# 29. The Organic Chemistry of Metabolic Pathways

- Based on McMurry's *Organic Chemistry*, 6th edition

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# 29.1 An Overview of Metabolism and Biochemical Energy

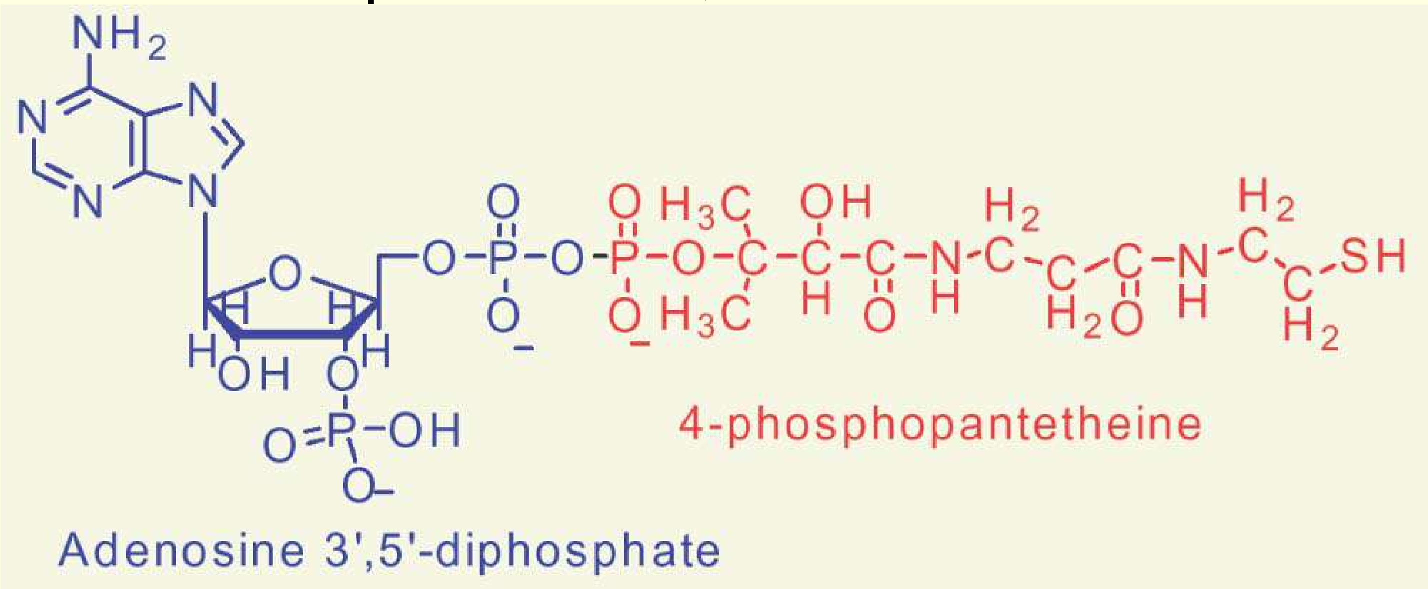
- Metabolism: The reactions in organisms
- Catabolism: Pathways that break down larger molecules into smaller ones
  - Usually release energy
- Anabolism: Pathways that synthesize larger biomolecules from smaller ones
  - Usually requires an input of energy

# The First Catabolic Stage: Digestion

- Food is broken down by hydrolysis of esters, glycosides, and peptide in the digestive system
- Yields fatty acids, simple sugars, and amino acids
- Smaller molecules are degraded in cells to acetyl groups attached to the large carrier molecule coenzyme A

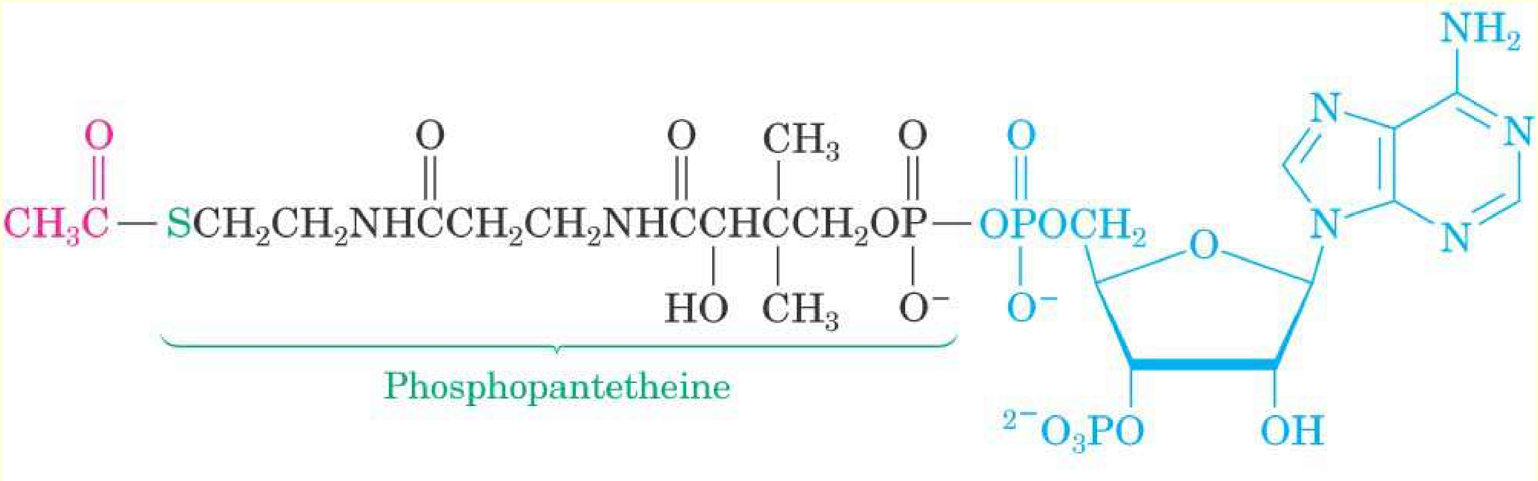
# Coenzyme A

- A complex thiol that transports acyl groups in enzyme reactions
- Most commonly as acetyl derivative
- Derived from pantetheine, a vitamin



# Acetyl Coenzyme A

- The acetyl ester of  Coenzyme A (acetyl CoA)
- Key substance in numerous other biological pathways




Acetyl CoA—a thioester

# Conversion of Food to Energy: Initial Digestion

- Digestion begins with enzymes that break down complex macromolecules
- For example, proteins are converted to peptides and then amino acids
- Complex carbohydrates are broken down to simple sugars
- Fats are hydrolyzed to acids and glycerol

# Breakdown of Smaller Molecules

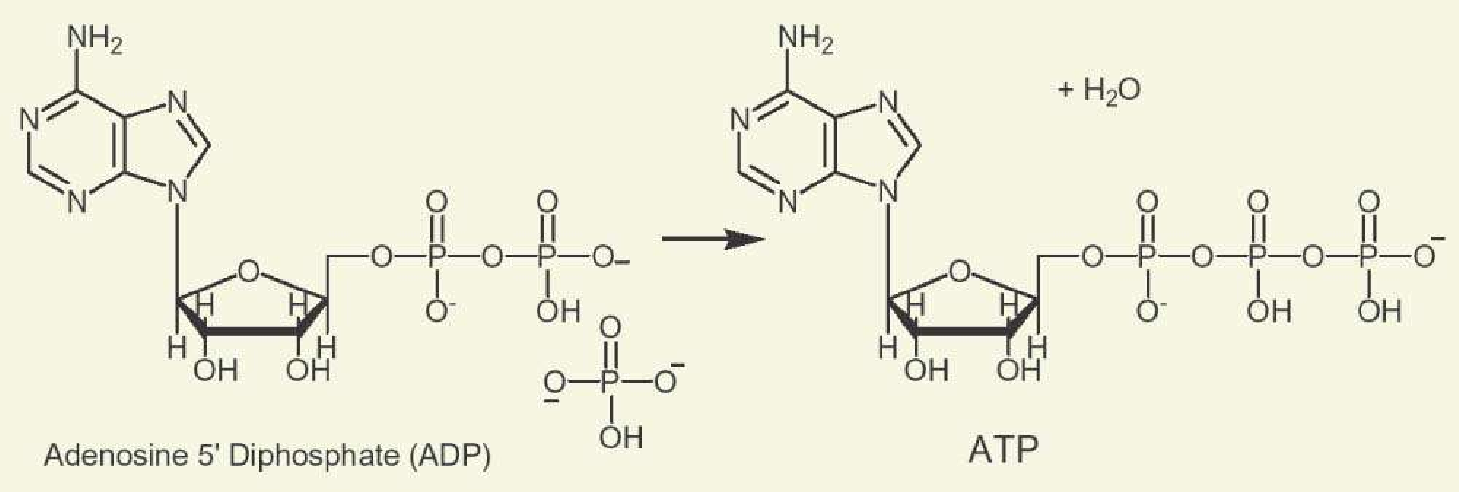
- Sugars and fat components are broken down in steps that lead to formation of acetate
- Some steps are coupled to formation of phosphate anhydrides
- Amino acids are recycled into proteins

# Oxidation of Acetyl Groups

- Acetyl groups are oxidized inside cellular mitochondria in the the citric acid cycle to yield $CO_2$
- The oxidation process releases energy in matched stages
- These reactions are coupled to an electron-transport chain (successive reduction)
- The energy available drives a dehydration reaction that forms  to produce molecules of the nucleotide *adenosine triphosphate*, *ATP* (shown on the next slide)

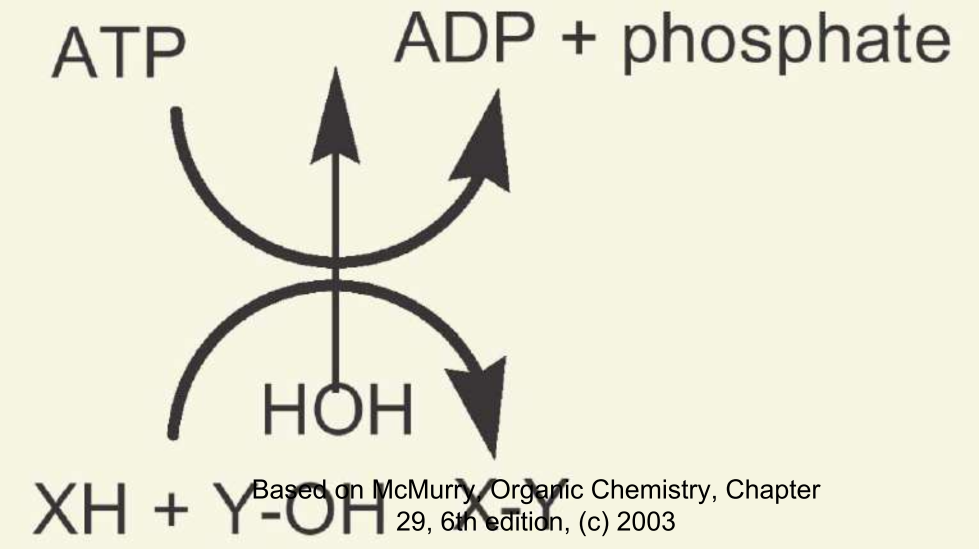
# Formation of ATP

- Energy in electron transport (stepwise oxidation) provides energy for formation of ATP from ADP with elimination of water



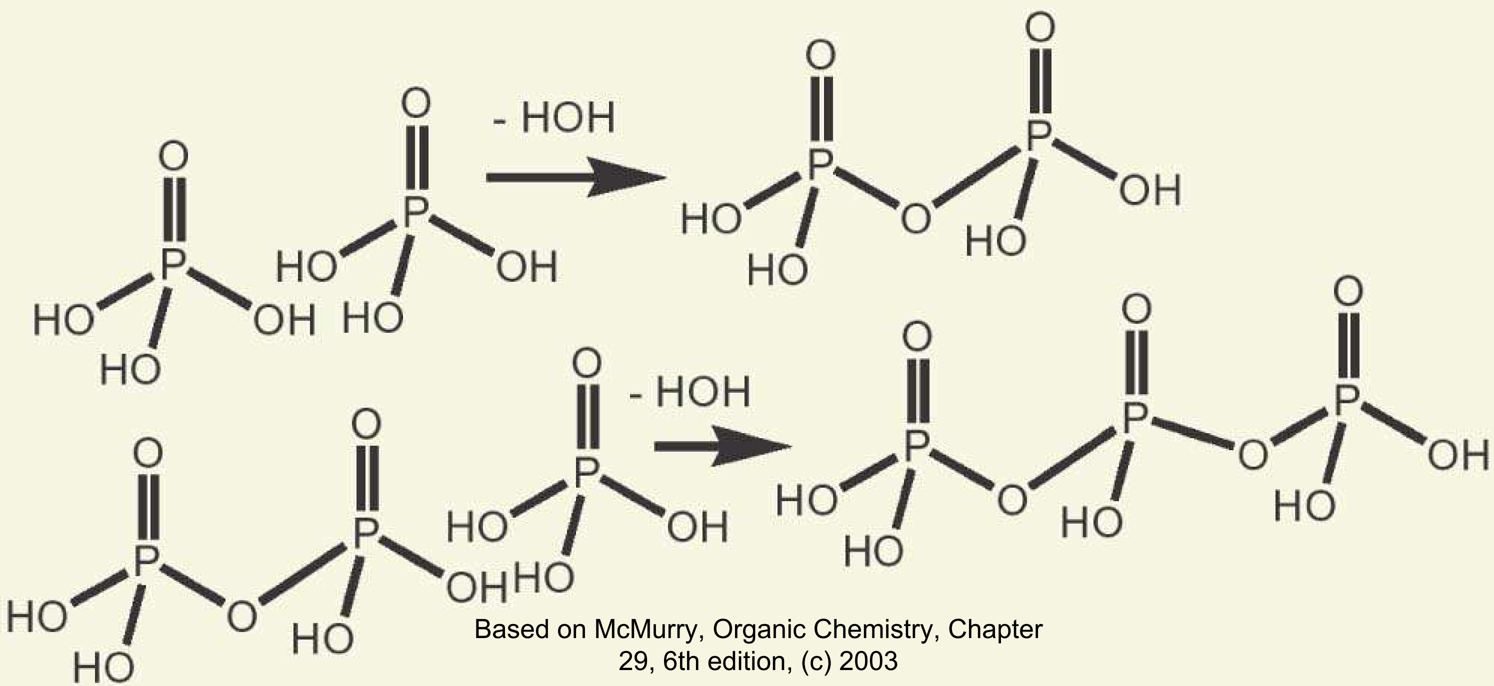
# The Role of ATP

- Catabolic reactions "pay off" in ATP by synthesizing it
- Anabolic reactions "spend" ATP by transferring the terminal phosphate group while regenerating ADP
- The transfer of phosphate from ATP to water (hydrolysis) gives off energy that can be used for another reaction!



