Syntheses of Gramicidin S Analogs Containing δ-Aminovaleric Acid.[1] [5–1′-δ-Aminovaleric Acid]-Gramicidin S and [5–1′, 5′–1-Bis-(δ-Aminovaleric Acid)]-Gramicidin S

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Gramicidin S (GS) analogs, [5–1′-δ-aminovaleric acid]-GS dihydrochloride (XIII) and [5–1′, 5′–1-bis(δ-aminovaleric acid)]-GS dihydrochloride (XIX), were synthesized. Both peptides are the analogs in which one or two L-prolyl-L-valyl residues of GS are replaced with one or two δ-aminovaleric acid residues. The spectra of optical rotatory dispersion (ORD) and circular dichroism (CD) of XIII and XIX were measured. The molar optical rotation of XIII is smaller than that of GS, but its ORD spectrum resembles that of GS. Analog XIII shows some antimicrobial activity, but not the analog XIX.

Analogs of gramicidin S (GS) containing δ-aminovaleric acid (δAva) residue have been synthesized in order to investigate the role of amide bond in the three-dimensional structure and antibiotic activity of GS. When one or two dipeptide residues of the antibiotic are replaced by δAva, one or two amide groups turn into ethylene groups, the chain length and ring size remaining unaltered. However, if the replaced amide group of GS participates in the hydrogen bond essential to the proper secondary structure of the original molecule, the replacement would lead to the impracticability of hydrogen bond formation, giving rise to the deformation of the ring structure and the loss of the biological activity.

In previous papers,[1,2] the syntheses and properties of [δAva$^{4-5,4'-5'}$]-GS and [δAva$^{4-5}$]-GS were reported. It has been deduced that the stabilization of the ring structure of GS needs the existence of D-phenylalanyl-L-prolyl residue, although it does not take part in intramolecular hydrogen bond formation. The NH groups of L-valyl residues seem to participate in the intramolecular hydrogen bonds, stabilizing the pleated sheet structure of GS.[3,4]

In this paper, we describe the syntheses of [δAva$^{5-1'}$]-GS and [δAva$^{5-1',5'-1}$]-GS, giving the results of antimicrobial assays and optical rotatory dispersion (ORD) and circular dichroism (CD) measurements. Both peptides are the analogs in which one or two L-prolyl–L-valyl residues participating in the hydrogen bonds in GS are replaced by δAva. The analogs are devoid of one or two amide groups in the corresponding residues of GS. The modification would be of use for a study of the conformation of GS.

