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Coenzyme A Analogues and Derivatives: Synthesis and Applications as Mechanistic Probes of Coenzyme A Ester-Utilizing Enzymes

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I. Introduction

A. Scope of This Review

This review presents the synthesis of coenzyme A (CoA), its esters, and analogues and the application of these compounds in studies of CoA ester-utilizing enzymes. The primary focus is on unnatural derivatives that have been designed for specific interaction with one or a group of CoA or CoA ester-utilizing enzymes. A range of enzymatic and nonenzymatic procedures is presented that has been developed for

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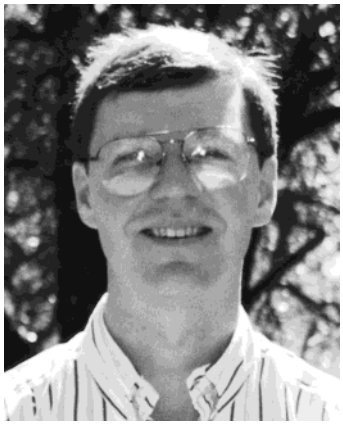
Pranab K. Mishra was born in Badanganj, India. He developed an interest in chemistry in high school, where he once saw his laboratory demonstrator make a fire by mixing chemicals. He received his B.S. in chemistry from the University of Calcutta in 1983 and his M. Tech. in polymer science and technology from the same university in 1988. While working toward his Ph.D. in polymer science from the Indian Institute of Technology, he moved to the United States. He received his M.A. in organic chemistry from the University of Delaware and his Ph.D. from Montana State University, both under the tutelage of Prof. Cynthia K. McClure. He then joined the laboratory of Dale Drueckhammer as a postdoctoral fellow. His research interests range from synthetic organic chemistry to molecular biology. He and his wife Soma have a daughter Ritobrita.



Dale Drueckhammer was born in Comanche, Texas, and was raised near Priddy, Texas. He received his B.S. in chemistry from Tarleton State University in 1983. He received his Ph.D. from Texas A & M University in 1987, where he worked with Prof. Chi-Huey Wong. He was a postdoctoral fellow in the laboratory of Prof. Christopher T. Walsh at Harvard Medical School. Since 1997 he has been on the faculty at the State University of New York at Stony Brook. His research interests include the design and synthesis of inhibitors and mechanistic probes for enzymes, the elucidation of enzyme mechanisms, the application of enzymes in synthesis, and molecular recognition. He lives in Setauket, New York, with his wife Maria, their children Rebecca and James, their golden retriever Potter, and pet goat Prudence. When not doing chemistry or attending his children's various activities, he does odd jobs around the house.

preparation of coenzyme A, its analogues, and derivatives. Also discussed is the utilization of these analogues in elucidating mechanistic details of enzymatic reactions of coenzyme A. The first sections of this review discuss the structure, enzymology, and biological importance of CoA and both biological and nonbiological methods for the synthesis of CoA. The third section presents methods for the synthesis of thioesters of CoA, a survey of the various types of CoA thioesters that have been prepared, and the use of these unnatural and/or uncommon CoA thioesters in mechanistic interpretations of CoA-related reactions. Subsequent sections present the synthesis and applications of CoA thioethers and other acyl-CoA analogues derived from CoA and finally the synthesis and applications of analogues that cannot be derived by simple derivatization of CoA. This review includes early examples starting in the 1960s, though most of the analogues and their applications have been reported since the late 1980s.

B. Structure and Biological Importance of Coenzyme A

Enzymes can catalyze a wide variety of chemical transformations by using functional groups of their component amino acids in various capacities, including acid−base reactions, formation of transient covalent bonds, and participation in charge−charge interactions. However, for certain reactions, enzymes require the participation of additional small molecules known as cofactors. Cofactors may be metal ions or organic molecules. Coenzyme A is a cofactor that aids in the activation and transfer of acyl groups in many enzymatic reactions. Unlike most cofactors which function by remaining bound to a single enzyme, CoA acts as a diffusible carrier of acyl groups from one enzyme-catalyzed reaction to another. CoA is used by an estimated 4% of all known enzymes and plays a role in a wide range of biological pathways.[1,2]

CoA was discovered by Lipmann in 1945 and its structure was reported in 1953.[3,4] The structure of CoA **1** (Figure 1) consists of a 3′-phosphoadenosine

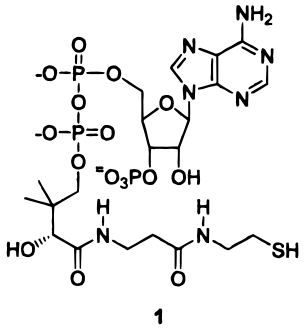


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Figure 1. The structure of coenzyme A.

moiety and pantetheine, linked by a pyrophosphate group. The pantetheine domain is derived from pantothenic acid, a member of the vitamin B family. While the structure of CoA is fairly complex, it is functionally a simple molecule. The enzymatic reactions of CoA involve only the thiol group, while reactions of CoA thioesters involve the thioester group and/or the acyl moiety.[5] The rest of the CoA molecule serves as a recognition element for binding by enzymes.

CoA esters are involved in fundamental processes such as fatty acid biosynthesis and degradation, transcription, cell−cell mediated recognition, hormone synthesis and regulation, nerve impulse conductance, and the tricarboxylic acid cycle.[1−4,6] Acetyl-CoA is involved in resistance to antibiotics such as chloramphenicol via enzyme-catalyzed acylation.[7] The critical acetylation of histones for chromatin remodeling and gene expression in eukaryotes is mediated by histone acetyl transferase.[8] In signal

transduction pathways, the 14-carbon myristoyl group is transferred from myristoyl CoA to proteins.[9] The myristoyl group serves as an anchor to localize the proteins at the plasma membrane. In addition to serving as substrates for enzymatic reactions, long chain CoA esters also function as regulators of various cellular functions.[10] Acyl-CoA binding protein sequesters long chain CoA esters from solution and, along with other metabolic factors, serves to control the free concentration of CoA esters in solution.[10] Thus CoA and its acyl derivatives play a very major and central role in many diverse areas of biology.

The conformation of CoA in solution and in the complex with crotonase has been studied in detail by NMR.[11−13] Furthermore, the crystal structures of more than 20 CoA ester-utilizing enzymes have been solved, most complexed with their CoA ester substrate and/or an analogue thereof.[14−18] Solution studies indicate a gauche conformation about the C−O bond at the point of attachment of the pantetheine moiety to the pyrophosphate group and suggest some interactions between protons of the pantetheine group with the adenine and 3′-phosphate group. The gauche conformation about the C−O bond is also observed in the enzyme complexes of CoA. Other conformational features appear to vary between enzymes, and there is no general structural motif or mode of binding of CoA between the different enzymes.[14,15]

C. Enzymatic Reactions of Coenzyme A and Coenzyme A Thioesters

Acylation of the thiol group of CoA gives an acyl derivative that is activated in two ways (Figure 2).

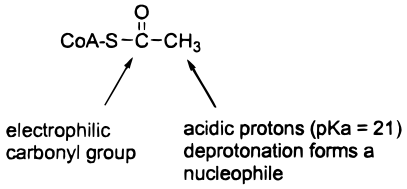

Figure 2. The two general modes of reactivity of acetyl-CoA.

The thioester carbonyl can react as an electrophile toward attack by a nucleophilic cosubstrate. The thioester α-carbon upon deprotonation can react as a nucleophile. Acetyl-CoA is the most common CoA thioester. Several enzymes catalyze the formation of acetyl-CoA including acetyl-CoA synthetase, phosphotransacetylase, ATP citrate lyase, and thiolase. The enzymes that utilize acetyl-CoA can be divided into two main classes. These are the Claisen enzymes, which catalyze reactions involving deprotonation of the α-carbon, and the acetyltransferases, which catalyze nucleophilic acyl substitution reactions at the carbonyl carbon. Some reactions of the more complex CoA esters involve simple acyl transfer or other reactions similar to those of acetyl-CoA, while some reactions involve functionality of the *β*-carbon and beyond.

1. Claisen Enzymes

Claisen enzymes utilize acetyl-CoA as a nucleophilic substrate via deprotonation of the methyl

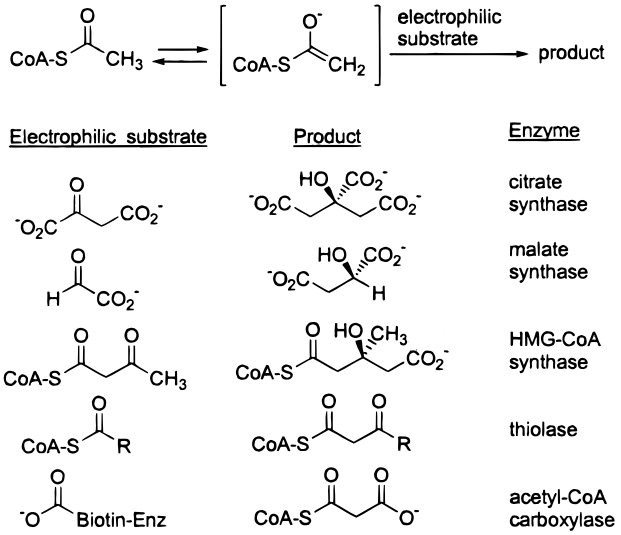

Figure 3. Examples of Claisen enzymes.

group. The electrophile is the carbonyl group of an aldehyde, ketone, or thioester or the carboxy group of carboxybiotin. The reactions of some representative Claisen enzymes are illustrated in Figure 3. Although many of these reactions are actually aldol reactions, this entire group of enzymes is commonly referred to as Claisen enzymes in the literature. In the reactions of citrate synthase and malate synthase, the initial aldol reaction is followed by enzymatic hydrolysis of the thioester.

The Claisen enzymes are involved in a variety of biological pathways. Citrate synthase catalyzes the key step in which acetyl-CoA is shuttled into the citric acid cycle. ATP-citrate lyase, which catalyzes the same aldol reaction as citrate synthase but functions in the opposite direction, controls the amount of cytosolic acetate available for fatty acid biosynthesis.[19] Thiolase is a key enzyme in steroid biosynthesis and fatty acid biosynthesis and degradation.[20] Acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA, which is the building block for fatty acid biosynthesis. This enzyme is an important pharmaceutical target in regulating hyperlipidemia in humans.[21]

The reactions catalyzed by Claisen enzymes could occur through either a stepwise or a concerted pathway. In a stepwise mechanism acetyl-CoA is first deprotonated to form an enolate or possibly an enol intermediate, followed by C−C bond formation in the second step. In a concerted pathway, deprotonation and bond formation occur simultaneously, without formation of an intermediate. Eggerer and co-workers provided evidence for a stepwise mechanism in citrate synthase.[22] This evidence was based on the observation of tritium incorporation from solvent into the methyl group of acetyl-CoA upon incubation with enzyme. This indicates that deprotonation is a distinct step from carbon−carbon bond formation. Eggerer extended these studies to malate synthase and obtained similar results. The exchange of methyl protons of acetyl-CoA was found to be enhanced by the presence of pyruvate and other α-keto acids that mimic natural cosubstrate glyoxalate.[23] Elegant isotope effect studies by Knowles and co-workers have

provided further evidence for a stepwise mechanism for malate synthase.[24]

The β-ketoacyl CoA thiolase enzymes catalyze a true Claisen reaction, and this reaction illustrates the two modes of reactivity of CoA thioesters. In the formation of acetoacetyl-CoA from 2 equiv of acetyl-CoA, 1 equiv of acetyl-CoA reacts as an electrophile as the acetyl group is initially transferred to an active site thiol group. The second equivalent of acetyl-CoA then reacts as a nucleophile, undergoing deprotonation followed by nucleophilic attack on the acetyl-enzyme. Exchange of the methyl protons of the substrate analogue dithioacetyl-CoA (in which the carbonyl oxygen was replaced with sulfur) with solvent was demonstrated by Anderson and co-workers, again supporting a stepwise mechanism.[25]

2. Acyltransferases

Acyltransferases catalyze the transfer of the acyl group from a CoA thioester to a nucleophilic acceptor, commonly an alcohol or amine or, in the thioesterases, water. Acyltransferases that specifically use acetyl-CoA are called acetyltransferases, which is the second major class of acetyl-CoA utilizing enzymes. Some representative acetyltransferase substrates are shown in Figure 4. The biological significance of

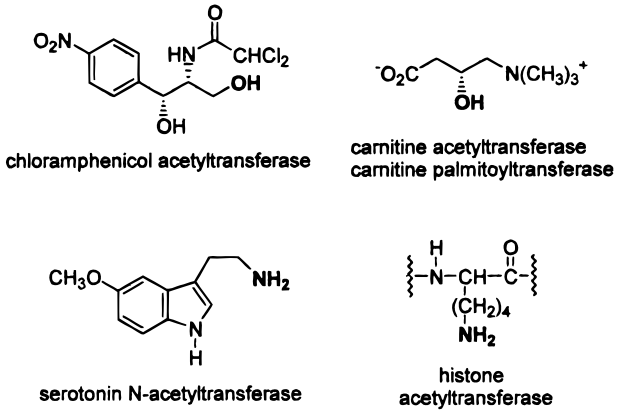

Figure 4. Examples of nucleophilic substrates for acetyltransferases.

acetyltransferases is broad. Bacterial acetylation of antibiotics such as chloramphenicol, gentamicin, and kanamycin renders the drugs inactive, thus confering antibiotic resistance to many bacteria.[26] The enzymes which catalyze these acetylation reactions thus act as resistance factors and can be exchanged at the genetic level on extrachromosomal elements or plasmids. Acetylation also plays a key role in the transmission of nerve impulses. Acetylcholine is a major neurotransmitter involved in learning processes, memory, and sleep.[27] The formation of acetylcholine from choline in response to nerve impulses near the end of presynaptic axon is catalyzed by choline acetyltransferase. Acetylation of histones catalyzed by histone *N*-acetyltransferase is a vital control element in gene transcription,[28] and several additional DNA-binding proteins may be acetylated as part of the transcriptional process.[29]

The acetyltransferase reactions are generally favorable thermodynamically due to the high energy of the thioester bond, which has a free energy of hydrolysis of 8.2 kcal/mol, about 2 kcal/mol greater than that of oxoesters.[30,31] Two general mechanisms may be followed for an acetyl-transfer reaction. The simplest mechanism is a direct transfer of the acetyl group from acetyl-CoA to the nucleophilic acceptor via a tetrahedral intermediate or transition state. The second mechanism involves initial acetyl transfer to an enzymatic residue to form an acetyl-enzyme intermediate, followed by transfer of the acetyl group from the enzyme to the nucleophilic substrate. In most of the acetyltransferase-catalyzed reactions characterized thus far, a direct acetyl-transfer mechanism not involving an acetyl-enzyme has been found. Shaw and co-workers have shown that chloramphenicol acetyltransferase operates by a direct transfer mechanism via a ternary complex,[32] and these results are further supported by the crystal structures of CAT with CoA and with chloramphenicol.[33] Gentamicin acetyltransferase has also been shown by kinetic analysis to form a ternary complex during catalysis.[34] Both choline acetyltransferase[35] and carnitine acetyltransferase[36] gave similar results. Arylamine acetyltransferase, like thiolase, forms an acetyl-enzyme intermediate.[37,38]

Other acetyltransferases are known which catalyze the transfer of the acyl groups from longer chain acyl-CoA. These include carnitine palmitoyltransferase, which catalyzes the transfer of palmitic acid to the hydroxyl group of carnitine[39,40] and protein *N*-myristoyltransferase, which catalyzes the transfer of myristic acid to the N-terminal amino group of a protein.[9] Acyl-CoA thioesterases are known that catalyze hydrolysis of CoA thioesters, which corresponds to acyl-transfer to water. These enzymes include the *p*-hydroxybenzoyl-CoA thioesterase involved in the pathway for *p*-chlorobenzoate metabolism.[41]

3. Other CoA Ester-Utilizing Enzymes

Enzymatic reactions of longer chain CoA esters may involve functionality beyond the α-carbon. Some examples are provided by the fatty acid β-oxidation cycle (Figure 5).[42,43] The reaction of acyl-CoA dehy-

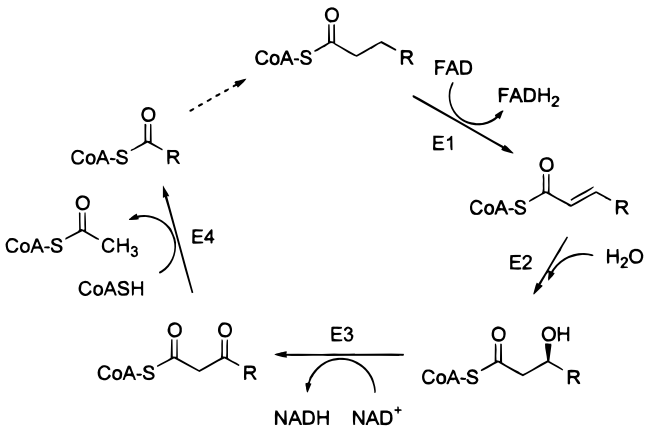

Figure 5. The fatty acid β-oxidation cycle. E1 = acyl-CoA dehydrogenase, E2 = crotonase, E3 = β-hydroxyacyl-CoA dehydrogenase, E4 = thiolase.

drogenase results in oxidation to the α,β-unsaturated CoA ester. Addition of water across the double bond

catalyzed by crotonase (also called enoyl-CoA hydratase) forms the β-hydroxy ester. The thioester functionality is assumed to facilitate both of these reactions by stabilization of negative charge at the α-carbon, as in the reactions of the Claisen enzymes. In contrast, the reaction of β-hydroxyacyl-CoA dehydrogenase does not involve the thioester functionality at all, though the CoA moiety is vital for substrate recognition. The reaction of the Claisen enzyme thiolase completes the cycle. Fatty acid biosynthesis by fatty acid synthase is the approximate reversal of this β-oxidation cycle. Another unique reaction of a CoA thioester is that of HMG-CoA reductase, which catalyzes reduction of the thioester carbonyl group to the corresponding alcohol using 2 equiv of NADH.[44]

II. Synthesis of Coenzyme A

While CoA can be isolated from natural sources, the synthesis of CoA has long been pursued as an alternative source of CoA. Synthesis has been achieved enzymatically, using the enzymes of CoA biosynthesis and by nonenzymatic total synthesis.

