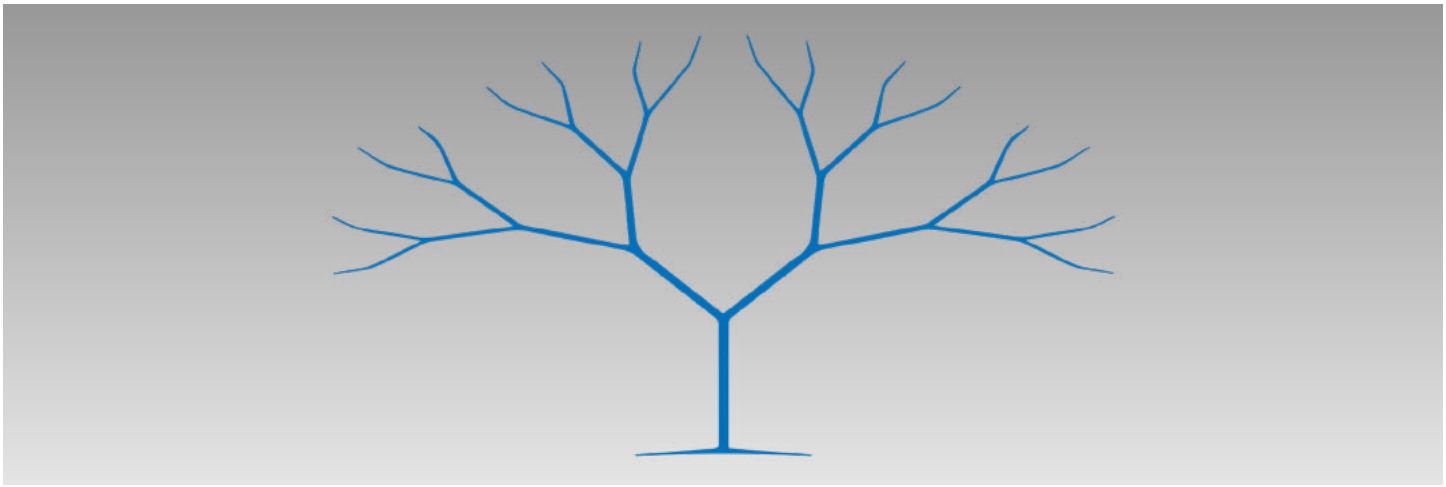
Microwave Assisted SPPS of Symmetrically Branched Peptides



Summary

Microwave-enhanced SPPS allows multiple antigenic peptides (MAPs) or peptide dendrimers with symmetrical lysine branching to be synthesized with drastically lowered synthesis times and improved purities. Synthesis of a tetra-branched acyl carrier protein (ACP) peptide was achieved in under 2 h with 70% purity. Synthesis of a tetra-branched M10 peptide[1] (the T-cell epitope of a *Paracoccidiodes brasiliensis* glycoprotein) was completed in under 4 h with 50% purity. Synthesis of an octameric, 3rd generation Lys-Leu antimicrobial peptide dendrimer (G3KL)[2] was carried out in under 2 h with 80% purity.

Introduction

Symmetrically branched peptides (**Figure 1**) represent a class of peptides with highly desirable physio-chemical and biological properties. In several cases, peptides with branched cores such as multiple antigenic peptides (MAPs) or peptide dendrimers have been shown to have increased biological activity compared to their linear counterparts due to multivalent binding to a protein target or due to an improved resistance to proteases.[3] As a result, branched peptides have found use in a variety of therapeutic applications including the development of antimicrobial and antiviral drugs[4,5], tumor-targeting agents[6,7], and drug delivery vehicles[8].

Symmetrically branched peptides can be synthesized via SPPS by using Fmoc-Lys(Fmoc)-OH to generate the branched position (**Figure 1**). Synthesis of branched peptides via SPPS is often challenging because of the inherent close proximity of the elongating peptide chains on a branched scaffold, which leads to steric clashes and poor peptide coupling. Application of microwave energy to the synthesis of branched peptides overcomes these steric challenges, allowing for more efficient coupling and the rapid synthesis of difficult branched peptides with fewer deletion products (CarboMAX™).[9]

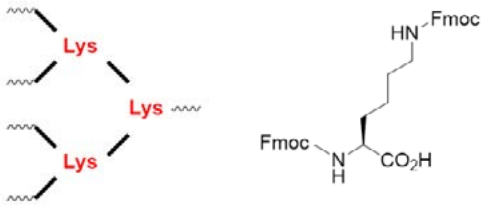
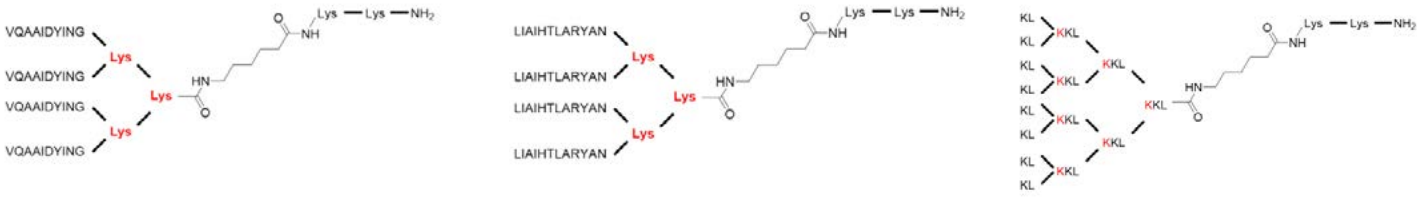


