Inhibition of γ-Butyrobetaine Hydroxylase by Cyclopropyl-Substituted γ-Butyrobetaines

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γ-Butyrobetaine hydroxylase (4-(trimethylamino)butyrate, 2-oxoglutarate: oxygen oxidoreductase (3-hydroxylating); EC 1.14.11.1) catalyzes the final step in the biosynthesis of carnitine. A stepwise, homolytic mechanism has been proposed (Englard, S.; et al. *Biochemistry* **1985**, *24*, 1110). Cyclopropyl-substituted substrate analogues **1**, **2**, and **3** were synthesized and evaluated as inhibitors of the enzyme with a view toward their potential as "free radical clock" probes for radical transients. Betaines **1** and **3** are competitive inhibitors and provide K_i values of 12.9 ± 4.6 mM and 7.9 ± 2.2 mM, respectively. Betaine **2** is a noncompetitive inhibitor with values for K_{ii} of 1.54 ± 0.31 mM and for K_{is} of 1.96 ± 1.36 mM. In each case enzyme activity was determined by measurement of (a) the $^{14}CO_2$ liberated from the coupled decarboxylation of [1-^{14}C]-2-oxoglutarate and (b) the ^{3}H released into the aqueous medium from [2,3-^{3}H]-γ-butyrobetaine added as substrate. None of the three cyclopropyl derivatives exhibited turnover, and preincubation of these substrate analogues with γ-butyrobetaine hydroxylase did not result in a time-dependent loss of activity greater than that of the controls. These results are discussed in terms of the conformation of the inhibitors relative to those of the substrate.

Introduction

Carnitine (vitamin B_T) functions as the carrier molecule in the transport of long-chain fatty acyl groups across the inner mitochondrial membrane.[1] The role of carnitine in metabolism,[1a,c] its nutritional aspects,[2] its relevance to certain disease states,[3] its pharmacological aspects,[4] and its possible involvement in peroxisomal β-oxidation of fatty acids[5] have been extensively reviewed. The preeminent role of carnitine in fatty acid metabolism makes it an attractive target for the development of new therapeutic agents.[6] Gandour and co-workers[7,8] have proposed a molecular mechanism to reconcile the structure and function of carnitine in the context of acetylcarnitine transferase activity.

γ-Butyrobetaine hydroxylase (4-(trimethylamino)-butyrate, 2-oxoglutarate: oxygen oxidoreductase (3-hydroxylating); EC 1.14.11.1) catalyzes the final step in the biosynthesis of carnitine from lysine[1a,c,2b,9] in humans and other organisms. The enzyme from *Pseudomonas* sp. AK 1 (MW 95 000 D) is composed of two nonidentical subunits of approximately equal size,[10] and it has been suggested that each subunit may house an active site.[10b] The 2-oxoglutarate-dependent dioxygenases, among which γ-butyrobetaine hydroxylase is numbered, share a common requirement for Fe(II), molecular oxygen, 2-oxoglutarate, and ascorbate.[11] An earlier step in the biosynthesis of carnitine is also mediated by a 2-oxoglutarate-dependent dioxygenase which hydroxylates 6-*N*-trimethyllysine,[1a,c,2b] and this has fueled interest in the metabolic relationship between vitamin C deficiency and carnitine.[12] γ-Butyrobetaine hydroxylase is currently thought to operate through a mechanism similar to that of prolyl 4-hydroxylase;[13] the evidence for this has been outlined in earlier reports of studies of stereospecifically radiolabeled γ-butyrobetaine by Englard and co-workers.[14] Thoughtful proposals for the mechanism of prolyl 4-hydroxylase have been put forward by Hanauske-Abel and Günzler[15] as well as Siegel[16] and Visser.[17] The common mechanistic theme of these proposals is a stepwise, homolytic process involving discrete, short-lived carbon radicals sequestered in the active site, in analogy to the mechanistic picture of hydroxylation by the cytochromes P-450 provided by Groves[18] and others.[19] However, there is a paucity of definitive evidence for the intermediacy of carbon radicals in the non-heme iron dioxygenases in general and γ-butyrobetaine hydroxylase in particular.

The experimental determination of the structure and

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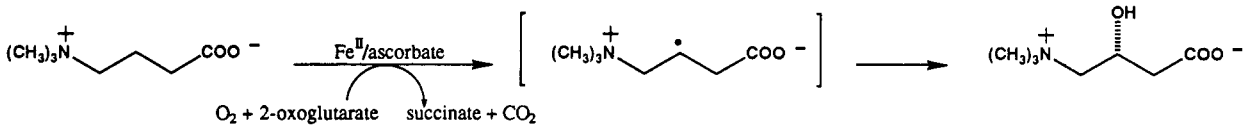
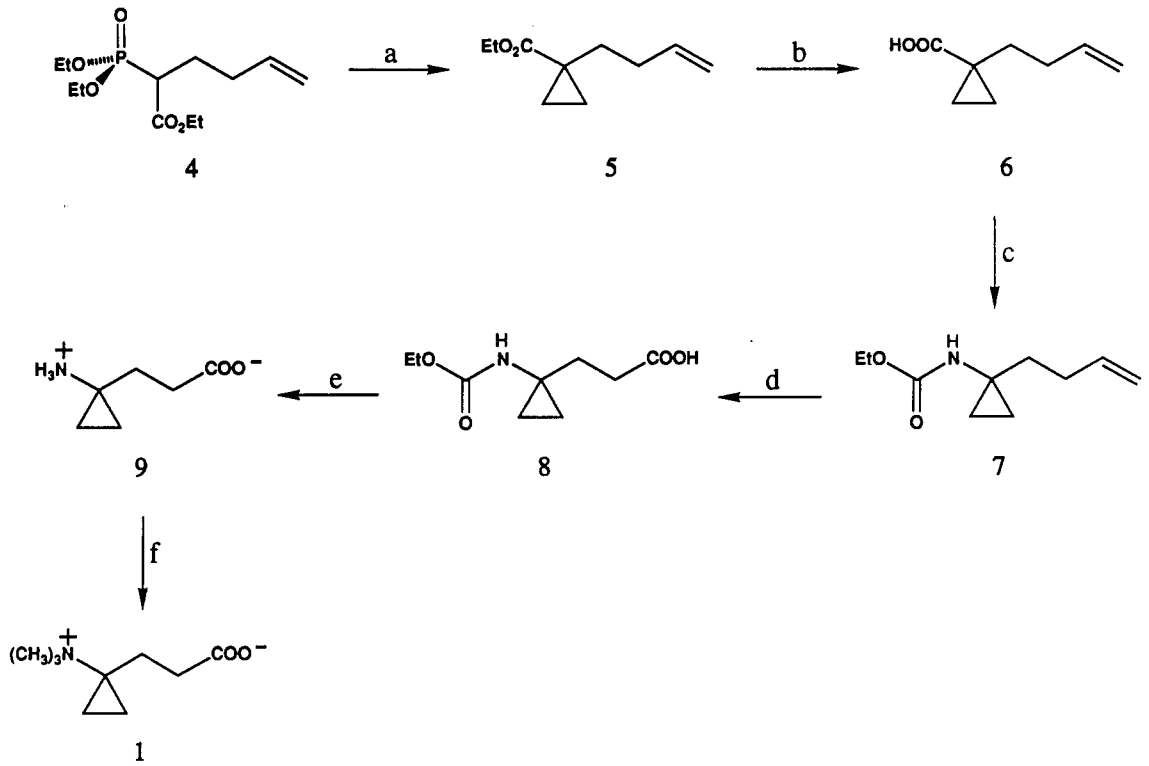


Figure 1. Proposed mechanism[14] for stereospecific hydroxylation of γ-butyrobetaine by γ-butyrobetaine hydroxylase involves formation of a carbon radical in the active site.

Scheme I[a]



[a] (a) NaH/C_6H_6/oxirane, 66%; (b) KOH/aqueous EtOH, 86%; (c) $(PhO)_2PON_3/PhCH_3$/TEA/80 °C, then EtOH, 69%; (d) $KMnO_4/SiO_2/C_6H_6$, 58%; (e) $ISiMe_3$/TEA/CH_3CN, 86% + starting material; (f) $Ba(OH)_2/CH_3I$/95% EtOH, 11%.

lifetimes of transient intermediates remains a fundamental problem in enzyme kinetics,[20] and radical transients present special challenges. The "free radical clock" strategy[21] offers an opportunity to probe the lifetime of a radical transient in an enzyme active site by introducing a rapid rearrangement which competes with radical quenching. The cyclopropylcarbinyl radical rearrangement, which proceeds with a first order rate constant of 1.3×10^8 s^{-1} at 25 °C,[22] has been used extensively in physical organic chemistry,[21,23] but its application to studying the mechanism and kinetics of radical enzymes has met with mixed success.[24-32] In light of the proposed mechanism, we synthesized three cyclopropyl-substituted analogues of γ-butyrobetaine (**1**, **2**, and **3**) and determined their effect on the reaction catalyzed by the bacterial hy-

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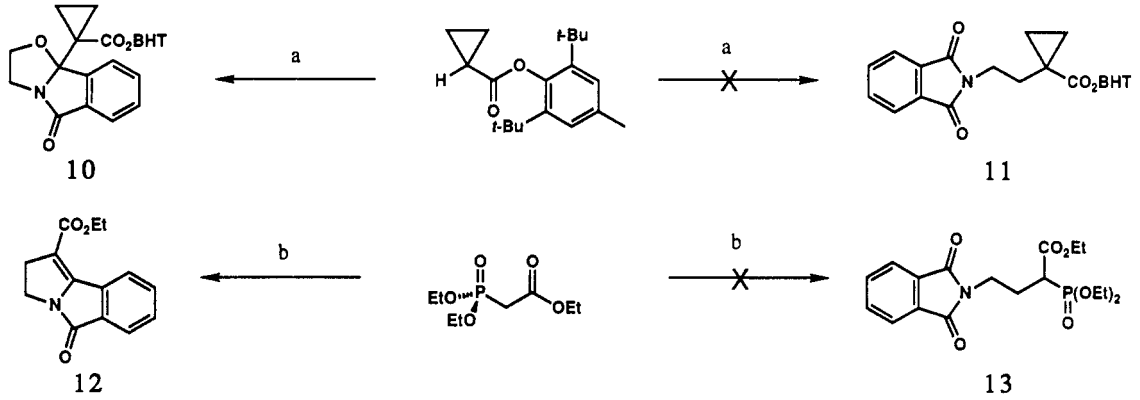
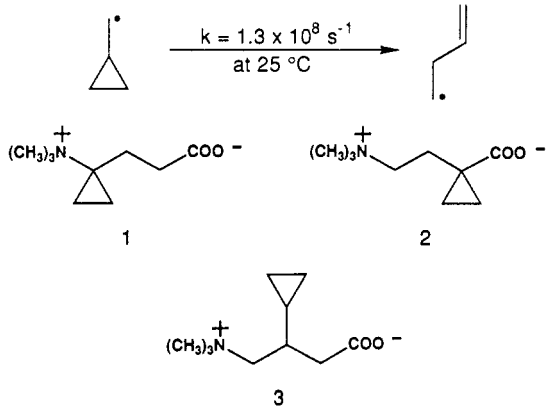
Scheme II[a]



