Reversible Hydrosulfide (HS^-) Binding using Exclusively C–H Hydrogen Bonding Interactions in Imidazolium Hosts

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Abstract

H_2S is a physiologically important signaling molecule with complex roles in biology and exists primarily as HS^- at physiological pH. Despite this anionic character, few investigations have focused on the molecular recognition and reversible binding of this important biological anion. Using a series of imidazole and imidazolium host molecules, we investigate the role of preorganization and charge on HS^- binding. Using a macrocyclic *bis*-imidazolium receptor, we demonstrate the unexpected 2:1 host:guest binding of HS^-, which was characterized both in solution and by X-ray crystallography. To the best of our knowledge, this is the first example of this binding stoichiometry for HS^- binding. Moreover, the short C–H···S distance of 2.53, 2.54, 2.76, and 2.79 Å are well within the sum of the van der Waal's radii of the interacting atoms, which is consistent with strong C–H···S interactions.

Introduction

Small reactive molecules play key roles in diverse areas of science ranging from environmental chemistry to chemical biology, and understanding both the fundamental chemistry and molecular recognition of such species poses a significant challenge. In many cases, synthetic host molecules have been used to further understand how these reactive analytes can be stabilized, recognized, and/or measured. In particular, the molecular recognition of anionic guests has made significant contributions to the stabilization and detection of environmental analytes, but the application to physiologically relevant anions remain underdeveloped. Prior work toward the recognition of biologically abundant anions has generally focused on Cl^-, Br^-, HPO_4^{2-}, SO_4^{2-}, and other relatively stable species.[1-7] These investigations have laid the foundation for further rational design of supramolecular receptors that can bind more reactive species and intermediates.[8-12]

In parallel with advancements in the molecular recognition of small anionic guests has been the expansion and recognition of small molecule signaling molecules in adjacent fields of chemical biology and pharmacology. Many of these species stem from endogenously produced signaling molecules like H_2S and related species, which have garnered significant attention over the last two decades due to their complex and intwined roles in ubiquitous biological processes.[13, 14] H_2S is established to reduce oxidative stress, promote vasodilation, and exert cardioprotection, in addition to other biological functions.[15, 16] What makes small molecules good cellular signaling agents also makes them difficult to study; specifically their propensity to react rapidly with other biomolecules or through different redox pathways.[17-21] Moreover, this high reactivity also suggests that specific molecular environments may stabilize and/or recognize these highly reactive species through specific types of interactions or recognition environments. Although generally written as its diprotic neutral form, H_2S exists primarily as hydrosulfide (HS^-) at physiological pH. Despite the prevalence of HS^- anion in biology, little is known about what fundamental motifs contribute to its molecular recognition, although such interactions undoubtedly play key roles in fundamental stabilization, reactivity, and crosstalk.

In designing molecular recognition approaches for anionic guests, supramolecular chemists often draw inspiration from how nature stabilizes reactive species. For example, electronegative hydrogen bond (H-bond) donors, such as O or N, with short and linear H-bonding interactions contribute to the defined tertiary structure of proteins and duplex structure in DNA. Analogously, supramolecular chemists incorporate the versatility of H-bonding interactions, often using abiotic H-bond donor motifs, to design different anion receptors, such as Bowman-James's polyamide cryptands,[22, 23] Flood's cyanostar,[24, 25] and Sindelar's bambusiril.[26, 27] In addition to more classical O–H and N–H H-bond donors, recent work establishes that C–H H-bond donors can contribute to strong anion binding, particularly for anions like Cl^-. Expanding to use of C–H H-bond donors, we have worked with the Johnson and Haley groups to demonstrate that the arylethynyl receptors containing a mixture of urea and C–H motifs can bind HS^- and HSe^-, and also that HS^- appears to have a particular preference for C–H donors (Figure 1a).[28-30] To the best of our knowledge, this arylethynyl receptor provides the sole example of a crystallographically characterized host-guest complex with HS^-. Additional examples of HS^- binding in anion receptors characterized in solution have primarily used C–H H-bond donors although halogen bond donors have also recently been employed.[26, 31-33] Further advancing the underappreciated role of C–H···S interactions, we recently analyzed C–H···S close contacts from the Cambridge Structural Database (CSD), which revealed that these interactions are a common yet widely-overlooked interaction in organic, inorganic, materials, and biological chemistry.[34]

