Synthesis of the Peptide Fragment of Pseudobactin

John F. Okonya, Teodozyj Kolasa,† and Marvin J. Miller*

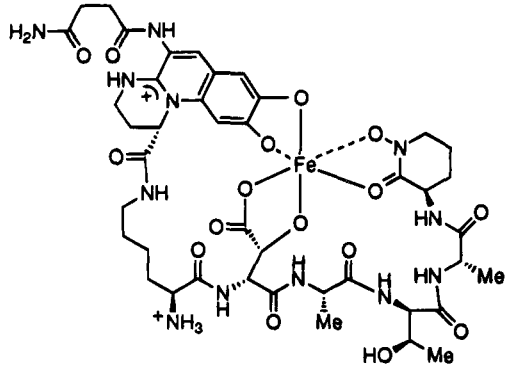
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Received October 10, 1994®

Synthesis of the peptide fragment, L-Lys-D-*threo*-β-OH-Asp-L-Ala-D-*allo*-Thr-L-Ala-*N*-OH-D-cOrn, of pseudobactin (**2**) is reported. By utilizing 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) as a coupling reagent, peptide bonds were constructed without requiring protection of hydroxyl groups. To access the D-*allo*-Thr residue in the peptide fragment of pseudobactin, the Thr residue in *N*-Cbz-L-Ala-D-Thr-L-Ala-O-*t*-Bu (**4b**) was converted to a peptidyl oxazoline using Burgess' reagent. Hydrolysis of the oxazoline with 1 N HCl followed by base-catalyzed acyl migration then provided the D-*allo*-Thr analog **4a**.

Introduction

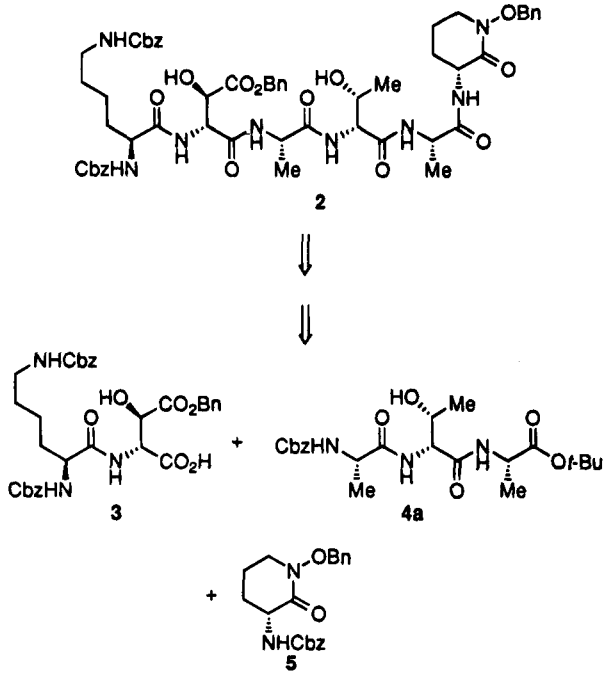
Specific strains of the *Pseudomonas fluorescens-putida* group, collectively called plant growth-promoting rhizobacteria (PGPR), rapidly colonize various plant roots and cause statistically significant yield increases.[1] PGPR promote plant growth in part by the production of extracellular siderophores (microbial iron-chelators) which strongly complex ferric ion in the soil and make this necessary nutrient unavailable to deleterious microorganisms. Pseudobactin (**1**) is one of the siderophores



1, Pseudobactin

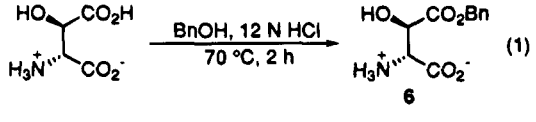
produced and utilized by PGPR. Pseudobactin (**1**) contains a fluorescent chromophore linked to an unusual linear peptide with alternating L- and D-amino acids which presumably minimizes its suceptibility to various proteases. The iron-chelating groups are incorporated as δ-*N*-hydroxycycloornithine, β-hydroxyaspartic acid, and a fluorescent dihydroxy quinoline component that is common to most pseudomonad siderophores. We have previously reported the syntheses of the fluorescent quinoline moiety[2] and δ-*N*-hydroxycycloornithine.[3] As part of our continuing effort to synthesize pseudobactin, its analogs, and drug conjugates we wish to report here the synthesis of the peptide fragment, L-Lys-D-*threo*-β-OH-Asp-L-Ala-D-*allo*-Thr-L-Ala-*N*-OH-D-cOrn, of pseudobactin.

