°C. Anal. ($C_{14}H_{22}N_2O_2$·HCl) C, H, N.

N-[3-[2-(Dipropylamino)ethyl]phenyl]acetamide (2b). A solution of 2.29 g of **13** in 150 mL of EtOH was hydrogenated at 60 psi over 200 mg of 10% Pd/C for 1 h. The catalyst was removed by filtration, etherial HCl was added, and the mixture was evaporated to a pale yellow oil. A solution of 848 mg of the amine hydrochloride in 35 mL of H_2O was warmed to 50 °C and treated with 475 mg of acetic anhydride and 576 mg of sodium acetate. It was stirred for 30 min, cooled, adjusted to pH 11 with 10% NaOH, and extracted with CH_2Cl_2. The extracts were washed with water, dried, and evaporated to give a yellow oil. This was taken up in Et_2O and treated with saturated etherial HCl to give 1.03 g of solid. This was recrystallized from MeOH–EtOAc to give 695 mg of white crystals (67%), mp 202–204 °C. Anal. ($C_{16}H_{26}N_2O_2$·HCl) C, H, N.

Registry No. **1c**, 91374-20-8; **2b**, 97351-98-9; **2b**·HCl, 97351-94-5; **3**, 1975-50-4; **4**, 23876-13-3; **5**, 60468-54-4; **6**, 23876-14-4; **7**, 23876-15-5; **8**, 91374-22-0; **9**, 91374-23-1; **10**, 97351-95-6; **11**, 91374-25-3; **12**, 1877-73-2; **13**, 97351-96-7; **13**·HCl, 97351-99-0; m-$ClCOCH_2C_6H_4NO_2$, 38411-41-5; m-$NO_2C_6H_4CH_2CONPr_2$, 97351-97-8; di-n-propylamine, 142-84-7; diethyl oxalate, 95-92-1.

Conformationally Restricted C-Terminal Peptides of Substance P. Synthesis, Mass Spectral Analysis and Pharmacological Properties

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Four cyclic analogues of the C-terminal hepta- or hexapeptide of substance P were prepared by the solution method. The cyclizations were obtained by substituting with cysteine the residues normally present in positions 5 or 6 or 11 of substance P and by subsequent disulfide bond formation. The final products were identified by ordinary analytical procedures and advanced mass spectroscopy. The biological activities were determined on three bioassays: the guinea pig ileum, the guinea pig trachea and the rabbit mesenteric vein. Results obtained with these assays indicate that all peptides with a disulfide bridgehead in position 11 are inactive and that a cycle between positions 5 and 6 already strongly reduces the biological activity. The acyclic precursors containing thiol protection groups display weak biological activities. These results further underline the importance of the side chain in position 11 of substance P and suggest that optimal biological activities may require a linear peptide sequence.

Conformationally restricted analogues of linear peptide hormones provide a useful approach to obtain hormone derivatives with increased affinities and/or durations of action. Successful cases in recent years are orally active somatostatin analogues,[1] cyclic hyperactive α-MSH analogues,[2] and cyclic enkephalins with increased activity[2] or increased selectivity.[3]

No such studies have been reported on substance P and other tachykinins. Generally, the structure–activity studies carried out on this neuropeptide concentrated rather on single substitution (e.g., the L-alanine series[4]) or on modifications of the C-terminal residue,[5] particularly the methionine side chain[6] or the C-terminal amide, which was methylated to reduce metabolic degradation.[7] Retro-inverso sequences[8] or substitutions of the aromatic residues[9] have also been reported.

Multiple substitutions with D-tryptophan have led to the obtainment of reasonably active competitive antagonists for substance P (for a review see ref 10). The affinities of these compounds are however still somewhat disappointing, because most of them do not exceed pA_2 values of 7.0. Compounds, both agonists and antagonists, with high affinities are urgently needed and could eventually be obtained by preparing cyclic analogues of SP or its active C-terminal fragments.

With this in mind, a series of analogues of the heptapeptide sequence SP,[5-11] containing a cyclic disulfide bridge between positions 5 and 6, 5 and 11, or 6 and 11, were prepared for their biological activities with the corresponding linear sequences.

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Syntheses

All compounds were synthesized by the classical solution method by combination of stepwise elongation from the C-terminal end with fragment couplings. The stepwise elongation was performed either with the formation of intermediate active esters of N-hydroxybenzotriazole, by direct activation with dicyclohexylcarbodiimide, by preformed N-hydroxysuccinimide-active esters, or by the mixed-anhydride method. The fragment couplings were performed with the dicyclohexylcarbodiimide and N-hydroxybenzotriazole technique. Generally, the *tert*-butyloxycarbonyl group was used for N protection, while the thiol side chains were protected either with the benzyl thioether or with the S-acetamidomethyl (S-Acm) group.

