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PAPER

Selective recognition of sulfate ions by tripodal cyclic peptides functionalised with (thio)urea binding sites†

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A tripodal urea and tripodal thiourea with the same cyclic peptide core have been synthesised and their anion binding ability investigated. In $CDCl_3$, the tripodal urea self-associates whereas the thiourea does not. Neither compound shows self-association in the more polar 10% v/v DMSO-d_6/$CDCl_3$. Both compounds bind strongly and selectively to sulfate ions in $CDCl_3$ and 10% v/v DMSO-d_6/$CDCl_3$. This selectivity is attributed to a unique binding mode for sulfate, in which this tetrahedral anion forms nine hydrogen bonds to the receptors, with three of these coming from the amide protons of the cyclic peptide.

Introduction

The design of host molecules capable of the selective recognition of anionic species is an area of intense current interest. In particular, significant effort has been directed towards the development of receptors capable of binding anions solely through hydrogen bonding interactions, in efforts to mimic natural anion receptors such as the sulfate and phosphate binding proteins. Neutral receptors with amide,[1] sulfonamide,[2] pyrrole,[3] indole[4] and thio(urea)[5] hydrogen bond donors (and combinations thereof) have successfully been employed in anion recognition. The thio(urea) class of receptors are particularly attractive because the two hydrogen bond donors of the thio(urea) can bind to either a single acceptor in a 6-membered chelate or to two atoms of a tetrahedral anion (*e.g.* sulfate) in an 8-membered chelate.[6] Careful positioning of several of these hydrogen bond donors allows receptors to be designed for specific anions. For example, calculations by Hay *et al.*[6] indicate that tripodal receptors will exhibit selectivity for tetrahedral anions (*e.g.* sulfate, phosphate) over trigonal planar anions (*e.g.* nitrate).

A number of tripodal (thio)ureas have been reported previously, with a variety of 'cores' including tren,[7,8] trisubstituted benzene derivatives,[9,10] cyanuric acid,[11] triindane[12] and calix[6]arenes[13] employed to provide the tripodal geometry, providing six hydrogen bond donors to the guest. Many of these have been found to favour the binding of tetrahedral anions. A logical step in the design of new tripodal receptors is the inclusion of additional hydrogen bond donor sites to fully complement target anions. For example, an optimal coordination number of twelve has been predicted for sulfate binding.[6] In an elegant example, Jia *et al.* have recently demonstrated the incorporation of additional hydrogen bond donors into the 'arms' of tripodal receptors[8] but with traditional scaffolds it is difficult to add additional hydrogen bond donors to the 'core' of such tripodal systems. Indeed, Gunnlaugsson and co-workers have recently demonstrated that for tripodal benzene derivatives bearing both amide and urea hydrogen bond donors in the arms, the amide protons do not participate in hydrogen bonds with anions.[10]

Backbone modified cyclic hexapeptides based on the lissoclinamide family of natural products[14,15] provide a unique 'core' for the synthesis of tripodal receptors, incorporating three hydrogen bond donors in the core which are available for guest binding. We have recently reported the anion binding abilities of two cryptands with a *Lissoclinum* type cyclic peptide 'core'.[16,17] We found that these receptors displayed strong affinity for sulfate anions with a unique recognition motif involving the cyclic peptide amide hydrogen bond donors. In comparison, spherical anions such as chloride appeared to bind solely through the thiourea binding sites, with no interaction from the peptide hydrogen bond donors. In efforts to further explore the anion binding ability of the lissoclinamide cyclic peptides, we report here an investigation of the related tripodal receptors, thiourea **1** and urea **2** (Fig. 1). These tripodal receptors are significantly more flexible than the related cryptands; it was therefore of interest to examine whether a similar binding motif would be adopted.

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C spectra of **1** and **2**; fitted curves for determination of K_as; Job plots; selected ^{1}H NMR titration spectra for dimerisation and anion binding experiments. See DOI: 10.1039/c2ob06964d

Results and discussion

Synthesis

The synthesis of the tris-(thio)urea receptors is outlined in Scheme 1. The *para*-^{t}butylphenyl substituents were chosen to

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provide solubility in organic solvents. Under optimised conditions, (thio)urea **1** was obtained by treating the known tripodal cyclic peptide scaffold **3a**[15] with triethylamine and *para*-^{t}butylphenyl isothiocyanate in chloroform at reflux. When **3a** was similarly treated with *para*-^{t}butylphenyl isocyanate, it was difficult to separate the desired tris-urea **2** from the triethylammonium bromide byproduct, providing **2** in only a modest yield of 61% after several attempts at column chromatography. Therefore, the free base tris-amine **3b** was treated with ^{t}butylphenyl isocyanate in chloroform, in the absence of triethylamine, to provide **2** in an improved yield of 80%.

Dimerisation studies

Given the propensity of urea and thiourea derivatives to self-assemble in solution,[18–20] we first investigated the dimerisation behaviour of **1** and **2** by obtaining their ^{1}H NMR spectra in $CDCl_3$ at 300 K at a range of concentrations between 0.174 and 22.9 mM. During the course of the titration with thiourea **1**, NH^a experienced a downfield shift of 0.25 ppm, whereas the change in chemical shift of NH^b was negligible ($\Delta\delta$ = 0.02 ppm). (See Scheme 1 for proton identification.) The changes in chemical shifts of all other proton environments of **1** (H^c–H^i) were also negligible. In contrast, a significant concentration dependence of the chemical shifts of the urea protons (NH^a and NH^b) of **2** was observed (Fig. 2). Signals attributable to both NH^a and NH^b underwent substantial downfield shifts from 6.78 to 7.41 ppm ($\Delta\delta$ = 0.63 ppm) and from 5.36 to 5.81 ppm ($\Delta\delta$ = 0.45 ppm), respectively, with increasing concentration of **2**. In contrast to the downfield shifts observed for NH^a and NH^b, all other proton environments (H^c–H^i) were observed to undergo small to negligible upfield shifts. Throughout the titration the molecule maintained C_3-symmetry and no additional resonances were observed, indicating the formation of well-defined aggregates. Fitting the data to a monomer–dimer equilibrium model (Fig. 3) was carried out by non-linear least squares regression,[21] giving dimerisation constants (K_{dimer}) of < 10 M^{-1} for (thio)urea **1** and 90 ± 19 M^{-1} for urea **2**.

Relative to the high dimerisation propensity often observed with urea-functionalised calixarene systems (up to 10^9 M^{-1}),[19] the K_{dimer}-value obtained for **2** suggests only a small tendency of the receptor to dimerise in solution. In analogy with these previously described systems, the large downfield shift of the urea

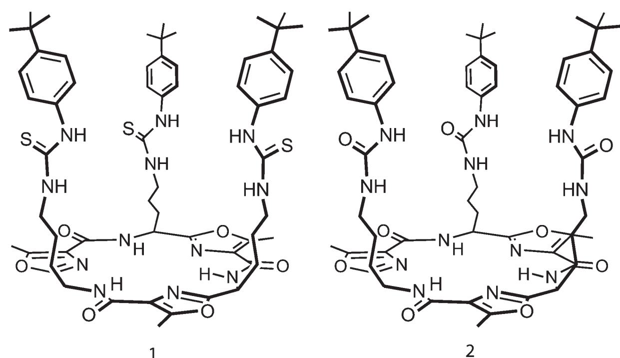


Fig. 1 Structures of tripodal receptors **1** and **2**.