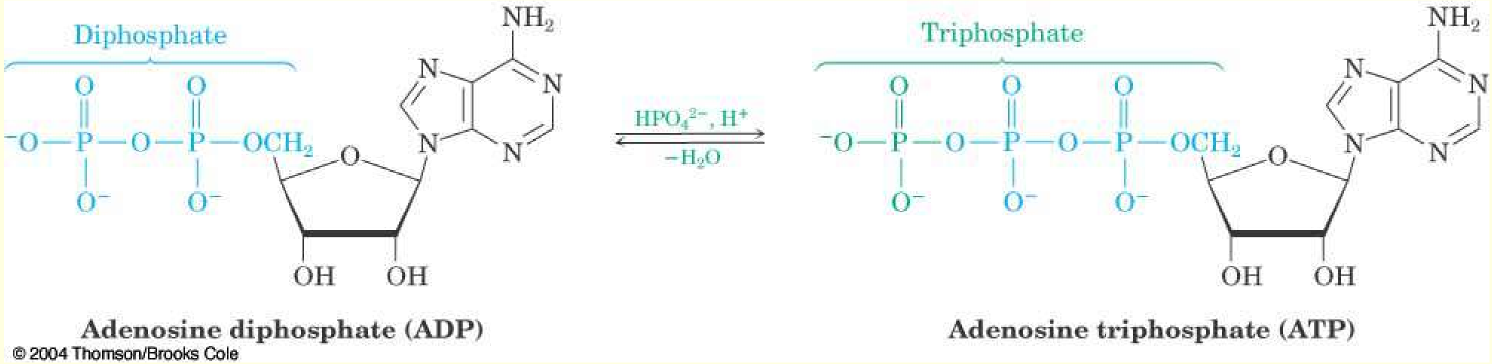
# Phosphoric Acid Anhydrides

- Phosphoric acid ($H_3PO_4$) forms anhydrides through the loss of water between two phosphate units
- This is analogous to carboxylic anhydrides
- A phosphate can form one or two anhydride bonds



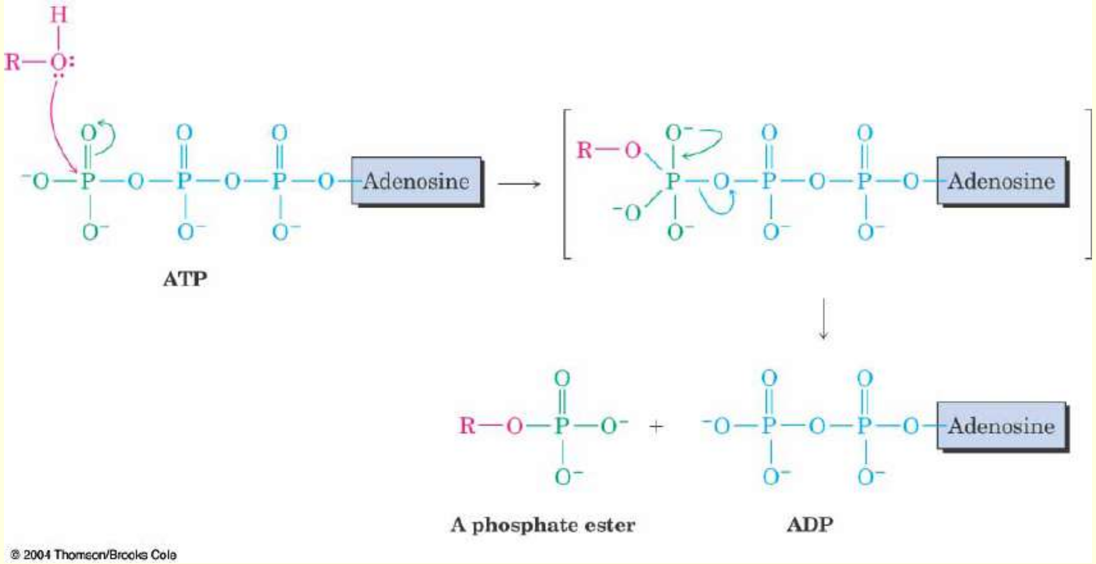
# ATP and ADP

- ATP is an ester of the linear anhydride of three equivalents of phosphoric acid
- The transfer of the terminal phosphate to an acceptor (including water) is an important part of metabolism
- Reaction with water produces ADP



# Phosphorylation

- ATP reacts with alcohols in enzyme reactions to produce esters of phosphoric acids, called phosphate esters
- The process is called phosphorylation

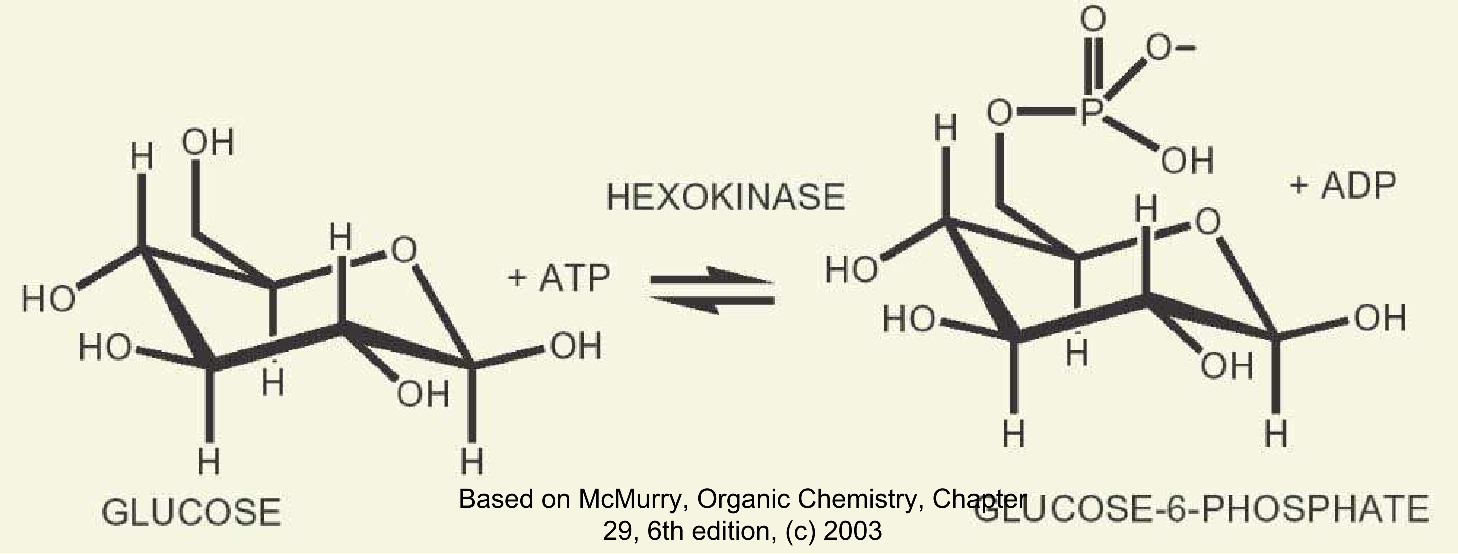


# Metabolic Use of ATP

- An endergonic reaction has a themrodynamic barrier in addition to a kinetic barrier
- Enzymes can cut kinetic barriers only
- Combining the reaction with an exergonic process (hydrolysis of ATP) converts the energetics of the total process
- The endergonic reaction is "coupled" to an energetically favorable reaction so that the *overall* free-energy change for the two reactions together is favorable

# Phosphorylation of Glucose

- The formation of glucose phosphate from glucose and $HPO_4^{2-}$ is energetically unfavorable: $\Delta G°' =$ +13.8 kJ/mol (3.3 kcal/mol)
- The formation of glucose phosphate from ATP is energetically favorable by 16.7 kJ/mol (4.0 kcal/mol)

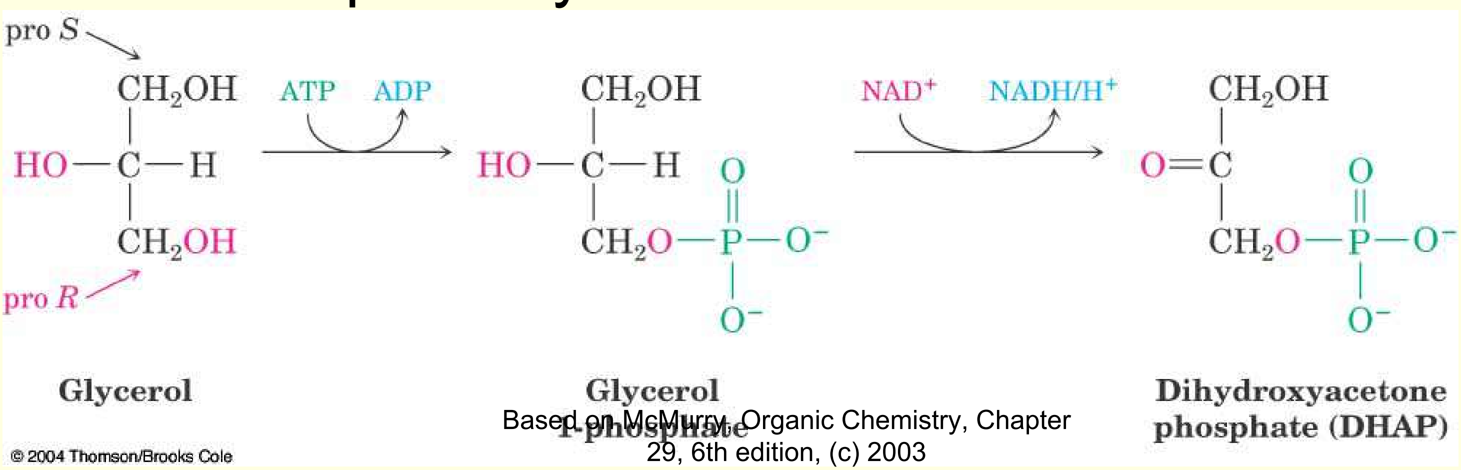


# ATP: the Driver of Biosynthesis

- Enzymes provide a means of coupling an unfavorable reaction to the conversion of ATP to ADP
- The phosphate esters that are formed are intermediates in further processes
- Nature uses phosphates the way chemists use tosylates (to make an OH into a leaving group)

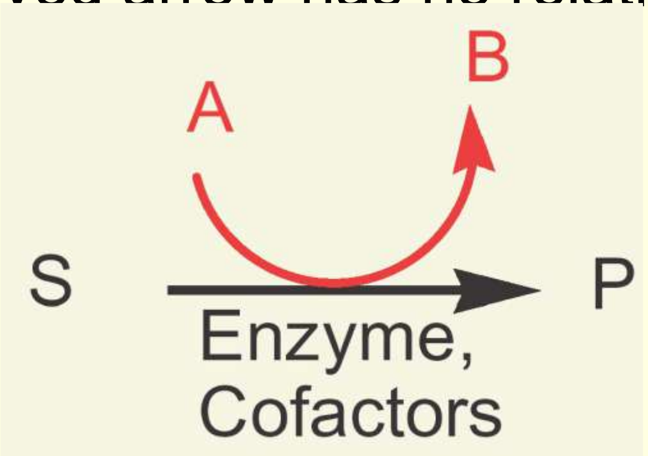
# 29.2 Catabolism of Fats: β-Oxidation

- Esters in fats are hydrolyzed, releasing fatty acids and glycerol
- The fatty acids are transported to cellular mitochondria and oxidized
- Glycerol is converted to dihydroxyacetone phosphate (DHAP), which enters the carbohydrate metabolic pathway



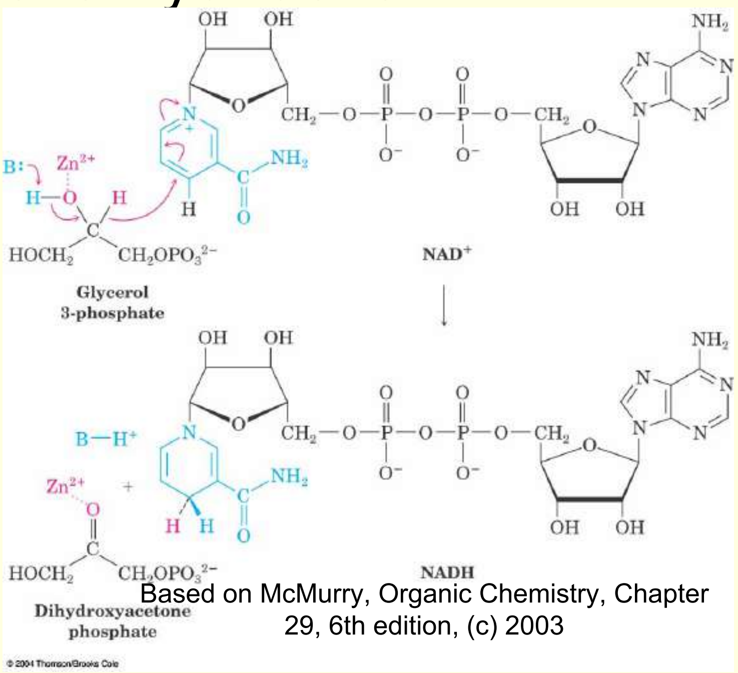
# Writing Metabolic Reactions

- Show the structures of the reactant and product
- Indicate the presence of enzymes and cofactors
- A curved arrow intersecting the usual straight shows a net conversion that is incidental (but essential) to the main reaction
- This type of curved arrow has no relationship to electron flow



# Oxidation With NAD+

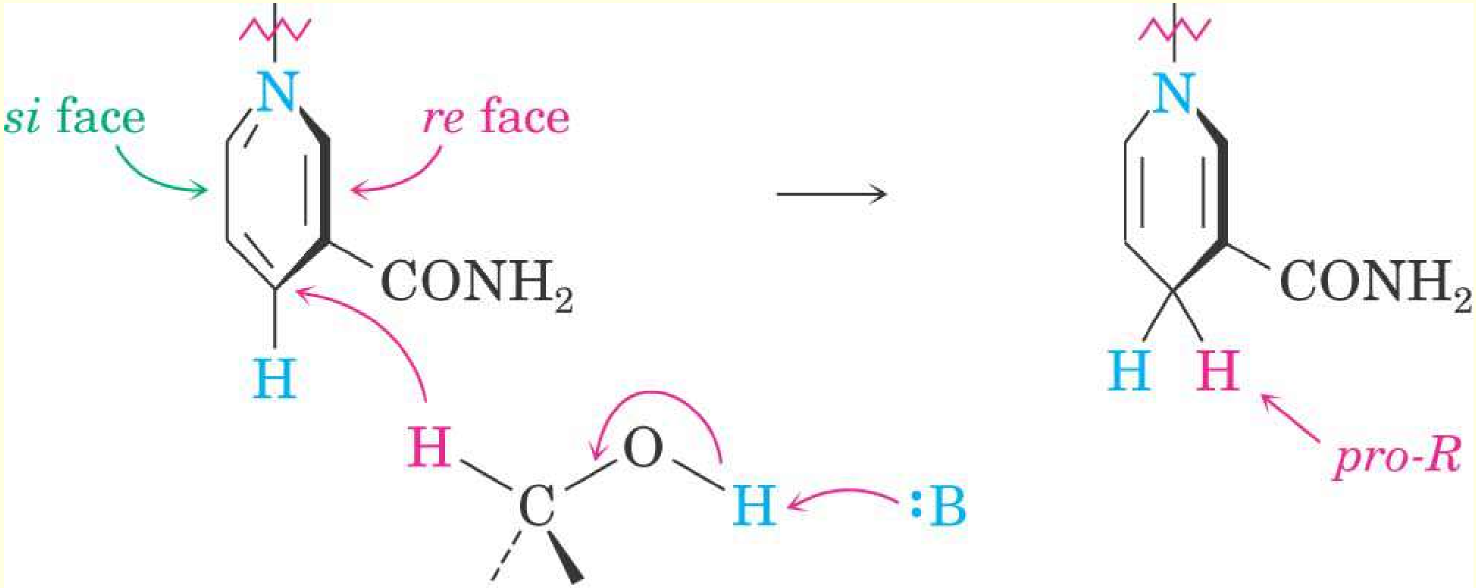
- Enzymes use NAD+ as the equivalent of a chemical oxidizing agent to accept the H from the C-H of an alcohol
- The H is transferred as to give NADH as the equivalent of a hydride ion




