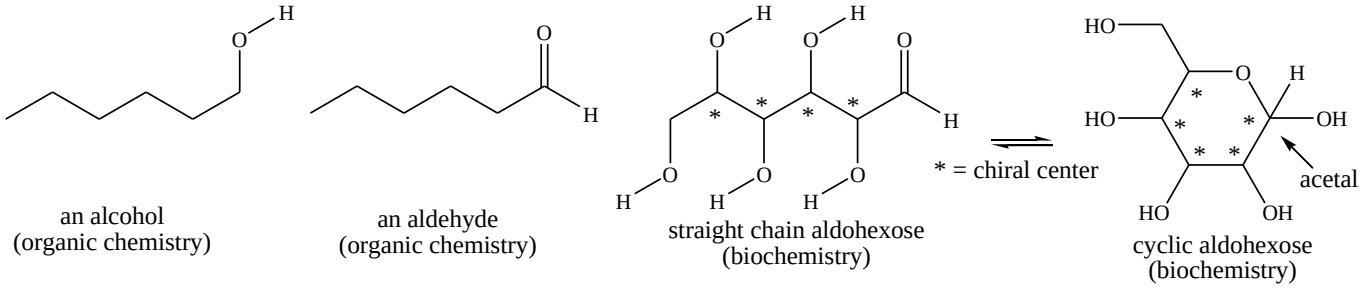
Molecules of Glycolysis – An Organic Chemistry Perspective

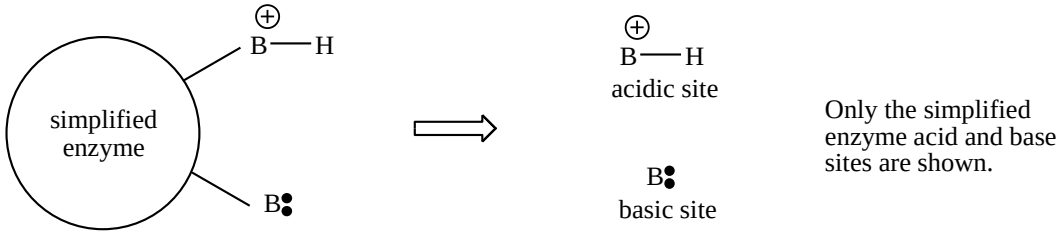
Biochemistry is a fascinating subject that provides us with insights into how life works. However, its molecules tend to be much more complicated than its sister science, organic chemistry. Introductory organic molecules usually show only one functional group at a time that is used to study simple functional group chemistry. Biochemical molecules often have multiple functional groups, some of which may be interacting with one another in complicated ways, while others are mere spectators. Compare two simple organic molecules with a typical aldohexose, like glucose, which has a functional group on all 6 of its carbon atoms and can exist as a straight chain or cyclic ring, with 4 or 5 chiral centers.



All of this greater complexity makes it more difficult to write out individual steps of biochemical mechanisms the way an organic chemist might write out a mechanism. This document presents an attempt to look at the individual steps of important biochemical pathways in a manner similar to organic chemistry. The goal is to analyze biochemical pathways and cycles, such as glycolysis, gluconeogenesis, the TCA cycle, fat metabolism, the Phosphopentose Pathway, the Shikimic acid pathway, cholesterol synthesis and more, using simplistic organic chemistry mechanisms to better understand how and why those reactions occur the way they do.

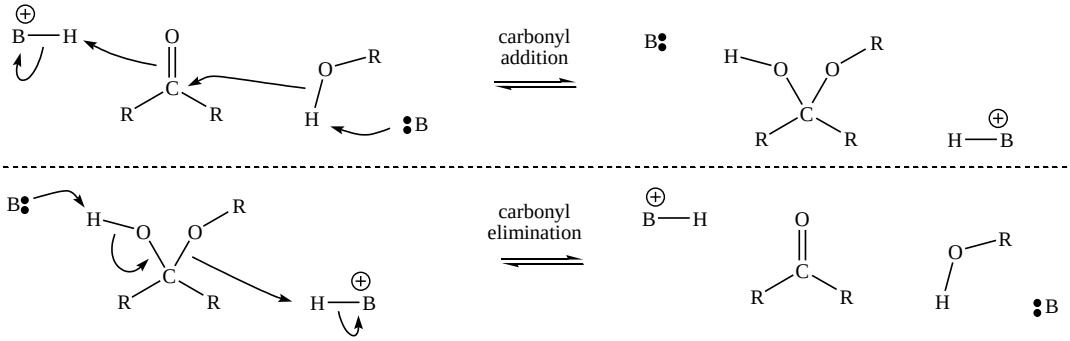
In this document, biomolecules are simplified to look more like simple organic molecules. The curved arrows are simplified so that multiple steps can be combined into a single reaction sequence. 'The Bio-Organic Game,' was created with the goal being to allow all students to write biochemical mechanisms in a manner that increases their insight into the reactions being studied. Simplified structures and arrow pushing make it easier to speculate how individual steps of biochemical pathways might occur, especially when there isn't any actual mechanism known, and it is fun and exciting to speculate. Speculation can expand your thinking to new insights and possibilities unknown.

Proton transfers are the most common reactions in biochemistry and occur as part of many biochemical reactions. The Bio-Organic Game greatly simplifies "enzyme" acids and bases and the arrow-pushing mechanisms, making it possible to show successive "biochemistry" steps in a reasonable way. These basic sites and acidic sites are simplified in the Bio-Organic Game to B: and B^+-H.

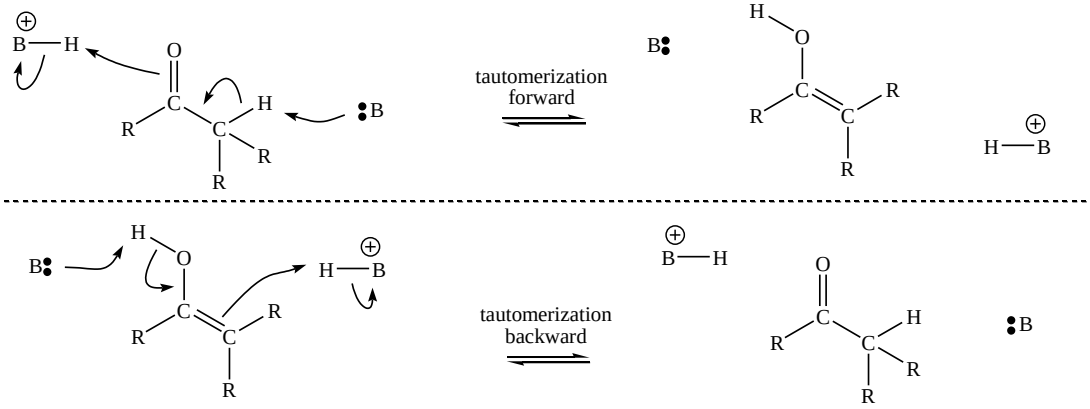


Using this approach, transformations that would require multiple steps in an organic mechanism are shown in a single overall transformation. Considering that enzymes tend to get all the reactants in just the right spot for faster reactions, this might not be so unreasonable. Only the bare-bones features are included in the biomolecules in order to show the essential proposed chemistry. Just below, four reactions that are common to organic and biochemistry are shown in this simplified format. The curved arrows show the bonds formed and broken in a concerted manner that normally would not happen in an organic mechanism, but might be possible in an efficient enzyme catalytic site.

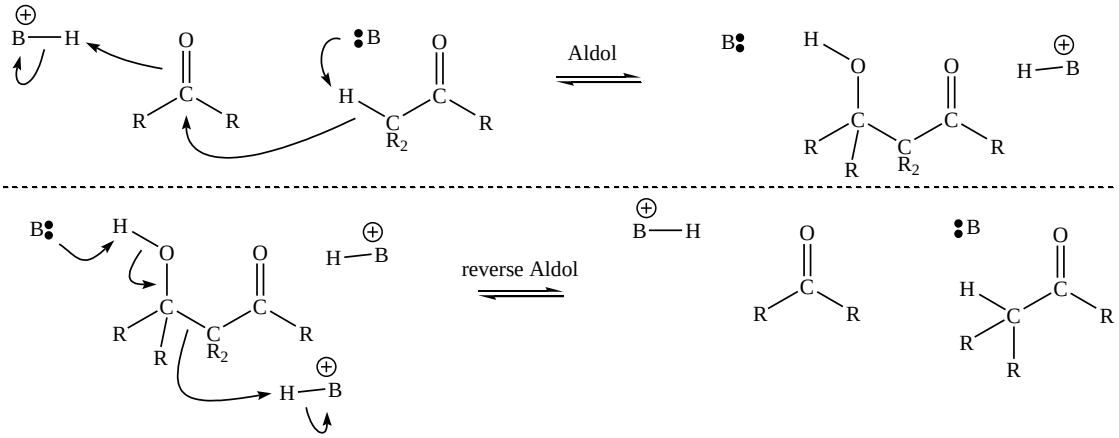
Carbonyl addition / elimination



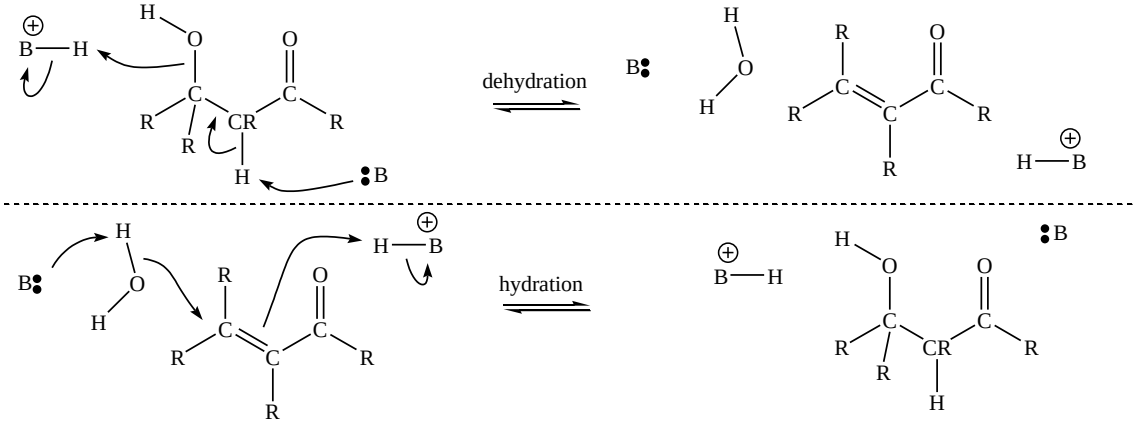
Enolizaton (tautomerization)



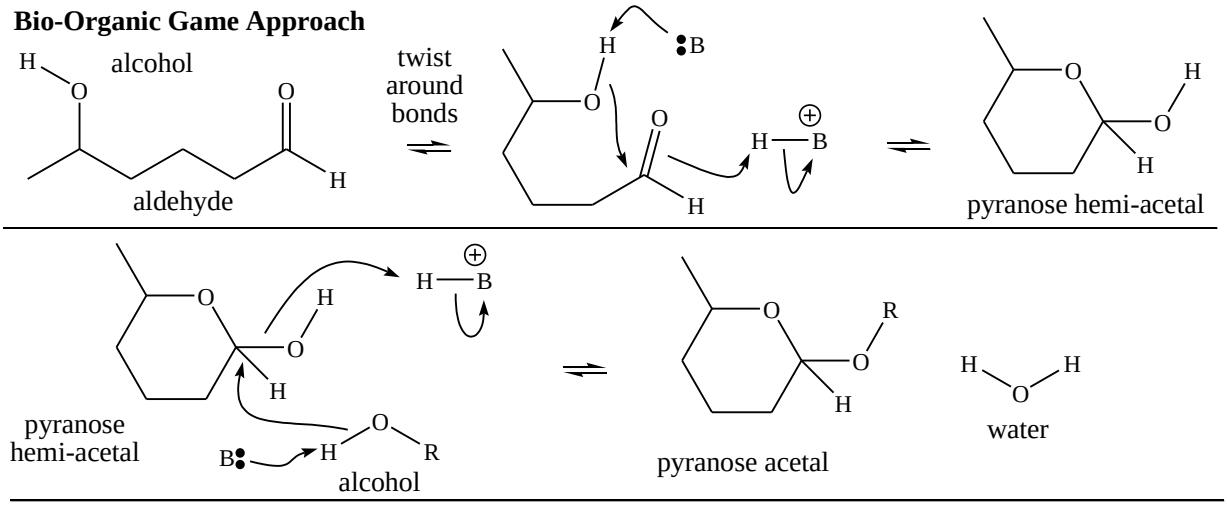
Aldol reactions / reverse Aldol

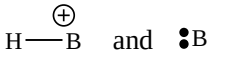


Dehydration /Hydration (reverse Michael / Michael)



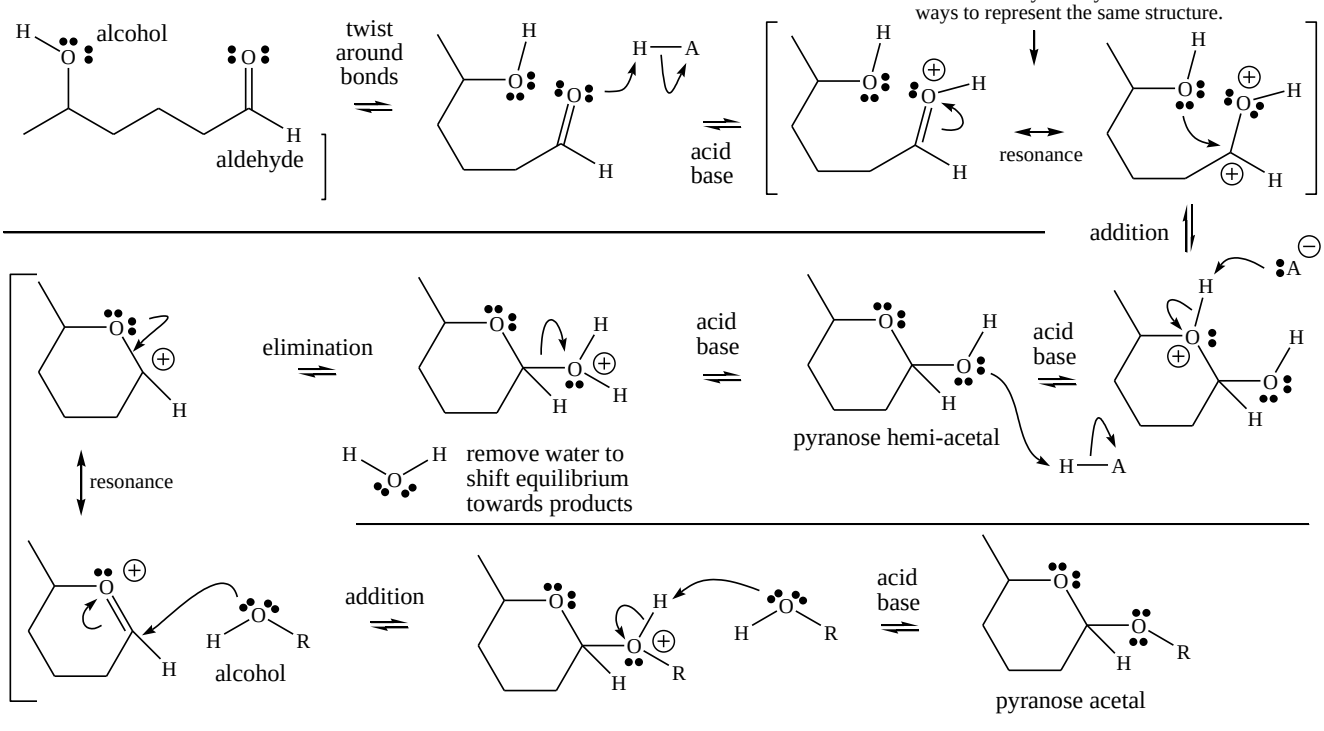
As a more elaborate example, a stripped down aldohexose is shown forming a cyclic hemi-acetal from an aldehyde and an alcohol, which can react with a second alcohol to form an acetal. This is shown two ways below. The first way shows the Bio-Organic game approach using the necessary biomolecules and enzyme acid sites, represented as B^+-H and enzyme base sites represented as B:. Two proton transfers are combined in a single reaction as might be possible in an enzyme catalytic site. The organic approach proposes to use an alcohol, ROH, and acidic conditions, H-A. The organic mechanisms take longer to react because each proton transfer occurs separately and sequentially, The Bio-Organic game approach is faster reacting and faster to write because multiple steps are combined in one, keeping the activation energies lower, yet the key details of the mechanism are retained.




and
...are assumed to be acidic and basic sites on an enzyme. Notice the proton transfers in both steps in addition to the chemical change.

Organic Approach - Acidic Alcohol and Remove Water (It's a lot longer.)

Resonance arrows are not a step in the reaction. They merely show different ways to represent the same structure.



A key goal of this introductory chapter is to show how important proton transfer reactions are in biochemical reactions. Also, proton transfer reactions are a good place to begin because they are just about the

easiest type of chemical reaction we can consider. While proton transfers may be the most common of all biochemistry reactions, they are usually part of the larger transformations that are shown above and in the examples that follow.

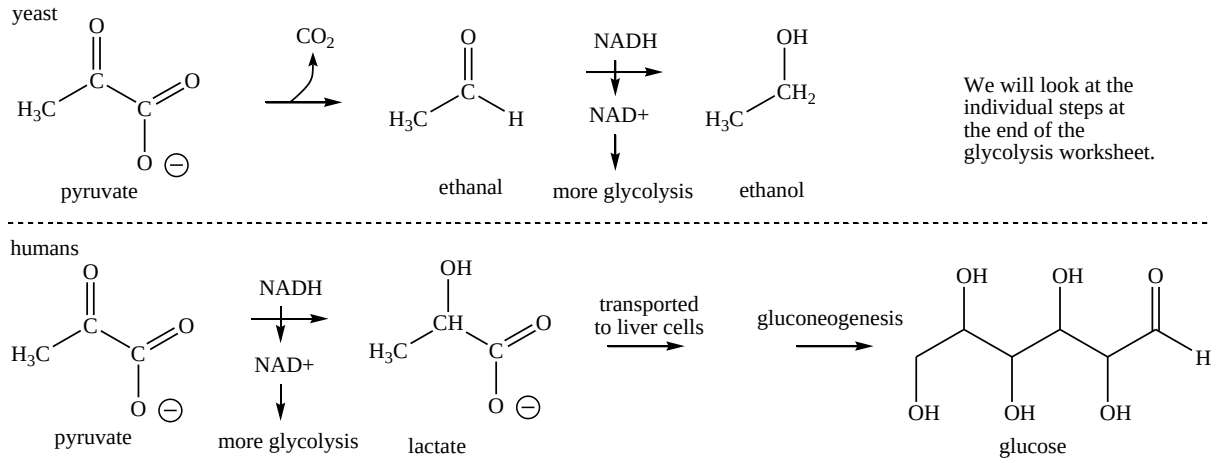
Introduction to Glycolysis

Glycolysis is one of Nature's most important pathways. Glycolysis is part of every living cell and very likely was in the first living cells billions of years ago, leading to life as we know it today. In books, glycolysis is presented as a 10 step pathway, starting with glucose and ending with pyruvate. Some of the proposed pathway steps actually have multiple chemical changes so there are more chemical steps than pathway steps in the scheme below. Some of those presented are my personal speculations. Proton transfer occurs in just about every chemical step of glycolysis, which makes glycolysis an excellent example to illustrate the importance of acid/base chemistry in biochemical pathways.

If you are not interested in this background discussion, you can jump to page 7 where the typical glycolysis pathway is shown, with connections to other pathways and cycles (that are not shown here). A second glycolysis pathway is proposed with numbered step-by-step chemical steps (pages 8-9). An individual mechanism is then proposed for every single numbered chemical step of glycolysis without any curved mechanism arrows on pages 10-19 (so you can fill them in). This is followed by a similar scheme with all of the mechanism arrows included (a key on pages 20-29). Following the glycolysis worksheets (blank reaction templates and the keys), essential biochemical cofactors are shown on pages 31-39 as their full structures and in simplified form as they are used in the Bio-Organic Game. Only the parts of the cofactors necessary to demonstrate their chemistry are used in the mechanism schemes throughout the workbook.

Along the pathway of glycolysis 2 high energy ATP molecules are initially required to get started, while 4 ATP are produced in the second half, for a net gain of 2 ATP per cycle. Also, in the middle of glycolysis, NAD+ is reduced to high energy NADH, while oxidizing glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. However, to keep the pathway running, the NADH has to be oxidized back to NAD+ or else glycolysis would stop. There are various ways to do that after pyruvate has been formed and some are shown at the end of the glycolysis pathway, along with their mechanisms at the very end of the worksheets.

Anaerobic organisms, like yeast, can decarboxylate pyruvate to carbon dioxide and acetaldehyde, which can be reduced with NADH to ethanol and regenerate NAD+ to keep glycolysis running. In our own cells, we can use NADH to reduce pyruvate to lactate and regenerate the necessary NAD+ to keep glycolysis going. Our cells can transport lactate out into the blood where it can be carried to the liver, reabsorbed into liver cells and made back into glucose using gluconeogenesis (also studied later, but not in this handout).



Our red blood cells do not have mitochondria (or a nucleus!) and only live 2-3 months. Neutrophils, in our own immune system, have very few mitochondria, and are the most abundant white blood cells, with about 100 billion being made every day! All of those cells must rely on glycolysis to survive.

In aerobic cells (when O_2 is present), pyruvate or its equivalent can be transported into the mitochondria and made into oxaloacetate or acetyl CoA, which can enter the TCA cycle producing lots of NADH, FADH2 and GTP, which feeds into the electron transport chain (ETC) and can make over 30 ATP equivalents per glucose molecule. The energy driving force to make this happen is the buildup of a proton imbalance between the inner mitochondrial space and the cytosol of the mitochondria. As the excess protons pass through a protein complex in the inner mitochondrial membrane back to the cytosol of the mitochondria, they convert ADP into ATP. The energy for the proton imbalance is created by transferring high energy electrons through a series of biomolecules (NADH, FADH2) that convert oxygen, O_2, into water, H_2O (requiring 4 electrons per oxygen molecule and 4 protons per 2 water molecules).

$$O_2 + 4H^+ + 4e^- \longrightarrow 2\,H_2O + \text{lots of energy}$$

The Tricarboxylic Acid Cycle (TCA cycle) is much more efficient at making high energy molecules that are used to make ATP, but it requires oxygen to keep it going. Early life on earth was anaerobic and did not make oxygen. The original function of the TCA cycle may have been to metabolize various biomolecules. It took, maybe 2 billion years, for cyanobacteria to develop photosynthesis, which could produce oxygen (some argue earlier). In anaerobic organisms today, and even our own cells when we go into oxygen debt, cells mostly use glycolysis to survive. We'll study the TCA cycle after glysolysis (but not in this handout).

Glycolysis is connected at many points along the way to other biochemical cycles and pathways. It is in equilibrium with glycogen (a glucose polymer), which releases essential glucose when glucose levels are low or stores glucose in glycogen when glucose levels are high. Glycolysis is also in equilibrium with the pentose phosphate pathway (PPP), which supplies various metabolic intermediates, such as ribose used to make RNA and DNA, erythrose used in the shikimic acid pathway to make aromatic rings and lots of NADPH, which helps in anabolic metabolism and helps protect against damaging free radical chemistry (studied later, but not in this handout).

Glycolysis also branches off to glycerol, used in making mono, di and triglycerides or fats. Many amino acids feed into glycolysis or are made from glycolysis intermediates (studied later). Biochemistry is a very complicated subject and even a year of study cannot teach you everything there is to know (and new things are constantly being discovered). For right now, our main goal is to understand how simple proton transfer reactions are used in the grand scheme of things, in combination with several steps in a biochemical cycle.

