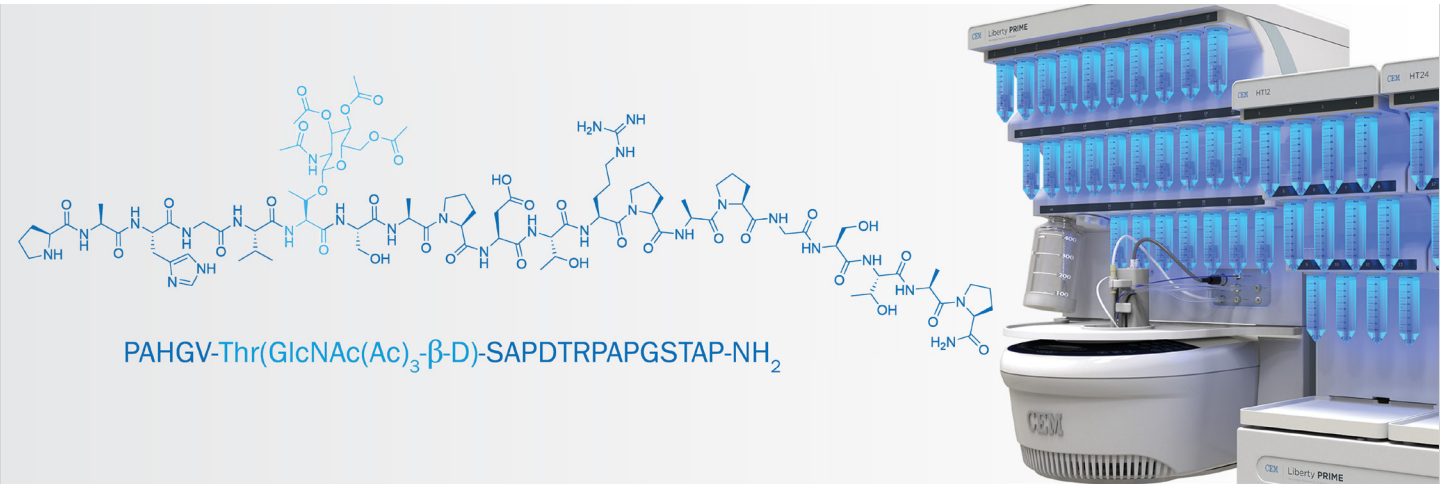
Automated Microwave-Enhanced Synthesis of Glycopeptides with O-Linked Glycans



Summary

Glycopeptides containing O-linked glycans can be synthesized quickly with good purity using microwave-enhanced SPPS. Preparation of a peptide substrate of WbwA[1] (a sialic acid glycosyltransferase), was completed in 3 h 10 min with 68% purity on the Liberty Blue™. Synthesis of an analog of APF (antiproliferative factor)[2] conjugated to the TUS nuclear localization sequence[3] was achieved in 1 h 8 min with 73% purity using the Liberty PRIME™.

Introduction

Glycosylation is an important post-translational and co-translational modification that involves the covalent modification of proteins or peptides with mono, di, or oligosaccharides.[4] Glycoproteins have diverse biological functions ranging from structural proteins and hormones (like collagen and thyroid-stimulating hormone) to enzymes and proteins involved in regulating immune response (such as various phosphatases and antibodies).[5] As the critical role of glycosylation becomes further illuminated, interest in the chemical synthesis of glycopeptides continues to grow steadily.

Peptides containing O-glycosylated amino acids can be prepared by SPPS by using Fmoc protected Ser or Thr (**Figure 1**). Application of microwave energy to the synthesis of glycopeptides containing O-linked glycans allows for more efficient coupling which leads to rapid synthesis times and high purity (CarboMAX™).[6]

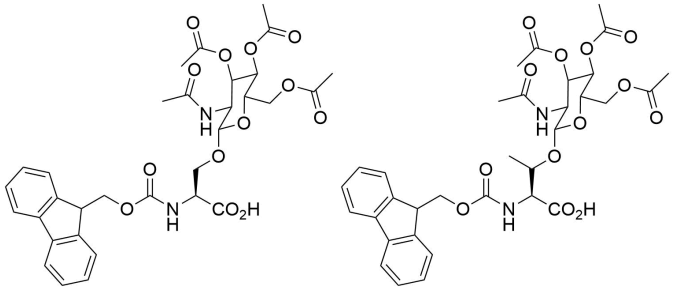


Figure 1: Fmoc-(S)-Ser(GlcNAc(Ac)$_3$-β-D)-OH (left) and Fmoc-(S)-Thr(GlcNAc(Ac)$_3$-β-D)-OH (right)

