Studies of Peptide Antibiotics. XXXVI.[1] Synthesis and Biological Activity of [5,5′-Leucine]-gramicidin S[2]

Okitoshi ABE, Yoko UTSUMI, and Nobuo IZUMIYA*

Laboratory of Chemistry, Fukuoka Dental College, Nishi-ku, Fukuoka 814

**Laboratory of Biochemistry, Fuculty of Science, Kyushu University 33, Hakozaki, Higashi-ku, Fukuoka 812*

(Received April 25, 1977)

An analog of gramicidin S, in which 5,5′-proline residues were replaced by leucine, was prepared in order to investigate the effect of the introduction of hydrophobic side groups on the antibacterial activity and on the conformation. Also the solubility problems encountered during the synthesis of this analog are described. No significant effect was observed on the cyclization reaction by the replacement of 5,5′-proline to leucine residues. This analog exhibited comparable biological activity and the similar conformation to the natural gramicidin S. These studies suggested that the imino groups of 5,5′-proline residues have no significant roles on both antibacterial activity and conformation of this antibiotic.

Synthetic analogs of gramicidin S (GS, Fig. 1) in which 5,5′-Pro residues are replaced by Gly,[3] Sar,[4] β-Ala,[5] α-aminoisobutyric acid (Aib),[6] or Phe[7] have been reported by Izumiya and his collaborators. Some of them, such as [5,5′-Gly]-,[3] [5,5′-Sar]-,[4] and [5,5′-Phe]-GS,[7] exhibited substantial biological activities. On the other hand, very weak or no antibacterial activity was observed on the other two analogs, *i.e.* [5,5′-β-Ala]-[5] and [5,5′-Aib]-GS.[6]

In this study was synthesized an analog in which 5,5′-Pro residues of the natural GS were replaced by Leu residues, namely [5,5′-Leu]-GS. Leucine residue possesses bulky hydrophobic side chain and its effect on the antibacterial activity has also been studied. In addition, as a strategy of the synthesis of this analog, the sequence of the linear intermediate was selected such a way that the 3- or 3′-Leu located to the carboxyl terminus in consideration of its similarity to the biosynthetic intermediate of GS.[8] The problems caused by this sequence are also discussed. Possibility of racemization during the cyclization reaction was overcome by the employment of the azide procedure.[9,10]

The intermediate peptides were protected on the α amino terminus by the *t*-butoxycarbonyl group (Boc–), on the δ amine of ornithines by the benzyloxycarbonyl group (Z–), and on α carboxyl groups by the ethyl ester (–OEt). Selective deprotection of Boc-group was carried out with hydrogen chloride in formic acid and of Z-group by hydrogenolysis in the presence of palladium black. As for the coupling reaction, the azide procedure of Curtius[9] and of Honzl and Rudinger[10] were mainly employed.

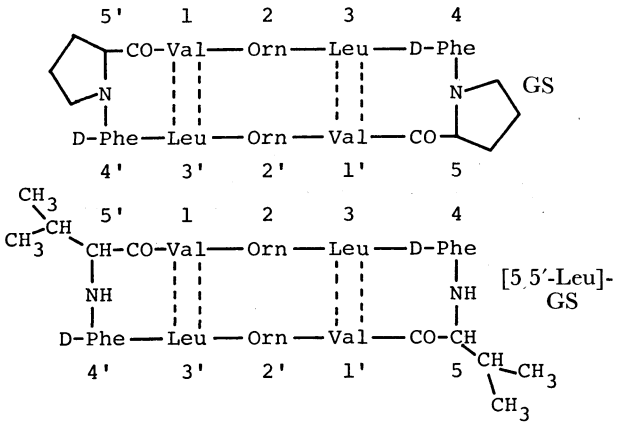
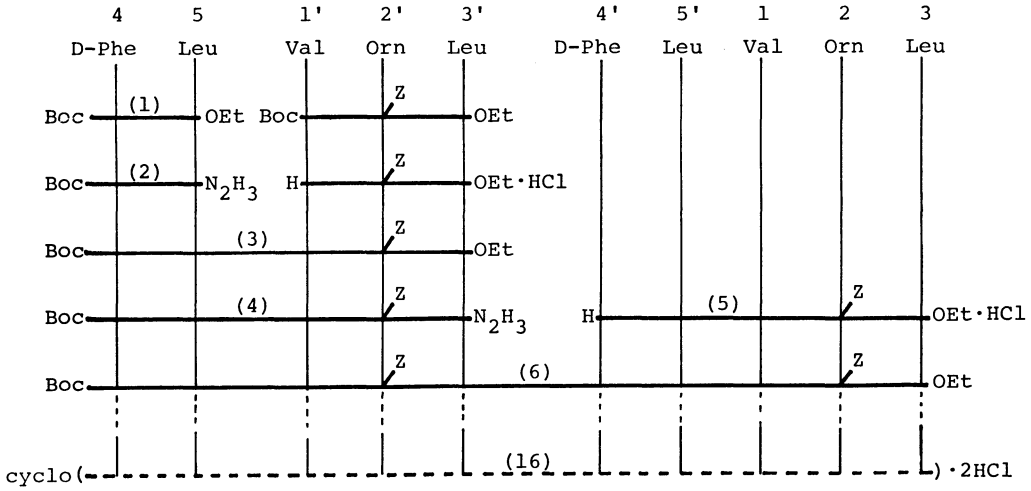


