Synthesis and Properties of Gramicidin S Analogs Containing Pro-D-Phe Sequence in Place of D-Phe-Pro Sequence in the β-Turn Part of the Antibiotic

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Two analogs of gramicidin S, [L-Pro^4, D-Phe^5]-gramicidin S and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-gramicidin S, were synthesized in order to investigate the relationships among positions of Pro residues, antibiotic activity and CD spectra. [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-gramicidin S showed little activity. On the other hand, the activity of [L-Pro^4, D-Phe^5]-gramicidin S against *Bacillus subtilis* and *Micrococcus flavus* was the same as that of gramicidin S, and its activity toward other microorganisms tested was 1/2 that of gramicidin S. The CD spectra of these analogs and gramicidin S in an aqueous solution differ from each other, indicating that these peptides have different conformations in aqueous solutions. The CD spectrum of [L-Pro^4, D-Phe^5]-gramicidin S resembles a graphical average of the CD spectra of gramicidin S and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-gramicidin S.

The secondary structure of gramicidin S (GS)[1] (Fig. 1) is established as an antiparallel β-sheet conformation containing two type-II′ β-turns derived from a D-Phe-Pro sequence.[2] In studies regarding the structure-activity relationship of GS, it has been proposed that a specific conformation is necessary in order to exhibit any antibiotic activity.[3,4] Recently, we synthesized two GS analogs containing D-Phe-Pro-D-Val or Phe-Pro-D-Val sequences in place of the D-Phe-Pro-Val sequence in the β-turn part of this antibiotic and discussed the role of the D-Phe-Pro-Val sequence for exhibiting the activity.[5] We also reported that during studies of an antibiotic cyclododecapeptide, gratisin, *cyclo*(-$\overset{1}{\text{Val}}$-$\overset{2}{\text{Orn}}$-$\overset{3}{\text{Leu}}$-D-$\overset{4}{\text{Phe}}$-$\overset{5}{\text{Pro}}$-D-$\overset{6}{\text{Tyr}}$-)$_2$, *cyclo*(-Val-Orn-Leu-D-Phe-D-Tyr-Pro-)$_2$ and *cyclo*(-Val-Orn-Leu-Pro-D-Phe-D-Phe-)$_2$ (in which the Pro residues ocuppy positions different from each other) possessed a strong activity.[6] In connection with these results, it is of interest to study the relationship among positions of Pro residues, secondary structures, and the antibiotic activity of GS.

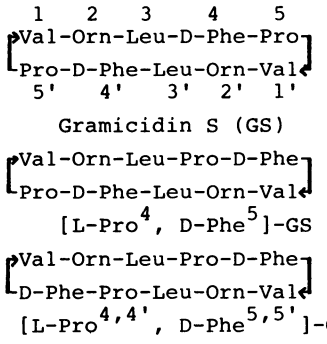


Fig. 1. Primary structure of gramicidin S and its analogs.

In the present paper, we wish to describe the synthesis, antibiotic activity and CD spectra of [L-Pro^4, D-Phe^5]- and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS (Fig. 1), in which one or two of D-Phe-Pro sequences in GS is replaced with a Pro-D-Phe sequence.

The synthetic routes of [L-Pro^4, D-Phe^5]- and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS are shown in Schemes 1 and 2. The synthetic methods are similar to those discussed in a previous paper of this series.[5] The yields, physical properties and analytical data of the intermediary products are summerized in Table 1. The homoge-

