[2.2.2]Urea cryptand: an easily accessible neutral organic cage for anion binding in water

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Abstract: Binding hydrophilic anions such as sulfate in water is a significant academic challenge, in particular for neutral receptors. To achieve this, typically a large molecular receptor is designed that wraps around a bound anion in a hydrophobic microenvironment. Such receptors require multi-step synthesis and often display selectivity towards hydrophobic anions such as iodide. We here report the one-pot synthesis of a [2.2.2]urea cryptand (cage) and its ability to bind sulfate in water with sub-millimolar affinity achievable by incorporation into micelles. Unlike existing neutral receptors, the cage has >10-fold selectivity for sulfate against hydrophobic anions even in pure water. The cage displays rare slow-exchange NMR responses to divalent anions in DMSO-water allowing for simultaneous analysis of multiple divalent anions in water analogous to an ion chromatography instrument. Our results demonstrate the pre-organization of strong directional NH hydrogen bond donors in a cage scaffold as a synthetically less-demanding, yet effective approach to achieving selective binding of hydrophilic anions in water.

Introduction

Anion binding in water is challenging[1] due to the significant energetic cost from anion dehydration [2] and the high dielectric constant of water diminishing electrostatic interactions.[3] In tackling those challenges, naturally occurring transmembrane proteins such as sulfate binding proteins,[4] phosphate binding proteins[5] and ClC channels/transporters[6] supply multiple hydrogen bonding donors from polar amino acid residues to encapsulate anions within a hydrophobic protein microenvironment, leading to modest-to-strong anion binding affinities. Synthetic chemists have sought to make molecular receptors that emulate the anion binding proteins. In the development of anion receptors that function in water or aqueous-organic mixed solvents, most of the successful examples are multiply positively charged metal complexes[7] or organic receptors.[8] In many areas of biological and industrial applications, neutral anion receptors are preferred to minimize off-target interactions with polyanionic materials such as DNA and avoid the drastic reduction of anion affinity under high ionic strength conditions as expected for cationic receptors.

Neutral organic anion receptors that function in water are rare. Notable examples including CH hydrogen-bonding bambusurils[9] and chalcogen-bonding foldamers[10] display strong selectivity for hydrophobic anions such as ClO_4^- or I^-as partly governed by low dehydration cost of those anions. To bind the hydrophilic SO_4^{2-} anion in water and aqueous-organic mixed solvents, Kubik and co-workers have developed biomimetic bis-cyclopeptides which wrap around SO_4^{2-} stabilized by several amide NH···anion interactions buried within a hydrophobic microenvironment.[11] The bis-cyclopeptides, however, require many synthetic steps and bind both SO_4^{2-} and I^- with comparable affinities in water.

Contrasting the abovementioned approaches, we here report the use of strong and directional NH hydrogen bonds in a neutral cage scaffold pre-organized for SO_4^{2-} binding in water, without the synthetic efforts to create a hydrophobic microenvironment. Such a strategy can be seen in the design of anion receptors for organic solvent or lipid membrane applications,[12] but surprisingly, was absent in the area of developing neutral anion receptors in water.[13] We have designed the [2.2.2]urea cryptand (cage **1**) simply by a functional group replacement of the ether-based [2.2.2]cryptand, previously developed by Lehn and coworkers as a high affinity K^+ receptor owing to the multivalency and pre-organization provided by the cage scaffold (Fig. 1).[14] Cage **1** is structurally simple, easily assembled in one pot from low-cost commercial reagents and yet displayed a remarkably selective SO_4^{2-} binding in water with a high-mM affinity that can be boosted to sub-mM levels by using micelles. We additionally show that the cage functions as an NMR-based "ion chromatography" for divalent anions with demonstrated applications in water and beverage analysis.