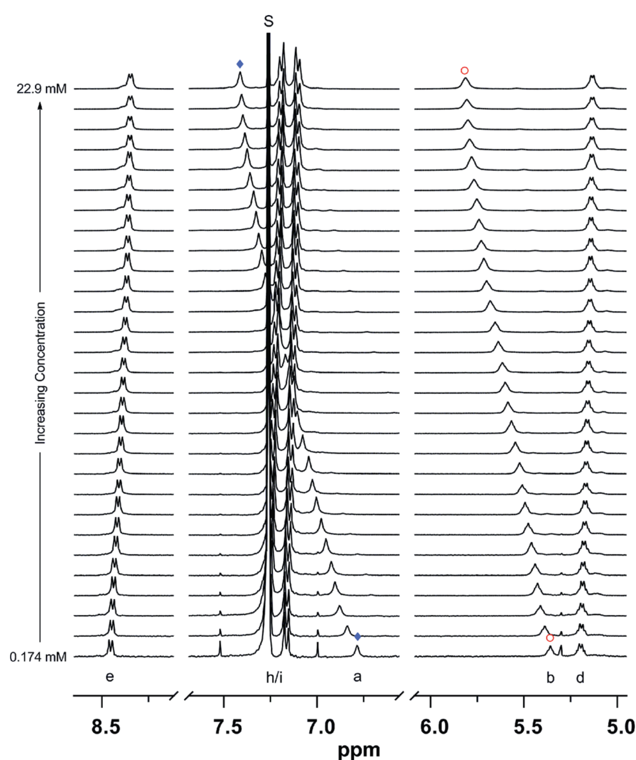
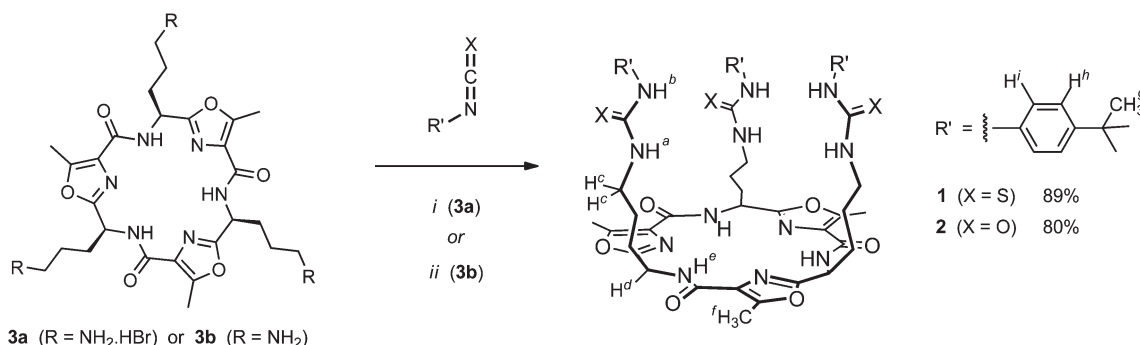


Fig. 2 ^{1}H NMR (400 MHz, 300 K) spectra of receptor **2** in $CDCl_3$ recorded at a range of concentrations.



Scheme 1 Synthesis of tripodal tris-(thio)urea receptors. *Reagents and conditions*: (*i*) **3a**, *para*-^{t}butylphenyl isothiocyanate (for **1**), anhydrous $CHCl_3$, triethylamine, reflux. (*ii*) **3b**, *para*-^{t}Butylphenyl isocyanate (for **2**), anhydrous $CHCl_3$, reflux.

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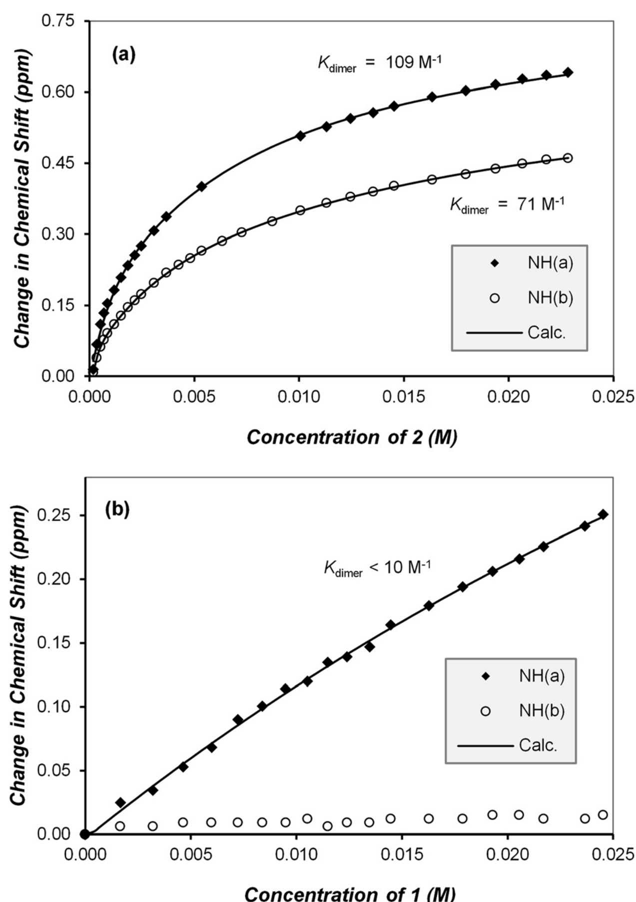
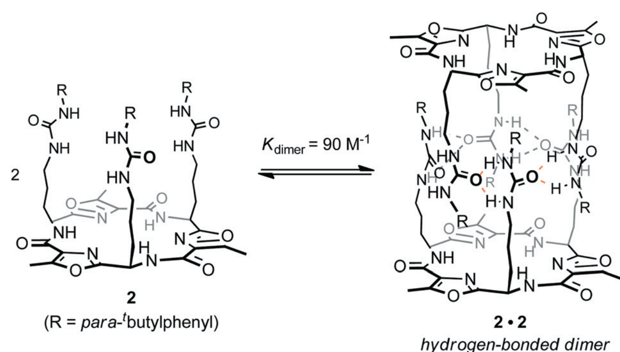


Fig. 3 Concentration dependence of the chemical shift of the (thio)urea protons (NH^a and NH^b) of (a) **2** and (b) **1** in $CDCl_3$. Solid lines are the calculated chemical shifts for 1 : 1 dimerisation. The chemical shift for NH^b of **1** could not be fitted to a 1 : 1 dimerisation model.



Scheme 2 Proposed dimerisation mode of tris-urea **2**.

protons of **2** implies that they participate in a band of hydrogen bonds which act to hold two molecules of **2** together (Scheme 2).[19] The relatively small upfield shifts of the other proton environments (H^c–H^i) are attributable to conformational changes upon dimerisation.

