Syntheses, Structures, and Enzymatic Evaluations of Conformationally Constrained, Analogue Inhibitors of Carnitine Acetyltransferase: (2*R*,6*R*)-, (2*S*,6*S*)-, (2*R*,6*S*)-, and (2*S*,6*R*)-6-(Carboxylatomethyl)-2-(hydroxymethyl)-2,4,4-trimethylmorpholinium

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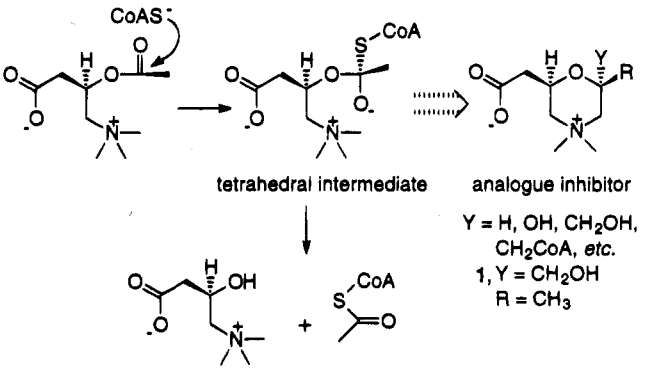
The syntheses and structures of the four stereoisomers of 6-(carboxylatomethyl)-2-(hydroxymethyl)-2,4,4-trimethylmorpholinium, **1**, are described. The key step in the synthetic strategy involves an intramolecular Michael addition reaction. Condensation of nonracemic 3-(methylamino)-2-methylpropane-1,2-diol, **3**, with methyl 4-bromo-2-butenoate followed by intramolecular Michael addition gives a mixture of two diastereomers of methyl 2-[4,6-dimethyl-6-(hydroxymethyl)morpholinyl]-acetate, **5**. The diastereomeric ratio of the products in this reaction changes from 6:1 to 1:1 with a change in solvent from diethyl ether:methanol (35:1, v:v) to methanol. The structures and absolute configurations of **1** were determined by single crystal X-ray analyses. In crystals and solution, the morpholinium rings adopt a chair conformation with carboxylatomethyl occupying an equatorial position. All four stereoisomers inhibit pigeon breast carnitine acetyltransferase (CAT). Of this series, (2*S*,6*R*)-**1** binds to CAT most strongly with a K_i of 190 ± 20 μM and an IC_{50} of 0.42 mM. The enzymatic assays of **1** confirm that CAT recognizes both configurations at C2 and C6 in the analogues. CAT has a different conformation when it binds carnitine or acetylcarnitine than when it binds **1**. This latter conformation may resemble that when CAT catalyzes acetyl transfer.

Introduction

Conformationally constrained, reaction-intermediate analogues can probe the topography of an enzymic active site even without three-dimensional structural data. This approach requires analogues in which the stereochemistry of the functional groups is varied and known. We design[1] and synthesize conformationally constrained analogues (Scheme 1) to mimic our proposed reaction intermediate[2] for acyl transfer between (*R*)-carnitine and coenzyme A (CoA), a reaction catalyzed by carnitine acyltransferases. By comparing the inhibition constants of stereoisomeric analogues, we can map the topography of the key recognition sites in these enzymes.

Carnitine acetyltransferase[3] (CAT) catalyzes the reversible transfer of short-chain acyl groups between (*R*)-carnitine and CoA (Scheme 1). This reaction affects the level of free CoA and acetyl-CoA in every eukaryotic cell.[4] Only recently, have the amino acid sequences of two CATs been deduced.[5,6] The three-dimensional structure of CAT and the bioorganic mechanism are unknown. We have proposed a mechanism[2] for acetyl transfer in CAT involving a tetrahedral intermediate (Scheme 1), which generates a new stereocenter. Molecular modeling shows that the most stable conformation of the tetrahedral intermediate has an *R* configuration at the new stereocenter. The thiolate ion would approach from the less-hindered side, *viz.*, the *re* face, of the ester.

Scheme 1



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[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1995.

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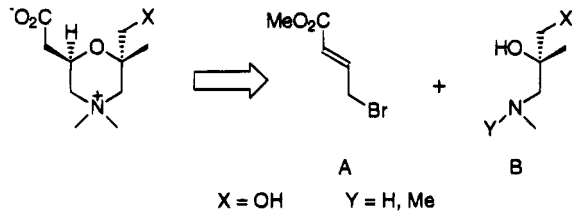
Scheme 2



Over the past few years, we have synthesized conformationally constrained meso and racemic morpholinium compounds,[7] which inhibit CAT. Our related nonracemic cyclic hemiketals,[8] which inhibit CAT more effectively, can open to the hydroxy ketone. In this paper, we report the syntheses and structures of the four stereoisomers of **1**, and we compare their CAT-inhibition constants.

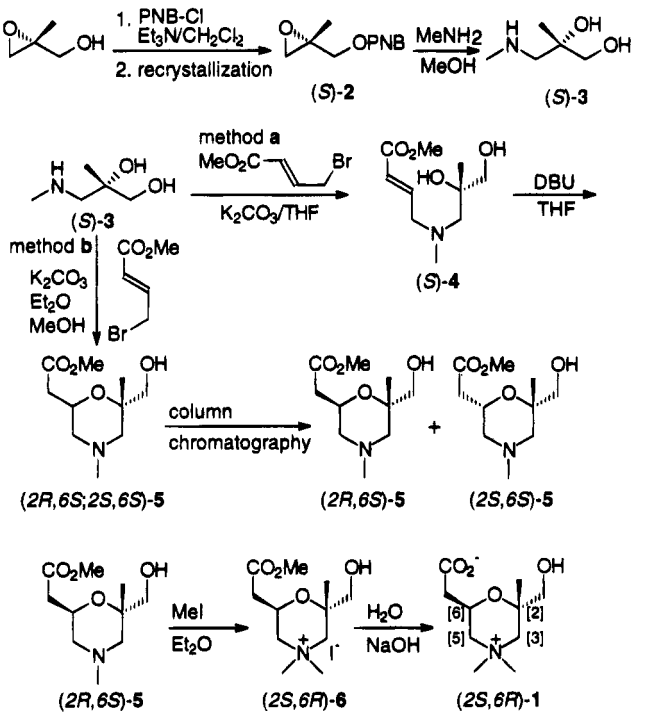
Strategy for Synthesis. Only a few of the many routes[9-13] for synthesizing morpholines will produce nonracemic compounds. Some six-membered heterocyclic compounds that contain two heteroatoms in the 1- and 4-positions have been synthesized via intramolecular Michael additions.[9b,14-17] We have previously used this approach to stereoselectively synthesize a series of racemic diasteromeric morpholiniums.[7]

In synthesizing the four stereoisomers of **1**, the key is to avoid forming racemates. An intramolecular Michael addition with a nonracemic nucleophile accomplishes this task. We disconnect **1** into two components, A and B, that may carry different functional groups (Scheme 2). For compound **1**, X represents a hydroxyl group. Component A is commercially available and B is made from 2-methylglycidol, which is commercially available for both *R* and *S* configurations with approximately 90% ee for both. Condensation of A with an enantiomer of B followed by intramolecular Michael addition gives two diastereomers, which separate on column chromatography. This approach obviates optical resolution but requires enriching optical purity.

