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Solid Phase Synthesis of Aromatic Oligoamides: Application to Helical Water-Soluble Foldamers

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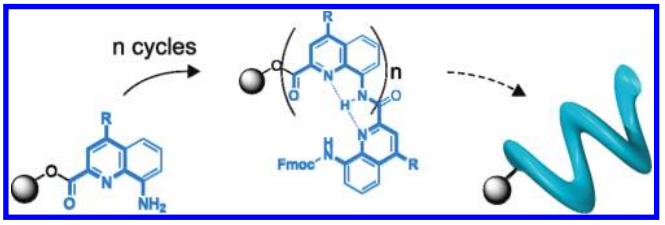

Synthetic helical aromatic amide foldamers and in particular those based on quinolines have recently attracted much interest due to their capacity to adopt bioinspired folded conformations that are highly stable and predictable. Additionally, the introduction of water-solubilizing side chains has allowed to evidence promising biological activities. It has also created the need for methods that may allow the parallel synthesis and screening of oligomers. Here, we describe the application of solid phase synthesis to speed up oligomer preparation and allow the introduction of various side chains. The synthesis of quinoline-based monomers bearing protected side chains is described along with conditions for activation, coupling, and deprotection on solid phase, followed by resin cleavage, side-chain deprotection, and HPLC purification. Oligomers having up to 8 units were thus synthesized. We found that solid phase synthesis is notably improved upon reducing resin loading and by applying microwave irradiation. We also demonstrate that the introduction of monomers bearing benzylic amines such as 6-aminomethyl-2-pyridinecarboxylic acid within the sequences of oligoquinolines make it possible to achieve couplings using a standard peptide coupling agent and constitute an interesting alternative to the use of acid chloride activation required by quinoline residues. The synthesis of a tetradecameric sequence was thus smoothly carried out. NMR solution structural studies show that these alternate aminomethyl-pyridine residues do not perturb the canonical helix folding of quinoline monomers in protic solvents, contrary to what was previously observed in nonprotic solvents.

Introduction

Solid phase synthesis (SPS) is commonly used to prepare different kinds of peptidic aliphatic foldamers using procedures similar to those developed for α-peptides. For example, Seebach et al. synthesized the first foldamers based on β^3-amino acids on solid support.[1] Gellman et al. used SPS to prepare 14-helical foldamers combining β^3-amino acids and constrained (cyclic) β-amino acids,[2] and Royo et al. described the SPS of constrained γ-peptides.[3] Guichard et al. reported the SPS of oligoureas[4] and the elucidation of their helical conformation on solid support.[5] Peptoids, which are

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also known to fold into helices, have been prepared on solid support as well.[6]

SPS has less commonly been applied to aromatic oligoamide sequences, and in all cases the reduced reactivity of aromatic amines as compared to aliphatic amines had to be overcome. In the synthesis of pyrrole- and imidazole-based oligoamides, Dervan et al. observed incomplete couplings to imidazole amines when using acids activated with the conventional coupling reagent HBTU. A complete acylation of the imidazole amine could be restored when the authors applied the mixed anhydride activations, in the presence of, e.g., Boc-pyrrole anhydride.[7] Similarly, Kilbinger et al. did not observe successful couplings of aromatic secondary amines when using traditional solid phase coupling procedures, and in their case, even mixed anhydride activation was inefficient.[8] They finally succeeded by using an activation method originally reported by Ueda et al.[9] involving $SOCl_2$ (1 equiv) in NMP leading to the formation of an acid chloride under mild conditions. This work was recently extended by Wilson et al., who prepared aromatic−aliphatic tertiary amides that project their side chains in a way similar to that of an α-helix.[10]

To the best of our knowledge, there has been no report on the SPS of stable *helical* aromatic amide foldamers. The need to develop and extend the scope of SPS of aromatic oligoamides is becoming obvious with the emergence of multiple potential biological applications of these compounds.[11−14] Indeed, the optimization of biological activities often proceeds through the parallel synthesis of multiple structural variants possessing different proteinogenic side chains, which is best achieved on solid phase, as recognized by others.[10] However, as mentioned above, synthesis of aromatic oligomers is rendered more difficult than for aliphatic peptides both in solution and in the solid phase because of the poor nucleophilicity of some aromatic amines. It is not uncommon that strong acid activation such as an acid chloride is required for coupling to proceed.[15] This difficulty is particularly serious for helically folded structures for which folding in the reaction mixture results in steric hindrance, leading to slower reactions and generally poorer yields, in particular when coupling together two long sequences.[16] Synthetic problems associated with folding and aggregation are notorious in the SPS of long aliphatic α-peptides as well.[17] In the case of aromatic oligoamide foldamers, these problems are not easy to solve because the folded structures are generally extremely stable and do not denature under ambient conditions.[14a,15a,16,18] A solution has been proposed that consists of temporarily changing some secondary amides into tertiary amides so as to disrupt the folded structures and reduce hindrance.[19] Alternatively, we have shown that some benzylic amines could be made compatible with the folding modes of aromatic oligoamides foldamers and bring both a higher nucleophilicity and a higher flexibility, which in turn allowed the solution synthesis of long oligomers.[20]

With the aim of speeding up the preparation of helically folded quinoline-derived aromatic amide oligomers and to facilitate the introduction of multiple proteinogenic side chains in their sequences in view of potential biological applications, we have investigated and now report the methods for their synthesis on solid phase. Three different side chains were introduced within the foldamer sequences: a leucine-like, an ornithine-like, and an asparate-like side chain. Protocols for the synthesis of rigid, purely aromatic backbones have been optimized using acid chloride activation. Alternate and easier routes that make use of some aliphatic (benzylic) amines in the sequences and conventional coupling agents have also been validated. Furthermore, because this later approach generates more flexible backbones, structural studies in solution have been carried out to verify the helical folding of the resulting oligomers in protic solvents and thus confirm the appropriateness of aliphatic amines in this context.

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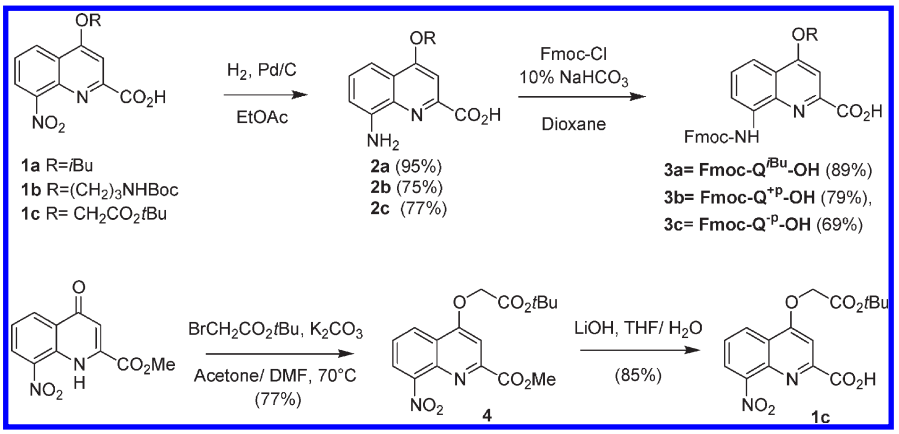
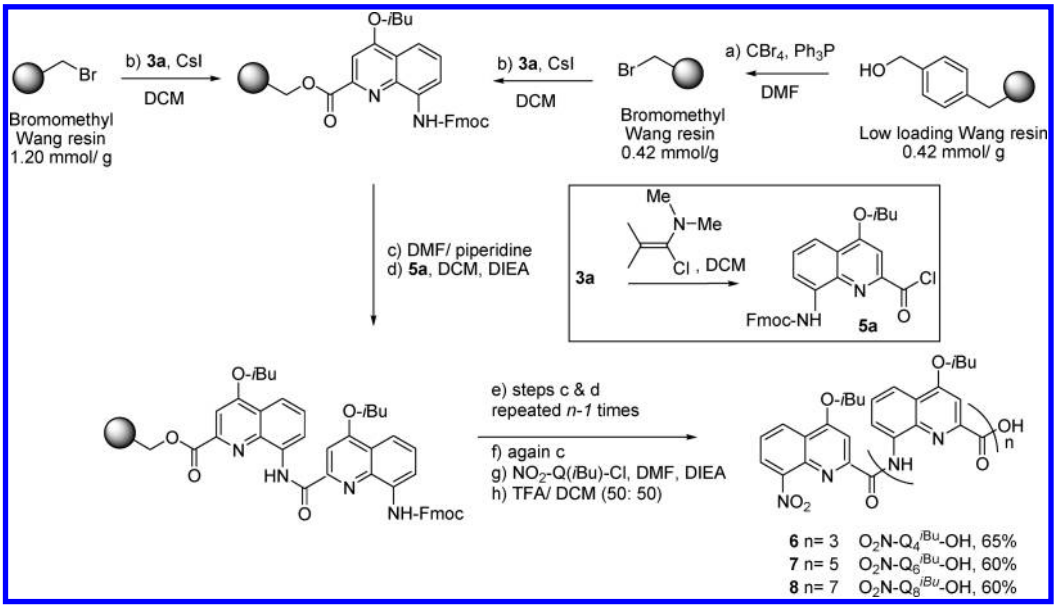
SCHEME 1. Synthesis of *N*-Fmoc-Quinoline Monomers 3a, 3b, and 3c[a]



