

Syntheses of Cyclic Deka-peptides with Four Ornithyl Residues Related to Gramicidin S¹⁾

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Three cyclic decapeptides with four L-ornithyl residues related to gramicidin S (GS), *cyclo*(-Orn-Leu-Orn-D-Phe-Pro-)₂ (**D-L-12**), *cyclo*(-Orn-Leu-Orn-Phe-Pro-)₂ (**L-L-12**), and *cyclo*(-Orn-Leu-Orn-Phe-D-Pro-)₂ (**L-D-12**), were synthesized to investigate the contribution of increase of the basic amino acid residues and of configurations of phenylalanyl and prolyl residues toward antibacterial activity. These GS-like analogs were synthesized by the solution method of peptide synthesis. Protected cyclic decapeptides were synthesized through a cyclization reaction of linear decapeptide azide in pyridine. Hydrogenolysis of the protected cyclic decapeptides afforded crystalline tetrahydrochlorides of the desired analogs. In the experiment of circular dichroism, **D-L-12** gave a curve similar to that of GS, while **L-L-12** and **L-D-12** gave different curves. Antibacterial assays showed **L-L-12** exhibited substantial activities against Gram-negative bacteria, whereas **D-L-12** and **L-D-12** negligible activity.

Gramicidin S (Fig. 1) is a cyclic decapeptide antibiotic, and exhibits the activity against Gram-positive bacteria. Its decapeptide backbone holds a rigid β -sheet structure stabilized with four hydrogen bonds as shown in Fig. 2. In the previous paper, we reported new function of a GS analog; [D-Dap^{4,4'}]GS exhibited the activity against Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhosa*, whereas GS shows no activity for these bacteria (Table 1).^{2,3)} We also observed that GS-like cyclic tetradecapeptide, *cyclo*(-Leu-Orn-Leu-Orn-Leu-D-Phe-Pro-)₂ (**LOP**), exhibited the activity against Gram-negative bacteria.⁴⁾ [D-Dap^{4,4'}]GS and LOP contain four basic amino acid residues and hold the β -sheet conformation similar to that of GS. It would be noteworthy that [D-Dap-(β -Z)^{4,4'}]GS, which contains two ornithyl residues and holds GS-like conformation, exhibited no activity against Gram-negative bacteria, but the activity against Gram-positive ones.³⁾

In the course of studies on the new function of GS analogs, we designed a GS-like analog contain-

ing four L-ornithyl residues, *cyclo*(-Orn-Leu-Orn-D-Phe-Pro-)₂ (**D-L-12** in Fig. 1). We assumed that **D-L-12** with D-Phe-L-Pro sequence holds GS-like conformation as shown in Fig. 2, then **D-L-12** possesses the activity against Gram-negative bacteria as [D-Dap^{4,4'}]GS or **LOP**. Additionally, we planned to synthesize two optical diastereomers of **D-L-12**, namely **L-L-12** with L-Phe-L-Pro and **L-D-12** with L-Phe-D-Pro sequence (Fig. 1); we were interested in examining possible activity for Gram-negative or positive bacteria though we could not speculate on the conformation for two GS-like analogs.

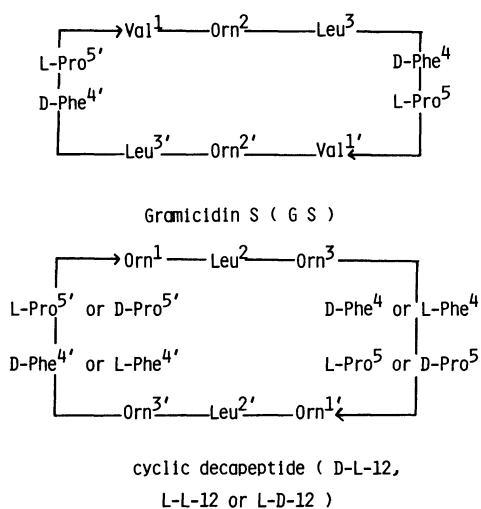


Fig. 1. Structures of GS and GS-like analogs. **D-L-12**, **L-L-12**, and **L-D-12** contain D-Phe-L-Pro, L-Phe-L-Pro, and L-Phe-D-Pro sequences, respectively.