Materials and Methods

Reagents

The following Fmoc amino acids were obtained from CEM Corporation (Matthews, NC) and contain the indicated side chain protecting groups: Ala, Arg(Pbf), Asp(OMpe), Gly, His(Boc), Ile, Leu, Lys(Boc), Pro, Ser(tBu), Thr(tBu), and Val. Rink Amide ProTide™ LL resin and Fmoc-Ala-Wang LL resin were also obtained from CEM Corporation. Fmoc-Ser(GlcNAc(Ac)$_3$-β-D)-OH and Fmoc-Thr(GlcNAc(Ac)$_3$-β-D)-OH were purchased from EMD Millipore (Burlington, MA). N,N'-Diisopropylcarbodiimide (DIC), piperidine, pyrrolidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), and triisopropylsilane (TIS) were obtained from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM), N,N-Dimethylformamide (DMF), anhydrous diethyl ether (Et_2O), and acetic acid were obtained from VWR (West Chester, PA). LC-MS grade water (H_2O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptide Synthesis: WbwA Substrate, PAHGV-Thr(GlcNAc(Ac)$_3$-β-D)-SAPDTRPAPGSTAP-NH$_2$

The peptide was synthesized on a 0.05 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide resin LL (0.18 meq/g substitution). Deprotection was performed with piperidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure (with DIEA) in DMF (CarboMAX).[6] Fmoc-Thr(GlcNAc(Ac)$_3$-β-D)-OH was used for the glycosylated amino acid. Cleavage was performed at room temperature for 2 h using TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

Peptide Synthesis: TUS-APF Analog, KLKIKRPVK-Ser(GlcNAc(Ac)$_3$-β-D)-VPAAVVVA-CO$_2$H

The peptide was synthesized on a 0.10 mmol scale using the CEM Liberty PRIME automated microwave peptide synthesizer on Fmoc-Ala-Wang LL resin (0.31 meq/g substitution). Deprotection was performed with pyrrolidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[6] Fmoc-Ser(GlcNAc(Ac)$_3$-β-D)-OH was used for the glycosylated amino acid. Cleavage was performed at room temperature for 2 h using TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with 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 0.05% TFA in (i) H_2O and (ii) MeCN.

Results

Microwave-enhanced SPPS of WbwA substrate on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 68% purity (**Figure 2**).

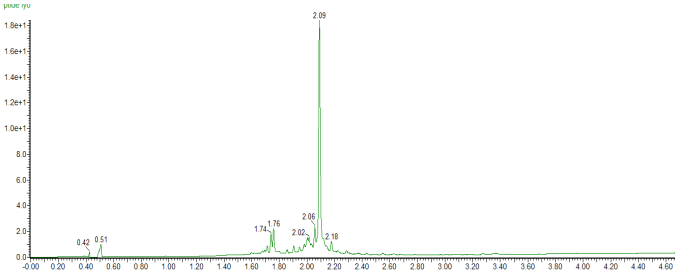


Figure 2: UPLC Chromatogram of WbwA Substrate

Microwave-enhanced SPPS of TUS-APF analog on the Liberty PRIME automated microwave peptide synthesizer produced the target peptide in 73% purity (**Figure 3**).

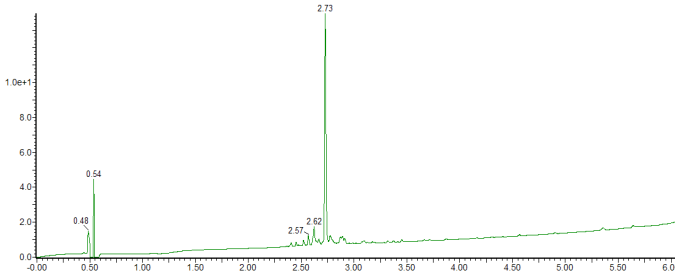


Figure 3: UPLC Chromatogram of TUS-APF analog

Conclusion

Glycopeptides with O-linked glycans can be synthesized rapidly and efficiently using automated microwave-enhanced SPPS. A WbwA peptide substrate was synthesized in 3 h 10 min with 68% purity using the Liberty Blue peptide synthesizer. On the Liberty PRIME, microwave-enhanced SPPS affords a TUS-APF analog peptide in 1 h 8 min with a purity of 73%. Additionally, this method reduces the overall synthesis cost for O-linked glycopeptides by using less than 2 equivalents of the very expensive Fmoc-protected glycoamino acids.

References

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(6) *CEM Application Note (AP0124) - "CarboMAX - Enhanced Peptide Coupling at Elevated Temperature."*

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