# Studies on the β-Turn of Peptides. VII.[1] Syntheses and Antibiotic Activities of Gramicidin S Analogs with L-Pro-L-Asn or L-Pro-D-Ala Sequence at the β-Turn Part

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Two analogs of gramicidin S (GS), [L-Pro$^{4,4'}$, L-Asn$^{5,5'}$]-GS (**10a**) and [L-Pro$^{4,4'}$, D-Ala$^{5,5'}$]-GS were synthesized to investigate the possibility of replacing the β-turn part of GS by a different type of β-turn keeping the biological activity. For the synthesis of **10a**, three procedures were examined and satisfactory results were obtained by the active ester method applied to cyclization of linear decapeptide with L-Pro at the C-terminus. Neither analog showed antibiotic activity indicating that the β-turn part of GS could not be replaced with L-Pro-L-Asn or L-Pro-D-Ala sequences without affecting on its activity. The CD and ORD spectra of the analogs and their 2,4-dinitrophenyl derivatives showed weaker Cotton effects than those of GS and its derivative, respectively. So, the analogs were considered not to take GS-like β-sheet conformation. The reason why the analogs did not take such conformations was investigated by the model tetrapeptides with chromophoric substituents.

Gramicidin S (GS) is a cyclic decapeptide antibiotic with the primary structure shown in Fig. 1.[2] Several models have been proposed for the conformation of GS in solid state and solution.[3] The most favorable model is the intramolecular antiparallel β-sheet with four hydrogen bonds between Val and Leu residues, and two β-turns (type II′) around the D-Phe–Pro sequences (Fig. 2).[4] The characteristic feature of this conformation is the orientation of side chains in which the charged Orn side chains are on one side and the hydrophobic Val and Leu side chains on the other side of the molecule.[5] Kato and Izumiya introduced the word "sidedness hypothesis" which means that the sidedness is important for the antibiotic activity of GS.[6,7] The hypothesis is supported by the results of many investigations of synthetic amino acid-substituted analogs.[8] The characteristic conformation of GS is considered to be stabilized not only by the four intramolecular hydrogen bonds but also by the stable β-turns formed by the two D-Phe–Pro sequences. The antibacterial activities of the GS analogs substituted both or either of 4,4′ and 5,5′ positions are summarized as follows:[8] The analogs in which D-Phe residues at the 4,4′ positions are replaced with other D-amino acids show antibacterial activity, while replacement with L-amino acids leads to loss of activity, and the analog replaced with Gly shows weaker activity than the D-amino acid analogs. The Pro residues at the 5,5′ positions can be replaced by a variety of amino acids (Gly, Sar, Leu, *etc.*) without loss of activity. One of the exceptions is [Aib$^{5,5'}$]-GS which shows no antibacterial activity. The activity of these analogs closely correlate to their conformations.[9] For example, the population of GS-type β-sheet conformer was in the order of GS≈[D-Ala$^{4,4'}$]-GS>[Gly$^{4,4'}$]-GS≫[L-Ala$^{4,4'}$]-GS≈0.[10]

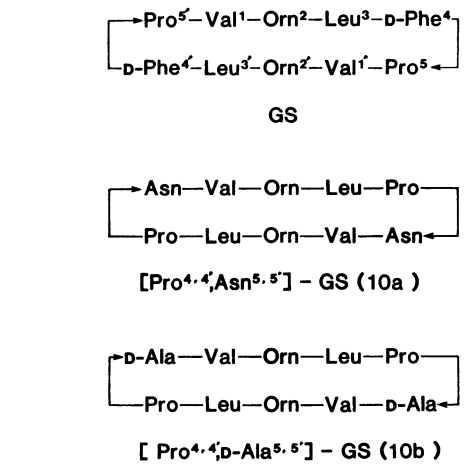


Fig. 1. Primary structures of GS and its analogs (**10a** and **10b**).

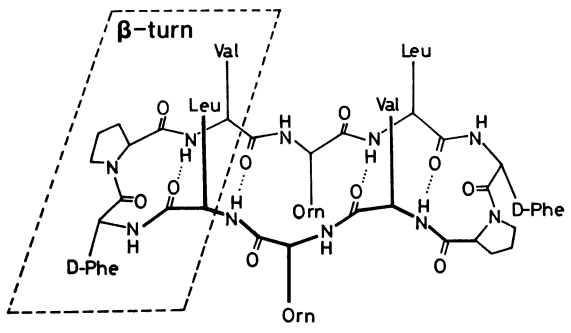


Fig. 2. β-Sheet conformation of GS.

In the course of studies on the β-turn of peptides, we proposed a new method to study the β-turn conformation of linear tetrapeptides.[1,11–15] *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp–tetrapeptide–pNA's) exhibit characteristic CD spectra above 250 nm when they take β-turn conformations. The Cotton effects are considered to be due to the exciton coupling of the transition moments in the two terminal chromophores. The magnitude of the Cotton effects near 350 and 310 nm were shown to reflect well the β-turn preference of the tetrapeptides.[11] In the case of model peptides related to the β-turn part of GS, β-turn preferences of the tetrapeptide derivatives had strong correlation with antibiotic activities of the GS analogs containing similar tetrapeptide sequences at their β-turn part.[12] Another series of compounds with a general structure Dnp–Gly–Pro–Y–Gly–pNA (Y=Asn, Gly, Ala, Gln, D-Ala) showed strong CD bands with opposite sign to those of GS-model peptides.[13] The

results indicated that the type of $\beta$-turn of the latter compounds were different from that of GS-model peptides (type II'). When Y was D-Ala, the strongest CD bands were observed and next ones were observed when Y was Asn (*cf.* Fig. 9).

It is of interest whether the $\beta$-turn part of GS can be replaced by a different type of $\beta$-turn keeping the biological activity. This paper describes the syntheses, antibiotic activities, and CD and ORD measurements of [Pro$^{4,4'}$, Asn$^{5,5'}$]-GS (**10a**) and [Pro$^{4,4'}$, D-Ala$^{5,5'}$]-GS (**10b**), in which the D-Phe–Pro sequences in GS were replaced by Pro–Asn and Pro–D-Ala sequences, respectively (Fig. 1). If Pro–Asn or Pro–D-Ala sequence could take stable $\beta$-turn conformation when incorporated in a cyclic decapeptide structure, the analogs would take GS-like $\beta$-sheet conformation, which would fulfil the "sidedness hypothesis," and would exhibit antibiotic activity.

## Results and Discussion

*Syntheses of Peptides.* First, we chose the route A shown in Fig. 3 for the synthesis of **10a**, because GS and its analogs had been synthesized in satisfactory results by cyclization of linear decapeptides with Leu at the C-terminus.[16,17] Stepwise chain elongation from C-terminal tripeptide H–Val–Orn(Z)–Leu–OEt[17] gave Boc–pentapeptide ester (**3**), which was converted into pentapeptide ester (**4**) and Boc–pentapeptide hydrazide (**5**) with the aid of hydrogen chloride in formic acid and hydrazine hydrate, respectively. Fragment condensation of **4** and **5** by azide method afforded Boc–decapeptide ester (**6**), which was converted into the hydrazide derivative (**7**) by treatment with hydrazine hydrate. Deblocking of Boc-group of **7** gave N-terminal-free decapeptide hydrazide (**8**). Cyclization of **8** by azide method was examined, but the results were not satisfactory because of low yields ($\leq$11%) of the crude cyclized peptide and many by-products contaminated. These results forced us to change the synthetic strategy.

