Synthesis of Novel Analogs of Acetyl Coenzyme A: Mimics of Enzyme Reaction Intermediates

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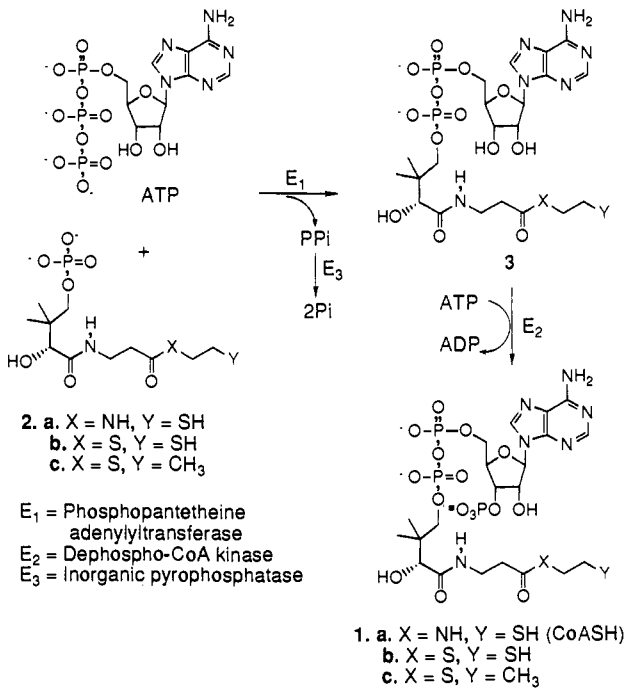
Abstract: An improved method for the synthesis of analogs of coenzyme A (CoA) and its thioesters, which are modified in the thiol or thioester moiety, has been developed using a combination of chemical and enzymatic reactions. The enzymes catalyzing the last two steps of CoA biosynthesis were used to prepare a CoA analog (**1c**) in which an amide bond is replaced by a thioester bond and the thiol group is replaced by a methyl group. Reaction of **1c** with a primary amine in aqueous solution results in aminolysis of the thioester linkage to form the desired CoA analog. Reaction with different amines permits the introduction of a variety of functional groups in place of the normal thiol or thioester group. This methodology has been used in the synthesis of five new analogs of acetyl-CoA in which the thioester sulfur is replaced by a methylene group and the acetyl group is replaced by carboxylate (**14a**), nitro (**14b**), carboxamide (**14c**), methyl sulfoxide (**14d**), and methyl sulfone (**14e**) groups. **14a–c** were designed to mimic the possible enolate or enol intermediate in the reaction of citrate synthase and related enzymes. **14a** and **14c** are potent inhibitors of citrate synthase, with K_i values 1000- and 570-fold lower than the K_m for acetyl-CoA, respectively. CD titrations indicate that **14a** and **14c** have low affinity for citrate synthase in the absence of oxaloacetate, consistent with their recognition as enol or enolate analogs. **14b** is a poor inhibitor of citrate synthase, with affinity slightly lower than that for acetyl-CoA. These results are consistent with generation of the enol form of acetyl-CoA as the nucleophilic intermediate in the reaction of citrate synthase. **14d** and **14e** were designed to mimic the tetrahedral intermediate or transition state in the reaction of chloramphenicol acetyltransferase and related acetyl-CoA-dependent acetyltransferases. Both compounds are poor inhibitors of chloramphenicol acetyltransferase, with affinities slightly lower than that of acetyl-CoA, indicating that these compounds are not good mimics of the enzyme-bound tetrahedral intermediate or transition state.

Cozenzyme A (CoA, **1a**) and its thioesters are involved in a variety of metabolic functions including glycolysis, fatty acid β-oxidation, and numerous biosynthetic pathways.[1] It has been estimated that 4% of the known enzymes require CoA or a CoA ester as substrate.[2] These include enzymes of pharmaceutical importance such as HMG-CoA reductase,[3] acyl-CoA:cholesterol acyltransferase,[4] myristoyl-CoA:protein *N*-myristoyltransferase,[5] and ATP citrate lyase.[6]

We recently reported a combined chemical and enzymatic synthesis of CoA analogs.[7] In this approach, the enzymes catalyzing the last two steps in CoA biosynthesis were used to prepare a coenzyme A analog **1b**, having an amide bond replaced by a thioester (Scheme 1). Aminolysis of the thioester bond of compound **1b** with the amine **5** formed the dethio (carba) analog of acetyl-CoA (acetonyldethio-CoA, **4**), as shown in Scheme 2. It was envisioned that by aminolysis with a variety of amines, **1b** could serve as a versatile synthon for a wide range of CoA and CoA ester analogs modified in the thiol/thioester portion of the molecule. Since the specific functionality of interest is introduced in a final chemical step, each analog would not be subject to substrate specificity limitations of the CoA biosynthetic enzymes.

Scheme 1



CoA esters are readily prepared by chemical[8] or enzymatic[9] acylation of CoA. Unnatural CoA esters have been useful in mechanistic studies of CoA ester utilizing enzymes.[10,11] Thioether analogs of CoA esters have been prepared by alkylation of CoA.[12]

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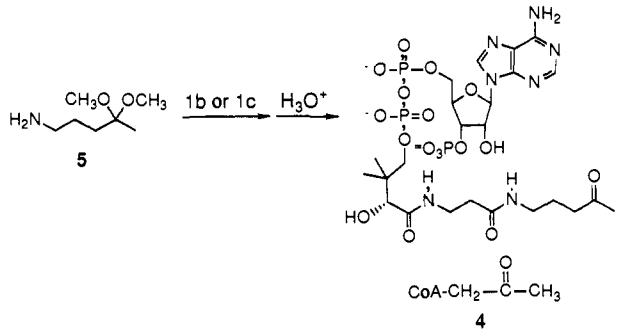
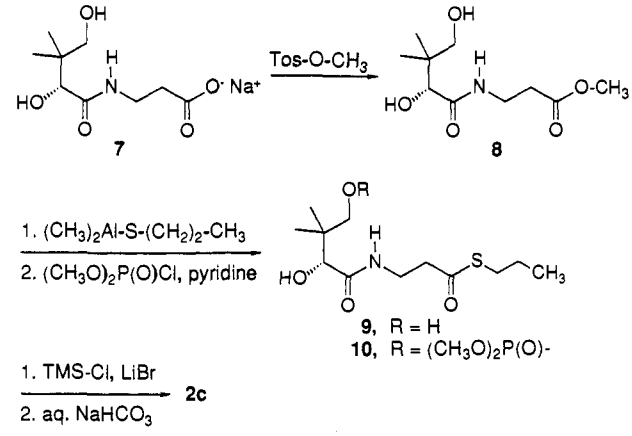
Scheme 2



Scheme 3



Such compounds have shown some utility in mechanistic studies but generally have lower binding affinities for the target enzyme than the natural substrate. A more interesting example of a CoA thioester is carboxymethyl-CoA, which is a potent inhibitor of citrate synthase.[12] This compound has been viewed as an analog of the enolate or enol form of acetyl-CoA **6**, despite its nonisosteric nature relative to acetyl-CoA.[12-15] Another very interesting class of CoA ester analogs is the dithioesters.[16,17] The dithioester analog of acetyl-CoA has greatly enhanced acidity of the acetyl methyl hydrogens (pK_a 12.5) and has been used to study the mechanism of citrate synthase.[16,17]

The thioester is intimately involved in most of the enzyme-catalyzed reactions of CoA esters. Functionalization of natural CoA provides access to a limited number of analogs modified in the thioester portion. Many other potentially valuable CoA ester analogs cannot be prepared directly from CoA and thus require assembly of the CoA moiety. A few examples of ketone analogs of CoA esters, having the thioester sulfur atom replaced with a methylene group,[18,19] have been prepared, as has an amide analog of propionyl-CoA.[20] These analogs have been synthesized using chemistry analogous to the original chemical synthesis of CoA.[21] These compounds have received very limited attention, probably due to the difficulty of their synthesis. The combined chemical and enzymatic CoA analog synthesis developed in this lab[7] permits for the first time the routine synthesis of CoA ester analogs modified in the thioester functionality.

In our initially reported synthetic strategy, the ethanedithiol thioester intermediates **1b** and **2b** were not isolable as they existed as a mixture of interconverting species due to disulfide bond formation and thioester thiolysis reactions.[7] We report here the improvement of our synthesis by deletion of the terminal thiol group. We also report the use of this methodology in the synthesis of five new analogs of acetyl CoA (**14a–e**) and studies of these compounds as inhibitors and mechanistic probes of acetyl-CoA utilizing enzymes.