To work through the full pathway of glycolysis we need to introduce several other biomolecules and co-factors. The full list of co-factors is shown on page 31-39 of this handout, along with a simplified version of each one, showing just enough to illustrate the chemistry. We don't need all of them to study glycolysis, but we will use all of them at some point in the other pathways and cycles discussed later (but not in this handout).

Why Study Glucose?

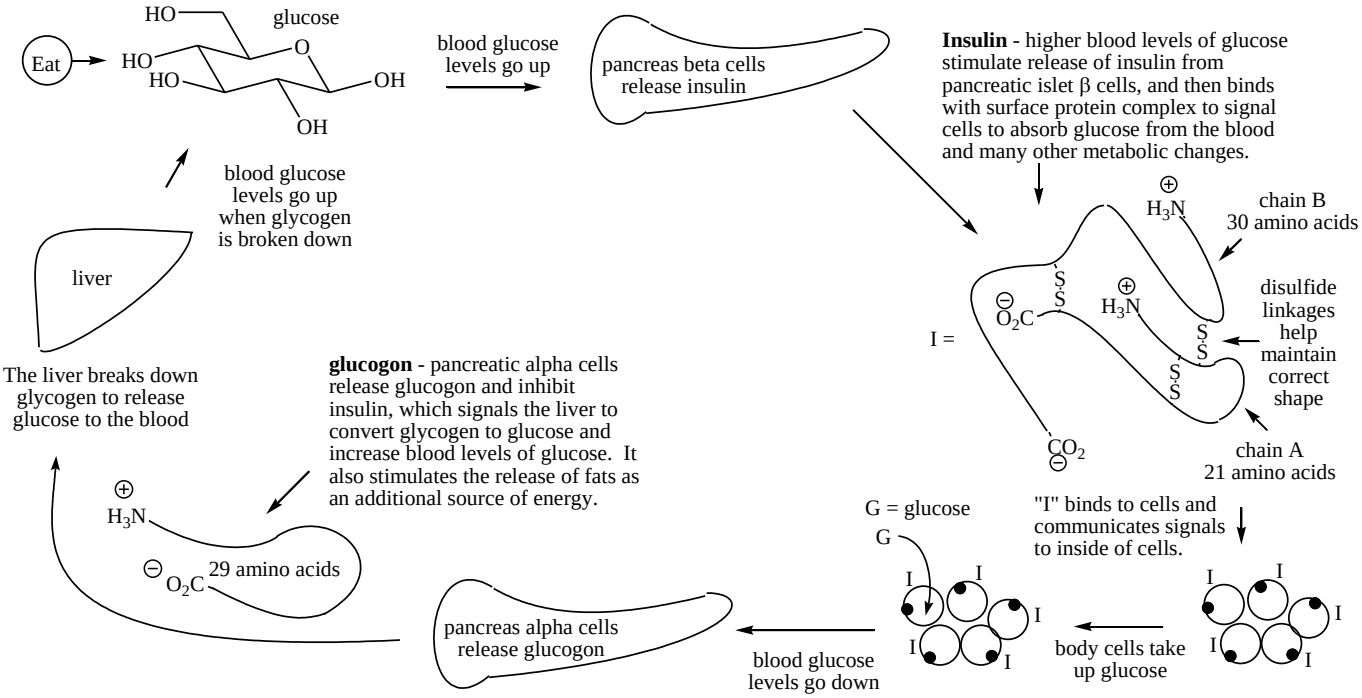
Glucose primes the glycolysis pathway and helps feed our brains. It is one of the central molecules of life. When blood glucose levels are high, pancreatic islet beta cells release insulin. Insulin is considered to be the main anabolic protein hormone of the body and regulates the metabolism of carbohydrates, fats and protein by binding to a membrane protein complex on the outside of the cell. This initiates a series of protein phosphorylations inside the cell that can continue all the way to DNA in the nucleus, turning on (or off) various genes that produce some of the necessary chemicals that promote the absorption and metabolism of glucose in liver, skeletal and fat cells.

Inside a cell, glucose can be converted to glucose-6-phosphate, which makes it ionic and prevents transport back out through the cell's hydrophobic membrane. This is the first step of glycolysis. Glucose-6-phosphate is metabolized to pyruvate in the glycolysis pathway when energy is needed, or made into glycogen or fats (both are possible in liver cells) when there is adequate energy available. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues.

Low insulin levels in the blood promote widespread catabolism (breakdown). Neighboring pancreatic alpha cells secrete glucagon into the blood when blood glucose is low, and decrease secretion when glucose concentration is high. Glucagon, stimulates the liver to release glucose into the blood by glycogenolysis

(breakdown of glycogen to glucose) and has the opposite effect of insulin. The secretion of insulin and glucagon into the blood in response to the blood glucose concentration is the primary mechanism of glucose homeostasis (balance). Enzymes are required for most reactions that occur in the body and their names end in "-ase", but are not emphasized in our discussions, except as simplified acids and bases.

Biochemical balance of insulin and glucogon



Glucose Disease – Diabetes

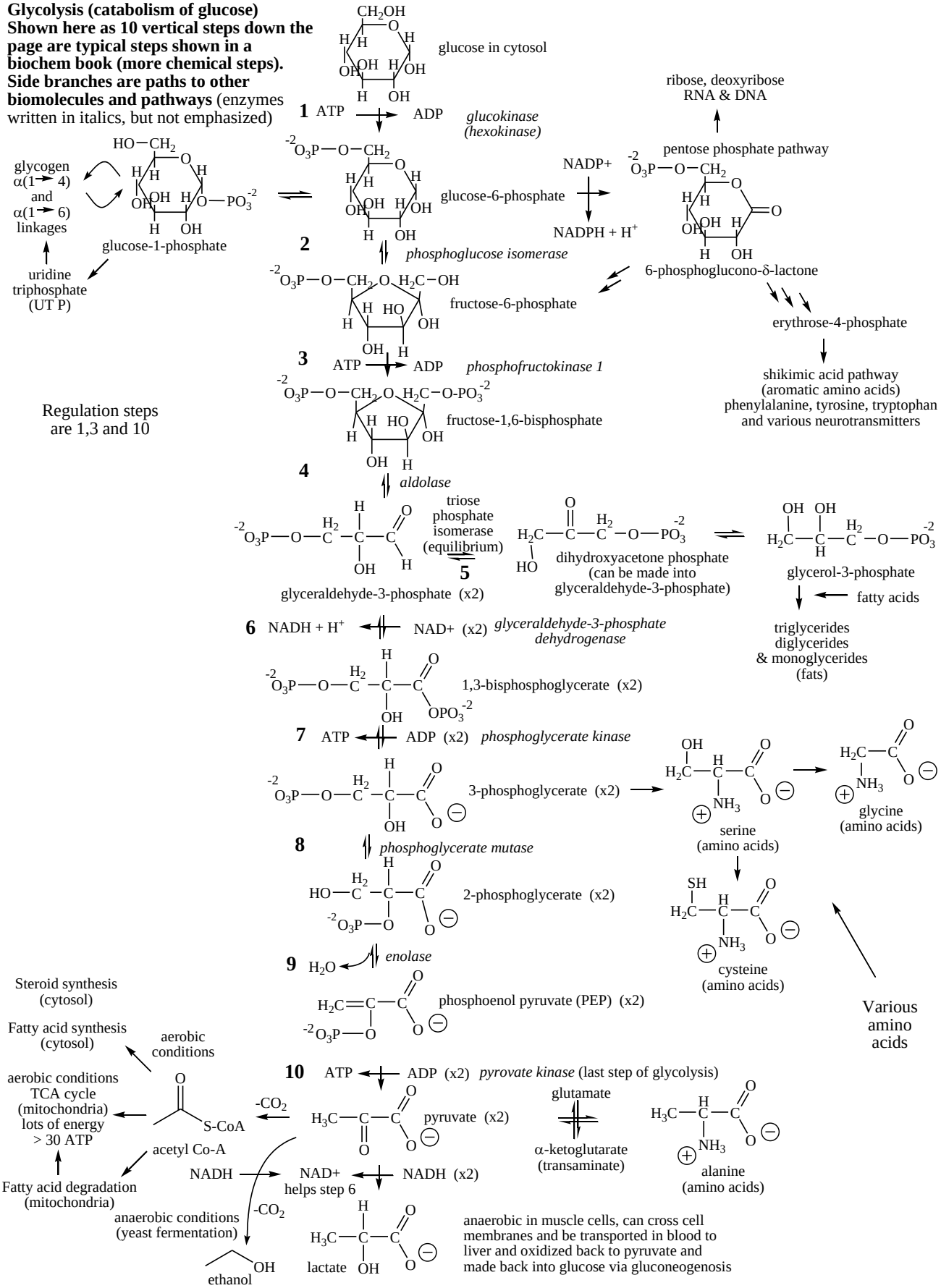
Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms include frequent urination, increased thirst, and increased hunger. Diabetes can cause many complications, such as cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the eyes and even death. About 9% of adults will get diabetes and rates seem to be increasing. Worldwide, about 500 million people have diabetes. Before the discovery of insulin (1922) people commonly died of diabetes. Treatment includes lifestyle changes in diet and exercise to maintain proper body weight and daily injections of biosynthetic human insulin using recombinant DNA methods.

Type 1 Diabetes Mellitus (juvenile diabetes or insulin dependent diabetes) results from the pancreas's failure to produce enough insulin. Type 1 represents about 10% of diabetes cases.

Type 2 Diabetes Mellitus (adult onset diabetes) begins with insulin resistance, a condition in which cells fail to respond to insulin properly (may be an autoimmune disease). As the disease progresses a lack of insulin may also develop. There is a strong genetic component, but contributing causes are thought to be excessive body weight and insufficient exercise. Type 2 represents about 90% of diabetes cases. Worldwide, about 5 million people die each year of diabetes and related complications.

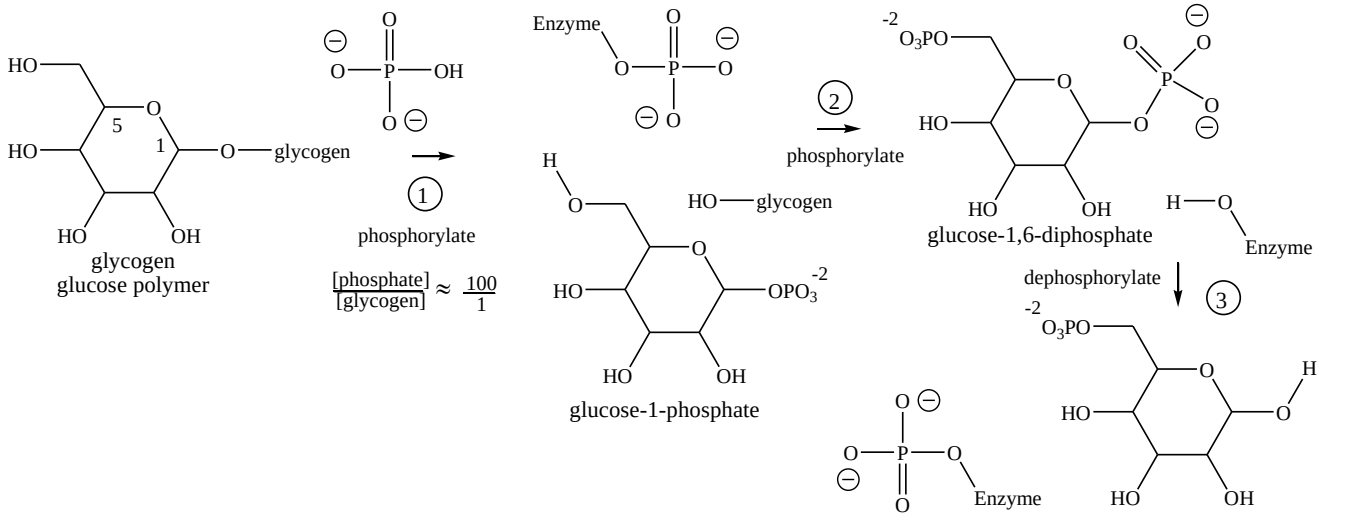
Gestational diabetes is a condition in which a woman without diabetes develops high blood sugar levels during pregnancy. Gestational diabetes increases the risk of pre-eclampsia, depression, and the necessity of a Caesarean section. Babies born to mothers with poorly treated gestational diabetes are at increased risk of being too large, having low blood sugar after birth, and jaundice. If untreated, it can also result in a stillbirth. It is most common in the last 3 months of pregnancy. About 3-9% of women will get it, but it goes away in about 90% of women after pregnancy.

Glycolysis (catabolism of glucose) Shown here as 10 vertical steps down the page are typical steps shown in a biochem book (more chemical steps). Side branches are paths to other biomolecules and pathways (enzymes written in italics, but not emphasized)



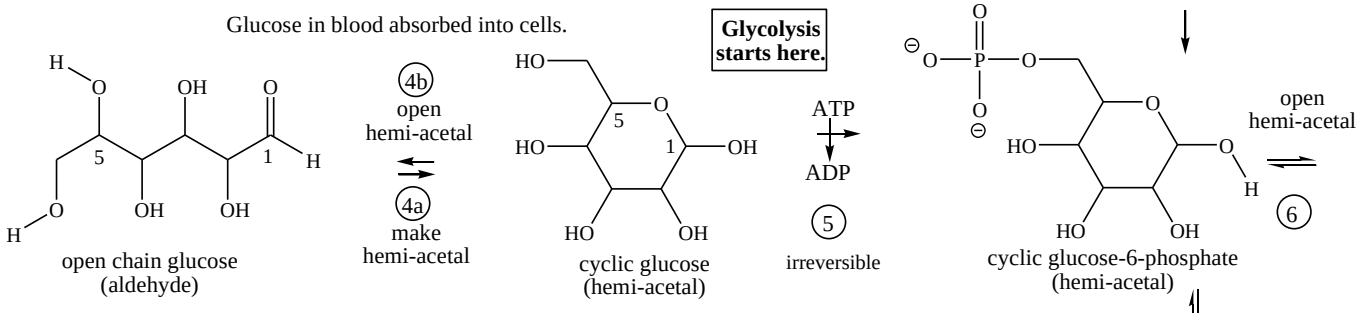
Overview of proposed "chemical" steps (2 pages), Each numbered step is presented separately below.

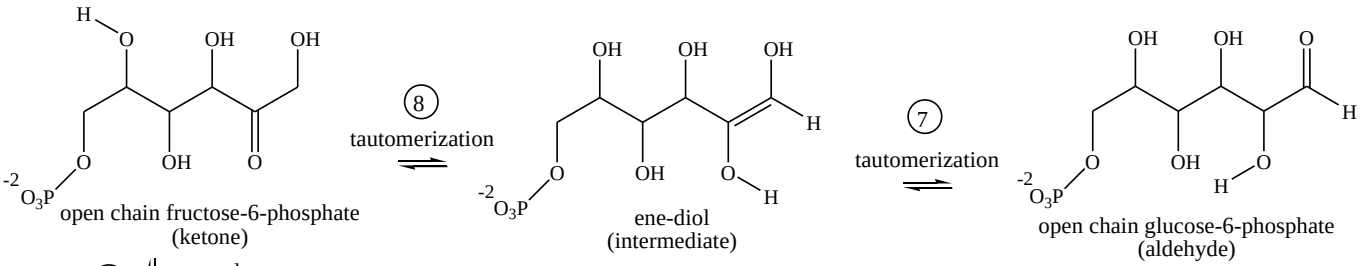
Glycolysis - step-by-step - Glucose in the blood can enter cells through various GLUT transporter proteins. It is immediately phosphorylated to glucose-6-phosphate to prevent it from exiting the cell and begin glycolysis. Also, glucose-1-phosphate can get clipped off of glycogen and converted into glucose-6-phosphate to enter glycolysis when blood glucose levels are low. Each number below represents a mechanism step without the details, for you to fill in the following scheme. Possible answers for each of those steps are provided in the next section.

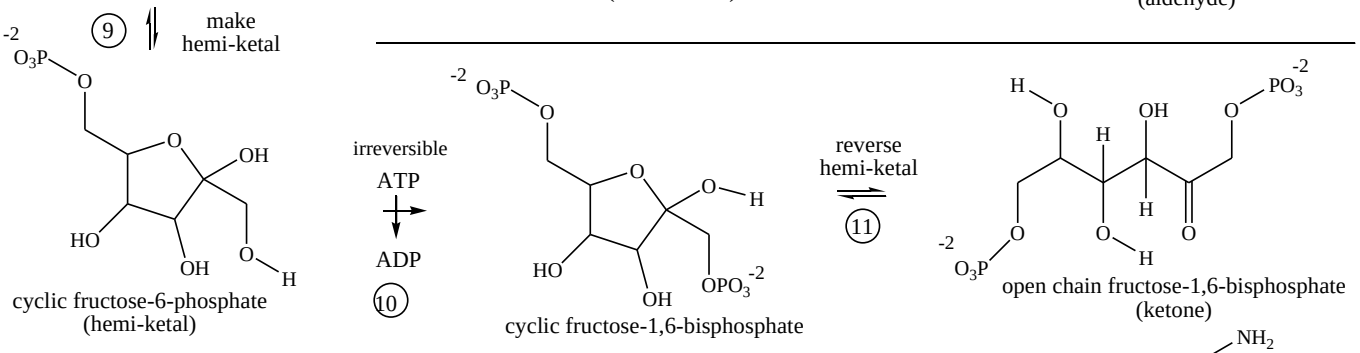


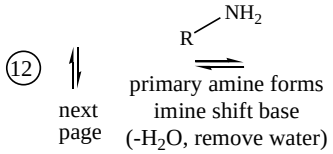
Glucose in blood absorbed into cells.

Glycolysis starts here.

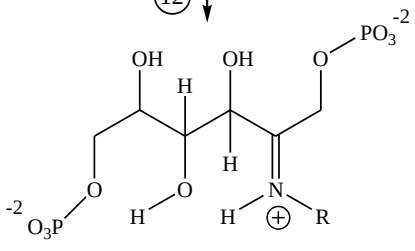




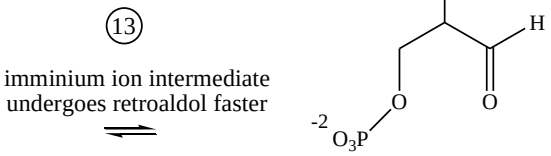




previous page

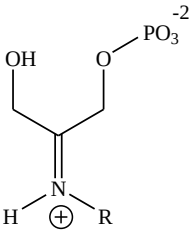


12

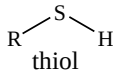


13 imminium ion intermediate undergoes retroaldol faster

glyceraldehyde-3-phosphate (aldehyde)



imminium ion of dihydroxyacetone phosphate



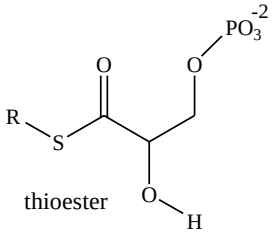
thiol
2x per glucose

16 make hemi-thioacetal

15a

15b via ene-diol tautomers

14 H_2O



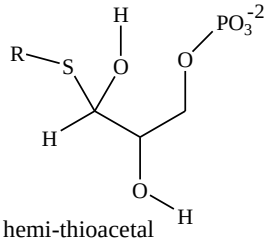
thioester

(x2)

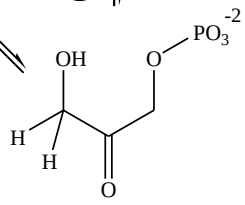
NAD+

NADH

17

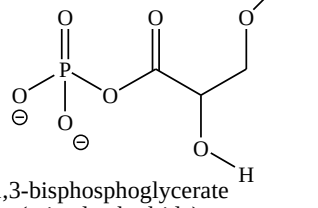


hemi-thioacetal



dihydroxyacetone phosphate (ketone)

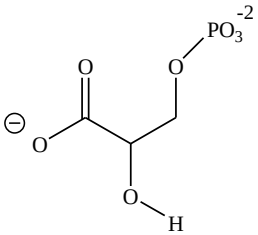
(x2) make mixed anhydride 18



1,3-bisphosphoglycerate (mixed anhydride)

19 irreversible
2x per glucose
ADP

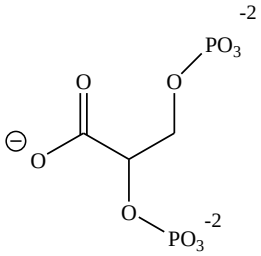
make ATP



3-phosphoglycerate

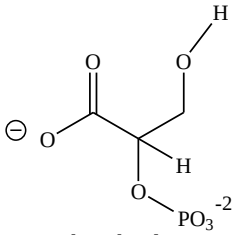
(x2) imidazole, phosphate on

20



2,3-bisphosphoglycerate

21 (x2) imidazole, phosphate off



2-phosphoglycerate

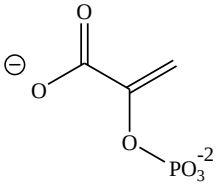
22

H_2O

elimination of water (Mg^{+2} helps)
(x2)

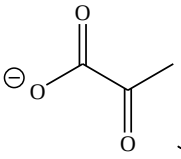
23 (x2) ADP

make ATP



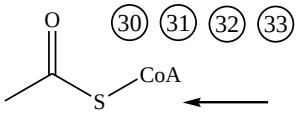
phosphoenol pyruvate (PEP)

glycolysis stops here



pyruvate

30 31 32 33



acetyl CoA

more chemistry

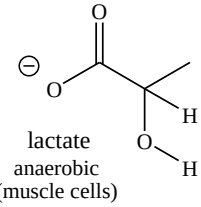
aerobic

1. Used to make ATP in Tricarboxylic Acid Cycle (TCA) in mitochondria

2. Used to make fatty acids, that make diglycerides in cell membranes and triglycerides stored as fat

3. Used to make the body's steroids and bile acids

NADH 24a 24b NAD+

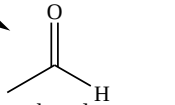


lactate
anaerobic (muscle cells)

more chemistry

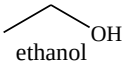
anaerobic (yeast)

25 26 27 28 29



ethanal

NADH → NAD+



ethanol

34 make alanine from pyruvate

35 phosphorylate carbonate with ATP

36 add CO2 to biotin

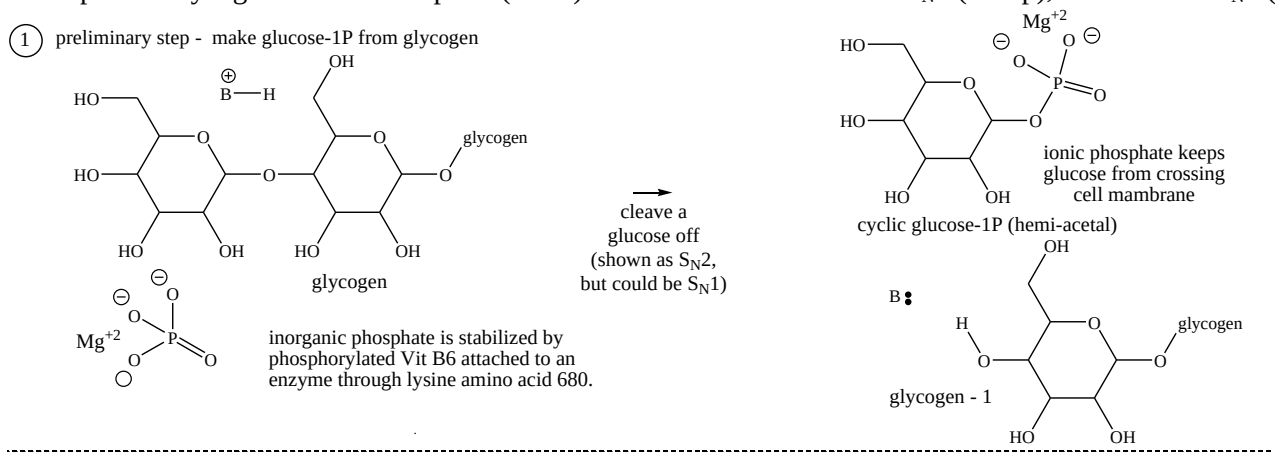
37 add CO2 to pyruvate to make oxaloacetate used in TCA cycle

38 decarboxylate oxaloacetate to make phosphoenol pyruvate (PEP) used in gluconeogenesis

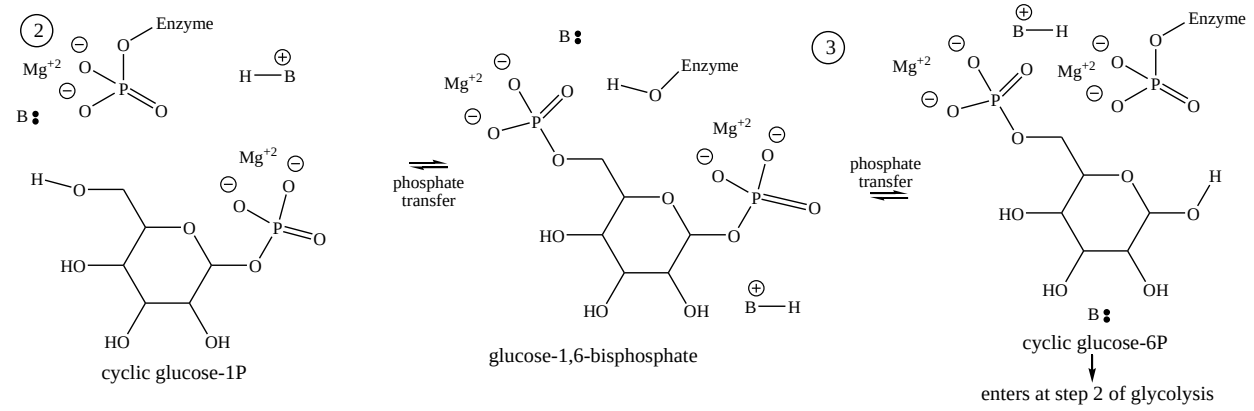
Shown below are my proposed arrow pushing mechanisms for glycolysis chemical steps and a few other biomolecules at the beginning and end of glycolysis. Some of my proposed steps are "speculative" since not every chemical step is shown in a biochemistry book. You supply the arrow pushing details for each step here and possible answers are provided in the following section.