(1) Freidinger, R. M.; Veber, D. F. In "Conformationally Directed Drug Design"; Vida, J. A., Gordan, M., Eds.; American Chemical Society: Washington, DC, 1984; pp 169–187.
(2) Hruby, V. J. Reference 1, pp 9–27.
(3) Schiller, P. W.; Eggimann, B.; DiMaio, J.; Lemieux, C.; Nguyen, T. M. D. *Biochem. Biophys. Res. Commun.* 1981, *101*, 337.
(4) Fournier, A.; Couture, R.; Regoli, D.; Gendreau, M.; St-Pierre, S. *J. Med. Chem.* 1982, *25*, 64.
(5) Escher, E.; Couture, R.; Poulos, C.; Pinas, N.; Mizrahi, J.; Theodoropoulos, D.; Regoli, D. *J. Med. Chem.* 1982, *25*, 1317.
(6) Theodoropoulos, D.; Poulos, C.; Gates, D.; Cordopatis, P.; Couture, R.; Mizrahi, J.; Regoli, D.; Escher, E. In "Peptides"; Blaha, K., Maley, P., Eds.; Wd. Gruyter: Berlin, 1983; p 521.
(7) Sandberg, B. E. B.; Lee, C. M.; Hanley, M. R.; Iversen, L. L. *Eur. J. Biochem.* 1981, *114*, 329.
(8) Chorev, M.; Rubini, E.; Gilon, C.; Wormser, U.; Selinger, Z. *J. Med. Chem.* 1983, *26*, 129.
(9) Escher, E.; Couture, R.; Champagne, G.; Mizrahi, J.; Regoli, D. *J. Med. Chem.* 1982, *25*, 470.
(10) Regoli, D.; Escher, E.; Mizrahi, J. *Pharmacology* 1984, *28*, 301.
(11) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* 1981, 325.

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Table I. Apparent Affinity of Heptapeptide and Hexapeptide Analogues of SP (5–11) and SP (6–11) on Three Pharmacological Preparations[a]

peptide	GPI			GPT			RMV		
	pD_2	AR	α^E	pD_2	AR	α^E	pD_2	AR	α^E
substance P	8.48	100	1.0	6.75	100	1.0	7.55	100	1.0
substance P (5–11)	8.51	107	1.0	7.10	225	1.12	7.32	60	1.0
[(S-MeOBzl)Cys[5],(S-Bzl)Cys[11]]-SP (5–11) (**1A**)	6.37	0.8	1.2	5.22	3.0	0.8	5.98	2.8	0.4
[Cys[5],Cys[11]]-SP (5–11) (**1**)		0.001	0.1	In			In		
[(S-Acm)Cys[5,6]]-SP (5–11) (**2A**)	7.70	16.6	1.4	6.45	50.0	1.1	6.52	9.3	0.6
[Cys[5],Cys[6]]-SP (5–11) (**2**)	7.13	4.5	1.1	5.27	3.7	1.0		0.001	0.1
[Cys[6],Cys[11]]-SP (6–11) (**3**)	4.83	0.02	0.7	In			In		
[$(CH_3)_2$Gln[5],Cys[6],Cys[11]]-SP (5–11) (**4**)		0.001	0.1	In			In		

[a] Key: GPI, guinea pig ileum treated with indomethacin (4.2×10^{-6} M), atropine (5.1×10^{-6} M), and diphenhydramine 5.9×10^{-6} M) (Regoli et al., 1984); GPT, guinea pig trachea treated with indomethacin (2.8×10^{-6} M); RMV, rabbit mesenteric vein; pD_2, –log of the concentration of peptide that produces 50% of the maximal effect; RA, relative affinity expressed as a fraction of that of SP; α^E, intrinsic activity; In, Inactive at 10^{-4} M. **1A** and **2A** were obtained from the N-protected precursor by deprotection with 20% HBr in active acid or 2 N HCl in dioxane, respectively, and are calculated as the hydrobromide and hydrochloride, respectively.

Disulfide cyclization was achieved in the first case by treatment with sodium in liquid ammonia followed by oxidation with 1,2-diiodoethane or for the S-Acm peptides with iodine in methanol. All peptides were purified by gel filtration and partition chromatography; they were identified with standard analytical procedures, and the structural identification of the cyclic disulfide analogues was achieved by fast atom bombardment and secondary ion mass spectroscopy (FAB and SIMS).

