and the combined extracts were washed with water and brine, dried over magnesium sulfate and activated charcoal, and concentrated to an orange oil. The oil was chromatographed on silica gel (1:1 diethyl ether/hexanes, then acetone) and distilled to give **9** as a pale yellow oil: yield 47.4 g (75%); bp 148–151 °C/0.35 mm; ^{1}H NMR ($CDCl_3$) δ 7.35 (d, 1 H, J = 8.1 Hz), 7.20 (t, 1 H, J = 8.1 Hz), 6.69 (m, 2 H), 4.65 (bs, 2 H), 3.61 (s, 3 H), 3.36 (s, 3 H). Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N. 15.55. Found: C, 59.72; H, 6.78; N, 15.47.

2′-(Benzyloxy)-2-aminobenzophenone (4a) (−78 °C procedure). To a mixture of **9** (2.00 g, 11.1 mmol) and **1a** (2.92 g, 11.1 mmol) in anhydrous tetrahydrofuran (65 mL) at −78 °C under nitrogen was added, with vigorous stirring, *n*-BuLi in hexanes (13.8 mL, 1.6 M, 22.2 mmol) at 0.6 mL/min. After 20 min, aqueous hydrochloric acid was added (1 N, 20 mL), the mixture was extracted with ethyl acetate (150 mL), and the ethyl acetate was washed with water and brine, dried over magnesium sulfate, and concentrated. Recrystallization from hexanes gave **4a** as yellow crystals: yield 2.29 g (68%); mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 7.60–7.00 (m, 11 H), 6.70 (d, 1 H, J = 8.2 Hz), 6.53 (t, 1 H, J = 8.2 Hz), 6.33 (bs, 2 H), 5.06 (s, 2 H). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.18; H, 5.65; N, 4.62. Found: C, 79.09; H, 5.64; N, 4.62.

3′-(Benzyloxy)-2-aminobenzophenone (4b). This compound was prepared in an identical fashion to **4a**: yield 67%; mp 106–107 °C; ^{1}H NMR ($CDCl_3$) δ 7.46–7.10 (m, 11 H), 6.73 (d, 1 H, J = 8.3 Hz), 6.57 (t, 1 H, J = 8.3 Hz), 6.08 (bs, 2 H), 5.05 (s, 2 H). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.18; H, 5.65; N, 4.62. Found: C, 78,89; H, 5.70; N, 4.61.

4′-(Benzoyloxy)-2-aminobenzophenone (4c). This compound was prepared in an identical fashion to **4a**: yield 70%; mp 99–101 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 2 H, J = 8.7 Hz), 7.38–7.25 (m, 7 H), 7.03 (d, 2 H, J = 8.7 Hz), 6.73 (d, 1 H, J = 8.3 Hz), 6.63 (t, 1 H, J = 8.3 Hz), 5.86 (bs, 2 H), 5.13 (s, 2 H). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.18; H, 5.65; N, 4.62. Found: C, 79.12; H, 5.66; N, 4.54.

2′-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-2-aminobenzophenone (4d). This compound was prepared in an identical fashion to **4a** and isolated by silica gel chromatography (sgc: hexanes/ethyl acetate); yield 47% as a viscous yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.63–7.22 (m, 16 H), 6.67 (d, 1 H, J = 8.1 Hz), 6.49 (t, 1 H, J = 8.1 Hz), 6.27 (bs, 2 H), 4.78 (s, 2 H), 0.95 (s, 9 H). An analytical sample was obtained by Kugelrohr distillation; bp ~210 °C/0.02 mm. Anal. Calcd for $C_{30}H_{31}NO_2Si$: C, 77.38; H, 6.71; N, 3.01. Found: C, 77.20; H, 6.74; N, 2.95.

3′-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-2-aminobenzophenone (4e). This compound was prepared and isolated in an identical fashion to **4d**: yield 50% as a viscous yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 4 H, J = 7.2 Hz), 7.60–7.23 (m, 12 H), 6.74 (d, 1 H, J = 8.1 Hz), 6.49 (t, 1 H, J = 8.1 Hz), 6.08 (bs, 2 H), 4.81 (s, 2 H), 1.09 (s, 9 H). An analytical sample was obtained by Kugelrohr distillation; bp ~210 °C/0.02 mm. Anal. Calcd for $C_{30}H_{31}NO_2Si$: C, 77.38; H, 6.71; N, 3.01. Found: C, 77.44; H, 6.73; N, 3.00.

