ORGANIC LETTERS

2007
Vol. 9, No. 17
3275−3278

Fit To Be Tied: Conformation-Directed Macrocyclization of Peptoid Foldamers

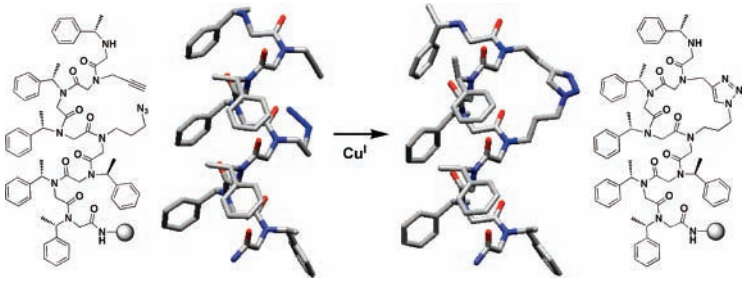
Justin M. Holub,[†] Hangjun Jang,[†,‡] and Kent Kirshenbaum*,[†]

Department of Chemistry, New York University, 100 Washington Square East, New York, New York 10003-6688, and Department of Medicine, Brookdale University Hospital and Medical Center, One Brookdale Plaza, Brooklyn, New York, 11212

kent@nyu.edu

Received May 18, 2007

ABSTRACT



Covalent macrocyclic constraints can be readily installed on N-substituted glycine "peptoid" oligomer substrates. Cu(I)-catalyzed [3+2] cycloaddition reactions were conducted on solid support to ligate peptoid side chain azide and alkyne functionalities. Intramolecular macrocycle formation is facilitated by preorganizing the reactive groups across one turn of the helical secondary structure. These results confirm that conformational ordering can be exploited to assist the macrocyclization of folded oligomers.

Macrocyclic structures play a prominent role in pharmaceutical and natural products chemistry. Cyclic peptides, for example, can display improved conformational stability and pharmacokinetic properties in comparison with their linear forms.[1] Accordingly, the macrocyclization of oligomers is a recurrent topic among bioorganic chemists. However, these reactions often proceed with poor yields and can be complicated by side reactions such as cyclodimerization and epimerization.[2] It is widely appreciated that proximity effects can facilitate the progress of otherwise sluggish reactions.[3] In this context, the rate of oligomer ring closure can be enhanced by preorganization of the reacting functionalities. Surprisingly, examples of the use of rational design to establish molecular architectures compatible with conformation-assisted cyclization are still uncommon.[2a]

In this report, we investigate the use of preorganization to assist the formation of foldamer macrocycles. Foldamers are oligomeric molecules that have a strong propensity to self-assemble into organized secondary structures in solution.[4] Due to their intrinsic capacity for structural organization, foldamers may prove to be an effective platform for performing intramolecular macrocyclization reactions.[5] Oligo-*N*-substituted glycines (termed "peptoids") are a well-known class of sequence-specific peptidomimetic foldamers that can be readily synthesized on solid support.[6] We have previously demonstrated that peptoid oligomers containing bulky α-chiral side chains can fold into a polyproline type I (PPI)-like

* To whom correspondence should be addressed.
[†] New York University.
[‡] Brookdale University Hospital.

(1) (a) Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654. (b) Reyes, S. J.; Burgess, K. *Tetrahedron: Asymmetry* **2005**, *16*, 1061. (c) Columbo, G.; Curnis, F.; De Mori, G. M. S; Gasparri, A.; Longoni, C.; Sacci, A.; Longhi, R.; Corti, A. *J. Biol. Chem.* **2002**, *277*, 47891. (d) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391. (e) Blackwell, H. E.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **1998**, *37*, 3281. (f) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891. (g) Chapman, R. N.; Dimartino, G.; Arora, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 12252.

(2) (a) Blankenstein, J.; Zhu, J. *Eur. J. Org. Chem.* **2005**, *2005*, 1949. (b) Yuan, L.; Feng, W.; Yamato, K.; Sanford, A. R.; Xu, D.; Guo, H.; Gong, B. *J. Am. Chem. Soc.* **2004**, *126*, 11120.

