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Small Head-to-Tail Macrocyclic α-Peptoids

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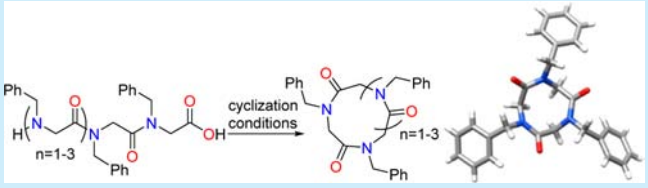
Supporting Information

ABSTRACT: A convenient and efficient methodology for the head-to-tail macrocyclization of small 3-mer, 4-mer, and 5-mer α-peptoid acids (9-, 12-, and 15-atom *N*-substituted glycine oligomers) is described. The cyclic trimer has a *ccc* amide sequence in the crystal structure, whereas the tetramer has *ctct* and the pentamer has *ttccc* stereochemistry. NMR analysis reveals rigid structures in solution. These synthetic macrocycles may prove useful in medicinal and materials applications.



Linear peptoids (*N*-substituted glycine oligomers), possessing tertiary amides, are rather "floppy" molecules since they are able to undergo *cis*−*trans* isomerization of the backbone *N*,*N*-disubstituted amides at ambient temperatures.[1] This is a distinct disadvantage for a protein interaction probe due to entropic loss upon binding, yet this disadvantage can be repurposed as a solid advantage for the synthesis of small, rigid peptide-isomer (α-peptoid) macrocycles in high yields.[2] The ideal peptoid head-to-tail macrocyclization (macrolactamization) concept is the tandem stepwise synthesis of the oligomer in a solid-phase protocol prior to simultaneous macrolactamizing cleavage from the resin. Typical resin loadings of ca. 1 mmol g^{-1} offer a built-in pseudodilution effect.[3] Although the introduction of the Kenner sulfamyl safety catch resin for cyclic peptide synthesis appeared to hold promise,[4] the recorded efficiencies in other's hands have been very low (typically 5%).[5] Park et al. demonstrated the complete nonreactivity of the Kenner resin with *N*-terminal secondary amines[6] (the situation present for linear peptoid oligomers), a result that we were able to confirm (results not shown). Fowler et al. introduced the tandem cyclization−cleavage from an alternative Wang resin for the production of 17-atom ring pseudopeptides[7] (there are three atoms per residue in an α-peptoid). However, they were only able to realize 15−36% yields after extensive optimization.[7] An alternate concept is to prepare the linear precursor peptoid acid on-resin, followed by cleavage from the resin and cyclization in solution.[2a,8] One advantage to this approach is the ability to intercept the peptoid acid postcleavage for precyclization purification by chromatography and drying. This also allows complete freedom to dictate solution conditions for macrocyclization. Further, the use of a syringe pump[9] for the consistent and slow addition of reactants permits the maintenance of a low concentration of reacting species in solution at any one time (another example of pseudodilution), thereby decreasing the need for large solvent volumes and concomitant water content that saps yield in condensation reactions. High reactant species dilution is absolutely required to allay linear dimer and cyclohomodimer product formation.

Some small natural product cyclotetrapeptides are known to be potently biologically active with histone deacetylase inhibitors trapoxin A and apicidin as representative examples.[10] We are interested in preparing small, rigid peptoid head-to-tail macrocycles with between three and five peptoid residues (9- to 15-atom rings) for their eventual application as protein interaction probe templates. Head-to-tail macrocyclization has the advantage of maintaining complete control of peptoid macrocycle design by allowing the widest possible range of amine submonomers to be considered in a synthesis so long as there is an *N*-terminal secondary amine and a *C*-terminal carboxylic acid in the linear peptoid acid precursor.

Benzylamine has been previously used to test peptoid synthetic conditions.[11] In addition, we considered that *N*-benzylglycine monomer units may be able to give rise to crystalline products amenably to single-crystal X-ray diffraction.[8a] Due to the bulk of the phenyl ring along with its inherent β-branching at the aromatic *ipso*-carbon, we also speculated that a benzylamine submonomer would serve as a sensitive measure of macrocyclization methodology, allowing us to easily discern robust and competent synthetic parameters. Finally, we surmised that the adoption of benzylamine as the submonomer amine in peptoid synthesis would provide NMR signal resolution in both proton and carbon modalities.

