

Design and Synthesis of Gramicidin S Analogs with High Antibiotic Activity

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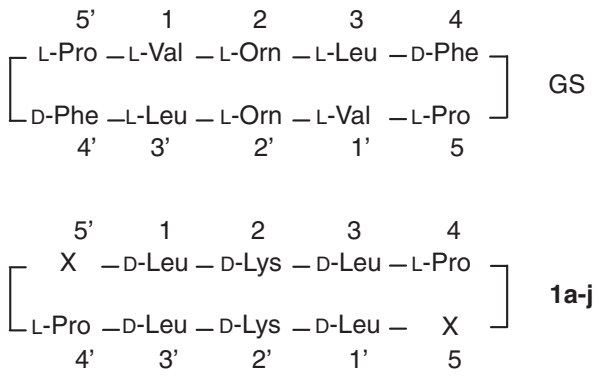
Based on the β-turn preference of tetrapeptide sequences as analyzed by CD spectra of their chromophoric derivatives, 10 analogs of gramicidin S (GS) were designed and synthesized with general formula *cyclo*(–D-Leu$^{1,1'}$–D-Lys$^{2,2'}$–D-Leu$^{3,3'}$–L-Pro$^{4,4'}$–X$^{5,5'}$–)$_2$, where X is L-Asn, L-Ala, L-Leu, L-Phe, L-cyclohexylalanine, Gly, D-Ala, D-Leu, D-Phe, or D-cyclohexylalanine. Several analogs with high hydrophobicity showed antibiotic activity as strong as GS. CD spectra of the analogs with D-amino acid or Gly at the X position and their dinitrophenyl derivatives suggested that they have β-sheet conformation that is antipodal to that of GS.

N-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp-tetrapeptide-pNA)[1] exhibit characteristic CD spectra when they take on β-turn conformation. Exciton coupling of electric transition dipoles of the two chromophores is thought to be the origin of the CD pattern observed, and intensities of the band near 310 and 350 mm were shown to reflect well the β-turn preferences of the parent tetrapeptides.[2,3] In a series of studies on the β-turn of peptides,[2–13] CD spectral analysis of Dnp-tetrapeptide-pNA showed that a) L–D–L–L (or D–L–D–D) sequence preferred β-turn, that b) only when Pro was at the 2nd position L–D–D–L (or D–L–L–D) sequence could also take β-turn, and that c) each amino acid (AA) in a turn-preferring sequence could be replaced with Gly with a little decrease of turn preference of its original sequence. Especially, it is of interest that tetrapeptide sequence with L-Pro at 2nd position and D-amino acid at 1st and 4th has the highest potential to take on β-turn conformation.

Based on the results mentioned above, we designed a series of compounds as shown in Figure 1 as the analogs of an antibiotic gramicidin S (GS). GS, *cyclo*(–Val$^{1,1'}$–Orn$^{2,2'}$–Leu$^{3,3'}$–D-Phe$^{4,4'}$–Pro$^{5,5'}$–)$_2$, is a typical amphiphilic peptide known to take on intramolecular antiparallel β-sheet conformation with two β-turns at D-Phe–Pro sequences (Figure 2). The characteristic feature of this conformation is the orientation of side chains in which charged Orn side chains are on one side and the hydrophobic Val and Leu side chains are on the other side of the molecule. From the study of many synthetic analogs in which these three residues were substituted, it was concluded that this orientation of side chains was essential for the activity of GS.[14]

In the series of synthetic analogs (Figure 1), we chose AA with increasing hydrophobicity as X, since we considered that not only the conformation but the hydrophobicity of the whole molecule was important for the activity of GS. If D-Leu–L-Pro–X–D-Leu sequence could take β-turn conformation in the



a (X=L-Asn), **b** (L-Ala), **c** (L-Leu), **d** (L-Phe), **e** (L-Cha), **f** (Gly), **g** (D-Ala), **h** (D-Leu), **i** (D-Phe), **j** (D-Cha)

Figure 1. Synthetic analogs of gramicidin S.

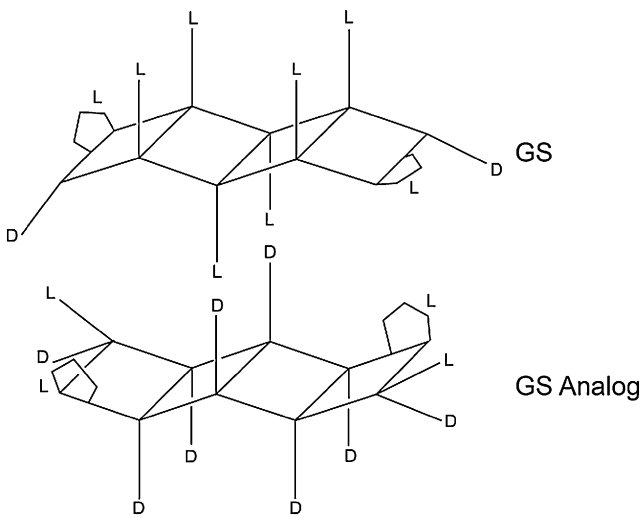


Figure 2. Models of β-sheet conformation of GS and its analogs.

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cyclic decapeptide structure, the analogs would take β-sheet conformation which is almost antipodal to that of GS as shown in Figure 2, and consequently the analogs would show antibiotic activity.

Results and Discussion

All the analogs were synthesized by conventional solution phase method according to schemes shown in Figures 3 or 4. For the synthesis of **1a**, a pentapeptide with Pro at the C-terminus was chosen as a precursor in a cyclization reaction, because many GS analogs had been synthesized satisfactorily by a similar strategy.[14,15] Boc–Asn–D-Leu–D-Lys(Z(Cl))–D-Leu–Pro–OH (**9a**) was prepared by active ester coupling of Boc–Asn–ONp and H–D-Leu–D-Lys(Z(Cl))–D-Leu–Pro–OH (**8**) which was prepared by stepwise chain elongation, and converted to its active ester **10a**. Cyclization of H–Asn–D-Leu–D-Lys(Z(Cl))–D-Leu–Pro–OSu (**11a**) derived from **10a** by acid treatment was carried out in anhydrous pyridine at the final concentration of 3 mM. After usual work-up,[8] a mixture of the crude cyclized peptides was applied to a column of Sephadex LH-20 (Figure 5). The product obtained from the major peak was determined to be cyclic dimer by FAB-MS measurement (M^+, 1219) after hydrogenolysis that afforded **1a**. It is interesting that cyclization of pentapeptide active ester afforded much dimeric products and almost no cyclic monomer, since there are few papers which reported the absence of cyclic monomerization of pentapeptides.[14,16] Analogs **1b**–**1d**, **1f**, and **1g** were synthesized by a similar procedure as described for **1a**. However, cyclization of pentapeptide precursors with X = Gly and D-Ala afforded much monomeric product as shown in Table 1 and Figure 5. This result suggests that Gly and D-Ala analogs are suitable for compact folded conformation. Therefore other analogs were synthesized by using decapeptide precursors as shown in Figure 4. By this strategy, D-Ala analog **1g** was obtained in a satisfactory yield (Figure 5). Table 1 summarizes the results of cyclization reactions. It is interesting that the analogs with L-AA afforded polymeric products, whereas those with Gly or D-AA afforded cyclic monomer only.

