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Synthesis and Properties of a Heterodetic Cyclic Peptide: Gramicidin S Analog Containing Disulfide Bond

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In order to investigate the relationship between the structure of a peptide backbone and the formation of an intramolecular disulfide bond (S-S) in the peptide, a linear analog (**1**) of gramicidin S and a heterodetic cyclic peptide containing an S-S bond (**2**) were synthesized by the conventional solution method. Since a disulfide bonded compound (**2**) was easily formed by a treatment of the acetamidomethyl(Acm)-protected linear peptide (**1**) with iodine in good yield (70%), it is suggested that the mutual position between Cys^1 and Cys^8 residues becomes approximate, owing to the contribution of -D-Phe-Pro- part of the sequence. A heterodetic cyclic peptide (**2**) showed about 1/16—1/8 fold activity compared to that of GS; **1** as inactive. By the construction of an S-S bridge on the peptide backbone (**1**), an inactive peptide derivative was effectively converted to an active analog (**2**). The CD spectrum pattern also suggests that the heterodetic cyclic peptide (**2**) has a GS-like structure.

The disulfide bond (S-S), comprising two cysteine residues, is one of the most important factors in the secondary structure of biologically active peptides. One can find extensive examples showing the essential role of intramolecular disulfide bonds for biological activity in heterodetic cyclic peptides such as oxytocin, somatostatin, insulin, etc.[1] The disulfide bond can bring parts of amino acid residues, which are apparently remote from each other in a linear peptide, into close proximity; as a result, the flexible peptide structure is constrained to form a biologically active molecule by the disulfide bond.

Gramicidin S (GS) is a homodetic cyclic decapeptide antibiotic which exhibits a strong activity against Gram-positive bacteria. Its secondary structure is well known to be a unique antiparallel β-pleated sheet including four intramolecular hydrogen bonds between Val and Leu residues.[2] A series of studies on structure-activity relationships for GS analogs indicates that the biological activity of GS analogs is due to the rigid GS ring conformation and the orientation of the side chains: the charged Orn side chains are on one side and the hydrophobic Val and Leu side chains on the other side of the β-pleated sheet structure.[3]

We therefore became interested in studying both the synthesis and properties of heterodetic cyclic peptide analogs of gramicidin S containing two cysteine residues. When the disulfide bond is formed in flexible linear GS analogs, the molecule would be rigidly constrained to the desired β-sheet conformation, and some effects of the disulfide bond on activity are expected.

We studied, the synthesis, antimicrobial activity and circular dichroism (CD) measurements of synthetic linear analog of GS (**1**) as well as the analog contain-

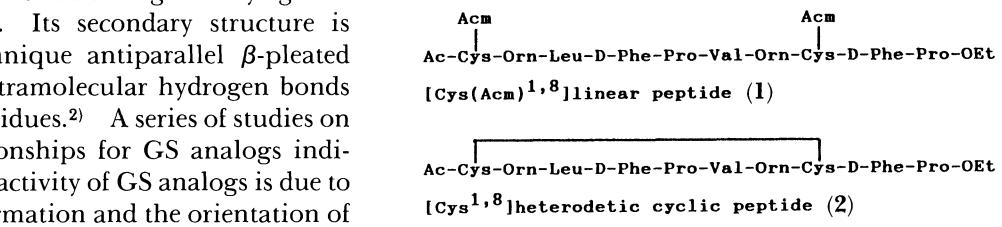

Fig. 2. Chemical structures of linear and heterodetic cyclic peptide analogs of gramicidin S.