Scheme 1



Results and Discussion

Our approach to the synthesis of the hexapeptide fragment (**2**) of pseudobactin which allowed for convenient manipulation of protecting groups is outlined in Scheme 1. D-*threo*-β-Hydroxyaspartic acid, required for the synthesis of dipeptide component **3**, was prepared by the method previously described.[4–6] Selective esterification of the β-carboxyl component of D-*threo*-β-hydroxyaspartic acid was achieved in 90% yield by heating D-*threo*-β-hydroxyaspartic acid in benzyl alcohol with concentrated HCl at 70 °C for 2 h (eq 1). Reaction of



(1)

β-hydroxyaspartic acid derivative (**6**) with the preformed *N*-hydroxysuccinimide (NHS) ester of bis-*N*,*N*′-(Cbz)-L-

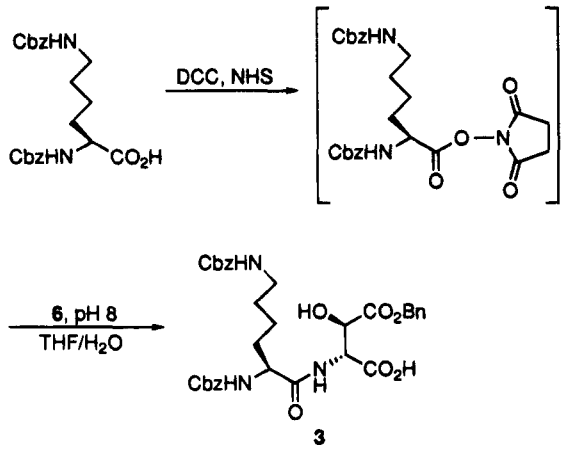
† Current address: Abbott Laboratories, Abbott Park, IL 60064.

® Abstract published in *Advance ACS Abstracts*, March 1, 1995.

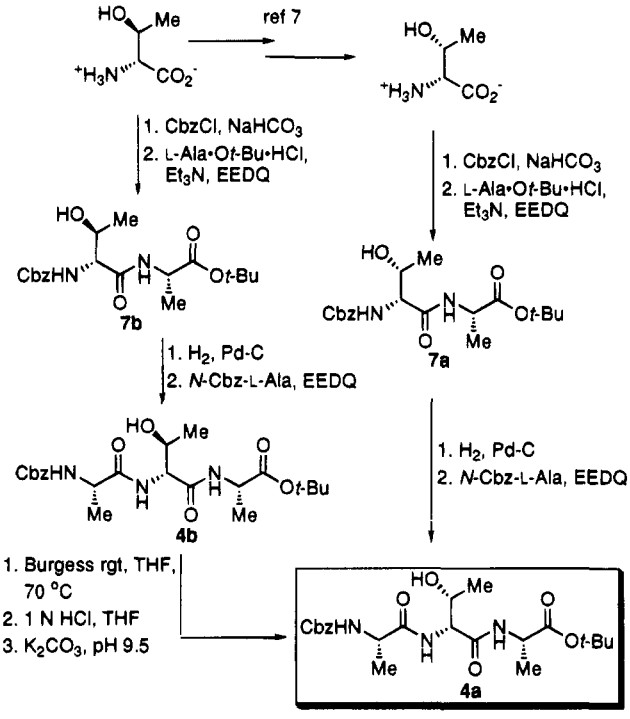
(1) Teintze, M.; Hossain, M. B.; Barnes, C. L.; Leong, J.; van der Helm, D. *Biochemistry* **1981**, *20*, 6446.
(2) Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1990**, *55*, 4246.
(3) Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1990**, *55*, 1711.
(4) Liwschitz, Y.; Rabinsohn, Y.; Haber, A. *J. Chem. Soc.* **1962**, 3589.
(5) Liwschitz, Y.; Editz-Pfeffermann, Y.; Singerman, A. *J. Chem. Soc. C* **1967**, 2104.

