Studies of Peptide Antibiotics. XXXIII.[1] Syntheses of Gramicidin S Analogs Containing *N*-Methyl-L-valine in Place of L-Valine

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Two analogs of gramicidin S, [1-*N*-methyl-L-valine]-gramicidin S and [1,1'-di-*N*-methyl-L-valine]-gramicidin S, were prepared for the purpose of investigating the contribution of NH group of L-valyl residue in gramicidin S to antibacterial activity. They exhibit weak activity as compared with gramicidin S in the antibacterial test toward microorganisms. The optical rotatory dispersions of these analogs and gramicidin S were measured in ethanol and 8 M urea. It was suggested that both analogs have a different molecular conformation from that of gramicidin S, and that the NH group of valyl residues is necessary for stabilizing the conformation to exhibit biological activity.

Several models have been proposed[2] for the conformation of gramicidin S (GS) in solid state and solution. The most favorable model is the intramolecular antiparallel β-form with four hydrogen bondings between the valyl and the leucyl residues.[3] A simplified structure of the model is shown in Fig. 1. In studies of the structure-activity relationship of GS, it has been proposed that the specific conformation is necessary for molecules of GS and its analogs to exhibit biological activity.[2]

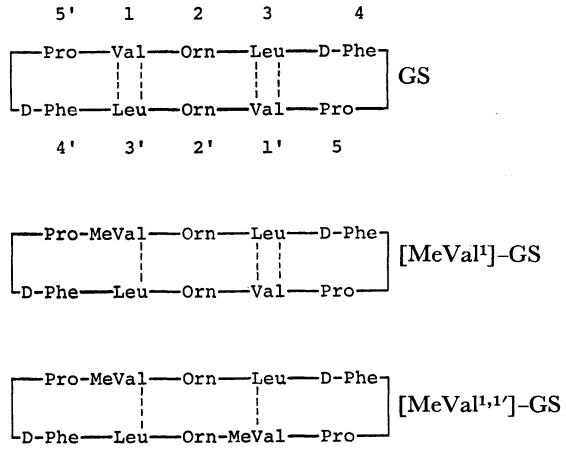

Fig. 1. Structure of GS and its analogs.

[3-*N*-Methyl-L-leucine]-gramicidin S ([MeLeu³]-GS) and [MeLeu³,³']-GS which lack some of the four hydrogen bondings in GS were synthesized in order to investigate to what extent the changes in the number of these hydrogen bondings vary the level of antibacterial activity and influence the conformations.[4] It was observed that the antibacterial activity of [MeLeu³]-GS and [MeLeu³,³']-GS is identical with that of GS and the optical rotatory dispersion (ORD) curves are similar to the curve of GS. Recent studies on the two analogs by proton magnetic resonance spectroscopy showed that the analogs might have a different conformation from that of GS in spite of the apparent resemblance between GS and the analogs in their ORD curves.[5] In other words, similarity of ORD patterns do not always guarantee similar conformation. The results led us to investigate other analogs which contain *N*-methylamino acid residue other than *N*-methyl-L-leucyl.

The present paper deals with the syntheses, antibacterial properties, and ORD measurements of [MeVal¹]-GS and [MeVal¹,¹']-GS, in which the L-valyl residues in positions 1 and 1' are replaced, singly or together, by *N*-methyl-L-valyl residues. These analogs lack one or two of the four hydrogen bondings in GS as shown in Fig. 1.

The syntheses of [MeVal¹]-GS and [MeVal¹,¹']-GS are outlined in Schemes 1 and 2.[6] The principle of the synthesis was to locate the leucyl residue at the C-terminal in order to achieve the cyclization in a good yield. In *B. brevis*, GS is synthesized enzymatically with the cyclization of a pentapeptide (H-D-Phe-Pro-Val-Orn-Leu-OH).[7] According to this finding, Tanaka *et al.* synthesized the protected GS chemically with a protected pentapeptide azide of the same amino acid sequence in a high yield of 30—36%.[8] Furthermore, the results of recent studies in this laboratory on the cyclization reaction of a pentapeptide *via* several active esters show that an *N*-hydroxysuccinimide method is excellent because of high yield, experimental simplicity, and no racemization.[1]

In order to avoid racemization of *N*-methyl-L-valyl residue, the stepwise elongation procedure was employed for the synthesis of X and XII for the following reason. McDermott and Benoiton observed that *N*-methyl-L-leucyl residue in Z-L-Ala-L-MeLeu-OH partially racemized in a coupling reaction with an amine-component by the DCC-HONSu method in spite of no racemization of Z-L-Ala-L-Leu-OH under the same conditions.[9] They reported absence of racemization of alkyloxycarbonyl-*N*-methyl-L-amino acid during the course of coupling reactions.[9]