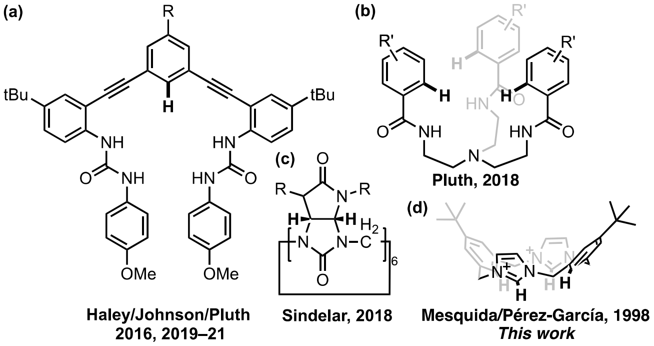

Figure 1. (a-c) Prior examples of H-bonding host molecules demonstrated to bind HS^{-} and (d) this work using exclusively C–H H-bond donors for HS^{-} recognition.

On the basis of prior work showing that anion receptors containing both N–H and C–H donors can bind HS^{-},[27,28,31] as well as prior crystallographic evidence suggesting the importance of C–H···S interactions,[34] we aimed to incorporate modular C–H H-bond donors into highly modifiable receptors. Building upon prior work on HS^{-} in the receptors described above, here we directly test the hypothesis that using strong C–H H-bond donor motifs in combination with electrostatic interactions can significantly enhance HS^{-} binding. By using a series of modifiable imidazolium host molecules, including a previously-reported *bis*-imidazolium macrocycle,[35] we demonstrate efficacious HS^{-} binding both in solution and in the solid state. To the best of our knowledge, this work provides the first example of differential HS^{-} and Cl^{-} binding stoichiometry and also provides the first examples of 2:1 host:guest binding for HS^{-} recognition.

Results and Discussion

To further advance our understanding of the molecular recognition of HS^{-} using H-bonding interactions, we chose to investigate HS^{-} using a modular class of receptors featuring solely C–H

H-bond donors. In choosing a donor motif, we focused on imidazole and imidazolium C–H H-bond donors due to the high directionality, prior incorporation into various anion receptors, and earlier work establishing binding polyatomic and halide anions in polar organic solvents.[35-38] In addition, imidazole and imidazolium-based receptors contain a highly directional, polarized and easily trackable C–H bond that can be used to investigate C–H···X$^-$ interactions. Additionally, multiple imidazole motifs can be incorporated into a single receptor to allow for direct investigations into how C–H···X$^-$ interaction multiplicity impacts guest binding. Alkylation of the imidazole nitrogen can further acidify and polarize the C–H H-bond donor, and the resultant positive charge can also favor anion binding due to Coulombic attraction. Utilizing these design principles, we prepared a small, modular series of imidazole/imidazolium receptors with different charge, directionality, and preorganization (Figure 2) and characterized each receptor by ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectroscopy.

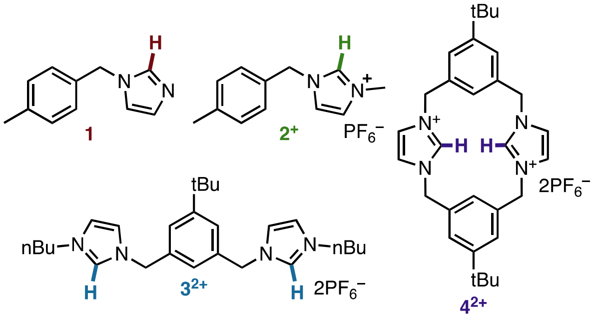

Figure 2. Imidazole/imidazolium receptors **1-4**.

We used neutral imidazole **1** as a control compound to better understand the impacts of the C–H bond polarization on guest binding. The mono-imidazolium **2**$^+$ and bis-imidazolium **3**$^{2+}$ receptors allow for investigations into how the number of cationic binding motifs influence guest binding. Additionally, the macrocycle **4**$^{2+}$ and its non-macrocyclic analogue **3**$^{2+}$ both contain two imidazolium groups, but **4**$^{2+}$ further restricts host rotation and provides a highly defined guest binding environment. For cationic receptors **2-4**, we prepared the non-coordinating PF_6^- salts to reduce potential counterion binding and to eliminate competing proton signals that could interfere with NMR resonances of interest on the host. Hosts **1** and **2**$^+$ are known compounds, but have not been used previously for anion binding, receptor **3**$^{2+}$ is a new host, and **4**$^{2+}$ has been reported previously as an anion-binding host.[35]