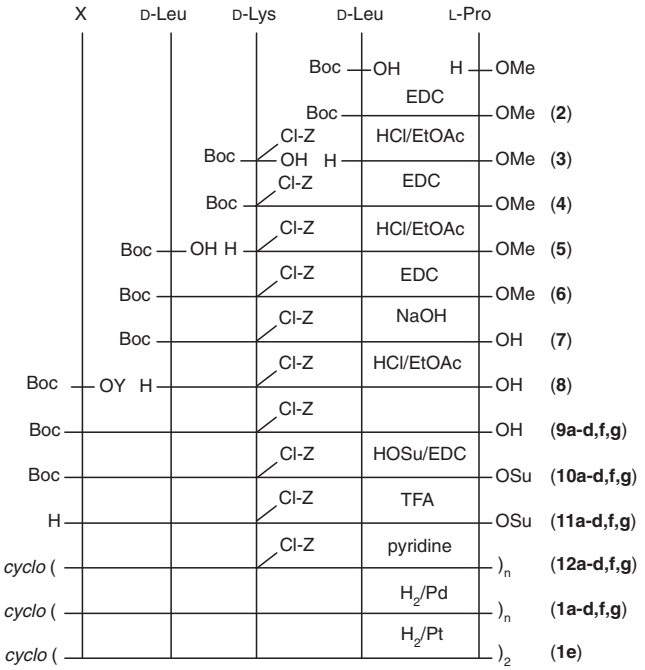

Figure 3. Synthetic scheme of peptides with pentapeptide precursors.

Minimum concentrations of the compounds necessary for the complete inhibition of growth of several microorganisms were determined to compare the antibiotic activity of the synthesized analogs to that of GS (Table 2). The activity increased as the hydrophobicity of X residues increased and the highest activity was observed when X was D-Cha, and was a little higher than that of natural GS against *Bacillus subtilis*. When X had the same hydrophobicity, D-AA analogs showed higher activity. Good correlation was observed between activity and the retention time (RT) on HPLC suggesting that RT could be a good index of the activity of analogs. For example **1h** (X = D-Leu) showed longer RT and higher activity than **1c** (X = L-Leu), although they had apparently the same hydrophobicity.

In many GS analogs, good correlation was observed between their CD spectra and activities.[17,18] CD spectra of the synthetic analogs are summarized in Figure 6 together with that of GS.

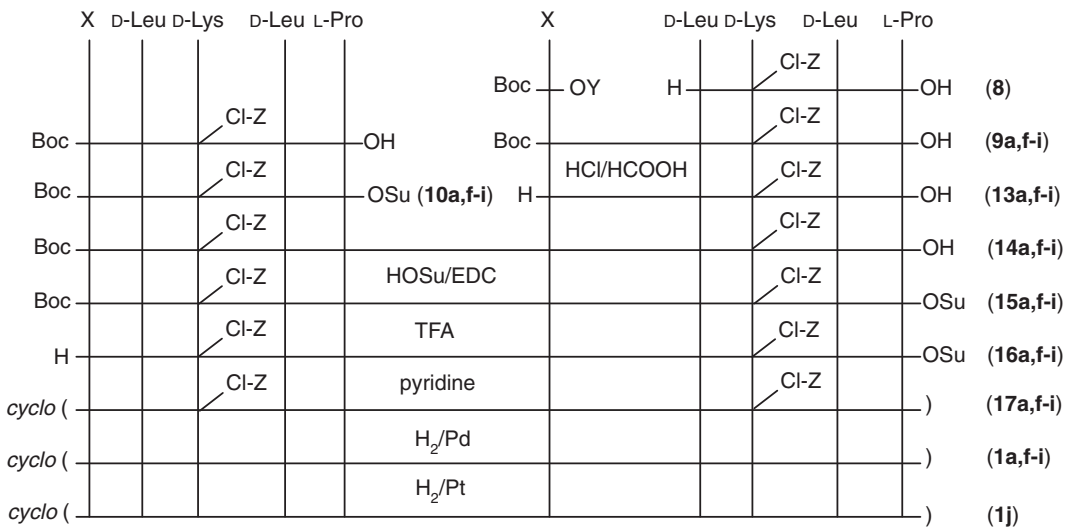

Figure 4. Synthetic scheme of peptides with decapeptide precursors.

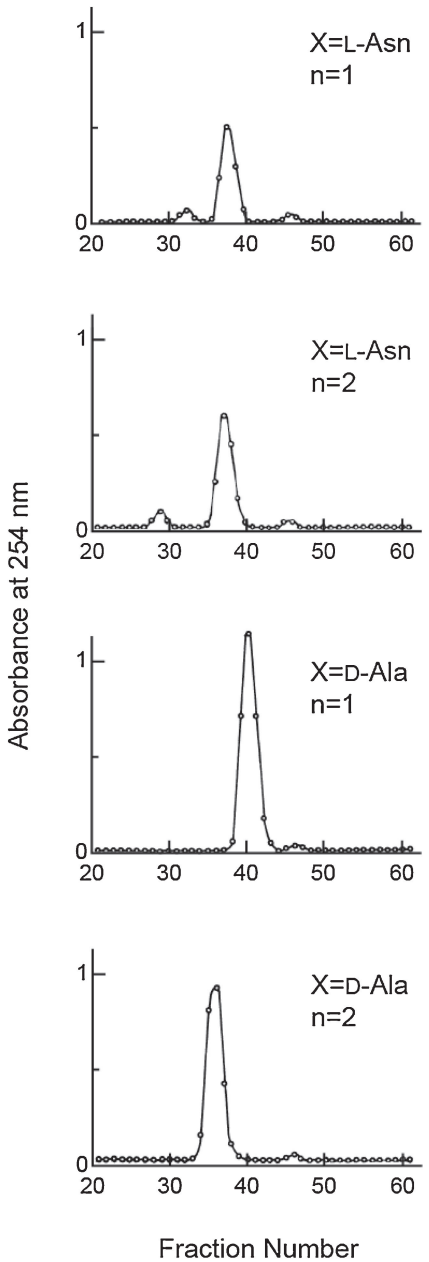

Figure 5. Elution profile of crude cyclized peptides of H–(D-Leu–D-Lys–D-Leu–L-Pro–X)$_n$–OSu from Sephadex LH-20 column. Each 17 mL fraction was collected.

Analogs with D-AA and Gly showed antipodal CD spectra to that of GS suggesting that these analogs had the mirror image conformation of GS-like β-sheet as expected in Figure 2. Difference between D-Phe analog and others may reflect the presence of aromatic amino acid, whereas the analogs with L-AA showed quite different CD patterns with weak intensity. As a consequence, we could not conclude that the analogs with L-AA took on a particular conformation from their CD spectra.

It was reported that the bis-Dnp derivatives of GS analogs with GS-like β-sheet conformation showed large CD bands above 250 nm due to the interaction of two Dnp chromophores.[17] Therefore we prepared bis-Dnp derivatives of the L-Ala (**18b**), L-Leu (**18c**), L-Cha (**18e**), Gly (**18f**), D-Ala (**18g**), and D-Leu (**18h**) analogs. Derivatives of the analogs **18f–18h** with D-AA and Gly at the X position showed large CD bands above 250 nm due to the interaction of two Dnp chromophores suggesting that they had β-sheet conformation (Figure 7b). The Cotton effects are even larger than that of GS. The difference of CD profile may be due to the presence of aromatic amino acid in GS derivative, since similar change was observed between GS and its saturated analogs [D-Cha$^{4,4'}$]GS.[17] The Dnp derivatization might not affect the backbone conformation of the analogs, since CD spectra at the shorter wavelength (<250 nm) did not change so significantly (Figure 6d). On the other hand, derivatives of the analogs **18b**, **18c**, and **18e** with L-AA at the X position showed weaker Cotton effects than that of GS derivative suggesting that they did not take on as stable β-sheet conformation as GS derivative did (Figure 7a). In addition, the back bone conformation of the analogs may somewhat affected by Dnp derivatization as shown in the CD spectra change at the shorter wavelength (Figure 6c).