Next, we examined two different routes (routes B and C) concurrently, in which the Pro residue was placed at the C-terminus and azide method and active ester method were employed for cyclization as shown in Figs. 4 and 5, respectively. In route B, Boc–pentapeptide ester (**17**) was prepared by stepwise elongation from C-terminal H–Pro–OBzl. Pentapeptide ester (**18**) and Boc–pentapeptide hydrazide (**19**) both derived from **17** were coupled by azide method to afford Boc–decapeptide ester (**20**), which was converted into the

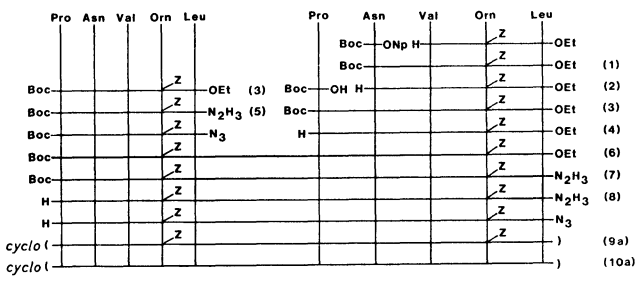


Fig. 3. Route A for the synthesis of **10a**.

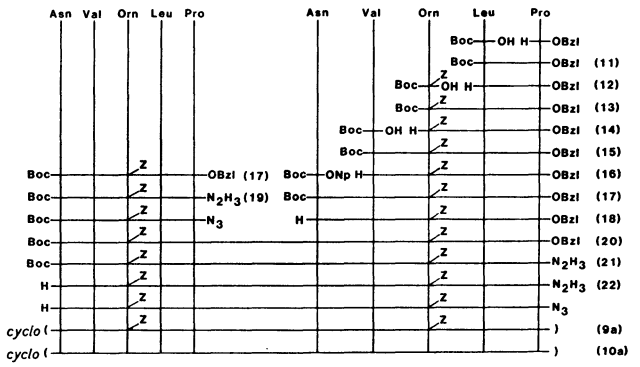


Fig. 4. Route B for the synthesis of **10a**.

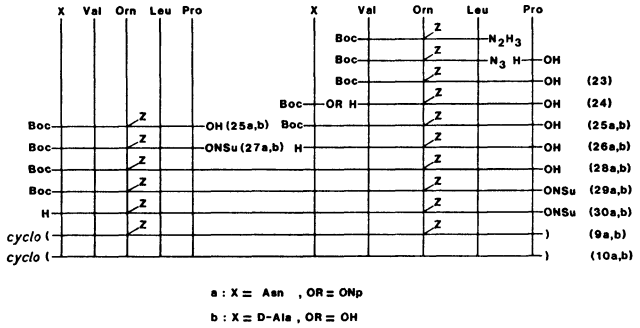


Fig. 5. Route C for the synthesis of **10a** and **10b**.

hydrazide derivative (**21**). Cyclization of **22** derived from **21** gave higher yield (38%) of crude cyclized peptide than that in route A. The crude product was purified by Sephadex LH-20 column chromatography and repeated recrystallization, however, small amount of by-product could not be removed. In route C, Boc-tetrapeptide with free carboxy terminus (**23**) was prepared by azide coupling of Boc–Val–Orn(Z)–Leu–$N_2H_3$[10] with H–Pro–OH. Compound **23** was treated with hydrogen chloride in formic acid, and subsequent active ester coupling of **24** with Boc–Asn–ONp afforded Boc–pentapeptide (**25a**). Pentapeptide (**26a**) and Boc–pentapeptide–ONSu (**27a**) both derived from **25a** were coupled to afford Boc–decapeptide (**28a**), which was converted into Boc–decapeptide–ONSu (**29a**). Boc-group of **29a** was removed with TFA to afford decapeptide–ONSu (**30a**). Cyclization of **30a** by active ester method gave satisfactory results: Yield of the crude cyclized peptide was 84%, and analytically pure **9a** was obtained after gel filtration with Sephadex LH-20 in 81% yield from **30a**. Compound **9a** prepared by route C was hydrogenated to afford [Pro$^{4,4'}$, Asn$^{5,5'}$]-GS (**10a**).

Another analog, [Pro$^{4,4'}$, D-Ala$^{5,5'}$]-GS (**10b**), was synthesized by the similar manner to that described for **10a** according to route C as shown in Fig. 5. The homogeneity of **10a** and **10b** was confirmed by thin-layer chromatography, paper electrophoresis, elemental analysis, and amino acid analysis.

It is interesting that the results of the cyclization largely depend on the sequence of the linear decapeptide precursor and on the activation method. Minematsu *et al.* reported high sequence dependence in the cyclization of linear pentapeptide precursor of GS.[18] They considered that steric character of terminal amino acids

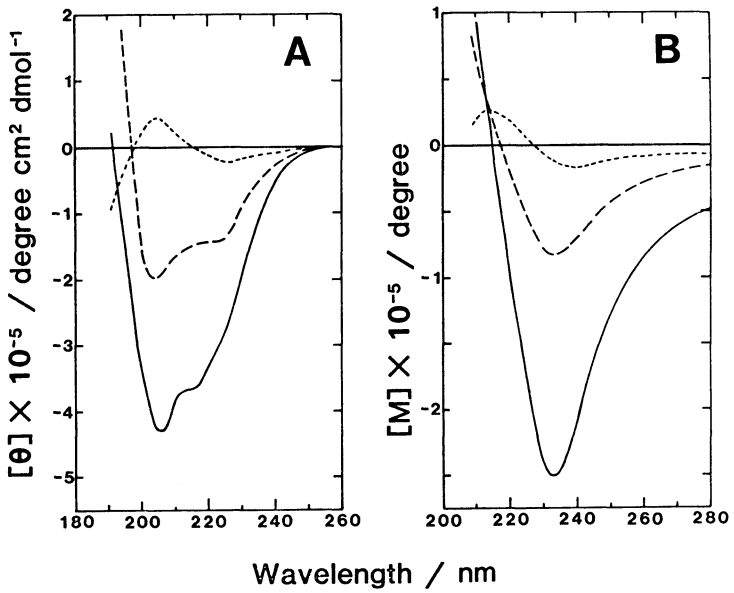


Fig. 6. CD (A) and ORD (B) spectra of GS and its analogs (**10a** and **10b**) in MeOH. ——: GS, - - -: **10a**, ·····: **10b**.

and conformation of peptide in the precursor might be important factors governing the marked differences in the mode of cyclization. Lee and Izumiya explained the sequence dependence in the cyclization of linear hexapeptide precursor of protodestruxin through the assumed conformation of the linear hexapeptide.[19] However, as far as we are aware, such a high sequence dependence as that shown in this work has not been reported in the case of cyclization of linear decapeptide precursor of GS and its analogs. Although the reason why such a high sequence dependence was observed is not clear, steric character of terminal amino acids is considered to play an important role for determining the mode of cyclization. Nucleophilic attack of amino group of Asn residue to carbonyl group of Pro residue (routes B and C) may be less hindered than that of imino group of Pro residue to carbonyl group of Leu residue (route A). As to activation method, active ester method seems to be more useful than azide method because of higher yield and experimental simplicity, when racemization-free amino acid such as Pro or Gly is used at the C-terminus of the linear precursor peptide.

