ORGANIC LETTERS

2011
Vol. 13, No. 1
34−37

Solvent-Free Thermocyclization of the Unactivated Linear Gramicidin S Precursor and Analogues

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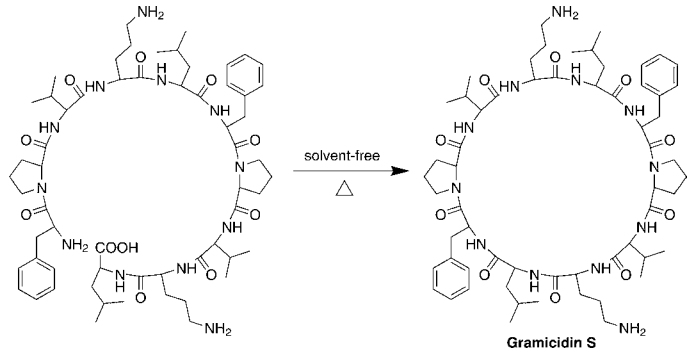
Received October 8, 2010

ABSTRACT



A convenient thermocyclization of the linear gramicidin S precursor and its analogues is demonstrated. With the preorganized β-sheet conformation, the unactivated linear precursors can cyclize into the corresponding head-to-tail cyclic products in high yield after being heated under solvent-free conditions.

Gramicidin S (**GS**) is a naturally occurring cationic antimicrobial peptide from *Bacillus brevis*[1] and is active against several bacteria and fungi.[2] Its synthesis has attracted intense efforts in the past decades due to its interesting bioactivity and its challenging structure.[3,4] The cyclization of the linear precursor is the major yield-limiting step, and this has been carried out in highly diluted solution with the assistance of activating agent or enzymes. The yield of cyclization is generally low to moderate, and it is difficult to get specific and high-yielding cyclized product. **GS**, cyclo(DPhe-Pro-Val-Orn-Leu)$_2$ (Figure 1), adopts a pleated β-sheet structure that is stabilized by four intramolecular hydrogen bonds between the Leu and Val residues. The Pro and DPhe residues hold two type II′ β-turns. In the most stable conformation, the hydrophobic residues of Leu and Val and the hydrophilic residues of Orn locate on opposite sides of the β-sheet

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10.1021/ol102800j
Published on Web 12/01/2010

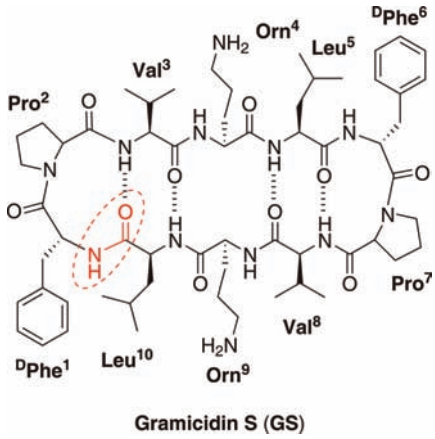
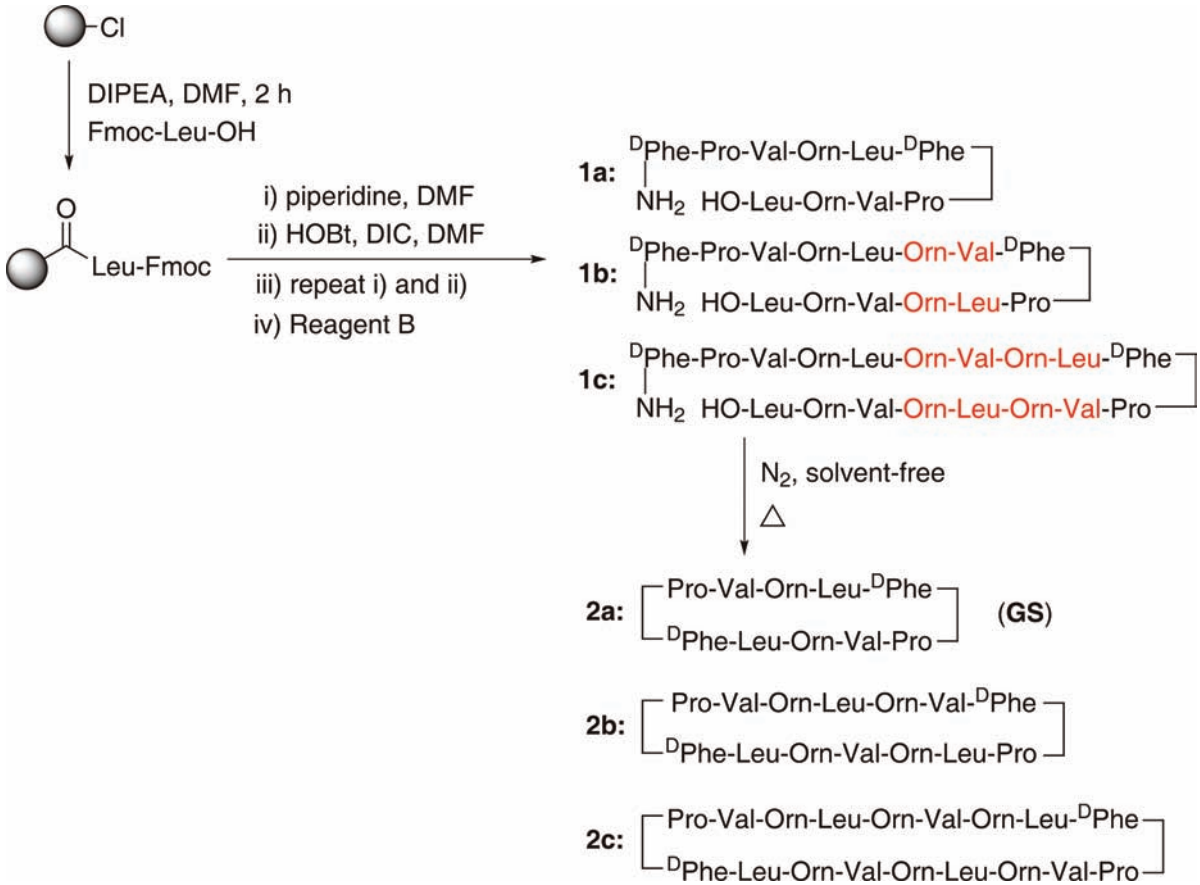