4′-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-2-aminobenzophenone (4f). This compound was prepared and isolated in an identical fashion to **4d**: yield 50% as a viscous yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 4 H, J = 7.2 Hz), 7.63 (d, 2 H, J = 8.0 Hz), 7.49–7.23 (m, 10 H), 6.75 (d, 1 H, J = 8.1 Hz), 6.63 (t, 1 H, J = 8.1 Hz), 6.05 (bs, 2 H), 4.82 (s, 2 H), 1.10 (s, 9 H). An analytical sample was obtained by Kugelrohr distillation; bp ~210 °C/0.02 mm; crystallized on standing; mp 106–108 °C. Anal. Calcd for $C_{30}H_{31}NO_2Si$: C, 77.38; H, 6.71; N, 3.01. Found: C, 77.28; H, 6.74; N, 3.00.

3′-Cyano-2-aminobenzophenone (4n) (−100 °C procedure). To a mixture of **9** (2.00 g, 11.1 mmol) and **1n** (2.02 g, 11.1 mmol) in anhydrous tetrahydrofuran (65 mL) at −100 °C under nitrogen was added, with vigorous stirring, *n*-BuLi in hexanes (13.8 mL, 1.6 M, 22.2 mmol) at 0.6 mL/min. After the addition was complete the reaction was allowed to warm to −70 °C (internal temperature), aqueous hydrochloric acid was added (1 N, 20 mL), the mixture extracted with ethyl acetate (150 mL), and the ethyl acetate was washed with water and brine, dried over magnesium sulfate, and concentrated. Purification by sgc (hexanes/ethyl acetate) and recrystallization from hexanes gave **4n** as yellow crystals: yield 0.84 g (34%); mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 7.94–7.77 (m, 3 H), 7.59 (t, 1 H, J = 8.0 Hz), 7.36–7.29 (m, 2 H), 6.75 (d, 1 H, J = 8.2 Hz), 6.63 (t, 1 H, J = 8.2 Hz), 6.19 (bs, 2 H). Anal. Calcd for $C_{14}H_{10}N_2O$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.57; H, 4.56; N, 12.53.

4′-Cyano-2-aminobenzophenone (4o). This compound was prepared in an identical fashion to **4n**: yield 40%; mp 157–159 °C; ^{1}H NMR ($CDCl_3$) δ 7.77 (d, 1 H, J = 7.5 Hz), 7.70 (d, 1 H, J = 7.5 Hz), 7.37–7.28 (m, 2 H), 6.76 (d, 1 H, J = 8.2 Hz), 6.61 (t, 1 H, J = 8.2 Hz), 6.24 (bs, 2 H). Anal. Calcd for $C_{14}H_{10}N_2O$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.39; H, 4.59; N, 12.51.

3′-(*tert*-Butoxycarbonyl)-2-aminobenzophenone (4p). This compound was prepared in an identical fashion to **4n** and isolated as an oil: yield 52%; ^{1}H NMR ($CDCl_3$) δ 8.23 (t, 1 H, J = 1.9 Hz), 8.14 (dt, 1 H, J = 7.8, 1.9 Hz), 7.76 (dt, 1 H, J = 7.8, 1.9 Hz), 7.51 (t, 1 H, J = 7.8 Hz), 7.39 (d, 1 H, J = 7.4 Hz), 7.31 (m, 1 H), 6.75 (d, 1 H, J = 7.8 Hz), 6.61 (t, 1 H, J = 7.8 Hz), 6.15 (bs, 2 H), 1.60 (s, 9 H). An analytical sample was obtained by Kugelrohr distillation; bp ~250 °C/0.02 mm; crystallized on standing; mp 129–130 °C. Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.60; H, 6.47; N, 4.69.

4′-(*tert*-Butoxycarbonyl)-2-aminobenzophenone (4q). This compound was prepared in an identical fashion to **4p**: yield 51%; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 2 H, J = 8.4 Hz), 7.64 (d, 2 H, J = 8.4 Hz), 7.39–7.26 (m, 2 H), 6.74 (d, 1 H, J = 8.3 Hz), 6.58 (t, 1 H, J = 8.3 Hz), 6.19 (bs, 2 H), 1.62 (s, 9 H). An analytical sample was obtained by Kugelrohr distillation; bp ~250 °C/0.02 mm. Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.74; H, 6.47; N, 4.68.