Based on McMurry, Organic Chemistry, Chapter 29, 6th edition, (c) 2003

# Stereochemistry of the Transfer of H to $NAD^+$

- A single isomer of NADH is produced with the creation of the chirality center that acquires H
- Note that the second hydrogen removed from the oxidized substrate enters the solution as $H^+$

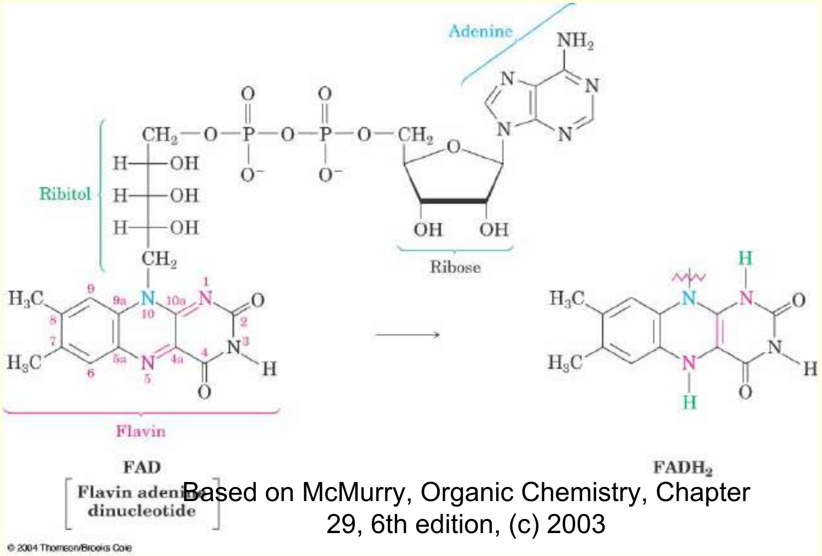


# Steps in Catabolism of Fatty Acids

- Fatty acids are cleaved and oxidized to a set of acetyl groups (as acetyl CoA)
- This is done in a repeating four-step sequence of enzyme-catalyzed reactions called the β-oxidation pathway
- The acetyl groups are then utilized in the citric acid cycle
- The pathway is summarized in Figure 29.2 in the text
- Note that every step requires an enzyme in order to proceed

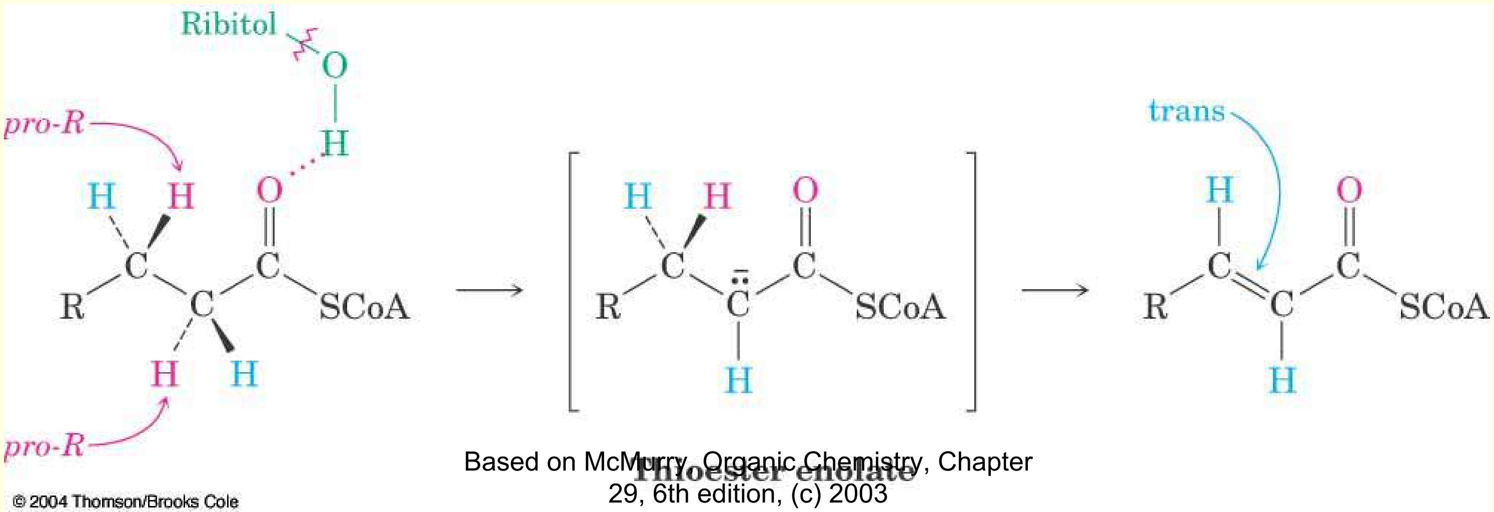
# β-Oxidation – Introduction of a αβ Double Bond

- The fatty acid reacts with ATP and CoA to give a fatty acyl CoA
- Hydrogen atoms are removed from carbons 2 and 3 (α and β positions)
- The coenzyme flavin adenine dinucleotide (FAD) is reduced to $FADH_2$



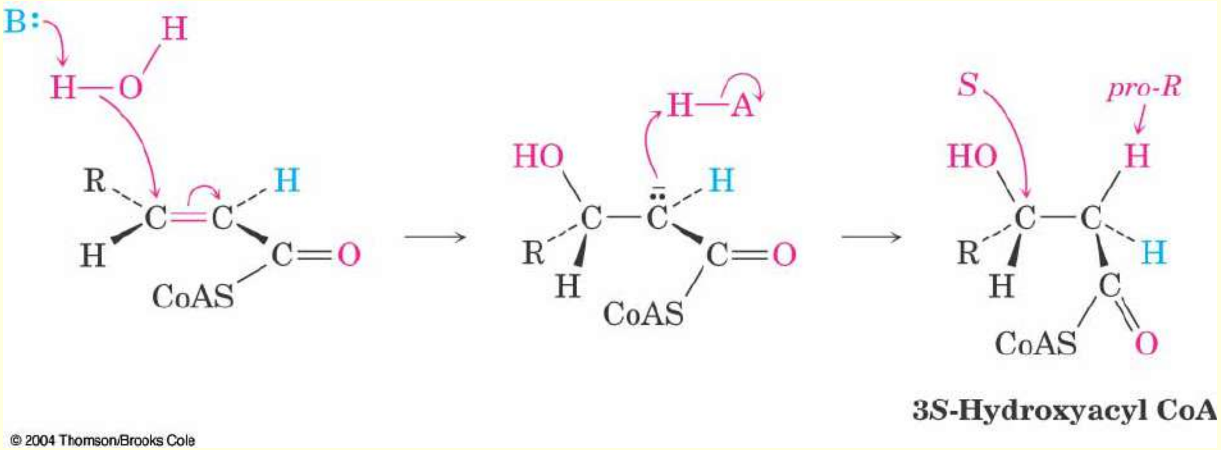
# Stereochemistry of Dehydrogenation

- The *pro-R* hydrogen is from the α position of the acyl CoA and transferred to the flavin
- The *pro-R* hydrogen at the β position is transferred to FAD
- The α,β-unsaturated acyl CoA that results has a trans double bond.



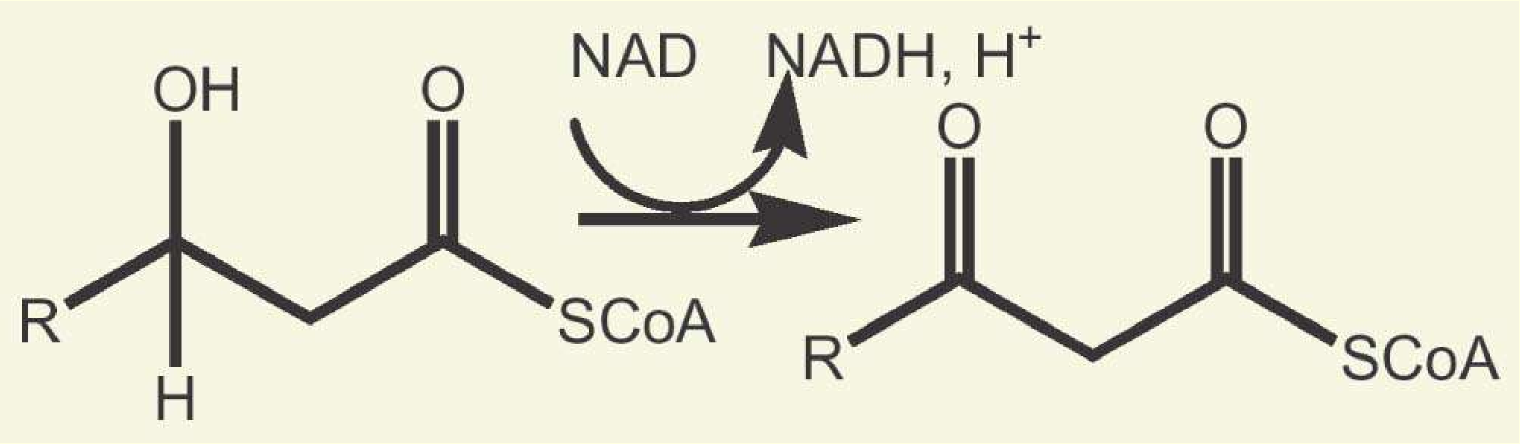
# Addition of Water

- Water adds to the α,β-unsaturated acyl CoA to yield a β-hydroxyacyl CoA
- Water adds to the β carbon of the double bond
- Hydrogen added at the α position resides in the *pro-R* site



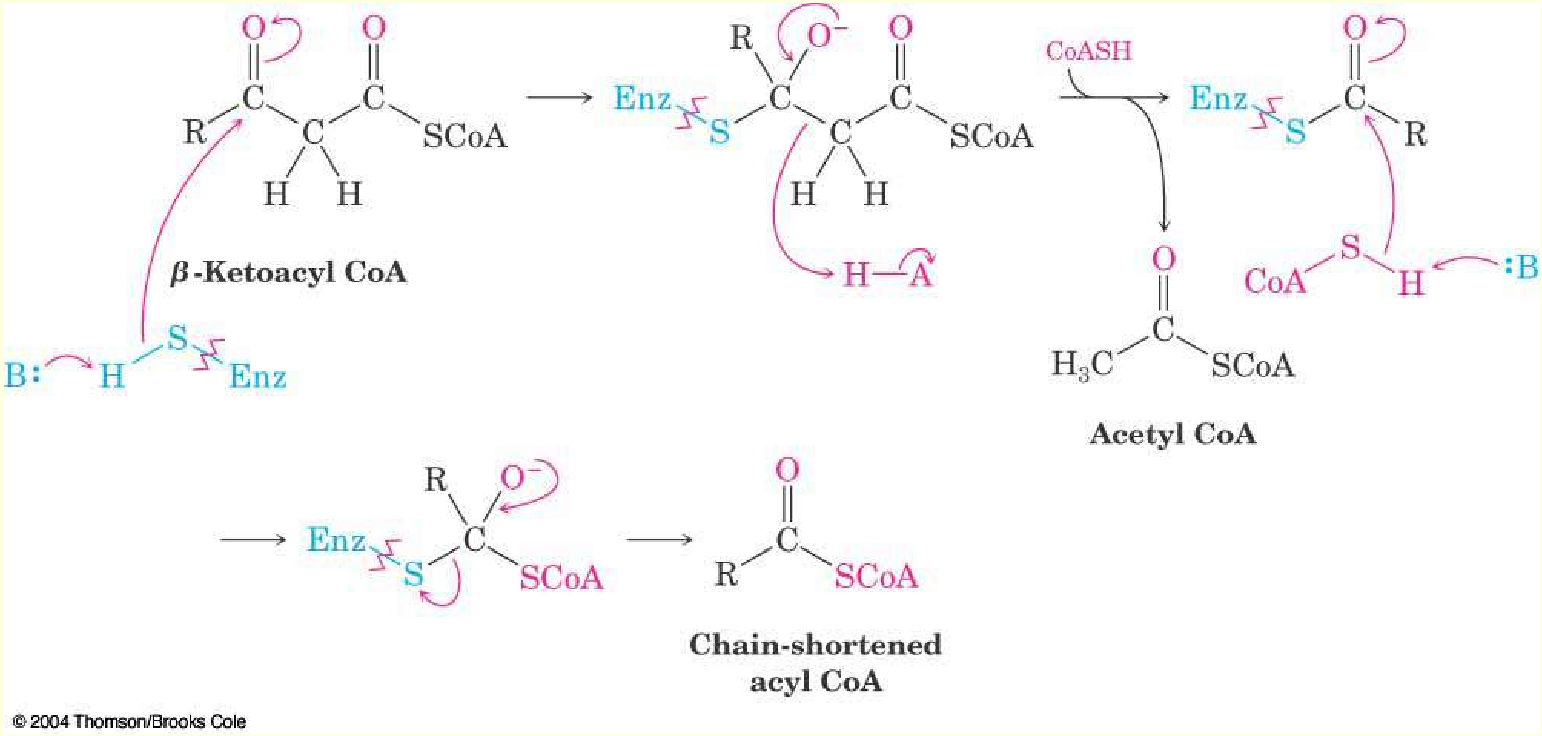
# Oxidation

- The β-hydroxyacyl CoA is oxidized to a β-ketoacyl CoA a dehydrogenase enzyme
- $NAD^+$ is the coenzyme and (accepts 2H to form NADH and $H^+$)



# Chain Cleavage

- Acetyl CoA is split off in the reverse of a Claisen condensation reaction (enzyme-catalyzed)

