Synthesis and Properties of Gramicidin S Analogs Consisting of Eight Amino Acid Residues

Makoto TAMAKI* and Sadatoshi AKABORI
Department of Chemistry, Faculty of Science, Toho University,
Miyama, Funabashi-shi, Chiba 274
(Received March 20, 1991)

Synopsis. Three cyclic octapeptides (des-Val$^{1,1'}$-gramicidin S, des-Leu$^{3,3'}$-gramicidin S and des-Val^{1},Leu$^{3'}$-gramicidin S), showed no antibiotic activity. Although their CD spectra resembled each other, they differed from that of gramicidin S. These results indicate that the removal of two of the Val and/or Leu residues at positions 1, 1', 3, and 3' of gramicidin S greately affects both the antibiotic activity and conformation.

Gramicidin S (GS)[1] is an antibiotic cyclodecapeptide, cyclo(–Val–Orn–Leu–D-Phe–Pro–)$_2$,[2] with a rigid β-pleated sheet conformation.[3] In order to investigate the contribution of the ring size to both the antibiotic activity and conformation, many GS analogs with a variety of ring sizes have been synthesized.[4–6] Recently, in synthetic studies of several cyclododeca- and cyclotetradecapeptides related to GS and gratisin (GR), we have reported that the primary structure of GS with ten residues is not always necessary for exhibiting strong activity, and that the CD spectra of these synthetic peptides mainly reflect the amino acid sequence around their β-turn part (–D-Phe–Pro–).[7–9] However, we have not yet evaluated the structure-activity correlation of its analogs with a smaller ring size than that of GS.

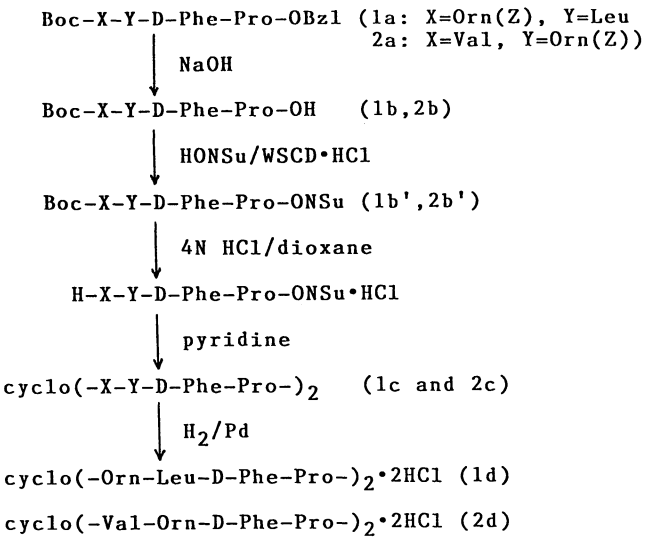

Fig. 1. Synthetic scheme of des-Val$^{1,1'}$- and des-Leu$^{3,3'}$-GS.

In this paper we describe the syntheses, antibiotic activity, and CD spectra of three cyclooctapeptides (cyclo(–Orn–Leu–D-Phe–Pro–)$_2$ (des-Val$^{1,1'}$-GS, **1d**), cyclo(–Val–Orn–D-Phe–Pro–)$_2$ (des-Leu$^{3,3'}$-GS, **2d**), and cyclo(–Orn–Leu–D-Phe–Pro–Val–Orn–D-Phe–Pro–) (des-Val^{1},Leu$^{3'}$-GS, **3d**)) which lack two residues of the Val or Leu residues at positions 1, 1', 3, and 3' of GS. We also discuss the contribution of the ring size to both the antibiotic activity and conformation.