A New Chemical Method for Synthesizing and Recycling Acyl Coenzyme A Thioesters

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Introduction

Coenzyme A functions as an acyl carrier in the biosynthetic pathways of most organisms. It participates in stereoselective carbon–carbon bond formation and is potentially useful for synthesizing complex organic molecules. Coenzyme A thioesters (acyl-CoA) serve as substrates for a wide variety of enzyme-catalyzed reactions including both fatty acid and polyketide biosynthesis.[1]

A variety of procedures have been described for preparing CoA thioesters of fatty acids. Those employed most frequently involve selective acylation of the CoA-thiol with acylating agents including acid anhydrides,[2] acyl chlorides,[3] a mixed anhydride of ethyl hydrogen carbonate,[4] and *N*-hydroxysuccinimide esters of fatty acids.[5] However all of these reagents are relatively nonspecific; they may react not only with the sulfhydryl group, but also with other functional groups present in CoA. Furthermore all of the methods employ organic solvents.

Enzymatic systems, on the other hand, have the advantage of working in aqueous buffers. The most commonly used enzyme for acyl-CoA synthesis is acyl-CoA synthetase (ACS). The disadvantage of this method is that the yields are low (50%) and products are contaminated with lipids present in the enzyme preparation[6,7] unless

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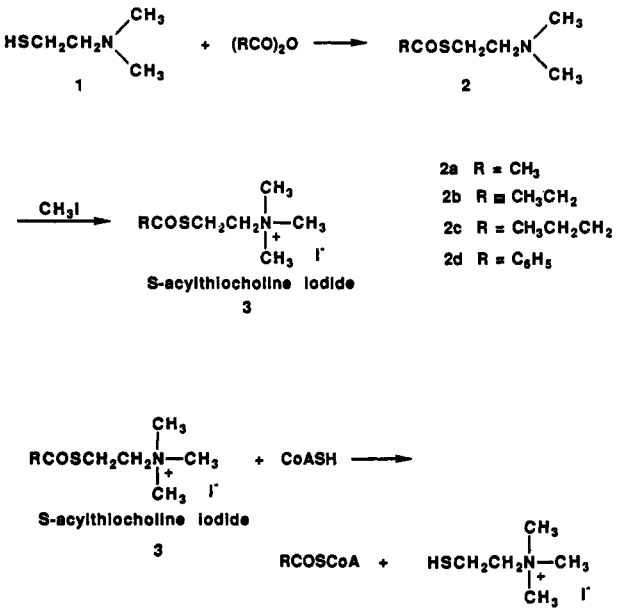
0022-3263/91/1956-3752$02.50/0

Table I. The Cost of CoA and CoA Thioesters (Sigma)

compound	$/100 mg
CoA	59.65
acetyl-CoA	220.05
propionyl-CoA	441.10
butyryl-CoA	426.00

Scheme I. Chemical Synthesis of Acyl-CoA



lengthy procedures are first employed to purify the enzyme.[8] ACS has been examined for its ability to accept various carboxylic acids as substrates in place of acetic acid and shows a limited substrate specificity.[9] Furthermore ACS is very expensive ($3275/1000 units).

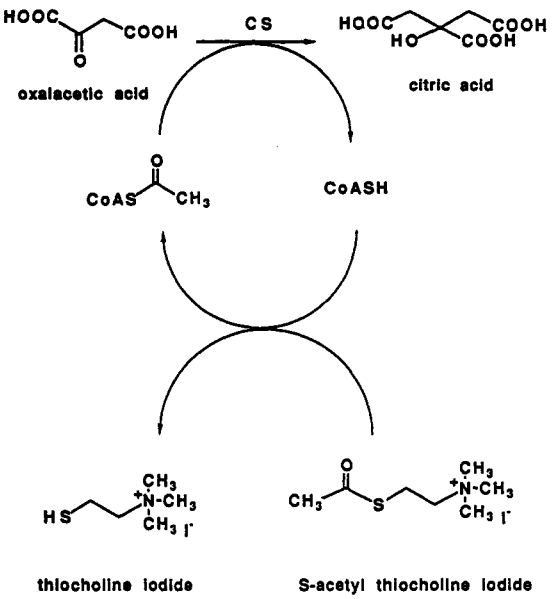
The expense of CoA and its acyl derivatives (Table I) places extreme demands on any method for regenerating them. Enzymes can be utilized for regenerating acyl-CoA thioesters in synthetic schemes where CoA is the byproduct of an enzyme-catalyzed reaction. Three enzymatic methods for regenerating acetyl-CoA have been demonstrated.[11,12] These enzymatic methods suffer from fairly narrow substrate limitations to regenerate unnatural acyl CoAs. A chemical method for regenerating acyl-CoAs has been reported utilizing a two-phase system of aqueous buffer and toluene.[10] This method does not have the substrate limitation for regenerating unnatural acyl-CoAs; however, some enzymes are denatured by the organic solvents employed.

