

**Fresh Quantitative Approaches for High-Throughput Characterization Using
ChemFETs and Statistical Analysis**

by

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A dissertation accepted and approved in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in Chemistry

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Summer 2024

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DISSERTATION ABSTRACT

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Doctor of Philosophy in Chemistry

Title: Fresh Quantitative Approaches for High-Throughput Characterization using ChemFETs and Statistical Analysis

Anion receptors are an increasingly important area of focus in synthetic organic chemistry, especially in areas such as environmental pollution detection and remediation. Many organic anion receptors are hydrophobic, limiting their utility for direct evaluation of aqueous anion affinity. Electrochemical sensors such as chemically-sensitive field effect transistors (ChemFETs) can bridge this gap. Incorporation of anion receptors into the chemically sensitive membrane of a ChemFET can facilitate direct measurement of aqueous anion affinity of hydrophobic sensors. One key piece of information that this can elucidate is the relative affinities of anions with the host by direct comparison of detection limits for each anion. Relative ranking of anion detection limits can be compared to the Hofmeister series, especially useful for determining the placement of relatively unknown, reactive species into the Hofmeister series.

Dodeca-n-butyl bambus[6]uril was used in the selective membrane of a ChemFET to produce the first reported placement of hydrosulfide in the Hofmeister series. The contribution of the binding pocket geometry on anion affinities was then explored by comparing anion detection limits of dodeca-n-butyl bambus[6]uril with dodecabenzyl bambus[6]uril. The utility of ChemFETs was then expanded to assess the anion affinity of metal organic frameworks (MOFs), to learn about the anion binding nature of a novel MOF.

After studying the nature of host-guest interactions using electrochemical sensors, research efforts expanded to include a statistically-based analytical method for characterization of synthetic pathways. Design of experiments (DOE) is generally used to characterize processes, and quantify

impacts of main and multi-factor interactions on desired outputs. In chemical applications, DOE can characterize syntheses, specifically the impacts of each factor (together or in isolation) on the resulting product. This information can then be used to provide optimization conditions to produce desired properties. Significantly, this evaluation technique can be applied to historical data in order to characterize reactions before running any new experiments. In this particular case, flat aluminum 13 ($f\text{-Al}_{13}$) cluster was analyzed via DOE in an effort to optimize desired properties. This data was then used to provide optimization conditions for the factors of size (minimize) and polydispersity index (minimize). Two different sets of optimization conditions were used as a validation run to synthesize aluminum particles, demonstrating a drastic improvement in one of the two optimization conditions.

Finally, other research efforts are examined and documented. These efforts include ChemFET characterization of anion receptors, synthetic challenges, and application of DOE to characterize and optimize reactions. Overall, this dissertation involves the coalescence of different areas of study in order to solve difficult problems.

This dissertation includes previously published and unpublished co-authored material.

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PUBLICATIONS:

D. H. Banning, G. M. Kuhl, S. A. Fontenot and D. W. Johnson, *Supramol. Chem.*, 2024, DOI: 10.1080/10610278.2024.2353565

D. H. Banning, G. M. Kuhl, M. M. Howell and D. W. Johnson, *Org. Biomol. Chem.*, 2024, **22**, 269–273.

G. M. Kuhl, D. H. Banning, H. A. Fargher, W. A. Davis, M. M. Howell, L. Zakharov, M. D. Pluth and D. W. Johnson, *Chem. Sci.*, 2023, **14**, 10273–10279.

ACKNOWLEDGMENTS

I thank God for blessing me beyond what I could ever imagine. And thanks to my girls: Bethany, Evelyn, Ingrid, and Zoe. This is my seventh Air Force assignment, and you all have followed me around the country and sacrificed more than anyone can imagine. I want to thank my Mom and Dad for all their love and support. Mom and Dad, you have always been there for me, you were my first teachers and instilled a love of learning even USAFA couldn't kill. I also wish to express my sincere appreciation to Professor Darren Johnson. Darren, you have been an incredible source of help, support, and mentorship ever since first welcoming me into the group back in 2017. I couldn't rotate, so you took a huge chance on me, and I am incredibly grateful. I also wish to extend special thanks to my Chair Professor Michael Haley, and committee members Professors Michael Pluth and Ghee Ong for all their help and support. Special thanks to Doctor Grace Kuhl and Doctor Sean Fontenot for being fantastic mentors and friends. You both helped and supported me more than I can adequately express during the beginning of both UO graduate school experiences. I also want to thank my friends Doctor Jacob Mayhugh and Doctor Jess Lohrman for all their mentorship. Special thanks to my friends Alex, Luca, Willow, Hannah, and Thaís for such a warm welcome back into the DWJ group. And special acknowledgement to my friends Madeline Howell, Natalie Lakanen, Cameron Moore, and Bronwen Link for all their incredible, thankless, and tireless efforts in the basement. After this I think I'm finally done with school.

My program was supported entirely by the Air Force Institute of Technology.

This is dedicated to my girls: my wife Bethany, and my daughters Evelyn, Ingrid, and double rainbow Zoe. Thanks for being such an unending source of love and support.

The views expressed in this dissertation are my own, and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

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CHAPTER I

INTRODUCTION TO THE ANALYTICAL METHODS USED: CHEMFET SENSORS AND DESIGN OF EXPERIMENTS

Part 1: Supramolecular methods: Utilising ChemFETs to evaluate aqueous anion affinity of hydrophobic receptors

From D. H. Banning, G. M. Kuhl, S. A. Fontenot, and D. W. Johnson, *Supramol. Chem.*, 2024, DOI: 10.1080/10610278.2024.2353565 (Table of Contents image depicted as Figure 1)

Abstract

The host-guest interaction and affinity of hydrophobic receptors towards aqueous anions can be evaluated by incorporating a receptor into a polymeric membrane and applying these membranes to FET-based sensing devices. A series of anions screened using these ChemFET (chemically-sensitive field effect transistor) sensors based on different receptors can also provide a Hofmeister-like ordering of anions based on their respective detection limits. Although the general trends of selectivity coefficients often follow the Hofmeister Series, the specific anion affinity profiles are generally unique to each receptor (Figure 1). The list of anion detection limits can be used to help define particular affinity and selectivity of receptors towards specific anions. In this methods/technique review paper, we provide a detailed example of how our lab uses

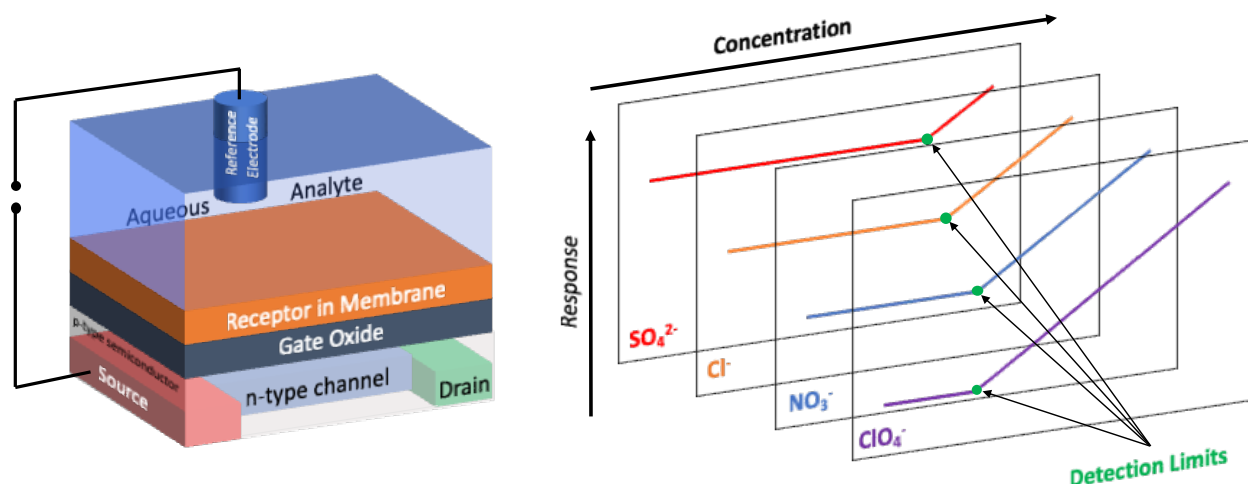


Figure 1. Abstract image of the paper. Depicted are a ChemFET (left), and an illustration of differing detection limits for common anions following a Hofmeister-like trend (right).

ChemFETs to evaluate host-guest interactions of hydrophobic host receptors with aqueous anions, and a detailed procedure for fabricating ChemFET sensor devices is presented along with select case studies for readers interested in applying related ion recognition strategies in sensors of this type.¹⁻⁴

Introduction

The fundamental basis of a chemically-sensitive field effect transistor (ChemFET) is similar to the ubiquitous metal oxide semiconductor field effect transistor (MOSFET), which features an insulated gate that controls the conductivity of the device through its applied voltage. The underlying design of a MOSFET can be changed by designing a FET with a physically-separated source and gate. This results in a FET that is sensitive to ions in solution, called an ion sensitive field effect transistor (ISFET). ISFETs can be further modified by functionalizing the gate oxide surface to interact selectively with analytes of interest. Imparting chemical sensitivity to the gate oxide surface of an ISFET results in a chemically-sensitive field effect transistor, or ChemFET. Many other FET-based approaches for a variety of ionic analytes exist, including BioFET,

Extended-Gate FET (EGFET), DNAFET, and others;⁵⁻⁷ this methods paper focuses on describing approaches to fabricate ChemFETs capable of detecting and quantitating aqueous anions.

ChemFETs are well-established for sensing applications.^{5,8-10} Supramolecular chemistry strategies using host-guest interactions between anions and membrane-bound receptors can be used to create a concentration gradient and therefore an electric potential, which can in turn be measured by the FET. This provides information on the concentration of analyte in solution, where the electric potential correlates directly to the concentration of the anion/analytes. ChemFET sensors are suitable for both anion and cation sensing, and indeed our lab has done both.¹¹ However, given the more challenging nature of sensing charge-diffuse anions, that has remained the primary focus of our work.¹² Although the principles remain similar, the remainder of this paper will reference anion sensing.⁵

The fundamental challenge of applications-focused anion sensing stems from the aqueous nature of the anions of interest. Practical sensing applications require working in aqueous conditions, while many organic anion receptors are water insoluble. In an effort to develop aqueous anion chemosensors, water soluble receptors for anions can be prepared; however, from a synthesis standpoint, using water-solubilizing groups can make the tuning of the receptor more challenging and may disrupt anion recognition (which is already quite challenging in water due to large anion solvation energies and competition from the polar solvent more generally). Ultimately, adding water solubilizing groups adds complexity to the molecule, which may diminish the ability to otherwise use these functional handles to tune receptor characteristics (such as including electron donating or withdrawing groups to tailor hydrogen/halogen bond strength, or adding groups with steric bulk to change the geometry of the binding pocket). The use of ChemFETs is therefore a

convenient way to repurpose organic-soluble anion receptors as components of a sensor membrane for aqueous measurements.

ChemFETs can be used in host/guest chemistry by coating an exposed gate oxide surface with a semipermeable polymer membrane containing ion receptors. The entire sensor can then be submerged into a solution containing the anion of interest, allowing interfacial interaction between these anions and the embedded anion receptors. This simplifies the receptor solubility problem immensely; the only solubility consideration is that the anion receptor, ionophore, and polymer components generally need to be soluble in miscible solvents for creation of a dropcast solution. Ionophore is typically a quaternary ammonium salt used to provide cationic nature to the membrane (described in more detail in the “Sensor Preparation” section). Also, special attention is paid to ensuring components are sufficiently insoluble in water to avoid leaching out or degrading during experiments.

Experimental Design

Given the complexity of setting up a ChemFET system, it is helpful to first understand exactly what specific information can be obtained through this approach to ascertain if the level of effort will fill a specific need in a current research area. Ultimately, a ChemFET provides a voltage reading that can be correlated to analyte concentration. The detection limit is calculated as the concentration at which the flat background line intercepts the Nernstian slope (Figure 2).¹³

The analyte concentration at which the signal can be detected above a competing species provides the selectivity coefficient, useful for quantifying preferential affinity between host receptor and potentially competing anions.¹³ Therefore, ChemFETs can quantitate anion concentration in solution, provide insight into selectivity profiles of different anion receptors, and

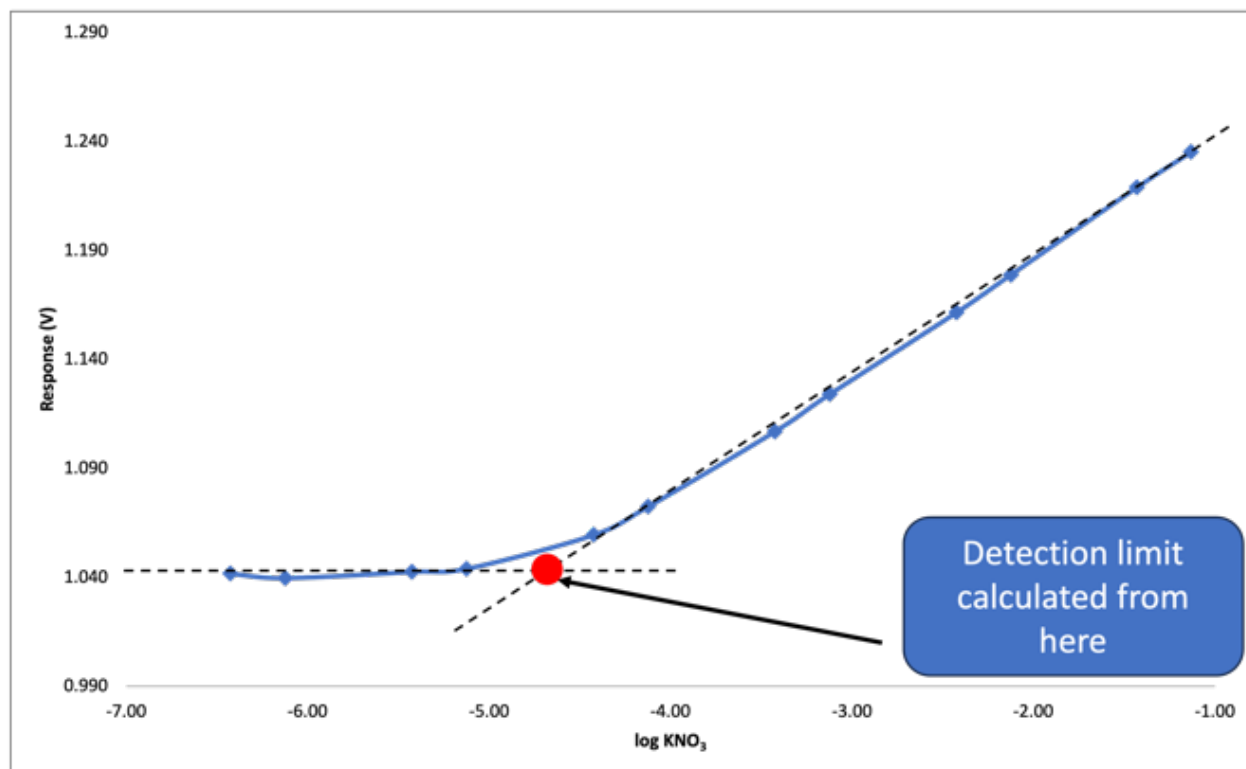


Figure 2. Typical ChemFET response curve. Detection limit is the concentration at which the analyte signal can be seen above the background. The curve in blue indicates response signal from the ChemFET. The flat region on the left indicates analyte concentration below the limit of detection, where increasing analyte concentration does not result in a measurable change in response. The area on the right indicates detectable region, where increasing analyte concentration results in measurable signals from the ChemFET. The interception of the two slopes highlighted in red defines the detection limit.¹³ Example calculation included in ESI for reference.

provide detection limits tuned by the anion receptors. In addition, a series of detection limits can provide a means for quantitative ordering of a series of anions of interest.^{1,14} We have observed that these generally follow a Hofmeister series-like trend.^{1,14} Affinity between receptor and a specific anion can result in a detection limit different than what would be expected based on the Hofmeister series trend generally observed with electrochemical sensors that tend to lack selectivity beyond the standard Hofmeister ordering typically observed. In other words, a ChemFET based on a well-designed chemical receptor will show a selectivity profile resembling the Hofmeister series but may feature an anion of interest "out of order" of the traditional series.

The sensitivity of a ChemFET is measured in mV change observed per order of magnitude change in concentration or pH unit (often termed ‘millivolts per decade’).

Electrochemical sensors like ion selective electrodes (ISE) and FETs have a potential voltage related to the chemical potential at the interface of a membrane and an electrolyte solution.^{6,15,16} The total cell potential is described using the Nikolsky-Eisenman equation^{16,17},

$$E_{cell} = E_{cons} + \frac{2.303RT}{z_A F} \log(a_A + K_{A,B} a_B^{z_A/z_B})$$

where E_{cons} is the sum of all constant potentials within the cell. Ions A and B have activities a_A and a_B , and z_A and z_B are ion charges. R is the ideal gas constant, T is temperature in Kelvin, and F is Faraday’s constant. Finally, $K_{A,B}$ is an experimentally derived selectivity coefficient defined by $K_{A,B}^{pot}$ using the following equation:

$$K_{A,B}^{pot} = \frac{a_A^{z_A/z_B}}{a_B}$$

where a represents the activities of the target analyte A and interferent B at the limit of detection, and z_A and z_B are the respective charges. If $K_{A,B}^{pot} < 1$, the membrane is selective towards the target analyte A. For example, if the selectivity coefficient $K_{A,B}^{pot}$ is 0.10, the system is detecting 10 target anions for every 1 interferent.^{18,19} In the absence of interfering ions, the Nikolsky-Eisenman equation simplifies to the Nernst equation:

$$E_{cell} = E_{cons} + \frac{2.303RT}{z_A F} \log(a_A)$$

At 25.00 °C a Nernstian slope is calculated as 59.17 mV/decade. Of note is that all calculations are performed after converting concentrations to activities (see ESI).

Setup

The system comprises ChemFET sensors, a reference electrode (RE), data acquisition unit, driver circuit, power supply, and laptop suitably configured with a digital data logging program such as National Instruments™ LabVIEW™. An exported executable of an example virtual instrument (VI) compatible with LabVIEW™ software is provided with the Electronic Supplementary Information (ESI).

ChemFET Construction

ChemFETs are available pre-fabricated from companies like Winsense™, Microsens™, and Sentron™ for example²⁰ (our evaluations have all used Winsense™ WIPS-C ChemFETs, www.winsense.co.th). Wires are then soldered to the source and drain leads. All the components and connections are then sealed, leaving the gate oxide exposed, using Loctite® Marine Epoxy 1919324. ChemFETs are then soaked for 10 minutes in H₂O₂ to ensure complete hydroxylation of the gate oxide. The ChemFET is then tested to ensure appropriate sensitivity. At this point, the device functions as a pH sensor, measuring the concentration of the H₃O⁺ ion. We ensure that the devices are sufficiently sensitive by performing a calibration using three pH solutions (pH 4,7,10), in duplicate. We retain devices having a sensitivity greater than 30 mV per pH unit. Repeated H₂O₂ treatments may be required to achieve this necessary level of sensitivity. See ESI for detailed ChemFET sensor construction diagram.

Reference Electrode Construction

Reference electrodes (REs) serve as the connection of the source-to-gate circuit. To construct these, we start with 0.5 mm silver wire, soldered to a length of conventional insulated 26-gauge electrical wire. The solder joint is then sealed with parafilm and heat shrink tubing. The exposed silver wire portion is then soaked in bleach for twelve hours to yield an Ag/AgCl surface. The silver wire is then placed into 1 mL syringe bodies, which have had the tip sealed by a 4 Å molecular sieve which had been soaking in 3 M KCl. The tip of the syringe is then filled with a warmed solution of 2 wt% agarose in 3 M KCl to seal it off, and then the remainder of the syringe body is filled with 3 M KCl. The silver wire is then placed in the syringe with the electrical wire coming out the back, and then sealed with parafilm and heat shrink tubing (see ESI). After construction, REs are then tested with a multimeter against a standard calomel electrode (SCE), with suitable readings between 20-40 mV. In our experience, a reading less than this window generally indicates a bubble in the syringe preventing the closing of the circuit, while a reading above this indicates oxidation of the silver. See ESI for detailed construction diagram and the “What to do if...” section for troubleshooting.

Different variants of REs can be constructed for specific applications, based on the chemistry of the aqueous environment to be evaluated. For example, when we were performing previous studies detecting aqueous hydrosulfide, the reference electrode silver wires were soaked in $(\text{NH}_4)_2\text{S}$ to form Ag/Ag₂S functionalized surface (see additional details in case study 2). This step was necessary for REs to function throughout the duration of the experiments.^{1,3} In lieu of these custom REs, a typical Ag/AgCl reference electrode can be used.

The design of our driver circuit is available in the ESI. The ChemFETs are driven by a DC power source at (4.7 V and 0.02 A) and a custom driver circuit. We use a Winsense™ WIPSK-

CB1 driver circuit, but this could also be simulated with a source measure unit (SMU). In operation, the drain voltage (V_{ds}) is held at 617.5 mV and the drain current (I_{ds}) at 100 mA. The external reference (typically Ag/AgCl) is held at ground, and the voltage between ground and the source (V_{gs}) terminal changes to maintain the values of V_{ds} and I_{ds} (Figure 3). V_{gs} is recorded as the measurement signal. An NI™ USB-6009 paired with a custom VI was used for data collection.¹

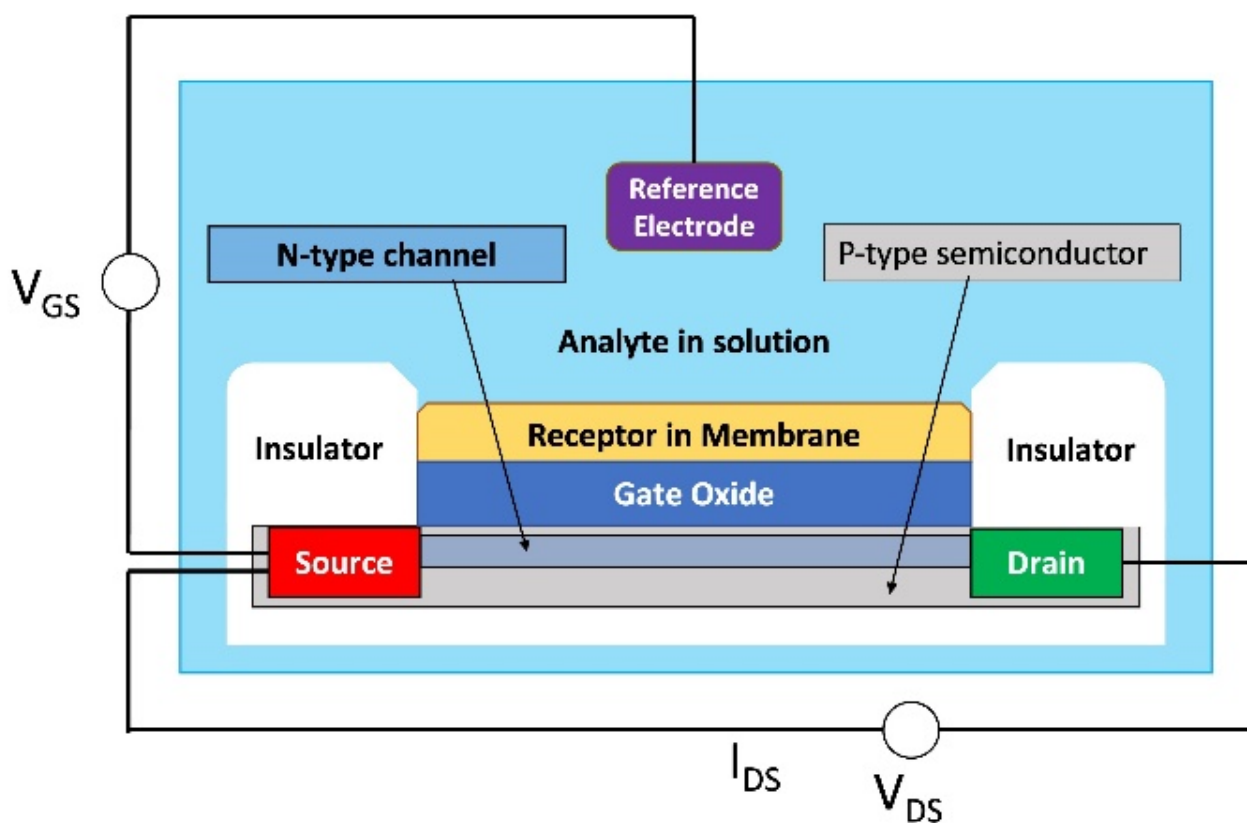


Figure 3. ChemFET diagram, with V_{GS} , V_{DS} , and I_{DS} indicated. V_{GS} is recorded as the measurement signal.

Sensor Preparation

We have found that drop-casting provides a suitable means of uniformly coating the ChemFET surface with the receptor-containing membrane. Drop-casting entails creating a solution containing a polymer, ionophore, and receptor. This solution is then applied by carefully controlled drops to the sensor surface, allowing solvent evaporation, and repeating until a membrane of required thickness (typically $\sim 50\text{ }\mu\text{m}$) is formed.

For neutral anion receptors, a quaternary ammonium salt is added as an ionophore to the dropcast solution to provide a fixed cationic nature to the membrane to facilitate anion mobility.^{1-3,11,16} Our lab has experienced best success using tetraoctylammonium nitrate (TOAN) for this, as the hydrophobic tetraoctylammonium does not leach out of the polymer membrane during ChemFET usage.^{1-3,11,14,16}

A suitable membrane has to be strong enough to withstand repeated immersion into analyte solutions, rinsing, and drying, while also remaining semipermeable to water and aqueous anions. In our typical experiments, we use 66 wt% high molecular weight polyvinyl chloride (PVC) (Sigma-Aldrich® 81387-250G), with 33 wt% *o*-nitrophenyl octyl ether (NPOE) (Sigma-Aldrich® 73732-5ML) included as plasticizer.

