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Oligourea Foldamers | *Hot Paper*

Anion Recognition by Aliphatic Helical Oligoureas

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Abstract: Anion binding properties of neutral helical foldamers consisting of urea type units in their backbone have been investigated. ^{1}H NMR titration studies in various organic solvents including DMSO suggest that the interaction between aliphatic oligoureas and anions (CH_3COO^-, $H_2PO_4^-$, Cl^-) is site-specific, as it largely involves the urea NHs located at the terminal end of the helix (positive pole of the helix), which do not participate to the helical intramolecular hydrogen-bonding network. This mode of binding parallels that found in proteins in which anion-binding sites are frequently found at the N-terminus of an α-helix. ^{1}H NMR studies suggest that the helix of oligoureas remains largely folded upon anion binding, even in the presence of a large excess of the anion. This study points to potentially useful applications of oligourea helices for the selective recognition of small guest molecules.

Introduction

Helices in proteins and α-peptides are highly modular secondary-structure elements that contribute to a wide range of molecular-recognition events critical to biological functions. Preorganization of a recognition motif through helical folding to achieve selective interactions with target molecules is, however, not restricted to natural biopolymers. Foldamers have recently made their way through to the field of molecular recognition. Helical systems highly diverse in terms of backbones, shapes, and appended functionalities can be created from a large repertoire of monomers, thus opening new opportunities for receptor design and guest recognition.[1] Some recent developments include the design of helical foldamers that interact with protein surfaces and inhibit protein–protein interactions[2] as well as the structural characterization of several foldamer–protein complexes at atomic resolution.[3] The design and chemical evolution of helical foldamers with an internal cavity or hollow interior for selective binding of small molecules of various size and shapes also represent a remarkable achievement.[4]

The development of foldamer-based receptors and channels for anions is another area of active research.[5] This interest stems from the importance of anion recognition and transmembrane anion transport in living systems but also from possible applications as stimuli-responsive materials. Anion-binding properties have been demonstrated for a number of aromatic oligomers equipped with hydrogen-bond donor groups (e.g., oligoguanidiniums,[6] oligoindoles and oligoindolocarbazoles,[7] aromatic oligoureas,[8] and oligotriazoles[9]). In most of these systems in which the main chain of the oligomer is wrapped around the anion, the helical folding and anion recognition are coupled processes, that is to say, folding is largely induced by the binding of the oligomer to the anion. In contrast, oligomers that adopt a stable helical secondary structure on their own may provide anion-binding sites with a high level of preorganization. In proteins for example, anion-binding sites are often located at the amino terminus of an α-helix in which the interaction with the positive pole of the helix dipole may contribute to the anion stabilization in addition to contacts with main chain amide NHs and some polar side chains (e.g., hydroxyl groups of Tyr and Ser).[10] In the case of anion channels (e.g., CLC chloride channels) this mode of anion recognition based on partial positive charges prevents the anion from binding too tightly, and thus permits a high conduction rate.[11]

Anion–macrodipole interactions (as well as other less common noncovalent interactions like halogen bonds and anion–π interactions) are equally useful for the design of synthetic ion transporters and other functional systems (e.g., catalysts).[12] Fine tuning of the anion-binding properties at the positive end of a helix dipole can be achieved by using helical backbones different from α-peptides in terms of helix parameters and hydrogen-bond donor groups. Here, we have investigated for the first time the anion-binding properties of a series of helical urea foldamers by focusing on the effects of the

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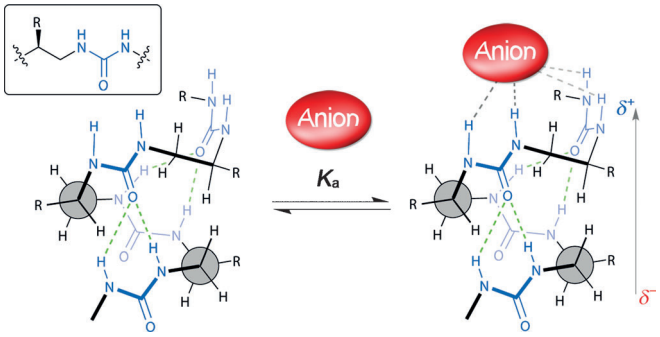
chain length and termination variations as well as on the nature of the solvent (Scheme 1).



Scheme 1. Schematic principle of the backbone-mediated anionic guest recognition by aliphatic oligourea helices.

The 2.5-helical structure formed by aliphatic N,N′-linked oligoureas[13] displays several features that make it suitable for anion recognition as illustrated in Scheme 1: 1) Ureas and thioureas have proven to be very effective hydrogen-bond donor groups for the construction of very diverse synthetic anion receptors and transport systems ranging from a relatively simple design,[14] to cyclohexane-based scaffolds,[15] macrocycles,[16] capsules, and cages.[17] 2) The four urea NHs at the positive end of the helix dipole do not participate in any intramolecular H-bond interactions and are therefore available for the anion recognition. 3) The geometry of the helix is well defined, thus providing significant preorganization for anion recognition.

