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Studies of Peptide Antibiotics. XLV.[1] Syntheses of Gramicidin S-like Analogs with Macro-ring Structures[2]

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Three cyclic tetradecapeptides related to gramicidin S (GS): *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Pro–)$_2$, *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Sar–)$_2$, and *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Gly–)$_2$, were synthesized to investigate the contributions of the ring size of cyclic peptides and of the number of the basic amino acid residues to the antibacterial activity. The protected cyclic tetradecapeptides were synthesized through a cyclization reaction of the linear tetradecapeptide azide in pyridine. Hydrogenolysis of the protected cyclic peptides afforded crystalline tetrahydrochlorides of the analogs. The homogeneity of the analogs was ascertained by TLC, paper electrophoresis, and elemental analysis. In the experiment of circular dichroism, the two analogs with 7,7′-Pro or 7,7′-Sar gave spectra similar in shape to that of GS, while the analog with 7,7′-Gly gave a slightly different spectrum from that of GS. The antibacterial assays showed that the analog with 7,7′-Pro exhibited considerable activities against the microorganisms tested, particularly against Gram-negative bacterias such as *Escherichia coli* and *Shigella sonnei*. On the other hand, the analogs with 7,7′-Sar or 7,7′-Gly exhibited weaker activities than that of GS.

For investigation of the relationship between the structure and the antibacterial activity, we have designed model compounds (Fig. 1). The following modifications to the Gramicidin S (GS) structure were made: (1) The number of amino acid residues in the cyclic peptides was increased from 10 to 14. (2) The number of ornithyl residues was increased from 2 to 4. (3) In addition to the prolyl residues at 7,7′-position, the prolyl residues were replaced by sarcocyl residues or glycyl residues.

Model compounds: *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Pro–)$_2$ (**P-12**), *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Sar–)$_2$ (**S-12**), and *cyclo*(–Leu–Orn–Leu–Orn–Leu–D-Phe–Gly–)$_2$ (**G-12**), have been synthesized. The present paper will describe the syntheses and the antibacterial properties of these compounds.

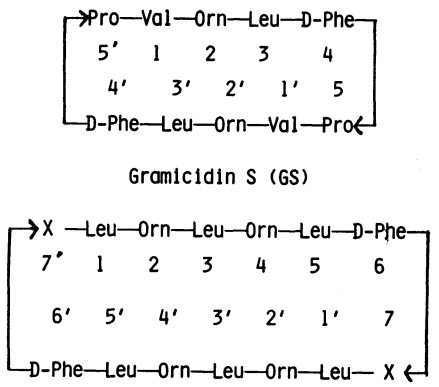

Fig. 1. Structures of GS and GS-like analogs [X=Pro (**P-12**), X=Sar (**S-12**), X=Gly (**G-12**)].

Routes for syntheses of **P-12·4HCl**, **S-12·4HCl**, and **G-12·4HCl** are outlined in Fig. 2. Boc-dipeptide ester (**1**) was prepared by means of the MA method[3] and the ester was converted into a hydrazide (**2**). Boc-pentapeptide esters (**S-3** and **G-3**) were prepared by the condensation of the azide derived from **2** with H–Leu–D-Phe–Sar–OEt·HCl[4] or H–Leu–D-Phe–Gly–OEt·HCl.[5] Pentapeptide ester hydrochlorides (**P-4**, **S-4**, and **G-4**) were derived from Boc–Leu–Orn(*Z*)–Leu–D-Phe–Pro–OEt[6] or Boc-pentapeptide ester (**S-3** and **G-3**) by the removal of the Boc group. Boc-heptapeptide esters (**P-5**, **S-5**, and **G-5**) were prepared by the condensation of the azide from **2** with the pentapeptide ester hydrochlorides (**P-4**, **S-4**, and **G-4**). The Boc-heptapeptide hydrazides (**P-6**. **S-6**, and **G-6**) were prepared from **P-5**, **S-5**, and **G-5** by treatment with hydrazine. Heptapeptide ester hydrochlorides (**P-7**, **S-7**, and **G-7**) were obtained from a part of **P-5**, **S-5**, and **G-5** by treatment with HCl in formic acid. Boc-tetradecapeptide esters (**P-8**, **S-8**, and **G-8**) were prepared by the condensation of the azide derived from **P-6**, **S-6**, and **G-6** with **P-7**, **S-7**, and **G-7**, respectively. For its cyclization, the compounds (**P-8**, **S-8**, and **G-8**) were converted into hydrazides (**P-9**, **S-9**, and **G-9**), and then by the removal of Boc group, the tetradecapeptide hydrazide dihydrochlorides (**P-10**, **S-10**, and **G-10**) were obtained. The azides derived from **P-10**, **S-10**, and **G-10** were treated with a large amount of pyridine, and the resulting *Z*-substituted cyclic tetradecapeptides (**P-11**, **S-11**, and **G-11**) were purified by passing them through columns of Dowex 50 and 1, and Sephadex LH-20. The compounds (**P-11**, **S-11**, and **G-11**) were confirmed to be the monomers by measuring molecular weights, CORONA Osmometer type 117 was used. The desired cyclic tetradecapeptides (**P-12**, **S-12**, and **G-12**) were afforded as crystalline tetrahydrochlorides by means of hydrogenolysis of **P-11**, **S-11**, and **G-11**. The compounds (**P-12**, **S-12**, and **G-12**) are more soluble in water than GS. The homogeneity o **P-12**, **S-12**, and **G-12** was ascertained by TLC, paper electrophoresis, and elemental analysis.

