56

Influences of Monomer Structural Elements in Hydrophilic Peptoids.

Eric Beausoleil, Kiet T. V. Truong, Kent Kirshenbaum, and Ronald N. Zuckermann

Chiron Technologies, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916 USA.

Introduction

Water solubility, side chain diversity [1], structural stability and rapid synthesis are desirable to build sequence-specific hydrophilic hetero-polymers that can be used as vehicles in gene [2], protein and small molecule delivery. *N*-Substituted glycines (peptoids) have been shown to possess many of these features. Previously, the presence of a helical secondary structure in peptoid oligomers was demonstrated in various solvents [3]. As determined by NMR studies, the peptoid helix possessing the enantiopure 1-*S*-phenylethylamine (N*s*pe) as a submonomer are characterized with a pitch of 6Å per turn where $\phi = -75$, $\psi = 170$, $\omega = 0$, and $\chi^1 = -120$ [4]. Although this was a significant first step, the high hydrophobic character of this side chain limits the solubility and the diversity of the helical peptoid. Therefore, we present here the synthesis of new classes of hydrophilic enantiopure submonomers that were incorporated into peptoid oligomers, analyzed using circular dichroism and evaluated for their propensities to form secondary structures.

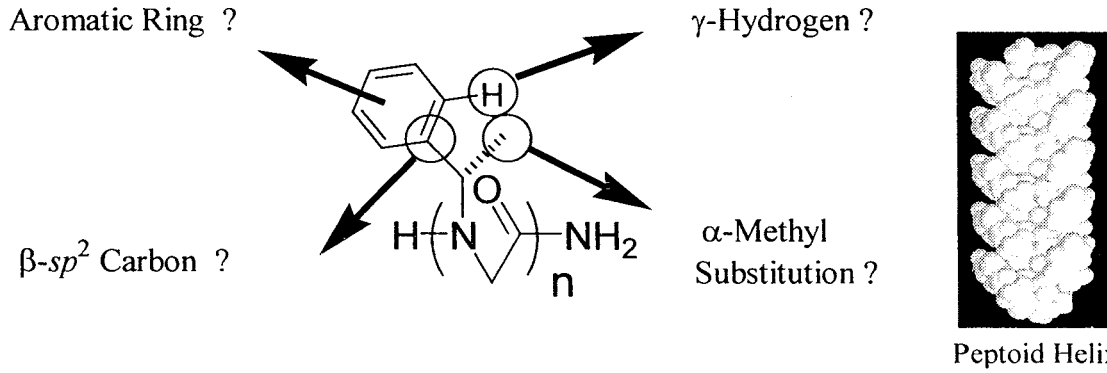

Figure 1. *Monomer structural elements of the peptoid helix. Each of these elements were investigated by the systematic synthesis of analogs.*

Results and Discussion

We have prepared a series of different peptoid 5mers and 9mers to evaluate the importance many of the structural elements of the N*s*pe monomer. Specifically, we directed our attention to the β-sp^2 carbon, the aromatic ring and the presence of γ-hydrogens in their propensity to form helical structures in aqueous solvent. Therefore,

different amines were prepared and used in the submonomer peptoid synthesis on solid support. The enantiopure 1-(3-furyl)-ethylamine submonomer was synthesized in three steps and one chiral resolution from (3)-furoic acid. The enantiopure 1-(2-furyl)-ethylamine submonomer was synthesized similarly in two steps from the commercially available 1-(2-furyl)-ethan-1-one. 2-*S*-Amino-*N*'-alkylpropanamide were synthesized in two step from *N*-Cbz-(L)-Alanine. Using GC-MS, submonomer enantiomeric purity was analyzed through the formation of their diastereomeric Mosher amide analogs and were found to be > 98% ee.

The peptoid oligomers were prepared using the previously reported submonomers peptoid synthesis [5] on Rink amide resin, except that amines were dissolved in NMP instead of DMSO. Peptoids synthesized using 1-(2-furyl)-ethylamine exhibited a very high lability in the acidic cleavage step (95 % TFA) and poor recovery of the desired pentamers was observed. In order to prevent this problem, we used a solid support with a photo-cleavable α-methyl-6-nitroveratryl linker [6]. All peptoids (Figure 2) were purified by reverse phase HPLC. Reliasil C4 BDX (5 μm, 300A) Column, 20 × 50, on a Rainin HPLC System using a linear gradient of 5-90% of solvent B in solvent A in 20 min. Solvent A = 0.1 % [v/v] TFA in H_2O, Solvent B = 0.1 % [v/v] TFA in CH_3CN.