[a] (a) *tert*-Butyllithium/THF/−78 °C/*N*-(2-bromoethyl)phthalimide; (b) NaH/THF/reflux/*N*-(2-bromoethyl)phthalimide.

droxylase. A similar approach has been pursued independently by Pascal.[33]



Results

Synthesis. Inhibitor **1** was prepared in seven steps from commercial material. Alkylation of triethyl phosphonoacetate (NaH/THF/4-bromo-1-butene) gave phosphonate **4** in 84% yield. Homologous Horner–Emmons reaction[34] (NaH/C_6H_6/oxirane) provided the cyclopropane in good yield. Subsequent hydrolysis could be performed in the same pot, but better yields were obtained when the ester was first isolated. Rearrangement of acid **6**, by a modified Curtius rearrangement[35] (diphenyl phosphorazidate/$PhCH_3$/TEA/90 °C), led to **7**[36] after quenching the isocyanate intermediate with ethanol. Oxidative cleavage of the olefin ($KMnO_4/SiO_2/C_6H_6$)[37] and hydrolysis of the carbamate **8** ($ISiMe_3/CH_3CN$) gave the previously reported amino acid **9**.[38] Methylation ($CH_3I/Ba(OH)_2$/95% EtOH) afforded the desired substrate (inhibitor **1**) (see Scheme I).

We initially tried to prepare **2** by alkylation of the lithium enolate of cyclopropanecarboxylic acid 2,6-di-*tert*-butyl-4-methylphenyl ester[39] with *N*-(2-bromoethyl)phthalimide (see Scheme II) to give **11**. Instead, preferential attack at the carbonyl produced **10**, which crystallized, allowing assignment of the structure by X-ray analysis (see the supplementary material). Alkylation of triethyl phosphonoacetate with *N*-(2-bromoethyl)phthalimide also gave an undesirable but interesting product: tricycle **12** (9% yield) instead of **13**. The reaction was clean but could not be driven to completion, even in the presence of excess base; the remainder of the material consisted of the two starting materials. Compound **12** has been previously obtained by condensation of potassium phthalimide with (carbethoxycyclopropyl)triphenylphosphonium tetrafluoroborate,[40] which also involves intramolecular Wittig-type attack on an imide carbonyl. However, Michael addition of triethyl phosphonoacetate (NaH/THF) onto *tert*-butyl acrylate gave the desired phosphonate **14** (49%), which, when subjected to the homologous Horner–Emmons reaction (NaH/C_6H_6/oxirane), provided the cyclopropane **15**. Selective cleavage of the *tert*-butyl ester (CF_3COOH) led to acid ester **16** and subsequent Curtius rearrangement followed by quenching the isocyanate intermediate with ethanol gave **17**. Urea **18** is the major byproduct (86%) when this reaction is quenched with water. Treatment of **17** with $Ba(OH)_2$ in methanol at reflux gave lactam **19**, which in turn was converted to amino acid **20** with $Ba(OH)_2$ in 1:1 aqueous dioxane. Exhaustive methylation and ion-exchange chromatography afforded inhibitor **2** (Scheme III).

Methyl cyclopropylacrylate (**21**) has been prepared by Marino[41] by condensation of lithium dicyclopropylcuprate with methyl propiolate and by Little[42] through base-catalyzed ring closure of (*E*)-6-chloro-2-hexenoic acid methyl ester. Nevertheless, condensation of trimethyl phosphonoacetate with cyclopropanecarboxaldehyde was more convenient, and vinylogous addition of nitromethane[43] (DBN with CH_3NO_2 as solvent) gave nitro ester **22**. The latter proved quite resistant to selective reduction, but adaptation of a procedure of Ganem's[44] (NiB_2/sonication) led directly to lactam **23**, which could be hydrolyzed and methylated in one pot to the betaine **3** (Scheme IV).

Molecular Modeling. The conformations of γ-butyrobetaines **1**, **2**, and **3** were analyzed using MacroModel

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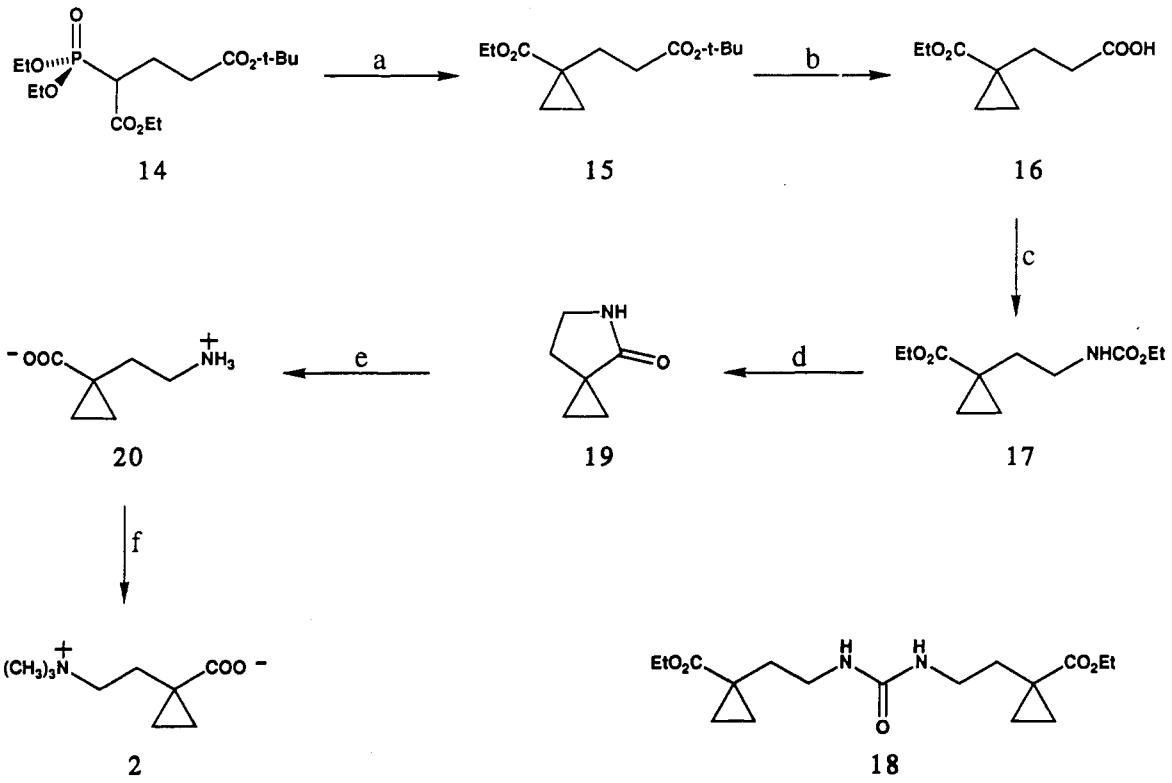
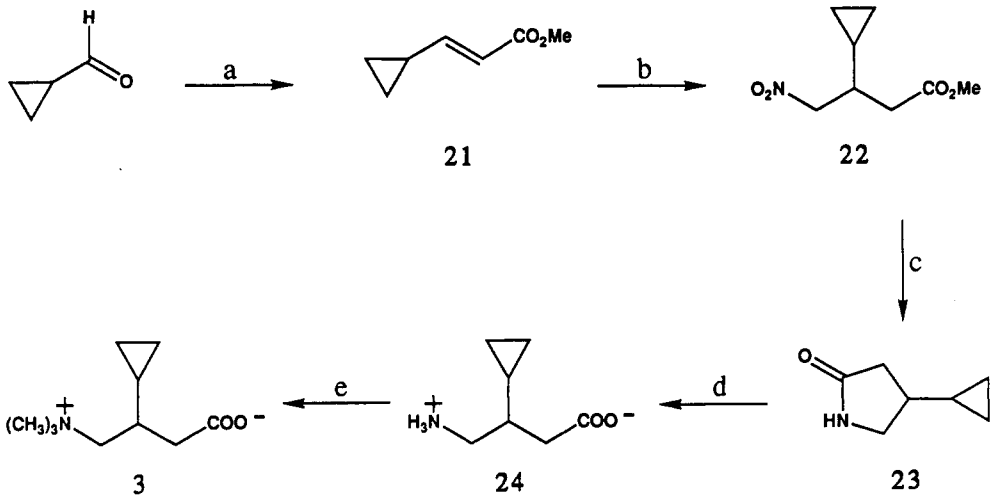
Scheme III[a]



14 15 16 17 19 20 2 18

[a] (a) NaH/C_6H_6/oxirane, 56%; (b) CF_3COOH, 81%; (c) $(PhO)_2PON_3/PhCH_3$/TEA/80 °C, then EtOH, 52%; (d) $Ba(OH)_2 \cdot 8H_2O/CH_3OH$/reflux/14 h, 34%; (e) $Ba(OH)_2 \cdot H_2O$/1:1 aqueous dioxane/reflux, 86%; (f) $CH_3I/Ba(OH)_2 \cdot 8H_2O$/1:1 aqueous dioxane, 44%.

Scheme IV[a]



21 22 23 24 3

[a] (a) $(MeO)_2POCH_2CO_2Me$/NaH/THF/reflux/30 min, 81%; (b) CH_3NO_2/DBN/reflux/12 h, 76%; (c) NiB_2/sonication/30 min/23 °C, 55%; (d) $Ba(OH)_2$/1:1 aqueous dioxane, 64%; (e) $Ba(OH)_2/CH_3I$/1:1 aqueous dioxane, 53%.

