HE-SPPS of Phosphopeptides

Summary

- The Liberty Blue™ automated microwave peptide synthesizer quickly and efficiently produces high quality phosphopeptides,
- CTEDQY(pS)LVED-NH_2 synthesized in 82% purity in under 2 hr.
- CPSPA(pT)DPSLY-NH_2 synthesized in 73% purity in under 2 hr.
- CSDGG(pY)MDMSK-NH_2 synthesized in 62% purity in just over 2 hr.

Introduction

Phosphorylation has elicited considerable interest in the investigation of post-synthetic modifications of proteins and peptides; it can be used to probe the purpose of phosphorylation of a protein or to identify the function of a particular phosphorylation. Originally, production of phosphopeptides required synthesis of the entire peptide followed by a post-synthetic phosphorylation step. This post-synthesis approach was often difficult to perform and frequently yielded impure peptides. The introduction of Fmoc-derived, monobenzyl-protected phosphoamino acids such as Fmoc-Thr(PO(OBzl)OH)-OH, Fmoc-Ser(PO(OBzl)OH)-OH, and Fmoc-Tyr(PO(OBzl)OH-OH (**Figure 1**) has significantly improved the synthesis process and enabled successful automation of SPPS for a wide range of phosphopeptides. Recently, microwave coupling steps have been shown to improve direct and subsequent couplings of phosphorylated residues as well as minimize dephosphorylation and deletion products.

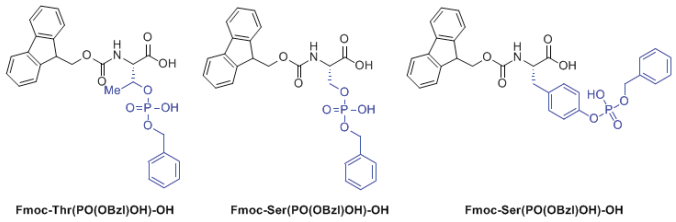

Figure 1. *Fmoc-Derived, Monobenzyl-Protected Phosphoamino Acids*

Materials and Methods

Reagents

N-α-Fmoc-O-benzyl-L-phosphoserine (pS), *N*-α-Fmoc-O-benzyl-L-phosphotyrosine (pY), and *N*-α-Fmoc-O-benzyl-L-phosphothreonine (pT) were obtained from CEM Corporation (Matthews, NC). All other amino acids were obtained from CEM Corporation (Matthews, NC) and contained the following side chain protecting groups: Asn(Trt), Asp(OMpe), Cys(Trt), Gln(Trt), Glu(OtBu), Lys(Boc), Ser(tBu), Thr(tBu), and Tyr(tBu). Oxyma Pure and Rink Amide ProTideTM LL resin were obtained from CEM Corporation (Matthews, NC). *N,N*-Diisopropylcarbodiimide (DIC) was obtained from CreoSalus (Louisville, KY). Piperidine was obtained from Alfa Aesar (Ward Hill, MA). Trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), triisopropylsilane (TIS), *N,N*-diisopropylethylamine (DIEA) thioanisole, and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), and anhydrous diethyl ether (Et_2O) were obtained from VWR (West Chester, PA). HPLC-grade water (H_2O), and HPLC-grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptide Syntheses

The peptides were prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.20 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with DIC in DMF, Oxyma Pure/DIEA in DMF, and a 5-fold excess of Fmoc-AA-OH. Additon of DIEA to Oxyma Pure solution employed to prevent dephosphorylation. Cleavage was performed with TFA/thioanisole/TIS/H_2O/DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Peptide Analysis

The peptides were analyzed on a Waters Acquity UPLC system with PDA detector equipped with an Acquity UPLC BEH C8 column (1.7 mm and 2.1 x 100 mm). The UPLC system was connected to a Waters 3100 Single Quad MS for structural determination. Peak analysis was achieved on Waters MassLynx software. Separations were performed with a gradient elution of 0.1% TFA in (i) H_2O and (ii) MeCN

Results

Microwave-enhanced SPPS of CTEDQY(pS)LVED-NH_2 on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 82% purity (**Figure 2**). No Tyr deletions were observed. Microwave-enhanced SPPS of CPSPA(pT) DPSLY-NH_2 produced the target peptide in 73% purity (**Figure 3**). Truncation of amino acids 7–11 was observed in a small quantity. Microwave-enhanced SPPS of CSDGG(pY)MDMSK-NH_2 produced the target peptide in 62% purity (**Figure 4**). Minimal aspartimide formation and methionine oxidation was observed.

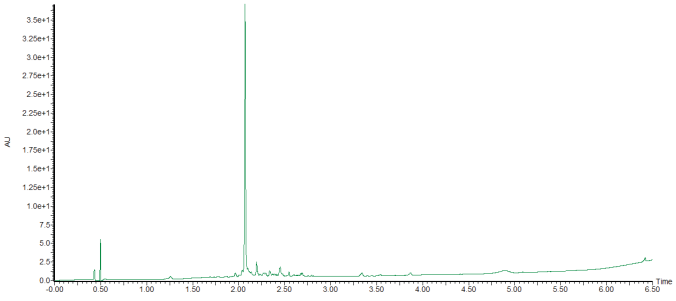

Figure 2. UPLC Chromatogram of CTEDQY(pS)LVED-NH_2

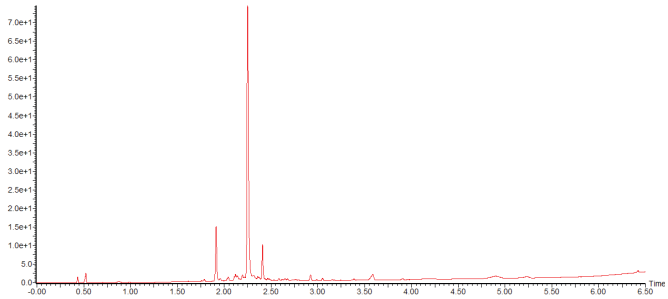

Figure 3. *UPLC Chromatogram of CPSPA(pT)DPSLY-NH_2*

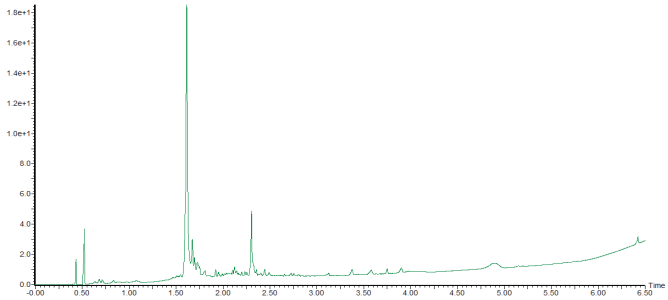

Figure 4. *UPLC Chromatogram of CSDGG(pY)MDMSK-NH_2*

Conclusion

Microwave-enhanced SPPS on the Liberty Blue automated microwave peptide synthesizer quickly and efficiently produces high quality phosphopeptides, such as CTEDQY(pS)LVED-NH_2, CPSPA(pT)DPSLY-NH_2, and CSDGG(pY)MDMSK-NH_2. Room temperature deprotection following the insertion of phosphoserine minimizes dephosphorylation in the synthesis of CTEDQY(pS) LVED-NH_2. Employment of Fmoc-Asp(OMpe)-OH minimizes the occurrence of aspartimide formation in susceptible sequences, especially CSDGG(pY)MDMSK-NH_2. Microwave-enhanced SPPS can improve direct and subsequent couplings of phosphorylated residues while minimizing undesired side reactions.

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