Facile Synthesis of Hydrocarbon-Stapled Peptides

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Introduction

The intracellular protein-protein interactions that govern many biological pathways are frequently mediated by α-helix structure of protein. Theoretically, helical peptides also can interfere with or stabilize protein-protein interactions, but native helical peptides have major shortcomings as experimental or therapeutic agents because of low potency, instability, and inefficient delivery to cells. Verdine's group [1-2] has shown that these problems could be overcome by a chemical modification of α-helical peptides they termed hydrocarbon stapling. They used (S)-α-(2'-pentenyl)alanine containing olefin-bearing tethers to generate an all-hydrocarbon "staple" by ruthenium-catalyzed olefin metathesis. The (S)-α-(2'-pentenyl)alanine peptides were made to flank three (substitution positions *l* and *l* + 4) or six (*l* and *l* + 7) amino acids within the peptide, so that reactive olefinic residues would reside on the same face of the α-helix. The modified hydrocarbon-stapled peptides are helical, relatively protease-resistant, and cell-permeable peptides that bind with increased affinity for its target, and may provide a useful strategy for experimental and therapeutic modulation of protein-protein interactions in many signaling pathways.

Here we report a versatile synthesis method for hydrocarbon-stapled peptides. Asymmetric synthesis of (S)-Fmoc-α-(2'-pentenyl)-alanine was successfully accomplished via an Ala-Ni (II)-BPB-complex [3] in three steps with a 40% total yield. The 12-mer peptide containing two α-pentenyl-alanines on positions 4 and 8 was synthesized by Fmoc solid phase synthesis method. After olefin metathesis and cleavage, the peptide was purified by HPLC to obtain the hydrocarbon-stapled peptide.

Results

In contrast with Verdine's method [2] for (S)-Fmoc-α-(2'-pentenyl)-alanine, we chose Ala-Ni (II)-BPB-complex method [3] for asymmetric synthesis. The Ala-Ni (II)-BPB-complex [4] was reacted with 5-bromo-1-pentene in acetone under basic conditions to give a mixture of a Ni(II) complex of Schiff base of (S)-BPB-(S)-trans-α-(2'-pentenyl)alanine [α-(S)-2] and Ni(II) complex of Schiff base of (S)-BPB-(R)-trans-α-(2'-pentenyl)-alanine [α-(R)-**2**] with ratio 6:1. After separation with silica gel column, diastereo-pure α-(S)-**2** complexes were obtained at 44% yield.

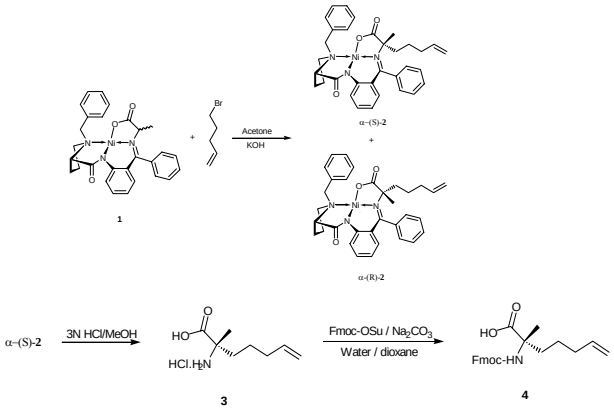
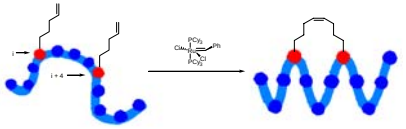


Figure 1. Synthesis of (S)-Fmoc-α-(2'-pentenyl)alanine

The α-(S)-2 complexes were decomposed with 3N HCl/MeOH to afford (S)-α-(2'-pentenyl)alanine (**3**) as well as a chiral ligand which was extracted with DCM. After work up, (S)-α-(2'-pentenyl)alanine (**3**) was protected with Fmoc-OSu to give the (S)-Fmoc-α-(2'-pentenyl)alanine (**4**) with 93% yield (two steps).

Peptide **1** was synthesized manually by Fmoc solid phase synthesis method using Rink amide MBHA resin. For normal amino acids, couplings were performed with fourfold excess of amino acids. Fmoc-amino acids were activated using the ratio of Fmoc-amino acid:HBTU:HOBt:DIEA, 1:1:1:2. For (S)-Fmoc-α-(2'-pentenyl)alanine , coupling was performed with twofold excess of amino acid which was activated with DIC:HOAt (1:1). For peptide olefin metathesis, the peptide resin with N-terminal protected by Fmoc group was treated with degassed 1, 2 dichloroethane containing Bis(tricyclohexyl-phosphine)-benzylidine ruthenium (IV) dichloride at room temperature for two hours and the reaction



Sequence of Peptide **1**. XXFZDLLZYYGX
Sequence of Peptide **2**. FITC-(βA)XXFZDLLZYYGX
Z = (S)-α-(2'-pentenyl)alanine

Figure 2. Strategy for hydrocarbon-stapled peptide with enhanced α helix structure.

was repeated once for completion. After de-Fmoc, the resin bound peptide was cleaved using standard protocols (95% TFA, 2.5% water, 2.5% TIS). The cleaved peptide was purified by RP-HPLC using 0.1% (v/v) TFA/water and 0.1% (v/v) TFA/acetonitrile. Chemical composition of the pure product was confirmed using MS. For fluorescently labeled Peptide **2,** the N-terminal group of Peptide **1** was further derivatized with β-Ala followed by FITC (DMF/DIEA) on the resin before the cleavage. The other cleavage, purification and confirmation steps were the same as above. Peptide **1** not only showed enhanced α-helicity and resistance to proteolysis, but also had antiviral activity (manuscript in preparation).

Conclusions

- Asymmetric synthesis of (S)-Fmoc-α-(2'-pentenyl)alanine was successfully prepared via an Ala-Ni (II)-BPB-complex with 40% total yield.
- Hydrocarbon-stapled peptides were synthesized.
- Peptide **1** not only showed enhanced α-helicity and resistance to proteolysis, but also had antiviral activity.

References:

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