0022-3263/95/1960-1932$09.00/0

Scheme 2



Scheme 3

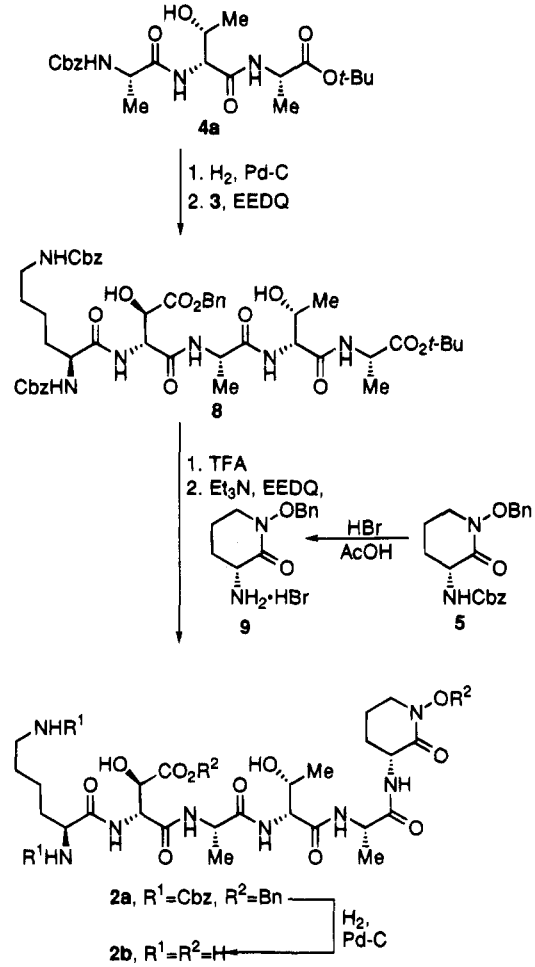


lysine at pH 8 (Scheme 2) allowed formation of dipeptide **3** in quantitative yield without requiring the protection of the α-carboxylate of **6**.

Tripeptide fragment **4a** was prepared in two ways (Scheme 3). In the first approach, D-Thr was first converted to D-*allo*-Thr in four steps with an overall yield of 36% by the method previously described by Morell and co-workers.[7] Protection of D-*allo*-Thr with CbzCl followed by 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) coupling to L-Ala-O-*t*-Bu provided dipeptide **7a** in 56% yield after column chromatography. Tripeptide **4a** was then obtained in 99% yield by deprotection of dipeptide **7a** by catalytic hydrogenolysis, followed by coupling to *N*-Cbz-L-Ala.

The second approach to tripeptide **4a** involved first the synthesis of its corresponding Thr tripeptide analog **4b** starting with D-Thr and following the same reaction

Scheme 4



sequence used for the preparation of **4a** (Scheme 3). To epimerize the Thr residue to the *allo*-Thr residue, tripeptide **4b** was treated with $CH_3O_2CNSO_2NEt_3$ (Burgess' reagent).[8,9] Mild hydrolysis of the resulting peptidyl oxazoline with 1 N HCl and base-catalyzed acyl migration afforded the desired protected tripeptide **4a** in over 70% yield from **4b**. This route to **4a** was efficient and avoided the multistep conversion of Thr to *allo*-Thr.

With methodology for the synthesis of α-*N*-Cbz-δ-*N*-OBn-D-cycloornithine (**5**) in place in our laboratory,[3] and with the peptide components **3** and **4a** in hand, we were able to complete the synthesis of the hexapeptide fragment of pseudobactin as shown in Scheme 4. Thus, deprotection of tripeptide **4a** by catalytic hydrogenolysis and subsequent coupling to dipeptide **3** provided protected pentapeptide **8** in 80% yield. Removal of the *t*-Bu ester of **8** with TFA and coupling of δ-*N*-OBn-D-cycloornithine•HBr, obtained by HBr/AcOH deprotection of **5**, provided protected hexapeptide **2a** in 60% yield after column chromatography. Deprotection of **2a** by hydrogenolysis provided the hexapeptide fragment of pseudobactin **2b** in quantitative yield.

Biological studies of the component peptides and efforts related to the completion of the total syntheses of pseudobactin and related analogs are in progress.

(6) Payne, B. P.; Williams, P. H. *J. Org. Chem.* **1959**, *24*, 54.
(7) Morell, J. L.; Fleckenstein, P.; Gross, E. *J. Org. Chem.* **1977**, *42*, 355.
(8) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 1575.
(9) Miller, M. J.; Maurer, P. J. *J. Am. Chem. Soc.* **1983**, *105*, 240.
(10) Teng, M.; Miller, M. J.; *J. Am. Chem. Soc.* **1993**, *115*, 548.

Experimental Section

General Methods. Instruments and general methods used have been described previously.[10] ^1H and ^{13}C NMR spectra were obtained on a General Electric GN-300 and a Varian VXR-500S. 1,4-Dioxane was used as reference in ^{13}C NMR recorded in D_2O.

Solvents used were dried and purified by standard methods.[11] The term "dried" refers to the drying of an organic layer over anhydrous $MgSO_4$.