Fig. 1. Structure of GS (upper) and [5,5′-Leu]-GS (bottom).

Scheme 1 indicates a route for the synthesis of the protected decapeptide (**6**) with 3-Leu as the carboxyl terminus. This route was designed to imply the similarity of the amino acid sequence to that of the bio-



Scheme 1. Synthesis of [5,5′-Leu]-GS with 3-Leu as carboxyl terminus.

synthetic intermediate of GS.[8]

The first dipeptide derivative (**1**) was obtained by the mixed anhydride method with isobutyl chloroformate as the coupling reagent. Product **1** was obtained in 65% yield and then converted without difficulty to its hydrazide (**2**) by the addition of 20 molar excess of hydrazine hydrate. Boc-pentapeptide ester (**3**) was obtained by the coupling of the product **2** with H–Val–Orn(Z)–Leu–OEt[11] using azide procedure[9] in 80% yield. First problem was encountered in the following step, conversion of the ester (**3**) to the hydrazide (**4**). At first the same condition as described for the preparation of **2** was employed, however, the reaction was incomplete. Therefore, the amount of hydrazine was increased to 40 molar excess and also the reaction time prolonged. On standing for eight days, precipitates were formed and the reaction mixture was revealed to contain almost no ester on a thin-layer plate, yielding 82% of the product **4**. Deblocking of Boc-group from **3** yielded the product **5** in 96%.

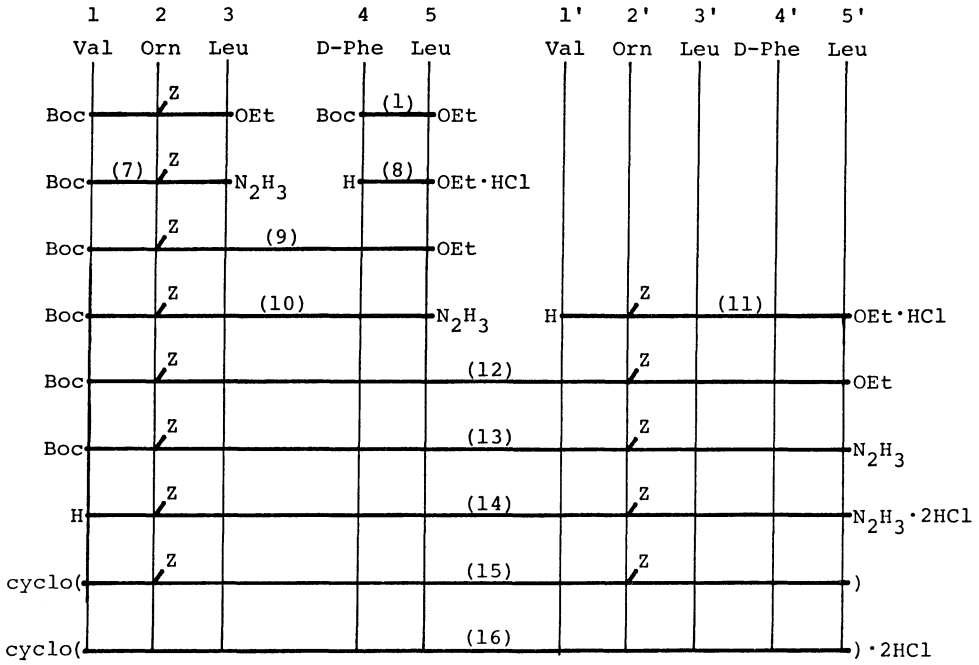
The second problem was in the coupling of the product **4** with **5**. Compound **4**, sparingly soluble in common organic solvents, was dissolved on two hours of stirring in the mixed solvent DMF–DMSO (1 : 1, v/v) and the coupling was carried out using the azide procedure of Honzl and Rudinger.[10] The product was revealed to be a mixture of several components and all the efforts for their separation and purification resulted in vain, mainly because of their insolubility. No better result was obtained when the coupling reaction was repeated several times. These facts forced to change the strategy of the synthetic route.

