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Solid-Phase Synthesis of Cyclic Depsipeptides Containing a Tyrosine Phenyl Ester Bond

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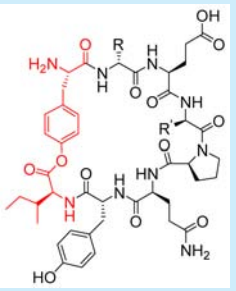
Supporting Information

ABSTRACT: The first solid-phase strategy for the synthesis of cyclic depsipeptides containing a phenyl ester linkage in their structure is described. The key steps of the synthesis were the formation of the phenyl ester bond and the on-resin head-to-side-chain cyclization. The amino acid configuration significantly influenced the formation and the stability of the cyclic depsipeptides. The presence of a L-Tyr[1] and a D-Tyr[7] led to the most stable sequences.



Fengycins are cyclic lipodepsipeptides isolated from several *Bacillus* spp. that were first described in 1986.[1] These cyclic compounds show interesting antifungal activity mainly against filamentous fungi, and unlike other lipopeptides produced by *Bacillus* spp., they are low hemolytic.[1,2] Moreover, they have been described as elicitors of defense responses in plants, making them a promising alternative for the protection of crops against phytopathogens.[3]

The fengycin family includes related compounds that share a common cyclic structure with exceptional features.[1,4−7] They are decapeptides acylated at the N-terminus with a β-hydroxy fatty acid tail (Figure 1). Eight of the amino acids form a macrolactone in which the ester bond occurs between the phenol group of a tyrosine (Tyr) and the carboxylic group of an isoleucine (Ile). Until now, three isoforms of fengycins have been isolated, A, B, and S, which differ on the amino acids at positions 4 and 6. There has been some confusion over the configuration of the Tyr residue at positions 3 and 9. Initially, fengycins were reported to have a D-Tyr[3]/L-Tyr[9] configuration,[1,8] whereas a L-Tyr[3]/D-Tyr[9] configuration was assigned to another family of related lipopeptides named plipastatins.[9] Nevertheless, a recent study showed that fengycins and plipastatins have the same primary structure, that is, a L-Tyr[3]/D-Tyr[9] configuration.[10] In this study, we name the peptides as fengycins because this term is more widely employed than plipastatins, and we use the correct configuration (Figure 1).

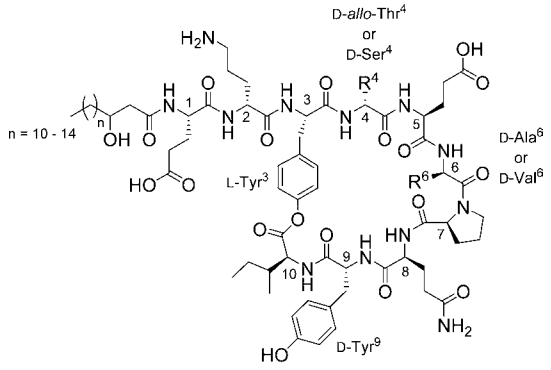


Figure 1. Structure of fengycins A, B, and S.

Solid-phase synthesis has been established as a good strategy for the obtention of cyclodepsipeptides.[11−15] However, to the best of our knowledge, the solid-phase synthesis of fengycins has not yet been described. Moreover, cyclodepsipeptides that have been synthesized on solid phase bear an ester bond involving the β-hydroxyl group of a Thr or Ser residue. No examples have been reported on the formation of ester bonds involving the phenol group of a Tyr, which can be attributed to the high lability of phenyl esters. In fact, until now, the preparation of fengycins was only addressed by the group of Marahiel and Essen, and it involves the solid-phase synthesis of the linear precursor followed by chemoenzymatic cyclization in solution.[16−18] Due to the structural requirements of this last step, this approach is restricted to the obtention of a limited number of analogues. Based on these considerations, the synthesis of fengycins constitutes an attractive synthetic challenge, not only for being constrained cyclopeptides but also for containing a phenyl ester in their structure.