Possible arrow pushing mechanisms for glycolysis steps shown above inside a cell, and a few other biomolecules at the end of glycolysis. Arrow pushing details for each step are presented here.

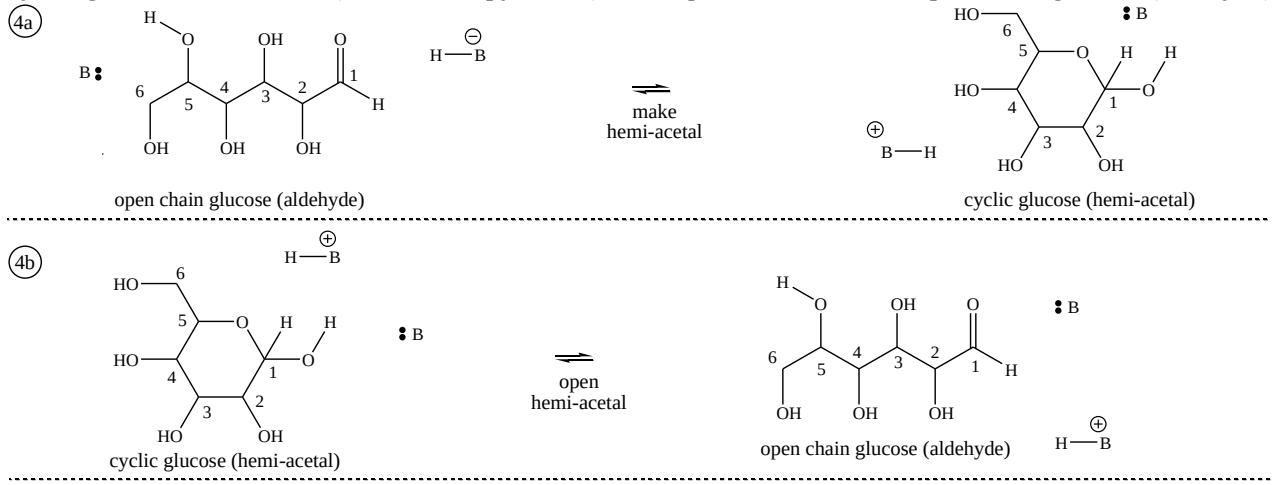
Cleavage of a glucose molecule from the end of a glycogen polymer of glucose, using an inorganic phosphate as a nucleophile. Glycogen is the electrophile (at C1). The reaction is shown as S_N2 (1 step), but could be S_N1 (2 steps).



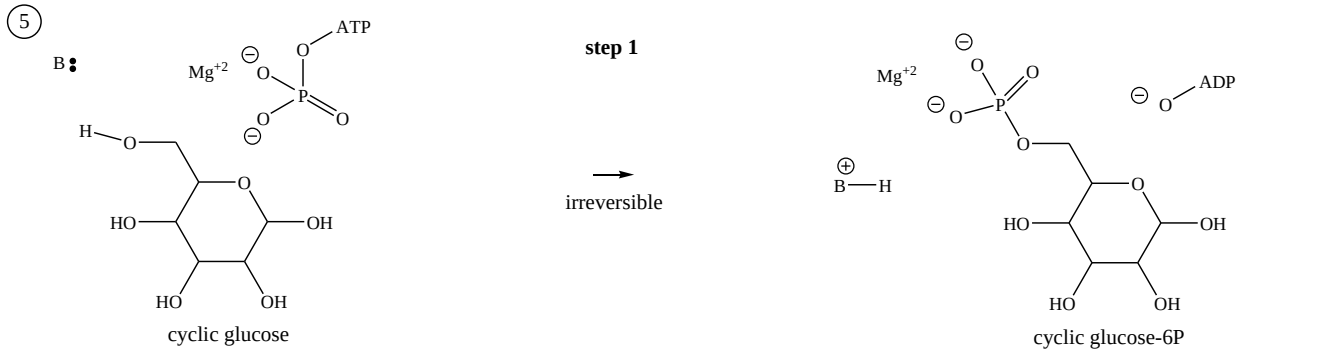
Glocose-1-phosphate is converted to glucose-6-phosphate. An enzyme phosphate phosphorylates C6 and then dephosphorylates C1, ready to do it again. Enters in the second step of glycolysis.



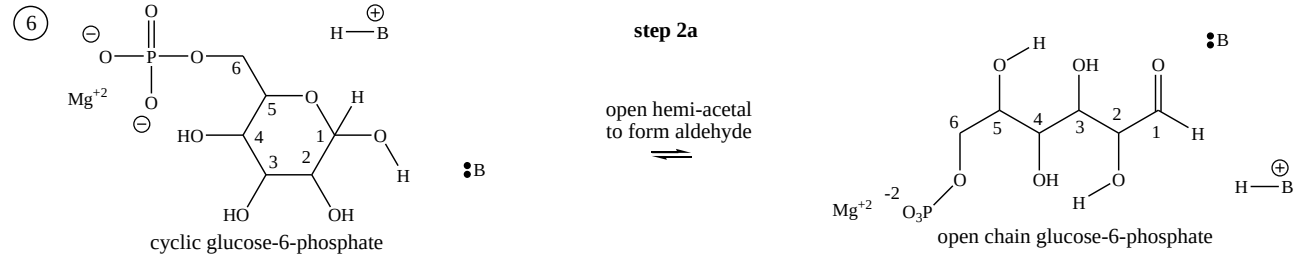
Cyclic glucose inside a cell (hemi-acetal pyranose) is in equilibrium with the open chain glucose (aldehyde).



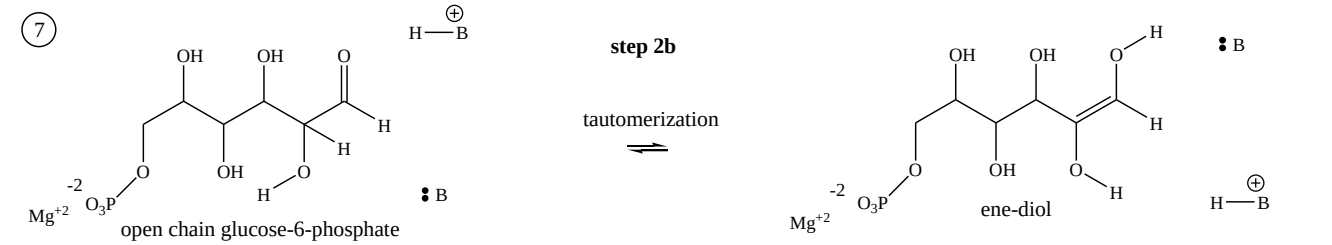
Glucose phosphorylates to glucose-6-phosphate, which prevents it from leaving the cell, and begins glycolysis.



Cyclic glucose (a pyranose) has to be opened to an aldehyde so that tautomerizetion can occur twice to form fructose (a furanose).

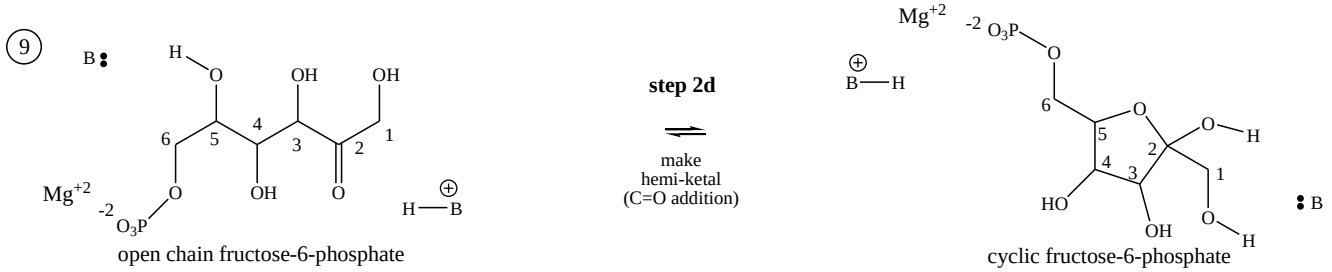


Two tautomerization reactions convert glucose-6-phosphate, an aldehyde, to fructose-6-phosphate, a ketone. The first tautomerization makes an ene-diol.

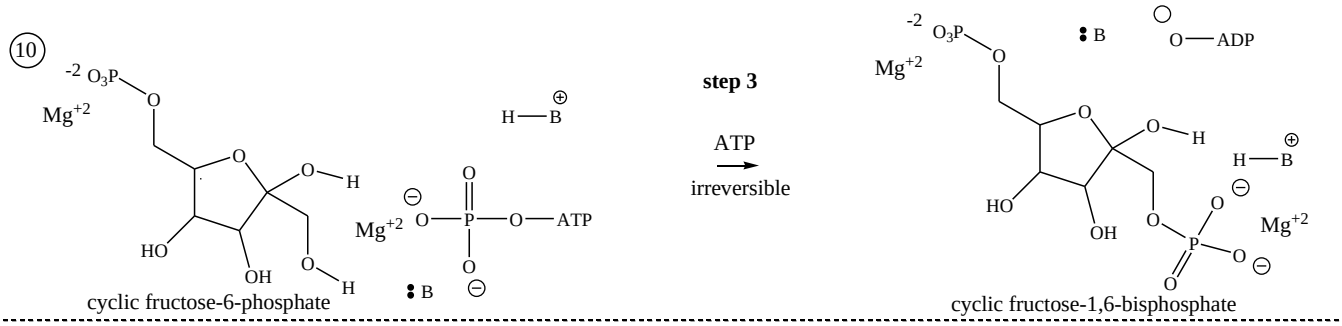


Second tautomer reaction makes fructose-6-phosphate, a ketone, from the ene-diol

Fructose-6-phosphate cyclizes to a hemi-ketal, a 5 atom furanose ring

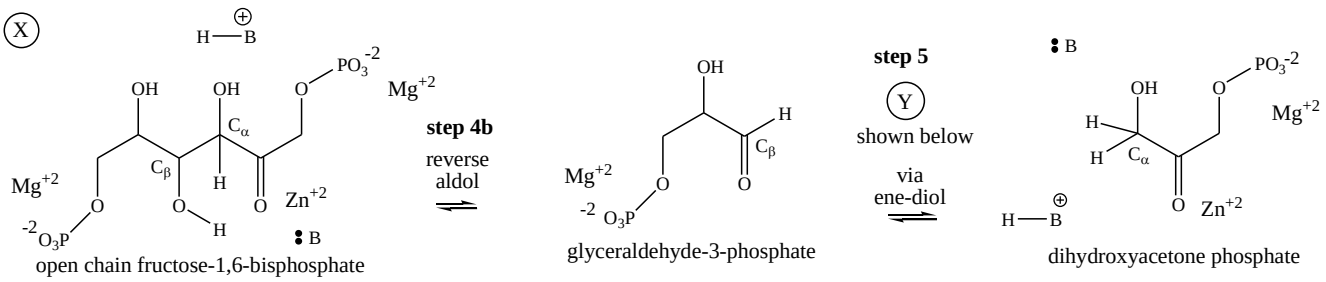


Fructose-6-phosphate phosphorylates a second time to form fructose-1,6-bisphosphate



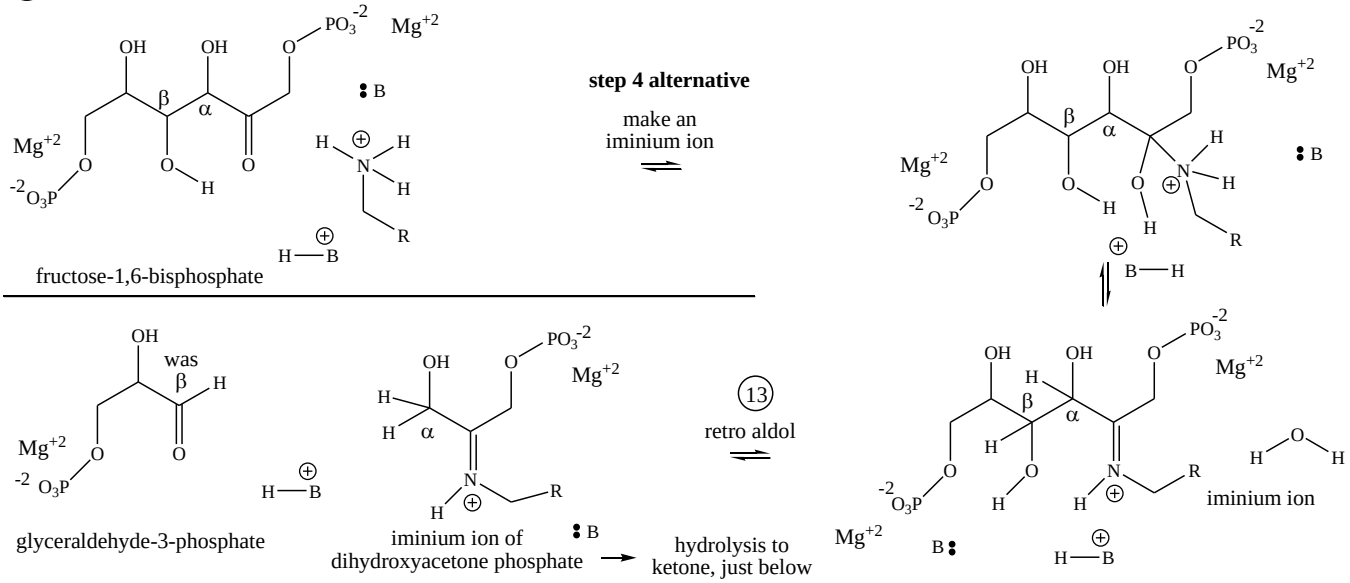
Cyclic fructose-1,6-bisphosphate, a hemi-ketal, opens (helped by charge repulsion?) to open chain fructose-1,6-bisphosphate, a ketone

The carbonyl group (C=O) and beta OH allow for a retro aldol reaction to cleave the 6C chain between C_α and C_β into two 3C chains. This is an abbreviated transformation that is much simpler to write, and possibly occurs when a Zn^{+2} ion complexes with the carbonyl group (C=O). A more involved transformation using a primary amine with the ketone to make an imine (also called a Schiff base) is shown just below, but it takes a lot more writing to show (reactions 10a and 10b). Even though it is slower for us to write, it is probably faster in the actual reaction since the Schiff base can be protonated to make a better C_α leaving group.

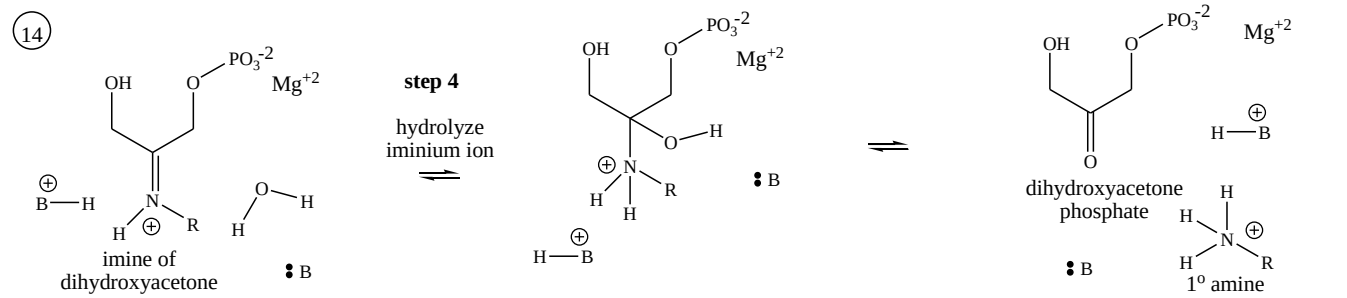


In a more likely mechanism, an imine is formed, combining the C=O with a primary amine and removing water. The imine is likely protonated at body pH. A protonated imine ($C=N^{+}HR$) is a better electron pair acceptor than the neutral carbonyl group (C=O) and possibly allows faster cleavage of the C_{α}-C_{β} bond in the retro aldol reaction. The imine would then have to be hydrolyzed (add water) to regenerate the carbonyl group. Because dihydroxy acetone and glyceraldehydes can easily interconvert via tautomerization (similar to glucose / fructose), this is equivalent to producing two glyceraldehydes molecules that can be carried forward in glycolysis.

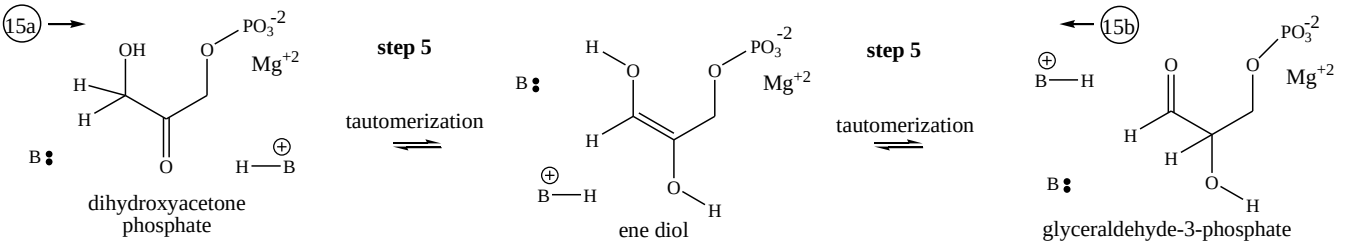
(12) imine alternative to keto retroaldol (actually protonated iminium ion)



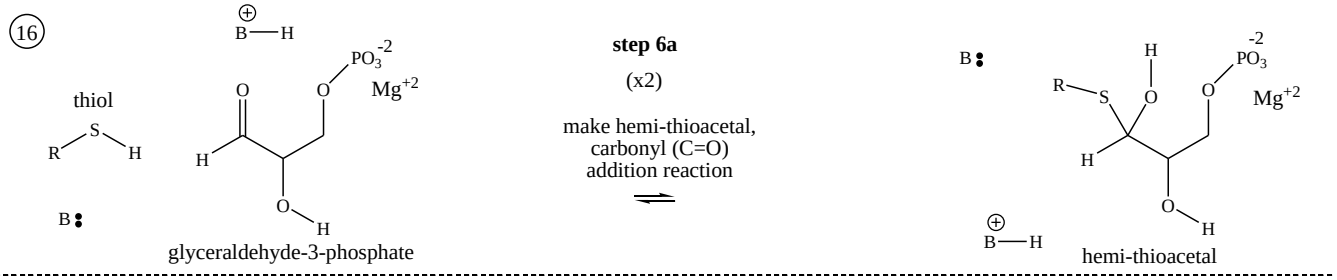
Hydrolyze iminium ion back to ketone.



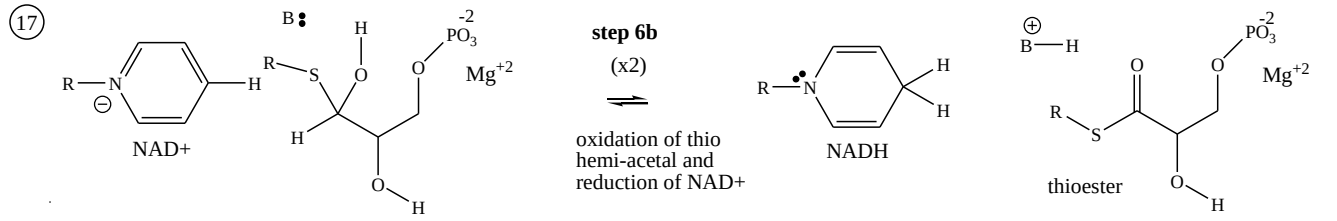
Two tautomerization reactions convert either 3C component into the other via an ene-diol. Similar to interconversions of glucose and fructose in steps 4 and 5 above. Because these 2 compounds can interconvert, it means that all 6 carbons of glucose can be carried forward in glycolysis as glyceraldehydes-3-phosphate, so all the reactions that follow can be multiplied by 2. It is also possible that dihydroxyacetone phosphate can be diverted off towards other biomolecules, such as glycerol used in fatty acid synthesis. It is also possible that glycerol can be converted into dihydroxyacetone phosphate and form glyceraldehydes-3-phosphate, which continues on in glycolysis to pyruvate.



