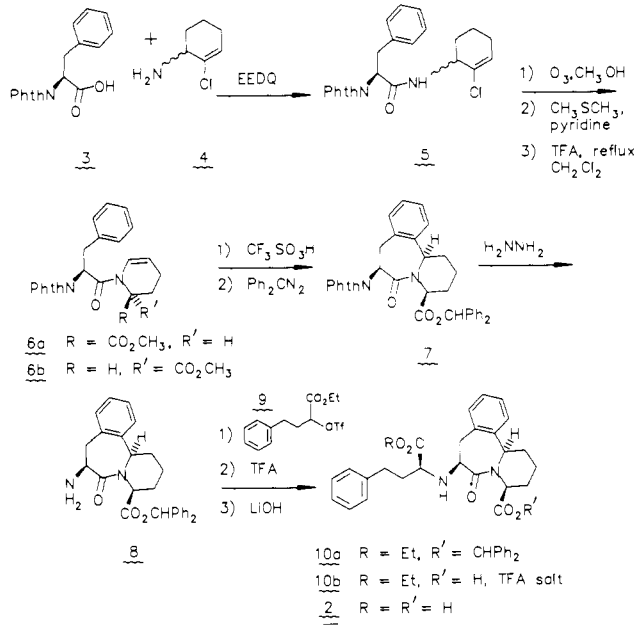
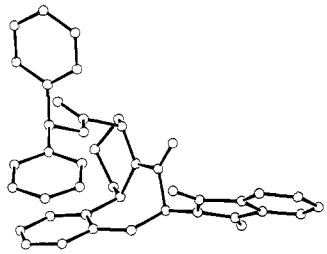
Scheme I



CH_3OH, -78 °C) followed by reduction with dimethyl sulfide (-78 → 25 °C, 12 h), workup, and mild dehydration (TFA, CH_2Cl_2, reflux, 3 h) gave a 1:1 ratio of isomeric acylenamines **6a** and **6b** which were separated by preparative HPLC (50% EtOAc/hexane) in 70% overall yield. Cyclization of acylenamine **6a** (CF_3SO_3H, CH_2Cl_2, 25 °C, 24 h) and re-esterification of the acidic product (Ph_2CN_2, CH_2Cl_2) gave the desired optically pure benzhydryl ester **7** (mp 156-157 °C, $[a]_D^{20}$ = -88.1°, 1.1, $CHCl_3$) in 77% yield. The relative stereochemistry and conformation of **7** have been confirmed by X-ray crystallography.



The stereospecificity of the acyl-iminium ion induced electrophilic aromatic substitution may be a result of a preference for the equatorial orientation of the phthalimide moiety in the transition state. Curiously, the other acylenamine isomer was resistant to cyclization under similar conditions. It is believed that the concurrent methyl ester hydrolysis which is observed during the cyclization reaction is facilitated by through-space participation of the proximal aromatic ring. Further mechanistic considerations will be discussed elsewhere.

Phthalimido ester **7** was converted to free amine **8** (H_2NNH_2, CH_3OH, Δ) which was then coupled to the 4-phenylbutyric ester side chain by S_N2 displacement of (*R*)-triflate[10] **9** (CH_2Cl_2, 1,8-bis(dimethylamino)naphthalene, 88% yield). Selective cleavage of the resulting benzhydryl ester **10a** (TFA, anisole, 25 °C, 91%) gave prodrug **10b** as a colorless analytically pure TFA salt ($[\alpha]_D^{20}$ = +25.5°, *c* 0.57, CH_3CN). Further hydrolysis with lithium hydroxide gave zwitterionic diacid **2** in 85% yield [mp 259-260 °C dec, $[\alpha]_D^{20}$ = +24°, 0.05, MeOH].

Diacid **2**, which inhibited rabbit lung ACE with a K_i of 1.2×10^{-11} M, is the most potent in vitro inhibitor of ACE we have examined.[11] Prodrug **10b** was orally active in the conscious spontaneously hypertensive rat as determined by reduction of angiotensin I-induced increase in blood pressure.[12] The potency of inhibition observed for hindered diacid **2** suggests that the region of ACE which binds the terminal carboxyl group is uncluttered and that significant binding affinity, over that realized by other inhibitors, may be the result of hydrophobic interactions near this site.

We have demonstrated the utility of acyl-iminium ion chemistry in the preparation of a useful conformationally restricted dipeptide mimic. The key cyclization step occurs in excellent yield with a high degree of stereospecificity. The potent activity of the angiotensin-converting enzyme inhibitor **2** is a strong indication of the usefulness of this design approach. Further applications of this methodology, which may shed important insights into protein structure and function, are currently being explored in our laboratory.