Results and Discussion

Analysis by Mass Spectroscopy. The new peptides, compounds **1–4** of Table I were analyzed by either fast atom bombardment (FAB)[11] or liquid secondary ion mass spectrometry (SIMS).[12] With glycerol as the matrix, the molecular weight measured on the protonated molecular ion was consistent with the cyclic disulfide structure. Thus, the protonated molecular ions were observed at m/z 814, 817, 686, and 842 for peptides **1–4**, respectively. Moreover, the peptide FAB-MS and liquid SIMS spectra provided complete sequence information for the cyclic and the reduced linear products through the series of fragment ions[13,14] and confirmed further the correct sequence and structure of the final products **1–4**.

Results of Biological Assays. All compounds were tested on three different bioassays: the guinea pig ileum, the guinea pig trachea, and the rabit mesenteric vein. Details of these bioassays have recently been extensively described.[15]

As shown in Table I, all cyclic analogues having a bridgehead in the C-terminal position were found to be inactive or very weak agonists or antagonists. The only exception is compound **2**, which has a disulfide bridge between residues 5 and 6 and an intact C-terminal end. These results support the findings of earlier investigations, in which it was observed that any modification of position 11 reduces the biological activities,[6] while changes in positions 5 and 6 are better tolerated.[16]

In conclusion, our negative results indicate that cyclizations of the sequence SP^{5-11} should not imply the residue in position 11. The present findings again stress the need of a free C-terminal side chain with the ideal proportions and chemistry of methionine for agonists and with more flexibility for the antagonists, which appear to tolerate fairly well Nle, Leu, or Phe.[10] For the agonists, even the replacement of the thioether by an oxo ether or a simple methylene group (Nle) reduces affinity: only the use of selenomethionine was tolerated without important loss of activity. A simple change of the position of the thioether (S-ethylhomocysteine) or the replacement of the δ-methylene by another sulfur [S-(methylthio)homocysteine] was not tolerated by the receptor of the guinea pig ileum.[6]

Experimental Section

Capillary melting points were determined on a Büchi SMP-20 apparatus and are reported uncorrected. Opitcal rotations were measured in a Carl Zeiss precision polarimeter (0.005°). Thin-layer chromatograms were carried out on silica gel plates with sample loads of 20–40 μg. The following solvent systems were used and allowed to ascend for 12–15 cm: A, 1-butanol–acetic acid–water (4:1:1, v/v): B, 1-butanol–pyridine–acetic acid–water (15:10:3:12); C, 1-butanol–acetic acid–water (4:1:5, upper phase); D, chloroform–methanol–acetic acid (95:5:3); E, chloroform–methanol (6:1), F, 1-butanol-pyridine–water (20:10:11). The compounds were visualized by reaction with ninhydrin or chlorine followed by toludine solution. Elemental analyses were performed by the Microanalytical Laboratory of the National Hellenic Research Foundation and the data (C, H, N) fall within ±0.4% of the theoretical value. Amino acid analyses were performed on peptide samples previously hydrolyzed in deaerated ampoules with 6 N HCl (110 °C, 24 h).

FAB mass spectral analyses were made with a JEOL HX100HF mass spectrometer equipped with a JEOL FAB source and gun. Spectra were taken at 5-keV acceleration potential and Xenon beam potential of 8 keV. Liquid SIMS mass spectral analyses were made on a CEC 21-110 mass spectrometer equipped with a focal plane electrooptical ion detector (x, x) and a Phrasor cesium ion gun. The cesium gun was mounted on a flange exterior to the source housing, and the cesium beam was focused in the center of the ion source by an Einzel lens mounted inside the source housing. Spectra were taken at 6-keV acceleration potential and a primary ion beam energy of 10 keV.

Samples were prepared by dissolving ~10 μg of the peptides in either acetic acid or dilute aqueous HCl applied directly to the end of the direct-insertion probe. Glycerol or α-monothioglycerol (~1 mL) was added to the solution, and the solvent was removed in the vacuum lock of the mass spectrometer. In some instances, a spectrum was recorded with the sample in glycerol, and then the probe was withdrawn and an equivalent amount of α-monothioglycerol was added for a second analysis.

