CHAPTER THIRTEEN

Solid Phase Synthesis of Helically Folded Aromatic Oligoamides

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Abstract

Aromatic amide foldamers constitute a growing class of oligomers that adopt remarkably stable folded conformations. The folded structures possess largely predictable shapes and open the way toward the design of synthetic mimics of proteins. Important examples of aromatic amide foldamers include oligomers of 7- or 8-amino-2-quinoline carboxylic acid that have been shown to exist predominantly as well-defined helices, including when they are combined with α-amino acids to which they may impose their folding behavior. To rapidly iterate their synthesis, solid phase synthesis (SPS) protocols have been developed and optimized for overcoming synthetic difficulties inherent to these backbones such as low nucleophilicity of amine groups on electron poor aromatic rings and a strong propensity of even short sequences to fold on the solid phase during synthesis. For example, acid chloride activation and the use of microwaves are required to bring coupling at aromatic amines to completion. Here, we report detailed SPS protocols for the rapid production of: (1) oligomers of 8-amino-2-quinolinecarboxylic acid; (2) oligomers containing 7-amino-8-fluoro-2-quinolinecarboxylic acid; and (3)

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heteromeric oligomers of 8-amino-2-quinolinecarboxylic acid and α-amino acids. SPS brings the advantage to quickly produce sequences having varied main chain or side chain components without having to purify multiple intermediates as in solution phase synthesis. With these protocols, an octamer could easily be synthesized and purified within one to two weeks from Fmoc protected amino acid monomer precursors.

1. INTRODUCTION

Foldamers, ie, artificial folded molecular architectures, are the object of very active research investigations (Guichard & Huc, 2011). While inspired by the folding behavior seen in natural biopolymers, they possess a fundamental difference, in that they are comprised of either nonnatural building blocks or natural building blocks arranged in a nonnatural sequence. Foldamers closely ressembling their natural counterpart have been termed "biotic" and include peptide nucleic acids (Nielsen, Egholm, Berg, & Buchardt, 1991), peptoids (Simon et al., 1992), β-peptides (Appella, Christianson, Karle, Powell, & Gellman, 1996; Seebach et al., 1996), γ-peptides, and δ-peptides. They also include peptide sequences where amides have been replaced by urea, hydrazide, or hydroxyamide linkages (Li & Yang, 2006; Salaun, Potel, Roisnel, Gall, & Le Grel, 2005; Semetey et al., 2002). In contrast, "abiotic" foldamers consist of entirely unnatural building blocks, giving rise to backbones, and folding modes which are inaccessible to natural motifs. These architectures may have unique physical properties, or may be capable of interacting with biomolecules in unforeseen and interesting ways. Many of these sequences are aromatic rich, examples including oligo-phenylene-ethynylenes (Nelson, Saven, Moore, & Wolynes, 1997), alternating aromatic electron donors and acceptors (Lokey & Iverson, 1995), aryl-oligomers (often based on aza-heterocycles: pyridines, pyrimidines, pyridazines, etc.; Bassani, Lehn, Baum, & Fenske, 1997), aromatic tertiary amide, imide, or urea oligomers, and aromatic oligoamides (Berl, Huc, Khoury, Krische, & Lehn, 2000; Hamuro, Geib, & Hamilton, 1996; Huc, 2004; Jiang, Léger, & Huc, 2003; Zhu et al., 2000). Oligoamide foldamers are particularly attractive, since the amide linkage offers a high level of synthetic feasibility; indeed, this is the motif which has been selected by nature.

Aromatic oligoamide foldamers consisting of 8-amino-2-quinolinecarboxylic acid (Fig. 1) form single helical architectures made up of 2.5 monomer units per turn, with a pitch of 3.4 Å that corresponds

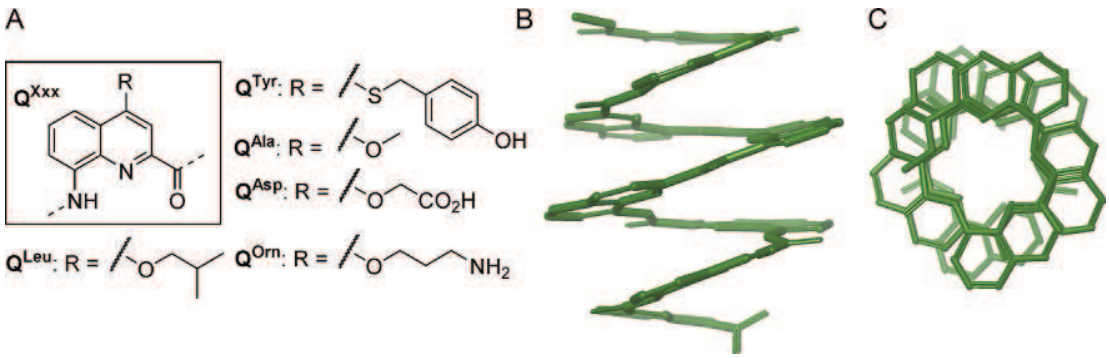


Fig. 1 (A) Structure of quinoline-based aromatic oligoamide foldamers with examples of available monomer side chains. Side (B) and top (C) views of the crystal structures of O_2N-$(Q^{Leu})_8$-OMe. Side chains and hydrogen atoms are omitted for clarity.

to the thickness of one aromatic ring (Fig. 1B and C) (Dolain et al., 2005; Jiang, Léger, Dolain, Guionneau, & Huc, 2003; Jiang, Léger, & Huc, 2003). They feature key properties which make them ideal candidates for the recognition of sizeable surface areas of biomolecules: they are medium sized (0.5–5.0 kDa), resistant to proteolytic degradation, and conformationally stable in a wide range of solvents and in particular in water, even at high temperatures (Gillies, Deiss, Staedel, Schmitter, & Huc, 2007; Qi et al., 2012). Indeed, to date no conditions have been found under which they do not adopt a helically folded conformation. Another advantage is that their helical shape is extremely predictable, and since monomer side chains may be positioned away from the backbone amide motifs, folding is essentially independent of R-group functionality. This allows the display of arrays of side chains to be tuned in order to optimize interactions with a specific biomolecule, affording foldamers with cell-penetrating properties (Gillies et al., 2007; Iriondo-Alberdi, Laxmi-Reddy, Bouguerne, Staedel, & Huc, 2010), high affinity for G-quadruplex DNA (Delaurière et al., 2012; Müller et al., 2014), or the potential to interact with protein surfaces (Buratto et al., 2014).

This high stability of helix shape also brings benefits in terms of synthetic availability. Once a trimer has been reached, addition of further monomer units only results in elongation of the helix under identical conditions regardless of helix length. This is in contrast to biopolymers such as peptides, where each synthetic intermediate may have a different conformation, potentially affecting reactivity in subsequent steps (eg, by aggregation).

A logical extension from homogenous backbones (ie, those consisting of exclusively one monomer type) is to combine a variety of different monomer types to produce a hybrid sequence. This concept highlights one of the advantages of synthetic foldamers, in that specific monomers can be included

at any desired point to globally or locally direct sequence architecture in a predictable manner, and thus potentially access unsuspected areas of structural and functional space. For quinoline-based oligoamide foldamers, considerable inroads have been made into understanding the effect on helical structure when a diverse array of aromatic building blocks are included (Fig. 2). Monomers such as those based on fluoroquinoline ("Q_F," **1**) or anthracene ("A," **2**) can be included to code for a wider helix diameter, which, combined in the centre of a quinoline sequence, can lead to the formation of a capsule shape. An example would be $Q_3(Q_F)_3A(Q_F)_3Q_3$. Through iterative design, cavity size, and functionality can be reliably tuned to encapsulate a specific guest, examples ranging from simple short-chain alkanes, to monosaccharides (Bao et al., 2008; Chandramouli et al., 2015; Garric, Léger, & Huc, 2005; Singleton, Pirotte, Kauffmann, Ferrand, & Huc, 2014). Short Q_3 quinoline sequences at each terminus of the capsule function as a type of "cap" effectively insulating the cavity from the exterior (Ferrand et al., 2012). If these quinoline caps are removed, the wider diameter of, for example, a homomeric fluoroquinoline sequence results in hybridization into double (Fig. 2B and C) and even quadruple helices (Gan et al., 2008). This behavior has even been shown to occur in aqueous conditions, representing one of the very few examples of water-soluble synthetic double helices reported in current literature (Shang et al., 2014).