After preparing each receptor, we measured the binding affinity (K_a) of receptors **1**–**4** with HS^- using ^1H NMR spectroscopy (Figure 3). We chose to use HS^- as a guest based on its biological relevance and also the surprising lack of receptors for this anion. Hydrogen sulfide and hydrosulfide are highly toxic and should be handled with caution to minimize exposure. We also chose to examine the binding affinity of Cl^- as a comparison for receptor binding affinity and selectivity. We used the tetrabutylammonium (TBA^+) salts to ensure anion solubility and performed each titration in anhydrous CD_3CN at 25 °C under N_2 to avoid potential unwanted HS^- oxidation.[39] For each titration, we observed a downfield shift of the imidazolium C–H and the phenyl C–H protons upon guest addition and subsequent binding. As expected, the more polarized imidazolium C–H resonances shift farther downfield upon guest addition than the less polarized aryl C–H resonances, which is consistent with a stronger interaction between the guest anion and the imidazolium motif. In addition, the hydrosulfide ^1H NMR signal can also be observed and shifts upfield during the course of the titration, which again is consistent with reversible binding to the host receptors. The chemical shifts of the interacting C–H resonances were measured after each addition of guest, plotted against guest equivalent and fit using the Nelder-Mead method in Bindfit to measure the K_a values.[40-42]

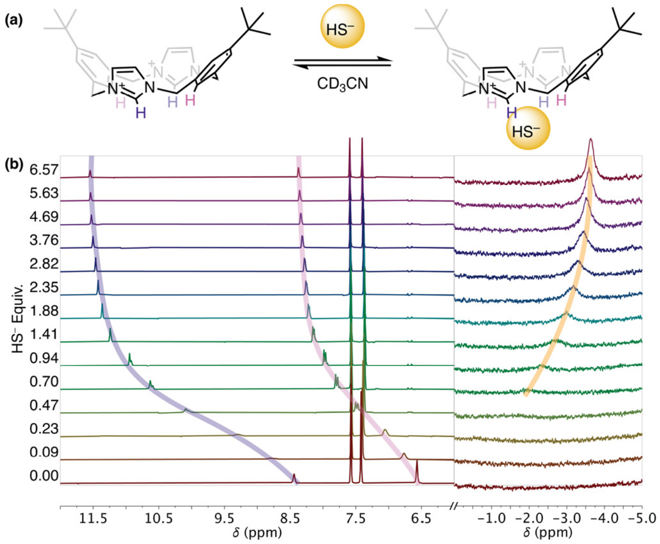

Figure 3. (a) General guest binding scheme for binding HS^- in $\mathbf{4}^{2+}$. (b) Representative ^1H NMR titration of $\mathbf{4}^{2+}$ with [TBA][SH]. General conditions: $[\mathbf{4}^{2+}]_i = 0.97$ mM, CD_3CN, 25 °C. The yellow highlighted peak at negative ppm in (b) corresponds to the HS^- signal.

The binding affinities for receptors **1-4** with HS^- are summarized in Table 1. Clear trends for HS^- guest binding were observed when comparing the K_a values for each receptor. First, no binding was observed for neutral imidazole **1**, but binding affinity increased to 60 M^{-1} upon alkylation of **1** to form cationic imidazolium receptor $\mathbf{2}^+$. This trend was maintained when an additional imidazolium motif was incorporated in receptor $\mathbf{3}^{2+}$, which resulted in an approximately two order of magnitude increase in K_a up to 1,000 M^{-1}. This increase in binding is likely due to a combination of both the increased number of C–H interactions and also the increase in Coulombic attraction from the dicationic receptor to monoanionic HS^-. We next investigated how further pre-organization of the imidazolium C–H donors would impact HS^- binding by investigating macrocycle $\mathbf{4}^{2+}$ and found significantly stronger anion binding with the sequential binding constants of 40,000 and 5,000 M^{-1}, respectively. Interestingly, the binding isotherm for $\mathbf{4}^{2+}$ with HS^- failed to fit cleanly to a 1:1 binding isotherm (Figure S28), but rather fit a 2:1 host:guest binding model (Figure S27). Further supporting this 2:1 binding mode, the Job Plot of $\mathbf{4^{2+}}$ binding HS^- shows a maximum at the mole fraction of 0.67, which is consistent with 2:1 host:guest binding (Figure S29). Notably, this apparent binding mode is different than what was observed from the binding isotherms from receptors $\mathbf{2^+}$ and $\mathbf{3^{2+}}$ that were fit to 1:1 models (Figures S15, 21, 27).

Table 1. Binding affinities for HS^- and Cl^- in receptors **1-4** in CD_3CN.

