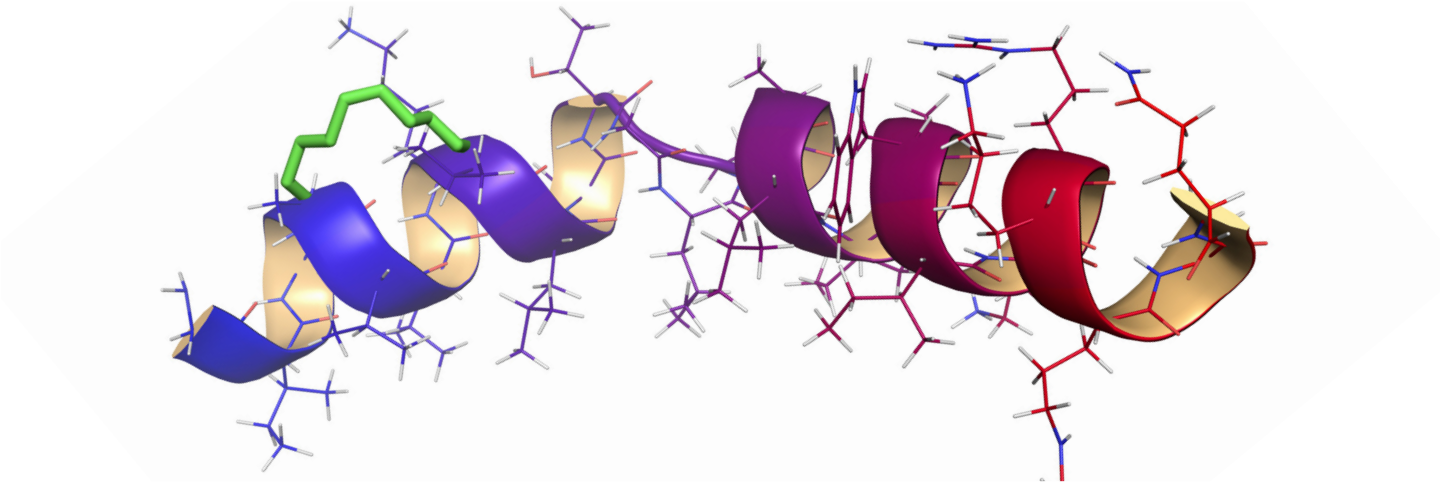
Automated Synthesis of Hydrocarbon-Stapled Peptides Via Microwave Assisted Ring-Closing Metathesis



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),[1] was achieved in under 4 h with 80% purity. Preparation of a pro-apoptotic BIM stapled peptide, BIM SAHB,[2] 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.[3] 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.[4] Additionally, some hydrocarbon-stapled peptides have been shown to have greater cell permeability and in vivo activity than their unstapled analogues,[2,5] which has further invigorated efforts to use α-helical peptides for therapeutic applications.[6]

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**).[7] 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**).[7] Conventional room temperature synthesis of stapled peptides is typically a lengthy process, with 20-mer peptides requiring well over 30 hours of synthesis time.[7] 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).[8]

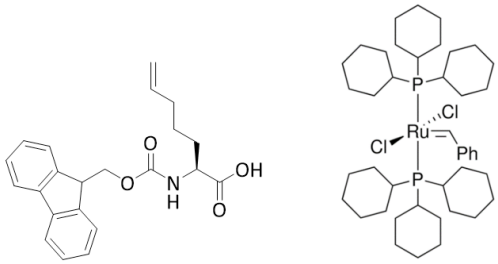


Figure 1: **(a)** Fmoc-(S)-2-(4-pentenyl)Ala-OH; **(b)** Grubbs Catalyst 1st Generation

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 (Ac_2O) 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 (Et_2O), acetic acid, HPLC grade water, and acetonitrile 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: BID SAHB, Ac-EDIIRNIARHLA(S5)VGD(S5)LDRSIW-NH_2

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% Ac_2O 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 92.5:2.5:2.5 TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

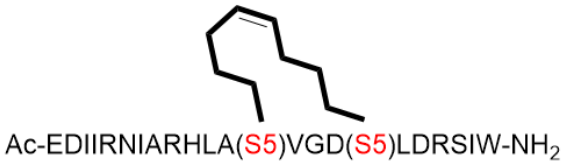


Figure 2: Hydrocarbon-stapled BID SAHB

Peptide Synthesis: BIM SAHB, Ac-IWIAQELR(S5)IGD(S5)FNAYYARR-NH_2

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% Ac_2O 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/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O 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) H_2O 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**).