In the alteration of the synthetic route, first to be considered was the use of the intermediate peptide derivatives synthesized in Scheme 1. As shown in Scheme 2, two peptide derivatives, Boc–Val–Orn(Z)–Leu–OEt and the dipeptide derivative (**1**), could be successfully used. Conversion of the Boc-tripeptide ester to its hydrazide (**7**) with 20 molar excess of hydrazine hydrate and deblocking of the Boc-group of the compound **1** were performed without trouble. Subsequent coupling of **7** with **8** was carried out by means of the azide procedure to produce Boc-pentapeptide ester (**9**) in the yield of 54%. The next step, conversion of ester (**9**) to its hydrazide (**10**), was much easier than the corresponding step (**3** to **4**) in Scheme 1. It took two days to complete the reaction with 40 molar excess of hydrazine hydrate. Decapeptide derivative (**12**) was also obtained without difficulty in pure form by the azide method and the yield was fairly good (88%).

In the synthetic route of Scheme 2, the most troublesome step was the conversion of **12** to the hydrazide (**13**). The amount of hydrazine was increased from 40 to 80 and finally to 200 molar equivalents. Under the final condition, the product **13** was obtained in good yield (96%).

After removal of Boc-group from **13**, the product **14** was cyclized by the azide procedure as described in Experimental part. Crude cyclic peptide thus obtained was dissolved in aqueous methanol and passed through a column of Dowex 50X8. Recrystallization from methanol–water gave the pure Z-protected cyclic petide (**15**) in the yield of 58%. Hydrogenolysis of **15** produced the final product, [5,5′-Leu]-GS (**16**), in the yield of 67%. Homogeneity of **16** was ascertained by paper and thin-layer chromatographies with various solvent systems, paper electrophoresis, elemental analysis and by amino acid analysis. Also the final product was subjected to antibacterial test and ORD measurements.

In general, from the standpoint of synthesis, deblocking of Boc-groups with hydrogen chloride in formic acid was performed without any trouble. On the contrary,



Scheme 2. Synthesis of [5,5′-Leu]-GS with 5′-Leu as carboxyl terminus.

Table 1. Antibacterial activity of the synthetic GS analog, [5,5′-Leu]-GS (**16**)
(Minimum inhibitory concentration, μg/ml)

Strain	[5,5′-Leu]-GS (**16**)		GS	
	A[a]	B[b]	A[a]	B[b]
Escherichia coli	>50	>50	>50	>50
Proteus vulgaris	>50	>50	>50	>50
Staphylococcus aureus	10	10	2	5
Bacillus subtilis	5	5	2	5
Mycobacterium 607	>50	>50	>50	>50
Mycobacterium avium	>50	>50	>50	>50

a) Nutrient agar medium, pH 7. b) Synthetic medium.

Table 2. Antibacterial activities of the 5,5′-position substituted GS analogs[a]

5,5′-Residues	*S. aureus*		*B. subtilis*		Reference No.
	A[b]	B[c]	A[b]	B[c]	
Gly	5 (2)	5 (5)	5 (2)	2—5 (5)	12)
Sar	10 (5)	10 (5)	5 (5)	5 (5)	4)
β-Ala[d]	100 (6)		100 (3)		5)
Aib[e]	>100 (3)		>100 (3)		6)
Phe	10 (5)	20 (5)	10 (2)	10 (5)	7)
Leu	10 (2)	10 (5)	5 (2)	5 (5)	This work

a) ( ): Activity of the natural GS used as the control. b) Bouillon agar medium. c) Synthetic agar medium. d) Assay conditions not clearly shown. e) Assayed by paper disk method.

the conversion of the esters to their hydrazides produced several difficulties in some cases. And the insolubility of some hydrazides made the subsequent reaction difficult. Although the imino acid proline was replaced by the amino acid leucine, the cyclization step by the azide method was carried out without trouble and resulted in good yield.

