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Oligoamide foldamers as helical chloride receptors – the influence of electron withdrawing substituents on anion binding interactions

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Abstract: The anion binding properties of three closely related oligoamide foldamers were studied using NMR spectroscopy, isothermal titration calorimetry and mass spectrometry, as well as DFT calculations. The ^{1}H NMR spectra of the foldamers in acetone-d_6 solution revealed partial preorganization by intramolecular hydrogen bonding, which creates a suitable cavity for anion binding. The limited size of the cavity, however, enabled efficient binding by the inner amide protons only for the chloride anion resulting in the formation of a thermodynamically stable 1:1 complex. All 1:1 chloride complexes displayed a significant favourable contribution of the entropy term. Most likely, this is due to the release of ordered solvent molecules solvating the free foldamer and the anion to the bulk solution upon complex formation. The introduction of electron withdrawing substituents in foldamers **2** and **3** had only a slight effect in the thermodynamic constants for chloride binding compared to the parent receptor. Remarkably, the binding of chloride to foldamer **3** not only produced the expected 1:1 complex but also open aggregates with 1:2 (host:anion) stoichiometry.

Introduction

Anion binding by synthetic receptors has many important biochemical and chemical applications in membrane transport, anion recognition and sensing, and template assisted catalysis of complex molecular architectures.[1] Anion binding has also been used to control the conformation and shape of anion responsive molecules and complexes, including synthetic self-folding molecules, foldamers.[2-7] Moreover, modulation of the foldamer-anion complexes to host multiple anions has been studied. For example, aryl-triazole foldamers showed sequence dependent equilibrium between single and double helices binding chloride in 1:1 or 2:2 stoichiometries.[8] Increasing the length of the aryl-triazole foldamer chain induced complexation of two halides into a single foldamer helix accompanied with an increase in the helical pitch in comparison to the 1:1 complex.[9] The significance of the preorganized binding site for the selectivity and affinity of anion complexation has been widely demonstrated with macrocyclic and cryptand-like receptors.[10,11] In neutral acyclic anion receptors preorganization has been induced by intramolecular hydrogen bonding to reduce the entropic penalty of the complex formation.[12,13] Nevertheless, solvation/desolvation effects are known to play a significant role in anion binding in polar media.[14]

The pyridine-2,6-dicarboxamide motif has been used in many acyclic anion receptors as a conformational lock to direct the *cis*-orientation of the adjacent NH groups through intramolecular hydrogen bonding interactions with the pyridyl nitrogen atom.[15-19] Examples of this construct include selective receptors for fluoride and chloride having the central pyridine-2,6-dicarboxamide motif equipped with indole groups.[18,19]

Aromatic oligoamide foldamers have been widely studied for their ease of synthesis and conformational stability.[20] In previous studies, we investigated the conformational features of a series of oligoamide foldamers incorporating the pyridine-2,6-dicarboxamide motif.[21-23] We showed that the foldamers adopted two different conformations. In one of them, the receptor takes a proto-helical shape (so-called @-conformation) and in the other, it arranges in a more open S-shape (Fig. S-1, ESI†). The two conformations display significant differences in intramolecular hydrogen bonding patterns. Moreover, these preferred conformational features remain even when the number of pyridine-2,6-dicarboxamide units in the foldamer are doubled or tripled.[24] Remarkably, the incorporation of an electron withdrawing nitro substituent at the *ortho*-positions of the terminal phenyl groups of the foldamer promoted the observation of a new type of conformation in the solid state.[15] This latter conformation was flatter and showed the establishment of intramolecular π···π stacking interaction between the A and B' phenyl rings of the foldamer, as well as two intramolecular NH···O=C bonds. The *cis*-preorganization of the two amides groups exerted by the pyridine-2,6-dicarboxamide motif was still present in this conformer. We surmised that the *cis*-arrangement of amide groups observed in the 2,6-carboxamidepyridyl unit in all conformations of the receptor provided a suitable host preorganization for the complexation of small anions.

Herein, we describe the results obtained in binding studies of anions in solution using three structurally related oligoamide foldamers **1**–**3** (Scheme 1). Our results demonstrate that the conformation (preorganization) of the host is not substantially altered during the binding processes of the anions resulting in helical 1:1 (host:anion) complexes. On the one hand, the

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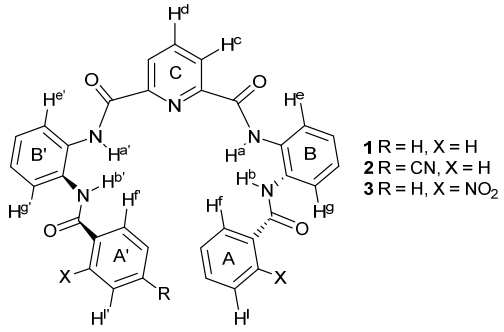
presence of an electron withdrawing CN substituent in the *para*-position of one terminal phenyl ring of the foldamer **2** provides a slight increase in the free energy of binding for the 1:1 complex compared to **1**. On the other hand, the introduction of an electron withdrawing nitro substituent at the *ortho*-position of the two terminal phenyl rings of **3** evidently favours the formation of an open complex with 1:2 (host:chloride) stoichiometry from the initially assembled 1:1 counterpart in the same range of concentrations used in the titrations of **1** and **2**.