The CD spectra of **P-12**, **S-12**, **G-12**, and GS were measured with methanol as a solvent (Fig. 3). The CD spectra of **P-12** and **S-12** have similar patterns to that of GS, but the troughs of **P-12** and **S-12** are shallower than that of GS. The result suggests that the conformation of **P-12**, and **S-12** may be similar to that of GS. For the comformation of **P-12** and **S-12**, we attempted to construct a model with the antiparallel β-form as a possible structure (Fig. 4). Since the CD spectrum of **G-12** is a little different from that of GS, the conformation of **G-12** may be different.

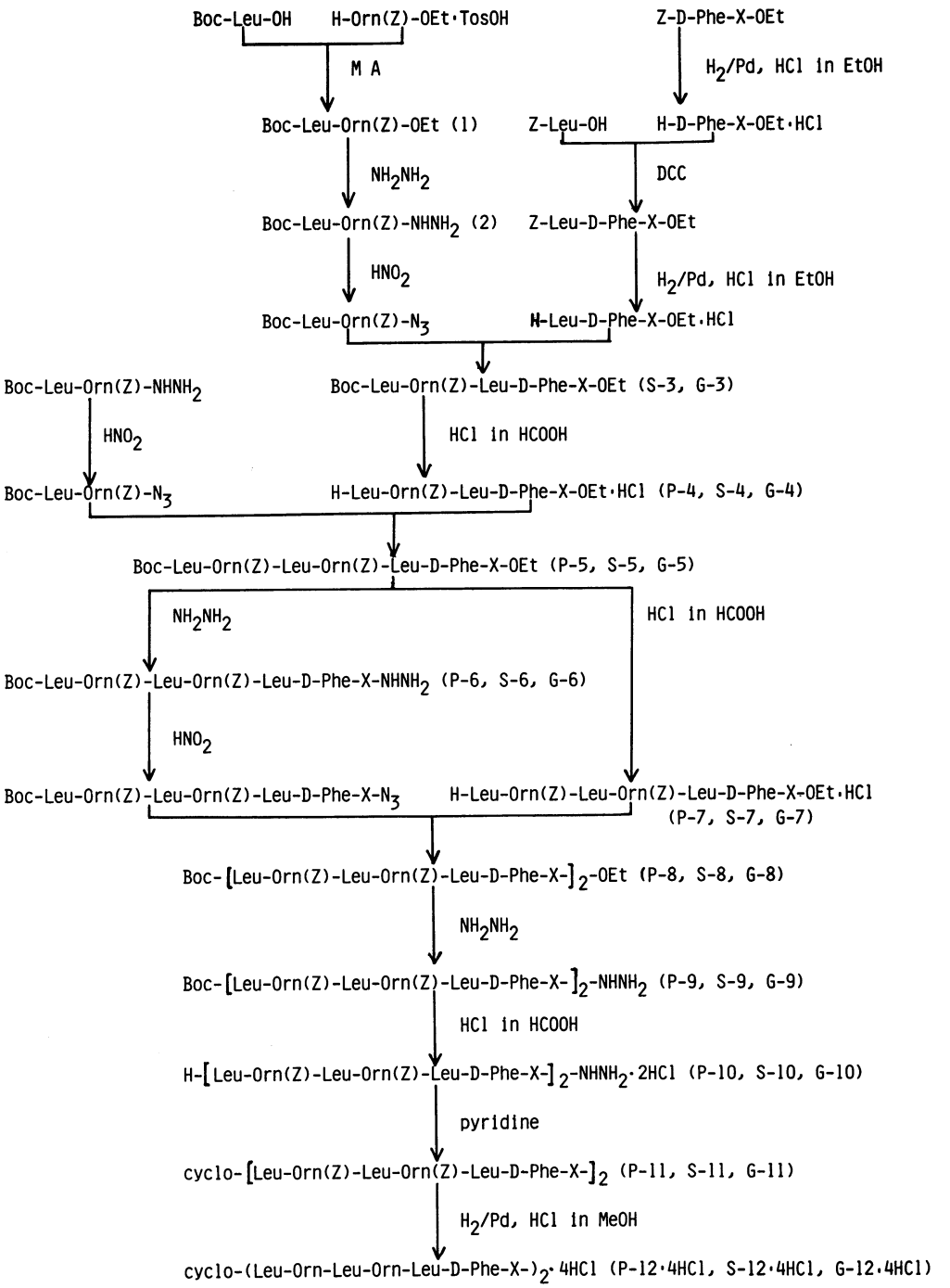

Fig. 2. Syntheses of GS-like analogs with four ornithyl residues [X=Pro (**P-12**), X=Sar (**S-12**), X=Gly (**G-12**)].

The antibacterial activities of **P-12**, **S-12**, **G-12**, and GS toward several microorganisms are listed in Table 1. It was found that the compound **P-12** exhibited considerable activity against the microorganisms tested, the compound **S-12** exhibited one-half activity compared with that of **P-12**, and the compound of **G-12** exhibited little activity. In particular, it is interesting that **P-12** has a marked inhibition against Gram-negative bacterias such as *E. coli* and *S. sonnei*. The ornithylleucyl copolymer[7] reported before exhibited a strong activity against *E. coli*. The result suggests that the ornithylleucyl residues contribute largely to the increase in activity against Gram-negative bacteria. [5,5'-Sar]-GS[4] and [5,5'-Gly]-GS[5,8] exhibited considerable antibacterial activity, but the synthesized analog (**S-12**) exhibited weaker activity, and **G-12** did not exhibit any activity. In particular, the contribution of prolyl residue was significant against the antibacterial activity. In addition, from the fact that the macro-ring analog such as **P-12** exhibited strong activities, it was found that the ring size is not always limited to 10 amino acid residues such as GS for the appearance of the activity.

Table 1. Antibacterial activities of GS-like analogs (**P-12**, **S-12**, and **G-12**) and GS[a]

Organism	P-12	S-12	G-12	GS[b]
Staphylococcus aureus	12.5	50	>100	3.13
Bacillus subtilis	6.25	25	100	3.13
Escherichia coli	12.5	100	>100	25
Salmonella typhosa	25	100	>100	>100
Shigella flexneri	6.25	25	100	3.13
Shigella sonnei	12.5	100	>100	25
Klebsiella pneumoniae	12.5	50	>100	6.25

a) This table shows the minimum inhibitory concentration (μg/ml) with a Bouillon agar medium. b) The natural GS was used as the control.

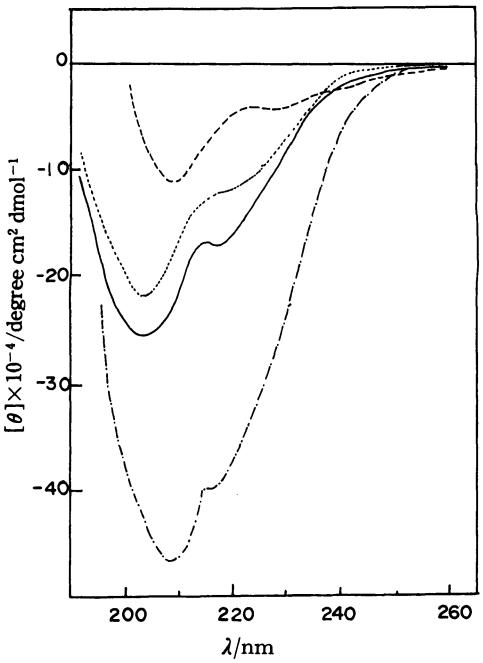

Fig. 3. CD Spectra of **P-12**, **S-12**, **G-12**, and GS in methanol.
GS: -·-·-, **P-12**: ——, **S-12**: ------, **G-12**: – – –.

Experimental

All melting points are uncorrected. Prior to analysis, compounds were dried over phosphorus pentaoxide at 80 °C and 2 mmHg††, except linear tetradecapeptide derivatives and cyclic tetradecapetides. TLC was carried out on the Merck silica gel G with the following solvent system: R_f, 1-butanol–acetic acid–pyridine–water (4 : 1 : 1 : 2, v/v).

