Thiol Esters and Lipid Biosynthesis

E.1 THIOL ESTERS

Thiol esters can be prepared by reaction of a thiol with an acyl chloride:

\[ RCOCl + R'\text{SH} \rightarrow RCO\text{SR'} + HCl \]

Although thiol esters are not often used in laboratory syntheses, they are of great importance in syntheses that occur within living cells. One of the important thiol esters in biochemistry is “acetyl-coenzyme A”:

\[ \text{Acetyl-coenzyme A} \]
The important part of this rather complicated structure is the thiol ester at the beginning of the chain; because of this, acetyl-coenzyme A is usually abbreviated as follows:

![Thiol Ester](image)

and coenzyme A is abbreviated CoA—SH.

In certain biochemical reactions, an acyl-coenzyme A operates as an acylating agent; it transfers an acyl group to another nucleophile in a reaction that involves a nucleophilic attack at the acyl carbon of the thiol ester. For example,

![Acyl Transfer Reaction](image)

This reaction is catalyzed by the enzyme *phosphotransacetylase*.

The α hydrogens of the acetyl group of acetyl-coenzyme A are appreciably acidic. Acetyl-coenzyme A, as a result, also functions as a nucleophilic alkylating agent. Acetyl-coenzyme A, for example, reacts with oxaloacetate ion to form citrate ion in a reaction that resembles an aldol addition:

![Aldol Addition Reaction](image)

One might well ask, “Why has nature made such prominent use of thiol esters?” Or, “In contrast to ordinary esters, what advantages do thiol esters offer the cell?” In answering these questions we can consider three factors:

1. Resonance contributions of type (b) in the following equation stabilize an ordinary ester and make the carbonyl group less susceptible to nucleophilic attack:

![Resonance Stabilization](image)

This structure makes an important contribution.

In contrast, thiol esters are not so effectively stabilized by a similar resonance contribution because structure (d) among the following ones requires overlap between the 3p orbital of sulfur and a 2p orbital of carbon. Since this overlap is not large, resonance stabilization by (d) is not so effective. Structure (e) does, however, make an important contribution—one that makes the carbonyl group more susceptible to nucleophilic attack.

![Resonance Overlap](image)

This structure is not an important contributor.

This structure makes the carbonyl carbon atom susceptible to nucleophilic attack.
2. A resonance contribution from the similar structure (g) makes the $\alpha$ hydrogens of thiol esters more acidic than those of ordinary esters:

![Chemical structure](image)

This structure's contribution stabilizes the anion of a thiol ester.

3. The carbon–sulfur bond of a thiol ester is weaker than the carbon–oxygen bond of an ordinary ester; SR is a better leaving group than OR.

Factors 1 and 3 make thiol esters effective acylating agents; factor 2 makes them effective nucleophilic alkylating agents. Therefore, we should not be surprised when we encounter reactions similar to the following one:

![Reaction](image)

In this reaction, 1 mol of a thiol ester acts as an acylating agent and the other acts as an alkylating agent (see Section E.2).

E.2 BIOSYNTHESIS OF FATTY ACIDS

Cell membranes, fats, and oils contain esters of long-chain (mainly C$_{14}$, C$_{16}$, and C$_{18}$) carboxylic acids, called fatty acids. Fatty acids are lipids, a largely hydrophobic family of biomolecules that we shall study in Chapter 23. An example of a fatty acid is hexadecanoic acid, also called palmitic acid:

![Chemical structure](image)

Palmitic acid

The fact that most naturally occurring fatty acids are made up of an even number of carbon atoms suggests that they are assembled from two-carbon units. The idea that these might be acetate (CH$_3$CO$_2^-$) units was put forth as early as 1893. Many years later, when radioactively labeled compounds became available, it became possible to test and confirm this hypothesis.

When an organism is fed acetic acid labeled with carbon-14 at the carboxyl group, the fatty acids that it synthesizes contain the label at alternate carbon atoms beginning with the carboxyl carbon:

![Feeding reaction](image)

yields palmitic acid labeled at these positions.
Conversely, feeding acetic acid labeled at the methyl carbon yields a fatty acid labeled at the other set of alternate carbon atoms:

Feeding $^{14}$C-methyl-labeled acetic acid . . .

![Diagram showing yield of palmitic acid labeled at these positions.]

The biosynthesis of fatty acids is now known to begin with acetyl-coenzyme A:

![Diagram showing bond-line formula for palmitic acid.]

The following bond-line formula shows the positions of the two-carbon units incorporated into palmitic acid from acetyl-coenzyme A:

The acetyl portion of acetyl-coenzyme A can be synthesized in the cell from acetic acid; it can also be synthesized from carbohydrates, proteins, and fats:

![Diagram showing biosynthesis of acetyl-CoA from various precursors.]

