

Synthesis of Gramicidin S (GS) Analogs, [3–4- δ Ava]-GS and [3–4,3'–4'-Bis(δ Ava)]-GS (δ Ava=5-Aminovaleric Acid)

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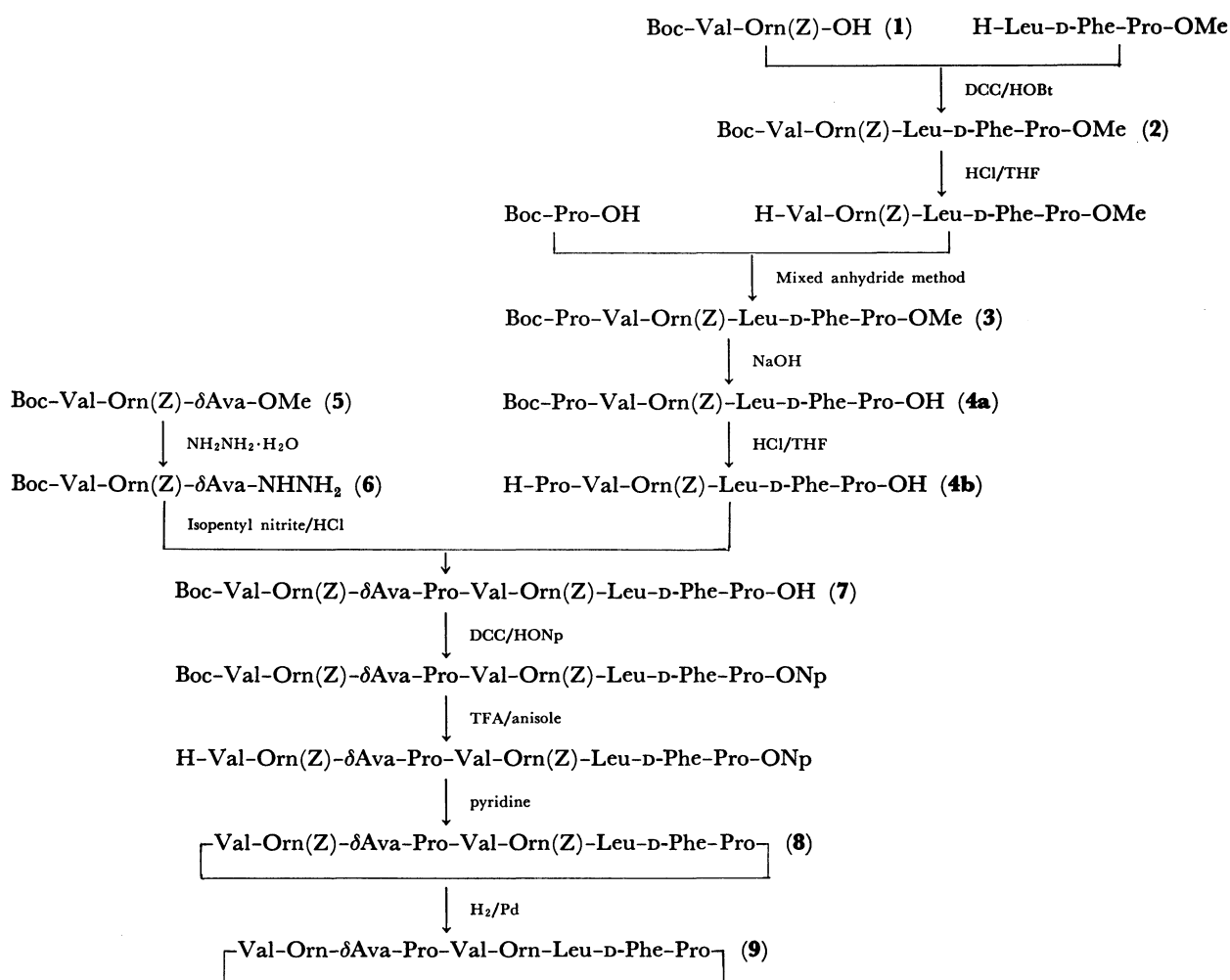
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Gramicidin S (GS) analogs, [δ Ava^{3–4}]-GS and [δ Ava^{3–4,3'–4'}]-GS, were synthesized. Both of these peptides are analogs in which one or two L-leucyl-D-phenylalanyl residues of GS are replaced by one or two 5-aminovaleric acid residues. The CD spectrum of the mono-substituted analog has two troughs as that of GS. However, this analog showed almost no antimicrobial activity. Moreover, the di-substituted analog showed a CD spectrum that is ascribed to a random structure and had no antimicrobial activity.