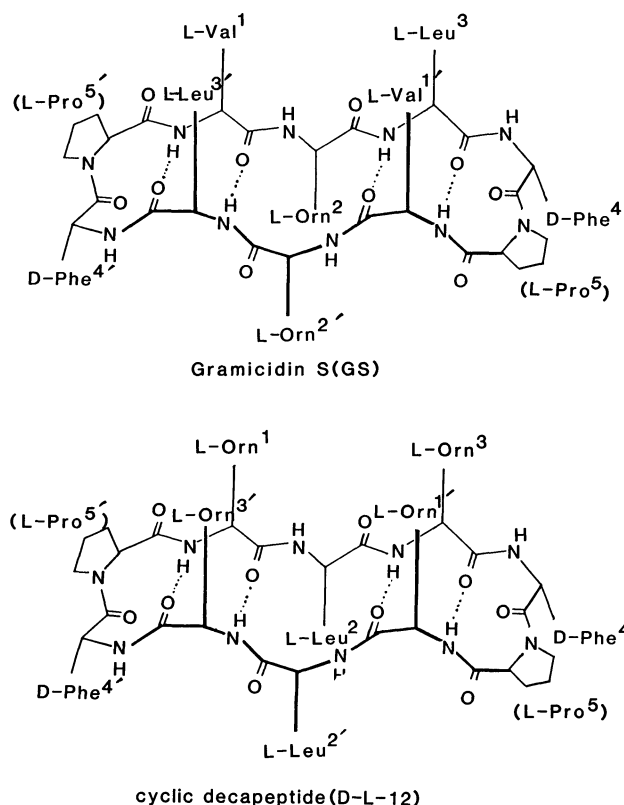


Fig. 2. Conformation of GS and assumed one of **D-L-12**.

Table 1. Antibacterial Activities of GS Analogs^{a)}

Organism	D-L-12	L-L-12	L-D-12	[D-Dap ^{4,4'}]GS ^{b)}	LOP ^{c)}	GS
<i>Staphylococcus aureus</i> FDA 209P	>100	50	>100	100	12.5	3.13
<i>Bacillus subtilis</i> PCI 219	50	12.5	12.5	12.5	6.25	3.13
<i>Escherichia coli</i> NIHJ JC-2	>100	50	>100	25	12.5	100
<i>Salmonella typhosa</i> Boxhill 58	>100	25	100	25	25	>100
<i>Shigella flexneri</i> EW-10	50	6.25	50	25	6.25	6.25
<i>Shigella sonnei</i> EW-33	100	25	>100	50	12.5	100
<i>Klebsiella pneumoniae</i> DT	100	25	>100	50	12.5	12.5

a) Numerals show the minimum inhibitory concentration ($\mu\text{g cm}^{-3}$). b) Data are cited from the literature.³⁾

c) Data are cited from the literature.⁴⁾ LOP represents *cyclo*(-Leu-Orn-Leu-Orn-Leu-D-Phe-Pro-)₂.

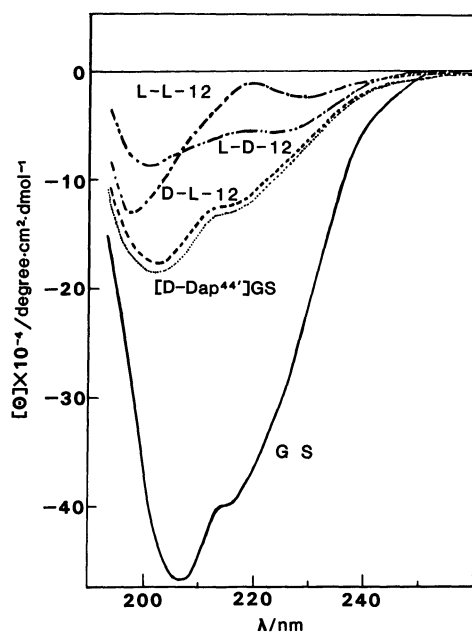


Fig. 4. CD spectra of GS-like analogs and GS in MeOH at 25°C. Curve of [D-Dap^{4,4'}]GS is cited from the literature.³⁾

by an osmometer for **11**, and by a fast atom bombardment (FAB) mass spectroscopy for **12**·4HCl.

