

Sulfate selective recognition using neutral dipeptide anion receptors in aqueous solution

Robert B. P. Elmes[‡], Karen K. Y. Yuen[‡] and Katrina A. Jolliffe*^[a]

Abstract: The synthesis of six small peptide anion receptors based on thiourea and squaramide recognition moieties is described. These new receptors bind to tetrahedral sulfate anions with remarkable affinity and

selectivity in aqueous solution as shown by NMR spectrometry. Molecular modelling suggests that selectivity is mediated by a hydrogen bond network incorporating the amide backbone protons in a manner similar

to that found in the sulfate binding protein. **Keywords:** Peptide • Anion Receptor • Squaramide • Thiourea • Sulfate

Introduction

The design of new chemosensors for anionic species is an ever expanding field due to the ubiquitous nature of anions in a wide range of environmental, chemical and biological processes.^{1,2} In particular, receptors capable of selectively recognising specific anionic guests in competitive solvents have potential uses in numerous biomedical and environmental applications. Sulfate anions are of particular relevance in this regard as they can interfere with proposed radioactive waste treatment processes^{3,4} and are often amongst a myriad of potentially toxic solutes found as ground water contaminants in mining associated water pollution.⁵ Indeed, a recent study evaluating the influence of mining on mine surrounded waterways observed SO_4^{2-} levels ranging from 11 mg L⁻¹ for unmined streams to 1187 mg L⁻¹ for streams in mined watersheds.⁶

In nature, the selective binding and transport of sulfate is achieved by the sulfate binding protein (SBP), which binds to sulfate through seven hydrogen bonds, five of which are provided by main chain amide groups.⁷ In this manner, the SBP is capable of highly selective binding of sulfate with an association constant of approximately 10^6 M^{-1} in water (pH 5–8.1).⁴ While a number of approaches have been taken towards the selective binding of SO_4^{2-} with synthetic receptors based on macrocyclic^{8,9} and interlocked structures,^{3,10} tripodal scaffolds,^{11–13} and metal ions,^{14–16} the majority of these systems are only capable of binding sulfate in organic

solvents, whereas for practical applications the binding of SO_4^{2-} in aqueous solutions is often desired.

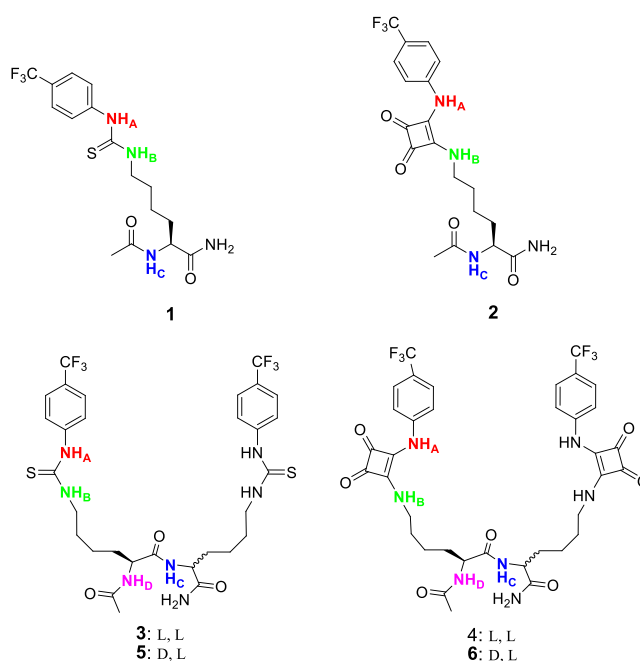


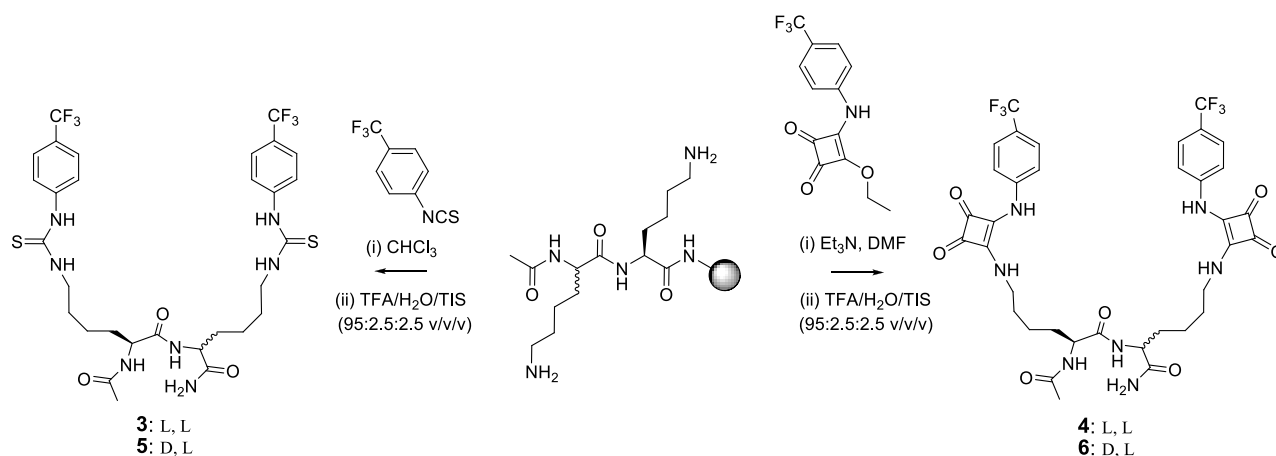
Figure 1. Structures of receptors 1 - 6.