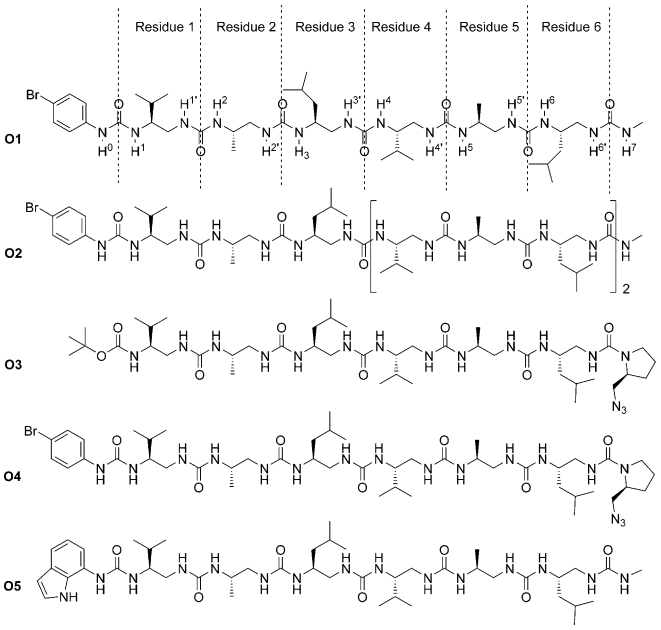
Results and Discussion

Design and synthesis of oligourea helices

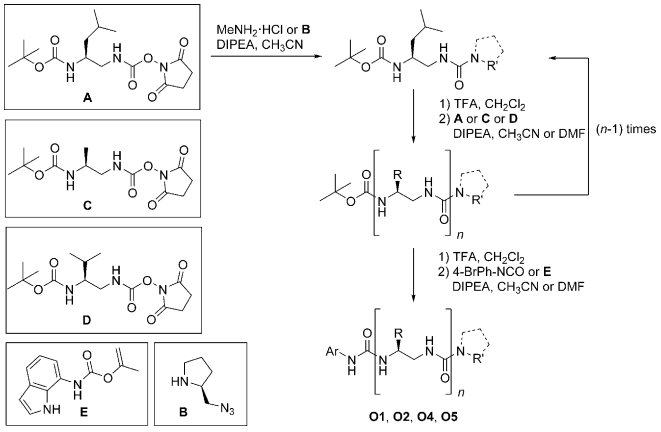
Five oligourea sequences (**O1**–**O5**) have been investigated in this study, all containing residues with side chains of the proteinogenic amino acids valine (Val^u), alanine (Ala^u), and leucine (Leu^u, Scheme 2).

They differ from one another either by the number of residues (**O1**, $n=6$; **O2**, $n=9$) or by their terminations (*tert*-butyl carbamate, 4-bromophenyl-urea or (1*H*-indol-7-yl)urea at the positive pole of the helix, and NHMe or 2-(azidomethyl)pyrrolidine (**B**) at the negative pole of the helix macrodipole). The synthesis of **O1** has been previously reported.[13b] All oligomers were synthesized in solution according to the iterative method shown in Scheme 3 using *N*-Boc protected activated monomers **A**, **C**, and **D**.[13c,18] The synthetic pathway that involves successive deprotection/coupling sequences allowed the introduction of each residue of the oligomer in a stepwise and sequence-controlled manner. Final capping of the amino group of the chain with either 4-bromophenylisocyanate or prop-1-en-2-yl 1*H*-indol-7-ylcarbamate **E**[19] provided compounds **O1**, **O2**, **O4** and **O5**.[20]

All oligomers were found to display spectroscopic features (by ^{1}H NMR and also by CD spectroscopy for **O1** and **O2**) characteristic of a helical conformation in solution (vide infra)[20].



Scheme 2. Formulae of the N,N′-linked oligoureas **O1**–**O5** used in this work.



Scheme 3. Stepwise synthetic approach leading to oligoureas **O1**–**O5** (DIEA = diisopropylethylamine).

The helical conformation of **O1** was further confirmed in the solid state by X-ray diffraction (XRD) analysis of crystals grown in a mixture of nitromethane and DMSO (Figure 1).[20,21] The structure shows a regular and complete network of intramolecular 12- and 14-membered H bonds and compares well with the crystal structure of the *N*-Boc protected precursor, the structure of which was previously solved[18b] (Figure 1 b).

Evidence for the chloride recognition at the positive pole of the helix of O1

We first investigated the anionic recognition properties of the 2.5-oligourea helix in solution by using **O1** as a representative compound in the series and chloride as anion. The ability of **O1** to bind anions was investigated by ^{1}H NMR spectroscopy in a mixture of [D$_6$]DMSO/CD$_3$CN 5:95 (v/v) as solvent.

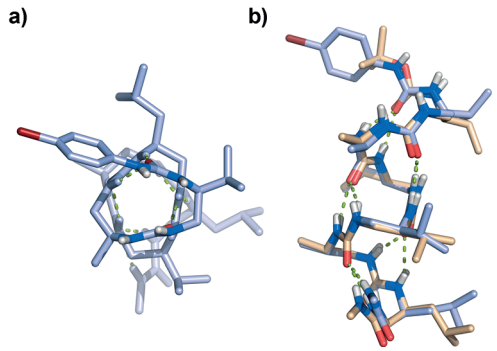


Figure 1. a) X-ray crystal structure of **O1** (crystals grown in a 1:1 mixture of nitromethane and DMSO) and b) overlay of the structures of **O1** (carbon atoms colored in light blue) and of the corresponding *N*-Boc protected precursor (CCDC 1026125,[18b] carbon atoms colored in beige). The RMSD (root-mean-square deviation of atomic positions) for this alignment performed by fitting the six pairs of β-carbons (CH(R) in the canonical units) is 0.246 Å.

[D_6]DMSO was added to overcome the low solubility of the molecule in pure acetonitrile. Note that DMSO is also expected to decrease the interaction with the anion, thus minimizing a possible error when calculating the binding constants from the experimental data. The sterically hindered and chemically inert tetrabutylammonium countercation was selected to limit possible interactions of the positive charge with the helix and for solubility reasons.

The addition of increasing concentrations (up to 5.1 equiv) of the anion to a solution of **O1** was found to cause a significant downfield shift of some but not all of the NMR signals of the 14 urea NHs present in the molecule (Figure 2a). This chemical shift variation is reflecting both a fast equilibrium between the free and bound forms of **O1**, and the magnitude of the interaction for each urea NH. Homonuclear COSY, TOCSY, and ROESY NMR experiments were required to unambiguously assign all urea NHs at each step of the titration. Chemical shift variations extracted from these NMR spectra are reported for each NH proton in Figure 2b.