Herein, we explore a suitable solid-phase approach for the preparation of the macrolactone of eight amino acids present in fengycins (Figure 1). In particular, we focused our attention on the synthesis of compounds **I** and **II** (Figure 2). The structure of

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I contains amino acids with the configuration described for fengycins, whereas **II** incorporates D-Tyr[1] and L-Tyr[7].

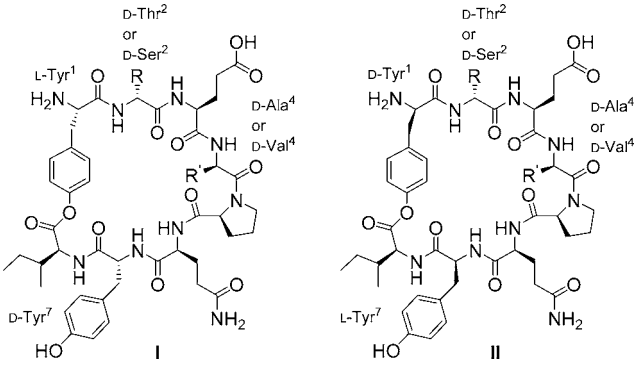


Figure 2. General structure of fengycin analogues **I** and **II**.

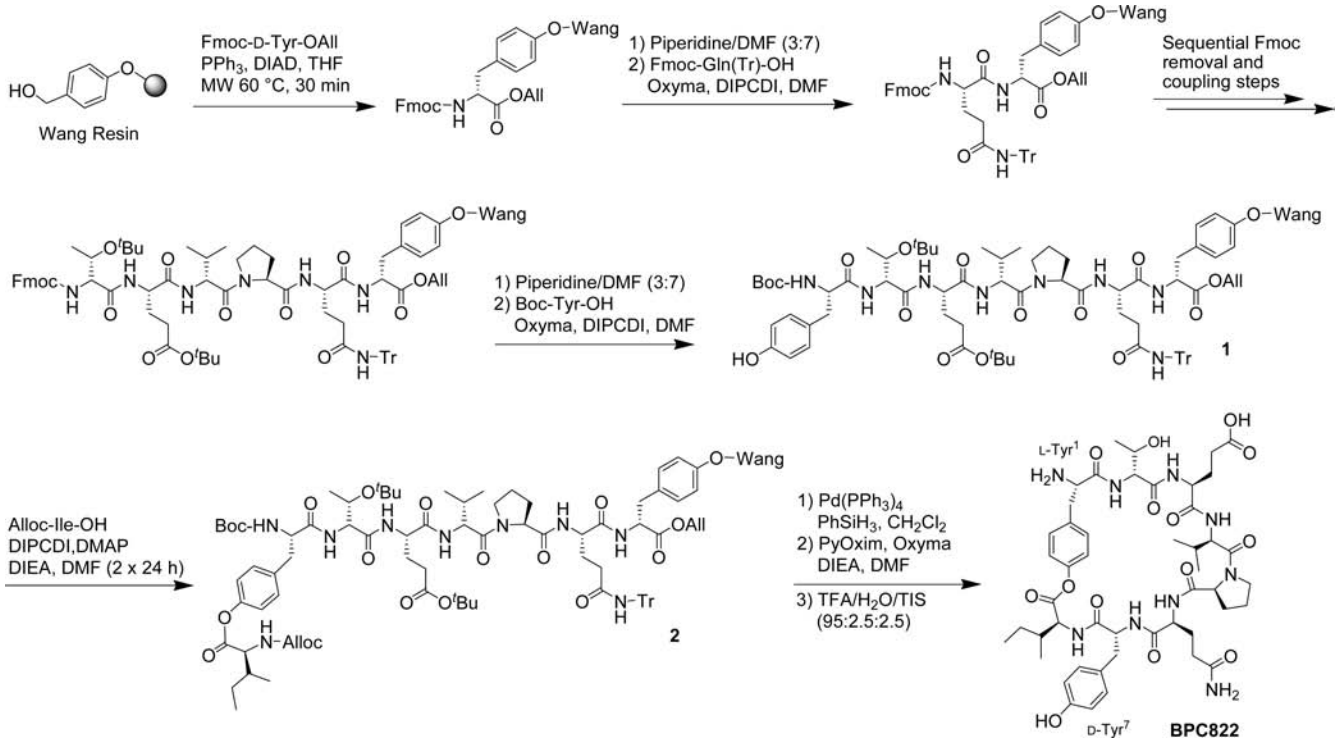
The main concerns when planning a retrosynthetic analysis for cyclopeptides **I** and **II** are the site of resin attachment and the point of cyclization. The selection of these issues was determined by the presence of the phenyl ester bond, whose formation is especially challenging. Thus, the side chain of Tyr[7] was selected as the anchoring point to the resin, and the cyclization would involve the formation of an amide bond between the carboxylic acid of Tyr[7] and the α-amino group of Ile[8]. Therefore, access to these cyclic compounds would require (i) preparation of a linear peptidyl resin with Tyr[7] linked to the solid support, (ii) ester bond formation, (iii) deprotection of the carboxylic and amino groups involved in the cyclization step, and (iv) final on-resin cyclization and cleavage.