In this paper, we report a new chemical method for preparing some unnatural CoA thioesters in aqueous buffer systems and for recycling acetyl-CoA thioesters in enzyme-catalyzed reactions.

Results and Discussion

Preparation of Acyl-CoAs. Various acyl-CoAs have been synthesized using *S*-acylthiocholine iodide as an acylating reagent (Scheme I). Acylthiocholines are very stable in pH 7–8 buffer (no hydrolysis observable over 3 days), are easy to prepare, and are inexpensive. The reaction is highly selective and provides high yields of a variety of derivatives. Compared with enzymatic methods, the method has the potential to make different acyl-CoA thioesters without substrate limitation. We successfully synthesized acetyl-CoA, propionyl-CoA, butyryl-CoA, and benzoyl-CoA from their respective *S*-acylthiocholines. Only *S*-benzoylthiocholine iodide is not available commercially but it is synthesized easily from benzoic anhydride and (*N*,*N*-dimethylamino)ethanethiol.[13]

Scheme II. Synthesis of Citric Acid Using *S*-Acetylthiocholine for CoA Recycling



The reaction is based on a thiol ester interchange. In order to determine the extent of acyl transfer from thiocholine to CoA, we performed the following experiment. One equivalent each of CoA and *S*-acylthiocholine were added to phosphate buffer (pH 7.4). Acyl-CoA was formed immediately as observed by HPLC. After several hours, the CoA peak disappeared, and the only peak observable corresponded to the acyl CoA. We conclude that the reaction between CoA and *S*-acylthiocholine in buffer is rapid and has an extremely large K_{eq}. In contrast, the reaction in distilled water is very slow with a $K_{eq} = 3.5 \times 10^{-3}$. Under acidic conditions, the nucleophilic thiol group in CoA is too weak to attack the carbonyl carbon in acylthiocholine. The trimethylammonium group of the acylating reagent plays three important roles in this reaction. First, it makes the reagent water soluble, so the reaction can be performed in aqueous solution. Second, it is electron withdrawing, which increases the electrophilicity of the carbonyl carbon and allows it to react with the thiol group of CoA to form acyl-CoA. Finally it decreases the nucleophility of the thiol group in the thiocholine byproduct relative to CoA, making the reaction essentially irreversible.

Acyl-CoA Regeneration. The expense of CoA and its derivatives requires that acyl-CoA regeneration methods possess exquisite selectivity for the formation of the enzymatically active cofactor, high rates of reaction with low concentrations of CoA, and high stability of all reagents. Chemical methods developed previously lack the necessary selectivity,[2-5] employ organic solvents that denature the

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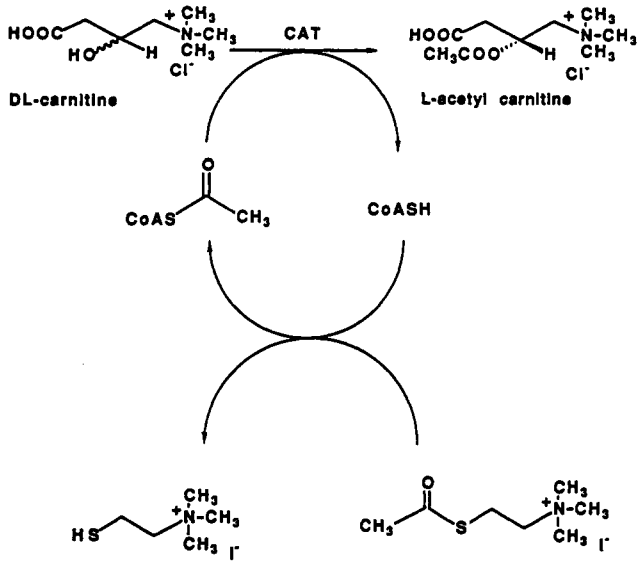
Scheme III. Synthesis of L-Acetylcarnitine Using *S*-Acetylthiocholine for CoA Recycling



enzymes[10] or employ easily hydrolyzable acylating agents. Furthermore, the enzymatic methods developed for recycling acetyl-CoA[11,12] all have limited substrate specificity and cannot make unnatural acyl-CoA derivatives. We successfully applied the new acyl-CoA synthesis to regenerate acetyl-CoA for an enzyme-catalyzed synthesis.