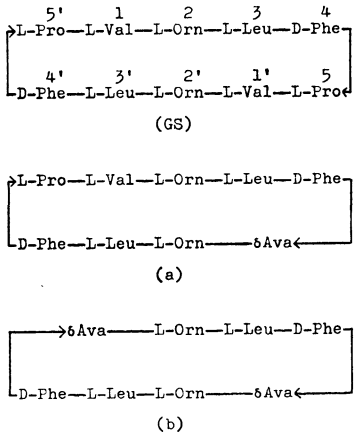
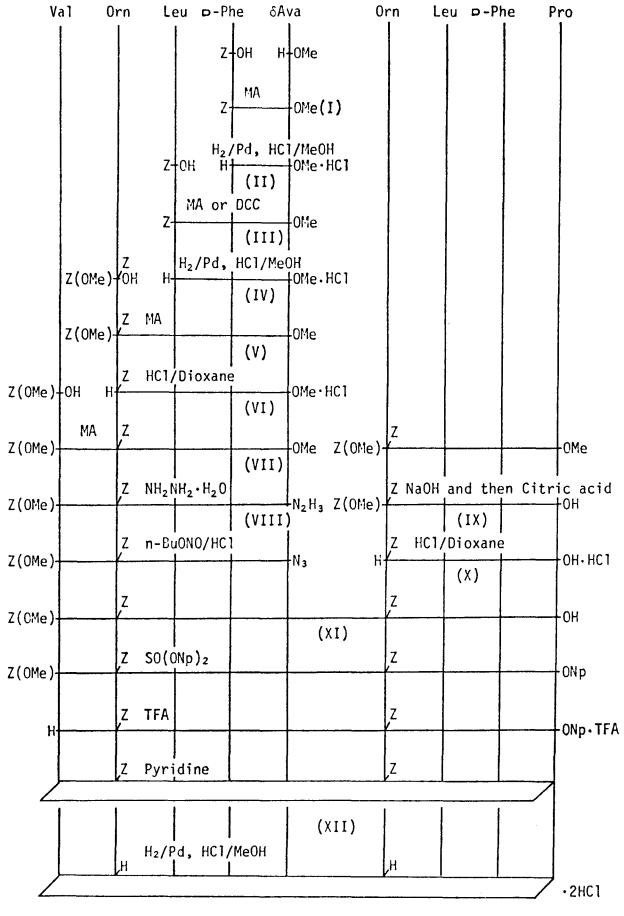
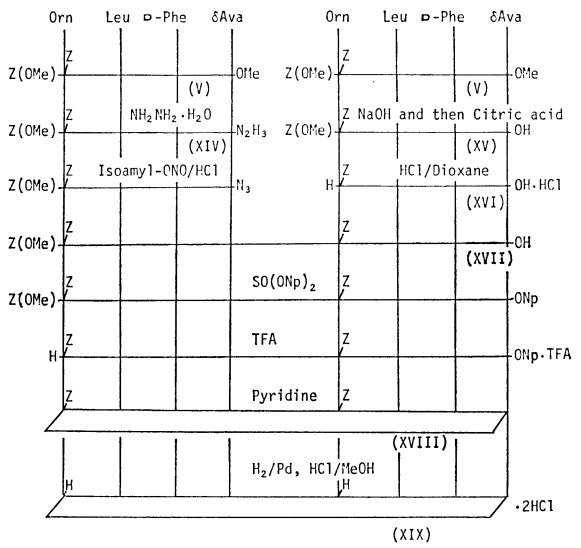


Fig. 1. Structures of gramicidin S (GS), [δAva$^{5-1'}$]-GS (a), and [δAva$^{5-1'}$, $^{5'-1}$]-GS (b).

The analogs were synthesized according to Schemes 1 and 2. The cyclization reactions were carried out by using the *p*-nitrophenyl esters of liner peptides XI and XVII. The reaction procedures were the same as in the cyclization.[2] The cyclic peptides XII and XVIII were obtained in 61 and 14% yields, respectively. In analogy with the yields of cyclization in the syntheses of [δAva$^{4-5}$]-GS (71%)[2] and [δAva$^{4-5,4'-5'}$]-GS



Scheme 1.



Scheme 2.

(32%),[1] the results might reflect the relative stability of the conformation favorable for cyclization of each active ester. [δAva$^{5-1'}$]-GS dihydrochloride (XIII) and [δAva$^{5-1',5'-1}$]-GS dihydrochloride (XIX) were obtained by hydrogenolysis of the benzyloxycarbonyl groups on the ornithyl residues of XII and XVIII. The purity of the synthetic analogs was confirmed by means of dansyl chloride (1-dimethylamino-5-naphthalenesulfonyl chloride) procedure,[2] paper electrophoresis, and amino acid analysis.

Table 1. Antimicrobial activity of GS and its analogs

Test organisms	Minimum inhibitory concentration[a] (μg/ml)		
	GS·2HCl	XIII[b]	XIX[c]
Staphylococcus aureus ATCC 6538p	6.3	50	>100
Streptococcus pyogenes N. Y. 5	6.3	25	>100
Sarcina lutea ATCC 9341	6.3	>100	100
Corynebacterium diphtheriae P. W. 8	1.6	3.2	>100
Bacillus subtilis ATCC 6633	6.3	25	>100
Escherichia coli B	>100	>100	>100
Proteus vulgaris OX 19	>100	>100	>100

a) Agar dilution method. b) [δAva$^{5-1'}$]-GS·2HCl. c) [δAva$^{5-1',5'-1}$]-GS·2HCl.