The dropcast solution is 1 wt% receptor, 2 wt% TOAN, and 97 wt% polymer blend (65 wt% PVC, 32 wt% NPOE). The dropcast solution is applied to the FET surface using micropipettes to deliver 1.6 μL at a time. Our technique generally requires 15-30 minutes between drops for drying, with typical drop-cast solutions comprising solvents such as acetone, tetrahydrofuran (THF), anisole, and chloroform. Higher boiling solvents such as dimethyl formamide (DMF) necessitate a much longer period between drops, and can benefit from being placed in an oven between drops to facilitate solvent removal. After the specified number of drops of drop-cast solution have been

applied, the ChemFETs are then placed in an oven overnight at 80 °C to ensure complete removal of the solvent. Our best experience is using 4 drops which results in a ~50 μm membrane.

Experimental Setup

Preparation of Test Solutions

The experimental setup for evaluating detection limits begins with solution preparation. This comprises the creation of a series of varying concentrations of analyte solution. Our lab's best practice for most anions is a series of 12 solutions of the following concentrations: 0.100 M, 50.0 mM, 10.0 mM, 5.00 mM, 1.00 mM, 500 μM , 100 μM , 50.0 μM , 10.0 μM , 5.00 μM , 1.00 μM , and 0.500 μM analyte, each with non-interfering buffer. Creating solutions with analyte in deionized (DI) water alone can misleadingly drop the signal at low concentrations due to low conductivity, resulting in an artificially low detection limit. To prevent this, a good practice is to incorporate a constant concentration of a non-interfering buffer to ensure the ionic strength of the solution never diminishes completely. After much evaluation, our lab has found that 50.0 mM piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES) buffer works well as a non-interfering buffer.

Selectivity Study

The experimental setup for a selectivity study will follow the previous section, with one important difference: each of the series of solutions of varying analyte concentration will also contain a constant concentration of competing interferent. The concentration of both analyte and interferent at the interception of flat background with Nernstian slope is used to calculate the selectivity coefficient (depicted in Figure 1) using the Nikolsky-Eisenman equation.

Procedure

Our lab defines a ChemFET evaluation as four sensors run through the full series of twelve solutions three times each. This triplicate evaluation also includes alternating runs that begin at the lowest and highest end of the solution series to account for possible drift in the voltage measurements. Additionally, this aids in identifying irregular sensor feedback and system troubleshooting.

Each run begins by connecting all sensors and reference electrode, and immersing in the first solution to be evaluated. In our experience, a five-minute run allows the sensor reading to stabilize, and provides consistent and reproducible data. After the first run is complete, the sensors and RE are removed from solution and submerged/rinsed in DI water. Then, they are dried and immersed in the next solution, after which the second run begins. This is repeated for each of the analyte solutions, and then repeated again for the number of runs desired.

Data Analysis

The major figures of merit in ChemFET sensor measurements are detection limit, selectivity, and sensitivity. The previous sections outlined how to mathematically calculate these figures, and an example spreadsheet is included for reference (see ESI).

Equipment

Equipment required to perform these measurements includes:

- Laptop
- Power supply
- Driver circuit

- FETs
- *LabVIEW™ license (if using LabVIEW™ compatible VI to run the ChemFETs)

What to do if...

The setup and operation of ChemFET sensors can at times necessitate troubleshooting techniques unfamiliar to those without prior electrochemical sensor experience. This section outlines common troubleshooting tips that have been identified and passed on within our lab to assist chemists in the setup and operation. Many commonly-encountered problems are listed, followed by a number of potential causes and/or solutions. As the title suggests, this section is organized by outlining common problems observed, what the possible reason might be, and subsequent troubleshooting/solution.

1. All voltages are at 0 V
 - a. No reference electrode plugged in
 - b. Power supply is switched off
 - c. Driver circuit is switched off
2. All voltages are at 3 V
 - a. Reference electrode went bad (can visually tell silver has turned lighter color from degradation, we have been unsuccessful at recycling them at this point)
 - b. If RE tests as reliable, then a bubble might be residing in the RE tip preventing closing of the circuit. Vigorous agitation of the RE in the solution can mitigate
 - c. All ChemFETs are dying
 - i. Note: This could also happen with old ChemFETs, as the plasticizer can leach out of the membrane over time, reducing/eliminating aqueous

permeability. Stripping membrane, re-treating with H_2O_2 , and re-dropcasting can recycle the sensors.

3. Individual sensor voltage is at 0 V
 - a. Leads switched (source and drain are flipped)
 - i. See ESI for how to mitigate this with color-coded electrical wiring
 - b. ChemFET disconnected
4. Individual sensor voltage is at 3 V
 - a. ChemFET is dying
 - b. Plasticizer has leached from polymer membrane, rendering it less permeable to water. This can result in the voltage reading approaching 3 V, indicating that the membrane needs to be stripped and the sensor re-dropcast.

Case Studies

Two case studies are presented showcasing the utility of ChemFET evaluations to solve unique problems. Both case studies outline some of the experimental design considerations that were made, based on the specific characteristics of the receptors and analytes involved. These examples should provide a useful reference in guiding future experiments, providing some general examples of how ChemFET sensors can be adapted and used.

Case Study 1

The first example to highlight is the use of ChemFETs to detect and measure aqueous concentrations of barbituric acid (BBA).² A number of Hamilton-type receptors had previously been reported to have displayed host-guest interactions with BBA.^{21–24} Two of these Hamilton-type receptors (pink and gray boxes in Figure 4) were integrated into selective membranes of ChemFET-based sensors to evaluate for selectivity of BBA over a series of common interferents in various aqueous media.² This evaluation consisted of determining detection limits for purposes of direct comparison of relative affinities, as well as a selectivity study to characterize interaction of the receptors with BBA in the presence of competing species.

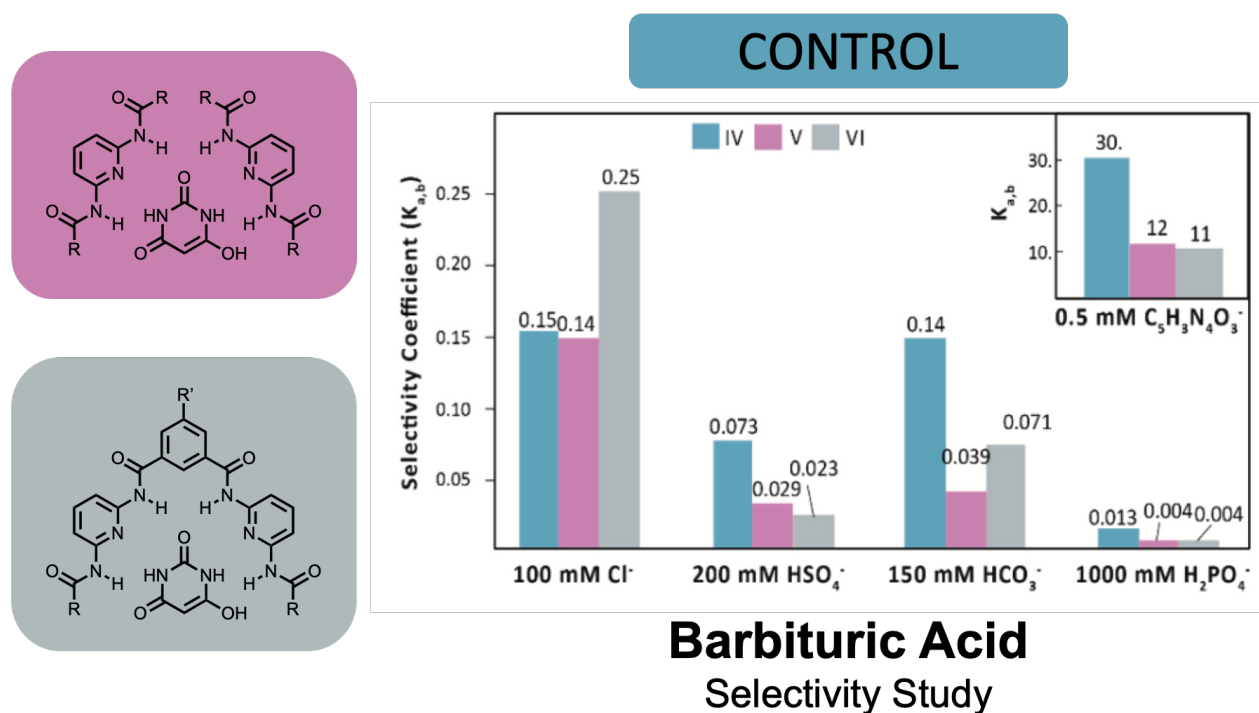


Figure 4. Fixed interferent evaluation to determine selectivity of Hamilton receptor for barbituric acid (BBA) over various anion interferents (right) including chloride, sulfate, carbonate, phosphate, and urate ($C_5H_3N_4O_3^-$). Blue bars labeled IV indicate sensors that contain no receptor (control), pink bars labeled V contain a Hamilton receptor (depicted in the pink box on the top left, where $R = C(CH_3)_3$), and blue bars labeled VI contain a different Hamilton receptor (depicted in the grey box on bottom left, where $R = C(CH_3)_3$, $R' = H$). Both Hamilton receptors depicted in a geometry with BBA in the binding pocket.

The construction of the sensors followed the previously outlined procedures, with nitrile butadiene rubber (NBR) chosen as membrane material, to which the TOAN ionophore and Hamilton-type receptors were added. Drop-casting sensor surfaces was accomplished as previously described, comprising ten 1.6 μL drops of the dropcast solution containing all the membrane components. The BBA ChemFET evaluation took place in four different aqueous media solution types: PIPES buffer, synthetic urine, river water, and 5 mM xanthine in PIPES buffer. These were chosen to provide a spread of media representative of real-world sensing applications in environments ranging from watersheds to urine samples.

The primary figures of merit reported in this paper were anion detection limits and selectivity coefficients for BBA over interferents. Both figures of merit were calculated as previously demonstrated, with results displayed in Figure 3. Of note is that this study demonstrated the ability for chemFET-based sensors incorporating Hamilton receptors to detect BBA in various media, and additionally this paper established the first known reported potentiometric method for direct determination and quantitation of BBA.²

Case Study 2

The second case study is the use of ChemFETs to detect and characterize aqueous hydrosulfide host-guest interactions (Figure 5).¹ Members of the bambus[6]uril family of anion receptors had been previously reported to bind with hydrosulfide.²⁵ A variant of the bambus[6]uril receptor family was chosen to characterize interactions with aqueous hydrosulfide using ChemFET sensors. Dodeca n-butyl bambus[6]uril was synthesized and integrated into the ChemFET selective membrane. This evaluation served to exploit interaction between the receptor and aqueous

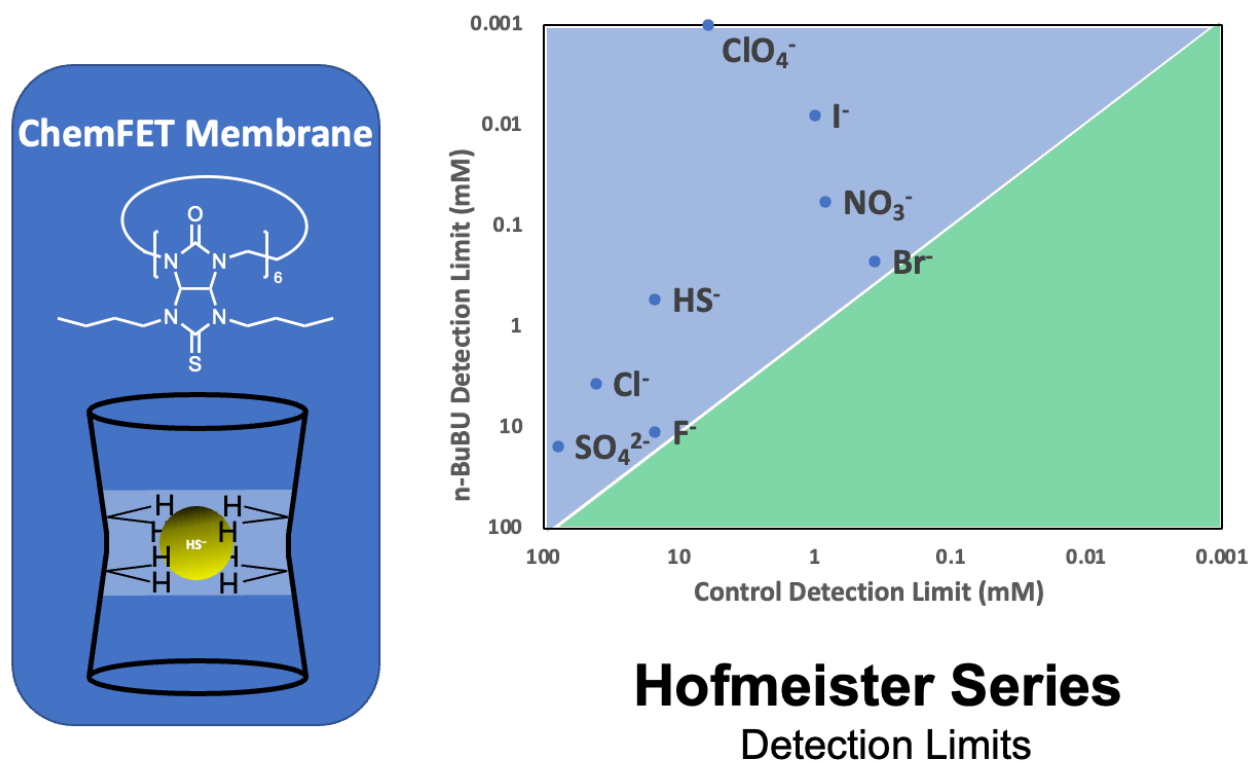


Figure 5. (left) Depiction of hourglass-shaped dodeca-*n*-butyl bambus[6]uril incorporated into ChemFET membrane to ascertain position of hydrosulfide anion in the Hofmeister Series. (right) Graph of detection limits in mM by anion towards *n*-butyl bambus[6]uril (y-axis) versus control (x-axis). Any anion appearing in the bottom right green triangle would indicate preferential affinity towards control, while any anion appearing in the top left blue triangle indicates preferential affinity towards to *n*-butyl bambus[6]uril. Any anions appearing on the dividing line (Br and F) showed no difference between *n*-butyl bambus[6]uril and control.

hydrosulfide to facilitate measuring concentration, as well as evaluate a number of other anions to establish the relative placement of hydrosulfide in the Hofmeister series.

The membrane chosen for this evaluation was PVC, with NPOE plasticizer and TOAN ionophore. A PVC membrane was chosen instead of the previously-used NBR due to adhesion issues that previous NBR-based sensor membranes suffered from. These three components, along with n-butyl bambus[6]uril receptor were dropcasted onto ChemFET sensors following the previously outlined procedures, using four 1.6 μL drops of dropcast solution containing all membrane components. Initial hydrosulfide detection limits were established, along with selectivity coefficients of hydrosulfide over competing species, chloride and L-cysteine. The sensors then were characterized for anion affinity over a number of anions in the Hofmeister series for reference. Standard Ag/AgCl REs were used for all anion evaluations except hydrosulfide, which degraded regular sensor components due to the reactivity of the analyte. This required special modification of the REs for the hydrosulfide evaluation. For this evaluation, REs were treated with $(\text{NH}_4)_2\text{S}$ to create Ag/Ag₂S REs that lasted the duration of the tests.

Detection limits of both control and n-butyl bambus[6]uril-containing sensors were plotted in Figure 5. Anions in the top left of the figure indicate preferential affinity for n-butyl bambusuril, anions in the bottom right would indicate preference for control, and anions located along the diagonal line between quadrants indicate no difference in affinity between n-butyl bambus[6]uril and control. This paper established an experimental placement of hydrosulfide in the Hofmeister series.¹

Conclusions

ChemFETs can fill a unique void in host-guest chemistry, particularly when researching specific aqueous anion affinity of hydrophobic receptors. The ability to functionalize ChemFET membranes provides an opportunity to characterize—or at least model interfacially—aqueous host-guest interactions using water-insoluble hosts without further host receptor modification. This approach is quite customizable to tailor to specific host-anion interactions, differing solubilities between receptor and analyte, etc. The use of anion receptors in ChemFETs merits much further study, especially for those interested toxic or reactive anions that are difficult to monitor stoichiometrically by traditional solution phase host-guest studies. Studying the interfacial interactions between ChemFET membranes and these challenging anions can help inform future detection efforts, host design, and ultimately remediation strategies, and ChemFETs are ideally suited for these studies. The following chapters describe numerous uses of ChemFETs to describe host-guest interactions and anion affinity profiles.

Outside the described electrochemical sensing research, an opportunity arose to incorporate a statistical technique based on the author's previous engineering experience in US Air Force flight testing. The world of flight testing is inherently hazardous and costly, driving constant improvement in test planning and data analysis. A widespread focus on integrating design of experiments (DOE) into flight testing was well documented in the early 2000s,^{26,27} and was eventually mandated by the US Office of the Secretary of Defense (OSD) Director of Operational Test & Evaluation (DOT&E) Dr. J. Michael Gilmore in 2010.²⁸ This resulted in extensive training and coursework for those involved in flight test planning and execution. The underlying principles of DOE involves the designing of tests for optimal data collection, characterizing a system and often able to uncover hidden relationships between inputs and output properties that may not be

apparent through traditional evaluations, similar to the use of ChemFET devices where traditionally inaccessible interfacial binding data can be acquired. This statistical methodology can also be applied to previously-recorded data, where similar relationships between input factors and output properties can be identified (although not as efficiently as when the evaluations are designed using DOE from the beginning).

This statistical characterization of a process lends itself very well to chemical syntheses, where often numerous experimental factors have specific impacts on the output properties that are not well understood. DOE can provide a means to design an experiment to fully characterize the specific impacts of input factors on output properties, and can additionally perform analysis on existing data to provide statistically-based characterization. Part 2 serves as an introduction to DOE, providing a case study and generally familiarizing the reader with the methodology prior to describing the author's use of DOE in Chapter 5.

Part 2: Design of Experiments analytical methodology for reaction characterization

Design of experiments (DOE) is a widely-used statistical tool that can design experiments to most efficiently glean statistically-significant data from a given set of experimental runs. Alternatively, the same DOE principles can be applied to historical data in order to characterize similar synthetic parameters, albeit less efficiently than a DOE-designed series of experiments. DOE will take a series of input factors and then characterize their influence on output factors. Often in chemistry these inputs are factors such as temperature, pressure, concentration, and pH. Common output factors include product yield, polydispersity, purity, and product distribution. DOE characterization of a system will provide statistically-ranked main factors and multi-factor interactions that influence system output properties. A common metric for these is p-value, based on the statistical probability of the null hypothesis (that the described factor has no impact on the output). P-values below 0.05 are generally considered to be statistically-significant. Following characterization of a system using DOE, predictively optimized conditions can then be ascertained and experiments ran to validate the model.

Case Study 3

One particularly notable example of DOE in pharmaceutical chemistry worked to optimize the drug delivery of the chemotherapy drug methotrexate (Figure 6).²⁹ This DOE analysis included evaluating historical drug delivery parameters, designing a series of experimental conditions to characterize the system, evaluation of the results, and validation of the predicted optimization conditions.

The factors of influence were predicted using previously-published historical data. The independent variables identified included polymer concentration, lipid concentration, and

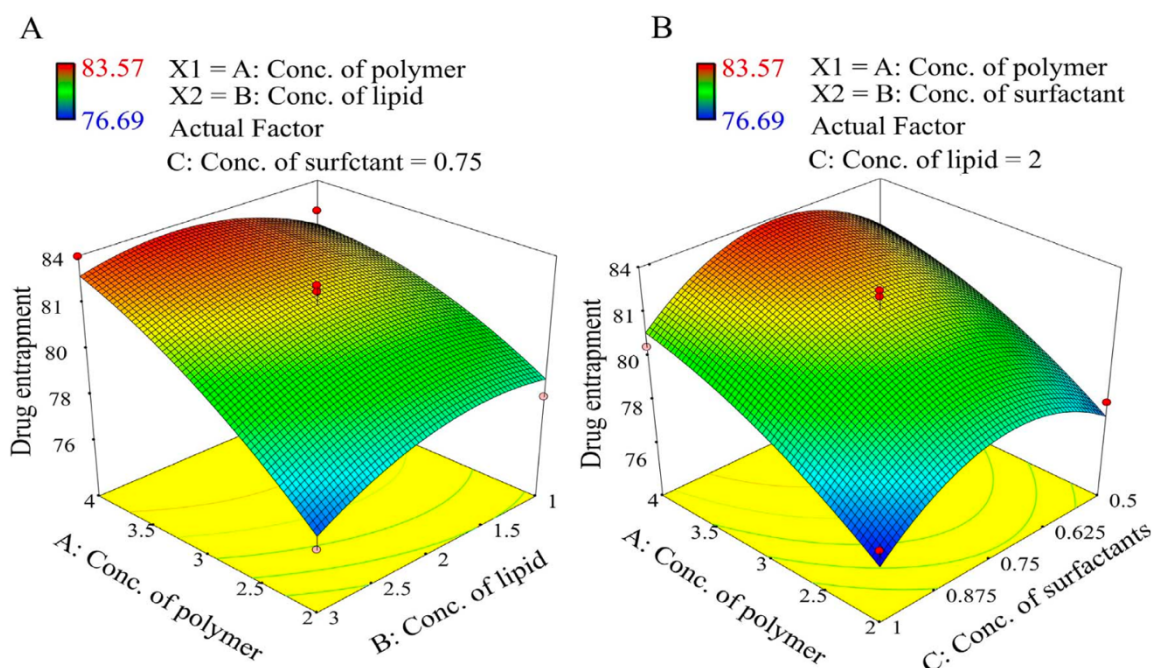


Figure 6. Design of Experiments optimization of methotrexate drug delivery. Depicted image includes two input factors (A: polymer and lipid concentrations, B: polymer and surfactant concentrations) on the base (X and Z axes), and output (drug entrapment) on the Y axis of both. Of particular importance is the center points, which helped evaluate the curvature present. If only the four corners had been evaluated, the assessed optimization condition (high on the Y axis) would have been predicted to lie outside the evaluated design space, whereas when the center point and subsequent curvature are included in the evaluation, the highest point is now found to lie within the design space, and parameters outside the design space would be predicted to degrade performance of desired output, in this case being drug entrapment.

surfactant concentration. The dependent variable outputs measured included particle size (minimize), entrapment efficiency (maximize), and drug release (maximize). Fifteen evaluation formulations of varying polymer concentration, lipid concentration, and surfactant concentration were evaluated to examine the resultant output properties, and a model was built using Design Expert® DOE software. The model included corners and center points (Figure 6) to determine curvature within the design space. The factors were analyzed with p value < 0.05 considered significant. This model was then used to predict 3 sets of optimization conditions, showing improvement in all facets of the desired optimization. Optimization formulation resulted in 181.3 ± 3.4 nm particles, $81.07\% \pm 2.11\%$ drug entrapment, and $73.95\% \pm 0.56\%$ drug release.

Of particular note in this project was the importance of widely varying evaluation conditions to accurately model the entire design space. If no center points had been evaluated to determine curvature, the desired optimization conditions could be incorrectly predicted to lie outside the evaluated design space. However, center points allowed for calculation of curvature, indicating the maxima lay within the evaluated design space, and parameters outside the design space in any direction would likely result in worsened results. This is best analyzed visually in Figure 6 by observation that the highest point of drug entrapment (most desirable) lies within the evaluated area, whereas if only the corners had been evaluated the direction for optimization would have lead outside the design space where the 3D graph indicates worsening (lowering) of the desired drug entrapment.

This case study serves as an illustration of the utility of using DOE to characterize a system and provide conditions to optimize the desired outputs. The following chapter 5 will serve to report the author's use of DOE to characterize an inorganic nanocluster synthetic system, as well as the validation results to highlight the utility of the methodology.

Co-Authored Work

Chapter 1 was written by myself with editorial assistance from coauthors Dr. Grace Kuhl, Dr. Sean Fontenot, and Prof. Darren Johnson. Chapter 2 was written by myself and Dr. Grace Kuhl, with assistance from Dr. Hazel Fargher, Willow Davis, Madeline Howell, Dr. Lev Zakharov, Prof. Michael Pluth, and Prof. Darren Johnson. Chapter 3 was written by myself, with assistance from coauthors Dr. Grace Kuhl, Madeline Howell, and Prof. Darren Johnson. Chapter 4 was written by myself, with assistance from Natalie Lakanen, Audrey Davenport, Dr. Jiawei Huang, Prof. Carl Brozek, and Prof. Darren Johnson. Chapter 5 was written by myself and Alex Rosen, with assistance from Victor Salpino and Prof. Darren Johnson. Chapter 6 was written entirely by myself, with editorial assistance from Dr. Jacob Mayhugh, Dr. Grace Kuhl and Willow Davis. All of the analysis and the majority of the synthesis was performed by myself. For the collaborative projects, synthesis was also performed by Dr. Grace Kuhl and Madeline Howell (bambus[6]uril, semithioglycoluril and semithio bambus[6]uril), Andy Davis in the Pluth Lab (imidazolium), Nathan Boone & Nolan McNeill in the Haley Lab (azaphosphinine and arylethynyl bisurea), and Allison LaSalvia in the Cook Lab (carbonylation).

Bridge

This chapter introduced the concept of using ChemFETs to characterize aqueous anion affinity of hydrophobic anion receptors, as well as the concept of using DOE to characterize and optimize synthetic systems. The remaining chapters will discuss the implementation of these concepts in a number of published and unpublished projects in order to solve difficult problems and meet challenging demands of host-guest characterization and reaction optimization. Chapter 2 will describe the use of ChemFETs in analyzing the host-guest anion affinity profile of dodeca-n-butyl

bambus[6]uril, as well as the utilization of that receptor to report the first ever placement of hydrosulfide in the Hofmeister series. Chapter 3 further describes host-guest characterization of two members of the bambus[6]uril family of anion receptors, examining the impact of steric bulk and binding pocket geometry on the anion affinity profile. Chapter 4 describes the first reported use of ChemFETs to characterize the host-guest affinity of metal organic frameworks (MOFs) with anions. Chapter 5 describes the use of DOE to characterize synthesis of an aluminum oxide nanoparticle, enabling the optimization of desired output parameters including particle size and polydispersity. Finally, Chapter 6 discusses all other unpublished work, including receptor synthesis and characterization using ChemFETs, as well as other DOE efforts. Any supplemental information referenced in-text can be found in the appendices of this manuscript.