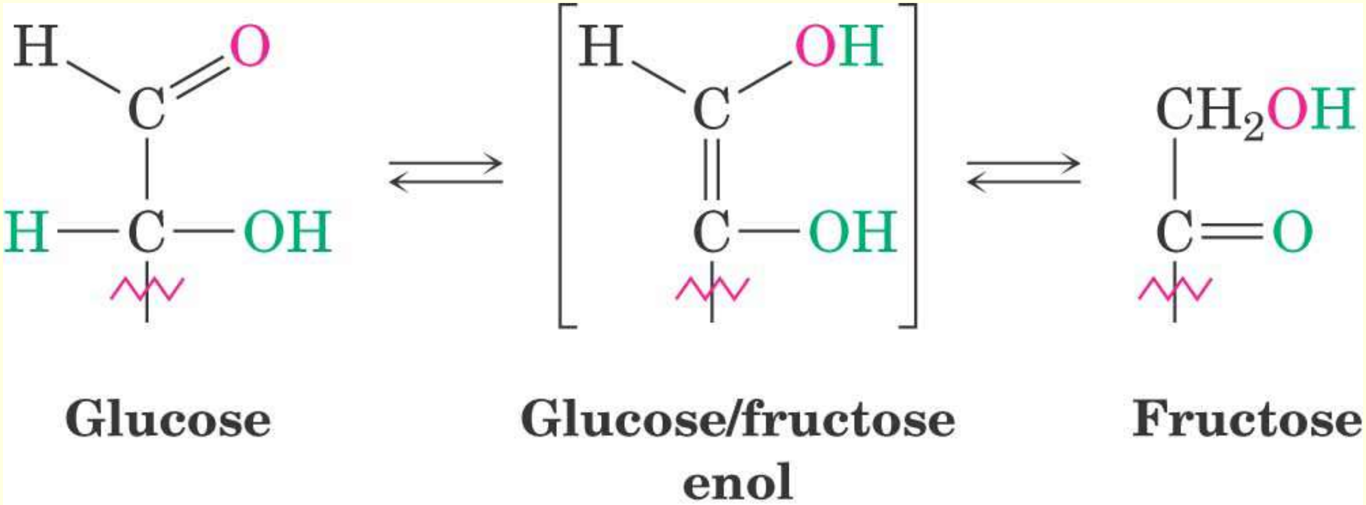


# 29.3 Catabolism of Carbohydrates: Glycolysis

- A pathway that converts glucose ($C_6H_{12}O_6$)into two equivalents of pyruvate, $CH_3COCO_2^-$
- The reactions are shown in Figure 29.4 of the text

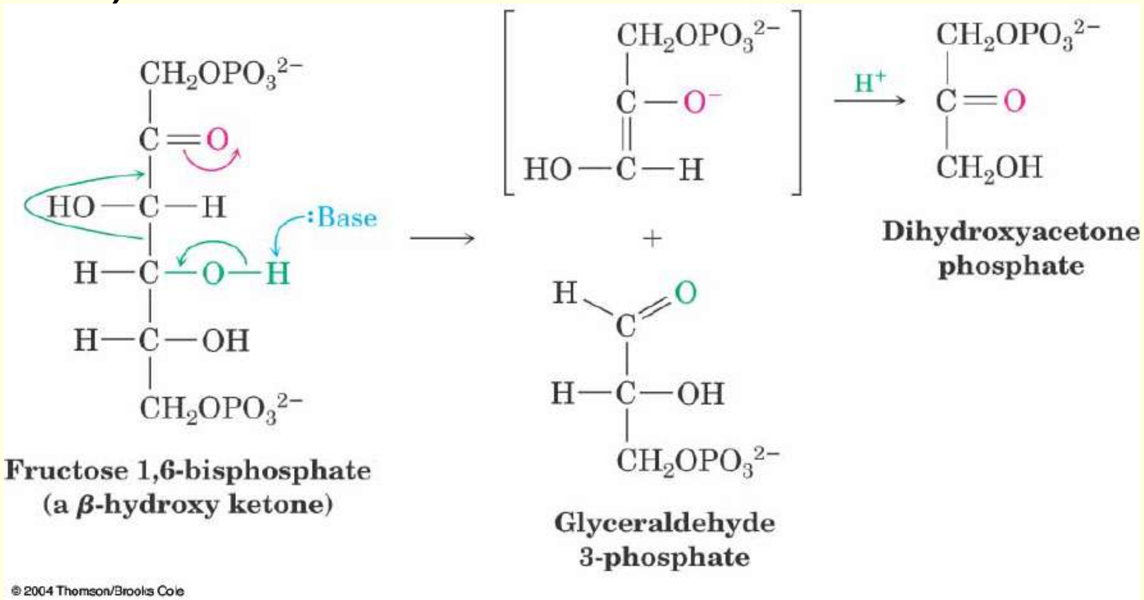
# Phosphorylation and Isomerization

- The $C_6$-OH of glucose is converted to a phosphate ester in a reaction with ATP and an enzyme
- Glucose 6-phosphate is converted to fructose 6-phosphate through an enzyme reaction that involes the enol that is common to the reactant and product



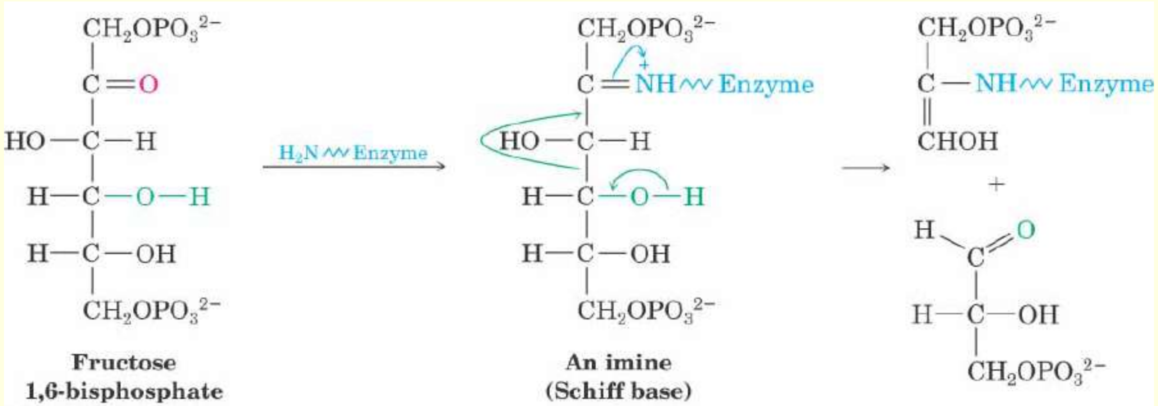
# Cleavage and Isomerization

- Fructose 6-phosphate (F6P) is converted to fructose 1,6-bisphosphate in a reaction with ATP (the prefix "bis-" means that there two of the item that follows)
- F6P is split into two 3 carbon compounds in a retro (= reverse) aldol reaction



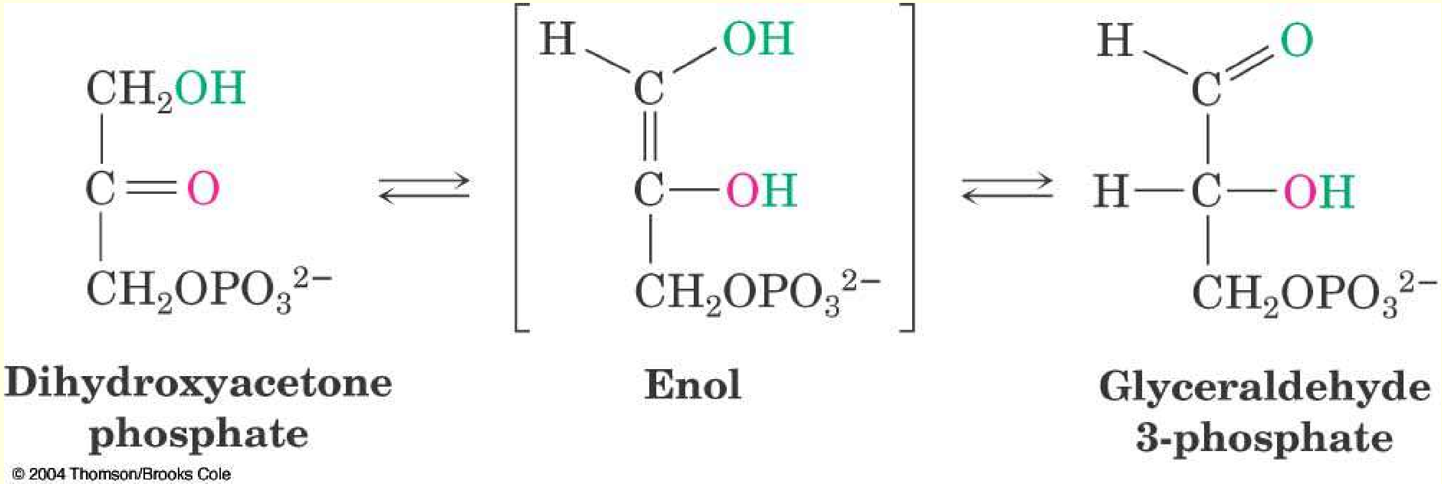
# Aldol Cleavage Mechanism

- Fructose 1,6-bisphosphate combines with the side-chain —$NH_2$ group of a lysine residue on the aldolase enzyme to yield an imine
- This splits the carbon chain into two three carbon species
- Addition of water to the imine regenerates the C=O



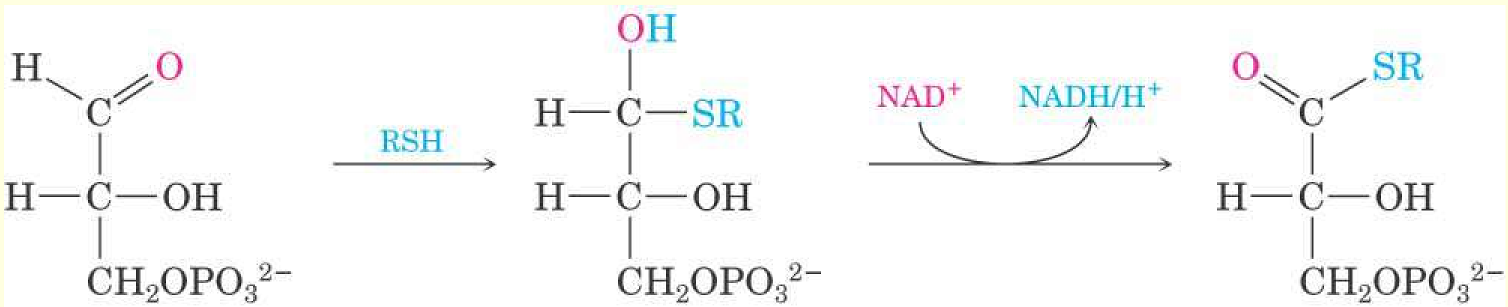
# Triose Phosphate Isomerase

- Glyceraldehyde 3-phosphate continues on in the glycolysis pathway
- Dihydroxyacetone phosphate is isomerized to glyceraldehyde 3-phosphate takes by keto–enol tautomerization through a common enol



# Oxidation and Phosphorylation

- The aldehyde of glyceraldehyde 3-phosphate is oxidized by an enzyme and $NAD^+$
- A thiol on the enzyme adds to the aldehyde
- The addition intermediate is oxidized to produce a thioester



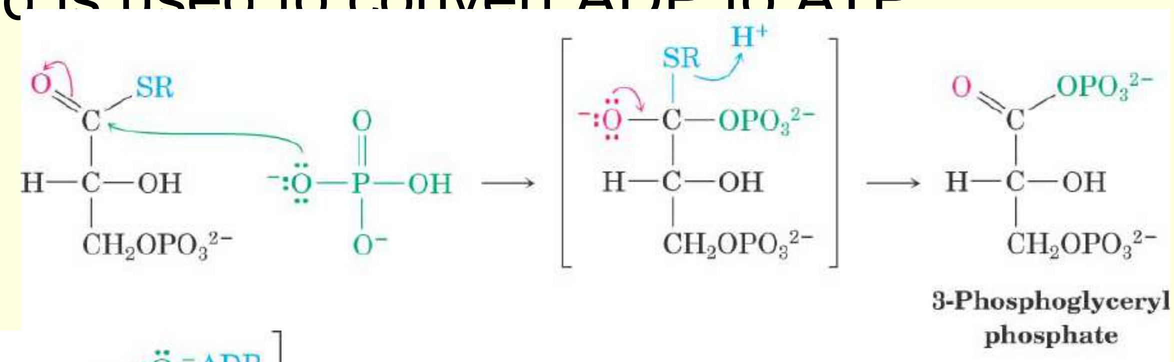

Glyceraldehyde 3-phosphate

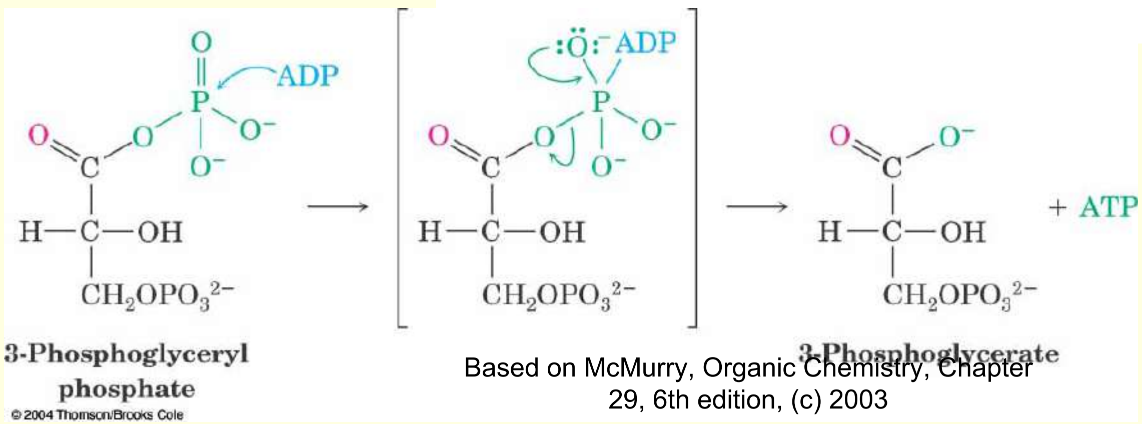
A hemithioacetal

A thioester

# Formation of an Acyl Phosphate to Produce ATP

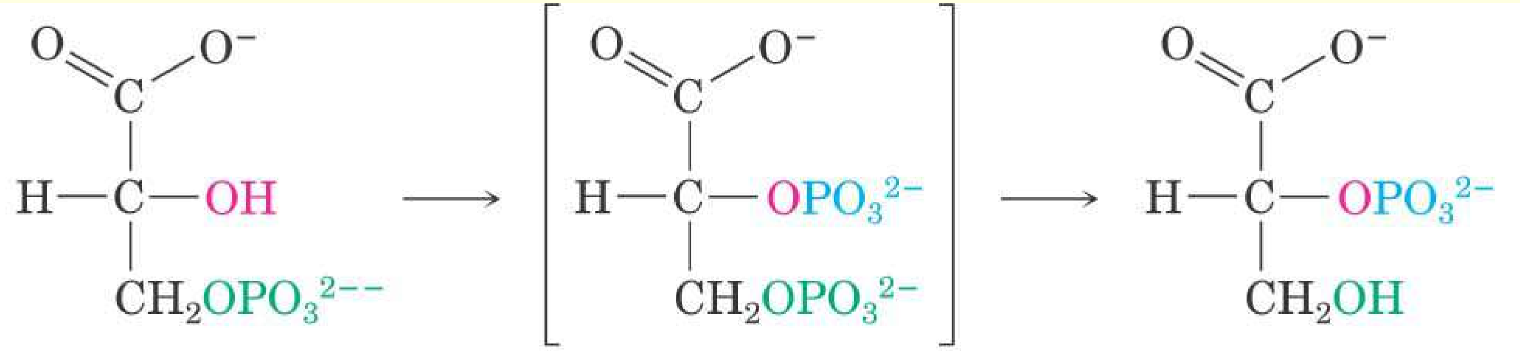
- The thiol is displaced by phosphate, resulting in an acyl phosphate intermediate on the enzyme
- This is at the same energy level as phosphate anhydrides and is used to convert ADP to ATP



