Asymmetric Synthesis of γ-D- and -L-Glutamyl-L-*meso*-diaminopimelic Acid Dipeptide

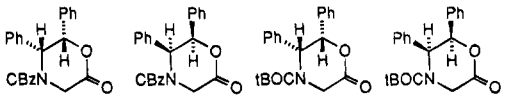
Robert M. Williams* and Chenguang Yuan

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received October 1, 1993®

A differentially protected form of *meso*-diaminopimelic acid suitable for peptide coupling is described. The synthesis of γ-D-glutamyl-L-*meso*-diaminopimelic acid dipeptide and γ-L-glutamyl-L-*meso*-diaminopimelic acid dipeptide has been achieved via this methodology.

In recent years, considerable attention has been focused on the peptidoglycan fragments of bacterial cell walls because of their unique immunostimulating activity.[1] Several recently isolated metabolites of peptidoglycans, FK-156 (**1**)[2,3] and a synthetic analog FK-565 (**2**),[4] revealed that these substances enhance host defense against microbial infections and exhibit strong antiviral activity[5] and remarkable antitumor potency.[6–12] It was found that FK-565 alone and in combination with zidovudine (AZT) inhibits retroviral infections by Friend leukemia virus in mice.[9] It is expected that FK-565 could be used not only in conjunction with cancer surgery and radiation therapy but it may also have potential for use in the treatment of human acquired immunodeficiency syndrome (AIDS).[12]



I *II* *III* *IV*

The unique biological activity of these compounds renders the synthesis of this class of peptides an attractive and worthy synthetic problem.[10] Very recently, Berner and associates reported the preparation of structurally modified peptidoglycans.[11] Kolodziejczyk and associates reported a synthesis of FK-156 and FK-565 by using selective enzymatic hydrolysis of the methyl ester group of the L-center of bis(benzyloxycarbonyl)-2,6-diaminopimelic dimethyl ester as a key step.[12] However, this approach is limited in accessing only *meso*-diaminopimelic acid (DAP)-containing peptides.

In this paper we report a new asymmetric and efficient synthesis of γ-D- and -L-glutamyl-L-*meso*-diaminopimelic acid peptides. The approach employed should allow the preparation of peptides containing all of the possible DAP stereoisomers in an unambiguous fashion. We have previously reported[13] on the utility of chiral, nonracemic diphenyloxazinones as versatile templates from which both electrophilic[14] and nucleophilic[15] C–C bond-forming strategies can be employed to access a variety of nonproteinogenic α-amino acids, including stereoisomers of DAP.[16] The key problem faced in preparing peptides of *meso*-DAP is the unambiguous and selective protection of the respective amino and carboxy termini suitable for standard peptide coupling chemistry. This problem has been solved in the present case by the remarkable, regioselective ring-opening of the differentially N-protected bis-lactone **4**. The synthesis of γ-D- and -L-glutamyl-L-*meso*-diaminopimelic acid peptides is detailed in Scheme I.

As previously reported,[16] the differentially N-protected DAP precursor **4** was prepared by employing selected diphenyloxazinones[17] carrying the requisite *N*-*t*-BOC and *N*-CBz protecting groups. The *N*-*t*-BOC group of **4** was removed with concomitant lactone ring-opening by treatment with concd hydrochloric acid in dioxane to give the regioselectively ring-opened amino acid; this substance was subsequently esterified with diazomethane furnishing **5** in 65% yield.

It is assumed that the secondary amino lactone (**11**, as the HCl salt) is the initially formed hydrolysis product; the protonated amino group of this lactone thus renders the proximal carbonyl moiety more reactive to hydrolytic ring-opening (giving **12**) than the partner lactone which still bears the *N*-CBz group (Scheme 2).

