# Microwave Assisted SPPS of Unsymmetrically Branched Peptides

## Summary

Microwave-enhanced SPPS allows unsymmetrically branched peptides to be synthesized rapidly and in high purity. Synthesis of a lactoferricin-lactoferrampin antimicrobial peptide (LF Chimera)[1] was achieved in under 5 h with 77% purity. Synthesis of a histone H2B-1A peptide fragment (residues 118-126)[2] conjugated to a ubiquitin peptide fragment (residues 47-76) was completed in under 5 h with 75% purity. Synthesis of tetra-branched analog of an antifreeze peptide[3] was carried out in under 5 h with 71% purity.

## Introduction

Unsymmetrically branched peptides can be synthesized via SPPS by using Fmoc-Lys(ivDde)-OH (**Figure 1**), which contains an orthogonally protected lysine. The ivDde group is stable under conditions necessary for Fmoc removal, but it is facilely cleaved by hydrazinolysis.[4] This allows for the selective generation of an unsymmetrical branch point where a peptide of a different sequence can be coupled at the ε-amine of the lysine sidechain. Unsymmetrical branching has been used to generate peptides with a wide variety of biological functions and chemical properties including compounds with antimicrobial activity[1], peptides with in vitro deubiquitinase resistance[2], and macromolecules with antifreeze properties[3].

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™).[5]

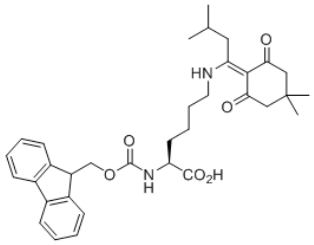


**Figure 1:** Fmoc-Lys(ivDde)-OH

## 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), Asp(OMpe), Asp(OtBu)-(Dmb)Gly-OH, Gln(Trt), Glu(OtBu), His(Boc), Lys(Boc), Ser(tBu), Tyr(tBu), Thr(tBu), and Trp(Boc). Rink Amide ProTide™ LL resin was also obtained from CEM Corporation (Matthews, NC). Boc-Phe-OH was purchased from Peptides International (Louisville, KY). Boc-Ala-OH was obtained from Alfa Aesar (Ward Hill, MA). Fmoc-Lys(ivDde)-OH was obtained from EMD Millipore (Billerica, MA). Anhydrous hydrazine, acetic anhydride, N,N'-Diisopropylcarbodiimide (DIC), piperidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), and triisopropylsilane (TIS) were obtained from

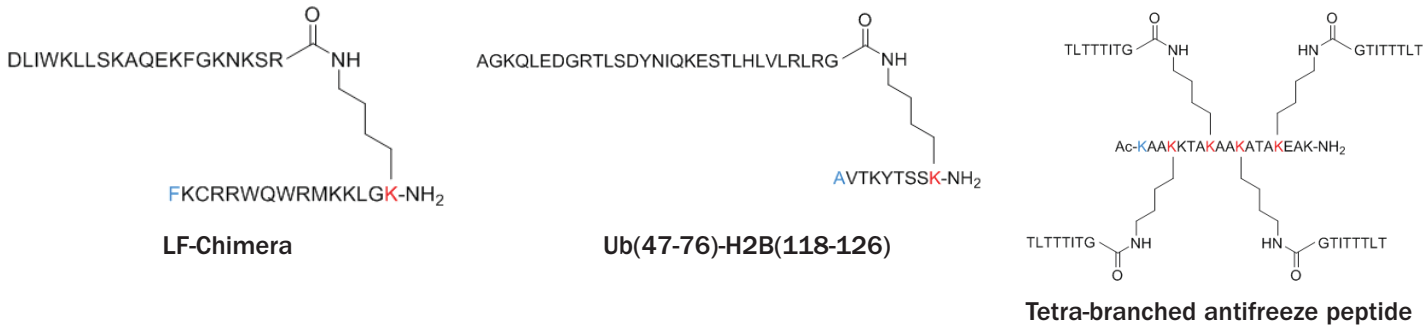


**Figure 2:** LF-Chimera, Ub(47-76)-H2B(118-126), and tetra-branched antifreeze peptide

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: LF-Chimera, (DLIWKLLSKAQEKFGKNKSR)-FKCRRWQWRNLLKGK-$NH_2$**

The peptide (**Figure 2**) was prepared on a 0.1 mmol scale using the CEM Liberty Blue™ automated microwave peptide synthesizer on 0.526 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with 1.0 M DIC and 1.0 M Oxyma Pure in DMF (CarboMAX™).[5] Fmoc-Lys(ivDde) was used for **K** at the branched position. A solution of 5% hydrazine in DMF was used to remove ivDde. Boc-Phe-OH was used for **F**. 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.

