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Synthesis and Circular Dichroism Spectroscopic Investigations of Oligomeric β-Peptoids with α-Chiral Side Chains

Anna S. Norgren,[†] Suode Zhang,[†] and Per I. Arvidsson*[,†,‡]

Department of Biochemistry and Organic Chemistry, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden, and Discovery CNS & Pain Control, AstraZeneca R&D Södertälje, SE-151 85 Södertälje, Sweden

per.arvidsson@astrazeneca.com

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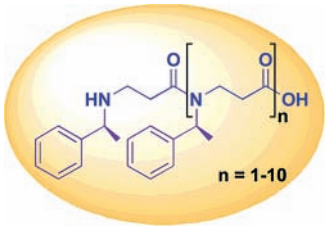
ABSTRACT



Biomimetic oligomers are of large interest both as targets for combinatorial and parallel synthetic efforts and as foldamers. For example, shorter peptoid derivatives of β-peptides, i.e., oligo-N-substituted β-Ala, have been described as potential lead structures. Herein, we describe a solid-phase synthetic route to β-peptoids with α-chiral aromatic N-substituents up to 11 residues long. Furthermore, the folding propensities of these oligomers were investigated by circular dichroism (CD) spectroscopy.

The design and exploration of novel peptidomimetic oligomers is currently an area of high interest in both medicinal chemistry and material science.[1] Depending on the application at hand, it may be desirable to develop oligomers with an unnatural backbone, e.g., for conformational rigidity and increased metabolic stability, or with chemically diverse side chains, e.g., for sampling molecular space and properties. The discovery of peptidomimetic oligomers capable of adopting ordered three-dimensional conformations has promoted a rapid growth of the research area devoted to foldamers.[2] Although unstructured oligomers of α-amino acids and nucleotides may be considered representative products of combinatorial efforts,[3] β- and γ-peptides,[4] oligoureas,[5] and oligopyrrolinones[6] are examples of recently

[†] Uppsala University.
[‡] AstraZeneca R&D Södertälje.

(1) (a) Zuckermann, R. N.; Barron, A. E. *Curr. Opin. Chem. Biol.* **1999**, *3*, 681. (b) *Pseudo-Peptides in Drug Discovery*; Nielsen, P. E., Ed.; Wiley-VCH: Weinheim, 2004; pp 1−31.

(2) (a) Cheng, R. P.; Gellman, S. H.; Degrado, W. F. *Chem. Rev.* **2001**, *101*, 3219. (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893.

(3) *Peptides: Chemistry and Biology*; Sewald, N., Jakubke, H.-D., Eds.; Wiley-VCH: Weinheim, 2002.

(4) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (c) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 6206. (d) Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1998**, *81*, 932. (e) Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043. (f) Seebach, D.; Gademann, K.; Schreiber, J. V.; Matthews, J. L.; Hintermann, T.; Jaun, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1997**, *80*, 2033. (g) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (h) Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717. (i) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiv.* **2004**, *1*, 1111. (j) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983. (k) Hanessian, S.; Luo, X. H.; Schaum, R. *Tetrahedron Lett.* **1999**, *40*, 4925.

(5) (a) Burgess, K.; Linthicum, K. S.; Shin, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 907. (b) Nowick J. S.; Powell, N. A.; Martinez, E. J.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763.

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studied foldamers. Of particular interest in this respect are peptoids, i.e., oligo-N-substituted glycines, as these biomimetic oligomers have been widely used both as products in combinatorial efforts[7] and as foldamers. Peptoids with α-chiral substituents on the nitrogen atom have been shown to fold into helical structures,[8] and several interesting biological applications have been reported on the basis of this property.[9]

