Multi-Turn Hollow Helices: Synthesis and Folding of Long Aromatic Oligoamides

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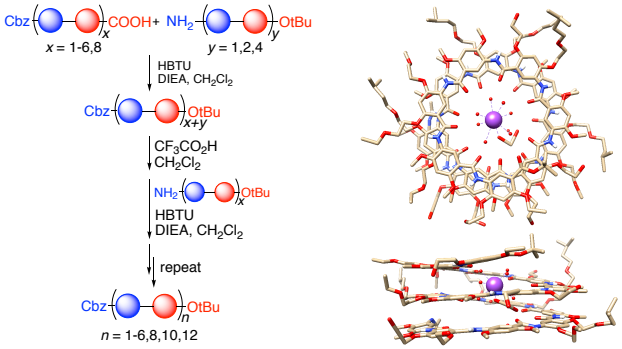
Supporting Information Placeholder



ABSTRACT: Aromatic oligoamides adopting helical conformations are synthesized by coupling carboxyl-terminated basic units having two, four, and eight residues to amine-terminated oligomer precursors. Coupling yields show no noticeable reduction with the size of the basic units or the final product. 1D and 2D NMR spectroscopy, along with computational studies demonstrate the reliable helical folding of these oligomers. The X-ray structure of 16mer **7** reveals a compact, multi-turn helix having a 9-Å inner pore.

The development of foldamers has attracted intense interests for more than two decades.[1] Among known systems, aromatic oligoamides with restricted conformational freedom represent a major class of foldamers having abiological backbones.[2] We and others created aromatic foldamers that adopt stably folded, cavity-containing conformations enforced by highly stable intramolecular hydrogen bonds.[3,4] General structures **A** and **B** (Figure 1) show the first series of oligoamide foldamers we developed. These oligoamides, consisting of *meta*-linked benzene residues, are forced to fold by localized three-center H-bonds.[5] Depending on its chain length, an oligoamide folds into either a flat crescent or a helical conformation that contains an inner cavity defined by inward-pointing amide carbonyl oxygens.

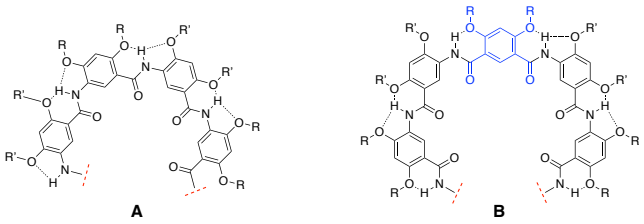


Figure 1. General structures of aromatic oligoamides having an unsymmetrical (*N*-to-*C*) backbone (**A**) and a symmetrical backbone (**B**) consisting of a central diacid residue (in blue) and two identical. Hydrogen bonds are shown as dashed lines.

The synthesis of oligoamides **A** typically involves coupling a monomeric unit carrying an acid chloride and a nitro group to another amine-bearing monomer or oligomer.[3,6] Reducing the nitro group and coupling with another monomer unit result in chain extension. Repetitive coupling leads to "unsymmetrical" oligoamides **A**, with up to eight residues.[3,6] Reacting amine-terminated A-type oligomers with a diacid chloride monomer led to "symmetrical" oligoamides **B**, in fewer coupling steps than those needed for preparing unsymmetrical oligomers of the same lengths. However, the yields of oligoamide **A** or **B** rapidly decrease with increasing oligomer length, which was believed to be caused by steric hindrance resulted from the folding of the oligoamides. By temporarily blocking the backbone-constraining three-center H-bonds with acid-labile 2, 4-dimethoxybenzyl (DMB) group, symmetrical oligoamides with 13 and 15 benzene residues were obtained, albeit at the cost of having to take extra steps to modify monomeric and oligomeric precursors.[7]

Reaching longer oligomers is highly desirable. Oligoamides of type **A** or **B** with sufficient length will form multi-turn helices and may provide "folding nanotubes" that are expected to have novel properties and offer new possibilities.[1e,2b] Such hollow helices, with their electrostatically negative pores of adjustable depth, may provide hosts that tailor the lengths of guests, or serve as ion and molecular channels spanning lipid bilayers.[8] The availability of such multi-turn helices would also address unanswered questions . For example, are the three-center intramolecular H-bonds shown in **A** or **B** strong enough to mediate the folding of a long oligoamide into an uninterrupted multi-turn helix? The X-ray structure of a type **B** symmetrical nonamer, the only known crystal structure showing a helical

conformation for this series of oligoamides,[3b] revealed a short (~1 turn) helix having a pitch of ~7.1 Å, which indicates the absence of aromatic stacking within this helix. If a multi-turn helix does form, is the helix compact or extended? How many residues constitute one turn in such a helix?

