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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 8475–8478

Biomimetic formation of gramicidin S by dimerization–cyclization of pentapeptide precursor on solid support

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Received 22 August 2006; revised 27 September 2006; accepted 28 September 2006
Available online 17 October 2006

Abstract—The biomimetic formation of gramicidin S, cyclo(-D-Phe-Pro-Val-Orn-Leu-)$_2$, by the dimerization and cyclization of pentapeptide precursor without the protection of δ-amino group of the Orn residue was examined on a solid support. The cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime on a resin with an oxime group of 0.62 mmol/g in 1,4-dioxane directly gave gramicidin S in a 50% yield. The dimerization–cyclization mode on the solid support was similar to that of the biosynthesis of gramicidin S on an enzyme.

Gramicidin S (GS),[1,2] cyclo(-D-Phe-Pro-Val-Orn-Leu-)$_2$, is an antibiotic cyclodecapeptide isolated from *Bacillus brevis* ATCC9999. In *Bacillus brevis*, GS is produced via the dimerization and cyclization of a pentapeptide fragment (-D-Phe-Pro-Val-Orn-Leu-) having an Leu residue at the C-terminus on GS synthetase.[3]

In 1957, the synthesis of GS by the cyclization of the pentapeptide precursor (H-Val-Orn(Tos)-Leu-D-Phe-Pro-*p*-nitrophenyl ester) was reported by Schwyzer and Sieber.[4] Since then, various analogous of GS have been synthesized by the dimerization and cyclization of pentapeptide precursors having a D-Phe, Pro, Val, Orn(Z), and Leu residue at the C-terminus.[5] In these cyclizations, *p*-nitrophenyl ester, *N*-hydroxysuccinimide ester, and azide methods are most often used for the cyclization of precursor peptides.[5] However, these methods are multistep processes suffering from low yields and racemization.[6] On the other hand, in the preparations of GS and its analogous using solid-phase peptide synthesis, many difficult challenges lie in the C-terminus activation and cyclization of the linear precursor on a solid support.[7] Recently, biomimetic syntheses of GS and its derivatives on a solid support have been reported by some research groups.[8] In these reported synthetic methods, the cyclizations of precursor peptides were carried out without the protection of the δ-amino group of the Orn residue on a solid support. However, the linear decapeptide (-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-) as a precursor peptide was used. Hence the mode of cyclization in the reported synthetic methods is significantly different from that of the biosynthesis of GS in *Bacillus brevis*.

In this study, we would like to report the novel biomimetic synthesis of GS via dimerization and cyclization of pentapeptide precursor (-D-Phe-Pro-Val-Orn-Leu-) without the protection of the δ-amino group of the Orn residue on a solid support.

Using Boc-solid phase peptide synthesis, a fully deprotected linear peptide oxime, H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) mimicking the biosynthetic precursor of GS was synthesized on a resin[9] (loading of oxime group: 0.48 mmol/g resins). The formation of the cyclic peptide by the cyclization-cleavage of oxime **1** on the resin was performed in several solvents (1,4-dioxane, CCl_4, benzene, CH_2Cl_2, pyridine, ethanol, and DMF) with 2 equiv of *N*,*N*-diisopropylethylamine and AcOH for 1 day at 25 °C (concentration of peptide in solution; 3×10^{-3} M).[10] The yields and contents of GS and semi-GS were determined by HPLC analysis (Table 1). The cyclization of oxime **1** on the resin in CCl_4 and benzene mainly gave GS (cyclic dimer) in 15% and 19% yields, respectively, and the production of semi-GS (cyclic monomer) was not detected by HPLC analysis. On the other hand, the cyclizations of oxime **1** on the resin in other solvents afforded semi-GS and GS. The cyclization in 1,4-dioxane gave the best yield among several

Keywords: Gramicidin S; Biomimetic synthesis; Dimerization–cyclization on solid phase; Oxime resin; Reaction mechanism.
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doi:10.1016/j.tetlet.2006.09.146

Table 1. Solvent effect on the cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on resin

Entry	Solvent	Ratio of cyclic products semi-GS:GS	Total yield (%) of semi-GS and GS
1	CCl_4	0:100	15
2	Benzene	0:100	19
3	1,4-Dioxane	18:82	49
4	CH_2Cl_2	20:80	24
5	Pyridine	20:80	43
6	DMF	25:75	32
7	Ethanol	18:82	11

solvents tried. The yields of semi-GS and GS were 9% and 40%, respectively (Table 1). Thus, the polarities of solvents significantly affect the cyclization mode of oxime **1** on the resin.

