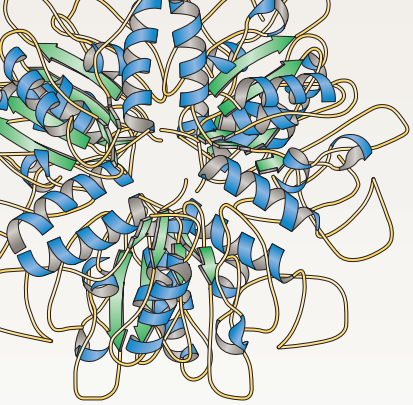
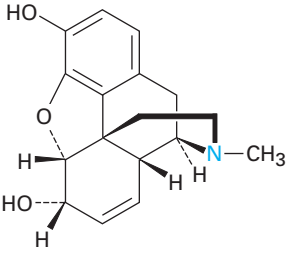


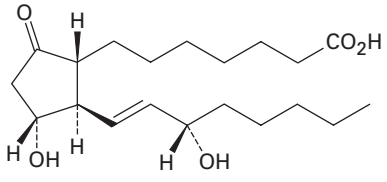
25 Secondary Metabolites: An Introduction to Natural Products Chemistry

In the past six chapters, we've looked at the chemistry and metabolism of the four major classes of biomolecules—proteins, carbohydrates, lipids, and nucleic acids. But there is far more to do, for all living organisms also contain a vast diversity of substances usually grouped under the heading *natural products.* The term **natural product** really refers to *any* naturally occurring substance but is generally taken to mean a **secondary metabolite**—a small molecule that is not essential to the growth and development of the producing organism and is not classified by structure.

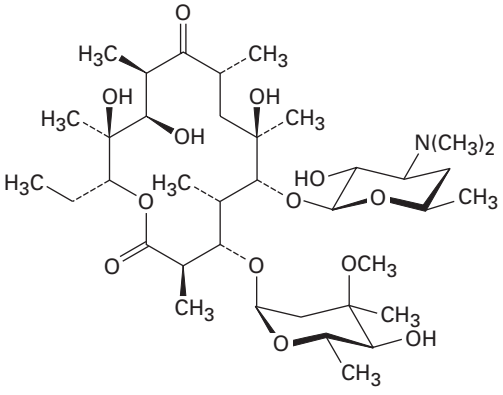
It has been estimated that well over 300,000 secondary metabolites exist, and it's thought that their primary function is to increase the likelihood of an organism's survival by repelling or attracting other organisms. Alkaloids, such as morphine; eicosanoids, such as prostaglandin E_1; and antibiotics, such as erythromycin and the penicillins, are examples.



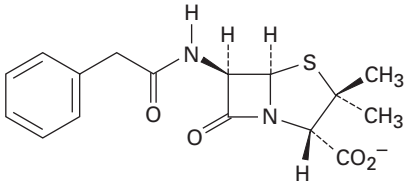
Morphine



Prostaglandin E_1



Erythromycin A



Benzylpenicillin

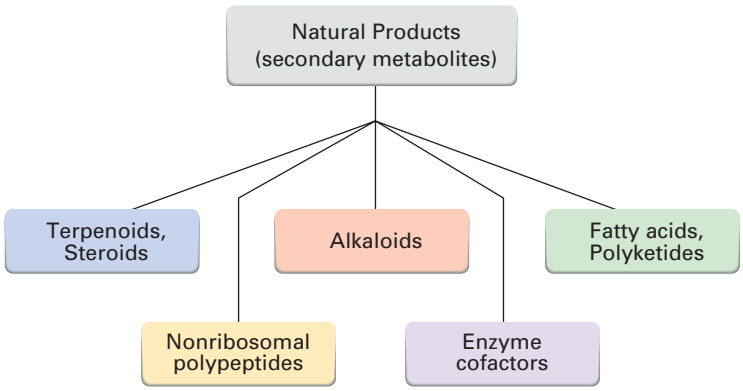
ORGANIC Chemistry Now™ Look for this logo in the chapter and go to OrganicChemistryNow at **http://now.brookscole.com/mcmurryorgbio1** for tutorials, simulations, and problems.

WHY THIS CHAPTER?

This small chapter merely tickles the surface of natural products chemistry, for hundreds, if not thousands, of books have been written on the subject. Rather than pretending to be comprehensive, this chapter is meant only to provide a brief introduction to a large and immensely important area of modern biochemistry, perhaps tempting you to learn more on your own. To provide that introduction, we'll look at the pathways by which several well-known natural products are synthesized in living organisms: pyridoxal phosphate (PLP), morphine, and erythromycin A. The molecules may appear complex (erythromycin A, in particular), but the individual chemical steps by which they are made should be familiar to you at this point.

25.1 Classification of Natural Products

There is no rigid scheme for classifying natural products—their immense diversity in structure, function, and biosynthesis is too great to allow them to fit neatly into a few simple categories. In practice, however, workers in the field often speak of five main classes of natural products: terpenoids and steroids, fatty acid–derived substances and polyketides, alkaloids, nonribosomal polypeptides, and enzyme cofactors.



- **Terpenoids** and **steroids,** as discussed previously in Chapter 23, are a vast group of substances—more than 35,000 are known—derived biosynthetically from isopentenyl diphosphate. Terpenoids have an immense variety of apparently unrelated structures, while steroids have a common tetracyclic carbon skeleton and are modified terpenoids that are biosynthesized from the triterpene lanosterol. We looked at terpenoid and steroid biosynthesis in Sections 23.8–23.10.
- **Alkaloids,** like terpenoids, are a large and diverse class of compounds, with more than 12,000 examples known at present. They contain a basic amine group in their structure and are derived biosynthetically from amino acids. We'll look at morphine biosynthesis as an example in Section 25.3.
- **Fatty acid–derived substances** and **polyketides,** of which more than 10,000 are known, are biosynthesized from simple acyl precursors such as acetyl CoA, propionyl CoA, and methylmalonyl CoA. Natural products

derived from fatty acids, such as the eicosanoid prostaglandin E_1, generally have most of the oxygen atoms removed, but polyketides, such as the antibiotic erythromycin A, often have many oxygen substituents remaining. We looked at eicosanoid biosynthesis as an example of a fatty acid–derived natural product in Section 23.7 and will look at erythromycin biosynthesis in Section 25.4.

- **Nonribosomal polypeptides** are peptidelike compounds that are biosynthesized from amino acids by a multifunctional enzyme complex without direct RNA transcription. The penicillins are good examples, but their chemistry is a bit complicated and we'll not discuss their biosynthesis.
- **Enzyme cofactors** don't fit one of the other general categories of natural products and are usually classed separately. We've seen numerous examples of coenzymes in past chapters (see the list in Table 19.3) and will look at the biosynthesis of pyridoxal phosphate (PLP) in Section 25.2.

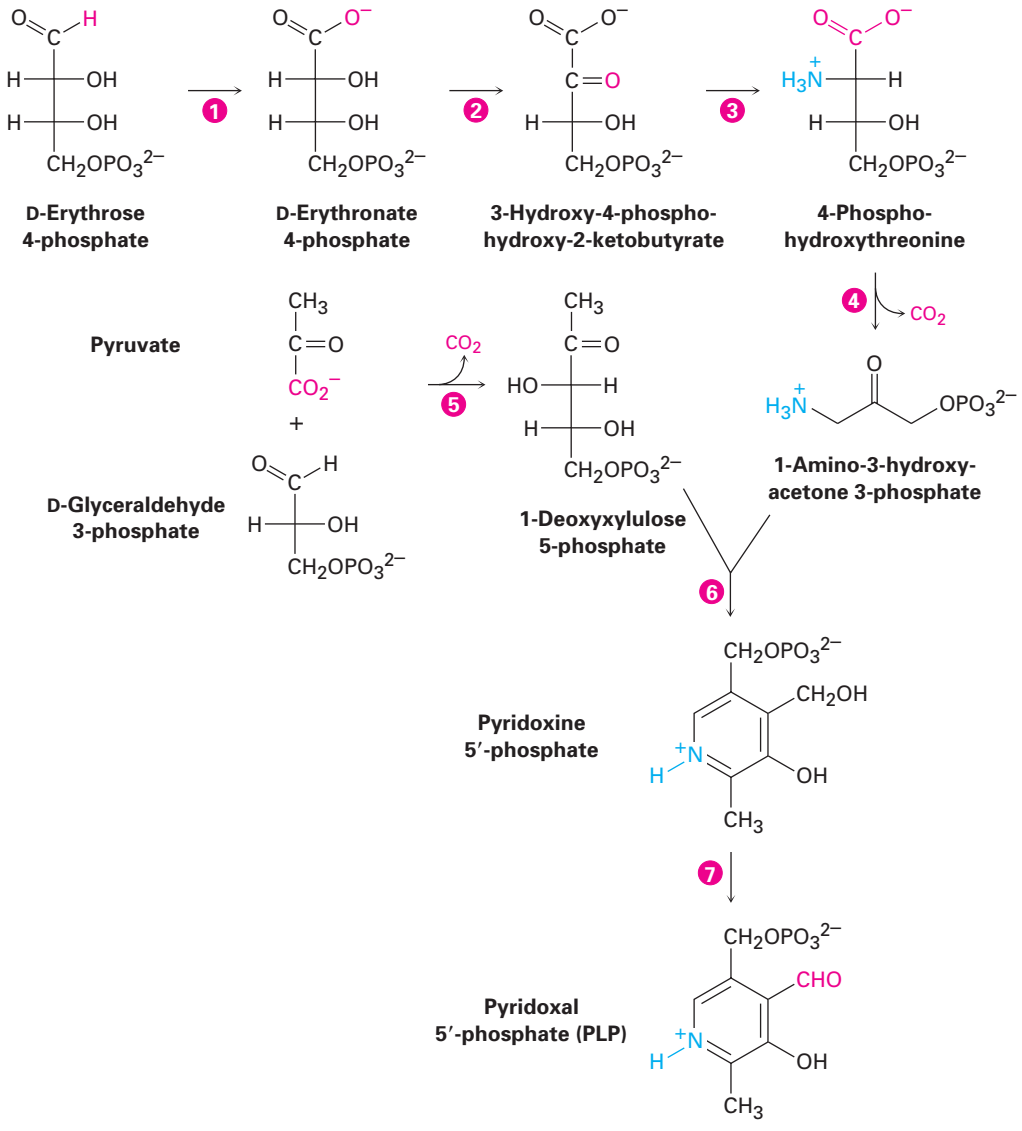


Figure 25.1 An overview of the pathway for pyridoxal 5′-phosphate biosynthesis. Individual steps are explained in the text.

As you might imagine, unraveling the biosynthetic pathways by which specific natural products are made is extremely difficult and time-consuming work. Small precursor molecules have to be identified, guesses about likely routes made, and individual enzymes that catalyze each step isolated, characterized, and mechanistically studied. The payoff for all this painstaking work is a fundamental understanding of how organisms function at the molecular level, an understanding that can be used to design new pharmaceutical agents.

25.2 Biosynthesis of Pyridoxal Phosphate

Let's begin this quick tour of natural products chemistry by looking at the biosynthesis of pyridoxal 5′-phosphate (PLP), a relatively simple enzyme cofactor we've encountered several times in different metabolic pathways. An overview of PLP biosynthesis is shown in Figure 25.1.

STEPS 1–2 OF FIGURE 25.1: OXIDATION Pyridoxal phosphate biosynthesis begins with oxidation of the aldehyde group in D-erythrose 4-phosphate to give the corresponding carboxylic acid, D-erythronate 4-phosphate. The oxidation requires NAD^+ as cofactor and occurs by a mechanism similar to that of step 6 in glycolysis, in which glyceraldehyde 3-phosphate is oxidized to the corresponding acid (see Section 22.2, Figure 22.6). A cysteine –SH group in the enzyme adds to the aldehyde carbonyl group of D-erythrose 4-phosphate to give an intermediate hemithioacetal, which is then oxidized by NAD^+ to a thioester. Hydrolysis of the thioester yields erythronate 4-phosphate, and a further oxidation of the –OH group at C2 by NAD^+ gives 3-hydroxy-4-phosphohydroxy-2-ketobutyrate (Figure 25.2).

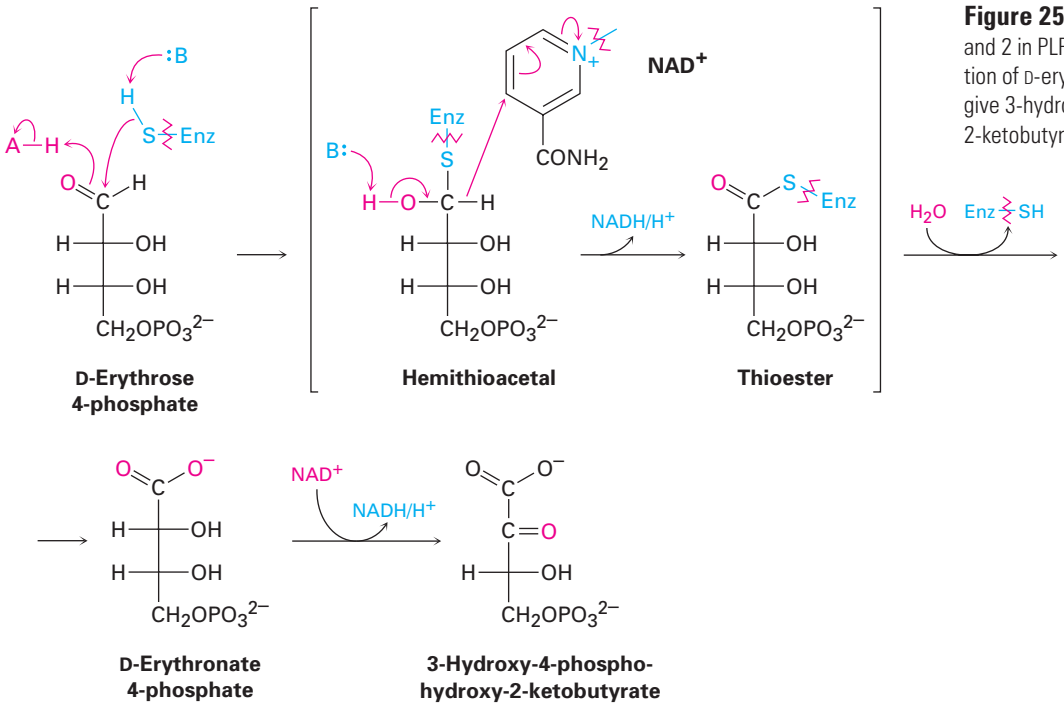


Figure 25.2 Mechanism of steps 1 and 2 in PLP biosynthesis, the oxidation of D-erythrose 4-phosphate to give 3-hydroxy-4-phosphohydroxy-2-ketobutyrate.

STEPS 3–4 OF FIGURE 25.1: TRANSAMINATION AND OXIDATION/DECARBOXYLATION 3-Hydroxy-4-phosphohydroxy-2-ketobutyrate undergoes a transamination in step 3 on reaction with α-ketoglutarate by the usual PLP-dependent mechanism, shown previously in Section 20.2, Figure 20.2. The product, 4-phosphohydroxythreonine, is then oxidized by NAD^+ to give an intermediate β-keto ester, which undergoes concurrent decarboxylation and yields 1-amino-3-hydroxyacetone 3-phosphate. The reactions are shown in Figure 25.3.