Results

Improved Synthesis of Coenzyme A Analogs. To overcome problems associated with the terminal thiol group of **2b**, the pantetheine phosphate analog **2c**, having the thiol group replaced with a methyl group, was prepared as shown in Scheme 3. The sodium salt of pantothenic acid **7** was reacted with methyl tosylate to form the methyl ester **8**.[22] **8** was converted to the propyl thioester **9** using a dimethyl(propylthio)aluminum reagent.[23] The aluminum reagent was prepared by reaction of trimethylaluminum with propanethiol. When this chemistry was performed with ethanethiol instead of propanethiol, separation of the ethyl thioester product from unreacted methyl ester **8** was difficult. This separation was much simpler with the propyl thioester **9**, thus this compound was used in further work. The primary hydroxyl group was selectively phosphorylated with dimethyl phosphorochloridate[24] to give the dimethyl phosphate **10**, which was demethylated using trimethylsilyl chloride and lithium bromide to form **2c**.[25]

Table 1. Kinetic Comparison of **2a**, **3a** vs **2c**, **3c** with CoA Biosynthetic Enzymes

	phosphopantetheine adenylyltransferase			dephosphocoenzyme A kinase	
	K_m (mM)	relative V_{max}		K_m (mM)	relative V_{max}
2a	0.19	1.0	**3a**	0.12	1.0
2c	0.38	0.45	**3c**	0.13	0.90

The enzymes used in this work were obtained from *Brevibacterium ammoniagenes* in a one-step purification (DEAE Sepharose chromatography) of the crude cell extract.[26,27] In kinetic analysis of phosphopantetheine adenylyltransferase in a coupled assay with pyrophosphate-dependent phosphofructokinase,[28] **2c** gave a V_{max} near half that obtained with the natural substrate **2a** and a 2-fold higher K_m, as indicated in Table 1. The intermediate dephospho-CoA analog **3c** was isolated from a preparative phosphopantetheine adenylyltransferase reaction and found to be almost as good as natural dephospho-CoA **3a** as a substrate for dephospho-CoA kinase, as also shown in Table 1. For preparative work, fractions from the DEAE Sepharose chromatography containing the two desired enzyme activities were combined and immobilized in polyacrylamide gel.[29] **2c** was efficiently converted to the corresponding CoA analog **1c** in a preparative reaction using the enzymes of Scheme 1 with 1 equiv of ATP. Phosphoenolpyruvate and pyruvate kinase were included

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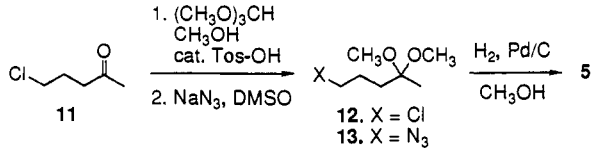
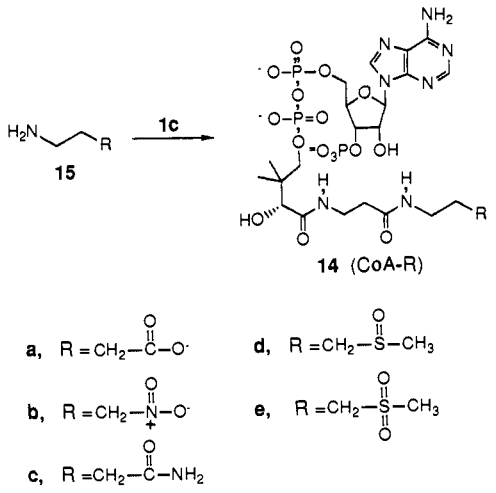
Scheme 4



Scheme 5

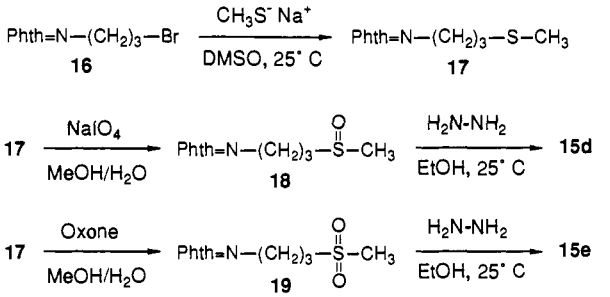


for regeneration of ATP from the ADP formed in the kinase step.[30] Inorganic pyrophosphatase was included to hydrolyze pyrophosphate, thus making the adenylyltransferase step irreversible. Upon completion of the reaction, the immobilized enzymes were recovered and reused. After three uses, the kinase activity was too low for further use of the immobilized enzyme. A total of about 1 g of **1c** was obtained in three preparative reactions using the enzymes obtained from a 12-L culture of *B. ammoniagenes*. The product **1c** was purified by anion-exchange chromatography followed by reverse-phase HPLC.

Synthesis of Analogs of Acetyl-CoA. The previously reported dethia analog of acetyl-CoA **4** was prepared from **1c** as shown in Scheme 2. The amine **5** was prepared as shown in Scheme 4.[7] This involved carbonyl protection of 5-chloro-2-pentanone (**11**), nucleophilic displacement of chloride by azide, and reduction of the azide to the primary amine **5**. The aminolysis reaction (Scheme 2) was performed with a 1.9 M concentration of the amine **5** at pH 10.2 at room temperature for 20 h. About 50% deprotection of the carbonyl occurred during the course of the aminolysis reaction. Carbonyl deprotection was completed upon acidification of the reaction mixture of pH 4.5. **4** was purified by reverse-phase HPLC and isolated in 50% yield.

Five additional new analogs of acetyl-CoA **14a–e** were prepared by reaction of **1c** with the amines **15a–e** as shown in Scheme 5. The nitro and amido amines **15b,c** were prepared according to literature methods.[31,32] The sulfoxide and sulfone **15d,e** were prepared via a common intermediate **17**, as shown in Scheme 6. **17** was prepared by reaction of **16** with thiomethoxide anion and converted to the sulfoxide **18** and sulfone **19** according to standard oxidation methods.[33,34] Deprotection by reaction with hydrazine in ethanol gave the amines **15d** and **15e**.[35] The aminolysis reactions of Scheme 5 were all carried out near pH 10 with an amine concentration near 1.0 M. Reaction times were typically about

Scheme 6



Scheme 7

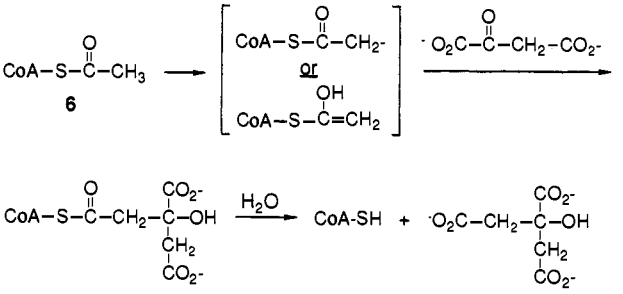


Table 2. Affinity of Citrate Synthase for Acetyl-CoA Analogs

compd	K_i (or K_m) (M)	K_d (binary complex) (M)
6[a]	1.6×10^{-5} (K_m)	1.0×10^{-4}
4[b]	1.6×10^{-5}	
14a	1.6×10^{-8}	1.4×10^{-4}
14b	1.6×10^{-5}	
14c	2.8×10^{-8}	1.3×10^{-4}

[a] References 40 and 15. [b] Reference 18.

24 h at room temperature. Analytical HPLC consistently showed >90% of the desired aminolysis products **14**, with the only other detectable product being the acid resulting from thioester hydrolysis, which represented <10% of the product. Since the sulfoxide **15d** was racemic, the corresponding CoA analog **14d** was formed as a mixture of epimers. Compounds **14a–e** were purified by reverse-phase HPLC, with typical purified yields ranging from 50 to 80%.

Binding Studies of Citrate Synthase to 14a–c. The acetyl-CoA analogs **14a–c** were tested as inhibitors of the enzyme citrate synthase, which catalyzes the reaction shown in Scheme 7. Citrate synthase activity was assayed by measuring the formation of coenzyme A by monitoring the increase in absorbance at 412 nm upon reaction of coenzyme A with DTNB.[36] Inhibition constants were determined from Lineweaver–Burk plots of 1/rate versus 1/acetyl-CoA concentration at three concentrations of inhibitor. The K_i values, which represent dissociation constants for **14a–c** from the citrate synthase oxaloacetate complex, are shown in the second column of Table 2. The carboxylate analog **14a** was found to be a potent slow binding inhibitor of citrate synthase, with a K_i 1000-fold lower than the K_m for acetyl-CoA. When the enzyme was preincubated with **14a** in the absence of oxaloacetate followed by initiation of the reaction by addition of oxaloacetate, the slow binding phenomenon was still observed, indicating that oxaloacetate is required for strong binding. The half-life for dissociation of the enzyme–inhibitor complex upon dilution was about 40 s, as determined by recovery of enzyme activity. The nitro analog **14b** was found to be a quite poor inhibitor, with binding affinity similar to that of acetyl-CoA. The amide **14c** was found to be a potent inhibitor, with affinity only slightly lower than that of the carboxylate **14a**. Unlike with **14a**, slow binding was not observed with either **14b** or **14c**.