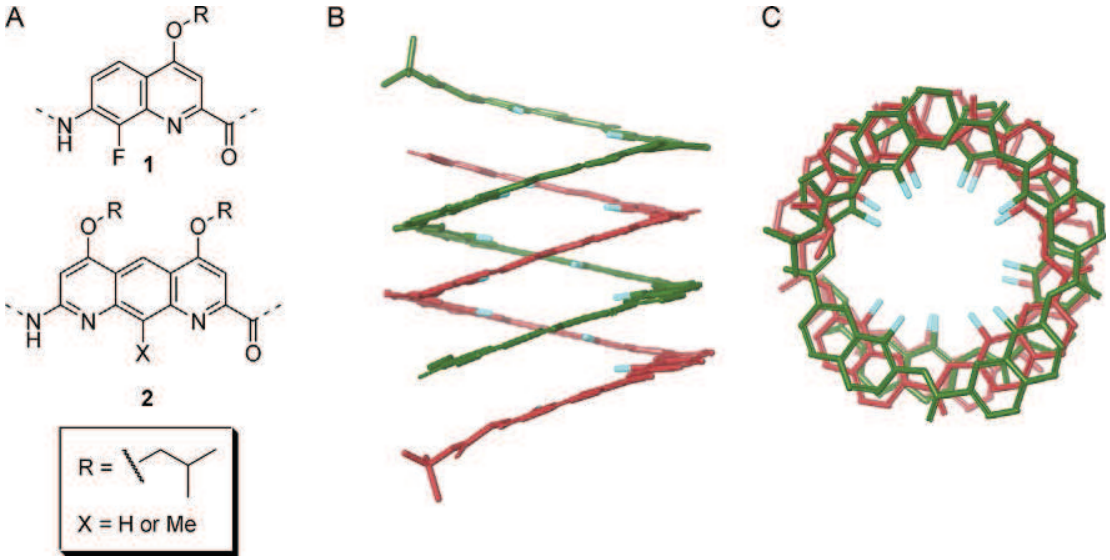


Fig. 2 (A) Fluoroquinoline (**1**) and anthracene (**2**)-based monomers. Side (B) and top (C) views of the crystal structure of double helical $\left(\text{Boc}-\left(Q_F{}^{Leu}\right)_8-\text{OMe}\right)_2$. The backbone of each strand is colored in *green* (*dark gray* in the print version) or *red* (*black* in the print version). Fluorine atoms are colored *blue* (*light gray* in the print version). Side chains and hydrogen atoms are omitted for clarity.

Recent work in the Huc group has also focused on the inclusion of aliphatic units into aromatic oligoamide foldamer sequences, in particular, α-amino acids. This is a natural progression from exclusively abiotic peptidomimetics, in that the exact functional groups which mediate biomolecule recognition (ie, α-amino acid side chains) are included. This also brings synthetic benefits, since a vast range of orthogonally protected α-amino acids are commercially available, avoiding the need for (a perhaps laborious) bespoke monomer synthesis.

In the foldamer world, a single amino acid may for example be added at the extremity of an α-helix mimetic (Barnard et al., 2015). When multiple α-amino acids are incorporated in an abiotic sequence to form a hybrid scaffold, the completely different folding principles of biotic and abiotic units may offer access to secondary structures distinct from those of biopolymers or synthetic homo-oligomers (Nair, Vijayadas, Roy, & Sanjayan, 2014). In other cases, the folding of abiotic units may be so effective that it forces α-amino acids to adopt conformations distinct from those found in peptides. We recently reported examples of this kind using helically folded quinoline oligoamides. Sequences combining α-amino acid (**X**) and quinoline (**Q**) units together in an $\mathbf{XQ_2}$ trimer repeat motif (Fig. 3) were found to adopt a single well-defined canonical aromatic helical conformation in both organic and aqueous conditions (Hu et al., 2016; Kudo, Maurizot, Kauffmann, Tanatani, & Huc, 2013). In contrast, hybrids based on an

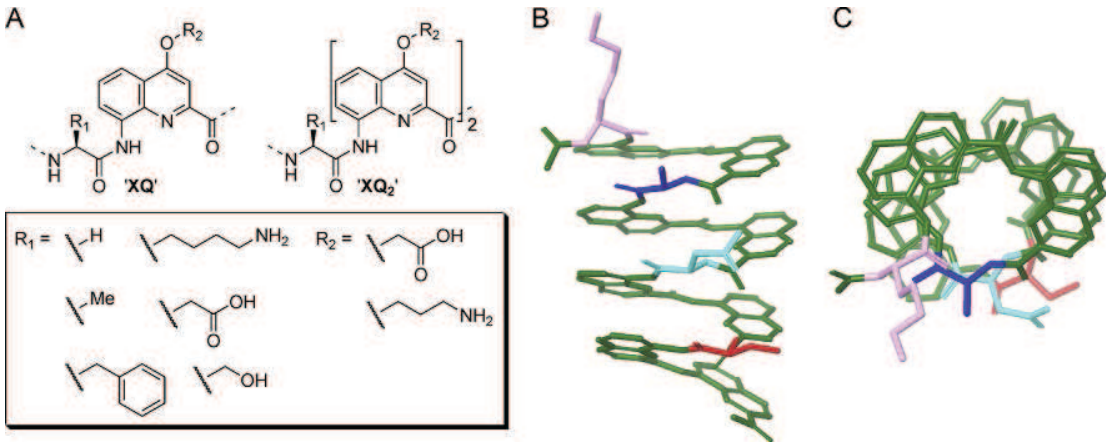


Fig. 3 (A) **XQ** and $\mathbf{XQ_2}$ motifs with α-amino acids currently validated for incorporation. Side (B) and top (C) views of NMR structure of Ac-K(Q^{Orn})$_2$A(Q^{Orn})$_2$D(Q^{Orn})$_2$S(Q^{Orn})$_2$-OH (Hu et al., 2016). The aromatic backbone is colored in *green* (*dark gray* in the print version) and the α-amino acid residues are in *pink* (*gray* in the print version) (Lys), *blue* (*black* in the print version) (Ala), *light blue* (*black* in the print version) (Asp), and *red* (*black* in the print version) (Ser). Quinoline side chains and hydrogen atoms are omitted for clarity.

XQ dimer repeat motif were found to adopt a partially folded zig–zag tape conformation with local conformational variability precluding long range order. This behavior was also evident in protic solvents, where the increased solvent accessibility of the hydrophobic aromatic surfaces appeared to drive nonspecific aggregation at lower temperatures (Hu et al., 2016; Kudo, Maurizot, Masu, Tanatani, & Huc, 2014). These types of hybrid foldamer sequences offer access to currently untapped areas of chemical space and may provide promising candidates for the recognition of "difficult" biological targets such as protein–protein interactions.

With foldamers becoming increasingly complex, organic chemists require synthetic methodologies capable of meeting the requirements of their designs. The production of oligomers by solid phase synthesis (SPS) is particularly attractive in that it offers a method for rapidly generating sequence analogues where any monomer unit can be substituted for another, without the laborious resynthesis of intermediates required by a more convergent solution phase approach. In addition, the pseudodilution effect of solid supported synthesis reduces intermolecular reactions between individual oligomer chains, and this "site–site isolation" phenomenon (Shi, Wang, & Yan, 2007) can help minimize reactivity issues which might occur otherwise in solution due to aggregation, or hybridization behavior (eg, as seen in the synthesis of fluoroquinoline double helical foldamers). While solid phase peptide synthesis methods are now widely standardized, the use of an SPS strategy for the production of aromatic foldamers based on 8-amino-2-quinolinecarboxylic acid is not without its challenges. The aromatic amine is a relatively poor nucleophile and thus coupling requires activation of monomers as acid chlorides and microwave assistance in order to be both rapid and essentially quantitative. Over the last number of years we have reported the SPS of increasingly elaborate oligomers, from homomeric quinoline sequences (Baptiste, Douat-Casassus, Laxmi-Reddy, Godde, & Huc, 2010) to heteromeric sequences containing fluoroquinoline monomers and α-amino acids (Hu et al., 2016; Shang et al., 2014). We are now in a position to report here optimized protocols for the microwave-assisted SPS of these foldamers, including variations and improvements from previously published work. These protocols are specific to the requirements of these classes of oligomers and are to be compared to SPS methods developed for other aromatic amide oligomers (König, Abbel, Schollmeyer, & Kilbinger, 2006; Murphy et al., 2013; Puckett, Green, & Dervan, 2012; Wurtz, Turner, Baird, & Dervan, 2001).

2. MATERIALS

2.1 Reagents

Acetyl chloride, acetic anhydride, CaH_2, CBr_4, 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez reagent), CsI, 1-methyl-2-(4′-nitrophenyl)-imidazo[1,2-a]pyrimidinium perchlorate (DESC), dimethylformamide (DMF), dichloromethane, *N*,*N*-diisopropylethylamine (DIEA), isopropanol, Fmoc α-amino acids, quinoline and fluoroquinoline monomers, methanol, nitrogen gas, piperidine, Sieber amide resin ("low loading" ~0.6–0.7 mmol g^{-1}), tetrahydrofuran (THF), trichloroacetonitrile (TCAN), trifluoroacetic acid (TFA), triisopropylsilane (TIS), triphenylphosphine, 2,4,6-collidine, Wang resin ("low loading" ~0.3–0.4 mmol g^{-1}), and water.