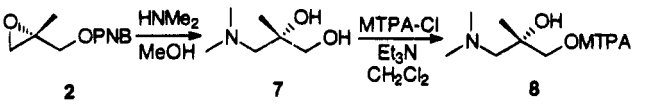
Results and Discussion

Syntheses. 1. Nonracemic Amino Diol 3. We used (*R*)- and (*S*)-2-methylglycidol to make (*S*)- and (*R*)-**3** by ring opening with methylamine (Scheme 3). Before starting our synthesis, we verified the 90% optical purities of (*R*)- and (*S*)-2-methylglycidol by ^{1}H NMR analysis of the Mosher's esters.[18] We modified the procedure of Gao et al.[19] to increase the optical purities of (*R*)- and (*S*)-methylglycidol by converting them into 4-nitrobenzoate esters, **2**, followed by recrystallizations. (Compounds (*R*)- and (*S*)-**2** with optical purities >98% are also commercially available.) Reaction of **2** with excess amine in methanol cleaved the ester and opened the epoxide in one step to give an amino diol. Methylamine gave **3** in 90% yield and dimethylamine gave **7** (Scheme 4), with which we determined the optical purity. The ester cleavage byproduct with methylamine was *N*-methyl 4-nitrobenzoate amide, while that with dimethylamine was methyl 4-nitrobenzoate, presumably formed by general-base-catalyzed methanolysis.

Scheme 3



Scheme 4



We determined the optical purity of **2** from the Mosher's ester of **7** (Scheme 4). Compound **7** reacted with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to give **8**. An ^{1}H NMR analysis of **8** indicated that the optical purities of both (*R*)- and (*S*)-**8** are greater than 98% (No minor isomer was detected.).

2. Morpholine 5. Two approaches yielded morpholine **5**. The first was a two-step procedure with isolation of **4** (Scheme 3, method a); the second a one-pot procedure (Scheme 3, method b). In the first approach, reaction of (*R*)- and (*S*)-**3** with methyl 4-bromo-2-butenoate gave (*R*)- and (*S*)-**4** in 83% yield. The 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU)-promoted ring closure[20] of **4** gave **5** in a 3:1 ratio of two diastereomers, which we separated by column chromatography for combined yield of 40%. We also separated and characterized methyl 4-oxobutanoate in 32% yield from the silica column. This aldehyde was

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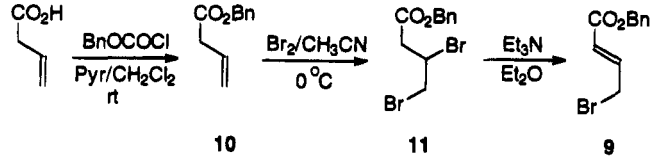
Scheme 5



absent in the NMR spectrum of the crude reaction product. The desired morpholine **5** thus decomposed during the chromatography.

This suggested that **5** fragmented[21] on silica gel in the presence of DBU. In THF, we tried potassium carbonate, a solid base, and triethylamine, a volatile base. We also tried quinine and quinidine, which could have affected the diastereomeric ratio. None of the four promoted ring closure. Treating **3** with methyl 4-bromo-2-butenoate and potassium carbonate in dry diethyl ether:methanol (35:1, v:v) gave **5** directly as a 6:1 diastereomeric mixture in quantitative yield (Scheme 3, method b). Eliminating the use of DBU saved one step in the overall synthesis.

In the ring closure, only a six-membered ring product formed with the major isomer having the (methoxycarbonyl)methyl and hydroxymethyl trans to each other. From (*S*)-**3**, we got (2*R*,6*S*)- and (2*S*,6*S*)-**5**; from (*R*)-**3**, we got (2*S*,6*R*)- and (2*R*,6*R*)-**5**. We assigned structures and absolute stereochemistry by single-crystal X-ray analyses of two of the corresponding stereoisomers of **6**.[31]

Given that the hydrolysis of a methyl ester might require treatment with strong acid or base and that our long-range target molecule, **1** (Y = CH_2CoA), contains many acid- and base-sensitive functional groups that may isomerize[22] or fragment under such harsh conditions, we tried replacing the methyl ester with a benzyl ester, which can be removed by hydrogenation. Benzyl 4-bromo-2-butenoate, **9**, had been synthesized by a side-chain bromination of the methyl group of benzyl 2-butenoate with NBS in carbon tetrachloride.[23] However, we obtained benzaldehyde as a major product, presumably because the α-bromobenzyl ester hydrolyzed. We then adopted the approach of Zindel and Meijere,[24] who recently synthesized 4-bromo-substituted Michael acceptors. We synthesized **9** in three high-yield steps: (a) benzylation of 3-butenoic acid, 88% yield; (b) bromination of benzyl 3-butenoate (**10**), 96% yield; and (c) dehydrobromination of dibromide **11**, 82% yield (Scheme 5).

The condensation of **3** with **9** proceeded more slowly than that of the condensation with the methyl ester

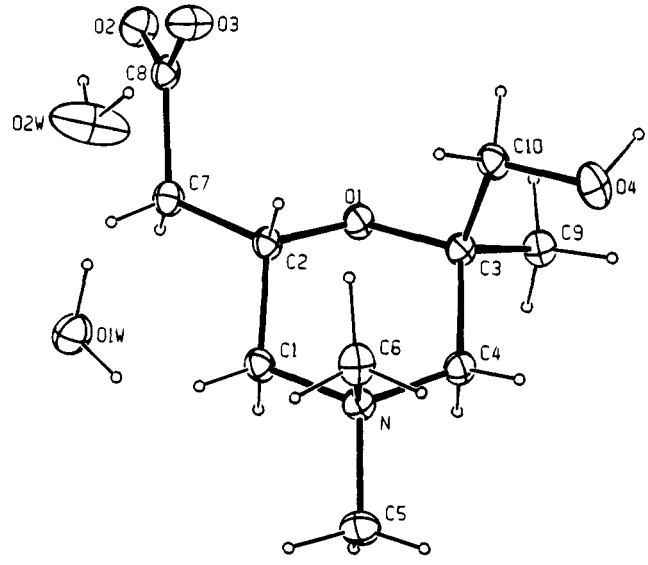


Figure 1. ORTEP drawing of (2*R*,6*S*)-**1**.

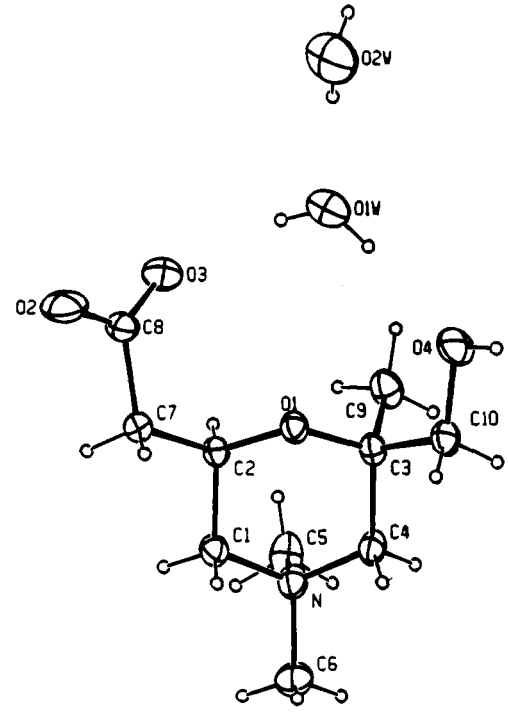


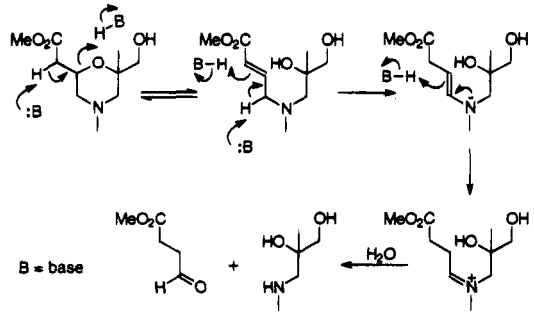
Figure 2. ORTEP drawing of (2*R*,6*R*)-**1**.

under identical conditions of method b. Chromatography on alumina, however, gave benzyl 4-oxobutanoate, indicating that the benzyl analogue of **5** was unstable.

