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Effect of spacer geometry on oxoanion binding by bis- and tetrakis-thiourea hosts

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Abstract

Hosts with thiourea groups bind anions by formation of multiple hydrogen bonds. This contribution discusses how spacers linking two or four thiourea groups affect the host affinity and selectivity. While most of the bis-thioureas bind $H_2PO_4^-$ preferentially, the extent of selectivity over chloride, acetate, and $H_2AsO_4^-$ is determined by the size of the binding cavity. A tetrakis-thiourea is shown to exhibit a unique $H_2AsO_4^-$ selectivity, and the discrimination of chloride is enhanced by specific solvation in dimethyl sulfoxide.

1. Introduction

The development of synthetic hosts for anions is a very active area of research.[1] In view of their important biological and environmental roles, oxoanions such as phosphate and sulfate have attracted particular attention. The design of most electrically neutral hosts for these anions is based on Lewis acid and/or hydrogen bond donor groups.[1] Encouraged by reports on mono-urea compounds that bind organic phosphates,[2] bis-ureas[3,4] that form complexes with dicarboxylates, disulfonates, and diphosphonates, and a host with two thiourea groups[3] that binds a dicarboxylate, we previously investigated hydrogen bond-mediated binding of inorganic phosphate by a number of bis-urea and bis-thiourea hosts (e.g., **1a**, **1b**, **2a**, **2b** in Fig. 1).[5–7] We showed that very simple hosts with a *m*-xylenyl-bis-thiourea subunit (**1a**, **1b**) are well suited for recognition of $H_2PO_4^-$ in non-aqueous solvents because they dissolve better, self-associate much less, and bind $H_2PO_4^-$ more strongly than corresponding bis-ureas.[5] Using a xanthene spacer (introduced by Rebek and co-workers)[6] to separate the two thiourea groups from one another, we also demonstrated that appropriate host preorganization as in **2a** and **2b** can drastically increase the affinity for $H_2PO_4^-$.[7,8] The preferential complexation of $H_2PO_4^-$ by these hosts was interpreted in terms of the hydrogen bond acceptor strength and geometry of the anion guests. Initially, only binding motifs **I** and **II** were considered,[5,7] and experimental data seemed to suggest that **I** is the better representation of the actual complex.

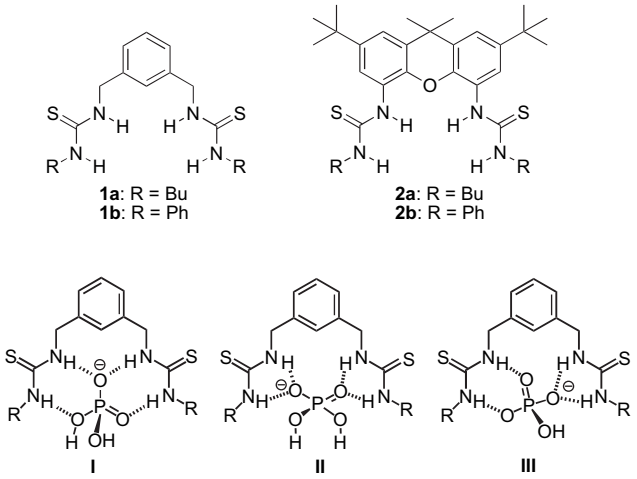

Figure 1. Bis-thiourea hosts with a *meta*-xylylene (**1a**, **1b**) and a xanthene (**2a**, **2b**) spacer, along with motifs **I**–**III** for $H_2PO_4^-$ binding.

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However, a recent computational study of bis-urea complexes suggests that binding motif **III** should also be considered.[9] Unfortunately, crystallographic evidence is not yet available.

