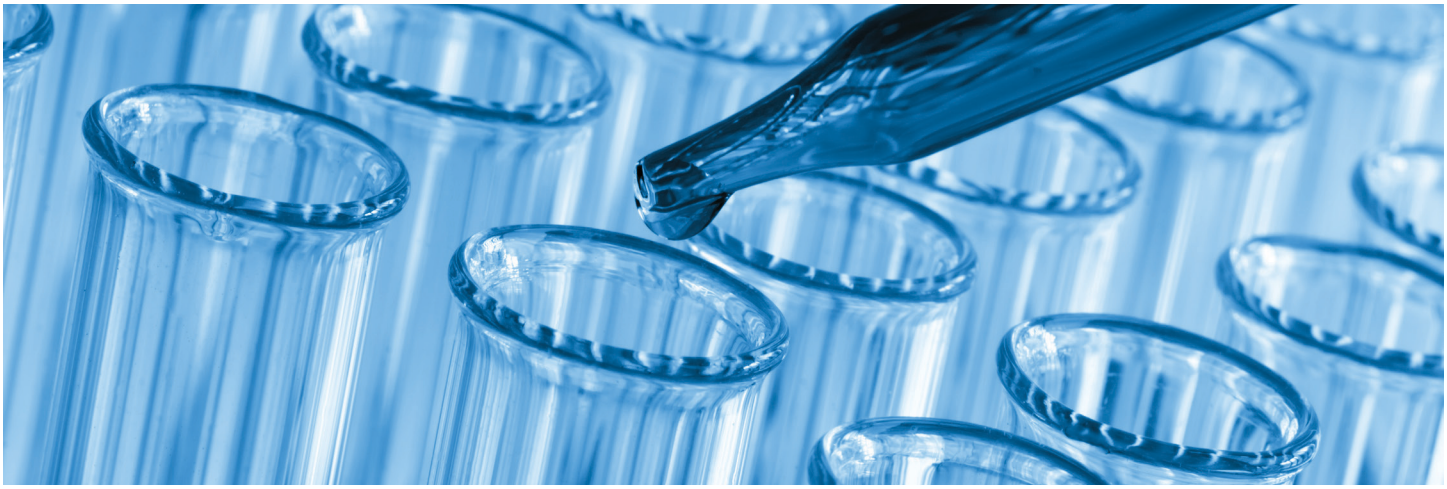
Automated Synthesis of Peptoids and Peptide-Peptoid Hybrids



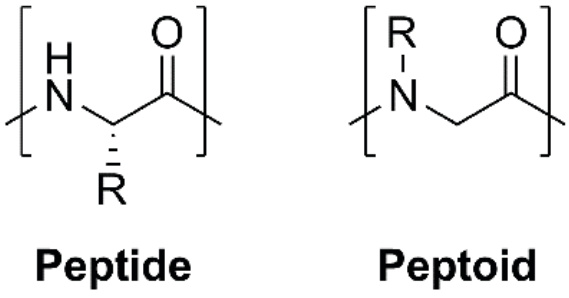
Summary

- The Liberty Blue™ automated microwave peptide synthesizer allows quick and efficient access to peptides, peptoids, and peptoid-peptide hybrids
- Peptide-peptoid hybrid Pro-Glu-(NLeu)-(NPhe)-Gly-(NLys)-NH_2 synthesized in 81% purity in under 2 hr.

Introduction

Peptoids are polymers of various N-substituted glycines. Though similar in structure to peptides (**Figure 1**), peptoids are resistant to proteolytic degradation, attributed to the complete substitution of their amide bonds. The increased stability of peptoids in vivo makes them an attractive peptidomimetic target for drug discovery and development.[1,2]

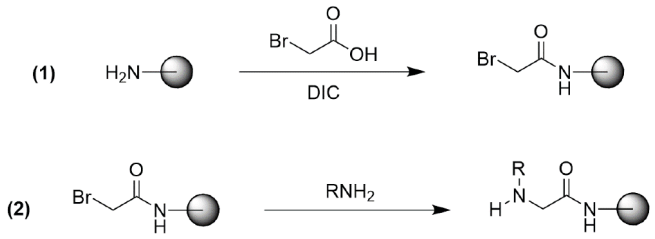
Figure 1



Comparison of peptide and peptoid structure

Peptoids and peptoid-peptide hybrids are typically synthesized through a "sub-monomer" process, which consists of two steps: (1) acylation with bromoacetic acid and N,N'diisopropylcarbodiimide (DIC) and (2) nucleophilic displacement with a monosubstituted amine (**Figure 2**).[1,3]

