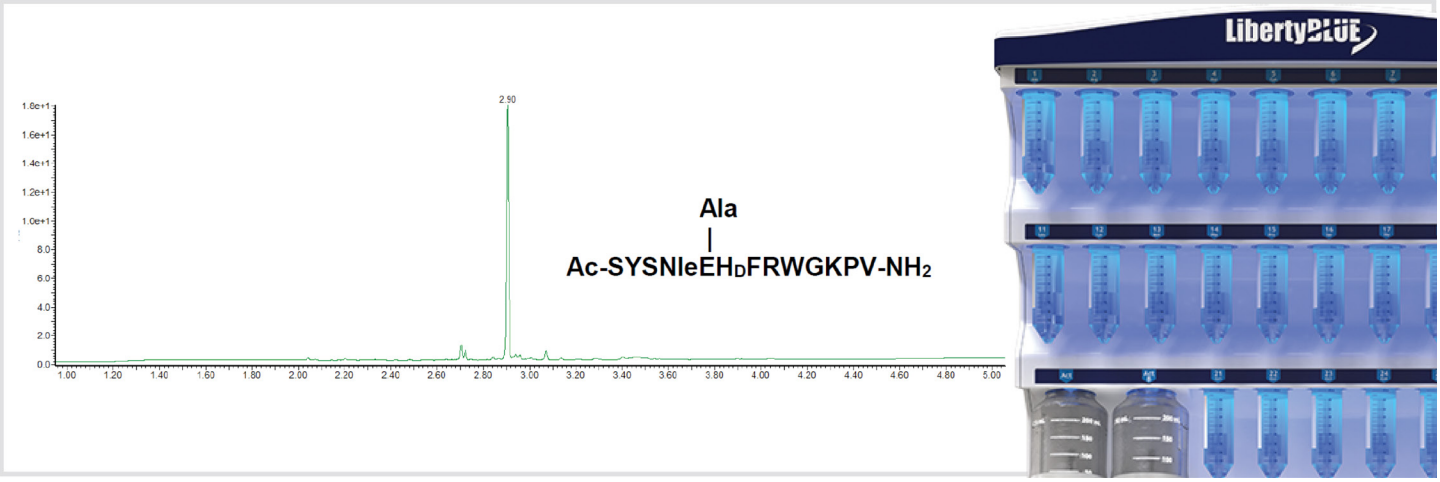
Automated Orthogonal Deprotection of Glu(OAllyl) and Peptide Stapling via Lactamization



Summary

Orthogonal deprotection of Glu(OAllyl) and peptide stapling via lactamization can be readily performed on the Liberty Blue™ automated microwave peptide synthesizer. Branched and lactam-stapled variants of both the HIV-1 antibody epitope gp41$_{659\text{-}671}$ and Afamelanotide were synthesized, with each of the four peptides synthesized between 80 – 87% purity and requiring 3 – 3.5 h of synthesis time, generating only 700 – 750 mL of total waste per synthesis.

Introduction

Peptidyl side-chain functionalizations, such as bioconjugation, branching, and cyclization, are impactful and essential synthetic tools, necessary for pharmaceutical development, medical imaging, materials research, and more.[1–4] Many amino acid residues can serve as sites for side-chain functionalization, including glutamic acid.

To perform side-chain functionalization successfully, the residue in question must be outfitted with an appropriately orthogonal protecting group, which readily undergoes selective deprotection while the rest of the peptide remains unaffected. In the case of glutamic acid, the allyl ester (OAllyl) is commonly used (**Figure 1**), requiring catalytic Pd(0) and phenylsilane for deprotection.

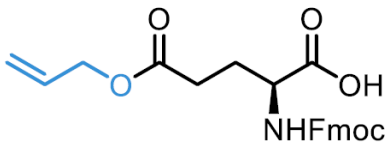


Figure 1: Fmoc-Glu(OAllyl)-OH

To demonstrate the Liberty Blue automated peptide synthesizer's ability to efficiently synthesize and functionalize peptides, branched and lactam-stapled variants of the HIV-1 antibody epitope gp41$_{659\text{-}671}$ (ELLELDKWASLWN) and Afamelanotide (Ac-SYSNleEHDFRWGKPV-NH$_2$) were synthesized. For branched variants, H-Ala-OtBu was coupled to the orthogonally deprotected Glu side-chain. For cyclized variants, Lys(Alloc) was incorporated into the linear synthesis and simultaneously deprotected with Glu(OAllyl) prior to lactam-stapling.

Materials and Methods

Reagents

Fmoc-Glu(OAllyl)-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Nle-OH, Fmoc-D-Phe-OH, and H-Ala-OtBu · HCl (free-based prior to use) were obtained from Novabiochem-MilliporeSigma (Burlington, MA). All other amino acids were obtained from CEM Corporation (Matthews, NC) and contained the following side chain protecting groups: Asn(Trt), Asp(OMpe), Arg(Pbf), Glu(OtBu), His(Boc), Lys(Boc), Ser(tBu), and Tyr(tBu). Oxyma Pure and Rink Amide ProTide™ LL resin were obtained from CEM Corporation (Matthews, NC). N,N-Diisopropylcarbodiimide (DIC) and hydroxybenzotriazole (HOBt) were obtained from CreoSalus (Louisville, KY). Piperidine was obtained from Alfa Aesar (Ward Hill, MA). Phenylsilane, tetrakis(triphenylphosphine)-palladium(0), acetic anhydride, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), triisopropylsilane (TIS), 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: ELLELDKWASLWN-NH_2 &
ELLELDKWASLWN-NH_2

The peptides were prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.18 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH, DIC in DMF and Oxyma Pure in DMF.[5] Alloc-Deprotection was performed with $Pd(PPh_3)_4$ in DCM and phenylsilane in DCM. Peptide stapling via lactamization was performed with a solution of DIC and HOBt in DMF. Cleavage was performed using the CEM Razor™ high-throughput peptide cleavage system with TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Note: The conditions employed in these Alloc-deprotection methods require less Pd than those in the following section, but longer heating periods.

