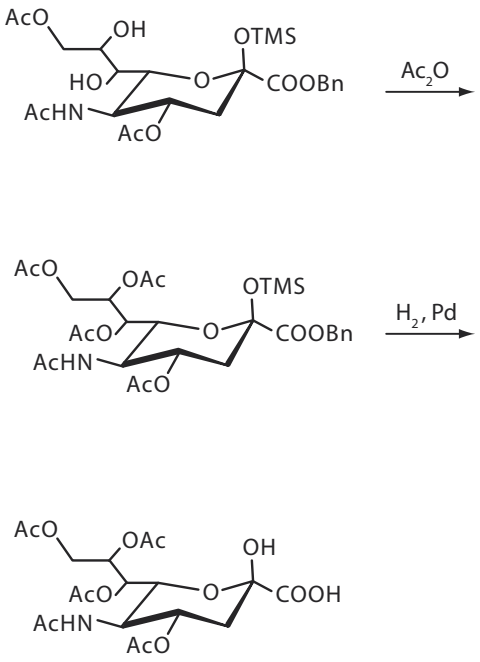


What change has occurred to the oxidation state of the hydroxyl bearing carbon of 3-phosphoglycerate as a result of this reaction?

A. –1 → +2
B. 0 → +2
C. 0 → +4
D. +1 → +2

6. Because postglycosylation acetylation of sialic acid is important to virus pathogenesis and mammalian immune response, the structural and functional understanding of these analogues is an area of active reasearch. Techniques for benchtop synthesis would afford the corresponding sialic acid analogues as useful research tools. This is one of the applications that make methodologies for selective modification of carbohydrate alcohols important synthetic tools in organic chemistry.

The figure below shows the final steps in a synthetic route to 5-N-Acetyl-4,7,8,9-tetra-O-acetylneuraminic acid.

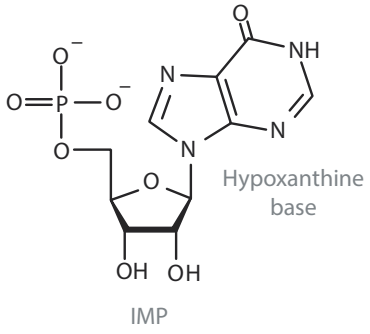


OTMS in the figure above represents . . .

A. **a** silyl ether
B. **a** sulfonic ester
C. **a** mercaptan
D. **a** purine

The following passage pertains to questions 7 - 9.

Bioynthesis of both of the purine ribonucleotides found in RNA, AMP and GMP, begins with a common pathway – the synthesis of inosine monophosphate from the starting material α-ribose-5-phosphate. α-Ribose-5-phosphate is a product of the pentose phosphate pathway.

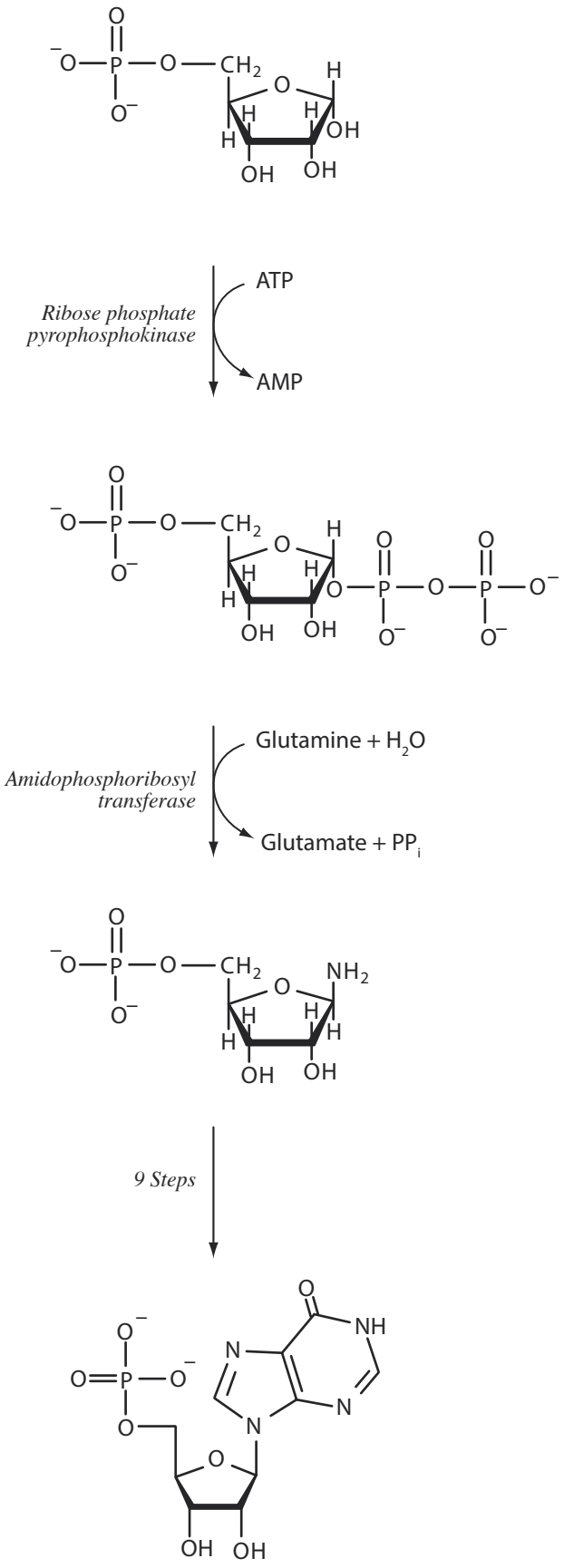


IMP

The pathway from α-ribose-5-phosphate to IMP is comprised of 11 steps. The first two steps are shown in the figure at right.

In the first step of IMP synthesis, ribose phosphate pyrophosphokinase reacts α-ribose-5-phosphate with ATP to form 5-phosphoribosyl-α-pyrophosphate (PRPP). A pyrophosphoryl group is transfered to the C1 carbon of ribose-5-phosphate.

In the second step, amidophosphoribosyl transferase catalyzes the conversion of 5-phosphoribosyl-α-pyrophosphate (PRPP) into 5-phosphoribosyl-β-amine (PRA), using the amine group from a glutamine side-chain. Amidophosphoribosyl transferase possesses two catalytic domains: a glutaminase domain that produces ammonia from glutamine by hydrolysis and a phosphoribosyltransferase domain that binds the ammonia to ribose-5-phosphate. Besides having their respective catalytic abilities, the two domains coordinate with one another to ensure that all the ammonia produced from glutamine is transferred to PRPP and no other nucleophile than ammonia attacks PRPP. This is achieved mainly by blocking formation of ammonia until PRPP is bound and channelling the ammonia to the PRTase active site.



7. A researcher carried out the phosphate pyrophosphokinase reaction with [1-^{18}O]ribose-5-phosphate and ATP in the reaction vessel. The reaction was run to completion. The PRPP product was isolated successfully and mass spectroscopy carried out. The mass spectrum fingerprint of the PRPP obtained showed

A. pure ^{18}O labeled PRPP
B. pure unlabeled PRPP
C. a mxture of labeled and unlabeled PRPP in 1:1 ratio
D. a mixture with unlabeled PRPP predominating

8. The role ATP plays in 'activating' ribose-5-phosphate in purine biosynthesis is most like a common benchtop purpose of which reagent?

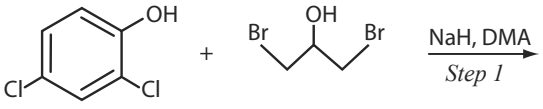
A. trimethylsilyl chloride
B. geranyl pyrophosphate
C. ***p***-toluenesulfonyl chloride
D. pyridinium chlorochromate

9. Students A, B, C, & D debated about the amidophosphoribosyl transferase reaction mechanism. Student A argued that SN2 was unequivocal due to the inversion of configiburation at C1. Student B countered that SN1 mechanism might be capable of producing a stereospecific result in the biochemical context. Student C argued for competing reactions consistent with the weak nucleophile. Student D argued that neither SN1 nor SN2 would describe the mechanism but imine formation instead. Which student is correct?

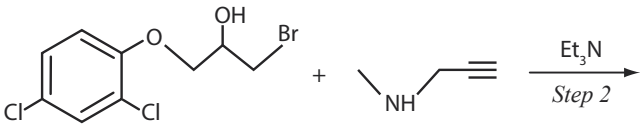
A. Student A
B. Student B
C. Student C
D. Student D

The following passage pertains to questions 10 - 14.

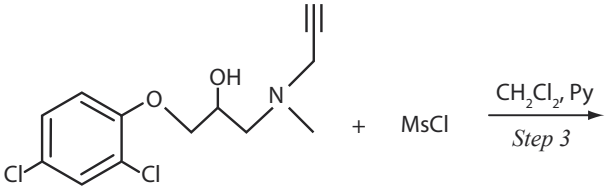
Chemists devised a synthesis of N-[3-(2',4'-dichlorophenoxy)-2-^{18}F-fluoropropyl]-N-methylpropargylamine (^{18}F-fluoroclorgyline) as a potential positron emission tomography (PET) radiotracer for monoamine oxidase A (MAO-A).



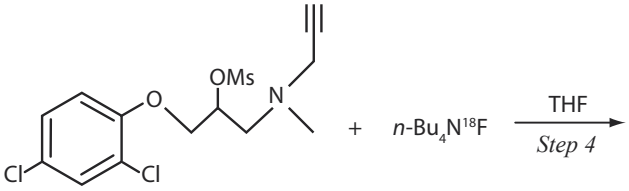
Compound **1** Compound **2**



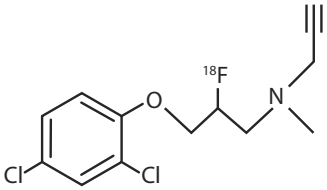
Compound **3** Compound **4**



Compound **5** Compound **6**



Compound **7** Compound **8**



18F-fluoroclorgyline

0. Which is the best description of the dimethylacetamide (DMA) solvent utilized in Step 1?

A. nonpolar
B. protic
C. aprotic
D. aromatic

11. Which step in the spathway includes the inversion of the configuration of a chiral center?

A. Step 1
B. Step 2
C. Step 3
D. Step 4

12. What is the rationale of Step 3?

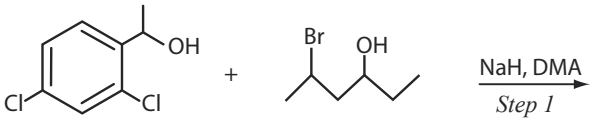
A. ensuring that synthesis is regiospecific
B. protecting the hydroxyl group from oxidation to a carbonyl group
C. preventing formation of precipitate
D. transforming the hydroxyl group into a good leaving group

13. Which of the following results from the action of NaH in Step 1?

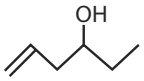
I. acid catalysis
II. activation of the nucleophile
III. stabilizing the transition state
IV. production of H_2 gas

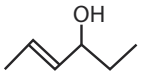
A. I only
B. I and II
C. II and IV
D. I, II, and III

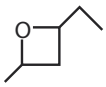
14. The alternative reagents shown below were utilized in an attempt to carry out a variation of the reaction in Step 1:

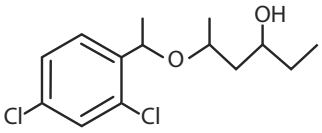


The product was quenched with acid and purified of residual 1-(2,4-dichlorophenyl)ethanol.What was the major product obtained?

A. 

B. 

C. 

D. 

Reactions of Alcohols
Answers and Explanations

1. A

The conversion of ethanol into acetaldehyde is an oxidation. As a consequence of the reaction, two electrons pass from ethanol to NAD^+, the oxidizing agent. The ethanol dehydrogenase mechanism, in other words, leads to production of NADH in the cytosol. This makes conditions in the cytosol too reducing. (It's important to understand that just like conditions in the cell can be acidic or basic based on the degree of protonation of solution components, an enviornment may be oxidizing or reducing based on the oxidation state of components.) Cytosol with a higher than normal NADH concentration is a reducing environment. Think of being within a reducing environment as a kind of 'electron pressure' onto the components of the solution. This is what happens to pyruvate in this case. Among other effects, the reducing environment produced by a great deal of ethanol dehydrogenase activity in the cytosol leads to reduction of pyruvate, transforming it into lactate. Thus, one of the effects of excessive alcohol consumption is inhibition of gluconeogenesis in liver cells.

2. A

When you have the structural formula of an organic compound, assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

The carbon of a primary alcohol gains two electrons that the two hydrogens brought and loses one to oxygen, so the oxidation state of the hydroxyl carbon at the start is –1.

After the reaction, the carbon will now have three electrons invested in bonds to oxygen (a double bond and a single bond), so its oxidation state in the histidine α carboxyl group has become +3. It has been oxidized by $2NAD^+$ in a four electron transfer.

3. C

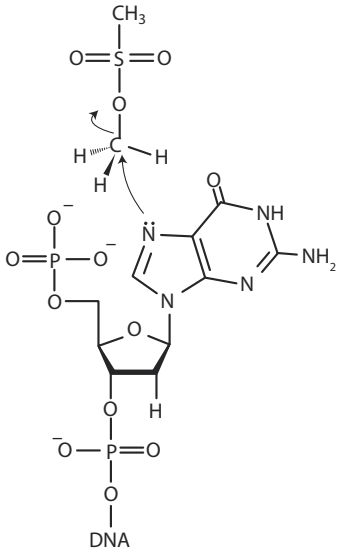
The threonine hydroxyl group is a somewhat less apt nucleophile for SN2 substitution because it is more hindered. (It also has a slightly *higher* pK_a, so it spends less time in the more reactive deprotonated form.)

4. D

As a figure of merit for MCAT preparation, the important thing for this question would be to recognize the mesylate leaving group in the structure of the reagent.

The reagent could have been formed by a prior treatment of methanol with methanesulfonyl chloride to convert the hydroxyl of methanol into a leaving group. Mesylate is an excellent leaving group in nucleophilic substitution reactions because the negative charge on the leaving group is stabilized by resonance.

Being a good substrate for SN2 substitution makes our reagent a tool for the convenient methylation of a nucleophlic moeity.



5. B

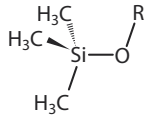
When you have the structural formula of an organic compound, assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

The carbon of a secondary alcohol gains one electron from a hydrogen and loses one to oxygen, so the oxidation state of the hydroxyl carbon at the start within 3-phosphoglycerate is 0.

After the reaction, the carbon will now have two electrons invested in its double bond to oxygen, so its oxidation state in 3-Phosphohydroxypyruvate has become +2.

6. A

TMSO stands for tetramethylsylyl ether.

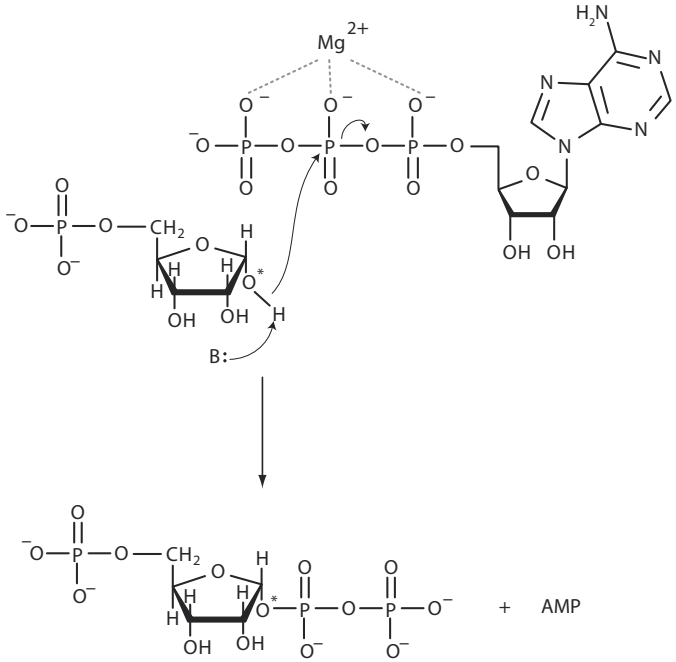


Silylating agents are often used in synthetic organic chemistry to protect hydroxyl groups from adverse reaction conditions. In the role of protection, they group forms a silyl ether with the substrate. Silyl groups are particularly useful for this purpose because they can be installed and removed very selectively under mild conditions.

7. A

You will often see a hydroxyl group serving as the target of phosphoryl transfer in biochemistry. Because the hydroxyl group serves as the nucleophile in a phosphoryl transfer reaction, it is the same extracyclic oxygen on the anomeric carbon in phosphoribosyl pyrophospate after the reaction as had been in that location prior to the reaction in ribose-5-phosphate.

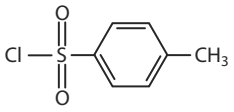
Note that transfer of pyrophosphate is very similar to what occurs in serine, threonine or tyrosine kinase except that the attack by hydroxyl occurs on the β phosphate of ATP, transfering a pyrophosphate, instead of on the γ phosphate of ATP in a kinase.



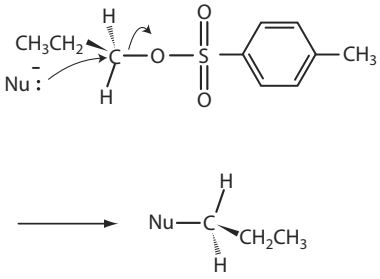
8. C

It is a very important thing about the hydroxyl group to understand that it is a *very poor leaving group*. One way to get a hydroxyl group to leave is by acid catalysis. This way it can leave as water.

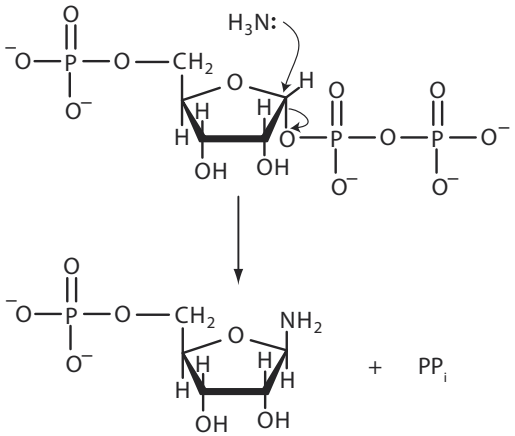
This is *p*-toluenesulfonyl chloride:



p-Toluenesulfonyl chloride can transfer its toluenesulfonyl moiety onto a hydroxyl group, transforming the hydroxyl group into a tosylate. Due to resonance stabilization, this an excellent leaving group.



p-toluenesulfonyl chloride does does appear on the life sciences benchtop, in drug synthesis, for example. However, it's more likely that AAMC decided to include the reagent on the MCAT outline because there are so many instances in biochemistry with the same logic involving ATP (or UTP). In purine biosynthesis, transfer of pyrophosphate onto ribose-5-phosphate transforms the C1 hydroxyl group into great leaving group.

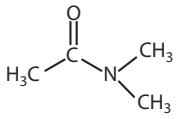


9. B

Student A would be correct for a benchtop reaction but not for a biochemical reaction. On the benchtop, absent a very special catalyst, stereospecific inversion of configuration with these reagents would be unequivocal evidence of SN2 substitution. However, enzyme catalysis is capable of producing stereospecific configurations through addition to a planar, achiral carbon. This is because the substrates are bound with multiple points of attachment within an active site that is itself asymmetric. It happens all the time in biochemistry that a pure optical isomer derives from an achiral, planar precursor. Even though the mechanism here *actually is* SN2 substitution, student B is correct that the inversion of configuration on its own is not enough evidence.

10. C

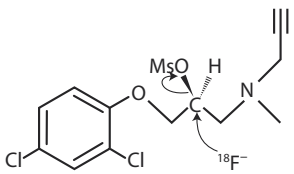
Dimethylacetamide (DMA) is a polar aprotic solvent. While having a high dielectric constant (polar), it does not possess any hydrogens bonded to electronegative elements, such as in hydroxyl or amine groups.



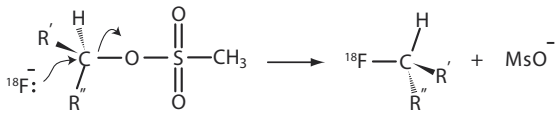
Polar aprotic solvents are the optimal type of solvent for SN2 substitution. Their dielectric property helps stabilize the charge separations of the transition state, but unlike a protic solvent, they don't carry out the hydrogen bonding that would cage and over-stabilize the nucleophile.

11. D

Steps 4 has SN2 substitution on a chiral center.

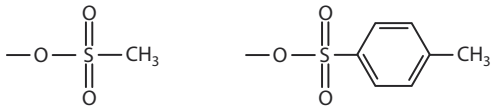


SN2 substitution leads to inversion of configuration. While Steps 1 and 2 are also SN2 substitution, inversion of configuration is only stereochemically dispositive with a chiral center.