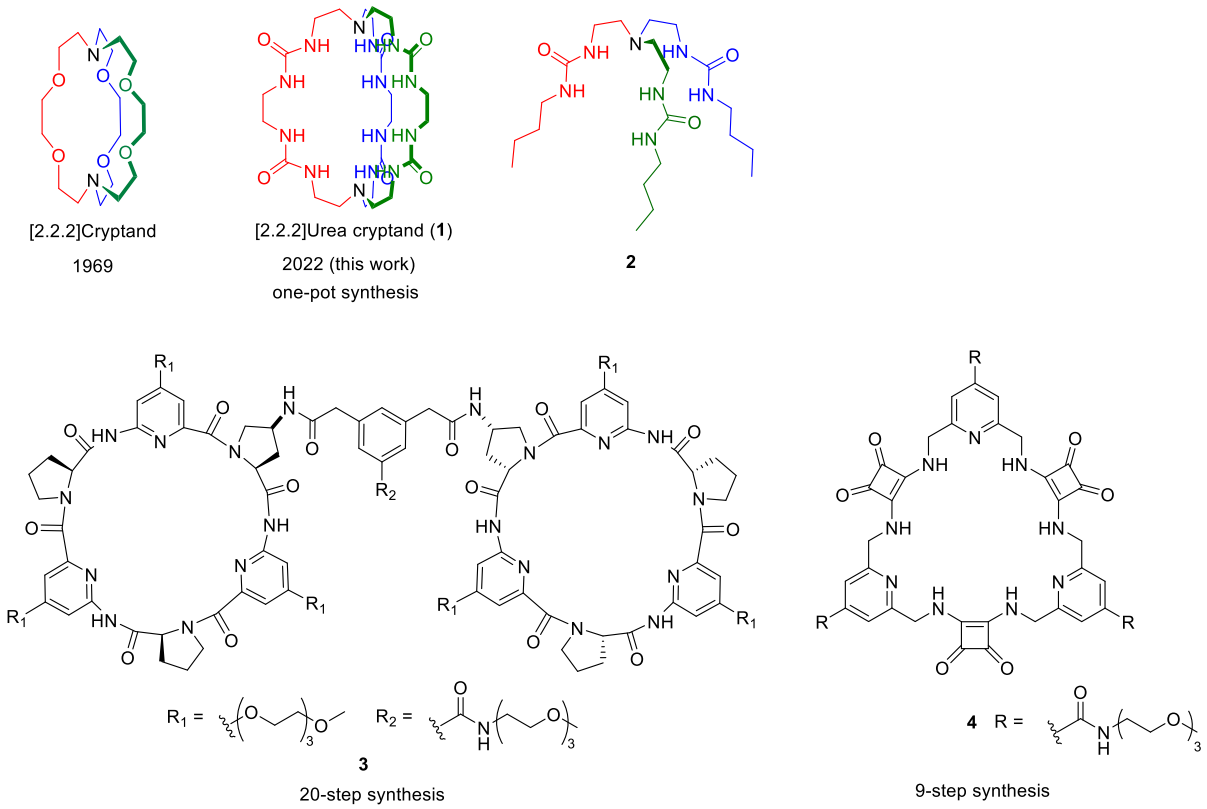

Fig. 1. Structures of [2.2.2]cryptand developed by Lehn et al., [2.2.2]urea cryptand (cage **1**) developed in this work, an acyclic tripodal tris-urea developed by Gale et al.,[15] a bis-cylopeptide developed by Kubik et al.[11a] and a macrocyclic tris-squaramide developed by Jolliffe et al.[16]

Results and Discussion

Synthesis and crystal structure of cage 1

Cage **1** was synthesized by a SO_4^{2-} templated one-pot [2+3] urea formation reaction between a carbonyl-imidazole derivative of tris(2-aminoethyl)amine (TREN) and ethylenediamine (EDA) in MeCN (Fig. 2). The tetrabutylammonium sulfate (TBA_2SO_4) template bound to the cage was removed by precipitation with $BaCl_2$, with the remaining TBACl removed by an ethanol wash. Purification of the cage simply by a water wash to remove the polymeric by-products afforded the cage in an overall 30% isolated yield. The SO_4^{2-} templated cage formation yield was determined to be 56% by NMR, a remarkable efficiency compared with other irreversible cage formation reactions reported.[17] By contrast, without the SO_4^{2-} template, the cage only formed in 8% and no improvement was observed (9%) by using Cl^- (as TBACl), a weaklier coordinating anion than SO_4^{2-} (SI).