We examined the enzyme-catalyzed aldol condensation of acetyl-CoA with oxalacetic acid to form citric acid to demonstrate acetyl-CoA recycling. This reaction is catalyzed by the enzyme citrate synthase which is commercially available. The reaction is shown in Scheme II. In this reaction, oxalacetic acid reacts with acetyl-CoA catalyzed by the enzyme citrate synthase to produce citric acid and the byproduct CoA. *S*-Acetylthiocholine iodide was introduced, acetylating CoA in situ to form acetyl-CoA, which is used in the aldol condensation and recycled. Therefore, only a catalytic amount of CoA was used for synthesizing citric acid. A recycling number of 1160 was obtained for acetylcoenzyme A. For practical purposes, it is desirable to use immobilized enzymes so that the enzyme activity can be recovered after the reaction. When citrate synthase immobilized on glass beads[14] was used we obtained a total turnover number of 700 for acetylcoenzyme A. At the end of the reaction, the enzyme was recovered by simple filtration and assayed. The enzyme retained 70% of its initial activity.

In 1955, Fritz[15] reported that L-carnitine stimulates the oxidation of long chain fatty acids in liver slices and homogenates. Later this effect of carnitine was observed in cell particle preparations from several tissues.[16] L-Carnitine has been found to be clinically useful in treatment of lipid storage myopathy and myocardial ischemia in animals.[18] D-Carnitine is an inhibitor and causes undesirable side effects when the patient is treated with a racemic mixture of DL-carnitine.[17] Hence, there is clinical interest in obtaining L-carnitine in an optically pure form. Present methods of preparing L-carnitine are expensive and involve chemical synthesis and enantiomer resolution.[19] The enzyme carnitine acetyltransferase catalyzes acetylation of carnitine with acetyl-CoA and is specific for the L isomer.[21] We successfully synthesized L-acetylcarnitine from DL-carnitine using acetyl-CoA recycling (Scheme III). The total turnover number obtained for this reaction is 340 and was determined by ^{1}H NMR using ethanol as an internal standard. The optical purity of L-acetylcarnitine was determined by ^{1}H NMR using a chiral shift reagent.

The present method for acetyl-CoA recycling has many advantages over other methods of regenerating acetyl-CoA. The method is not limited in its ability to regenerate unnatural acyl-CoA as is the case with enzymatic methods.[11,12] Furthermore the method does not require organic solvents, which may cause some enzymes to denature. More generally, this method for acyl-CoA synthesis is superior to all previous methods in terms of its selectivity and its use of only aqueous buffers.

In conclusion, we developed a new chemical method for synthesizing acylcoenzyme A thioesters and for recycling acetyl-CoA thioesters in enzyme-catalyzed reactions. The acylating reagent is *S*-acylthiocholine, which is highly selective, specific, rapid, and produces high yields of acyl-CoAs. Acetyl-, propionyl-, butyryl- and benzoyl-CoA were synthesized from the corresponding *S*-acylthiocholine iodides. *S*-Acetylthiocholine was also employed in enzyme-catalyzed reactions for acetyl-CoA recycling. Citric acid and L-acetylcarnitine were both synthesized by this method.

Experimental Section

Oxalacetic acid, D,L-carnitine and D,L-acetylcarnitine, *S*-acetylthiocholine iodide, *S*-propionylthiocholine iodide, and butyrylthiocholine iodide were purchased from Aldrich (Milwaukee, WI). Coenzyme A, citrate synthase, and carnitine acetyltransferase were purchased from Sigma (St. Louis, MO). (*N,N*-Dimethylamino)ethanethiol was purchased from Research Organics, Inc. (Cleveland, OH). An HPLC instrument with a C-18 reverse-phase silica column and an absorbance detector were used to monitor the reaction.