*CD and ORD Studies.* Figure 6 shows the CD and ORD spectra of GS and its analogs (**10a** and **10b**). The analog **10a** shows similar curves to those of GS in shape, but the Cotton effects were much weaker than those of GS. In the case of **10b**, the shapes of the curves were also much different from those of GS. Usually, active analogs of GS having GS-like β-sheet conformation are considered to show similar ORD curves to that of GS.[9] It is possible for the analogs, **10a** and **10b**, to show the different CD and ORD spectra from those of GS even when they have β-sheet conformations, because the type of the β-turn of these analogs is considered to differ from that of GS. However, weak Cotton effects shown by the analogs suggest that they have some unordered conformations. The CD spectrum of the bis(Dnp) derivative of GS, [Orn(Dnp)$^{2,2'}$]-GS (**31c**), shows strong Cotton effects

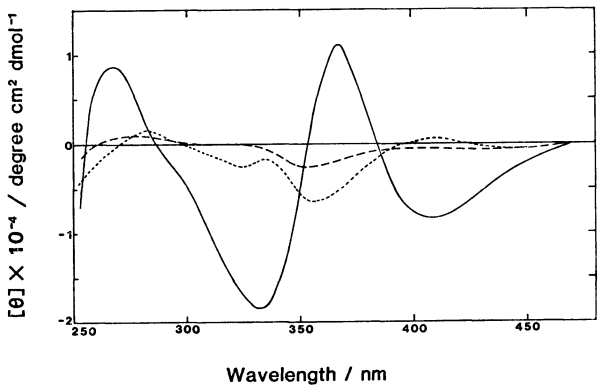


Fig. 7. CD spectra of bis(Dnp) derivatives of GS (**31c**), **10a** (**31a**), and **10b** (**31b**) in MeOH. ——: **31c**, - - -: **31a**, ·····: **31b**.

above 250 nm due to the interaction of the two Dnp chromophores, because the side chains of the two Orn residues in GS are close to each other owing to the β-sheet conformation of GS (Fig. 2).[10] Both of the bis(Dnp) derivatives of **10a** and **10b** (**31a** and **31b**, respectively) showed much weaker Cotton effects than that of GS, suggesting that neither **10a** nor **10b** took GS-like β-sheet conformation (Fig. 7).

*Biological Activities.* Neither **10a** nor **10b** showed antibiotic activities even at the concentration of 100 μg/ml. At such higher concentrations as 400 μg/ml and 200 μg/ml, respectively, **10a** and **10b** showed antibiotic activities on some Gram-positive bacteria, but practically they were inactive. The results indicate that the β-turn part of GS cannot be replaced with Pro–Asn or Pro–D-Ala sequences without affecting on its activity. Certainly, both analogs were hard to be absorbed on the cells of GS-sensitive strains (*B. subtilis* and *S. aureus*).[20]

*Conformations and β-Turn Preferences.* The inactivities of the analogs (**10a** and **10b**) seemed to result

from the fact that they did not take GS-like β-sheet conformation on the basis of the CD and ORD studies. So, the reason why they did not take such conformations was investigated. Heretofore, the high β-turn preferences of Pro–Asn and Pro–D-Ala sequences have been discussed by the model peptides with Gly residues at the first and the fourth positions of tetrapeptides,[13] however in the GS analogs these positions are occupied by Leu and Val residues, respectively. So, we synthesized Dnp–Leu–Pro–Asn–Val–pNA (**37a**) and Dnp–Leu–Pro–D-Ala–Val–pNA (**37b**), in which the terminal positions of Dnp–Gly–Pro–Y–Gly–pNA (Y=Asn or D-Ala) were replaced with Leu and Val residues, respectively (Fig. 8). Both of **37a** and **37b** showed weak Cotton effects similar to those of Dnp–Leu–Ala–Pro–Val–pNA which is considered to take random conformation (Figs. 9A and 9B).[11] The results indicated the importance of the terminal amino acid residues for the tetrapeptide sequences to take β-turn conformation. So, reluctance of the tetrapeptide sequences to take β-turn is considered to be the reason why **10a** and **10b** do not take GS-like β-sheet conformation. It is interesting to note that in the case of GS-model peptides, similar replacement of the terminal residues do not affect the β-turn preference (Fig. 9C).[12]

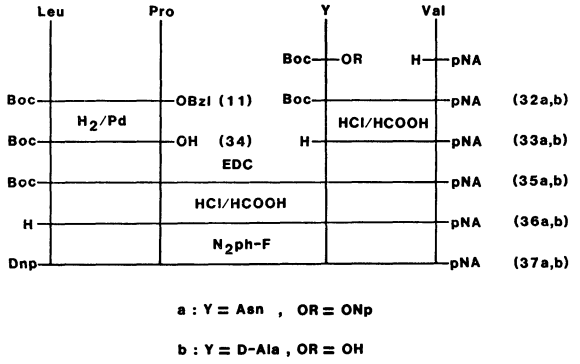


Fig. 8. Synthetic route of Dnp-Leu-Pro-Y-Val-pNA (**37a**: Y=Asn, **37b**: Y=D-Ala).

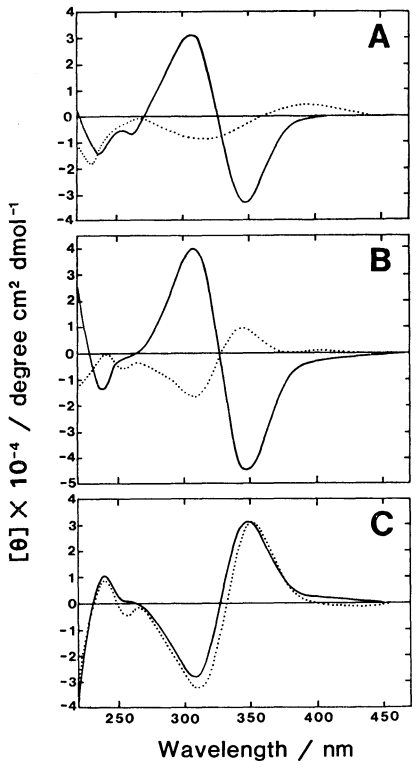


Fig. 9. CD spectra of Dnp-X′-Pro-Asn-Y′-pNA (A), Dnp-X′-Pro-D-Ala-Y′-pNA (B), and Dnp-X′-D-Ala-Pro-Y′-pNA (C)[12] in MeOH. ——: X′=Y′=Gly, ……: X′=Leu, Y′=Val.

## Experimental

*Syntheses of Peptides.* All the melting points were measured on a Yanagimoto micro melting point apparatus and were uncorrected. TLC's were carried out on Merck silica gel 60 $F_{254}$ plates with the following solvent systems: $R_f^1$, $CHCl_3$–MeOH (5:1, v/v); $R_f^2$, $CHCl_3$–MeOH–AcOH (95:5:1, v/v); $R_f^3$, *n*-BuOH–AcOH–pyridine–$H_2O$ (4:1:1:2, v/v). Optical rotations were measured on an Union automatic polarimeter PM-201. Amino acid analyses of peptides were performed by Durrum D-500 Amino Acid Analyzer after hydrolyses in 6 M (1 M=1 mol dm$^{-3}$) HCl at 105 °C for 20 h.

*Boc–Asn–Val–Orn(Z)–Leu–OEt (**1**).* To a solution of H–Val–Orn(Z)–Leu–OEt·HCl[17] (2.72 g, 5 mmol) and TEA (0.7 ml, 5 mmol) in DMF (20 ml) was added Boc–Asn–ONp (1.77 g, 5 mmol). The reaction mixture was stirred at room temperature overnight and evaporated to leave a solid which was collected by filtration with the aid of water and washed successively with 10% citric acid, 4% $NaHCO_3$, and water, and dried *in vacuo* over $P_2O_5$. The crude product was recrystallized from MeOH; yield, 2.67 g (74%); mp 214 °C; $[\alpha]_D^{22}$ −57.2° (*c* 1, MeOH); $R_f^1$ 0.69, $R_f^2$ 0.24, $R_f^3$ 0.97.