It is noteworthy that the resonances of the NH protons along the backbone are differentially affected by the presence of the chloride guest. Marked downfield shifts ($\Delta\delta$) of 1.11 and 0.75 ppm were observed for the NH0 and NH1 protons (corresponding to the terminal urea close to the positive pole of the helix), respectively. This variation was less important in the case of NH1′ and NH2 ($\Delta\delta = 0.28$ and 0.31 ppm, respectively). Although all four protons are in principle available to participate to the anion-recognition process, the data qualitatively suggest that the interaction of chloride with the diurea recognition site of **O1** is unsymmetrical and the binding event appears to be more centered on the first urea function. Chemical shifts of the protons of the ureas 4–7 (i.e., NH3′, NH4, NH4′, NH5, NH5′, NH6, NH6′, and NH7) did not shift significantly ($\Delta\delta < 0.08$ ppm) upon chloride addition in agreement with the binding mode we proposed. When the oligomer is folded, the protons of the ureas 3–7 are expected to be engaged in a network of ten C=O···HN bonds that maintain the overall foldamer helical conformation, thus precluding interactions with the anionic guest. However, the signals of the NHs of urea 3 (NH2′ and NH3) were found to exhibit a shift upon chloride addition similar to that of NH1′ and NH2 ($\Delta\delta = 0.28$ and 0.31 ppm, respectively). This observation is not necessarily in conflict with an anion-binding mode involving only the first two ureas of the oligomer as proposed. NH2′ and NH3 are H bonded to the first urea moiety, which exhibits the highest affinity for the chloride ion. The spectroscopic behavior of NH2′ and NH3 could, thus, rather result from the massive electronic changes affecting this neighboring urea moiety.

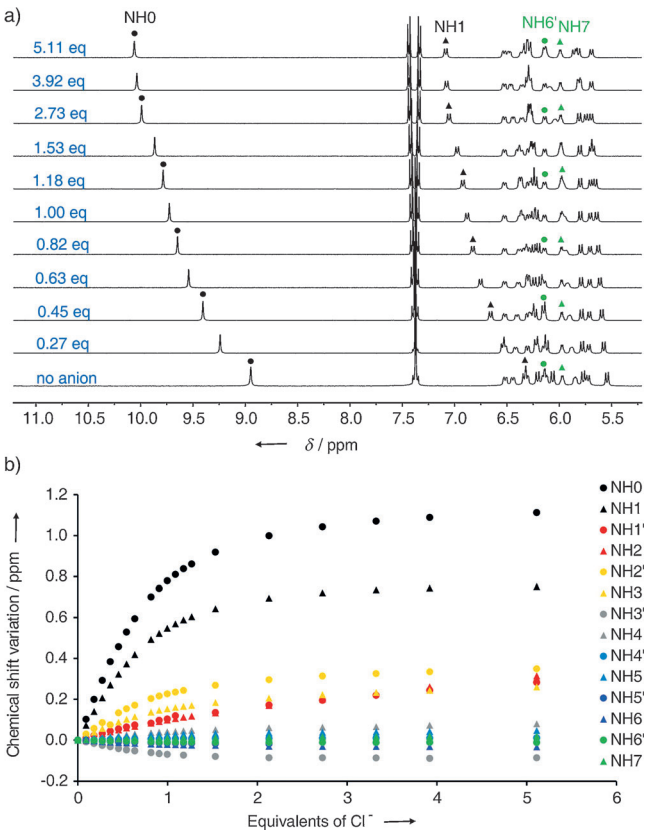


Figure 2. a) Part of the ^{1}H NMR spectra (aromatic and NH protons) of **O1** (2 mM) in the absence and in the presence of increasing concentrations of tetrabutylammonium chloride. Data were recorded at 25 °C in a mixture of [D_6]DMSO/CD_3CN (5:95, v/v). b) Chemical shift variations ($\Delta\delta$) of **O1** NH protons upon addition of increasing amounts of tetrabutylammonium chloride. NH protons are colored per urea function according to their position in the sequence.

Examination of the ^{1}H NMR spectra also showed that the aromatic signals of **O1** are similarly affected by the addition of chloride (Figure 3). There is an inversion of the AB system detected by ^{1}H NMR spectroscopy around 7.37 ppm at low chloride concentrations, and an increase of the splitting between the two signals of the AB system at higher concentrations. This splitting reflects the different electronic properties of the substituents in the 1 and 4 positions of the aromatic ring, namely the first urea function of the helix and the bromine atom. As previously discussed, anion binding to the helix substantially modifies the electronic properties of the terminal urea function. It is noteworthy that the experimental splitting variation of the AB system and the downfield shift of NH0 with changing concentrations of the anion are nearly superimposable.[20]

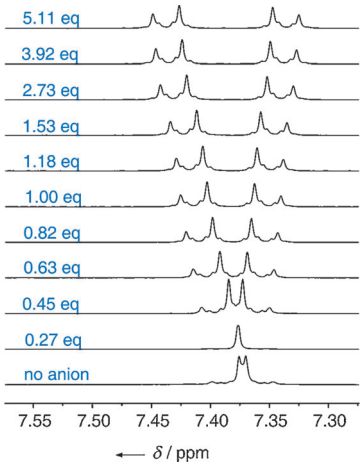


Figure 3. Overlay of the aromatic region of the ^{1}H NMR spectra of **O1** in the absence and in the presence of increasing concentrations of tetrabutylammonium chloride. Data were recorded at 20 °C in a [D$_6$]DMSO/CD$_3$CN mixture (5:95, v/v).