***N-t*-Boc-Leu-(*p*-MeOBzl)Cys-OMe.** Boc-Leu·H_2O (1.4 g, 5.6 mmol) and dicyclohexylcarbodiimide (DCC) (1.15 g, 5.6 mmol) were added to a chilled solution of (p-MeOBzl)Cys-OMe hydrochloride (1.63 g, 5.6 mmol) and N-methylmorpholine (NMM) (0.61 mL, 5.6 mmol) in CH_2Cl_2 (20 mL). After 8 h at 0 °C and another

(12) Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* 1982, *54*, 2029.
(13) Barber, M.; Bardoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Biomed. Mass Spectrom.* 1982, *9*, 208.
(14) Hunt, D. F.; Bako, A. M.; Ballard, J. M.; Shabanowitz, J.; Giordani, A. B. *Biomed. Mass Spectrom.* 1981, *8*, 397.
(15) Regoli, D.; D'Orléans-Juste, P.; Escher, E.; Mizrahi, J. *Eur. J. Pharmacol.* 1984, *97*, 161.
(16) Theodoropoulos, D.; Pinas, N.; Poulos, C.; Couture, R.; Mizrahi, J.; Regoli, D.; Escher, E. *Eur. J. Med. Chem.* 1982, *17*, 527.

12 h at room temperature, the reaction mixture was filtered from the precipitated dicyclohexylurea (DCU) and the solvent evaporated in vacuo. The remaining residue was taken up with ethyl acetate, washed with 5% $NaHCO_3$ solution, water, 10% citric acid solution, and water, and finally dried over Na_2SO_4. The solvent was evaporated under vacuum and the residue crystallized upon addition of petroleum ether. Recrystallization from ethyl acetate–petroleum ether gave 1.74 g (66%): mp 83–85 °C; $[\alpha]^{25}{}_D$ −40.2° (*c* 1, DMF); TLC R_f(B) 0.94, R_f(C) 0.94, R_f(D) 0.91.

***N-t*-Boc-Leu-(*p*-MeOBzl)Cys-NH₂.** A methanolic solution (40 mL) of *N-t*-Boc-Leu-(*p*-MeOBzl)Cys-OMe (1.10 g, 2.35 mmol saturated with dry ammonia was left for 3 days at room temperature. Then, the solvent was removed under vacuum and the resulting product solidified with ether–petroleum ether (1:1 v/v). The yield, after recrystallization from ethyl acetate–petroleum ether (2:1 v/v), was 0.74 g (69%): mp 123–125 °C; $[\alpha]^{25}{}_D$ −45.2°(*c* 1, DMF); TLC R_f(A) 0.80, R_f(B) 0.093, R_f(D) 0.73.

***N-t*-Boc-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH₂.** *N-t*-Boc-Leu-(*p*-MeOBzl)Cys-NH_2 (0.45 g, 1.0 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA) (3 mL) and CH_2Cl_2 (3 mL). After 45 min at 20 °C, the solvents were removed under vacuum. The evaporation was repeated after the addition of MeOH, and the product was precipitated with dry ether and dried in vacuo over P_2O_5 and KOH pellets. This trifluoracetate salt (0.43 g, 0.92 mmol) was dissolved in DMF (3 mL), neutralized with NMM (0.1 mL, 0.93 mmol) and cooled to −5 °C (solution A).

DCC (0.206 g, 1.0 mmol) was added to a chilled solution of 0.55 g (0.93 mmol) of *N-t*-Boc-Gln-Phe-Phe-Gly-OH (1) and 1-hydroxybenzotriazole (HOBT) (0.245 g, 1.65 mmol) in DMF (4 mL). The mixture was kept for 20 min at −4 °C and another 15 min at room temperature and was then mixed with solution A. After 37 h at room temperature the solvent was evaporated in vacuo, and the remaining residue solidified upon addition of water. The solid product was filtered, washed copiously with 5% $NaHCO_3$ solution, water, 10% citric acid solution, and water, and finally dried over P_2O_5. Trituration with ethanol (10 mL) under reflux gave 0.78 g (89%) of pure product: mg 216–217 °C; $[\alpha]^{25}{}_D$ −13.7° (*c* 0.5, DMF); TLC R_f(B) 0.92, R_f(C) 0.96, R_f(D) 0.27.

***N*-Z-(*S*-Bzl)Cys-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH₂, Z-1A.** An aliquot of *N-t*-Boc-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH_2 (670 mg, 0.71 mmol) was deprotected with TFA. After 45 min, the acid evaporated and the residue was triturated to a solid with ether, filtered, washed with dry ether, and dried in vacuo. It was then dissolved in DMF (5 mL), neutralized with NMM, and allowed to react with 575 mg (1.3 mmol) of *N*-Z-(*S*-Bzl)Cys-OSu. After 48 h at room temperature, the solvent was removed under reduced pressure and the oily residue was solidified by the addition of water and cooling. The product was filtered, washed several times with 5% $NaHCO_3$ solution, water, 10% citric acid solution, and water, and dried over P_2O_5. It was then washed with EtOH under reflux and finally recrystallized from DMF–ether (1:2 v/v): yield 600 mg (72%); mp 241–244 °C; $[\alpha]^{25}{}_D$ −17.8° (*c* 0.25, DMF); TLC R_f(B) 0.91, R_f(C) 0.90.