12. D

The hydroxyl group is a poor leaving group. A solution to the problem is to turn the alcohol into a sulfonic ester. A commonly employed method is to form an organic mesylate or an organic tosylate by treatment of the alcohol with either methanesulfonyl chloride or para-toluene sulfonyl chloride. Mesylate (-OMs) and tosylate (-OTs) groups are excellent leaving groups in nucleophilic substitution reactions because the negative charge on the leaving group is stabilized by resonance.



Mesylate (-OMs)

Tosylate (-OTs)

13. C

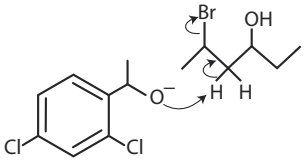
Sodium hydride (NaH) is is a very strong base (a superbase) capable of deprotonating even very weak Brønsted acids. NaH has utility in organic chemistry where typical substrates contain O-H, N-H, S-H bonds. The abstraction of the proton from 2,4-dichlorophenol in Step 1 of our synthesis converts the molecule into a phenolate anion, being charged, a much more aggressive nucleophile (choice II) for SN2 substitution.

Additionally, the basicity of NaH is driven by the high reduction potential of the hydride ion (H^-). Hydride reduces the abstracted proton yielding H_2 (choice IV).

$$H^- + H^+ \rightharpoonup H_2$$

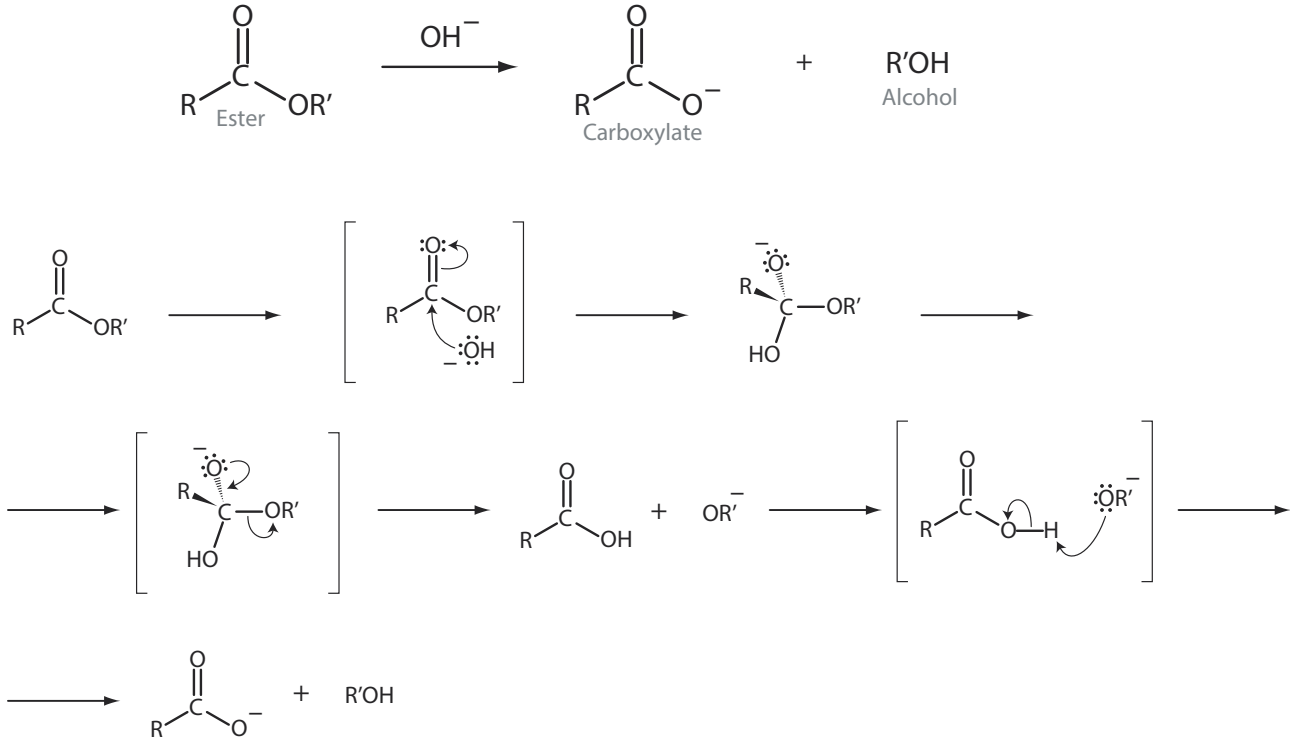
14. B

Abstraction of a proton by NaH from our new reagent yeilds an alkoxide anion, which is a much stronger base than the phenolate anion, a weak base, produced in the original reaction. The favored reaction with a hindered strong base, especially with a secondary alkyl halide, will be E2 elimination, not SN2 substitution.

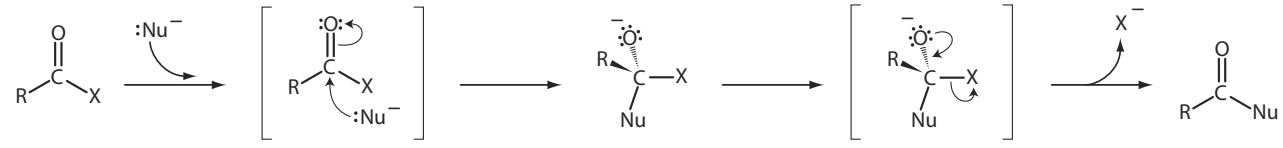


Note that the proton taken by the base is the one which produces the most highly substituted alkene (so not a proton from the end carbon).

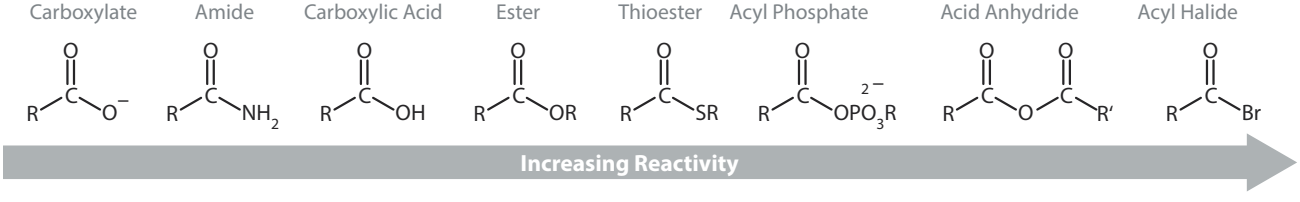
Nucleophilic Acyl Substitution - Hydrolysis of an Ester



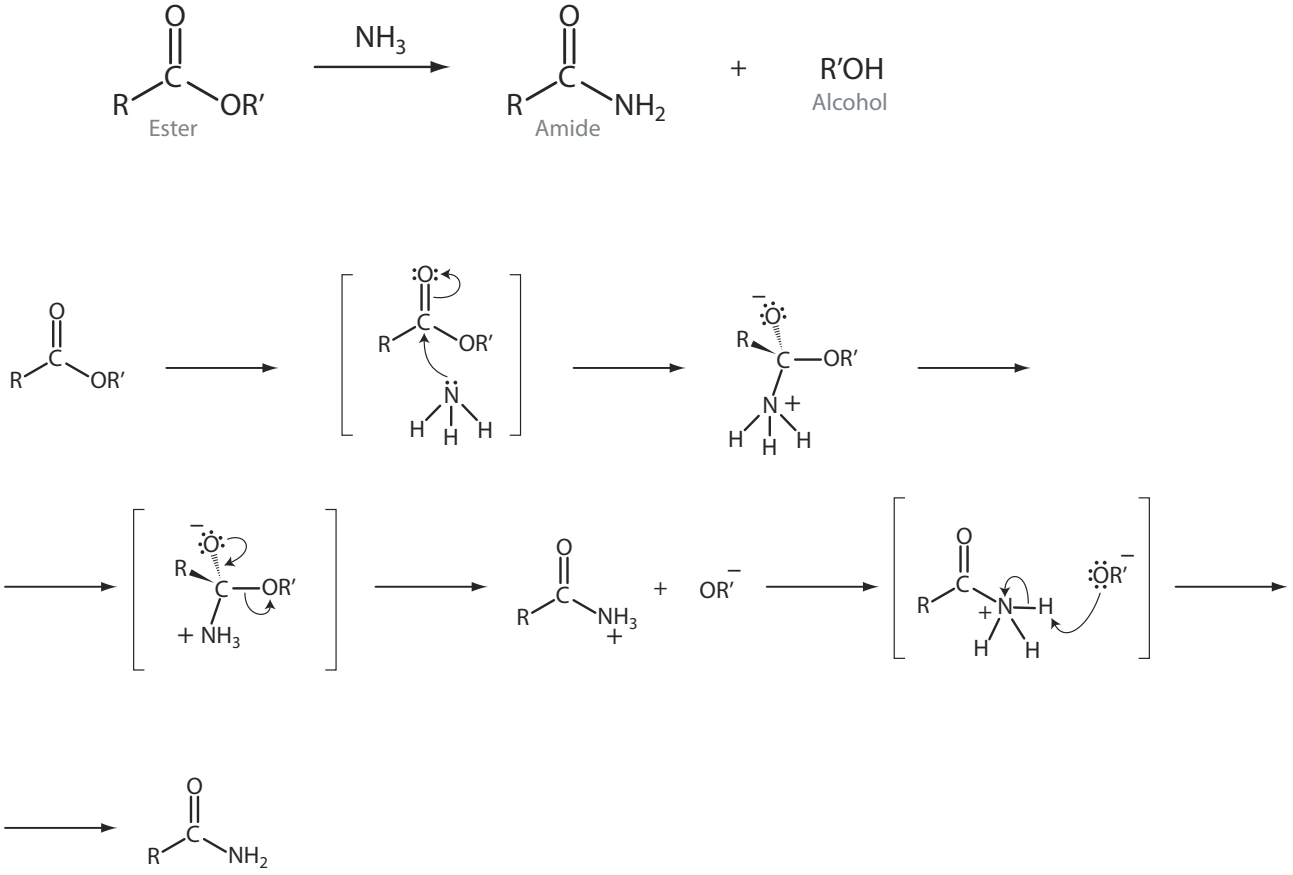
Hydrolysis of an ester under basic conditions is called saponification because it is the chemical reaction underlying the traditional method of making soap from glycerol triesters. In saponification, a hydroxide anion adds to the carbonyl group of the ester to form a tetrahedral intermediate. The intermediate then collapses as alkoxide anion departs from the ester. A carboxylic acid forms, which is soon deprotonated by the alkoxide anion. (Note that ester hydrolysis can also be promoted by acid catalysis). Saponification of an ester is an example of an acyl substitution mechanism. In these reactions, attack of the nucleophile forms a reactive intermediate. The tetrahedral intermediate collapses. The carbon-oxygen double bond re-forms, and the original acyl X group is expelled. This is how carboxylic acid derivatives are changed from one type to another.



As a general rule the weaker the basicity of the leaving group of a carboxylic acid derivative, the more reactive it is for acyl substitution. Remember the order of reactivity below, and take special note that thioster and acyl phosphate are the most reactive (highest energy) biologically important forms of those shown. In biochemistry, thioesters and acyl phosphates are your 'activated carboxylates.'



Nucleophilic Acyl Substitution - Aminolysis of an Ester

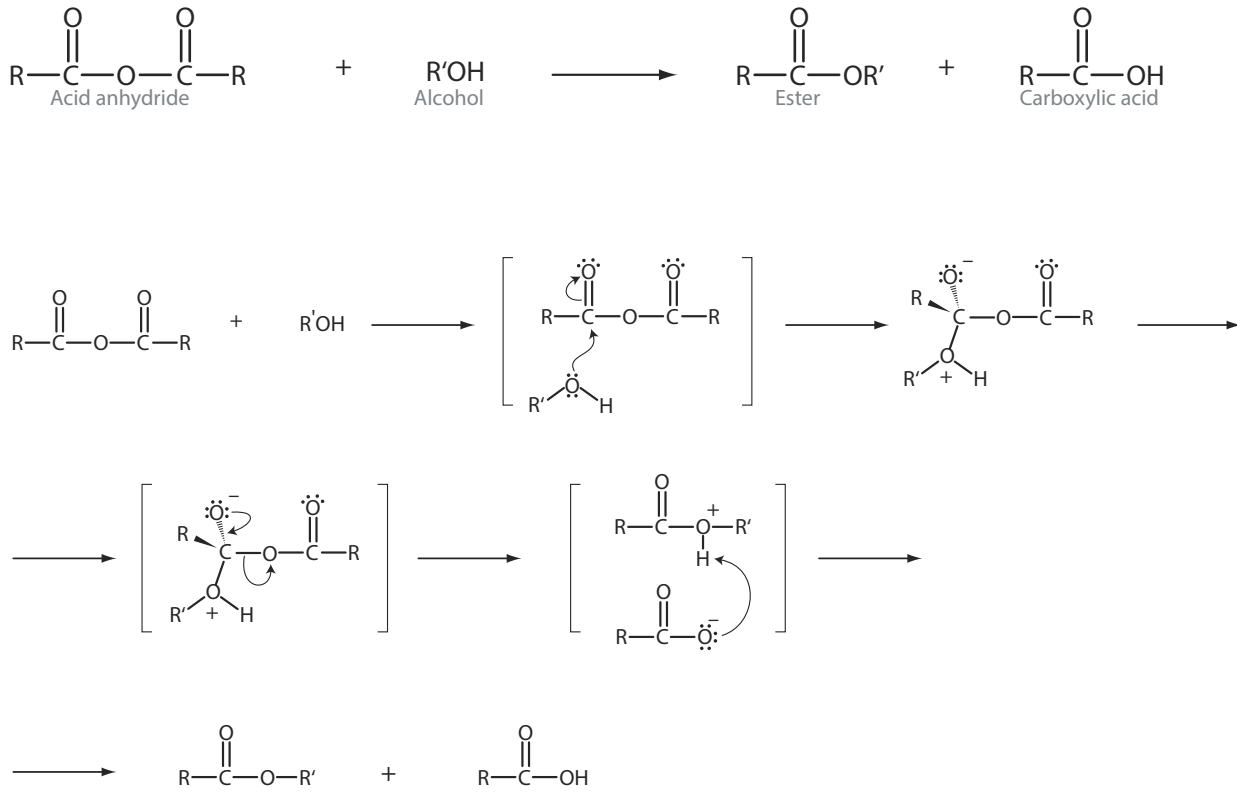


Ammonia and amines are good nucleophiles. When nucleophilic acyl substitution is carried out with an amine as the nucleophile, the product of the reaction is an amide, one of the most stable types of carboxylic acid derivatives. Aminolysis of carboxylic acid derivatives is widely used, especially in the synthesis of peptides.

If one tried to carry out aminolysis of a carboxylic acid, an ammonium carboxylate salt would be formed instead. Only proton exchange would occur, not acyl substitution. To overcome this, the carboxylic acid would first need to be converted into another carboxylic acid derivative such as an ester, anhydride, or acid halide and then aminolysis carried out.

In the aminolysis of an ester, as in the figure above, alkoxide anion handles removing the proton in the deprotonation step. However, when aminolysis is carried out upon a compound such as acid anhydride or acid halide, there is nothing to prevent the proton lost in the deprotonation step from being taken up by another amine nucleophile, rendering it useless as a nucleophile. For this reason, two equivalents of amine must be used in some aminolysis reactions.

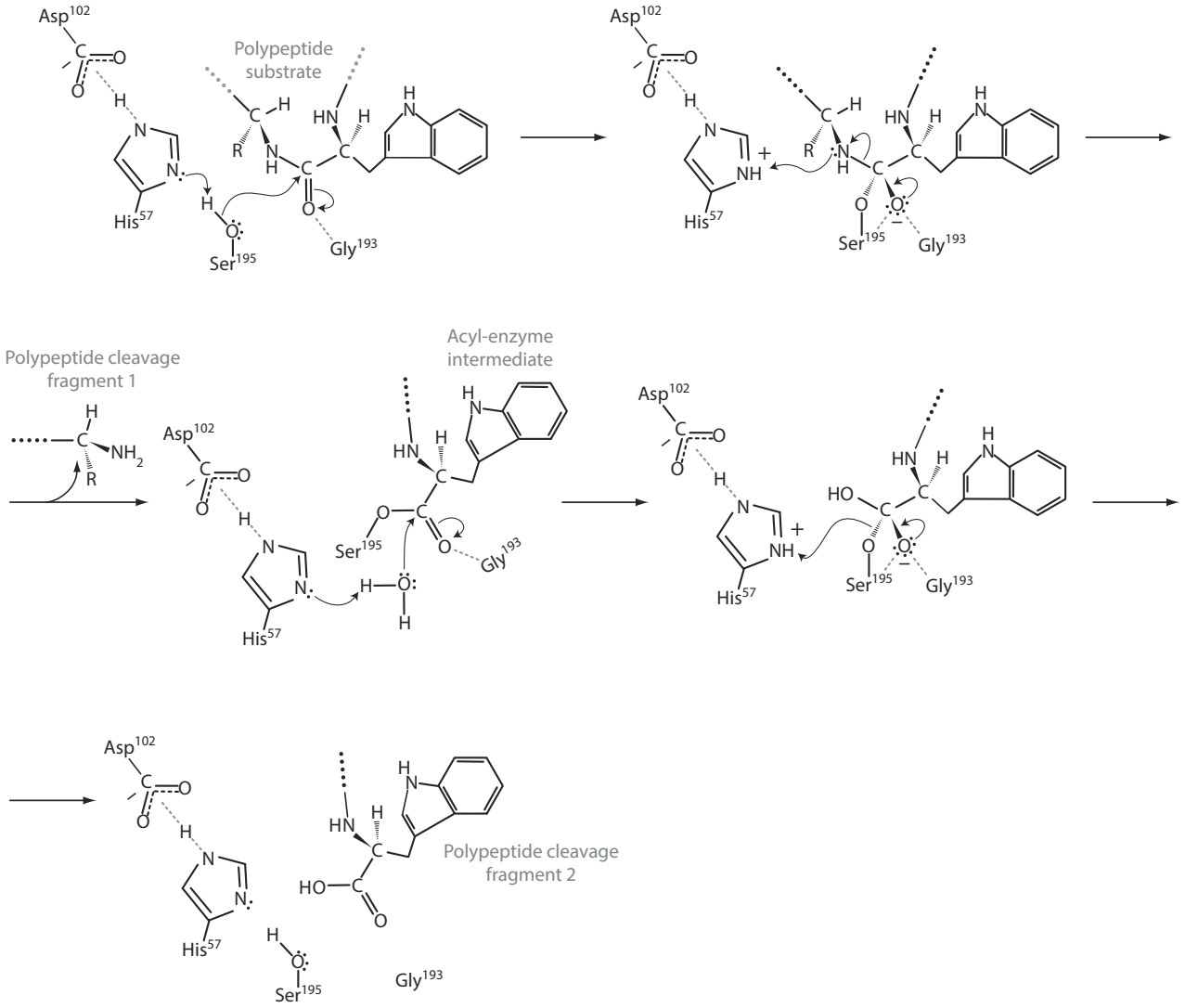
Nucleophilic Acyl Substitution - Esterification of an Anhydride



Acid anhydrides are on the unstable end of the spectrum of carboxylic acid derivatives. Like acyl halides, acyl phosphates, and thioesters, acid anhydrides may be readily hydrolyzed, aminolyzed, or esterified through the acyl substitution pathway.