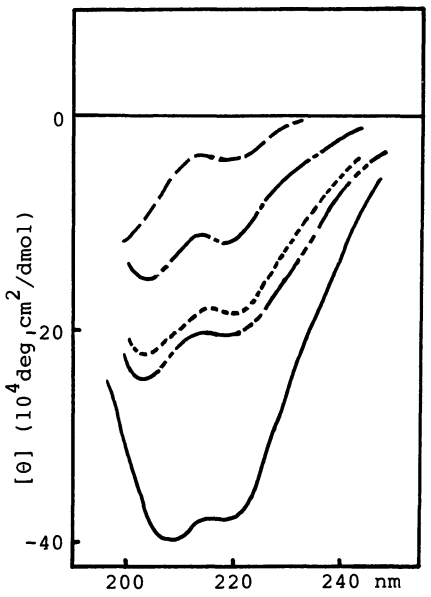
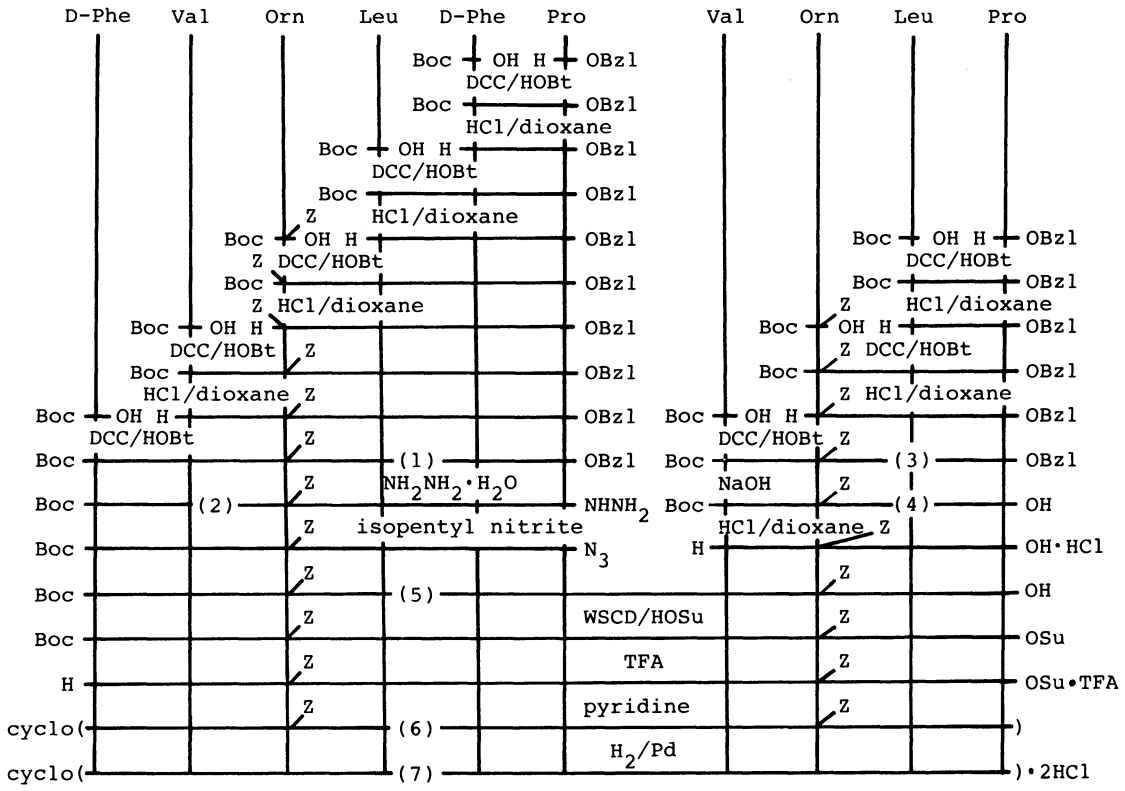