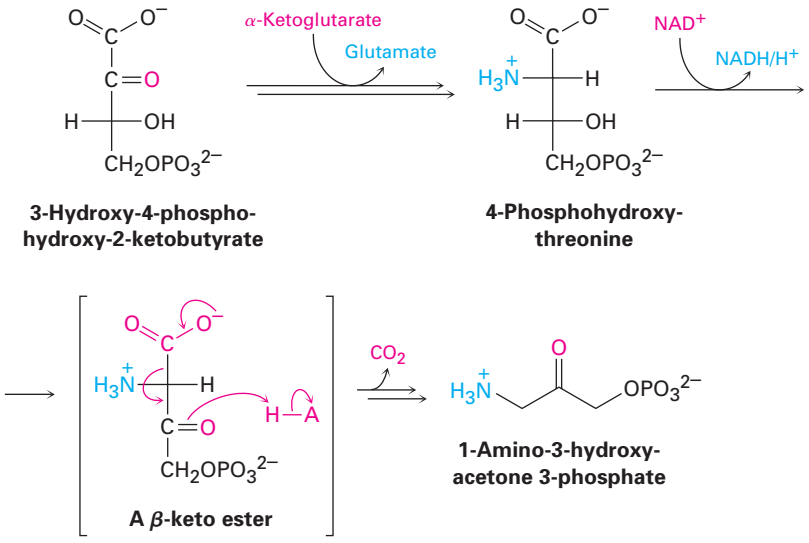


Figure 25.3 Mechanism of steps 3 and 4 in PLP biosynthesis.

STEP 5 OF FIGURE 25.1: FORMATION OF 1-DEOXYXYLULOSE 5-PHOSPHATE The 1-amino-3-hydroxyacetone 3-phosphate formed in step 4 of PLP biosynthesis reacts in step 6 with 1-deoxyxylulose 5-phosphate (DXP). DXP arises in step 5 by an aldol-like condensation of D-glyceraldehyde 3-phosphate with pyruvate in a thiamin-dependent reaction catalyzed by DXP synthase.

You might recall from Section 22.3, Figure 22.7, that pyruvate is converted to acetyl CoA by a process that begins with addition of thiamin diphosphate (TPP) ylide to the ketone carbonyl group, followed by decarboxylation to give hydroxyethylthiamin diphosphate (HETPP). Exactly the same reaction occurs in DXP biosynthesis, but instead of reacting with lipoamide to give a thioester, as in the formation of acetyl CoA, HETPP adds to glyceraldehyde 3-phosphate in an aldol-like reaction. The tetrahedral intermediate that results expels TPP ylide as leaving group and yields DXP. The mechanism is shown in Figure 25.4.

STEP 6 OF FIGURE 25.1: CONDENSATION AND CYCLIZATION 1-Deoxy-D-xylulose 5-phosphate is dephosphorylated and then condenses with 1-amino-3-hydroxyacetone 3-phosphate in step 6 to give pyridoxine 5′-phosphate. The reaction begins with formation of an enamine, followed by loss of water to form an enol that also contains a ketone group six atoms away. The enol adds to the ketone in an intramolecular aldol reaction (see Section 17.7) to

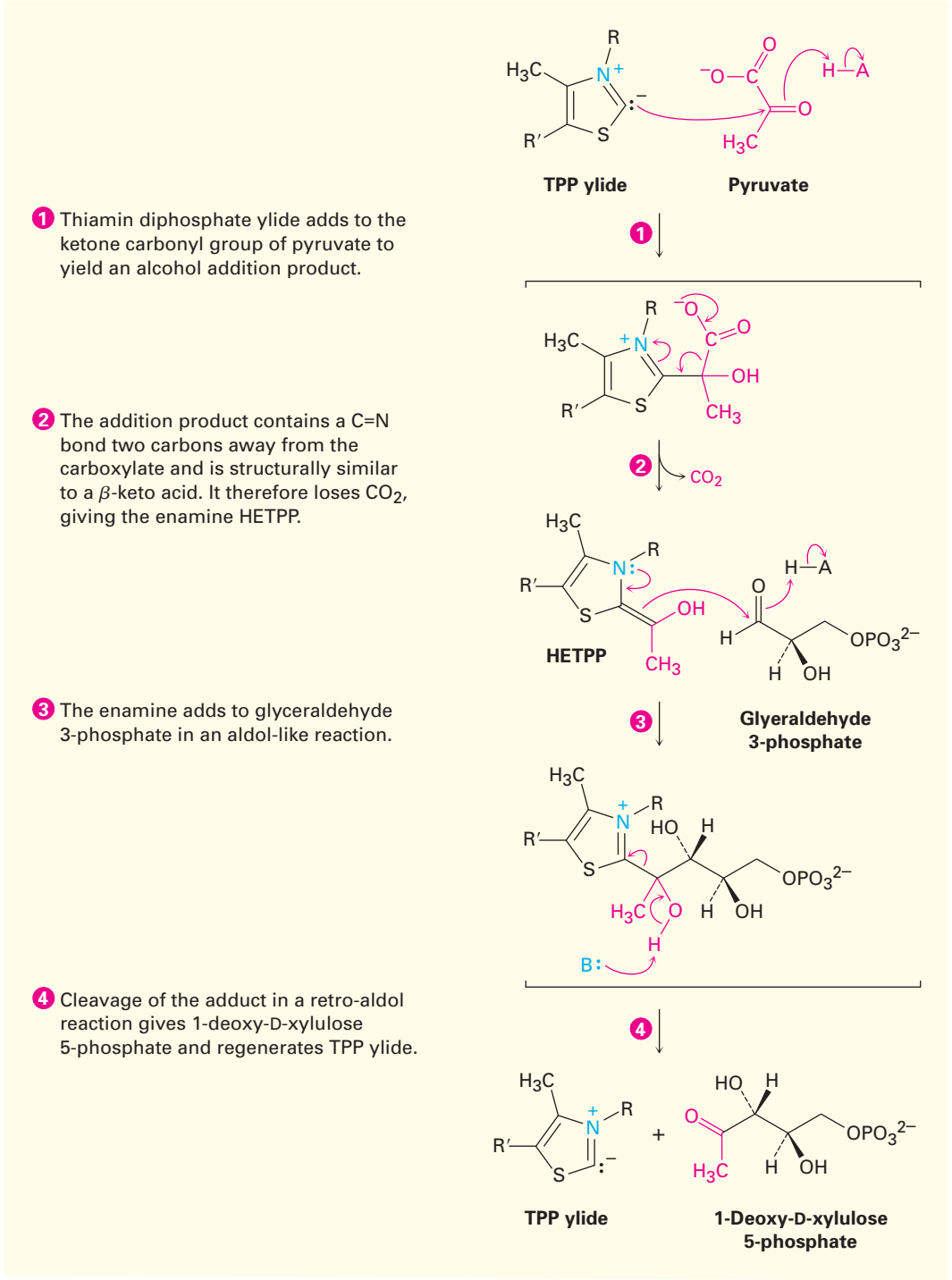


Figure 25.4 **MECHANISM:** Mechanism of step 5 in pyridoxal phosphate biosynthesis, the thiamin-dependent aldol reaction of D-glyceraldehyde 3-phosphate with pyruvate to give 1-deoxyxylulose 5-phosphate.

form a six-membered ring, which then loses water. Tautomerization of the resultant unsaturated ketone gives an aromatic pyridine ring. Note that a loss of phosphate ion occurs at some point in the process, although the exact point at which this happens is not known. The mechanism is shown in Figure 25.5.

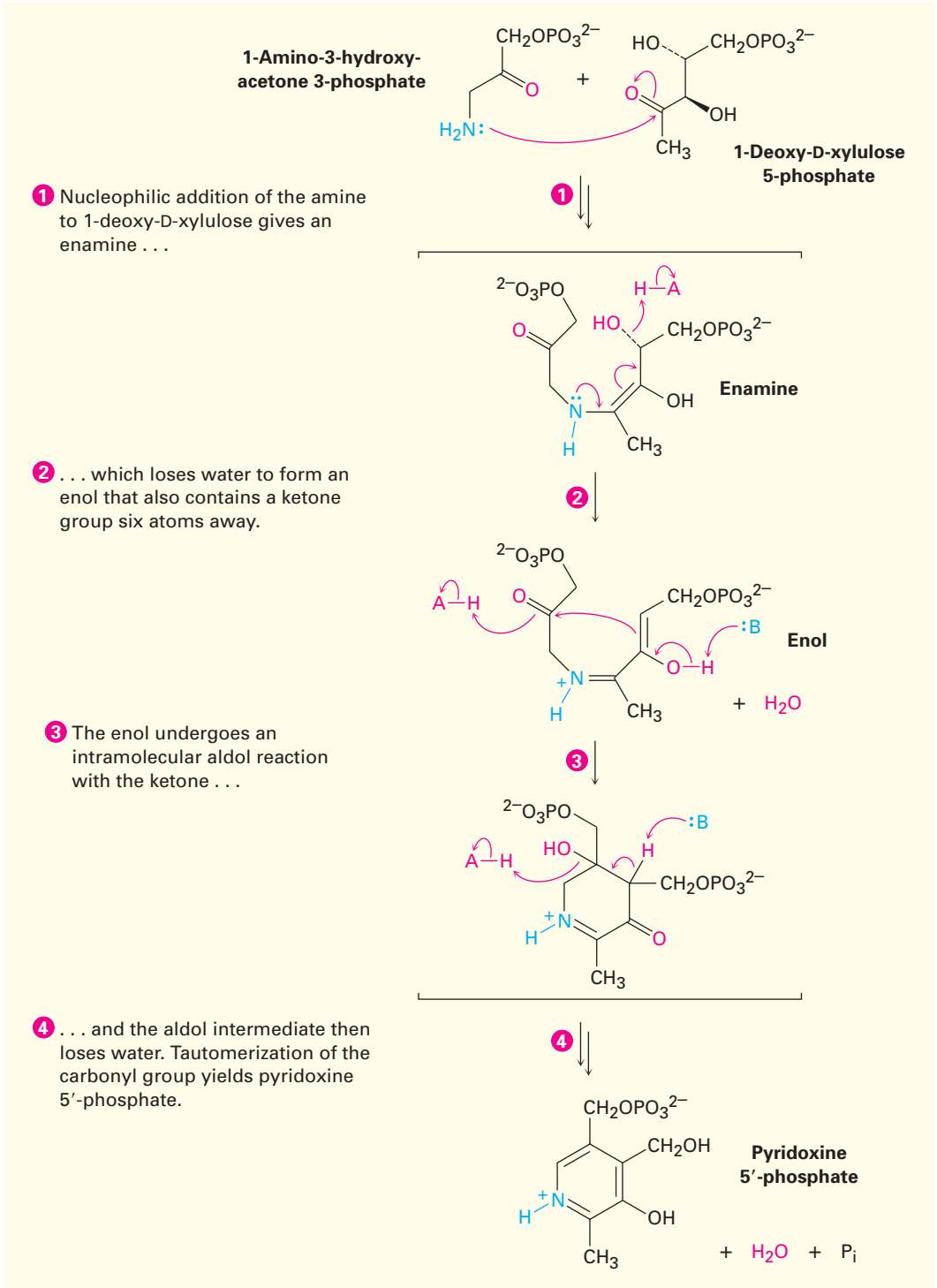
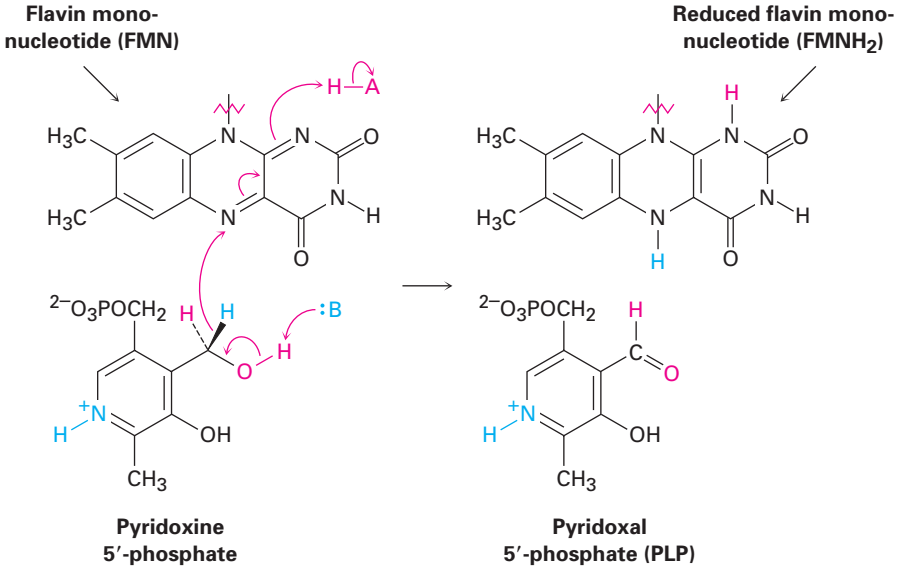


Figure 25.5
MECHANISM: Mechanism of step 6 in PLP biosynthesis, the reaction of 1-amino-3-hydroxyacetone 3-phosphate with 1-deoxy-D-xylulose 5-phosphate to give pyridoxine 5′-phosphate.

STEP 7 OF FIGURE 25.1: OXIDATION The final step in PLP biosynthesis is oxidation of the primary alcohol group in pyridoxine 5′-phosphate to the corresponding aldehyde. Typically, as we've seen on numerous occasions, alcohol oxidations are carried out by either NAD^+ or $NADP^+$. In this instance, however, flavin mononucleotide (FMN) is involved as the oxidizing coenzyme and reduced flavin mononucleotide ($FMNH_2$) is the by-product. The details of the reaction are not clear, but evidence suggests that a hydride transfer is involved, just as in NAD^+ oxidations.



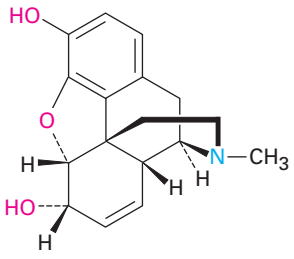
Problem 25.1
In the addition of HETPP to glyceraldehyde 3-phosphate shown in Figure 25.4, does the reaction take place on the *Re* face or the *Si* face of the glyceraldehyde carbonyl group?

Problem 25.2
Show a likely mechanism for the final tautomerization in the reaction of 1-amino-3-hydroxyacetone 3-phosphate with 1-deoxy-D-xylulose to give pyridoxine 5′-phosphate (Figure 25.5).

