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Receptors for sulfate that function across a wide pH range in mixed aqueous–DMSO media†

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Water soluble squaramide macrocycles (MSQs) display high sulfate binding affinities in aqueous DMSO mixtures. The introduction of pyridine spacers into the macrocycles resulted in increased sulfate binding affinity in comparison to compounds with benzene spacers. [3]MSQ 6 was found to be a selective ligand for SO_4^{2-} in highly competitive conditions and over a wide pH range (3.2–14).

Sulfate recognition in aqueous media is of significant interest for many potential applications. For example, sulfate removal from seawater is required to prevent the buildup of scale in injection operations in the oil and gas industry.[1] In the nuclear industry, the selective extraction of sulfate could have significant benefits for nuclear waste remediation.[2–4] In a biological context, sulfate is essential for the formation of synovial membranes in joints,[5] and mucin proteins[6] and unusually low levels of sulfate have been found in the plasma of patients with rheumatoid arthritis[5] and irritable bowel disease.[6,7] Selective sulfate detection by molecular receptors could provide a means for the straightforward and rapid analysis for diagnosis of these conditions.

Despite the clear need, the selective binding of sulfate in aqueous solution in the presence of other anions remains a significant challenge. The high hydration energy of sulfate ($\Delta G_{hyd} = -1080$ kJ mol^{-1})[8] means that it is difficult to achieve strong host-sulfate interactions through hydrogen bonding in water. The presence of other anions poses a further difficulty for selective binding of sulfate in water, because other anions that are frequently present in sulfate rich solutions, *e.g.* chloride ($\Delta G_{hyd} = -340$ kJ mol^{-1}) and nitrate ($\Delta G_{hyd} = -306$ kJ mol^{-1}), have much lower hydration energies, as reflected by the relative positions of these anions in the Hofmeister series.[8–10] Additionally, other tetrahedral anions, *e.g.* selenate, chromate, and phosphates have similar geometries, charge and size to sulfate.[11–15] Therefore, the structures of synthetic sulfate binding receptors need to be carefully designed to retain strong binding, whilst providing high selectivity to distinguish sulfate from other potential interferants.

Many sulfate binding receptors have been developed for the recognition of this ion *via* hydrogen bonding interactions using ureas, thioureas, pyrroles, indoles, and squaramides amongst others as the hydrogen bond donating motif,[16–31] but very few of these show both significant affinity and selectivity towards sulfate in aqueous solution. Increasing the hydrogen bond donor ability of the receptors in an attempt to provide improved competition with water frequently leads to receptor deprotonation at neutral or basic pH.[21,32–35] Other effects have been exploited to overcome the high hydration energy of sulfate, such as the use of electrostatic binding interactions[36,37] or hydrophobic effects.[38,39] However, while these approaches can provide increased sulfate binding affinity, this is frequently at the expense of selectivity. Very few receptors are able to bind selectively to sulfate in aqueous solution[38–42] and sulfate binding across a wide pH range has not yet been explored, although this is necessary to further develop sulfate binding receptors for potential applications.

We have previously reported the neutral macrocyclic squaramides (MSQs) **1** and **2** (Chart 1) that exhibited remarkable SO_4^{2-} binding selectivity *via* hydrogen bonding interactions in aqueous media, including selectivity over other divalent tetrahedral anions including SeO_4^{2-} and CrO_4^{2-} anions.[31] In efforts to further increase the sulfate binding affinity of these systems while retaining the high sulfate selectivity and increasing the pH range across which selective sulfate binding occurs, we have designed analogous macrocycles in which the benzene spacer units have been replaced by pyridines. It was anticipated that this might enhance receptor preorganisation through the formation of intramolecular hydrogen bonds between the pyridine nitrogen lone pair and the amide protons, as has previously been observed for a range of linear and macrocyclic anion receptors.[43–49] We also replaced the ester linkages that attach the solubilising triethyleneglycol chains to the periphery of the receptors with amides to increase receptor stability at