Our investigations started with the solid-phase preparation of cyclic depsipeptides **I** (Figure 2 and Scheme 1). In particular, we assayed the synthesis of compound **BPC822** (L-Tyr[1], D-Thr[2], D-Val[4], D-Tyr[7]). A three-dimensional orthogonal 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (^{t}Bu)/allyl (All) protecting strategy was selected. According to our methodology, the linear resin Boc-Tyr[1]-D-Thr[2](^{t}Bu)-Glu(O^{t}Bu)-D-Val[4]-Pro-Gln(Tr)-D-Tyr[7](Wang)-OAll (**1**) was considered a precursor of **BPC822**. Wang resin was used as solid support because it enables incorporation of Tyr[7] using a Mitsunobu reaction.[19,20] Thus, Fmoc-D-Tyr-OAll was anchored to the resin by treatment with PPh_3 and diisopropylazodicarboxylate (DIAD) in anhydrous THF under stirring at room temperature for 24 h.[19,20] As determined with the Fmoc method, resin Fmoc-D-Tyr(Wang)-OAll was obtained with a loading of 0.33 mmol/g. Moreover, according to Marfey's test, the Tyr residue did not racemize under these conditions. Reaction time was considerably shortened when the anchoring was carried out under microwave irradiation. After only 30 min irradiation at 60 °C, the Fmoc-D-Tyr(Wang)-OAll resin was obtained in a similar loading and no racemization of the D-Tyr was observed. To the best of our knowledge, this is the first example of a microwave-assisted Mitsunobu reaction for the anchoring of Tyr onto a solid support.

Elongation of the peptide sequence was performed by sequential Fmoc removal and coupling steps. Fmoc group removal was performed with piperidine/DMF (3:7). Amino acid couplings were mediated by *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) in DMF for 1 h. After completion of the sequence, an aliquot of the resulting resin Boc-Tyr[1]-D-Thr[2](^{t}Bu)-Glu(O^{t}Bu)-D-Val[4]-Pro-Gln(Tr)-D-Tyr[7](Wang)-OAll (**1**) was cleaved with TFA/H_2O/triisopropylsilane (TIS) (95:2.5:2.5) for 2 h, affording the expected linear peptide in 85% HPLC purity, which was characterized by mass spectrometry.

The unprotected phenol group of Tyr was esterified with Alloc-Ile-OH. The allyloxycarbonyl (Alloc) group was chosen for