Scheme 1. Oligoamide foldamers **1**–**3** showing the corresponding proton assignment (for the symmetrical **1** and **3** A = A' etc.).

Results and Discussion

ESI-MS experiments

The oligoamide foldamer **1** has five aromatic six-membered rings and four primary amide groups. We have previously shown that in the solid-state receptor **1** forms a 1:1 complex with fluoride.[15] The bound receptor adopts a helical-like conformation featuring a sizable polar cavity suitable for the inclusion of the fluoride with the simultaneous formation of four NH···F^- hydrogen bonds and two additional CH··· F^- interactions.

Mass spectrometry was used in the screening of other potential anions that might bind to host **1** in solution. The (-)ESI-MS spectra (negative ion mode) of separate acetonitrile solutions of **1** containing three equivalents of the ammonium salts of F^-, Cl^-, Br^-, I^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-}, HSO_4^- or CO_3^{2-} showed intense signals for the peaks corresponding to 1:1 complexes with chloride, bromide and nitrate, and ions [**1**+$Cl]^-$, [**1**+$Br]^-$, [**1**+$NO_3]^-$ appeared in the spectra. Less intense peaks were detected for the ion-peaks of the 1:1 complexes of iodide and hydrogen sulfate ([**1**+$I]^-$ and [**1**+$HSO_4]^-$). The mass spectra from solutions with Cl^- and Br^- also showed the peak of the deprotonated receptor [**1**-$H]^-$. This might result from the basicity of the anions (A^-) in a non-protic organic solvent and elimination of HA from the complexes. In ESI-MS spectra measured from iodide solution [M-$H]^-$ was barely detectable, but interestingly from the solution containing F^- the peak of the deprotonated receptor [**1**-$H]^-$ was exclusively observed. We interpret this result as evidence of the high basicity of F^- in a non-protic organic solvent.

We performed bilateral competition experiments involving receptor **1** and a series of halides, which included Cl^-, Br^- and I^-. Three equivalents of each competing anion (Cl^- and Br^-, Br^- and I^-) was added to an acetonitrile solution of **1**. The ESI-MS spectra showed significant differences in intensities for the 1:1 complexes [**1**+$A]^-$. In the competition between chloride and bromide, the [**1**+$Cl]^-$ complex was clearly more abundant with almost 90% relative intensity. Respectively, competition between bromide and iodide showed more abundant complex formation with bromide. Assuming that the ESI response of the three anionic 1:1 complexes is similar, the observed difference in relative intensities of the peaks can be correlated with the relative abundance of the anionic complexes in solution. In short, the abundance of the complexes with receptor 1 in competing conditions is clearly highest for chloride followed by bromide and iodide.

The evaluation of binding properties using MS was also performed for the foldamers **2** and **3**, having a cyano substituent in the *para*-position of one terminal phenyl ring and nitro groups in the *ortho*-position of both terminal phenyl rings, respectively. We limited the binding analyses to the halides that were successfully complexed by host **1**, namely, Cl^-, Br^-, and I^-. The MS spectra showed the expected peaks for the 1:1 complexes [**2**+$Cl]^-$, [**2**+$Br]^-$, [**2**+$I]^-$, [**3**+$Cl]^-$, [**3**+$Br]^-$ and [**3**+$I]^-$. These results indicated that the incorporation of the substituents in the terminal phenyl rings of the foldamers did not affect their anion binding significantly.

^{1}H NMR titrations of foldamer 1

Next, we undertook the evaluation of the anion binding properties of the three receptors using ^{1}H NMR spectroscopy. All ^{1}H NMR spectroscopy titrations of **1** with chloride, bromide, iodide and nitrate anions, as tetrabutylammonium salts, were carried out using millimolar solutions of the host in acetone-d_6.[25] The incremental addition of the anion induced significant chemical shift changes in several signals of the protons of foldamer **1**.

In particular, the gradual addition of TBACl to a millimolar solution of **1** produced significant downfield shifts in the two signals of its amide protons (Figure 1a). This observation suggests their involvement in the formation of hydrogen-bonding interactions with the chloride. The addition of 1 equiv of the TBACl induced the saturation of the chemical shift changes. These observations indicate that the binding process of the foldamer **1** with chloride shows fast dynamics on the chemical shift timescale and a complex with 1:1 stoichiometry (**1**·Cl) is formed for which a binding constant over 10^4 M^{-1} can be estimated. This value is too large to be measured accurately using ^{1}H NMR spectroscopy techniques. The complexation induced shift (CIS) experienced by the amide protons in the central 2,6-biscarboxamide pyridyl unit (H^a) and the amides connecting the terminal phenyl groups (H^b) were very similar, $\Delta\delta$ = 0.96 and $\Delta\delta$ = 1.03 ppm, respectively. This result suggested that they were involved in the binding of the anion to the same extent.[26] In addition to the amide protons, the *ortho*-protons H^f of the terminal phenyl rings (A) also shifted downfield ($\Delta\delta$ = 0.48 ppm). In contrast, the *meta*- and *para*-protons of the A phenyl ring moved slightly upfield (0.01–0.04 ppm). We interpreted these observations considering the *selective* establishment of CH···Cl^- interactions with the *ortho*-aromatic protons (H^f).