Methods. Preparation of *S*-Benzoylthiocholine Iodide. The procedure was adapted from ref 13. (Dimethylamino)ethanethiol (10 mmol) and benzoic anhydride (15 mmol) were mixed and heated to reflux temperature for 10 min. Heating was then stopped, and the mixture was allowed to stand overnight. The mixture was then diluted with acetone (3 mL), and methyl iodide (1 mL, 15 mmol) was added in one portion. After standing for 4 h at room temperature, the solid product was filtered, air-dried, and recrystallized from propanol to give off-white small plates in 95% yield (mp 252–254 °C): ^{1}H NMR (D_2O) δ 3.15 ppm (s, 9 H, NCH_3), 7.4–7.9 ppm (m, 5 H, C_6H_5), 3.4 ppm (m, 4 H, CH_2CH_2); FAB/MS M^+ 224.03 (100) $C_{12}H_{18}ONS$ = 224; (MI) M^+ 575.45.

Preparation of Acyl-CoA Thioesters (Scheme I). A general procedure to synthesize acyl-CoA thioesters is described: CoA (1 mg, 1.3×10^{-2} mmol) and *S*-acetylthiocholine iodide (6 mg, 2.0×10^{-2} mmol) were dissolved in 0.1 M phosphate buffer pH 7.4. The reaction was incubated at room temperature and monitored for formation of acyl-CoA using HPLC. Typically, the reaction was complete after 3 h. The product acyl-CoA was purified on a reverse-phase preparative C-18 column using 20–30% methanol in 0.05 M phosphate buffer, pH 5.5, as the mobile phase. ^{1}H NMR spectra of CoA and the thioesters were taken in D_2O. The ^{1}H NMR of acetyl-CoA and propionyl-CoA were consistent with the ref 9 data. The ^{1}H NMR of butyryl-CoA was consistent with the ^{1}H NMR of the standard product from Sigma, and the ^{1}H NMR

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of benzoyl-CoA was compared with the ^{1}H NMR of other acyl-CoA derivatives; the chemical shift of the phenyl group appeared at δ 7.6–7.9 ppm (m, 5 H, C_6H_5).

Synthesis of Citric Acid Using Acetyl-CoA Recycling (Scheme II). A typical reaction was carried out as follows: Oxalacetic acid (500 mg, 3.8 mmol) was dissolved and neutralized with 6 mL of 2 M Tris base to pH 7.8. To start the reaction and every 2 or 3 h, 50-mg portions of *S*-acetylthiocholine iodide (500 mg, 1.7 mmol) and 200-μL aliquots of oxalacetate solution were added to CoA (1 mg, 1.3 μmol) and the enzyme citrate synthase (EC 4.1.3.7) (1000 units). The mixture was left to react in a shaker incubator at 40 °C. The progress of the reaction was monitored by detecting the formation of citric acid using ^{1}H NMR. After 3 days, the amount of citrate formed was determined by ^{1}H NMR using ethanol as an internal standard. Citric acid was purified from the reaction mixture by acidifying the mixture to pH 1 and then lyophilizing. The resulting white powder was extracted with methanol–acetone (1:50). Solvents were removed under reduced pressure to provide an oil. Citric acid was determined by ^{1}H NMR. The amount of citric acid was determined by using absolute ethanol as the internal standard. The total turnover number of 1160 was obtained for acetylcoenzyme A. Yield 88% based on acetylthiocholine or 38% based on oxalacetic acid.

Acetyl-CoA Recycling Using Immobilized Enzymes. The procedure was repeated using citrate synthase (1000 units) immobilized on glass beads.[14] At the end of the reaction, the immobilized enzyme was removed by filtration. The enzyme was assayed after adding the substrates oxalacetic acid and acetyl-CoA using HPLC to monitor the formation of CoA.

