Aromatic Foldamer Helices as α-Helix Extended Surface Mimetics


Abstract: Helically folded aromatic oligoamide foldamers have a size and geometrical parameters very distinct from those of α-helices and are not obvious candidates for α-helix mimicry. Nevertheless, they offer multiple sites for attaching side chains. It was found that some arrays of side chains at the surface of an aromatic helix make it possible to mimic extended α-helical surfaces. Synthetic methods were developed to produce quinoline monomers suitably functionalized for solid phase synthesis. A dodecamer was prepared. Its crystal structure validated the initial design and showed helix bundling involving the α-helical-like interface. These results open up new uses of aromatic helices to recognize protein surfaces and to program helix bundling in water.

Protein–protein interactions (PPIs) often involve α-helices as key, if not the only, components of the binding interface. Great effort has thus been devoted to develop α-helix mimetics as PPI competitive inhibitors all the way to the clinic. This includes methods to enhance α-helix conformational stability through macrocycles involving main chain or side chain functionalities, the use of conformational templates and helix stabilizing monomers, or the design of peptidic analogues or homologues that adopt helical conformations more stable than α-helices of similar length. In those cases, side chains may be displayed on all faces of the helix. In contrast, other α-helix mimetics consist of more or less rigid linear scaffolds, for example, terphenyl, aromatic amides, oxopiperazines, pyrrololone-pyrrolidine, carrying proteinogenic side chains at distance intervals similar to those of an α-helix, typically following the i, i + 4, i + 7 arrangement. The functionalization of these scaffolds on opposite edges to include other side chain positions has also been proposed. All these mimics have distinct degrees of freedom and may not perfectly reproduce a desired side chain arrangement, yet they represent good starting points for rational design. Here, we introduce the concept that helical aromatic foldamers may provide original solutions to α-helix mimicry, even in the case of long helices.

Foldamers with aryl rings in their main chain may adopt stable helical conformations in solution. These helices feature a pitch of 3.5 Å and a diameter of at least 1.3 nm that do not relate to those of an α-helix. They can be decorated with proteinogenic side chains and may interact with protein surfaces but, at first sight, they appear to be unsuitable for α-helix mimicry (see Figure 1 in ref. [14]). Instead, some have been used as duplex B-DNA charge surface mimetics. Nevertheless, aromatic foldamers possess many sites for functionalization and thus for creating diverse side chain patterns.

Oligoamides of δ-peptidic 8-amino-2-quinolinocarboxylic acid Q adopt helical conformations with 2.5 units per turn (Figure 1a,b). Q helices have until now been built with low side chain density, mainly in position 4, occasionally in position 5, and not in position 6. Upon careful examination of the Q helix (Figure S1), we realized that a single side chain arrangement involving 4- and 6-functionalized quinoline rings potentially matches with that of the face of an α-helix. The actual curvature of α-helices may vary between the ideal 3.66 Å α-helix, with 3 turns for 11 residues (Figure 1d), and the more classical 3.60 Å α-helix with 5 turns for 18 units (Figure 1e, Figure S2). We found that one can match four side chains (i, i + 3, i + 4, i + 7) of a 3.60 Å α-helix and four residues on a P-helical aromatic backbone with an RMSD of 0.70–0.95 Å (Figure 1e), which fares well compared to reported values. The match can be extended to eight side chains (the above plus i + 11, i + 14, i + 15, i + 18)—an entire face—of an octadecapeptide (Fig-
Figure 1. a) Some previously described 4-substituted Q\textsuperscript{xx} monomers and some of the new 6-substituted Q\textsuperscript{xx} monomers reported in this study. b) Molecular model and helical wheel of a (Q\textsuperscript{xx}Q\textsuperscript{xx}Q\textsuperscript{xx})\textsubscript{4} octade-ca-arylamide helix showing substituents in position 6 and 4 as blue and green balls, respectively. c) Structure of an ideal (computed) 3.66\texttimes-helix showing substituents in position 6 or 4 as red spheres. d) Overlay of the side chains in b) and c) with an RMSD of 1.50 Å (see Figure S2). e) Overlay of four side chains of Q\textsuperscript{xx}Q\textsuperscript{xx}Q\textsuperscript{xx}Q\textsuperscript{xx} (same color code as b) with four side chains of a short 3.6 \alpha-helix (red spheres). Note the position of the side chains in the 3.6 helix slightly differ from those of the 3.66 helix above. An RMSD of 0.92 Å is calculated if the overlay concerns the peptide \beta-carbon and a quinoline exocyclic carbon in position 4 or 6 (shown). An RMSD of 0.79 Å is calculated if the overlay concerns the peptide \alpha-carbon and the quinoline endocyclic carbon in position 4 or 6 (not shown).