Additional concentration dependent studies with **2** were carried out in a more competitive solvent mixture (10% v/v DMSO-d_6/$CDCl_3$). Only negligible changes in chemical shift ($\Delta\delta$ = 0.02 ppm) were observed over a similar concentration range to that above, indicating that in this more polar environment the equilibria can be completely shifted to the monomer.

The difference in the dimerisation behaviours of receptors **1** and **2** can be explained by the differences in the strengths of the intermolecular hydrogen bonding interactions between the thiourea and urea moieties. In general, the thiocarbonyl of a thiourea is a poor hydrogen bond acceptor owing to the lower electronegativity of sulfur in relation to the oxygen of a urea.[20] As such, the oxygen of a urea will more readily accept hydrogen bonds than the sulfur of a thiourea, thus reflecting the trend observed with **1** and **2**.

Anion binding studies

The anion binding properties of **1** and **2** were quantitatively determined by ^{1}H NMR spectroscopic titrations with a range of anions as their tetrabutylammonium salts in $CDCl_3$. As a representative example, ^{1}H NMR spectra of receptor **1** recorded over the course of the titration with a solution of tetrabutylammonium chloride are shown in Fig. 4. Throughout the titration the molecule maintained C_3-symmetry and fast exchange processes were observed. Significant downfield shifts of the (thio)urea protons, NH^a and NH^b, were observed over the titration range ($\Delta\delta$ = 2.39 and 1.87 ppm, respectively) which was a strong indication that all six (thio)urea protons were cooperatively involved in hydrogen bonding to the anions in solution.

The apparent stability constants (K_a) of receptors **1** and **2** with a range of anions in $CDCl_3$ are presented in Table 1. In all cases, except for the titration with receptor **1** and sulfate, fast exchange processes were observed. For thiourea **1**, data were fitted to a 1 : 1 binding model by non-linear curve fitting (see ESI† for titration curves).[21] The 1 : 1 stoichiometry of the complexes was confirmed by Job plots (see ESI†). In the case of the urea **2**, the dimerisation behaviour (K_{dimer} = 90 M^{-1}), was incorporated into the binding model to account for its ability to self-associate in $CDCl_3$. In contrast to all other systems investigated, when sulfate ions were added to receptor **1**, slow exchange processes were observed with the appearance of new signals corresponding to a host–guest complex in the ^{1}H NMR spectra.

The data obtained suggests that both **1** and **2** are selective for sulfate as they both bind the tetrahedral oxoanion extremely strongly with stability constants (K_a) > 10^4 M^{-1}. Receptor **1** favours benzoate over acetate, chloride and bromide while **2** favours chloride over bromide, benzoate and nitrate, indicating that the relative acidities[22] of the two receptors influence the binding selectivities. Both receptors showed selectivity towards smaller halides, with both **1** and **2** displaying the lowest affinity for iodide. The experimental data obtained from the titration of hydrogen sulfate and **2** suggests strong binding, however, the data did not fit to a suitable binding model which suggests a proton transfer reaction may be occurring.[23] Peak broadening during the titration of **1** with dihydrogenphosphate prevented an association constant from being determined. Notably, the data presented in Table 1 suggests that the receptors have selectivity for tetrahedral oxoanions, and sulfate in particular.

In all titrations, the addition of anions resulted in restricted rotation of the receptor arms, as evidenced by changes to the signals attributed to the CH_2^c environments of **1** and **2** (see

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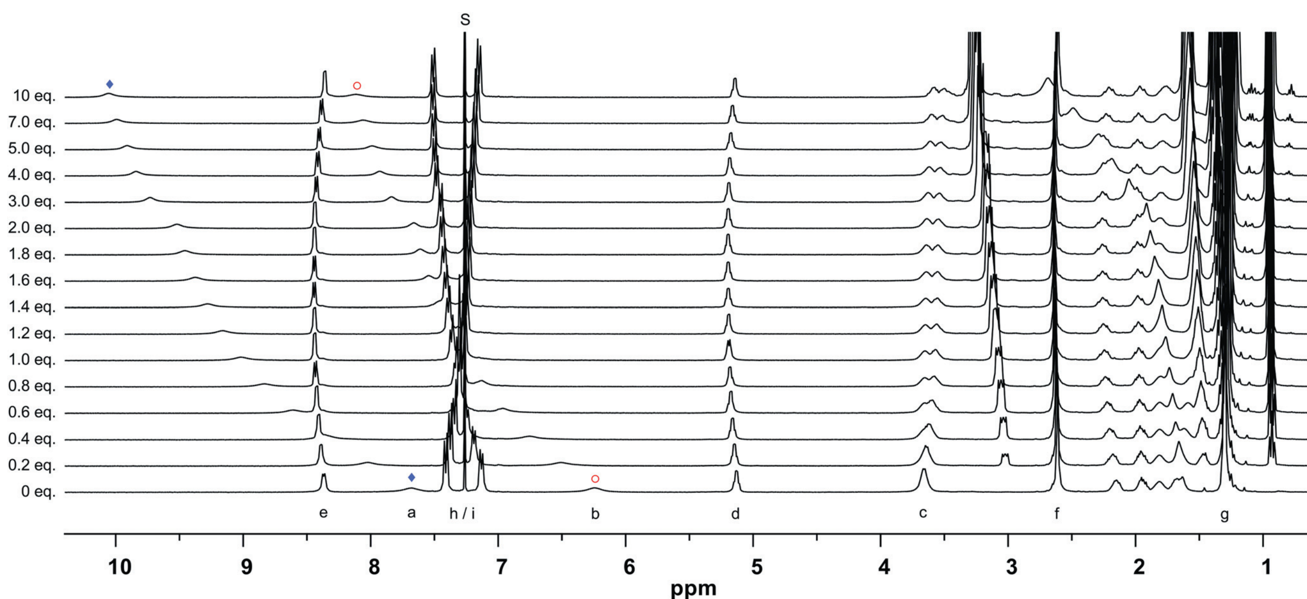
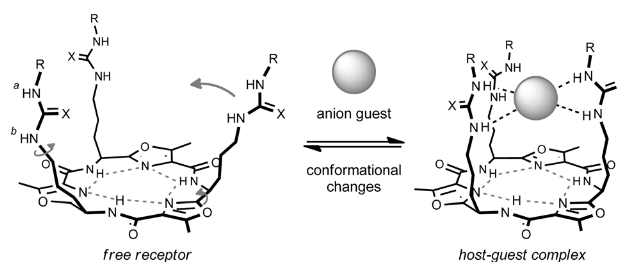


Fig. 4 ^{1}H NMR (400 MHz, 300 K) spectra from the titration of **1** with tetrabutylammonium chloride in $CDCl_3$. S: solvent residual. See Scheme 1 for proton assignments (H^{a-i}).