3. Carnitine Analogue 1. Methylation of **5** with excess of iodomethane gave **6** in 74% yield. Saponification of **6** with *0.1* N sodium hydroxide solution gave **1** in 67% yield. We assigned structures and absolute stereochemistry of the four stereoisomers of both **6** and **1** by single-crystal X-ray analyses of one enantiomer from each diastereomeric pair of enantiomers[31] (Figures 1–4). The two diastereomers of zwitterion **1** both crystallized as dihydrates. We determined absolute configurations by parallel refinement of the mirror image. The reported configurations gave significantly better fits than the mirror image configurations.

Crystal Structures of 6 and 1. In crystals, the morpholinium rings adopt chair conformations. For (2*S*,6*R*)-**6** and (2*R*,6*S*)-**1**, the (methoxycarbonyl)methyl or

(21) One of the possible mechanisms for the degradation is shown below:



(22) We have observed very slow isomerization (<10%) of **5** upon prolonged storage (ca. 9 months).

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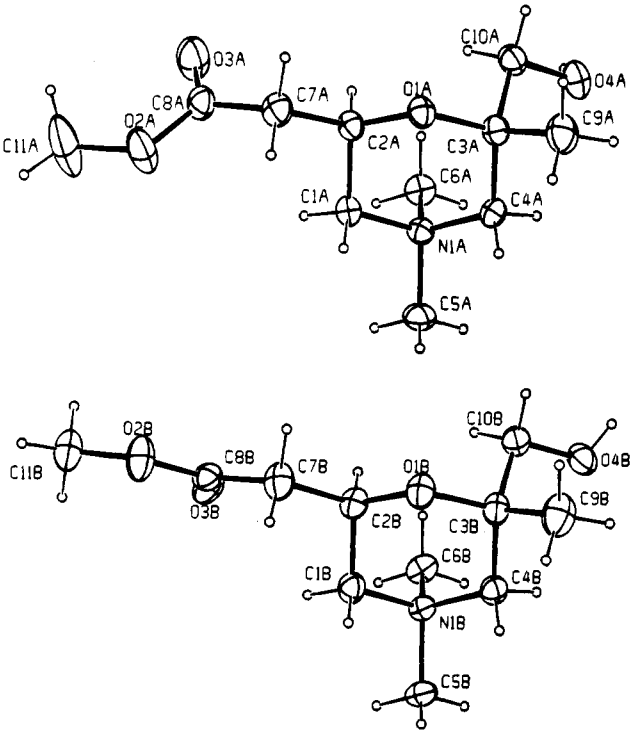


Figure 3. ORTEP drawing of (2*S*,6*R*)-**6**.

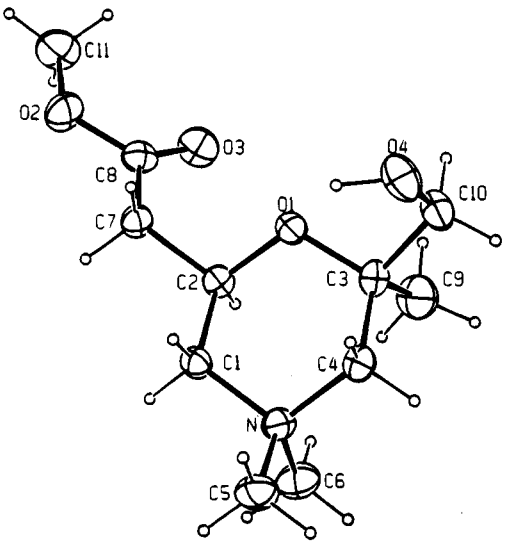


Figure 4. ORTEP drawing of (2*R*,6*R*)-**6**.

carboxylatomethyl and methyl on C3 are cis;[25] both occupy equatorial positions. For (2*R*,6*R*)-**6** or (2*R*,6*R*)-**1**, the (methoxycarbonyl)methyl or carboxylatomethyl and hydroxymethyl are cis; both occupy equatorial positions. The (methoxycarbonyl)methyl or carboxylatomethyl occupies an equatorial position in all four compounds. The torsion angles of C1–C2–C7–C8 in (2*R*,6*S*)-**1** (169.5°) and (2*R*,6*R*)-**1** (−173.7°) are similar, but different in (2*S*,6*R*)-**6** (-72.9 and −71.4° for two independent molecules) and (2*R*,6*R*)-**6** (−165.2°).

The conformations and patterns of hydrogen bonding are similar for the two diastereomers of **1**, but different for the two diastereomers of **6**. In both diastereomers of **1**, the hydroxyl group donates an *inter*molecular hydrogen bond to the carboxylate group and accepts one from a water molecule. The carboxylate group accepts three hydrogen bonds, two of which are from the water molecules. All donors form *inter*molecular hydrogen bonds, all of which have O–H···O angles 150.4° or greater. In contrast, the two diastereomers of **6** form quite different patterns of hydrogen bonding. In (2*R*,6*R*)-**6**, the hydroxyl group forms a bifurcated hydrogen bond to the morpholine oxygen O1 and an iodide, with respective distances, 2.762(3) and 3.617(3) Å. In (2*S*,6*R*)-**6**, the hydroxyl group of independent molecule (A) forms a linear OH···I hydrogen bond of length 3.408(3) Å, while that of molecule (B) forms a linear OH···O hydrogen bond of length 2.773(4) Å to O4A.

Solution NMR. NMR data support the assumption that the chair conformations of the morpholinium rings in the crystals also predominate in solution. All ring protons in **6** and **1** are very well resolved by 400 MHz ^{1}H NMR and assigned from DEPT, 2D ^{13}C–^{1}H correlation, 2D ^{1}H COSY, and 2D ^{1}H NOESY experiments. The chemical-shift ranges include both diastereomers of both **6** and **1**. The equatorial protons on C[5][25] are deshielded relative to the axial protons by 0.37–0.48 ppm; the J_{gem}'s are 11.74–12.82 Hz. The equatorial protons on C[3] are deshielded relative to the axial protons by 0.12–0.70 ppm; the J_{gem}'s are 11.90–13.74 Hz. The protons (axial) on C[6] couple to axial and equatorial protons on C[5] with J_{vic} of 11.26–12.05 and <0.5–2.18 Hz, respectively. Carboxylatomethyl and (methoxycarbonyl)methyl are, thus, equatorial in **1** and **6**, respectively. We observe weak long-range couplings between equatorial protons on C[3] and C[5], except in spectra of (2*R*,6*R*)-**1** and the enantiomer (2*S*,6*S*)-**1**, in which the long-range couplings might be too weak to be resolved; the coupling constants are 1.56–1.84 Hz. The chemical shifts of the protons of axial methyl groups on nitrogen are 0.06–0.12 ppm downfield relative to the equatorial ones. The chemical shifts of the protons of axial methyl groups on C[2] in (2*R*,6*S*)-**6**, (2*R*,6*S*)-**1**, and the enantiomers (2*S*,6*R*)-**6** and (2*S*,6*R*)-**1** are 0.16–0.18 ppm downfield relative to the equatorial ones in (2*R*,6*R*)-**6**, (2*R*,6*R*)-**1**, and the enantiomers (2*S*,6*S*)-**6** and (2*S*,6*S*)-**1**. In (2*S*,6*R*)-**1**, we observe NOEs from the (axial) proton on C[6] only to the methylene protons on the 2-hydroxymethyl group, the protons on the axial methyl of the quaternary nitrogen, the equatorial proton on C[5], and the methylene protons of the carboxylatomethyl group. In (2*S*,6*S*)-**1**, we observe NOEs from the (axial) proton on C[6] only to the protons of the methyl group on C[2], the protons on the axial methyl of the quaternary nitrogen, the equatorial proton on C[5], and the methylene protons of the carboxylatomethyl group. From these data, we infer chair conformations for both diastereomers.

