DEVELOPMENT OF A SYNTHETIC SUPRAMOLECULAR RECEPTOR FOR THE HYDROSULFIDE ANION

by

SAMUEL S. PRAKEL

A THESIS

Presented to the Department of Chemistry and Biochemistry
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

March 2018

An Abstract of the Thesis of

Samuel S. Prakel for the degree of Bachelor of Science
in the Department of Chemistry and Biochemistry to be taken March 2018

Title: Development of a Synthetic Supramolecular Receptor for the Hydrosulfide Anion

Approved: ____

Dr. Michael D. Pluth

Hydrogen sulfide (H_2S) has recently emerged as an important biomolecule in cellular signaling. As its significance in various physiological processes in the cardiovascular, digestive, nervous, and immune systems is still being uncovered, reversible detection techniques for H_2S and its predominant form, hydrosulfide (HS^-), are necessary to properly research the binding and activity of this gasotransmitter. This thesis reports the first synthetic receptor capable of reversibly binding HS^-. In addition to hydrogen bonding from urea NH groups to HS^-, this receptor also uses a notable aromatic CH---S hydrogen bond to achieve binding constants of up to 90,300 ± 8700 M^{-1} in acetonitrile. This fundamental study should pioneer work toward developing new, synthetic HS^- receptors and increase the understanding of biological HS^- receptors and synthetic H_2S recognition techniques.

Acknowledgements

I would first like to thank Professor Michael Pluth for helping me fully explore and examine the field of hydrogen sulfide research. By opening the doors of his lab to me, Prof. Pluth sent me on a journey of learning and discovery that challenged me and helped me grow in my skills and knowledge in chemistry. There were also many other important key mentors along the way. I would like to start with Dr. Matthew Hartle who first took me under his wing in the Pluth lab and taught me the basics needed to perform the experiments in this thesis and many other skills dealing with chemistry and science. Dr. Hartle showed great patience in my training and was an incredible teacher and scientist to look up to. I would like to also thank all the other graduate students and post-docs who spent time in the Pluth lab during my thesis work. Everyone's friendliness and helpfulness made it an enjoyable and easy place to work.

Additionally, two other professors were instrumental in my thesis: Prof. Michael Haley and Prof. Darren Johnson. These two showed the innovations in anion detection that were needed to work on my thesis and opened up their labs to me so I could gain more experience in the realm of supramolecular chemistry. During this time in their labs, Ryan Hansen mentored me in synthesis and other skills in chemistry, which I am forever grateful. Ryan Hansen also played a very important role in assisting my thesis as he performed various experiments to make this study complete and initiated a lot of the work before Dr. Hartle and I joined his mission in hydrosulfide binding. I would like to also thank the NSF for supporting this research through grant number CHE-1454747.

I am also very thankful for Dr. Nathanael Lau as he served on my thesis committee and mentored me in the Pluth lab my last few terms. Nathan's guidance in the research I performed after finishing work on this thesis was top-notch and his patience in helping me learn new things was much appreciated. Dr. Casey Shoop also served on my thesis committee, as my Clark Honors College advisor, and I want to thank him for his assistance ever since freshman year when I had him my first term in a CHC Literature course. Dr. Shoop offered advice along the way and was a great resource my five years of college. I would also like to thank my cross country and track coach, Andy Powell, for allowing me to not only balance my thesis, school, and running, but also succeed in all three.

Finally, I would like to thank my parents, Mike and Christy Prakel, for their unwavering support during this process. Mom and Dad, you helped me through the struggles of balancing this project, school, and running. Thanks for listening and for all your advice and trust as I worked through this experience. I would like to also thank my sisters: Mary, Margaret, Caroline, and Lucy for their support and advice and for all the traveling you, Mom, and Dad did when coming to visit me. Thank you to everyone for making this experience so rewarding and worthwhile.

Table of Contents

List of Figures vi
List of Tables vii
Background 1
- Hydrogen Sulfide 1
- HS^- Binding in the Literature 2
  - Reversible Receptors 2
- Supramolecular Chemistry 3
- A New Method for H_2S Detection 7

Methods and Materials 8
- Methods 8
  - Synthesis of the HS^- Receptor 8
  - Modulating the Receptor 10
  - UV-vis Titrations 10
  - ^1H NMR Titrations 12
  - X-Ray Crystallography 13
- Materials 13
  - Spectrometers 13
  - Chemicals and Glassware 13

Experimental Results and Discussion 14
- Receptor Selectivity 14
- Receptor Reversibility 18
- Crystal Structure 18
- Conclusions 20

Future Directions 21
Supporting Information 23
- UV-Vis Titrations 23

Glossary 35
References 37

List of Figures

Figure 1. a) Bacterial HS^- protein channel b) Amino acid residues hydrogen bonding to hydrosulfide anion 3

Figure 2. Supramolecular receptor non-covalently binding the HS^- anion 5

Figure 3. Derivatives of the HS^- receptor 6

Figure 4. The different structures in the receptor: red) receptor core, blue) ethynylaniline groups, green) phenylisocyanate groups 9

Figure 5. Synthetic scheme for the supramolecular receptor 9

Figure 6. UV/vis spectrometer 11

Figure 7. UV/vis spectrum and binding curve of a titration with receptor **2** and HS^- 15

Figure 8. UV/vis difference plot of an HS^- titration with receptor **2** 15

Figure 9. ^{1}H NMR spectra showing the reversible binding as $Zn(OCOCH_3)_2$ is added to the solution. 19

Figure 10. Crystal structure of receptor **1** binding HS^-. Carbon is black, oxygen is red, nitrogen is blue, hydrogen is white, and sulfur is yellow. 19

Figure 11. Other recently developed receptors for non-covalent HS^- binding 21

Figure S1. UV-Vis titration of **1** with HS^- 24

Figure S2. UV-Vis titration of **1** with Cl^- 26

Figure S3. UV-Vis titration of **2** with HS^- 28

Figure S4. UV-Vis titration of **2** with Cl^- 30

Figure S5. UV-Vis titration of **3** with HS^- 32

Figure S6. UV-Vis titration of **3** with Cl^- 34

List of Tables

Table 1. log(K_a) for chloride and hydrosulfide for all three receptors 17
Table S1. Titration of **1** with HS^- 23
Table S2. Titration of **1** with Cl^-.. 25
Table S3. Titration of **2** with HS^-. 27
Table S4. Titration of **2** with Cl^-.. 29
Table S5. Titration of **3** with HS^-. 31
Table S6. Titration of **3** with Cl^-.. 33

Background

Hydrogen Sulfide

Hydrogen sulfide (H_2S) is notorious for being a foul-smelling and toxic gas, as it is known to cause the odor of rotten eggs and is lethal to humans between 500 and 1,000 ppm.[1] This colorless gas can be found in volcanic gases, septic tanks, sewers, and water treatment plants, and is produced when certain bacteria break down matter during anaerobic respiration.[2] H_2S can also be harmful at lower levels, causing respiratory issues, eye irritation, and loss of smell at just 150 ppm.[3]

Despite its toxic reputation, H_2S has been shown to be vital to human life. H_2S is produced in the human body by three main enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST). H_2S was originally believed to be insignificant metabolic waste, but it is now recognized as one of the three endogenously produced gasotransmitters alongside carbon monoxide (CO) and nitric oxide (NO).[4] A gasotransmitter is a small, dissolved gas molecule that is freely permeable to membranes and has well defined signaling functions in various physiological pathways.[5]

H_2S features prominently in the signaling pathways associated with the cardiovascular, immune, gastrointestinal, and nervous systems. For example, H_2S is known to be a crucial vasodilator and is thus important in the prevention of

---

[1]National Research Council (US) Committee on Acute Exposure Guideline Levels. Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9. Washington (DC): National Academies Press (US); **2010**, *4*, Hydrogen Sulfide Acute Exposure Guideline Levels.
[2] Wang, R. *Physiol Rev.* **2012**, *92*, 791-896.
[3] National Research Council. Acute Exposure Guidelines: Volume 9. Washington (DC): National Academies Press (US); **2010**, *4*, Hydrogen Sulfide.
[4] Wang, R. *Physiol Rev.* **2012**, *92*, 791-896.
[5] Wang, R. *Trends Biochem Sci* **2014**, *39*, 227.

hypertension.[6] Other studies have investigated the effect of H_2S levels in mice, and shown that such modulations influence inflammation, diabetes, and neurological function.[7] What makes H_2S different from the other gasotransmitters, however, is its ability to lose a proton, changing its protonation state. At the physiological pH of 7.4, H_2S exists mainly as the anionic form known as hydrosulfide (HS^-). Since the pKa of H_2S is 7.0, about 70% of H_2S is present as HS^- at physiological pH. Little is known about how HS^- binds and interacts in physiological pathways. Thus, the binding of HS^- needs to be better understood. This could help discover protein targets for HS^- and develop ways to detect HS^-, advancing what is known about this important biomolecule and how it acts in nature.

HS^- Binding in the Literature

Reversible Receptors

In 2012, Czyzewski and Wang discovered an ion channel for HS^- transport in bacteria (Figure 1).[8] This ion channel (PDB: 3TDX) works by non-covalently interacting with HS^- in a reversible manner. Certain amino acid residues use hydrogen bonding to interact with the HS^- anion to regulate the function of the channel. This protein is a biological analog to the synthetic reversible hydrosulfide receptors developed in this study, and emulating similar interactions could provide insight into the mechanism of protein function.

[6] Wang, R. *Physiol Rev.* **2012**, *92*, 791-896.
[7] Wang, R. *Physiol Rev.* **2012**, *92*, 791-896.
[8] Czyzewski, B., Wang, D. *Nature* **2012**, *483*, 494–497.