2.2 Equipment

Balloons, CEM discover SPS microwave oven (CEM Corporation, Matthews, USA) equipped with an infrared optical fiber probe internal to the reaction mixture linked to an IR detector for temperature control, CEM SPS vacuum station with membrane vacuum pump (Vacuubrand model ME1, VACUUBRAND GMBH, Wertheim, Germany), CEM SPS reactor vessel (25 mL, polypropylene) with end cap, vial heating block, micropipettes (Gilson, 20 and 100 μL, Gilson, Middleton, USA), high vacuum pump (Vacuubrand model RC6) with glass vacuum manifold and cold finger, microsyringe (Hamilton, 100 μL, Hamilton, Reno, USA), needles (disposable, 2.5 cm and 10 cm), Pasteur pipettes, plastic syringes (disposable, 2, 5, and 10 mL), round bottomed flasks (10 mL) with corresponding rubber septa, sample vials (approx. 1–2 mL), sintered glass filter funnel (approx. 2 mL, porosity grade 3), squeeze solvent wash bottles, stoppered vacuum adapters, rotary evaporator (Büchi, model R-3000, Büchi, Flawil, Switzerland) with vacuum pump (Büchi, model V-500), and vacuum controller (Büchi, model V-800).

2.3 Reagent Setup

Resins: "low loading" resins are to be preferred, in order to avoid potential steric crowding when synthesizing longer oligoamide sequences.

Dichloromethane: dry dichloromethane is obtained by filtration through activated alumina using a dedicated purification system (MBRAUN SPS-800, M. Braun Inertgas-Systeme GmbH, Garching, Germany) and should be used immediately.

THF: dry THF is obtained by filtration through activated alumina using a dedicated purification system (MBRAUN SPS-800) and should be used immediately.

N,*N*-diisopropylethylamine: should be freshly distilled over CaH_2 to remove traces of water and used immediately.

20% (v/v) piperidine in DMF: solution should be freshly prepared.

0.1 M DESC in anhydrous DMF: solution should be freshly prepared.

Where not stated as anhydrous, all solvents used for rinsing the resin and reaction vessel can be delivered using plastic squeeze bottles.

The synthesis of various Fmoc protected 8-amino-2-quinolinecarboxylic acid and 7-amino-8-fluoro-2-quinolinecarboxylic acid has been reported before (Baptiste et al., 2010; Buratto et al., 2014; Shang et al., 2014).

2.4 Equipment Setup

Dry syringes and needles: Plastic syringes and needles directly used from the packaging are considered dry. When they are reused, they should be cleaned with acetone and dried under vacuum (at least for 1 h).

Microwave: As of publication, these protocols have not been validated on microwave systems other than the CEM discover. Follow manufacturer's instructions for setup of microwave cavity and vacuum station for SPS. The microwave should be run in "open vessel" mode, with temperature control via an internal fiber optic probe. A standard temperature program should be used, with a 5 min ramp time and medium stirring speed. Hold times vary (see specific protocol).

3. SYNTHETIC PROTOCOLS

3.1 8-Amino-2-quinolinecarboxylic Acid-Based Oligoamides

Notes: We show here as an example the synthesis of a water-soluble octameric quinoline oligoamide (Fig. 4) on a 19 μmol scale. In our experience, this protocol can be reliably scaled to approx. 80 μmol with no decrease in efficiency. We have successfully synthesized oligomers up to 24 units in length with these protocols. Protocols 3–6 and 8 have also been validated using low-loading Sieber amide resin.

3.1.1 Protocol 1: Bromination of Low-Loading Wang Resin

Notes: Method based on the procedure of Morales and coworkers (Morales, Corbett, & DeGrado, 1998) where it is reported at larger scales. We

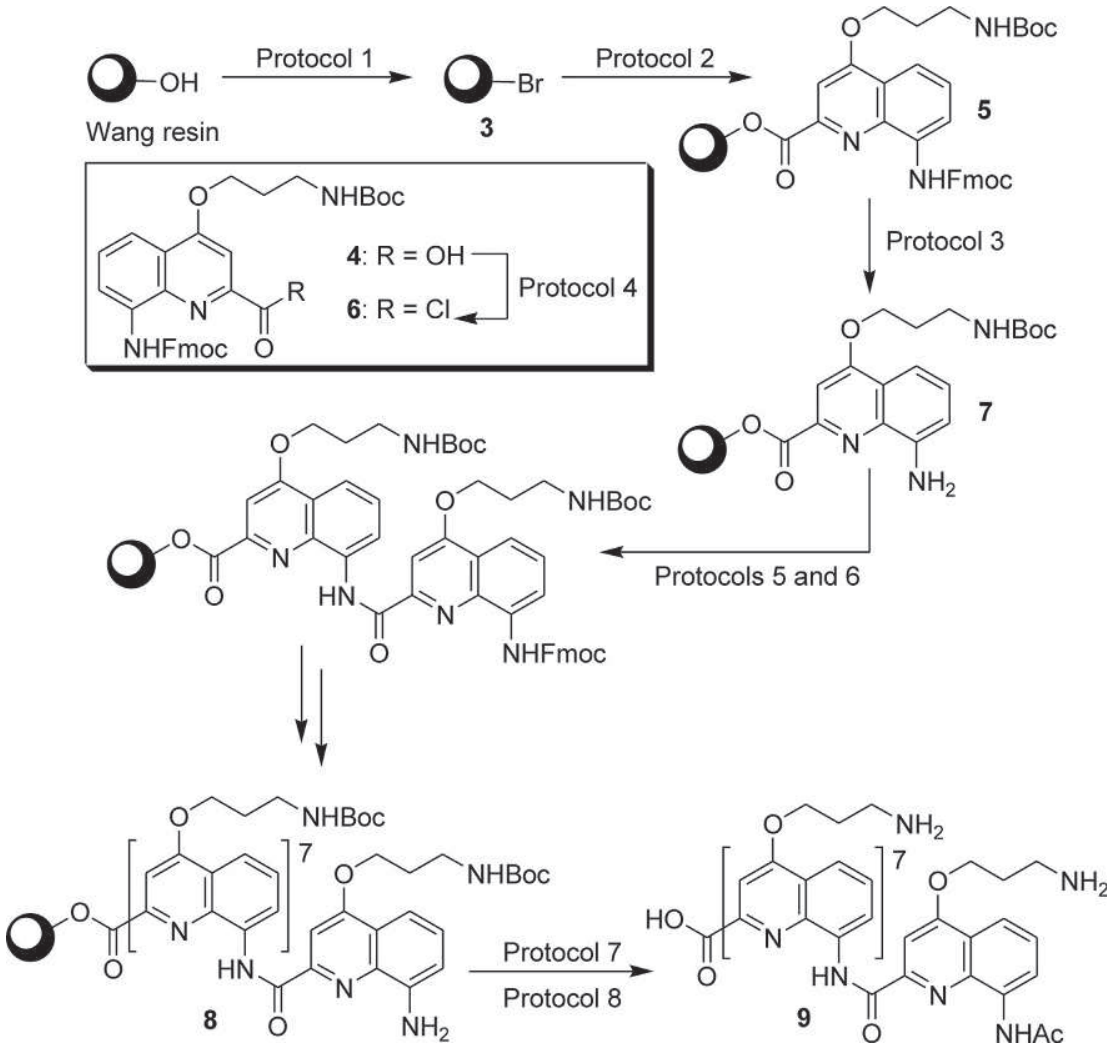


Fig. 4 Summary of SPS protocols exemplified by synthesis of acetylated Q^{Orn} octamer **9**.

exemplify here the procedure on a 76 μmol scale, which is routinely carried out in our laboratory.

Time required: 17 h

(1) Prepare a clean, dry 10 mL round bottomed flask equipped with small magnetic stirring bar, septum, and nitrogen balloon.

(2) Add Wang resin (200 mg, 76 μmol for 0.38 mmol g^{-1} loading) to the reaction flask, followed by 2 mL anhydrous DMF via dry syringe through the septum. Ensure all resin is rinsed from the sides of the flask, then flush with N_2. Note that success of this reaction is highly dependent on the quality of the anhydrous DMF.

(3) Leave resin to swell for 1 h under N_2 atmosphere.

(4) Remove the septum. While stirring the resin, add PPh_3 (99.7 mg, 0.38 mmol, 5 equiv.), and CBr_4 (126.1 mg, 0.38 mmol, 5 equiv.) rapidly in that order. Then equip with the septum and nitrogen balloon.

Note that flask should warm noticeably, and the solution should develop a lasting yellow–orange coloration.

(5) Stir for 15 h under N_2 atmosphere.

(6) Collect the resin by filtration and wash sequentially with anhydrous DMF (5 mL), anhydrous CH_2Cl_2 (5 mL), and isopropanol (5 mL). Repeat the washing cycle three times in total.