Selectivity of Ring Closure. We used two methods for the preparation of **5** from **3**. In Scheme 3 method a, DBU-promoted ring closure of **4** into **5** in THF with a diastereomeric ratio of 3:1. In methanol, the ring closure of **4** into **5** gave a diastereomeric ratio of ca. 1:1. In method b, **5** formed in diethyl ether:methanol, (35:1, v:v) with a diastereomeric ratio of 6:1. In all cases, the major isomer had the (methoxycarbonyl)methyl trans to hydroxymethyl.

We attribute the resulting stereoselectivities to competition between *intra*- and *inter*molecular hydrogen bonding. In our proposed chair transition structures for the ring closures (Figure 5), the (methoxycarbonyl)methyl

(25) The numbering system for the ORTEP drawings of the crystal structures is different from IUPAC nomenclature. For the former, we use C no. for crystallographic numbering in the discussion of crystal structures; for the latter, we use C[no.] for IUPAC numbering in the discussion of solution NMR spectra and enzymatic inhibition.

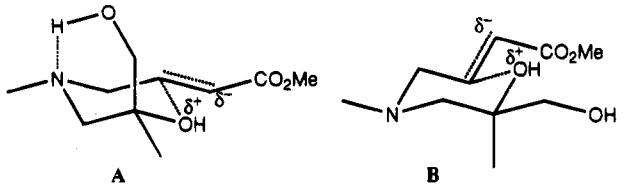
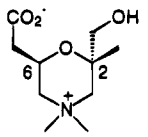
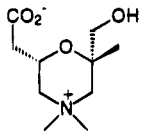
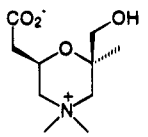
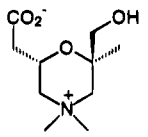


Figure 5. Proposed transition structures for ring closure reaction of **4**.

Table 1. Inhibition of the Four Stereoisomers of 1 on Pigeon Breast CAT (0.01 units)

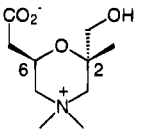
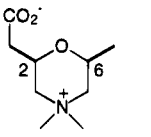
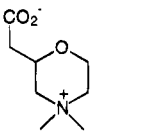
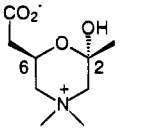
Inhibitor	Structure	IC_{50} (mM)
(2*S*,6*R*)-**1**		0.42
(2*S*,6*S*)-**1**		4.04
(2*R*,6*R*)-**1**		1.36
(2*R*,6*S*)-**1**		3.41

is equatorial. Transition structure A is probably more stable than B because the primary hydroxyl group *intra*molecularly hydrogen bonds to the nitrogen. Only an axial hydroxymethyl group can form this *intra*molecular hydrogen bond. This bond fixes the $N-CH_2-C-CH_2OH$ torsion angle in a gauche conformation. In low polarity, aprotic solvents, transition structure A leads to the major product, which has the (methoxycarbonyl)-methyl trans to hydroxymethyl. The product ratio of the ring closure in methanol support this speculation. Under such conditions, *inter*molecular hydrogen bonding between methanol and both the nitrogen and the hydroxyl group competes with the *intra*molecular hydrogen bonding. The loss of *intra*molecular hydrogen bonding allows the $N-CH_2-C-CH_2OH$ torsion angle to be gauche or anti.

Enzymatic Evaluations of 1. Pigeon breast CAT binds both enantiomers of carnitine and acetylcarnitine equally well.[26,27] (*R*)-Carnitine and (*R*)-acetylcarnitine are substrates in the forward and reverse reactions, respectively, while the *S* enantiomers are competitive inhibitors.[26,27] This stereospecificity implies that the acetyl-CoA binding site must be closer to the hydroxyl on (*R*)-carnitine than the one on (*S*)-carnitine.[2]

Table 1 reports the IC_{50}s of four stereoisomers of **1** with commercial pigeon breast CAT (0.01 units) at 250 μM (*R*)-acetylcarnitine (K_m = 350 μM). CAT recognizes changes in configurations at both C[6] and C[2] in the inhibitors. Compound (2*S*,6*R*)-**1** inhibits CAT 10-fold better than (2*S*,6*S*)-**1**, and (2*R*,6*R*)-**1** inhibits 2.5-fold better than (2*R*,6*S*)-**1**. The pairs have the same configurations at C[2] but different configurations at C[6]. CAT prefers the *R* configuration at C[6] as it does for C[3] in carnitine and acetylcarnitine in acetyl transfer. Because CAT binds *R* better than *S* in the carnitine fragment of the inhibitors, but binds both *R*- and *S*-carnitine(acetylcarnitine) equally well, these inhibitors are not substrate analogues. Compound (2*S*,6*R*)-**1** inhibits better than (2*R*,6*R*)-**1**. CAT prefers the *S* configuration at C[2], which is the same relative configuration as *R* at C[5] in the proposed tetrahedral intermediate (Scheme 1).

Table 2. Comparison of (2*S*,6*R*)-1 with Other Morpholinium Inhibitors

Inhibitor	Structure	K_i (μM)
(2*S*,6*R*)-**1**		190 +/- 20
(2*S*,6*R*:2*R*,6*S*)-**12**		1080
(*RS*)-**13**		1000
(2*S*,6*R*)-**14**		59

To compare with other inhibitors, we have determined the K_i for (2*S*,6*R*)-**1** (Table 2). As expected, (2*S*,6*R*)-**1** competitively inhibits CAT and K_i is lower than IC_{50}. Compound (2*S*,6*R*)-**1** is more active than the racemates[7] **12** and **13**, but is almost 3-fold less active than nonracemic **14**[3] (Table 2). We estimate K_i values of 540 and 500 μM for the more active enantiomers, (2*R*,6*S*)-**12** and (*R*)-**13**, respectively. Inhibition improves with substitution at C[2] in the sequence of OH > CH_2OH > H. Whether the open or closed form of **14** is the active structure remains unresolved,[8] but we note that the closed form of **14** has the same relative configuration of the most active stereoisomer of **1**.

The results confirm that CAT recognizes configurations at both C[2] and C[6] in the tetrahedral-intermediate analogues. CAT catalyzes by a random Bi-Bi mechanism.[26] The conformation of CAT when it binds substrates must differ from that when it binds **1**. CAT discriminates the configuration when it binds **1**, but not when it binds substrates. Perhaps this occurs because the conformational constraint in **1** is absent in acetylcarnitine or because **1** resembles the putative reaction intermediate or both. The conformation of CAT when it binds **1** may resemble that when it catalyzes the reaction. Hydroxymethyl does not mimic CoA, but may provide a point for conjugation to a CoA analogue. We are working toward this goal.

(26) Chase, J. F. A.; Tubbs, P. K. *Biochem. J.* **1966**, *99*, 32–40.
(27) Tipton, K. F.; Chase, J. F. A. *Biochem. J.* **1969**, *115*, 517–521.