The stereogenic integrity of the synthesized amino acids and peptides was confirmed by HPLC. The protected peptides were analyzed using a Beckman HPLC system consisting of a Model 110 A pump, a Model 420 controller, a Model 332 injector, and an Alltech Econosil column (25 cm × 4.6 mm, 5 μ silica). The diastereomeric purity of the peptides was also confirmed by preparing their *o*-phthalaldehyde (OPA) derivatives and then analyzing them by reversed-phase (C-18) HPLC with fluorescence detection.[12] Similarly, the optical purity of the amino acids were confirmed by analysis of their OPA-*N*-acetyl-L-cysteine (NAC) derivatives[13] and by measurement of their optical rotations.

***β*-Benzyl D-*threo*-*β*-Hydroxyaspartate (6).** To D-*threo*-*β*-hydroxyaspartic acid[4-6] (1.20 g, 8.05 mmol) suspended in 12 mL of benzyl alcohols was added 1.4 mL of concentrated HCl. The mixture was stirred at 70 °C for 2 h. Water was removed by evaporation under reduced pressure, 0.75 mL of concd HCl was added, and the mixture was concentrated again. The pH of the residue was adjusted to 6 using saturated $NaHCO_3$ solution, and then 60 mL of EtOH was added to precipitate ester **6** (1.73 g, 90%) as a white solid: mp 198–200 °C; ^1H NMR (DMSO-d_6) δ 3.50 (d, J = 4.5 Hz, 1H), 4.52 (d, J = 4.5 Hz, 1H), 5.10 (dd, J = 17.36, 12.55 Hz, 2H), 7.20 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 55.9, 66.0, 69.7, 128.0, 128.4, 136.0, 167.6, 171.6; IR (KBr) 3650-2200 (br), 1735 (br), 1690 cm^{-1}; HRMS (FAB) calcd for $C_{11}H_{14}NO_5$ (MH^+) 240.0872, found 240.0862. Anal. Calcd for $C_{11}H_{13}NO_5 \cdot 1/2H_2O$: C, 53.22; H, 5.68; N, 5.64. Found: C, 53.69; H, 5.72; N, 5.45.

Bis-*N*,*N*′-(Cbz)-L-lysyl-*β*-benzyl D-*threo*-*β*-Hydroxyaspartate (3). To a mixture of bis-*N*,*N*′-(Cbz)-L-lysine (Sigma, 0.82 g, 2.00 mmol) and *N*-hydroxysuccinimide (0.23 g, 2.00 mmol) in 3 mL of anhydrous THF at 0 °C under N_2 was added dropwise a solution of DCC (0.41 g, 2.00 mmol) in 2 mL of anhydrous THF. The solution was stirred overnight at rt. The precipitated DCU was removed by filtration, and the filtrate was added to a solution of *β*-benzyl D-*threo*-*β*-hydroxyaspartate (**6**) (0.48 g, 2.00 mmol) and $KHCO_3$ (~0.42 g) in 40 mL of THF and 20 mL of H_2O maintained at pH 8. The mixture was stirred for 24 h at rt. The THF was removed by evaporation under reduced pressure. The pH of the mixture was adjusted to 4 with 10% citric acid, and the solution was extracted with EtOAc. The EtOAc extracts were washed with H_2O and saturated NaCl, dried, filtered, and then concentrated. The residue was chromatographed on silica gel eluting with a gradient of MeOH in EtOAc (5%–15%) to provide 1.23 g (100%) of dipeptide **3** as a white solid: mp 160–164 °C; ^1H NMR (CD_3OD) δ 1.20–1.80 (m, 6H), 3.09 (t, J = 6.5 Hz, 2H), 4.14 (d, J = 5.0 Hz, 1H), 4.80 (d, J = 2.4 Hz, 1H), 4.98 (d, J = 2.4 Hz, 1H), 5.00–5.10 (m, 6H), 7.30 (m, 15H); ^{13}C NMR (CD_3OD) δ 20.9, 30.5, 33.0, 41.4, 56.3, 56.5, 67.3, 67.8, 68.5, 72.1, 128.8, 128.9, 129.0, 129.4, 129.5, 129.6, 136.9, 138.1, 138.4, 158.4, 159.0, 172.0, 172.8, 175.1; IR (KBr) 3650–3180 (br), 1850–1600 (br) cm^{-1}; HRMS (FAB) calcd for $C_{33}H_{38}H_3O_{10}$ (MH^+) 636.2557, found 636.2552. Anal. Calcd for $C_{33}H_{38}N_3O_{10} \cdot H_2O$: C, 60.64; H, 6.01; N, 6.43. Found: C, 61.03; H, 5.80; N, 6.16.

