

## Synthesis and Multibromination of Nanosized Helical Aromatic Amide Foldamers via Segment-Doubling Condensation

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## S Supporting Information

**ABSTRACT:** The synthesis of very long helical aromatic amide foldamers was thought to be limited by steric hindrance associated with stable folded conformations. This difficulty may be overcome by using pure reagents, relatively high concentrations, and long reaction times. Bromine substituents and careful identification and elimination of anhydride byproducts both greatly improve chromatographic purification, giving access to pure products amenable to a segment-doubling synthesis of sequences composed of up to 96 monomers. An efficient one-pot multibromination of helical oligomers is also reported.



Much interest is currently devoted to artificial folded molecular architectures, also termed foldamers.<sup>1</sup> A wide variety of organic backbones that adopt folded conformations in solution has been described, opening the prospect to mimic and possibly go beyond biopolymer structures and functions. However, while the functions of biopolymers, in particular those of proteins, are generally achieved by large tertiary or quaternary folded motifs, foldamers produced by stepwise synthesis most often consist of relatively small objects in the 1–5 kDa range.<sup>2</sup> A major challenge in foldamer chemistry thus lies in the development of synthetic methods to prepare substantially larger folded structures that would give access to more elaborate functions. For this purpose, a possible strategy is to adapt or to evolve molecular biology tools so that they accommodate non-natural monomers. For example, the tolerance of DNA polymerases to non-natural nucleobases is being enhanced,<sup>3</sup> and the ribosome machinery has been shown to accept monomers different from natural  $\alpha$ -amino acids.<sup>4</sup> Nevertheless, backbones that significantly differ from biopolymers (i.e., abiotic backbones) still escape this approach, and their preparation rests solely on stepwise synthetic chemistry. In this respect, great achievements have been reported in the chemical synthesis of large peptidic chains (>300 amino acids)<sup>5</sup> through the combination of solid-phase synthesis and native chemical ligation methods.<sup>6</sup> Related strategies are being applied to oligonucleotide synthesis.<sup>7</sup>

Given this background, we have been seeking viable chemical approaches to access to abiotic folded molecular strands significantly longer than currently available. In the following

report, we describe the gram-scale preparation of quinoline-based helical aromatic oligoamide foldamers up to 25 kDa via a segment-doubling approach. We show that pure reagents, high reaction concentration, and long reaction times allow us to carry out high-yielding iterative couplings between long folded segments.

Helical aromatic oligoamide foldamers are an increasingly popular class of molecules possessing easy-to-predict and remarkably stable conformations.<sup>8</sup> As a drawback, conformational stability has been thought to result in synthetic difficulties. Helical folding occurs even for short sequences in all types of solvents,<sup>9</sup> causing steric hindrance at terminal main-chain reactive functions. This, together with the intrinsically poor nucleophilicity of some aromatic amines, has often been observed to result in low coupling yields and difficult to purify reaction mixtures, precluding the routine synthesis of long sequences<sup>10</sup> despite the use of strong activation of the terminal carboxylic groups as acid chloride.

Strategies to circumvent these difficulties have been proposed. For example, the insertion of removable alkoxybenzyl substituents resulted in the formation of tertiary amides that locally disrupt folding and alleviate steric hindrance during synthesis<sup>10</sup> and also prevent aggregation.<sup>11</sup> Alternatively, including some reactive monomers bearing, e.g., aliphatic instead of aromatic amines, allowed the use of standard peptide coupling agents, and gave easy access to large foldamers that retained their compact

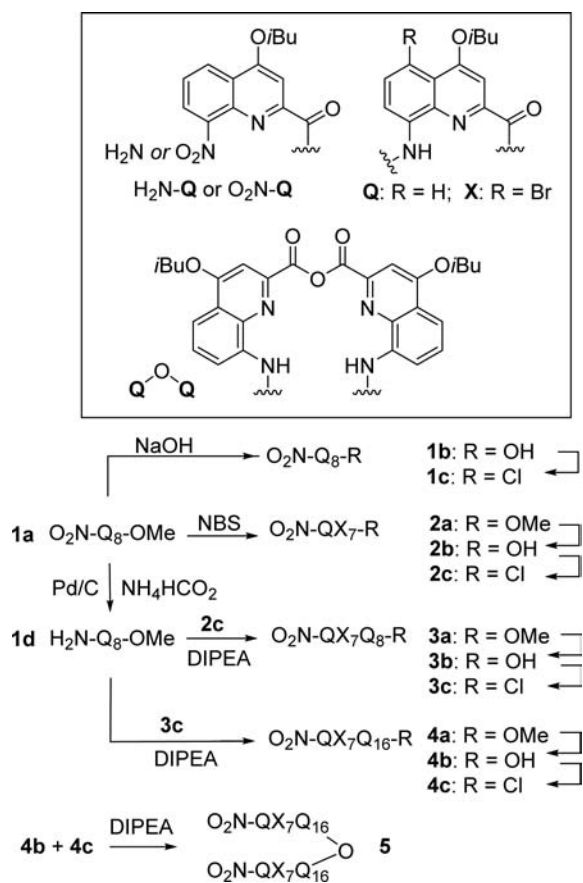
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structure despite the flexibility imparted by the aliphatic linkages, the aromatic units dictating the overall folding behavior.<sup>2e,g,12</sup> As shown in the following, the recourse to these strategies may be avoided as direct couplings between large folded segments can be carried out directly and efficiently giving access to large and well-defined abiotic foldamers.