In the field of synthetic anion receptors, amides, ureas, thioureas and, more recently, squaramides have been extensively employed to provide hydrogen-bond donor sites,^{17–21} and the use of either ureas or thioureas in combination with amides has been shown to provide significant enhancements in anion binding affinity.^{22,23} Indeed, we recently described a number of tripodal anion receptors based on a cyclic peptide scaffold functionalized with either urea or thiourea binding sites which were highly selective for SO_4^{2-} and bound this ion with high affinities in aqueous solvent mixtures.^{24–26} This selectivity and affinity is proposed to

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Scheme 1. General synthetic route to receptors **1** - **6**.

arise from a synergistic effect brought about by hydrogen bond donation from both the (thio)urea protons and the cyclic peptide backbone amides. Similarly, Kubik and co-workers have recently reported that a combination of cyclic peptide backbone hydrogen bond donors and charged side chain ammonium groups provides high affinity and selectivity for sulfate in aqueous buffer.²⁷ While such cyclic peptide scaffolds have shown a high degree of selectivity towards their target anions, their synthesis is laborious and involves numerous purification steps. We envisaged that linear peptide scaffolds functionalised with suitable anion recognition motifs would be readily synthesised and may also function as charge neutral sulfate selective receptors in competitive solvents through a combination of peptide backbone and sidechain binding sites, in a manner analogous to that of the SBP. Our recent observation that linear peptide-based bis[Zn(II) dipicolylamino] receptors²⁸ showed similar anion selectivity to related cyclic peptide analogues²⁹⁻³¹ despite their lack of preorganisation, suggested that the excellent sulfate selectivity previously observed for cyclic peptide scaffolds could be maintained for simplified linear analogues. In order to investigate this hypothesis we set about synthesising a small family of linear peptide based receptors in which we (a) varied the peptide length and number of binding sites available, (b) varied the nature of the recognition moiety, replacing thiourea groups with squaramides to provide enhanced hydrogen-bond donor strength and (c) varied the stereochemistry of the amino acid side chains (Receptors **1** - **6**; Figure 1). To enable rapid access to these and other linear peptide derivatives we designed a synthetic approach to enable the entire synthesis to be performed on the solid phase. The anion binding properties of these receptors were then investigated.

Results and Discussion

The synthesis of **1** - **6** was carried out using an Fmoc solid phase peptide synthesis (Fmoc-SPPS) strategy on Rink amide resin (Scheme 1) with orthogonal allyloxycarbonyl (Alloc) protection of the side chain amino groups. Loading was achieved by treatment with L-Lys in the presence of HBTU and DIPEA. Iterative deprotection (20% Piperidine/DMF) and coupling (amino acid, HBTU/DIPEA) steps were followed by acetyl capping of the N-terminal amino acid by treatment with 20% acetic anhydride/pyridine. After assembly of the desired linear peptide scaffold, the Alloc groups were removed by treatment with Pd(PPh₃)₄ in the presence of acetic acid and morpholine.³²

Subsequent functionalisation of the side chain amino groups was achieved by reaction with either 4-(trifluoromethyl)phenyl isothiocyanate (**1**, **3** and **5**) or 3-ethoxy-4-(4-(trifluoromethyl)phenylamino)cyclobut-3-ene-1,2-dione (**2**, **4** and **6**) to install the thiourea and squaramide moieties respectively. The entire assembly was finally cleaved from the solid support by treatment with a solution of TFA/TIS/H₂O (95/2.5/2.5) to afford the desired anion receptor peptides (**1** - **6**) in isolated yields of 28 - 36% and 58 - 76% for the thiourea and squaramide based receptors, respectively. While it was possible to purify squaramide receptors **2**, **4** and **6** by simple trituration with MeOH, the solubility of **1**, **3** and **5** did not allow for this and HPLC purification was necessary for these analogues resulting in lower isolated yields of **1**, **3** and **5**.

In order to assess the anion binding properties of this family of receptors a number of ¹H NMR spectroscopic titration experiments were conducted, using the tetrabutylammonium salts of the anions to ensure their complete solubility. With the exception of the complex between compound **1** and acetate (data for this complex could not be fit to a suitable binding model), in all cases the observed changes to NH_A and NH_B were fitted to a 1:1 binding model using Hyperquad^{®33} to give apparent stability constants which are summarised in Table 1 (see supporting information for fitted titration data). We first evaluated the binding ability of modified amino acids **1** and **2** to assess the effect that changing the binding motif (thiourea vs squaramide) would have on binding affinity. Fabbrizzi *et al.* have recently reported that anion binding affinities of squaramides are significantly higher than those for the analogous ureas³⁴ and as expected, squaramide derivative **2** was found to bind anions significantly more strongly than its thiourea counterpart (e.g. **1** + BzO⁻ $K_a = 76 \text{ M}^{-1}$ while **2** + BzO⁻ $K_a = 1300 \text{ M}^{-1}$). ¹H NMR spectroscopic titrations of both **1** and **2** revealed that they were capable of binding SO₄²⁻ with high affinity while AcO⁻, BzO⁻ and Cl⁻ were bound less strongly (Table 1). Notably, for both **1** and **2**, the addition of sulfate ions resulted in significant downfield shifts ($\Delta\delta$ 1.77 and 1.67 ppm, respectively) of the amide protons (NH_C), indicating the formation of hydrogen bonds from these protons to sulfate. In contrast, the addition of other anions to **1** and **2** resulted in only minor shifts of these protons (< 0.2 ppm), suggesting that the incorporation of additional amide hydrogen bond donors might result in increased selectivity for sulfate over other anions. We therefore investigated dipeptide receptors **3** - **6** which contain additional hydrogen bond donor sites.