[a]Throughout the manuscript, quinoline monomers are symbolized by "Q" and their side chains in position 4 by superscript *i*Bu, +p, and −p for isobutoxy, -O(CH$_2$)$_3$-NHBoc, and -OCH$_2$-CO$_2$*t*Bu, respectively, where p stands for "protected".

SCHEME 2. Solid Phase Oligoquinoline Synthesis on Bromomethyl Wang Resin



Results and Discussion

Solid Phase Synthesis of Aromatic, Quinoline-Based Oligoamides. Helically folded quinoline oligoamides bearing isobutoxy side chains (Q^{iBu}_n) have been synthesized, thoroughly characterized, and introduced in multiple foldamer architectures.[15a,16,20] This motif was thus chosen as a model system for first investigating SPS. The TFA-labile Wang resin (normal loading of 1.20 mmol/g) was selected as a starting resin as it offers the possibility to synthesize oligomers using an Fmoc-type strategy. For this purpose, we needed to first prepare an Fmoc-protected 8-amino-2-quinolinecarboxylic acid monomer bearing an isobutoxy side chain in position 4 (monomer **3a** in Scheme 1). Hence, the previously reported quinoline acid **1a**[15a] was hydrogenated to give amine **2a**, which was then easily protected in the presence of Fmoc-chloride to give **3a** in 89% yield.

The first monomer **3a** was anchored to the commercial bromomethyl Wang resin in the presence of cesium iodide using conditions reported by Morales et al.[21] (Scheme 2). The Fmoc protecting group was easily removed under standard deprotection conditions (20% piperidine in DMF). The introduction of the second monomer gave rise to the problem of choosing a suitable acid activation method. Our experience with solution synthesis has been that coupling agents (e.g., HBTU, PyBOP) give satisfactory results only when coupling two monomers and do not perform as well when one of the reagents is a dimer or longer. Acid chloride activation, however, though less convenient, is generally high-yielding. Additionally, it can be carried out under neutral conditions compatible with the presence of acid-labile side-chain protecting groups when using the Ghosez reagent (1-chloro-*N*,*N*,2-trimethylpropenylamine).[22] Activation via acid chloride

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formation thus remains well adapted to quinoline amino acids presenting functional side chains with protecting groups, such as Boc or *tert*-butyl ester. Activation of **3a** using the Ghosez reagent and coupling the resulting acid chloride **5a** to the amine residue of the first resin-bound monomer was complete when using 2 equiv of acid chloride. Cycles of piperidine deprotection followed by acid chloride coupling were applied smoothly up to the tetramer, the last residue bearing a nitro group at the N-terminus. After TFA cleavage, the tetramer ($n = 3$ in Scheme 2) $Q^{iBu}{}_4$ was obtained in good yield (65% from the resin loading) and good purity of the crude product. Product purity was conveniently assessed using reverse phase HPLC analysis, even though this technique is unconventional for such highly hydrophobic compounds (see Supporting Information for details). Product identity was confirmed by comparison with a genuine sample obtained via solution phase synthesis, by LC−MS analysis, and by ^{1}H NMR (see Supporting Information).

This result prompted us to extend the use of SPS to the synthesis of longer sequences. However, when this methodology was first applied to the synthesis of the octamer (O_2N-$Q^{iBu}{}_8$-OH), HPLC analysis revealed the presence of oligomers with one or more deletions in non-negligible quantity (penta-, hexa-, and heptamers). Even when double couplings were carried out (i.e., 2 × 2 equiv of acid chloride **5a** without any deprotection in between), deletions persisted beyond the fourth residue, and the yield and purity of the octamer were unsatisfactory. It has been previously reported that the solid phase synthesis of foldamers differs from standard α-peptides in the sense that during the course of the synthesis, the increased folding onto the support is expected to influence the efficiency of coupling and/or deprotection reactions.[23] One possibility to circumvent these problems of steric hindrance, accessibility, and/or poor reactivity related to the aggregation/folding of the oligomer onto the support is to use a resin with a lower loading and a higher bead size. Thus, we decided to use the commercially available low loading Wang resin (0.48 mmol/g). First, the commercial hydroxymethyl group of the Wang resin was converted to a bromomethyl. Acylation steps were again optimized by doing a double coupling of each monomer after the formation of the tetramer. This proved successful as hexamer **7** and octamer **8** were obtained in this way with satisfactory yields (60%) and high purities, as confirmed by HPLC analysis and ^{1}H NMR (Scheme 2). These first results demonstrate two clear advantages of SPS over solution phase: the perspective of parallel synthesis of foldamer libraries for biological screening and rapidity. Indeed, each deprotection/coupling cycle amounts to 1 h (or 2 for the double couplings), which makes it possible to prepare an octamer in only 1 day.

Inspired by a report by Gellman et al.[2a] describing that microwaves improve the synthesis of 14-helical β-peptides on solid phase, we decided to examine the effects of microwaves on aromatic amine coupling. Microwaves were applied only during coupling steps and not during deprotection since the latter is carried out without any difficulty. THF was used as solvent in place of DCM as it makes it possible to heat to higher temperatures. The best microwave conditions found involved heating at 50 °C for 10 min with 50 W microwave power. Moreover, to reduce truncated oligomer formation, capping of potentially uncoupled amines in the presence of acetic anhydride was implemented after each coupling. HPLC analysis of $Q^{iBu}{}_6$ and $Q^{iBu}{}_8$ synthesized under these conditions did not reveal significant improvements in yield or purity. Nevertheless, the coupling times could be diminished by a factor of 3, thus rendering possible the synthesis of an octamer in half a day. The only limitation of microwave-based synthesis is the resin amount that can be loaded in the microwave glass reactor, which cannot be scaled up at will.

Encouraged by the successful SPS of Q^{iBu} oligomers, we tested this methodology to prepare oligomers bearing protected water-solubilizing side chains that may have biological applications.[14] Monomers bearing ornithine-like side chains (Q^+) protected with a Boc group (Q^{+p}) have already been described;[14] Fmoc-protected monomer **3b** was prepared using the same conditions as for **3a** (Scheme 1). In addition, a new anionic monomer (Q^-) bearing an aspartate-like side chain protected as a *t*Bu ester (Q^{-p}) was also prepared (Scheme 1). Fmoc-Q^{-p}-OH monomer **3c** was prepared starting with a Williamson alkylation of a nitro-quinolinone precursor in 77% yield. The delicate selective saponification of the methyl ester in the presence of the *t*Bu ester was conducted under mild conditions in the presence of LiOH to give **1c** in 85% yield, which was then converted to **3c** in 69% yield. Acid chloride activation of **3b** and **3c** was achieved with the Ghosez reagent, which left intact the *t*Bu and Boc side-chain protections, giving **5b** and **5c**, respectively.