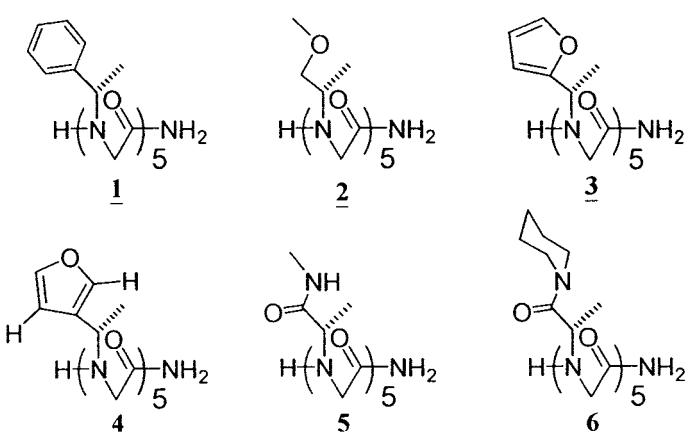

Figure 2. *5mers peptoid synthesized.*

In order to uncover circular dichroism spectra that indicate the presence of secondary structure, we searched for peptoid pentamers that exhibited a variation of their CD signature upon heating. We reasoned that a variation of the temperature should alter the orientation of the backbone amides chromophore and therefore modify their corresponding CD spectra. The peptoid homopentamers formed using enantiopure (*2*)-methoxy-(1)-methylethylamine **2**, 1-(2-furyl)-ethylamine **3**, 1-(3-furyl)-ethylamine **4** and L-Ala-(*N'*,*N'*)-dialkylamides **6** did not exhibit an helix like signature (i.e. two minima of negative ellipticity) or any variation of their circular dichroism spectra upon heating. Therefore we did not determined the absolute configuration of the 1-(furyl)-ethylamines. On the other hand, homopentamer **5** formed using 2-*S*-amino-*N*'-methylpropanamide demonstrated both a helix-like signature and a remarkable variation in their circular dichroism spectra upon heating. These results suggest that a secondary structure is present in **5** and that this secondary structure is lost upon heating. However, it is still possible that peptoids **2**,**3**,**4**,and **6** posses a secondary structures, but it is not

possible to detect their presence using this method. Furthermore, it was intriguing to find that **5** is the only pentamer to exibit a CD signature similar to the one observed for **1**(Figure 3).

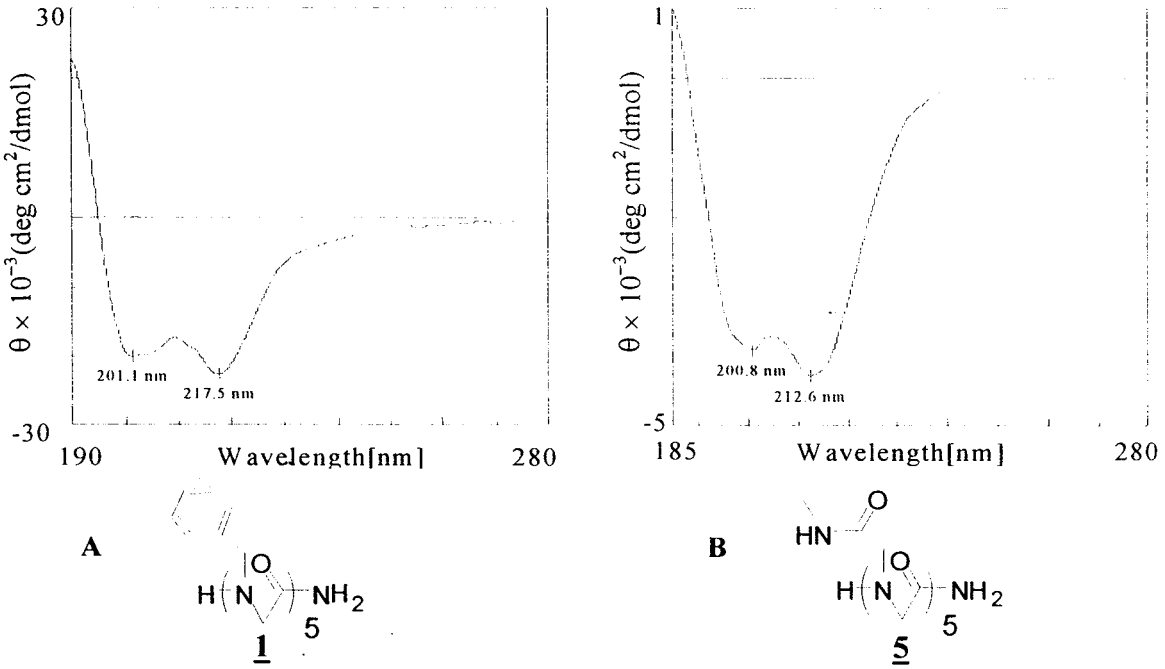

Figure 3. *Circular dichroism of homopentamers **1** and **5** in 5% methanol v/v in H_2O reported in terms of mean amide molar ellipticity.*

