Syntheses of Cyclic Decapeptides with Four Ornithyl Residues Related to Gramicidin S[1)]

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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 containing 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.

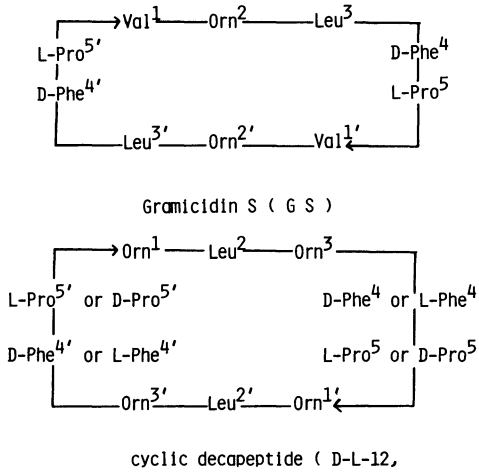

Fig. 1. Structures of GS and GS-like analogs. **D-L-12**, **L-L-12**, and **L-D-12** contain D-Phe-L-Pro, L-Phe-L-Pro, and L-Phe-D-Pro sequences, respectively.

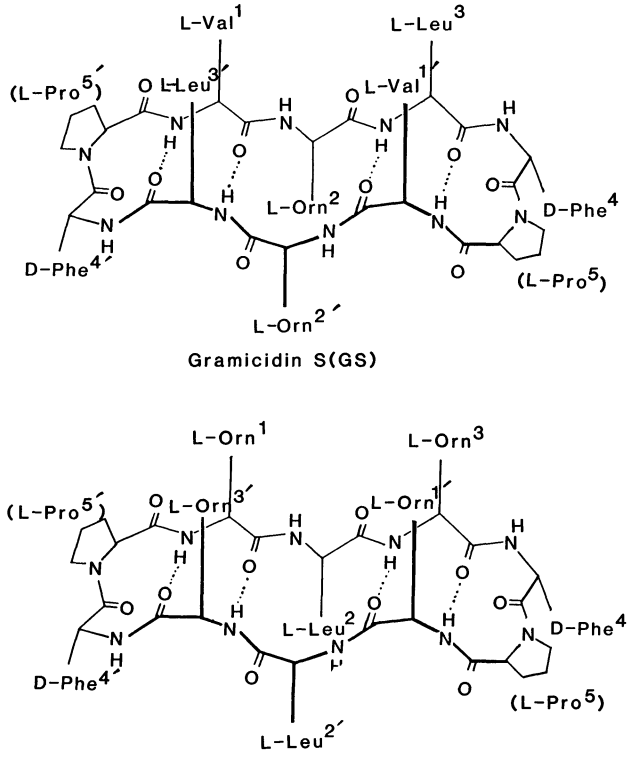

Fig. 2. Conformation of GS and assumed one of **D-L-12**.