Table 1. Apparent association constants (K_a , M^{-1}) and $\Delta\delta$ values (ppm) for **1** - **6** with various anions (added as their tetrabutylammonium salts) as determined by 1H NMR titrations monitoring the thiourea or squaramide resonances NH_A and NH_B in 0.5% H_2O in $DMSO-d_6$.^[a]

| | SO_4^{2-} | | | AcO^- | | | BzO^- | | | Cl^- | | |
|----------|-------------------|----------------------|----------------------|-------------------|----------------------|----------------------|---------|----------------------|----------------------|--------|----------------------|----------------------|
| | K_a | $\Delta\delta_{NHA}$ | $\Delta\delta_{NHC}$ | K_a | $\Delta\delta_{NHA}$ | $\Delta\delta_{NHC}$ | K_a | $\Delta\delta_{NHA}$ | $\Delta\delta_{NHC}$ | K_a | $\Delta\delta_{NHA}$ | $\Delta\delta_{NHC}$ |
| 1 | $> 10^4$ | 2.51 | 1.77 | ND ^[b] | 2.95 | 0.19 | 76 | 2.84 | 0.18 | 38 | 0.56 | 0.03 |
| 2 | $> 10^4$ | 2.94 | 1.67 | 1724 | 3.00 | 0.12 | 1300 | 3.03 | 0.12 | 130 | 1.37 | 0.01 |
| 3 | ND ^[b] | 2.08 | 1.40 | 330 | 2.80 | 0.22 | 314 | 2.64 | 0.16 | 53 | 0.47 | 0.04 |
| 4 | ND ^[b] | 2.81 | 1.58 | 483 | 3.00 | 0.17 | 514 | 3.07 | 0.15 | 284 | 1.29 | 0.03 |
| 5 | ND ^[b] | 2.32 | 1.34 | 235 | 2.82 | 0.17 | 369 | 2.64 | 0.12 | 63.8 | 0.49 | 0.02 |
| 6 | ND ^[b] | 2.96 | 1.49 | 421 | 2.98 | 0.12 | 479 | 3.07 | 0.10 | 186 | 1.29 | 0.01 |

^[a] Determined at 300 K. Where possible 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] Titration data suggests strong binding however it could not be fitted to a suitable binding model.

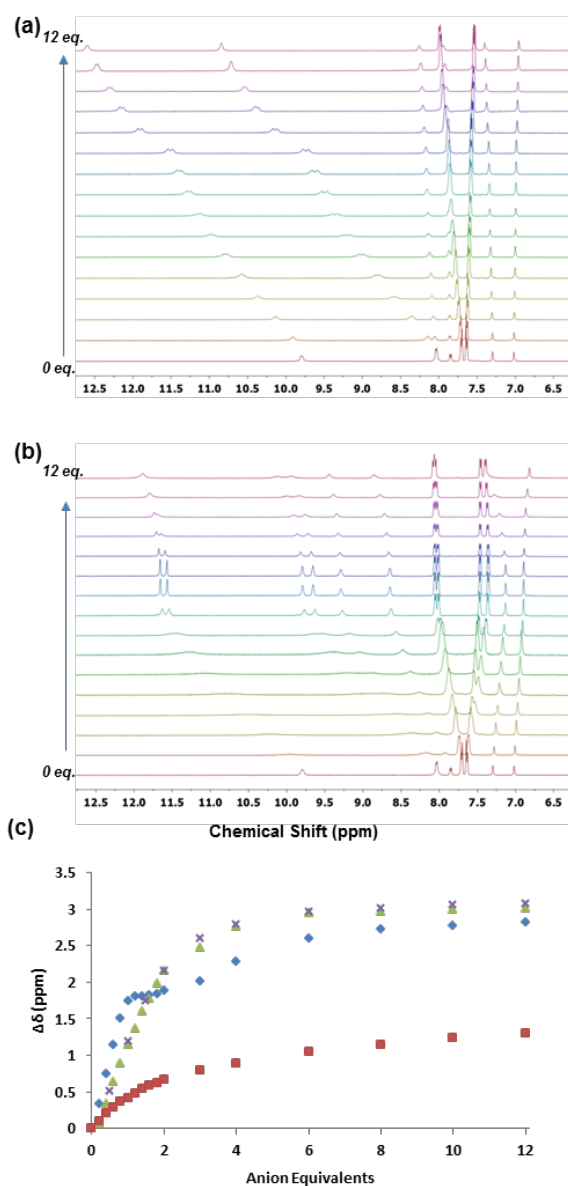


Figure 2. Stack plots of 1H NMR spectra of **3** ($2.5 \times 10^{-3} M$) upon addition of (a) TBAOAc and (b) $(TBA)_2SO_4$ (0 – 12 eq.) in 0.5% H_2O in $DMSO-d_6$ at 25 $^{\circ}C$. (c) Comparison isotherms of **3** in the presence of increasing concentrations of SO_4^{2-} (\blacklozenge), Cl^- (\blacksquare), BzO^- (\blacktriangle) and AcO^- (\times)

