Automated Synthesis of Head-to-Tail Cyclic Peptides via Microwave-Enhanced SPPS

Summary

Fully automated synthesis of head-to-tail cyclized peptides can be performed rapidly and with excellent purity using the Liberty Blue™ and Liberty PRIME™ peptide synthesizers. Microwave SPPS benefits not only the linear assembly but also the subsequent cyclization step with exceptional purity achieved on a variety of difficult biologically important peptides. The one-pot Fmoc SPPS cycles used on the Liberty PRIME provide even further benefit for synthesis time and waste reduction.

Table 1: Fully automated synthesis of head-to-tail cyclized peptides.

Instrument	Sequence	Crude Purity	Synthesis Time
Liberty Blue	cyclo-[GVYLHIE]	78%	2 h 13 min
Liberty Blue	cyclo-[WTaRRR-nal-R-Fpa-nle-Q]	75%	3 h 1 min
Liberty PRIME	cyclo-[WTaRRR-nal-R-Fpa-nle-Q]	75%	2 h
Liberty PRIME	cyclo-[WTaR-NMeGly-NMePhe-nal-NMeGly-Fpa-nle-E]	66%	2 h 5 min
Liberty PRIME	cyclo-[KA-NMeIle-NMeGly-NMeLeu-A-NMeGly-NMeGly-E]	73%	2 h 12 min

Table 2: Comparison of Liberty Blue and Liberty PRIME for the Synthesis of Cyclorasin A[1]

Instrument	Peptide	Synthesis Time	Total Waste Volume
Liberty Blue	Cyclorasin A	3 h 1 min	674 mL
Liberty PRIME	Cyclorasin A	2 h	584 mL

Introduction

Cyclic peptides are capable of bridging the gap of chemical space between small molecules and antibodies, allowing for the design of molecules with high binding affinity, remarkable selectivity, low toxicity, and the ability to access intracellular targets.[2] As a result, macrocyclic peptides hold considerable promise as therapeutics for targeting traditionally undruggable biological targets.[3] As of 2017, more than 40 cyclic peptides are used clinically.[4] This encouraging trend for the development of cyclic peptides as drug candidates has provided an impetus for more robust synthetic methods for their preparation.

Head-to-tail cyclized peptides can be prepared by SPPS by using Fmoc-Glu-ODmab as the C-terminal amino acid (**Figure 1**). After synthesis of the linear peptide backbone, the Dmab group can be selectively deprotected using a dilute hydrazine solution. Afterwards, head-to-tail cyclization can be achieved using microwave-enhanced coupling. Application of microwave energy to the synthesis of head-to-tail cyclized peptides allows for more efficient coupling which leads to rapid synthesis times and high purity (CarboMAX™).[5]

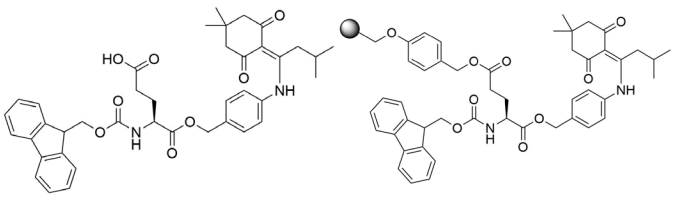

Figure 1: Fmoc-Glu-ODmab (left); Fmoc-Glu(Wang resin LL)-ODmab (right)

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), Gly, His(Boc), Ile, Leu, Lys(Boc), Thr(tBu), Trp(Boc), Tyr(tBu), and Val. Rink Amide ProTideTM LL resin was also obtained from CEM Corporation. Fmoc-Glu-ODmab, Fmoc-Glu(Wang)-ODmab LL resin, Fmoc-D-Ala-OH and Fmoc-4-fluoro-L-phenylalanine were purchased from EMD Millipore (Burlington, MA). Fmoc-D-2-Nal-OH, Fmoc-D-Nle-OH, and Fmoc-N-methyl-L-phenylalanine were obtained from Bachem (Torrance, CA). Fmoc-N-methyl-isoleucine-OH was purchased from Advanced ChemTech (Louisville, KY). Fmoc-N-methyl-leucine-OH was obtained from Alfa Aesar (Haverhill, MA). Hydrazine hydrate, N,N-Diisopropylethylamine (DIEA), Fmoc-N-methyl-glycine-OH, 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). N,N-Dimethylformamide (DMF), anhydrous diethyl ether (Et_2O), and acetic acid were obtained from VWR (Radnor, PA). LC-MS grade water (H_2O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific (Hampton, NH).

Peptide Synthesis: CEM 7-mer, cyclo-[GVYLHIE]

The peptide was synthesized on a 0.10 mmol scale (Dmab deprotection was performed on a 0.05 mmol scale, and head-to-tail cyclization was performed on a 0.025 mmol scale) using the CEM Liberty Blue automated microwave peptide synthesizer on Fmoc-Glu(Wang)-ODmab resin (0.25 meq/g substitution). Deprotection was performed with piperidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure (CarboMAX).[5] A solution of hydrazine in DMF was used to deprotect the ODmab group. Head-to-tail cyclization was performed using DIC/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 with Et_2O and lyophilized overnight.