Figure 4 shows CD curves of synthesized analogs and GS. The analog **D-L-12** exhibited the similar pattern for GS as expected, though the troughs (203 and 216 nm) were shallower than those of GS; the result suggested that the conformation of peptide-backbone is not changed significantly by the presence of L-Orn-L-Leu-L-Orn instead of L-Val-L-Orn-L-Leu sequence in GS. The CD curve of **D-L-12** is very similar to that of [D-Dap^{4,4'}]GS (Fig. 4).³⁾ Other analogs, **L-L-12** and **L-D-12**, exhibited the CD curves different from that of GS; the result suggests that the changes in conformation are due to presence of L-Phe-L-Pro and L-Phe-D-Pro sequence.

Results of the antibacterial assay are shown in Table 1. Unexpectedly, **D-L-12** showed negligible activity against Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhosa* and also against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. On the other hand, **L-L-12** showed substantial activity against several Gram-negative

bacteria, and even some activity for Gram-positive bacteria. Level of the activities for Gram-negative and -positive bacteria by **L-L-12** is similar to those by [D-Dap^{4,4'}]GS. Analog **L-D-12** showed weak activity against a Gram-positive bacteria, *Bacillus subtilis* PCI 219.

In the previous papers, we reported that [D-Dap^{4,4'}]GS and LOP containing four basic amino acid residues possess the activity against Gram-negative bacteria and hold GS-like conformation.^{3,4)} However, present cyclic decapeptide **D-L-12** satisfying two requirements (four basic amino acid residues and GS-like conformation) should no activity against Gram-negative bacteria. Whereas, the **L-L-12** holding a certain conformation other than that of GS exhibited the activity. At present, we can not give definite explanation why **D-L-12** shows no activity while **L-L-12** the activity. In this connection, it would be noteworthy that cyclic polymyxins, known for their activity against Gram-negative bacteria, contain six basic amino acid residues of all L-configuration out of ten component amino acid residues.⁶⁾

Experimental

All melting points are uncorrected. Prior to analysis, compounds were dried over P₂O₅ at 80°C and 2 mmHg[†]. Linear and cyclic decapeptide derivatives were dried over P₂O₅ at 25°C in a desiccator. TLC was carried out on Merck silica gel G with following solvent system: R_f 1-butanol-acetic acid-pyridine-water (4:1:1:2, v/v).

Z-Phe-D-Pro-OEt (L-D-1). To a solution of Z-Phe-OH (14.93 g, 50 mmol) in chloroform (70 cm³) was added DCC (11.57 g, 55 mmol) at -5°C. After several min, H-D-Pro-OEt·TsOH (15.77 g, 50 mmol) and triethylamine (7 cm³, 50 mmol) in chloroform (70 cm³) was added to the solution. After the mixture was stirred for 45 h at 5°C, resulting N,N'-dicyclohexylurea was filtered off and the filtrate was evaporated to dryness in vacuo. Oily residue was dissolved in ethyl acetate (200 cm³), and the solution was washed successively with 4% NaHCO₃, 2% HCl, water, and dried (Na₂SO₄). The filtrate from the salt was evaporated, and product was obtained as an oil; yield, 19.24 g (90%); R_f=0.97.

H-Phe-Pro-OEt·HCl (L-L-2). Z-Phe-Pro-OEt⁷⁾ (16.88 g, 40 mmol) in 1.7 M^{††} HCl in ethanol (34 cm³) was hydrogenated in the presence of Pd black. The filtrate was

[†]1 mmHg≈133.322 Pa.

^{††}1 M=1 mol dm⁻³.

evaporated, and the resulting crystals were collected by filtration with the aid of ether; yield, 10.3 g (87%); mp 153–154°C; $[\alpha]_D^{25} - 22^\circ$ (*c* 1, DMF); $R_f = 0.78$.