Based on the β-turn preference of tetrapeptide sequences, we designed two series of analogs of GS in which D–L–D–D and D–L–L–D sequence would be located at the β-turn position. In the case of the analogs with D–L–D–D sequence, the analogs

Table 1. Cyclization of H–[X–D-Leu–D-Lys(Z(Cl))–D-Leu–L-Pro]$_n$–OSu

Precursor		Yield of crude product/%	Isolation yield after gel filtration/%		
X	n		$n=1$	$n=2$	$n \geqq 3$
L-Asn	1	79	0	58	5
L-Ala	1	82	0	50	6
L-Leu	1	76	0	34	12
L-Phe	1	89	0	57	14
Gly	1	92	68	0	0
D-Ala	1	91	79	0	0
L-Asn	2	76	—	57	6
Gly	2	92	—	84	0
D-Ala	2	90	—	77	0
D-Leu	2	92	—	88	0
D-Phe	2	93	—	82	0

Table 2. Antibiotic Activity and Physicochemical Properties of Synthetic Peptides

Peptide	MIC/μg mL^{-1}		Retention time /min
	Bacillus subtilis	*Staphylococcus aureus*	
1a (L-Asn)	>100	>100	5.2
1b (L-Ala)	>100	>100	6.7
1c (L-Leu)	25	100	11.7
1d (L-Phe)	3.13	3.13	13.7
1e (L-Cha)	1.56–3.13	1.56–3.13	19.8
1f (Gly)	25	>100	8.0
1g (D-Ala)	25	>100	10.1
1h (D-Leu)	6.25	25	17.3
1i (D-Phe)	1.56–3.13	3.13–6.25	19.9
1j (D-Cha)	0.78–1.56	3.13–6.25	23.2
GS	1.56–3.13	1.56–3.13	23.3

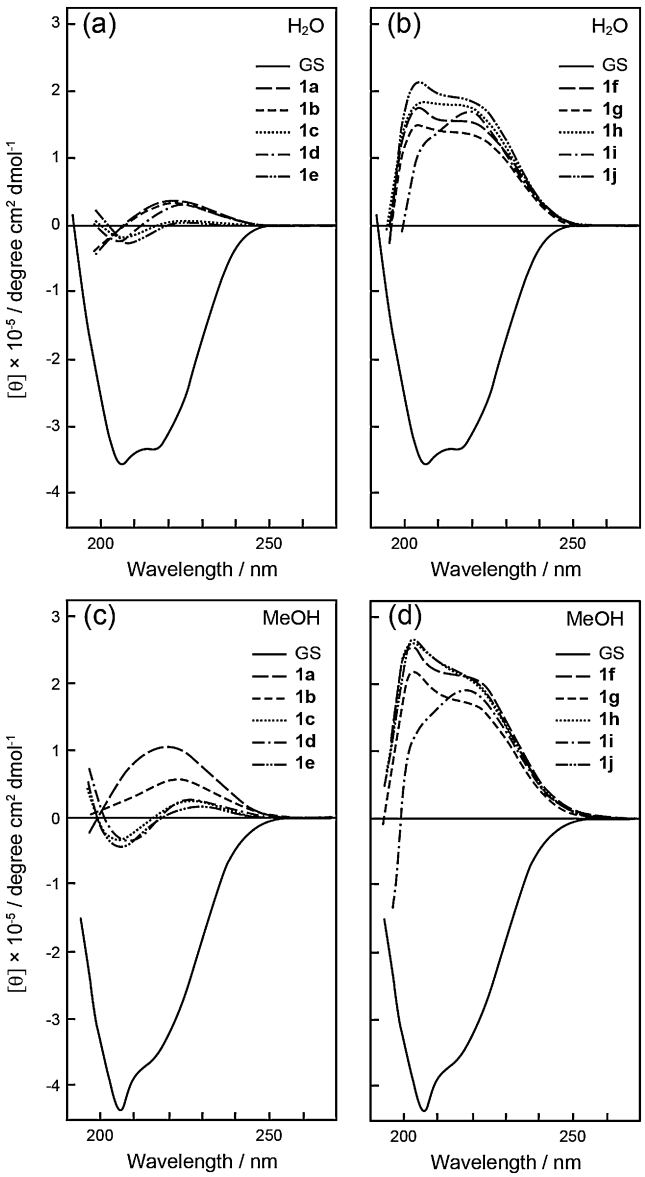

Figure 6. CD spectra of GS and its analogs measured in H_2O and in MeOH solutions.

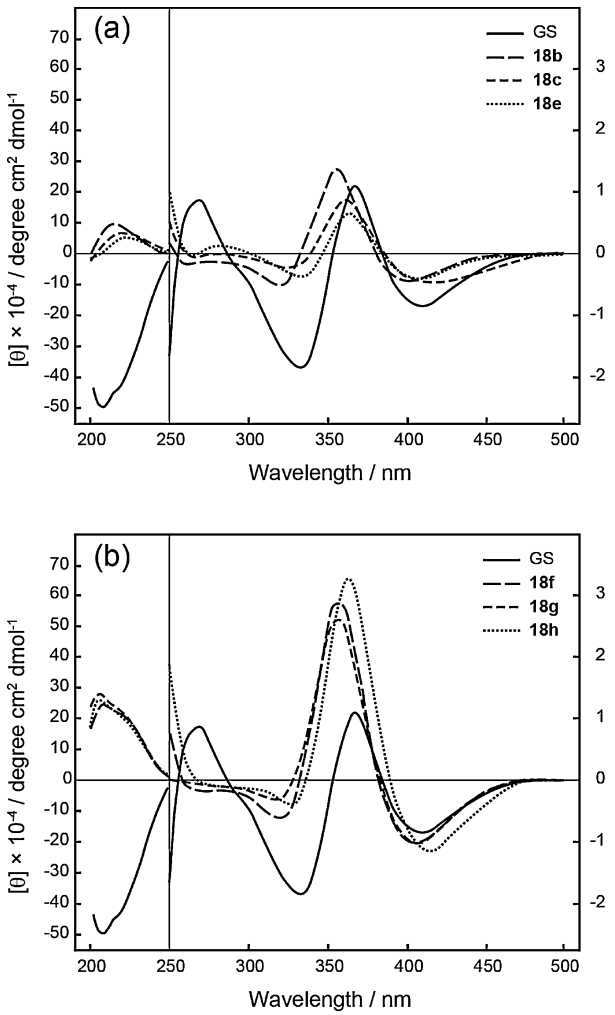

Figure 7. CD spectra of bis-Dnp derivatives of GS and its analogs in MeOH solution.

were suggested to take on the β-sheet conformation that is antipodal to that of GS and showed antibacterial activity that is closely related to the hydrophobicity of component amino acid. In the case of the analogs with D–L–L–D sequences, we could not prove that they had β-sheet conformation from their CD spectra. However, the fact that some of the analogs showed strong antibacterial activity suggesting that they had amphiphilic β-sheet conformation as expected in Figure 2. It was noteworthy that retention time on HPLC had some relation to the activity of GS analogs. The overall molecular hydrophobicity caused by the difference of conformation may be reflected in HPLC retention time. For the precise conformational analysis of the analogs with D–L–L–D sequence, it would be necessary to apply NMR spectroscopy. This would be our future experiment. As for the structure–activity relationships of GS, recently, several papers have been published on the unique analogs of GS.[19–25]

Experimental

All the melting points were uncorrected. Thin-layer chromatography was carried out on Merck silica gel 60 F_{254} plates with the following solvent systems, the ratio in parentheses after the solvent system being indicated by vol: R_f^1, $CHCl_3$–MeOH (5:1); R_f^2, $CHCl_3$–MeOH–AcOH (95:5:1); R_f^3, *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2); R_f^4, *n*-BuOH–AcOH–H_2O (4:1:5, organic phase). Optical rotations were measured on a JASCO DIP-360 digital polarimeter. FAB/MS spectra were measured on a JEOL HX-100 mass spectrometer. MALDI-TOF-MS was measured on an Applied Biosystems Voyager-DE Pro Biospectrometry. Amino acid analysis was performed on an IRICA model A-5500 amino acid analyzer after hydrolysis in 6 M HCl in a sealed tube at 110 °C for 24 h. HPLC analysis was performed on a Shimadzu LC-6A system with Cosmosil $5C_{18}$-AR-II ($4.6 \times 250\,mm^2$) column. Solvent system was a linear gradient from 40% to 70% CH_3CN in 0.1% TFA for 30 min, and the flow rate was 1 mL min^{-1}. Eluent was monitored by absorbance at 230 nm. CD spectra were recorded on a JASCO J-40 spectropolarimeter in 0.1 mM solution using a cell of 1 mm pass length.