(7) Dry the resin under vacuum in the desiccator.

3.1.2 Protocol 2: Loading of Wang-Bromide Resin

Time required: 1.5 h

(1) Prepare a clean, dry 10 mL round bottomed flask, and equip with septum and nitrogen balloon.

(2) Add Wang-bromide resin (**3**, 50 mg, 19 μmol for 0.38 mmol g^{-1} loading) to the flask, followed by approximately 1 mL of anhydrous DMF via dry syringe through the septum. Ensure all resin is rinsed from the sides of the flask, then flush with N_2.

(3) Leave resin to swell for 1 h under N_2 atmosphere.

(4) Equip a 25 mL polypropylene SPS microwave reaction vessel with the appropriate size magnetic stirring bar.

(5) Transfer the swollen resin to the microwave reaction vessel as a slurry in anhydrous DMF. Rinse the round bottomed flask with small quantities of anhydrous DMF to allow transfer of residual resin. Remove residual anhydrous DMF from the reaction vessel using the vacuum station.

(6) Remove reaction vessel from the vacuum station and fit end cap to avoid leakage.

(7) To the reaction vessel, add 0.45 mL anhydrous DMF, followed by Fmoc-$Q^{Orn\text{-}Boc}$-OH (**4**, 33.2 mg, 0.057 mmol, 3 equiv.), and CsI (14.8 mg, 0.057 mmol, 3 equiv.). Add DIEA (9.9 μL, 0.057 mmol, 3 equiv.) using a micropipette.

(8) Rinse the inside surface of the reaction vessel with a further 0.45 mL anhydrous DMF, to ensure all reagents are at the resin bed.

(9) Insert optical fiber probe into the reactor vessel ensuring the tip is immersed in the reaction mixture and then place the vessel into the microwave cavity with the appropriate insert. Treat with microwaves: 50 W, ramp to 50°C, with a 5 min hold time.

(10) Remove reaction vessel from microwave cavity, remove end cap, and remove reagent solution on vacuum station. Rinse the inside surfaces of the reaction vessel with anhydrous DMF (5 mL) to remove reagents

and ensure all displaced resin is moved down to the resin bed. Remove DMF on vacuum station and fit end cap to the vessel.

(11) Repeat steps (7) to (9).

(12) Remove reaction vessel from microwave cavity, remove fiber optic probe and end cap, then remove reagent solution using the vacuum station. Using DMF thoroughly rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

(13) Loading efficiency can be calculated using the same UV spectroscopic methods (based on liberated dibenzofulvene adducts from Fmoc deprotection) reported widely in the literature for solid phase peptide synthesis (White & Chan, 2000).

3.1.3 Protocol 3: Fmoc Deprotection

Time required: allow 40 min.

(1) To the loaded resin **5** in reaction vessel, add approximately 2–3 mL of a 20% (v/v) solution of piperidine in DMF. Stir at medium speed at room temperature for 10 min (can be carried out on a normal stirring plate if desired).

(2) Remove end cap from reaction vessel and remove reagent solution on vacuum station. Rinse the inside surfaces of the reaction vessel with anhydrous DMF (5 mL) to remove reagents and ensure all displaced resin is moved down to the resin bed. Remove DMF on vacuum station and fit end cap to the vessel.

(3) Repeat steps (1) and (2) twice more.

(4) Using DMF thoroughly rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

3.1.4 Protocol 4: Conversion of N-Fmoc Quinoline Carboxylic Acid Monomer *4* to the Corresponding Acid Chloride, *6*

Time required: allow 3.5 h.

(1) Prepare a clean dry 10 mL round bottomed flask and equip with septum and nitrogen balloon.

(2) To the reaction flask, add monomer **4** (66.4 mg, 0.114 mmol, 6 equiv. relative to resin loading) followed by 1.7 mL anhydrous CH_2Cl_2 via dry syringe through the septum. Note that full dissolution is not required for reaction success. For the cases where solubility is low, sonication is required to obtain a homogeneous slurry before starting next step.

(3) While stirring, add 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (30.2 μL, 0.228 mmol, 12 equiv. relative to resin loading) via dry microsyringe through the septum. Stir for 1 h at room temperature, under N_2.

(4) Fit flask with vacuum adaptor and, while stirring, remove solvent, and reagents on a vacuum manifold equipped with liquid N_2 cold finger. Once evaporated, leave the product (**6**) to dry for at least a further 2 h on the vacuum line.

(5) Turn off the vacuum and fill the flask back with nitrogen using the dual manifold of the vacuum line or with a nitrogen-filled balloon. Remove the vacuum adaptor, replace septum, and ensure that from this point the product remains under positive nitrogen pressure (or under vacuum) until usage.

3.1.5 Protocol 5: Coupling of N-Fmoc Quinoline Carboxylic Acid Chloride (6) to Resin Bound Amine (7)

Note: This procedure can also be used for the loading of Sieber amide resin.

Time required: allow 20–40 min.

(1) On the vacuum station thoroughly rinse the inside surfaces of the reaction vessel and the resin with anhydrous THF (approx. 10 mL) using a dry syringe, ensuring that no DMF remains. Remove all remaining THF, then remove the vessel from the vacuum station, and replace end cap.

(2) To the round bottomed flask containing acid chloride **6** (quantity corresponding to 0.114 mmol, 6 equiv.) add 2 mL anhydrous THF via dry syringe through the septum.

(3) Add 0.3 mL anhydrous THF to the resin using a dry syringe, followed by DIEA (20 μL, 0.114 mmol, 6 equiv.) using a micropipette. Note that it is **essential** that DIEA is added prior to the addition of the acid chloride.

(4) Using a dry syringe, remove 1 mL of the acid chloride solution (ie, corresponding to 0.057 mmol, 3 equiv.) and add to the resin.

(5) Insert fiber optic probe into the reaction vessel (ensuring the tip is immersed in the reaction mixture) and then place the vessel into the microwave cavity with the appropriate insert. Treat with microwaves: 50 W, ramp to 50°C. For acid chlorides with poor THF solubility (eg, **6**) we recommend setting the hold time to 15 min. Otherwise hold time should be set at 5 min.

(6) Remove reaction vessel from microwave cavity, remove end cap, and remove reagent solution on vacuum station. Using a dry syringe, rinse the inside surfaces of the reaction vessel with anhydrous THF (5 mL) to

remove reagents, and ensure all displaced resin is moved down to the resin bed. Remove THF on vacuum station and fit end cap to the vessel.

(7) Repeat steps (3) to (5).

(8) Remove reaction vessel from microwave cavity, remove fiber optic probe and end cap, then remove reagent solution on vacuum station. Using a dry syringe, rinse the inside surfaces of the reaction vessel, and the resin itself with anhydrous THF (approx. 10 mL). Then, using DMF, thoroughly rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

3.1.6 Protocol 6: Assessment of Coupling Completion Using a Modified DESC Test

Note: DESC (Fig. 5) should be synthesized using the procedure reported in the literature (Claerhout, Ermolat'ev, & Van der Eycken, 2008).

Time required: 7 min.

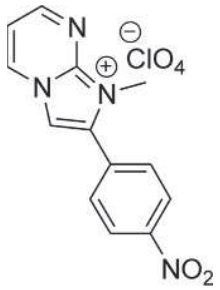
(1) Remove a very small quantity of resin from the reaction vessel (approx. 20–30 beads) using a Pasteur pipette or spatula, and place in a small sample vial.

(2) Using a Pasteur pipette, add to the sample vial of resin five drops of a 0.1 M solution of DESC in DMF, followed by two drops of a 20% (v/v) solution of DIEA in DMF.

(3) Heat the sample vial at 60°C for 5 min.

(4) Carefully remove supernatant using a Pasteur pipette and add fresh DMF. Repeat in this manner until the supernatant is colorless.

(5) Visualize the resin beads under a microscope. Any red–orange coloration indicates that coupling is incomplete (ie, presence of aromatic amine). The coupling procedure should thus be repeated until no orange coloration is observed with this test.



DESC

Fig. 5 Structure of DESC color test agent.

3.1.7 Protocol 7: Acetylation of the N-Terminal Aromatic Amine

Time required: 40 min.

(1) On the vacuum station thoroughly rinse the inside surface of the reaction vessel and the resin-bound foldamer **8** with anhydrous THF (approx. 10 mL) using a dry syringe, ensuring that no DMF remains. Remove all remaining THF, then remove the vessel from the vacuum station, and replace end cap.

(2) Using a dry syringe, add 1.3 mL anhydrous THF to the resin, followed by DIEA (20 μL, 0.114 mmol, 6 equiv.) using a micropipette. Then add a diluted acetyl chloride solution (10%, v/v) in anhydrous THF (41 μL, 0.057 mmol, 3 equiv.) using a dry syringe.

(3) Insert fiber optic probe into the reaction vessel (ensuring the tip is immersed in the reaction mixture) and then place the vessel into the microwave cavity with the appropriate insert. Treat with microwaves: 50 W, ramp to 60°C with a 15 min hold time.