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Scheme 8

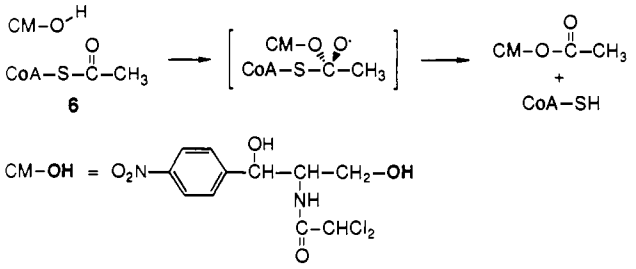

Table 3. Inhibition of Chloramphenicol Acetyltransferase by Acetyl-CoA Analogs

compd	K_i (or K_m) (M)	compd	K_i (or K_m) (M)
6	9.8×10^{-5} (K_m)	**14d**	2.6×10^{-4}
4	9.5×10^{-5}	**14e**	1.4×10^{-4}
14a	6×10^{-4}		

The affinity of CoA analogs **14a** and **14c** for citrate synthase without the cosubstrate oxaloacetate bound were determined by CD measurements of the change in ellipticity at 260 nm, as described by Kurz et al.[13] These binding constants are shown in the third column of Table 2. Both **14a** and **14c** showed greatly decreased affinity for citrate synthase in the absence of oxaloacetate, with K_d values almost identical to that of acetyl-CoA.

Inhibition Studies of Chloramphenicol Acetyltransferase with 4 and 14a,d, and e. The ketone (**4**), carboxylate (**14a**), sulfoxide (**14d**), and sulfone (**14e**) analogs were tested as inhibitors of chloramphenicol acetyltransferase, which catalyzes the reaction shown in Scheme 8. Chloramphenicol acetyltransferase was assayed by monitoring formation of coenzyme A by reaction with DTNB.[37] The results are shown in Table 3. All four compounds were poor inhibitors, with K_i values equal to or higher than the K_m for acetyl-CoA.

Discussion

The initially reported CoA analog synthesis procedure[7] has been substantially improved by replacement of the terminal thiol group with a methyl group. Synthesis of the phosphopantetheine analog **2c** is much simpler than the synthesis of **2b** described previously.[7] Furthermore, unlike the ethanedithiol monoesters, which were not isolable in pure form due to disulfide formation and thioester thiolysis reactions, the propyl thioesters **2c** and **1c** are readily isolated and purified. This facilitates better monitoring of the enzyme-catalyzed conversion of **2c** to **3c** and subsequently to **1c** for better optimization of conditions. As the concentration in the preparative enzyme reactor is more than 100-fold higher than the K_m for phosphopantetheine, the reaction proceeds at V_{max}, and thus the observed rate of the reaction with **2c** is almost half that obtained with the natural substrate **2a**. Since precise kinetic data have not been determined for **2b** due to its nonisolability, it is not clear what degree of the loss in rate is due to replacement of the amide bond with thioester and how much is due to replacement of the terminal thiol with a methyl group.

An initial obstacle to this synthsis was the isolation of two non-commercially available enzymes. Fortunately, for this work both enzymes were obtained in a simple one-step purification from *B. ammoniagenes* crude cell extract, thus the enzyme isolation is now a quite routine procedure. Enzymatic synthesis of CoA has been accomplished using *B. ammoniagenes* whole cells as catalyst, thus avoiding enzyme purification entirely.[27] However, it is expected that the thioester bonds central to the analog synthesis described here would not tolerate the high concentration of cellular nucleophiles of whole cells or crude cell extract, thus the partial purification is worthwhile and probably essential. Immobilization in polyacrylamide gel permits recovery and reuse of the enzymes.[29] However, the kinase gradually lost its activity, so its use was limited to three sequential preparative reactions, each requiring about 2 days reaction time. This was more efficient that a single larger scale reaction requiring a longer reaction time because longer reaction times resulted in a significant amount of thioester hydrolysis.

The aminolysis reactions are very efficient, resulting in high yields of the desired products. The only byproduct detected by analytical reverse-phase HPLC was the acid resulting from hydrolysis of the thioester, which consistently accounted for less than 10% of the product. This is not surprising given the established high reactivity of thioesters toward aminolysis relative to hydrolysis.[38] Introduction of the specific functionality of interest in a final chemical step avoids any possible substrate specificity limitations, demonstrating a useful concept in enzymatic synthesis which may have applications in other systems. Furthermore, this approach requires that only a single compound be carried through the multistep synthesis, with production of a sufficient quantity of the versatile CoA analog synthon **1c** for synthesis of a series of CoA analogs. Each individual analog requires only a single synthetic step from **1c** and the appropriate primary amine, except for the dethia analog **4** (Scheme 2), which requires an additional deprotection step. Another attractive feature of this synthetic approach is that the transformations performed enzymatically are highly analogous to what are probably the two major challenges in the chemical synthesis of CoA and CoA analogs, these being coupling of the phosphopantetheine and adenylate moieties and regiospecific introduction of the 3′-phosphate.[18-21]

Compounds **14a–e** were designed to mimic high-energy intermediates or transition states in the enzymatic-catalyzed reactions of acetyl-CoA. Most of the enzyme-catalyzed reactions of acetyl-CoA fit into two general classes. One is carbon–carbon bond-forming reactions represented by citrate synthase in Scheme 7.[39,40] These reactions are generally perceived to proceed through an enol or enolate intermediate. The other class of reactions of acetyl-CoA is acetyl transfer reactions, represented by chloramphenicol acetyltransferase in Scheme 8.[41-43] These reactions proceed through a tetrahedral transition state or intermediate, which is generated upon nucleophilic attack on the carbonyl carbon of the acetyl group. Some of these reactions proceed via acetyl-enzyme intermediates, with the initial nucleophilic group being an enzyme amino acid side chain.[10] It is expected that acetyl-CoA analogs which mimic the intermediates or transition states in enzymatic reactions of CoA esters may act as inhibitors of acetyl-CoA utilizing enzymes.[44,45]

14a–c are much better analogs of the enol and enolate forms of acetyl-CoA than compounds previously prepared by alkylation of the thiol group of CoA.[12] Replacement of the sulfur atom by a methylene group results in substitution of two carbon–sulfur bonds by shorter carbon–carbon bonds and may introduce conformational restrictions. However, these compounds are still better isosteric matches with acetyl-CoA than with carboxy-

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methyl-CoA and carbamoylmethyl-CoA, which have an extra atom between the CoA and acetate mimicking moiety, but have been utilized previously as presumed analogs of the enol or enolate form of acetyl-CoA.[12-15]

The nature of the intermediate in the reaction of citrate synthase and other enzymes catalyzing proton abstraction from carbon acids has received much recent attention. Gerlt et al. have presented arguments for an enol mechanism, pointing out that the rate of deprotonation of acetyl-CoA (pK_a 19) by an active site base with a pK_a of 7 or less should be too slow to account for the observed rates in reactions of acetyl-CoA.[46] Carboxymethyl-CoA, a presumed enol/enolate analog of acetyl-CoA formed by reaction of CoA with iodoacetic acid, is believed to bind to citrate synthase in protonated form, also suggesting an enol mechanism.[13,14] However, the significance of results based on carboxymethyl-CoA have been questioned due to its nonisosteric nature relative to acetyl-CoA.[12] Model studies have been reported presenting evidence for the feasibility of an enolate mechanism.[47,48] An enzyme-bound intermediate has been observed upon reaction of citrate synthase with the dithioester analog of acetyl-CoA, but it is unclear if this species is the enethiol or enethiolate form.[16,17] More recent explanations offer what may be viewed as a compromise mechanism in which the pK_a of the enol form of acetyl-CoA is matched by an amino acid acting as a hydrogen bond donor to the carbonyl oxygen of acetyl-CoA.[49,50] A proton is thus shared between the oxygen of the "enolic" intermediate and the mildly acidic amino acid (His-274 in citrate synthase[14]) in a very strong short hydrogen bond.

The 1000-fold greater binding affinity of **14a** for the citrate synthase–oxaloacetate complex than that of the substrate acetyl-CoA suggests that **14a** is a good mimic of a high-energy intermediate formed in the reaction. As **14a** is viewed as a mimic of the enolate form of acetyl-CoA, this might appear to support an enolate mechanism. However, **14a** may alternatively bind in protonated form as an enol mimic. The K_i for **14a** with citrate synthase has been found to decrease with decreasing pH. This is consistent with binding of **14a** in protonated form as an enol mimic but could also be explained by protonation of an amino acid residue in the enzyme active site.

The nitro analog **14b** is expected to mimic an enolate and not an enol, though it is a less ideal enolate mimic than the carboxylate due to its net neutral charge and the lower basicity and hydrogen bond accepting ability. The lack of strong inhibition of **14b** provides evidence against the formation of an enolate intermediate. The amide analog **14c** may be viewed as an enol mimic, with C=O in place of $C{=}CH_2$ and NH_2 in place of OH, though it is also possible that it binds as a ground-state analog with NH_2 in place of the methyl group of acetyl-CoA. **14c** is not expected to be sufficiently acidic (pK_a 15)[52] to form an enzyme-bound anion as an enolate mimic. The potent inhibition observed by **14c** may be further evidence for an enol intermediate.