These complex-induced shifts can be interchangeably used as a reporter to monitor the anionic recognition process taking place exclusively at the helix terminus.

Job plot analysis reveals a maximum at an equimolar ratio of **O1** and tetrabutylammonium chloride, confirming the 1:1 binding stoichiometry of the interaction (Figure 4). The stability of the complex was, thus, determined by fitting the titration data for NH0, NH1, and the aromatic protons to a 1:1 binding

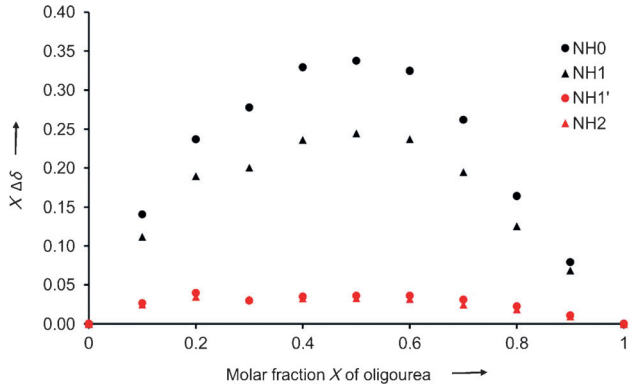


Figure 4. Job plot for the binding of **O1** with NBu$_4$Cl determined by ^{1}H NMR in a mixture of [D$_6$]DMSO/CD$_3$CN 5:95 at 20 °C ([anion] + [oligourea] = 5 mM). The results are given based on the chemical shift variation $\Delta\delta$ of NH0, NH1, NH1′, and NH2.

model using the WinEQNMR2 program.[22] The resulting K_a values in the range 1700–2900 M^{-1} (Table 1) support a predominant interaction of the chloride with the first urea moiety in **O1**. Corresponding binding constants calculated from NH1′ and NH2 are reduced by a factor of 17.[20]

As already mentioned, the signals of the protons corresponding to those urea functions involved in the helical H-bond network are hardly influenced by the presence of tetrabutylammonium chloride, which suggests that the helical conformation is only weakly modified upon increasing anion

Table 1. Binding constants (K_a) of anionic guests to **O1** in [D$_6$]DMSO/CD$_3$CN (5:95, v/v) measured by ^{1}H NMR titrations at 25 °C for NH0, NH1, and the aromatic protons Ar–H.[a]

Protons monitored	K_a of anions [M^{-1}][b] Cl$^-$	CH_3COO^-	$H_2PO_4^-$
NH0	2400	3700	$\log(\beta_1) = 5.7$[d] $\log(\beta_2) = 9.8$
NH1	2900	3500	$\log(\beta_1) = 5.0$[d] $\log(\beta_2) = 8.5$
Ar–H[c]	1700	3300	$\log(\beta_1) = 5.9$[d] $\log(\beta_2) = 9.9$

[a] Errors are estimated to be < 10%. [b] Anions added as tetrabutylammonium salts. [c] Splitting between the two signals of the AB system was monitored during the titration. [d] Chemical shift data were fitted to a 1:2 binding model. $\beta_1 = K_{11}$ (binding constant of one anionic guest), $\beta_2 = K_{11}K_{12}$ (binding constant of two anionic guests).

concentrations. Additional information about the extent of the helical perturbation or stabilization induced by the presence of anions was gained from further analyses of the ^{1}H NMR spectra. When placed in a helical environment, the main-chain methylene protons of a given residue exhibit a high degree of anisochronicity, which can be used as an empirical parameter to locally interrogate the helicity of the oligomer. The anisochronicity values were extracted for each residue from the ^{1}H NMR spectra of **O1** measured at increasing anion concentrations and compared to the corresponding values for anion-free **O1** (Table 2). These values are only slightly affected by the addition of chloride (up to 5.5 equivalents), suggesting that the organization of the binding site and the 2.5-helical conformation are largely maintained during the anion-recognition process.

Table 2. Anisochronicity of the backbone geminal αCH_2 protons in **O1** (Res 1–6) with increasing concentrations of tetrabutylammonium chloride.

Anion [equiv]	$\Delta\delta$ [ppm][a] Res 1	Res 2	Res 3	Res 4	Res 5	Res 6
0	1.04	1.26	1.31	1.25	1.35	1.12
0.7	0.92	1.21	1.26	1.23	1.33	1.12
2.0	0.99	1.24	1.32	1.24	1.33	1.08
5.5	–	1.22	1.30	1.22	1.33	–

[a] Determined by ^{1}H NMR spectroscopy at 20 °C in a mixture of [D$_6$]DMSO/CD$_3$CN (5:95, v/v).

Overall, these NMR data are consistent with an interaction between the oligomer and the chloride anion taking place at the end of the main chain, in which the four free NHs corresponding to the first two urea linkages are located. The dissymmetry of the binding site that seems to be rather centered on the first urea group is likely to reflect the intrinsically lower pK_a value of the aryl-substituted urea moiety.[23]

Anion recognition properties of O1

We next screened a variety of other anions for binding to **O1** to evaluate how the affinity and the binding mode are affected by the nature of the anion. Figure 5 shows an overlay of the

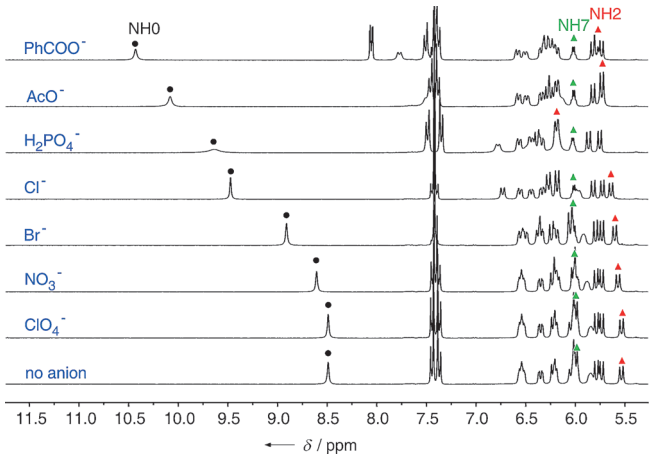


Figure 5. Part of the ^{1}H NMR spectra (NH region) of oligomer **O1** (2 mM) in the presence of 1 equiv of different tetrabutylammonium salts NBu_4X ($X = ClO_4^-$, NO_3^-, Br^-, Cl^-, $H_2PO_4^-$,AcO^-, and $PhCOO^-$). ^{1}H NMR data were recorded at 20 °C in a mixture of [D_6]DMSO/CD_3CN (5:95, v/v).

