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Syntheses and Properties of [1,1′-Bis(α-Aminoisobutyric Acid)]Gramicidin S and [1-α-Aminoisobutyric Acid]Semigramicidin S

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Analogs of gramicidin S, [1,1′-bis(α-aminoisobutyric acid)]gramicidin S (**18**) and [1-α-aminoisobutyric acid] semigramicidin S (**11**), have been synthesized by a conventional solution method. The cyclization reactions of a linear pentapeptide active ester and a linear decapeptide active ester yielded exclusively a protected cyclic pentapeptide and a protected cyclic decapeptide, respectively. After deprotection of these peptides, we obtained the final products (**11** and **18**). The mobility of **18** in paper electrophoresis was the same as that of gramicidin S, however, the CD spectrum of **18** in a methanol solution showed a markedly different pattern from that of gramicidin S. Both analogs (**11** and **18**) are inactive against the Gram-positive microorganisms tested. The results suggest that the positions of the valine residues in gramicidin S require a hydrophobic amino acid having L-configuration to show activity.

Gramicidin S (GS) is a cyclic peptide antibiotic which exhibits a strong activity against Gram-positive bacteria. Its secondary structure is well-known to be an antiparallel β-pleated sheet including four intramolecular hydrogen bonds with C_2 symmetry as shown in Fig. 1.

Regarding the contribution of the side chains of the Val residues in GS to the antibacterial activity, several GS analogs have been synthesized by one of the authors (M.K) and it has been reported that [Ala$^{1,1'}$]GS and [Leu$^{1,1'}$]GS were as active as natural GS toward several microorganisms, whereas [Gly$^{1,1'}$]GS exhibited no antibacterial activity.[1,2] Thus, it is suggested that side chains as bulky or hydrophobic as methyls might be required at positions 1 and 1′ for the interaction between GS and sensitive bacteria.[3] On the other hand, in the syntheses of the above analogs using a dimeric cyclization reaction of the corresponding pentapeptide active esters, the ratio of a cyclic dimer to a cyclic monomer decreases in the order of steric hindrance of an N-terminal amino acid in each pentapeptide active ester substituted with Val,[4] Leu,[2] and Ala.[1] Finally Gly substitution[1] results in only a cyclic monomer as shown in Table 1. From these previous investigations it appears that the steric nature of the side chain of the N-terminal amino acid in a pentapeptide active ester greatly influences the cyclization reaction and consequently induces ring closure to a dimer by dimerization.[5]

In this connection, an analog of GS containing α-aminoisobutyric acid (Aib) in place of Val is of interest in demonstrating the specific contribution of Aib to the activity. This Aib is found in several antibiotics, alamethicin,[6] suzukacillin,[7] antiameobins,[6] emerimicins,[6] trichotoxin A-40,[8] and hypelcins.[9] They are peptide ionophores functioning as transmembrane channels for ion transport.[10] The Aib includes two methyl groups as side chains which have similar bulkiness or hydrophobicity to that of Ala, and is optically inactive as Gly. In addition, the steric hindrance caused by the two methyl groups of Aib sometimes prevents introducing a protecting group, converting to the corresponding active ester, and coupling with other amino acids or peptide.[11–13] Accordingly a moderate yield of a dimer was expected from the cyclization reaction of the pentapeptide active ester containing Aib at the N-terminus. Furthermore, such a synthetic [Aib$^{1,1'}$]GS analog should exhibit biological activity because the side chains of Aib, two methyl

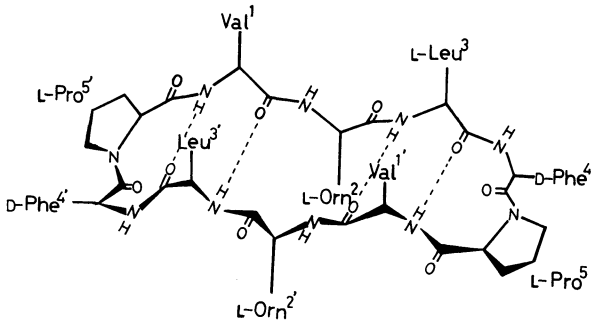

Fig. 1. A model with β-pleated sheet conformation of gramicidin S.

Table 1. Ratio of Protected Monomer and Dimer after Cyclization of Linear Pentapeptide *p*-Nitrophenyl Ester[a)]

p-Nitrophenyl ester of pentapeptide					Ratio of compounds in product[b)]		Ref.
					Z-cyclic monomer	Z_2-cyclic dimer	
1	2	3	4	5			
Val	Orn(Z)	Leu	D-Phe	Pro	32	68	4
Leu					78	22	2
Ala					91	9	1
Gly					100	0	1

a) The concentration was approximately $3\times10^{-3}\,\mathrm{mol\,dm^{-3}}$. b) The ratios shown are weight by weight.