Scheme 1. Synthetic Strategy of Cyclodepsipeptide BPC822



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the protection of the α-amino group of Ile^8 as an alternative to the standard Fmoc or Boc protection because, on the one hand, once the phenyl ester is formed the use of bases such as piperidine is precluded due to the lability of the phenyl ester. On the other hand, the Boc group cannot be removed without releasing the peptide from the Wang resin. Moreover, the conditions of the Alloc group removal using Pd(0) would allow the simultaneous cleavage of the allyl ester group of Tyr^7 before the cyclization step. Esterification of resin **1** with Alloc-Ile-OH was carried out, employing DIPCDI, DMAP, and *N*,*N*′-diisopropylethylamine (DIEA) in DMF for 24 h. This reaction was repeated twice to obtain the linear depsipeptidyl resin Boc-Tyr^1(O-Ile^8-Alloc)-D-Thr^2(^{t}Bu)-Glu(O^{t}Bu)-D-Val^4-Pro-Gln-(Tr)-D-Tyr^7(Wang)-OAll (**2**). An aliquot of this resin was treated under acidolytic conditions, yielding the expected linear peptide in 84% HPLC purity. Next, the Alloc and allyl protecting groups were removed using a catalytic amount of $Pd(PPh_3)_4$ in the presence of $PhSiH_3$ in CH_2Cl_2 for 4 h. After acidolytic cleavage of an aliquot of Boc-Tyr^1(O-Ile^8-H)-D-Thr^2(^{t}Bu)-Glu(O^{t}Bu)-D-Val^4-Pro-Gln(Tr)-D-Tyr^7(Wang)-OH, the corresponding peptide was obtained in 82% HPLC purity.

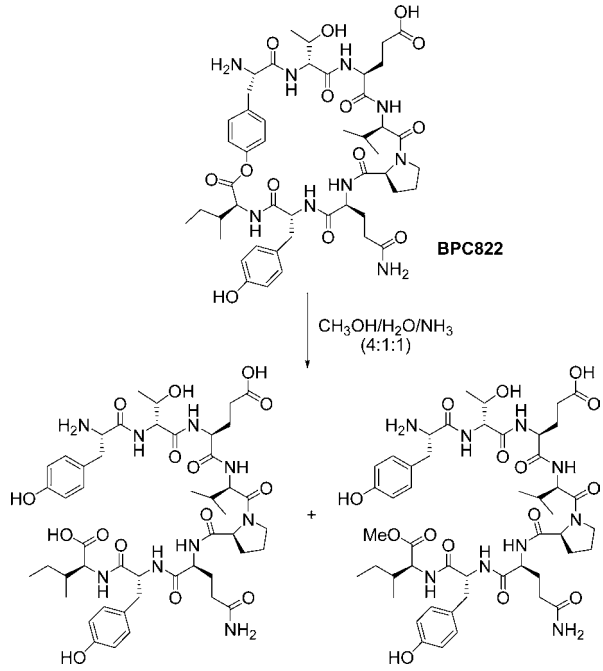
On-resin cyclization of this linear peptidyl resin was assayed using [ethyl cyano(hydroxyimino)acetato-*O*^{2}]tri-1-pyrrolidinyl-phosphonium hexafluorophosphate (PyOxim), Oxyma, and DIEA in DMF for 24 h. Acidolytic cleavage yielded the cyclic depsipeptide **BPC822** in 31% HPLC purity, as shown by ESI-MS, where a peak at m/z 994.6 corresponding to $[M + H]^+$ was observed. The linear peptide precursor was not detected either by HPLC or by mass spectrometry. To confirm the cyclic structure of **BPC822**, the crude reaction mixture resulting from acidolytic cleavage was treated with $CH_3OH/H_2O/NH_3$ (4:1:1), conditions that are known to hydrolyze ester bonds.[21] HPLC and mass spectrometry analysis of the resulting crude revealed only the presence of the linear peptide resulting from the hydrolysis of the ester bond in **BPC822**, H-Tyr^1-D-Thr^2-Glu-D-Val^4-Pro-Gln-D-Tyr^7-Ile^8-OH, and of the corresponding methyl ester H-Tyr^1-D-Thr^2-Glu-D-Val^4-Pro-Gln-D-Tyr^7-Ile^8-OMe (Scheme 2). Therefore, this result demonstrated the formation of the cyclic depsipeptide **BPC822**. Moreover, the structure of this compound was verified by 1D and 2D NMR experiments.