Figure 2



Typical synthesis of peptoids

Because many structurally diverse monosubstituted amines are commercially available, peptoids with a wide variety of side chains can be readily synthesized.[2,3] However, conventional synthesis of peptoids can take up to three hours per residue.[1] Microwave irradiation has been shown to significantly reduce this time, making production of peptoid libraries and peptoid-peptide hybrids much more viable.[1–3]

Materials and Methods

Reagents

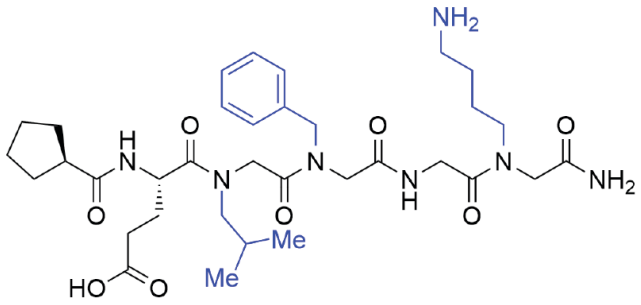
All amino acids were obtained from CEM Corporation (Matthews, NC) and contained the following side chain protecting groups: Glu(OtBu) and Lys(Boc). Oxyma Pure and Rink Amide ProTideTM LL resin were obtained from CEM Corporation (Matthews, NC). Bromoacetic acid, *N,N*-diisopropylcarbodiimide (DIC), benzylamine, β-alanine t-butyl ester hydrochloride, piperidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), triisopropylsilane (TIS), and acetic acid were obtained from

Sigma-Aldrich (St. Louis, MO). N-Boc-1,4-diaminobutane and isobutylamine were obtained from Alfa Aesar (Ward Hill, MA). Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), and anhydrous diethyl ether (Et2O) were obtained from VWR (West Chester, PA). HPLC-grade water (H2O), and HPLC-grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptoid-Peptide Hybrid Synthesis: Pro-Glu-(*N*Leu)-(*N*Phe)-Gly-(*N*Lys)-NH_2

The peptoid-peptide hybrid (**Figure 3**) was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.556 g Rink Amide ProTide LL resin (0.18 meq/g substitution). For peptoid residues, deprotection was performed with 20% piperidine in DMF, acylation was performed with 2 M bromoacetic acid and 2.4 M DIC, and nucleophilic displacement was performed with 1 M monosubstituted amine in DMF. For peptide residues, deprotection was performed with 20% piperidine in DMF, and coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH, 0.5 M DIC in DMF and 1.0 M Oxyma Pure in DMF. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with 92.5:2.5:2.5:2.5 TFA/H2O/TIS/ DODT. Following cleavage, the peptide was precipitated in Et2O and lyophilized overnight.

Figure 3



Target Peptoid-Peptide Hybrid: Pro-Glu-(NLeu)-(NPhe)-Gly-(NLys)-NH_2

Method Programming

Resin to Peptoid Monomer Coupling

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 5 mL). Then, bromoacetic acid (2.5 mL) and DIC (2.5 mL) were added to the reaction vessel and the solution was microwaved for 5 min at 75 °C, whereupon the reaction vessel was drained, a Wash Through Manifold performed, and the peptide washed with DMF (4 x 5 mL). Amine (5 mL) was added and the solution microwaved for 5 min at 75 °C. Upon completion, the reaction vessel was drained, a Wash Through Manifold performed, and the peptoid washed with DMF (4 x 5 mL), preparing the vessel for the next coupling reaction. (Pro-Glu-(NLeu)-(NPhe)-Gly-(**NLys**)-NH_2)