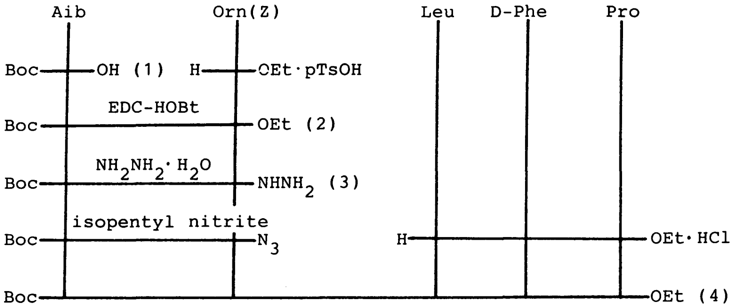

Fig. 2. Synthesis of Boc-pentapeptide.[14]

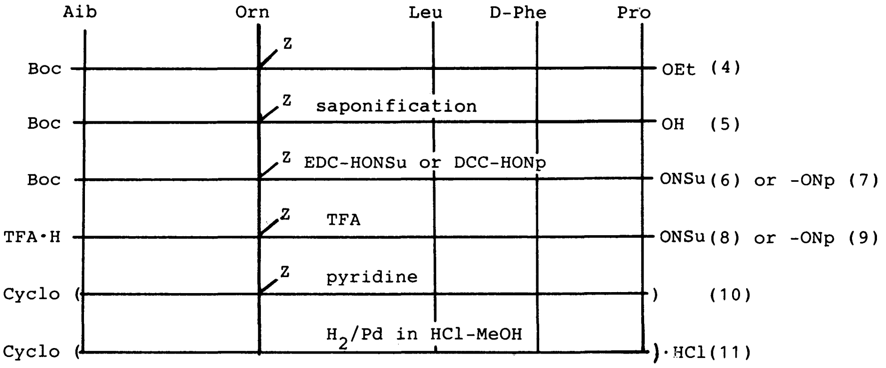

Fig. 3. Cyclization of linear pentepeptide active esters.

groups, in the analog have the same hydrophobicity as that of Ala in [Ala$^{1,1'}$]GS of which the biological activity is as strong as natural GS.[1)]

The present paper will describe the syntheses, antibacterial properties, and CD measurements of [Aib$^{1,1'}$]GS and [Aib^1]semiGS.

The route for the synthesis of a protected pentapeptide is indicated in Fig. 2. The dipeptide derivative (**2**) was effectively synthesized by the EDC–HOBt method involving stirring for a long time and extracting with ether, followed by conversion to the hydrazide (**3**). The condensation of the azide derived from **3** with a tripeptide ester gave an acylpentapeptide ester (**4**). The synthetic route for cyclization reactions of two pentapeptide active esters is shown in Fig. 3. The Boc-pentapeptide ester (**4**) was converted to the corresponding Boc-pentapeptide (**5**) by saponification. To investigate the best method for the cyclization reaction, two active esters using *N*-hydroxysuccinimide ester (**6**) and *p*-nitrophenyl ester (**7**) were prepared, and their Boc-groups were removed by the action of TFA at 0 °C. The cyclization of **8** or **9** at a concentration of 3×10^{-3} mol dm^{-3} in pyridine and subsequent purification of the crude cyclopeptide with Dowex 1 and 50 yielded exclusively the Z-substituted cyclic monomer (**10**), instead of the expected bis(Z-substituted) cyclic dimer. Its molecular weight was confirmed by mass spectrometry. The strong tendency of **8** or **9** to ring closure by monomerization indicates that the structural restriction imposed by Aib results in a favorable conformation of the cyclic pentapeptide and in this case of a cyclization of the pentapeptide active ester, L-configuration of the N-terminal amino acid plays basically a more important role than the steric nature in regard to its dimerization reaction. The Z-[Aib^1]semiGS (**10**) thus obtained was hydrogenated to afford [Aib^1]semiGS · HCl (**11**) as crystals.

In order to obtain the cyclodecapeptide, a cyclization reaction of a decapeptide active ester was employed for the preparation of Z$_2$-[Aib$^{1,1'}$]GS. The route outlined in Fig. 4 was employed for synthesis of Z$_2$-[Aib$^{1,1'}$]GS. The Boc-decapeptide ester (**13**) was synthesized by the EDC–HOBt method from **5** and a pentapeptide ester trifluoroacetate, and **13** was saponified to give the Boc-decapeptide (**14**). The compound **14** was converted to the Boc-decapeptide succinimide ester (**15**). The bis(Z-substituted) cyclodecapeptide (**17**) was obtained through the cyclization reaction at a high dilution in pyridine after treatment of **15** with TFA, and the crude product was then purified with Dowex 1 and 50, gel chromatography on Sephadex LH-20 and silica-gel columns. Its molecular weight determination with a vapor pressure osmometer demonstrated that the molecular size of **17** corresponds to that of the bis(Z-substituted) cyclic decapeptide. The compound **17** was subjected to hydrogenolysis, and the final product **18** was obtained as crystals. Its homogeneity was confirmed by paper and thin-layer chromatographies and paper electrophoresis.