Found: C, 58.58; H, 7.48; N, 11.47%. Calcd for $C_{35}H_{56}O_{10}N_6$: C, 58.32; H, 7.83; N, 11.66%.

*H–Asn–Val–Orn(Z)–Leu–OEt·HCl (**2**·HCl).* Compound **1** (2.16 g, 3 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (36 ml). The solution was allowed to stand at room temperature for 30 min and evaporated. The residue was solidified by the addition of ether; yield, 1.97 g (100%); mp 210—212 °C; $[\alpha]_D^{22}$ −33.0° (*c* 1, MeOH); $R_f^1$ 0.25, $R_f^3$ 0.71.

Found: C, 54.62; H, 7.51; N, 12.77%. Calcd for $C_{30}H_{49}O_8N_6Cl$: C, 54.83; H, 7.52; N, 12.79%.

*Boc–Pro–Asn–Val–Orn(Z)–Leu–OEt (**3**).* To a chilled solution of Boc–Pro–OH (344 mg, 1.6 mmol) and TEA (0.22 ml, 1.6 mmol) in THF (5 ml) was added isobutyl chloroformate (0.21 ml, 1.6 mmol) at −15 °C. After 10 min, a chilled solution of **2**·HCl (1.05 g, 1.6 mmol) and TEA (0.22 ml, 1.6 mmol) in DMF (5 ml) was added. The mixture was stirred at 0 °C for 1 h and at room temperature overnight and evaporated *in vacuo*. After the addition of water, the solid formed was collected by filtration and washed as described for **1**. The crude product was recrystallized from EtOH; yield, 1.11 g (85%); mp 193 °C; $[\alpha]_D^{22}$ −75.8° (*c* 1, MeOH); $R_f^1$ 0.67, $R_f^2$ 0.13, $R_f^3$ 0.93.

Found: C, 58.54; H, 7.66; N, 11.71%. Calcd for $C_{40}H_{63}O_{11}N_7$: C, 58.73; H, 7.76; N, 11.99%.

*H–Pro–Asn–Val–Orn(Z)–Leu–OEt·HCl(**4**·HCl).* Compound **3** (409 mg, 0.5 mmol) was treated with 0.1 M hydrogen chloride in formic acid (6 ml) as described for **2**·HCl; yield, 362 mg (96%); mp 220—222 °C; $[\alpha]_D^{22}$ −62.8° (*c* 0.5, MeOH); $R_f^1$ 0.12, $R_f^3$ 0.69.

Found: C, 55.58; H, 7.17; N, 13.00%. Calcd for $C_{35}H_{56}O_9N_7Cl$: C, 55.73; H, 7.48; N, 13.00%.

*Boc–Pro–Asn–Val–Orn(Z)–Leu–$N_2H_3$ (**5**).* A solution of **3** (491 mg, 0.6 mmol) and hydrazine hydrate (0.58 ml,

12 mmol) in DMF (3 ml) was allowed to stand at room temperature for 2 d. The solution was poured dropwise into chilled water (100 ml), and the precipitate formed was collected by filtration, washed with water, and dried. The crude product was recrystallized from EtOAc; yield, 357 mg (74%); mp 235—236 °C; $[\alpha]_D^{22}$ −63.0° (*c* 0.2, MeOH); $R_f^1$ 0.47, $R_f^3$ 0.83.

Found: C, 56.33; H, 7.59; N, 15.65%. Calcd for $C_{38}H_{61}O_{10}N_9 \cdot 1/2\ H_2O$: C, 56.13; H, 7.69; N, 15.51%.

*Boc–(Pro–Asn–Val–Orn(Z)–Leu)$_2$–OEt (**6**).* To a solution of **5** (322 mg, 0.4 mmol) in DMF (2 ml) were added 2 M hydrogen chloride in EtOAc (0.8 ml) and isopentyl nitrite (0.056 ml, 0.4 mmol) at −60 °C. After being stirred at −20 °C for 10 min, the solution was cooled again to −60 °C and neutralized with TEA (0.22 ml, 1.6 mmol). To the solution was added a chilled solution of **4**·HCl (302 mg, 0.4 mmol) and TEA (0.056 ml, 0.4 mmol) in DMF (1 ml). The reaction mixture was stirred at 0 °C for 2 d and then evaporated. Addition of water to the residue gave a white precipitate, which was filtered, washed successively with 10 % citric acid, 4% $NaHCO_3$, and water, and dried over $P_2O_5$. The crude product dissolved in MeOH (5 ml) was applied to a column (3×170 cm) of Sephadex LH-20 and eluted with MeOH. The fractions with the desired product detected by UV absorption and TLC were collected and evaporated to leave a solid which was recrystallized from EtOH; yield, 439 mg (74%); mp 227—228 °C; $[\alpha]_D^{22}$ −96.0° (*c* 0.2, MeOH); $R_f^1$ 0.64, $R_f^3$ 0.86.

Found: C, 58.05; H, 7.33; N, 12.96%. Calcd for $C_{73}H_{112}O_{19}N_{14} \cdot H_2O$: C, 58.15; H, 7.62; N, 13.01%.

*Boc–(Pro–Asn–Val–Orn(Z)–Leu)$_2$–$N_2H_3$ (**7**).* A solution of **6** (402 mg, 0.27 mmol) and hydrazine hydrate (0.65 ml, 13.5 mmol) in DMF (5 ml) was treated as described for **5**. The crude product was recyrstallized from DMF–ether; yield, 325 mg (82%); mp 238—240 °C; $[\alpha]_D^{22}$ −76.0° (*c* 0.2, MeOH); $R_f^1$ 0.31, $R_f^3$ 0.83.

Found: C, 57.14; H, 7.41; N, 15.11%. Calcd for $C_{71}H_{110}O_{18}N_{16} \cdot H_2O$: C, 57.08; H, 7.56; N, 15.01%.

*H–(Pro–Asn–Val–Orn(Z)–Leu)$_2$–$N_2H_3$·2HCl (**8**·2HCl).* Compound **7** (295 mg, 0.2 mmol) was treated with 0.1 M hydrogen chloride in formic acid (5 ml) as described for **2**·HCl; yield, 284 mg (98%); mp 215—217 °C; $[\alpha]_D^{22}$ −89.0° (*c* 0.2, MeOH); $R_f^1$ 0.02, $R_f^3$ 0.66.

Found: C, 54.41; H, 7.11; N, 15.21%. Calcd for $C_{66}H_{104}O_{16}N_{16}Cl_2$: C, 54.72; H, 7.24; N, 15.47%.

*cyclo(–(Pro–Asn–Val–Orn(Z)–Leu)$_2$–) (**9a**).* To a solution of **8**·2HCl (174 mg, 0.12 mmol) in DMF (2 ml) were added 2 M hydrogen chloride in EtOAc (0.24 ml) and isopentyl nitrite (0.017 ml, 0.12 mmol) at −60 °C. After 20 min, the reaction mixture was added to pyridine (60 ml) at 0 °C. After being stirred at 0 °C for 3 d, the solution was evaporated and the residue was dissolved in a mixture (120 ml) of MeOH–$H_2O$ (5:1, v/v). The solution was applied to columns (1.6×10 cm each) of Dowex 50 ($H^+$ form) and Dowex 1 ($OH^-$ form). The columns were washed with the same solvent (200 ml) and the effluent was evaporated to leave a white solid, which was collected by filtration with the aid of water. Because of the low yield, 17 mg (11%), and the contaminated by-products ($R_f^1$, 0.40 and 0.35) estimated to 50% of the crude product, further treatment of the crude product was not carried out. Cyclizations examined further two times gave similar results.