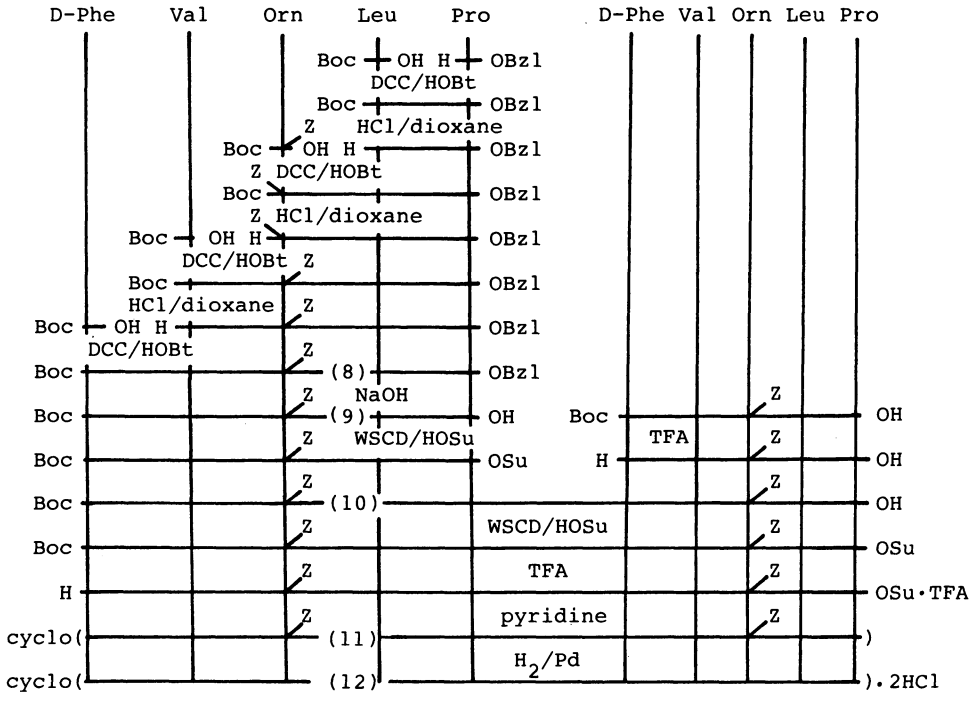
Fig. 2. CD spectra of GS and its analogs in aqueous solution.
——, GS: – – –, [L-Pro^4, D-Phe^5]-GS: ———, [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS: – – –, equimolar mixture of GS and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS: — — —, graphical average of the CD spectra of GS and [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS.

neity of the analogs was confirmed by thin-layer chromatography, cellulose plate electrophoresis, elemental analysis, amino acid analysis and fast-atom-bomberdment (FAB) mass spectrometry (Table 2).

The CD spectra of [L-Pro^{4}, D-Phe^{5}]-GS, [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS and GS in aqueous solutions are shown in Fig. 2. The shapes of [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS and GS differ from each other, indicating that these peptides have different conformations in aqueous solutions. On the other hand, the feature of the CD spectrum of



Scheme 1. Synthesis of [L-Pro^{4}, D-Phe^{5}]-GS.



Scheme 2. Synthesis of [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS.

[L-Pro⁴, D-Phe⁵]-GS resembles the graphical average of the spectra of GS and [L-Pro⁴,⁴′, D-Phe⁵,⁵′]-GS. The latter was also very similar to the spectrum of an equimolar mixture of both peptides. The difference found in the ellipticities of these compounds seems to reflect the difference of the stability of their structures in aqueous solutions. The existence of the additivity in a CD spectra of these analogs and GS suggests that D-Phe–Pro and Pro–D-Phe sequences of each β-turn part contribute separately to their spectra of these peptides.

The antibiotic activity of GS and these two analogs

Table 1. Yields, physical properties and analytical data of intermediary products of GS analogs[a]

