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FIRST TOTAL SYNTHESIS OF ANTIFUNGAL CYCLOPEPTIDE TUNICYCLIN D BY A SOLID-PHASE METHOD

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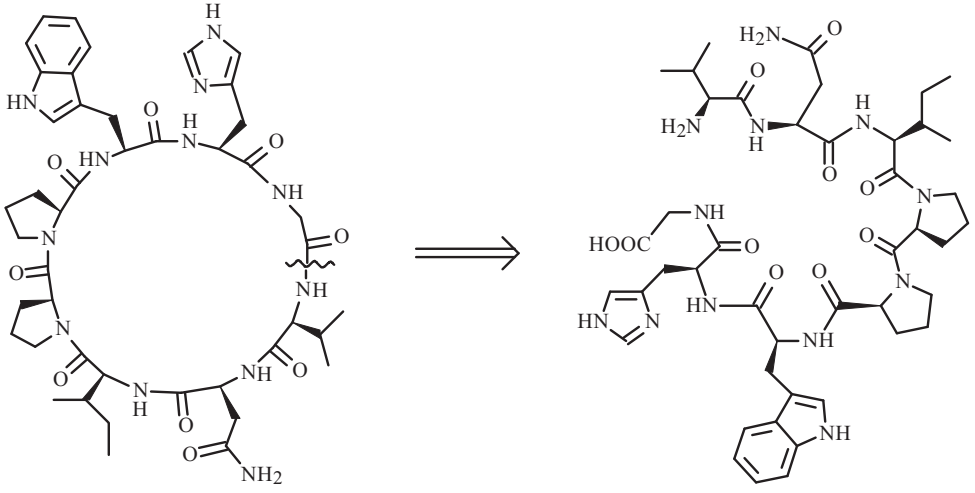
The present study deals with the first total synthesis of a new antifungal cyclic octapeptide, tunicyclin D, cyclo*[VNIPPWHG], where all the amino acids are L-configuration, by a two-step solid-phase/solution synthesis strategy. The linear octapeptide was assembled by a solid-phase peptide synthesis (SPPS) method. Subsequently cyclization and deprotection were achieved in solution with high efficiency and reproducibility. The final product was purified by preparative RP-HPLC, and its structure was identified by ESI-MS, ^{1}H NMR, ^{13}C NMR, and HR-QTOF-MS.*

Keywords: antifungal, cyclic octapeptide, solid-phase synthesis, cyclization, deprotection, purification.

Recently, a number of cyclopeptides with pharmacological and biochemical activities, such as cytostatic, antifungal, antiviral, antibacterial, and so on [1], have been isolated from plants, fungi, and lower sea animals [2]. These bioactive cyclic peptides have been considered as promising resources for lead compounds or drug candidates. As potential drug candidates, cyclic peptides have several advantages. Unlike linear peptides, they do not have charges at the peptide amino and carboxyl termini and lack zwitterionic character; therefore, they are more lipophilic and membrane permeable [3]. Furthermore, restricted bond rotation makes these molecules retain a rigid backbone conformation resulting in higher affinity and selectivity to certain specific target molecules and relevant biological activities [4].