*Boc–Leu–Pro–OBzl (**11**).* Boc–Leu–OH (4.63 g, 20 mmol) and H–Pro–OBzl·HCl (4.83 g, 20 mmol) were coupled by mixed anhydride method as described for **3**. The reaction mixture was evaporated and the residue was dissolved in EtOAc. The solution was washed successively with 10% citric acid, 4% $NaHCO_3$, and water, dried over $Na_2SO_4$, and evaporated to leave an oil; yield, 7.61 g (91%); $R_f^1$ 0.89, $R_f^2$ 0.76.

*H–Leu–Pro–OBzl·HCl (**12**·HCl).* Compound **11** (7.61 g, 18 mmol) was treated with 0.1 M hydrogen chloride in formic acid as described for **2**·HCl; yield, 6.36 g (90%); mp 150—151 °C; $[\alpha]_D^{22}$ −67.8° (*c* 1, MeOH); $R_f^1$ 0.48, $R_f^2$ 0.04, $R_f^3$ 0.70.

Found: C, 60.52; H, 7.05; N, 8.01%. Calcd for $C_{18}H_{27}O_3N_2Cl$: C, 60.92; H, 7.67; N, 7.89%.

*Boc–Orn(Z)–Leu–Pro–OBzl (**13**).* This compound was prepared from Boc–Orn(Z)–OH (1.72 g, 4.7 mmol) and **12**·HCl (1.67 g, 4.7 mmol) as described for **11**; yield of an oil, 2.44 g (78%); $R_f^1$ 0.88, $R_f^2$ 0.58, $R_f^3$ 0.92.

*H–Orn(Z)–Leu–Pro–OBzl·HCl (**14**·HCl).* A solution of **13** (2.44 g, 3.7 mmol) in 0.1 M hydrogen chloride in formic acid was allowed to stand at room temperature for 30 min and evaporated to leave an oil, which was kept over KOH pellets under reduced pressure overnight and used for the next reaction without further treatment; yield, 2.21 g (100%); $R_f^1$ 0.71, $R_f^2$ 0.09, $R_f^3$ 0.75.

*Boc–Val–Orn(Z)–Leu–Pro–OBzl (**15**).* This compound was prepared from Boc–Val–OH (0.08 g, 3.7 mmol) and **14**·HCl (2.21 g, 3.7 mmol) as described for **11**; yield of an oil, 2.35 g (84%); $R_f^1$ 0.89, $R_f^2$ 0.54, $R_f^3$, 0.98.

*H–Val–Orn(Z)–Leu–Pro–OBzl·HCl (**16**·HCl).* Compound **15** (2.35 g, 3.1 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid, and the solution was allowed to stand at room temperature for 30 min and evaporated. The residue dissolved in a mixture (5 ml) of $CHCl_3$–MeOH–AcOH (95:5:1, v/v) was applied to a column (3.3×35 cm) of silica gel 60 (Merck) and the column was washed with the same solvent. The desired product was eluted with a mixture of $CHCl_3$–MeOH (9:1, v/v); yield of an oil, 1.98 g (92%); $R_f^1$ 0.36, $R_f^2$ 0.07.

*Boc–Asn–Val–Orn(Z)–Leu–Pro–OBzl (**17**).* Boc–Asn–ONp (0.99 g, 2.8 mmol) and **16**·HCl (1.98 g, 2.8 mmol) were treated as described for **1**. The crude product dissolved in MeOH (5 ml) was applied to a column (3×170 cm) of Sephadex LH-20 and eluted with MeOH. The fractions with the desired product were collected and evaporated. Addition of ether to the residue gave a solid which was recrystallized from MeOH–ether; yield, 1.91 g (77%); mp 186—188 °C; $[\alpha]_D^{22}$ −90.0° (*c* 1, MeOH); $R_f^1$ 0.75, $R_f^2$ 0.25, $R_f^3$ 0.95.

Found: C, 61.29; H, 7.52; N, 11.08%. Calcd for $C_{45}H_{65}O_{11}N_7$: C, 61.41; H, 7.45; N, 11.14%.

*H–Asn–Val–Orn(Z)–Leu–Pro–OBzl·HCl (**18**·HCl).* Compound **17** (409 mg, 0.46 mmol) was treated with 0.1 M hydrogen chloride in formic acid (5.5 ml) as described for **2**·HCl; yield, 376 mg (100%); mp 185—188 °C; $[\alpha]_D^{22}$ −72.2° (*c* 1, MeOH); $R_f^1$ 0.31, $R_f^3$ 0.72.

Found: C, 56.68; H, 7.02; N, 11.73%. Calcd for $C_{40}H_{58}O_9N_7Cl \cdot 3/2\ H_2O$: C, 56.96; H, 7.29; N, 11.62%.

*Boc–Asn–Val–Orn(Z)–Leu–Pro–$N_2H_3$ (**19**).* Compound **17** (491 mg, 0.6 mmol) was treated with hydrazine hydrate (1.16 ml, 24 mmol) as described for **5**; yield, 302 mg (68%); mp 176—178 °C; $[\alpha]_D^{22}$ −96.8° (*c* 0.5, MeOH); $R_f^1$ 0.43, $R_f^2$ 0.02, $R_f^3$ 0.85.

Found: C, 56.58; H, 7.74; N, 15.35%. Calcd for $C_{38}H_{61}O_{10}N_9$: C, 56.77; H, 7.65; N, 15.68%.

*Boc–(Asn–Val–Orn(Z)–Leu–Pro)$_2$–OBzl (**20**).* This compound was prepared from **19** (260 mg, 0.35 mmol) and **18**·HCl (264 mg, 0.35 mmol) as described for **6**; yield, 410 mg (76%); mp 218—220 °C; $[\alpha]_D^{22}$ −90.4° (*c* 0.5, MeOH); $R_f^1$ 0.58, $R_f^3$ 0.89.

Found: C, 60.12; H, 7.37; N, 12.58%. Calcd for

$C_{78}H_{114}O_{19}N_{14}$: C, 60.37; H, 7.41; N, 12.64%.

*Boc-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-$N_2H_3$ (**21**).* Compound **20** (372 mg, 0.24 mmol) was treated with hydrazine hydrate (0.58 ml, 12 mmol) as described for **4**. The crude product was recrystallized from EtOH; yield, 307 mg (87%); mp 201—203 °C; $[\alpha]_D^{22}$ −104° (*c* 0.2, MeOH); $R_f^1$ 0.21, $R_f^3$ 0.82.

Found: C, 57.07; H, 7.51; N, 15.06%. Calcd for $C_{71}H_{110}O_{18}N_{16}\cdot H_2O$: C, 57.09; H, 7.56; N, 15.00%.

*H-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-$N_2H_3$·2HCl (**22**·2HCl).* Compound **21** (138 mg, 0.094 mmol) was treated with 0.1 M hydrogen chloride in formic acid (2.3 ml) as described for **2**·HCl; yield, 133 mg (98%); mp 168—169 °C; $[\alpha]_D^{22}$ −68.0° (*c* 0.2, MeOH); $R_f^1$ 0.03, $R_f^3$ 0.69.