Conclusion

We have prepared four stereoisomers of **1**, conformationally constrained analogues, which inhibit CAT. Compound (2*S*,6*R*)-**1** inhibits CAT better than the other three stereoisomers. The key step is to construct a morpholine via *N*-alkylation followed by an intramolecular Michael addition. The diastereomeric ratio of the products in this reaction changes from 6:1 to 1:1 with a change in solvent from diethyl ether:methanol (35:1, v:v) to methanol. From the hydroxymethyl group on this morpholine, we will attach additional moieties as we synthesize our long-range target **1**, Y = CH_2CoA.

Experimental Section

General Methods. Uncorrected melting points were measured on a digital melting-point apparatus equipped with multistage ramping rates from 0.1 to 10.0 °C/min. ^1H NMR spectra were recorded at 500, 400, 270, and 200 MHz, respectively. ^{13}C NMR spectra were recorded at 67.5 and 100 MHz, respectively. Unless noted otherwise, all NMR spectra were recorded in $CDCl_3$. Proton chemical shifts are expressed in ppm downfield from internal TMS; coupling constants that were verified using PANIC (parameter adjustment in NMR by iteration calculation) are listed as *J*; observed coupling constants not verified are listed as J_{app}; all coupling constants are reported in hertz. The ^{13}C chemical shifts are also expressed in ppm relative to the solvent chemical shift. Assignments of the ^1H and ^{13}C NMR signals were made by comparison with similar compounds and using DEPT, 2D ^{13}C–^1H correlation, 2D ^1H COSY, and 2D ^1H NOESY experiments. IR spectra were recorded as thin films on KBr cells and are reported in cm^{-1}. FAB MS samples were prepared by suspending in glycerol. Elemental analyses were performed by Oneida Research Services of Whitesboro, NY, and Atlantic Microlabs of Norcross, GA. The optical rotations were recorded in a 3.5 × 10 mm or 10 × 100 mm cell, respectively. The organic solutions were dried over $MgSO_4$ and concentrated by rotary evaporation unless otherwise noted.

Materials. Unless otherwise noted, materials obtained from commercial sources were used without further purification. THF was distilled from K. Diethyl ether was distilled from Na–K alloy. Triethylamine was distilled from CaH_2 and stored over Linde molecular sieves type 3A. CH_2Cl_2 was purified by shaking with concd H_2SO_4, washing with H_2O and brine, drying with CaH_2, distilling, and storing over 4A molecular sieves. Methanol was distilled over a small amount of Mg. Methyl 4-bromo-2-butenoate was purified by vacuum distillation.

(*R*)-3-(Methylamino)-2-methylpropane-1,2-diol ((*R*)-3). To a solution of (*R*)-**2** (7.13 g, 30.1 mmol) in MeOH (60 mL) was added H_2NMe (9.13 g, 300 mmol) in MeOH (60 mL) was added dropwise in 30 min. The mixture was stirred at rt for 4 h and then cooled to 0 °C for 1 h. The precipitate was removed by filtration through Celite. The solution was concentrated. Kugelrohr distillation of the crude product gave 3.21 g (90%) of (*R*)-**3** as a colorless oil (bp 80 °C/0.2 Torr, 94 °C/1 Torr, 160 °C/17 Torr). ^1H NMR (200 MHz): 1.10 (s, 3 H), 2.44 (s, 3 H), 2.66 (d, 1 H, J_{app} = 12.1), 2.74 (dd, 1 H, J_{app} = 12.2, 1.4), 3.48 (dd, 1 H, J_{app} = 11.2, 1.4), 3.65 (d, 1 H, J_{app} = 11.0). ^{13}C NMR (50 MHz): 23.2, 36.9, 60.9, 70.9, 71.2. IR: 3321 (OH), 1055 (C-N). MS *m*/*e* (relative intensity): 88 (11.4), 75 (3.6), 70 (7.3), 58 (10.4), 57 (4.3), 45 (5.1), 44 (100), 43 (10.2), 42 (11.1). Anal. Calcd for $C_5H_{13}NO_2$: C, 50.42; H, 10.92; N, 11.76. Found: C, 50.14; H, 11.02; N, 11.57.

Compound (*S*)-**3** was prepared from (*S*)-**2** in the same manner as (*R*)-**3**. Anal. Found: C, 50.29; H, 10.89; N, 11.56.

(*R*)-Methyl 4-[Methyl-(2,3-dihydroxy-2-methylpropyl)-amino]-2-butenoate ((*R*)-4). A solution of methyl 4-bromo-2-butenoate (7.22 g, 40.3 mmol) in THF (30 mL) was added to a mixture of (*R*)-**3** (4.80 g, 40.3 mmol) and K_2CO_3 (7.4 g, 54 mmol) in THF (50 mL). The reaction mixture was stirred overnight and then filtered. The solution was concentrated and then placed under vacuum. After adding dry Et_2O (40 mL), the precipitate was removed by filtration. Concentrating the solution gave 7.26 g (83%) of a light yellow oil, which was used for the next reaction without further purification. ^1H NMR (200 MHz): 1.07 (s, 3 H), 2.38 (s, 3 H), 2.49 (d, 1 H, J_{app} = 13.8), 2.62 (d, 1 H, J_{app} = 13.8), 3.13–3.63 (m, 4 H), 3.73 (s, 3 H), 5.90–6.00 (m, 1 H), 6.85–6.99 (m, 1 H). ^{13}C NMR (50 MHz): 23.7, 45.0, 51.6, 60.4, 65.4, 70.2, 71.5, 123.2, 144.9, 166.4. IR: 3425 (OH), 1724 (C=O), 1660 (C=C). MS, FAB, *m*/*e*: 218 ($M^+ + 1$).

Compound (*S*)-**4** was prepared from (*S*)-**3** in the same manner as (*R*)-**4**.

Methyl (2*S*,6*R*)-2-[4,6-Dimethyl-6-(hydroxymethyl)-morpholinyl]acetate ((2*S*,6*R*)-5) and Methyl (2*R*,6*R*)-2-[4,6-Dimethyl-6-(hydroxymethyl)morpholinyl]acetate ((2*R*,6*R*)-5). Method a. DBU (2.5 mL, 16 mmol) was added to a solution of crude (*R*)-**4** (7.26 g, 33.4 mmol) in THF (500 mL). The reaction mixture was stirred at rt for 24 h. Concentration of the solution gave a brown liquid, which was purified by column chromatography (SiO_2, 70–230 mesh, sample:SiO_2 = 1:25, EtOAc:MeOH = 100:5), yielding a mixture of two diastereomers. The diastereomers were separated by column chromatography (SiO_2, 230–400 mesh, sample:SiO_2 = 1:70, hexanes:CH_2Cl_2:EtOH = 10:10:3), giving (2*S*,6*R*)-**5** (2.18 g, 30%) R_f 0.38 and (2*R*,6*R*)-**5** (0.70 g, 9.6%) R_f 0.30.

