Mechanisms in biological chemistry 50

Connections

Building on:

- Acidity and basicity ch8
- Carbonyl chemistry ch12 & ch14
- Stereochemistry ch16
- Conformational analysis and elimination ch18–ch19
- Enolate chemistry and synthesis ch24–ch30
- Pericyclic reactions ch35–ch36
- Determining mechanisms ch13 & ch41
- Heterocycles ch42–ch44
- Asymmetric synthesis ch45
- Sulfur chemistry ch46
- Chemistry of life ch49

Arriving at:

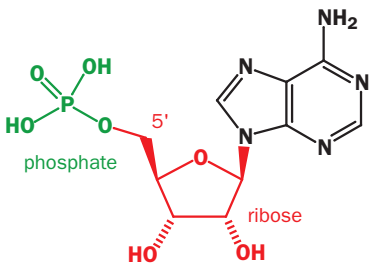
- How Nature makes small molecules using ordinary organic mechanisms
- Enzymes are Nature's catalysts, speeding up reactions by factors of 10^6 or more
- Coenzymes and vitamins are Nature's versions of common organic reagents
- Reductions with NADH
- Reductive amination, deamination, and decarboxylation with pyridoxal
- Enol chemistry with lysine enamines, with coenzyme A, and with phosphoenolpyruvate
- Umpolung chemistry with thiamine as a d^1 reagent
- Carboxylation with biotin
- Oxidations with FAD
- How Nature makes aromatic amino acids

Looking forward to:

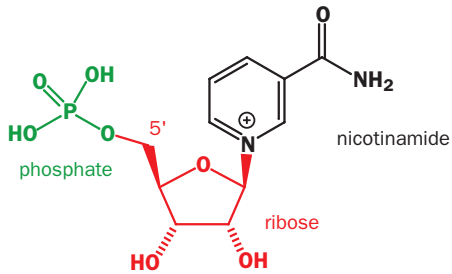
- Natural products ch51

Nature's $NaBH_4$ is a nucleotide: NADH or NADPH

In Chapter 49 we spent some time discussing the structure of nucleotides and their role as codons in protein synthesis. Now we shall see how Nature uses different nucleotides as reagents. Here is the structure of AMP, just to remind you, side by side with a new pyridine nucleotide.

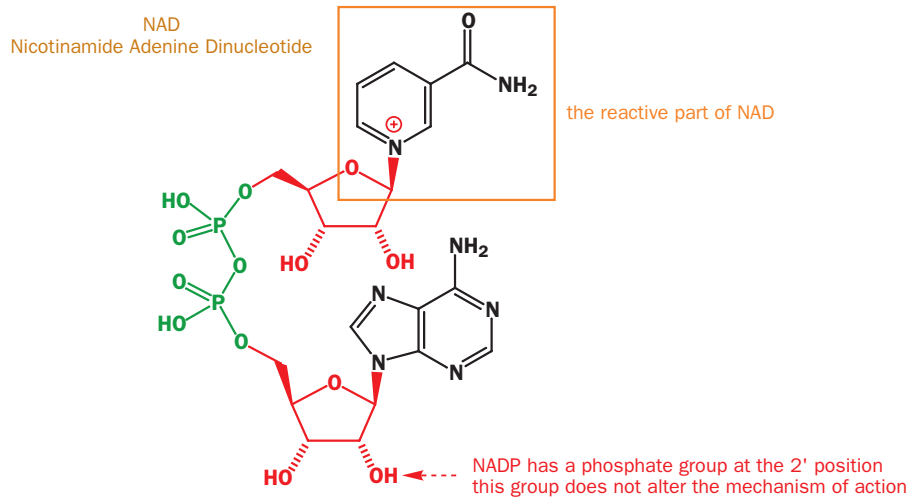


AMP—an adenine nucleotide

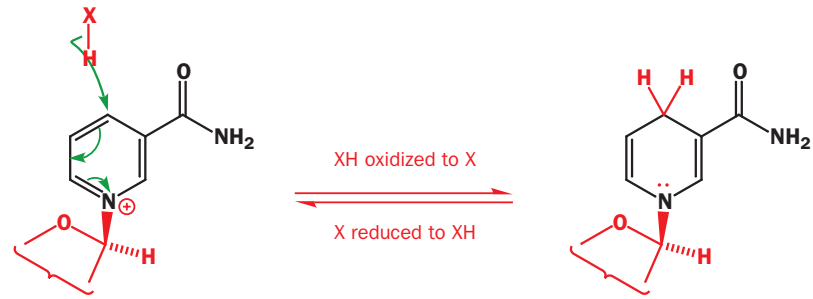


a nicotinamide nucleotide

These two nucleotides can combine together as a pyrophosphate to give a dinucleotide. Notice that the link is not at all the same as in the nucleic acids. The latter are joined by one phosphate that links the 3′–5′ positions. Here we have a *pyro*phosphate link between the two 5′-positions.



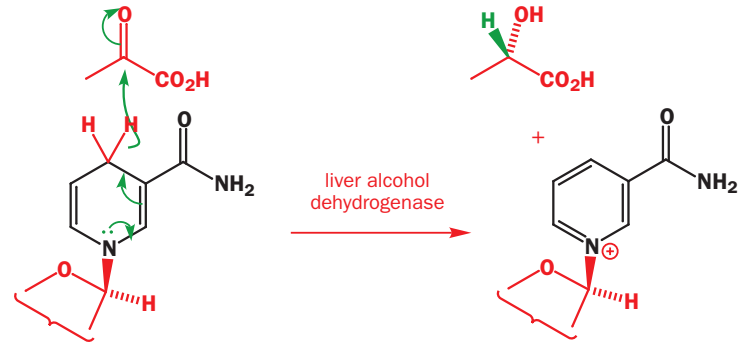
Notice also the positive charge on the nitrogen atom of the pyridine ring. This part of the molecule does all the work and from now on we will draw only the reactive part for clarity. This is **NAD$^+$**, **nicotinamide adenine dinucleotide**, and it is one of Nature's most important oxidizing agents. Some reactions use NADP instead but this differs only in having an extra phosphate group on the adenosine portion so the same part structure will do for both. NAD$^+$ and NADP both work by accepting a hydrogen atom and a pair of electrons from another compound. The reduced compounds are called NADH and NADPH.



NAD$^+$—Nature's oxidizing agent NADH—Nature's reducing agent

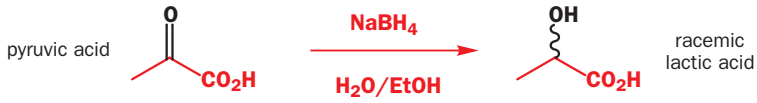
The reduction of NAD$^+$ (and NADP) is reversible, and NADH is itself a reducing agent. We will first look at one of its reactions: a typical reduction of a ketone. The ketone is pyruvic acid and the reduction product lactic acid, two important metabolites. The reaction is catalysed by the enzyme liver alcohol dehydrogenase.

▶ The names of enzymes are usually chosen to tell us where they come from and what job they do and the name ends '-ase'. A **dehydrogenase** is clearly a redox enzyme as it removes (or adds) hydrogen.

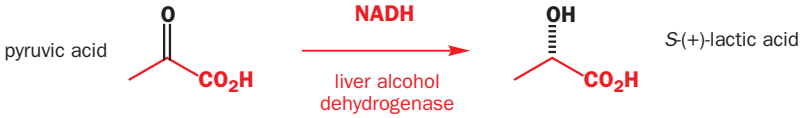


NADH—Nature's reducing agent NAD$^+$—Nature's oxidizing agent

This is a reaction that would also work in the laboratory with $NaBH_4$ as the reducing agent, but there is a big difference. The product from the $NaBH_4$ reaction *must* be racemic—no optical activity has been put in from compound, reagent, or solvent.

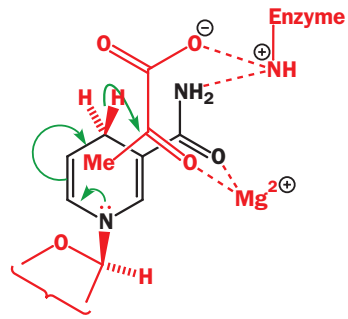


But the product from the enzymatic reaction is optically active. The two faces of pyruvic acid's carbonyl group are enantiotopic and, by controlling the addition so that it occurs from one face only, the reaction gives a single enantiomer of lactic acid.

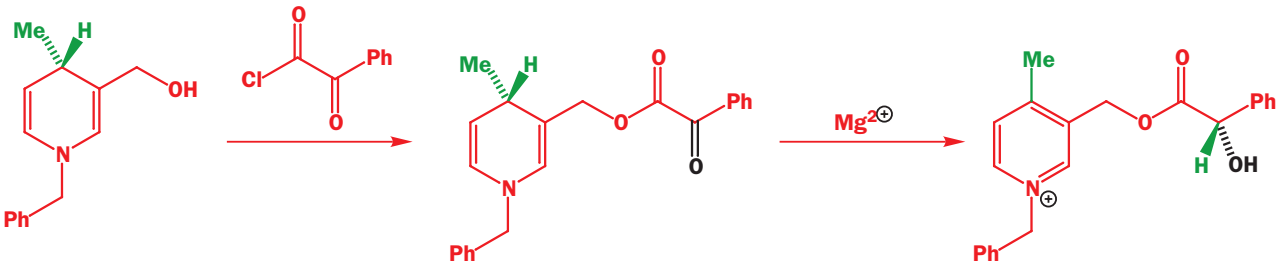


■ If you are not clear about enantioselective reactions and why $NaBH_4$ must give a racemic mixture, reread Chapter 45. If you are not clear about the terms 'enantiotopic' and 'prochiral' reread Chapters 32 and 34. If you are not clear about what enantiomers are, you must reread Chapter 16 now.

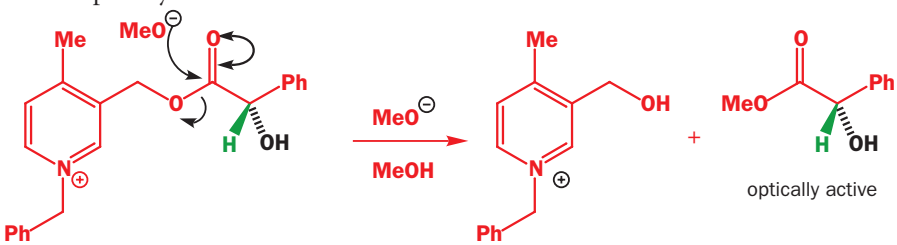
Both the enzyme and the reagent NADH are single enantiomers and they cooperate by binding. The enzyme binds both the substrate (pyruvic acid) and the reagent (NADH) in a specific way so that the hydride is delivered to one enantiotopic face of the ketone. Pyruvic acid under physiological conditions will be the anion, pyruvate, so it is held close to the positively charged amino group of a lysine residue on the enzyme that also binds the amino group of NADH. A magnesium(II) cation, also held by the enzyme, binds the carbonyl group of the amide of NADH and the ketone in pyruvate. If this model is correct, only the top H atom (as drawn) of the diastereotopic CH_2 group in NADH should be transferred to pyruvate. This has been proved by deuterium labelling.



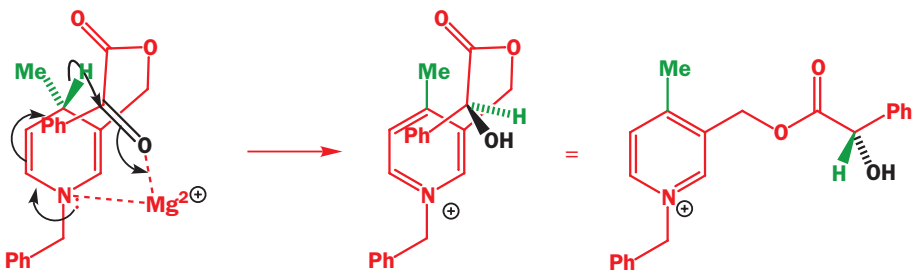
Supporting evidence comes from a model system using a much simpler reducing agent. A dihydropyridine with a primary alcohol replacing the amide group in NADH and a simple benzyl group replacing the nucleotide forms stable esters with keto-acids. As soon as the ester is treated with magnesium(II) ions, intramolecular and stereospecific reduction occurs. The hydride ion is transferred from a stereogenic centre, which replaces the diastereotopic CH_2 group in NADH.



When the ester is cleaved by transesterification with methoxide ion, the newly released hydroxy-ester is optically active.

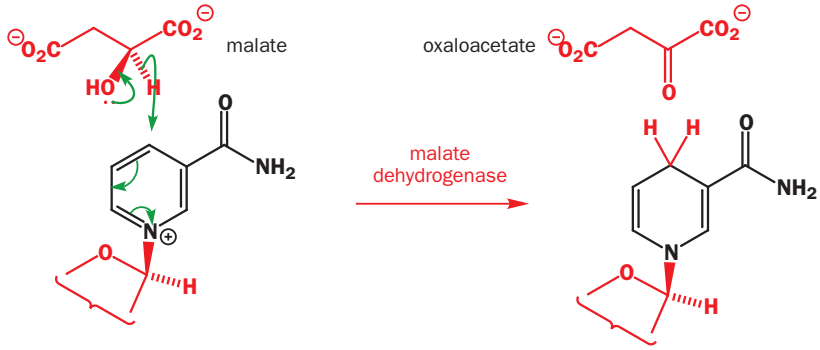


The details of the reaction are probably a good model for the NADH reaction even down to the activation by magnesium(II) ions. A possible transition state would be very similar to the NADH transition state above.



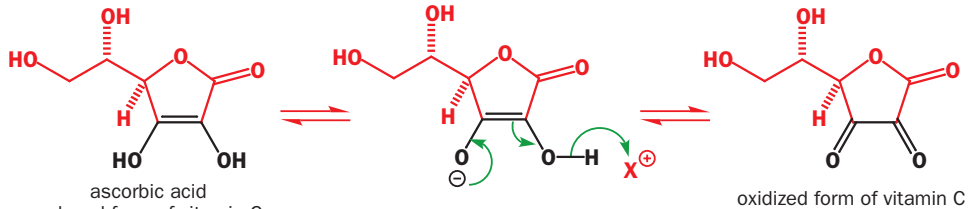
▶ The other two reactions are of a more complex type that we will meet soon when we show how acetyl coenzyme A is a key reagent in the building of carbon–carbon chains.

Many other reactions use NADH as a reducing agent or NAD^+ as oxidizing agent. Three molecules of NAD^+ are used in the citric acid cycle (see the chart on p. 1393). One of these oxidations is the simple transformation of a secondary alcohol (malate) to a ketone (oxaloacetate).



▶ Ascorbic acid is usually described as an antioxidant rather than a reducing agent though mechanistically they are the same.

Other redox reagents include dinucleotides such as FAD (flavine adenine dinucleotide), lipoic acid, which we will meet when we discuss the chemistry of thiamine, and ascorbic acid (vitamin C), which you met in Chapter 49. Ascorbic acid can form a stable enolate anion that can transfer a hydride ion to a suitable oxidant.



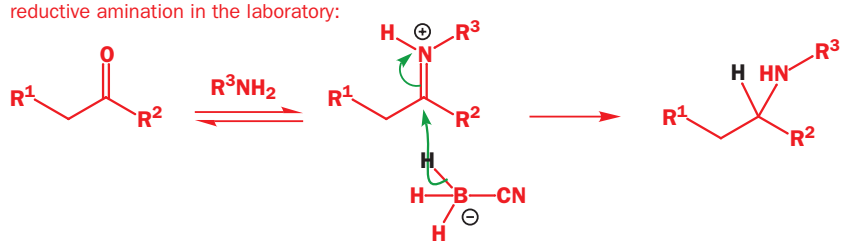
In this mechanism 'X^+' represents an oxidant—a dangerously reactive peroxide perhaps, or even Fe(III) which must be reduced to Fe(II) as part of the reaction cycle of many iron-dependent enzymes.

Reductive amination in nature

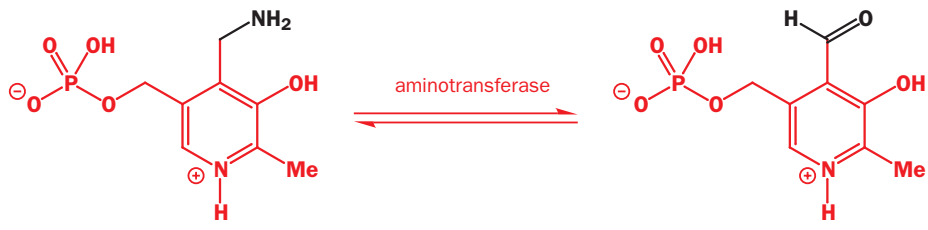
■ For more on reductive amination, see Chapter 14.

One of the best methods of amine synthesis in the laboratory is **reductive amination**, in which an imine (formed from a carbonyl compound and an amine) is reduced to a saturated amine. Common reducing agents include $NaCNBH_3$ and hydrogen with a catalyst.

reductive amination in the laboratory:

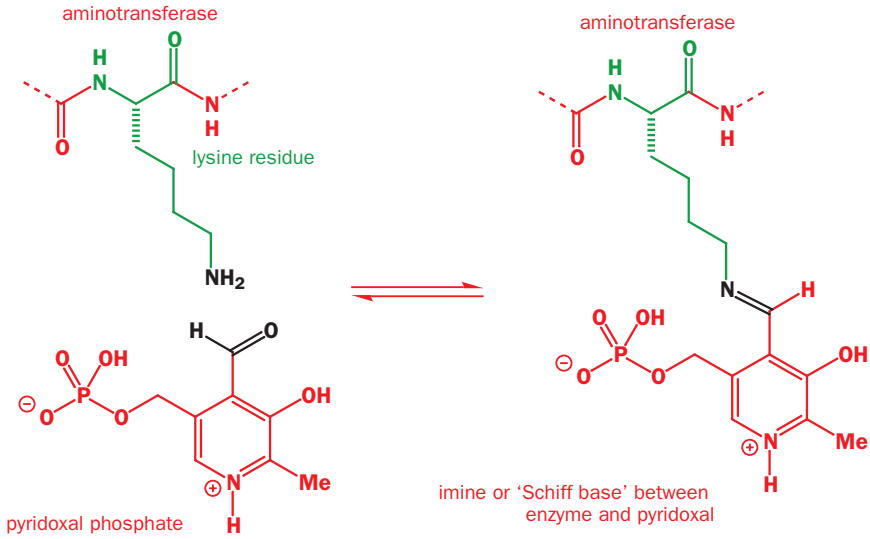


This reaction, of course, produces racemic amines. But Nature transforms this simple reaction into a stereospecific and reversible one that is beautiful in its simplicity and cleverness. The reagents are a pair of substituted pyridines called **pyridoxamine** and **pyridoxal**.

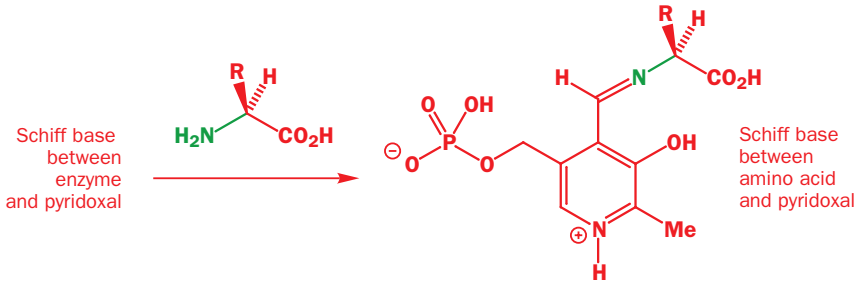


You might imagine that pyridoxamine is a product of reductive amination of pyridoxal with ammonia. In practice it doesn't work like that. Nature uses an amine transfer rather than a simple reductive amination, and the family of enzymes that catalyse the process is the family of **aminotransferases**.