		Yield %	Mp (θ_m/°C)	$[\alpha]_D^{25}$ (°) (c 1, DMF)		Elemental analysis (%)			R_f^1	R_f^3
						C	H	N		
3	Boc-Val-Orn(Z)-Leu-Pro-OBzl	73	101—102	−55.0	$C_{41}H_{59}O_9N_5$	C: 64.29 F: 64.19	7.76 7.85	9.15 8.88	0.66	0.60
4	Boc-Val-Orn(Z)-Leu-Pro-OH[b]	62	105—109	−45.6	$C_{34}H_{53}O_9N_5 \cdot 1/2H_2O$	C: 59.63 F: 59.38	7.95 7.75	10.23 10.36	0.45	0.48
5	Boc-D-Phe-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Leu-Pro-OH	56	161—165	−55.8	$C_{81}H_{114}O_{17}N_{12}$	C: 63.68 F: 63.23	7.52 7.60	11.00 11.01	0.49	0.56
6	*cyclo*[-D-Phe-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-Leu-Pro-]	46	250—253	−90.3 (c 0.5)	$C_{76}H_{104}O_{14}N_{12}$	C: 64.75 F: 64.43	7.44 7.49	11.92 11.95	0.64	0.58
8	Boc-D-Phe-Val-Orn(Z)-Leu-Pro-OBzl	54	158—160	−25.5	$C_{50}H_{68}O_{10}N_6$	C: 65.77 F: 65.43	7.51 7.55	9.20 9.09	0.65	0.50
9	Boc-D-Phe-Val-Orn(Z)-Leu-Pro-OH	86	192—193	−18.3	$C_{43}H_{62}O_{10}N_6 \cdot 1/2H_2O$	C: 62.08 F: 62.14	7.63 7.63	10.10 10.10	0.38	0.42
10	Boc-[D-Phe-Val-Orn(Z)-Leu-Pro-]$_2$-OH	49	143—147	−18.4	$C_{81}H_{114}O_{17}N_{12} \cdot 2H_2O$	C: 62.21 F: 62.28	7.60 7.49	10.75 10.58	0.46	0.45
11	*cyclo*[-D-Phe-Val-Orn(Z)-Leu-Pro-]$_2$	61	289—290	−12.2 (c 0.5)	$C_{76}H_{104}O_{14}N_{12} \cdot 2H_2O$	C: 63.14 F: 63.38	7.53 7.47	11.63 11.46	0.65	0.53

a) Compounds **1** and **2** have been reported in the literature.[6] b) Mp 158—160 °C; $[\alpha]_D^{22}$ −62.2° (c 1, MeOH) in the literature.[8]

Table 2. Yields, physical properties and analytical data of GS analogs

7 *cyclo*(-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-Pro-D-Phe-)·2HCl

Yield, 56%; mp 262—265 °C (dec); $[\alpha]_D^{28}$ −140.0° (c 0.2, EtOH) R_f^3 0.55, R_f^4 0.75. Amino acid ratios: Val, 1.01; Orn, 0.95; Leu, 1.01; Phe, 1.04; Pro, 1.00. MS (FAB), *m/z* 1141 ($C_{60}H_{93}O_{10}N_{12}$, MH+). Fonnd: C, 55.97; H, 7.98; N, 12.96%. Calcd for $C_{60}H_{92}O_{10}N_{12} \cdot 2HCl \cdot 4H_2O$: C, 56.01; H, 7.99; N, 13.06%.

12 *cyclo*(-Val-Orn-Leu-Pro-D-Phe-)$_2$·2HCl

Yield, 61%.; mp 262—265 °C (dec); $[\alpha]_D^{28}$ −43.4° (c 0.2, EtOH) R_f^3 0.50, R_f^4 0.77. Amino acid ratios: Val, 1.00; Orn, 0.95; Leu, 1.01; Phe, 1.02; Pro, 1.03. MS (FAB), *m/z* 1141 ($C_{60}H_{93}O_{10}N_{12}$, MH+). Found: C, 55.16; H, 8.07; N, 12.80%. Calcd for $C_{60}H_{92}O_{10}N_{12} \cdot 2HCl \cdot 5H_2O$: C, 55.25; H, 8.04; N, 12.89%.