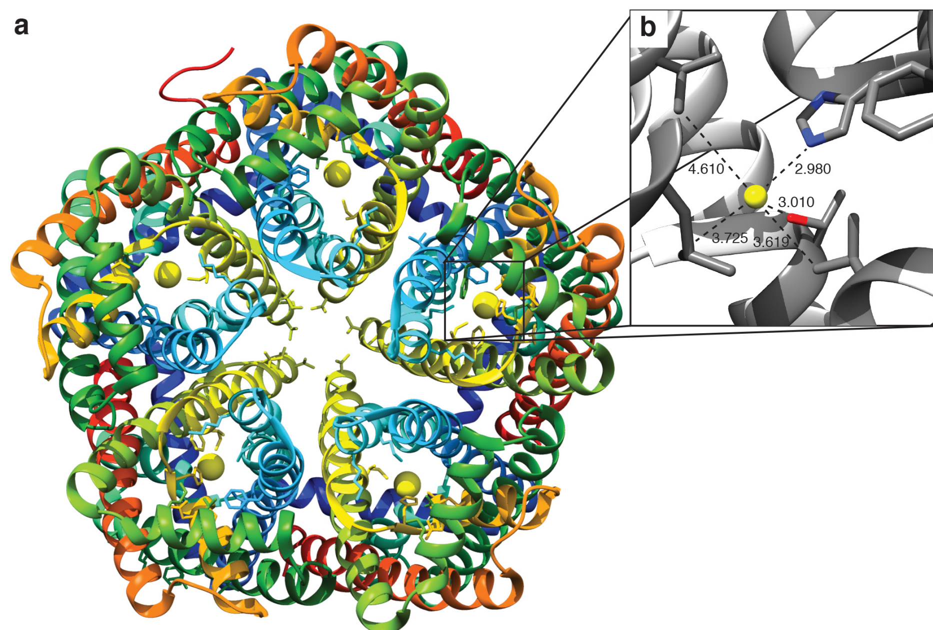

Figure 1. a) Bacterial HS^- protein channel b) Amino acid residues hydrogen bonding to hydrosulfide anion

Another study by Jennings demonstrating the non-covalent binding of HS^- in biological systems shows HS^- to be a viable substrate for Cl^-/HCO_3^- anion-exchange proteins.[9] This work describes the transport of HS^- across human red blood cell membranes, a process that also relies on non-covalent, reversible interactions. These biological examples of HS^- binding suggest that HS^- needs to be included when thinking about the complex landscape of biologically relevant anions.

Supramolecular Chemistry

One field of study that offers new tools toward studying HS^- binding is supramolecular chemistry. Whereas traditional chemistry tends to focus on covalent

[9] Jennings, M. *Am. J. Physiol. Cell Physiol.* **2013**, *305*, C941–C950.

bonding, supramolecular chemistry instead focuses on non-covalent bonding. Practically, traditional approaches toward visualizing HS^- in solution usually require an irreversible reaction with the HS^- probe where the new species formed has altered physical properties that can be contrasted to the parent probe. This is problematic when trying to study biological processes that use HS^-, as the act of detecting the anion will alter its concentration. In contrast, since supramolecular chemistry utilizes weaker, non-covalent interactions such as hydrogen bonding, probes based on this chemistry should avoid covalent modification in favor of allowing reversible interactions when binding HS^-. This should more closely mimic the interactions occurring in nature

Supramolecular receptors have previously been developed for a variety of different anions. Such compounds are generally large molecules that contain many substructures that can interact with anions with moieties such as NH groups or aromatic CH groups. In this thesis, the HS^- anion is encapsulated by the receptor by weak, non-covalent, hydrogen bonds that are represented by the dashed lines in Figure 2. This binding event produces a change in the receptor that is observable by techniques such as nuclear magnetic resonance (NMR) and UV-visible (UV-vis) spectroscopies. This receptor, unlike other probes, is not permanently altered and the reversible interaction with HS^- allows the original form of the receptor to be restored.

When deciding how to design an initial synthetic HS^- receptor, synthetic receptors that had been made for other anions offered viable options. The Haley and Johnson labs at the University of Oregon developed a bis(ethynylaniline) receptor, a

supramolecular receptor for the chloride anion (Cl^-).[10] This class of receptors can bind Cl^- and elicit a fluorescent response. As Cl^- has a similar ionic radius to HS^-,[11] we hypothesized that this type of receptor could potentially be repurposed to study the binding of HS^-.

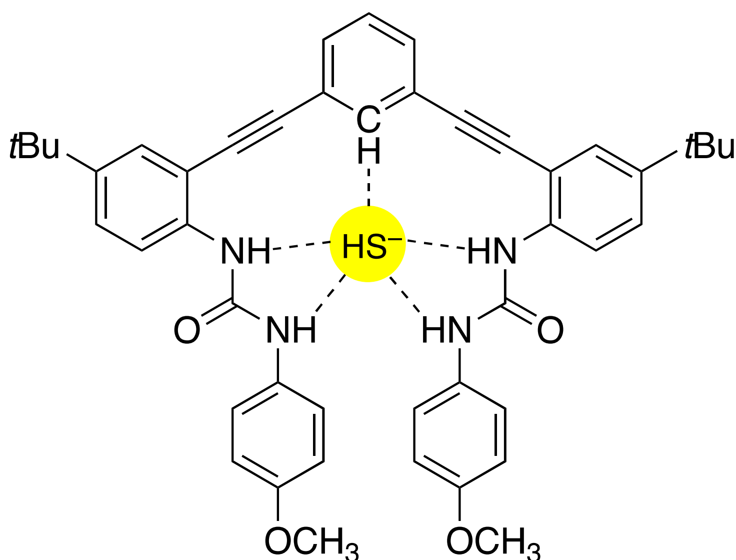

Figure 2. Supramolecular receptor non-covalently binding the HS^- anion

In general, these receptors are designed to have a binding pocket in the center where the HS^- hydrogen bonds with the receptor. Another way of thinking of it is imagining the receptor as a baseball glove and HS^- the baseball. The glove can surround the baseball to detect it but can then release the baseball under certain conditions. The challenge is making the binding pocket specific for just HS^- and not other molecules

[10] Carroll, C.; Coombs, B.; McClintock, S.; Johnson II, C.; Berryman, O.; Johnson, D.; Haley, M. *Chem. Commun*. **2011**, *47*, 5539–5541.
[11] Hartle, M.D., Hansen, R.J., Tresca, B.W., Prakel, S.S., Zakharov, L.N., Haley, M. M., Pluth, M.D., Johnson, D.W. *Angew. Chem. Int. Ed.* **2016**, *55*, 11480 –11484.

that may be present in the solution. This can be accomplished by altering the different substructures, or functional groups, that make up the receptor (Figure 3).

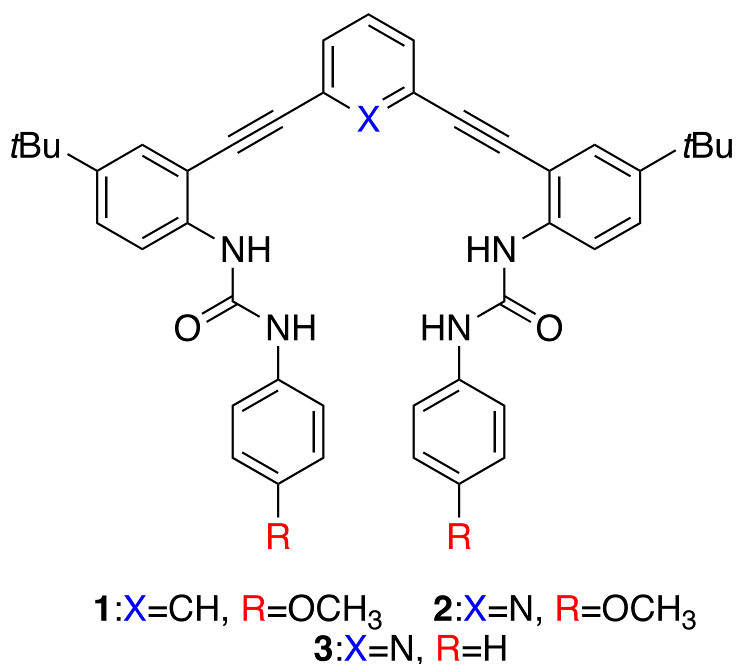

Figure 3. Derivatives of the HS^- receptor

By modulating the functional groups in the receptor, different binding environments can be tailored to promote HS^- selectivity. Selective HS^- receptors would not only provide significant insights into potential HS^- binding environments in biological contexts but also foster new strategies for developing reversible and real-time HS^- detection methods.

A New Method for H_2S Detection

The increasing biological relevance of H_2S as a gasotransmitter present in various pathways as HS^- has resulted in a need for better methods for detecting these species. The irreversible H_2S probes are impractical for understanding biological processes since they permanently react with H_2S and disturb its concentration. A reversible receptor would solve this issue and the process of developing it could also provide insights into how certain channels and proteins interact with HS^- in biology. The reversible synthetic receptor described in this thesis is the first of its kind in what it binds and how it binds. By non-covalently binding HS^-, these receptors provide a new paradigm of H_2S detection and uncover how this anion might be interacting in nature.

Methods and Materials

Methods

Synthesis of the HS^- Receptor

The preparation of receptors **1**, **2**, and **3** (Figure 3) was carried out by Ryan Hansen of the Johnson lab at the University of Oregon (UO). The same general bis(ethynylaniline) structure was the basis for all receptors synthesized in this study. As shown in Figure 4, these receptors contain an aromatic core (in red) with two symmetric ethynyl arms (in blue) in the *ortho* positions. These arms support two aromatic urea moieties (in green), which complete the binding pocket. The receptor can be modified at several positions, as denoted by the 'X' and 'R' groups.

Each synthesis of the receptors followed the reaction scheme seen in Figure 5.[12] In the first step, tert-butyl aniline is iodinated in the 2 position, followed by a Sonogashira cross-coupling reaction to produce the ethynylaniline. A further Sonogashira cross-coupling reaction incorporates these arms to the core. The attack of the aniline moieties on phenylisocyanates yields the complete receptor.

[12] Hartle, M.D., Hansen, R.J., Tresca, B.W., Prakel, S.S., Zakharov, L.N., Haley, M. M., Pluth, M.D., Johnson, D.W. *Angew. Chem. Int. Ed.* **2016**, *55*, 11480 –11484.