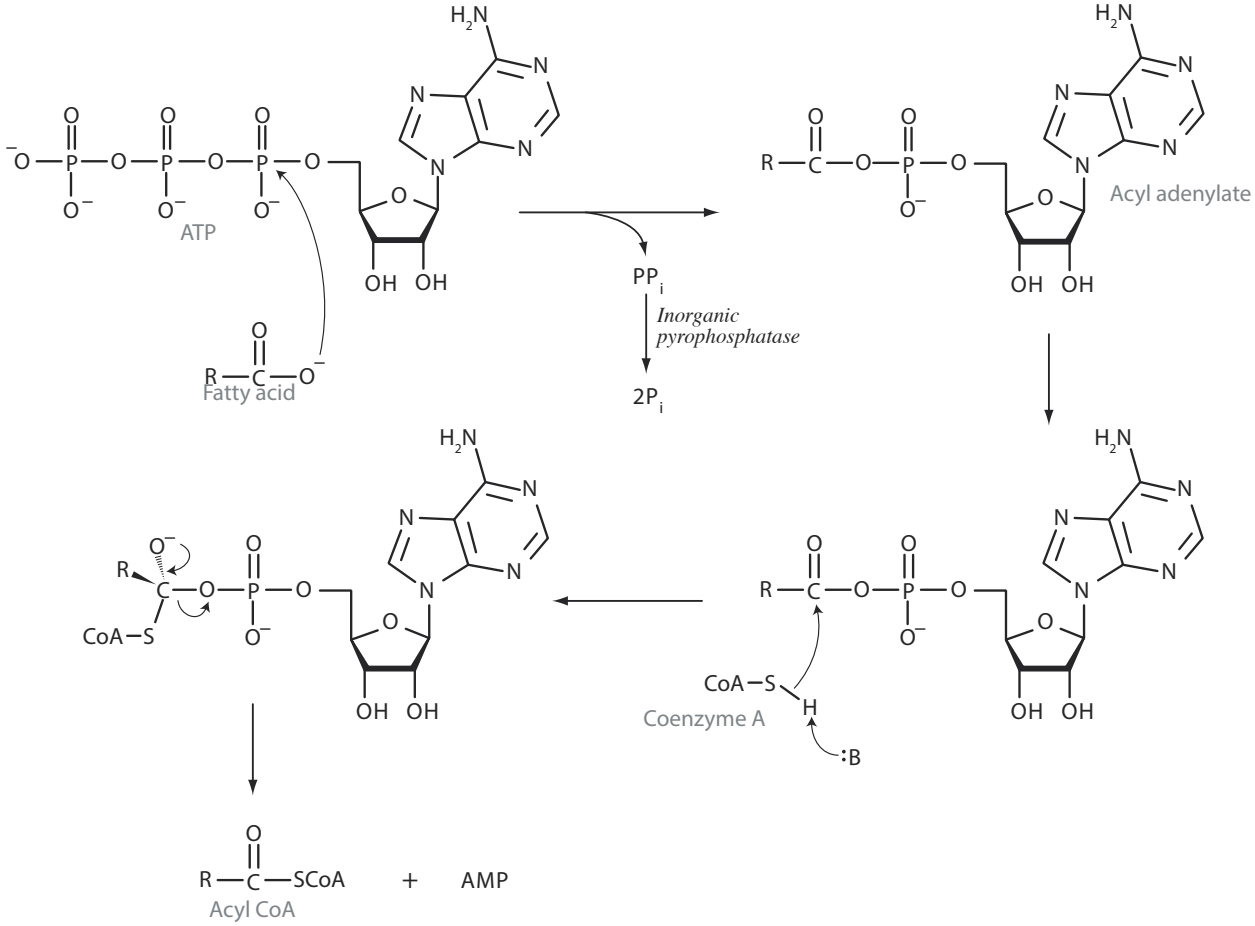
In the case of reaction of an acid anyhydride with an alcohol, the product is an ester with a carboxylate anion as the leaving group. Attack of the alcohol forms a tetradral intermediate with the anhydride. The tetrahedral intermediate collapses. The carbon-oxygen double bond re-forms, and the carboxylate anion is expelled. The tetrahedral intermediate favors collapse in this direction, instead of expelling the recently joined alkoxide because carboxylate anions are more stable in solution than alkoxide. Just as carboxylate is the weaker base, it's also the better leaving group. Because carboxylate is a more stable anion than alkoxide, acid anhydrides are more reactive than esters.

Chymotrypsin



Chymotrypsin is a serine protease. It employs a particularly reactive serine residue to cleave a polypeptide. The chymotrypsin mechanism is one of the classics of enzyme mechanics. Acyl substitution occurs twice in the mechanism. First, the serine residue attacks an amide peptide linkage shared on the N-terminus side by a large nonpolar residue that fits in chymotrypsin's specificity pocket. Serine is assisted through charge relay by a nearby histidine residue and a further aspartate residue, which heighten the nucleophilicity of serine's hydroxyl group by involving it in a particularly strong hydrogen bond. When the tetrahedral intermediate forms, another pocket in the enzyme, the oxyanion hole, stabilizes the negatively charged oxygen atom. Then the intermediate collapses along the acyl substitution pathway, and chymotrypsin releases the C-terminal fragment of the polypeptide with the N-terminal fragment remaining bound to serine by an ester linkage. This serine-peptide ester is next hydrolyzed in the enzyme deacylation step, another acyl substitution with water as the nucleophile.

Acyl CoA Synthetase

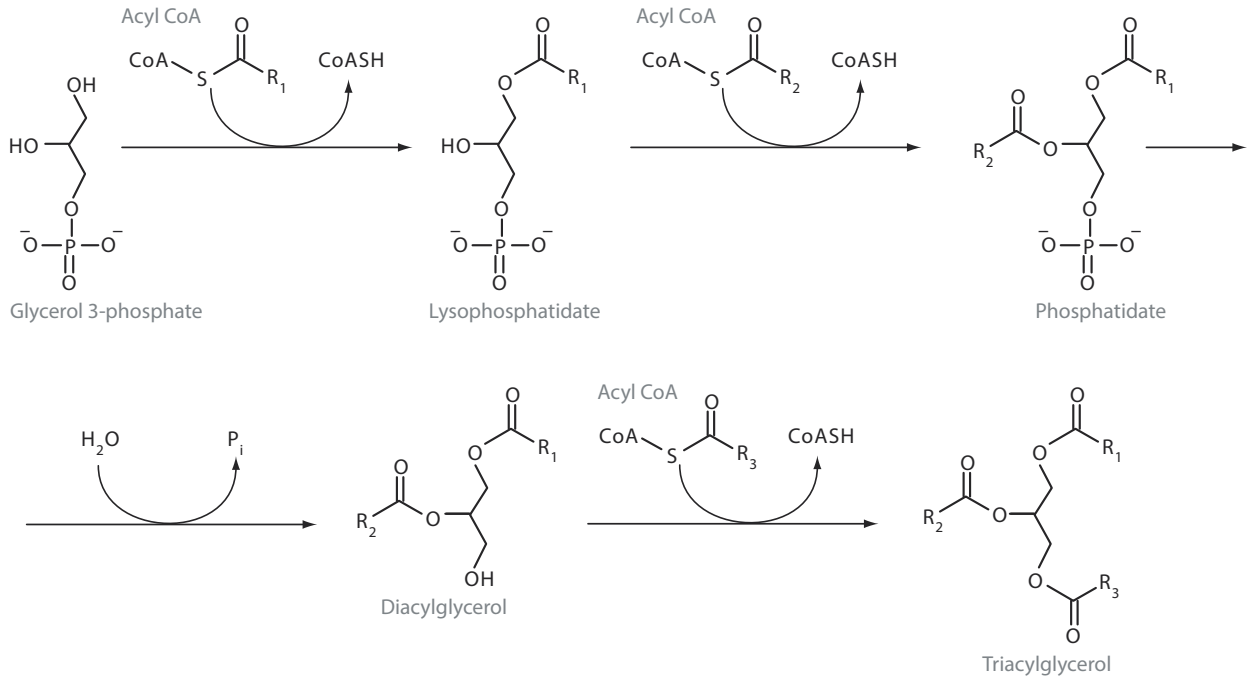


Acyl phosphates and thioesters are high energy carboxylic acid derivatives (pg 58), a fact of profound importance in biochemistry. Acyl phosphates and thioesters are 'activated.' They can be spontaneously converted to esters, carboxylic acids and amides without the additional input of energy.

One example of the importance of these concepts in biochemistry is in the purpose of the cofactor coenzyme A (CoA). By forming a thioester with acyl groups, coenzyme A provides them with a high transfer potential. Coenzyme A is a carrier of activated acyl groups.

Prior to β-oxidation, fatty acids are linked to coenzyme A by acyl CoA synthetase. ATP cleavage drives the formation of acyl CoA. The enzyme uses ATP to first transform the fatty acid into an even higher energy carboxylic acid derivative than a thioester, a type of a type of phosphate ester called an acyl adenylate. Formation of acyl adenylate occurs by transfer of adenyl phosphate onto carboxylate, which breaks the bond between the alpha and beta phosphate groups of ATP and releases pyrophosphate (PP_i). Subsequent cleavage of pyrophosphate by pyrophosphatase drives this reaction forward. The sulfhydral group of CoA then attacks the acyl adenylate to form a tetrahedral intermediate. Proceeding through the acyl substitution pathway, the intermediate then collapses to form acyl CoA and release AMP.

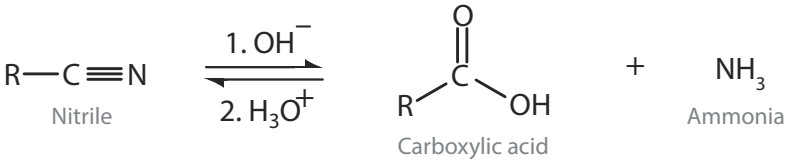
Triglyceride Synthesis

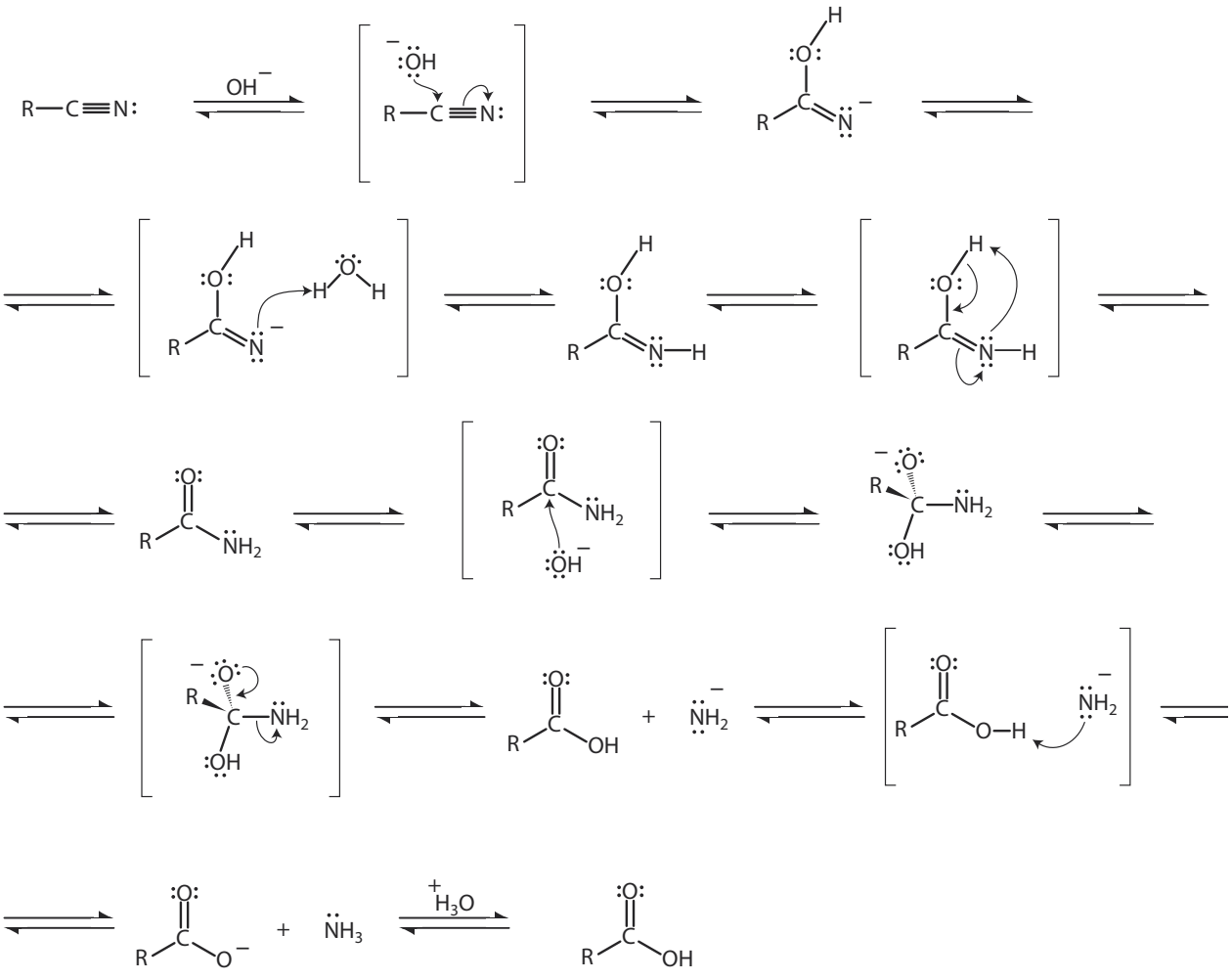


With their high energy thioester bonds, acyl CoAs are activated for acyl substitution. Triglyceride synthesis, which takes place primarily in the liver, employs three acyl CoAs to form the three ester linkages of a triglyceride.

The process of triglyceride synthesis begins with transfer of the acyl group of acyl CoA to glycerol-3-phosphate, forming lysophosphatidate. Lysophosphatidate is next acylated once again in like manner with acyl CoA to yield phosphatidate, the common precursor of both triglycerides and phosphoglycerides. These two acylations were catalyzed by glycerol phosphate acyltransferase, but for the rest of triglyceride synthesis, the process will continue through the activity of the enzymes of the endoplasmic reticulum-bound triacylglycerol synthetase complex. Phosphatidate is hydrolyzed on the endoplasmic reticulum to yield a diacylglycerol and then acylated to form the triacylglycerol.

Nitrile Hydrolysis

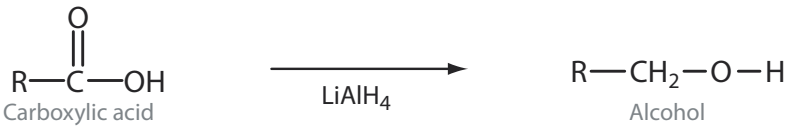


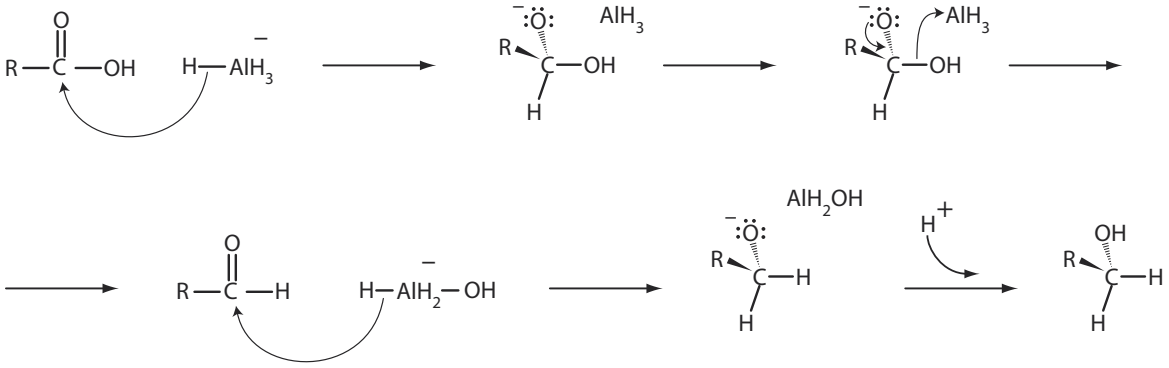


The electropositive nitrile carbon is similar to a carbonyl carbon in that it can accept the approach of a nucleophile for addition, although the overall process is significantly different with nitriles. Nitrile hydrolysis begins with the nucleophilic addition of hydroxide anion to the nitrile. The first intermediate formed then takes a proton from water and subsequently undergoes an intramolecular rearrangement to form an amide. This amide, however, is temporary in a strong base environment, becoming transformed through hydrolysis along the acyl substitution pathway to form the carboxylate anion.

* Nitrile hydrolysis plays an important part in the Strecker amino acid synthesis (pg 117.)

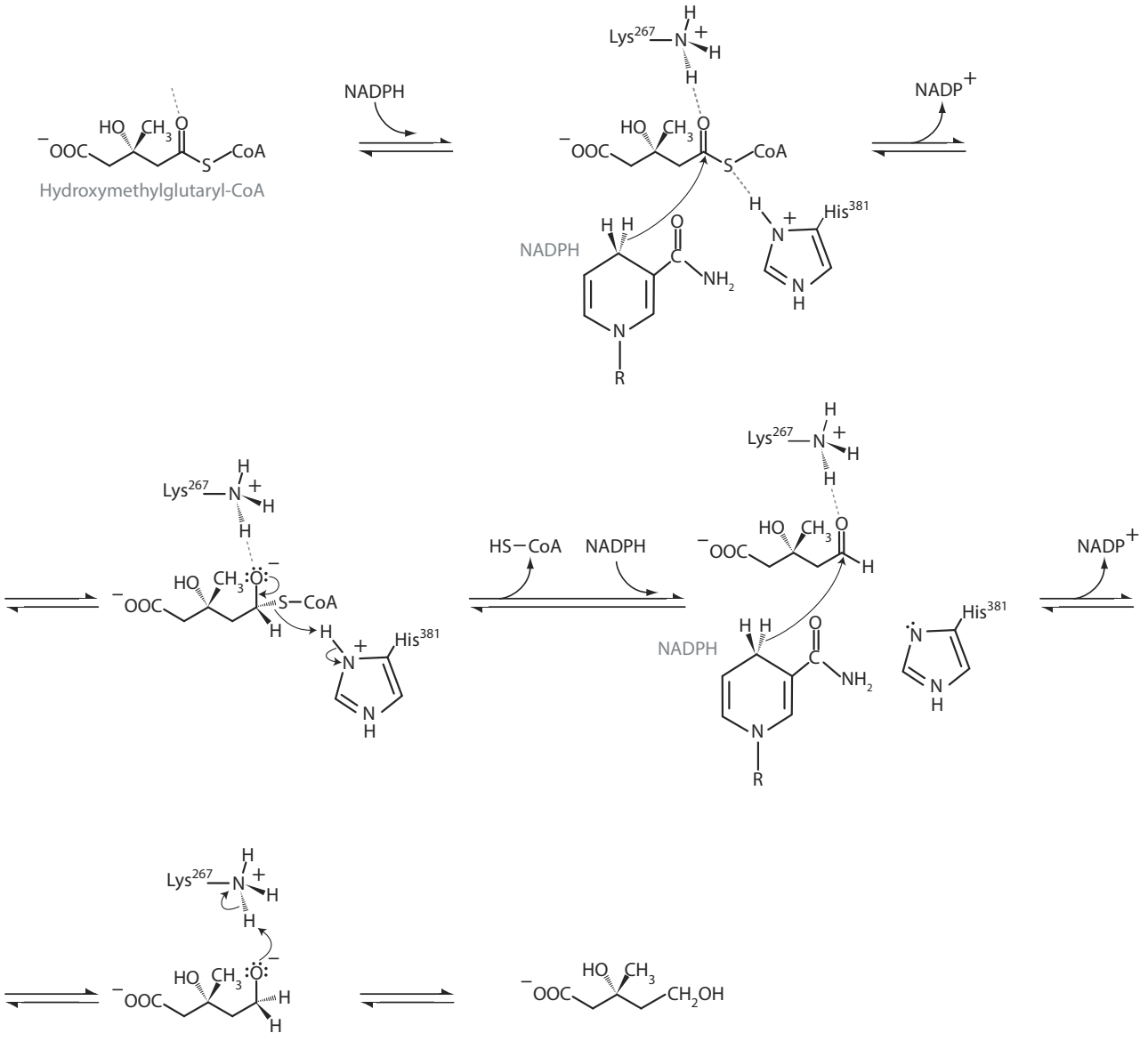
Reduction of Carboxylic Acids





Carbohydrates can be reduced to primary alcohols by lithium aluminum hydride ($LiAlH_4$). The reaction happens in two stages, first forming an aldehyde then the primary alcohol. The reaction begins with a hydride (H^-) from $LiAlH_4$ acting like an acyl substitution nucleophile, attacking the carbonyl of the carboxylic acid to form a tetrahedral intermediate. The tetrahedral intermediate collapses with departure of the hydroxyl group. An aldhehyde is formed. However, the aldehyde does not persist because aldehydes themselves may be reduced. Another hydride anion adds to the carbonyl group of the aldehyde with electrons from the carbonyl group moving up onto the carbonyl oxygen to form an alkoxide. Protonation of the alkoxide creates the primary alcohol.