Antibacterial activities of [5,5′-Leu]-GS measured in both nutrient agar and synthetic medium are listed in Table 1. Compared to natural GS, the synthetic analog exhibited considerable inhibitory activities against some organisms tested. From these data, the 5,5′-Pro residues of the natural GS seem to be exchangeable to bulky hydrophobic amino acid residues such as Leu without any significant effect on the antibacterial activity. In Table 2 are listed antibacterial activities of the 5,5′-position substituted GS analogs reported thus far. These data also indicate the insignificance of 5,5′-Pro residues of GS on its antibacterial activity.

The ORD curves of [5,5′-Leu]-GS measured in both ethanol and 8 M urea were presented in Fig. 2. In both media the synthetic analog exhibited similar curves as those of the natural GS with a negative trough at 234—235 nm. The fact that no shift of the trough had been observed even in the strong denaturing reagent such as 8 M urea suggested the rigid and stable conformation of the synthetic analog as well as the natural GS molecule, which has the intramolecular antiparallel β-form with four hydrogen bondings between the valyl and the leucyl residues.[13] Correlation between the rigid structure and the antibacterial activity is also suggested.

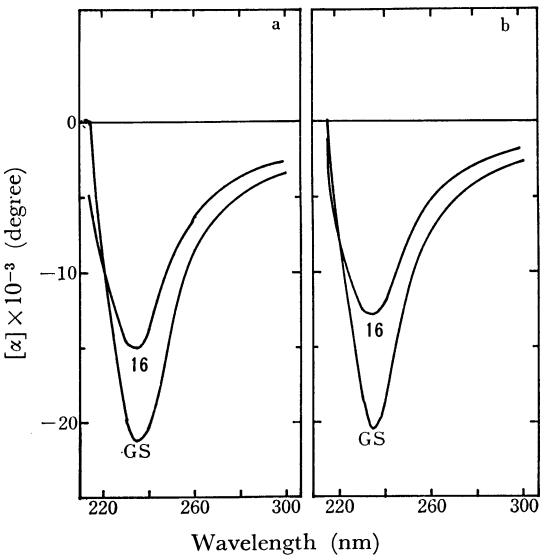


Fig. 2. ORD curves of [5,5′-Leu]-GS (**16**) and natural GS.
Solvent: a, EtOH; b, 8 M urea.

Experimental

All melting points are uncorrected. Prior to elemental analysis, all the compounds were dried over P_2O_5 to a constant weight at 80 °C and 2 Torr. TLC was carried out on silica gel G (Merck) with the following solvent systems: R_f^1, chloroform–MeOH (5 : 1, v/v), R_f^2, chloroform–MeOH–AcOH (95 : 5 : 1, v/v), R_f^3, chloroform–MeOH (9 : 1, v/v), R_f^4, 1-butanol (BuOH)–AcOH–pyridine–H_2O (4 : 1 : 1 : 2, v/v), R_f^5, BuOH–AcOH–pyridine–H_2O (15 : 3 : 10 : 12, v/v). Paper chromatography (PPC) was performed on Toyo Roshi No. 52 paper with the solvent system, either R_f^4 or R_f^5. The optical rotations were determined with a polarimeter at the 589 sodium line. Amino acid analysis was carried out on the sample which had been hydrolyzed for 24 h in a evacuated sealed tube at 110 °C.

*Boc–D-Phe–Leu–OEt (**1**).* To a solution of Boc–D-Phe–OH (2.65 g, 10 mmol) in THF (20 ml), were added triethylamine (TEA, 1.40 ml) and isobutyl chloroformate (1.31 ml, 10 mmol) at 0—4 °C. The mixture was stirred for 10 min at 0—4 °C and to this was added a solution of H–Leu–OEt·*p*TsOH[14] (3.31 g, 10 mmol) and TEA (1.4 ml) in chloroform (20 ml). After the mixture was stirred overnight at a room temp, the solvent was evaporated *in vacuo* and the residue was taken up in AcOEt. The AcOEt solution was then washed successively with 0.5 M citric acid, 4% sodium hydrogen carbonate and water, and then dried (Na_2SO_4). The solvent was evaporated *in vacuo* and the product was precipitated by the addition of ether and petroleum ether. Recrystallization was carried out from ether–petroleum ether; yield, 2.64 g (65%); mp 111—112 °C; $[\alpha]_D^{25}$ −4.6° (*c* 1, DMF); R_f^1 0.96.
Found: C, 64.96; H, 8.44; N, 7.04%. Calcd for $C_{22}H_{34}N_2O_5$: C, 65.00; H, 8.43; N, 6.89%.