Herein we report the synthesis of type-A oligoamides by coupling differently-sized building blocks to a growing amine-terminated oligoamide chain. Oligoamides with up to 24 residues are obtained. The synthesized oligoamides were examined with 1D NMR spectroscopy, which revealed upfield shifts of aromatic proton resonances that indicate the involvement of intramolecular stacking in the folding of these oligomers. The folded structures were computationally optimized, which provided helices of different lengths. These oligoamides were analyzed with 2D (ROESY or NOESY) NMR spectroscopy, results from which are consistent with the presence of helical conformations. In addition, single crystals of a 16-residue oligomer were obtained and led to the determination of the first crystal structure of a multi-turn helix for this series of aromatic oligoamides.

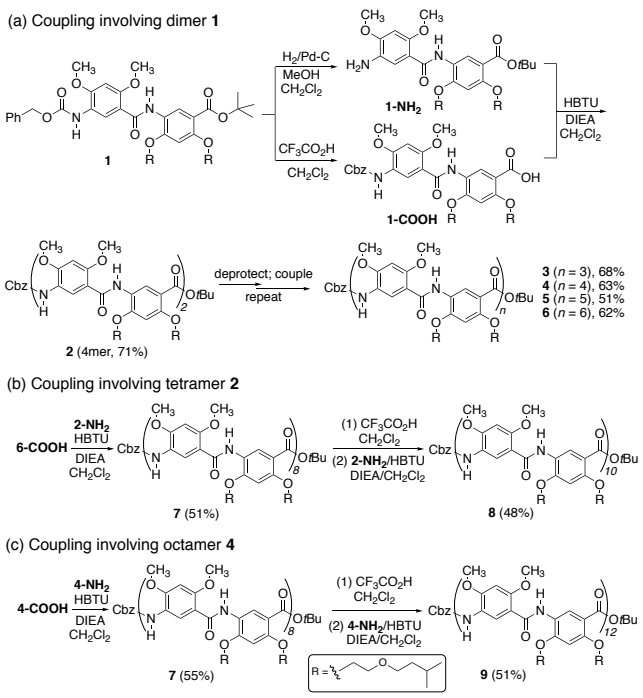
Scheme 1 shows the synthetic routes for preparing tetramer **2** through 24mer **9**. Coupling steps based on building blocks derived from dimer **1** (Supporting Information), which bears the orthogonal protecting carboxybenzyl (Cbz) and *t*-butyl groups at its *N*- and C-termini, respectively (Scheme 1a). Reacting acid **1-COOH** and amine **1-NH$_2$**, obtained by removing the Cbz and *t*-butyl groups of dimer **1**, in the presence of the coupling reagent HBTU and *N*,*N*-diisopropylethylamine (DIEA) in CH_2Cl_2 for at least 12 hours afforded tetramer **2** which bears an *N*-Cbz and a *t*-butyl ester group. Removing the *t*-butyl group of **2** gives the corresponding 4-residue acid **2-COOH** which was coupled with dimer amine **1-NH$_2$** to give hexamer **3**. Chain extension from the *C*- to *N*-temini proceeded smoothly by repeating the steps of removing the *t*-butyl group and coupling with **1-NH$_2$**, which led to octamer **4**, decamer **5**, and 12mer **6** in satisfactory yields after extensive purification.

Compared to coupling monomers or dimers, chain elongation based on building blocks derived from longer oligomers allows products of the same length to be obtained in fewer steps. A potential disadvantage of using longer oligomers as basic coupling units is the likely reduced reactivity and decreased yields.

Scheme 1b shows chain extension using the four-residue **2-NH$_2$** derived from tetramer **2**. Reacting the 12-residue acid **6-COOH** with **2-NH$_2$** gave 16mer **7** in 51% yield. Coupling the 16-residue acid **7-COOH** with the four-residue amine **2-NH$_2$** gave 20mer **8** in 48% yield. As shown in Scheme 1c, coupling building blocks **4-COOH** and **4-NH$_2$**, derived from octamer **4** by removing the Cbz and *t*-butyl groups, respectively, led to 16mer **7** in 55% yield after extensive purification. Removing the *t*-butyl group of 16mer **7** gave the carboxyl-terminated **7-COOH** which was then coupled with eight-residue amine **4-NH$_2$** to give 24mer **9** in 51% yield after extensive purification.

