Helical Structures

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Parallel and Antiparallel Triple Helices of Naphthyridine Oligoamides**

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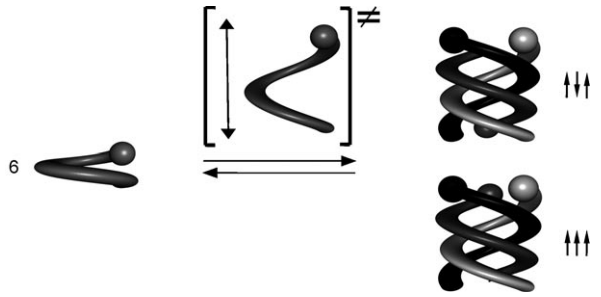
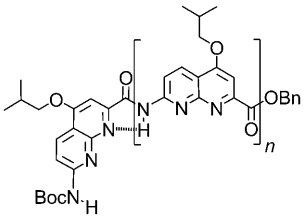
The hybridization of organic strands into double helices is a common structural pattern of biopolymers. In recent years, a number of synthetic oligomers and polymers have also been reported to form stable double helices[1] following original strand–strand recognition motifs as, for example: aromatic oligoamides based on pyridine[2] or fluoroquinoline rings;[3] oligoresorcinols;[4] ethynylhelicene oligomers;[5] *m*-terphenyl backbone oligomers exploiting amidinium–carboxylate salt bridges;[6] and alternate sequences of aromatic hydrogen-bond donors and acceptors.[7] However, the occurrence of triple helices formed from the direct recognition of three organic strands is much less common.[8] Natural triple helices include those of collagen,[9] nucleic acids,[10] and some polysaccharides,[11] but aside from nucleic acid analogues (e.g. PNA),[12] artificial triple helices have not been described to date. Herein, we report the serendipitous discovery of stable parallel and antiparallel triple helices formed by 1,8-naphthyridine oligoamides (Scheme 1). We found that these oligomers can spontaneously assemble in triply stranded structures in solution at the exclusion of any other species and thus emerge as a robust and unprecedented triplex motif.

We and others have embarked on a systematic program aiming at exploring the structures and functions of folded aromatic oligoamides[1,13] driven by the hypothesis that the chemical space offered by these foldamers may be as vast as that of their aliphatic counterparts, namely α-peptides and their homologues. We have reported on sequences comprised of pyridine,[2] quinoline,[14] 8-fluoroquinoline,[3] and pyrido-quinoline[15] monomers, as well as combinations thereof,[15,16] and their ability to form a variety of single-helical and double-helical architectures. Our interest for 1,8-naphthyridine stemmed from its ability to serve as a hydrogen-bonding unit in molecular recognition and large self-assemblies,[17] and as a potential precursor of hollow helices.[18] We thus designed and prepared tetrameric oligomer **1**, which is composed of



Scheme 1. Hybridization of three single-helical strands into a triple helix through a springlike extension. Triple helices may adopt parallel (bottom right) or antiparallel (top right) configurations. The balls at the end of each strand discriminate the strand ends and symbolize the strand polarity.



1: $n = 3$

2: $n = 1$

four 2-amino-5-isobutoxy-1,8-naphthyridine-7-carboxylic acid units. The synthesis of amidonaphthyridine derivatives is notoriously tedious owing to the poor solubility and weak reactivity of aminonaphthyridine precursors as well as the relative instability of amide functions.[19] As shown in the Supporting Information, these difficulties could be overcome, and a new protected naphthyridine amino acid monomer was prepared in four steps from 2,6-diaminopyridine on a 30 g scale without any chromatographic purification (Scheme S1). Key synthetic steps consisted of the addition of an amino-pyridine onto dimethylacetylene dicarboxylate, thermal cyclization of the resulting fumarate into a naphthyridone, and the introduction of the isobutoxy side chains under Mitsunobu conditions. Isobutoxy groups provided excellent solubility of the monomers and oligomers in organic media as well as high crystallinity. Amine and acid functions were protected as *tert*-butyl carbamate and benzyl esters, respectively. Oligomer synthesis was performed through selective deprotections and couplings using coupling agents (e.g. 1-benzotriazolyloxytris(pyrollidino)phosphonium hexafluorophosphate) to yield naphthyridine tetramer **1**.