2.0[45] to generate predictions for two dihedral angles of interest: θ (C1–C2–C3–C4) and ϕ (C2–C3–C4–N^+). In the case of **3**, an additional dihedral angle (ψ) was examined: H–C3–C5–H, where C5 is the cyclopropyl carbon attached to the betaine backbone. Each permutation of gauche and anti was entered and minimized until the root mean square of the gradient vectors was ≤0.002 kJ/Å using the Amber force field parameters[46] with a distance-dependent dielectric constant of 4.0, which has been shown to approximate the electrostatic contribution to the energy of small charged molecules in polar solvents.[47] The values obtained are summarized in Table I and are relative to the conformer with the lowest calculated energy for each betaine. γ-Butyrobetaine exhibited three minima with the *g,a* (θ,ϕ) being lowest and the *a,a* only 0.8 kcal/mol above it. Inhibitor **1** gave four minima, *a,a* being lowest. Inhibitor **2** only showed three energy minima, and *a,g* was again the lowest. Inhibitor **3** required more extensive analysis since the rotation of the cyclopropyl group must be considered; we were able to find 20 energy minima, with a,g^-,g^+ (ψ,θ,ϕ)

(45) MacroModel 2.0, W. C. Still, Department of Chemistry, Columbia University.

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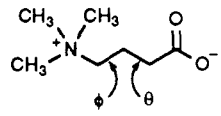
Table I. Relative Energies (kcal/mol) of Betaine Conformers



θ,ϕ	γBB	1	2	3[a]
a,a	0.80	2.24	3.24	2.90
g,a	0.00	1.38	0.00	0.66, 0.95[b]
a,g	2.94	1.42	nam[c]	0.64, 3.93[d]
g,g	nam[e]	0.00	1.83	0.00[f]

[a] In each of the minima recorded here the dihedral angle defined by H3–C3–C5–H (rotation about the bond connecting the cyclopropyl substituent to the backbone of 3) was anti. [b] These values are for the g^+,a and g^-,a conformers, respectively. [c] This conformer does not correspond to an energy minimum; the program finds the *g,a* conformation. [d] These values are for the a,g^+ and a,g^- conformers, respectively. [e] This conformer does not correspond to an energy minimum; the program finds the *g,a* conformation. [f] The lowest energy conformer is g^-,g^+. Calculations were performed using MacroModel 2.0[45] on a MicroVAX II computer equipped with a Pericom terminal. Using the Amber force field,[46] a distance-dependent dielectric of 4.0 was used to approximate solvent water.[47] Each structure was minimized until the root mean square of the gradient vectors was ≤0.002 kJ/Å.

the lowest and four others within 1.0 kcal/mol of that value. In all of these last five instances, ψ was anti (ψ = 180 ± 15°). Of particular interest is that the lowest energy conformer of **2** (*g,a*), which binds relatively well to the protein, corresponds to the lowest energy conformer (also *g,a*) of γ-butyrobetaine, the natural substrate.

Enzyme Assays. All three substances were tested as potential inhibitors of γ-butyrobetaine hydroxylase. In each case enzyme activity was determined by measurement of (a) the $^{14}CO_2$ liberated from the coupled decarboxylation of [1-^{14}C]-2-oxoglutarate and (b) the ^{3}H released into the aqueous medium from [2,3-^{3}H]-γ-butyrobetaine added as substrate. While the former method is convenient and is commonly used in the assay of 2-oxoglutarate-dependent dioxygenases,[13] it suffers from two shortcomings: in crude preparations of the enzymes there is the potential for nonspecificity and inhibitory substances might uncouple the decarboxylation from substrate hydroxylation. The latter technique, developed by one of us,[48] obviates these interferences by specifically assaying for the utilization of labeled γ-butyrobetaine.

When γ-butyrobetaine hydroxylase activity was determined in the presence of increasing concentrations of cyclopropyl-substituted substrate analogues, both **1** and **3** were found to be relatively weak inhibitors, while **2** was, by comparison, strongly inhibitory (see Figure 2). The degree of inhibition of γ-butyrobetaine hydroxylase by various concentrations of **2** was essentially independent of the nature of the enzyme preparation examination (crude bacterial extract vs essentially homogeneous enzyme) or of the assay procedure used to measure catalytic activity: decarboxylation of [1-^{14}C]-2-oxoglutarate vs release of ^{3}H from [2,3-^{3}H]-γ-butyrobetaine; data in the supplementary material.

Double-reciprocal plots of the kinetic data obtained for inhibitors **1** and **3** conformed to eq 1 for competitive inhibition and provided K_i values of 12.9 ± 4.6 and 7.9 ± 2.2 mM, respectively (see Figure 3). The K_M value for the substrate γ-butyrobetaine was 1.04 ± 0.14 mM. It should be noted that inhibitor **3** was added to the assay reaction

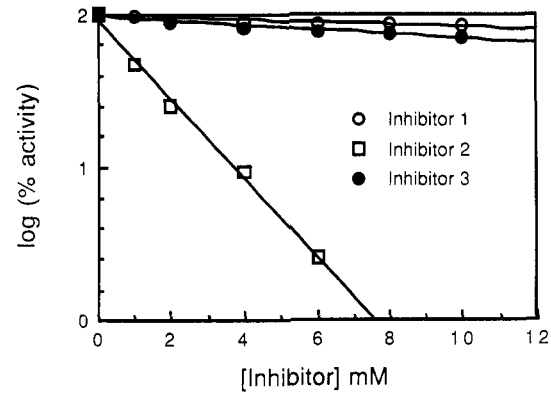


Figure 2. Inhibition of purified γ-butyrobetaine hydroxylase by **1**, **2**, and **3**; assayed by decarboxylation of [1-^{14}C]-2-oxoglutarate (0.107 mM) in the presence of 1.5 mM γ-butyrobetaine.

mixture as a racemate. Accordingly, the magnitude of the derived K_i represents an average value for the two enantiomers, which may be variably inhibitory.

$$V = \frac{V_{max}[S]}{K_M\left[1 + \frac{[I]}{K_{is}}\right] + [S]} \quad (1)$$

None of the three cyclopropyl derivatives exhibited turnover, as determined by their inability to promote decarboxylation of [1-^{14}C]-2-oxoglutarate in the presence of γ-butyrobetaine. In addition, preincubation of these substrate analogues with γ-butyrobetaine hydroxylase in the presence of all other components necessary for hydroxylation did not result in a time-dependent loss of activity greater than that of the control. The results of such a preincubation experiment with the more effective analogue **2** are shown in Figure 4. In separate experiments, crude bacterial extract and purified enzyme were added to the standard assay reaction mixture containing **2** (0.714 and 5.0 mM, respectively) and incubated at 37 °C. At the indicated time intervals, aliquots were removed, placed on ice, and following appropriate dilution assayed for residual hydroxylase activity by measurement of $^{14}CO_2$ release from [1-^{14}C]-2-oxoglutarate. As shown in Figure 4, there was no significant deviation from the controls (**2** absent) for either of two enzyme preparations, suggesting that **2** is not a time-dependent inactivator of γ-butyrobetaine hydroxylase.

In contrast to **1** and **3**, the kinetic data obtained for the inhibition of γ-butyrobetaine hydroxylase by **2** obeyed eq 2. The double-reciprocal plots reveal noncompetitive inhibition kinetics with values for K_{ii} of 1.54 ± 0.31 mM and for K_{is} of 1.96 ± 1.36 mM (see Figure 5).

$$V = \frac{V_{max}[S]}{K_M\left[1 + \frac{[I]}{K_{is}}\right] + \left[1 + \frac{[I]}{K_{ii}}\right][S]} \quad (2)$$

Discussion

Betaines **1** and **3** are weak competitive inhibitors of γ-butyrobetaine hydroxylase, whereas **2** is a noncompetitive inhibitor with K_{is} and K_{ii} values that closely approximate the K_M value determined for γ-butyrobetaine. Although the complete γ-butyrobetaine skeleton is present in each of the cyclopropyl-substituted substrate analogues, at least two factors are likely to contribute to the relatively low affinities of **1** and **3** for the enzyme: (1) The bulky cyclopropane groups may result in steric hindrance that precludes optimal interaction between the inhibitors and

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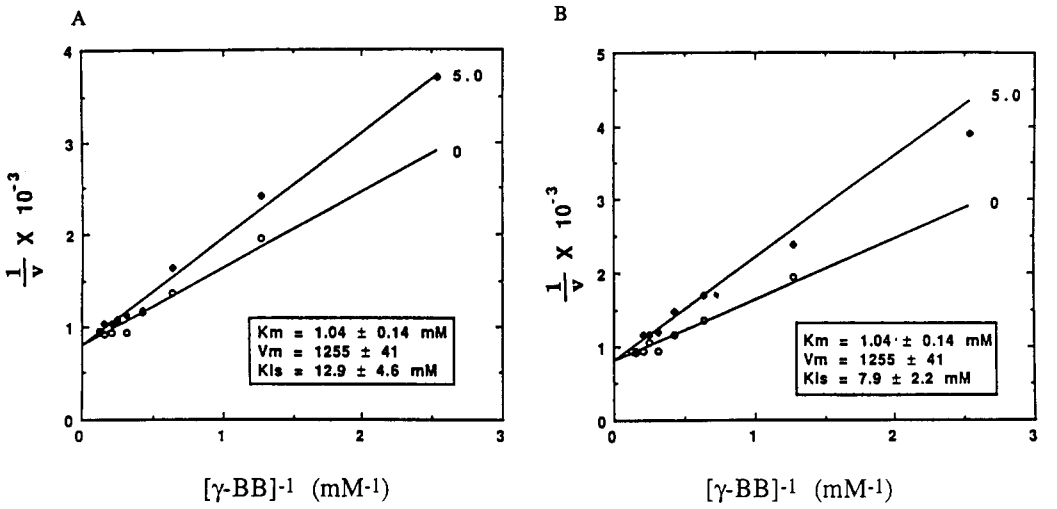

Figure 3. Double reciprocal plots of v^{-1} vs $[\gamma\text{-BB}]^{-1}$ with added 1 (A, 5.0 mM) and 3 (B, 5.0 mM). Enzyme velocities determined by measurement of $^{14}CO_2$ released from [1-^{14}C]-2-oxoglutarate.

