Synthesis of Gramicidin S Analogues Consisting of Fourteen Amino Acid Residues

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Four cyclotetradecapeptides related to gramicidin S, *cyclo*(-Val-Orn-Leu-Leu-D-Phe-Pro-Leu-)$_2$ (14-peptide-**1**), *cyclo*(-Val-Orn-Leu-D-Leu-D-Phe-Pro-Leu-)$_2$ (14-peptide-**2**), *cyclo*(-Val-Orn-Leu-Leu-D-Phe-Pro-D-Leu-)$_2$ (14-peptide-**3**), *cyclo*(-Val-Orn-Leu-D-Leu-D-Phe-Pro-D-Leu-)$_2$ (14-peptide-**4**), were prepared in order to investigate the contribution of the ring size and the amino acid sequences around the Pro residue to the antibiotic activity and conformation. The CD spectra of these synthetic peptides in ethanol solutions were different from each other, indicating that they possess various conformations in ethanol solutions. 14-Peptides-**1**, -**2**, and -**3** showed 1/4—1/8 of the antibiotic activity of gramicidin S against the gram-positive microorganisms tested, whereas 14-peptide-**4** showed no activity. A clear relationship between CD spectra and the antibiotic activity of these synthetic peptides could not be found.

Gramicidin S-1 (GS)[1] is an antibiotic cyclodecapeptide, *cyclo*(-Val-Orn-Leu-D-Phe-Pro-)$_2$,[2] with a rigid β-pleated sheet conformation.[3] From numerous investigations of the relationship between the structure and the antibiotic activity of this antibiotic, it has been proposed that the D-Phe-Pro-Val sequence in the β-turn part is essential for its antibiotic activity.[4,5] On the other hand, we found during studies of an antibiotic cyclododecapeptide, gratisin (GR),[6] that *cyclo*(-Val-Orn-Leu-D-Phe-Pro-D-Tyr-)$_2$, *cyclo*(-Val-Orn-Leu-D-Phe-D-Tyr-Pro-)$_2$, and *cyclo*(-Val-Orn-Leu-Pro-D-Phe-D-Phe-)$_2$ possess a strong activity, although their sequences in the β-turn part are different from each other.[7] These results suggest that the presence of an amino acid residue, D-Tyr or D-Phe,

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                          1   2   3   4    5    6   7
14-peptide-1  cyclo(-Val-Orn-Leu-Leu-D-Phe-Pro-Leu-)2
14-peptide-2  cyclo(-Val-Orn-Leu-D-Leu-D-Phe-Pro-Leu-)2
14-peptide-3  cyclo(-Val-Orn-Leu-Leu-D-Phe-Pro-D-Leu-)2
14-peptide-4  cyclo(-Val-Orn-Leu-D-Leu-D-Phe-Pro-D-Leu-)2
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Fig. 1. Primary structures of synthetic cyclotetradecapeptides.