Biological assay of [Aib$^{1,1'}$]GS and [Aib^1]semiGS shows no antibiotic activity against all the Gram-positive microorganisms listed in Table 2. The bio-

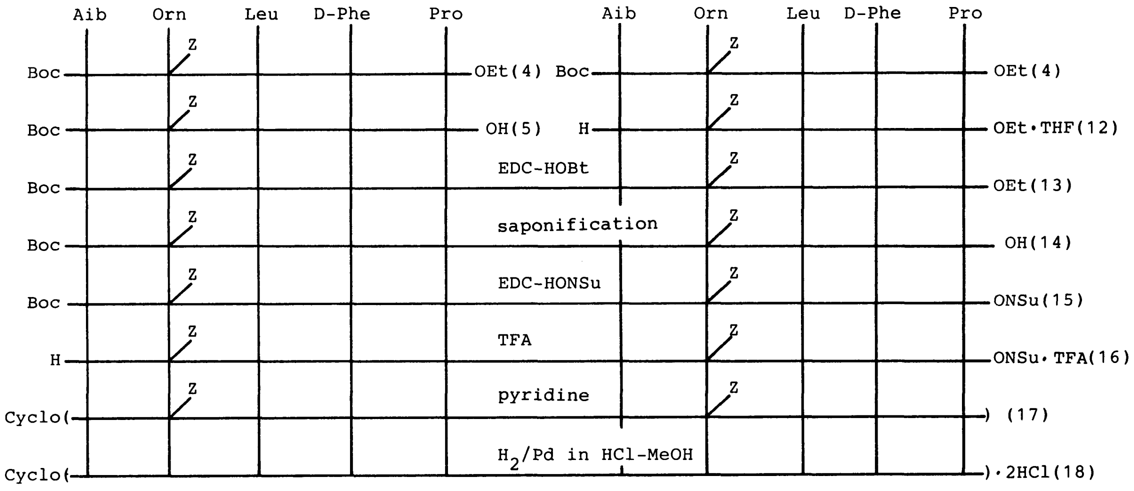

Fig. 4. Synthesis of [Aib$^{1,1'}$] GS·2HCl (**18**).

Table 2. Inhibitory Activity of [Aib^1] semiGS (**11**), [Aib$^{1,1'}$] GS (**18**) and GS on Microorganisms

Test organisms	GS	11	18
S. Aureus	3	>100	>100
B. subtilis	3	>100	100
E. coli	>100	>100	>100
S. flexneri	6	>100	>100
P. vulgaris	>100	>100	>100
P. aeruginosa	>100	>100	>100

Minimum inhibitory concentration, μg ml^{-1}.

logical data demonstrate that the Val residues in GS can not be substituted by the Aib residues without affecting the biological activity. Further, it has been reported that synthetic GS analogs such as [Aib$^{4,4'}$]GS and [Aib$^{5,5'}$]GS exhibited no antibiotic activity against all the microorganisms tested.[12,13] These results and our study of CPK model indicate that the rigid β-sheet structure of the GS molecule might be destabilized by the steric hindrance of the Aib residue in the GS analog. Hence the loss of sidedness structure affects the biological activity of the analog.[15]

The CD spectra of Boc-pentapeptide-ONSu and the protected cyclopentapeptide were measured to obtain information on a conformational change of the active ester just before ring closure, as shown in Fig. 5. A negative maximum and a shoulder of the protected cyclic pentapeptide (**10**) are about twice as great as those of the protected linear peptide active ester (**6**). Their characteristic shapes resemble each other. This seems to support the view that the conformation of the peptide backbone before ring closure is similar to that after ring formation and the cyclization reaction of the pentapeptide-ONSu afforded a cyclic monomer in good yield (73%) and no cyclic dimer. As shown in Fig. 6, the ellipticities of the negative troughs near 200 nm of three analogs, [Gly^1], [Ala^1], and [Aib^1]semiGS, are considerably smaller than that of GS, and the negative maximum bands of the analogs show blue shifts from that of GS. The shoulder at 218 nm is observed in

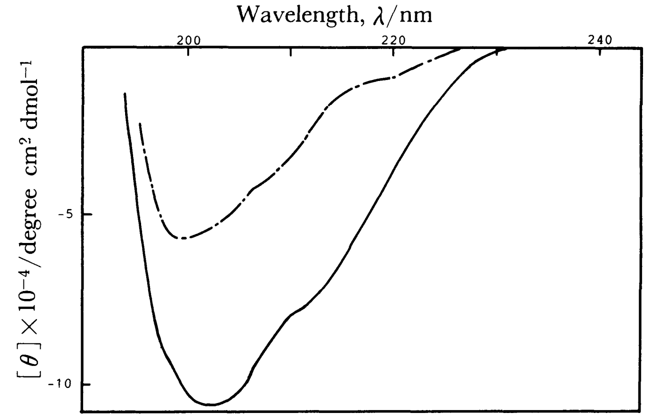

Fig. 5. CD spectra of Boc-pentapeptide-ONSu (**6**) and Z-substituted cyclopentapeptide (**10**) in MeOH. —·—·—: **6**, ——: **10**.

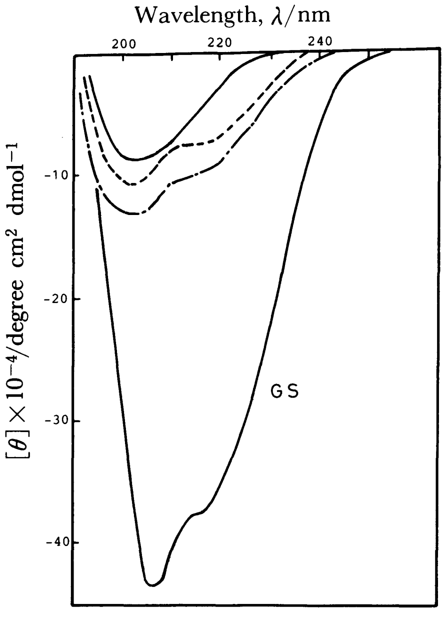

Fig. 6. CD spectra of semiGS analogs and GS. ——: [Aib^1] semiGS, ----: [Gly^1] semiGS, —·—·—: [Ala^1] semiGS.