Herein, we present convenient and efficient methodologies for the creation of these valuable macrocycles in high yield and purity. Linear peptoid acids **1**−**3** were prepared using a submonomer protocol under solid-phase synthetic conditions[11] using protocols developed on an automated microwave-assisted peptide synthesizer (stepwise yields of 97% in 6−8 h; see the Supporting Information for details). Hexafluoro-2-propanol[2a]

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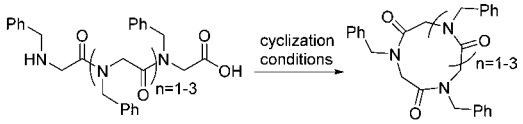
was used to cleave the peptoid acids from the 2-chlorotrityl resin.[12] Our results are presented in Table 1.

Table 1. Cyclization of Small *N*-Benzyl α-Peptoids



Compounds: **1**, **4**, n = 1; **2**, **5**, n = 2; **3**, **6**, n = 3.

entry	linear precursor	cyclization conditions	cyclic peptoid	yield[a] (%)
1	1	HATU[b]/ DIEA	4	24
2	1	HATU/HOAt/DIEA	4	80
3	1	EDC/HOAt/TEA	4	**90**
4	2	HATU/DIEA	5	28
5	2	HATU/HOAt/DIEA	5	41
6	2	EDC/HOAt/TEA	5	38
7	2	HATU/HOAt/DIEA; 50 °C	5	**80**
8	3	Py-BOP/DIEA/DMF[c]	6	0
9	3	Mukaiyama reagent	6	0
10	3	HATU/DIEA	6	30
11	3	pyBrOP	6	30
12	3	EDC/DMAP	6	52
13	3	EDC/HOBt/TEA	6	71
14	3	HATU/HOAt/DIEA	6	83
15	3	EDC/HOAt/TEA	6	**97**

[a]Isolated yields (%) following C_{18}-reversed-phase chromatographic purification, lyophilization, and weighing. [b]HATU = 1-[Bis-(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate. [c]Repeated twice with the same result, 0% isolated yield. Typical reaction scale was 100 μmol of α-peptoid acid starting material. See the Supporting Information for details.

We employed a range of macrolactamization methods at room temperature that we considered would be expedient for cyclic peptoid synthesis and having either not been applied to peptoids before or having not been optimized for small cyclic peptoids. We split the reacting species into two compartments with a stirred solution of the linear peptoid acid and organic base in one (5−10 mM) and a concentrated solution of the coupling activator and coreagent in a syringe pump. In this way, the reacting species concentration was kept at a low level, providing exclusive cyclomonomer formation. Initial attempted cyclization of **3** with the Mukaiyama[13] reagent was not successful (Table 1, entry 9). Even though a major protocol reported for peptoid cyclizations (head-to-tail, side chain-to-side chain, and side-chain-to-tail) uses pyBOP,[2a,e,f,8c,14] this coupling reagent has not been useful in our hands (entry 8), and this is an observation echoed by Roy et al.[8b] Indeed, Kwon et al.[14d] stated that peptoids need to be larger than hexamers (6-mers giving 18-atom rings) to be candidates for pyBOP macrocyclization. Since we have 3- to 5-mer peptoid acids **1**−**3** as cyclization precursors, we had to construct alternate synthetic methodology. A related reagent, pyBrOP enjoys some distinct advantages in comparison with pyBOP: useful for *N*-methyl peptide (peptoid-like) synthesis and formation of an acyl bromide offers the possibility of amide bond formation in an uncluttered reaction intermediate complex.[15] We achieved a moderate 30% yield of **6** with this reagent (entry 11). A few HATU-mediated peptoid and peptide macrocyclizations have accumulated in the recent literature reporting low to good yields of 15−78% for 9-atom to 18-atom rings,[8a,b,16] comparing well with the 24% yield of **4**, 28% of **5**, and 30% of **6** that we were able to achieve (entries 1, 4, and 10). Fernández-Llamazares et al. have used the *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) reagent with DMAP additive for peptide macrocyclization, achieving a mean of 50% yield over 11 *N*-methylcyclopentapeptides.[17] Applying this methodology to **3**, we reached a similarly modest 52% yield of **6** (entry 12). Recently, Caumes et al. have introduced the EDC with the 1-hydroxybenzotriazole (HOBt) additive method of Le Grel et al.[18] to β-peptoid macrocyclization for a useful increase in yield to 73% for a 14-atom $\alpha_2\beta_2$-tetrapeptoid macrocycle.[19] We accomplished a comparable 71% yield of **6**, a 15-atom cyclopenta-α-peptoid (entry 13). Swapping out the HOBt coreagent for the 7-aza analogue, 1-hydroxy-7-azabenzotriazole (HOAt),[20] we attained an astonishing increase in isolated yield to 97% of **6**, with 90% for **4** but a disappointing 38% for **5** (entries 3, 6, and 15). We attribute this gain to the ability of HOAt to simultaneously assemble the *C*- and *N*-terminii of a linear peptoid in close proximity while the carboxyl function is activated to nucleophilic attack leading directly to macrocyclization. Given the observed benefit ascribed to HOAt, we revisited the HATU-mediated cyclizations and added HOAt which gave 80% and 83% for **4** and **6** (entries 2 and 14), respectively (up from 24% and 30%), with, again, a low 41% for **5** (entry 5), yet still increased from the previous 28% observed for HATU/DIEA alone (entry 4). We considered that a higher yield might be attainable if we increased the rapidity of *cis*−*trans* amide geometry interchange in **2** caused by a rise in temperature. Finally, by increasing the reaction temperature from 22 to 50 °C (in a water bath), we were able to secure an 80% yield of **5** (entry 7).