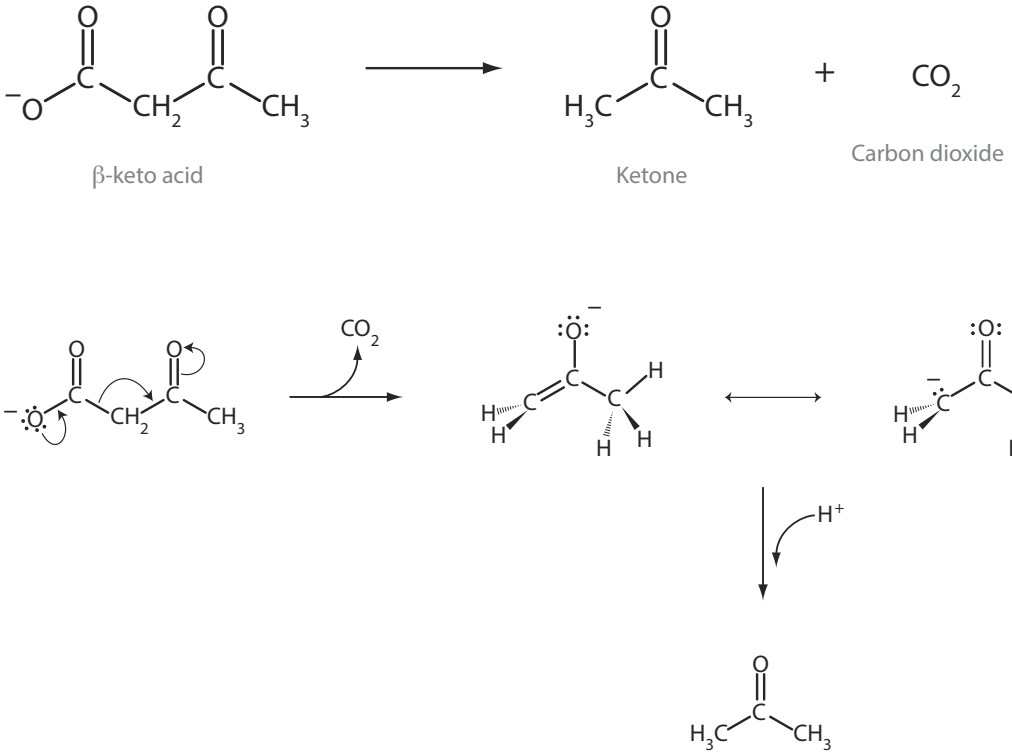
HMG-CoA Reductase



Hydroxymethylglutaryl-CoA (HMG-CoA) is the precursor of isopentenyl pyrophosphate, which is the building block of the isoprenoid lipids as well as steroids.* HMG-CoA is reduced to mevalonate to start the pathway to isopentenyl pyrophosphate. The HMG-CoA reductase mechanism is similar to the organic benchtop reduction of a carboxylic acid to an alcohol with $LiAlH_4$ (pg 71). Reduction occurs in two stages, first forming an aldehyde then forming the primary alcohol. The HMG-CoA reductase reaction begins with a hydride (H^-) from NADPH attacking the carbonyl of the HMG-CoA thioester to form a tetrahedral intermediate. The tetrahedral intermediate collapses with departure of the thiol group, forming an aldehyde. Hydride from another NADPH adds to the carbonyl group of the aldehyde. Electrons from the carbonyl group move up onto the carbonyl oxygen to form an alkoxide. Protonation of the alkoxide creates the primary alcohol of mevalonate.

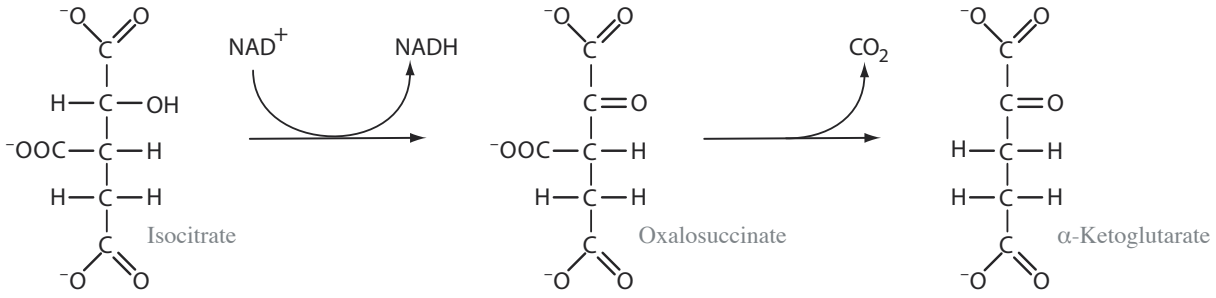
* Steroid synthesis is just past the borderline of required knowledge for the MCAT. It's helpful to have a good general sense about this material, but you don't need to anticipate questions on specifics. Here, the focus is on the behavior of NADPH which IS required.

Decarboxylatin of a β-Keto Acid



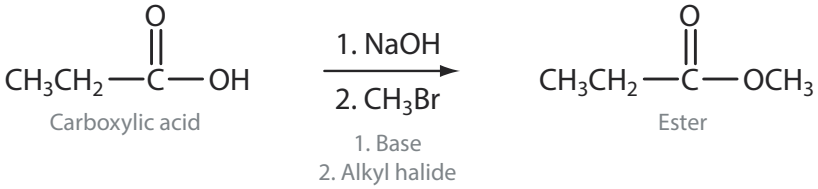
A β-keto acid is a carboxylic acid containing a carbonyl group two bonds away from the carboxyl group. β-keto acids undergo thermal decarboxylation (lose carbon dioxide) quite easily, because the immediate product of decarboxylation will be a resonant stabilized enolate anion. Tautomerism of the enolate leads to a ketone.

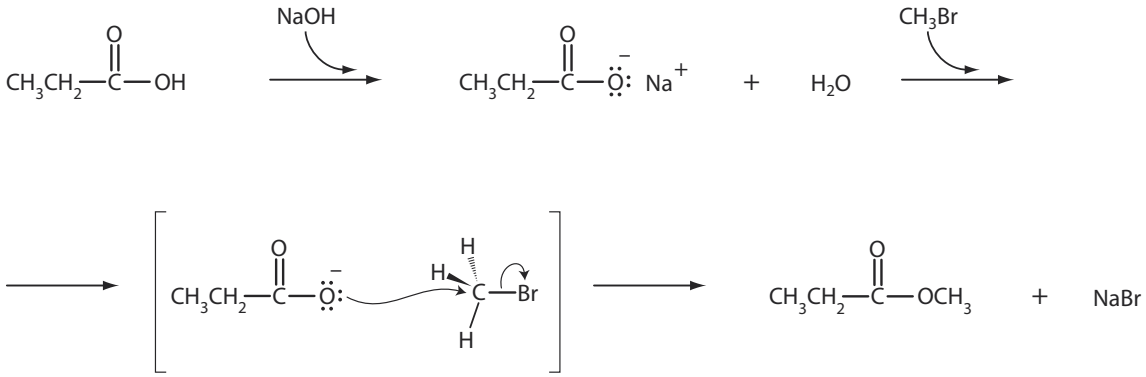
Isocitrate Dehydrogenase



In the isocitrate dehydrogenase mechanism of the citric acid cycle, oxidation of isocitrate by NAD^+ converts a hydroxyl group within isociatrate into a carbonyl group in oxalosuccinate. This carbonyl group is located two positions from a carboxyl group. Therefore, oxalosuccinate is a β-keto acid. Because β-keto acids are extremely labile to decarboxylation, oxalosuccinate immediately decarboxylates to form α-ketoglutarate.

Formation and Use of a Carboxylate Nucleophile

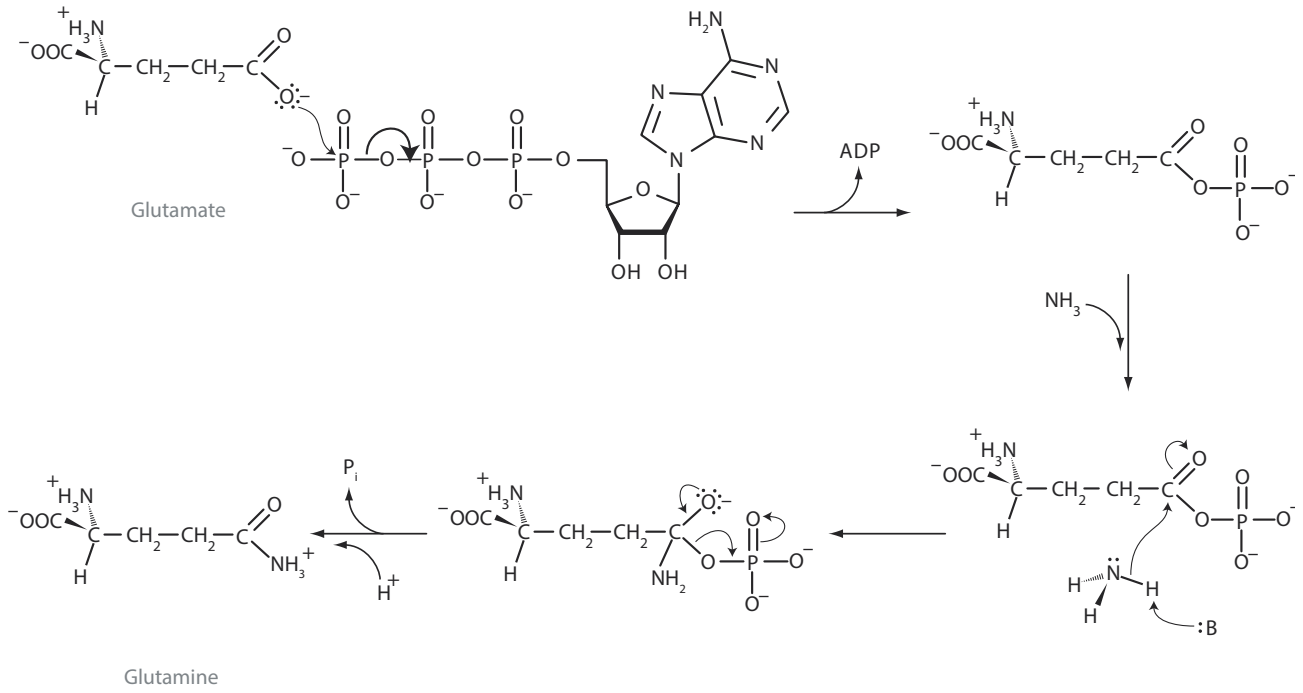




Titrating carboxylic acid with a strong base forms a carboxylate salt. The carboxylate anion can then serve as a nucleophile in an SN2 reaction upon a primary or secondary alkyl halide to form an ester.

Watch out for mechanisms on the MCAT where a carboxylate nucleophile is being employed in this manner because it's easy to fall into the assumption that a pathway from one carboxylic acid derivative to another must be following an acyl substitution pattern (pg 58).

Glutamine Synthetase

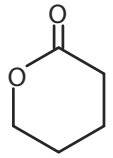


The biosynthesis of glutamine from glutamate by glutamine synthetase begins by phosphorylation of the glutamate side-chain to form an acyl phosphate. Acyl phosphates are high energy carboxylic acid derivatives (pg 58). Therefore, subsequent aminolysis of the acyl phosphate to form the amide side-chain of glutamine can occur with a negative free energy change.

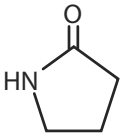
Notice that the initial step of the phosphorylation of glutamate occurs via the phosphoryl transfer mechanism (pg 99) using a carboxylate nucleophile, not acyl substitution. As a consequence, in the acyl phosphate product of this step, the anhydride oxygen originates with the carboxylate not the phosphate.

* While we are focusing here on the use of a carboxylate nucleophile in phosphoryl transfer, we should point out that, as a general topic, amino acid biosynthesis will not be test on the new MCAT. In our opinion, the basics are fundamental to metabolic integration, so you should definitely know your way around a few simple syntheses. Be able to describe the synthesis of aspartate by transamination of oxaloacetate, for example, or glutamine synthesis shown here. However, the exam will definitely not expect you to know every step of the difficult ones like tryptophan synthesis or the synthesis of histidine, for example.

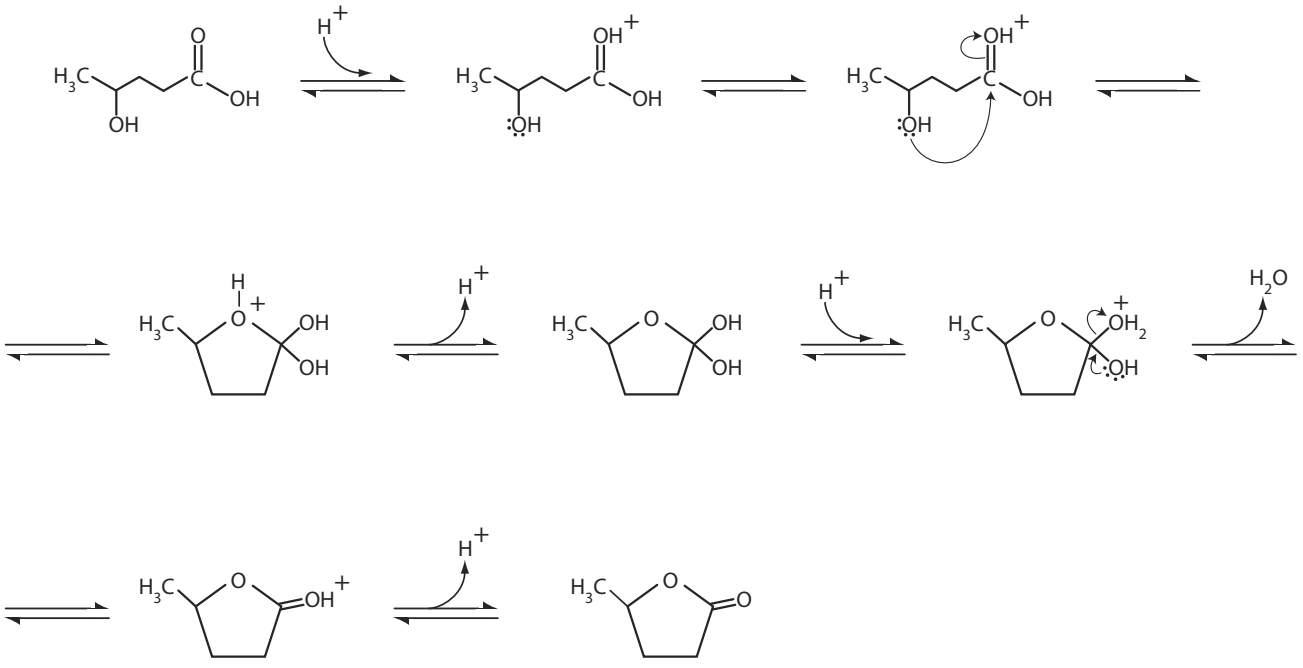
Lactones and Lactams



Lactone



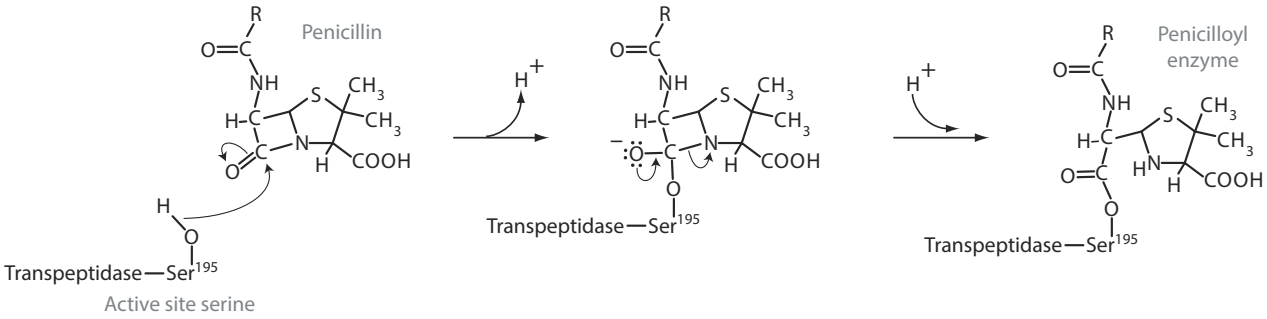
Lactam



A molecule that contains a carboxyl group and a hydroxyl group can undergo an intramolecular esterification forming a cyclic ester called a lactone. A molecule with a carboxyl group and an amine can undergo intramolecular aminolysis to form a cyclic amide called a lactam.

Cyclization is often so favorable for γ- or δ- hydroxy acids, which form five- or six-membered lactones (and γ- or δ- amino acids that form five- or six-membered lactams) that the straight chain form may be difficult to isolate. For carboxylic acids with hydroxyl groups in other positions, though, the free energy change is not so favorable due to ring strain. In those cases equilibrium favors the straight chain form.

Irreversible Inhibition of Transpeptidase by Penicillin



Penicillin belongs to the class of β-lactam antibiotics, possessing a β-lactam moeity in their molecular structure. A β-lactam is cyclic amide formed between a carboxyl group and an amine group two carbons away, making a four membered ring. Penicillin acts by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls by targeting transpeptidase, which catalyzes the final cross-linking step in peptidoglycan synthesis. The β-lactam moeity of penicillin irreversibly binds to a serine residue in the transpeptidase active site.

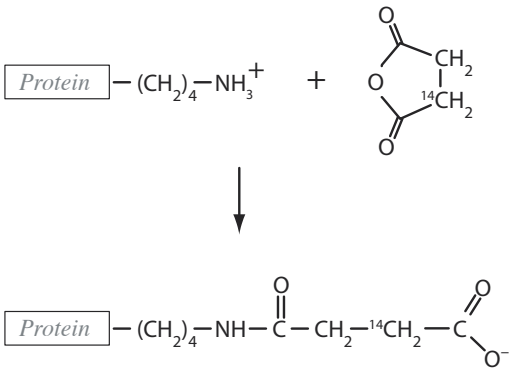
Unlike γ- or δ-lactams, which form five- and six- membered rings, respectively, the four-membered ring of a β-lactam suffers under a great deal of angle strain. The lactam ring of penicillin is primed to break open, which is what occurs when the pencillin molecule encounters the transpeptidase active site. Through the acyl substitution pathway, the lactam carbon forms a covalent ester linkage with a reactive serine residue, breaking the amide, and the ring opens. Penicillin is now bound within the active site of the enzyme, an irreversible inhibitor of transpeptidase.

Carboxylic Acid Derivatives Practice Items

1. What type of linkage is a peptide bond?

A. ester
B. amino
C. anhydride
D. amide

2. A method was devised to quantify free ε-amino groups of proteins using [^{14}C]succinic anhydride for measurement in scintillation counting.



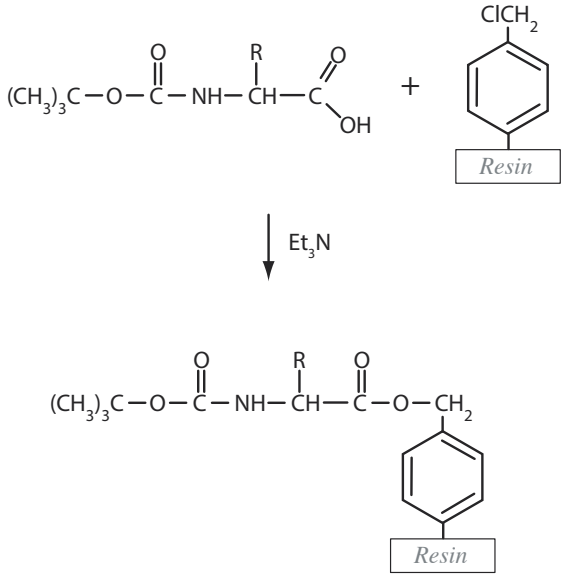
In the depiction above, what type of reaction mechanism has occurred between [^{14}C]succinic anhydride and the lysine side chain?

A. SN2 substitution
B. imine formation
C. nucleophilic acyl substitution
D. cyanohydrin formation

3. Which of the following general class of carboxylic acid derivatives is most reactive towards nucleophiles in acyl substitution?

A. amides
B. thioesters
C. carboxylates
D. carboxylic acids

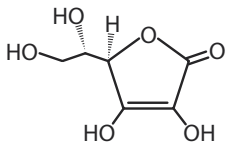
4. A technique for solid phase polypeptide synthesis begins with anchoring a chemically protected C-terminal amino acid to a chloromethyl substituted polystyrene resin.



What type of reaction is depicted in the above figure?

A. SN2 substitution
B. Michael addition
C. nucleophilic acyl substitution
D. oxidation

5. Vitamin C (ascorbate) functions as a cofactor in many enzymatic reactions in humans.

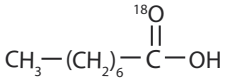


Ascorbate

Vitamin C is a . . .

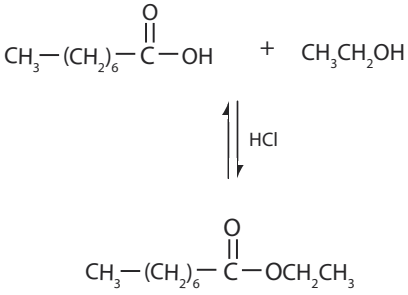
A. β-lactone
B. γ-lactone
C. β-lactam
D. δ-lactone

6. To form ethyl caprylate, Fischer esterification was carried out upon ^{18}O labeled caprylic acid, a medium-chain saturated fatty acid.



^{18}O labeled caprylic acid

Caprylic acid is sparingly soluble in water. The reaction was carried out with excess ethanol in aqueous solution.



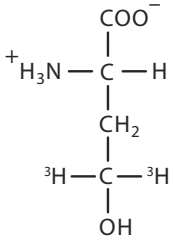
Upon completion of the reaction the supernatant was evaporated and the ethyl caprylate and unreacted caprylic acid were recovered as precipitates. The supernatant was separately recovered by means of a condensing column.

The various components were subsequently analyzed to confirm the presence of the ^{18}O label. In which of the following reaction components were ^{18}O labeled molecules confirmed to be present?

I. ethyl caprylate
II. caprylic acid
III. water
IV. ethanol

A. I and II
B. II and III
C. I, II and III
D. III and IV

7. A method for characterization of phosphorylated aspartate residues on enzymes involves cleavage of the acyl phosphate bond with sodium [^3H]borohydride. Analysis of the acid hydrolysate of the reacted protein of will show labeled homoserine if phosphorylated aspartate residues had been present.