Results of the antimicrobial assay of these analogs for several microorganisms are given in Table 1. ORD and CD spectra are shown in Figs. 2 and 3. The minimum value in ORD spectrum of XIII (1.4×10^{-4} M) is observed at 232 nm. Although the molar optical rotation of XIII is smaller than that of GS, its ORD spectrum shape resembles that of GS. The analog XIII exhibits some antimicrobial activity against several microorganisms. CD spectrum of XIII has two troughs near 200 nm. The trough at 203 nm corresponds to that

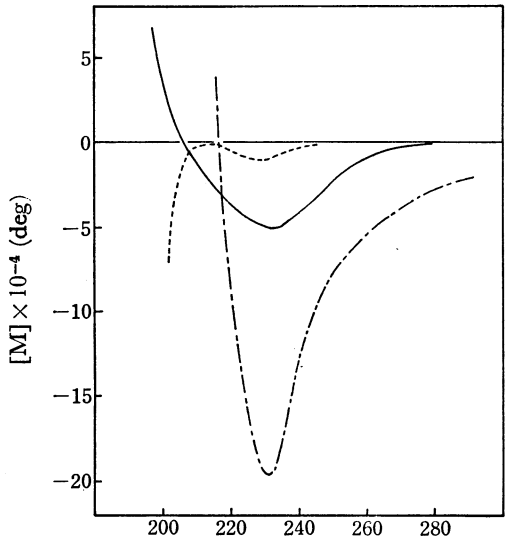


Fig. 2. ORD spectra of [δAva$^{5-1'}$]-GS·2HCl (XIII), [δAva$^{5-1',5'-1}$]-GS·2HCl (XIX), and gramicidin S 2HCl (GS) in water; XIII (——), XIX (----), and GS (- · -). Measurements were made using a 1 mm quartz cell at room temperature.

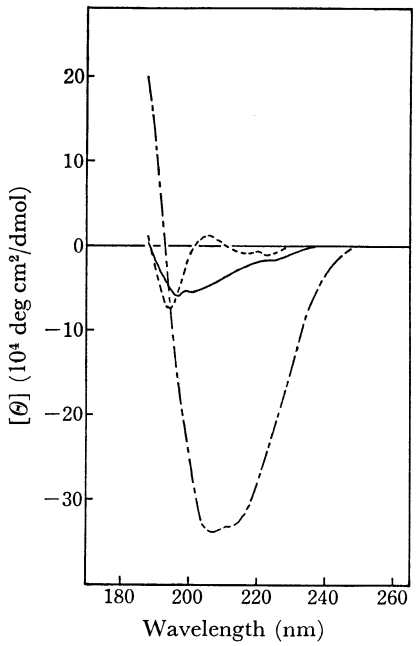


Fig. 3. CD spectra of [δAva$^{5-1'}$]-GS·2HCl (XIII), [δAva$^{5-1',5'-1}$]-GS·2HCl (XIX), and gramicidin S 2HCl (GS) in water; XIII(——), XIX(----), and GS(- · -). Measurements were made using 0.5 mm and 0.1 mm (XIX) quartz cells at room temperature.

of CD spectrum of [δAva$^{4-5}$]-GS,[2] which has a GS-like conformation. The trough in shorter wave length region is not observed in the spectrum of GS; it might arise from the partially flexible structure of XIII. The ORD and CD spectra of XIX (9.5×10^{-4} M) differ from both those of GS (1.3×10^{-4} M) and those of other GS analogs containing δAva.[1,2] Analog XIX shows no antimicrobial activity.

