Peptidomimetics

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Development and Biological Assessment of Fully Water-Soluble Helical Aromatic Amide Foldamers**

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Much effort is being devoted to develop oligomers that adopt stable helical conformations and mimic the structures of folded biopolymers.[1] For example, bio-inspired helical oligomers based on aliphatic backbones such as β-peptides[1b,c,2–5] and peptoids[1e,6–9] may adopt helical conformations related to peptide α helices and polyproline type I helices, respectively. These compounds have attracted considerable interest because of their promising biological activity in a number of areas including the development of antibiotics[3,7] and gene delivery systems,[4,8] and the inhibition of protein–protein interactions.[5,9] This success was anticipated based on the close structural resemblance of the synthetic oligomers to α-peptides and their improved properties, such as a decreased susceptibility to proteolytic degradation.[10] Linear aromatic oligomers with amphipathic arene faces also show promising biological activity[11] and may be designed to mimic one face of a peptide α helix.[12] However, numerous classes of helical oligomers possess structures remote from those of peptides and may not, a priori, feature any peptide-like biological activity. For instance, secondary amide aromatic oligomers[1b] developed by us[13–16] and by others,[17] for example, oligomers of 8-amino-2-quinoline carboxylic acid, form helices that are wider, with a smaller helical pitch, and much more stable than those of peptides (Figure 1). These oligomers are receiving increasing attention because of the high predictability and tunability of their conformations. However, none has been made highly water-soluble, and their biological potential has remained unexplored. Here, we show that multiple ammonium side chains confer helical aromatic oligoamides with high water-solubility, and that some of their biological properties compare favorably with those of aliphatic peptide mimics even though their conformations differ considerably. Our results provide the groundwork for promising future biological applications of this and similar classes of molecules.

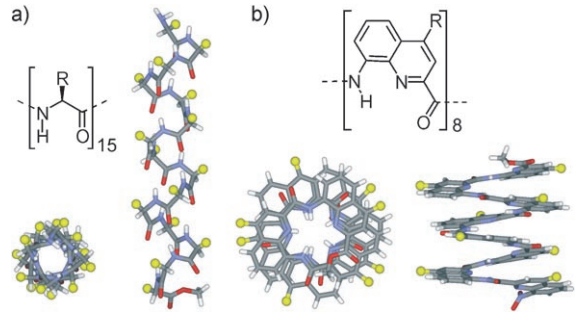

Figure 1. Top view and side views of fragments shown at the same scale of crystal structures of: a) a peptide α helix and b) a helix of an aromatic oligoamide foldamer. Side chains (R) are shown as yellow spheres. These structures illustrate the striking differences between the two motifs in terms of side-chain density, diameter, aspect ratio (at comparable molecular weight), helical pitch, and overall chemical composition.

Our first attempt to prepare water-soluble oligoamides of 8-amino-2-quinoline carboxylic acid focused on amphipathic helices bearing both hydrophilic and hydrophobic residues.[15] These molecules allowed us to demonstrate that the stability of the helical conformation is actually enhanced in the presence of MeOH and H_2O, but their solubility in aqueous buffers and cell culture media proved too low to assess their biological behavior. Thus, oligomers **1**–**6** (Figure 2) were equipped with multiple cationic side chains to improve their hydrosolubility and because such side chains are known to assist in processes such as DNA transfection[4,8] and membrane transport.[18] The multiple synthetic manipulations carried out to control the oligomer length, side-chain functionality, charge density, and hydrophilicity are described in the Supporting Information. A major improvement over previous syntheses was that monomers were activated as acid chlorides using 1-chloro-*N*,*N*,2-trimethylpropenylamine;[19] this makes the synthesis compatible with most usual α-amino acid side-chain protecting groups. Thus, the *tert*-butoxycarbonyl (Boc)-protected precursor of octamer **1** could be prepared on a 650-mg scale. A portion was converted to an even longer oligomer **3**, which spans almost six helical turns, using a 2,6-bis(aminomethyl)pyridine spacer.[16] Oligomers **2** and **4**, which possess higher charge density, were prepared from the deprotected methyl esters of **1** and **3**, respectively. The molecular weight of **3** (4193 Da) is comparable to that of a 30-residue peptide. Oligomer **4** bears 32 cationic amine groups and has a molecular weight of 6242 Da. A polyethylene oxide (PEO) residue was introduced in **5** to

