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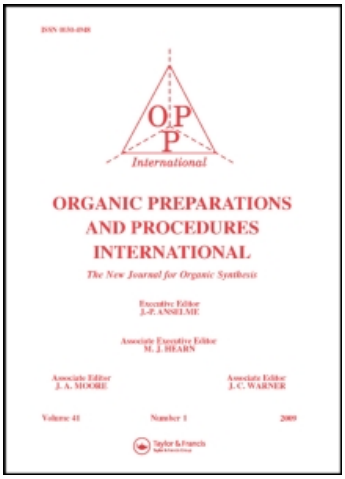

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SYNTHESIS OF PSEUDOPEPTIDES

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SYNTHESIS OF PSEUDOPEPTIDES

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Variation of one or two amino acids within a polypeptide chain frequently leads to significantly different biological properties for that polypeptide.[1] The changes which have been introduced include replacement with residues of differing hydrophobicity, substitution of D for L-amino acids, and substitution of positions in the native molecule which might be expected to undergo proteolysis with residues that are resistant to such cleavage. A set of peptide analogs, "peptide-gap inhibitors" or "pseudopeptides", in which the peptide amide bond has been replaced with a thioether linkage, was recently introduced by Yankeelov and coworkers[2] who described the preparation of some of these thioethers and outlined general procedures for preparing others. However, few experimental details appear in the literature.

$$BrCH_2CO_2H + HSCH_2CH_2NH_2\cdot HCl + NaHCO_3 \longrightarrow H_2NCH_2CH_2SCH_2CO_2H \ (=Gly\text{-}\Psi\text{-}Gly)$$

$$BocNHCHRCH_2OH + TsCl \xrightarrow{pyridine} BocNHCHRCH_2OTs$$

$$H_2NCHRCO_2H \xrightarrow[H_2SO_4]{NaNO_2,\ KBr} BrCHRCO_2H$$

$$BrCHRCO_2H + Na_2CS_3 \longrightarrow HSCHRCO_2H$$

$$BocNHCHRCH_2OTs + HSCHRCO_2H \longrightarrow BocNHCHRCH_2SCHRCO_2H$$

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This paper describes the synthesis and characterization of some pseudopeptides which are expected to be hydrophobic, namely Gly-Ψ-Gly, (S,R)- and (S,S)-Phe-Ψ-Phe, and (S,R)- and (S,S)-Ala-Ψ-Ala. The stereochemical integrity of these pseudopeptides was not established in the present work. One of these analogs, (S,S)-Phe-Ψ-Phe, has been elaborated via its p-nitrophenyl ester into a peptide related to the C-terminal pentapeptide of Epidermal Growth Factor (EGF). Further incorporation of this pseudopentapeptide into a larger fragment of the EGF molecule is now being undertaken and will be reported at a later time. The scheme above summarizes the Yankeelov reactions that were used for the pseudopeptide syntheses.

EXPERIMENTAL

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^{1}H NMR spectra were determined by using a Varian HR 220 spectrometer with $(CH_3)_4Si$ as internal standard unless otherwise indicated. IR spectra were measured by using a Perkin Elmer 727 B or a Beckmann 4220 spectophotometer and UV spectra by using a Carey Model 17 spectrophotometer. Optical rotations were determined by using a Perkin Elmer 141 polarimeter. Melting points, measured on a Thomas-Hoover capillary melting point apparatus, are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of NIADDK, National Institutes of Health.

Glycyl-Ψ-gylcine.- Aqueous sodium bicarbonate (0.5 M, 530 mL) was purged with argon for several minutes. Bromoacetic acid (3.75 g, 27 mmol) and 2-mercaptoethylamine hydrochloride (9.2 g, 81 mmol) were added. The argon purge was continued for 1 hr, and the flask then stoppered and left at room temperature for 3 days. The solution was acidified with 6 N HCl, then washed twice with ether. The aqueous layer was adjusted to pH 7 with 2 N NaOH, diluted to 2 L, and desalted on Bio Rad AG 2-X 8 resin (37 x 5 cm), the product finally eluted with 1 N acetic acid. The eluent was stripped, and the residue taken up in 50 mL ethanol. Crystalline material which deposited on standing was collected and washed with ethanol, then with ether to yield 1.8 g (50 %) of pseudopeptide, mp. 165-166°.