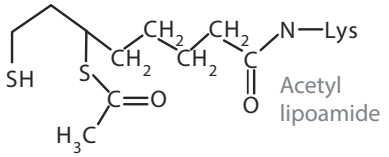


Labeled Homoserine

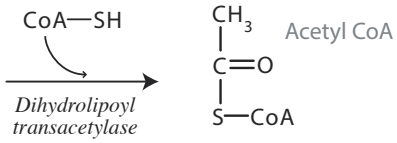
This technique is possible only because

A. $NaBH_4$ is a strong acid.
B. $NaBH_4$ doesn't reduce carboxylic acids.
C. Acyl phosphates are strong oxidizers.
D. Tritide is more nucleophilic than hydride.

8. The figure below shows a step in the dihydrolypoyl transcetylase mechanism in the pyruvate dehydrogenase complex. Which of the following describes this reaction?



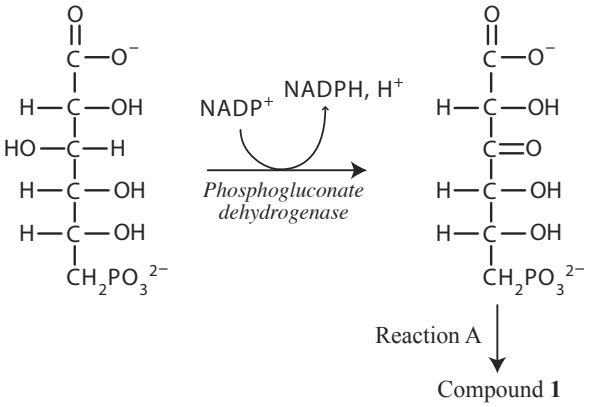
Acetyl lipoamide



Acetyl CoA

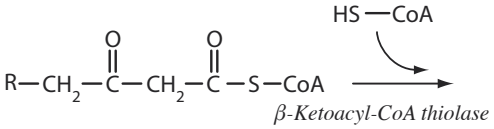
A. transthioesterification
B. transsulfuration
C. transthiolation
D. transcarboxylation

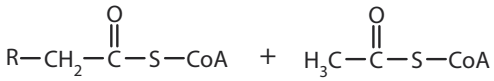
9. 6-Phosphogluconate dehydrogenase is an enzyme in the pentose phosphate pathway. The enzyme converts 6-phospho-D-gluconate into the intermediate 6-phospho-2-dehydro-D-gluconate. Still bound to the enzyme, 6-phospho-2-dehydro-D-gluconate then undergoes Reaction A to form Compound 1. What is reaction A?



A. oxidation
B. reduction
C. decarboxylation
D. esterificaiton

10. β-Ketoacyl-CoA thiolase catalyzes the final step of fatty acid oxidation in which acetyl-CoA is released and the CoA ester of a fatty acid two carbons shorter is formed.

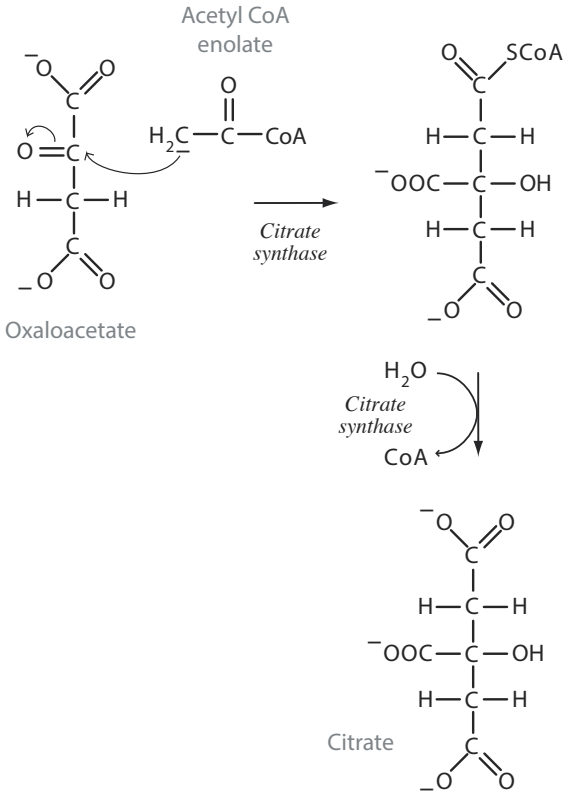




What is the best description of the mechanism of this reaction?

A. retro-Claisen condensation
B. SN2 substitution
C. aldol cleavage
D. thioesterificaiton

11. Citrate synthase catalyzes the first reaction of the citric acid cycle: the condensation of acetyl-CoA and oxaloacetate to form citrate.

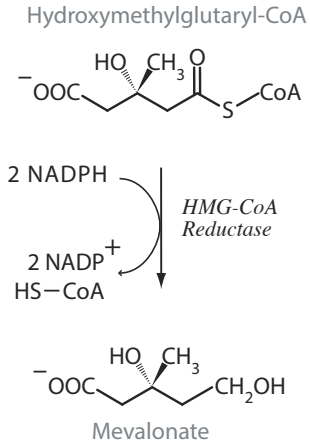


The standard free energy change (ΔG°) for the citrate synthase reaction is -31.5kJ/mol, and the enzyme functions far from equilibrium under physiological conditions.

What is the primary factor determining the negatative standard free energy change of the citrate synthase reaction?

A. hydration of the tri-carboxylate citrate product
B. resonance stabilization of the acetyl Coa enolate
C. hydrolysis of the thioester
D. electrostatic repulsion between the carboxylate groups of oxaloacetate

12. HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate, a necessary step in the biosynthesis of cholesterol and other isoprenoid lipids.



What change has occurred to the oxidation state of the thioester carbon of HMG-CoA as a result of this reaction?

A. –3 → +1
B. +2 → 0
C. +2 → –1
D. +3 → –1

13. Glutamine synthetase (GS) catalyzes the ATP-dependent condensation of glutamate with ammonia to yield glutamine. The hydrolysis of ATP drives the first step of a two-part mechanism. In the first step (Step 1), ATP phosphorylates glutamate to form γ-glutamyl phosphate. In the second step (Step 2), γ-glutamyl phosphate reacts with ammonia, forming glutamine and inorganic phosphate.

Which of the two respective steps in the glutamine synthetase mechanism described above has a negative standard free energy change (ΔG°)?

A. Step 1
B. Step 2
C. neither Step 1 nor Step 2
D. both Step 1 and Step 2

14. In polypeptide synthesis, the C-terminal residue of the growing polypeptide is sequentially esterified to the 3' hydroxyl of a t-RNA adenosine ribose (peptidyl-tRNA). In chain termination, a release factor binds to the stop codon leading to hydrolysis of peptidyl-tRNA by the peptidyl transferase ribozyme forming polypeptide and free tRNA. If an experiment were conducted with ^{18}O labeled water ($H_2{}^{18}O$) employed for hydrolysis, after liberation of the free polypeptide, ^{18}O label would be located on

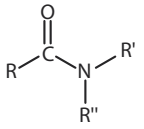
A. the polypeptide
B. the tRNA
C. neither the polypeptide nor the tRNA
D. both the polypeptide and the tRNA

Carboxylic Acid Derivatives

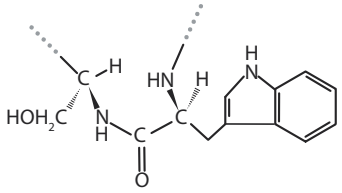
Answers and Explanations

1. D

The peptide bond is a secondary amide linkage. Amides are carboxylic acid derivatives with the general structure below, where R, R', and R" represent organic groups or hydrogen atoms.

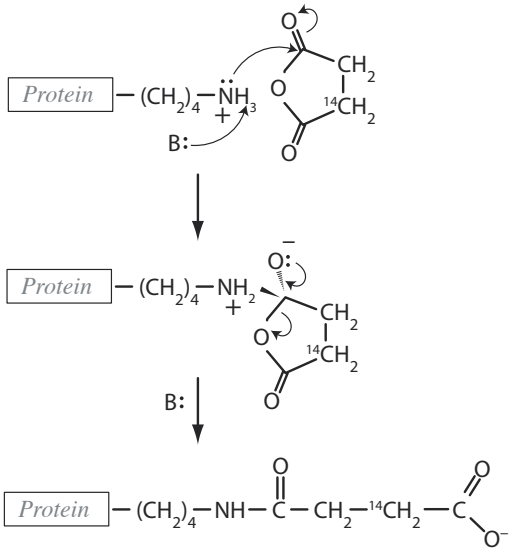


The figure below depicts the peptide bond between serine and tryptophan residues within a polypeptide:



2. C

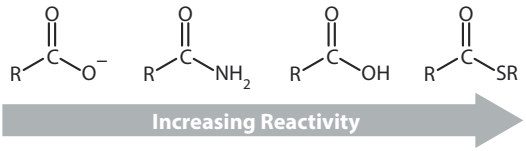
Aminolysis of an anhydride is a type of nucleophilic acyl substitution reaction. In this type of reaction, a nucleophile – such as an alcohol, amine, or enolate – displaces the leaving group of an acyl derivative – such as an acid halide, anhydride, or ester. In the resulting product, the nucleophile has taken the place of the leaving group in the original acyl derivative. In the mechanism, the nucleophile attacks the carbonyl carbon, forming a tetrahedral intermediate. After the tetrahedral intermediate forms, it collapses, recreating the carbonyl C=O bond and ejecting the leaving group in an elimination reaction.



3. B

Among the choices, thioesters are the most reactive towards nucleophiles, followed by esters and carboxylic acids. Carboxylate ions are essentially unreactive towards nucleophilic substitution, since they possess no leaving group.

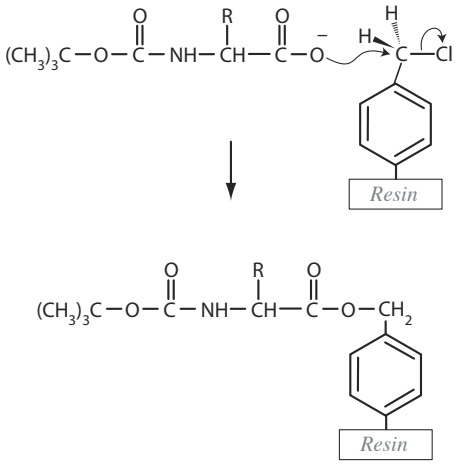
A major factor in determining the reactivity of acyl derivatives is leaving group ability, which is related to basicity. This is a thumbnail heuristic for comparing the reactivity of various carboxylic acid derivatives. Weak bases are better leaving groups than strong bases. Thiolate will be a better leaving group than amide or hydroxide. Therefore, thioesters are more reactive than esters or carboxylic acids.



The reactivity of thioesters (and phosphate anhydrides) towards nucleophilic acyl substitution is a *major* theme in biochemistry. For example, the role of coenzyme A as a carrier of 'activated' acyl groups depends on the reactivity of thioesters for nucleophilic acyl substitution. Thioesters are involved in the synthesis of many biomolecules including triglycerides, fatty acids, sterols, terpenes, porphyrins, and others.

4. A

The deprotonating agent triethylamine (Et_3N) transforms the α-carboxyl of the amino acid into its carboxylate form. The carboxylate anion then serves as a nucleophile in an SN2 reaction upon the chloromethyl substituent to form an ester.

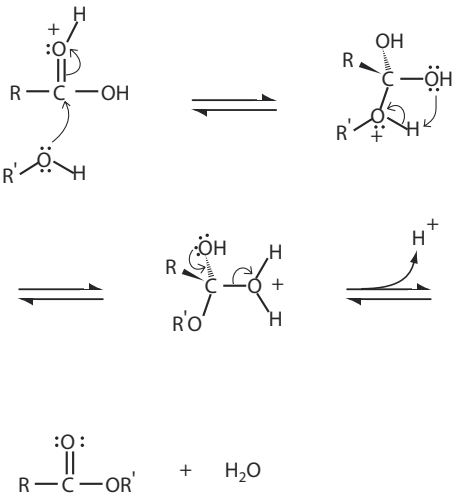


5. B

Lactones are cyclic esters. (Lactams are cyclic amides.) The Greek letter prefixes indicate the size of the ring: α-lactone = 3-membered ring, β-lactone = 4-membered, γ-lactone = 5-membered, and δ-lactone = 6-membered.

6. C

Fischer esterification is, basically, acid catalyzed nucleophilic acyl substitution. The acid catalyst protonates the carbonyl group to increasing its electron withdrawing character. In the mechanism, the alcohol nucleophile attacks the carbonyl carbon, forming a tetrahedral intermediate. The tetrahedral intermediate then collapses, recreating the carbonyl C=O bond and ejecting the water leaving group in an elimination reaction.



To keep track of the ^{18}O label, it's important to understand that *the two carboxyl group oxygens are equivalent*. Acidic protons come and go many thousands of time per second. Even under acidic conditions, the identity of the protonated oxygen changes frequently, so it will be a 50% chance whether the water leaving group carries the ^{18}O label, so there will be label present in the water, the ethyl caprylate product, as well as the unreacted caprylic acid (also some of the caprylate reformed at equilibrium through the reverse reaction).

7. B

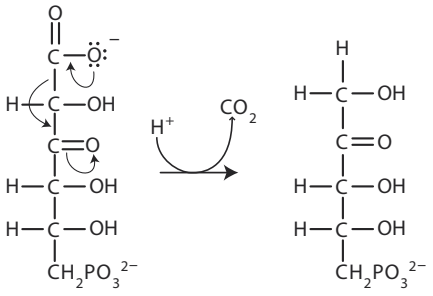
$NaBH_4$ is not powerful enough to reduce carboxylic acids but will reduce phosphate anhydrides (acyl phosphates). The presence of the alcohol product of reduction, homoserine, confirms the presence of phosphorylated aspartate residues in the enzyme being tested.

8. A

Transthioesterification is the process of exchanging the organic group SR″ of an thioester with the organic group SR′ of a thiol. The reaction is nucleophilic acyl substitution with $\Delta G \sim 0$. The reaction is driven forward by mass action as the acetyl CoA product is removed for citric acid cycle in the mitochondrion or shuttled to the cytosol for fatty acid synthesis.

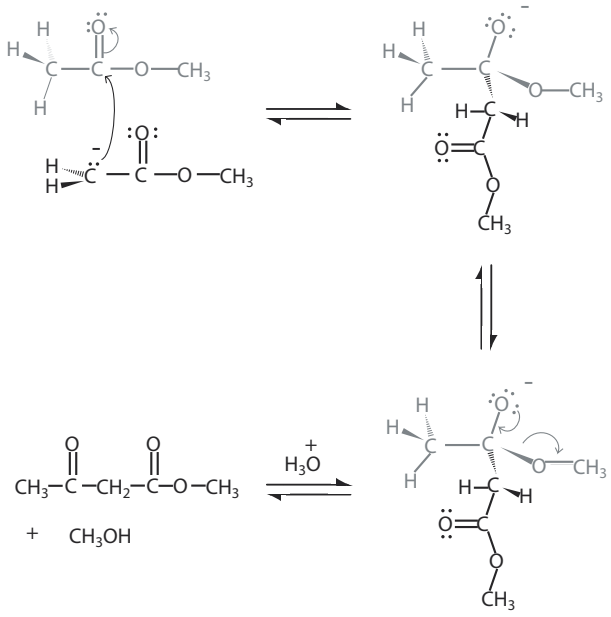
9. C

6-phospho-2-dehydro-D-gluconate is a β-keto acid, a molecule in containing a carbonyl group on the β-carbon of a carboxylic acid. β-Keto acids are very unstable and readily undergo decarboxylation with elimination of carbon dioxide under mild conditions.



10. A

Claisen condensation is like aldol addition for the world of esters. Instead of the nucleophilic addition pattern you see with aldehydes and ketones, the reaction follows the pattern of nucleophilic acyl substitution.



There are a number of reactions that appear frequently in biochemistry that did not appear on the AAMC MCAT topic outline. These reactions include E1 & E2 elimination, Michael addition, and Claisen condensation. Prior knowledge is better, but the test won't necessarilly expect it.

11. C

Thioesters are among the more reactive carboxylic acid derivatives. Hydrolysis of a thioester is thermodynamically favorable. To see thioesters (and phosphate anhydrides) as a form of metabolic energy is a big theme in biochemistry.

12. D

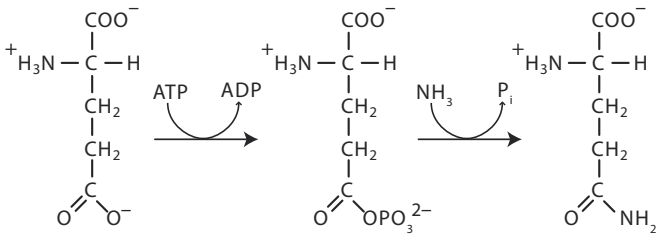
Assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

In redox accounting, the thioester carbon at the start has lost two electrons to the double bond to oxygen and an additional electron to sulfur, which is slightly more electronegative than carbon (2.6 vs. 2.5). Therefore, the oxidation state of the thioester carbon in HMG-CoA is +3. This is the univeral oxidation state of the carboxylic acid derivatives.

After the reaction, the carbon will now have one electron invested in a bond with oxygen while it gained two from the bonds to hydrogens, so carbon's oxidation state in mevalonate has become −1.

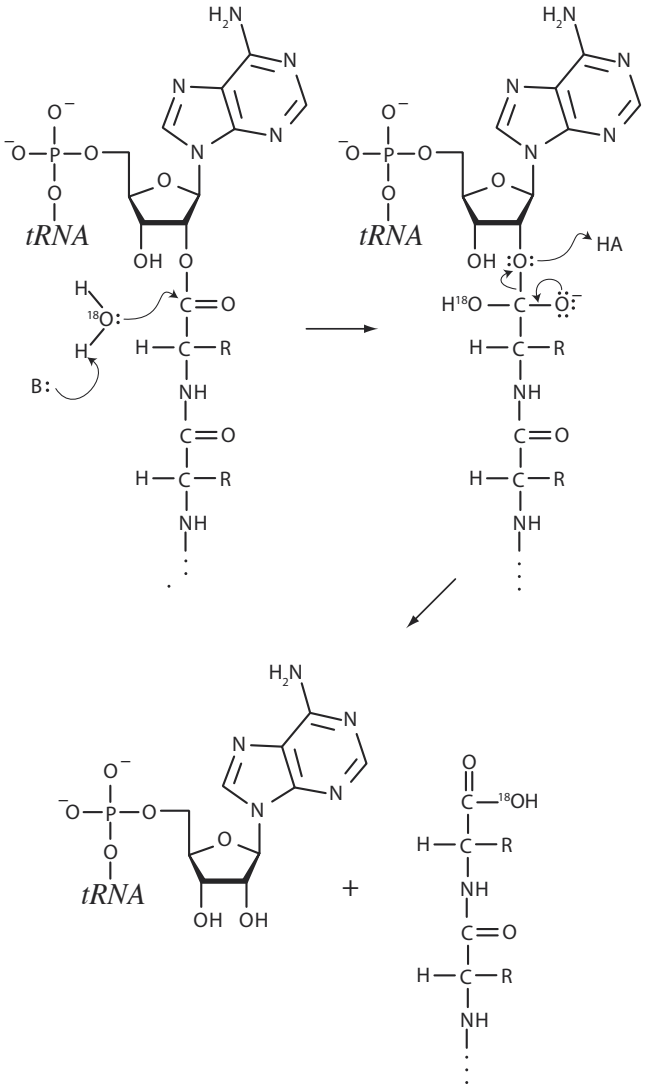
13. D

Both steps have a negative standard free energy change (ΔG°). Phosphorylation of glutamate is powered by ATP cleavage. The second step, aminolysis of phosphate anhydride, transforms a higher energy carboxylic acid derivative into a lower energy carboxylic acid derivative. This is one of the most basic functions of ATP, activating a carboxylate for nucleophilic acyl substitution by first transforming it into a phosphate anhydride.