Boc–Leu–Orn(Z)–OEt (1). To a solution of Boc–Leu–OH (7.63 g, 33 mmol) and triethylamine (4.67 ml, 33 mmol) in tetrahydrofuran (40 ml) was added isobutyl chloroformate (4.37 ml, 33 mmol) at −5 °C. After 15 min, a mixture of H–Orn(Z)–OEt·TsOH (13.7 g, 29.3 mmol) and triethylamine (4.15 ml, 29.3 mmol) in chloroform (50 ml) was added. The mixture was left to stand overnight at room temperature, evaporated *in vacuo*, and the oily residue was dissolved in ethyl acetate (150 ml). The solution was washed successively with 4% $NaHCO_3$, 0.5 M††† citric acid, and water, and then dried (Na_2SO_4). The filtrate which separated from the salt was concentrated *in vacuo*. The product which precipitated upon the addition of ether and petroleum ether was collected and washed with the same solvent; yield, 14.4 g (97%); mp 78—80 °C; $[\alpha]_D^{25}$ −14° (*c* 1, DMF); R_f 0.95.

Found: C, 61.22; H, 8.42; N, 8.07%. Calcd for $C_{26}H_{41}O_7N_3$: C, 61.52; H, 8.14; N, 8.28%.

Boc–Leu–Orn(Z)–NHNH₂ (2). A solution of **1** (14.4 g, 28 mmol) and hydrazine hydrate (27.6 ml, 568 mmol) in DMF (60 ml) was allowed to stand at room temperature for 7 d. The excess hydrazine was evaporated *in vacuo*, and then water (150 ml) was added to the residue. The resulting solid was collected by filtration; yield, 11.9 g (85%); mp 141—142 °C; $[\alpha]_D^{25}$ −24° (*c* 1, AcOH); R_f 0.85.

Found: C, 57.41; H, 8.25; N, 13.93%. Calcd for $C_{24}H_{39}O_6N_5 \cdot 1/2\ H_2O$: C, 57.35; H, 8.02; N, 13.93%.

Boc–Leu–Orn(Z)–Leu–D-Phe–Sar–OEt (S-3). A solution of **2** (6.43 g, 13 mmol) dissolved in DMF (30 ml) was cooled to −22 °C. To this solution, 3.40 M HCl in dioxane (11.5 ml, 39 mmol) and isopentyl nitrite (1.95 ml, 14.3 mmol) were added. After 10 min, the solution was neutralized with *N*-methylmorpholine (4.31 ml, 39 mmol). To this solution was added a chilled solution of H–Leu–D-Phe–Sar–OEt·HCl[4] (5.46 g, 13.2 mmol) and *N*-methylmorpholine (1.61 ml, 14.5 mmol) in DMF (30 ml). The reaction mixture was stirred at 0 °C for 7 d and evaporated *in vacuo*. The residual solid was triturated with 0.5 M citric acid, and then allowed to stand in a refrigerator. The solid was collected by filtration and washed successively with 4% $NaHCO_3$, 0.5 M citric acid and water; yield, 9.59 g (88%); mp 140—141 °C; $[\alpha]_D^{25}$ −15° (*c* 1,

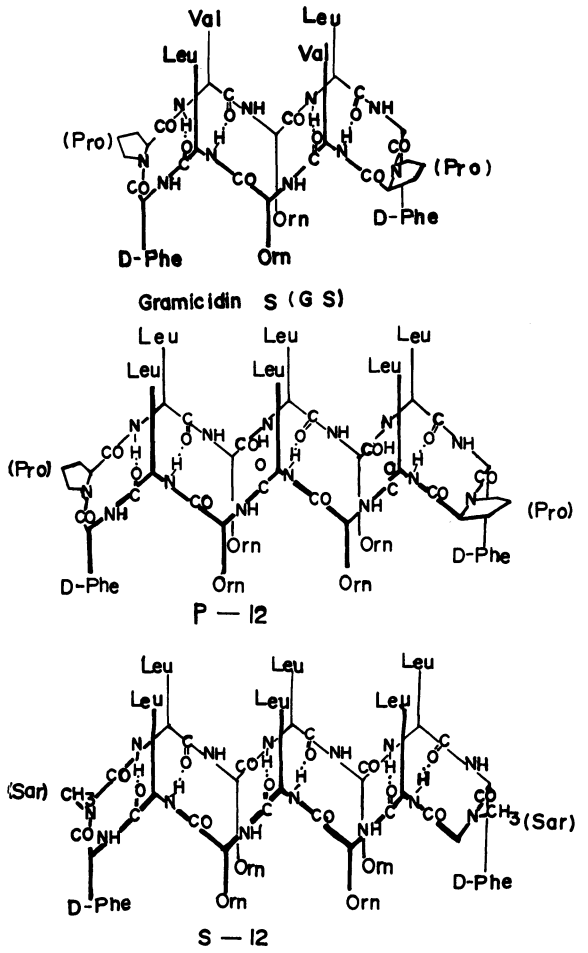

Fig. 4. Structure of GS and possible ones of **P-12** and **S-12**.