Table 3. Antibiotic activity of GS and its analogs[a]

Test organisms	GS	[L-Pro⁴, D-Phe⁵]-GS	[L-Pro⁴,⁴′, D-Phe⁵,⁵′]-GS
Staph. aureus ATCC 6538 P	1.6	3.1	50
Strept. pyogenes N.Y. 5	1.6	3.1	25
Micrococcus flavus ATCC 10240	0.8	0.8	25
Corynebact. diphtheriae P.W. 8	0.8	1.6	50
Bac. subtilis ATCC 6633	3.1	3.1	25
E. coli NIHJ-JC2	>100	>100	>100
Proteus vulgaris OX 19	>100	>100	>100

a) Minimum inhibitory concentration in μg/ml: The minimum amount of the compounds necessary for the complete inhibition of growth was determined by a agar dilution method with 10^6 organisms per milliter.

toward several microorganisms are summerized in Table 3. [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS showed little activity. This indicates that the positions of the two Pro residues of GS can not be transposed without a loss of activity. Several gratisin isomers, in which Pro residues occupy positions different from esch other, showed strong activity.[6] The significant difference in this regard may result from the distinction of their rings.

The activity of [L-Pro^4, D-Phe^5]-GS against *Bacillus subtilis* and *Micrococcus flavus* was the same as that of GS, and the activity toward other organisms tested was a little less compared to GS. This shows that a single D-Phe–Pro sequence in the β-turn part of GS can be inverted without affecting the activity. Similar results have been reported by Sofuku *et al.* for [δ-Ava$^{4-5}$]- and [δ-Ava$^{4-5,4'-5'}$]-GS, in which D-Phe–Pro sequences of GS are replaced, singly or together, with a δ-Ava residue.[7] That is, [δ-Ava$^{4-5}$]-GS showed antibiotic activity, but [δ-Ava$^{4-5,4'-5'}$]-GS not active. Recently, in studies on [L-Pro$^{4,4'}$, D-Ala$^{5,5'}$]-GS, in which D-Phe–Pro sequences in GS were replaced with Pro–D-Ala sequence, it was reported that this analog showed no activity,[8] and that it could take a GS-like conformation in an aqueous solution, but its population decreased in a less-polar environment.[9] In the present studies, it is likely that when the synthetic analogs interact with a cell membrane of target microorganisms, the presence of a D-Phe–Pro sequence in [L-Pro^4, D-Phe^5]-GS makes this analog feasible to adopt the GS-like conformation, while [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-GS cannot take the GS-like conformation.

Experimental

The synthetic manners of the analogs are similar to those reported in a previous paper of this series.[5] The yields, physical properties and analytical data of the intermediary and final products are summerized in Tables 1 and 2. All melting points are uncorrected. The molecular weights of GS analogs were determined by fast-atom-bomberdment (FAB) mass spectrometry using a JMS D-300 mass spectrometer. Amino acid analyses were carried out using a Hitachi 835 amino acid analyzer, after the hydrolysis of peptides in 6 M† HCl at 110 °C for 24 h. Thin-layer chromatography was performed on Merck silica-gel F_{254} plates with the following solvent systems (v/v): R_f^1, $CHCl_3$–MeOH (9:1); R_f^2, $CHCl_3$–MeOH–AcOH (95:5:3); R_f^3, *n*-BuOH–AcOH–H_2O (4:1:1); R_f^4, *n*-BuOH–pyridine–AcOH–H_2O (4:1:1:2). The yields of peptides **3** and **8** were calculated on the basis of the amount of Pro-OBzl as a starting material. The CD spectra shown in Fig. 2 were obtained using a JASCO spectropolarimeter (model J-500). The CD spectroscopy of GS and the analogs was carried out with an aqueous solution of their dihydrochlorides. Cellulose plate electrophoresis was carried out with a cellulose (Avicel) plate and with a solvent system of HCOOH–AcOH–MeOH–H_2O (1:3:6:10 v/v, PH 1.4) for 2 h at 500 V/20 cm. Each of the analogs revealed a single spot, the mobility being the same as that of GS.

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† 1 M=1 mol dm^{-3}.

References

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4) Amino acid residues with no plefix are of L-configuration. The abbreviations for amino acids and peptides are in accordance with the rules of IUPAC-IBU Commission of Biological Nomencalture. Abbreviations used are as follows: Boc, *t*-butoxycarbonyl; Z, benzyloxycarbonyl; OBzl, benzyloxy; WSCD, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOBt, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid.

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