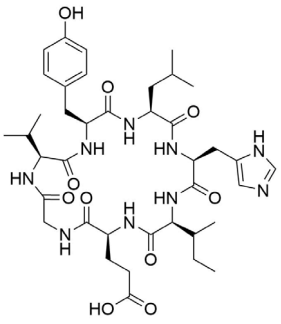

Figure 2: CEM 7-mer

Peptide Synthesis: Cyclorasin A, cyclo-[WTaRRR-nal-R-Fpa-nle-Q] *(Liberty Blue)*

The peptide was synthesized on a 0.05 mmol scale (Dmab deprotection was performed on a 0.05 mmol scale, and head-to-tail cyclization was performed on a 0.025 mmol scale) using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.19 meq/g substitution). Deprotection was performed with piperidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure (CarboMAX).[5] Fmoc-Glu-ODmab was used for the first amino acid, (Q). A solution of hydrazine in DMF was used to deprotect the ODmab group. Head-to-tail cyclization was performed using DIC/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 with Et_2O and lyophilized overnight.

Peptide Synthesis: Cyclorasin A, cyclo-[WTaRRR-nal-R-Fpa-nle-Q] *(Liberty PRIME)*

The peptide was synthesized on a 0.05 mmol scale (Dmab deprotection was performed on a 0.05 mmol scale, and head-to-tail cyclization was performed on a 0.025 mmol scale) using the CEM Liberty PRIME automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.19 meq/g substitution). Deprotection was performed with pyrrolidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure (CarboMAX).[5] Fmoc-Glu-ODmab was used for the first amino acid (Q). A solution of hydrazine was used to deprotect the ODmab group. Head-to-tail cyclization was performed using DIC/HOBt 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 with Et_2O and lyophilized overnight.

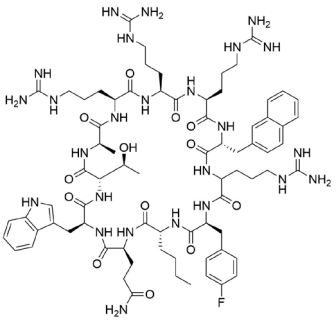

Figure 3: Cyclorasin A

Peptide Synthesis: N-Methyl Cyclorasin Analog, cyclo-[WTaR-NMeGly-NMePhe-nal-NMeGly-Fpa-nle-E]

The peptide was synthesized on a 0.10 mmol scale (Dmab deprotection was performed on a 0.05 mmol scale, and head-to-tail cyclization was performed on a 0.025 mmol scale)

using the CEM Liberty PRIME automated microwave peptide synthesizer on Fmoc-Glu(Wang)-ODmab resin (0.25 meq/g substitution). Deprotection was performed with pyrrolidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[5] A solution of hydrazine was used to deprotect the ODmab group. Head-to-tail cyclization was performed using DIC/HOBt in DMF. Cleavage was performed at room temperature using TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

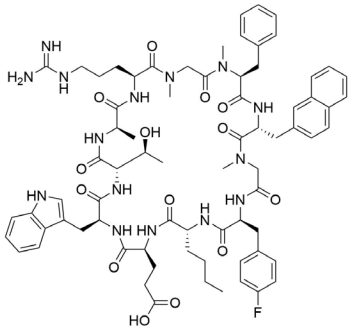

Figure 4: N-Methyl Cyclorain Analog

Peptide Synthesis: Poly N-Methyl Peptide, cyclo-[KA-NMelle-NMeGly-NMeLeu-A-NMeGly-NMeGly-E]

The peptide was synthesized on a 0.10 mmol scale (Dmab deprotection was performed on a 0.05 mmol scale, and head-to-tail cyclization was performed on a 0.025 mmol scale) using the CEM Liberty PRIME automated microwave peptide synthesizer on Fmoc-Glu(Wang)-ODmab resin (0.25 meq/g substitution). Deprotection was performed with pyrrolidine in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[5] A solution of hydrazine was used to deprotect the ODmab group. Head-to-tail cyclization was performed using DIC/HOBt in DMF. Cleavage was performed at room temperature using TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

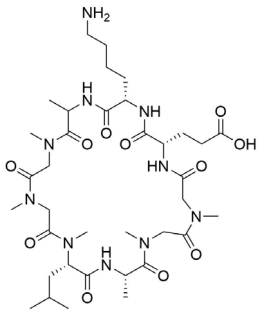

Figure 5: Poly N-Methyl Peptide

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 CEM 7-mer on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 78% purity (**Figure 6**).

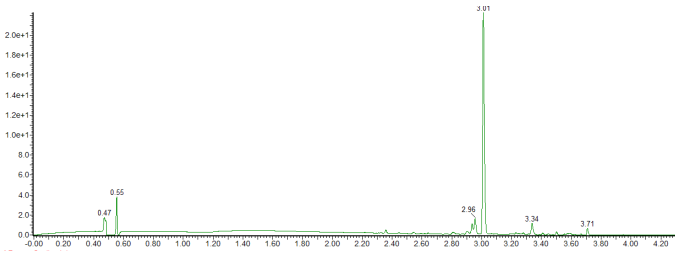

Figure 6: UPLC Chromatogram of CEM 7-mer

Microwave-enhanced SPPS of Cyclorasin A on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 75% purity (**Figure 7**).