We had planned to carry out SPS of oligoamides using these monomers on a resin that would allow resin cleavage under very mildly acidic conditions to avoid side-chain deprotection. Among the large variety of resins commercially available, the 2-chlorotrityl resin appeared to be well suited for this purpose. Oligomers of monomer **3c** were prepared to investigate the efficiency of protected oligoquinoline synthesis on this support. Monomer **3c** was introduced on a chlorotrityl resin under the conditions reported by Lipton et al.[24] After removal of Fmoc protecting groups, couplings were carried out as for the Wang resin using, e.g., acid chloride **5c** (Scheme 3). However, oligomer release in AcOH/TFE/DCM showed that sequences hardly grow beyond four units and that yields are very poor. It might be that acidity is too high during couplings, even in the presence of excess DIEA, or that the aromatic acid−resin bond is simply too labile and resin cleavage occurs during synthesis. In any case, the trityl resin proved unpractical, and we returned to the synthesis on low loading Wang resin of homologous oligomer $Q^-{}_6$ (**9**) and heterologous oligomers $(Q^+Q^-)_n$ (**10**, $n = 2$; **11**, $n = 3$). Syntheses were performed under the conditions optimized for $Q^{iBu}{}_8$ via acid chloride activation (Scheme 3). In the case of monomer **3b**, activation was carried out following the same procedure, but once formed the corresponding acid chloride **5b** was no longer soluble in DCM, and dry THF was used instead as the solvent for solid phase coupling (Scheme 3). We noticed that double couplings were useful as early as the third residue. Cleavage from the resin and side-chain deprotection was achieved using a TFA/TIS/H_2O mixture (95:2.5:2.5 v/v/v), which proved to be more efficient than the previously used TFA/DCM (1:1) mixture. Indeed, standard

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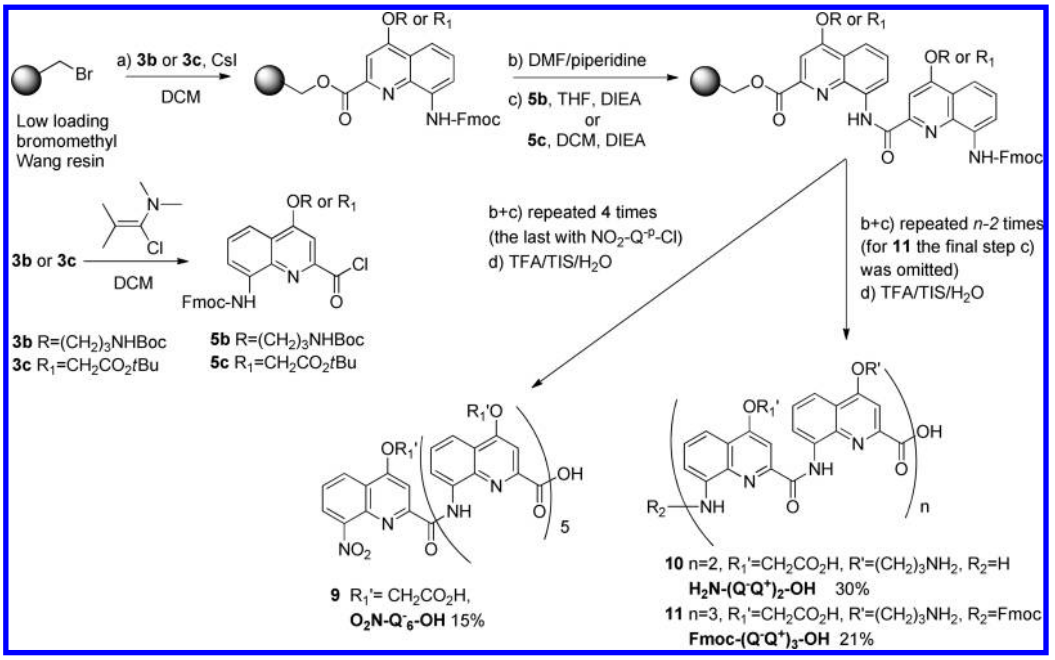
SCHEME 3. Solid Phase Synthesis of Water-Soluble Oligoquinolines



peptide RP-HPLC analysis revealed fewer byproducts when the tri-isopropyl-silane (TIS) scavenger was added. The method was validated to deliver various water-soluble oligomers possessing nitro (**9**), amino (**10**), or Fmoc (**11**) termini, in 20−30% isolated yields after HPLC purification.

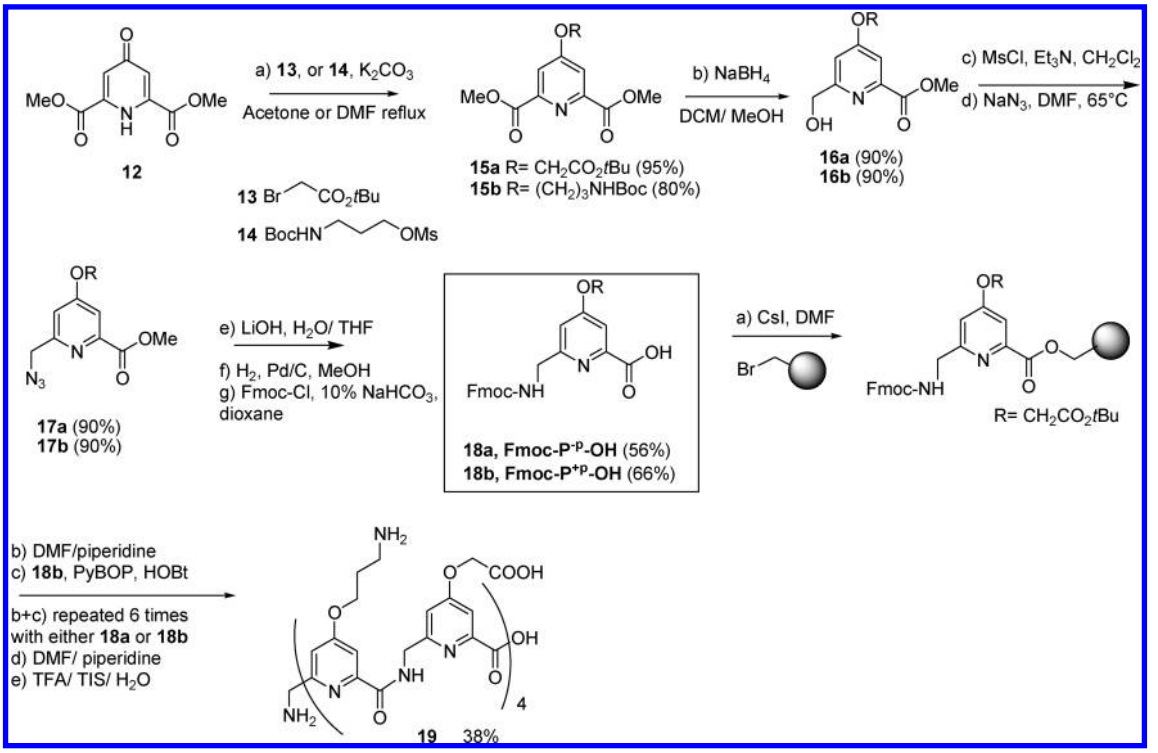
This first set of results makes it possible, in principle, to prepare libraries of short helical quinoline oligoamide foldamers via parallel synthesis on solid phase. Limitations reside in the need to use acid chloride activation, which is not the most convenient, and in average to low yields that do not allow easy access to oligomers longer than 8−10 units. To circumvent these limitations, we decided to explore the SPS of hybrid sequences composed of both fully aromatic aminoquinolinecarboxylic acid monomers and benzylic 6-aminomethyl-2-pyridinecarboxylic acid monomers (P), which we have shown to much improve solution phase synthesis while being able to adopt folded conformations compatible with the helices formed by Q_n monomers.[20]

Solid Phase Synthesis with 6-Aminomethyl-2-Pyridinecarboxylic Acid Units (P). P units (Scheme 4) are comparable to Q monomers in that they are δ-amino acids displaying the same set of atoms toward the inner rim of the folded helices. However, they introduce two major differences. Firstly, they provide more conformational flexibility than Q units. They thus decrease helix stability when introduced into Q_n sequences because of the loss of conjugation and additional rotatable bond about the methylene group and because of the reduced surface for $\pi-\pi$ stacking interactions provided by the pyridine ring as compared to the quinoline ring. Such a decrease in helix stability is expected to reduce steric hindrance during synthesis. Secondly, benzylic amines as in P units are considerably more nucleophilic than those of 8-aminoquinoline monomers, which allows facile synthesis using standard coupling reagents as was shown in the solution preparation of sequences composed of up to 40 units. [20]

Hence, following the previously reported solution synthesis of $P^{iBu}{}_8$, we first considered the SPS of a hydrophilic $(P^+P^-)_4$ oligomer. Starting from chelidamic dimethyl ester **12**, a reaction scheme similar to that of Boc-P^{iBu}-OH monomer was applied to the synthesis of Fmoc-P-OH monomers **18a** and **18b** bearing either an asparate-like side chain (Fmoc-P^{-p}-OH) or an ornithine-like side chain (Fmoc-P^{+p}-OH) (Scheme 4). These monomers were purified using preparative scale HPLC; without this extra purification, we found that SPS worked very poorly. SPS was then carried out using classical peptide synthesis conditions. Once the first monomer **18b** was anchored on a low loading Wang resin, Fmoc-deprotection/coupling cycles were repeated alternatively with **18a** and **18b**. PyBOP, in combination with HOBt, was selected as a coupling agent. After TFA cleavage, the $(P^+P^-)_4$ oligomer **19** was easily isolated in relatively good yield (38%) and with a good purity as illustrated in the Supporting Information.