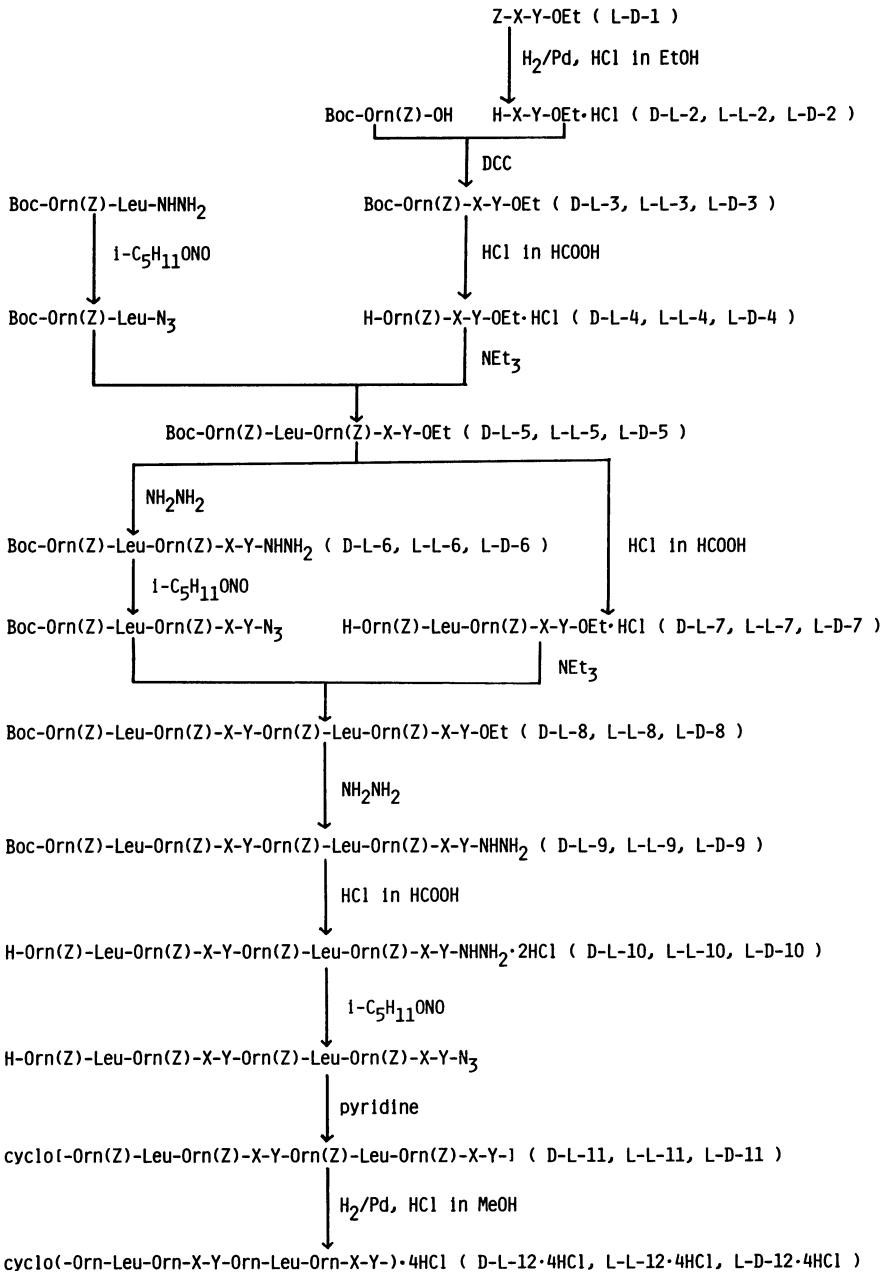

Fig. 3. Syntheses of GS-like analogs. **D-L**-series; X=D-Phe, Y=L-Pro. **L-L**-series; X=L-Phe, Y=L-Pro. **L-D**-series; X=L-Phe, Y=D-Pro.

Syntheses of tetrahydrochlorides of three analogs are outlined in Fig. 3. Boc-pentapeptide ester (**5**) was prepared by the coupling of Boc-dipeptide azide with H-tripeptide ester (**4**). A part of **5** was converted into Boc-pentapeptide hydrazide (**6**), and **6** was changed to the corresponding azide. Coupling of the azide and H-pentapeptide ester (**7**) afforded Boc-decapeptide ester (**8**) which was converted to the corresponding hydrazide (**9**). The Boc-hydrazide **9** was converted to H-decapeptide hydrazide (**10**), and **10** was changed to H-decapeptide azide. Then, the azide was subjected to cyclization in pyridine, and the protected cyclic decapeptide (**11**) was obtained in 49% yield for **D-L-11**, 45% **L-L-11** or 55% **L-D-11**. It should be noted that we could obtain pure cyclic decapeptide (**L-L-11**) with all L-amino acid residues in a good yield (45%) from the cyclization product of H-decapeptide azide though several investigators only isolated cyclic decapeptide in poor yield by the cyclization of H-peptide active ester with all L-amino acid residues.[5] Desired crystalline **D-L-12·4HCl**, **L-L-12·4HCl** or **L-D-12·4HCl** was obtained by hydrogenolysis of each **11** in methanol containing hydrogen chloride.