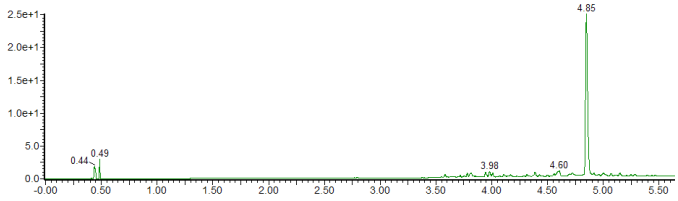


Figure 4: UPLC Chromatogram of BID SAHB

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

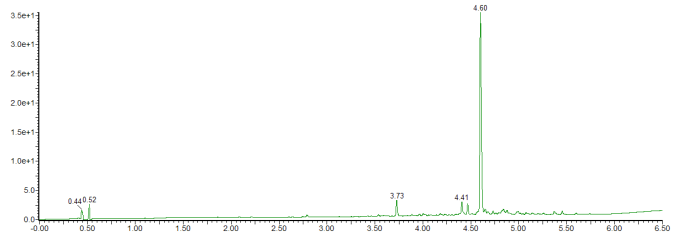


Figure 5: UPLC Chromatogram of BIM SAHB

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.[7] 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.[7] On the other hand, microwave-enhanced SPPS affords the stapled peptide in under 4 h with a purity of 80%.

References

(1) Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. Science 2004, 305 (5689), 1466–1470.

(2) LaBelle, J. L.; Katz, S. G.; Bird, G. H.; Gavathiotis, E.; Stewart, M. L.; Lawrence, C.; Fisher, J. K.; Godes, M.; Pitter, K.; Kung, A. L.; Walensky, L. D. J. Clin. Invest. 2012, 122 (6), 2018–2031.

(3) Bock, J. E.; Gavenonis, J.; Kritzer, J. A. ACS Chem. Biol. 2013, 8 (3), 488–499.

(4) Walensky, L. D.; Bird, G. H. J. Med. Chem. 2014, 57 (15), 6275–6288.

(5) Walensky, L. D.; Pitter, K.; Morash, J.; Oh, K. J.; Barbuto, S.; Fisher, J.; Smith, E.; Verdine, G. L.; Korsmeyer, S. J. Mol. Cell 2006, 24 (2), 199–210.

(6) Chang, Y. S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K.-H.; Olson, K. A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; Baker, T.; Darlak, K.; Elkin, C.; Filipovic, Z.; Qureshi, F. Z.; Cai, H.; Berry, P.; Feyfant, E.; Shi, X. E.; Horstick, J.; Annis, D. A.; Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. Proc. Natl. Acad. Sci. U. S. A. 2013, 110 (36), E3445-54.

(7) Bird, G. H.; Crannell, W. C.; Walensky, L. D. Curr. Protoc. Chem. Biol. 2011, 3 (3), 99–117.

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

United States (Headquarters)
800-726-3331
704-821-7015
Fax: 704-821-7894
info@cem.com

France
33 (01) 69 35 57 80
Fax: 33 (01) 60 19 64 91
info.fr@cem.com

Germany, Austria, Switzerland
(49) 2842-9644-0
Fax: (49) 2842-9644-11
info@cem.de

Ireland
+353 (0) 1 885 1752
Fax: +353 (0) 1 885 1601
info.ireland@cem.com

Italy
(39) 35-896224
Fax: (39) 35-891661
info.srl@cem.com

Japan
+81-3-5793-8542
Fax: +81-3-5793-8543
info@cemjapan.co.jp

United Kingdom
(44) 1280-822873
Fax: (44) 1280-822873
info.uk@cem.com

www.cem.com