

# Self-Association of Aromatic Oligoamide Foldamers into Double Helices in Water

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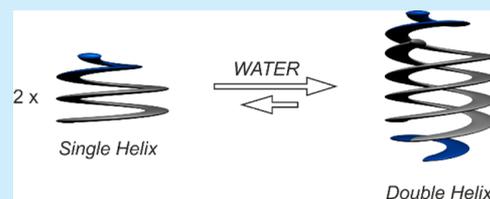
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## Supporting Information

**ABSTRACT:** Heteromeric oligoamide foldamers composed of 8-amino-2-quinolinecarboxylic acid and 7-amino-8-fluoro-2-quinolinecarboxylic acid bearing cationic water-solubilizing side chains were prepared using solid-phase synthesis (SPS). The sequences were designed to adopt a single- or a double-helical motif depending on the nature of the solvent, DMSO or water, respectively. Self-association was demonstrated by NMR and mass spectrometry. Dimerization in water was found to be much stronger than observed previously in organic solvents for analogous oligoamide sequences.

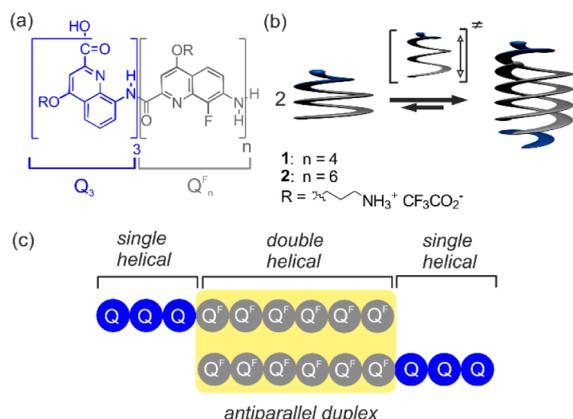


The self-aggregation of organic oligomeric or polymeric molecular strands into multiple helices is a commonly observed phenomenon in nature.<sup>1</sup> Chemists have mimicked this process in a variety of systems and shown that artificial organic oligomers are also able to associate into multistranded structures using original recognition motifs (a vast array of helicates involving metal coordination also exist and are not being considered here). As prime examples, multiple helical structures have been evidenced in oligoamides composed of pyridine,<sup>2</sup> chloroaromatic or fluoroaromatic,<sup>3</sup> or naphthyridine<sup>4</sup> rings; in oligoresorcinols;<sup>5</sup> in ethynylhelixene oligomers;<sup>6</sup> in *m*-terphenyl backbone oligomers exploiting amidinium carboxylate salt bridges;<sup>7</sup> and in alternate sequences of aromatic hydrogen-bond donors and acceptors.<sup>8</sup> However, most of these multistranded structures only form in organic solvents. The transposition of such architectures into water is far less common, and no family of organic double helices has been reported to form in essentially any solvent. To our knowledge, apart from the notable exception of Yashima's oligoresorcinols,<sup>5,9</sup> no other example of double helix association in water based on non-natural motifs has been described so far. In this context, worth mentioning also are Iverson's aedamers which represent an original class of aromatic oligomers able to fold and aggregate as discrete heteroduplexes in water, though they do not possess a helical architecture.<sup>10</sup> The latter two systems both rely extensively on aromatic–aromatic stacking interactions between (self)-complementary oligomeric strands. In line with this background, we now report the association of quinoline oligoamide strands into remarkably stable antiparallel helical duplexes in water.

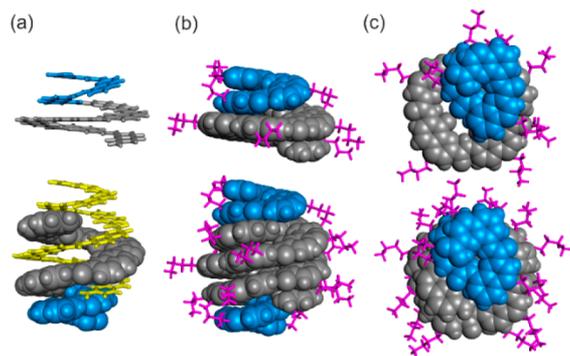
The prospect of associating helical aromatic oligoamide foldamers in water potentially brings concerns about their solubility and about their ability to remain folded in this medium.<sup>11</sup> However, it has recently been shown that, in spite of their aromatic nature, they can be easily solubilized in water provided polar side chains diverge from the structure.<sup>12</sup> Moreover, it was demonstrated that their conformational stability can in fact be dramatically enhanced in this solvent, presumably due to the strength of hydrophobic effects.<sup>13</sup> We thus speculated that the introduction of ornithine-like side chains at the periphery of a sequence prone to double helix formation in organic solvents might lead to self-association in water. Inspired by a previous design,<sup>14</sup> we targeted oligoamide sequences comprised of both single- and double-helical segments so as to promote exclusively antiparallel duplex formation (Figure 1). Specifically, a quinoline trimer ( $Q_3$ ) at the C-terminus codes for a short single-helical segment that has no propensity to associate. In contrast, the fluoroquinoline segment ( $Q^F_n$ ) at the N-terminus was expected to self-associate, as previously shown in organic solvents, but only in an antiparallel fashion.<sup>14</sup> This is because the  $Q_3$  segment serves to prevent parallel duplex formation which would require severe steric hindrance or disfavored association to occur at one end. Energy-minimized molecular models indicated a helix pitch of 3.5 Å in single-helical monomeric **1**, whereas the models showed the helix pitch to be 3.5 and 7 Å for the  $Q_3$  and  $Q^F_4$  segments of (**1**)<sub>2</sub>, respectively (Figure 2). In the modeled duplex, interstrand