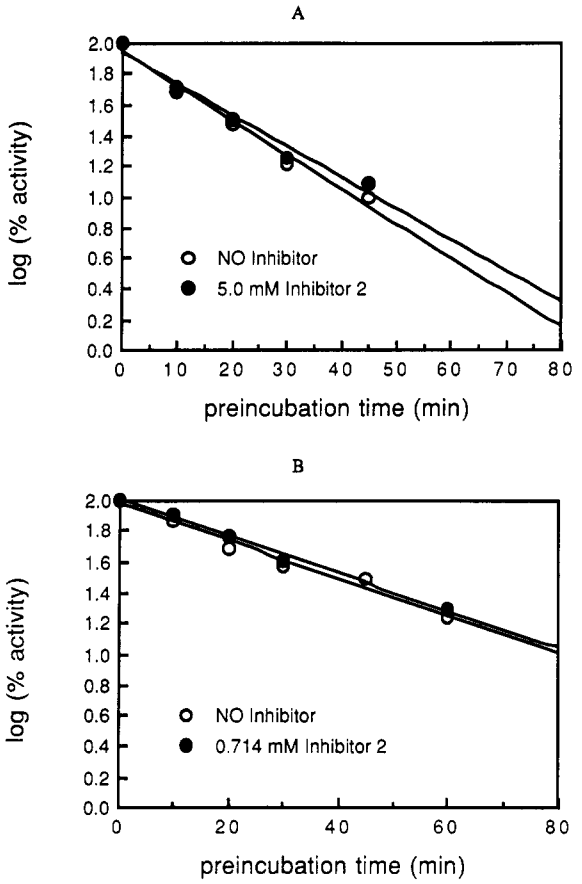

Figure 4. Time-dependent inactivation of γ-butyrobetaine hydroxylase in the absence and presence of **2**. (A) Crude bacterial extract, [**2**] = 5.0 mM; (B) purified enzyme, [**2**] = 0.714 mM. Residual enzyme activity following preincubation with **2** determined as indicated in Figure 2.

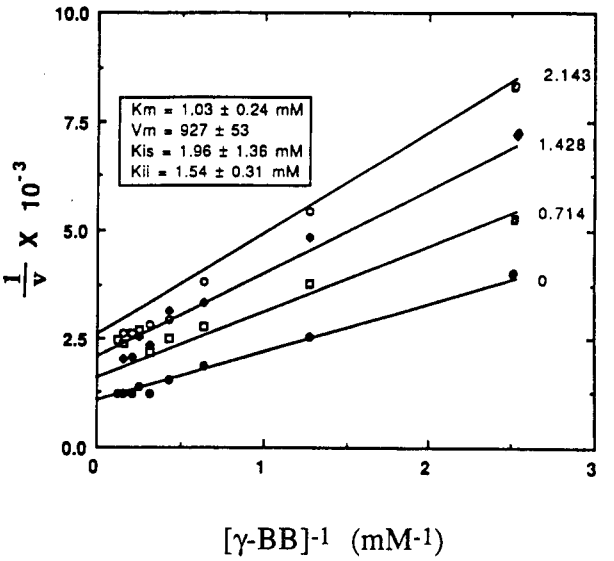

Figure 5. Double reciprocal plot of v^{-1} vs $[\gamma\text{-BB}]^{-1}$ with added **2** (0.714, 1.428, and 2.143 mM). Enzyme velocities determined as indicated in Figure 3.

critical active site amino acid residues, and (2) the cyclopropane substituents likely alter the distribution of conformations of the inhibitors, compared to the distribution of enzymatically relevant conformations found in solutions of γ-butyrobetaine. Molecular mechanics calculations indicate that γ-butyrobetaine has two energetically preferred conformers: *g,a* and *a,a*, the latter being only 0.8 kcal/mol higher in energy than the former. At a relative energy of 2.9 kcal/mol, the *a,g* conformer of γ-butyrobetaine would be present in negligible concentrations. If the lowest energy conformer of γ-butyrobetaine (*g,a*) is the form that is bound by the enzyme, this might account for the differences in inhibitory potentials between **1** and **2**. Of the four conformational minima available to **1**, *g,a* is predicted by these calculations to be 1.4 kcal/mol about *g,g*. This 1.4 kcal/mol must be reflected in the ΔG for binding of **1** relative to γ-butyrobetaine if *g,a* is the bound form; this would account for the observation that $K_i(1)/K_M = 12$. While a similar line of reasoning might be brought to bear on **3**, the large number of conformational possibilities discourages meaningful analysis. In contrast, inhibitor **2** has *g,a* as its lowest energy form, the other two minima being *g,g* and *a,a*, 1.8 and 3.2 kcal/mol higher in energy, respectively. Thus, more than 90% of this betaine will populate the *g,a* minimum which corresponds to the posited enzyme-bound substrate conformation. While this conclusion is tentative, the agreement between our observed inhibitory potentials and calculated conformational preferences encourage us to advance the hypothesis that γ-butyrobetaine assumes the *g,a* conformation when bound to γ-butyrobetaine hydroxylase.

It is somewhat surprising therefore that although **2** is predicted to assume a conformation similar to that of γ-butyrobetaine, it is not a substrate for the enzyme, nor

is its inhibition of the hydroxylase competitive with respect to γ-butyrobetaine. This is consistent, however, with the high substrate specificity of γ-butyrobetaine hydroxylase[49] and other 2-oxoglutarate-dependent dioxygenases.[11,13]

A recent publication by Lardy et al.[50] describing studies that relate to the interaction of rat liver γ-butyrobetaine hydroxylase with substrate, cosubstrate, and various other ligands reported moderate substrate inhibition by γ-butyrobetaine at concentrations near its K_M. Since the inhibition was not accompanied by uncoupling of 2-oxoglutarate decarboxylation from substrate hydroxylation, binding of the substrate at a second site appeared to be consistent with the data. Analogue **2**, in its energetically favored *g,a* conformation, although inactive as a substrate because of the absolute catalytic specificity of the hydroxylase, may nonetheless interact, as does γ-butyrobetaine, with such a secondary site. Indeed, this may account for the observed noncompetitive inhibition exhibited by **2**.

Experimental Section

General Methods. Chemical shifts in D_2O are reported relative to internal sodium 3-(trimethylsilyl)propionate-d_4 (TSP, for ^1H NMR), or dioxane (for ^{13}C NMR). Melting points are uncorrected. Tetrahydrofuran was distilled from Na with benzophenone as an indicator; benzene, CH_2Cl_2, and toluene were distilled from CaH_2. EtOAc and hexanes for chromatography were both distilled. Flash chromatography[51] was performed with Kieselgel 60 SiO_2 (230–400 mesh) from E. Merck. The purity of all compounds was judged to be >95% by ^1H and ^{13}C NMR data; copies of spectra of new compounds are included in the supplementary material. γ-Butyrobetaine hydroxylase was isolated and purified from *Pseudomonas* sp. AK1 by previously reported procedures.[14]

Conditions for Measurement of γ-Butyrobetaine Hydroxylase Activity. (1) Assays Based on Decarboxylation of [1-^{14}C]-2-Oxoglutarate. Assays were carried out in 16 × 100 mm test tubes provided with 1.5 × 3.0 cm rolled filter paper strips impregnated with 200 μL of 0.1 N NaOH placed in plastic holders attached to the rubber stoppers (Kontes Scientific Glassware/Instruments, Vineland, NJ; catalog numbers 882320-0000 and 882310-0000) sealing the tubes. For the routine measurement of enzyme activity, reaction mixtures (700 μL) contained 50 mM Tris at pH 7.8, 1.4 mg of bovine serum albumin (BSA), 140 μg of catalase, 50 μM dithiothreitol, 1 mM sodium ascorbate, 10 μM $Fe(NH_4)_2SO_4$, 6 mM γ-butyrobetaine, enzyme, and 110 μM [1-^{14}C]-2-oxoglutarate (~0.75 μCi/μmol) to initiate the reaction. Following incubations at 37 °C for 20 min with moderate shaking, reactions were terminated by injection of 1.0 mL of 10% trichloroacetic acid through rubber stoppers. The test tubes were than incubated at 37 °C for an additional 90 min to allow for maximal diffusion of the released $^{14}CO_2$ to the filter papers. The filter paper strips were transferred to counting vials and after addition of 5 mL of Dimiscint scintillation fluid (National Diagnostic) radioactivity was determined in an LKB Rackbeta 1219 scintillation counter.

(2) Assays Based on Tritium Release from [2,3-^{3}H]-γ-Butyrobetaine. Assays were carried out in 1.5-mL Eppendorf tubes with reaction mixtures (250 μL) that contained 50 mM Tris at pH 7.8, 0.5 mg of BSA, 50 μg of catalase, 50 μM dithiothreitol, 1 mM sodium ascorbate, 150 μM $Fe(NH_4)_2SO_4$, 1.48 μM [2,3-^{3}H]-γ-butyrobetaine (~0.52 μCi/μmol), enzyme, and 3.0 mM 2-oxoglutarate to initiate the reaction. Following incubations at 37 °C for 20 min with moderate rotatory shaking, reactions were terminated by addition of 50 μL of 30% trichloroacetic acid, and the reaction mixtures were centrifuged for 2 min in a high-speed microfuge. Aliquots (200 μL) of the clear supernates were applied to small columns (1-mL total volume) of Dowex-50 (H^+) (X8, 200–400 mesh) and the columns eluted with 1 mL of H_2O. The column effluents, collected directly in the counting vials, were analyzed for tritium radioactivity following the addition of 5 mL of Liquiscint scintillation fluid (National Diagnostic).

Time-Dependent Inactivation Studies. Reaction mixtures (100 μL) contained 50 mM Tris at pH 7.8, 0.2 mg of BSA, 20 μg of catalase, 50 μM dithiothreitol, 1 mM sodium ascorbate, 150 μM $Fe(NH_4)_2SO_4$, 1.5 mM 2-oxoglutarate, and either (A) 1.46 μg of pure γ-butyrobetaine hydroxylase, (B) 1.46 μg of pure γ-butyrobetaine hydroxylase + 0.714 mM inhibitor **2**, (C) 397.6 μg of crude *Pseudomonas* sp. AK1 extract, (D) 397.6 μg of crude *Pseudomonas* sp. AK1 extract + 5.0 mM inhibitor **2**.