A. Biosynthesis and Enzymatic Synthesis of Coenzyme A

Coenzyme A is synthesized in mammalian cells by the pathway shown in Figure 6. Brown et al. first established the CoA biosynthetic pathway in *Proteus morganni*[45] and later it was verified in rat liver[46] and subsequently in other mammals[46] and a variety of microorganisms.[47,48] Pantothenic acid **2** is first phosphorylated by pantothenate kinase with the consumption of ATP to yield 4′-phosphopantothenic acid **3**.[46,48] **3** is then coupled with L-cysteine by 4′-phosphopantethenoylcysteine synthase.[45] The intermediate 4′-phosphopantethenoyl cysteine **4** is decarboxylated by 4′-phosphopantethenoyl cysteine decarboxylase to yield 4′-phosphopantetheine **5**.[49] Dephospho-CoA **6** is formed by phosphopantetheine adenylyltransferase, which couples **5** to the α-phosphate of ATP with the concomitant formation of inorganic pyrophosphate.[50] The 3′-hydroxyl group of **6** is regioselectively phosphorylated by dephospho-CoA kinase to yield CoA **1**. The overall process requires 4 equiv of ATP, one of which provides the adenylate moiety of CoA.

Hoagland and Novelli showed that pigeon liver extracts convert pantothenoylcysteine (**4** without the phosphate) to CoA and argued that the first step of CoA biosynthesis was the coupling of pantothenic acid **2** with cysteine rather than the phosphorylation of **2**.[51] However, the enzyme pantethenoyl cysteine synthase, which is required for Novelli's route, is not present in rat liver. Further work proved that both pantethenoylcysteine and pantetheine **7**, in addition to pantothenic acid **2**, are substrates for pantothenate kinase. However, the true CoA biosynthetic pathway involves phosphorylation of pantothenic acid, as the phosphate group is required for subsequent steps. The genes for pantothenate kinase[52] and phosphopantetheine adenylyltransferase[53] have been identified, and the crystal structure of phosphopantetheine adenylyltransferase has been solved.[53] The biosynthesis of CoA and several other cofactors has recently been reviewed.[54]

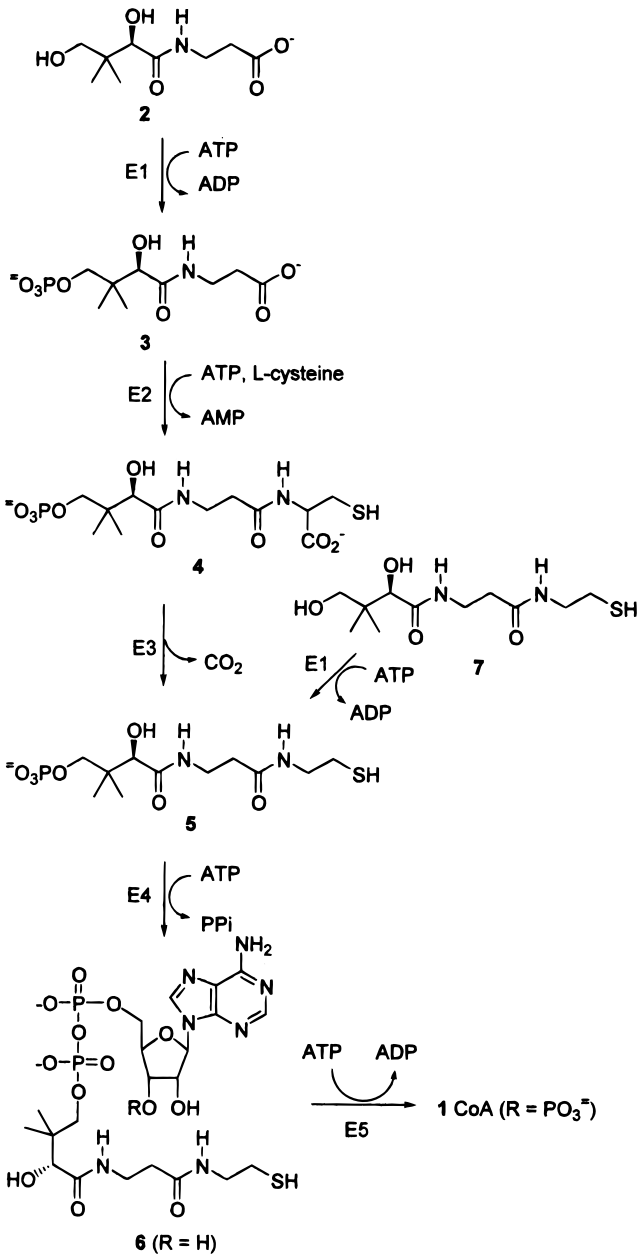

Figure 6. The biosynthetic pathway of coenzyme A. E1 = pantothenate kinase, E2 = phosphopantothenoylcysteine synthetase, E3 = phosphopantothenoylcysteine decarboxylase, E4 = phosphopantetheine adenylyltransferase, E5 = dephosphocoenzyme A kinase.

CoA is generally obtained by use of the machinery of CoA biosynthesis. In the simplest approach, CoA can be isolated from natural sources. Alternatively, the enzymes of CoA biosynthesis may be used for the in vitro enzymatic synthesis of CoA from suitable starting materials. In 1979 Shimizu et al. reported the microbial system of *Brevibacterium ammoniagenes* (more recently renamed *Corynebacterium ammoniagenes*) for CoA synthesis.[55,56] Microbial culture was grown and dry cells produced that were used as a crude catalyst for the synthesis of CoA from pantothenic acid, cysteine, and ATP. The yield and efficiency were fairly low, largely because of feedback inhibition of pantothenate kinase by CoA. This

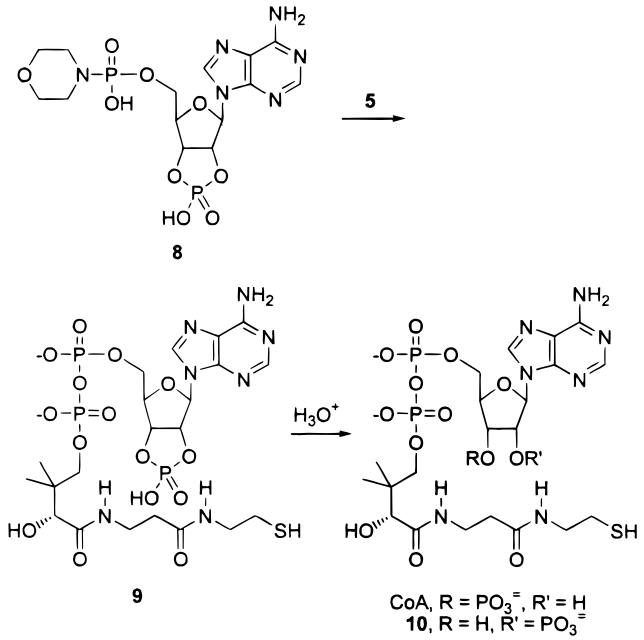

Figure 7. The original coenzyme A synthesis of Moffatt and Khorana.

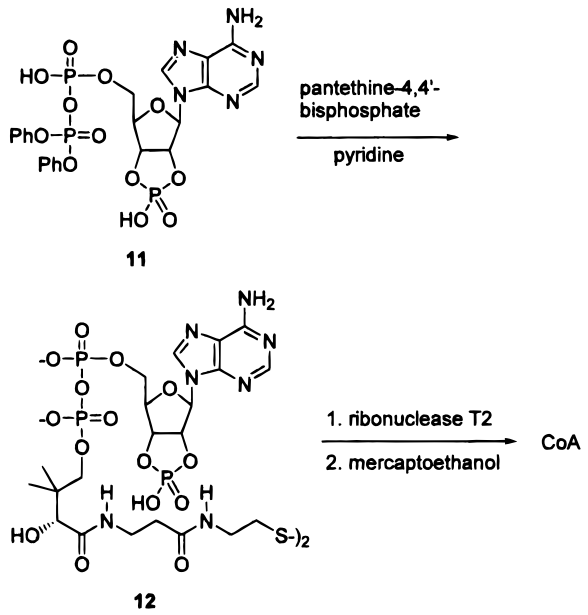

Figure 8. The coenzyme A synthesis of Michelson.

feedback inhibition was partially circumvented by utilizing a mutant strain of *C. ammoniagenes* selected for resistance to oxypantetheine (an analogue of pantetheine with oxygen in place of sulfur).[57] This mutant was used to synthesize CoA from pantothenic acid, cysteine, and ATP in the presence of sodium dodecyl sulfate. The mutant cells exhibited 3-fold enhanced pantothenate kinase activity relative to the parent strain. In subsequent work, the feedback inhibition of pantothenate kinase was avoided by utilizing nonenzymatically prepared 4′-phosphopantethenic acid **3** or 4′-phosphopantetheine **5** as a starting material.[58] By this approach, a 10-fold increase in final CoA concentration was achieved relative to the synthesis using pantothenic acid as starting material. Dephospho-CoA kinase has also been used to prepare ^{32}P-labeled CoA by phosphorylation of dephospho-CoA with γ-^{32}P-ATP.[59−61]

B. Nonenzymatic Synthesis of Coenzyme A

In 1959 Moffatt and Khorana reported the first total synthesis of CoA (Figure 7).[62] The establishment of the high-energy pyrophosphate bridge between the adenosine and pantetheine portions of the molecule and the regioselective phosphorylation of the 3′-hydroxyl group on the ribose sugar were the primary challenges of the synthesis. The pyrophosphate linkage was formed by coupling of an activated phosphomorpholidate derivative of 2′,3′-cyclic ADP **8** with phosphopantetheine **5** to form **9**. Compound **8** was prepared in three steps from adenosine, while **5** was also prepared from simple starting materials. Regioselective phosphorylation was not achieved as acid-catalyzed hydrolysis of the cyclic phosphate of **9** yielded approximately equal amounts of the natural 3′-phosphate (CoA, **1**) and unnatural 2′-phosphate (**10**). CoA was purified by two steps of ion exchange chromatography in about 15% yield.

A modified synthetic method was developed by Michelson as shown in Figure 8.[63] P^1-Adenosine (2′,3′-cyclic phosphate)-5′-P^2-diphenylpyrophosphate **11** was prepared by reaction of a mixture of 2′,5′-ADP and 3′,5′-ADP with diphenylphosphorochloridate and tributylamine. Reaction of **11** with pantetheine 4′,4′-bisphosphate (the disulfide of **5**) formed **12**, the disulfide form of the final intermediate **9** in the Moffatt and Khorana synthesis of Figure 7. The synthesis further differed from the original synthesis in that an enzymatic cleavage step was used to selectively hydrolyze the 2′,3′-cyclic phosphate to form CoA without contamination by the 2′-phosphate isomer. This selective cyclic phosphate hydrolysis was catalyzed by ribonuclease T2. Reduction of the disulfide with β-mercaptoethanol and ion exchange chromatography gave CoA in 63% yield from the starting 2′,5′-ADP and 3′,5′-ADP mixture used in the preparation of **11**. The enzymatic step suffers from the poor activity of the enzyme toward compound **12** and the cost of the enzyme. The ready availability of other ribonucleases would appear to make this method more efficient. However, despite the apparent improvements over the Moffatt and Khorana method, the original method has still been largely utilized in more recent syntheses of CoA and analogues. Gruber and Lynen reported a route to CoA based on reaction of a protected pantetheine derivative and 3′-AMP with pyrophosphoryl tetrachloride. Deprotection and hydrolysis gave CoA, though only in very poor yield.[64] Direct phosphorylation of a dephospho-CoA analogue using [^{32}P]H_3PO_4 has been reported, though the reported yield was only 10% and the crude product is sure to contain a mixture of 2′- and 3′-phosphate products.[65]

III. Coenzyme A Thioesters

A. Synthesis of Coenzyme A Thioesters

CoA thioesters are prepared relatively easily by modifying natural CoA and are reported extensively in the literature. The terminal thiol group is very reactive and can be selectively acylated with an

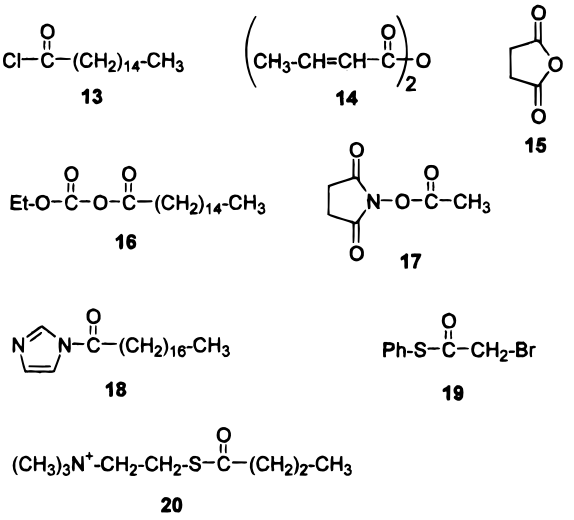

Figure 9. Examples of reagents used for acylation of coenzyme A.

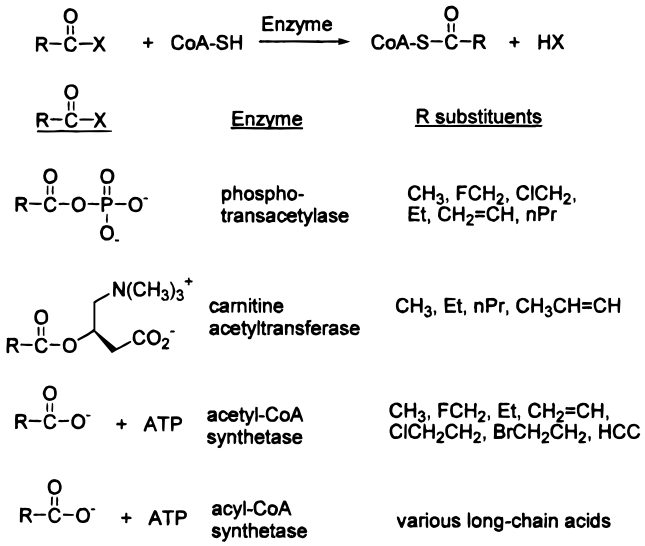

Figure 10. Examples of enzymatic syntheses of coenzyme A thioesters.

appropriate acylating reagent. Because of enormous literature on preparation of thioesters of CoA, only selected examples are reported here. Other examples of specific CoA esters can be found in the references in the following sections. Acylation of CoA has been achieved by both enzymatic and nonenzymatic methods. Nonenzymatic routes involve coupling of an activated acid derivative with CoA in aqueous solution or in an aqueous/organic mixture. Figure 9 gives some representative reagents that have been used for the S-acylation of CoA. Acid chlorides have been used for the synthesis of a wide range of CoA esters, such as palmitoyl-CoA derived from **13**.[66] While such acylation reactions using acid chlorides have been successful, less reactive derivatives are likely to give fewer side products and to result in a greater ratio of desired S-acylation product to hydrolysis product. Symmetric anhydrides have been used for the synthesis of a range of CoA esters, including unsaturated compounds such as crotonyl-CoA derived from **14**.[67] Similarly succinic anhydride **15** has been used to prepare succinyl-CoA.[68] Anhydrides have also been used to acylate CoA in a biphasic system with (dimethylamino)pyridine used to transfer activated acyl groups to the aqueous layer.[69] Mixed anhydrides such as **16** formed from reaction of the acid with ethyl chloroformate have been used.[70] The CoA esters of *E*-2-hexenoic acid and *E*,*E*-2,4-hexadienoic acid were prepared similary by activation with isobutyl chloroformate.[71] Also used have been acyloxysuccinimides **17** and acyl imidazoles **18**, the latter formed by reaction of the acid with carbonyl diimidazole.[72,73] Acyl imidazoles have also been used for the preparation of radiolabeled acetyl- and propionyl-CoA.[74] A recent paper has demonstrated the practical utility of the acylimidazole method in the synthesis of CoA thioesters of 3-oxohexanoic acid, 3(*R*)-hydroxy-4(*R*)-methyl-6(*E*)-octanoic acid, 2(*R*)-methyl-4(*E*)-hexenoic acid, and 4(*E*)-hexenoic acid, which were used for analysis of the reaction sequence in a polyketide pathway.[71] Thiophenyl esters have been widely used, including the bromoacetyl derivative **19**, which requires selective acylation over alkylation.[75] The thiophenyl esters used for the synthesis of CoA esters of 3-keto acids have been prepared from acylated Meldrum's acids while pantetheine esters of 3-keto acids have been prepared directly from acylated Meldrum's acids.[76] A special example is the *S*-acyl thiocholine **20**.[77] This derivative is not practical for preparative synthesis of CoA esters but has been used for in situ recycling of acyl-CoA in a preparative enzyme-catalyzed reaction using a CoA ester-utilizing enzyme. An organic-soluble polysilylated CoA derivative has been used for the synthesis of long chain acyl-CoA esters from acid chlorides, anhydrides, acyl imidazoles, and hydroxysuccinimide esters in organic solvents.[78]

Several enzymatic routes to CoA esters have been explored as summarized in Figure 10. The enzymes employed have exhibited the ability to accept a limited range of substrates in addition to their natural substrates. The enzymes phosphotransacetylase and carnitine acetyltransferase have been used to acylate CoA using acyl phosphates and acylcarnitines, respectively, as acyl donors.[79] The equilibrium of the phosphotransacetylase reactions strongly favor acylation of CoA, while the equilibrium constant for the carnitine acetyltransferase reaction is near unity. Phosphotransacetylase has been shown to transfer several acyl groups in addition to the natural acetyl group. Activites relative to the natural substrate are >90% for propionyl and fluoroacetyl transfer, though only 2% for butyryl transfer, with activities of 17% and 7% for chloroacetyl and acryloyl transfer. Relative activities for carnitine acetyltransferase are 78% and 50% for propionyl and butyryl transfer, respectively, but only 5% for crotonyl transfer. Neither phosphotransacetylase nor carnitine acetyltransferase are practical for preparative synthesis of CoA esters, as the acyl phosphate and acylcarnitine substrates are no more easily prepared than the CoA esters themselves. However, both of these enzymes are potentially useful for in situ regeneration systems for CoA esters in preparative reactions of CoA ester-utilizing enzymes utilizing a catalytic amount of CoA.