The geometry adopted by foldamer **1** in the **1**·Cl complex was inferred from the chemical shift changes experienced by the *ortho*-aromatic protons, H^e and H^g, of the diamino-phenyl ring B and the *ortho*-proton, H^c, of the 2,6-biscarboxamide pyridyl unit.

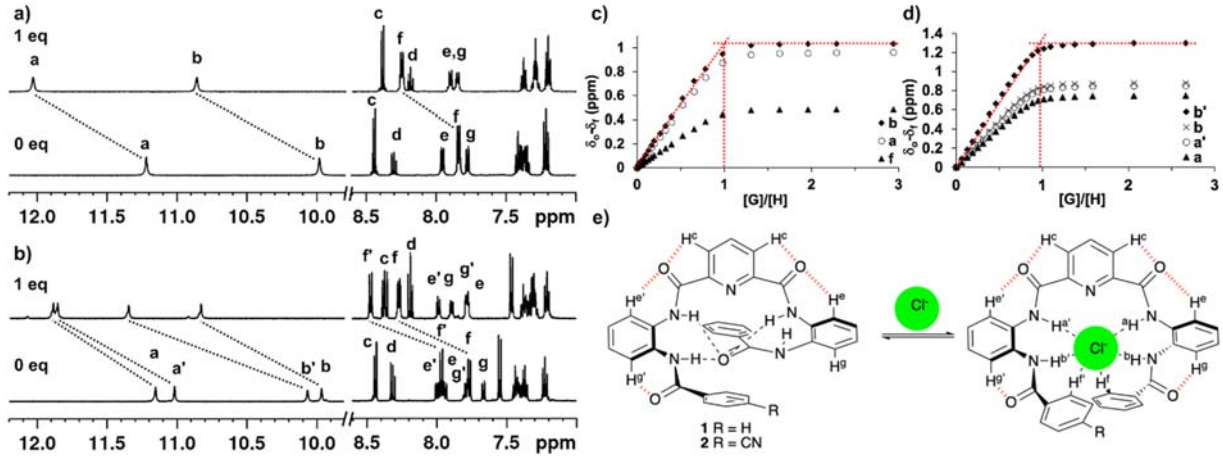


Figure 1. ^{1}H NMR spectra of foldamer 1 (a, 5 mM host) and 2 (b, 2.5 mM host) in acetone-d_6 with 0 and 1 equiv of TBACl. Chemical shift changes experienced by protons a, b and f in 1 (c) and protons a, a', b and b' in 2 (d) during the titrations. Scheme of free host (in a protohelical @-conformation) and the suggested helical chloride complexes for 1 and 2 (e). The anisotropic field induced by the oxygen lone-pairs is indicated with red dashed lines.

Protons H^e and H^c moved slightly upfield ($\Delta\delta$ = - 0.06 ppm) while proton H^g shifted minimally downfield ($\Delta\delta$ = 0.09 ppm). It is well known that the electron lone-pairs of the oxygen atom of carbonyl groups in diphenyl ureas produce an anisotropic field inducing large upfield shifts to the syn-*ortho*-aromatic proton ($\Delta\delta$ > 1.00 ppm). Owing to the reduced chemical shift changes observed for the aromatic protons *ortho* to the amide groups of **1** upon complex formation, we conclude that the bound receptor experiences a reduced conformational change to wrap around the bound anion, in comparison to the conformation adopted in solution in the free state. We putatively assign a helicoidally-shaped conformation for free **1** in solution based on our solid-state studies.

We also performed a ^{1}H NMR spectroscopy titration of **1** with the bromide TBA salt. The incremental addition of the salt produced the expected chemical shift changes for the proton signals of foldamer **1** (Figure 2). Remarkably, the addition of 1 equivalents of the bromide salt did not provoke the saturation of the chemical shift changes. The mathematical analysis of the titration data using a 1:1 theoretical binding isotherm produced a good fit and returned a binding constant value K = 281 M^{-1} (Table 1) and the complexation induced shift (CIS) values of the analysed proton signals. The downfield CIS calculated for the terminal amide protons, H^b, was $\Delta\delta$ = 0.64 ppm. The internal amide protons in **1**, H^a, also shifted downfield but to a lesser extent $\Delta\delta$ = 0.41 ppm. The *ortho*-proton, H^g, in the diamino-phenyl ring B moved downfield $\Delta\delta$ = 0.14 ppm, whereas the chemical shift change experienced by the other *ortho*-proton in the same ring, H^e, was negligible. It is worthy to note that the *ortho*-proton, H^f, in the terminal phenyl groups showed a significant downfield CIS, $\Delta\delta$ = 0.38 ppm. Altogether, the small values calculated for the CIS of the amide protons in the **1**·Br complex suggest that owing to the larger size of the bromide anion the hydrogen bonding interactions are substantially reduced. Nevertheless, the trends of the CIS support that the receptor **1** adopts a bound conformation in the **1**·Br complex that is similar to the one present in the **1**·Cl analogue.