Found: C, 52.67; H, 6.91; N, 14.73%. Calcd for $C_{66}H_{104}O_{16}N_{16}Cl_2\cdot 3H_2O$: C, 52.76; H, 7.37; N, 14.91%.

cyclo(-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-) (**9a**). Cyclization of **22**·2HCl (123 mg, 0.085 mmol) was examined by the similar manner to that of **8**·2HCl. The crude product (43 mg, 38%) eluted from columns of Dowex 50 and Dowex 1 showed one major spot ($R_f^1$ 0.66) and two minor spots ($R_f^1$ 0.40 and 0.55) on TLC. All the crude product was dissolved in MeOH (5 ml) and applied to a column (3×170 cm) of Sephadex LH-20, and eluted with MeOH. The fractions containing the desired product were collected and evaporated to leave a white solid, which was collected by filtration with the aid of MeOH–ether; yield of semi-purified product, 34 mg (30%). By-product with $R_f^1$ 0.40 was removed by this treatment but that with $R_f^1$ 0.55 was not. Repeated recrystallization did not give satisfactory results.

*Boc-Val-Orn(Z)-Leu-Pro-OH (**23**).* To a solution of Boc-Val-Orn(Z)-Leu-$N_2H_3$[10] (2.37 g, 4 mmol) in DMF (10 ml) were added 2 M hydrogen chloride in EtOAc (6 ml) and isopentyl nitrite (0.56 ml, 4 mmol) at −60 °C. After being stirred at −20 °C for 10 min, the solution was cooled again to −60 °C and neutralized with TEA (1.68 ml, 12 mmol). To the solution was added a chilled solution of H-Pro-OH (0.59 g, 6 mmol) and TEA (0.84 ml, 6 mmol) in $H_2O$ (4 ml). The reaction mixture was stirred at 0 °C for 3 days and evaporated. To the residue were added 3% $NH_4OH$ and EtOAc. The aqueous layer was washed with EtOAc and acidified with 10% citric acid, and the oily product separated was extracted with EtOAc. The organic layer was washed with water, dried ($Na_2SO_4$), and evaporated. The residue was solidified by successive addition of ether and petroleum ether, and the crude product was dissolved in a mixture of $CHCl_3$–MeOH (5:1, v/v), applied to a column (3.3×35 cm) of silica gel 60 (Merck), and eluted with the same solvent. The fractions containing the desired product were collected and evaporated. Addition of a small amount of EtOAc to the residue gave colorless crystals; yield, 2.03 g (73%); mp 158—160 °C; $[\alpha]_D^{22}$ −62.2° (*c* 1, MeOH); $R_f^1$ 0.44, $R_f^2$ 0.24, $R_f^3$ 0.83.

Found: C, 58.27; H, 7.51; N, 10.00%. Calcd for $C_{34}H_{53}O_9N_5\cdot 3/2\ H_2O$: C, 58.10; H, 8.03; N, 9.96%.

*H-Val-Orn(Z)-Leu-Pro-OH·HCl (**24**·HCl).* Compound **23** (946 mg, 1.4 mmol) was treated with 0.1 M hydrogen chloride in formic acid (15.4 ml) as described for **2**·HCl. The product was used for the next reaction without further treatment; yield, 857 mg (100%); $R_f^3$ 0.71.

*Boc-Asn-Val-Orn(Z)-Leu-Pro-OH (**25a**).* To a chilled solution of **24**·HCl (857 mg, 1.4 mmol) and TEA (0.39 ml, 2.8 mmol) in DMF (10 ml) was added Boc-Asn-ONp (742 mg, 2.1 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature overnight and evaporated. To the residue were added 10% citric acid and EtOAc. When the organic layer was being washed with water, crystallization of the product occurred. So, the crystals were filtered and dried *in vacuo* over $P_2O_5$. The crude product dissolved in MeOH (5 ml) was applied to a column (3×170 cm) of Sephadex LH-20 and eluted with MeOH. The fractions containing the desired product were collected and evaporated. The residue was crystallized by successive addition of MeOH and ether; yield, 867 mg (78%); mp 172—173 °C; $[\alpha]_D^{22}$ −78.0° (*c* 1, MeOH); $R_f^1$ 0.61, $R_f^2$ 0.03, $R_f^3$ 0.80.

Found: C, 56.65; H, 7.47; N, 12.16%. Calcd for $C_{38}H_{59}O_{11}N_7\cdot H_2O$: C, 56.49; H, 7.61; N, 12.14%.

*H-Asn-Val-Orn(Z)-Leu-Pro-OH·HCl (**26a**·HCl).* Compound **25a** (158 mg, 0.2 mmol) was treated with 0.1 M hydrogen chloride in formic acid (3 ml) as described for **2**·HCl. The product was used for the next reaction without further treatment; yield, 145 mg (100%); $R_f^3$ 0.61.

*Boc-Asn-Val-Orn(Z)-Leu-Pro-ONSu (**27a**).* To a chilled solution of **25a** (237 mg, 0.3 mmol) and HONSu (59 mg, 0.6 mmol) in a mixture of DMF (2 ml) and $CH_2Cl_2$ (2 ml) was added EDC·HCl (115 mg, 0.6 mmol), and the reaction mixture was stirred at 0 °C overnight and evaporated. The residue was triturated with the aid of chilled water, filtered, and dried *in vacuo* over $P_2O_5$. The product was used for the next reaction without further treatment; yield, 263 mg (99%); $R_f^1$ 0.58, $R_f^2$ 0.11, $R_f^3$ 0.81.

*Boc-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-OH (**28a**).* To a chilled solution of **26a**·HCl (145 mg, 0.2 mmol) and TEA (0.056 ml, 0.4 mmol) in DMF (2 ml) was added **27a** (222 mg, 0.25 mmol), and the mixture was stirred at 0 °C for 2 days and evaporated. The residue was solidified by addition of 10% citric acid, filtered, washed with water, and dried. The crude product was purified by column chromatography with Sephadex LH-20 as described for **6**; yield, 276 mg (94%); mp 210—211 °C; $[\alpha]_D^{22}$ −94.0° (*c* 0.5, MeOH); $R_f^1$ 0.14, $R_f^3$ 0.83.

Found: C, 57.25; H, 7.46; N, 13.15%. Calcd for $C_{71}H_{108}O_{19}N_{14}\cdot 3/2\ H_2O$: C, 57.28; H, 7.51; N, 13.17%.

*Boc-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-ONSu (**29a**).* Compound **28a** (248 mg, 0.17 mmol) and HONSu (39 mg, 0.34 mmol) was treated as described for **27a**; yield, 265 mg (100 %); $R_f^1$ 0.45, $R_f^3$ 0.80.

*H-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-ONSu·TFA (**30a**·TFA).* Compound **29a** (265 mg, 0.34 mmol) was dissolved in TFA (5 ml), and the solution was allowed to stand at 0 °C for 1 h and evaporated. The residue was solidified by addition of ether, filtered, dried *in vacuo* over KOH. The product was used for the next reaction without further treatment; yield, 243 mg (91%); $R_f^1$ 0.35, $R_f^3$ 0.74.

cyclo(-(Asn-Val-Orn(Z)-Leu-Pro)$_2$-) (**9a**). A solution of **30a**·TFA (243 mg, 0.16 mmol) in DMF (8 ml) was added dropwise to pyridine (80 ml) at room temperature, and the mixture was stirred overnight and evaporated. The residue was dissolved in a mixture (60 ml) of MeOH–$H_2O$ (5:1, v/v), and the solution was applied to columns (1.6×10 cm each) of Dowex 50 ($H^+$ form) and Dowex 1 ($OH^-$ form). The columns was washed with the same solvent (150 ml.) and the combined effluent was evaporated to leave a white solid, which was collected with the aid of water. The crude product was purified by column chromatography with Sephadex LH-20 as described for **6**. The product was recrystallized from MeOH–ether; yield, 168 mg (81%); mp 241—243 °C; $[\alpha]_D^{22}$ −102° (*c* 0.5, MeOH); $R_f^1$ 0.66, $R_f^2$ 0.08, $R_f^3$ 0.83. Amino acid ratios in acid hydrolyzate: Asp (1.00), Val (0.95), Orn (1.00), Leu (1.00), Pro (1.06).