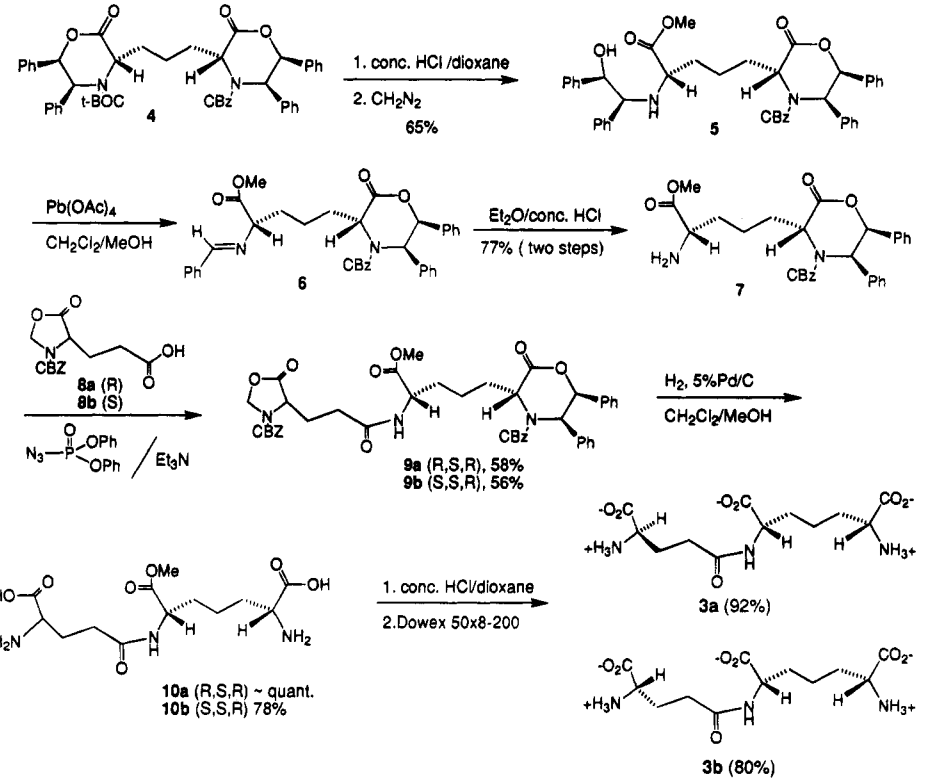
There was no detectable racemization of compound **5** as evidenced by inspection of the proton NMR. Amino alcohol **5** was treated with lead tetraacetate forming Schiff base **6**. Without purification, the Schiff base was

® Abstract published in *Advance ACS Abstracts*, September 15, 1994.

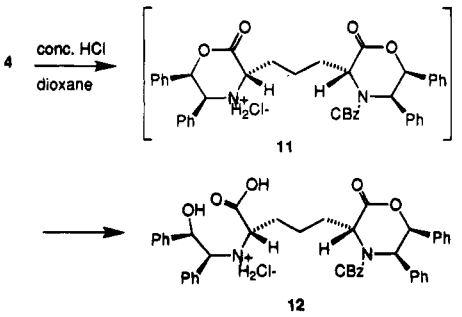
(1) Ledered, E. *J. Med. Chem.* **1980**, *23*, 819.
(2) Takeno, H.; Okada, S.; Hemmi, K.; Aratami, M.; Kitaura, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1984**, *32*, 2925.
(3) Kitama, Y.; Nakguchi, O.; Takeno, H.; Okada, S.; Yonishi, S.; Hemmi, K.; Mori, Y.; Hashimoto, M. *J. Med. Chem.* **1982**, *25*, 335.
(4) Mine, Y.; Yokota, Y.; Wakai, Y.; Fukuda, S.; Nishida, M.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1983**, *36*, 1045.
(5) Oku, T.; Imanishi, J.; Kishida, T. *Antiviral Res.* **1986**, *6*, 733–739.
(6) Li, W.; Kaplan, S.; Whiteside, T.; Herberman, R. H. *Immunopharmacology* **1989**, *18*, 213.
(7) Talmadge, J. E.; Lenz, B.; Schneider, M.; Philips, H.; Long, C. *Cancer Immunol. Immunother.* **1989**, *28*, 93.
(8) Inamura, N.; Nakahara, K.; Kino, T.; Gotoh, T.; Kawamura, I.; Aoki, H.; Imanaka, H.; Sone, S. *J. Biol. Response Modif.* **1985**, *41*, 408.
(9) Yokota, Y.; Wakai, Y.; Watanabe, Y.; Mine, Y. *J. Antibiot.* **1988**, *41*, 1479.
(10) Suskovic, B.; Vajtner, Z.; Naumski, R. *Tetrahedron* **1991**, *47*(39), 8407. Kitaura, Y.; Takeno, M.; Aratani, S.; Okada, S.; Yonishi, K.; Hemmi, O.; Nakaguchi, O.; Hashimoto, M. *Experentia* **1982**, *38*, 1101. Hemmi, K.; Hidekazu, T.; Okada, S.; Nakaguchi, O.; Kitaura, Y.; Hashimoto, M. *J. Am. Chem. Soc.* **1981**, *103*, 7026.
(11) Schneider, H.; Sigmund, G.; Schricker, B.; Thirring, K.; Berner, H. *J. Org. Chem.* **1993**, *58*, 683.
(12) Kolodziejczyk, A. M.; Kolodziejczyk, A. S.; Stoev, S. *Int. J. Peptide Progein Res.* **1992**, *39*, 382.
(13) (a) Williams, R. M. *Aldrichim. Acta* **1992**, *25*, 11. See also: (b) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon: Oxford, 1989.
(14) (a) Sinclair, P. J.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103. (b) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547. (c) Sinclair, P. J. Ph.D. Thesis, Colorado State University, 1987. (d) Williams, R. M.; Zhai, D.; Sinclair, P. J. *J. Org. Chem.* **1986**, *51*, 5021. (e) Williams, R. M.; Sinclair, P. J.; Zhai, W. *J. Am. Chem. Soc.* **1988**, *110*, 482. (f) Williams, R. M.; Zhai, W. *Tetrahedron* **1988**, *44*, 5425. (g) Zhai, D.; Zhai, W.; Williams, R. M. *J. Am. Chem. Soc.* **1988**, *110*, 2501. (h) Williams, R. M.; Hendrix, J. A. *J. Org. Chem.* **1990**, *55*, 3723.
(15) (a) Williams, R. M.; Im, M.-N. *Tetrahedron Lett.* **1989**, *29*, 6075. (b) Williams, R. M.; Im, M.-N.; Cao, J. *J. Am. Chem. Soc.* **1991**, *113*, 9276.
(16) (a) Williams, R. M.; Yuan, C. *J. Org. Chem.* **1992**, *57*, 6520. (b) Baldwin, J. E.; Lee, V.; Schofield, C. J. *Synlett* **1992**, 249.