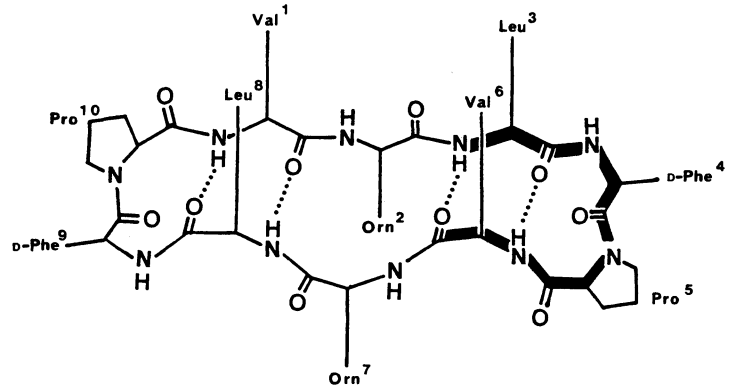

Fig. 1. A β-sheet conformation of gramicidin S. (The bold line indicates β-turn).

ing disulfide bond (**2**). In order to discuss the heterodetic cyclic structure of peptides, a temperature-dependent NMR study was also performed.

Results and Discussion

Synthesis. The syntheses of a [Cys(Acm)^{1,8}] linear decapeptide (**1**) and [Cys^{1,8}] heterodetic cyclic peptide containing a disulfide bond (**2**) are outlined in Fig. 3. The peptide synthesis was carried out by the conventional solution method. The mercapto group of cysteine was protected with an acetamidomethyl (Acm) group, which was easily removed to form a disulfide bond by iodine in MeOH.[4,5] Boc-pentapeptides (**5** and **9**) were prepared by coupling the corresponding Boc-dipeptide azide and H-tripeptide ester, respectively. Azide coupling of Boc-pentapeptide hydrazide (**6**) derived from Boc-pentapeptide ester (**5**), with **10** gave Boc-decapeptide (**11**). The crude product was purified by the column chromatography of Sephadex LH-20. The Boc-group of **11** was removed by trifluoroacetic acid (TFA) to give H-decapeptide ester (**12**). The N-terminal protecting groups of the final product of linear peptide (**1**) and heterodetic cyclic peptide (**2**) were converted to an acetyl (Ac) group which can be found in natural biological peptides and proteins. After acetylation of **12**, performed by *p*-nitrophenyl acetate, we obtained Ac-decapeptide ester (**13**).[6] The Z-group of **13** was removed by hydrogen bromide in AcOH, and the liberated decapeptide salt (**1**) was purified by gel filtration chromatography of Sephadex G-25.

The disulfide bond formation in the linear decapeptide (**13**) was carried out by treating a very dilute solution of **13** in MeOH (3×10^{-3} mol dm^{-3}) with iodine; the thus-obtained disulfide peptide (**14**) was purified by a column of Sephadex LH-20. The elution pattern of Sephadex LH-20 filtration indicated that the main peak contained the desired monomer, and two minor peaks before the main peak (hence, smaller retention times) could be attributed to compounds with higher molecular weights possessing intermolecular S-S bonds. The desired product from the main fraction was obtained in 70% yield.

A small amount of the main product was deprotected, and the sample was found to have the same molecular weight as that calculated for a heterodetic cyclic monomer. Fast atom bombardment (FAB) mass spectroscopy was used and the mentioned compound migrated the same distance as GS on paper electrophoretogram. The presence of an S-S bond in the compound (**2**) was ascertained by Chales' method, in which a peptide containing an S-S bond is reduced with $NaBH_4$ and detected by DTNB reagent.[7] The homogeneity of **1** and **2** was confirmed by several analytical experiments such as TLC, paper electrophoresis, and amino acid analysis.

Measurement of CD and NMR. The CD spectra of peptides (**2**, **13**, and **14**) with GS-like amino acid sequences and GS were measured in 2,2,2-trifluoroethanol at 25 °C. The thus-obtained infor-

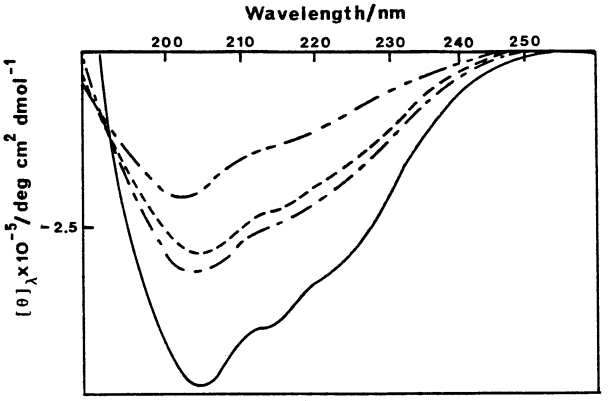

Fig. 4. CD spectra of linear peptide **13** (— - - —), protected heterodetic cyclic peptide **14** (— · —), (heterodetic cyclic peptide **2** (— —) and gramicidin S (——) in 2,2,2-trifluoroethanol at 25 °C.