*Boc–D-Phe–Leu–N_2H_3 (**2**).* Hydrazine hydrate (2.91 ml, 60 mmol) was added with stirring to the solution of **1** (1.22 g, 3 mmol) in DMF (6 ml) and the mixture was left to stand at a room temp. After stirring for 48 h, the reaction mixture showed a single spot, which was distinguished from that of **1**, on a TLC plate. Excess hydrazine was evaporated

in vacuo, and then the product was precipitated with water (200 ml). The precipitates were washed with water and desiccated overnight (P_2O_5); yield, 1.03 g (87%); mp 163—164 °C; $[\alpha]_D^{25}$ −12.0° (*c* 1, DMF); R_f^1 0.77.

Found: C, 61.13; H, 8.30; N, 14.28%. Calcd for $C_{20}H_{32}N_4O_4$: C, 61.20; H, 8.22; N, 14.28%.

Boc–D-Phe–Leu–Val–Orn(Z)–Leu–OEt (**3**). In a mixture of glacial AcOH (40 ml) and 1 M HCl (3 ml) was dissolved **2** (0.59 g, 1.5 mmol) and the solution was cooled to −5 °C. To the solution was added, with stirring, a cold concentrated aq solution of sodium nitrite (0.103 g, 1.5 mmol). After 10 min, the azide was precipitated by the addition of cold water (200 ml) and the precipitates were filtered, washed successively with ice-cold water, 5% sodium hydrogen carbonate, and again with water. After brief drying (P_2O_5), the azide was added to the solution of H–Val–Orn(Z)–Leu–OEt·HCl[11] (0.815 g, 1.5 mmol) and TEA (0.21 ml, 1.5 mmol) dissolved in DMF (10 ml). After standing overnight at 0 °C and then for 2 days at a room temp, the reaction mixture was concentrated *in vacuo* and the product was precipitated on adding water. The crude product was recrystallized with EtOH–ether; yield, 1.04 g (80%); mp 237—238 °C (dec); $[\alpha]_D^{20}$ −14.0° (*c* 1, DMF); R_f^1 0.98, R_f^2 0.98.

Found: C, 63.45; H, 8.19; N, 9.71%. Calcd for $C_{46}H_{70}N_6O_{10}$: C, 63.72; H, 8.14; N, 9.69%.

Boc–D-Phe–Leu–Val–Orn(Z)–Leu–N_2H_3 (**4**). A solution of **3** (0.867 g, 1 mmol) and hydrazine hydrate (1.94 ml, 40 mmol) in DMF (20 ml) was left to stand for 8 days at a room temp and hydrazide precipitated in the reaction mixture was filtered, washed with water and with a small amount of EtOH, and then dried (P_2O_5); yield ,0.72 g (82%); mp 237—238 °C (dec); $[\alpha]_D^{20}$ −11.8° (*c* 1, DMF); R_f^1 0.98, R_f^2 0.94, R_f^5 0.98.

Found: C, 60.55; H, 9.86; N, 12.50%. Calcd for $C_{44}H_{68}N_8O_9$: C, 60.52; H, 10.16; N, 12.83%.

H–D-Phe–Leu–Val–Orn(Z)–Leu–OEt·HCl (**5**). Compound **4** (434 mg, 0.5 mmol) was dissolved in 0.075 M HCl in formic acid (8.0 ml) and allowed to stand for 2 h at a room temp. After the reaction mixture was evaporated to dryness *in vacuo*, the product was solidified by the addition of ether; yield, 387 mg (96%); mp 231 °C (dec); $[\alpha]_D^{20}$ +4.0° (*c* 1, DMF); R_f^1 0.66, R_f^2 0.10, R_f^5 (PPC) 0.95.