Table 1 Apparent stability constants (K_a, M^{-1}) of **1** and **2** towards various anions as determined by monitoring the urea or (thio)urea proton resonances, NH^a and NH^b, during ^{1}H NMR titrations in $CDCl_3$[a]

Anion[b]	Receptor **1**	Receptor **2**
Cl^-	298	1920
Br^-	104	1210
I^-	36	619
NO_3^-	74	944
AcO^-	486	919
BzO^-	579	1170
$H_2PO_4^-$	[c]	1500
TsO^-	189	1250
HSO_4^-	1700	[d]
SO_4^{2-}	$>10^4$[e,f]	$>10^4$

[a] Determined at 300 K. Data was fitted to a 1 : 1 binding model as confirmed by Job plot titrations. K_a values are an average obtained from monitoring NH^a and NH^b. Errors < 15%. [b] Anions added as their tetrabutylammonium salts. [c] Peak broadening prevented an association constant from being determined. [d] Titration data suggests strong binding however it could not be fitted to a suitable binding model. [e] Titration displayed slow exchange on the NMR timescale. [f] K_1 value only.



Scheme 3 Proposed conformational changes to the tripodal receptors incurred during anion binding. Such changes are expected to lead to changes in the nature of the hydrogen-bond network of the macrocycle.

ESI†). Based on these observations, it is hypothesised that co-operative anion binding by the three (thio)urea moieties of the receptors results in restricted conformational freedom of the binding arms (Scheme 3). The three binding arms of the receptors become fixed in place due to the formation of six hydrogen-bonds from the (thio)urea groups which converge on a central anion. Notably, larger splittings of signals for these diastereotopic protons were found to correlate with larger K_a values.

The binding titrations for sulfate with each receptor exhibited distinct behaviour which merits comment. The chemical shift data for the urea protons (NH^a and NH^b) of **2** with increasing equivalents of sulfate is shown in Fig. 5. Addition of 0.2 to 0.6 equivalents of anion caused both proton signals to broaden significantly into the baseline of the spectra so that they were hardly observed (see ESI†). However, upon further addition of sulfate both urea signals were observed significantly downfield from their original positions and continued to shift downfield until one equivalent of anion had been added.

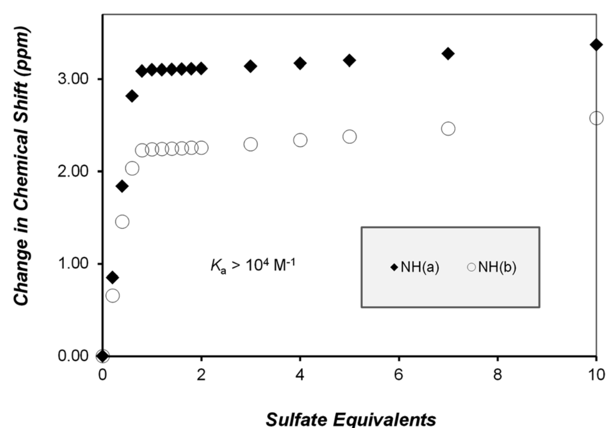


Fig. 5 Change in chemical shift (ppm) of the urea protons, NH^a and NH^b, of **2** in $CDCl_3$ with the addition of bis(tetrabutylammonium) sulfate.

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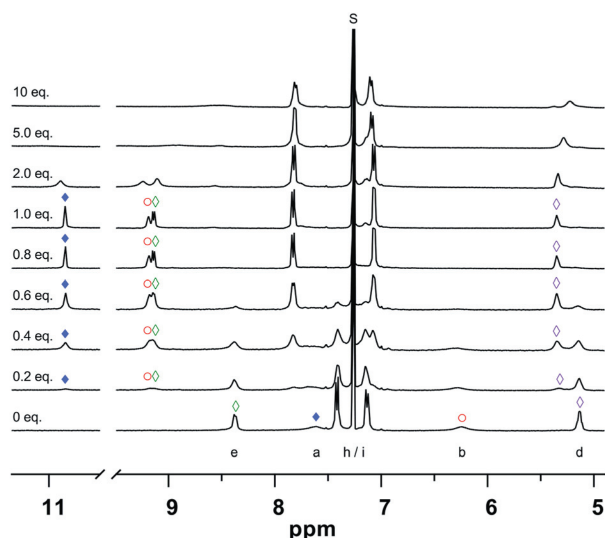

Fig. 6 ^{1}H NMR (400 MHz, 300 K) spectra from the titration of **1** with bis(tetrabutylammonium) sulfate in $CDCl_3$. The titration resulted in slow exchange up to approximately 2 equivalents of sulfate. See Scheme 1 for proton assignments (H^{a-i}).

The titration of sulfate with receptor **1** resulted in dissimilar behaviour indicative of slow exchange on the NMR timescale (Fig. 6). New signals for each proton environment appeared significantly downfield from their original positions upon the addition of anion; these were attributed to the formation of a new host–guest complex in solution. A steady increase in the population of the new signals was observed as the titration progressed until, after one equivalent of anion had been added, the original signals could no longer be observed indicating very strong 1 : 1 binding ($K_a > 10^4$ M^{-1}). Beyond the addition of two equivalents of sulfate, the (thio)urea (NH^a and NH^b) and amide (NH^e) signals broadened to such an extent that they could no longer be observed. In contrast, the signal attributable to the ornithine α-protons (CH^d) underwent a gradual upfield shift with fast exchange. This indicated that the receptor was undergoing further conformational changes in the presence of higher equivalents of anion. These observations suggest that **1** initially forms strong 1 : 1 complexes at low concentrations of sulfate (*i.e.* less than two equivalents of sulfate), which display slow exchange on the NMR timescale, while at higher concentrations of anion, more complex binding equilibria exist which result in fast exchange processes. Similar behaviour has recently been observed by Gale and co-workers with acyclic indole and carbazole-based receptors.[23,24]

In order to gain further insight into the complex behaviour **1** exhibited at high concentrations of sulfate, and to further investigate the specific binding interactions involved in anion recognition, the chemical shifts of the thiourea or urea protons (NH^a) and the amide protons (NH^e) of **1** and **2** were examined in closer detail. Table 2 summarises the maximum changes in the chemical shift ($\Delta\delta_{max}$) of these protons over the course of titrations carried out with a variety of anions.

For both **1** and **2** the more basic anions, such as acetate, benzoate, dihydrogenphosphate and sulfate, were found to induce the largest downfield shifts in the thiourea or urea protons (Table 2). However, the same trend was not observed in the case

Table 2 Maximum changes in the chemical shifts ($\Delta\delta_{max}$, ppm) of the thiourea or urea NH^a protons and the amide protons (NH^e) of **1** and **2** over the course of titrations with a variety of anions in $CDCl_3$

	NH^a [thiourea]		NH^e [amide]	
Anion[a]	Receptor **1**	Receptor **2**	Receptor **1**	Receptor **2**
Cl^-	2.385	3.369	0.076	0.109
Br^-	1.696	2.173	0.044	0.079
I^-	0.680	0.618	0.002	0.049
NO_3^-	1.364	1.411	0.012	0.032
AcO^-	3.472	2.565	0.092	0.115
BzO^-	3.169	3.235	0.019	0.050
$H_2PO_4^-$	[b]	2.997	0.285	0.260
TsO^-	1.149	0.804	0.052	0.053
HSO_4^-	1.430	1.029	0.150	0.219
SO_4^{2-}	3.271	3.263	0.765	0.703

[a] Anions added as their tetrabutylammonium salts. [b] Titration resulted in broadening of the thiourea protons.

