Stereochemical Correlation of Proclavaminic Acid and Syntheses of *erythro*- and *threo*-L-β-Hydroxyornithine from an Improved Vinylglycine Synthon

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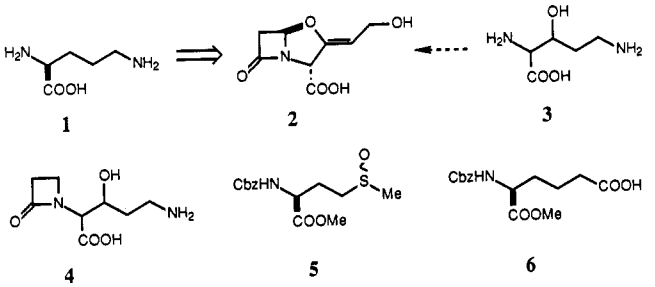
The Hanessian method to prepare the *N*-Cbz-L-vinylglycine methyl ester has been improved to obtain reproducibility an alternately protected version of this useful synthon in optically pure, crystalline form. A nitrone cycloaddition route has been developed to synthesize *erythro*- and *threo*-L-β-hydroxyornithine having stereoisomeric purities of >99%. A parallel route described recently, proceeding from Hanessian's methyl vinylglycinate, gives these oxidatively modified α-amino acids in inferior enantiomeric excess owing to partial racemization that occurs in the oxidative decarboxylation to the protected vinylglycines themselves. The intermediate isoxazolidine generated in the present synthesis was separately converted to proclavaminic acid, a key intermediate in the biosynthesis of the β-lactamase inhibitor clavulanic acid, and its absolute configuration was established as L-*threo* by unambiguous correlation to L-glutamic acid.

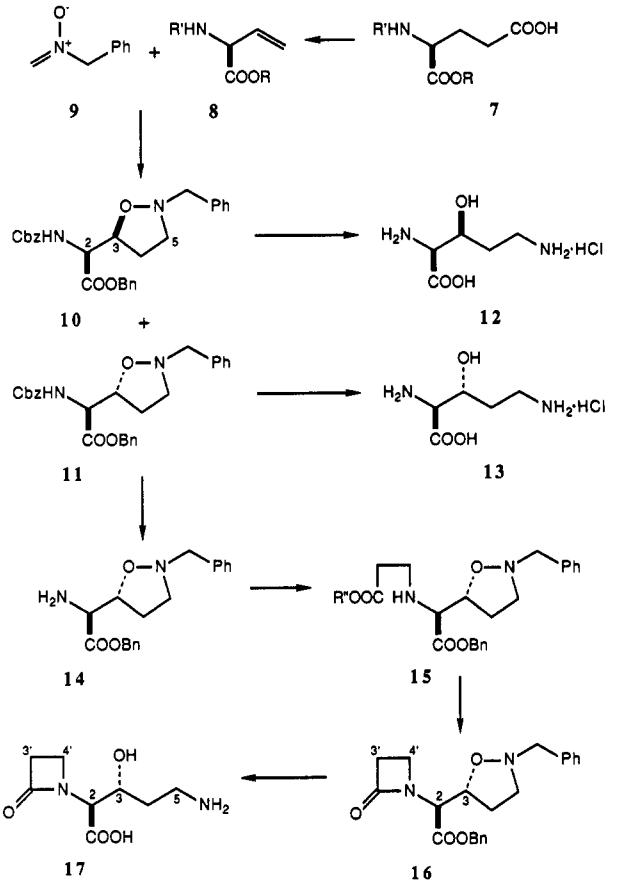
L-Ornithine (1) has been established to be one of two fundamental building blocks drawn from primary metabolism in *Streptomyces clavuligerus* to construct the β-lactamase inhibitor clavulanic acid (2).[1] The oxidation state at C-2 of the latter suggested that β-hydroxyornithine (3) might be an intermediate in the biosynthetic pathway.