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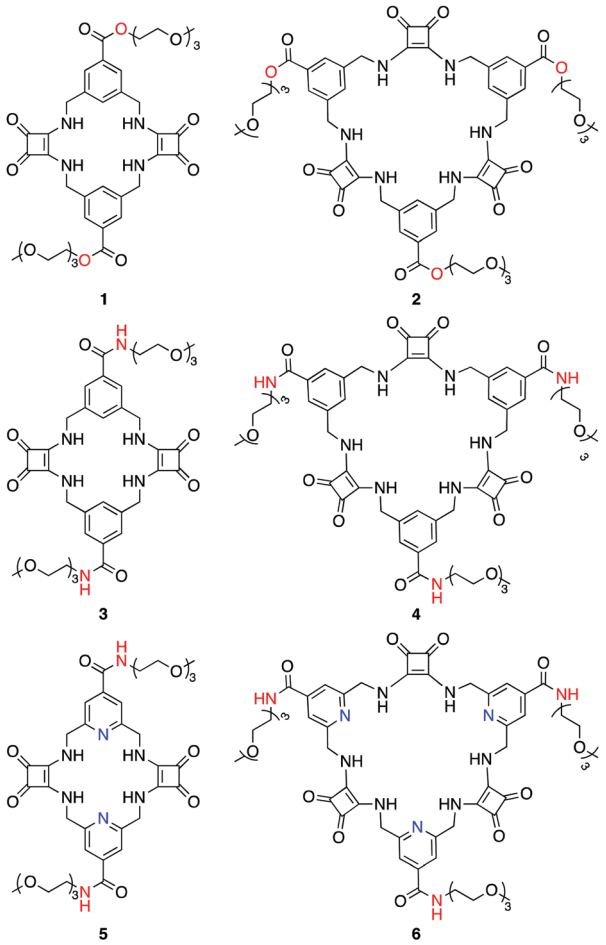

Chart 1 Structures of MSQs **1–6**.

high pH. We report here the synthesis of receptors **3–6** and their ability to bind to sulfate ions across a wide pH range in aqueous DMSO.‡

Given that MSQs **1** and **2** were found to be highly selective for SO_4^{2-}, we initially focused our investigations on the impact that the newly introduced structural modifications had on sulfate binding affinity. Quantitative NMR binding studies were conducted by the addition of 0.2–12.0 equivalents of tetrabutylammonium sulfate to MSQs **1–6** in H_2O/DMSO-d_6 mixtures of varying composition with the aid of WATERGATE methods or pre-saturation pulses to suppress the H_2O signal. A 1 : 1 binding model provided the best fits for the determination of all association constants (Table 1). All MSQs exhibited high affinities for SO_4^{2-} and the 3[MSQ]s displayed higher affinities than the analogous [2]MSQs, as was previously observed for **1** and **2**. All 2[MSQ]s bound sulfate with $K_a > 10^4$ M^{-1} in 1 : 9 v/v H_2O/DMSO-d_6 mixtures, whereas the 3[MSQ]s displayed sulfate binding affinities $K_a > 10^4$ M^{-1} in more competitive 1 : 2 v/v H_2O/DMSO-d_6 mixtures, but a decrease of the binding affinities for all MSQs was observed as the proportion of water was increased, as a result of the high hydration energy of sulfate.

