Boc Synthesis of a Biotinylated Peptide Thioester on the Prelude®

Application Note 11

D0029148/B

Introduction

YIYGSFK is a substrate for the protein tyrosine kinase. Tyrosine kinase activity is increased in several human tumors, so the study of its substrates may lead to a greater understanding of cancer. In this application, a tyrosine kinase substrate was synthesized and modified with a biotinylated lysine and a racemic amino acid thioester on a **Prelude®** peptide synthesizer using Boc chemistry. The **Prelude** peptide synthesizer is ideal for the synthesis of peptides containing special monomers. With its Single-Shot delivery feature, the **Prelude** can deliver the entire contents of an amino acid vial to any reaction vessel without priming or wasting a drop!

Special thanks to Dr. Laurie Parker, Assistant Professor at Purdue University for the synthesis data.

Sequence[1]**:** YIYGSFK-Kb-X-L

Method

Peptide Synthesis: The peptide was synthesized at the 100 µmol scale on a Prelude peptide synthesizer using MBHA resin (0.46 mmol/g). Deprotection was performed with 100% TFA for 2 x 2 min. The resin was then neutralized with 2M DIPEA in DMF for 5 minutes. Coupling was performed with a ratio of 1:0.95:2 AA/HCTU/DIPEA in DMF, 4x excess for 15 minutes. Washing with DMF and DCM was performed between all steps. Cleavage was performed for 1h with anhydrous HF starting at -72°C and gradually increasing to 0°C at the end of the hour.

Analysis: The peptide was analyzed on a C18 column (2.1 x 50 mm) on an Agilent 1100/XCT LC/MS using an aqueous acetonitrile, 0.1% TFA buffer system with an increasing gradient of 5-65% acetonitrile at 4% per minute. A flow rate of 0.5 mL/min was used, and detection was at 215 nm.

Results

The results are shown in Figures 1 and 2.

The final product (1) was successfully obtained as a diastereomeric mixture because a racemic amino acid thioester was used. p-cresol leftover from the cleavage reaction formed a peak which can easily be separated from the peptide product.

The lysine deletion (2) was due to running the synthesis under unoptimized conditions. This peak can easily be minimized in the future by optimizing that coupling step.

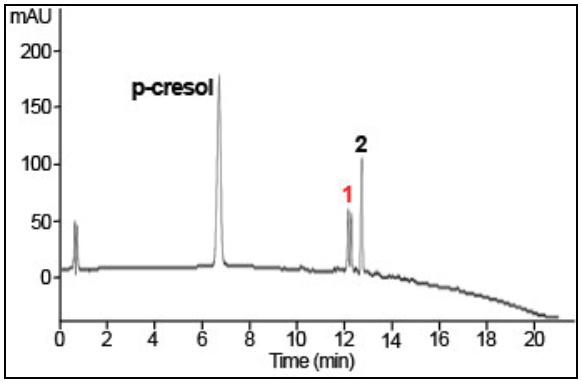

Figure 1: HPLC results for biotinylated peptide thioester. 1 is the target peptide, 2 is a lysine deletion, and p-cresol is leftover from the cleavage reagent.

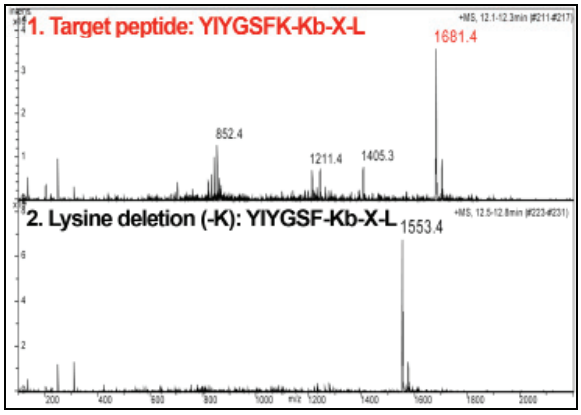

Figure 2: Mass spectrometry results for biotinylated peptide thioester product and lysine deletion.is the target peptide, 2 is a lysine deletion, and p-cresol is leftover from the cleavage reagent.

CONCLUSION

Boc chemistry was successfully performed on the **Prelude** to synthesize a biotinylated peptide thioester. The **Prelude**'s Single-Shot delivery feature was used to deliver the special monomers without any priming or waste.

[1]Kb = biotinylated lysine, X = racemic amino acid thioester

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