Initially qualitative measurements with the dipeptide derivatives **3** and **4** were undertaken using 1H NMR screening experiments in which 10 eq. of a range of anions (AcO^- , BzO^- , $H_2PO_4^-$, SO_4^{2-} , Br^- , HSO_4^- , NO_3^- , TsO^- and Cl^- as their tetrabutylammonium salts) were added to the receptors in solution (0.5% H_2O in $DMSO-d_6$). These preliminary results indicated significant changes of the spectra of **3** and **4** in the presence of AcO^- , BzO^- , $H_2PO_4^-$, SO_4^{2-} and Cl^- culminating in large downfield shifts of both of the thiourea/squaramide NH protons and, in some cases, the amide NH protons. Moreover, for the squaramide derivative **4**, there was also a large degree of peak broadening observed indicating the occurrence of slow exchange processes on the NMR timescale. Conversely only minor changes were observed in the presence of Br^- , HSO_4^- , NO_3^- and TsO^- suggesting little interaction of these anions with either **3** or **4**. In order to investigate these effects and to probe the binding mode and affinities more closely we conducted additional quantitative binding studies with **3** and **4** in the presence of AcO^- , BzO^- , SO_4^{2-} and Cl^- . Unfortunately, titration of **3** and **4** with $H_2PO_4^-$ led to peak broadening, preventing an association constant from being determined. This behavior has also been observed for our (thio)urea functionalised tripodal cyclic peptides^{24,25} and for diindolylureas reported by Gale *et. al.* and may be indicative of a deprotonation process.³⁵

The addition of AcO^- , BzO^- , SO_4^{2-} and Cl^- to both **3** and **4** resulted in downfield shifts of the thiourea/squaramide NH proton signals (NH_A and NH_B) as well as varying degrees of downfield shifts for the backbone amides (NH_C and NH_D). Representative spectra for titration of **3** with AcO^- are shown in Figure 2 (a), illustrating the significant downfield shifts of the thiourea proton signals. Similar effects were observed for squaramide derivative **4** in the presence of each of the anions measured, however, as seen for the amino acid receptors **1** and **2**, higher affinity for all anions was observed for **4** compared to the thiourea derivative **3** (e.g. **3** + Cl^- $K_a = 53 M^{-1}$ while **4** + Cl^- $K_a = 284 M^{-1}$). As observed for **1** and **2** above, on addition of AcO^- , BzO^- and Cl^- the signals attributable to the peptide amide NH protons (NH_C and NH_D) were not significantly shifted, suggesting minimal hydrogen bonding is occurring at these sites. The binding titrations for SO_4^{2-} with **3** and **4**, however, exhibited distinct behaviour from the other anions measured and resulted in significant changes to NH_A , NH_B , NH_C

and NH_D as well as the aromatic phenyl protons. The ^1H NMR titration for thiourea **3** with increasing equivalents of SO_4^{2-} is shown

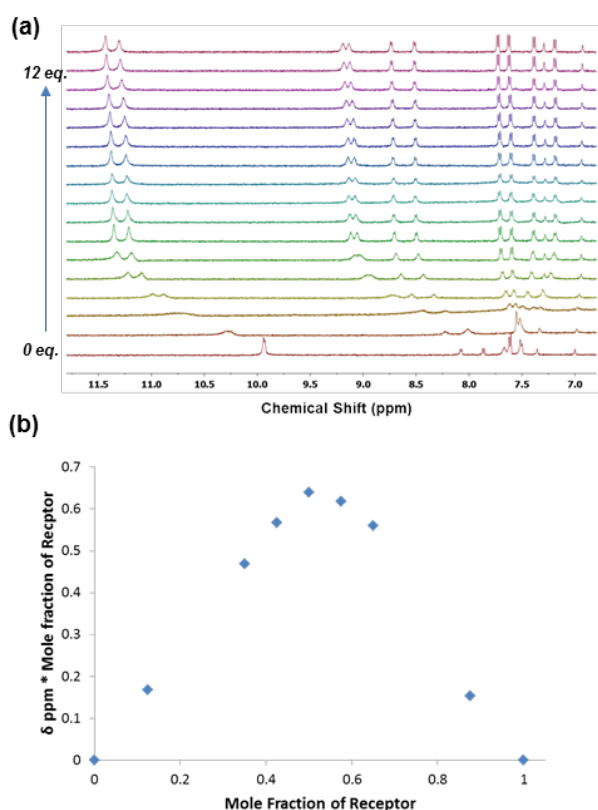


Figure 3. (a) Stack plot of ^1H NMR spectra of **4** (2.5×10^{-3} M) upon addition of $(\text{TBA})_2\text{SO}_4$ (0 – 12 eq.) in 20% H_2O in DMSO-d_6 at 25 $^\circ\text{C}$. (b) The corresponding Job plot analysis of **4** in the presence of $(\text{TBA})_2\text{SO}_4$. Figure 4. Comparison isotherm of **4** in the presence of increasing concentrations of SO_4^{2-} (\square), BzO^- (\square) and AcO^- (\times) in 20% H_2O in DMSO-d_6 .

in Figure 2(b). Unfortunately, as has been previously observed for (thio)urea functionalised tripodal cyclic peptides,^{24,25} an accurate binding constant could not be calculated from the titration data due to a two-step binding profile. Such behaviour has also been observed with acyclic indole and carbazole-based receptors and indicates the occurrence of more complex binding equilibria depending on the concentration of SO_4^{2-} present.³⁶ Addition of 0.2 to 1.4 equivalents of SO_4^{2-} resulted in significant signal broadening,