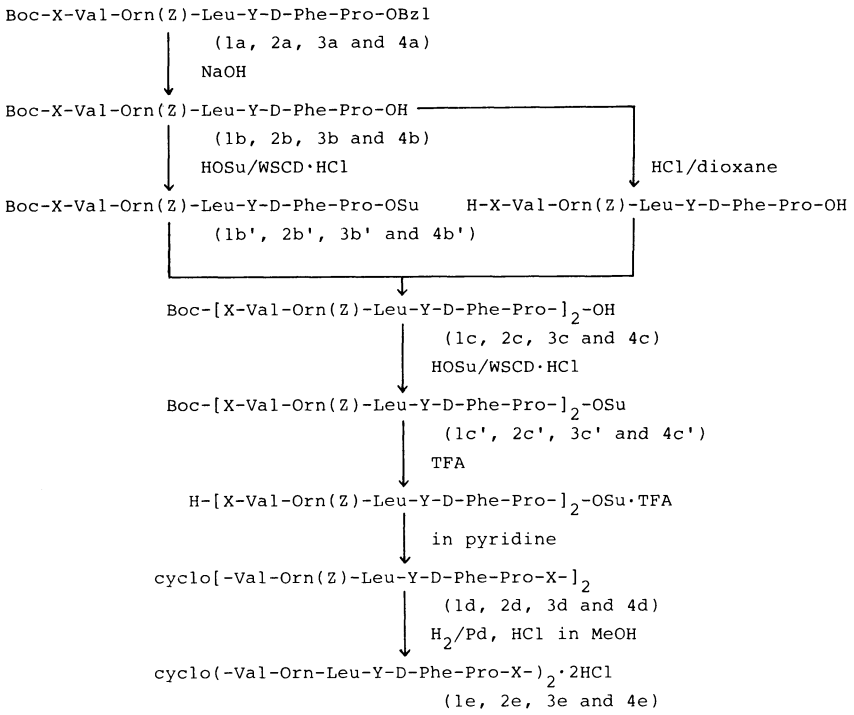

Fig. 2. Synthesis of cyclotetradecapeptides.
[X=Leu, Y=Leu (14-peptide-**1**), X=Leu, Y=D-Leu (14-peptide-**2**), X=D-Leu, Y=Leu (14-peptide-**3**), X=D-Leu Y=D-Leu (14-peptide-**4**)].

in gratisin peptides makes it possible for them to adopt a suitable conformation for antibiotic activity, regardless of the position of the Pro residue. Thus, it is of interest to study the structure-activity relationship of a cyclic peptide with a larger ring size than those of GS and GR. The configuration and the hydrophobic side chain of the amino acid residues around the D-Phe-Pro sequence are known to play an important role in stabilizing the β-turn formed by the D-Phe-Pro sequence.[4,7] In order to further investigate the contribution of the ring size and the amino acid sequence around the β-turn to the secondary structure and the antibiotic activity, we synthesized four cyclotetradecapeptides (Fig. 1) containing, respectively, partial sequences: Leu-D-Phe-Pro-Leu, D-Leu-D-Phe-Pro-Leu, Leu-D-Phe-Pro-D-Leu, and D-

Table 1. Yields, Physical Properties, and Analytical Data of Intermediary Products of 14-Peptide-**1**—**4**

		Yield/%	Mp θ_m/°C
1a	Boc-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OBzl	47	213—217
1b	Boc-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OH	88	196—198
1c	Boc-[Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-]$_2$-OH	83	255—260
1d	*cyclo*[-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-Leu-]$_2$	55	>340
2a	Boc-Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-OBzl	68	210—215
2b	Boc-Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-OH	82	206—207
2c	Boc-[Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-]$_2$-OH	87	223—225
2d	*cyclo*[-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-Leu-]$_2$	60	323—325 (decomp)
3a	Boc-D-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OBzl	71	204—207
3b	Boc-D-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OH	90	199—202
3c	Boc-[D-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-]$_2$-OH	30	209—210
3d	*cyclo*[-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-D-Leu-]$_2$	69	235—239
4a	Boc-D-Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-OBzl	44	190—194
4b	Boc-D-Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-OH	92	159—164
4c	Boc-[D-Leu-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-]$_2$-OH	67	197—201
4d	*cyclo*[-Val-Orn(Z)-Leu-D-Leu-D-Phe-Pro-D-Leu-]$_2$	67	303—304