Incubations were carried out at 37 °C with moderate rotatory shaking, and at indicated time intervals aliquots were removed and stored on ice until all time points were collected. Aliquots (25 μL) of each sample, following appropriate further dilution (1:10 for A and B and 1:40 for C and D), were than assayed for enzymatic activity using the standard assay procedure based on the decarboxylation of [1-^{14}C]-2-oxoglutarate.

Triethyl 2-Phosphono-5-hexenoate (4).[36] NaH (8.91 g of a 60% dispersion in mineral oil, 0.22 mol) was washed twice with pentane (40 mL each) and then suspended in 40 mL of dry THF under an atmosphere of N_2. Triethyl phosphonoacetate (28.9 g, 0.129 mol) was added dropwise over 30 min at 23 °C. After mechanical stirring for an additional 30 min, 4-bromo-1-butene (30.8 g, 0.23 mol) was added dropwise over 30 min. A mildly exothermic reaction ensued, accompanied by deposition of a precipitate. The reaction was brought to reflux for 5 h, cooled to 23 °C, and quenched by the addition of 40 mL of 1 N aqueous NH_4Cl. Concentration by rotary evaporation gave a residue which was partitioned between 200 mL of H_2O and diethyl ether (200 mL). The aqueous layer was saturated with NaCl and extracted twice with 100 mL of diethyl ether. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give 31.2 g of a yellow oil. Distillation at reduced pressure (92–98 °C/0.05 mmHg) gave 30.3 g (0.109 mol, 84%) of a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 5.74 (m, 1 H), 5.03 (m, 2 H), 4.3–4.0 (m, 6 H), 2.97 (ddd, J = 3.6, 10.5, $^3J_{H-P}$ = 22.7 Hz, 1 H), 2.2–1.9 (m, 4 H), 1.3 (m, 9 H), ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 168.8 (s), 136.5 (d, J = 152.5 Hz), 115.9 (t, J = 156.2 Hz), 62.4 (t, J = 145.8 Hz), 61.6 (t, J = 152.7 Hz), 44.7 (dd, J_{C-H} = 130.2, J_{C-P} = 131.2 Hz), 32.0 (t, poorly resolved), 25.9 (t, J = 135.5 Hz), 16.1 nq, J = 124.1 Hz), 13.9 (q, J = 127.6 Hz) ppm; ^{31}P NMR 121.5 MHz, $CDCl_3$, 85% aqueous H_3PO_4 as external reference) δ 23.4 ppm; IR (thin film) ν 3480 (br), 3077, 2984, 2938, 1734 (C=O), 1646, 1254 (P=O), 1163, 1051, 968 cm^{-1}; MS (EI) m/z 278 (M^+), 237, 224, 197, 179 amu; HRMS m/z calcd for $C_{12}H_{23}O_5P$ 278.1283, m/z obsd 278.1284.

Ethyl 1-(1-But-3-enyl)cyclopropanecarboxylate (5).[36] NaH (6.47 g of a 60% dispersion in mineral oil, 0.27 mol), in a flame-dried flask equipped with a magnetic stir bar, dropping funnel, and a Dewar-type condenser, was washed with pentane (30 mL) and suspended in 120 mL of dry benzene under an atmosphere of N_2. Phosphonate **4** (62.94 g, 0.226 mol), in 30 mL of benzene, was added dropwise to the stirring suspension at 23 °C over 60 min. Catalytic ethanol (160 μL, 0.0027 mol) was added to speed the deprotonation. After the addition was complete the reaction was cooled to ~0 °C, and the condenser was charged with dry ice and acetone. Oxirane (46 g, 1.04 mol) was condensed in a separate flask and then transferred via cannula into the cooled reaction mixture. The ice bath was removed, and the reaction mixture was warmed to a gentle reflux (oil bath temperature 40 ± 5 °C) for 6 h. The reaction was quenched carefully at 23 °C by addition of 40 mL of 1 N aqueous NH_4Cl. The reaction was poured into 200 mL of aqueous NH_4Cl, the organic layer was separated, and the aqueous layer was extracted four times with 200 mL of diethyl ether. The combined organic layers were washed with 150 mL each of saturated $NaHCO_3$, H_2O, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give an oily residue. Distillation at reduced pressure (48–60 °C/0.3 mmHg) gave a clear oil (25.0 g, 0.149 mol, 66%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.82 (ddt, J = 17.0, 10.3, 6.7 Hz, 1 H), 5.04–4.90 (m, 2 H), 4.11 (q, J = 7.1 Hz, 2 H), 2.22 (m, 2 H), 1.62 (m, 2 H), 1.24 (t, J = 7.1 Hz, 3 H), 1.19 (AB q, J = 6.7, 3.9 Hz, 2 H), 0.68 (AB q, J = 6.7, 3.9 Hz, 2 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.14 (s), 138.63 (d, J = 149.0 Hz), 114.38 (t, J = 154.4 Hz), 60.34 (t,

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(50) Wehbie, R. S.; Punekar, N. S.; Lardy, H. A. *Biochemistry* **1988**, *27*, 2222.

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J = 147.2 Hz), 33.52 (t, J = 127.5 Hz), 32.06 (s), 15.15 (t, J = 164.2 Hz), 14.22 (q, J = 125.2 Hz) ppm; IR (thin film) ν 3079, 2980, 2936, 2855, 1723 (C=O), 1642, 1449, 1372, 1345, 1156, 1032, 911, 756 cm^{-1}; MS (EI) m/z 168 (M^+), 153, 140, 123, 99 amu; HRMS m/z calcd for $C_{10}H_{16}O_2$ 168.1150, m/z obsd 168.1150.

1-(1-But-3-enyl)cyclopropanecarboxylic Acid (6).[36] Ester **5** (18.66 g, 111 mmol) was dissolved in 200 mL of ethanol and added to KOH (16.24 g, 289 mmol) in 100 mL of H_2O; the reaction mixture was stirred and heated at reflux for 12 h. After cooling to 23 °C the ethanol was removed by rotary evaporation and the aqueous residue added to ~500 mL of crushed ice. The resulting slurry was acidified to pH ~1 by dropwise addition of concentrated H_2SO_4 while stirring and maintaining the temperature at ≤5 °C. The aqueous suspension was extracted three times with 150 mL of $CHCl_3$ and once with 100 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated by rotary evaporation. Distillation of the residue gave a clear oil (76 °C/0.05 mmHg), 13.29 g (94.9 mmol, 86%): ^1H NMR (300 MHz, $CDCl_3$) δ 10.55 (br, 1 H), 5.82 (ddt, J = 17.0, 10.3, 6.7 Hz, 1 H), 5.05–4.91 (m, 2 H), 2.28–2.21 (m, 2 H), 1.64–1.59 (m, 2 H), 1.29 and 0.78 (AB q, J = 7.0, 4.0 Hz, 4 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.49 (s), 138.44 (d, J = 150.7 Hz), 114.56 (t, J = 155.4 Hz), 33.07 (t, J = 128.0 Hz), 31.85 (t, J = 127.0 Hz), 23.03 (s), 16.61 (t, J = 164.8 Hz) ppm; IR (thin film) ν 3080, 2980, 2928 (br, OH), 2748, 2742, 2619, 1692 (C=O), 1642 (C=C), 1443, 1424, 1224, 1032, 912 cm^{-1}; MS (EI) m/z 140 (M^+), 125, 111, 99, 95, 86, 81 amu; HRMS m/z calcd for $C_8H_{12}O_2$ 140.0837 m/z obsd 140.0830. Anal. Calcd for $C_8H_{12}O_2$: C, 68.28; H, 8.84. Found: C, 68.55; H, 8.63.

***N*-(Ethoxycarbonyl)-1-(1-but-3-enyl)cyclopropanamine (7).** A solution of **6** (1.40 g, 10.0 mmol) in dry toluene (15 mL) was purged with N_2, triethylamine (1.01 mL, 10.0 mmol) was added by syringe, followed by diphenyl phosphorazidate[35] (2.75 g, 10.0 mmol) in 5 mL of toluene. The reaction mixture was stirred at 75 °C (bath temperature) for 16 h followed by addition of 25 mL of absolute ethanol and warming the solution to reflux for 5 h. The reaction was cooled and concentrated in vacuo to a viscous oil, which was purified by flash chromatography (SiO_2, 3:1 hexanes–EtOAc) to give 1.26 g (69%) of an oil: ^1H NMR (500 MHz, room temperature, $CDCl_3$) δ 5.75 (m, 1 H), 5.1–4.9 (m, 2 H), 4.1 (br s, 2 H), 2.18 (dt, J = 7.7, 6.9 Hz, 2 H), 1.65 (br s, 2 H), 1.25 (br s, 2 H), 0.75 (s, 2 H), 0.63 (s, 2 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1 (s), 138.7 (d, J = 152 Hz), 114.6 (t, J = 155 Hz), 60.5 (t, J = 146 Hz), 35.9 (t, J = 128 Hz), 33.2, 30.9 (t, J = 123 Hz), 14.7 (q, J = 126 Hz), 14.0 (t, J = 158 Hz) ppm; IR (thin film) ν 3328, 3079, 2980, 2934, 1704, 1520, 1247, 1084, 911 cm^{-1}; MS (EI) m/z 182 (M^+ – H), 168, 154 ($M^+ - C_2H_5$), 142, 129, 110, 82 amu; HRMS m/z calcd for $C_{10}H_{17}NO_2$ 183.1259, m/z obsd 183.1259.

1-(Ethoxycarbonylamino)cyclopropanepropanoic Acid (8). $KMnO_4$ (25 g, 0.158 mol) was dissolved in H_2O; silica gel (105 g) was added, and the water was removed in vacuo to give a fine flowing powder, which was packed into a chromatography column.[37] Olefin **7** (2.93 g, 16.0 mmol) was dissolved in 200 mL of benzene, and this solution was percolated through the column. After eluting with another 200 mL of benzene the column was washed with 600 mL of H_2O and the eluate acidified to pH ~1 with 25% aqueous HCl. The purple color of the solution was discharged with solid $NaHSO_3$. After extraction with 3 × 75 mL of diethyl ether and 2 × 100 mL of CH_2Cl_2, the combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and concentrated in vacuo to give a white solid (1.9 g, 9.5 mmol, 58%): mp 101–103 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 4.1 (q, J = 6.6 Hz, 2 H), 2.5 (t, J = 6.8 Hz, 2 H), 1.9 (t, J = 6.7 Hz, 2 H), 1.23 (t, J = 5.1 Hz, 3 H), 0.95–0.86 (m, 2 H), 0.85–0.72 (m, 2 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 156.7, 60.80, 32.14, 31.51, 28.80, 14.48, 13.96 ppm and smaller peaks at 158.8, 61.72, 33.03, 32.61 which we assign to the minor amide isomer; IR (thin film) ν 3324, 3091, 2984, 2936, 1709, 1524, 1422, 1255, 1096 cm^{-1}; MS (EI) m/z 201 (M^+), 183, 172, 155, 141, 128 (100), 110, 100, 84, 82, 69, 56 amu; HRMS m/z calcd for $C_9H_{15}NO_4$ 201.1001, m/z obsd 201.1001. Anal. Calcd for $C_9H_{15}NO_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.90; H, 7.48; N, 9.97.