Although the methyl group of acetyl-coenzyme A is already activated toward condensation reactions by virtue of its being a part of a thiol ester (Section E.1), nature activates it again by converting it to malonyl-coenzyme A:

![Diagram showing conversion of acetyl-CoA to malonyl-CoA.]

The next steps in fatty acid synthesis involve the transfer of acyl groups of malonyl-CoA and acetyl-CoA to the thiol group of a coenzyme called acyl carrier protein or ACP—SH:

*This step also requires 1 mol of adenosine triphosphate (Section 22.1B) and an enzyme that transfers the carbon dioxide.
Acetyl-S-ACP and malonyl-S-ACP then condense with each other to form acetoacetyl-S-ACP:

\[
\text{Acetyl-S-ACP} + \text{Malonyl-S-ACP} \rightarrow \text{Acetoacetyl-S-ACP} + \text{CO}_2
\]

The molecule of CO₂ that is lost in this reaction is the same molecule that was incorporated into malonyl-CoA in the acetyl-CoA carboxylase reaction.

This remarkable reaction bears a strong resemblance to the malonic ester syntheses that we saw earlier (Section 18.7) and deserves special comment. One can imagine, for example, a more economical synthesis of acetoacetyl-S-ACP, that is, a simple condensation of 2 mol of acetyl-S-ACP:

\[
\text{Acetyl-S-ACP} + \text{Acetyl-S-ACP} \rightarrow \text{Acetoacetyl-S-ACP} + \text{ACP-SH}
\]

Studies of this last reaction, however, have revealed that it is highly endothermic and that the position of equilibrium lies very far to the left. In contrast, the condensation of acetyl-S-ACP and malonyl-S-ACP is highly exothermic, and the position of equilibrium lies far to the right. The favorable thermodynamics of the condensation utilizing malonyl-S-ACP comes about because the reaction also produces a highly stable substance: carbon dioxide. Thus, decarboxylation of the malonyl group provides the condensation with thermodynamic assistance.

The next three steps in fatty acid synthesis transform the acetoacetyl group of acetoacetyl-S-ACP into a butyryl (butanoyl) group. These steps involve (1) reduction of the keto group (utilizing NADPH⁺ as the reducing agent), (2) dehydration of an alcohol, and (3) reduction of a double bond (again utilizing NADPH).

**Reduction of the Keto Group**

\[
\text{Acetoacetyl-S-ACP} + \text{NADPH} + \text{H}^+ \rightarrow \text{β-Hydroxybutyryl-S-ACP} + \text{NADP}^+
\]

**Dehydration of the Alcohol**

\[
\text{β-Hydroxybutyryl-S-ACP} + \text{H}_2\text{O} \rightarrow \text{Crotonyl-S-ACP}
\]

**Reduction of the Double Bond**

\[
\text{Crotonyl-S-ACP} + \text{NADPH} + \text{H}^+ \rightarrow \text{Butyryl-S-ACP} + \text{NADP}^+
\]

These steps complete one cycle of the overall fatty acid synthesis. The net result is the conversion of two acetate units into the four-carbon butyrate unit of butyryl-S-ACP. (This conversion requires, of course, the crucial intervention of a molecule of carbon dioxide.) At this point, another cycle begins and the chain is lengthened by two more carbon atoms.

*NADPH is nicotinamide adenine dinucleotide phosphate (reduced form), a coenzyme that is very similar in structure and function to NADH (see “The Biochemistry of… Alcohol Dehydrogenase” in Section 12.3 and Section 14.10).
Subsequent turns of the cycle continue to lengthen the chain by two-carbon units until a long-chain fatty acid is produced. The overall equation for the synthesis of palmitic acid, for example, can be written as follows:

\[
\text{SCoA} + 7 \text{HO} - \text{SCoA} + 14 \text{NADPH} + 14 \text{H}^+ \rightarrow \text{SCoA} + 7 \text{CO}_2 + 8 \text{CoA-SH} + 14 \text{NADP}^+ + 6 \text{H}_2\text{O}
\]

One of the most remarkable aspects of fatty acid synthesis is that the entire cycle appears to be carried out by a dimeric multifunctional enzyme. The molecular weight of this enzyme, called fatty acid synthetase, has been estimated as 2,300,000.* The synthesis begins with a single molecule of acetyl-S-ACP serving as a primer. Then, in the synthesis of palmitic acid, for example, successive condensations of seven molecules of malonyl-S-ACP occur, with each condensation followed by reduction, dehydration, and reduction. All of these steps, which result in the synthesis of a C\textsubscript{16} chain, take place before the fatty acid is released from the enzyme.