Found: C, 58.01; H, 7.39; N, 8.35%. Calcd for $C_{16}H_{23}O_3N_2Cl \cdot 1/4H_2O$: C, 58.00; H, 7.15; N, 8.45%.

H-Phe-D-Pro-OEt·HCl (L-D-2). This compound was prepared from **L-D-1** (19.24 g, 45 mmol) as described for the preparation of **L-L-2**. Product was obtained as an oil; yield, 14.71 g (100%); $R_f = 0.77$.

Boc-Orn(Z)-D-Phe-Pro-OEt (D-L-3). To a solution of Boc-Orn(Z)-OH (13.0 g, 35.5 mmol) in chloroform (60 cm³) was added DCC (7.21 g, 35.5 mmol) at -5°C. After several min, **D-L-2**⁹ (12.9 g, 35.5 mmol) and triethylamine (4.97 cm³, 35.5 mmol) in chloroform (60 cm³) was added to the solution. The mixture was stirred for 3 d at 5°C, the filtrate from *N,N'*-dicyclohexylurea was evaporated, and oily residue was dissolved in ethyl acetate (150 cm³). The solution was washed successively with 4% NaHCO₃, 0.5 M citric acid, water, and dried (Na₂SO₄). The filtrate was evaporated and product was obtained as an oil; yield, 17.2 g (76%); $R_f = 0.97$.

Boc-Orn(Z)-Phe-Pro-OEt (L-L-3). This compound was obtained from Boc-Orn(Z)-OH (10.5 g, 28.7 mmol) and **L-L-2** (10.4 g, 28.7 mmol) as described for the preparation of **D-L-3**; yield of an oil, 17.4 g (95%); $R_f = 0.93$.

Boc-Orn(Z)-Phe-D-Pro-OEt (L-D-3). This compound was obtained from Boc-Orn(Z)-OH (11.0 g, 30 mmol) and **L-D-2** (9.93 g, 30 mmol) as an oil; yield, 17.4 g (90%); $R_f = 0.95$.

H-Orn(Z)-D-Phe-Pro-OEt·HCl (D-L-4). Compound **D-L-3** (17.3 g, 26.9 mmol) was dissolved in 0.183 M HCl in formic acid (177 cm³). After standing at room temperature for 20 min, the solution was evaporated to dryness. Product was obtained as an oil; yield, 15.5 g (100%); $R_f = 0.81$.

H-Orn(Z)-Phe-Pro-OEt·HCl (L-L-4). This compound was obtained from **L-L-3** (16.9 g, 25.0 mmol) as described for the preparation of **D-L-4**. Product was obtained as an oil; yield, 13.7 g (90%); $R_f = 0.85$.

H-Orn(Z)-Phe-D-Pro-OEt·HCl (L-D-4). This compound was obtained from **L-D-3** (17.4 g, 27.0 mmol) as an oil; yield, 15.5 g (100%); $R_f = 0.84$.

Boc-Orn(Z)-Leu-Orn(Z)-D-Phe-Pro-OEt (D-L-5). To a solution of Boc-Orn(Z)-Leu-NHNH₂⁹ (11.3 g, 22.9 mmol) in DMF (70 cm³) at -20°C were added 2.02 M HCl in dioxane (34 cm³) and isopentyl nitrite (3.44 cm³, 25.2 mmol). After 5 min, the solution was neutralized with *N*-methylmorpholine (7.56 cm³, 68.7 mmol). To this solution was added a chilled solution of **D-L-4** (14.0 g, 22.9 mmol) and *N*-methylmorpholine (2.77 cm³, 25.2 mmol) in DMF (70 cm³). The mixture was stirred at 0°C for 7 d and evaporated in vacuo. Residual solid was dissolved in ethyl acetate (150 cm³), and the solution was washed successively with 0.5 M citric acid, 4% NaHCO₃, water, and dried (Na₂SO₄). The filtrate was evaporated and oily residue solidified upon the addition of ether. Product was obtained as crystalline solid; yield, 15.9 g (71%); mp 108–110°C; $[\alpha]_D^{25} - 64^\circ$ (*c* 0.1, MeOH); $R_f = 0.98$.