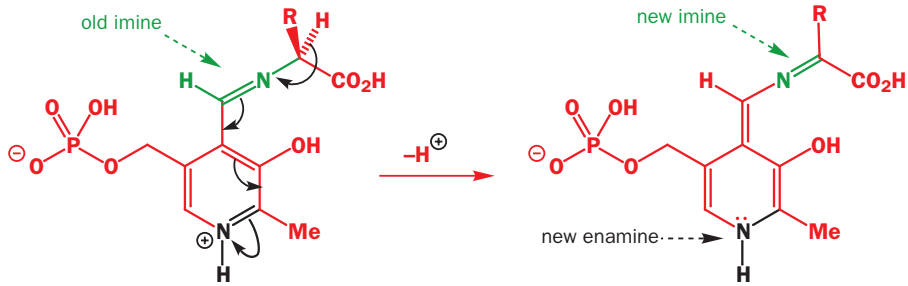
Pyridoxal is a coenzyme and it is carried around on the side chain of a lysine residue of the enzyme. Lysine has a long flexible side chain of four CH_2 groups ending with a primary amine (NH_2). This group forms an imine (what biochemists call a 'Schiff base') with pyridoxal. An imine is a good functional group for this purpose as imine formation is easily reversible.



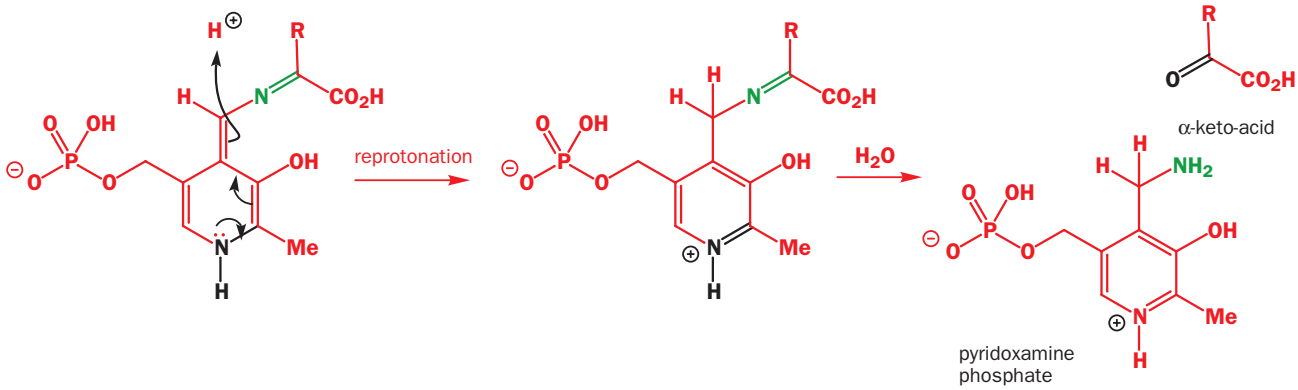
When reductive amination or its reverse is required, the pyridoxal is transferred from the lysine imine to the carbonyl group of the substrate to form a new imine of the same sort. The most important substrates are the amino acids and their equivalent α-keto-acids.



Now the simple but amazing chemistry begins. By using the protonated nitrogen atom of the pyridine as an electron sink, the α proton of the amino acid can be removed to form a new imine at the top of the molecule and an enamine in the pyridine ring.

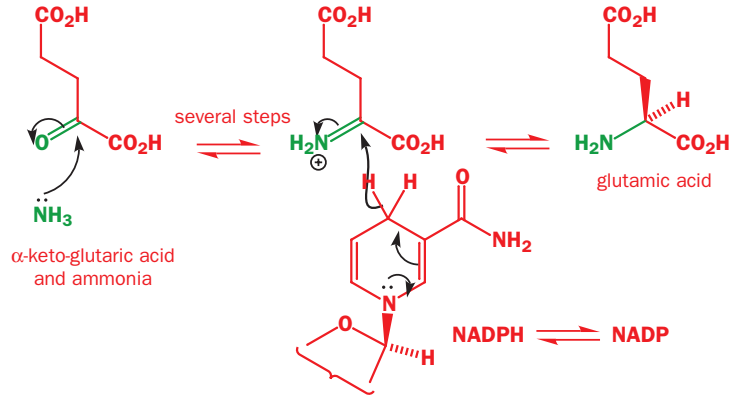


Now the electrons can return through the pyridine ring and pick up a proton at the top of the molecule. The proton can be picked up where it came from, but more fruitfully it can be picked up at the carbon atom on the other side of the nitrogen. Hydrolysis of this imine releases pyridoxamine and the keto-acid. All the natural amino acids are in equilibrium with their equivalent α-keto-acids by this mechanism, catalysed by an aminotransferase.



Reversing this reaction makes an amino acid stereospecifically out of an α-keto-acid. In fact, a complete cycle is usually set up whereby one amino acid is converted to the equivalent α-keto-acid while another α-keto-acid is converted into its equivalent amino acid. This is true transamination.

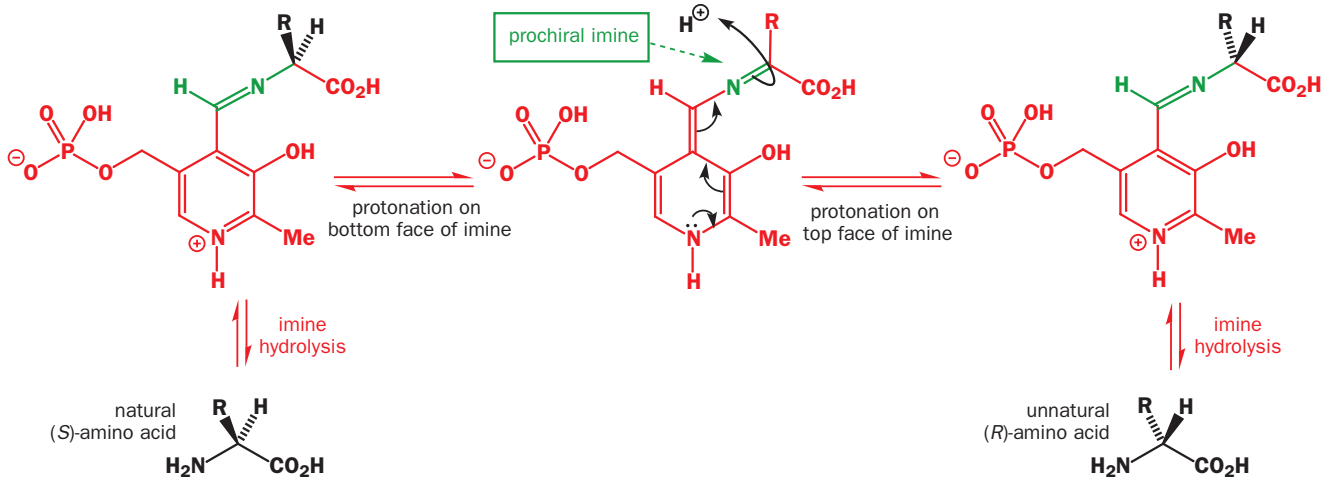
Amino acids get used up (making proteins, for example) so, to keep life going, ammonia must be brought in from somewhere. The key amino acid in this link is glutamic acid. A true reductive amination using NADPH and ammonia builds glutamic acid from α-keto-glutaric acid.



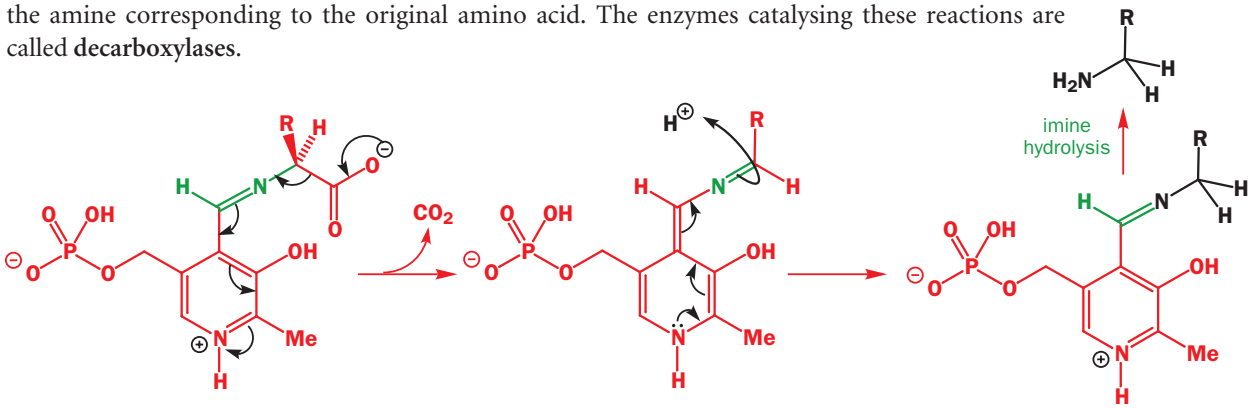
The other amino acids can now be made from glutamic acid by transamination. At the end of their useful life they are transaminated back to glutamic acid which, in mammals at least, gives its nitrogen to urea for excretion.

Pyridoxal is a versatile reagent in the biochemistry of amino acids

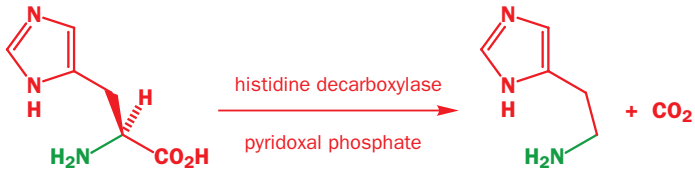
Pyridoxal is the reagent in other reactions of amino acids, all involving the imine as intermediate. The simplest is the racemization of amino acids by loss of a proton and its replacement on the other face of the enamine. The enamine, in the middle of the diagram below, can be reprotonated on either face of the prochiral imine (shown in green). Protonation on the bottom face would take us back to the natural amino acid from which the enamine was made in the first place. Protonation on the top face leads to the unnatural amino acid after 'hydrolysis' of the imine (really transfer of pyridoxal to a lysine residue of the enzyme).



A very similar reaction is decarboxylation. Starting from the same imine we could lose carbon dioxide instead of a proton by a very similar mechanism. Reprotonation and imine transfer releases the amine corresponding to the original amino acid. The enzymes catalysing these reactions are called **decarboxylases**.

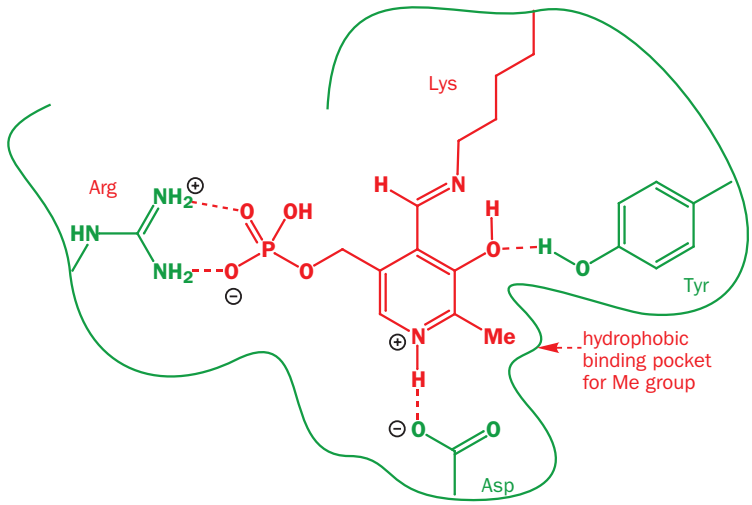


In Chapter 43 we mentioned the role of histamine in promoting acid secretion in the stomach, and its role in causing gastric ulcers. The drug cimetidine was designed to counteract the effect of histamine. Histamine is produced in the body by decarboxylation of histidine using the mechanism you have just seen.



histidine histamine

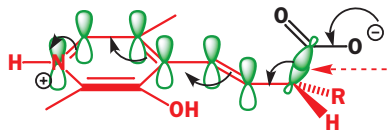
How is it possible for the same reagent operating on the same substrate (an amino acid) to do at will one of two quite different things—removal and/or exchange of a proton and decarboxylation? The answer, of course, lies in the enzymes. These hold pyridoxal exceptionally tightly by using all the available handles: the hydroxy and phosphate groups, the positively charged nitrogen atom, and even the methyl group. The diagram shows the proposed binding of the lysine imine of pyridoxal by an aminotransferase.



The green line shows an imaginary shape of the enzyme chain into which fit acidic groups and basic groups forming hydrogen bonds to groups on the coenzyme. Around the methyl group are alkyl-substituted amino acids, which form a hydrophobic region. Even when the lysine attachment is exchanged for the substrate, all these interactions remain in place. The substrate is bound by similar interactions with other groups on the enzyme.

Control over the choice of reaction arises because the different enzymes bind the substrate–pyridoxal imine in different ways. Decarboxylases bind so that the C–C bond to be broken is held orthogonal to the pyridine ring and parallel to the p orbitals in the ring. Then the bond can be broken and CO_2 can be lost.

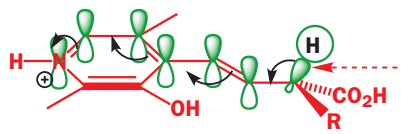
decarboxylases bind the substrate–pyridoxal imine so that the C–C bond is parallel to the p orbitals in the pyridoxal ring



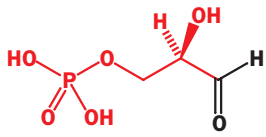
the σ bond to be broken is parallel to the p orbitals in the pyridine ring

Racemases and transaminases bind the substrate–pyridoxal imine so that the C–H bond is parallel to the p orbitals in the ring so that proton removal can occur. Enzymes do not speed reactions up indiscriminately—they can selectively accelerate some reactions at the expense of others, even those involving the same reagents.

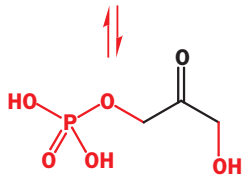
racemases and transaminases bind the substrate–pyridoxal imine so that the C–H bond is parallel to the p orbitals in the pyridoxal ring



the σ bond to be broken is parallel to the p orbitals in the pyridine ring



glyceraldehyde-3-phosphate

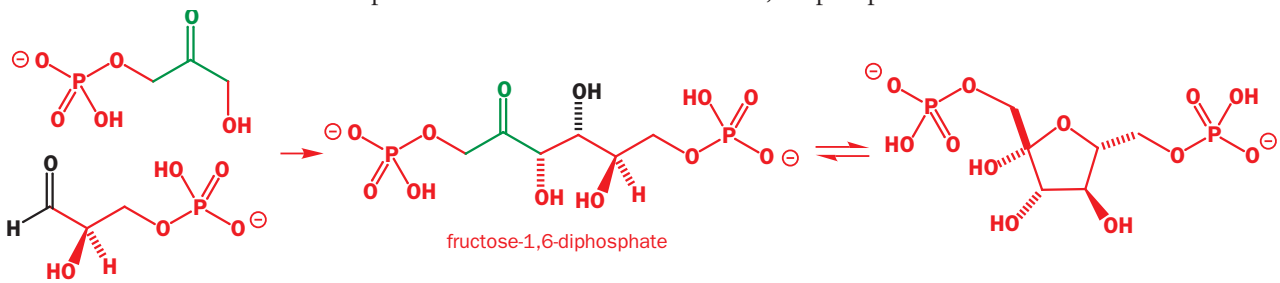


dihydroxyacetone-3-phosphate

Nature's enols—lysine enamines and coenzyme A

The glycolysis pathway breaks down glucose to produce energy, and in doing so produces smaller molecules for use in the citric acid cycle. In reverse, it allows the synthesis of the six-carbon sugar fructose from two three-carbon fragments. A key reaction is the step in which these two C_3 sugars combine. They are glyceraldehyde and dihydroxyacetone and we met them and their interconversion in the last chapter.

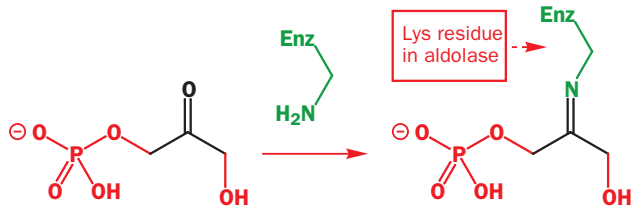
The reaction is effectively an aldol condensation between the enol of the keto-sugar phosphate and the electrophilic aldehyde of glyceraldehyde phosphate and the enzyme is named appropriately **aldolase**. The product is the keto-hexose fructose-1,6-diphosphate.



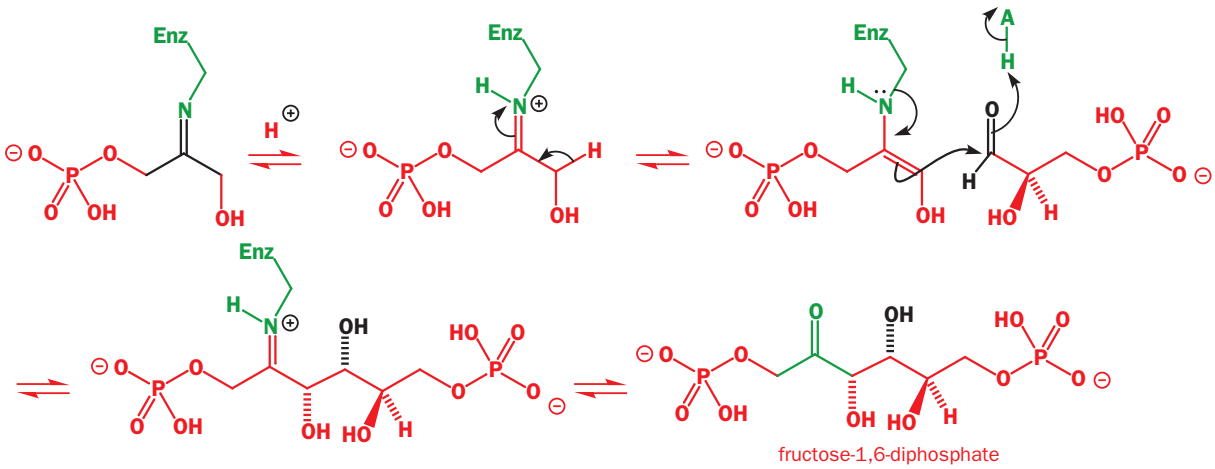
fructose-1,6-diphosphate

■ The rest of the aldolase molecule is represented by 'Enz'.

No enolate ion is formed in this aldol. Instead a lysine residue in the enzyme forms an imine with the keto-triose.