In previous reports,^{1–3)} we have described the syntheses and properties of four gramicidin S (GS) analogs containing 5-aminovaleric acid residue (δ Ava). All of them were cyclic peptides in which each sequence of two adjacent amino acid residues is replaced by one residue of 5-aminovaleric acid. Consequently, each amide group was substituted by an ethylene group which had the same number of members in the ring as that of natural GS, *cyclo*-(Val-Orn-Leu-D-Phe-Pro)₂, although the side chains inevitably were lacking. Thus, it can be elucidated

whether or not the original amide group in the natural peptide takes part in the intramolecular hydrogen bond. One of the analogs, [δ Ava^{4–5}]-GS, had a similar CD spectrum to that of GS, and its conformation was confirmed by ¹H-NMR measurement.⁴⁾ This analog showed significant antimicrobial activity against some microorganisms.⁵⁾

In view of these results, it became of interest to obtain information about the contributions of the amide bond and side chains in Leu-D-Phe sequence of GS. Hence we undertook the synthesis and

Fig. 1. Synthetic scheme for [δ Ava^{3–4}]-GS.

evaluation of the properties of $[\delta\text{Ava}^{3-4}]$ -GS and $[\delta\text{Ava}^{3-4,3'-4'}]$ -GS, as described in this paper.

Results and Discussion

The synthesis of $[\delta\text{Ava}^{3-4}]$ -GS is outlined in Fig. 1.⁶⁾ H-Leu-D-Phe-Pro-OMe was obtained by hydrogenolysis of oily Z-Leu-D-Phe-Pro-OMe which had been synthesized stepwise by the mixed anhydride method.⁸⁾ Peptides **2** and **3** were prepared by the conventional methods.⁹⁾ On the other hand, Boc-Val-Orn(Z)- δ Ava-OMe (**5**) was synthesized from Boc-Val-Orn(Z)-NHNH₂¹⁰⁾ and H- δ Ava-OMe¹⁾ by the azide method¹¹⁾ and converted to **6** by hydrazinolysis. Peptide **7** was synthesized from **4b** and **6** by the azide method.

The synthetic scheme of $[\delta\text{Ava}^{3-4,3'-4'}]$ -GS is also shown in Fig. 2. Peptide **10** was synthesized from **6** and H-Pro-OMe by the azide method. Deprotections of **10** gave **11** and **12**, respectively, which were then coupled with each other to afford **13** by the DCC-HOBt method. After purification by gel filtration on a Sephadex LH-20 with MeOH, the saponification of **13** gave **14**.

The intramolecular cyclizations of **7** and **14** were performed by the active ester method in dilute pyridine solution and the products (**8** and **15**) were purified by column chromatography. $[\delta\text{Ava}^{3-4}]$ -GS

(**9**) and $[\delta\text{Ava}^{3-4,3'-4'}]$ -GS (**16**) were obtained by hydrogenolysis of **8** and **15**, respectively, and were confirmed by means of the DNSC (5-dimethylamino-1-naphthalenesulfonyl chloride) procedure¹²⁾ and paper electrophoresis.¹⁾

To obtain information on the conformation of $[\delta\text{Ava}^{3-4}]$ -GS and $[\delta\text{Ava}^{3-4,3'-4'}]$ -GS, CD and ORD spectra of the aqueous solutions were measured. The CD spectra of these analogs are shown in Fig. 3. The CD spectrum of the former analog has two troughs (207 and 215 nm) which are similar to those of GS although the molar ellipticity is smaller than that of GS, and the spectrum of the latter suggests a random structure. The ORD spectra of these analogs gave the same information. The minimum value in the ORD spectrum of $[\delta\text{Ava}^{3-4}]$ -GS was observed at 230 nm, the same as that of GS. The sequence of $[\delta\text{Ava}^{3-4}]$ -GS is the same as half that of GS, and this half can make the hydrogen bond maintain the β -turn, which has been indicated from the CD spectrum. From these results we predicted that $[\delta\text{Ava}^{3-4}]$ -GS may show antimicrobial activity against Gram-positive bacteria. However, as shown in Table 1, the mono-substituted analog had almost no activity, and the di-substituted analog had no activity at all. The partial sequence, -NH-(CH₂)₄-CO-Pro-Val, of these analogs corresponds to Gly-Pro-Val or D-X-Pro-Y of Type II' in the rules for

