Automated Synthesis of Peptoids and Peptoid-Peptide Hybrids

Introduction

Peptoids are polymers of various (N-alkyl) glycines.[1] While they are similar in structure to peptides (Figure 1), peptoids lack a peptide bond making them resistant to proteolytic activity *in vivo*[1,2]. This stability makes peptoids an attractive peptidomimetic target for drug discovery and development.

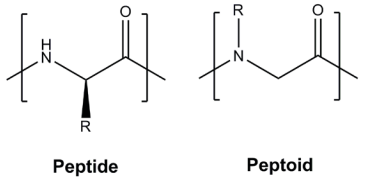


Figure 1. Comparison of peptide and peptoid structure.

Peptoids and peptide-peptoid hybrids are generally synthesized through a "sub-monomer" process consisting of two steps; an acylation step using bromoacetic acid and N,N'-diisopropylcarbodiimide (DIC) followed by reaction with a primary amine via nucleophilic displacement of the bromide (Figure 2).[1]

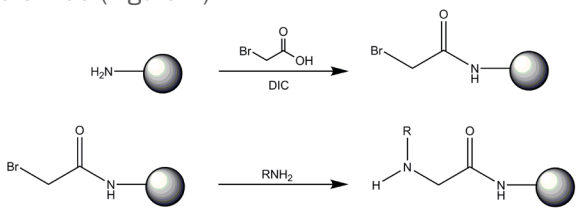


Figure 2. General synthesis of peptoids.

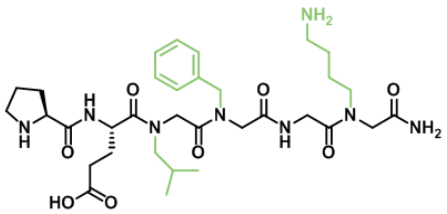
Because many structurally diverse monosubstituted amines are commercially available, peptoids with a wide variety of side chains can be readily synthesized.[2,3] However, conventional synthesis of peptoids can take up to three hours per residue.[1] Microwave irradiation has been shown to significantly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

Materials and Methods

Reagents

Bromoacetic acid, N,N-diisopropylcarbodiimide (DIC), benzylamine, β-Alanine t-butyl ester hydrochloride, piperidine, and trifluoroacetic acid (TFA), were obtained from Sigma Aldrich (St. Louis, MD). N-Boc-1,4-diaminobutane and isobutylamine were obtained from Alfa Aesar (Haverhill, MA). Amino acids, Oxyma Pure, and Rink Amide ProTide™ LL resin were obtained from CEM Corporation (Matthews, NC). Dichloromethane (DCM), N,N-dimethyl-formamide (DMF), anhydrous diethyl ether, acetic acid, HPLC grade water and acetonitrile were obtained from VWR (West Chester, PA).

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



The peptoid 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). Acylation was performed with Bromoacetic acid and DIC in a 1:1.2 ratio for 5 min at 75 °C. Nucelophilic displacement was performed with various amines for 5 min at 75 °C. For non-peptoid residues (Pro, Gly, and (NAla)), deprotection with 20% piperidine in DMF was performed in a single step of 1 min at 90 °C, and coupling reactions were performed with a 5 fold excess of Fmoc-AA-OH with 1:1:1 AA/DIC/Oxyma Pure for 2 min at 90 °C. Cleavage was performed using 92.5:2.5:2.5:2.5 TFA/H2O/TIS/DODT for 30 min at 42 °C. Following cleavage the peptoid was precipitated in diethyl ether, then lyophilized overnight.

Method Programming

Single Peptoid to Peptide Coupling: The initial peptoid coupling was performed by adding deprotection solution (4 mL) and microwaving for 1 min at 90 °C. Deprotection was followed by 3 consecutive washes. Bromoacetic acid (2.5 mL) and DIC (2.5 mL) were added from external positions to the reaction vessel and microwaved for 5 min at 75 °C. A wash through manifold was executed, followed by four consecutive reaction vessel washes. The peptoid residue was added (5 mL from method) and microwaved for 5 min at 75 °C. A wash through manifold was executed, followed by four consecutive reaction vessel washes.

Single Peptide to Peptoid Coupling: The peptide to peptoid coupling was performed by adding the natural amino acid (2.5 mL from method), DIC (1 mL from position), and Oxyma (0.5 mL from position) to the reaction vessel and microwaved for 4 min at 90 °C. A wash through manifold was executed, followed by 3 consecutive reaction vessel washes.

Single Peptoid to Peptoid Coupling: The peptoid to peptoid coupling was performed by adding Bromoacetic acid (2.5 mL) and DIC (2.5 mL) from external positions to the reaction vessel and microwaved for 5 min at 75 °C. A wash through manifold was executed, followed by four consecutive reaction vessel washes. The peptoid residue was added (5 mL from method) and microwaved for 5 min at 75 °C. A wash through manifold was executed, followed by four consecutive reaction vessel washes.

Peptoid-Peptide Hybrid Analysis

The peptoid 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 gradients elution of 0.1% TFA in (i) water and (ii) ACN.

Results

The peptoid was synthesized with a purity of **81.4%** using the Liberty Blue automated microwave peptide synthesizer.

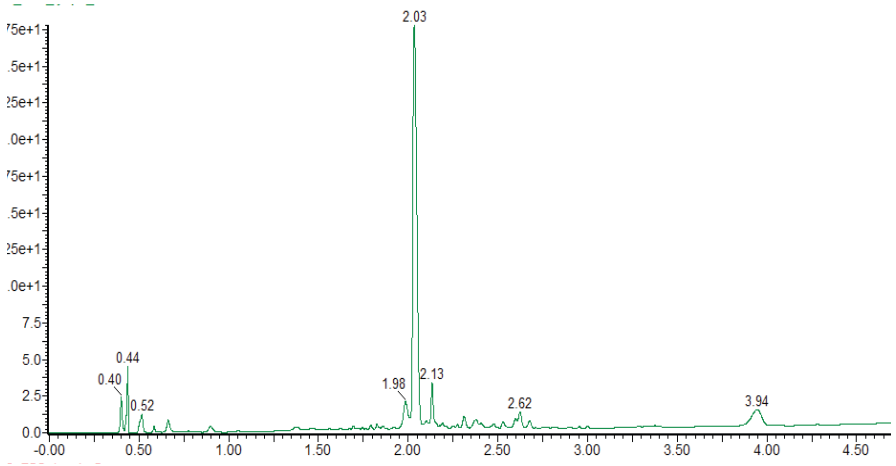


Fig 3. UPLC Chromatogram of Model Peptoid-Peptide Hybrid

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