Boc–D-Leu–Pro–OMe (2). To a chilled solution of H–Pro–OMe·HCl (1.66 g, 10 mmol) in CH_2Cl_2 (40 mL) were added TEA (1.4 mL, 10 mmol), Boc–D-Leu–OH (2.31 g, 10 mmol) and EDC·HCl (1.92 g, 10 mmol). After being stirred overnight at room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed successively with 10% citric acid, 4% $NaHCO_3$, and H_2O, and the solution was dried over anhydrous Na_2SO_4. Evaporation of the solvent left an oil; yield, 3.08 g (90%); R_f^1 0.65, R_f^2 0.38, R_f^3 0.65, and R_f^4 0.68.

H–D-Leu–Pro–OMe·HCl (3·HCl). Compound **2** (3.08 g, 9 mmol) was dissolved in 2 M hydrogen chloride in EtOAc (90 mL, 180 mmol). The solution was allowed to stand at room temperature for 30 min and evaporated. The desired product was collected by filtration with the aid of ether and recrystallized from MeOH–ether; yield 2.36 g (94%); mp 146–148 °C; $[\alpha]_D^{24}$ −52.3° (*c* 1, MeOH); R_f^1 0.49, R_f^2 0.03, R_f^3 0.61, and R_f^4 0.41. Found: C, 46.78; H, 8.14; N, 9.94%. Calcd for $C_{11}H_{20}N_2O_3$·HCl·H_2O: C, 46.72; H, 8.20; N, 9.90%.

Boc–D-Lys(Cl-Z)–D-Leu–Pro–OMe (4). Compound **3**·HCl (0.95 g, 3.4 mmol) and Boc–D-Lys(Cl-Z)–OH (1.41 g, 3.4 mmol) were treated as described for **2** to leave an oil; yield, 1.60 g (73%); R_f^1 0.69, R_f^2 0.39, R_f^3 0.68, and R_f^4 0.69.

H–D-Lys(Cl-Z)–D-Leu–Pro–OMe·HCl (5·HCl). Compound **4** (1.60 g, 2.5 mmol) was treated as described for **3** to leave an oil; yield, 1.42 g (100%); R_f^1 0.69, R_f^2 0.39, R_f^3 0.68, and R_f^4 0.69.

Boc–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OMe (6). Compound **5**·HCl (1.42 g, 2.5 mmol) and Boc–D-Leu–OH (0.58 g, 2.5 mmol) were treated as described for **2**. The crude product was purified by silica gel chromatography with 2% MeOH in CH_3Cl; yield, 1.47 g (79%); mp 102–103 °C; $[\alpha]_D^{24}$ 20.7° (*c* 0.9, MeOH); R_f^1 0.76, R_f^2 0.34, R_f^3 0.90, and R_f^4 0.93. Found: C, 59.04; H, 7.78; N, 9.33%. Calcd for $C_{37}H_{58}N_5O_9Cl$: C, 59.07; H, 7.77; N, 9.31%.

Boc–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (7). Compound **6** (1.47 g, 2 mmol) was dissolved in MeOH (20 mL) and 1 M NaOH (3.9 mL, 3.9 mmol), and the solution was stirred at room temperature for 3 h. H_2O (20 mL) was added to the solution and the MeOH was removed by evaporation under reduced pressure. The solution was washed with ether and acidified with 10% citric acid to leave an oil which was extracted with EtOAc. After being dried over anhydrous Na_2SO_4, the solvent was removed by evaporation under reduced pressure to leave a solid; yield, 1.17 g (81%); mp 95–100 °C; $[\alpha]_D^{24}$ 18.6° (*c* 0.3, MeOH); R_f^1 0.32, R_f^2 0.17, R_f^3 0.73, and R_f^4 0.84. Found: C, 57.73; H, 7.67; N, 9.33%. Calcd for $C_{36}H_{56}N_5O_9Cl$·0.5H_2O: C, 57.86; H, 7.69; N, 9.37%.

H–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (8·HCl). Compound **7** (1.71 g, 1.6 mmol) was treated as described for **3**; yield 1.03 g (96%); mp 143–149 °C; $[\alpha]_D^{24}$ 0.5° (*c* 0.6, MeOH); R_f^1 0.05, R_f^2 0.00, R_f^3 0.67, and R_f^4 0.50. Found: C, 53.77; H, 7.26; N, 10.12%. Calcd for $C_{31}H_{48}N_5O_7Cl$·HCl·H_2O: C, 53.75; H, 7.42; N, 10.11%.

Boc–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9a). To a chilled solution of **8**·HCl (270 mg, 0.4 mmol) in DMF (4 mL) were added TEA (0.11 mL, 0.8 mmol) and Boc–Asn–ONp (159 mg, 0.45 mmol). The reaction mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. Addition of 10% citric acid to the residue gave a precipitate. The crude product dissolved in MeOH (3 mL) was applied to a column (3 × 170 cm^2) of Sephadex LH-20 and eluted with MeOH. The fractions containing the desired product detected by UV absorption and TLC were collected and evaporated, and the residue was crystallized from ether–petroleum ether; yield, 297 mg (87%); mp 197–200 °C; $[\alpha]_D^{24}$ 12.6° (*c* 0.5, MeOH); R_f^1 0.08, R_f^2 0.00, R_f^3 0.80, and R_f^4 0.78. Found: C, 55.44; H, 7.36; N, 11.33%. Calcd for $C_{40}H_{62}N_7O_{11}Cl$·H_2O: C, 55.19; H, 7.41; N, 11.26%.

Boc–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OSu (10a). To a chilled solution of **9a** (255 mg, 0.3 mmol) and HOSu (69 mg, 0.6 mmol) in DMF (5 mL) was added a solution of EDC·HCl (115 mg, 0.6 mmol) in $CHCl_3$ (2 mL). After being stirred at 5 °C overnight, the solution was evaporated under reduced pressure. The precipitate formed by the addition of chilled H_2O was collected by filtration and dried in vacuo over P_2O_5. The product was used for the next reaction without further treatment; yield 247 mg (98%); R_f^1 0.30 and R_f^2 0.12.

H–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OSu·TFA (11a·TFA). Compound **10a** (247 mg, 0.3 mmol) was dissolved in TFA (2 mL). The solution was allowed to stand at 0 °C for 30 min and evaporated to leave an oil which was crystallized by addition of ether. The product was used for the next reaction without further treatment; yield 283 mg (100%), R_f^1 0.18 and R_f^2 0.00.

***cyclo*[–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (12a).** A solution of **11a**·TFA (283 mg, 0.3 mmol) in DMF (5 mL) was added dropwise into pyridine (95 mL) at room temperature. The final concentration was 3 mM. The reaction mixture was stirred overnight and evaporated. The residue was dissolved in mixture (10 mL) of MeOH–H_2O (5:1, by vol), and 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 (100 mL) and the combined effluent was evaporated to leave a white solid, which was collected with the aid of H_2O. The crude product (174 mg, 79%) dissolved in MeOH (2 mL) was applied to a column (3 × 170 cm^2) of Sephadex LH-20 and eluted with MeOH (Figure 5). The fractions containing the desired product were collected and evaporated. The product was recrystallized from MeOH–ether; yield, 126 mg (58%); mp 161–167 °C; $[\alpha]_D^{24}$ 87.0° (*c* 0.5, MeOH); R_f^1 0.53, R_f^2 0.02, R_f^3 0.87, and R_f^4 0.90. Found: C, 55.42; H, 6.98; N, 12.91%. Calcd for $C_{70}H_{104}N_{14}O_{16}Cl_2$·3$H_2O$: C, 55.22; H, 7.28; N, 12.88%.

