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Design and Facile Solid-Phase Synthesis of Conformationally Constrained Bicyclic Peptoids

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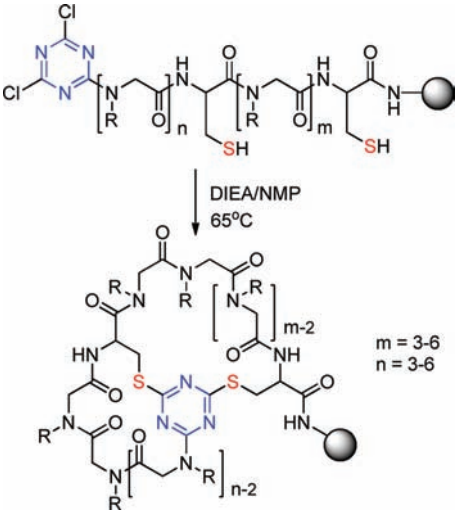
ABSTRACT



Triazine-bridged bicyclic peptoids as conformationally constrained peptidomimetics are described. Bicyclic peptoids composed of 6−12 peptoid residues (*m*, *n* = 3−6) were synthesized in excellent yields using a highly efficient solid-phase synthetic route.

Peptoids are oligomers of N-substituted glycines and have desirable features as peptidomimetics; they are easily synthesized,[1] resistant to proteolytic degradation,[2] and importantly far more cell permeable than peptides.[3] They have been shown to be a rich source of protein-binding molecules.[4] Hence, peptoids have a great potential both as chemical tools to interrogate protein functions and as therapeutic candidates.[4a] However, peptoids are relatively flexible. Such a flexible nature of peptoids may limit the development of peptoid-based protein ligands with high affinity and specificity.

One potentially promising way to overcome the limitation is cyclization of linear peptoids. Macrocyclization has been a successful strategy used by nature and chemists to restrict the conformational freedom in peptides.[5] It is generally considered that cyclic peptides may have enhanced binding affinity, selectivity, and metabolic stability compared to their linear counterparts. Indeed, a number of cyclic peptides have been shown to exhibit highly potent

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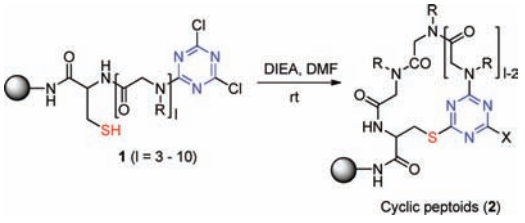
biological activities, and some of them are clinically used.[5a] Similarly, cyclic peptoids are expected to have increased conformational rigidity and preorganized structures, potentially enabling them to bind more tightly to target proteins without a major entropy loss. Thus, cyclic peptoids are of great interest as a promising class of peptidomimetics. Not surprisingly, there have been a growing number of reports on synthesis of cyclic peptoids and cyclic peptoid/peptide hybrids.[6]

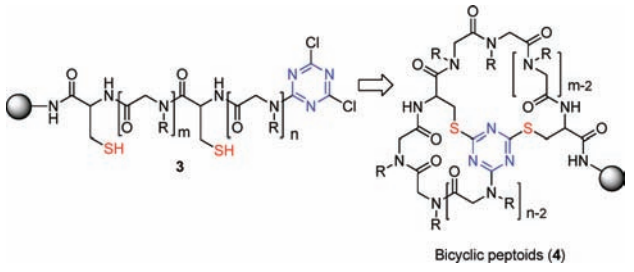
Scheme 1. Solid-Phase Synthesis of Triazine-Bridged Cyclic Peptoids