The enzymes acetyl-CoA synthetase and acyl-CoA synthetase both catalyze the formation of a CoA thioester from an acid and CoA, coupled with the hydrolysis of ATP to AMP and pyrophosphate. Acetyl-CoA synthetase has been shown to accept acrylic acid and propionic acid with activity 60% or greater relative to acetic acid, though activities with other substrates tested were 10% or less relative to acetate.[80] Acyl-CoA synthetase accepts long chain acids as substrates and may have more general utility.[81,82] The acyl-CoA synthetase from *Pseudomonas* is commercially available, though it is more expensive than the other enzymes which have been used to prepare CoA esters. This enzyme has been used for the preparation of CoA esters of a wide range of carboxylic acids, including radiolabeled acids.[83]

While nonenzymatic methods generally provide the most practical and versatile routes for prepararation of CoA esters, enzymatic methods offer advantages in some cases. For example, acetyl-CoA synthetase and acyl-CoA synthetase permit direct coupling of an acid to CoA in aqueous solution without isolation and activation of the acid. Enzymatic methods may also provide a more pure product than nonenzymatic methods and thus may avoid purification steps. This minimization of chemical manipulations and purification steps may be especially useful in the synthesis of CoA esters of radiolabeled acids.

Certain CoA esters may be best prepared by enzymatic modification of a more readily prepared or available CoA ester. This may be especially valuable for example in the synthesis of CoA esters of α,β-unsaturated acids, the synthesis of which is complicated by conjugate addition reactions by the thiol group of CoA or other nucleophiles. Such α,β-unsaturated CoA esters have been prepared by enzymatic oxidation of the saturated CoA esters.[84] Furthermore, isomerases have been used to interconvert unsaturated acyl-CoA isomers.[84] As discussed by Thorpe, acetoacetyl-CoA may be prepared by reaction of CoA with diketene, but the synthesis of other 3-ketoacyl-CoA esters is often problematic.[85] A novel method for the preparation of these compounds was demonstrated that involves the enzymatic hydration of 2-alkynoyl-CoA esters by crotonase.[85]

B. Unsaturated Acyl-Coenzyme A Thioesters

A number of CoA thioesters of unsaturated acids have been prepared and used as inactivators and mechanistic probes of CoA ester utilizing enzymes. An example is 3-keto-4-pentenoyl CoA **21**, which was shown to inactivate carnitine acetyltransferase.[86] This analogue exhibited slight inhibition of the enzyme in the absence of carnitine **22** and increased inactivation in the presence of carnitine. Apparently **21** acts as an affinity label via conjugate addition of a nucleophilic active site residue to the 4,5 double bond in the absence of carnitine. In presence of carnitine, transfer of the 3-keto-4-pentenoyl functionality from CoA to carnitine occurs and the resulting 3-keto-4-pentenoyl carnitine **23** is the true inactivating species, reacting via conjugate addition of an active site nucleophile to the 4,5 double bond to form **24** as shown in Figure 11.

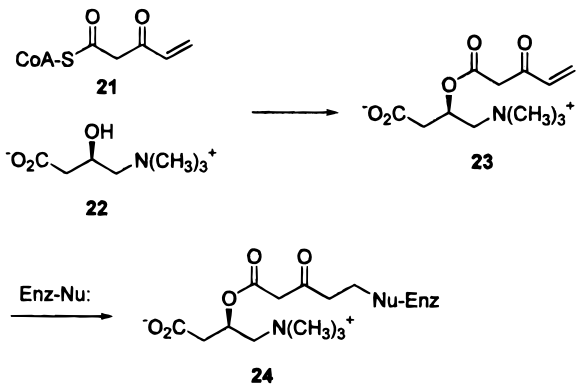

Figure 11. Inactivation of carnitine acetyltransferase by 3-keto-4-pentenoyl-CoA.

A number of 3-alkynyl (**25**) and 2,3-dienoyl **26** CoA thioesters have been studied as inhibitors of thiolase and of the acyl-CoA dehydrogenases. Holland et al. studied 3-pentynoyl CoA **25b** as a mechanism-based inhibitor of thiolase.[87,88] The proposed mechanism of inactivation involves isomerization of **25** to the 2,3-dienoyl thioester **26**, which undergoes conjugate addition by an active site cysteine to form an alkylated, inactivated enzyme **27** (Figure 12). **26b** was also prepared and found to irreversibly inactivate the enzyme at the same rate as **25b**. The rate of inactivation by **25b** was faster than the rate of buffer-catalyzed isomerization of **25** to **26**, supporting the proposed enzyme-catalyzed isomerization of **25** to **26**. Simplified pantetheine esters analogous to **25** and **26** and a corresponding acrylate ester were later used to identify the active site base in the biosynthetic thiolase from *Zoogloea ramigera*.[89,90]

The related analogues 3-butynoyl CoA **25a**, 3-octynoyl CoA **25c**, and 2,3-octadienoyl CoA **26c** (Figure 12) were studied as inhibitors of the acyl CoA dehydrogenase from pig liver.[91] Time dependent inhibition of the enzyme was observed with each analogue, with 1 equiv of inhibitor becoming covalently attached to the protein. As with thiolase, the 2,3-dienoyl analogue **26** apparently undergoes conjugate addition of an active site nucleophile at C-2, and **26** is also believed to be the true inactivating species in the inactivation by **25**. Isomerization of **25** to **26** by initial deprotonation of **25** at C-2 followed by reprotonation at C-4 is consistent with α-deprotonation of substrate as the first step in the natural reaction of the acyl-CoA dehydrogenases. The unsaturated acyl-CoA derivatives **25** and **26** and related compounds have been used in similar studies of other acyl-CoA dehydrogenases.[92]

2-Alkynoyl derivatives of CoA (**28**) have also been studied as inhibitors of the pig kidney medium-chain acyl CoA dehydrogenase.[93,94] It was expected that enzyme inactivation could occur by conjugate addition of an active site nucleophile to C-3 of the alkynoyl-CoA. However, these compounds were found to be mechanism-based inactivators, with the inactivating species again being the 2,3-allenoyl-CoA **26**. Isomerization of the alkyne to the allene is catalyzed by enzymatic deprotonation at C-4 and subsequent reprotonation at C-2. Edman degradation of a peptide isolated from a tryptic digestion of the pig kidney

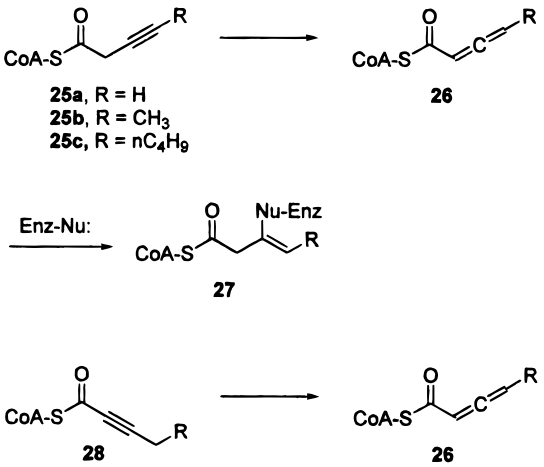

Figure 12. Enzyme inactivation by 2- and 3-alkynoyl and 2,3-allenoyl-CoA.

acyl-CoA dehydrogenase inactivated by radiolabeled inhibitor identified Glu-401 as the residue covalently modified during inactivation. Subsequent structural and mutagenesis studies have confirmed this residue as the base responsible for α-proton abstraction in the natural reaction. The 2-alkynoyl-CoA derivatives have also been shown to inactivate both the short chain and long chain acyl-CoA dehydrogenases and the rat liver enoyl-CoA reductase.[95−97]

2-Butynoyl-CoA has been shown to inactivate HMG-CoA lyase, forming a covalent adduct with an active site cysteine.[98] It appears that the inactivation does not involve isomerization to the allene but occurs by direct conjugate addition of the cysteine thiol on the alkyne. Conjugate addition is also the basis for inhibition of HMG-CoA synthase, fatty acid synthase, HMG-CoA lyase, and thiolase by acryloyl-CoA and simpler acryloyl thioesters.[90,99]

Another class of unsaturated CoA esters is represented by 3-methyl-2-butenoyl-CoA **29a** and 3-methylenebutyryl-CoA **30a**. These compounds and their longer chain analogues are also inactivators of the acyl-CoA dehydrogenases.[100,101] Inactivation by **29** is proposed to proceed by deprotonation of the α-carbon to form a nucleophilic enolate **31**, which reacts with the electrophilic flavin cofactor to form a covalent adduct (Figure 13). Inactivation by **30** is proposed to

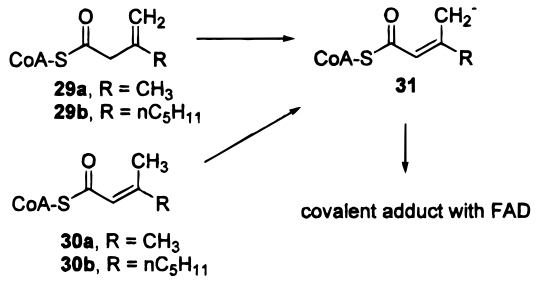

Figure 13. Inactivation of acyl-CoA dehydrogenases by 3-methyl-2-enoyl-CoA and 3-methylene acyl-CoA.

proceed by deprotonation of the γ-carbon to form the same enolate **31** formed upon α-deprotonation of **29** followed by nucleophilic attack of the enolate on the flavin. Both **29** and **30** inactivate the short-chain acyl-CoA dehydrogenase. Compound **29** also inactivates the related enzyme isovaleryl-CoA dehydrogenase, though **30** does not inactivate this enzyme. These results indicate that isovaleryl-CoA dehydrogenase cannot remove a proton from the γ-carbon. Similar results were observed in acyl-CoA dehydrogenase inactivation by 2-alkynoyl-CoA esters, for which γ-deprotonation is required to form the inactivating 2-allenoyl ester. The 2-alkenoyl esters **30** inactivate the short- and medium-chain acyl-CoA dehydrogenases and a related glutaryl-CoA dehydrogenase but do not inactivate isovaleryl-CoA dehydrogenase or the long-chain acyl-CoA dehydrogenase. These results have been attributed to the position of the active site base, which serves to remove the α-proton in the natural reactions of the different dehydrogenases. In the short- and medium-chain dehydrogenases and in the glutyryl-CoA dehydrogenase, the glutamate is positioned such that it can remove either an α- or γ-proton from substrate or a substrate analogue. In contrast, the long-chain and isovaleryl-CoA dehydrogenases have the base positioned such that it can remove only the α-proton and not a γ-proton. Analogues which require enzyme-catalyzed removal of a γ-proton thus do not inactivate the latter two enzymes. The results and interpretation of the inhibition studies are supported by molecular modeling and mutagenesis results. A related 3-methylene analogue of myristoyl-CoA was prepared, but it exhibited no inactivation of the protein *N*-myristoyltransferase.[102]

A series of *p*-substituted phenylacetyl-CoAs (substituent = NO_2, CN, or $CH_3C(=O)$) were used to probe transition state stabilization and pK_as of functional groups in the acyl-CoA dehydrogenases.[103] Upon binding to the enzyme, the analogues all underwent partial deprotonation of the α-carbon. It was shown that the pK_a of the α-proton of these substrate analogues were lowered by 8 to ≥11 pH units, close to the pK_a of the base responsible for α-deprotonation of the substrate. β-Aryl-substituted propionyl-CoA thioesters have been utilized as substrates for the acyl-CoA dehydrogenases, including β-indolylpropionyl-CoA **32a**, β-2-furylpropionyl-CoA **32b**, and 4-(dimethylamino)phenylpropionyl-CoA **32c** (Figure 14).[104−107] These act as chromogenic sub-

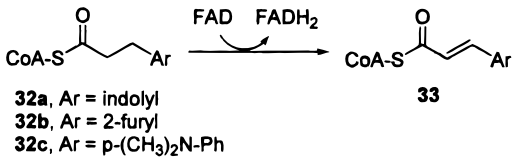

Figure 14. Acyl-CoA dehydrogenase-catalyzed oxidation of 3-arylpropionyl-CoA.

strates due to the long wavelength absorbance of the highly conjugated product. The acyl-CoA dehydrogenases have been shown to have significant oxidase activity using this type of substrate.[104,105] The β-arylacryloyl-CoA products **33** of these reactions have been used as spectroscopic probes to study substrate polarization by the acyl-CoA dehydrogenases, which occurs in a slower step after rapid initial binding of the product analogue to the enzyme.[108]

Acryloyl-CoA derivatives having extended conjugation have also been used as spectroscopic probes of

other enzymes. The complex of 2,4-hexadienoyl-CoA **34** (Figure 15) bound to crotonase was studied by

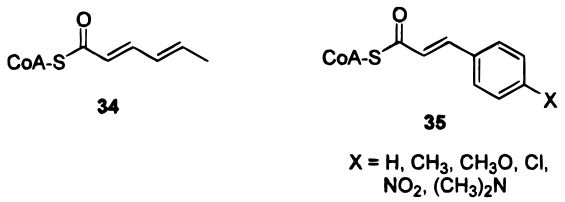

Figure 15. Reactions catalyzed by cyclohex-1-enecarbonyl-CoA reductase.

Raman difference spectroscopy.[109] This highly conjugated analogue of the natural 2-alkenoyl substrate is not hydrated by the enzyme which facilitates spectral analysis of the enzyme−substrate complex. Comparison with spectra of an ethyl thioester model compound indicated that the enzyme binds a single rotamer of the substrate analogue. Hexadienoyl-CoA was also prepared with ^{18}O in the C=O group and with ^{13}C at the C2 position. Comparison of Raman data for the free and bound ligands indicated strong π-electron polarization in the carbonyl and C2−C3 bond in the enzyme complex but little polarization of the C4−C5 double bond. This substrate polarization is one of the means by which the enzyme facilitates catalysis. In related work, a series of *p*-substituted cinnamoyl-CoA esters **35** was prepared.[110] Like hexadienoyl-CoA, these compounds are not hydrated by the enoyl-CoA hydratase and have been useful in spectroscopic studies of the enzyme substrate analogue complex. Other unsaturated acyl-CoA thioesters which have been used as spectroscopic probes of substrate polarization include benzoyl-CoA and 3-thiaacryloyl-CoA thioesters (also see section III.D.).[111,112]

The 3-fluoro analogues of cyclohex-1-enecarbonyl-CoA were studied as substrates for the NADPH-dependent cyclohex-1-enecarbonyl CoA reductase from *Streptomyces collinus*.[113] The fluorine substitution was found to have a significant effect on the reaction, as the fluorinated analogues were processed with a 5-fold increase in V_{max} relative to the natural substrate. The enzyme also showed a small kinetic preference for axial over equatorial fluorine at C-3 of the product.

C. Haloacyl-Coenzyme A Thioesters

Among the unnatural CoA thioesters that have been used in probing mechanisms of CoA ester utilizing enzymes are those in which the acyl group contains a halide substituent. An interesting and simple example is bromoacetyl-CoA **36**, which was first studied as an inhibitor of carnitine acetyltransferase.[75,114] While **36** was initially used for affinity labeling in the absence of the cosubstrate carnitine **37**, incubation of enzyme with both **36** and carnitine resulted in increased inhibition. Furthermore, inactivation by (bromoacetyl)carnitine **38** in the absence of CoA was slow, but was extremely rapid in the presence of CoA. Substitution of desulfoCoA for CoA resulted in a sharp decrease in the rate of inactivation by **38**. Further investigation showed that while bromoacetyl-CoA and (bromoacetyl)carnitine both acted as slow-reacting affinity labels for the enzyme, the reaction of (bromoacetyl)carnitine with CoA forms the bisubstrate adduct **39** that binds tightly to the enzyme via noncovalent interactions (Figure 16).

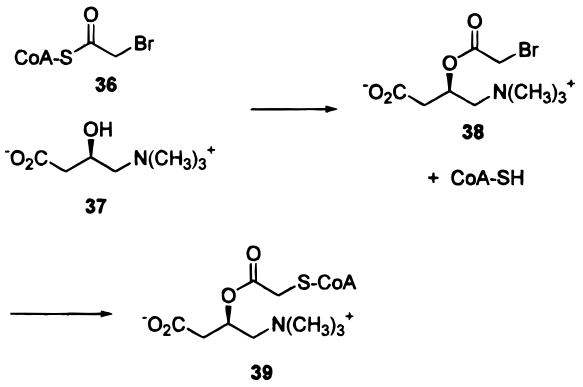

Figure 16. Inactivation of carnitine acetyltransferase by bromoacetyl-CoA.