	$[\alpha]_D^{20}$/° (DMF)			Elemental analysis (%) C	H	N	R_f^1	R_f^2
1a	−39.09	$C_{62}H_{90}O_{12}N_8 \cdot 0.5H_2O$	C:	64.84	7.99	9.76	0.69	0.56
	(*c* 1.1)		F:	64.86	8.05	9.76		
1b	−42.25	$C_{55}H_{84}O_{12}N_8 \cdot 0.5H_2O$	C:	62.42	8.10	10.59	0.36	0.42
	(*c* 1.1)		F:	62.32	8.18	10.52		
1c	−32.72	$C_{105}H_{158}O_{21}N_{16} \cdot 2.5H_2O$	C:	62.26	8.11	11.06	0.29	0.31
	(*c* 1.1)		F:	62.18	8.10	11.21		
1d	−137.12	$C_{100}H_{148}O_{18}N_{16} \cdot 2H_2O$	C:	63.27	8.07	11.81	0.55	0.57
	(*c* 0.7)		F:	63.47	8.00	11.93		
2a	−23.73	$C_{62}H_{90}O_{12}N_8$	C:	65.36	7.96	9.83	0.63	0.51
	(*c* 1.2)		F:	65.12	8.14	9.51		
2b	−20.89	$C_{55}H_{84}O_{12}N_8 \cdot 0.5H_2O$	C:	62.42	8.10	10.59	0.36	0.43
	(*c* 1.5)		F:	62.32	7.93	10.56		
2c	−18.38	$C_{105}H_{158}O_{21}N_{16} \cdot 2.5H_2O$	C:	62.26	8.11	11.06	0.34	0.32
	(*c* 1.2)		F:	62.48	8.18	10.80		
2d	−37.10	$C_{100}H_{148}O_{18}N_{16} \cdot H_2O$	C:	62.88	8.04	11.92	0.55	0.42
	(*c* 0.6)		F:	63.90	7.89	11.93		
3a	−22.58	$C_{62}H_{90}O_{12}N_8$	C:	65.36	7.96	9.83	0.60	0.48
	(*c* 1.2)		F:	65.11	7.97	9.66		
3b	−23.62	$C_{55}H_{84}O_{12}N_8 \cdot H_2O$	C:	61.89	8.12	10.50	0.28	0.34
	(*c* 1.0)		F:	61.92	8.07	10.09		
3c	−18.72	$C_{105}H_{158}O_{21}N_{16} \cdot 2.5H_2O$	C:	62.26	8.11	11.06	0.20	0.24
	(*c* 0.9)		F:	62.59	8.03	10.78		
3d	−38.60	$C_{100}H_{148}O_{18}N_{16} \cdot 2.5H_2O$	C:	62.97	8.08	11.75	0.55	0.48
	(*c* 0.9)		F:	62.71	7.95	11.88		
4a	−2.57	$C_{62}H_{90}O_{12}N_8$	C:	65.36	7.96	9.83	0.71	0.61
	(*c* 1.2)		F:	65.10	8.02	9.90		
4b	+1.17	$C_{55}H_{84}O_{12}N_8 \cdot 0.5H_2O$	C:	62.42	8.10	10.59	0.40	0.50
	(*c* 1.0)		F:	62.36	8.00	10.91		
4c	−2.74	$C_{105}H_{158}O_{21}N_{16} \cdot 1.5H_2O$	C:	62.82	8.08	11.16	0.40	0.52
	(*c* 1.0)		F:	62.88	8.00	11.14		
4d	+28.88	$C_{100}H_{148}O_{18}N_{16} \cdot 2H_2O$	C:	63.27	8.07	11.81	0.68	0.61
	(*c* 0.7)		F:	63.36	7.99	11.90		

Table 2. Yields, Physical Properties, and Analytical Data of 14-Peptide-**1**—**4**

1e *cyclo*(-Val-Orn-Leu-Leu-D-Phe-Pro-Leu-)$_2$·2HCl
Yield, 85%; mp 291—294 °C (decomp); $[\alpha]_D^{20}$ −95.28° (*c* 0.45, EtOH); R_f^3 0.76, R_f^4 0.68.
Amino acid ratios: Val, 1.01; Orn, 0.88; Leu, 3.04; Phe, 1.09; Pro, 0.97.
MS (FAB), *m/z* 1594 ($C_{84}H_{136}O_{14}N_{16}$, MH^+). Found: C, 57.01; H, 8.20; N, 12.12%.
Calcd for $C_{84}H_{136}O_{14}N_{16}$·2HCl·6$H_2O$: C, 56.84; H, 8.52; N, 12.62%.

2e *cyclo*(-Val-Orn-Leu-D-Leu-D-Phe-Pro-Leu-)$_2$·2HCl
Yield, 83%; mp 281—283 °C (decomp); $[\alpha]_D^{20}$ −76.72° (*c* 0.37, EtOH); R_f^3 0.76, R_f^4 0.65.
Amino acid ratios: Val, 1.03; Orn, 0.88; Leu, 3.00; Phe, 1.20; Pro, 0.90.
MS (FAB), *m/z* 1594 ($C_{84}H_{136}O_{14}N_{16}$, MH^+). Found: C, 57.41; H, 8.21; N, 12.33%.
Calcd for $C_{84}H_{136}O_{14}N_{16}$·2HCl·5$H_2O$: C, 57.42; H, 8.49; N, 12.75%.

3e *cyclo*(-Val-Orn-Leu-Leu-D-Phe-Pro-D-Leu-)$_2$·2HCl
Yield, 88%; mp 234—238 °C; $[\alpha]_D^{20}$ −52.0° (*c* 0.3, DMF); R_f^3 0.77, R_f^4 0.66.
Amino acid ratios: Val, 1.03; Orn, 0.93; Leu, 3.02; Phe, 1.05; Pro, 0.97.
MS (SIMS), *m/z* 1594 ($C_{84}H_{136}O_{14}N_{16}$, MH^+). Found: C, 57.77; H, 8.04; N, 12.91%.
Calcd for $C_{84}H_{136}O_{14}N_{16}$·2HCl·5$H_2O$: C, 57.42; H, 8.49; N, 12.75%.