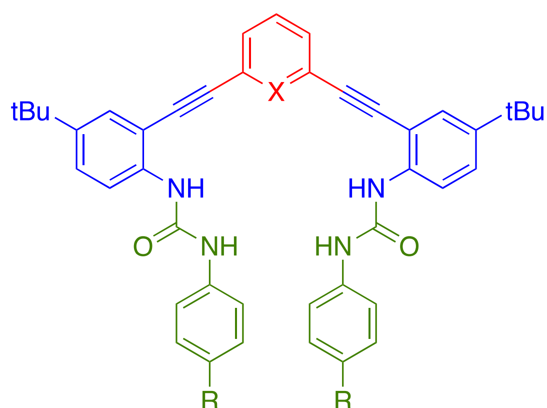

Figure 4. The different structures in the receptor: red) receptor core, blue) ethynylaniline groups, green) phenylisocyanate groups

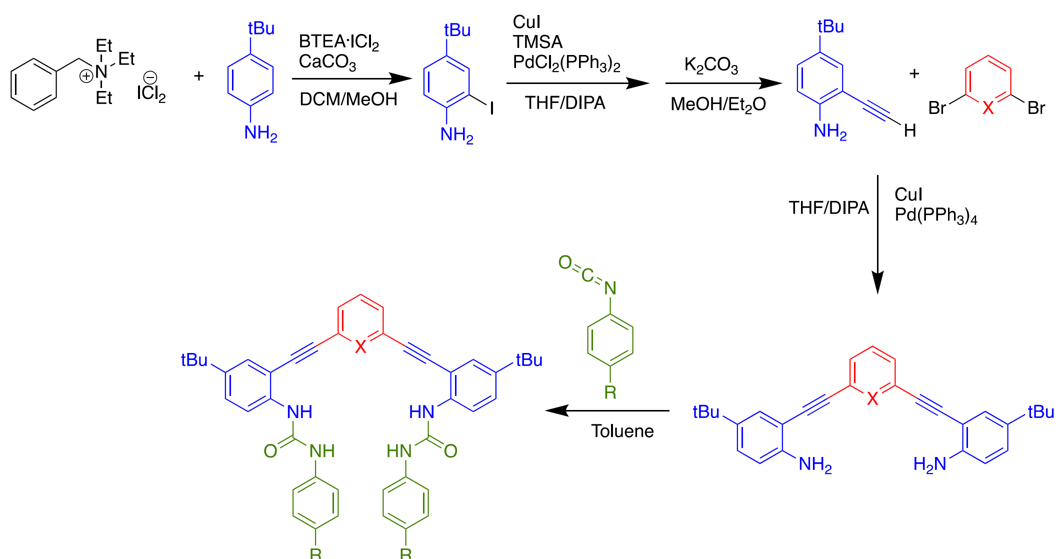

Figure 5. Synthetic scheme for the supramolecular receptor

Modulating the Receptor

As previously stated, this receptor could be modified in several positions. In this study, two of these positions were targeted with the aim of increasing HS^- binding selectivity. Figure 3 in the introduction displayed the different derivatives of the receptor and showed the 'X' and 'R' points that were modulated to yield a family of three receptors. By altering the 'X' position to be either a nitrogen atom or a carbon-hydrogen bond, we find that the additional hydrogen bond donor in that position increases the HS^- binding ability of the receptor. Furthermore, modifying the 'R' group to be either a hydrogen atom or a more electron-donating methoxy group tunes the strength of the hydrogen bonding interactions of the urea N-H groups to the HS^- anion.

UV-vis Titrations

One method for detecting and measuring the binding of a receptor and the HS^- anion is monitoring titrations by UV-vis spectroscopy. UV-vis titrations observe a change in the light absorption of the receptor molecule as HS^- is titrated into the solution. A UV-vis spectrometer emits light waves both in the visible and ultraviolet parts of the spectrum. These various wavelengths of light then individually pass through a sample solution containing the receptor, and the spectrometer records the wavelengths of light that the receptor absorbs and the amount of absorption at those wavelengths (Figure 6).

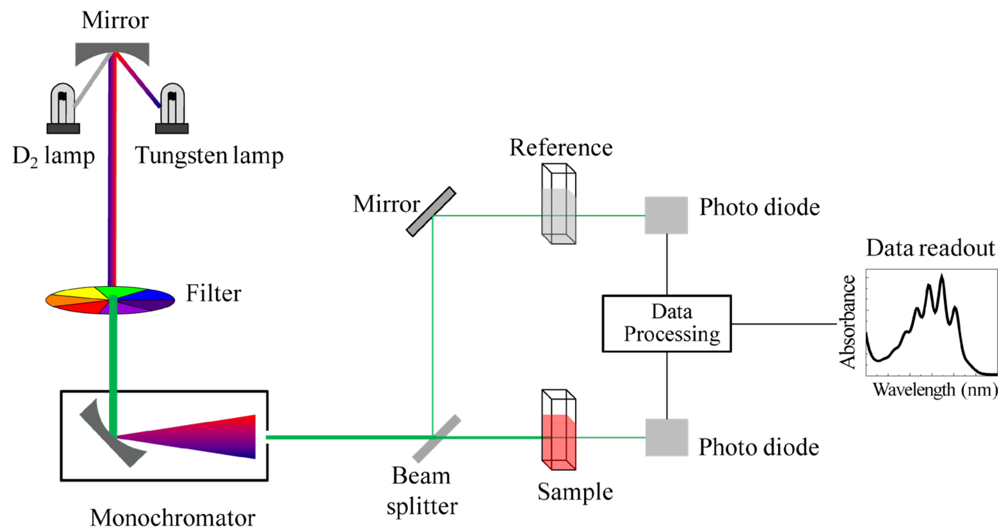

Figure 6. Schematic depicting the operation of a UV/vis spectrometer

As HS^- is slowly titrated into a solution with the receptor, the absorption at specific wavelengths changes, and this can be tracked by collecting a UV/vis spectrum after each addition. This change in absorption happens because the receptor binding the anion alters the electronic environment of the whole molecule, thus changing the absorption at specific wavelengths. The concentrations of both the receptor and the anion are used with the change in absorption to calculate an association constant (K_a). These association constants are obtained by fitting the titration data to a 1:1 receptor :HS^- binding model using a method developed by the Thordarson research group.[13] Fitting the data to a 1:1 binding equation means that we are hypothesizing that one receptor is binding one HS^- molecule and not any other combination of receptors and anions are interacting.

[13] Thordarson, P. Determining Association Constants from Titration Experiments in Supramolecular Chemistry. *Chem. Soc. Rev.* **2011**, *40* (3), 1305–1323.

^{1}H NMR Titrations

Another method for detecting and measuring the binding of a receptor and the HS^{-} anion uses NMR spectroscopy. Ryan Hansen performed the ^{1}H NMR titrations for these specific receptors. NMR stands for nuclear magnetic resonance, and this type of spectroscopy reveals the structures of molecules by measuring how specific atoms in molecules respond to a magnetic field. ^{1}H NMR spectroscopy produces a spectrum that describes the environment of every hydrogen atom in a molecule. This method is not only useful for determining the structure of molecules, but also observing changes in the electronic environment around the molecule.

When taking an ^{1}H NMR of a receptor, a spectrum of the hydrogen atoms in that receptor is produced. As soon as HS^{-} anions enter the solution and bind to the receptor, the environment around the hydrogen atoms in the receptor involved in the binding of HS^{-} will change and a slightly different spectrum will be produced. This is the basis around ^{1}H NMR titrations. HS^{-} anions are slowly titrated into a solution with the receptor and the change in the receptor is measured using an NMR spectrometer. The concentrations of the receptor and the HS^{-} anion in the solution and the change in the spectra are used to calculate the K_a.

^{1}H NMR spectroscopy is also used in measuring the reversibility of the interaction between the receptor and HS^{-}. Four equivalents of zinc acetate, $Zn(OCOCH_3)_2$, were added to the solution of the receptor and HS^{-} to precipitate out ZnS and restore the original NMR spectrum of the receptor. Ryan Hansen also performed this experiment.

X-Ray Crystallography

To further confirm that the binding interaction was 1:1, Ryan Hansen grew X-ray diffraction quality crystals of the receptor/HS^- adduct. These crystals were obtained by dissolving an equimolar amount of the receptor and HS^- in a solution of tetrahydrofuran (THF) and then layering *n*-hexane on top of this solution.

Materials

Spectrometers

^{1}H NMR titrations were carried out on an Inova 500 MHz spectrometer. UV-vis titrations were carried out on a Cary 100 spectrophotometer in the Pluth lab.

Chemicals and Glassware

NBu_4SH was synthesized by Matt Hartle and used as a source of HS^- for the titration experiments.[14] This was important because NBu_4SH is soluble in the organic solvents used for these experiments. The receptors made by Ryan Hansen were dissolved in acetonitrile, and the UV-vis titrations were done in 100% acetonitrile in an air-free environment. Acetonitrile was degassed by sparging with argon followed by passage through a Pure Process Technologies solvent purification system to remove water and stored over 4Å molecular sieves in an Innovative Atmospheres N_2-filled glove box. Titrations were prepared in the same glove box, and septum-sealed cuvettes were used to perform the UV-vis experiments. 10 µL Hamilton syringes were used to administer the small amounts of NBu_4SH into the cuvettes.

[14] Hartle, Matthew; Meininger, Daniel; Zakharov, Lev; Tonzetich, Zachary; Pluth, Michael. *Dalton Transactions* **2015**, *44*.

Experimental Results and Discussion

Receptor Selectivity

^{1}H NMR and UV-vis titrations were all repeated in triplicate to produce consistent binding data. As previously mentioned, Ryan Hansen performed the ^{1}H NMR titrations, and I performed the UV-vis titrations. Consistent association constants were achieved in both methods; therefore, I will only refer to and discuss the results from the UV-vis titrations for this section.

During the UV-vis titrations, scans were taken after each addition of HS^-, producing spectra, of which Figure 7 is a characteristic example. The black line is the initial scan representing just the receptor, or host. The red line is the last scan in the titration representing the receptor bound to HS^-, also known as the host-guest complex where HS^- is the guest. The gray lines between the black and red lines monitor the process of the titration and show the changes in absorbance as HS^- is added to the solution. The inset of Figure 7 plots the change of the wavelength of maximum absorbance as a function of concentration of HS^-. This curve is then fitted using the aforementioned Thordarson method to yield an association constant. The red dots represent the residuals, and a good experiment should have a random scatter of small residuals.

The change in absorbance at each wavelength can be plotted into what is known as a difference plot. Figure 8 displays a difference plot for the titration in Figure 7. The change in absorbance is more clearly represented and creates a binding curve at an accurate wavelength. The binding curve in Figure 8 shows the change in absorbance

at 333 nm, so a decreasing absorbance is observed. This can be compared to the binding curve in Figure 7, which tracks the absorbance at 360 nm and shows an increase in absorbance. Both plots represent receptor **2** binding HS^{-}. When calculating the K_a four different wavelengths are used as seen in Table S1.