Found: C, 63.59; H, 7.58; N, 9.71%. Calcd for $C_{53}H_{73}O_{12}N_7$: C, 63.65; H, 7.36; N, 9.80%.

Boc-Orn(Z)-Leu-Orn(Z)-Phe-Pro-OEt (L-L-5). Azide derived from Boc-Orn(Z)-Leu-NHNH₂⁹ (9.87 g, 20 mmol) was coupled with **L-L-4** (13.7 g, 22.4 mmol) as described for the preparation of **D-L-5**. Product was recrystallized from ethyl acetate-ether-petroleum ether; yield, 14.1 g (68%); mp 98–99°C; $[\alpha]_D^{25} - 68^\circ$ (*c* 0.2, MeOH); $R_f = 0.93$.

Found: C, 62.96; H, 7.65; N, 9.55%. Calcd for $C_{53}H_{73}O_{12}N_7 \cdot 1/2H_2O$: C, 63.08; H, 7.39; N, 9.72%.

Boc-Orn(Z)-Leu-Orn(Z)-Phe-D-Pro-OEt (L-D-5). Azide derived from Boc-Orn(Z)-Leu-NHNH₂⁹ (6.18 g, 12.6 mmol) was coupled with **L-D-4** (7.67 g, 12.6 mmol) as described for the preparation of **D-L-5**. Product was obtained as crystalline solid; yield, 12.2 g (97%); mp 107–108°C; $[\alpha]_D^{25} - 3.58^\circ$ (*c* 0.2, DMF); $R_f = 0.93$.

Found: C, 62.87; H, 7.55; N, 10.06%. Calcd for $C_{53}H_{73}O_{12}N_7$: C, 63.65; H, 7.36; N, 9.80%.

Boc-Orn(Z)-Leu-Orn(Z)-D-Phe-Pro-NHNH₂ (D-L-6). A solution of **D-L-5** (8.29 g, 8.3 mmol) and hydrazine hydrate (16.1 cm³, 332 mmol) in DMF (40 cm³) was allowed to stand at room temperature for 7 d. Excess hydrazine was evaporated in vacuo, and water (60 cm³) was added to the residue. Resulting solid was collected by filtration; yield, 7.93 g (97%); mp 136–138°C; $[\alpha]_D^{25} - 45^\circ$ (*c* 0.3, AcOH); $R_f = 0.97$.

Found: C, 61.77; H, 7.50; N, 12.83%. Calcd for $C_{51}H_{71}O_{11}N_9$: C, 62.11; H, 7.26; N, 12.78%.

Boc-Orn(Z)-Leu-Orn(Z)-Phe-Pro-NHNH₂ (L-L-6). This hydrazide was obtained from **L-L-5** (10.4 g, 10 mmol) as described for the preparation of **D-L-6**; yield, 6.38 g (95%); mp 111–112°C; $[\alpha]_D^{25} - 47^\circ$ (*c* 0.5, AcOH); $R_f = 0.93$.

Found: C, 61.37; H, 7.55; N, 12.61%. Calcd for $C_{51}H_{71}O_{11}N_9 \cdot 1/2H_2O$: C, 61.66; H, 7.29; N, 12.67%.

Boc-Orn(Z)-Leu-Orn(Z)-Phe-D-Pro-NHNH₂ (L-D-6). This hydrazide was obtained from **L-D-5** (8.24 g, 8.24 mmol); yield, 7.92 g (96%); mp 119–120°C; $[\alpha]_D^{25} - 19.7^\circ$ (*c* 0.5, AcOH); $R_f = 0.93$.

Found: C, 60.07; H, 7.07; N, 12.63%. Calcd for $C_{51}H_{71}O_{11}N_9 \cdot 3/2H_2O$: C, 60.46; H, 7.33; N, 12.44%.

H-Orn(Z)-Leu-Orn(Z)-D-Phe-Pro-OEt·HCl (D-L-7). This compound was obtained from **D-L-5** (5.18 g, 5 mmol) as described for the preparation of **D-L-4**; yield of an oil, 4.68 g (100%); $R_f = 0.83$.