Previous structural studies had shown that $P^{iBu}{}_n$ oligomers do not adopt stable folded conformations in $CDCl_3$ despite the structural similarity between P and Q units, because of the greater flexibility imparted by the methylene bridges.[20a] We sought indications of folding of the $(P^+P^-)_4$ oligomer **19** in protic solvents (CD_3OH and H_2O/D_2O) with the idea that solvophobic effects could make aromatic stacking stronger in these media and promote folding. However, we could not find any evidence of it. The typical features which indicate folding of such oligomers were not observed, in particular upfield shifts of NMR resonances due to ring current effects and the spreading of NMR signals over a wide chemical shift range. On the other hand, previous studies have also shown that P units adopt the same folding mode as Q units provided that the fraction of the latter in the sequences is sufficiently large.[20a] We thus decided to explore the SPS of $(PQ)_n$ oligomers in which PQ segments are first prepared in solution and then coupled on solid support, presuming that coupling to aliphatic amines was sufficiently efficient to incorporate dimeric units at once, hence dividing the number of coupling steps on solid support by two to reach sequences of the same length.

SCHEME 4. Synthesis of Fmoc-Aminomethylpyridine Monomers and Rapid Synthesis of 2-Aminomethylpyridine Oligomers on Solid Support from Fmoc-Aminomethylpyridine Monomers



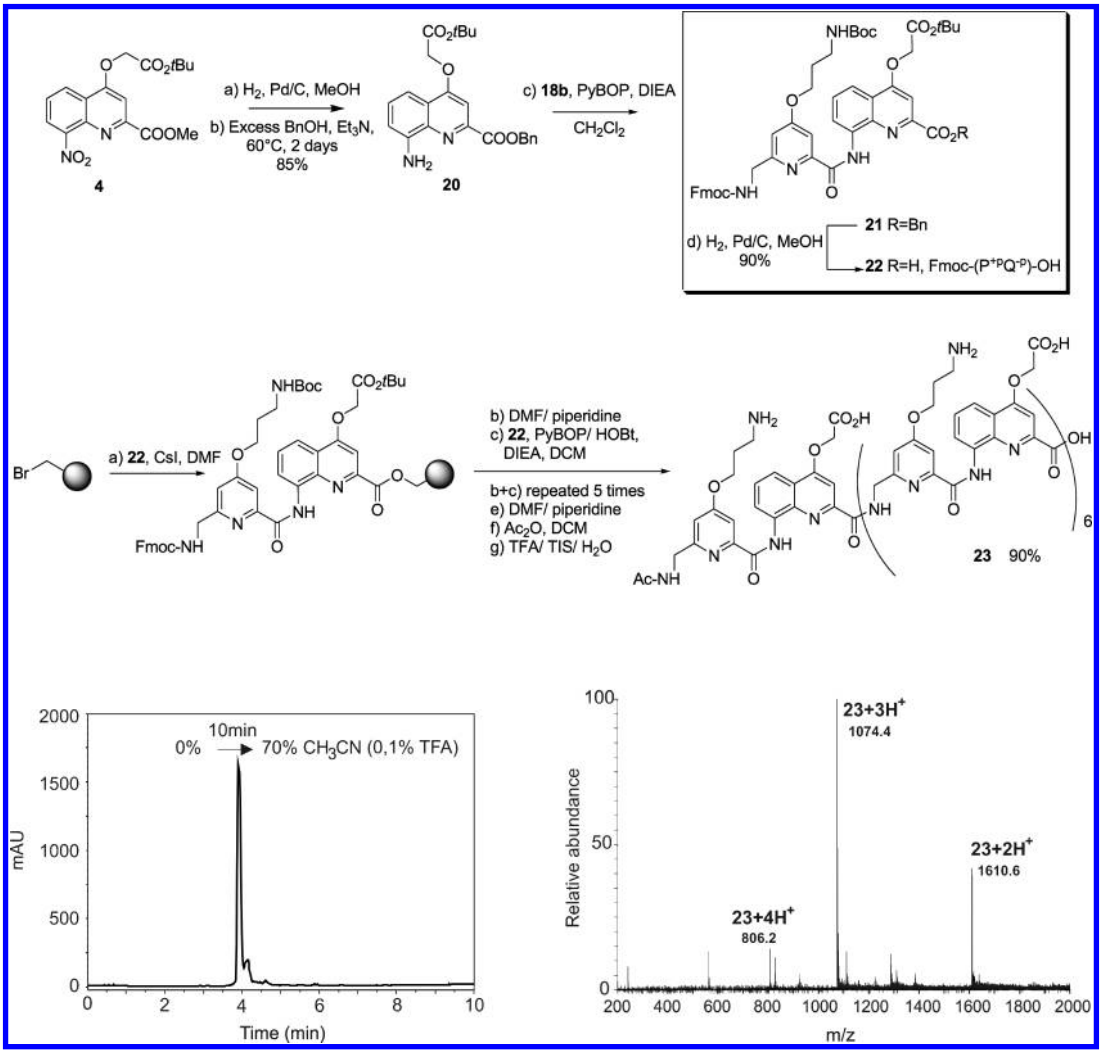
For this purpose, the water-soluble dimer Fmoc-$P^{+p}Q^{-p}$-OH **22** was synthesized as a new building block for SPS (Scheme 5). It was necessary to change the protection of the quinolinecarboxylic acid function as we encountered difficulties in selectively saponifying the methyl ester of Fmoc-$P^{+p}Q^{-p}$-OMe in the presence of the *t*Bu ester (not shown). We thus introduced a benzyl ester via a transesterification under mildly basic conditions (Scheme 5). PQ dimer **22** was obtained in good yield in two steps from amino-quinoline **20** and was engaged in SPS. As for P monomers, dimer **22** was HPLC purified on a preparative scale prior to SPS. After loading the first dimer onto the resin, six standard couplings proceeded smoothly up to $(P^+Q^-)_7$ (Scheme 5). The final amine was then acetylated. We noticed that the coupling of aminomethylpyridine residues was remarkably efficient, as only 2 equiv of Fmoc-$P^{+p}Q^{-p}$-OH was sufficient for each coupling step. After TFA cleavage, the 14-mer **23** was obtained with a crude purity of 90%. The isolated yield after HPLC purification was only 19%, suggesting that some material was lost in the process and that room for optimization still exists. Hence, despite the multiple steps for the preparation of PQ building block, this strategy allows the preparation of a 14-mer in only 6 efficient coupling steps on solid phase.

Solution Structural Investigation of $P^{+p}Q^{-p}$ Oligomers. We have previously described the very high stability of the helical conformation of sequences consisting exclusively of Q monomers in chlorinated or aromatic solvents[15a,25,26] and also found indirect evidence that these helices are at least as stable in protic media.[14a,b] This is for example illustrated by the sharpness, wide chemical shift distribution, and strong upfield shifts of ^{1}H NMR signals. Presumably, the solvophobic effects associated with intramolecular stacking of aromatic rings prevails in water and compensates for potentially weaker effects of hydrogen bonds or electrostatic repulsions due to competing interactions with the solvent. However, as mentioned above, solvophobic effects are not strong enough to promote the folding of oligomers consisting of P units exclusively. We thus considered it important to assess the solution conformation of $(PQ)_n$ oligomers in protic solvents to evaluate the extent to which P units may replace Q monomers in the sequences, thus making the SPS method described above a valid alternative to the more difficult SPS of Q_n oligomers. In chlorinated solvents, $(P^{i\mathrm{Bu}}Q^{i\mathrm{Bu}})_4$ has been shown to undergo a rapid equilibrium between several folded conformations: a canonical helix similar to that of Q_n oligomers and so-called "herringbone helices" in which consecutive PQ segments are perpendicular to each other (Figure 1).[20a] Each of these conformations gives rise to some unambiguous intramolecular NOE correlations, which makes it possible to distinguish them.