^{1}H NMR (NH region) spectra recorded for a solution of **O1** in [D_6]DMSO/CD_3CN (5:95, v/v) in the absence and in the presence of one equivalent of the TBA salts of ClO_4^-, NO_3^-, Br^-, Cl^-, $H_2PO_4^-$, AcO^-, and $PhCOO^-$. The complexation-induced downfield shift of the NH0 resonance that is easily identified on all spectra ($\delta \approx 8.5$ ppm in the absence of an anion) was found to be strongly dependent on the nature of the anion and was used to examine the general anion-binding behavior of **O1**.

The anions can be classified in two groups. Those like ClO_4^-, NO_3^-, and Br^- that induce no or only a weak downfield shift of the NH0 signal (and all other urea resonances in the oligomer) are likely to be poor anionic guests. The complexation-induced shift of the NH0 signal (and some other NH resonances) upon the addition of chloride, phosphate, acetate, and benzoate salts is much more pronounced in the order $C_6H_5CO_2^- > CH_3CO_2^- > H_2PO_4^- > Cl^-$. The NMR titration curves upon addition of the acetate salt (Figure 6a) were reminiscent of those obtained for chloride (Figure 2b) with a strong downfield shift of both NH0 and NH1 resonances ($\delta = 2.28$ and 2.16 ppm, respectively) and moderate complexation-induced shifts of the NH1′ and NH2 resonances ($\delta = 0.46$ and 0.39 ppm, respectively), which suggests a mode of binding centered on the first urea. The binding constant K_a (≈ 3300–$3700\ M^{-1}$) that is calculated by fitting the experimental titration data for NH0, NH1, and the aromatic protons to a 1:1 binding model indicated that the terminal urea function of the oligomer **O1** has a slightly higher affinity for AcO^- than for Cl^- (Table 1). A CD analysis of **O1** in MeCN and in the presence of increasing amounts of tetrabutylammonium acetate (up to 8 equiv) confirmed that the oligomer remains largely folded upon anion binding.[20]

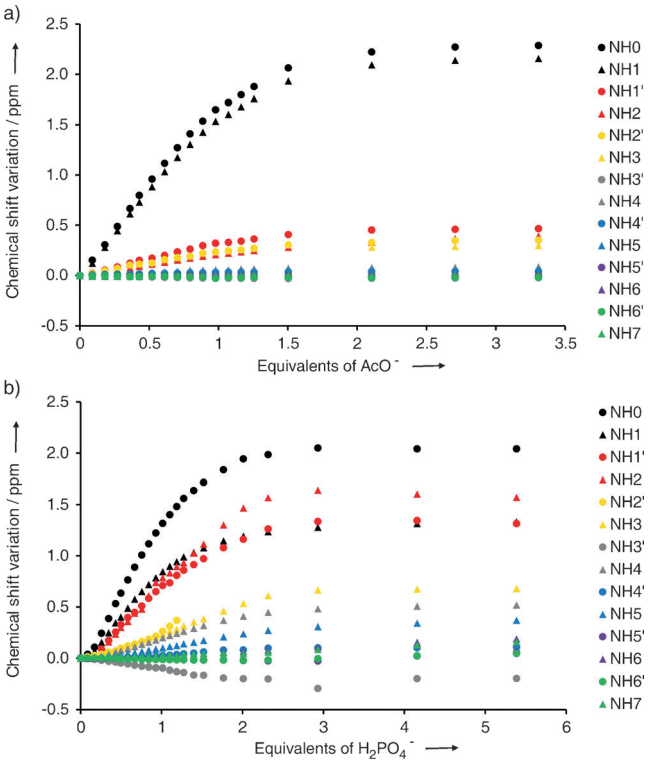


Figure 6. Chemical shift variations ($\Delta\delta$) of **O1** NH protons upon addition of increasing amounts of tetrabutylammonium salts with a) AcO^- and b) $H_2PO_4^-$ as anions. Data were recorded at 25 °C in a [D_6]DMSO/CD_3CN mixture (5:95, v/v). NH protons are colored per urea function according to their position in the sequence.

The binding mode appears to be more complex in the case of $H_2PO_4^-$, which shows a marked downfield shift of the NH1′ and NH2 resonances ($\Delta\delta = 1.33$ and 1.28 ppm, respectively) in addition to strong shifts of the NH0 and NH1 signals ($\Delta\delta =$ 2.05 and 1.64 ppm, respectively), revealed by NMR titration curves (Figure 6b).

Job plot analysis[20] suggests the formation of a 1:2 complex with two phosphate molecules bound to **O1**, which confirms that the first two ureas in the sequence are both involved in the binding process. The stability constants of each equilibrium step, expressed as $\log(K_{11})$ and $\log(K_{12})$, were estimated by fitting the titration curves of NH0, NH1, NH1′, and the aromatic protons to a 1:2 model with WinEQNMR2. The best fit was obtained by using the splitting variation of the AB system, which gave $\log(K_{11}) = 5.9$ and $\log(K_{11}K_{12}) = 9.9$ for the binding of one and two anions, respectively. The $\log(K_{12})$ for the second $H_2PO_4^-$ therefore equals $\log(K_{11}K_{12}) - \log(K_{11}) = 4$.