Since the first report of phosphate binding by bis-thioureas, a large number of new anion hosts with thiourea groups have been developed. Effects of electron withdrawing and hydrogen-bonding substituents on the selectivity and spectroscopic properties of a variety of mono-thioureas were reported, and iminoyl- as well as *N*-benzamido-substituted mono-thioureas with similar binding patterns were described.[10] Complexation of dicarboxylates with bis-ureas and bis-thioureas in dimethyl sulfoxide (DMSO) was shown to be enthalpically driven,[11] and ditopic carriers with two thiourea groups were shown to permit spectroscopic phosphate detection and phosphate ester binding in water.[12] Moreover, anion binding properties of macrocycles, resorcinarene cavitands, bis-crown ethers, and tripodal benzene derivatives with three or four thiourea groups were reported.[13]

Interestingly, the *m*-xylenyl-bis-thiourea subunit was utilized in several hosts. It was applied in several macrocyclic bis- and tris-thioureas,[14] and in linear tri-, tetra-, and hexa-thiourea hosts.[15] Bis-thiourea hosts with a *m*-xylenyl-bis-thiourea subunit were used as ionophores for ion-selective electrodes to facilitate ion transfer at the liquid–liquid interface and to promote helicity in carbohydrate-containing foldamers.[16] Moreover, the *m*-xylenyl spacer was used in closely related bis-isothiouronium hosts,[17] and a geometrically similar spacer based on indoaniline was used for colorimetric detections.[18] A bis-thiourea based on a 2,2′-disubstituted biphenyl spacer represents a rare departure from the *m*-xylenyl spacer approach.[19] Rotation around the single bond connecting the two phenyl units permits the two thiourea groups to approach one another to the extent that steric repulsion may occur. Not surprisingly, this host was found to bind the small fluoride ion very strongly.

However, in view of the need for selective oxoanion hosts and the rather extensive literature on anion hosts with thiourea groups, it has surprised us and others[9] how little experimental effort has been spent in the past to systematically investigate the relationship between the shape of the host cavity formed by thiourea compounds and the resulting affinity and selectivity for different anions. A computational study suggests that doubly charged oxoanions such as sulfate may bind up to six urea groups at a time, and it appears likely that the same would also be true for thiourea groups.[20] Unfortunately, spacers that connect the urea groups with an optimum geometry were not proposed.[20] Also, besides the above mentioned xanthene and biphenyl spacer, little is known experimentally about alternatives to the popular *m*-xylenyl-bis-thiourea spacer. Therefore, we determined binding strengths and selectivities of hosts with four new spacers linking two thiourea groups.

2. Results and discussion

Bis-thiourea hosts **3**–**6** (see Fig. 2) were prepared from phenyl isothiocyanate and the appropriate diamines. 2,7-Bis-(aminomethyl)naphthalene required for the synthesis of **5** was obtained by dibromination of 2,7-dimethylnaphthalene, conversion to the diazide, and reduction to the diamine.[21] The linear tetrakis-thiourea **7** was synthesized by mono-protection of *m*-xylenediamine,[22] conversion of the unprotected amino group into a isothiocyanate, reaction with *m*-xylenediamine to give **8**, deprotection, and conversion to the tetrakis-thiourea with phenyl isothiocyanate (Fig. 3).

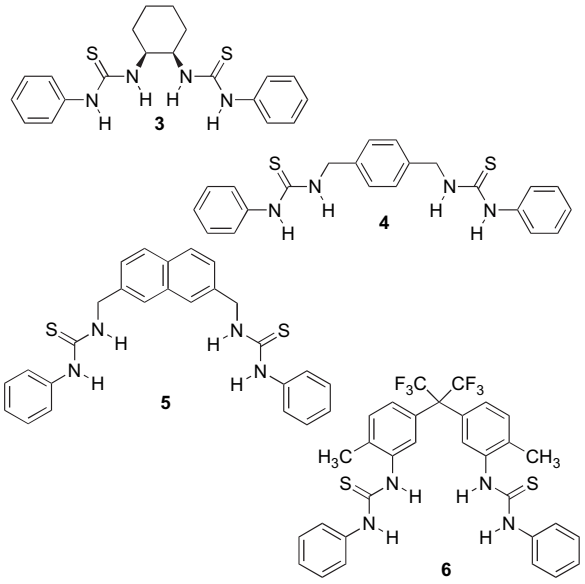

Figure 2. New bis-thiourea hosts.

