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FACILE SYNTHESIS OF GRAMICIDIN S VIA CYCLIZATION OF A LINEAR PENTAPEPTIDE

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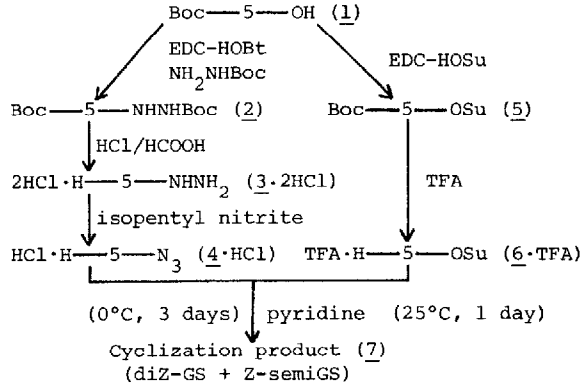
Abstract: Azide and *N*-hydroxysuccinimide ester of five pentapeptides related to gramicidin S (cyclic decapeptide) were cyclized to determine the ratio of dimer to monomer in the cyclization product, the pentapeptide derivative with D-Phe as *C*-terminus giving the dimer in good yield.

Gramicidin S (GS) is a cyclic decapeptide antibiotic with the repeated pentapeptide sequence of -Val-Orn-Leu-D-Phe-Pro-.[1] Waki and Izumiya observed the formation of a mixture composed of cyclic dimer (diZ-GS) and cyclic monomer (Z-semiGS) in 7:3 ratio by cyclization of pentapeptide active ester with Pro as *C*-terminus; they isolated the desired diZ-GS by fractional crystallization.[2] Recently, Nonaka *et al.* cyclized H-D-Phe-Pro-Val-Orn(Z)-Leu-OSu (6b) expecting to obtain only the cyclic dimer because 6b has same pentapeptide sequence as found in the biosynthetic precursor of GS, but they observed the formation of a mixture with diZ-GS and Z-semiGS in 4:6 ratio.[3] Thus it is interesting to examine how variation in amino acid sequence in linear pentapeptide precursors related to GS influences the composition and yield of the cyclization product. We describe here the mode of cyclization of pentapeptides in which Val, Orn(Z), D-Phe or Pro occupies the *C*-terminus to search the most favorable precursor among five pentapeptides for the chemical synthesis of GS.

A key intermediate, Boc-pentapeptide acid (Boc-5-OH) (1),[4] was prepared by stepwise elongation method using Boc-amino acid-OSu. Compound 1 was subjected to cyclization by either the azide or OSu method as shown in Scheme. The weight ratio of diZ-GS and Z-semiGS in 7 was determined by carboxymethylcellulose column chromatography of the hydrogenated material of 7.[2] The results of the cyclization of five pentapeptides by the two activation methods are summarized in Table 1.



Scheme

Table 1. Cyclization of pentapeptide precursors related to GS.[a)]

Pentapeptide precursor[b)]	Cyclization method					
	Azide			OSu		
	Dimer : Monomer	Yield(%)[c)]		Dimer : Monomer	Yield(%)[d)]	
H-Orn(Z)-Leu-D-Phe-Pro-Val-X	35 : 65	90		62 : 38	89	
H-Leu-D-Phe-Pro-Val-Orn(Z)-X	67 : 33	75		77 : 23	57	
H-D-Phe-Pro-Val-Orn(Z)-Leu-X[e)]	25 : 75	45		43 : 57	60	
H-Pro-Val-Orn(Z)-Leu-D-Phe-X	81 : 19	78		89 : 11	46	
H-Val-Orn(Z)-Leu-D-Phe-Pro-X	67 : 33	55		81 : 19	75	

a) Concentration of precursors in pyridine: 3×10^{-3} M. b) X = N_3 or OSu.
c) Calculated from 2. d) Calculated from 5. e) From Ref. 3.

Table 1 shows the most predominant formation of diZ-GS by cyclization of H-Pro-Val-Orn(Z)-Leu-D-Phe-N_3 (4a) or H-Pro-Val-Orn(Z)-Leu-D-Phe-OSu (6a). The highest yield (63%) of diZ-GS can be calculated in the cyclization product, 7a, derived from 4a. In the cases of the pentapeptide precursors with Orn(Z) and Pro as *C*-terminus, dimeric cyclization was also predominant. On the contrary, preferential monomerization was observed in the cases of the precursors with *C*-terminal Leu (both activation methods) and with *C*-terminal Val (the azide method). Steric nature of terminal amino acids and conformation of peptide in these precursors may be important factors governing these marked differences in the mode of cyclization.

To demonstrate the facile synthesis of GS, 4a was subjected to cyclization on a preparative scale (0.5 mmol) at 30 mM reactant concentration in pyridine. DiZ-GS was isolated from 7a by fractional crystallization or Sephadex LH-20 column chromatography.[2)] Hydrogenolysis of the diZ-GS afforded GS·2HCl in 50% yield from 2a: mp 273-275°C (dec); $[\alpha]_D^{20}$ -270° (*c* 0.1, EtOH) (lit.[2)] mp 274-276°C (dec); $[\alpha]_D^{20}$ -271° (EtOH)). Analysis of the diZ-GS by the modified Manning-Moore procedure[5)] showed no racemization of D-Phe residue, but slight racemization of D-Phe in diZ-GS obtained from 6a.

Thus the cyclization of linear pentapeptide precursors related to GS with D-Phe as *C*-terminus by the azide method will provide an effective route for the synthesis of GS or its analogs without racemization.

References and Notes

1) Abbreviations according to IUPAC-IUB Commission, *J. Biol. Chem.*, 247, 977 (1972), are used throughout. Other abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOBt, *N*-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; Orn(Z), δ-benzyloxycarbonyl-L-ornithyl; OSu, *N*-hydroxysuccinimide ester; TFA, trifluoroacetic acid. All amino acids are of L-configuration except for D-Phe.

2) M. Waki and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 40, 1687 (1967).

3) K. Nonaka, K. Kamekawa, K. Sato, M. Waki, T. Kato and N. Izumiya in "Peptide Chemistry 1977" ed. by T. Shiba, p. 135, Protein Research Foundation, Osaka (1978).

4) Satisfactory elemental analyses were obtained for all crystalline compounds.

5) Y. Shimohigashi, S. Lee and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 49, 3280 (1976).

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