β-Peptoids, i.e., oligomers with a backbone composed of an unsubstituted *β*-amino acid *β*-Ala and with a side chain anchored to the amide nitrogen, were first described by Hamper et al. in 1998.[10] This report described the synthesis of *β*-peptoid dimers and trimers with nonchiral substituents on the nitrogen atom. To the best of our knowledge, no synthesis or structural investigation of *β*-peptoids with α-chiral N-substituents has been reported. Although the resulting *β*-peptoid would have three rotatable bonds in the backbone, and consequently an expected low tendency to adopt a folded conformation, we found it valuable to investigate the influence of α-chiral N-substituents on the structure of *β*-peptoids, especially considering the large impact of such groups on the structure of normal α-peptoids and the large interest in *β*-peptides.[2,4] Herein, we report our efforts to develop a practical solid-phase synthesis of *β*-peptoids with α-chiral aromatic N-substituents (Figure 1)

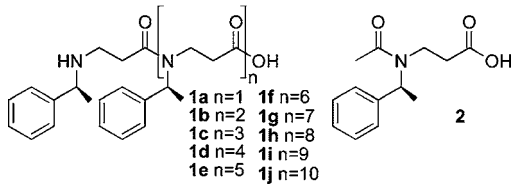


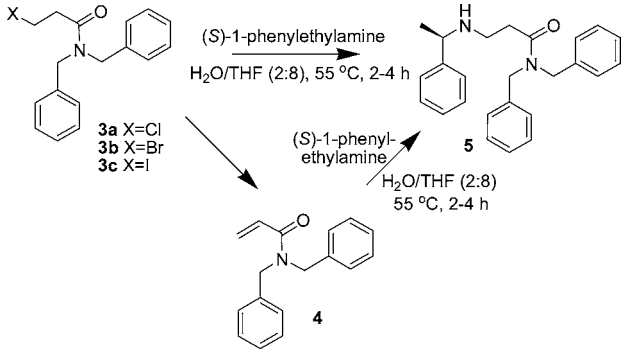
Figure 1. Structures of *β*-peptoids **1a**−**j** and N-acetylated monomer **2**, all possessing α-chiral aromatic N-substituents.

and an investigation concerning the folding propensities of such molecules by circular dichroism (CD) spectroscopy.

For the synthesis of *β*-peptoids, Hamper developed a two-step solid-phase methodology involving first acryloyl chloride as acylating agents to generate acrylamide resins, followed by Michael addition of a primary amine to the α,*β*-unsaturated system to generate the secondary amine.[10] This method was applied successfully for the synthesis of *β*-peptoids containing 2−3 residues; still, this procedure required a high excess of amines (up to 20 equiv) and very long reaction times (up to 72 h) that preclude synthesis of longer derivatives in a reasonable time. In contrast, Zuckermann and co-workers[11] invented a submonomer method for synthesis of α-peptoids where each cycle of monomer addition consists of two steps: acylation of the secondary amine by an α-halogenated acyl donor followed by nucleophilic displacement of the halogen by the next amine. Both these steps require short reaction times (90 min), and relatively long α-peptoid chains can be synthesized by this methodology.

β-Peptoids with α-chiral N-substituents are expected to be more difficult to synthesize due to low reactivity of the secondary amine in the acylation step as well as to steric repulsions during the nucleophilic addition of the α-branched side chain. In the search of good experimental procedures to synthesize *β*-peptoids with α-branched aromatic side chains, solution-phase chemistry was initially employed. First, we focused on utilizing the nucleophilic displacement reaction of Zuckerman in the synthesis of *β*-peptoids (Scheme 1, top). Consequently, *β*-halogenated amides

Scheme 1. Solution-Phase Synthesis of Protected *β*-Peptoid **5**



3a−**c**[12] were treated with (*S*)-1-phenyl ethylamine in 1 mL of solvent[12] and stirred at 55 °C for 2−4 h. The reactions were monitored by analytical reversed-phase HPLC-MS, and the conversions were determined by integration of the UV trace (Table 1). As expected, the chloride derivative **3a** was less reactive than the bromine and iodine analogues **3b** and **3c** (which had similar reactivity). Variation of the reaction media revealed that 20% H_2O in THF was one of the best solvent systems tested. The HPLC-MS analyses also revealed that the reaction conditions caused major elimination of the *β*-halogen to give the α,*β*-unsaturated amide **4**[12] in situ. Employing **4** itself as a reactant provided the product **5** in rates and yields similar to those for the halogen derivatives. Thus, we next focused our attention on the Hamper method, using acryloyl chloride as the acylating agent, and tried to

(6) Smith, A. B.; Favor, D. A.; Sprengler, P. A.; Guzman, M. C.; Carroll, P. J.; Furst, G. T.; Hirschmann, R. *Bioorg. Med. Chem.* **1999**, *7*, 9.