Figure 1: Left: Symmetrically branched lysine core; Right: Fmoc-Lys(Fmoc)-OH

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(OtBu), Gln(Trt), His(Boc), Lys(Boc), Tyr(tBu), and Thr(tBu). Rink Amide ProTide LL resin was also obtained from CEM Corporation. Fmoc-6-Ahx-OH was purchased from AnaSpec (Fremont, CA). Fmoc-Lys(Fmoc)-OH was obtained from CreoSalus (Louisville, KY). N,N'-Diisopropylcarbodiimide (DIC), piperidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), and triisopropylsilane



Tetra-branched ACP **Tetra-branched M10** **G3KL Octamer**

Figure 2: Tetra-branched ACP, tetra-branched M10, and G3KL Octamer.

(TIS) were obtained from Sigma-Aldrich (St. Louis, MO). 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: Tetra-Branched ACP, (VQAAIDYING)$_4$-(K)$_2$-K-Ahx-KK-NH$_2$

The peptide (**Figure 2**) was prepared on a 0.1 mmol scale (resin at 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 and Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[9] Fmoc-Lys(Fmoc)-OH was used for **K** at the branched position. 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: Tetra-Branched M10, (LIAIHTLAIRYAN)$_4$-(K)$_2$-K-Ahx-KK-NH$_2$

The peptide (**Figure 2**) was synthesized on a 0.1 mmol scale (resin at 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 and Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[9] Fmoc-Lys(Fmoc)-OH was used for **K** at the branched position. 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: G3KL Octamer, (KL)$_8$-(KKL)$_4$-(KKL)$_2$-KKL-Ahx-KK-NH$_2$

The peptide (**Figure 2**) was prepared on a 0.25 mmol scale (resin at 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 and Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).[9] Fmoc-Lys(Fmoc)-OH was used for **K** at the branched position. 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 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 tetra-branched ACP on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 70% purity (**Figure 3**).

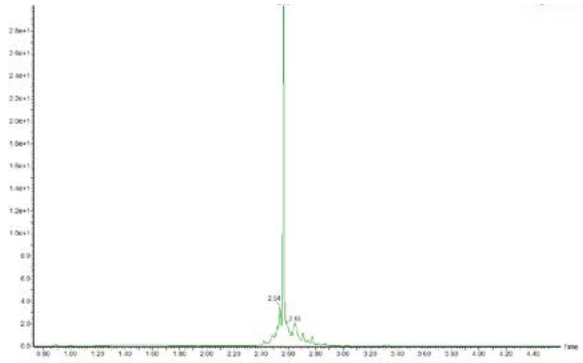


Figure 3: UPLC Chromatogram of Tetra-branched ACP

Microwave-enhanced SPPS of a tetra-branched M10 on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 50% purity (**Figure 4**).

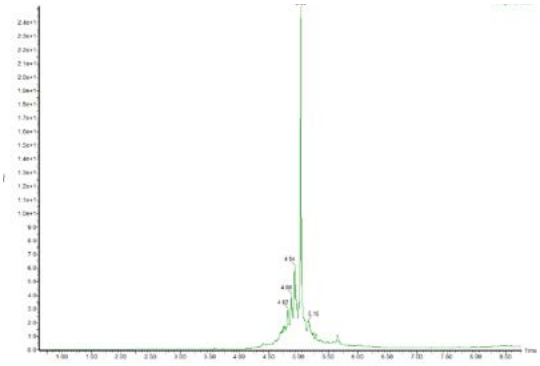


Figure 4: UPLC Chromatogram of Tetra-branched M10

Microwave-enhanced SPPS of G3KL octamer on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (**Figure 5**).

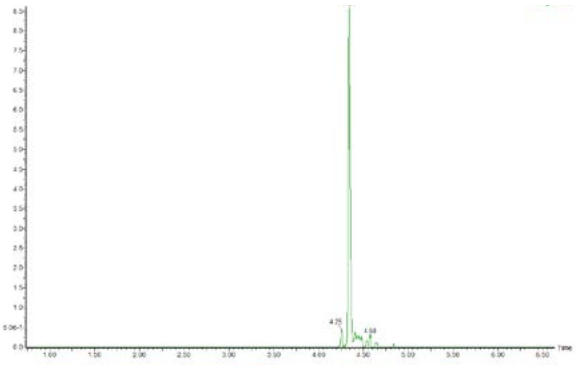


Figure 5: UPLC Chromatogram of G3KL Octamer

Conclusion

Symmetrically branched peptides can be prepared more rapidly and in considerably higher purities using microwave-enhanced SPPS compared to conventional SPPS methods. Using microwave-enhanced SPPS, tetra-branched ACP was synthesized in under 2 h with 70% purity. Conventional room temperature synthesis of a tetra-branched M10 peptide requires over 42 h of manual labor time with a 4% isolated yield of target peptide.[1] On the other hand, microwave-enhanced SPPS affords the peptide in under 4 h with a purity of 50%. Conventional synthesis of the G3KL octamer requires over 35 h of manual labor time and yields the target peptide in 8% isolated yield.[2] Application of microwave energy to the synthesis of the G3KL octamer provides the target peptide in under 2 h and in 80% purity.

References

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

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