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Modular Tripodal Receptors for the Hydrosulfide (HS^-) Anion

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Hydrogen sulfide (H_2S) is an endogenously-produced gasotransmitter and is predominantly speciated as HS^- at physiological pH. Despite this importance, reversible binding of HS^- to synthetic receptors remains rare and confined to highly-engineered receptor systems. Here we demonstrate the generality of reversible HS^- binding in a family of tren-based receptors.

Anions play vital roles in biological and environmental processes,[1] and thus considerable effort has been directed toward the detection and recognition of specific anions.[2] For example, many supramolecular hosts have been developed to detect different types of anions, such as monovalent halides and multivalent phosphates and sulfates.[2] Such receptors primarily rely on non-covalent interactions to bind anionic guests. Additionally, these receptors can often be structurally or electronically tuned to target specific anion properties such as shape, basicity, and hard/soft characteristics. Despite advances in this field, only one well-characterized class of synthetic supramolecular receptor capable of binding the hydrosulfide anion (HS^-) has been reported.[3]

The hydrosulfide anion is the conjugate base of the important biological signaling molecule hydrogen sulfide (H_2S).[4-5] H_2S is involved in the regulation of cellular processes and responses in the cardiovascular, immune, and nervous systems and is one of three recognized gasotransmitters alongside carbon monoxide (CO) and nitric oxide (NO).[6-7] At physiological pH, H_2S exists primarily as HS^-, suggesting that HS^- is an important, and almost completely overlooked, biological anion.[4-5] Thus, the development and application of supramolecular HS^- receptors is poised to fill a gap in the field of anionic recognition, and also provide insights into factors influencing HS^- binding.

Our group hypothesized that supramolecular receptors capable of binding Cl^- should also be able to bind HS^- due to their similar ionic radii.[8-9] Supporting this hypothesis, HS^- has been shown to interact with Cl^- ion channels and anion-exchange proteins in biological systems.[10-13] To this end, on collaboration with the Johnson and Haley labs, we recently reported that a series of *bis*(ethynylaniline)-based Cl^- receptors could also be used to bind HS^-.[14-15] These receptors were able to reversibly bind HS^- through non-covalent hydrogen bonding interactions between the host urea N–H and aromatic C–H moieties and HS^-.[3] Despite this report, we are unaware of subsequent examples of well-characterized HS^- binding to synthetic receptors, which raised the question of the generality of HS^- binding in synthetic motifs.

To directly address this question and to demonstrate the generality of HS^- binding, we prepared a family of readily-modifiable tripodal receptors capable of binding HS^-. Based on *N*,*N*',*N*''-(nitrilotris(ethane-2,1-diyl))tribenzamide (baTren, Figure 1), which has a binding affinity for Cl^- in acetonitrile (CH_3CN) of ~100 M^{-1},[16] these receptors contain N–H and aromatic C–H moieties that were previously used in the *bis*(ethynylaniline) system to bind HS^-.[3] One benefit of the baTren system is its modularity; substituted versions of baTren can be prepared in one step by reacting different commercially available benzoyl chlorides with tris(2-aminoethyl)amine (tren) in the presence of base.[16-17]

We initially prepared baTren and measured its binding affinity for HS^- in anhydrous CD_2Cl_2 by titrating 1.0 – 2.0 mM solutions of baTren with $NBu_4(SH)$ and monitoring by ^1H NMR spectroscopy (Figure 2). Pronounced shifts in the resonances associated with the amide N–H protons and *ortho*-aromatic C–H protons (Figure 2b) were observed, indicating that these protons were involved in HS^- binding as predicted. Only minor shifts were observed in the resonances associated with the ethylene groups of the tren backbone (Figure 2c), suggesting that HS^- did not interact with the tren backbone and ruling out a strong hydrogen bonding interaction between the S–H proton

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and the lone pair of the tertiary amine nitrogen of the tren backbone.