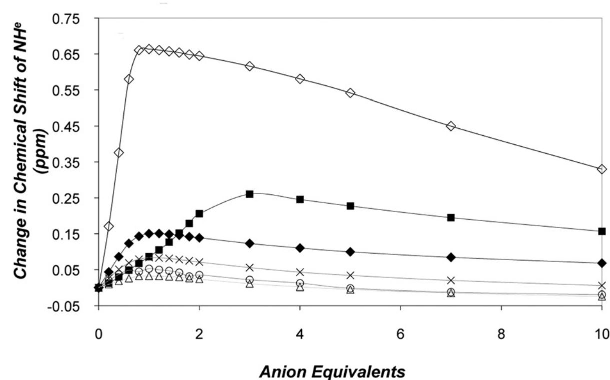

Fig. 7 Plot of the change in chemical shift ($\Delta\delta$, ppm) of the cyclic peptide amide protons (NH^e) of **2** in $CDCl_3$ with the addition of various anions. ◇ sulfate; ◆ hydrogensulfate; ■ dihydrogenphosphate; × bromide; ◯ tosylate; △ nitrate.

of the amide protons. For instance, for both **1** and **2**, the maximum downfield shift of the amide protons incurred during the titration with benzoate was small (0.019 and 0.050 ppm, respectively). Small downfield shifts for the amide protons were also observed for the titrations performed with chloride, bromide, iodide, nitrate, acetate and tosylate indicating that the predominant hydrogen bonding interactions to these guests in solution were from the (thio)urea or urea binding sites only. In sharp contrast to these observations, relatively large $\Delta\delta_{max}$-values for the amide protons of both **1** and **2** were observed for the titrations involving dihydrogenphosphate, hydrogensulfate and in particular for sulfate indicating that the peptide backbone is involved with binding to these anions to varying extents.

The titration profiles of the amide protons (NH^e) of **2** with a variety of anions are shown in Fig. 7. Although the magnitude of the downfield shift was different for each anion, the signal in each case first underwent a downfield shift with the addition of anion which was then followed by an upfield shift for the remainder of the titration. All titrations resulted in similar profiles, irrespective of the binding strength to the anions. With the exception of dihydrogenphosphate, the downfield shifts reach a maximum value upon the addition of approximately one

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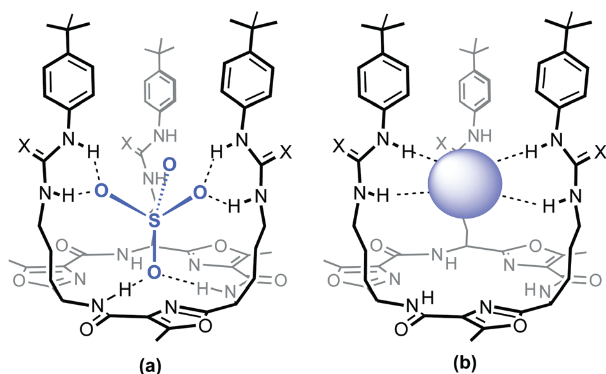

Fig. 8 Proposed binding modes of receptors **1** (X = S) and **2** (X = O) for (a) sulfate anions involving nine hydrogen bonding interactions and (b) spherical anions involving six hydrogen bonding interactions.

equivalent of anion, which further suggests a 1 : 1 binding stoichiometry. In the case of dihydrogenphosphate, the curve reached a maximum value with the addition of three equivalents of anion, however, Job's plot titrations of the urea protons indicate 1 : 1 binding (see ESI†).

Small changes in chemical shift of the amide hydrogens are considered to be a result of conformational changes of the scaffolds. However, the large changes observed upon sulfate addition strongly suggest the formation of hydrogen-bond interactions with this anion.[25] Similar shifts previously observed for the amide proton signals of related cryptand-like receptors upon binding sulfate ions have been attributed to the formation of hydrogen bonds between the receptors and this anion.[17] Due to a combination of the hydrogen bonding and conformational effects, the chemical shift data for the amide proton signals of **1** and **2** could not be fitted to an appropriate binding model, preventing the determination of stability constants using these protons. Indeed, the titration profiles observed drastically differ from those reported previously during anion titrations with other *Lissoclinum* type receptors.[26]

The significant and rapid downfield shift of the amide proton signals of **1** and **2** observed in the titration with sulfate (Table 2, Fig. 7), in contrast to the smaller shifts observed for all other anions, strongly suggests that the amide hydrogens contribute significantly to binding this particular anion. Based on the titration data obtained, a proposed binding mode for sulfate with receptors **1** and **2** is shown in Fig. 8a. It is postulated that a total of nine hydrogen bonding interactions (six from the (thio)urea groups and three from the amide protons) act to selectively and strongly hold a central sulfate anion in place. In contrast, the proposed binding mode for spherical anions features only six binding contributions from the three urea or (thio)urea groups (Fig. 8b). The observed selectivity of the receptors for sulfate may be attributed to these additional hydrogen bonding interactions. This is in agreement with the proposed binding modes we have previously reported for related cryptands.[17] The smaller changes to the amide protons observed in the titrations with dihydrogenphosphate and hydrogensulfate also suggest a binding association. However, these other tetrahedral anions are presumably not able to adopt a binding orientation like that represented in Fig. 8a due to their higher protonation states and, as such, are not bound as strongly.

Table 3 Apparent stability constants (K_a, M^{-1}) of **1** and **2** towards various anions as determined by monitoring the urea or thiourea proton resonances, NH[a] and NH[b], during ^1H NMR titrations in 10% v/v DMSO-d_6/$CDCl_3$[a]

Anion[b]	Receptor **1**	Receptor **2**
Cl^-	477	593
NO_3^-	89	129
HSO_4^-	289	521
SO_4^{2-}	>10[4c,d]	>10[4]

[a] Determined at 300 K. Data was fitted to a 1 : 1 binding model. K_a values are an average obtained from monitoring NH[a] and NH[b]. Error <15%. [b] Anions added as their tetrabutylammonium salts. [c] Titration displayed slow exchange on the NMR timescale. [d] K_1 value only.

Notably, with the exception of sulfate, the stability constants obtained for tris-urea **2** were several times larger than those found for tris-thiourea **1**. In the most extreme case with iodide, binding with **2** is more than 17 times stronger than with **1**. While thiourea is more acidic than urea (pK_a = 21.1 and 26.9, respectively, in DMSO)[22] and therefore expected to establish stronger hydrogen bonding interactions with anions, it has recently been shown that the conformational preferences of urea and thiourea groups can be an overriding factor which dictates binding behaviour. While ureas frequently adopt the *cis–cis* conformation required for binding, thioureas have been found to favour a *cis–trans* conformation and require rotational reorganisation to adopt the required geometry for anion binding.[27] In this regard, **2** has an added degree of pre-organisation compared to **1**, and this is reflected in the lower binding constants observed for **1**.