The synthetic routes of these analogs are shown in Figs. 1 and 2. These methods are similar to those described in previous papers of this series.[7,10] In the syntheses of des-Val$^{1,1'}$- and des-Leu$^{3,3'}$-GS, cyclizations of H–Orn(*Z*)–Leu–D-Phe–Pro–ONSu (**1b'**) and H–Val–Orn(*Z*)–D-Phe–Pro–ONSu (**2b'**) were carried out in pyr-

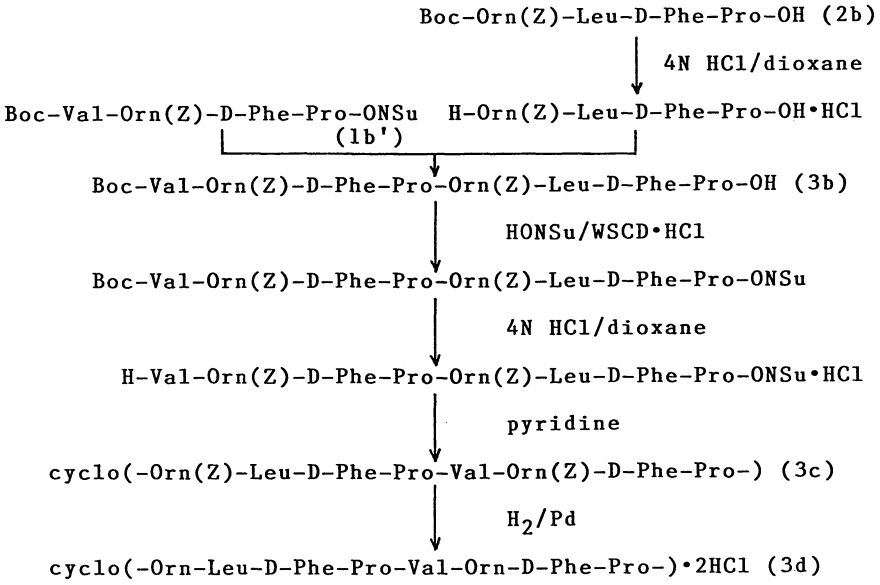

Fig. 2. Synthetic scheme of des-Val^{1}, Leu$^{3'}$-GS.

idine at 25 °C for 1 d. The final concentration of the active esters was 3 mM (1 M=1 mol dm^{-3}). High-performance liquid chromatography (HPLC) profiles of the crude cyclic products are shown in Fig. 3. The molecular weight of each cyclic product isolated by semipreparative HPLC was determined by FAB mass spectrometry. The cyclic products from the tetrapeptide active esters have widely different molecular weights. Compound **1b′** produced the cyclic dimer (30%), trimer (4%), and tetramer (0.5%); Compound **2b′** produced the cyclic dimer (35%), trimer (6%), and tetramer (0.5%). These active esters, however, did not produce a cyclic monomer. The numbers in parentheses are the yields of each cyclic product calculated from **1b** and **2b**. Recently, regarding the cyclization of H–Val–Orn(*Z*)–Leu–D-Phe–Pro–ONSu and H–Tyr(Bzl)–Val–Orn(*Z*)–Leu–D-Phe–Pro–ONSu containing the D-Phe–Pro sequence at the C-terminal, we reported that the former yields exclusively the cyclic dimer, while the latter produces the cyclic monomer and dimer.[10] The present results indicate that the lengths of these active esters greatly affect their mode of cyclization.