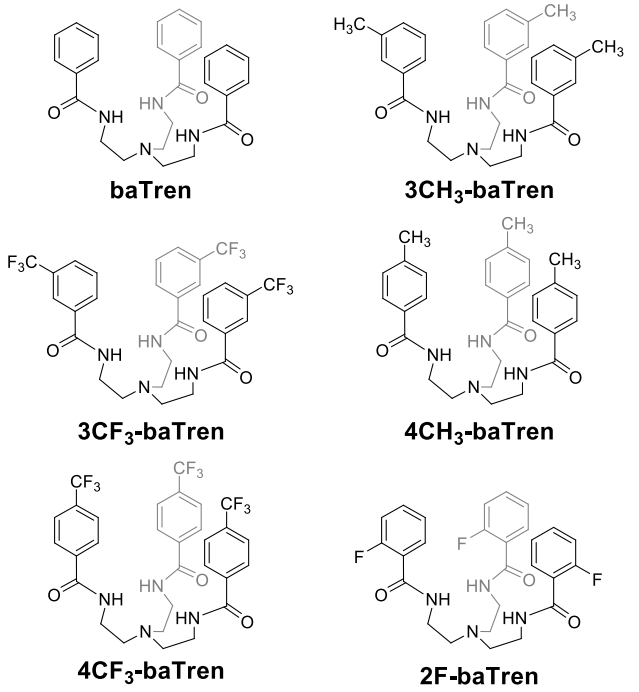

Figure 1. Tripodal receptors used in this study: baTren, $3CF_3$-baTren, $4CF_3$-baTren,[18] $3CH_3$-baTren, $4CH_3$-baTren,[19] and 2F-baTren.

Previous studies of baTren-type systems with halides suggested that the binding stoichiometry of this family of receptors should be limited to a simple 1:1 model.[17] To determine whether similar binding was present for HS^-, we constructed a Job plot for HS^- binding to baTren (Figure 2b). This plot supported a 1:1 binding stoichiometry as evidenced by the plot maximum near 0.5, which is further substantiated by direct fitting of the titration data to a 1:1 binding isotherm model.[20] Building from this binding stoichiometry, we measured the 1:1 binding constants for baTren and both HS^- and Cl^- in CD_2Cl_2 using the Thordarson method (Table 1).[21] The binding affinities for HS^- (149 ± 8 M^{-1}) and Cl^- (160 ± 20 M^{-1}) were equivalent within error, suggesting a lack of selectivity between the two anions for this receptor. These values in CD_2Cl_2 were similar to the previously reported value between baTren and Cl^- in CH_3CN;[16] we found baTren to be poorly soluble in CD_3CN and D_2O and thus conducted our experiments in CD_2Cl_2. Additionally, the NBu_4^+ counter cation did not appear to influence anion binding in this system as evidenced by the identical binding affinities obtained from titrations using $NEt_4(SH)$ to those using $NBu_4(SH)$.

To determine whether the binding affinity of HS^- could be modulated within the baTren scaffold, variants of baTren containing electron-withdrawing CF_3 substituents on the aryl rings were prepared ($3CF_3$-baTren and $4CF_3$-baTren, Figure 1). Such modifications should increase the acidity and therefore the H-bond donating ability of the amide N–H and aromatic C–H moieties, resulting in stronger interactions with anions. These modifications should also modify the intramolecular hydrogen-bonding within the receptor in the absence of the guest, thus changing the ground state stability of the receptors (Figure S2). In addition to increasing binding affinity, these modified receptors should provide insight into the relative importance of amide N–H versus aromatic C–H groups toward HS^- binding. We expected that $4CF_3$-baTren should increase the acidity of the amide N–H moieties *via* inductive effects, whereas $3CF_3$-baTren should increase the acidity of the aromatic C–H moieties oriented towards the anion.