Based upon the separate observations of Doyle[2] and Ganem,[3] racemic *erythro*- and *threo*-[5-^{14}C]-β-hydroxyornithine, 12 and 13, respectively, were prepared as shown in Scheme I where [^{14}C]formaldehyde provided a ready source of radiolabel. Unfortunately, attempted incorporation of these oxidatively modified amino acids under whole-cell conditions that had been used successfully earlier[1] failed to show uptake of the labeled material (<5%) by the organism and no significant incorporation (<0.1%) of radiolabel into clavulanic acid.[4]

An important advance in identifying intermediates beyond ornithine in clavulanic acid biosynthesis came with the isolation of proclavaminic acid (4).[5] Initially it was not possible to establish the relative or absolute stereochemistry of 4. However, recently workers at Beecham have assigned the absolute configuration of the biochemically active stereoisomer of 4 as L-*threo* based on the probable stereochemical course of an enzymic resolution step.[6] In this paper we confirm this structural assignment

Scheme I



in an independent correlation of proclavaminic acid ultimately to L-glutamic acid by way of a stereochemically homogeneous intermediate 11 (Scheme I). In the course of this work we have extended the well-known Hanessian method[7] of vinylglycine preparation to the benzyl ester (8; R = CH_2Ph,R′ = Cbz), which can be readily obtained by

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(2) Vyas, D. M.; Chiang, Y.; Doyle, T. W. *Tetrahedron Lett.* **1984**, *25*, 487-490.
(3) Tice, C. M.; Ganem, B. *J. Org. Chem.* **1983**, *48*, 5048-5050.
(4) The biosynthesis of acivicin provides an interesting comparison: Gould, S. J.; Ju, S. *J. Am. Chem. Soc.* **1989**, *111*, 2329-2331.
(5) Elson, S. W.; Baggaley, K. H.; Gillett, J.; Holland, S.; Nicholson, N. H.; Sime, J. T.; Woroniecki, S. R. *J. Chem. Soc., Chem. Commun.* **1987**, 1736-1738 and 1739-1740.
(6) Baggaley, K. H.; Nicholson, N. H.; Sime, J. T. *J. Chem. Soc., Chem. Commun.* **1988**, 567-568. See in particular: Rossi, D.; Romeo, A.; Lucente, G. *J. Org. Chem.* **1978**, *43*, 2576-2581. Full papers have recently appeared: Baggaley, K. H.; Elson, S. W.; Nicholson, N. H.; Sime, J. T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1513-1520 and 1521-1533.
(7) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425-1428.

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crystallization in optically pure form owing to its particularly favorable physical properties. The previously described methyl ester,[7] on the other hand, can be difficult to obtain optically pure as we illustrate below.

Syntheses of L-vinylglycine have been reported from *N*-Cbz-L-vinylglycine methyl ester (8; R = Me, R′ = Cbz), an oil, which can in turn be derived by dehydrosulfenylation of the appropriately protected L-methionine sulfoxide 5,[8] or by oxidative decarboxylation of 6.[7] To avoid the necessity of hydrolyzing the methyl ester present in 8 (R = Me) at later stages in the planned syntheses shown in Scheme I, we sought to modify the Hanessian oxidative decarboxylation route so that the benzhydryl (7; R = $CHPh_2$, R′ = *t*-Boc to allow differential N,O-deprotection) or benzyl (7; R = CH_2Ph, R′ = Cbz) ester could be obtained. In both cases, contrary to expectation,[7] racemization, occasionally substantial, was seen in the conversion of 7 to 8 (40–90% ee) despite the precautions[7] of using freshly recrystallized lead tetraacetate and not adding pyridine to the reaction mixtures. The extent of racemization was readily established by catalytic hydrogenation of 8 to α-aminobutyric acid and measurement of its optical rotation.[9] The benzhydryl ester 8 (R = $CHPh_2$, R′ = *t*-Boc) was obtained as an oil from which the racemate was isolated by crystallization. In contrast the benzyl ester 8 (R = CH_2Ph, R′ = Cbz) gave a crystalline mixture or conglomerate[10] of broad melting range (again owing to partial racemization), which on recrystallization readily afforded the optically pure enantiomer of 8. It is notable that the effectiveness of this procedure depends upon a significant melting point difference that exists between the racemate of 8 (R = CH_2Ph, R′ = Cbz), mp 37–38 °C, and the L- and D-antipodes, mp 79.5–80.0 °C such that the stereoisomerically pure forms can be easily isolated on a small or large scale.

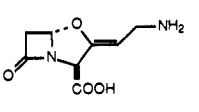
With the optically pure vinylglycine synthon 8 (R = CH_2Ph, R′ = Cbz) in hand, the nitrone 9 underwent cycloaddition in a single regiochemical sense as anticipated[3] to give the chromatographically separable *erythro*- and *threo*-isoxazolidines 10 and 11 in a 2:3 ratio. Hydrogenolysis of 10 and 11 gave correspondingly *erythro*-β-hydroxyornithine (12) and *threo*-β-hydroxyornithine (13), isolated as their optically pure L-hydrochlorides in an 80% combined yield from protected vinylglycine 8.[11] The stereochemical integrity of 12 and 13 was established by HPLC analysis using Marfey's reagent[12] and shown to have stereoisomeric purities of greater than 99%.