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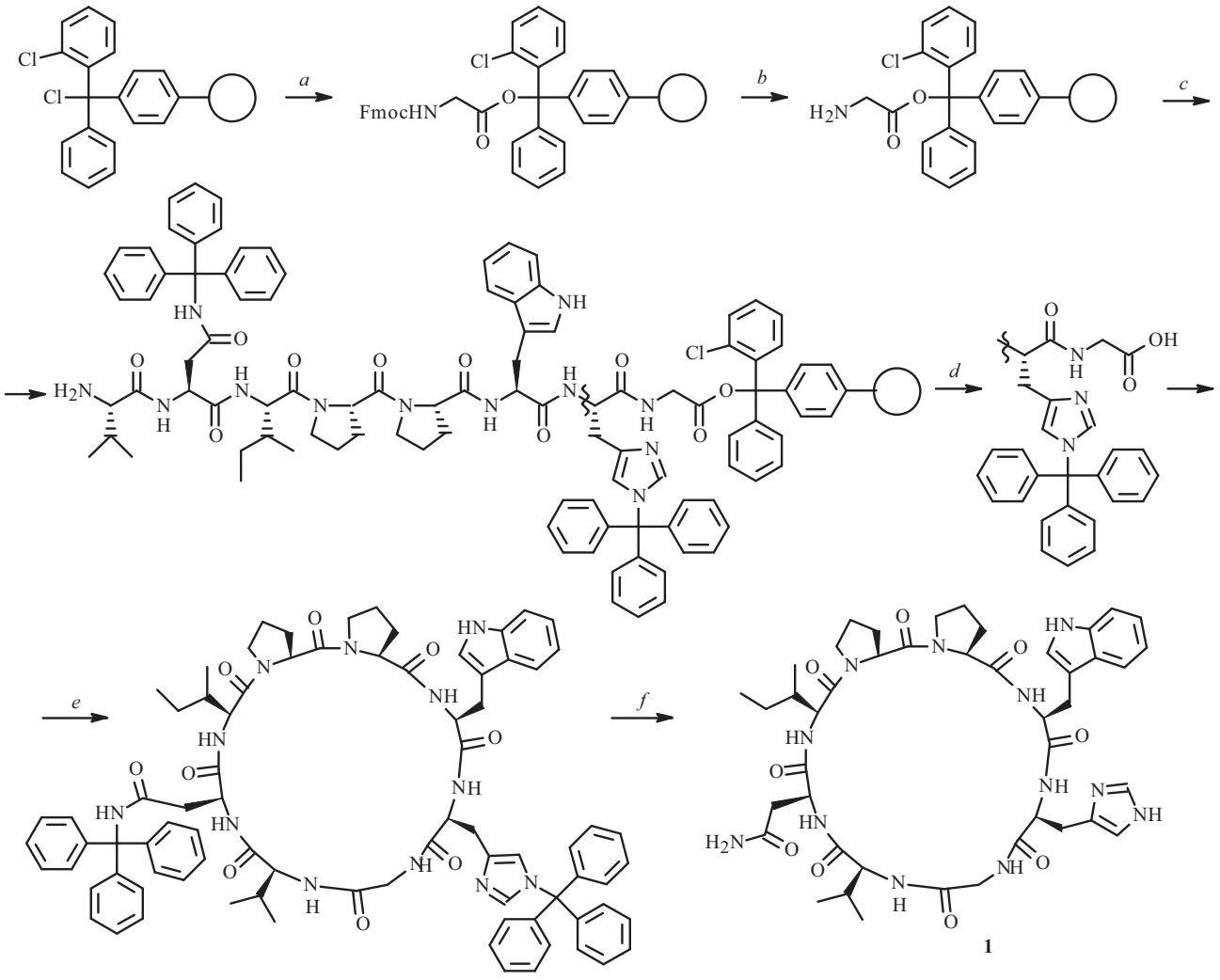

a. Fmoc-Gly-OH/DCM, DIPEA, 1 h; *b*. 20% piperidine/DMF, 2h; *c*. Fmoc-AA-OH/HOBt/HBTU/DIPEA/DCM, 2 h; *d*. AcOH/TFE/DCM (1:2:16), 1 h; *e*. PyBOP/HOBt/DIPEA/DCM, 0°C – r.t., overnight; *f*. TFA/Et_3SiH/DCM (2:1:8), 1.5 h

Scheme 1

Tunicyclin D is a new antifungal cyclooctapeptide isolated from the root of *Psammosilene tunicoides* by Wei-Dong Zhang et al. in 2010. It exhibited antifungal activity against *Candida albicans* (SC5314), *Candida albicans* (Y0109), *Candida tropicalis*, *Candida parapsilosis*, and *Cryptococcus neoformans* (BLS108) with MIC_{80} values of 4.0, 16.0, 0.25, 1.0 and 1.0 μg mL^{-1}, respectively [5]. But its content in the plant is very low, and only 68 mg tunicyclin D could be obtained from 40 kg dry roots of *Psammosilene tunicoides*. So the total synthesis is meaningful for its further study. Because our discussion group was interested in synthesizing natural cyclopeptides by a solid-phase method [6], which has the advantages of easy removal of the reagents and reduction in total reaction time, the present study was aimed at the first total synthesis of the new antifungal cyclopeptide tunicyclin D by the solid-phase method.

