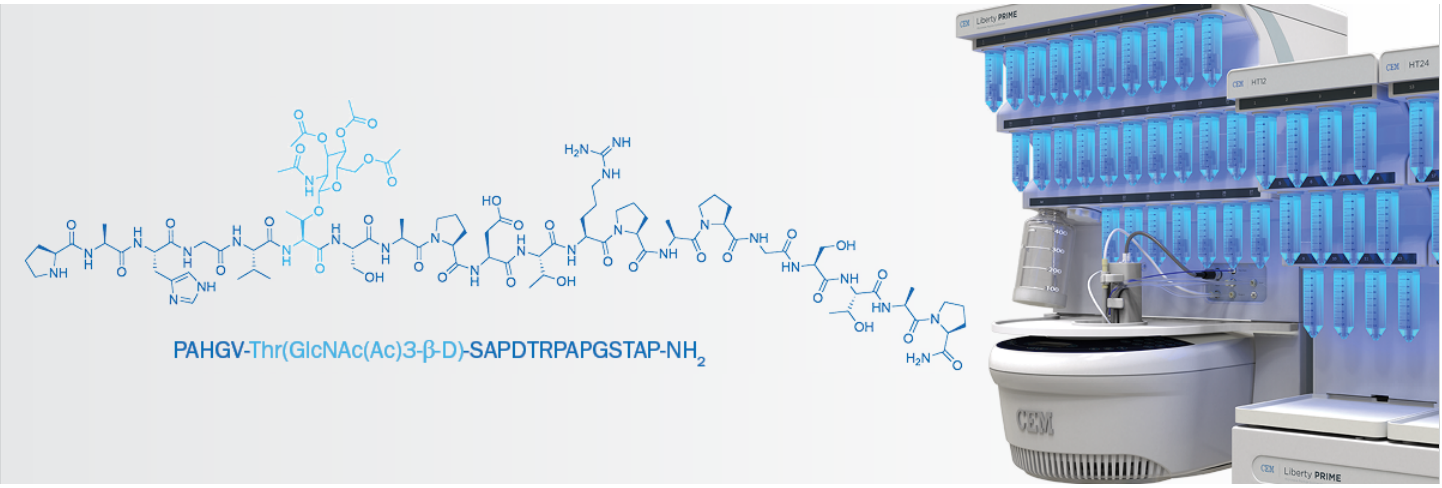
Automated Microwave-Enhanced Synthesis of Glycopeptides with O-Linked Glycans



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]

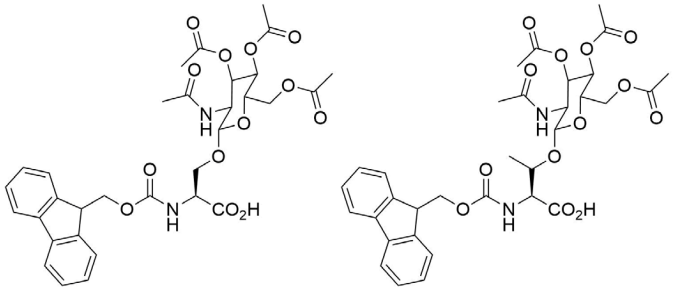


Figure 1: Fmoc-(S)-Ser(GlcNAc(Ac)3-β-D)-OH (left) and Fmoc-(S)-Thr(GlcNAc(Ac)3-β-D)-OH (right)

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).

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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 0.278 g Rink Amide ProTide resin LL (0.18 meq/g substitution). Deprotection was performed with 20% piperidine in DMF. Coupling reactions were performed in 5-fold excess of 0.2 M Fmoc-AA with 0.5 M DIC and 0.5 M Oxyma Pure (with 0.4 equiv DIEA) in DMF (CarboMAX).6 Fmoc-Thr(GlcNAc(Ac)3-β-D)-OH was used for the glycosylated amino acid (2-fold excess for glycoamino acid, 0.08 M in DMF). Cleavage was performed at room temperature for 2 h using 92.5:2.5:2.5:2.5 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_2H

The peptide was synthesized on a 0.10 mmol scale using the CEM Liberty PRIME automated microwave peptide synthesizer on 0.323 g Fmoc-Ala-Wang LL resin (0.31 meq/g substitution). Deprotection was performed with 25% pyrrolidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with 2.0 M DIC and 0.25 M Oxyma Pure in DMF (CarboMAX).[6] Fmoc-Ser(GlcNAc(Ac)3-β-D)-OH was used for the glycosylated amino acid (1.7-fold excess for glycoamino acid, 0.15 M in DMF). Cleavage was performed at room temperature for 2 h using 92.5:2.5:2.5:2.5 TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

Method Programming: WbwA Substrate

Peptide coupling, PAHGV-Thr(GlcNAc(Ac)3-β-D)-SAPDTRPAPGSTAP-NH_2

Deprotection (3 mL) was added to the reaction vessel containing the peptidyl resin, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the resin was washed with DMF (4 x 4 mL). Then, amino acid (1.25 mL), DIC (1 mL), and Oxyma Pure w/ 0.4 equiv DIEA (0.5 mL) were added to the reaction vessel, and the solution was microwaved for 4 min at 90 °C. Upon completion, the reaction vessel was drained, preparing the peptidyl resin for the next coupling reaction.