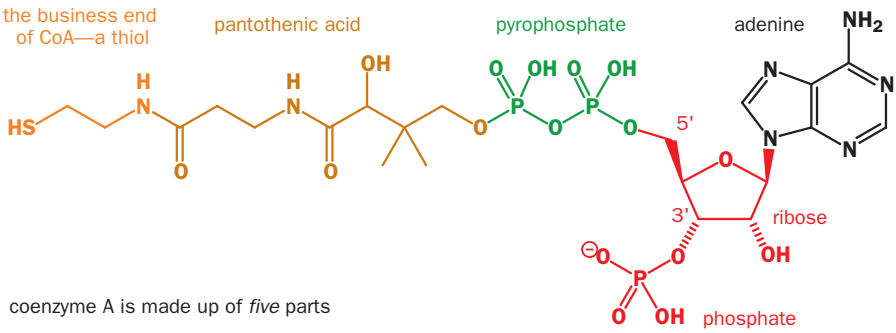
Proton transfers allow this imine to be converted into an enamine, which acts as the nucleophile in the aldol reaction. Stereochemical control (it's a *syn* aldol) comes from the way in which the two molecules are held by the enzyme as they combine. The product is the imine, which is hydrolysed to the open-chain form of fructose-1,6-diphosphate.



Many other reactions in nature use enamines, mostly those of lysine. However, a more common enol equivalent is based on thiol esters derived from coenzyme A.

Coenzyme A and thiol esters

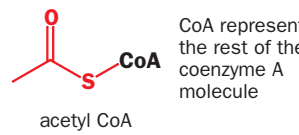
Coenzyme A is an adenine nucleotide at one end, linked by a 5′-pyrophosphate to pantothenic acid, a compound that looks rather like a tripeptide, and then to an amino thiol. Here is the structure broken down into its parts.

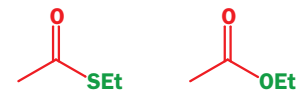


coenzyme A is made up of *five* parts

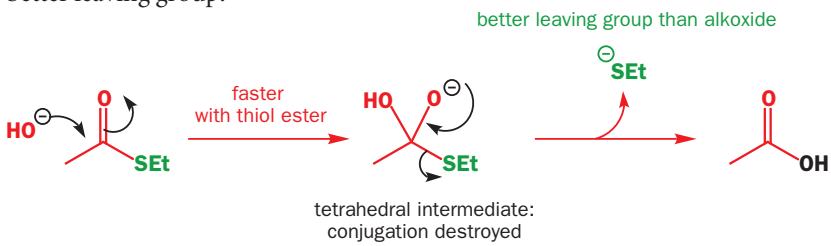
▶ Compare this structure with that of NAD—the adenine nucleotide is the same, as is the 5′-pyrophosphate link. The difference is at the other end of that link where we find this new tripeptide-like molecule and not another nucleotide. There is also a 3′-phosphate on the ribose ring not present in NAD.

By now you will realize that most of this molecule is there to allow interaction with the various enzymes that catalyse the reactions of coenzyme A. We will abbreviate it from now on as CoASH where the SH is the vital thiol functional group, and all the reactions we will be interested in are those of esters of CoASH. These are **thiol esters**, as opposed to normal 'alcohol esters', and the difference is worth a few comments.



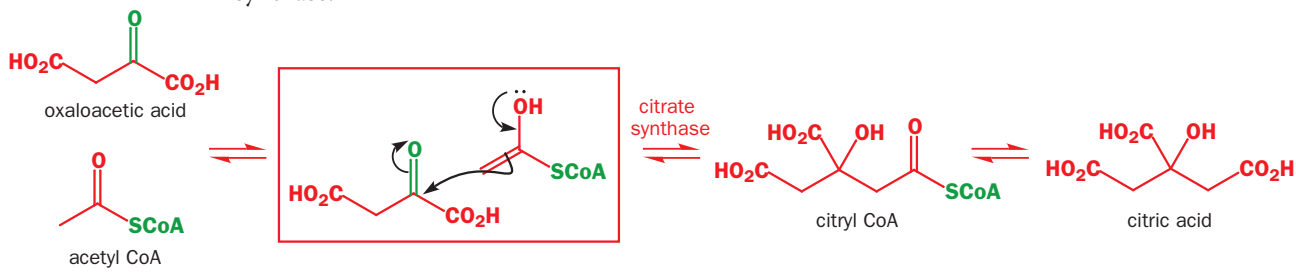


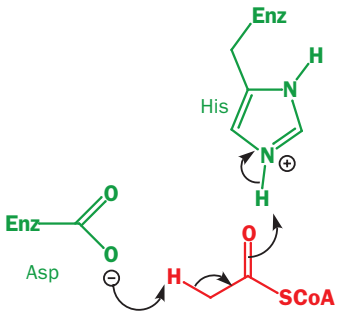
Thiol esters are less conjugated than ordinary esters (see Chapter 28, p. 744), and ester hydrolysis occurs more rapidly with thiol esters than with ordinary esters because in the rate-determining step (nucleophilic attack on the carbonyl group) there is less conjugation to destroy. The thiolate is also a better leaving group.



Another reaction that goes better with thiol esters than with ordinary esters is enolization. This is an equilibrium reaction and the enol has lost the conjugation present in the ester. The thiol ester has less to lose so is more enolized. This is the reaction of acetyl CoA that we are now going to discuss. We have mentioned the citric acid cycle several times and it has appeared in two

diagrams but we have not so far discussed the chemistry involved. The key step is the synthesis of citric acid from oxaloacetate and acetyl CoA. The reaction is essentially an aldol reaction between the enol of an acetate ester and an electrophilic ketone and the enzyme is known as **citrate synthase**.

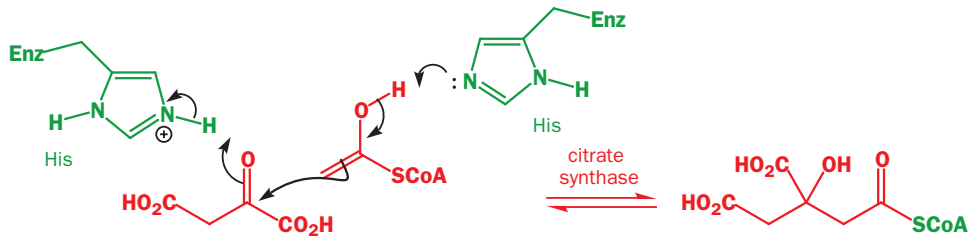




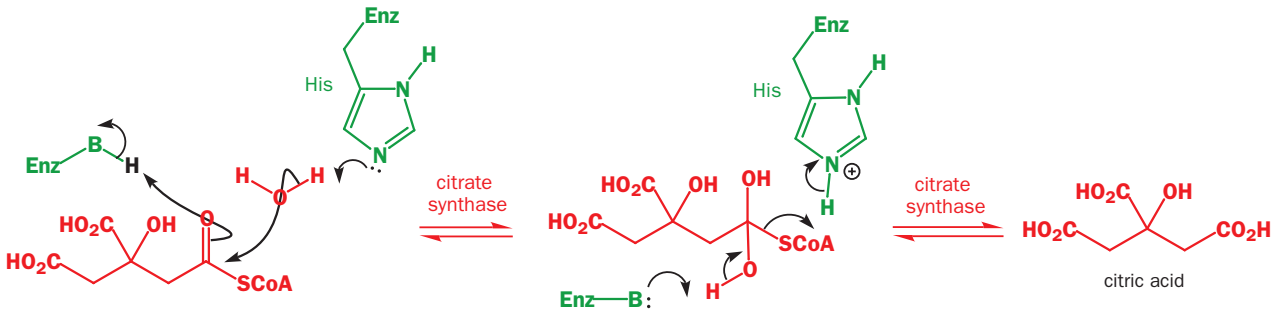
▶ This is **general acid catalysis**, as described in Chapter 41.

The mechanism in the frame shows the enol of acetyl CoA attacking the reactive ketone. In nature the enolization is catalysed by a basic carboxylate group (Asp) and an acidic histidine, both part of the enzyme, so that even this easy reaction goes faster.

In the C–C bond-forming step, the same histidine is still there to remove the enol proton again and another histidine, in its protonated form, is placed to donate a proton to the oxygen atom of the ketone. You should see now why histidine, with a pK_{aH} of about 7, is so useful to enzymes: it can act either as an acid or as a base.



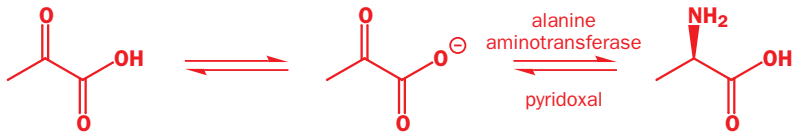
Even the hydrolysis of the reactive thiol ester is catalysed by the enzyme and the original histidine again functions as a proton donor. Acetyl CoA has played its part in all steps. The enolization and the hydrolysis in particular are better with the thiol ester.



CoA thiol esters are widely used in nature. Mostly they are acetyl CoA, but other thiol esters are also used to make enols. We will see more of this chemistry in the next chapter. The two enol equivalents that we have met so far are quite general: lysine enamines can be used for any aldehyde or ketone and CoA thiol esters for any ester. Another class of enol equivalent—the enol ester—has just one representative but it is a most important one.

Phosphoenolpyruvate

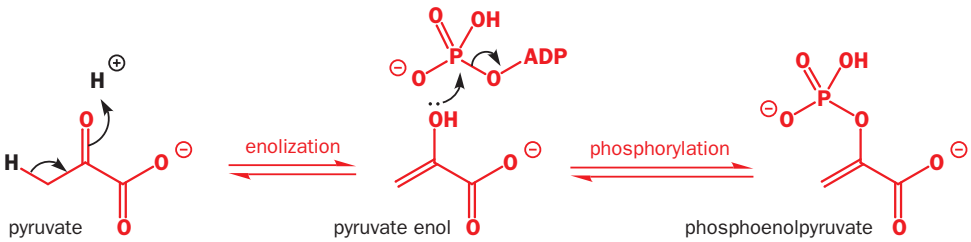
Pyruvic acid is an important metabolite in its own right as we shall see shortly. It is the simplest α-keto-acid (2-oxopropanoic acid). Having the two carbonyl groups adjacent makes them more reactive: the ketone is more electrophilic and enolizes more readily and the acid is stronger. Pyruvate is in equilibrium with the amino acid alanine by an aminotransferase reaction catalysed by pyridoxal (above).



pyruvic acid pyruvate alanine

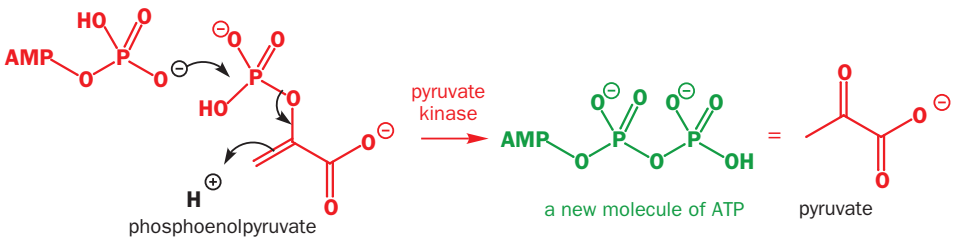
■ For an explanation of the effect of two adjacent carbonyl groups, see Chapter 28, p. 728.

Nature uses the enol phosphate of pyruvic acid (**phosphoenolpyruvate** or **PEP**) as an important reagent. We might imagine making this compound by first forming the enol and then esterifying on oxygen by some phosphorylating agent such as ATP.



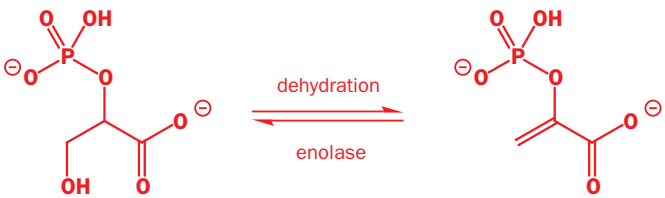
pyruvate pyruvate enol phosphoenolpyruvate

Now, in fact, this reaction does occur in nature as part of the glycolysis pathway, but it occurs almost entirely in reverse. PEP is used as a way to make ATP from ADP during the oxidation of energy-storing sugars. An enol is a better leaving group than an ordinary alcohol especially if it can be protonated at carbon. The reverse reaction might look like this.



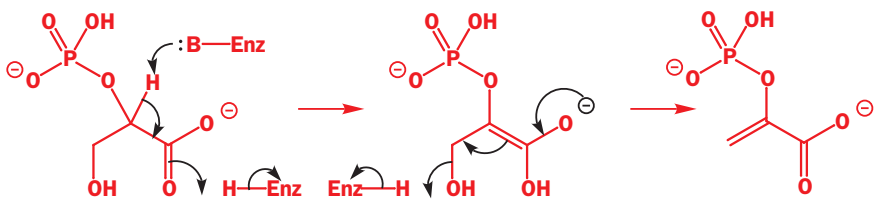
phosphoenolpyruvate a new molecule of ATP pyruvate

PEP is also used as an enol in the making of carbon–carbon bonds when the electrophile is a sugar molecule and we will see this reaction in the next chapter. So, if PEP is not made by enolization of pyruvate, how is it made? The answer is by **dehydration**. The phosphate is already in place when the dehydration occurs, catalysed by the enzyme enolase.



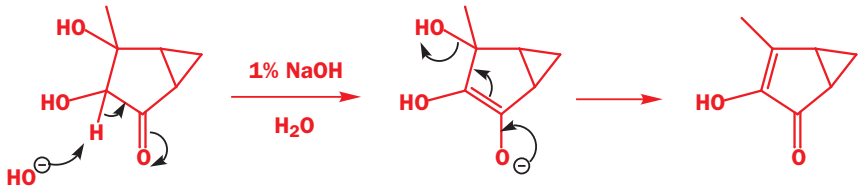
2-phosphoglycerate phosphoenolpyruvate

You saw in Chapter 19 how simple OH groups could be lost in dehydration reactions. Either the OH group was protonated by strong acid (this is not an option in living things) or an enol or enolate pushed the OH group out in an E1cB-like mechanism. This must be the case here as the better leaving group (phosphate) is ignored and the worse leaving group (OH) expelled.



2-phosphoglycerate elimination step phosphoenolpyruvate

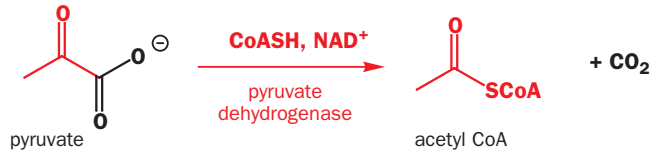
This would be an unusual way to make an enol in the laboratory but it can be used, usually to make stable enols. An example that takes place under mildly basic conditions is the dehydration of the bicyclic keto-diol in dilute sodium hydroxide—presumably by an E1cB mechanism.



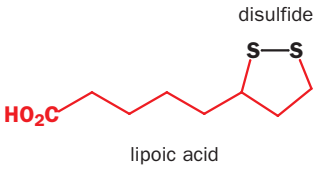
Pyruvic acid and acetyl CoA: the link between glycolysis and the citric acid cycle

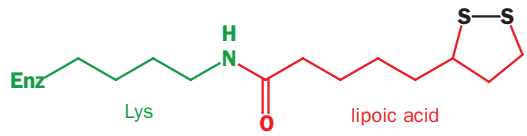
We have now examined the mechanism of several steps in glycolysis and one in the citric acid cycle and we have seen enough to look at the outline of these two important processes and the link between them (see opposite).

You have already seen that citric acid is made from acetyl CoA. The acetyl CoA comes in its turn from pyruvic acid. Pyruvic acid comes from many sources but the most important is glycolysis: acetyl CoA is the link between glycolysis and the citric acid cycle. The key reaction involves both CoASH and pyruvate and carbon dioxide is lost. This is an oxidation as well and the oxidant is NAD^+. The overall reaction is easily summarized.



This looks like a simple reaction based on very small molecules. But look again. It is a very strange reaction indeed. The molecule of CO_2 clearly comes from the carboxyl group of pyruvate, but how is the C–C bond cleaved, and how does acetyl CoA join on? If you try to draw a mechanism you will see that there must be more to this reaction than meets the eye. The extra features are two new cofactors, thiamine pyrophosphate and lipoic acid, and the reaction takes place in several stages with some interesting chemistry involved.



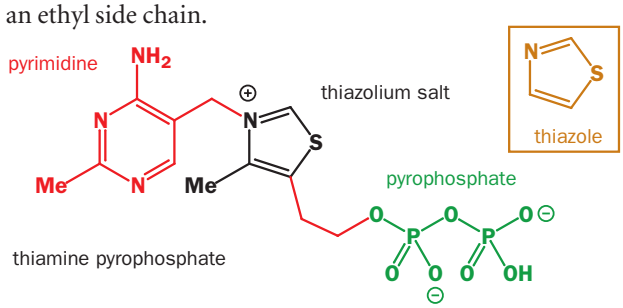


lipoic acid attached to the enzyme as a lysine amide

Lipoic acid is quite a simple molecule with a cyclic disulfide as its main feature. It is attached to the enzyme as an amide with lysine. Our first concern will be with the much more complex coenzyme thiamine pyrophosphate.

Nature's acyl anion equivalent (d^1 reagent) is thiamine pyrophosphate

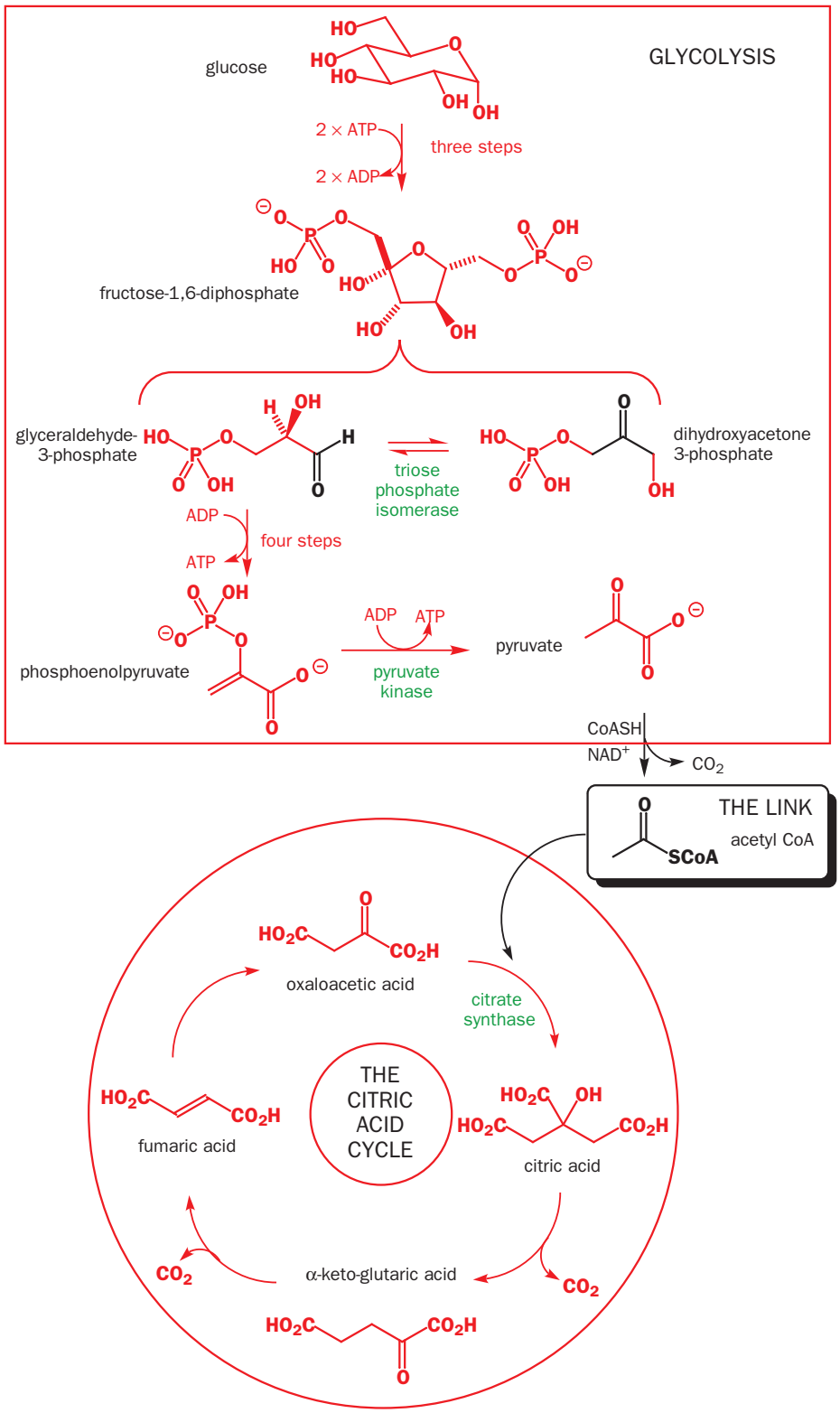
Thiamine pyrophosphate looks quite like a nucleotide. It has two heterocyclic rings, a pyrimidine similar to those found in DNA and a thiazolium salt. This ring has been alkylated on nitrogen by the pyrimidine part of the molecule. Finally, there is a pyrophosphate attached to the thiazolium salt by an ethyl side chain.