Found: C, 60.48; H, 7.95; N, 10.23%. Calcd for $C_{41}H_{63}N_6O_8Cl\cdot 1/2H_2O$: C, 60.61; H, 7.94; N, 10.34%.

Boc–(D-Phe–Leu–Val–Orn(Z)–Leu)$_2$–OEt (**6**). To a solution of **4** (175 mg, 0.2 mmol) in a mixed solvent of DMF (25 ml) and DMSO (25 ml) were added 3.97 M HCl in dioxane and then isoamyl nitrite (0.031 ml, 0.22 mmol) at −30 °C. After 30 min of stirring at −10 °C, hydrazide test[15] of the reaction mixture turned negative. And the mixture was neutralized by the addition of TEA (0.084 ml, 0.6 mmol). After 5 min at 0 °C, a solution of **5** (176 mg, 0.22 mmol) was added to the mixture. After stirring for 3 days at 4 °C, 0.5 M citric acid (100 ml) was added to the reaction mixture concentrated *in vacuo* to *ca.* 30 ml, and the mixture was kept at 4 °C for 3 h. The colloidal product, which was hard to filter, was collected by centrifugation at 4000×*g* for 10 min and washed with water several times. The resulting residue was dried over P_2O_5.

Boc–Val–Orn(Z)–Leu–N_2H_3 (**7**). To a solution of Boc–Val–Orn(Z)–Leu–OEt[11] (1.03 g, 1.7 mmol) in DMF (3 ml), hydrazine hydrate (1.65 ml, 34 mmol) was added and allowed to stand overnight at a room temp. After the excess hydrazine had been evaporated *in vacuo*, water (50 ml) was added to the mixture.Precipitates formed were filtered, washed with water, and dried (P_2O_5); yield ,988 mg (98%); mp 209—210 °C; $[\alpha]_D^{20}$ −19.2° (*c* 0.5, AcOH); R_f^1 0.70, R_f^5 0.95.

Found: C, 59.05; H, 8.22; N, 14.21%. Calcd for $C_{29}H_{48}O_7N_6$: C, 58.76; H, 8.16; N, 14.18%.

H–D-Phe–Leu–OEt·HCl (**8**). The compound **1** (813 mg, 2 mmol) was dissolved in 0.113 M HCl in formic acid (21.2 ml) and allowed to stand at a room temp. After 30 min, the solution was evaporated *in vacuo*. The addition of ether and subsequent evaporation were repeated several times and the oily product was obtained; yield of the oil, 686 mg (100%); R_f^1 0.73.

Boc–Val–Orn(Z)–Leu–D-Phe–Leu–OEt (**9**). The compound was obtained from **7** (890 mg, 1.5 mmol) and **8** (686 mg, 2 mmol) by the azide procedure[10] as described for the preparation of **6**. The product was crystallized twice from AcOEt–ether–petroleum ether; yield, 701 mg (54%); mp 211—213 °C; $[\alpha]_D^{20}$ −12.9° (*c* 1, DMF); R_f^3 0.73.

Found: C, 63.47; H, 8.10; N, 9.63%. Calcd for $C_{46}H_{70}O_{10}N_6$: C, 63.72; H, 8.14; N, 9.69%.

Boc–Val–Orn(Z)–Leu–D-Phe–Leu–N_2H_3 (**10**). The compound **9** (607 mg, 0.7 mmol) was dissolved in DMF (10 ml) and hydrazine hydrate (1.36 ml, 28 mmol) was added. After standing for two days at a room temp, the solvent was evaporated *in vacuo* to a small volume and water was added to the residue. After brief standing at 4 °C, the precipitates were filtered and dried over P_2O_5; yield, 622 mg (96%); mp 207—209 °C; $[\alpha]_D^{20}$ −11.3° (*c* 0.5, DMF); R_f^1 0.67.

Found: C, 61.61; H, 8.21; N, 13.00%. Calcd for $C_{44}H_{68}O_9N_8$: C, 61.95; H, 8.04; N, 13.14%.

H–Val–Orn(Z)–Leu–D-Phe–Leu–OEt·HCl (**11**). Deblocking of Boc-group from **9** (780 mg, 0.9 mmol) was performed by the similar method described in the preparation of **5** by the action of 0.13 M HCl in formic acid (7.6 ml); yield, 694 mg (96%); mp 211—213 °C; $[\alpha]_D^{20}$ +3.8° (*c* 1, DMF) R_f^3 0.17.