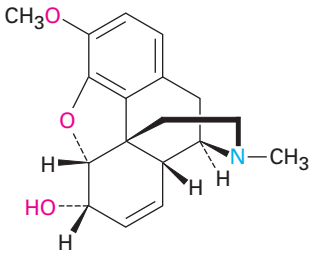
25.3 Biosynthesis of Morphine

Having looked at the biosynthesis of pyridoxal 5′-phosphate in the previous section, let's now go up a level in complexity by looking at morphine biosynthesis. Morphine, perhaps the oldest and best known of all alkaloids, is obtained from the opium poppy *Papaver somniferum,* which has been cultivated for more than 6000 years. Medical uses of the poppy have been known since the early 1500s, when crude extracts, called *opium,* were used for the relief of pain. Morphine was the first pure compound to be isolated from opium, but its close relative codeine also occurs naturally. Codeine, which is simply the methyl ether of morphine and is converted to morphine in the

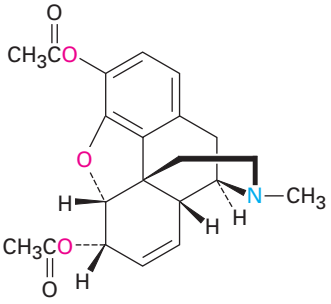
body, is used in prescription cough medicines and as an analgesic. Heroin, another close relative of morphine, does not occur naturally but is synthesized in the laboratory by diacetylation of morphine.



Morphine



Codeine

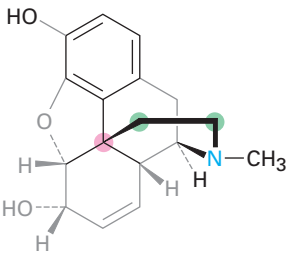


Heroin

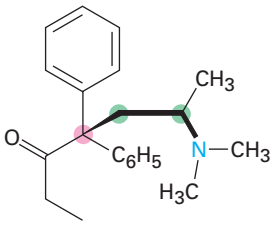
Chemical investigations into the structure of morphine occupied some of the finest chemical minds of the 19th and early 20th centuries, and it was not until 1924 that the puzzle was finally solved by Robert Robinson, who received the 1947 Nobel Prize in Chemistry for this and other work with alkaloids.

Morphine and its relatives are extremely useful pharmaceutical agents, yet they also pose an enormous social problem because of their addictive properties. Much effort has therefore gone into understanding how morphine works and into developing modified morphine analogs that retain the analgesic activity but don't cause physical dependence. Our present understanding is that morphine functions by binding to so-called mu opioid receptor sites in both the spinal cord, where it interferes with the transmission of pain signals, and brain neurons, where it changes the brain's reception of the signal.

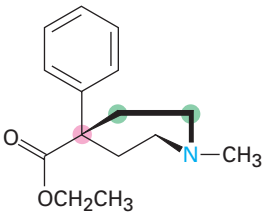
Hundreds of morphine-like molecules have been synthesized and tested for their analgesic properties. Research has shown that not all the complex framework of morphine is necessary for biological activity. According to the "morphine rule," biological activity requires (1) an aromatic ring attached to (2) a quaternary carbon atom, followed by (3) two more carbon atoms and (4) a tertiary amine. Meperidine (Demerol), a widely used analgesic, and methadone, a substance used in the treatment of heroin addiction, are two compounds that fit the morphine rule.



The morphine rule



Methadone



Meperidine

An aromatic ring
attached to a quaternary carbon (●)
followed by two or more carbons (●)
and a tertiary amine (N)

Morphine is biosynthesized from two molecules of the amino acid tyrosine. One tyrosine is converted into dopamine, the second is converted into *p*-hydroxyphenylacetaldehyde, and the two are coupled to give morphine. The entire pathway is a bit complex at several points, but an abbreviated scheme is given in Figure 25.6.

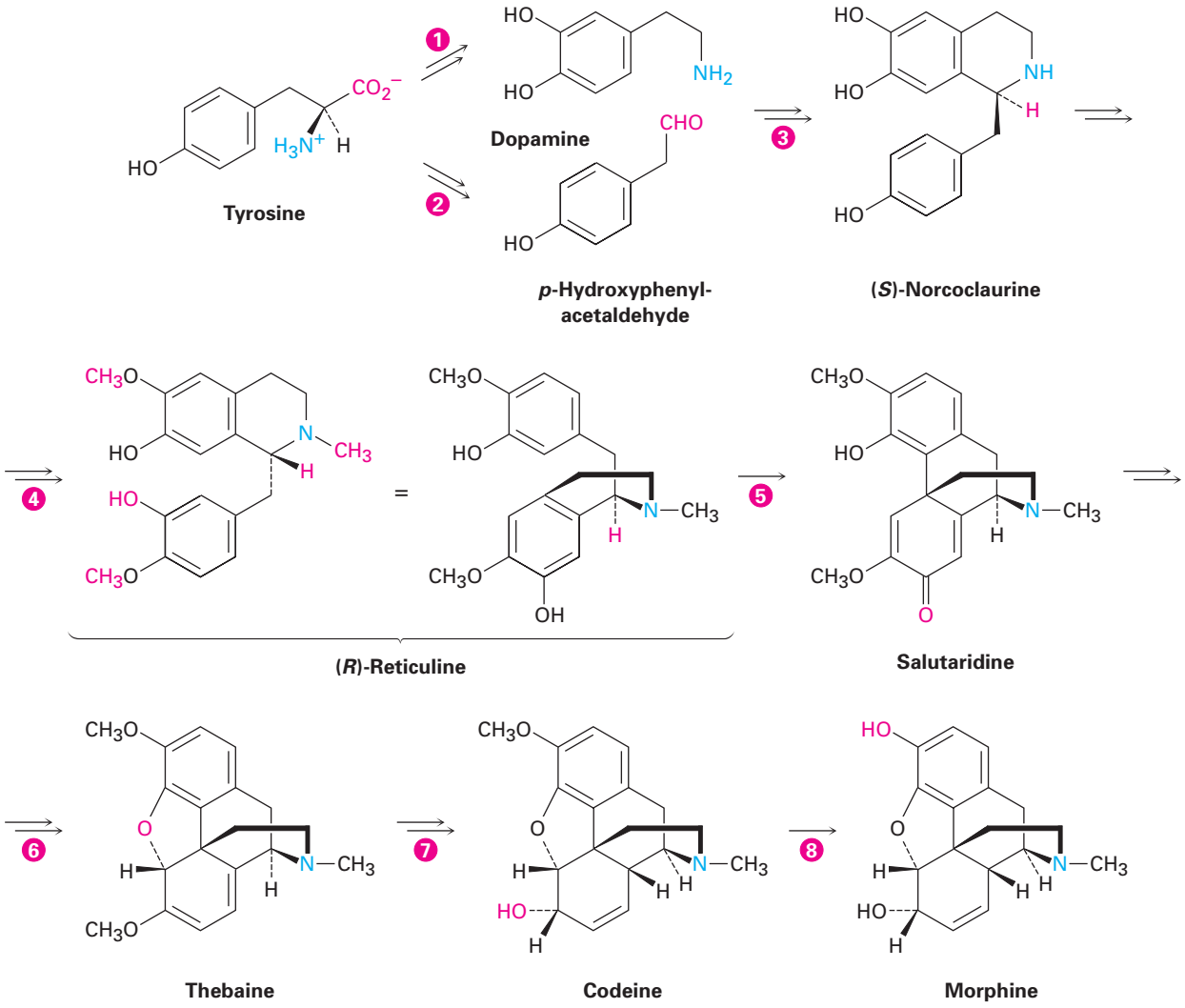
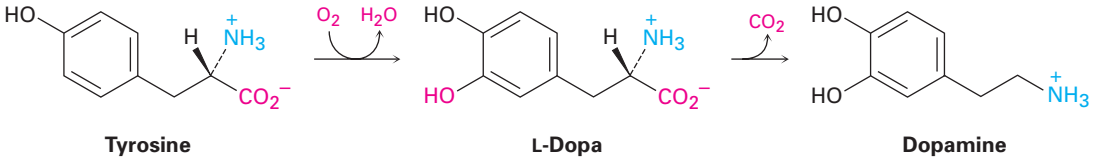


Figure 25.6 An abbreviated pathway for the biosynthesis of morphine from two molecules of tyrosine. The individual steps are explained in more detail in the text.

STEP 1 OF FIGURE 25.6: DOPAMINE BIOSYNTHESIS Dopamine is formed from tyrosine in two steps: an initial hydroxylation of the aromatic ring, followed by decarboxylation. The hydroxylation is catalyzed by tyrosine 3-monooxygenase, requires a cofactor called tetrahydrobiopterin, and occurs through a somewhat complex pathway that involves an iron–oxo (Fe=O) complex analogous to that involved in prostaglandin biosynthesis (see Figure 7.9). The

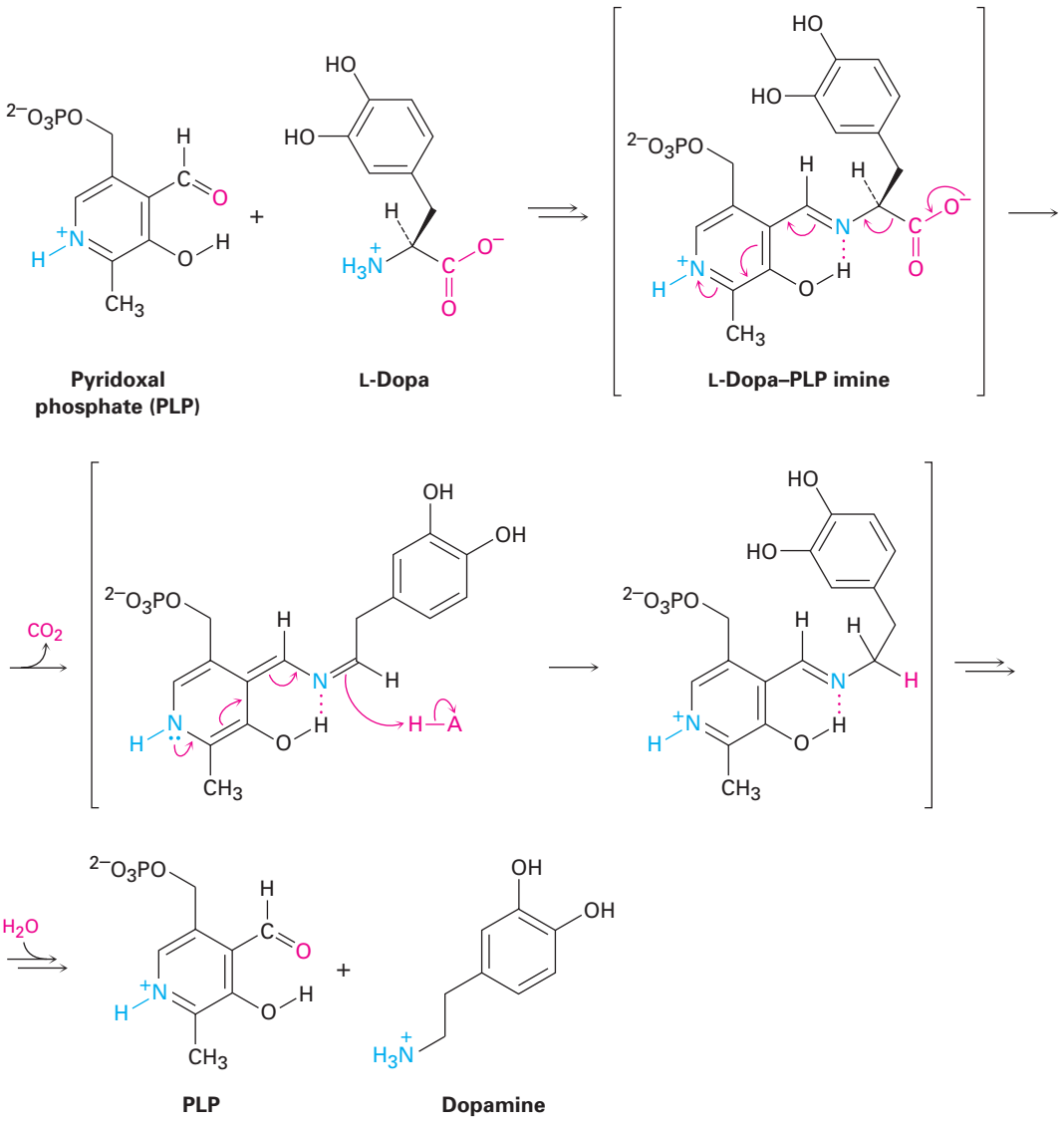
decarboxylation is catalyzed by the PLP-dependent enzyme aromatic L-amino acid decarboxylase.



Tyrosine **L-Dopa** **Dopamine**

Recall from Section 20.2 that pyridoxal 5′-phosphate reacts with the α amino group of an α-amino acid to form an imine, or Schiff base. When L-dopa reacts with PLP, the resultant imine undergoes decarboxylation, with the pyridinium ion of PLP acting as the electron acceptor. Hydrolysis then gives dopamine and regenerated PLP. The mechanism is shown in Figure 25.7.

Figure 25.7
Mechanism of step 1 in morphine biosynthesis, the PLP-dependent decarboxylation of L-dopa to give dopamine.



Pyridoxal phosphate (PLP) **L-Dopa** **L-Dopa–PLP imine**

PLP **Dopamine**

STEP 2 OF FIGURE 25.6: *p*-HYDROXYPHENYLACETALDEHYDE BIOSYNTHESIS *p*-Hydroxyphenylacetaldehyde, the second tyrosine-derived precursor of morphine, is also formed in two steps: an initial PLP-dependent transamination with α-ketoglutarate to give *p*-hydroxyphenylpyruvate, followed by decarboxylation of the α-keto acid. The transamination occurs by the mechanism previously shown in Section 20.2, Figure 20.2. The decarboxylation requires thiamin diphosphate as coenzyme and occurs by a slight variant of the mechanism described previously in Section 22.3, Figure 22.7, for the formation of acetyl CoA from pyruvate. As in the pyruvate decarboxylation, thiamin diphosphate is required as coenzyme.

Decarboxylation of *p*-hydroxyphenylpyruvate begins with nucleophilic addition of TPP ylide to the ketone carbonyl group, followed by loss of CO_2 to give an enamine in the usual way. But whereas the enamine formed from pyruvate decarboxylation reacts with lipoamide to give a thioester and regenerated TPP ylide, the enamine from *p*-hydroxyphenylpyruvate decarboxylation is simply protonated to give an aldehyde plus TPP ylide. The mechanism is shown in Figure 25.8.