Found: C, 58.27; H, 7.44; N, 14.28%. Calcd for $C_{66}H_{98}O_{16}N_{14}\cdot H_2O$: C, 58.22; H, 7.40; N, 14.40%.

cyclo(-(Asn-Val-Orn-Leu-Pro)$_2$-)·2HCl (**10a**·2HCl). Compound **9a** (67 mg, 0.05 mmol) dissolved in 0.05 M hy-

drogen chloride in MeOH (4 ml) was hydrogenated with paradium black as a catalyst. After removal of the catalyst by filtration, the filtrate was evaporated to leave a solid, which was recrystallized from MeOH–ether; yield, 49 mg (86%); mp 228—230 °C; $[\alpha]_D^{22}$ −98° (*c* 0.5, MeOH); $R_f^3$ 0.38. Amino acid ratios in acid hydrolyzate: Asp (0.98), Val (0.94), Orn (1.00), Leu (1.00), Pro (1.05).

Found: C, 49.48; H, 7.56; N, 15.84%. Calcd for $C_{50}H_{88}O_{12}N_{14}Cl_2 \cdot 4H_2O$: C, 49.21; H, 7.93; N, 16.07%.

*Boc–D-Ala–Val–Orn(Z)–Leu–Pro–OH (**25b**).* This compound was prepared from Boc–D-Ala–ONSu (401 mg, 1.4 mmol) and **24**·HCl (857 mg, 1.4 mmol) as described for **25a**; yield, 544 mg (52%); mp 174—175 °C; $[\alpha]_D^{22}$ −52.0° (*c* 0.5, MeOH); $R_f^1$ 0.31, $R_f^2$ 0.09, $R_f^3$ 0.83.

Found: C, 58.84; H, 7.73; N, 11.09%. Calcd for $C_{37}H_{58}O_{10}N_6 \cdot 1/2\ H_2O$: C, 58.78; H, 7.87; N, 11.12%.

*H–D-Ala–Val–Orn(Z)–Leu–Pro–OH·HCl (**26b**·HCl).* Compound **25b** (0.185 mg, 0.25 mmol) was treated with 0.1 M hydrogen chloride in formic acid (4 ml) as described for **2**·HCl. The product was used for the next reaction without further treatment; yield, 177 mg (100%); $R_f^3$ 0.68.

*Boc–D-Ala–Val–Orn(Z)–Leu–Pro–ONSu (**27b**).* This was prepared from **25b** (299 mg, 0.4 mmol) and HONSu (69 mg, 0.6 mmol) as described for **27a**. The product was used for the next reaction without further treatment; yield, 214 mg (63%); $R_f^1$ 0.78, $R_f^2$ 0.30.

*Boc–(D-Ala–Val–Orn(Z)–Leu–Pro)$_2$–OH (**28b**).* This was prepared from **26b**·HCl (150 mg, 0.22 mmol) and **27b** (186 mg, 0.22 mmol) as described for **28a**; yield, 162 mg (54%); mp 143—145 °C; $[\alpha]_D^{22}$ −52.0° (*c* 1, MeOH); $R_f^1$ 0.48, $R_f^2$ 0.07, $R_f^3$ 0.83.

Found: C, 59.77; H, 7.74; N, 12.31%. Calcd for $C_{69}H_{106}O_{17}N_{12} \cdot 1/2\ H_2O$: C, 59.85; H, 7.79; N, 12.14%.

*Boc–(D-Ala–Val–Orn(Z)–Leu–Pro)$_2$–ONSu (**29b**).* This was prepared from **28b** (138 mg, 0.1 mmol) and HONSu (23 mg, 0.2 mmol) as described for **27a**. The product was used for the next reaction without further treatment; yield, 134 mg (91%); $R_f^1$ 0.76, $R_f^2$ 0.29.

*H–(D-Ala–Val–Orn(Z)–Leu–Pro)$_2$–ONSu·TFA (**30b**·TFA).* Compound **29b** (134 mg, 0.091 mmol) was treated with TFA (3 ml) as described for **30a**. The product was used for the next reaction without further treatment; yield, 136 mg (100 %); $R_f^1$ 0.54, $R_f^2$ 0.13.

cyclo(–(D-Ala–Val–Orn(Z)–Leu–Pro)$_2$–) (**9b**). This was prepared from **30b**·TFA (136 mg, 0.091 mmol) as described for **9a**; yield; 74 mg (65%); mp >300 °C; $[\alpha]_D^{22}$ −44.9° (*c* 0.5, MeOH); $R_f^1$ 0.66, $R_f^2$ 0.13, $R_f^3$ 0.85. Amino acid ratios in acid hydrolyzate: Ala (1.07), Val (1.04), Orn (1.06), Leu (1.00), Pro (1.11).

Found: C, 60.55; H, 7.68; N, 13.13%. Calcd for $C_{64}H_{96}O_{14}N_{12} \cdot H_2O$: C, 60.26; H, 7.74; N, 13.18%.

cyclo(–(D-Ala–Val–Orn–Leu–Pro)$_2$–)·2HCl (**10b**·2HCl). Compound **9b** (50 mg, 0.04 mmol) was hydrogenated as described for **10a**·2HCl; yield, 33 mg (78%); mp 261—263 °C; $[\alpha]_D^{22}$ −36.1° (*c* 0.5, MeOH); $R_f^3$ 0.44. Amino acid ratios in acid hydrolyzate: Ala (1.00), Val (0.95), Orn (1.00), Leu (1.00), Pro (1.05).

Found: C, 52.11; H, 8.04; N, 15.04%. Calcd for $C_{48}H_{86}O_{10}N_{12}Cl_2 \cdot 2H_2O$: C, 52.49; H, 8.26; N, 15.30%.

*Bis(Dnp) Derivatives of **10a** and **10b** (**31a** and **31b**).* A small amount of **10a** and **10b** were treated with $N_2$ph–F as usual way[10] and purified by silica-gel (Merck) column chromatography; **31a**: $R_f^1$ 0.63, $R_f^2$ 0.08, $R_f^3$ 0.78; **31b**: $R_f^1$ 0.68, $R_f^2$ 0.22, $R_f^3$ 0.82.

*Boc–Asn–Val–pNA (**32a**).* Boc–Asn–ONp (706 mg, 2 mmol) and H–Val–pNA (476 mg, 2 mmol) were treated as described for **1**. The crude product was recrystallized from EtOH; yield, 666 mg (74%); mp 225—227 °C; $[\alpha]_D^{22}$ −121.0° (*c* 0.2, MeOH); $R_f^1$ 0.63, $R_f^2$ 0.16, $R_f^3$ 0.92.

Found: C, 52.89; H, 6.36; N, 15.32%. Calcd for $C_{20}H_{29}O_7N_5$: C, 53.20; H, 6.47; N, 15.51%.

*H–Asn–Val–pNA·HCl (**33a**·HCl).* Compound **32a** (451 mg, 1 mmol) was treated with 0.1 M hydrogen chloride in formic acid (12 ml) as described for **2**·HCl; yield, 413 mg (100%); mp 160—162 °C; $[\alpha]_D^{22}$ −18.4° (*c* 1, MeOH); $R_f^1$ 0.16, $R_f^3$ 0.73.