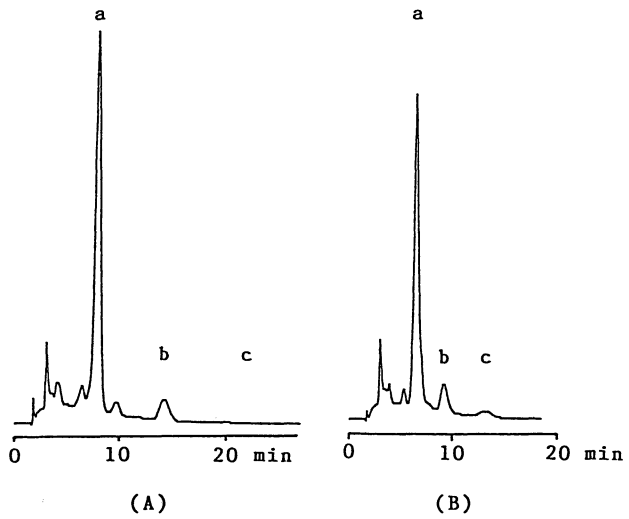

Fig. 3. HPLC profiles[a] of products in cyclization of H–Orn(*Z*)–Leu–D-Phe–Pro–ONSu (A) and H–Val–Orn(Z)–D-Phe–Pro–ONSu (B).
(a: cyclic dimer, b: cyclic trimer, c: cyclic tetramer)
a) Elution solvent used in these HPLC analysis was MeOH–H_2O (6 : 1).

The yields, physical properties and analytical data of intermediary products and GS analogs are summarized in Tables 1 and 2.

Des-Val$^{1,1'}$-GS, des-Leu$^{3,3'}$-GS and des-Val^{1}, Leu$^{3'}$-GS showed no antibiotic activity against all of the microorganisms tested. We also found that cyclo(–Orn–D-Phe–Pro–)$_2$ shows no activity.[11] On the other hand, we have reported that some cyclododecapeptides related to GR with various seqeunces at the β-turn part show strong activity,[7,8] and that several cyclotetradecapeptides related to GS possess 1/4—1/8 activity of GS.[9] These results indicate that removing either two or four of the Val and/or Leu residues at positions 1, 1′, 3, and 3′ from GS results in a loss of activity, while the addition of two or four amino acid residues to GS makes it feasible to take a suitable conformation for exhibiting antibiotic activity.

The CD spectra of these synthetic peptides and GS in an aqueous solution are shown in Fig. 4. These CD

Table 1. Yields and Analytical Data of Intermediary Products of GS Analogs

	Yield	Mp	$[\alpha]_D^{25}$/°		Elemental analysis/%						
	%	°C	(*c* 1, DMF)			C	H	N		C	H	N
1a.	60[a]	89—92	−35.4	$C_{45}H_{59}O_9N_5 \cdot 0.5H_2O$	Found:	65.79	7.34	8.70	Calcd:	65.67	7.35	8.51
1b.	92	94—96	−40.7	$C_{38}H_{53}O_9N_5 \cdot 0.5H_2O$	Found:	62.59	7.70	9.02	Calcd:	62.28	7.43	9.35
1c.	30	120—126	−6.7[b]	$C_{66}H_{86}O_{12}N_{10} \cdot H_2O$	Found:	64.18	7.24	11.13	Calcd:	64.47	7.21	11.39
2a.	67[a]	72—75	−21.7	$C_{44}H_{57}O_9N_5 \cdot 0.5H_2O$	Found:	65.27	7.20	8.82	Calcd:	65.33	7.23	8.66
2b.	97	73—78	−27.3	$C_{37}H_{51}O_9N_5$	Found:	62.25	7.28	9.38	Calcd:	62.61	7.24	9.87
2c.	35	131—137	+8.7[b]	$C_{64}H_{82}O_{12}N_{10} \cdot 1.5H_2O$	Found:	63.12	6.97	11.65	Calcd:	63.51	7.08	11.57
3b.	87	116—122	−36.0	$C_{70}H_{94}O_{15}N_{10} \cdot 1.5H_2O$	Found:	62.74	7.22	10.42	Calcd:	62.62	7.28	10.43
3c.	64	120—125	−0.3[b]	$C_{65}H_{84}O_{12}N_{10} \cdot 2H_2O$	Found:	63.37	7.03	11.49	Calcd:	63.29	7.19	11.36

a) The yields of **1a** and **2a** were calculated on the basis of the amount of Pro–OBzl · HCl as a starting material.
b) *c* 0.5.