CHAPTER II

BENCHMARKING THE PLACEMENT OF HYDROSULFIDE IN THE HOFMEISTER SERIES USING A BAMBUS[6]URIL-BASED CHEMFET SENSOR

From G. M. Kuhl, D. H. Banning, H. A. Fargher, W. A. Davis, M. M. Howell, L. Zakharov, M. D. Pluth and D. W. Johnson, *Chem. Sci.*, 2023, **14**, 10273–10279.

Abstract

Hydrosulfide (HS^-) is the conjugate base of gasotransmitter hydrogen sulfide (H_2S) and is a physiologically-relevant small molecule of great interest in the anion sensing community. However, selective sensing and molecular recognition of HS^- in water remains difficult because, in addition to the diffuse charge and high solvation energy of anions, HS^- is highly nucleophilic and readily oxidizes into other reactive sulfur species. Moreover, the direct placement of HS^- on the Hoffmeister series remains unclear. Supramolecular host-guest interactions provide a promising platform on which to recognize and bind hydrosulfide, and characterizing the placement of HS^- in the Hofmeister series would facilitate the future design of selective receptors for this challenging anion. Few examples of supramolecular HS^- binding have been reported, but the Sindelar group reported HS^- binding in water using bambus[6]uril macrocycles in 2018. We used this HS^- binding platform as a starting point to develop a chemically-sensitive field effect transistor (ChemFET) to facilitate assigning HS^- to a specific place in the Hofmeister series. Specifically, we prepared dodeca-n-butyl bambus[6]uril and incorporated it into a ChemFET as the HS^- receptor motif. The resultant device provided an amperometric response to HS^- , and we used this device to

measure the response of other anions, including SO_4^{2-} , F^- , Cl^- , Br^- , NO_3^- , ClO_4^- , and I^- . Using this response data, we were able to experimentally assign that HS^- lies between Cl^- and Br^- in the Hofmeister series, which matches recent theoretical computational work that predicted a similar placement on the Hofmeister series. Taken together, these results highlight the potential of using molecular recognition coupled with ChemFET architectures to develop new approaches for direct and reversible HS^- detection and measurement in water and further advance our understanding of different recognition approaches for this challenging anion.

Overview

Hydrosulfide (HS^-) is the conjugate base of the gasotransmitter hydrogen sulfide (H_2S), and is a physiologically-relevant small molecule of emerging interest in the anion sensing community.^{1–}
¹⁰ The selective recognition and detection of HS^- in water, however, remains difficult due to the diffuse charge and high solvation energy of anions in general,⁴ as well as the high nucleophilicity and redox activity of HS^- .⁵ Different detection and quantification methods for H_2S have been reported and typically rely on electrophilic trapping or reaction with activity-based probes to generate a fluorescent response.^{5,11,12} An alternative approach to activity-based methods that irreversibly couple an H_2S -mediated reaction with an optical response is to use host-guest chemistry to enable reversible $\text{H}_2\text{S}/\text{HS}^-$ detection. Early examples of this approach have included investigations into the ability of hosts to bind HS^- as an anion of interest.^{1–3,6,7,10} A key and unmet need for advancing reversible HS^- detection is a better understanding of the placement of HS^- within the Hofmeister series, which would facilitate the development of receptors with engineered specificity for HS^- over other competing anions. Prior work has described the HS^- position in the Hofmeister series as “unclear”² and “rarely reported.”¹³ Previously, our lab has used chemically-

sensitive field effect transistors (ChemFETs) monitor reactive species that are otherwise difficult to characterize. Here we report the translation of a reversible HS⁻ binding receptor to develop a ChemFET sensor for the hydrosulfide anion. Moreover, we use this system to provide an experimental benchmark for the placement of HS⁻ in the Hofmeister series, which we anticipate can be used to further advance new approaches for selective HS⁻ binding in synthetic receptors.

ChemFET Design and Response

Electrochemical potentiometric sensors, which incorporate simple instrumentation that is easily integrated with chip-based devices,^{14,15} are one option for HS⁻ detection. Potentiometric sensors interface different membrane designs with sensor components, including inorganic crystal lattices,¹⁶ lipophilic ionophores,¹⁷ or metal-ligand coordination to impart analyte selectivity.^{18,19} The selectivity of an electrochemical sensor is derived from the membrane, and different components that impart selectivity can be incorporated into these membranes. Such components are generally ionophores, which are often either biological or synthetic structures with tailored noncovalent intermolecular interactions, such as hydrogen bonding and related electrostatic forces, that interact selectively with specific analytes.²⁰ Given the well-characterized nature of sensor construction, lipophilic ionophore sensors are therefore a viable option for anion sensing since membrane compositions and properties are well known and easy to process.

ChemFETs have a different internal construction than ion-selective electrodes (ISEs), but they provide a logarithmic potentiometric response to changes in analyte concentration. ChemFETs are useful for aqueous ion sensing due to their fast response, stability, reusability,^{15,20} high signal-to-noise ratios, and low sample volume requirements.²¹ The ChemFET response relies upon the

change in potential at the sensing gate, which is covered by an ionophore-doped semi-permeable polymeric membrane to impart selectivity towards the analyte of interest (Figure 1).

In general, ChemFETs exploit intermolecular interactions (in this case, host-guest interactions between bambusuril and a target anion) to generate a concentration gradient of charged species, which results in a shifting surface potential that impacts a depletion layer between the source and drain terminals.²² This layer allows electrons to flow and a current to be measured, and the magnitude is altered by the field effect and directly relates to the concentration of target analyte in the sample solution.²³

ChemFETs are particularly useful to facilitate measurements and characterization of target analytes that are reactive or otherwise difficult to work with. This allows for characterization of species, including assessing the placement of an analyte within the Hofmeister series, that are otherwise inaccessible.

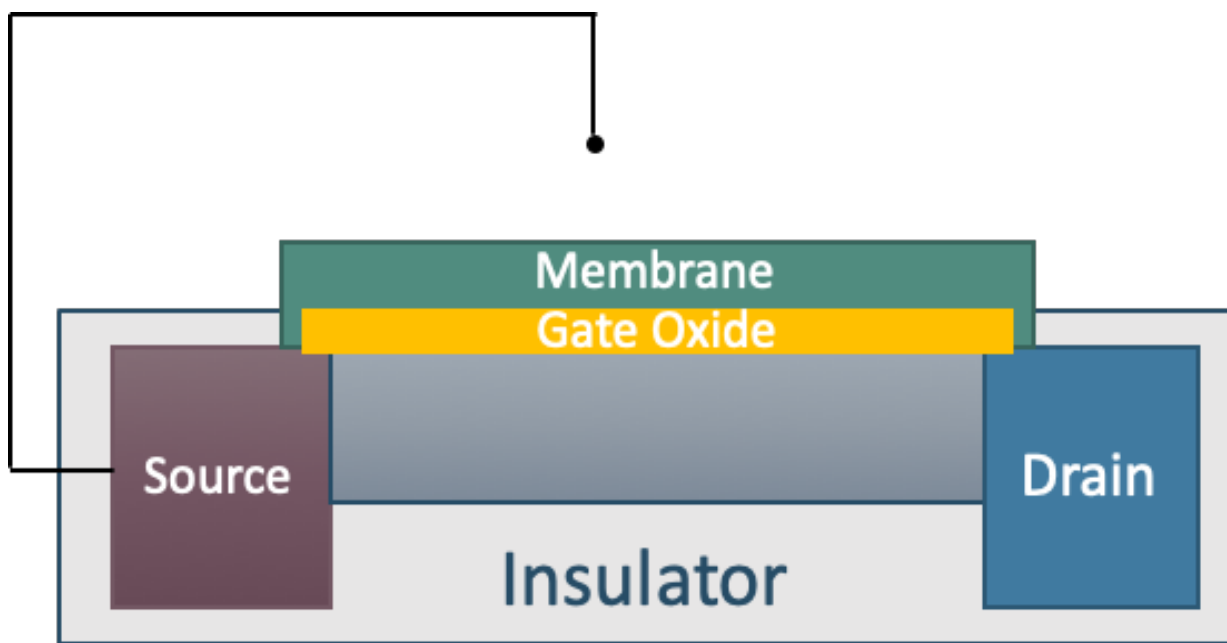


Figure 1. Chemically selective field effect transistor (ChemFET) general diagram. For sensor construction, pre-constructed FETs are covered with insulating epoxy, except for the gate nitride/oxide surface, which is coated with the ionophore-containing membranes. Reference electrodes are constructed separately.

Ionophores for Hydrosulfide

Recent work in our labs introduced the first proof-of-concept HS^- ChemFET sensor using a nitrile butadiene rubber (NBR) coating and tetraoctylammonium nitrate as a simple, lipophilic ionic additive, or ionophore.⁸ Although this approach did not contain a specific receptor for HS^- , the lipophilic cation yielded modest selectivity for HS^- over competing species in water, with selectivity coefficients of 0.12 and 0.13 over Cl^- and L-cysteine, respectively. We expected that direct incorporation of a supramolecular cavitand receptor as a neutral ionophore in a polymer membrane would impart selectivity for aqueous HS^- and improve the figures of merit of this device.

When considering available systems for HS^- binding^{1,2,6,24} and the above criteria, we chose to use the bambus[6]uril system reported by the Sindelar group in 2018.⁷ Bambus[6]urils contain four or six repeating bicyclic glycoluril units connected by a bridging methylene (Figure 2).²⁵

These receptors have a defined cavity size, tunable functional groups, and strong anion affinity in water and organic solvents due to strong C–H hydrogen bonding interactions in the cavity.^{25–27} We surmised that integrating a bambus[6]uril receptor into a ChemFET polymer membrane would increase sensitivity and selectivity toward HS^- . Further supporting this idea, recent work reported a bambus[6]uril based ionophore used to develop a perchlorate-selective ISE-based sensor.²⁸

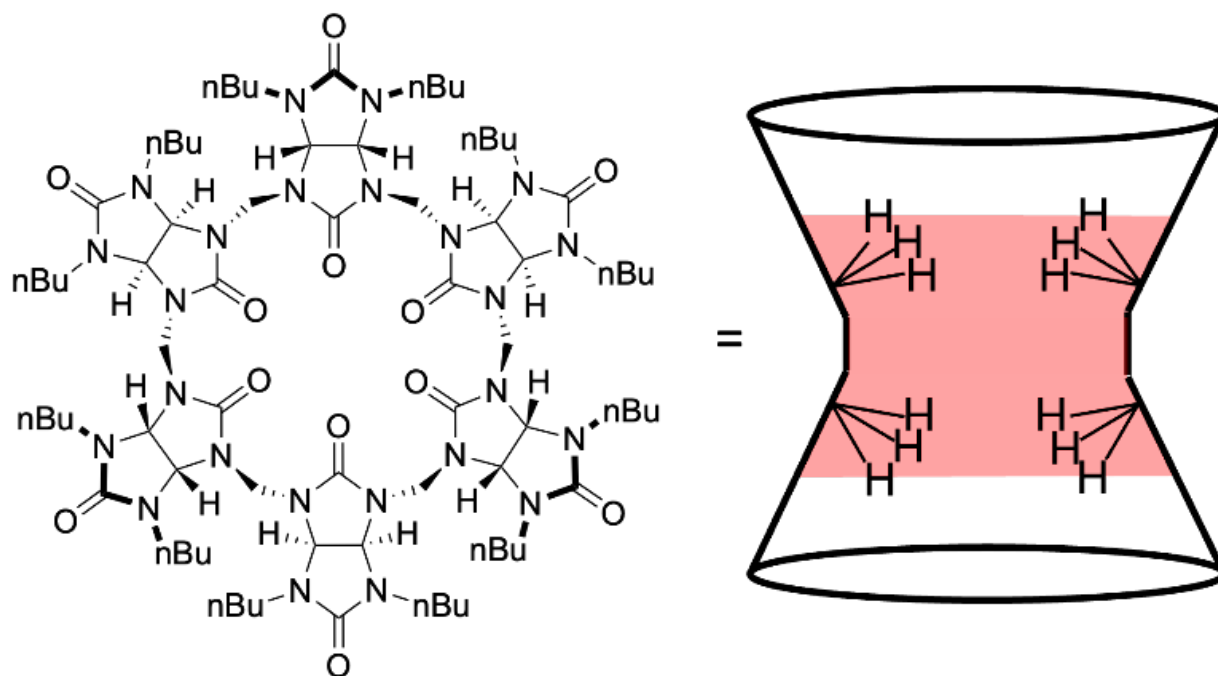


Figure 2. Three-dimensional representation of dodeca-n-butyl bambus[6]uril (left). Simplified figure depicting the hourglass-type shape common to bambus[6]urils, with binding pocket depicted in pink (right). As with the rest of bambus[6]uril family of macrocycles, this receptor is comprised of repeating glycoluril subunits of alternating orientation.

Hofmeister Series

Originally discovered while studying salt effects on protein solubility, Hofmeister effects remain an active area of research in interfacial and host-guest chemistry.^{29,30} The Hofmeister series is an experimentally-derived ranking of ions based on their ability to salt proteins in or out of solution.²⁹ Interestingly, other physical phenomena also follow this same ranking, including surface tension and potential.^{29,31} Anions on the chaotropic (or *charge diffuse*) end of this series, such as ClO_4^- and I^- , increase protein solubility and decrease surface tension and potential, whereas anions on the kosmotropic (or *charge dense*) end of the series, such as SO_4^{2-} and F^- , increase surface tension and potential while lowering protein solubility (Figure 3).²⁹⁻³²

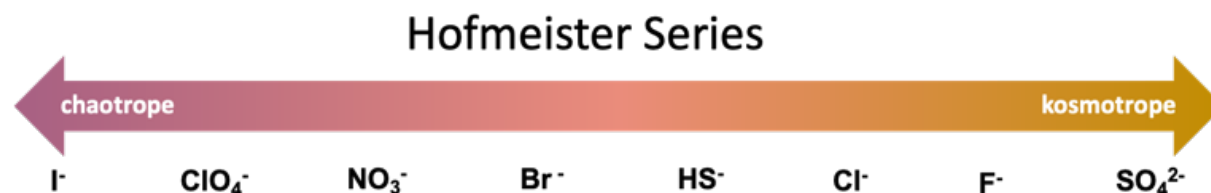


Figure 3. Common anions in the Hofmeister Series. This represents a common reference framework for electrochemical sensors. Electrochemical sensors are commonly more sensitive to **charge diffuse** chaotropes (left) than **charge dense** kosmotropes (right). In this study, we place HS^- in the series between Cl^- and Br^- (vide infra).

Although many of the phenomena that follow the Hofmeister series trend were studied in bulk solution, the Hofmeister trend can also be observed at aqueous interfaces³² such as those found at ISE or ChemFET membranes in an electrolyte solution.³³ The fundamental interactions that influence the Hofmeister effect are not fully understood, but are thought to be a combination of ion size, ion hydration energy, polarization, electrostatics, as well as other phenomena.³¹ Recent work by Page et al. used computationally-derived linear charge density of different anions to rank anion positions on the Hofmeister series.³⁴ In potentiometry, the Hofmeister effect is displayed even in the absence of a membrane-bound ionophore, which leads to an even greater sensitivity to chaotropes than kosmotropes.^{35,36}

Results and discussion

Hofmeister Series Comparison

To investigate whether host incorporation into the ChemFET membrane could be used to place HS^- in the Hofmeister series, we first prepared control sensors without bambusuril ionophore and tested these devices with eight representative anions in the Hofmeister series (Figure 4).

The eight anions were then ranked by detection limit to produce a qualitative Hofmeister-type ranking order. We then performed a second set of experiments under identical conditions, but included the dodeca-*n*-butyl bambusuril ionophore in the gate nitride membrane. This second set of experiments served a twofold purpose: 1) to examine if bambusuril ionophore would increase sensitivity toward HS^- , and 2) to determine whether any re-ordering of anions in reference to the Hofmeister series that would indicate either increasing or decreasing binding preference and selectivity. By comparing these two ChemFET devices we were able to measure the relative affinity and determine whether there was a Hofmeister bias in ion sensitivity in the sensor. In addition to informing on the Hofmeister series, these experiments also inform on the performance

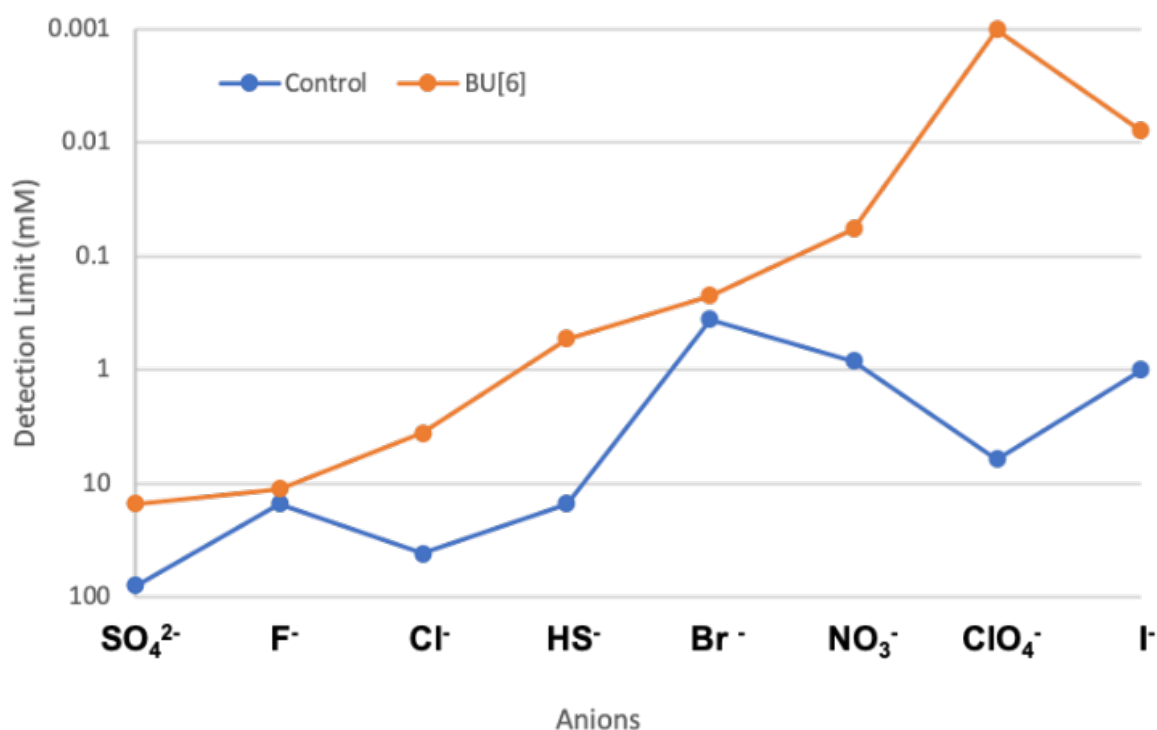


Figure 4. Results of control (no ionophore) sensors in blue, and *n*-butyl bambusuril sensors in orange. A lower detection limit (up on the y-axis) indicates stronger recognition. F^- and Br^- show little difference between control and ionophore-containing sensors, whereas ClO_4^- shows the largest difference. The overall trend shows the most sensitivity to the chaotrope end of the Hofmeister series (I^- and ClO_4^-) and the least sensitivity to the kosmotrope end (SO_4^{2-} and F^-).

of n-butyl bambusuril as an HS^- ionophore. Upon incorporation of 1 wt. % dodeca-n-butyl bambus[6]uril into the PVC-based ChemFET membrane, a lower detection limit was observed for all anions measured, which supports the importance of the host-guest interaction between the bambusuril ionophore and the anion.

Hofmeister Series Computations

Initially, the ChemFET sensor runs were intended to provide a qualitative ranking of common anions in a Hofmeister-like series for evaluation of relative affinities. However, the measured detection limits also provide a quantitative means of comparison.[‡] One key benefit of using ChemFETs to evaluate anion receptors is that they provide quantitative binding data on highly reactive species within minutes. Recently, Page et al. published a Hofmeister series study indicating that theoretical calculation of linear charge density compared very favorably with the experimental placement of anions on the Hofmeister series.³⁴ We were able to use our ChemFET binding data to validate these theoretical computations, including for anions that had not been previously validated experimentally. Specifically, we used the experimentally-derived ChemFET detection limits to place HS^- on the Hofmeister series experimentally. Gratifyingly, this experimental placement of HS^- in the series, specifically between Cl^- and Br^- , matched the theoretical placement by Page et al. (Table 1).

Moreover, this placement also corroborates experimental data on $\text{CH}\cdots\text{S}$ interactions that indicate HS^- most closely resembles Cl^- in its binding interactions with related hosts.^{34,37,38} Of particular interest is that the detection limit and binding constant trends mirrored each other.[‡]

		I ⁻	ClO ₄ ⁻	NO ₃ ⁻	Br ⁻	HS ⁻	Cl ⁻	F ⁻	SO ₄ ²⁻
Computations (linear charge density, C·M ⁻¹)	Theoretical (spherical)	-4.90	-5.53	-6.26	-5.63	-5.68	-6.25	-8.64	-10.25
	Theoretical (polyatomic)		-6.15	-5.78		-6.39			-8.19
No Bambusuril (control)	Detection Limit (mM)	1.0	6.1	0.83	0.36	15	41	15	78
n-butyl Bambusuril	Detection Limit (mM)	0.0078	0.0010	0.057	0.22	0.53	3.6	11	15

Table 1. Comparison of Page et al. Hofmeister computational data (top box) with ChemFET experimental sensor data (bottom two boxes). Of particular note is one of the only reported Hofmeister series placements of hydrosulfide agrees with the computational placement between bromide and chloride.

In addition to the experimental validation of the theoretical Hofmeister placement of hydrosulfide, these results highlight the utility of using electrochemical sensors in this application. ChemFETs appear to be uniquely suited to quickly and efficiently measure Hofmeister trends for highly reactive species that are otherwise unsuitable for other measurement techniques.

Hydrosulfide Selectivity

To further investigate whether the ChemFET approach could be used for future HS⁻ detection, we also evaluated the affinity for HS⁻ over other competing anions to provide a selectivity coefficient. The selectivity coefficient is calculated via a fixed interferent experiment, where the concentration of an interfering anion is kept fixed, and the concentration of target analyte HS⁻ is varied. The concentration at which hydrosulfide is detected above the level of the fixed interferent

is used to calculate the selectivity coefficient $K_{A,B}^{pot}$ using the following equation: $K_{A,B}^{pot} = \frac{a_A}{a_B^{z_A/z_B}}$ where a represents the activities of the target analyte A and interferent B at the limit of detection, and z_A and z_B are the respective charges. If $K_{A,B}^{pot} < 1$, the membrane is selective towards the target analyte A. For example, if the selectivity coefficient $K_{A,B}^{pot}$ is 0.10, the system is detecting 10 target anions

for every 1 interferent.^{19,20} After observing the Hofmeister effect and assessing trends based on bambusuril affinity toward a series of representative anions, we next investigated the specific affinity toward HS⁻ over competitive anions. In the previous section, reported results were experimentally observed in solutions with non-competing piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) buffer. However, this selectivity evaluation involved the examination of HS⁻ affinity in the presence of competing anions. The buffer pH was adjusted to pH 8 to ensure sufficient HS⁻ and cysteine thiolate speciation for accurate evaluation of anion interaction with bambusuril receptors in chemFET sensors.

Selectivity experiments were designed to evaluate interferences of practical interest when attempting to use electrochemical sensors in HS⁻ detection applications. Chloride is similar in size to HS⁻, which is often an important factor in determining selectivity in host-guest chemistry and supramolecular interactions. Cysteine is a thiol-containing biomolecule that introduces a competing -SH group. We screened both as interferences, since they should be among the most competitively-binding anions based on similarities to hydrosulfide.

In both cases, incorporation of n-butyl bambusuril resulted in a selectivity coefficient of less than one. Calculated selectivity coefficients were 0.4 for chloride, and 0.037 for cysteine. Selectivity coefficients less than one indicates preference for HS⁻ over both Cl⁻ and cysteine thiolate. We attribute the large preference for HS⁻ binding over cysteine to the size limit of the bambusuril binding cavity.

Conclusions

ChemFETs provide a useful approach to exploit supramolecular host-guest interactions between hydrophobic hosts and aqueous anion guest. Highlighting the utility of this platform, we

have demonstrated that receptor-containing ChemFETs can be designed to bind HS^- , which is often difficult to work with and characterize due to its toxicity and reactivity. Incorporation of selective ionophores into the gate oxide membrane of a ChemFET provides the ability to measure aqueous anion concentration in real time, facilitating direct comparison to other anions of interest. We found that incorporation of dodeca-n-butyl bambus[6]uril into the ChemFET gate membrane improved competitive HS^- selectivity over size-similar Cl^- , and thiol-containing cysteine. Further characterization of the bambus[6]uril ChemFETs against common anions in the Hofmeister series provided the relative affinity differences compared to the unaltered Hofmeister series arrangement. We found our electrochemical sensors to generally be more sensitive to charge diffuse chaotropes and generally less sensitive to charge dense kosmotropes. This trend held for both the control sensors as well as bambus[6]uril ionophore-containing sensors, with the ionophore further improving (or lowering) detection limits.

More broadly, our approach allowed for experimental validation of the previously reported theoretical calculations by Page et al. of HS^- placement in the Hofmeister series. Not only does this study serve to experimentally validate the Hofmeister placement of this one particular anion of interest, but it sets a precedent for the use of electrochemical sensors for similar types of benchmarking that may not be otherwise feasible due to challenges with synthesis, toxicity, reactivity, etc. Furthermore, these results provide an approach for designing an anion receptor with preferential binding for one particular target analyte over competing species based on tailored receptor size and hydrogen bond donor configuration, which is well-known for cationic analytes but far less studied for their negatively charged counterparts.

Materials and Methods

Electrode and Membrane Preparation

Silicon nitride-gated field effect transistors (FETs) were purchased from Winsense (<http://www.winsense.co.th>, WIPS-C) and cleaned with ethanol and soaked in H_2O_2 for 10 minutes prior to functionalization. Polyvinyl chloride (PVC), 2-nitrophenyl octyl ether (NPOE), and tetraoctylammonium nitrate (TOAN) were obtained from Fisher Scientific and TCI Chemicals. Chemically selective membranes were deposited onto the FET surface by manual drop-casting. Four aliquots of 1.6 μL dropcast solution were applied at 15-minute increments before being placed in an oven at 60 °C for at least 4 hours, yielding an approximate film thickness of 50 μm . Polymer membrane dropcast solutions were formulated as follows:

Control Sensor Membranes:

- 66 wt. % PVC (69.0 mg), 32 wt. % NPOE (33.0 mg), and 2 wt. % TOAN (2.0 mg) in THF (2 mL).