For the synthesis of protected [MeVal¹]-GS (XIX), Boc-pentapeptide-ONSu (IX) and pentapeptide (XVI) were prepared by the stepwise elongation from the carboxyl toward the amino end as shown in Scheme 1. Coupling of these IX and XVI afforded Boc-decapeptide acid (XVII) which was converted into a decapeptide-ONSu. A key intermediate (XIX) was derived by the cyclization of the decapeptide-ONSu in a satisfactory yield of 56%. As shown in Scheme 2, Boc-decapeptide acid (XXII) was converted into the corresponding decapeptide-ONSu. A Z-substituted intermediate (XXIV) was derived by the cyclization of the decapep-

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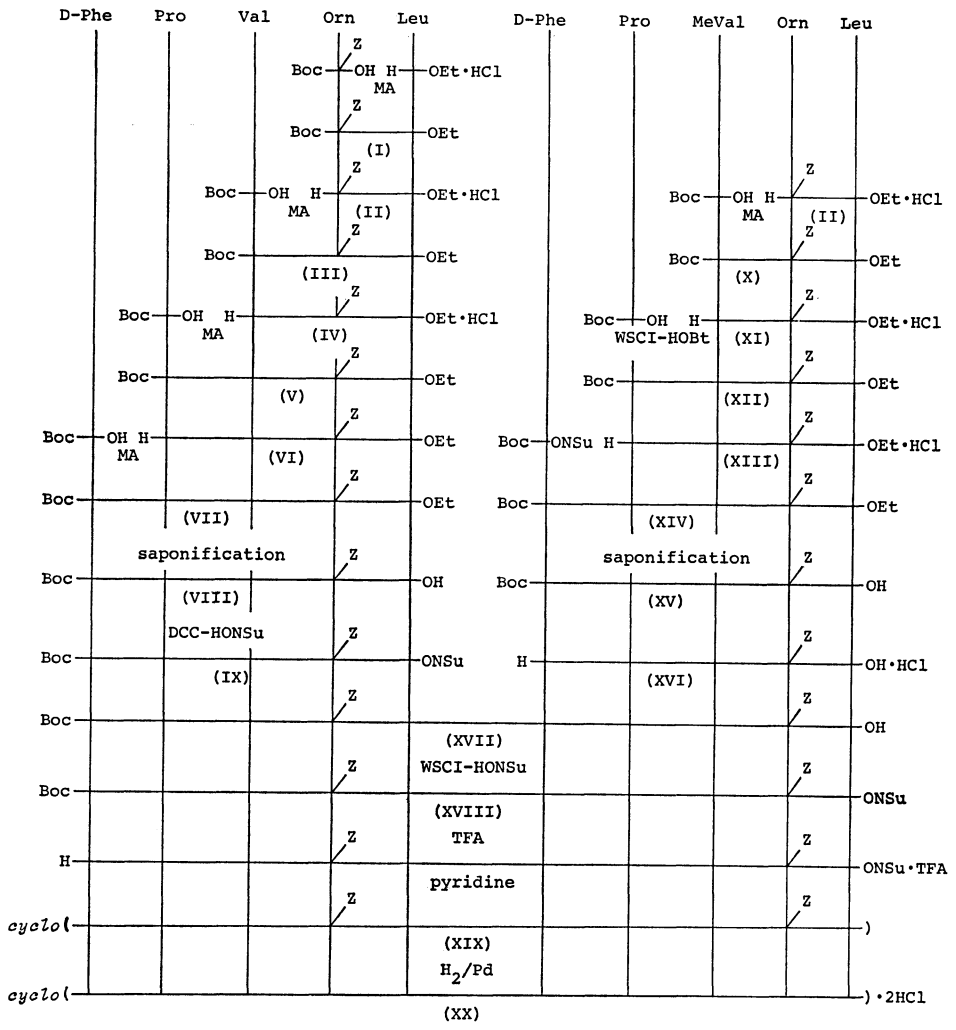
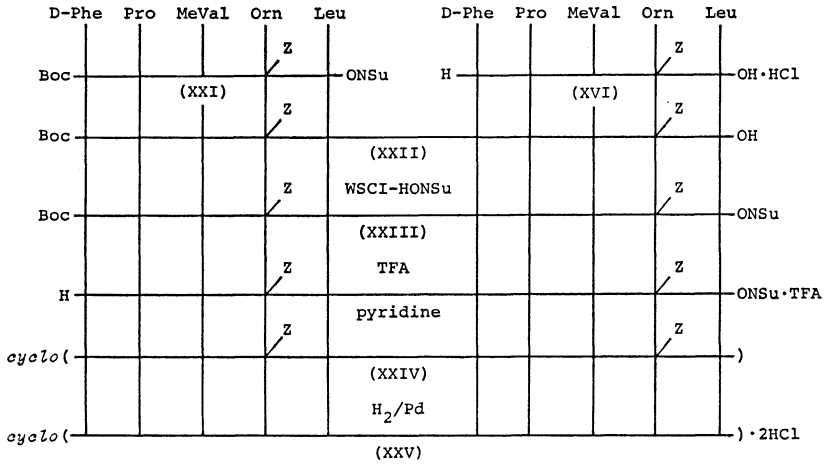


Scheme 1. Synthesis of [MeVal^{1}]-GS.



