5.84 g of a yellow oil. Passage through 220 g of silica gel 60 with ether/petroleum ether (1:10) as eluent afforded 2.16 of oily **1a** and 1.90 g of oily **1b**, both of which solidified upon standing. Recrystallization of crude **1a** from 20 mL of ether and 30 mL of petroleum ether afforded 1.82 g of pure **1a**: mp 61–62 °C; NMR ($CDCl_3$) δ 0.96 (t, 3 H, CH_3CH_2), 4.02 (q, 2 H, CH_3CH_2), 5.11 (s, 2 H, CH_2), 7.00 (s, 1 H, NH), 7.20 (s, 5 H, Phe aromatic), 7.30 (s, 5 H, aromatic), 7.57 (s, 1 H, vinyl proton); IR (KBr) 3340 (NH), 1720 (urethane and ester carbonyls), 1655 (C=C), 1530 (amide II), 1250 and 1230 cm^{-1} (CO). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.88; N, 4.30. Found: C, 70.16; H, 5.90; N, 4.30. From the mother liquor was obtained an additional 0.11 g for a combined yield of 26%. Recrystallization of crude **1b** from 20 mL of ether and 30 mL of petroleum ether afforded 1.39 g of pure **1b**: mp 58–59 °C;[16] NMR ($CDCl_3$) δ 1.32 (t, 3 H, CH_3CH_2), 4.32 (q, 2 H, CH_3CH_2), 5.17 (s, 2 H, CH_2), 6.41 (s, 1 H, NH), 7.42–7.73 (m, 11 H, Ar H and CH=C); IR (KBr) 3310 (NH), 1730 (urethane C=O), 1700 (ester C=O), 1650 (C=C), 1500 (amide II), 1280 and 1230 cm^{-1} (CO). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.88; N, 4.30. Found: C, 70.08; H, 5.89; N, 4.32. From the mother liquor was obtained an additional 0.39 g for a combined yield of 24.1%.

(*Z*)-*N*-Me-Δ^E-PheOEt (7a). To a solution of 200 mg (0.615 mmol) of **1a** dissolved in 2 mL of dry DMF were added 0.20 mL (5.2 equiv) of methyl iodide and 292 mg of anhydrous K_2CO_3. The reaction mixture was stirred for 24 h, diluted with 20 mL of dry $CHCl_3$, filtered, and concentrated in vacuo to a yellow oil. Elution through 30 g of silica gel 60 with ether/petroleum ether (1:10) as eluent afforded 122 mg (61%) of **6a** as a colorless oil: NMR ($CDCl_3$) δ 0.93 (br t, 3 H, CH_3CH_2), 3.30 (s, 3 H, CH_3), 3.91 (br, q, 2 H, CH_3CH_2), 5.12 (s, 2 H, CH_2), 6.68 (s, 1 H, vinyl proton), 7.24 and 7.30 (2 s, 10 H, Ar H); IR (NaCl plates) 1730 (urethane C=O), 1715 (ester C=O), 1645 (C=C), 1450 (NCH_3), 1220 and 1160 cm^{-1} (CO). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.68; H, 6.29; N, 4.06.

(*Z*)-*N*-Me-Δ^Z-PheOEt (7b). By use of the procedure given for **7a**, the crude oily product was crystallized from 20 mL of ether/petroleum ether (1:1) to afford 154 mg (74%) of **7b**: mp 78–79 °C; NMR ($CDCl_3$) δ 1.67 (t, 3 H, CH_3CH_2), 3.02 (s, 3 H, NCH_3), 4.13 (q, 2 H, CH_3CH_2), 5.07 (s, 2 H, CH_2), 7.17 (s) and 7.3 (m, 11 H, Ar H and vinyl proton); IR (KBr) 1720 (urethane C=O), 1710 (ester C=O), 1645 (C=C), 1450 (NMe), 1275 and 1205 cm^{-1} (CO). An analytical sample was recrystallized from diethyl ether and petroleum ether; mp 77–78 °C. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.59; H, 6.31; N, 4.07.