(3) Deslongchamps, P. *Pure Appl. Chem.* **1992**, *64*, 1831.

(4) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252.

10.1021/ol071169l CCC: $37.00
Published on Web 07/18/2007

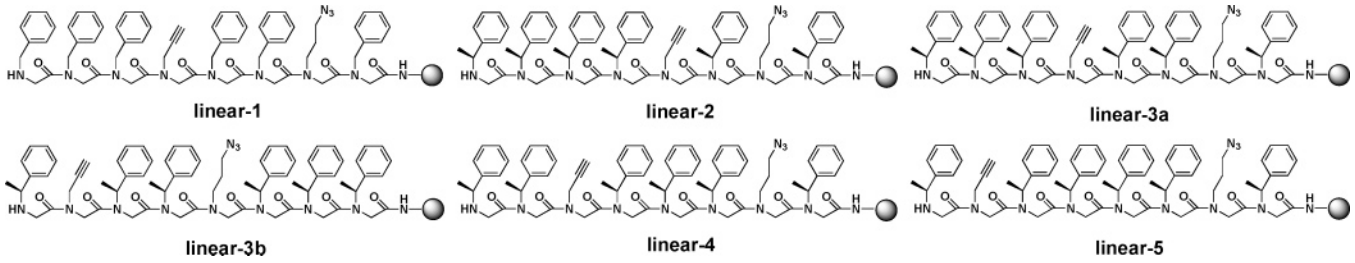


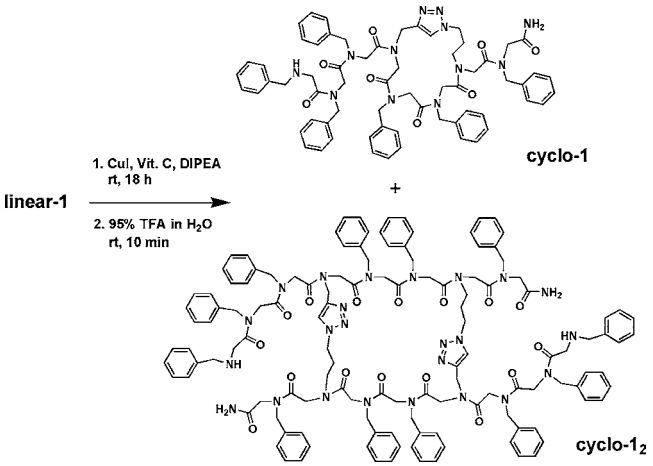
Figure 1. Peptoid oligomers used in macrocyclization reactions. Peptoid **linear-1** is an unstructured oligomer, whereas peptoids **linear-2** to **linear-5** contain helix-inducing α-chiral side chains.

helical conformation with a periodicity of three residues per turn and a helical pitch of ~6.7 Å.[7] Additionally, we have recently reported that intermolecular Cu-catalyzed azide−alkyne [3+2] cycloaddition reactions proceed efficiently on peptoid substrates bearing azidoalkyl or propargyl side chains to form conjugates through 1,4-substituted triazole linkages.[8] We sought to determine if peptoid helical propensity could be exploited to enhance the efficiency of *intramolecular* azide−alkyne [3+2] cycloaddition reactions by placing the reactive species in close proximity within the foldamer architecture.

Our studies were initiated by employing standard solid-phase peptoid synthesis techniques[6a] to generate one unstructured (Figure 1, **linear-1**) peptoid octamer and five structured (Figure 1, **linear-2** to **linear-5**) peptoid octamers including bulky α-chiral side chains. All peptoid oligomers shown in Figure 1 incorporated azide and alkyne-functionalized side chains at varying positions along the oligomer scaffold. Peptoids were synthesized on a commercially available low-loading level resin that would allow for site isolation (NovaSynTGR, 0.23 mmol g^{-1}).