Scheme 2. Synthesis of [MeVal$^{1,1'}$]-GS.

tide–ONSu in a satisfactory yield of 66%.

For the syntheses of final [MeVal^{1}]-GS and [MeVal$^{1,1'}$]-GS, each Z-substituted cyclodecapeptide (XIX and XXIV) was subjected to hydrogenolysis, and the desired analogs were obtained as crystalline dihydrochlorides (XX and XXV). Their homogeneity was confirmed by paper and thin-layer chromatography, paper electrophoresis and elemental analysis.

Antibacterial activity toward several microorganisms was examined, the results being given in Table 1. The two analogs were found to exhibit weak activity for Gram-positive microorganisms, whereas GS showed strong activity. The antibacterial potency of [MeVal^{1}]-GS is slightly higher than that of the [MeVal$^{1,1'}$]-

Table 1. Antibacterial activity of GS and its analogs
(Minimum inhibitory concentration, μg/ml)

Strain	GS·2HC	[MeVal[1]]-GS·2HCl	[MeVal[1,1']]-GS·2HCl
Staphylococcus aureus 209p	12.5	25	50
Staphylococcus aureus Terajima	1.56	12.5	25
Enterococcus hemolyticus Hoshi	6.25	50	50
Bacillus subtillis ATCC 6633	1.56	25	50
Escherichia coli K 12	50	50	50
Proteus vulgaris OX 19	50	50	50

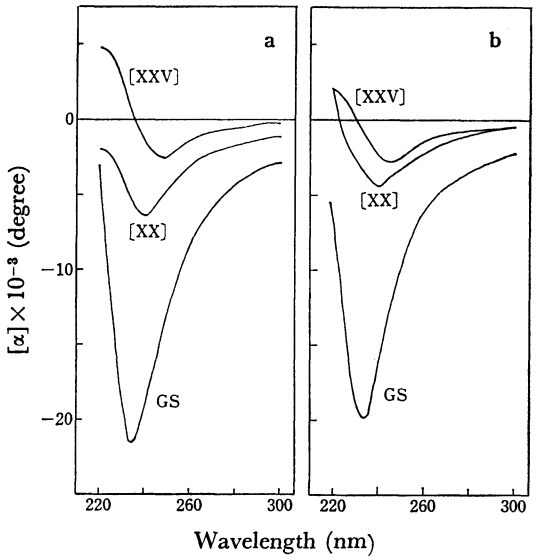


Fig. 2. ORD curves of [MeVal[1]]-GS (XX), [MeVal[1,1']]-GS (XXV), and GS.
Solvent: a, ethanol; b, 8 M aqueous urea.

analog (Table 1). The results indicate the importance of the NH's of L-valyl residues in GS for exhibiting the activity.

The ORD curves of the two analogs in ethanol as well as of GS are shown in Fig. 2-a. The trough of [MeVal[1]]-GS and of [MeVal[1,1']]-GS is at 240 and 248 nm, respectively, whereas that of GS is at 236 nm. The troughs of the analogs are shallower than the trough of GS. [MeVal[1,1']]-GS shows a greater shift in position and a shallower trough than [MeVal[1]]-GS. In 8 M aqueous urea which causes denaturation of various polypeptides, the position of the trough of [MeVal[1,1']]-GS shifts from 248 to 245 nm, but that of [MeVal[1]]-GS at 240 nm remains unchanged as in the case of GS (Fig. 2-b). The results of ORD measurements indicate that the conformation of the analogs differs from that of GS, and the stability of the analogs decreases as the number of *N*-methylvalyl residues increases. The tendency is comparable with the biological activity. We proposed that the biologically active analogs of GS show a similar ORD curve to that of GS.[10] The two analogs containing *N*-methylvalyl residues show a different ORD curve from that of GS and exhibit weak biological activity as had been expected. These results indicate that the destruction of intramolecular hydrogen bondings by substitution of valyl residues for *N*-methylvalyl residues cause the change and instabilization of conformation and decrease the biological activity consequently.

