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\(^{10}\) 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.

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

Figure 1. Structures of receptors 1 - 6.

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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. 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] receptors showed similar anion selectivity to related cyclic peptide analogues 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 allyloxy carbonyl (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. 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-(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 v/v/v) 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 NH2 and NHδ were fitted to a 1:1 binding model using Hyperquad 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 ureas and as expected, squaramide derivative 2 was found to bind anions significantly more strongly than its thiourea counterpart (e.g. 1 + BrO− Ks = 76 M−1 while 2 + BrO− Ks = 1300 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 (Δδ 1.77 and 1.67 ppm, respectively) of the amide protons (NH2), 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 $^1$H NMR titrations monitoring the thiourea or squaramide resonances NH$_A$ and NH$_B$ in 0.5% H$_2$O in DMSO-d$_6$.$^{35}$

<table>
<thead>
<tr>
<th>Anion</th>
<th>$K_a$</th>
<th>$\Delta \delta$ NH$_A$</th>
<th>$\Delta \delta$ NH$_B$</th>
<th>$\Delta \delta$ O$_A$</th>
<th>$\Delta \delta$ O$_B$</th>
<th>$\Delta \delta$ S$_A$</th>
<th>$\Delta \delta$ S$_B$</th>
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<td>&gt; 10$^4$</td>
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<tr>
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<td>421</td>
<td>2.98</td>
<td>0.12</td>
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</table>

$^{35}$ 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 $< 15\%$. $^{35}$ Titrination data suggests strong binding however it could not be fitted to a suitable binding model.

Initially qualitative measurements with the dipeptide derivatives 3 and 4 were undertaken using $^1$H NMR screening experiments in which 10 eq. of a range of anions (AcO$^-$, BzO$^-$, H$_2$PO$_4^-$, 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$_2$O in DMSO-d$_6$). These preliminary results indicated significant changes of the spectra of 3 and 4 in the presence of AcO$^-$, BzO$^-$, H$_2$PO$_4^-$, 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$_2$PO$_4^-$ 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$_C$.

Figure 2. Stack plots of $^1$H NMR spectra of 3 (2.5 x 10$^{-3}$ M) upon addition of (a) TBAOAc and (b) (TBA)$_2$SO$_4$ (0 - 12 eq.) in 0.5% H$_2$O in DMSO-d$_6$ at 25 °C. (c) Comparison isotherms of 3 in the presence of increasing concentrations of SO$_4^{2-}$ (+), Cl$^-$ (■), BzO$^-$ (▲) and AcO$^-$ (○).
and NH₂ as well as the aromatic phenyl protons. The 1H NMR titration for thiourea 3 with increasing equivalents of SO₄²⁻ is shown however, further additions brought about well resolved thiourea and amide signals that were significantly shifted downfield from theirs original positions (Δδ NH₂ = 1.83 ppm and Δδ NH₆ = 1.26 ppm). Moreover, addition of SO₄²⁻ 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 SO₄²⁻ 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 SO₄²⁻ (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 SO₄²⁻; however, the resolution of the squaramide signals occurred after just 1 eq. had been added (Δδ NH₂ = 1.81 ppm and Δδ NH₆ = 1.71 ppm) and these signals had completely disappeared before the addition of 2 equivalents of SO₄²⁻ suggesting a stronger interaction between squaramide 4 and SO₄²⁻ compared to that of the thiourea analogue 3. As was the case with 3, large Δδ values were measured for the amide protons of 4 over the course of the titration (Δδ NH₂ = 1.58 ppm and Δδ NH₆ = 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 SO₄²⁻ 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.

Figure 3. (a) Stack plot of 1H NMR spectra of 4 (2.5 × 10⁻³ M) upon addition of (TBA)₂SO₄ (0 – 12 eq.) in 20% H₂O in DMSO-d₆ at 25 °C. (b) The corresponding Job plot analysis of 4 in the presence of (TBA)₂SO₄.

Figure 4. Comparison isotherm of 4 in the presence of increasing concentrations of SO₄²⁻ ( ), BrO⁻ ( ) and AcO⁻(x) in 20% H₂O in DMSO-d₆.

Table 2. Apparent association constants (Kₐ, M⁻¹) and Δδ 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 NH₂ and NH₆ in 20% H₂O in DMSO-d₆.

<table>
<thead>
<tr>
<th></th>
<th>SO₄²⁻</th>
<th>AcO⁻</th>
<th>BrO⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δδ NH₂</td>
<td>Δδ NH₆</td>
<td>Kₐ</td>
</tr>
<tr>
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<td>1116</td>
<td>0.68</td>
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</tr>
<tr>
<td>3</td>
<td>1282</td>
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<tr>
<td>4</td>
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<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>775</td>
<td>1.31</td>
<td>0.75</td>
</tr>
<tr>
<td>6</td>
<td>&gt; 10[^4]</td>
<td>1.49</td>
<td>0.40</td>
</tr>
</tbody>
</table>

[^4] Determined at 300 K. Where possible data was fitted to a 1:1 binding model as confirmed by Job plot titrations. Kₐ values are an average obtained from monitoring NH₂ and NH₆ Errors < 15%. Anions added as their tetrabutylammonium salts.[^5] Peak broadening prevented a Δδ value from being determined.[^1] Titration displayed slow exchange on the NMR timescale and thus Kₐ values were obtained from monitoring NH₂.
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 ∆δ values observed for the D,L derivatives 5 and 6 are very similar to those measured for their 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 SO₄²⁻ ions in 0.5% v/v H₂O/DMSO-d₆, we chose to investigate these systems in more competitive media by conducting further binding studies with AcO, BzO and SO₄²⁻. These studies were conducted in 20% v/v H₂O/DMSO-d₆ as the receptors were not soluble at higher H₂O 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 SO₄²⁻ for receptors 2 – 6.