Structural studies on water-soluble $(PQ)_n$ oligomers were conducted on the fully cationic octamer H_2N-$(P^+Q^+)_4$-OMe to allow direct comparison with the structure of Boc-$(P^{i\mathrm{Bu}}Q^{i\mathrm{Bu}})_4$-OMe, whose structure was previously solved in $CDCl_3$ and in the solid state.[20a] H_2N-$(P^+Q^+)_4$-OMe was in fact prepared using solution phase synthesis, and its conformation was studied prior to optimizing SPS. Details for the solution phase protocols are reported in the Supporting Information. The structures of $Q^{i\mathrm{Bu}}{}_n$, $(P^{i\mathrm{Bu}}Q^{i\mathrm{Bu}})_4$, and $(P^{i\mathrm{Bu}}Q^{i\mathrm{Bu}}{}_4)_n$ oligomers have been consistently investigated in the solid state using X-ray crystallography, taking advantage of the high crystallizability of these compounds.[15,20,25] Solid state structural investigation was thus attempted on water-soluble oligomers but proved unsuccessful. Crystals with suitable

(25) Jiang, H.; Léger, J.-M.; Huc, I. *J. Am. Chem. Soc.* **2003**, *125*, 3448–3449.
(26) Dolain, C.; Grélard, A.; Laguerre, M.; Jiang, H.; Maurizot, V.; Huc, I. *Chem.—Eur. J.* **2005**, *11*, 6135–6144.

SCHEME 5. Preparation of an Fmoc-$P^{+p}Q^{-p}$-OH Building Block and Preparation of Ac-$(P^+Q^-)_7$-OH Using Standard SPS Procedures; RP-HPLC Chromatogram and ESI-MS Spectra of Ac-$(P^+Q^-)_7$-OH Oligomer after Release from the Support



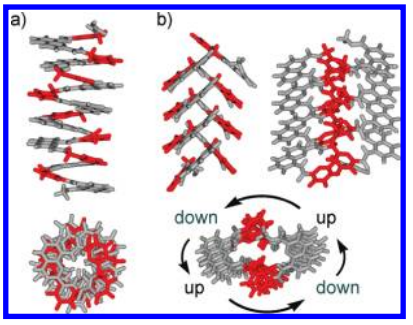


FIGURE 1. Side view and top view of energy minimized conformations (MacroModel v8.6; force field MM3) of $(PQ)_8$ as a canonical helix (a) and as a noncanonical "herringbone" helix (b). Amide and quinoline moieties are shown in gray; 6-aminomethyl-pyridine units are shown in red.

size and shape were obtained for Ac-$(P^+Q^-)_7$-OH **23** using various crystallization conditions, but diffraction was too poor to solve the structure, even under synchrotron radiation. Similar negative results were obtained with water-soluble Q_n oligomers. This might originate from the flexibility of the multiple ornithine-like residues susceptible to create entropic barriers to crystallization and to long-range order in the solid state. The structure of H_2N-$(P^+Q^+)_4$-OMe was thus studied in solution by NMR, both in CD_3OH and in H_2O/D_2O (90:10 v/v). Spectra were all measured at 50 °C where they were sharper, some slight broadness being observed at 25 °C. Results are similar in the two solvents; spectra recorded in H_2O/D_2O are slightly complicated by the slow hydrolysis of the methyl ester function (evidenced by NMR and mass spectrometry) and thus the emergence of a second set of signals that does not occur in methanol.

We initially attempted to carry out a full solution structural investigation. The spin systems of the different residues were identified in each solvent from combined HMQC, HMBC, COSY, and TOCSY experiments as we have previously reported.[20,26] For example, HMQC experiment allowed us to assign protons to either P or Q monomers (Figure SI-NMR 1 in Supporting Information). COSY and TOCSY experiments revealed scalar couplings between H5, H6, and H7 protons of the quinoline rings and some cross-peaks between H3 and H5 protons of the pyridine moieties (Figure 2a). HMBC experiments (Figure 2b and Figure SI-NMR 3−8 in Supporting Information) were performed to distinguish H5 from H7 protons on each quinoline and H3 from H5 on each pyridine ring.

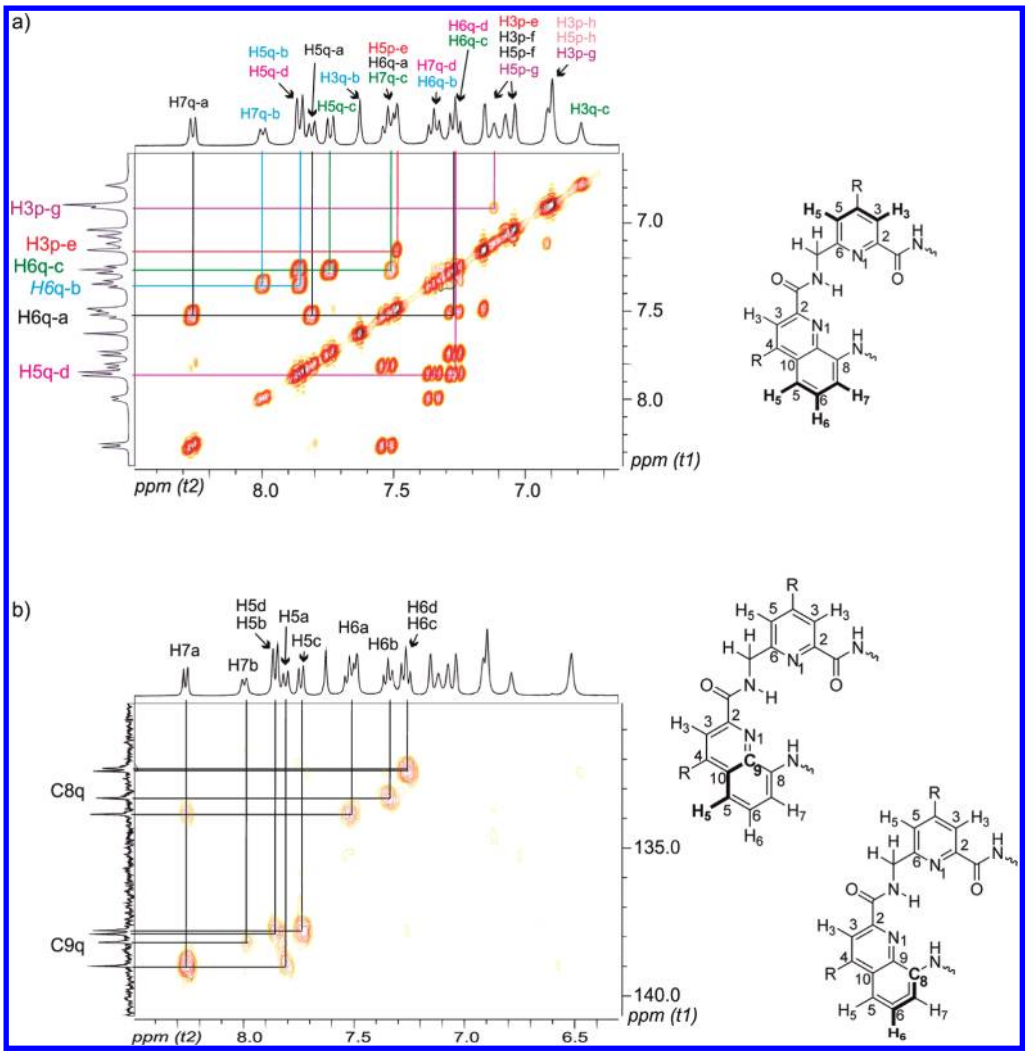


FIGURE 2. 2D NMR spectra of H_2N-$(P^+Q^+)_4$-OMe in CH_3OD at 323 K from a COSY (a) and an HMBC experiment (b). The structures on the right show the observed correlations (in bold).