The effect of replacement of the ester linkages by amides on sulfate binding was best observed in the 2[MSQ] series,

Table 1 Association constants $K_a[SO_4^{2-}]$ (M^{-1}) of MSQs **1–6** in various H_2O/DMSO-d_6 mixtures (pH 7)[a]

	1 : 9 (v/v)	1 : 2 (v/v)	1 : 1 (v/v)	2 : 1 (v/v)
[2]MSQ **1**	$>10^4$ [b]	1820[b]	—[c]	—[c]
[3]MSQ **2**	—[d]	$>10^4$ [b]	—[c]	—[c]
[2]MSQ **3**	$>10^4$	3170	—[c]	—[c]
[3]MSQ **4**	—[d]	$>10^4$	1340	130
[2]MSQ **5**	$>10^4$	—[c]	—[c]	—[c]
[3]MSQ **6**	—[d]	$>10^4$	4870	480

[a] Estimated errors ±15%. [b] Data reported previously.[31] [c] Not soluble. [d] Not determined.

where in 1 : 2 v/v H_2O/DMSO-d_6, a very slight increase (1.7 fold) in sulfate binding affinity was observed for **3** in comparison to **1**, indicating that this structural change at the periphery had little impact on the ability of the macrocycle to bind to sulfate. The low solubility of **5** in 1 : 2 v/v H_2O/DMSO-d_6 prevented a comparison of the effect of incorporation of the pyridyl spacer in the 2[MSQ] series. For the 3[MSQ] series, the increased solubility of compounds **4** and **6** in comparison to that of **2** in mixtures containing higher proportions of water enabled sulfate binding to be investigated in more competitive solvent mixtures than those used in our previous work.[31] In 1 : 1 v/v H_2O/DMSO-d_6, MSQ **6**, with pyridine spacers, bound sulfate approximately 3 times more strongly than MSQ **4**, with benzene spacers, indicating that this change, which directly impacts on the sulfate binding site, leads to a modest increase in the sulfate binding affinity of these MSQs that is maintained in the more competitive 2 : 1 v/v H_2O/DMSO-d_6 mixture. Given the loss of additional C–H hydrogen bonding interactions (as observed by downfield shifts of the signals attributed to the CH protons in **2** and **4** upon addition of sulfate[31]) as a result of replacing the benzene spacers with pyridine rings, we speculate that this enhanced binding is a result of the greater preorganization resulting from the formation of intramolecular hydrogen bonds between the pyridine N atoms and the squaramide NH protons, as suggested by the higher chemical shift of the signal attributable to the squaramide NH protons of **6** (DMSO-d_6: δ = 8.00 ppm), compared to that of **4** (DMSO-d_6: δ = 7.44 ppm). DFT calculations (B3LYP/6-31G*), further support this, suggesting that **6** maintains a similar conformation in both the presence and absence of sulfate ions, with all squaramide protons projecting into the centre of the macrocyclic structure (Fig. 1). In contrast, these calculations suggest that **4** is less preorganised and must undergo conformational changes in order to bind to sulfate through all six NH protons. Unfortunately, despite the solubility of **4** and **6** in 100% water (both MSQs soluble up to 30 μM), no measurable sulfate binding was observed in solvent mixtures containing higher proportions of water (97 : 3 v/v H_2O–DMSO-d_6).

Given that **6** exhibited the highest sulfate binding affinity of this series of receptors, we further investigated anion binding of this 3[MSQ] to determine whether the changes in receptor structure impacted on the high sulfate selectivity previously observed for 3[MSQ] **2**. We first screened binding of **6** towards a range of anions in 1 : 1 v/v H_2O/DMSO-d_6. No changes to the ^{1}H NMR spectrum of **6** were observed upon addition of 5 equiv.

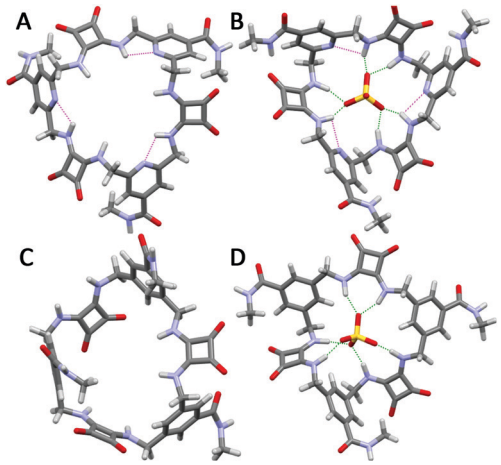

Fig. 1 Minimised structures (DFT, B3LYP/6-31G*, Spartan'14, Wavefunction®) of (A) [3]MSQ-**6**; (B) [3]MSQ-[**6**·SO_4]$^{2-}$ complex; (C) [3]MSQ-**4** and (D) [3]MSQ-[**4**·SO_4]$^{2-}$ complex. The TEG-ester functional group has been replaced with a methanamide group for computational ease and clarity.