(*Z*)-Δ^Z-PheOH (6b). Method A. To 528 g (1.62 mmol) of **1b** dissolved in 5 mL of methanol was added 3.0 mL of 4 N NaOH, and the reaction mixture was stirred 1 h. The solvent was removed in vacuo, and the residue was dissolved in 15 mL of water and extracted (2 × 10 mL) with diethyl ether. The aqueous solution was acidified to pH 3 (pH paper) with solid citric acid and extracted (3 × 20 mL) with ethyl acetate, and the organic phase was dried with anhydrous $MgSO_4$. The solvent was removed in vacuo to afford a white solid which was recrystallized from 50 mL of ethyl acetate/petroleum ether (2:3) to afford 338 mg of pure **6b**: mp 157–159 °C;[17] NMR ($CDCl_3$) δ 5.07 (s, 2 H, CH_2), 6.53 (s, 1 H, NH), 7.10–7.60 (m, 11 H, Ar H and vinyl proton), 9.70 (s, 1 H, CO_2H); IR (KBr) 3290 (NH), 2940 (OH), 1690 (acid and urethane C=O), 1650 (C=C), 1510 (amide II), 1250 cm^{-1} (CO). Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.08; N, 4.71. Found: C, 68.76; H, 5.13; N, 4.68. From the mother liquor was obtained an additional 79.0 mg for a combined yield of 86.5%.

Method B. Hydrolysis of 1b with 3.25 Equiv of NaOH. To 200 mg (0.615 mmol) of **1b** dissolved in 2.0 mL of methanol was added 0.50 mL of 4 N NaOH. The reaction mixture was stirred for 1.5 h and the methanol removed in vacuo. The gellike residue was dissolved in 20 mL of water and extracted twice with 15-mL portions of ether. The aqueous layer was acidified to pH 2 (pH paper) with solid citric acid and extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried (anhydrous $MgSO_4$) and evaporated to dryness in vacuo. The colorless oil obtained (170 mg) solidified upon standing. Recrystallization from 4 mL of ethyl acetate and 4 mL of hexanes afforded 74.5 mg of pure **7b**, mp 157–158 °C. An additional 44.2 mg was obtained from the mother liquor for a total yield of 65%. Recrystallization from benzene–cyclohexane gave a melting point of 155.5–156.5 °C. This product was identical (IR, NMR, TLC) with that obtained by method A.

Method C. Hydrolysis of 1a with 3.25 Equiv of NaOH. Compound **1a** (200 mg, 0.615 mmol) was treated as described above. The crude colorless oil obtained was purified by preparative TLC using Whatman PK6F plates with chloroform–methanol–acetic acid (25:5:1) as eluent. Recrystallization from benzene–cyclohexane afforded 80.2 mg of pure **7b**, mp 154–155 °C. An additional 25.6 mg was obtained from the mother liquor for a total yield of 58%. This product was identical (IR, NMR, TLC) with that obtained by method A, and a mixture melting point was not depressed.

Acknowledgment. We are grateful to Dr. Gerald Morton and the National Science Foundation Regional NMR Facility at the University of South Carolina, Columbia, SC, for ^{13}C spectroscopy on **1a** and **1b**.

Registry No. **1a**, 50685-12-6; **1b**, 50685-13-7; **2**, 5292-53-5; **3**, 24302-10-1; **4**, 77416-48-9; **5a**, 77416-49-0; **5b**, 52157-07-0; **6b**, 72015-61-3; **7a**, 77416-50-3; **7b**, 77416-51-4; DMAP, 1122-58-3; Dabco, 280-57-9; TEA, 121-44-8; DIPEA, 7087-68-5; DBU, 6674-22-2; NMM, 109-02-4; Pyr, 110-86-1.

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Synthesis of Dehydrothyroliberin, Δ^ZPhe^2-TRF

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The first use of the N-chlorination–dehydrochlorination method in the synthesis of a dehydrodipeptide is reported in the preparation of the title compound.

Due to the biological importance of thyroliberin (TRF, 1) numerous analogues have been synthesized and their bioactivities examined. Of particular interest among these is the Phe^2 analogue (2) which has about 10% the potency

pGlu-His-ProNH$_2$ (1) pGlu-Phe-ProNH$_2$ (2)

pGlu-Δ^2Phe-ProNH$_2$ (3)