Hence bromoacetyl-CoA **36** acts as a mechanism-based inhibitor rather than as a simple affinity-labeling reagent in this mode of inhibition. **36** was also shown to inactivate the thiolase from pig heart.[87,88] The simplified analogue (bromoacetyl)-pantetheine pivalate was also used as an affinity label of the thiolase from *Zoogloea ramigera* and shown to react with the thiol group of Cys-89 in competing acylation and alkylation reactions.[115] Other experiments, including mutagenesis, have identified Cys-89 as the residue involved in acyl-enzyme formation in the natural reaction of thiolase.

Chloroacetyl-CoA has also been employed in mechanistic and inactivation studies. This compound was shown to irreversibly inactivate the chicken liver fatty acid synthase.[116] It was also shown to alkylate the pantetheinyl thiol group of the bovine mammary fatty acid synthase, but without effecting the transacylase activity.[117]

Fluoroacetyl-CoA **40** has been studied and, being less reactive than the chloro and bromo compounds, tends to be a substrate rather than an inhibitor (Figure 17). It has been shown to be a substrate for

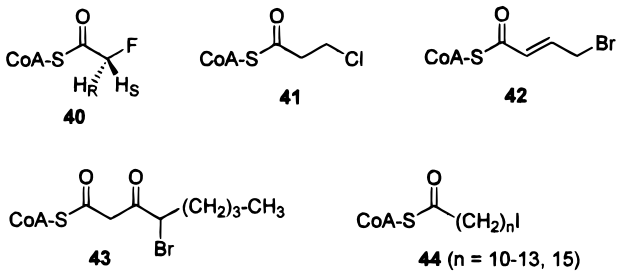

Figure 17. Examples of haloacyl-CoA thioesters.

the acetyl-CoA utilizing Claisen enzymes citrate synthase, malate synthase, and acetyl-CoA carboxylase.[118,119] Utilization of **40** by citrate synthase forms fluorocitrate, which is a potent inhibitor of aconitase. This conversion to fluorocitrate and resulting inhibition of aconitase is responsible for the high toxicity of fluoroacetate.[118] Citrate synthase is highly selective for removal of the pro-*S* proton of **40** while malate

synthase removes both the pro-*S* and pro-*R* protons with almost equal efficiency.[120−122] On the basis of computational studies, it has recently been proposed that the high stereoselectivity exhibited by citrate synthase is due to formation of the more stable *E*-enol.[123,124] The low stereoselectivity of malate synthase was attributed to formation of both the *E*- and *Z*-enolates, which computational studies predict to have a much smaller difference in energy relative to the enols.

The 2-halo derivatives of a number of other CoA thioesters have been reported. 2-Bromo-, 2-chloro-, and 2-fluorooctanoyl-CoA were shown to be substrates for the pig kidney medium-chain acyl-CoA dehydrogenase with activitites 6.6%, 33%, and 3.5% of that with octanoyl-CoA.[125] It was further shown that the activity was attributed primarily to the (*S*)-isomers. It was more recently shown that the shorter chain analogue 2-bromohexanoyl-CoA and the branched-chain analogue 2-bromo-4-methylpentanoyl-CoA act as both substrates and active site-directed inhibitors of the medium-chain acyl-CoA dehydrogenase.[126]

2-Bromopalmitoyl-CoA has been used in numerous studies of carnitine palmitoyltransferase.[127−130] Inactivation was observed in the presence of carnitine, forming a tightly but noncovalently bound complex. This apparently results from formation of a bisubstrate adduct, as in the inactivation of carnitine acetyltransferase by bromoacetyl-CoA. 2-Bromo- and 2-fluoromyristoyl-CoA have been prepared enzymatically using acyl-CoA synthetase.[131] The 2-fluoro compound was a substrate for the protein *N*-myristoyltransferase, while the bromo compound was a competitive inhibitor.[131]

3-Chloropropionyl-CoA **41** has been shown to inactivate HMG-CoA synthase and fatty acid synthase.[99] Inactivation of HMG-CoA synthase apparently proceeds via enzyme-catalyzed elimination to form acryloyl-CoA, which is the true inactivating species. In contrast, inactivation of fatty acid synthase appears to occur by direct alkylation of an active-site cysteine. 4-Bromocrotonyl-CoA **42** has also been shown to inactivate thiolase, though the mechanism of inactivation appears to not have been studied in detail.[87,88] 4-Bromo-2-octenoic acid has been shown to be metabolized in rat liver mitochondria to 3-keto-4-bromooctanoyl-CoA **43**, which irreversibly inactivates 3-ketoacyl-CoA thiolase.[132]

Several CoA esters of long chain fatty acids having halides at or near the terminal methyl group have also been prepared. These include several fatty acyl-CoAs which have an ^{125}I-label at the terminal carbon **44**. These compounds have been used to identify fatty acylated proteins and offer much greater sensitivity than methods using ^{14}C- or ^{3}H-labeled acids.[133,134] They have also been used as substrates for assay of acyltransferase activities, as illustrated in the detection and partial purification of a putative palmitoyltransferase enzyme from bovine brain.[135] Several terminal mono- and trihalogenated CoA esters and terminal halophenyl esters have been prepared using acyl-CoA synthetase and studied as substrates for the protein *N*-myristoyltransferase.[136]

D. Other Heteroatom-Substituted Acyl Coenzyme A Thioesters

CoA esters of acids having heteroatomic groups other than halides have also been reported, most commonly having a nitrogen, oxygen, or sulfur atom replacing one of the methylene groups of the acyl chain. Carbamate and carbonate analogues in which the α-carbon is replaced with nitrogen or oxygen are shown in Figure 18. The 2-aza acetyl- (**45a**) and

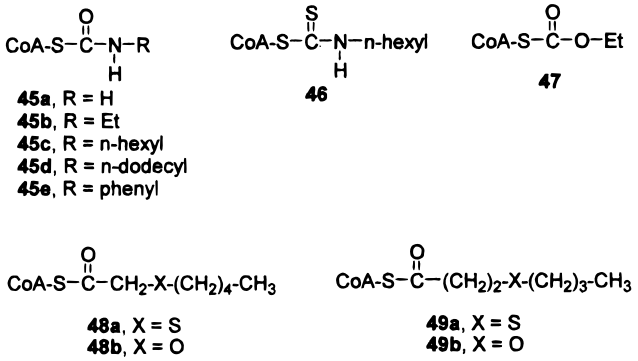

Figure 18. Examples of heteroatom-substituted acyl-CoA thioesters.

octanoyl-CoA (**45c**) analogues and the 2-aza-dithiaoctanoyl- (**46**) and 2-oxabutyryl-CoA (**47**) analogues were used as redox-inactive substrate analogues to investigate the structural requirements for the protection of the reduced flavin of the medium-chain acyl-CoA dehydrogenase from reaction with oxygen.[137] All exhibited a significant slowing of the reoxidation of reduced enzyme, the effect being largest with **45c** and smallest with the dithiocarbamate **46**. Binding of **45c** to the carboxymethylated form of medium-chain acyl-CoA dehydrogenase was also studied.[138] Binding of **45c**, like that of *trans*-2-octenoyl-CoA, is greatly weakened upon carboxymethylation of the enzyme. Binding of **45c** and **45d** to the medium-chain acyl-CoA dehydrogenase causes a positive shift in the redox potential which is 65% of the shift caused by binding of the natural substrate/product couple.[139] Binding of **45b** or **45c** to the short-chain acyl-CoA dehydrogenase caused a negative change in the reduction potential and resulted in preferential stabilization of the semiquinone radical anion.[140] Both **45c** and **45e** were used to study the role of the carbonyl group in chain length recognition by the medium-chain acyl-CoA dehydrogenase.[141]

3-Thiaoctanoyl-CoA **48a** (the analogue of octanoyl-CoA in which C-3 is replaced with sulfur) and the corresponding 3-oxa analogue **48b** as well as the 4-thia **49a** and 4-oxa **49b** analogues were prepared and their interaction with the medium-chain acyl-CoA dehydrogenase was studied.[142] The 3-thia compound **48a** formed a long-wavelength (804 nm) absorption band attributed to a charge-transfer complex between the enolate, formed upon α-deprotonation and the active site FAD. This demonstrates that α-deprotonation can be uncoupled from *β*-hydride transfer, even though the reaction is believed to be concerted with normal substrates. The 3-oxa analogue **48b** formed a similar band at 780 nm, though it was weaker and less stable than the complex with

the 3-thia analogue. The 4-thia **49a** and 4-oxa **49b** analogues reacted as normal substrates for the dehydrogenation reaction of medium-chain acyl-CoA dehydrogenase, with the 4-thia analogue being a 1.5-fold better substrate than octanoyl-CoA, while the 4-oxa analogue reacted at a rate 10% that with the natural substrate. Binding of the 3-thia compound to the carboxymethylated medium-chain acyl-CoA dehydrogenase has been studied,[138] and both the 3-thia and 3-oxa compounds have been shown to protect the reduced flavin form of medium-chain acyl-CoA dehydrogenase from reaction with oxygen.[137] The 3-thia analogue has also been used to show the decreased carbanion-stabilization by the medium-chain acyl-CoA dehydrogenase in which the FAD cofactor is replaced with the 2′-deoxy analogue.[143] The products **50a,b** of the acyl-CoA dehydrogenase reactions of **49** are substrates for enoyl-CoA hydratase, forming the hemithioacetal **51a** and hemiacetal **51b** adducts which fragment to malonylsemialdehyde-CoA **52** and the thiol **53a** or alcohol **53b** (Figure 19).[112]

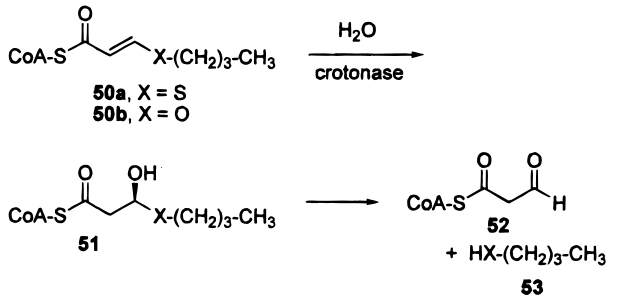

Figure 19. Crotonase-catalyzed hydration and subsequent elimination of 4-thia- and 4-oxaacryloyl-CoA.

The CoA ester **54** of the cytotoxic agent 5,6-dichloro-4-thia-5-hexenoic acid undergoes competing dehydrogenation to form **55** and β-elimination to form acryloyl-CoA **56** and the enethiol **57** catalyzed by the medium-chain acyl-CoA dehydrogenase (Figure 20)

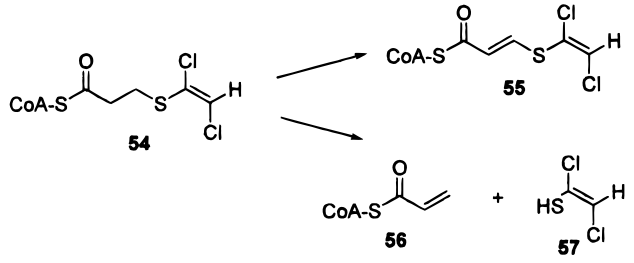

Figure 20. Acyl-CoA dehydrogenase-catalyzed reactions of 5,6-dichloro-4-thia-5-hexenoyl-CoA.

and over time the enzyme is inactivated.[144] The inactivation is attributed to the reaction of a reactive electrophilic hydrolysis product of **57** with a nucleophilic group on the enzyme. Generation of this reactive species may be the basis for the cytotoxicity of 5,6-dichloro-4-thia-5-hexenoic acid. Several additional *S*-vinyl, *S*-alkyl, and *S*-aryl 4-thiaacyl-CoA esters were prepared.[145] Elimination was shown to compete with dehydrogenation with compounds having sufficiently good thiolate leaving groups, while compounds with relatively poor leaving groups gave only products of dehydrogenation. A number of other mechanistic studies have also utilized 3-thia[139,141,146] and 4-thia[141] acyl-CoA analogues.

A series of myristoyl-CoA analogues similar to **48** and **49** were prepared in which the C-6, C-11, C-12, or C-13 methylene groups were replaced with an ether oxygen or sulfide sulfur atom.[147] These myristoyl-CoA analogues were generally good substrates for the yeast protein *N*-myristoyltransferase. These results suggest that the myristoyltransferase selects its acyl-CoA substrate primarily on the basis of chain length and not on hydrophobicity. More recently, additional myristoyl-CoA analogues including analogues with oxygen or sulfur in the C-9 and/or C-12 positions were prepared and again these were generally good substrates for the myristoyltransferase.[148,149]

In addition to natural CoA esters containing hydroxyl groups, several hydroxyacyl-CoA's have been utilized as unnatural substrates. Of special note are the detailed studies of a number of hydroxy analogues of myristoyl-CoA which have been tested as substrates for myristoyltransferase.[136] Some terminal nitro-substituted acyl-CoAs have also been prepared and studied as myristoyltransferase substrates.[136] 3-Ketoacyl-CoAs, while natural intermediates of fatty acid oxidation and biosynthesis, also bind in anionic form and form charge-transfer complexes with the acyl-CoA dehydrogenases and have been viewed as transition state analogues in this context.[143]

Acyl-CoA analogues having epoxide groups in the acyl chain have also been studied. Much work has ensued from the discovery of 2-oxiranecarboxylic acids as hypoglycemic agents.[128,129,150−152] This activity results from the in vivo conversion of these acids to the CoA thioesters which inactivate carnitine palmitoyltransferase I, an enzyme which controls access of long-chain fatty acids to sites of β-oxidation.[153,154] Among the most well-studied of these compounds are etomoxiryl-CoA **58a**, clomoxiryl-CoA **58b**, and palmoxiryl-CoA (tetradecylglycidyl-CoA) **58c** (Figure 21). The (*R*)-enantiomer of **58a** is responsible for the inactivation of carnitine palmitoyltransferase by formation of a covalent adduct with the enzyme, presumably by epoxide opening by attack of a nucleophilic group of the enzyme.[153] The biological and clinical effects of these epoxy acids have been extensively studied.[155]

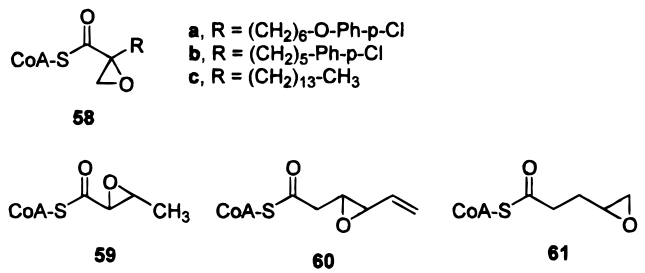

Figure 21. Examples of epoxide-substituted acyl-CoAs.

Some more recently reported epoxyacyl-CoAs are the α,β-epoxide **59**, the β,γ-epoxide **60**, and the γ,δ-epoxide **61**.[156] **59** was found to be a time-dependent active-site directed inactivator of the short chain acyl-CoA dehydrogenase from *Megasphaera elsdenii*. In-

activation by **59** having ^3H at C-3 resulted in incorporation of tritium into the protein. The covalent inactivation has been attributed to nucleophilic epoxide opening by the carboxylate of Glu-367, which is the apparent base responsible for α-deprotonation of the substrate. **59** did not inactivate the medium-chain acyl-CoA dehydrogenase from pig kidney but was only a competitive inhibitor. Compounds **60** and **61** exhibited no time-dependent inactivation of either acyl-CoA dehydrogenase but were competitive inhibitors of both.

E. Cyclopropylacyl and Related Coenzyme A Thioesters

The CoA thioester of methylenecyclopropyl acetate **63** is derived from metabolism of the unusual amino acid hypoglycin **62**.[157] Hypoglycin is a toxic component of the unripe ackee fruit responsible for Jamaican Vomiting Sickness. **63** is a mechanism-based inhibitor of pig kidney medium-chain acyl CoA dehydrogenase and has also been shown to inactivate the short-chain acyl-CoA and isovaleryl-CoA dehydrogenases.[158] The mechanism of inactivation of the medium-chain dehydrogenase by **63** has been studied by several groups.[159−165] Spectral changes observed upon incubation of the dehydrogenase with **63** and analysis of the modified chromophores released upon protein denaturation indicate that the inhibitor is covalently attached to the flavin rather than to an amino acid functionality of the enzyme. Wenz and co-workers proposed a mechanism of inactivation involving initial α-proton abstraction, followed by cyclopropyl ring opening of the resulting enolate **64** and reaction of the ring-opened carbanion with the active site flavin to form a covalent adduct.[159] Baldwin and co-workers showed that the CoA esters of both enantiomers of **63** inactivated the medium-chain acyl-CoA dehydrogenases from pig kidney, though inactivation was faster by the natural *R*-isomer.[160,161] The same group showed that the inactivation exhibited only small kinetic isotope effects, indicating that inactivation does not involve initial oxidation of **63** to the α,*β*-unsaturated derivative.[162] Liu and co-workers have suggested a mechanism involving oxidation of the enolate **64** via single electron transfer to form the radical **65** and a flavin semiquinone radical, with **65** rapidly undergoing ring opening to form **66** (Figure 22).[164,165] Reaction of **66** with the flavin semiquinone radical forms the covalent adduct **67**. The nonstereospecific bond rupture at the *β*-carbon of **63** appears most consistent with spontaneous ring-opening of a cyclopropylcarbinyl radical rather than an enzyme-catalyzed step.