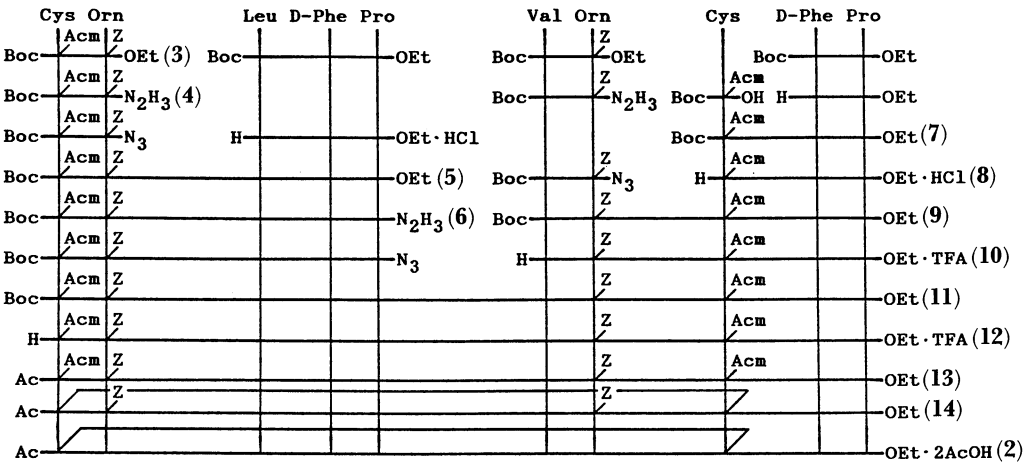

Fig. 3. Synthetic routes of linear and heterodetic cyclic peptide anlogs of gramicidin S.

mation made a comparison between the conformational behavior of heterodetic cyclic peptide (**14**) and the S-S bond free linear peptide (**13**) possible. The presence of a trough at the 205 nm region is usually observed in natural GS. Though these troughs in the spectra of the peptides (**2**, **13**, and **14**) were almost located in the same region as GS, they were shallower than that of GS. The CD curves of the heterodetic cyclic peptides (**2** and **14**), containing an S-S bond, show a characteristic shoulder (215 nm) similar to that of GS. However, the CD curve of the linear peptide (**13**) has no characteristic shoulder. Furthermore, the intensity of the trough of peptide (**14**), having a larger heterodetic ring, increased by two times in comparison with that of the corresponding open linear peptide (**13**). This result indicates that a flexible linear peptide is constrained by S-S bond formation between positions 1 and 8, and that the essential β-turn structure, thus made, in **14** results in a GS-like structure.

In a buffer solution the CD curves of peptides (**1**) and (**2**) were quite different from that of GS; in liposomes containing acidic phospholipid,[8] however, the intensity of the troughs of these analogs were markedly increased and the characteristic curves of these peptides were similar to that of GS. This seems to indicate that peptides (**1**) and (**2**) hold GS-like conformations in the presence of liposome. Also, the studies of Kaiser et al.[9] on peptide-lipid interactions suggest that the activity of biologically active peptides correlates with the behavior of the molecules in biological membranes, such as phospholipid membranes, rather than that of in an aqueous solution.

The low-temperature coefficients (3.1, 3.9, and 4.1) of amide proton chemical sifts in the ^{1}H NMR-experiments suggest that the three NH protons of heterodetic cyclic peptide (**14**) are solvent-shielded and probably participate in hydrogen-bonding.