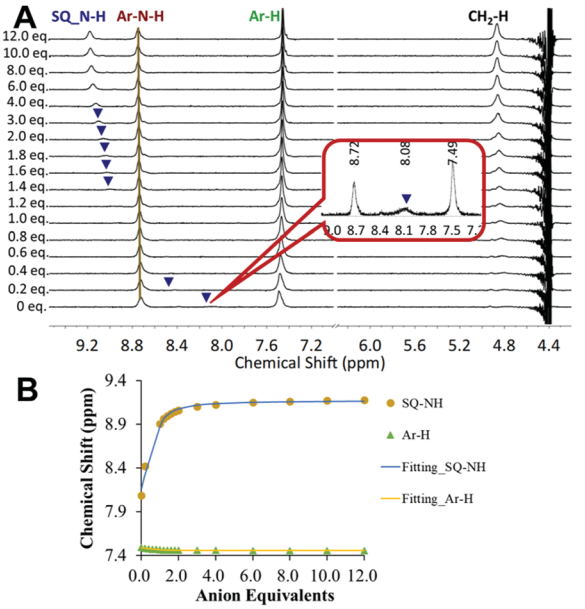

Fig. 2 (A) ^{1}H NMR titration of [3]MSQ **6** with $(TBA)_2SO_4$ in 1 : 1 (v/v) H_2O/DMSO-d_6 mixture (pH 3.2) containing 2.5 mM $H_3PO_4/H_2PO_4^-$, 10 mM TBACl, 250 mM $TBANO_3$ at 300 K. (B) Comparison isotherms of squaramide NH, and aromatic protons Ar–H in the presence of increasing concentrations of SO_4^{2-}.

of AcO^-, NO_3^-, Cl^-, HCO_3^- or $H_2PO_4^-$ indicating that, in this solvent mixture, binding to these anions was negligible. We performed NMR titrations of **6** with both SeO_4^{2-} and CrO_4^{2-} ions, given that these ions have similar shape, charge and size to SO_4^{2-}, and also evaluated the ability of **6** to bind to sulfate ions in anion mixtures that mimic the composition present in either nuclear waste or mammalian plasma. Titrations of **6** with SeO_4^{2-} and CrO_4^{2-} were performed in 1 : 1 v/v H_2O/DMSO-d_6 and titration data fit to a 1 : 1 binding model as for the sulfate titrations above. As was previously observed for **2**,[31] 3[MSQ] **6** was found to exhibit significantly higher affinity for sulfate than for selenate ($K_{a(sulfate)}/K_{a(selenate)}$ = 5) or chromate ($K_{a(sulfate)}/K_{a(chromate)}$ = 483). In a 1 : 1 mixture of DMSO-d_6 and simulated aqueous plasma electrolytes (20 mM Tris buffer, 1.5 mM $H_2PO_4^-/HPO_4^{2-}$, 106 mM Cl^-, 28 mM H_2CO_3/HCO_3^-) at pH 7.4, [3]MSQ **6** was capable of binding to sulfate ions with titration data able to be fit to a 1 : 1 binding model to give K_a = 2490 M^{-1} (±15%). This is only slightly lower than the sulfate binding affinity displayed by **6** in the absence of competing anions (K_a = 4870 M^{-1} (±15%)) indicating the high sulfate selectivity of **6** even in the presence of both phosphate and a high concentration of chloride and bicarbonate. Furthermore, titration of [3]MSQ **6** with sulfate (1 equiv. SO_4^{2-} = 2.5 mM, final SO_4^{2-} concentration = 30 mM) in mock nuclear waste solution (1 : 1 v/v H_2O/DMSO-d_6; 2.5 mM $H_3PO_4/H_2PO_4^-$, 10 mM TBACl, 250 mM $TBANO_3$) at pH 3.2 gave an apparent K_a for SO_4^{2-} = 8910 M^{-1} (±15%) (Fig. 2), approximately double that observed in 1 : 1 v/v H_2O/DMSO-d_6 at neutral pH in the absence of competing anions. We attribute this surprising increase in binding affinity in a more complex environment to the protonation of at least one of the isonicotinamide units in the macrocycle (pK_a[isonicotinamide] = 3.3[50]) providing additional electrostatic interactions with the anion.