An important aspect of these synthetic methods was the development of supporting analytical methods. Initial normal-phase chromatographic purification furnished oversimplistic single peak chromatograms with consequent exaggerated yields (data not shown). Preparative C_{18}-reversed-phase chromatography furnished the pure cyclic peptoids as well-resolved peaks, free of contamination (Supporting Information). This allowed us to obtain pure materials for subsequent applications and for the accurate recording of isolated product yield.

Purified compounds **4**−**6** were prepared as single crystals suitable for X-ray analysis by slow evaporation of 10:1 (v/v) chloroform/methanol solutions. This is the first time that a cyclopentapeptoid structure has been analyzed by X-ray crystallography, and such small macrocycles are rare in the areas of peptide and peptoid chemistry. The three amide bonds of **4** have a *ccc* geometry, and the benzyl side chains lie on one face of the circular peptoid ring which has a diameter of about 0.38 nm (Figure 1). Two independent molecules of **4** exist in

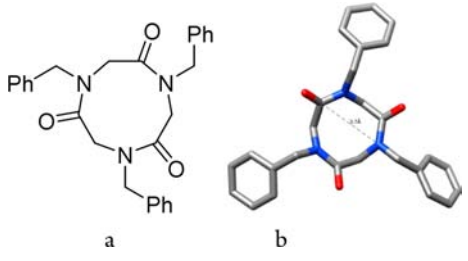


Figure 1. X-ray structure of **4**: (a) structure of **4** showing the *ccc* amide geometry; (b) view of crystal structure (H atoms omitted for clarity).

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the asymmetric unit. The crystal structures of cyclo(*N*,*N*′,*N*″-triallylglycine)[21a] and cyclo(dibenzylglycyl-L-proline)[21b] exhibit similar *ccc* crown conformations (overlays in Supporting Information).

Cyclotetrapeptoid **5** is oblong in shape having alternate benzyl substituents above and below the plane of the ring, which has dimensions of about 0.63 nm by 0.29 nm. Compound **5** provided two polymorph X-ray structures that differed in the rotation about two of the four side-chain phenyl rings, yet leaving the macrocyclic ring unchanged (Figure 2).

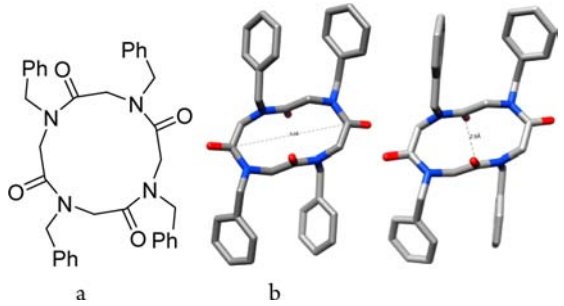

Figure 2. X-ray structure of **5**: (a) structure of **5**; (b) view of polymorph crystal structures showing the *ctct* amide geometry and phenyl ring rotation (H atoms omitted for clarity).