Experimental

Melting points are uncorrected. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck) with the solvent system: chloroform–methanol (5: 1, v/v). The NMR spectra were recorded on a Hitachi R-20B spectrometer at 60 MHz with tetramethylsilane as an internal standard. The optical rotations were measured on a Yanagimoto polarimeter, OR-20.

Boc-Orn(Z)-Leu-OEt (I). To a solution of Boc-Orn(Z)-OH[11] (10.99 g, 30 mmol) and TEA (4.20 ml, 30 mmol) in THF (30 ml) was added isobutyl chloroformate (3.93 ml, 30 mmol) at −5 °C. After 15 min, a mixture of H-Leu-OEt·HCl (5.87 g, 30 mmol) and TEA (30 mmol) in chloroform (30 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. The solution was washed successively with 4% sodium hydrogencarbonate, 10% citric acid and water, and then dried over sodium sulfate. The solvent was evaporated under reduced pressure. The residual oil was solidified by the addition of ether and petroleum ether. The product was recrystallized from methanol–ether–petroleum ether; yield, 13.15 g (86%); mp 100—101 °C, $[\alpha]_D^{20}$ −21.6° (*c* 1, ethanol).

Found: C, 61.65; H, 8.02; N, 8.32%. Calcd for $C_{26}H_{41}O_7N_3$: C, 61.52; H, 8.14; N, 8.28%.

H-Orn(Z)-Leu-OEt·HCl (II). Compound I (12.69 g, 20 mmol) was dissolved in 96 ml of 2.6 M hydrogen chloride in ethyl acetate. The solution was allowed to stand for 1 h at room temperature and then evaporated. The oily residue was solidified by the addition of ether and petroleum ether, and the crude product was recrystallized from ethanol–ether–petroleum ether; yield, 10.66 g (96%); mp 126—129 °C; $[\alpha]_D^{20}$ −1.4° (*c* 1, ethanol).

Found: C, 57.13; H, 7.69; N, 9.43%. Calcd for $C_{21}H_{34}O_5N_3Cl$: C, 56.81; H, 7.72; N, 9.47%.

Boc-Val-Orn(Z)-Leu·OEt (III). A reaction mixture of Boc-Val-OH (1.87 g, 8.6 mmol), TEA (8.6 mmol) and isobutyl chloroformate (8.6 mmol) in THF (9 ml) was treated with a solution of II (3.84 g, 8.6 mmol) and TEA (8.6 mmol) in chloroform (9 ml) as described for I. The crude product was recrystallized from methanol–ether–petroleum ether; yield, 4.80 g (92%); mp 152—154 °C; $[\alpha]_D^{20}$ −38.6° (*c* 1, ethanol).

Found: C, 60.65; H, 8.13; N, 9.41%. Calcd for $C_{31}H_{50}O_8N_4 \cdot 1/2H_2O$: C, 60.46; H, 8.35; N, 9.10%.

H-Val-Orn(Z)-Leu-OEt·HCl (IV). Compound III (4.41 g, 7.3 mmol) was dissolved in 2.6 M hydrogen chloride in ethyl acetate (28 ml) and treated as described for II. The product was recrystallized from ethanol–ether–petroleum ether; yield, 3.57 g (90%); mp 205—207 °C; $[\alpha]_D^{20}$ −19.6° (*c* 0.5, ethanol).

Found: C, 56.72; H, 7.88; N, 10.27%. Calcd for $C_{26}H_{43}O_6N_4 \cdot 1/2H_2O$: C, 56.56; H, 8.03; N, 10.15%.

Boc-Pro-Val-Orn(Z)-Leu-OEt (V). A mixture of Boc-Pro-OH (1.29 g, 6 mmol), TEA (6 mmol) and isobutyl chloroformate (6 mmol) in THF (6 ml) was treated with IV (3.26 g, 6 mmol) and TEA (6 mmol) in chloroform (6 ml) as described for I. The product was recrystallized from

methanol–ether–petroleum ether; yield, 3.65 g (90%); mp 158—160 °C; $[\alpha]_D^{20}$ −69.0° (*c* 1, ethanol).

Found: C, 61.12; H, 8.04; N, 10.00%. Calcd for $C_{36}H_{57}O_9N_5$: C, 61.43; H, 8.16; N, 9.95%.

H–Pro–Val–Orn(Z)–Leu–OEt·HCl (VI). Compound V (3.52 g, 5 mmol) was treated with 2.6 M hydrogen chloride in ethyl acetate (19 ml) as described for II. The product was recrystallized from ethanol–ether–petroleum ether; yield, 3.07 g (96%); mp 187—189 °C; $[\alpha]_D^{20}$ −60.0° (*c* 0.5, ethanol).