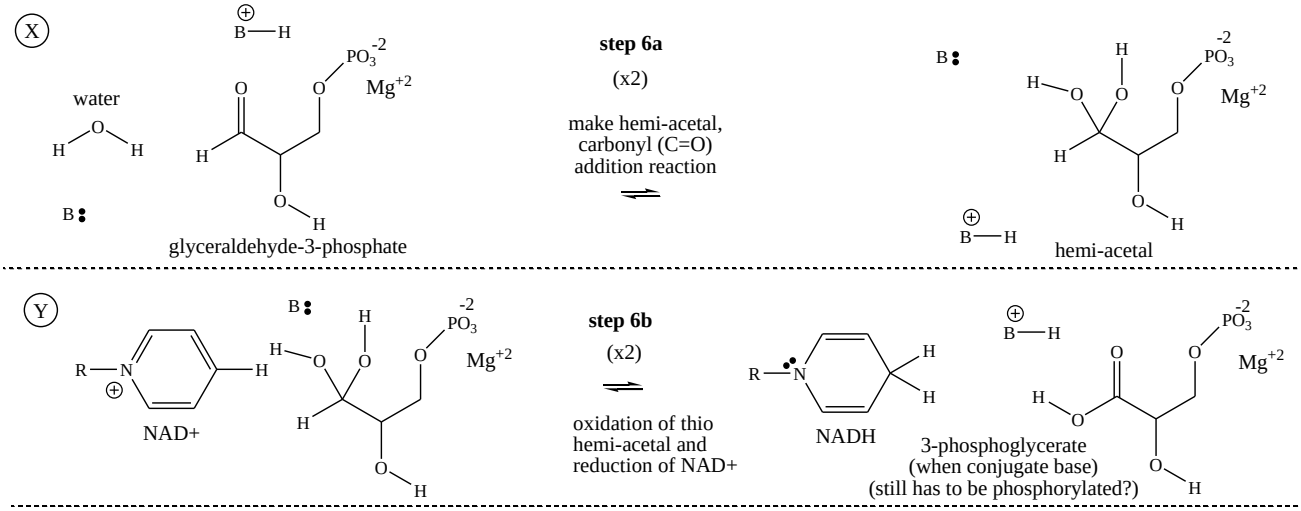
It wasn't real clear to me what form glyceraldehye-3-phosphate gets oxidized by NAD+. So I'm proposing 2 possibilities that seem reasonable to me. In the first, a thiohemi-acetal is formed, which allows for oxidation with NAD+ to a highly reactive thioester in the next step. NAD+ is formed in either mechanism.



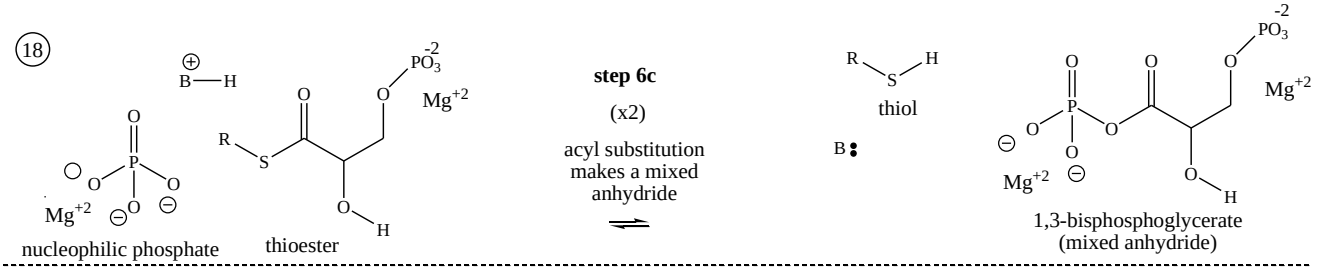
NAD+ accepts a hydride (reduced) to form NADH and a highly reactive thioester used in the next step to make ATP.



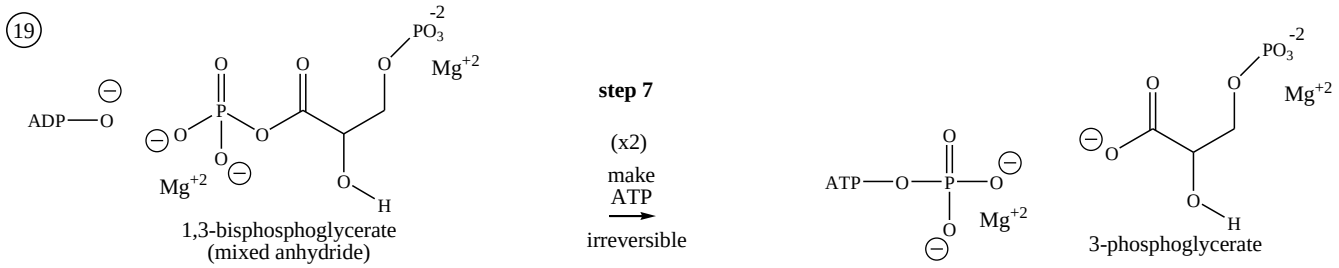
The second possibility is a carbonyl hydrate forms and gets oxidized by NAD+. That would require a very high energy phosphorylating agent (ATP?) to form the 1,3-bisphosphoglycerate and that does not seem to be occurring. So I favor the first mechanism.



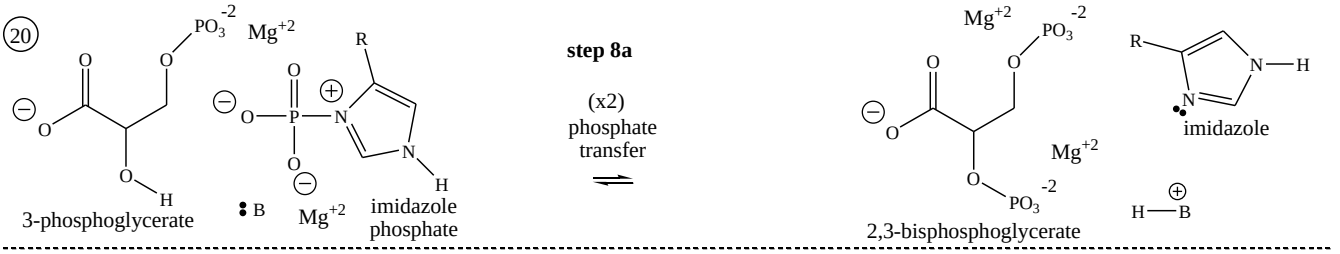
Inorganic phosphate does an acyl substitution reaction with the highly reactive thioester to form a highly reactive mixed anhydride that allows formation of a high energy ATP molecule (an inorganic anhydride) in the next step. The good thiol leaving group helps make this possible. Acyl substitution is really 2 steps: addition and elimination.

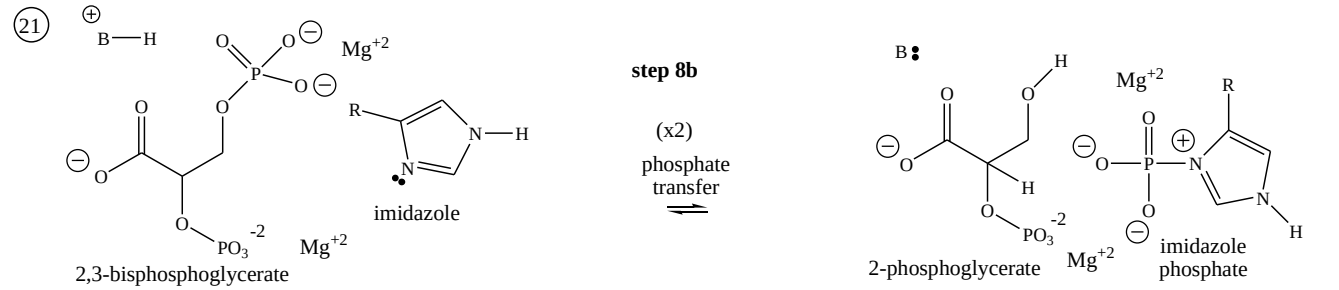


An ADP molecule adds a phosphate from the highly reactive mixed anhydride to form ATP (2x from glucose).

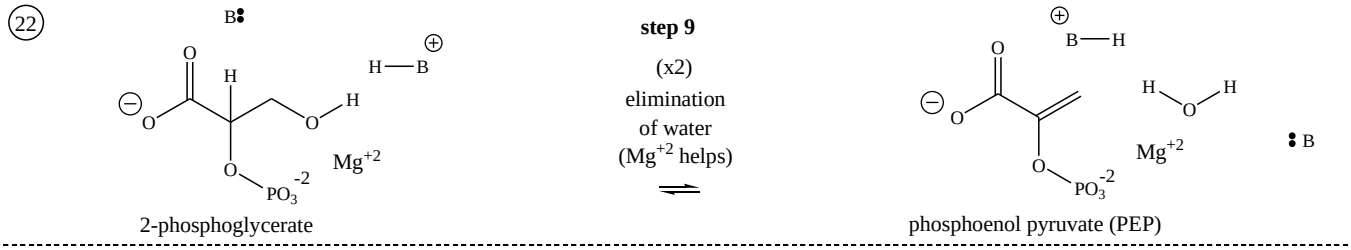


In 2 steps, an imidazole phosphate adds a phosphate at the 2-OH, forming the bisphospho-2,3-glycerate, and then removes the 3-phosphate. This probably makes the phosphate more reactive as it moves the anionic phosphate closer to the anionic carboxylate.

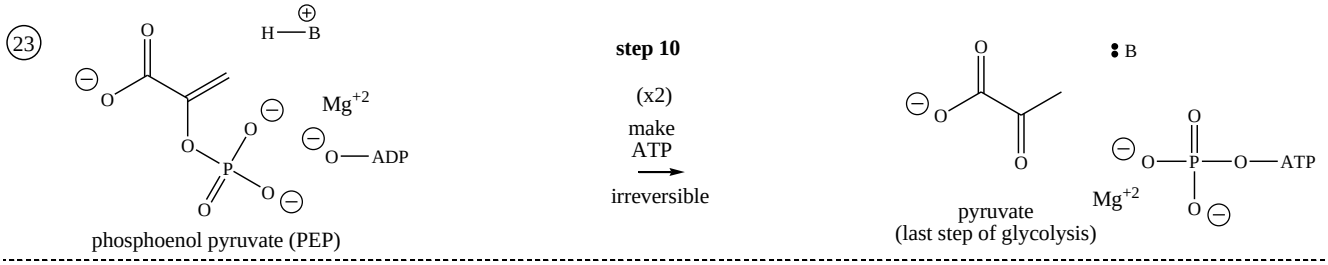




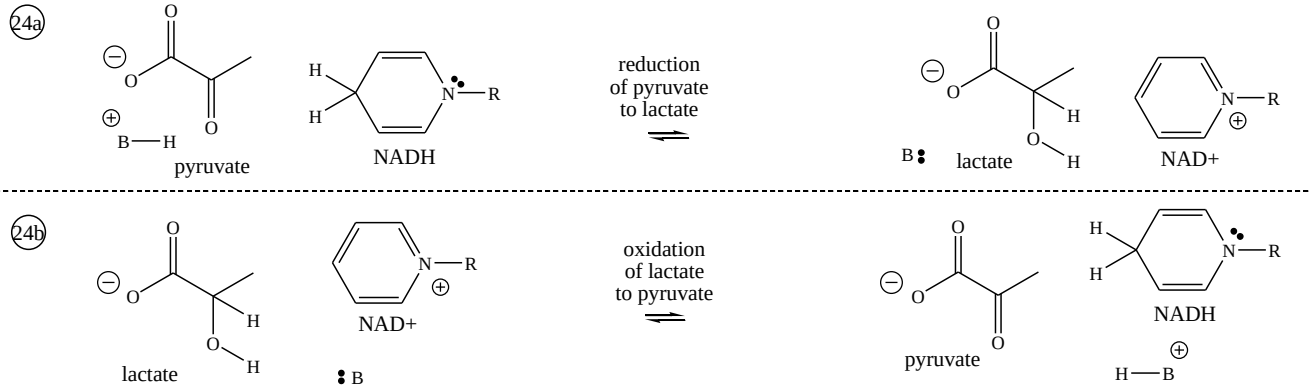
Dehydration forms a highly reactive phosphoenol pyruvate (PEP), which has enough energy to form ATP. Mg+2 is thought to make the HO into a better leaving group (shown as water leaving group in the mechanism below).



An ADP molecule adds a phosphate from the very high energy phosphoenol pyruvate (PEP) to form ATP and pyruvate, which represents the end of the line for glycolysis. In the presence of oxygen pyruvate can migrate to a mitochrondiron, make oxaloacetate and enter the TCA cycle to make NADH and FADH2, used to make large amounts of ATP using the electron transport chain (ETC = electron transport chain = respiration). In low oxygen conditions, pyruvate and NADH can be converted to lactate and NAD+, and the lactate exported outside the cell and carried to the liver to be reoxidized to pyruvate and made back into glucose (gluconeogenesis), or in yeast, in the absence of oxygen, pyruvate can be decarboxylated (-CO_2) to make ethanal, then ethanol and NAD+, via fermentation. Under the low oxygen conditions, the oxidation of NADH makes more NAD+ which is needed to oxidize glyceraldehydes-3-phosphate to keep the glycolysis running.

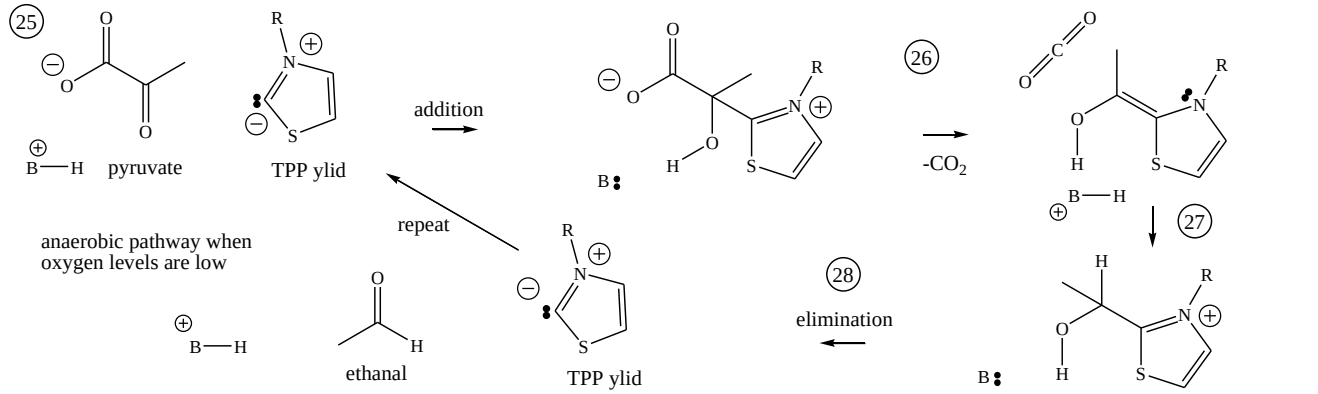


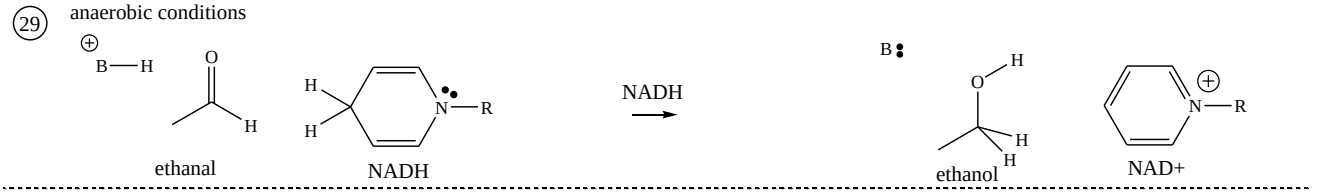
Make lactate from pyruvate under anaerobic conditions. Make pyruvate from lactate in liver cell for gluconeogenesis.



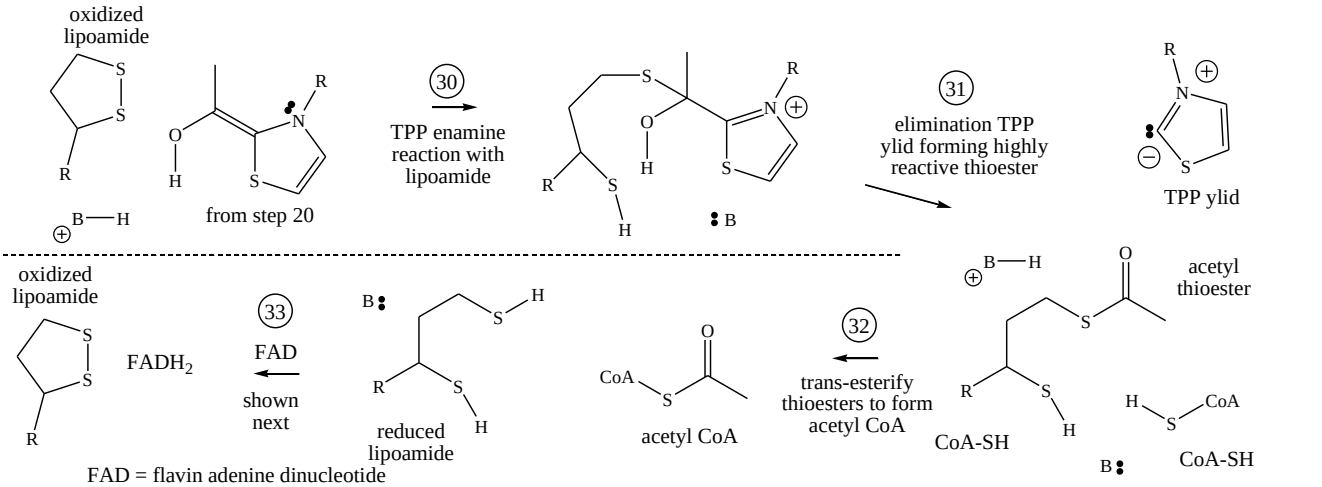
Pyruvate can also be made into acetyl CoA, a very central and important chemical in all living organisms. Acetyl CoA is used in the Tricarboxylic Acid Cycle (TCA cycle = Kreb's cycle) to make citrate from oxaloacetate. Citrate continues through the TCA cycle, making NADH and $FADH_2$ that are used to make about 90% of the body's ATP in the Electron Transport Chain (ETC) at the inner mitochondrial membrane. Acetyl CoA is also a building block to make fatty acid chains in the cytosol, which are used in diglycerides present in cell membranes and triglycerides used in fat storage. Also, acetyl is the starting point for synthesis of all of the body's steroids and bile acids. The steps to make acetyl CoA from pyruvate are shown below. An alternate ending path in anaerobic yeast is to make ethanol (also shown below). Pyruvate can also make alanine, an amino acid and oxaloacetate, also shown below.

Reaction of pyruvate with TPP ylid (thyamine pyrophosphate = tyamine diphosphate = vitamin B1), which decarboxylates ($-CO_2$) to make an enamine-like intermediate that can protonate and eliminate the TPP ylid and ethanal. Ethanal can be reduced by NADH to form ethanol and NAD+ to keep glycolysis going.

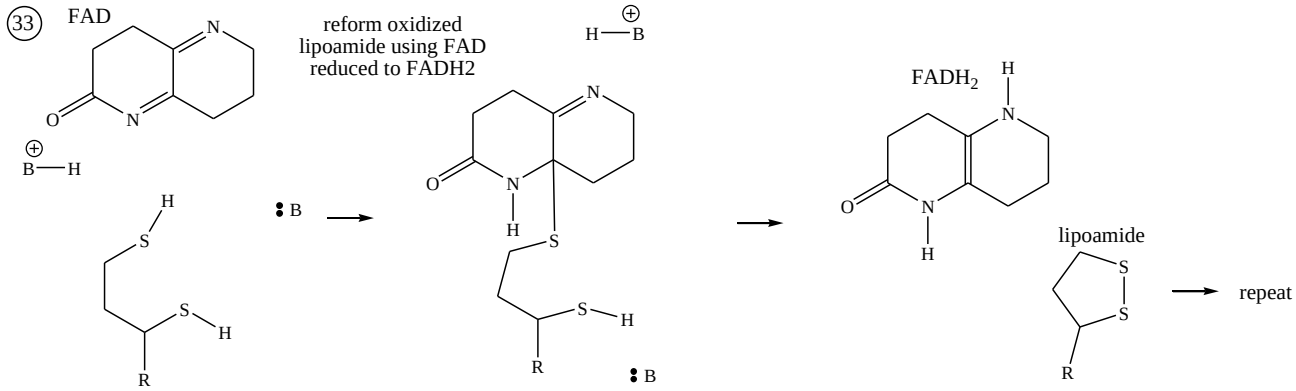




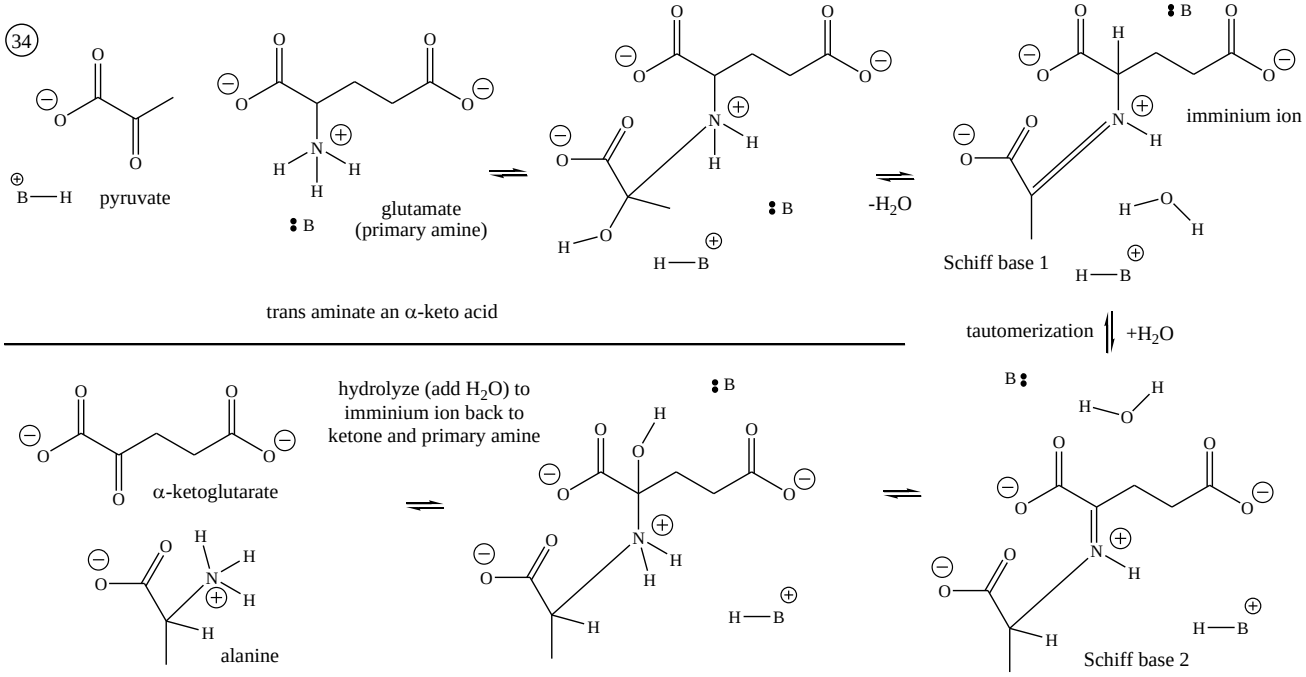
Alternatively, the TPP intermediate can react with lipoamide and then eliminate to form a thioester. The thioester can trans-thioesterify with CoA-SH to form acetyl CoA which is central to many biological pathways, a fuel for the TCA cycle (generates ATP), and a building block for fatty acids and steroid synthesis.



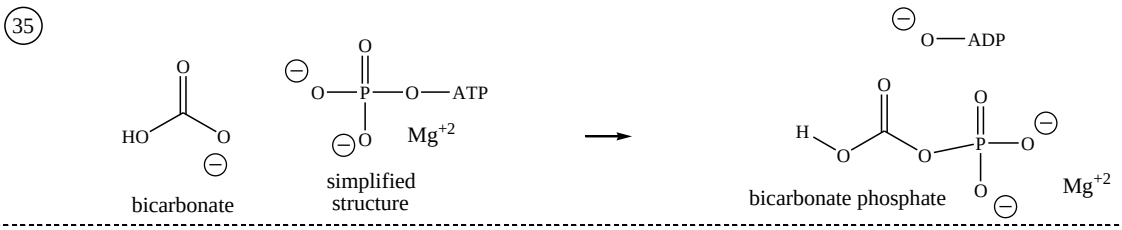
Regenerating Lipoamide using FAD (flavin adenine dinucleotide) This is necessary to continue making acetyl CoA.