The association of the new hosts with various anionic guests in DMSO-d_6 was studied by ^{1}H NMR spectroscopy, as described previously.[5,7] This solvent was chosen because it is a strong hydrogen bond acceptor itself, and thereby effectively suppresses ionophore self-association. To diminish possible effects of ion-pair formation on the formation constants,

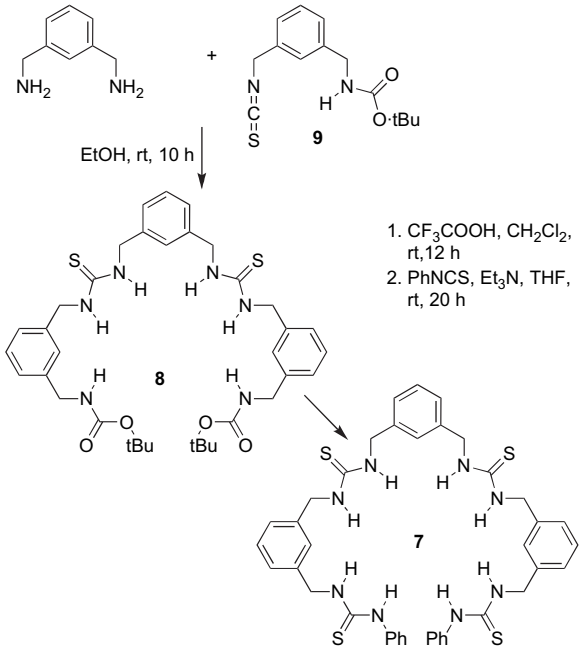

Figure 3. Synthesis of tetrakis-thiourea **7**.

K_{11}, of the 1:1 complexes, the bulky tetrabutylammonium ion was chosen as counterion.

Representative titration curves are shown in Figure 4 for the naphthylene derivative **5**. As for all other host–guest systems, binding of the anion guests to this host resulted in changes in the chemical shifts of more than one hydrogen. For most hosts, not only nitrogen-bound hydrogens of the thiourea groups but also carbon-bound hydrogens exhibited chemical shift changes upon anion binding. Even though the changes in the chemical shifts of the carbon-bound hydrogens (e.g., bottom panel of Fig. 4) were always significantly smaller than for nitrogen-bound hydrogens (e.g., top panel, Fig. 4), the sharpness of the signals provided for titration curves of high quality.

Figure 4 shows titration curves for only two hydrogens of host **5**, but for each of the three guest anions titration curves could be obtained for four different host hydrogens. Due to the different structures of the hosts and occasional signal overlap, the number of individual titration curves for each of the 23 newly investigated host–guest systems varied. However, for 22 of the 23 systems, titration curves for at least three different hydrogens could be obtained. The resulting data were fitted with an appropriate binding isotherm model, as reported previously.[5,7]

While the chemical shifts of different hydrogens of a given host changed to a different extent upon addition of a given anion, these changes are the result of the same macroscopic binding event. Therefore, fitting of the titration curves of different host hydrogens for any given host–guest pair is expected to give within error the same binding constant. Indeed, experimentally determined binding constants obtained for different host hydrogens of any given host–guest system did not differ much from one another. This is reflected by the relative standard deviation of the K_{11} values for the different hydrogens of a given host–guest system. Relative standard deviations were obtained for each host–guest system from the standard deviation of the experimental log K_{11} values by division with the average log K_{11} of the respective host–guest system. The relative standard deviation of the log K_{11} values was thus found to be for all host–guest systems within the range of 8±4%. Indeed, all fits were of high quality and showed no evidence of 1:2 or 2:2 complex formation, as it was described for other hosts previously.[5]

