Fully Automated Synthesis of a Stapled Peptide on the Prelude®

Application Note 14

D0029151/C

Introduction

Cyclization is known to improve the stability, potency, and selectivity of many peptides. One method for peptide cyclization which has generated tremendous interest is the use of olefin metathesis to create "stapled" peptides.[1] This all-hydrocarbon constraint has been shown to stabilize alpha-helical secondary structures and promote cell-penetrating properties.

Recently the synthesis (**Figure 1**) and properties of a novel stapled peptide, NYAD-1, which likely targets the capsid and inhibits HIV-1 in cell culture, have been described.[2]

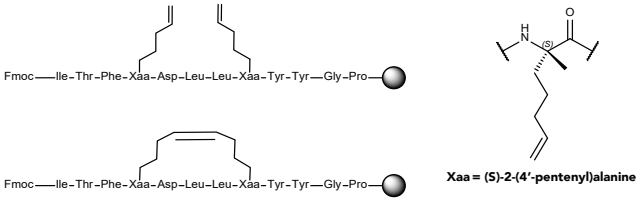

Figure 1. Metathesis reaction for NYAD-1.

In this application note we demonstrate the fully automated synthesis of NYAD-1 on the **Prelude®** peptide synthesizer, using the **Prelude**'s **Single-Shot™ Delivery Feature** to deliver expensive unnatural amino acid and perform the metathesis reaction completely on resin using Grubbs catalyst. The **Prelude**'s **Single-Shot Delivery Feature** is perfect for special reagent additions. It can deliver the entire contents of the 10 mL **Single-Shot** delivery vial to the reaction vessel of your choice without priming or wasting a drop!

Method

Materials: Fmoc-protected natural amino acids were supplied by Protein Technologies, Inc. (Tucson, AZ). (S)-2-(4'-pentenyl)alanine was purchased from Anaspec (Fremont, CA). Grubbs 1st generation catalyst and 1,2-dichloroethane were purchased from Sigma-Aldrich (St. Louis, MO).

Peptide Synthesis: The peptide was synthesized on Rink Amide MBHA resin (0.33 mmol/g) at 10 µmol scale. Deprotection: 20% piperidine/DMF for 2 x 5 min. Washes: DMF 6 x 30 sec. **Coupling:** 1:1:4 0.05M AA/0.05M HATU/0.2M NMM in DMF for 2 x 20 min with ten-fold excess for natural amino acids, 1 x 30 min with five-fold excess for (S)-2-(4'-pentenyl) alanine. (S)-2-(4'-pentenyl)alanine was added to the reaction vessel using the **Single-Shot Delivery Feature**. **Cleavage:** 95/2.5/2.5 TFA/TIS/water for 2 hours. The cleavage cocktail was precipitated in cold ethyl ether and washed with ethyl ether three times, with centrifuging and decanting. The resulting solid residues were allowed to dry overnight and then analyzed by HPLC and LC/MS.

Metathesis Reactions: The peptide on resin (with Fmoc protection intact on the N-terminus) was treated with a 10 mM solution of Grubbs catalyst (bis(tricyclohexylphosphine)benzylidine ruthenium(IV) dichloride) in degassed 1,2-dichloroethane (2 mL) for 2 x 2 hours. The catalyst solution was prepared immediately prior to the metathesis reaction and added using the **Single-Shot Delivery Feature**.

Analysis: Crude peptides (after precipitation) were dissolved in 1:1 water:acetonitrile at a concentration of 3 mg/mL and analyzed on a Varian ProStar HPLC using a C18, 300 Å, 5 µm, 250 x 4.6 mm column (Varian Microsorb-MV), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A is 0.1% TFA in water, and Buffer B is 0.1% TFA in acetonitrile. Detection was at 214 nm. Prior to LC/MS analysis, 60 µL of the 3 mg/mL solution was diluted to 600 µL with 540 µL of water. Mass analysis was performed on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 µm, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 minutes with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A is 0.1% formic acid in water and Buffer B is 0.1% formic acid in acetonitrile.

[1] Drahl, C. Chem. Eng. News, 2008, 86, 18-23.
[2] Zhang et al. J. Mol. Bio. 2008, 378, 565-580.

Result/Discussion

Only one major peak is observable in the HPLC trace of the crude peptide (**Figure 2**). This peak is resolved from others in the chromatogram, which would facilitate purification by RP-HPLC. The correct mass is found in LC/MS in both positive (726, corresponding to $[M+2H]^{2+}$) and negative (1449, corresponding to $[M-H]^{-}$) modes (data not shown).

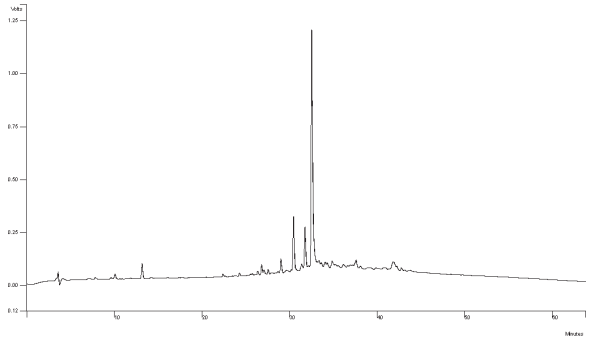

Figure 2. HPLC chromatogram of crude NYAD-1 synthesized on the Prelude® peptide synthesizer.

Conclusion

This application note demonstrates the successful fully automated synthesis of the stapled peptide, NYAD-1, on the **Prelude** peptide synthesizer using the **Single-Shot Delivery Feature** to deliver special expensive amino acids and Grubb's catalyst for the on resin metathesis reaction.

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