

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

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 are 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_{S1} -Casein (70-79)¹: H-EIVPNSVEQK-NH₂ and (2) SIN1s315CP²: H-CRRKGSQKVS-NH₂. Phosphorylated serines are underlined in each sequence.

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. 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 1 and 2.

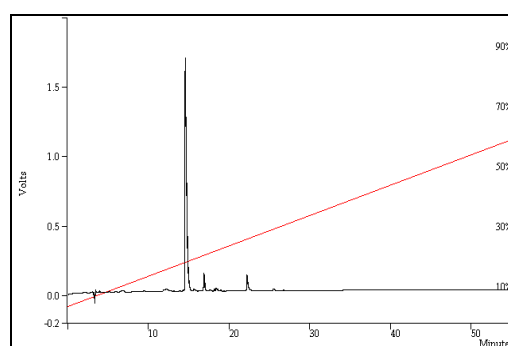


Figure 1: HPLC results for H-EIVPNpSVEQK-NH₂.

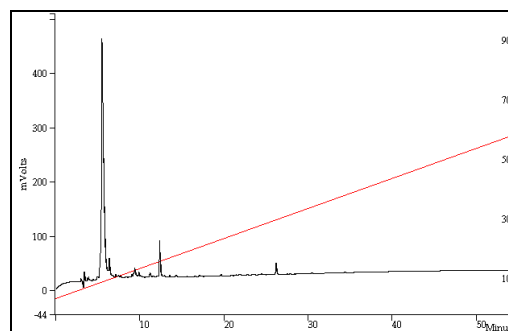


Figure 2: HPLC results for H-CRRKGpSQKVS-NH₂.

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.