Peptide Synthesis: Ac-SYSNIeEH$_D$FRWGKPV-NH_2 &
Ac-SYSNIeEH$_D$FRWGKPV-NH_2

The peptides were prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.18 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH, DIC in DMF and Oxyma Pure in DMF.[5] Alloc-Deprotection was performed with $Pd(PPh_3)_4$ in DCM and phenylsilane in DCM. Peptide stapling via lactamization was performed with a solution of DIC and HOBt in DMF. N-terminal acetylation was performed with acetic anhydride in DMF. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H_2O/TIS/ DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Note: The conditions employed in these Alloc-deprotection methods require more Pd equivalents than those in the previous section, but shorter heating periods.

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 the Glu-branched variant of the HIV-1 antibody epitope $gp41_{659–671}$ on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 82% purity (**Figure 2**). Microwave-enhanced SPPS of the lactam-stapled variant of the HIV-1 antibody epitope $gp41_{659–671}$ on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 87% purity (**Figure 3**). Microwave-enhanced SPPS of the Glu-branched variant of Afamelanotide on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 85% purity (**Figure 4**). Microwave-enhanced SPPS of the lactam-stapled variant of Afamelanotide on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (**Figure 5**).

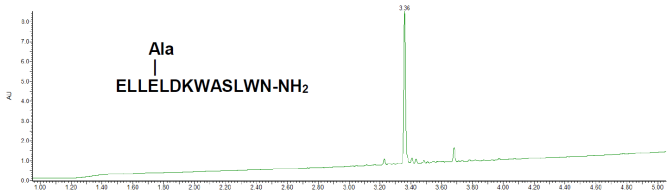


Figure 2: UPLC Chromatogram of the Glu-branched variant of HIV-1 antibody epitope $gp41_{659–671}$.

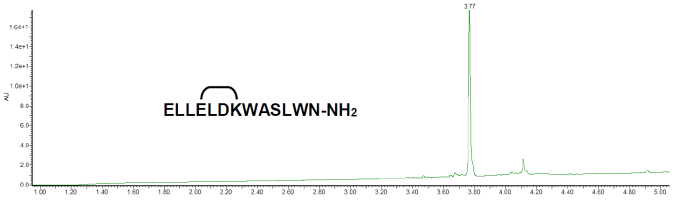


Figure 3: UPLC Chromatogram of the lactam-stapled variant of HIV-1 antibody epitope $gp41_{659–671}$.

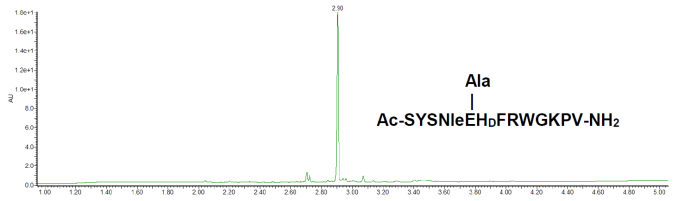


Figure 4: UPLC Chromatogram of the Glu-branched variant of Afamelanotide.

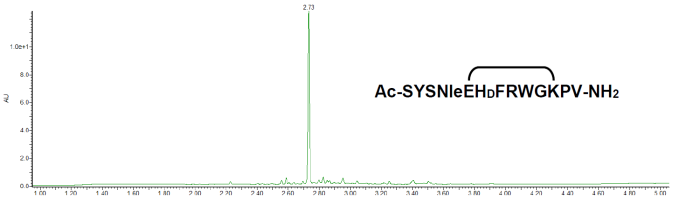


Figure 5: UPLC Chromatogram of the lactam-stapled variant of Afamelanotide.

Conclusions

The Liberty Blue automated peptide synthesizer enables the simple and efficient synthesis of variously functionalized peptides. With the capability of performing automated orthogonal deprotections (and contingent synthetic manipulations), branched and lactam-stapled variants of both the HIV-1 antibody epitope $gp41_{659–671}$ and Afamelanotide were synthesized. Each of the 4 peptides were synthesized between 80–87% purity and required 3–3.5 h of synthesis time, generating only 700 – 750 mL of total waste per synthesis.

References

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

(6) Automated Alloc-deprotection adapted from: Wilson, K. R.; Sedberry, S.; Pescatore, R.; Vinton, D.; Love, B.; Ballard, S.; Wham, B. C.; Hutchison, S. K.; Williamson, E. J. *J. Pep. Sci.* **2016**, *22*, 622–627.

(7) *CEM Application Note (AP0114) - "Automated N-Terminal Acetylation."*

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