A Stereochemical Probe of the Tetrahedral Intermediate in the Reactions of Acetyl-Coenzyme A Dependent Acetyltransferases

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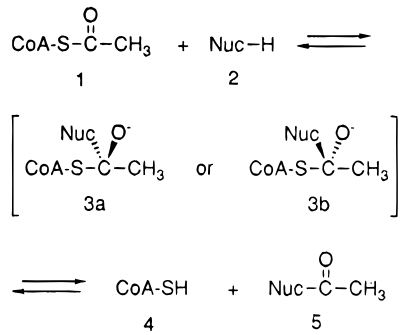
Abstract: A pair of isomeric acetyl-coenzyme A (acetyl-CoA) analogs have been prepared in which the thioester group is replaced with a secondary alcohol. The two isomers differ in stereoconfiguration of the secondary alcohol and were designed to mimic the two possible configurations of the tetrahedral intermediate or transition state in the reactions of acetyl-CoA dependent acetyltransferases. These two isomers were tested as inhibitors of chloramphenicol acetyltransferase and carnitine acetyltransferase, both of which have previously been predicted to form a tetrahedral intermediate which matches the configuration of the (*S*)-alcohol. The (*S*)-isomer was the more potent inhibitor of both enzymes, with K_i values 12-fold and 6-fold lower than the K_i values for the (*R*)-isomer. The (*S*)-isomer was also the more potent inhibitor of phosphate acetyltransferase, acetyl-CoA synthetase, and arylamine acetyltransferase, for which the stereochemistry of the tetrahedral intermediate was previously unknown. These results suggest a common stereoconfiguration of the tetrahedral intermediate among acetyl-CoA dependent acetyltransferases.

The interpretation of stereochemical details of enzyme-catalyzed reactions has been useful in addressing issues of mechanism, function, and evolution in enzyme catalysis.[1] Stereochemical features of many enzyme-catalyzed reactions can be deduced from the stereochemistry of the product, often with the use of a substrate containing one or more stereospecifically incorporated isotopic labels. Enzymes which catalyze acyl transfer reactions are presumed to proceed via a tetrahedral intermediate or transition state which has one of two possible stereoconfigurations, depending on the face of attack of the acyl group acceptor on the acyl donor.[2] These include a number of acetyl-coenzyme A (acetyl-CoA) dependent acetyltransferases, which catalyze the transfer of the acetyl group from acetyl-CoA **1** to an oxygen, nitrogen, or carbon nucleophile (Scheme 1).[3−9] The configuration of the tetrahedral intermediate **3a** or **3b** cannot be deduced from product analysis, as the product **5** like the substrate **1** has an achiral sp^2 carbon at the site of reaction.

The two most well studied of the acetyl-CoA dependent acetyltransferases are carnitine acetyltransferase[3] and chloramphenicol acetyltransferase,[4] which catalyze acetyl transfer to a hydroxyl group of an alcohol. The tetrahedral intermediate in the reaction of carnitine acetyltransferase has been predicted to be that represented by **3a** in Scheme 1, based on molecular modeling analysis.[10] The configuration of the tetrahedral intermediate in the reaction of chloramphenicol acetyltransferase has also been predicted to be that indicated by **3a**, by computer modeling of the reaction based on the known crystal structure of the enzyme.[11] Extensive work with carnitine acetyltransferase has demonstrated the value of knowledge of the stereochemistry of the tetrahedral intermediate in inhibitor design, and these studies have provided further support for the predicted stereochemistry of this intermediate.[12] Similar observations have been made in the design of hydroxyethylene isosteres as inhibitors of proteases, with the configuration at a chiral secondary alcohol center corresponding to the site of hydrolysis being important for optimum inhibition.[13,14] As part of our program in the synthesis[15] and applications[16] of CoA analogs as mechanistic tools in enzymology, we have designed a pair of secondary alcohol analogs of acetyl-CoA **6** as stereochemical probes of

Scheme 1



[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.