We first investigated the capability of the Cu-catalyzed azide−alkyne [3+2] cycloaddition reaction to generate an intramolecular cross-link within unstructured peptoid oligomer **linear-1**. We anticipated that peptoids could potentially form the desired intramolecular macrocycle products or intermolecular cyclodimerization products, as observed for polypeptides[9] (Scheme 1). Peptoid **linear-1** was synthesized

Scheme 1. Cyclization of **linear-1** by Azide-Alkyne [3+2] Cycloaddition



in high purity (>85%) on solid support and subjected to macrocyclization in the presence of CuI, ascorbic acid (Vit. C), and *N*,*N*′-diisopropylethylamine[8] (DIPEA). Conducting the reactions on solid support facilitated the use of a large excess of Cu ions.[10] Following extensive washing of the resin to remove residual Cu, the products were cleaved from solid support with 95% trifluoroacetic acid (TFA) in H_2O. High-

(5) (a) Bru, M.; Alfonso, I.; Burguete, M. I.; Luis, S. V. *Tetrahedron Lett.* **2005**, *46*, 7781. (b) Zhang, A.; Han, Y.; Yamato, K.; Zeng, X. C.; Gong, B. *Org. Lett.* **2006**, *8*, 803. (c) Rotger, C.; Pina, M. N.; Vega, M.; Ballester, P.; Deya, P. M.; Costa, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 6844. (d) Böhme, F.; Kunert, C.; Komber, H.; Voigt, D.; Friedel, P.; Khodja, M.; Wilde, H. *Macromolecules* **2003**, *35*, 4233. (e) Xing, L.; Ziener, U.; Sutherland, T. C.; Cuccia, L. A. *Chem. Commun.* **2005**, 5751. (f) Hui, J. K.-H.; MacLachlan, M. J. *Chem. Commun.* **2006**, 2480. (g) Jiang, H.; Leger, J.-M.; Guionneau, P.; Huc, I. *Org. Lett.* **2004**, *6*, 2985. (h) Le Grel, P.; Salaün, A.; Potel, M.; Le Grel, B.; Lassagne, F. *J. Org. Chem.* **2006**, *71*, 5683. (i) Semetey, V.; Didierjean, C.; Briand, J.-P.; Aubry, A.; Guichard, G. *Angew. Chem. Int. Ed.* **2002**, *41*, 1895. (j) Amorín, M.; Castedo, L.; Ganja, J. *J. Am. Chem. Soc.* **2003**, *125*, 2844.

(6) (a) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646. (b) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367. (c) Patch, J. A.; Barron, A. E. *Curr. Opin. Chem. Biol.* **2002**, *6*, 872.

(7) (a) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. R.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303. (b) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, K. T. V.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4309. (c) Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 13525. (d) Farfarman, A.; Borbat, P. P.; Freed, J. H.; Kirshenbaum, K. *Chem. Commun.* **2007**, 377.

(8) (a) Jang, H.; Farfarman, A.; Holub, J. M.; Kirshenbaum, K. *Org. Lett.* **2005**, *7*, 1951. (b) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Biomol. Chem.* **2006**, *4*, 1497.

(9) (a) Punna, S.; Kuzelka, J.; Wang, Q.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2215. (b) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503. (c) Angell, Y.; Burgess, K. *J. Org. Chem.* **2005**, *70*, 9595.

(10) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Breinbauer, R.; Kohn, M. *ChemBioChem.* **2003**, *4*, 1147. (c) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.