We recently reported the chromatography-free, large-scale (i.e., 10 g) synthesis of octameric strand **1a** (Scheme 1).<sup>13</sup> This

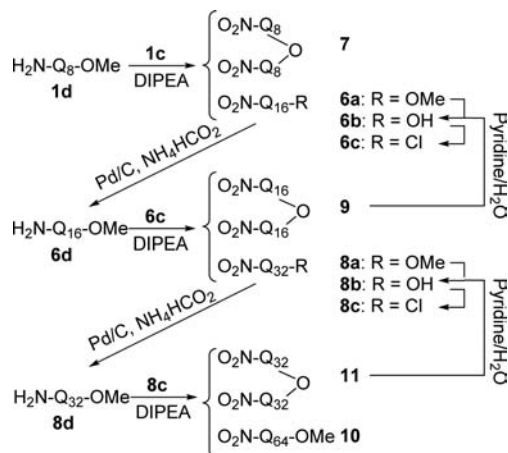
**Scheme 1. Incremental Synthesis of Bromo-Substituted Oligomers**



compound has been shown previously to fold in a helix spanning over three turns with a pitch of 3.5 Å that was characterized both in the solid state<sup>14</sup> and in solution.<sup>15</sup> The available quantities of **1a** prompted us to use this octamer as a starting material and to attempt the synthesis of much longer oligomers. The saponification of the terminal methyl ester to give **1b** and the palladium-catalyzed hydrogenation of the N-terminal nitro group with ammonium formate ( $\text{NH}_4\text{HCO}_2$ ) to give amine **1d** both require harsher conditions than for a monomer, typically longer reaction time, higher reagent concentration or higher temperatures, due to the hindrance of these groups associated with folding. Activation of **1b** with oxalyl chloride to give acid chloride **1c** proceeded quantitatively as judged by  $^1\text{H}$  NMR spectra. The coupling of **1c** and **1d** was relatively slow unless concentrations in the 150 mM range were used which, due to the high molecular weight of the reagents (approximately 2 kDa), translated in ca. 0.3 g mL<sup>-1</sup> and required sufficiently high solubility. Under such conditions, this reaction yielded a mixture containing the expected 16mer **6a**, and variable amounts of anhydride **7** and of remaining amine **1d** (Scheme 2). The

separation of **1d**, **6a** and **7** by chromatographic methods proved to be extremely delicate, if feasible at all, and in any case incompatible with large scales.

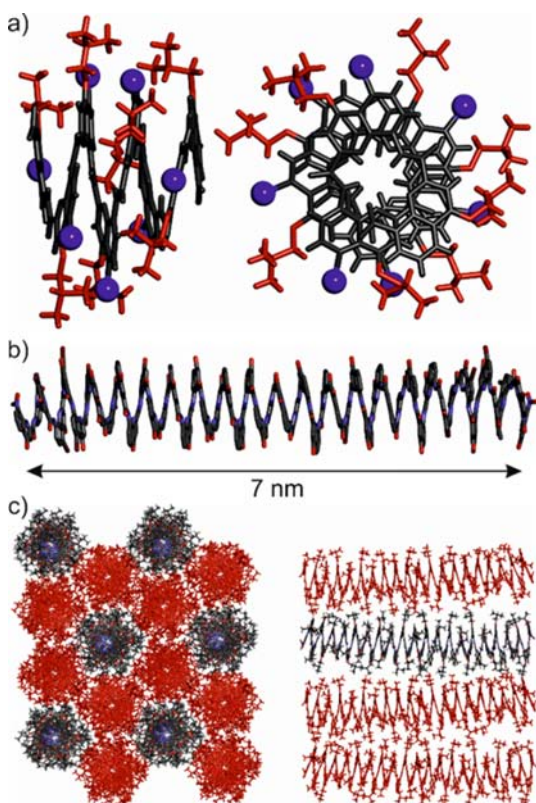
**Scheme 2. Segment-Doubling Synthesis of Long Bromine-Free Oligomers**



The formation of anhydrides such as **7** reflects the greater reactivity of acid **1b** as compared to amine **1d** toward acid chloride **1c** in the presence of base. Because of their folded structure, these anhydrides are particularly stable and do not react readily with water or methanol.<sup>16</sup> Multiple attempts showed that the anhydride constitute a recurrent byproduct: rigorously anhydrous conditions that would prevent acid chloride hydrolysis and anhydride formation were rarely achieved. As shown below, these difficulties were eventually overcome in two distinct ways.