Receptor	Anion	K_a (M^{-1})[a]	ΔG (kcal/mol)
1	**HS^-**	NBO[b]	-
	Cl^-	NBO[b]	-
2^+	**HS^-**	60 ± 4	-2.4 ± 0.1
	Cl^-	63 ± 3	-2.5 ± 0.1
3^{2+}	**HS^-**	1,000 ± 90	-4.1 ± 0.1
	Cl^-	1,350 ± 75	-4.3 ± 0.1
4^{2+}	**HS^-**	40,000 ± 8,000[c] 5,000 ± 1,000[c]	-6.3 ± 0.1 -5.0 ± 0.2
	Cl^-	6,600 ± 800	-5.2 ± 0.1

[a]From fitting ^1H NMR titration data to 1:1 binding unless otherwise noted. Reported error is the standard deviation of triplicate titrations, or 5% of the K_a, whichever was greater. [b]NBO = no measurable binding observed. [c]Fit to 2:1 binding; the first value is 1:1 binding followed by the 2:1 binding event.

In an effort to further contextualize the selectivity and binding affinities of the receptors, we also measured the binding affinity of Cl^-. Both HS^- and Cl^- are similar in size, with van der Waal's radii of 2.05 and 2.06 Å for Cl and S respectively,[43]. The noted trends and K_a values that were observed for HS^- were also observed for Cl^- with the exception of receptor $\mathbf{4^{2+}}$, which exhibited unexpected binding stoichiometry of $\mathbf{4^{2+}}$ with HS^- compared to that of Cl^-. We found that Cl^- binding to $\mathbf{4^{2+}}$ fit to a 1:1 binding isotherm with a K_a value of 6,600 M^{-1} in CD_3CN. Moreover, this binding stoichiometric is further supported by the Job plot, which showed a maximum at a mole fraction of ~0.5 (Figure S32). To the best of our knowledge, this is the first observed difference in binding stoichiometry between HS^{-} and Cl^{-} in a host-guest system. In addition, the measured K_a value for Cl^{-} binding is significantly lower than the measured K_a for $\mathbf{4}^{2+}$ with HS^{-}, which further supports the potential importance of C–H···S interactions in HS^{-} recognition.

To further investigate the binding stoichiometry and strong affinity of $\mathbf{4}^{2+}$ for HS^{-}, we also investigated the solid-state binding of HS^{-} in $\mathbf{4}^{2+}$. Crystals suitable for X-ray diffraction were grown by combining $\mathbf{4}^{2+}$ and 2 equiv. of [TBA][SH] in MeCN under N_2. After mixing, the solvent was then removed under vacuum, and the resultant solid was washed with CH_2Cl_2 to remove [TBA][PF_6]. This remaining residue was redissolved in minimal MeCN, and Et_2O was diffused into the solution at room temperature in an N_2-filled drybox to yield crystals suitable for X-ray diffraction. The resultant molecular structure confirmed the 2:1 host:guest complex, with one HS^{-} bound through exclusively C–H···S interactions and three PF_6^{-} counteranions (Figure 4; CCDC# 2270610).[44] The structure crystallized in P2/c, with one PF_6^{-} on a special position. The hydrogen atom on HS^{-} was not located directly from the residual electron density, but the presence of 3 PF_6^{-} counter ions is consistent with a monoanionic HS^{-} guest. These data are also supported by the presence of an observable HS^{-} signal in the ^{1}H NMR spectrum during the titrations used to measure K_a values. To the best of our knowledge, this complex is one of two crystal structures of HS^{-} bound in a host molecule. Consistent with our hypothesis that C–H···S interactions should drive strong host-guest binding, the C–H···S interactions in the structure are all significantly shorter than the sum of the van Der Waal radii (3.00 Å) for H and S. The two imidazolium H···S distances are 2.53 and 2.54 Å with corresponding angles of 159.5 and 157.7°, respectively. The two phenyl H···S distances are 2.76 and 2.79 Å, with corresponding and nearly linear C–H···S angles of 171.6 and

174.8°, respectively. The imidazolium hydrogen atoms were refined using a riding model, but separate positional refinement of the H atoms did not significantly impact the measured distances. These short distances and near linear angles are suggestive of strong H-bonds, which supports a strong enthalpic interaction leading to the observed large K_a and ΔG measured from the solution binding experiments. Considering that the majority of reported C–H···S contacts in the CSD are longer than the sum of the van Der Waals radii and that very few contacts are found with a distance below 2.75 Å, the imidazolium C–H···S contacts observed in $(\mathbf{4}^{2+})_2{\cdot}HS^-$ are unusually short .[32] Further analyzing the shortest intermolecular C–H···S contacts reported in the CSD, the are 2.53 and 2.54 Å distances are among the shortest 0.34% of reported C–H···S distances.