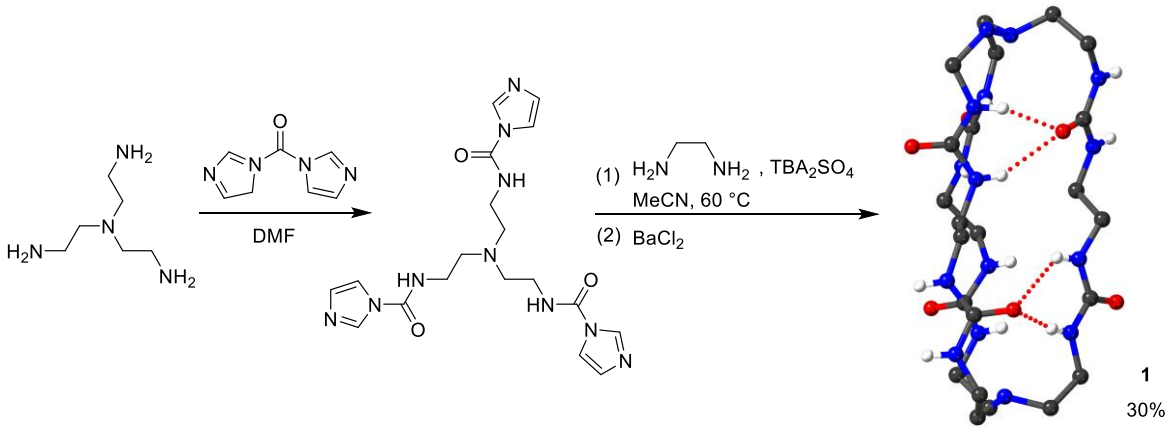

Fig. 2. Synthesis and crystal structure of cage **1** (CCDC: 2220230, with water solvates and non-acidic hydrogen atoms omitted). Note that the carbonyl-imidazole derivative of TREN was used in the next step without purification.

Unlike most neutral organic cages that require the decoration of hydrophilic substituents to achieve water solubility, cage **1** is water-soluble up to ~15 mM at room temperature, due to the high polarity of the dialkyl-substituted urea motifs. Single crystals of the free cage were obtained by slow evaporation of an aqueous solution of the cage. The free cage (Fig. 2) shows an extended capsule-like conformation stabilized by two sets of bifurcated urea NH···O=C intramolecular hydrogen bonds.

Anion binding studies

Initially anion binding of cage **1** was accessed by ^{1}H NMR titrations in DMSO-d_6/0.5% H_2O and compared with a previously reported TREN-based tripodal anion receptor **2** (Table 1).[15] Surprisingly, the hexa-urea cage **1**, which is expected to be a strong anion receptor, shows a similar Cl^- affinity to acyclic tris-urea **2**. This is attributed to strong intramolecular hydrogen bonds[18] in **1** that competes with anion binding, as supported by the crystal structure of free **1** (Fig. 2) and the observation of Cl^--induced upfield shift of the TREN-NH (SI) indicating the dissociation of intramolecular hydrogen bonds after anion binding. Despite the lack of improvement in Cl^- binding, SO_4^{2-} binding was enhanced by over four orders of magnitude going from **2** to **1**, as estimated by a host competition experiment between **1** and **2**. Therefore, in the case of binding the more strongly coordinating SO_4^{2-} anion, the enthalpic gains by forming more NH···anion hydrogen bonds and the entropic benefit from a cage scaffold in **1** overcompensate the energetic loss due to intramolecular hydrogen bonds. Notably, cage **1** features a slow exchange response to SO_4^{2-} (but not to Cl^-) showing separate sets of signals for the free and the SO_4^{2-} bound cage (Fig. 3a), in contrast to the fast exchange response (peak shifting) observed for **2** (SI). Cage **1** can pick up µM concentrations of SO_4^{2-} impurity from commercial DMSO-d_6, an indication of its strong SO_4^{2-} affinity.