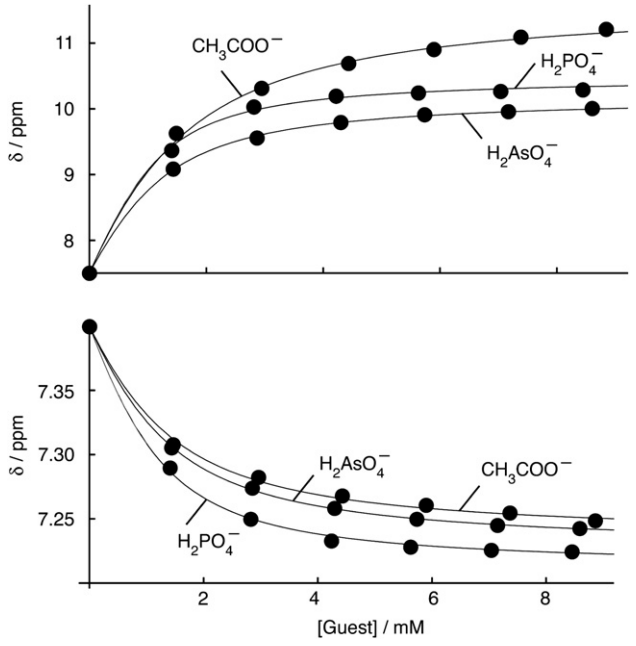

Figure 4. Representative ^{1}H NMR titrations: observed chemical shifts of NH hydrogens (top) and the phenyl hydrogens in *meta* position to the thiourea substituents (bottom) of bis-thiourea **5** (1 mM, in DMSO-d_6) upon addition of tetrabutylammonium salts of various anion guests. Data for chloride binding are not included since binding constants for this weakly binding anion had to be determined at higher host and guest concentrations.

Table 1 shows K_{11} values for the new hosts and four previously described bis-thioureas.[5,7] As reported earlier, the higher acidity of the thiourea hydrogens correlates with stronger anion binding.[5,7] This explains the higher K_{11} values for the more acidic phenyl-substituted hosts **1b** and **2b** in comparison to the butyl-substituted **1a** and **2a** analogues, respectively. Phenyl groups are well known to increase the acidity of the thioureas (pK_a of $(H_2N)_2CS$: 21.0; pK_a of $(PhNH)_2CS$: 13.5; in DMSO at 25 °C).[23] To make a meaningful comparison possible, all other bis-thiourea hosts investigated in this study are also phenyl-substituted.

Host **1b** is a *meta*-substituted phenylene derivative, and host **4** is its *para*-substituted analogue. Binding of $H_2PO_4^-$ to **4** is weaker by two orders of magnitude than in the case of **1b**. Host **4** was tested with the expectation that it might discriminate better against smaller anions such as chloride. CPK models[24] suggested that—despite the flexibility imparted by the two methylene groups—the two thiourea groups could not converge to bind small anions such as chloride. Indeed, chloride binding is weak, but so is $H_2PO_4^-$, $H_2AsO_4^-$, and acetate binding. This may be the result not of geometric constraints but rather of electrostatic repulsion. In a complex in which both thiourea groups interact with an oxoanion, the latter has to be located in the electrostatically unfavorable position above the phenyl ring.

The previously reported, less preorganized host **1b** is found to have a smaller affinity for $H_2PO_4^-$ than the new hosts **3** and **5** with their cyclohexylene and naphthylene-based spacers,

Table 1
Association constants (K_{11}, M^{-1}, in DMSO-d_6) of hosts **1**–**8** with various anions

Host	$H_2PO_4^-$	$H_2AsO_4^-$	CH_3COO^-	Cl^-
1a[a]	820	—[c]	470	9
1b[a]	4600	—[c]	2300	10
2a[a]	55,000	—[c]	3800	—[c]
2b[a]	195,000	—[c]	840	1000
3	5500	640	1200	68
3[b]	2600	240	370	6500
4	47	47	33	19
5	5800	1100	520	16
6	330	23	350	20
7[b]	1700	2700	1200	—[c]
8	440	—[c]	—[c]	140

[a] Refs. 5 and 7.
[b] Solvent: THF-d_8/DMSO-d_6 90:10.
[c] Not determined.

respectively. Importantly, the selectivities of anion binding of **1b**, **3**, and **5** clearly show size selectivity. On the one hand, the low discrimination of chloride by **3** is consistent with the ability to form a rather small binding cavity. On the other hand, the naphthylene-based spacer of host **5** puts a larger distance between the two thiourea groups, resulting in the highest $H_2PO_4^-$ vs acetate selectivity of **1b**, **3**, and **5**.