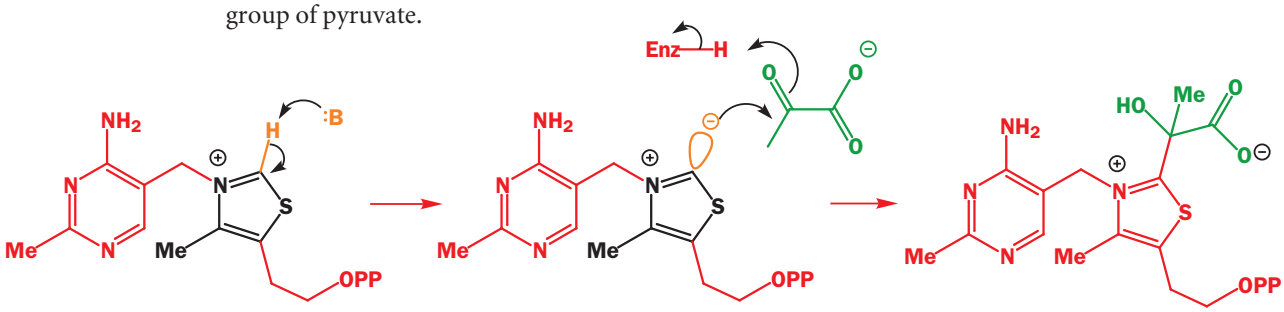
▶ We will abbreviate pyrophosphate to 'OPP' in structures.

▶ Do not confuse thiamine with thymine, one of the pyrimidine bases on DNA. The DNA base thymine is just a pyrimidine; thymidine is the corresponding nucleoside. The coenzyme thiamine is a more complicated molecule, that contains a different pyrimidine.

the link between glycolysis and the citric acid cycle

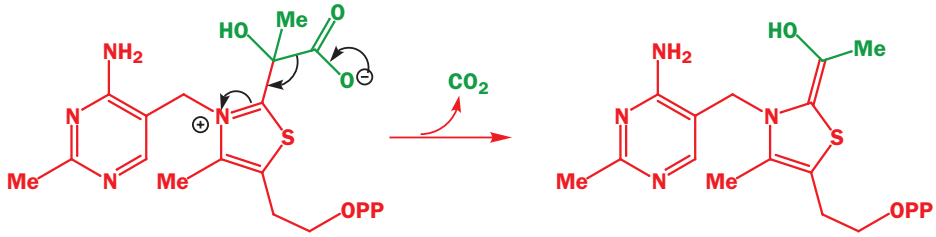


The key part of the molecule for reactivity is the thiazolium salt in the middle. The proton between the N and S atoms can be removed by quite weak bases to form an ylid. You saw sulfonium ylids in Chapter 46, and there is some resemblance here, but this ylid is an ammonium ylid with extra stabilization from the sulfur atom. The anion is in an sp^2 orbital, and it adds to the reactive carbonyl group of pyruvate.

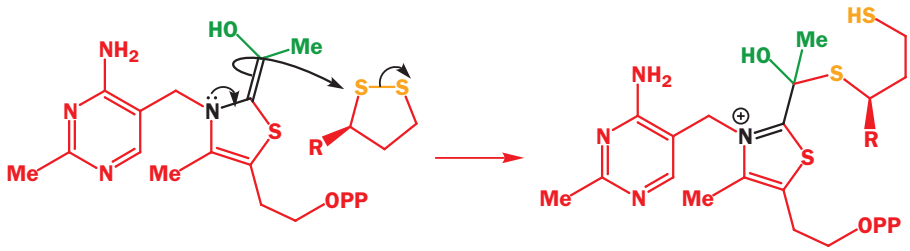


■ For more on fragmentation reactions see Chapter 38.

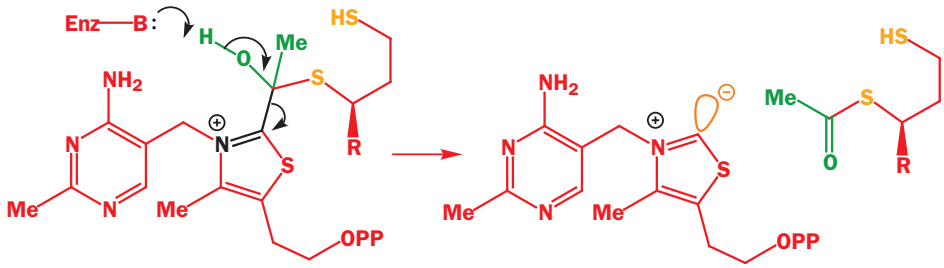
Now the carboxylate can be lost from the former pyruvate as the positively charged imine in the thiamine molecule provides a perfect electron sink to take away the electrons from the C–C bond that must be broken.



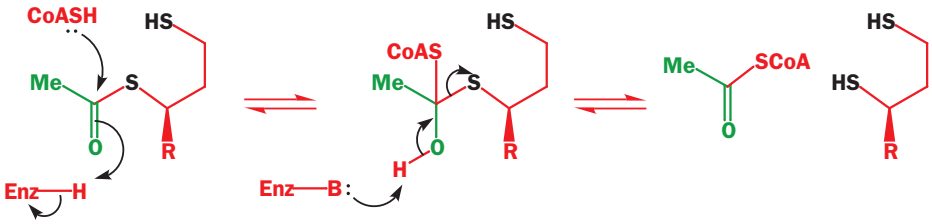
This new intermediate contains a new and strange C=C double bond. It has OH, N, and S substituents making it very electron-rich. As the nitrogen is the most electron-donating you can view it as an enamine, and it attacks the disulfide functional group of lipoic acid, the other cofactor in the reaction.



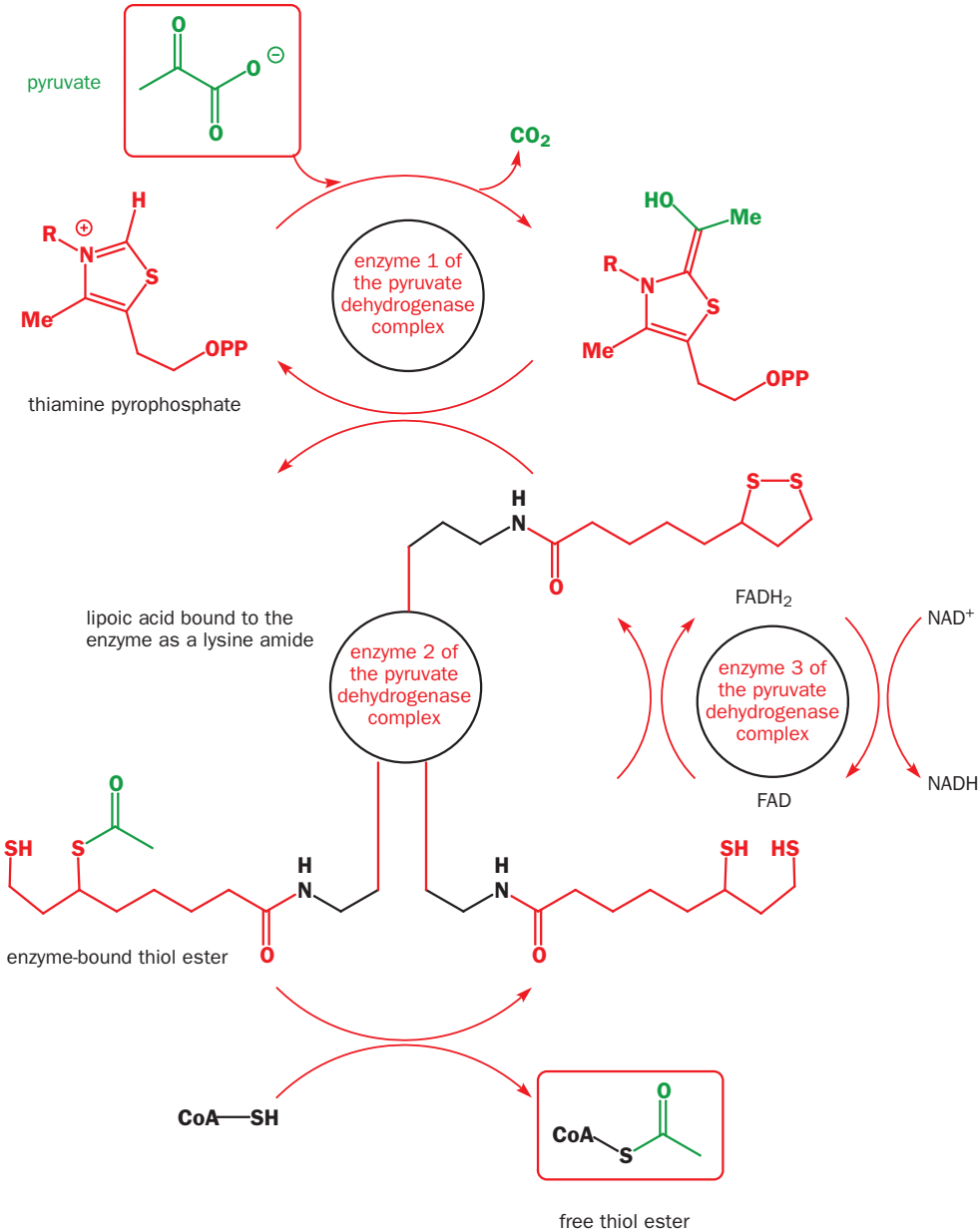
Now the thiamine can be expelled using the green OH group. The leaving group is again the ylid of thiamine, which functions as a catalyst.



The product is a thiol ester and so can exchange with CoASH in a simple ester exchange reaction. This is a nucleophilic attack on the carbonyl group and will release the reduced form of lipoic acid. All that is necessary to complete the cycle is the oxidation of the dithiol back to the disulfide. This is such an easy reaction to do that it would occur in air anyway but it is carried out in nature by FAD, a close relative of NAD^+.



This is one of the most complicated sequences of reactions that we have discussed so far. It is critical to living things because it links glycolysis and the citric acid cycle. Nature has provided not one enzyme but three enzymes to catalyse this process. In the cell they are massed together as a single protein complex.

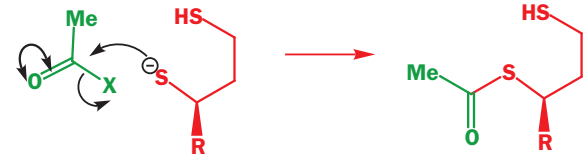


At the centre is 'enzyme 2' which binds the acetyl group through a lipoic acid–lysine amide. On the one side this acetyl group is delivered from pyruvate by the ministrations of thiamine pyrophosphate and 'enzyme 1' and on the other it is delivered to CoA as the free thiol ester. Enzyme 3 recycles

the reduced lipoic acid using FAD and then NAD^+. This remarkable assembly of proteins maintains stocks of acetyl CoA for use in the citric acid cycle and for building complex organic molecules by enol chemistry, as we will see in the next chapter.

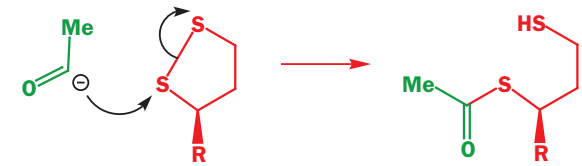
One reaction in this sequence is worth detailed analysis. The enzyme-bound lipoic thiol ester is a perfectly normal thiol ester and we would expect it to be formed by acylation of the thiol.

thiol ester formation with normal polarity: nucleophilic S electrophilic carbonyl

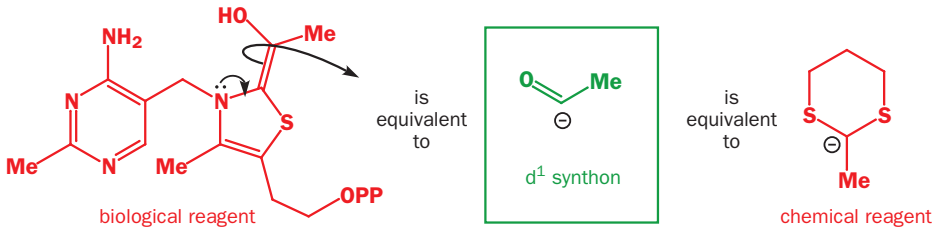


But this thiol ester is not formed by the expected mechanism in the enzymatic reaction. Thiamine delivers a *nucleo*philic acetyl group to an *electro*philic sulfur atom—the reverse polarity to normal ester formation.

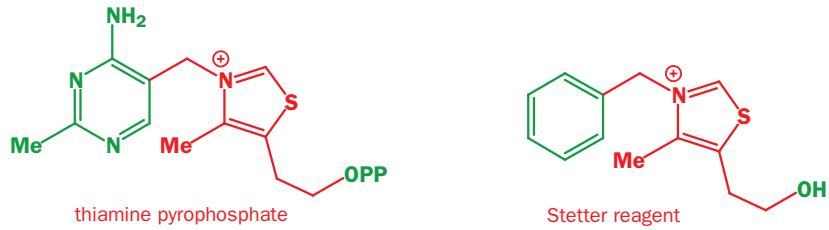
thiol ester formation with reverse polarity: electrophilic S nucleophilic carbonyl



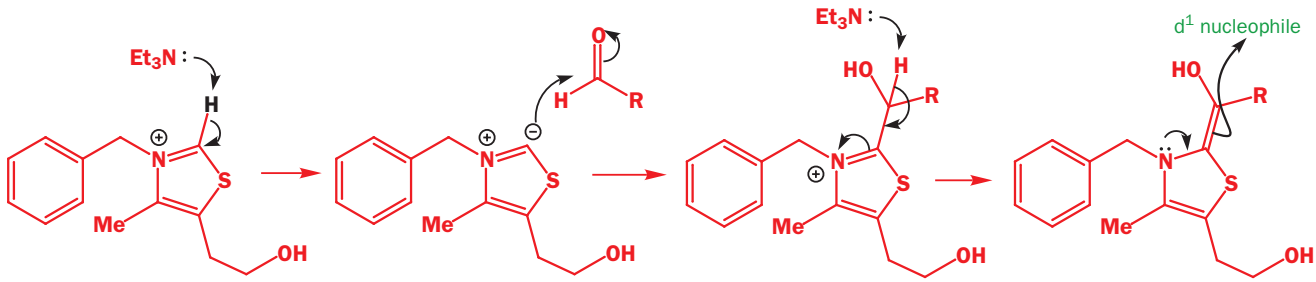
The compound formed from thiamine pyrophosphate and pyruvic acid is Nature's nucleophilic acetyl group. This is a d^1 reagent like the dithiane anion you met in Chapter 46.



If this is really true and not just a theoretical analogy, it ought to be possible to learn from Nature and design useful d^1 reagents based on thiamine. This was done by Stetter using simplified thiamines. The pyrimidine is replaced by a benzene ring and the pyrophosphate is removed. This leaves a simple thiazolium salt called a **Stetter reagent**.

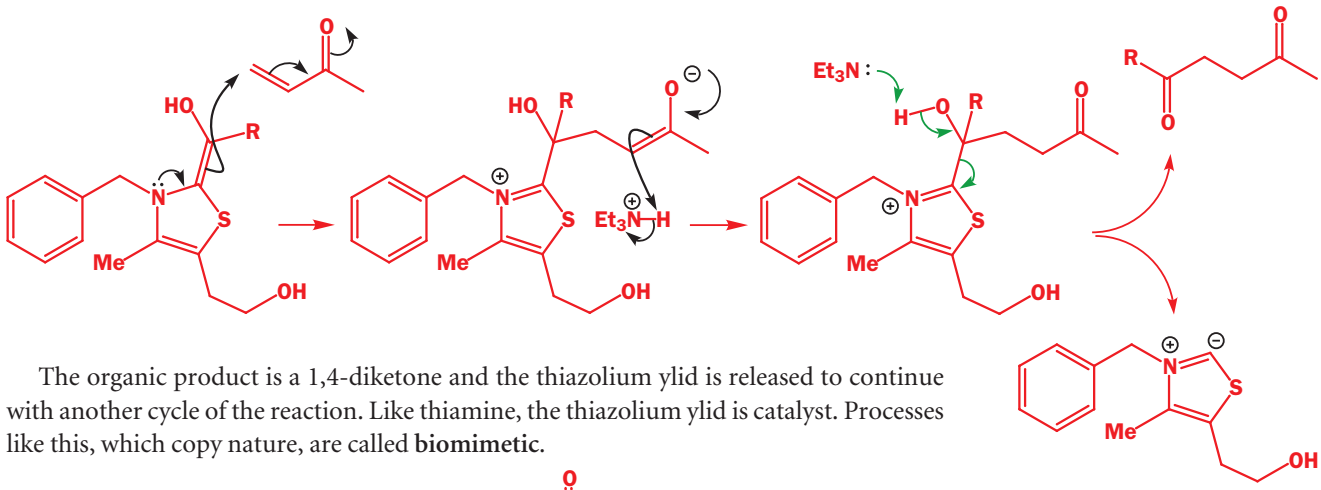


By analogy with the biological reaction, we need only a weak base (Et_3N) to make the ylid from the thiazolium salt. The ylid adds to aldehydes and creates a d^1 nucleophile equivalent to an acyl anion.

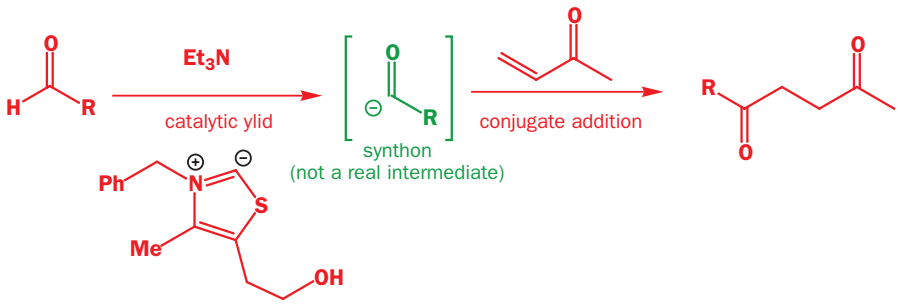


A useful application of these reagents is in conjugate addition to unsaturated carbonyl compounds. Few d^1 reagents will do this as most are very basic and prefer to add directly to the carbonyl

group. Notice that a tertiary amine, pK_{aH} about 10, is strong enough to remove both protons in this sequence.