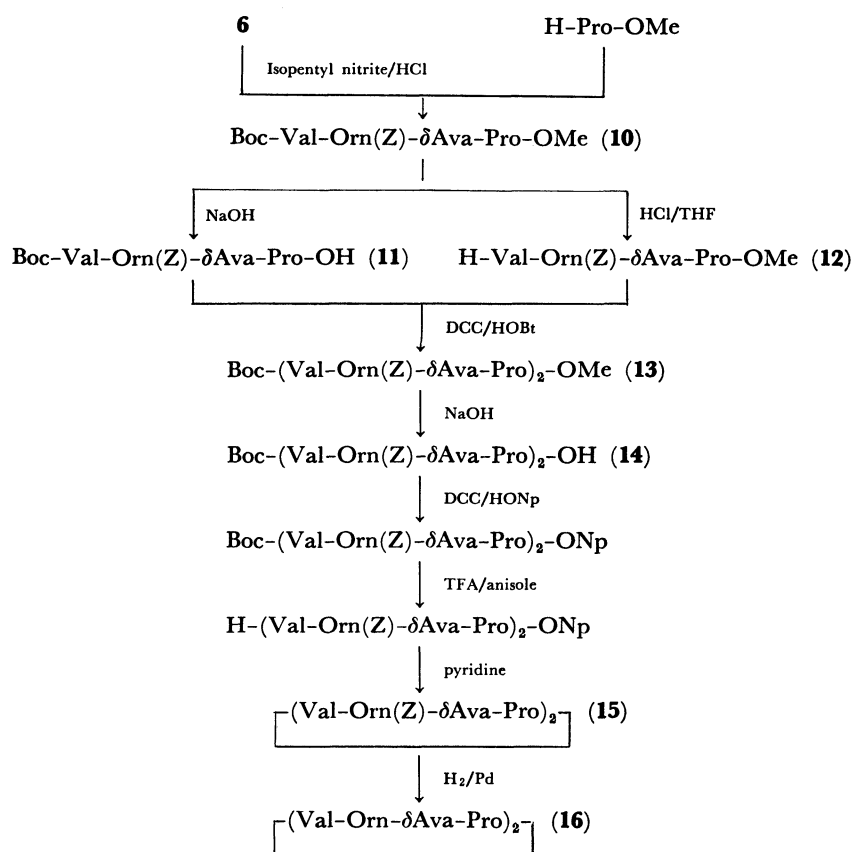


Fig. 2. Synthetic scheme for $[\delta\text{Ava}^{3-4,3'-4'}]$ -GS.

Table 1. Atimicrobial Activity of GS and its Analogs

Test organism	Minium inhibitory concentration ^{a)}		
	GS	[δ Ava ³⁻⁴]-GS	[δ Ava ^{3-4,3'-4'}]-GS
<i>Sarcina lutea</i> ATCC 9341	6.3	>100	>100
<i>Micrococcus flavus</i> ATCC 10240	3.1	50	>100
<i>Corynebacterium diphtheriae</i> P.W.8	1.6	50	>100
<i>Bacillus subtilis</i> ATCC 6633	6.3	>100	>100
<i>Escherichia coli</i> NIHJ-JC2	>100	>100	>100

a) The minimum concentration ($\mu\text{g/ml}$) was determined by the agar dilution method.