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(2) For brevity, the tetrahedral species is referred to as an intermediate hereafter, though it is unclear if it is indeed an intermediate or a transition state. Reactions of some of the acetyltransferases are believed to involve an acyl enzyme, the formation of which would also proceed via a tetrahedral intermediate such as **3**.[5,9]

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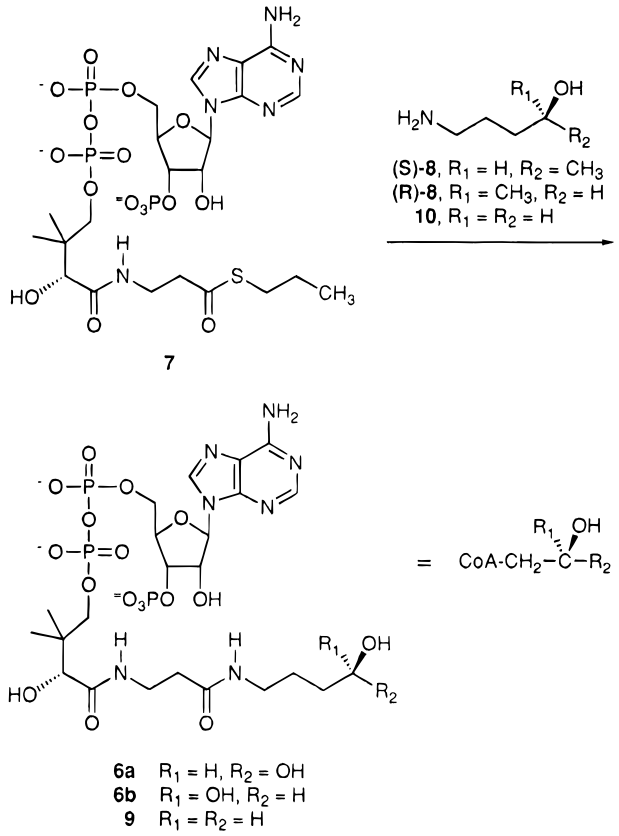
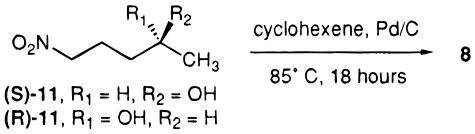
Scheme 2



Scheme 3

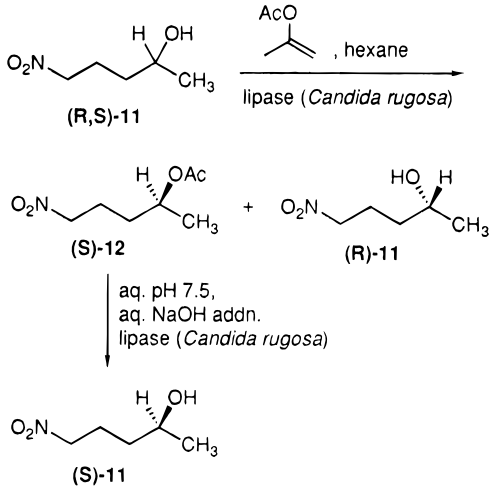


the tetrahedral intermediate in reactions catalyzed by the acetyl-CoA dependent acetyltransferases. We report here the synthesis of these analogs and their inhibition of the enzymes carnitine acetyltransferase, chloramphenicol acetyltransferase, phosphate acetyltransferase, acetyl-CoA synthetase, and arylamine acetyltransferase. All five enzymes exhibit more potent inhibition by the (*S*)-alcohol, suggesting a common configuration of the tetrahedral intermediate among these enzymes.