Anion binding in more competitive solvent mixtures

In order to investigate the effect of solvent polarity on the binding affinities of **1** and **2**, further binding studies with selected anions were carried out in a more competitive medium, namely 10% v/v DMSO-d_6/$CDCl_3$ (Table 3). It was previously established that **2** does not self-associate in this solvent system; as such a pre-dimerisation equilibrium was not taken into account. In all cases, except for titration of **1** with sulfate, fast exchange processes were observed.

The binding data obtained reveals that moving to this more polar solvent mixture had a significant influence on the binding behaviour of receptor **2**. For example, the stability constants obtained for chloride and nitrate with **2** are 3 and 7 times smaller, respectively, than those values obtained previously in $CDCl_3$. In addition, in this more polar solvent, the binding of **2** and hydrogen sulfate was simplified with good fits obtained to a 1 : 1 binding model. In contrast, the stability constants found for the tris-thiourea **1** are not as dramatically affected upon moving to the more competitive solvent system. In fact, surprisingly the stability constant obtained for chloride and **1** increased in moving to this more polar solvent system (K_a = 477 M^{-1} in 10% v/v DMSO-d_6/$CDCl_3$ *versus* 298 M^{-1} in $CDCl_3$). Such behaviour suggests that the rotational barriers of the thiourea and urea binding groups are influenced to different extents by the polarity of the solvent system employed and that in more polar solvents,

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the higher acidity of the thiourea protons outweighs the conformational preferences of these groups.[28]

Both **1** and **2** bind sulfate selectively and strongly in this more competitive medium (Table 3). The results clearly reflect the importance of the highly complementary binding network on binding affinity and selectivity of this tripodal system. As with the data obtained in $CDCl_3$, titrations with sulfate and tris-thiourea **1** resulted in slow exchange on the NMR timescale and at high concentrations of anion the NMR data suggested that more complex equilibria existed. The titration with tris-urea **2**, on the other hand, resulted in fast exchange processes and fitted well to a 1 : 1 binding model.

In comparison to the cryptand-like thiourea systems previously reported,[16,17] **1** and **2** show similar selectivity for sulfate ions over all other anions tested, attributable to a binding mode involving the amide hydrogen bond donors of the cyclic peptide scaffold. Notably, binding affinity for other anions (in particular for chloride) is much lower in these more flexible tripodal systems providing greater selectivity for sulfate. However, direct comparisons of the binding constants of the tripodal and cryptand-like systems can not be made as measurements were made in different solvents for solubility reasons.

Conclusions

Quantitative binding studies carried out with the tripodal receptors indicate that both **1** and **2** bind sulfate ions strongly and selectively in $CDCl_3$ and 10% v/v DMSO-d_6/$CDCl_3$ (K_a values > 10^4 M^{-1} in both solvents). Notably, in $CDCl_3$ the tris-urea **2** exhibited higher association constants for all anions than the tris-thiourea **1**. This can be attributed to the relative reorganisational energies required for the thiourea and urea groups of **1** and **2**, respectively, to adopt the *cis–cis* conformation necessary for anion binding. In the more polar solvent, 10% v/v DMSO-d_6/$CDCl_3$, both receptors bound anions with similar affinities suggesting that the higher acidity of the thiourea protons outweighs the reorganisational energies in more polar media.

The observed selectivity for sulfate is attributed to a network of hydrogen bonds which is highly complementary for the tetrahedral oxoanion. ^{1}H NMR evidence indicates that sulfate accepts a total of nine hydrogen bonds, six from the (thio)urea groups and three from the amide NH groups of the peptide backbone, while spherical anions, *e.g.* chloride, only form six hydrogen bonds to the (thio)urea groups. Presumably, the selectivity for sulfate over the other tetrahedral anions examined (hydrogensulfate and dihydrogenphosphate) is due to the inability of these anions to adopt a similar orientation to sulfate due to their higher protonation states. In this regard, the selectivity mechanism of the receptors mimics that of biological systems. We are currently extending our investigations of the anion binding affinities and selectivities of these and structurally similar receptors.

Experimental

General remarks

Melting points were measured using a Stanford Research Systems Optimelt apparatus and are uncorrected. Optical rotations were performed using a Perkin Elmer Model 341 polarimeter using the indicated spectroscopic grade solvents. ^{1}H NMR spectra were recorded using a Bruker Avance DPX 400 at a frequency of 400.13 MHz or a Bruker Avance DPX 300 at a frequency of 300.13 MHz and are reported as parts per million (ppm) with $CDCl_3$ (δ_H 7.26 ppm) as an internal reference. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (J Hz) and relative integral. ^{13}C NMR spectra were recorded using a Bruker Avance DPX 400 at a frequency of 100.61 MHz or a Bruker Avance DPX 300 at a frequency of 75.47 MHz and are reported as parts per million (ppm) with $CDCl_3$ (δ_C 77.16 ppm) as an internal reference. High resolution ESI spectra were recorded on a Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) with an Analytica ESI source, operating at 4.7 T or a Bruker Daltonics Apex Ultra FTICR with an Apollo Dual source, operating at 7 T. Analytical TLC was performed using precoated silica gel plates (Merck Kieselgel 60 F254). Preparative column chromatography was carried out using either Merck Kieselgel 60 silica gel (SiO_2; 0.040–0.063 mm) or Davisil® Chromatographic Silica Media (SiO_2; 0.040–0.063 mm), with the indicated solvents which were mixed v/v as specified. Triethylamine was distilled from calcium hydride prior to use. Chloroform was distilled from calcium chloride and passed through a column of basic alumina prior to use. Tetrabutylammonium salts were used as supplied and were stored in a vacuum desiccator over silica drying beads and phosphorous pentoxide. Compound **3** was synthesised according to our previously reported procedures.[15,16] All other reagents were commercially available and were used as supplied.

Receptor 1

Under an atmosphere of nitrogen, anhydrous $CHCl_3$ (6.5 mL) was added to tris-hydrobromide salt **3a** (261 mg, 0.315 mmol) to provide a suspension. Triethylamine (0.141 mL, 1.01 mmol) was then added and the resulting mixture was allowed to reflux for 30 min followed by cooling to room temperature. *para*-^{t}Butylisothiocyanate (193 mg, 1.01 mmol) was then added and the reaction mixture was returned to reflux for a further 19 h. The mixture was cooled to room temperature, diluted with $CHCl_3$ (10 mL), quenched with 0.5 M HCl (5 mL) and the layers separated. Further extraction of the aqueous layer with $CHCl_3$ (2 × 20 mL) was followed by washing the combined organic layers with water (15 mL) and saturated brine (15 mL). The organic layers were then dried ($MgSO_4$) and concentrated under reduced pressure to give the crude product as a brown oil. Subsequent purification by flash chromatography (silica gel; EtOAc:hexanes [1 : 4] then CH_2Cl_2:MeOH [20 : 1]) gave the desired tris-(thio)urea **1** as an off-white solid (325 mg, 89%). m.p. 160–165 °C; $[\alpha]_D^{20}$ −33.6 (c 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.33 (d, J = 6.5 Hz, 3 H), 8.12 (br s, 3 H), 7.44–7.31 (m, 6 H), 7.17–7.06 (m, 6 H), 6.27 (br s, 3 H), 5.16–5.03 (m, 3 H), 3.75–3.51 (m, 6 H), 2.57 (s, 9 H), 2.20–2.02 (m, 3 H), 2.00–1.54 (m, 9 H), 1.27 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 180.7, 161.1, 160.7, 154.1, 150.3, 133.6, 128.4, 127.0, 125.1, 47.6, 44.8, 34.7, 32.1, 31.3, 24.5, 11.7; HRMS (ESI) m/z calcd for $C_{60}H_{79}N_{12}O_6S_3$ $[M + H]^+$: 1159.5408, found: 1159.5433.