Chain-length effect on anion recognition

The length of the main chain is an important parameter that may influence the ability of the terminal end of the helix to bind anions. The folding propensity of oligoureas is known to increase with their chain length,[13c,24] yet it is not clear whether this is accompanied by a local rearrangement of the anion-binding site. In addition, the helix dipole is expected to in-

crease with the length of the helix, which would suggest a more favorable contribution to the anion binding of longer oligomers. To evaluate this effect, we have prepared **O2,** a nonamer with one additional Val^{u}Ala^{u}Leu^{u} repeat for direct comparison with **O1**. The lower solubility of **O2** precluded the use of CD_3CN/[D_6]DMSO (95:5, v/v) and we decided to use [D_6]DMSO to compare the two sequences. Because conformational variations imposed by the solvent may affect the anion-binding site of **O1** and the recognition process, we have first evaluated whether the ability of **O1** to fold and to bind chloride was maintained in a more competitive solvent such as DMSO. Previous studies have shown that the 2.5-helix folding propensity of oligoureas, though more populated in apolar or weakly polar solvents, is largely maintained in more polar ones.[24b] The anisochronicity values measured for the diastereotopic protons within **O1** in [D_6]DMSO are reported in Table 3 and were found to be significantly smaller than those measured in CD_3CN/[D_6]DMSO (95:5), indicating some perturbation of the helical conformation. Helix fraying appears to be more pronounced at the helix terminus in which the anion-binding site is located.

The hexaurea **O1** was then titrated with increasing amounts of tetrabutylammonium chloride in [D_6]DMSO.[20] Although the chemical shift variations of the NH protons are much smaller than in CD_3CN containing 5% [D_6]DMSO, the observed trend was the same; the NH0 and NH1 protons show a more pronounced downfield shift, which suggests that the anion-binding mode is conserved. As expected, the binding constant calculated from the NMR-chemical-shift data using NH0 (K_a = 89 M^{-1}) was significantly smaller in DMSO (Table 4). The anisochronicities measured in residues 1–5 of **O2** (Table 3) match those in **O1**, which indicates that the binding site at the helix end is unlikely to be modified (i.e., rigidified) upon chain lengthening. Titration curves were similar to those of **O1**,[20] which suggests that the anion-binding mode is also conserved. The binding affinity of **O2** for chloride was also calculated by fitting the titration curves using NH0 and was found to be more than 1.5 fold higher (K_a = 140 M^{-1}), consistent with a role of the helix dipole in the anion-recognition process (Table 4).

Table 4. Binding constants (K_a) of anionic guests to **O1**, **O2**, and **O5** measured by ^{1}H NMR titrations at 25 °C.[a]

Compound	Anion[b]	K_a [M^{-1}][c]	
		CD_3CN/[D_6]DMSO[d]	[D_6]DMSO
O1 (6-mer)	Cl^-	2400 (2900)	89[h]
O2 (9-mer)	Cl^-	–[e]	140[i]
O5 (6-mer)	Cl^-	>10 000[f]	212 (222)[j]
O5 (6-mer)	AcO^-	–[g]	3800 (4200)[k]

[a] Errors are estimated to be <10%. [b] Anions added as tetrabutylammonium salts. [c] K_a was calculated by fitting the titration data (NH0) to a 1:1 binding model (binding constant calculated from NH1 data in brackets). [d] [D_6]DMSO/CD_3CN (5:95, v/v). [e] Not soluble. [f] K_a value has to be considered with caution as it is above the upper determination limit. [g] The titration data (NH0 and NH1) gave a sigmoidal curve that could not be fitted to a suitable binding model. [h] K_a calculated from the variation of the aromatic proton splitting is 49 M^{-1}. [i] K_a calculated from the variation of the aromatic proton splitting is 107 M^{-1}. [j] K_a calculated by fitting of the titration data for the indole NH is 217 M^{-1}. [k] Error are estimated to be ≈15%.

Influence of the end group on anion recognition

Studies with **O1** and **O2** revealed a key role of the first urea group in the binding of anions, with NH0 and NH1 signals showing the most pronounced downfield shifts upon anion titration. To more specifically investigate the contribution of the second urea linkage of **O1** to the anion-binding process, we analyzed oligomer **O3** in which the 4-bromophenyl-urea group has been replaced by a *tert*-butyl carbamate. To maximize the anion-binding properties of **O3** we performed titrations in CD_3CN. The 2-(azidomethyl)pyrrolidine residue at the other helix end was introduced to increase the solubility in CD_3CN and facilitate the determination of a binding constant. This structural change has no significant effect on the helicity at the recognition site.[20,25] The signals of the urea NHs between residues 1 and 2 (i.e., NH1′ and NH2) were strongly downfield shifted upon titration of **O3** with tetrabutylammonium acetate ($\Delta\delta$ = 2.21 ppm for NH1′ and $\Delta\delta$ = 2.06 ppm for NH2), thus indicating a site for anion binding at the helix end. The NH1 (Boc protected) protons were only moderately involved in the recognition process ($\Delta\delta$ = 0.64 ppm). The titration curves of NH1′ and NH2 were nicely fitted to a 1:1 binding model[20] and gave a binding constant K_a of roughly 830 M^{-1}. For a direct comparison, we titrated **O4** under the same conditions. As expected, the signals of NH0 and NH1 were downfield shifted to a higher extent than those of NH1′ and NH2, and the affinity constants calculated by fitting these curves to a 1:1 binding model (K_a = 22 000 M^{-1} from the NH0 and NH1 data) were higher than those calculated for **O3** by more than one order of magnitude.[20] This increased affinity of acetate for **O4** compared to **O3** is also evident when comparing the binding constants calculated for the second urea (K_a = 11 000 M^{-1} and 4500 M^{-1} from NH1′ and NH2 in **O4**, respectively). Overall,

Table 3. Anisochronicity of the backbone geminal αCH_2 protons in **O1** and **O2**.