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**Figure 1.** (a) Formula of **1** and **2**; (b) schematic representation of a single-helix–double-helix equilibrium requiring springlike extension; (c) simplified representation of double helix ( $2$ )<sub>2</sub>. Quinolines are denoted in blue, whereas fluoroquinolines are shown in light gray.

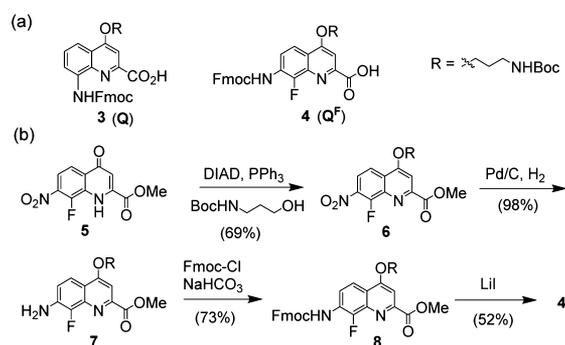


**Figure 2.** Side (a,b) and top (c) views of energy-minimized models of single helical **2** and double helical ( $2$ )<sub>2</sub>. Aromatic backbones are color coded in blue (quinoline) and gray (fluoroquinoline). In (a), one strand of ( $2$ )<sub>2</sub> is shown in a yellow tube representation. Side chains were omitted for clarity. In (b) and (c), **2** and ( $2$ )<sub>2</sub> are shown as CPK models. Side chains are shown as purple tubes.

contacts consist mainly of face-to-face aromatic stacking of the  $Q^F$  units. The aromatic surface exposed to solvent was found to be strongly reduced in the duplex compared to the single helix, which potentially represents a driving force in a solvophobic context (see the Supporting Information). Specifically, the calculated solvent accessible surface for double-helical ( $2$ )<sub>2</sub> and single-helical **2** were found to be 1655 and 1021 Å<sup>2</sup>, respectively. Thus, one can estimate that a double helix ( $2$ )<sub>2</sub> exposes to the solvent only about 80% of the surface exposed by two individual single helices.

We opted for the solid-phase synthesis (SPS) of oligomers **1** and **2**.<sup>15</sup> SPS brings the benefits of speed, and in the present instance, elongation from the surface of resin beads also advantageously prevents duplex formation from occurring during synthetic steps. Indeed, our experience is that the formation of stable duplexes in solution seriously hampers strand elongation reaction. The TFA-labile Wang resin was selected as it offered the possibility to synthesize oligomers using an Fmoc-type strategy.<sup>15a</sup> For this purpose, we needed to prepare both the previously described Fmoc-protected 8-amino-2-quinolinecarboxylic acid monomer bearing a Boc-protected aminopropoxy side chain **3** and the new Fmoc-protected 7-amino-8-fluoro-2-quinolinecarboxylic acid monomer **4**, the synthesis of which is shown in Scheme 1. Starting from

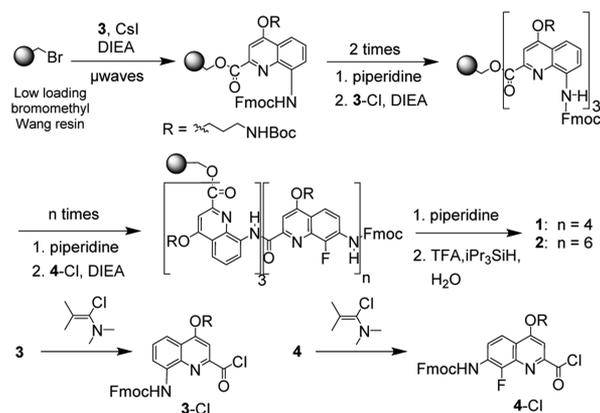
### Scheme 1. Formulas and Synthesis of Fmoc-quinoline Derivatives **3** and **4**



quinolone **5**, Boc-protected aminopropanol was introduced under Mitsunobu conditions (69%). The nitro group was then reduced via catalytic hydrogenation with Pd/C. The resulting amine was protected using Fmoc-Cl and the Fmoc quinoline acid derivative **4** subsequently obtained in moderate yield (52%) through the cleavage of its methyl ester using a LiI-mediated nucleophilic dealkylation. High purity of this material is essential for successful SPS.