We next exploited the ease of synthesis of these submonomers to prepare a more complex oligomer with the objective to show that this class of submonomers expands the number possible peptoids with a defined secondary structure. As shown on the HPLC trace of the crude material after cleavage (figure 4) the synthesis of a hetero-9mer peptoid **7** proceeds very cleanly and the desired product was easily purified by reverse phase HPLC.

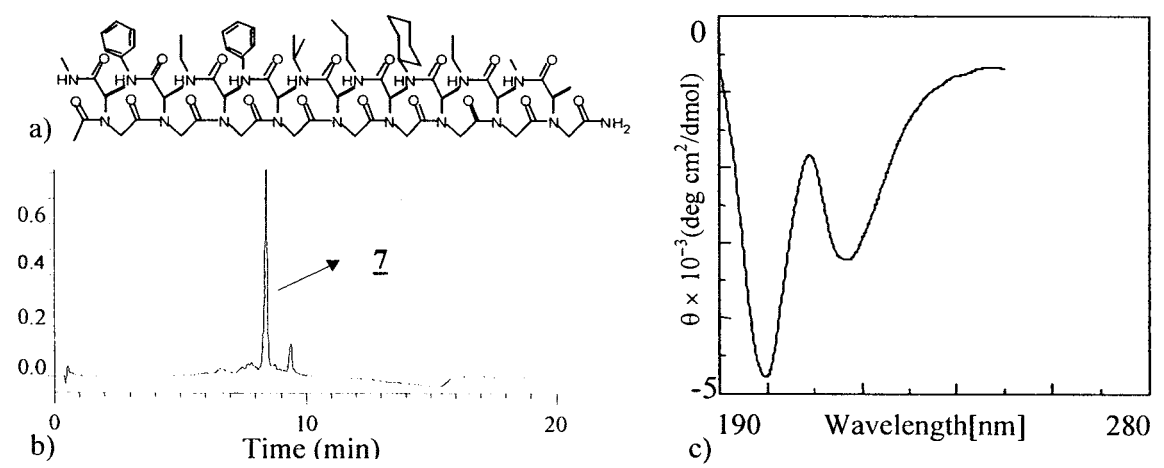

Figure 4. a) *Peptoid 7 structure, b) Peptoid 7 crude HPLC trace, c) Peptoid 7 circular dichroism spectra in methanol reported in terms of mean amide molar ellipticity.*

Conclusion

These results suggest that peptoids prepared using analogs of (L)-Ala-N-Alkylamide could be used as a general scaffold for generation of a variety of hydrophilic peptoids with a defined secondary structure.

References

1. Murphy, J.E., Uno, T., Hamer, J.D., Cohen, F.E., Dwarki, V. and Zuckermann, R.N., *Proc. Natl. Acad. Sci. U.S.A.*, 95 (1998) 1517.
2. Uno, T., Beausoleil, E., Goldsmith, R., Levine, B.H. and Zuckermann, R.N., *Tet. Lett.*, 40 (1999) 1475.
3. Kirshenbaum, K., Barron, A.E., Goldsmith, R.A., Armand, P., Bradley, E.K., Truong, K.T.V, Dill, K.A., Cohen, F.E. and Zuckermann, R.N., *Proc. Natl. Acad. Sci. U.S.A.*, 95 (1998) 4303.
4. Armand, P., Kirshenbaum, K., Falicov, A., Dunbrack, R.L., Jr., Dill, K.A. and Zuckermann, R. N. and Cohen, F.E., *Folding Des.*, 2 (1997) 369.
5. Figliozzi, G.M., Goldsmith, R., Ng, S.C., Banville, S.C., Zuckermann, R.N., *Methods Enzymol.*, 267 (1996) 437.
6. Holmes, C.P. and Jones, D.G., *J. Org. Chem.*, 60 (1995) 2318.