†† 1 mmHg≈133.322 Pa.
††† 1 M=1 mol dm⁻³.

DMF); R_f 0.98.

Found: C, 63.08; H, 8.26; N, 10.02%. Calcd for $C_{44}H_{66}O_{10}N_6$: C, 62.99, H, 7.93, N, 10.02%.

*Boc-Leu-Orn(Z)-Leu-D-Phe-Gly-OEt (**G-3**).* This compound was prepared from **2** (1.58 g, 3.4 mmol) and H-Leu-D-Phe-Gly-OEt·HCl[5] (1.39 g, 3.5 mmol) as described for the preparation of **S-3**. The product was obtained as an oil; yield, 1.67 g (63%); R_f 0.95.

*H-Leu-Orn(Z)-Leu-D-Phe-Pro-OEt·HCl (**P-4**).* The compound Boc-Leu-Orn(Z)-Leu-D-Phe-Pro-OEt[6] (4.33 g, 50 mmol) was dissolved in 0.183 M HCl in formic acid. After being left to stand at room temperature for 30 min, the solution was evaporated to dryness. The residual oil was treated with ether, but still was obtained as an oily substance; yield, 4.01 g (100%); R_f 0.83.

*H-Leu-Orn(Z)-Leu-D-Phe-Sar-OEt·HCl (**S-4**).* This compound was prepared from **S-3** (8.39 g, 10 mmol) as described for the preparation of **P-4**. The product was obtained as an oil; yield, 7.75 g (100%); R_f 0.77.

*H-Leu-Orn(Z)-Leu-D-Phe-Gly-OEt·HCl (**G-4**).* This compound was prepared from **G-3** (1.72 g, 2 mmol) as described for the preparation of **P-4**. The product was obtained as an oil; yield, 1.52 g (100%); R_f 0.93.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro-OEt (**P-5**).* A solution of **2** (2.47 g, 5.0 mmol) dissolved in DMF (20 ml) was cooled to −22 °C. To this solution, 3.40 M HCl in dioxane (4.41 ml, 15 mmol) and isopentyl nitrite (0.74 ml, 5.5 mmol) were added. After 15 min, the solution was neutralized with *N*-methylmorpholine (1.67 ml, 15 mmol). To this solution was added a chilled solution of **P-4** (4.01 g, 5.0 mmol) and *N*-methylmorpholine (0.61 ml, 5.5 mmol) in DMF (20 ml). The reaction mixture was stirred at 0 °C for 7 d and evaporated *in vacuo*. The residual solid was triturated with 0.5 M citric acid, and then allowed to stand in a refrigerator. The solid was collected by filtration and dissolved in ethyl acetate (200 ml). The solution was washed successively with 4% $NaHCO_3$, 0.5 M citric acid, and water, and then dried (Na_2SO_4). The filtrate separated from the salt was evaporated *in vacuo*. The resulting crystals were collected by filtration with the aid of ether–petroleum ether; yield, 3.60 g (59%); mp 190—195 °C; R_f 0.98.

Found: C, 63.38; H, 7.91; N, 10.07%. Calcd for $C_{65}H_{95}O_{14}N_9$: C, 63.65; H, 7.81; N, 10.28%.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar-OEt (**S-5**).* This compound was prepared from **2** (4.94 g, 10 mmol) and **S-4** (7.75 g, 10 mmol) as described for the preparation of **S-3**; yield, 8.92 g (74%); mp 148—153 °C; $[\alpha]_D^{25}$ −28° (*c* 1, DMF); R_f 0.95.

Found: C, 58.21; H, 7.87; N, 9.94%. Calcd for $C_{63}H_{93}O_{14}N_9\cdot 1/2\ H_2O$: C, 58.23; H, 8.07; N, 9.70%.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly-OEt (**G-5**).* This compound was prepared from **2** (4.94 g, 10 mmol) and **G-4** (8.37 g, 10 mmol) as described for the preparation of **S-3**; yield, 11.47 g (97%); mp 130—132 °C; $[\alpha]_D^{25}$ 19° (*c* 1, DMF); R_f 0.95.

Found: C, 62.53; H, 7.48; N, 10.89%. Calcd for $C_{62}H_{91}O_{14}N_9$: C, 62.77; H, 7.73; N, 10.63%.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro-NHNH$_2$ (**P-6**).* A solution of **P-5** (1.65 g, 1.34 mmol) and hydrazine hydrate (2.60 ml, 53.6 mmol) in DMF (20 ml) was allowed to stand for 7 d at room temperature. The solution was concentrated *in vacuo* to a small volume. The hydrazide precipitated upon the addition of water (100 ml) was collected by filtration; yield, 1.62 g (100%); mp 140—143 °C; $[\alpha]_D^{25}$ −83° (*c* 0.4, AcOH).