Table 2. Yields and Analytical Data of GS Analogs

1d.	Yield, 80%; mp, 232—235 °C; $[\alpha]_D^{25}$−97.1° (*c* 0.5, EtOH); MS(FAB) *m/z* 944 (MH^+). Amino acid analysis: Orn, 1.96; Leu, 2.07; Phe, 1.97; Pro, 2.00. Found: C, 55.82; H, 7.60; N, 12.96%. Calcd for $C_{50}H_{74}O_8N_{10} \cdot 2HCl \cdot 3.5\ H_2O$: C, 55.65; H, 7.75; N, 12.98%.
2d.	Yield, 80%; mp, 251—254 °C; $[\alpha]_D^{25}$−42.2° (*c* 0.5, EtOH); MS(FAB); *m/z* 915 (MH^+). Amino acid analysis: Val, 2.09; Orn, 2.03; Phe, 1.95; Pro, 1.92. Found: C, 53.31; H, 7.29; N, 12.80%. Calcd for $C_{48}H_{70}O_8N_{10} \cdot 2HCl \cdot 5H_2O$: C, 53.47; H, 7.67; N, 12.99 %.
3d.	Yield, 90%; mp, 226—230 °C; $[\alpha]_D^{25}$−108.8° (*c* 0.5, EtOH); MS(FAB) *m/z* 929 (MH^+). Amino acid analysis: Val, 1.05; Orn, 2.00; Leu, 1.07; Phe, 1.90; Pro, 1.99. Found: C, 54.30; H, 7.41; N, 12.89 %. Calcd for $C_{49}H_{72}O_8N_{10} \cdot 2HCl \cdot 4.5H_2O$: C, 54.34; H, 7.72; N, 12.93%.

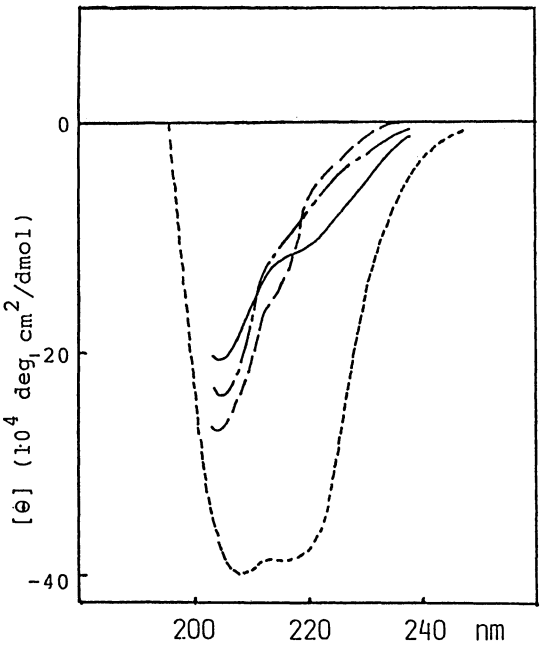

Fig. 4. CD spectra of GS and its analogs in aqueous solution.
des-Val$^{1,1'}$-GS, ——; des-Leu$^{3,3'}$-GS, ——; des-Val^{1}, Leu$^{3'}$-GS, — - —; GS, ------.

spectra resemble each other; although a trough is observed at 204 nm and a shoulder near 217 nm, their CD patterns differ from that of GS, even though these peptides possess the D-Phe–Pro seqeunce. We recently reported that the CD spectra of cyclo(–Val–Orn–Leu–D-Phe–Pro–Tyr–)$_2$ and cyclo(–Val–Orn–Leu–Leu–D-Phe–Pro–Leu–)$_2$ having the D-Phe–Pro sequence are similar to that of GS.[7,9] On the other hand, the CD spectrum of cyclo(–Orn–D-Phe–Pro–)$_2$ resembles those of the synthetic cyclooctapeptides, rather than GS; a trough was observed near 200 nm and a shoulder near 220 nm.[12] Kopple et al. reported from NMR studies that this cyclohexapeptide possesses a β-turn caused by a D-Phe–Pro seqeunce as GS.[13] Further, the same β-turn was also found in cyclo(–Ala–Gly–D-Phe–Pro–)$_2$, which possesses the Ala–Gly sequence in place of the Orn–Leu or Val–Orn sequence of the cyclooctapeptides synthesized in our studies.[14] These facts suggest that the synthetic cyclooctapeptides have a β-turn similar to that of GS, and that the difference between these synthetic cyclooctapeptides and GS in CD spectra may result from removing two of Val and / or Leu residue from GS, in other words, a distinction of the ring size.