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Receptor 2

Under an atmosphere of nitrogen, *para*-^{t}butylisocyanate (48.6 μL, 0.273 mmol) was added to a solution of tris-amine **3b** (50.0 mg, 85.4 μmol) in anhydrous $CHCl_3$ (5 mL). The reaction mixture was refluxed for 15 h after which time it was concentrated under reduced pressure to give a pale yellow oil. Subsequent purification by flash chromatography (silica gel; CH_2Cl_2:MeOH [12 : 1]) provided the desired tris-urea **2** as an off-white solid (74.5 mg, 80%). m.p. 186 °C (decomp.); $[\alpha]_D^{20}$ −12.4 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.33 (br s, 3 H), 7.59 (br s, 3 H), 7.23–6.99 (m, 12 H), 5.97 (br s, 3 H), 5.12 (br s, 3 H), 3.25–2.97 (m, 6 H), 2.55 (s, 9 H), 2.15–1.99 (m, 3 H), 1.97–1.78 (m, 3 H), 1.69–1.51 (m, 3 H), 1.49–1.33 (m, 3 H), 1.21 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.9, 156.9, 154.1, 146.0, 136.3, 128.4, 125.9, 120.1, 47.8, 39.6, 34.3, 32.0, 31.5, 25.4, 11.7 (one quaternary carbon signal obscured or overlapping); HRMS (ESI) *m*/*z* calcd for $C_{60}H_{79}N_{12}O_9$ $[M + H]^+$: 1111.6093, found: 1111.6072.

Determination of dimerisation constants (K_{dimer}, M^{-1}) by ^{1}H NMR spectroscopic titrations

In a typical dimerisation experiment a solution of receptor **1** or **2** was prepared in the stated deuterated solvents (v/v) of known concentration (of the order of 40 mM) using a volumetric flask. In each case, aliquots of these stock solutions were added to 500 μL of the same deuterated solvent in an NMR tube. After each addition the solution was thoroughly mixed and the ^{1}H NMR (400 MHz, 300 K) spectrum was recorded. Titrations were performed in duplicate. Non-linear curve fitting[21] of the experimentally obtained titration isotherms (concentration of receptor *versus* chemical shift of (thio)urea NH protons) enabled the calculation of dimerisation constants (K_{dimer}, M^{-1}).

Determination of association constants (K_a, M^{-1}) for various anions by ^{1}H NMR spectroscopic titrations

In a typical anion titration experiment a 2–5 mM stock solution of receptor was accurately prepared in the stated deuterated solvents (v/v) using a volumetric flask. Solutions of anions (as their tetrabutylammonium salts) to be titrated were then prepared in separate volumetric flasks using the same host solution so that the concentration of the host remained constant throughout a given titration experiment. The concentration of anion solutions were made 50 times that of the host (*i.e.* 0.1–0.25 M). In each case, 500 μL of host solution in an NMR tube was titrated with aliquots of anion stock solution and after each addition the ^{1}H NMR (400 MHz, 300 K) spectrum was recorded after thorough mixing. Typically this was performed in the following order: 10 × 2 μL, 3 × 10 μL, 20 μL then 30 μL (total 100 μL). Titrations were performed in duplicate. Non-linear curve fitting[21] of the experimentally obtained titration isotherms (equivalents of anion *versus* chemical shift of the (thio)urea NH protons) enabled the calculation of association constants (K_a, M^{-1}). In all cases complete dissociation of the tetrabutylammonium salts was assumed and the data was fitted to a 1 : 1 binding model as confirmed by Job's plot titrations (see below).

Job's plot titrations

Separate stock solutions of receptor (2.00 mM) and an anion guest (2.00 mM) were prepared in $CDCl_3$ using volumetric flasks. ^{1}H NMR spectra were recorded for eight different solutions containing a total volume of 500 μL in the following receptor : anion ratios: 500 : 0, 450 : 50, 375 : 125, 325 : 175, 250 : 250, 175 : 325, 125 : 375 and 50 : 450. The molar fraction of the receptor (X_R) was then plotted as a function of $\Delta\delta \times X_R$, where $\Delta\delta = \delta_{obs} - \delta_{free}$ (δ_{obs} is the observed chemical shift and δ_{free} is the chemical shift of the free receptor). In each case the chemical shifts of both the (thio)urea protons, NH^a and NH^b, were examined.

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References

1 For reviews see: J. L. Sessler, P. A. Gale and W.-S. Cho, *Anion receptor chemistry*, Royal Society of Chemistry, Cambridge, 2006, ch. 4; S. Kubik, *Chem. Soc. Rev.*, 2009, **38**, 585; S. O. Kang, R. A. Begum and K. Bowman-James, *Angew. Chem., Int. Ed.*, 2006, **45**, 7882; C. R. Bondy and S. J. Loeb, *Coord. Chem. Rev.*, 2003, **240**, 77.

2 For recent examples see: Á. L. Fuentes de Arriba, M. G. Turiel, L. Simón, F. Sanz, J. F. Boyero, F. M. Muñiz, J. R. Morán and V. Alcázar, *Org. Biomol. Chem.*, 2011, **9**, 8321; V. Amendola, L. Fabbrizzi, L. Mosca and F.-P. Schmidtchen, *Chem.–Eur. J.*, 2011, **17**, 5972; M. V. López, M. R. Bermejo, M. E. Vásquez, A. Taglietti, G. Zaragoza, R. Pedrido and M. Martínez-Calvo, *Org. Biomol. Chem.*, 2010, **8**, 357; C. Caltagirone, G. W. Bates, P. A. Gale and M. A. Light, *Chem. Commun.*, 2008, **44**, 61; O. B. Berryman, C. A. Johnson II, L. N. Zakharov, M. M. Haley and D. W. Johnson, *Angew. Chem., Int. Ed.*, 2008, **47**, 117.

3 For a review see: J. L. Sessler, P. A. Gale and W.-S. Cho, *Anion receptor chemistry*, Royal Society of Chemistry, Cambridge, 2006, ch. 5.

4 For a review see: P. A. Gale, *Chem. Commun.*, 2008, 4525.

5 For reviews see: A.-F. Li, J.-H. Wang, F. Wang and Y.-B. Jiang, *Chem. Soc. Rev.*, 2010, **39**, 3729; V. Amendola, L. Fabbrizzi and L. Mosca, *Chem. Soc. Rev.*, 2010, **39**, 3889.