Bambusuril Sensor Membranes:

- 65 wt. % PVC (68.6 mg), 32 wt. % NPOE (33.2 mg), 2 wt. % TOAN (2.0 mg), and 1 wt. % dodeca-n-butyl bambus[6]uril (1.02 mg) in 50:50 anisole/THF (2 mL).

Ag/Ag₂S reference electrodes (REs) were used in measurements using NaSH, and Ag/AgCl REs are used in all other potentiometric experiments. All REs in this report were made in-house following previously-reported procedures.⁸

Synthetic Procedures

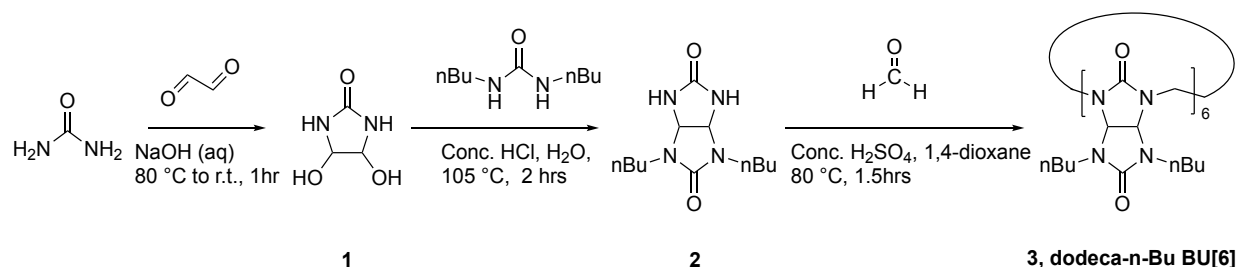
Reagents and solvents were purchased from Fisher Scientific, Tokyo Chemical Industry or Sigma Aldrich. We note that NaSH and NH₄SH evolve H₂S gas in water and should be handled in

a fume hood. Additionally, a $\text{Zn}(\text{OAc})_2$ quench solution and a personal H_2S monitor are encouraged when there is potential for exposure outside of a glove box or fume hood. Scheme 1 outlines the synthesis the *N,N'*-dibutyl glycoluril and dodeca-*n*-butyl bambus[6]uril, which was adapted from literature.

4,5-Dihydroxyimidozoline (DHI, 1). 4,5-dihydroxyimidozoline was synthesized according to literature procedure.²⁵

***N,N'*-Dibutyl glycoluril (n-butyl GLY, 2).** *N,N'*-Dibutylurea (2.39 g, 13.9 mmol) was added to a solution of 4,5-dihydroxyimidozoline (1.63 g, 13.8 mmol) in 35 mL of DI water.^{25,39} Concentrated hydrochloric acid (12.1 M, 1.1 mL) was added dropwise to the reaction mixture and stirred at reflux for 2 hours, at which point the reaction turned a cloudy yellow color. The reaction mixture was then cooled to room temperature and a light-yellow solid formed. The precipitate was collected via vacuum filtration and dried under vacuum. The crude product was dissolved in acetone and purified by column chromatography (SiO_2 , 95:5 EtOAc:MeOH) to produce a fluffy white solid (1.54 g, 44%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 7.50 (s, 2H), 5.22 (s, 2H), 3.19 (m, 2H), 2.98 (m, 2H), 1.42 (m, 4H), 1.25 (m, 4H), 0.89 (t, $J = 7.3$ Hz, 6H).

Dodeca-*n*-butyl bambus[6]uril (dodeca-*n*-Bu BU[6], 3). Compound 2 (1.14 g, 4.40 mmol) was added to a mixture of paraformaldehyde (129 mg, 4.60 mmol) in 30 mL of 1,4-dioxane. While stirring, concentrated sulfuric acid (1 mL) was slowly added dropwise. The reaction mixture was heated at 80 °C for 1.5 hours. Upon heating, the reaction turned a clear light-yellow color, and



Scheme 1. Synthesis of dodeca-*n*-butyl bambus[6]uril (3).²⁵

darkened to an orange color as the reaction proceeded. Following the allotted time, the solution was cooled to room temperature and precipitation occurred. The precipitate was collected via vacuum filtration yielding an orange powder. The crude product was recrystallized in hot EtOH and acetonitrile (95:5 v/v) to yield colorless crystals (430 mg, 37% yield). Connectivity of the host structure was confirmed through a low-resolution x-ray crystal structure (see ESI), ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ : 5.44 (s, 2H), 4.80 (s, 2H), 3.44 (m, 4H), 1.50 (m, 4H), 1.23 (m, 4H), 0.86 (t, $J = 7.3$ Hz, 6H). ^{13}C NMR (126 MHz, Chloroform- d) δ : 159.3, 159.1, 69.1, 48.7, 44.0, 30.2, 20.1, 13.9. and HRMS (ESI+): m/z calculated for $[\text{C}_{78}\text{H}_{132}\text{N}_{24}\text{O}_{12}]$: 1598.0636, found: 1598.0383 (see Supporting Information).

Potentiometric Measurements

The ChemFETs were driven by a benchtop power source, and ISFETs obtained from Winsense were used as the device base. In operation, the drain voltage (V_{ds}) is held at 617.5 mV and the drain current (I_{ds}) at 100 mA. The external reference (Ag/AgCl or Ag/Ag $_2$ S) is held at ground, and the voltage between ground and the source (V_{gs}) terminal changes to maintain the values of V_{ds} and I_{ds} . V_{gs} is recorded as the measurement signal. NI-DAQ 6009 at a rate of 1 kHz was used for data acquisition paired with a custom Labview program for collection. Hydrosulfide experiments employed 180-second measurement periods to minimize electrode fouling. The other potentiometric tests were recorded for 300 seconds.

Solutions used in these experiments were prepared and used at ambient temperature. Previously reported procedures were employed to prepare samples for hydrosulfide measurement.¹⁵ All solutions are based on a 50.0 mM PIPES buffer in DI water fixed to pH 7 using 4.0 M KOH. The hydrosulfide measurements were performed in pH 8 buffer to reduce H_2S

release.⁸ Potassium or sodium salts containing the target anion were used to make 0.100 M stock solutions which were further diluted in 50 mL polypropylene centrifuge tubes for sensor calibration. Potassium was used as the counter cation for all evaluated analyte solutions (except for NaSH). To evaluate counter cation impact on anion sensitivity, a number of cations varying in formal charge, molecular geometry, and atomic radii were evaluated with a common counter anion (chloride) using ChemFET sensors. The results (Supporting Information) indicated none of the counter cations screened provided a statistically-significant impact to the relevant figures of merit in anion sensing. This information validated the reported anion sensing data between various salts. All reported results are the average of four sensors run in triplicate through the entire analyte solution series of 12 solutions spanning six orders of magnitude range of concentration.

Notes

‡ Binding Constants

The primary means of measuring host-guest interaction between dodeca-n-butyl bambusuril with the target anions was via ChemFET evaluation and relative ranking of anions by respective detection limits. Although initial ¹H NMR titrations were performed to measure the binding constants of dodeca-n-butyl bambusuril with common halides, unfortunately many of the binding constants were too high to measure accurately using BindFit.⁴⁰ Thus, the lower bound of Cl⁻ K_a was estimated to be ~10⁷ M⁻¹ in CDCl₃. We also note that the similar dodecapropyl and dodecamethyl bambusurils were evaluated by Havel for solution state binding (Cl⁻, Br⁻, I⁻) using ¹H NMR titrations and isothermal titration calorimetry (ITC). Both techniques revealed the same Hofmeister trend when association constants were ranked: Cl⁻ < Br⁻ < I⁻.⁴¹

Bridge

The previous work demonstrated an applications-focused utility of using ChemFETs for anion receptor evaluations, and the usefulness of the fast ChemFET responses to provide a quantitative means of comparison (i.e. detection limits) for highly reactive species such as HS⁻ towards other anions for the purposes of determining first ever placement of HS⁻ in the Hofmeister Series. The use of the novel dodeca-n-butyl bambus[6]uril facilitated this due to its marked anion affinity as demonstrated in the preceding chapter. While this work was ongoing, thoughts ran to the potential impact of changing the functionality of the bambus[6]uril to change the steric bulk, and subsequently the physical size and configuration of the binding pocket, and explore the impacts on anion affinities. Literature had reported that dodecamethyl bambus[6]uril formed a more cylindrical shape, binding anions 1:1, and dodecabenzyl bambus[6]uril formed a more hourglass conformation, binding anions 1:2 (one each in each end of the bambus[6]uril). Dodecamethyl bambus[6]uril and dodecabenzyl bambus[6]uril were both synthesized following literature procedures. Interestingly, a detailed examination of crystal structures indicated that n-butyl bambus[6]uril formed a very similar cylindrical shape to dodecamethyl bambus[6]uril. Therefore, the dodeca-n-butyl bambus[6]uril anion binding data, as well as the control (no receptor) data was used from the previous chapter, and only dodecabenzyl bambus[6]uril was evaluated in ChemFETs to perform the steric evaluation in the following chapter.

CHAPTER III

EVALUATING IMPACTS OF BAMBUSURIL POCKET SIZE AND STERICS ON ANION BINDING TRENDS USING CHEMFET SENSORS

From D. H. Banning, G. M. Kuhl, M. M. Howell, and D. W. Johnson, *Org. Biomol. Chem.*, 2024, **22**, 269-273. (Cover artwork depicted in Figure 1)

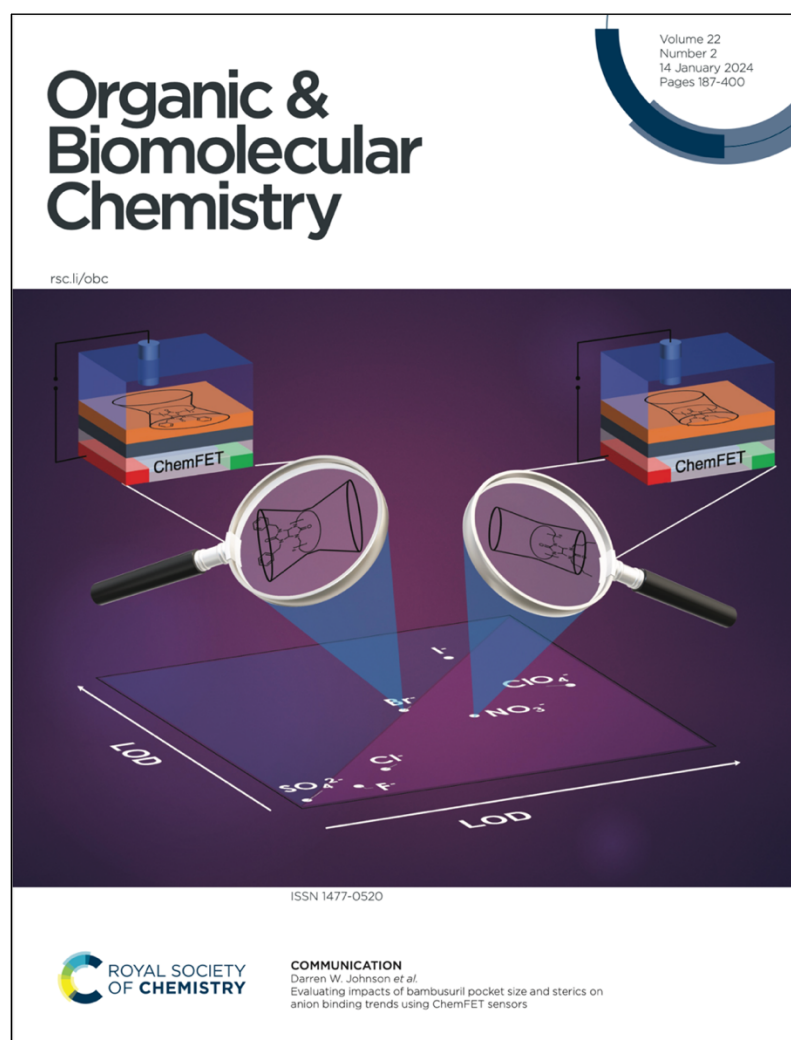


Figure 1. This paper was selected for the cover of *Organic & Biomolecular Chemistry*, Volume 22, published January 14, 2024. Cover artwork depicted here.

Abstract

Chemically-sensitive Field Effect Transistors (ChemFETs) are a useful tool to evaluate aqueous anion affinity of hydrophobic supramolecular scaffolds. More specifically, ChemFETs can be used to probe impacts of receptor modification to aqueous anion affinity. In this study, ChemFETs are used to evaluate the anion affinity of both dodeca-n-butyl bambus[6]uril and dodecabenzyl bambus[6]uril to assess steric effects in the chemical selectivity of the sensor membrane. The ChemFETs were evaluated through a series of common anions in the Hofmeister series in order to ascertain the difference in detection limit imparted by the specific functionalization of the bambus[6]uril macrocycles, which are quite sensitive to modest steric effects. Significant improvements to perchlorate and nitrate detection limits were observed via n-butyl bambusuril-containing sensor membranes over detection limits recorded with benzyl bambusuril sensors.

Overview

The size and configuration of supramolecular host motifs have long been recognized as influential factors for target analyte affinity in host-guest chemistry.¹⁻⁸ Bambus[6]urils are a known supramolecular host scaffold comprising six glycoluril subunits cyclized around methylene bridges.⁹⁻¹³ Two of the first reported bambusurils by Sindelar et al. contained n-propyl and benzyl functionality, respectively.¹³ Bambusuril crystal structures confirmed the shape of the binding pocket was drastically altered by the different steric bulk of these functional groups.¹³ The dodeca-n-propyl bambusuril was roughly cylindrical in shape, binding a single chloride (Figure 2, bottom left).¹⁰ However, replacing each propyl group with bulkier benzyl groups changed the shape from

cylindrical to a more hourglass-like shape, resulting in the bambusuril binding two chlorides, one each in two binding pockets (Figure 2, bottom right).¹³

The current study seeks to evaluate the impacts of bambusuril sterics and binding pocket shape/configuration on anion sensing when the macrocycles are used as an additive in a ChemFET

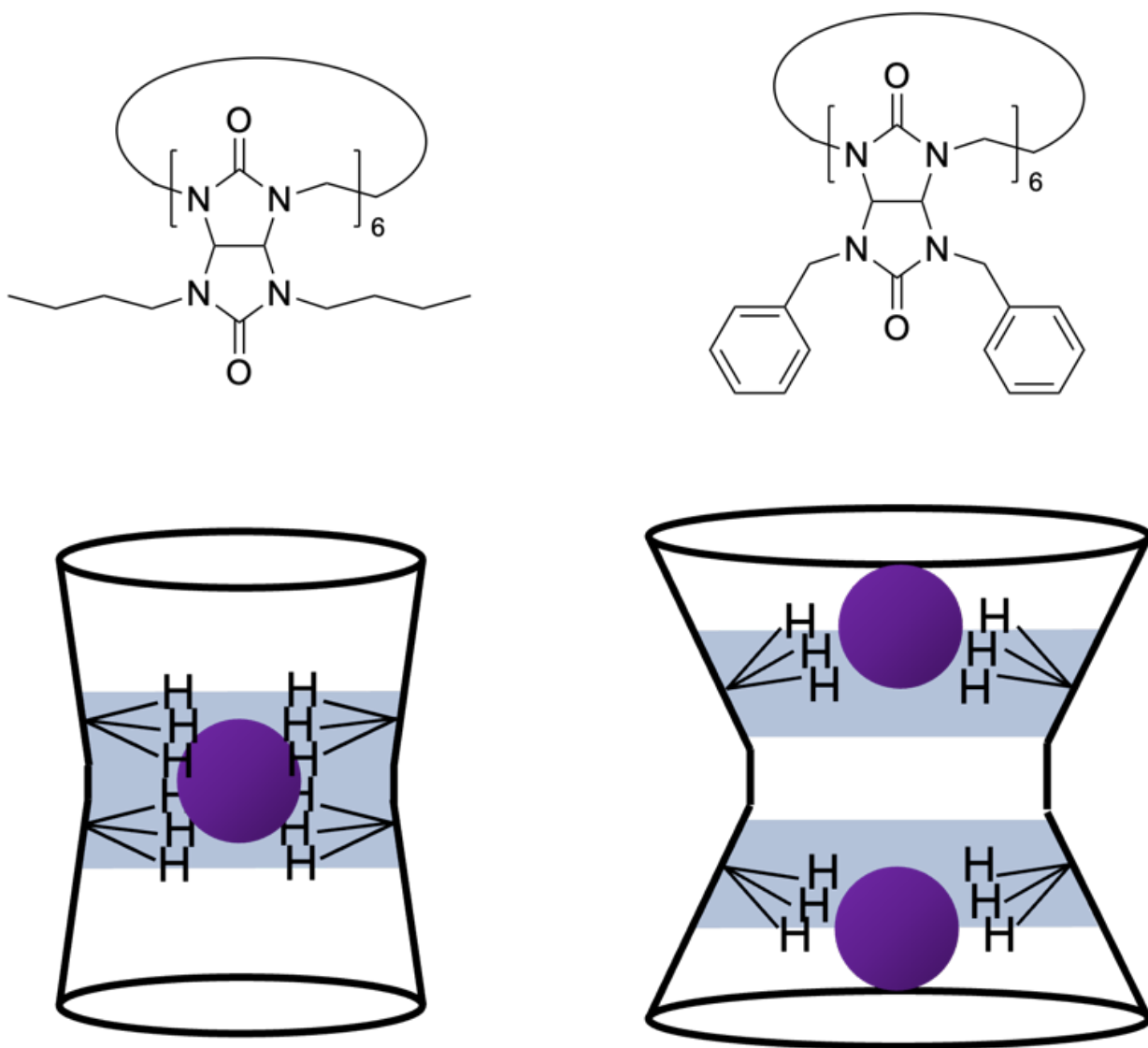


Figure 2. Simplified structural comparison of (left) dodeca-*n*-butyl bambusuril and (right) dodecabenzyl bambusuril. Anions are depicted as purple spheres. (Left) The lower steric bulk of the *n*-butyl groups results in a more cylindrical host shape, a single binding pocket, a 1:1 binding stoichiometry, and directs all 12 CH hydrogen bond donors into the pocket. (Right) The increased steric bulk of the benzyl groups results in an hourglass shape for the host, two binding pockets, 1:2 binding, and only six hydrogen bond donors inwardly directed to each pocket.

sensor membrane. ChemFETs are similar to ISFETs (Ion-selective FETs), with the addition of a chemically-selective membrane to introduce specificity towards anions of interest. ChemFETs can thus take advantage of host-guest interactions by incorporating supramolecular host scaffolds in the gate oxide membrane, where the affinity for the analyte of interest results in a concentration gradient and resulting potential change that is measurable by the FET (Figure 3).^{14–16} Incorporation of the supramolecular host into the gate oxide membrane facilitates direct measurement of hydrophobic host interaction with aqueous anion guests.

In a ChemFET application, the differences between the more accessible binding pockets found in dodecabenzyl bambusuril and the less accessible central interior binding pocket of dodeca-*n*-butyl bambusuril are of particular interest (Figure 2). For numerous sensing applications, the ability to tune detection limits by modifying the receptor binding pocket size, shape, and configuration is of great interest and could lead to sensors with improved anion binding selectivities and detection limits.^{17,18}

The two bambusurils chosen were the previously characterized dodeca-*n*-butyl bambus[6]uril¹⁶ and dodecabenzyl bambus[6]uril.^{9,12–14} These two bambusurils were chosen due to marked differences in sterics and anion binding pockets resulting from the different external substituents. Additionally, crystal structures of dodeca-*n*-butyl bambusuril showed that, like dodeca-*n*-propyl bambusuril, it was also cylindrical in shape and binds small anions in a 1:1 host-guest stoichiometry.¹⁶ Both bambusurils were evaluated via ChemFETs for affinity to seven common anions found on the Hofmeister series, with careful attention paid to differing aqueous anion detection limits. Any trends in this key figure of merit were expected to be based primarily, if not solely, on steric differences (and the resulting host-guest binding geometry differences)

between the otherwise identical bambusurils and ChemFET gate oxide membranes. Affinity was assessed via millimolar detection limit determination.

ChemFET construction was accomplished via previously reported methods (Figure 3).¹⁴⁻¹⁶ A polyvinyl chloride (PVC)-based membrane system was developed, with the PVC made semi-permeable by the inclusion of *ortho*-nitrophenyl octyl ether (NPOE) as plasticizer. Cationic character was imparted into the semi-permeable membrane by inclusion of tetraoctylammonium nitrate (TOAN).

Synthesis of the dodeca-*n*-butyl bambus[6]uril and dodecabenzyl bambus[6]uril was accomplished via previously reported methods¹⁶ and literature procedures¹³ using commercially

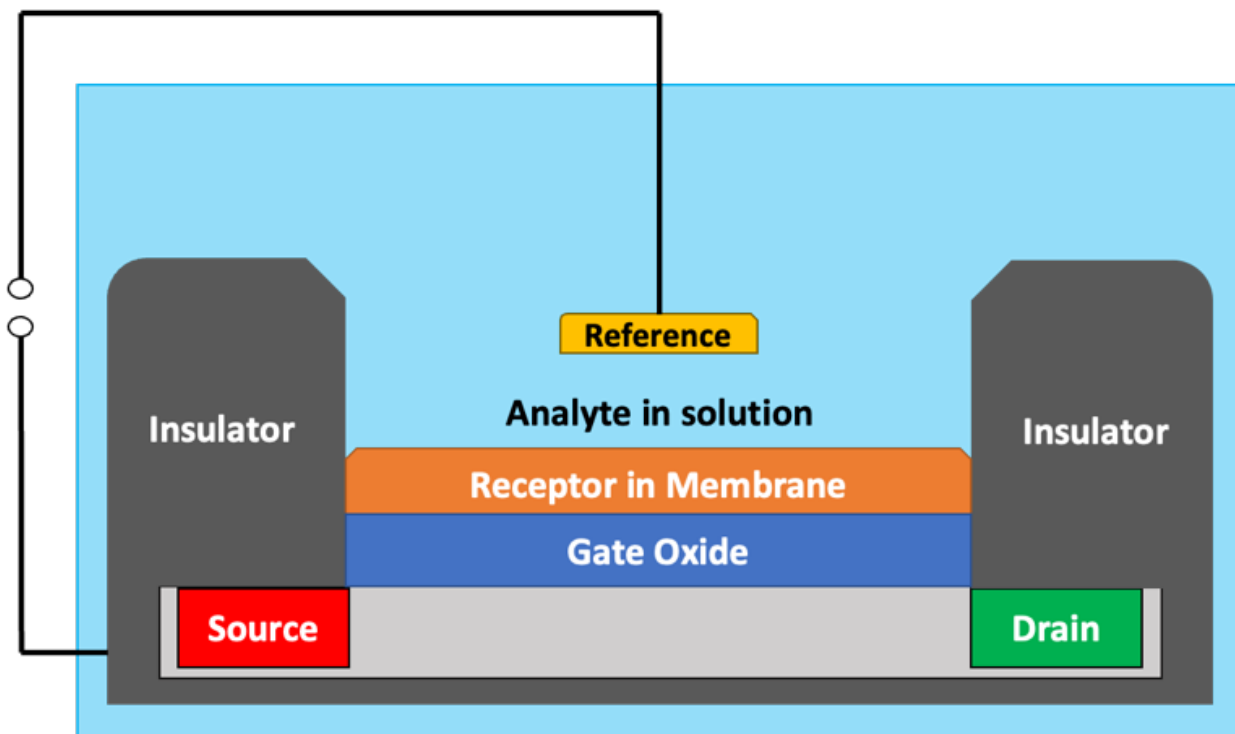


Figure 3. Chemically-sensitive Field Effect Transistor (ChemFET) diagram. FETs are purchased pre-constructed. FET modification includes covering the entire FET with insulating epoxy (aside from gate nitride/oxide surface), development of ionophore-containing membrane to cover the gate oxide surface, and construction of reference electrode. Entire ChemFET and reference electrode are submerged in analyte solution for analysis. Construction and operation follows previously published procedures.¹⁴⁻¹⁶

available precursors. The sensor membranes were then prepared according to the following formulas, where the only variable changed between the three formulations was the host macrocycle used (none used in the control membrane):

Control Sensor Membrane (no macrocycle):

- 66 wt. % PVC (69.0 mg), 32 wt. % NPOE (33 mg), and 2 wt. % TOAN (2 mg) in THF (2 mL).

Dodeca-n-butyl Bambus[6]uril Sensor Membranes:

- 65 wt. % PVC (68.6 mg), 32 wt. % NPOE (33.2 mg), 2 wt. % TOAN (2 mg), and 1 wt. % dodeca-n-butyl bambus[6]uril (1.02 mg) in 50:50 anisole/THF (2 mL).

Dodeca-benzyl Bambus[6]uril Sensor Membranes:

- 65 wt. % PVC (64.7 mg), 32 wt. % NPOE (32.3 mg), 2 wt. % TOAN (2 mg), and 1 wt. % dodeca-benzyl bambus[6]uril (1 mg) in 50:50 anisole/THF (2 mL).

ChemFET evaluation was accomplished via previously reported methods.^{14–16} Each ChemFET evaluation involved the synthesis of the respective bambusuril ionophore, the creation of the membrane formulation, construction of ChemFET sensors, construction of reference electrodes, and performance of the sensor during experimental runs (see Appendix C for details). Analyte solutions were developed from commercially-obtained reagents. Each anion was evaluated as the potassium salt, using a common counteranion for the sake of consistency. Each salt was used to create a series of 12 solutions, each with a constant concentration of piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES) buffer and varying analyte concentration. The concentrations used

were 0.100 M, 50.0 mM, 10.0 mM, 5.00 mM, 1.00 mM, 500 μ M, 100 μ M, 50.0 μ M, 10.0 μ M, 5.00 μ M, 1.00 μ M, and 0.500 μ M analyte, each with constant 50.0 mM PIPES. All solutions were fixed to pH 7 using 4.0 M KOH. Each sensor evaluation comprised sensors run through the 12 analyte solutions, in triplicate, using four different identically-constructed sensors. Actual detection limits were calculated using these results, after conversion of concentration to activity (using Davies activity coefficients).¹⁹

Results and Discussion

The two bambusuril binding motifs appears to engender some significant differences in ChemFET evaluation results (Table 1). Most notably, N-butyl bambusuril sensors display one and two orders of magnitude improvement over benzyl bambusuril sensors for nitrate and perchlorate, respectively, while the remaining anions remained very similar between the two membranes featuring the host macrocycle as well as the control sensor lacking any supramolecular host.