Results

Compounds **6a** and **6b** were prepared by reaction of **7**[15] with each enantiomer of 5-amino-2-pentanol (**8**) as shown in Scheme 2 and as described previously for the synthesis of other CoA analogs.[15] The related primary alcohol **9** was prepared similarly by reaction of **7** with 4-amino-1-butanol (**10**). The individual isomers of **8** were prepared by catalytic transfer hydrogenation of the corresponding nitro compounds **11** as shown in Scheme 3.[17] Individual isomers of **11** were in turn prepared in >98% ee by enzymatic resolution of racemic material[18] using the lipase from *Candida rugosa* (Scheme 4).[19] The (*S*)-isomer was obtained by isolation of the enzymatically produced (*S*)-acetyl derivative **12**, which was subsequently subjected to enzymatic hydrolysis using the same enzyme to form the further enantiomerically enriched (*S*)-alcohol. The (*R*)-isomer was obtained by isolation of unreacted starting material after selective enzymatic acylation of the (*S*)-isomer of the racemic alcohol. The enantiomeric purity of resolved products was determined by ^{1}H-NMR analysis of the corresponding MTPA esters.[19,20] The absolute stereochemistry predicted from the lipase reaction was confirmed by optical rotation[19] ($[\alpha]^{20}_D = -16.1°$ for the (*R*)-isomer) and was further supported by analysis of relative chemical shifts of the MTPA esters. In the most stable conformation of MTPA esters, the methine proton, ester carbonyl, and trifluoromethyl groups are aligned in one plane. Using this model for the (*R*)-(+)-MTPA ester, the configuration in which the terminal methyl group of **11** is syn to the phenyl group ((*R*)-**11**) is expected to display upfield NMR shifts for the methyl group relative to the isomer in which the methyl group is syn to the methoxy group ((*S*)-**11**).[20] The terminal methyl of the (*R*)-(+)-MTPA ester of (*R*)-**11** had a chemical shift of 1.30 ppm while the methyl group from the (*S*)-**11** ester had a shift of 1.37 ppm, confirming predictions of absolute stereochemistry from optical rotation.

Scheme 4



Compounds **6a** and **6b** were tested as inhibitors of chloramphenicol acetyltransferase, carnitine acetyltransferase, phosphate acetyltransferase (phosphotransacetylase), acetyl-CoA synthetase, and arylamine acetyltransferase. Results, along with K_m values for acetyl-CoA **1** are shown in Table 1. The primary alcohol **9** was also tested as an inhibitor of chloramphenicol acetyltransferase. For chloramphenicol acetyltransferase, **6a** was found to be a 12-fold better inhibitor than **6b**, with **9** having a K_i equivalent to that for **6b**. The K_i for **6a** at pH 8.0 was 12 μM, 1.5-fold higher than the K_i at pH 7.0. With the other four enzymes, **6a** was also the more potent inhibitor. In general, the K_i for **6b** was fairly near the K_m for acetyl-CoA, with the K_i for **6a** ranging from 6-fold to about 27-fold lower than the K_m for **6b**.

Discussion

The secondary alcohols **6a** and **6b** were chosen as simple analogs of the tetrahedral intermediate represented by **3a** and **3b**. This choice was based largely on the utility of hydroxyethylene isosteres of peptides as protease inhibitors.[13,14] For both chloramphenicol acetyltransferase and carnitine acetyltransferase, **6a**, which matches the predicted stereochemistry of the tetrahedral intermediate, was a better inhibitor than the

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Table 1. Michaelis and Inhibition Constants for Substrates and Inhibitors of Acetyltransferases

	K_m or K_i (mM)				
compd	chloramphenicol acetyltransferase	carnitine acetyltransferase	phosphate acetyltransferase	acetyl-CoA synthetase	arylamine acetyltransferase
1	0.03	0.04	0.4	1.1	0.6
6a	0.008	0.01	0.02	0.18	0.03
6b	0.1	0.06	0.35	2.1	0.8
9	0.1				

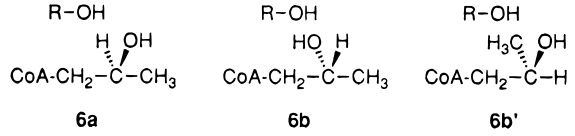

Figure 1.

epimer **6b**. **6a** was also the better inhibitor of the phosphate and arylamine acetyltransferases and of acetyl-CoA synthetase, which in the reverse direction catalyzes the transfer of an acetyl group from acetyl-CoA to AMP. For these three enzymes, the stereoconfiguration of the tetrahedral intermediate had not been previously predicted. The results reported here suggest that these three enzymes also proceed through a tetrahedral intermediate which matches the stereochemistry of the (*S*)-alcohol **6a**.