# Isomerization of 3-Phosphoglycerate

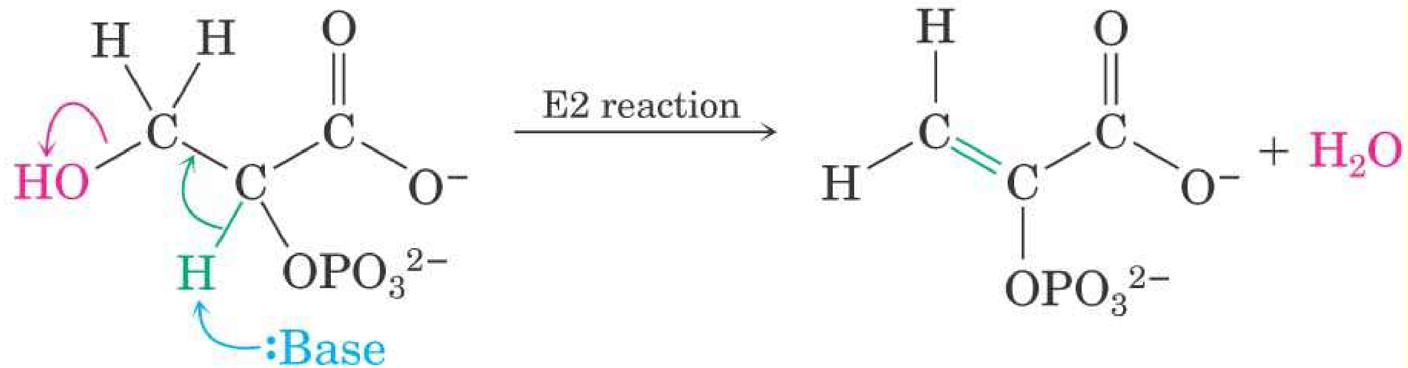
- The resulting 3-phosphoglycerate is converted first to 2,3-bisphosphoglycerate and then to 2-phospho-glycerate




3-Phosphoglycerate 2,3-Bisphosphoglycerate 2-Phosphoglycerate

# Enol Ester Formation

- Water is eliminated from 2-phosphoglycerate, producing a phosphate ester of an enol, phosphoenolpyruvate (PEP)

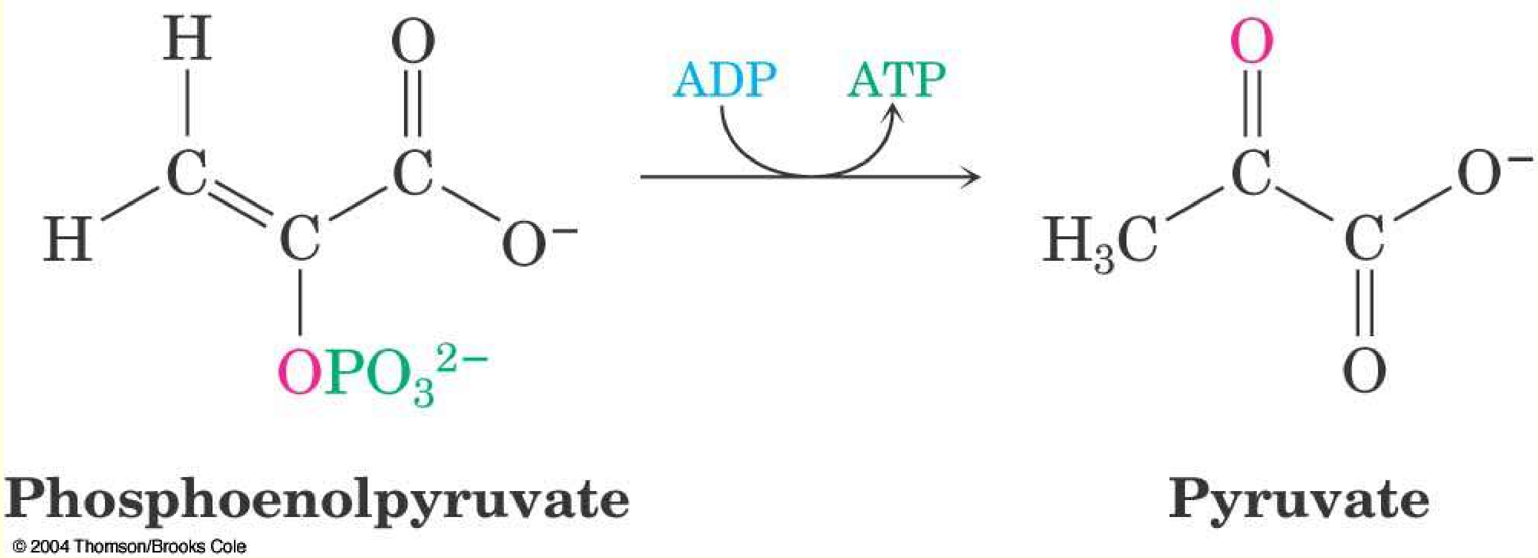



2-Phosphoglycerate
(a β-hydroxy carbonyl compound)

Phosphoenolpyruvate (PEP)

# Formation of ATP from PEP

- The terminal phosphate of ADP adds to the phosphate of PEP, producing ATP and pyruvate (catalyzed by pyruvate kinase)



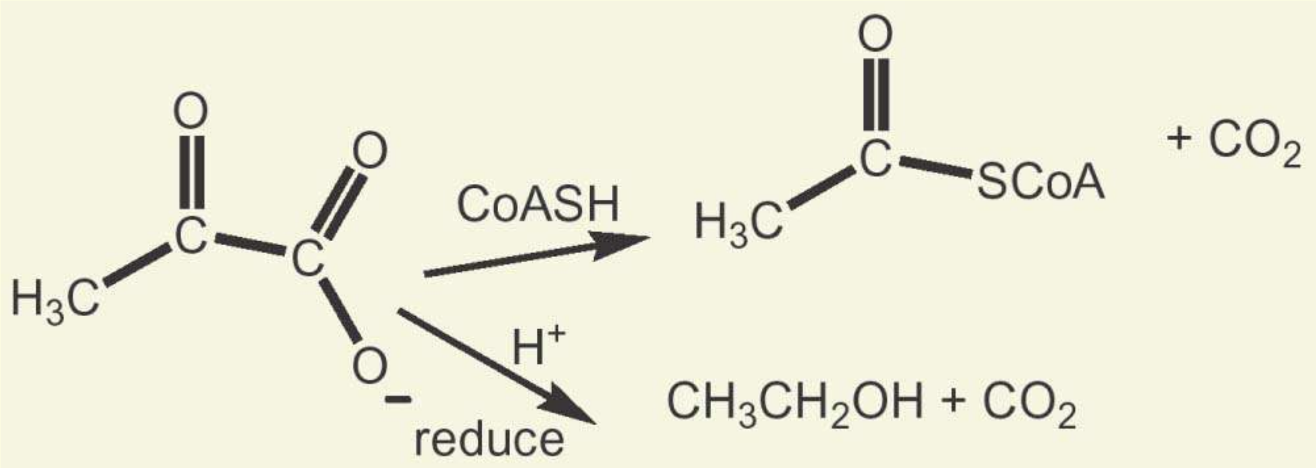
# The Overall Result of Glycolysis

$$C_6H_{12}O_6 + 2\,NAD^+ + 2\,HPO_4^{2-} + 2\,ADP \longrightarrow 2\,CH_3\overset{\displaystyle O}{\overset{\|}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}O^- + 2\,NADH + 2\,ATP + 2\,H_2O + 2\,H^+$$

**Glucose** **Pyruvate**

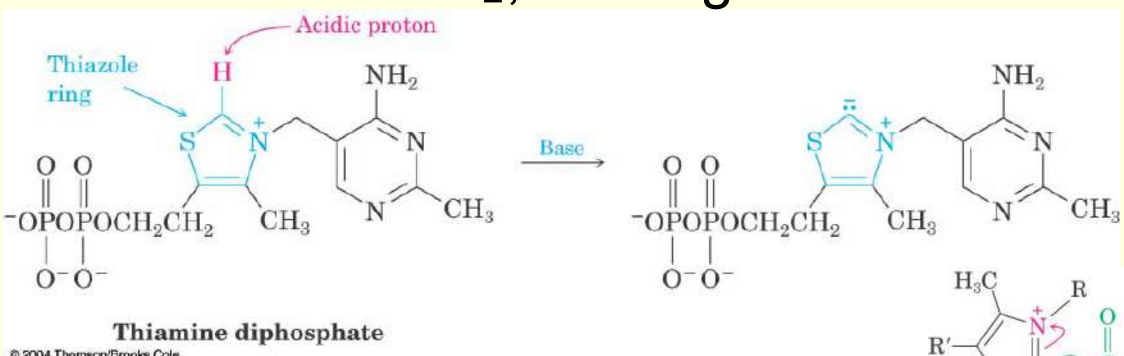
# 29.4 The Conversion of Pyruvate to Acetyl CoA

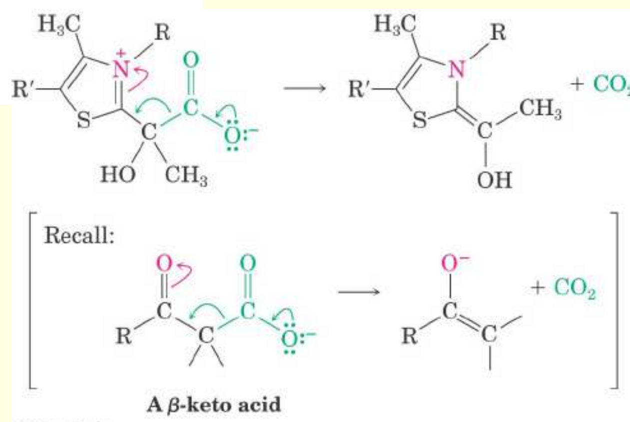
- In higher organisms, pyruvate is converted to acetyl CoA and $CO_2$ in the *pyruvate dehydrogenase complex* (steps are shown in Figure 29.5)
- In yeast, pyruvate is converted to ethanol and $CO_2$ (the basis of making beer, for example)



# Thiamine Diphosphate (TDP) Reacts With Pyruvate

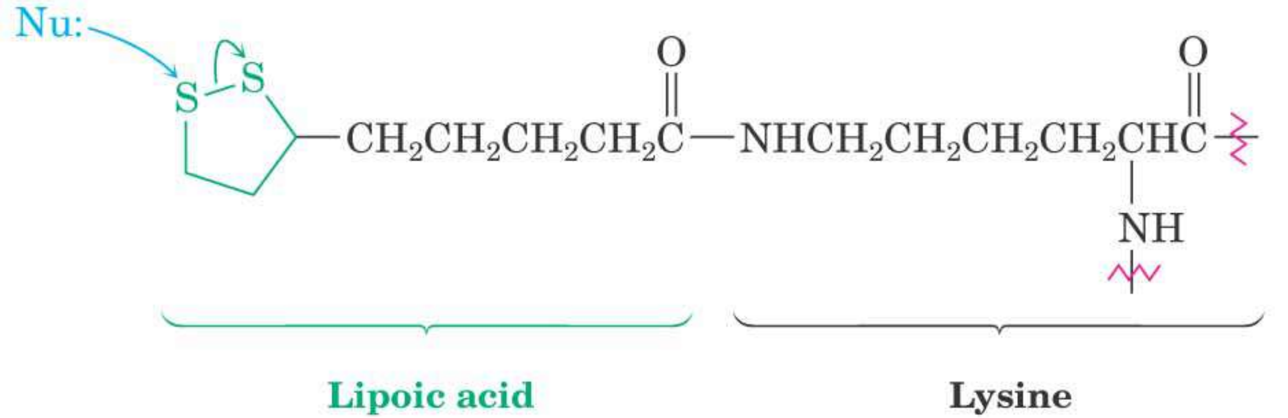
- The proton on C2 of the thiazolium ring of TDP dissociates
- The ylide anion at C2 adds to the keto group of pyruvate covalent addition product on the enzyme, lactyl-TDP, which loses $CO_2$, forming an enamine



# Reaction with Lipoamide

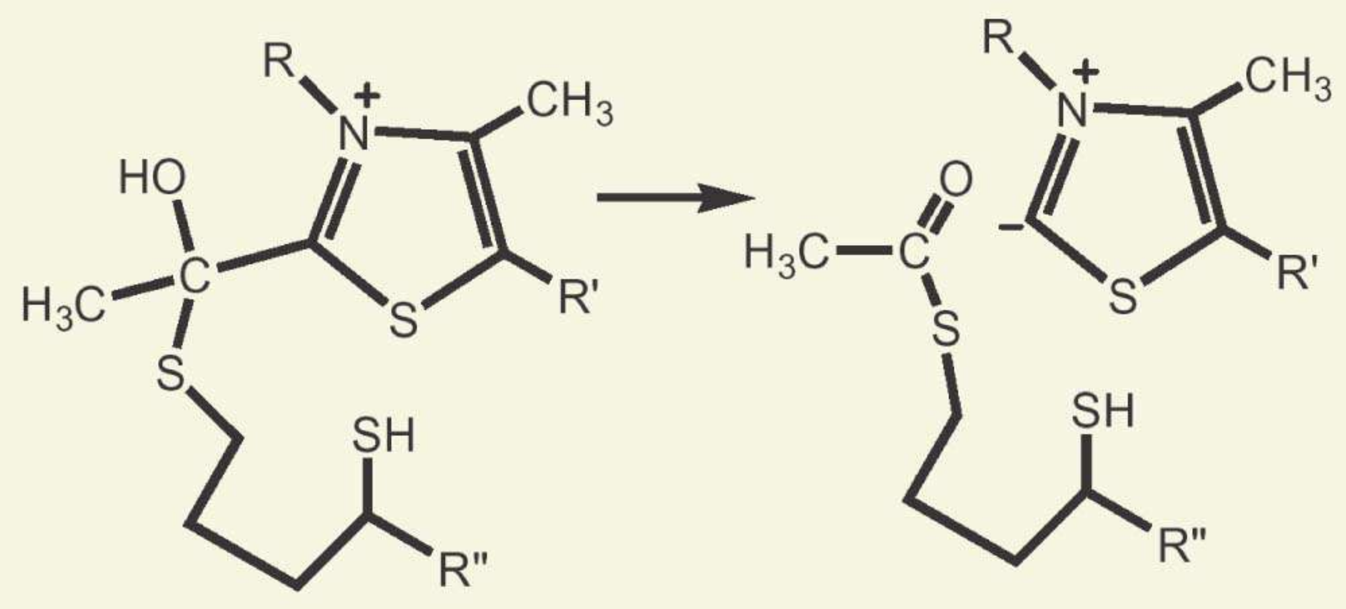
- The enamine adds to the cyclic disulfide in lipoamide, resulting in the ring opening through departure of the second S as a thiolate




**Lipoamide:** Lipoic acid is linked through an amide bond to the side-chain $NH_2$ group of a lysine residue in dihydrolipoyl transacetylase