H-Cys-Gln-Phe-Phe-Gly-Leu-Cys-NH₂ (1). A sample of the protected heptapeptide Z-1A (150 mg, 0.13 mmol) was deprotected by sodium in liquid ammonia. After evaporation of the ammonia by a nitrogen stream, the residue was dissolved in deaerated 50% aqueous methanol and the disulfide was formed by oxidation with 1 equiv of 1,2-diiodoethane. The product was subjected to gel chromatography on a 52 × 1.9 cm column of Sephadex G-15 eluted with 25% acetic acid; it was further purified by partition chromatography on a 52 × 1.9 cm column of Sephadex G-15 that had been equilibrated with both phases of the solvent system 1-butanol–acetic acid–water (4:1:5 v/v), followed by a gel chromatography on a 52 × 1.9 cm column of Sephadex G-15 (fine) with 0.2 M acetic acid. Peptide material was detected by monitoring the absorbancy at 280 and 254 nm: yield following lyophilization 12 mg; TLC (single spot) R_f(A) 0.28, R_f(B) 0.75, R_f(C) 0.29; mp 250–255 °C dec; $[\alpha]^{25}{}_D$ −17.8° (*c* 0.25, DMF). Amino acid anal.: Cys, 1.91; Gln, 1.06; Phe, 2.08; Gly, 1.02; Leu, 0.98; NH_3, 1.88. FAB-MS *m/z* 814 (M^+).

***N-t*-Boc-Phe-Phe-Gly-Leu-Met-NH₂.** A portion of the dipeptide Boc-Leu-Met-NH_2 (4) (2.0 g, 5.52 mmol) was deprotected with 1 N HCl in CH_3COOH. The resulting hydrochloride salt was dissolved in DMF (5 mL), neutralized with NMM, and allowed to react with a sample of Boc-Phe-Phe-Gly-OH (2.2 g, 4.7 mmol) dissolved in DMF (8 mL) and preactivated at 0 °C for 0.5 h with HOBt (1.25 g, 8.4 mmol) and DCC (0.97 g, 4.7 mmol). The reaction mixture was left to stand for 2 h at 0 °C and then for 24 h at room temperature. The precipitated DCU was filtered off, and the solvent was removed in vacuo. The remaining residue was solidified upon trituration with water; the resulting solid was washed with 5% $NaHCO_3$, 10% citric acid, and water and dried over P_2O_5. After recrystallization from DMF–ether (1:5 v/v), 2.33 g of the desired product was obtained: 70%; mp 163–166 °C; $[\alpha]^{20}{}_D$ −32° (*c* 0.5, DMF); TLC R_f(A) 0.68, R_f(B) 0.94, R_f(C) 0.84.

***N-t*-Boc-(*S*-Acm)Cys-Phe-Phe-Gly-Leu-Met-NH₂.** *N-t*-Boc-Phe-Phe-Gly-Leu-Met-NH_2 (1.2 g, 1.7 mmol) was dissolved in 4 mL of 1.7 N HCl/CH_3COOH. After 45 min at 20 °C the solvent was removed in vacuo, and the hydrochloride was precipitated with dry ether and dried in vacuo over P_2O_5 and KOH pellets. The hydrochloride salt was dissolved in DMF (3 mL), neutralized with NMM (0.18 mL, 1.7 mmol), and cooled to −5 °C (solution A).

DCC (0.35 g, 1.7 mmol) was added to a chilled solution of 0.49 g (1.7 mmol) of *N-t*-Boc-(*S*-Acm)-Cys-OH and HOBt (0.41 g, 2.7 mmol) in DMF (3 mL). The mixture was kept for 20 min t −4 °C and another 15 min at room temperature and was then mixed with solution A. After 22 h at room temperature, the reaction mixture was filtered from the precipitated DCU and the solvent was evaporated in vacuo. The remaining residue was solidified upon addition of water, filtered, washed copiously with 5% $NaHCO_3$ solution, water, 10% citric acid solution, and water, and dried over P_2O_5. Recrystallization from ethanoo–ether (4:1 v/v) gave 0.75 g (50%) of product: mp 199–202 °C; $[\alpha]1^{20}{}_D$ −38.4° (*c* 0.5, DMF); TLC R_f(B) 0.84, R_f(C) 0.80.