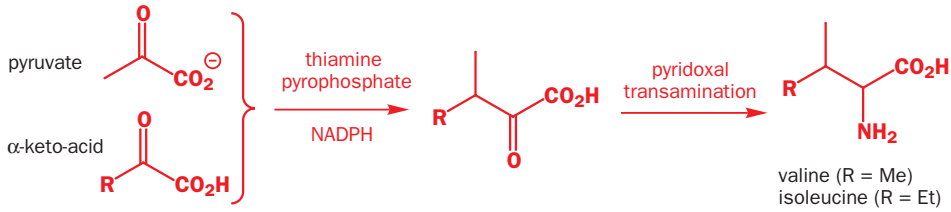


The organic product is a 1,4-diketone and the thiazolium ylid is released to continue with another cycle of the reaction. Like thiamine, the thiazolium ylid is catalyst. Processes like this, which copy nature, are called **biomimetic**.

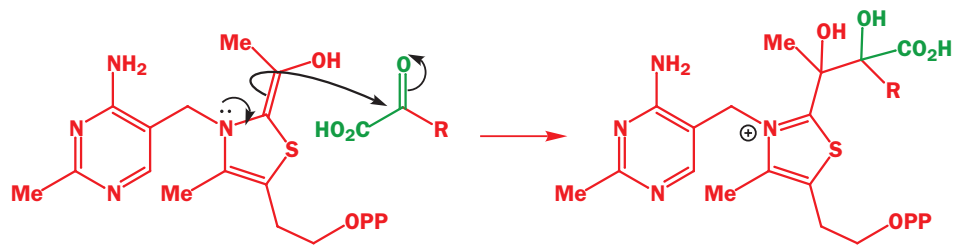


Rearrangements in the biosynthesis of valine and isoleucine

In nature, thiamine pyrophosphate also catalyses reactions of α-keto-acids other than pyruvic acid. One such sequence leads through some remarkable chemistry to the biosynthesis of the branched-chain amino acids valine and isoleucine.

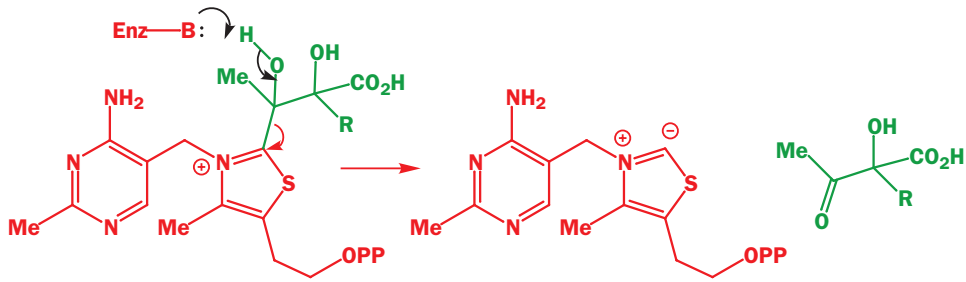


The remarkable aspect of this chemistry is that it involves 1,2-alkyl shifts in pinacol-like rearrangements (Chapter 37). The sequence starts as before and we will pick it up after the addition and decarboxylation of pyruvate and as the resulting d^1 reagent adds to the new α-keto-acid.

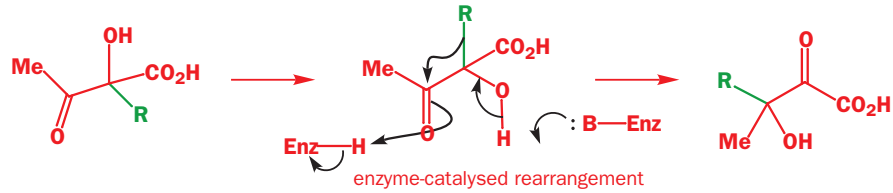


Decomposition of this product with the release of the thiazolium ylid also releases the product of coupling between the two keto-acids: a 1-hydroxy-2-keto-acid (in green). The original keto group of

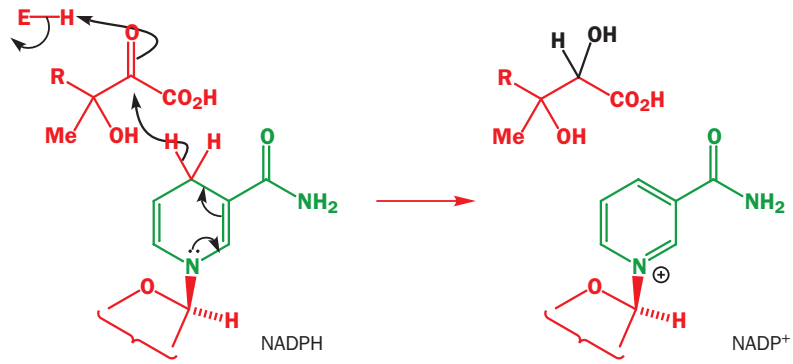
the pyruvate reappears—it's clear that an acetyl anion equivalent (the d^1 reagent) has added to the keto group of the new keto-acid. The thiazolium ylid is free to catalyse the next round of the reaction.



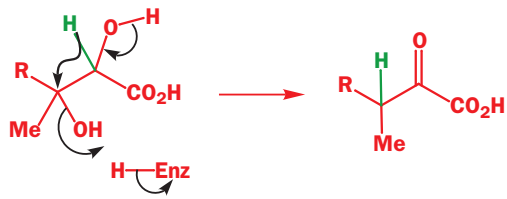
The green hydroxy-keto-acid is now primed for rearrangement. The migration of the group R is pushed by the removal of a proton from the OH group and pulled by the electron-accepting power of the keto group. Notice that the group R (Me or Et) migrates in preference to CO_2H. Usually in rearrangements the group better able to bear a positive charge migrates (Chapter 37).



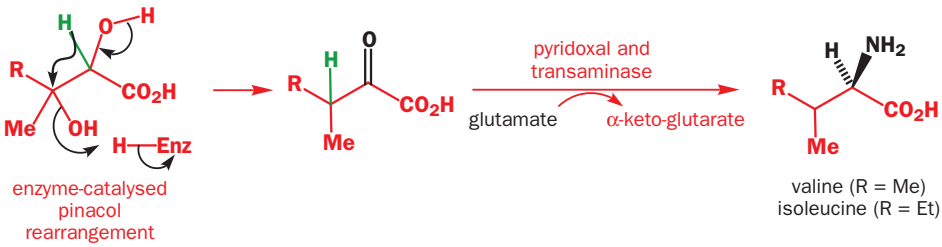
Control in this reaction is likely to be exerted stereoelectronically by the enzyme as it was in the pyridoxal reactions above. Since the C–R bond is held parallel to the p orbitals of the ketone, R migration occurs, but if the CO_2H group were to be held parallel to the p orbitals of the ketone, decarboxylation would occur. Next, a simple reduction with NADPH converts the ketone into an alcohol and prepares the way for a second rearrangement.



The second rearrangement is even more like a pinacol rearrangement because the starting material is a 1,2-diol. The tertiary alcohol is protonated and leaves, and again the CO_2H group does not migrate even though the alternative is merely hydride.

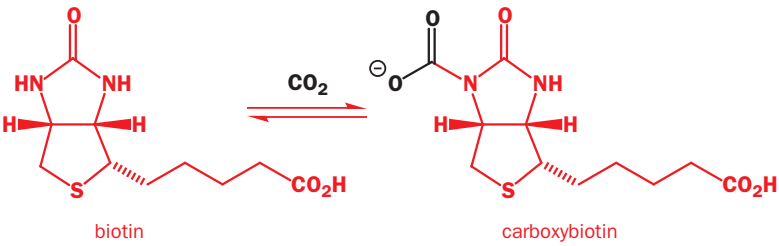


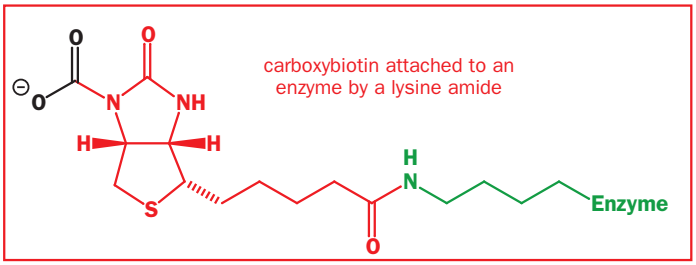
Finally, a pyridoxal transamination converts the two keto-acids stereospecifically to the corresponding amino acids, valine (R = Me) and isoleucine (R = Et). The donor amino acid is probably glutamate—it usually is in amino acid synthesis.



Carbon dioxide is carried by biotin

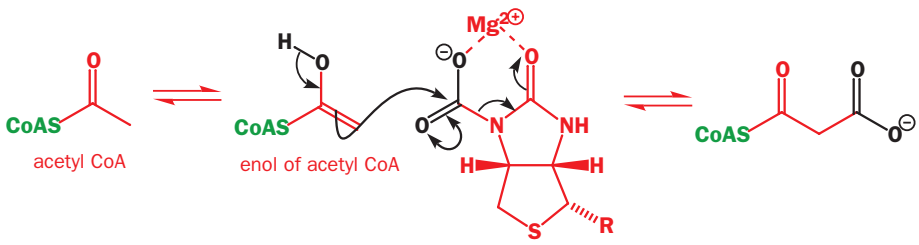
We have added and removed carbon dioxide on several occasions in this chapter and the last but we have not until now said anything about how this happens. You would not expect gaseous CO_2 to be available inside a cell: instead CO_2 is carried around as a covalent compound with another coenzyme—**biotin**.





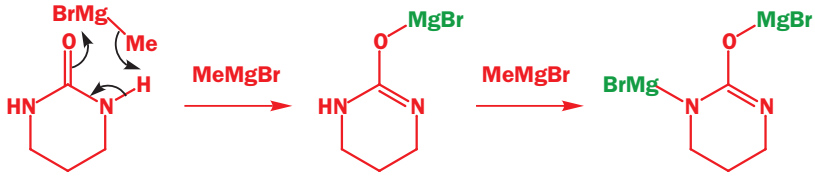
Biotin has two fused five-membered heterocyclic rings. The lower is a cyclic sulfide and has a long side chain ending in a carboxylic acid for attachment—yes, you've guessed it—to a lysine residue of a protein. The upper ring is a urea—it has a carbonyl group flanked by two nitrogen atoms. It is this ring that reversibly captures CO_2, on the nitrogen atom opposite the long side chain. The attachment to the enzyme as a lysine amide gives it an exceptionally long flexible chain and allows it to deliver CO_2 wherever it's needed.

One of the important points at which CO_2 enters as a reagent carried by biotin is in fatty acid biosynthesis where CO_2 is transferred to the enol of acetyl CoA. A magnesium(II) ion is also required and we may imagine the reaction as a nucleophilic attack of the enol on the magnesium salt of carboxybiotin. Most of the CO_2 transfers we have met take place by mechanisms of this sort: nucleophilic attack on a bound molecule of CO_2, usually involving a metal ion.

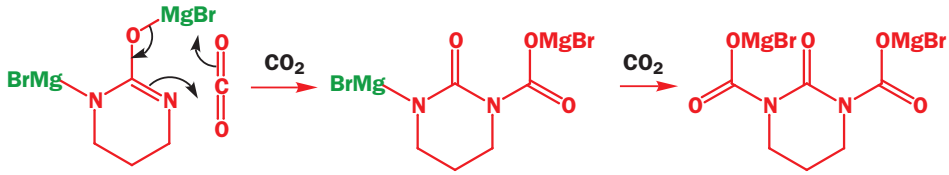


Very similar reactions can be carried out in the laboratory. This simple cyclic urea reacts twice with the Grignard reagent MeMgBr to give a dimagnesium derivative, probably having the structure shown with one O–Mg and one N–Mg bond.

■ We will see in the next chapter how acetoacetyl CoA is used in the biosynthesis of fatty acids and polyketides.

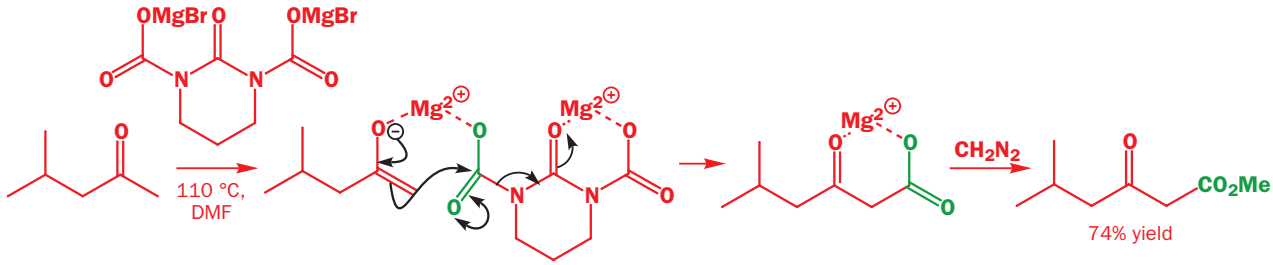


This magnesium derivative reacts with two molecules of CO_2 to give a double adduct with both nitrogens combining with CO_2. The product is stable as the double magnesium salt, which is a white powder.

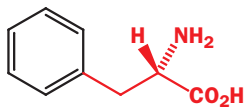


■ Diazomethane esterification appeared in Chapter 40, p. 1053.

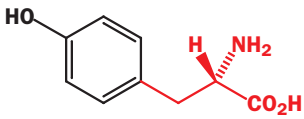
Simply heating this white powder with a ketone leads to efficient carboxylation and the unstable keto-acid may be trapped with diazomethane to form the stable methyl ester. The mechanism is presumably very like that drawn above for the transfer of CO_2 from carboxybiotin to acetyl CoA. Reactions like this *prove* nothing about the biochemical reaction but they at least show us that such reactions are possible and help us to have confidence that we are right about what Nature is doing.



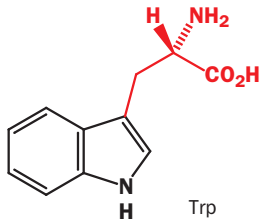
The shikimic acid pathway



Phe



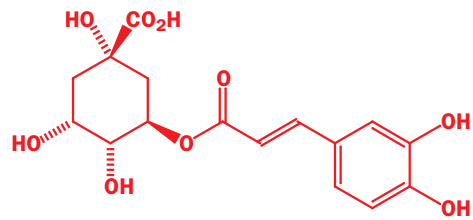
Tyr



Trp

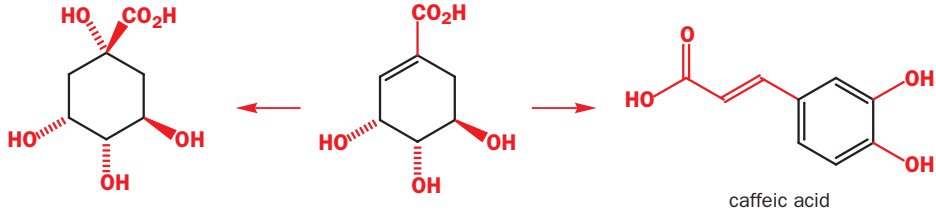
We have described reactions from various different pathways in this chapter so far, but now we are going to look at one complete pathway in detail. It is responsible for the biosynthesis of a large number of compounds, particularly in plants. Most important for us is the biosynthesis of the aromatic amino acids Phe (phenylalanine), Tyr (tyrosine), and Trp (tryptophan). These are 'essential' amino acids for humans—we have to have them in our diet as we cannot make them ourselves. We get them from plants and microorganisms.

So how do plants make aromatic rings? A clue to the chemistry involved comes from the structure of caffeyl quinic acid, a compound that is present in instant coffee in some quantity. It is usually about 13% of the soluble solids from coffee beans.



caffeyl quinic acid

This ester has two six-membered rings—one aromatic and one rather like the sugar alcohols we were discussing in the last chapter. You might imagine making an aromatic ring by the dehydration (losing three molecules of water) of a cyclohexane triol and the saturated ring in caffeyl quinic acid looks a good candidate. It is now known that both rings come from the same intermediate, shikimic acid.

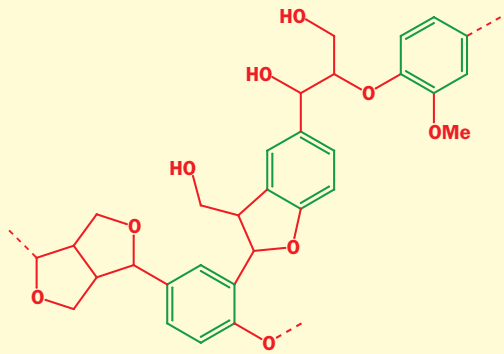


quinic acid shikimic acid caffeic acid

This key intermediate has given its name to Nature's general route to aromatic compounds and many other related six-membered ring compounds: **the shikimic acid pathway**. This pathway contains some of the most interesting reactions (from a chemist's point of view) in biology. It starts with an aldol reaction between phosphoenol pyruvate as the nucleophilic enol component and the C_4 sugar erythrose 4-phosphate as the electrophilic aldehyde.

Wood

Even the structural material of plants, lignin, comes from the shikimic acid pathway. Lignin—from which wood is made—has a variable structure according to the plant and the position in the plant. A typical splinter is shown here.

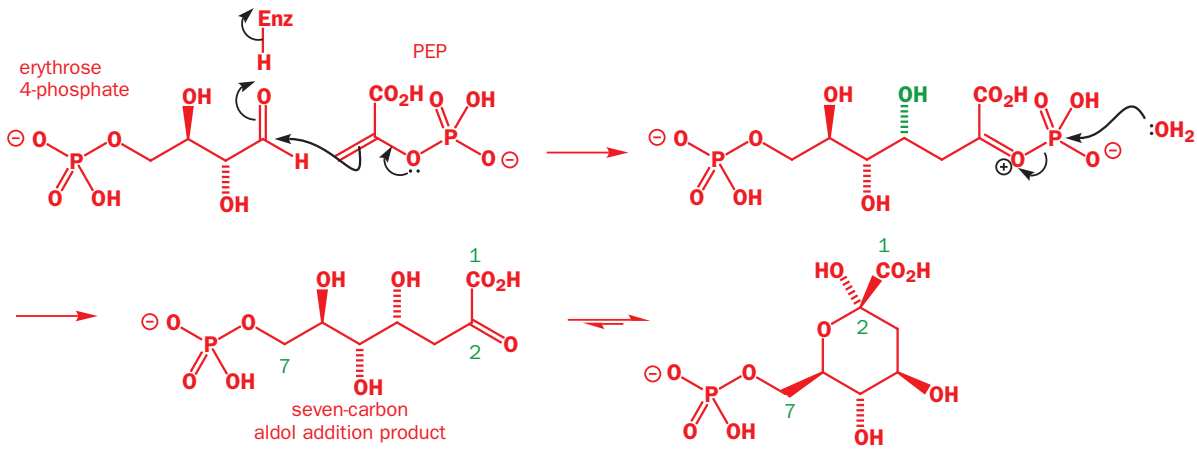


a typical lignin fragment

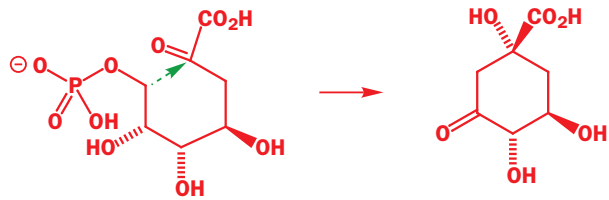
the aromatic rings are made from shikimic acid

the various structural parts and functional groups might be assembled in many different ways

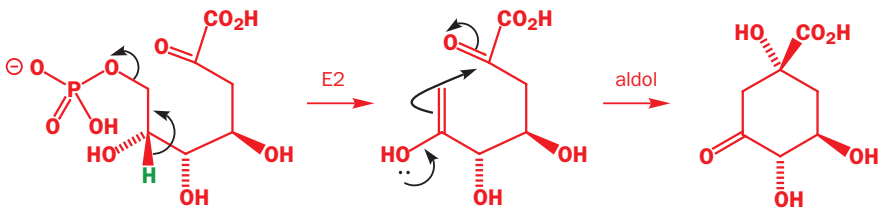
the dotted bonds show where the structure might continue



Hydrolysis of the phosphate releases the aldol product, a C_7 α-keto-acid with one new stereogenic centre, which is in equilibrium with a hemiacetal, just like a sugar. This intermediate has the right number of carbon atoms for shikimic acid and the next stage is a cyclization. If we redraw the C_7 α-keto-acid in the right shape for cyclization we can see what is needed. The green arrow shows only which bond needs to be formed.