Table 2. Apparent association constants (K_a , M^{-1}) and $\Delta\delta$ values (ppm) for **2** – **6** with various anions (added as their tetrabutylammonium salts) as determined by ^1H NMR titrations monitoring the thiourea or squaramide resonances NH_A and NH_B in 20% H_2O in DMSO-d_6 .^[a]

| | SO_4^{2-} | | | AcO^- | | | BzO^- | | |
|----------|--------------------|-----------------------------|-----------------------------|----------------|-----------------------------|-----------------------------|----------------|-----------------------------|-----------------------------|
| | K_a | $\Delta\delta_{\text{NHA}}$ | $\Delta\delta_{\text{NHC}}$ | K_a | $\Delta\delta_{\text{NHA}}$ | $\Delta\delta_{\text{NHC}}$ | K_a | $\Delta\delta_{\text{NHA}}$ | $\Delta\delta_{\text{NHC}}$ |
| 2 | 1116 | ND ^[b, c] | 0.68 | 12.3 | ND ^[b, c] | 0.03 | 26.9 | ND ^[b, c] | 0.02 |
| 3 | 1282 | 1.27 | 0.60 | 23.3 | 0.27 | 0.02 | 21.3 | 0.26 | 0.03 |
| 4 | $> 10^4$ | 1.44 | 0.66 | 44.9 | ND ^[b, c] | 0.07 | 17.1 | ND ^[b, c] | 0.04 |
| 5 | 775 | 1.31 | 0.75 | 17.3 | 0.36 | 0.06 | 17.4 | 0.35 | 0.05 |
| 6 | $> 10^4$ | 1.49 | 0.40 | 72.6 | ND ^[b, c] | 0.08 | 29.7 | ND ^[b, c] | 0.05 |

^[a] Determined at 300 K. Where possible 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%. Anions added as their tetrabutylammonium salts. ^[b] Peak broadening prevented a $\Delta\delta$ value from being determined. ^[c] Titration displayed slow exchange on the NMR timescale and thus K_a values were obtained from monitoring NH_C

however, further additions brought about well resolved thiourea and amide signals that were significantly shifted downfield from

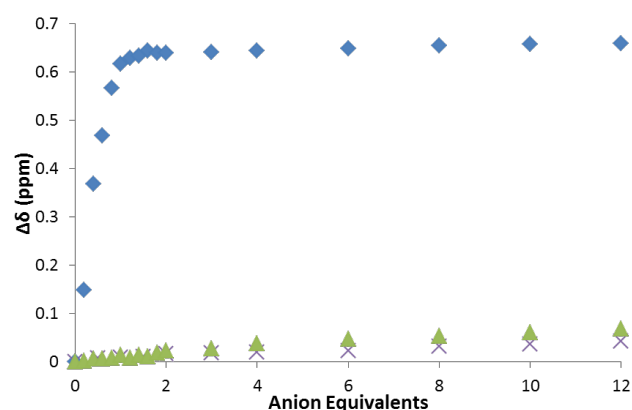


Figure 4. Comparison isotherm of **4** in the presence of increasing concentrations of SO_4^{2-} (\square), BzO^- (\square) and AcO^- (\times) in 20% H_2O in DMSO-d_6 .

their original positions ($\Delta\delta_{\text{NH}_A} = 1.83$ ppm and $\Delta\delta_{\text{NH}_C} = 1.26$ ppm). Moreover, addition of SO_4^{2-} results in the poorly resolved aromatic and thiourea signals separating into two clearly distinct sets of peaks and strongly suggests the formation of a host–guest complex in solution. Subsequent additions induced no further changes to the spectra until, after 4 equivalents of SO_4^{2-} had been added, the thiourea and amide signals began to shift downfield and broaden once again. This two stage process suggests that initially **3** forms a strong 1:1 complex at low concentrations of SO_4^{2-} (i.e. less than two equivalents) while at higher concentrations of anion, more complex binding equilibria exist. Comparable behaviour was observed for **4** in the presence of SO_4^{2-} however, the resolution of the squaramide signals occurred after just 1 eq. had been added ($\Delta\delta_{\text{NH}_A} = 1.81$ ppm and $\Delta\delta_{\text{NH}_B} = 1.71$ ppm) and these signals had completely disappeared before the addition of 2 equivalents of SO_4^{2-} suggesting a stronger interaction between squaramide **4** and SO_4^{2-} compared to that of the thiourea analogue **3**. As was the case with **3**, large $\Delta\delta$ values were measured for the amide protons of **4** over the course of the titration ($\Delta\delta_{\text{NH}_C} = 1.58$ ppm and $\Delta\delta_{\text{NH}_D} = 1.33$ ppm), providing further evidence to suggest that the peptide backbone of these linear peptide scaffolds has a major role to play in the selective recognition of SO_4^{2-} in solution. Titrations were next carried out using **5** and **6**, to probe the effect of alternating stereochemistry on the binding behavior of the linear peptide

scaffold. This structural change had only a minor influence on binding behavior as summarised in Table 1. Similar binding constants were determined for **5** and **6** to those obtained for **3** and **4**, respectively. Moreover, the $\Delta\delta$ values observed for the D,L derivatives **5** and **6** are very similar to those measured for their L,L counterparts providing further evidence that the chirality of these particular receptors has little effect on their binding affinity for anionic species. Due to the large apparent stability constants obtained for these receptors with SO_4^{2-} ions in 0.5% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$, we chose to investigate these systems in more competitive media by conducting further binding studies with AcO^- , BzO^- and SO_4^{2-} . These studies were conducted in 20% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$ as the receptors were not soluble at higher H_2O concentrations. The binding data obtained reveals that moving to this more polar solvent mixture had a significant influence on the binding behaviour of **1** – **6** (Table 2). Large decreases in the apparent stability constants were observed for binding of **1** – **6** to both AcO^- and BzO^- , while high affinity was retained for SO_4^{2-} for receptors **2** – **6**.