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Anal. Calcd for $C_4H_9NO_2S$: C, 35.55; H, 6.71; N, 10.37; S, 23.69

Found: C, 35.49; H, 6.73; N, 10.20; S, 23.78

^1H nmr (D_2O, DSS): δ 2.89 (t, J = 6.5 Hz, 2H), 3.22 (t, J = 6.5 Hz, 2H), 3.26 (s, 2H). IR (KBr): 1636, 1612, 1568, 1468, 1389, 1372 cm^{-1}.

S,R-(+)-Phenylalanyl-ψ-phenylalanine.

S(-)-t-Butoxycarbonylphenylalanol.- S(-)-2-Amino-3-phenyl-1-propanol (3.02 g, 20 mmol), triethylamine (4.2 mL, 30 mmol), and BOC-ON (2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile, 5.4 g, 22 mmol, Aldrich) in 12.5 mL dioxane and 12.5 mL water was stirred at room temperature for 5 hr. The yellow mixture was diluted with water and ethyl acetate, and the aqueous layer then extracted 3 times with ethyl acetate. The combined organic extract was washed with brine (3 times), dried (Na_2SO_4), filtered, and evaporated. The resulting oil crystallized from ether-petroleum ether to yield 4.3 g (85 %) of product, mp. 94-95°; $[\alpha]_D^{25}$ -28.9° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 1.40 (s, 9H, t-Bu), 2.82 (d, J = 7 Hz, 2H, CH_2O), 3.38 (br s, 1H, OH), 3.53 (d of d, J = 11.5 Hz, J' = 5 Hz, 1H, benzylic), 3.64 (d of d, J = 11.5 Hz, J' = 4.5 Hz, 1H, benzylic), 3.85 (m, 1H, α-CH), 4.92 (m, 1H, N-H), 7.24 (m, 5H, aromatic). IR ($CHCl_3$): 3425 (OH,NH); 1695 (C=O) cm^{-1}.

S-(-)-t-Butoxycarbonylphenylalanyl Tosylate.- S(-)-t-Butoxycarbonylphenylalanol (10.2 g, 40.6 mmol) and freshly recrystallized tosyl chloride (15.5 g, 81 mmol) dissolved in 150 mL dry pyridine was let stand at 4° for 4 days. The mixture was poured into ice and water, stirred until the product crystallized, then collected, washed with water and dried in a vacuum desiccator to yield 14.5 g of crude product which was recrystallized from ether-petroleum ether affording 12 g (73 %) of tosylate which melted over a variable range with decomposition; $[\alpha]_D^{25}$ -24.2° (c = 1.1, MeOH). ^1H nmr ($CDCl_3$): δ 1.39 (s, 9H, t-Bu), 2.45 (s, 3H, CH_3), 2.80 (m, 2H, benzylic), 3.98 (m, 3H, CH_2O and α-CH), 4.77 (br d, J = 8 Hz, 1H, NH), 7.07 (m, 2H, aromatic), 7.20

(m, 3H, aromatic), 7.34 (d, J = 8.5 Hz, 2H, tosyl), 7.77 (d, J = 8.5 Hz, 2 H, tosyl). IR ($CHCl_3$): 3425 (NH), 1705 (C=O) cm^{-1}.