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The new naphthyridine oligomer was expected to adopt a helical conformation stabilized by conjugation between amide and aryl units, intramolecular hydrogen bonding, and electrostatic repulsions between amide oxygen and endocyclic nitrogen atoms, as for other aromatic oligoamide foldamers.[13] Additionally, the helix cavity in **1** was expected to be larger than of the related 8-fluoroquinoline oligoamides,[3] whose cavities are partly filled with fluorine atoms. These predictions were validated by the solid-state structure of **1** obtained by X-ray diffraction analysis of single crystals grown from a pyridine/hexane mixture. In this structure, tetramer **1** adopts a single-helical conformation spanning just over one turn and with a helix pitch of 3.5 Å (Figure 1a). The helix cavity has a diameter of 5 Å and accommodates an included pyridine solvent molecule (not shown). The single-helical conformation also appears to prevail in solution in pyridine, a solvent known to disfavor hybridization of aromatic oligoamides.[20] The presence of a single set of sharp ^{1}H NMR signals, the low-field resonance of amide protons ($\delta > 12$ ppm; Figure 2b), and the absence of a diastereotopic motif of the side chains and benzylic methylene protons are consistent with a single helix that rapidly inverts its handedness.[21] Cooling the sample or increasing the concentration (Figure S1 and S2) only led to slight variations in chemical shift, suggesting a poor ability of **1** to aggregate in this solvent.

Two different crystal types of **1** were also obtained in two separate batches of a chloroform/pyridine/hexane ternary solvent mixture. Crystallographic analysis revealed two distinct and unprecedented triple-helical structures. The first triplex displays a parallel orientation of the three strands (Figure 1b); three *tert*-butyloxycarbonyl groups protrude at one extremity and the three benzyl ester groups at the other. This triplex has a perfect C_3 symmetry axis coinciding with the helix axis. In the second triplex, one strand is oriented antiparallel to the two others, and the helix has no symmetry element. The two structures are almost superimposable except for the one strand that has inverted its orientation. Interstrand interactions consist of extensive π–π contacts between both sides of each strand with its neighbors. Triplex formation requires a springlike extension of each strand from its single-helical conformation to reach a triple helical pitch of around 10.5 Å. This extension is accommodated by an increase of the twist angle between adjacent naphthyridine rings in each strand up to an average 26° (see Table S1 in the Supporting Information). The triplex cavities are slightly narrower (4.3 Å) than that of the single helix as a result of the springlike extension.