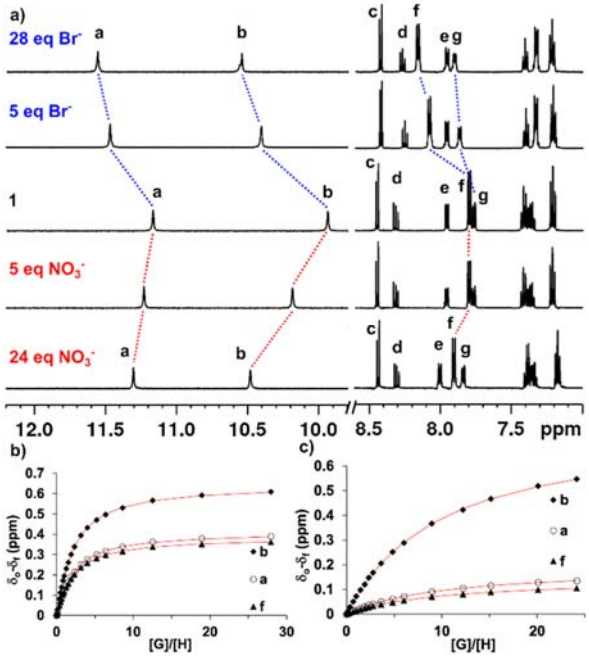


Figure 2. a) The ^{1}H NMR spectra of foldamer **1** in acetone-d_6 (2.5 mM) with incremental amounts of anions. The chemical shift changes observed by protons with addition of b) TBABr, c) $TBANO_3$ fitted to 1:1 binding isotherms (red lines).

Table 1. Binding constants for **1** and TBA salts in 1:1 stoichiometry determined by NMR titration in acetone-d_6 at 25 ˚C.

Guest	K_a (M^{-1})[a]
TBACl	> 10^4
TBABr	281[b]
TBAI	13[b]
$TBANO_3$	46[b]

[a] Global fit of 3-4 signals. [b] The average of two NMR titrations, estimated error < 25 %.

Similarly, we determined the binding constant values for the 1:1 complexes of **1** with nitrate and iodide as 46 and 13 M^{-1}, respectively (Figure 2, Fig. S-24). These results are in complete agreement with the findings of the mass spectrometry experiments (vide supra). The trend in binding constant values is the expected one for the binding of the anions using mainly hydrogen-bonding interactions. The increase in the size of the mono-charged anion has a detrimental effect in the strength of the hydrogen-bonding interaction, which is primarily of electrostatic nature (charge-dipole). Overall, the observed chemical shift changes for the protons of **1** during the titrations with iodide and nitrate suggest a change in the binding geometry adopted by the receptor in the complexes with the larger anions compared to the one expressed in the binding of chloride and bromide. Alternatively, complexes of other stoichiometry than the simple 1:1 might be assembled with the larger anions. Unfortunately, the low thermodynamic stabilities of the complexes hampered further studies.

^{1}H NMR titrations of foldamer 2

The chloride anion was selected for further binding studies with foldamer **2**. Foldamer **2** has one electron withdrawing cyano substituent at the *para*-position of one of its terminal phenyl group A' (Scheme 1). This substitution is expected to increase the anion binding affinity of **2** due to the electron withdrawing effect of the substituent, which is expected to favour NH···anion, CH···anion and anion···π interactions. Moreover, the lack of C_{2v} symmetry for foldamer **2** should allow a direct comparison of the contribution of the two different halves of the host in the chloride binding.

The complete assignment of the proton signals of foldamer **2** was based on a set of high-resolution 1D and 2D NMR spectra (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HSQC, and HMBC NMR). The ^{1}H NMR spectroscopy titration of foldamer **2** with TBACl in acetone-d_6 showed the diagnostic chemical shift changes of the protons of the host for the formation of the 1:1 complex (Figure 1) with an estimated binding constant value larger than 10^4. The amide proton, $H^{b'}$, corresponding to the *p*-cyanophenyl substituent (ring A') showed the largest CIS ($\Delta\delta$ = 1.30 ppm). In contrast, the amide proton, H^b, attached to the phenyl ring moved downfield to a lesser extent ($\Delta\delta$ = 0.88 ppm), but still in line with the shift experienced by the analogous amide protons of **1**. This observation suggested that the electron-withdrawing -CN substituent had a noticeable effect on the NH···Cl^- interaction for $H^{b'}$. Similarly, the internal amide protons H^a and $H^{a'}$ displayed reduced downfield shifts, $\Delta\delta$ = 0.75 and 0.86 ppm, compared to their counterparts in foldamer **1**. The *ortho*-protons, H^r and H^f, of the two the terminal aryl groups of **2** moved downfield ($\Delta\delta$ = 0.51 ppm) to a similar extent. We observed analogous chemical shift changes for the corresponding *ortho*-protons in foldamer **1**. These observations suggest that **2** adopts a similar conformation than **1** for chloride binding.