[MeVal^{1}]-GS and [MeVal$^{1,1'}$]-GS[5] were synthesized by Abe *et al.*[6] From the lack of amide hydrogen between L-prolyl and L-valyl residues, these analogs are comparable with analogs XIII and XIX. XIII shows antimicrobial activity and ORD spectrum similar to those of [MeVal^{1}]-GS.[6] If the part of ornithyl-leucyl-D-phenylalanyl-prolyl-valyl-ornithyl-leucyl[7] in the primary structure of XIII has a fixed secondary structure containing two hydrogen bonds and β-turn (D-phenylalanyl-prolyl),[4] analog XIII possibly exists in a GS-like conformation which can be illustrated by use of CPK model.

Experimental[5]

The purity of the synthetic compounds was confirmed by thin-layer chromatography (TLC) on silica gel plates using the following solvent systems (v/v): Solv. 1, $CHCl_3$–MeOH–AcOH–pyridine (95: 5: 3: 4); Solv. 2, $CHCl_3$–MeOH–AcOH (95: 5: 3); Solv. 3, $CHCl_3$–MeOH (9: 1); Solv. 4, *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2); Solv. 5, *n*-BuOH–AcOH–H_2O (4: 1: 1). The molecular weight was determined with a Hitachi molecular weight apparatus model 115, using methanol. Amino acid analyses were performed with a JEOL automatic amino acid analyzer, after hydrolyzing samples with 6 M HCl in evacuated sealed ampoules for 20 h at 110 °C. ORD and CD spectra were measured with a JASCO model J-20 spectrometer and are represented in terms of molar optical rotation and molar ellipticity, respectively.

Z-D-Phe-δAva-OMe (I). The peptide was synthesized according to the method of Anderson *et al.*[8] Et_3N (1.39 ml, 10 mmol) was added to a solution of isobutyl chloroformate (1.44 ml, 11 mmol) in THF (20 ml) at −10 °C. After 3 min, a solution of Z-D-Phe-OH (2.99 g, 10 mmol) in THF was added dropwise to the above solution at −15 °C. After the reaction mixture had been stirred for 15 min, a mixture of H-δAva-OMe·HCl[2] (1.68 g, 10 mmol) and Et_3N (1.39 ml) in $CHCl_3$ (20 ml) was added. The reaction mixture was further stirred at −15 °C for 1 h and at room temperature overnight. After filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed successively with 0.5 M HCl, water, 5% $NaHCO_3$, and water, and then dried over Na_2SO_4. After removal of the drying agent, the solution was concentrated *in vacuo* and the residue was recrystallized from ethyl acetate and petroleum ether. Yield, 3.38 g (81.9%); mp 103—104 °C; $[\alpha]_D^{20}$ +3.2° (*c* 4, MeOH).

Found: C, 67.14; H, 6.87; N, 6.99%. Calcd for $C_{23}H_{28}N_2O_5$: C, 66.97; H, 6.84; N, 6.79%.

H-D-Phe-δAva-OMe·HCl (II). I (4.13 g, 10 mmol) in MeOH (30 ml) containing methanolic 1.2 M HCl (10 ml) was hydrogenolyzed in the presence of Pd black (0.5 g) for 2 h. After removal of the catalyst and concentration of the filtrate *in vacuo*, white precipitate was obtained by trituration of the oily residue with diethyl ether in a theoretical yield.

Z-Leu-D-Phe-δAva-OMe (III). *a*) DCC (2.06 g, 10 mmol) was added to a mixture of II (3.15 g, 10 mmol) and Z-Leu-OH·DCHA[9] (4.48 g, 10 mmol) in $CHCl_3$ (50 ml) at 2—3 °C in an ice-salt bath.[10] The reaction mixture was stirred at this temperature for 3 h and then at room temperature overnight. After evaporation of the solvent *in vacuo* and addition of ethyl acetate to the residue, insoluble substance was removed by filtration. The filtrate was treated in a way similar to that for the synthesis of I. The pure product was obtained by recrystallization from ethyl acetate, EtOH and hexane. Yield, 3.7 g (70.3%); mp 145.5 °C; $[\alpha]_D^{20}$ +10.5° (*c* 2, MeOH). *b*) Z-Leu-OH obtained from the corresponding DCHA salt (14.73 g, 33 mmol) was combined with 30 mmol of II by a method similar to that for the synthesis of I. The product was recrystallized from ethyl acetate and petroleum ether. Yield, 12.42 g (77.8%); mp 144—145 °C; $[\alpha]_D^{22}$ +11.6° (*c* 2, MeOH).