Found: C, 60.95; H, 7.67; N, 12.73%. Calcd for $C_{63}H_{93}O_{13}N_{11}\cdot 3/2\ H_2O$: C, 61.05; H, 7.81; N, 12.43%.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar-NHNH$_2$ (**S-6**).* This compound was prepared from **S-5** (5.28 g, 4.4 mmol) as described for the preparation of **P-6**; yield, 5.01 g (96%); mp 147—150 °C; $[\alpha]_D^{25}$ −30° (*c* 0.7, AcOH).

Found: C, 61.55; H, 7.83; N, 13.11%. Calcd for $C_{61}H_{91}O_{13}N_{11}$: C, 61.75; H, 7.73; N, 12.99%.

*Boc-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly-NHNH$_2$ (**G-6**).* This compound was prepared from **G-5** (7.09 g, 6.0 mmol) as described for the preparation of **P-6**; yield, 2.63 g (37%); mp 120—122 °C; $[\alpha]_D^{25}$ −7° (*c* 1, AcOH); R_f 0.84.

Found: C, 59.66; H, 7.51; N, 12.71%. Calcd for $C_{60}H_{89}O_{13}N_{11}\cdot 2H_2O$: C, 59.63; H, 7.76; N, 12.75%.

*H-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro-OEt·HCl (**P-7**).* This compound was prepared from **P-5** (1.52 g, 1.2 mmol) as described for the preparation of **P-4**. The product was obtained as an oil; yield, 1.44 g (100%); R_f 0.85.

*H-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar-OEt·HCl (**S-7**).* This compound was prepared from **S-5** (3.60 g, 3.0 mmol) as described for the preparation of **P-4**. The product was obtained as an oil; yield, 3.37 g (100%); R_f 0.84.

*H-Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly-OEt·HCl (**G-7**).* This compound was prepared from **G-5** (2.37 g, 2.0 mmol) as described for the preparation of **P-4**. The product was obtained as an oil; yield, 2.24 g (100%); R_f 0.78.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro]$_2$-OEt (**P-8**).* This compound was prepared from **P-6** (1.50 g, 1.2 mmol) and **P-7** (1.44 g, 1.2 mmol) as described for the preparation of **P-5**. The product was obtained as a solid; yield, 2.72 g (88%); mp 192—195 °C; $[\alpha]_D^{25}$ −66° (*c* 0.2, MeOH); R_f 0.94.

Found: C, 60.45; H, 7.66; N, 10.25%. Calcd for $C_{123}H_{176}O_{26}N_{18}\cdot 7H_2O$: C, 60.72; H, 7.87; N, 10.36%.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar]$_2$-OEt (**S-8**).* This compound was prepared from **S-6** (3.56 g, 3.0 mmol) and **S-7** (3.37 g, 3.0 mmol) as described for the preparation of **P-5**. The product was obtained as a solid; yield, 4.95 g (74%); mp 145—148 °C; $[\alpha]_D^{25}$ −33° (*c* 0.1, MeOH); R_f 0.98.

Found: C, 61.13; H, 7.81; N, 11.15%. Calcd for $C_{119}H_{172}O_{25}N_{18}\cdot 4H_2O$: C, 61.43; H, 7.80; N, 10.84%.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly]$_2$-OEt (**G-8**).* This compound was prepared from **G-6** (2.63 g, 2.2 mmol) and **G-7** (2.47 g, 2.2 mmol) as described for the preparation of **P-5**. The product was obtained as an oil; yield, 4.90 g(100%); R_f 0.96.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro]$_2$-NHNH$_2$(**P-9**).* This compound was prepared from **P-8** (2.72 g, 1.2 mmol) as described for the preparation of **P-6**; yield, 2.47 g (92%); mp 140—144 °C; $[\alpha]_D^{25}$ −79° (*c* 0.4, AcOH).

Found: C, 62.18; H, 7.77; N, 11.73%. Calcd for $C_{121}H_{174}O_{24}N_{20}\cdot 5/2\ H_2O$: C, 62.16; H, 7.72; N, 11.98%.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar]$_2$-NHNH$_2$(**S-9**).* This compound was prepared from **S-8** (4.95 g, 2.2 mmol) as described for the preparation of **P-6**; yield, 4.79 g (98%); mp 156—158 °C; $[\alpha]_D^{25}$ −34° (*c* 0.5, AcOH).