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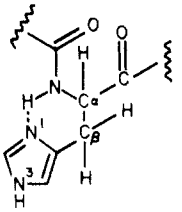
of TRF and, significantly, was inhibited by triiodothyronine (T_3) and serum, characteristics of the natural hormone not found in most other analogues.[2] Since we had previously synthesized several dehydrophenylalanine (ΔPhe) peptides,[3] it seemed attractive to prepare the Δ^ZPhe[2] analogue (3) of TRF, especially in view of earlier work on the active conformation of TRF. In 1972, Vale reported[4] that the N^{im3}-Me-His[2] analogue of TRF had eight times the potency of TRF while the N^{im1}-Me-His derivative showed only a trace of activity. These facts support a tertiary structure in which N^1 of the imidazole ring is hydrogen bonded to the amide NH of the histidine moiety, causing the imidazole ring to appear approximately coplanar to the N^α-C_α-C_β plane (4). Later workers however, questioned[5a,b] these results and postulated an "extended" conformation for TRF and its N^{im1}-Me-His[2] analogue[5c] based on careful nuclear magnetic resonance studies. Since the thermodynamically stable conformation of a ΔPhe residue is that in which the phenyl ring and N^α are cis oriented (*Z* configuration, 5) we felt that 3 might show interesting bioactivity and possibly shed light on the question of the bioactive conformation of TRF.



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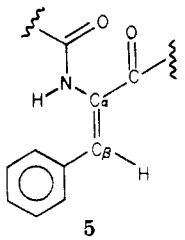

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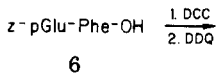
The two most widely used approaches to dehydropeptide synthesis entail either direct introduction of the unsaturated site into a dipeptide derivative or synthesis of a dehydroamino acid followed by coupling into the peptide chain. Since ΔPhe is particularly difficult to couple,[6] we first attempted to prepare the dehydroazlactone (7) using



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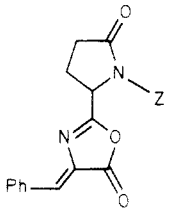

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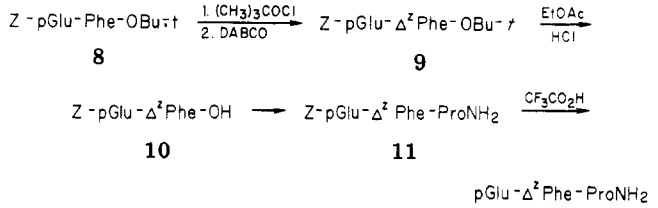
our quinone dehydrogenation method[7] on the corresponding saturated azlactone prepared by treatment of the dipeptide 6 with dicyclohexylcarbodiimide. After a difficult purification, 7 was obtained in only ~15% yield. The Bergmann reaction,[8] in which Z-pGlu-DL-Phe (β-OH)-OH is treated with an acetic anhydride–sodium acetate mixture, gave a mixture of products which we were unable to separate.

Since dipeptides containing Z-pGlu have only one amide NH, it seemed feasible to try the N-chlorination–dehydrochlorination sequence reported[9] in 1975 as useful in the synthesis of dehydro amino acids. Accordingly, the *tert*-butyl ester (8) was N-chlorinated and dehydrochlorinated to form the dehydrodipeptide in 28% yield. Small amounts of starting peptide (8) and a presumed α-methoxy compound (δ 3.66, 3 H) accompanied the dehydro compound (9) in the reaction mixture. Even though N-chlorination was complete (TLC), the starting material (8) was always present after treatment with base. The *tert*-butyl ester function was removed in 90% yield and the resulting acid 10 was obtained as a powder which held recrystallization solvents vehemently. This compound was



coupled with proline amide to form 11 in 44% yield. The final product, Δ^ZPhe[2]-TRF (3), was obtained as a white powder after gel filtration and partition chromatography in 89% yield by decarbobenzoxylation with trifluoroacetic acid. It was pure as shown by thin-layer chromatography in five solvent systems and showed an $R_{Lys} = 0.28$ on paper electrophoresis. The compound was completely stable to α-chymotrypsin at an enzyme–substrate ratio of 1:100 in tris buffer, 38 °C. Bioassays of 3 will be reported elsewhere.

Experimental Section

Materials and Instrumentation. L-Glutamic acid, L-phenylalanine, and L-prolineamide hydrochloride were purchased from Sigma Chemical Co. and used without further purification. Tetrahydrofuran was distilled over potassium and stored over sodium. All other solvents were reagent grade and were used without purification unless specified.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were measured with a Perkin-Elmer Model 297 recording spectrophotometer with polystyrene as standard. Proton NMR spectra were recorded on either a Varian T-60 or Varian EM-390 NMR spectrometer with Me_4Si as internal reference. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter and amino acid analyses were obtained on a Beckman 119-C amino acid analyzer. Elemental analyses were provided by Atlantic Microlab, Atlanta, GA.