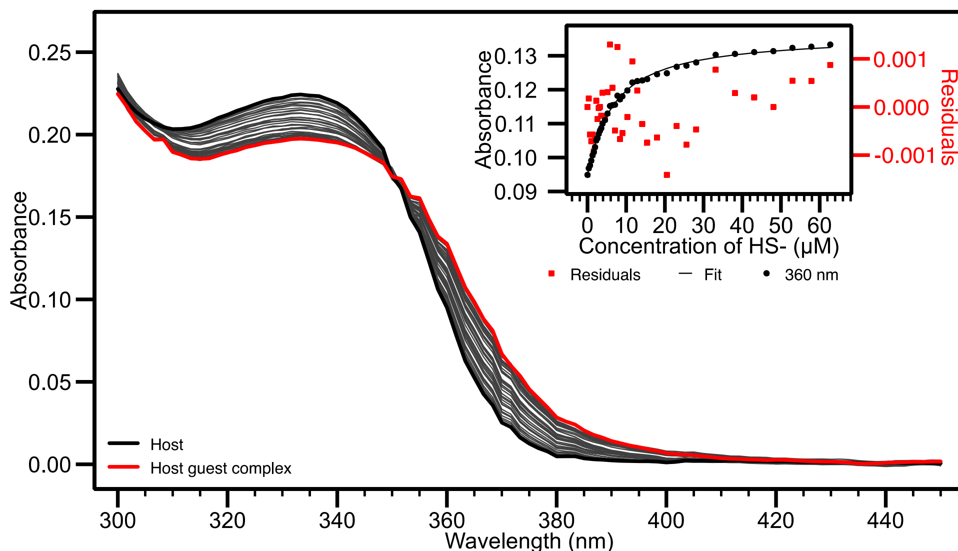

Figure 7. UV/vis spectrum and binding curve of a titration with receptor **2** and HS^{-}

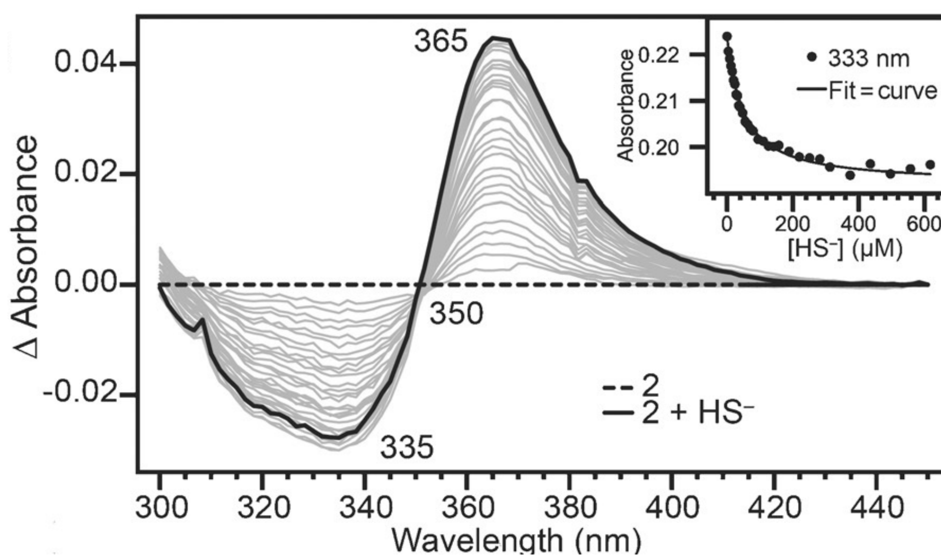

Figure 8. UV/vis difference plot of an HS^{-} titration with receptor **2**

Table 1 contains the UV-vis association constants for the three receptors that were first presented in Figure 3. K_a values were obtained for both Cl^- and HS^- in order determine any increased selectivity toward HS^-, meaning they bound HS^- stronger than Cl^-. All UV-vis titrations were done in 100% acetonitrile solvent in an air-free environment. The K_a's are reported as $\log(K_a)$ to stay consistent with the formatting in the literature. The units of K_a values for 1:1 binding systems is M^{-1}.

As seen in the table, each receptor is more selective for HS^- over Cl^-. Receptor **1** is the least selective, and receptors **2** and **3** show the most selectivity with differences in $\log(K_a)$ of 1.11 and 1.37, respectively. This means that these two receptors bind to HS^- over a whole order of magnitude more strongly than they bind to Cl^-. Even though receptor **1** has the highest K_a for HS^-, it is intriguing because it has the least selectivity toward HS^- when compared to receptors **2** and **3**.

There are two areas to look at to determine what is causing this increased selectivity in receptors **2** and **3**: the 'X' and the 'R' groups on the receptor. Since the 'X' group is so directly involved in the binding pocket, it is the best place to perform analysis on this selectivity. Receptors **2** and **3** both have a pyridine core, meaning they have a nitrogen atom in the 'X' position. This pyridine core assists in the selectivity because the nitrogen can act as a hydrogen bond acceptor for the hydrogen in the HS^- anion. The Cl^- anion does not have a hydrogen atom to donate, so the electronegative nitrogen atom creates an unfavorable interaction. Therefore, receptors **2** and **3** see a much lower K_a for Cl^- when compared to HS^-.

Host	Guest	Log(K_a)
1	Cl^-	4.53±0.07
	HS^-	4.96±0.04
2	Cl^-	3.19±0.07
	HS^-	4.30±0.07
3	Cl^-	3.08±0.06
	HS^-	4.45±0.07

Table 1. log(K_a) for chloride and hydrosulfide for all three receptors

The 'X' group in receptor **1** is a carbon atom bonded to a hydrogen atom, giving the receptor what is called a phenyl core. As seen in the table, this phenyl core produces the highest binding constants for both Cl^- and HS^-. This is likely due to the ability of the phenyl carbon to donate a slightly positive hydrogen atom when binding the negatively charged anions. Since this interaction produces the highest K_a's, it must be crucial in establishing high binding constants for both anions.

While the phenyl core produces the highest binding constants for both anions, the pyridine cores produce the highest selectivity for HS^-. This is contrary to an initial hypothesis that the pyridine core would also produce the highest binding constants. This was based on the knowledge that the pyridine receptors had the hydrogen bond accepting nitrogen atom in the core that was thought to might stabilize the interaction with HS^- more than the hydrogen bond donating phenyl core. This hypothesis was wrong as the phenyl core proved to be more important in achieving high K_a's while the pyridine core played a bigger role in the selectivity between HS^- and Cl^-.

Receptor Reversibility

Ryan Hansen provided data that confirmed the reversibility of these receptors. After taking an initial NMR of just the receptor, he added HS^- to generate the host-guest adduct. The NMR of the host-guest complex showed a shift in peaks as seen in Figure 9. The addition of zinc acetate restored the original receptor peaks, showing the interaction does not covalently modify the receptor and is thus reversible.

Crystal Structure

Figure 10 shows the crystal structure obtained by Ryan Hansen confirming the binding and orientation of the receptor-HS^- complex. This was obtained through X-ray crystallography, a method that reveals a snapshot of the host-guest complex in solution. The five hydrogen bonds from receptor **1** are represented with the dotted lines in Figure 10. The bond lengths involved in binding HS^- are very similar to the same receptors binding Cl^-. This suggests that other Cl^- binding motifs have potential to bind HS^-.

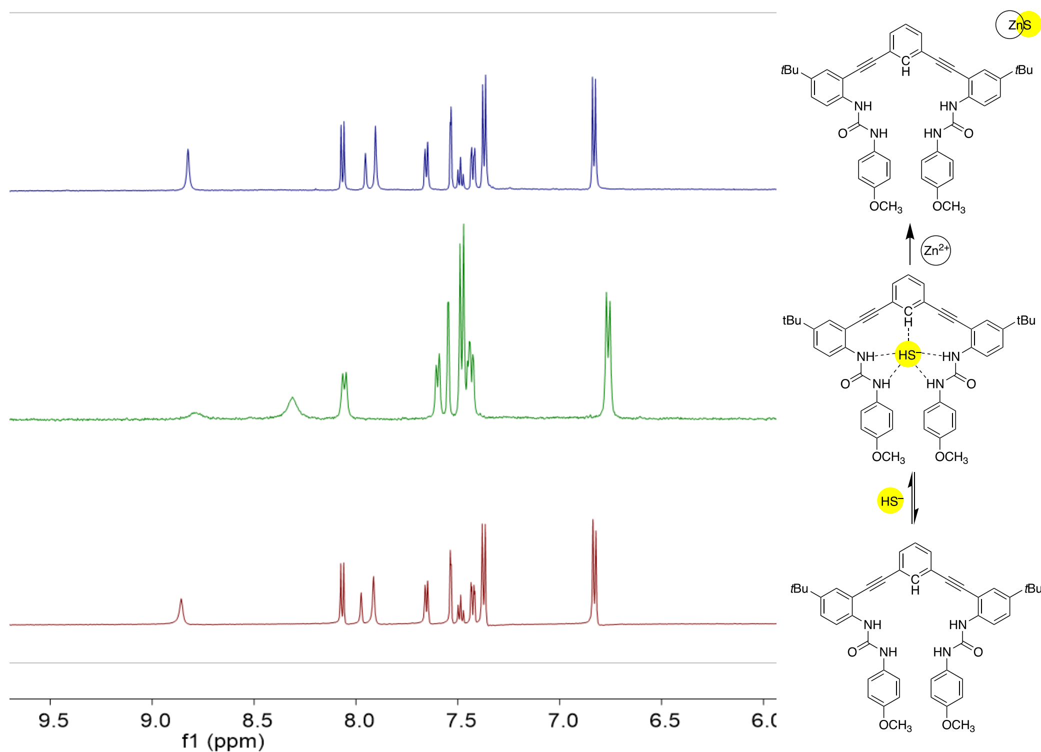

Figure 9. ^{1}H NMR spectra showing the reversible binding as $Zn(OCOCH_3)_2$ is added to the solution.