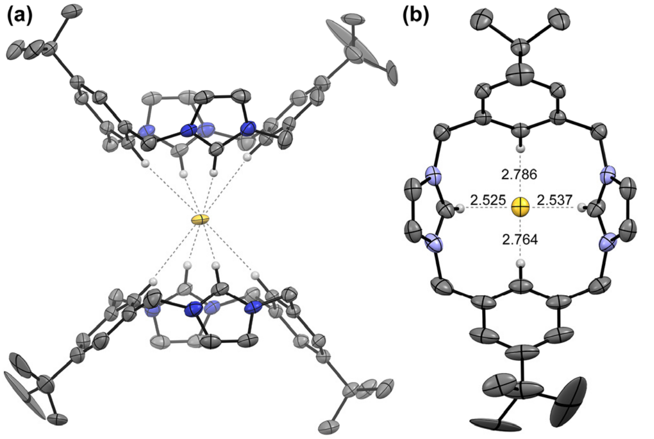

Figure 4. (a) Side on and (b) top views of the molecular structure of the $[(\mathbf{4}^{2+})_2{\cdot}HS^-][PF_6]_3$. Thermal ellipsoids are drawn at 50% probability level. Noninteracting H atoms, solvent molecules, and PF_6^- counter anions are omitted for clarity.

Furthering the comparison with Cl^-, the molecular structure of $\mathbf{4}^{2+}{\cdot}Cl^-$ was previously reported by Mesquida and Perez-García and shows solely 1:1 host:guest binding.[39] This stoichiometry from the $\mathbf{4}^{2+}{\cdot}Cl^-$ structure also matches our solution binding stoichiometry measured for Cl^- binding in $\mathbf{4}^{2+}$. Moreover, the imidazolium H···Cl distances (2.61 and 2.65 Å) and phenyl

H···Cl distances (2.77 and 3.02 Å) are mostly below the sum of the van der Waal radii for H and Cl (2.95 Å), but are significantly longer than the H···S observed in the HS^- structure. This difference suggests that electrostatic attraction and the strong C–H···S interactions may be sufficient to not only increase the binding affinity for HS^- over Cl^-, but also to overcome both the increased entropic penalty for forming a 2:1 host:guest complex and also the Coulombic repulsion of assembling two dicationic receptors in solution.

Conclusions

We demonstrated that simple imidazolium receptors can be used to directly investigate the roles of C–H H-bond donor multiplicity, cationic charge, and preorganization for binding HS^-. Surprisingly, we observed an unprecedented 2:1 binding complex with $(\mathbf{4}^{2+})_2\cdot HS^-$, in which two dicationic hosts come together to strongly bind HS^-. This stoichiometry was supported by both solution and solid-state investigations. The molecular structure of this host-guest complex is one of only two known structures of hosts binding HS^- and revealed short C–H···S H-bond interactions that drive HS^- binding. More broadly, these results contribute to the rational design of anion receptors for the stabilization of reactive sulfur species. Building from these design principles, we anticipate that the rational design of anion receptors for related and more reactive anionic small molecule bioregulators is now within reach, and can further inform on the fundamental stability, reactivity, and crosstalk of these species in in both supramolecular chemistry and biology.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures, NMR spectra, X-ray collection details and parameters, CCDC database search parameters, and representative titration data (PDF).

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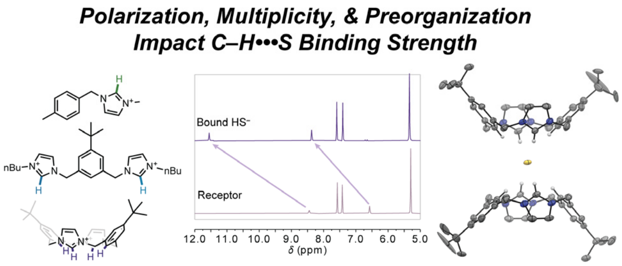

TOC Synopsis:

H_2S is a physiologically important signaling molecule with complex roles in biology. H_2S exists primarily as HS^- at physiological pH, and little is known about its fundamental molecular recognition. Here we investigate HS^- binding to a series of C–H hydrogen bonding imidazolium receptors. The macrocyclic receptor results in unexpected 2:1 host:guest binding of HS^- with short C–H···S distance well within the sum of the van der Waal's radii of the interacting atoms.