Activity. The antibacterial activities of **1** and **2** are listed in Table 1. The linear decapeptide (**1**), which has no intramolecular S-S bond, shows no activity against the tested microorganisms. On the other hand, the [Cys^{1,8}]heterodetic cyclic peptide (**2**), which has a large heterodetic ring, was found to exhibit about 1/16—1/8 fold activity, compared with that of GS.

Relationship between the Structure and Activity. Schrage et al. reported that the formation of the

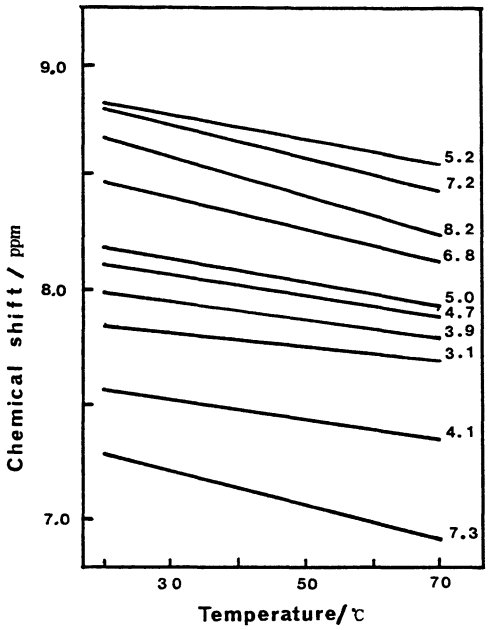

Fig. 6. Temperature dependences of amide proton chemical shifts of protected heterodetic cyclic peptide (**14**) in dimethyl-d_6 sulfoxide.
Side numbers of the lines refer to slopes (temperature coefficients; $\times 10^{-3}$ ppm °C^{-1}).

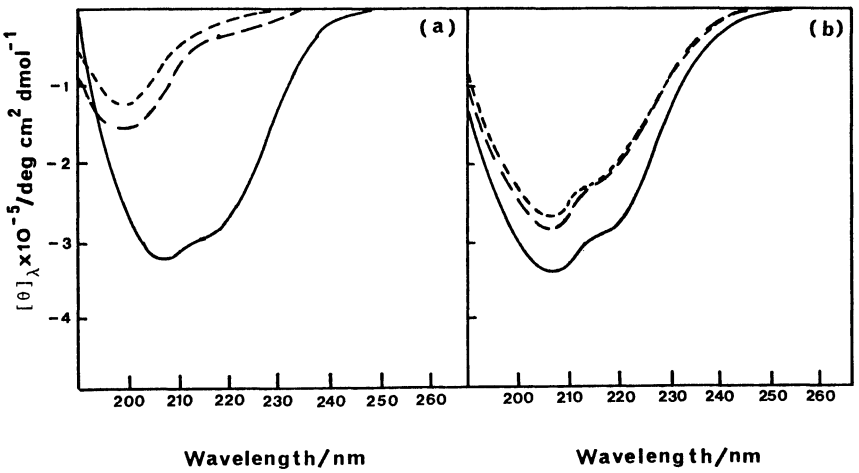

Fig. 5. CD spectra of gramicidin S (——) and linear peptide analogs **1** (------) and heterodetic cyclic peptide **2** (— —) in 20 mM Tris-HCl (pH 7.4) (a); and in presence of liposome of DPPC-DPPG (3:1) in the same buffer (b).

Table 1. Antimicrobial Activities of Gramicidin S and Linear Peptide (**1**) and Heterodetic Cyclic Peptide (**2**) (Minimum Inhibitory Concentration, $\mu g\,ml^{-1}$)