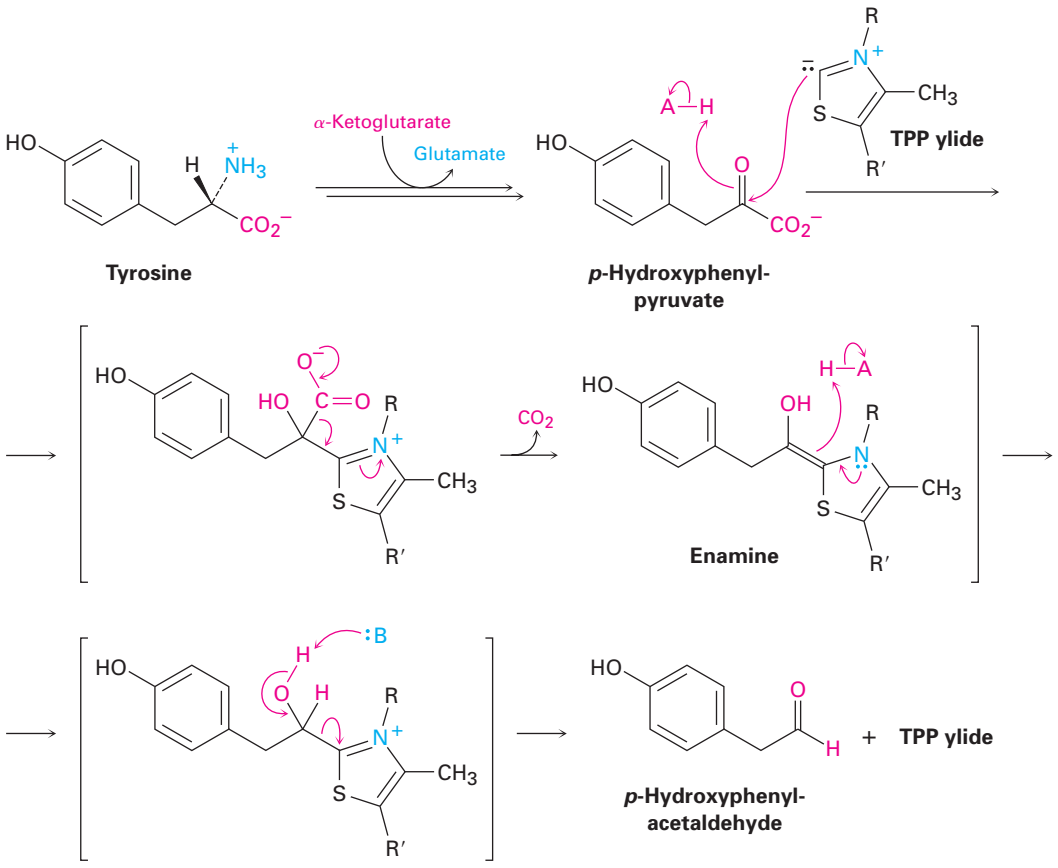


Figure 25.8
Mechanism of step 2 in morphine biosynthesis, the TPP-dependent decarboxylation of *p*-hydroxyphenylpyruvate to give *p*-hydroxyphenylacetaldehyde.

STEP 3 OF FIGURE 25.6: COUPLING The coupling of dopamine and *p*-hydroxyphenylacetaldehyde is catalyzed by (*S*)-norcoclaurine synthase and is relatively straightforward. The reaction proceeds through initial formation of

an intermediate iminium ion, followed by intramolecular electrophilic aromatic substitution at a position para to one of the hydroxyl groups (Figure 25.9).

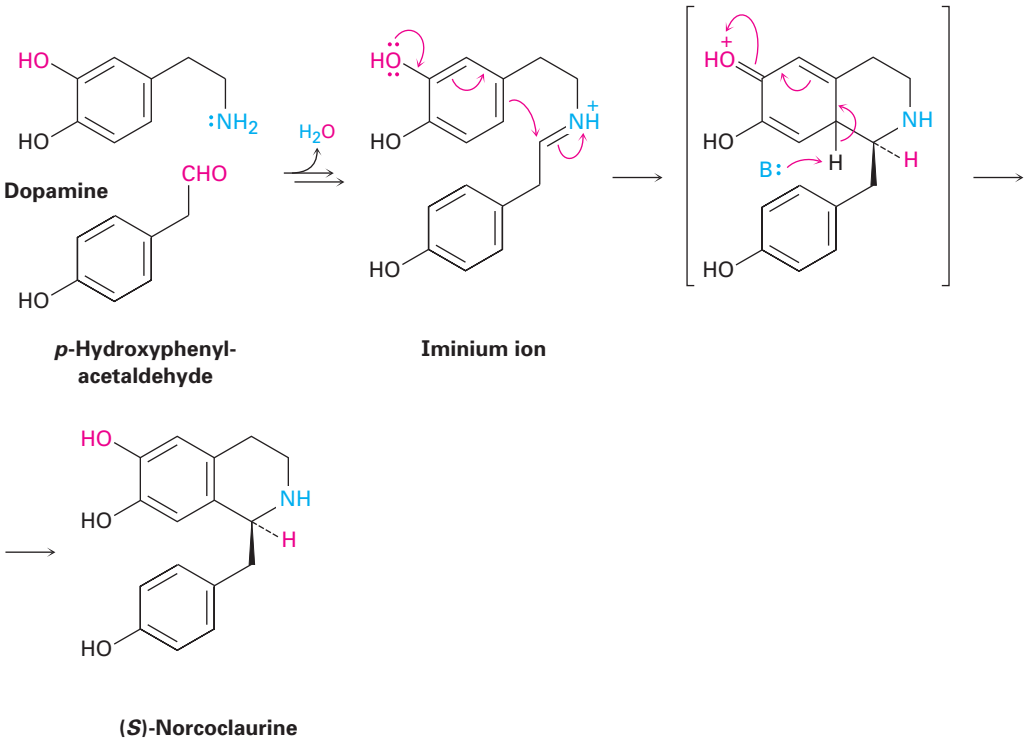


Figure 25.9 Mechanism of step 3 in morphine biosynthesis, the coupling of dopamine and *p*-hydroxyphenylacetaldehyde to give (*S*)-norcoclaurine.

STEP 4 OF FIGURE 25.6: METHYLATION, HYDROXYLATION, AND EPIMERIZATION (*S*)-Norcoclaurine next undergoes two methylations and a hydroxylation to give (*S*)-3′-hydroxy-*N*-methylcoclaurine, which is methylated a third time to produce (*S*)-reticuline. Epimerization of (*S*)-reticuline then yields (*R*)-reticuline (Figure 25.10).

Both initial methylations use *S*-adenosylmethionine (SAM) as the methyl donor, as discussed in Section 10.9. *S*-Adenosylhomocysteine (SAH) is the by-product in each case, and the reactions occur by the usual S_N2 substitution pathway. The first methylation occurs on a phenol oxygen, and the second takes place on the amine nitrogen.

The hydroxylation of (*S*)-*N*-methylcoclaurine to give (*S*)-3′-hydroxy-*N*-methylcoclaurine is superficially similar to the hydroxylation of tyrosine in step 1 in that both involve an iron–oxo complex as the active hydroxylating agent. Unlike the enzyme in the tyrosine hydroxylation, however, that responsible for hydroxylation of *N*-methylcoclaurine is a so-called cytochrome P450 enzyme. These enzymes, of which more than 500 are known, contain an iron–heme cofactor ligated to the sulfur atom of a cysteine residue in the enzyme. The details of the hydroxylation itself are not clear, although

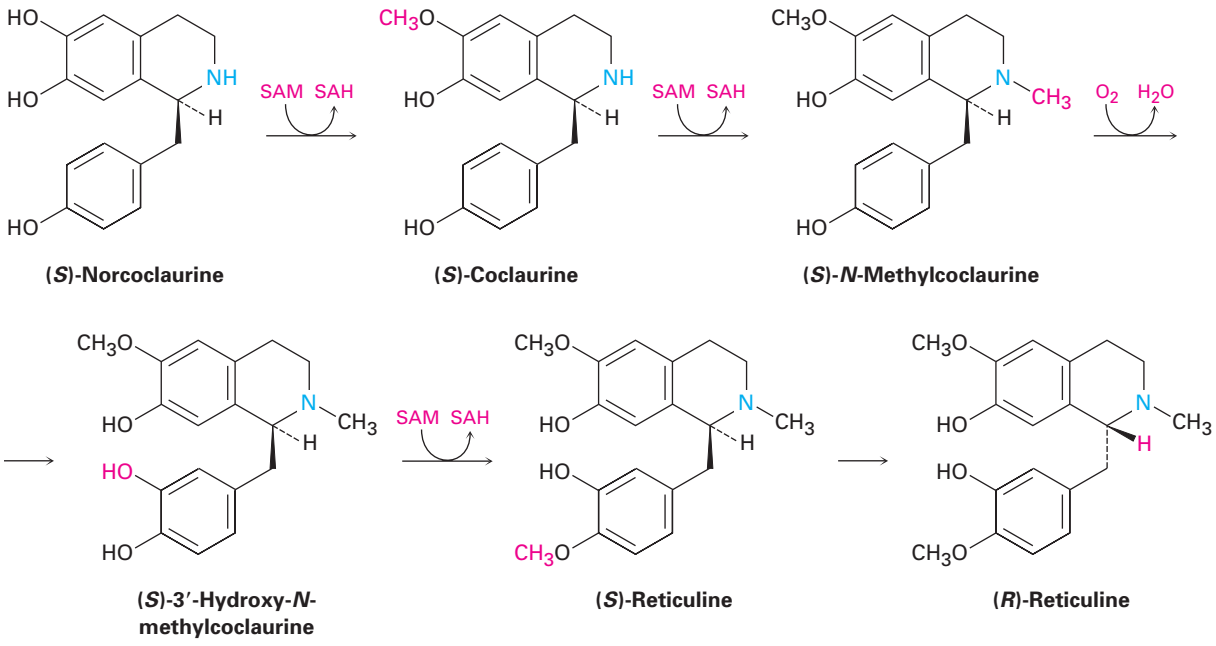
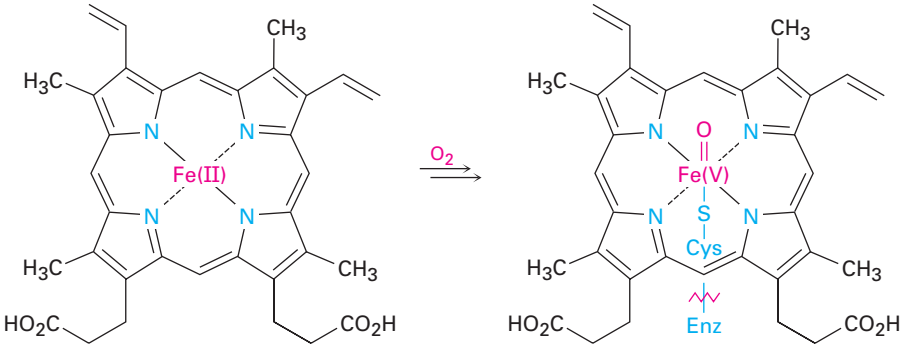


Figure 25.10 An overview of the reactions in step 4 of morphine biosynthesis, the conversion of (*S*)-norcoclaurine to (*R*)-reticuline.

it may occur through a straightforward electrophilic aromatic substitution mechanism.



Methylation of a phenolic –OH group in (*S*)-3′-hydroxy-*N*-methylcoclaurine by SAM gives (*S*)-reticuline through the usual S_N2 pathway, and epimerization of the chirality center forms (*R*)-reticuline. The epimerization is a two-step process, the first an oxidation of the tertiary amine to an intermediate iminium ion and the second a hydride reduction of

the iminium ion. The mechanism of the oxidation step is not yet known, but the reduction of the iminium ion requires NADPH as cofactor (Figure 25.11).

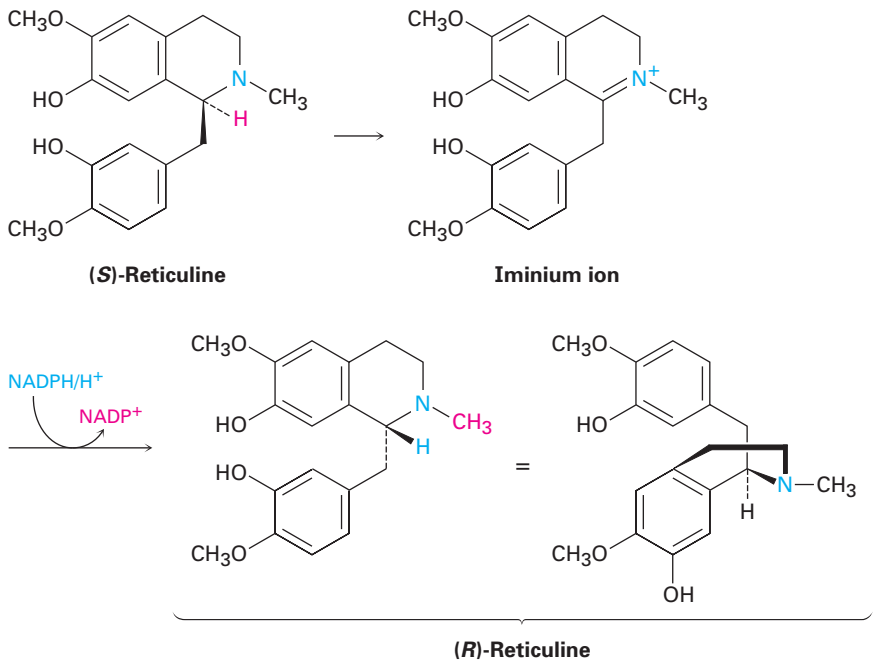


Figure 25.11 Mechanism of the epimerization of (*S*)-reticuline to (*R*)-reticuline in step 4 of morphine biosynthesis.

Why does morphine biosynthesis proceed through initial formation of (*S*)-reticuline as an intermediate, followed by epimerization, rather than through (*R*)-reticuline directly? There is no obvious answer other than to say that many metabolic pathways contain such small inefficiencies, probably as a result of the evolutionary development of the responsible enzymes—what some people have called "unintelligent design."

STEP 5 OF FIGURE 25.6: OXIDATIVE COUPLING (*R*)-Reticuline is converted into salutaridine in step 5 by an oxidative coupling between the ortho position of one phenol ring and the para position of the other. The reaction is catalyzed by another cytochrome P450 enzyme like that involved in the hydroxylation of (*S*)-*N*-methylcoclaurine in step 4. Formation of the phenoxide ions and abstraction of a nonbonding electron from each oxygen atom to give radicals occurs, followed by radical coupling and a keto–enol tautomerization to yield salutaridine (Figure 25.12).

STEP 6 OF FIGURE 25.6: REDUCTION AND CYCLIZATION Reduction of salutaridine to salutaridinol is catalyzed by salutaridine reductase, with NADPH as cofactor. This alcohol then undergoes a nucleophilic acyl substitution reaction with acetyl CoA to give a doubly allylic acetate, which spontaneously eliminates acetate ion in an S_N1-like process and cyclizes to thebaine (Figure 25.13).