A parallel route to 12 and 13 has been described by Gould[13] using the vinylglycine methyl ester (8; R = Me, R′ = Cbz) prepared by the Hanessian method.[7] While we find good agreement for the optical rotation of the L-*erythro* isomer 12, $[\alpha]_D$ = 17.8° (*c* = 2.0, 6 N HCl), with that reported by Gould, $[\alpha]_D$ = 18.0° (*c* = 2.2, 6 N HCl),[13] our observed rotation for the L-*threo* diastereomer 13 is substantially higher, $[\alpha]_D$ = 17.9° (*c* = 2.0, 6 N HCl) versus $[\alpha]_D$ = 9.6° (*c* = 2.0, 6 N HCl).[13] Stereochemical analysis of a sample of the latter, kindly provided by Professor Gould, using Marfey's reagent[12] as above revealed the presence of a substantial enantiomeric impurity.[14] The racemization event could be traced to the vinylglycine preparation itself. It is evident from the further experience of Gould's laboratory and ours noted above for the benzhydryl and benzyl esters that, despite careful preparation of the lead tetraacetate and the absence of added pyridine in the oxidative decarboxylation of 7 to 8, variable extents of racemization can occur. The melting point behavior of the *N*-Cbz benzyl ester of 8, however, allows enantiomerically pure vinylglycine to be reproducibility obtained.

Having secured the stereoisomeric identities[11] and purities of the *erythro*- and *threo*-L-β-hydroxyornithines 12 and 13, and hence of their isoxazolidine precursors 10 and 11, the latter was partially deprotected with HBr in acetic acid[15] to give the free amine 14 in 66% yield after flash chromatography. Treatment of 14 with neat *tert*-butyl acrylate gave in 8 days an equilibrium mixture of starting material and the β-addition product 15 (R″ = *t*-Bu), which could be isolated in 51% yield as a low melting solid, mp 60–61 °C. Brief treatment of the latter with trifluoroacetic acid gave the β-amino acid 15 (R″ = H), which was closed to the azetidinone 16 using the Ohno procedure.[16] Careful high-field NMR analysis revealed that the stereochemical integrity of C-2 and C-3 remained unaltered through the three steps from 14. Hydrogenation of 16 over 2 days and removal of minor impurities by preparative HPLC gave proclavaminic acid (17). Incubation of this material with purified clavaminate synthase and comparisons with the other stereoismers of 4 clearly showed the L-*threo* form 17 to be the kinetically significant biosynthetic intermediate.[17]

In conclusion, we have developed syntheses of *erythro*- and *threo*-β-hydroxyornithine in both their racemic and optically pure forms. Success in preparation of the latter depended upon the availability of an optically pure, protected form of vinylglycine 8. Extention of the Hanessian oxidative decarboxylation protocol[7] from appropriately protected L-glutamic acid 7, however, gave 8 (R = CH_2Ph, $CHPh_2$) in which variable extents of racemization were observed. However, for the *N*-Cbz benzyl ester (8; R = CH_2Ph, R′ = Cbz) fortuitous melting point behavior was found to afford the desired synthon as a highly crystalline solid in optically pure form. A nitrone cycloaddition route established earlier for the racemic series gave the isoxazolidines 10 and 11 as a separable mixture of diastereomers. Hydrogenolysis of these gave *erythro*- and *threo*-L-β-hydroxyornithines 12 and 13 in >99% stereoisomeric purity. Proceeding from 11, N-deprotection and elaboration