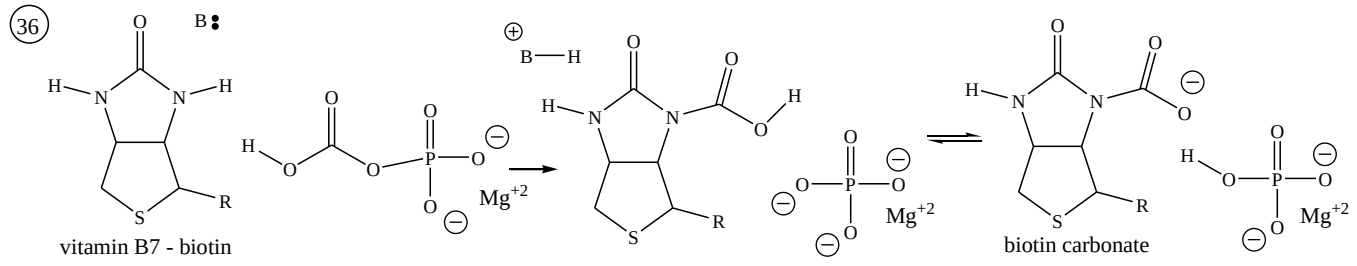
Pyruvate (α-ketoacid) can make the amino acid alanine by transamination with amino acid glutamate into α-ketoglutarate. It involves formation of a Schiff base by condensation (removal of water) of an α-ketone and a primary amine. Tautomerization switches a proton and the pi bond of the Schiff base to the other nitrogen and then hydration (addition of water) reforms a different primary amine (alanine) and α-ketone (α-ketoglutarate).



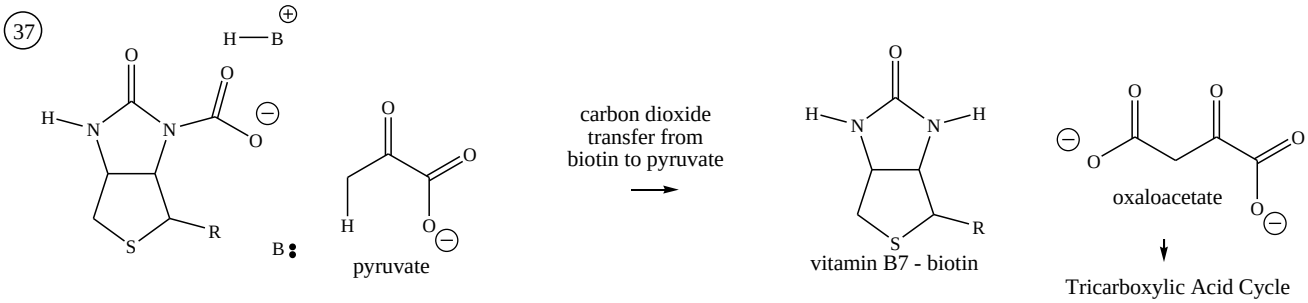
ATP can phosphorylate bicarbonate to make a mixed anhydride with a good phosphate leaving group to add CO_2 to biotin.



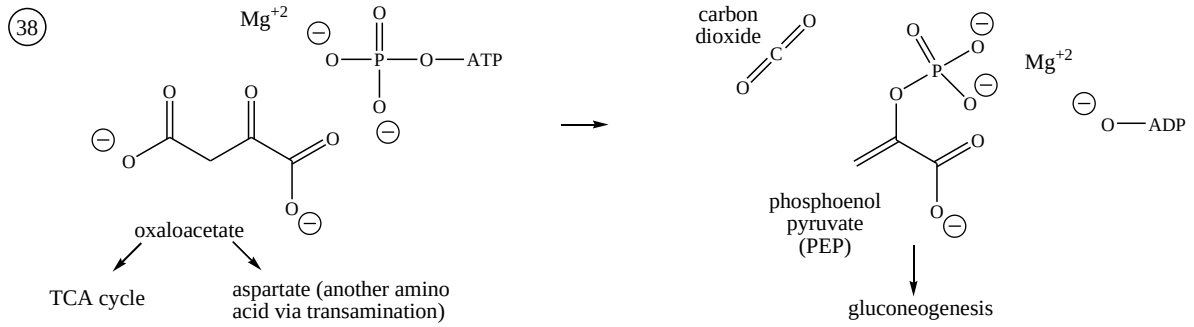
Biotin can make biotin-CO_2 which carboxylates other biomolecules.



Biotin-CO_2 can carboxylate pyruvate to make oxaloacetate used in TCA cycle and make PEP for gluconeogenesis.

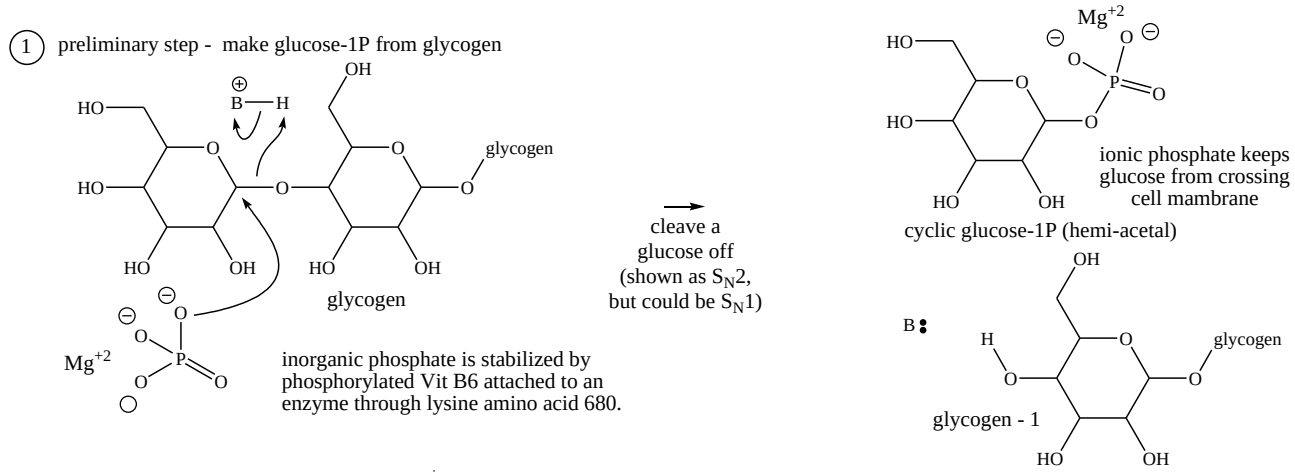


Oxaloacetate, begins the Tricarboxylic Acid Cycle (Kreb's cycle) and can also make phosphoenol pyruvate, which starts gluconeogenesis

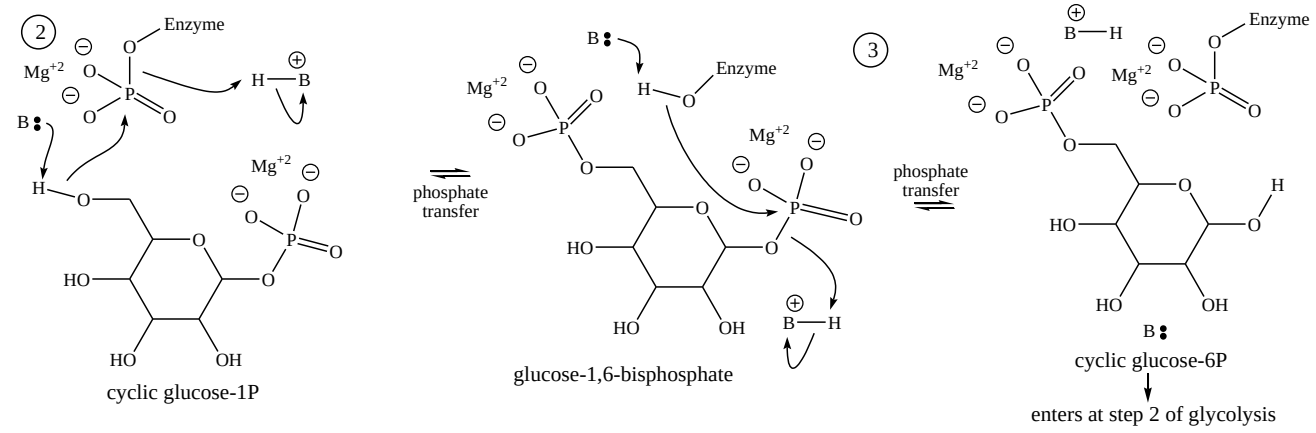


Possible arrow pushing mechanisms for glycolysis steps shown above inside a cell, and a few other biomolecules at the beginning and end of glycolysis. Arrow pushing details for each step are presented here.

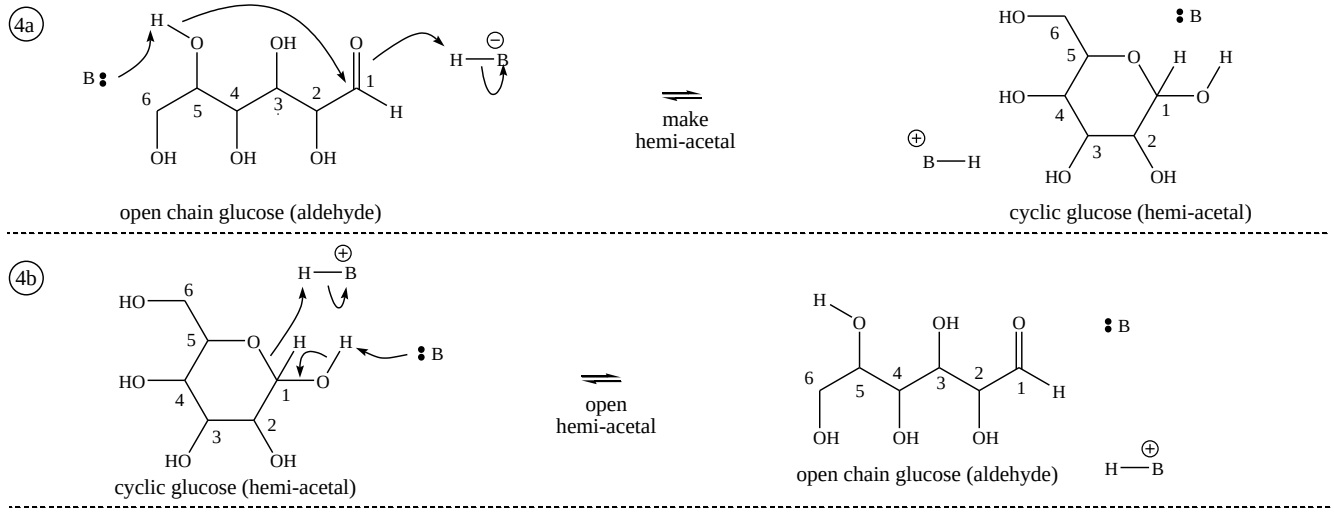
Cleavage of a glucose molecule from the end of a glycogen polymer of glucose, using an inorganic phosphate as a nucleophile. Glycogen is the electrophile (at C1). The reaction is shown as S_N2 (1 step), but could be S_N1 (2 steps).



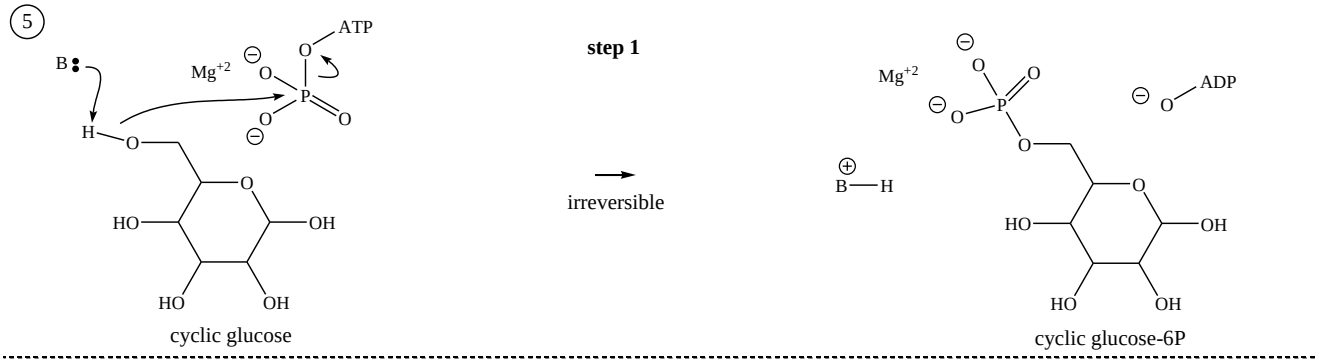
Glocose-1-phosphate is converted to glucose-6-phosphate. An enzyme phosphate phosphorylates C6 and then dephosphorylates C1, ready to do it again. Enters in the second step of glycolysis.



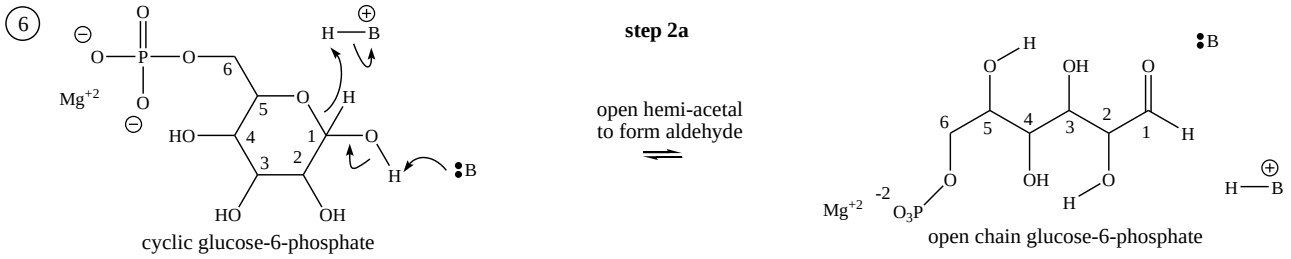
Cyclic glucose inside a cell (hemi-acetal pyranose) is in equilibrium with the open chain glucose (aldehyde).



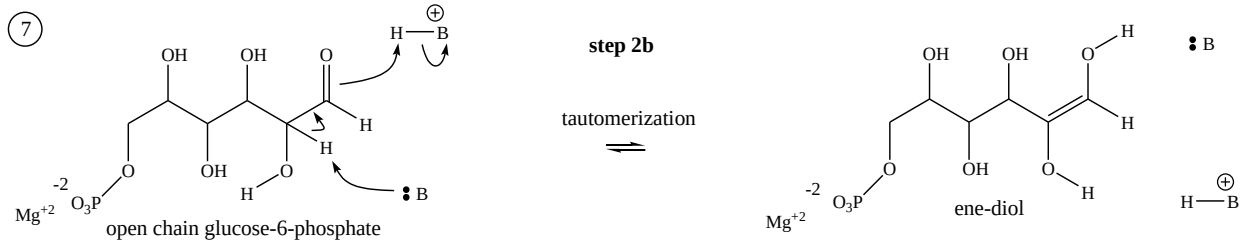
Glucose phosphorylates to glucose-6-phosphate, which prevents it from leaving the cell, and begins glycolysis.



Cyclic glucose (a pyranose) has to be opened to an aldehyde so that tautomerizetion can occur twice to form fructose (a furnaose).

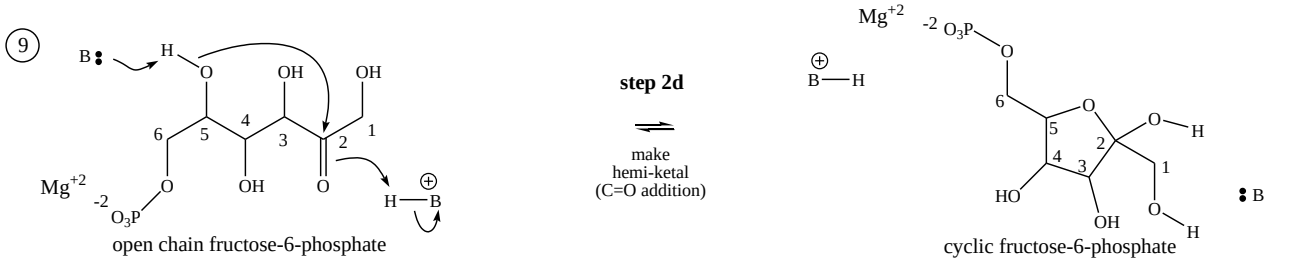


Two tautomerization reactions convert glucose-6-phosphate, an aldehyde, to fructose-6-phosphate, a ketone. The first tautomerization makes an ene-diol.

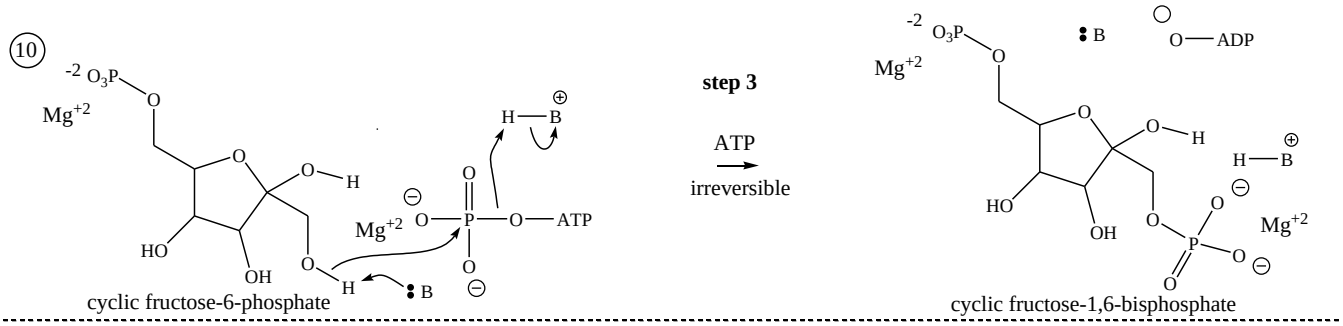


Second tautomer reaction makes fructose-6-phosphate, a ketone, from the ene-diol

Fructose-6-phosphate cyclizes to a hemi-ketal, a 5 atom furanose ring

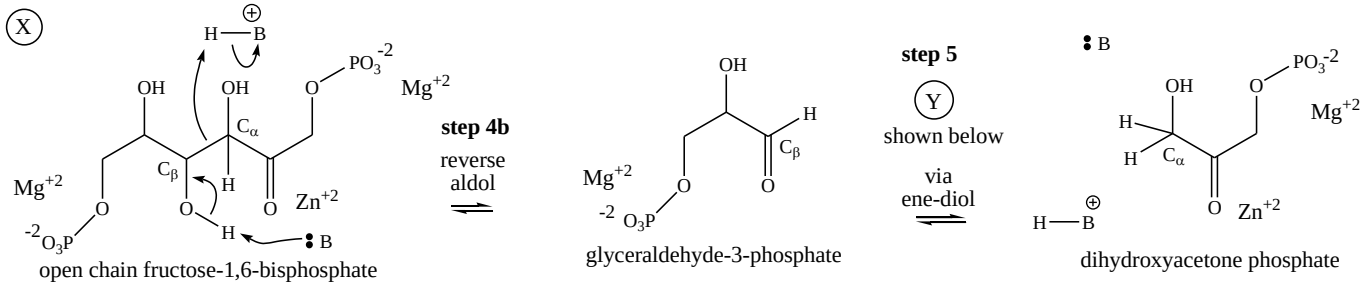


Fructose-6-phosphate phosphorylates a second time to form fructose-1,6-bisphosphate

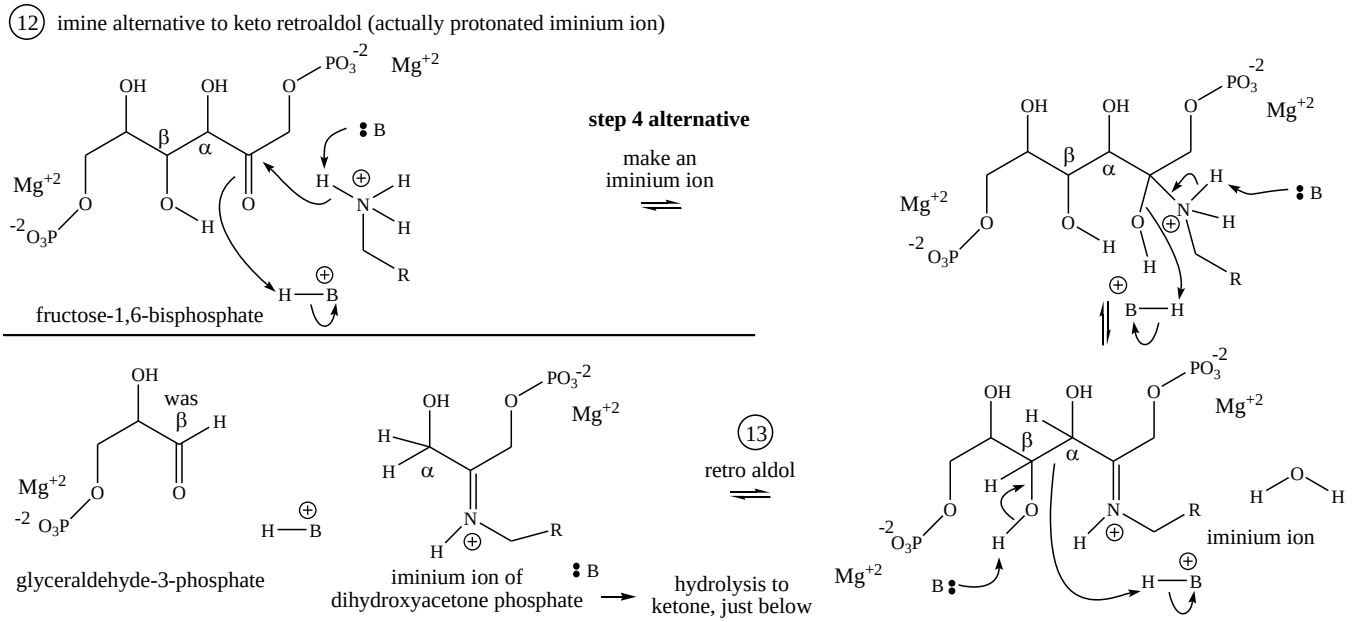


Cyclic fructose-1,6-bisphosphate, a hemi-ketal, opens (helped by charge repulsion?) to open chain fructose-1,6-bisphosphate, a ketone

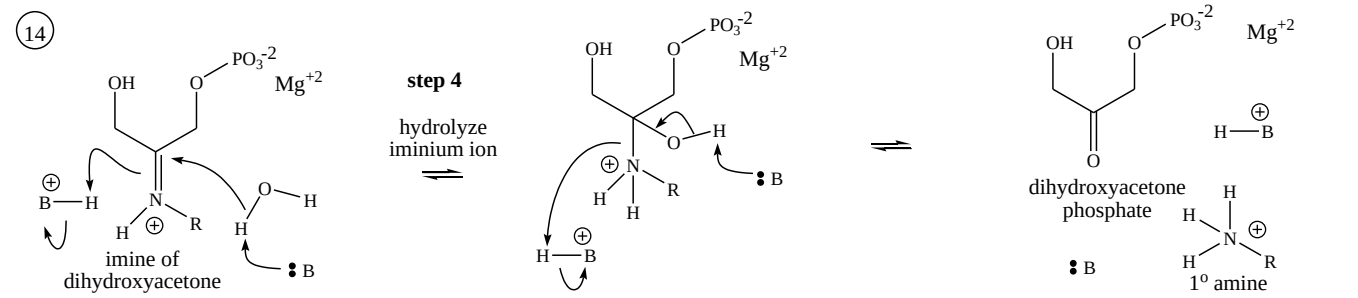
The carbonyl group (C=O) and beta OH allow for a retro aldol reaction to cleave the 6C chain between C_α and C_β into two 3C chains. This is an abbreviated transformation that is much simpler to write, and possibly occurs when a Zn^{+2} ion complexes with the carbonyl group (C=O). A more involved transformation using a primary amine with the ketone to make an imine (also called a Schiff base) is shown just below, but it takes a lot more writing to show (reactions 10a and 10b). Even though it is slower for us to write, it is probably faster in the actual reaction since the Schiff base can be protonated to make a better C_α leaving group.