Thin-layer chromatography was performed on Whatman precoated silica gel plates, using the following solvent systems: (I) *n*-BuOH–AcOH–H_2O–EtOAc (1:1:1:1); (II) $CHCl_3$–MeOH–AcOH (50:10:1); (III) $CHCl_3$–MeOH–AcOH (95:5:1); (IV) $CHCl_3$–MeOH (5:1); (V) *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2); (VI) *n*-BuOH–AcOH–H_2O (7:1:2). Thin-layer plates were visualized by using UV light, 1% ninhydrin/acetone v/v, I_2 vapor, and 10% H_2SO_4.

N-pGlu-Phe-O-*t*-Bu (8). A solution of 8.0 g (304 mmol) of N-Z-pGlu[10] in 100 mL of dry THF was cooled in a dry ice/2-propanol bath to −20 to −30 °C. The cold solution was treated with 3.36 mL (304 mmol) of *N*-methylmorpholine and 3.96 mL (304 mmol) of isobutyl chloroformate. After being stirred at −20 to −30 °C for 30 min, the reaction mixture was treated with 8.6 g (336 mmol) of Phe-OBu-*t*·HCl[11] which had been neutralized with an equivalent amount of *N*-methylmorpholine in a mixture of

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dioxane/water. After the mixture was stirred at room temperature for 23 h, the solvents were removed under vacuum. The amorphous white solid residue was dissolved in $CHCl_3$, washed twice with 5% citric acid, washed with water, and dried over anhydrous $MgSO_4$. The $MgSO_4$ was filtered and the solvent was removed under vacuum to give 13.62 g (96%) of **8** as a white amorphous solid. Two crystallizations from $CHCl_3$/petroleum ether afforded 8.32 g (58.7%) of **8** as a white crystalline solid: mp, decomposes slowly above 134 °C; $[\alpha]_D^{25}$ +22.78° (*c* 1.1, $CHCl_3$); R_f (IV) 0.69 (UV and I_2), R_f (I) 0.82 (UV and I_2); IR (KBr) 3310 (NH) 3025 and 3010 (aromatic CH), 1760–1700 (Cbz, lactam, ester C=O's), 1650 (amide I); NMR ($CDCl_3$) δ 7.4 (s, 5 H, aromatic), 7.24 (s, 5 H, aromatic), 6.5 (m, 1 H, NH), 5.3 (s, 2 H, $PhCH_2O$), 4.7 (m, 2 H, α-CH_2), 3.05 (d, 2 H, β-CH_2), 2.36 (m, 4 H, CH_2CH_2), 1.4 (s, 9 H, *tert*-butyl ester).

Anal. Calcd for $C_{26}H_{30}O_6N_2$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.89; H, 6.48; N, 6.00.

N-Z-pGlu-ΔᶻPhe-O-*t*-Bu (9). A solution of 4.0 g (8.6 mmol) of **8** in 30 mL of MeOH was cooled to 10 °C and treated with 2.06 mL (17 mmol) of freshly prepared *tert*-butylhypochlorite[12] and 0.33 g (0.86 mmol) of $Na_3B_4O_7{\cdot}10H_2O$ in the absence of direct light. After the mixture stood in a refrigerator at 10 °C in the dark for 15 h, TLC in ether of the reaction mixture showed that no starting material remained. The solution was concentrated to an oil under vacuum in the dark and the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed quickly with saturated NaCl solution and dried over anhydrous $MgSO_4$ in an ice bath. The cold CH_2Cl_2 solution was filtered directly into a stirred CH_2Cl_2 solution of 0.96 g (8.6 mmol) of DABCO (1,4-diazabicyclo[2.2.2]octane). The resulting solution was stirred rapidly at room temperature for 10 min and then heated to reflux for 10 min. Up to this point the procedure was carried out as much as possible in the dark. The reaction mixture was washed twice with 5% citric acid, washed with saturated NaCl solution and dried over anhydrous $MgSO_4$. Removal of the solvent under vacuum afforded 3.7 g (92.7%) of a pale yellow amorphous solid. TLC of the material in Et_2O showed it to be a mixture of four components among which were **8**, **9**, and an α-methoxyphenylalanine derivative (δ 3.66, s, 3 H). The compounds were separated on a 2.2 × 40 cm column of 230–400-mesh silica gel. The column was eluted with ether, using a flow rate of 0.6 mL/min.; 18-mL fractions were collected. Fractions 31–40 were combined and concentrated under vacuum. Crystallization of the residue from ether afforded 1.13 g (28%) of **9** as small, white, needle-like crystals: mp 118–120 °C; $[\alpha]_D^{25}$ +46.6° (*c* 1.0, $CHCl_3$); R_f (Et_2O) 0.30 (UV and I_2), R_f (II) 0.71 (UV and I_2), R_f (III) 0.48; IR (KBr) 3300 (NH), 3025 and 3010 (aromatic CH), 2980 and 2910 (aliphatic CH), 1785 (ester C=O), 1750 (Cbz C=O), 1720 (lactam C=O), 1670 (amide C=O); NMR ($CDCl_3$) δ 7.42 (br s, 12 H, aromatic, vinyl, NH), 5.46 (s, 2 H, $PhCH_2O$), 4.73 (m, 1 H, α-CH), 2.40 (m, 4 H, CH_2CH_2-pGlu), 2.54 (s, 9 H, *tert*-butyl ester); NMR (TFA) δ 8.12 (s, 1 H, vinyl), 7.60, 7.50 (2 overlapping s, 10 H, aromatic), 6.70 (br d, 1 H, NH), 5.53 (s, 2 H, $PhCH_2$), 4.93 (br, 1 H, α-CH), 2.73 (br, 4 H, CH_2CH_2), 2.66 (5, 9 H, *tert*-butyl ester); UV (CH_3OH) λ_{max} 279 (ϵ_{max} 18 400).

