Automated Synthesis of Hydrocarbon-Stapled Peptides Via Microwave Assisted Ring-Closing Metathesis

Summary
Hydrocarbon-stapled peptides can be synthesized rapidly with excellent purity using microwave enhanced SPPS on the Liberty Blue™ automated microwave peptide synthesizer. Synthesis of a pro-apoptotic BID stapled peptide derivative, BID SAHB (stabilized alpha-helix of BCL-2 domain), was achieved in under 4 h with 80% purity. Preparation of a pro-apoptotic BIM stapled peptide, BIM SAHB, was completed in under 4 h with 80% purity.

Introduction
Peptide stapling is an effective strategy for stabilizing α-helices, which are important structural motifs that dictate the biological activity of various peptides and proteins. Hydrocarbon stapling in particular has emerged as a powerful method for stabilizing α-helices and has produced several examples of peptides with higher target affinity and with dramatically increased protease resistance. Additionally, some hydrocarbon-stapled peptides have been shown to have greater cell permeability and in vivo activity than their unstapled analogues, which has further invigorated efforts to use α-helical peptides for therapeutic applications.

Hydrocarbon stapled peptides can be prepared by SPPS using amino acids bearing a terminal alkene in the sidechain, such as Fmoc-(S)-2-(4-pentenyl)Ala-OH (Figure 1a). After the pre-stapled peptide has been synthesized, the stapled variant can be prepared via ring-closing metathesis (RCM) using Grubbs Catalyst™ 1st Generation (Figure 1b). Conventional room temperature synthesis of stapled peptides is typically a lengthy process, with 20-mer peptides requiring well over 30 hours of synthesis time. Application of microwave energy to the synthesis of hydrocarbon stapled peptides allows for more efficient coupling which leads to rapid synthesis times and high purity (CarboMAX).

Materials and Methods
Reagents
The following Fmoc amino acids were obtained from CEM Corporation (Matthews, NC) and contain the indicated side chain protecting groups: Arg(Pbf), Asn(Trt), Asp(OMpe), Glu(OtBu), Gln(Trt), His(Boc), Ser(tBu), Trp(Boc), and Tyr(tBu). Rink Amide ProTide™ LL resin was also obtained from CEM Corporation. Grubbs Catalyst™ 1st Generation, Fmoc-(S)-2-(4-pentenyl)Ala-OH, N,N'-Diisopropylcarbodiimide (DIC), piperidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT),
triisopropylsilane (TIS), and acetic anhydride (Ac₂O) were obtained from Sigma-Aldrich (St. Louis, MO). 1,2-dichloroethane (DCE) was purchased from Alfa Aesar (Haverhill, MA). Dichloromethane (DCM), N,N-Dimethylformamide (DMF), anhydrous diethyl ether (Et₂O), acetic acid, HPLC grade water, and acetonitrile were obtained from VWR (West Chester, PA). LC-MS grade water (H₂O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptide Synthesis: BID SAHB, Ac-EDIIRNIHLA(S5)VGD(S5)LDRSIW-NH₂

The peptide (Figure 2) was prepared on a 0.05 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.263 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Fmoc deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure 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 in DMF (CarboMAX).⁸ Fmoc-(S)-2-(4-pentenyl)Ala-OH was used for S₅. Acetyl capping using 10% Ac₂O in DMF was performed after Fmoc deprotection of E. A 10 mM solution of Grubbs Catalyst 1st generation (58 mg) in DCE (7 mL) was used for the ring-closing metathesis stapling reaction. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H₂O/TIS/DODT. Following cleavage, the peptide was precipitated with Et₂O and lyophilized overnight.

Figure 2: Hydrocarbon-stapled BID SAHB

Peptide Synthesis: BIM SAHB, Ac-IWIAQELR(S5)IGD(S5)FNAYYARR-NH₂

The peptide (Figure 3) was synthesized on a 0.05 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.263 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Fmoc deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure 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 in DMF (CarboMAX).⁸ Fmoc-(S)-2-(4-pentenyl)Ala-OH was used for S₅. Acetyl capping using 10% Ac₂O in DMF was performed after Fmoc deprotection of I. A 10 mM solution of Grubbs Catalyst 1st generation (58 mg) in DCE (7 mL) was used for the ring-closing metathesis stapling reaction. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H₂O/TIS/DODT. Following cleavage, the peptide was precipitated with Et₂O and lyophilized overnight.

Figure 3: Hydrocarbon-stapled BIM SAHB

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) H₂O and (ii) MeCN.

Results

Microwave-enhanced SPPS of BID SAHB on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (Figure 4).

Figure 4: UPLC Chromatogram of BID SAHB

Microwave-enhanced SPPS of BIM SAHB on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (Figure 5).

Figure 5: UPLC Chromatogram of BIM SAHB
Conclusion

Hydrocarbon-stapled peptides can be synthesized rapidly and efficiently using microwave-enhanced SPPS. Conventional room temperature synthesis of a BID SAHB peptide requires over 35 h of synthesis time to generate the unstapled peptide and an additional 3-6 h for stapling. Using microwave-enhanced SPPS, the stapled peptide was synthesized in under 4 h with 80% purity. Conventional room temperature synthesis of BIM SAHB requires 33 h of manual labor time and an additional 3-6 h for stapling. On the other hand, microwave-enhanced SPPS affords the stapled peptide in under 4 h with a purity of 80%.

References


(8) CEM Application Note (AP0124) - “CarboMAX - Enhanced Peptide Coupling at Elevated Temperature.”