S(-)-2-Bromo-1-phenylpropanoic acid.- Potassium bromide (40.2 g, 0.34 mol) was dissolved in 200 mL of 2.5 N H_2SO_4 in a 500 mL round bottom flask equipped with magnetic stirrer and thermometer. L-Phenylalanine (16.5 g, 0.1 mol) was added, and the solution cooled to 2-4°. Solid sodium nitrite (10.5 g, 0.15 mol) was added in portions during 30 min. After stirring an additional 50 min at 2-4° and 1 hr at ambient temperature, the product was extracted into ether (3 times), the ether extract washed with water (once), then brine (once). It was dried (Na_2SO_4), filtered, and evaporated leaving a light yellow oil. This was taken up in ether, diluted to the cloud point with petroleum ether, and let stand overnight at 4° . Colorless needles of 2-hydroxy-1-phenylpropanoic acid (1.89 g, 11 %) were collected and washed with petroleum ether; mp. 122-125°; $[\alpha]_D^{25}$ -19.3° (c = 1, MeOH), -29.5° (c = 1.3, acetone); lit[3] mp. 125-126°, $[\alpha]_D$ -28.1° (c = 1.13, acetone). The filtrate was evaporated and the residue distilled bulb-to-bulb at 0.6 mm, oven temperature = 160°, to give 16.5 g of distilled bromide (72 %); $[\alpha]_D^{25}$ -10.0° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 3.20 (d of d, J = 14 Hz, J' = 7 Hz, 1H, benzylic), 3.43 (d of d, J = 14 Hz, J' = 8 Hz, 1H, benzylic), 4.39 (ca. t, J = 7.5 Hz, 1H, α-CH), 7.25 (m, 5H, aromatic), 11.4 (br s, 1H, COOH). IR (neat): 1720 (C=O).

R(-)-2-Mercapto-1-phenylpropanoic acid.- S(-)-2-Bromo-1-phenylpropanoic acid (13.5 g, 59 mmol) dissolved in 7 mL dioxane was added by dropper to a mechanically stirred 33 % aqueous solution of sodium thiocarbonate[4] (30 g of solution, ca. 65 mmol) at room temperature. An additional 12 g of sodium thiocarbonate solution was added and stirring continued for 20 min at ambient temperature, then at 60° for 1 hr. Water (100 mL) was added, and the aqueous solution washed with ether, then acidified with 2 N H_2SO_4.

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The product was extracted into ether (3 times), the extract dried (Na_2SO_4), filtered, evaporated, and distilled to yield 5.82 g (54 %) of thiol, bp. 134-144° (0.4 mm), $[\alpha]_D^{25}$ -7.84° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 2.25 (d, J = 9 Hz, 1H, SH), 2.98 (d of d, J = 14 Hz, J' = 7 Hz, benzylic), 3.23 (d of d, J = 14 Hz, J' = 8 Hz, 1H, benzylic), 3.59 (ca. quartet, J = 9 Hz, 1H, α-CH), 7.25 (m, 5H, aromatic), 11.5 (br s, 1H, COOH). IR (neat): 1710 (C=O) cm^{-1}.

S,R-t-Butoxycarbonylphenylalanyl-ψ-phenylalanine.- Sodium ethoxide was prepared by dissolving sodium metal (130 mg, 5.6 mg-atom) in 3 mL absolute ethanol. R(-)-2-Mercapto-1-phenylpropanoic acid (368 mg, 2.0 mmol) in 5 mL absolute ethanol was added. The solution was warmed to 60° under argon, and S(-)-t-butoxycarbonylphenylalanyl tosylate (810 mg, 2.0 mmol) dissolved in 20 mL abs. ethanol and 20 mL dry THF was added by syringe pump during 2 hr. After stirring an additional 3.5 hr at 60°, solvent was stripped and the residue taken up in 15 mL water. The aqueous solution was washed with ether (3 times), acidified with 2 N H_2SO_4, and the product extracted into ethyl acetate (3 times). After drying and removal of solvent, 870 mg of a yellow oil was obtained. This material was passed through silica gel (34 x 3 cm, ethyl acetate) to give a green eluent (850 mg). HPLC (Waters C-18 μ-Bondapak, Pump A = water + 0.1 % TFA, Pump B = 9:1 CH_3CN : H_2O + 0.1 % TFA, 50 % B, 1 mL/min, 210 nm) indicated a major product with retention time 47.9 min as well as several minor more polar impurities, one of which is starting thiol. ^1H nmr ($CDCl_3$): δ 1.42 (s, 9H, t-Bu), 2.65 and 2.79 (m's, 4H, CH_2's and 2 benzylic), 2.91 and 3.18 (d of d's, J = 14 Hz, J' = 8 Hz, 2 H, benzylic), 3.58 (m, 1H, methine), 4.07 (m, 1H, methine), 4.54 (m, 1H, NH), 7.24 (m, 10 H, aromatic), 11.17 (br s, 1H, COOH).