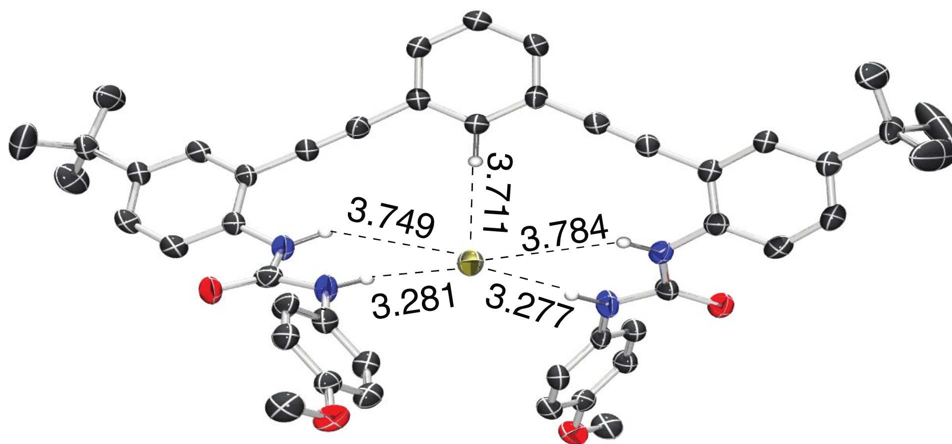

Figure 10. Crystal structure of receptor **1** binding HS^-. Carbon is black, oxygen is red, nitrogen is blue, hydrogen is white, and sulfur is yellow.

Conclusions

The work presented in this thesis details the preparation and characterization of the first reported example of reversible HS^- binding. As seen in the solid-state structure, there are similarities between this receptor binding HS^- and Cl^-. The number of hydrogen bonds involved in the binding interaction is the same for both anions, and the lengths of these hydrogen bonds are also very similar. The orientation in which this receptor wraps around HS^- is almost identical to the geometry it uses in binding Cl^-.

Despite these similarities, there are differences in how these receptors bind these two anions once certain functional groups on the receptor are modulated. The pyridine core showed a greater selectivity of the HS^- anion over the Cl^- anion than the phenyl core. The open hydrogen bond accepting nitrogen in the pyridine receptor allowed for this selectivity of HS^- over Cl^- since Cl^- does not have a hydrogen atom to donate to this interaction. With just a negative charge, the Cl^- is also slightly repelled by the electronegative nitrogen, contributing even more to this selectivity for HS^-.

With these factors contributing to a stronger interaction with HS^-, it seems logical that a pyridine core would be the best way to bind HS^-. However, the phenyl core displayed higher association constants for both anions. This demonstrates that the hydrogen bond donating carbon in the phenyl core is a key factor in binding anions of this size and should be considered when strong binding is desired over selectivity.

Future Directions

This first example of reversible HS^- binding and has already inspired more research in the field of H_2S and anion binding. Another family of tripodal receptors (Figure 11) has recently been added to the library of reversible HS^- receptors,[15] and other receptors using similar binding motifs are currently under investigation.

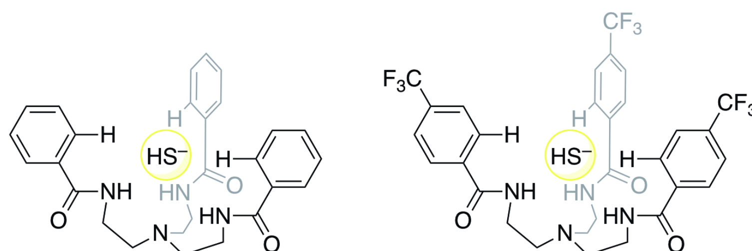

Figure 11. Other recently developed receptors for non-covalent HS^- binding[16]

Future studies in this field could cover multiple different areas. One area involves further research into the binding of HS^-. Variations of the receptor in this thesis could be synthesized and other functional groups could be added on to study how those affect HS^- binding. While there is much more to be learned with these specific receptors and HS^-, other forms of receptors, like the aforementioned tripodal receptors that were just published, have shown potential. Many other receptors, like Cl^- receptors, are viable options for reversible HS^- binding as well and are currently being looked at in the Pluth lab. Receptors with promising hydrogen bond donors and tunable functional groups will result in the best options for future binding studies.

[15] Lau N., Zakharov L., Pluth M. *Chem Commun (Camb).* **2018**, Advance Article.
[16] Ibid.

Another area that this research could cover is exploring the binding of other little explored anions. The hydrogenselenide (HSe^-) anion is an obvious candidate for further studies with the bis(ethylnylaniline) based receptors. This anion also has biological relevance as it is contained in the antioxidant amino acid selenocysteine. The Pluth lab is currently investigating how receptors not only bind HS^- but also HSe^-. As the library of receptors expand, the knowledge of how these anions operate in pathways and proteins will develop to a new level.

After these short-term goals are achieved, the long-term goal is to create a receptor that detects HS^- in biological environments. Involving the right receptors and anions in this research will ideally lead to a reversible receptor that senses biological HS^- concentrations in real-time. This tool would be fundamental in exploring potential treatment options in diseases that involve H_2S pathways and would display that basic science research is imperative in supporting advancements in applied science.

Supporting Information

UV-Vis Titrations

Table S1. Titration of **1** with HS^{-}

#	[**1**] (M)	[HS^{-}] (M)	Equiv.	Absorbance (324 nm)	Absorbance (333 nm)
0	1.07E-05	0.00E+00	0.00	0.2286	0.2370
1	1.07E-05	1.44E-06	0.13	0.2285	0.2347
2	1.07E-05	2.87E-06	0.27	0.2283	0.2350
3	1.07E-05	4.31E-06	0.40	0.2279	0.2351
4	1.07E-05	5.74E-06	0.54	0.2267	0.2337
5	1.07E-05	7.17E-06	0.67	0.2254	0.2321
6	1.07E-05	8.60E-06	0.80	0.2263	0.2326
7	1.07E-05	1.00E-05	0.93	0.2260	0.2329
8	1.07E-05	1.15E-05	1.07	0.2253	0.2312
9	1.07E-05	1.29E-05	1.21	0.2255	0.2315
10	1.07E-05	1.43E-05	1.34	0.2243	0.2301
11	1.07E-05	1.57E-05	1.47	0.2238	0.2285
12	1.07E-05	1.72E-05	1.61	0.2243	0.2294
13	1.07E-05	1.86E-05	1.74	0.2243	0.2290
14	1.07E-05	2.00E-05	1.87	0.2229	0.2277
15	1.07E-05	2.14E-05	2.00	0.2232	0.2271
16	1.07E-05	2.43E-05	2.27	0.2221	0.2259
17	1.07E-05	2.71E-05	2.53	0.2212	0.2248
18	1.07E-05	3.00E-05	2.80	0.2200	0.2237
19	1.07E-05	3.28E-05	3.07	0.2188	0.2225
20	1.07E-05	3.56E-05	3.33	0.2189	0.2219
21	1.07E-05	3.85E-05	3.60	0.2200	0.2228
22	1.07E-05	4.13E-05	3.86	0.2194	0.2222
23	1.07E-05	4.69E-05	4.38	0.2190	0.2211
24	1.07E-05	5.25E-05	4.91	0.2159	0.2171
25	1.07E-05	5.82E-05	5.44	0.2191	0.2208
26	1.07E-05	6.37E-05	5.95	0.2183	0.2194
27	1.07E-05	6.93E-05	6.48	0.2179	0.2196
28	1.07E-05	8.05E-05	7.52	0.2150	0.2167
29	1.07E-05	9.15E-05	8.55	0.2159	0.2173
30	1.07E-05	1.03E-04	9.58	0.2145	0.2149
31	1.07E-05	1.13E-04	10.61	0.2127	0.2132
32	1.07E-05	1.24E-04	11.63	0.2142	0.2150

33	1.07E-05	1.35E-04	12.64	0.2113	0.2104
34	1.07E-05	1.46E-04	13.65	0.2138	0.2143
35	1.07E-05	1.57E-04	14.65	0.2130	0.2133
36	1.07E-05	1.67E-04	15.65	0.2125	0.2126
37	1.07E-05	1.78E-04	16.65	0.2142	0.2151
38	1.07E-05	1.89E-04	17.64	0.2130	0.2135
39	1.07E-05	1.99E-04	18.62	0.2116	0.2116
40	1.07E-05	2.10E-04	19.60	0.2120	0.2122

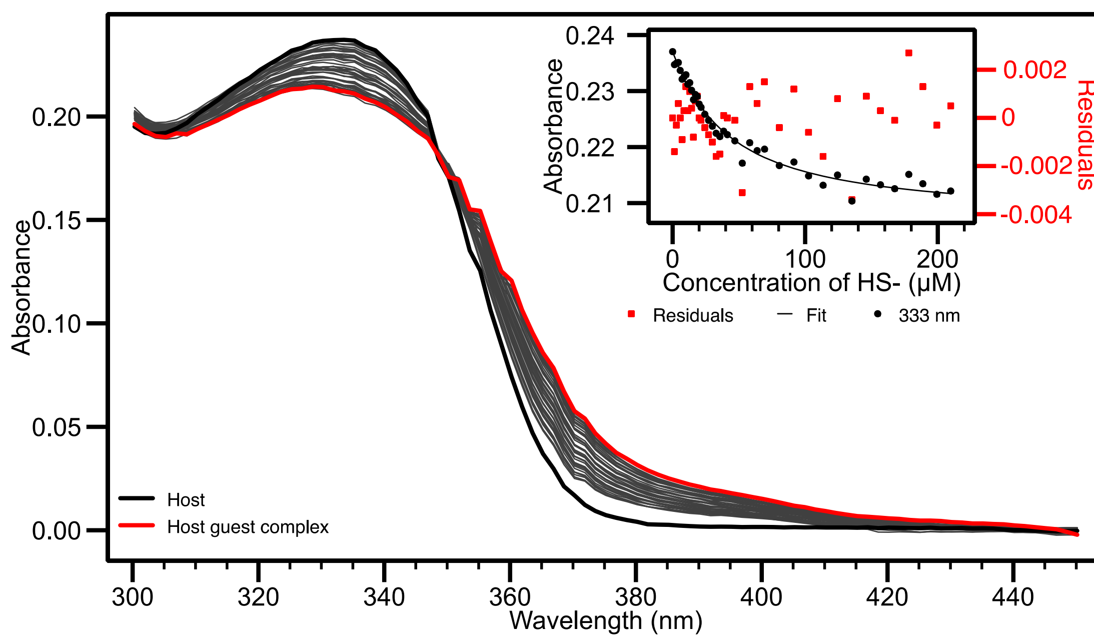

Figure S1. UV-Vis titration of **1** with HS^-

Table S2. Titration of **1** with Cl^-.