***N-t*-Boc-(*S*-Acm)Cys-(*S*-Acm)Cys-Phe-Phe-Gly-Leu-Met-NH₂, Boc-2A.** A portion of *N-t*-Boc-(*S*-Acm)Cys-Phe-Phe-Gly-Leu-Met-NH_2 (0.31 g, 0.35 mmol) was deprotected (1.7 N HCl/CH_3COOH), then dissolved in DMF (3 mL), neutralized (NMM), and allowed to react with *N-t*-Boc-(*S*-Acm)Cys-OH in the presence of DCC (0.072 g, 0.35 mmol) and HOBt (0.093 g, 0.63 mmol), for 24 h at room temperature as above. After the same workup as for the preceding compound, the product was obtained in a yield of 0.33 g (90%: mp 198–202 °C; $[\alpha]^{25}{}_D$ −29.2° (*c* 1, DMF); TLC R_f(B) 0.83, R_f(C) 0.72.

After deprotection with 2 N HCl in dioxane for 1 h followed by evaporation and lyophilization from H_2O, this compound, **2A**, was found to exert on the guinea pig ileum (RA 16.6) and particularly on the guinea pig trachea (RA 50.0) a fairly good, full agonistic effect (α^E 1.1). However, it remains a weak, partial agonist on the rabbit mesenteric vein (RA 9.3, α^E 0.6; see Table I).

***N-t*-Boc-Cys-Cys-Phe-Phe-Gly-Leu-Met-NH₂, Boc-2.** A solution of Boc-**2A** (320 mg, 0.3 mmol) in methanol (400 mL) was added to a solution of I_2 (0.38 g, 1.5 mmol) in methanol (120 mL) over a period of 30 min under vigorous stirring and at room temperature. After a further 30 min of agitation, the reaction mixture was cooled to 0 °C and a 1 N solution of sodium thiosulfate was added dropwise, until the deep brown color had disappeared. The solvent was reduced in vacuo to a volume of 10 mL, and the desired product was precipitated by addition of water (50 mL) and NaCl (0.5 g). The solid compound was filtered off, washed several times with water, and dried over (P_2O_5): yield 0.23 g (83%); mp 201–204 °C; $[\alpha]^{25}{}_D$ −41.2° (*c* 1, DMF); TLC R_f(B) 0.85, R_f(C) 0.79.

H-Cys-Cys-Phe-Phe-Gly-Leu-Met-NH₂ (2). A portion of boc-2 (200 mg, 0.22 mmol) was dissolved in formic acid 98% (10 mL) and left for 1 h at room temperature. The solvent was removed in vacuo, and the residue was triturated to a solid with ether, filtered, washed with dry ether, and dried in vacuo over P_2O_5 and KOH pellets. The yield was 171 mg (90%) of crude product which was subjected to gel chromatography on a 82 × 2.2 cm column of Sephadex G-15 eluted with 3 M acetic acid. It was further purified by partition chromatography on a 1.9 × 86 cm column of Sephadex G-25F that had been equilibrated with both phases of the solvent system 1-butanol–acetic acid–water (4:1:5 v/v). Peptide material was detected in the eluate by monitoring the absorbance at 280 and 254 nm: yield following

lyophilization 45 mg; TLC (single spot) R_f(B) 0.90, R_f(C) 0.62; mp 214–217 °C dec; $[\alpha]^{20}$D −20.8° (*c* 0.3, 2 M AcOH). Amino acids anal.: Cys, 1.85; Phe, 2.05; Gly, 1.03; Leu, 0.95; Met, 0.90; NH_3, 0.93. FAB-MS *m/z* 817 (M^+).

***N*-*t*-Boc-Leu-Cys(Acm)-NH$_2$.** NMM (0.45 mL, 4.12 mmol) and isobutyl chloroformate (0.53 mL, 4.12 mmol) were added to a solution of *N*-*t*-Boc-Leu-OH·H_2O (1.02 g, 4.12 mmol) in THF (20 mL) cooled to −10 °C. A solution of H-Cys(Acm)-NH$_2$ in THF (10 mL), prepared by deprotection of *N*-*t*-Boc-Cys(Acm)-NH$_2$ (1.20 g, 4.12 mmol) with TFA and neutralization of the resulting salt with NMM (0.45 mL, 4.12 mmol), was added to the anhydride. The reaction mixture was left to stand for 30 min at 0 °C and then for 1.5 h at room temperature. After filtration, the solvent was removed under reduced pressure and the oily residue was taken up in ethyl acetate, washed with 5% $NaHCO_3$ (3 × 20 mL) and water (2 × 20 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the oily residue was solidified upon trituration with ethyl acetate–petroleum ether (1:5 v/v): yield 810 mg (49%); melting from 71 °C (unclear); $[\alpha]^{20}$D −68.3° (*c* 1, MeOH); TLC R_f(E) 0.33.