6 B. P. Hay, T. K. Firman and B. A. Moyer, *J. Am. Chem. Soc.*, 2005, **127**, 1810.

7 R. Custelcean, B. A. Moyer and B. P. Hay, *Chem. Commun.*, 2005, 5971; R. Custelcean, P. Remy, P. V. Bonnesen, D.-E. Jiang and B. A. Moyer, *Angew. Chem., Int. Ed.*, 2008, **47**, 1866; B. Wu, J. Liang, J. Yang, C. Jia, X.-J. Yang, H. Zhang, N. Tang and C. Janiak, *Chem. Commun.*, 2008, 1762; Y. Li, K. M. Mullen, T. D. W. Claridge, P. J. Costa, V. Felix and P. D. Beer, *Chem. Commun.*, 2009, 7134; I. Ravikumar, P. S. Lakshminarayanan, M. Arunachalam, E. Suresh and P. Ghosh, *Dalton Trans.*, 2009, 4160; R. Custelcean, A. Bock and B. A. Moyer, *J. Am. Chem. Soc.*, 2010, **132**, 7177; N. Busschaert, P. A. Gale, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis and W. A. Harrell Jr., *Chem. Commun.*, 2010, **46**, 6252; S. K. Dey and G. Das, *Dalton Trans.*, 2011, **40**, 12048; M. Li, Y. Hao, B. Wu, C. Jia, X. Huang and X.-J. Yang, *Org. Biomol. Chem.*, 2011, **9**, 5637; N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás and P. A. Gale, *J. Am. Chem. Soc.*, 2011, **133**, 14136; Y. Hao, C. Jia, S. Li, X. Huang, X.-J. Yang, C. Janiak and B. Wu, *Supramol. Chem.*, DOI: 10.1081/10610278.2011.622389.

8 C. Jia, B. Wu, S. Li, X. Huang, Q. Zhao, Q.-S. Li and X.-J. Yang, *Angew. Chem., Int. Ed.*, 2011, **50**, 486.

9 H. R. Seong, D.-S. Kim, S.-G. Kim, H.-J. Choi and K. H. Ahn, *Tetrahedron Lett.*, 2004, **45**, 723; I. Hisaki, S. Sasaki, K. Hirose and Y. Tobe, *Eur. J. Org. Chem.*, 2007, **2007**, 607; S. Sasaki, D. Citterio, S. Ozawa and K. Suzuki, *J. Chem. Soc., Perkin Trans. 2*, 2001, 2309; D. R. Turner, M. J. Paterson and J. W. Steed, *J. Org. Chem.*, 2006, **71**, 1598.

10 C. M. Gomes dos Santos, E. M. Boyle, S. De Solis, P. E. Kruger and T. Gunnlaugsson, *Chem. Commun.*, 2011, **47**, 12176.
11 I. Ravikumar and P. Ghosh, *Chem. Commun.*, 2010, **46**, 6741.
12 H.-J. Choi, Y. S. Park, S. H. Yun, H.-S. Kim, C. S. Cho, K. Ko and K. H. Ahn, *Org. Lett.*, 2002, **4**, 795.
13 M. Hamon, M. Ménand, S. Le Gac, M. Luhmer, V. Dalla and I. Jabin, *J. Org. Chem.*, 2008, **73**, 7067; J. Scheerder, J. F. J. Engbersen, A. Casnati, R. Ungaro and D. N. Reinhoudt, *J. Org. Chem.*, 1995, **60**, 6448.
14 D. Mink, S. Mecozzi and J. Rebek Jr., *Tetrahedron Lett.*, 1998, **39**, 5709; A. J. Lucke, J. D. A. Tyndall, Y. Singh and D. P. Fairlie, *J. Mol. Graphics Modell.*, 2003, **21**, 341; Y. Singh, M. J. Stoermer, A. J. Lucke, T. Guthrie and D. P. Fairlie, *J. Am. Chem. Soc.*, 2005, **127**, 6563; K. A. Jolliffe, *Supramol. Chem.*, 2005, **17**, 81; A. Pintér, G. Haberhauer, I. Hyla-Kryspin and S. Grimme, *Chem. Commun.*, 2007, 3711; A. Pintér and G. Haberhauer, *Synlett*, 2009, 3082.
15 R. J. G. Black, V. J. Dungan, R. Y. T. Li, P. G. Young and K. A. Jolliffe, *Synlett*, 2010, 551.
16 P. G. Young, J. K. Clegg, M. Bhadbhade and K. A. Jolliffe, *Chem. Commun.*, 2011, **47**, 463–465.
17 P. G. Young, J. K. Clegg and K. A. Jolliffe, *Supramol. Chem.*, DOI: 10.1081/10610278.2011.622388.
18 M. George, G. Tan, V. T. John and R. G. Weiss, *Chem.–Eur. J.*, 2005, **11**, 3243 and references therein.
19 V. Rudzevich, Y. Rudzevich and V. Boehmer, *Synlett*, 2009, 1887; K. D. Shimizu and J. Rebek Jr., *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 12403; P. Ballester and G. Gil-Ramirez, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10455; M. Alajarín, A. Pastor, R.-A. Orenes and J. W. Steed, *J. Org. Chem.*, 2002, **67**, 7091.
20 A. Asadi, D. Ajami and J. Rebek Jr., *J. Am. Chem. Soc.*, 2011, **133**, 10682.
21 All curve fitting was performed using the Equilibria program, C. E. Marjo, University of New South Wales Analytical Centre, Sydney, Australia, http://www.sseau.unsw.edu.au/Index.htm, 2009.
22 F. G. Bordwell, *Acc. Chem. Res.*, 1988, **21**, 456.
23 P. A. Gale, J. R. Hiscock, S. J. Moore, C. Caltagirone, M. B. Hursthouse and M. E. Light, *Chem.–Asian J.*, 2010, **5**, 555.
24 P. A. Gale, J. R. Hiscock, C. Z. Jie, M. B. Hursthouse and M. E. Light, *Chem. Sci.*, 2010, **1**, 215.
25 G. Wagner, A. Pardi and K. Wüthrich, *J. Am. Chem. Soc.*, 1983, **105**, 5948.
26 M. Schnopp, S. Ernst and G. Haberhauer, *Eur. J. Org. Chem.*, 2009, 213; L. Molina, E. Moreno-Clavijo, A. J. Moreno-Vargas, A. T. Carmona and I. Robina, *Eur. J. Org. Chem.*, 2010, 4049.
27 J. R. Hiscock, P. A. Gale, C. Caltagirone, M. B. Hursthouse and M. E. Light, *Supramol. Chem.*, 2010, **22**, 647; V. S. Bryantsev and B. P. Hay, *J. Phys. Chem. A*, 2006, **110**, 4678–4688; B. P. Hay, *Chem. Soc. Rev.*, 2010, **39**, 3700; V. S. Bryantsev, T. K. Firman and B. P. Hay, *J. Phys. Chem. A*, 2005, **109**, 832.
28 K. A. Haushalter, J. Lau and J. D. Roberts, *J. Am. Chem. Soc.*, 1996, **118**, 8891; J. B. Gerken and S. Ross, *J. Phys. Org. Chem.*, 2010, **23**, 806.

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