Of all the bis-thioureas, the highly preorganized hosts **2a** and **2b** with their xanthene spacers bind $H_2PO_4^-$ most strongly. Both compounds have a spacer between their thiourea groups with similar atom connectivity as host **6**, which binds this oxoanion much more weakly. Steric repulsion between the hydrogens bound to the two methylsubstituted aromatic rings of **6** prevent the near coplanarity that is possible for the two C_6 rings in the xanthene unit of **2a** and **2b**.

To test whether the introduction of two additional thiourea groups can result in strong oxoanion binding without the need for a highly rigid host structure, tetrakis-thiourea **7** was prepared. This host was found to be rather insoluble in DMSO-d_6. Therefore, the stability of its complexes was determined with THF-d_8/DMSO-d_6 9:1 as solvent. For comparative purposes, the stabilities of complexes of **3** were determined in the same solvent mixture. As Table 1 shows, the difference between the stabilities of the $H_2PO_4^-$ complexes of **3** in pure DMSO-d_6 and in THF-d_8/DMSO-d_6 9:1 is rather small. This suggests that even if the experiment could be performed, tetrakis-thiourea **7** would not bind $H_2PO_4^-$ in DMSO-d_6 more strongly than **2a** or **2b** do, and is more evidence for the importance of the high level of host preorganization in **2a** and **2b**. However, this does not mean that the third and fourth thiourea groups are not affected by $H_2PO_4^-$ binding. In the NMR titrations, the chemical shifts of their NH hydrogens clearly increased by several parts per million with addition of anions. Moreover, tetrakis-thiourea **7** has a notably larger $H_2PO_4^-$ affinity than its synthetic precursor **8**, which has an identical structure around its two central thiourea groups but two urethane groups replacing the two peripheral thiourea groups. An explanation for these findings may be that **7** and **8** form intramolecular hydrogen bonds that must be broken to permit binding of $H_2PO_4^-$, thereby lowering the $H_2PO_4^-$ affinity of these hosts. The fact that the carbonyl group of **8** is a stronger hydrogen bond acceptor than the thiocarbonyl group of **7** would be consistent with the weaker binding of $H_2PO_4^-$ by **8**. Indeed, even conformations of bis-thiourea hosts with intramolecular hydrogen bonds between the two thiourea groups are conceivable. While they would not affect binding selectivities, they would lower anion binding affinities overall.

Tetrakis-thiourea **7** is remarkable in that it has a strong affinity for $H_2AsO_4^-$, which it binds preferentially over $H_2PO_4^-$. While the *cis*-1,2-cyclohexylene host **3** and naphthylene derivative **5** exhibit $H_2PO_4^-$ vs $H_2AsO_4^-$ selectivity, these two bis-thioureas also bind $H_2AsO_4^-$ quite strongly. The formation of complexes between thiourea hosts and these oxoanions is not surprising. H_3AsO_4 (pK_a 2.26) and H_3PO_4 (pK_a 2.16) have very similar pK_a values,[25] suggesting similar properties of the corresponding mono-anions as hydrogen bond acceptors. Since the hydrogen bond acceptor properties of $H_2PO_4^-$ are well confirmed, it can be expected that $H_2AsO_4^-$ is also a good hydrogen bond acceptor and will form stable complexes with hydrogen bond donating hosts. Because $H_2PO_4^-$ and $H_2AsO_4^-$ have very similar basicities, it appears that the $H_2AsO_4^-$ selectivity evident from Table 1 is caused by the larger size of this oxoanion. This is illustrated by the crystal structures of $Ca[H_2AsO_4]_2$ and $Ca[H_2PO_4]_2$.[26,27] In the former, the average As–O bond is 169 pm long, while the average P–O bond in the latter is only 154 pm. Similarly, in $H_2AsO_4^-$ the average distance between two hydrogen-bonded oxygens is 275 pm, while the corresponding average O···O distance in $H_2PO_4^-$ is 251 pm. Interestingly, there is indication from crystal structures that the AsO_4^- tetrahedron is more easily distorted than the PO_4^- tetrahedron.[28] This suggests that size selectivity may be used to prepare even more selective $H_2AsO_4^-$ hosts using a higher degree of host preorganization. This is particularly interesting in view of the toxicity of arsenate and the fact that even nature has not developed systems with a more than marginal $H_2AsO_4^-$ vs $H_2PO_4^-$ binding selectivity.