Organism	GS	(**1**)	(**2**)
S. aureus FDA 209P	3.18	>100	50
S. aureus 1840	3.18	>100	50
B. subtilis PCI 219	1.59	>100	12.5
S. flexneri EW-10	6.25	>100	100
S. sonnei EW-33	100	>100	>100
K. pneumoniae DT	50	>100	>100
E. coli NIHJ JC-2	>100	>100	>100
E. coli O-111	>100	>100	>100

desired S-S bond in a linear hexapeptide gave an intramolecular S-S bond in 20—30% yield.[10] In the case of the synthesis of [Cys^{2,7}]GS, which is a bicyclic peptide containing an intramolecular S-S bond by Schwyzer et al.,[11] S-S bond formation is carried out in 65% yield, since the conformational behavior of the decapeptide backbone is limited due to the cyclic structure of the β-turn peptide backbone. In our case, the S-S bond formation reaction of the open-chain decapeptide (**13**) at the 1 and 8 positions afforded a heterodetic cyclic monomer (**14**) exclusively in a high yield (70%). It is therefore apparent that the disulfide bond formation and β-turn are highly cooperative.

When an S-S bond is formed in protected linear peptide (**13**), its structural flexibility in the heterodetic cyclic peptide (**14**) is constrained to form a β-pleated sheet structure. The S-S bond between two cysteine residues at the 1- and 8-positions far from the β-turn portion should be suitable for taking a heterodetic cyclic structure. These considerations lead to the notion that the structure of heterodetic cyclic peptide (**14**) reflects an efficient maintenance of the GS-like conformation. Indeed, we observed a deeper trough of the S-S peptide (**14**) in 205 nm region of the CD spectra in trifluoroethanol than that of the linear peptide (**13**). The antibacterial activity was also compatible with the above-mentioned considerations. The heterodetic cyclic peptide (**2**) was partially active against several test microorganisms. Even though a lack of hydrophobic side chains (Val and Leu) in S-S peptide (**2**) might reduce the full activity, it is of interest that an inactive analog, such as linear peptide (**1**), could be converted to an active analog (**2**) through S-S bond formation at the effective positions for the β-turn in a linear peptide.

Experimental

General. All of the melting points were uncorrected. The molecular weight of the final compound (**2**) was determined by an FAB-Mass spectrometer using a JEOL JMS-D300 mass spectrometer. The CD spectra were obtained using a JASCO spectropolarimeter (Model J-20). Dipalmitoyl-DL-phosphatidylcholine (DPPC) and dipalmitoyl-DL-phosphatidylglycerol (DPPG) were purchased from Sigma. ^{1}H NMR spectra were recorded on a JEOL JNM GX-270 spectrometer, operating at 270 MHz using Fourier-transfer techniques. Chemical shifts were determined using tetramethylsilane as an internal standard. Thin-layer chromatography was carried out on silica gel (Merck 60 GF_{254}) with the following solvent systems: R_f^1, chloroform-methanol (5 : 1, v/v); R_f^2, chloroform-methanol (9 : 1); R_f^3, chloroform-methanol-acetic acid (8 : 1 : 1); R_f^4, chloroform-methanol-acetic acid (95 : 5 : 1); R_f^5, 1-butanol-acetic acid-water (4 : 1 : 2). The symbols and abbreviations used follow the IUPAC-IUB recommendations.[12] The other abbreviations used are as follows: Et_3N, triethylamine; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, *N,N*-dimethylformamide; NMM, *N*-methylmorpholine; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol.

All amino acid residues are of L-configuration unless specifically stated otherwise.

Boc-Cys(Acm)-Orn(Z)-OEt (3): To a solution of H-Orn(Z)-OEt·*p*-TosOH[13] (6.13 g, 13.2 mmol) in DMF (19.0 ml) and Et_3N (2.03 ml, 14.5 mmol) were added Boc-Cys(Acm)-OH (3.84 g, 13.2 mmol), HOBt (2.13 g, 15.8 mg), and DCC (2.72 g, 13.2 mmol) at 0 °C. After stirring for 2 days at room temperature, an insoluble material was removed by filtration and the filtrate evaporated in vacuo. The residue was dissolved in EtOAc (200 ml) and the solution successively washed with aqueous 10% citric acid, aqueous 4% $NaHCO_3$, and water. After being dried, the solution was evaporated in vacuo to leave an oil; the residue was solidified by the addition of ether and petroleum ether; yield 5.22 g (70%); mp 101—103 °C; $[\alpha]_D^{20}$ −15.3° (*c* 1.0, MeOH); R_f^1, 0.69; R_f^3, 0.80.