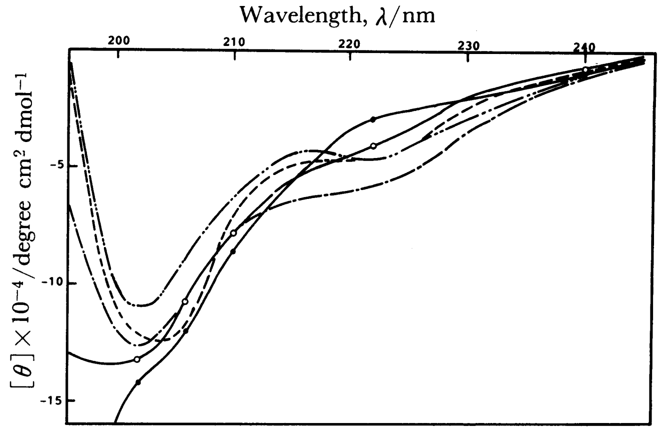

Fig. 7. CD spectra of protected linear **13**, **14**, **15**, **16**, and cyclic decapeptide (**17**) in MeOH. ----: **13**, -·-·-: **14**, -··-··-: **15**, —○—○—: **16**, —●—●—: **17**.

[Gly^1]semiGS and [Ala^1]semiGS, but it is not detectable in [Aib^1]semiGS (**11**). The small and simple Cotton effect of **11** might be due to the influence of the large steric hindrance of Aib residue on the peptide backbone. The CD spectra of the linear decapeptide derivatives before ring closure and the protected cyclic decapeptide in MeOH are shown in Fig. 7. Each of the linear peptides shows a negative trough at 203 nm and a shoulder near 223 nm. However, the spectrum of the cyclic peptide shows only a shoulder instead of a trough at 203 nm, and the trough gives a blue shift from that of the linear decapeptide derivatives. It is obvious that the backbone conformations of the linear peptides and that of the cyclic peptide are different. The CD curves of [Aib^1]semiGS (**11**) and [Aib$^{1,1'}$]GS (**18**) in MeOH as well as that of GS are shown in Fig. 8. The spectral shapes of these compounds indicate that these molecular conformations of **11** and **18** become significantly different from that of GS. The CD pattern of **18** exhibits no characteristic negative Cotton effect shown in GS at 206 and 217 nm, but its CD spectrum is similar to that of [D-Val$^{1,1'}$]GS recently synthesized by Tamaki et al.[16] It is also worthy to note that the [D-Val$^{1,1'}$]GS has practically no activity. On the basis of these results and observations, several reasonable explanations for the structure-activity relationship of **18** are considered as follows. (a) The Aib residues in **18** behave like D-amino acid residues at positions 1 and 1′. (b) The presence of geminal methyl groups at an α-carbon of Aib greatly restricts the range of accessible conformation for a β-turn of the cyclic peptide containing Aib. (c) This restrictive effect results in a distortion of the molecular conformation of a sidedness feature which is required for exhibiting the activity of antibiotic GS.

Conformational study of the β-turn part in GS analogs containing Gly, Ala, or Aib in place of Val is in progress to elucidate the effect of the amino acid residues at positions 1 and 1′ in the peptide backbone on the antibacterial activity.

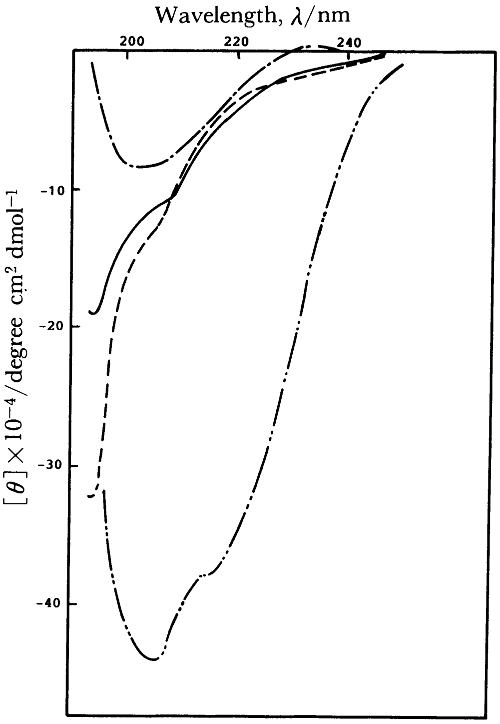

Fig. 8. CD spectra of [Aib^1] semiGS (**11**), Z_2- [Aib$^{1,1'}$] GS (**17**), [Aib$^{1,1'}$] GS (**18**), and GS in MeOH. -·-·-: **11**, ----: **17**, ——: **18**, -··-··-: GS.