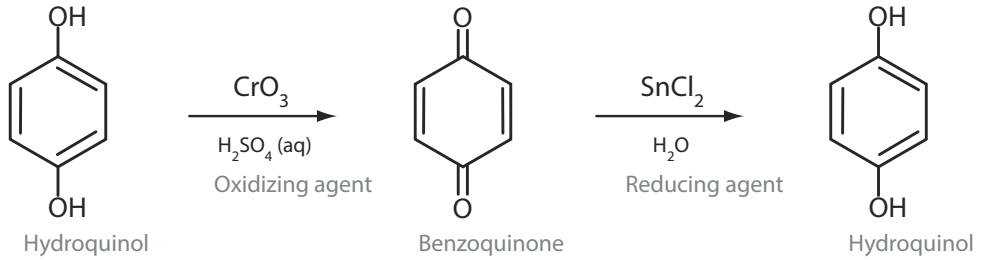


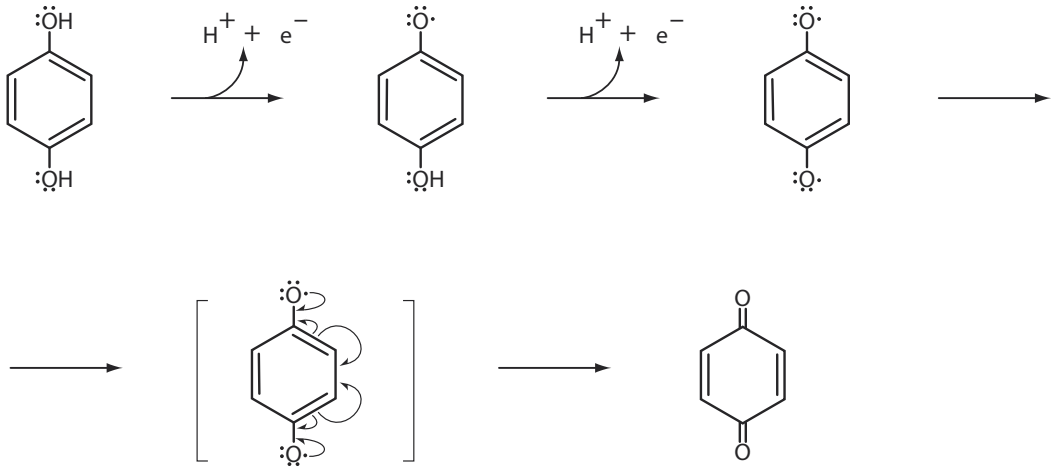
14. A

After the reaction the ^{18}O label will be on the polypeptide. This type of question, which tests understanding of the nucleophilic acyl substitution mechanism, is a long-standing tradition with AAMC.



Oxidation of Dihydroxybenzene

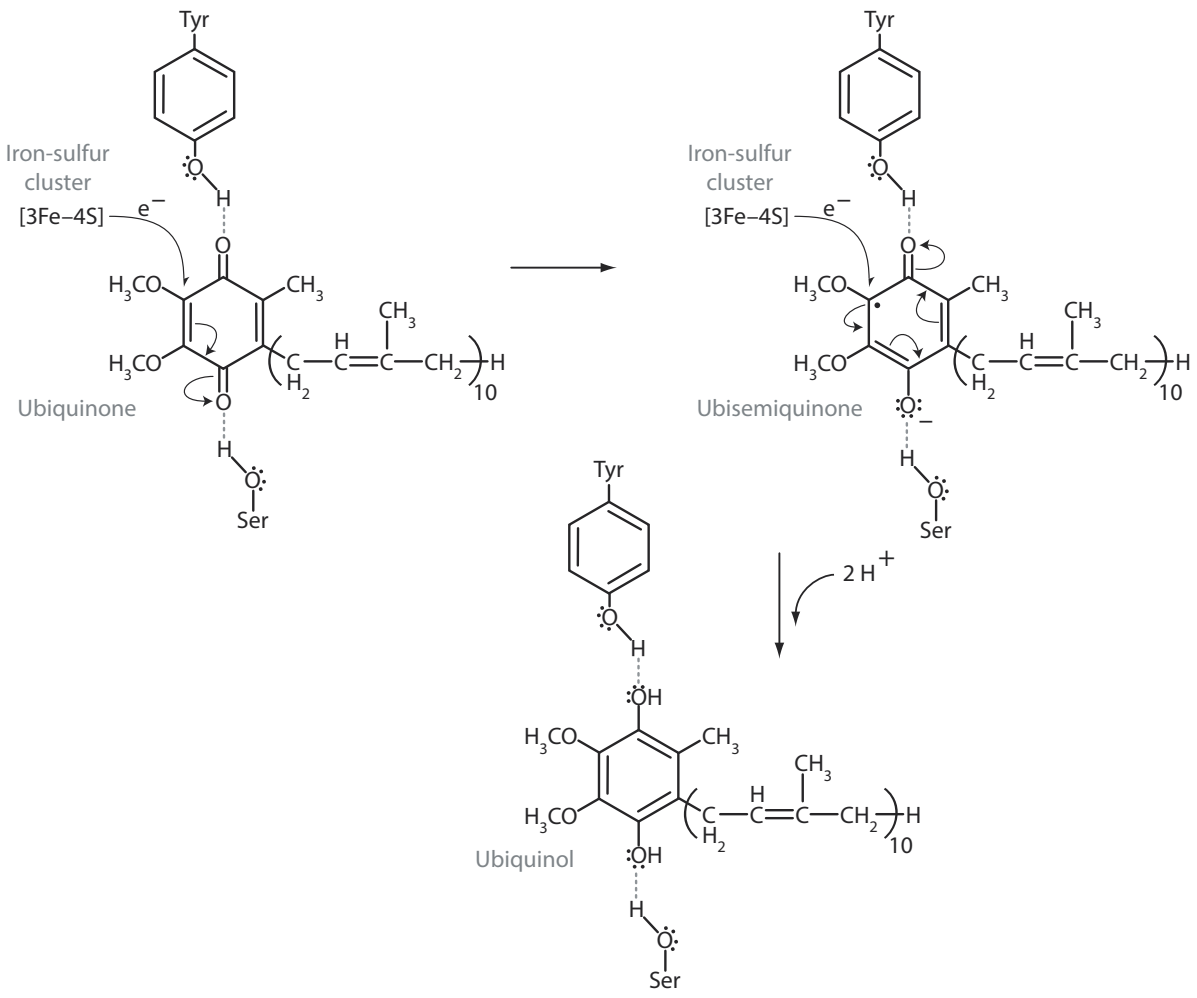




Hydroquinol is a type of phenol with hydroxyl groups bonded to a benzene ring in the *para* position. Hydroquinol is reversibly oxidized to the dicarbonyl compound benzoquinone. A quinol represents the reduced end of a redox couple with a quinone. While hydroquinol is aromatic, benzoquinone is merely conjugated, so the powerful reduction potential of oxygen favoring oxidation is balanced by the loss of the aromaticity in the quinol. This balancing act explains why the reaction is reversible.

The mechanism for the oxidation of hydroquinol passes through a free radical intermediate, a semiquinone, on the way to benzoquinone. Semiquinones are relatively stable free radicals due to resonance stabilization. The formation of the semiquinone free radical intermediate promotes one of the most important aspects of the oxidation of a quinol to a quinone, that it occurs with the transfer of a single electron at a time.

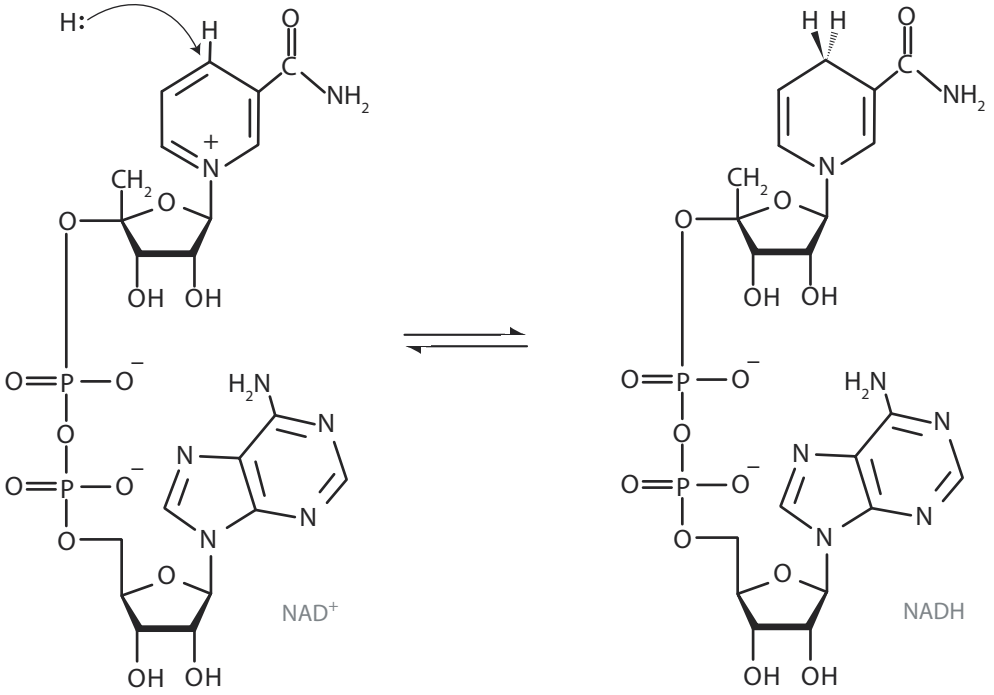
Reduction of Ubiquinone by Succinate Dehydrogenase



Penicillin belongs to the class of β-lactam antibiotics, possessing a β-lactam moeity in their molecular structure. A β-lactam is cyclic amide formed between a carboxyl group and an amine group two carbons away, making a four membered ring. Penicillin acts by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls by targeting transpeptidase, which catalyzes the final cross-linking step in peptidoglycan synthesis. The β-lactam moeity of penicillin irreversibly binds to a serine residue in the transpeptidase active site.

Unlike γ- or δ-lactams, which form five- and six- membered rings, respectively, the four-membered ring of a β-lactam suffers under a great deal of angle strain. The lactam ring of penicillin is primed to break open, which is what occurs when the pencillin molecule encounters the transpeptidase active site. Through the acyl substitution pathway, the lactam carbon forms a covalent ester linkage with a reactive serine residue, breaking the amide, and the ring opens. Penicillin is now bound within the active site of the enzyme, an irreversible inhibitor of transpeptidase.

Reduction of NAD^+

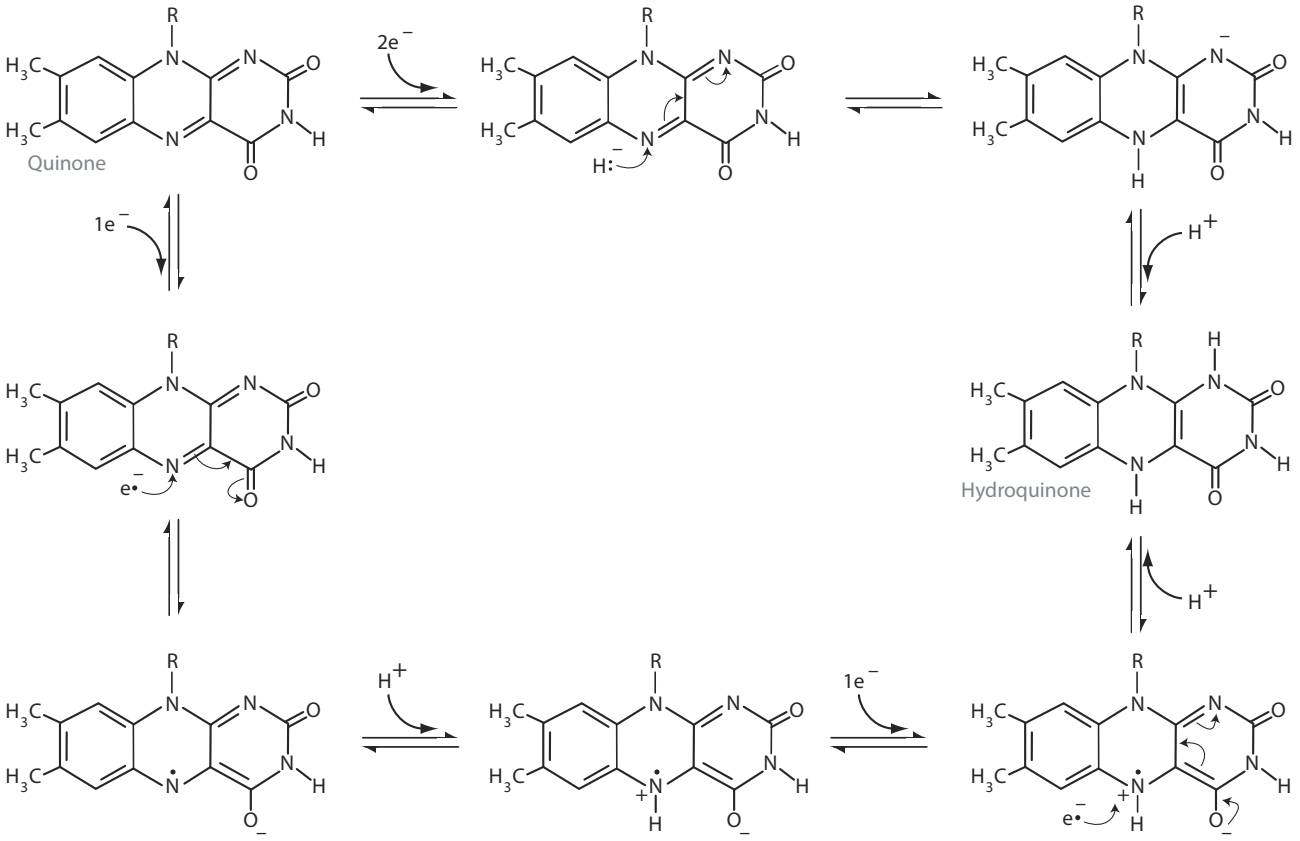


An essential coenzyme of many oxidoreductases, NADH (nicotinamide adenine dinucleotide) is the primary carrier of reducing equivalents for energy metabolism, carrying electrons from one redox reaction to another. NAD^+ is an oxidizing agent, accepting electrons from other molecules, forming NADH, which can then be used as a reducing agent. Another coenzyme, NADPH, differs from NADH in having a phosphate group on C-2 carbon of its adenosine ribose. NADPH performs chemically in virtually the same way as NADH. NADPH is the primary source of reducing equivalents for biosynthesis.

Nicotinamide adenine dinucleotide is a hydride (H^-) donor and receiver. Reactivity is restricted to the nicotinamide portion of the molecule. When NAD^+ receives a hydride to form NADH, the nicotinamide ring loses aromaticity. The electrons are kept in an elevated energy. This is why NADH is such a good electron transfer agent. When it donates its electrons it regains aromaticity.

Note that the C-4 carbon of nicotinamide is prochiral. Using deuterium labeling it has been possible to distinguish the direction of hydride approach for many oxidoreductase reactions. This tells us how the nicotinamide ring of the coenzyme is positioned in the active site vis-a-vis the substrate. Depending on the enzyme, the hydride donor will be positioned either above or below the plane of the C4 carbon and donate to either the "re" face of NAD^+ or the "si" face, respectively.

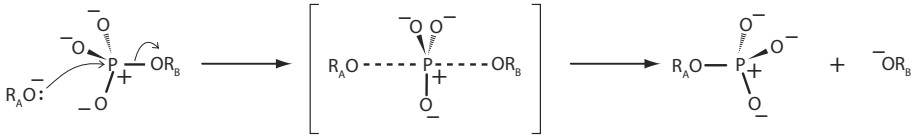
Reduction of Flavin



A flavoprotein contains a flavin moiety in the form of one of the prosthetic groups flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN). Important flavoproteins in energy metabolism include succinate dehydrogenase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and acyl-CoA dehydrogenase. There are many flavoproteins in metabolism. Humans have 90 flavoprotein encoded genes. Unlike the other redox cofactors in metabolism, such as NADH (pg 95), which transfers electrons two at a time, or Coenzyme Q_{10} (pg 93), which transfers electrons 1 at a time, flavins are capable of carrying out either 1 or 2 electron transfers. Represented in the clock-wise path from the the quinone above left, FAD can accept 2 electrons and 2 protons to become $FADH_2$ (hydroquinone form). Alternatively, the free radical semiquinone (FADH·) can be formed by a single electron reduction of FAD, as shown in the counter-clockwise path, followed by another single electron transfer to form $FADH_2$. Both pathways are reversible, so $FADH_2$ can also donate 1 or 2 electrons.

The flavin moeity has a more positive reduction potential than the nicotinamide ring of NAD+. Flavin is a strong oxidizing agent. The cell utilizes its high reduction potential in many energetically difficult oxidation reactions such as dehydrogenation of a C-C bond to an alkene (pg 112).

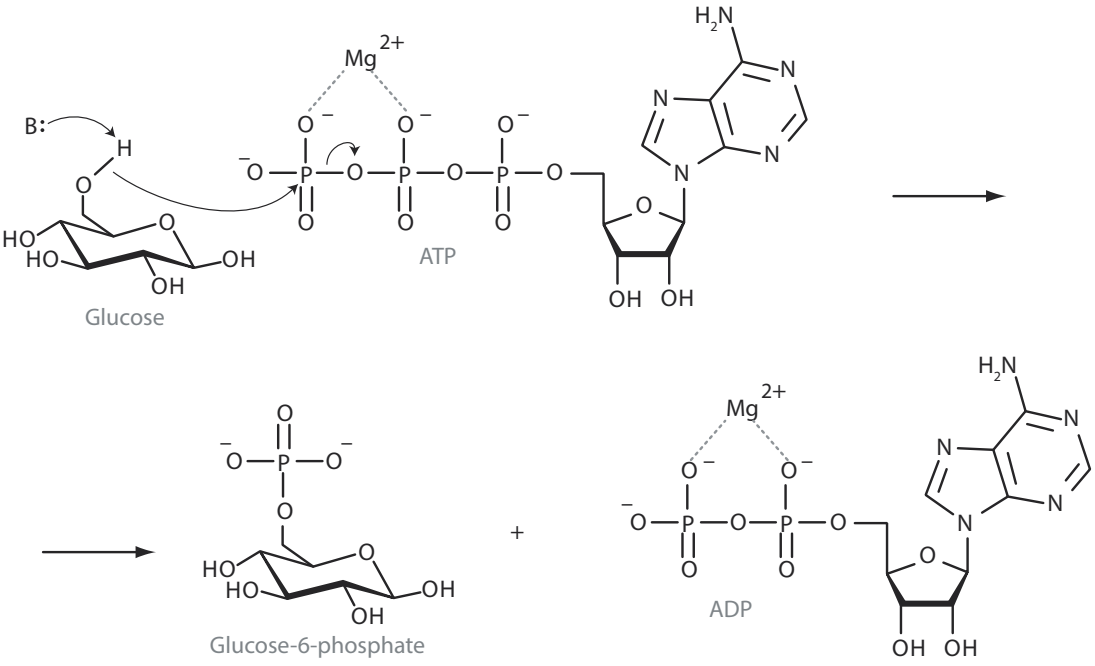
Phosphoryl Transfer



Because phosphate esters play a central role in biochemistry, phosphoryl transfer reactions are correspondingly important. The study of chiral phosphate derivatives has demonstrated that several mechanistic variants of the phosphoryl transfer reaction occur. In the most common variant, the in-line mechanism shown above, the phosphoryl transfer reaction occurs along a pathway that is very similar to an SN2 reaction at a carbon (pg 47), but across a phosphorus atom instead. In the in-line phosphoryl transfer mechanism, the nucleophile approaches the phosphorus from the backside, opposite the leaving group. As the nucleophile begins to attach and the leaving group begins its departure, the bonding geometry at the phosphorus atom changes from the tetravalent reagent to a pentavalent transition state. As in SN2, the stereochemistry of the substituent groups flips when the leaving group departs and there is an inversion of configuration.

Note that this in-line SN2-type mechanism is not the only possible mechanism of phosphoryl transfer. A dissocciative mechanism may also occur, similar to the SN1 reaction (pg 45) in which elimination of the leaving group occurs first, forming an unstable intermediate which then captures the nucleophile. There is also a concerted variation that involves a pentavalent transition state which forms by nucleophilic attack from the same side as the leaving group.

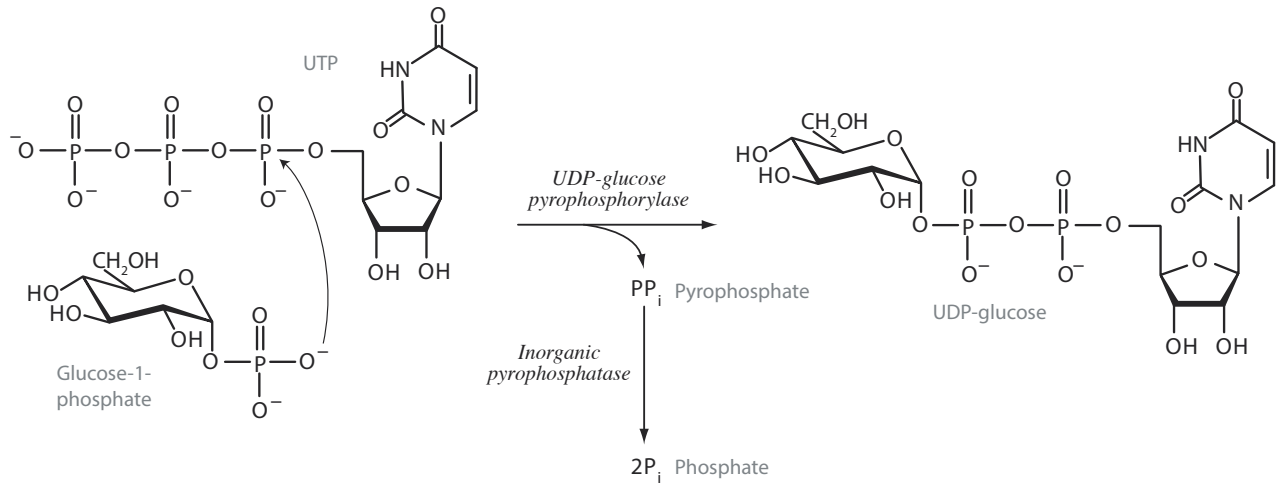
Hexokinase



Hexokinase catalyzes the phosphorylation of hexoses such as glucose, mannose and fructose. Phosphorylation by hexokinase commits glucose to one of a number of intracellular processes.