As it is known, cyclic head-to-tail connected peptides can easily be synthesized in good purity using standard procedures and orthogonally protected amino acid residues. However, the cyclization step is critical and can be very long depending on the peptide sequence, structural constraints, and the resulting ring size [7–9]. Tunicyclin D is a new antifungal cyclooctapeptide with seven different amino acids. Due to our previous experience in synthesizing natural cyclopeptides, we know that the cyclization-point of amino acid residues should be small and simple. So we chose a point of cyclization between Gly and Val to give the linear precursor **2** with Val as the *N*-terminal amino acid and Gly as the C-terminal amino acid. The synthesis of the linear precursor **2** was carried out by standard solid-phase peptide synthesis (SPSS), using a 9-fluorenylmethyloxycarbonyl/triphenylmethyl (Fmoc/Trt) protecting scheme and 2-chlorotrityl chloride resin (CLTR-Cl) as solid support on a Selecta automated peptide synthesizer. The synthetic approach produced the linear precursor **2** with high efficiency and reproducibility, which is exemplary for the synthesis of other cyclic peptides.

Scheme 1 shows the total synthetic process of tunicyclin D, including the linear octapeptides synthesis, cyclization, and deprotection. The synthesis of the linear octapeptides was conducted by the standard solid-phase peptide synthesis (SPSS) method. Cyclization and deprotection were carried out in solution with high efficiency and reproducibility. The final residue was purified by preparative reversed-phase HPLC to give the target compound tunicyclin D as a white solid powder with a total yield of 54.7% and HPLC purity of over 95%.

The 2-chlorotrityl chloride resin was chosen as the solid support because we can enable cleavage of the linear peptide from the solid phase with the side chain protecting groups intact. As anticipated, the cyclization was not straightforward. In order to reduce side reactions, cyclization was performed under highly diluted conditions in dichloromethane (DCM) with a cyclic peptide concentration of 0.5 mg mL^{-1} at 0°C. Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-hydroxy-benzotriazole (HOBT) were chosen as the coupling reagents on account of the higher coupling efficiency. Before deprotection, the residue was initially purified by Sephadex LH-20. The side chain deprotection time should not be too long to avoid conformational changes of the cyclic peptide.

Finally, the purication was performed on recycling preparative RP-HPLC. Acetonitrile with 0.01 M trifluoroacetic acid (TFA) was used as the mobile phase under a linear gradient elution mode (acetonitrile, 30–70%, 30 min). The flow rate was 10 mL min^{-1} with detection at 220 nm by ultraviolet detection. The HR-QTOF-MS spectrum of the major peak in HPLC showed the target cyclopeptide molecular ion peak at 901.4683 $[M + H]^+$.

The synthetic tunicyclin D was tested for antifungal effects *in vitro*. The results showed that the cyclopeptide exhibited antifungal activity against *Candida albicans* (SC5314), *Candida albicans* (Y0109), *Candida tropicalis*, *Candida parapsilosis*, and *Cryptococcus neoformans* (BLS108) with MIC_{80} values of 4.0, 4.0, 4.0, 8.0, and 4.0 μg mL^{-1}, respectively.

In summary, we have successfully completed the first total synthesis of the naturally occurring cyclic peptide tunicyclin D, which may serve as a good lead compound in the development of antifungal drugs, by a two-step solid-phase/solution synthesis strategy with good yield and high purity. The antifungal effective date of the synthetic product is consistent with literature date of the natural product. The synthesis of its glycosyl derivatives and their biological study is in progress and will be reported in due course.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectrum were obtained on a Bruker Avance 600 MHz NMR spectrometer. The chemical shifts of protons are given on the δ scale, ppm, with tetramethylsilane (TMS) as the internal standard. NMR spectra were recorded in D_2O with several drops of C_5D_5N. ESI-MS was measured on a Finngan LCQ Deca XP Max mass spectrometer. HR-QTOF-MS was measured on an Agilent 6538 UHD Accurate Mass Q-TOF LC/MS mass spectrometer.

Synthesis of the Linear Octapeptide. Under anhydrous conditions, the first amino acid (Fmoc–Gly–OH, 1.5 equiv) was loaded on the resin (1.0 equiv) by treatment with *N*,*N*-diisopropylethylamine (DIPEA, 7.5 equiv) in DCM for 1 h; then the solvent was drained and the resin was washed sequentially with MeOH and DCM (3 × 5 mL) before being dried. The Fmoc group was removed with 20% piperidine in *N*,*N*-dimethylformamide (DMF) for 2 h, and the resin was washed with DMF (3 × 5 mL), then dried under vacuum. After the first of the amino acids were loaded, all the other protected amino acids (1.5 equiv) were coupled by treatment with HOBT (2 equiv), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 2 equiv), and DIPEA (3 equiv) in DMF for 2 h at room temperature, then the solvent was drained and the resin washed sequentially with DMF and DCM (3 × 5 mL). The protected linear octapeptide was split from the resin by treatment with 10% AcOH in trifluoroethanol (TFE) and DCM (1:8, 5 mL) for 1 h at room temperature; then the resin was washed with DCM (3 × 5 mL), filtered, and concentrated *in vacuo* to give the protected linear octapeptide as a white solid powder with a yield of 89.8%.