We performed a 2D NOESY experiment of the **2**·Cl complex in acetone-d_6, which showed strong NOE cross peaks between the NHs of the *ortho*-substituted amides in the two diamino rings of **2**, H^a and H^b, and $H^{a'}$ and $H^{b'}$, respectively, (Fig. S-7, ESI†). This observation supports the *syn* conformation adopted by the amides in the **2**·Cl complex.

^{1}H NMR titrations of foldamer 3

The complexation of Cl^- with foldamer **3**, possessing one strong electron-withdrawing substituent at the *ortho* position of its two terminal aryl rings (A and A'), was expected to provide a significant boost in binding affinity. The ^{1}H NMR spectroscopy titration, however, revealed that the downfield shifts experienced by the two terminal amide protons, NH^b, were similar to the one measured for foldamer **2** (Figure 3). Intriguingly, the internal amide protons, NH^a, and the *ortho*-aryl proton, H^f, of the terminal phenyl groups in **3** experienced reduced downfield shifts (0.67 and 0.11 ppm) in comparison to the ones observed for foldamers **1** and **2**. Surprisingly, both *ortho*-aryl protons H^g and H^e of the diaminophenyl ring B experienced significant downfield shifts (0.14–0.25 ppm), which can be interpreted as a change in foldamer conformation upon chloride binding compared to **1** and **2**. Furthermore, the saturation of the chemical shift changes experienced by the protons of receptor **3** was achieved after the addition of 2 equivalents of the TBACl. This finding is in striking difference to the observations made in the analogous titrations of **1** and **2** with chloride requiring only one equivalent to reach saturation. These results suggested that the binding of chloride to foldamer **3** might produce a complex with higher stoichiometry than the simple 1:1. The mathematical analysis of the titration data using a 1:1 theoretical binding isotherm produced an acceptable fit, returning a binding constant value lower than expected for the 1:1 complex, K_1 = 5800 M^{-1}. This value is one order of magnitude smaller than the ones measures for the 1:1 complexes of chloride with the **1** and **2** counterparts. As could be anticipated, a better fit of the titration data was obtained using a 1:2 (H:G) binding model containing more variables to optimise ($K_{1:1}$, $K_{1:2}$, $\delta_{1:1}$ and $\delta_{1:2}$). Remarkably, the new fit returned K_1 value for the stability of the 1:1 complex larger than 10^4 M^{-1}. This calculated magnitude for K_1 is more in line with our expectations. The fit assigned a value of 10^8 M^{-2} to the 1:2 complex. Most likely, a relative increase in the thermodynamic stability of the 1:2 complex is responsible for its formation to a significant extent during the titration rather than a destabilisation of the 1:1 precursor (see DFT calculations). We carried out a statistical F-test of the data fit to the two theoretical models to support our preferential selection of the 1:2 model.

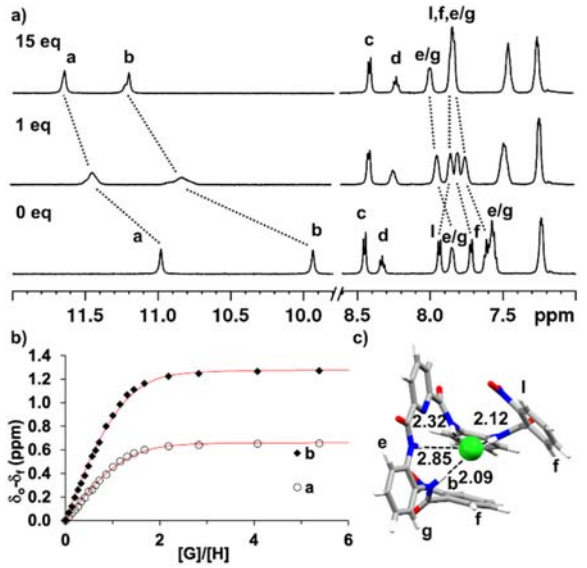


Figure 3. a) The ^{1}H NMR spectra of foldamer **3** with 0, 1 and 15 eq of TBACl in acetone-d_6 (1.2 mM host). b) The chemical shift changes observed by protons with addition of guest (up to 6 eq shown for clarity) fitted to a 1:2 binding isotherm. c) The DFT minimized structure of **3**·Cl showing hydrogen bond distances in Ångströms and NMR labels.