0022-3263/94/1959-6190$04.50/0

Scheme 1



Scheme 2



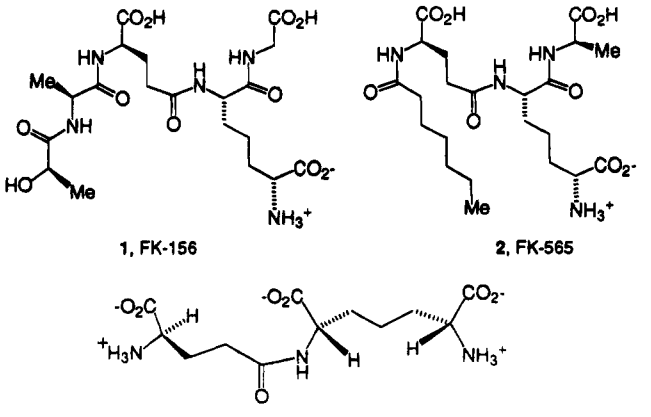
converted to the free amine (**78**, 77% from **5**) by treatment with concentrated hydrochloric acid in ether at ice bath temperature. Peptide coupling between the free amine of **7** and the protected D-glutamic acid derivative **8a** or L-glutamic acid derivative **8b** was accomplished using diphenyl phosphorazidate in DMF affording the desired peptides (**9a**, 58% yield; **9b**, 56% yield). The fully protected peptides were unmasked by simple catalytic hydrogenation to give the methyl esters; **10a** was obtained in essentially quantitative yield, and **10b** was obtained in 78% yield. Hydrolysis of the methyl ester could be accomplished by treatment of **10a** or **10b** with concentrated HCl in dioxane followed by ion-exchange chromatography affording the free dipeptides **3a** (92% yield) and **3b** (80% yield).

Since all stereochemical variations of differentially N-protected DAP precursors corresponding to **4** are readily accessible by the methods previously published,[16] strategies to access many different N-terminal and C-terminal DAP-containing peptides can be easily envisioned. Efforts to synthesize additional subunits of bacterial peptidoglycan using the approach reported herein are under study in these laboratories and will be reported on in due course.

Experimental Section

General Information. Visualization on TLC was achieved with ultraviolet light, I_2 developing chamber, and/or heating of TLC plates dipped in a 5% solution of phosphomolybdic acid in 95% ethanol. Preparative chromatography was performed by the following methods. Column chromatography was performed using Merck silica gel grade 60, 230–240 mesh, 60 Å. Radical chromatography was done on 1, 2, and 4 mm silica gel plates using E. Merck silica gel 60 PF-254 containing gypsum. Reagents and solvents were commercial grades and were used as supplied with the following exceptions. Tetrahydrofuran (THF) was freshly distilled from sodium benzophe-

(17) The requisite diphenyloxazinones utilized to prepare compound **4** and stereochemical variants (**i**–**iv**) are commercially available from Aldrich Chemical Co.: (**i**) catalog no. 33-185-6 (CAS registry no. 105228-46-4); (**ii**) catalog no. 33,187-2 (CAS registry no. 100516-54-9); (**iii**) catalog no. 33-181-3 (CAS registry no. 112741-50-1); (**iv**) catalog no. 33-184-8 (CAS registry no. 112741-49-8). Registry numbers are supplied by the author.



