Fully Automated Synthesis of Cyclic Disulfide-Bridged Peptides

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Introduction

Cyclic peptides containing disulfides represent a class of compounds with a profound array of biological functions ranging from venoms to integral hormones.[1] The disulfide bonds help stabilize the secondary structure and conformation of peptides, which can contribute favorably to proteolytic stability and target affinity.[2] Because of their promising therapeutic potential, interest in the synthesis of cyclic disulfide-bridged peptides has grown steadily. Peptides with disulfide bridges can be prepared with SPPS by using orthogonally-protected cysteine amino acids such as Fmoc-(S)-Cys(Mmt)-OH and Fmoc-(S)-Cys(STmp)-OH (**Figure 1**). The Cys(Mmt) group can be selectively deprotected using a dilute solution of trifluoroacetic acid (TFA), whereas the Cys(STmp) group is orthogonally deprotected using dithiothreitol (DTT) as a reducing agent. After deprotection, selective oxidation of the Cys thiol groups to form a disulfide bond can be achieved using N-chlorosuccinimide (NCS) as a mild oxidant.[3]

Here, we report the fully automated synthesis of cyclic disulfide-bridged peptides in good purity using the Liberty Blue™ microwave peptide synthesizer. Preparation of a peptide agonist of BMP receptor activin-like kinase 3 (Alk3), THR-123[4], was completed in 3 h with 77% purity. Finally, a peptide venom from cone snails containing two disulfide bridges (Conotoxin-SI)[5] was synthesized in under 4 h with 67% purity. Application of microwave energy to the synthesis of disulfide-bridged peptides allows for more efficient coupling which leads to rapid synthesis times and high purity (CarboMAX™).6

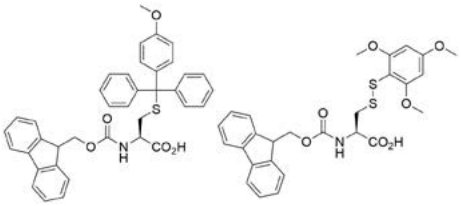

Figure 1. Left: Fmoc-(S)-Cys(Mmt)-OH; Right: Fmoc-(S)-Cys(STmp)-OH

Experimental

HE-SPPS Materials and Methods: All peptides were synthesized on the CEM Liberty Blue™ automated microwave peptide synthesizer using Rink Amide ProTide™ LL resin (0.19 mmol/g substitution) or Cl-MPA ProTide™ LL resin (0.18 mmol/g substitution). Post-deprotection washing with DMF was followed by coupling using a DIC/Oxyma activation method. The peptide resin was cleaved with TFA/TIS/H_2O/DODT (92.5/2.5/2.5/2.5) on the CEM Razor® peptide cleavage system. The peptide was precipitated in cold ether, and the crude material was lyophilized prior to analysis.

Analysis: Crude 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 2100 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 & Discussion

A) Synthesis of THR-123, CYFDDSSNVLCKKYRS-CO_2H

THR-123 (**Figure 2**) was chosen to demonstrate the synthesis of a single disulfide-bridged peptide containing a C-terminal acid. The peptide was synthesized on a 10 µmol scale on Cl-MPA ProTide™ LL resin (0.18 mmol/g substitution). The first amino acid was automatically loaded using CEM's previously reported chloride loading cycle. All other amino acid cycles used 1 min/90 °C deprotection and a single 2 min/90 °C coupling with DIC/Oxyma (Fmoc-(S)-Cys(Mmt)-OH was used for C). A solution of 2% TFA in DCM was used for deprotection of Cys(Mmt). The reaction was carried out at room temperature for 1 min and was repeated five times. Disulfide formation was achieved using a 25 mM solution of NCS in DMF. The reaction was performed at room temperature for 15 min. Microwave-enhanced SPPS of THR-123 on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 77% purity (**Figure 3**).

Figure 2. THR-123

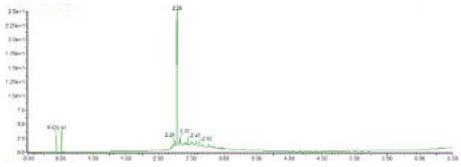

Figure 3. UPLC Chromatogram of THR-123

B) Synthesis of Conotoxin-SI, ICCNPACGPKYSC-NH_2

Conotoxin-SI (**Figure 4**) was chosen to demonstrate the synthesis of a cyclic peptide containing two disulfide bonds. The peptide was synthesized on a 10 µmol scale on Rink Amide ProTide™ LL resin (0.19 mmol/g substitution). All amino acid cycles used 1 min/90 °C deprotection and a single 2 min/90 °C coupling with DIC/Oxyma (Fmoc-(S)-Cys(Mmt)-OH was used for C; Fmoc-(S)-Cys(STmp)-OH was used for C). A solution of 2% TFA in DCM was used for deprotection of Cys(Mmt). The reaction was carried out at room temperature for 1 min and was repeated five times. Disulfide formation was achieved using a 25 mM solution of NCS in DMF. The reaction was performed at room temperature for 15 min. A solution of 5% DTT + 0.1 M NMM in DMF was used for deprotection of Cys(STmp). The reaction was carried out at room temperature for 5 min and was repeated three times. Finally, the second disulfide bond was formed using a solution of 25 mM NCS in DMF (room temperature for 15 min). Microwave-enhanced SPPS of Conotoxin-SI on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 67% purity (**Figure 5**).

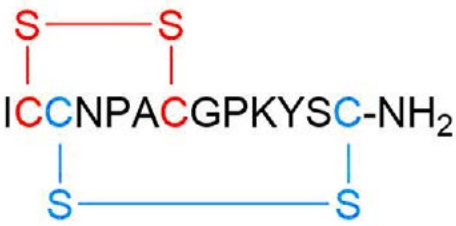

Figure 4. Conotoxin-SI

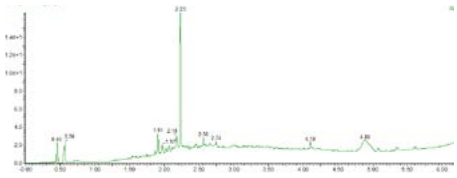

Figure 5. UPLC Chromatogram of Conotoxin-SI

Entry	Synthesis Scale	Resin	Disulfide Bonds	Protecting Groups	Crude Purity (%)
A	10 µmol	Cl-MPA ProTide LL	1	Cys(Mmt)	77
B	10 µmol	Rink Amide ProTide LL	2	Cys(Mmt) Cys(STmp)	67

Conclusion

The fully automated rapid synthesis of cyclic disulfide-bridged peptides was successfully performed in good purity. CarboMAX™ [6] chemistry allows for more efficient coupling which leads to rapid synthesis times and high purity. A cyclic, disulfide-bridged peptide with a C-terminal acid,THR-123, was quickly synthesized in 77% purity in under 3 h. Conventional room temperature synthesis of Conotoxin-SI, which contains two disulfide bridges, requires 20 h.[3] On the other hand, microwave-enhanced SPPS affords the peptide in under 4 h with a purity of 67%.

References

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