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Introduction of a Triazole Amino Acid into a Peptoid Oligomer Induces Turn Formation in Aqueous Solution

Jonathan K. Pokorski,[§] Lisa M. Miller Jenkins,[†] Hanqiao Feng,[‡] Stewart R. Durell,[†] Yawen Bai,[‡] and Daniel H. Appella*[,§]

Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, DHHS, Bethesda, Maryland 20892, Laboratory of Cell Biology, NCI, National Institutes of Health, DHHS, Bethesda, Maryland 20892, and Laboratory of Biochemistry and Molecular Biology, NCI, National Institutes of Health, DHHS, Bethesdsa Maryland 20892.

appellad@niddk.nih.gov

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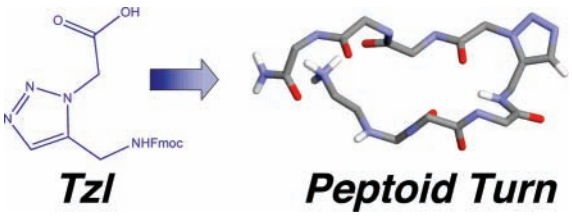
ABSTRACT



Peptoids are a non-natural class of oligomers that are composed of repeating N-substituted glycine units and are capable of folding into helices that mimic peptide structure and function. In this letter, we report the concise synthesis of a 1,5-substituted triazole amino acid (Tzl) and its subsequent incorporation into a short peptoid. The Tzl amino acid was shown to induce turn formation in aqueous solution, thus expanding the structural repertoire available to peptoid chemists.

Peptoids are a class of synthetic oligomers that consist of repeating N-substituted glycine units and are capable of folding into discrete structures.[1] Peptoids bearing α-branched side chains can fold into helices resembling the polyproline type I (PPI) helix[2] and are attractive pharmaceutical candidates because of their enhanced proteolytic stability[3] and cellular uptake.[4] In this context, peptoids' propensity for helix formation has been used to develop mimics of the antibacterial magainin peptides[5] and lung surfactant proteins.[6] More recently, macrocyclic peptoids were designed to mimic *β*-turn formation in organic solvents.[7] Expanding the structural repertoire of peptoids to include turns, loops, and sheets should aid the development of biologically active derivatives where such motifs are relevant. Progress in the development of noncyclic peptoid structure has shown that certain nonamers can fold into loops in organic solvent,[8] which can be stabilized through the introduction of electron-poor

[§] Laboratory of Bioorganic Chemistry.
[†] Laboratory of Cell Biology.
[‡] Laboratory of Biochemistry and Molecular Biology.

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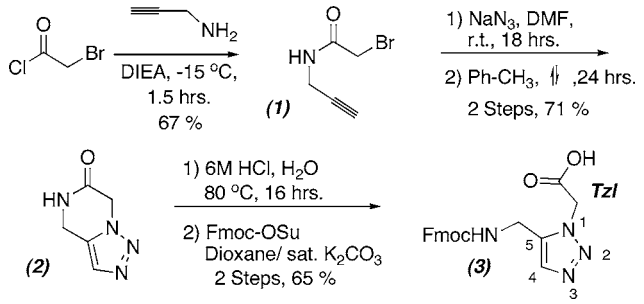
aromatic residues.[9] Aqueous conditions, alternatively, induce a conformational change favoring the PPI helix owing to the necessity of hydrogen bonding between the peptoid termini for loop formation.

Recent research has begun to elucidate the rules for hairpin formation in peptides.[10] Conformational constraint about the loop region has proven to be a powerful method for enforcing a hairpin structure in model peptides[11] and has been extended to the design of hairpin mimics of protegrins.[12] However, turn constraints have not yet been applied in peptoid design. We hypothesized that the incorporation of a 1,5-substituted triazole amino acid (**3**) into peptoid oligomers would induce turn formation in a peptoid backbone. The triazole introduces a constraint that is geometrically similar to a cis double bond, while acting as an amide isostere with respect to polarity. Furthermore, this amino acid would not disrupt the registry of the peptoid, maintaining molecular spacing equivalent to two peptoid monomers. In this communication we report the synthesis of a 1,5-substituted triazole amino acid (Tzl) and its effects upon the secondary structure of short peptoids.