Anal. Calcd for $C_{26}H_{28}O_6N_2$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.26; H, 6.08; N, 6.04.

N-Z-pGluΔᶻPheOH (10). To 1.8 g (3.88 mmol) of **9** was added 58.3 mL of 2.66 M HCl/EtOAc at room temperature. The reaction was followed by TLC in system II. After the mixture stood at room temperature for 2.5 h, the EtOAc was removed under vacuum. The gelatinous residue was suspended in EtOAc and crushed and concentrated under vacuum twice to remove HCl. The final residue was suspended in EtOAc and ether and petroleum ether added. The residue was crushed to obtain a powder and the suspension allowed to stand at ca. 0 °C overnight. The product was isolated by filtration to give 1.59 g (100%) of **10** as a white powder. Recrystallization from MeOH/Et_2O afforded 1.42 g (89.9%) of **10** as a white powder: mp 198–201 °C dec.; $[\alpha]_D^{25}$ +215.8° (*c* 0.53, MeOH); R_f (II) 0.29 (UV and I_2), R_f (I) 0.72 (UV and I_2), R_f (VI) 0.62; NMR (Me_2SO-d_6) δ 9.83 (s, 1 H, OH), 7.56 (2 overlapping s, 2 H, vinyl, NH), 7.30 (s, 10 H, aromatic), 5.20 (d, 2 H, $PhCH_2$), 4.73 (br, 1 H, α-CH), 2.6–1.8 (br, 4 H, CH_2CH_2).

N-Z-pGluΔᶻPhe-ProNH₂ (11). A solution of 500 mg (1.2 mmol) of **10** in 15 mL of dry THF was cooled to −5 to 0 °C with a NaCl/ice bath. The cold solution was treated with 0.17 mL (1.2 mmol) of triethylamine and 0.16 mL (1.2 mmol) of isobutyl chloroformate. The resulting suspension was stirred magnetically at −5 to 0 °C for 30 min. The reaction mixture was then treated with 235 mg (1.56 mmol) of Pro-NH_2·HCl, which had been neutralized in 5 mL of $CHCl_3$ with 0.218 mL of triethylamine. The reaction mixture was then allowed to warm to room temperature gradually. After the mixture was stirred at room temperature for 14 h, the solvents were removed under vacuum. The residue was dissolved in $CHCl_3$, extracted with saturated NaCl, 1 M HCl, 5% $NaHCO_3$, and saturated NaCl, and dried over anhydrous $MgSO_4$. Filtration of the drying agent and evaporation of the solvent afforded an oily residue. The residue was dissolved in $CHCl_3$ and Et_2O and petroleum ether added to give 480 mg (79.4%) of **11** as a white powder. TLC showed this material to contain a small amount of impurity. Precipitation of the impure compound from $CHCl_3$ with Et_2O yielded 265.6 mg (44%) of pure **11** as a white powder: mp 119 °C dec; $[\alpha]_D^{25}$ −15.9° (*c* 0.56, $CHCl_3$), $[\alpha]_D^{25}$ +257.8° (*c* 0.55, MeOH); R_f (I) 0.62 (UV and 10% H_2SO_4), R_f (II) 0.5 (UV and 10% H_2SO_4), R_f (III) 0.14 (UV and 10% H_2SO_4); NMR ($CDCl_3$) δ 9.25 (s, 1 H, ΔPheNH), 7.33 (s, 10 H, aromatic), 6.93 (s, 1 H, $CONH_2$), 6.06 (s, 1 H, vinyl), 5.43 (s, 1 H, $CONH_2$), 5.16 (s, 2 H, $PhCH_2O$), 4.73 (br, 1 H, α-CH), 4.40 (br, 1 H, α-CH), 3.63 (br t, 2 H, δCH_2Pro), 2.66–1.70 (very br, 8 H, CH_2CH_2pGlu, β,γ-CH_2CH_2-Pro).