Found: C, 57.58; H, 8.04; N, 11.01%. Calcd for $C_{31}H_{50}O_7N_5Cl$: C, 58.15; H, 7.87; N, 10.94%.

Boc–D–Phe–Pro–Val–Orn(Z)–Leu–OEt (VII). A mixture of Boc–D–Phe–OH (1.19 g, 4.5 mmol), TEA (4.5 mmol) and isobutyl chloroformate (4.5 mmol) in THF (4.5 ml) was treated with VI (2.88 g, 4.5 mmol) and TEA (4.5 mmol) in chloroform (4.5 ml) as described for I. The product was recrystallized from methanol–ether–petroleum ether; yield, 3.25 g (84%); mp 94—98 °C; $[\alpha]_D^{20}$ −76.0° (*c* 0.5, ethanol).

Found: C, 62.75; H, 8.05; N, 9.65%. Calcd for $C_{45}H_{66}O_{10}N_6\cdot 1/2H_2O$: C, 62.84; H, 7.85; N, 9.77%.

Boc–D–Phe–Pro–Val–Orn(Z)–Leu–OH (VIII). To a solution of VII (1.72 g, 2 mmol) in a mixture of methanol (10 ml) and dioxane (5 ml) was added 1 M sodium hydroxide (3.4 ml) and the solution was allowed to stand for 3 h at room temperature. After the addition of water (30 ml), the solution was concentrated *in vacuo* at a low temperature, and the residual solution was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residual oil was triturated with a mixture of ether and petroleum ether, and the powder was recrystallized from methanol–ether–petroleum ether; yield, 1.50 g (90%); mp 120—123 °C; $[\alpha]_D^{20}$ −80.1° (*c* 1, methanol).

Found: C, 61.96; H, 7.67; N, 9.98%. Calcd for $C_{43}H_{62}O_{10}N_6\cdot 1/2H_2O$: C, 62.07; H, 7.63; N, 10.10%.

Boc–D–Phe–Pro–Val–Orn(Z)–Leu–ONSu (IX). To a solution of VIII (832 mg, 1 mmol) in dioxane (5 ml) were added HONSu (115 mg, 1 mmol) and DCC (206 mg, 1 mmol) at 0 °C. After being left to stand at room temperature for 12 h, dicyclohexylurea formed was filtered off, the filtrate was evaporated, and the residual oil was solidified by the addition of ether. The product was recrystallized from dioxane–ether; yield, 643 mg (69%); mp 136—138 °C; $[\alpha]_D^{20}$ −74.1° (*c* 0.54, ethyl acetate).

Found: C, 60.83; H, 7.18; N, 10.50%. Calcd for $C_{47}H_{65}O_{12}H_7\cdot 1/2H_2O$: C, 60.76; H, 7.16; N, 10.55%.

Boc–MeVal–Orn(Z)–Leu–OEt (X). A reaction mixture of Boc–MeVal–OH[12] (4.63 g, 20 mmol), *N*-methylmorpholine (2.20 ml, 20 mmol) and isobutyl chloroformate (20 mmol) in THF (20 ml) was treated with II (8.88 g, 20 mmol) and *N*-methylmorpholine (20 mmol) in THF (20 ml) as described for I. The product was obtained as an oil; yield, 11.42 g (92%).

H–MeVal–Orn(Z)–Leu–OEt·HCl (XI). Compound X (11.17 g, 18 mmol) was treated with 2.6 M hydrogen chloride in ethyl acetate (139 ml) as described for II. The product was recrystallized from methanol–ether; yield, 7.42 g (74%); mp 174—175 °C; $[\alpha]_D^{20}$ −29.0° (*c* 0.52, methanol).

Found: C, 57.87; H, 8.15; N, 10.13%. Calcd for $C_{27}H_{45}O_6N_4Cl$: C, 58.20; H, 8.14; N, 10.06%.

Boc–Pro–MeVal–Orn(Z)–Leu–OEt (XII). To a solution of Boc–Pro–OH (2.80 g, 13 mmol), HOBt (1.76 g, 13 mmol), XI(7.24 g, 13 mmol) and TEA (13 mmol) in DMF (40 ml) was added WSCI (2.49 g, 13 mmol) at −5 °C. After stirring had been continued at −5—0 °C for 2 h, the mixture was left to stand overnight at room temperature. It was then evaporated and the residue was diluted with ethyl acetate (300 ml). The solution was washed with 4% sodium hydrogencarbonate, 10% citric acid and water, and dried over sodium sulfate. The solvent was evaporated to leave an oil; yield, 3.64 g (39%). Amino acid ratios in acid hydrolyzate; Pro 1.06, Orn 0.96, Leu 1.00.

