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Total synthesis of cyclic heptapeptide euryjanicin B

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Abstract

The first synthesis of the naturally occurring cyclic peptide euryjanicin B has been achieved. A general method was described to synthesize the cyclic peptide by a two-step solid-phase/solution synthesis strategy. All the amino acids in this study are *L*-configuration, The linear heptapeptide was assembled by standard Fmoc chemistry on solid-phase and subsequently cyclization was carried out by solution method.

Keywords: Cyclic peptide; Solid-phase synthesis; Cyclization; Purification; Euryjanicin B

An emerging class of proline-rich cyclic peptides was isolated mainly from Puerto Rican marine sponge *Prosuberites laughlini*. Homodetic cyclopeptides of the ''proline-rich'' class are mainly distributed in marine environments, but are found also in higher plants [1–3]. Cyclic peptides are of biological and chemico-pharmaceutical interest [4,5]. Structurally their mobility is more restricted than that of their linear counterparts so that they adopt fewer conformations and, as a result, show higher specificity for the target receptors [6]. In addition they are often more stable *in vivo*. Therefore, they present promising drug candidates. Because of these advantages, many laboratories have focused on the syntheses of cyclic peptides and cyclic depsipeptides, especially by solid-phase peptide synthesis methodologies [7].

In this paper we report the synthesis of euryjanicin B (Fig. 1) from the Puerto Rican marine sponge. The compound is characterized by the presence of seven amino acids with three proline residues [8]. When tested against the National Cancer Institute 60 tumor cell line panel, the purified euryjanicin B [9] displayed weak cytotoxicity.

We planned to obtain the cyclopeptide by combining solid-phase and conventional solution methods. The synthesis of linear euryjanicin B was approached by using a Fmoc/tBu protecting scheme and 2-chorotrityl chloride resin as a solid support. The synthetic approach produced the linear precursor and the target cyclic peptide with high efficiency and reproducibility, which is exemplary for the synthesis of other cyclic peptides.

Scheme 1 shows the procedures of solid-phase synthesis of the linear heptapeptide. The starting Fmoc-protected amino acid (Fmoc-Phe-OH) was loaded on 2-chorotrityl chloride resin by the treatment with *N*,*N*-diisopropyl-ethylamine

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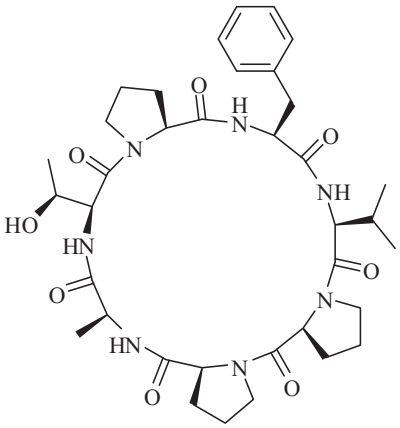
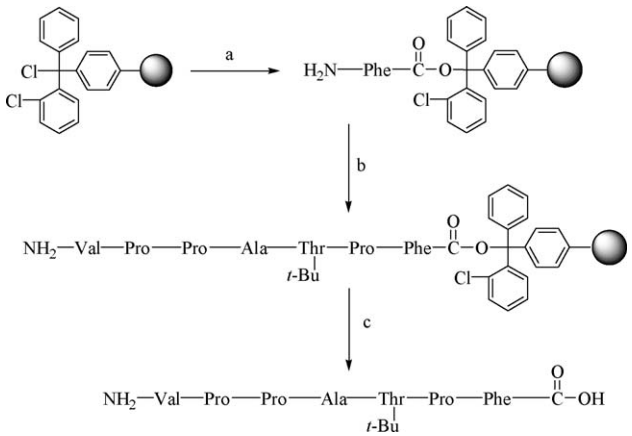


Fig. 1. Structure of euryjanicin B, cyclo[FPTAPPL] (all the amino acids are *L*-configuration).



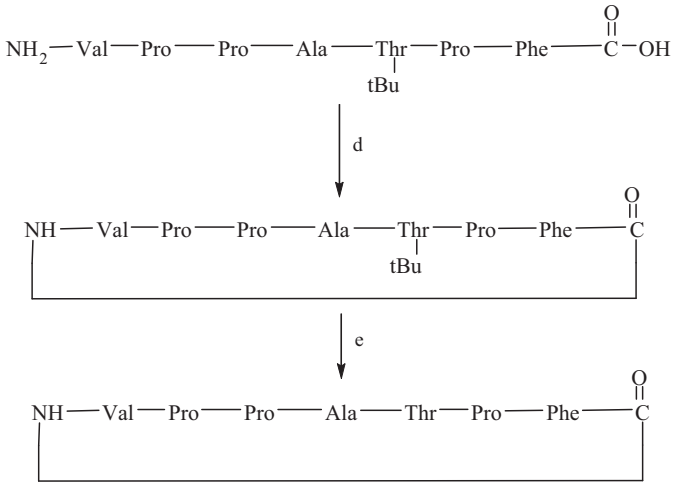
Scheme 1. Synthesis of the linear precursor of euryjanicin B: (a) (i) Fmoc-Phe-OH/DCM, DIPEA, 1 h and (ii) 20% piperidine/DMF, 2 h; (b) (i) Fmoc-aa-OH/NMP, DCC, HOBt, 4 h and (ii) 20% piperidine/DMF, 2 h; and (c) AcOH/TFE/DCM 1:2:16, 1 h (70%).