1-Ammoniocyclopropanepropanoate (9). A solution of carbamate **8** (0.520 g, 2.59 mmol) in 10 mL of dry CH_3CN and triethylamine (570 μL, 4.12 mmol) was purged with Ar and cooled in an ice bath. Iodotrimethylsilane (520 mg, 2.6 mmol, in 0.5 mL of CH_3CN) was added slowly by syringe, and the reaction mixture was allowed to warm to 23 °C and stir for 2 h. Water was added (5 mL) followed by concentrated NH_4OH (until the reaction mixture was basic by indicating paper) and a crystal of sodium thiosulfate. The resulting solution was charged onto a column (1 × 18 cm) of Rexyn-101 (H^+) and eluted with 25 mL each of H_2O, dioxane, H_2O, and 50 mL of 50% NH_4OH. The H_2O and dioxane fractions were combined, rendered basic with concentrated NH_4OH, and lyophilized to give recovered starting material (250 mg, 1.24 mmol). The 50-mL eluate was lyophilized, purified again by passage through a Rexyn-101 column, and the off-white solid after lyophilization was the desired product (150 mg, 1.16 mmol, 86% based on recovered material): mp 148–152 °C dec; ^1H NMR (300 MHz, D_2O) δ 2.38 (t, J = 7.4 Hz, 2 H), 1.91 (t, J = 7.4 Hz, 2 H), 0.93 (m, 2 H), 0.81 (m, 2 H) ppm; ^{13}C NMR (125 MHz, D_2O, dioxane ref, gated decoupling) δ 184.57 (1 C), 36.83 (1 C), 36.33 (1 C), 33.34 (1 C), 12.33 (2 C) ppm; MS (FAB) m/z 222 (M^+ + glycerol), 130 (M^+ + H), 112, 84 amu.

1-(Trimethylammonio)cyclopropanepropanoate (1). To a solution of amino acid **9** (2.36 g, 18.26 mmol) in 100 mL of 95% ethanol was added $Ba(OH)_2 \cdot 8H_2O$ (14.4 g, 45.7 mmol), followed by iodomethane (40 g, 0.283 mol). After 30 min at room temperature, the flask was warmed to reflux (~50 °C) for 15 h, cooled to ~0 °C, and acidified with 25% H_2SO_4 to precipitate $BaSO_4$. The mixture was filtered through Celite, the filtrate was neutralized at ~0 °C with concentrated aqueous NH_4OH, and concentrated by rotary evaporation, and the resulting liquid was charged onto a column (2 × 15 cm) of Rexyn-101 (H^+). The column was eluted with H_2O and then 50% aqueous NH_4OH; the latter eluate was lyophilized. Repetition of the ion-exchange procedure gave a white powder (342 mg, 2.0 mmol; 11%): mp, decomposes without melting up to 300 °C; ^1H NMR (300 MHz, D_2O, dioxane ref, room temperature) δ 3.04 (s, 9 H), 2.28 (br t, 2 H), 2.11 (br t, 2 H), 1.37 (br t, 2 H), 0.88 (br t, 2 H); ^{13}C NMR (75 MHz, D_2O, TSP ref) δ 183.4 (s), 69.1 (t, J = 145 Hz), 54.0 (q, J = 145 Hz), 36.4 (t, J = 129 Hz), 28.7 (t, J = 129 Hz), 10.35 (t, J = 164.5 Hz) ppm; IR (KBr) ν 3278 (br), 1680, 1571 (C=O, carboxylate), 1492, 1400, 1169, 966, 727 cm^{-1}; MS (FAB) m/z 186 (methyl ester + H^+, methanol used as matrix), 172 (M^+ + H), 94, 74, 58 amu; HRMS (FAB) m/z calcd for $C_9H_{18}NO_2$ (M^+ + H) 172.1337, m/z obsd 172.1355.

9b-[1-[[2,6-Bis(1,1-dimethylethyl)-4-methylphenoxy]carbonyl]cyclopropyl]-2,3,5,9b-tetrahydrooxazolo[3,2-*a*]isoindol-5-one (10). The 2,6-di-*tert*-butyl-4-methylphenol (BHT) ester of cyclopropanecarboxylic acid[39] (1.15 g, 3.99 mmol) was placed in dry THF and cooled to −78 °C. *tert*-Butyllithium (2.6 mL of a 1.7 mM solution in hexanes) was added by syringe, and the reaction mixture was stirred at reduced temperature for 30 min; *N*-(2-bromoethyl)phthalimide (1.01 g, 3.98 mmol) in THF was then added by syringe, and the reaction mixture was allowed to warm slowly to 23 °C. When complete the reaction was diluted with diethyl ether, washed with saturated aqueous NH_4Cl and brine, dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by flash chromatography (1% CH_3OH/C_6H_6 through SiO_2) to give a crystalline solid (0.98 g, 53%): mp 175–177 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (m, 2 H), 7.52–7.40 (m, 2 H), 7.03 (s, 1 H), 7.00 (s, 1 H), 4.25 (m, 1 H), 4.15 (m, 1 H), 3.95 (m, 1 H), 3.4 (m, 1 H), 2.24 (s, 3 H), 1.9 (m, 1 H), 1.8 (m, 1 H), 1.7 (m, 1 H), 1.3 (m, 3 H), 1.23 (s, 9 H), 1.20 (s, 9 H) ppm; MS (EI) m/z 446 ($M^+ - CH_3$), 242, 174 amu.

The structure of **10** ($C_{29}H_{35}NO_4$, MW 461.604) was confirmed by X-ray crystallography. Crystals of **10** were monoclinic ($P2_1/n$), Z = 4, $F(000)$ = 992, λ(Mo Kα) = 0.71069 Å, 2601 observed reflections gave final R = 0.048, a = 16.486 (8) Å, b = 9.501 (5) Å, c = 17.187 (8) Å. Further details are included in the supplementary material.

Ethyl 2,5-Dihydro-5-oxo-3*H*-pyrrolo[2,1-*a*]isoindole-1-carboxylic Acid (12).[40] NaH (266 mg of a 60% suspension in mineral oil, 6.65 mmol) was washed with 3 mL of petroleum ether under an atmosphere of N_2 and suspended in dry THF (30 mL). Triethyl phosphonoacetate (1.0 g, 4.4 mmol) in THF (10 mL) was added dropwise, and the resulting suspension was stirred for 30 min at 23 °C. *N*-(2-Bromoethyl)phthalimide (1.6 g, 6.6 mmol) in THF (10 mL) was added, and the reaction mixture was stirred at 23 °C for 30 min and then brought to a reflux for 12 h. After

addition of 5 mL of 1 N aqueous NH_4Cl, the mixture was concentrated in vacuo, and the solid yellow residue was partitioned between EtOAc and H_2O. The layers were separated, the aqueous layer was extracted with EtOAc, the combined organic layers washed with brine, dried (Na_2SO_4), and concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2, 10% EtOAc/petroleum ether) to give 100 mg of a yellow solid (9% yield): mp 280–285 °C dec; ^1H NMR (500 MHz; $CDCl_3$) δ 8.53 (d, J = 7.2 Hz, 1 H), 7.83 (d, J = 7.2 Hz, 1 H), 7.6 (m, 2 H), 4.35 (q, J = 7.2 Hz, 2 H), 3.95 (t, J = 8.4 Hz, 2 H), 3.56 (t, J = 8.4 Hz, 2 H), 1.40 (t, J = 7.2 Hz, 3 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7, 163.8, 148.8, 136.3, 132.0, 131.3, 129.6, 126.7, 123.4, 110.5, 60.83, 39.91, 34.09, 14.54 ppm; IR (thin film) ν 2960, 2903, 1707, 1691, 1650, 1470, 1386, 1262, 1108 cm^{-1}; MS (EI) m/z 243 (100, M^+), 214, 198, 170, 142, 115 amu; HRMS m/z calcd for $C_{14}H_{13}NO_3$ = 243.0895, m/z obsd 243.0895.

2-(Diethylphosphono)glutarate 5-*tert*-Butyl 1-Ethyl Diester (14). NaH (12.0 g of a 60% suspension in mineral oil, 0.30 mol) was washed with ~50 mL of pentane and then suspended in 200 mL of dry THF under an atmosphere of N_2. Triethyl phosphonoacetate (56.0 g, 0.25 mol) in 50 mL of THF was added over 30 min at 23 °C. After a further 90 min the solution had become homogeneous and *tert*-butyl acrylate (32.0 g, 0.25 mol) in 50 mL of THF was added slowly. After addition was complete the reaction mixture was brought to reflux for 5 h. The reaction was cooled to 23 °C, quenched carefully with 20 mL of 1 N aqueous NH_4Cl, and concentrated in vacuo. The residue was partitioned between 75 mL each of H_2O and diethyl ether, and the aqueous layer was extracted with 3 × 75 mL of diethyl ether. The combined organic layers were washed with 100 mL of brine, dried (Na_2SO_4), filtered, and concentrated, and the crude product was distilled under reduced pressure (130–140 °C at 0.25 mmHg) to yield an oil (42.2 g, 0.12 mol, 49%): ^1H NMR (300 MHz, $CDCl_3$) δ 4.28 (m, 6 H), 3.13–2.93 (m, 1 H), 2.49–2.11 (m, 4 H), 1.44 (s, 9 H), 1.39–1.21 (m, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 168.8, 88.60, 62.79, 61.46, 45.17, 44.12, 33.58, 33.47, 28.06, 22.52, 16.37, 14.13 ppm; IR (thin film) ν 2982, 2936, 1731 (C=O), 1369, 1256, 1152, 1025, 968 cm^{-1}; MS (EI) m/z 296 ($M^+ - (CH_3)_2C{=}CH_2$), 279, 251, 237, 224, 86 amu; HRMS m/z calcd for $C_{11}H_{21}O_7P$ ($M^+ - (CH_3)_2C{=}CH_2$) 296.1025, m/z obsd 296.1024.