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

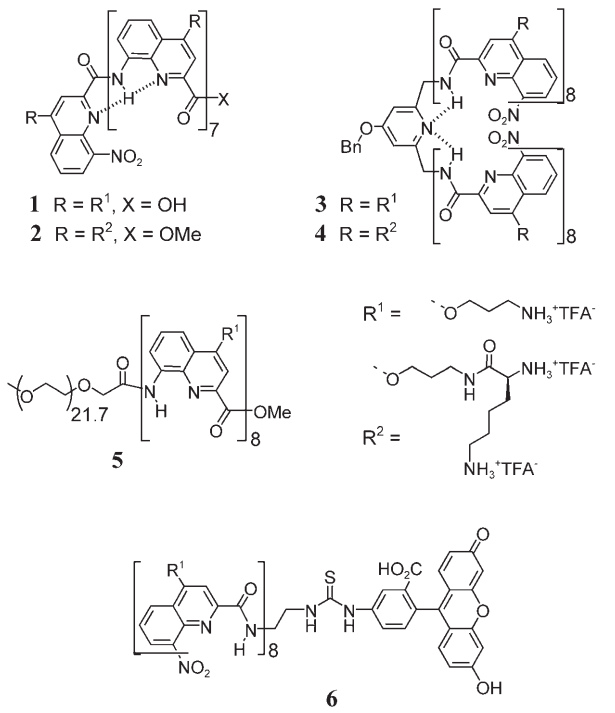

Figure 2. Water-soluble helical aromatic amide foldamers. Bn = benzyl, TFA = trifluoroacetate.

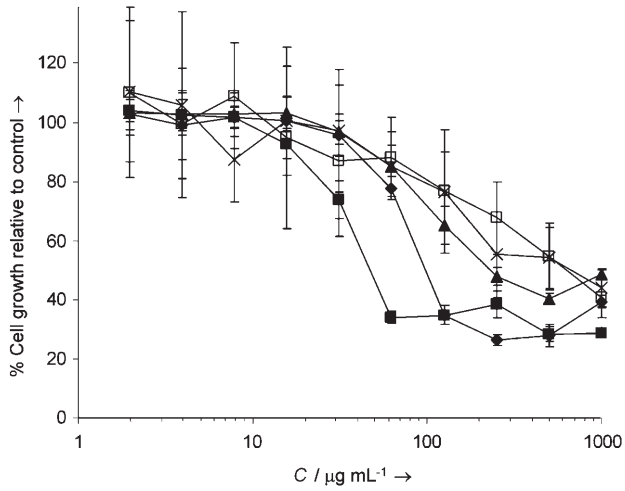

Figure 3. Growth of HeLa cells after 24 h incubation with cationic helices **1** (◆), **2** (□), **3** (■), **4** (▲), **5** (×) as measured by the MTS assay. Error bars represent standard deviations on three measurements. IC_{50} values (the concentration at which 50% growth inhibition is observed) are in the 50–500 $\mu g\,mL^{-1}$ range.

further increase helix solubility and to possibly reduce helix toxicity; a fluorescein probe was attached to octamer **6** to monitor its fate within cells. It should also be noted that all these oligomers exist as equimolar mixtures of interconverting right- and left-handed helices. In the case of **1**, **3**, **5**, and **6**, these helices are enantiomers, whilst in the case of **2** and **4**, they are diastereomers owing to the chirality of the side chains. Unlike in peptides, side-chain chirality in these oligomers is remote from the backbone and does not favor one or the other handedness.

To test the toxicity of this new class of molecules, the growth of HeLa cells in the presence of cationic helices **1–5** was evaluated using the MTS assay (see the Supporting Information for details).[20] Results are given in Figure 3. Oligomers **1** and **3** appear to be slightly more toxic than other derivatives, with IC_{50} values of approximately 100 $\mu g\,mL^{-1}$ and 50 $\mu g\,mL^{-1}$, respectively. This toxicity is comparable to that of other polycations[21] such as polylysine, polyethyleneimine (PEI), chitosan, and polyamidoamine (PAMAM) dendrimers, which have been frequently used in applications such as DNA transfection. The higher relative toxicity of the longer oligomer **3** may result from the increased number of cationic charges on the molecule or perhaps its higher aspect ratio.[22] Lysine-functionalized oligomers **2** and **4** have IC_{50} values greater than 500 $\mu g\,mL^{-1}$ and 200 $\mu g\,mL^{-1}$, respectively. Again, somewhat higher toxicity was observed for the longer oligomer **4**. The lower toxicity of these molecules relative to **1** and **3** is notable given their higher density of positive charges. It is possible that toxicity is decreased by shielding of the hydrophobic aromatic backbone by the lysine residues. Indeed, in the development of antimicrobial amphipathic aryl amide oligomers, it has been demonstrated that selective toxicity towards bacterial over mammalian cells requires a delicate balance of hydrophobicity and cationic charge.[11a] Alternatively, the lower aspect ratio resulting from the derivatization of the side chains with the lysine groups may provide increased biocompatibility.[22] The PEO-functionalized octamer **5** has an IC_{50} greater than 500 $\mu g\,mL^{-1}$, which shows that **5** has significantly lower toxicity than octamer **1**, even when taking into account the contribution of PEO to the molecular weight of **5**. Thus, the high biocompatibility of PEO helps lower toxicity in oligocations.

Studies of the degradation of aromatic amide oligomers were performed on oligomer **1** using proteases both unspecific and specific to positively charged residues (proteinase K, pepsin, subtilisin, and trypsin) under conditions optimized for each protease. Monitoring of a time-course experiment was performed by HPLC with UV spectrophotometric detection and mass spectrometry (ESI-LC-MS and MALDI-MS). After incubation for 24 h, complete resistance to degradation (see the Supporting Information) was observed for the four proteases using conditions under which a reference protein (horse heart cytochrome C was used for this control) is completely degraded in a few hours.