The yields of the isolated oligoamides from coupling 4-residue amine **2-NH$_2$** and 8-residue amine **4-NH$_2$** show no noticeable difference. In fact, the yields of the coupling reactions involving the 2-, 4- and 8-residue amines **1-NH$_2$**, **2-NH$_2$**, and **4-NH$_2$** do not show a correlation with the size of the basic coupling units. Under the adopted conditions, the final products, i.e., oligoamides with 4 to 24 residues, were prepared in satisfactory yields, suggesting that our synthetic method is suitable for preparing even longer oligoamides. In a recently published work on synthesis of a different series of aromatic oligoamides,[9] Huc *et al.* concluded that coupling of long sequences is slower, but does not stop the coupling. Increasing reaction times helps, and increasing concentration is critical.

Scheme 1. Synthetic routes of aromatic oligoamides



Oligoamides **4** through **9** were fully optimized using a density-functional-theory (DFT) method implemented in the CP2K package (see Supporting Information). The optimized structures reveal structural parameters including ~6.3 residues per helical turn,[10] a diameter of ~8.3 Å (O to O) for the inner cavity, and a helical pitch of ~3.5 Å indicating that the helical structures are stabilized by effective aromatic stacking involving the benzene residues and amide groups of adjacent turns (Figure S1).

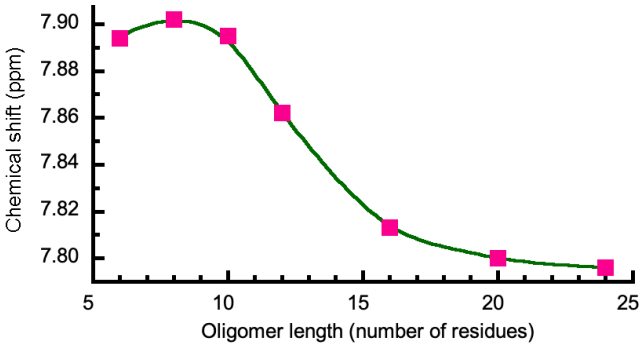


Figure 2. Average chemical shift of aromatic protons versus chain length for hexamer **3** through 24mer **9** (1 mM). The ^{1}H NMR spectra were measured in DMSO-d_6/$CDCl_3$ (1/1, v/v) at 45 °C.

The ^{1}H NMR spectra of oligoamides **4** through **9** (Figure S2) have three distinct regions, i.e., from 9.62 to 10.30 ppm, 8.68 to 9.30 ppm, and 6.24 to 6.86 ppm, which show the signals of the backbone amide protons, the "internals" aromatic protons, i.e., those placed inside the cavities, and the "external" aromatic protons flanked by the phenolic ether sidechains. The overall sharpness and wide resonance dispersion of the amide and aromatic signals are consistent with the well-defined conformations adopted by these oligomers.[3c]

The average chemical shift of the aromatic protons of hexamer **3** through 24mer **9** was determined by dividing the sum of

the chemical shift values of these protons with the total number of such hydrogens in each oligomer. The aromatic proton signals exhibit an upfield shift with increasing oligomer length (Figure 2), indicating stacking of the aromatic residues due to the adoption of helical conformations.[11] From hexamer **3** to decamer **5**, which adopt conformations with less than one turn to about 1.5 turns, the average chemical shift of the aromatic protons exhibits negligible upfield shift. From decamer **5** to 24mer **9**, the average chemical shift of aromatic proton resonances shows a noticeable upfield shift, from 7.90 to 7.80 ppm. This indicates that, with increasing chain length, aromatic stacking is enhanced by the adoption of helical conformations. The fact that the upfield shift of the aromatic proton resonances continues as oligomer length extends implies that the intramolecular aromatic stacking may be cooperative, i.e., such interaction gets strengthened as the number of aromatic units, i.e., the number of helical turns, increases. Thus, a longer oligoamide that folds into multiple turns experiences stronger inter-turn aromatic stacking than a shorter one does.

The ROESY spectrum of octamer **4** and NOESY spectra of 12mer **6**, 16mer **7**, and 20mer **8** were recorded (Figure S3). One ROE is detected between protons *b2* and *d* of **4** (Figure S3a), which is consistent with the folding of **4** into a helical conformation that brings these remote protons into close proximity.