H–Pro–MeVal–Orn(Z)–Leu–OEt·HCl (XIII) Compound XII (2.52 g, 3.5 mmol) was treated with 2.6 M hydrogen chloride in ethyl acetate (27 ml) as described for II. The oily product was purified with a column (4.9×33 cm) with silica gel 60 (Merck) as follows. The oily product was applied on the column, and the column was washed with ethyl acetate (1000 ml) and eluted with a mixture of ethyl acetate and ethanol (1: 1, v/v) (500 ml). Each fraction was assayed by TLC, and the fractions containing the desired product were evaporated to leave an oil which was crystallized by the addition of ether and petroleum ether; yield, 1.75 g (76%); mp 85—90 °C; $[\alpha]_D^{20}$ −86.6° (*c* 1.1, methanol).

Found: C, 58.28; H, 8.00; N, 10.55%. Calcd for $C_{32}H_{52}O_7N_5Cl$: C, 58.74; H, 8.01; N, 10.71%.

Boc–D–Phe–Pro–MeVal–Orn(Z)–Leu–OEt (XIV). To a solution of XIII (1.64 g, 2.5 mmol) and TEA (2.5 mmol) in dioxane (15 ml) at 5 °C was added Boc–D–Phe–ONSu[13] (1.09 g, 3 mmol) in dioxane (5 ml). The mixture was left to stand for 30 min at 5 °C and overnight at room temperature, and a few drops of 1-(2-aminoethyl)piperazine were added. After 30 min, the solution was evaporated and the residue was diluted with ethyl acetate (100 ml). The solution was washed with 4% sodium hydrogencarbonate, 10% citric acid and water, and dried over sodium sulfate. The solvent was evaporated to leave an oil; yield, 1.99 g (92%). Amino acid ratios in acid hydrolyzate; Phe 1.02, Pro 1.05, Orn 0.93, Leu 1.00.

Boc–D–Phe–Pro–MeVal–Orn(Z)–Leu–OH (XV). A solution of XIV (1.73 g, 2 mmol) in methanol (12 ml) was treated with 1 M sodium hydroxide (2.4 ml) as described for VIII. The product was recrystallized from methanol–ether–petroleum ether; yield, 1.37 g (82%); mp 95—97 °C; $[\alpha]_D^{20}$ −97.4° (*c* 0.51, methanol).

Found: C, 62.85; H, 7.72; N, 10.14%. Calcd for $C_{44}H_{64}O_{10}N_6$: C, 63.13; H, 7.71; N, 10.01%. NMR ($CDCl_3$); 1.36 (s, 9H, $(CH_3)_3C$–), 2.78 (s, 3H, CH_3N–), 5.06 (s, 2H, –O–CH_2–C_6H_5), 7.18—7.28 (m, 10H, phenyl protons).

H–D–Phe–Pro–MeVal–Orn(Z)–Leu–OH·HCl (XVI). Compound XV (840 mg, 1 mmol) was treated with 2.6 M hydrogen chloride in ethyl acetate (8 ml) as described for II. The oily product was purified with a column (4.9×38 cm) with silica gel 60 (Merck) as follows. The oily product was applied on the column, and the column was washed with a mixture of chloroform and methanol (19: 1, v/v) (500 ml) and eluted with a mixture of chloroform and methanol (9: 1, v/v) (1000 ml). The fraction with the desired product were evaporated and the residual oil was crystallized by the addition of ether; yield, 557 mg (72%); mp 136—140 °C; $[\alpha]_D^{20}$ −48.4° (*c* 0.49, methanol).

Found: C, 59.59; H, 7.32; N, 10.61%. Calcd for $C_{39}H_{57}O_8N_6Cl\cdot H_2O$: C, 59.19; H, 7.51; N, 10.62%.

Boc–D–Phe–Pro–Val–Orn(Z)–Leu–D–Phe–Pro–MeVal–Orn(Z)–Leu–OH (XVII). To a solution of XVI (162 mg, 0.21 mmol) and TEA (0.42 mmol) in DMF (1 ml) at 0 °C was added IX (195 mg, 0.21 mmol) in DMF (1 ml). The mixture was left to stand for 30 min at 0 °C and overnight at room temperature, a few drops of 1-(2-aminoethyl)piperazine were added, the solution was evaporated, and the residue was diluted with ethyl acetate (100 ml). The solution was washed with 4% sodium hydrogencarbonate, 10% citric acid and

water, dried over sodium sulfate, and the solvent was evaporated to leave an oil, which was solidified by the addition of ether. The product was recrystallized from methanol–ether; yield, 215 mg (66%); mp 133—134 °C; $[\alpha]_D^{20}$ −118° (*c* 0.33, methanol). Amino acid ratios in acid hydrolyzate; Phe 1.96, Pro 2.02; Val 0.98; Orn 1.98; Leu 2.00.