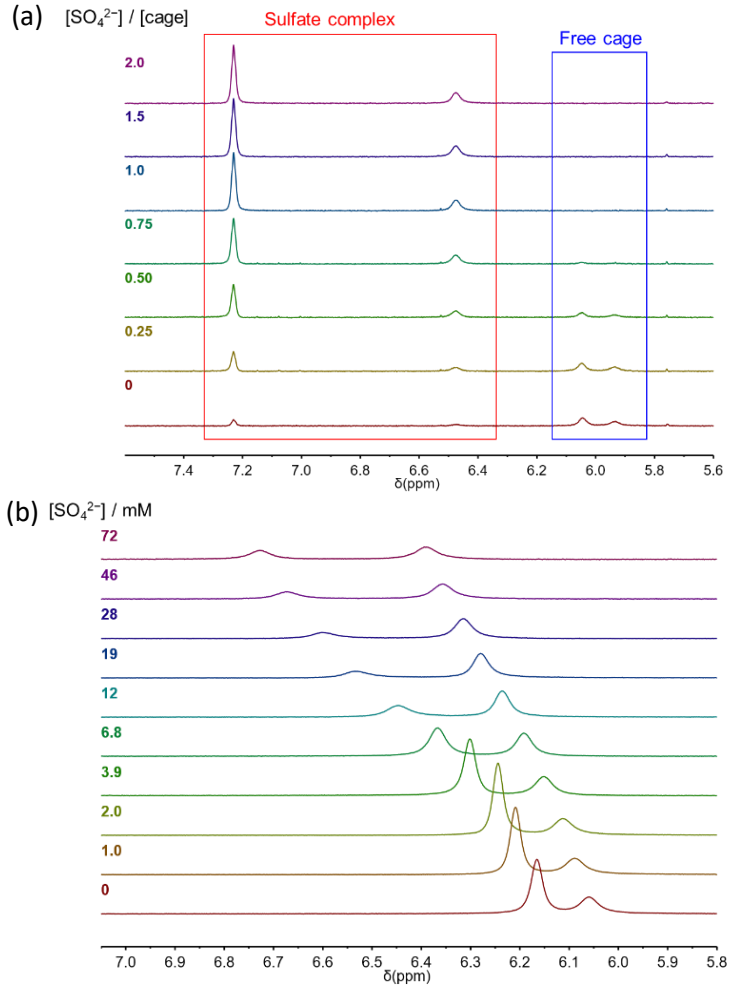

Fig. 3. (a) ^{1}H NMR (700 MHz) titration of cage **1** (50 µM) with TBA_2SO_4 in DMSO-d_6/0.5% H_2O at 298 K. Note that because of SO_4^{2-} impurity in DMSO-d_6, the **1**-SO_4^{2-} complex can be observed before TBA_2SO_4 addition. (2) ^{1}H NMR (700 MHz) titration of cage **1** (0.9 mM) with Na_2SO_4 in 9:1 H_2O/D_2O at 298 K. The more downfield signal corresponds to the urea NH from the TREN motif and the more upfield signal corresponds to the urea NH from the EDA motif.

Table 1 Cl^- and SO_4^{2-} binding affinities of cage **1** and acyclic tris-urea **2** in DMSO-d_6/0.5% H_2O at 298 K.

K (anion) / M^{-1}	Cage **1**	Acyclic tris-urea **2**
Cl^-	560	670 [a]
SO_4^{2-}	> 5×10^9 [b]	8.9×10^4 [c]

[a] Reported by Busschaert et al.[15]

[b] Estimated by a host competition experiment between **1** and **2** (SI).

[c] Re-determined in this work. Previously reported to be > 10^4 M^{-1} (SI).

The DFT-optimized structure of the **1**-SO_4^{2-} complex shows encapsulation of SO_4^{2-} inside the cage by 12 strong NH···O (SO_4^{2-}) hydrogen bonds. The binding of tetrahedral SO_4^{2-} contracts the cage from a capsule to a more globular shape. The positioning and orientation of the six urea groups around SO_4^{2-} in the cage deviate from an ideal octahedral coordination geometry previous observed in crystal structures of 2:1 **2**-SO_4^{2-} complex[15] and 1:1 SO_4^{2-} complexes of TREN-based acyclic hexa-ureas.[19] Nonetheless, the pre-organization of six urea groups in cage **1** is crucial to achieving the ultra-strong SO_4^{2-} affinity in DMSO and allowing SO_4^{2-} binding to be observed in water (vide infra).

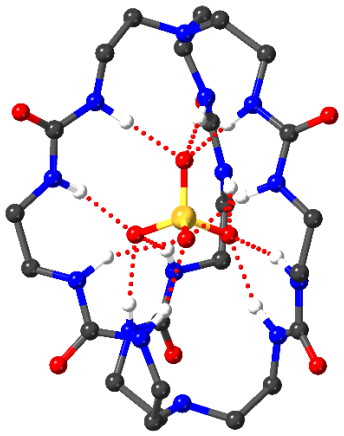

Fig. 4. Optimized structure of the **1**-SO_4^{2-} complex (B3LYP/6-31G*). Non-acidic hydrogen atoms are omitted.

The water content of the solvent was then increased to 50% and 100%, with water suppression techniques applied to avoid the use of D_2O as the water component and allow for the observation of exchangeable urea NHs, the most sensitive protons to anion binding. Downfield shifting of NH signals were observed for **1** upon titrating Na_2SO_4 under those conditions, giving SO_4^{2-} binding constants of 3.8×10^4 and 66 M^{-1} in 50% DMSO-d_6/H_2O and 100% water (9:1 H_2O/D_2O), respectively (Table 2). We also performed Na_2SO_4 titration of **1** in 10 mM sodium carbonate buffer at pH 9.5, giving a SO_4^{2-} affinity of 52 M^{-1} similar to that determined without the buffer (SI, the slight reduction due to competitive CO_3^{2-} binding). The drastically attenuated SO_4^{2-} binding affinities with increasing water contents are expected and caused by the heavy dehydration energetic cost of SO_4^{2-} in water.[11a] It should be noted that even SO_4^{2-} binding in an aqueous-organic binary solvent with at least 50% water content is highly challenging and currently the only two classes of neutral molecular receptors capable of that (excluding biphasic[20] and polymeric[21] systems) are Kubik's cyclopeptides[11] (e.g., compound **3**) and Joliffe's squaramides[16, 22] (e.g., compound **4**). Cage **1** demonstrates a 7-fold enhanced SO_4^{2-} affinity than macrocyclic squaramide **4**, despite the squaramide being a stronger anion binding motif than the urea[23], attributed to the higher levels of multivalency and pre-organization in cage **1**. The strongest SO_4^{2-} affinity of bis-cyclopeptide **3** among the three receptors likely owes to the hydrophobic anion binding microenvironment that is absence from **1** and **4**, which, however requires formidable synthetic efforts. Among the three receptors, cage **1**, despite not being the champion in SO_4^{2-} affinity, demonstrates advantages in its extremely low synthetic cost, no chromatographic purification and high water-solubility, rendering cage **1** suitable for industry-scale production and applications in water.