***N*-Cbz-D-*allo*-threonyl-L-alanine, *tert*-Butyl Ester (7a).** To a mixture of D-*allo*-Thr[7] (2.38 g, 20.00 mmol) and $NaHCO_3$ (3.36 g, 40.00 mmol) in 35 mL of H_2O was added a solution of CbzCl (3.58 g, 21.00 mmol) in 35 mL of THF. The mixture was stirred for 18 h at rt. The THF was removed under reduced pressure. The aqueous layer was washed with EtOAc, acidified to pH 3 with 10% citric acid, and then extracted three times with EtOAc. The combined EtOAc extracts were washed with saturated NaCl, dried, filtered, and concentrated. The residue was dissolved in 65 mL of CH_3CN. EEDQ (5.31 g, 21.48 mmol), L-Ala-O-*t*-Bu·HCl (Sigma, 3.55 g, 19.53 mmol), and Et_3N (1.98 g, 2.72 mL, 19.53 mmol) were added, and the solution was stirred overnight at rt. The solvent was evaporated under reduced pressure and the residue dissolved in EtOAc, washed with 10% citric acid, 10% $NaHCO_3$, H_2O, and saturated NaCl, dried, filtered, and concentrated. The residue was chromatographed on silica gel eluting with EtOAc/hexanes (1:1) and recrystallized from EtOAc/hexanes to provide 4.28 g (56%) of dipeptide **7a** as a white solid: mp 104–105 °C; HPLC t_R 28 min (2.5% 2-propanol in hexanes); $[\alpha]^{22}_D$ +17.8 (c = 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.27 (d, J = 6.4 Hz, 3H), 1.38 (d, J = 7.1 Hz, 3H), 1.43 (s, 9H), 3.96 (dq, J = 6.4, 4.7 Hz, 1H), 4.12 (dd, J = 7.5, 4.1 Hz, 1H), 4.42 (q, J = 7.2 Hz, 1H), 5.12 (s, 2H), 5.80 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 6.8 Hz, 1H), 7.35 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 18.1, 19.7, 27.9, 48.9, 58.8, 67.3, 69.1, 82.3, 128.1, 128.2, 128.5, 136.0, 156.5, 170.2, 171.9; IR (KBr) 3300 (br), 1735, 1695, 1650, cm^{-1}; MS (CI, isobutane) m/z 381 (MH^+). Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 59.99; H, 7.42; N, 7.36. Found: C, 60.04, H, 7.28; N, 7.42.

***N*-Cbz-D-threonyl-L-alanine, *tert*-Butyl Ester (7b).** The procedure used above to prepare **7a** was repeated using D-Thr (Aldrich) to afford 5.38 g (71%) of dipeptide **7b** as a white solid: mp 76–78 °C; HPLC t_R 21 min (2.5% 2-propanol in hexanes); $[\alpha]^{22}_D$ +29.9 (c = 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.18 (d, J = 6.4 Hz, 3H), 1.37 (d, J = 7.2 Hz, 3H), 1.49 (s, 9H), 3.50 (br s, 1H), 4.15 (d, J = 7.58 Hz, 1H), 4.40 (m, J = 7.3 Hz, 2H), 5.15 (s, 2H), 5.85 (d, J = 6.4 Hz, 1H), 6.95 (d, J = 6.4 Hz, 1H), 7.35 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 17.9, 18.3, 27.9, 48.9, 59.1, 67.0, 67.3, 82.3, 128.0, 128.2, 128.5, 136.0, 156.8, 170.7, 172.0; IR (KBr) 3600–3150 (br), 1730, 1700, 1645 cm^{-1}; HRMS (isobutane CI) calcd for $C_{19}H_{29}N_2O_6$ (MH^+) 381.2026, found 381.2010. Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 59.99; H, 7.42; N, 7.36. Found: C, 60.16; H, 7.36; N, 7.28.