A general trend that emerges is that our sensors displayed the most sensitivity to the chaotrope end of Hofmeister series (I^- and ClO_4^-), and the least sensitivity to the kosmotrope end (SO_4^{2-} and F^-) (Figure 4). However, the difference in physical configuration of bambusuril binding pockets

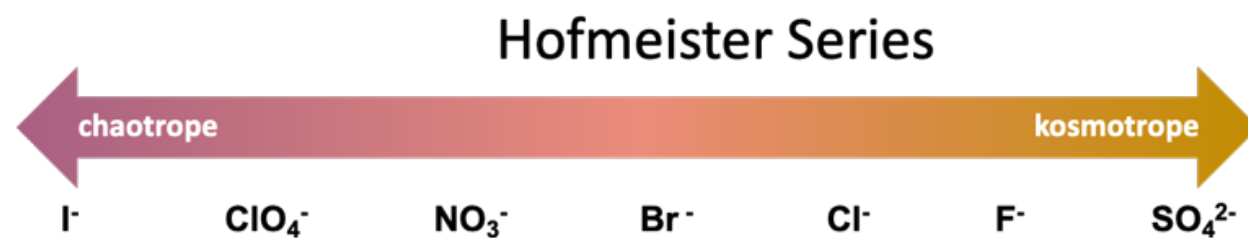


Figure 4. Common anions in the Hofmeister Series. This represents a common reference framework for electrochemical sensors. Electrochemical sensors are commonly more sensitive to chaotropes (left) than kosmotropes (right).

caused by bulky functional groups caused clear impacts on binding trends in the Hofmeister anions evaluated (Figure 5).

The larger size of the binding pocket, coupled with less available hydrogen bond donors, resulted in clear differences in Hofmeister trends typically seen in electrochemical sensor evaluations, which tend to simply select for more chaotropic (~lipophilic) anions within the membranes.

The bambus[6]uril family contains 12 hydrogen bond donors; 2 from each of the 6 glycoluril subunits. The more sterically-bulky benzyl bambusuril, which binds anions in a 1:2 host:guest stoichiometry, has only half the number of hydrogen bond donors available in each binding pocket. While the more spacious physical configuration of the binding pocket may be more conducive to certain anions, that is countered by the presence of fewer hydrogen bond donors.

This feature may shed light on the impact of the bambusuril structural differences on detection limits for various anions (Figure 5). Of particular interest is that the dodeca-*n*-butyl bambusuril is nearly equal or superior to dodecabenzyl bambusuril as measured by detection limits across the series of anions measured. This result was quite unexpected, as it was thought the binding pockets more at the periphery of the benzyl variant might be more accessible to anions in the semipermeable PVC membrane, and thus show improvement in binding (as measured by detection limits). However, along with the more accessible binding pocket comes the added complication

Receptor	Figure of Merit	SO ₄ ²⁻	F ⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	ClO ₄ ⁻	I ⁻
n-BuBU[6]	Detection Limit (mM)	34	6.3	1.9	0.16	0.039	0.0013	0.0086
BnBU[6]	Detection Limit (mM)	30	19	7.5	0.20	0.43	0.14	0.0055

Table 1. ChemFET evaluation results of *n*-butyl and benzyl bambusuril-containing sensors. Both sensors contain 1 weight percent bambusuril. Detection limits measured in mM.

that the number of hydrogen bond donors per pocket is halved, which may play a significant role in the differences observed. Additionally, the more accessible peripheral binding pockets found in benzyl bambusuril may also leave the binding pockets more susceptible to intrusion by the membrane material (PVC) and plasticizer (NPOE).

Other particularly noteworthy observations were that fluoride and bromide show almost no difference between control and ionophore-containing sensors, and perchlorate shows the largest difference (3-4 orders of magnitude, Figure 5). Significantly, although the general trends between n-butyl and benzyl bambusuril-containing sensors are similar, benzyl bambusuril shows a marked

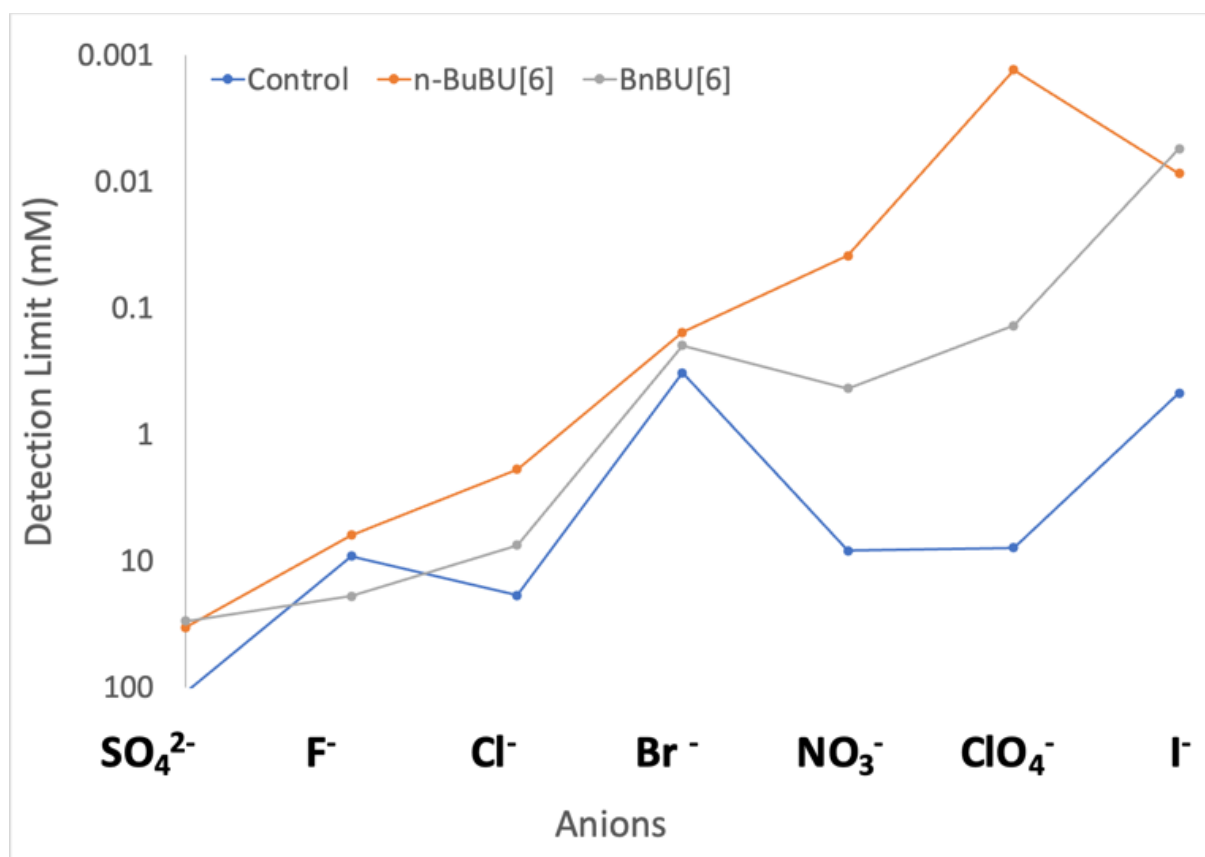


Figure 5. Graphically-depicted results of control (no ionophore) sensors in grey, n-butyl bambusuril sensors in orange, and benzyl bambusuril in blue. Lower detection limit (higher on the y-axis) is better. Of particular note is the significant differences in detection limits between the control and both bambusuril derivative-containing sensors at the more lipophilic end of the anion range.

degradation towards sensing perchlorate (and, to a lesser extent, nitrate). Given that nitrate and perchlorate are larger anions that feature more hydrogen bond acceptors, it appears the more open binding pocket and additional hydrogen bond donors provides a markedly enhanced detection limit for these two anions.

This evaluation also provides a good illustration of the utility of “turning off” sensitivity towards particular analytes through functionalization of the host scaffold. The change of bambusuril functional groups significantly “turned off” (or at least greatly diminished) perchlorate sensitivity. The corollary is likely true also, where strengthening the molecular-level interactions (e.g., strong CH hydrogen bonds) between host and guest would provide even better sensitivity for tightly bonded anions. Taken in tandem, that might suggest this approach could be well-suited towards array-based sensing platforms, where subtle differences in analyte binding within an array can provide robust anion speciation and concentration information in solution.^{20–24}

Much of host-guest chemistry stems from ideas around the lock and key principle, which emphasizes the size, shape, and non-covalent interactions between host and guest. Varying the configuration of the binding pocket thus results in different binding affinities. This concept holds especially true for the shape-persistent bambus[6]uril family of supramolecular host scaffolds, where R groups with different steric bulk result in different anion affinities, and changing bambusuril functionality even allows changing between 1:1 or 1:2 binding configurations. Differences were measured in a ChemFET sensor motif, where both types of bambusuril (n-butyl 1:1 binding, and benzyl 1:2 binding) were evaluated against a series of anions to determine the resulting impact of the different binding configurations.

Of particular note was the response to perchlorate, which was demonstrated to be quite sensitive to sensors with one bambusuril configuration, with that same affinity greatly toned down

merely by changing the functional group of the bambusuril. This also illustrates the differences between electrochemical sensing and solution state binding, where Sindelar et al. demonstrated remarkably high solution state binding of dodecabenzyl bambus[6]uril with perchlorate.²⁵ We attribute one possible explanation of this difference to the intrusion of polymer and/or plasticizer into the more exposed dual binding pockets of dodecabenzyl bambus[6]uril, a complication not present in solution state ¹H NMR titrations or Isothermal Titration Calorimetry (ITC) determinations of binding constants.

Conclusion

In the field of electrochemical perchlorate sensing, the current best detection limit was reported by Filip and coworkers of 0.46 nM.^{26,27} This ISE-based sensor system required pre-treatment of the sensor membrane with perchlorate to attain this record detection limit, and without such pretreatment attained a diminished detection limit of 280 nM. The next lowest reported perchlorate detection limit with an ISE-based sensing system and perchlorate conditioning of the membrane is 3.09 nM by Bhatti and coworkers.^{26,28} In light of this, the 1 μ M detection limit of the n-butyl bambus[6]uril ChemFET system stands out given ChemFETs are robust and low-cost, and importantly, they require no pretreatment or perchlorate-containing reference electrolyte, so that identical sensors can be used for all evaluated anions. Overall, this shows promise for future studies in evaluating impacts of subtle structural changes to host receptors and their effects on sensor performance.

Bridge

The previous chapter demonstrated the impacts of anion receptor binding pocket geometry of chemFET-based sensors on aqueous anion affinities throughout the Hofmeister series. However, aqueous anion affinity evaluation via ChemFETs are not limited to organic anion receptors. The next chapter dives into an evaluation of the aqueous anion affinity profiles of a number of triazolate (TA) metal organic frameworks (MOFs) developed by the Brozek lab. Previous cyclic voltammetry (CV) studies on this MOF family suggested a size-based affinity of anions in the pores, so a collaboration was undertaken to further explore the host-guest interactions of the MOF by developing anion affinity profiles for the MOF via ChemFET-based sensors. The next chapter highlights the integration of MOFs into the selective membrane of chemFET sensors for use in profiling anion affinity trends.

CHAPTER IV

CHARACTERIZING THE ANION AFFINITY PROFILE OF COPPER TRIAZOLATE METAL ORGANIC FRAMEWORK USING CHEMFET SENSORS

From D. H. Banning, A. M. Davenport, N. M. Lakanen, J. Huang, C. K. Brozek and D. W. Johnson, *Manuscript in Prep.*

Abstract

Nanoparticles of metal-organic frameworks (nanoMOFs) possess the unusual combination of both internal and external surfaces. While internal surfaces have been the focus of fundamental and applications-based MOF studies, the chemistry of the external surfaces remains scarcely understood. Herein we report that specific ion interactions with nanoparticles of Cu(1,2,3-triazolate)₂ (Cu(TA)₂) resemble the Hofmeister behavior of proteins and the supramolecular chemistry of synthetic macromolecules. Inspired by these anion-selective interactions, we tested the performance of Cu(TA)₂ nanoparticles as chemical field effect transistor (ChemFET) anion sensors. Rather than size-based selectivity, the detection limits of the devices exhibit a Hofmeister trend, with the greatest sensitivity toward anions similar in size to the MOF pore cavities—perchlorate, iodide, and nitrate. These results highlight the importance of the pore-based supramolecular interactions, rather than localized donor-acceptor pairs, in designing MOF-based technologies.

Overview

Metal-organic frameworks (MOFs) are an exciting area of materials chemistry with a wide range and continually expanding array of interesting properties¹⁻³ and applications that including gas capture,^{4,5} remediation,^{6,7} catalysis,^{8,9} nanocrystal formation,^{10,11} thin films,^{12,13} and sensing.¹³⁻¹⁷ Specifically in chemically-sensitive field effect transistor (ChemFET) sensing applications, MOFs have been most commonly used for gas detection.^{14,18} ChemFETs are an electrochemical sensing tool that has previously been well-characterized in developing affinity profiles for host-guest interactions.¹⁹⁻²⁶ ChemFETs utilize host incorporation into a semipermeable membrane to provide a response specific to the functionalized membrane and the analyte of interest. In the cases of MOF evaluations via ChemFETs, a MOF-functionalized gate oxide is one of the more common methods of integrating MOFs into ChemFET-based sensor systems.^{14,18} Despite the impressive sensitivity of MOF-based ChemFET sensors, little is known about the underlying guest-host chemical interactions between the analytes and MOFs. Additionally, deployment of these technologies at-scale will require facile fabrication of uniform MOF thin films or polymer-hybrids, as with chemical separation membranes or other recent MOF-based industrial technologies.²⁷ Whereas typical reports of MOF sensors employ bulk powder, nanoparticles (nanoMOFs) offer the ability to use solution processable techniques ranging from drop-casting and doctor-blading, to spin- and spray-coating.²⁸ Here, we report the design of nanoMOF-based ChemFET anion sensors based on chemical insight into the specific guest-host chemistry underlying the MOF-analyte interactions.

In search of MOFs with the potential for selective anion interactions, we identified Cu(1,2,3-triazolate)₂ as having a similar pore size (ca. 5-6 Å in diameter) and chemical environment to the bambus[6]uril family of anion receptors.^{25,26,29} Whereas most MOF pores exceed 10 Å, *M*(TA)₂ *M*

= Mg, Cr, Mn, Fe, Co, Zn, Cd possess unusually small pores.^{30,31} Previously, we demonstrated that such narrow pore apertures frustrate the ability of anions to intercalate into electrochemical thin films of $M(\text{TA})_2$ $M = \text{Cr, Fe}$ nanoparticles.^{10,32} The oxidation of interior Fe sites anodically shifts ca. 1.5 V due to the additional anion desolvation and solvent reorganization in comparison to the charge compensation mechanism involving exterior Fe sites. The larger pore apertures of $\text{Cr}(\text{TA})_2$ permitted redox intercalation of a range of anions, but where the associated redox potentials varied by hundreds of mV depending on the anion size.³³ This electrochemical behavior suggested that constricted pore environments of $\text{Cu}(\text{TA})_2$ would display anion-specific interactions due to the close proximity of anions to the pore walls, especially for anions with similar diameters to the pores.

The synthesis of the $\text{Cu}(\text{TA})_2$ followed previously published procedures for $M(\text{TA})_2$, $M = \text{Co, Fe, Cr}$.¹⁰ Briefly, $\text{Cu}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ was dissolved in anhydrous DMF to afford a 10 mM solution, to which was added 1,2,3-triazole with vigorous stirring. This mixture was heated at 120 °C for 2 h on a dry bath. Particles were collected by centrifugation, washed twice with fresh DMF and twice with fresh methanol, and desolvated under reduced pressure via standard Schlenk techniques. Particle sizes and dispersities were measured by Scherrer analysis of PXRD patterns, dynamic light scattering (DLS), and scanning electron microscopy (SEM).

Results and Discussion

For microscopic insight into the interactions of $\text{Cu}(\text{TA})_2$ nanoparticles with anions, zeta potentials and DLS sizes were recorded in tandem during the titration of potassium salts. Such measurements have been critical in understanding the surface chemistry of proteins and conventional, nonporous colloidal nanoparticles.^{34,35} Salts that cause zeta potentials to reach 0 mV

at lower concentrations interact more strongly with the nanoparticle surface, typically causing the particles or proteins to “salt out”. Interestingly, in DMF $\text{Cu}(\text{TA})_2$ particles have a remarkably high zeta potential which suggests not only excellent colloidal stability, but also the presence of open metal sites at the surface of the particles (Figure 1). In general, 3 mg/mL 30-nm $\text{Cu}(\text{TA})_2$ nanoparticles in DMF were treated with stock solutions of 10 μM and 100 μM of DMF solutions of each electrolyte using appropriate volumetric amounts to reach the desired electrolyte concentrations. Measurements were collected in triplicate. The resulting data are shown in Figure 2. Although all salts eventually caused zeta potentials to reach a “point of zero charge” (PZC), only KI induced nanoparticle precipitation. Interestingly, the order of electrolyte concentrations required to reach the PZC resembles the ordering observed for proteins, now well-known as the Hofmeister series.^{25,26,36} It is important to note that zeta potentials report on the “slipping plane” of a colloid, which, depending on the system, corresponds to the inner solvation shell or several

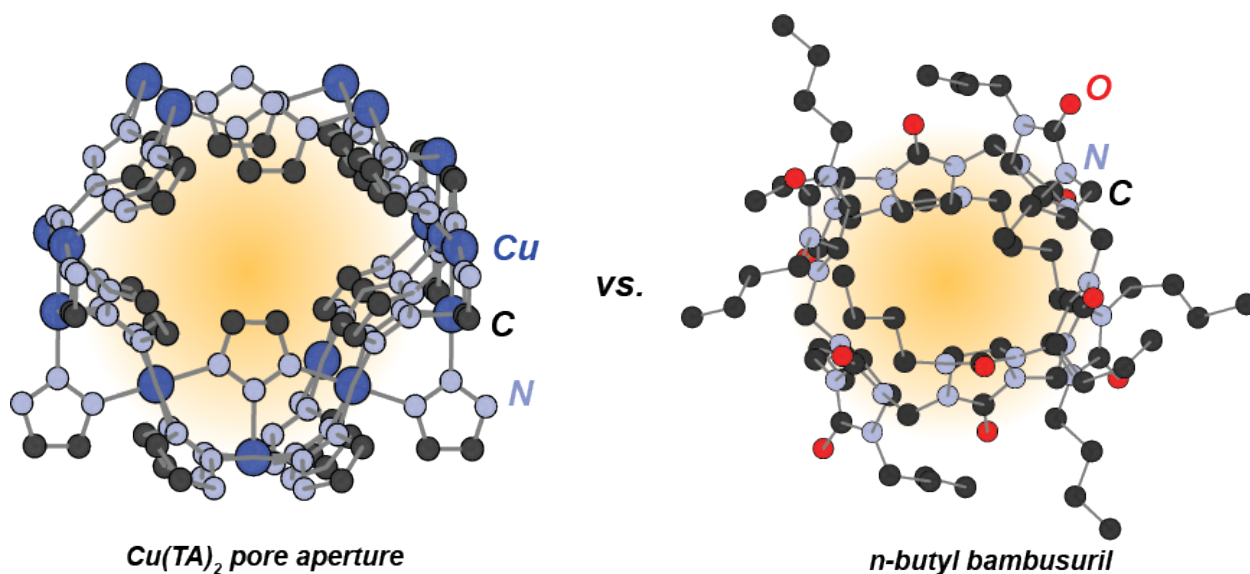


Figure 1. Representation of a portion of the $\text{Cu}(1,2,3\text{-triazolate})_2$ ($\text{Cu}(\text{TA})_2$) pore aperture versus *n*-butyl bambus[6]uril, a previously-characterized anion receptor. Hydrogen atoms omitted for clarity.

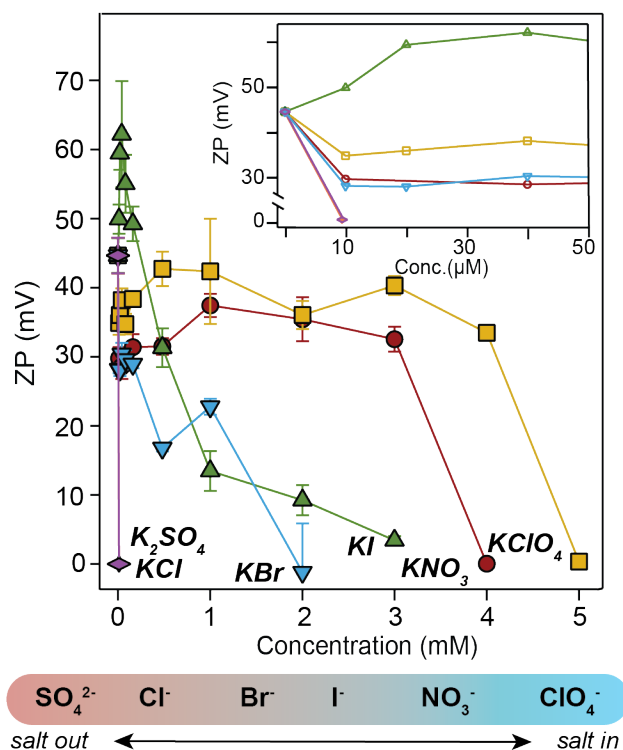


Figure 2. Zeta potentials of 30-nm Cu(TA)₂ colloidal nanoparticles suspended in DMF solutions. Electrolyte salts were added sequentially in increasing concentrations as indicated in mM. All measurements were recorded in triplicate. Solvation diameters were recorded simultaneously by dynamic light scattering and are included in the ESI. Inset: Zeta potentials recorded at low electrolyte concentrations.

nanometers from the nanoparticle surface. Therefore, these results suggest a considerable selectivity of anion interactions with the external surface of Cu(TA)₂ nanoparticles.

Given the structural similarity between the Cu(TA)₂ pores and the binding pockets of previously-characterized anion receptors, a plan was developed to follow previous electrochemical sensing methodology to characterize the “host”-like nature of the pores interacting with aqueous anion “guests.” These previous studies involved the use of chemically sensitive field-effect transistors (ChemFETs) to evaluate the host-guest interaction of host receptors with anion guests. Since the applications of interest for previous projects involved aqueous anion affinity, they

necessitated some methodology tolerant of characterizing interactions between both hydrophobic hosts and aqueous guests. ChemFETs indeed demonstrated suitable bridging of this solubility gap.

ChemFETs are a useful tool that have been used in sensing applications to evaluate aqueous anion affinity of hydrophobic anion receptors.^{23–26} Previous work evaluated various organic anion receptors for aqueous anion affinity trends.^{25,26} A series of control sensors, polymer membranes without added receptors, are also used to contrast with the receptor results, for comparison with

Hofmeister Series to characterize the specific effect the receptors have, if any, on the anion affinity profiles.

Related studies in ChemFET sensors using MOFs have investigated functionalizing the gate oxide surface of FETs with MOFs to provide chemical selectivity.^{14,18} Our initial attempt at using Cu(TA)₂ to bias selectivity in ChemFET sensors followed these literature precedents. However, in this case all attempts at functionalizing the gate oxide surface with Cu(TA)₂ failed. Therefore, we pivoted to emulate aspects of previous studies by us and others that used organic anion receptors within ChemFET membranes to impart selectivity.^{23–26} This well-established approach involves integration of receptors into a polyvinyl chloride (PVC)-based membrane to facilitate aqueous anion affinity evaluation.^{23–26} Another benefit of this approach is that integrating MOF particles into the ChemFET membrane facilitates the direct comparison of sensors with MOF-containing membrane to sensors containing a control membrane that lacks the MOF but still contains all of the other components of the ChemFET (polymer, ionophore/immobilized cation, plasticizer). These control sensors are created identically in all respects, with the exception of addition of any anion receptor, and therefore, these control receptors will simply respond to anions with Hofmeister trend selectivity. This facilitates creation of a control profile for comparison purposes that is unavailable to ChemFET evaluations with a functionalized gate oxide. Therefore, the detection limit of each anion with MOF-containing sensors was able to be compared to a corresponding “blank” run, further isolating the difference in response entirely to inclusion of the MOF. Thus, the host-guest interaction of the anion with the MOF accounts for the entire difference between control and MOF-containing sensor results.

Sensor evaluation was performed following previously published reports.^{23–26} The MOF was incorporated into a ChemFET membrane dropcast solution for application to the FET. The MOF was incorporated as 1 wt% of the dropcast solution, with 2 wt% tetraoctylammonium nitrate (TOAN), 32 wt% *o*-nitrophenyl octyl ether (NPOE) plasticizer, and 65 wt% high molecular weight PVC. The sensors were then coated by drop-casting four 1.6 μL drops of the receptor onto the surface of the FETs. The drops were applied in thirty-minute intervals to allow for solvent removal. Following the completion of the dropcasting, the ChemFET sensors were then placed in a 60 $^{\circ}\text{C}$ oven overnight to ensure complete solvent removal. The sensors were then allowed to cool over another 24 hours before the evaluation began.