Table 2 SO_4^{2-} binding affinities of cage **1**, along with reported values for compounds **3** and **4** in DMSO-water binary solvents and in water.

K (SO_4^{2-}) / M^{-1}, solvent conditions	Cage **1**	Compound **3**	Compound **4**
50% DMSO/water	3.8×10^4	7.8×10^5	4900
100% water	66	2000	No binding

Anion selectivity of cage **1** was investigated in 9:1 H_2O/D_2O. Table 3 demonstrates a >10-fold selectivity of cage **1** for SO_4^{2-} than hydrophobic anions such as ReO_4^- and I^-, contrasting bis-cyclopeptide **3** showing comparable SO_4^- and I^- (4200 M^{-1}) affinities in water.[11a] This can be rationalized by the stronger hydrogen bond donating abilities and the greater quantities of urea NH donors in **1** than the amide NH donors in **3**, which leads to more favorable binding of charge-dense SO_4^{2-} than charge-diffuse I^-. Selectivity for SO_4^{2-} against other divalent anions including HPO_4^{2-} and SeO_4^{2-} were also observed for **1**. This likely arises from the heavier dehydration penalty[2] and the presence of a repulsive hydrogen atom for HPO_4^{2-}, and weaker coordinating ability for SeO_4^{2-}. The strong SO_4^{2-} selectivity of **1** in water is expected to benefit certain applications, e.g., in nuclear waste treatment. A future direction is to incorporate the cage into a polymeric adsorbent material for selective adsorption and removal of SO_4^{2-} from nuclear waste,[24] where the presence of high concentrations of SO_4^{2-} is known to hamper vitrification due to the phase separation of sulfate salts in borosilicate glass. For this purpose, a strong SO_4^{2-} selectivity against hydrophobic anions would minimize co-adsorption of radioactive anions $^{129}I^-$ [25] and $^{99}TcO_4$ [26], thus improving the efficiency and safety of the procedure.

Table 3 Affinities of cage **1** for various anions in 9:1 H_2O/D_2O, along with anion hydration energies compiled by Marcus.[2]

Anion	$-\Delta G_{hydr}$ / kJ mol^{-1}	*K* for cage **1** / M^{-1}
SO_4^{2-}	975	66
SeO_4^{2-}	909	8.6
HPO_4^{2-}	1366	5.3
CH_3COO^-	374	1.7
Cl^-	344	3.9
Br^-	318	3.9
NO_3^-	286	< 4 [a]
I^-	280	< 4 [a]
ClO_4^-	229	< 4 [a]
ReO_4^-	226	< 4 [a]

[a] The affinities of these anions cannot be determined from the ^1H NMR titration data due to weak or negligible responses. We estimated a higher limit of 4 M^{-1} by competitive experiments with Cl^- (SI).

Enhancement of sulfate binding by micelles

Anion binding is expected to be enhanced within a low-polarity environment compared with in bulk aqueous solutions,[27] and this would be the situation where in future work, cage **1** is incorporated into an insoluble polymeric matrix to adsorb SO_4^{2-} from water. To assess this potential, we conducted SO_4^{2-} binding studies in 9:1 H_2O/D_2O in the presence of cetyltrimethylammonium bromide (CTAB) micelles. A dramatically enhanced apparent SO_4^{2-} affinity of 1000 M^{-1} was determined by ^1H NMR titrations (Fig. 5a). This value is only an apparent affinity because as confirmed by DOSY NMR experiments, cage **1** was in the bulk solution in the absence of SO_4^{2-} (showing a diffusion coefficient of 4.1×10^{-10} m^2 s^{-1} identical to that determined without micelles) and only migrated into the micelles upon SO_4^{2-} binding (diffusion coefficient reduced to 1.6×10^{-10} m^2 s^{-1}). We found negligible partitioning of the free cage in micelles likely due to the highly hydrophilic nature of the cage. After taking into account the unfavorable partitioning of cage **1** in the micellar phase (which reduces the apparent SO_4^{2-} affinity) and the positive surface charge of CTAB micelles (which enhances the apparent SO_4^{2-} affinity), we

estimate the intrinsic SO_4^{2-} binding constant of cage **1** within the micellar phase to be > 10^4 M^{-1} (see SI for details). Therefore, we anticipate cage **1** to be capable of removing SO_4^{2-} below 0.1 mM with future incorporation into polymers.