(2*S*,6*R*)-**5**. ^1H NMR (500 MHz): 1.11 (s, 3 H), 1.74 (dd, 1 H, *J* = 10.87, 10.73), 1.94 (d, 1 H, *J* = 11.83), 2.20 (s, 3 H), 2.37 (dd, 1 H, *J* = 16.35, 4.06), 2.49 (dd, 1 H, *J* = 16.35, 8.93), 2.65 (d, 1 H, *J* = 11.83), 2.70 (d(br), 1 H, *J* = 10.87), 3.40 (d, 1 H, *J* = 11.58), 3.70 (s, 3 H), 4.20 (d, 1 H, *J* = 11.58), 4.29–4.37 (m, 1 H). ^{13}C NMR (50 MHz): 23.6, 38.2, 45.9, 51.7, 58.8, 61.3, 65.8, 66.6, 73.5, 171.5. IR: 3506 (OH), 1741 (C=O). MS *m*/*e* (relative intensity): 217 (19.5), 186 (54.5), 144 (13.7), 143 (26.5), 142 (31.6), 128 (17.6), 114 (10.5), 98 (18.4), 70 (25.1), 59 (16.9), 58 (17.6), 57 (21.5), 44 (47.9), 43 (100), 42 (60.4), 41 (27.0). $[\alpha]^{23}_D$ −20.2° (*c* 9.95, $CHCl_3$, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.30; H, 8.76; N, 6.45. Found: C, 54.98; H, 8.73; N, 6.47.

(2*R*,6*R*)-**5**. ^1H NMR (500 MHz): 1.29 (s, 3 H), 1.68 (dd, 1 H, *J* = 11.08, 10.96), 2.09 (d, 1 H, *J* = 10.95), 2.24 (s, 3 H), 2.39 (dd, 1 H, *J* =15.30, 5.74), 2.42 (d, 1 H, *J* = 10.95), 2.48 (dd, 1 H, *J* = 15.30, 7.11), 2.75 (d(br), 1 H, *J* = 11.08), 3.33 (d, 1 H, *J* = 11.23), 3.47 (d, 1 H, *J* = 11.23), 3.69 (s, 3 H), 4.21–4.27 (m, 1 H). ^{13}C NMR (50 MHz): 18.7, 38.9, 46.4, 51.6, 59.3, 59.7, 66.5, 69.2, 74.3, 171.1. IR: 3452 (OH), 1741 (C=O). MS *m*/*e* (relative intensity): 217 (21.1), 186 (56.2), 144 (16.1), 143 (26.5), 142 (37.4), 128 (15.0), 114 (13.3), 98 (17.7), 84 (84.8), 70 (26.0), 59 (15.6), 58 (12.7), 57 (19.2), 44 (48.3), 43 (100), 42 (40.7), 41 (15.3). $[\alpha]^{23}_D$ +2.24° (*c* 11.6, $CHCl_3$, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.30; H, 8.76; N, 6.45. Found: C, 54.90; H, 8.45; N, 6.33.

Compounds (2*R*,6*S*)-**5** and (2*S*,6*S*)-**5** were prepared from (*S*)-**4** in the same manner as (2*S*,6*R*)-**5** and (2*R*,6*R*)-**5**, respectively.

(2*R*,6*S*)-**5**. $[\alpha]^{23}_D$ +20.6° (*c* 5.93, $CHCl_3$, 3.5 × 10 mm cell). Anal. Found: C, 54.98; H, 8.45; N, 6.46.

(2*S*,6*S*)-**5**. $[\alpha]^{23}_D$ −2.26° (*c* 9.28, $CHCl_3$, 3.5 × 10 mm cell). Anal. Found: C, 54.92; H, 8.50; N, 6.34.

Methyl (2*S*,6*R*)-2-[4,6-Dimethyl-6-(hydroxymethyl)-morpholinyl]acetate ((2*S*,6*R*)-5) and Methyl (2*R*,6*R*)-2-(4,6-Dimethyl-6-(hydroxymethyl)morpholinyl]acetate ((2*R*,6R)-5). Method b. A solution of methyl 4-bromo-2-butenoate (4.04 g, 22.5 mmol) in dry Et_2O (30 mL) was added in 1 h to a stirred solution of (*R*)-**3** (2.68 g, 22.5 mmol) and K_2CO_3 (9.34 g, 67.6 mmol) in dry Et_2O (40 mL) containing 2 mL of dry MeOH. The reaction mixture was stirred for 24 h at rt under N_2. The pale yellow reaction mixture was filtered through Celite and then concentrated to give 4.88 g (100%) of a diastereomeric mixture of (2*S*,6*R*)-**5** and (2*R*,6*R*)-**5** in a ratio of 6:1 as estimated by integration of ^1H NMR absorptions.

(2*R*,6*S*)-2-(Hydroxymethyl)-6-[(methoxycarbonyl)methyl]-2,4,4-trimethylmorpholinium Iodide ((2*R*,6*S*)-6). To a solution of (2*S*,6*R*)-**5** (0.868 g, 4.00 mmol) in dry Et_2O (30 mL) was added CH_3I (5 mL, 80 mmol). The mixture was placed in the dark and stirred for 3 d. The solution was decanted, and the precipitate was washed with dry Et_2O (3 ×

5 mL). The yellow paste was dried under vacuum to give 1.06 g (74%) of a light yellow solid, which was used for the next reaction without further purification. Crystals for X-ray analysis were obtained by recrystallization from MeOH by vapor diffusion with Et_2O.

(2*R*,6*S*)-**6** (mp 136.5–140 °C, dec). ^{1}H NMR (400 MHz, CD_3OD): 1.27 (s, 3 H), 2.60 (dd, 1 H, J = 16.13, 7.08), 2.65 (dd, 1 H, J = 16.13, 5.04), 3.13 (d, 1 H, J = 13.39), 3.26 (dd, 1 H, J = 11.91, 12.82), 3.30 (s, 3 H), 3.36 (s, 3 H), 3.56 (d, 1 H, J = 11.69), 3.63 (ddd, 1 H, J = 12.82, 1.56, 1.49), 3.71 (s, 3 H), 3.83 (dd, 1 H, J = 13.39, 1.56), 3.95 (d, 1 H, J =11.69), 4.63–4.70 (m, 1 H). ^{13}C NMR (50 MHz, CD_3OD): 26.94, 38.31, 51.52, 52.56, 59.53, 63.74, 63.96, 64.10, 74.98, 171.72. IR: 3346 (OH), 1734 (C=O), 1058 (C–O–C). MS, FAB, *m/e*: 232 ($M - I^-$). $[\alpha]^{22}_D$ −21.3° (*c* 7.10, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{11}H_{22}NO_4I$: C, 36.77; H, 6.13; N, 3.90. Found: C, 36.64; H, 6.02; N, 3.87.

Compounds (2*R*,6*R*)-**6**, (2*S*,6*S*)-**6**, and (2*S*,6*R*)-**6** were prepared from (2*R*,6*R*)-**5**, (2*S*,6*S*)-**5**, and (2*R*,6*S*)-**5**, respectively, in the same manner as (2*R*,6*S*)-**6**.

(2*R*,6*R*)-**6** (mp 136.2–140 °C, dec). ^{1}H NMR (400 MHz, CD_3OD): 1.45 (s, 3 H), 2.63 (dd, 1 H, J = 15.38, 8.41), 2.67 (dd, 1 H, J = 15.38, 4.91), 3.20 (dd, 1 H, J = 12.05, 11.74), 3.31 (s, 3 H), 3.34 (d, 1 H, J = 11.71), 3.43 (s, 3 H), 3.45 (d, 1 H, J =13.74), 3.46 (d, 1 H, J = 11.71), 3.57 (dd, 1 H, J = 13.74, 1.84), 3.64 (ddd, 1 H, J = 11.74, 2.18, 1.84), 3.71 (s, 3 H), 4.61–4.68 (m, 1 H). ^{13}C NMR (50 MHz, CD_3OD): 19.93, 38.13, 51.90, 52.49, 59.74, 63.21, 64.34, 65.39, 69.92, 74.77, 171.62. IR: 3359 (OH), 1735 (C=O), 1066 (C–O–C). MS, FAB, *m/e*: 232 ($M - I^-$). $[\alpha]^{22}_D$ +15.1° (*c* 9.90, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{11}H_{22}NO_4I$: C, 36.77; H, 6.13; N, 3.90. Found: C, 36.67; H, 6.08; N, 3.85.