Found: C, 66.46; H, 7.39; N, 8.01%. Calcd for $C_{29}H_{39}N_3O_6$: C, 66.26; H, 7.48; N, 7.99%.

H-Leu-D-Phe-δAva-OMe-HCl (IV). III (10.51 g, 20 mmol) was hydrogenolyzed by the method for II. The product was obtained in a theoretical yield.

Z(OMe)-Orn(Z)-Leu-D-Phe-δAva-OMe (V). This was prepared from IV (20 mmol) and Z(OMe)-Orn(Z)-OH obtained from the corresponding DCHA salt[11] (13.47 g, 22 mmol). The coupling method was similar to that for the preparation of I. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$, and the solution was washed successively with water, 5% citric acid, water, 5% $NaHCO_3$, and water, and then dried over Na_2SO_4. After removal of the drying agent, the filtrate was concentrated *in vacuo*, and the residue was recrystallized from MeOH (600 ml). Yield, 12.52 g (77.9%); mp 182—183 °C; $[\alpha]_D^{22}$ +5.5° (*c* 1, DMF); TLC: R_f 0.64 (Solv. 3).

Found: C, 64.30; H, 7.10; N, 8.79%. Calcd for $C_{43}H_{57}N_5O_{10}$: C, 64.24; H, 7.15; N, 8.71%.

H-Orn(Z)-Leu-D-Phe-δAva-OMe·HCl (VI). V (1.85 g, 2.3 mmol) was added to 2 M HCl–dioxane (25 ml) containing anisole (0.6 ml). The solution was stirred at room temperature for 3 h and then evaporated *in vacuo*. The oily residue was triturated with diethyl ether and the resulting white precipitate was collected by filtration. After drying, the product weighed 1.42 g (89.1%).

Z(OMe)-Val-Orn(Z)-Leu-D-Phe-δAva-OMe (VII). Z(OMe)-Val-OH obtained from the corresponding DCHA salt[11] (1.16 g, 2.5 mmol) was introduced to a mixed anhydride in a way similar to that for the preparation of I. To this THF solution (19 ml) was added a solution of VI (1.42 g, 2.1 mmol) and Et_3N (0.29 ml) in DMF (15 ml) at −15 °C. The reaction mixture was stirred at this temperature for 1 h and at room temperature overnight and then filtered. The filtrate was concentrated to dryness *in vacuo*. The residue was washed on a filter funnel with water, 5% citric acid, water, 5% $NaHCO_3$, and water. The dried crude product was recrystallized from MeOH and diethyl ether. Yield, 1.2 g (63.2%); mp 193.5—195 °C; $[\alpha]_D^{18}$ +6.5° (*c* 1, DMF).

Found: C, 63.97; H, 7.41; N, 9.46%. Calcd for $C_{48}H_{66}N_6O_{11}$: C, 63.84; H, 7.36; N, 9.31%.

Z(OMe)-Val-Orn(Z)-Leu-D-Phe-δAva-NHNH$_2$ (VIII). $NH_2NH_2·H_2O$ (80%, 12.5 g) was added to a solution of VII (2.72 g, 3.0 mmol) in DMF (50 ml) and the resulting solution was stirred at room temperature for 4.5 d. The hydrazide precipitated by addition of water (400 ml) was filtered, washed with water, and dried. Yield, 2.68 g (98.9%); mp 213.5—215 °C; $[\alpha]_D^{20}$ +7.0° (*c* 1, HMPA).