Table 1 also shows a very distinct difference in the selectivities of host **3** in pure DMSO-d_6 and in THF-d_8/DMSO-d_6 9:1. While in DMSO-d_6 chloride is discriminated by nearly two orders of magnitude, chloride is the preferred ion in the solvent mixture. Even though not anticipated to this extent, this effect is expected. Strong ion–dipole interactions between chloride and DMSO are known (ΔG^0 in the gas phase for $Cl^- + DMSO \Leftrightarrow Cl^- \cdot DMSO$: 52.3 kJ/mol).[29] Therefore, specific solvation of chloride in DMSO lowers the observed affinity of bis-thiourea hosts in DMSO-d_6. Also, since pK_a values are biased by solvation effects, chloride is a better hydrogen bond acceptor than its pK_a of −6.1 might suggest. The free energies of hydration $\Delta G^0_{0,1}$ in the gas phase (in kJ/mol; CH_3COO^-: 39; Cl^-: 34; $H_2PO_4^-$: 32)[30] show that chloride is a very good hydrogen bond acceptor. This explains at least partly the high chloride selectivity of ion-selective electrodes based on host **2a**.[8]

3. Conclusion

In conclusion, the results from this study put the popularity of the *meta*-xylylene spacer into perspective. While the limited added advantages of the naphthylene spacer may not justify the extra synthetic effort, the *cis*-1,2-cyclohexylene spacer is readily available and offers, in view of selectivity, an interesting alternative. This study also confirms the exceptional advantages of the highly preorganized xanthene spacer. Knowing the effect of cavity size on the anion selectivities of these bis-thioureas will be useful in the design of hosts with multiple thiourea groups. Interestingly, tetrakis-thiourea **7** is uniquely $H_2AsO_4^-$ selective. To the best of our knowledge, this is the first synthetic host that preferentially binds $H_2AsO_4^-$ vs $H_2PO_4^-$.

4. Experimental

4.1. Synthesis of bis-thiourea hosts 3–6

Bis-thiourea hosts **3**–**6** were prepared in good yields from the appropriate diamines according to a procedure previously

described for **1b**.[5,7] Specifically, solutions of the diamines in EtOH (dried over molecular sieves) were cooled to 0 °C. A solution with 2 equiv of phenyl isothiocyanate was then added slowly. The reaction solutions were stirred for at least another 12 h, at first for about 3 h at 0 °C and subsequently at room temperature. 2,7-Bis(aminomethyl)naphthalene required for the synthesis of **5** was obtained by dibromination of 2,7-dimethylnaphthalene, conversion to the diazide, and reduction to the diamine.[21] The bis-thioureas were purified by chromatography on silica gel using methylene chloride/ethyl acetate as eluent.

*4.1.1. N,N″-cis-1,2-Cyclohexanediylbis[N′-phenylthiourea], **3***

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.66 (br, 2H, NH), 7.57 (d, 2H, J=4.2 Hz, NH), 7.50 (d, 4H, J=4.5 Hz, Ph), 7.28 (t, 4H, J=4.8 Hz, Ph), 7.06 (t, 2H, J=4.5 Hz, Ph), 4.66 (br, 2H, C*H*NH), 1.80−1.40 (m, 8, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.3, 139.7, 128.9, 124.3, 122.8, 52.8, 28.6, 22.2. ESI-MS, *m/z* calcd for $C_{20}H_{25}N_4S_2$, $[M+H]^+$: 385.1521; found: 385.1497.