In 20% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$ the addition of SO_4^{2-} initially resulted in peak broadening of all of the signals. However, after 1 equivalent of the anion had been added, fast exchange processes on the NMR timescale were restored and well resolved thiourea/squaramide signals were observed that were also shifted significantly downfield from their original positions. Interestingly, the two stage binding process observed for **3** – **6** with SO_4^{2-} in 0.5% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$ was completely suppressed in this more polar solvent (Figure 3a). This solvent dependent behaviour has previously been observed for cyclic peptides with thiourea arms and also with indole based sulfate receptors.^{25,36} Significant shifts were again observed for the backbone amide protons, suggesting that they are involved in hydrogen bonding to the SO_4^{2-} ions even in this highly competitive solvent. As was observed in the titrations carried out in 0.5% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$, the addition of SO_4^{2-} results in increased resolution of the aromatic and thiourea/squaramide signals giving rise to four clearly resolved peaks. This observation suggests that anion:receptor complex formation significantly reduces the flexibility of the peptide, thereby placing these functional groups in chemically inequivalent environments. This effect was not observed upon addition of either AcO^- or BzO^- , indicative of the higher degree of flexibility of these complexes, which can be attributed to the comparatively weak binding event (Figure 4). Notably, affinity for sulfate by the squaramide receptors **2**, **4**, and **6** was significantly higher than that observed for the analogous thioureas, **1**, **3** and **5**, respectively in this more competitive solvent mixture. The strong binding interaction with SO_4^{2-} was particularly evident in the cases of the dipeptide squaramide derivatives **4** and **6** which displayed characteristic evidence to support the formation of a 1:1 receptor anion complex in solution. These observations were supported by Job's plot analysis (Figure 3b) and when fitted to a 1:1 binding model apparent stability constants of $> 10^4 \text{ M}^{-1}$ were calculated. Interestingly, under the same conditions, the stability constants obtained for AcO^- with **4** and **6** were seen to be at least an order of magnitude lower than the values obtained previously in 0.5% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$ demonstrating the high degree of selectivity that these receptors exhibit towards SO_4^{2-} even in highly competitive media.

In order to gain insight into possible modes of binding of SO_4^{2-} with this class of receptor, molecular modeling of **4** was performed using Spartan 10 for Windows (Wavefunction, Inc.). The structure

of **4** was energy minimized using molecular mechanics before a SO_4^{2-} molecule was placed into the centre of the receptor and the resulting complex was optimized by density functional theory (DFT) calculations at the B3LYP/6-31G* level of theory. Although such modeling of molecular docking cannot provide any quantitative

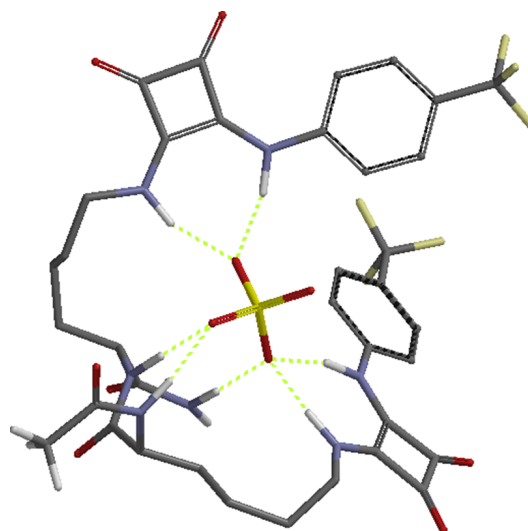


Figure 5. DFT calculated structure of the SO_4^{2-} complex of **4** showing the anion inserted into the binding cavity stabilised by 7 N-H \cdots O hydrogen-bond interactions.

results for interaction energies, it affords reasonable qualitative evidence for possible host:guest orientation in molecular complexes. As shown in Figure 5, modelling indicates that **4** wraps around the sulfate ion, binding through seven hydrogen bonds in a manner similar to the binding of SO_4^{2-} to the SBP. Two H-bonds are provided by each of the squaramide moieties, two further interactions from the backbone amide protons and a final H-bond from the carboxamide terminus. Moreover, the H-bond lengths observed between SO_4^{2-} and the squaramide NHs were calculated as being between 1.740 and 1.928 Å in length; values that highlight the strong H-bond interaction with SO_4^{2-} and are shorter than distances recently observed in crystal structures of squaramides with Cl^- and DMSO .^{34,37,38} Although not a conclusive result, the calculated structure corroborates the results observed in the NMR measurements, accounting for the observed shifts of all of the NH protons as well as the increased degree of chemical inequivalence seen between the squaramide NHs and the aromatic protons upon SO_4^{2-} addition.

Conclusion

In summary, we have developed a solid phase synthetic strategy that is readily applicable to the preparation of libraries of peptide based receptors and allows facile functionalisation with different recognition motifs. We have synthesized and evaluated the anion binding affinity and selectivity of a small family of such peptide based anion receptors, all of which show remarkable binding to SO_4^{2-} in 0.5% v/v $\text{H}_2\text{O}/\text{DMSO-}d_6$ with significantly higher affinity for this anion than for AcO^- , BzO^- , H_2PO_4^- , SO_4^{2-} , Br^- , HSO_4^- , NO_3^- , TsO^- and Cl^- . The inclusion of a squaramide anion recognition motif was found to give rise to stronger anion affinity than the thiourea analogues, with 1:1 receptor anion complexes formed after the addition of just one equivalent of SO_4^{2-} . Studies to probe the

effect of side chain stereochemistry revealed little preference for one diastereoisomer over the other, suggesting that with a flexible lysine sidechain the dipeptide stereochemistry is not a major contributor to selectivity or affinity. Receptors **1** and **2**, which comprise a single amino acid, exhibited significant binding affinity for SO_4^{2-} . However, receptors **3** – **6** with additional hydrogen bond donor sites exhibited enhanced binding affinity and sulfate selectivity in comparison to the amino acid analogues, especially when measured in 20% v/v $\text{H}_2\text{O}/\text{DMSO}-d_6$. This suggests that at least two amino acid residues are required for optimal sulfate binding. The selectivity for sulfate appears to arise from a synergistic interaction between both the amide backbone and the thiourea/squaramide NH protons that is not observed with AcO^- , BzO^- or Cl^- . Such results, taken in combination with the recent observations of Kubik *et al.* that oxygen atoms of the sulfate anion can form hydrogen bonds to the NH groups along a cyclopeptide ring²⁷ suggest that exploiting such interactions is a viable new approach to the future design of sulfate selective receptors. Taken together, the NMR and molecular modeling studies suggest a 1:1 receptor: SO_4^{2-} complex for these flexible dipeptide receptors which is brought together by a network of hydrogen bonds in a manner similar to that of the SBP. Future work will focus on analogues with increased water solubility and the introduction of additional binding sites for the fourth O atom of the sulfate residue to provide the maximum number of 12 hydrogen bonds ideal for coordination of sulfate.