The expected basis for selective inhibition by the isomer of **6** which matches the configuration of the tetrahedral intermediate is illustrated in Figure 1. In the ternary complex of enzyme, nucleophilic substrate (represented by ROH, as in the chloramphenicol and carnitine acetyltransferases), and **6a**, the hydroxyl group of **6a** is expected to bind in the oxyanion binding site of the enzyme. The absence of increased inhibition of chloramphenicol acetyltransferase at higher pH supports inhibition by the neutral form of the alcohol rather than the alkoxide. The potential steric interaction between the hydrogen atom on the chiral carbon of the inhibitor and the nucleophilic oxygen is probably fairly small. Binding of **6b** in the active site as shown in Figure 1 would result in more severe steric interaction between the hydroxyl oxygens of **6b** and the nucleophilic substrate and would not benefit from interactions of the hydroxyl oxygen of **6b** with the oxyanion binding site. Alternatively, binding as illustrated by **6b′** would allow the hydroxyl group to bind in the oxyanion binding site, with the hydrogen and methyl groups reversed relative to that shown for the structure of the complex with **6a**. This binding mode would also be expected to result in unfavorable steric interactions, in this case between the hydroxyl oxygen of the nucleophile and the methyl group of the **6b**, and would result in a loss of favorable binding interactions for the methyl group. The fact that the isomer of **6** which matches the predicted stereochemistry of the tetrahedral intermediate for chloramphenicol acetyltransferase and carnitine acetyltransferase is the better inhibitor for both enzymes supports the above analysis.

For further support of the significance of interactions with the methyl group in inhibitor binding, the primary alcohol **9** was tested as an inhibitor of chloramphenicol acetyltransferase. The K_i 12-fold higher than the K_i for **6a** but near the K_i for **6b** indicates that binding of the methyl group by the enzyme contributes about 1.5 kcal/mol to the binding of **6a**, which matches the stereochemistry of the tetrahedral intermediate, while the methyl group has little effect on binding by the mismatched isomer **6b**. The additional binding imparted by the methyl group of **6a** is above the value of 0.9 kcal/mol originally predicted by Pauling,[21] though values up to 3.6 kcal/mol have been observed in methyl group enhancement of substrate binding by certain aminoacyl-tRNA synthetases.[22]

While all five enzymes studied in this work exhibit selective inhibition by the (*S*)-isomer of **6**, the inhibition is fairly modest. The K_i for **6a** ranges from 4-fold to 20-fold lower than the K_m for acetyl-CoA. A number of factors could contribute to this modest inhibition relative to the inhibition of proteases by the related peptide isosteres.[10,11] Steric interactions between the hydroxyl group or other nucleophilic atom of the nucleophilic substrate and the hydrogen atom at the alcohol carbon may have a small negative effect on binding, even with the configurationally preferred inhibitor, though a similar effect might be seen in the related protease inhibitors. Furthermore, efficient recognition of a tetrahedral intermediate or mimic vs a planar trigonal species requires recognition of three corners of the tetrahedron. In the peptide isosteres, the hydroxyl group and the two peptide moieties attached to the tetrahedral alcohol center may all serve as good recognition elements. While the hydroxyl and CoA moieties of the acetyl-CoA analogs may serve as good recognition elements, the small and unfunctionalized methyl group may be a poor third recognition element so that greatly enhanced affinity relative to acetyl-CoA is not observed. Perhaps more importantly, less enzymatic stabilization of the tetrahedral intermediate may be necessary in acyl transfer reactions involving CoA thioesters than in protease-catalyzed amide hydrolysis. This differential stabilization is predicted on the basis of lower ground state energies of amide bonds vs thioester bonds due to the significant π orbital delocalization found in amides but similar energies for the tetrahedral intermediates. The protease-catalyzed reactions thus involve a greater activation energy, requiring these enzymes to provide more stabilization of the tetrahedral species. Indeed, site-directed mutagenesis studies of the serine protease subtilisin predict approximately 10 kcal/mol stabilization provided by the oxyanion hole of these enzymes,[23,24] compared to only 3 kcal/mol predicted by similar studies of chloramphenicol acetyltransferase.[25,26] Weaker binding of the tetrahedral intermediate by the acetyltransferases relative to the proteases could translate to weaker binding of an analog of this intermediate. However, despite the modest inhibition of acetyltransferases by **6a** and **6b**, the stereodiscrimination of the two isomers **6a** and **6b** is clear and provides a prediction of the stereochemistry of the tetrahedral intermediate.