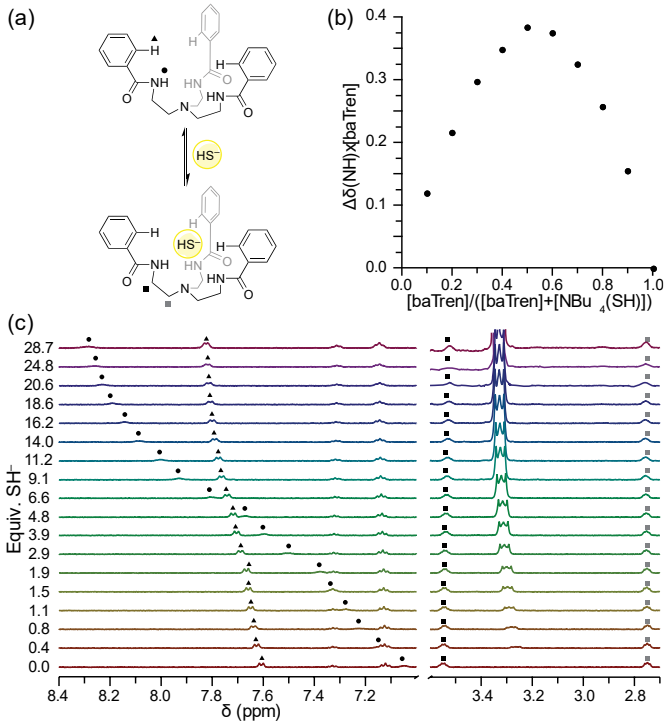

Figure 2. (a) Representation of the HS^- host-guest equilibrium with baTren, (b) representative ^1H NMR titration of 1.0 mM baTren with $NBu_4(SH)$ in anhydrous CD_2Cl_2, and (c) Job plot of baTren with $NBu_4(SH)$ in anhydrous CD_2Cl_2.

Supporting our hypothesis, the measured binding affinity of $4CF_3$-baTren (3,500 ± 300 M^{-1}) toward HS^- was approximately three times greater than that of $3CF_3$-baTren (1160 ± 90 M^{-1}) (Table 1). This result suggested that N–H groups in the baTren scaffolds are more influential in anion binding than aromatic C–H groups, a conclusion consistent with the fact that N–H bonds are more polarized than those of aromatic C–H bonds. Additionally, variable temperature ^1H NMR studies on a solution of $4CF_3$-baTren with 2 equivalents of $NBu_4(SH)$ in CD_2Cl_2 revealed that the amide N–H peak shifted downfield at –35 °C. In contrast, the *ortho*-aromatic C–H peak shifted slightly upfield and was not further resolved. Thus, decreasing the temperature increased the anionic interaction with the N–H groups but decreased the interaction with the C–H moieties as the aromatic rings continued to rotate freely despite the lowered temperature. We note, however, that the inductive effects of the CF_3 groups, as measured by Hammett parameters, are not equivalent for the *para* (σ_p = 0.54) and *meta* (σ_m = 0.43) position.[22] Also, since substituent positions are relative, the inductive effect of a given substitution cannot exclusively target either the N–H or C–H groups; both will experience inductive

effects, which complicates deriving a direct correlation between substituent position and the hydrogen bonding ability of either the N–H and C–H groups in this system. Nevertheless, the substantial difference in binding affinity between the *para* and *meta* substituted receptors supports our initial hypothesis that HS^- affinities can be modulated by electronic effects.

Table 1. HS^- binding parameters for the baTren based receptors. All values were obtained by fitting ^1H NMR spectroscopic data to 1:1 binding isotherm models, using the Thordarson method, in triplicate.[21]

Host (Guest)	K_a (M^{-1})	ΔG (kcal/mol)
baTren	149 ± 8	–2.96
baTren (Cl^-)	160 ± 20	–2.99
$3CF_3$-baTren	1160 ± 90	–4.18
$4CF_3$-baTren	3500 ± 300	–4.83
$3CH_3$-baTren	130 ± 20	–2.88
$4CH_3$-baTren	140 ± 30	–2.92

To further probe the effect of electronic modulation on HS^- binding, we prepared *para* and *meta* CH_3 substituted versions of baTren ($3CH_3$-baTren and $4CH_3$-baTren, Figure 1). These receptors, although sterically similar to the CF_3 substituted receptors, should have lower binding affinity for HS^- than even unsubstituted baTren due to their electron-donating CH_3 substituents. As expected, the binding affinities for $3CH_3$-baTren and $4CH_3$-baTren were indeed lower than those of the CF_3 substituted receptors (Table 1), but surprisingly these values were approximately equivalent to that of unsubstituted baTren. This observation suggested that the modest electron-donating characteristics of the CH_3 groups does not significantly impact the overall binding affinity. Additionally, the similar binding affinity between $3CH_3$-baTren and $4CH_3$-baTren implied that the *meta* CH_3 substituent does not sterically encumber anionic binding.