Finally, the following long-range correlations allowed the complete assignment of each spin system: H6−C10 (J^3), H6−C8 (J^3), H5−C4 (J^3) for quinolines and H3-CO (J^3) for pyridines (Figure SI-NMR 3−7 in Supporting Information).

However, a few missing or overlapping correlations, in particular correlations between the amide carbon and amide proton, did not allow us to reconstitute the sequence of spin systems and fully assign ^1H and ^{13}C spectra, even when crossing information collected in water and methanol. Changing mixing times and using a 700 MHz instrument were unsuccessful. Information from NOE maps could only be used partially (see below). However, sufficient indirect evidence of folding could be collected to point with a high confidence to folding into a canonical helix of this sequence.

First of all, ^1H NMR signals spread over a wide chemical shift range and indicate substantial and selective upfield shifts as expected for a folded structure (Figure 3). Chemical shift values indicate that ring current effects are stronger in protic solvents than in $CDCl_3$. The signal of the terminal CH_3 ester is found at 3.51 ppm in $CDCl_3$ for $(P^{i\mathrm{Bu}}Q^{i\mathrm{Bu}})_4$-OMe and at 3.31 and 3.01 ppm for H_2N-$(P^+Q^+)_4$-OMe in methanol and water, respectively. The signals of the main chain benzylic CH_2 signals are found in the 2.60−5.00 ppm range in protic solvents, compared with 4.7−5.6 in $CDCl_3$. These large differences may simply result from a better folding in protic solvents that would give rise to a more compact conformation but may also be the result of the prevalence of the canonical helix in which stacking interactions are more extensive throughout the structure, particularly for the benzylic CH_2 residues.

Most importantly, the benzylic CH_2 protons of the backbone show a diastereotopic pattern (Figure 3) both in water and methanol, indicating a slow exchange between right-handed and left-handed conformations, in contrast with the fast exchange observed in $CDCl_3$ between the right- and left-handed forms of both canonical and herringbone helices.[20a] Diastereotopicity is expressed by large $\Delta\delta$ values of 0.40, 1.02, and 1.17 ppm for the inner methylene units of the sequence in MeOH-d_3 at 323 K. These values compare well with those observed in $Q^{i\mathrm{Bu}}{}_4P^{i\mathrm{Bu}}{}_nQ^{i\mathrm{Bu}}{}_4$ oligomers, which have been shown to fold exclusively as canonical helices in chloroform.[20b] The diastereotopic patterns of H_2N-$(P^+Q^+)_4$-OMe are very sharp in water and broader in methanol, suggesting a slower exchange and so a higher stability in water. The folded structure thus appears to be substantially more stable in protic solvents than in $CDCl_3$. It is unlikely, though formally not impossible, that fast equilibria might exist between several types of folded structures (e.g., a

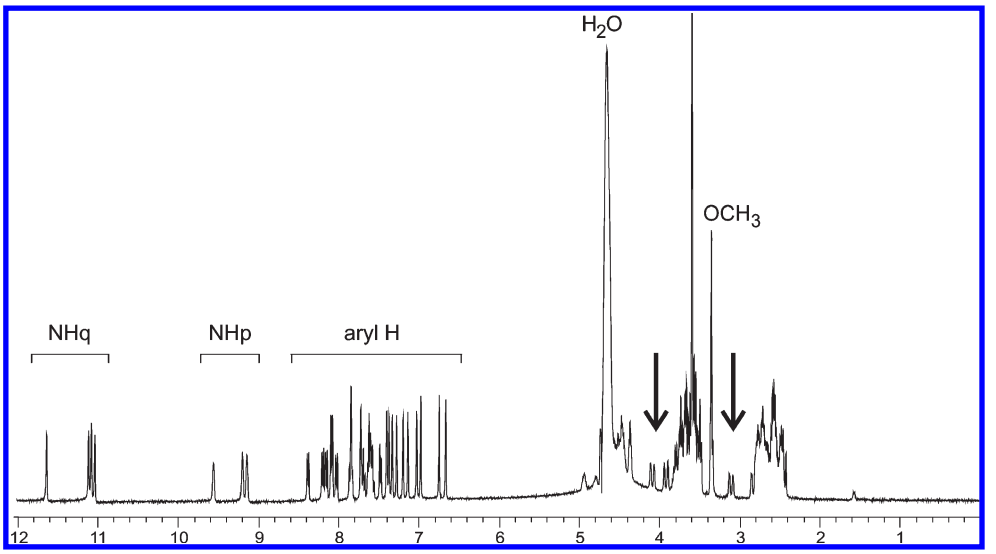


FIGURE 3. ^{1}H NMR spectrum of H_2N-$(P^+Q^+)_4$-OMe in H_2O/D_2O (90:10 v/v) at 323 K. The arrows indicate clear diastereotopic patterns of the signals of the backbone benzylic CH_2 protons.

canonical helix and herringbone helices), while slow equilibrium is observed between the right- and left-handed conformations. The diastereotopic patterns therefore hint at the strong prevalence of one stable conformation in protic solutions, contrary to the mixture of conformers observed in $CDCl_3$. Further support comes from an intense NOE correlation between the terminal CH_3 ester and a single H3 proton belonging to a quinoline residue remote in the sequence. Such a correlation is, in principle, compatible with both a herringbone helix and a canonical helix conformation, but it should occur with two distinct H3 protons. The observation of a single correlation again suggests the prevalence of one conformation.

Finally, another indication that the canonical helix prevails in protic solvents was provided by the pattern of NOE correlations between aromatic and aliphatic amide signals (Figure 4). Two amide protons consecutive in a sequence are expected to produce an NOE correlation when folded in a canonical helix, whereas in herringbone helices, this is true only across Q monomers for which both neighbor amides are in the plane of the quinoline ring and not across P monomers. The pattern of observed correlations, even though they cannot be precisely assigned, is fully consistent with a canonical helix. It shows that two aromatic amides correlate with two aliphatic amides, whereas another two aromatic amides (the N- and C-terminal aromatic amides) correlate with only one aliphatic amide. Conversely, each aliphatic amide correlates with two aromatic amides. Again, if both canonical and herringbone helices were coexisting, a larger number of correlations would be expected, and the same set of correlations could not be produced by a single herringbone helix alone.

Altogether, these data demonstrate that the folded structure of $(PQ)_n$ oligomers in protic solvents is both more stable and better defined than in aprotic medium. All patterns identified so far support the claim that this folded structure is a canonical helix. The insertion of P units in Q_n sequences without disturbing the overall helix conformation is thus better tolerated in water. It remains to be demonstrated whether changing a Q unit into a P unit having the same side chain would result in the same potential biological

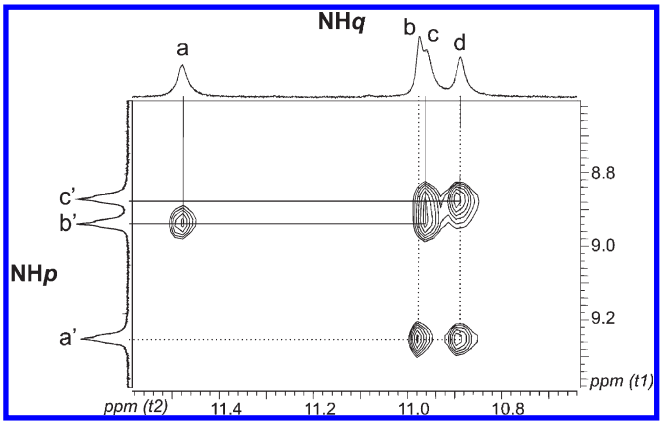


FIGURE 4. NOE correlations between the amide protons derived from aliphatic amines (NH_p) and amide protons derived from aromatic amines (NH_q). The following correlations are identified: a′−b, a′−d, b′−a, b′−c, c′−c, c′−d).

activity. In any case, the possibility to combine P and Q monomers expands the range of tools available for solid phase synthesis and provides highly modular sequences in which side chains can be modified without changing folding propensity and in which backbone stability can be modified without changing the overall conformation and the projection in space of the side chains.