Found: C, 54.78; H, 7.23; N, 9.60%. Calcd for $C_{26}H_{40}N_4O_8S$: C, 54.91; H, 7.09; N, 9.85%.

Boc-Cys(Acm)-Orn(Z)-N_2H_3 (4): Hydrazine hydrate (5.5 ml, 110 mmol) was added to a solution of Boc-Cys(Acm)-Orn(Z)-OEt (3.13 g, 5.5 mmol) in DMF (6.5 ml) and the reaction mixture was kept at room temperature for 3 h. After the solution was evaporated, the residue was solidified by water (10 ml), then collected by filtration and then washed with water. The crude product was dried over P_2O_5 and recrystallized from EtOAc-ether-petroleum ether; yield 2.72 g (89%); mp 131—133 °C; $[\alpha]_D^{20}$ −20.9° (*c* 0.9, MeOH); R_f^1 0.51.

Found: C, 51.57; H, 7.00; N, 14.91%. Calcd for $C_{24}H_{38}N_6O_7S$: C, 51.97; H, 6.91; N, 15.15%.

Boc-Cys(Acm)-Orn(Z)-Leu-D-Phe-Pro-OEt (5): To a solution of Boc-Cys(Acm)-Orn(Z)-N_2H_3 (**4**) (2.22 g, 4.0 mmol) in DMF (9 ml) were added 4.1 mol dm^{-3} HCl in dioxane (1.94 ml, 8.0 mmol) and isopentyl nitrite (0.61 ml, 4.4 mmol) at −50 °C. After being left to stand at −20 °C for 10 min, the solution was recooled to −50 °C and neutralized with Et_3N (1.12 ml, 8.0 mmol). To this solution were added a chilled solution of H-Leu-D-Phe-Pro-OEt·HCl[14] (1.77 g, 4.0 mmol) in DMF (8 ml) and Et_3N (0.56 ml, 4.0 mmol). The reaction mixture was stirred for 4 h at −20 °C and for 6 days at −4 °C; it was then concentrated to a small volume of solution. The residue was dissolved in chloroform (370 ml) and washed as described for **3**. The solution was then evaporated in vacuo. The residual oil was solidified by the addition of ether-petroleum ether. The product

was recrystallized from EtOAc-ether-petroleum ether; yield 3.13 g (85%); mp 149—151 °C; $[\alpha]_D^{20}$ −47.4° (*c* 1.05, MeOH); R_f^1 0.64, R_f^2 0.73, R_f^3 0.64.

Found: C, 59.03; H, 7.28; N, 10.30%. Calcd for $C_{46}H_{67}N_7O_{11}S \cdot 1/2H_2O$: C, 59.08; H, 7.33; N, 10.48%.

Boc-Cys(Acm)-Orn(Z)-Leu-D-Phe-Pro-N_2H_3 (6): Compound **5** (2.78 g, 3.00 mmol) was treated with hydrazine hydrate (6 ml, 120 mmol) as described for **4**. The product was recrystallized from EtOAc-ether-petroleum ether; yield 2.16 g (79%); mp 116—119 °C; $[\alpha]_D^{20}$ −53.9° (*c* 0.8, MeOH); R_f^3 0.48.

Found: C, 58.23; H, 7.39; N, 13.89%. Calcd for $C_{44}H_{65}N_9O_{10}S$: C, 57.94; H, 7.18; N, 13.82%.

Boc-Cys(Acm)-D-Phe-Pro-OEt (7): This compound was prepared from Boc-Cys(Acm)-OH (3.83 g, 13.1 mmol) and H-D-Phe-Pro-OEt·HCl (4.28 g, 13.1 mmol) as described for **3**. The product was yielded as a foamy residue; yield 5.82 g (79%); mp 58—61 °C; $[\alpha]_D^{20}$ −56.5° (*c* 1.0, MeOH); R_f^1 0.92, R_f^3 0.73.