In a first approach, we exploited the efficient conversion of octamer **1a** into its heptabromo analogue **2a** (Scheme 1). We previously reported the regioselective monobromination of **1a**.<sup>17</sup> Upon prolonged exposure to excess *N*-bromosuccinimide, seven bromines are quantitatively introduced in position 5 of the seven C-terminal quinolines as demonstrated by NMR and mass spectrometry (Figures S5 and S6). A hexabromomono-chloro analogue of **2a** was the only byproduct detected in trace amounts by MS, presumably resulting from interference with the chlorinated solvent. Compounds **1a** and **2a** possess markedly different retention coefficients ( $R_f$ ) on silica gel (0.1 and 0.8, respectively, in dichloromethane/cyclohexane 4:1, vol/vol). This was eventually exploited in the purification of longer oligomers. Thus, when acid chloride **2c** was reacted with amine **1d**, a mixture of hexadecamer **3a**, the anhydride of acid **2b**, and remaining **1d** was obtained. These compounds bear 7, 14, and 0 bromine atoms, respectively, resulting in  $R_f$  differences that made their separation by chromatographic methods relatively easy. Hexadecamer **3a** was thus obtained in pure form in 80% yield.

The bromine substituents also have the merit of making crystallogenes easier. In the case of **2a** and **5** (see below), this allowed validation of the structure assignment and helical conformation in the solid state by single crystal X-ray diffraction analysis (Figure 1). However, in most cases, these crystals were fragile and rarely allowed the collection of high quality data sets. Partial occupancy factors of bromine atoms were observed in structures solved from these poor quality data, hinting at a decomposition of carbon–bromine bonds under X-ray irradiation. Thus, in most cases, X-ray structures were not refined.



**Figure 1.** Crystal structures of octamer **2a** (a, side and top views) and 48mer anhydride **5** (b, c) shown as sticks. Included solvent molecules have been removed for clarity. (a) Bromine atoms are shown as blue spheres. (b) Isobutoxy side chains have been removed for clarity. (c) Pseudohexagonal packing of **5**.

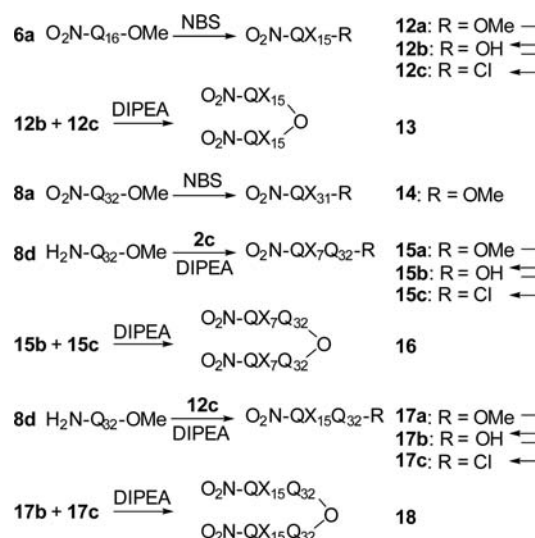
We did not look for conditions that would allow the reduction of the nitro group of **3a** in the presence of its seven bromine atoms. Instead, we carried out its saponification into **3b** which was in turn activated and coupled to **1d** to yield 24mer **4a**. Again, the purification of **4a** by chromatography on silica gel caused no particular difficulty. The process was iterated once more with saponification of **4a** to **4b**. The reaction of **4b** and **4c** led to the isolation of anhydride **5** which comprises 48 quinoline monomers (FW = 11.7 kDa). The structure of **5** in the solid state could be solved by crystallographic analysis (Figure 1). As for **2a**, the helical structure is consistent with those of shorter sequences in terms of helix pitch (3.5 Å).<sup>14,15</sup> The bromine atoms appear to slightly influence the number of units per turn which does not exactly match with the value (2.5) observed without bromines.<sup>2e,14</sup> The 48 monomers of **5** eventually fold into a 7 nm long helix spanning 19 turns and represents the largest single molecular structure deposited in the Cambridge Crystallographic Data Centre. The high aspect ratio of the helices was found to result in a pseudohexagonal packing.