Rational receptor design can also be used to prevent anion binding in the baTren system, as demonstrated by 2F-baTren (Figure 1). By incorporating fluorine atoms in the *ortho*-positions of the receptor, we aimed to promote strong intramolecular H-bonding at the expense of intermolecular interactions. Stable six-membered rings can be formed if hydrogen bonds are formed between the amide N–H groups and the F atoms of the same arm, which we hypothesized would prevent HS^- binding. Indeed, the N···F distances of 2.713, 2.740, 2.967 Å are indicative of strong intramolecular H-bonds (Figure S2c).[23] The addition 10 equivalents of either $NBu_4(SH)$ or $NBu_4(Cl)$ to a solution of 2F-baTren in CD_2Cl_2 resulted in virtually no shifts in any ^1H NMR resonances, suggesting that 2F-baTren is not able to bind anions with appreciable affinity. These experiments again illustrate how important receptor modifications are to the binding affinity of this system.

Finally, to examine and confirm the reversibility of HS^- binding in this system, we treated a solution of $3CF_3$-baTren in 7% DMSO-d_6/CD_2Cl_2 (Figure 3b) with 2 equivalents $NBu_4(SH)$ to form the HS^- bound complex (Figure 3c). This mixed solvent system was required for the dissolution of $Zn(OAc)_2$, and the addition of 6 equivalents of $Zn(OAc)_2$ restored the spectrum to that of free $3CF_3$-baTren (Figure 3d). Further additions of $NBu_4(SH)$ could reform the HS^- complex, demonstrating the reversibility of this binding event.

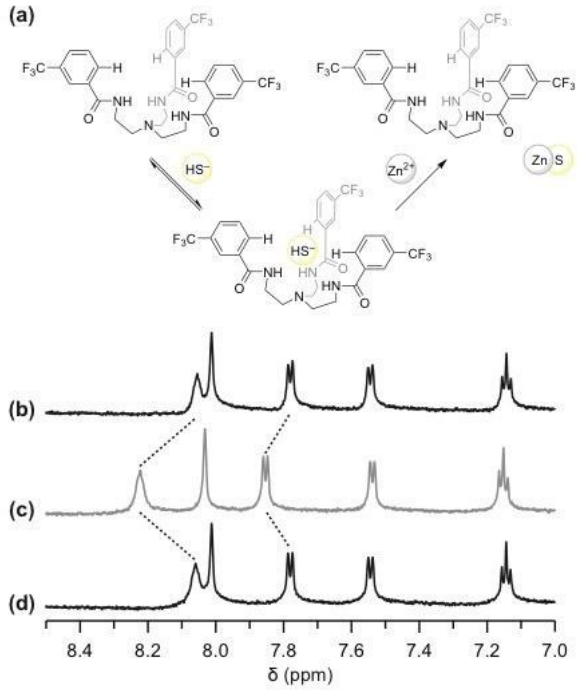

Figure 3. (a) Scheme for the $3CF_3$-baTren anion binding reversibility experiment. ^1H NMR spectrum of (b) 0.5 mM $3CF_3$-baTren in 7% DMSO-d_6/CD_2Cl_2, (c) after treatment with 2 equivalents $NBu_4(SH)$, and (d) after treatment of 6 equivalents of $Zn(OAc)_2$.

Conclusions

In conclusion, we have expanded the library of supramolecular HS^- receptors by repurposing the known tripodal Cl^- receptor baTren towards HS^- binding. Importantly, this study demonstrates the generality of using simple synthetic receptors for reversible HS^- binding. ^1H NMR titrations suggested that these receptors utilize their amide N–H and aromatic C–H groups to non-covalently bind HS^- and that the binding affinity could be tuned by electronic modulation. Additionally, the observation that the HS^- binding affinity of $4CF_3$-baTren was substantially higher than that of $3CF_3$-baTren suggested that in these simple systems, the amide N–H moieties may be more influential to HS^- binding than the aromatic C–H groups. Moreover, the ability of these simple receptors, which only use amide N–H and aromatic C–H bonds to bind HS^-, points to the generality of HS^- binding in molecular receptors.

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Conflicts of interest

There are no conflicts to declare.

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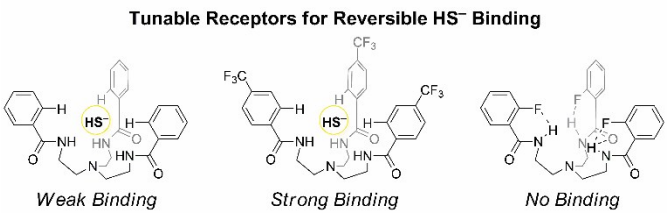

3-fold symmetric receptors with N-H and C-H bond donors enable reversible HS^- binding.