***N*-Cbz-L-alanyl-D-threonyl-L-alanine, *tert*-Butyl Ester (4b).** Dipeptide **7b** (1.00 g, 2.62 mmol) in 10 mL of MeOH was hydrogenolyzed over 10% Pd–C (0.20 g) at atmospheric pressure for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was dissolved in 30 mL of $CHCl_3$ and treated with *N*-Cbz-L-Ala (0.65 g, 2.62 mmol) and EEDQ (0.71 g, 2.88 mmol). The solution was stirred for 48 h at rt. The reaction mixture was washed with 10% citric acid, 10% $NaHCO_3$, H_2O, and saturated NaCl, dried, and filtered. The solution was concentrated, and the residue was chromatographed on silica gel eluting with EtOAc/hexanes (9:1) and recrystallized from EtOAc/hexanes to afford 1.02 g (86%) of tripeptide **4b** as a white solid: mp 121–123 °C; R_f 0.41 (EtOAc/hexanes [9:1]); R_f 0.67 (EtOAc/MeOH [25:1]); HPLC t_R 17 min (5% 2-propanol in hexanes); $[\alpha]^{22}_D$ +28.0 (c = 1, $CHCl_3$); ^1H NMR ($CDCl_3$, data does not include hydroxyl H) δ 1.14 (d, J = 6.2 Hz, 3H), 1.36 (d, J = 7.3 Hz, 3H), 1.40 (d, J = 7.0 Hz, 3H), 1.44 (s, 9H), 4.05 (br s, 1H), 4.33 (m, 1H), 4.41 (2 overlapping q, J = 7.2 Hz, 2H), 5.06 (dd, J_{AB} = 24.2, 12.1 Hz, 2H), 5.50 (br s, 1H), 7.05 (br s 1H), 7.25 (br s, 1H), 7.30 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 17.4, 18.3, 19.5, 27.9, 48.9, 50.9, 57.7, 66.8, 66.9, 82.1, 127.9, 128.1, 128.4, 136.0, 156.2, 170.3, 172.3, 173.6; IR (KBr) 3300(br), 1730, 1680, 1640 (br) cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{34}N_3O_7$ (MH^+) 452.2397, found 452.2410. Anal. Calcd. for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C, 58.10; H, 7.22; N, 8.98.

***N*-Cbz-L-alanyl-D-*allo*-threonyl-L-alanine, *tert*-Butyl Ester (4a).** The procedure used above to prepare **4b** was repeated starting with dipeptide **7a** to afford 1.17 g (99%) of protected tripeptide **4a** as a white solid: mp 144–146 °C; R_f 0.31 (EtOAc/hexanes (9:1)); R_f 0.60 (EtOAc/MeOH [25:1]); HPLC t_R 21 min (5% 2-propanol in hexanes); $[\alpha]^{22}_D$ +16.2 (c = 1, $CHCl_3$); ^1H NMR ($CDCl_3$, data does not include a hydroxyl H) δ 1.24 (d, J = 7.1 Hz, 3H), 1.37 (d, J = 7.2 Hz, 3H), 1.40 (d, J = 7.1 Hz, 3H), 1.44 (s, 9H), 3.98 (br s, 1H), 4.27 (t, J = 7.0 Hz, 1H), 4.40 (2 overlapping t, J = 7.3 Hz, 2H), 5.08 (dd, J_{AB} = 25.0, 12.1 Hz, 2H), 5.62 (br s, 1H), 7.15 (2 overlapping br s,

(11) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon Press: New York, 1986.
(12) Lotz, B. T.; Garsparski, C. M.; Peterson, K.; Miller, M. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1107.
(13) Aswad, D. W. *Anal. Biochem.* **1984**, *137*, 405.

2H), 7.35 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 17.7, 18.2, 19.6, 27.9, 48.9, 50.8, 57.4, 67.1, 68.1, 82.2, 128.1, 128.2, 128.5, 136.0, 156.1, 170.0, 172.1, 173.0; IR (KBr) 3300 (br), 1730, 1690, 1645 cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{34}N_3O_7$ (MH^+) 452.2397, found 452.2397. Anal. Calcd for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C, 58.76; H, 7.50; N, 9.19.

Protected Tripeptide 4a by Epimerization of 4b. A solution of *N*-Cbz-L-Ala-D-Thr-L-Ala-O-*t*-Bu (**4b**, 1.00 g, 2.22 mmol) in 20 mL of THF was flushed with N_2 and treated with $CH_3O_2CNSO_2NEt_3$ (Burgess' reagent)[8,9] (Fluka, 0.61 g, 2.54 mmol). The mixture was stirred at 70 °C for 2 h and diluted to 100 mL with THF. This solution was treated with 100 mL of aqueous 1 N HCl and stirred for 30 min at rt. The pH of the solution was adjusted to 9.5 by addition of saturated K_2CO_3, and the mixture was stirred for 2 h. The solution was neutralized to pH 7 with aqueous 1 N HCl, and the THF was removed under reduced pressure. The aqueous layer was extracted with EtOAc (3 × 80 mL), and the combined organic extracts were washed with brine (80 mL), dried, filtered, and concentrated. The crude product was recrystallized from EtOAc/hexanes to provide 0.70 g (70%) of tripeptide **4a** as a white solid, fully identical with the sample obtained above. Anal. Calcd for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C, 58.21; H, 7.24; N, 9.12.