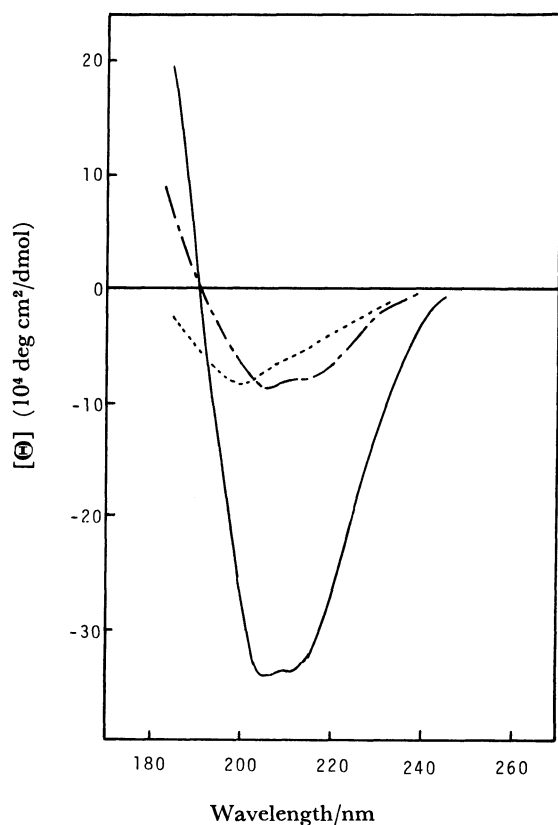


Fig. 3. CD spectra of GS·2HCl (—), [δ Ava³⁻⁴]-GS·2HOAc (---), and [δ Ava^{3-4,3'-4'}]-GS·2HOAc (····) in water. Measurements were made using a 0.5mm quartz cell at room temperature.

β -turn proposed by Blout.¹³⁾ However, δ Ava has no carbonyl group at the γ -position and hence this part can not form the hydrogen bond as in the case of GS. [δ Ava^{5-1'}]-GS, in spite of the lack of an amide bond between Pro⁵ and Val^{1'}, did exhibit some antimicrobial activity.³⁾ In the case of [δ Ava⁴⁻⁵]-GS, this analog showed significant antimicrobial activity even though one of the side chains of D-phenylalanyl residues was lacking. The present results, together with these earlier findings, suggest the importance of both leucyl residue side chains in GS for the exhibition of antimicrobial activity.

Experimental

The melting points are uncorrected. The homogeneity of the synthetic compounds was confirmed by TLC on Merck silica gel F₂₅₄ plates with following solvent systems (v/v): R_f^1 , CHCl₃-MeOH-HOAc (9:1:1); R_f^2 , *n*-BuOH-HOAc-pyridine-H₂O (4:1:1:2); R_f^3 , *n*-BuOH-HOAc-H₂O (4:1:1); R_f^4 , CHCl₃-MeOH-EtOAc (95:7:5). Amino acid analyses were performed with a JEOL automatic amino acid analyzer, after the samples had been hydrolyzed with constant-boiling HCl in evacuated sealed ampoules for 20 h at 110 °C. The molecular weight was determined with a Hitachi molecular weight apparatus model 115, using MeOH. CD spectrum was measured with a JASCO model J-20 spectrometer and is represented in term of molar ellipticity.

Boc-Val-Orn(Z)-OH (1). Boc-Val-Orn(Z)-OEt⁷⁾ (14.0 g, 28.4 mmol) was saponified in MeOH (150 ml) with 2.6 M[†] NaOH (44 ml) at room temperature for 20 min. The solution was neutralized with 5% citric acid and concentrated in vacuo to remove the MeOH. The aqueous phase was acidified with citric acid to pH 3 and extracted with EtOAc. The extract was dried over anhydrous Na₂SO₄ and evaporated in vacuo. The residue was recrystallized from EtOAc and hexane. Yield, 12.8 g (97.0%); mp 66–68 °C; [α]_D²⁰ +16.2° (*c* 0.7, DMF).

Found: C, 59.27; H, 7.88; N, 9.32%. Calcd for C₂₃H₃₅N₃O₇: C, 59.34; H, 7.58; N, 9.03%.

Boc-Val-Orn(Z)-Leu-D-Phe-Pro-OMe (2). H-Leu-D-Phe-Pro-OMe·HCl was obtained in the theoretical yield by hydrogenolysis (Pd black in 50 ml of MeOH containing 1.8 ml of concd HCl) of oily Z-Leu-D-Phe-Pro-OMe¹⁴⁾ (7.8 g, 15 mmol) which had been synthesized stepwise by the mixed anhydride method from C-terminal. This deprotected peptide (15 mmol) and compound **1** (7.0 g, 15 mmol) were dissolved in THF (80 ml) containing *N*-methylmorpholine (1.75 ml, 15 mmol). Coupling was performed with DCC (3.1 g, 15 mmol) and HOBt (4.1 g, 30 mmol) at 0 °C for 2 h and room temperature overnight. After filtration of the insoluble materials, the filtrate was concentrated in vacuo. The EtOAc solution of the residue was washed successively with 5% citric acid, water, 5% NaHCO₃, and water, and dried over anhydrous Na₂SO₄. The solution was concentrated in vacuo and the residue was recrystallized from EtOAc and hexane. Yield, 7.3 g (58.3%); mp 159–162 °C; [α]_D²⁰ –31.0° (*c* 1.0, DMF).