Found: C, 56.37; H, 7.32; N, 9.37%. Calcd for $C_{27}H_{40}N_4O_7 \cdot 1/2H_2O$: C, 56.53; H, 7.20; N, 9.77%.

H-Cys(Acm)-D-Phe-Pro-OEt·HCl (8): Compound **7** (2.26 g,4.0 mmol) was dissolved in formic acid (16 ml) and added 4.1 mol dm^{-3} HCl (1.9 ml) in dioxane at 0 °C. After 1 h, the solution was evaporated in vacuo. The addition of ether to the oily residue gave a hygroscopic product. The product was used for the next reaction without further purification; yield 1.81 g (90%); R_f^1 0.55, R_f^3 0.17, R_f^4 0.55, R_f^5 0.87.

Boc-Val-Orn(Z)-Cys(Acm)-D-Phe-Pro-OEt (9): This compound was prepared from Boc-Val-Orn(Z)-N_2H_3 (1.42 g, 2.96 mmol) and **8** (1.41 g, 2.96 mmol) by the same procedure as that for **5**. The crude product was recrystallized from MeOH-ether-petroleum ether; yield 1.79 g (70%); mp 148—150 °C; $[\alpha]_D^{20}$ −51.2° (*c* 1.05, MeOH); R_f^1 0.65, R_f^3 0.77.

Found: C, 58.51; H, 7.21; N, 10.28%. Calcd for $C_{45}H_{65}N_7O_{11}S \cdot H_2O$: C, 58.11; H, 7.26; N, 10.54%.

H-Val-Orn(Z)-Cys(Acm)-D-Phe-Pro-OEt·TFA (10): Compound **9** (1.37 g, 1.50 mmol) was dissolved in TFA (3 ml); the solution was allowed to stand for 30 min at 0 °C, and was then evaporated to dryness at 0 °C. The residue was solidified by the addition of ether, and dried over KOH. The product was used for the next reaction without further purification; yield 1.35 g (98%); R_f^3 0.33.

Boc-Cys(Acm)-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Cys(Acm)-D-Phe-Pro-OEt (11): This compound was prepared from **10** (0.68 g, 0.73 mmol) and **6** (0.67 g, 0.73 mmol), as described for **5**. The crude product was applied to a Sephadex LH-20 column (3.6×120 cm) and eluted with DMF. Fractions containing the pure compound were collected, and the product recrystallized from MeOH-ether-petroleum ether; yield 645 mg (48%); mp 116—119 °C; $[\alpha]_D^{20}$ −73.7° (*c* 0.17, TFE); R_f^2 0.46, R_f^3 0.72, R_f^4 0.19.

Found: C, 58.37; H, 7.05; N, 11.46%. Calcd for $C_{84}H_{118}N_{14}O_{19}S_2 \cdot 2H_2O$: C, 58.38; H, 7.12; N, 11.35%.

H-Cys(Acm)-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Cys(Acm)-D-Phe-Pro-OEt·TFA (12): Compound **11** (494 mg, 0.29 mmol) was treated with TFA (1.5 ml), as described for **10**. The product was used for the next reaction without further purification; yield 461 mg (93%); R_f^3 0.44, R_4^5 0.59.

Ac-Cys(Acm)-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Cys(Acm)-D-Phe-Pro-OEt (13): To a chilled solution of **12** (415 mg, 0.246 mmol) and Et_3N (0.051 ml, 0.369 mmol) in DMF (1.1 ml) was added *p*-nitrophenyl acetate (53 mg, 0.295 mmol), and the reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The solution was evaporated, leaving an oil; the residue was solidified by the addition of water. The product was collected by filtration and thoroughly washed with aqueous 10% citric acid, aqueous 4% $NaHCO_3$, and water. It was recrystallized from MeOH-ether-petroleum ether; yield 349 mg (85%); mp 109—112 °C; $[\alpha]_D^{20}$ −96.2° (*c* 0.08, TFE); R_f^2 0.73, R_f^3 0.73. Amino acid ratios in hydrolysate of **13**; Val 1.1, Orn 1.7, Leu 1.0, Phe 1.9, Pro 1.8, Cys 0.6.