Strong binding of **14a** and **14c** to citrate synthase occurs only in the presence of oxaloacetate. In the binary complex, the affinity for these analogs is virtually identical to the affinity for acetyl-CoA (Table 2). Citrate synthase catalyzes exchange of the methyl protons of acetyl-CoA with solvent only in the presence of oxaloacetate or the analog L-malate, indicating that citrate synthase forms the enolate or enol form of acetyl-CoA only when oxaloacetate is bound.[51] The high affinity for the amide and carboxylate analogs only when oxaloacetate is present further supports the recognition of **14a** and **14c** as enol or enolate mimics by citrate synthase.[53]

The sulfoxide **14d** and the sulfone **14e** were chosen as easily accessible analogs of acetyl-CoA designed to mimic the tetrahedral intermediate or transition state in the reaction of acetyltransferases. However, they were found to have binding affinities for chloramphenicol acetyltransferase lower than that of acetyl-CoA, suggesting that **14d** and **14e** are not good mimics of the tetrahedral species. Other tetrahedral functional groups such as sulfoximines and phosphonates have served as useful inhibitors of other enzymatic reactions proceeding through tetrahedral intermediates or transition states.[54,55] A possible explanation for the lack of enhanced affinity of the sulfoxide **14d** relative to aceyl-CoA for chloramphenicol acetyltransferase is that the methyl group is a poor recognition element for binding. Chloramphenicol acetyltransferase may thus not recognize the CoA moiety, the sulfoxide oxygen, and the methyl group as being in a pyramidal arrangement vs the planar-trigonal arrangement of acetyl-CoA. With the sulfone **14e**, potential hydrogen bonding of one oxygen atom to the base, which serves to deprotonate the hydroxyl nucleophile, and the other to the oxyanion binding site was expected to lead to enhanced binding. An explanation for the relatively low affinities of **14d** and **14e** for chloramphenicol acetyltransferase is that the sulfoxide and sulfone oxygens are not sufficiently basic or good enough hydrogen bond acceptors to form strong interactions with the enzyme. The carboxylate analog **14a** was tested as an inhibitor of chloramphenicol acetyltransferase since it was expected to be a good hydrogen bond acceptor, though it is not tetrahedral. However, **14a** was found to be an even weaker inhibitor than the sulfoxide and sulfone. This indicates that a hydrogen bond-accepting carboxylate group is not sufficient to mimic the tetrahedral intermediate or transition state. The ketone analog **4** was also tested for inhibition of chloramphenicol acetyltransferase. Not surprisingly, the binding affinity was almost identical to that of acetyl-CoA. The ketone is presumably not sufficiently electrophilic to form a stable hemiacetal with chloramphenicol in the enzyme active site.

This work has demonstrated a convenient and versatile synthetic approach to a variety of analogs of acetyl-CoA. The ability to design mimics of the intermediates of enzyme-catalyzed reactions of acetyl-CoA has also been demonstrated. The design and synthesis of analogs of the enol and enolate forms of acetyl-CoA as inhibitors of citrate synthase have been quite successful. These compounds are expected to be very useful for elucidating the mechanisms and structures of enzyme-bound intermediates of the reactions of citrate synthase and related acetyl-CoA utilizing enzymes. Initial efforts toward analogs of the tetrahedral intermediate or transition state in the reaction of chloramphenicol acetyltransferase have been less successful. However, the results appear to provide some insights into requirements for efficient mimics of the tetrahedral species. Studies are in progress to use the acetyl-CoA analogs described here and additional analogs to investigate the mechanisms of enzyme-catalyzed reactions of acetyl-CoA.

Experimental Section

General Experimental. Reagents were obtained from Aldrich, Sigma, or Mallinckrodt and used as supplied. Pyridine, methylene chloride (CH_2Cl_2), and acetonitrile (MeCN) were distilled from calcium hydride. For ^1H NMR experiments, TMS (0 ppm) and HOD (4.8 ppm) were used as internal references when $CDCl_3$ and D_2O were used as solvents, respectively. For ^{13}C NMR experiments, $CDCl_3$ (central peak at 77 ppm) and external dioxane (67.6 ppm) were used as references when

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$CDCl_3$ and D_2O were used as solvents, respectively. 200-MHz ^1H NMR and 50-MHz ^{13}C NMR experiments were performed using a Varian Gemini-200 spectrometer and 400-MHz ^1H, 100-MHz ^{13}C, and 161.9-MHz ^{31}P NMR experiments using a Varian XL-400. IR experiments were performed on a Perkin-Elmer 1605 FTIR. Analytical and preparative HPLC experiments were carried out using a Perkin-Elmer 250 HPLC with a PE LC-235 diode array detector and a gradient of methanol in aqueous potassium phosphate (50 mM, pH 4.5). Analytical HPLC was done using a Dynamax C-18 column (4.6 × 250 mm^2, 5 μm) with monitoring at 215 and 260 nm. The compound were eluted at a flow rate of 1 mL/min with 5% methanol for 2 min, followed by a linear gradient of methanol increasing to 60% over 12 min, and then maintained at 60% methanol. C-8 preparative HPLC was done using a Merck LiChroprep RP-8 column (2.5 × 31 cm^2, 40–63 μm) with monitoring at 215 and 285 nm. C-18 preparative HPLC was done on an Altex Ultrasil-ODS column (10 × 250 mm^2, 10 μm) with monitoring at 215 and 285 nm. Mass spectra analysis were performed at the University of California-Riverside Mass Spectrometry Facility, Riverside, CA, and UCSF Mass Spectrometry Facility, San Francisco, CA. Elemental analyses were performed at E + R Microanalytical Laboratory, Inc., Corona, NY, and Robertson Laboratory, Madison, NJ. The concentrations of acetyl CoA and CoA analog solutions were determined by ultraviolet absorbance using $\epsilon_{260} = 16.4 \times 10^3\ M^{-1}\ cm^{-1}$.

Pantothenic Acid Methyl Ester (8). The sodium salt of pantothenic acid (20 g, 83 mmol) was suspended in 150 mL of methanol. Methyl *p*-toluenesulfonate (15.4 g, 83.7 mmol) was added to yield a homogeneous solution.[32] The solution was heated to reflux overnight, concentrated to 50 mL *in vacuo*, and filtered to remove the precipitate. The filtrate was concentrated *in vacuo* to yield a viscous liquid. The liquid was dissolved in 100 mL of chloroform and 15 mL of ether, refiltered, and concentrated *in vacuo* to yield 20 g of crude material as a viscous yellow liquid. An analytical sample was purified by chromatography on silia gel using ethyl acetate/hexane (2:1) to yield a colorless viscous oil, R_f 0.14. ^1H NMR (200 MHz, $CDCl_3$): δ 0.91 (s, 3H), 1.02 (s, 3H), 2.55–2.61 (m, 2H), 3.18 (t, 1H, J = 5.9 Hz, exchanges with CD_3OD), 3.49–3.63 (m, 3H), 3.71 (s, 3H), 3.87 (d, 1H, J = 5.0 Hz, exchanges with CD_3OD), 4.02 (d, 1H, J = 5.1 Hz), 7.23 (br, 1H, exchanges with CD_3OD). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.18, 20.65, 33.65, 34.51, 39.02, 51.73, 70.70, 76.85, 172.56, 173.77. Anal. Calcd for $C_{10}H_{19}NO_5$: C, 51.49; H, 8.21; N, 6.00. Found: C, 51.68; H, 8.47; N, 6.11.

Pantothenic Acid Propyl Thioester (9). In a dry, round-bottom flask under a nitrogen atmosphere, dimethyl(propylthio)aluminum was generated by the slow addition of propanethiol (2.74 g, 35.9 mmol) to a cooled (0 °C) solution of trimethylaluminum (2 M in hexanes, 18 mL, 36 mmol) in 55 mL of dry CH_2Cl_2.[33] The dimethyl(propylthio)aluminum solution was stirred at 0 °C for 5 min and then at room temperature until gas evolution stopped (about 20 min). In a separate dry flask, compound **8** (3.0 g, 13 mmol) was dissolved in about 10 mL of dry toluene. The toluene was evaporated *in vacuo* to remove residual water, and compound **8** was redissolved in 20 mL of dry CH_2Cl_2. The dimethyl(propylthio)-aluminum solution was cannulated into the solution of **8**, and the reaction mixture was stirred overnight at room temperature under nitrogen. A solution of brine (10 mL) and 1 M HCl (35 mL) was added slowly. The reaction mixture was stirred for 5 min and was then extracted with ethyl acetate (3 × 75 mL). The combined organic extracts were washed sequentially with 1 M NaOH (50 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated *in vacuo* to yield a viscous oil. The product was purified by silica gel chromatography using ethyl acetate/hexane (2:1), R_f 0.21, to give a viscous, colorless oil (1.25 g, 35% yield). ^1H NMR (200 MHz, $CDCl_3$): δ 0.91 (s, 3H), 0.97 (t, 2H, J = 7.3 Hz), 1.03 (s, 3H), 1.51–1.69 (m, 2H), 1.98 (br, 1H, exchanges with CD_3OD), 2.79–2.91 (m, 4H), 3.49–3.61 (m, 5H), 4.02 (s, 1H), 7.20 (t, 1H, J = 5.6 Hz, exchanges with CD_3OD). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.06, 20.02, 21.05, 22.63, 30.70, 34.89, 39.12, 43.11, 71.18, 77.54, 173.45, 199.00. Anal. Calcd for $C_{12}H_{23}NO_4S$: C, 51.96; H, 8.36; N, 5.05; S, 11.56. Found: C, 51.99; H, 8.29; N, 5.14; S, 11.31.