This synthetic methodology was extended to the preparation of the cyclic depsipeptides with general structure **I**: **BPC824** (L-Tyr^1, D-Ser^2, D-Val^4 and D-Tyr^7), **BPC826** (L-Tyr^1, D-Thr^2, D-Ala^4 and D-Tyr^7), and **BPC828** (L-Tyr^1, D-Ser^2, D-Ala^4 and D-Tyr^7) (Figure 3). Mass spectrometry analysis showed that these cyclic depsipeptides were the only products of the synthesis. Similarly to **BPC822**, hydrolysis of the crude reaction mixtures confirmed the cyclic structure of these compounds. Cyclic depsipeptides **BPC822**, **BPC824**, **BPC826**, and **BPC828** were purified by column chromatography and obtained in purities ranging from 93 to >99%. These peptides were fully characterized by NMR.

Finally, the synthesis of cyclic depsipeptides type **II** bearing a D-Tyr^1 and a L-Tyr^7 was also attempted following the above methodology. This set included **BPC830** (D-Tyr^1, D-Thr^2, D-Val^4, L-Tyr^7), **BPC832** (D-Tyr^1, D-Ser^2, D-Val^4, L-Tyr^7), **BPC834** (D-Tyr^1, D-Thr^2, D-Ala^4, L-Tyr^7), and **BPC836** (D-Tyr^1, D-Ser^2, D-Ala^4, L-Tyr^7) (Figure 3). Contrary to our expectations, the cyclic depsipeptides were obtained together with a high amount of the corresponding dimeric product and a linear peptide that did not contain Ile^8. The formation of the latter product revealed that the cyclization was not complete and that the ester bond of the linear precursor hydrolyzed, prompting the release of the Ile residue. This result demonstrated that the cyclization of cyclic depsipeptides with D-Tyr^1 and L-Tyr^7 is difficult and that they are not stable, reinforcing the hypothesis by Honma and co-workers that postulated a L and D configuration for these residues in natural fengycins.[10]

Herein, we established a suitable strategy for the solid-phase synthesis of head-to-side-chain cyclic depsipeptides containing a phenyl ester linkage. Using a Fmoc/^{t}Bu/Alloc/allyl strategy, a set of cyclic octadepsipeptides derived from fengycins was successfully prepared. Our studies revealed the significance of

Scheme 2. Hydrolysis of the Ester Bond of BPC822



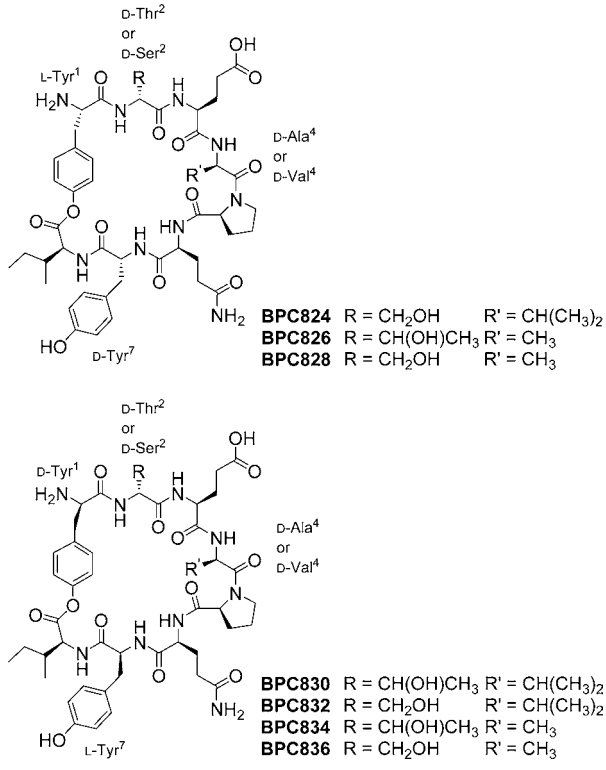


Figure 3. Structure of cyclodepsipeptides.

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the amino acid configuration on the stability of the cyclic depsipeptides, with those containing a L-Tyr[1] and a D-Tyr[7] being the most stable. This study is the first example on the synthesis of cyclic depsipeptides incorporating a phenyl ester bond and represents a promising approach for the future total synthesis of fengycins and of other natural compounds including this moiety.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02281.

Experimental procedures and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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