*4.1.2. N,N″-[1,4-Phenylenebis(methylene)]bis[N′-phenylthiourea], **4***

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.66 (br, 2H, NH), 8.20 (br, 2H, NH), 7.44 (d, 4H, J=7.5 Hz, Ph), 7.35 (t, 4H, J=7.5 Hz, Ph), 7.33 (s, 4H, phenylene), 7.11 (t, 2H, J=7.5 Hz), 4.72 (d, 4H, J=5.1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.0, 139.5, 138.0, 129.0, 127.7, 124.6, 123.7, 47.2. ESI-MS, *m/z* calcd for $C_{22}H_{23}N_4S_2$, $[M+H]^+$: 407.1364; found: 407.1333.

*4.1.3. N,N″-[2,7-Naphthalenediylbis(methylene)]bis[N′-phenylthiourea], **5***

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.71 (br, 2H, NH), 8.31 (br, 2H, NH), 7.90 (d, 2H, J=8.5 Hz, naphthyl), 7.80 (s, 2H, naphthyl), 7.51 (d, 2H, J=8.5 Hz, naphthyl), 7.47 (d, 4H, J=7.8 Hz, Ph), 7.36 (t, 4H, J=7.5 Hz, Ph), 7.14 (t, 2H, 7.5 Hz, Ph), 4.92 (d, 4H, J=5.1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.2, 139.5, 137.3, 133.1, 131.6, 129.0, 128.0, 126.0, 125.7, 124.7, 123.7, 47.6. ESI-MS, *m/z* calcd for $C_{26}H_{25}N_4S_2$, $[M+H]^+$: 457.1521; found: 457.1495.

*4.1.4. 1,1′-[[2,2,2-Trifluoro-1-(trifluoromethyl)-ethylidene]bis(6-methyl-3,1-phenylene)]-bis[N′-phenylthiourea], **6***

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (s, 2H, NH), 9.37 (s, 2H, NH), 7.45 (d, 4H, J=7.8 Hz, Ph), 7.33 (m, 8H, overlap of two signals from trisubstituted phenyl ring and one signal from Ph), 7.15 (m, 4H, overlap of triplet from Ph and doublet from trisubstituted phenyl ring), 2.28 (s, 6H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 180.1, 139.6, 138.3, 136.4, 130.8, 130.0, 129.6, 128.8, 127.8, 124.9, 124.1, 17.8. Due to ^{19}F–^{13}C coupling, the signals for $C(CF_3)_2$ and CF_3 are expected to consist of 7 and 16 peaks, respectively, and were too small to be observed. ESI-MS, *m/z* calcd for $C_{31}H_{27}F_6N_4S_2$, $[M+H]^+$: 633.1581; found: 633.1602.

*4.2. tert-Butyl 3-(isothiocyanatomethyl)benzylcarbamate, **9***

Isothiocyanate **9** was prepared in a modification of a literature procedure.[22] The mono-protected diamine *tert*-butyl 3-(aminomethyl)benzylcarbamate (0.99 g, 4.1 mmol) in ethyl acetate (20 mL) was added dropwise to a solution of 1,1′-thiocarbonyldiimidazole (0.77 g, 4.3 mmol) in ethyl acetate (20 mL). After stirring for 16 h at room temperature and dilution with ethyl acetate (50 mL), the reaction mixture was washed with water and brine. The resulting solution was dried over $MgSO_4$, concentrated, and purified by column chromatography with hexane/ethyl acetate (3:1) as eluent to yield a white solid (0.79 g, 69% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (t, 1H, J=7.8 Hz, ArCH), 7.28 (m, 3H, ArCH), 4.98 (br, 1H, NH), 4.74 (s, 2H, CH_2NCS), 4.35 (br, 2H, CH_2NH), 1.49 (s, 9H, CH_3).