Found: C, 45.26; H, 5.58; N, 17.56%. Calcd for $C_{15}H_{22}O_5N_5Cl \cdot 1/2\ H_2O$: C, 45.39; H, 5.84; N, 17.65%.

*Boc–Leu–Pro–OH (**34**).* Compound **11** (1.38 g, 3.3 mmol) dissolved in MeOH (10 ml) was hydrogenated with paradium black as a catalyst. After removal of the catalyst by filtration, the filtrate was evaporated to a foam, which was used for the next reaction without further treatment; yield, 1.08 g (100%); $R_f^1$ 0.69, $R_f^2$ 0.47, $R_f^3$ 0.67.

*Boc–Leu–Pro–Asn–Val–pNA (**35a**).* To a chilled solution of **33a**·HCl (116 mg, 0.3 mmol) in $CHCl_3$ (3 ml) were added TEA (0.042 ml, 0.3 mmol), **34** (99 mg, 0.3 mmol), and EDC·HCl (58 mg, 0.3 mmol). The reaction mixture was treated as described for **11**, and the crude product obtained as a solid was purified by LH-20 column chromatography as described for **17**; yield, 75 mg (38%); mp 128—130 °C; $[\alpha]_D^{22}$ −130.0° (*c* 0.5, MeOH); $R_f^1$ 0.67, $R_f^2$ 0.15, $R_f^3$ 0.81.

Found: C, 55.70; H, 7.13; N, 14.77%. Calcd for $C_{31}H_{47}O_9N_7 \cdot 1/2\ H_2O$: C, 55.51; H, 7.21; N, 14.62%.

*H–Leu–Pro–Asn–Val–pNA·HCl (**36a**·HCl).* Compound **35a** (47 mg, 0.07 mmol) was treated with 0.1 M hydrogen chloride in formic acid (0.8 ml) as described for **2**·HCl, and the residue was used for the next reaction without further treatment; yield was quantitative; $R_f^1$ 0.18, $R_f^3$ 0.74.

*Dnp–Leu–Pro–Asn–Val–pNA (**37a**).* To a solution of **36a**·HCl (42 mg, 0.07 mmol) and TEA (0.029 ml, 0.21 mmol) in DMF (2 ml) was added $N_2$ph–F (26 mg, 0.14 mmol), and the reaction mixture was stirred at room temperature for 3 h and evaporated. The residue dissolved in $CHCl_3$ (5 ml) was applied to a column (1.8×20 cm) of silica gel 60 (Merck), and the column was washed with $CHCl_3$. The fractions containing the desired product eluted with a mixture of $CHCl_3$–MeOH (19:1, v/v) were evaporated to leave a solid, which was recrystallized from EtOAc–ether; yield, 42 mg (83%); mp 145—148 °C; $[\alpha]_D^{22}$ −90.2° (*c* 1, MeOH); $R_f^1$ 0.67, $R_f^2$ 0.13, $R_f^3$ 0.79.

Found: C, 52.44; H, 5.72; N, 17.11%. Calcd for $C_{32}H_{41}O_{11}N_9$: C, 52.82; H, 5.68; N, 17.32%.

*Boc–D-Ala–Val–pNA (**32b**).* This compound was prepared from Boc–D-Ala–OH (189 mg, 1 mmol) and H–Val–pNA (238 mg, 1 mmol) as described for **35a**. The crude product was recrystallized from EtOH–ether; yield, 306 mg (76%); mp 160 °C; $[\alpha]_D^{22}$ −47.0° (*c* 1, MeOH); $R_f^1$ 0.82, $R_f^2$ 0.45, $R_f^3$ 0.91.

Found: C, 55.77; H, 6.98; N, 13.68%. Calcd for $C_{19}H_{28}O_6N_4$: C, 55.87; H, 6.91; N, 13.72%.

*H–D-Ala–Val–pNA·HCl (**33b**·HCl).* Compound **32b** (245 mg, 0.6 mmol) was treated with 0.1 M hydrogen chloride in formic acid (7 ml) as described for **2**·HCl; yield, 209 mg (100%); mp 170—173 °C; $[\alpha]_D^{22}$ −42.2° (*c* 1, MeOH); $R_f^1$ 0.33, $R_f^2$ 0.02, $R_f^3$ 0.75.

Found: C, 48.01; H, 6.03; N, 16.19%. Calcd for $C_{14}H_{21}O_4N_4Cl \cdot 1/4\ H_2O$: C, 48.14; H, 6.20; N, 16.04%.

*Boc–Leu–Pro–D-Ala–Val–pNA (**35b**).* This compound was prepared from **33b**·HCl (209 mg, 0.6 mmol) and **34** (197 mg, 0.6 mmol) as described for **35a**; yield, 209 mg (56%); mp 140—142 °C; $[\alpha]_D^{22}$ −20.6° (*c* 1, MeOH); $R_f^1$ 0.79, $R_f^2$ 0.38, $R_f^3$ 0.86.

Found: C, 57.36; H, 7.37; N, 13.09%. Calcd for $C_{30}H_{48}O_8N_6 \cdot 1/2\ H_2O$: C, 57.40; H, 7.55; N, 13.39%.

*H–Leu–Pro–D-Ala–Val–pNA·HCl (**36b**·HCl).* Compound **35b** (62 mg, 0.1 mmol) was treated with 0.1 M hydrogen chloride in formic acid (1.5 ml) as described for **2**·HCl, and the residue was used for the next reaction without further treatment; yield was quantitative; $R_f^1$ 0.27, $R_f^3$ 0.69.

*Dnp–Leu–Pro–D-Ala–Val–pNA (**37b**).* This compound was prepared from **36b**·HCl (56 mg. 0.1 mmol) as described for **37a**; yield, 59 mg (86%); mp 231 °C; $[\alpha]_D^{23}$ −38.4° (*c* 1, MeOH); $R_f^1$ 0.77, $R_f^2$ 0.36, $R_f^3$ 0.82.

Found: C, 54.26; H, 5.78; N, 16.18%. Calcd for $C_{31}H_{40}O_{10}N_8$: C, 54.37; H, 5.89; N, 16.39%.

*Paper Electrophoresis.* Electrophoresis was carried out with Whatman No. 1 paper and a solvent system of HCOOH–AcOH–MeOH–$H_2O$ (1:3:6:10, v/v, pH 1.8) for 2 h at 500 V/30 cm. Each of the analogs (**10a** and **10b**) showed single spot and their mobilities were as follows: **10a**, 7.3 cm; **10b**, 7.5 cm; GS, 7.1 cm.

*CD and ORD Measurements.* These spectra were recorded on a JASCO J-20 spectropolarimeter in MeOH solution at room temperature (23±2 °C).

*Microbiological Assays.* The minimum concentration of the compounds necessary for the complete inhibition of growth of several microorganisms was determined by a dilution method using a nutrient agar. Natural GS used as a reference showed complete inhibition at 3.13 and 6.25 μg/ml on *B. Subtilis* and *S. Aureus*, respectively, and was inactive on *E. Coli* at 100 μg/ml.

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## References

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2) The abbreviations used in this paper are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Boc, *t*-butoxycarbonyl; Z, benzyloxycarbonyl; Dnp, 2,4-dinitrophenyl; pNA, *p*-nitroanilide; OBzl, benzyl ester; ONp, *p*-nitrophenyl ester; HONSu, *N*-hydroxysuccinimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; $N_2$ph-F, 1-fluoro-2,4-dinitrobenzene;TEA, triethylamine; DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran, MA, mixed anhydride; TLC, thin-layer chromatography; GS, gramicidin S. Symbols of optically active amino acids denote the L-configuration unless otherwise stated.

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