Cyclization of the Linear Octapeptide. Cyclization was performed with PyBOP (5 equiv), HOBT (5 equiv), and DIPEA (10 equiv) in pure DCM. First, the reagents PyBOP, HOBT, and DIPEA were dissolved in DCM in a round-bottom flask; then the protected linear peptide was diluted in DCM to a concentration of 0.5 mg mL^{-1} and dropped into the flask slowly at 0°C. After the dropping was completed, the reaction mixture was stirred at room temperature overnight. DCM was removed by rotary evaporation and the resulting oil was initially purified by Sephadex LH-20 to give the protected cyclopeptide as a white solid powder with a yield of 82.7%.

Removal of the Trt Protecting Group. The deprotection was performed with TFA–Et_3SiH–DCM (2:1:8) at room temperature for 1.5 h, and the reaction was monitored by ESI-MS. After the reaction was completed, toluene was added, and

the mixture was concentrated in vacuum. The final residue was purified by preparative RP-HPLC to give the target compound tunicyclin D as a white solid powder with a total yield of 54.7% and HPLC purity of over 95%. Its structure was identified by ESI-MS, ^{1}H NMR, ^{13}C NMR, and HR-QTOF-MS.

Spectroscopic Data of Tunicyclin D. $C_{44}H_{61}N_{12}O_9$. ESI-MS *m/z* 901.93 $[M + H]^+$. HR-QTOF-MS *m/z* 901.4683 $[M + H]^+$. ^{1}H NMR (600 MHz, in D_2O with several drops of C_5D_5N, δ, ppm, J/Hz): 7.50 (2H, t, J = 7.8), 7.29 (1H, d, J = 6.6), 7.06 (1H, m), 6.92 (1H, d, J = 6.6), 6.81 (1H, s), 6.78 (1H, m), 6.61 (2H, m), 4.42 (3H, m), 4.07 (1H, m), 3.98 (1H, m), 3.92 (1H, m), 3.83 (1H, d, J = 16.8), 3.43 (2H, m), 3.32 (1H, m), 2.92–2.89 (3H, m), 2.78 (2H, m), 2.68 (2H, m), 2.54–2.50 (3H, m), 1.87 (1H, m), 1.73 (3H, m), 1.56 (2H, m), 1.30 (1H, m), 1.14 (2H, m), 0.66–0.54 (9H, m), 0.43 (3H, d, J = 9.6).

^{13}C NMR (150 MHz, in D_2O with several drops of C_5D_5N, δ, ppm): 172.52, 172.08, 171.90, 171.17, 170.98, 170.51, 170.22, 170.06, 168.41, 135.66, 133.62, 133.24, 131.63, 126.62, 120.11, 117.73, 117.54, 116.10, 110.33, 109.51, 59.74, 58.24, 54.78, 54.30, 50.88, 50.48, 46.25, 45.29, 42.06, 39.86, 35.71, 35.45, 29.65, 29.12, 28.61, 27.90, 27.22, 23.53, 22.96, 19.85, 17.99, 16.58, 14.66, 10.18.

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REFERENCES

1. A. B. Pomilio, M. E. Battista, and A. A. Vitale, *Curr. Org. Chem*., **10**, 2075 (2006).
2. U. Schmidt, *Pure Appl. Chem*., **58**, 295 (1986).
3. L. Ali, S. G. Musharraf, and F. Shaheen, *J. Nat. Prod*., **71**, 1059 (2008).
4. H. Morita and K. Takeya, *Heterocycles*, **80**, 739 (2010).
5. J. Tian, Y. Shen, X. Yang, S. Liang, L. Shan, H. Li, R. Liu, and W. Zhang, *J. Nat. Prod*., **73**, 1987 (2010).
6. H. Hu, J. Xue, B. M. Swarts, Q. Wang, Q. Wu, and Z. Guo, *J. Med. Chem*., **52**, 2052 (2009).
7. K. A. Fairweather, N. Sayyadi, C. Roussakis, and K. A. Jolliffe, *Tetrahedron*, **66**, 935 (2010).
8. L. Yang and G. Morriello, *Tetrahedron Lett*., **40**, 8197 (1999).
9. P. Li, P. P. Roller, and J. Xu, *Curr. Org. Chem*., **6**, 411 (2002).