The cyclizations were carried out in different amounts of 1,4-dioxane (peptide concentration: 3×10^{-3} M and 0.6×10^{-3} M) at 25 °C for 1 day. The amounts of reaction solvents slightly affect the total yield and the ratios of GS and semi-GS. This result indicates that the three steps of dimerization, cyclization, and cleavage of precursor pentapeptide occur on the solid support.

Next, the effect of concentration of oxime **1** on the resin for the formation of GS was examined on resins with oxime of 0.48, 0.68, and 0.88 mmol/g. The ratios of GS and semi-GS depended directly on the concentration of oxime **1** on the resin. However, the use of the resin with 0.88 mmol/g gave very low yields (7.5%) of cyclic peptides. On the other hand, the use of the resin with 0.68 mmol/g gave GS and semi-GS with the best yield among three resins tried in this study (Fig. 1). The yields of semi-GS and GS were 7% and 50%, respectively. Thus, the peptide concentration on the resin significantly affects the yield of GS.

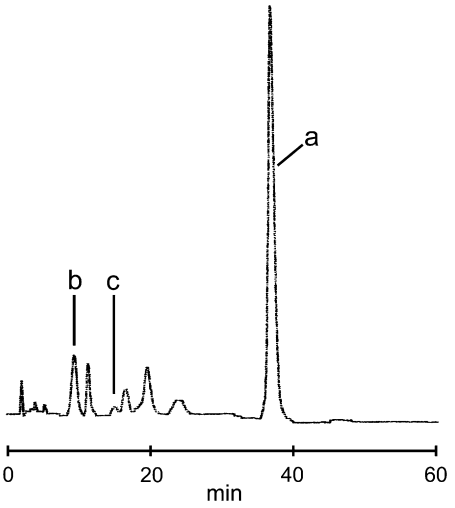

Figure 1. HPLC profile of crude product of the cyclization of peptide-oxime **1** on resin with oxime group of 0.68 mmol/g in 1,4-dioxane. a = GS, b = semi-GS, c = H-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu.

To investigate the mode of the cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on the resin, the cyclizations of several derivatives **2**–**4**, Z-D-Phe-Pro-Val-Orn-Leu-oxime (**2**), H-D-Phe-Pro-Val-Orn(Z)-Leu-oxime (**3**), and H-(D-Phe-Pro-Val-Orn-Leu-)$_2$-oxime (**4**), were performed on the resin with oxime groups of 0.68 mmol/g at 25 °C for 1 day using 1,4-dioxane as a solvent.

The main products of each cyclization were confirmed on HPLC analysis by direct comparison with authentic samples.[5e–g] In the cyclization of oxime **2**, cyclic dimer, Z-D-Phe-Pro-Val-Orn-Leu-Leu-Orn-Val-Pro-D-Phe-Z,[5f,h] produced in a 56% yield, but the produce of cyclic monomer, Z-D-Phe-Pro-Val-Orn-Leu,[5f,h] having a highly strained nine-membered ring, could not be found on HPLC. In addition, the cyclization of oxime **3** gave diZ-GS (the benzyloxycarbonyl derivative of GS)[5e] and Z-semi-GS[5e] in 53% and ~3%, respectively. These results suggested that in the intramolecular cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on the resin, the C-terminal oxime reacts very slowly with the α-amino group of the D-Phe residue to give the semi-GS, but does not react with the δ-amino group of the Orn residue under the condition used.

