

Cost-Effective Automated Phosphopeptide Synthesis on the Prelude[®] Using Single-Shot[™] Deliveries

Application Note 3

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Introduction

The use of specially modified amino acids is available for a wide variety of peptide synthesis applications. These modified monomers are frequently extremely expensive and their implementation is limited by these costs. A **Single-Shot Delivery** feature has been designed into the **Prelude[®]** peptide synthesizer to ensure greater utilization of costly monomers. The entire contents of a Single-Shot amino acid vessel is delivered to a designated reaction vessel at a specified coupling step, eliminating any reagent loss. All other automated synthesizers waste some quantity during the delivery process. In this Application Note, the Single-Shot feature is used in the synthesis of the following phosphopeptides: (1) Bovine α_{S_1} -Casein (70-79)¹: H-EIVPNSSVEQK-NH₂ and (2) SIN1s315CP²: H-CRRKGSQKVS-NH₂. Phosphorylated serines are underlined in each sequence.

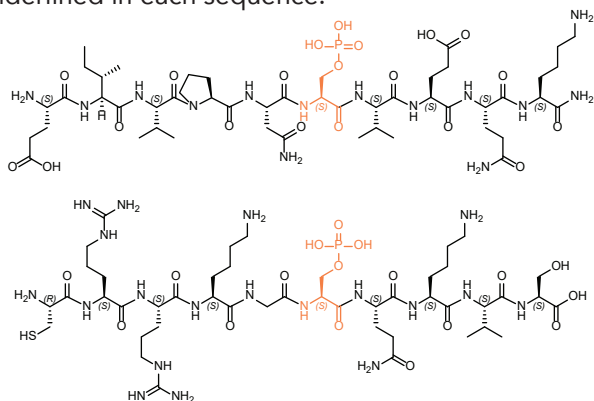


Figure 1: Structures of phosphorylated peptides.

Method

Peptide Synthesis: Peptides were synthesized on a **Prelude** peptide synthesizer at the 40 μ mol scale using Rink-amide-MBHA resin (0.47 mmol/g). Deprotection was performed with 20% piperidine in DMF for 2 x 15 minutes. Coupling was performed with 200 mM amino acid with a ratio of 1:1:2 amino acid/HCTU/NMM in DMF for 2 x 45 minutes for all couplings except phosphoserine, which was coupled

for 2 x 1 hour. Online cleavage was performed with 95:2:2:1 TFA/EDT/anisole/water for 2 hours. 1 mL aliquots of 200 mM phosphoserine were placed in Single-Shot vessels for each sequence.

Analysis: Peptides were analyzed on a Varian Microsorb C-18 column (4.5 x 50 mm) on a Varian Pro-Star HPLC using an aqueous acetonitrile, 0.1% TFA buffer system with an increasing gradient of 5-95% acetonitrile over 60 minutes. Detection was at 214 nm.

Results

The results are shown in Figures 2 and 3.

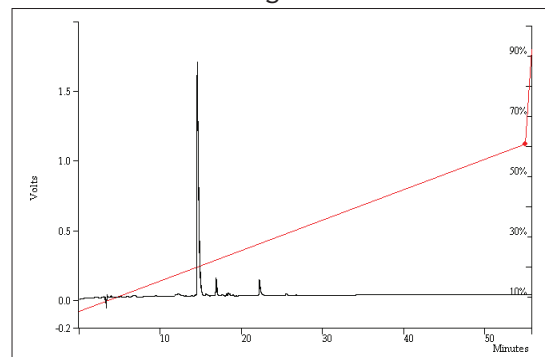


Figure 2: HPLC results for H-EIVPNS(S)VEQK-NH₂.

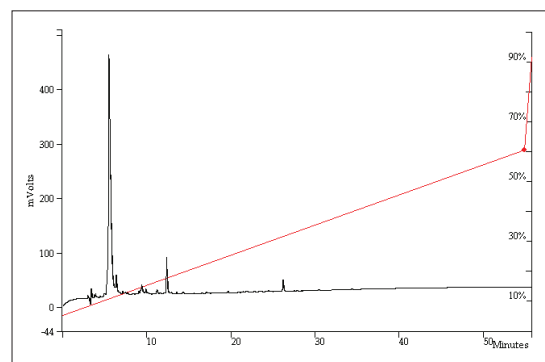


Figure 3: HPLC results for H-CRRKGS(Q)KVS-NH₂.

Conclusion

The **Prelude** produces high quality crude phosphopeptides without wasting expensive phosphorylated amino acid.

¹ P. Ferranti, S. Lilla, L. Chianese, and F. Addeo, J. Prot. Chem., 18, 595 (1999).

² Sequence provided by Cancer Research UK.

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