H-Orn(Z)-Leu-Orn(Z)-Phe-Pro-OEt·HCl (L-L-7). This compound was obtained from **L-L-5** (5.11 g, 5 mmol); yield of an oil; 4.68 g (100%); $R_f = 0.83$.

H-Orn(Z)-Leu-Orn(Z)-Phe-D-Pro-OEt·HCl (L-D-7). This compound was obtained from **L-D-5** (3.80 g, 3.8 mmol); yield of an oil; 3.50 g (100%); $R_f = 0.83$.

Boc[Orn(Z)-Leu-Orn(Z)-D-Phe-Pro]₂OEt (D-L-8). Azide derived from **D-L-6** (5.11 g, 5 mmol) was coupled with **D-L-7** (4.88 g, 5 mmol) as described for the preparation of **D-L-5**. Product was recrystallized from ethyl acetate-ether; yield, 5.78 g (62%); mp 139–142°C; $[\alpha]_D^{25} - 33^\circ$ (*c* 0.2, MeOH); $R_f = 0.95$.

Found: C, 63.61; H, 7.42; N, 10.26%. Calcd for $C_{99}H_{132}O_{21}N_{14} \cdot 3/2H_2O$: C, 63.61; H, 7.15; N, 10.31%.

Boc[Orn(Z)-Leu-Orn(Z)-Phe-Pro]₂OEt (L-L-8). Azide derived from **L-L-6** (5.11 g, 5 mmol) was coupled with **L-L-7** (4.88 g, 5 mmol). Product was obtained as crystalline solid; yield, 6.60 g (70%); mp 151–154°C; $[\alpha]_D^{25} - 53^\circ$ (*c* 0.2, MeOH); $R_f = 0.98$.

Found: C, 62.87; H, 7.40; N, 10.39%. Calcd for $C_{99}H_{132}O_{21}N_{14} \cdot 3H_2O$: C, 62.72; H, 7.21; N, 10.16%.

Boc[Orn(Z)-Leu-Orn(Z)-Phe-D-Pro]₂OEt (L-D-8). Azide derived from **L-D-6** (2.76 g, 2.8 mmol) was coupled with **D-L-7** (2.62 g, 2.8 mmol); yield of crystalline solid, 4.34 g (82%); mp 129–132°C; $[\alpha]_D^{25} - 8.36^\circ$ (*c* 0.2, MeOH); $R_f = 0.97$.

Found: C, 63.32; H, 7.47; N, 10.42%. Calcd for $C_{99}H_{132}O_{21}N_{14} \cdot 2H_2O$: C, 63.31; H, 7.17; N, 10.26%.

Boc[–Orn(Z)–Leu–Orn(Z)–D-Phe–Pro–]₂NHNH₂ (D-L-9). This compound was obtained from **D-L-8** (5.58 g, 2.98 mmol) as described for the preparation of **D-L-6**. Product was recrystallized from ethyl acetate–ether; yield, 4.94 g (90%); mp 107–110 °C; $[\alpha]_D^{25} -46^\circ$ (*c* 0.5, AcOH); $R_f=0.98$.

Found: C, 61.54; H, 7.49; N, 12.13%. Calcd for C₉₇H₁₃₀O₂₀N₁₆·3H₂O: C, 61.51; H, 7.24; N, 11.83%.

Boc[–Orn(Z)–Leu–Orn(Z)–Phe–Pro–]₂NHNH₂ (L-L-9). This hydrazide was obtained from **L-L-8** (3.72 g, 2.0 mmol); yield, 3.65 g (99%); mp 145–147 °C; $[\alpha]_D^{25} -49^\circ$ (*c* 0.5, AcOH); $R_f=0.98$.

Found: C, 61.49; H, 7.18; N, 12.04%. Calcd for C₉₇H₁₃₀O₂₀N₁₆·3H₂O: C, 61.51; H, 7.24; N, 11.83%.