(4) Remove reaction vessel from microwave cavity, remove end cap, and remove reagent solution on vacuum station. Using a dry syringe, rinse the inside surfaces of the reaction vessel with anhydrous THF (5 mL) to remove reagents and ensure all displaced resin is moved down to the resin bed. Remove THF on vacuum station and fit end cap to the vessel.

(5) Repeat steps (2) and (3).

(6) Remove reaction vessel from microwave cavity, remove fiber optic probe and end cap, then remove reagent solution on vacuum station. Using a dry syringe, rinse the inside surfaces of the reaction vessel, and the resin itself with anhydrous THF (approx. 10 mL). Then, using DMF, thoroughly rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF), and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

3.1.8 Protocol 8: Cleavage of Foldamer from the Resin

Note: This procedure can be used for cleavage of the foldamer from both Wang and Sieber amide resins.

Time required: Drying resin: 15 h; cleavage: 2 h; workup: 2.5 h.

(1) Using the vacuum station, rinse the resin sequentially with DMF (10 mL), CH_2Cl_2 (10 mL), and (1:1) CH_2Cl_2/MeOH (10 mL). Use the vacuum station to dry the resin briefly (approx. 5 min), then transfer the reaction vessel to a desiccator attached to a vacuum manifold and dry the resin under vacuum for approx. 15 h.

(2) Transfer the resin to a clean, dry 10 mL round bottomed flask equipped with a magnetic stirring bar.

(3) Prepare 1 mL of TFA/TIS/H_2O (95:2.5:2.5, v/v/v) solution, and add to the resin. Stopper the flask, and stir the mixture for 2 h at room temperature.

(4) Filter the reaction mixture using a clean, dry sintered glass filter funnel and collect the supernatant. Rinse the resin three times with TFA (3 × 0.5 mL) and combine the supernatants.

(5) Concentrate the TFA solution on a rotary evaporator with a bath temperature of 40°, to obtain a viscous oil. Add diethyl ether to precipitate the product (**9**) and triturate. Filter the product using a clean, dry sintered glass filter funnel, dry and desiccate (approx. 2 h on a vacuum manifold). An alternative to filtration consists in centrifugating, removing the supernatant, redissolving the sticky solid in water or a water–acetonitrile mixture, and freeze-drying.

(6) Purify the product using preparative RP-HPLC.

3.2 7-Amino-8-fluoro-2-quinolinecarboxylic Acid-Based Oligoamides

Advice on SPS of 7-amino-8-fluoro-2-quinolinecarboxylic acid-based oligoamides

Note: SPS of **12** (Fig. 6) can be carried out using the same methods as described in protocols for 8-amino-2-quinolinecarboxylic acid-based oligoamides, with the following exceptions and advice:

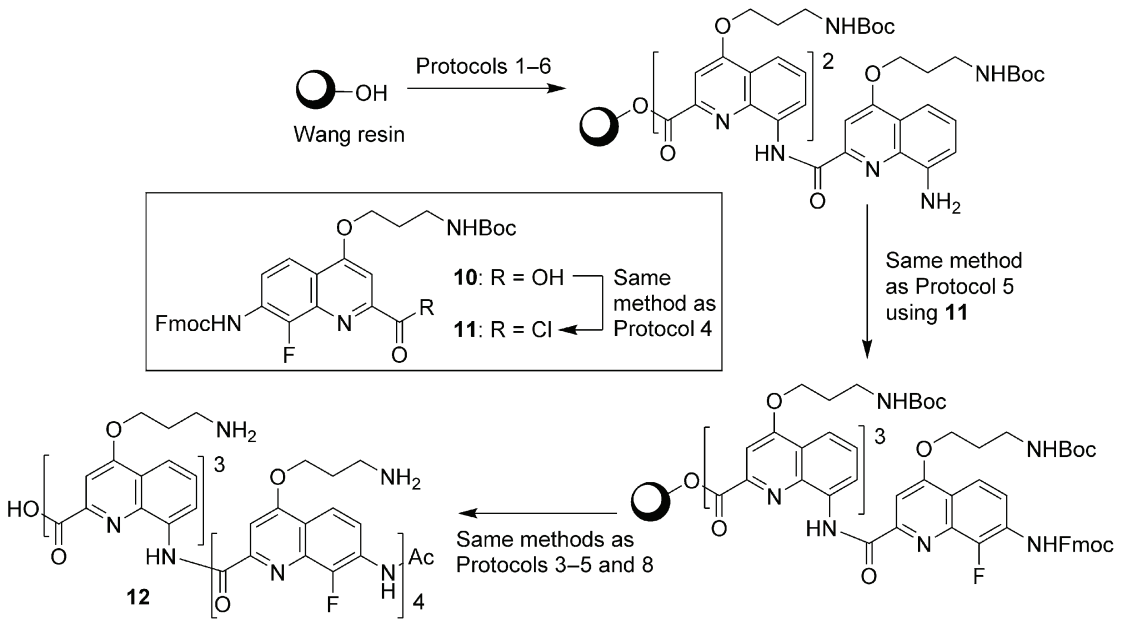


Fig. 6 SPS of 7-amino-8-fluoro-2-quinolinecarboxylic acid-based oligoamides exemplified by synthesis of the antiparallel double helix-forming sequence **12** (Shang et al., 2014).

(1) *Protocol 6*: In contrast with the 8-amino-quinoline, the 7-amino-8-fluoro-quinoline was found to afford a pale yellow coloration using the DESC test, which was difficult to observe. Therefore, we advise that coupling completion should be confirmed by cleaving a small aliquot of resin and analyzing the resulting product(s) via ^{1}H NMR and LC-MS.

(2) *Protocol 8*: The fluoroquinoline amine was found to be particularly susceptible to trifluoroacetylation when undergoing cleavage from the resin (Shang et al., 2014). We therefore advise that if possible, this functionality is blocked (for example, via acetylation, see Protocol 7) during this step.

(3) *Purification/characterization*: It should be noted that products from SPS of these sequences are potentially capable of self- and cross-hybridization (both parallel and antiparallel) after cleavage from the resin. Therefore to avoid difficulties in purification it is recommended that:

- **a.** Each coupling step should be verified as complete in order to avoid as much as possible the presence of deletion sequences (see also point 1).
- **b.** Where possible, the sequence should be designed with a quinoline trimer motif at one terminus which codes for a single helical segment. Since this has no propensity to self-associate, it will force exclusively antiparallel duplex formation.

Final purification should be carried out by RP-HPLC as for the 8-amino-2-quinolinecarboxylic acid-based oligoamides.

3.3 Quinoline/α-Amino Acid Hybrid Oligoamides

Note: Methods described in Protocols 1–8 can be used unchanged for the synthesis of quinoline/α-amino acid hybrid oligoamides (Fig. 7). Coupling of quinoline monomers to the α-amino acid aliphatic amine can be carried out using Protocols 4 and 5 even though other activation methods work as well. Note that for these sequences, all protocols have only been currently validated using Wang resin. They are also not suitable for use with cysteine or 2-aminoisobutyric acid, due to racemization and poor reactivity, respectively.

3.3.1 Protocol 9: Coupling of α-Amino Acid to the Quinoline Amine via In Situ Acid Chloride Formation

Time required: 40 min.

(1) On the vacuum station thoroughly rinse the inside surfaces of the reaction vessel and the resin with anhydrous THF (approx. 10 mL) using a