Found: C, 58.16; H, 6.88; N, 11.93%. Calcd for $C_{81}H_{112}N_{14}O_{18}S_2 \cdot 2H_2O$: C, 58.26; H, 7.00; N, 11.74%.

Ac-Cys(Acm)-Orn-Leu-D-Phe-Pro-Val-Orn-Cys(Acm)-D-Phe-Pro-OEt·2AcOH (1): To a solution of compound **13** (102 mg, 62.8 μmol) in anisole (0.60 ml) and AcOH (0.06 ml) was added 25% HBr in AcOH (0.81 ml). The solution was left for 2 h at room temperature and then solidified by the addition of ether. The product was washed with ether by decantation and dried over KOH. The crude product was purified by column chromatography of Sephadex G-25 (aqueous 30% AcOH). The fractions containing the desired compound were collected and evaporated. The product was obtained as a hygroscopic powder; yield 90 mg (92%); R_f^5 0.21. Amino acid ratio in hydrolysate of **1**; Val 0.9, Orn 1.9, Leu 1.0, Phe 2.1, Pro 1.9, Cys 0.8.

Ac-Cys-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Cys-D-Phe-Pro-OEt (14): To a solution of iodine (365 mg, 0.144 mmol) in MeOH (480 ml) was gradually added a solution of **13** (237 mg, 0.144 mmol) in MeOH (480 ml) in 3 h under nitrogen gas atmosphere at room temperature; the solution was stirred for an additional 3 h. After 6 h, the reaction mixture was cooled to 0 °C and decolorized by the dropwise addition of 1 mol dm^{-3} $Na_2S_2O_3$ (2.8 ml). The solution was evaporated to dryness at room temperature, and the residue solidified by the addition of water. The product was collected and washed thoroughly with water. The product was applied to a Sephadex LH-20 column (3.6×120 cm) and eluted with DMF. The fractions containing the crude product were collected and evaporated. The residue was solidified by the addition of ether; yield 143 mg (67%); mp 143—145 °C; $[\alpha]_D^{20}$ −210° (*c* 0.12, TFE); R_f^2 0.39. R_f^4 0.32, R_f^6 0.94.

Found: C, 58.42; H, 6.80; N, 10.69%. Calcd for $C_{75}H_{100}N_{12}O_{16}S_2 \cdot 5/2H_2O$: C, 58.69; H, 6.90; N, 10.95%.

Ac-Cys-Orn-Leu-D-Phe-Pro-Val-Orn-Cys-D-Phe-Pro-OEt·2AcOH (2): To a solution of **14** (47.8 mg, 32 μmol) in anisole (0.03 ml) and AcOH (0.3 ml) was added with 25% HBr in AcOH (0.41 ml) as described for **1**. The product was applied to a Sephadex G-25 column and eluted with aqueous 30% AcOH. The fractions containing the pure compound and the crude material were collected and evaporated; yield 43.6 mg (80%); mp 128—131 °C; $[\alpha]_D^{20}$ −195° (*c* 0.19, TFE); R_f^5 0.63. Amino acid ratio in hydrolysate of **2**; Val 0.8, Orn 1.9, Leu 1.0, Phe 2.0, Pro 2.1, Cys 0.9.

Found: C, 51.46; H, 7.38; N, 11.34%. Calcd for $C_{59}H_{88}N_{12}O_{12}S_2 \cdot 2AcOH \cdot 7H_2O$: C, 51.55; H, 7.55; N, 11.45%.

Fast atom bombardment mass spectrum of **2** was obtained. A value of *m/z* equal to 1222 was observed, whereas the calculated value was 1221.

Microbiological Assays. The microorganisms employed are listed in Table 1. The minimum amount of the com-

pound necessary for the complete inhibition of growth was determined by a dilution method using a trypticase soy agar.

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