The mechanism of acyl-CoA dehydrogenase inactivation by a related compound, (spiropentyl)acetyl-CoA **68** was also explored. **68** was shown to be a mechanism-based inhibitor of pig kidney medium-chain and short-chain acyl-CoA dehydrogenases.[166] The inactivation was time-dependent, active-site directed, and irreversible. Both (*R*)- and (*S*)-**68** were effective inactivators of the medium-chain dehydrogenase. The *R*-isomer was also an irreversible inhibitor of the short chain enzyme, though the *S*-isomer

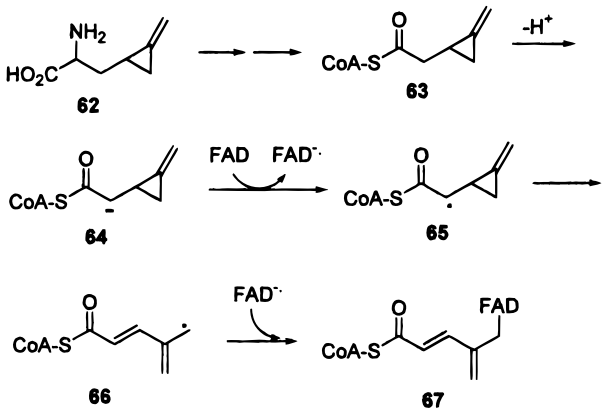

Figure 22. Inactivation of acyl-CoA dehydrogenase by methylenecyclopropyl acetyl-CoA.

was only a competitive inhibitor. NMR analysis of the inhibitor−FAD adducts formed using ^{13}C-labeled inactivator indicated that one of the three-membered rings of the spiropentyl moiety was cleaved. It was argued that the nonsterospecificity in the C−C bond scission again indicates radical-initiated ring opening. Since radical-induced ring fragmentation is very facile, it could easily bypass any enzyme-induced chiral discrimination. A proposed mechanism is shown in Figure 23. As in Figure 22, the mechanism

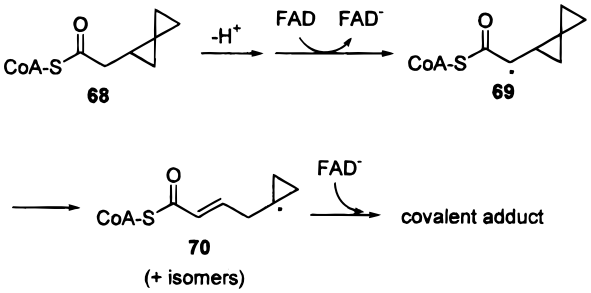

Figure 23. Inactivation of acyl-CoA dehydrogenase by (spiropentyl)acetyl-CoA.

involves deprotonation followed by oxidation of the resulting enolate to form the radical **69**. Multiple ring-opening pathways for **69** are possible to form **70** or an isomer thereof, which can form a covalent adduct with the flavin.

An isomer of **63**, *β*-(cyclopropylidene)propionyl-CoA **71** (Figure 24), was prepared but was found to not

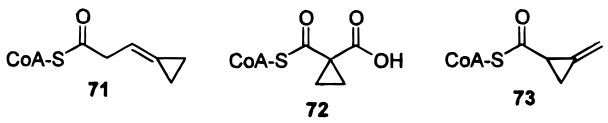

Figure 24. Other cyclopropane-containing acyl-CoAs.

inactivate the acyl-CoA dehydrogenase from pig kidney.[167] Both **63** and cyclopropylcarbonyl-CoA carboxylate **72** were found to be fairly weak reversible inhibitors of human methylmalonyl-CoA mutase.[168] Methylenecyclopropylformyl-CoA **73**, a probable metabolite of methylenecyclopropylglycine from litchi fruits, has been shown to irreversibly and covalently inactivate crotonase from both pig kidney and bovine liver.[169]

A related acyl-CoA analogue is cyclobutaneacetyl-CoA **74**. **74** is a substrate for medium-chain acyl-CoA dehydrogenase, forming the expected α,β-unsaturated product **75**.[170] In contrast, **74** exhibited time-dependent inactivation of the short-chain acyl-CoA dehydrogenase. Using tritium-labeled **74**, it was shown that the inhibitor became covalently attached to the flavin. A mechanism similar to those for the cyclopropyl ring-containing analogues was proposed (Figure 25), involving ring opening of a radical intermediate **76** and reaction of the resulting radical **77** with the flavin to form the covalent adduct **78**.

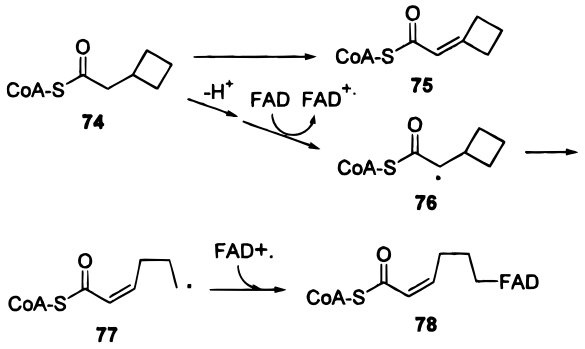

Figure 25. Inactivation of acyl-CoA dehydrogenase by cyclobutaneacetyl-CoA.

F. Other Coenzyme A Thioesters

Several acyl-CoA analogues containing azido groups have been prepared as photoaffinity labels for CoA ester-utilizing enzymes (Figure 26). A summary of

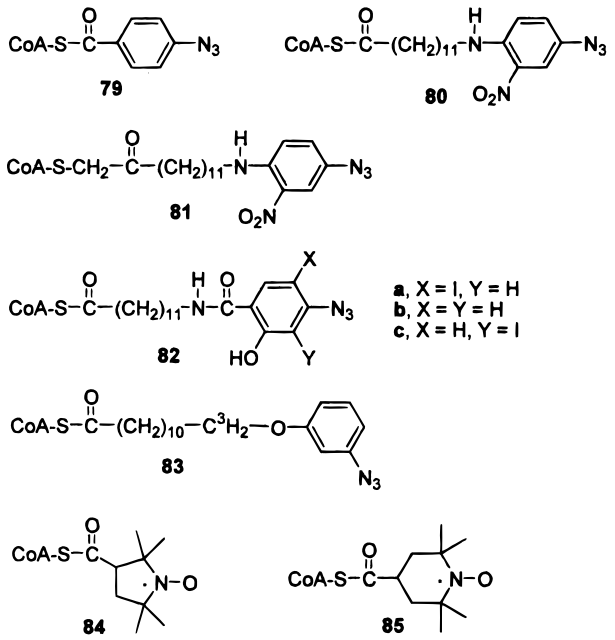

Figure 26. CoA ester-based photoaffinity labels and spin labels.

the preparation and use of photolabile acyl-CoA analogues was published in 1983.[171] Early photolabile analogues included the CoA thioesters *p*-azidobenzoyl-CoA (PAB-CoA) **79**, *S*-[12-*N*-(4-azido-2-nitrophenyl)aminododecanoyl]-CoA (AND-CoA) **80**, and the thioether homologue of AND-CoA, *S*-[13-*N*-(4-azido-2-nitrophenyl)amino-2-oxotridecyl]-CoA (NAT-CoA) **81** (see section IV.B. for a general discussion of analogues having this type of thioether linkage). These analogues, usually possessing ^{3}H or ^{35}S in the CoA moiety, have been used as photoaffinity labels for a large number of enzymes including acyl-CoA:glycine *N*-acyltransferase, citrate synthase, transcarboxylase, pyruvate carboxylase, and carnitine acetyltransferase.[171] In more recent work, **80** and a related analogue in which the aryl group is attached to the alkyl chain via an ether linkage were used in the photoaffinity labeling of the acyl-CoA binding protein from bovine liver.[172] Due to the lability of the thioester bond in **79** and **80**, the radiolabeled CoA moiety was sometimes lost during attempted isolation of radiolabeled protein or peptide. The thioether analogue **81** was prepared to eliminate this labile thioester bond.[171]

In other more recent work, ^{125}I-labeled 12-[(5-iodo-4-azido-2-hydroxybenzoyl)amino]dodecanoyl-CoA **82a** was prepared.[173] This photoaffinity label, upon photolysis, inactivated the microsomal acyl-CoA:cholesterol *O*-acyltransferase[173] and has been shown to inhibit the diacyl glycerol acyltransferase in liver microsomes and to photolabel several microsomal membrane proteins.[174] A detailed study of the photoaffinity labeling of acyl-CoA oxidase was reported.[175] 12-Azidooleoyl-CoA and 12-[(4-azidosalicyl)-amino]dodecanoyl-CoA (ASD-CoA) **82b** were prepared having ^{32}P-labeled 3′-phosphate in the CoA moiety. These compounds were prepared using acyl-CoA synthetase to couple the acid with the radiolabeled CoA, with the labeled CoA prepared by enzymatic phosphorylation of dephospho-CoA with γ-^{32}P-ATP catalyzed by the dephospho-CoA kinase from pig liver. The acyl-CoA analogues were substrates for acyl-CoA oxidase in the absence of UV light, but irreversibly inactivated the enzyme in the presence of UV light. These two analogues were also used to photolabel soybean membrane proteins.[176] In other recent work, **82b** was used to identify a plant acyl-CoA:fatty alcohol acyltransferase by photoaffinity labeling.[177,178] The related 12-[3-iodo-4-azidosalicyl)-amino]dodecanoyl-CoA **82c** was used as a photoprobe for the lysophosphatidylcholine acyltransferase from castor bean.[179] Photoaffinity labeling of peroxisomal proteins of *Candida tropicalis* using 11-*m*-diazirinophenoxy-[11-^{3}H]undecanoyl-CoA **83** resulted in radiolabeling of 70 and 55 kDa proteins, with the 70 kDa protein identified as an acyl-CoA oxidase.[180] The CoA ester of 18-(4′-azido-2′-hydroxybenzoyl-amino)oleic acid upon photolysis inactivated lysophosphatidylcholine:acyl-CoA *O*-acyltransferase.[181] A series of 11 terminal *m*- or *p*-azidophenyl- or (azidophenoxy)acyl-CoAs were prepared and tested as substrates for myristoyltransferase, though they were not actually used as photoaffinity labels.[136] CoA analogues having an azido group in the adenine base are described in section VI.B.7.[65,182]

Other reported CoA thioesters include a pair of compounds having nitroxide spin labels in the acyl group **84** and **85**.[183−185] Compound **84** was used in EPR studies of the complexes with citrate synthase[183] and HMG-CoA synthase[184] and more recently with the pyruvate dehydrogenase complex.[185]

IV. Coenzyme A Thioethers

A. Analogues of Coenzyme A Thioesters Having C=O Replaced with CH_2

Another class of readily prepared analogues of natural CoA thioesters is the CoA thioethers. These analogues are prepared by alkylation of the thiol group of CoA with an alkyl halide or other alkylating agent. Many CoA thioethers have been prepared which differ from a natural CoA ester simply by replacement of the carbonyl group with a methylene group. These compounds are structurally similar to the corresponding CoA thioesters, but they cannot undergo any of the reactions that involve cleavage of the thioester or deprotonation of the α-carbon. The simplest CoA thioethers are those represented by **86** (Figure 27), in which CoA is alkylated with a simple

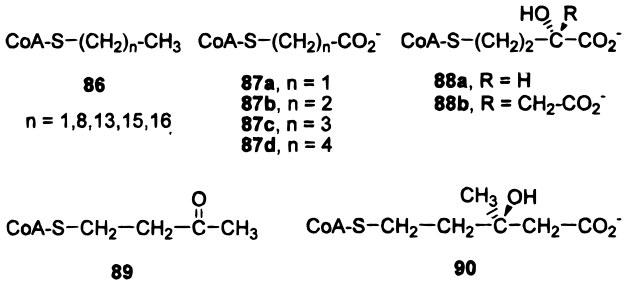

Figure 27. Examples of CoA thioethers.

alkyl group. For example, *S*-ethyl-CoA (**86**, $n = 1$), the thioether analogue of acetyl-CoA, is an inhibitor of the acetyl-CoA-utilizing enzymes citrate synthase,[186] malate synthase,[187] and phosphotransacetylase[188] and can replace acetyl-CoA as an effector of pyruvate carboxylase.[188] However, this compound does not inhibit acetyl-CoA carboxylase or fatty acid synthase, indicating that the carbonyl group is important in acetyl-CoA binding to these enzymes.[187] *S*-Octyl-CoA (**86**, $n = 7$) and *S*-heptadecyl-CoA (**86**, $n = 16$) both inhibit acyl-CoA dehydrogenase and the latter is also an inhibitor of carnitine palmitoyltransferase.[189] The myristoyl-CoA analogue tetradecyl-CoA (**86**, $n = 13$) and the palmitoyl-CoA analogue hexadecyl-CoA (**86**, $n = 15$) were both tested as inhibitors of protein *N*-myristoyltransferase.[190] There was little difference in affinity for these two analogues for the enzyme. This is in contrast to the corresponding thioesters, as the enzyme exhibits high selectivity for myristoyl-CoA over palmitoyl-CoA, indicating that the carbonyl group is important in chain-length recognition by the myristoyltransferase. These and other simple alkyl-CoA thioethers have been employed in numerous other studies.[135,137,138,140,141,146,191] Similar CoA thioethers have been prepared in which the alkyl chain contains a heteroatom (e.g., 3-thiaoctyl-CoA)[137,138,146] or a double bond (e.g. allyl-CoA).[140]

Another general group of CoA thioethers are those in which the *S*-alkyl substituent bears a terminal carboxylate group (**87**). Carboxymethyl-CoA **87a** could be viewed as an analogue of oxalyl-CoA but has actually been utilized as an acetyl-CoA enolate mimic (see section VI.B.4).[186,192,193] The malonyl-CoA analogue **87b** was not an inhibitor of acetyl-CoA carboxylase or fatty acid synthetase.[187] Both **87b** and the isomeric 1-carboxyethyl-CoA were modest inhibitors of methylmalonyl-CoA mutase and were used in EPR studies of the enzyme complex.[194] The succinyl-CoA analogue **87c** is a competitive inhibitor of methylmalonyl-CoA mutase with a K_i near the K_m for succinyl-CoA.[195] Compound **87d**, an analogue of glutaryl-CoA or of HMG-CoA lacking the 3-hydroxyl and methyl groups, was a very weak inhibitor of HMG-CoA reductase.[196]

The thioether analogue of malyl-CoA **88a** is an inhibitor of malate synthase, an enzyme which forms malyl-CoA as an enzyme-bound intermediate.[197] Inhibition studies showed that malate synthase exhibits affinity for CoA thioethers approximately equal to that for the corresponding CoA esters. The thioether analogues of both malyl-CoA **88a** and citryl-CoA **88b** are inhibitors of citrate synthase.[197,198] **88b** has been useful in X-ray crystallographic studies of the enzyme−inhibitor complex as a model for the transient citryl-CoA complex.[199,200] **88b** is also a strong inhibitor of ATP-citrate lyase, exhibiting affinity similar to that of citryl-CoA.[187]

S-(3-Oxobutyl)-CoA **89**, the thioether analogue of acetoacetyl-CoA, is an inhibitor of acetyl-CoA:acetoacetate CoA-transferase and is a substrate for 3-hydroxy-3-methylglutaryl-CoA synthase and 3-hydroxyacyl-CoA dehydrogenase.[201] *S*-(4-Carboxy-3-hydroxy-3-methylbutyl)-CoA **90**, the product of the HMG-CoA synthase reaction of **89**, is an inhibitor of HMG-CoA lyase.[201] The other diastereomer of **90** was made nonenzymatically and was shown to inhibit HMG-CoA-reductase.[202] Each pair of thioester and thioether derivatives of 3-hydroxy-3-methylglutarate displayed almost equal affinity toward HMG-CoA reductase.[203] Additional thioether analogues of HMG-CoA related to **90** were prepared in which the hydroxyl and/or methyl substituents at C-3 were deleted.[196,204] Inhibition studies of HMG-CoA reductase indicated that the hydroxyl group but not the methyl group are critical for high-affinity enzyme binding.

B. Analogues of Coenzyme A Thioesters Having CH_2 Inserted between S and C=O

The other large class of CoA thioethers differ from the natural CoA thioesters by insertion of a methylene group between the sulfur atom and the carbonyl carbon (Figure 28). These compounds are prepared by alkylation of the thiol group of CoA with an

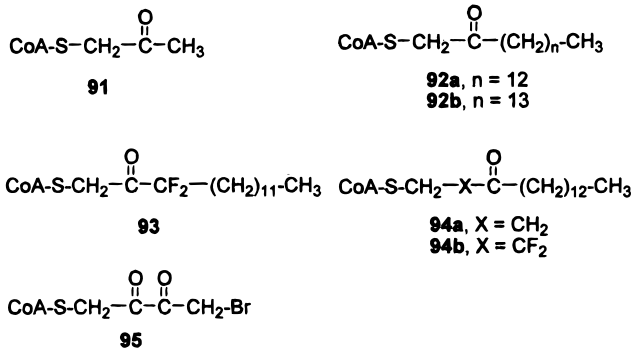

Figure 28. 2-Ketoalkyl-CoA thioethers.

