

## Boc Synthesis of a Biotinylated Peptide Thioester on the *Prelude*<sup>TM</sup>

### 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<sup>1</sup>: YIYGSFK-Kb-X-L

### METHOD

**Peptide Synthesis:** The peptide was synthesized at the 100  $\mu$ mol 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.

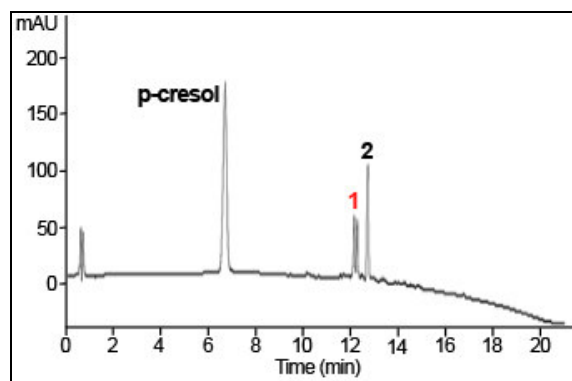
**Analysis:** The peptide was analyzed on a C18 column (2.1 x 50 mm) on an Agilent 1100/XCT LC/MS using an aqueous acetonitrile, 0.1% TFA

<sup>1</sup> Kb = biotinylated lysine, X = racemic amino acid thioester

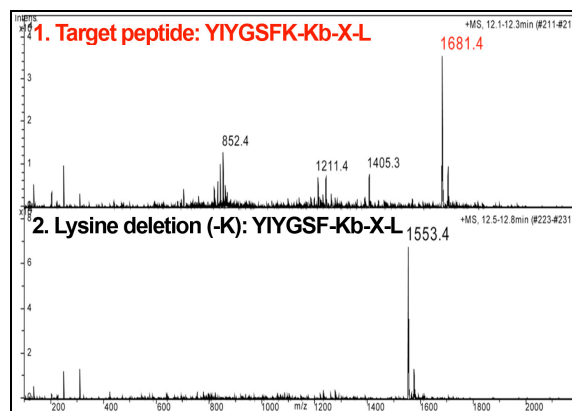
buffer system with an increasing gradient of 5-65% acetonitrile at 4% per minute. A flow rate of 0.5 mL/min was used, and detection was at 215 nm.

### RESULTS

The results are shown in Figures 1 and 2.



**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.

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.

## **CONCLUSION**

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