[†] 1 M=1 mol dm⁻³.

Found: C, 62.92; H, 7.76; N, 10.50%. Calcd for $C_{44}H_{64}N_6O_{10}$: C, 63.14; H, 7.71; N, 10.04%.

Boc-Pro-Val-Orn(Z)-Leu-D-Phe-Pro-OMe (3). Compound **2** (5.57 g, 6.65 mmol) was dissolved in 2.3 M HCl/THF (29 ml) to remove the Boc-group. The solution was stirred at room temperature for 2 h and evaporated in vacuo. The oily residue was dried over NaOH pellets in vacuo and dissolved in DMF (20 ml) containing *N*-methylmorpholine (0.75 ml). The solution was added to the mixed anhydride solution which was prepared from Boc-Pro-OH (1.5 g, 7.0 mmol), ethyl chloroformate (0.83 ml, 8.6 mmol) and *N*-methylmorpholine (0.96 ml, 8.7 mmol) in DMF (30 ml) at -15°C for 4 min. This reaction mixture was stirred at 0°C 2 h and at room temperature overnight. After filtration of the mixture, the filtrate was evaporated in vacuo and the residue was triturated with water. The product was collected by filtration and washed on a funnel successively with 5% citric acid, water, 5% NaHCO_3 and water. The crude product was recrystallized from DMF and water. Yield, 5.45 g (87.0%); mp $208-209^{\circ}\text{C}$; $[\alpha]_D^{20} -54.0^{\circ}$ (c 1.0, DMF).

Found: C, 62.29; H, 7.60; N, 10.50%. Calcd for $C_{49}H_{71}N_7O_{11} \cdot 0.5\text{H}_2\text{O}$: C, 62.40; H, 7.69; N, 10.40%.

Boc-Pro-Val-Orn(Z)-Leu-D-Phe-Pro-OH (4a). Into the solution of **3** (5.2 g, 5.5 mmol) in THF (60 ml), 3.6 M NaOH (6.2 ml) was added. The reaction solution was stirred at room temperature for 3 h and neutralized with 5% citric acid. After removal of the solvent in vacuo, the residue was treated with 5% citric acid. The precipitate was filtered and washed with cold water on a funnel. The dried product weighed 5.15 g (100%). Mp $158-164^{\circ}\text{C}$; $[\alpha]_D^{20} -52.5^{\circ}$ (c 0.3, DMF).

Found: C, 61.98; H, 7.82; N, 10.05%. Calcd for $C_{48}H_{69}N_7O_{11} \cdot 0.5\text{H}_2\text{O}$: C, 62.05; H, 7.59; N, 10.55%.

Boc-Val-Orn(Z)- δ Ava-OMe (5). Boc-Val-Orn(Z)-NHNH $_2^{10}$ (2.5 g, 5.2 mmol) was converted to the corresponding azide with isopentyl nitrite (0.82 ml, 6.1 mmol) and 2.9 M HCl/dioxane (11.3 ml) in DMF (26 ml) at -30°C for 15 min. Into the azide solution neutralized with Et_3N (4.8 ml) at -55°C , the solution of H- δ Ava-OMe $\cdot\text{HCl}^{11}$ (0.92 g, 5.5 mmol) and Et_3N (1.5 ml) in DMF (26 ml) was added and the reaction mixture was stirred at 0°C for 3 d. After filtration of the insoluble materials, the filtrate was concentrated in vacuo. The AcOEt solution of the residue was treated in the same manner as described for **2**. The product was recrystallized from AcOEt and diethyl ether. Yield, 2.21 g (73.4%); mp $148-152^{\circ}\text{C}$; $[\alpha]_D^{20} -21.3^{\circ}$ (c 0.5, DMF).

Found: C, 59.78; H, 8.06; N, 10.22%. Calcd for $C_{29}H_{46}N_4O_8$: C, 60.19; H, 8.01; N, 9.68%.