The evaluation comprised four identically constructed chemFET sensors with the $\text{Cu}(\text{TA})_2$ incorporated into the PVC-based selective membrane (Figure 3). The four sensors were then run through a series of 12 solutions of increasing analyte concentration: 0.500 μM , 1.00 μM , 5.00 μM , 10.0 μM , 50.0 μM , 100 μM , 500 μM , 1.00 mM, 5.00 mM, 10.0 mM, 50.0 mM, and 0.100 M, with each containing a constant 50.0 mM piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) acting as a non-interfering buffer to ensure consistent ionic strength of the solution even at low analyte concentrations (this avoids a

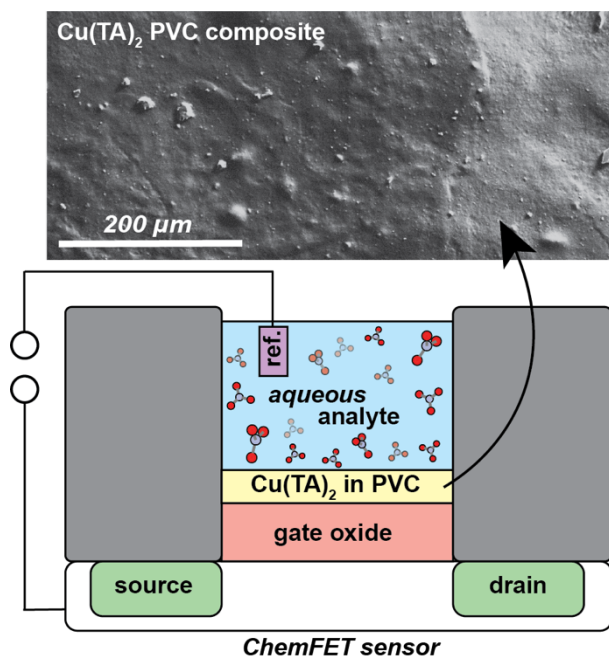


Figure 3. ChemFET sensor construction for MOF evaluation. Sensor depicted contains $\text{Cu}(\text{TA})_2$ in the PVC membrane. Control sensors were identically constructed with PVC membrane, just without $\text{Cu}(\text{TA})_2$ providing selectivity. An SEM image of the control is included the ESI.

misleadingly low detection limit stemming from the low ionic strength of the low concentration solutions). A set of four sensors was run through the 12 series of analyte solutions in triplicate: from low concentration to high, then high to low, and then finally low to high, and the data averaged to provide the reported metrics. An anion affinity profile was evaluated for a series of seven common anions in the Hofmeister series to illuminate whether the $\text{Cu}(\text{TA})_2$ -containing sensors followed a Hofmeister-like trend or a size exclusion trend (Figure 4).³⁷

The ChemFET sensor evaluation of $\text{Cu}(\text{TA})_2$ indicated an affinity profile consistent with a Hofmeister trend rather than a size-based trend. Chaotropes (or more lipophilic anions) such as

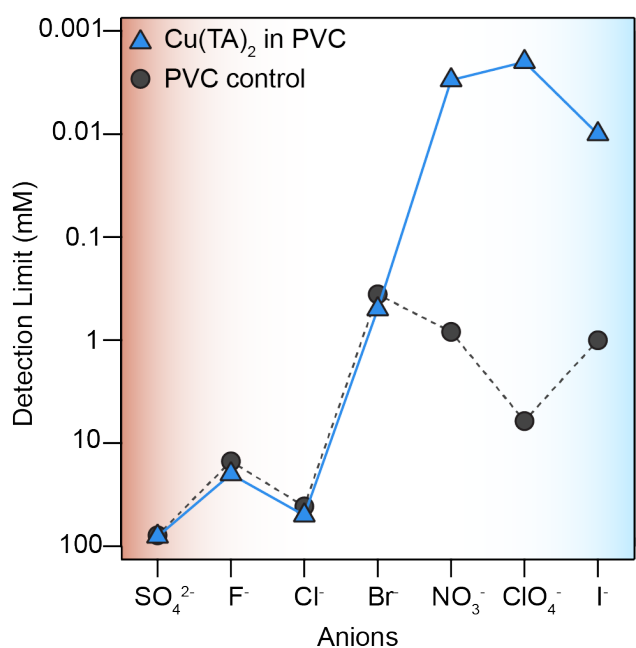


Figure 4. Anion affinity profile as evaluated via $\text{Cu}(\text{TA})_2$ -containing ChemFET sensors. Detection limits are presented on a log scale, with lower detection limits (better) located higher on the chart. Detection limits are in units of mM on the primary Y axis. Affinity profile of $\text{Cu}(\text{TA})_2$ in blue and control in black. Anions ordered per the computational work by Page et al. for direct comparison to the Hofmeister Series trend. All counter cations are potassium to eliminate influence of cation on the anion affinity profile.

iodide, perchlorate, and nitrate produced low detection limits (μM), while kosmotropes (harder, more hydrophobic anions) such as sulfate and fluoride produced high detection limits (M). Ordering of the Hofmeister series follows the computational work of Page et al. based on linear charge density of each anion.³⁷ Therefore, in the context of ChemFET-based sensors, the affinity profile clearly does not

follow the size trend first suggested by electrochemical measurements of the MOF nanocrystals in solution.³⁸ Interestingly, the evaluation of $\text{Cu}(\text{TA})_2$ host-guest interactions with a series of anions paralleled the same Hofmeister trend found for organic anion

receptors that featured a similar pore size to the MOF.^{25,26} It is also important to note that the counter cation remained constant during these experiments to rule out any impact on the anion affinity profile: potassium was used as the counter cation for all anions evaluated.

Conclusion

In conclusion, we report an anion-dependent affinity of MOF nanoparticles that follows a Hofmeister trend of Lewis acid/base hard/soft theory rather than a simple size-exclusion mechanism. These results suggest the complex surface of MOF pores—the alternating regions of hydrophobicity/hydrophilicity, polarizability, and nanoconfined topologies—resembles the supramolecular chemistry of proteins and macrocyclic ion receptors. Beyond anion sensing, these results provide design principles for gas sorption, catalysis and other MOF applications involving guest-host chemistry. In addition to their practical utility, the ChemFET sensors are critical to establishing this fundamental connection between MOF and macromolecular chemistry.

Bridge

The previous chapter outlined the utility of ChemFET sensing systems to characterize host-guest interactions of non-water-soluble receptors with aqueous anions. An affinity profile was developed for comparison to the well-known Hofmeister series. This was the first-reported use of ChemFET sensors to characterize anion affinity of MOF nanoparticles. This successful study prompted further investigation on nanoparticle synthesis occurring within the DWJ lab.

One possible approach to optimizing nanoparticle synthesis was to take a statistically-based approach to characterize the reaction parameters. Previous experience with statistical evaluation of processes provided a foundation to apply this type of approach to chemical synthesis. The author's flight test engineering work had involved the test design and execution for operational testing of Air Force Special Operations Command (AFSOC) aircraft, weapons, and systems. A key component of process is the integration of Design of Experiments (DOE), a statistical analysis technique that produces test design strategies to best gather statistically-rigorous data and conclusions from flight testing. Additionally, DOE tools were able to evaluate historical data to help bolster characterization efforts with no additional testing required. This second facet of DOE was especially attractive in a research environment, where established research projects may have years or decades of research data that has often never been statistically analyzed to better characterize the relationship between reaction conditions and products/outputs. This next chapter explores the use of DOE to perform a statistical analysis of a vast quantity of historical research data on $f\text{-Al}_{13}$ cluster synthesis to optimize desired output properties. These predicted conditions were then put to the test to validate the soundness of the predicted model by running predicted optimization conditions.

CHAPTER V

OPTIMIZATION OF f - Al_{13} CLUSTER SYNTHESIS USING COMPLEMENTARY SYNTHESIS AND DATA ARCHIVES ANALYZED BY DESIGN OF EXPERIMENTS

From A. Q. Rosen, D. H. Banning, V. M. Salpino and D. W. Johnson, *Submitted Manuscript*

Abstract

The synthesis of a ~ 1 nm “flat- Al_{13} ” (f - Al_{13}) nanoscale aqueous aluminum cluster species was optimized through statistical analysis. Through examination of historic data sets, improved synthesis conditions were elucidated by design of experiments and validated experimentally. These results were confirmed through DLS measurements, allowing the experimental results to be fed back into the model for further refinement resulting in a 4- to 12-fold increase in DLS peak intensity for the 1 nm cluster and an essentially quantitative conversion of % mass to the small f - Al_{13} cluster over larger oligomers and agglomerates.

Overview

Aluminum oxide is a versatile material that has many well-known applications in thin-films or as a coating, such as hydrophobicity and resistance to corrosion or abrasion.¹⁻³ These coatings are classically deposited through techniques such as atomic layer deposition, which is both energy intensive and requires the use of toxic precursors. Additionally, these techniques are usually

restricted to small, laboratory scale preparations, making them suboptimal for industrial scale use.⁴ Previous work from our lab has shown that the use of the “flat” tridecameric aluminium aquo/hydroxo cluster ($f\text{-Al}_{13}$) constitutes a scalable, green, and inexpensive alternative precursor for alumina films.^{1,5} The most scalable synthetic method for the $f\text{-Al}_{13}$ cluster utilizes an acid facilitated mineral dissolution process, resulting in an aqueous solution suitable for direct use in thin-film coating applications.⁶ However, these synthetic methods have proven quite sensitive to reaction conditions, which have an influence on the cluster’s properties and the aluminum ion speciation. This has led to an effort to more fully characterize the system to ascertain how to better optimize product properties and improve the preparation to yield a single, pure stable solution of $f\text{-Al}_{13}$.

A major development that arose during the exploration of $f\text{-Al}_{13}$ cluster solutions was the observation of nanoscale agglomerates.⁷ The presence of agglomerates could impact the usability of the coating solution in applications, causing issues such as settling, clogging, or flocculation of other additives. It is known that formation of agglomerates is a complex process, although simplified models like the LaMer nucleation model can describe (with some modern updates) the relation between solution aging and agglomerate formation.^{8,9} The growth of such particulates in precursor solutions can have a large effect on the roughness and performance of the resultant film.¹⁰ Thus, understanding the growth, presence, and methods of controlling agglomerate formation are essential to the use of these solutions on a large scale and to the preparation of analytically pure, shelf-stable samples of cluster solutions.

Over the course of research on the synthesis and speciation of $f\text{-Al}_{13}$, a large library of historical reaction conditions and resulting data were collected from our published papers and lab notebooks (see Figure 1).⁷ In order to target specific sizes of species in solution, it was concluded that the

historic data sets should be evaluated to inform future reaction parameters. However, to date little statistical analysis had been accomplished to evaluate the specific impacts of varying reaction conditions on the resulting size distribution of product species or the yield of $f\text{-Al}_{13}$. Statistical analysis was chosen to analyse historical data with the goals of being able *i*) to predict optimized reaction conditions for synthesizing clusters with smaller particle sizes (near the $f\text{-Al}_{13}$ radius of 0.7 nm) and *ii*) to minimize polydispersity index.

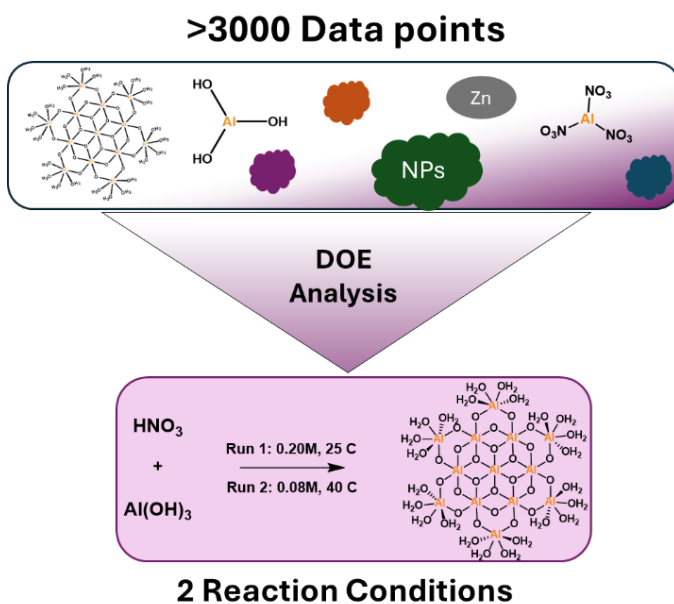


Figure 1. Pictogram showing the workflow of this project. A large group of historical data and results was passed through a DOE “filter” to find and test optimized conditions. The $f\text{-Al}_{13}$ cluster is a charged ionic specie with chemical formula $[\text{Al}_{13}(\mu_3\text{-OH})_6(\mu\text{-OH})_{18}(\text{H}_2\text{O})_{24}]^{15+}$ with 15 nitrate counterions (not pictured).

Design of Experiments (DOE) is a statistical tool that is often used in industry for process characterization and improvement, synthetic chemistry reaction optimization, optimizing drug delivery, and many other applications.^{11–13} DOE can be used in two broad ways: first, to design a series of experiments with specifically varying conditions to provide information on main factor and multi-factor interactions, using statistically rigorous quantification based on p-values. This allows the most efficient use of experimental time, materials, and efforts, producing the most statistically-significant information with the fewest possible runs. The second major use of DOE, and the focus of this paper, is the post-analysis of prior data to determine statistical significance of main and multi-factor interactions on the outputs of the experiment. One of the drawbacks of the

second approach is the limitation of how much of the design space previous experiments covered, potentially limiting the utility of the evaluation. One significant benefit, however, is the ability to glean some amount of statistically significant information without the need to run additional experiments. That utility is demonstrated here, where a decade of synthetic conditions and resultant product properties were evaluated to characterize the reaction and the specific impacts of each factor. These optimized conditions were then validated by running a series of syntheses to evaluate the efficacy of the optimized synthesis and quantify its efficacy by DLS.

The purpose of this project was twofold. The first aim was to evaluate f -Al₁₃ cluster synthesis to characterize main and multi-factor interactions for optimization of both particle size and polydispersity. The second aim was to perform an exhaustive analysis to determine how much statistically significant information could be gleaned by statistical analysis of historical data. Such an intersection of DOE approaches with inorganic chemical synthesis remains relatively unexplored, although not unknown, and it is commonly studied in organic process optimization.^{11,14,15}

Results and Discussion

The initial DOE analysis was conducted by evaluating pH, temperature, and initial concentration of the aluminium salt starting material as the dependent variables. All three input factors were categorized as continuous, with the bounds set equal to the highest and lowest values seen in the historical data. Over 3000 historical data points were evaluated, all from f -Al₁₃ reactions performed by various members of our lab at the University of Oregon. Of these data points, 525 different measurements were identified that led to successful

synthesis of $f\text{-Al}_{13}$, comprising 125 different batches of reaction conditions and 25 sets of reaction conditions that resulted in desired outputs (see Table S1). The range of evaluated parameters included pH from 3 to 3.9, starting temperature ranges from 22 °C to 40 °C (all reactions eventually reached 40 °C), and concentration from 0.0002 M to 0.2 M. The desired outputs included low polydispersity (below 20%) and low particle size (below 5 nm).

Results from the initial analysis are presented in Table 1, indicating factors ordered from most to least significant. Notably, only half the analysed main factors and 2-factor interactions were statistically significant ($P < 0.05$), and of these three factors considered to be statistically significant, two were 2-factor interactions which cannot be reliably elucidated without DOE or related advanced statistical processing.

The next step was to take the analysis results and use predictive modelling to optimize outputs.

Optimization consists of minimizing both polydispersity and particle size outputs. Due to the interactive nature of the factors, a number of different conditions were identified to optimize both low polydispersity and low particle size. The “old” reaction listed in Table 2 was our workhorse procedure for $f\text{-Al}_{13}$ synthesis and featured a concentration of 1 M	Factors	P Value
	Temperature*Concentration	0.00019
	pH*Temperature	0.00119
	Temperature	0.0192
	Concentration	0.102
	pH*Concentration	0.103
	pH	0.137

[Al], and a starting temperature of 25 °C, that was raised to 40 °C as the reaction progressed. These conditions facilitate the

Table 1. Results of statistical analysis of factors of significance. Of particular note is that only three of the six interactions describe factors/multifactor interactions that are statistically significant (p value below 0.05) to the desired output parameters.

formation of the $f\text{-Al}_{13}$ cluster, while also forming a range of agglomerates.

Syntheses with new DOE-optimized conditions summarized in the SI (see Table 3 for key reaction details) were then carried out and characterized by DLS to validate the predictive power of the DOE processing. These synthesis alterations, in brief, include drastic decreases in the concentration (from 1 M [Al] to 0.2 and 0.08 M) and changes in the starting temperature, including using a “hot injection” method for one run, where additions were performed at elevated temperature rather than ramping the temperature during synthesis.

	Radius (nm)	% Intensity	% Mass	% Number	PDI
Old Data	0.76	5.3	99.97	100	0.2
	63.3	94.7	0.03	0	0.6
Validation Run 1	0.57	23.6	98.9	100.0	0.2
	846	47.6	0.0	0.0	3.6
Validation Run 2	1.5	66.2	100.0	100.0	0.7
	2966	33.8	0.0	0.0	2.7

Table 2. Results from the DLS. Old run is indicative of the previous synthetic methods performed. All runs indicate 100% small particle by number and mass. The old run results in a small DLS peak intensity of 5.3%; the validation runs result in 24% and 66% peak intensity, respectively. This metric offers an indicator of the improvement in the desired synthesis output.

Conclusion	pH Temp. Conc.			
		(°C)		(M)
The results of the validation run were shockingly successful. DLS measurements as shown in Table 2 revealed a ~20-60% increase in peak intensity, indicating a large	Run	3.2	25	0.2
	1			
	Run	3.2	40	0.08
	2			

Table 3. *Optimized reaction conditions. Determined by the JMP interactive optimization tool. Two sets of reaction conditions were identified that produced desired output properties of low polydispersity index and low particle size.*

due to the fact that even though the small particle size peak started with low intensity, it still represents >98% of particles by number since large particles scatter light disproportionately more.^{16,17} After optimization, the small particle peak is more intense than the large peak indicating that the small particles are even more predominant after optimization (and thus the resulting solution is much higher purity).

Of secondary interest is the large radii of the second (>100 nm) peak in each of the validation runs, which is significantly greater than the filter size used (see ESI) and any previously-reported particulates (~60 nm radii). This is likely an artifact (dust, cuvette scratches, filter fibers, etc.) or post-filtration agglomeration. However, due to the extremely small population represented by this peak (which was actually reported as ~0% by mass and only detectable due to light scattering overrepresentation by DLS software), this was unable to be fully characterized. While the source

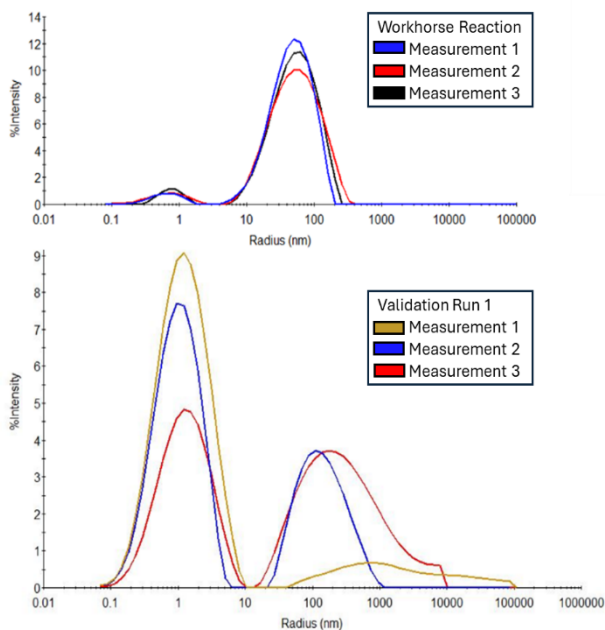


Figure 2. DLS characterization of the $f\text{-Al}_{13}$ cluster in solution. The top chart indicates pre-DOE synthesis particle size distribution, where <1 nm peak has a much lower intensity, even while containing the majority of particle mass. The bottom chart shows the DOE-optimized reaction conditions effectively selected for small particles, with the large peak becoming so sparse that the intensity fell below that of the first peak.

was 0.125. Although the PDI did not experience the drastic shift observed in particle size distribution, the values recorded still fall in the range of monodisperse PDIs.¹⁸ The extremely high PDIs for the large peaks in the validation runs support the hypothesis that those peaks are trace artifacts that are negligible in the real solution.

This highlights an important consideration that when trying to optimize multiple factors, it is possible that they can come into conflict and only one factor will display the desired improvement. In some cases, this may necessitate compromise in the event of mutually exclusive or inversely-

of the >100 nm peak remains uncertain, the point of the project was to optimize the <1 nm species, which was successfully increased in both mass percent and peak intensity.

The polydispersity index (PDI) of the small particle peaks did not shift in the desired direction as drastically as particle size shifted, presumably because our workhorse synthesis had already been optimized by traditional means to provide a single product. Although PDI measurements observed included values on the lower end near the desired minima (0.2 and 0.7), the lowest observed previously

related factors. Follow on work would include the iteration of the DOE model by feeding these validation run results back in, further refining our optimization parameters.

Of particular note regarding the significant factors outlined in Table 1 is the fact that the DOE optimization does not necessarily take the chemistry into account. For example, the entirety of the sample data used to develop the DOE model was taken at pH between 3.0 and 3.9. This is due to the very specific speciation of the $f\text{-Al}_{13}$ cluster, which preferentially only forms in this pH range.¹⁹ Although listed as the least important factor, if exploring outside this pH range, the pH would clearly become the most practically important factor, since the desired product would not be formed at all. Therefore, this example is a reminder that DOE software serves to help supplement the synthesis-characterization process but does not serve as a replacement for chemical intuition. There are a few major takeaways from this project: first, the ability to use historical data to generate optimized reaction conditions. It is common for academic research laboratories to generate a repository of experimental results over the years that could be further analysed to generate new leads or optimize reaction conditions. This could be a powerful technique that allows for re-examination of prior experiments and lead to fresh ideas.

Secondly, the importance of using chemical intuition in conjunction with statistical analysis cannot be understated. The initial DOE results at face value would have obfuscated the importance of pH in this reaction. The knowledge to hold the pH value within a very tight range came from a detailed understanding of the thermodynamics of the system under study. Additionally, unrealistic reaction conditions (such as a ridiculously high or low concentration, or a temperature far above the tolerance of the reactants), could be immediately filtered out. The validation runs demonstrate the importance of dilute solutions on precluding agglomeration, which makes chemical sense given the concentrations effects inherent to aggregation. However, the researcher must also understand

the effect of dilution on DLS experiments, which can cause error in highly concentrated or dilute samples.

Finally, the importance of recording and using data from unsuccessful experiments are equally important to positive results in building an accurate model for design of experiments. Data from a failed experiment can be fed back into the model, improving the statistical analysis and potentially revealing new reaction conditions and optimizations. This method provides a rigorous technique for the analysis of many data sets, allowing for a greater understanding of historic data and providing new insight to more easily build on the results of previous experiments. In this paper, just such a process was undertaken to improve the synthesis of a well-studied inorganic cluster species and improve the effective yield to nearly qualitative values (>99.9% species in solution). As this cluster becomes further developed as a reagent for bulk aluminum oxide film formation, this insight is critical for quality control of the films.¹

Bridge

The previous chapter outlined the use of DOE to characterize f -Al₁₃ cluster synthesis in order to optimize desired reaction output properties, demonstrating the successful characterization and optimization of the cluster synthesis through validation runs. The preceding chapters outlined organic anion receptor synthesis and ChemFET-based sensing characterization of host-guest interactions. All previous chapters are either submitted or published papers in various journals. However, numerous other receptors were synthesized (or attempted to be synthesized), other receptors were characterized using ChemFET-based sensor systems, and other DOE analysis was performed that never made it into a published paper. However, important strategies were developed, difficult problems were overcome, and significant steps made that need to be documented for others who may be exploring this space.

The next chapter outlines attempts to synthesize numerous novel bambus[6]uril derivatives (especially those pursued in the Ch. 3 comparison project), ChemFET characterization of various receptors, and DOE analysis of historical reaction data. Chapter 6 serves as a repository of lessons-learned, especially relating to the development of new synthetic approaches for novel bambus[6]uril derivatives. This chapter also outlines strategies employed to conduct DOE analysis of historical data to characterize chemical syntheses (separate from Chapter 4). ChemFET-based anion affinity characterization of other anion receptors is documented. Finally, Chapter 6 serves as a conclusion of the lessons learned, and lays out the path forward for each of these research efforts.

CHAPTER VI

UNPUBLISHED ANION RECEPTOR SYNTHESIS, CHEMFET CHARACTERIZATIONS, AND DESIGN OF EXPERIMENTS ANALYSIS PROJECTS

Abstract

Two of the previous chapters comprise previously published work surrounding the successful synthesis and anion affinity characterization of the bambus[6]uril family of anion receptors. Another previous chapter indicates collaborative work evaluating other anion receptors. This chapter summarizes synthetic efforts, sensor-facilitated receptor characterization, and statistical analysis & characterization that does not otherwise fit into previously published work or previous chapters. Specific synthetic efforts involved other bambus[6]uril derivatives that were intended to display distinct differences (steric or electronic) from other already-synthesized bambus[6]urils. Narrative of the collaborations provides insights into the diverse utility that ChemFET sensors provide, allowing for a unique method of aqueous anion affinity characterization for hydrophobic receptors. This chapter provides important insights into overcoming synthetic challenges and highlighting which barriers still remain at time of publication.

I am the primary and only author of this chapter. All of the analysis and the majority of the synthesis was performed by myself. For the collaborative projects, synthesis was also performed by Dr. Grace Kuhl and Madeline Howell (semithioglycoluril and semithio bambus[6]uril), Andy Davis in the Pluth Lab (imidazolium), Nathan Boone & Nolan McNeill in the Haley Lab

(azaphosphinine and arylethynyl bisurea), and Allison LaSalvia in the Cook Lab (carbonylation). At the time of writing, the entirety of this chapter remains unpublished.