S,R-(+)-Phenylalanyl-ψ-phenylalanine.- Crude Boc derivative (above) (1 g) was taken up in 6 mL methylene chloride, and 3 mL TFA added. The brown

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solution was stirred at room temperature for 1 hr. Solvents were then stripped. The residual oil was redissolved in methylene chloride, then stripped, then dissolved in abs. ethanol and stripped. The material was stirred in 10 mL of 0.5 N HCl with slight warming for 30 min. The mixture was extracted with ether (3 times) and the ether extract dried ($MgSO_4$), filtered and stripped leaving 460 mg of yellow oil. The aqueous layer was adjusted to pH 7.5 with 1 N NaOH, and the precipitated product (234 mg) collected and washed with water, ethanol, and ether. Treatment of the initial ether extract with 0.5 N HCl and repeated work-up (2 times) gave an additional 288 mg of product. Total yield, 522 mg (69 %). A sample was recrystallized from a mixture of hot water, ethanol and acetone, mp. 200° (dec.); $[\alpha]_D^{25}$ +64.2° (c = 1, 1 N HCl).

Anal. Calcd for $C_{18}H_{21}NO_2S$: C, 68.54; H, 6.71; N, 4.44; S, 10.17

Found: C, 68.23; H, 6.38; N, 4.19; S, 10.13

^1H nmr (TFA): δ 3.02 (m, 5H), 3.27 (d of d, J = 14 Hz, J' = 6.5 Hz, 1H), 3.59 (m, 1H), 3.70 (m, 1H), 6.54 (br s, 3H, NH_3), 7.14 - 7.45 (m, 10 H, aromatic). IR (KBr): 1640, 1560, 1525 cm^{-1}. λ_{max} (0.1 N HCl): 267 nm (ε 225), 264 (381), 258 (537), 250 (537), 245 (503), 240 (468).

S,S-(-)-Phenylalanyl-ψ-phenylalanine.

R(+)-2-Bromo-1-phenylpropanoic acid.- This enantiomer was prepared from D-phenylalanine using the procedure described above for the S isomer. Crystallization afforded a 13.5 % yield of (+)-2-hydroxy-1-phenylpropanoic acid, mp. 122-125°, $[\alpha]_D^{25}$ +19.0° (c = 1.2, MeOH). Distillation of the filtrate yielded 67 % of the (+) bromide, bp. 126-136° (0.4 mm); $[\alpha]_D^{25}$ +11.2° (c = 1, MeOH).

S(+)-2-Mercapto-1-phenylpropanoic acid.- This enantiomer was prepared using the same procedure described above for the R(-) isomer. Distillation gave an 83 % yield of thiol, bp. 135-145° (0.5 mm); $[\alpha]_D^{25}$ +7.7° (c = 1, MeOH).

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S,S-t-Butoxycarbonylphenylalanyl-ψ-phenylalanine.- This diastereomer was prepared from S(-)-t-butoxycarbonylphenylalanyl tosylate and S(+)-2-mercapto-1-phenylpropanoic acid in a procedure identical to that described above for the S,R-diastereomer. The crude product was characterized by HPLC only and was used without purification for further transformations.