Boc[–Orn(Z)–Leu–Orn(Z)–Phe–D-Pro–]₂NHNH₂ (L-D-9). This hydrazide was obtained from **L-D-8** (3.72 g, 2.0 mmol); yield, 3.39 g (80%); mp 139–142 °C; $[\alpha]_D^{25} -10.8^\circ$ (*c* 0.5, AcOH); $R_f=0.98$.

Found: C, 61.53; H, 7.38; N, 12.76%. Calcd for C₉₇H₁₃₀O₂₀N₁₆·3H₂O: C, 61.51; H, 7.24; N, 11.83%.

H[–Orn(Z)–Leu–Orn(Z)–D-Phe–Pro–]₂NHNH₂·2HCl (D-L-10). This compound was obtained from **D-L-9** (1.84 g, 1 mmol) as described for the preparation of **D-L-4**; yield of an oil, 1.81 g (100%); $R_f=0.82$.

H[–Orn(Z)–Leu–Orn(Z)–Phe–Pro–]₂NHNH₂·2HCl (L-L-10). This compound was obtained from **L-L-9** (1.81 g, 1.0 mmol); yield of an oil, 1.81 g (100%); $R_f=0.82$.

H[–Orn(Z)–Leu–Orn(Z)–Phe–D-Pro–]₂NHNH₂·2HCl (L-D-10). This compound was obtained from **L-D-9** (1.10 g, 0.6 mmol); yield of an oil, 1.10 g (100%); $R_f=0.82$.

cyclo[–Orn(Z)–Leu–Orn(Z)–D-Phe–Pro–]₂ (D-L-11). To a solution of **D-L-10** (1.81 g, 1 mmol) in DMF (30 cm³) at –20 °C, were added 1.71 M HCl in dioxane (1.75 cm³) and isopentyl nitrite (0.15 cm³, 1.1 mmol). After stirred at –20 °C until a hydrazine test became negative, this solution was added dropwise into pyridine (500 cm³) at 0 °C for 5 min, and stirring was continued for 2 h at 0 °C and for 65 h at 5 °C. After the solvent was removed, the residue was dissolved in a mixture (280 cm³, 6:1 v/v) of methanol and water, the solution was passed through the columns of Dowex 1 (OH[–] form) and 50 (H⁺ form). The columns were washed with the same solvent (560 cm³), and effluent was evaporated to dryness. The residue was collected by filtration with the aid of water. For purification, solution of the crude product (1.02 g) in DMF (6 cm³) was applied to a column (25×845 mm) with Sephadex LH-20, and development continued with DMF. Elution was carried out at room temperature at flow rate of 20 cm³/h; a 3 cm³ fraction was collected in each test tube. Peptide content in the fractions was determined with LKB 2138 Uvicord S at 245 nm. Fractions 55–65 containing **D-L-11** were evaporated, and product was collected by filtration with the aid of water; yield, 837 mg (49%); mp 107–109 °C; $[\alpha]_D^{25} -71^\circ$ (*c* 0.1, MeOH); $R_f=0.98$.

Found: C, 61.15; H, 7.03; N, 11.27%. Calcd for C₉₂H₁₁₈O₁₈N₁₄·5H₂O: C, 61.45; H, 7.18; N, 10.91%.

cyclo[–Orn(Z)–Leu–Orn(Z)–Phe–Pro–]₂ (L-L-11). This compound was prepared from **L-L-10** (1.81 g, 1 mmol) as described for the preparation of **D-L-11**. Product was collected by filtration with the aid of water; yield, 775 mg (45%); mp 135–140 °C; $[\alpha]_D^{25} -43^\circ$ (*c* 0.1, MeOH); $R_f=0.97$.

Found: C, 64.80; H, 7.22; N, 11.18%. Calcd for C₉₂H₁₁₈O₁₈N₁₄: C, 64.49; H, 7.22; N, 11.48%.

cyclo[–Orn(Z)–Leu–Orn(Z)–Phe–D-Pro–]₂ (L-D-11). This compound was prepared from **L-D-10** (1.09 g, 0.6 mmol);

yield, 568 mg (55%); mp 139–141 °C; $[\alpha]_D^{25} -37.5^\circ$ (*c* 0.1, MeOH); $R_f=0.98$.