(8) Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817–4820.
(9) Greenstein, J. P.; Birnbaum, S. M.; Otey, M. C. *J. Biol. Chem.* **1953**, *204*, 307–312.
(10) Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; Lahav, M.; Leiserowitz, L. *Top. Stereochem.* **1986**, *16*, 1–129.
(11) Preliminary experiments with D,L-8 (R = CH_2Ph, R′ = Cbz) gave D,L-*erythro*- and D,L-*threo*-β-hydroxyornithine whose properties were in accord with those previously reported for the racemates and, therefore, unambiguously established the relative configurational identities of 12 and 13: Wakamiya, T.; Teshima, T.; Kubota, I.; Shiba, T.; Kaneko, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2292–2296.
(12) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596. Aberhart, D. J.; Cotting, J. A.; Lin, H.-J. *Anal. Biochem.* **1985**, *151*, 88–91.
(13) Wityak, J.; Gould, S. J.; Hein, S. J.; Keszler, D. A. *J. Org. Chem.* **1987**, *52*, 2179–2183.
(14) We are grateful to Professor Gould for providing this sample for comparison and for generously allowing us to report these findings in the present context. It is evident that the variable extents of racemization that we observed for the benzhydryl and benzyl esters apply to the methyl ester as well.
(15) Ben-Ishai, D.; Berger, A. *J. Org. Chem.* **1952**, *17*, 1564–1570.
(16) Kobayashi, S.; Iimori, T.; Izawa, T.; Ohno, M. *J. Am. Chem. Soc.* **1981**, *103*, 2406–2408.
(17) Clavaminate synthase catalyzes the double oxidative cyclization of 17 to clavaminic acid (i) in the presence of α-ketoglutaric acid, Fe(II) and molecular oxygen.[5] This enzyme has been purified to homogeneity and used in the assay of the possible stereoisomeric forms of proclavaminic acid (4): Salowe, S. P.; Marsh, E. N.; Townsend, C. A. *Biochemistry* **1990**, *26*, 6499–6508.



(*i*)

of the azetidinone ring established the absolute configuration of proclavaminic acid as the L-*threo* diastereomer **17** in accord with the stereochemical assignment of the Beecham group.[6]

Experimental Section

Melting points were determined in open capillaries using a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian XL-400 instrument; cm indicates complex multiplet, sym m represents symmetrical multiplet, and app means apparent.

L-2-[[(Benzyloxy)carbonyl]amino]-3-butenoic Acid Benzyl Ester (8, R = CH_2Ph, R′ = Cbz). To a solution of *N*-Cbz-L-glutamic acid monobenzyl ester[18] (16.58 g, 44.6 mmol) in dry benzene (500 mL) was added cupric acetate monohydrate (2.25 g, 11.25 mmol), and the suspension was stirred for 1 h at room temperature under argon. Lead tetraacetate (39.9 g, 90 mmol; recrystallized from glacial acid and rinsed with freshly distilled anhydrous diethyl ether in a sintered glass funnel immediately prior to use) was added, and the mixture was heated to reflux for 17 h.[7] After cooling and filtration of precipitated salts on Celite, the benzene solution was diluted with ethyl acetate (250 mL), washed with water (3 × 400 mL) and brine (400 mL), and dried (Na_2SO_4). Filtration and removal of the solvents in vacuo gave a yellow oil from whch **8** (R = CH_2Ph, R′ = Cbz) was obtained by flash chromatography (230 g silica gel, 9:1 hexanes/ethyl acetate) as a partially racemized solid [6.0 g, 18.4 mmol, 41%; mp 73–79 °C, $[\alpha]_D$ = −14.9° (*c* = 1.0, MeOH)]. Recrystallization from ethyl acetate/hexanes gave optically pure material (4.06 g, 12.5 mmol, 28%) as fine white needles: mp 79.5–80 °C (racemate mp 37–38 °C); $[\alpha]_D$ = −15.9° (*c* = 1.0, MeOH); IR ($CHCl_3$) 3420, 3026, 1723, 1333 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.99 (br t, *J* = ca. 7 Hz, 1 H, H-2), 5.12 (s, 2 H, CH_2Ph), 5.19 (s, 2 H, CH_2Ph), 5.27 (dd, *J* = 1.8, 10.4 Hz, 1 H, H-4*Z*), 5.36 (dd, *J* = 1.8, 17.1 Hz, 1 H, H-4*E*), 5.47 (br d, *J* = 7.0 Hz, 1 H, NH), 5.92 (ddd, *J* = ca. 7, 10.4, 17.1 Hz, 1 H, H-3), 7.16–7.39 (m, 10 H, Ph).

Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.22; H, 5.94; N, 4.30.

***N*-Benzylnitrone (9).** To a solution of *N*-benzylhydroxylamine[19] (1.53 g, 12.45 mmol) in absolute ethanol (30 mL) was added dropwise 37% aqueous formaldehyde (950 μL, 12.6 mmol) in absolute ethanol (30 mL) with vigorous stirring over 1 h, and the nitrone **9** was generated according to the method of Ganem[3] (1.66 g, 12.2 mmol, 98%): ^{1}H NMR ($CDCl_3$) δ 4.92 (s, 2 H, CH_2Ph), 6.22 (d, *J* = 7.4 Hz, 1 H, vinyl), 6.55 (d, *J* = 7.4 Hz, 1 H, vinyl), 7.42 (s, 5 H, Ph).