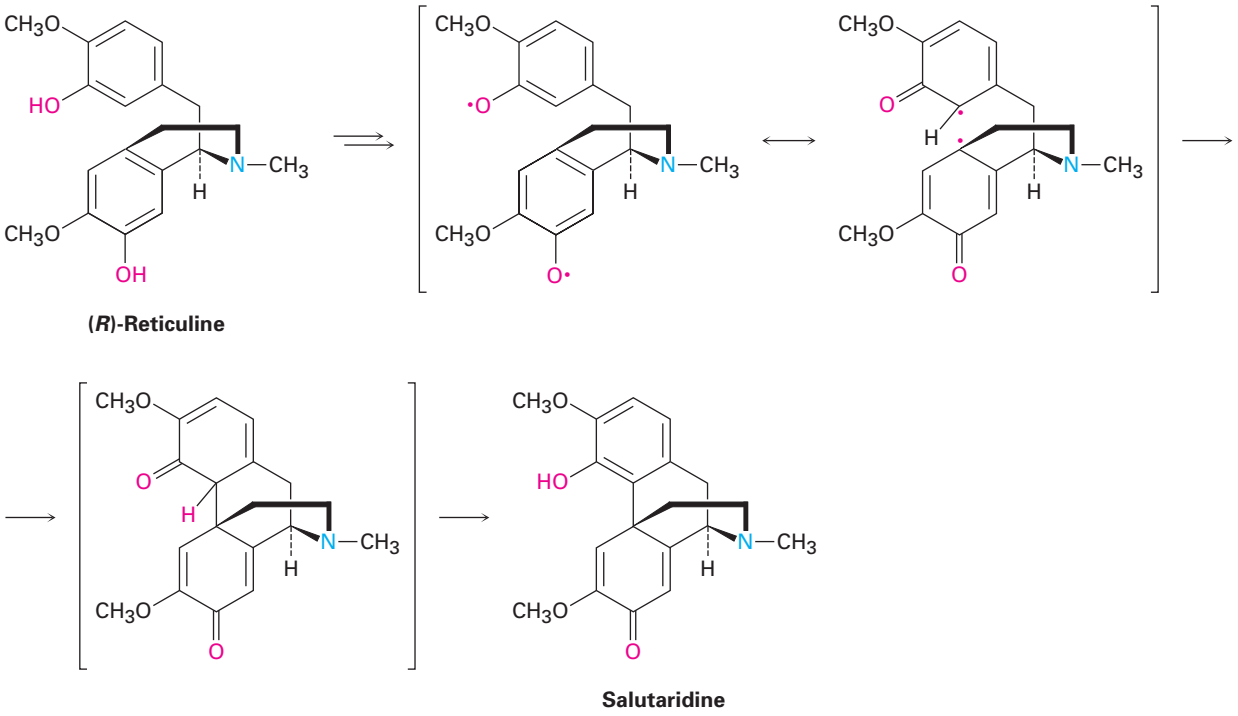


Figure 25.12 Mechanism of step 5 in morphine biosynthesis, the oxidative phenol coupling of (*R*)-reticuline to salutaridine.

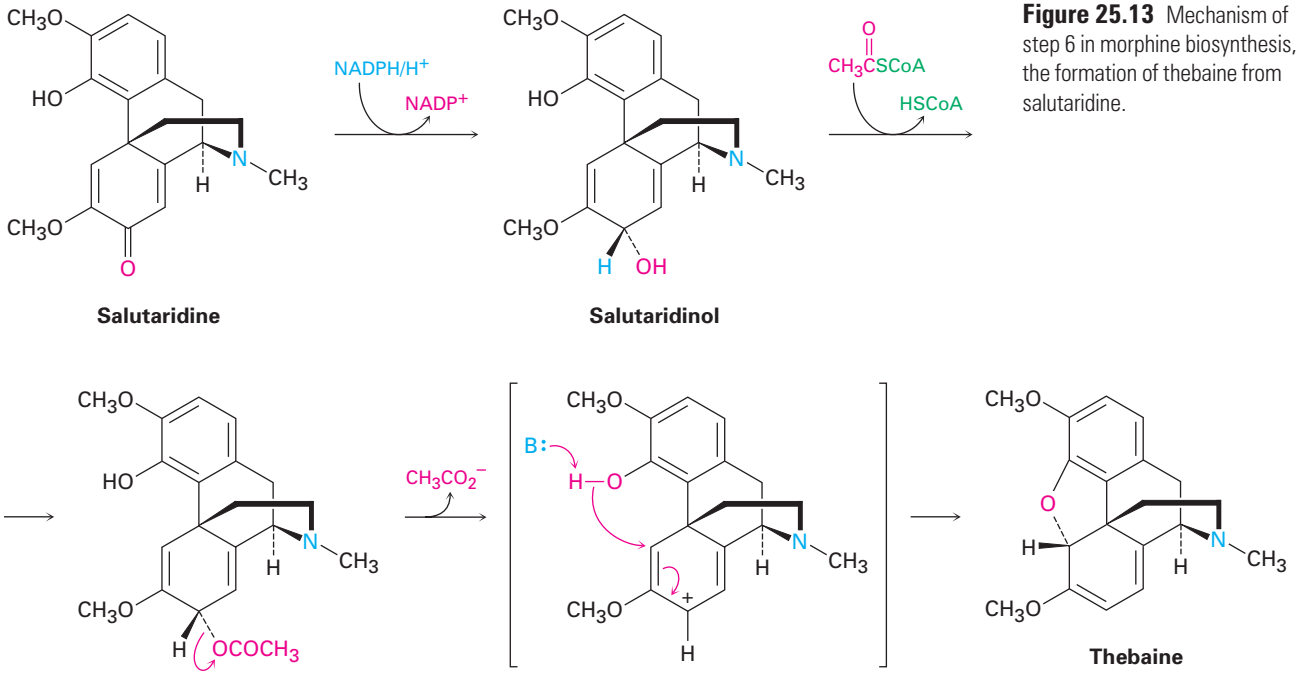


Figure 25.13 Mechanism of step 6 in morphine biosynthesis, the formation of thebaine from salutaridine.

STEPS 7–8 OF FIGURE 25.6: DEMETHYLATION AND REDUCTION The remaining steps in the biosynthesis of morphine involve two demethylation reactions and a reduction. The first demethylation is catalyzed by a cytochrome P450 enzyme, which hydroxylates the $-OCH_3$ group of thebaine to form $-OCH_2OH$, a hemiacetal. Loss of formaldehyde then gives an enol that tautomerizes to codeinone. Reduction of the resultant ketone by NADPH yields codeine, and demethylation by a P450 enzyme produces morphine (Figure 25.14).

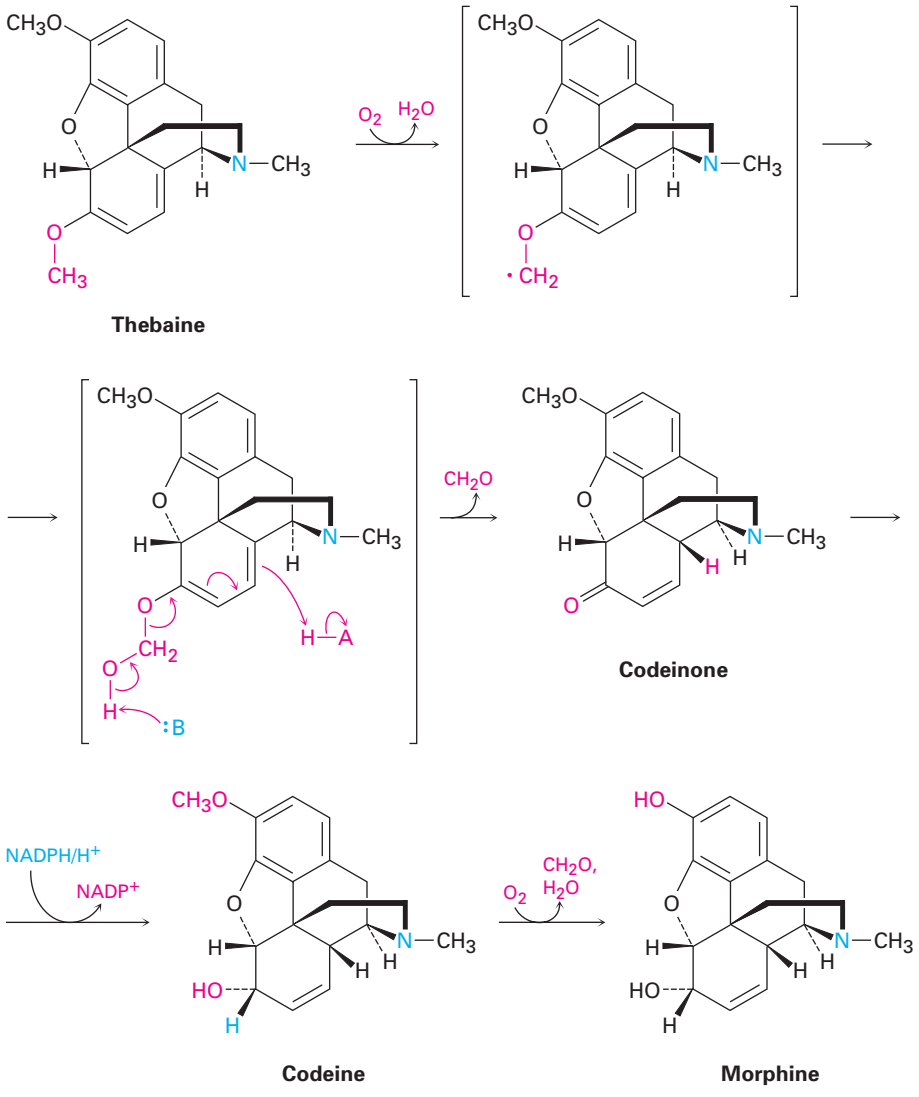


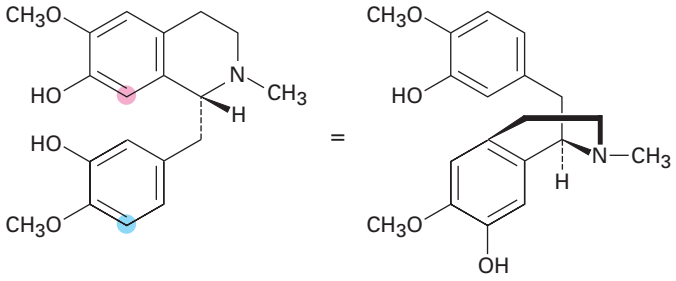
Figure 25.14 Mechanism of step 7 in morphine biosynthesis, the demethylation of thebaine to give codeinone, catalyzed by a P450 enzyme. Reduction of codeinone with NADPH then yields codeine, and a final demethylation produces morphine.

Problem 25.3

Show the mechanism of the reaction of (*S*)-norcoclaurine with *S*-adenosylmethionine to give (*S*)-coclaurine (Figure 25.10).

Problem 25.4

Convince yourself that the following two structures both represent (*R*)-reticuline. Which carbon atoms in the structure on the right correspond to the two carbons indicated in the structure on the left?



25.4 Biosynthesis of Erythromycin

Having discussed the biosynthesis of pyridoxal phosphate and morphine in the preceding two sections, we'll end this chapter on natural products chemistry by going up yet one more level in complexity and looking at polyketide biosynthesis. Unlike what happens in many metabolic pathways, where each separate step is catalyzed by a separate, relatively small enzyme, erythromycin and other polyketides are assembled by a single massive enzyme called a *synthase.* The synthase contains many enzyme domains linked together, with each domain catalyzing a specific biosynthetic step in sequence.

Polyketides are an extraordinarily valuable class of natural products numbering over 10,000 compounds. Commercially important polyketides include antibiotics (erythromycin A, tetracycline) and immunosuppressants (rapamycin), as well as anticancer (doxorubicin), antifungal (amphotericin B), and cholesterol-lowering (lovastatin) agents (Figure 25.15). It has been estimated that the sales of these and other polyketide pharmaceuticals total more than $15 billion per year.

Polyketides are biosynthesized by the joining together of the simple acyl CoA's acetyl CoA, propionyl CoA, methylmalonyl CoA, and (less frequently) butyryl CoA. The key carbon–carbon bond-forming step in each joining is a Claisen condensation (see Section 17.8). Once the carbon chain is assembled and released from the enzyme, further transformations take place to give the final product. Erythromycin A, for instance, is prepared from one propionate and six methylmalonate units by the pathway outlined in Figure 25.16. Following initial assembly of the acyl units into the macrocyclic lactone 6-deoxyerythronolide B, two hydroxylations, two glycosylations, and a final methylation complete the biosynthesis.

The initial assembly of seven acyl CoA precursors to build a polyketide carbon chain is carried out by a multienzyme complex called a *polyketide synthase,* or PKS. The 6-deoxyerythronolide B synthase (DEBS) is a massive structure of greater than 2 million molecular weight that contains more than 20,000 amino acids. Furthermore, it is a *homodimer,*

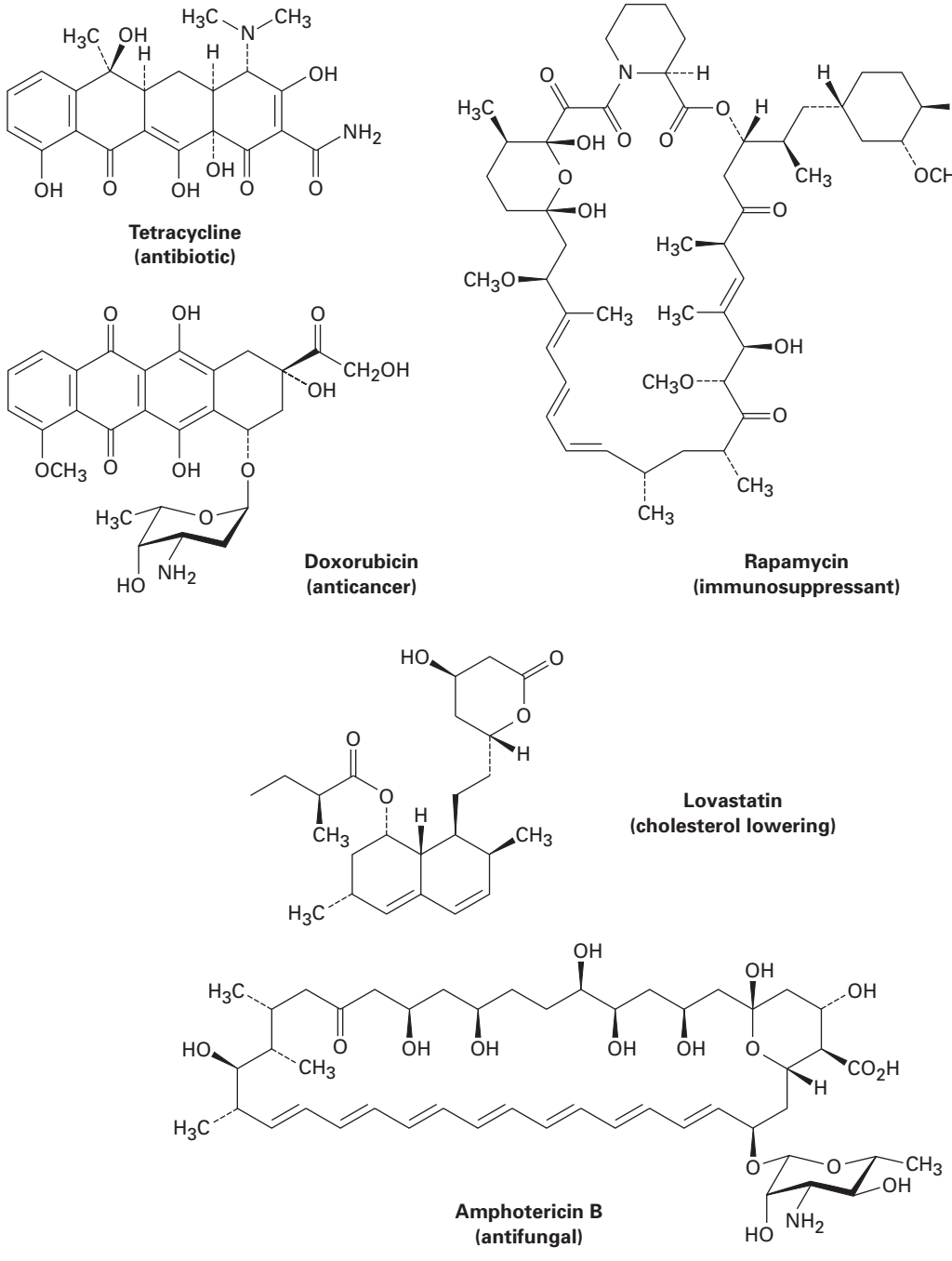


Figure 25.15 Structures of some polyketides used as pharmaceutical agents.