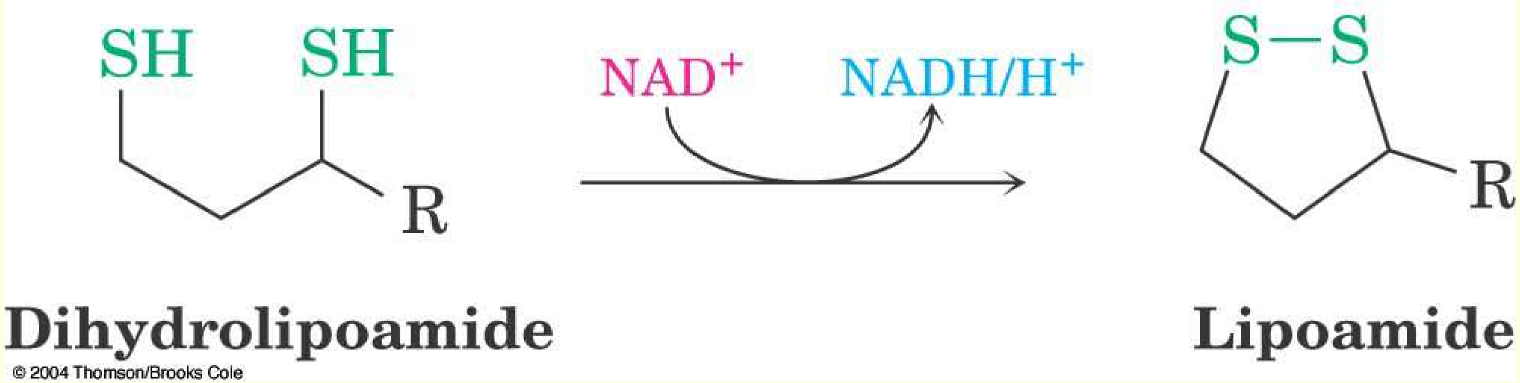
# Elimination of TDP

- The product of the enamine reaction with lipoamide is the TDP conjugate of acetyl dihydrolipoamide
- Elimination of the ylide of TDP from the conjugate gives acetyl dihydrolipoamide



# Acyl transfer

- Acetyl dihydrolipoamide exchanges its thioester for the thiol of coenzyme A to yield acetyl CoA and dihydrolipoamide
- Dihydrolipoamide is oxidized to lipoamide by FAD ($FADH_2$ is in turn oxidized to FAD by $NAD^+$)



# 29.5 The Citric Acid Cycle

- The eight steps of the citric acid cycle are given in Figure 29.6
- This cycle of reactions converts acetyl CoA to two equivalent of $CO_2$ and CoA with transfer of energy to numerous acceptors and formation of reduced coenzymes

# The Cycle Requires Oxygen

- Oxidizing coenzymes $NAD^+$ and FAD are needed for key reduction steps
- The reduced coenzymes NADH and FADH2 are reoxidized via the electron-transport chain
- This relies on oxygen as the ultimate electron acceptor

# Steps 1 and 2: Addition to Oxaloacetate

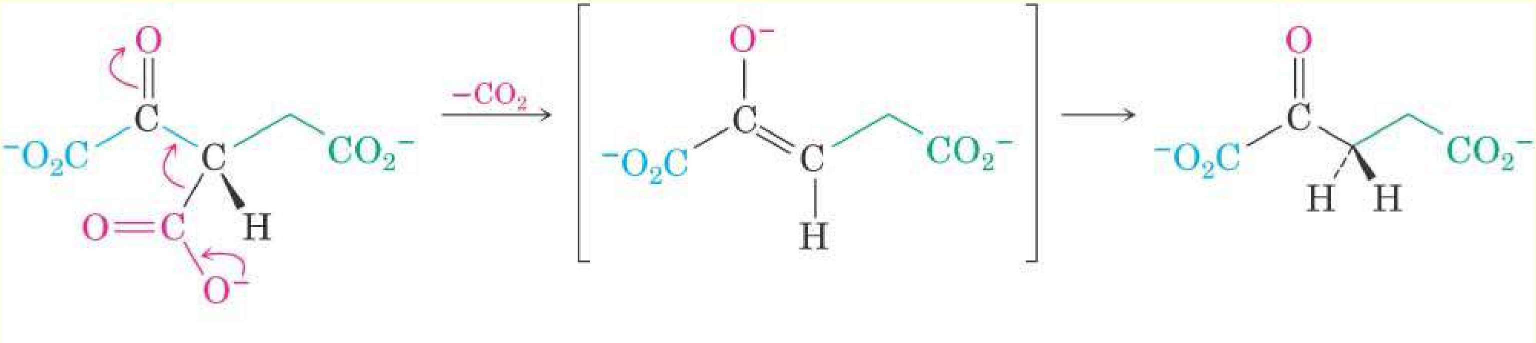
- The carbon of the $CH_3$ of the acetyl group of acetyl CoA adds to ketone carbonyl group of oxaloacetate to give (*S*)-citryl CoA
- (*S*)-Citryl CoA hydrolyzes to citrate and CoA

# Conversion of Citrate to Isocitrate

- The isomerization occurs in two steps and is catalyzed by aconitase
- Dehydration of citrate (in the pro-*R* branch sel) gives *cis*-aconitate
- Addition of water to *cis*-aconitate gives isocitrate
- The net effect is a 1,2 shift od the OH

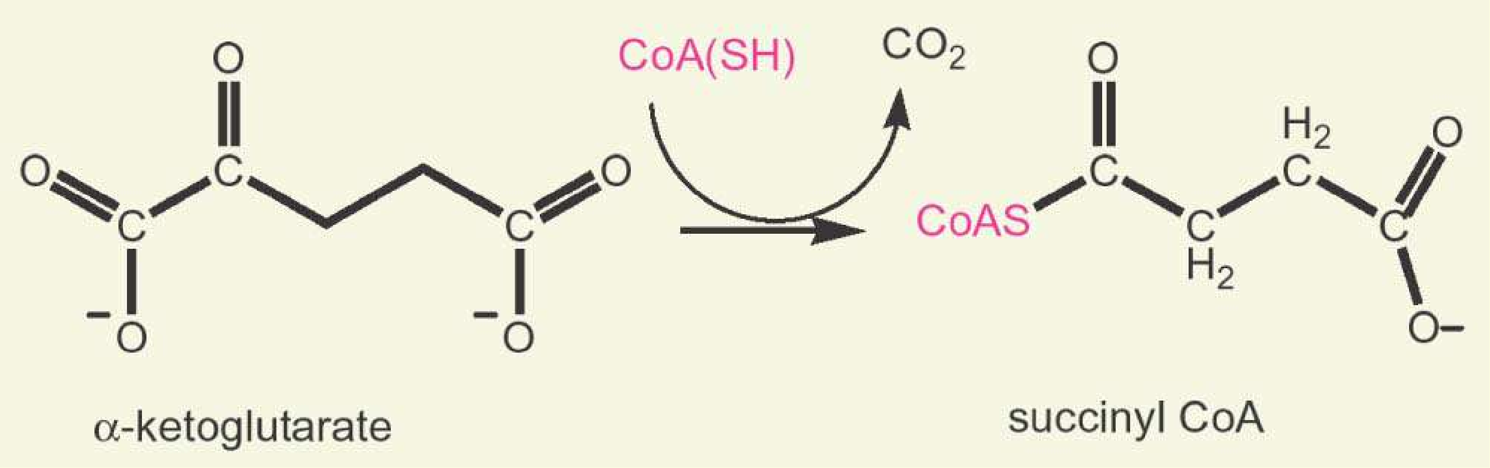
# Oxidative Decarboxylation

- The OH in isocitrate is oxidized ($H^-$ from CH(OH) is transferred to $NAD^+$) to C=O (oxalosuccinate)
- Loss of $CO_2$ gives α-ketoglutarate



# Formation of Succinyl CoA from α-Ketoglutarate

- Multi-step process catalyzed by an enzyme complex
- The α-keto acid loses $CO_2$ in a step with TDP as a cofactor

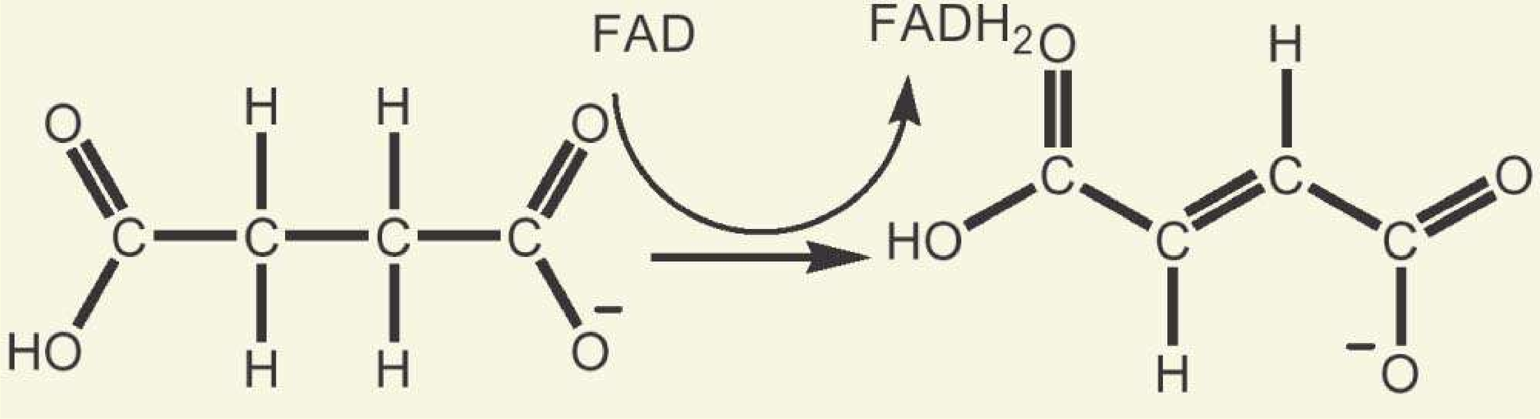


# Hydrolysis and Dehydrogenation of Succinyl CoA

- Succinyl CoA is hydrolyzed to succinate coupled with formation of guanosine triphosphate (GTP) from GDP
- Reaction is via an acyl phosphate

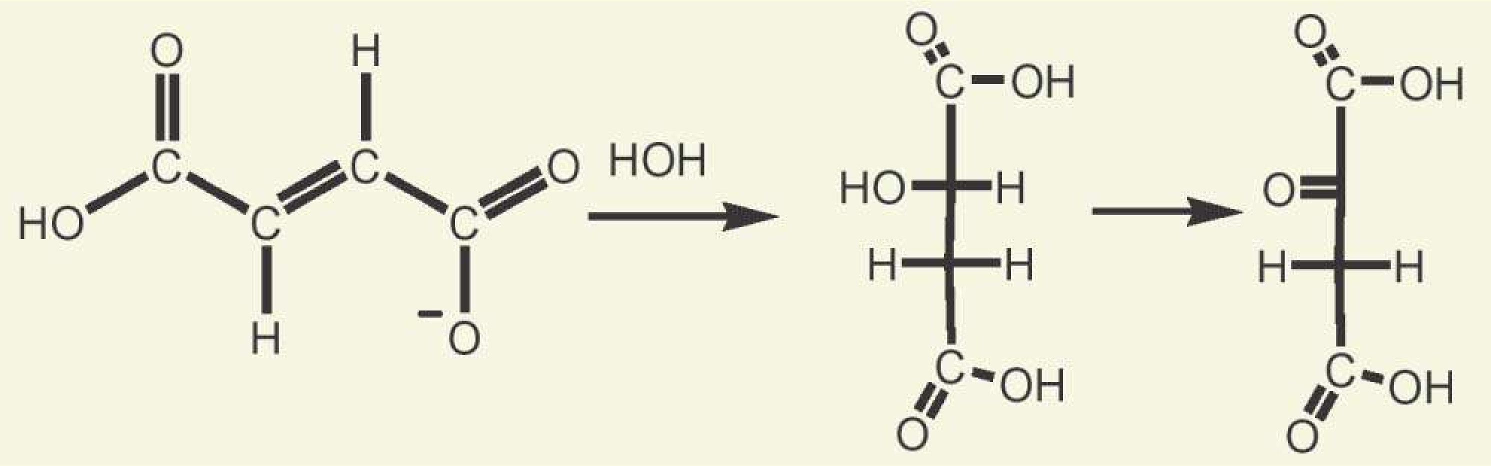
# Dehydrogenation of Succinate

- H's are transferred to FAD by succinate dehydrogenase to give fumarate
- Note that the ends of succinate cannot be distinguished



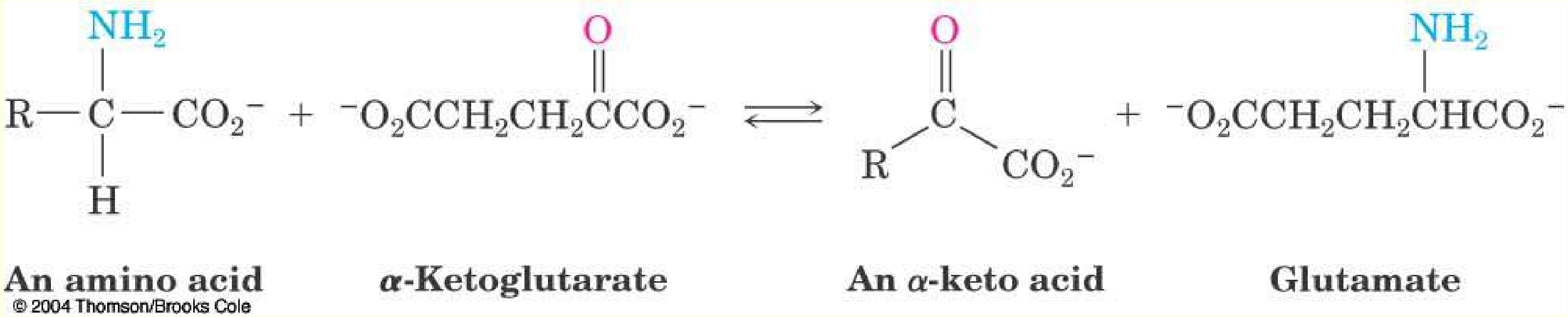
# Formation of Malate and Oxaloacetate

- Fumarase catalyzes the addition of HOH to fumarate to give L-malate
- Malate dehydrogenase and $NAD^+$ oxidize the C-OH to C=O, forming oxaloacetate, which continues the cycle again



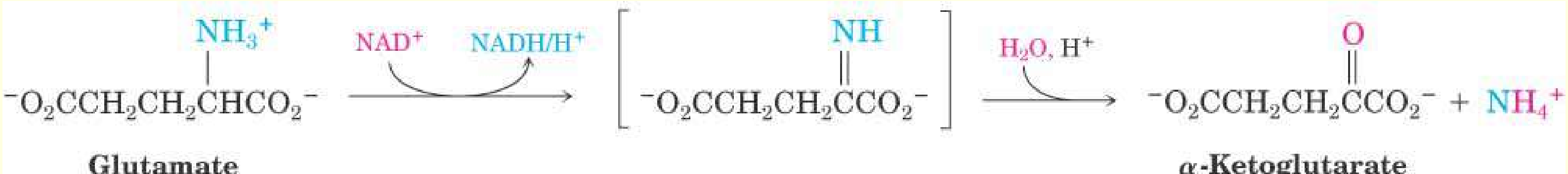
# 29.6 Catabolism of Proteins: Transamination