Ethyl 1-[2-(*tert*-Butoxycarbonyl)ethyl]cyclopropanecarboxylate (15). NaH (6.0 g of a 60% dispersion in mineral oil, 0.15 mol) was washed three times with 20 mL of dry pentane and suspended in dry benzene under an atmosphere of N_2 in a flask equipped with a Dewar-type condenser. Phosphonate **14** (3.52 g, 0.10 mol) in 50 mL of benzene was injected by syringe over 20 min with accompanying evolution of gas. After 2 h of stirring at 23 °C the reaction mixture was homogeneous and was cooled in an ice bath for 30 min prior to addition of oxirane. The Dewar-type condenser was charged with dry ice and acetone, and oxirane (10 g, 0.22 mol, previously condensed into a separate flask) was cannulated into the reaction mixture. The contents of the flask were brought to a gentle reflux (bath temperature ~40 °C) for 3 h and then cooled to 23 °C and quenched by careful addition of 40 mL of 1 N aqueous NH_4Cl and 60 mL of H_2O. The was aqueous layer extracted with 3 × 50 mL of diethyl ether, the organic layers were combined, washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo, and the crude product was distilled under reduced pressure (90–100 °C at 0.10 mmHg) to give 13.6 (0.056 mol, 56%) of a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 4.11 (q, J = 7.1 Hz, 2 H), 2.43 (m, 2 H), 1.80 (m, 2 H), 1.43 (s, 9 H), 1.23 (t, J = 7.1 Hz, 3 H), 1.19 (m, 2 H), 0.72 (m, 2 H) ppm; ^{13}C NMR (125 MHz, CH_3OH-d_4) δ 177.07, 176.28, 61.70, 33.23, 30.24, 23.82, 16.14, 14.50 ppm; IR (thin film) ν 2981, 2936, 1726, 1447, 1370, 1257, 1152, 1033 cm^{-1}; MS (EI) m/z 242 (M^+), 214, 197, 186, 169, 158, 141, 140, 127, 113, 112, 71, 57 (100) amu; HRMS m/z calcd for $C_{11}H_{18}O_4$ ($M^+ - CH_2{=}CH_2$) = 214.1205; m/z obsd = 214.1205. Anal. Calcd for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.65; H, 9.00.

Ethyl 1-(2-Carboxyethyl)cyclopropanecarboxylate (16). Diester **15** (9.68 g, 40 mmol) was dissolved in 40 mL of CF_3CO_2H and heated to reflux for 12 h. After the mixture was cooled to 23 °C the CF_3CO_2H was removed in vacuo to afford 6.3 g (32.5 mmol, 81%) of a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 11.1 (s, 1 H), 4.12 (q, J = 7.1 Hz, 2 H), 2.61 (t, J = 7.7 Hz, 2 H), 1.87 (t, J = 7.6 Hz, 2 H), 1.25 (m, 2 H), 1.24 (t, J = 7.1 Hz, 3 H), 0.75 (m, 2 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.7, 174.8, 60.7, 32.5, 29.1, 22.8, 15.9, 14.2 ppm; IR (KBr) ν 3210 (br), 2985, 2938, 1714, 1189, 1146, 1032 cm^{-1}; MS (EI) m/z 185 (M^+ – H), 169 (M^+ – OH), 140, 127, 112, 99 amu; HRMS m/z calcd for $C_9H_{13}O_4$ (M^+ – H) 185.0813; m/z obsd 185.0814.

Ethyl *N*-Carbethoxy-1-(2-aminoethyl)cyclopropanecarboxylate (17). Acid **16** (3.0 g, 16.1 mmol) in 25 mL of dry toluene and triethylamine (1.626 g, 16.1 mmol) were mixed at room temperature under an atmosphere of N_2. Diphenyl phosphorazidate (4.43 g, 16.1 mmol) in 15 mL of toluene was added by syringe, and the contents of the flask were warmed to 75 °C (bath temperature) for 4 h. EtOH (absolute, 20 mL) was added, and the reaction mixture was maintained at reflux for 12 h, the reaction mixture was cooled to 23 °C, and the remaining EtOH was removed in vacuo. Water was added to the organic residue, the layers were separated, the aqueous layer was extracted with diethyl ether (2 × 40 mL) and EtOAc (40 mL), the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2, 25% EtOAc/hexanes) to give 1.9 g (8.3 mmol, 52% yield) of an oil: ^1H NMR (300 MHz, $CDCl_3$) δ 4.96 (br s), 4.1 (m, 4 H), 3.32 (m, 2 H), 1.74 (t, J = 7.0 Hz, 2 H), 1.23 (m, 7 H), 0.75 (m, 2 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0 (s), 156.6 (s), 60.5 (t, J = 147 Hz), 60.4 (t, J = 147 Hz), 39.6 (t, J = 139 Hz), 33.9 (t, J = 128 Hz), 21.5 (s), 15.5 (t, J = 168 Hz), 14.6 (q, J = 126 Hz), 14.1 (q, J = 126 Hz) ppm; IR (thin film) ν 3364 (br), 2983, 2937, 1715, 1532, 1250, 1156, 1032 cm^{-1}; MS (EI) m/z 229 (M^+), 201, 183, 156, 138, 110, 102 amu; HRMS (EI) m/z calcd for $C_{11}H_{19}NO_4$ (M^+) 229.1314, m/z obsd 229.1315.

***N*,*N*'-Bis[2-(1-carbethoxycyclopropyl)ethyl]urea (18).** When the above procedure is followed with the exception that 20 mL of H_2O is added instead of EtOH, a dimer is obtained in which the two amines are linked by a urea carbonyl (86% yield): mp 71–72 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 4.82 (br s, 2 H), 4.10 (q, J = 7.1 Hz, 4 H), 3.30 (m, 4 H), 1.75 (t, J = 7.3 Hz, 4 H), 1.24 (t overlapping a m, J = 7.1 Hz, 10 H), 0.77 (m, 4 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 158.5, 60.65, 39.27, 34.32, 21.70, 15.57, 14.23 ppm; IR (thin film) ν 3355, 2986, 2938, 2879, 1712, 1625, 1569, 1454, 1373, 1219, 1165 cm^{-1}; MS (EI) m/z 340 (M^+), 312, 289, 248, 184, 156, 138 amu; HRMS (EI) m/z calcd for $C_{17}H_{28}N_2O_5$ (M^+) 340.1998; m/z obsd 340.1997. Anal. Calcd for $C_{17}H_{28}N_2O_5$: C, 59.72; H, 8.33; N, 8.17. Found: C, 59.98; H, 8.29; N, 8.23.

4-Oxo-5-azaspiro[2.4]heptane (19). Ester–carbamate **17** (1.7 g, 7.42 mmol) was dissolved in CH_3OH (30 mL) and $Ba(OH)_2{\cdot}8H_2O$ (2.4 g, 7.62 mmol) was added. The solution was heated to reflux for 14 h, cooled with ice, and acidified with concentrated H_2SO_4, and the resulting $BaSO_4$ precipitate was removed by filtration. The aqueous filtrate was extracted with EtOAc, and the organic extract was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography (SiO_2, 20% EtOAc/hexanes) to give 280 mg (2.52 mmol, 34%) of the solid lactam: ^1H NMR (300 MHz, $CDCl_3$) δ 6.35 (br s, 1 H), 3.48 (t, J = 7.3 Hz, 2 H), 2.19 (t, J = 7.3 Hz, 2 H), 1.11 (m, 2 H), 0.74 (m, 2 H) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 181.04 (s), 39.66 (t, J = 143 Hz), 28.82 (t, J = 132 Hz), 21.85, 13.31 (t, J = 163 Hz) ppm; IR (thin film) ν 3204, 3092, 1686; MS (EI) m/z 111 (M^+), 82, 67, 54 amu; HRMS (EI) m/z calcd for C_6H_9NO (M^+) 111.0684, m/z obsd 111.0682.

1-(2-Aminoethyl)cyclopropanecarboxylic Acid (20). Lactam **19** (110 mg, 0.99 mmol) was dissolved in 20 mL of 1:1 aqueous dioxane, and $Ba(OH)_2{\cdot}8H_2O$ (350 mg, 2.05 mmol) was added. The flask was heated to 90 °C (oil bath temperature) for 24 h and then cooled to ~0 °C; concentrated H_2SO_4 was added to precipitate $BaSO_4$. After filtration, the filtrate was rendered alkaline with concentrated NH_4OH and the solution was charged onto a Rexyn-101 (H^+) column (1 × 5 cm), which was eluted with H_2O and then 50% aqueous NH_4OH. The latter eluate was lyophilized to give 110 mg (0.85 mmol, 86%) of a white powder: mp 200 °C dec; ^1H NMR (300 MHz, CD_3OD) δ 3.03 (t, J = 5.9 Hz, 2 H), 1.74 (t, J = 5.9 Hz, 2 H), 1.10 (dd, J = 3.6, 6.3 Hz, 2 H), 0.55 (dd, J = 3.6, 6.3 Hz, 2 H) ppm; ^{13}C NMR (75 MHz, D_2O) δ 186.0, 41.25, 34.96, 25.87, 16.81 ppm; MS (FAB) m/z 222 (M^+ + glycerol), 130 (M^+ + H), 112, 86 amu.

1-[2-(Trimethylammonio)ethyl]cyclopropanecarboxylic Acid (2). Amino acid **19** (70 mg, 0.54 mmol) was dissolved in a solution of 2 mL of 1:1 H_2O–dioxane, 2 mL of CH_3I (4.56 g, 32 mmol), and $Ba(OH)_2$ (278 mg, 1.62 mmol). The reaction mixture

was warmed to 60 °C for 18 h and quenched at 23 °C with concentrated H_2SO_4, and the precipitated $BaSO_4$ was removed by filtration. The filtrate was charged onto a column of Rexyn-101 (1 × 15 cm) and eluted with H_2O and 50% aqueous NH_4OH. The latter eluate was lyophilized to give 40 mg (0.23 mmol, 43%) of pasty solid: ^1H NMR (300 MHz, D_2O, TSP ref) δ 3.47 (m, 2 H), 3.09 (s, 9 H), 1.95 (m, 2 H), 1.09 (dd, J = 4.1, 6.8 Hz, 2 H), 0.74 (dd, J = 4.1, 6.8 Hz, 2 H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 181.6, 66.9, 53.5, 30.0, 23.0, 14.7 ppm; IR (KBr) ν 3432 (br), 3013, 1578 (C=O), 1483, 1399, 1244, 967, 932, 907, 776 cm^{-1}; MS (FAB) m/z 186 (methyl ester + H^+, methanol used as matrix), 172 (M^+ + H), 127, 117, 103, 94, 74, 58 amu; HRMS (FAB) m/z calcd for $C_9H_{18}NO_2$ (M^+ + H) 172.1337, m/z obsd 172.1334.