(DIPEA). For the remaining linear peptide synthesis, the standard Fmoc protocol was followed, and the resin was submitted to coupling and deprotection cycles to build the linear heptapeptide as the precursor for the cyclic euryjanicin B. All of the Fmoc protected amino acids were activated by 1-hydroxybenzotriazole/*N*,*N*-dicyclohexylcarbodiimide (HOBt/DCC) in the presence of *N*-methyl-2-pyrrolidone (NMP) before the next coupling was carried out. The progress of the amino acid coupling was checked through the ESI-MS test. Fmoc deprotection before each coupling step was achieved by the treatment of the resin-bound peptide with a 20% solution of piperidine in DMF. The linear heptapeptide was obtained by the treatment of the peptide on resin with AcOH/TFE/DCM (1:2:16) for 1 h with stirring.

Scheme 2 shows the procedure of conventional solution method synthesis of the cyclization and deprotection. The cyclization was accomplished by using PyBOP/HOBt/DIPEA in DMF at 0 °C. In order to reduce side reactions, cyclization was performed under highly diluted conditions with cyclic peptide concentration of 0.65×10^{-3} mol/L. PyBOP was chosen to be the coupling reagent on account of the higher coupling efficiency. The side chain deprotection was carried out by the treatment with TFA/Et_3SiH/DCM (2:1:8) for 3 h with stirring.

Finally, the purification was carried out on recycling preparative reversed-phase HPLC (RP-HPLC) using 1:1 ratio of water/acetonitrile as a solvent system. The HR-QTOF-MS spectrum of the major peak in HPLC showed the molecular ion peak of the cyclized product at 710.3871 $[M+H]^+$.

The first total synthesis of the cyclic heptapeptide euryjanicin B was accomplished in good yield *via* coupling reactions utilizing DCC/HOBt method. A general synthesis method was developed to prepare cyclic peptide by combining solid-phase and conventional solution methods with a good yield and high purity. This strategy is an effective method to synthesize cyclic peptides especially when there is not suitable anchoring side chain to be linked to the resin.



Scheme 2. Synthesis of the euryjanicin B. (d) PyBop, HOBt, DIEA, DCM, 0 °C (82%, calculated by crude protected euryjanicin B) and (e) TFA/Et_3SiH/DCM (1:2:16) (78%, calculated by crude euryjanicin B).

1. Experimental

1.1. Synthesis of the linear precursor of euryjanicin B

Synthesis was performed in a peptide synthesizer. The first amino acid was loaded on the resin by the treatment with DIPEA for 1 h, and then the Fmoc was subsequently removed by the treatment with piperidine (20%, v/v, in DMF, 2 h). After the first amino acid had been loaded, all protected amino acids were coupled by using HOBt/DCC chemistry. Typically, the amino acid (AA) (1.5 equiv.) was treated for 2 min with HOBt (2 equiv.) and DCC (2 equiv.) in NMP. The solution was added to the resin, which was then agitated for 4 h at room temperature. After coupling and Fmoc deprotection with 20% piperidine/DMF the resin was washed three times with NMP and DCM, respectively. Then the resin was treated with AcOH/TFE /DCM (1:2:16, v/v) at room temperature for 1 h. The protected linear heptapeptide was obtained.

1.2. Synthesis of the euryjanicin B

The protected cyclic heptapeptide was cyclized by the treatment with 5 equiv. PyBOP, 5 equiv. HOBt and 6 equiv. DIPEA in pure DCM. PyBOP, HOBt and DIPEA were dissolved in DCM in a round-bottom flask. The protected linear peptide was dissolved in DCM (0.65 mol/L) and dropped into the flask at 0 °C. The mixture was stirred and the reaction was monitored by ESI-MS. After the reaction was completed, DCM was removed by rotary evaporation and the resulting oil was initially purified by Sephedex LH-20. Then the protected cyclic heptapeptide was treated with TFA/Et_3SiH/DCM (2:1:8) at room temperature for 3 h. The reaction was monitored by ESI-MS. After the reaction was completed, toluene was added and the mixture was concentrated in a vacuum. The residue was purified by RP-HPLC to give cyclic heptapeptide as a white solid powder with a total yield of 48% and HPLC purity of over 95%.

Acknowledgments

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[9] Spectroscopic data of euryjanicin B. HR-QTOF-MS: $[M+H]^+$ = 710.3871, calcd. for $C_36H_{51}N_7O_8$: $[M+Na]^+$ = 732.3710; ^1H NMR ($CDCl_3$, 600 MHz): δ 0.93 (d, 3H, J = 6.8 Hz), 0.98 (d, 3H, J = 6.5 Hz), 1.14 (d, 3H, J = 6.0 Hz), 1.53 (m, 1H), 1.56 (d, 3H, J = 6.7 Hz), 1.76 (m, 2H), 1.87–1.95 (m, 4H), 2.05 (m, 2H), 2.14 (m, 2H), 2.35–2.43 (m, 2H), 3.05–3.15 (ddd, 2H, J = 13.8 Hz, J = 20.6 Hz), 3.43 (m, 2H), 3.61–3.71 (m, 4H), 4.05–4.38 (m, 5H), 4.60 (m,1H), 4.77–4.83 (m, 2H), 6.21 (d, 1H, J = 9.1 Hz), 6.68 (d, 1H, J = 8.6 Hz), 7.19 (d, J = 7.1 Hz, 2H), 7.30–7.36 (m, 3H), 7.61 (d, 1H, J = 5.9 Hz), 7.80 (d, 1H, J = 5.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.9, 18.9, 19.9, 20.0, 22.0, 24.8, 25.7, 28.6, 29.6, 30.3, 31.9, 37.8, 46.6, 47.8, 47.9, 52.6, 53.9, 55.6, 56.8, 59.5, 60.9, 62.4, 69.0, 127.5, 128.8, 128.9, 129.8, 129.9, 136.8, 169.5, 169.8, 171.2, 171.3, 171.6, 171.8, 172.0.