- In general the $NH_2$ is removed first
- This is usually done through transamination in which the —$NH_2$ group substitutes for the O in a C=O, creating a C=O where the C-$NH_2$ had been



# Pyridoxal Phosphate

- This is a cofactor that is functionally an aldehyde
- It accepts the $NH_2$, forming an imine
- The conversion of glutamate to $\alpha$-keto-glutarate is typical
- The mechanism is shown in Figure 29.7

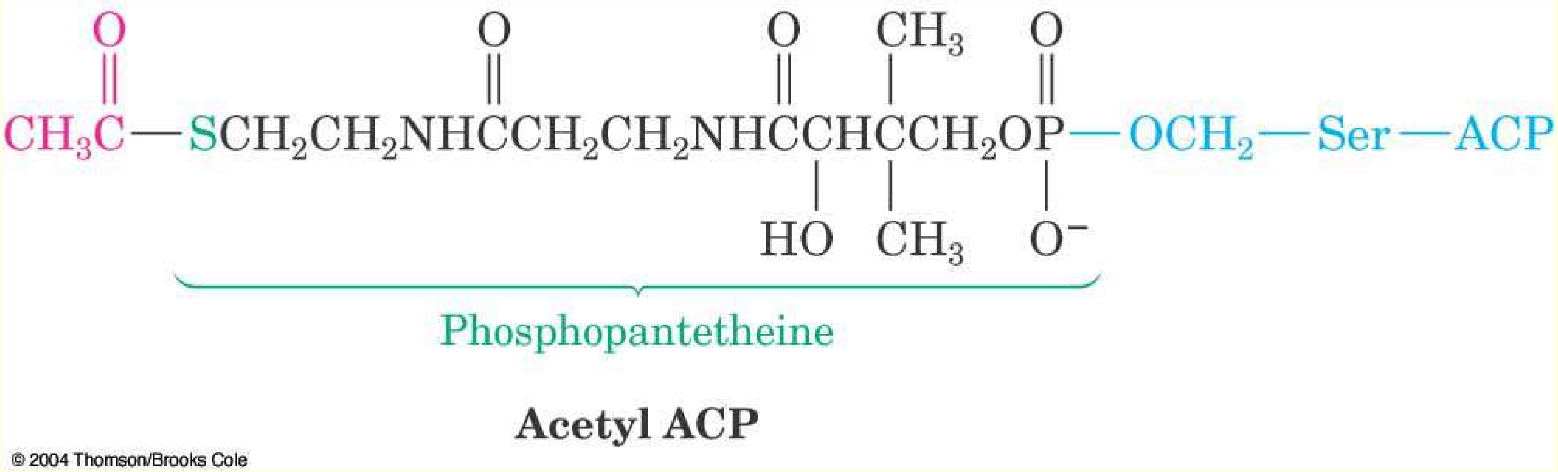


# 29.7 Fatty-Acid Biosynthesis

- An overview is shown in Figure 29.8
- Note that the anabolic pathway differs from the catabolic pathway in more ways than just being opposite in direction

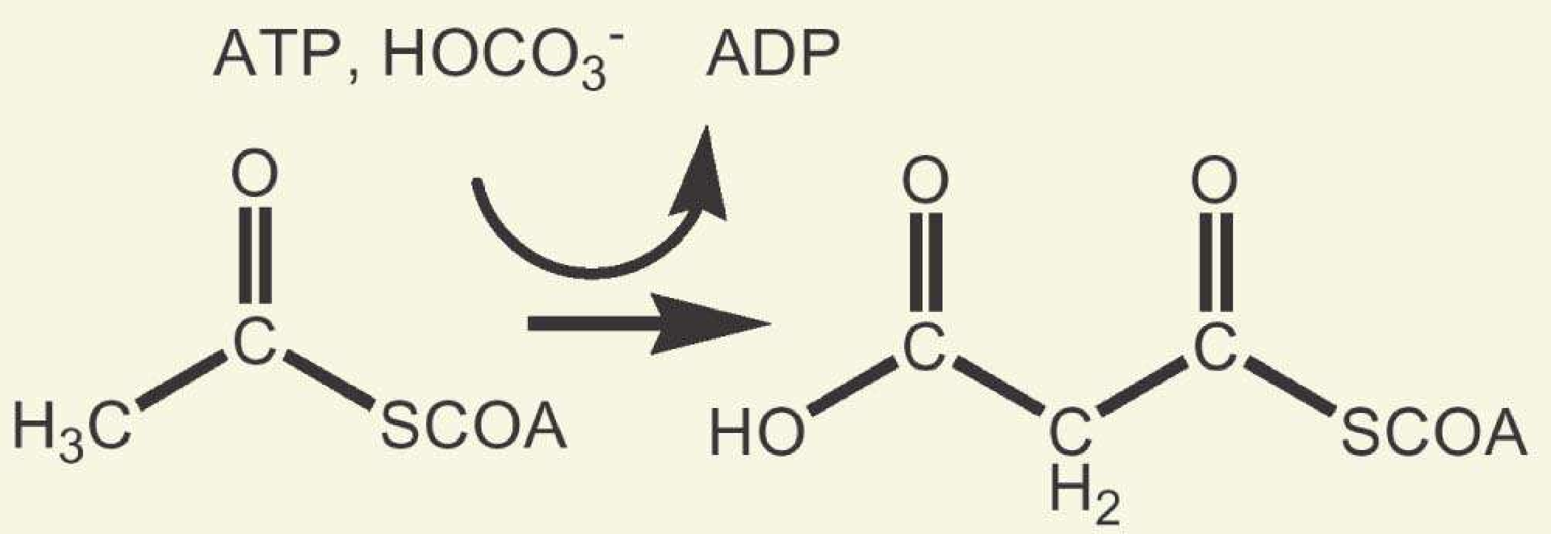
# Fatty-Acid Synthesis Begins WithAcyl Transfers

- The acetyl group of acetyl CoA is transferred to the ACP (acyl carrier protein thioester) via phosphopantetheine (details vary with species)
- The thioester is switched to a cysteine



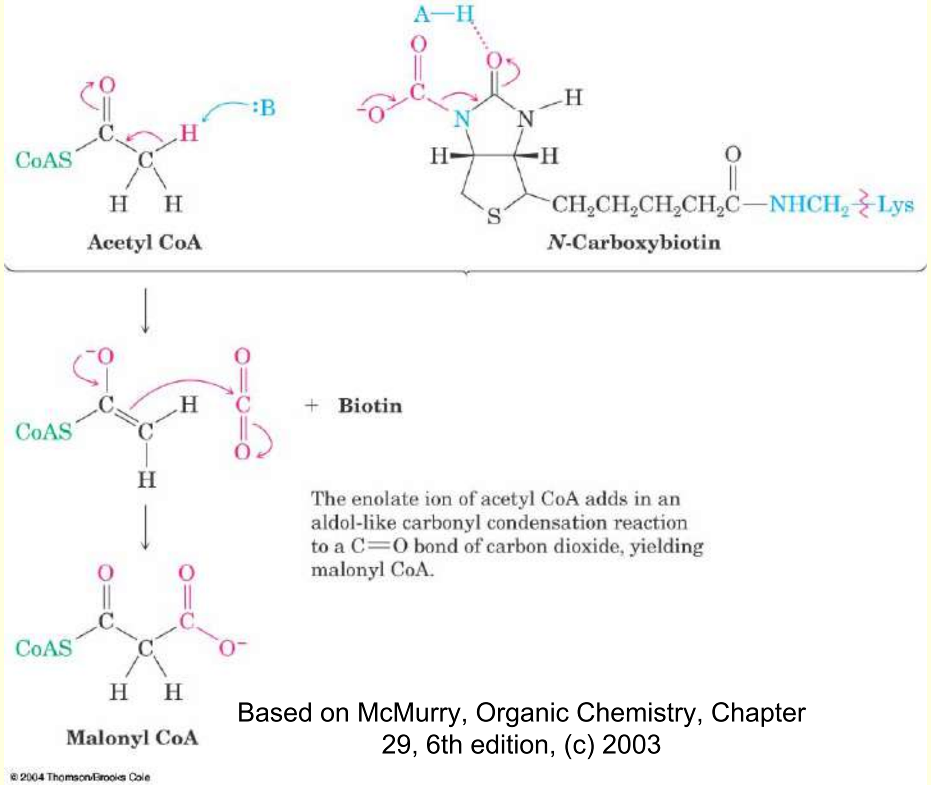
# Fatty Acid Biosynthesis -Carboxylation and Acyl transfer

- Acetyl CoA is converted to malonyl CoA by replacement of a proton by a carboxyl
- The reaction uses $HCO_3^-$ and ATP to yield malonyl CoA plus ADP



# Biotin – Coenzyme for Carboxylation Enzymes

- Biotin first reacts with bicarbonate ion to give N-carboxybiotin
- This transfers the $CO_2$ group



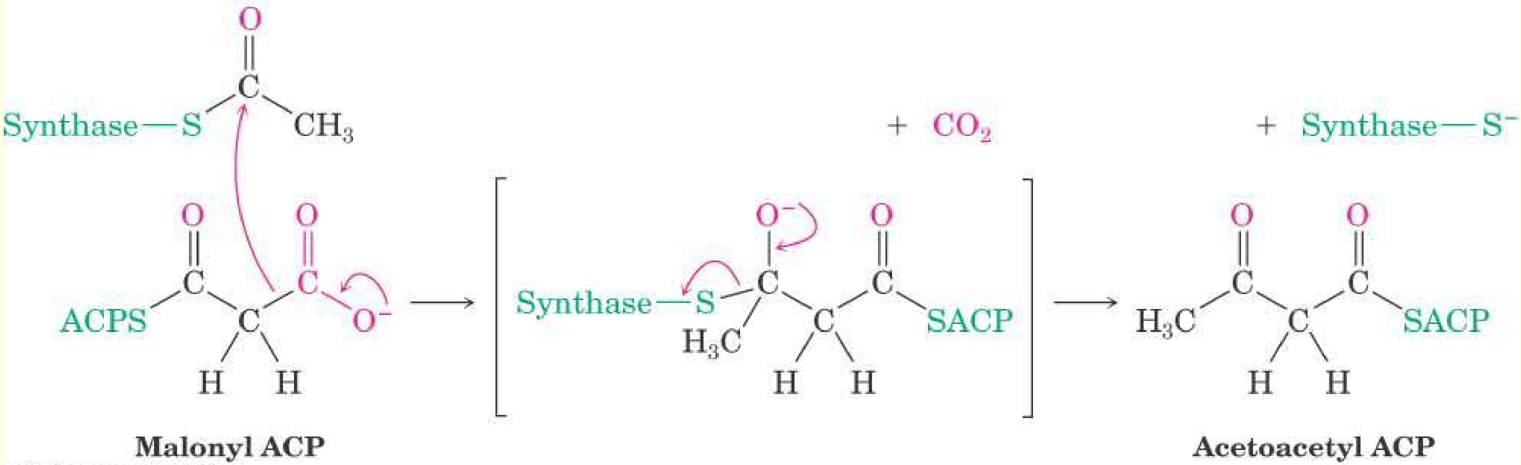

Based on McMurry, Organic Chemistry, Chapter 29, 6th edition, (c) 2003

# Formation of Malonyl ACP

- The malonyl group is transferred from malonyl CoA to ACP
- This places the malonyl group on an ACP arm of the multienzyme synthase complex
- Acetyl and malonyl groups are bound to the same enzyme

# Condensation – the Key Step

- Decarboxylation of malonyl ACP gives an enolate ion that adds to the acetyl carbonyl, producing acetoacetyl ACP



# Reduction and dehydration

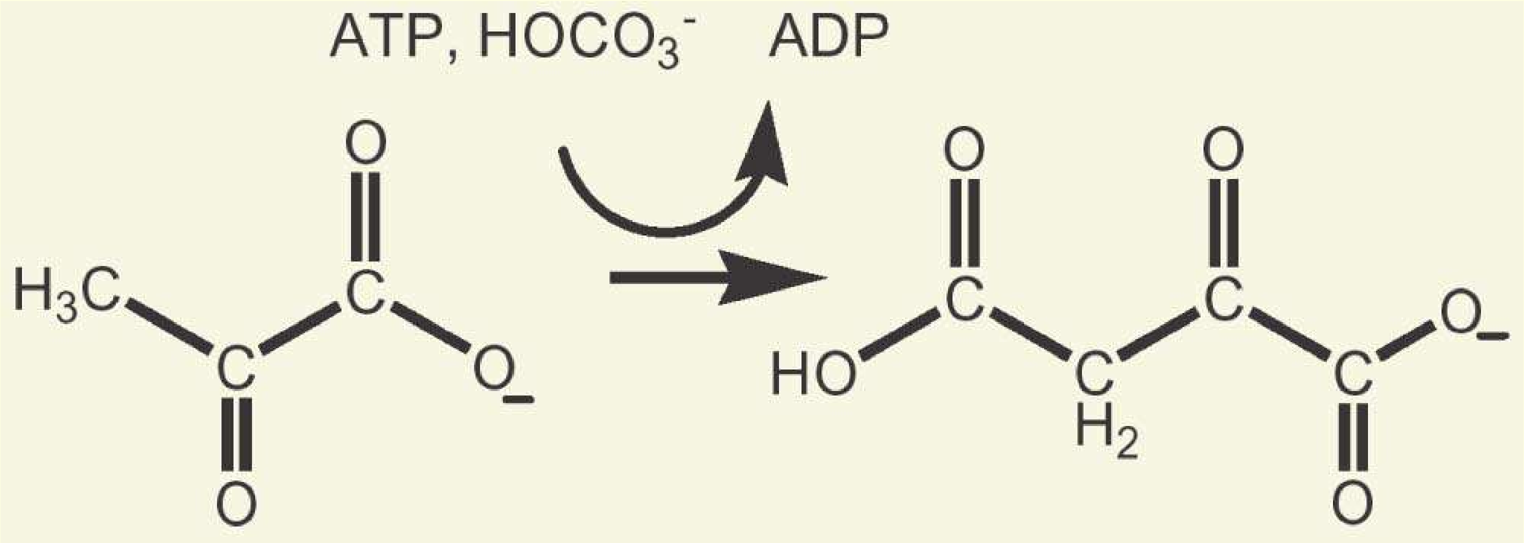
- The C=O in acetoacetyl ACP is reduced to C-OH with NADPH, an analogue of NADH having an additional phosphate
- Dehydration of the β-hydroxy thioester yields trans-crotonyl ACP
- The C=C of crotonyl ACP is reduced with NADPH to yield butyryl ACP
- The reaction is repeated until the appropriate length is obtained

# 29.8 Carbohydrate Biosynthesis: Gluconeogenesis

- The biosynthetic pathway by which organisms make glucose from pyruvate
- The gluconeogenesis pathway is shown in Figure 29.9