Experimental Section

Synthesis

As the syntheses for all the compounds were similar, general outlines of the procedures used and example characterisation of receptors **3** and **4** are given below. Full details of all synthetic procedures and anion binding studies are provided in the supporting information

Iterative peptide assembly (Fmoc-SPPS): Rink amide resin (0.41 mmol/g as stated) was swollen in dry CH_2Cl_2 for 1 h. The resin was drained, then washed with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). The resin was treated with 10% piperidine/DMF (2×3 min) and subsequently washed with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). A solution of appropriate Fmoc-protected amino acid (2 eq. or 4 eq. relative to resin capacity for Lys or other amino acids respectively), HBTU (1.1 eq. relative to peptide) and $^i\text{Pr}_2\text{NET}$ (2 eq. relative to peptide) in dry DMF (0.1 M) was added and the mixture was agitated at rt for 2 h. The resin was then washed with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). **Deprotection:** The resin was treated with 10% piperidine/DMF (2×3 min) and washed with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). **Amino acid coupling:** A preactivated solution of protected amino acid (2 eq. or 4 eq. relative to resin capacity for Lys or other amino acids respectively), HBTU (1.1 eq. relative to peptide) and $^i\text{Pr}_2\text{NET}$ (2 eq. relative to peptide) in dry DMF (0.1 M) was added to the resin and agitated at rt for 2 h. The resin was then washed with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). **Acetylation:** Upon removal of the Fmoc protecting group, the resin was treated with 20% acetic anhydride/pyridine (3×4 min), followed by washing with DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$).

Allyloxycarbonyl (Alloc) deprotection: All Alloc-deprotected peptides were prepared following a modification of the method described by Kates *et al.*³² The resin was swollen at rt or 15 min in CHCl_3 /morpholine/acetic acid (90:5:5). Tetrakis(triphenylphosphine)palladium (1.05 eq. relative to peptide) was added to the suspension, and the syringe was shielded from light and agitated for 2 h. The resin was drained then washed with CHCl_3 ($\times 5$) and a palladium chelating cocktail (DMF/diethyldithiocarbamic acid-3-water/triethylamine 25 mL:225 mg:250 μL). Traces of the chelating cocktail were removed *via* a basic wash (0.5% triethylamine in DMF, $\times 5$). The resin was then washed with MeOH ($\times 5$), DMF ($\times 5$), CH_2Cl_2 ($\times 5$) and DMF ($\times 5$). **Thiourea functionalisation:** The resin was swollen in dry DMF at room temp. for 30 min before the addition of 4-(trifluoromethyl)phenyl isothiocyanate in CHCl_3 (12 equiv. relative to loading). The suspension was agitated at room temp. for 24 hr, drained and washed sequentially with DMF (5×10 mL), DCM (5×10 mL), DMF (5×10 mL) and DCM (5×10 mL). **Squaramide functionalisation:** The resin was swollen in dry DMF at room temp. for 30 min before the addition of 3-(4-trifluoromethylphenylamino)-4-ethoxycyclobut-3-ene-1,2-dione³⁸ (3 equiv. relative to loading) and triethylamine (6 equiv. relative to loading). The suspension was agitated at room temp. for 24 hr, drained and washed sequentially with DMF (5×10 mL), DCM (5×10 mL), DMF (5×10 mL) and DCM (5×10 mL). **Cleavage:** The resin was treated with a solution of trifluoroacetic acid/ H_2O /triisopropylsilane (95:2.5:2.5 v/v/v) for 1 h. The resin was drained and then washed with trifluoroacetic acid ($\times 4$). The cleavage solution and acid washes were combined and concentrated *in vacuo*.

Ac-Lys(thiourea)-Lys(thiourea)-NH₂, (3)

Receptor **3** was synthesised on Rink amide resin (0.610 g, 0.250 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and thiourea functionalisation. Cleavage from the resin was then achieved through treatment with a solution of TFA, triisopropylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 50% B over 40 min). The appropriate fractions were lyophilised, affording the linear dipeptide receptor **3** as a TFA salt, which was treated with basic anion exchange resin to give the desirredipeptide receptor **3** ($t_{\text{R}} = 18.6$ min) as a white solid. Yield: 58 mg, (32%); $[\alpha]_{\text{D}} - 11.7$ (c 0.05 in MeOH); $^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$) δ 9.81 (br s, 2H, $2 \times$ thiourea), 8.03-8.05 (m, 3H, amide and $2 \times$ thiourea), 7.84-7.86 (d, J 8.2 Hz, 1H, amide), 7.70-7.72 (d, J 8.5 Hz, 4H, ArH), 7.62-7.64 (d, J 8.6 Hz, 4H, ArH), 7.30 (s, 1H, carboxamide), 7.02 (s, 1H, carboxamide), 4.15-4.25 (m, 2H, $2 \times \alpha\text{-CH}$), 1.85 (s, 3H, CH_3), 1.51-1.75 (m, 10H, CH_2), 1.23-1.39 (m, 6H, CH_2); $^{13}\text{C NMR}$ (100 MHz, $\text{DMSO}-d_6$) δ 180.2, 173.6, 171.6, 169.5, 143.4, 125.7, 125.6, 123.0, 121.7, 52.8, 52.2, 43.7, 31.7, 31.5, 28.0, 27.9, 22.9, 22.8, 22.5; IR (thin film) ν_{max} 3280, 3269, 3250, 1660, 1502, 1325, 1207, 1164, 1123 cm^{-1} ; HRMS (ESI) $m/z = 744.2196$ $[\text{M} + \text{Na}]^+$, 744.2196 calcd. for $\text{C}_{30}\text{H}_{37}\text{F}_6\text{N}_7\text{O}_5\text{S}_2\text{Na}^+$.