Peptoid-Terminus to Amino Acid Coupling

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 4 min at 90 °C. Upon completion, the reaction vessel was drained, a Wash Through Manifold performed, and the peptoid-peptide hybrid washed with DMF (4 x 5 mL), preparing the vessel for the next coupling reaction. (Pro-**Glu**-(NLeu)-(NPhe)-**Gly**-(NLys)-NH_2)

Peptide-Terminus to Peptoid Monomer Coupling

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 5 mL). Then, bromoacetic acid (2.5 mL) and DIC (2.5 mL) were added to the reaction vessel and the solution was microwaved for 5 min at 75 °C, whereupon the reaction vessel was drained, a Wash Through Manifold performed, and the peptide washed with DMF (4 x 5 mL). Amine (5 mL) was added and the solution microwaved for 5 min at 75 °C. Upon completion, the reaction vessel was drained, a Wash Through Manifold performed, and the peptoid-peptide hybrid washed with DMF (4 x 5 mL), preparing the vessel for the next coupling reaction. (Pro-Glu-(NLeu)-(**NPhe**)-Gly-(NLys)-NH_2)

Peptide-Terminus to Amino Acid Coupling

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Peptoid-Peptide Hybrid Analysis

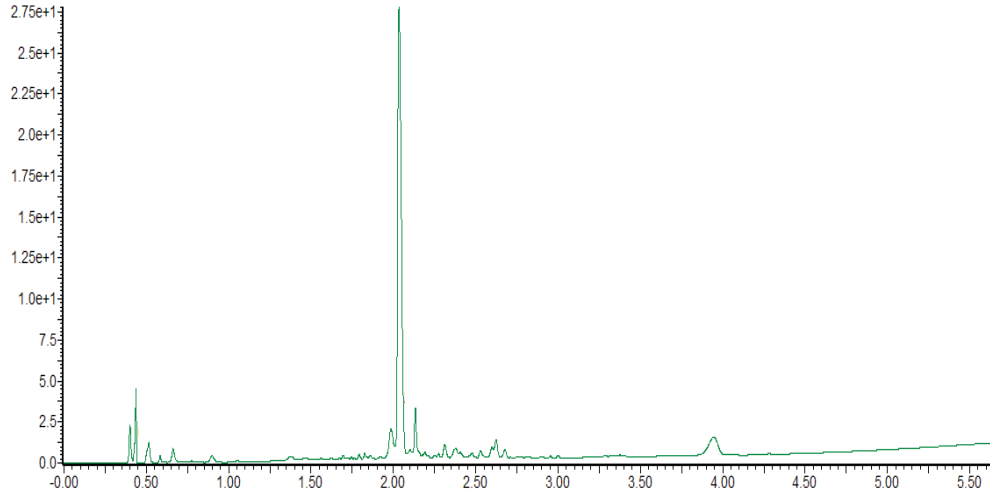
The peptoid-peptide hybrid was analyzed on a Waters Acquity UPLC system with PDA detector equipped with an Acquity UPLC BEH C8 column (1.7 mm and 2.1 x 100 mm). The UPLC system was connected to a Waters 3100 Single Quad MS for structural

determination. Peak analysis was achieved on Waters MassLynx software. Separations were performed with a gradient elution of 0.1% TFA in (i) H2O and (ii) MeCN.

Results

Microwave-enhanced SPPS of Pro-Glu-(NLeu)-(NPhe)-Gly-(NLys)-NH_2 on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 81% purity (**Figure 4**).

Figure 4



UPLC Chromatogram of Pro-Glu-(NLeu)-(NPhe)-Gly-(NLys)-NH_2

Conclusion

The CEM Liberty Blue automated microwave peptide synthesizer allows quick and efficient access to peptides, peptoids, and peptoid-peptide hybrids. Microwave-enhanced SPPS produced peptoid-peptide hybrid, Pro-Glu-(NLeu)-(NPhe)-Gly-(NLys)-NH_2, in 81% purity.

References

(1) Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057–4059.

(2) Unciti-Broceta, A.; Diezmann, F.; Ou-Yang, C. Y.; Fara, M. A.; Bradley, M. Bioorg. *Med. Chem.* **2009**, *17*, 959–966.

(3) Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521–1524.

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