Boc-Val-Orn(Z)- δ Ava-NHNH $_2$ (6). Compound **5** (20.2 g, 34.9 mmol) in MeOH (100 ml) was treated with 80% hydrazine hydrate (44 ml) at room temperature overnight. After evaporation of the solvent, the crystalline residue was collected on a funnel and washed with water several times. Dried crystals weighed 18.9 g (92.1%). Mp $163-165^{\circ}\text{C}$.

Found: 57.03; H, 8.33; N, 13.88%. Calcd for $C_{28}H_{46}N_6O_7 \cdot 0.5\text{H}_2\text{O}$: C, 57.22; H, 8.06; N, 14.30%.

Boc-Val-Orn(Z)- δ Ava-Pro-Val-Orn(Z)-Leu-D-Phe-Pro-OH (7). Into the solution of **6** (3.47 g, 6 mmol) in DMF (80 ml), 7.3 ml of 3.3 M HCl/THF was added and followed by isopentyl nitrite (0.9 ml, 6.7 mmol) at -15°C . After 15 min, the reaction mixture was neutralized with

Et_3N (3.4 ml) and the solution of the hydrochloride of **4b** obtained from **4a** (4.8 g, 5.2 mmol) by treatment with 2.5 M HCl/THF as described for **3** and Et_3N (0.77 ml) in DMF (40 ml) was added into the above azide solution. After the stirring for 3 d at 0°C , the filtrate was evaporated in vacuo and the residue was triturated with cold water. The product was washed on a funnel successively with 5% citric acid, 5% NaHCO_3 , and water. This product was purified by column chromatography on silica gel (Wakogel C-200, 3.6×40 cm) with CHCl_3 -MeOH (97:3). The combined eluates were concentrated in vacuo and the residue was recrystallized from EtOH and diethyl ether. Yield, 2.55 g (35.9%); mp $197-200^{\circ}\text{C}$; R_f 0.40; $[\alpha]_D^{20} -28.71^{\circ}$ (c 0.4, EtOH).

Found: C, 62.10; H, 7.87; N, 11.18%. Calcd for $C_{71}H_{103}N_{11}O_{16}$: C, 62.40; H, 7.60; N, 11.27%.

Cyclo-(Val-Orn(Z)- δ Ava-Pro-Val-Orn(Z)-Leu-D-Phe-Pro-) (8). Into the solution of **7** (700 mg, 0.51 mmol) in DMF (25 ml), *p*-nitrophenol (219 mg, 1.6 mmol) and DCC (126 mg, 0.61 mmol) were added at 0°C and the reaction mixture was stirred at room temperature overnight.

After removal of the DCC, urea, and the solvent, the residue was recrystallized from MeOH-water and washed with diethyl ether. The *p*-nitrophenyl ester of **7** weighed 550 mg (72.5%), R_f 0.89. Boc-group of this active ester (500 mg, 0.34 mmol) was removed with TFA (5 ml) containing anisole (0.8 ml) at 0°C for 20 min. Evaporation of the TFA was carried out at 0°C . An oily residue was crystallized by treatment with diethyl ether and decantation. A solution of this ester in DMF (10 ml) containing HOAc (0.1 ml) was added dropwise into pyridine (350 ml) at $58-60^{\circ}\text{C}$ for 2.5 h. After the stirring of the solution at $58-60^{\circ}\text{C}$ for 5 h, it was concentrated in vacuo. The residue was dissolved in MeOH-water (7:1) and passed successively through columns of Dowex 1 (OH^-) and Dowex 50 (H^+). The oily product was obtained by concentration of the eluate and crystallized with diethyl ether. The recrystallization from MeOH and diethyl ether gave 77 mg (17.9%). R_f 0.79; mp 165°C ; $[\alpha]_D^{18} -166.9^{\circ}$ (c 0.6, MeOH); mol wt, found: 1240 (calcd for $C_{66}H_{93}N_{11}O_{13}$: 1247).

Found: C, 62.43; H, 7.76; N, 12.02%. Calcd for $C_{66}H_{93}N_{11}O_{13} \cdot \text{H}_2\text{O}$: C, 62.59; H, 7.56; N, 12.16%.