***N*-[(Benzyloxy)carbonyl]-L-(2-benzyl-(5*R*,*S*)-isoxazolidin-5-yl)glycine Benzyl Esters (10 and 11).** Solutions of *N*-Cbz-L-vinylglycine benzyl ester (2.66 g, 8.3 mmol), benzene (30 mL), and freshly prepared *N*-benzylnitrone **9** (1.66 g, 12.2 mmol) in benzene (30 mL) were combined and heated to reflux for 16 h and cooled to room temperature, and the solvent was removed in vacuo. Purification and separation of the diastereomeric isoxazolidine products was achieved by sequential flash chromatography on silica gel [9:1 benzene/diethyl ether; R_f = 0.13 (minor, *erythro*), R_f = 0.17 (major, *threo*)], affording **10** (1.26 g, 2.74 mmol) and **11** (2.33 g, 5.06 mmol) as clear oils (total 3.6 g, 7.8 mmol, 94%). L-*erythro*-**10**: $[\alpha]_D$ = +3.3° (*c* = 2.2, MeOH); IR ($CHCl_3$) 3032, 3014, 1722, 1508, 1190, 912 cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.14 (sym m, 6 lines, 1 H, H-4), 2.32 (sym m, 6 lines, 1 H, H-4), 2.78–2.85 (br m, 1 H, H-5), 2.86–2.99 (br m, 1 H, H-5), 3.84 (AB q, *J* = 14.0 Hz, 2 H, NCH_2Ph), 4.28 (br app t, *J* = ca. 7–8 Hz, 1 H, H-2), 4.31–4.38 (br m, 1 H, H-3), 5.04 (s, 2 H, OCH_2Ph), 5.09 (AB q, *J* = 11.1 Hz, OCH_2Ph), 7.01–7.35 (m, 15 H, Ph); {^{1}H} ^{13}C NMR ($CDCl_3$) δ 30.3, 54.2, 57.5 (br), 61.8, 66.9, 67.0, 127.2, 127.9, 128.0, 128.1, 129.0, 135.1, 136.0, 136.4, 152.8, 169.7; MS *m*/*z* 460 (M^+, 6), 369, 162, 120, 91 (100); accurate mass 460.2007, calcd for $C_{27}H_{28}N_2O_5$ 460.1998. L-*threo*-**11**: $[\alpha]_D$ = −44° (*c* = 2.2, MeOH); IR ($CHCl_3$) 3032, 3013, 1747, 1721, 1510, 1224, 912 cm^{-1}; ^{1}H NMR (DMSO-d_6, 80°C) δ 2.08 (sym m, 6 lines, 1 H, H-4*S*), 2.38 (sym m, 6 lines, 1 H, H-4*R*), 2.75–2.85 (br m, 1 H, H-5), 2.85–3.00 (br m, 1 H, H-5), 3.85 (br s, 2 H, NCH_2Ph), 4.35 (dd, *J* = 4.9, 8.55 Hz, 1 H, H-2), 4.45–4.53 (br m, 1 H, H-3), 5.05 (br s, 2 H, OCH_2Ph), 5.12 (br s, 2 H, OCH_2Ph), 7.70–7.37 (m, 15 H, Ph); {^{1}H} ^{13}C NMR ($CDCl_3$) δ 31.6, 54.8, 58.5 (br), 62.0, 67.0, 67.2, 127.9, 128.0, 128.2, 128.5, 129.0, 135.5, 136.4, 157.0, 170.1; MS *m*/*z* 460 (M^+, 7), 369, 162, 120, 91 (100); accurate mass 460.2003, calcd for $C_{27}H_{28}N_2O_5$ 460.1998.