The homogeneity of **11** and **12·4HCl** was ascertained by several analytical experiments such as TLC, paper electrophoresis and amino acid analysis. The fact that **11** and then **12·4HCl** are a cyclic monomer was confirmed by determination of molecular weight

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
Staphylococcus aureus FDA 209P	>100	50	>100	100	12.5	3.13
Bacillus subtilis PCI 219	50	12.5	12.5	12.5	6.25	3.13
Escherichia coli NIHJ JC-2	>100	50	>100	25	12.5	100
Salmonella typhosa Boxhill 58	>100	25	100	25	25	>100
Shigella flexneri EW-10	50	6.25	50	25	6.25	6.25
Shigella sonnei EW-33	100	25	>100	50	12.5	100
Klebsiella pneumoniae DT	100	25	>100	50	12.5	12.5

a) Numerals show the minimum inhibitory concentration ($\mu g\,cm^{-3}$). 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-)$_2$.

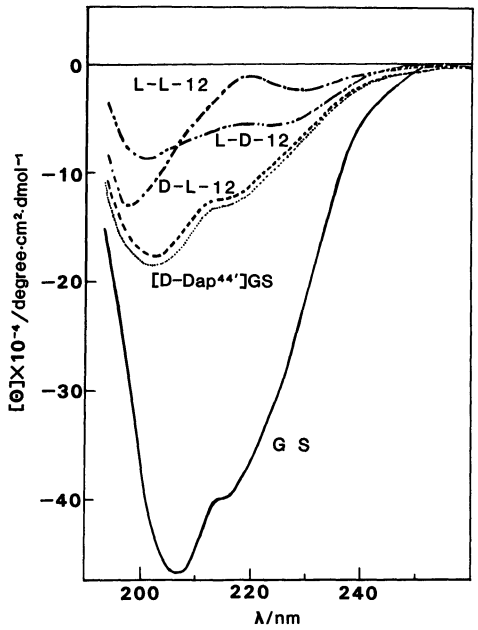

Fig. 4. CD spectra of GS-like analogs and GS in MeOH at 25 °C. Curve of [D-Dap$^{4,4'}$]GS is cited from the literature.[3)]

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 P_2O_5 at 80 °C and 2 mmHg†. Linear and cyclic decapeptide derivatives were dried over P_2O_5 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 cm^3) 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 cm^3, 50 mmol) in chloroform (70 cm^3) 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 cm^3), and the solution was washed successively with 4% $NaHCO_3$, 2% HCl, water, and dried (Na_2SO_4). 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-OEt[7)] (16.88 g, 40 mmol) in 1.7 M†† HCl in ethanol (34 cm^3) 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° (*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**[8] (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% $NaHCO_3$, 0.5 M citric acid, water, and dried (Na_2SO_4). 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-$NHNH_2$[9] (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% $NaHCO_3$, water, and dried (Na_2SO_4). 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° (*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; N, 7.36; N, 9.80%.

Boc-Orn(Z)-Leu-Orn(Z)-Phe-Pro-OEt (L-L-5). Azide derived from Boc-Orn(Z)-Leu-$NHNH_2$[9] (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° (*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-$NHNH_2$[9] (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° (*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-$NHNH_2$ (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° (*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-$NHNH_2$ (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° (*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-$NHNH_2$ (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° (*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-]$_2$OEt (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° (*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-]$_2$OEt (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° (*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-]$_2$OEt (L-D-8). Azide derived from **L-D-6** (2.76 g, 2.8 mmol) was coupled with **L-D-7** (2.62 g, 2.8 mmol); yield of crystalline solid, 4.34 g (82%); mp 129—132 °C; $[\alpha]_D^{25}$ −8.36° (*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-]$_2$NHNH$_2$ (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° (*c* 0.5, AcOH); R_f=0.98.