α-haloketone and act as noncleavable ketone homologues of CoA thioesters. The simplest of these analogues is acetonyl-CoA **91**, the homologue of acetyl-CoA.[205] This compound can replace acetyl-CoA in the exchange of α-protons by solvent catalyzed by citrate synthase in the presence of oxaloacetate, though no condensation reaction is observed and the rate of proton exchange (0.03 μmol min^{-1} mg^{-1}) is much slower than the V_{max} (150−200 μmol min^{-1} mg^{-1}) of the natural reaction.[192]

Several thioether homologues of myristoyl-CoA have been reported and studied as inhibitors of *N*-myristoyltransferase. *S*-(2-Ketopentadecyl)-CoA **92a** was prepared by reaction of 1-chloro-2-pentadecanone with CoA in aqueous solution.[206] **92a** was a competitive inhibitor of the *N*-myristoyltransferase from mouse brain with a K_i of 24 nM, compared to K_m for the natural substrate of 400 nM.[206] **92a** exhibited similar K_i values for the yeast and human myristoyltransferases[207,208] and was used for the identification and characterization of multiple forms of bovine brain *N*-myristoyltransferase.[209] The corresponding alcohol *S*-(2-hydroxylpentadecyl)-CoA was a poor inhibitor of the myristoyltransferase.[210] *S*-(3,3-Difluoro-2-oxopentadecyl)-CoA **93** and *S*-(2,2-difluoro-3-oxohexadecyl)-CoA **94b** exhibited IC_{50} values for *N*-myristoyltransferase of 80 and 110 nM, respectively,[211] while *S*-(2-oxopentadecyl)-CoA **94a** exhibited a K_i of 7 nM.[212]

A related compound is 4-bromo-2,3-dioxobutyl-CoA **95**, which was shown to inactivate fatty acid synthase[213] and the HMG-CoA reductases from both yeast and rat liver.[214] The latter enzyme was protected from inactivation by the substrate HMG-CoA but not by the potent inhibitor mevinolin.[215] **95** was also shown to inhibit a malonyl-CoA sensitive form of rat liver carnitine palmitoyltransferase but not the malonyl-CoA insensitive form.[216]

C. Other Coenzyme A Thioethers

Other enzyme inhibitors and probes based on CoA thioethers include some bisubstrate analogues, which incorporate CoA, the acyl group, and the acyl-acceptor in a stable covalent complex. One such example is the bisubstrate adduct **39** formed enzymatically in the reaction of carnitine acetyltransferase with bromoacetyl-CoA (section III.C., Figure 16).[114] A similar adduct formed between CoA and bromopyruvate is proposed to be responsible for the inhibition of pyruvate:ferredoxin oxidoreductase.[217] A bisubstrate adduct formed between CoA and (bromoacetyl)spermidine is an inhibitor of histone acetyltransferase.[28,218,219] An interesting recent example is the thioether bisubstrate adduct of the melatonin rhythm enzyme serotonin *N*-acetyltransferase.[220] This enzyme catalyzes the transfer of the acetyl group from acetyl-CoA to the primary amino group of serotonin (see Figure 4). This enzyme follows an ordered BiBi kinetic mechanism in which both substrates must bind to the enzyme prior to the release of either product. The acetyl group is believed to be transferred directly from acetyl-CoA to serotonin without the involvement of a covalent acetyl-enzyme intermediate.[221] The mechanism provides the basis for possible inhibition by a bisubstrate analogue containing the components of serotonin and acetyl-CoA covalently linked. The bisubstrate analogue **98** was prepared by reaction of the alternate acceptor substrate tryptamine **96** with bromoacetyl bromide to afford the bromoacetamide derivative **97**, followed by alkylation of CoA with this derivative in mildly basic conditions (Figure 29). **98** was found to inhibit

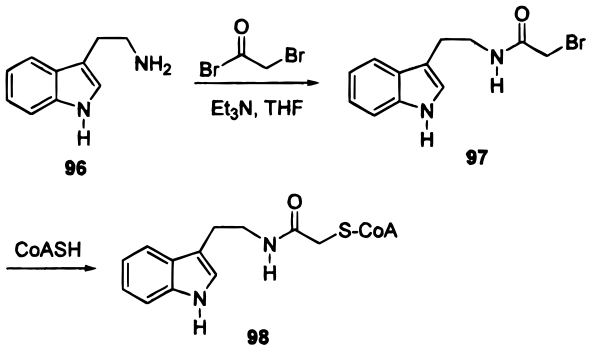

Figure 29. Synthesis of a bisubstrate analogue inhibitor of serotonin-*N*-acetyltransferase.

the recombinant sheep serotonin *N*-acetyl transferase with an IC_{50} of 150 nM. This is approximately 1000-fold lower than the substrate K_m values or the IC_{50} of all other inhibitors reported to date. **98** was shown to be a competitive inhibitor versus acetyl-CoA (K_i = 90nM) and a noncompetitive inhibitor versus tryptamine. This pattern of inhibition further supports an ordered BiBi mechanism with acetyl-CoA binding before tryptamine (or serotonin).[222]

V. Other Analogues Derived from Coenzyme A

A. Desulfo-Coenzyme A

A variety of other CoA and CoA ester analogues have been prepared by direct modification of the thiol group of CoA. Perhaps the simplest example is desulfo-CoA **99**, prepared by the Raney nickel reductive removal of the terminal thiol of CoA (Figure 30).[223] **99** is an effective competitive inhibitor of CoA- and CoA ester-requiring enzymes such as carnitine acetyl transferase, phosphotransacetylase, citrate synthase, β-hydroxy-β-methylglutaryl CoA synthase, and α-ketoglutarate dehydrogenase.[223] The K_i for desulfo-CoA with each of the enzymes is close to the K_m value for CoA or acetyl-CoA.

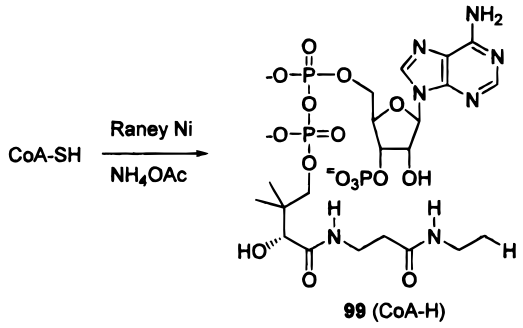

Figure 30. Synthesis of desulfo-CoA.

B. Coenzyme A Dithioesters

Dithioester derivatives of acetyl-CoA **102a** and fluoroacetyl-CoA **102b** and the corresponding oc-

tanoyl-CoA analogue have been prepared in which the thioester carbonyl oxygen is replaced with sulfur. The phenyl thioesters **100a** and **100b** were first converted to the corresponding dithioesters **101a** and **101b** by reaction with Lawesson's reagent (Figure 31). Transesterification reactions between CoA and

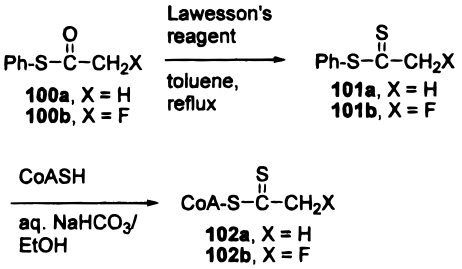

Figure 31. Synthesis of CoA dithioesters.

the phenyl dithioesters formed the CoA dithioesters **102**.[224] The dithioesters have special spectral properties, with a λ_{max} of 306 nm. The α-protons of **102a** have a pK_a of 12.5, compared to 21.5 for the natural thioester.[225] Compound **102a** underwent citrate synthase-catalyzed exchange of the α-protons with solvent at a rate 250-fold faster than the same exchange reaction with acetyl-CoA. However, **102a** was a poor substrate in the citrate synthase-catalyzed formation of thiocitrate, with a V_{max} 2×10^{-6} times the V_{max} with acetyl-CoA. The fluoro compound **102b** gave a 10-fold higher V_{max} than **102a** in the citrate synthase reaction. The absorbance at 306 nm of **102a** decreased upon binding to citrate synthase.[226] This decrease was attributed to conversion of **102a** to the deprotonated enethiolate form when bound to citrate synthase. Resonance Raman studies of the citrate synthase complex of **102a** indicate that the enzyme binds **102a** in at least two rotational isomeric forms. **102a** was a fairly good substrate for choline acetyltransferase, with a V_{max} 7.2% the V_{max} with acetyl-CoA. **102a** is a substrate for 3-oxoacyl-CoA thiolases and has been used in a novel spectrophotometric assay for these enzymes in the thermodynamically unfavorable condensation direction.[227]

Compound **102a** has also been used in studies of the role of the metal ion in HMG-CoA lyase.[228] This enzyme requires a divalent metal ion such as Mg or Mn for activity. The results suggest a direct interaction between the metal ion and the carbonyl oxygen of acetyl-CoA and the thiocarbonyl sulfur of **102a** in catalysis. The dithioester of HMG-CoA is a potent inhibitor of the HMG-CoA reductase from *Pseudomonas mevalonii*.[229]

C. Coenzyme A Sulfoxides and Sulfones

A few CoA sulfoxides and sulfones have been prepared by oxidation of the corresponding CoA thioethers using chloramine T or oxone, respectively. These include the acetyl-CoA **103**, malyl-CoA **104**, and HMG-CoA **105** analogues (Figure 32). These analogues are isosteric with the corresponding CoA esters, but the oxidation is moved toward the CoA moiety by one atom, from carbon to sulfur. The acetyl-CoA analogues **103** were competitive inhibitors of citrate synthase, the K_i for the sulfone (12 μM)

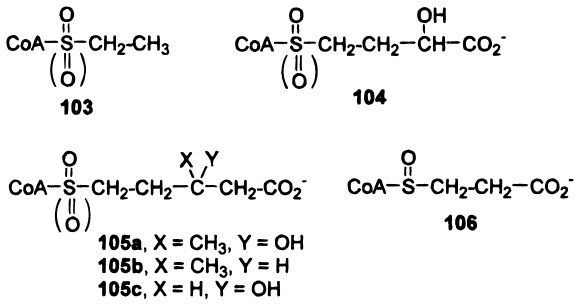

Figure 32. CoA sulfoxides and sulfones.

being near the K_m for acetyl-CoA, while the K_i for the sulfoxide was 40 μM.[204] The malyl-CoA analogues **104** were competitive inhibitors of the citrate synthase-catalyzed hydrolysis of malyl-CoA (a reaction similar to the hydrolysis of the natural intermediate citryl-CoA), with K_i values of 350 and 800 μM for the sulfoxide and sulfone, respectively, both higher than the K_m for malyl-CoA of 96 μM.[204] The HMG-CoA analogues **105a** were competitive inhibitors of the human HMG-CoA reductase. The K_i values for the sulfoxide and sulfone were 6 and 18 nM, much lower than the K_m for HMG-CoA and the K_i for the corresponding CoA thioether (both about 2 μM).[204] As with the previously reported thioethers, deletion of the methyl group (**105c**) had almost no effect on affinity for the enzyme, while deletion of the hydroxyl group (**105b**) resulted in a more than 1000-fold increase in K_i.[204] 2-Carboxyethyl-CoA sulfoxide **106** was a modest inhibitor of methylmalonyl-CoA mutase and gave an EPR active complex, while the isomeric 1-carboxyethyl-CoA sulfoxide was a weaker inhibitor and did not induce an EPR signal upon binding to the enzyme.[194]

Recently, *S*-3-oxobutylsulfoxyl-CoA **107** was prepared by alkylation of CoA with methyl vinyl ketone followed by oxidation of the resulting thioether to the sulfoxide with Chloramine T.[230] This analogue is an inhibitor of HMG-CoA synthase, HMG-CoA lyase, and β-keto thiolase. The postulated mechanism is shown in Figure 33. A reactive sulfenic acid CoA

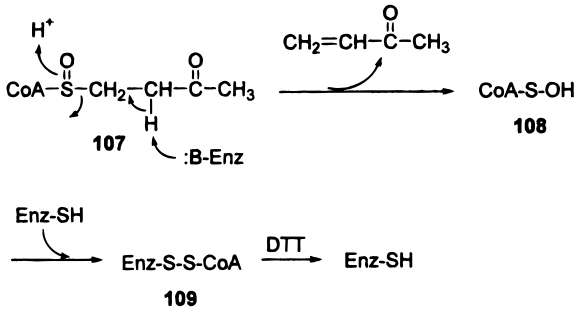

Figure 33. Enzyme inactivation by *S*-3-oxobutylsulfoxyl-CoA.

species **108** is formed by deprotonation of the analogue at the β-position and release of methyl vinyl ketone. Attack of an active site thiol on the sulfenic acid forms an inactive enzyme-CoA disulfide (**109**). Treatment with DTT to reduce the disulfide restored the enzymatic activity and released free CoA.

D. Coenzyme A Hemithioacetals

The most easily derived analogues of CoA esters are the hemithioacetals **111**. Four hemithioacetals of CoA were generated by mixing CoA with the appropriate aldehyde **110** (Figure 34).[231] The rates

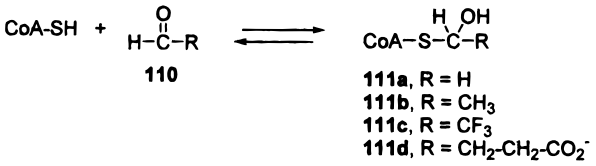

Figure 34. Formation of CoA hemithioacetals.

and equilibria of hemithioacetal formation and dissociation were studied. Formation and dissociation of the hemithioacetals with acetaldehyde (**111b**) and succinic semialdehyde (**111d**) were too fast to measure by simple methods, while the formaldehyde (**111a**) and trifluoroacetaldehyde (**111c**) hemithioacetals had rate constants for dissociation of 1.3×10^{-2} and 3.0×10^{-3} s^{-1}, respectively. **111a**−**c** were tested as inhibitors of chloramphenicol acetyltransferase.[231] Conditions were chosen such that inhibition by CoA and aldehyde was small relative to inhibition by the hemithioacetal. The K_i for **111a** was 6-fold higher than the K_m for acetyl-CoA (13 μM at pH 7), while the K_i values for **111b** and **111c** were 2.4- and 10-fold lower than the K_m for acetyl-CoA. **111d** was an inhibitor of succinic thiokinase (succinyl-CoA synthetase), with a K_i of 4.3 μM, 4-fold lower than the K_m for succinyl-CoA. Inhibition by the hemithioacetals is attributed to their analogy to the tetrahedral intermediate or transition state of acyl transfer.

E. Other Thiol-Modified CoA Derivatives

Other CoA analogues have been prepared by derivatization of the thiol group of CoA. These include *S*-methanesulfonyl-CoA, prepared by reaction of CoA with methanesulfonyl chloride.[232] This compound rapidly inactivates succinate thiokinase and fatty acid synthase. An oxidized form of CoA disulfide was shown to inactivate phosphotransacetylase and carnitine acetyltransferase,[233] and methoxycarbonyl-CoA disufide (CoA-S−S−C(=O)OCH_3) was shown to inactivate carnitine acetyltransferase.[234] With each of these three compounds, enzyme inactivation is believed to result from formation of a mixed disulfide between CoA and a cysteine thiol group in the enzyme active site. Activity is recovered upon reduction of the disulfide with DTT.

S-Dimethylarsino-CoA (CoAS−As$(CH_3)_2$) was prepared by reaction of CoA with dimethylchloroarsine.[235] This compound was shown to irreversibly inactivate phosphotransacetylase, apparently by transfer of the dimethylarsine group to a cysteine thiol group of the enzyme. This compound did not inactivate carnitine acetyltransferase or citrate synthase but was a reversible inhibitor of both enzymes, with K_i values near the K_m for acetyl-CoA.

F. Adenine-Modified Coenzyme A Derivatives

CoA has also been modified by reagents specific for the adenine moiety. Reaction of CoA disulfide with chloroacetaldehyde followed by disulfide reduction forms etheno-CoA, in which two nitrogens of the adenine base are linked by an ethylene group.[236] The oleoyl thioester of etheno-CoA is a better detergent than oleoyl-CoA but is a 10-fold poorer inhibitor of citrate synthase.[236] Inosino-CoA, in which the adenine base is replaced with inosine, was prepared by deamination of CoA disulfide using nitrite.[237] CoA has also been converted to derivatives suitable for affinity chromatography following general methods developed for the equivalent functionalization of other adenine nucleotides. An analogue of desulfo-CoA **112** was prepared having a 6-aminohexylamine group at the C-8 position of the adenine ring which was used to attach the analogue to a solid support (Figure 35).[238]

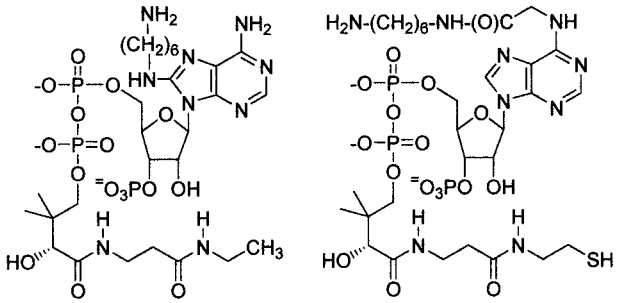

Figure 35. Adenine-modified CoA derivatives for affinity chromatography.

Synthesis of **112** involved bromination of desulfo-CoA at the C-8 position followed by displacement of bromide with 1,6-diaminohexane. N^6-(*N*-(6-aminohexyl)carbamoylmethyl)-CoA **113** was prepared by a procedure which involved alkylation with iodoacetate, heating to induce migration of the acetyl group to the N^6 position and carbodiimide coupling to 1,6-diaminohexane.[239] **113** was also attached to a solid support to make a CoA-based affinity column.

VI. Analogues that Cannot Be Made from Coenzyme A

Some analogues of CoA require replacement of the terminal thiol group with a different functionality. Except for the desulfo-CoA prepared by Raney nickel reduction of CoA, these analogues cannot be made by simple modification of CoA but instead require assembly of the CoA moiety from simpler starting materials. Both nonenzymatic and more recently enzymatic methods have been developed for the synthesis of such analogues.