Experimental

All the melting points are uncorrected. All the $[\alpha]_{589}$ measurements were obtained using a JASCO spectropolarimeter model J-20. The molecular weight of Z-[Aib^1]semiGS was determined by mass spectrometry using a JMS-D300 mass spectrometer. Amino acid analyses were performed with a Hitachi amino acid analyzer KLA-5. The ratio in parentheses after a solvent system was indicated by volume. Thin-layer chromatography was performed on silica gel (Merck 60 GF_{254}) with the following solvent systems: R_f^1, $CHCl_3$–MeOH (5 : 1); R_f^2, $CHCl_3$–MeOH (9 : 1); R_f^3, $CHCl_3$–MeOH–AcOH (8 : 1 : 1); R_f^4, $CHCl_3$–MeOH–98% NH_3 (8 : 4 : 1); R_f^5, *n*-BuOH–AcOH–H_2O (4 : 1 : 2); R_f^6 *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2).

Boc-Aib-OH (1): To a solution of Aib (3.35 g, 32.5 mmol) in 2 mol dm^{-3} NaOH (15.9 ml, 32.5 mmol) were added Et_3N (4.55 ml, 32.5 mmol) and $(Boc)_2O$ (5.46 g, 25.0 mmol) in dioxane (35 ml). After stirring for 40 h at room temperature, the solution was evaporated in vacuo to remove dioxane and diluted with water (200 ml). After extraction twice with ether (200 ml), the aqueous layer was cooled to 0 °C and adjusted to pH 2.5 with citric acid solution containing NaCl. The reaction product was then extracted twice with EtOAc (200 ml). The combined organic layers were washed twice with water (100 ml), dried over sodium sulfate, and triturated by the addition of petroleum ether. The resulting solid was recrystallized from ether; yield, 4.98 g (98% calculated from $(Boc)_2O$); mp 114—116 °C (lit, 119—120 °C); R_f^1 0.58, R_f^4 0.32.

Found: C, 52.98; H, 8,30; N, 6.93%. Calcd for $C_9H_{17}O_4N$: C, 53.19; H, 8.43; N, 6.89%.

Boc-Aib-Orn(δ-Z)-OEt (2): To a solution of **1** (3.25 g, 15.0 mmol) in DMF (35.0 ml) were added NMM (1.73 g, 15.8

mmol), Orn(δ-Z)-OEt·*p*-TosOH (7.35 g, 15.8 mmol), HOBt (2.43 g, 18.0 mmol), and EDC·HCl (3.22 g, 16.8 mmol) at 0 °C. After stirring for 3 d at room temperature, the reaction mixture was evaporated in vacuo. The residue was dissolved in ether (800 ml). The solution was washed successively with water, 10% citric acid, water, 0.5 mol dm^{-3} $NaHCO_3$ and water, and then dried over sodium sulfate. After removal of the drying agent, the solution was evaporated in vacuo to a small volume. The oily residue was crystallized by letting it stand for 2 h at room temperature. The product was collected by filtration; yield, 6.35 g (88%); mp 87—89 °C; $[\alpha]_{589}$ −7.7°(*c* 2.1, MeOH); R_f^1 0.71, R_f^2 0.58, R_f^4 0.80.

Found: C, 58.87; H, 7.57; N, 8.37%. Calcd for $C_{24}H_{37}N_3O_7\cdot 1/2H_2O$: C, 59.00; H, 7.84; N, 8.60%.

Boc-Aib-Orn(δ-Z)-$NHNH_2$ (3): A solution of **2** (1.50 g, 3.14 mmol) and hydrazine hydrate (1.57 ml, 31.4 mmol) in MeOH (6.0 ml) was allowed to stand for 16 h at 28 °C. The solution was concentrated in vacuo. The foamy residue was dissolved in CH_2Cl_2 (300 ml), washed with water (200 ml), and then dried over sodium sulfate. After removal of the drying agent, the solution was evaporated in vacuo. The precipitate which formed upon the addition of ether was collected by filtration; yield, 1.34 g (92%); mp 75—76 °C; $[\alpha]^{22}_{589}$ −15.9° (*c* 2.0, MeOH); R_f^1 0.50.

Found: C, 56.64; H, 7.52; N, 15.03%. Calcd for $C_{22}H_{35}N_5O_6$: C, 56.76; H, 7.58; N, 15.04%.

Boc-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-OEt (4): To a solution of **3** (883 mg, 1.90 mmol) in DMF (8.0 ml) were added 4.14 mol dm^{-3} HCl/dioxane (0.916 ml, 3.80 mmol) and isopentyl nitrite (0.290 ml, 2.08 mmol) at −50 °C. After stirring for 10 min at −20 °C, Et_3N (0.531 ml, 3.80 mmol) was added at −50 °C. The reaction mixture was combined with a chilled solution of H-Leu-D-Phe-Pro-OEt·HCl (959 mg, 2.18 mmol)[17] and Et_3N (0.305 ml, 2.18 mmol) in DMF (6.0 ml). The solution was stirred for the 5 d at 0 °C and concentrated to a small volume. The residue was dissolved in EtOAc (300 ml) and it was washed as described for the preparation of **2**. After removal of the drying agent, the solution was evaporated in vacuo. The precipitate which was formed upon the addition of petroleum ether was collected by filtration. The product was recrystallized from EtOAc–petroleum ether; yield, 1.16 g (73%); mp 74—78 °C; $[\alpha]^{23}_{589}$ −43.8°(*c* 2.0, MeOH); R_f^1 0.70, R_f^3 0.91.