***cyclo*[–Asn–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1a·2HCl).** Compound **12a** (147 mg, 0.1 mmol) dissolved in 0.02 M hydrogen chloride in MeOH (5 mL) was hydrogenated in the presence of Pd-black (ca. 100 mg) for 3 h at room temperature. After filtration of catalyst, the filtrate was evaporated and the residue was crystallized from MeOH–ether; yield 119 mg (99%); mp 273–274 °C; $[\alpha]_D^{24}$ 71.9° (*c* 0.5, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.45, and R_f^4 0.20; MS (FAB), *m*/*z* 1131 ($C_{54}H_{94}N_{14}O_{12}$, M^+). Amino acid ratios in acid hydrolysate: Asp (0.95), Lys (1.00), Leu (1.90), and Pro (1.00). Found: C, 52.48; H, 8.03; N, 15.77%. Calcd for $C_{54}H_{94}N_{14}O_{12}$·2HCl·2H_2O: C, 52.29; H, 8.13; N, 15.81%.

Boc–Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9b). Compound **8**·HCl (337 mg, 0.5 mmol) was coupled with

Boc–Ala–OSu (172 mg, 0.6 mmol) as described for **9a**; yield, 366 mg (90%); mp 120–122 °C; $[\alpha]_D^{24}$ 11.8° (*c* 0.8, MeOH); R_f^1 0.22, R_f^2 0.15, R_f^3 0.76, and R_f^4 0.94. Found: C, 57.40; H, 7.63; N, 10.25%. Calcd for $C_{39}H_{61}N_6O_{10}Cl \cdot 0.5H_2O$: C, 57.24; H, 7.64; N, 10.27%.

***cyclo*[–Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (12b).** Active ester **10b** (357 mg, 98%, R_f^1 0.73, and R_f^2 0.23) prepared from **9b** (324 mg, 0.4 mmol) and HOSu (92 mg, 0.8 mmol) was treated with TFA to afford **11b** (395 mg, 100%, R_f^1 0.42, and R_f^2 0.08), which was cyclized in anhydrous pyridine (126 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **12b** was obtained as colorless powder; yield, 135 mg (50%); mp 227–228 °C; $[\alpha]_D^{24}$ 65.0° (*c* 0.4, MeOH); R_f^1 0.75, R_f^2 0.31, R_f^3 0.91, and R_f^4 0.90. Found: C, 59.08; H, 7.49; N, 12.01%. Calcd for $C_{68}H_{102}N_{12}O_{14}Cl_2$: C, 59.08; H, 7.44; N, 12.16%.

***cyclo*[–Ala–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1b·2HCl).** Compound **12b** (97 mg, 0.07 mmol) was hydrogenated as described for **1a**; yield 75 mg (96%); mp 290–293 °C; $[\alpha]_D^{24}$ 46.2° (*c* 0.7, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.57, and R_f^4 0.27; MS (FAB), *m*/*z* 1044 ($C_{52}H_{92}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolyzate: Ala (0.96), Lys (1.06), Leu (2.01), and Pro (1.00). Found: C, 52.59; H, 8.37; N, 13.90%. Calcd for $C_{52}H_{92}N_{12}O_{10} \cdot 2HCl \cdot 4H_2O$: C, 52.47; H, 8.64; N, 14.12%.

Boc–Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9c). Compound **8**·HCl (270 mg, 0.4 mmol) was coupled with Boc–Leu–OSu (149 mg, 0.48 mmol) as described for **9a**; yield, 249 mg (73%); mp 127–130 °C; $[\alpha]_D^{24}$ 10.4° (*c* 0.6, MeOH); R_f^1 0.35, R_f^2 0.22, R_f^3 0.81, and R_f^4 0.88. Found: C, 58.85; H, 7.95; N, 9.79%. Calcd for $C_{42}H_{67}N_6O_{10}Cl$: C, 59.24; H, 7.93; N, 9.87%.

***cyclo*[–Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (12c).** Active ester **10c** (240 mg, 100%, R_f^1 0.78, and R_f^2 0.29) prepared from **9c** (213 mg, 0.25 mmol) and HOSu (58 mg, 0.5 mmol) was treated with TFA to afford **11c** (252 mg, 100%, R_f^1 0.49, and R_f^2 0.14), which was cyclized in anhydrous pyridine (80 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **12c** was obtained as colorless powder; yield, 63 mg (34%); mp 237–241 °C; $[\alpha]_D^{24}$ 36.1° (*c* 0.2, MeOH); R_f^1 0.81, R_f^2 0.32, R_f^3 0.98, and R_f^4 0.98. Found: C, 59.91; H, 7.81; N, 11.24%. Calcd for $C_{74}H_{114}N_{12}O_{14}Cl_2 \cdot H_2O$: C, 59.86; H, 7.88; N, 11.32%.

***cyclo*[–Leu–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1c·2HCl).** Compound **12c** (44 mg, 0.03 mmol) was hydrogenated as described for **1a**; yield 36 mg (100%); mp 230–233 °C; $[\alpha]_D^{24}$ 4.7° (*c* 0.2, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.75, and R_f^4 0.44; MS (FAB), *m*/*z* 1129 ($C_{58}H_{104}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Leu (2.87), Lys (1.01), and Pro (1.00). Found: C, 54.21; H, 8.82; N, 12.83%. Calcd for $C_{58}H_{104}N_{12}O_{10} \cdot 2HCl \cdot 4.5H_2O$: C, 54.27; H, 9.03; N, 13.10%.

Boc–Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9d). Compound **8**·HCl (337 mg, 0.5 mmol) was coupled with Boc–Phe–OSu (218 mg, 0.6 mmol) as described for **9a**; yield, 383 mg (86%); mp 198–200 °C; $[\alpha]_D^{24}$ 22.6° (*c* 0.4, MeOH); R_f^1 0.29, R_f^2 0.17, R_f^3 0.76, and R_f^4 0.85. Found: C, 60.20; H, 7.36; N, 9.34%. Calcd for $C_{45}H_{65}N_6O_{10}Cl \cdot 0.5H_2O$: C, 60.42; H, 7.44; N, 9.40%.

***cyclo*[–Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (12d).** Active ester **10d** (406 mg, 100%, R_f^1 0.77, and R_f^2 0.37) prepared from **9d** (355 mg, 0.4 mmol) and HOSu (92 mg, 0.8 mmol) was treated with TFA to afford **11d** (407 mg, 100%, R_f^1 0.48, and R_f^2 0.11), which was cyclized in anhydrous pyridine (128 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **12d** was obtained as colorless powder; yield, 175 mg (57%); mp 145–147 °C; $[\alpha]_D^{24}$ 23.6° (*c* 0.2, MeOH); R_f^1 0.82, R_f^2 0.33, R_f^3 0.98, and R_f^4 0.96. Found: C, 61.41; H, 7.13; N, 10.73%. Calcd for $C_{80}H_{110}N_{12}O_{14}Cl_2 \cdot 2H_2O$: C, 61.17; H, 7.32; N, 10.70%.

***cyclo*[–Phe–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1d·2HCl).** Compound **12d** (77 mg, 0.05 mmol) was hydrogenated as described for **1a**; yield 65 mg (100%); mp 215–220 °C; $[\alpha]_D^{24}$ 1.9° (*c* 0.4, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.74, and R_f^4 0.46; MS (FAB), *m*/*z* 1196 ($C_{64}H_{112}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Phe (0.90), Lys (1.00), Leu (1.91), and Pro (1.00). Found: C, 56.65; H, 8.04; N, 12.04%. Calcd for $C_{64}H_{112}N_{12}O_{10} \cdot 2HCl \cdot 5H_2O$: C, 56.50; H, 8.30; N, 12.35%.