Acknowledgment. We express gratitude to Douglas W. Beight for the preparation of precursors to compound **4**. High field NMR studies provided by Dr. Michael Whalon and Edward Huber as well as analytical separations performed by Nicholas W. Brake were particularly helpful. Single-crystal X-ray structures were provided by Dr. John C. Huffman, Indiana University.

(7) Amine **4** was prepared from 2,3-dichlorocyclohexene (Bergman, E. *J. Org. Chem.* **1963**, *28*, 2210) in 70% yield by displacement with potassium phthalimide and potassium iodide in DMF followed by dephthaloylation with hydrazine.
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(12) A 3 mg/kg dose of **10b** caused half-maximal inhibition at 1 h post oral dosing, and significant inhibition was sustained for 4 h.

A "Siameso" Inhibitor: Chiral Recognition of a Prochiral Bilaterally Symmetric Molecule by Carnitine Acetyltransferase

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Fascination with chiral recognition by enzymes began in 1858 with Pasteur's report[1] of stereoselective fermentation of tartaric acid. Explanations of the process of chiral recognition later appeared, with notable contributions from Bergmann[2] in the 1930s, Ogston[3] in 1948, and Hirschmann[4] in 1960 as well as reviews by Popjak[5] and Alworth[6] in the early 1970s. Ogston[3] proposed that chiral recognition requires only a three-point contact between enzyme and substrate. Alworth[6] emphasized that chemical nonequivalence of enantiotopic groups is the critical factor for chiral recognition and clearly illustrated this for recognition of succinic acid.

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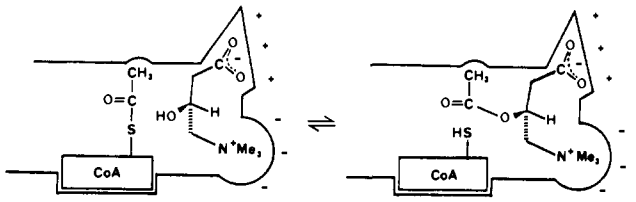
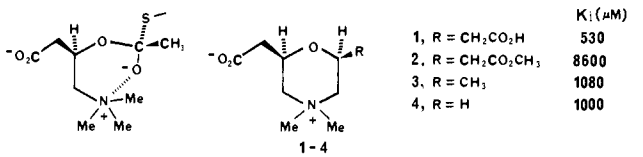


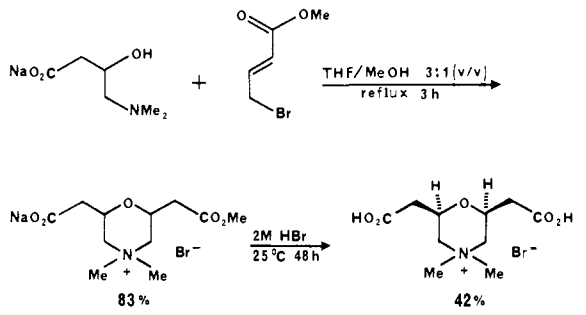
Figure 1. Mode of substrate recognition in CAT.



	K_i (μM)
1, R = CH_2CO_2H	530
2, R = $CH_2CO_2CH_3$	8600
3, R = CH_3	1080
4, R = H	1000

Figure 2. Conformationally rigid analogues (right) of the proposed tetrahedral intermediate (left) for acetyl transfer in CAT and their K_is with respect to (*R*)-carnitine.

Scheme I



83% 42%

Carnitine needs only two-point recognition by carnitine acetyltransferase (CAT) (Figure 1), because acetyl coenzyme A is the third locus required for chiral recognition.[7] Pigeon breast CAT binds enantiomers of both carnitine and acetylcarnitine equally well. (*R*)-Carnitine (K_m, 120 μM) and (*R*)-acetylcarnitine (K_m, 350 μM) are substrates in the forward and reverse reactions, respectively, while the (*S*)-enantiomers are competitive inhibitors,[8,9] K_is 106 and 256 μM, respectively. CATs from other sources[10-12] stereoselectively bind these substrates. In all CATs, the (*R*)-enantiomers are crucial for acetyl transfer.

We have synthesized conformationally rigid analogues of the tetrahedral intermediate proposed for acetyl transfer in CAT[7] (Figure 2). These analogues map the topography of the binding site for carnitine (and acetylcarnitine). We propose that our analogues must have the same relative configuration as (*R*)-carnitine, if CAT stereoselectively binds them as it binds the tetrahedral intermediate. To verify that pigeon breast CAT recognizes only one configuration of our inhibitors, we have devised a unique approach that utilizes only racemic compounds and a prochiral molecule with an achirotopal plane.