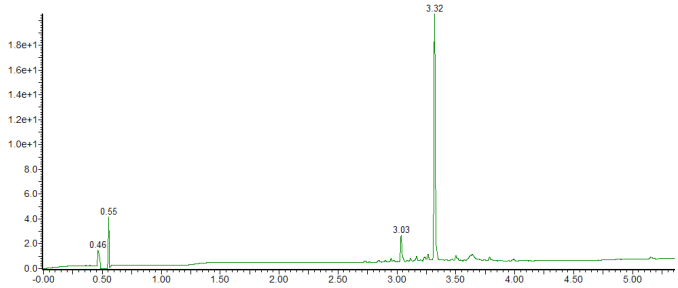

Figure 7: UPLC Chromatogram of Cyclorasin A (Liberty Blue)

Microwave-enhanced SPPS of Cyclorasin A on the Liberty PRIME automated microwave peptide synthesizer produced the target peptide in 75% purity (**Figure 8**).

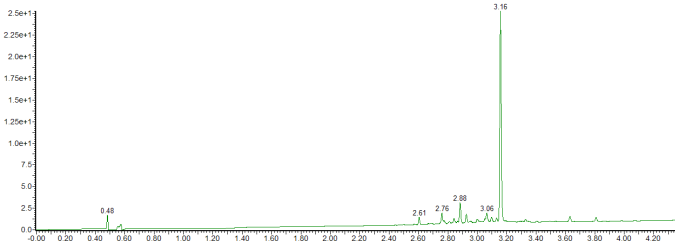

Figure 8: UPLC Chromatogram of Cyclorasin A (Liberty PRIME)

Microwave-enhanced SPPS of a poly N-Methyl peptide on the Liberty PRIME automated microwave peptide synthesizer produced the target peptide in 73% purity (**Figure 9**).

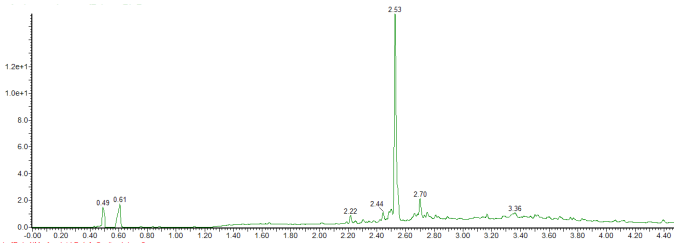

Figure 9: UPLC Chromatogram of Poly N-Methyl Peptide

Microwave-enhanced SPPS of N-methyl Cyclorasin Analog on the Liberty PRIME automated microwave peptide synthesizer produced the target peptide in 66% purity (**Figure 10**).

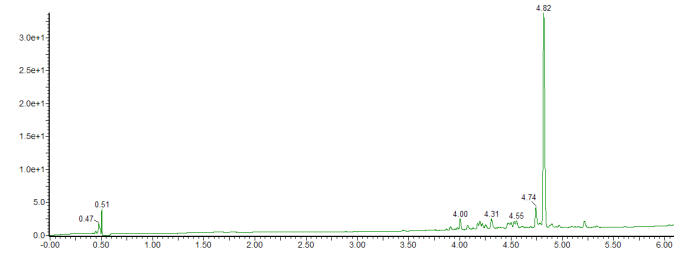

Figure 10: UPLC Chromatogram of N-Methyl Cyclorasin Analog

Conclusions

Head-to-tail cyclic peptides can be synthesized rapidly and efficiently using automated microwave-enhanced SPPS. Additionally, the easy-to-use Liberty Blue and Liberty PRIME software allows for quick and straightforward programming of the cyclic peptide sequences. A 7-mer peptide was synthesized in 2 h 13 min with 78% purity using the Liberty Blue peptide synthesizer. Cyclorasin A was synthesized in high purity (75%) in 3 h 1 min on the Liberty Blue. The same peptide was synthesized on the Liberty PRIME in just 2 h with excellent purity (75%) and roughly 100 mL less waste. On the Liberty PRIME, microwave-enhanced SPPS affords a synthetically challenging N-methyl cyclorasin analog in 2 h 5 min with a purity of 66%. Finally, a poly N-methylated 11-mer peptide was prepared in 2 h 12 min in 73% purity on the Liberty PRIME.

References

(1) Upadhyaya, P; Qian, Z.; Selner, N. G.; Clippinger, S. R.; Wu, Z.; Briesewitz, R.; Pei, D. Angew. *Chem. Int. Ed. Engl.* **2015**, *54* (26), 7602–7606.

(2) White, A. M.; Craik, D. J. Expert Opin. *Drug Discov.* **2016**, *11* (12), 1151–1163.

(3) Hurtley, S. M. *Science*. **2018**, *361* (6407), 1084.4-1085.

(4) Zorzi, A.; Deyle, K.; Heinis, C. *Curr. Opin. Chem. Biol*. **2017**, *38*, 24–29.

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

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