Found: C, 61.68; H, 8.43; N, 12.55%. Calcd for $C_{117}H_{170}O_{24}N_{20}\cdot 2H_2O$: C, 61.72; H, 7.70; N; 12.30%.

*Boc-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly]$_2$-NHNH$_2$ (**G-9**).* This compound was prepared from **G-8** (4.90 g, 2.2 mmol) as described for the preparation of **P-6**; yield, 2.51 g (54%); mp 127—130 °C; $[\alpha]_D^{25}$ −7° (*c* 0.5, AcOH); R_f 0.82.

Found: C, 60.67; H, 7.58; N, 12.49%. Calcd for $C_{115}H_{166}O_{24}N_{20}\cdot 3H_2O$: C, 60.94; H, 7.65; N, 12.36%.

*H-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro]$_2$-NHNH$_2$·2HCl (**P-10**).* The compound **P-9** (1.38 g, 0.6 mmol) was dissolved in 0.183 M HCl in formic acid (7.90 g, 1.44 mmol). After being left to stand at room temperature for 30 min, the solution was evaporated to dryness. The product was obtained as an oily form; yield, 1.36 g (100%); R_f 0.90.

H-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar]$_2$-NHNH$_2$·2HCl (**S-10**). This compound was prepared from **S-9** (2.33 g, 1.0 mmol) as described for the preparation of **P-10**. The product was obtained in an oily form; yield, 2.20 g (100%); R_f 0.90.

H-[Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly]$_2$-NHNH$_2$·2HCl (**G-10**). This compound was prepared from **G-9** (2.51 g, 1.1 mmol) as described for the preparation of **P-10**. The product was obtained as an oily substance; yield, 2.31 g (96%); R_f 0.79.

cyclo[-*Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Pro-*]$_2$ (**P-11**). A solution of **P-10** (1.36 g, 0.66 mmol) in DMF (40 ml) was chilled to −22 °C. To this solution 3.40 M HCl in dioxane (0.41 ml) and isopentyl nitrite (0.09 ml, 0.66 mmol) were added. After 5 min, the solution was added dropwise into pyridine (300 ml) at 0 °C for 5 min, and stirring was continued for 3.5 h at 0 °C. It then was stirred for 55 h at 5 °C. After the solvent was removed, the residue was dissolved in a mixture of methanol and water (450 ml, 8 : 1, v/v). The solution was passed through two columns, of Dowex 1 (OH^- form) and 50 (H^+ form). The columns were washed with the same solvent (900 ml), and the effluent was evaporated to dryness. The residue was collected by filtration with the aid of water. For purification, the solution of the crude product in DMF (3 ml) was applied to a column (2.5×84.5 cm) of Sephadex LH-20, and the development continued with DMF. Elution was carried out at room temperature, at a flow rate of 24 ml per h. The peptide content in the fractions was determined with LKB 2138 Uvicord S (at 254 nm). The fractions containing the desired product were evaporated, and the product was collected by filtration with the aid of water; yield, 128 mg (53%); mp 184—188 °C; $[\alpha]_D^{25}$ −141° (*c* 0.1, MeOH); R_f 0.98.

Found: C, 60.63; H, 7.57; N, 10.79%. Calcd for $C_{116}H_{162}O_{22}N_{18}\cdot 3H_2O$: C, 60.69; H, 7.77; N, 10.98%.

cyclo(-*Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Sar-*]$_2$ (**S-11**). This compound was synthesized from **S-10** (2.20 g, 1.0 mmol) as described for the preparation of **P-11**; yield, 127 mg (29%); mp 170—175 °C; $[\alpha]_D^{25}$ −47° (*c* 0.1, MeOH); R_f 0.98.

Found: C, 61.30; H, 7.75; N, 11.75%. Calcd fro $C_{112}H_{158}O_{22}N_{18}\cdot 9/2\ H_2O$: C, 61.44; H, 7.69; N, 11.51%.

cyclo[-*Leu-Orn(Z)-Leu-Orn(Z)-Leu-D-Phe-Gly-*]$_2$ (**G-11**). This compound was prepared from **G-10** (2.11 g, 0.9 mmol) as described for the preparation of **P-11**; yield, 100 mg (21%); mp 103—105 °C; $[\alpha]_D^{25}$ −43° (*c* 0.1, MeOH); R_f 0.98.