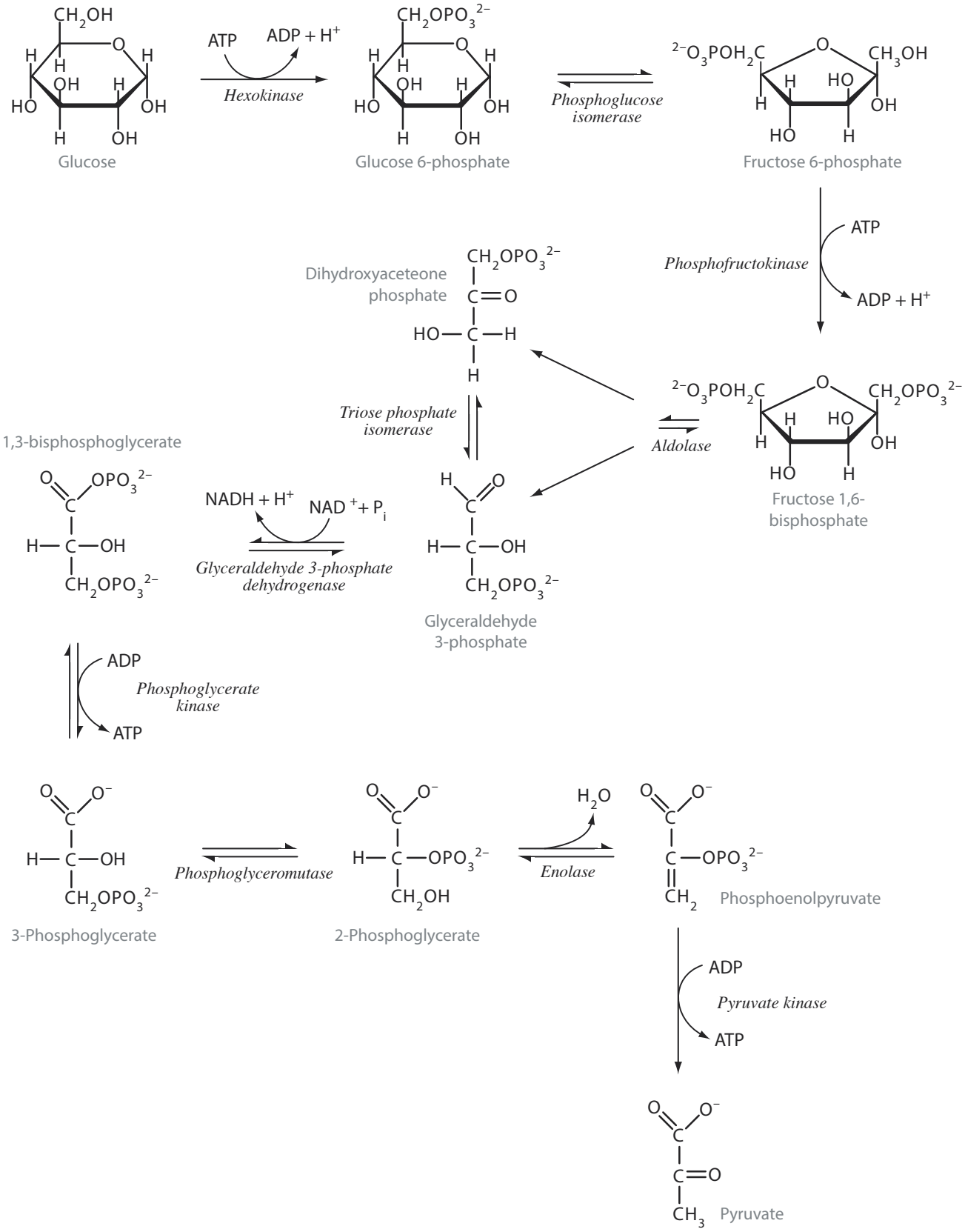
An important feature in the general kinase mechanism is the role of magnesium (II) ion as a cofactor. Magnesium ion forms a complex with ATP which helps to stabilizes the transition state during phosphoryl transfer.

UDP-Glucose Pyrophosphorylase

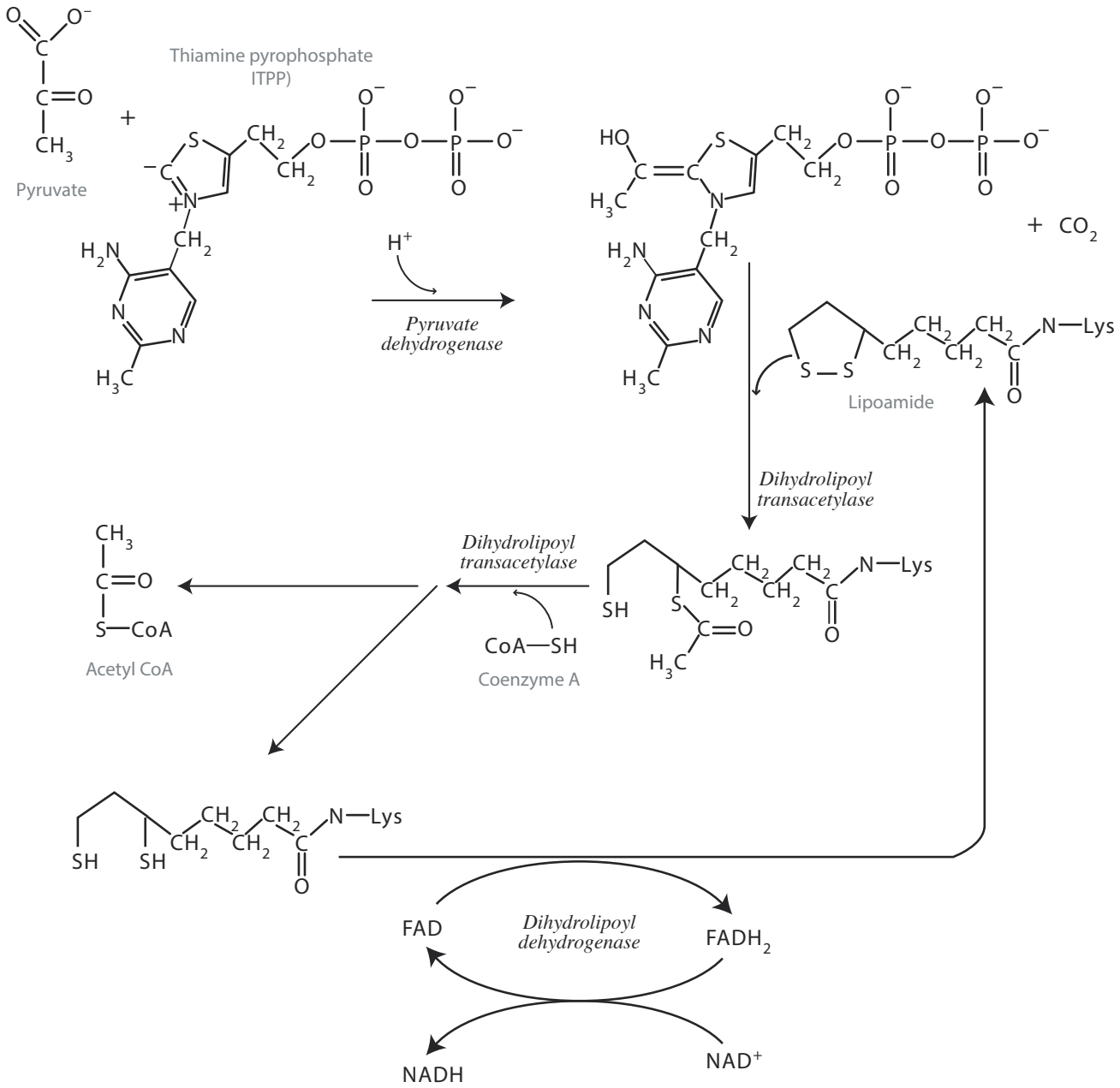


In the UDP-glucose pyrophosphorylase reaction, a phosphoryl oxygen of glucose-1-phosphate attacks the α phosphorus of UTP to form a new phosphoanhydride linkage while releasing pyrophosphate. Because this reaction is merely a phosphoanhydride exchange, there is not much to push this reaction forward thermodynamically. However, the pyrophosphate formed is subsequently cleaved by inorganic pyrophosphatase, a highly exergonic process. Subsequent breakdown of pyrophosphate drives the reaction forward.

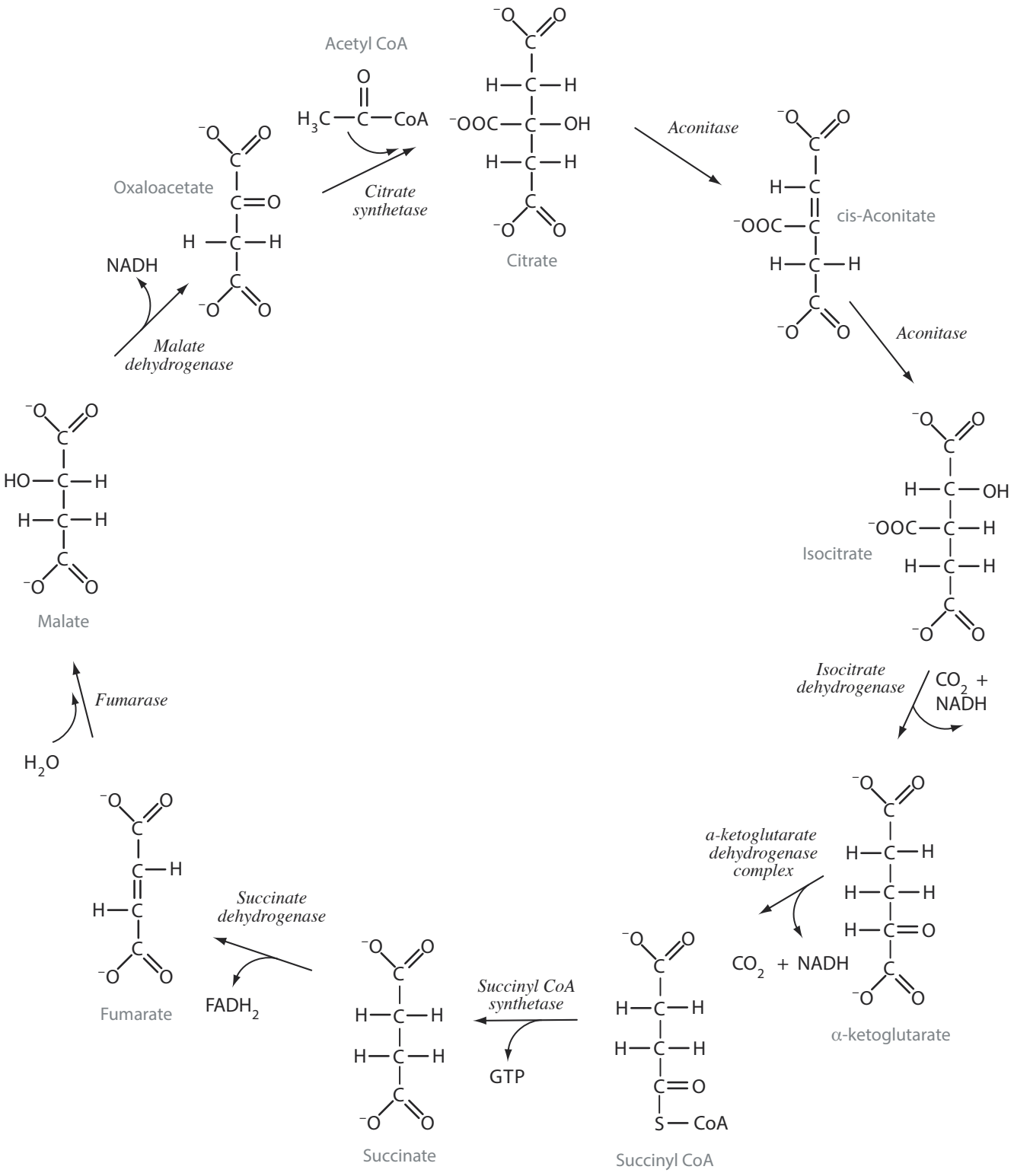
Glycolysis


Glucose
ATP
ADP + H+
Hexokinase
Glucose 6-phosphate
Phosphoglucose isomerase
Fructose 6-phosphate
ATP
Phosphofructokinase
ADP + H+
Dihydroxyaceteone phosphate
Triose phosphate isomerase
Aldolase
Fructose 1,6-bisphosphate
1,3-bisphosphoglycerate
NADH + H+
NAD + + Pi
Glyceraldehyde 3-phosphate dehydrogenase
Glyceraldehyde 3-phosphate
ADP
Phosphoglycerate kinase
ATP
3-Phosphoglycerate
Phosphoglyceromutase
2-Phosphoglycerate
H2O
Enolase
Phosphoenolpyruvate
ADP
Pyruvate kinase
ATP
Pyruvate