Ac-Lys(squaramide)-Lys(squaramide)-NH₂, (4)

Receptor **4** was synthesised on Rink amide resin (0.254 g, 0.105 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and squaramide functionalisation. Cleavage from the resin was then achieved through treatment with a solution of TFA, triisopropylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by titration with MeOH affording the linear dipeptide receptor **4** as a white solid. Yield: 48 mg, (58%); $[\alpha]_{\text{D}} - 1.1$ (c 0.54 in DMF); $^1\text{H NMR}$ (500 MHz, $\text{DMSO}-d_6$) δ 9.91 (s, 2H, NH), 8.02 (d, $J = 7.3$ Hz, 1H, NH), 7.83 (d, $J = 8.0$ Hz, 1H, NH), 7.72 (s, 2H, NH), 7.66 (d, $J = 8.3$ Hz, 4H, ArH), 7.59 (d, $J = 7.6$ Hz, 4H, ArH), 7.30 (s, 1H, NH), 7.02 (s, 1H, NH), 4.15-4.25 (m, 2H, $2 \times \alpha\text{CH}$), 3.58 (d, $J = 5.3$ Hz, 4H, $2 \times \text{CH}_2$), 1.84 (s, 3H, CH_3), 1.2-1.76 (m, 12H, $6 \times \text{CH}_2$); $^{13}\text{C NMR}$ (125 MHz, $\text{DMSO}-d_6$) δ 184.7, 180.0, 173.5, 171.6, 169.7, 169.6, 162.9, 142.6, 126.7, 125.6, 123.4, 122.3 (q, $J = 32.0$ Hz, CF_3), 117.9, 52.7, 52.1, 43.7, 31.5, 31.4, 30.3, 22.5, 22.4, 22.3; IR (thin film) ν_{max} 3173, 1657, 1557, 1455, 1336, 1116, 1073, 834 cm^{-1} ; HRMS (ESI) $m/z = 816.2558$ $[\text{M} + \text{Na}]^+$, 816.2556 calcd. for $\text{C}_{36}\text{H}_{37}\text{F}_6\text{N}_7\text{O}_7\text{Na}^+$.

NMR Binding Studies

NMR titrations were performed by additions of aliquots of the putative anionic guest as the tetrabutylammonium (TBA) salt (0.15 – 0.2 M), in a solution of the receptor (2.5×10^{-3} M) in either 0.5% H_2O in $\text{DMSO}-d_6$ or 20% H_2O in $\text{DMSO}-d_6$ to a 2.5×10^{-3} M solution of the receptor in either 0.5% H_2O in $\text{DMSO}-d_6$ or 20% H_2O in $\text{DMSO}-d_6$. Typically, up to 9-12 equivalents of the anion were added to the solution. Both salt and receptor were dried under high vacuum prior to use. $^1\text{H NMR}$ spectra were recorded on a Bruker Avance III 500 spectrometer at a frequency of 500.13 MHz and calibrated to the residual protio solvent peak in $\text{DMSO}-d_6$ ($\delta = 2.50$ ppm). Stack plots were made using MestReNova Version 6.0. Where possible and when the change in chemical shift was larger than 0.02 ppm, non-linear curve fitting of the experimentally obtained titration isotherms (equivalents of anion vs. chemical shift of the squaramide NH protons or amide NH protons) using the commercially available software program HypNMR[®] (Hyperquad[®] package) enabled the calculation of association constants (K_{a}/M^{-1}) using a 1:1 model.

Molecular Modelling

Molecular modelling of **4** was performed using Spartan 10 for Windows (Wavefunction, Inc. Irvine, CA). The structure was energy minimized using molecular mechanics before a SO_4^{2-} molecule was placed into the centre of the receptor and optimized by density functional theory (DFT) calculations at the B3LYP/6-31G* level of theory.

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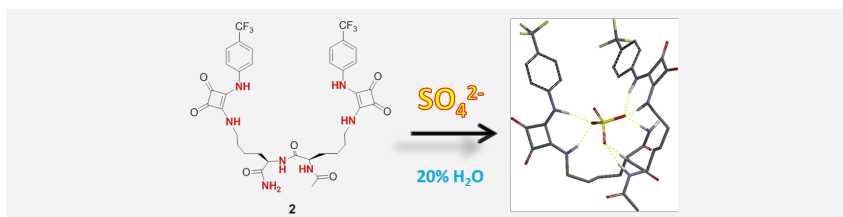
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Sulfate is all wrapped up

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Sulfate selective recognition with neutral dipeptide anion receptors in aqueous solution



Dipeptides bearing hydrogen bond donating side chains bind sulfate ions with high selectivity and affinity in aqueous solution through a network of seven hydrogen bonds from both the sidechains and backbone amide groups .