(Bis-*N,N'*-(Cbz)-L-lysyl-D-*threo*-β-hydroxyaspartyl β-benzyl ester)-L-alanyl-D-*allo*-threonyl-L-alanine, *tert*-Butyl Ester (8). Tripeptide **4a** (0.90 g, 2.00 mmol) in 40 mL of MeOH was hydrogenolyzed over 10% Pd–C (0.18 g) at atmospheric pressure for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was dissolved in 10 mL of $CHCl_3$ and added to a solution of dipeptide **3** (1.27 g, 2.00 mmol) in 15 mL of $CHCl_3$. To the solution was then added EEDQ (0.54 g, 2.20 mmol). The resulting mixture was stirred for 20 h at rt. The reaction mixture was washed with 10% citric acid, 10% $NaHCO_3$, and saturated NaCl and then dried, filtered, and concentrated. The crude product was purified by column chromatography on silica gel with $CHCl_3$/EtOH (19:1) as eluent and recrystallized from MeOH/EtOAc to afford 1.50 g (80%) of pentapeptide **8** as a white solid: mp 173–175 °C; HPLC t_R 16 min (10% 2-propanol in hexanes); $[\alpha]^{22}_D$ +9 (c = 0.5, CH_3OH); ^{1}H NMR (CD_3OD) δ 1.18 (d, J = 6.5 Hz, 3H), 1.33 (d, J = 7.3 Hz, 3H), 1.37 (d, J = 7.3 Hz, 3H), 1.26–1.48 (m, 4H), 1.42 (s, 9H), 1.55 (m, 2H), 3.07 (m, 2H), 4.02 (m, 2H), 4.25 (d, J = 7.0 Hz, 1H), 4.35 (m, 2H), 4.80 (1H, obscured by H_2O peak), 4.92 (d, J = 2.3 Hz, 1H), 5.02–5.20 (m, 6H), 7.30 (m, 15H); ^{13}C NMR (CD_3OD) δ 17.4, 17.5, 19.5, 24.0, 28.2, 30.5, 31.8, 41.4, 50.4, 51.5, 57.0, 57.3, 59.7, 67.4, 67.7, 68.3, 68.7, 71.6, 82.8, 128.7, 128.8, 129.0, 129.3, 129.4, 129.5, 129.6, 136.9, 138.1, 138.4, 158.6, 158.9, 171.3, 171.9, 173.0, 173.4, 175.0, 175.7; IR (KBr) 3300 (br), 1730–1610 (br) cm^{-1}; HRMS (FAB) calcd for $C_{47}H_{63}N_6O_{14}$ (MH^+) 935.4402, found 935.4407. Anal. Calcd for $C_{47}H_{62}N_6O_{14}$: C, 60.37; H, 6.68; N, 8.99. Found: C, 60.69; H, 6.60; N, 9.17.

δ-*N*-(Benzyloxy)-D-cycloornithine·HBr (9). α-*N*-Cbz-δ-*N*-(benzyloxy)-D-cycloornithine (**5**)[3] (0.35 g, 1.00 mmol) was suspended in 8 mL of anhydrous CH_2Cl_2 under N_2 and treated with 8 mL of 33% HBr in acetic acid. The reaction mixture was stirred at rt for 30 min. The solvent was evaporated under reduced pressure. Chloroform was added, and the solvent was evaporated once more under reduced pressure. The salt **9** was then precipitated out of chloroform as a white solid (0.30 g, 99%) by addition of hexanes: ^{1}H NMR (300 MHz, D_2O) δ 1.90 (m, 2H), 2.10 (m, 1H), 2.28 (m, 1H), 3.60 (dd, J = 9.8, 4.0 Hz, 2H), 4.08 (dd, J = 11.4, 5.7 Hz, 1H), 5.00 (s, 2H), 7.00 (m, 5H); ^{13}C NMR (75 MHz, D_2O) δ 20.6, 25.5, 50.5, 51.2, 76.7, 129.7, 130.3, 130.9, 134.9, 165.6; HRMS (FAB, nitrobenzyl alcohol) calcd for $C_{12}H_{17}N_2O_2$ (MH^+) 221.1290, found 221.1729.