The acyl carrier protein from Escherichia coli has been isolated and purified; its molecular weight is approximately 10,000. In animals the carrier is part of the larger multifunctional enzyme. Both types of carrier protein contain a chain of groups called a phosphopantetheine group that is identical to that of coenzyme A (Section E.1). In ACP this chain is attached to a protein (rather than to an adenosine phosphate as it is in coenzyme A):

The length of the phosphopantetheine group is 20.2 Å, and it has been postulated that it acts to transport the growing acyl chain from one active site of the enzyme to the next (Fig. E.1).

*As isolated from yeast cells. Fatty acid synthetases from different sources have different molecular weights; that from pigeon liver, for example, has a molecular weight of 450,000.
Isoprenoid compounds are another class of lipid biomolecules. Among them are natural products such as α-terpineol, geraniol, vitamin A, β-carotene, steroids (e.g., cholesterol, cortisone, the estrogens, and testosterone), and many related compounds. We shall study terpenes further in Chapter 23. Now, however, let us consider aspects of their biosynthesis that involve reactions parallel to some that we have recently studied as well as reactions that we have seen in earlier chapters:

The basic building block for the synthesis of terpenes and terpenoids is 3-methyl-3-butenyl pyrophosphate. The five carbon atoms of this compound are the source of all the “isoprene units” in isoprenoid compounds. (Isoprene units in the preceding structures are shown in blue and red.)

We consider how 3-methyl-3-butenyl pyrophosphate is biosynthesized in Section E.4. First, however, let us look at the way C₅ isoprene units are joined together. A necessary first step is enzymatic formation of 3-methyl-2-butenyl pyrophosphate from 3-methyl-3-butenyl pyrophosphate. This isomerization establishes an equilibrium that makes both compounds available to the cell:

The joining of 3-methyl-2-butenyl pyrophosphate and 3-methyl-3-butenyl pyrophosphate involves enzymatic formation of an allylic cation. Here, the pyrophosphate group functions as a natural leaving group. This is one of many instances where nature relies on the pyrophosphate group for biochemical
processes. Condensation of the two \( \text{C}_5 \) units yields a \( \text{C}_{10} \) compound called geranyl pyrophosphate:

![Diagram of the condensation of two \( \text{C}_5 \) units to form \( \text{C}_{10} \) geranyl pyrophosphate]

Geranyl pyrophosphate is the precursor of the monoterpenes; hydrolysis of geranyl pyrophosphate, for example, yields geraniol:

![Diagram showing the hydrolysis of geranyl pyrophosphate to form geraniol]

Geranyl pyrophosphate can also condense with 3-methyl-3-butenyl pyrophosphate to form the \( \text{C}_{15} \) precursor for sesquiterpenes, farnesyl pyrophosphate:

![Diagram of the condensation of geranyl pyrophosphate with 3-methyl-3-butenyl pyrophosphate to form farnesyl pyrophosphate]

Farnesol is a common component in the essential oils of plants and flowers. It has been isolated from roses, lemon and citronella grasses, and ambrette oil. It has the odor of lily of the valley. Farnesol also functions as a hormone in certain insects and initiates the change from caterpillar to pupa to moth. It is released by female mites as a sex attractant for male mites.

Similar condensation reactions yield the precursors for all of the other terpenes (Fig. E.2). In addition, a tail-to-tail reductive coupling of two molecules of farnesyl pyrophosphate produces squalene, the precursor for the important group of isoprenoids known as steroids (see Sections 23.4 and E.4).
When farnesol is treated with sulfuric acid, it is converted to bisabolene. Outline a possible mechanism for this reaction.

\[ \text{Farnesol} \stackrel{\text{H}_2\text{SO}_4}{\rightarrow} \text{Bisabolene} \]

**PRACTICE PROBLEM E.1**

When farnesol is treated with sulfuric acid, it is converted to bisabolene. Outline a possible mechanism for this reaction.

We saw in the previous section that the C₅ compound 3-methyl-3-butenyl pyrophosphate is the actual “isoprene unit” that nature uses in constructing terpenoids and carotenoids. We can now extend that biosynthetic pathway in two directions. We can show how 3-methyl-3-butenyl pyrophosphate (like the fatty acids) is ultimately derived from acetate units and how cholesterol, the precursor of most of the important steroids, is synthesized from 3-methyl-3-butenyl pyrophosphate.