***erythro*- and *threo*-L-3-Hydroxyornithine Monohydrochlorides (12 and 13).** L-Isoxazolidine **10** or **11** (200 mg, 0.43 mmol) in ethanol (5 mL) and 6 N hydrochloric acid (150 μL) was hydrogenolyzed over Pearlman's catalyst [$Pd(OH)_2$/C, 250 mg] in a Parr shaker (50 psi) for 3 days. The catalyst was removed by filtration through Celite and washed with water (100 mL). The filtrate was concentrated to ca. 10 mL and then lyophilized to a pale yellow foam. Recrystallization from water/ethanol/pyridine gave the monohydrochloride as a white powder in 85% yield. L-*erythro*-**12**: mp 231–232 °C dec (lit.[13] mp 232 °C dec); $[\alpha]_D$ +17.8° (*c* = 2.0, 6 N HCl) [lit.[13] $[\alpha]_D$ = +18.0° (*c* = 2.2, 6 N HCl)]; IR (1% KBr) 3073 (br), 3000 (br), 1614, 1575, 1530, 1430, 1356, 1324, 1139, 1064, 921 cm^{-1}; ^{1}H NMR (D_2O/DSS) δ 1.75–1.89 (m, 10 lines, 1 H, H-4), 1.89–2.05 (m, 10 lines, 1 H, H-4), 3.16 (sym m, 8 lines, 2 H, H-5), 3.87 (d, *J* = 3.7 Hz, 1 H, H-2), 4.24 (br dt, *J* = ca. 3.2, 10.7 Hz, 1 H, H-3). L-*threo*-**13**: mp 195 °C (dec with gas evolution) (lit.[13] 123 °C dec); $[\alpha]_D$ = +17.9° (*c* = 2.0, 6 N HCl) [lit.[13] $[\alpha]_D$ = +9.6° (*c* = 2.0, 6 N HCl); IR (1% KBr) 3345 (br), 2950, 1639, 1616, 1572, 1540, 1413, 1152, 1096, 1054, 915 cm^{-1}; ^{1}H NMR (D_2O/DSS) δ 1.85–1.98 (sym m, 1 H, H-4), 2.02–2.13 (sym m, 1 H, H-4), 3.20 (sym m, 8 lines, 2 H, H-5), 3.66 (d, *J* = 5.2 Hz, 1 H, H-2), 4.16 (br sym m, 5 lines, 1 H, H-3).

Stereochemical Analysis of 12 and 13. β-Hydroxyornithine samples were reacted with Marfey's reagent as described.[12,20] HPLC analyses were carried out using a Spherisorb ODS II 5 μm cartridge column (250 × 4.6 mm, Alltech Associates) with UV peak detection at 240 and 340 nm. A nonlinear binary gradient elution was performed (convex gradient no. 4, flow rate 1.5 mL/min) over 45 min starting at 10% acetonitrile/90% 50 mM triethylammonium phosphate buffer, pH 3.0, and ending at 40% acetonitrile. Under these conditions the stereoisomers of β-hydroxyornithine were observed to have the following retention times: L-*erythro* 23.9 min, L-*threo* 22.5 min, D-*erythro* 25.9 min, D-*threo* 21.5 min.

L-(2-Benzyl-(5*R*)-isoxazolidin-5-yl)glycine Benzyl Ester (14). A mixture of L-*threo*-isoxazolidine **11** (1.32 g, 2.87 mmol) and 30 wt % HBr in acetic acid (6 mL) under argon was stirred at room temperature until the isoxazolidine had fully dissolved and evolution of CO_2 had ceased (ca. 0.5 h). The solvent was removed in vacuo at 30 °C, and the black residue thus obtained was dried under vaccum for 1 h and triturated with diethyl ether (3 × 30 mL) to afford an off-white free-flowing powder. This powder was partitioned between ethyl acetate (100 mL) and water (100 mL) at 0 °C and carefully adjusted to pH 7.8 by the dropwise addition of 0.1 N sodium hydroxide. The aqueous phase was decanted and washed with ethyl acetate (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated to a yellow oil, which was purified by flash chromatography (30 g of silica gel, ethyl acetate) to afford **14** (615 mg, 1.8 mmol, 66% from **11**) as a clear oil. The amine was found to be unstable and was typically prepared and used directly in the next reaction: $[\alpha]_D$ = −62° (*c* = 2.0, CH_2Cl_2); IR ($CHCl_3$) 3389, 3331, 3002, 2966, 1731, 1600, 1230, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.69 (br s, 2 H, NH_2), 2.21–2.49 (br m, 2 H, H-4), 2.89 (br s, 2 H, H-5), 3.51 (d, *J* = 4.3 Hz, 1 H, H-2), 3.84 (AB q, *J* = 13.3 Hz, 2 H, NCH_2Ph), 4.35 (br s, 1 H, H-3), 5.12 (s, 2 H, OCH_2Ph), 7.13–7.34 (m, 10 H, Ph); {^{1}H}^{13}C NMR ($CDCl_3$) δ 31.1 (br), 54.4 (br), 58.0 (br), 61.8 (br), 66.8, 78.2, 127.3, 128.1, 128.2, 128.3, 128.5, 135.6, 136.9, 173.3; CIMS *m*/*z* 327 (MH^+, 51%; methane as reagent gas), 220, 164, 108, 91 (100%); accurate mass 327.1712, calcd for $C_{19}H_{23}N_2O_3$ 327.1709.