***cyclo*[–Cha–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1e·2HCl).** Compound **1d**·2HCl (64 mg, 0.05 mmol) dissolved in AcOH (5 mL) was hydrogenated in the presence of Pt-black (ca 150 mg) for 7 h at room temperature. After filtration of catalyst, the filtrate was evaporated and the residue was crystallized from MeOH–ether; yield 62 mg (97%); mp 220–225 °C; $[\alpha]_D^{24}$ 1.4° (*c* 0.5, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.75, and R_f^4 0.47; MS (FAB), *m*/*z* 1208 ($C_{64}H_{112}N_{12}O_{10}$, M^+). Found: C, 58.56; H, 8.88; N, 12.51%. Calcd for $C_{64}H_{112}N_{12}O_{10} \cdot 2HCl \cdot 2H_2O$: C, 58.30; H, 9.02; N, 12.75%.

Boc–Gly–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9f). Compound **8**·HCl (270 mg, 0.4 mmol) was coupled with Boc–Gly–OSu (122 mg, 0.45 mmol) as described for **9a**; yield, 290 mg (91%); mp 115–119 °C; $[\alpha]_D^{24}$ 23.7° (*c* 0.4, MeOH); R_f^1 0.22, R_f^2 0.09, R_f^3 0.78, and R_f^4 0.80. Found: C, 56.78; H, 7.49; N, 10.40%. Calcd for $C_{38}H_{59}N_6O_{10}Cl \cdot 0.5H_2O$: C, 56.74; H, 7.52; N, 10.45%.

***cyclo*[–Gly–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–] (12f-monomer).** Active ester **10f** (289 mg, 98%, R_f^1 0.77, and R_f^2 0.23) prepared from **9f** (262 mg, 0.33 mmol) and HOSu (76 mg, 0.66 mmol) was treated with TFA to afford **11f** (295 mg, 100%, R_f^1 0.34, and R_f^2 0.02), which was cyclized in anhydrous pyridine (101 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **12f-monomer** was obtained as colorless powder; yield, 148 mg (68%); mp 150–151 °C; $[\alpha]_D^{24}$ 76.9° (*c* 0.6, MeOH); R_f^1 0.48, R_f^2 0.07, R_f^3 0.73, and R_f^4 0.66. Found: C, 56.95; H, 7.18; N, 12.05%. Calcd for $C_{33}H_{49}N_6O_7Cl \cdot H_2O$: C, 57.01; H, 7.39; N, 12.09%.

***cyclo*[–Gly–D-Leu–D-Lys–D-Leu–Pro–]·HCl (1f-monomer·HCl).** Compound **12f-monomer** (68 mg, 0.05 mmol) was hydrogenated as described for **1a**; yield 55 mg (100%); mp 187–192 °C; $[\alpha]_D^{24}$ 104.9° (*c* 0.6, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.67, and R_f^4 0.37; MS (FAB), *m*/*z* 508 ($C_{25}H_{44}N_6O_5$, M^+). Amino acid ratios in acid hydrolysate: Gly (0.94), Lys (1.00), Leu (1.90), and Pro (1.00). Found: C, 52.06; H, 8.22; N, 14.38%. Calcd for $C_{25}H_{44}N_6O_5 \cdot HCl \cdot 1.5H_2O$: C, 52.48; H, 8.46; N, 14.69%.

Boc–D-Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9g). Compound **8**·HCl (337 mg, 0.5 mmol) was coupled with Boc–D-Ala–OSu (172 mg, 0.6 mmol) as described for **9a**; yield, 370 mg (91%); mp 122–124 °C; $[\alpha]_D^{24}$ 34.5° (*c* 0.5, MeOH); R_f^1

0.25, R_f^2 0.22, R_f^3 0.74, and R_f^4 0.86. Found: C, 57.50; H, 7.64; N, 10.22%. Calcd for $C_{39}H_{61}N_6O_{10}Cl \cdot 0.5H_2O$: C, 57.24; H, 7.64; N, 10.27%.

***cyclo*[–D-Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–] (12g-monomer).** Active ester **10g** (349 mg, 96%, R_f^1 0.73, and R_f^2 0.23) prepared from **9g** (324 mg, 0.4 mmol) and HOSu (92 mg, 0.8 mmol) was treated with TFA to afford **11g** (369 mg, 100%, R_f^1 0.41, and R_f^2 0.05), which was cyclized in anhydrous pyridine (123 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **12g-monomer** was obtained as colorless powder; yield, 211 mg (79%); mp 192–193 °C; $[\alpha]_D^{24}$ 105.8° (*c* 0.8, MeOH); R_f^1 0.53, R_f^2 0.09, R_f^3 0.70, and R_f^4 0.63. Found: C, 58.69; H, 7.47; N, 12.08%. Calcd for $C_{34}H_{51}N_6O_7Cl$: C, 59.08; H, 7.44; N, 12.16%.

***cyclo*[–D-Ala–D-Leu–D-Lys–D-Leu–Pro–]·HCl (1g-monomer·HCl).** Compound **12g-monomer** (138 mg, 0.2 mmol) was hydrogenated as described for **1a**; yield 106 mg (95%); mp 198–200 °C; $[\alpha]_D^{24}$ 118.7° (*c* 0.7, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.67, and R_f^4 0.37; MS (FAB), *m/z* 522 ($C_{26}H_{46}N_6O_5$, M^+). Amino acid ratios in acid hydrolyzate: Ala (0.90), Lys (0.99), Leu (1.85), and Pro (1.00). Found: C, 53.25; H, 8.32; N, 14.31%. Calcd for $C_{26}H_{46}N_6O_5 \cdot HCl \cdot 1.5H_2O$: C, 53.28; H, 8.61; N, 14.34%.

H–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (13a·HCl). Compound **9a** (255 mg, 0.3 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (4.5 mL, 0.45 mmol). The solution was allowed to stand at room temperature for 1 h and evaporated. The desired product was collected by filtration with the aid of ether. The product was used for the next reaction without further treatment; yield 237 mg (100%), R_f^1 0.00, R_f^2 0.00, R_f^3 0.56, and R_f^4 0.41.

Boc–[Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro]$_2$–OH (14a). To a chilled solution of **13a**·HCl (237 mg, 0.3 mmol) in DMF (4 mL) were added TEA (0.084 mL, 0.6 mmol) and **10a** (285 mg, 0.3 mmol). The reaction mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. Addition of 10% citric acid to the residue gave a precipitate. The crude products 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 detected by UV absorption and TLC were collected and evaporated, and the residue was crystallized from EtOAc; yield, 314 mg (66%); mp 146–151 °C; $[\alpha]_D^{24}$ 19.1° (*c* 0.9, MeOH); R_f^1 0.18, R_f^2 0.00, R_f^3 0.80, and R_f^4 0.80. Found: C, 54.25; H, 7.07; N, 11.83%. Calcd for $C_{75}H_{114}N_{14}O_{19}Cl_2 \cdot 4H_2O$: C, 54.31; H, 7.41; N, 11.82%.

***cyclo*[–Asn–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (17a).** Active ester **15a** (280 mg, 98%, R_f^1 0.66, and R_f^2 0.21) prepared from **14a** (270 mg, 0.17 mmol) and HOSu (35 mg, 0.3 mmol) was treated with TFA to afford **16a** (282 mg, 100%, R_f^1 0.51, and R_f^2 0.05), which was cyclized in anhydrous pyridine (50 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **17a** was obtained as colorless powder; yield, 139 mg (57%). The product was chromatographically identical to **12a**.

H–Gly–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (13f·HCl). Compound **9f** (382 mg, 0.48 mmol) was treated as described for **13a**; yield 338 mg (96%), R_f^1 0.00, R_f^2 0.00, R_f^3 0.69, and R_f^4 0.47.