Found: C, 62.05; H, 7.28; N, 12.07%. Calcd for $C_{47}H_{66}N_8O_{10}$: C, 62.51; H, 7.36; N, 12.41%.

Z(OMe)-Orn(Z)-Leu-D-Phe-Pro-OH (IX). Z(OMe)-Orn(Z)-Leu-D-Phe-Pro-OEt[2] (1.63 g, 2.0 mmol) was saponified in a mixture of MeOH (10 ml), dioxane (10 ml), and 1 M NaOH (4 ml) at room temperature for 3 h. After filtration of the reaction mixture, an oily product resulting by the addition of 5% citric acid (25 ml) in an ice bath was separated from the aqueous layer by decantation. The product was reprecipitated from MeOH, 5% citric acid, and excess water, washed with water, and dried. Yield, 1.46 g (92.5%); TLC: R_f 0.64 (Solv. 1).

H-Orn(Z)-Leu-D-Phe-Pro-OH·HCl (X). HCl-dioxane (8.6 M, 5 ml) was added to a solution of IX (1.46 g, 1.85 mmol) in dioxane (10 ml) containing anisole (0.22 ml). The solution was stirred at room temperature for 1 h. After the solvent had been evaporated *in vacuo*, the product was obtained by trituration with diethyl ether and decantation; yield, 1.19 g (97.5%).

Z(OMe)-Val-Orn(Z)-Leu-D-Phe-δAva-Orn(Z)-Leu-D-Phe-Pro-OH (XI). VIII (1.63 g, 1.8 mmol) was suspended in DMF (20 ml), followed by addition of 8.16 M HCl-dioxane (0.88 ml) and butyl nitrite (0.25 ml, 2.2 mmol) at −40 °C.[12] After the reaction mixture had been stirred at −20——30 °C for 15 min, Et_3N (1 ml) was added at −60 °C, followed by the addition of a mixture of X (1.19 g, 1.8 mmol) and Et_3N (0.5 ml) in DMF (15.5 ml) at −20——30 °C. The reaction mixture was stirred at this temperature for 1 h and at 0 °C for 66 h and then poured into cold water (500 ml). The resulting precipitate was collected by filtration, and washed with water, 5% citric acid, and water. The dried crude product (2.61 g) was recrystallized from DMF and diethyl ether. Yield, 1.53 g (56.7%); mp 226—227 °C; $[\alpha]_D^{24}$ −21.6° (*c* 0.5, DMF); TLC: R_f 0.52 (Solv. 1).

Found: C, 63.00; H, 7.25; N, 10.23%. Calcd for $C_{80}H_{107}N_{11}O_{17}\cdot 2H_2O$: C, 62.77; H, 7.31; N, 10.06%.

cyclo(-Val-Orn(Z)-Leu-D-Phe-δAva-Orn(Z)-Leu-D-Phe-Pro-) (XII). The cyclic peptide was prepared in the same way as described.[2] The reaction of XI (1.05 g, 0.7 mmol) with bis(*p*-nitrophenyl)sulfite (1.59 g) in a mixture of DMF (18 ml) and pyridine (8 ml) at room temperature for 43 h gave the *p*-nitrophenyl ester of XI. After the removal of Z(OMe)-group of this ester with TFA (7 ml) containing anisole (1.12 ml), cyclization of the resulting peptide ester was carried out in pyridine (467 ml) at 58—60 °C. The solution was concentrated *in vacuo*. The residue was dissolved in aq MeOH and passed through Dowex-1 (OH^- form) and Dowex-50 (H^+ form). After evaporation of the effluent, the product (628 mg) was recrystallized from aq MeOH. Yield, 561 mg (61.1%); mp 126—130 °C; $[\alpha]_D^{24}$ −158.0° (*c* 0.3, EtOH); TLC: R_f 0.5 (Solv. 2); mol wt, found: 1331 (calcd for $C_{71}H_{97}N_{11}O_{13}$: 1313).