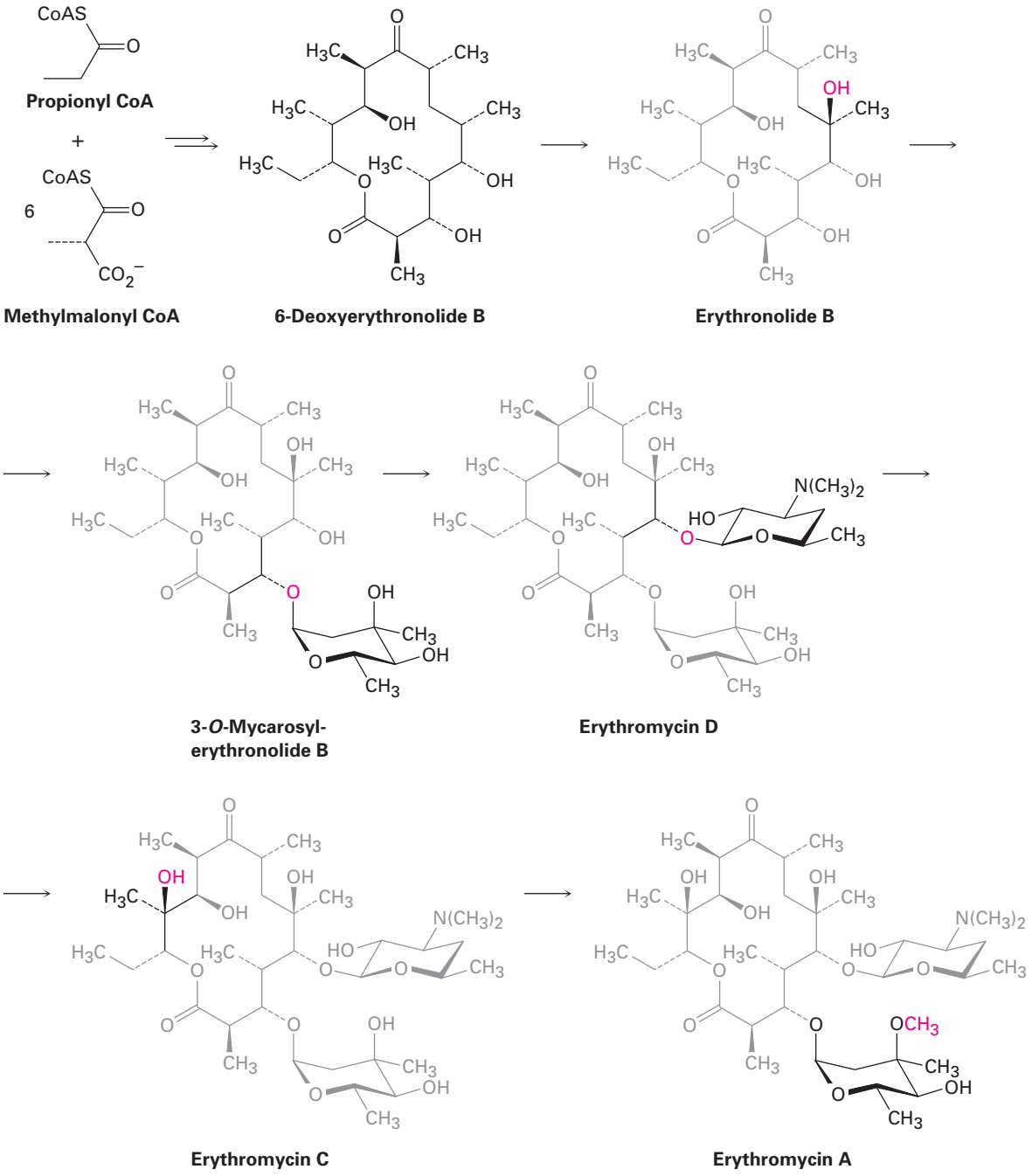


Figure 25.16 An outline of the pathway for the biosynthesis of erythromycin A. One propionate and six methylmalonate units are first assembled into the macrocyclic lactone 6-deoxyerythronolide B, which is then hydroxylated, glycosylated by two different sugars, hydroxylated again, and finally methylated.

meaning that it consists of two identical protein chains held together by noncovalent interactions, with each chain containing all the enzymes necessary for constructing the polyketide.

Each separate enzyme domain in the erythromycin synthase is a folded, globular region within a huge protein chain that catalyzes a specific biosynthetic step. The domains are grouped into modules, where each module carries out the sequential addition and processing of an acyl CoA to the growing polyketide. In addition, adjacent modules form three larger groups (DEBS 1, DEBS 2, and DEBS 3) that are linked by peptide spacers. As shown in Figure 25.17, the erythromycin PKS consists of an initial *loading module* to attach the first acyl group, six *extension modules* to add six further acyl groups, and an *ending module* to cleave the thioester bond and release the polyketide. The ending module also catalyzes cyclization to give a macrocyclic lactone.

The loading module has two domains: an acyl transfer (AT) domain and an acyl carrier protein (ACP) domain. The AT selects the first acyl CoA (propionyl CoA in the case of erythromycin) and transfers it to the adjacent ACP, which binds it through a thioester linkage and holds it for further reaction. Each extension module has a minimum of three domains: an AT, an ACP, and a ketosynthase (KS), which catalyzes the Claisen condensation reaction that builds the polyketide chain. In addition to the three minimum domains, some extension modules also contain a ketoreductase (KR) to reduce a ketone carbonyl group and produce an alcohol, a dehydratase (DH) to dehydrate the alcohol and produce a C=C bond, and an enoyl reductase (ER) to reduce the C=C bond. Finally, the ending domain is a thioesterase (TE), which releases the product by catalyzing a lactonization.

Polyketide chain extension occurs when an extension module AT selects a new acyl CoA, transfers it to the ACP, and the KS then catalyzes a Claisen condensation reaction between the newly bonded acyl group and the acyl group of the previous module. Figure 25.18 shows the steps occurring in the first extension cycle; other extension cycles take place similarly.

STEP 1 OF FIGURE 25.18: LOADING The loading AT domain begins the erythromycin biosynthesis by binding a propionyl CoA through a thioester bond to the –SH of a cysteine residue. The AT then transfers the propionyl group to the adjacent ACP. Each ACP in the synthase contains a phosphopantetheine bonded to the hydroxyl of a serine residue, and bonding of the acyl group to the enzyme occurs by thioester formation with the phosphopantetheine –SH (Figure 25.19). The phosphopantetheine effectively acts as a long, flexible arm to allow movement of the acyl group from one catalytic domain to another.

STEPS 2–4 OF FIGURE 25.18: CHAIN EXTENSION Polyketide chain extension begins (step 2) when the acyl ACP of the loading module transfers the propionyl group to the ketosynthase of module 1 (KS1), again forming a thioester bond to a cysteine residue. At the same time (step 3), the AT and ACP of module 1 load a (2*S*)-methylmalonyl CoA onto the thiol terminus of the ACP1 phosphopantetheine. The key carbon–carbon bond formation occurs (step 4) when KS1 catalyzes a Claisen condensation and decarboxylation to form an

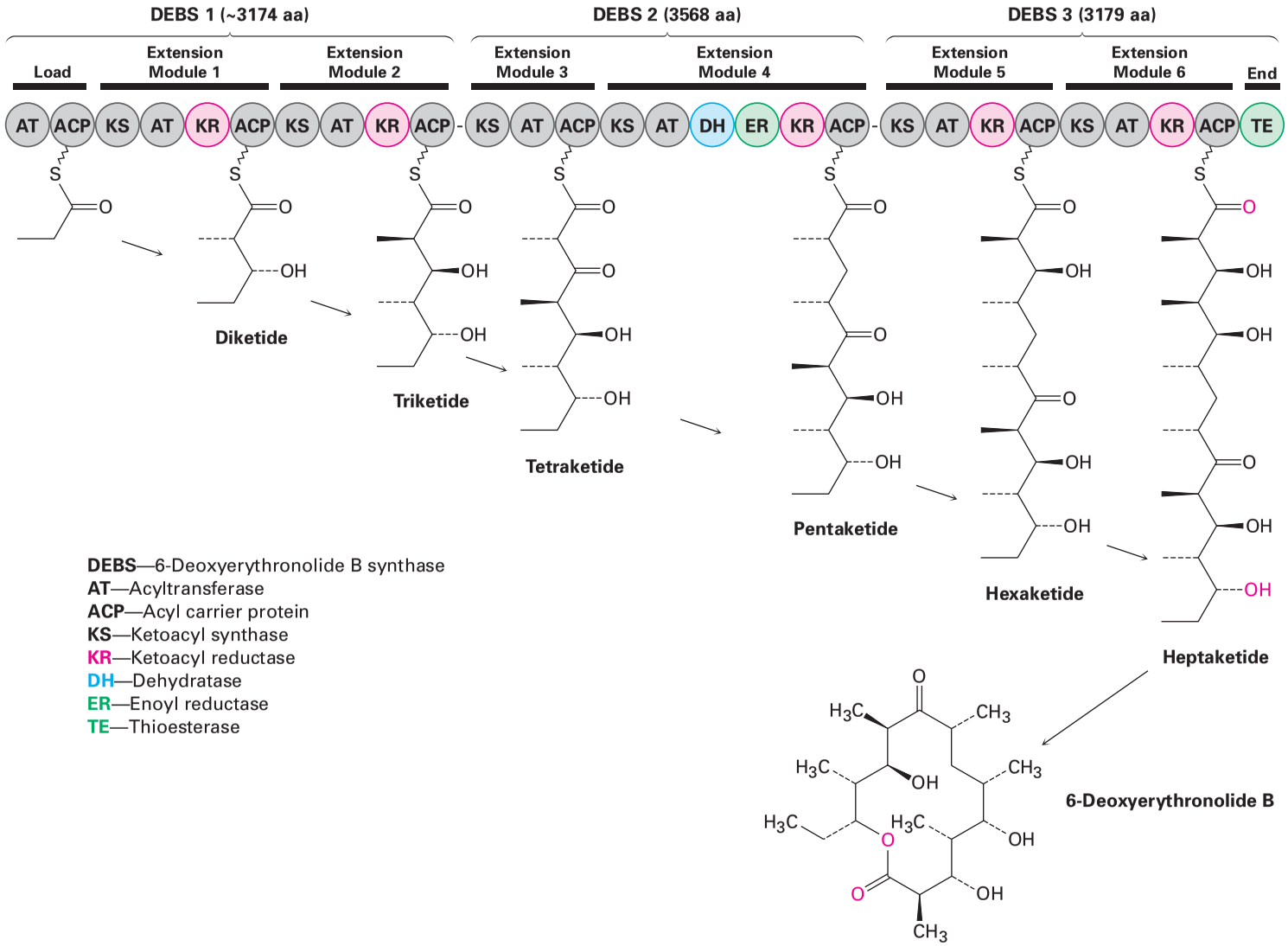


Figure 25.17 A schematic view of the 6-deoxyerythronolide B synthase (DEBS), showing the locations of the enzyme domains within the loading module and the six extension modules. The figure is explained in detail in the text.

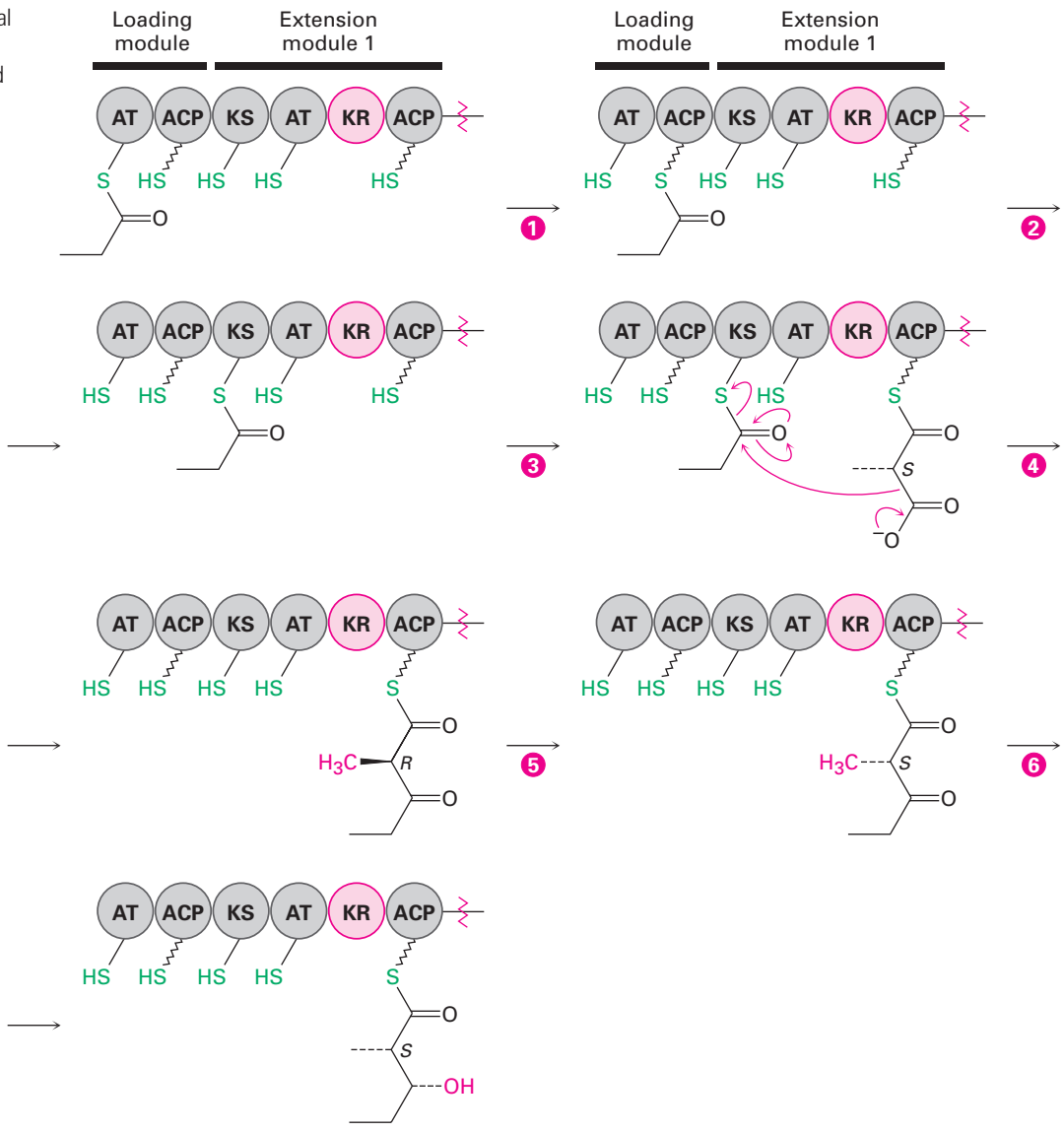
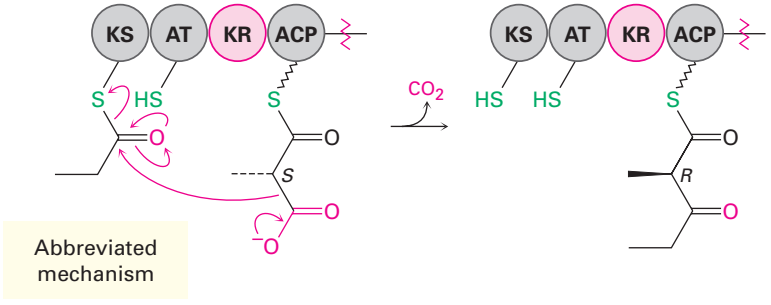


Figure 25.18 The initial loading and first chain-extension cycle catalyzed by the erythromycin PKS. Individual steps are explained in the text.

enzyme-bound β-keto thioester. It's likely that the decarboxylation occurs simultaneously with the Claisen condensation, giving the enolate ion necessary for nucleophilic addition to the second thioester.