Found: C, 62.81; H, 7.79; N, 9.91%. Calcd for $C_{44}H_{64}N_6O_{10}$: C, 63.14; H, 7.71; N, 10.04%.

Boc-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-OH (5): To a solution of **4** (853 mg, 1.02 mmol) in MeOH (5.0 ml) was added 0.94 mol dm^{-3} NaOH (2.17 ml, 2.04 mmol) at 0 °C. The solution was allowed to stand for 5 h at 32 °C, evaporated in vacuo to a small volume, and diluted with water (15 ml). After extraction of the unreacted compound (**4**) with EtOAc (5 ml), the aqueous layer was cooled to 0 °C and adjusted to pH 3 with citric acid, and then extracted twice with CH_2Cl_2 (25 ml). The combined CH_2Cl_2 layers were washed twice with saturated aqueous NaCl (20 ml), dried over sodium sulfate, and then evaporated in vacuo. The precipitate which formed upon the addition of petroleum ether was collected by filtration; yield, 794 mg (96%); mp 103—105 °C; $[\alpha]^{23}_{589}$ −37.6° (*c* 2.0, MeOH); R_f^1 0.47; R_f^3 0.70; R_f^4 0.68.

Found: C, 61.16; H, 7.43; N, 9.85%. Calcd for $C_{42}H_{60}N_6O_{10}\cdot H_2O$: C, 61.00; H, 7.56; N, 10.16%.

Boc-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-ONSu (6): To a solution of **5** (657 mg, 0.812 mmol) in DMF (3.0 ml) were added HONSu (188 mg, 0.812 mmol) and EDC·HCl (315 mg, 1.62 mmol) at 0 °C, and the mixture was then stirred for 26 h at room temperature. The reaction mixture was evaporated in vacuo and diluted with $CHCl_3$ (100 ml). This was washed with 0.5 mol dm^{-3} $NaHCO_3$ and water, and dried over sodium salfate. After removal of the drying agent, the solution was evaporated in vacuo. The residue was solidified with petrolem ether; yield, 599 mg (82%); mp 98—100 °C; $[\alpha]^{23}_{589}$ −40.5° (*c* 1.1, MeOH); R_f^1 0.60.

Found: C, 60.00; H, 7.37; N, 10.18%. Calcd for $C_{46}H_{63}N_7O_{12}\cdot H_2O$: C, 59.79; H, 7.09; N, 10.61%.

Boc-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-ONp (7): To a solution of **5** (649 mg, 0.80 mmol) in pyridine (7.0 ml) were added *p*-nitrophenol (222 mg, 1.60 mmol) and DCC (189 mg, 0.92 mmol) at 0 °C. After stirring for 25 h at room temperature, the precipitated urea was filtered off and the filtrate was concentrated. The residue was worked up as described for the synthesis of **6**; yield, 500 mg (67%); mp 67—70 °C; R_f^1 0.75.

H-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-ONSu·TFA (8): Compound **6** (588 mg, 0.649 mmol) was dissolved in TFA (4.0 ml) at 0 °C. After 30 min, the solution was evaporated, and the residue was triturated with a mixture of ether and petroleum ether. The crystals which remained were collected by filtration; yield, 585 mg (98%); mp 110—112 °C; $[\alpha]^{23}_{589}$ −48.6° (*c* 1.0, MeOH); R_f^1 0.62, R_f^3 0.47, R_f^5 0.57.

Found: C, 54.30; H, 6.20; N, 10.23%. Calcd for $C_{43}H_{56}N_7O_{12}F_3\cdot 2H_2O$: C, 54.02; H, 6.32; N, 10.26%.

H-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-ONp·TFA (9): This compound was prepared from **7** (476 mg, 0.512 mmol) as described for the preparation of **8**; yield, 350 mg (72%); mp 100—103 °C; R_f^1 0.57, R_f^3 0.38.

***Cyclo*[Aib-Orn(δ-Z)-Leu-D-Phe-Pro] (10):** This compound was synthesized by (1) ONSu method and (2) ONp method.

(1) A solution of **8** (374 mg, 0.406 mmol) in DMF (2.6 ml) was added dropwise into pyridine (120 ml) at room temperature. After stirring for 5 d at room temperature, the solution was evaporated in vacuo. The residue was dissolved in a mixture of MeOH and water (2 : 1) and applied to columns (1.8×12 cm) of Dowex 1 and 50. The eluate (ca. 500 ml) was evaporated to dryness. A few mg of the crude product were subjected to hydrogenolysis, and paper electrophoresis of the hydrogenated material showed one spot. The crude product was chromatographed over a silica-gel column (Wako silica gel CQ-3, 2×30 cm) using hexane–$CHCl_3$–MeOH (2 : 3 : 1) as eluent; yield, 204 mg (73%, 59% from **9**); mp 100—102 °C; $[\alpha]^{23}_{589}$−64.8° (*c* 2.0, MeOH); R_f^2 0.50, R_f^3 0.85, R_f^5 0.72, R_f^6 0.78.

Found: C, 63.13; H, 7.39; N, 11.46%; M^+, 690. Calcd for $C_{37}H_{50}N_6O_7\cdot H_2O$: C, 62.69; H, 7.39; N, 11.86%.