ITC experiments

Isothermal titration calorimetry (ITC) experiments were carried out to assess and compare the binding affinities of foldamers **1**–**3** for chloride accurately. These experiments also provided the thermodynamic enthalpy and entropy parameters of the complexation processes (Table 2, Figure 4). The measurements were performed at 25 °C by placing 0.8–2.0 mM acetone solutions of the host in the calorimeter cell and adding incremental amounts of 7.2–19 mM acetone solution of TBACl using a computer controlled microsyringe. The binding constant values determined for the **1**·Cl and **2**·Cl complexes are similar, and their binding enthalpies identical. Thus, the effect provided by incorporating one -CN group (electron withdrawing substituent) in the terminal phenyl substituent of **2** is minimal based on the ITC results.

Table 2. Thermodynamic parameters for the 1:1 foldamer:Cl^- complexes in acetone at 25 °C. The parameters are an average of 3-6 ITC measurements.

Host	K_a ($\times 10^4$ M^{-1})	$\Delta H^{[a]}$	$T\Delta S^{[a]}$	$\Delta G^{[a]}$
1	2.3 ± 0.4	-2.5 ± 0.2	3.4 ± 0.3	-5.9 ± 0.1
2	4.6 ± 0.9	-2.5 ± 0.2	3.9 ± 0.3	-6.4 ± 0.1
3	3.0 ± 0.3	-1.4 ± 0.1	4.7± 0.1	-6.1 ± 0.1

[a] in kcal×mol^{-1}

Surprisingly to us, when the interaction of foldamer **3** with chloride was probed using an ITC experiment, a single sigmoidal isotherm with an inflexion point centred at a 1:1 molar ratio was observed. Thus, the integrated and normalised heat values were fit to a theoretical 1:1 binding model. The returned K_a value was in complete agreement with the one determined using ^{1}H NMR spectroscopy titrations and considering a 1:2 binding model. On the other hand, the binding enthalpy measured for the **3**·Cl complex is slightly smaller than that obtained for the chloride complexes with the foldamers **1** and **2**. Even though only one binding event is detected by ITC we cannot rule out the formation of the 1:2 complex. Assuming that the enthalpy gain for the formation of the 1:2 complex is almost identical to the enthalpy loss during the disassembly of the 1:1 complex the net enthalpy of the binding process will be athermic and thus not detectable by ITC.

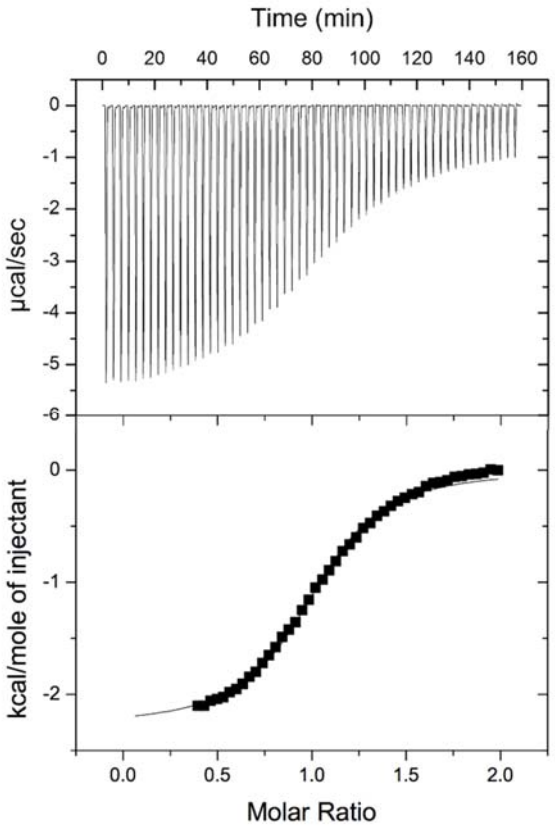


Figure 4. The ITC titration of foldamer **1** with TBACl in acetone at 25 ˚C, c(host) = 0.97 mM. Data below molar ratio 0.4 was omitted from the fit due to the heat of dilution of the guest.

The binding processes of chloride with the foldamer series are highly favoured by entropy. In fact, the entropic term is the main contributor to complex formation suggesting that significant solvation/desolvation processes are taking place during the binding processes. The observation of a very large and favourable entropic term for binding processes of anions with neutral receptors in polar solvents is not unprecedented.[13] The formation of 1:1 complexes of the foldamers in which the anion is almost fully solvated by the polar groups and the hydrogen atoms of the receptor, requiring a complete desolvation of the chloride and the polar groups of the receptor, would provide a sensible explanation to our observations.

These results demonstrate the important - and difficult to predict - contribution of the entropic term in binding experiments of anions performed in polar solvents. Clearly, the solvation/desolvation processes experienced by receptors **1** and **2** and their chloride complexes are different from those of the dinitro-foldamer **3**. The existence of prominent solvation/desolvation processes makes the analysis and dissection of the contributions of the enthalpy and entropic terms to binding difficult. Nevertheless, the reduced conformational change experienced by the foldamers upon chloride binding, which we derived from the ^{1}H NMR experiments, constitutes an additional factor to reduce the entropic cost of complex formation.

