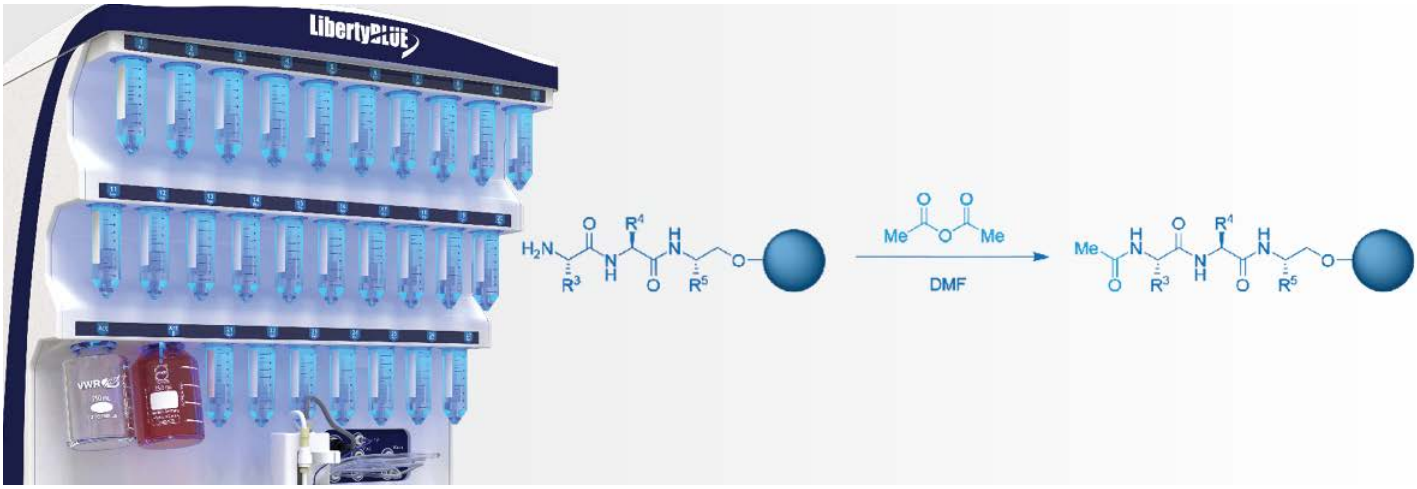
Automated N-Terminal Acetylation



Summary

- The Liberty Blue™ can accommodate a wide range of post synthetic modifications such as N-terminal acetylation.
- Microwave-enhanced SPPS on the Liberty Blue produced nonacetylated and acetylated peptides at 78% and 72% purity respectivly.
- Both peptides synthesized with a combined time of 2 hr 16 min and a total of < 200 mL of main wash (DMF) consumed.

Introduction

Synthetic peptides make promising drug candidates because their structural similarity to endogenous peptides and proteins grants them high levels of selectivity and specificity. Unfortunately, serum proteases rapidly break down peptides in the body, limiting their efficacy as drugs. To extend serum half-life, a number of peptide modifications have been developed, with N-terminal acetylation being one of the simplest (**Figure 1**). For example, Vogel et al. demonstrated that N-terminal acetylation of the small antimicrobial peptide, Lfc, extends the half-life in human serum from 0.5 to 1.5 hours.[1] Synthesis of N-terminally acetylated peptides can be easily automated using the CEM Liberty Blue automated microwave peptide synthesizer.

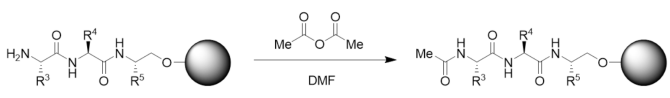


Figure 1. N-terminal acetylation

Materials and Methods

Reagents

All amino acids were obtained from CEM Corporation (Matthews, NC) and contained the following side chain protecting groups: Arg(Pbf), Gln(Trt), Trp(Boc). Oxyma Pure and Rink Amide ProTide™ 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), acetic anhydride 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 Synthesis: RRWQWR-NH_2 (Lfc2)

The peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.164 g Rink Amide ProTide resin (0.61 meq/g substitution). Deprotection was performed with 20% piperidine in DMF. Coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH, 0.5 M DIC in DMF and 1.0 M Oxyma Pure in DMF. Cleavage was performed using the CEM Razor™ high-throughput peptide cleavage system with 92.5:2.5:2.5:2.5 TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Peptide Synthesis: Ac-RRWQWR-NH_2 (Lfc4)

The peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.164 g Rink Amide ProTide Resin (0.61 meq/g substitution). Deprotection was performed with 20% piperidine in DMF. Coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH, 0.5 M DIC in DMF and 1.0 M Oxyma Pure in DMF. N-terminal acetylation was performed with 10% acetic anhydride in DMF. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with 92.5:2.5:2.5:2.5 TFA/H_2O/TIS/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 nonacetylated Lfc2 (RRWQWR-NH_2) on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 78% purity (**Figure 2**).

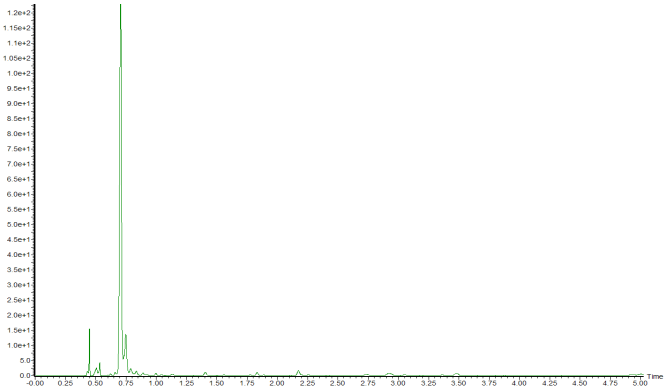


Figure 2: UPLC Chromatogram of RRWQWR-NH_2

Microwave-enhanced SPPS of acetylated Lfc4 (RRWQWR-NH_2) on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 72% purity (**Figure 3**). No unacetylated peptide was detected in the analysis of Lfc4.

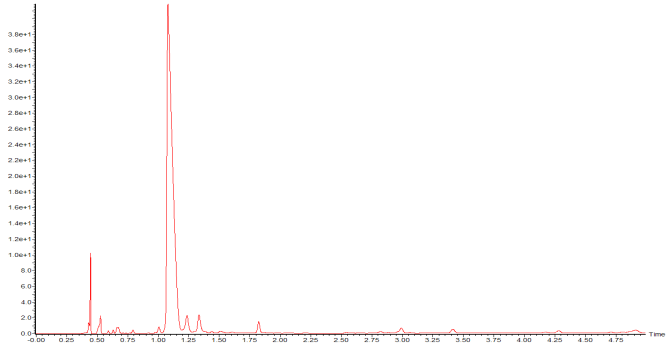


Figure 3: UPLC Chromatogram of Ac-RRWQWR-NH_2

Conclusion

The CEM Liberty Blue automated microwave peptide synthesizer allows quick and efficient access to synthetic peptides and effortlessly facilitates peptide modifications, like N-terminal acetylation. Microwave-enhanced SPPS produced nonacetylated peptide, Lfc2 (RRWQWR-NH_2), in 78% purity and acetylated peptide, Lfc4 (RRWQWR-NH_2), in 72% purity. Both peptides were synthesized with a combined time of 2 hr 16 min and a total of < 200 mL of main wash (DMF) consumed.

References

(1) Nguyen, L.; Chau, J.; Perry, N.; de Boer, L.; Vogel, H. *PLoS ONE*. **2010**, *5*, e12684.

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