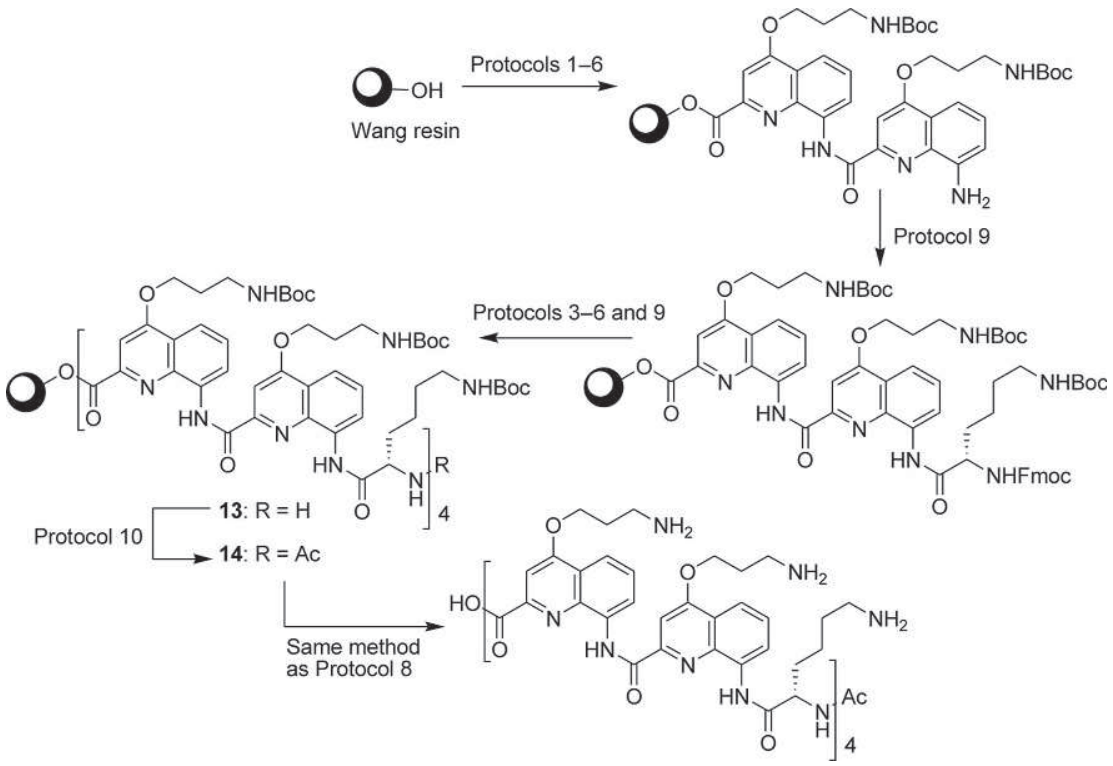


Fig. 7 SPS of quinoline/α-amino acid hybrid oligoamides exemplified by the XQ_2-based sequence $Ac-\left(KQ^{Orn}{}_2\right)_4-OH$.

dry syringe, ensuring that no DMF remains. Remove remaining THF, then remove the vessel from the vacuum station and replace end cap.

(2) Add 0.3 mL anhydrous THF to the resin using a dry syringe, followed by 2,4,6-collidine (21.8 μL, 0.165 mmol, 8.7 equiv.) using a micropipette.

(3) Prepare a clean dry 10 mL round bottomed flask and equip with septum and nitrogen balloon.

(4) To the flask, add Fmoc-Lys(Boc)-OH (17.8 mg, 0.038 mmol, 2 equiv. relative to resin loading) and PPh_3 (54.8 mg, 0.21 mmol, 11 equiv.) followed by 1 mL anhydrous THF via syringe through the septum. While stirring, add TCAN (16.5 μL, 0.165 mmol, 8.7 equiv.) via dry microsyringe through the septum. Using a dry syringe, immediately transfer the resulting mixture to the resin.

(5) Insert optical fiber probe into the reaction vessel (ensuring the tip is immersed in the reaction mixture) and then place the vessel into the microwave cavity with the appropriate insert. Treat with microwaves: 50 W, ramp to 50°C with a 15 min hold time.

(6) Remove reaction vessel from microwave cavity, remove end cap, and remove reagent solution on vacuum station. Using DMF, thoroughly

rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed. Then, using a dry syringe, rinse the inside surfaces of the reaction vessel and the resin itself again with anhydrous THF (approx. 10 mL) to remove the remaining DMF. Remove THF on vacuum station and fit end cap to the vessel.

(7) Repeat steps (2–6). Note: the number of repeat cycles may depend on the Fmoc α-amino acid used. Coupling completion can be determined using Protocol 6. For further details, see Hu et al. (2016).

(8) Remove reaction vessel from microwave cavity, remove fiber optic probe and end cap, then remove reagent solution on vacuum station. Using DMF, thoroughly rinse the inside surfaces of the reaction vessel (approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

3.3.2 Protocol 10: Acetylation of Aliphatic N-Terminal Amine

Time required: 50 min.

(1) On the vacuum station thoroughly rinse the inside surface of the reaction vessel and the resin with anhydrous DMF (approx. 10 mL) using a dry syringe. Remove residual anhydrous DMF from the reaction vessel using the vacuum station. Then remove the vessel from the vacuum station and replace end cap.

(2) Using a dry syringe, add 1.3 mL anhydrous DMF to the resin, followed by DIEA (66.7 μL, 0.38 mmol, 20 equiv.) using a micropipette. Then add acetic anhydride (17.9 μL, 0.19 mmol, 10 equiv.) using a micropipette.

(3) Insert optical fiber probe into the reaction vessel (ensuring the tip is immersed in the reaction mixture) and then place the vessel into the microwave cavity with the appropriate insert. Treat with microwaves: 25 W, ramp to 25°C with a 20 min hold time.

(4) Remove reaction vessel from microwave cavity, remove end cap, and remove reagent solution on vacuum station. Using a dry syringe, rinse the inside surfaces of the reaction vessel with anhydrous DMF (5 mL) to remove reagents and ensure all displaced resin is moved down to the resin bed. Remove residual anhydrous DMF on vacuum station and fit end cap to the vessel.

(5) Repeat steps (2) and (3).

(6) Remove reaction vessel from microwave cavity, remove fiber optic probe and end cap, then remove reagent solution on vacuum station. Using DMF, thoroughly rinse the inside surfaces of the reaction vessel

(approx. 10 mL DMF) and the resin itself (approx. 10 mL DMF) to ensure all reagents have been removed.

4. CHARACTERIZATION OF FOLDAMERS

Section 4.1 shows analytical RP-HPLC and ^{1}H NMR characterization of examples of 8-amino-2-quinolinecarboxylic acid-based oligoamides, 7-amino-8-fluoro-2-quinolinecarboxylic acid-based oligoamides, and quinoline/α-amino acid hybrid oligoamides. Analytical RP-HPLC was carried out on a Macherey–Nagel Nucleodur C_{18} gravity column (4.6×100 mm^2, 3 μm) at 1.5 mL/min, running solvents: MilliQ water containing 0.1% (v/v) TFA (solvent 1), CH_3CN containing 0.1% (v/v) TFA (solvent 2). Gradients were as follows: for 8-amino-2-quinolinecarboxylic acid-based oligoamides, 20% to 28% solvent 2 over 10 min (System A); for 7-amino-8-fluoro-2-quinolinecarboxylic acid-based oligoamides, 5% to 100% solvent 2 over 15 min (System B); and for quinoline/α-amino acid hybrid oligoamides, 13% to 18% solvent 2 over 10 min (System C).

4.1 8-Amino-2-quinolinecarboxylic Acid-Based Oligoamides

See Figs. 8 and 9.

4.2 7-Amino-8-fluoro-2-quinolinecarboxylic Acid-Based Oligoamides

See Figs. 10 and 11.

4.3 Quinoline/α-Amino Acid Hybrid Oligoamides

See Figs. 12 and 13.

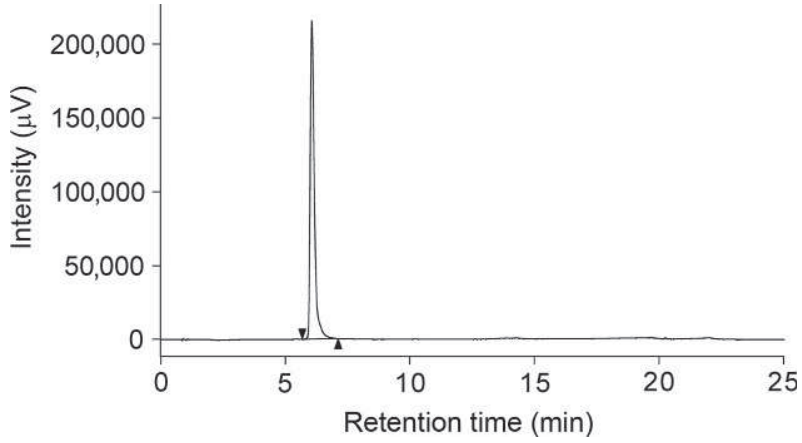


Fig. 8 Analytical RP-HPLC chromatogram (System A) of the 12mer Ac-($Q^{Asp}(Q^{Orn})_2$ $Q^{Ala}Q^{Orn})_2Q^{Asp}Q^{Orn}$-OH after purification by preparative RP-HPLC.

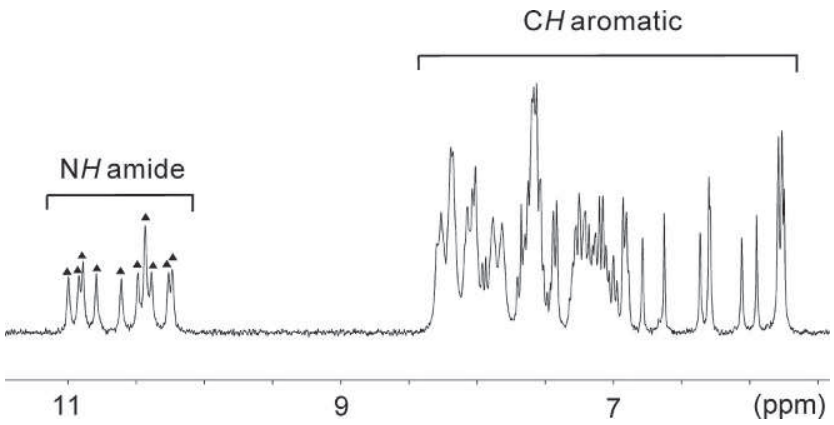


Fig. 9 Excerpt of the ^{1}H NMR spectrum (300 MHz) at 298 K of Ac-($Q^{Asp}(Q^{Orn})_2$ $Q^{Ala}Q^{Orn})_2Q^{Asp}Q^{Orn}$-OH in DMSO-$d_6$. *Solid triangles* indicate the 11 amide NH signals of the 12mer.