(2*S*,6*S*)-**6**. $[\alpha]^{22}_D$ −14.8° (*c* 9.25, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 36.76; H, 6.08; N, 3.84.

(2*S*,6*R*)-**6**. $[\alpha]^{22}_D$ +21.5° (*c* 6.40, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 36.66; H, 6.05; N, 3.84.

(2*R*,6*S*)-6-(Carboxylatomethyl)-2-(hydroxymethyl)-2,4,4-trimethylmorpholinium ((2*R*,6*S*)-1). A solution of (2*R*,6*S*)-**6** (0.95 g, 2.6 mmol) in 0.1 M NaOH (26 mL, 2.6 mmol) was stirred at rt overnight. The reaction mixture was concentrated and then dried under vacuum. The residual solid was dissolved in CH_3OH (30 mL) and filtered. The liquid was concentrated and dried under vacuum. The resulting light yellow solid was dissolved in a minimum amount of CH_3OH, and acetone (40 mL) was added. The solution was decanted, and the precipitate was dried under vacuum. The solid obtained was dissolved in CH_3OH (80 mL). Then acetone (900 mL) was added. The solution was left open to the air on the bench, yielding 0.44 g (67%) of colorless crystals.

(2*R*,6*S*)-**1** (mp 230–231 °C). ^{1}H NMR (400 MHz, CD_3OD): 1.26 (s, 3 H), 2.30 (dd, 1 H, J = 15.34, 6.44), 2.46 (dd, 1 H, J = 15.34, 6.61), 3.06 (d, 1 H, J = 13.40), 3.11 (dd, 1 H, J = 12.55, 11.79), 3.23 (s, 3 H), 3.30 (s, 3 H), 3.56 (ddd, 1 H, J = 12.55, 1.79, 1.68), 3.64 (d, 1 H, J = 11.80), 3.75 (dd, 1 H, J = 13.40, 1.79), 3.87 (d, 1 H, J = 11.80), 4.49–4.57 (m, 1 H). ^{13}C NMR (50 MHz, CD_3OD): 26.84, 42.21, 51.24, 59.25, 63.89, 64.92, 64.99, 74.65, 176.94. IR: 3387 (OH), 1586 (C=O), 1061 (C–O–C). MS, FAB, *m/e*: 218 ($M^+ + 1$). $[\alpha]^{22}_D$ −23.1° (*c* 6.50, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4{\cdot}2H_2O$: C, 47.43; H, 9.09; N, 5.53. Found: C, 47.78; H, 8.93; N, 5.46.

Compounds (2*R*,6*R*)-**1**, (2*S*,6*S*)-**1**, and (2*S*,6*R*)-**1** were prepared from (2*R*,6*R*)-**6**, (2*S*,6*S*)-**6**, and (2*S*,6*R*)-**6**, respectively, in the same manner as (2*R*,6*S*)-**1**.

(2*R*,6*R*)-**1** (mp 235.5–236.5 °C). ^{1}H NMR (400 MHz, CD_3OD): 1.42 (s, 3 H), 2.34 (dd, 1 H, J = 14.82, 6.43), 2.45 (dd, 1 H, J = 14.82, 6.26), 3.09 (dd, 1 H, J = 12.26, 11.26), 3.24 (s, 3 H), 3.30 (d, 1 H, J = 11.90), 3.35 (s, 3 H), 3.42 (s, 2 H), 3.46 (d, 1 H, J = 11.90), 3.57 (d, 1 H, J = 12.26), 4.51 (m, 1 H). ^{13}C NMR (50 MHz, CD_3OD): 20.00, 42.36, 51.65, 59.53, 64.48, 65.23, 65.43, 70.06, 74.44, 177.09. IR: 3583 (OH), 1587 (C=O), 1078 (C–O–C). MS, FAB, *m/e*: 218 ($M^+ + 1$). $[\alpha]^{22}_D$ +15.6° (*c* 4.8, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4{\cdot}2H_2O$: C, 47.43; H, 9.09; N, 5.53. Found: C, 47.31; H, 8.83; N, 5.47.

(2*S*,6*S*)-**1**: $[\alpha]^{22}_D$ −16.0° (*c* 5.00, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 47.29; H, 8.77; N, 5.57.

(2*S*,6*R*)-**1**: $[\alpha]^{22}_D$ +23.1° (*c* 5.40, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 47.05; H, 8.89; N, 5.34.

Benzyl 3-butenoate (10). To a stirred solution of 3-butenoic acid (5.0 g, 60 mmol) and anhyd pyridine (11.32 g, 145.2 mmol) in dry CH_2Cl_2 (40 mL) was added a solution of CBZ-Cl[28] (10.86 g, 63.89 mmol) in dry CH_2Cl_2 (20 mL) dropwise at rt. After being stirred at rt for 24 h, the cloudy solution was filtered through Celite. The filtrate was washed with saturated aqueous $CuSO_4$ (3 × 20 mL) and brine (25 mL). The organic phase was dried and concentrated. The residue was purified by flash chromatography (10% Et_2O–hexanes) to afford 8.9 g (88%) of **10** as a colorless oil. An identical mass spectrum was obtained as reported.[29]

Benzyl 3,4-Dibromobutanoate (11). To a solution of **10** (10 g, 57 mmol) in dry CH_3CN (10 mL) at 0 °C in the dark was added Br_2 (2.93 mL, 56.8 mmol) dropwise in 10 min and stirred for 5 min. Saturated aqueous $Na_2S_2O_3$ (20 mL) was added. The solution was extracted with Et_2O (3 × 20 mL). The ethereal extract was washed with saturated $NaHSO_3$ (20 mL), saturated $NaHCO_3$ (15 mL), and brine (25 mL). The extract was dried and concentrated to afford 18.3 g (96%) of a pale yellow oil, which was used for the next reaction without purification. ^{1}H NMR (270 MHz): 2.91 (dd, 1 H, J_{app} = 8.8, 16.4), 3.37 (dd, 1 H J_{app} = 4.2, 16.7), 3.71 (dd, 1 H, J_{app} = 9.8, 10.1), 3.90 (dd, 1H, J_{app} = 4.4, 10.2), 4.52 (m, 1 H), 5.18 (s, 2 H), 7.27–7.38 (m, 5 H); ^{13}C NMR (67.5 MHz): 35.2, 41.7, 44.7, 66.9, 128.3, 128.6, 135.3, 169.5; MS *m/e* (EI): 338/336 (M^+), 257/255 (M^+ − Br), 229/227 (M^+ − Br − C_2H_4), 91 (CH_2Ph); HRMS calcd for $C_{11}H_{12}O_2Br_2$ 333.9204, found 333.9195.