1, FK-156 2, FK-565

3, γ-D-glutamyl-*meso*-L-diaminopimelic acid

none ketyl. Dry methylene chloride and carbon tetrachloride were obtained by distillation over CaH_2. DMF and HMPA were dried over activated 4 Å molecular sieves. The amino acids furnished crude from the hydrogenation were always obtained in greater than the theoretical amount due to a certain fraction of HCl salt resulting from the $PdCl_2$ catalyst. TBAHS is tetrabutylammonium hydrogen sulfate (97%, Aldrich).

Methyl Ester 5. To a solution of the substrate **4** (prepared as previously described[16]) (1.558 g, 2.0 mmol, 1.0 equiv) in dioxane (100 mL) was added concd HCl (50 mL) at 0 °C. The resulting mixture was stirred for 3 days at room temperature and was concentrated and dried under reduced pressure overnight to yield a white, solid residue. The residue was treated with EtOAc (200 mL) and phosphate buffer (pH = 7). The organic phase was separated and washed with saturated NH_4Cl aqueous solution and dried over anhydrous $MgSO_4$. After filtration, the mixture was concentrated under reduced pressure to afford a white solid residue. The residue was treated with dry THF (50 mL) and CH_2Cl_2 (20 mL). To this solution was added a solution of diazomethane/ether (generated from MNNG), and the solution was stirred overnight at room temperature. The resulting mixture was concentrated to dryness and separated by column chromatography (silica gel, eluted with CH_2Cl_2:hexane:EtOAc, 5:4:1) to give 926 mg (65%) of **5** as a white solid.

^{1}H NMR (300 MHz, 393 K, DMSO-d_6 vs TMS) δ: 1.43−1.61 (4H, m); 2.05 (2H, m); 3.12 (1H, m); 3.45 (3H, s); 3.81 (1H, d, J = 5.53 Hz); 4.77 (1H, t, J = 7.44 Hz); 4.80 (1H, d, J = 5.52 Hz); 5.00 (2H, s); 5.27 (1H, d, J = 3.16 Hz); 6.15 (1H, d, J = 3.06 Hz); 6.59 (2H, d, J = 7.35 Hz); 7.03−7.26 (23H, m) ppm. IR (NaCl, CH_2Cl_2): 3498, 3031, 2949, 1755, 1703 cm^{-1}. $[\alpha]^{25}_D$: +8.1 (c = 1.0, CH_2Cl_2). Mp: 188−9 °C. Anal. Calcd for $C_{44}H_{44}N_2O_7$: C, 74.13; H, 6.22; N, 3.93. Found: C, 74.08, H, 6.38; N, 3.83.

Amino Methyl Ester 7. To a stirred solution of **5** (895 mg, 1.25 mmol, 1.0 equiv) in CH_2Cl_2/MeOH (1:1, 70 mL) was added $Pb(OAc)_4$ (583 mg, 1.32 mmol, 1.05 equiv) at 0 °C. The mixture was stirred for 5 min at 0 °C and was quenched with saturated aqueous sodium bicarbonate solution (30 mL). A yellow precipitate formed which was filtered off and the residue partitioned between CH_2Cl_2 (3 × 30 mL) and saturated NaCl solution (30 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, concentrated, and dried under reduced pressure to give an oily residue of Schiff base **6** that was used directly for the next step without further purification.

The crude product obtained above was treated with concd HCl/ether (20:30 mL) at 0 °C and was stirred at room temperature for 1 h. The organic phase was separated, and the aqueous phase was concentrated to dryness at room temperature to give a white solid residue that was treated with phosphate buffer (pH = 7) and extracted with CH_2Cl_2 (2 × 100 mL). The organic extract was washed with aqueous NaCl and dried over anhydrous $MgSO_4$, filtered, concentrated, and separated by column chromatography (silica gel, eluted with CH_2Cl_2:hexane:EtOAc, 5:4:1) to give 500 mg (77.4%) of product **7** as a colorless oil.