(7) (a) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A. et al. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367. (b) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R. et al. *J. Med. Chem.* **1994**, *37*, 2678.

(8) (a) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303. (b) 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.

(9) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092.

(10) Hamper, B. C.; Kolodziej, S. A.; Scates, A. M.; Smith, R. G.; Cortez, E. *J. Org. Chem.* **1998**, *63*, 708.

(11) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(12) For further details, see Supporting Information.

(13) Some examples: (a) Bartoli, G.; Bartolacci, M.; Bosco, M.; Foglia, G.; Giuliani, A.; Marcantoni, E.; Sambri, L.; Torregiani, E. *J. Org. Chem.* **2003**, *68*, 4594. (b) Enders, D.; Wallert, S.; Runsink, J. *Synthesis* **2003**, *12*, 1856. (c) Yadav, J. S.; Abraham, S.; Reddy, B. V. S.; Sabitha, G. *Synthesis* **2001**, *14*, 2165.

Table 1. Conversion of Reagents **3a**−**c** and **4** after Reaction with (*S*)-1-Phenylethylamine in Various Solvents after 4 h of Reaction at 25 °C

entry	reagent	solvent	conversion (%)
1	**3a**	DMSO	43
2	**3b**	DMSO	66
3	**3c**	DMSO	66
4	**4**	DMSO	2
5	**3c**	THF	97
6	**3c**	*i*-PrOH	98
7	**3c**	*i*-PrOH/THF[a]	96
8	**3c**	H_2O/THF[b]	95
9	**4**	THF	4
10	**4**	*i*-PrOH	95
11	**4**	*i*-PrOH/THF[a]	68
12	**4**	H_2O/THF[b]	90

[a] 50% v/v. [b] 20% v/v H_2O in THF.

optimize the Michael addition step to allow synthesis of longer β-peptoid derivatives in a reasonable time.

As the Lewis acid catalyst is known to accelerate the Michael addition,[13] we tested 14 different potential catalysts [e.g., $FeNO_3$, CF_3CO_2Ag, and $CeCl_3$] for the conversion of **4** to **5** (see Supporting Information, Table S1). The reaction temperature was lowered to 35 °C, and all other reaction conditions were the same as those described previously, using 20% H_2O in THF as the reaction solvent. The reactions was again followed by HPLC-MS. In our system, CF_3CO_2Ag proved to be the best catalyst.[12] Upon varying the solvent composition (H_2O/THF) in the absence of a catalyst, it was found that a high percentage of water (>50%) itself effectively catalyzed the Michael addition (Supporting Information, Table S2).

In a parallel optimization on solid phase, we established that a PEGylated resin, i.e., Tentagel, provided superior yields and reaction times as compared to a polystyren-based resin (Supporting Information, Table S3).

Therefore, it was concluded that the optimal conditions for solid-phase synthesis were the use of Tentagel resin in high water content solution without additional catalysts. In case a cheaper PS resin needs to be used, we suggest that the reaction be performed with catalytic CF_3CO_2Ag in 20% H_2O/THF as solvent.

With the optimized procedure at hand, the solid-phase synthesis of N-chiral β-peptoids **1a**−**j** was initiated. β-Tripeptoid **1b** was the first sequence to be synthesized on solid support, and once again, the conditions needed certain adjustments as the route went from solution to solid phase.[12] For the reaction on solid phase, Tentagel S PHB (0.26 mmol/g) resin at 55 °C for 9 h was the best choice for the Michael addition, and the acylation step only required 2 × 30 min.

The optimized conditions were then used as a repetitive cycle in the synthesis of β-peptoids **1a**−**j** (Scheme 2), resulting in a new efficient method of synthesizing longer-chain β-peptoids.