A. Synthetic Methods

1. Nonenzymatic Synthesis

The methods developed by Moffatt and Khorana[62] and Michelson[63] for the synthesis of coenzyme A (section II.B.) have been used for the preparation of several CoA analogues. The most common route involves coupling of a phosphopantetheine analogue with the phosphomorpholidate derivative of 2′,3′-cyclic ADP **8** used in the original CoA synthesis of Moffatt and Khorana, followed by acid-catalyzed

hydrolysis of the 2′,3′-cyclic phosphate to form a mixture of the desired 3′-phosphate and the isomeric 2′-phosphate ester. The pantetheine analogues are commonly prepared by reaction of a cysteamine analogue with an activated derivative of pantothenic acid or by reaction of pantolactone with a β-alanylcysteamine analogue. Conversion to the phosphopantetheine analogue is commonly performed by reaction with dibenzylphosphorochloridate in pyridine to form the dibenzyl phosphate triester followed by debenzylation with sodium in liquid ammonia or by reaction with DCC-activated cyanoethyl phosphate followed by base-catalyzed elimination of the cyanoethyl group.[240] This general method has been used in the synthesis of several CoA analogues and is highly versatile but involves several steps, gives fairly low overall yield, and requires the careful separation of the 2′- and 3′-phosphate isomers.

2. Enzymatic Synthesis

The first introduction of enzymes into the synthesis of CoA analogues was the use of ribonuclease T2 to hydrolyze the 2′,3′-cyclic phosphate formed in the coupling of phosphopantetheine analogues with the activated derivative of 2′,3′-cyclic ADP. This enzyme catalyzes hydrolysis of the cyclic phosphate to the 3′-phosphate with no contamination by the 2′-phosphate, thus avoiding the tedious separation of the 2′- and 3′-phosphate isomers. In one example of this method, the phosphomorpholidate **8** was coupled with phosphopantethenonitrile **114** followed by ribonuclease-catalyzed hydrolysis of the 2′,3′-cyclic phosphate to form **115** (Figure 36).[240] The nitrile **115** was

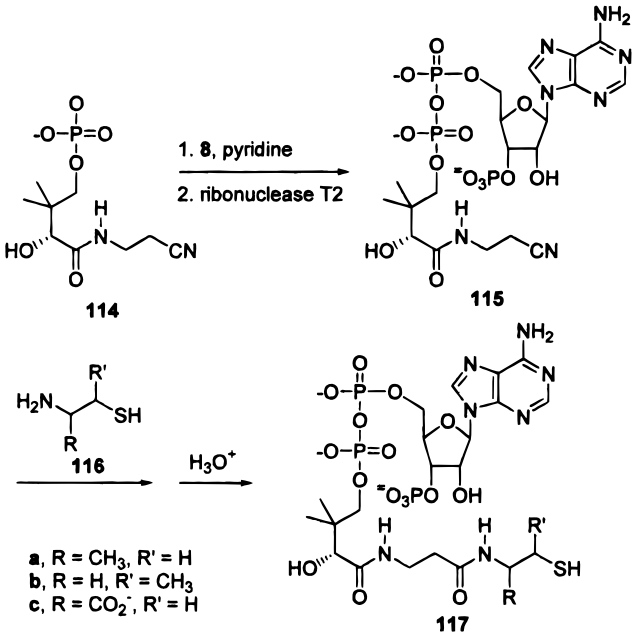

Figure 36. Synthesis of CoA analogues modified in the cysteamine moiety.

reacted with cysteamine analogues **116** to form a thiazoline, which upon hydrolysis in aqueous acid formed the CoA analogues **117** modified in the cysteamine region. This synthetic approach provides some versatility, though it is useful only for the synthesis of analogues bearing substituents on the cysteamine carbons.

The enzymes of CoA biosynthesis have also been used to prepare CoA analogues. Initial work involved nonenzymatic synthesis of the phosphopantetheine equivalent **118** of the desired CoA analogue followed by conversion to the CoA analogue **119** using the enzymes which convert phosphopantetheine to CoA. The oxy and desulfo analogues of CoA (**119a,b**) were prepared by this approach (Figure 37).[241,242] The final

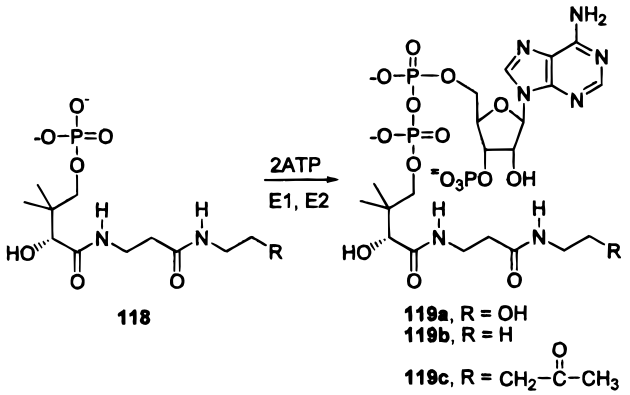

Figure 37. Enzymatic synthesis of CoA analogues. E1 = phosphopantetheine adenylyltransferase, E2 = dephosphocoenzyme A kinase.

steps were achieved by incubation of oxy- (**118a**) or desulfophosphopantetheine (**118b**) with ATP and the enzymes dephospho-CoA kinase and phosphopantetheine adenyltransferase, which were isolated from beef liver as a bifunctional complex. Acetonyldethio-CoA **119c** has also been prepared utilizing the enzymes of CoA biosynthesis from pig liver.[243] **119c** has an acetonyl group in place of the sulfhydryl group of phosphopantetheine that is tolerated by the CoA biosynthetic enzymes, indicating that these two enzymes have somewhat liberal substrate specificity for the phosphopantetheine moiety.

A more recent synthetic approach involves the enzymatic synthesis of a versatile CoA analogue synthon (**121b**) from the pantetheine phosphate analogue **120b**, in which the amide bond nearest the thiol group of the natural substrate **5** is replaced with a thioester (Figure 38).[244] Aminolysis of the thioester

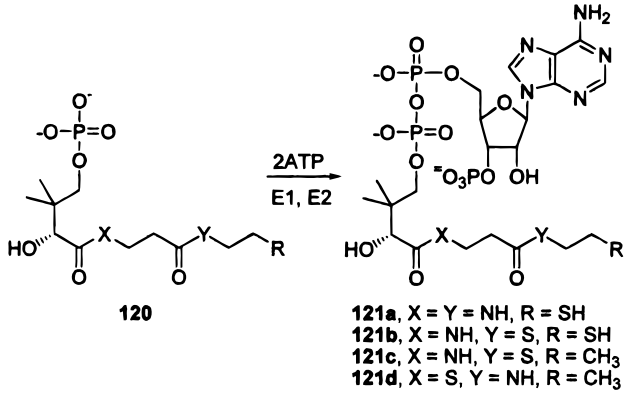

Figure 38. Enzymatic synthesis of versatile CoA analogue synthons. E1 = phosphopantetheine adenylyltransferase, E2 = dephosphocoenzyme A kinase.

bond of **121b** with an appropriate amine **122** (Figure 39) reinstates the amide bond present in CoA and introduces functionality contained in the simple

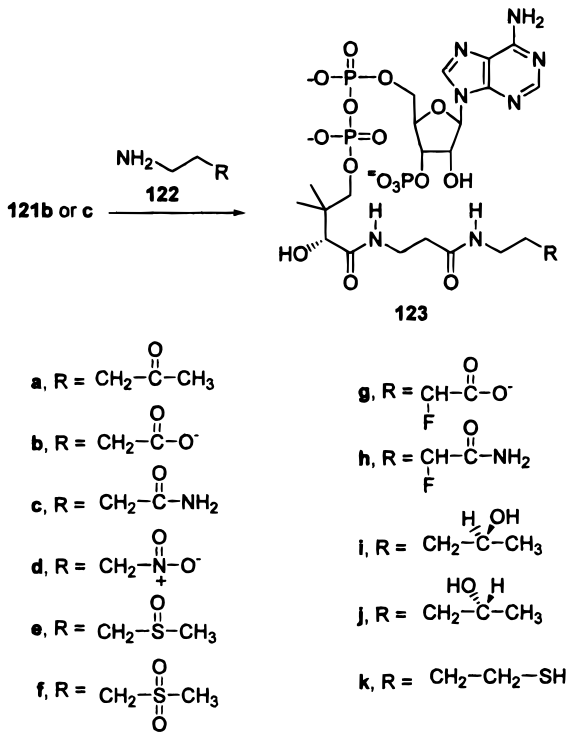

Figure 39. CoA analogues prepared by aminolysis of a versatile CoA analogue synthon.

amine in place of the thiol group of CoA. The phosphopantetheine analogue **120b** used in initial work was replaced in subsequent work with the analogue **120c**, in which the terminal thiol group is replaced with a methyl group.[245] This simplifies the synthesis by avoiding undesired reactions of the thiol group. This propyl thioester **120c** was prepared in four nonenzymatic steps from pantothenic acid. **120c** was converted to the CoA analogue **121c** by reaction with ATP, catalyzed by the enzymes phosphopantetheine adenylyltransferase and dephospho-CoA kinase, which were isolated from *Corynebacterium ammoniagenes* and coimmobilized in polyacrylamide gel. The commercially available enzyme inorganic pyrophosphatase was included to hydrolyze the pyrophosphate formed in the adenylyltransferase reaction.

The general CoA analogue synthon **121c** has now been used to prepare CoA analogues **123** having a range of functionality in place of the thiol group by reaction with primary amines **122** bearing the appropriate group (Figure 39). These include the previously reported acetonyl dethia analogue **123a** and the initial five new analogues of acetyl-CoA in which the thioester sulfur is replaced by a methylene group and the acetyl group is replaced by carboxylate (**123b**), carboxamide (**123c**), nitro (**123d**), methyl sulfoxide (**123e**), and methyl sulfone (**123f**) groups.[245] More recently the α-fluoro analogues **123g** and **123h**, the secondary alcohol analogues **123i** and **123j**, and others have been prepared similarly.[246−248] As the specific functionality of interest is introduced in the final nonenzymatic step, synthesis of each analogue is free of any substrate specificity limitations of the CoA biosynthetic enzymes.

More recently the CoA analogue **121d** has been prepared in which the more internal amide bond is replaced with a thiol group. **121d** was also prepared enzymatically from the corresponding phosphopantetheine analogue **120d** (Figure 38).[249] **121d** has been used to prepare analogues **125** having modifications more remote from the thiol group by formation of the more internal amide bond in a final aminolysis step (Figure 40). Applications of **121d** suffer from the

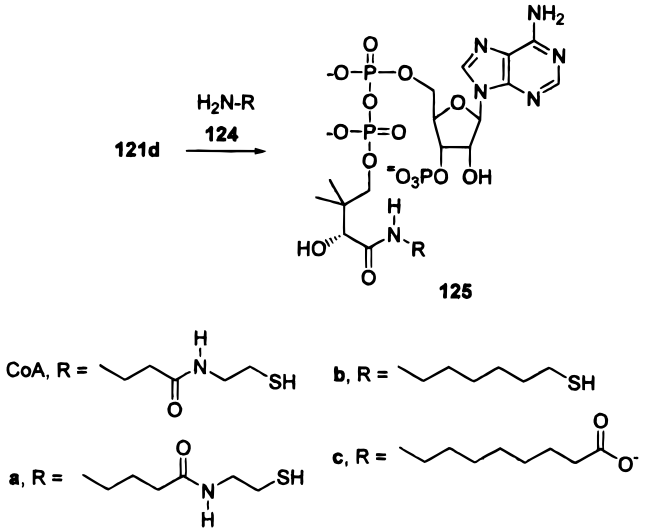

Figure 40. CoA analogue synthesis from a second-generation CoA analogue synthon.

more laborious synthesis of **120d** relative to **120c** and the lower reactivity of the thioester bond of **121d** toward aminolysis.

B. Analogues

1. Thiol-Modified Analogues: Oxy-Coenzyme A, Selenol-Coenzyme A, and Amino-Coenzyme A Derivatives

The selenol analogue of CoA in which the thiol sulfur atom is replaced with selenium was prepared nonenzymatically using the Moffatt and Khorana method.[250,251] After coupling of the phosphopantetheine analogue to the phosphomorpholidate **8** and hydrolysis of the 2′,3′-cyclic phosphate, separation of the resulting mixture of 2′- and 3′-phosphates was successful only after protection of the selenol as the benzoate ester followed by chromatography on EC-TEOLA cellulose. The oxy analogue of CoA (**119a**), in which the thiol group is replaced with a hydroxyl group, was prepared similarly,[252] though this analogue has also been prepared enzymatically (Figure 37). 3′-Dephosphooxy-CoA was prepared by condensing D-oxyphosphopantetheine with the adenosine-5′-phosphomorpholidate lacking the 2′,3′-cyclic phosphate.[252] The propionyl amide of amino-CoA has been prepared similarly.[253]

Seleno-CoA was neither a substrate nor an inhibitor of phosphotransacetylase, while oxy-CoA was a competitive inhibitor of this enzyme with a K_i of 3.5 $\times 10^{-7}$ M.[242,250,251] Oxypantetheine is also a competitive growth inhibitor of pantetheine requiring microorganisms.[254] The propionamide analogue was not carboxylated by propionyl-CoA oxaloacetate transcarboxylase, presumably because the α-protons are

not sufficiently acidic, though this analogue did induce decarboxylation of oxaloacetate by the enzyme.[253]

2. Dethia Analogues of Coenzyme A Thioesters

Several dethia analogues of CoA esters have been prepared in which the thioester sulfur atom is replaced with a methylene group (Figure 41), and

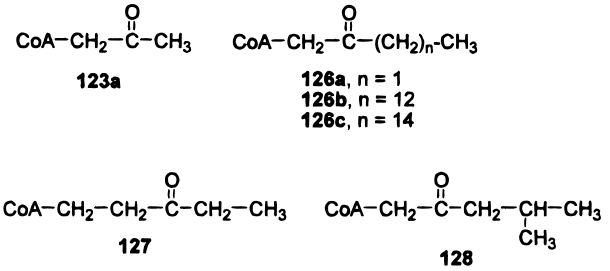

Figure 41. Dethia analogues of CoA esters.

these analogues have been recently reviewed.[255,256] These analogues like the CoA thioethers are nonhydrolyzable but are generally a better isosteric match with the natural CoA thioesters. Initial examples of dethia analogues were prepared nonenzymatically from the phosphomorpholidate **8** and an appropriate phosphopantetheine analogue as in Figure 7,[257] though the dethia analogue of acetyl-CoA **123a** (same as **119c**) has more recently been prepared using enzymatic methods.[243−245] **123a** was found to be a competitive inhibitor of citrate synthase and phosphotransacetylase, and for both enzymes the K_i for **123a** was found to be very close to the K_m for acetyl-CoA.[243,257] **123a** has been shown to be a substrate for acetyl-CoA carboxylase[258] and for the deprotonation step of citrate synthase, as indicated by rapid exchange of the α-protons with solvent.[259]

The dethia analogues of several long-chain fatty acyl-CoA's (**126**) including the palmitoyl-CoA analogue **126c**, were prepared nonenzymatically and were found to be inhibitors of the acyl-CoA dehydrogenases, being competitive against the natural substrates.[260] The myristoyl-CoA analogue **126b** has been shown to inhibit protein myristoyltransferase.[212]

The dethia analogue of propionyl-CoA **126a** was prepared and used as an alternate substrate for propionyl-CoA in a sequence of enzymatic reactions.[261] **126a** was a substrate for the propionyl-CoA oxaloacetate transcarboxylase to form the dethia analogue of methylmalonyl CoA **129** (Figure 42). The

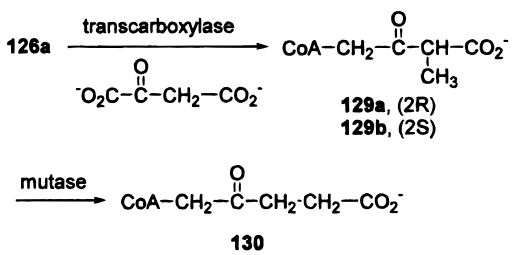

Figure 42. Enzymatic transformations of dethia-acetyl-CoA.

product, (2*R*)-methylmalonylcarba(dethio)-CoA **129a**, underwent base-catalyzed epimerization to form (2*S*)-methylmalonylcarba(dethio)-CoA **129b**, a reaction which was also catalyzed by methylmalonyl-CoA epimerase. The B_{12}-dependent methylmalonyl-CoA mutase then catalyzed the rearrangement of the 2*S*-isomer to the dethia analogue of succinyl-CoA **130**. The dethia analogues in this reaction sequence exhibited almost the same K_m and V_{max} values as the corresponding natural CoA ester substrates.[261] NMR studies of the methylmalonyl-CoA mutase reaction of **129** in D_2O showed the nonstereospecific incorporation of deuterium into the product.[262] The value of the dethia analogue was that the reaction could be monitored for a much longer period, since the dethia substrate and product, unlike the natural CoA thioester substrates, are stable to hydrolysis. The propionyl-CoA analogue **127**, in which the sulfur atom is replaced by an ethylene group, has also been prepared.[263] **127** was also accepted as a substrate by the enzymes of Figure 42, being converted to the corresponding methylmalonyl derivative by propionyl-CoA oxaloacetate transcarboxylase and to the corresponding succinyl-CoA analogue upon incubation with the methylmalonyl-CoA epimerase and the methylmalonyl-CoA mutase.

The dethia analogue of *isobutanoyl*-CoA **128** was prepared and used in similar studies of the coenzyme B_{12}-dependent isobutanoyl-CoA:*n*-butanoyl-CoA mutase.[264] The dethia analogue again was found to be an excellent substrate for the enzyme to give the dethia *n*-butanoyl-CoA product.

3. A Reversed Thioester Analogue of Acetyl-Coenzyme A

An analogue of acetyl-CoA **133** has been prepared in which the orientation of the thioester is reversed.[248] Reaction of the general CoA analogue synthon **121c** with 1,1,1-tris(methylthio)-4-aminobutane **131** formed the CoA analogue **132** (Figure 43).

