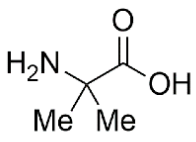
Microwave Assisted SPPS of Hindered, Non-Standard Amino Acids

Summary

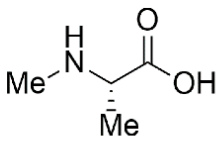
- Microwave-enhanced SPPS enables conventionally-difficult couplings of bulky amino acids, like Aib and *N*-Me-A, to occur quickly and efficiently.
- Syntheses of acyl carrier protein derivatives VQAibAibIDYING-OH and VQ(*N*-Me-A)(*N*-Me-A)IDYING-OH are completed in under 2 h and in 95% and 86% purities respectively
- Synthesis of GEQKLGAibAibAibASEESLG-NH_2 is completed in under 3 hr with an 89% purity.

Introduction

Hindered, non-standard amino acids such as α-aminoisobutyric acid (Aib) and *N*-methyl alanine ((*N*-Me)-A) (**Figure 1**) can be found in many biologically relevant compounds.[1–3] The synthesis of peptides including Aib or *N*-methylated amino acids has proved challenging, however; the steric hindrance introduced by the second methyl group, whether on the α-carbon or the amide nitrogen, makes coupling these amino acid derivatives difficult in conventional SPPS.



α-Aminoisobutyric Acid



***N*-Methyl Alanine**

Figure 1 Sterically-Hindered, Non-Standard Amino Acids

Through the use of microwave-enhanced SPPS, though, difficulties associated with hindered, non-standard amino acids have been minimized. The employment of microwave energy in SPPS drives conventionally-difficult couplings of bulky amino acids, like Aib and N-methyl alanine, quickly and efficiently to completion.[4,5]

Materials and Methods

Reagents

N-α-Fmoc-α-aminoisobutyric acid was obtained from AnaSpec (Freemont, CA). Fmoc-*N*-Me-Ala-OH was obtained from Peptides International (Louisville, KY). All other amino acids were obtained from CEM Corporation (Matthews, NC) and contained the following side chain protecting groups: Asn(Trt), Asp(OMpe), Gln(Trt), Glu(OtBu), Lys(Boc), Ser(OtBu), and Tyr(tBu). Oxyma Pure and Rink Amide ProTide™ LL resin were obtained from CEM Corporation (Matthews, NC). *N,N*-Diisopropylcarbodiimide (DIC) was obtained from CreoSalus (Louisville, KY). Fmoc-Gly-Wang Resin LL was obtained from NovaBiochem (St. Louis, MO). Piperidine was obtained from Alfa Aesar (Ward Hill, MA). Trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), triisopropylsilane (TIS), and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), and anhydrous diethyl ether (Et_2O) were obtained from VWR (West Chester, PA). HPLC-grade water (H_2O), and HPLC-grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptide Synthesis: GEQKLGAibAibAibASEESLG-NH_2

The peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue™ automated microwave peptide synthesizer

on Rink Amide ProTide LL resin (0.18 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with DIC in DMF, Oxyma Pure in DMF, and a 5-fold excess of Fmoc-AA-OH. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H_2O/TIS/ DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Peptide Synthesis: VQAibAibIDYING-OH

The peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Fmoc-Gly-Wang LL Resin (0.33 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with DIC in DMF, Oxyma Pure in DMF, and a 5-fold excess of Fmoc-AA-OH. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H_2O/TIS/ DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Peptide Synthesis: VQ(*N*-Me-A)(*N*-Me-A)IDYING-OH

The peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Fmoc-Gly-Wang LL Resin (0.19 meq/g substitution). Deprotection was performed with piperidine and Oxyma Pure in DMF. Coupling reactions were performed with DIC in DMF, Oxyma Pure in DMF, and a 5-fold excess of Fmoc-AA-OH. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H_2O/TIS/ DODT. Following cleavage, the peptide was precipitated in 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 0.1% TFA in (i) H_2O and (ii) MeCN.