Found: C, 61.17; H, 7.92; N, 10.36%. Calcd for $C_{41}H_{63}O_8N_6Cl$: C, 61.29; H, 7.90; N, 10.46%.

Boc–(Val–Orn(Z)–Leu–D-Phe–Leu)$_2$–OEt (**12**). This compound was prepared from **10** (512 mg, 0.6 mmol) and **11** (627 mg, 0.78 mmol) by the similar method described for the preparation of **3**, except that a mixed solvent, DMF–DMSO (5 : 1, v/v), was used in this case. Crude product was recrystallized from MeOH; yield, 839 mg (88%); mp 239—241 °C; $[\alpha]_D^{20}$ −13.4° (*c* 0.5, DMF); R_f^1 0.93.

Found: C, 63.57; H, 7.97; N, 10.58%. Calcd for $C_{85}H_{126}O_{17}N_{12}\cdot H_2O$: C, 63.57; H, 8.08; N, 10.53%.

Boc–(Val–Orn(Z)–Leu–D-Phe–Leu)$_2$–N_2H_3 (**13**). To the compound **12** (250 mg, 0.157 mmol) dissolved in DMF (10 ml), was added 200 molar equivalents of hydrazine hydrate (1.52 ml, 31.4 mmol) and allowed to stand at a room temp for 2 days and treated as described for the preparation of **2**; yield, 226 mg (96%); mp >250 °C; $[\alpha]_D^{20}$ −14.6° (*c* 0.5, DMF–DMSO (2 : 1, v/v)); R_f^2 0.01, R_f^3 0.41.

Found: C, 63.20; H, 8.01; N, 12.20%. Calcd for $C_{83}H_{124}O_{16}N_{14}$: C, 63.33; H, 7.94; N, 12.46%.

H–(Val–Orn(Z)–Leu–D-Phe–Leu)$_2$–N_2H_3·2HCl (**14**). The compound **13** (200 mg, 0.127 mmol) was treated with 0.12 M HCl in formic acid (2.5 ml) at a room temp. After an hour, the reaction mixture was evaporated *in vacuo*, AcOEt added to the residue and evaporation repeated. To the residue was added ether and the mixture was kept at 4 °C; yield, 199 mg (100%); mp >250 °C; $[\alpha]_D^{20}$ −32.2° (*c* 0.2, DMF–DMSO (2 : 1, v/v)); R_f^1 0.41.

Found: C, 60.11; H, 7.74; N, 12.53%. Calcd for $C_{78}H_{116}O_{14}N_{14}\cdot 2HCl\cdot H_2O$: C, 59.86; H, 7.73; N, 12.53%.

cyclo(*–(Val–Orn(Z)–Leu–D-Phe–Leu)$_2$–*) (**15**). To the solution of **14** (193 mg, 0.13 mmol) in DMF–DMSO (2 : 1, v/v, 6 ml) was added at −30 °C, 2.7 M HCl in AcOEt (0.096 ml) and then 10% isoamyl nitrite in DMF (0.182 ml,

0.13 mmol). The reaction mixture was stirred at −20 °C until hydrazide test[15] became negative. After 15 min of stirring, the resulting pale yellow solution was added at 0 °C to pyridine (65 ml) and the mixture was kept stirring at 0 °C. After 3 days, pyridine and DMF were evaporated *in vacuo* from the mixture and 5 M citric acid (80 ml) was added. White precipitates formed were filtered, washed with water and dried (P_2O_5). The crude product (199 mg) was dissolved in a mixture of MeOH (100 ml) and H_2O (10 ml), and passed through a column (1.5×7.0 cm) of Dowex 50x8 and the elution was performed with the same solvent. The combined eluates were evaporated *in vacuo*. The white residue was filtered with the aid of small amount of water and dried over P_2O_5. The product (125 mg) was recrystallized from MeOH–H_2O; yield, 109 mg (58%); mp >250 °C; $[\alpha]_D^{20}$ −122° (*c* 0.2, DMF); R_f^3 0.53.