***S*-Propyl Thiopantothenate 4′-(Dimethyl phosphate) (10).** Dimethyl phosphorochloridate was generated from dimethyl phosphite (2.5 mL, 27 mmol) and *N*-chlorosuccinimide (3.63 g, 27.3 mmol) in 35 mL of dry toluene.[34] The mixture was stirred under nitrogen for about 2 h, during which time heat was generated and succinimide precipitated from solution. The solution was cooled in an ice bath as necessary to prevent refluxing. The resulting solution was used immediately in the next step.

Compound **9** (2.52 g, 9.10 mmol) was dissolved in dry pyridine (10 mL). The pyridine was evaporated *in vacuo* to remove residual water. The residue was dissolved in 40 mL of dry pyridine and cooled in –40 °C bath (MeCN–dry ice). The dimethyl phosphorochloridate solution was cannulated into the reaction vessel over 10 min. The reaction mixture was allowed to warm slowly to room temperature and was stirred overnight. Water (15 mL) was added, and the mixture was concentrated under reduced pressure to yield a brown oil, which was dissolved in 100 mL of ethyl acetate and washed sequentially with 1 M sulfuric acid (2 × 25 mL), 1 M sodium bicarbonate (2 × 25 mL), and saturated sodium sulfate (1 × 25 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by flash chromatography on silica gel using ethyl acetate, R_f 0.15, in ethyl acetate/hexane (2:1), yielding 1.78 g of a colorless oil in 51% yield. ^1H NMR (200 MHz, $CDCl_3$): δ 0.85 (s, 3H), 0.96 (t, 3H, J = 7.3 Hz), 1.15 (s, 3H), 1.51–1.69 (m, 2H), 2.78–2.91 (m, 4H), 3.53–3.69 (m, 3H), 3.76–3.84 (m, 6H), 4.00 (d, 1 H, J = 5.6 Hz), 4.15 (dd, 1H, J = 8.1, 10.2 Hz), 4.58 (d, 1Hz, J = 5.8 Hz, exchanges with CD_3OD), 7.32 (br, 1H, exchanges with CD_3OD). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.07, 18.14, 20.80, 22.68, 30.63, 34.83, 39.49 (d, J = 4.8 Hz), 43.25, 54.59 (d, J = 6.1 Hz), 73.19, 73.77 (d, J = 6.3 Hz), 172.29, 198.49. HRMS (FAB): $[M + H]^+$ calcd for $C_{14}H_{29}NO_7PS$ m/z 386.1402, found 386.1408. Anal. Calcd for $C_{14}H_{28}NO_7PS$: C, 43.63; H, 7.32; N, 3.63; P, 8.04; S, 8.32. Found: C, 43.53; H, 7.54; N, 3.79; P, 7.81; S, 8.12.

***S*-Propyl Thiopantothenate 4′-Phosphate (2c).** Compound **10** (1.20 g, 3.12 mmol) and lithium bromide (0.56 g, 6.5 mmol) was added to 4 mL of dry MeCN. The MeCN was evaporated under reduced pressure to remove residual water. Dry MeCN (6.0 ML) and trimethylsilyl chloride (4.0 mL, 31 mmol) were added to the residue to yield a clear solution. The reaction mixture was heated overnight with stirring at 50 °C under nitrogen. White crystals formed and pervaded the solution. ^1H NMR analysis of the reaction mixture showed complete disappearance of the starting material. The reaction mixture was filtered, evaporated, treated with methanol, and reevaporated. The residue was dissolved in water, brought to pH 4.5 with lithium hydroxide (0.5 M), and filtered though cotton. Lyophilization yielded a tan foam containing inorganic salts. The crude material was dissolved in water, centrifuged to remove insolubles, and purified by preparative C-18 reverse-phase HPLC using a gradient of methanol in aqueous phosphate (50 mM, pH 4.5). While being monitored at 215 and 275 nm, compound **2c** was eluted at a flow rate of 3 mL/min with 10% methanol for 5 min, followed by a linear gradient of methanol increasing to 70% over 40 min. Fractions containing **2c** were pooled and lyophilized. Compound **2c** was assayed with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, $\epsilon_{412} = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$, in 0.1 M Tris buffer, pH 8, 0.1 M hydroxylamine hydrochloride, 30 mM $NaHCO_3$), and the yield was 0.11 mmol (34% yield). The material containing inorganic salts was used for the enzymatic synthesis of compound **1c**. For analysis, residual phosphates were removed by solid-phase extraction on a C-18 reverse-phase SPICE cartridge from Rainin to yield the free acid. An aqueous solution (2 mL) of the HPLC residue of compound **2c** was acidified with 1 M H_3PO_4 to pH 2 (vs pH paper) and was loaded onto the SPICE pack (prewashed with methanol and then 3 mM HCl). The SPICE pack was washed with 2 mL of 3 mM HCl, and **2c** was eluted with 25% methanol in water and lyophilized. Analytical HPLC: t_R = 19.5 min, λ_{max} = 235 nm. ^1H NMR (as free acid, 400 MHz, D_2O): δ 0.84 (s, 3H), 0.88 (t, 3H, t, J = 7.5 Hz), 0.92 (s, 3H), 1.48–1.57 (m, 2H), 2.80–2.85 (m, 4H), 3.47–3.51 (m, 2H), 3.58 (dd, 1H, J = 4.5, 9.5 Hz), 3.79 (dd, 1H, J = 4.5, 9.2 Hz), 3.97 (s, 1H). ^{13}C NMR (as free acid, 100 MHz, D_2O): δ 12.09, 18.08, 20.22, 21.78, 30.37, 34.87, 37.86 (d, J = 7.9 Hz), 42.33, 71.07 (d, J = 5.6 Hz), 73.88, 174.33, 202.17. HRMS (FAB): $[M + H]^+$ calcd for $C_{12}H_{25}NO_7PS$ m/z 358.1089, found 358.1078.

Phosphopantetheine Adenylyltransferase (PPAT) and Dephospho-CoA Kinase (DPCK) Preparation and Immobilization. A 12-L culture of *B. ammoniagenes* was grown and the crude extract prepared and chromatographed on DEAE Sepharose as described previously.[36] The fractions containing PPAT activity were pooled (0.40–0.43 M NaCl, 63 mL, 0.026 unit/mL, 0.019 unit/mg), and the Tris buffer was replaced with HEPES buffer (50 mM, pH 7.5, 5 mM DTT) by two serial dilutions via ultrafiltration (PM10 diaflo membrane from Amicon). The fractions containing DPCK activity (0.36–0.39 M NaCl, 56 mL, 0.08 unit/mL, 0.028 unit/mg) were pooled and dialyzed vs HEPES buffer (20 mM, pH 8, 5 mM DTT, 0.1 mM PMSF, 10 mM $MgCl_2$, 1 L). Each enzyme solution was concentrated to 1 mL (ultrafiltration PM10 membrane, followed by Centriprep 10 from Amicon). The PPAT and DPCK were then incubated separately in 0.1 M HEPES buffer, pH 8, 0.07 mM dephospho-CoA, 0.7 mM $MgCl_2$, 2 mM DTT, 0.07 mM ATP (DPCK only), and 0.07 mM inorganic pyrophosphate (PPAT only) to protect the active sites, and the enzymes were coimmobilized in 1.5 g of PAN-950[39]

(yielding 36 mL of gel suspension; PPAT, 0.012 unit/mL, 27%; DPCK, 0.021 unit/mL, 17%).