We have recently reported on triazine-bridged cyclic peptoids **2**.[6j] In this study, we developed a highly efficient on-resin cyclization of peptoids with various ring sizes (with 3 to 10 peptoid residues) via a triazine-derived linker (Scheme 1). This method provides the convenient synthesis of cyclic peptoids in a combinatorial fashion. Additionally, this method allows for direct analysis of cyclic peptoid structures. That is, oxidative ring-opening reaction with *m*-CPBA/NaOH converts the cyclic peptoids into linearized peptoids, which can be sequenced by mass spectrometry. Thus, there is no need for encoding when constructing one-bead-one-compound combinatorial libraries. Notably, incorporation of a triazine ring into the backbone could impose additional conformational restrictions on cyclic peptoids, enabling them to have preorganized structures, as demonstrated in a number of aryl-bridged cyclic peptides and cyclic peptidomimetics.[6b,7]

As part of our ongoing efforts to develop novel peptidomimetics,[6j,8] here we describe the design and facile synthesis of triazine-bridged bicyclic peptoids **4** as a new class of macrocyclic peptoids. As demonstrated by many bicyclic peptides (either naturally occurring or synthesized),[9] bicyclic peptoids would have significantly enhanced conformational rigidity, compared to monocyclic peptoids, as well as linear peptoids. Accordingly, bicyclic peptoids should be able to bind more tightly and specifically to target proteins. Given the constrained scaffold and relatively large size, bicyclic peptoids could act like protein epitope mimetics or small protein functional domains involved in protein−protein interactions, thus potentially serving as an excellent source of modulators of protein−protein interactions.[9d,10]

Scheme 2. Synthetic Strategy of Triazine-Bridged Bicyclic Peptoids **4**



In our previous work,[6j] macrocyclization was accomplished by a nucleophilic attack by the cysteine sulfhydryl group on a reactive chloride on the triazine (Scheme 1). Based on this work, we envisaged that if two cysteine residues are incorporated in a peptoid sequence, the triazine core with two reactive chlorides can be anchored by the two cysteines, resulting in triazine-bridged bicyclic peptoid frameworks (Scheme 2). To test this, we first synthesized a peptoid **3a** containing two cysteine residues spaced by three peptoid residues and a dichlorotriazine on the N-terminal (Scheme 3).

TentaGel S NH_2 beads (90 μm) were used for solid-phase synthesis. First, we conjugated 3-amino-3-(2-nitrophenyl)-propionic acid (Fmoc-ANP-OH) as a photocleavable linker, which can be cleaved from beads by UV irradiation (360 nm, 1 h).[6j] Then, 4-aminobutanoic acids (Fmoc-Abu-OH) as a spacer and a *p*-methoxytriphenylmethyl (Mmt)-protected cysteine (Fmoc-Cys(Mmt)-OH) were subsequently

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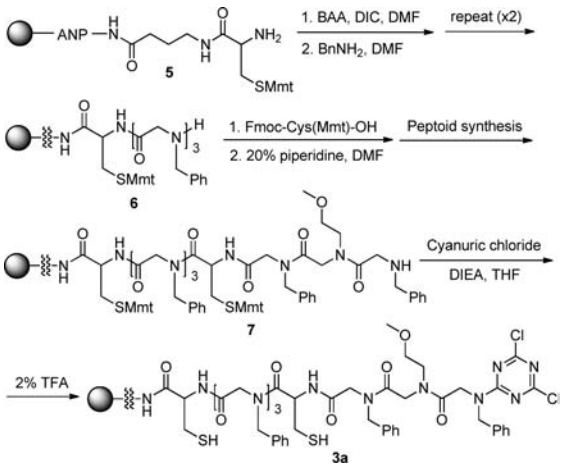
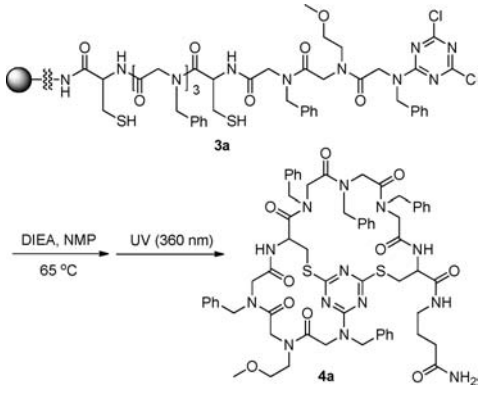
Scheme 3. Solid-Phase Synthesis of a Peptoid **3a** with Two Cysteines and a Triazine