Methyl (*E*)-3-Cyclopropylpropenoate (21).[41] NaH (5.60 g of a 60% dispersion in mineral oil) was washed with 3 × 20 mL of dry pentane and suspended in 50 mL of dry THF under an atmosphere of N_2. Trimethyl phosphonoacetate (25.5 g, 0.14 mol) in 75 mL of THF was added dropwise over 2 h at 23 °C with evolution of gas. Cyclopropanecarboxaldehyde (9.80 g, 0.14 mol) was dissolved in 25 mL of THF and added rapidly. The reaction mixture was brought to reflux for 30 min, cooled to 23 °C, and stirred for 12 h. After quenching with 15 mL of 1 N aqueous NH_4Cl, the THF was removed in vacuo, and the residue was partitioned between 50 mL of H_2O and 50 mL of diethyl ether. The aqueous layer was extracted with 4 × 75 mL of diethyl ether, and the combined organic layers were washed with brine, dried (Na_2SO_4) filtered, and concentrated, and the resulting residue was distilled at reduced pressure (28 °C at 0.25 mmHg) to afford a clear oil (14.3 g, 0.11 mol, 81%): ^1H NMR (300 MHz, $CDCl_3$) δ 6.42 (dd, J = 10.1, 15.4 Hz, 1 H), 5.89 (d, J = 15.4 Hz, 1 H), 3.71 (s, 3 H), 1.6 (m, 1 H), 0.95 (m, 2 H), 0.65 (m, 2 H) ppm; IR (thin film) ν 2952, 1719 (C=O), 1653, 1437, 1377, 1308, 1269, 1150, 1188, 1019, 980, 943 cm^{-1}; MS (EI) m/z 126 (M^+), 111, 98, 95, 67, 55 amu.

Methyl 4-Nitro-3-cyclopropylbutanoate (22). To a solution of ester **21** (10.24 g, 81.3 mmol) in nitromethane (30 mL) was added DBN (1,5-diazabicyclo[4.3.0]non-5-ene, 12.2 g, 80.1 mmol) in 20 mL of nitromethane dropwise by syringe. The contents of the flask were heated to reflux for 12 h. After cooling to 23 °C the reaction was quenched with 0.1 N aqueous HCl, and the reaction mixture was partitioned between 30 mL each of H_2O and diethyl ether. The aqueous layer was extracted with 4 × 50 mL of diethyl ether, and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo, and the residue was distilled under reduced pressure (80 °C at 0.5 mmHg) to yield 11.5 g (61.5 mmol, 76%) of a clear oil: ^1H NMR (300 MHz; $CDCl_3$) δ 4.61 and 4.54 (overlapping dd's, J = 6.8, 11.9 Hz and J = 6.3, 11.8 Hz, respectively, 2 H), 3.70 (s, 3 H), 2.57 (d, J = 6.6 Hz, 2 H), 1.85 (m, 1 H), 0.8 (m, 1 H), 0.6–0.5 (m, 2 H), 0.25 (m, 2 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 79.2, 51.8, 40.1, 36.6, 13.5, 4.3, 3.9 ppm; IR (thin film) ν 3006, 2955, 1734 (C=O), 1553, 1437, 1379, 1177 cm^{-1}; MS (EI) m/z 156 (M^+ - OMe), 129, 109, 95, 84, 48 amu; HRMS m/z calcd for $C_7H_{10}NO_3$ 156.0661, m/z obsd 156.0661. Anal. Calcd for $C_7H_{10}NO_3$: C, 51.33; H, 6.0; N, 7.48. Found: C, 51.30; H, 7.03; N, 7.77.

3-Cyclopropyl-2-pyrrolidinone (23).[44] To a solution of $NiCl_2 \cdot 6H_2O$ (7.13 g, 30.0 mmol) in 100 mL of CH_3OH was added $NaBH_4$ (3.40 g, 90.0 mmol), and the resulting solution was sonicated (immersion horn) for 30 min at ambient temperature. A solution of nitro ester **22** (11.2 g, 60.0 mmol) in 50 mL of CH_3OH was added dropwise followed by a second portion of $NaBH_4$ (7.94 g, 210 mmol). The mixture was stirred for 3 h, filtered through Celite, and concentrated in vacuo. The residue was partitioned between 75 mL each of H_2O and diethyl ether, and the aqueous layer was extracted 3 × 50 mL of diethyl ether. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to yield a solid. Flash chromatography yielded 4.16 g (33.3 mmol, 55%) of a white solid: mp 101–104 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 5.9 (br s, 1 H), 3.50 (m, 1 H), 3.20 (dd, J = 6.3, 9.5 Hz, 1 H), 2.44 (dd, J = 8.7, 16.9 Hz, 1 H), 2.17 (dd, J = 7.3, 16.7 Hz, 1 H), 1.85 (m, 1 H), 0.95–0.75 (m, 1 H), 0.60–0.45 (m, 1 H), 0.15–0.10 (m, 1 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 47.9, 39.9, 36.4, 15.0, 3.34 ppm; IR (thin film) ν 3184, 3078, 2872, 1685 (C=O) cm^{-1}; MS (EI) m/z 125 (M^+), 96, 84, 67, 54 amu; HRMS m/z calcd for $C_7H_{11}NO$ 125.0841, m/z obsd 125.0841. Anal. Calcd for $C_7H_{11}NO$: C, 67.17; H, 8.86; N, 11.19. Found: C, 66.81; H, 8.75; N, 10.86.

4-Amino-3-cyclopropylbutanoic Acid (24). Lactam **23** (3.0 g, 24.0 mmol) was dissolved in 50 mL of 1:1 dioxane–H_2O with $Ba(OH)_2 \cdot 8H_2O$ (15.14 g, 48.0 mmol), and the resulting suspension was heated to reflux for 12 h. The reaction was cooled in an ice bath and acidified with 25% H_2SO_4 to precipitate $BaSO_4$, which was removed by filtration through Celite. The filtrate was kept cold and basified with concentrated NH_4OH, concentrated in vacuo, and the aqueous residue was eluted through Rexyn-101 (H^+) first with H_2O, then 20% NH_4OH. The NH_4OH fractions were combined and lyophilized to give 2.20 g (15.4 mmol, 64%) of a solid: mp 177–178 °C; ^1H NMR (300 MHz, D_2O, TSP ref) δ 3.13 (dd, J = 6.0, 12.8 Hz, 1 H), 3.06 (dd, J = 8.3, 12.8 Hz, 1 H), 2.41 (dd, J = 6.9, 14.2 Hz, 1 H), 2.32 (dd, J = 7.2, 14.1 Hz, 1 H), 1.36 (m, 1 H), 0.7–0.5 (m, 3 H), 0.3–0.2 (m, 2 H) ppm; ^{13}C NMR (75 MHz, D_2O, dioxane ref 67.6 ppm) δ 182.0, 45.3, 42.4, 40.6, 13.9, 4.8, 3.3 ppm; MS (FAB) m/z 144 (M^+ + H), 126, 93, 75, 57 amu (higher mass ions derived from reaction with the glycerol matrix were also observed). Anal. Cald for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.56; H, 9.04; N, 9.63.

4-(Trimethylammonio)-3-cyclopropylbutanoic Acid (3). Amino acid **24** (1.37 g, 9.60 mmol) was dissolved in 100 mL of 1:1 dioxane–H_2O along with $Ba(OH)_2 \cdot 8H_2O$ (24.13 g, 76.5 mmol). CH_3I (2 mL, 32 mmol) was added by syringe, and the solution was brought to a gentle reflux (bath temperature 50 °C). After 12 h the reaction was cooled to ~0 °C and acidified with 25% H_2SO_4 to precipitate the $BaSO_4$, which was removed by filtration through Celite. The cold filtrate was basified with concentrated NH_4OH and concentrated by rotary evaporation. The aqueous residue was passed through a column of Rexyn-101 (H^+), eluting first with H_2O and then with 20% NH_4OH. The NH_4OH fractions were combined and lyophilized to yield a solid (0.95 g, 5.14 mmol, 53%): mp 149–155 °C dec; ^1H NMR (500 MHz, D_2O, TSP ref) δ 3.56 (dd, J = 5.8, 13.6 Hz, 1 H), 3.46 (dd, J = 4.9, 13.6 Hz, 1 H), 3.19 (s, 9 H), 2.38 (dd, J = 7.0, 14.7 Hz, 1 H), 2.33 (dd, J = 6.6, 14.7 Hz, 1 H), 1.7 (m, 1 H), 0.85 (m, 1 H), 0.7–06 (m, 2 H), 0.4–0.3 (m, 2 H) ppm; ^{13}C NMR (125 MHz, D_2O, dioxane ref 67.6 ppm) δ 179.1 (s), 70.8 (t, J = 144.6 Hz), 52.7 (q, J = 144.5 Hz), 41.9 (t, J = 128.4 Hz), 35.6 (d, J = 129.3 Hz), 15.1 (d, J = 158.8 Hz), 4.74 (t, J = 160.5 Hz), 3.64 (t, J = 161.3 Hz) ppm; IR (KBr) ν 3016, 2929, 1725 (CO_2^-), 1480, 1406, 1187, 971 cm^{-1}; MS (FAB) m/z 186 (M^+ + H) amu.

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Supplementary Material Available: Tables of atomic coordinates, isotropic thermal parameters, bond lengths, bond angles, and anisotropic thermal parameters for the X-ray structure determination of compound **10**; graph comparing inhibitory effect of **2** on different preparations of γ-butyrobetaine hydroxylase; ^1H and ^{13}C NMR spectra of new compounds (49 pages). Ordering information is given on any current masthead page.