As various polycations are known to be effective DNA transfection agents, preliminary studies were carried out to investigate the potential of **1–5** in this application. Initial studies were carried out using HeLa and Huh7 cells and a plasmid DNA encoding for green fluorescent protein (GFP). Most of the evaluated helices exhibit minimal fluorescence due to quenching by the terminal nitro groups; however, it was found in control experiments that this fluorescence interferes with evaluation of GFP expression as cells exposed to helices without plasmid DNA exhibited significant fluorescence in both fluorescence microscopy and flow cytometry. Therefore, preliminary transfection experiments were also conducted with **1**, **3**, and **4** using HeLa cells and plasmid DNA encoding for luciferase. Thus far, only **4** has provided a

modest degree of transfection (about 10 times less than that of a lipofectamine positive control), indicating that a significant number of positive charges may be required. This is consistent with the results of studies of PEI where a molecular weight greater than 3000 Da was needed for stable complexation of DNA.[23] Even longer oligomers or polymers may be a worthwhile pursuit to achieve high transfection activity.

The significant fluorescence of cells exposed to the helices alone indicates that they readily cross membranes. To investigate this behavior, studies were carried out using the fluorescein-functionalized octamer **6**. The helices were found to localize within the cytoplasm and in nucleoli within the nucleus (Figure 4). This distribution is similar to that

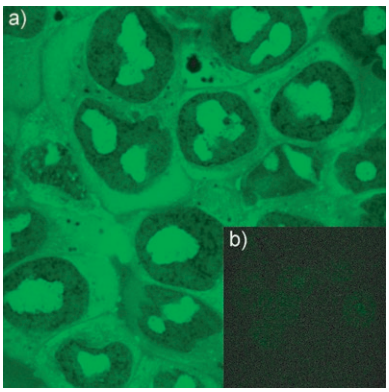

Figure 4. a) Fluorescence confocal microscopy image of HeLa cells after incubation in a 10 μM solution of **6** for 1 h. b) Inset illustrates the absence of background fluorescence in cells not exposed to **6**.

observed for the well-known transporter HIV Tat peptide, oligoarginines, and analogues based on foldamers such as β-peptides, and oligoprolines.[18] The efficiency of uptake was quantified and compared to that of a fluorescein-labeled $Tat_{47–57}$ oligopeptide (FITC-LC-YGRKKRRQRRR-NH_2). The effects of both incubation time and concentration were investigated. The uptake of **6** and FITC-$Tat_{47–57}$ are remarkably similar considering the significant differences in their structures (Figure 5). In the case of helical β-peptides and oligoprolines,[18] it has been necessary to place the side-chain residues in specific sequences and orientations about the helix in order to achieve efficient cell uptake. In the case of **6**, comparable results are achieved using a simple cationic helix, even without guanidine groups. After longer incubation periods (1 h), the percentage of fluorescent cells is higher following incubation with **6** than with $Tat_{47–57}$. This may result from the gradual degradation of FITC-$Tat_{47–57}$, which is an α-peptide and is therefore susceptible to proteases, in contrast to the aromatic oligoamide helices.

We believe that the scope of abiotic foldamers is much extended by our finding that peptides and helical aromatic oligoamides show apparently similar behavior with respect to cell penetration despite their important structural differences. The results described here were obtained using unoptimized molecules. As many possibilities exist to modify side-chain composition and backbone constitution, our results bode well for various biological applications of aromatic amide oligomers and related families of foldamers. One potential advantage of these oligomers over peptides is that changing side chains does not concomitantly affect helix structure or stability. Thus, unlike in peptides, structure–activity relationships may be established based on the sole effects of a side-chain or of a main-chain modification.

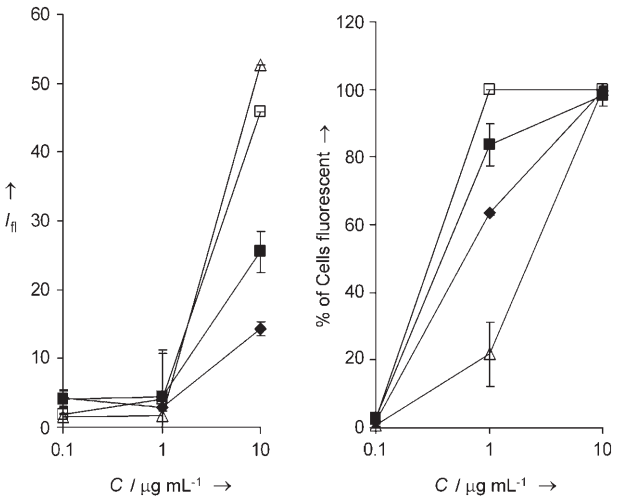

Figure 5. Concentration and time-dependent cell uptake of **6** after 10 min (◆) and after 1 h (■), and of FITC-$Tat_{47–57}$ after 10 min (□) and after 1 h (△) determined by flow cytometry: a) mean fluorescence intensity (I_{fl}) of cells versus concentration, b) proportion of cells exhibiting fluorescence versus concentration.

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