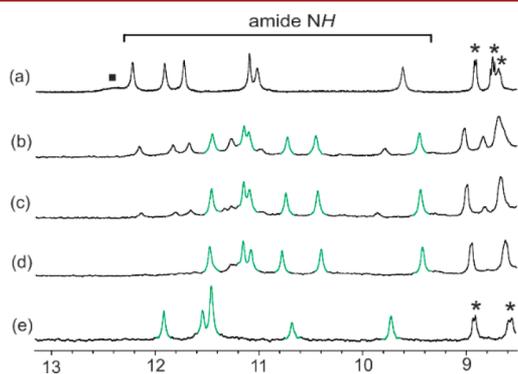
Consistent with the structures of **1** and **2** and to exploit our previous experience with SPS of quinoline oligoamides,<sup>15a</sup> we decided to elongate the oligomer from a quinoline C-terminus ( $Q$ ) to a fluoroquinoline N-terminus ( $Q^F$ ) (Scheme 2).

### Scheme 2. Solid-Phase Synthesis (SPS) of **1** and **2**



However, the reverse sequence where synthesis would start with the ( $Q^F$ )<sub>n</sub> segment and the N-terminus would be at the end of the  $Q_3$  segment may have also been envisaged. Quinoline acid monomer **3** was introduced onto low loading (0.38 mmol g<sup>-1</sup>) Wang bromide resin in the presence of cesium iodide under microwave heating. Fmoc deprotection was carried out at room temperature by treatment with piperidine, and the second  $Q$  unit was incorporated via the acid chloride  $3\text{-Cl}$  under microwave heating (Scheme 2). Another deprotection–coupling sequence was performed to add a third quinoline to the resin. Fluoroquinoline monomers were then introduced successively using a similar deprotection–coupling protocol via the acid chloride  $4\text{-Cl}$  (Scheme 2). Concomitant side-chain deprotection and cleavage from the resin was carried out in 95:2.5:2.5 TFA/ $i\text{-Pr}_3\text{SiH}$ / $\text{H}_2\text{O}$  (vol/vol/vol). The water soluble oligomers **1** and **2** were then purified by reversed-phase HPLC prior to physicochemical studies. Purification was difficult in the case of **2**.

Oligomers **1** and **2** were designed to be soluble both in polar organic solvents and pure (i.e., nonbuffered) water. The aggregation behavior of **1** was initially studied in DMSO- $d_6$ , a solvent known to disfavor the aggregation of aromatic oligoamides. In this solvent, a single set of signals was observed at 2 mM and assigned to a single helix (Figure 3a). Upon



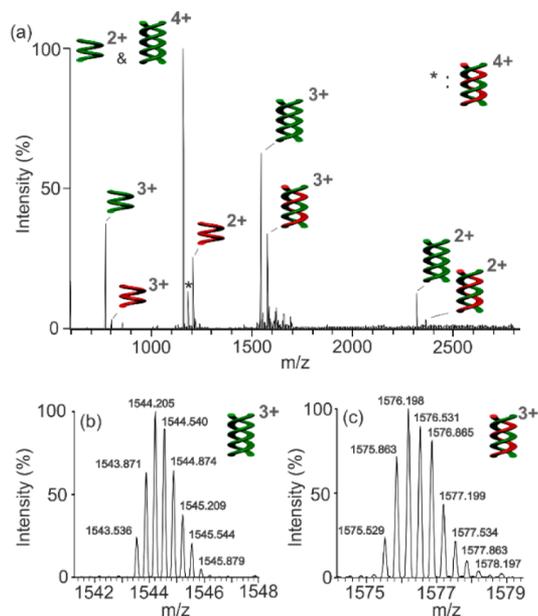
**Figure 3.** Excerpts of the  $^1\text{H}$  NMR (400 MHz) titration at 298 K of **1** (2 mM) in DMSO- $d_6$ /H $_2$ O mixtures in the following vol/vol ratios: (a) 100:0; (b) 80:20; (c) 76:24; (d) 70:30. (e)  $^1\text{H}$  NMR (400 MHz) spectrum of (**1**) $_2$  in water (H $_2$ O/D $_2$ O 90:10 vol/vol). Black and green signals stand for single and double helix, respectively. Some aromatic resonances are denoted with stars. The signal of the carboxylic acid is marked with a black square.