attached using standard peptide coupling conditions. After Fmoc deprotection, peptoid residues were added using the submonomer route developed by Zuckermann and colleagues.[1] Briefly, the free NH_2 group on the cysteine residue of **5** was bromoacetylated by treating with bromoacetic acid (BAA) and *N*,*N*′-diisopropylcarbodiimide (DIC) in DMF. The resulting bromide was displaced by benzyl amine. This bromoacetylation and amination process was repeated to afford **6**. Subsequently, the second cysteine (Fmoc-Cys(Mmt)-OH) was coupled to the peptoids using DIEA/HOBt/HBTU in DMF. After Fmoc was removed, three more peptoid residues were introduced to give **7**. The N-terminus of the peptoid **7** was capped with a triazine by reacting with cyanuric chloride in the presence of DIEA in THF at room temperature. Next the Mmt protecting groups on the cysteine residues were deprotected by treating with 2% trifluoroacetic acid (TFA) in CH_2Cl_2.

Scheme 4. On-Resin Macrocyclization for a Triazine-Bridged Bicyclic Peptoid **4a**



Finally, on-resin macrocyclization was carried out using DIEA in *N*-methylpyrrolidone (NMP) at 65 °C (Scheme 4). The resulting bicyclic peptoid was then cleaved from the beads upon UV irradiation. The crude product was analyzed by HPLC and MALDI-TOF MS for the identity and purity (Figure 1 and Supporting Information). The HPLC trace of the crude product shows that our solid-phase synthesis was strikingly efficient, furnishing the desired bicyclic peptoid **4a** in high yield (94% as determined by HPLC) without detectable byproducts (e.g., a cyclodimer) or an unreacted starting material (Figure 1).

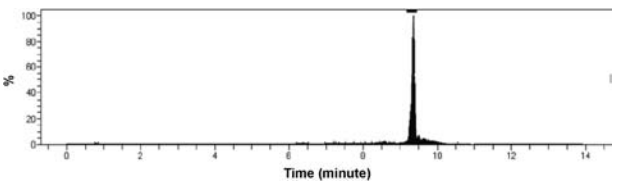


Figure 1. HPLC chromatogram of crude bicyclic peptoid **4a**.

To further assess the feasibility of our solid-phase synthesis, we synthesized a series of bicyclic peptoids that have varying lengths (6 to 12 peptoid residues) and different side chains randomly selected from 11 primary amines shown in Table 1. The bicyclic peptoids were prepared using the same solid-phase synthetic route depicted in Schemes 3 and 4. After the cleavage reaction, the released crude bicyclic peptoids **4b**−**j** were characterized by HPLC and MALDI-TOF MS (see the Supporting Information). The sequence and purity data for the cyclic peptoids **4b**−**j** are shown in Table 1. In all cases examined, bicyclic peptoids were efficiently synthesized in excellent yields (89−96%) regardless of the ring sizes and monomers used. This result indicates that our solid-phase synthetic method is robust and amenable to large library construction.

Next, we examined whether the identity of bicyclic peptoids on a single bead can be obtained. To this end, beads displaying bicyclic peptoids (**4b**, **4e**, and **4g**) were treated with mCPBA/NaOH followed by exposure to UV irradiation (Scheme 5). The crude products released from a single bead were analyzed by MS and MS/MS. Indeed, linearized peptoids **8** were efficiently generated by the ring-opening reaction, and their sequences were unambiguously determined (Figures S10−S12, Supporting Information).