Compound	Solvent	$\Delta\delta$ [ppm][a]								
		Res 1	Res 2	Res 3	Res 4	Res 5	Res 6	Res 7	Res 8	Res 9
O1	[D_6]DMSO/CD_3CN[b]	1.04	1.26	1.31	1.25	1.35	1.12	–	–	–
O1	[D_6]DMSO	0.59	0.92	1.19	1.19	1.20	0.88	–	–	–
O2	[D_6]DMSO	0.60	0.88	1.18	1.28	1.26	1.37	1.20	1.23	0.82

[a] Determined by ^{1}H NMR spectroscopy at 20 °C. [b] [D_6]DMSO/CD_3CN (5:95, v/v).

these data show that the more acidic protons of the first urea group largely dictate the recognition process and confirm the dissymmetry of the anion-binding site in **O1**.

We next sought to further modify the anion-binding site of the oligoureas by replacing the terminal 4-bromophenyl-urea moiety by a capping group that could directly participate to the anion-binding process. We selected 1*H*-indol-7-yl-urea based on earlier work from Gale and coworkers, which showed that such derivatives have excellent anion-binding properties including an enhanced ability to bind oxo anions,[14b,26] and therefore prepared oligomer **O5**. The anion-binding properties of **O5** were first studied by NMR spectroscopy in CD_3CN/[D_6]DMSO (95:5, v/v). The examination of the titration curves with tetrabutylammonium chloride shows that the NH signals of the first three ureas (i.e., NH0, NH1, NH2′, and NH3; NH1′and NH2 to a lesser extent) and the indole NH all shift downfield and reach a plateau upon addition of 1 equiv of chloride, thus suggesting a contribution of all these NHs in the binding process. Fitting the titration data for these NHs to a 1:1 binding mode revealed that **O5** binds chloride strongly with a stability constant ($K_a > 10^4\ M^{-1}$) about 4–5 fold higher than **O1** in the same solvent mixture (Table 4). The increased binding of **O5** to chloride compared to **O1** was also confirmed in [D_6]DMSO (see Figure 7a for the titration data) with a calculated constant of $K_a = 212\ M^{-1}$ (NH0). Similarly, **O5** was found to tightly bind to tetrabutylammonium acetate (see Figure 7b for the titration data) with an estimated binding constant of $K_a = 3800\ M^{-1}$ (NH0) in [D_6]DMSO (Table 4).

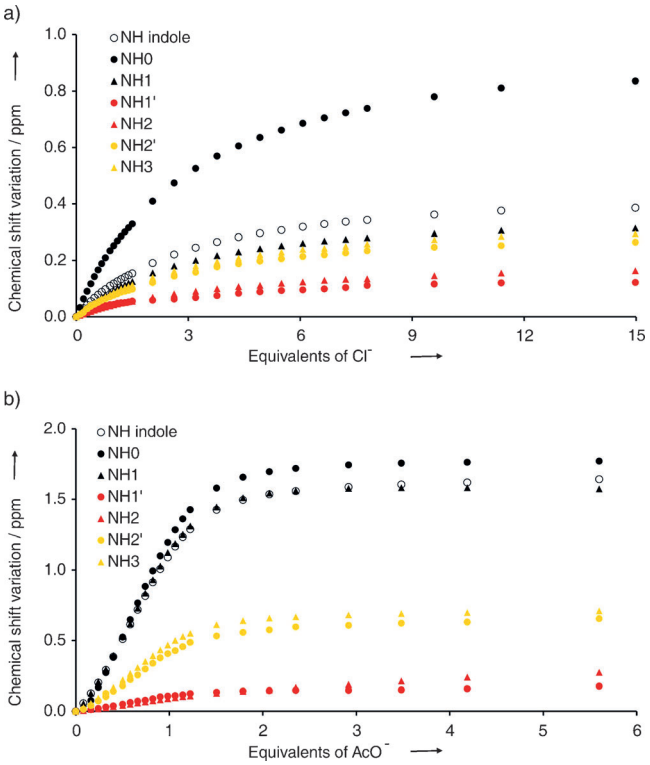


Figure 7. Chemical shift variations ($\Delta\delta$) of **O5** NH protons upon addition of increasing amounts of tetrabutylammonium salts with a) Cl^- and b) AcO^- as anions. Data were recorded at 25 °C in [D_6]DMSO. NH protons are colored per urea function according to their position in the sequence.

Structural insight into the recognition properties of the oligoureas

Attempts to grow crystals of the anion complexes of the oligoureas and elucidate their structure by XRD have failed thus far. The engagement of the first two ureas in the anion binding as well as the presence of the counter ion are believed to prevent the ability of oligourea helices to pack axially by H bonds as usually observed in the crystals of oligoureas grown in organic solvents. However, single crystals of **O1** and **O5** suitable for XRD were grown from CH_3CN/DMSO and DMSO solutions. The structures determined by XRD analysis (Figure 8) reveal that one solvent molecule is bound to the anion-binding site at the terminus of the fully folded helical structure.[20,21] In the case of the **O1**·DMSO solvate (Figure 8a), the DMSO molecule is hydrogen bonded to NH0 and NH1 (N···O distances of 2.878 and 3.078 Å, respectively) whilst NH1′ and NH2 of the second urea group are each intermolecular H bonded to one of the last two carbonyl groups of a second helix (Figure 8c). The structure of **O5** shows that the binding site at the end of the helix is significantly remodeled by the indole group, the nitrogen of which points towards the helix interior (Figure 8b). The sulfoxide oxygen in the solvate of **O5** is engaged in three hydrogen bonds with the NHs (NH1′ and NH2) of the second urea (N···O distances of 3.089 Å and 2.973 Å, respectively) and of the indole (N···O distance of 2.893 Å). The NHs of the first urea are now engaged in intermolecular bonds with the penultimate carbonyl group of a neighboring helix. Altogether,