Found: C, 64.09; H, 7.56; N, 11.03%. Calcd for $C_{71}H_{97}N_{11}O_{13}\cdot CH_3OH$: C, 64.31; H, 7.57; N, 11.46%.

cyclo(-Val-Orn-Leu-D-Phe-δAva-Orn-Leu-D-Phe-Pro-)·2HCl (XIII). Benzyloxycarbonyl groups of XII (394 mg, 0.3 mmol) were hydrogenolyzed in MeOH (10 ml) containing 1 M HCl (0.9 ml) in the presence of Pd-black (100 mg) for 7.5 h. After removal of the catalyst, the filtrate was concentrated *in vacuo*. The residue was washed with diethyl ether by decantation and then dissolved in water. The solution was filtered through active charcoal and the filtrate was lyophilized. Yield, 293 mg (77.4%); mp 228—231 °C (dec); $[\alpha]_D^{24}$ −157.0° (*c* 1, EtOH); TLC: R_f 0.82 (Solv. 4). Paper electrophoresis: migration distance −12 cm, *cf.* −12 cm for GS [HCOOH: AcOH: H_2O=4: 15: 180 v/v (pH 1.9), 600 V, 12—14.5 mA, 2 h, Toyo no. 50 (15×40 cm)]. Amino acid ratios: Val, 0.95; Orn, 2.00; Leu, 2.07; Phe, 2.00; Pro, 1.05; δAva, 0.96 (recovery 90%).

Found: C, 52.57; H, 7.15; N, 11.90%. Calcd for $C_{55}H_{87}N_{11}O_9Cl_2\cdot 6H_2O\cdot HCl$: C, 52.35; H, 7.99; N, 12.21%.

Z(OMe)-Orn(Z)-Leu-D-Phe-δAva-NHNH₂ (XIV). A solution of V (2.01 g, 2.5 mmol) and 80% $NH_2NH_2\cdot H_2O$ (4.7 g) in DMF (15 ml) was stirred at room temperature for 3 d. The solution was concentrated *in vacuo* to a volume of *ca.* 10 ml. White precipitate was obtained by the addition of excess water to the concentrate. The filtered and dried product weighed 1.94 g (96.5%). Mp 203.5—205.5 °C. A sample for analysis was obtained by crystallization from MeOH–ethyl acetate–diethyl ether. Mp 205.5—207.5 °C; $[\alpha]_D^{21}$ +8.4° (*c* 1, DMF).

Found: C, 62.47; H, 7.25; N, 12.46%. Calcd for $C_{42}H_{57}N_7O_9$: C, 62.75; H, 7.15; N, 12.19%.

Z(OMe)-Orn(Z)-Leu-D-Phe-δAva-OH (XV). A solution of V (2.41 g, 3 mmol) in a mixture of dioxane (50 ml) and MeOH (30 ml) containing 1 M NaOH (9 ml) was stirred at room temperature for 2.5 h. To this was added 1 M NaOH (3 ml) and the stirring was continued. Another portion (3 ml) of the alkali solution was added 1 h later. Four h after the last addition, the solution was acidified with citric acid (3.15 g) and concentrated *in vacuo*. On the addition of water to the residue, precipitate appeared immediately. After filtration and drying, the precipitate was recrystallized from MeOH. Yield, 1.80 g (75.9%); mp 165—167 °C; $[\alpha]_D^{21}$ +5.0° (*c* 1, DMF); TLC: R_f 0.18 (Solv. 3).

Found: C, 64.03; H, 7.19; N, 9.18%. Calcd for $C_{42}H_{55}N_5O_{10}$: C, 63.86; H, 7.02; N, 8.87%.