***N*-(3-*tert*-Butoxy-3-oxopropyl)-L-(2-benzyl-(5*R*)-isoxazolidin-5-yl)glycine (15).** A solution of the amine **14** (615 mg, 1.8

(18) Nefkens, G. H. L.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas* **1964**, *83*, 199–207.

(19) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904. Isoma, Y.; Kurita, H. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 720–722.

(20) Pierce, 1988 Handbook & General Catalog, pp 129 and 131.

mmol) and *tert*-butyl acrylate (30 mL, 205 mmol) was stirred for 8 days under an argon atmosphere in the dark at which time TLC (2:1 hexanes/EtOAc) showed 2 spots, R_f = 0.13 (major, corresponding the β-addition product) and R_f = 0.07 (minor, corresponding to unreacted **14**). The *tert*-butyl acrylate was removed in vacuo, and the residue was purified by radial chromatography (2:1 hexanes/EtOAc) to afford unreacted starting material (138 mg, 0.41 mmol) and **15** (418 mg, 0.91 mmol, 51%) [65% based upon recovered starting material] as a clear oil, which solidified on standing: mp 61-62 °C; $[\alpha]_D$ = -43.7° (c = 2, CH_2Cl_2); IR ($CHCl_3$) 3028, 3011, 1724 (br), 1378, 1258, 1216, 1156, 910 cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.39 (s, 9 H, *t*-Bu), 2.20 (sym 6 lines, 1 H, H-4*S*), 2.27 (t, 2 H, J = 6.7 Hz, CH_2COO*t*-Bu), 2.66 (sym 6 lines, 1 H, H-4*R*), 2.84 (br m, 2 H, H-5), 3.03 (m, 2 H, partially obscured by H_2O, NHCH_2CH$_2$), 3.40 (br s, 1 H, H-2), 3.82 (AB q, 2 H, J = 13.4 Hz, NCH_2Ph), 4.33 (br t, 1 H, H-3), 5.13 (s, 2 H, OCH_2Ph), 7.21-7.34 (m, 10 H, Ph); {^{1}H}^{13}C NMR ($CDCl_3$) δ 28.1, 31.0 (br), 36.5, 44.2, 54.2 (br), 62.0 (br), 64.2 (br), 66.7, 78.0 (br), 127.4, 128.2, 128.4, 128.5, 129.1, 135.7 (br), 171.7, 172.7; MS m/z 454 (M$^+$, <0.005), 381 (M$^+$ - O-*t*-Bu, 4.2), 329, 162, 120, 91 (100); accurate mass 454.2465, calcd for $C_{26}H_{34}N_2O_5$ 454.2468, (M$^+$ - O-*t*-Bu) accurate mass 381.1821, calcd for $C_{22}H_{25}N_2O_4$ 381.1814.

(*S*)-(2-Benzyl-(5*R*)-isoxazolidin-5-yl)(2-oxoazetidin-1-yl)acetic Acid Benzyl Ester (16). A solution of **15** (481 mg, 0.91 mmol) and trifluoroacetic acid (TFA, 12 mL; freshly distilled from P_2O_5) was stirred under argon at room temperature for 15 min, and the TFA was removed in vacuo. The unstable clear oil thus obtained was dried under vacuum for 0.5 h and used directly in the next reaction.

A solution of the β-amino acid in acetonitrile (150 mL) was treated with triphenylphosphine (300 mg, 1.1 mmol, 1.2 equiv) and dipyridyl disulfide (250 mg, 1.1 mmol, 1.2 equiv). The mixture was heated at reflux for 18 h, cooled to room temperature, and concentrated in vacuo, and the product was separated from the triphenylphosphine, triphenylphosphine oxide, and 2-mercaptopyridine by repeated radial chromatography (1:1 hexanes/EtOAc, R_f = 0.13, followed by 1:1 CH_2Cl_2/Et_2O for separation from the 2-mercaptopyridine) to afford **16** (159 mg, 0.42 mmol, 46% through 2 steps) as a slightly yellow oil: $[\alpha]_D$ = -30° (c = 2.0, CH_2Cl_2); IR ($CHCl_3$) 3012, 2919, 1739 (br), 971 cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.05 (sym 6 lines, 1 H, H-4*S*), 2.43 (sym 6 lines, 1 H, H-4*R*), 2.85 (br m, 2 H, H-3'), 2.87-2.90 (br m, 1 H, H-5), 2.93-2.97 (br m, 1 H, H-5), 3.36 (br m, 2 H, H-4'), 3.89 (s, 2 H, NCH_2Ph), 4.53 (d, 1 H, J = 5.12 Hz, H-2), 4.57 (sym app 3 line m, 1 H, H-3), 5.17 (s, 2 H, OCH_2Ph), 7.21-7.38 (m, 10 H, Ph); {^{1}H}^{13}C NMR ($CDCl_3$) δ 31.6, 37.2, 40.2, 54.4, 56.4, 62.1 (br), 67.3, 75.8, 127.5, 128.1, 128.3, 128.5, 128.6, 129.1, 135.1, 136.8, 168.9, 177.7; MS m/z 380 (M$^+$, 5.4), 289, 162, 120, 91 (100); accurate mass 380.1740, calcd for $C_{22}H_{24}N_2O_4$ 380.1736.