Conclusion

We have shown that the judicious application of solid phase synthesis allows the rapid preparation of quinoline oligoamides equipped with different peptide-like side chains, thus paving the way for the parallel synthesis of oligomers relevant to screening assays. The poor nucleophilicity of aromatic amines combined with the stable folding of Q oligomers on the resin makes complete acylation difficult beyond the fourth residue, thus diminishing the purity of the crude material. Reducing the loading of the Wang resin circumvented this problem, and several Q_n oligomers were obtained in good yields. Furthermore, microwave irradiation efficiently speeds up the SPS of aromatic

oligoamides with a complete synthesis of $Q^{iBu}{}_8$ oligomer in only half a day. As a future prospect, the automation of this procedure would offer the possibility of rapidly generating libraries of Q_n oligomers.

Moreover, replacing some quinoline residues by 6-aminomethyl-2-pyridinecarboxylic acid type monomers P rendered possible the use of classical coupling agents, and the higher conformational flexibility of this unit abrogates the risk to observe truncated oligomers, thus extending the scope of SPS to oligomers much longer than 8 residues. Hence, by directly coupling presynthesized PQ dimers, we were able to synthesize a 14-mer with high efficiency as only 2 equiv of PQ were necessary for each coupling. Finally, a careful NMR investigation of a hydrophilic $(PQ)_4$ oligomer in protic solvents highlighted that a stable canonical helix prevails, unlike what was previously found in nonprotic solvents. This conformational behavior makes it possible to insert P units in place of Q without perturbing the conformation of the generated oligomers in protic solvents and thus validates the use of SPS with sequences combining both P and Q units bearing different side chains for biological purposes.

Experimental Section

General Procedures. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in deuterated solvents on 300, 400, or 700 MHz spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to the signal of the NMR solvent used. ^{1}H NMR splitting patterns with observed first-order coupling are designated as singlet (s), doublet (d), triplet (t), or quartet (q). Coupling constants (J) are reported in hertz. Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m) or broad (br). ^{13}C NMR spectra were recorded on 75 or 100 MHz spectrometers. Chemical shifts are reported in ppm (δ) relative to carbon resonances of the NMR solvent. Mass spectra (MS) were obtained using electrospray ionization

Dichloromethane was distilled from CaH_2. THF was dried by passing through a neutral alumina column system. Triethylamine (Et_3N) and *N*,*N*-diisopropylethylamine (DIEA) was distilled from CaH_2. All reagents and solvents were purchased from commercial suppliers and used without further purification. Wang resins, HOBT and PyBOP were obtained from Novabiochem (Switzerland). Trifluoroacetic acid (TFA) from Sigma-Aldrich was of spectrophotometric grade (99%). HPLC-quality acetonitrile (CH_3CN) and Milli-Q water were used for HPLC analyses and purification. Evaporations were conducted under reduced pressure at temperatures less than 40 °C unless otherwise noted. Reactions were monitored by analytical thin-layer chromatography using Merck silica gel 60 F254 plates. Compounds were visualized with a UV lamp (λ 254 nm). Flash column chromatography was performed under positive pressure using 40−60 μm silica gel (Merck) and the indicated solvents. Solid phase reactions were performed manually in glass reactor with mechanic stirring.

RP-HPLC analyses were carried out on a Thermo Spectra system using a Chromolith Performance RP-18e column (4.6 mm × 100 mm, 5 μm) at a flow rate of 3.0 mL min^{-1} with P1000 XR pumps, an AS3000 autosampler and a UV 6000 LP diode array detector. The solvent system was A = 0.1% TFA in H_2O and B = 0.1% TFA in CH_3CN using the indicated HPLC conditions. Column effluent was monitored by UV detection at 214 and 254 nm using a diode array detector. Semipreparative purifications of oligomers were performed on a semi-preparative HPLC using a C18 column (21.4 mm × 250 mm, 100 Å pore size, 5 μm). Column effluent was monitored by UV detection at 214 and 254 nm using a diode array detector.

Standard Protocol for Fmoc Removal. The Fmoc protecting group was removed with a solution of piperidine in DMF (1:4) for 3 min. After filtration, the procedure was repeated for 7 min, followed by washing (2× each) with DMF, MeOH, and DCM. The chloranil test showed the presence of free amine giving a green coloration to the resin.

General Procedure for Acid Chloride Activation. To a solution of **3a** (0.270 g, 0.56 mmol) in dry DCM (5 mL) under inert atmosphere was added 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (0.115 mL, 1,12 mmol). The reaction was stirred at room temperature for 2 h after which time the solvent was removed, and the sample was dried under vacuum line to give the corresponding acid chloride in quantitative yield, which was used without further purification. A proton NMR was generally recorded to check purity. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.16 (d, J = 6.7, 6H), 2.31 (m, 1H), 4.06 (d, J = 6.7, 2H), 4.39 (t, J = 7.3, 14.9, 1H), 4.55 (d, J = 7.3, 2H), 7.35 (td, J = 7, 14.4, 12H), 7.45 (td, J = 7, 14.4, 2H), 7.65 (td, J = 8.2, 16.1, 1H), 7.72 (d, J = 7.4, 2H), 7.80 (d, J = 7.4, 2H), 7.88 (dd, J = 1.2, 7.3, 1H), 8.46 (bp, 1H, NH), 9.34 (s, 1H).

Standard Protocol for TFA Cleavage. The resin was cleaved with a TFA/DCM mixture (5 mL, 1:1, v/v) for 1 h in the case of *i*Bu-type oligoquinolines and with a TFA/TIS/H_2O mixture (5 mL, 95:2.5:2.5, v/v/v) for oligoquinolines equipped with proteogenic-like side chains. After solvent coevaporation the oligomers were precipitated with MeOH except for oligoquinolines bearing ornithine-like side chains, where the precipitation was carried out with methyl-*tert*-butyl ether.

SPS of O_2N-$(Q^{iBu})_8$-OH (8). The Wang bromo resin (0.1 g, 0.046 mmol) was reacted with Fmoc-Q^{iBu}-OH **3a** (0.080 g, 0.092 mmol, 2 equiv relative to the resin loading) in the presence of cesium iodide (0.024 g, 0.092 mmol) and DIEA (16 μL, 0.092 mmol). The resin was gently shaken overnight. The reaction solution was removed by filtration. The resin was washed (2× each) with DMF, MeOH, and DCM and dried under vacuum. The Fmoc protecting group was removed according to the standard protocol. The resin was preswelled with DCM and acylated with Fmoc-Q^{iBu}-Cl **5a** (0.090 g, 0.092 mmol) in the presence of DIEA (33 μL, 0.19 mmol). After 90 min, the resin was washed (2× each) with DMF, MeOH, and DCM and then controlled with the chloranil test. The same acylation step was repeated five times, and finally the NO_2-Q^{iBu}-Cl acid chloride was introduced following the same procedure described above.

Microwave-Assisted SPS of O_2N-$(Q^{iBu})_8$-OH. The Wang bromo resin (0.1 g, 0.046 mmol) was placed in a modified polypropylene SPE tube (4 mL, Varian, top rim removed with a razor blade), suspended in DMF (1 mL), and Fmoc-Q^{iBu}-OH **3a** (0.043 g, 0.092 mmol, 2 equiv relative to resin loading) following by cesium iodide (0.024 g, 0.092 mmol) and DIEA (16 μL, 0.092 mmol) were added. The tube was then placed inside a glass 10 mL microwave reaction vessel. The vessel was placed in the microwave reactor (CEM Discover) and irradiated (50 W maximum power, 50 °C, ramp 5 min, hold 5 min). All microwave experiments were conducted at atmospheric pressure. The temperature was controlled by modulation of power, and the sample was cooled with compressed air. The tube was then removed from the reactor and washed as for the classical SPS procedure (see above). The removal of the Fmoc protecting group was done without the assistance of microwave as described in the standard protocol. The only difference is that the last washing was done twice with anhydrous THF. The following acylation steps were achieved by adding to the resin a solution of Fmoc-Q^{iBu}-Cl **5a** (0.045 g, 0.092 mmol) and DIEA (39 μL, 0.23 mmol) in anhydrous THF (2 mL). The tube containing the resin was again placed inside the glass 10 mL microwave reaction vessel, itself introduced in the microwave reactor and again irradiated (50 W maximum power, 50 °C, ramp 5 min, hold 5 min). After coupling, the resin was washed (2× each) with DMF, MeOH, DCM, and anhydrous THF. The same microwave

conditions were applied for each acylation step. The efficiency of coupling was monitored using the chloranil test. After each coupling, a capping step was carried out with a solution of acetic anhydride in DCM (2 mL, 1:1, v/v) for 5 min, and the resin was washed as before. From the tetramer and for longer oligomers, a double coupling was required to ensure good yields of acylation. The final coupling was carried out with NO_2-Q^{iBu}-Cl acid chloride.