Recent studies have shown two areas of sequence homology between an arylalkylamine acetyltransferase and several other

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acetyl-CoA dependent acetyltransferases. This suggests an evolutionary relationship among these enzymes, though this homology is not shared by many other acetyl-CoA dependent acetyltransferases. Evolutionarily related enzymes would be expected to proceed through a tetrahedral intermediate of the same stereoconfiguration. The results of this work support a possible evolutionary relationship among acetyltransferases, as the five enzymes studied all show inhibition by the same isomer of **6**. Further studies of additional acetyltransferases using this pair of probes will permit further investigation of whether the stereochemistry of the tetrahedral intermediate is conserved among all of this class of enzymes or whether other acetyltransferases will be found for which the tetrahedral intermediate has the opposite stereochemistry.

Compounds **6a** and **6b** should serve as general probes for predicting the stereochemistry of the tetrahedral intermediate in reactions catalyzed by acetyl-CoA dependent acetyltransferases. This information may be useful in guiding the design of inhibitors of this class of enzymes. These probes may also serve as new nonhydrolyzable acyl-CoA analogs for use in studies of enzyme−ligand complexes by X-ray crystallography.[16] Such studies may provide additional insights into the means of stabilization of the tetrahedral intermediate in these enzymes and how it relates to stabilization of the analogous intermediates in reactions of the protease and esterase enzymes. These and related studies are currently underway.

Experimental Section

Materials. Chloramphenicol acetyltransferase from *Escherichia coli*, carnitine acetyltransferase from pigeon breast muscle, phosphate acetyltransferase (phosphotransacetylase) from *Bacillus stearothermophilus*, acetyl-CoA synthetase from Bakers yeast, and arylamine acetyltransferase from pigeon liver were all obtained from Sigma and used without further purification.

(*R*)-5-Nitro-2-pentanol. To a solution of racemic 5-nitro-2-pentanol (6.0 g, 45.0 mmol, prepared as described previously[18]) suspended in hexanes (800 mL) was added isopropenyl acetate (11.0 mL, 250 mmol) and lipase (10.0 g from *C. rugosa*). The heterogeneous reaction was stirred vigorously with aliquots removed once an hour for analysis by ^{1}H-NMR. After 30% conversion was observed, the reaction was filtered and concentrated *in vacuo* to remove hexanes and excess isopropenyl acetate. The acetate and alcohol were separated on silica gel (800 mL) with 1:1 hexane−ethyl acetate (R_f = 0.60 for acetate, 0.27 for alcohol; visualized with $KMnO_4$). The acetate was used for preparation of the (*S*)-alcohol (see below). The isolated alcohol (3.9 g, 29.3 mmol) was resuspended in hexanes (500 mL), and isopropenyl acetate (8.6 mL, 194 mmol) and lipase (11.3 g) were added. The reaction was monitored as before, and was filtered after 54% conversion to acetate. The acetate and alcohol were separated as before to yield the (*R*)-nitro alcohol as a clear oil (2.1 g, 15.8 mmol). ^{1}H-NMR for 5-nitro-2-pentanol ($CDCL_3$): δ 1.22, d, 2H, J_{H-H} = 6.6 Hz; δ 1.4−1.6, m, 2H; δ 1.9−2.0, br s, 1H; δ 2.0−2.3, m, 2H; δ 3.8−4.0, m, 1H; δ 4.45, t, 2H, J_{H-H} = 7 Hz. ^{1}H-NMR for acetate ($CDCL_3$): δ 1.25, d, 3H, J_{H-H} = 6.4 Hz; δ 1.6−1.7, m, 2H; δ 2.05, s, 3H; δ 2.0−2.1, m, 2H; δ 4.42, t, 2H, J_{H-H} = 7.0 Hz; δ 4.9−5.0, m, 1H.