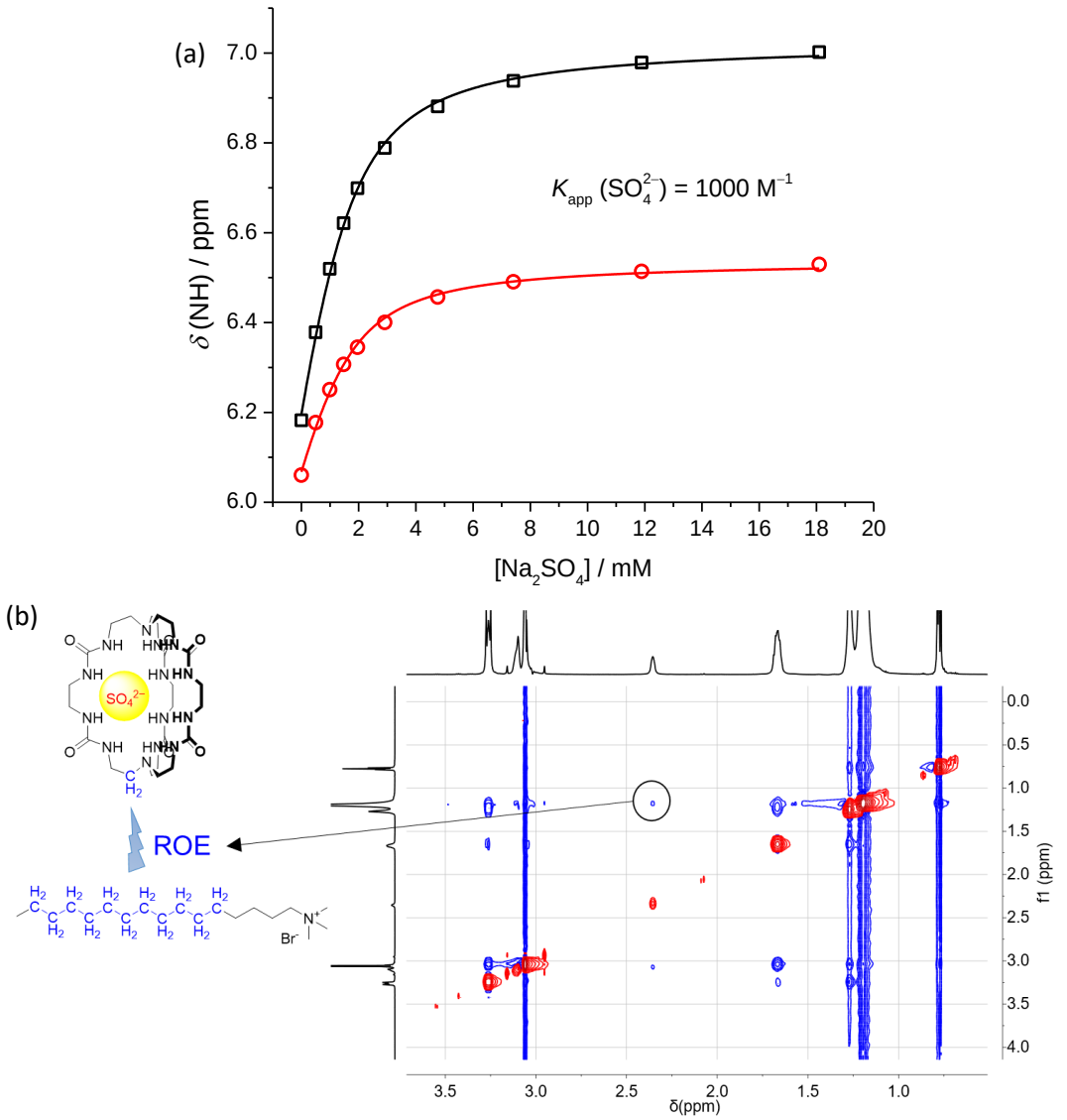

Fig. 5. (a) ^{1}H NMR titration of cage **1** (0.9 mM) with Na_2SO_4 in the presence of CTAB (20 mM) in 9:1 H_2O/D_2O at 298 K. (b) 2D EASY-ROESY (700 MHz) spectrum of cage **1** (0.9 mM) in the presence of Na_2SO_4 (20 mM) and CTAB (20 mM) in 9:1 H_2O/D_2O at 298 K, showing ROE cross-peak between the cage and backbone CH_2 of CTAB.