Boc–[Gly–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro]$_2$–OH (14f). Compound **13f**·HCl (338 mg, 0.46 mmol) and **10f** (410 mg, 0.46 mmol) were treated as described for **14a**; yield, 443 mg (65%); mp 145–148 °C; $[\alpha]_D^{24}$ 71.3° (*c* 0.8, MeOH); R_f^1 0.44, R_f^2 0.16, R_f^3 0.84, and R_f^4 0.91. Found: C, 57.19; H, 7.40; N, 11.24%. Calcd for $C_{71}H_{108}N_{12}O_{17}Cl_2 \cdot H_2O$: C, 57.21; H, 7.44; N, 11.28%.

***cyclo*[–Gly–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (17f).** Active ester **15f** (421 mg, 99%, R_f^1 0.75, and R_f^2 0.27) prepared from **14f** (398 mg, 0.27 mmol) and HOSu (62 mg, 0.54 mmol) was treated with TFA to afford **16f** (402 mg, 94%, R_f^1 0.62, and R_f^2 0.10), which was cyclized in anhydrous pyridine (80 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **17f** was obtained as colorless powder; yield, 290 mg (84%); mp 163–166 °C; $[\alpha]_D^{24}$ 158.5° (*c* 0.7, MeOH); R_f^1 0.83, R_f^2 0.33, R_f^3 0.93, and R_f^4 0.92. Found: C, 57.78; H, 7.28; N, 12.32%. Calcd for $C_{66}H_{98}N_{12}O_{14}Cl_2 \cdot H_2O$: C, 57.76; H, 7.34; N, 12.25%.

***cyclo*[–Gly–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1f·2HCl).** Compound **17f** (135 mg, 0.1 mmol) was hydrogenated as described for **1a**; yield 105 mg (96%); mp 292–296 °C; $[\alpha]_D^{24}$ 187.9° (*c* 0.6, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.53, and R_f^4 0.23; MS (FAB), *m/z* 1017 ($C_{50}H_{88}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Gly (0.95), Lys (1.01), Leu (1.92), and Pro (1.00). Found: C, 52.32; H, 8.41; N, 14.40%. Calcd for $C_{50}H_{88}N_{12}O_{10} \cdot 2HCl \cdot 3H_2O$: C, 52.48; H, 8.46; N, 14.69%.

H–D-Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (13g·HCl). Compound **9g** (243 mg, 0.3 mmol) was treated as described for **13a**; yield 217 mg (97%), R_f^1 0.04, R_f^2 0.00, R_f^3 0.73, and R_f^4 0.45.

Boc–[D-Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro]$_2$–OH (14g). Compound **13g**·HCl (217 mg, 0.29 mmol) and **10g** (289 mg, 0.32 mmol) were treated as described for **14a**; yield, 266 mg (61%); mp 150–152 °C; $[\alpha]_D^{24}$ 75.8° (*c* 0.8, MeOH); R_f^1 0.49, R_f^2 0.26, R_f^3 0.84, and R_f^4 0.92. Found: C, 57.68; H, 7.60; N, 11.02%. Calcd for $C_{73}H_{112}N_{12}O_{17}Cl_2 \cdot H_2O$: C, 57.73; H, 7.57; N, 11.07%.

***cyclo*[–D-Ala–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (17g).** Active ester **15g** (278 mg, 100%, R_f^1 0.75, and R_f^2 0.22) prepared from **14g** (255 mg, 0.17 mmol) and HOSu (35 mg, 0.3 mmol) was treated with TFA to afford **16g** (257 mg, 94%, R_f^1 0.53, and R_f^2 0.08), which was cyclized in anhydrous pyridine (50 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **17g** was obtained as colorless powder; yield, 170 mg (77%); mp 268–270 °C; $[\alpha]_D^{24}$ 182.0° (*c* 0.8, MeOH); R_f^1 0.71, R_f^2 0.29, R_f^3 0.95, and R_f^4 0.94. Found: C, 58.44; H, 7.50; N, 12.07%. Calcd for $C_{68}H_{102}N_{12}O_{14}Cl_2 \cdot H_2O$: C, 58.31; H, 7.49; N, 12.00%.

***cyclo*[–D-Ala–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1g·2HCl).** Compound **17g** (138 mg, 0.1 mmol) was hydrogenated as described for **1a**; yield 108 mg (97%); mp 278–280 °C; $[\alpha]_D^{24}$ 176.6° (*c* 0.7, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.58, and R_f^4 0.27; MS (FAB), *m/z* 1044 ($C_{52}H_{92}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Ala (0.97), Lys (0.98), Leu (1.94), and Pro (1.00). Found: C, 52.61; H, 8.52; N, 13.83%. Calcd for $C_{52}H_{92}N_{12}O_{10} \cdot 2HCl \cdot 4H_2O$: C, 52.47; H, 8.64; N, 14.12%.

Boc–D-Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9h). Compound **8**·HCl (371 mg, 0.55 mmol) was coupled with Boc–D-Leu–OSu (205 mg, 0.66 mmol) as described for **9a**;

yield, 408 mg (87%); mp 128–131 °C; $[\alpha]_D^{24}$ 36.4° (*c* 1, MeOH); R_f^1 0.43, R_f^2 0.25, R_f^3 0.84, and R_f^4 0.90.

H–D-Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (13h·HCl). Compound **9h** (179 mg, 0.21 mmol) was treated as described for **13a**; yield 164 mg (100%), R_f^1 0.00, R_f^2 0.23, R_f^3 0.78, and R_f^4 0.49.

Boc–[D-Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro]$_2$–OH (14h). Active ester **10h** (198 mg, 100%, R_f^1 0.79, and R_f^2 0.36) prepared from **9h** (179 mg, 0.21 mmol) and HOSu (48 mg, 0.42 mmol) was coupled with **13h**·HCl (164 mg, 0.21 mmol) as described for **14a**; yield, 181 mg (54%); mp 153–155 °C; $[\alpha]_D^{24}$ 78.1° (*c* 0.5, MeOH); R_f^1 0.56, R_f^2 0.28, R_f^3 0.86, and R_f^4 0.93. Found: C, 59.23; H, 8.02; N, 10.36%. Calcd for $C_{79}H_{124}N_{12}O_{17}Cl_2 \cdot H_2O$: C, 59.20; H, 7.92; N, 10.49%.

***cyclo*[–D-Leu–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (17h).** Active ester **15h** (168 mg, 100%, R_f^1 0.82, and R_f^2 0.38) prepared from **14h** (158 mg, 0.1 mmol) and HOSu (23 mg, 0.2 mmol) was treated with TFA to afford **16h** (150 mg, 88%, R_f^1 0.64, and R_f^2 0.11), which was cyclized in anhydrous pyridine (26 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **17h** was obtained as colorless powder; yield, 114 mg (88%); mp 223–224 °C; $[\alpha]_D^{24}$ 160.8° (*c* 0.4, MeOH); R_f^1 0.83, R_f^2 0.30, R_f^3 0.94, and R_f^4 0.95. Found: C, 60.34; H, 7.92; N,11.17%. Calcd for $C_{74}H_{114}N_{12}O_{14}Cl_2$: C, 60.60; H, 7.84; N, 11.46%.

***cyclo*[–D-Leu–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1h·2HCl).** Compound **17h** (88 mg, 0.06 mmol) was hydrogenated as described for **1a**; yield 70 mg (97%); mp 264–268 °C; $[\alpha]_D^{24}$ 179.5° (*c* 0.6, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.75, and R_f^4 0.45; MS (FAB), *m/z* 1128 ($C_{58}H_{104}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Lys (1.01), Leu (2.87), and Pro (1.00). Found: C, 55.09; H, 8.82; N, 12.99%. Calcd for $C_{58}H_{104}N_{12}O_{10} \cdot 2HCl \cdot 3.5H_2O$: C, 55.05; H, 9.00; N, 13.28%.