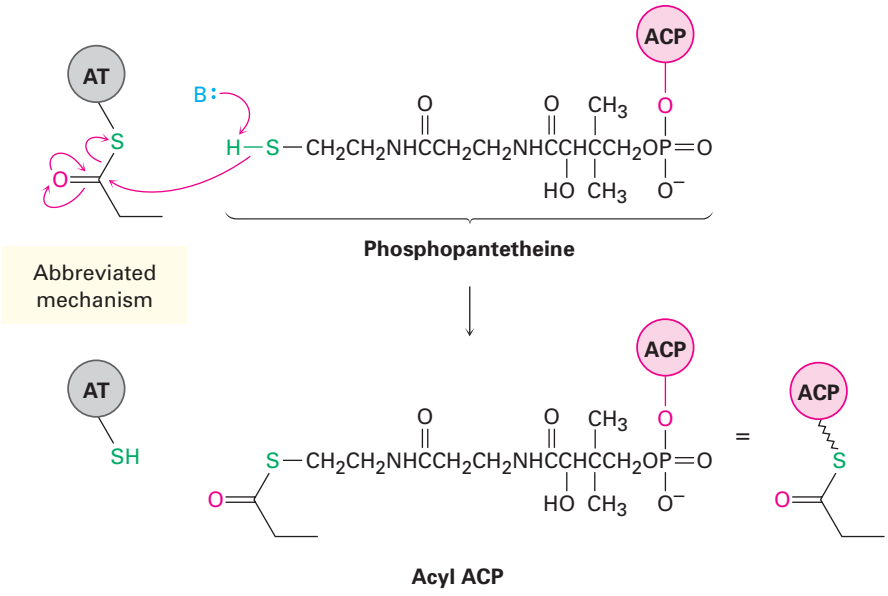
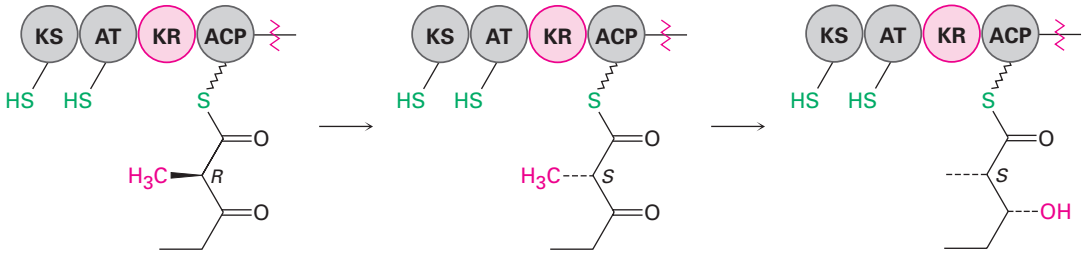


Figure 25.19 Formation of an acyl ACP during polyketide biosynthesis. Phosphopantetheine, symbolized by a zigzag line between S and ACP, acts as a long, flexible arm to allow the acyl group to move from one catalytic domain to another.

STEPS 5–6 OF FIGURE 25.18: EPIMERIZATION AND REDUCTION Interestingly, the Claisen condensation occurs with inversion of configuration at the methyl-bearing chirality center so that the initially formed diketide has (*R*) stereochemistry. Base catalyzed epimerization of the (*R*) product, an acidic β-diketone, occurs in step 5, however, so the product that goes on to the next step regains the (*S*) configuration. Finally, KR1 reduces the ketone to a β-hydroxy thioester in step 6 by transfer of the *pro-S* hydrogen from NADPH as cofactor. Module 1 is now finished, so the diketide is transferred to KS2 for another chain extension.



The reactions catalyzed by extension modules 2, 5, and 6 are similar to those of module 1, although the stereochemistries of the Claisen condensation and reduction steps may differ. The reactions in modules 3 and 4, however, are different. Module 3 lacks a KR domain, so no reduction occurs and the tetraketide product contains a ketone carbonyl group (Figure 25.17). Module 4 contains a KR and two additional enzyme domains, so it catalyzes a ketone reduction plus two additional reactions. Following the reduction by KR4 of the pentaketide, a dehydratase (DH) dehydrates the pentaketide alcohol to an α,β-unsaturated thioester and the double bond is then reduced by an enoyl reductase (ER) domain (Figure 25.20).

Note that the complete sequence of reactions carried out by module 4—Claisen condensation, ketone reduction, dehydration, and double-bond reduction—is identical to the series of reactions found in fatty-acid biosynthesis (see Section 23.6, Figure 23.6). In fact, all fatty-acid synthases have the same set of AT, ACP, KS, KR, DH, and ER domains as the polyketide synthases.

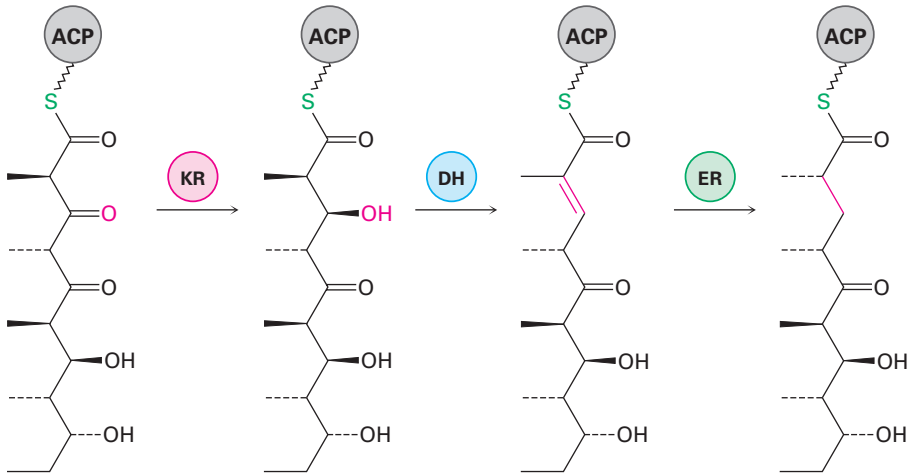


Figure 25.20 Additional processing of the pentaketide intermediate in module 4 removes a carbonyl group by a reduction–dehydration–reduction sequence.

Release of 6-deoxyerythronolide B from the PKS is catalyzed by the ending thioesterase module. A serine residue on the TE module first carries out a nucleophilic acyl substitution on the ACP-bound heptaketide, and the acyl enzyme that results undergoes lactonization. A histidine residue in the TE acts as base to catalyze nucleophilic acyl substitution of the serine ester by the terminal –OH group in the heptaketide (Figure 25.21).

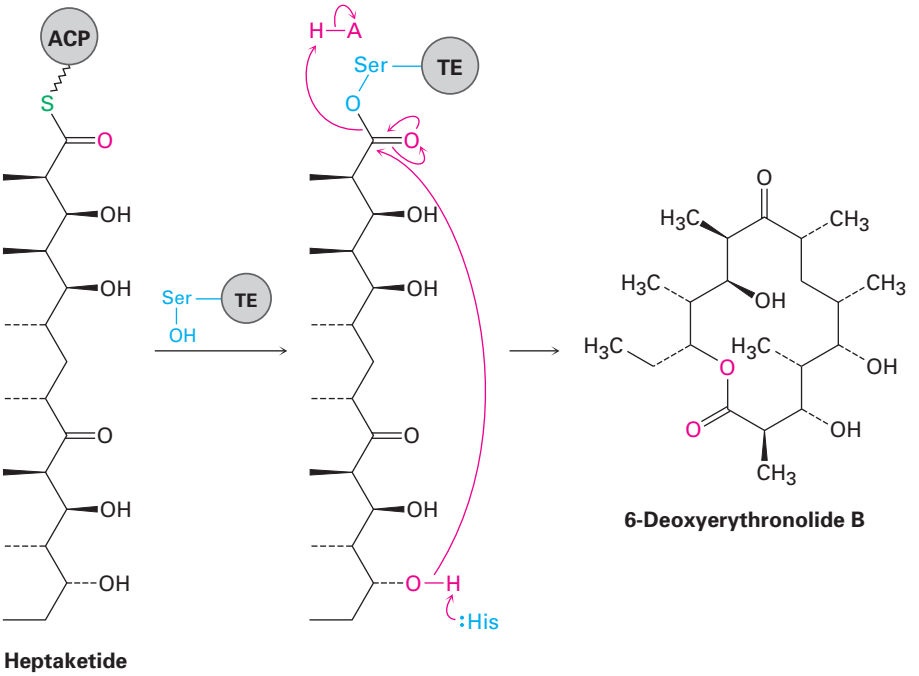


Figure 25.21 Release of 6-deoxyerythronolide from the PKS occurs by lactonization of an acyl enzyme, formed by reaction of a serine residue in the TE module with the heptaketide.

Following its release from the PKS, 6-deoxyerythronolide B is hydroxylated at C6 with retention of configuration to give erythronolide B. The reaction is catalyzed by a P450 hydroxylase analogous to that involved in morphine biosynthesis (Section 25.3, Figure 25.14). L-Mycarose is then attached to the C3 hydroxyl group by reaction with thymidyl diphosphomycarose through an S_N1-like process that proceeds by initial formation of the mycarosyl carbocation (Figure 25.22).

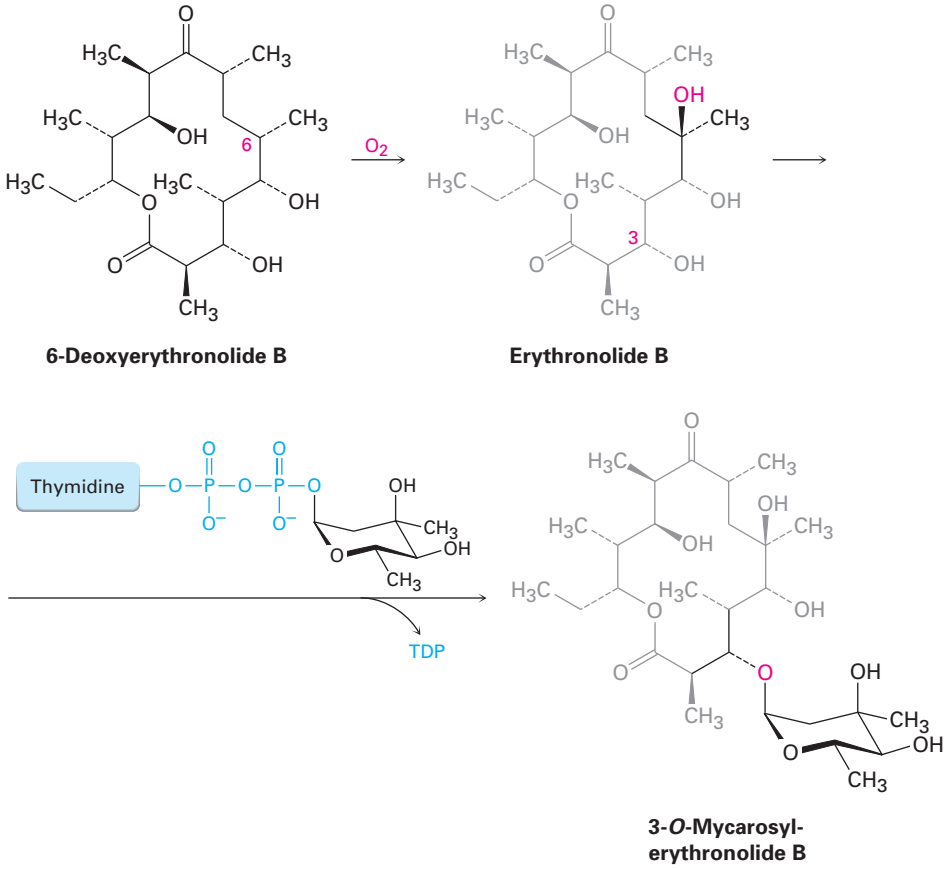


Figure 25.22 Hydroxylation and glycosylation of 6-deoxyerythronolide B to give 3-*O*-mycarosylerythronolide B.

The final steps in erythromycin A biosynthesis are a further glycosylation, a further hydroxylation, and a methylation (Figure 25.23). As in the attachment of mycarose, the attachment of the amino sugar D-desosamine also takes place by transfer from a thymidyl diphosphosugar. C12 hydroxylation by another P450 enzyme occurs with retention of configuration to give erythromycin C, and methylation of the C3′′ hydroxyl group of the mycarose unit by reaction with *S*-adenosylmethionine gives erythromycin A.