Cyclo-(Val-Orn- δ Ava-Pro-Val-Orn-Leu-D-Phe-Pro) (9). Hydrogenolysis of **8** (80 mg, 0.063 mmol) was carried out in a mixture of MeOH (6 ml) containing 0.1 ml of 3 M HCl/THF and Pd black for 10 h at room temperature. After removal of the catalyst, an oily product was obtained by concentration of the filtrate in vacuo, and it was purified by chromatography on a Sephadex G-10 with 4% HOAc. The homogeneous fractions were collected and lyophilized and white crystalline peptide was obtained. Yield, 69 mg (94.9%); mp $168-169^{\circ}\text{C}$; $[\alpha]_D^{22} -171.2^{\circ}$ (c 0.3, EtOH); R_f 0.63. Amino acid ratio: Val 1.89, Orn 1.91, Leu 1.02, Phe 1.02, Pro 2.07, δ Ava 1.09.

Found: C, 55.80; H, 8.07; N, 13.41%. Calcd for $C_{50}H_{81}N_{11}O_9 \cdot 2\text{HOAc} \cdot 3\text{H}_2\text{O}$: C, 56.18; H, 8.29; N, 13.35%.

Boc-Val-Orn(Z)- δ Ava-Pro-OMe (10). Hydrazide **6** (3.86 g, 6.57 mmol) was converted to the corresponding azide with isopentyl nitrite (1.2 ml, 9 mmol) and 5 M HCl/THF (5.3 ml) in DMF (30 ml) at -20°C for 40 min. A solution of H-Pro-OMe $\cdot\text{HCl}$ (1.32 g, 8.0 mmol) and Et_3N (1.2 ml, 8.6 mmol) in DMF (20 ml) was added to the above azide solution previously neutralized with Et_3N (3.7 ml

26.6 mmol) at -50°C . This reaction mixture was stirred at 0°C for 3 d and then added into 0.4% citric acid (750 ml), liberating an oily product. The product extracted in AcOEt was washed with water and dried over anhydrous Na_2SO_4 . The solution was concentrated in vacuo and the residue was recrystallized from AcOEt and hexane. Yield, 3.93 g (88.5%); mp 87°C ; $[\alpha]_D^{20} -18.1^{\circ}$ (c 0.7, DMF).

Found: C, 59.94; H, 8.09; N, 10.51%. Calcd for $\text{C}_{34}\text{H}_{53}\text{N}_5\text{O}_9$: C, 60.43; H, 7.90; N, 10.36%.

Boc-(Val-Orn(Z)- δ Ava-Pro)₂-OH (14). Saponification of **10** (1.96 g, 2.9 mmol) was carried out with 2.2 M NaOH (5.2 ml) in MeOH (20 ml) at room temperature for 6 h. After evaporation of the MeOH, the residue was treated with 5% citric acid and extracted with AcOEt. The oily product **11** (1.25 g, 1.9 mmol) was obtained in a 64% yield by concentration of the extract which was washed with water and dried over anhydrous Na_2SO_4 . On the other hand, removal of the Boc-group of **10** (1.66 g, 2.5 mmol) was carried out with 2.3 M HCl/THF (24.5 ml) at room temperature for 2.5 h. After concentration of the reaction solution, the residual oily compound (**12**, 2.5 mmol) was dried over NaOH pellets in vacuo and used directly in the next step. The solution of above **11** and **12**·HCl in THF (30 ml) was neutralized with *N*-methylmorpholine (0.96 ml), followed by the additions of HOBT (0.5 g, 3.7 mmol) and DCC (0.42 g, 2.0 mmol). The coupling reaction proceeded at 0°C overnight. After filtration of the insoluble materials, the filtrate was concentrated in vacuo. The residue was crystallized by treatment with water and washed successively with 5% citric acid, water, 5% Na_2CO_3 , and water on a funnel. The crude product was purified by gel filtration on a Shephadex LH-20 with MeOH. The yield of **13** was 2.22 g (95.7%) and the amino acid analysis showed the composition within experimental error. Saponification of **13** (1.36 g, 1.1 mmol) was carried out with 1.3 M NaOH (4.4 ml) in DMF (30 ml) at room temperature for 2.5 h. The reaction solution was added into 0.5% citric acid (500 ml) at 0°C . The precipitate was filtered off and recrystallized from MeOH and diethyl ether. Yield, 1.30 g (93.9%); mp $205.5\text{--}206.5^{\circ}\text{C}$; $[\alpha]_D^{20} -23.8^{\circ}$ (c 0.8, DMF); R_f^1 0.56.