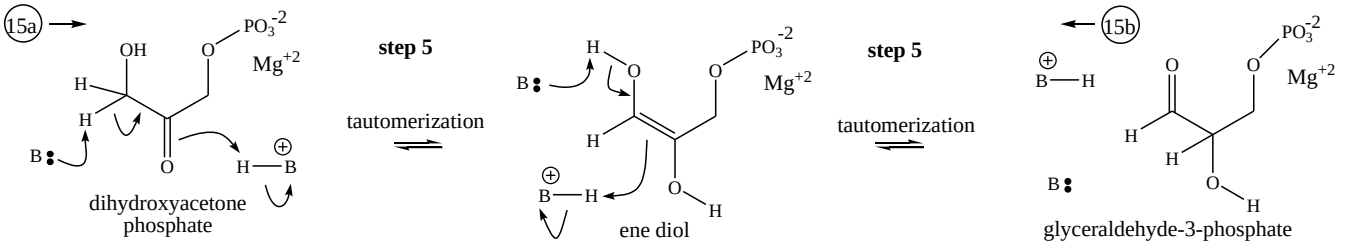
In a more likely mechanism, an imine is formed, combining the C=O with a primary amine and removing water. The imine is likely protonated at body pH. A protonated imine ($C=N^{+}HR$) is a better electron pair acceptor than the neutral carbonyl group (C=O) and possibly allows faster cleavage of the C_{α}-C_{β} bond in the retro aldol reaction. The imine would then have to be hydrolyzed (add water) to regenerate the carbonyl group. Because dihydroxy acetone and glyceraldehydes can easily interconvert via tautomerization (similar to glucose / fructose), this is equivalent to producing two glyceraldehydes molecules that can be carried forward in glycolysis.



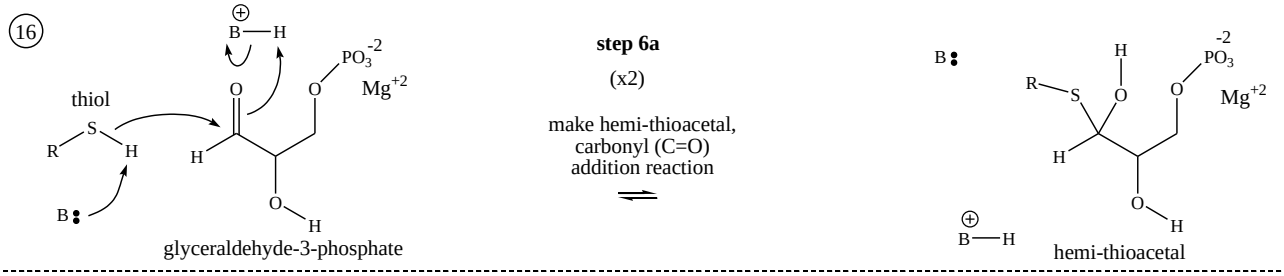
Hydrolyze iminium ion back to ketone.



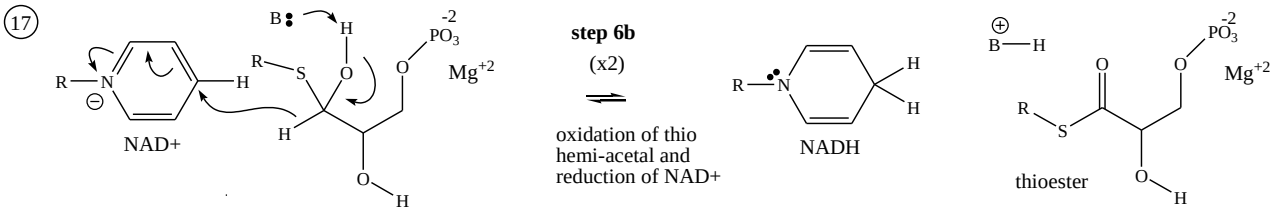
Two tautomerization reactions convert either 3C component into the other via an ene-diol. Similar to interconversions of glucose and fructose in steps 4 and 5 above. Because these 2 compounds can interconvert, it means that all 6 carbons of glucose can be carried forward in glycolysis as glyceraldehydes-3-phosphate, so all the reactions that follow can be multiplied by 2. It is also possible that dihydroxyacetone phosphate can be diverted off towards other biomolecules, such as glycerol used in fatty acid synthesis. It is also possible that glycerol can be converted into dihydroxyacetone phosphate and form glyceraldehydes-3-phosphate, which continues on in glycolysis to pyruvate.



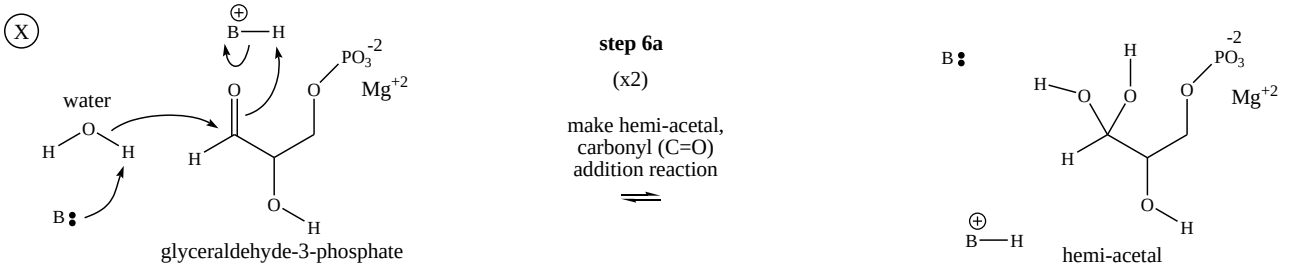
It wasn't real clear to me what form glyceraldehye-3- phosphate gets oxidized by NAD+. So I'm proposing 2 possibilities that seem reasonable to me. In the first, a thiohemi-acetal is formed, which allows for oxidation with NAD+ to a highly reactive thioester in the next step. NAD+ is formed in either mechanism.

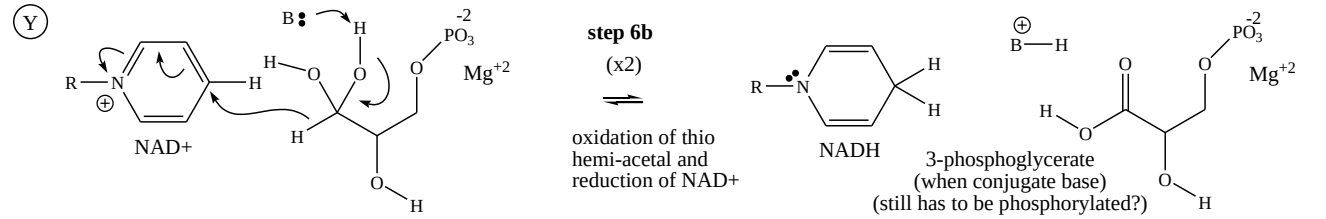


NAD+ accepts a hydride (reduced) to form NADH and a highly reactive thioester used in the next step to make ATP.

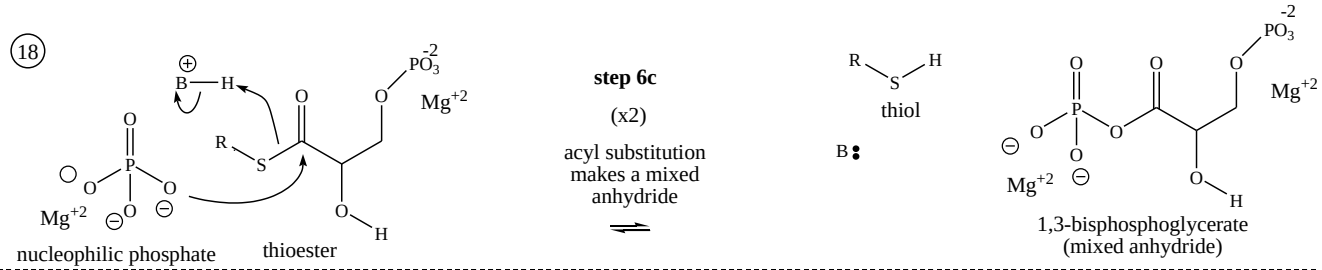


The second possibility is a carbonyl hydrate forms and gets oxidized by NAD+. That would require a very high energy phosphorylating agent (ATP?) to form the 1,3-bisphosphoglycerate and that does not seem to be occurring. So I favor the first mechanism.

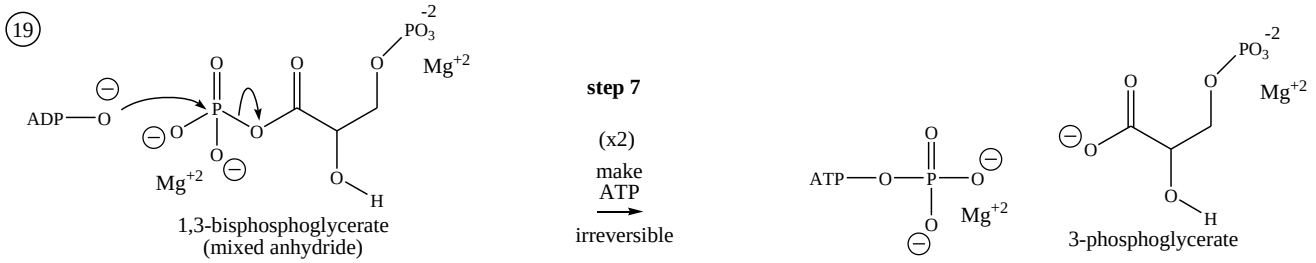




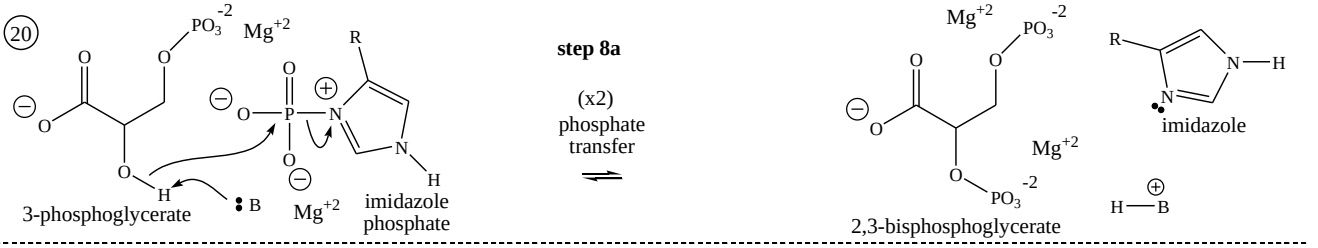
Inorganic phosphate does an acyl substitution reaction with the highly reactive thioester to form a highly reactive mixed anhydride that allows formation of a high energy ATP molecule (an inorganic anhydride) in the next step. The good thiol leaving group helps make this possible. Acyl substitution is really 2 steps: addition and elimination.

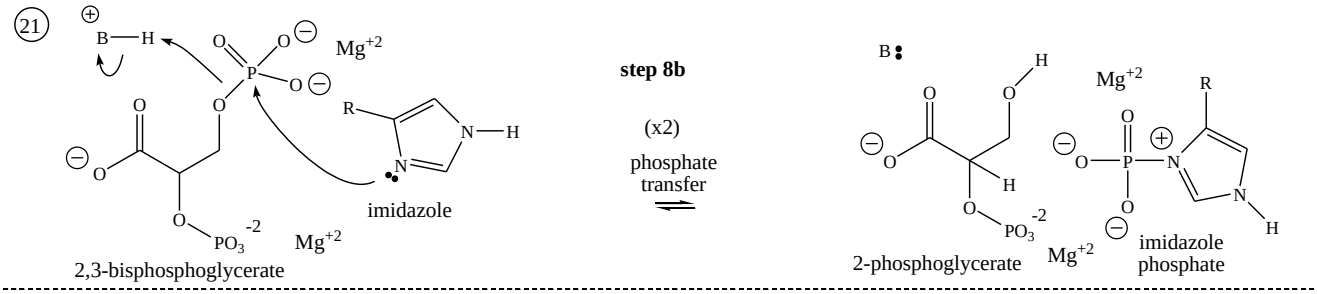


An ADP molecule adds a phosphate from the highly reactive mixed anhydride to form ATP (2x from glucose).

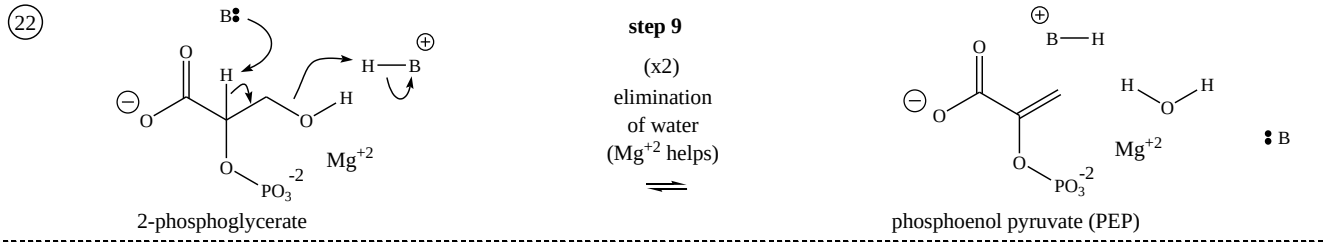


In 2 steps, an imidazole phosphate adds a phosphate at the 2-OH, forming the bisphospho-2,3-glycerate, and then removes the 3-phosphate. This probably makes the phosphate more reactive as it moves the anionic phosphate closer to the anionic carboxylate.

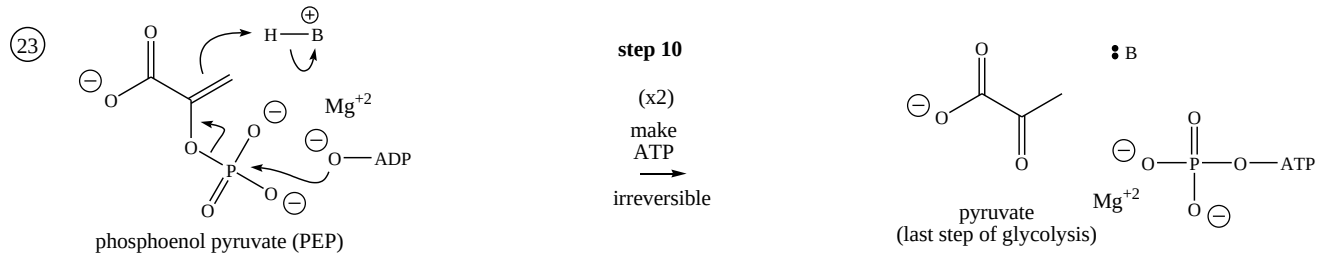




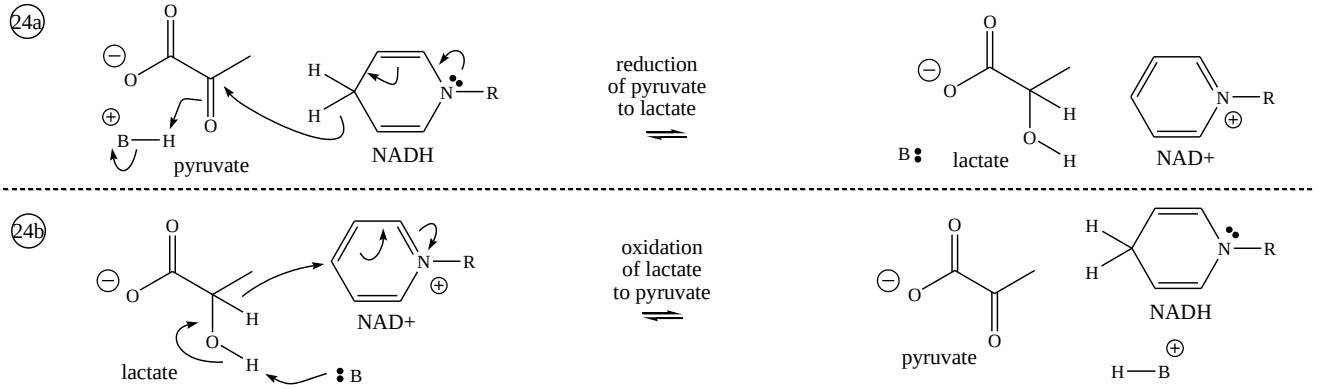
Dehydration forms a highly reactive phosphoenol pyruvate (PEP), which has enough energy to form ATP. Mg+2 is thought to make the HO into a better leaving group (shown as water leaving group in the mechanism below).



An ADP molecule adds a phosphate from the very high energy phosphoenol pyruvate (PEP) to form ATP and pyruvate, which represents the end of the line for glycolysis. In the presence of oxygen pyruvate can migrate to a mitochrondiron, make oxaloacetate and enter the TCA cycle to make NADH and FADH2, used to make large amounts of ATP using the electron transport chain (ETC = electron transport chain = respiration). In low oxygen conditions, pyruvate and NADH can be converted to lactate and NAD+, and the lactate exported outside the cell and carried to the liver to be reoxidized to pyruvate and made back into glucose (gluconeogenesis), or in yeast, in the absence of oxygen, pyruvate can be decarboxylated ($-CO_2$) to make ethanal, then ethanol and NAD+, via fermentation. Under the low oxygen conditions, the oxidation of NADH makes more NAD+ which is needed to oxidize glyceraldehydes-3-phosphate to keep the glycolysis running.

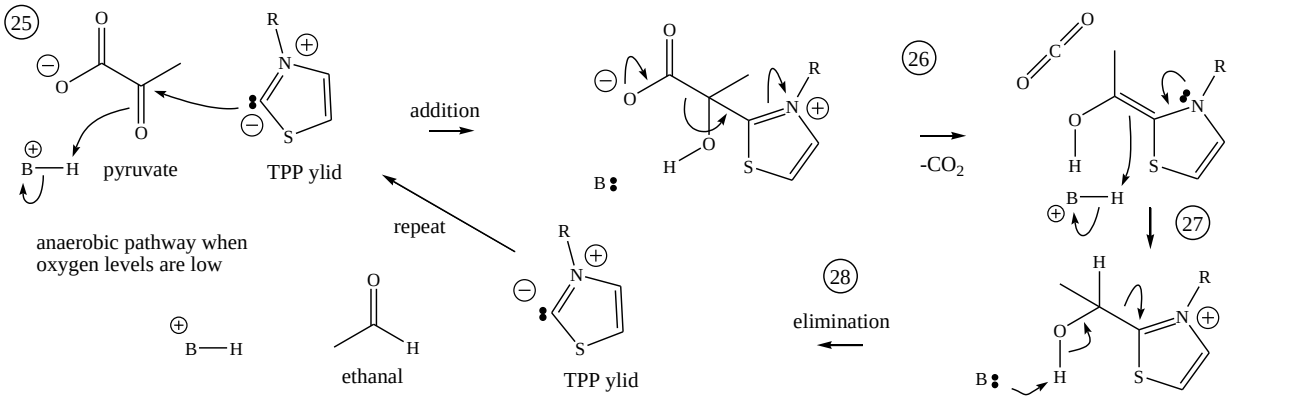


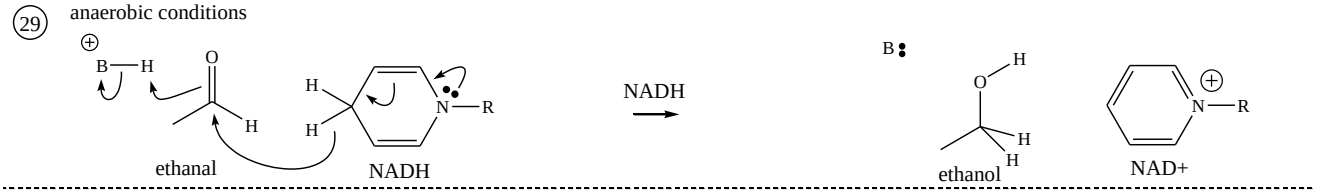
Make lactate from pyruvate under anaerobic conditions. Make pyruvate from lactate in liver cell for gluconeogenesis.



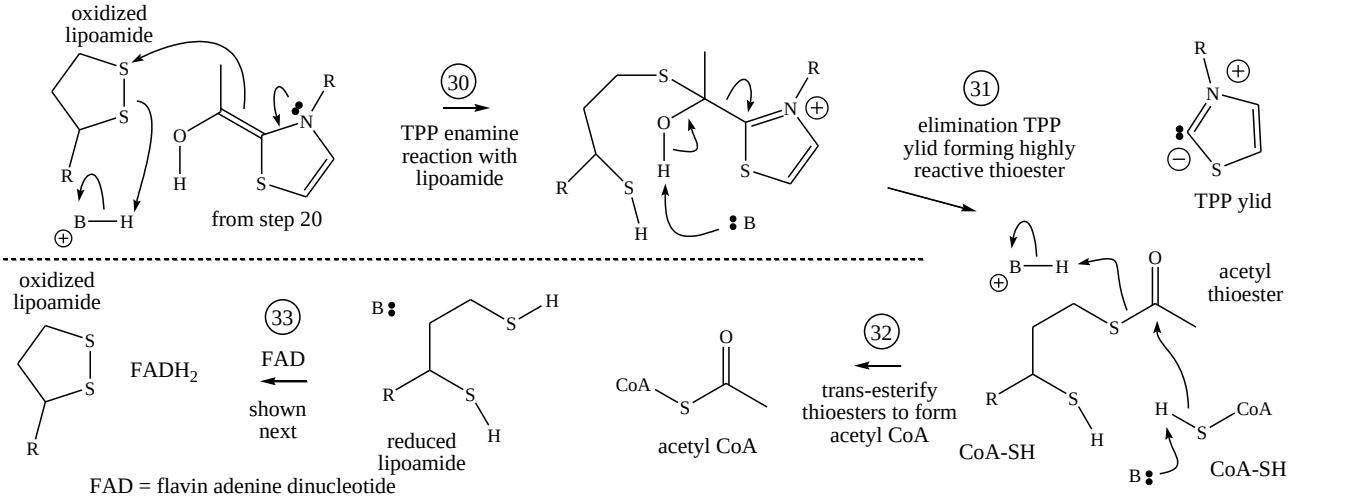
Pyruvate can also be made into acetyl CoA, a very central and important chemical in all living organisms. Acetyl CoA is used in the Tricarboxylic Acid Cycle (TCA cycle = Kreb's cycle) to make citrate from oxaloacetate. Citrate continues through the TCA cycle, making NADH and $FADH_2$ that are used to make about 90% of the body's ATP in the Electron Transport Chain (ETC) at the inner mitochondrial membrane. Acetyl CoA is also a building block to make fatty acid chains in the cytosol, which are used in diglycerides present in cell membranes and triglycerides used in fat storage. Also, acetyl is the starting point for synthesis of all of the body's steroids and bile acids. The steps to make acetyl CoA from pyruvate are shown below. An alternate ending path in anaerobic yeast is to make ethanol (also shown below). Pyruvate can also make alanine, an amino acid and oxaloacetate, also shown below.

Reaction of pyruvate with TPP ylid (thyamine pyrophosphate = tyamine diphosphate = vitamin B1), which decarboxylates ($-CO_2$) to make an enamine-like intermediate that can protonate and eliminate the TPP ylid and ethanal. Ethanal can be reduced by NADH to form ethanol and NAD+ to keep glycolysis going.