The **1**-SO_4^{2-} complex locates at the low-polarity hydrocarbon tail region of CTAB micelles, as determined by 2D ROESY NMR experiments showing ROE of **1** with the backbone CH_2 of CTAB, but not with other protons in CTAB (Fig. 5b). This supports our hypothesis that the micelles enhance anion binding of the cage by providing a hydrophobic microenvironment.

An “ion-chromatography” for divalent anions

The ultra-strong anion binding of **1** leads to slow-exchange ^{1}H NMR responses giving sharp peaks even in DMSO-d_6 that contains a significant water content. Inspired by previous work by Jeong[28] and Sindelar[29], we explored this feature to develop NMR-based anion sensing method for simultaneous analysis of multiple anions. While the previous systems were investigated in CD_3CN or DMSO-d_6/D_2O, we were able to use DMSO-d_6/5% H_2O as the solvent, allowing real-world water samples to be analyzed simply by dilution into DMSO-d_6. Another distinctive feature of cage **1** is that slow exchange response was only observed for divalent anions (e.g., SO_4^{2-})

but not monovalent anions (e.g., Cl^-) in DMSO-d_6/5% H_2O, unlike the previous systems applicable for monovalent anions.

In the current preliminary study of the NMR sensing method, we investigated six divalent anions. As shown in Fig. 6, the addition of 1 eqv. of SO_4^{2-}, SeO_4^{2-}, HPO_4^{2-} and $C_2O_4^{2-}$ (oxalate) lead to the quantitative formation of the respective anion complexes, indicating strong affinities of the cage for these anions. By contrast, for 1 eqv, of $S_2O_3^{2-}$ and CO_3^{2-}, a mixture of the free cage and the anion complex co-existed, indicating comparatively weaker binding. This can be explained by the geometry mismatch of $S_2O_3^{2-}$ and CO_3^{2-} for the cage cavity that prefers more symmetrical tetrahedral anions or elongated anions like oxalate.