#	[**1**] (M)	[Cl^-] (M)	Equiv.	Absorbance (350 nm)	Absorbance (353 nm)	Absorbance (355 nm)	Absorbance (360 nm)
0	1.08E-05	0.00E+00	0.00	0.180	0.145	0.128	0.081
1	1.08E-05	2.03E-05	1.88	0.182	0.148	0.130	0.082
2	1.08E-05	4.07E-05	3.77	0.178	0.149	0.133	0.083
3	1.08E-05	6.10E-05	5.65	0.185	0.151	0.133	0.084
4	1.08E-05	8.12E-05	7.52	0.181	0.152	0.136	0.087
5	1.08E-05	1.01E-04	9.40	0.181	0.153	0.136	0.087
6	1.08E-05	1.42E-04	13.14	0.184	0.155	0.139	0.089
7	1.08E-05	1.82E-04	16.87	0.186	0.157	0.140	0.091
8	1.08E-05	2.22E-04	20.60	0.193	0.159	0.141	0.091
9	1.08E-05	2.63E-04	24.31	0.187	0.159	0.143	0.092
10	1.08E-05	3.03E-04	28.02	0.189	0.161	0.145	0.094
11	1.08E-05	3.82E-04	35.40	0.198	0.163	0.145	0.094
12	1.08E-05	4.62E-04	42.75	0.195	0.166	0.150	0.098
13	1.08E-05	5.41E-04	50.06	0.196	0.168	0.152	0.100
14	1.08E-05	6.19E-04	57.33	0.200	0.172	0.155	0.102
15	1.08E-05	6.97E-04	64.57	0.200	0.172	0.155	0.103
16	1.08E-05	7.75E-04	71.78	0.201	0.173	0.157	0.103
17	1.08E-05	8.52E-04	78.95	0.202	0.173	0.157	0.104
18	1.08E-05	9.30E-04	86.08	0.207	0.174	0.156	0.103
19	1.08E-05	1.01E-03	93.18	0.205	0.176	0.160	0.106
20	1.08E-05	1.10E-03	102.01	0.205	0.178	0.161	0.107
21	1.08E-05	1.20E-03	110.78	0.209	0.180	0.164	0.109
22	1.08E-05	1.29E-03	119.50	0.207	0.180	0.163	0.109
23	1.08E-05	1.38E-03	128.17	0.208	0.181	0.165	0.110
24	1.08E-05	1.48E-03	136.79	0.209	0.182	0.165	0.110
25	1.08E-05	1.57E-03	145.35	0.214	0.186	0.170	0.115
26	1.08E-05	1.66E-03	153.87	0.212	0.185	0.168	0.114
27	1.08E-05	1.75E-03	162.33	0.212	0.184	0.168	0.113
28	1.08E-05	1.84E-03	170.75	0.214	0.187	0.170	0.115
29	1.08E-05	1.93E-03	179.11	0.214	0.188	0.171	0.116
30	1.08E-05	2.02E-03	187.43	0.216	0.189	0.171	0.116

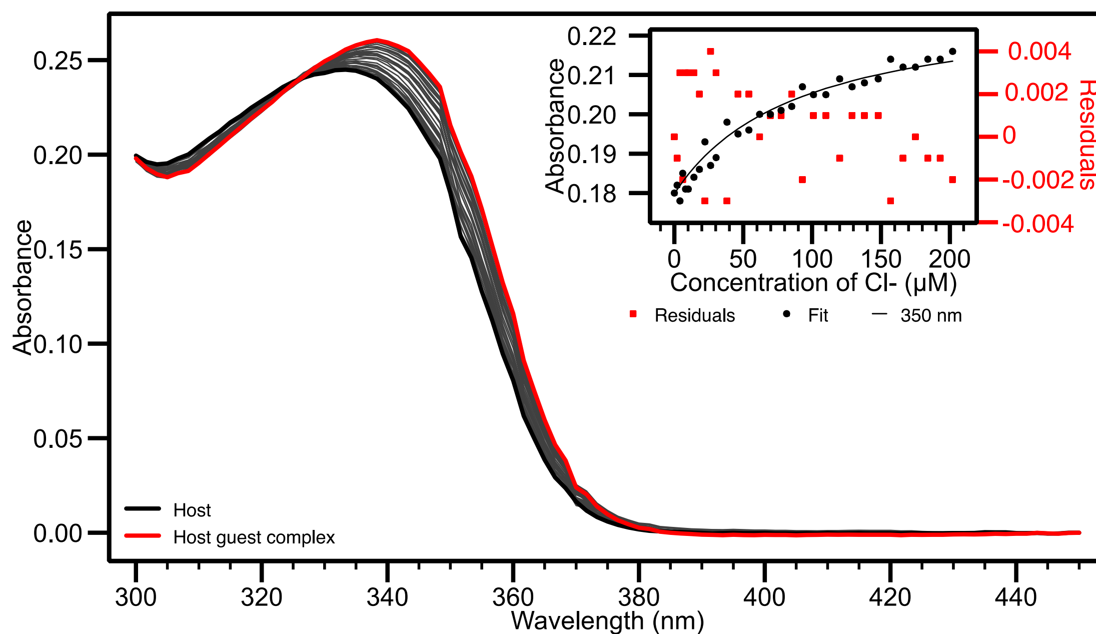

Figure S2. UV-Vis titration of **1** with Cl⁻

Table S3. Titration of **2** with HS^-.

#	[**2**] (M)	[SH^-] (M)	Equiv.	Absorbance (327 nm)	Absorbance (333 nm)	Absorbance (360 nm)	Absorbance (375 nm)
0	1.06E-05	0.00E+00	0.00	0.220	0.224	0.095	0.012
1	1.06E-05	3.24E-06	0.31	0.219	0.223	0.097	0.013
2	1.06E-05	6.47E-06	0.61	0.218	0.222	0.098	0.014
3	1.06E-05	9.70E-06	0.92	0.218	0.221	0.099	0.015
4	1.06E-05	1.29E-05	1.22	0.216	0.219	0.101	0.016
5	1.06E-05	1.62E-05	1.53	0.215	0.219	0.102	0.016
6	1.06E-05	1.94E-05	1.83	0.214	0.217	0.103	0.018
7	1.06E-05	2.26E-05	2.14	0.214	0.217	0.105	0.020
8	1.06E-05	2.59E-05	2.44	0.213	0.216	0.106	0.021
9	1.06E-05	2.91E-05	2.75	0.212	0.215	0.107	0.021
10	1.06E-05	3.23E-05	3.05	0.212	0.214	0.108	0.023
11	1.06E-05	3.55E-05	3.36	0.211	0.213	0.109	0.023
12	1.06E-05	3.88E-05	3.66	0.210	0.213	0.110	0.024
13	1.06E-05	4.52E-05	4.27	0.208	0.210	0.111	0.026
14	1.06E-05	5.16E-05	4.88	0.207	0.210	0.113	0.027
15	1.06E-05	5.81E-05	5.48	0.207	0.210	0.115	0.029
16	1.06E-05	6.45E-05	6.09	0.206	0.208	0.115	0.029
17	1.06E-05	7.09E-05	6.70	0.205	0.207	0.116	0.030
18	1.06E-05	7.74E-05	7.31	0.206	0.208	0.118	0.033
19	1.06E-05	8.38E-05	7.91	0.204	0.206	0.117	0.031
20	1.06E-05	9.02E-05	8.52	0.203	0.205	0.118	0.032
21	1.06E-05	1.03E-04	9.73	0.200	0.203	0.120	0.035
22	1.06E-05	1.16E-04	10.94	0.200	0.202	0.122	0.036
23	1.06E-05	1.29E-04	12.15	0.200	0.203	0.123	0.036
24	1.06E-05	1.41E-04	13.35	0.199	0.201	0.123	0.038
25	1.06E-05	1.54E-04	14.56	0.198	0.201	0.123	0.037
26	1.06E-05	1.80E-04	16.96	0.198	0.200	0.125	0.039
27	1.06E-05	2.05E-04	19.36	0.197	0.200	0.125	0.040
28	1.06E-05	2.30E-04	21.75	0.198	0.200	0.127	0.040
29	1.06E-05	2.56E-04	24.14	0.197	0.199	0.127	0.041
30	1.06E-05	2.81E-04	26.52	0.197	0.200	0.128	0.042
31	1.06E-05	3.31E-04	31.26	0.197	0.200	0.130	0.042
32	1.06E-05	3.81E-04	35.98	0.196	0.198	0.131	0.043
33	1.06E-05	4.31E-04	40.68	0.196	0.198	0.131	0.044
34	1.06E-05	4.80E-04	45.35	0.195	0.198	0.131	0.044
35	1.06E-05	5.30E-04	50.00	0.195	0.197	0.132	0.044
36	1.06E-05	5.79E-04	54.62	0.194	0.196	0.133	0.044
37	1.06E-05	6.27E-04	59.23	0.194	0.197	0.133	0.044

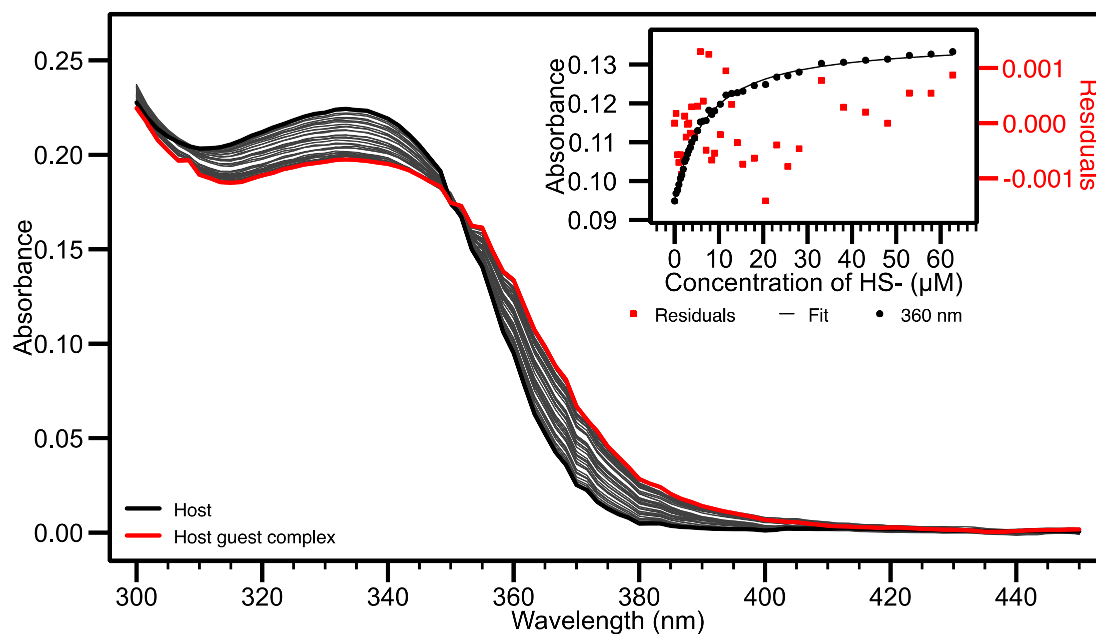

Figure S3. UV-Vis titration of **2** with HS^{-}

Table S4. Titration of **2** with Cl^{-}.