Adenosine 5'-(Trihydrogen diphosphate) 3'-(Dihydrogen phosphate) 5'-[(*R*)-3-Hydroxy-4-[[3-[propylthio]-3-oxopropyl]amino]-2,2-dimethyl-4-oxobutyl] ester (1c). Compound **2c** (0.45 mmol) was dissolved in HEPES buffer (0.1 M, 16 mL, pH 7.5). Dephospho-CoA pyrophosphorylase and dephospho-CoA kinase (0.13 and 0.23 unit, respectively), coimmobilized in polyacrylamide gel, were added to the solution, which was then sparged with nitrogen for 15 min. ATP (0.54 mmol, dissolved in 2.7 mL of water, pH adjusted to 7 with KOH), $MgCl_2$ (0.54 mmol), and inorganic pyrophosphatase (10 units) were added, and the reaction mixture was swirled at 30 °C. After 5 h, pyruvate kinase (22 units) and phosphoenol pyruvate (0.45 mmol as the potassium salt, pH adjusted to 7 with KOH) were added. The progress of the reaction was monitored via HPLC and was judged complete by the disappearance of compound **2c** (about 2 days). The reaction mixture was centrifuged and the supernatant decanted. The immobilized enzymes were washed twice with HEPES buffer (0.1 M, 5 mL). The combined washes and supernatant were filtered through a 0.45-μm nylon filter and lyophilized. The crude material (2.6 g) was dissolved in 3 mM HCl (80 mL), and the solution was adjusted to pH 3.5 with 1 M phosphoric acid. At 4 °C, the solution was loaded onto a DEAE cellulose column (2.5 × 17 cm^2) which had been equilibrated with 3 mM HCl. The column was washed with 100 mL of 3 mM HCl and then with a linear gradient of lithium chloride (0–0.2 M) in 3 mM HCl (total volume of gradient 1 L). Individual fraction of 18 mL each were collected and analyzed by HPLC. Compound **1c** eluted between 0.11 and 0.14 M lithium chloride. Fractions containing compound **1c** were adjusted to pH 4 with 1 M KH_2PO_4 and were lyophilized (0.11 g of **1c**, 31% yield). Compound **1c** was further purified by preparative C-8 reverse-phase HPLC using a gradient of methanol in aqueous phosphate (50 mM, pH 4.5). Compound **1c** was eluted at a flow rate of 3 mL/min with 5% methanol for 10 min, followed by a linear gradient of methanol increasing to 60% over 60 min. Fractions containing **1c** were pooled and lyophilized. For analysis, residual phosphates were removed by solid-phase extraction on a SPICE cartridge (as outlined for compound **2c**) to yield the free acid. Analytical HPLC: t_R = 16.6 min, λ_{max} = 260 nm. ^{1}H NMR (as free acid, 400 MHz, D_2O): δ 0.80 (s, 3H), 0.87 (t, 3H, J = 7.3 Hz), 0.93 (s, 3H), 1.43–1.52 (m, 2H), 2.78–2.84 (m, 4H), 3.44–3.48 (m, 2H), 3.54–3.58 (m, 1H), 3.81–3.86 (m, 1H), 4.01 (s, 1H), 4.28 (br, 2H), 4.60 (br, 1H), 6.19 (d, 1H, J = 5.2 Hz), 8.42 (s, 1H), 8.65 (s, 1H). ^{13}C NMR (as free acid, 100 MHz, D_2O): δ 12.03, 17.72, 20.40, 21.73, 30.32, 34.82, 37.90 (d, J = 7.0 Hz), 42.30, 64.68 (d, J = 2.5 Hz), 71.66 (d, J = 5.9 Hz), 73.53 (d, J = 4.5 Hz), 73.68–73.76 (m, 2 carbons), 82.94–83.05 (m, 1 carbon), 87.01, 118.00, 142.00, 144.35, 147.99, 149.37, 202.12. HRMS (FAB): $[M + H]^+$ calcd for $C_{22}H_{38}N_{16}O_{16}P_3S$ m/z 767.1278, found 767.1323.

5-Chloro-2,2-dimethoxypentane (12). Technical grade 5-chloro-2-pentanone (**11**) (5.0 g, 41 mmol) was converted to its dimethyl ketal (**12**) by stirring overnight in methanol with trimethyl orthoformate (9.0 g, 85 mmol) and a catalytic amount of *p*-toluenesulfonic acid (~10 mg). The material was concentrated under reduced pressure and vacuum filtered through a Celite and silica gel mixture to remove dark material. The Celite/silica gel mixture was washed with ether. Concentration of the combined filtrate and washings under reduced pressure yielded **12** (6.3 g, 92% yield) as a dark liquid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (s, 3H), 1.71–1.89 (m, 4H), 3.18 (s, 6H), 3.56 (t, 2H, J = 6.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.75, 27.46, 33.73, 45.00, 47.90, 101.17.

5-Azido-2,2-dimethoxypentane (13). 5-Chloro-2,2-dimethoxypentane (**12**) (3.21 g, 19.3 mmol) was dissolved in dimethyl sulfoxide (20 mL) with sodium azide (2.34 g, 36.0 mmol) and stirred at 50 °C overnight. Water (45 mL) and ether (60 mL) were added to the reaction mixture, and the phases were separated. The aqueous phase was extracted with ether (2 × 45 mL), and the combined organic layers were washed with water (30 mL) and dried over $MgSO_4$. Concentration under reduced pressure yielded an orange oil. The oil was purified by silica gel chromatography in hexane/ether (4:1), R_f 0.39, to yield 2.68 g of **13** (80% yield) as a slightly yellow-colored liquid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (s, 3H), 1.58–1.72 (m, 4H), 3.18 (s, 6H), 3.31 (t, 2H, J = 6.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.76, 23.83, 33.18, 47.97, 51.48, 101.27.

5-Amino-2,2-dimethoxypentane (5). To a solution of 5-azido-2,2-dimethoxypentane (**13**) (1.63 g, 9.42 mmol) in methanol was added palladium on activated carbon (0.21 g, Degussa type, 10% Pd content, 50% water content). The mixture was stirred at room temperature under hydrogen overnight, after which TLC analysis (hexane/ether (4:1), R_f azide 0.39) showed no residual azide. The reaction mixture was filtered through sintered glass and concentrated under reduced pressure to yield **5** (1.24 g, 89% yield) as a colorless liquid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (s, 3H), 1.43–1.69 (m, 6H), 2.71 (t, 2H, J = 6.8 Hz), 3.18 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.77, 28.41, 33.66, 42.31, 47.92, 101.61.

3-Nitropropylamine (15b). Compound **15b** was prepared as the hydrochloride as described previously.[41] ^{1}H NMR (200 MHz, D_2O): δ 2.28–2.42 (m, 2H), 3.10–3.18 (m, 2H), 4.66 (t, 2H, J = 6.4 Hz).

4-Aminobutanamide (15c). At 100 °C, sodium cyanide (4.56 g, 93.0 mmol) was stirred in 25 mL of dimethylformamide with 3-chloropropionamide (10 g, 93 mmol) for 24 h. The solution was filtered, concentrated to 15 mL, and refiltered. Toluene (50 mL) was added, and the crude solid was removed by filtration. The product was recrystallized from ethyl acetate and pentane to yield 3-cyanopropanamide (2.41 g, 26% yield), mp 92–94 °C (lit.[56] mp 94 °C). ^{1}H NMR (200 MHz, D_2O): δ 2.66–2.71 (m). 4-Aminobutanamide hydrochloride was prepared from 3-cyanopropionamide as described previously.[42] ^{1}H NMR (200 MHz, D_2O): 1.87–2.02 (m, 2H), 2.41 (t, 2H, J = 7.4 Hz), 3.03 (t, 2H, J = 7.7 Hz).

Methyl 3-(*N*-Phthalylamino)propyl Sulfide (17). *N*-(3-Bromopropyl)-phthalimide (8.69 g, 32.4 mmol) was dissolved in 25 mL of DMSO. Sodium thiomethoxide (2.50 g, 35.7 mmol) was added, and the mixture was allowed to stir at 25 °C overnight. Water (5 mL) was added, and the mixture was stirred for 10 min. The mixture was then diluted with 100 mL of water and extracted with diethyl ether (5 × 50 mL). The organics were combined and washed with water (50 mL), dried over $MgSO_4$, filtered, and evaporated to give the sulfide (7.00 g, 29.8 mmol) as a white solid in 92% yield with no further purification. IR ($CDCl_3$): 2919.6, 1773.1, 1712.8, 1438.4, 1397.0, 1367.1 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.98 (quintet, 2H, J = 7.2 Hz), 2.10 (s, 3H), 2.55 (t, 2H, J = 7.3 Hz), 3.79 (t, 2H, J = 7.1 Hz), 7.70–7.87 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.99, 27.50, 30.99, 36.66, 122.80, 131.69, 133.58, 167.85. Anal. Calcd for $C_{12}H_{13}NO_2S$: C, 61.25; H, 5.57; N, 5.95; S, 13.63. Found: C, 61.25; H, 5.75; N, 6.16; S, 13.39.

Methyl 3-(*N*-Phthalylamino)propyl Sulfoxide (18). Compound **17** (2.44 g, 10.4 mmol) was dissolved in 20 mL of MeOH, and water was added until the solution began to get cloudy. Sodium periodate (2.33 g, 10.9 mmol) was added, and the solution was allowed to stir at 25 °C overnight. The solution was diluted with 100 mL of water and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried with $MgSO_4$, filtered, and evaporated to give the sulfoxide (2.48 g, 9.87 mmol) as a white solid in 95% yield with no further purification required. IR ($CDCl_3$): 2943.9, 1773.4, 1712.7, 1438.5, 1397.7, 1367.7, 1043.2 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.18 (quintet, 2H, J = 7.6 Hz), 2.58 (s, 3H), 2.71–2.79 (m, 2H), 3.87 (t, 2H, J = 6.6 Hz), 7.72–7.89 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.01, 36.55, 38.47, 51.63, 123.10, 131.60, 133.95, 167.01. Anal. Calcd for $C_{12}H_{13}NO_3S$: C, 57.35; H, 5.21; N, 5.57; S, 12.76. Found: C, 57.19; H, 5.41; N, 5.79; S, 12.44.