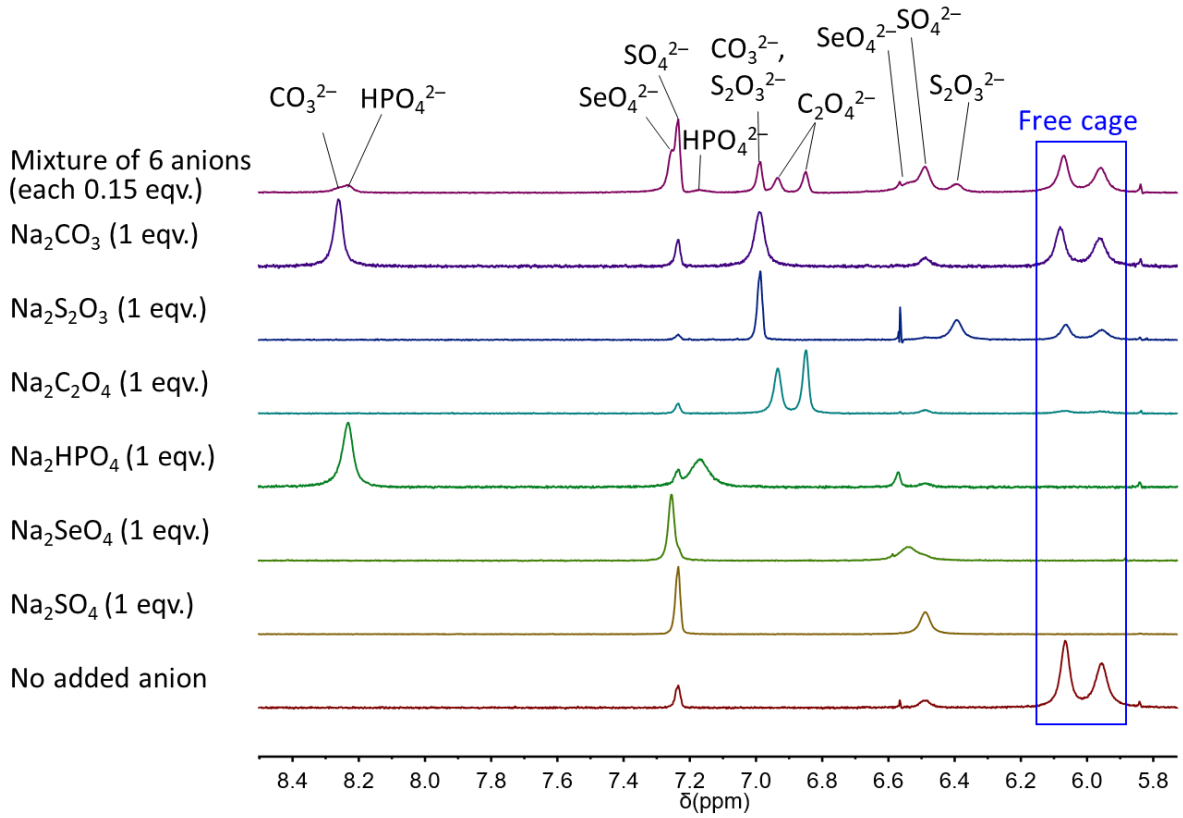

Fig. 6. ^1H NMR (700 MHz) spectrum of cage **1** (106 µM, the additional 6 µM being used to complex the SO_4^{2-} impurity in DMSO-d_6) in the absence and presence of six anions at 100 µM or a mixture in DMSO-d_6/5% H_2O at 298 K, showing the urea NH signals. Note that signals of **1**-SO_4^{2-} complex were observed in all spectra due to SO_4^{2-} impurity in DMSO-d_6.

The six anions induced different chemical shifts in the two urea NH signals depending on the anion charge density and structural fit for the cage. In this regard, it is interesting to note that SeO_4^{2-}, despite being less charge dense than SO_4^{2-}, induced slightly stronger downfield shifts in both NHs, likely due to its large size leading to better fit for the cage cavity than SO_4^{2-}. Contrasting other anion complexes where the two urea NHs were separated by > 0.8 ppm, the chemical shifts of two urea NHs were close for the $C_2O_4^{2-}$ complex, consistent with the elongated shape of $C_2O_4^{2-}$ leading to more balanced perturbations of the two urea NH signals. Other tested anions that are globular or planar are bound at the center of the cage resulting in more pronounced downfield shifts of the EDA NH than the TREN NH.

Because of the broad range of NH chemical shift changes induced by the anions, all the six divalent anions were observed (by either or both NH signals) in a ^1H NMR spectrum of their mixture, although the NH signals from HPO_4^{2-} and CO_3^{2-} may be too broad for quantification purposes at low concentrations (Fig. 6.). The NH signals from SO_4^{2-} and SeO_4^{2-} complexes overlap and yet better resolution between the two anions can be achieved using the CH_2 connected to the nitrogen bridgeheads (SI). Overall, these results demonstrate the functional analogy of cage **1** to an ion-chromatography (IC) instrument.

We used cage **1** to determine the SO_4^{2-} concentrations in Brisbane River (0.097 mM), UQ Lake (0.37 mM) and a tap water sample (0.61 mM), giving results in good agreement with those determined by IC methods (0.092, 0.37, and 0.65 mM, respectively, for the above three samples). Additionally, the cage can simultaneous quantify SO_4^{2-} and $C_2O_4^{2-}$ in more complex samples including an iced tea drink ($[SO_4^{2-}]$ = 0.13 mM, $[C_2O_4^{2-}]$ = 0.25 mM) and a beer ($[SO_4^{2-}]$ = 2.2 mM, $[C_2O_4^{2-}]$ = 0.035 mM) sample (SI). These analyses require minimum sample preparation that involves adding the cage to the samples and if necessary, adjusting the pH to ensure that the anion to be analyzed exists in the divalent form (the latter is not necessary for analyzing non-basic anions such as SO_4^{2-}). High-throughput screening is achievable by using cryo-probe NMR instruments that can complete data acquisition for each sample in 2–3 min. In comparison, IC analysis typically takes 10–20 min per sample and may require time-consuming sample preparation such as centrifugation and degassing for complex samples.

Conclusion

In summary, we have implemented a classical strategy of pre-organizing strong, directional NH hydrogen bonds to develop an easily accessible SO_4^{2-} binding cage functioning in water. Cage **1** demonstrates a SO_4^{2-} affinity of 66 M^{-1} that can be boosted to sub-mM levels by using micelles in water. Benefiting from the use of multiple strong urea anion binding motifs, a >10-fold selectivity for SO_4^{2-} against hydrophobic anions such as I^- was observed even in pure water contrasting the hydrophobic selectivity observed with existing neutral anion receptors in water. Cage **1** functions as an "ion chromatography" for analyzing divalent anions due to its slow exchange ^{1}H NMR responses in DMSO-d_6/5% H_2O. The anion-templated one-pot synthesis and potent, selective anion binding of cage **1** opens the path to using easily synthesized classical NH hydrogen-bond based anion binding architectures for industrial, environmental and biological applications where compatibility to the aqueous environment is required.

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