The *ctct* amide sequence of **5** mirrors that seen in some cyclotetra-α-peptides[22a−f] as well as the only other example of a cyclotetra-α-peptoid.[8a] However, cyclotetrapeptides apicidin and trapoxin A, which possess bioactivity, display *tttc* stereochemistry.[22g,h] Comparison of **5** with De Riccardis' cyclotetrapeptoid and cyclotetrasarcosyl[23] revealed a very similar amide backbone structure (Supporting Information).

Cyclopentapeptoid **6** has a diameter of about 0.55 nm and *ttccc* (*N*- to *C*-direction) amide geometry. Benzyl substituents 1, 2, and 4 occupy one side of the ring whereas benzyl groups 3 and 5 are disposed to the other face of the ring (*N*- to *C*-direction) (Figure 3). Synthetic cyclopentapeptides have been

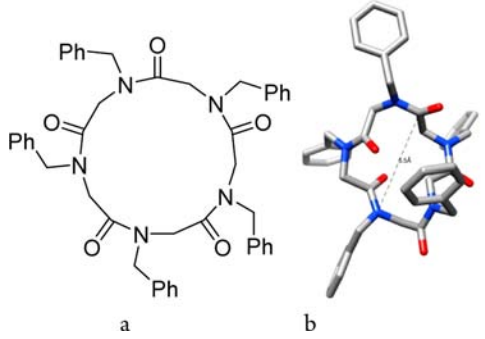

Figure 3. X-ray structure of **6**: (a) structure of **6**; (b) view of crystal structure showing the *ttccc* amide geometry (H atoms omitted for clarity).

extensively studied as models of β-turns in peptides and proteins.[24a,b] Most X-ray structures exhibit all-*trans* amide geometry, are proline and glycine-rich, and have achiral or D-amino acid residue(s) and one trans-annular hydrogen bond.[24a−h] Alternate *cis*-amide geometry is observed adjacent to proline in some structures.[24i−k] We compared **6** with all-*trans* *cyclo*-(glycylprolylglycyl-D-alanylprolyl)[24c] as this cyclopentapeptide has been extensively studied by a range of analytical modalities.[24l] An overlay revealed reasonable overlap for three of the five peptoid residues (Supporting Information).

Torsion angle analyses showed that classical Ramachandran-type plots are not as useful for cyclic peptoid structure descriptions (Supporting Information).[2a,d] However, a simple distance measurement parameter of $C_{\alpha i} \rightarrow C_{\alpha i+2}$ in **6** reveals 0.48 nm (C5−C11) and 0.46 nm (C2−C8) that are within the 0.50 nm limit for describing a peptide β-turn.[2a]

A suite of 1D and 2D NMR experiments[25] (^{1}H, ^{13}C, double quantum filtered-COSY, HSQC, HMBC, phase-sensitive NOESY; Supporting Information) was used to obtain complete assignments for **4** and **5** with partial assignments being possible for **6** due to the rapid increase in NMR signal complexity. Unique NOESY crosspeaks, strategic long-range $^4J_{HH}$ W-coupling, and differential methylene proton resonances of the peptoid side chain in *cis*-amide bonds, caused by magnetic anisotropy of the amide carbonyl bond, were novel circumstances aiding NMR signal assignment in these small, rigid cyclopeptoids. Since these NMR effects originate from the methylene groups surrounding the tertiary amide nitrogen, it is likely that these will be useful in characterizing other small macrocyclic peptoids as these particular methylenes are commonly observed in peptoid structures. The backbone structures are rigid in solution (at room temperature) and are devoid of conformational interchange as evidenced from the narrow line widths of the observed NMR signals and the lack of antiphase NOESY (chemical exchange) crosspeaks.

These new data regarding the well-defined shape, stereochemistry, and conformation of these unique molecules will be essential inputs in future applications of α-peptoid macrocycles.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental procedures; characterization of compounds **1**−**6**; flash chromatographic traces; ^{1}H, ^{13}C, and DQF-COSY NMR spectra; crystallographic data for **4**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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