Methyl 3-Aminopropyl Sulfoxide Hydrochloride (15d). Compound **18** (0.78 g, 3.10 mmol) was dissolved in 2.5 mL of EtOH. Hydrazine monohydrate (0.158 mL, 3.25 mmol) was added and the solution stirred at 25 °C for 4 h. Concentrated HCl (0.31 mL, 3.72 mmol) was added, and stirring was continued for 30 min. Water (10 mL) was added, and the precipitate was removed by filtration. The solution was concentrated *in vacuo* to 5 mL and refiltered. An additional 5 mL of water was added, and the solution was concentrated to 5 mL and refiltered. This process was repeated until no precipitate formed. The solution was then lyophilized, and the resulting oil was recrystallized from EtOH/acetone to give the hydrochloride salt (0.11 g, 0.67 mmol) as a clear oil in 36% yield. IR (thin film): 3357.0, 3292.0, 2929.8, 2862.5, 1664.9, 1602.6, 1024.2 cm^{-1}. ^{1}H NMR (200 MHz, D_2O): δ 2.14 (quintet, 2H, J = 7.7 Hz), 2.73 (s, 3H), 2.86–3.07 (m, 2H), 3.16 (t, 2H, J = 7.6 Hz). ^{13}C NMR (50 MHz, D_2O): δ 25.59, 38.05, 40.26, 51.37. Anal. Calcd for $C_4H_{12}ClNOS$: C, 30.47; H, 7.67; N, 8.88; S, 20.34. Found: C, 30.18; H, 7.90; N, 8.62; S, 19.99.

Methyl 3-(*N*-Phthalylamino)propyl Sulfone (19). Compound **17** (2.44 g, 10.4 mmol) was dissolved in 40 mL of MeOH. Oxone (9.56 g, 15.6 mmol) dissolved in 40 mL of water was added, and the solution was allowed to stir at 25 °C overnight. The solution was diluted with 100 mL of water and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried with $MgSO_4$, filtered, and evaporated to give the sulfone (2.48 g, 9.87 mmol) as a white solid in 95% yield with no further purification. IR ($CDCl_3$): 2924.3, 1772.9, 1715.2, 1438.3, 1398.4, 1362.3, 1313.1, 1140.5 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.17–2.32 (m, 2H), 2.93 (s, 3H), 3.07–3.15 (m, 2H), 3.86 (t, 2H, J = 6.6 Hz),

(56) Berther, C. *Chem. Ber.* **1959**, *92*, 2616.

7.72–7.89 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.87, 36.21, 40.60, 52.28, 123.35, 131.73, 134.17, 168.12. Anal. Calcd for $C_{12}H_{13}NO_4S$: C, 53.92; H, 4.90; N, 5.24; S, 12.00. Found: C, 53.73; H, 5.19; N, 5.01; S, 12.01.

Methyl 3-Aminopropyl Sulfone Hydrochloride (15e). Compound **19** (0.71 g, 2.67 mmol) was dissolved in 2.5 mL of EtOH. Hydrazine monohydrate (0.133 mL, 2.74 mmol) was added, and the solution was stirred at 25 °C for 4 h. Concentrated HCl (0.31 mL, 3.72 mmol) was added, and stirring was continued for 30 min. Water (10 mL) was added, and the precipitate was removed by filtration. The solution was concentrated *in vacuo* to 5 mL and refiltered. An additional 5 mL of water was added, and the solution was concentrated to 5 mL and refiltered. This process was repeated until no precipitate formed. The solution was then lyophilized, and the resulting white powder was recrystallized from EtOH/acetone to give the hydrochloride salt (0.16 g, 0.92 mmol) as a white powder in 34% yield. Ir (KBr): 3239.3, 3006.1, 2918.4, 1592.5, 1285.4, 1127.7 cm^{-1}. ^{1}H NMR (200 MHz, D_2O): δ 2.13–2.29 (m, 2H), 3.14 (s, 3H), 3.14–3.22 (m, 2H), 3.39 (t, 2H, J = 7.6 Hz). ^{13}C NMR (50 MHz, D_2O): 22.38, 40.42, 42.44, 53.18. Anal. Calcd for $C_4H_{12}ClNO_2S$: C, 27.67; H, 6.96; N, 8.07; S, 18.46. Found: C, 27.81; H, 6.94; N, 8.31; S, 18.14.

Acetonyldethio-Coenzyme A (4). Amine **5** (1 mL, 1.9 M, pH adjusted to 10.5 with 1 M H_3PO_4) was added to compound **1c** (5 μmol, 4 mg as an HPLC residue containing KH_2PO_4). The pH was found to be 10.2. The solution was allowed to stand for 20 h. HPLC analysis showed the disappearance of **1c** and the appearance of three new peaks corresponding to product **4** (11.7 min), its dimethyl acetal (13.2 min), and the carboxylic acid (9.5 min) resulting from hydrolysis of the thioester bond of **1c**. The reaction mixture was neutralized to pH 4.5 with 1 M H_3PO_4 and lyophilized. The material was purified by reverse-phase C-18 HPLC as outlined for compound **14a** to yield 2 mg of compound **4** (2.5 μmol, 50%). For analysis, residual phosphates were removed using a SPICE cartridge as outlined for compound **2c** to yield the free acid. Analytical HPLC: t_R = 11.7 min, λ_{max} = 260 nm. ^{1}H NMR (as lithium salt, 400 MHz, D_2O): δ 0.74 (s, 3H), 0.87 (s, 3H), 1.63 (quintet, 2H, J = 7.1 Hz), 2.13 (s, 3H), 2.40 (t, 2H, J = 6.4 Hz), 2.51 (t, 2H, J = 7.3 Hz), 3.07 (t, 2H, J = 6.9 Hz), 3.41–3.44 (m, 2H), 3.54 (dd, 1H, J = 4.1, 9.6 Hz), 3.81 (dd, 1H, J = 4.1, 9.6 Hz), 3.98 (s, 1H), 4.22 (br, 2H), 4.57 (br, 1H), 6.13 (d, 1H, J = 5.6 Hz), 8.23 (s, 1H), 8.53 (s, 1H). ^{31}P NMR (161.9 MHz, D_2O, H_3PO_4 external reference at 0 ppm): δ 0.19 (s), –10.43 (m), –10.92 (m). ^{13}C NMR (as free acid, 100 MHz, D_2O): δ 18.71, 21.18, 23.06, 29.71, 35.76, 35.90, 38.78 (d, J = 7.9 Hz), 39.01, 40.72, 65.58 (d, J = 4.9 Hz), 72.62 (d, J = 6.4 Hz), 74.22 (d, J = 5.1 Hz), 74.60, 74.82 (d, J = 5.1 Hz), 83.60–83.71 (m, 1 carbon), 87.89, 118.85, 142.83, 145.17, 148.82, 150.20, 174.22, 175.13, 216.04. HRMS (FAB): [M – H]$^-$ calcd for $C_{24}H_{39}N_7O_{17}P_3$ m/z 790.1615, found 790.1567. Anal. Calcd for $C_{24}H_{40}N_7O_{17}P_3 \cdot 2H_2O$: C, 34.83; H, 5.36; N, 11.85. Found: C, 34.48; H, 5.16, N, 11.81.

Carboxymethyldethio-Coenzyme A (14a). Large Scale. Compound **1c** (0.078 mmol, 60 mg as an HPLC residue containing KH_2PO_4) was dissolved in a solution of γ-aminobutyric acid (6.5 mL, 1 M, pH adjusted to 10.2 with NaOH), and the final pH was adjusted to 9.9 with 1 M phosphoric acid. The reaction was stirred at room temperature and monitored by analytical HPLC. After disappearance of the starting material (23 hours), the reaction mixture was acidified to pH 5 with 1 M phosphoric acid. The product was purified by preparative C-18 reverse-phase HPLC at 2.5 mL/min using the solvent gradient outlined for the purification of compound **1c**. For analysis, residual phosphates were removed using a SPICE cartridge as outlined for compound **2c** to yield the free acid (31 mg, 0.039 mmol, 50%). Analytical HPLC: t_R = 10.7 min, λ_{max} = 260 nm. ^{1}H NMR (as free acid, 400 MHz, D_2O): δ 0.77 (s, 3H), 0.89 (s, 3H), 1.71 (quintet, 2H, J = 7.1 Hz), 2.32 (t, 2H, J = 7.2 Hz), 2.41 (t, 2H, J = 6.4 Hz), 3.13 (t, 2H, J = 6.8 Hz), 3.43 (t, 2H, J = 6.4 Hz), 3.56 (dd, 1H, J = 3.9, 9.4 Hz), 3.83 (dd, 1H, J = 3.8, 9.2 Hz), 3.99 (s, 1H), 4.24 (br, 2H), 4.57 (br, 1H), 6.14 (d, 1H, J = 5.2 Hz), 8.31 (s, 1H), 8.58 (s, 1H). ^{13}C NMR (as free acid, 100 MHz, D_2O): δ 17.50, 20.20, 23.22, 30.73, 34.70, 34.79, 37.66 (d, J = 7.7 Hz), 37.94, 64.52 (d, J = 3.9 Hz), 71.23 (d, J = 5.2 Hz), 73.32 (d, J = 4.8 Hz), 73.44 (d, J = 5.0 Hz), 73.53, 82.82–82.90 (m, 1 carbon), 86.43, 117.76, 140.95, 146.56, 148.00, 150.83, 173.17, 174.06, 177.65. HRMS (FAB): [M – H]$^-$ calcd for $C_{23}H_{37}N_7O_{18}P_3$ m/z 792.1408, found 792.1434.