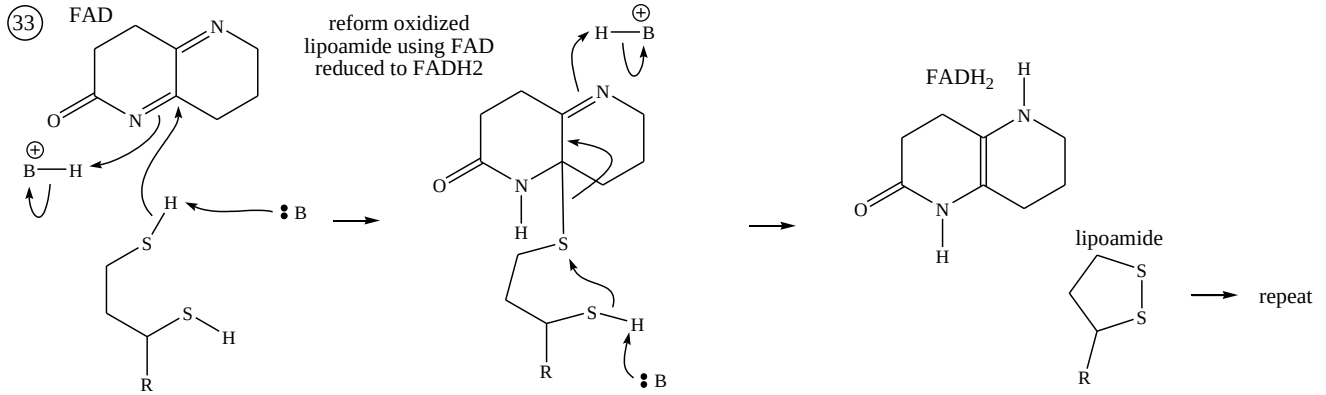




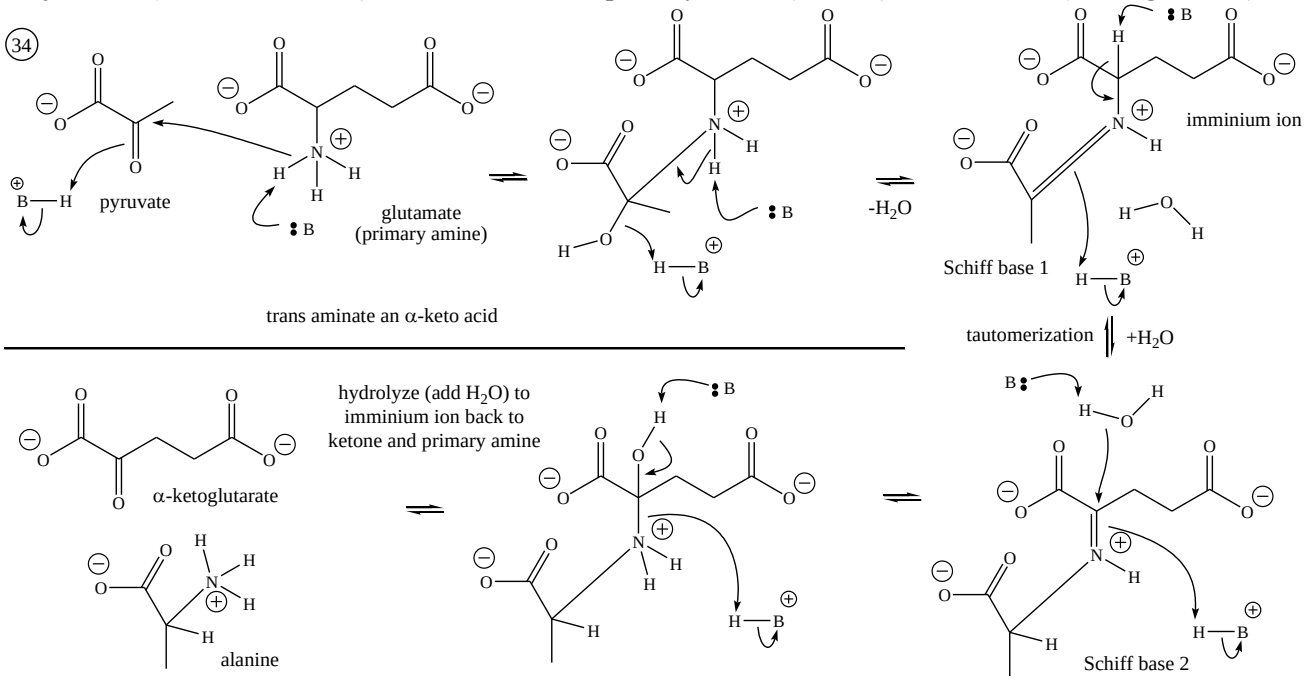
Alternatively, the TPP intermediate can react with lipoamide and then eliminate to form a thioester. The thioester can trans-thioesterify with CoA-SH to form acetyl CoA which is central to many biological pathways, a fuel for the TCA cycle (generates ATP), and a building block for fatty acids and steroid synthesis.



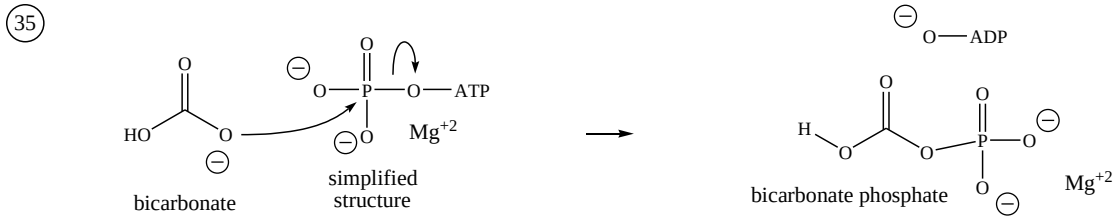
Regenerating Lipoamide using FAD (flavin adenine dinucleotide) This is necessary to continue making acetyl CoA.



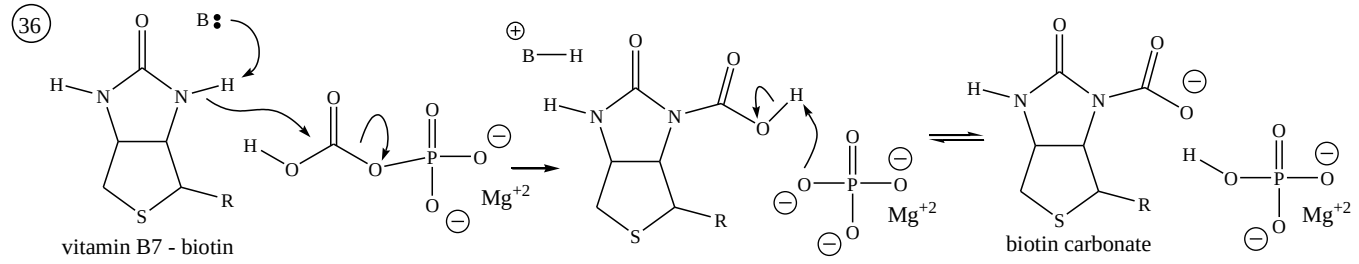
Pyruvate (α-ketoacid) can make the amino acid alanine by transamination with amino acid glutamate into α-ketoglutarate. It involves formation of a Schiff base by condensation (removal of water) of an α-ketone and a primary amine. Tautomerization switches a proton and the pi bond of the Schiff base to the other nitrogen and then hydration (addition of water) reforms a different primary amine (alanine) and α-ketone (α-ketoglutarate).



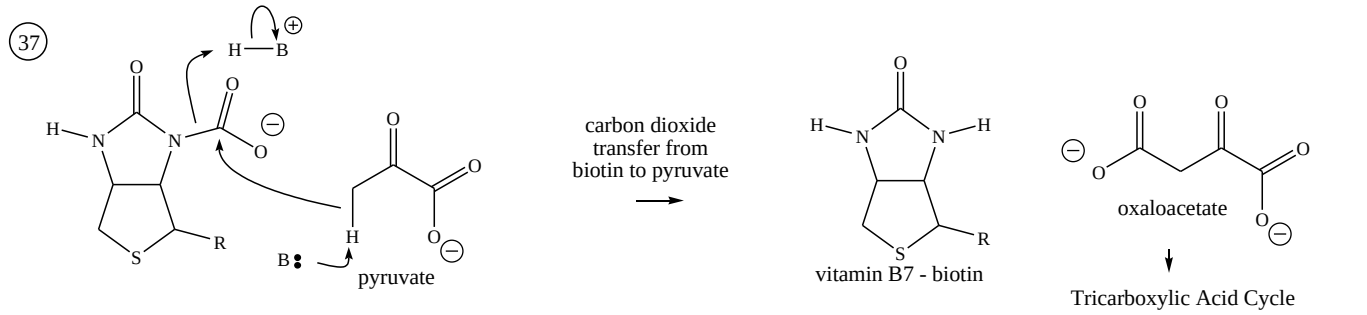
ATP can phosphorylate bicarbonate to make a mixed anhydride with a good phosphate leaving group to add CO_2 to biotin.



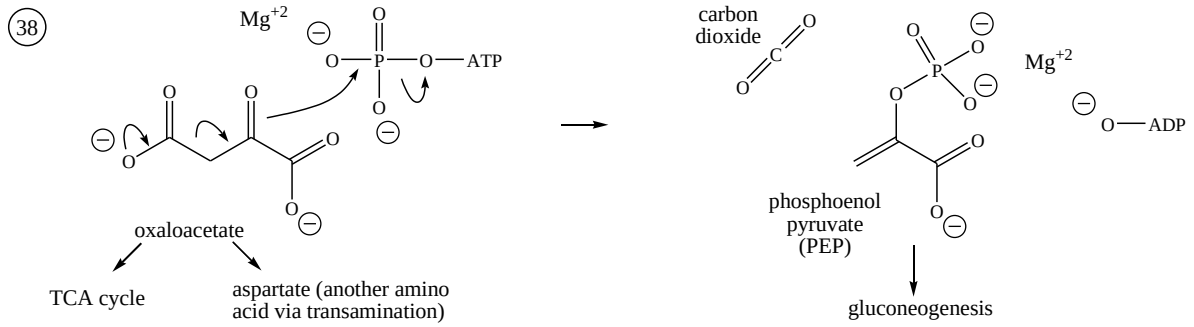
Biotin can make biotin-CO_2 which carboxylates other biomolecules.



Biotin-CO_2 can carboxylate pyruvate to make oxaloacetate used in TCA cycle and make PEP for gluconeogenesis.

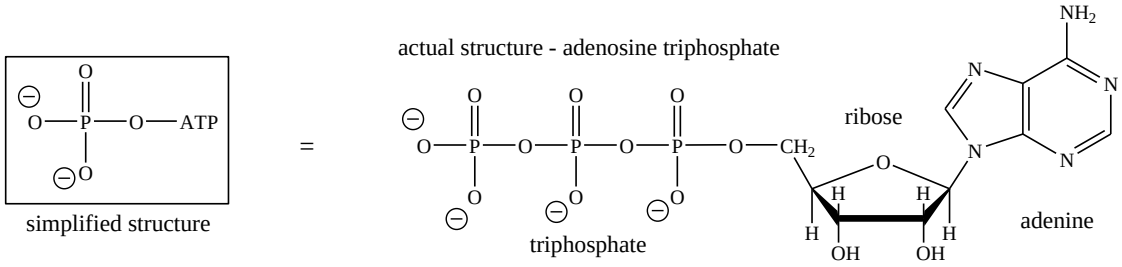


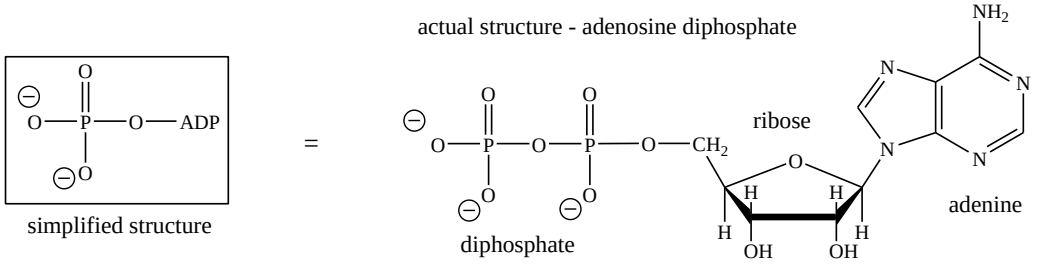
Oxaloacetate, begins the Tricarboxylic Acid Cycle (Kreb's cycle) and can also make phosphoenol pyruvate, which starts gluconeogenesis

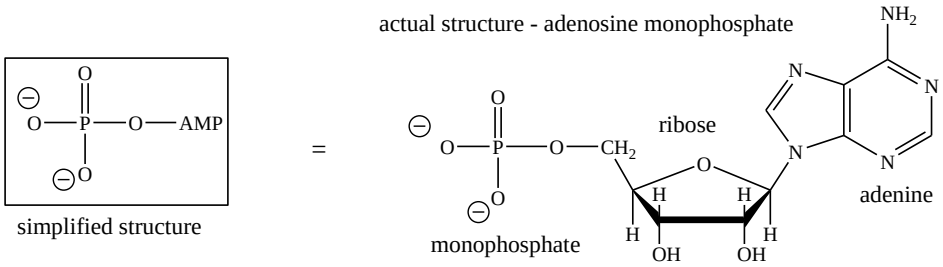


Chapter # - Various Cofactors (Full and Simplified), Glycolysis worksheet begins on p. #.

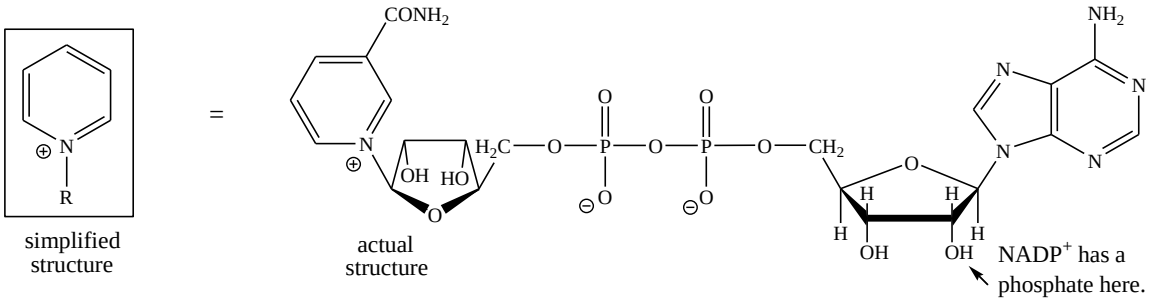
1. ATP – adenosine triphosphate – phosphorylation, source of high energy bonds



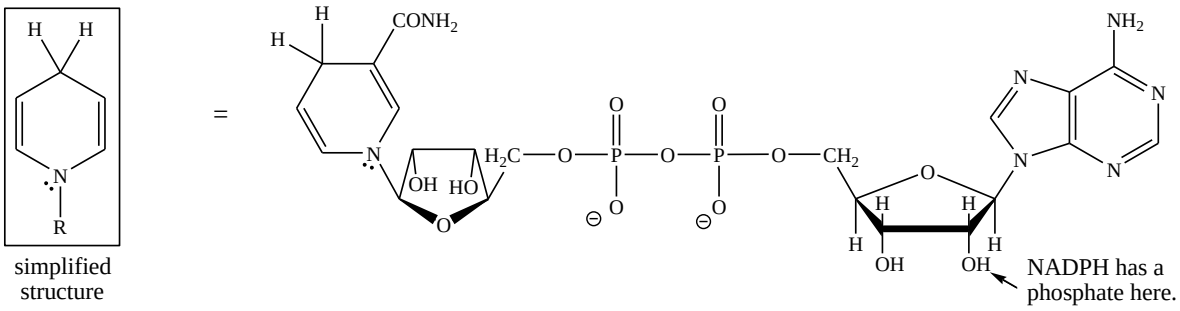




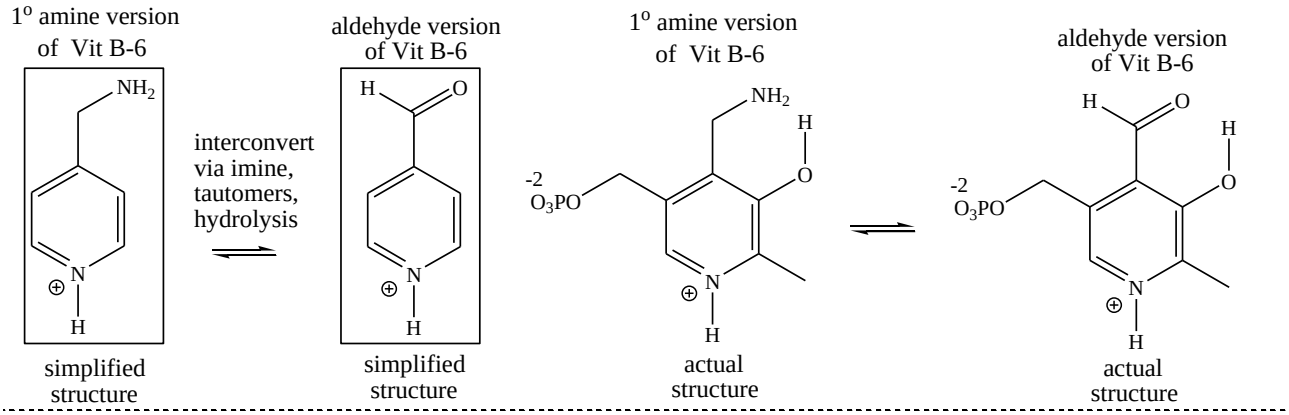
2. NAD^+ (catabolism) and $NADP^+$ (anabolism) - nicotinamide adenine dinucleotide (hydride acceptors) The extra phosphate on the C3 ribose position of NADP structures probably helps to keep the cycles and pathways where each is used separate from one another.



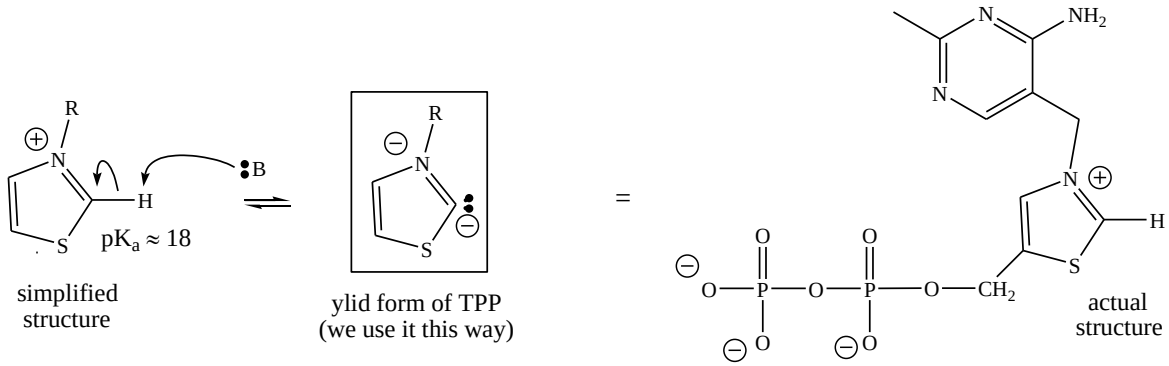
3. NADH and NADPH - nicotinamide adenine dinucleotide (hydride donors)



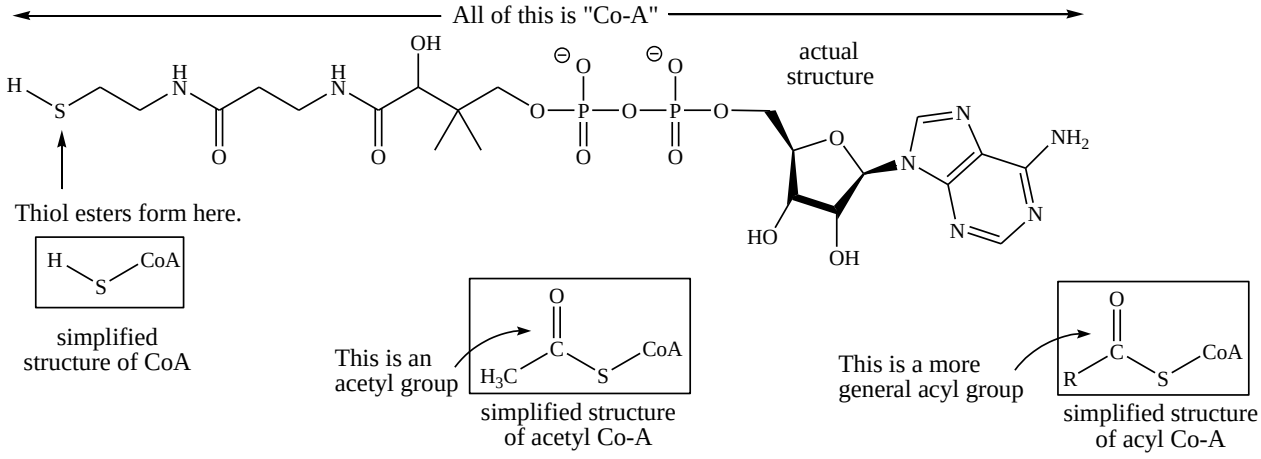
4. Vitamin B-6 – pyridoxal phosphate (amino acid metabolism, transamination with α-ketoacids, decarboxylation, removal of some amino acid side chains, epimerizations)



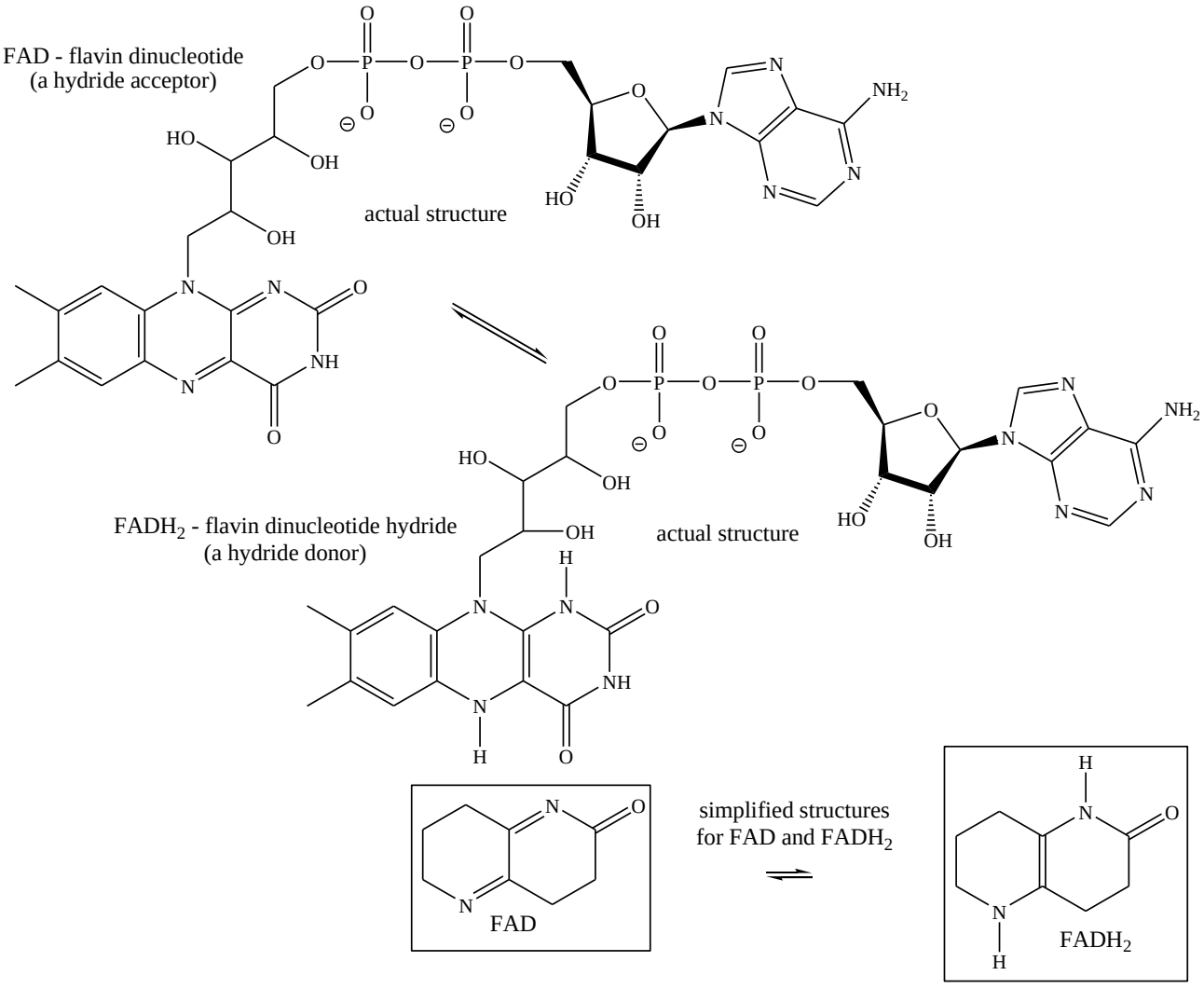
5. TPP – Thiamine diphosphate (decarboxylation and enamine chemistry with proton or carbohydrates, used to be called thiamine pyrophosphate = TPP)