Results

Microwave-enhanced SPPS of GEQKLGAibAibAibASEEDLG-NH_2 on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 89% purity (**Figure 2**).

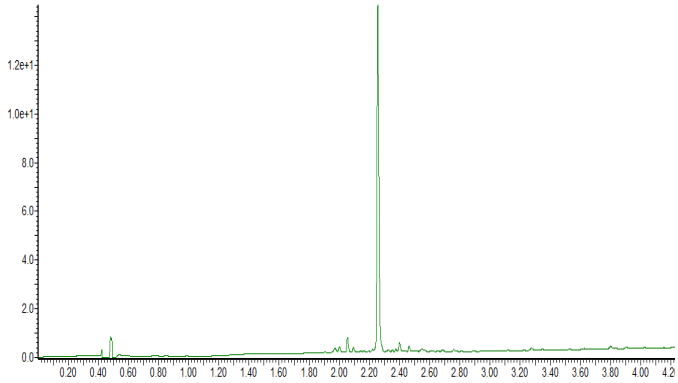


Figure 2 UPLC Chromatogram of GEQKLGAibAibAibASEEDLG-NH_2

Microwave-enhanced SPPS of VQAibAibIDYING-OH on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 95% purity (**Figure 3**).

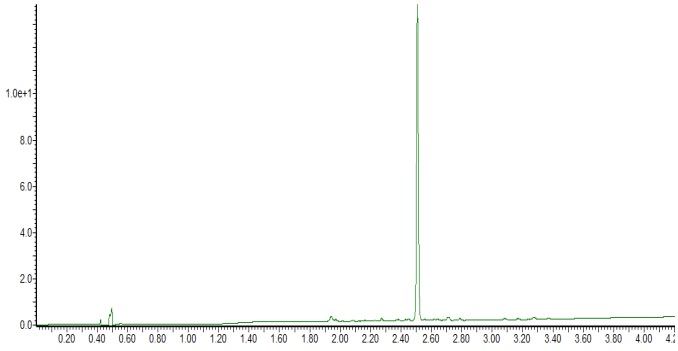


Figure 3 UPLC Chromatogram of VQAibAibIDYING-OH

Microwave-enhanced SPPS of VQ(*N*-Me-A)(*N*-Me-A)IDYING-OH on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 86% purity (**Figure 4**).

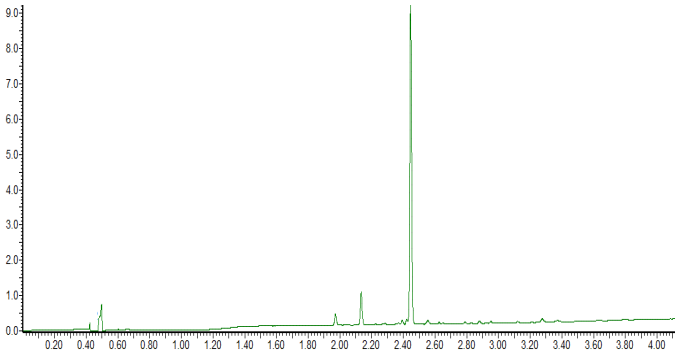


Figure 4 UPLC Chromatogram of VQ(N-Me-A)(N-Me-A)IDYING-OH

Conclusion

Microwave-enhanced SPPS enables conventionally-difficult couplings of bulky amino acids, like Aib and *N*-Me-A, to occur quickly and efficiently. Though conventional synthesis produces GEQKLGAibAibAibASEEDLG-NH_2 in 40 h and < 10% purity, microwave-enhanced SPPS produces the target peptide in under 3 h and in 89% purity. Additionally, syntheses of acyl carrier protein derivatives VQAibAibIDYING-OH and VQ(*N*-Me-A)(*N*-Me-A)IDYING-OH are completed in under 2 h and in 95% and 86% purities respectively. Microwave-enhanced SPPS has proven an effective tool in minimizing the difficulties associated with hindered, non-standard amino acids in SPPS.

References

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