The most common method for accessing the triazole framework is the copper-catalyzed Huisgen cycloaddition.[13] This methodology, though, was not suitable for our needs because the favored product is the 1,4-substituted regioisomer. There are few methods in the literature available to access the 1,5-substitution pattern, but those proved either unreliable or costly because of the use of organometallic reagents.[14] Thus, we developed a gram-scale synthesis that utilizes a ring-constrained Huisgen cycloaddition to introduce the 1,5-substitution pattern (Scheme 1). Synthesis of the Tzl

Scheme 1. Tzl Synthesis



monomer is accomplished in three steps, with no chromatographic purification required. Propargyl amine is first acylated using bromoacetyl chloride. The resulting bromoacetamide (**1**) is converted to an azide upon treatment with NaN_3.

The key synthetic step in the sequence is the Huisgen cycloaddition that yields bicyclic triazole **2** as a single regioisomer. The lactam is then opened under strongly acidic conditions followed by Fmoc protection of the primary amine to yield Tzl monomer **3** in good yield. We anticipate that Tzl will prove useful for a number of studies that require the use of a ring-constrained amino acid.

The Tzl monomer was readily incorporated into peptoid oligomers using a modified version of the submonomer synthesis.[15] Peptoids were designed so that the Tzl monomer was located in an analogous position to the turn region of a peptide in a hairpin conformation. The folded conformation of Tzl peptoids was initially assessed using circular dichroism. Peptoid pentamer **4** showed both a global minimum at 200 nm, and a local minimum at 220 nm (Figures 1 and 2).

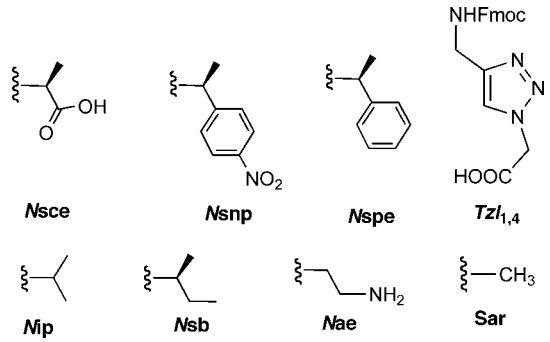


Figure 1. Peptoid side-chain structures and nomenclature.

Previous work with peptoids has attributed the negative peak at 200 nm to a loop structure, while the minimum at 220 nm suggests a helical conformation.[8] We speculated that **4** was in conformational equilibrium between two folded states. This hypothesis was confirmed by the observation of two equally intense sets of resonances in TOCSY spectra. To

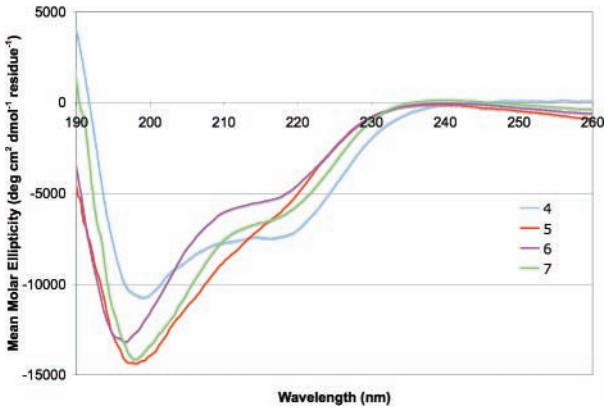


Figure 2. CD spectra of peptoid oligomers **4**−**7**. Peptoid sequences are as follows. **4**: *N*sce*N*spe*N*Tzl*N*spe*N*ae, **5**: *N*sce*N*ip*N*spe*N*Tzl*N*snp*N*sb*N*ae, **6**: *N*sce*N*ip*N*spe*N*Tzl$_{1,4}$*N*snp*N*sb*N*ae, **7**: *N*sce*N*ip*N*spe*N*Sar*N*Sar*N*snp*N*sb*N*ae.