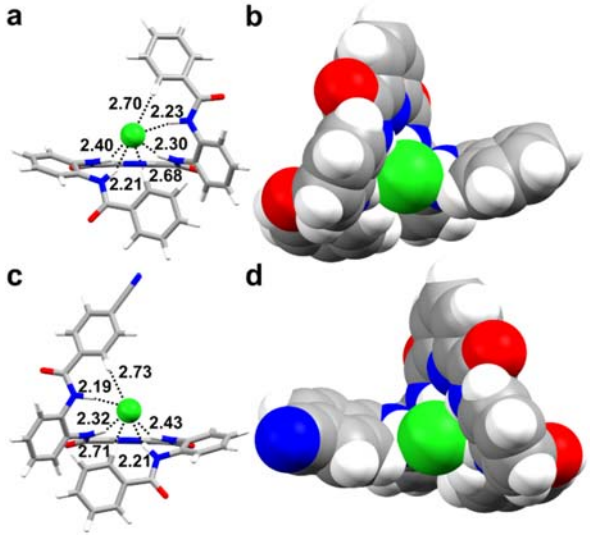


Figure 5. DFT minimized (solution state) geometries for **1**·Cl and **2**·Cl: a,c) a view showing the hydrogen bonding distances in Ångströms, b,d) a side view as a space-filling model. Atom colors: blue = nitrogen, green = chlorine, grey = carbon, red = oxygen, white = hydrogen.

DFT calculations of foldamers 1–3

The conformation adopted by the receptor **1** upon chloride binding, **1**·Cl complex, was also investigated using DFT calculations in the gas phase and in solution (see ESI† for the details and comparison of gas phase and solution state studies). The initial geometry for the energy minimization of the **1**·Cl complex consisted of the atomic coordinates of the corresponding fluoride-foldamer complex crystal structure.[15] The energy-minimized structure of the **1**·Cl complex in solution coincided well with the helical conformation deduced from the ^{1}H NMR titration data. The chloride anion is located within the polar binding site of **1** establishing four hydrogen-bonds with the amide NHs and two CH···Cl interactions with the *ortho*-protons of the terminal phenyl group (Figure 5). This confirms that the radius of the chloride is too big for the anion to be symmetrically inserted in the cavity of the helix defined by bound **1**. For this reason, the chloride shifts away from the plane defined by the atoms of the pyridine ring. The average hydrogen bond lengths for the NH^b protons (2.23 and 2.21 Å) is shorter than for the NH^a analogues (2.30 and 2.40 Å). The C-H^f···Cl^- distances of 2.68 and 2.70 Å support the existence of weak interactions with the *ortho*-protons of the terminal phenyl rings of **1**.

The DFT energy-minimized structure of the **2**·Cl complex is very similar to the calculated structure of **1**·Cl. The most significant finding is that during energy-minimization the Cl^- shifts closer to the *para*-cyano phenyl ring of foldamer **2** in contrast to the symmetrical starting geometry used in the calculation. This finding coincides well with the observed large CIS of $NH^{b'}$ in the NMR titration.

The crystal structure of **3**·F used as starting coordinates for the energy-minimized 1:1 **3**·Cl complex showed a similar helical conformation of the foldamer (Figure 3). The halide establishes four NH hydrogen bonds, a H^f···F^- interaction and a F^-···π interaction with the terminal nitrophenyl rings A (Fig. S-30). The DFT minimized structure of **3**·Cl, however, showed a π···π interaction between rings A and B and further Cl^-···π interactions between the anion and ring A. One of the NH^a···Cl^- hydrogen bonds is extended (2.85 and 2.32 Å) in comparison to **1**·Cl, whereas the NH^b···Cl^- distances are shortened (2.09 and 2.12 Å) in perfect agreement with the observed CIS in the NMR titration. Remarkably, the H^f···Cl^- interaction has been eliminated, which partially coincides with the reduced CIS observed in the NMR titration.

We also used DFT calculations to assign a putative structure to the **3**·Cl_2 complex. The resulting energy-minimized structure (Figure 6) shows two intramolecular NH^a···O=C hydrogen bonds, and two binding clefts for the anions, in which they are experiencing hydrogen bonds with NH^b and Cl^-···π interactions with the nitrophenyl rings.

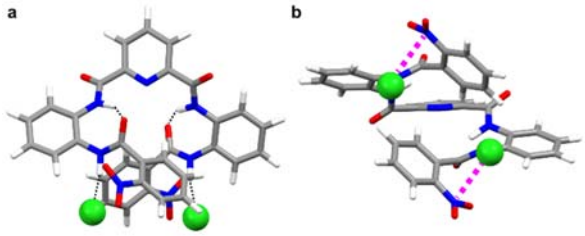


Figure 6. An energy-minimized structure of **3**·Cl_2 showing a) hydrogen bonds with black dashed lines, b) Cl^-···π interactions with magenta dashed lines. Atom colors: blue = nitrogen, green = chlorine, grey = carbon, red = oxygen, white = hydrogen.