**Peptide Synthesis: Ub(47-76)-H2B(118-126), (AGKQLEDGRTLSDYNIQKESTLHLVLRLRG)-AVTKYTSSK-$NH_2$**

The peptide (**Figure 2**) was synthesized on a 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.526 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of Fmoc-AA with 1.0 M DIC and 1.0 M Oxyma Pure in DMF (CarboMAX).[5] For the Asp and Asp-Gly residues, Asp(OMpe) and an Asp(OtBu)-(Dmb) Gly-OH dipeptide were used, respectively to lower aspartimide formation. To prevent epimerization, His(Boc) was used instead of His(Trt). Fmoc-Lys(ivDde) was used for **K** at the branched position. A solution of 5% hydrazine in DMF was used to remove ivDde. Boc-Ala-OH was used for **A**. 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.

**Peptide Synthesis: Tetra-Branched Antifreeze Peptide, $(TLTTTITG)_4$-Ac-KAAKKTAKAAKATAKEAK-$NH_2$**

The peptide (**Figure 2**) was prepared on a 0.1 mmol scale (resin at 0.1 mmol scale for 1st strand; resin at 0.025 mmol scale for 2nd strand) using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide LL resin (0.526 g for the 1st strand; 0.132 g for the 2nd strand; 0.19 meq/g substitution). Deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure in DMF (CarboMAX).[5] Coupling reactions were performed in 5-fold excess of Fmoc-AA with 1.0 M DIC and 1.0 M Oxyma Pure in DMF. Fmoc-Lys(ivDde) was used for **K** at the branched position. A solution of 5% hydrazine in DMF was used to remove ivDde. Acetyl capping using 10% $Ac_2O$ in DMF was performed after deprotection of **K**. 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.

**Method Programming: LF-Chimera, (DLIWKLLSKAQEKFGKNKSR)-FKCRRWQWRNLLKGK-$NH_2$**

Deprotection (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the peptide was washed with DMF (4 x 4 mL). Then, amino acid (2.5 mL), DIC (1 mL), and Oxyma Pure (0.5 mL) were added to the reaction vessel, and the solution was microwaved for 2 min at 90 °C. Upon completion, the reaction vessel was drained, preparing the peptide for the next coupling reaction. This deprotection and coupling procedure was repeated to prepare the first peptide

strand up to **Phe**. Boc-Phe-OH was used to prevent further coupling to the N-terminus of the first strand.

After coupling Boc-Phe-OH, deprotection of Lys(ivDde) was performed. A solution of 5% v/v hydrazine in DMF (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 3 min at 90 °C. Upon completion, the peptide was washed with DMF (4 x 4 mL) using a wash through the manifold. After ivDde deprotection and washing, the second peptide strand was synthesized using the same coupling and deprotection conditions detailed above for preparing the first strand of the peptide.

**Method Programming: Ub(47-76)-H2B(118-126), (AGKQLEDGRTLSDYNIQKESTLHLVLRLRG)-AVTKYTSSK-$NH_2$**
Deprotection (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the peptide was washed with DMF (4 x 4 mL). Then, amino acid (2.5 mL), DIC (1 mL), and Oxyma Pure (0.5 mL) were added to the reaction vessel, and the solution was microwaved for 2 min at 90 °C. Upon completion, the reaction vessel was drained, preparing the peptide for the next coupling reaction. This deprotection and coupling procedure was repeated to prepare the first peptide strand up to **Ala**. Boc-Ala-OH was used to prevent further coupling to the N-terminus of the first strand.

After coupling Boc-Ala-OH, deprotection of Lys(ivDde) was performed. A solution of 5% v/v hydrazine in DMF (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 3 min at 90 °C. Upon completion, the peptide was washed with DMF (4 x 4 mL) using a wash through the manifold. After ivDde deprotection and washing, the second peptide strand was synthesized using the same coupling and deprotection conditions detailed above for preparing the first strand of the peptide.

**Method Programming: Tetra-Branched Antifreeze Peptide, $(TLTTTITG)_4$-Ac-KAAKKTAKAAKATAKEAK-$NH_2$**
Deprotection (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the peptide was washed with DMF (4 x 4 mL). Then, amino acid (2.5 mL), DIC (1 mL), and Oxyma Pure (0.5 mL) were added to the reaction vessel, and the solution was microwaved for 2 min at 90 °C. Upon completion, the reaction vessel was drained, preparing the peptide for the next coupling reaction. This deprotection and coupling procedure was repeated to prepare the first peptide strand up to **Lys**. After coupling Fmoc-Lys(Boc)-OH, an acetyl capping procedure was performed. Deprotection (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 1 min at 90 °C. Following deprotection, the peptide was washed with DMF (4 x 4 mL). Then, a solution of 10% acetic anhydride in DMF (4 mL) was added to the reaction vessel, and the solution was microwaved using the following microwave cycle: heat at 60 °C for 30 s; no heating for 30 s; heat at 60 °C for 30 s; no heating for 30 s. Upon completion, the peptide was washed with DMF (5 x 4 mL) using a wash through the manifold.