In the 1940s, Konrad Bloch of Harvard University used labeling experiments to demonstrate that all of the carbon atoms of cholesterol can be derived from acetic acid. Using methyl-labeled acetic acid, for example, Bloch found the following label distribution in the cholesterol that was synthesized:

Bloch also found that feeding carboxyl-labeled acetic acid led to incorporation of the label into all of the other carbon atoms of cholesterol (the unstarrred carbon atoms of the formula just given).
Subsequent research by a number of investigators has shown that 3-methyl-3-butenyl pyrophosphate is synthesized from acetate units through the following sequence of reactions:

\[
\begin{align*}
\text{(C}_2\text{)} & \quad \text{Acetyl-CoA} \\
\text{(C}_4\text{)} & \quad \text{Acetoacetyl-CoA} \\
\text{(C}_6\text{)} & \quad \beta\text{-hydroxy-}\beta\text{-methylglutaryl-CoA}
\end{align*}
\]

The first step of this synthetic pathway is straightforward. Acetyl-CoA (from 1 mol of acetate) and acetoacetyl-CoA (from 2 mol of acetate) condense to form the \( \text{C}_6 \) compound, \( \beta\text{-hydroxy-}\beta\text{-methylglutaryl-CoA} \). This step is followed by an enzymatic reduction of the thiol ester group of \( \beta\text{-hydroxy-}\beta\text{-methylglutaryl-CoA} \) to the primary alcohol of mevalonic acid. The enzyme that catalyzes this step is called HMG-CoA reductase (HMG is \( \beta\text{-hydroxy-}\beta\text{-methylglutaryl} \)), and this step is the rate-limiting step in cholesterol biosynthesis. The key to finding this pathway was the discovery that mevalonic acid was an intermediate and that this \( \text{C}_6 \) compound could be transformed into the five-carbon 3-methyl-3-butenyl pyrophosphate by successive phosphorylations and decarboxylation.

As we saw earlier (Section E.3), 3-methyl-3-butenyl pyrophosphate isomerizes to produce an equilibrium mixture that contains 3-methyl-2-butenyl pyrophosphate, and these two compounds condense to form geranyl pyrophosphate, a \( \text{C}_{10} \) compound. Geranyl pyrophosphate subsequently condenses with another mole of 3-methyl-3-butenyl pyrophosphate to form farnesyl pyrophosphate, a \( \text{C}_{15} \) compound. (Geranyl pyrophosphate and farnesyl pyrophosphate are the precursors of the mono- and sesquiterpenes; see Section E.3.)
Two molecules of farnesyl pyrophosphate then undergo a reductive condensation to produce squalene:

Squalene is the direct precursor of cholesterol. Oxidation of squalene yields squalene 2,3-epoxide, which undergoes a remarkable series of ring closures accompanied by concerted methanide and hydride migrations to yield lanosterol. Lanosterol is then converted to cholesterol through a series of enzyme-catalyzed reactions:

(continues on next page)
E.5 CHOLESTEROL AND HEART DISEASE

Because cholesterol is the precursor of steroid hormones and is a vital constituent of cell membranes, it is essential to life. On the other hand, deposition of cholesterol in arteries is a cause of heart disease and atherosclerosis, two leading causes of death in humans. For an organism to remain healthy, there has to be an intricate balance between the biosynthesis of cholesterol and its utilization, so that arterial deposition is kept at a minimum.

For some individuals with high blood levels of cholesterol, the remedy is as simple as following a diet low in cholesterol and in fat. For those who suffer from the genetic disease familial hypercholesterolemia (FH), other means of blood cholesterol reduction are required. One remedy involves using the drug lovastatin (also called Mevacor):
Lovastatin, because part of its structure resembles mevalonate ion, can apparently bind at the active site of HMG-CoA reductase (Section E.4), the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis. Lovastatin acts as a competitive inhibitor of this enzyme and thereby reduces cholesterol synthesis. Reductions of up to 30% in serum cholesterol levels are possible with lovastatin therapy.

Cholesterol synthesized in the liver either is converted to bile acids that are used in digestion or is esterified for transport by the blood. Cholesterol is transported in the blood, and taken up in cells, in the form of lipoprotein complexes named on the basis of their density. **Low-density lipoproteins (LDLs)** transport cholesterol from the liver to peripheral tissues. **High-density lipoproteins (HDLs)** transport cholesterol back to the liver, where surplus cholesterol is disposed of by the liver as bile acids. High-density lipoproteins have come to be called “good cholesterol” because high levels of HDL may reduce cholesterol deposits in arteries. Because high levels of LDL are associated with the arterial deposition of cholesterol that causes cardiovascular disease, it has come to be called “bad cholesterol.”

Bile acids that flow from the liver to the intestines, however, are efficiently recycled to the liver. Recognition of this process has led to another method of cholesterol reduction, namely, the ingestion of resins that bind bile acids and thereby prevent their reabsorption in the intestines.