Bambus[6]uril Synthesis Overview

Much of the previously published work surrounds the anion characterization of the bambus[6]uril family of anion receptors. Dodeca-n-butyl bambus[6]uril was synthesized and characterized in chapter 2, with its hydrosulfide affinity being used to place the elusive anion in the Hofmeister series. To evaluate modulation of the bambus[6]uril's binding pocket, the synthesis of a more sterically bulky bambus[6]uril was performed and discussed in Chapter 3. The impact of steric bulk to the anion binding pocket geometry was first documented through initial work by Sindelar, evaluating crystal structures of dodeca-n-propyl bambus[6]uril and dodecabenzyl bambus[6]uril.¹ The less bulky dodeca-n-propyl groups on the bambus[6]uril yielded a cylindrical cavity (not dissimilar to a segment of bamboo, which originally prompted the name "bambusuril");¹ In contrast, the bulkier benzyl groups biased an hourglass-type scaffold. Consequently, dodeca-n-propyl bambus[6]uril and dodecabenzyl bambus[6]uril chloride complexes exhibit different binding motifs in the solid state.¹ The cylindrical dodeca-n-propyl bambus[6]uril was found to bind a single chloride in the center of the bambus[6]uril binding pocket, with all 12 hydrogen bond donors facing inward. In contrast, dodecabenzyl bambus[6]uril's hourglass shape resulted in a chloride in each of the two conical ends, with each chloride being subsequently coordinated by half (six) the total number of hydrogen bond donors (Figure 1, also see Figure 1 from Chapter 3). As discussed in previous chapters, dodeca-n-butyl and dodeca-n-propyl bambus[6]urils display identical cylindrical geometries, and, therefore, also prefer 1:1 anion binding.² Initial work on the steric comparison project involved the synthesis of

two bambus[6]urils with varying steric bulk to assess the impact to aqueous anion binding of the different binding pockets.³ Here, initial planning involved the synthesise dodeca-tert-butyl bambus[6]uril (bulkier than the benzyl derivative) to study in contrast with dodeca-n-butyl bambus[6]uril (similar in shape to the n-propyl derivative). The steric impacts of tert-butyl groups represented the largest immediate steric bulk that could feasibly be added to the precursor

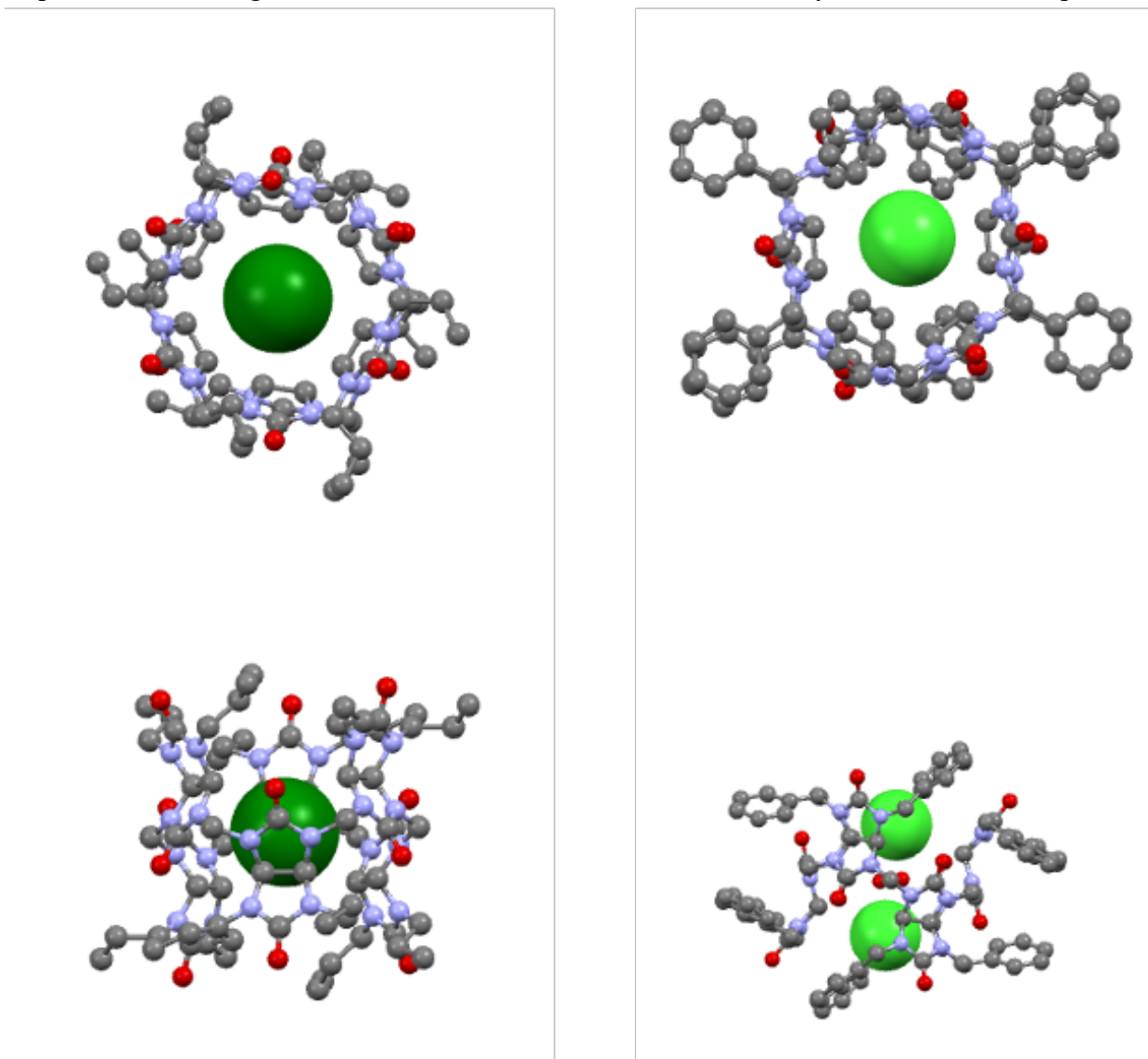


Figure 1. Crystal structures of dodeca-*n*-propyl bambus[6]uril (left) with dodecabenzyl bambus[6]uril (right) binding chloride. As depicted, dodeca-*n*-propyl bambus[6]uril binds a single chloride in the middle, with all 12 hydrogen-bond donors coordinating to the single central binding pocket. In contrast, dodecabenzyl bambus[6]uril binds in a 1:2 host:guest motif, with each of the two chlorides in its own binding pocket, with six hydrogen-bond donors coordinating to each pocket.

glycoluril nitrogen, facilitating exploration of the far end of the hourglass spectrum. Of note is that, while bambus[6]urils with bulkier groups have been synthesized,^{4,5} in these examples the bulk of the pendent R groups are somewhat removed from the glycoluril core, and therefore do not “pry open” the bambus[6]uril the way the immediate steric bulk of benzyl bambus[6]urils did as initially reported by Sindelar et al.¹ Thus, tert-butyl bambus[6]uril became the initial focus of the steric comparison project.

Synthetic Efforts for dodeca-tert-butyl bambus[6]uril

Initial attempts to synthesize dodeca-tert-butyl bambus[6]uril began from following the synthetic scheme in chapter 1, with a simple replacement of di-n-butyl urea with di-tert-butyl urea under conditions identical to those published. However, solubility immediately became an issue, with di-tert-butyl urea’s water insolubility forcing the first deviation from previous bambus[6]uril synthetic conditions. Other solvents including ethanol, isopropanol, and dioxane were explored that facilitated solvation of both reactants: functionalized urea (forming the bambus[6]uril pendant R-groups) and 4,5-dihydroxyimidozoline (4,5-DHI). Unfortunately, even once the solubility hurdle was overcome repeated attempts at this synthesis were unsuccessful, prompting an examination as to the underlying reason. Examinations of the pertinent three-dimensional structures in Avogadro⁶ suggested the underlying problem lay with the steric bulk of the tert-butyl groups blocking the lone pairs of electrons on both urea nitrogens from performing nucleophilic attack on the 4,5-DHI to form the desired glycoluril. Even if one nitrogen lone pair was able to bond to the DHI, it was suspected that the steric bulk of the urea’s second tert-butyl group would serve to further block the second nitrogen lone pair from closing the second ring forming the desired glycoluril.

As soon as issues arose with attempts to synthesize dodeca-tert-butyl bambus[6]uril, parallel efforts were directed at finding other suitable members of the bambus[6]uril family with bulky substituents. Dodecabenzyl bambus[6]uril was identified as an alternate, and the synthesis proved straightforward (see Chapter 3 for details). Thus, the need to synthesize the tert-butyl variant was obviated. However, any future efforts to synthesize dodeca-tert-butyl bambus[6]uril may consider beginning by reordering the traditional bambus[6]uril synthetic scheme comprised of reacting urea with glyoxal to form 4,5-DHI, which is then reacted with functionalized urea to form glycoluril. Instead, efforts may instead focus on first reacting the substituted urea with glyoxal to form a functionalized DHI derivative, then reacting with urea to form the glycoluril. This is more likely to succeed because the steric bulk of the tert-butyl groups that blocked reaction of nitrogen lone pairs with DHI may not block those same lone pairs from reacting with the much smaller glyoxal. Or, tert butyl amine can be used to stepwise create the desired glycoluril following the synthetic pathway. Regardless, identification and synthesis of a suitable bulky alternative obviated the need to continue synthetic efforts relating to tert-butyl bambus[6]uril.³

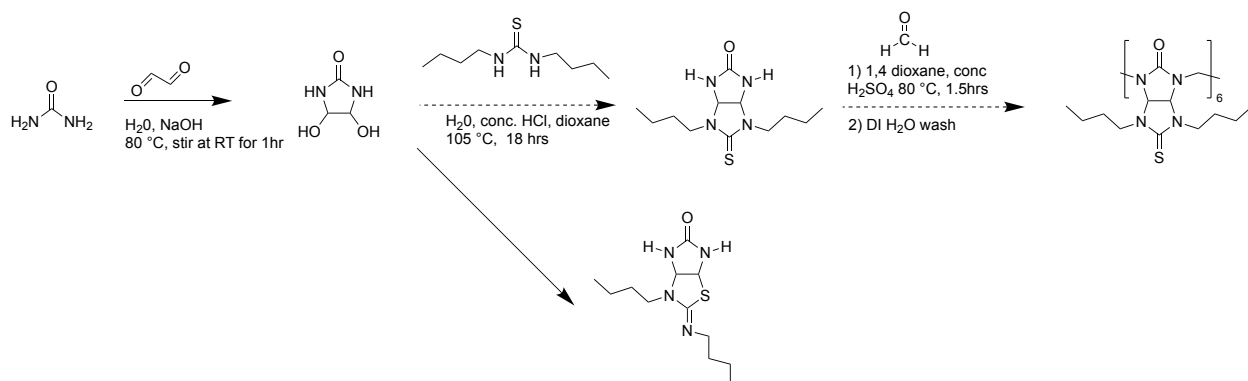
Synthetic efforts for dodeca-n-butyl semithiobambus[6]uril

Another effort involved comparison of electronically different bambus[6]uril derivatives. At this point, dodeca-n-butyl bambus[6]uril and dodecabenzyl bambus[6]uril had both been characterized in published work.³ One follow-on project idea was to take an already-characterized bambus[6]uril and substituting the axial glycoluril carbonyl oxygen with sulfur to change the hydrogen bond donor strength of the glycoluril subunits of the bambus[6]uril. Although oxygen is more electronegative than sulfur, a thiocarbonyl ends up being more polarized than carbonyl.⁷ This is attributed to poor bond overlap between carbon 2p and sulfur 3p orbitals in thiocarbonyl carbon-

sulfur pi bonds, compared to the carbon 2p and oxygen 2p overlap in carbonyl carbon-oxygen pi bonds. Therefore, thiocarbonyls can be drawn with a formal positive charge on the carbon, drawing more electron density from the surrounding nitrogens, resulting in less hyperconjugation between neighboring nitrogen lone pairs and C-H hydrogen bond donors. This presumably results in longer, weaker C-H bonds, and subsequently better hydrogen bond donors. Indeed, literature reports thiobambus[6]urils are stronger hydrogen bond donors than bambus[6]urils.⁸

Therefore, this project began with the intent to mirror the chapter 3 project comparison of bambus[6]urils with differing sterics, but in this case to evaluate impacts of different electronic environments and subsequently different hydrogen bond donor strength. Initial attempts involved axial-semithio-dodeca-n-butyl bambus[6]uril to serve as a direct comparison to dodeca-n-butyl bambus[6]uril, where sterics are essentially identical, and all differences in binding affinities can be solely attributed to the replacement of oxygen with sulfur.

Synthesis began by following the synthetic scheme of dodeca-n-butyl bambus[6]uril found in chapter 2. However, this approach proved unsuitable, as the thiourea nitrogen lone pairs evidently did not prove as effective a nucleophile as the lone pairs on the sulfur, resulting in S-alkylation product rather than the expected/desired N-alkylation product (Scheme 1).



Scheme 1. Initial semithiobambus[6]uril synthetic attempts, along with undesired S-alkylation product. This reaction was almost entirely starting material (4,5 DHI and thiourea), but S-alkylation product discovered by isolation of a crystal and subsequent x-ray crystallography.

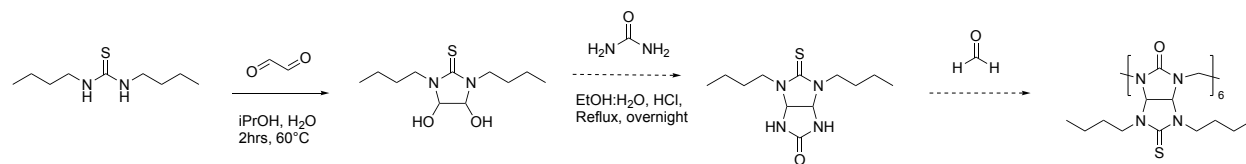
Reordering Bambus[6]uril Synthetic Steps

A literature review uncovered a possible solution, where the reaction steps could be re-ordered to react the thiourea with glyoxal forming a thiocarbonyl-functionalized DHI (denoted “DHIT” in literature⁹) heterocycle, which could then be reacted with urea to form a semithioglycoluril with the pendant R groups on the thiocarbonyl side. If the thiocarbonyl appeared on the unsubstituted side of the glycoluril, the thiocarbonyl sulfurs would be inside the binding pocket, where literature reports they fill the pocket, rendering it unsuitable for anion binding.¹⁰ The semithioglycoluril could then be reacted with formaldehyde to form semithio bambus[6]uril (Scheme 2). Since dodeca-n-butyl bambus[6]uril had been well characterized, the goal was to follow this alternative synthetic pathway to make axial semithio dodeca-n-butyl bambus[6]uril for comparison.

However, although relevant literature reported successful axial semithio dodecamethyl bambus[6]uril synthesis following this reordered synthetic pathway, attempts to make axial semithio dodeca-n-butyl bambus[6]uril using this pathway proved unsuccessful.

Protecting groups for semithio bambus[6]uril synthesis

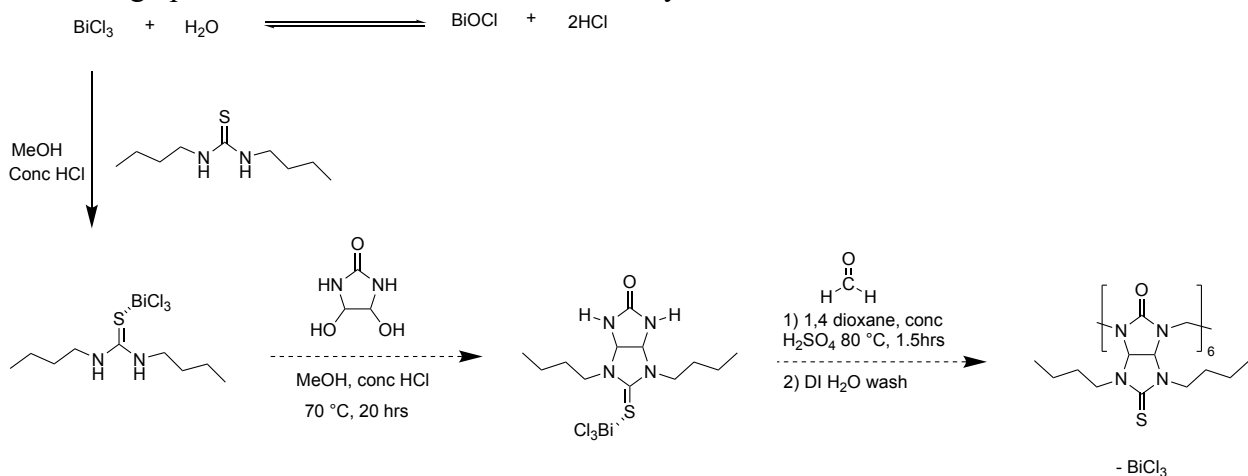
An alternative pathway to synthesis the desired semithio bambus[6]urils was to use a protecting (or coordinating) group to interact with the thiourea sulfur, leaving the nitrogen lone pairs free for nucleophilic attack on 4,5-DHI, facilitating the synthesis of the desired semithioglycoluril while



Scheme 2. Re-ordering of semithiobambus[6]uril synthesis steps to avoid S-alkylation. This involves reacting the substituted urea (or in this case, thiourea) with glyoxal to form a DHI derivative, and then further react with urea to end up at the desired glycoluril, and eventually bambus[6]uril.

avoiding the undesired S-alkylation product. Initial approach involved the introduction of thiophilic bismuth trichloride to coordinate with the thiourea sulfur. Initial investigation of the feasibility of this approach began with adding BiCl₃ to N,N' di-n-butyl thiourea in CD₃OD. A corresponding shift of thiourea N-H proton peaks in ¹H NMR indicated some amount of interaction between the BiCl₃ and thiourea sulfur. This interaction was thought to be enough to occupy the nucleophilic sulfur enough to prevent S-alkylation. Over time this also produced the expected insoluble BiOCl precipitating out of solution, indicative of the presence of water. Addition of HCl brought the BiOCl back into solution, indicating a reversible equilibrium that could be controlled by keeping the pH low. Thus, the planned reaction order was derived from Scheme 3, starting by adding BiCl₃ with HCl, then thiourea to coordinate at low pH, and then finally to 4,5-DHI to create the desired N-alkylated glycoluril product.

Unfortunately, this did not work. This was presumably due to the weak interaction of BiCl₃ with the thiourea sulfur, which had only demonstrated ¹H NMR shifts in the absence of any interfering species. This would be more accurately described as a “coordination” rather than a



Scheme 3. Implementation of protecting group methodology to avoid S-alkylation. Thiophilic bismuth was chosen, and the reaction carried forward with BiCl₃. Acid added was to push the top equilibrium to the left, avoiding bismuth oxychloride precipitation by lowering the pH. Then the thiourea could be added, and reacted with 4,5 DHI with the intent to ultimately synthesize the dodeca-*n*-methyl axial-semithiobambus[6]uril.

more conventional protecting group. Upon addition of DHI, the sulfur was still able to react with the 4,5-DHI to some extent, resulting in a messy ^1H NMR indicating large portion of unreacted starting material as well as S-alkylation/N-alkylation/partial alkylation mixtures.

Given the experience of previous work, future work should focus on alternative reaction pathways for synthesizing DHIT and semithioglycoluril leading to semithio bambus[6]uril derivatives.

ChemFET Collaborative Characterization Efforts

Imidazolium Macrocycle

An imidazolium-based macrocycle (Figure 2) was synthesized by the Pluth research group, who reported hydrosulfide affinity.¹¹ In parallel with the publication of the initial communication, the imidazolium macrocycle was simultaneously being evaluated via ChemFET sensors to

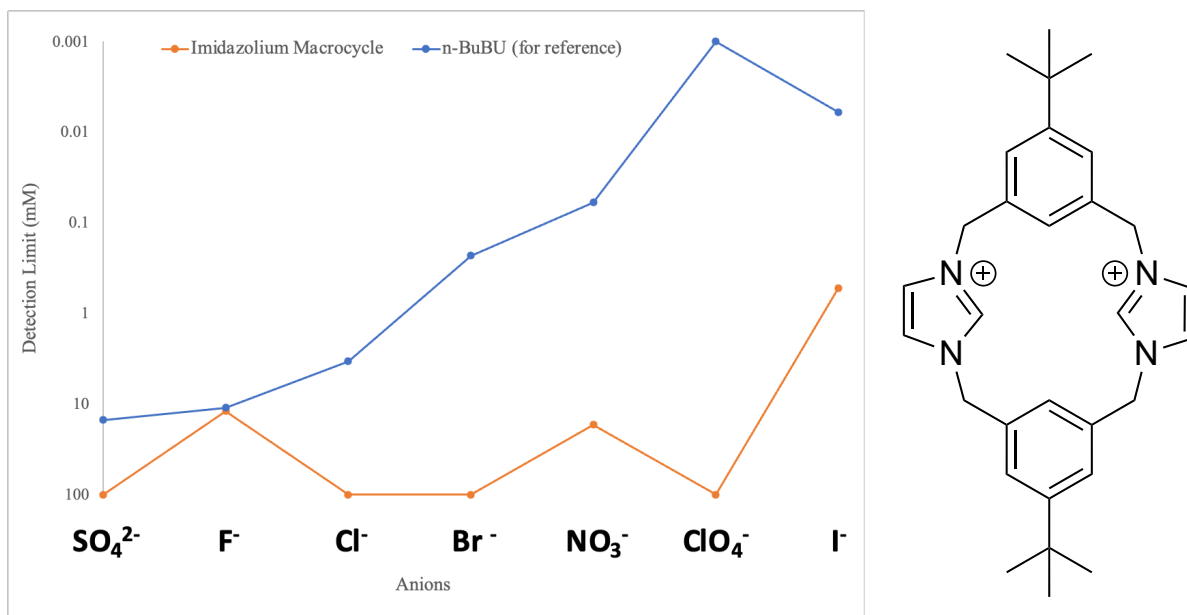


Figure 2. Imidazolium anion receptor (right) evaluated for aqueous anion affinity via ChemFETs (left). Chart includes characterization of dodeca-*n*-butyl bambus[6]uril for reference. Affinity profile was remarkable in not appearing to have any interaction with perchlorate, highly unusual compared to other anion receptors evaluated.

establish an aqueous anion affinity profile. After overcoming solubility issues, the anion affinity profile demonstrated overall poor anion affinity with a weak Hofmeister-like trend.

Azaphosphinines

The inclusion of phosphorous and nitrogen into the backbone of a macrocycle has long been studied by the DWJ and Haley research groups at UO.^{12,13} Much of the previous characterization of the azaphosphinine family included NMR and spectroscopic data, but no in-depth electrochemical characterization of their aqueous anion interactions.^{12,13} Some of these azaphosphinines demonstrated initial anion affinity via ¹H NMR titrations or other means, leading them as promising candidates for aqueous anion affinity evaluation via ChemFETs. An arylethynyl bisurea receptor scaffold has also been extensively studied in the DWJ research group, both via solution-state methodology (i.e. ¹H NMR titrations) and via electrochemical sensors (ChemFETs).^{14–18} An azaphosphinine derivative of the arylethynyl bisurea scaffold (Figure 3) proved an ideal candidate for ChemFET characterization of host-guest interaction with aqueous anions. This receptor was analyzed through the standard series of seven common anions in the Hofmeister series to establish an aqueous anion affinity profile.

Future work on this project by Nathan Boone and Bronwen Link will involve comparison of this anion affinity profile to one derived from a control receptor identical to the non-PN containing half of the azaphosphinine. Thus, any difference in results stem solely from the incorporation of the PN moiety in the receptor.

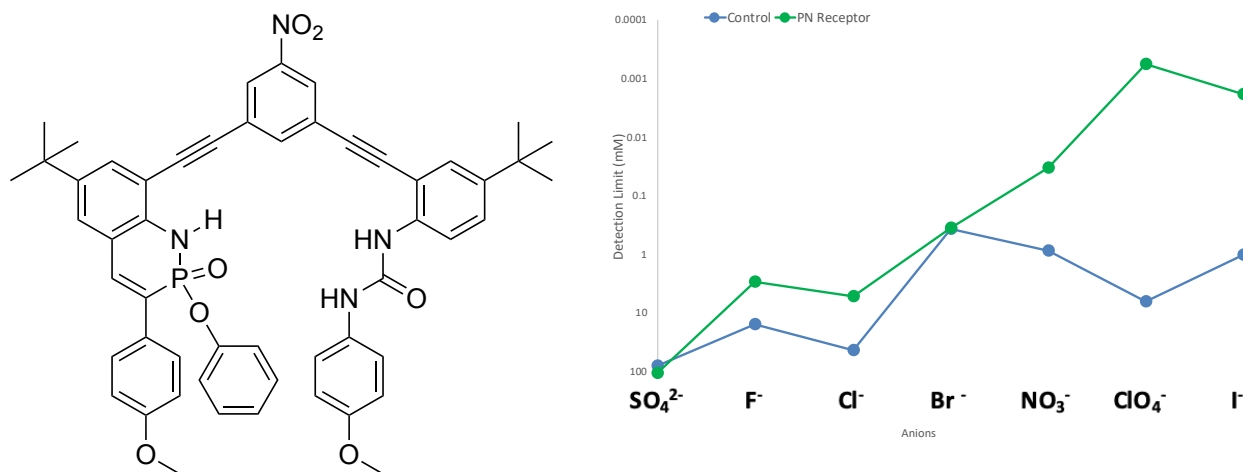


Figure 3. Azaphosphinine anion receptor (left) evaluated for aqueous anion affinity via ChemFETs. Detection limits depicted on the right, indicating similar general Hofmeister trend. Of note is that this is the first evaluated receptor with higher fluoride response than control. Future work will likely evaluate arylethynyl bisurea receptor with similar functional groups (nitro/*t*-butyl/methoxy) as a sort of control to establish the impact of the hydrogen bond acceptors present on one arm of the azaphosphinine, potentially related to shape persistency in the membrane via intramolecular interactions. Shape persistency is very influential in solid-state evaluations (such as ChemFETs), where once embedded in the membrane the receptor may have little or no motion to adjust shape or configuration as is feasible in solution-state studies. This is thought to be a major contributor to the exceptional anion detection limits present in the cavitand-type bambus[6]uril receptors characterized in chapters 2 and 3.

Design of Experiments Collaborative Efforts

Carbonylation reactions often use metal catalysts and carbon monoxide as a means of forming carbonyl groups in larger structures. One obvious hazard and environmental concern is the (often) required liberal use of carbon monoxide (CO) gas as a reactant, which prompted an effort to explore characterizing the reaction with a goal of minimizing CO usage. This project largely followed Chapter 5 methodology in using design of experiments (DOE) to characterize the reaction with a goal of minimizing CO pressure, as well as optimizing yield and product distribution. An analysis of historical data from three sources^{19–21} was accomplished following many of the

procedures in Chapter 5 (including determination of statistically-significant p-values as those less than 0.05). This historical analysis was accomplished using JMP software, using an iterative approach to characterizing the impacts of reaction inputs on product. This evaluation specifically considered all main factors and (initially) all two factor interactions (Table 1). Following standard best practices and previous work,^{22,23} DOE Model 1 was developed by evaluating historical data to characterize all main and two-factor interactions that influence product properties. An immediate problem arose that often appears when conducting historical analyses, where the experimental data does not adequately fill the design space to provide enough degrees of freedom to allow a model to thoroughly evaluate all main and two-factor interactions. All 5 main interactions ended up with p-values of 1, indicating the analysis was unable to attribute statistical significance to their influence on the output properties. DOE Model 2 was then developed after manually removing the five least significant 2-factor interactions from Model 1 (Table 1, Model 1, lines 6-10) to provide enough degrees of freedom to adequately evaluate the main factors. Once a second evaluation was accomplished, Model 2 now indicated that three of the five main interactions now had p-values indicating statistical significance: temperature, CO pressure, and catalyst type. Since CO pressure was now included as a statistically-significant model parameter, Model 2 was now suitable for one of the initially-stated goals of predictively optimizing reaction conditions to minimize CO pressure. This work is ongoing by Allison LaSalvia of the Cook research group at UO, with results iteratively used to improve the model and predict new optimization conditions.