*4.3. [[1,3-Phenylenebis(methylene)]bis[thioureylene[1,3-phenylenebis(methylene)]]]biscarbamic acid dibutyl ester, **8***

A solution of *m*-xylylenediamine (0.137 g, 1.01 mmol) in dried EtOH (70 mL) was chilled in an ice bath. A solution of isothiocyanate **9** (0.531 g, 1.91 mmol) in dry EtOH (10 mL) was added dropwise to the *m*-xylylenediamine solution. The reaction was allowed to reach room temperature and stirred for 10 h. The cloudy white solution was concentrated by evaporation in vacuo at 45 °C, and the compound was purified by column chromatography with CH_2Cl_2/EtOAc/EtOH (6:2:1) to yield a white solid (0.332 g, 50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (br, 4H, NH), 7.41 (t, H, J=6.3 Hz, ArCH), 7.27 (m, 2H, ArCH), 7.16 (m, 9H, ArCH), 4.68 (br, 10H, CH_2 and NHCO), 4.11 (d, 4H, J=6.0 Hz, CH_2NHCO), 1.41 (s, 18H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 140.7, 139.7, 128.7, 126.7, 126.1, 125.9, 75.6, 43.8, 47.7, 28.7. ESI-MS, *m/z* calcd for $C_{36}H_{49}N_6O_4S_2$, $[M+H]^+$: 693.3257; found: 693.3292.

*4.4. N,N″-[1,3-Phenylenebis(methylene)]bis[N′-[3-[N′-phenylthioureylene]-1,3-phenylenebis(methylene)]-thiourea], **7***

Bis-thiourea **8** (0.189 g, 0.27 mmol) was suspended in CH_2Cl_2 (20 mL) to form a cloudy white solution, and trifluoroacetic acid (3.0 mL) was added dropwise. The reaction solution was allowed to stir for 12 h before evaporation of the solvent. To remove traces of trifluoroacetic acid, 10 mL aliquots of THF were added three times, which was each time followed by complete evaporation of the solvent. The raw product of the resulting diamine was used without further purification. It was dissolved in THF (20 mL), and triethylamine (0.160 mL, 3.0 mmol, dried over molecular sieves) and phenyl isothiocyanate (0.128 mL, 2.8 mmol) were added. After stirring the reaction solution for 20 h, the solvent was evaporated and the raw product was purified by column chromatography with CH_2Cl_2/EtOAc/EtOH (18:4:1) to yield **7** as a white solid (0.081 g, 64%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.66 (br, 2H, NH), 8.19 (br, 2H, NH), 7.99 (br, 4H, NH), 7.45 (d, 2H,

J=7.8 Hz, ArCH), 7.23 (m, 20H, ArCH), 4.71 (m, 12H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.1, 139.5, 139.2, 138.9, 128.7, 128.6, 126.7, 126.5, 126.4, 124.7, 123.7, 47.8. ESI-MS, m/z calcd for $C_{40}H_{41}N_8S_4$, $[M-H]^-$: 761.2337; found: 761.2356.

4.5. Binding studies

4.5.1. Reagents

Tetrabutylammonium dihydrogen arsenate was prepared by titration of an aqueous solution of arsenic(V) oxide with tetrabutylammonium hydroxide to the first equivalence point, washing of the resulting solution with chloroform, and freeze drying of the aqueous phase.[31] All other inorganic anions were commercially available as tetrabutylammonium salts and were thoroughly dried in vacuo prior to use. DMSO-d_6 was dried over molecular sieves (3 Å).

4.5.2. Determination of complexation constants by ^1H NMR spectroscopy

^1H NMR spectra were obtained on a Varian Inova 300 or Varian Unity 300 spectrometer (300 MHz; Varian, Palo Alto CA). All chemical shift values (δ) are reported in parts per million (ppm). For ^1H NMR titrations, two stock solutions were prepared in DMSO-d_6, one of them containing host only and the second one host of the same concentration and an appropriate concentration of guest. Aliquots of the two solutions were mixed directly in NMR tubes, minimizing experimental error, and H_2O contamination. Insufficient mixing would be readily recognizable in the resulting titration curves by broad signals and poor fits. It was carefully avoided, giving data of the type shown in Figure 4 and in Ref. 5. All experimental data obtained by these methods were analyzed using Mathematica® 6.0 with an appropriate binding isotherm model.[32]

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