Next, the cyclization of oxime **4** under conditions similar to those in the case of the reaction of oxime **1** on the resin gave GS and H-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu[5f] in 56% and 15% yields, respectively. These results indicated that in the cyclization of decapeptide precursor on resin, the C-terminal oxime reacts with both the α-amino group of D-Phe residue and the δ-amino group of the Orn residue near the N-terminal. On the other hand, the cyclization of oxime **1** on the resin gave GS in a 50% yield, but the produce of H-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu[5f] be slightly found on HPLC (Fig. 1). These results indicated that in the synthetic process of GS from H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on the resin, the formation of decapeptide precursor, H-(D-Phe-Pro-Val-Orn-Leu-)$_2$-oxime, as an intermediate is for a moment.

On the basis of the above results, the mode of biomimetic synthesis of GS from H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on the resin is proposed as follows. When GS is synthesized by the dimerization–cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on the resin, the two pentapeptide-oximes (**1**) on the solid support take an anti-parallel type of conformation that brings the N- and C-terminals into close proximity (Fig. 2). Next, in an intermolecular reaction, the decapeptide-oxime was formed by the coupling of the two H-D-Phe-Pro-Val-Orn-Leu-oximes (**1**) on the resin and then cyclized to give GS with a great facility. Finally, the head-to-tail cyclization of the decapeptide precursor directly separates GS from the solid support.

In summary, the present protocol provides a practical method for the biomimetic synthesis of GS and its analogous from their fully deprotected pentapeptide precursors oxime on a resin. It is interesting that both in

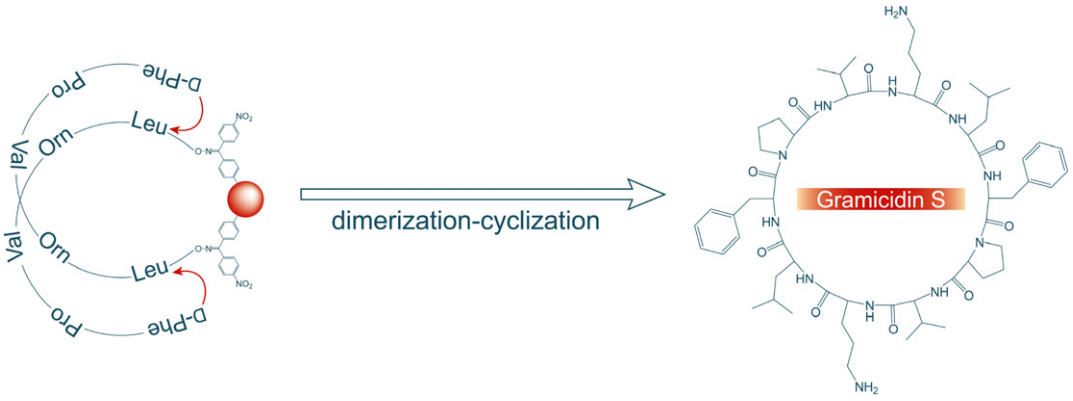

Figure 2. Biomimetic formation of gramicidin S by the dimerization–cyclization of H-D-Phe-Pro-Val-Orn-Leu-oxime (**1**) on resin.

biological synthesis and in chemical synthesis on a solid phase, the formations of the cyclic peptides are subjected to a similar regiospecific control, although the conditions of their cyclizations are different from each other. This novel dimerization–cyclization method may be applicable to the synthesis of other natural cyclic peptides and its derivatives with a C_2 symmetry.

Acknowledgments

We are grateful to the staff of the Research Laboratories of Asahi Chemical Industry Company (Ohito, Shizuoka, Japan) for elemental analysis.

Supplementary data

Details of synthesis and cyclization of penta- and decapeptide-oxime on resin (**1**–**4**); HPLC profiles of the crude products of biomimetic cyclization of penta- and decapeptide-oxime on resin (**1**–**4**); CD, NMR and LR-Mass (FAB) spectra of GS synthesized in preparative scale on resin (13 pages). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.09.146.

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