Table 1. Macrocyclization Reactions on Peptoids

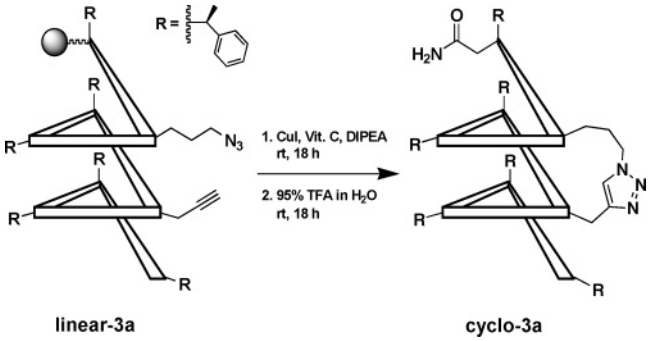
entry	peptoid	reactive group positions[a]	yield[b] (%)	cyclic product monomer:dimer[c]
1	**linear-1**	$i, i+3$	50	2:1
2	**linear-2**	$i, i+2$	40	1:1
3	**linear-3a**	$i, i+3$	80	4:1
4	**linear-3b**	$i, i+3$	70	4:1
5	**linear-4**	$i, i+4$	60	3:1
6	**linear-5**	$i, i+5$	30	1:2

[a] Relative position of alkyne and azide side chain on the linear peptoid oligomers. [b] Approximate yields of cyclic monomer products evaluated by HPLC. [c] Conversion ratio was based on the relative peak integration area of HPLC chromatograms monitored at 214 nm. See the Supporting Information for characterization details.

Performance Liquid Chromatography (HPLC) profiles of the crude products showed two distinct product peaks with minimal impurities (see the Supporting Information). Liquid Chromatography/Mass Spectrometry (LC/MS) analysis of the two products confirmed formation of cyclic monomer **cyclo-1** and cyclic homodimer **cyclo-1$_2$** (vide infra). It was found that **linear-1** forms **cyclo-1** and **cyclo-1$_2$** at an approximate ratio of 2:1 with a 50% yield of **cyclo-1**, as evaluated by HPLC (Table 1, entry 1). These results demonstrate that peptoid oligomers can be cyclized on solid support by using a Cu-catalyzed azide−alkyne [3+2] cycloaddition reaction.

We then sought to exploit the structural organization of helical peptoid foldamers for positioning reactive side chains in close proximity to favor the rapid formation of the cyclic monomer product (Scheme 2). Peptoid oligomers containing

Scheme 2. Schematic Helical Representation of **linear-3a** Showing Triazole Linkage Formation between Reactive Side Chains i and $i + 3$



bulky α-chiral side chains can fold into PPI-like helices that contain roughly three residues per turn.[7a,b] We evaluated a structured peptoid octamer (Figure 1, **linear-3a**) containing an azide and an alkyne side chain functionality at respective sequence positions i and $i + 3$. To induce helix propensity, (*S*)-1-phenylethyl residues were incorporated at the remaining six positions. When subjected to the reaction conditions outlined in Scheme 2, compound **linear-3a** forms cyclic monomer and cyclic homodimer at a ratio of 4:1, with an 80% yield of **cyclo-3a** (Table 1, entry 3). Despite greater steric congestion, **linear-3a** showed enhanced yield of cyclic monomer when compared to its unstructured counterpart **linear-1** (Table 1, entries 1 and 3), indicating that structural preorganization can be used to favor the formation of cyclic monomer in this system. Varying the placement of the reactive moieties toward the N terminus of the foldamer sequence had little effect on the cyclic monomer to cyclic homodimer ratio (compounds **linear-3a** and **linear-3b**; Table 1, entries 3 and 4), suggesting that the proximity of the reactive groups to the solid support has little influence on the overall reaction.

Because the peptoid helix exhibits a helical pitch of three residues per turn,[7a,b] we anticipated that cyclic monomer yields would decrease as we placed the reactive side chains at varying positions other than i and $i + 3$ along the helical scaffold. To test this hypothesis, we evaluated three structured peptoid octamers (Figure 1, compounds **linear-2**, **linear-4**, and **linear-5**) each containing azide and alkyne moieties at positions i and $i + 2$, i and $i + 4$, and i and $i + 5$, respectively. We observed that when the reactive side chains were located at positions other than i and $i + 3$ along the oligomer scaffold, the ratio of cyclic monomer to cyclic homodimer decreased substantially (Table 1, entries 2, 5, and 6). These results support the notion that the foldamer conformation plays a beneficial role of preorganization in compounds **linear-3a** and **linear-3b** by positioning the reactive functionalities in proximity across one face of the peptoid helix.