increasing the water content, a new set of signals appeared at lower field (Figure 3b–d, green) which is characteristic of duplex formation.<sup>2</sup> This observation was consistent with a slow exchange between **1** and (**1**) $_2$  on the NMR time scale. Similar upfield shifts of amide signals have been observed in organic solvents and represent a typical signature of ring current effects arising from extended intermolecular  $\pi$ – $\pi$  stacking within double-helical aggregates. Although a full structural elucidation of (**1**) $_2$  was not carried out, its assignment to a double-helical structure such as that shown in Figure 2 could be inferred by analogy to related sequences in organic solvents.<sup>14</sup> Importantly, in agreement with our design to favor antiparallel duplexes, a single set of signals was observed for the aggregate. In addition, the multiplicity of these signals indicated that the molecules of **1** involved in the aggregate are magnetically equivalent. Given the previous characterization of related antiparallel duplexes in organic solvents,<sup>3a</sup> and in agreement with molecular models (see above), these data make it very likely that an antiparallel duplex also prevails in water.

To assess its dimerization strength, **1** was dissolved in DMSO- $d_6$  containing varying proportions of water. Upon increasing the water content from 0 to 30% vol/vol, the signals of single helical **1** were progressively replaced by peaks corresponding to the double helix (**1**) $_2$ . The dimerization constant could be calculated through the integration of the signals of the two species at a total concentration of 2 mM and was found to be  $10^3 \text{ L mol}^{-1}$  in a H $_2$ O/DMSO- $d_6$  20:80 (v/v) mixture. A slight increase of this ratio (26:74 vol/vol) led to an increase of the  $K_{\text{dim}}$  by 2 orders of magnitude ( $10^5 \text{ L mol}^{-1}$ ). Above 30% of water in DMSO oligomer **1** proved to exist solely as a double helix as judged by the unique set of resonances by  $^1\text{H}$  NMR (Figure 3d). The dimerization constant of **1** in pure water was too high to be measured by NMR. Dimerization was also observed upon adding a 20 mM aqueous solution of D $_{11}$ -Tris buffer at pH 6.2. In comparison, an organic soluble (Q $^F$ ) $_4$  analogue was

previously shown to only weakly aggregate in chloroform.<sup>3a</sup> Double helix formation of aromatic oligoamides thus appears to be strongly enhanced in water, possibly due to the same factors which also enhance single helix stability,<sup>13</sup> i.e., hydrophobic effects.

The self-association of longer oligomer **2** was investigated by electrospray mass spectrometry (ESI-MS). This method allows us to unambiguously determine the stoichiometry of supramolecular aggregates.<sup>16</sup> ESI-MS measurements of **2** were performed in water. The oligomer appeared as multiple positively charged species, corresponding to the mass of the single and double helices (Figure 4a, green helices). Monomeric



**Figure 4.** (a) Electrospray ionization mass spectrum (ESI-MS) of a solution of 20  $\mu\text{M}$  of oligomer **2** in water. The homoduplex of **2** (green helices) and heteroduplex of **2**–COCF $_3$  (red helices) are detected. Excerpts of the high resolution ESI mass spectrum of: (b) the homoduplex (**2**) $_2$  and (c) the heteroduplex **2**–**2**–COCF $_3$ .

**2** was detected at  $m/z = 772.6$ , 1158.4 (3+ and 2+ species, respectively), and the double helix (**2**) $_2$  was detected at  $m/z = 1158.4$ , 1544.2, 2315.8 (4+, 3+, and 2+ species, respectively). A closer examination of the mass spectrum allowed us to identify a molecular adduct of **2**, the mass of which was larger than that of **2** by 95.97 mass units (red helices). This adduct presumably corresponds to a trifluoroacetylation (2-COCF $_3$ ) which likely occurs at the N-terminal Q $^F$  aromatic amine function during TFA-mediated resin cleavage as a result of incomplete protonation of this amine. As a consequence of the presence of this species, the mass spectrum also shows peaks corresponding to a cross-hybrid between **2** and 2-COCF $_3$ , a species that can be regarded as additional evidence of the strong aggregation propensity of these oligomers into duplexes in water.

In summary, we have shown that water permits and greatly enhances aromatic oligoamide foldamers double helix formation. This family of double helices appear to be the only one that can form in virtually any solvent. SPS was shown to give rapid and easy access to oligomers comprised of different aromatic amide units and able to associate strongly in aqueous media. This may make water-soluble (Q $^F$ ) $_n$  oligomers become a convenient “molecular glue” for the noncovalent conjugation of

biomolecules or for use in biomaterials. In this respect, cross-hybridization of distinct sequences could prove particularly useful.<sup>3b</sup>

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental procedures and compound characterization data including NMR spectra and relevant HPLC chromatograms. 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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