S,S-(-)-Phenylalanyl-ψ-phenylalanine.- Crude t-Boc-phenylalanyl-ψ-phenylalanine (ca. 5 g, derived from 18 mmol each of S(-)-t-butoxycarbonylphenylalanyl tosylate and S(+)-2-mercapto-1-phenylpropanoic acid) in 25 mL of methylene chloride and 6 mL of TFA was stirred at room temperature for 2 hr. Workup similar to that described above for the S,R diastereomer afforded 1.63 g of the S,S diastereomer, mp. 185-186°, $[\alpha]_D^{25}$ -10.1° (c = 1.15, 1 N HCl).

Anal. Calcd for $C_{18}H_{21}NO_2S$: C, 68.54; H, 6.71; N, 4.44; S, 10.17

Found: C, 68.26; H, 6.18; N, 4.34; S, 10.22

^1H nmr (TFA): δ 3.00 (m, 5H), 3.33 (d of d, J = 14 Hz, J' = 7 Hz, 1H), 3.68 (m, 1H), 3.74 (m, 1H), 6.79 (br s, 3H, NH_3), 7.10 - 7.52 (m, 10 H, aromatic). IR (KBr): 1630, 1570, 1525 cm^{-1}.

S,R-(+)-t-Butoxycarbonylalanyl-ψ-alanine.

S-(-)-t-Butoxycarbonylalanol.- Di-t-butyl dicarbonate (32 g, 0.15 mol) was dissolved in 110 mL of methylene chloride. A solution of L-2-amino-propanol (10 g, 0.13 mol) and triethylamine (20 mL, 0.14 mol) in 60 mL methylene chloride was added during 20 min. The mixture was stirred overnight at ambient temperature, then washed with 3 N HCl (twice) and with water (once). After drying ($MgSO_4$), filtering, and removing solvent, the residue was taken up in 30 mL of ether, filtered through Celite, and diluted to 400 mL with petroleum ether. After standing at 0° for several hours, the product was collected and washed with petroleum ether: 14.6 g (63 %), mp. 53-56°; $[\alpha]_D^{25}$ -9.5° (c = 1.2, MeOH). ^1H nmr ($CDCl_3$): δ 1.14 (d, J = 6.8 Hz, 3H, CH_3), 1.45 (s, 9H, t-Bu), 3.52 (m, 3H, CH_2 and OH), 3.75 (m, 1H, α-CH), 4.92 (m,

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1H, NH). IR ($CHCl_3$): 3450 (NH, OH), 1705 (C=O), 1500, 1375, 1170 cm^{-1}.

S-(-)-t-Butoxycarbonylalanyl Tosylate.- This was prepared using the procedure described above for the phenylalanine analog. Recrystallization from ether-petroleum ether afforded a 68 % yield, mp. 74-76°; $[\alpha]_D^{25}$ -35.4° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 1.15 (d, J = 7 Hz, 3H, CH_3), 1.40 (s, 9H, t-Bu), 2.45 (s, 3H, tosyl CH_3), 3.95 (m, 3H, CH_2 and α-CH), 4.65 (br s, 1H, NH), 7.34 (d, J = 8.5 Hz, 2H, aromatic), 7.78 (d, J = 8.5 Hz, 2H, aromatic). IR ($CHCl_3$): 3440 (NH), 1710 (C=O), 1500, 1370, 1170, 970 cm^{-1}.

S-(-)-2-Bromopropanoic Acid.- A procedure similar to that described for the phenylalanine analog afforded a 61 % yield of bromide, bp. 92° (aspirator), $[\alpha]_D^{25}$ -33.8° (c = 1, MeOH). No alcohol by-product was obtained. ^1H nmr ($CDCl_3$): δ 1.85 (d, J = 7 Hz, 3H, CH_3), 4.41 (quart, J = 7 Hz, 1H, α-CH), 10.70 (s, 1H, COOH). IR (neat): 1720 (C=O) cm^{-1}.