Conclusions

Oligoamide foldamers **1**–**3** formed anionic complexes with chloride, bromide, iodide and nitrate while showing the highest binding affinity towards chloride in the order of $K = 10^4$ M^{-1}. The *cis*-preorganization exerted by pyridine-2,6-dicarboxamide motif was clearly assigned from the NMR experiments in solution. Based on the NMR and DFT studies, a helicoid structure for the 1:1 complexes in solution was proposed. The electron withdrawing *para*-cyano substituent at one terminal phenyl group in foldamer **2** slightly increased the binding affinity towards chloride, yet the effect was minimal. The *ortho*-nitro substituents

in foldamer **3** had an unexpected contribution to the anion binding since besides a thermodynamically stable 1:1 complex, we suggest the formation of a 1:2 host:guest complex.
All chloride complexes had a large positive entropy term, which demonstrates the important, and difficult to predict, contribution of the entropic term in binding experiments performed in polar solvents. Remarkably, the **3**·Cl complex showed the highest gain in entropy, which indicates different solvation/desolvation processes of dinitro-foldamer **3** and its complexes. This is reflected in the slightly different binding geometry supported by anion···π interactions and the likely presence of a 1:2 complex in comparison to the two counterparts. Clearly, the increase in the electrostatic interactions for the binding of charged substrates with a neutral receptor in polar solvent does not warrant a net gain in free energy of binding owing to the complex solvation/desolvation process that takes place in solution.

Experimental Section

Materials

All starting materials and chemicals were commercially available and used as such unless otherwise noted. Analytical grade solvents were used for the complexation studies. Compounds **1-3** were prepared as previously reported.[15,21-23]

Mass spectrometric studies

Mass spectrometry experiments were performed with Micromass LCT ESI-TOF and Qstar Elite ESI-Q-TOF mass spectrometers. 10 mM stock solutions of foldamers **1**, **2** and **3** were prepared in THF and 10 mM stock solutions of ammonium salts of guests were prepared in MeOH. In all measurements acetonitrile was used as solvent and the concentration of hosts **1**, **2** and **3** was 20 μM and the concentration of guests 60 μM. Competition experiments were performed with foldamer **1** and ammonium salts of halide ions Cl^- and Br^-, and Br^- and I^-, respectively.

NMR titration

The NMR spectra were measured with a Bruker Avance DRX 500 or Bruker Avance 400 spectrometer at 25 °C, and the chemical shifts were calibrated to the residual proton resonance of the deuterated solvent. For NMR titration 1.2–5.0 mM host stock solution was prepared in acetone-d_6 using Hamilton glass syringes. The guest solutions were prepared from TBAF·$(H_2O)_3$, TBACl, TBABr, TBAI or $TBANO_3$ in host solution keeping the total concentration of the host constant during titration. Titration was performed using 650 μl of host solution and adding 0.02–35 equivalents of guest in 2–240 μl aliquots. Each titration was performed twice except for foldamer **3** the titration was done once in acetone-d_6 and once in a 9:1 mixture of acetone-d_6 and $CDCl_3$. The titration data were analysed with non-linear regression of 2–4 proton signals to 1:1 binding model using MATLAB (MATLAB and Statistics Toolbox Release R2014b).[27] The analysis of **3**·Cl was made with HypNMR 2008 software Version 4.0.66.[28] The fitting of the titration data was made by optimizing the chemical shifts of the complex species ($\delta_{1:1}$, or $\delta_{1:1}$ and $\delta_{1:2}$ of the same four protons) by non-linear regression.

ITC measurements

The ITC measurements were performed on a MicroCal VP-ITC microcalorimeter at 25 °C. A host stock solution of 0.8–2.0 mM in acetone was titrated with a TBACl solution of a concentration of $c_{guest} = 9 \times c_{host}$. Each titration was performed 3–6 times. A blank experiment was done by titrating the guest solution to the pure solvent, and the heat of dilution was reduced from the experiment before fitting the data to a binding isotherm.

Calculations

The gas phase and solution state geometry optimisations were performed for the foldamer complexes **1**·Cl, **2**·Cl and **3**·Cl with the Turbomole program package[29] using the PBE1PBE density functional[30-33] together with Ahlrichs' def2-TZVP basis sets[34] and Grimme's GD3BJ empirical dispersion correction.[35,36] The effect of the solvent (acetone, ε = 20.5) was taken into account using a conductor-like continuum solvation model COSMO.[37] The initial geometries of complexes **1**·Cl, **2**·Cl, and **3**·Cl were taken directly from the single-crystal X-ray diffraction data of the corresponding fluoride complexes.[15] The initial geometry for **3**·Cl_2 was envisaged by analysing the observed CIS in the NMR titration. Full frequency calculations were performed for the optimised structures in the gas phase to ensure that they correspond to true minima on the potential energy hypersurface.

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