Glycoamino acid coupling, PAHGV-Thr(GlcNAc(Ac)3-β-D)-SAPDTRPAPGSTAP-NH_2

Deprotection (3 mL) was added to the reaction vessel containing the peptidyl resin, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the resin was washed with DMF (8 x 4 mL). Then, glycoamino acid (1.25 mL), DIC (1 mL), and Oxyma Pure w/ 0.4 equiv DIEA (0.5 mL) were added to the reaction vessel, and the solution was microwaved for 10 min at 90 °C. Upon completion, the reaction vessel was drained, preparing the peptidyl resin for the next coupling reaction.

Method Programming: TUS-APF Analog

Peptide coupling, KLKIKRPVK-Ser(GlcNAc(Ac)3-β-D)-VPAAVVVA-CO_2H

Deprotection (0.75 mL) was added to the reaction vessel containing the peptidyl resin, and the solution was microwaved for 40 sec at 110 °C (for the first deprotection step only, 4 mL of DMF is added to the reaction vessel before the addition of deprotection solution). Following deprotection, the resin was washed with DMF (2 x 4 mL). Then, amino acid (1 mL), DIC (0.5 mL), and Oxyma Pure (2 mL) were added to the reaction vessel, and the solution was allowed to bubble without microwave irradiation for 30 sec before being microwaved for 1 min at 105 °C. Upon completion, one-pot deprotection was performed by adding deprotection (0.75 mL) to the coupling solution to begin the next cycle (the RV was drained after deprotection).

Glycoamino acid coupling, KLKIKRPVK-Ser(GlcNAc(Ac)3-β-D)-VPAAVVVA-CO_2H

Deprotection (0.75 mL) was added to the reaction vessel containing the peptidyl resin, and the solution was microwaved for 40 sec at 110 °C. Following deprotection, the resin was washed with DMF (6 x 4 mL). Then, glycoamino acid (1 mL), DIC (0.5 mL), and Oxyma Pure (2 mL) were added to the reaction vessel, and the solution was allowed to bubble without microwave irradiation for 30 sec before being microwaved for 4 min at 105 °C. Afterwards, the coupling solution was drained, and DMF (4 mL) and deprotection (0.75 mL) were added to the reaction vessel, and the solution was microwaved for 40 sec at 110 °C. Following deprotection, the resin was washed with DMF (2 x 4 mL). Then, 0.5 M Fmoc-Lys(Boc)-OH in DMF (1 mL), DIC (0.5 mL), and Oxyma Pure (2 mL) were added to the reaction vessel, and the solution was allowed to bubble without microwave irradiation for 30 sec before being microwaved for 1 min at 105 °C. The reaction vessel was drained, and additional 0.5 M Fmoc-Lys(Boc)-OH in DMF (1 mL), DIC (0.5 mL), and Oxyma Pure (2 mL) were added. The solution was allowed to bubble without microwave irradiation for 30 sec before being microwaved for another 1 min at 105 °C. Upon completion, one-pot deprotection was performed by adding deprotection (0.75 mL) to the coupling solution to begin the next cycle (the RV was drained after deprotection).

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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**).

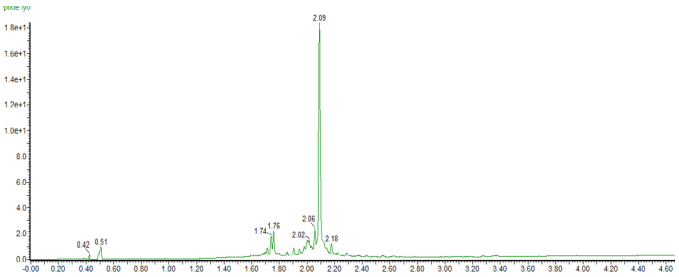

Figure 2: UPLC Chromatogram of WbwA Substrate

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

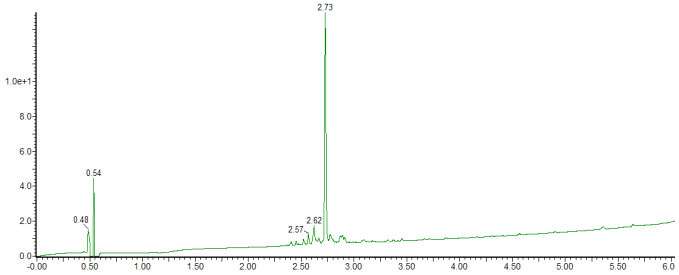

Figure 3: UPLC Chromatogram of TUS-APF analog

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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