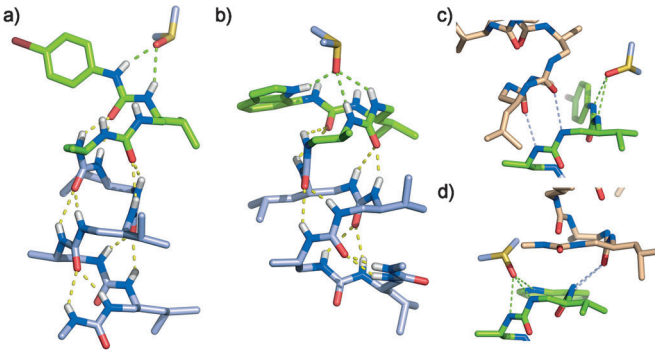


Figure 8. (a, b) X-ray crystal structures of a) **O1**·DMSO and b) **O5**·DMSO solvates. (c, d) Details of the intermolecular H bonds formed between c) two helices of **O1** and d) two helices of **O5**. The C atoms defining the anion-binding site at the positive pole of the helix dipole are shown in green.

these two structures give some useful information about possible ways to modulate the geometry of the binding site as well as some molecular insight into host–guest interactions at the helix terminus. However, the structures of the DMSO solvates in the solid state reported herein only imperfectly account for host–guest interactions in solution, because half of the binding sites are used to pack and align the helices in the crystal.

Conclusion

The recognition properties of aliphatic N,N′-linked oligoureas, like other peptidomimetic foldamers,[2a,b,27] can be easily tuned by projection of requested side chains at the surface of the helix in a sequence-dependent manner.[28] In this work, we have shown that the 2.5-helical oligourea backbone possesses intrinsic features that make it useful as a receptor for small guest molecules. The NMR titration studies in solvents ranging from the moderately polar CD_3CN to the more competitive DMSO reported herein, reveal for the first time the ability of N,N′-linked oligourea foldamers to interact with various anions (oxyanions and halides) as well as some details about possible binding modes. The proton signals corresponding to the terminal urea moieties located at the positive pole of the helical macrodipole experience marked downfield shifts upon titration with the different anions (as their tetrabutylammonium salt). This trend confirms the hypothesis of a preorganized anion-binding site at the terminal end of the helix. Indeed, the structures at atomic resolution of the 2.5-helix of oligoureas shows that the four NHs of the first and second urea groups are not involved in intramolecular H bonding and are, thus, free to interact with guest molecules (see Figure 1). Calculations from the titration curves suggest that the selectivity, the affinity, and the anion-binding mode can be finely tuned by varying the nature of the capping group and through further differentiation of the two urea moieties forming the binding site (e.g., by increasing the acidic strength). These findings pave the way for new developments of aliphatic oligourea helices in areas such as chiral ion recognition, sensing, anion transport, and catalysis.[12a,16d,29] One noteworthy example in this direction is the recent finding that a screw-sense preference may be induced in achiral *meso*-oligourea helices by selective formation of a 1:1 hydrogen-bonded complex with a chiral carboxylate anion.[30]

The higher affinity for chloride observed upon increasing the chain length of the oligoureas from a hexamer to a nonamer (**O1** vs. **O2**) is another noteworthy feature. Although it would be tempting to attribute this difference to a direct contribution of the helix macrodipole,[10b–d] it is however difficult to rule out the consequence of a possible conformational rearrangement resulting from the helix rigidification upon increasing the length of the main chain, which could lead to an increased preorganization of the binding site. To address this particular aspect, future studies could concentrate on more sophisticated architectures like tertiary and quaternary structures, whereby charged species could be stabilized by multiple helix dipoles similar to what is commonly observed among proteins involved in the anion recognition and transport[10b,c] (e.g., ClC chloride channels[11]). A hint in this direction was provided by the recent structure of an amphiphilic water soluble oligourea determined at atomic resolution, which revealed a hydrogen-bonded complex between one chloride and the NHs of two oligourea helices oriented head-to-head with their positive poles pointing towards the anion.[31] The observation of such a complex in the crystal state suggests that the anion-binding ability of oligoureas reported here could likely extend to aqueous conditions, thus, expanding the range of possible applications of these foldamers. In particular, the tight binding of phosphate to **O1** may suggest a complementary mechanism to account for the remarkable interaction properties of antimicrobial oligourea helices with negatively charged model phospholipid membranes as well as bacterial membranes.[28a,32]

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Keywords: anions · foldamers · helical structures · oligourea · X-ray diffraction

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FULL PAPER

Oligourea Foldamers

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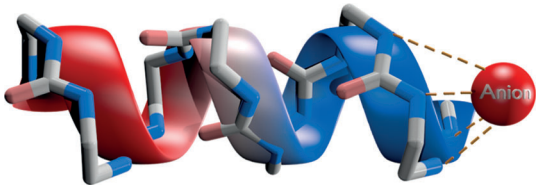
Anion Recognition by Aliphatic Helical Oligoureas



Free to interact! The four backbone urea NHs located at the positive pole of homo-oligourea helices are ideally preorganized to interact with anionic guests. ^{1}H NMR titration studies with various anions reveal that binding to oligoureas is site-selective and does not cause a helix unfolding. This anion-binding mode is reminiscent of that observed in proteins in which anion-binding sites are frequently found at the N-terminus of an α-helix.