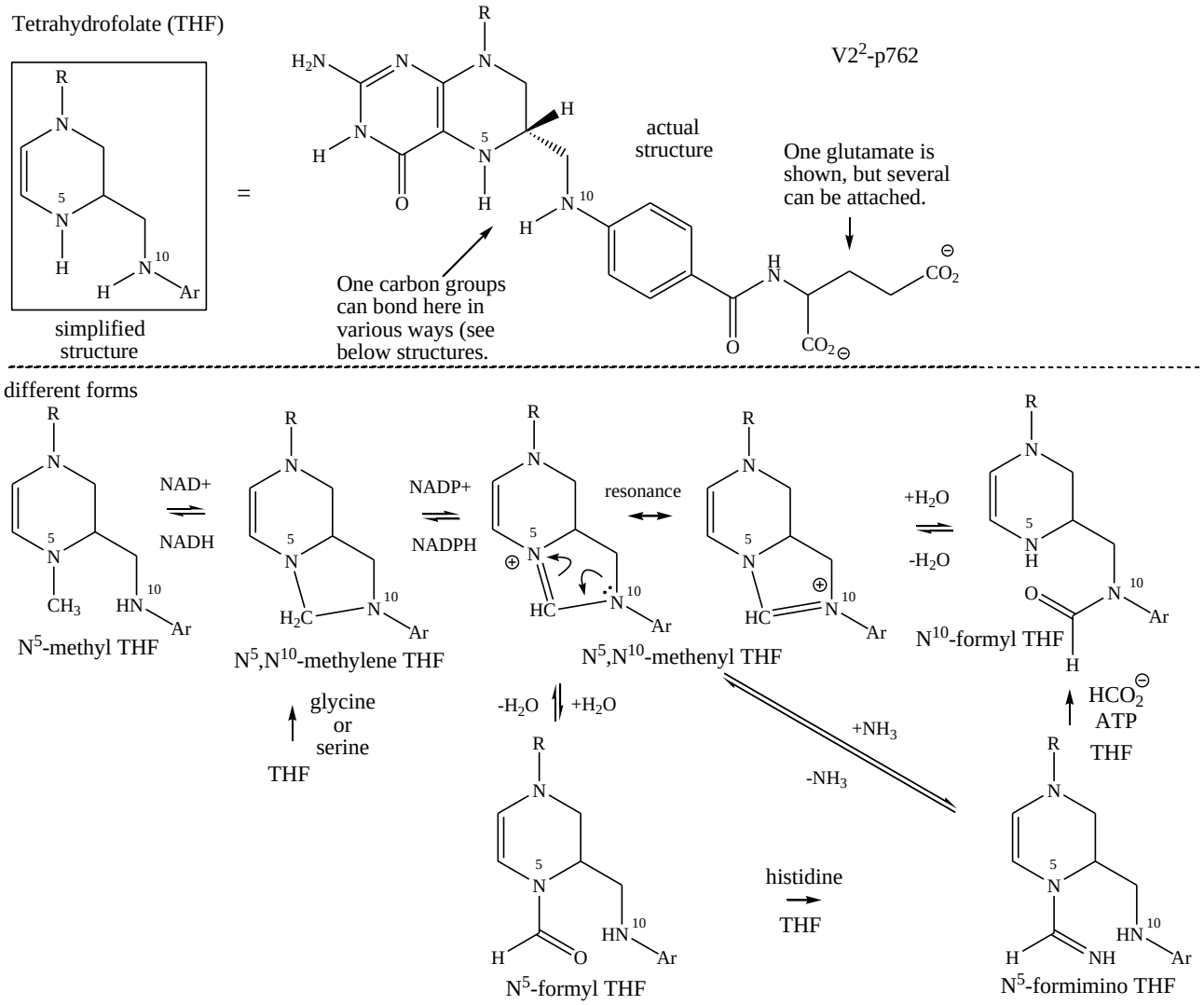
6. Coenzyme A and acetyl CoA (more general acyl CoA's are possible) (acyl transfers)



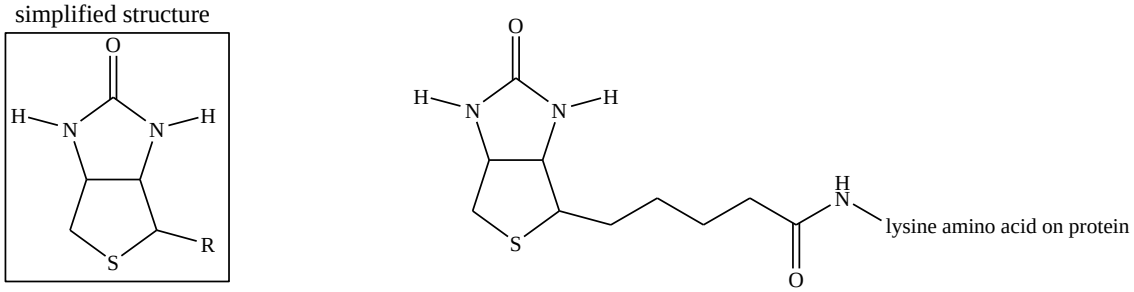
7. FAD / $FADH_2$ – Flavin adenine dinucleotide (oxidation – reduction) – used to deliver hydride to C=C or take hydride from CH-CH (fatty acid metabolism, etc.). Can also be used in 1 electron transfers.



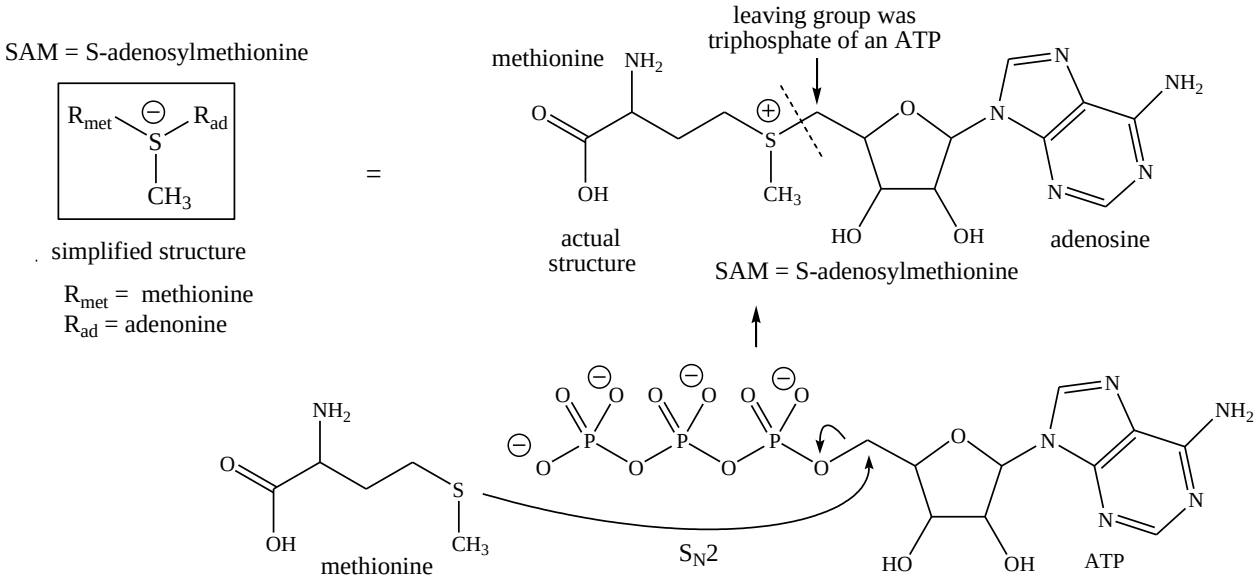
8. THF – tetrahdrofolate (transfer of one carbon units in various forms as CH_3, CH_2, CH) –recycles cysteine to methionine and other 1C metabolic functions, many variations.



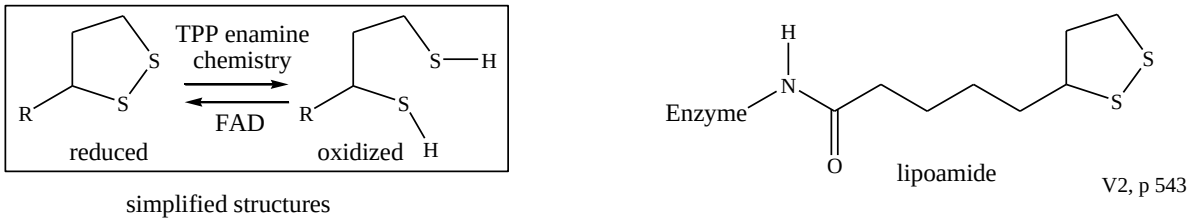
9. Biotin –Involved in 1C transfers (CO_2 as an activated carbonate derivative is added to activate C=O for aldol type chemistry and then taken off when no longer needed). Helps at the end of odd fatty acid chain metabolism (propanoyl CoA → succinate conversion).



10. SAM = S-adenosylmethionine (methyl transfer agent), The methyl group (CH_3) attached to the methionine sulfur atom in SAM is chemically reactive. This allows transfer of a methyl group to an acceptor substrate in transmethylation reactions. More than 40 metabolic reactions involve the transfer of a methyl group from SAM to various substrates, such as nucleic acids (epigenetics), proteins, neurotransmitters, lipids and secondary metabolites. SAM can be made from methionine, which is made from homocysteine and N^5-methyl THF (just above).

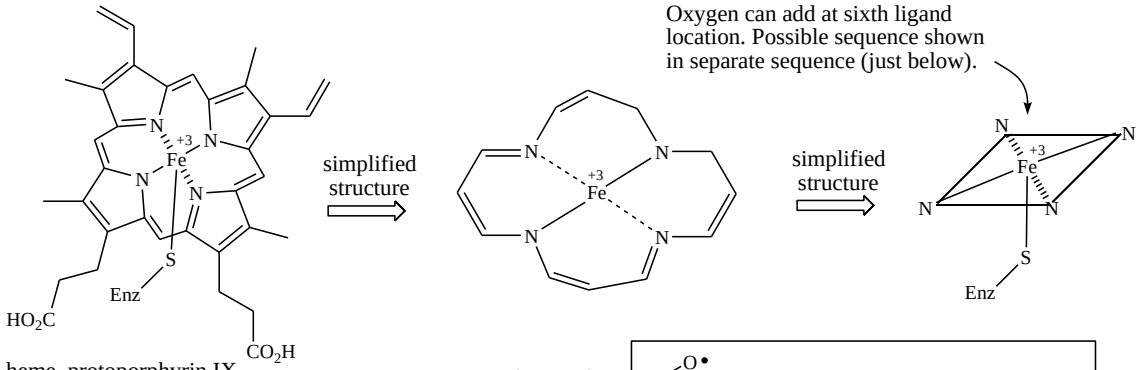


11. Lipoyl amide – important in fat metabolism



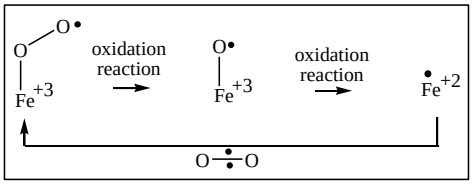
12. Cytochrom P-450 enzymes are oxidizing agents in the body. They can convert inert alkane sp^3 C-H bonds into C-OH bonds and they can make epoxide groups at alkenes and aromatic pi bonds. Iron loads up with oxygen by reacting with O_2 and subsequent reactions (shown later) to form the simplified structure that we use. The iron occurs in several oxidation states. In the Bio-Organic Game we only use the simplified forms below.

Oxidations in the body often use cytochrom P-450 enzymes (free radical chemistry).



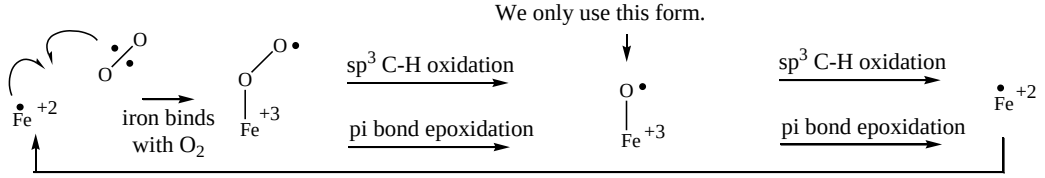
heme, protoporphyrin IX, found in cytochrom P-450 oxidative enzymes

Both oxidized forms of iron will work in the Bio-Organic Game reactions, but we will only use the second form to keep things simpler.



simplified structures, oxygen reacts like a diradical

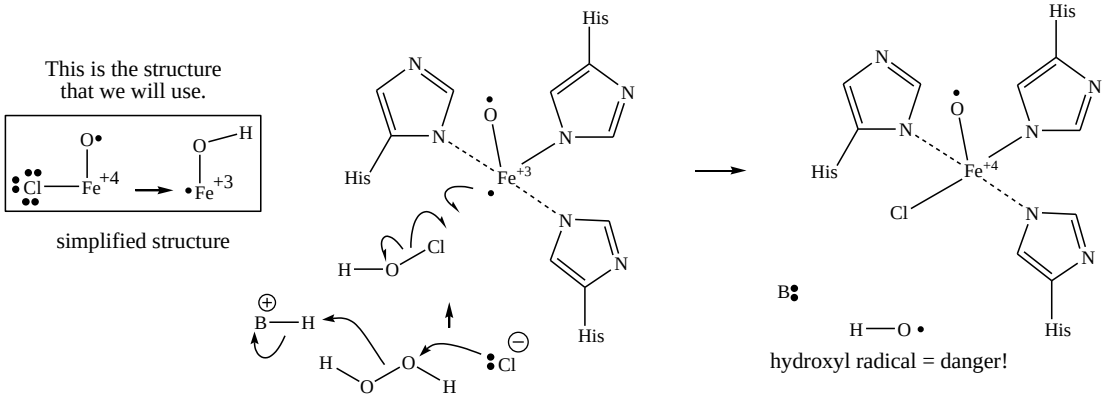
Cytochrom P-450 free radical reactions used in the Bio-Organic Game - view an oxygen molecule as a diradical



---

13. Halogenase Enzymes (related to cytochrom P-450 enzymes). The 'full' structure below only shows a simplified form complexed with imidazole ligands from a protein's histadine amino acids and a halogen as one of the ligands. The further simplified form used in the Bio-Organic Game only shows the iron, halogen and oxygen.

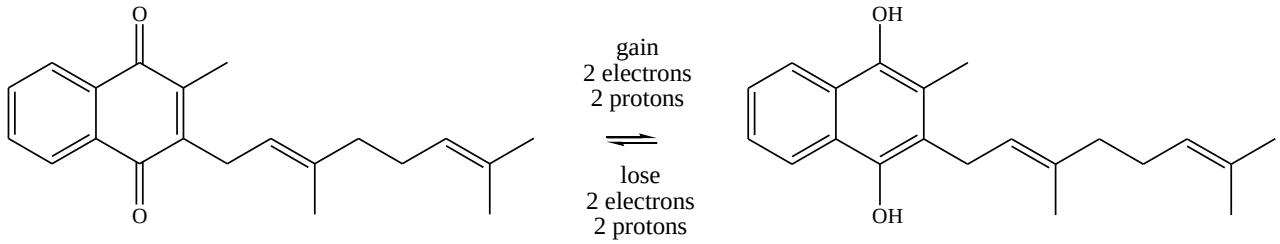
Halogenations in an organism can occur using iron halogen bonds.



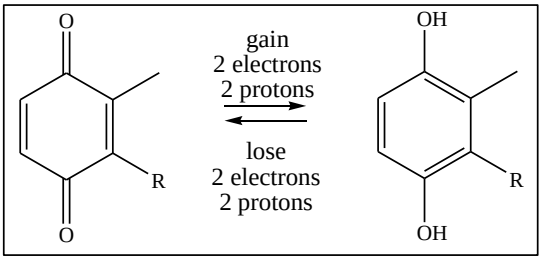
---

14. Vitamin K (quinone and hydroquinone) – 1 electron transfers such as in the Electron Transport Chain in a mitochondrial membrane.

Vit K (many versions) important in electron transport.

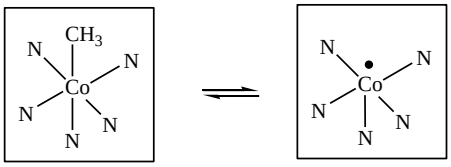


simplified structures

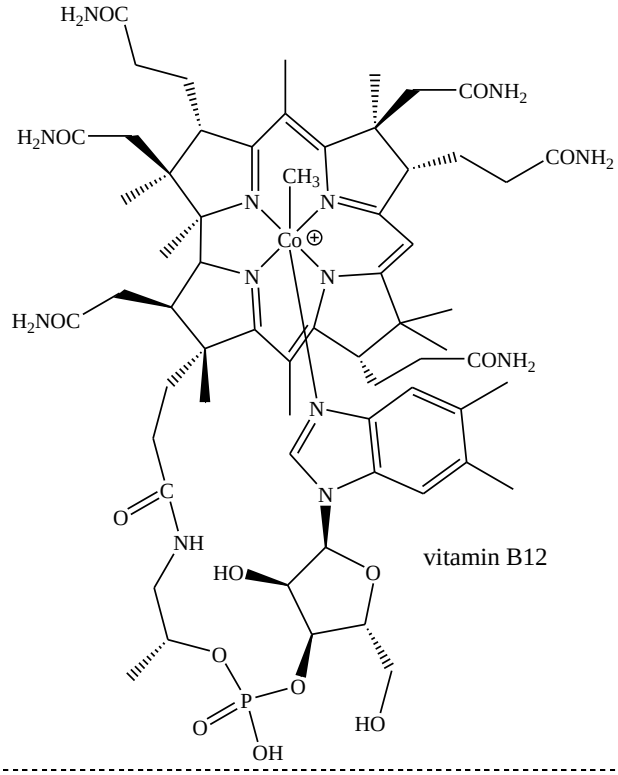


15. Vitamin B-12 organocobalt compound, free radical isomerizations in odd fatty acid chains, synthesis of methionine, maintenance of folate levels, crucial to DNA synthesis and cells with a high turnover (blood cells), mylene sheath of nerve cells

simplified vitamin B12
(free radical chemistry)



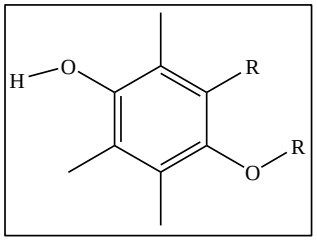
Cobalt is a very uncommon metal in biochemistry. Vitamin B12 is not made by animals, plants or fungi, only by bacteria. It has nitrogen at 5 ligand sites and either OH or CN at the sixth site. Once in a human body that site can be switched for a methyl group. The ring system is not common either and called a 'corrin' ring. Without vitamin B12, fatal pernicious anemia occurs. It is necessary for conversion of homocysteine to methionine, which also requires N5 methyl tetrahydrofolate. The recommended daily intake is about 2 μg. Deficiency can cause irreversible damage to the brain and nervous system. Vitamin B12 is used to regenerate folate to produce thiamine. When not present folate gets trapped as N5 methyl and cannot make 5,10-methylene tetrahydrofolate used to make thiamine so fast growing cells (blood cells) cannot make enough DNA. It is also needed to make SAM used in the synthesis of neurotransmitters, catecholamines and mylin sheath phospholipids. It helps in the metabolism of odd carbon fatty acid chains.



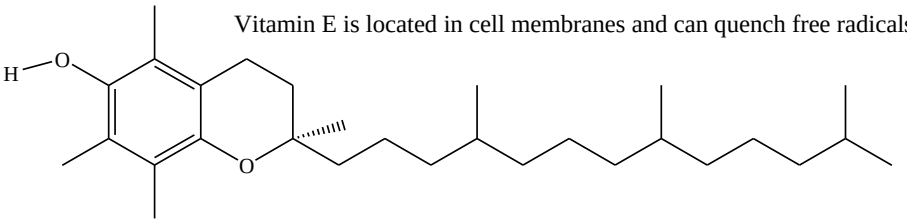
vitamin B12

16. Vitamin E – hydrophobic antioxidant structure is likely found in membranes

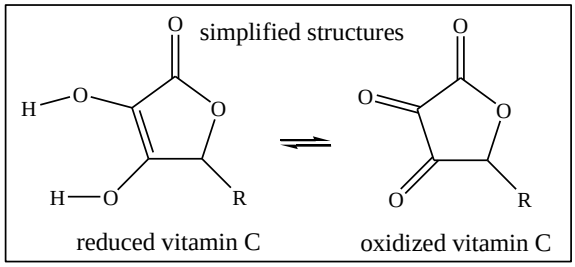
simplified structure

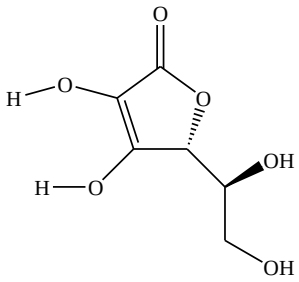


Vitamin E is located in cell membranes and can quench free radicals.



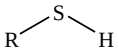
17. vitamin C – hydrophilic antioxidant structure is likely found in blood or cytosol. Vitamin C can reduce vitamin E back to its reduced state in the membrane. Vitamin C then washes out of the body because it is water soluble.



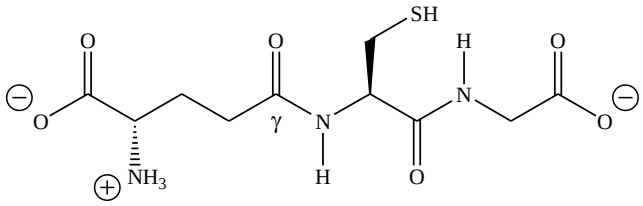


17. Glutathione – antioxidant tripeptide, helps prevent free radical damage by reactive oxygen species (ROS), heavy metals and conjugates with hydrophobic molecules, making them water soluble to flush out of the body. It can reduce disulfide linkages and make disulfide linkages and its proton is acidic enough to act as a proton donor in some instances. It has a gama amide linkage in the glutamate amino acid. Concentration levels in solid tissue can be as high as 50 μM and 5 μM in the blood.

Simplistic Version



Tripeptide = glutamate - cysteine - glycine



Summary table of several simplified biochemical structures above.