To exploit the new design, we first needed a robust synthetic route to 6-functionalized monomers suitably protected for solid phase synthesis. The eight monomers 1a–h shown in Scheme 1 were targeted, which bear protected hydrophobic, polar neutral and cationic side chains. The synthetic route relies on key intermediates 2 and 3, which were obtained on a multigram scale. Compound 2 was prepared in three steps from commercially available 4-bromo-2-nitroaniline (see Supporting information) and 3 in two steps from 2. These initial steps were all chromatography-free and scalable. Both 2 and 3 bear different halogen atoms at C-4 and C-6. Selective functionalization at C-6 was achieved, but this route also makes it possible to further functionalize position 4 if additional side chains are required, that is, in both positions 4 and 6 of the same quinoline ring. Depending on the nature of the side chain and availability of the corresponding synthons, regioselective reactions at C-6 involved sp\textsuperscript{2}–sp\textsuperscript{2} (Suzuki), sp\textsuperscript{2}–sp\textsuperscript{3} (Suzuki) or sp\textsuperscript{2}–sp (Sonogashira) cross-couplings. Side chains could thus be introduced having a C–C triple, C–C double or C–C single bond. We therefore had the choice of a wide range of potential coupling conditions. The loop through trifluoroacetamide intermediate 3 was required because the nitro function was found to be incompatible with certain sp\textsuperscript{2}–sp\textsuperscript{2} Suzuki couplings. For that, the nitro group was carefully reduced over Raney-Ni without dehalogenation prior to trifluoroacetylation.

With commercial or easily available alkyne precursors, Sonogashira couplings were first attempted. Conditions, particularly the temperature, were optimized for each coupling to enhance regioselectivity. While 4a–d were obtained in satisfactory yields, it was not the case with benzylacrylene. Instead, the phenylpropenyl side chain of 4e was introduced in fair yield.
through an $sp^2$-$sp^2$ Suzuki coupling using the commercially available boronic acid. Another approach was developed where acetylene or vinyl boronate precursors would have been difficult to obtain. The corresponding alkenes were hydroborated to yield alkylboranes quantitatively, which were directly utilized in a one-pot, $sp^2$-$sp^3$ Suzuki coupling with 3 to obtain 4f–h. In the case of 4f the alkene precursor was synthesized in one step (see supporting information). Conditions were optimized in each case to obtain good selectivity and suppress dehalogenation.

Following side chain installation, the double (or triple) bond was saturated, the nitro group was reduced, and the aryl-Cl bond was hydrogenolized in the same hydrogenation step. Optimization and screening were required to find ideal conditions. Eventually, HCOONH$_4$ proved to be an excellent transfer hydrogenation agent and, in most cases, side reactions were suppressed. Finally, methyl ester and trifluoroacetamide hydrolysis followed by Fmoc installation afforded 1a–h on a 0.57–1.30 g scale. The final products were purified by normal phase flash chromatography and preparative RP-HPLC to reach the desired purity (> 99%) for solid phase foldamer synthesis.

Sequence 5 (Figure 2a) was designed to validate side chain positions using the new $Q^{XXX}$ monomers and to possess some side chain diversity (eight different aromatic monomers). Manual solid phase synthesis provided outstanding crude purity (Figure 2b). The spreading of $^1H$ NMR signals of aromatic amide protons in [D$_6$]DMSO (Figure 2c) is characteristic of helix folding. Derivative 6 was prepared to have a slightly better water solubility and was set to crystallize from water/acetonitrile.

Scheme 1. General synthetic strategy of 6-substituted quinoline amino acid building blocks 1a–h with protected proteinogenic side chains suitable for solid phase foldamer synthesis (SPFS). 9-BBN: 9-borabicyclo[3.3.1]nonane.
trile using the hanging drop method. Crystals that diffracted up to a resolution of 1.2 Å were obtained and the structure was solved in the P-1 space group with two independent molecules in the asymmetric unit (Figure 2d-2f). Figure 2d shows an array of hydrophobic QXXX and 6-substituted QXXX monomer side chains arranged as predicted in Figure 1b. An overlay with the predicted model demonstrates an RMSD of 0.4 Å of the first atom of each side chain, establishing the good prediction of side chain positions by a simple molecular modelling tool, and the weak deviation of the structure of 6 from an ideal calculated helix (Figure S5). Furthermore, pairs of parallel helices are observed in which two arrays of hydrophobic groups cluster and interdigitate (Figure 2e), as in α-helix peptide bundles.[21] The two helices nevertheless have opposite handedness and opposite N→C polarity, an assembly mode that has already been observed in helix bundles.[22] In solution, water rich samples only show broad H NMR lines (Figure S5), suggesting that no well-defined aggregate prevails. Nevertheless, the structure of 6 clearly hints at the possibility to design knob-into-hole complementary hydrophobic interfaces similar to those of α-helices. Notwithstanding a certain disorder of the side chains resulting in partial occupancy factors, additional remarkable features of this structure include likely side chain-main chain hydrogen bonding (Figure 2f left) and also some proximity between Orn and 6Tyr side chains hinting at possible intramolecular cation–π interactions (Figure 2f right). Side chain-side chain interactions are indeed well-known to influence α-helix stability.[23] Q helices are intrinsically very stable and need no further stabilization. Nevertheless, such interactions would help to fine-tune the stability of related sequences having stronger dynamics.[24]

Altogether, these results establish the suitability of substituted Q oligomers as scaffolds to mimic some extended arrays of side chains at the surface of long α-helices. Interestingly, sequences that combine Q monomers and α-amino acids do not provide such a good match.[25] Prospective applications of these mimics include the recognition of protein surfaces and the construction of peptide bundles. Progress in these directions is being made in our laboratories and will be reported in due course.

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Conflict of interest

The authors declare no conflict of interest.

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