Benzyl 4-bromo-2-butenoate (9).[23,30] To a solution of **11** (14.6 g, 43.6 mmol) in dry Et_2O (50 mL) at 0 °C was added freshly distilled Et_3N (8.8 g, 87 mmol) in dry Et_2O (30 mL) over a period of 30 min. The turbid reaction mixture was allowed to warm to rt (2 h) and was filtered through a Celite pad. The filtrate was washed with 1 N HCl (25 mL) and brine (20 mL). The extract was dried and concentrated. The residue was dissolved in Et_2O and filtered through a short silica column eluting with 10% Et_2O in hexanes. Material with R_f 0.2 was collected. After evaporation of the solvent, recrystallization from pentane gave 11.9 g (82%) of **9** as colorless needles (mp 41.8–42.4 °C). ^{1}H NMR (270 MHz): 4.01 (d, 2 H, J_{app} = 7.3), 5.20 (s, 2 H), 6.08 (dd, 1 H, J_{app} = 1.3, 15.3), 7.05 (dt, 1 H, J_{app} = 7.5, 15.3), 7.27–7.33 (m, 5 H); ^{13}C NMR (67.5 MHz): 28.9, 66.5, 124.4, 128.3, 128.6, 142.2, 165.3; MS *m/e* (EI): 255 (M^+), 91 (CH_2Ph); Anal. Calcd for $C_{11}H_{11}O_2Br$: C, 51.79; H, 4.35; Br, 31.32. Found: C, 51.96; H, 4.33; Br, 31.11.

Determination of Optical Purity of 2-Methylglycidol by ^{1}H NMR. To a solution of 2-methylglycidol (13.2 mg, 0.150 mmol) and Et_3N (0.06 mL) in CH_2Cl_2 (1 mL) was added (*R*)-(+)-MTPA-Cl[15] (0.10 mL). After placing in a refrigerator (0 °C) for 10 h, the reaction mixture was washed with 5% H_2SO_4 (2 mL), filtered, and concentrated affording the Mosher's ester. ^{1}H NMR (200 MHz): (a) Mosher's ester of (*R*)-(+)-2-methylglycidol: 1.36 (s, 3 H, CCH_3), 2.64 (d, 1 H, *H*CH-oxirane, J_{app} = 4.6), 2.77 (d, 1 H, *H*CH-oxirane, J_{app} = 4.6), 3.56 (s, 3 H, OCH_3), 4.23 (d, 1 H, *H*CHO-MTPA, J_{app} = 11.9), 4.47 (d, 1 H, *H*CHO-MTPA, J_{app} = 11.8), 7.35–7.68 (m, 5 H, aromatic). (b) Mosher's ester of (*S*)-(−)-2-methylglycidol: 1.33 (s, 3 H, CCH_3), 2.64 (d, 1 H, *H*CH-oxirane, J_{app} = 4.7), 2.74 (d, 1 H, *H*CH-oxirane, J_{app} = 4.7), 3.56 (s, 3 H, OCH_3), 4.16 (d, 1 H, *H*CHO-MTPA, J_{app} = 11.8), 4.50 (d, 1 H, *H*CHO-MTPA, J_{app} = 11.9), 7.35–7.68 (m, 5 H, aromatic).

The optical purities of (*R*)- and (*S*)-2-methylglycidols were determined by integration of the signals (dd) of H_2CO-(+)-

(28) Kim, S.; Lee, J. I.; Kim, Y. C. *J. Org. Chem.* **1985**, *50*, 560–565.

(29) Corina, D. L.; Wright, J. N.; Ballard, K. E. *Org. Mass Spectrom.* **1983**, *18*, 60–63.

(30) No spectroscopic or analytical data for (**9**) were given in the reference 23.

(31) The author has deposited atomic coordinates for these structures with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

MTPA. The ee values were 88.4% for (*R*)-2-methylglycidol and 91.8% for (*S*)-2-methylglycidol.

Enrichment and Determination of Optical Purity of 2. The optical purities of commercial (*S*)- and (*R*)-2-methylglycidol were enriched by converting them into (*R*)- and (*S*)-**2** as described[19] with the following modifications: (a) the reaction was run in Et_2O for 1 h; (b) we washed the reaction solution with 1 N HCl; (c) the product was recrystallized twice from Et_2O; and (d) the yields were 80−82%. The optical purities of (*R*)- and (*S*)-**2** were determined by the following procedure.

A solution of **2** (0.711 g, 3.00 mmol) in MeOH (9 mL) was added to a solution of $HNMe_2$ (0.54 g, 12 mmol) in MeOH (3 mL). The reaction mixture was stirred for 2 h and then concentrated. MeOH (3 mL) was added, and the precipitate was removed by filtration. The solution was concentrated. Again, MeOH (2 mL) was added, and the precipitate was removed by filtration. The solution was placed in freezer for 0.5 h. The liquid was pipetted out and concentrated and then placed under vacuum. Vacuum distillation of the residue gave 0.30 g (75%) of 3-(dimethylamino)-2-methylpropane-1,2-diol, **7**, as a colorless oil (bp: 150 °C/17 Torr). ^{1}H NMR (200 MHz): 1.07 (s, 3 H, C*H*$_3$C), 2.37 (s, 6 H, C*H*$_3$N), 2.43 (dd, 1 H, *H*CHN, J_{app} = 13.7, 1.8), 2.57 (d, 1 H, *H*CHN, J_{app} = 13.6), 3.46 (dd, 1 H, *H*CHOH, J_{app} = 11.0, 1.7), 3.67 (d, 1 H, *H*CHOH, J_{app} = 11.1). ^{13}C NMR (50 MHz): 23.7, 47.9, 67.9, 70.7, 71.2. IR: 3405 (OH), 1044 (C−N). MS, FAB, *m*/*e*: 134 ($M^+ + 1$). Anal. Calcd for $C_6H_{15}NO_2$: C, 54.14; H, 11.28; N, 10.53. Found: C, 53.82; H, 11.10; N, 10.41.

To a solution of **7** (24 mg, 0.18 mmol) and Et_3N (0.05 mL) in CH_2Cl_2 (1 mL) was added (*R*)-(+)-MTPA-Cl (0.10 mL) at 0 °C. The reaction mixture was placed in a freezer for 10 h. NaOH solution (5%) was added until pH ~ 12. The phases were separated, and the aqueous solution was extracted with CH_2Cl_2 (3 × 2 mL). The combined CH_2Cl_2 extracts were washed with H_2O (2 × 5 mL), dried, and concentrated to give Mosher's ester, **8**, as an oil.

The optical purities of (*R*)- and (*S*)-**2** were determined by integration of the ^{1}H NMR signals of C*H*$_3$C of Mosher's ester **8**. ^{1}H NMR (400 MHz): 1.086 (s, C*H*$_3$C) for Mosher's ester from (*S*)-**2**, 1.069 (s, C*H*$_3$C) for Mosher's ester from (*R*)-**2**. The optical purities of both (*R*)- and (*S*)-**2** were greater than 98%. The minor isomer was undetectable.

Enzymatic Assays. Materials. Pigeon breast CAT, acetyl-(*R*)-carnitine, and CoA were used as received from Sigma.

Methods. 1. Assay of CAT. CAT activity was measured at 30 °C by monitoring the CoA ester formation directly at 232 nm using $\epsilon = 4500\ M^{-1}\ cm^{-1}$ for the thioester bond.[26] The assay contained in a volume of 1 mL: 0.01 units (0.1 μg) CAT, 20 mM $KHPO_4$ buffer, pH = 7.4, [acetyl-(*R*)-carnitine] ranged from 100−2000 μM, and [CoA] = 200 μM.

IC_{50} Determinations. CAT activity was measured as described above with [acetyl-(*R*)-carnitine] = 250 μM (K_m = 350 μM) and [CoA] = 200 μM (K_m = 20 μM). The IC_{50} value given for each compound is the concentration of inhibitor that gives 50% inhibition compared to a control without inhibitor under these conditions.

K_i Determination. CAT activity was measured as described above with [CoA] = 200 μM. For each [acetyl-(*R*)-carnitine], which was systematically varied from 100−2000 μM, the [(2*R*,6*S*)-**1**] varied as follows: 50, 100, 250, 500, and 750 μM. A value of $K_i = 190 \pm 20$ was found.

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