One feature confounding the characterization of the products is that intramolecular azide−alkyne [3+2] cycloaddition reactions do not produce mass changes between the linear reactant and the macrocyclic products. Our previous reports have shown that LC/MS^n ion trap fragmentation gives reliable characterization of linear peptoid oligomers similar to that of peptide sequencing.[8] We observed that macrocyclic peptoids are selectively resistant to MS/MS fragmentation, giving rise to distinct MS signatures when compared to the linear forms. These ions, specific to cyclic monomer peptoids, were utilized to characterize peptoid oligomers that contain macrocyclic constraints. In addition, LC/MS profiles of cyclic homodimer compounds revealed the presence of distinct $(M+3H^+)/3$ ionization profiles not observed in corresponding intramolecular cycloaddition products (see the Supporting Information).

To further evaluate the formation of macrocyclic products, spectroscopic comparisons of linear and cyclic peptoid counterparts were performed. We investigated variations in circular dichroism (CD) spectra between linear peptoid oligomers and their respective cyclic monomer forms. Peptoids were purified to >95% by HPLC and CD scans were performed at 25 °C in acetonitrile (Figure 2, and the Supporting Information). When compared to the CD spectrum of **linear-3b**, **cyclo-3b** showed a marked increase in signal intensity around 200 nm and a slight decrease in signal intensity around 220 nm (Figure 2). In contrast, **linear-2** and **linear-4** showed only slight variations in signal strength upon

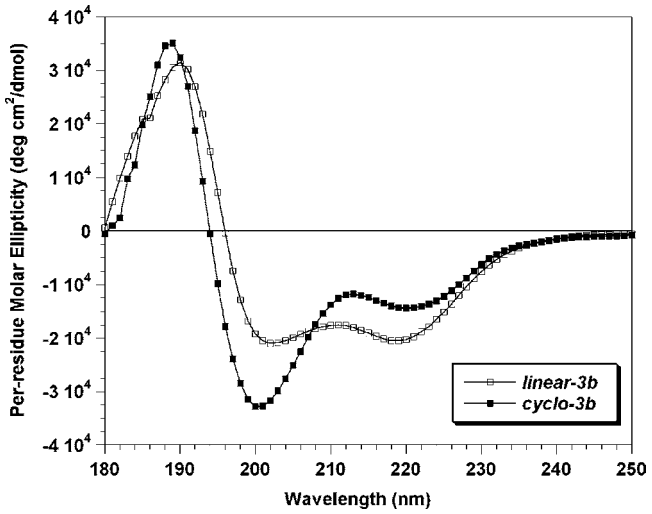


Figure 2. Circular dichroism spectra of **linear-3b** and **cyclo-3b**, (each at 82 μM in acetonitrile). Scans perfomed at 25 °C.

conversion to the macrocylic forms, while **linear-5** exhibited a significant diminishment in ellipticity around 200 nm.

The enhancement in CD signal strength upon cyclization of **linear-3b** indicates a significant alteration in the overall conformational ensemble of the peptoid oligomer. The increased ellipticity at 200 nm seen for **cyclo-3b** suggests an overall reduction in conformational heterogeneity upon cross-linking of side chains located at positions i and $i + 3$. We also examined patterns of proton−carbon cross-peaks in two-dimensional heteronuclear single quantum coherence (2D-HSQC) NMR spectra of **linear-3b** and **cyclo-3b**. Purified peptoid oligomers were dissolved in deuterated acetonitrile at a concentration of 3.0 mg mL^{-1} (2.5 mM) and experiments were conducted on a 500 MHz NMR spectrometer at 25 °C. 2D-HSQC NMR showed complete disappearance of alkyne proton−carbon cross-peaks in the $^1H-^{13}C$ HSQC spectral regions of 80.0 to 90.0 ppm following peptoid cyclization. Additionally, the $^1H-^{13}C$ HSQC spectral regions corresponding to the side chain methyl groups of **linear-3b** show significantly broader distribution of cross-peaks compared to **cyclo-3b**. Similarly, 2D-HSQC NMR spectra show backbone methylene protons of **linear-3b** give a broader distribution of $^1H-^{13}C$ cross-peaks in the H^1 chemical shift range of 3.0 to 4.4 ppm when compared to the same region of **cyclo-3b** (see the Supporting Information). These results indicate a decrease in conformational heterogeneity of the peptoid foldamer macrocycle when compared to its linear counterpart.