Anal. Calcd for $C_{27}H_{28}N_4O_6{\cdot}H_2O$: C, 62.06; H, 5.79; N, 10.72. Found: C, 62.14; H, 5.78; N, 10.75.

pGluΔᶻPhe-Pro-NH₂ (3). To 400 mg (0.79 mmol) of **11** was added 16 mL of trifluoroacetic acid (TFA) at room temperature. After the reaction mixture was allowed to stand at room temperature for 29 h, the TFA was removed under vacuum. The residue was dissolved in CH_2Cl_2 and concentrated to an oil under vacuum, the procedure being repeated three times. The final oil was dried under vacuum over KOH. The oil was dissolved in $CHCl_3$ and Et_2O was added, yielding 335 mg of **3** as a white powder; TLC in $CHCl_3$–MeOH–AcOH (50:10:1) showed three minor impurities. Partition chromatography on Sephadex G-10, using *n*-BuOH–AcOH–H_2O (4:1:5) upper phase as eluent, afforded pure **3** in fractions 88–108 (R_f 0.51). The fractions were combined and the solvent was removed under vacuum. The residue was dissolved in 20 mL of H_2O and lyophilized to yield 262 mg (89.5%) of **3** as a white powder: mp 130 °C dec; $[\alpha]_D^{25}$ +67.6° (*c* 0.25, MeOH); R_f (II) 0.20 (UV and I_2), R_f (I) 0.58 (UV and I_2), R_f (VI) 0.62 (UV and I_2), R_f (VII) 0.25 (UV and I_2), R_f (V) 0.32 (UV and I_2); paper electrophoresis [buffer HCOOH–CH_3COOH–CH_3OH–H_2O (1:3:10 v/v) pH 1.9] 500 V, 8 mA, 2 h, Whatman 3MM chromatography paper, visualized with Cl_2/*o*-tolidine, one spot, R_{lys} = 0.28; NMR ($CDCl_3$) δ 10.36 (s, 1 H, ΔPheNH), 8.2 (br s, 1 H, NH), 7.46 (s, 5 H, aromatic), 6.7 (br s, 1 H, $CONH_2$), 6.16 (br s, 2 H, vinyl, $CONH_2$) 4.4 (m, 2 H, αCHpGlu, α-CHPro), 3.8 (m, 2 H, δCH_2Pro), 2.16 (very br m, 8 H, CH_2CH_2pGlu, β,γ-CH_2CH_2Pro); amino acid analysis Glu-OH–Pro-OH (1.00:1.05).

Anal. Calcd for $C_{19}H_{22}O_4N_4{\cdot}0.5AcOH$: C, 59.99; H, 6.34; N, 13.99. Found: C, 59.62; H, 6.54; N, 13.92.

Description of Enzyme Test. A 1 mM solution of pGluΔᶻPheProNH₂ (**3**) in 0.05 M tris buffer was treated with 0.01 μmol of α-chymotrypsin. Both tris and α-chymostrypsin were purchased from Sigma Chemical Co. The solution was maintained at 38 °C in a constant-temperature bath and checked periodically by thin-layer chromatography. TLC in *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2) and in *n*-BuOH–AcOH–H_2O–EtOAc (1:1:1:1) showed no ninhydrin positive spots with R_f values corresponding to $ProNH_2$ or UV-positive spots other than **3** after 30 h at 38 °C.

Registry No. **3**, 77400-59-0; **8**, 77400-60-3; **9**, 77400-61-4; **10**, 77400-62-5; **11**, 77400-63-6; *N*-Z-pGlu, 32159-21-0; Phe-OBu-*t*·HCl, 15100-75-1; Pro-NH_2·HCl, 42429-27-6.

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