R-(+)-2-Mercaptopropanoic Acid.- Treatment of the above bromide with Na_2CS_3 as described for the phenylalanine analog afforded 64 % of thiol, bp. 94° (aspirator); $[\alpha]_D^{25}$ +44.8° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 1.55 (d, J = 7 Hz, 3H, CH_3), 2.25 (d, J = 8.5 Hz, 1H, SH), 3.53 (ca. quintet, J = 7 Hz, 1H, α-CH), 10.3 (br s, 1H, COOH). IR (neat): 1710 (C=O), 1455, 1415, 1285, 1242, 1208 cm^{-1}.

S,R-(+)-t-Butoxycarbonylalanyl-ψ-alanine.- Sodium ethoxide was prepared from sodium metal (583 mg, 25 mg-atom) and 20 mL abs. ethanol. R-(+)-2-Mercaptopropanoic acid (836 mg, 7.89 mmol) in 15 mL ethanol was added. A solution of S-(-)-t-butoxycarbonylalanyl tosylate (2.6 g, 7.9 mmol) in 50 mL ethanol was added during 5 hr at 60°. Stirring at 60° was continued overnight. Solvent was stripped from the white suspension, and the residue taken up in 50 mL water. This solution was washed 3 times with ether, then acidified with 2 N H_2SO_4. The product was extracted into ethyl acetate (3 times), dried ($MgSO_4$), filtered, concentrated, and passed through silica gel (40 x 3 cm, ethyl acetate) to yield a colorless oil which crystallized

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on pumping at 0.1 mm (1.64 g). Recrystallization from ether-petroleum ether afforded 1.36 g (65 %) of product, mp. 102-104°; $[\alpha]_D^{25}$ +102.5° (c = 0.75, MeOH).

Anal. Calcd for $C_{11}H_{21}NO_4S$: C, 50.17; H, 8.04; N, 5.32; S, 12.17

Found: C, 49.84; H, 8.10; N, 5.17; S, 12.07

^1H nmr ($CDCl_3$): δ 1.20 (d, J = 6.8 Hz, 3H, CH_3), 1.45 (s superimposed on d, J = 6.8 Hz, 12 H, t-Bu and CH_3), 2.77 (m, 2H, CH_2), 3.46 (m, 1H, α-CH), 3.91 (m, 1H, α-CH), 4.65 (m, 1H, NH). IR ($CHCl_3$): 3425 (NH), 1705 (C=O), 1490, 1360, 1165 cm^{-1}.

S,S-(-)-t-Butoxycarbonylalanyl-ψ-alanine.

R-(+)-2-Bromopropanoic Acid.- This enantiomer was prepared in 63 % yield from D-alanine using the procedure described previously, bp. 102-104° (aspirator); $[\alpha]_D^{25}$ +36.1° (c = 1.25, MeOH).

S-(-)-2-Mercaptopropanoic Acid.- This enantiomer was prepared from the R-(+)-bromide using the procedure described previously; bp. 114° (aspirator); $[\alpha]_D^{25}$ -46.8° (c = 1, MeOH).

S,S-(-)-t-Butoxycarbonylalanyl-ψ-alanine.- This diastereomer was prepared in 54 % yield from sodium ethoxide, S-(-)-2-mercaptopropanoic acid, and S-(-)-t-butoxycarbonylalanyl tosylate using the procedure described for the S,R isomer above, mp. 76-78.5°; $[\alpha]_D^{25}$ -96.1° (c = 0.83, MeOH).