Cu-catalyzed azide−alkyne [3+2] cycloaddition reactions have been used to synthesize peptide macrocycles.[9] For reasons presently under investigation, Cu-catalyzed azide−alkyne [3+2] macrocyclization reactions involving peptide substrates result in high yields of cyclic homodimer products.[9a,b] It has been suggested that backbone amide N−H functionalities may complex Cu ions, leading to unwanted side reactions.[9a] The results provided here substantiate the role of the amide NH groups in influencing Cu-catalyzed azide−alkyne [3+2] cycloaddition reactions and indicate that the conjugations can be efficiently performed on oligomers containing only tertiary amide bonds with minimal side reactions.[8,11] Further studies are ongoing to elucidate aspects related to the mechanism of Cu-catalyzed cycloaddition reactions in peptides and peptidomimetics.

In summary, we have successfully generated peptoid macrocycles in good yield using a Cu-catalyzed azide−alkyne [3+2] cycloaddition reaction. In addition, we have shown that the peptoid foldamer structure can be utilized to preorganize reactive functionalities in close proximity, facilitating the generation of cyclic monomer products. In the context of the peptoid helical structure, placing the azide and alkyne side chain functionalities at sequence positions i and $i + 3$ orients the reactive groups on the same face of the helix and results in the enhanced yield of cyclic monomer in comparison with oligomers that place the reactive side chains on alternate faces of the helix or with a similar unstructured peptoid. In this manner, we may establish a means to obtain an indirect reactivity-based evaluation of peptoid helicity. Comparison of the linear and macrocyclic forms of a peptoid oligomer by CD and 2D-HSQC spectrometry reveals marked differences in conformation, indicating that the macrocyclic constraint alters the heterogeneity of the peptoid structural ensemble. The techniques outlined herein may complement approaches for constraining peptoid oligomers into more ordered conformations that would potentially facilitate specific binding interactions between peptoids and biomolecular targets.[12]

Acknowledgment. We thank Dr. Chin Lin (New York University) for technical assistance. This work was supported by a New Investigator Award from the Alzheimer's Association and by a National Science Foundation CAREER Award (CHE-0645361). We thank the NCRR/NIH for a Research Facilities Improvement Grant (C06RR-165720) at NYU. We acknowledge helpful discussions with M. G. Finn and Reshma Jagasia (Scripps Research Institute, La Jolla CA) and Ronald Zuckermann (The Molecular Foundry, Lawrence Berkeley National Laboratory, CA).

Supporting Information Available: Additional information regarding the synthesis, cyclization, and full structural characterization of peptoid oligomers by HPLC, LC/MS^n, CD, and NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

OL071169L

(11) Kumin, M.; Sonntag, L. S.; Wennemers, H. *J. Am. Chem. Soc.* **2007**, *129*, 466.

(12) (a) Shin, S.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2007**, *129*, 3218. (b) Nnanabu, E.; Burgess, K. *Org. Lett.* **2006**, *8*, 1259. (c) Gorske, B. C.; Blackwell, H. E. *Org. Biomol. Chem.* **2006**, *4*, 1441. (d) Pokorski, J. K.; Miller Jenkins, L. M.; Feng, H.; Durell, S. R.; Bal, Y.; Appella, D. H. *Org. Lett.* **2007**, *9*, 2381.