Finally, we prepared a large, one-bead-one-compound combinatorial library. A standard split-and-pool synthesis[11] was used to create a library of bicyclic peptoids containing six peptoid residues ($m = 3$, $n = 3$) employing seven different amines (theoretical diversity; $7^6 = 117649$) (Figure S13, Supporting Information). After synthesis, 32 beads were picked randomly from the library and subjected to MS for quality control. On the basis of the mass data, all of the tested library molecules showed high

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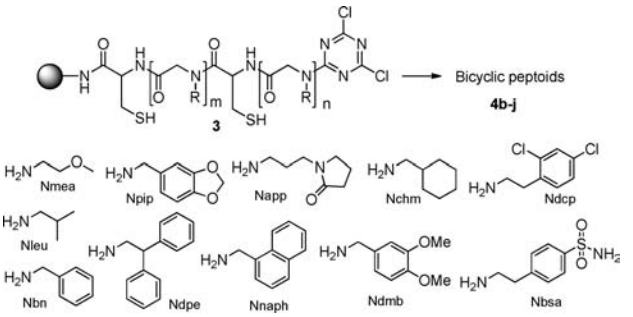
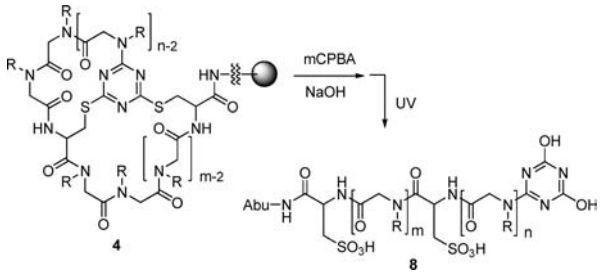
Table 1. Sequence and Purity Data for the Triazine-Bridged Bicyclic Peptoids **4b**–**j**



compd	*m*	*n*	sequence	purity[a] (%)
4b	3	3	Cys-(Nbn-Napp-Nbsa)-Cys-(Nmea-Nnaph-Nchm)	89
4c	3	4	Cys-(Nbn-Napp-Nbsa)-Cys-(Nmea-Nnaph-Nchm-Ndcp)	91
4d	3	5	Cys-(Nbn-Napp-Nbsa)-Cys-(Nmea-Nnaph-Nchm-Ndcp-Napp)	92
4e	4	3	Cys-(Nmea-Ndcp-Nchm-Nnaph)-Cys-(Nbn-Napp-Npip)	96
4f	4	4	Cys-(Nmea-Ndcp-Nchm-Nnaph)-Cys-(Nbn-Napp-Npip-Nleu)	96
4g	5	3	Cys-(Nnaph-Nleu-Ndpe-Nmea-Npip)-Cys-(Nmea-Ndcp-Ndmb)	95
4h	5	4	Cys-(Nnaph-Nleu-Ndpe-Nmea-Npip)-Cys-(Nmea-Ndcp-Ndmb-Nbn)	96
4i	5	5	Cys-(Nnaph-Nleu-Ndpe-Nmea-Npip)-Cys-(Nmea-Ndcp-Ndmb-Nbn-Nchm)	92
4j	6	6	Cys-(Nmea-Ndcp-Nchm-Nnaph-Nleu-Ndmb)-Cys-(Nchm-Npip-Napp-Ndpe-Nmea-Nnaph)	89

[a] Purity of the crude products assessed by HPLC.

Scheme 5. Ring-Opening of Bicyclic Peptoids **4** into Sequenceable Linearized Peptoids **8**



purity (Figure S14, Supporting Information). Given the highly efficient synthesis and simple sequencing method, our bicyclic peptoids can be readily used in high-throughput screening of combinatorial libraries.

In summary, we designed triazine-tethered bicyclic peptoids, which are expected to have a high degree of conformational rigidity and preorganized structures compared to monocyclic peptoids. To the best of our knowledge, there have been no previous reports of bicyclic peptoids. We also developed an efficient and convenient solid-phase synthetic route, providing exclusively the bicyclic peptoids in excellent yields. Given the rigid scaffold and straightforward solid-phase synthesis, in addition to the well-known advantages of peptoids such as better cell permeability and proteolytic stability, our bicyclic peptoids should find a wide range of applications in the field of chemical biology and biomedicine.

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Supporting Information Available. Detailed experimental procedures for the synthesis and characterization data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.