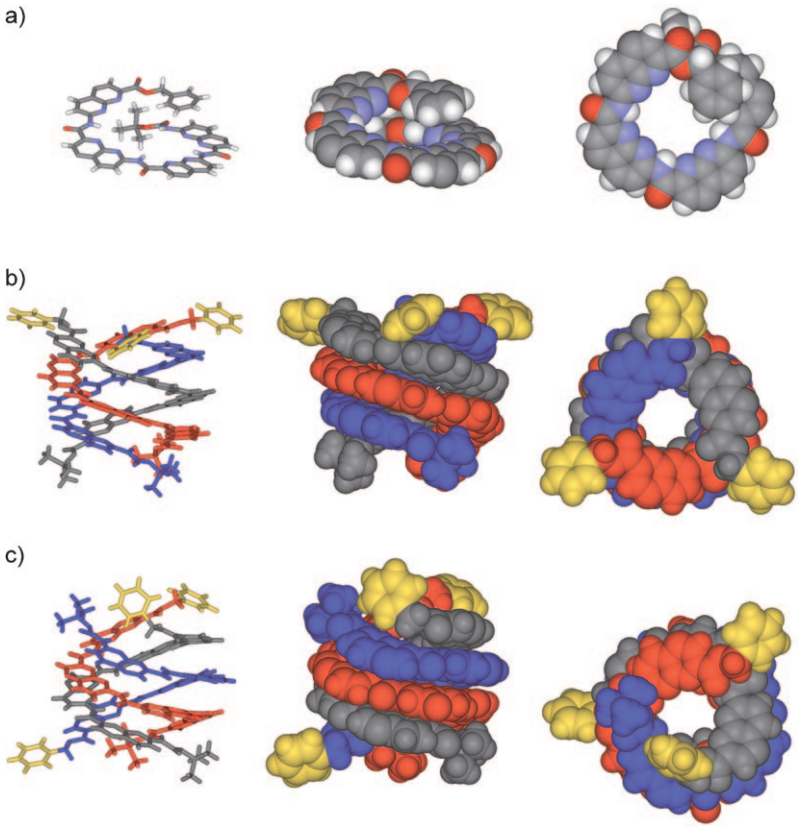

Figure 1. Side views and top views in cylindrical and CPK representations of crystal structures of **1** determined by X-ray diffraction of three independent crystals: a) **1** as a single helix; b) $(\mathbf{1})_3$ as parallel triple helix; c) $(\mathbf{1})_3$ as an antiparallel triple helix. Atoms in (a) are color coded as follows: carbon gray, hydrogen white, nitrogen blue, oxygen red. In (b) and (c), each strand of the triplex is color coded in red, blue, and gray. The terminal phenyl groups are shown in gold to highlight the relative orientation of the strands in the triplexes. Included solvent molecules and isobutoxy residues are omitted for clarity.

Solution studies carried out in $CDCl_3$ and CD_3CN supported the prevalence of a mixture of the parallel and antiparallel triplexes in both media, at the exclusion of any other species (Figure 2c,d). In particular, no duplex was found, unlike in many other aromatic oligoamides.[2,3] Evidence in support of this conclusion is as follows: 1) ESI mass spectra showed the almost exclusive presence of a trimer $(\mathbf{1})_3$ (Figure S7). 2) ^{1}H NMR spectra feature two sets of signals corresponding to one species having a single strand as its smallest asymmetric unit (three distinct amide resonances), and to another species having three strands as its smallest asymmetric unit (nine distinct amide resonances). This latter pattern strongly supports the presence of the antiparallel triplex, whereas the former might arise from a variety of species. 3) NMR spectra measured in $CD_3CN/CDCl_3$ mixtures show chemical shift variations and reveal that the two species with high and low symmetry are the same in the two solvents (Figure S6). 4) ^{1}H DOSY experiments of **1** in CD_3CN show that the two sets of signals belong to species having the same diffusion coefficient, that is, the same size (Figure S8). 5) Dilution studies showed no change between the proportion of the two species, suggesting identical molecularities. 6) Unlike in pyridine (Figure 2b), signals of the side-chain benzylic methylene protons in $CDCl_3$ and CD_3CN show diastereotopic patterns (Figure 2c,d).[21]

