

Peptoid Synthesis on the Symphony

INTRODUCTION

Peptides are studied because of their range of biological activities in the body. As receptor-binding molecules, peptides have been investigated for therapeutic uses. Unfortunately, in the body peptides are easily broken down by proteases (enzymes that digest proteins and peptides) and have difficulty crossing cell membranes (1). This has spurred the development of peptidomimetic compounds that mimic the biological activity of peptides, but show greater stability in the body and greater ability to cross cell membranes (2).

Peptoids are a class of peptidomimetics with an identical backbone structure to peptides but a different placement of side chain groups. In a peptide, the side chain is attached to the alpha-carbon. In a peptoid, the side chain is attached to the nitrogen (Figure 1). This seemingly

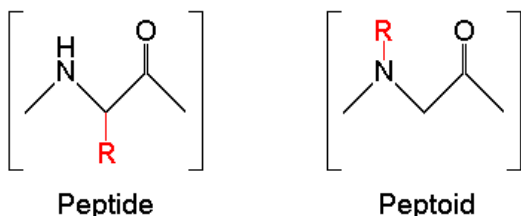


Figure 1: Peptide and peptoid structures. R side chain is shown in red.

small change gives peptoids greater stability in the body as their structure is not recognized by proteases. In addition, it has been found that peptoids containing cationic side chains efficiently cross cell membranes (3).

Peptoids are synthesized using the submonomer method developed by Zuckermann, *et al.* (4). This method consists of two main steps: Acylation and displacement. Following pre-swelling of Rink amide MBHA resin, the resin is acylated using bromoacetic acid activated with DIC in DMF. Primary amines are used in the displacement step to replace the bromine with a substituted nitrogen. Acylation and displacement

are repeated until the peptoid chain is completely assembled.

The advantage of this method is there are a large variety of commercially available primary amines with different side chains to choose from. This increases the diversity available for library synthesis. The two-step process is also easily automated.

Three peptoids were synthesized from various primary amines on the *Symphony*[®] peptide synthesizer, and analyzed using HPLC and mass spectroscopy.

METHOD

Peptoid Synthesis: N-butylamine, cyclopropylamine, 4-fluorobenzylamine, bromoacetic acid and DIC were purchased from Sigma-Aldrich. A modification of the original submonomer method (4) was employed to synthesize homopentamers from three primary amines on a Protein Technologies, Inc. *Symphony*[®] peptide synthesizer. The peptoids were synthesized at the 25 μ mol scale on Rink Amide MBHA resin. Acylation was performed using 1 M bromoacetic acid/1.2 M DIC in DMF (50x excess) for 2 x 30 minutes. Displacement was performed with a 1 M solution of the primary amine in DMF coupled for 1 hour (100x excess). 6 x 30 second washes with DMF were performed between reaction steps. Cleavage was performed by treatment with 95% TFA in water for 1 hour. Following cleavage, the filtrate was evaporated under nitrogen. N-butyl and cyclopropyl pentamers were dissolved in 5%ACN/0.1% TFA in water, while the 4-fluorobenzyl pentamer was dissolved in 2/1 ACN/water.

Analysis: Samples were analyzed using RP-HPLC on a Rainin Dynamax HPLC System using a Varian Microsorb C18 column (5 mm, 300 Å , 4.6 x 50.0 mm), Buffer A: water, 0.1% TFA, Buffer B: ACN, 0.1% TFA. Samples were separated using a gradient of 5-95% B over 7

min with a flow rate of 2 mL/min and detection at 214 nm. Samples were submitted to the University of Arizona Mass Spectrometry Facility for ESI-MS analysis

RESULTS/DISCUSSION

Peptoids were synthesized successfully on the *Symphony*[®] peptide synthesizer (Figures 2-4) and their identities verified by mass spectrometry (Table 1).

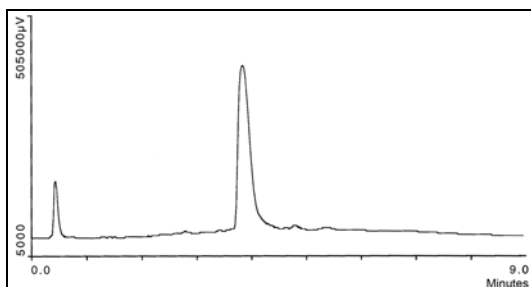


Figure 2: HPLC of peptoid pentamer with n-butyl side chains.

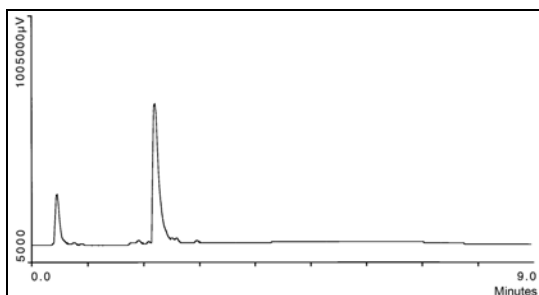


Figure 3: HPLC of peptoid pentamer with cyclopropyl side chains.

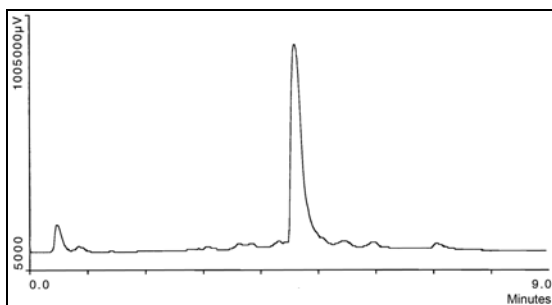


Figure 4: HPLC of peptoid pentamer with 4-fluorobenzyl side chains.

Table 1: Mass spectrometry analysis of pentamer peptoids.

Peptoid	Expected m/z	Observed m/z
n-butyl	583	583
cyclopropyl	503	503
4-fluorobenzyl	843	843

CONCLUSION

This application note demonstrates that peptoids can be successfully synthesized on the *Symphony*[®] peptide synthesizer.

REFERENCE

- (1) Farmer, P.S. and Ariens E.J. "Speculations on the design of nonpeptidic peptidomimetics," *TIPS* **1982**, 3, 362-365.
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