Found: C, 62.16; H, 6.99; N, 11.32%. Calcd for C₉₂H₁₁₈O₁₈N₁₄·4H₂O: C, 62.08; H, 7.13; N, 11.02%.

cyclo[–Orn–Leu–Orn–D-Phe–Pro–]₂·4HCl (D-L-12·4HCl). Compound **D-L-11** (200 mg, 0.12 mmol) in 0.094 M HCl in methanol (6.2 cm³) was hydrogenated in presence of Pd black. The filtrate was evaporated and resulting crystals were collected by filtration with the aid of ether. Product was recrystallized from methanol–ether; yield, 129 mg (82%); mp 209–211 °C; $[\alpha]_D^{25} -68^\circ$ (*c* 0.1, MeOH); $R_f=0.78$. Amino acid ratios: Orn, 1.98; Leu, 1.04; Phe, 1.00; Pro, 0.97.

Found: C, 48.40; H, 7.72; N, 12.90%. Calcd for C₆₀H₉₈O₁₀N₁₄Cl₄·10H₂O: C, 48.12; H, 7.94; N, 13.09%.

cyclo[–Orn–Leu–Orn–Phe–D-Pro–]₂·4HCl (L-L-12·4HCl). This compound was prepared from **L-L-11** (200 mg, 0.12 mmol); yield, 101 mg (65%); mp 192–194 °C; $[\alpha]_D^{25} -46^\circ$ (*c* 0.1, MeOH); $R_f=0.75$. Amino acid ratios: Orn, 1.96; Leu, 1.06; Phe, 1.00; Pro, 0.99.

Found: C, 50.66; H, 7.88; N, 13.50%. Calcd for C₆₀H₉₈O₁₀N₁₄Cl₄·6H₂O: C, 50.56; H, 7.78; N, 13.76%.

cyclo[–Orn–Leu–Orn–Phe–D-Pro–]₂·4HCl (L-D-12·4HCl). This compound was prepared from **L-D-11** (200 mg, 0.12 mmol); yield, 104 mg (68%); mp 194–196 °C; $[\alpha]_D^{25} -30^\circ$ (*c* 0.1, MeOH); $R_f=0.73$. Amino acid ratios: Orn, 2.03; Leu, 0.98; Phe, 1.00; Pro, 0.96.

Found: C, 50.64; H, 7.83; N, 13.60%. Calcd for C₆₀H₉₈O₁₀N₁₄Cl₄·6H₂O: C, 50.56; H, 7.78; N, 13.76%.

Molecular Weight Determination. Molecular weight (MW) of Z-substituted cyclic decapeptides was determined by the use of CORONA osmometer type 117 in DMF as a solvent. The values of MW for three analogs (**D-L-11**, **L-L-11**, and **L-D-11**) were 1788, 1698, and 1720, respectively, whereas the calculated value is 1708. FAB mass spectra of three analogs were obtained by the use of JEOL JMS-DX300. The values of *m/z* 1172 were observed, whereas the calculated value as [C₆₀H₉₅O₁₀N₁₄ (M+H)⁺] is 1172.

Amino Acid Analysis. Analyses were carried out using JASCO HPLC amino acid analysis system, after hydrolysis of the peptides in 6 M HCl at 110 °C for 24 h.

Electrophoresis. Electrophoresis on Toyo Roshi No. 52 paper was carried out with the solvent system, formic acid–acetic acid–methanol–water (1:3:6:10, v/v; pH 1.8), for 2 h at 500 V/30 cm. Each of three analogs gave single spot. Ratios of mobility of **D-L-12**, **L-L-12**, and **L-D-12** toward GS were 1.43, 1.44, and 1.44, respectively.

CD Measurement. Measurement of CD spectra was performed with JASCO Model J-40 over a wavelength range of 190 to 260 nm in methanol as a solvent at 25 °C. Cell of 0.01 and 0.1 cm path length was used. In Fig. 5 are shown CD spectra of the analogs and GS.

Antibacterial Assays. Minimum amount of peptides necessary for the complete inhibition of growth was determined by a dilution method with Bouillon agar medium. Results are shown in Table 1.

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