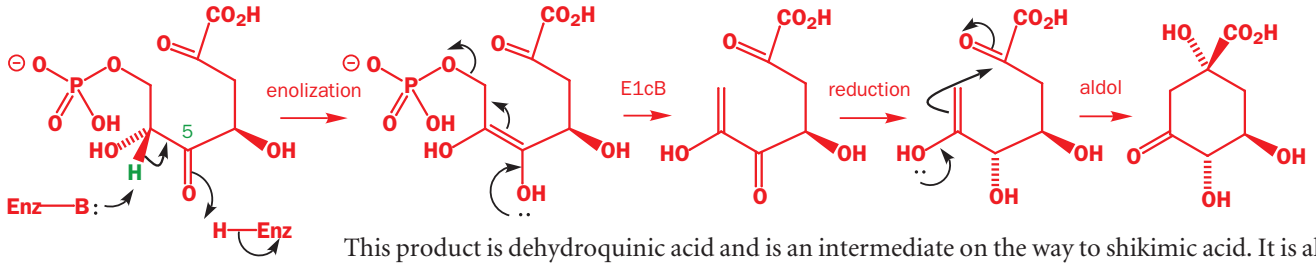


This reaction looks like an aldol reaction too and there is an obvious route to the required enol by elimination of phosphate. This would require the removal of a proton (green in the diagram) that is not at all acidic.

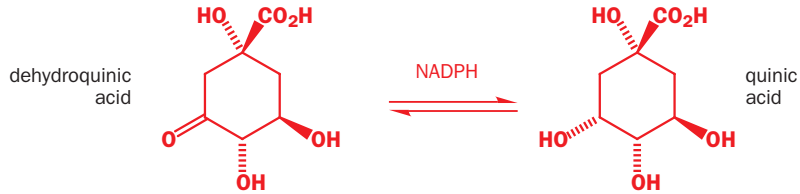


The problem can be avoided if the hydroxyl group at C5 is first oxidized to a ketone (NAD^+ is the oxidant). Then the green proton is much more acidic, and the elimination becomes an E1cB

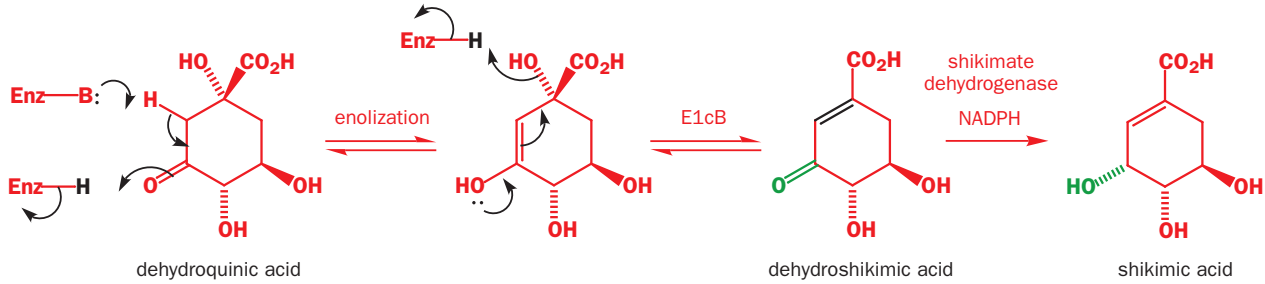
reaction, similar to the one in the synthesis of PEP. True, the ketone must be reduced back to the alcohol afterwards but Nature can deal with that easily.



This product is dehydroquinic acid and is an intermediate on the way to shikimic acid. It is also in equilibrium with quinic acid, which is not an intermediate on the pathway but which appears in some natural products like the coffee ester caffeyl quinic acid.

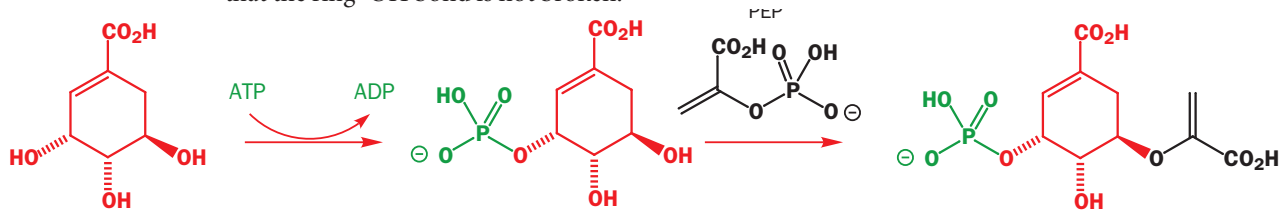


The route to shikimic acid in plants involves, as the final steps, the dehydration of dehydroquinic acid and then reduction of the carbonyl group. Doing the reactions this way round means that the dehydration can be E1cB—much preferred under biological conditions. This is what happens.

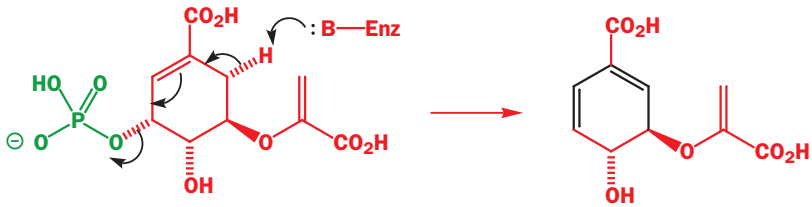


The final reduction uses NADPH as the reagent and is, of course, totally stereoselective with the hydride coming in from the top face of the green ketone as drawn. At last we have arrived at the halfway stage and the key intermediate, shikimic acid.

The most interesting chemistry comes in the second half of the pathway. The first step is a chemoselective phosphorylation of one of the three OH groups by ATP—as it happens, the OH group that has just been formed by reduction of a ketone. This step prepares that OH group for later elimination. Next, a second molecule of PEP appears and adds to the OH group at the other side of the molecule. This is PEP in its enol ether role, forming an acetal under acid catalysis. The reaction occurs with retention of stereochemistry so we know that the OH group acts as a nucleophile and that the ring–OH bond is not broken.



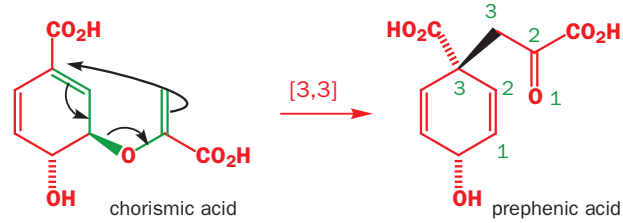
Now a 1,4 elimination occurs. This is known to be a *syn* elimination on the enzyme. When such reactions occur in the laboratory, they can be *syn* or *anti*. The leaving group is the green phosphate added two steps before.



EPSP—5-enolpyruvylshikimate 3-phosphate

chorismic acid

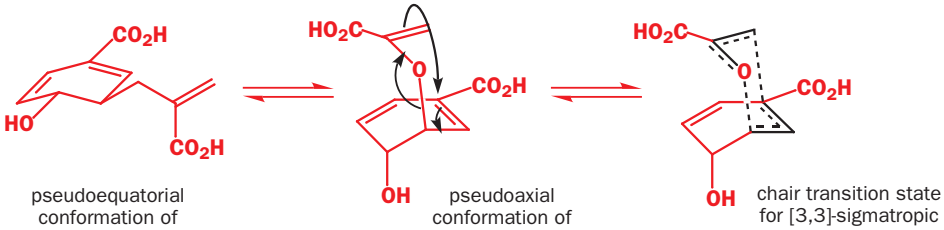
The product is chorismic acid and this undergoes the most interesting step of all—a [3,3]-sigmatropic rearrangement. Notice that the new (black) σ bond forms on the same face of the ring as the old (green) σ bond: this is, as you should expect, a suprafacial rearrangement.



chorismic acid prephenic acid

■ For more on sigmatropic rearrangements, see Chapter 36.

The most favourable conformation for chorismic acid has the substituents pseudoequatorial but the [3,3]-sigmatropic rearrangement cannot take place in that conformation. First, the diaxial conformation must be formed and the chair transition state achieved. Then the required orbitals will be correctly aligned.



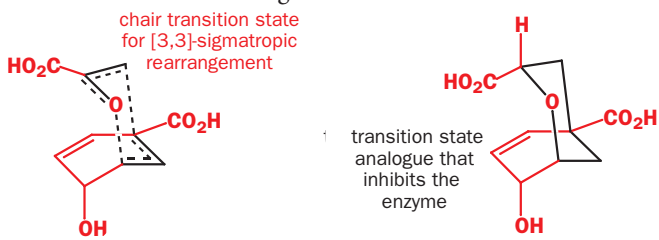
pseudoequatorial conformation of chorismic acid

pseudoaxial conformation of chorismic acid

chair transition state for [3,3]-sigmatropic rearrangement

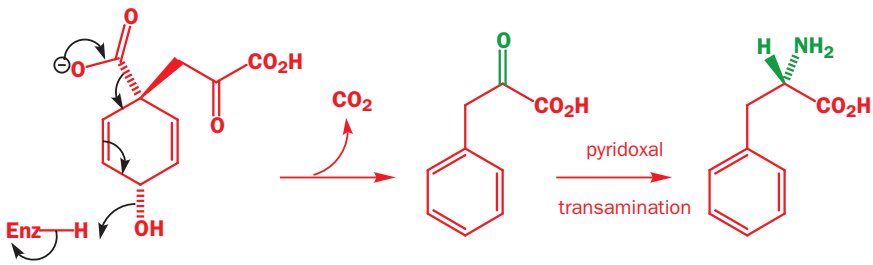
These reactions occur well without the enzyme (Chapter 36) but the enzyme accelerates this reaction by about a 10^6 increase in rate. There is no acid or base catalysis and we may suppose that the enzyme binds the transition state better than it binds the starting materials. We know this to be the case, because close structural analogues of the six-membered ring transition state also bind to the enzyme and stop it working. An example is shown alongside—a compound that resembles the transition state but can't react.

▶ By binding the transition state (not the starting materials) strongly, the enzyme lowers the activation energy for the reaction.



transition state analogue that inhibits the enzyme

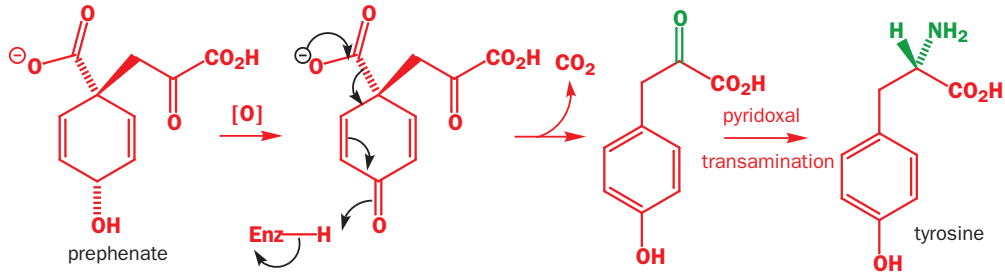
We have arrived at prephenic acid, which as its name suggests is the last compound before aromatic compounds are formed, and we may call this the end of the shikimic acid pathway. The final stages of the formation of phenylalanine and tyrosine start with aromatization. Prephenic acid is unstable and loses water and CO_2 to form phenylpyruvic acid. This α-keto-acid can be converted into the amino acid by the usual transamination with pyridoxal.



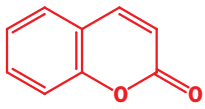
prephenate phenylpyruvic acid phenylalanine

The route to tyrosine requires a preliminary oxidation and then a decarboxylation with the

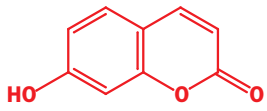
electrons of the breaking C–C bond ending up in a ketone group. Transamination again gives the amino acid.



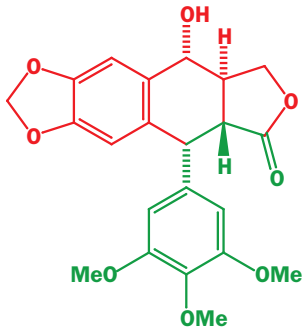
prephenate



coumarin



umbelliferone



podophyllotoxin

Other shikimate products

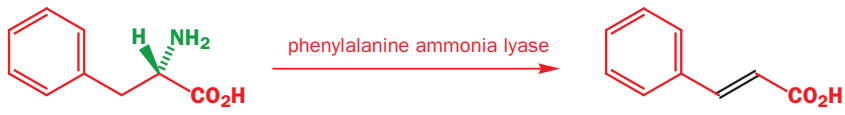
Many natural products are formed from the shikimate pathway. Most can be recognized by the aromatic ring joined to a three-carbon atom side chain. Two simple examples are coumarin, responsible for the smell of mown grass and hay, and umbelliferone, which occurs in many plants and is used in suntan oils as it absorbs UV light strongly. These compounds have the same aryl-C_3 structure as Phe and Tyr, but they have an extra oxygen atom attached to the benzene ring and an alkene in the C_3 side chain.

An important shikimate metabolite is podophyllotoxin, an antitumour compound—some podophyllotoxin derivatives are used to combat lung cancer. The compound can be split up notionally into two shikimate-derived fragments (shown in red and green). Both are quite different and there is obviously a lot of chemistry to do after the shikimic acid pathway is finished.

Among the more interesting reactions involved in making all three of these natural products are the loss of ammonia from phenylalanine to give an alkene and the introduction of extra OH groups around the benzene rings. We know how a *para* OH of Tyr is introduced directly by the oxidation of prephenic acid before decarboxylation and it is notable that the extra oxygen functionalities appear next to that point. This is a clue to the mechanism of the oxidation.

Alkenes by elimination of ammonia—phenyl ammonia lyase

Many amino acids can lose ammonia to give an unsaturated acid. The enzymes that catalyse these reactions are known as **amino acid ammonia lyases**. The one that concerns us at the end of the shikimic acid pathway is phenylalanine ammonia lyase, which catalyses the elimination of ammonia from phenylalanine to give the common metabolite cinnamic acid.

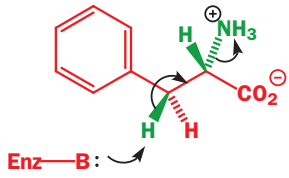


Phe
phenylalanine

cinnamic acid

This reaction gives only *E*-cinnamic acid and the proton *anti* to the amino group is lost. This might make us think that we have an E2 reaction with a base on the enzyme removing the required proton. But a closer look at this mechanism makes it very unconvincing. The proton that is removed has no acidity and ammonia is not a good leaving group. It is very unusual for Nature to use an enzyme to make a reaction happen that doesn't happen at all otherwise. It is much more common for Nature to make a good reaction better.

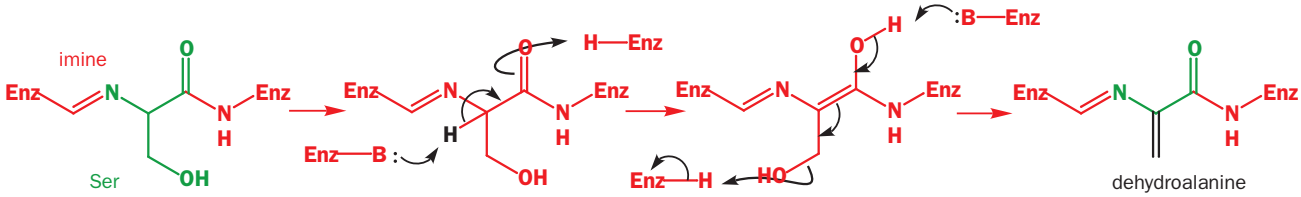
So how does an ammonia lyase work? The enzyme makes the ammonia molecule into a much better leaving group by using a serine residue. This serine is attached to the protein through its carboxyl group by the usual amide bond but its amino group is bound as an imine. This allows it to eliminate water to form a double bond before the phenylalanine gets involved. The elimination converts serine into a dehydroalanine residue. This is an E1cB elimination using only general acid and base catalysis as the proton to be lost is acidic and an enol can be an intermediate.



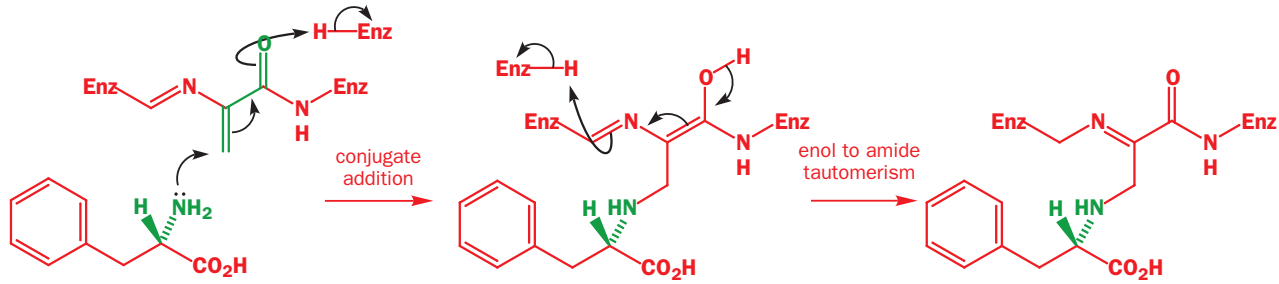
possible E2 mechanism for phenylalanine ammonia lyase?

▶ A *lyase* is an enzyme that catalyses *lysis*: it breaks something down.

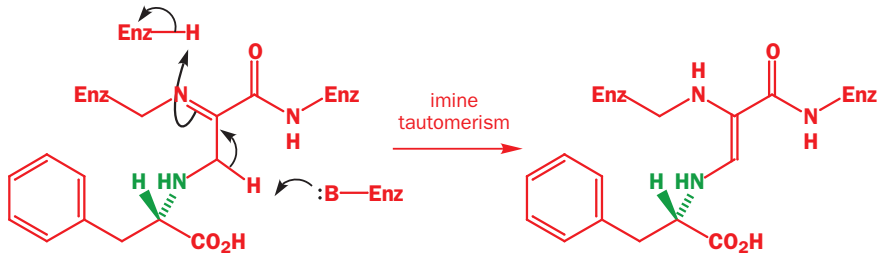
▶ Eliminations of ammonium salts (Chapter 19, p. 484) require very strong bases—much stronger than those available to enzymes—and fully alkylated amines. You can't protonate an amine in the presence of strong base.