H-Orn(Z)-Leu-D-Phe-δAva-OH·HCl (XVI). XV (1.58 g, 2 mmol) was treated with a solution of 2.2 M HCl–dioxane (20 ml) containing anisole (0.65 ml) at room temperature for 2 h. The product was obtained in a theoretical yield following the procedure in the preparation of X.

Z(OMe)-Orn(Z)-Leu-D-Phe-δAva-Orn(Z)-Leu-D-Phe-δAva-OH (XVII). XIV (1.61 g, 2 mmol) and XVI (1.32 g, 2 mmol) were subjected to the same treatment as that for XI, except for the use of isopentyl nitrite (0.42 ml) instead of butyl nitrite. The reaction mixture was stirred at 0 °C for 70 h and filtered. The precipitate and the residue obtained from the filtrate after concentration *in vacuo* were combined, and washed with 5% citric acid and water. Recrystallization of the dried crude product from DMF and MeOH gave 1.65 g (59.0%) of the pure product. Mp 218—220 °C; $[\alpha]_D^{24}$ +3.4° (*c* 0.6, DMF).

Found: C, 64.10; H, 7.48; N, 9.79%. Calcd for $C_{75}H_{100}N_{10}O_{16}$: C, 64.45; H, 7.21; N, 10.02%.

cyclo(-Orn(Z)-Leu-D-Phe-δAva-Orn(Z)-Leu-D-Phe-δAva-) (XVIII). This cyclic peptide was synthesized following the same procedure as described for the prepararion of XII. From XVII (1.40 g, 1 mmol), 194 mg of crude product was obtained. Upon recrystallization from aq MeOH, 174 mg (14.3%) of the pure product was obtained. Mp 274—275 °C; $[\alpha]_D^{24}$ −20.7° (*c* 0.1, EtOH); TLC: R_f 0.46 (Solv. 3); mol wt, found: 1142 (calcd for $C_{66}H_{90}N_{10}O_{12}$: 1216).

Found: C, 64.71; H, 7.33; N, 11.60%. Calcd for $C_{66}H_{90}N_{10}O_{12}$: C, 65.22; H, 7.46; N, 11.52%.

cyclo(-Orn-Leu-D-Phe-δAva-Orn-Leu-D-Phe-δAva-)·2HCl (XIX). XVIII (146 mg, 0.12 mmol) was hydrogenolyzed in MeOH containing 1 M HCl (0.4 ml) in the presence of Pd-black (50 mg) for 6 h. After removal of the catalyst, the filtrate was concentrated *in vacuo*. After trituration of the residue with diethyl ether and decantation, it was dissolved in water. The solution was filtered through active charcoal. By lyophilization of the filtrate the product was obtained in a 71.2% yield (94.4 mg). Mp 199—202 °C (dec); $[\alpha]_D^{25}$ −20.7° (*c* 0.1, H_2O); TLC: R_f 0.59 (Solv. 4) and 0.69 (Solv. 5). Paper electrophoresis: migration distance −10 cm, *cf.* −10.5 cm for GS [HCOOH: AcOH: H_2O=4: 15: 180 v/v (pH 1.9), 600 V, 11.8—14.8 mA, 2 h, Toyo no. 50 (15×40 cm)]. Amino acid ratios: Orn, 0.96; Leu, 1.00; Phe, 0.90; δAva, 1.06 (recovery 96%).

Found: C, 52.88; H, 7.34; N, 12.03%. Calcd for $C_{50}H_{80}N_{10}O_8Cl_2\cdot 4H_2O\cdot HCl$: C, 53.21; H, 7.95; N, 12.41%.

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5) Abbreviations: MeVal, *N*-methylvaline; Z–, benzyloxycarbonyl; Z(OMe)–, *p*-methoxybenzyloxycarbonyl; –ONp, *p*-nitrophenoxy; –OMe, methoxy; –OEt, ethoxy; DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran; Et_3N, triethylamine; DCHA, dicyclohexylamine; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; HMPA, hexamethylphosphoramide; MA, mixed anhydride method.

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