(2) A solution of **9** (148 mg, 0.156 mmol) in DMF (5.0 ml) was added dropwise to pyridine (70 ml) heated to 62 °C. After stirring for 6 h at 62 °C and for 10 h at room temperature, the reaction mixture was worked up as described above. The hydrogenated material from a few mg of the crude showed one spot in paper electrophoresis. The crude oily product was crystallized from ether–hexane; yield, 67.0 mg (62%, 30% from **9**); mp 101—103 °C; $[\alpha]^{23}_{589}$ −65.2° (*c* 2.0, MeOH); R_f^3 0.85, R_f^5 0.72, R_f^6 0.78.

Found: C, 62.51; H, 7.30; N, 11.67%; M^+, 690. Calcd for $C_{37}H_{50}N_6O_7\cdot H_2O$: C, 62.69; H, 7.39; N, 11.86%; M^+, 690.

***Cyclo*(Aib-Orn-Leu-D-Phe-Pro)·HCl (11):** A solution of **10** (162 mg, 0.230 mmol) in 1.67 mol dm^{-3} HCl/EtOH

(0.155 ml, 0.250 mmol) and MeOH (10 ml) was subjected to hydrogenolysis in the presence of Pd-black. After 3 h, the catalyst was filtered off and the filtrate was evaporated to dryness; yield, 132 mg (95%); mp 202—206 °C; $[\alpha]^{23}_{589}$ −67.5°(*c* 2.0, MeOH); R_f^1 0.21, R_f^3 0.40, R_f^4 0.94, R_f^5 0.41, R_f^6 0.65.

Found: C, 54.17; H, 7.64; N, 12.59%. Calcd for $C_{29}H_{45}N_6O_5Cl \cdot 3H_2O$: C, 53.82; H, 7.94; N, 12.98%.

Amino acid ratios in acid hydrolyzate of **11**; Aib 1.0, Orn 1.1, Leu 1.0, Phe 1.0, Pro 1.05.

H-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-OEt·TFA (12): This compound was synthesized from **4** (152 mg, 0.169 mmol) as described for the synthesis of **8**; yield, 140 mg (98%); mp 99—106 °C; $[\alpha]^{23}_{589}$ −48.1° (*c* 2.0, MeOH); R_f^1 0.60, R_f^3 0.50, R_f^4 0.75.

Found: C, 56.87; H, 6.76; N, 9.70%. Calcd for $C_{41}H_{57}N_6O_{10}F_3 \cdot H_2O$: C, 56.67; H, 6.84; N, 9.67%.

Boc[-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-]$_2$OEt (13): To a solution of **12** (508 mg, 0.596 mmol) in DMF (4.0 ml) were added Et_3N (0.083 ml, 0.596 mmol), **5** (482 mg, 0.596 mmol), HOBt (96.9 mg, 0.715 mmol), and EDC·HCl (172 mg, 0.896 mmol) at 0 °C. The reaction mixture was stirred for 3 d at room temperature and worked up as described for the synthesis of **4**. The product was recrystallized from EtOAc-ether-petroleum ether; yield, 735 mg (81%); mp 93—96 °C; $[\alpha]^{23}_{589}$ −34.9°(*c* 2.0, MeOH); R_f^1 0.73, R_f^2 0.72, R_f^3 0.90, R_f^4 0.78, R_f^5 0.84, R_f^6 0.81.

Found: C, 62.69; H, 7.53; N, 10.49%. Calcd for $C_{31}H_{114}N_{12}O_{17} \cdot H_2O$: C, 62.93; H, 7.56; N, 10.87%.

Boc[-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-]$_2$OH (14). To a solution of **13** (658 mg, 0.430 mmol) in MeOH (3.0 ml) was added 3.7 mol dm^{-3} NaOH (0.346 ml, 1.29 mmol) at 0 °C. After the solution was allowed to stand for 11 h at 30 °C, 1 mol dm^{-3} HCl (1.29 ml) was added to the solution. Subsequently, the solution was evaporated in vacuo and the oily residue was acidified with 10% citric acid. After the residue had been stored in a refrigerator over night, the precipitate was collected by filtration. The product was recrystallized from EtOAc-ether-petroleum ether; yield, 609 mg (94%); mp 111—112 °C; $[\alpha]^{23}_{589}$ −32.2° (*c* 2.0, MeOH); R_f^4 0.86, R_f^6 0.67.

Found: C, 61.49; H, 7.34; N, 10.66%. Calcd for $C_{79}H_{110}N_{12}O_{17} \cdot 2H_2O$: C, 61.78; H, 7.48; N, 10.94%.

Boc[-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-]$_2$ ONSu (15): This compound was synthesized from **14** (546 mg, 0.346 mmol) as described for the synthesis of **6**; yield, 553 mg (95%); mp 110—111 °C; $[\alpha]^{22}_{589}$ −32.6° (*c* 1.3, MeOH); R_f^1 0.78, R_f^3 0.83, R_f^4 0.96.

Found: C, 60.84; H, 7.10; N, 11.04%. Calcd for $C_{83}H_{113}N_{13}O_{19} \cdot 2H_2O$: C, 61.05; H, 7.22; N, 11.15%.