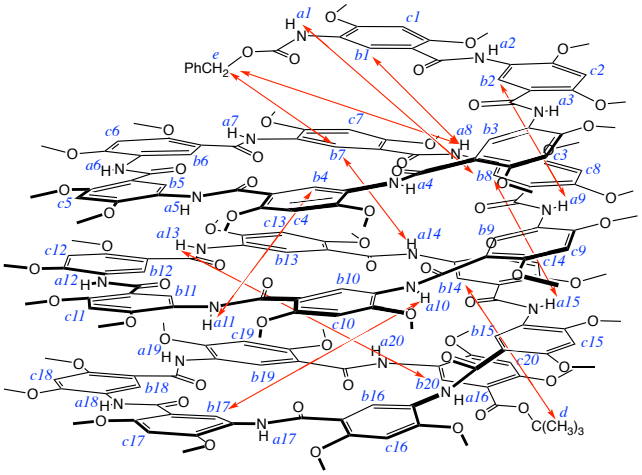


Figure 3. Conformation of 20mer **8** drawn based on energy-minimized helical structure, is supported by NOEs (red arrows) revealed by the NOESY spectrum of **8**. The numbering of amide and aromatic hydrogen atoms starts from the *N*-terminus of each oligomer. Sidechains are shown as methyl groups.

A large number of NOE contacts consistent with the helical folding of **6** (Figure S3b), **7** (Figure S3c), and **8** (Figures 3 and S3d) are found. Major NOEs revealed with 20mer **8** are between protons *a1* and *b8*, *a9* and *b2*, *a10* and *b17*, *a11* and *b4*, *a13* and *b20*, *a14* and *b7*, and *a15* and *b8* (Figure 3). The observed NOEs involve protons on residues that are six and seven basic units apart, while no NOE between protons on residues that are inside or beyond this range is detected, indicating that the two groups of protons being placed into close proximity belong to adjacent turns of a helix. Thus, the number of residues per helical turn is between six and seven turns, consistent with the computationally revealed ~6.3 residues per turn.

Single crystals of 16mer **7** were obtained by slow liquid-liquid diffusion of a solution of **7** in chloroform into methanol. As shown in Figure 4, the crystal structure of **7** reveals a well-defined helix, in which some of the side chains and the Cbz protecting group are disordered. With about 6.6 residues per turn, oligomer **7** folds into a helix of ~2.5 turns. The helical pitch of ~3.4 Å indicates that the aromatic residues engage in strong intramolecular aromatic stacking interaction, which results in a compact helix. The inner cavity of this helix, defined by 16 centripetally pointed carbonyl oxygens, has a diameter of 9.0 Å. Interestingly, a Na^+ ion is complexed in the cavity, with water and methanol molecules serving as the first sphere of coordination, and the inward-pointing oxygen atoms as the second sphere.[12] This hints at possible binding of guests having multiple hydrogen bond donors.

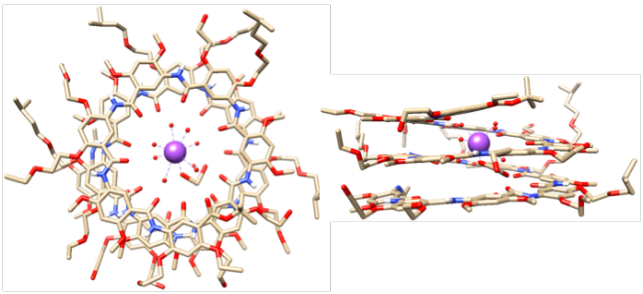


Figure 4. Top and side views of the crystal structure of 16mer **7**. Hydrogen atoms, other than those of the amide groups, along with PF_6^- ions, are removed for clarity.

In summary, type-A oligoamides with lengths up to 24 residues have been synthesized. Coupling differently-sized building blocks to a growing oligomer chain gives oligoamides of desired length in satisfactory yields. The synthesized oligoamides were examined with NMR spectroscopy, along with computational studies, which demonstatrated the helical folding of these molecules. The X-ray structure of 16mer **7** reveals a compact helix of ~2.5 turns with structural parameters including helical pitch, number of residues per turn, and diameter of the inner pore, based on which the folded structures of other oligomers of this series can be accurately modeled. This study has not only demonstrated the reliable folding of these oligoamides, but also have, for the first time, confirmed that the longer oligomers of this series fold into multi-turn helices that are stable, compact and contain large hydrophilic inner pores.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supporting figures, experimental details, 1D/2D NMR, MS spectra (PDF), X-Ray crystallography data, and computational methods

Accession Codes

CCDC 1971430 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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