Small Scale. Compound **1c** (7.8 μmol, 5.9 mg as an HPLC residue containing KH_2PO_4) was dissolved in a solution of γ-aminobutyric acid (1 M, 3 mL, pH adjusted to 10.5). The pH was adjusted to about 10, and the reaction mixture was allowed to stand overnight. The reaction progress was monitored via HPLC analysis. When all of compound **1c** was consumed (1–2 days), the pH was adjusted to 5 with 1 M phosphoric acid, and the reaction mixture was lyophilized. The crude material was redissolved in a minimum of water, and the pH was adjusted to 3 with 1 M phosphoric acid prior to purification by C-18 reverse-phase HPLC as outlined above for the large-scale synthesis to yield 2.4 mg of **14a** (3.0 μmol, 39%). Analytical data shown above for the large-scale preparation.

Nitromethyldethio-Coenzyme A (14b). Compound **14b** was prepared from compounds **1c** (2.0 μmol, 1.5 mg as an HPLC residue containing KH_2PO_4) and **15b** (25 mg as the hydrochloride salt, 0.18 mmol, dissolved in 0.4 mL at pH 10) as shown above for the small-scale synthesis of compound **14a**. The crude material was purified by reverse-phase C-18 HPLC as outlined for compound **14a** to yield 0.74 mg of **14b** (0.93 μmol, 47%). Analytical HPLC: t_R 11.7 min, λ_{max} = 260 nm. ^{1}H NMR (as potassium salt, 400 MHz, D_2O): δ 0.70 (s, 3H), 0.83 (s, 3H), 2.10 (quintet, 2H, J = 6.7 Hz), 2.38 (t, 2H, J = 6.6 H), 3.19 (t, 2H, J = 6.7 Hz), 3.39 (t, 2H, J = 6.5 Hz), 3.50 (dd, 1H, J = 4.8, 9.8 Hz), 3.77 (dd, 1H, J = 5.0, 9.8 Hz), 3.98 (s, 1H), 4.17–4.19 (m, 2H), 4.47 (t, 2H, J = 6.7 Hz), 4.54–4.55 (m, 1H), 6.13 (d, 1H, J = 6.5 Hz), 8.24 (s, 1H), 8.52 (s, 1H). HRMS (FAB): [M – H]$^-$ calcd for $C_{22}H_{36}N_8O_{18}P_3$ m/z 793.1360, found 793.1318.

Carbamoylmethyldethio-Coenzyme A (14c). Compound **14c** was prepared from compounds **1c** (2.6 μmol, 2.0 mg as an HPLC residue containing KH_2PO_4) and **15c** (70 mg as the hydrochloride salt, 0.51 mmol dissolved in 1.1 mL at pH adjusted to 10.1) as shown above for the small-scale synthesis of compound **14a**. The crude material was purified by reverse-phase C-18 HPLC as outlined for compound **14a** to yield 1.3 mg of **14c** (1.7 μmol, 65%). Analytical HPLC: t_R = 10.9 min, λ_{max} = 260 nm. ^{1}H NMR (as potassium salt, 400 MHz, D_2O): δ 0.73 (s, 3H), 0.86 (s, 3H), 1.71 (quintet, 2H, J = 7.3 Hz), 2.22 (t, 2H, J = 7.6 Hz), 2.41 (t, 2H, J = 6.5 Hz), 3.12 (t, 2H, J = 6.9 Hz), 3.41–3.44 (m, 2H), 3.53 (dd, 1H, J = 4.9, 9.8 Hz), 3.79 (dd, 1H, J = 5.0, 9.8 Hz), 3.97 (s, 1 H), 4.20–4.21 (m, 2H), 4.56–4.57 (m, 1H), 6.16 (d, 1H, J = 6.4 Hz), 8.28 (s, 1H), 8.54 (s, 1H). HRMS (FAB): [M – H]$^-$ calcd for $C_{23}H_{38}N_8O_{17}P_3$ m/z 791.1568, found 791.1550.

(Methylsulfinyl)methyldethio-Coenzyme A (14d). Compound **14d** was prepared from compounds **1c** (2.6 μmol, 2.0 mg as an HPLC residue containing KH_2PO_4) and **15d** (158 mg as the hydrochloride salt, 1.0 mmol dissolved in 0.9 mL) as shown above for the small-scale synthesis of compound **14a**. The crude material was purified by reverse-phase C-18 HPLC as outlined for compound **14a** to yield 1.4 mg of **14d** (1.7 μmol, 65%). Analytical HPLC: t_R = 10.7 min, λ_{max} = 260 nm. ^{1}H NMR (as potassium salt, 400 MHz, D_2O): δ 0.61 (s, 3H), 0.72 (s, 3H), 1.71–1.78 (m, 2H), 2.30 (t, 2H, J = 6.4 Hz), 2.54 (s, 3H), 2.65–2.78 (m, 2H), 3.13 (t, 2H, J = 6.7 Hz), 3.30 (t, 2H, J = 6.4 Hz), 3.41 (dd, 1H, J = 4.9, 9.8 Hz), 3.66 (dd, 1H, J = 4.9, 9.8 Hz) 3.84 (s, 1H), 4.08 (br, 2H), 4.45 (br, 1H), 6.06 (d, 1H, J = 6.1 Hz), 8.22 (s, 1H), 8.46 (s, 1H).

(Methylsulfonyl)methyldethio-Coenzyme A (14e). Compound **14e** was prepared from compounds **1c** (2.6 μmol, 2 mg as an HPLC residue containing KH_2PO_4) and **15e** (87 mg as the hydrochloride salt, 0.5 mmol dissolved in 1.0 mL) as shown above for the small-scale synthesis of compound **14a**. The crude material was purified by reverse-phase C-18 HPLC as outlined for compound **14a** to yield 1.7 mg of **14e** (2.1 μmol, 80%). Analytical HPLC: t_R = 11.1 min, λ_{max} = 260 nm. ^{1}H NMR (as potassium salt, 400 MHz, D_2O): δ 0.66 (s, 3H), 0.77 (s, 3H), 1.82–1.88 (m, 2H), 2.35 (t, 2H, J = 6.5 Hz), 2.97 (s, 3H), 3.11–3.15 (m, 2H), 3.17 (t, 2H, J = 6.8 Hz), 3.35 (t, 2H, J = 6.4 Hz), 3.45 (dd, 1H, J = 4.9, 9.8 H) 3.71 (dd, 1H, J = 4.9, 9.7 Hz), 3.89 (s, 1H), 4.12 (br, 2H), 4.49 (br, 1H), 6.10 (d, 1H, J = 6.1 Hz), 8.25 (s, 1H), 8.49 (s, 1H). FABMS: m/z 826 (M – H$^+$, 22), 497 (10), 417 (14), 399 (23), 219 (22). HRMS (FAB): [M – H]$^-$ calcd for $C_{23}H_{39}N_7O_{18}P_3S$ m/z 826.1285, found 826.1321.

Inhibition Studies of Citrate Synthase. For inhibition studies of citrate synthase with the acetyl-CoA analogs **14a**, **14b**, and **14c**, assays were conducted as described previously[46] in 0.1 M Tris, pH 8, containing 0.1 mM DTNB, 0.5 mM oxaloacetone, and 0.03 units of citrate synthase (from porcine heart). The reactions were monitored at 412 nm, using $\epsilon_{412} = 13.6 \times 10^3$ M^{-1} cm^{-1} for DTNB. The concentration of acetyl-CoA was varied from 0.14 to 0.032 mM. K_i values were calculated from double-reciprocal plots of $1/v$ vs $1/$[acetyl CoA] at varying concentrations of the inhibitors: 1.2–0.20 μM for **14a**, 48–12 μM for **14b**, and 1.8–0.5 μM for **14c**.

***K*$_d$ Determinations by Circular Dichroism.** CD spectra were collected at 25 °C at 260 nm, and each data point was time-averaged over 720 s. A 0.2-cm cell was used containing 100 μM citrate synthase in 50 mM Tris buffer, pH 7.5. Measurements were made at concentrations of **14a**

or **14c** ranging from 0 to 300 μM. Data were fitted by nonlinear regression to the following equation for determination of K_d values as described by Kurz et al.[13]

$$-\Delta q_{obs} = a([L] + [CS] + K_d - (([L] + [CS] + K_d)^2 - 4[CS][L])^{1/2})$$

Inhibition Studies of Chloramphenicol Acetyltransferase. For inhibition studies of chloramphenicol acyltransferase with the acetyl-CoA analogs **14d** and **14e**, assays were conducted in 0.1 M Tris, pH 8, containing 0.1 mM DTNB, 0.5 mM chloramphenicol, and 4.0 units of chloramphenicol acyltransferase (from *Escherichia coli*). The reactions were monitored at 412 nm, using $\epsilon_{412} = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$ for DTNB. The concentration of acetyl-CoA was varied from 300 to 15.0 mM. K_i values were calculated from double-reciprocal plots of $1/v$ vs $1/$[acetyl CoA] at varying concentrations of the inhibitors: 100–10 μM.

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