Found: C, 61.54; H, 7.49; N, 12.13%. Calcd for $C_{97}H_{130}O_{20}N_{16}\cdot 3H_2O$: C, 61.51; H, 7.24; N, 11.83%.

Boc[-Orn(Z)-Leu-Orn(Z)-Phe-Pro-]$_2$NHNH$_2$ (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° (*c* 0.5, AcOH); R_f=0.98.

Found: C, 61.49; H, 7.18; N, 12.04%. Calcd for $C_{97}H_{130}O_{20}N_{16}\cdot 3H_2O$: C, 61.51; H, 7.24; N, 11.83%.

Boc[-Orn(Z)-Leu-Orn(Z)-Phe-D-Pro-]$_2$NHNH$_2$ (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° (*c* 0.5, AcOH); R_f=0.98.

Found: C, 61.53; H, 7.38; N, 12.76%. Calcd for $C_{97}H_{130}O_{20}N_{16}\cdot 3H_2O$: C, 61.51; H, 7.24; N, 11.83%.

H[-Orn(Z)-Leu-Orn(Z)-D-Phe-Pro-]$_2$NHNH$_2\cdot$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-]$_2$NHNH$_2\cdot$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-]$_2$NHNH$_2\cdot$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 cm^3) at −20 °C, were added 1.71 M HCl in dioxane (1.75 cm^3) and isopentyl nitrite (0.15 cm^3, 1.1 mmol). After stirred at −20 °C until a hydrazine test became negative, this solution was added dropwise into pyridine (500 cm^3) 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 cm^3, 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 cm^3), 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 cm^3) 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 cm^3/h; a 3 cm^3 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° (*c* 0.1, MeOH); R_f=0.98.

Found: C, 61.15; H, 7.03; N, 11.27%. Calcd for $C_{92}H_{118}O_{18}N_{14}\cdot 5H_2O$: 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° (*c* 0.1, MeOH); R_f=0.97.

Found: C, 64.80; H, 7.22; N, 11.18%. Calcd for $C_{92}H_{118}O_{18}N_{14}$: 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° (*c* 0.1, MeOH); R_f=0.98.

Found: C, 62.16; H, 6.99; N, 11.32%. Calcd for $C_{92}H_{118}O_{18}N_{14}\cdot 4H_2O$: C, 62.08; H, 7.13; N, 11.02%.

***cyclo*(-Orn-Leu-Orn-D-Phe-Pro-)$_2\cdot$4HCl (D-L-12·4HCl).** Compound **D-L-11** (200 mg, 0.12 mmol) in 0.094 M HCl in methanol (6.2 cm^3) 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° (*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 $C_{60}H_{98}O_{10}N_{14}Cl_4\cdot 10H_2O$: C, 48.12; H, 7.94; N, 13.09%.

***cyclo*(-Orn-Leu-Orn-Phe-Pro-)$_2\cdot$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° (*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 $C_{60}H_{98}O_{10}N_{14}Cl_4\cdot 6H_2O$: C, 50.56; H,7.78; N, 13.76%.

***cyclo*(-Orn-Leu-Orn-Phe-D-Pro-)$_2\cdot$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° (*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 $C_{60}H_{98}O_{10}N_{14}Cl_4\cdot 6H_2O$: 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 [$C_{60}H_{95}O_{10}N_{14}$ $(M+H)^+$] 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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2) Abbreviations: Z, benzyloxycarbonyl; Boc, *t*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; TsOH, *p*-toluenesulfonic acid; DMF, *N,N*-dimethylformamide; Dap, 2,3-diaminopropionic acid; Dap(β-Z), 3-benzyloxycarbonyl-2,3-diaminopropionic acid. Amino acid symbols except D-Phe and D-Pro denote the L-configuration.

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