Boc–D-Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH (9i). Compound **8**·HCl (540 mg, 0.8 mmol) was coupled with Boc–D-Phe–OSu (349 mg, 0.96 mmol) as described for **9a**; yield, 610 mg (86%); mp 121–125 °C; $[\alpha]_D^{24}$ 21.9° (*c* 1, MeOH); R_f^1 0.29, R_f^2 0.17, R_f^3 0.76, and R_f^4 0.81.

H–D-Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–OH·HCl (13i·HCl). Compound **9i** (284 mg, 0.32 mmol) was treated as described for **13a**; yield 264 mg (100%), R_f^1 0.00, R_f^2 0.21, R_f^3 0.71, and R_f^4 0.68.

Boc–[D-Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro]$_2$–OH (14i). Active ester **10i** (322 mg, 100%, R_f^1 0.83, and R_f^2 0.05) prepared from **9i** (284 mg, 0.32 mmol) and HOSu (74 mg, 0.64 mmol) was coupled with **13i**·HCl (264 mg, 0.32 mmol) as described for **14a**; yield, 371 mg (67%); mp 142–145 °C; $[\alpha]_D^{24}$ 46.1° (*c* 0.8, MeOH); R_f^1 0.89, R_f^2 0.29, R_f^3 0.84, and R_f^4 0.79. Found: C, 61.21; H, 7.30; N, 10.18%. Calcd for $C_{85}H_{120}N_{12}O_{17}Cl_2 \cdot H_2O$: C, 61.10; H, 7.36; N, 10.06%.

***cyclo*[–D-Phe–D-Leu–D-Lys(Cl-Z)–D-Leu–Pro–]$_2$ (17i).** Active ester **15i** (354 mg, 97%, R_f^1 0.80, and R_f^2 0.44) prepared from **14i** (344 mg, 0.2 mmol) and HOSu (46 mg, 0.4 mmol) was treated with TFA to afford **16i** (304 mg, 85%, R_f^1 0.63, and R_f^2 0.14), which was cyclized in anhydrous pyridine (50 mL) as described for **12a**. After usual work-up including gel filtration with Sephadex LH-20, **17i** was obtained as colorless powder; yield, 219 mg (82%); mp 156–159 °C; $[\alpha]_D^{24}$ 92.4° (*c* 0.5, MeOH); R_f^1 0.84, R_f^2 0.33, R_f^3 0.98, and R_f^4 0.95. Found: C, 62.26; H, 7.21; N, 10.73%. Calcd for $C_{80}H_{110}N_{12}O_{14}Cl_2$: C, 62.61; H, 7.22; N, 10.95%.

***cyclo*[–D-Phe–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1i·2HCl).** Compound **17i** (192 mg, 0.12 mmol) was hydrogenated as described for **1a**; yield 142 mg (93%); mp 253–256 °C; $[\alpha]_D^{24}$ 111.2° (*c* 0.5, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.75, and R_f^4 0.43; MS (FAB), *m/z* 1196 ($C_{64}H_{100}N_{12}O_{10}$, M^+). Amino acid ratios in acid hydrolysate: Phe (0.91), Lys (1.00), Leu (1.92), and Pro (1.00). Found: C, 58.04; H, 8.05; N, 12.36%. Calcd for $C_{64}H_{100}N_{12}O_{10} \cdot 2HCl \cdot 3H_2O$: C, 58.04; H, 8.22; N, 12.69%.

***cyclo*[–D-Cha–D-Leu–D-Lys–D-Leu–Pro–]$_2$·2HCl (1j·2HCl).** Compound **1i** (64 mg, 0.05 mmol) dissolved in AcOH (5 mL) was hydrogenated in the presence of Pt-black (ca. 150 mg) for 4 h at room temperature. After filtration of catalyst, the filtrate was evaporated and the residue was crystallized from MeOH–ether; yield 59 mg (92%); mp 273–277 °C; $[\alpha]_D^{24}$ 152.7° (*c* 0.5, MeOH); R_f^1 0.00, R_f^2 0.00, R_f^3 0.76, and R_f^4 0.59; MS (FAB), *m/z* 1208 ($C_{64}H_{112}N_{12}O_{10}$, M^+). Found: C, 58.43; H, 8.94; N, 12.29%. Calcd for $C_{64}H_{112}N_{12}O_{10} \cdot 2HCl \cdot 2H_2O$: C, 58.30; H, 9.02; N, 12.75%.

***cyclo*[–Ala–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18b).** To a solution of **1b** (11 mg, 0.01 mmol) in DMF (2 mL) were added TEA (0.006 mL, 0.04 mmol) and N_2Ph-F (8 mg, 0.04 mmol). The reaction mixture was stirred at room temperature overnight in the dark and evaporated under reduced pressure. The residue dissolved in CH_3Cl (3 mL) was applied to a column (1.8 × 18 cm^2) of silica gel 60 (Merck) and the column was washed with CH_3Cl. The desired product was eluted with a mixture of CH_3Cl and MeOH (19:1, v/v). The fractions containing the desired product were evaporated and the product was crystallized from MeOH–ether; yield 14 mg (100%); mp 264–266 °C; R_f^1 0.78 and R_f^2 0.27; MS (MALDI-TOF), 1400 $[C_{64}H_{96}N_{16}O_{18} + Na]^+$.

***cyclo*[–Leu–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18c).** Compound **1c** (9 mg, 0.0075 mmol) was treated as described for **18b**; yield 7 mg (67%); mp 185–187 °C; R_f^1 0.77 and R_f^2 0.35; MS (MALDI-TOF), 1484 $[C_{70}H_{108}N_{16}O_{18} + Na]^+$.

***cyclo*[–Cha–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18e).** Compound **1e** (13 mg, 0.01 mmol) was treated as described for **18b**; yield 8 mg (50%); mp 168–170 °C; R_f^1 0.78 and R_f^2 0.56; MS (MALDI-TOF), 1565 $[C_{74}H_{112}N_{16}O_{18} + Na]^+$.

***cyclo*[–Gly–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18f).** Compound **1f** (11 mg, 0.01 mmol) was treated as described for **18b**; yield 10 mg (73%); mp 284–286 °C; R_f^1 0.72 and R_f^2 0.25; MS (MALDI-TOF), 1372 $[C_{62}H_{92}N_{16}O_{18} + Na]^+$.

***cyclo*[–D-Ala–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18g).** Compound **1g** (11 mg, 0.01 mmol) was treated as described for **18b**; yield 6 mg (46%); mp >300 °C; R_f^1 0.78 and R_f^2 0.28; MS (MALDI-TOF), 1400 $[C_{64}H_{96}N_{16}O_{18} + Na]^+$.

***cyclo*[–D-Leu–D-Leu–D-Lys(Dnp)–D-Leu–Pro–]$_2$ (18h).** Compound **1h** (12 mg, 0.01 mmol) was treated as described for **18b**; yield 10 mg (70%); mp >300 °C; R_f^1 0.78 and R_f^2 0.37; MS (MALDI-TOF), 1484 $[C_{70}H_{108}N_{16}O_{18} + Na]^+$.

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 "Eiken" Sensitivity Test Agar E-MC10.

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References

1 The abbreviations used in this paper are those recommended by IUPAC-IUB: *Eur. J. Biochem.* **1984**, *138*, 9. Additional abbreviations: Boc, *tert*-butoxycarbonyl; Cha, cyclohexylalanine; DMF, *N*,*N*-dimethylformamide; Dnp, 2,4-dinitorphenyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxy-1*H*-benzotriazole; HOSu, *N*-hydroxysuccinimide; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; N_2ph-F, 1-fluoro-2,4-dinitrobenzene; ONp, *p*-nitrophenyl ester; OSu, *N*-hydroxysuccinimide ester; pNA, *p*-nitroanilide; TEA, triethylamine; TLC, thin-layer chromatography.

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