(Bis-*N,N'*-(Cbz)-L-lysyl-D-*threo*-β-hydroxyaspartyl β-benzyl ester)-L-alanyl-D-*allo*-threonyl-L-alanyl-δ-*N*-(benzyloxy)-D-cycloornithine (2a). (Bis-*N,N'*-(Cbz)-L-lysyl-D-*threo*-β-hydroxyaspartyl β-benzyl ester)-L-alanyl-D-*allo*-threonyl-L-alanine, *tert*-butyl ester (**8**, 0.47 g, 0.50 mmol) was suspended in 5 mL of anhydrous CH_2Cl_2 under N_2. The suspension was cooled to 0 °C, and 5 mL of TFA was added. The resulting solution was stirred at rt for 3 h. The solvent was evaporated under reduced pressure. Toluene (5 mL) was added to the residue and evaporated under reduced pressure to completely remove TFA. The residue was dissolved in 15 mL of $CHCl_3$. EEDQ (0.15 g, 0.55 mmol), Et_3N (0.06 g, 0.08 mL, 0.55 mmol), and δ-*N*-(benzoyl)-D-cycloornithine·HBr (**9**) (0.15 g, 0.50 mmol) were added, and the solution stirred at rt for 48 h. The solvent was evaporated under reduced pressure and the residue chromatographed on silica gel eluting with 7% EtOH in $CHCl_3$ to provide protected hexapeptide **2a** as white solid (0.32 g, 60%): mp 125–130 °C; HPLC t_R 8 min (10% 2-propanol in CH_2Cl_2); $[\alpha]^{22}_D$ −1.4 (c = 0.5 CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.20 (d, J = 6.5 Hz, 3H), 1.35 (d, J = 8.0 Hz, 3H), 1.37 (d, J = 7.5 Hz, 3H), 1.26–1.46 (m, 4H), 1.55 (q, J = 7 Hz, 2H), 1.68–1.97 (m, 4H), 3.06 (t, J = 7.0 Hz, 2H), 3.37 (m, 1H), 3.47 (td, J = 11.0 Hz, 5.0 Hz, 1H), 4.04 (m, 2H), 4.21 (d, J = 6.5 Hz, 1H), 4.35 (q, J = 7.0 Hz, 1H), 4.40 (m, 2H), 4.90 (m, 4H), 5.00–5.18 (m, 6H), 7.22–7.45 (m, 20H); ^{13}C NMR (75 MHz, CD_3OD) δ 17.2, 17.9, 20.1, 22.0, 24.0, 28.5, 30.5, 41.4, 50.7, 51.2, 51.6, 52.0, 56.9, 57.3, 60.4, 65.2, 67.3, 67.7, 68.3, 68.6, 71.7, 76.7, 127.9–130.6 (multiple overlapping peaks), 136.7, 136.9, 138.1, 138.4, 158.7, 158.9, 168.6, 171.2, 172.2, 173.0, 174.6, 174.9, 175.9; IR (KBr) 3300 (br), 1780–1630 (br) cm^{-1}; HRMS (FAB, nitrobenzyl alcohol) calcd for $C_{55}H_{69}N_8O_{15}$ (MH^+) 1081.4882, found 1081.4880. Anal. Calcd for $C_{55}H_{68}N_8O_5 \cdot 1/2H_2O$: C, 60.6; H, 6.38; N, 10.28. Found: C, 60.68; H, 6.24; N, 10.26.

L-Lysyl-D-*threo*-β-(hydroxyaspartyl)-L-alanyl-D-*allo*-threonyl-L-alanyl-δ-*N*-hydroxy-D-cycloornitine (2b). Bis-(*N,N'*-(Cbz)-L-lysyl-D-*threo*-β-hydroxyaspartyl β-benzyl ester)-L-alanyl-D-*allo*-threonyl-L-alanyl-δ-*N*-(benzyloxy)-D-cycloornithine (**2a**, 0.10 g, 0.09 mmol) in 20 mL of MeOH was hydrogenolyzed over 10% Pd–C (0.02 g) at atmospheric pressure for 3 h. The catalyst was removed by filtration and washed with H_2O. The filtrate was concentrated and lyophilized to give **2b** as a white fluffy solid (0.06 g, 100%): $[\alpha]^{22}_D$ +36 (c = 0.3, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.20 (d, J = 6.4 Hz, 3H), 1.26–1.46 (m, 2H), 1.39 (d, J = 6.8 Hz, 3H), 1.40 (d, J = 7.3 Hz, 3H), 1.75 (m, 5H), 1.95 (m, 3H), 3.02 (t, J = 7.0 Hz, 2H), 3.60 (m, 2H), 4.09 (quintet, J = 6.4 Hz, 1H), 4.14–4.22 (m, 5H), 4.23 (d, J = 2.5 Hz, 1H), 4.84 (d, J = 2.5 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 17.2, 17.5, 19.5, 20.8, 22.5, 27.2, 27.6, 33.3, 40.0, 50.7, 50.9, 51.0, 52.7, 54.5, 57.4, 59.9, 67.8, 72.9, 166.0, 172.3, 172.5, 175.2, 175.7, 175.8, 177.1; HRMS (FAB, nitrobenzyl alcohol) calcd for $C_{25}H_{45}N_8O_{11}$ (MH^+) 633.3208, found 633.3213.

Acknowledgment. We gratefully acknowledge the NIH for support of this research.

Supplementary Material Available: Copies of ^{1}H and ^{13}C NMR spectra of **2a**, **4b**, and **6** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO941692A