4e *cyclo*(-Val-Orn-Leu-D-Leu-D-Phe-Pro-D-Leu-)$_2$·2HCl
Yield, 76%; mp 280—282 °C (decomp); $[\alpha]_D^{20}$ −9.15° (*c* 0.27, EtOH); R_f^3 0.79, R_f^4 0.67.
Amino acid ratios: Val, 1.06; Orn, 0.91; Leu, 3.00; Phe, 1.22; Pro, 0.90.
MS (SIMS), *m/z* 1594 ($C_{84}H_{136}O_{14}N_{16}$, MH^+). Found: C, 58.89; H, 8.42; N, 13.29%.
Calcd for $C_{84}H_{136}O_{14}N_{16}$·2HCl·2.5$H_2O$: C, 58.93; H, 8.42; N, 13.09%.

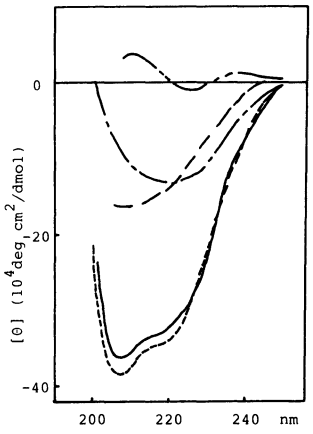

Fig. 3. CD spectra of synthetic cyclotetradecapeptides and GS in ethanol.
14-Peptide-**1** ——, 14-peptide-**2** — —, 14-peptide-**3** —·—, 14-peptide-**4** —··—, and GS ------.

Leu-D-Phe-Pro-D-Leu. The Leu residue was chosen as an amino acid residue having a hydrophobic side chain for an enlargement of the ring size.

These peptides were synthesized by conventional methods, as shown in Fig. 2. The yields, physical properties and analytical data regarding intermediary products are summarized in Table 1. The homogeneity of 14-peptide **1**—**4** was confirmed by means of thin-layer chromatography (TLC), amino acid analysis, high-performance liquid chromatography (HPLC), elemental analysis, and fast atom bombardment (FAB) or secondary ion (SI) mass spectrometry (Table 2).

The CD spectra of these synthetic peptides and GS in ethanol solutions are shown in Fig. 3. The spectral shapes of 14-peptide-**1**, -**2**, -**3**, and -**4** differ from each other, indicating that their peptides have different conformations in ethanol solutions. Recently, in CD spectroscopic studies of GS and GR, we reported that the CD spectra of their synthetic isomers could be classified into four groups, and that the isomers belonging to each group possess analogous partial sequences around the Pro residues.[5,7] 14-Peptide-**1**, having a D-Phe-Pro-Leu sequence, showed a similar CD curve to that of GS and GR-peptides having a D-Phe-Pro-X (X=Val or Tyr) sequence at the β-turn part, although the number of ring members of this peptide is different from that of GS or GR, and its ellipticity is almost the same as that of GS. This result indicates that 14-peptide-**1** possesses a conformation similar to that of GS in an ethanol solution, and that the amino acid sequence at the β-turn part makes a large contribution to the conformation. On the other hand, 14-peptides-**2**, -**3**, and -**4** showed CD spectra different from GS analogs and GR-peptides possessing Phe-Pro-D-X or D-Phe-Pro-D-X (X=Val or Tyr). These results suggest that it might be impossible to evaluate the contribution of the conformation around the Pro residue to the CD spectra of these 14-peptides by comparing their CD curves.

The antibiotic activity of these synthetic peptides against several microorganisms is summarized in Table 3. 14-Peptide-**1**, having a D-Phe-Pro-Leu sequence, showed 1/4—1/8 of the activity of GS against the gram-positive microorganisms tested. Recently, we reported that a GR-peptide having a D-Phe-Pro-Tyr sequence at the β-turn part possessed 1/4—1/16 of the activity of GS, and that its CD spectrum is similar to that of GS.[7] Supposing that the mode of action of these peptides having a -D-Phe-Pro- sequence at the β-turn part is the same as that of GS, the present results indicate that the ring size of a molecule is important in order to exhibit a strong and characteristic antibiotic activity, though it is not essential to exhibit activity. 14-Peptide-**2** and -**3** showed almost the same activity as that of 14-peptide-**1** toward gram-postive microorganisms, although their