Found: C, 58.11; H, 7.75; N, 11.10%. Calcd for $\text{C}_{61}\text{H}_{92}\text{N}_{10}\text{O}_{15}\cdot 3\text{H}_2\text{O}$: C, 58.17; H, 7.84; N, 11.12%.

Cyclo-(Val-Orn(Z)- δ Ava-Pro)₂ (15). a) *p*-Nitrophenol (104 mg, 0.75 mmol) and DCC (152 mg, 0.74 mmol) were added into the solution of **14** (440 mg, 0.35 mmol) in DMF (10 ml) at 0°C . After the stirring of the reaction mixture at room temperature overnight, it was filtered and the filtrate was concentrated in vacuo. The residual oily product was treated with a mixture of diethyl ether-hexane (1:1) and the crystalline *p*-nitrophenyl ester (292 mg, 0.22 mmol) of **14** was collected by decantation. In the same way as in the preparation of **8**, this active ester was used in the cyclization reaction after removal of the Boc-group. The product was dissolved in MeOH-water (7:1) and passed successively through columns of Dowex 1 (OH^-) and Dowex 50 (H^+). The eluent was removed under reduced pressure to give an oil. Chromatography on Sephadex LH-20 eluting with MeOH gave after recrystallization from aqueous MeOH 29 mg (11.5%) of **15**. Mp $202\text{--}204^{\circ}\text{C}$; $[\alpha]_D^{22} -39.5^{\circ}$ (c 0.3, MeOH); R_f^3 0.77, R_f^4 0.42; mol wt, found 1050 (calcd for $\text{C}_{56}\text{H}_{82}\text{N}_{10}\text{O}_{12}$: 1086).

Found: C, 58.87; H, 7.59; N, 11.33%. Calcd for

$\text{C}_{56}\text{H}_{82}\text{N}_{10}\text{O}_{12}\cdot 3.5\text{H}_2\text{O}$: C, 58.46; H, 7.79; N, 12.17%. b) Compound **14** (1.25 g, 0.99 mmol), HOSu (245 mg, 2.13 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (320 mg, 2.06 mmol) and 3 M HCl/dioxane (0.67 ml) were mixed in DMF (8 ml) at 0°C and the reaction mixture was stirred at room temperature overnight. After concentration of this mixture under reduced pressure, an oily residue was crystallized by the treatment with water. The dried compound weighed 750 mg (58%). Removal of the Boc-group of this *N*-hydroxysuccinimide ester, the subsequent cyclization reaction and the purification were carried out just as described in the method a. This compound gave the same properties mentioned above. Yield, 87 mg (13.1%).

Cyclo-(Val-Orn- δ Ava-Pro)₂ (16). Z-groups of **15** (64.8 mg, 0.056 mmol) in MeOH (20 ml) with 3.1 M HCl/THF (0.05 ml) were removed by hydrogenolysis over Pd black for 5 h. In the same manner as with **9**, crystalline product was obtained in a 62.1% yield (36.4 mg). Mp $182\text{--}190^{\circ}\text{C}$; $[\alpha]_D^{23} -66.7^{\circ}$ (c 0.2, H_2O); R_f^2 0.37. Amino acid ratio: Val 2.03, Orn 2.04, δ Ava 2.14, Pro 1.78.

Found: C, 50.29; H, 8.18; N, 13.76%. Calcd for $\text{C}_{40}\text{H}_{70}\text{N}_{10}\text{O}_8\cdot 2\text{HOAc}\cdot 6\text{H}_2\text{O}$: C, 50.46; H, 8.66; N, 13.37%.

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- 6) Abbreviations with no prefix indicate the L-amino acid residue and follow the IUPAC-IUB tentative nomenclature described in *J. Biol. Chem.*, **247**, 977 (1972). Abbreviations used are: Z, benzyloxycarbonyl; Boc, *t*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; HONp, *p*-nitrophenol; HOSu, *N*-hydroxysuccinimide; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; TFA, trifluoroacetic acid.
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14) This compound was synthesized from H-Pro-OMe·HCl by a procedure reported by Ohno et al.¹⁰⁾ although the ethyl ester had been used in their procedure. Yield, 81%; oil; R_f 0.59 (CHCl₃-MeOH, 9:1).
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