^{1}H NMR (300 MHz, 393 K, DMSO-d_6 vs TMS) δ: 1.62−1.74 (4H, m); 2.15 (2H, q, J = 6.66 Hz); 3.45 (1H, t, J = 8.76 Hz); 3.67 (3H, s); 4.82 (1H, t, J = 7.05 Hz); 5.00 (2H, d, J = 5.89 Hz); 5.28 (1H, d, J = 3.11 Hz); 6.20 (1H, d, J = 3.11 Hz); 6.60 (2H, d, J = 7.43 Hz); 7.08−7.30 (13H, m) ppm. IR (NaCl, CH_2Cl_2): 3382, 3063, 2950, 1754, 1704, 1641, 1603 cm^{-1}. $[\alpha]^{25}_D$: +4.2 (c = 1.0, CH_2Cl_2). HRMS: calcd for $C_{30}H_{32}N_2O_6$ 517.2339, found 517.2364.

Protected Dipeptide 9a. To a mixture of **7** (190 mg, 0.37 mmol, 1.0 equiv) and **8a** (216 mg, 0.74 mmol, 2.0 equiv) in DMF (5 mL) was added diphenyl phosphorazidate (203 mg, 0.74 mmol, 2.0 equiv) at 0 °C. Et_3N (156 μL, 1.11 mmol, 3 equiv) was then added to the reaction mixture. The mixture was stirred for 6 h at 0 °C. The resulting mixture was poured into water and extracted with CH_2Cl_2 (3 × 30 mL), washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered, concentrated, and separated by column chromatography (silica gel, eluted with CH_2Cl_2:EtOAc:MeOH, 5:1:0.2) to give 170 mg (58%) of **9a** as an oil.

^{1}H NMR (300 MHz, 393 K, DMSO-d_6 vs TMS) δ: 1.57 (2H, m); 1.80 (2H, m); 2.19 (6H, m); 3.66 (3H, s); 4.37 (2H, t, J = 6.11 Hz); 4.83 (1H, t, J = 7.26 Hz); 5.01 (2H, d, J = 4.29 Hz); 5.18 (2H, s); 5.27 (2H, dd, J = 4.29, 3.12 Hz); 5.47 (1H, d, J = 4.17 Hz); 6.19 (1H, d, J = 3.07 Hz); 6.60 (2H, d, J = 7.23 Hz); 7.05−7.73 (18H, m); 7.73 (1H, d, J = 7.00 Hz) ppm. IR (NaCl, CH_2Cl_2): 3356, 3063, 2952, 1799, 1749, 1705 cm^{-1}. $[\alpha]^{25}_D$: +70.0 (c = 0.55, CH_2Cl_2). HRMS: calcd for $C_{44}H_{45}N_3O_{11}$ (M + H^+) 792.3132, found 792.3148. Anal. Calcd for $C_{44}H_{45}N_3O_{11}$: C, 66.74; H, 5.73; N, 5.31. Found: C, 66.60; H, 6.00; N, 5.14.

Protected Dipeptide 9b. The same procedure was used as that described above for **9a**: from **7** (300 mg, 0.58 mmol, 1.0 equiv) and **8b** (341 mg, 1.16 mmol, 2.0 equiv) in DMF (5 mL), diphenyl phosphorazidate (319 mg, 1.46 mmol, 2.0 equiv), and Et_3N (220 μL, 1.74 mmol, 3 equiv) was obtained 256 mg (56%) of **9b** as an oil.

^{1}H NMR (300 MHz, 393 K, DMSO-d_6 vs TMS) δ: 1.57 (2H, m); 1.79 (2H, m); 2.08−2.47 (6H, m); 3.65 (3H, s); 4.37 (2H, t, J = 5.92 Hz); 4.83 (1H, t, J = 7.21 Hz); 5.00 (2H, d, J = 4.39 Hz); 5.25 (1H, s); 5.26 (2H, dd, J = 0.67, 0.76 Hz); 5.46 (1H, d, J = 4.27 Hz); 6.19 (1H, d, J = 3.08 Hz); 6.59 (2H, d, J = 7.25 Hz); 7.04−7.40 (18H, m); 7.80 (1H, J = 7.00 Hz) ppm. IR (NaCl, CH_2Cl_2): 3355, 3064, 2952, 1799, 1749, 1706, 1674 cm^{-1}. $[\alpha]^{25}_D$: +58.5 (c = 1.6, CH_2Cl_2). HRMS; calcd for $C_{44}H_{45}N_3O_{11}$ 792.3132, found 792.3139.