Found: C, 62.92; H, 7.54; N, 10.80%. Calcd for $C_{82}H_{116}O_{17}N_{12}\cdot H_2O$: C, 63.13; H, 7.63; N, 10.78%.

*Boc–*D*–Phe–Pro–Val–Orn(Z)–Leu–*D*–Phe–Pro–MeVal–Orn(Z)–Leu–ONSu (XVIII).* To a solution of XVII (185 mg, 0.12 mmol) in DMF (2 ml) were added HONSu (28 mg, 0.24 mmol) and WSCI (46 mg, 0.24 mmol) at 0 °C. After being left to stand at room temperature for 12 h, ethyl acetate (50 ml) was added, and the solution was washed with 4% sodium hydrogencarbonate, 10% citric acid and water. After drying over sodium sulfate, the solvent was evaporated to leave an oil which was solidified by the addition of ether. The product was reprecipitated from ethyl acetate–ether; yield, 186 mg (95%); mp 130—132 °C; $[\alpha]_D^{20}$ −146° (*c* 0.13, ethyl acetate).

Found: C, 61.93; H, 7.34; N, 10.95%. Calcd for $C_{86}H_{119}O_{19}N_{13}\cdot 2H_2O$: C, 61.66; H, 7.40; N, 10.87%.

cyclo(–*D*–*Phe–Pro–Val–Orn(Z)–Leu–D–Phe–Pro–MeVal–Orn(Z)–Leu–*) *(XIX).* Compound XVIII (164 mg, 0.1 mmol) was dissolved in TFA (3 ml) at 0 °C. After 20 min, the solution was evaporated, the residual oil was triturated with ether, and the precipitate was collected by filtration (yield, 160 mg). The *N*-hydroxysuccinimide ester trifluoroacetate thus obtained was dissolved in DMF (2 ml), the solution was added drop by drop to pyridine (50 ml) at room temperature, and the mixture was stirred for 12 h and evaporated. The residual oil was dissolved in a mixture (25 ml) of methanol and water (5: 1, v/v), and the solution was passed through columns (1.8×20 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (500 ml), and the combined effluent was evaporated to leave an oily product which was precipitated by the addition of water (yield, 98 mg). A part (63 mg) of the precipitate dissolved in methanol (1 ml) was applied on a column (1.8×110 cm) with Sephadex LH-20 and the developing was continued with methanol. The fractions with the desired product were evaporated and the residual solid was recrystallized from methanol–ether–petroleum ether; yield, 51 mg (56% from XVIII); mp 218—219 °C; $[\alpha]_D^{20}$ −211° (*c* 0.17, methanol).

Found: C, 63.19; H, 7.45; N, 11.39%; mol wt, 1455.[14] Calcd for $C_{77}H_{106}O_{14}N_{12}\cdot 2H_2O$: C, 63.35; H, 7.60; N, 11.52%; mol wt, 1460.

cyclo(–*D*–*Phe–Pro–Val–Orn–Leu–D–Phe–Pro–MeVal–Orn–Leu–*)·*2HCl ([MeVal*[1]*]-GS·2HCl) (XX).* Compound XIX (40 mg, 0.028 mmol) dissolved in 0.01 M hydrogen chloride in methanol (7 ml) was hydrogenated using palladium black as a catalyst. After removal of the catalyst, the filtrate was evaporated and the residual oil was precipitated by the addition of 1 M hydrochloric acid (1 ml). The product was recrystallized from ethanol–1 M hydrochloric acid; yield, 24 mg (70%); 226—228 °C; $[\alpha]_D^{20}$ −141° (*c* 0.12, ethanol). Amino acid ratios in acid hydrolyzate; Phe 2.06, Pro 2.10, Val 1.08, Orn 1.92, Leu 2.00.

Found: C, 57.75; H, 7.85; N, 13.21%. Calcd for $C_{61}H_{94}O_{10}N_{12}\cdot 2HCl\cdot 2H_2O$: C, 57.94; H, 7.97; N, 13.30%.

*Boc–*D*–Phe–Pro–MeVal–Orn(Z)–Leu–ONSu (XXI).* Compound XV (670 mg, 0.8 mmol) in dioxane (4 ml) was treated with HONSu 184 mg, 1.6 mmol) and WSCI (307 mg, 1.6 mmol) as described for XVIII. The product was reprecipitated from ethyl acetate–ether–petroleum ether; yield, 677 mg (91%); mp 105—107 °C; $[\alpha]_D^{20}$ −67.8° (*c* 0.26, ethyl acetate).