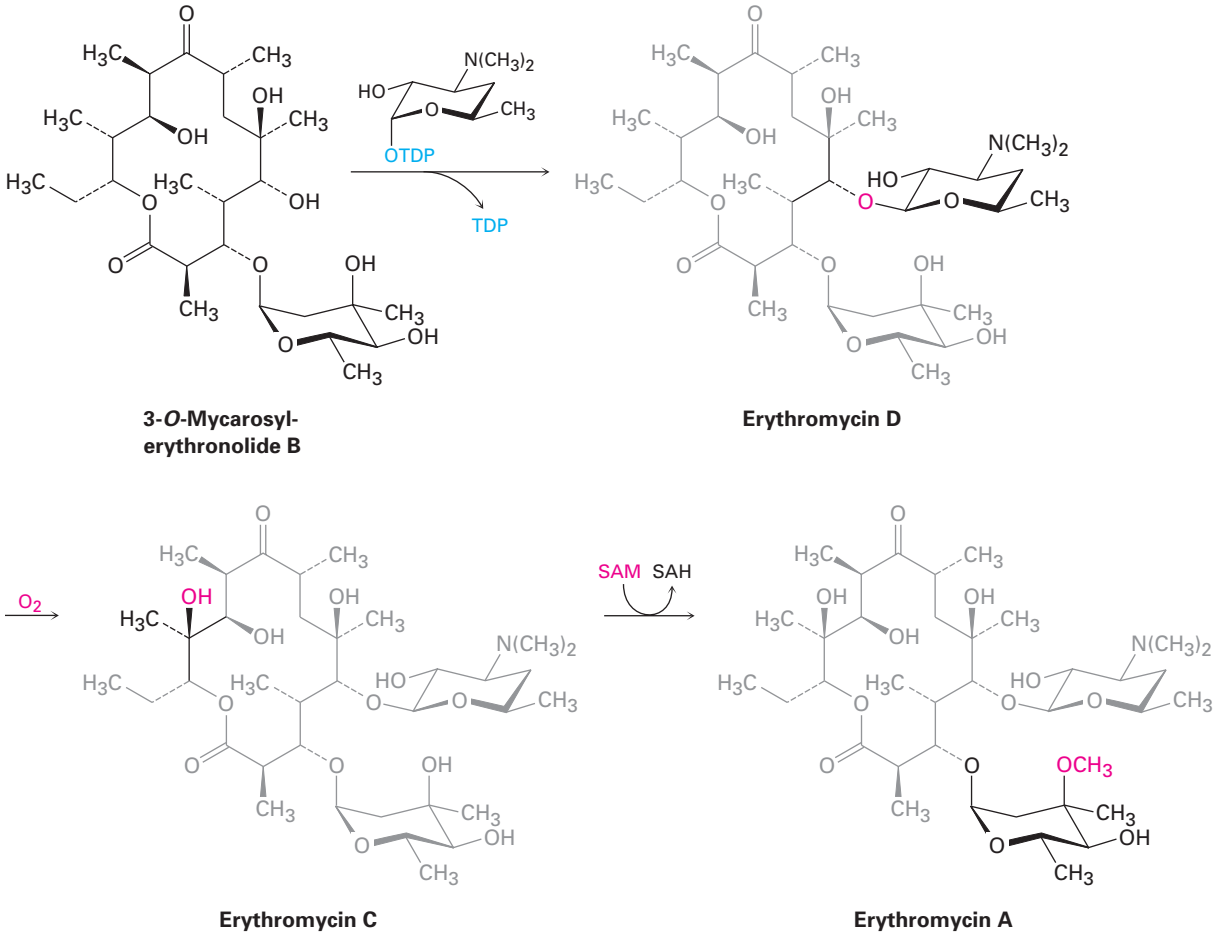
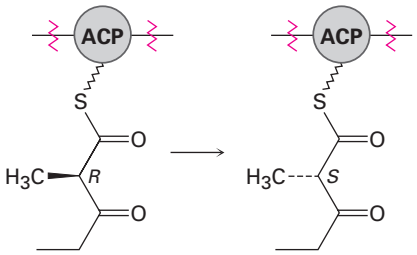


Figure 25.23 Final steps in the biosynthesis of erythromycin A.

Problem 25.5

Show a likely mechanism for the epimerization that occurs in step 5 of Figure 25.18.



Problem 25.6

Propose a mechanism for the reaction of erythronolide B with thymidyl diphosphomycarose to give 3-*O*-mycarosylerythronolide B (Figure 25.22).

Lagniappe

Bioprospecting: Hunting for Natural Products

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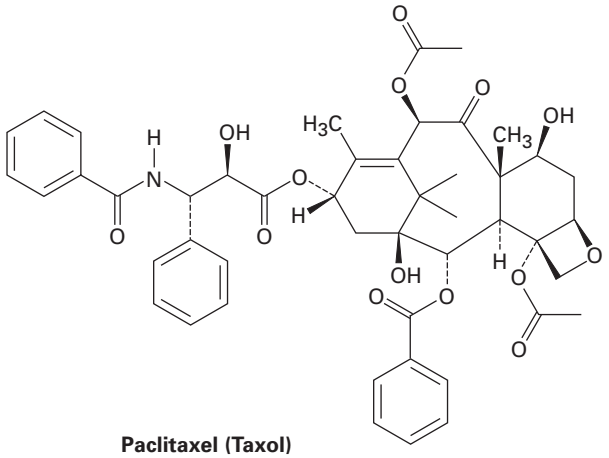
Rapamycin, an immunosuppressant natural product used during organ transplants, was originally isolated from a soil sample found on Easter Island, or Rapa Nui, an island 2200 miles off the coast of Chile known for its giant Moai statues.

Most chemists and biologists spend the majority of their time in the laboratory. A few, however, spend their days scuba diving on South Pacific islands or trekking through the rain forests of South America and Southeast Asia. They aren't on vacation, though; they're at work as bioprospectors, and their job is to hunt for new and unusual natural products that might be useful as drugs.

As noted in the Chapter 5 *Lagniappe,* more than half of all new drug candidates come either directly or indirectly from natural products. All four natural products shown in the introduction to this chapter, for instance, are used as drugs: morphine from the opium poppy, prostaglandin E_1 from sheep prostate glands, erythromycin A from a *Streptomyces erythreus* bacterium cultured from a Philippine soil sample, and benzylpenicillin from *Penicillium notatum.* Still other examples include rapamycin (Figure 25.15), an immunosuppressant isolated from a *Streptomyces hygroscopicus* bacterium first found in a soil sample from Easter Island (Rapa Nui), and paclitaxel (Taxol), an anticancer drug isolated from the bark of the Pacific yew tree found in the American Northwest.



Paclitaxel (Taxol)

With less than 1% of living organisms yet investigated, bioprospectors have a lot of work to do, but there is a race going on. Rain forests throughout the world are being destroyed at an alarming rate, causing many species of both plants and animals to become extinct before they can even be examined. Fortunately, the governments in many countries seem aware of the problem, but there is as yet no international treaty on biodiversity that could help preserve vanishing species.

Summary

The term **natural product** is generally taken to mean a **secondary metabolite**—a small molecule that is not essential to the growth and development of the producing organism and is not classified by structure. Well over 300,000 secondary metabolites probably exist, generally classified into five categories: **terpenoids** and **steroids, fatty acid–derived substances** and **polyketides, alkaloids, nonribosomal polypeptides,** and **enzyme cofactors.**

Unraveling the biosynthetic pathways by which natural products are made is difficult and time-consuming work, but the payoff is a fundamental understanding of how organisms function at the molecular level. The molecules are sometimes complex, but the individual chemical steps by which they are made are familiar.

Exercises

ORGANIC Chemistry Now™ Assess your understanding of this chapter's topics with additional quizzing and conceptually based problems at **http://now.brookscole.com/mcmurryorgbio1**

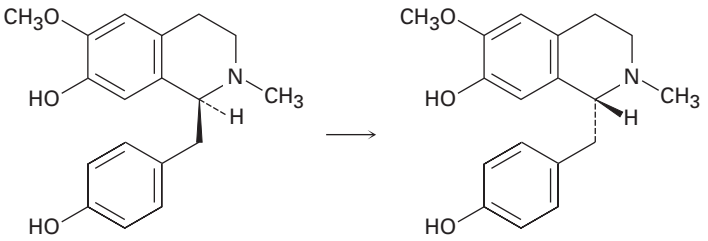
25.7 Which hydrogen, *pro-R* or *pro-S,* is removed from pyridoxine 5′-phosphate in the final step of PLP biosynthesis?

Pyridoxine 5′-phosphate **Pyridoxal 5′-phosphate (PLP)**

25.8 Does the ketone reduction step catalyzed by KR1 in erythromycin biosynthesis occur on the *Re* or the *Si* face of the substrate carbonyl group? (See Figure 25.18.)

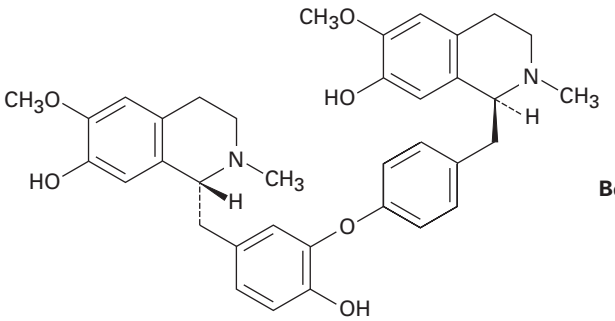
25.9 When the enoyl reductase domain (ER4) in the erythromycin PKS is deactivated by gene mutation, all further steps still occur normally. What is the structure of the lactone that results?

25.10 One of the steps in the biosynthesis of the alkaloid berbamunine is an epimerization of (*S*)-*N*-methylcoclaurine. Review the morphine biosynthesis in Figure 25.6, and propose a mechanism for the epimerization.



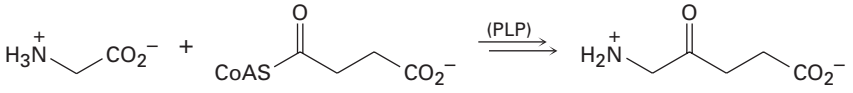
(*S*)-*N*-Methylcoclaurine **(*R*)-*N*-Methylcoclaurine**

25.11 The final step in the biosynthesis of berbamunine is a coupling reaction of (*S*)-*N*-methylcoclaurine with (*R*)-*N*-methylcoclaurine (Problem 25.10). Propose a mechanism.



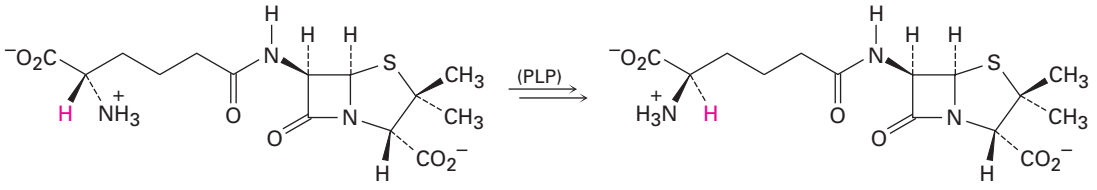
Berbamunine

25.12 5-Aminolevulinate is the precursor from which the large class of alkaloids called *tetrapyrroles* are biosynthesized. It arises by a PLP-dependent reaction of glycine and succinyl CoA. Review the mechanism of the formation of dopamine from L-dopa in Figure 25.7, and propose a mechanism for 5-aminolevulinate biosynthesis.



Glycine **Succinyl CoA** **5-Aminolevulinate**

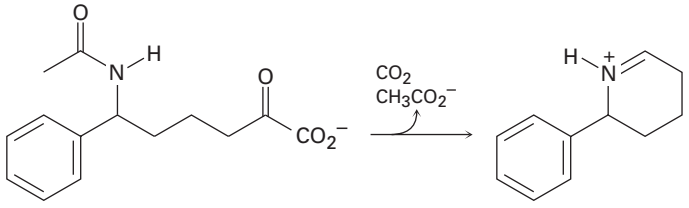
25.13 One of the steps in the biosynthesis of penicillins is a PLP-dependent epimerization of isopenicillin N to penicillin N.



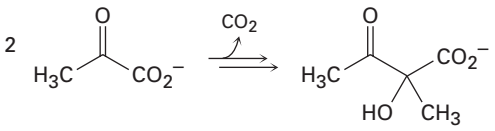
Isopenicillin N **Penicillin N**

The reaction occurs by initial formation of an imine, followed by a base-catalyzed isomerization. Propose a mechanism.

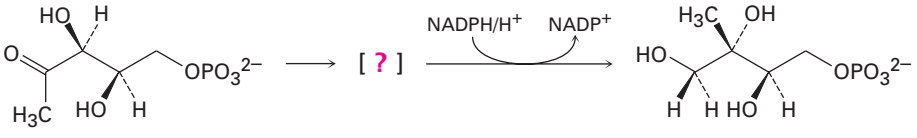
25.14 Propose a mechanism for the following biosynthetic conversion. What cofactors are likely to be involved?



25.15 The enzyme acetolactate synthase catalyzes the thiamin-dependent conversion of two molecules of pyruvate to acetolactate. Propose a mechanism.

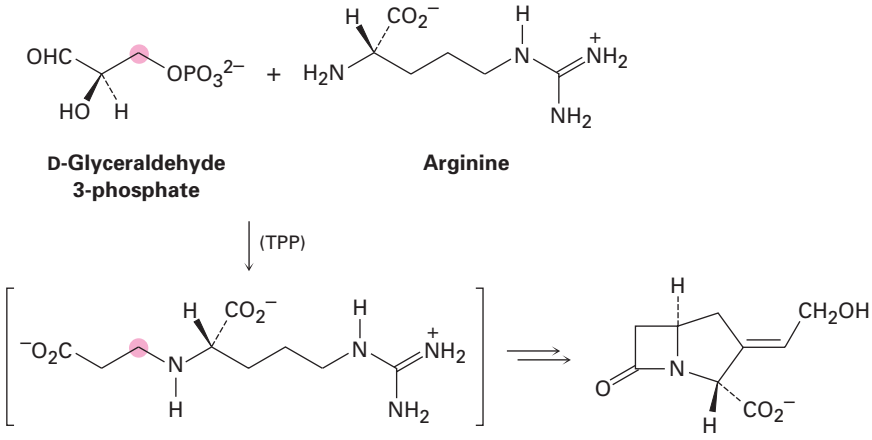


25.16 1-Deoxy-D-xylulose 5-phosphate (DXP), in addition to being a precursor to PLP, is also a precursor to isopentenyl diphosphate in terpenoid biosynthesis. The initial step in the pathway is a base-catalyzed rearrangement, followed by reduction with NADPH to give 2*C*-methyl-D-erythritol 4-phosphate. Show the structure of the rearranged intermediate, and propose a mechanism for its formation.



1-Deoxy-D-xylulose 5-phosphate | **2*C*-Methyl-D-erythritol 4-phosphate**

25.17 Biosynthesis of the β-lactam antibiotic clavulanic acid begins with a TPP-dependent reaction between D-glyceraldehyde 3-phosphate and arginine.



D-Glyceraldehyde 3-phosphate | **Arginine**

Clavulanic acid

(a) The first step is the reaction of D-glyceraldehyde 3-phosphate with TPP ylide, followed by dehydration to give an enol. Show the mechanism, and draw the structure of the product.

(b) The second step is loss of hydrogen phosphate from the enol to give an unsaturated carbonyl compound. Show the mechanism, and draw the structure of the product.

(c) The third step is a conjugate of arginine to the unsaturated carbonyl compound. Show the mechanism, and draw the structure of the product.

(d) The final step is a base-catalyzed hydrolysis to give the final product and regenerate TPP ylide. Show the mechanism.