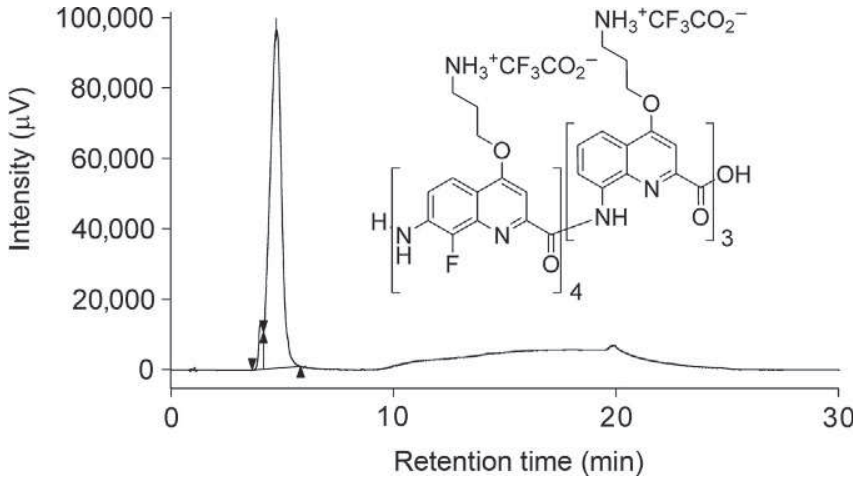


Fig. 10 Analytical RP-HPLC chromatogram (System B) of the 7mer $\left(Q_F{}^{Orn}\right)_4\left(Q^{Orn}\right)_3-OH$ (structure shown in insert) after purification by preparative RP-HPLC.

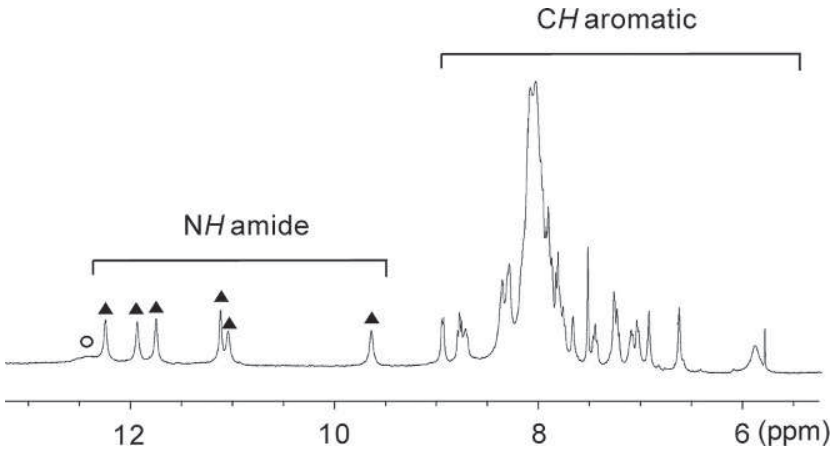


Fig. 11 Excerpt of the ^{1}H NMR spectrum (400 MHz) at 298 K of $H_2N-\left(Q_F{}^{Orn}\right)_4\left(Q^{Orn}\right)_3-$ OH in DMSO-d_6. *Solid triangles* and the *blank circle* indicate the six amide signals and the carboxylic acid signal of the 7mer, respectively. Note in this solvent, the oligomer is in single helical form (Shang et al., 2014).

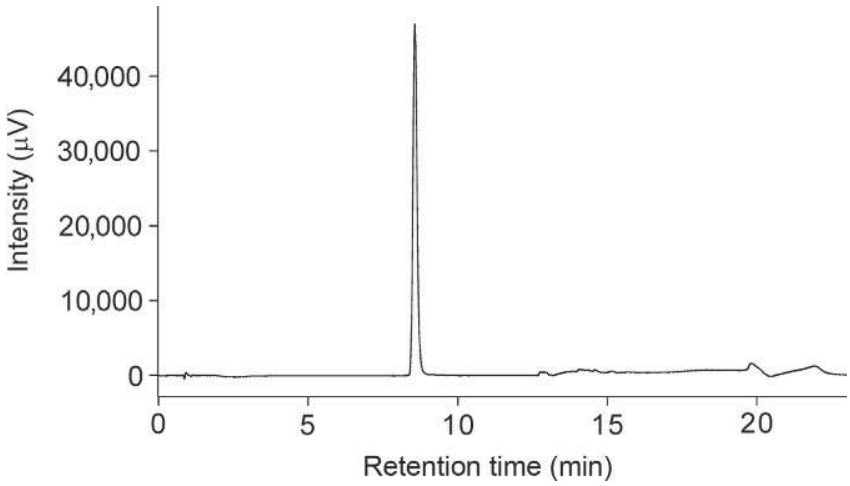


Fig. 12 Analytical RP-HPLC chromatogram (System C) of Ac-K(Q^{Orn})$_2$A(Q^{Orn})$_2$D(Q^{Orn})$_2$S(Q^{Orn})$_2$-OH after purification by preparative RP-HPLC.

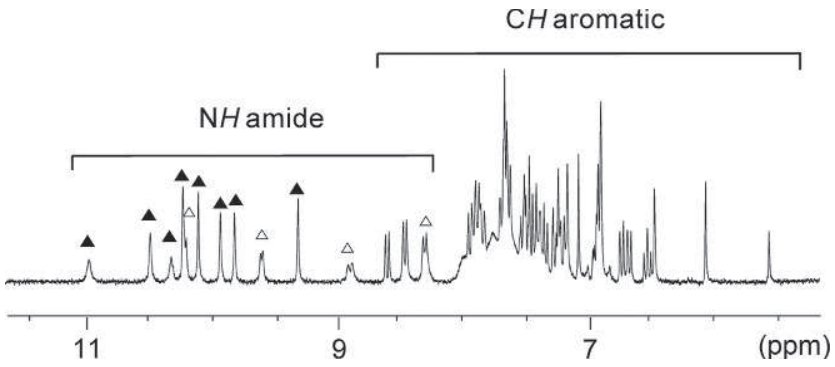


Fig. 13 Excerpt of the ^{1}H NMR spectrum (800 MHz) at 298 K of Ac-K(Q^{Orn})$_2$A(Q^{Orn})$_2$D(Q^{Orn})$_2$S(Q^{Orn})$_2$-OH in D_2O/H_2O (1:9). *Solid* and *hollow triangles* indicate amide signals from eight aromatic and four aliphatic amines of the 12mer, respectively.

REFERENCES

Appella, D. H., Christianson, L. A., Karle, I. L., Powell, D. R., & Gellman, S. H. (1996). β-peptide foldamers: Robust helix formation in a new family of β-amino acid oligomers. *Journal of the American Chemical Society*, *118*, 13071–13072.

Bao, C., Kauffmann, B., Gan, Q., Srinivas, K., Jiang, H., & Huc, I. (2008). Converting sequences of aromatic amino acid monomers into functional three-dimensional structures: Second-generation helical capsules. *Angewandte Chemie International Edition*, *47*, 4153–4156.

Baptiste, B., Douat-Casassus, C., Laxmi-Reddy, K., Godde, F., & Huc, I. (2010). Solid phase synthesis of aromatic oligoamides: Application to helical water-soluble foldamers. *The Journal of Organic Chemistry*, *75*, 7175–7185.

Barnard, A., Long, K., Martin, H. L., Miles, J. A., Edwards, T. A., Tomlinson, D. C., et al. (2015). Selective and potent proteomimetic inhibitors of intracellular protein–protein interactions. *Angewandte Chemie International Edition*, *54*, 2960–2965.

Bassani, D. M., Lehn, J.-M., Baum, G., & Fenske, D. (1997). Designed self-generation of an extended helical structure from an achiral polyheterocylic strand. *Angewandte Chemie International Edition in English*, *36*, 1845–1847.

Berl, V., Huc, I., Khoury, R., Krische, M., & Lehn, J.-M. (2000). Interconversion of single and double helices formed from synthetic molecular strands. *Nature*, *407*, 720–723.

Buratto, J., Colombo, C., Stupfel, M., Dawson, S. J., Dolain, C., Langlois d'Estaintot, B., et al. (2014). Structure of a complex formed by a protein and a helical aromatic oligoamide foldamer at 2.1 Å resolution. *Angewandte Chemie International Edition*, *53*, 883–887.