Figure 1. Conformation of GS.

molecule.[5] Molecular dynamics (MD) simulations showed that the linear precursor with a break between the ^{D}Phe^{1} and Leu^{10} residue, ^{D}Phe^{1}-Pro^{2}-Val^{3}-Orn^{4}-Leu^{5}-^{D}Phe^{6}-Pro^{7}-Val^{8}-Orn^{9}-Leu^{10} (**1a**, Scheme 1), keeps the β-sheet structure as a result of four intramolecular hydrogen bonds available in the **GS** molecule. The ^{D}Phe^{6} and Pro^{7} residues remain a typical II′ β-turns structure, and the C- and N-termini are close to each other.[6] The characteristic feature of linear peptide **1a** led us to study on the cyclization of the linear precursor into the corresponding cyclized peptide. In this study, we wish to report our preliminary results of the solvent-free head-to-tail thermocyclizations of the linear Gramicidin S precursor and its analogues.

As shown in Scheme 1, the linear precursor peptides were synthesized manually according to the standard Fmoc solid-phase peptide synthesis protocol.[7] The first amino acid, Fmoc-Leu-OH, was attached to the 2-chlorotrityl chloride resin. The linear peptides were coupled using HOBt and DIC as the coupling agents. The Fmoc deprotection and coupling efficiencies were detected with Kaiser's test.[8] The final linear peptides were cleaved from the resin with a mixture of trifluoroacetic acid/phenol/water/triisopropylsilane (88:5:5:2)[9] and were purified with HPLC. MD stimulations indicated that the β-sheet conformations of linear **GS** analogues could be stabilized by the intramolecular hydrogen bond between the Leu and Val residues.[3,6] On the basis of this observation, the linear peptide would still maintain the β-sheet structure if a pair of Orn-Val/Orn-Leu fragments (hydrogen bond formtion unit, red residues in Scheme 1) were inserted into the corresponding site of the linear strand. To further investigate the effect of extra hydrogen bonds on the conformation and the

Scheme 1. Syntheses and Thermocyclizations of Linear Peptides



efficiency of thermocyclization, linear precursors **1b** and **1c**, with one and two extra hydrogen bond formation units, respectively, were synthesized. The structures of the linear precursors were confirmed by mass and ^{1}H NMR spectra (Supporting Information).

Solid-phase circular dichroism (CD) spectra of **1a**−**c** were measured to investigate the conformation of synthesized linear peptides (Figure 2). CD spectra of **1a** and **1c** exhibited

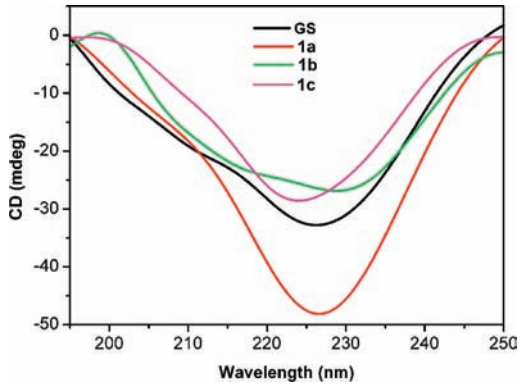


Figure 2. CD spectra of **GS** standard sample and the synthesized linear precursor peptides **1a**−**c** in the solid phase.