(*S*)-5-Nitro-2-Pentanol. The acetate isolated after 30% enzymatic conversion of the racemic alcohol (1.73 g, 9.9 mmol) was dissolved in phosphate buffer (10 mM, 100 mL, pH 7.5). Lipase (500 mg) was added, and the pH was maintained by the addition of sodium hydroxide (0.5 M) with a pH Stat. After 50% hydrolysis (as measured by the addition of 10 mL of base), the reaction was extracted with methylene chloride (3 × 25 mL) and the combined organics were washed with water (2 × 25 mL). The methylene chloride layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo* to yield the (*S*)-nitro alcohol as a clear oil (0.40 g, 2.9 mmol).

(+)-α-Methoxy-α-(trifluoromethyl)phenylacetate (MTPA Ester) of (*R*)- and (*S*)-5-Nitro-2-pentanol. To a solution of (−)-MTPA chloride (26 uL, 0.14 mmol) in dry pyridine (300 uL) and carbon tetrachloride (300 uL) was added either the (*R*)- or (*S*)-5-nitro-2-pentanol (13.3 mg, 0.10 mmol). The reaction was stirred until the formation of precipitate ceased, after which 3,3′-diamino-*N*-methyldipropylamine (32.2 uL, 0.20 mmol) was added to quench. After 5 min, the solution was diluted with ether (5 mL) and washed with cold, dilute hydrochloric acid (2 × 5 mL), cold, saturated sodium bicarbonate (5 mL), and saturated brine (5 mL). The ether was dried over magnesium sulfate, filtered, and concentrated *in vacuo* to yield a clear oil (40 mg, 0.11 mmol). ^{1}H-NMR for the (+)-MTPA ester of (*S*)-5-nitro-2-pentanol ($CDCL_3$): δ 1.37, d, 3H, J_{H-H} = 6.4 Hz; δ 1.6−1.8, m, 2H; δ 2.0−2.1, m, 2H; δ 3.52, d, 3H, J_{H-H} = 1.2 Hz; δ 4.36, t, 2H, J_{H-H} = 6.8 Hz; δ 5.1−5.3, m, 1H; δ 7.3−7.6, m, 5H. ^{1}H-NMR for the (+)-MTPA ester of (*R*)-5-nitro-2-pentanol ($CDCL_3$): δ 1.30, d, 3H, J_{H-H} = 6.4 Hz; δ 1.6−1.8, m, 2H; δ 2.0−2.1, m, 2H; δ 3.52, d, 3H, J_{H-H} = 1.2 Hz; δ 4.36, t, 2H, J_{H-H} = 6.8 Hz; δ 5.1−5.3, m, 1H; δ 7.3−7.6, m, 5H.

(*R*)-5-Amino-2-pentanol. To a solution of (*R*)-5-nitro-2-pentanol (0.60 g, 4.5 mmol) in cyclohexene (9.0 mL) was added palladium (10% on carbon, 30 mg). After heating overnight at 85 °C, the reaction was filtered through Celite and concentrated *in vacuo* to yield a clear oil (0.37 g, 3.6 mmol). The amine was used without further purification. ^{1}H-NMR (D_2O): δ 1.15, d, 3H, J_{H-H} = 3.0 Hz; δ 1.4−1.6, m, 4H; δ 2.6−2.8, t, 2H; δ 3.7−3.9, m, 1H.