We made (*meso*)-2,6-bis(carboxymethyl)-4,4-dimethylmorpholinium, **1**, in two steps from condensation of sodium (*RS*)-norcarnitine[13] and methyl (*E*)-4-bromo-2-butenoate in THF/MeOH (Scheme I). Only one pair of enantiomers, presumably the cis diastereomer of **2**, was present by ^{1}H NMR, indicating that the ring formed stereoselectively. Hydrolysis of **2** yielded the anticipated diequatorial meso diacid, whose structure was verified by single-crystal X-ray analysis.[14] The solid-state

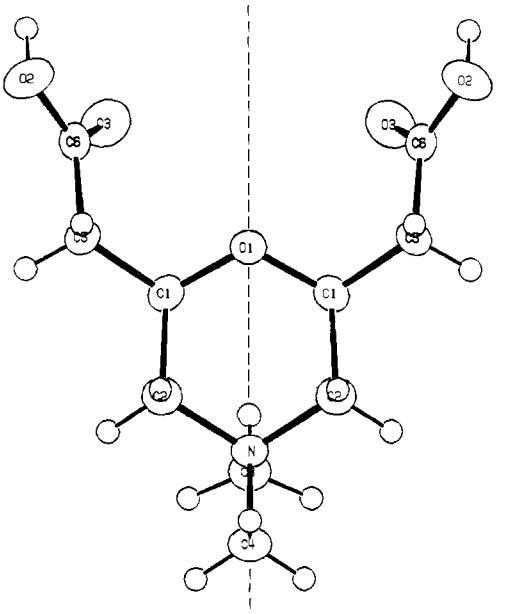


Figure 3. Perspective drawing of the crystal structure of **1**.

structure of **1** (Figure 3), in fact, displays meso symmetry, with a crystallographic mirror plane containing the atoms O1, N, C3, and C4. Hence, we call it a "Siameso" inhibitor[15] because of its morphological similarity to Siamese twins. Compounds **3** and **4** were prepared by similar reactions of the appropriate dimethylamino alcohol and bromoalkenoate. Compound **3** was identified as the cis diastereomer by single-crystal X-ray analysis.[16]

We have measured[17] the K_is of **1**–**4** with pigeon breast CAT (Figure 2). Of this series, **1** binds most strongly, with a K_i half that of the racemic compounds **3** and **4**. This is because every molecule has one side with the correct configuration of (*R*)-carnitine. This twofold improvement in binding for **1** suggests that CAT is selectively binding one configuration of these inhibitors. Compound **2** does not bind well because of the increased size of the ester or the polarity change from acid to ester. The key features of these inhibitors are their rigidity and their similarity to the tetrahedral intermediate. Rigidity in the inhibitor reduces binding because only the enzyme can adjust, but rigidity is essential for identifying the topographical arrangement of recognition points on the enzyme as well as the conformation of the substrate fragment of the tetrahedral intermediate. For example, the N–C–C–O torsion angle in the inhibitors is locked in a gauche conformation, which is predicted for carnitine bound to CAT.[18]

In conclusion, we have prepared three inhibitors, two racemates and a "Siameso", whose relative K_is with pigeon breast CAT suggest chiral recognition. We propose that CAT selectively binds one configuration in these inhibitors, because they are analogues of the proposed tetrahedral intermediate in the stereospecific acetyl transfer.

Acknowledgment. L.J.B. is grateful to the American Diabetes Association and the National Institutes of Health (DK39285) for support of the biological work. We thank the referees for their valuable suggestions.

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(14) Diffraction data were collected, and structure solution was performed by using an Enraf-Nonius CAD4 diffractometer, equipped with Mo Kα radiation. $C_{10}H_{18}NO_5Br$: fw = 312.2, orthorhombic space group *Pnma*, a = 11.422 (2) Å, b = 15.051 (2) Å, c = 7.762 (1) Å, V = 1334.4 (6) Å^3, Z = 4, λ = 0.71073 Å, D_{calcd} = 1.554 g cm^{-3}, R = 0.048 for 694 observed data with $\theta < 25°$, 123 variables.
(15) In searching for a suitable name for our meso inhibitor, we first talked with Dick Wolfenden, who suggested "Siamese". Next was Paul A. Bartlett, who eruditely offered "Janus". Our dilemma was resolved by Julius Rebek, who neologized the hybrid, "Siameso".
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