Found: C, 61.33; H, 7.17; N, 10.47%. Calcd for $C_{18}H_{67}O_{12}N_7$: C, 61.72; H, 7.23; N, 10.50%.

*Boc–(*D*–Phe–Pro–MeVal–Orn(Z)–Leu)*$_2$*–OH (XXII).* A solution of XVI (600 mg, 0.78 mmol) and TEA (1.56 mmol) in DMF (3 ml) was treated with XXI (654 mg, 0.7 mmol) in DMF (3 ml) as described for XVII. The product was recrystallized from methanol–ether–petroleum ether; yield, 943 mg (87%); mp 125—127 °C; $[\alpha]_D^{20}$ −46.8° (*c* 0.80, methanol). Amino acid ratios in acid hydrolyzate; Phe 1.02, Pro 1.01, Orn 1.12, Leu 1.00.

Found: C, 63.13; H, 7.73; N, 10.71%. Calcd for $C_{83}H_{118}O_{17}N_{12}\cdot H_2O$: C, 63.33; H, 7.69; N, 10.68%.

*Boc–(*D*–Phe–Pro–MeVal–Orn(Z)–Leu)*$_2$*–ONSu (XXIII).* Compound XXII (902 mg, 0.58 mmol) was converted into the active ester (XXIII) by the method described for XVIII; yield, 865 mg (90%); mp 115—117 °C $[\alpha]_D^{20}$ −70.6° (*c* 0.28, ethyl acetate).

Found: C, 63.00; H, 7.42; N, 10.92%. Calcd for $C_{87}H_{121}O_{19}N_{13}$: C, 63.21; H, 7.38; N, 11.02%.

cyclo(–(*D*–*Phe–Pro–MeVal–Orn(Z)–Leu)*$_2$–) *(XXIV).* Compound XXIII (826 mg, 0.5 mmol) was treated with TFA (10 ml) and pyridine (500 ml), successively, as described for XIX. The crude product dissolved in a mixture of methanol and water (5: 1, v/v) was treated with Dowex 1 and 50, and an oily product was precipitated by the addition of water (yield, 552 mg). A part (100 mg) of the precipitate dissolved in methanol (20 ml) was purified by column chromatography using Sephadex LH-20 (2.8×158 cm) and the residual solid was recrystallized from methanol–ether–petroleum ether; yield, 86 mg (66% from XXIII); mp 280—285 °C; $[\alpha]_D^{20}$ −50.6° (*c* 0.29, methanol).

Found: C, 64.32; H, 7.56; N, 11.51%; mol wt, 1427.[14] Calcd for $C_{78}H_{108}O_{14}N_{12}\cdot H_2O$: C, 64.35; H, 7.62; N, 11.55%; mol wt, 1438.

cyclo(–(*D*–*Phe–Pro–MeVal–Orn–Leu)*$_2$–)·*2HCl ([MeVal*$^{1,1'}$*]-GS·2HCl) (XXV).* Compound XXIV (50 mg, 0.035 mmol) was hydrogenated as in the case of XX; yield, 41 mg (95%); mp 218—220 °C; $[\alpha]_D^{20}$ −51.9° (*c* 0.18, ethanol). Amino acid ratios in acid hydrolyzate; Phe 1.00, Pro 1.05, Orn 1.10, Leu 1.00.

Found: C, 54.25; H, 7.74; N, 11.92%. Calcd for $C_{62}H_{96}O_{10}N_{12}\cdot 2HCl\cdot 7H_2O$: C, 54.40; H, 8.25; N, 12.28%.

Paper Electrophoresis. In order to confirm the purity of the analogs of gramicidin S synthesized, paper electrophoresis was carried out.[15] The peptide (XX and XXV) revealed a single spot, and their mobility were comparable with that of GS·2HCl.

Microbiological Assays. The minimum amount of the compounds necessary for the complete inhibition of growth of several microorganisms was determined by a dilution method using a nutrient agar, the results being shown in Table 1.

ORD Measurements. The measurements were performed on a JASCO spectropolarimeter model ORD/UV-5 over the wavelength range 220—300 nm at an ambient temperature. A cell of path length 0.1 cm was used. Patterns in ethanol and 8 M urea are shown in Figs. 2-a and 2-b, respectively.

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6) The abbreviations recommended by the IUPAC-IUB Commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used throughout. Additional abbreviations: DCC, dicyclohexylcarbodiimide; DMF, *N,N*-dimethylformamide; HOBt, 1-hydroxy-benzotriazole; HON-Su, *N*-hydroxysuccinimide; MA, mixed anhydride method; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; WSCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide. Amino acid symbols except D-Phe denote the L-configuration.

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