Table 3. Antibiotic Activity of 14-Peptides-**1**—**4** and GS[a]

Test organisms	GS	**1**	**2**	**3**	**4**
Staph. aureus MS353 C36 C	3.13	25	25	12.5	>50
Strept. pyogenes N.Y. 5	3.13	12.5	12.5	12.5	>50
Corynebact. diphtheriae P.W. 8	1.56	12.5	12.5	25	>50
Bac. subtilis ATCC 6633	3.13	12.5	25	25	>50
E. coli NIHJ-JC2	>100	>50	>100	>50	>50

a) Minimum inhibitory concentration ($\mu g\,ml^{-1}$) was determined by an agar dilution method with 10^6 organisms per milliliter.

sequences at the β-turn part are different from each other. Similar results were obtained in studies of peptides related to GR.[7] These results indicate that, for exhibiting activity, the amino acid sequence at the β-turn part is not limited to a D-Phe-Pro-Val sequence, which is of GS. On the other hand, 14-peptide-**4** showed no activity. This result indicates that Leu residues at positions 7 and 7′ in 14-peptide-**2**, or at positions 4 and 4′ in 14-peptide-**3**, could not be substituted by D-Leu residues without affecting the activities.

Experimental

All melting points are uncorrected. The CD spectra were obtained by use of a JASCO spectropolarimeter, model J-500, using a 0.5-mm quartz cell at room temperature. The CD spectroscopy of GS and four cyclotetradecapeptides were carried out with ethanol solutions of their dihydrochlorides at a concentration of 1.5—2.0×10^{-4} M (1 M=1 mol dm^{-3}). The molecular weights of these synthetic peptides were determined by FAB mass spectrometry using a JEOL JMS-D-300 mass spectrometer and SI mass spectrometry using a HITACHI M-80. Amino acid analyses were carried out by a Hitachi 835 amino acid analyzer, after hydrolysis of the peptides in 6 M HCl at 110 °C for 24 h. HPLC was performed by an ODS column (φ 4.6×250 mm) using MeOH–5% aq $NaClO_4$(5:1) as an elution solvent. TLC was performed on Merck silica-gel F_{254} plates with the following solvent systems (v/v): R_f^1, $CHCl_3$–MeOH (9:1); R_f^2, $CHCl_3$–MeOH–AcOH (95:5:3); R_f^3, *n*-BuOH–AcOH–H_2O (4:1:1); R_f^4, *n*-BuOH–pyridine–AcOH–H_2O (4:1:1:2). The yields of **1a**, **2a**, **3a**, and **4a** were calculated on the basis of the amount of Pro-OBzl as a starting material.

Boc-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OBzl (1a). HCl·Pro-OBzl (1.70 g, 7 mmol) was dissolved in $CHCl_3$ (30 ml); the solution was then washed with 5% Na_2CO_3 and water under cooling in an ice bath. To this solution were added Boc-D-Phe (1.85 g, 7 mmol), HOBt (1.08 g, 8 mmol) and WSCD·HCl (1.33 g, 7 mmol) at 0 °C. This solution was stirred for 1 h at 0 °C and 2 h at room temperature. The reaction mixture was washed successively with 5% citric acid, water, and 5% Na_2CO_3 and water; the solvent was then evaporated in vacuo. The residue was dissolved in 4 M HCl/dioxane (20 ml) at 0 °C. After stirring for 30 min at room temperature, the solution was concentrated in vacuo. The residue was dissolved in $CHCl_3$ (30 ml); the solution was then washed with 5% Na_2CO_3 and water under cooling in an ice bath. To this solution were added Boc-Leu(1.68 g, 7 mmol), HOBt (1.08 g, 8 mmol) and WSCD·HCl (1.33 g, 7 mmol) at 0 °C. The same procedure as described above was repeated for this reaction mixture. Further, Boc-Leu, Boc-Orn(Z), Boc-Val, Boc-Leu were successively coupled by the same method. All reactions were followed by TLC on a silica-gel plate. The crude protected heptapeptide obtained from the final reaction mixture was purified by reprecipitation from AcOEt–ether; overall yield, 3.73 g (47% from Pro-OBzl).

Compounds **2a**, **3a**, and **4a** were prepared in a similar manner. Compounds **2a** and **4a** were purified by chromatography on a silica-gel column (φ 1.2×40 cm) using a solvent system of $CHCl_3$–MeOH (50:1) and reprecipitation from AcOEt–ether.

