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 small

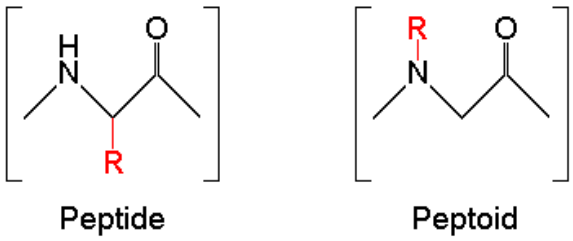

Figure 1: Peptide and peptoid structures. R side chain is shown in red.

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 and deprotection of Fmoc-Rink amide 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 that 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. In this application, three peptoid homopentamers were synthesized with various primary amines on the Symphony® peptide synthesizer.

METHOD

Materials: n-butylamine, cyclopropylamine, 4-fluorobenzylamine, diisopropylcarbodiimide (DIC), and bromoacetic acid were purchased from Aldrich. All other chemicals were provided by Protein Technologies, Inc.

Synthesis: A modification of the original submonomer method (4) was employed to synthesize homopentamers from three primary amines (n-butylamine, cyclopropylamine, and 4-fluorobenzylamine) on the Symphony. Peptoids were synthesized at the 50 μmol scale on Rink Amide-MBHA resin (0.47 mmol/g loading). The synthesis and cleavage programs are listed in Tables 1-3. Following cleavage, the filtrate was evaporated under nitrogen, resuspended in 1:1 ACN/water (v/v), then lyophilized 2x.

Analysis: Samples were analyzed using RP-HPLC on a Rainin Dynamax HPLC System using a Varian Microsorb C18 column (5 □m, 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. Mass analysis was performed using liquid chromatography/electrospray ionization mass spectrometry (LC/MS).

RESULTS/DISCUSSION

The peptoid homopentamers were successfully synthesized and verified by mass analysis (Figures 1-3).

Table 1: Swelling and deprotection program

Step	Operation	Solvent	Vol	Mix Time	Drain	Rep
1	Wash	DMF	2	0:10:00	ON	3
2	Deprotection	20% piperidine/DMF	2	0:02:00	ON	2
3	Wash	DMF	2	0:00:30	ON	6

Table 2: Modified peptoid synthesis program.

Step	Operation	Solvent	Vol	Mix Time	Drain	Rep
1	Acylation	1M Bromoacetic Acid/DMF	1	0:00:00	OFF	1
2	Acylation	1.2M DIC/DMF	1	0:30:00	ON	1
3	Acylation	1M Bromoacetic Acid/DMF	1	0:00:00	OFF	1
4	Acylation	1.2M DIC/DMF	1	0:30:00	ON	1
5	Wash	DMF	2	0:00:30	ON	6
6	Displacement	1M Amine/DMF	2	1:00:00	ON	1
7	Wash	DMF	2	0:00:30	ON	3

Table 3: Peptoid cleavage program.

Step	Solvent	Vol	Mix Time	Drain	Rep
1	DMF	2	0:00:30	ON	3
2	DCM	2	0:00:30	ON	6
3	Dry	1	0:10:00	ON	1
4	95% TFA/water	3	1:00:00	ON	1
5	95% TFA/water	2	0:00:30	ON	1
6	DCM	2	0:00:30	ON	3
7	Dry	1	0:02:00	ON	1

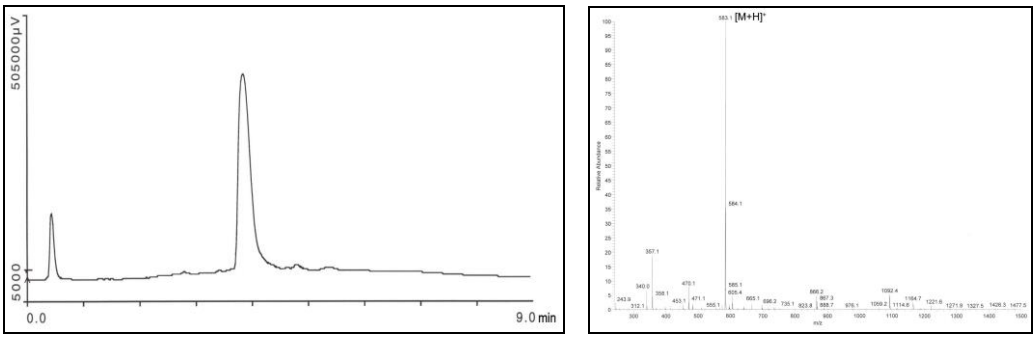

Figure 1: (a) HPLC and (b) mass spectrometry analysis of peptoid homopentamer synthesized with n-butylamine.