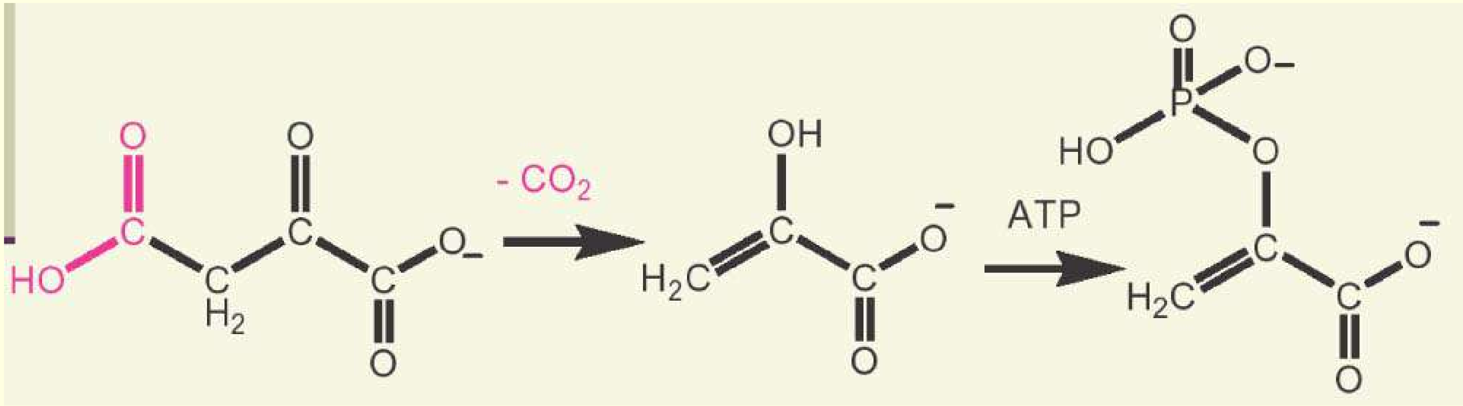
# Gluconeogenesis Carboxylation(Glucose from Pyruvate)

- Carboxylation of pyruvate yields oxaloacetate
- Requires ATP and biotin as a carrier of CO2



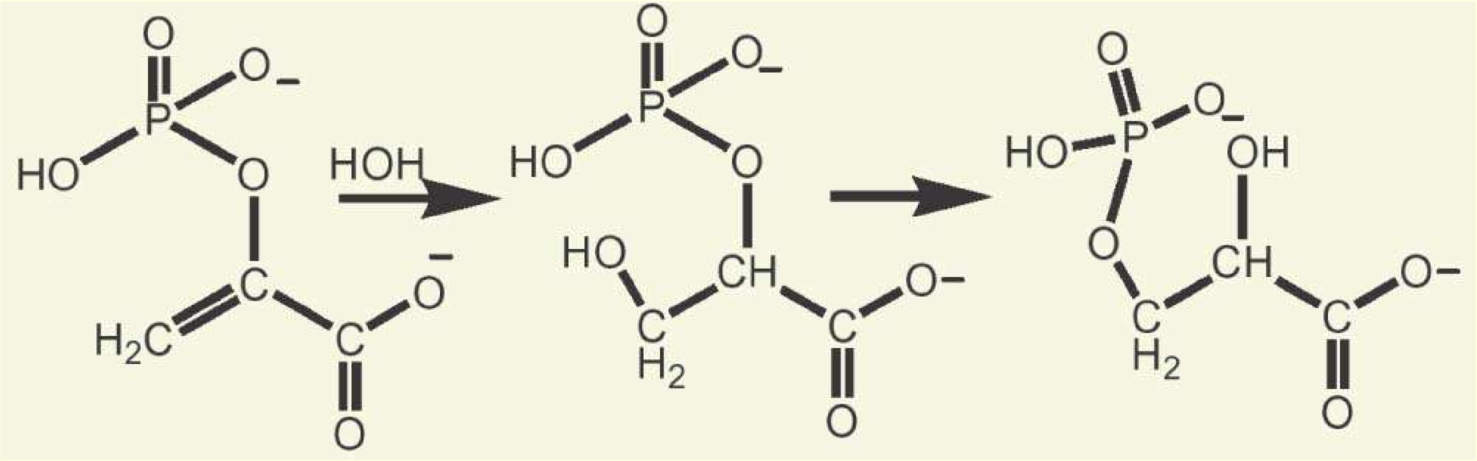
# Decarboxylation of Oxaloacetate

- Loss of $CO_2$ from the β-ketoacid and phosphorylation of the resultant pyruvate enolate occur concurrently to give phosphoenolpyruvate



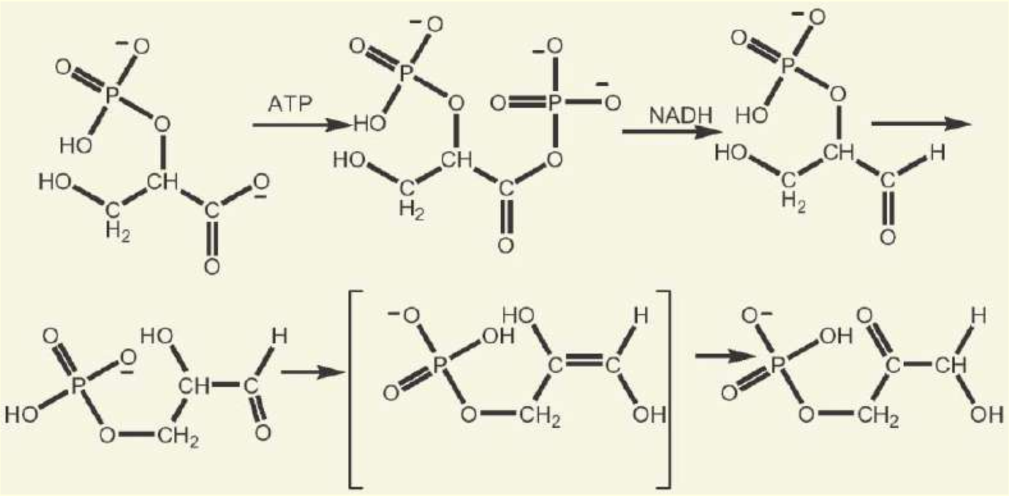
# Hydration and Isomerization

- Addition of water to the double bond of phosphoenolpyruvate produces 2-phosphoglycerate
- The phosphate migrates from C2 to C3, yielding 3-phosphoglycerate



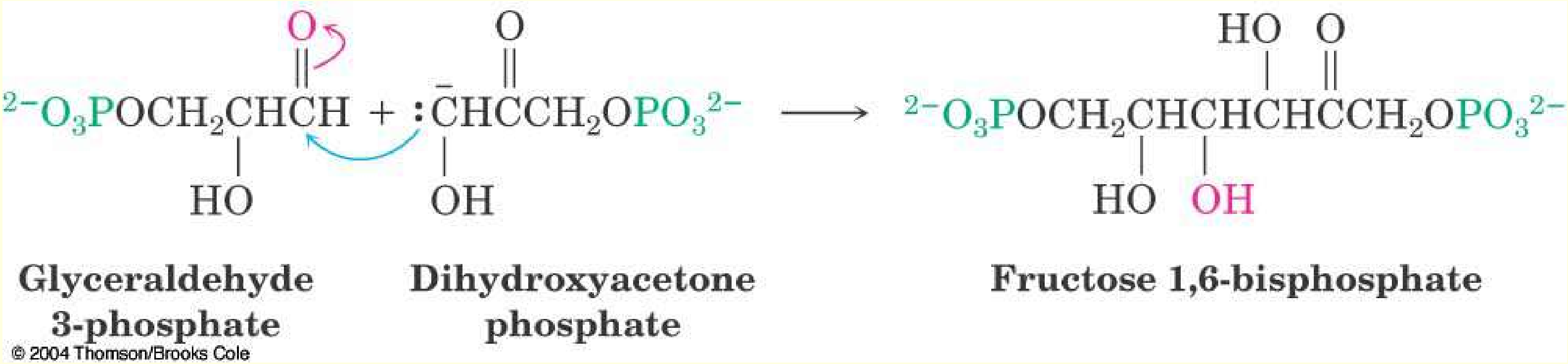
# Phosphorylation, reduction, and tautomerization

- 3-Phosphoglycerate and ATP produce an acyl phosphate
- This is reduced with NADH/$H^+$ to an aldehyde
- The aldehyde enol gives dihydroxyacetone phosphate



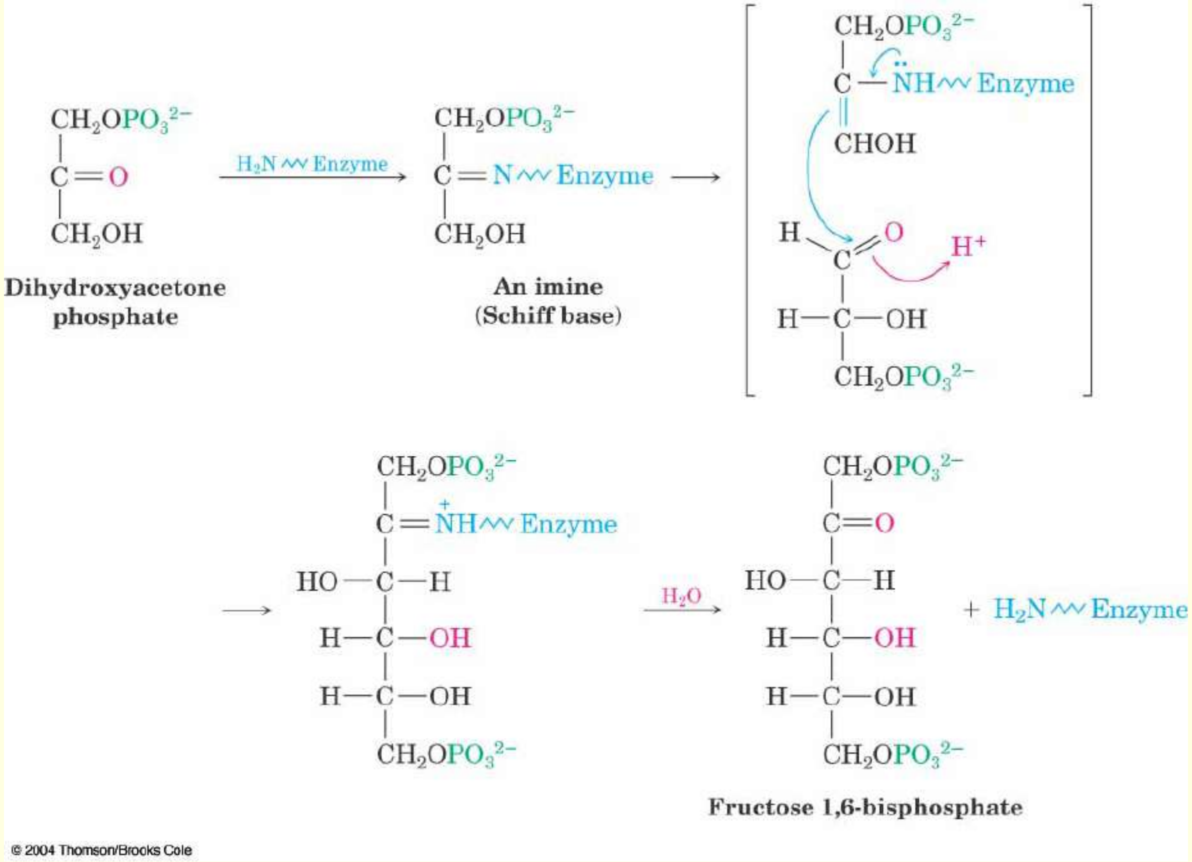
# Aldol Condensation: the Formation of a Hexose

- Dihydroxyacetone phosphate and glyceraldehyde 3-phosphate combine to give the diphosphate of fructose
- The reaction is an enzymic aldol condensation



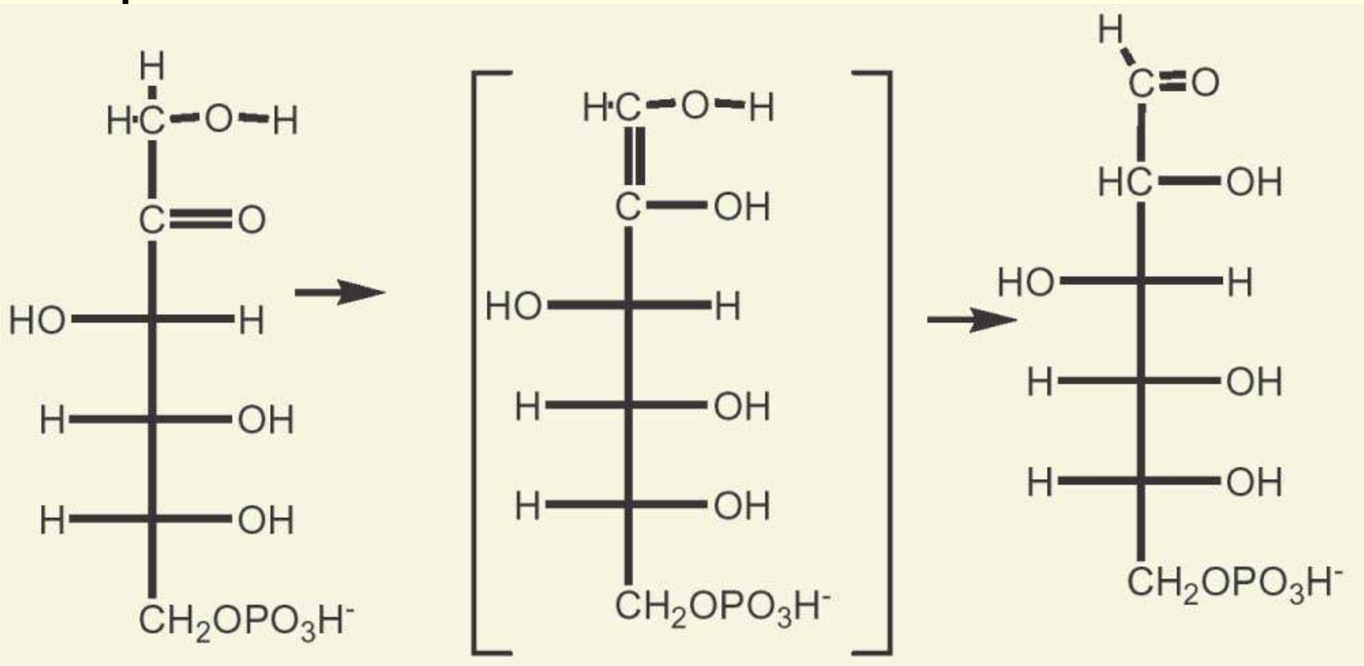
# Mechanism of the Condensation

- Proceeds via an imine from the enzyme



# Hydrolysis and Isomerization

- Hydrolysis of the phosphate group at C1
- Keto–enol isomerization produces glucose-6-phosphate



# 29.9 Some Conclusions About Biological Chemistry

- The reactions in metabolism follow the same rules of chemistry that we have learned throughout the course
- Mechanisms are just as important for understanding how biochemistry operates as they are in organic chemistry
- The ability of enzymes to catalyze reactions under conditions compatible with life is a clear indication that complex and specific processes do not require toxic reagents