For some applications, *e.g.* processing nuclear waste at the Hanford Site (USA), sulfate is present in solutions that are extremely basic (pH 14).[51] Sulfate binding in these conditions presents additional challenges as the receptors must be stable at basic pH; the high concentrations of hydroxide (OH^-) provide added competition and may result in deprotonation of the receptors thereby removing hydrogen bonds and introducing negative charge onto receptors; and interferants such as phosphate species (mainly PO_4^{3-}) are very competitive at this pH because of their higher charge. While the previously reported MSQs **1** and **2** were not stable at pH 14, decomposing within minutes; replacement of the ester functionality with the amides as in MSQs **3–6** resulted in increased stability at high pH. In 1 : 1 v/v H_2O–DMSO-d_6 at pH 14, these compounds were stable for up to 5 hours, but slowly decomposed on standing for longer periods, presumably as a result of alkaline hydrolysis of the squaramides.[52] We therefore tested the ability of [3]MSQ to bind to sulfate in mimicked nuclear waste at pH 14 with the same anion composition previously tested at low pH (2.5 mM phosphates, 10 mM TBACl, 250 mM $TBANO_3$).

While the squaramide protons were too broad to observe in NMR experiments under these conditions, upon addition of sulfate, small downfield shifts of the signals attributable to the isonicotinamide and methylene protons were observed, suggesting an interaction between sulfate and [3]MSQ **6**. The fitting of the titration data to a 1 : 1 binding model gave an apparent K_a = 610 M^{-1} (±20%). While sulfate binding affinity of **6** is reduced at this high pH (approx. 8 fold lower compared to that observed in 1 : 1 v/v H_2O–DMSO-d_6, pH 7), these results indicate that 3[MSQ] **6** is able to bind sulfate across a very wide pH range (pH 3.2–14.1) and in the presence of a range of other anions, including phosphates.

In summary, we have successfully synthesized a new class of water soluble squaramide based macrocycles (MSQs), and shown that these can be readily functionalized to modulate their solubility and binding affinity. Evaluation of the anion-binding ability of

[2]- and [3]-MSQs revealed high sulfate binding affinities and selectivities in a range of H_2O/DMSO-d_6 mixtures. [3]MSQs **2**, **4** and **6** all exhibited higher sulfate binding affinity than the [2]MSQ analogs, **1**, **3** and **5**. A comparison of [3]MSQs **4** and **6** suggests that the replacement of the benzene spacers with pyridine units increases binding affinity, as a result of enhanced receptor preorganization. [3]MSQ **6** was found to be a highly selective ligand for SO_4^{2-} in the presence of a broad range of interferants in aqueous mixtures and across a pH window from 3.2 to 14.1, demonstrating the potential for applications of [3]MSQs in sulfate separation from nuclear wastes and in plasma sulfate assays.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ pH values in aqueous/organic mixtures are not well defined, so pH refers to the measured pH of the aqueous phase before mixing with DMSO.

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