***N*-*t*-Boc-Phe-Phe-Gly-Leu-Cys(Asm)-NH$_2$.** *N*-*t*-Boc-Leu-Cys(Acm)-NH$_2$ (0.69g, 1.7 mmol) was deprotected with a mixture of TFA–anisole (9:1 v/v; 10 mL). The resulting trifluoroacetate salt was dissolved in DMF (5 mL), neutralized with NMM, and allowed to react with *N*-*t*-Boc-Phe-Phe-Gly-OH (0.80 g, 1.7 mmol) in the presence of DCC (0.39 g, 1.9 mmol) and HOBt (0.47 g, 3.07 mmol), for 24 h at room temperature. During the reaction, the product precipitated progressively. After the solvent was removed in vacuo, the remaining residue was solidified upon addition of water, filtered, and washed copiously with 5% $NaHCO_3$, water, 10% citric acid solution, and water, and dried over P_2O_5. Trituration with ethanol under reflux gave 0.74 g (58%) of pure product: mp 230–231.5 °C; $[\alpha]^{25}$D −43.4° (*c* 0.5, DMF); TLC R_f(A) 0.71, R_f(B) 0.83.

***N*-*t*-Boc-Cys(Asm)-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$.** A portion of *N*-*t*-Boc-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$ (0.72 g, 0.95 mmol) was deprotected with TFA–anisole (9:1 v/v; 10 mL). The resulting trifluoroacetate salt was dissolved in DMF (3 mL), neutralized with NMM, and allowed to react with *N*-*t*-Boc-Cys(Asm)-OH (0.29 g, 1.0 mmol) in the presence of DCC (0.226 g, 1.1 mmol) and HOBt (0.27 g, 1.8 mmol) for 48 h at room temperature. After the same workup as for the preceding compound, the resulting pure product was obtained in a yield of 488 mg (55%): mp 219–220 °C; $[\alpha]^{20}$D −52° (*c* 0.5, DMF); TLC R_f(A) 0.63, R_f(E) 0.26, R_f(F) 0.75.

***N*-*t*-Boc-Cys-Phe-Phe-Gly-Leu-Cys-NH$_2$, Boc-3.** A solution of *N*-*t*-Boc-Cys(Acm)-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$ (200 mg, 0.21 mmol) in methanol (200 mL) was added to a solution of I_2 (275 mg, 1.08 mmol) in methanol (125 mL) over a period of 30 min, under vigorous stirring and at room temperature. After another 30 min of agitation, the reaction mixture was cooled to 0 °C and a 1 N solution of sodium thiosulfate was added dropwise, until the deep brown had disappeared. The solvent was evaporated under reduced pressure (to a volume of 5 mL), and the product was precipitated by addition of water. The solid compound was filtered off, washed with water, and dried over P_2O_5; TLC showed the presence of a small quantity of uncyclized *N*-*t*-Boc-Cys(Acm)-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$: yield 153 mg; mp 216–217 °C; $[\alpha]^{25}$D −61.9° (*c* 0.5, DMF); R_f(E) 0.35.

H-Cys-Phe-Phe-Gly-Leu-Cys-NH$_2$ (3). Compound Boc-**3** (330 mg) was dissolved in formic acid (98%; 10 mL) and left standing for 1.5 h at room temperature. The solvent was removed under reduced pressure, the residue was triturated to a solid with dry ether, filtered, washed with dry ether, and dried in vacuo over P_2O_5 and KOH pellets. The product (300 mg) was subjected to gel chromatography on a 70 × 2.5 cm column of Sephadex G-10 with 2 M acetic acid as eluent. The fractions were spotted on TLC, and the pure peptide fractions were pooled and yielded 225 mg of pure material: mp 198–200 °C; $[\alpha]^{25}$D −71.2° (*c* 0.2, MeOH); TLC R_f(A) 0.50, R_f(B) 0.67. Amino acids anal.: Cys, 1.89; Phe, 2.09; Gly, 1.02; Leu, 1.00, NH_3, 0.92. FAB-MS *m/z* 686 (M^+).