(*S*)-5-Amino-2-pentanol. To a solution of (*S*)-5-nitro-2-pentanol (0.28 g, 2.1 mmol) in cyclohexene (5.0 mL) was added palladium (10% on carbon, 20 mg). After heating overnight at 85 °C, the reaction was filtered through Celite and concentrated *in vacuo* to yield a clear oil (0.17 g, 1.7 mmol). The amine was used without further purification. ^{1}H-NMR (D_2O): δ 1.15, d, 3H, J_{H-H} = 3.0 Hz; δ 1.4−1.6, m, 4H; δ 2.6−2.8, t, 2H; δ 3.7−3.9, m, 1H.

(*R*)- and (*S*)-(2-Hydroxypropyl)dethio-Coenzyme A. (*R*)-5-Amino-2-pentanol (0.35 g, 3.4 mmol) was dissolved in water (1.5 mL), and the pH was adjusted to 10.0 with 4 M HCl. A 40 mg sample of adenosine 5′-(trihydrogen diphosphate) 3′-(dihydrogen phosphate) 5′-[(*R*)-3-hydroxy-4-[[3-(propylthio)-3-oxopropyl]amino]-2,2-dimethyl-4-oxobutyl] ester[15] was added, and the pH was again adjusted to 10.0. The reaction was followed by analytical reversed-phase HPLC using conditions previously descibed.[15] The product eluted with a retention time of 12.2 min, and the side product due to hydrolysis of the thiopropyl ester eluted at 10.3 min. After the complete disappearance of the thioester starting material (retention time 17.8 min) was observed, the reaction was acidified with 4 M HCl to pH 5.0 and purified by preparative reversed-phase HPLC, and residual phosphates were removed using a SPICE cartridge. Similar conditions were used to prepare the (*S*)- and (*R*/*S*)-(2-hydroxypropyl)dethio-coenzyme A. ^{1}H-NMR (D_2O): δ 0.67, s, 3H; δ 0.79, s, 3H; δ 1.06, d, 3H, J_{H-H} = 6.0 Hz; δ 1.35−1.45, m, 2H; δ 1.5−1.7, m, 2H; δ 2.41, t, 2H, J_{H-H} = 6.8 Hz; δ 2.93, t, 2H, J_{H-H} = 7.6 Hz; δ 3.32, t, 2H, J_{H-H} = 6.8 Hz; δ 3.4−3.5, m, 0.5H; δ 3.6−3.7, m, 1.5H; δ 3.89, s, 1H; δ 4.1−4.2, m, 2H; δ 6.03, d, 1H, J_{H-H} = 5.2 Hz; δ 8.21, s, 1H; δ 8.44, s, 1H.

(2-Hydroxyethyl)dethio-Coenzyme A. 4-Amino-1-butanol (0.3 g, 4.0 mmol, purchased from Aldrich) was dissolved in water (1.0 mL), and the pH was adjusted to 10.0 with 4 M HCl. A 15 mg sample of adenosine 5′-(trihydrogen diphosphate) 3′-(dihydrogen phosphate) 5′-[(*R*)-3-hydroxy-4-[[3-(propylthio)-3-oxopropyl]amino]-2,2-dimethyl-4-oxobutyl] ester was added, and the pH was again adjusted to 10.0. The reaction was followed by analytical reversed-phase HPLC. The product eluted with a retention time of 11.4 min, and the side product due to hydrolysis of the thiopropyl ester eluted at 10.2 min. After the complete disappearance of the thioester starting material (retention time 17.8 min) was observed, the reaction was acidified with 4 M HCl to pH 5.0 and purified as (2-hydroxypropyl)dethio-coenzyme A. ^{1}H-NMR (D_2O): δ 0.56, s, 3H; δ 0.68, s, 3H; δ 1.2−1.4, m, 4H; δ 2.25, t, 2H, J_{H-H} = 6.4 Hz; δ 2.93, t, 2H, J_{H-H} = 6.0 Hz; δ 3.26, t, 2H, J_{H-H} = 6.0 Hz; δ 3.35−3.45, m, 1.5H; δ 3.6−3.7, m, 0.5H; δ 3.79, s, 1H; δ 4.0−4.1, m, 2H; δ 6.02, d, 1H, J_{H-H} = 6.0 Hz; δ 8.18, s, 1H; δ 8.41, s, 1H.