Boc-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OH (1b). To a solution of **1a** (3.00 g, 2.63 mmol) in MeOH (20 ml) and dioxane (10 ml), 1 M NaOH (5.3 ml) was added. The solution was stirred for 10 h at room temperature. 5% citric acid (100 ml) was added to the reaction mixture. The resulting solid was collected by filtration, washed with water and dried. The product was recrystallized from MeOH–ether–hexane; yield 2.42 g (88%).

Compounds **2b**, **3b**, and **4b** were prepared in a similar manner.

Boc-[Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-]$_2$-OH (1c). To a solution of **1b** (1.05 g, 1.0 mmol) in DMF (10 ml) were added HOSu (0.23 g, 2.0 mmol) and WSCD·HCl (0.38 g, 2.0 mmol) at 0 °C, and then the mixture was stirred for 5 h at room temperature. After the solution was evaporated, water was added to the residue. The resulting solid, Boc-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OSu (**1b′**), was collected by filtration, washed with water and then dried. Another crop of **1b** (1.05 g, 1.0 mmol) was dissolved in 4 M HCl/dioxane (10 ml) at 0 °C. After stirring a room temperature for 30 min, the solution was concentrated in vacuo. To a solution of H-Leu-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-OH (prepared as mentioned above) and TEA (0.28 ml, 2.00 mmol) in DMF (15 ml) at 0 °C was added **1b′** derived from **1b**. The mixture was left to stand for 2 h at 0 °C and overnight at room temperature. The solution was evaporated; then 5% citric acid was added to the residue. The resulting solid was collected by filtration, washed with water and then dried. The product was recrystallized from MeOH–ether–hexane; yield, 1.64 g (83%).

Compounds **2c**, **3c**, and **4c** were prepared in a similar manner.

***cyclo*[-Val-Orn(Z)-Leu-Leu-D-Phe-Pro-Leu-]$_2$ (1d).** Compound **1c** (0.80 g, 0.40 mmol) was converted into the *N*-hydroxysuccinimide ester (**1c′**) by the method described for **1c**. It was then dissolved in TFA (10 ml) at 0 °C. The mixture was stirred for 40 min at room temperature and then concentrated in vacuo. The residue, the trifluoroacetate of

the tetradecapeptide active ester, was triturated with ether and collected by filtration and dissolved in DMF (10 ml). The solution was poured, dropwise, into pyridine (350 ml) at 45 °C. After stirring for 3 h at 45 °C, the solution was concentrated. The addition of water to the residue afforded precipitates, which were filtered and washed with water. The purification of this compound was performed by chromatography on a silica-gel column (ϕ 1×20 cm) using a solvent system of $CHCl_3$–MeOH (50:1), followed by reprecipitation from MeOH–ether; yield, 0.41 g (55%).

Compounds **2d**, **3d**, and **4d** were prepared in a similar manner.

***cyclo*(-Val-Orn-Leu-Leu-D-Phe-Pro-Leu-)$_2$·2 HCl (1e).** Compound **1d** (121 mg, 0.065 mmol) was dissolved in 90% aq MeOH (15 ml) and dioxane (10 ml); then, 1 M HCl (0.15 ml) was added to the solution. This mixture was hydrogenolyzed in the presence of palladium black for 15 h. After removing the catalyst, the filtrate was concentrated in vacuo. The product was purified by gel filtration on a Sephadex LH-20 column (ϕ 1.2×120 cm) using MeOH as a solvent, and by reprecipitation from MeOH–ether; yield, 92 mg (85%).

Compounds **2e**, **3e**, and **4e** were prepared in a similar manner. However, in **2e**, the gel-filtration procedure was not required.

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References

1) Recently, it was reported that natural GS is a mixture of several homologs. The GS signified in the present paper corresponds to gramicidin S-1 in the report: S. Nozaki and I. Muramatsu, *J. Antibiot.*, **37**, 689 (1983).

2) Amino acid residues with no prefix are of L-configuration unless otherwise noted. The abbreviations for amino acids and peptides are in accordance with the rules of IUPAC-IBU Commission of Biological Nomenclature. Abbreviations used are as follows: Boc, *t*-butoxycarbonyl; Z, benzyloxycarbonyl; OBzl, benzyloxy; HOBt, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; WSCD·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimidehydrochloride; DMF, *N*,*N*-dimethylformamide; AcOEt, ethyl acetate; TEA, triethylamine; TFA, trifluoroacetic acid.

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