γ-D-Glutamyl-L-*meso*-diaminopimelic Acid Dipeptide Methyl Ester 10a. A mixture of substrate **9a** (83 mg, 0.105 mmol) and 86 mg of 5% Pd/C in CH_2Cl_2/MeOH (1:1, 6 mL) was stirred under 60 psi H_2 for 24 h at room temperature. After the catalyst was filtered off, the clear solution was concentrated under reduced pressure to afford a crude residue. The residue was exhaustively triturated with hexane to remove the bibenzyl yielding 35 mg (100%) of **10a** as a white, amorphous solid.

^{1}H NMR (300 MHz, D_2O vs DOH) δ: 1.30 (2H, m); 1.71 (4H, m); 1.97 (2H, m); 2.32 (2H, m); 3.40 (1H, m); 3.58 (1H, m); 3.62 (3H, s); 4.27 (1H, m) ppm. IR (NaCl, CH_2Cl_2): 3380, 3246, 2954, 1730, 1638 cm^{-1}. $[\alpha]^{25}_D$: −13.9 (c = 1.6, MeOH). HRMS: calcd for $C_{13}H_{23}N_3O_7$ (M^+) 334.1614, found 334.1614.

γ-L-Glutamyl-L-*meso*-diaminopimelic Acid Dipeptide Methyl Ester 10b. The same procedure was used as that described above for **10a**: from **9b** (73 mg, 0.092 mmol) and 70 mg of 5% Pd/C in CH_2Cl_2/MeOH (1:1, 6 mL) was obtained 24 mg (78%) of **10b** as a white, amorphous solid.

^{1}H NMR (300 MHz, D_2O vs HOD) δ: 1.18 (2H, m); 1.38−1.71 (4H, m); 1.90 (2H, m); 3.41 (3H, s); 3.54 (1H, m); 3.77 (1H, m); 4.07 (1H, m) ppm. IR (NaCl, CH_2Cl_2): 3575, 3324, 2918, 1740, 1724, 1703, 1625 cm^{-1}. $[\alpha]^{25}_D$: −11.7 (c = 1.2, MeOH). HRMS: calcd for $C_{13}H_{23}N_3O_7$ 334.1614, found 334.1614.

γ-D-Glutamyl-L-*meso*-diaminopimelic Acid Dipeptide 3a. To the suspension of **10a** (17 mg, 0.051 mmol) in dioxane (3 mL) was added concd HCl (1 mL) at room temperature. The mixture was stirred for 2 h at 80 °C. The residue was dissolved in MeOH and heated to reflux. To this reflux solution was added propylene oxide (0.1 mL), and the solution was stirred for 20 min at reflux. The resulting mixture was concentrated in *vacuo* to give an oily residue. The residue was purified on Dowex 50x8−200 (eluted with 1.5% aqueous NH_4OH solution) to yield 15 mg (92%) of product **3a** as a semisolid.

^{1}H NMR (300 MHz, D_2O vs HOD) δ: 1.23 (2H, m); 1.49−2.08 (6H, m); 2.27 (2H, m); 3.37 (1H, m); 3.59 (1H, m); 3.96 (1H, m) ppm. IR (NaCl, CH_2Cl_2): 3342 (br), 2941, 1638 cm^{-1}. $[\alpha]^{25}_D$: +8.4 (c = 0.5, MeOH).

γ-L-Glutamyl-L-*meso*-diaminopimelic Acid Dipeptide 3b. The same procedure was used as that described above for **3a**: from **10b** (16 mg, 0.048 mmol) in dioxane (3 mL) and concd HCl (1 mL) was obtained 12 mg (80%) of product **3b** as a semisolid.

^{1}H NMR (300 MHz, D_2O vs HOD) δ: 1.19 (2H, m); 1.40−1.72 (4H, m); 1.91 (2H, m); 2.23 (2H, q, J = 7.76 Hz); 3.64

(2H, m); 3.73 (1H, m); 4.02 (1H, m) ppm. IR (NaCl, CH_2Cl_2): 3412, 3252, 3045, 2945, 1723, 1633 cm^{-1}. $[\alpha]^{25}_D$: −6.4 (c = 0.5, H_2O).

Acknowledgment. We are indebted to the National Science Foundation (CHE 8717017), the National Institutes of Health (GM 40988), The Herman Frasch Foundation, and Hoffman-La Roche, Inc., for providing financial support.

Supplementary Material Available: ^1H NMR spectra of compounds **7**, **9a**, **9b**, **10a**, **10b**, **3a**, and **3b** (7 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.