In 20% v/v H₂O/DMSO-d₆, the addition of SO₄²⁻ 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 SO₄²⁻ in 0.5% v/v H₂O/DMSO-d₆ was completely suppressed in this more polar solvent mixture (Figure 3a). This solvent dependent behaviour has previously been observed for cyclic peptides with thiourea arms and also with indole based squaramide receptors. Significant shifts were again observed for the backbone amide protons, suggesting that they are involved in hydrogen bonding to the SO₄²⁻ ions even in this highly competitive solvent. As was observed in the titrations carried out in 0.5% v/v H₂O/DMSO-d₆, the addition of SO₄²⁻ 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 SO₄²⁻ 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 > 10⁷ 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 H₂O/DMSO-d₆, demonstrating the high degree of selectivity that these receptors exhibit towards SO₄²⁻ even in highly competitive media.

In order to gain insight into possible modes of binding of SO₄²⁻ 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 SO₄²⁻ 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 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 SO₄²⁻ 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 carbamoyl terminus. Moreover, the H-bond lengths observed between SO₄²⁻ 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 SO₄²⁻ and are shorter than distances recently observed in crystal structures of squaramides with Cl and DMSO. 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 SO₄²⁻ 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 SO₄²⁻ in 0.5% v/v H₂O/DMSO-d₆ with significantly higher affinity for this anion than for AcO⁻, BzO⁻, H₂PO₄⁻, SO₄²⁻, Br⁻, HSO₄⁻, NO₃⁻, 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 SO₄²⁻. Studies to probe
Ac-Lys(thioeic)lys(thioeic)-NH₂(3) receptor was synthesised on Rink amide resin (0.610 g, 0.250 mmol, resin capacity 0.41 mmol g⁻¹), using the general methods for Fmoc-SPPS. Alloc deprotection and thioeic 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 w

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⁻¹ as stated) was swollen in dry CH₂Cl₂ for 1 h. The resin was drained, then washed with DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×). The resin was treated with 10% piperidine/DMF (2 × 3 min) and subsequently washed with DMF (5 ×), CHCl₃ (5 ×) and DMF (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 PyrN(T) (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 (5 ×), CHCl₃ (5 ×) and DMF (5 ×). A similar reaction mixture was prepared with piperidine (0.15 M) and washed with DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×). Amino acid coupling: A preactivation solution of protected amino acid (2 eq. or 4 eq. relative to resin capacity for Lys or other amino acid respectively), HBTU (1.1 eq. relative to peptide) and PyrN(T) (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 (5 ×), CHCl₃ (5 ×) and DMF (5 ×). Alloc deprotection: Upon removal of the Alloc protecting group, the resin was treated with 20% acetic anhydride/pyridine (3 × 4 min), followed by washing with DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×).

Alylsalicylcarbonyl (Alloc) deprotection: All Alloc-deprotected peptides were prepared following a modification of the method described by Kates et al.³ The resin was swollen at rt or at 15 min in CHCl₃/morpholine/acetic acid (90:5:5). Tetraakis(triphenylphosphine)palladium(II) (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 CHCl₃ (5 ×) and palladium chelating cocktail (DMF/diethylidithiocarbamic acid-3-water/triethylamine 25 mL:225 mg:250 mL). Traces of the chelating cocktail were removed via a basic wash (0.5% triethylamine in DMF, 5 ×). The resin was then washed with MeOH (5 ×), DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×). The reaction mixture was concentrated in vacuo, and the residue was stirred with 5 × 10 mL of 2 ml of DMSO. The resin was then washed with DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×). Amino acid coupling: A preactivation 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 PyrN(T) (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 (5 ×), CHCl₃ (5 ×) and DMF (5 ×). Alloc deprotection: Upon removal of the Alloc protecting group, the resin was treated with 20% acetic anhydride/pyridine (3 × 4 min), followed by washing with DMF (5 ×), CHCl₃ (5 ×) and DMF (5 ×).


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 × 10⁻⁵ M) in either 0.5% H₂O in DMSO-d₄ or 20% H₂O in DMSO-d₄, to a 2.5 × 10⁻⁵ M solution of the receptor in either 0.5% H₂O in DMSO-d₄ or 20% H₂O in DMSO-d₄. Typically, up to 9-12 equivalents of the anion were added to the solution. Both salt and anion were dried under high vacuum prior to use. ¹H NMR spectra were recorded on a Bruker Avance III 500 spectrometer at a frequency of 500.13 MHz and calibrated to the residual deutero solvent peak in DMSO-d₄ (δ = 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 squarimide NH protons or amide NH protons) using the commercially available software program HypNMR® (Hyperquad® package) enabled the calculation of association constants (Kₐ,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 30 ns molecular dynamics run 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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**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.