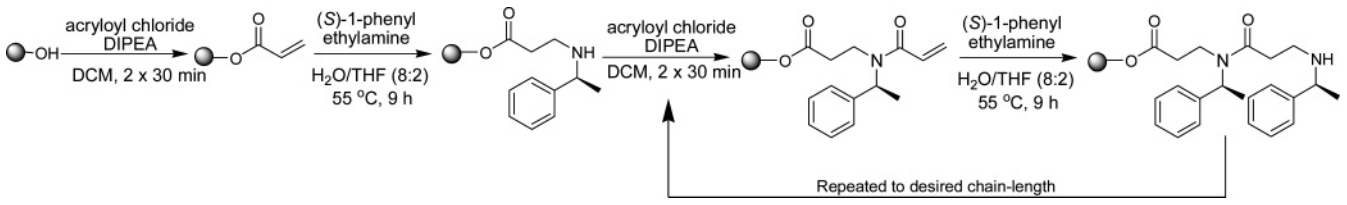
Cleavage from the resin using 95% TFA/H_2O at room temperature for 1 h gave crude products which were purified by preparative reversed-phase HPLC. The dimer **1a** and trimer **1b** were isolated in good yields of 70% and 62%, respectively, and the isolated yields of β-peptoids **1c**−**i** were determined to be in the range of 50−33%.[12] We found these yields acceptable as the synthesis includes nucleophilic addition of the sterically demanding α-branched N-substituent (*S*)-1-phenylethylamine. Further, shorter and/or longer oligomers could be collected as byproducts upon purification, thus increasing the overall yields. As an example, **1j** was collected as a byproduct from the synthesis of **1i**, representing an amount corresponding to a 22% yield of oligomer **1j**. The presence of shorter derivatives may readily be explained by incomplete coupling; however, the longer derivatives are more difficult to rationalize. Most likely, these results are from incomplete washing of the reagents.

Interestingly, a doubling of the peaks was seen for the shorter-chain peptoids **1a**−**f** when the purified peptoids were analyzed on analytical HPLC. Analysis by MS, as well as by NMR spectroscopy, allowed us to establish that the two peaks originated from the same peptoid and thus represent conformers undergoing slow cis/trans isomerization around the tertiary amide linkage; similar observations have been made for N-alkylated α-peptides.[14]

The ability of the β-peptoids with the α-chiral side chains (*S*)-1-phenylethyl **1a**−**j** to adopt an ordered secondary structure was evaluated by CD spectroscopy. In previous studies by Barron and co-workers,[8a] α-peptoids as short as five residues in length, possessing chiral aromatic side chains, were reported to show intense CD, revealing the presence of a regularly repeating, chiral secondary structure.[8a] Further, this class of peptoids has been both predicted and observed to adopt a helical polyproline type I-like conformation.

The folding propensities of the β-peptoids **1a**−**j** were first analyzed using methanol as the solvent at 25 °C (c = 0.1 mM) (Figure 2a). All 10 β-peptoid oligomers gave rise to CD spectra almost identical to those arising from right-

Scheme 2. General Pathway of Solid-Phase Synthesis of β-Peptoids



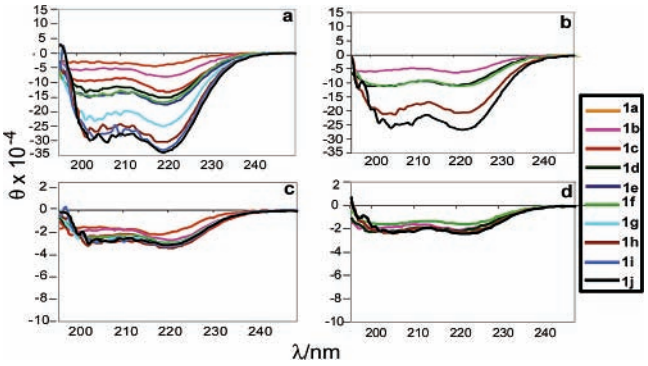


Figure 2. CD spectra of β-peptoids **1a**−**j** in methanol (a) and of **1b**,**d**,**f**,**h**,**j** in acetonitrile (b). Included also are the normalized CD spectra in methanol (c) and acetonitrile (d), respectively, taking the number of chromophores into account.

handed helical secondary α-peptoids, with the same side chains, recorded in acetonitrile, both regarding shape and intensity.[8a,15] The CD spectra are characterized by double minima near 204 and 218 nm. CD spectra of **1b**,**d**,**f**,**h**,**j** in acetonitrile at 25 °C (c = 0.1 mM) gave rise to similar CD spectra (Figure 2b).