Anal. Calcd for $C_{11}H_{21}NO_4S$: C, 50.17; H, 8.04; N, 5.32; S, 12.17

Found: C, 50.34; H, 7.98; N, 5.20; S, 12.10

^1H nmr ($CDCl_3$): δ 1.21 (d, J = 7 Hz, 3H, CH_3), 1.44 (s superimposed on d, J ca. 7 Hz, 12 H, t-Bu and CH_3), 2.71 (m, 1H, methylene), 2.83 (d of d, J = 13.5 Hz, J' = 5.5 Hz, 1H, methylene), 3.46 (m, 1H, α-CH), 4.76 (m, 1H, NH), 8.35 (br s, 1H, COOH). IR ($CHCl_3$): 3425 (NH), 1705 (C=O), 1490, 1365, 1162 cm^{-1}.

Boc-Leu Arg(NO_2)OCH_3.- A solution of Arg(NO_2)OCH_3·HCl (4.94 g, 18.3 mmol,

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Vega), BocLeuONp (6.44 g, 18.3 mmol) and triethylamine (2.6 mL, 18.6 mmol) in 105 mL ethyl acetate and 10 mL DMF was stirred overnight at room temperature. The yellow solution was washed with water (twice), then brine (twice). After drying ($MgSO_4$) and removal of solvent, the residue was flash chromatographed[5] twice on 600 mL of silica gel eluting with ethyl acetate to give 6.8 g (83 %) of protected dipeptide; $[\alpha]_D^{25}$ -32.1° (c = 1, MeOH). ^1H nmr ($CDCl_3$): δ 0.92 (m, 6H, i-Pr CH_3's), 1.38 (s, 9H, t-Bu), 1.61 (m, 8H, CH_2's), 2.27 (m, 1H, i-Pr CH), 3.20 (m, 1H, α-CH), 3.40 (m, 1H, α-CH), 3.66 (s, 3H, OCH_3), 4.10 (m, 1H, NH), 4.50 (m, 1H, NH), 5.22 (m, 1H, Boc-NH), 7.60 (m, 1H, NH), 8.60 (m, 1H, NH). IR ($CHCl_3$): 1735, 1695, 1665, 1620, 1600 cm^{-1}.

BocGlu(OBz) Leu Arg(NO_2)OCH_3.- The Boc group was removed from 4.86 g (10.9 mmol) of Boc-Leu Arg(NO_2)OCH_3 by stirring in 10 mL of 1:1 TFA : CH_2Cl_2 for 3.25 hr at room temperature. The dipeptide was precipitated with ether, collected, and washed with ether. This material was dissolved in 60 mL of ethyl acetate and 6 mL of DMF. Boc-Glu(OBz)ONp (4.8 g, 10.5 mmol) was added, followed by 3.5 mL (20 mmol) of N,N-di-i-propylethylamine. After stirring at room temperature overnight, the solution was washed with water (once), then with brine (4 times), dried ($MgSO_4$), filtered, concentrated, and flash chromatographed twice on silica gel (550 mL, dry volume, ethyl acetate) to give 5.65 g (81 %) of protected tripeptide; $[\alpha]_D^{25}$ -29° (c = 0.9, MeOH). ^1H nmr ($CDCl_3$): δ 1.09 (m, 6H, Leu(CH_3)$_2$), 1.36 (s, 9H, t-Bu), 1.45 to 2.2 (m, ca. 11 H, 5 x CH_2 and i-Pr CH), 2.43 (m, 2H, CH_2CO_2Ph), 3.14 and 3.27 (m, 2H, 2 α-CH), 3.60 (s, 3H, CO_2CH_3), 4.08 (m, 1H, α-CH), 4.42 (m, 2H, 2 x NH), 4.97 (s, 2H, CH_2Ph), 5.53 (m, 1H, BocNH), 7.12 (s superimposed on m, ca. 7H, Ph + 2 x NH), 7.50 (m, 1H, NH). IR ($CHCl_3$): 1735, 1690 cm^{-1}.