#	[**2**] (M)	[Cl^{-}] (M)	Equiv.	Absorbance (352 nm)	Absorbance (355 nm)	Absorbance (357 nm)	Absorbance (360 nm)
0	1.06E-05	0.00E+00	0.00	0.169	0.150	0.137	0.115
1	1.06E-05	3.59E-06	0.34	0.170	0.150	0.137	0.115
2	1.06E-05	7.18E-06	0.68	0.170	0.150	0.137	0.116
3	1.06E-05	1.43E-05	1.35	0.171	0.151	0.138	0.116
4	1.06E-05	2.15E-05	2.03	0.170	0.151	0.138	0.116
5	1.06E-05	2.87E-05	2.71	0.173	0.154	0.140	0.118
6	1.06E-05	3.58E-05	3.38	0.172	0.152	0.139	0.117
7	1.06E-05	4.29E-05	4.05	0.171	0.152	0.139	0.117
8	1.06E-05	5.00E-05	4.73	0.173	0.153	0.140	0.118
9	1.06E-05	5.72E-05	5.40	0.172	0.153	0.140	0.118
10	1.06E-05	6.43E-05	6.07	0.173	0.154	0.141	0.119
11	1.06E-05	7.14E-05	6.74	0.174	0.155	0.142	0.120
12	1.06E-05	7.84E-05	7.41	0.174	0.154	0.141	0.119
13	1.06E-05	9.26E-05	8.74	0.175	0.155	0.142	0.120
14	1.06E-05	1.07E-04	10.07	0.175	0.156	0.143	0.121
15	1.06E-05	1.21E-04	11.40	0.176	0.157	0.143	0.121
16	1.06E-05	1.35E-04	12.73	0.176	0.157	0.144	0.121
17	1.06E-05	1.49E-04	14.05	0.177	0.158	0.145	0.122
18	1.06E-05	1.63E-04	15.37	0.178	0.159	0.146	0.123
19	1.06E-05	1.77E-04	16.69	0.178	0.159	0.146	0.124
20	1.06E-05	1.91E-04	18.00	0.178	0.159	0.146	0.124
21	1.06E-05	2.18E-04	20.61	0.179	0.160	0.147	0.125
22	1.06E-05	2.46E-04	23.21	0.180	0.162	0.149	0.126
23	1.06E-05	2.73E-04	25.80	0.181	0.162	0.149	0.126
24	1.06E-05	3.01E-04	28.38	0.182	0.163	0.150	0.127
25	1.06E-05	3.28E-04	30.94	0.184	0.165	0.151	0.129
26	1.06E-05	3.55E-04	33.49	0.183	0.165	0.152	0.129
27	1.06E-05	3.82E-04	36.03	0.185	0.166	0.153	0.131
28	1.06E-05	4.08E-04	38.56	0.185	0.166	0.153	0.130
29	1.06E-05	4.35E-04	41.07	0.186	0.168	0.155	0.132
30	1.06E-05	4.61E-04	43.57	0.186	0.168	0.155	0.132
31	1.06E-05	4.88E-04	46.06	0.186	0.168	0.155	0.132
32	1.06E-05	5.14E-04	48.53	0.186	0.168	0.155	0.132
33	1.06E-05	5.40E-04	51.00	0.188	0.169	0.156	0.133
34	1.06E-05	5.66E-04	53.45	0.188	0.169	0.156	0.133

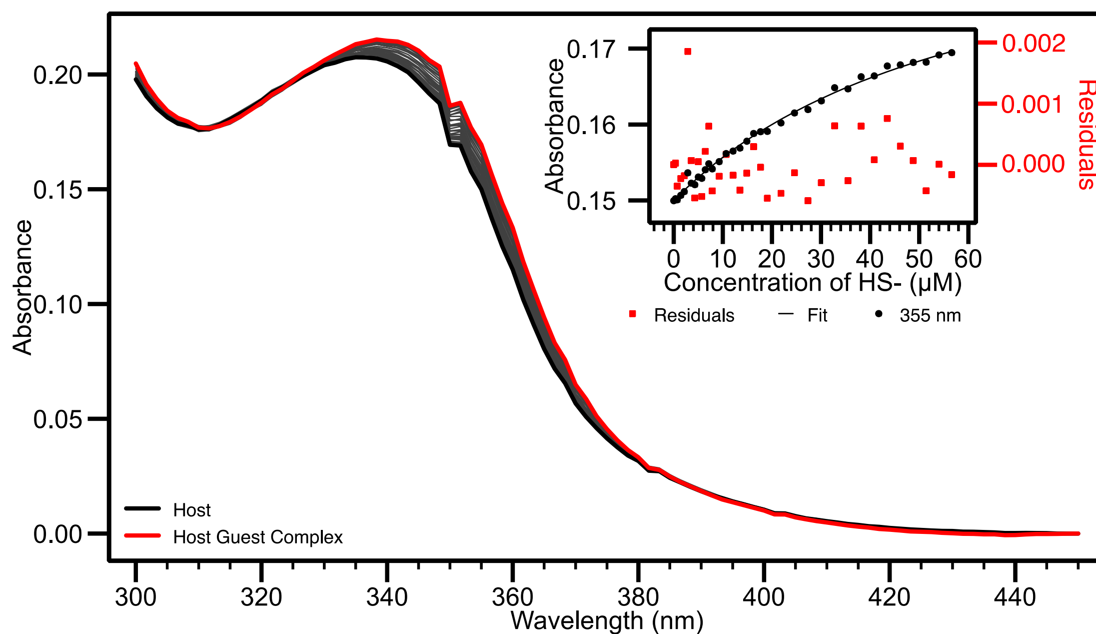

Figure S4. UV-Vis titration of **2** with Cl^{-}

Table S5. Titration of **3** with HS^-.

#	[**3**] (M)	[SH^-] (M)	Equiv.	Absorbance (310 nm)	Absorbance (322 nm)	Absorbance (330 nm)	Absorbance (347 nm)
0	1.03E-05	0.00E+00	0.00	0.189	0.215	0.203	0.095
1	1.03E-05	3.30E-06	0.32	0.187	0.212	0.205	0.105
2	1.03E-05	6.59E-06	0.64	0.183	0.211	0.206	0.110
3	1.03E-05	9.89E-06	0.96	0.181	0.208	0.206	0.113
4	1.03E-05	1.32E-05	1.28	0.180	0.208	0.206	0.117
5	1.03E-05	1.65E-05	1.60	0.179	0.207	0.207	0.120
6	1.03E-05	1.98E-05	1.92	0.176	0.205	0.207	0.122
7	1.03E-05	2.30E-05	2.24	0.175	0.204	0.207	0.122
8	1.03E-05	2.63E-05	2.56	0.175	0.204	0.208	0.125
9	1.03E-05	2.96E-05	2.87	0.174	0.204	0.208	0.126
10	1.03E-05	3.29E-05	3.19	0.175	0.205	0.209	0.128
11	1.03E-05	3.94E-05	3.83	0.175	0.204	0.210	0.130
12	1.03E-05	4.60E-05	4.46	0.174	0.203	0.210	0.131
13	1.03E-05	5.25E-05	5.10	0.174	0.204	0.210	0.133
14	1.03E-05	5.90E-05	5.73	0.172	0.203	0.209	0.134
15	1.03E-05	6.56E-05	6.37	0.173	0.204	0.211	0.136
16	1.03E-05	7.86E-05	7.63	0.174	0.205	0.213	0.137
17	1.03E-05	9.15E-05	8.89	0.170	0.201	0.209	0.135
18	1.03E-05	1.04E-04	10.14	0.173	0.204	0.214	0.140
19	1.03E-05	1.17E-04	11.40	0.174	0.204	0.213	0.141
20	1.03E-05	1.30E-04	12.65	0.173	0.204	0.213	0.140
21	1.03E-05	1.56E-04	15.14	0.173	0.203	0.213	0.140
22	1.03E-05	1.81E-04	17.62	0.174	0.205	0.213	0.141
23	1.03E-05	2.07E-04	20.08	0.176	0.206	0.216	0.143
24	1.03E-05	2.32E-04	22.54	0.173	0.204	0.213	0.141
25	1.03E-05	2.57E-04	24.98	0.174	0.204	0.213	0.141