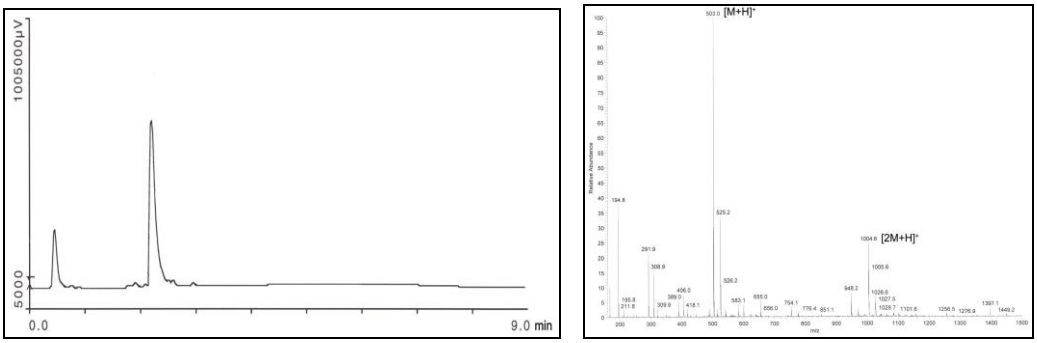

Figure 2: (a) HPLC and (b) mass spectrometry analysis of peptoid homopentamer synthesized with cyclopropylamine.

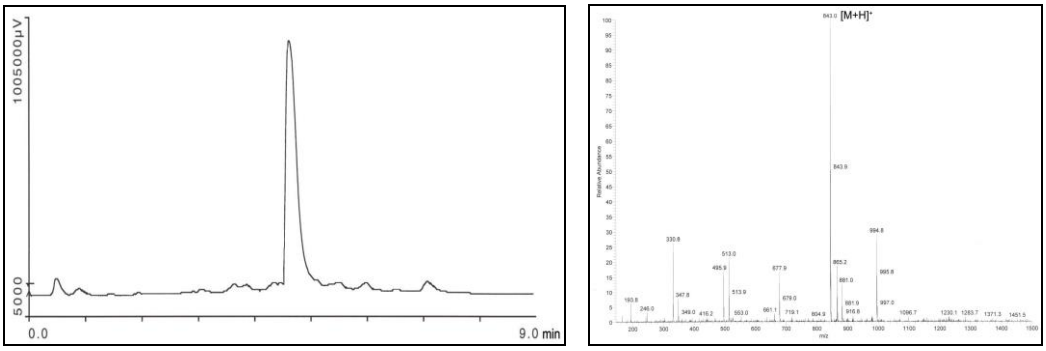

Figure 3: (a) HPLC and (b) mass spectrometry analysis of peptoid homopentamer synthesized with 4-fluorobenzylamine.

CONCLUSION

This application note demonstrates a method for the successful synthesis of various peptoids on the Symphony.

REFERENCES

1. Farmer, P.S. and Ariens E.J. “Speculations on the design of nonpeptidic peptidomimetics,” *TIPS* **1982**, 3, 362-365.
2. Burkoth, T.S.; Fafarman, A.T.; Charych, D.H.; Conolly, M.D.; Zuckermann, R.N. “Incorporation of Unprotected Heterocyclic Side Chains into Peptoid Oligomers via Solid-Phase Submonomer Synthesis,” *JACS* **2003**, 125, 8841-8845.
3. Wender, P.A.; Mitchell, D.J.; Pattabiraman, K.; Pelkey, E. T.; Steinman, L.; Rothbard, J.B. “The Design, Synthesis, and Evaluation of Molecules That Enable or Enhance Cellular Uptake: Peptoid Molecular Transporters,” *PNAS* **2000**, 97, 13003-13008.
4. Zuckermann, R.N.; Kerr, J.M.; Kent, S.B.H.; Moos, W.H. “Efficient Method for the Preparation of Peptoids [Oligo(N-substituted glycines)] by Submonomer Solid-Phase Synthesis,” *JACS* **1992**, 114, 10646-10647.
5. Chan, WC & White PD, Ed. “Fmoc Solid Phase Peptide Synthesis: A Practical Approach” Oxford University Press, Oxford © 2000, p. 62.