Inhibition Studies of Chloramphenicol Acetyltransferase. Inhibition of CAT by the acetyl-CoA analogs was determined as previously described[15] in 0.8 mL of Tris buffer (0.1 M, pH 7.0) with 0.01 unit of chloramphenicol acetyltransferase, 0.1 mM chloramphenicol, 0.2 mM DTNB, 0.01−0.08 mM acetyl-CoA, inhibitor, and water to bring the final volume to 1.0 mL per assay. The reactions were monitored at 412 nm using $\epsilon_{412} = 13.6 \times 10^3$ M^{-1} cm^{-1} for DTNB. K_i values were

calculated from double-reciprocal plots of $1/v$ vs 1/[acetyl-CoA] at four concentrations of the inhibitors.

Inhibition Studies of Carnitine Acetyltransferase. Inhibition was determined as previously described[27] in 0.8 mL of potassium phosphate buffer (25 mM, pH 7.5) with 0.026 unit of carnitine acetyl transferase, 2.0 mM L-carnitine, 0.2 mM DTNB, 0.01−0.1 mM acetyl-CoA, inhibitor, and water to bring the total volume to 1.0 mL per assay. The reactions were monitored at 412 nm using $\epsilon_{412} = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$ for DTNB. K_i values were calculated from double-reciprocal plots of $1/v$ vs 1/[acetyl-CoA] at four concentrations of the inhibitors.

Inhibition Studies of Phosphotransacetylase. Inhibition was determined in 0.8 mL of Tris buffer (0.1 M, pH 7.5) with 0.17 unit of phosphotransacetylase, 20 mM potassium phosphate, 20 mM ammonium chloride, 0.035−0.14 mM acetyl-CoA, inhibitor, and water to bring the total volume to 1.0 mL per assay. The reactions were monitored at 240 nm using $\epsilon_{240} = 5.4 \times 10^3\ M^{-1}\ cm^{-1}$ for the thioester. K_i values were calculated from double-reciprocal plots of $1/v$ vs 1/[acetyl-CoA] at four concentrations of the inhibitors.

Inhibition Studies of Acetyl-CoA Synthetase. Inhibition was determined in 0.8 mL of Tris buffer (0.05 M, pH 8.0) with 0.007 unit of acetyl-CoA synthetase, 1 mM adenosine 5′-monophosphate (AMP), 1 mM sodium pyrophosphate, 1 mM magnesium chloride, 0.058−0.58 mM acetyl-CoA, inhibitor, and water to bring the total volume to 1.0 mL per assay. The reactions were monitored at 240 nm using $\epsilon_{240} = 5.4 \times 10^3\ M^{-1}\ cm^{-1}$ for the thioester. Concentrations of AMP, magnesium chloride, and pyrophosphate were limited due to the formation of precipitates in the assay. Vigorous mixing before the addition of acetyl-CoA ensured solubilization of all components. K_i values were calculated from double-reciprocal plots of $1/v$ vs 1/[acetyl-CoA] at four concentrations of the inhibitors.

Inhibition Studies of Arylamine Acetyltransferase. Inhibition was determined using a modified procedure of that previously described[28] in 0.8 mL of potassium phosphate buffer (0.0625 M, pH 7.8) with 0.04 unit of arylamine acetyltransferase, 1 mM EDTA, 0.1 mM aniline, 1 mM DTNB, 0.112−1.12 mM acetyl-CoA, inhibitor, and water to bring the total volume to 1.0 mL per assay. The reactions were monitored at 412 nm using $\epsilon_{412} = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$ for DTNB. The large amount of enzyme used per assay and the replacement of *p*-nitroaniline with aniline were necessary due to the failure to detect activity using the previously described procedure.[28] K_i values were calculated from double-reciprocal plots of $1/v$ vs 1/[acetyl-CoA] at three concentrations of the inhibitors.

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