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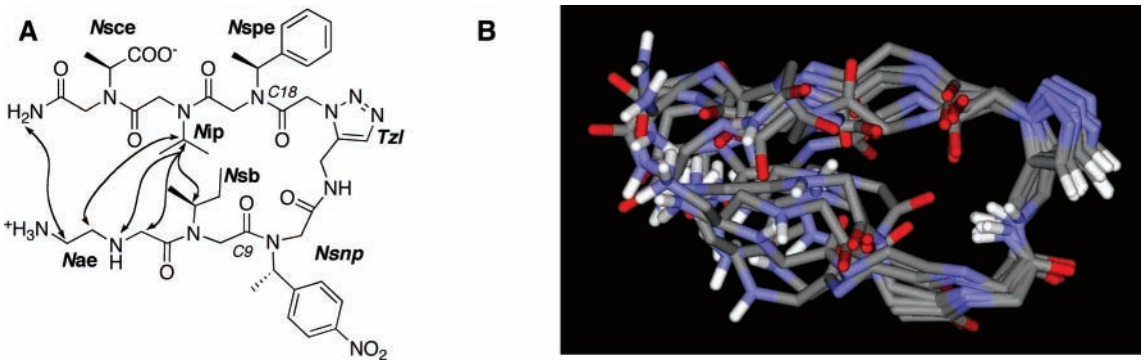

Figure 3. Structure of peptoid **5**: (a) chemical structure of **5** showing long-range NOEs and side-chain nomenclature; (b) ensemble of 9 modeled structures of the major conformation of **5** derived from the NOE restraints. The role of the Tzl unit is seen in the upper right portion of the figure. Average atomic rmsd's: all atoms, 5.26 Å; backbone turn atoms, 2.55 Å. The mean violation per structure for 26 restraints is 8.3 Å. All NMR experiments were performed in aqueous buffer; 10 mM sodium phosphate, pH 7.0.

further bias the peptoid toward a hairpin conformation, two hydrophobic residues were incorporated in peptoid **5** (Figures 2 and 3) to encourage hydrophobic collapse as seen in hairpin peptides.[16] The CD spectrum of this peptoid showed only a single intense global minimum at 200 nm. Subsequent control experiments where the Tzl unit was replaced with the analogous 1,4-substituted triazole ($Tzl_{1,4}$) (**6**) or with sarcosine (**7**) maintained a local minimum at ~220 nm, indicating that the Tzl unit induces a unique fold.

Peptoid **5** was further characterized by NMR spectroscopy. The 2D TOCSY spectrum of **5** in aqueous solution produced a high quality spectrum with well-dispersed resonances corresponding to a single peptoid conformation. Assignments were confirmed and distance constraints were obtained from a 2D ROESY spectrum. Refinement yielded a major and minor conformation that both converged to show a compact hairpin structure, resembling a peptide hairpin (Figure 3). The major structure is characterized by a highly conserved turn region and flexible termini. The Tzl residue initiates a turn in the backbone, of which the carbonyl carbons of Tzl (C18) and *N*sb (C9) represent the ends of a 10-atom turn. These carbon atoms are ~5 Å apart, well within the 7 Å maximum prescribed to peptide turns,[17] suggesting that the backbone geometry is a competent turn mimic. In all solutions, the N- and C-termini maintain a close proximity that may be facilitated by a salt bridge or hydrogen bond. The termini though, are inherently flexible and can orient themselves either above or below the plane of the turn region.

The findings reported here represent the first account of hairpin formation by a peptoid in aqueous solution. The synthesis and subsequent incorporation of the Tzl monomer in concert with principles derived from model hairpin peptides were critical to successful peptoid design. These results have major implications toward the de novo design of functional nonnatural polymers and effectively extend the range of secondary structures available to the peptoid community.

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Supporting Information Available: Detailed procedures for synthesis of all compounds, CD studies, and NMR stucture determination. NMR TOCSY and ROESY data for peptoid **5** are also present, along with information on the minor peptoid structural conformation. This material is available free of charge via the Internet at http://pubs.acs.org.

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