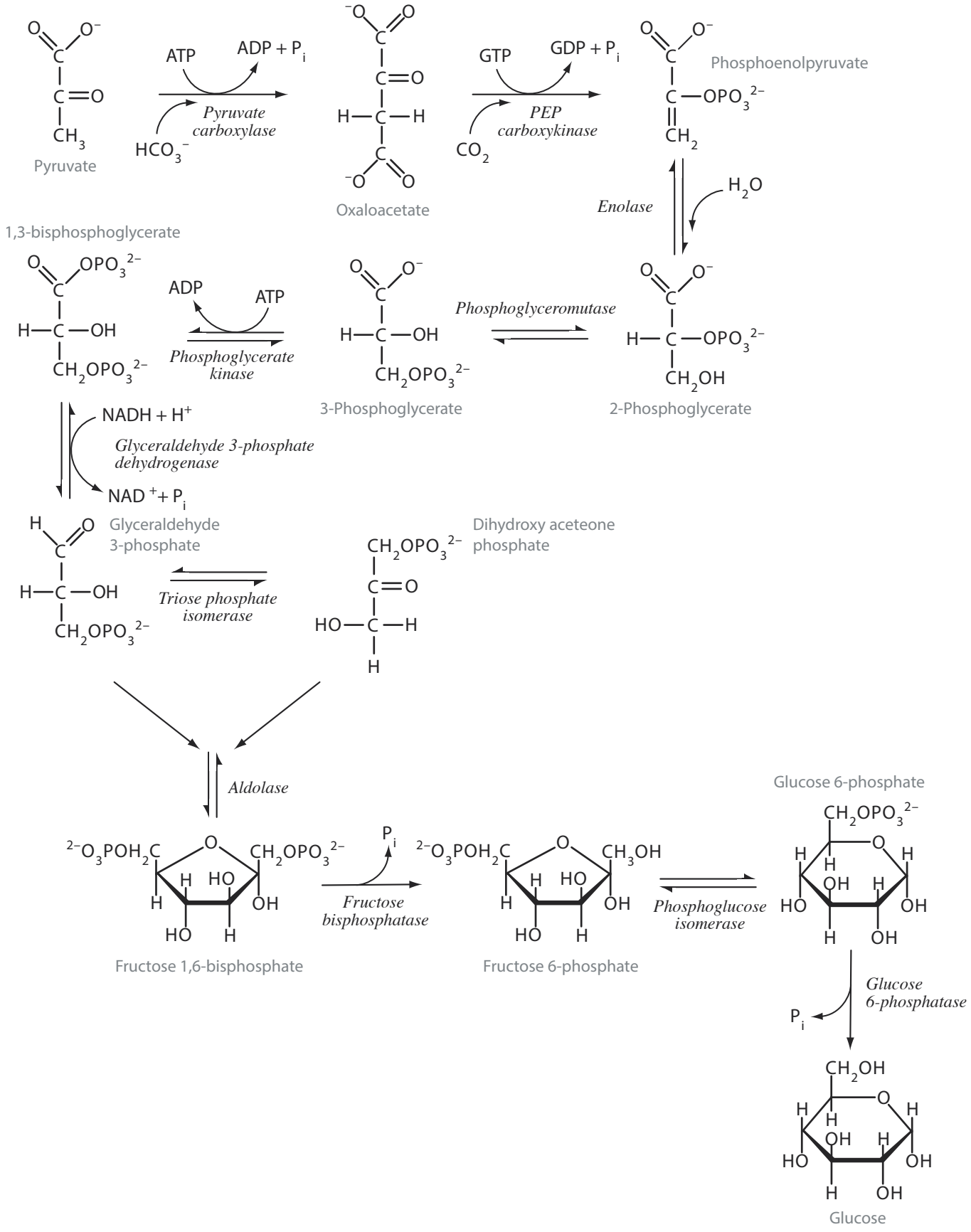
Pyruvate Dehydrogenase Complex



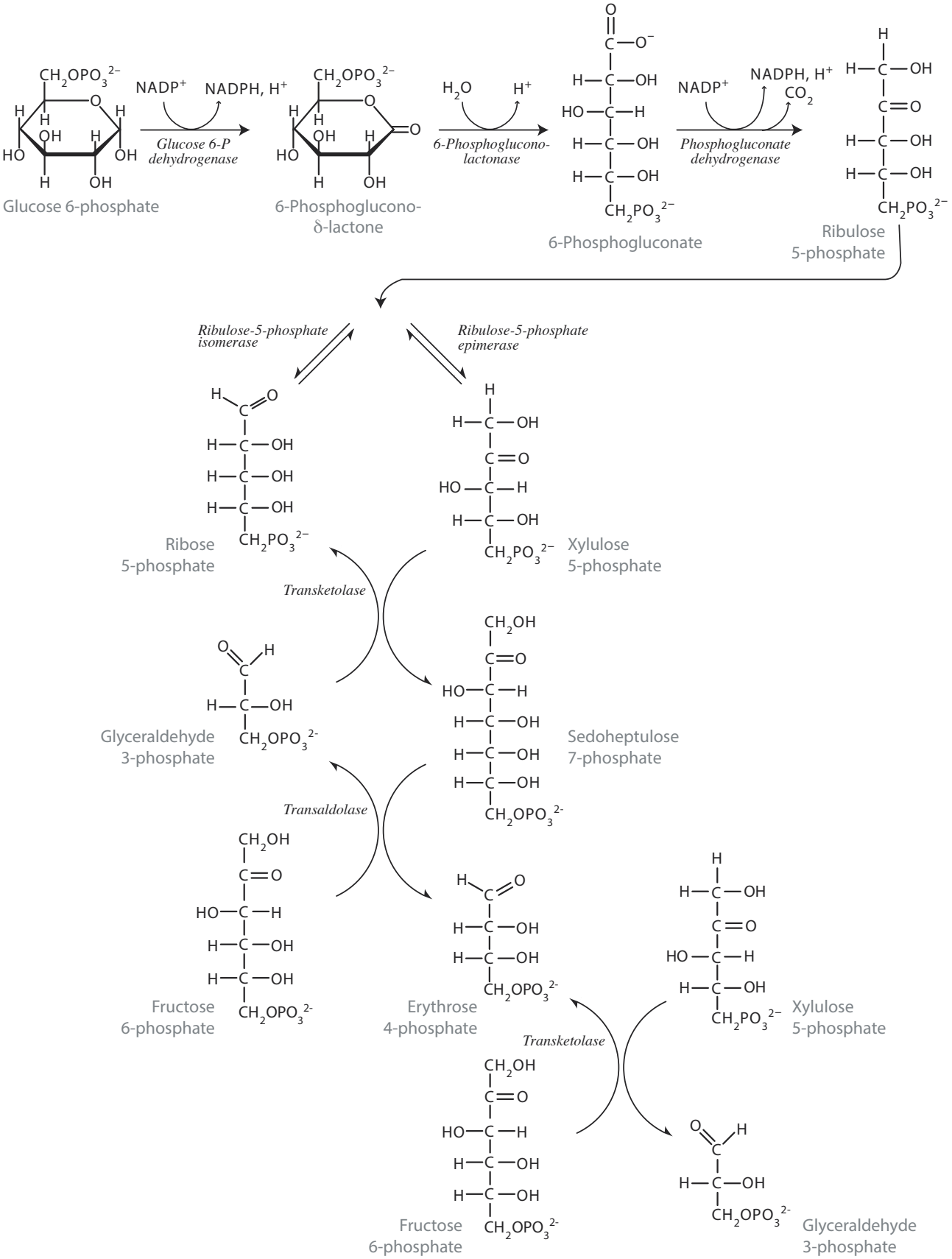
Citric Acid Cycle



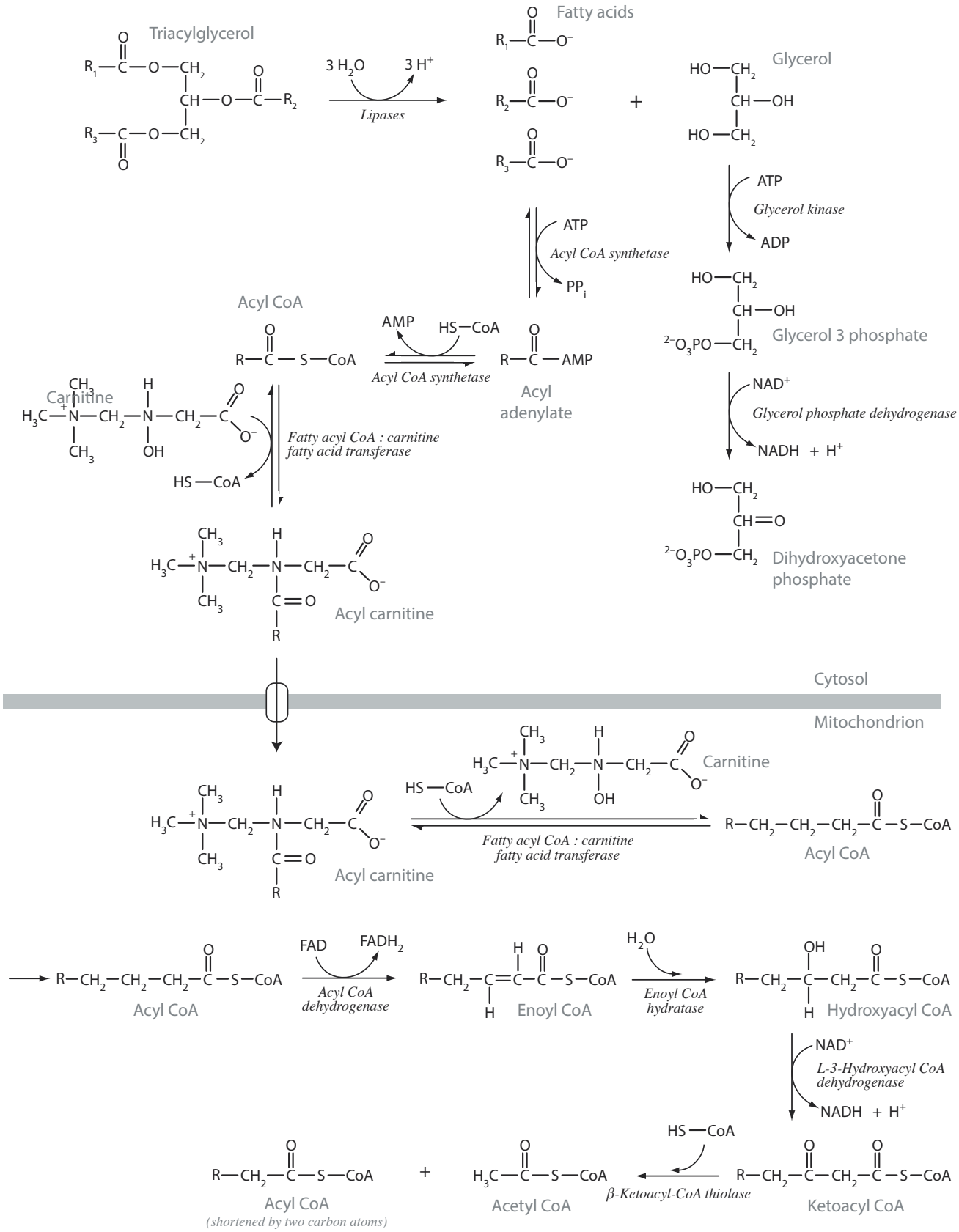
Gluconeogenesis



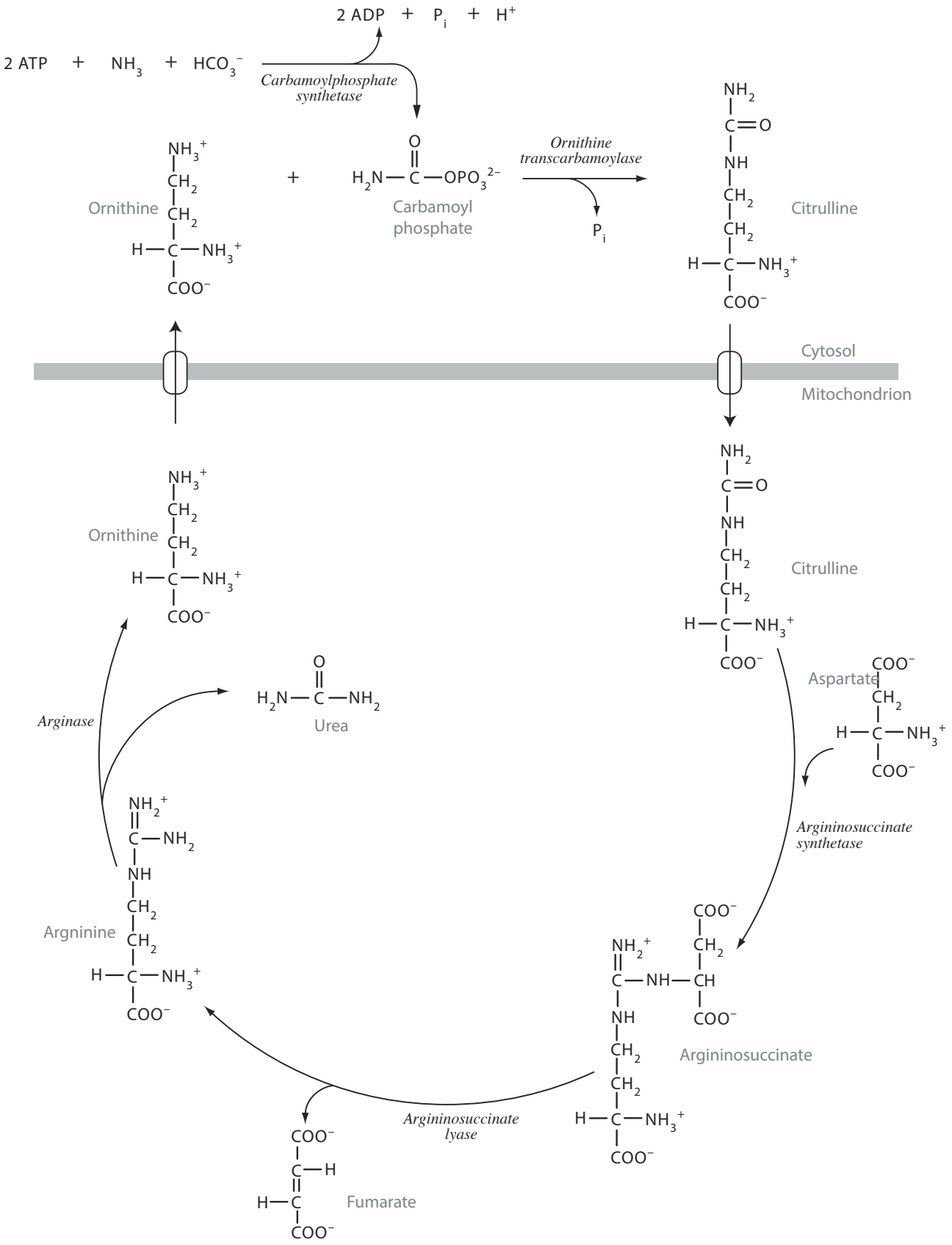
Pentose Phosphate Pathway



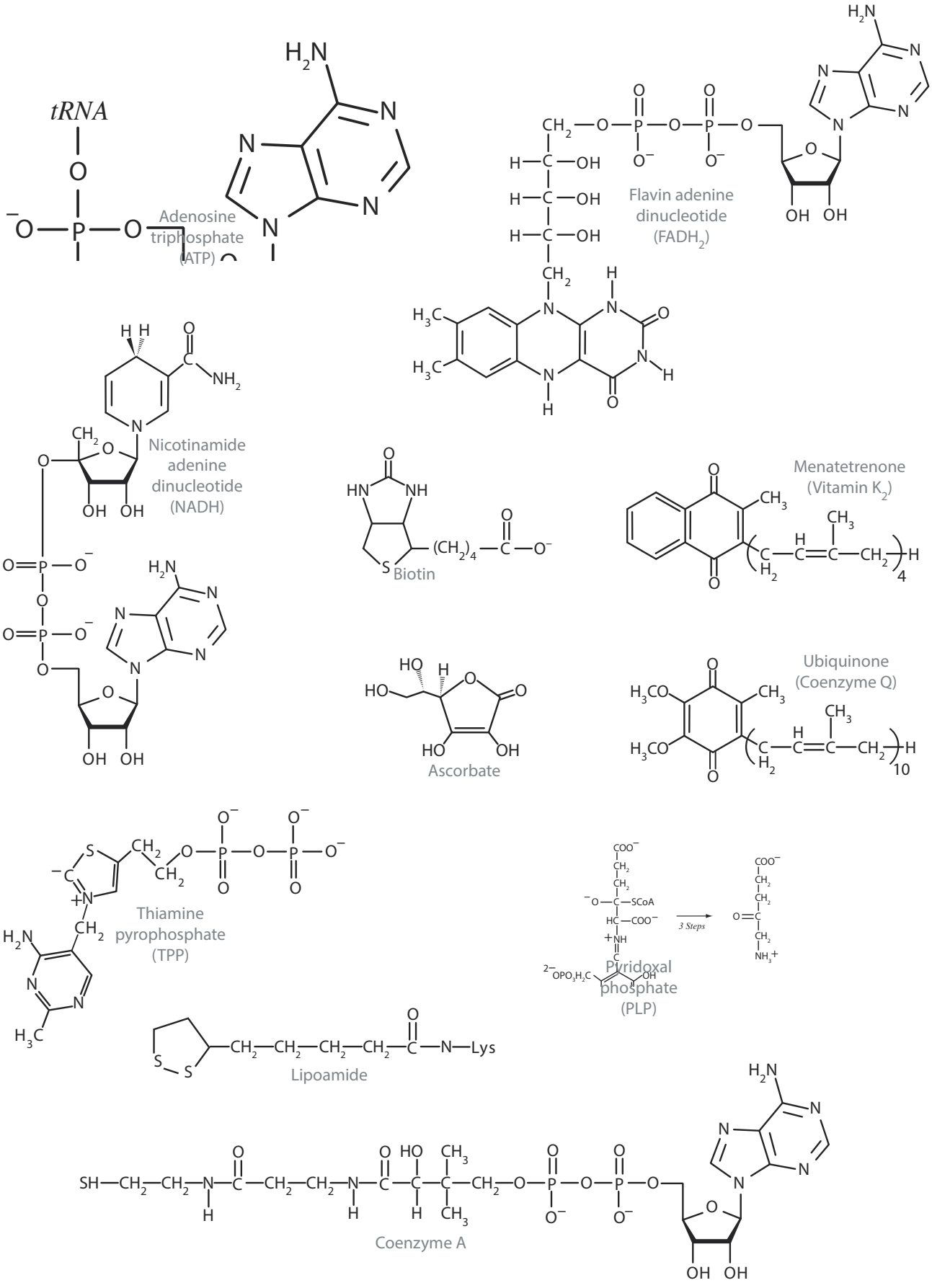
Triglyceride Breakdown & Fatty Acid Oxidation



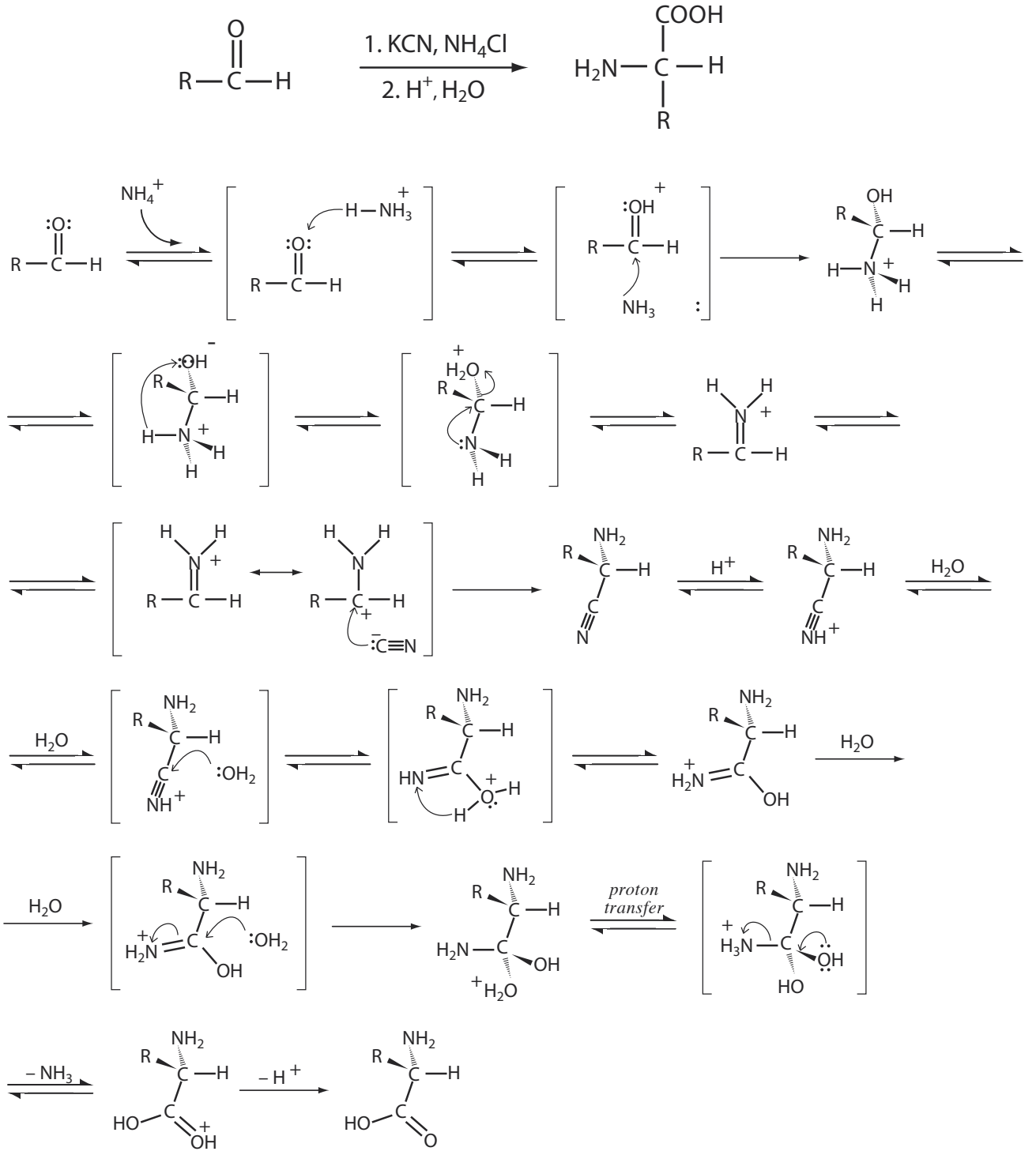
Urea Cycle


2 ADP + P_i + H^+
2 ATP + NH_3 + HCO_3^-
Carbamoylphosphate synthetase
Ornithine
Carbamoyl phosphate
Ornithine transcarbamoylase
P_i
Citrulline
Cytosol
Mitochondrion
Ornithine
Citrulline
Aspartate
Argininosuccinate synthetase
Arginase
Urea
Argninine
Argininosuccinate
Argininosuccinate lyase
Fumarate

Coenzymes

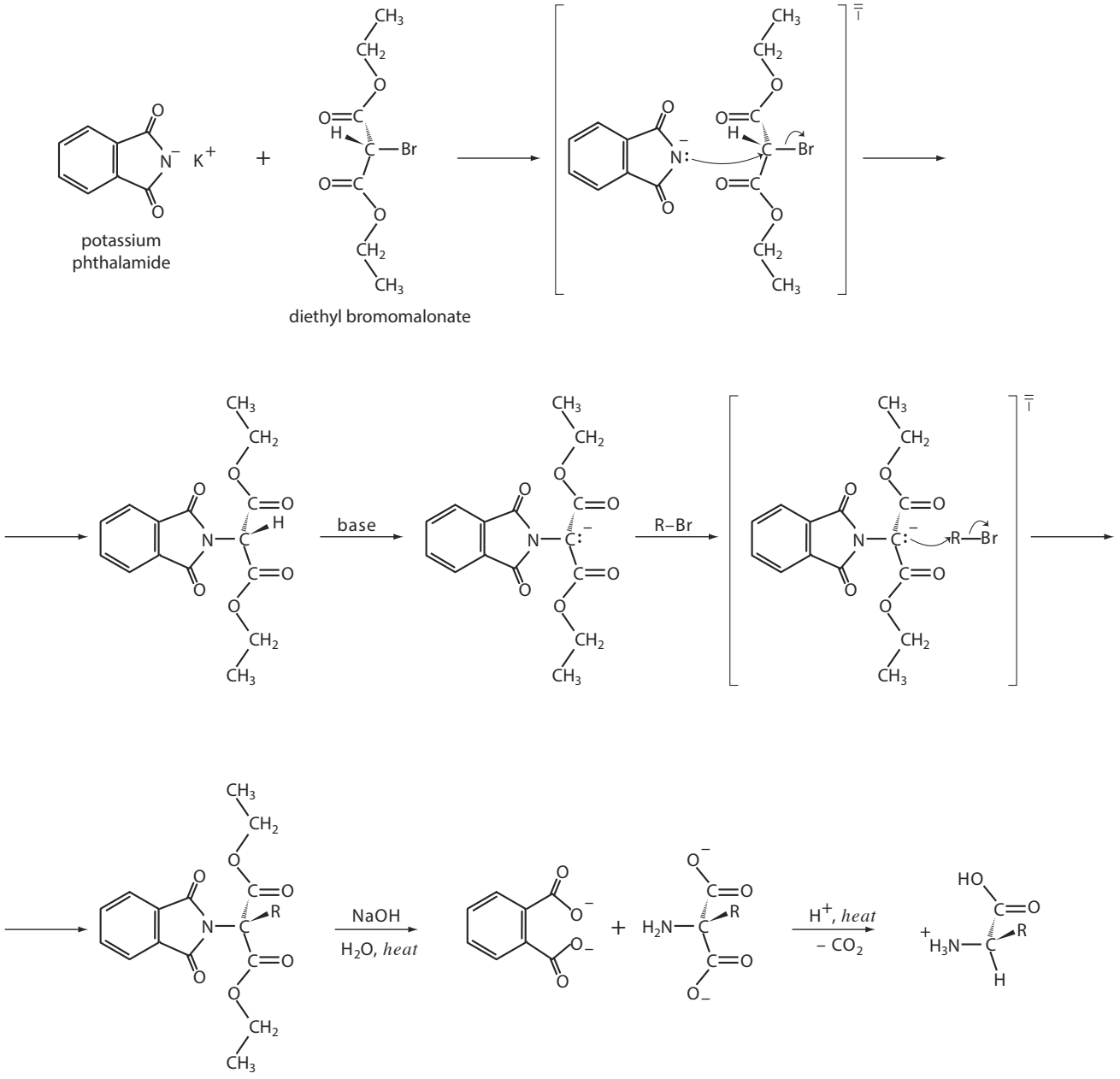

tRNA
Adenosine triphosphate (ATP)
Flavin adenine dinucleotide ($FADH_2$)
Nicotinamide adenine dinucleotide (NADH)
Biotin
Menatetrenone (Vitamin K_2)
Ascorbate
Ubiquinone (Coenzyme Q)
Thiamine pyrophosphate (TPP)
3 Steps
Pyridoxal phosphate (PLP)
Lipoamide
Coenzyme A

Strecker Synthesis



In the Strecker amino acid synthesis, ammonia serves as the amine precursor in the synthesis and cyanide as the carboxyl precursor. The first step involves nucleophilic addition of ammonia to an aldehyde to form an imine. Next, reaction of the imine with a cyanide nucleophile is carried out to form an α-aminonitrile. Subsequent hydrolysis of the nitrile group of the α-aminonitrile transforms it into the carboxyl group of the finished α-amino acid. The traditional Strecker synthesis yields a racemic mixture of L- and D- amino acids, but several procedures utilizing asymmetric catalysts have been developed.

Gabriel Synthesis



The Gabriel synthesis of α-amino acids begins with phthalimide anion reacting with diethylbromomalonate in an SN2 process. Malonic esters are distinctive in having a significantly acidic α hydrogen, so a carbanion can be easily formed and used in another SN2 substitution, this time to add what will become the amino acid side-chain onto the growing molecule. Base catalyzed hydrolysis then removes the pthalamide moeity as well as the alkoxy portions of the esters. The final step is acidification and thermal decarboxylation to remove one of the two carboxyl groups, yielding the α-amino acid in racemic mixture.