Synthesis of L-Acetylcarnitine Using Acetyl-CoA Recycling (Scheme III). DL-Carnitine (1 g, 5 mmol) dissolved in distilled water and neutralized with 2 M K_3PO_4 to pH 7.8 was added to CoA (1 mg, 1.3×10^{-3} mmol). The enzyme carnitine acetyltransferase (EC 2.3.1.7) (500 units) was added in 60-unit aliquots, and *S*-acetylthiocholine (500 mg, 1.7 mmol) was added in 50-mg portions to the reaction mixture every 2 or 3 h. The mixture was left to react in a shaker incubator at 40 °C for 3 days. The formation of L-acetylcarnitine was monitored using either 300-MHz ^{1}H NMR or HPLC with reverse-phase C-18 column and an UV detector at 208 nm. The amount of L-acetylcarnitine formed was determined by ^{1}H NMR using ethanol as an internal standard and corresponded to a recycling number of 340 for acetyl-CoA, corresponding to a 26% yield based on acetyl thiocholine or 18% based on L-carnitine in the starting racemate. The L-acetylcarnitine was purified by HPLC, on a reverse-phase preparative C-18 column using 0.1 M phosphate buffer, pH 5.5, as mobile phase and detected at 208 nm. L-Acetylcarnitine was confirmed by ^{1}H NMR and its optical purity determined by using the chiral shift reagent, tris[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium(III).[20] When an equimolar quantity of the chiral shift reagent was added to DL-acetylcarnitine, resolution of the two enantiomers was observed on the ^{1}H NMR. The acetyl group appears as two singlets at 2.04 and 2.05 ppm and the trimethylammonium group as two singlets at 3.5 and 3.6 ppm. Addition of the chiral shift reagent to the acetylcarnitine purified from the above reaction showed only one isomer in the ^{1}H NMR. To verify, 5 mg of DL-acetylcarnitine was added to the NMR tube producing a small new peak at 2.04 ppm belonging to D-acetylcarnitine.

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Registry No. **1**, 108-02-1; **2b**, 24395-12-8; **2c**, 63512-62-9; **3a**, 1866-15-5; **3d**, 10561-14-5; CoA, 85-61-0; acetyl-CoA, 72-89-9; propionyl-CoA, 317-66-8; butyryl-CoA, 2140-48-9; benzoyl-CoA, 6756-74-7; $(PhCO)_2O$, 93-97-0; $(HO_2CCH_2)_2C(OH)CO_2H$, 77-92-9; $HO_2CCOCH_2CO_2H$, 144-62-7; citrate synthase, 9027-96-7; L-acetylcarnitine, 3040-38-8; carnitine acetyltransferase, 9029-90-7; DL-carnitine, 406-76-8.

Supplementary Material Available: ^{1}H NMR spectrum of *S*-benzoylthiocholine iodide **3d** (1 page). Ordering information is given on any current masthead page.

Strained Heterocyclic Systems. 20.[1] Basicities of Bicyclic Quinoxalines

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The influence of ring strain effects on the basicity of quinolines was first reported in 1967.[3] The initial studies were extended to quinoxalines[4] and, more recently, to 1-azatriptycene.[5] In this report a similar correlation is applied to a series of bicyclic quinoxalines.

Strain effects in bicyclic alkanes are well known[6] and are readily reflected in an NMR parameter such as the $J(^{13}C–H)$ value for bridgehead protons. For instance, the one-bond coupling constants for that position in the closely related series bicyclo[2.2.2]octane, bicyclo[2.2.1]heptane, and bicyclo[2.1.1]hexane are 134.3, 140.1, and 150.5 Hz, respectively,[7] reflecting the increased s character in the C–H bond due to orbital rehybridization.[8] For comparison, the same parameter for cyclopentane is 128 Hz.[9]

With this in mind, the following compounds were chosen for study: 2,3-dihydro-1*H*-cyclopenta[*b*]quinoxaline (**1**), 1,2,3,4-tetrahydro-1,4-ethanophenazine (**2**), 1,2,3,4-tetrahydro-1,4-methanophenazine (**3**), and 2,3-dihydro-1,3-methano-1*H*-cyclopenta[*b*]quinoxaline (**4**). Compounds **1–3** were prepared by literature methods; a preliminary account of **4** has been reported.[10] The pK_a values of the conjugate acids were determined by spectrophotometric titration, and the results in order of decreasing basicity are summarized in Table I, along with values for model compounds 2,3-dimethylquinoxaline (**5**) and quinoxaline (**6**).

Compounds **1–4** were all less basic than **5** and more basic than **6**. The latter fact was somewhat unexpected, although strain effects in ortho-annelated quinoxalines were previously observed to be more compressed than in analogous quinolines.[4,13] The basicities of **3** and **4** were essentially the same, and both compounds were the least basic of the series studied. Such order was consistent with

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