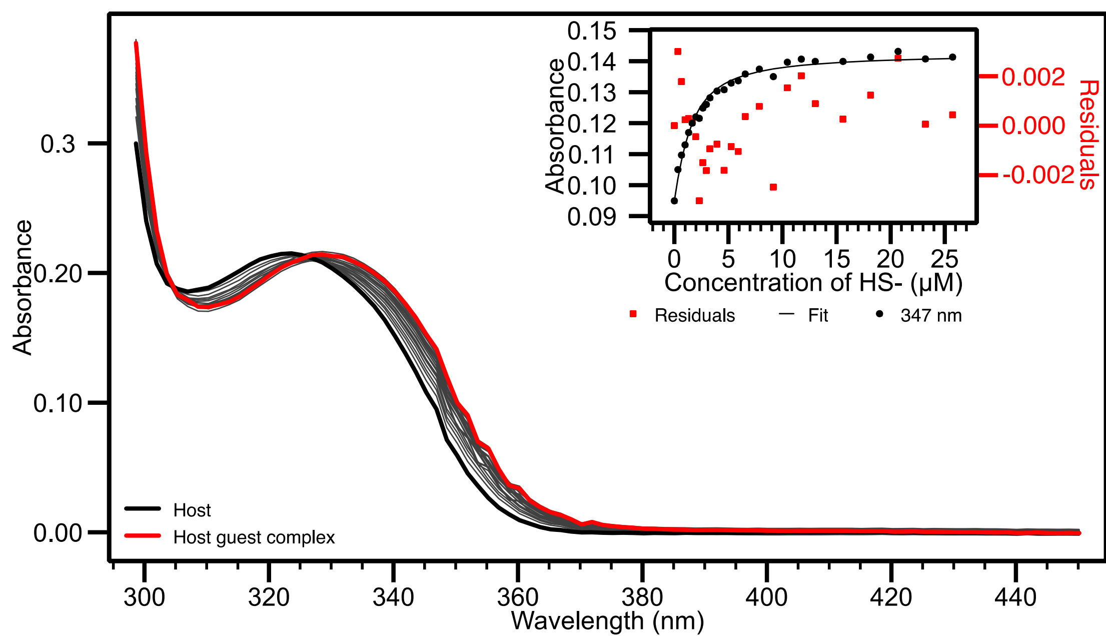

Figure S5. UV-Vis titration of **3** with HS^{-}

Table S6. Titration of **3** with Cl^{-}.

#	[**3**] (M)	[Cl^{-}] (M)	Equiv.	Absorbance (335 nm)	Absorbance (338 nm)	Absorbance (340 nm)	Absorbance (343 nm)
0	1.03E-05	0.00E+00	0.00	0.192	0.172	0.160	0.132
1	1.03E-05	1.56E-06	0.15	0.193	0.174	0.161	0.133
2	1.03E-05	3.12E-06	0.30	0.194	0.175	0.163	0.134
3	1.03E-05	4.67E-06	0.45	0.195	0.176	0.164	0.136
4	1.03E-05	6.23E-06	0.60	0.197	0.177	0.166	0.137
5	1.03E-05	7.79E-06	0.75	0.196	0.177	0.165	0.137
6	1.03E-05	1.09E-05	1.05	0.197	0.178	0.166	0.138
7	1.03E-05	1.40E-05	1.35	0.200	0.181	0.169	0.141
8	1.03E-05	1.71E-05	1.65	0.202	0.183	0.172	0.143
9	1.03E-05	2.02E-05	1.95	0.204	0.185	0.174	0.146
10	1.03E-05	2.33E-05	2.25	0.204	0.186	0.174	0.146
11	1.03E-05	2.64E-05	2.55	0.206	0.187	0.176	0.149
12	1.03E-05	3.25E-05	3.15	0.208	0.190	0.179	0.151
13	1.03E-05	3.87E-05	3.74	0.210	0.192	0.181	0.154
14	1.03E-05	4.48E-05	4.34	0.211	0.194	0.182	0.155
15	1.03E-05	5.09E-05	4.93	0.213	0.196	0.185	0.158
16	1.03E-05	5.70E-05	5.52	0.214	0.197	0.186	0.158
17	1.03E-05	6.31E-05	6.11	0.214	0.198	0.187	0.160
18	1.03E-05	6.92E-05	6.69	0.216	0.200	0.188	0.161
19	1.03E-05	7.52E-05	7.28	0.216	0.200	0.189	0.162
20	1.03E-05	8.73E-05	8.45	0.218	0.202	0.191	0.164
21	1.03E-05	9.93E-05	9.61	0.219	0.203	0.192	0.166
22	1.03E-05	1.11E-04	10.76	0.219	0.203	0.192	0.165
23	1.03E-05	1.23E-04	11.91	0.221	0.205	0.195	0.168
24	1.03E-05	1.35E-04	13.06	0.221	0.206	0.195	0.169
25	1.03E-05	1.47E-04	14.19	0.221	0.205	0.195	0.168
26	1.03E-05	1.58E-04	15.33	0.222	0.206	0.196	0.169
27	1.03E-05	1.70E-04	16.45	0.221	0.206	0.195	0.168
28	1.03E-05	1.82E-04	17.57	0.222	0.206	0.196	0.170
29	1.03E-05	1.93E-04	18.69	0.222	0.207	0.196	0.170
30	1.03E-05	2.05E-04	19.80	0.222	0.207	0.196	0.170

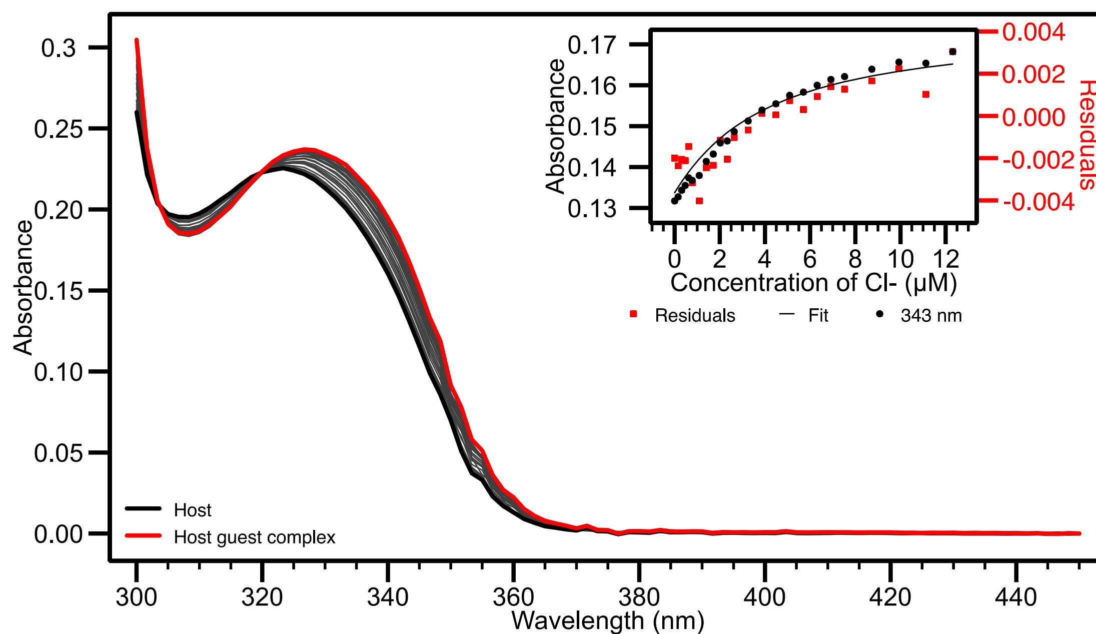

Figure S6. UV-Vis titration of **3** with Cl^{-}

Glossary

Absorption: when electrons of a molecule capture electromagnetic radiation from a certain wavelength of light

Association constant: a value calculated to represent the concentration of guest that is bound to the host divided by the total concentration of host

Covalent Bonding: the strong, electron-sharing interaction between atoms to form a molecule

Electronegative: a characteristic of certain atoms like oxygen and nitrogen that pull electrons slightly tighter and thus have a slightly negative charge in molecules

Electronic environment: describing the make-up and behavior of the electrons in a molecule

Functional groups: small chemical structures that have unique chemical characteristics and make up larger supramolecular structures

Gasotransmitters: small, dissolved gas molecules that are freely permeable to membranes and have well defined signaling functions in various physiological pathways

Hydrogen bond: a weak, non-covalent interaction between a slightly positive hydrogen atom and an electronegative oxygen or nitrogen atom

Iodination: a chemical reaction used in organic synthesis to create a carbon-iodine bond

Non-covalent bonding: weak, electromagnetic interactions between molecules such as hydrogen bonding and Van der Waals forces

Organic: carbon-based

pKa: the pH at which a molecule is 50% deprotonated

Residual: the difference between a value measured in a scientific experiment and the theoretical value

Sonogashira cross-coupling reaction: a type of chemical reaction used in organic synthesis that uses palladium to catalyze the formation of carbon-carbon bonds

Supramolecular Chemistry: the field of chemistry dealing with non-covalent interactions between large, host molecules and smaller, guest molecules

Titration: an experiment consisting of the incremental addition of some chemical to a solution containing another chemical to determine unknown concentrations

References

Czyzewski, B., Wang, D. *Nature* **2012**, *483*, 494–497.

Carroll, C.; Coombs, B.; McClintock, S.; Johnson II, C.; Berryman, O.; Johnson, D.; Haley, M. *Chem. Commun*. **2011**, *47*, 5539–5541.

Hartle, Matthew; Meininger, Daniel; Zakharov, Lev; Tonzetich, Zachary; Pluth, Michael. "NBu_4SH provides a convenient source of HS(-) soluble in organic solution for H_2S and anion-binding research." *Dalton Transactions* **2015**, *44*.

Hartle, M.D., Hansen, R.J., Tresca, B.W., Prakel, S.S., Zakharov, L.N., Haley, M. M., Pluth, M.D., Johnson, D.W. *Angew. Chem. Int. Ed.* **2016**, *55*, 11480 –11484.

Jennings, M. *Am. J. Physiol. Cell Physiol.* **2013**, *305*, C941–C950.

Lau N., Zakharov L., Pluth M. *Chem Commun (Camb).* **2018**, Advance Article.

Montoya, L.; Pearce, T.; Hansen, R.; Zakharov, L.; Pluth, M. *J Org Chem*. **2013**, *78*, 6550–6557.

Montoya, L.; Shen, X.; McDermott, J.; Kevil, C.; Pluth, M. *Chem Sci.* **2015**, *6*, 294-300.

National Research Council (US) Committee on Acute Exposure Guideline Levels. *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9*. Washington (DC): National Academies Press (US); **2010**, *4*, Hydrogen Sulfide Acute Exposure Guideline Levels.

Thordarson, P. Determining Association Constants from Titration Experiments in Supramolecular Chemistry. *Chem. Soc. Rev.* **2011**, *40* (3), 1305–1323.

Wang, R. *Physiol Rev.* **2012**, *92*, 791-896.