Found: C, 64.01; H, 7.82; N, 11.17%; mol wt,[16] 1441. Calcd for $C_{78}H_{112}O_{14}N_{12}\cdot H_2O$: C, 64.17; H, 7.87; N, 11.51 %; mol wt, 1460.

cyclo*(-(Val–Orn–Leu–*D*-Phe–Leu)$_2$–)·2HCl* (**16**).
The compound **15** was dissolved in 0.07 M HCl in EtOH (1.2 ml) and hydrogenolysis was performed in the presence of palladium black. After 2 h, additional 0.07 M HCl was added to the reaction mixture and the hydrogenolysis was continued overnight at 4 °C. After the catalyst had been filtered and washed several times with EtOH, the filtrate was evaporated to dryness and the residual solid was collected with the aid of ether. The crude product was recrystallized from EtOH–ether; yield, 29 mg (67%); mp 251—255 °C; $[\alpha]_D^{20}$ −156° (*c* 0.2, DMF); R_f^3 0.01, R_f^4 0.91, R_f^4 (PPC) 0.92. Amino acid ratios in acid hydrolyzate; Val 2.16, Orn 2.00, Leu 4.00, Phe 2.30.

Found: C, 56.52; H, 8.24; N, 12.33%. Calcd for $C_{62}H_{100}O_{10}N_{12}\cdot 2HCl\cdot 4H_2O$: C, 56.47; H, 8.40; N, 12.75%.

Electrophoresis Electrophoresis on Toyo Roshi No. 52 paper was carried out with a solvent system, formic acid–AcOH–MeOH–H_2O (1 : 3 : 6 : 10, v/v, pH 1.8) at 500 V/30 cm for 3 h. As shown in Fig. 3, [5,5′-Leu]-GS migrated more slowly toward cathode than the control GS, *i.e.* the former migrated 7.4 cm and the latter 8 cm by length.

ORD Measurements These were performed with a JASCO spectropolarimeter Model ORD/UV-5 over a range 215—300 nm. A cell of the path length 0.1 cm was used and the runs were at ambient temperature. Patterns in EtOH and 8 M urea are shown in Fig. 2.

Microbiological Assays. The results of the assay and the microorganisms employed are listed in Table 1. The minimum amount of the compound necessary for the complete inhibition of growth was determined by a dilution method using a bouillon agar medium and a synthetic medium. As shown in Tables 1 and 2, [5,5′-Leu]-GS (**16**) was found to exhibit a considerable antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

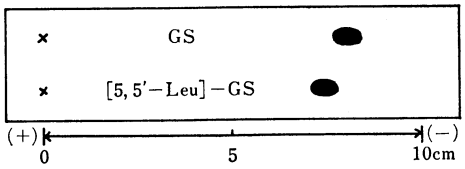


Fig. 3. Paper electrophoresis of [5,5′-Leu]-GS (**16**) and natural GS.

The authors are indebted to Dr. K. Kuromizu for his useful suggestions and discussions, Mr. Y. Shimohigashi for ORD measurements ,and the staffs of Takeda Chemical Industries, Ltd., for the biological assays.

References

1) Part XXXV of this series: K. Sato, H. Abe, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **50**, 1999 (1977).
2) All optically active amino acids, except D-Phe, are of the L configuration.
3) H. Aoyagi, T. Kato, M. Ohno, M. Kondo, M. Waki, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **38**, 2139 (1965).
4) H. Aoyagi and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **39**, 1747 (1966).
5) S. Matsuura, M. Waki, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **43**, 1197 (1970).
6) H. Takiguchi, H. Nishikawa, K. Matsudai, and N. Izumiya, *Fukuoka Univ. Sci. Reports*, **3**, 31 (1974).
7) H. Takiguchi, H. Nishikawa, and N. Izumiya, *Fukuoka Univ. Sci. Reports*, **5**, 139 (1975).
8) I. W. Pollard, N. V. Bhagavan, and J. B. Hall, *Biochemistry*, **7**, 1153 (1968).
9) T. Curtius, *Ber.*, **35**, 3226 (1902).
10) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
11) H. Abe, K. Sato, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **49**, 3113 (1976).
12) S. Matsuura, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **45**, 863 (1972).
13) D. C. Hodgkin and B. M. Oughton, *Biochem. J.*, **65**, 752 (1957).
14) T. Kato, S. Makisumi, M. Ohno, and N. Izumiya, *Nippon Kagaku Zasshi*, **83**, 1151 (1962).
15) H. Ertel and L. Horner, *J. Chromatogr.*, **7**, 268 (1962).
16) Molecular weight was determined on a Hitachi Osmometer, type 115, with methanol as a solvent.