Chandramouli, N., Ferrand, Y., Lautrette, G., Kauffmann, B., Mackereth, C. D., Laguerre, M., et al. (2015). Iterative design of a helically folded aromatic oligoamide sequence for the selective encapsulation of fructose. *Nature Chemistry*, *7*, 334–341.

Claerhout, S., Ermolat'ev, D. S., & Van der Eycken, E. V. (2008). A new colorimetric test for solid-phase amines and thiols. *Journal of Combinatorial Chemistry*, *10*, 580–585.

Delaurière, L., Dong, Z., Laxmi-Reddy, K., Godde, F., Toulmé, J.-J., & Huc, I. (2012). Deciphering aromatic oligoamide foldamer–DNA interactions. *Angewandte Chemie International Edition*, *51*, 473–477.

Dolain, C., Grélard, A., Laguerre, M., Jiang, H., Maurizot, V., & Huc, I. (2005). Solution structure of quinoline- and pyridine-derived oligoamide foldamers. *Chemistry A European Journal*, *11*, 6135–6144.

Ferrand, Y., Chandramouli, N., Kendhale, A. M., Aube, C., Kauffmann, B., Grélard, A., et al. (2012). Long-range effects on the capture and release of a chiral guest by a helical molecular capsule. *Journal of the American Chemical Society*, *134*, 11282–11288.

Gan, Q., Bao, C., Kauffmann, B., Grélard, A., Xiang, J., Liu, S., et al. (2008). Quadruple and double helices of 8-fluoroquinoline oligoamides. *Angewandte Chemie International Edition*, *47*, 1715–1718.

Garric, J., Léger, J.-M., & Huc, I. (2005). Molecular apple peels. *Angewandte Chemie International Edition*, *44*, 1954–1958.

Gillies, E., Deiss, F., Staedel, C., Schmitter, J.-M., & Huc, I. (2007). Development and biological assessment of fully water-soluble helical aromatic amide foldamers. *Angewandte Chemie International Edition*, *46*, 4081–4084.

Guichard, G., & Huc, I. (2011). Synthetic foldamers. *Chemical Communications*, *47*, 5933–5941.

Hamuro, Y., Geib, S. J., & Hamilton, A. D. (1996). Oligoanthranilamides. Non-peptide subunits that show formation of specific secondary structure. *Journal of the American Chemical Society*, *118*, 7529–7541.

Hu, X., Dawson, S. J., Kudo, M., Nagaoka, Y., Tanatani, A., & Huc, I. (2016). Solid-phase synthesis of water-soluble helically folded hybrid α-amino acid/quinoline oligoamides. *The Journal of Organic Chemistry*, *81*, 1137–1150.

Huc, I. (2004). Aromatic oligoamide foldamers. *European Journal of Organic Chemistry*, 17–29.

Iriondo-Alberdi, J., Laxmi-Reddy, K., Bouguerne, B., Staedel, C., & Huc, I. (2010). Cellular internalization of water-soluble helical aromatic amide foldamers. *ChemBioChem*, *11*, 1679–1685.

Jiang, H., Léger, J.-M., Dolain, C., Guionneau, P., & Huc, I. (2003a). Aromatic delta-peptides: Design, synthesis, and structural studies of helical, quinoline-derived oligoamide foldamers. *Tetrahedron*, *59*, 8365.

Jiang, H., Léger, J.-M., & Huc, I. (2003b). Aromatic delta-peptides. *Journal of the American Chemical Society*, *125*, 3448–3449.

König, H. M., Abbel, R., Schollmeyer, D., & Kilbinger, A. F. (2006). Solid-phase synthesis of oligo(p-benzamide) foldamers. *Organic Letters*, *8*, 1819–1822.

Kudo, M., Maurizot, V., Kauffmann, B., Tanatani, A., & Huc, I. (2013). Folding of a linear array of a-amino acids within a helical aromatic oligoamide frame. *Journal of the American Chemical Society*, *135*, 9628–9631.

Kudo, M., Maurizot, V., Masu, H., Tanatani, A., & Huc, I. (2014). Structural elucidation of foldamers with no long range conformational order. *Chemical Communications*, *50*, 10090–10093.

Li, X., & Yang, D. (2006). Peptides of aminoxy acids as foldamers. *Chemical Communications*, *2006*, 3367–3379.

Lokey, R. S., & Iverson, B. L. (1995). Synthetic molecules that fold into a pleated secondary structure in solution. *Nature*, *375*, 303–305.
Morales, G. A., Corbett, J. W., & DeGrado, W. F. (1998). Solid-phase synthesis of benzopiperazinones. *The Journal of Organic Chemistry*, *63*, 1172–1177.
Müller, S., Laxmi-Reddy, K., Jena, P. V., Baptiste, B., Dong, Z., Godde, F., et al. (2014). Targeting DNA G-quadruplexes with helical small molecules. *ChemBioChem*, *15*, 2563–2570.
Murphy, N. S., Prabhakaran, P., Azzarito, V., Plante, J. P., Hardie, M. J., Kilner, C. A., et al. (2013). Solid-phase methodology for synthesis of O-alkylated aromatic oligoamide inhibitors of α-helix-mediated protein–protein interactions. *Chemistry A European Journal*, *19*, 5546–5550.
Nair, R. V., Vijayadas, K. N., Roy, A., & Sanjayan, G. J. (2014). Heterogeneous foldamers from aliphatic–aromatic amino acid building blocks: Current trends and future prospects. *European Journal of Organic Chemistry*, 7763–7780.
Nelson, J. C., Saven, J. G., Moore, J. S., & Wolynes, P. G. (1997). Solvophobically driven folding of nonbiological oligomers. *Science*, *277*, 1793–1796.
Nielsen, P. E., Egholm, M., Berg, R. H., & Buchardt, O. (1991). Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science*, *254*, 1497–1500.
Puckett, J. W., Green, J. T., & Dervan, P. B. (2012). Microwave assisted synthesis of Py-Im polyamides. *Organic Letters*, *14*, 2774–2777.
Qi, T., Maurizot, V., Noguchi, H., Charoenraks, T., Kauffmann, B., Takafuji, M., et al. (2012). Solvent dependence of helix stability in aromatic oligoamide foldamers. *Chemical Communications*, *48*, 6337–6339.
Salaun, A., Potel, M., Roisnel, T., Gall, P., & Le Grel, P. (2005). Crystal structures of aza-beta3-peptides, a new class of foldamers relying on a framework of hydrazinoturns. *The Journal of Organic Chemistry*, *70*, 6499–6502.
Seebach, D., Overhand, M., Kühnle, F. N. M., Martinoni, B., Oberer, L., Hommel, U., et al. (1996). β-peptides: Synthesis by *Arndt-Eistert* homologation with concomitant peptide coupling. Structure determination by NMR and CD spectroscopy and by X-ray crystallography. Helical secondary structure of a β-hexapeptide in solution and its stability towards pepsin. *Helvetica Chimica Acta*, *79*, 913–941.
Semetey, V., Rognan, D., Hemmerlin, C., Graff, R., Briand, J.-P., Marraud, M., et al. (2002). Stable helical secondary structure in short-chain N,N′-linked oligoureas bearing proteinogenic side chains. *Angewandte Chemie International Edition*, *41*, 1893–1895.
Shang, J., Gan, Q., Dawson, S. J., Rosu, F., Jiang, H., Ferrand, Y., et al. (2014). Self-association of aromatic oligoamide foldamers into double helices in water. *Organic Letters*, *16*, 4992–4995.
Shi, R., Wang, F., & Yan, B. (2007). Site–site isolation and site–site interaction—Two sides of the same coin. *International Journal of Peptide Research and Therapeutics*, *13*, 213–219.
Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., et al. (1992). Peptoids: A modular approach to drug discovery. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 9367–9371.
Singleton, M. L., Pirotte, G., Kauffmann, B., Ferrand, Y., & Huc, I. (2014). Increasing the size of an aromatic helical foldamer cavity by strand intercalation. *Angewandte Chemie International Edition*, *53*, 13140–13144.
White, P. D., & Chan, W. C. (2000). Basic procedures. In P. D. White & W. C. Chan (Eds.), *Fmoc solid phase synthesis: A practical approach*.Oxford: Oxford University Press. p. 44.
Wurtz, N. R., Turner, J. M., Baird, E. E., & Dervan, P. B. (2001). Fmoc solid phase synthesis of polyamides containing pyrrole and imidazole amino acids. *Organic Letters*, *3*, 1201–1203.
Zhu, J., Parra, R. D., Zeng, H., Skrzypczac-Jankun, E., Zeng, X. C., & Gong, B. (2000). A new class of folding oligomers: Crescent oligoamides. *Journal of the American Chemical Society*, *122*, 4219–4220.