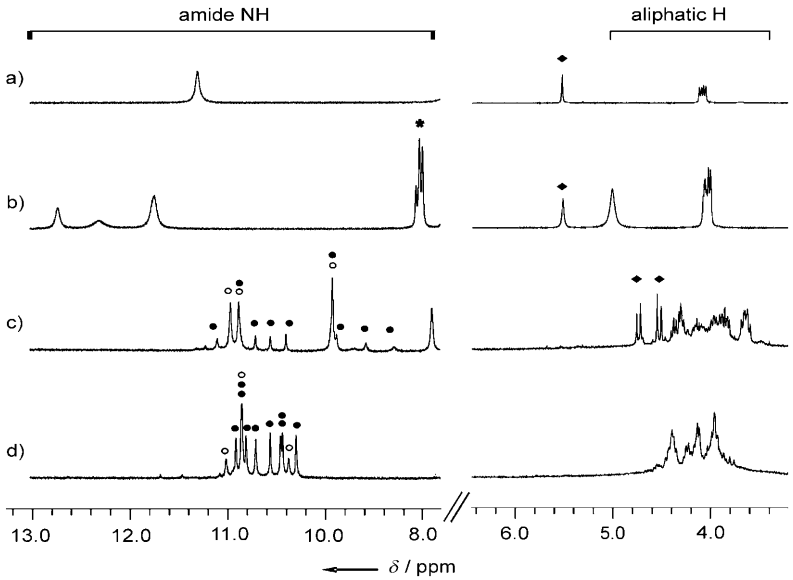

Figure 2. Excerpts of ^{1}H NMR spectra at 298 K of: a) **2** (5 mM) in $CDCl_3$; b) **1** (5 mM) in [D_5]pyridine; c) **1** (5 mM) in $CDCl_3$; d) **1** (5 mM) in CD_3CN. Signals of the triplex in parallel and antiparallel configurations are marked with open (○) and filled circles (●), respectively. Methylene protons of benzyl ester groups are indicated by diamonds (◆). The water peak in pyridine appears at around $\delta = 5.1$ ppm. A few aromatic signals are marked with a star.

Diluting CD_3CN or $CDCl_3$ solutions of **1** down to 0.1 mM did not give any sign of new lower molecularity species (e.g. single or double helices). These experiments provided a minimal estimate of the constant of formation of $(\mathbf{1})_3$ as $K_{trim} > 10^8\ L^2 mol^{-2}$. Considerable broadening of NMR signals occurred upon heating the solution or adding pyridine, suggesting a faster exchange of the two triplexes and presumably the emergence of single helices. In CD_3CN, the molar ratio between parallel and antiparallel triplexes is 15:85, reflecting a deviation from a statistical distribution (i.e., equal stability) of the two species, in favor of the antiparallel triplex. Interestingly, this ratio is almost reversed in chloroform (75:25), revealing a strong bias in favor of the parallel triplex.[22]

These findings bring up two questions. What factors favor the triplex structure of **1** to such a dramatic extent, and why are triplex structures rare in general and had never been observed in other aromatic oligoamide foldamers? It was demonstrated that the hybridization of multistranded helical oligoamides is an enthalpically driven process that reflects a balance between strong attractive interstrand π–π interactions and a very unfavorable springlike extension of single helical monomers to accommodate the other strand(s) (Scheme 1).[15b] It was also shown that the energy cost of springlike extension is much lower for helices with large diameters because it can be achieved with smaller twist angles of aryl–amide bonds than in helices with small diameters.[3,15] The emergence of $(\mathbf{1})_3$ is in agreement with these observations: a larger diameter permits an easy springlike extension and the reciprocal intercalation of not two but three strands. Nevertheless, oligomers related to **1** based on 8-fluoroquinoline did not form any triplex but only duplex and poorly stable quadruplex structures.[3] A potential factor that can make any triple helix a less likely architecture is that it requires at least two adjacent strands to adopt a parallel orientation (Scheme 1). Triple helices may thus not be favored by strongly polar sequences possessing a large macrodipole. We propose that the very structure of naphthyridine rings is crucial to triplex formation because, although naphthyridines possess a large dipole, these dipoles are oriented in planes perpendicular to the triple helix axis of **1** and thus do not contribute to the helix macrodipole. In contrast, 8-fluoroquinoline oligoamides contribute to the helix macrodipole and only antiparallel arrangements are observed in their multiple helices, de facto excluding triply stranded structures.

In summary, we have characterized a unique and robust artificial triple helix architecture. The principles governing these assemblies may allow the design of multistranded structures with even higher multiplicity resembling protein β-barrel architectures, for example, upon further increasing the monomer size and helix diameter.

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[20] See reference [15b] for another example. This behavior is presumably due to very favorable interactions between aromatic strands and the solvent, which competes with intermolecular stacking within duplexes.

[21] The *P* and *M* enantiomers of a multiple helix are expected to undergo slow exchange on the NMR timescale and thus result in diastereotopic motifs of the methylene protons. Handedness inversion is slower for a multiple helix because it can hardly occur without strand dissociation.

[22] It is unclear what factors come into play to stabilize the parallel triple helix in $CDCl_3$. Acetonitrile has a larger dipole moment than chloroform and might have been expected to stabilize the triple helix better. However, the C_3 symmetry of chloroform molecules may provide an interface more suited to solvate the C_3 symmetrical parallel triplex. Nevertheless, it should be pointed that only a small amount of energy is involved in shifting the proportions from 15:85 to 75:25.