This approach allowed the incremental elongation of bromo-substituted oligomers via iterative couplings to octamer amine **1d**. To implement a more convergent and higher yielding synthesis, we sought for conditions to hydrolyze anhydride byproducts and eventually recycle the recovered carboxylic acids. We found that clean hydrolysis of the helically folded anhydrides could be achieved upon refluxing in a pyridine–water mixture. This treatment was thus included in the workup of coupling reactions. In addition, in order to avoid difficult chromatographic separation between the coupling products and the starting

unreacted amine, we included a control by <sup>1</sup>H NMR of the presence of remaining amine and its elimination by adding excess acid chloride until it had completely disappeared. This procedure afforded hexadecamer **6a** (Scheme 2) in 87% isolated yield on a ca. 6 g scale following a relatively easy purification as the main byproduct was the starting acid **1b** which is readily removed and recycled by chromatographic purification on silica gel (Figure S1). The procedure was efficiently iterated. Hexadecamer amine **6d** and acid chloride **6c** were coupled to yield 2 g of 32mer **8a** (92% yield, Figure S2). However, the coupling of two 32mers into 64mer **10** met a limitation as this compound was poorly soluble, allowing the chromatographic purification of a few milligrams at a time (Figures S3 and S4). Nevertheless, no barrier was found in the coupling reactions themselves provided that acid chloride and amine were of high purity, concentration was sufficiently high, and reaction time was sufficiently long.

The syntheses described above were expanded to a number of other derivatives (Scheme 3). Quite remarkably, hexadecamer **6a**

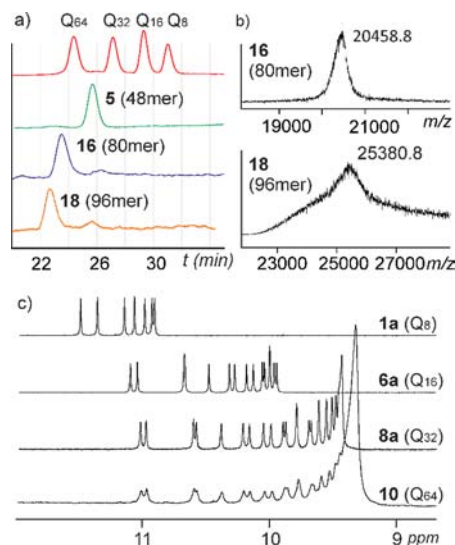
**Scheme 3**



underwent a quantitative pentadecabromination to produce **12a**. In principle, this reaction allows the formation of exactly 32766 distinct partially brominated intermediates<sup>18</sup> which eventually all lead to the same pentadecabrominated product. The NMR spectra of the intermediate crude mixtures contain so many species that no clear set of signals can be distinguished. Instead broad lines are observed. But these eventually resolve into one set of sharp signals upon completion of the reaction (Figure S7). Mass spectrometry confirmed completion of the reaction (Figure S8). Yet, bromination appears to occur with some selectivity. Indeed, when NBS was added sequentially to octamer **1a**, the distribution of product was not as broad as expected for a random bromination (Figure S5), suggesting that remote substituent effects operate as shown previously for the first and second brominations.<sup>17</sup> The introduction of 31 bromine atoms on 32mer **8a** involves 2,147,483,646 distinct theoretical intermediates.<sup>18</sup> The reaction was also eventually completed after 80 days (Figure S11) but was not as clean as for **1a** and **6a**, as some chlorinated products were also observed (Figure S9). The reaction was also impractically long. Finally, the bromine substituents of 40mer **15a** and 48mer **17a** endowed these compounds with good solubility, allowing their saponification and conversion into anhydrides **16** (20.5 kDa) and **18** (25.7



kDa), composed of 80 and 96 quinoline units, respectively. The acceptable purity of the compounds was supported by sharp NMR spectra and GPC profiles (Figure 2) as well as mass



**Figure 2.** Representative examples of GPC traces (a), MALDI mass spectra (b), and excerpts of  $^1\text{H}$  NMR spectra (amide resonances) of long helical aromatic amide foldamers.

spectrometry. Chemical shift values were shown to depend on length even for very long structures. For example, the main peak of central, most-shielded amide protons is found at 9.45 ppm in 32mer **8a** and at 9.33 ppm in 64mer **10**. This reflects the addition of multiple weak (and generally considered short-range) ring currents effects over long distances due to the high structural organization of the helices in solution.

In conclusion, we have shown that a segment-doubling strategy provides a viable preparative-scale synthetic approach to helically folded oligoamides having a size well above that currently met in the field. The length of these oligomers is typical of polymerization reaction products, yet they possess an exact number of monomers and thus have no molecular weight distribution. These results bode well for the chemical synthesis of more complex structures composed of distinct secondary folded motifs. An intriguing question associated with these syntheses is whether any enantioselectivity exists when coupling helices have either *P* or *M* handedness as in the racemic mixtures handled here.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00165.

Experimental procedures, full characterization of new compounds, and crystallographic data (PDF)  
X-ray data for compound **2a** (CIF)  
X-ray data for compound **5** (CIF)

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## Notes

The authors declare no competing financial interest.

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