N^α-*t*-Boc-Gln[N^γ-$(CH_3)_2$]-Cys-Phe-Phe-Gly-Leu-Cys-NH$_2$ Boc-4. A portion of compound **3** (200 mg, 0.27 mmol) was dissolved in DMF (2 mL), neutralized with NMM, and allowed to react with N^α-*t*-Boc-Gln[N^γ-$(CH_3)_2$]-OH (81 mg, 0.29 mmol) in the presence of DCC (67 mg, 0.32 mmol) and HOBt (92 mg, 0.53 mmol) for 48 h at room temperature. After the same workup as for *N*-*t*-Boc-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$, the product was obtained in a yield of 150 mg (59%): mp 229–231 °C dec; $[\alpha]^{25}$D −65.7° (*c* 0.5, DMF); TLC R_f(E) 0.39.

H-Gln[N^γ-$(CH_3)_2$]-Cys-Phe-Phe-Gly-Leu-Cys-NH$_2$ (4). A sample of compound Boc-**4** (100 mg) was deprotected by treating with 4 N HCl in dioxane (3 mL) for 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was treated twice with methanol. The oily product was dissolved in 2 M CH_3COOH (5 mL), filtered through a Millipore filter, and subjected to gel chromatography on a 66 × 2.5 cm column of Sephadex G-15 with 2 M acetic acid as eluent. After lyophilization, 40 mg of pure peptide was recovered: mp 232 °C dec; $[\alpha]^{25}$D −95.3° (*c* 0.5, MeOH); TLC R_f(A) 0.32, R_f(B) 0.64. Amino acid anal.: Cys, 1.85; Phe, 2.07; Gly, 1.03; Glu 1.05; Leu, 1.00; NH_3, 0.94. FAB-MS *m/z* 842 (M^+).

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Registry No. 1, 96048-59-8; **1A**, 97721-54-5; **2**, 97731-60-7; Boc-**2**, 97731-61-8; **2A**, 97721-55-6; Boc-**2A**, 97721-56-7; **3**, 97731-62-9; Boc-**3**, 97731-63-0; **4**, 97731-64-1; Boc-**4**, 97731-65-2; Boc-Leu, 13139-15-6; (*p*-MeOBzl)Cys-OMe, 61314-87-2; *N*-*t*-Boc-Leu-(*p*-MeOBzl)Cys-OMe, 97721-57-8; *N*-*t*-Boc-Leu-(*p*-MeOBzl)Cys-NH$_2$, 97721-58-9; H≥Leu-Cys(*p*-MeOBzl)-NH$_2$·TFA, 97721-60-3; *N*-*t*-Boc-Gln-Phe-Phe-Gly-OH, 64699-00-9; *N*-*t*-Boc-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH$_2$, 97721-61-4; H-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH$_2$, 97731-66-3; *N*-Z-(*S*-Bzl)Cys-OSu, 3401-57-8; *N*-Z-(*S*-Bzl)Cys-Gln-Phe-Phe-Gly-Leu-(*p*-MeOBzl)Cys-NH$_2$, 97721-62-5; Boc-Leu-Met-NH$_2$, 2280-68-4; H-Leu-Met-NH$_2$·HCl, 2131-00-2; Boc-Phe-Phe-Gly-OH, 82816-76-0; *N*-*t*-Boc-Phe-Phe-Gly-Leu-Met-NH$_2$, 73148-99-9; H-Phe-Phe-Gly-Leu-Met-NH$_2$·HCl, 55572-15-1; *N*-*t*-Boc-(*S*-Acm)-Cys-OH, 19746-37-3; *N*-*t*-Boc-(*S*-Acm)Cys-Phe-Phe-Gly-Leu-Met-NH$_2$, 97721-63-6; *N*-*t*-Boc-Cys(Acm)-NH$_2$, 88530-31-8; H-Cys(Acm)-NH$_2$, 88530-32-9; *N*-*t*-Boc-Leu-Cys(Acm)-NH$_2$, 97721-64-7; H-Leu-Cys(Acm)-NH$_2$·TFA, 97721-66-9; *N*-*t*-Boc-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$, 97731-67-4; *N*-*t*-Boc-Cys-(Acm)-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$, 97721-67-0; H-Phe-Phe-Gly-Leu-Cys(Acm)-NH$_2$, 97721-68-1; N^2-*t*-Boc-Gln[N^γ-$(CH_3)_2$]-OH, 72449-42-4.