Found: C, 62.17; H, 7.50; N, 11.61%. Calcd for $C_{110}H_{154}O_{22}N_{18}\cdot 5/2\ H_2O$: C, 62.16; H, 7.54 N, 11.86%.

cyclo(-*Leu-Orn-Leu-Orn-Leu-D-Phe-Pro-*)$_2$·4HCl (**P-12**·4HCl). A solution of **P-11** (100 mg, 0.05 mmol) in 0.094 M HCl in methanol (2.4 ml) was hydrogenated in the presence of palladium black. After removal of the catalyst the filtrate was evaporated, and the resulting crystals were collected by filtration with the aid of ether; yield, 57 mg (70%); mp 232—238 °C; $[\alpha]_D^{25}$ −89° (*c* 0.05, MeOH); R_f 0.73.

Found: C, 51.88; H, 8.18; N, 12.67%. Calcd for $C_{84}H_{142}O_{14}N_{18}Cl_4\cdot 10H_2O$: C, 51.74; H, 8.37; N, 12.93%.

cyclo(-*Leu-Orn-Leu-Orn-Leu-D-Phe-Sar-*)$_2$·4HCl (**S-12**·4HCl). This compound was prepared from **S-11** (100 mg, 0.05 mmol) as described for the preparation of **P-12**; yield, 57 mg (70%); mp 180—184 °C; $[\alpha]_D^{25}$ −136° (*c* 0.05 MeOH); R_f 0.81.

Found: C, 46.82; H, 8.53; N, 12.17%. Calcd for $C_{80}H_{138}O_{14}N_{18}Cl_4\cdot 18H_2O$: C, 47.05; H, 8.59; N, 12.35%.

cyclo(-*Leu-Orn-Leu-Orn-Leu-D-Phe-Gly-*)$_2$·4HCl (**G-12**·4HCl). This compound was prepared from **G-11** (96 mg, 0.05 mmol) as described for the preparation of **P-12**; yield, 44.6 mg (68%); mp 141—143 °C; $[\alpha]_D^{25}$ −48° (*c* 0.05, MeOH); R_f 0.77.

Found: C, 49.82; H, 8.53; N, 13.25%. Calcd for $C_{78}H_{134}O_{14}N_{18}Cl_4\cdot 10H_2O$: C, 50.10; H, 8.30; N, 13.48%.

Electrophoresis. Electrophoresis on Toyo Roshi No. 52 paper was carried out with a 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. The mobilities toward the cathode of **P-12**, **S-12**, and **G-12** were comparable with that of GS; the ratios of the mobilities of **P-12**, **S-12**, and **G-12** *vs.* GS were 1.10, 1.16, and 1.20, respectively.

CD Measurement. The measurement of CD was performed with a JASCO Model J-40 over a wavelength range of 195 to 260 nm in methanol as a solvent. In Fig. 3 are shown the CD spectra of **P-12**, **S-12**, **G-1 2**, and GS.

Microbiological Assays. The minimum amount of the compounds (**P-12**, **S-12**, **G-12**, and GS) necessary for the complete inhibition of growth was determined by a dilution method. The results are shown in Table 1.

Molecular Weight Measurement. The molecular weight measurement of the *Z*-substituted cyclic tetradecapeptides (**P-11**, **S-11**, and **G-11**) was carried out by the use of a CORONA Osmometer type 117, in DMF as a solvent. The values of molecular weight **P-11**, **S-11**, and **G-11** were 2022, 1997, and 1958; the calculated values were 2160, 2108, and 2080 respectively.

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References

1) Part XLIV of series: H. Yonezawa, K. Okamoto, M. Kaneda, N. Tominaga, and N. Izumiya, *Int. J. Peptide Protein Res.*, in press.

2) A part of this work has been already communicated: S. Ando, H. Nishikawa, H. Takiguchi, and N. Izumiya, *Fukuoka University Science Reports*, **13**, 49 (1983).

3) Abbreviation: Z, benzyloxycarbonyl; Boc, *t*-butoxycarbonyl; MA, mixed anhydride method; DCC, dicyclohexylcarbodiimide; TsOH, *p*-toluenesulfonic acid; DMF, *N,N*-dimethylformamide, MeOH, methanol; AcOH, acetic acid. Amino acid symbols except D-Phe denote the L-configuration.

4) H. Aoyagi and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **39**, 1747 (1966).

5) H. Aoyagi, T. Kato, M. Ohno , M. Kondo, M. Waki, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **38**, 2139 (1965); H. Aoyagi, T. Kato, M. Ohno, M. Kondo, and N. Izumiya, *J. Am. Chem. Soc.*, **86**, 5700 (1964).

6) M. Kondo and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **43**, 1850 (1970).

7) L. Bichowsky-Slomnicki, A. Berger, J. Kurts, and E. Katchalski, *Arch. Biochem. Biophys.*, **65**, 400 (1956).

8) H. Aoyagi, M. Kondo, T. Kato, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **40**, 1685 (1967).