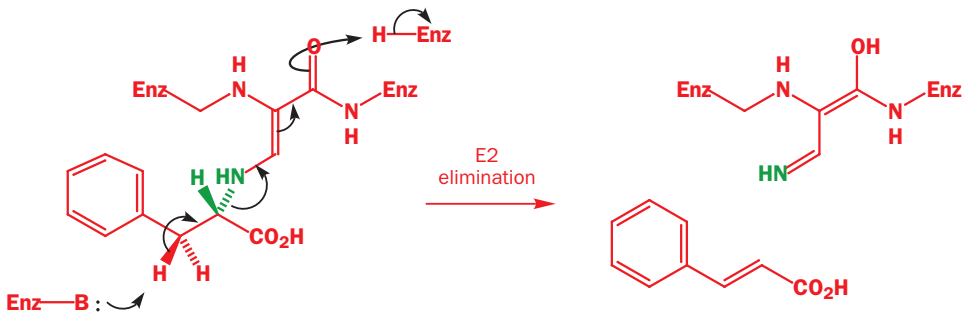
The alkene of the dehydroenzyme is conjugated with a carbonyl group—it's electrophilic and the amino group of Phe can add to it in conjugate fashion. When the enol tautomerizes back to a carbonyl compound, it can be protonated on the imine carbon because the imine is conjugated to the enol. This might remind you of pyridoxal's chemistry (p. 1384).



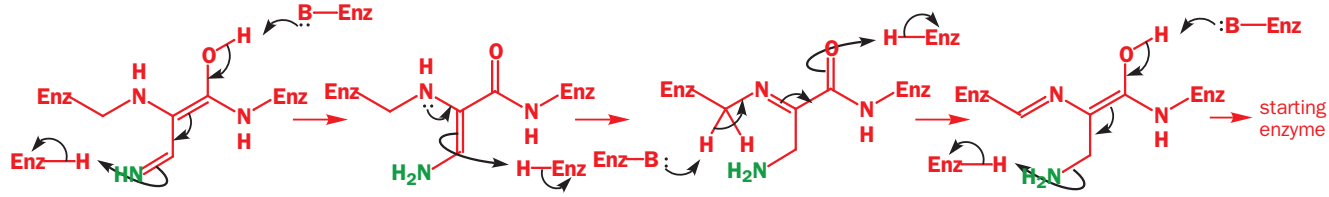
A second tautomerism makes an enamine—again very like the pyridoxal mechanisms you saw earlier.



Now at last the secret is revealed. We can break the C–N bond and use the carbonyl group as an electron sink. The acidity of the proton that must be lost is no greater but the nitrogen atom has become a very much better leaving group.

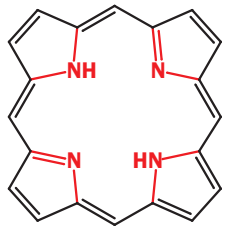


The difficult elimination is accomplished by making it an ammonia transfer reaction rather than an elimination of ammonia. Recycling the enzyme does eventually require elimination of ammonia but in an easy E1cB rather than a difficult E2 reaction. Overall, a difficult reaction—elimination of ammonia—is accomplished in steps that involve no strong acids or strong bases, and most of the steps are simple proton transfers, often tautomerisms between imines, enols, and amides.



Haemoglobin carries oxygen as an iron(II) complex

porphyrin

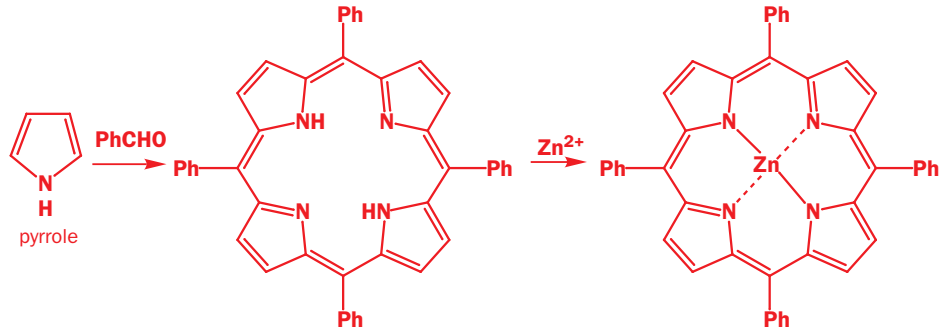


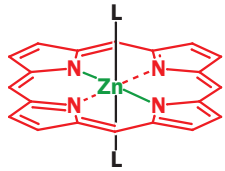
18 electrons in a conjugated ring = $4n + 2$ ($n = 4$)

Porphyrins appeared in Chapters 43 and 44, pp. 1178 and 1189.

Biological oxidations are very widespread. Human metabolism depends on oxidation, and on getting oxygen, which makes up 20% of the atmosphere, into cells. The oxygen transporter, from atmosphere to cell, is **haemoglobin**.

The reactive part of haemoglobin is a **porphyrin**. These are aromatic molecules with 18 electrons around a conjugated ring formed from four molecules of a five-membered nitrogen heterocycle. Chemically, symmetrical porphyrins are easily made from pyrrole and an aldehyde.

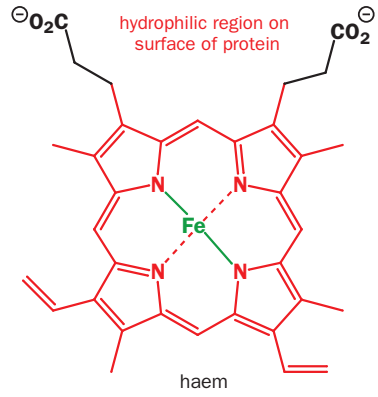




octahedral zinc(II) porphyrin with two extra ligands

The hole in the middle of a porphyrin is just the right size to take a divalent transition metal in the first transition series, and zinc porphyrins, for example, are stable compounds. Once the metal is inside a porphyrin, it is very difficult to get out. Two of the nitrogen atoms form normal covalent bonds (the ones that were NH in the porphyrin) and the other two donate their lone pairs to make four ligands around the metal. The complexed zinc atom is square planar and still has two vacant sites—above and below the (more or less) flat ring. These can be filled with water molecules, ammonia, or other ligands.

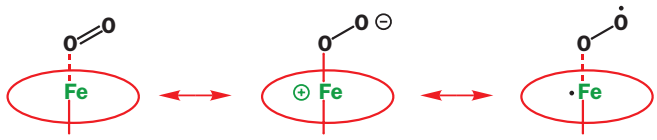
The porphyrin part of haemoglobin is called **haem**, and it is an iron(II) complex. It is unsymmetrically substituted with carboxylic acid chains on one side and vinyl groups on the other.

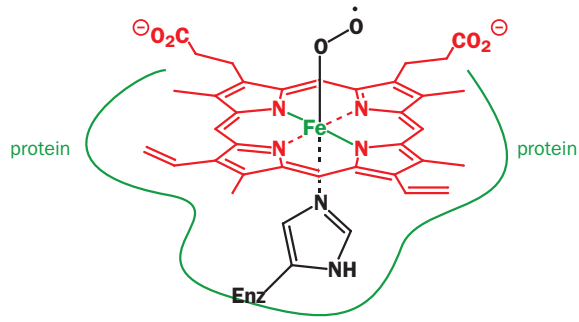


haem

Haem is bound to proteins to make haemoglobin (in blood) and myoglobin (in muscle). The hydrophilic carboxylate groups stick out into the surrounding medium, while the majority of the molecule is embedded in a hydrophobic cleft in the protein, lined with amino acids such a leucine and valine. The octahedral coordination sphere of the iron(II) is completed with a histidine residue from the protein and an oxygen molecule.

The oxygen complex can be drawn like this or, alternatively, as an Fe(III) complex of an oxyanion (below).

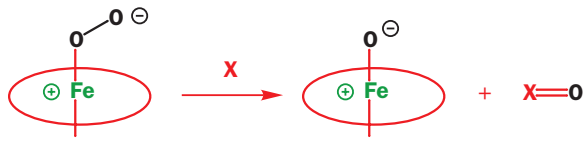




oxyhaemoglobin

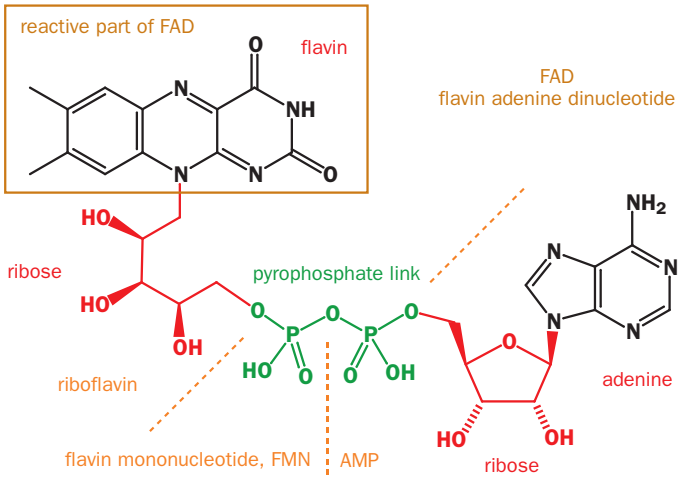
It is difficult to draw detailed mechanisms for oxidations by iron complexes but it is the oxygen atom further from Fe that reacts. You can see in principle how breakage of the weak O–O bond could deliver an oxygen atom to a substrate and leave an Fe(III)–O^- complex behind.

Oxygen molecules are transferred from haemoglobin to other haems, such as the enzyme P450, and to a wide range of oxidizing agents. Almost any molecule we ingest that isn't a nutrient—a drug molecule, for example—is destroyed by oxidation. The details of the mechanisms of these oxidations have proved very difficult to elucidate, but the hydroxylation of benzene is an exception. We do know how it happens, and it's another case of Nature using enzymes to do some really remarkable chemistry.



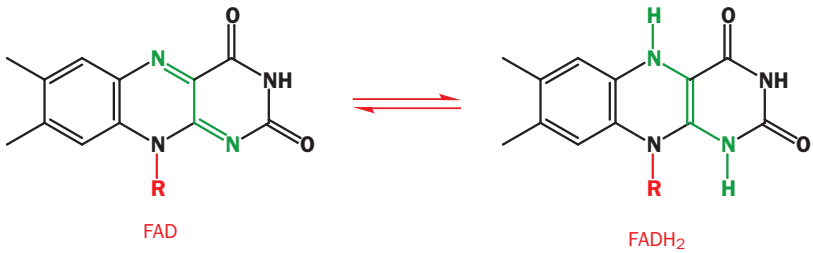
Aromatic rings are hydroxylated via an epoxide intermediate

The oxidizing agents here are related to FAD. We said little about $FADH_2$ as a reducing agent earlier in this chapter because it is rather similar to NADH which we have discussed in detail. FAD is another dinucleotide and it contains an AMP unit linked through the 5′ position by a pyrophosphate group to another nucleotide. The difference is that the other nucleotide is flavin mononucleotide. Here is the complete structure.



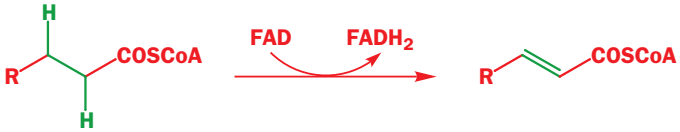
The whole thing is FAD. Cutting FAD in half down the middle of the pyrophosphate link would give us two nucleo*tides*, AMP and FMN (flavin mononucleotide). The sugar in each case is ribose (in its furanose form in AMP but in open-chain form in FMN) so the flavin nucleo*side* is riboflavin. We can abbreviate this complex structure to the reactive part, which is the flavin. The rest we shall just call 'R'.

▶ Riboflavin is also known as vitamin B_2 as you may see on the side of your cornflakes packet.

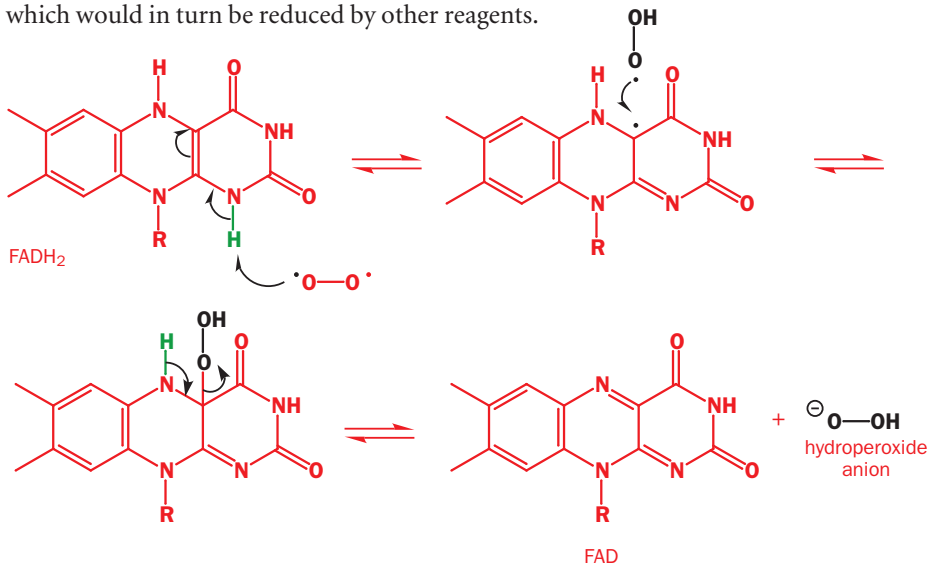


Redox reactions with FAD involve the transfer of two hydrogen atoms to the part of the molecule shown in green. Typical reactions of FAD involve dehydrogenations—as in double bond formation from single bonds. Of course, one of the H atoms can be transferred to FAD as a proton—only one need be a hydride ion H^-, though both could be transferred as radicals ($H^\bullet$).

▶ You should contrast this with the redox reactions of NAD where only one hydrogen atom is transferred.

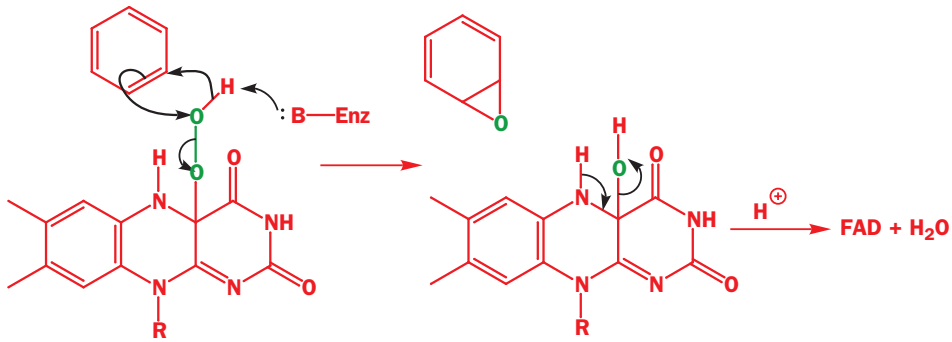


After FAD has been used as an oxidant in this fashion, the $FADH_2$ reacts with molecular oxygen to give a hydroperoxide, which decomposes back to FAD and gives an anion of hydrogen peroxide, which would in turn be reduced by other reagents.

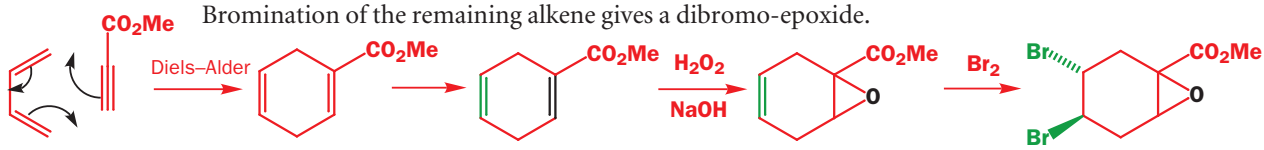


▶ Note the radical steps in this sequence. The reactions of oxygen, whose ground state is a triplet diradical (see Chapter 4), are typically radical processes.

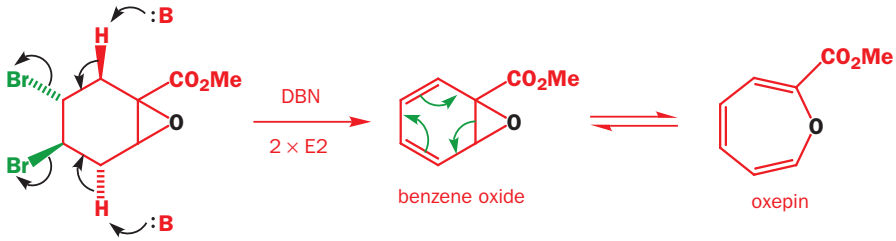
In the reactions we are now concerned with, the hydroperoxide intermediate itself is the important reagent, before it loses hydroperoxide anion. This intermediate is an oxidizing agent—for example, it reacts quite dramatically with benzene to give an epoxide.



This benzene oxide may look very dubious and unstable, but benzene oxides can be made in the laboratory by ordinary chemical reactions (though not usually by the direct oxidation of benzene). We can instead start with a Diels–Alder reaction between butadiene and an alkyne. Epoxidation with a nucleophilic reagent ($HO–O^-$ from H_2O_2 and NaOH) occurs chemoselectively on the more electrophilic double bond—the one that is conjugated to the electron-withdrawing carbonyl group. Bromination of the remaining alkene gives a dibromo-epoxide.

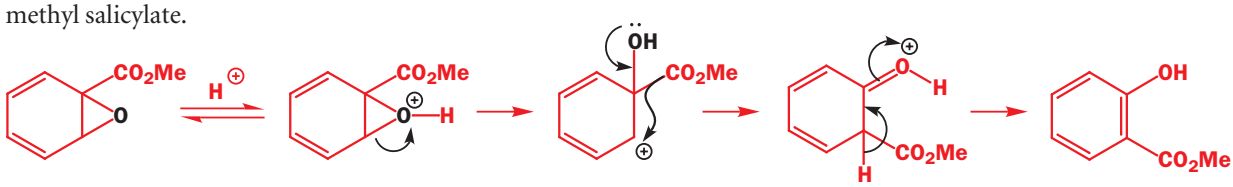


This is an ordinary electrophilic addition to an alkene so the two bromine atoms are *anti* in the product. Elimination under basic conditions with DBN gives the benzene oxide.

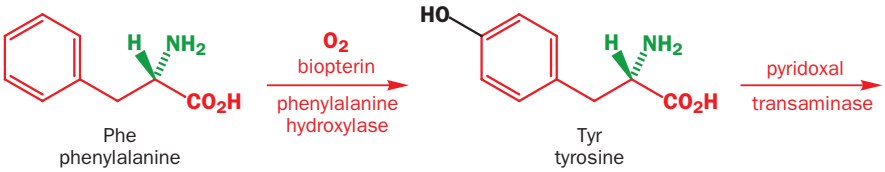


At least, it ought to have given the benzene oxide! The compound turned out to have a fluxional structure—it was a mixture of compounds that equilibrate by a reversible disrotatory electrocyclic reaction.