After acetyl capping the N-terminus of Lys, deprotection of Lys(ivDde) was performed. A solution of 5% v/v hydrazine in DMF (4 mL) was added to the peptide-containing reaction vessel, and the solution was microwaved for 3 min at 90 °C. Upon completion, the peptide was washed with DMF (4 x 4 mL) using a wash through the manifold. After ivDde deprotection and washing, the second peptide strand was synthesized using the same coupling and deprotection conditions detailed above for preparing the first strand of the 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 LF-Chimera on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 77% purity (**Figure 3**).

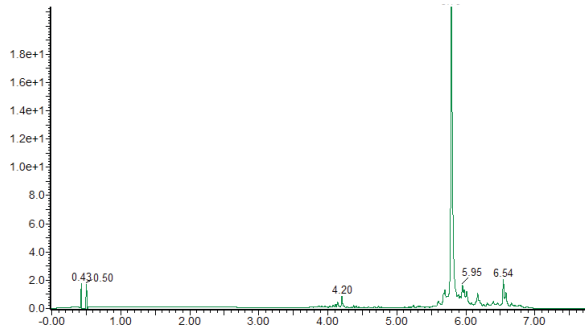


**Figure 3:** UPLC Chromatogram of LF-Chimera

Microwave-enhanced SPPS of Ub(47-76)-H2B(118-126) on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 75% purity (**Figure 4**).

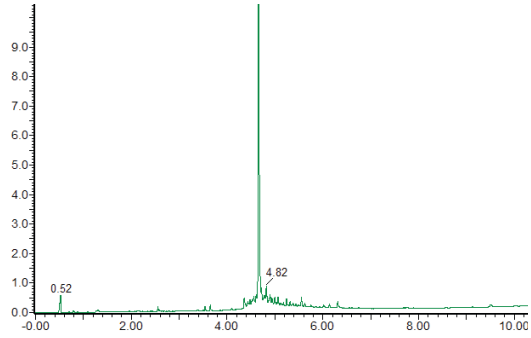


**Figure 4:** UPLC Chromatogram of Ub(47-76)-H2B(118-126)

Microwave-enhanced SPPS of tetra-branched antifreeze peptide on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 71% purity (**Figure 5**).

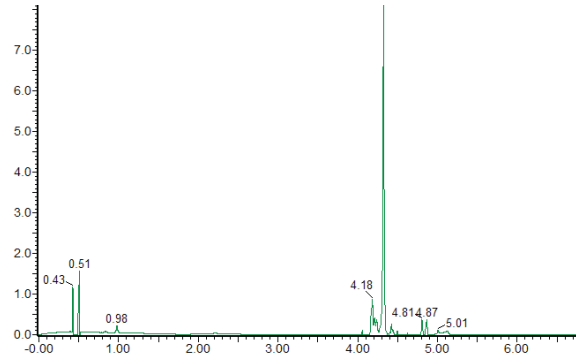


**Figure 5:** UPLC Chromatogram of Tetra-Branched Antifreeze Peptide

## Conclusion

Using microwave-enhanced SPPS, unsymmetrically branched peptides can be synthesized rapidly and in excellent purity. Application of microwave energy to the synthesis of a chimeric lactoferricin-lactoferrampin peptide provides the target peptide in under 5 h and in 77% purity. Using microwave-enhanced SPPS, a histone H2B fragment (residues 118-126) conjugated to a ubiquitin fragment (residues 47-76) was synthesized in under 5 h with 75% purity. Conventional room temperature synthesis of Ub(47-76)-H2B(118-126) requires over 53 h of manual labor time and gives a 10-20% isolated yield of target peptide.[2] Conventional synthesis of a dendrimeric antifreeze peptide requires over 72 h of manual labor time and yields the target peptide in 40% isolated yield.[3] Using microwave-enhanced SPPS, a tetra-branched antifreeze peptide was synthesized in under 5 h in 71% purity.

## References

(1) Haney, E. F.; Nazmi, K.; Bolscher, J. G. M.; Vogel, H. J. Biochim. Biophys. Acta - Biomembr. 2012, 1818 (3), 762–775.

(2) Haj-Yahya, M.; Eltarteer, N.; Ohayon, S.; Shema, E.; Kotler, E.; Oren, M.; Brik, A. Angew. Chemie Int. Ed. 2012, 51 (46), 11535–11539.

(3) Vera-Bravo, R.; Scotter, A. J.; Davies, P. L.; Blanco, L. H. Rev. Colomb. Química 2012, 41 (1), 133–157.

(4) Isidro-Llobet, A.; Álvarez, M.; Albericio, F. Chem. Rev. 2009, 109 (6), 2455–2504.

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

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