S,S-(-)-p-Nitrophenyl t-butoxycarbonylphenylalanyl-ψ-phenylalanate

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[S,S-(-)-BocPhe-ψ-PheONp].- A solution of S,S-t-butoxycarbonylphenylalanyl-ψ-phenylalanine (2.4 g, 5.7 mmol), p-nitrophenol (800 mg, 5.7 mmol), and DCC (1.2 g, 5.8 mmol) in 35 mL of ethyl acetate was stirred overnight at room temperature. The urea was removed by filtration, the filtrate concentrated and then flash chromatographed on 550 mL silica gel (dry volume) eluting with methylene chloride. The product was then recrystallized from ether-petroleum ether yielding 1.02 g (33 %) of p-nitrophenyl ester, mp. 104-107° ; $[\alpha]_D^{25}$ -46.3° (c = 1.04, MeOH).

Anal. Calcd for $C_{29}H_{32}N_2O_6S$: C, 64.90; H, 6.01; N, 5.22; S, 5.98

Found: C, 64.75; H, 5.90; N, 5.04; S, 6.38

^1H nmr ($CDCl_3$): δ1.36 (s, 9H, t-Bu), 2.74, 3.05, and 3.15 (m's, 6H, 3 x CH_2), 3.68 (m, 1H, α-CH), 3.93 (m, 1H, α-CH), 4.49 (m, 1H, BocNH), 6.87 (d, J = 9 Hz, 2H, p-nitrophenyl), 6.84 (m, 10 H, 2 x C_6H_5), 7.98 (d, J = 9 Hz, 2H, p-nitrophenyl). IR ($CHCl_3$): 1750, 1705, 1490, 1345, 1180, 1110 cm^{-1}.

Boc-S,S-Phe-ψ-PheGlu(OBz) Leu Arg(NO_2)OCH_3.- Boc-Glu(OBz) Leu Arg(NO_2)OCH_3 (822 mg, 1.3 mmol) was stirred for 3 hr at room temperature in 4 mL methylene chloride and 4 mL TFA. The tripeptide was precipitated with ether, collected, washed with ether and dried in a vacuum desiccator. A mixture of this tripeptide (810 mg), S,S-(-)-p-nitrophenyl t-butoxycarbonylphenylalanyl-ψ-phenylalanine (628 mg, 1.17 mmol), and N,N-di-i-propylethylamine (210 μL, 1.2 mmol) in 10 mL ethyl acetate and 1 mL DMF was stirred at room temperature for 2.5 hr. An additional 210 μL of tertiary amine was added, and stirring continued overnight. The yellow slurry was washed with water (5 times), then brine (once), dried ($MgSO_4$), filtered, concentrated, flash chromatographed on 600 mL silica gel (ethyl acetate), and finally recrystallized from ethyl acetate-ether yielding 808 mg (71 %) of ψ-peptide, mp. 100° ; $[\alpha]_D^{25}$ -37.1° (c = 1, MeOH).

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Anal. Calcd for $C_{48}H_{66}N_8O_{11}S$: C, 59.85; H, 6.91; N, 11.64; S, 3.33

Found: C, 59.64; H, 6.56; N, 11.57; S, 3.36

^1H nmr ($CDCl_3$): δ 0.86 (m, 6H, Leu $(CH_3)_2$), 1.30 (s, 9H, t-Bu), 1.57 (m, 5H), 1.91 (m, 5H), 2.35 (m, 2H, CH_2CO_2Bz), 2.5 - 2.9 (m, 5H), 3.16 (m, 3H), 3.48 (m, 1H), 3.62 (s, 3H, CO_2CH_3), 3.75 (m, 1H), 4.20 (m, 2H), 4.48 (m, 1H), 4.73 (m, 1H), 4.97 (s, 2H, benzylic), 6.8 - 7.6 (m, 20 H, Ar + NH).

IR ($CHCl_3$): 1735, 1665, 1625 cm^{-1}.

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