H[-Aib-Orn(δ-Z)-Leu-D-Phe-Pro-]$_2$ONSu·TFA (16). This compound was synthesized from **15** (526 mg, 0.329 mmol) as described for the synthesis of **8**; yield, 510 mg (96%); mp 123—127 °C; $[\alpha]^{22}_{589}$ −52.6° (*c* 0.63, MeOH); R_f^1 0.78, R_f^3 0.60.

Found: C, 58.95; H, 6.85; N, 10.75%. Calcd for $C_{80}H_{106}N_{13}O_{19}F_3 \cdot H_2O$: C, 58.99; H, 6.68; N, 11.18%.

***Cyclo*[Aib-Orn(δ-Z)-Leu-D-Phe-Pro]$_2$ (17).** Compound **16** (503 mg, 0.312 mmol) was subjected to a cyclization reaction, in a similar manner as described for the preparation of **10**. The crude product was applied to a Sephadex LH-20 column (3.6×114 cm). The column was eluted with MeOH. The main fractions were rechromatographed over a silica-gel column (Wako silica gel CQ-3, 2×30 cm) using a solvent system of EtOAc-$CHCl_3$-MeOH (9 : 5 : 1). The purified product was recrystallized from EtOAc-ether-petroleum ether; yield, 208 mg (48%; 44% from **14**); mp 131—134 °C. $[\alpha]^{23}_{589}$ −88.5° (*c* 1.0, MeOH); R_f^1 0.75, R_f^2 0.64, R_f^3 0.82, R_f^4 1.00, R_f^5 0.79, R_f^6 0.83.

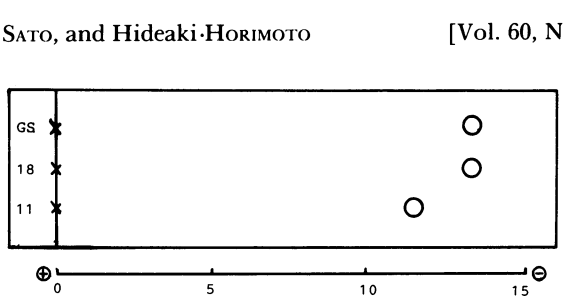

Fig. 9. Paper electrophoresis of [Aib$^{1,1'}$] GS (**18**), and [Aib^1] semiGS (**11**).

Found: C, 63.24; H, 7.37; N, 11.71%. Calcd for $C_{74}H_{100}N_{12}O_{14} \cdot H_2O$: C, 63.50; H, 7.34; N, 12.01%.

The molecular weight was determined by a Hitachi osmometer, type 117 (solvent; MeOH). Found: 1340. Calcd for $C_{74}H_{100}N_{12}O_{14}$: 1381.7.

***Cyclo*(Aib-Orn-Leu-D-Phe-Pro)$_2$·2HCl (18).** Compound **17** (52.8 mg, 38.2 mmol) was hydrogenated as described for the preparation of **11**; yield, 43.9 mg, (97%); mp 209—212 °C; $[\alpha]^{23}_{589}$ −76.7° (*c* 0.49, MeOH); R_f^1 0.16, R_f^3 0.37, R_f^4 0.94, R_f^5 0.38, R_f^6 0.65. Amino acid ratios in hydrolysate of **18**; Aib 2.0, Orn 2.03, Leu 2.0, Phe 2.2, Pro 2.1.

Found: C, 53.56; H, 7.84; N, 12.60%. Calcd for $C_{58}H_{90}N_{12}O_{10}Cl_2 \cdot 6H_2O$: C, 53.82; H, 7.94; N, 12.98%.

Paper Electrophoresis. This was carried out on Toyo Roshi No. 51A paper with a HCOOH-AcOH-MeOH-H_2O solvent system (2 : 9 : 18 : 30, pH 1.8) for 3 h at 500 V/30 cm. Each of these analogs showed a single spot. Figure 9 shows that [Aib$^{1,1'}$]GS (**18**) migrates faster toward the cathode than [Aib^1]semiGS (**11**), and that the mobility of **18** is indistinguishable from that of the natural GS.

Microbiological Assay and CD Measurements. The microorganisms employed are listed in Table 2. The minimum amount of the compound necessary for the complete inhibition of growth was determined by a dilution method using a trypticase soy agar. As is shown in Table 2, [Aib$^{1,1'}$]GS (**18**) and [Aib^1]semiGS (**11**) exhibited no antibacterial activity against any of the microorganisms tested. The CD spectra were obtained using a JASCO spectropolarimeter model J-20 in MeOH solution at room temperature.

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14) Amino acid residues with no prefix are of L-configuration. The abbreviations for amino acids and peptides are in accordance with the rules of IUPAC-IBU Commission of Biological Nomenclature [*Eur. J. Biochem.*, **138**, 9 (1984)]. Additional abbreviations used are as follows: DCC, dicyclohexylcarbodiimide; HONSu, *N*-hydroxysuccinimide; NMM, *N*-methylmorpholine; TFA, trifluoroacetic acid; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride.

15) The terms of sidedness was used for a specific conformation of GS molecule in which the charged side chains are on one side and the hydrophobic side chains on the other side. K. Sato, K. Ueda, M. Kondo, H. Aoyagi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **51**, 1830 (1978) and references cited herein.

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