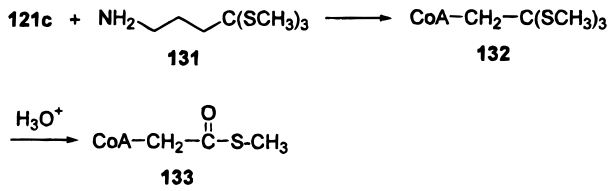

Figure 43. Synthesis of a reversed thioester analogue of acetyl-CoA.

Hydrolysis of the trithioortho ester formed the thioester **133**. **133** is actually an isomer of propionyl-CoA, having the sulfur atom and methylene group on each side of the carbonyl group interchanged. **133** is a time dependent inhibitor of thiolase. This inhibition is attributed to formation of an acyl-enzyme **134**, in which the CoA moiety rather than an acetyl group is esterified to an active-site cysteine thiol group of the enzyme (Figure 44). The enzyme was slowly

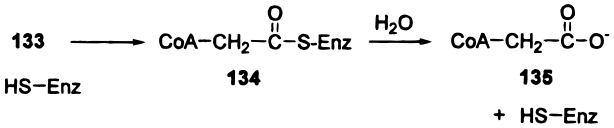

Figure 44. Inactivation of thiolase by a reversed thioester analogue.

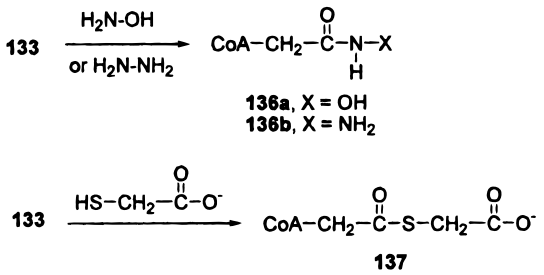

Figure 45. Use of the reversed thioester analogue as a synthon for other analogues.

reactivated upon dilution or treatment with hydroxylamine, which is attributed to hydrolysis or aminolysis of the thioester linkage. **133** was a weak competitive inhibitor of carnitine acetyltransferase, with a K_i value about double the K_m for acetyl-CoA.

133 has also been used as a synthon for other CoA ester analogues.[248] As shown in Figure 45, reaction with hydroxylamine or hydrazine formed the hydroxamate **136a** and hydrazide **136b** analogues. These compounds are potent inhibitors of citrate synthase. Reaction of **133** with mercaptoacetic acid formed the reversed thioester isomer of succinyl-CoA **137**. **137** was a weak competitive inhibitor of succinyl-CoA synthetase with a K_i of 40 μM, higher than the K_m for acetyl-CoA of 7 μM.

4. Analogues of the Enol and Enolate Forms of Acetyl-Coenzyme A

123b−**d**, **g**, and **h** (Figure 39) were designed to mimic the possible enolate or enol intermediate in the reaction of citrate synthase and related enzymes. **123b** and **123c** were potent inhibitors of citrate synthase from pig heart, with K_i values 1000- and 570-fold lower than the K_m for acetyl-CoA, respectively.[245] **123b** is a somewhat better inhibitor than the homologue carboxymethyl-CoA (**87a**, Figure 27), which has been studied as an inhibitor of the citrate synthases from pig heart and *Escherichia coli*.[192,193] CD titrations indicated that **123b** and **123c** have low affinity for citrate synthase in the absence of oxaloacetate, consistent with their recognition as enol or enolate analogues, as citrate synthase deprotonates acetyl-CoA only in the presence of oxaloacetate.[245] **123d** was a poor inhibitor of citrate synthase, with affinity near that of acetyl-CoA. The α-fluoro carboxylate analogue **123g** was a 50-fold poorer inhibitor than **123b**, though its K_i at pH 8 is still 20-fold lower than the K_m for acetyl-CoA and the K_i for the dethia analogue **123a**.[246] The K_i for the α-fluoroamide **123h** was only 1.5-fold greater than that of **123c**.[246]

The structures of the ternary complexes of **123b**,**c**,**g**, and **h** with oxaloacetate and citrate synthase have been determined.[145,265] An interesting feature of these structures are the short hydrogen bonds formed between the inhibitors and Asp-375, which is the base responsible for deprotonation of acetyl-CoA. These short hydrogen bonds are not a result of pK_a matching, as discussed by several authors,[266−268] because there is no difference in lengths of the hydrogen bonds for the α-fluoro analogues **123g** and **123h** compared to the nonfluorinated analogues **123b** and **123c**.

5. Secondary Alcohol Analogues of Acetyl-CoA

A pair of secondary alcohol analogues of acetyl-CoA, **123i** and **123j**, were prepared to mimic the tetrahedral intermediate or transition state in the acetyltransferase reactions.[247] The two analogues differ in the configuration of the secondary alcohol carbon. These analogues were tested as inhibitors of five enzymes which catalyze acetyltransfer from acetyl-CoA. The *S*-isomer **123i** was found to be the better inhibitor of all five enzymes, the K_i for the *S*-isomer being 4- to 20-fold lower than the K_m for acetyl-CoA, while the K_i for the *R*-isomer **123j** was near or above the K_m for acetyl-CoA.[247] The *S*-isomer matches the configuration of the tetrahedral intermediate in the reaction of chloramphenicol acetyltransferase, as predicted by the crystal structure of this enzyme. It is proposed that the greater inhibition by the *S*-isomer of the other enzymes indicates that the configuration of the tetrahedral intermediate is conserved among this group of enzymes, despite the lack of sequence homology.

6. Other Analogues Modified in the Acylthio Moiety

The sulfoxide and sulfone analogues **123e** and **123f** were designed to mimic the tetrahedral intermediate or transition state in the reaction of chloramphenicol acetyltransferase and related acetyl-CoA dependent acetyltransferases.[245] These analogues have an additional methylene group between the CoA moiety and the sulfur atom relative to the sulfoxides and sulfones derived from CoA (section V.C., Figures 32 and 33), thus placing the sulfur atom in a position equivalent to the carbonyl group of a CoA ester. Both **123e** and **123f** were poor inhibitors of chloramphenicol acetyltransferase, with K_i values slightly higher than the K_m for acetyl-CoA.[245]

7. Analogues Having Modifications Remote from the Acylthio Moiety

A limited number of analogues of CoA have been made having modifications remote from the thiol group. Most of the examples are simplified analogues, in which various amounts of the CoA molecule have been deleted. The intermediates of CoA biosynthesis (Figure 6), dephospho-CoA **6** and phosphopantetheine **4**, as well as pantetheine **7**, have been used as simplified analogues as well as the further simplified analogues *N*-acetylaletheine **138** and *N*-acetylcysteamine **139** (Figure 46). All of these simplified analogues have the portion of the molecule closest to the thiol group intact.

The acceptance of simplified derivatives of CoA as substrates varies greatly among different CoA-utilizing enzymes. For example, dephospho-CoA, lacking only the 3′-phosphate group, is a very poor substrate for phosphotransacetylase,[269] while the acetyl derivatives of CoA and dephospho-CoA are virtually indistinguishable as substrates for acetyl-CoA carboxylase.[270] Acetylphosphopantetheine and acetylpantetheine exhibit values of V_{max} with acetyl-CoA carboxylase of 15% and 1.5%, respectively, relative to acetyl-CoA, with K_m values increased about 10- and 30-fold.[270] Pantetheine and phosphopantetheine es-

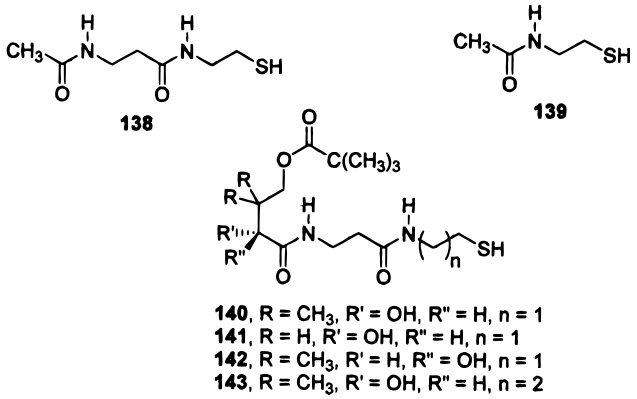

Figure 46. Simplified analogues of CoA.

ters are very poor substrates for crotonase, with activity less than 0.1% the activity with the natural CoA ester as substrate, though some increased activity can be induced by including AMP or better yet 3′,5′-ADP in the assay mixture.[271,76] On the basis of the crystal structure, this increased activity may be attributed to the hydrogen bonding of the adenine amino group to an amino acid involved directly in catalysis,[272] a structural feature which is also observed in the *p*-chlorobenzoyl-CoA dehalogenase.[273] Palmitoyl dephospho-CoA exhibited a K_i value 40-fold higher than that for palmitoyl CoA in the inhibition of acetyl-CoA carboxylase, while the K_i value for palmitoyl-4′-phosphopantetheine was similar to that for palmitoyldephospho-CoA.[191] 3′-Dephospho-CoA esters have also been studied as substrates for the medium-chain acyl-CoA dehydrogenase.[274,275] It was found that deletion of the 3′-phosphate slowed the reductive half-reaction 2−10-fold but increased the rate of dissociation of the enoyl-CoA product 2−13-fold. The enthalpic contribution of the 3′-phosphate to binding was similar in the ground and transition states.[275] The 3′-phosphate has also been shown to be critical for the inhibition of UDP-glucuronosyltransferase by fatty acyl-CoA.[276] Crystal structures of CoA-utilizing enzymes have shown that the 3′-phosphate is always directed toward solution in the enzyme complex, suggesting that the 3′-phosphate may not be critical for binding to most CoA-utilizing enzymes.[14] In recent work, a simple synthesis of the *N*-acetylcysteamine thioester of methylmalonic acid has been reported and this compound has been used as a substrate for a polyketide synthase.[277]

The most extensive study of the utilization of simplified CoA analogues by a CoA-utilizing enzyme has been the work of Jencks and co-workers on the 3-oxoacid CoA transferase.[278] This enzyme catalyzes the transfer of the CoA moiety from acetoacetyl-CoA **144** to succinate **147** to form succinyl-CoA **148**, proceeding via the covalent enzyme-CoA thioester adduct **146** (Figure 47). Desulfo-CoA is a 40-fold better inhibitor of the CoA transferase than desulfopantetheine, indicating that the nucleotide domain of CoA contributes 2.2 kcal/mol of binding energy in stabilization of noncovalent complexes with the enzyme. However, studies of the CoA and pantetheine thioesters as substrates for the enzyme indicate that the nucleotide domain contributes 7.2 kcal/mol to

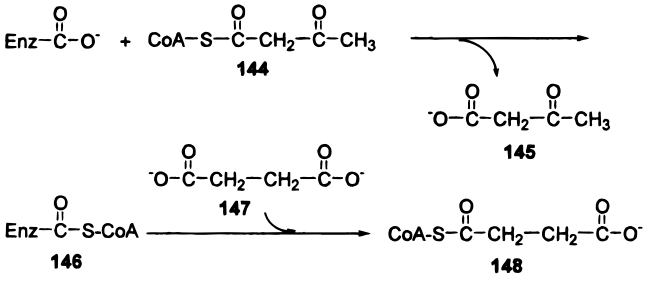

Figure 47. The reaction catalyzed by 3-oxoacid CoA transferase.

stabilization of the covalent intermediate **146** and 8.9 kcal/mol to stabilization of the transition state of the reaction. Studies of the further simplified *N*-acetylaletheine (**138**) and *N*-acetylcysteamine (**139**) thioesters have shown that the covalent enzyme adducts with these analogues are actually more stable than the adduct with pantetheine, indicating that the pantoic acid domain (deleted in going from pantetheine to **138**) actually destabilizes this intermediate. However, the covalent adduct with pantetheine is more reactive toward succinate or acetoacetate than the adduct with **138** or **139** by a factor of 10^7, indicating that the destabilizing interactions of the pantoic acid domain observed in the intermediate **146** are relieved and replaced with stabilizing interactions in the transition state.

A series of pantetheine derivatives was tested as substrates for the thiolase from *Zoogloea ramigera*, and thioesters of pantetheine pivalate **140** were found to be good substrates and were used extensively in studies of this enzyme.[20] The acetoacetate thioesters of a series of pantetheine pivalate analogues were prepared to determine the importance of specific functionality in the reaction of thiolase. The analogue **141** having the geminal dimethyl groups of the pantoate moiety replaced with hydrogens exhibited a 2.5-fold decreased V_{max} and a 3.4-fold increased K_m relative to the parent compound. The analogue **142**, in which the configuration of the secondary alcohol carbon of the pantoate moiety was inverted, exhibited a 2-fold decreased V_{max} and a 9-fold increased K_m. The homocysteamine analogue **143**, having an extra methylene group in the cysteamine moiety, had a 3-fold increased K_m, while V_{max} was 60% that with the parent compound.

Only a few analogues of CoA have been prepared in which the CoA moiety has been modifed in ways not involving truncation to a pantetheine or simpler analogue. The oldest examples include the series of CoA analogues **117** modifed in the cysteamine moiety described in section VI.A.2. (Figure 36). Analogues in which the hydroxyl group of the pantoate moiety is oxidized or inverted were prepared using the general coupling methodology of Moffatt and Khorana, followed by the enzymatic hydrolysis of the cyclic phosphate as developed by Michelson.[279] These two analogues inhibited acetyl-CoA carboxylase with almost no change in K_i value relative to palmitoyl-CoA.[191] Guano-CoA and inosino-CoA, in which the adenine base is replaced with guanine or inosine, have also been prepared using the general Moffatt and Khorana method.[237,280] The K_i of palmitoyl-

inosino-CoA for acetyl-CoA carboxylase was only 2-fold higher than the K_i for palmitoyl-CoA. 2′-Azido-S-palmitoyl[^{32}P]CoA, having an azido substituent on the adenine base, was prepared and used as a photoaffinity label for proteins from beef heart mitochondria.[65] 2-Azidodephospho-CoA was initially prepared using the general method of Michelson and acylated using the N-hydroxysuccinimide ester of palmitic acid. Phosphorylation was achieved in about 10% yield using [^{32}P]H_3PO_4, though the product was not characterized.[65] *S*-Benzoyl-8-azidodephospho-CoA was prepared from 8-azido-AMP and *S*-benzoylphosphopantetheine using the Michelson method and was used as a photoaffinity label for acyl-CoA:glycine N-acyltransferase.[182]

Analogues of CoA have also been reported in which an extra methylene group is present in the cysteamine[281,282] or the β-alanine moiety[249] and an analogue in which the amide bond has been replaced with a pair of methylene groups.[249] The acetyl derivative of the homocysteamine analogue is a poor substrate for carnitine acetyltransferase with a V_{max} less than 1% that with acetyl-CoA and exhibited no measurable activity with chloramphenicol acetyltransferase or citrate synthase, though it bound to all three enzymes with affinity decreased only 2−4-fold relative to acetyl-CoA.[283] The deamide carboxylate analogue was found to be a more than 10^4-fold poorer inhibitor of citrate synthase than the previously reported carboxylate analogue **123b** (Figure 39), from which it differs only in replacement of an amide bond with an ethylene group.

VII. Acyl–Acyl Carrier Protein and Analogues

Coenzyme A analogues and derivatives have also been used in the preparation of the corresponding acyl carrier protein (ACP) analogues and derivatives, using the *E. coli* holo-ACP synthase. This enzyme catalyzes transfer of the phosphopantetheine moiety from CoA to a serine hydroxyl group of the apo-form of ACP to form the active holo-form of ACP **150a** (Figure 48).[284] The enzyme has been shown to catalyze the transfer of modified phosphopantetheine groups from CoA analogues **149** to form the corresponding ACP analogues, including the homocysteamine (**150b**) and acetonyl dethia (**150c**) analogues.[281] The enzyme has also been shown to accept acyl derivatives of CoA including α-aminoacyl thioesters to form the corresponding ACP thioesters **150d** and α-aminothioesters **150e**.[285,286] Acyl-ACPs prepared by this method have been used as substrates for polyketide synthases and nonribosomal peptide synthetases.[285,286]

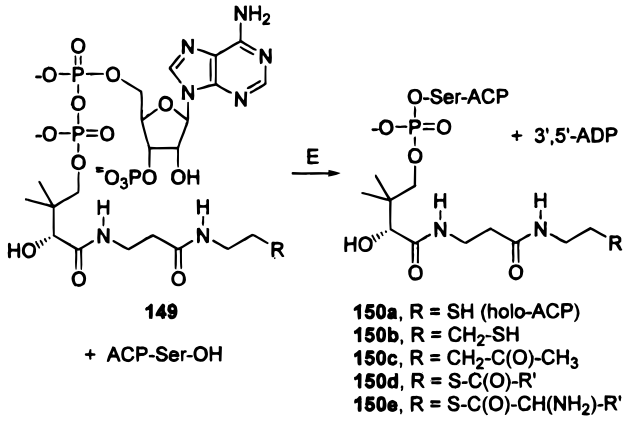

Figure 48. Enzymatic synthesis of holo acyl carrier protein (ACP) and analogues. E = holo-ACP synthase.

VIII. Summary

The use of various thioesters, thioethers, and other derivatives of CoA as inhibitors and mechanistic probes of CoA ester-utilizing enzymes is fairly well developed and has been highly valuable in the elucidation of mechanistic details. The synthesis and study of analogues which cannot be made by simple derivatization of CoA is much less well developed, in part because convenient methods for the synthesis of such analogues are only beginning to be developed. It is expected that both new and existing analogues of natural CoA esters will continue to be very valuable in the study of the enzymes that use CoA or CoA esters as substrates.

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X. References

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