SPS of NO_2-$(Q^{-p})_6$. The same procedure as described for the synthesis of **8** was applied with the exception that the coupling time was increased to 4 h. **NO_2-$(Q^{-p})_6$-OH (9)**: ^{1}H NMR (CD_3OD, 300 MHz) δ 5.02 (2s, 2H), 5.10 (4s, 4H), 5.11 (4s, 4H), 5.28 (s, 2H), 6.39 (s, 1H), 6.42 (s, 1H), 6.51 (s, 1H), 6.71 (s, 1H), 6.82 (s, 1H), 7.12 (s, 1H), 7.38−7.57 (m, 4H), 7.60−7.81 (m, 4H), 7.90 (d, J = 9.82, 1H), 7.99−8.18 (m, 5H), 8.32 (d, J = 6.80, 1H), 8.55 (d, 1H), 8.75 (d, J = 7.74, 1H), 8.90 (d, J = 7.37, 1H), 11.38 (2s, 2H), 11.46 (s, 1H), 11.57 (s, 1H), 11.73 (s, 1H). HRMS (ESI) m/z calcd for $C_{72}H_{46}N_{12}O_{27}^{2-}$, 1510.2606; found Mass 755.1354 $[M - 2H]^{2-}$.

SPS of H_2N-$(Q^{-p}Q^{+p})_2$. The same procedure as described for the synthesis of **8** was applied with the exception that THF was used as coupling solvent in acylation step involving Fmoc-Q^{+p}-Cl **5c** and the coupling time was increased to 4 h. Double couplings were conducted starting from the third residue. **H_2N-$(Q^-Q^+)_2$-OH (10)**: ^{1}H NMR (CD_3OD, 300 MHz) δ 2.22−2.56 and 3.20−3.57 (m, 12H), 4.05−4.33 (m, 4H), 5.04 (d, J = 7.05, 1H), 5.99 (d, J = 7.60, 1H), 6.28 (s, 1H), 6.53 (s, 1H), 7.00 (1s, 1H), 7.06 (t, J = 7.57, 15.94, 1H), 7.19 (t, J = 7.92, 15.80, 1H), 7.44 (1d, J = 8.12), 7.61 (1s, 1H), 7.76 (1 m, 4H), 8.12 (1d, J = 8.32, 1H), 8.87 (1 m, 1H), 11.19 (s, 1H), 11.48 (s, 1H), 12.09 (s, 1H). HRMS (ESI) m/z calcd for $C_{50}H_{45}N_{10}O_{13}^+$, 993.3162; found Mass 993.3240 $[M + H]^+$.

SPS of Fmoc-$(Q^{-p}Q^{+p})_3$. The same procedure as described for the preparation of resin-bound **10** was applied. **Fmoc-$(Q^-Q^+)_3$-OH (11)**: ^{1}H NMR (DMSO-d_6/acetone-d_6 (80:20), DMSO was used as reference, 300 MHz) δ 1.99−2.11 (m, 12H), 3.07−3.16 (m, 12H), 3.82−5.33 (m, 24H), 6.15 (s, 1H), 6.44 (s, 1H), 6.67 (s, 2H), 6.75 (s, 1H), 6.86 (s, 1H), 6.98 (t, 1H), 7.10 (t, J = 7.37, 1H), 7.19 (s, 1H), 7.24−8.28 (m, 23H), 8.49 (d, 1H), 11.16 (s, 1H), 11.32 (s, 1H), 11.38 (s, 1H), 11.52 (s, 1H), 11.60 (s, 1H). HRMS (ESI) m/z calcd for $C_{90}H_{76}N_{15}O_{21}^+$, 1702.5335; found Mass 1702.5239 $[M + H]^+$.

SPS of Fmoc-$(P^{-p}P^{+p})_4$. The Wang bromo resin (0.1 g, 0.048 mmol) was acylated with Fmoc-P^{+p}-OH **18b** (0.05 g, 0.096 mmol, 2 equiv relative to the resin loading) in the presence of cesium iodide (0.024 g, 0.096 mmol) and DIEA (16 μL, 0.096 mmol). The resin was gently shaken overnight. Solvents were removed by filtration and the resin was washed (2× each) with DMF, MeOH, and DCM. The Fmoc protecting group was removed following the standard protocol The trinitrobenzenesulfonylchloride (TNBS) test shows the presence of free primary amine giving a red coloration to the resin. The resin was then acylated with Fmoc-P^{-p}-OH **18a** (0.046 mg, 0.096 mmol) in the presence of PyBOP (0.096 g, 0.192 mmol), HOBt (0.025, 0.192 mmol), and DIEA (33 μL, 0.19 mmol). After 4 h, solvents were removed by filtration. The resin was washed as before, and then controlled with the TNBS test. The same acylation step was alternatively repeated six times with either Fmoc-P^{+p}-OH **18b** or Fmoc-P^{-p}-OH **18a**. Finally, the Fmoc protecting group was removed before TFA cleavage. **H_2N-$(P^-P^+)_4$-OH (19)**: ^{1}H NMR (D_2O/H_2O 10:90 v/v, 300 MHz) δ 1.79−2.15 (m, 8H), 2.86−3.17 (m, 8H), 3.61−3.85 (m, 8H), 3.86−4.11 (m, 6H), 4.18 (s, 1H), 4.25 (s, 1H), 6.24 (d, J = 1.8, 1H), 6.27 (d, 2H), 6.38 (d, J = 1.9, 1H), 6.49 (d, J = 2, 1H), 6.58 (d, J = 1.8, 1H), 6.68 (d, 2H), 6.74 (d, J = 1.9, 1H), 6.76 (d, 1H), 6.80 (d, 1H), 6.83 (d, 1H), 7.00 (d, J = 1.9, 1H), 7.13 (d, 1H), 7.33 (d, J = 1.9, 1H), 7.61 (d, 1H), 9.01−9.13 (m, 3H), 9.43 (d, 1H), 9.63 (d, 1H), 9.80 (d, 1H), 9.86 (d, 1H). MS (ESI) m/z calcd for $C_{76}H_{87}N_{20}O_{25}^+$, 1679.61; found Mass 1680.81 $[M + D]^+$ (deuterated compound, see Supporting Information).

SPS of Ac-$(P^{+p}Q^{-p})_7$. The same procedure as described for the preparation of resin-bound **19** was applied, and Fmoc-$P^{+p}Q^{-p}$-OH **22** was used as the monomer to be coupled. After removal of the last Fmoc protecting group the free amine was acetylated with a solution of acetic anhydride in DCM (1:1, v/v) for 1 h at room temperature. **Ac-$(P^+Q^-)_7$-OH (23)**: ^{1}H NMR (D_2O/acetonitrile-d_6 (70:30), H_2O was used as reference, 300 MHz, 25 °C) δ 1.86 (s, 3H), 1.99−2.69 (m, 22H), 2.69−3.29 (m, 3H) 3.29−3.75 (m, 23H), 4.10−5.42 (m, 22H), 6.56 (s, 1H), 6.58 (2s, 2H), 6.68 (3s, 3H), 6.75 (2s, 2H), 6.78 (2s, 2H), 6.87 (2s, 2H), 6.93 (3s, 3H), 6.99 (2s, 2H), 7.01 (s, 1H), 7.08 (2s, 2H), 7.14 (s, 1H), 7.44−7.71 (13H), 7.79 (t, J = 8.12, 7.37, 1H), 7.99−8.23 (7H), 8.31 (3s, 3H), 8.44 (s, 1H), 8.59 (s, 1H), 8.76 (s, 1H), 9.36 (s, 1H), 10.46 (2s. 2H), 10.53 (s, 1H), 10.66 (s, 1H), 10.74 (s, 1H), 11.21 (s, 1H). MS (ESI) m/z calcd for $C_{156}H_{154}N_{35}O_{44}^{3+}$, 3221.09; found Mass 1074.17 $[M + 3H]^{3+}$.

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Supporting Information Available: Experimental details for the preparation of the different monomers, solution-phase synthesis of $(P^{iBu}Q^{iBu})_4$-OMe, structural NMR investigation, and characterization data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.