Experimental

All melting points are uncorrected. The CD spectra were measured by a JASCO J-500 spectropolarimeter at a concentration of 1.5—2.0×10^{-4} M. The molecular weights of these synthetic peptides were determined by FAB mass spectrometry using a JEOL JMS-D-300 mass spectrometer. Amino acid analyses were carried out using a Hitachi 835 amino acid analyzer after hydrolysis in 6 M HCl at 110 °C for 24 h. HPLC analysis was carried out using an 800 series (JASCO). A Finepak SIL C18 column (10 μm, 250×4.6 mm I.D., or 250×6.7 mm I.D., JASCO) was used: flow rate, 1 ml min^{-1}; solvent, MeOH–H_2O (6 : 1) or MeOH-5%–$NaClO_4$ (4 : 1); monitoring wavelength, 220 nm.

Microbiological Assays. The microorganisms employed were *Staphylococcus aureus* ATCC 6538, *Streptomyces pyogenes* N.Y.5, *Corynebacterium diphtheriae* P.W.8, *Micrococcus pyogenes* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ-JC2, and *Proteus vulgaris* OX 19. The minimum concentration of compounds necessary to completely inhibit the growth of these microorganisms was determined by an agar dilution method with 10^6 organisms per milliliter.

We thank Professor Ichiro Muramatsu, Rikkyo University, for helpful discussion. We also thank the staff of the Research Laboratories of Toyo Jozo Co. for elemental analyses, microbiological assays, and measurement of FAB mass spectra.

References

1) A. R. Battersby and L. C. Craig, *J. Am. Chem. Soc.*, **73**, 1887 (1951).
2) The abbreviations used in this paper are those recommended by IUPAC-IBU: *Eur. J. Biochem.*, **138**, 9 (1984). Additional abbreviations: HONSu, *N*-hydroxysuccinimide; WSCD · HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride.
3) D. C. Hodgkin and B. M. Oughton, *Biochem. J.*, **65**, 752 (1957).
4) N. Izumiya, T. Kato, H. Aoyagi, M. Waki, and N. Kondo, "Synthetic Aspects of Biologically Active Cyclicpeptide-Gramicidin S and Tyrocidines," Kodansha, Tokyo, Halsted Press, New York (1979), pp. 49—70.
5) M. Waki and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **40**, 1687 (1967).
6) S. Matsuura, M. Waki, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **46**, 977 (1973).
7) M. Tamaki, *Bull. Chem. Soc. Jpn.*, **57**, 3210 (1984).
8) M. Tamaki, M. Takimoto, and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, **60**, 2101 (1987).
9) M. Tamaki, M. Takimoto, and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, **61**, 3925 (1988).
10) M. Tamaki, M. Takimoto, M. Hayashi, and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, **62**, 594 (1989).
11) Cyclo(–Orn–D-Phe–Pro–)$_2$ was synthesized by Kopple et al.,[13] but its antibiotic activity has not been reported yet. We synthesized this peptide according to the similar method described in the synthesis of **3d**, and measured the antibiotic activity of this peptide.
12) C. A. Bush, S. K. Sarkar, and K. D. Kopple, *Biochemistry*, **17**, 4951 (1978).
13) K. D. Kopple, A. Co, T. J. Schauper, and C. S. Wilcox, *J. Am. Chem. Soc.*, **95**, 6090 (1973).
14) E. Blout, *Biopolymers*, **20**, 1901 (1981).