(2*S*,3*R*)-5-Amino-3-hydroxy-2-(2-oxoazetin-1-yl)pentanoic Acid (17, Proclavaminic Acid). To a 250-mL Parr hydrogenation bottle was added L-*threo*-isoxazolidine **16** (82 mg, 0.22 mmol), absolute ethanol (8 mL), water (8 mL), and 20% $Pd(OH)_2$/C (160 mg, Pearlmans catalyst). The mixture was degassed and mechanically shaken under an atmosphere of hydrogen (initially 50 psi, Parr hydrogenation apparatus) for 2 days. The mixture was filtered through a bed of Celite to remove the catalyst, rinsing with water (50 mL). The filtrate was lyophylized to afford a slightly brown powder, which was purified by preparative HPLC (Whatman ODS-3 C18 R.P.; 9.4 × 250 mm; detection at 220 nm; mobile phase: H_2O, 3.0 mL/min; retention time, 4.1 min) to afford **17** (38.0 mg, 0.19 mmole; 85%) as clear white prisms after lyophilization: mp 127-130 °C (dec with gas evolution); $[\alpha]_D$ = +7.3° (c = 1.3, H_2O); IR (1% in KBr) 3373, 2940 (v br), 2088, 1703 (br), 1634 (br), 1378, 773 cm^{-1}; ^{1}H NMR (D_2O) δ 1.77 (m, 2 H, H-4), 2.93 (t, J = 3.97 Hz, 2 H, H-3'), 3.07 (m, 2 H, CH_2NH$_2$), 3.43 (m, 1 H, H-4'), 3.50 (m, 1 H, H-4'), 4.00 (d, J = 5.5 Hz, 1 H, H-2), 4.22 (m, 1 H, H-3); {^{1}H}^{13}C NMR (D_2O, dioxane internal reference at 67.4 ppm) δ 31.68, 36.16, 37.89, 41.02, 63.27, 69.61, 172.83, 174.89; CIMS m/z 203 (MH$^+$, 100; methane as reagent gas), 185, 167, 143, 115; accurate mass (MH$^+$) 203.1037, calcd for $C_8H_{15}N_2O_4$ 203.1037.

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Registry No. **2**, 58001-44-8; **7** (R = Bn, R' = Cbz), 3705-42-8; **8**, 130096-70-7; **9**, 74635-18-0; **10**, 130096-71-8; **11**, 130096-72-9; **12**, 107942-04-1; **13**, 107942-03-0; **14**, 130096-73-0; **15**, 130096-74-1; **16**, 130096-75-2; **17**, 112240-59-2; H_2C=CHCOOBu-*t*, 1663-39-4; BnNHOH, 622-30-0.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra for **10**, **11**, and **14-17** (12 pages). Ordering information is given on any current masthead page.

Nucleophile-Dependent Substitution Reactions of 5-Halovaleric Acid Esters: Synthesis of 6,12-Dioxamyristic Acid

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The reaction of 5-ethoxypentan-1-ol with a variety of 5-halovalerate alkyl esters afforded ester exchange products rather than the expected products of Williamson ether synthesis. The virtually unknown reaction of an alkoxide with a 5-halovalerate ester contrasts strongly with literature reports of reactions involving other nucleophiles in which the halogen substitution products are nearly always isolated. An explanation is offered for this behavior in terms of a chelation-induced conformation of the substrate. Although the direct synthetic approach failed, the hitherto unknown title compound could still be prepared, albeit in six steps in 6% overall yield. The approach used is discussed along with the interesting chemistry of this system.

Myristoyl-CoA:protein *N*-myristoyl transferase (NMT, E.C. 2.3.1.97) catalyzes the co-translational attachment of myristate via an amide bond to the NH_2-terminal glycine residues of a number of cellular and viral proteins (reviewed in ref 1). *N*-Myristoylation of certain retroviral

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