Barron[15] noted an increase in the per residue CD intensity up to a certain chain length, after which no further change was observed. We see no such cooperative effect (Figure 2c and 2d) with the β-peptoids of chain lengths between 3 and 11 residues investigated here, suggesting that these oligomers do not adopt helical secondary structures or, alternatively, that an ordered strucure is present already in the trimer. It should be noted that cooperative folding is not expected for conformations resulting exclusively from adjacent interacting sites;[1b] thus, β-peptoid folding could still result from propagation of local conformational preferences.

To further establish if the CD spectra in Figure 2 arose from a possible secondary structure or if the CD pattern was inherent of the side chain functionalized amide linkage itself, we prepared the acetylated "monomer" **2**. Analysis by CD spectroscopy in both methanol and acetonitrile (Figure 3) showed a CD pattern similar to those originating from the oligomers. On the basis of this result, it appears most reasonable to conclude that β-peptoids with α-chiral side chains lack the high tendency of helical secondary structure formation characteristic of the analogous α-peptoids, as the simple monomer would not be expected to adopt an ordered conformation.

The solvent dependence of the β-peptoid oligomers was also investigated by running additional CD spectra of

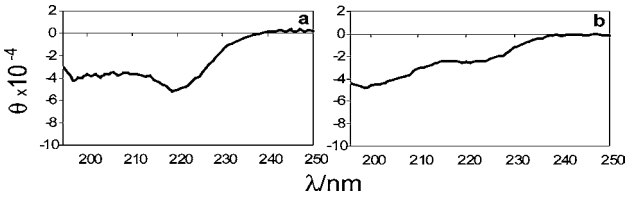


Figure 3. CD spectra of N-acetylated monomer **2** in methanol (a) and acetonitrile (b).

β-pentapeptoid **1d** in trifluoroethanol (TFE) at 25 °C (c = 0.1 mM).[12] TFE is known to stabilize helical secondary structures in polypeptides but to have marginal effects for α-peptoids.[16] Similarly, we did not observe a significant change in the CD pattern of **1d** upon changing the solvent from methanol and acetonitrile to TFE.

In conclusion, a systematically developed and optimized strategy for the challenging solid-phase synthesis of β-peptoids with α-chiral aromatic side chains has been obtained. The present methodology uses Tentagel S PHB as a solid support and 80% H_2O/THF as a mixed solvent system. Although not perfect, these improvements now allow for the addition of one residue every 12 h, making the synthesis of longer β-peptoids within a reasonable time span feasible. The synthetic pathway was demonstrated in the synthesis of nine novel β-peptoids **1a**−**j** that were also analyzed by CD spectroscopy. The spectra closely resembled those reported for α-peptoids with α-chiral ((*S*)-1-phenylethyl) side chains, proven to fold into helical secondary structures;[8a] still, our results suggest that the homologated β-peptoid analogues investigated here do not form ordered secondary structures, although additional studies by high-resolution methods would be needed to fully ascertain this conclusion. Nonetheless, longer-chain β-peptoids with α-chiral side chains still represent an interesting class of biomimetic oligomers that may find use in biomedical and material science applications that do not neccessarily require secondary structure formation function.

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Supporting Information Available: Experimental details and characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Teixidó, M.; Albericio, F.; Giralt, F. *J. Pept. Res.* **2005**, *65*, 153.
(15) Wu, C. W.; Sanborn, T. J.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 2958.
(16) Sanborn, T. J.; Wu, C. W.; Zuckermann, R. N.; Barron, A. E. *Biopolymers* **2002**, *63*, 12.