Treatment with acid turns the benzene oxide/oxepin into an aromatic ring by a very interesting mechanism. The epoxide opens to give the cation, which is *not* conjugated with the electron-withdrawing CO_2Me group, and then a migration of that CO_2Me group occurs. This has been proved by isotope labelling experiments. The final product is the *ortho*-hydroxy-ester, known as methyl salicylate.

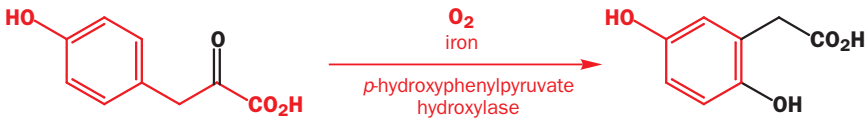


This chemistry seems rather exotic, but in the degradation of phenylalanine two benzene oxide intermediates and two such rearrangements occur one after the other. This is the initial sequence.



Phe
phenylalanine

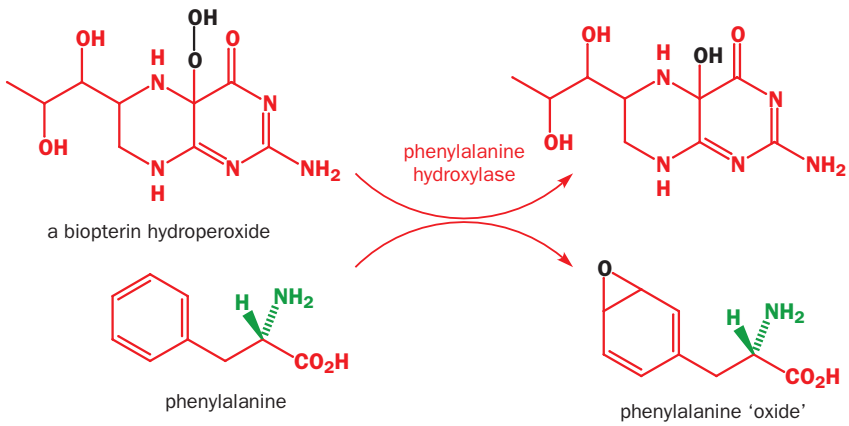
Tyr
tyrosine



p-hydroxyphenylpyruvic acid

homogentisic acid

The first reaction involves a hydroperoxide related to the FAD hydroperoxide you have just seen but based on a simpler heterocyclic system, a biopterin. The reaction is essentially the same and a benzene oxide is formed.

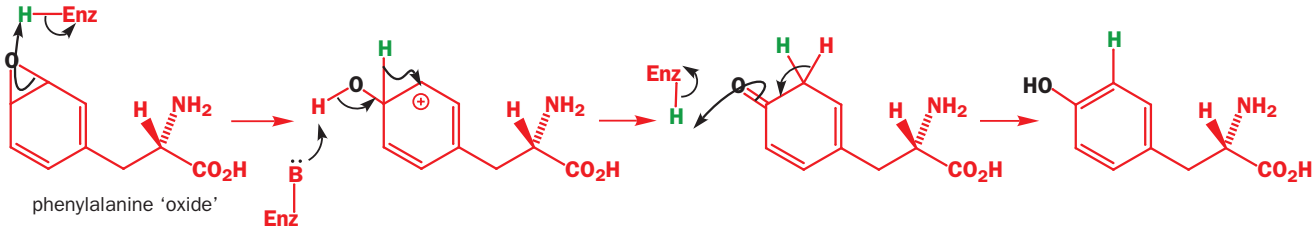


a biopterin hydroperoxide

phenylalanine

phenylalanine 'oxide'

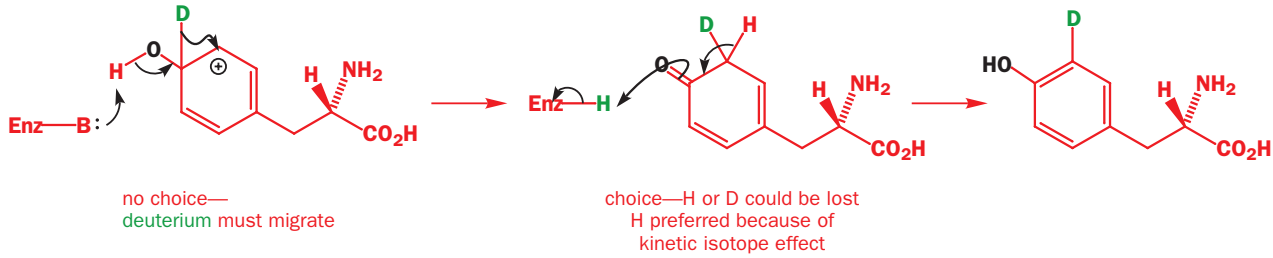
The biopterin product is recycled by elimination of water, reduction using NADPH as the reagent, and reaction with molecular oxygen. The other product, the phenylalanine oxide, rearranges with a hydride shift followed by the loss of a proton to give tyrosine.



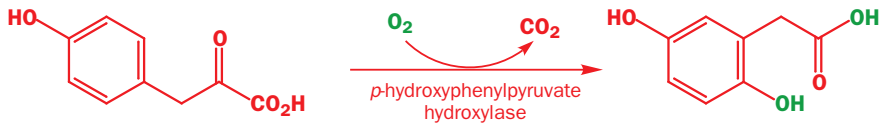
phenylalanine 'oxide'

▶ This rearrangement is known as the 'NIH shift', after its discovery at the National Institutes of Health at Bethesda, Maryland.

We know that this is the mechanism because we can make the green H a deuterium atom. We then find that deuterium is present in the tyrosine product *ortho* to the phenolic hydroxyl group. When the migration occurs, the deuterium atom must go as there is no alternative, but in the next step there is a choice and H loss will be preferred to D loss because of the kinetic isotope effect (Chapter 19). Most of the D remains in the product.

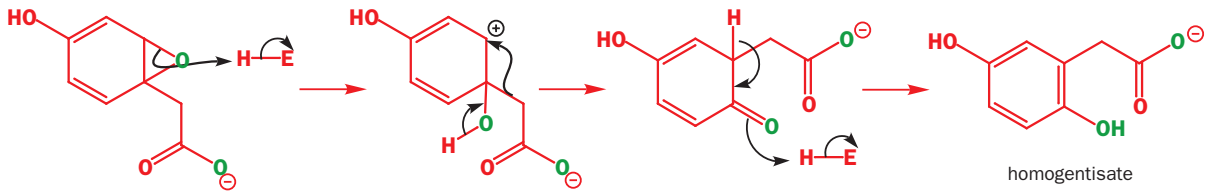


A shift of a larger group comes two steps later in the synthesis of homogentisic acid. Another labelling experiment, this time with $^{18}O_2$, shows that both atoms of oxygen end up in the product.

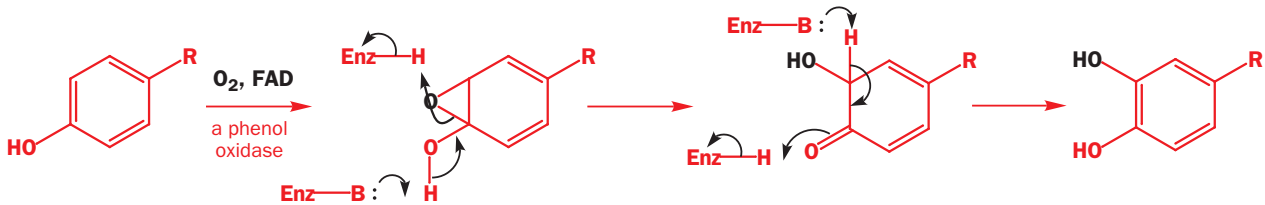


The key intermediate is a peroxy-acid formed after decarboxylation. The peroxy-acid is perfectly placed for an intramolecular epoxidation of a double bond in the benzene ring next to the side chain.

The epoxide can now rearrange with the whole side chain migrating in a reaction very similar to the laboratory rearrangement to give methyl salicylate that you saw on p. 1409.



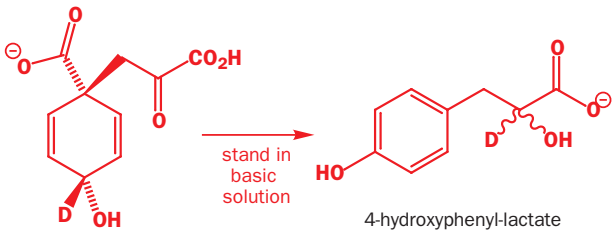
When hydroxylation occurs next to an OH group that is already there, no NIH shift occurs. This is because the epoxide is opened by the push of electrons from the OH group and there is only one H atom to be lost anyway. The cofactor for these enzymes is slightly different, being again the hydroperoxide from FAD, but the principle is the same.



In the next chapter you will see how hydroxylation of benzene rings plays an important part in the biosynthesis of alkaloids and other aromatic natural products.

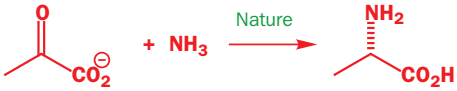
Problems

1. On standing in alkali in the laboratory, prephenic acid rearranges to 4-hydroxyphenyl-lactic acid with specific incorporation of deuterium label as shown. Suggest a mechanism, being careful to draw realistic conformations.

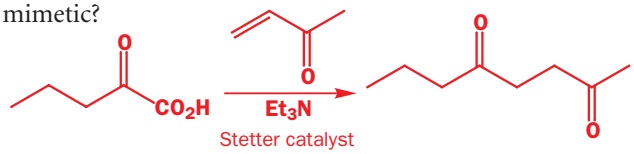


this product is racemic. why?

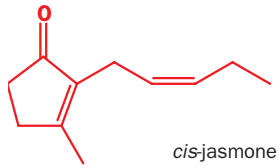
2. Write a full reaction scheme for the conversion of ammonia and pyruvate to alanine in living things. You will need to refer to the section of the chapter on pyridoxal to be able to give a complete answer.



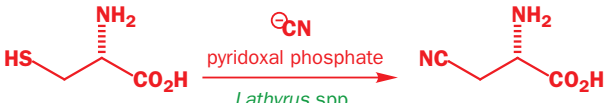
3. Give a mechanism for this reaction. You will find the Stetter catalyst described in the chapter. How is this sequence biomimetic?



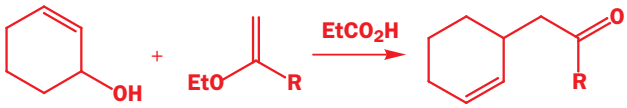
What starting material would be required for formation of the natural product *cis*-jasmone by an intramolecular aldol reaction (Chapter 27). How would you make this compound using a Stetter reaction?



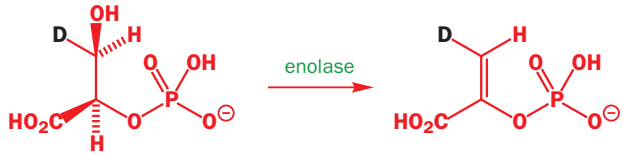
4. The amino acid cyanoalanine is found in leguminous plants (*Lathyrus*) but not in proteins. It is made in the plant from cysteine and cyanide by a two-step process catalysed by pyridoxal phosphate. Suggest a detailed mechanism.



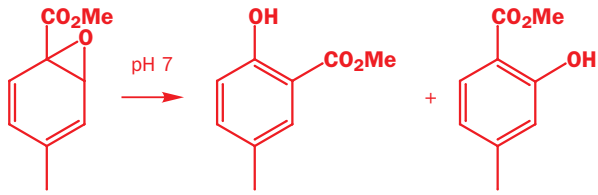
5. This chemical reaction might be said to be similar to a reaction in the shikimic acid pathway. Compare the two mechanisms and suggest how the model might be made closer and more interesting.



6. Stereospecific deuteration of the substrate for enolase, the enzyme that makes phosphoenol pyruvate, gives the results shown below. What does this tell us definitely about the reaction and what might it suggest about the mechanism?

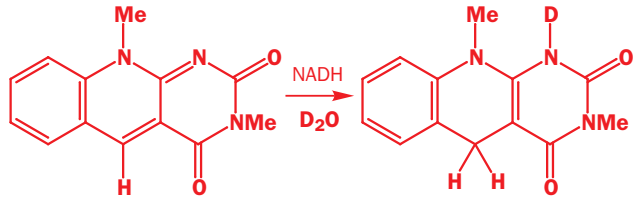


7. This rearrangement was studied as a biomimetic version of the NIH shift. Write a mechanism for the reaction. Do you consider it a good model reaction? If not, how might it be made better?

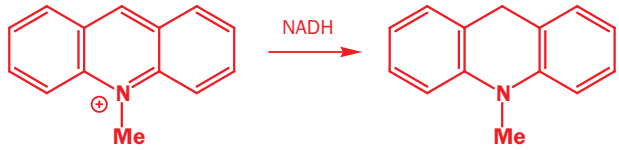


8. The following experiments relate to the chemical and biological behaviour of NADH. Explain what they tell us.

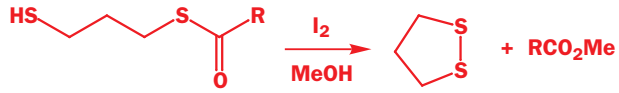
(**a**) This FAD analogue can be reduced *in vitro* with NADH in D_2O with deuterium incorporation in the product as shown.



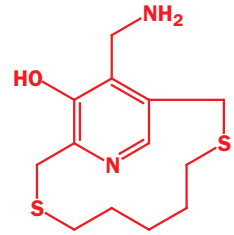
(**b**) NADH does not reduce benzaldehyde *in vitro* but it does reduce this compound.



9. Oxidation of this simple thiol ester gives a five-membered cyclic disulfide. The reaction is proposed as a model for the behaviour of lipoic acid in living things. Draw a mechanism for the reaction and make the comparison.

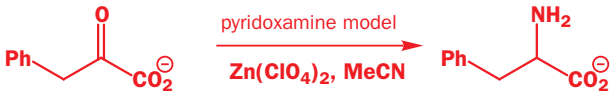


10. This curious compound is chiral—indeed it has been prepared as the (–) enantiomer. Explain the nature of the chirality.

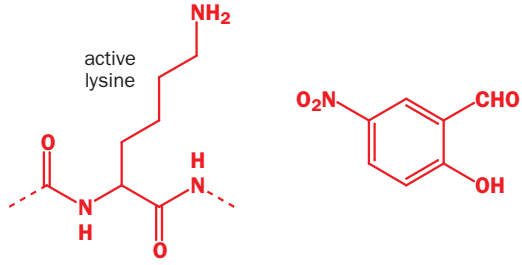


enantiomerically enriched (–) enantiomer

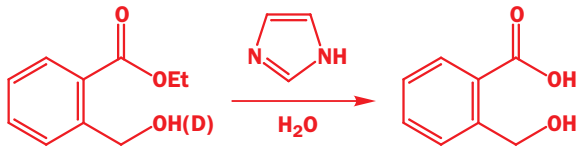
This compound has been used as a chemical model for pyridoxamine. For example, it transaminates phenylpyruvate under the conditions shown here. Comment on the analogy and the role of Zn(II). In what ways is the model compound worse and in what ways better than pyridoxamine itself?



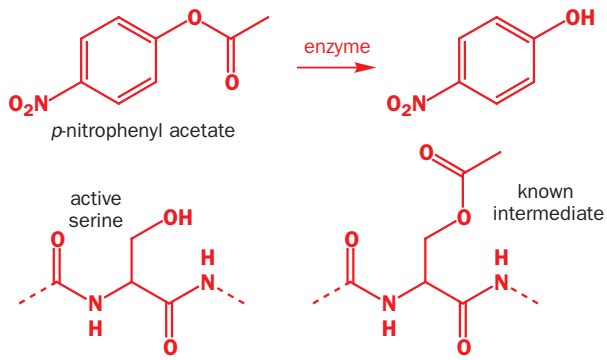
11. Enzymes such as aldolase, thought to operate by the formation of an imine and/or an enamine with a lysine in the enzyme, can be studied by adding $NaBH_4$ to a mixture of enzyme and substrate. For example, treatment of the enzyme with the aldehyde shown below and $NaBH_4$ gives a permanently inhibited enzyme that on hydrolysis reveals a modified amino acid in place of one of the lysines. What is the structure of the modified amino acid, and why is this particular aldehyde chosen?



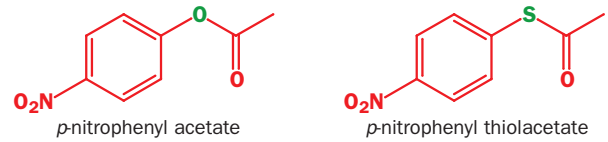
12. This question is about the hydrolysis of esters by 'serine' enzymes. First, interpret these results: The hydrolysis of this ester is very much faster than that of ethyl benzoate itself. It is catalysed by imidazole and then there is a primary isotope effect (Chapter 41) $k_{(OH)}/k_{(OD)} = 3.5$. What is the mechanism? What is the role of the histidine?



The serine enzymes have a serine residue vital for catalysis. The serine OH group is known to act as a nucleophilic catalyst. Draw out the mechanism for the hydrolysis of *p*-nitrophenyl acetate.



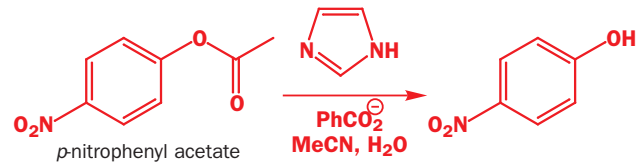
The enzyme also has a histidine residue vital for catalysis. Use your mechanism from the first part of the question to say how the histidine residue might help. The histidine residue is known to help both the formation and the hydrolysis of the intermediate. The enzyme hydrolyses both *p*-nitrophenyl acetate and *p*-nitrophenyl thiolacetate at the same rate. Which is the rate-determining step?



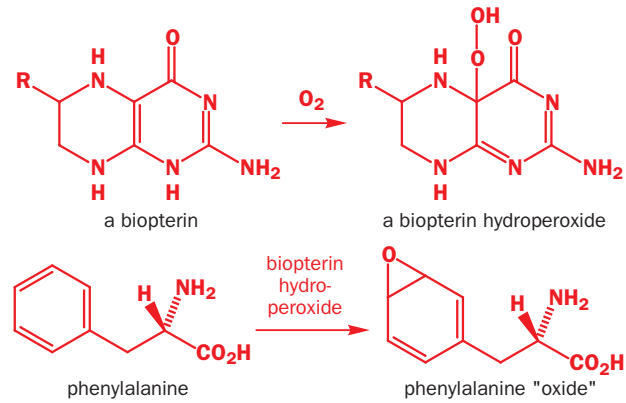
Finally, an aspartic acid residue is necessary for full catalysis and this residue is thought to use its CO_2^- group as a general base. A chemical model shows that the hydrolysis of *p*-nitrophenyl acetate in aqueous acetonitrile containing sodium benzoate and imidazole follows the rate law:

rate = k[*p*-nitrophenyl acetate] [benzoate] [imidazole].

Suggest a mechanism for the chemical reaction.



13. Give mechanisms for the biological formation of biopterin hydroperoxide and its reaction with phenylalanine. The reactions were discussed in the chapter but no details were given.



14. Revision of Chapter 48. How many electrons are there on the iron atom in the oxyhaemoglobin structure shown in the chapter? Does it matter if you consider the complex to be of Fe(II) or Fe(III)? Why are zinc porphyrins perfectly stable *without* extra ligands (L in diagram)?