minima at ∼227 and ∼225 nm, respectively, which is similar to that of **GS**. The CD results indicated that **1a** and **1c** mainly adopt the β-sheet conformation like **GS** in solid state, which was also consistent with the MD simulation results.[3,6] The CD spectrum of **1b** exhibited a minimum at ∼229 nm with a shoulder at ∼220 nm because of its lower β-sheet content than **GS**.[10]

The linear precursor could cyclize by heating under solvent-free conditions in a nitrogen atmosphere (Table 1). After being heated at 150 °C for 4 h, linear precursor **1a** cyclized into cyclic peptide **2a** in 87% yield, and no **1a** could be detected by HPLC analysis (Figure 3). Compound **2a**

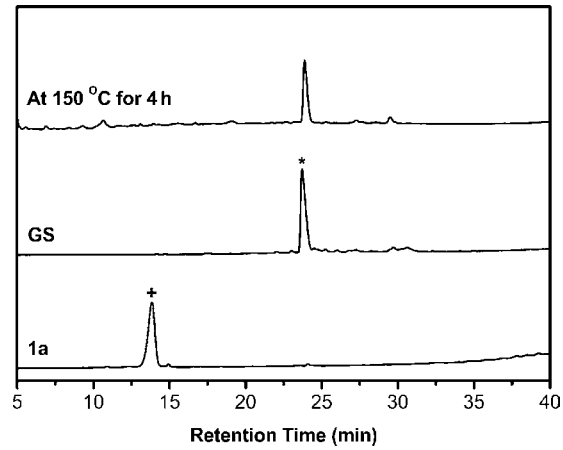


Figure 3. HPLC analysis of the thermocyclization product of **1a** at 150 °C for 4 h under solvent-free conditions: ∗, standard **GS** sample; +, **1a**.

showed the same HPLC retention time compared to the authentic **GS** sample. Mass and ^{1}H NMR spectra of **2a** were also identical to those of the authentic sample,[11] which could be regarded as the head-to-tail cyclized product. The thermocyclization occurred between the N- and the C-terminal functional residues of the linear precursor, which should be attributed to the preorganized β-sheet conformation of **1a**. This preorganization of the starting linear peptide would allow the maximum number of four intramolecular hydrogen bonds and place the N- and C-termini in a relatively close position, which further facilitated the cyclization reaction.[3] A range of experimental conditions for the thermocyclization were assayed (Table 1). Best results were obtained when the cyclizations were performed at 150 °C for 4 h. Lower

Table 1. Effect of Reaction Conditions on the Thermocyclization of Linear Peptides

linear peptides					cyclized products				
precursors	t_R[a] (min)	purity[b] (%)	reaction temp (°C)	reaction time (h)	calcd mass	$[M + H]^{+,c}$	t_R[a] (min)	yield[d] (%)	ratio (linear/cyclic)[e]
			180	2				41	–[f]
			150	4				87	–
			150	3				71	1:10.0
1a	14.2	97.1	150	2	1140.7	1141.4	23.9	51	1:2.8
			150	1				34	1:2.3
			130	4				53	1:1.1
			130	2				9	1:0.1
1b	15.2	91.4	150	4	1581.0	1582.2	26.7	56	1:1.7
1c	11.3	92.7	150	4	2022.3	505.6[4][g] 675.1[3][h]	35.3	93	1:15.1

[a] t_R means the HPLC retention time of the peptide peak. [b] Percentage of the peptide peak area over the total peak area between 5 and 40 min on the HPLC chromatogram. [c] Molecular ion from FAB-MS or ESI-MS. [d] The yield of the cyclized product is detected by HPLC chromatogram and calculated relative to the amount of pure linear precursor. [e] Ratio of the remaining linear precursor peak area to the cyclic product peak area of the reaction mixture on the HPLC chromatogram. [f] "–" means no linear precursor is found by HPLC in the reaction mixture. [g] The molecular ion is $[M + 4H]^{4+}$. [h] The molecular ion is $[M + 3H]^{3+}$.

reaction times or temperature resulted in lower yields, whereas higher temperature resulted in partial product degradation. Compounds **1b** and **1c** were then cyclized under the established conditions. The lower yields of compound **2b** can be accounted for by considering that its precursor **1b**, unlike **1a** and **1c**, is not a head-to-tail close peptide and has lower β-sheet content.[3]

In summary, we have shown on three examples that linear peptides that are structurally preorganized with a high content of β-sheet that makes the N- and C-termini close in space can be efficiently thermocyclized, without a need for activation. In particular, this method provides an economical and convenient method to obtain macrocyclic structures such as **GS**.

Acknowledgment. We acknowledge the financial support from the National Natural Science Foundation of China (No. 30801425). We thank Dr. Z. H. Guo, Hong Kong University of Science and Technology, for his helpful instruction and Dr. X. Z. Bu, Sun Yat-sen University, for his kind gift of an authentic sample of gramicidin S.

Supporting Information Available: Experimental procedures, MS and ^{1}H NMR spectra data of linear and cyclic peptides, and HPLC analysis of thermocyclization of **1b** and **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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