Model 1		Model 2	
Factors	PValue	Factors	PValue
1 Catalyst (mol %)*Metal	0.00000	1 Catalyst (mol %)(0.1,2.7)	0.00000
2 Catalyst (mol %)*Promoter	0.00000	2 Catalyst (mol %)*Metal	0.00000
3 Temperature (C)*Catalyst (mol %)	0.00014	3 Pressure (bar)*Temperature (C)	0.00000
4 Metal*Promoter	0.00057	4 Pressure (bar)(11,100)	0.00001
5 Pressure (bar)*Temperature (C)	0.00223	5 Temperature (C)(160,230)	0.00006
6 Pressure (bar)*Promoter	0.00299	6 Temperature (C)*Catalyst (mol %)	0.00278
7 Pressure (bar)*Metal	0.00662	7 Catalyst (mol %)*Promoter	0.23630
8 Temperature (C)*Metal	0.01159	8 Metal	1.00000
9 Pressure (bar)*Catalyst (mol %)	0.02959	9 Promoter	1.00000
10 Temperature (C)*Promoter	0.60467	10 Metal*Promoter	1.00000
11 Pressure (bar)(11,100)	1.00000		
12 Temperature (C)(160,230)	1.00000		
13 Catalyst (mol %)(0.1,2.7)	1.00000		
14 Metal	1.00000		
15 Promoter	1.00000		

Table 1. Design of Experiments iterative optimizations for characterization of significant factors influencing carbonylation reaction outputs. Model 1 was developed through an analysis of all available historical data, which unfortunately did not provide enough degrees of freedom to thoroughly analyze the design space, resulting in all five main factors (Catalyst, Metal, Pressure, Promoter, and Temperature) having insignificant p-values, meaning the software could not fit their impacts into a model with statistical significance. In an attempt to counter this, the bottom five two-factor interactions (lines 6-10 in Model 1) were manually removed from the DOE software evaluation, and the data re-analyzed. This new model now incorporated Catalyst, Pressure, and Temperature into the model with statistically-significant p-values. Model 2 was then used for validation studies.

Conclusion

ChemFET sensors provide a unique means to evaluate the aqueous anion affinity of hydrophobic receptors. This manuscript documents numerous successful utilizations of ChemFETs to detect anions, provide detection limits as a quantitative means of receptor analysis, comparison, and characterization (Chapter 1). ChemFETs are an incredibly useful tool to provide primary or supporting characterization of receptors in challenging conditions, such as when the

analyte is toxic and/or reactive (Chapter 2). The Hofmeister series is a useful point of reference for which to compare ordered detection limits of receptors as characterized using ChemFETs. Perturbations to detection limits and the Hofmeister ordering can provide insight into the host-guest interactions of different receptors for purposes of direct comparison (Chapter 3). ChemFET aqueous anion affinity characterization is not limited to more traditional organic anion receptors, but other porous material that may also display host-guest interactions (Chapter 4). Then, statistical analysis can be applied to historical data to characterize syntheses and provide optimization conditions (Chapters 5 and 6). Throughout this manuscript, rarely are entirely new processes developed. However, what provides useful data is the synergistic combination of disparate fields, such as electrochemical sensor-based analysis of anion receptors and MOFs, or the application of DOE to characterize reactions for optimization.

A final note to the reader: The collaboration between two labs studying very different chemistry that resulted in Chapter 4 largely happened because of the interesting MOF results noticed by Prof. Carl Brozek, who was able to walk two offices over and ask Prof. Darren Johnson about how the host-guest interaction could be characterized. The collaboration resulting in Chapter 5 would likely never have happened had the author not happened to sit next to Alex Rosen during his entire PhD program. Two chapters, or a full third of this dissertation, is thus comprised of collaborative efforts that happened thanks to the members of the chemistry department taking an interest in the research of those around them, and being willing to collaboratively solve difficult problems.

APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 1: INTRODUCTION TO THE ANALYTICAL METHODS USED: CHEMFET SENSORS AND DESIGN OF EXPERIMENTS

ChemFET Construction

Following are the images for how to construct a ChemFET (Figure A1):

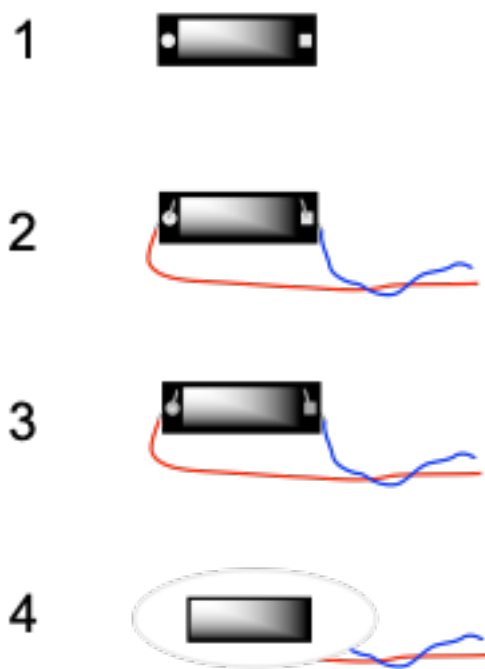


Figure A1. 1. Commercially available ChemFET. 2. Thread wires through the source and drain leads, ensuring standardization of color to know how to connect them to the system. Square hole indicates drain; circle hole indicates source. For standardization, our lab always uses red wires for source and blue for drain. 3. Solder the wires into the leads. 4. Cover the entire ChemFET (minus the gate oxide surface) with epoxy for waterproofing.

Reference Electrode Construction

Following are the images for how to construct a reference electrode (RE) (Figure A2):

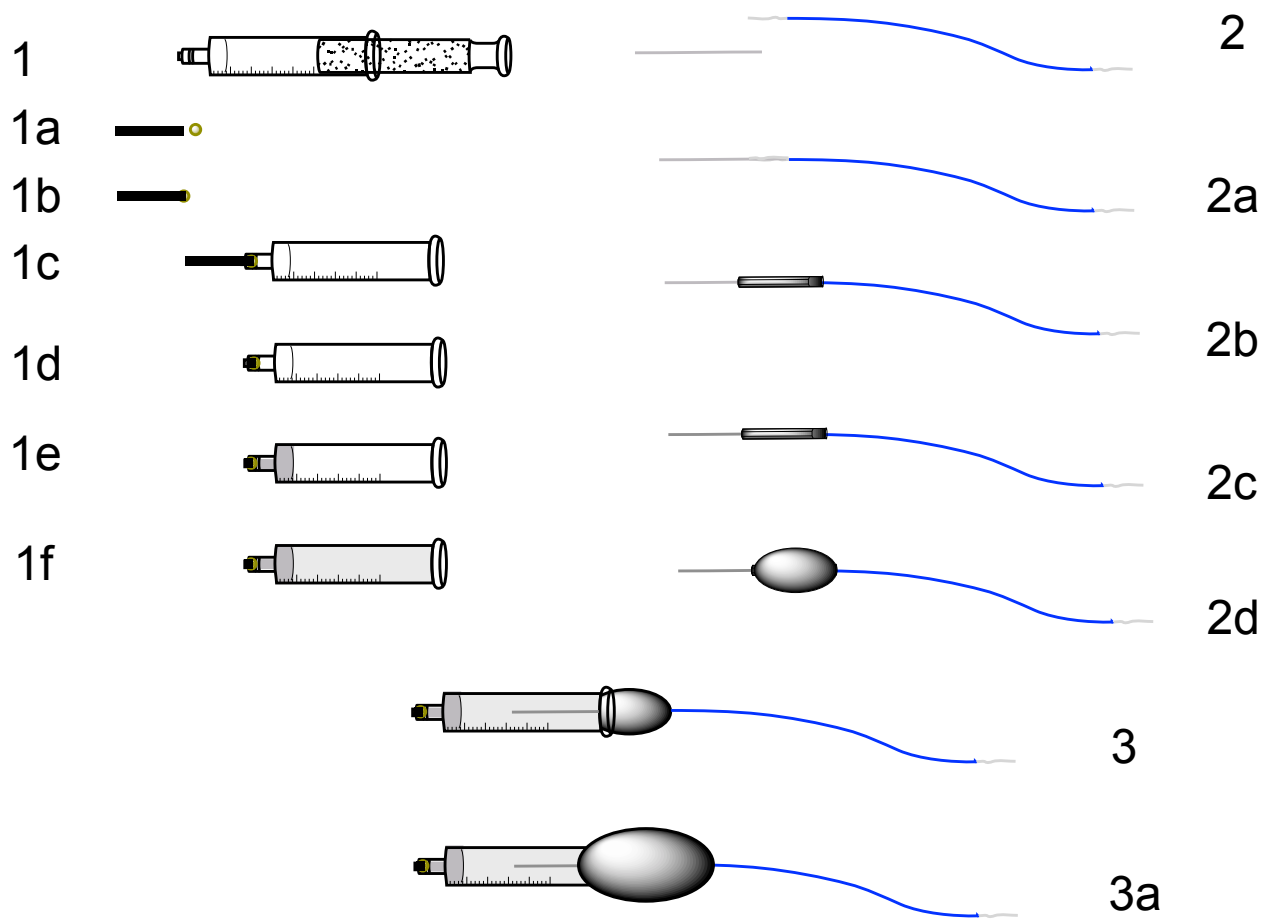


Figure A2. (1) 1 mL syringe. (1a) 4 Å molecular sieve soaked in 3 M KCl, and heat shrink tubing. (1b) place sieve into heat shrink tubing. (1c) pull heat shrink tubing through syringe tip until sieve in the syringe tip. (1d) cut the excess heat shrink tubing and use a heatgun to seal heat shrink tubing “collar” around the sieve in the syringe tip. (1e) fill tip of syringe with 3 M KCl in agarose. (1f) fill remainder of syringe with 3 M KCl. (2) take length of 20-gauge wire along with section of bare silver wire. (2a) solder silver wire to one end of electrical wire. (2b) use heat shrink tubing to seal soldered section (note: this may take several portions of heat shrink tubing; it is critical that the soldered portion is not exposed to the KCl in the syringe body, or the Ag/AgCl will oxidize and

RE will no longer function). (2c) submerge the exposed silver in bleach for 6-12 hours to create Ag/AgCl surface (or other functionality as dictated by analyte). (2d) parafilm to widen the base of the electrode to seal within the syringe body. (3) place the silver electrode in the syringe body (note: it is important to completely fill the syringe body with 3 M KCl, let excess drain out when silver wire inserted, but there cannot be any air bubbles present or the silver will have to be removed, the KCl solution topped off, and the wire re-inserted). (3a) the reference electrode join is then parafilmed and finally sealed with electrical tape.

Following is the diagram necessary to construct a driver circuit to drive a reference electrode and ChemFET sensors (Figure A3):

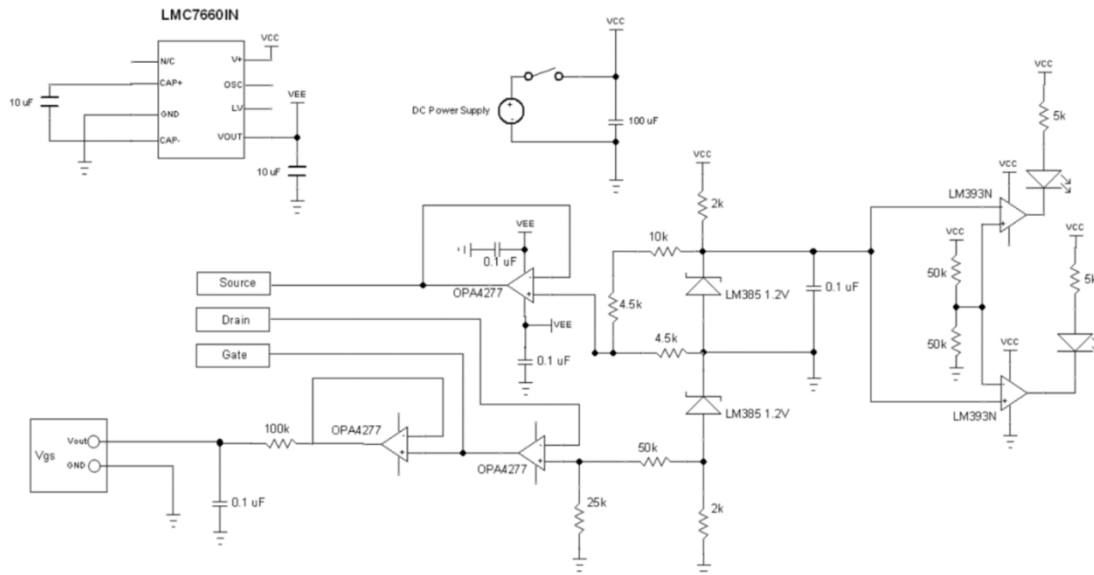


Figure A3. The driver circuit maintains a constant drain current (I_D) of 99.6 μA and drain voltage (V_{DS}) of 617 mV in a feedback mode in which that the gate voltage (V_{GS}) is changed as necessary to maintain the constant drain current and voltage. V_{GS} is taken as the measurement signal. This circuit is based on an ISFET analog driver circuit available from Winsense (Winsense.co.th, WIPSK-CB1)

Data Analysis Spreadsheet

An example spreadsheet is included with the ESI for detailed analysis of ChemFET sensor data (Figure A4):

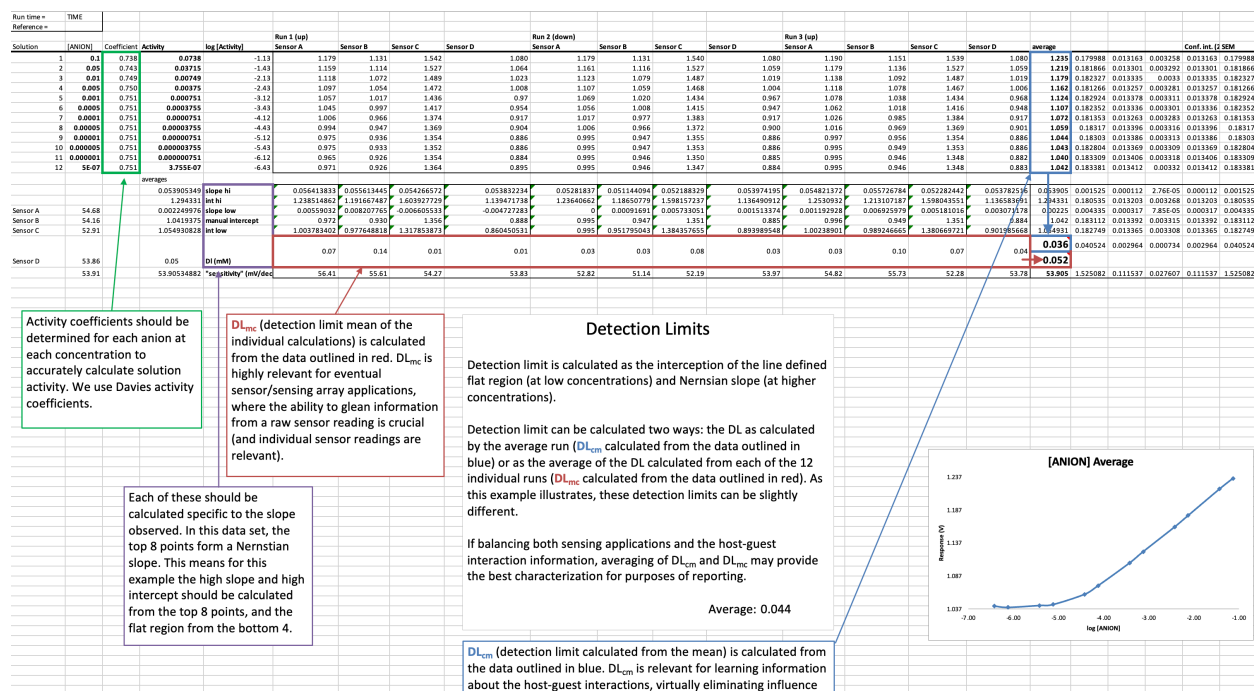


Figure A4. This interactive spreadsheet includes detailed labeling that outlines how to calculate figures of merit such as sensitivity and detection limit, and also outlines different ways to calculate detection limits and the relative merits of each. This spreadsheet is entitled Anion Example Data Analyzed.xlsx and is included with the ESI.

For purposes of calculations, concentrations have been converted to activity. We have done this using the Davies activity calculation,¹ and examples of this in use are shown in previously published works.²⁻⁶ For example, the activity of a solution containing PIPES, KOH (used to tune pH to 7), and analyte salt are all included, and the activity coefficient of the anion noted. In the

above example, the activity coefficient at 0.1 M was calculated as 0.738, and at 0.05 M was calculated to be 0.743. This activity coefficient is calculated for each concentration, and the resulting coefficient multiplied against the concentration to produce a corrected activity for each of the 12 solutions. These 12 activities are then used for all further analysis and graphical depiction. Although all our work has been done converting to Davies activities, other options also exist, including Debye-Huckel activity coefficients.⁷

Appendix A References

- 1 C. W. Davies, *J. Chem. Soc.*, 1938, 2093–2098.
- 2 T. J. Sherbow, G. M. Kuhl, G. A. Lindquist, J. D. Levine, M. D. Pluth, D. W. Johnson and S. A. Fontenot, *Sensing and Bio-Sensing Research*, 2021, **31**, 100394.
- 3 G. M. Kuhl, D. T. Seidenkranz, M. D. Pluth, D. W. Johnson and S. A. Fontenot, *Sensing and Bio-Sensing Research*, 2021, **31**, 100397.
- 4 G. M. Kuhl, Doctoral Dissertation, University of Oregon, 2022.
- 5 G. M. Kuhl, D. H. Banning, H. A. Fargher, W. A. Davis, M. M. Howell, L. Zakharov, M. D. Pluth and D. W. Johnson, *Chem. Sci.*, 2023, **14**, 10273–10279.
- 6 D. H. Banning, G. M. Kuhl, M. M. Howell and D. W. Johnson, *Org. Biomol. Chem.*, 2024, **22**, 269–273.
- 7 G. G. Manov, R. G. Bates, W. J. Hamer and S. F. Acree, *J. Am. Chem. Soc.*, 1943, **65**, 1765–1767.

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 2. BENCHMARKING THE PLACEMENT OF HYDROSULFIDE IN THE HOFMEISTER SERIES USING A BAMBUS[6]URIL-BASED CHEMFET SENSOR

N,N'-dibutyl glycoluril ^1H NMR (Figure B1)

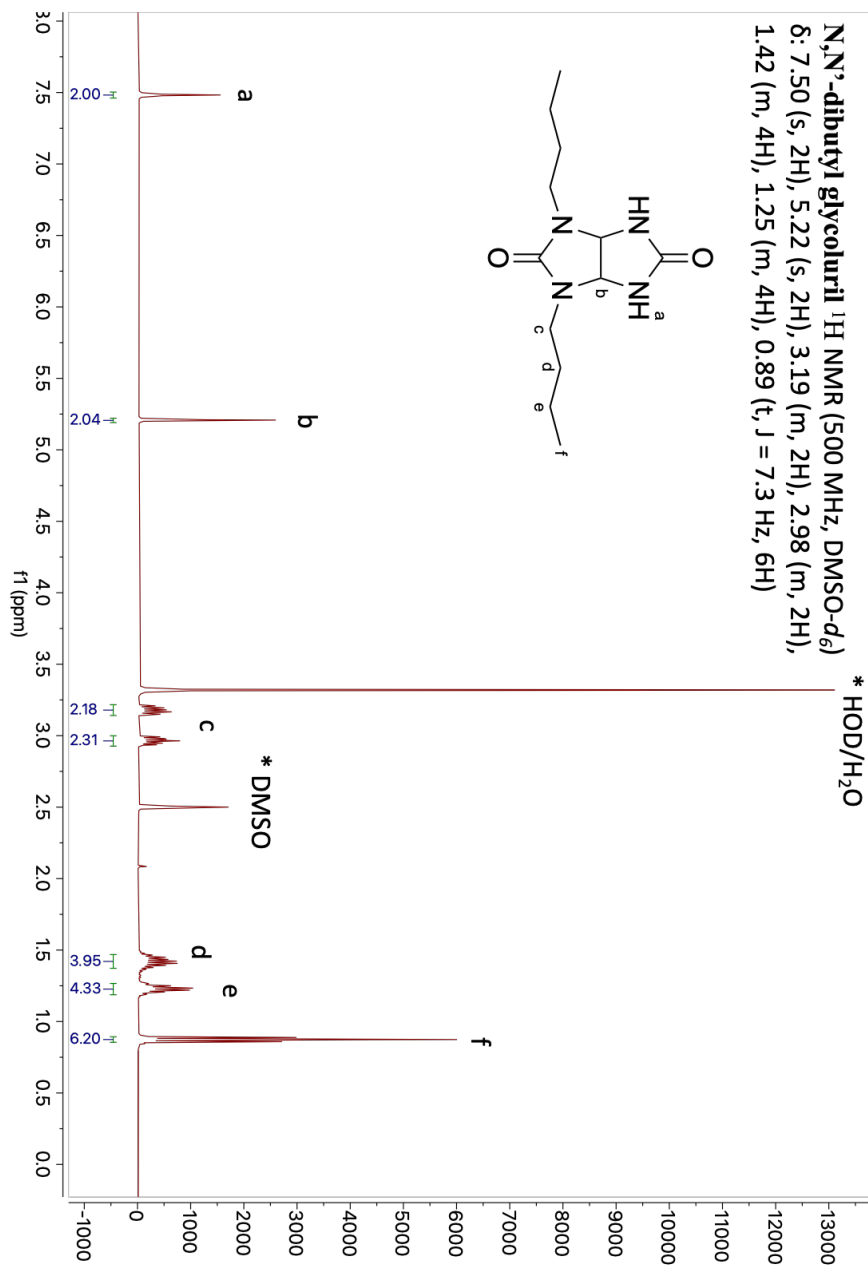


Figure B1. *N,N'*-dibutyl glycoluril ^1H NMR for purposes of structure confirmation

Dodeca-n-butyl bambus[6]uril Mass Spectrometry(Figure B2)

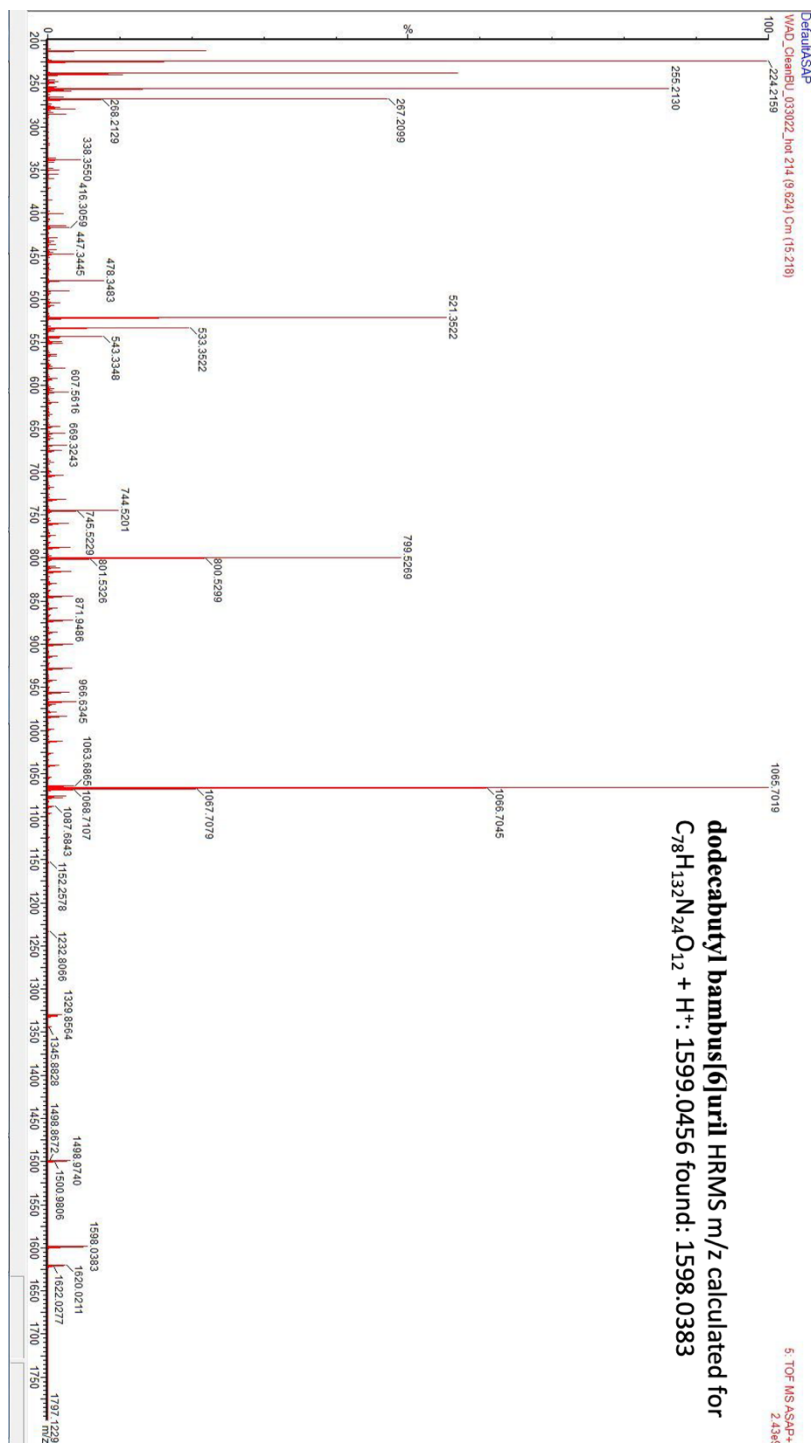


Figure B2. Dodeca-n-butyl bambus[6]uril Mass Spectrometry for purposes of structure confirmation

Dodeca-n-butyl bambus[6]uril ^1H NMR (Figure B3)

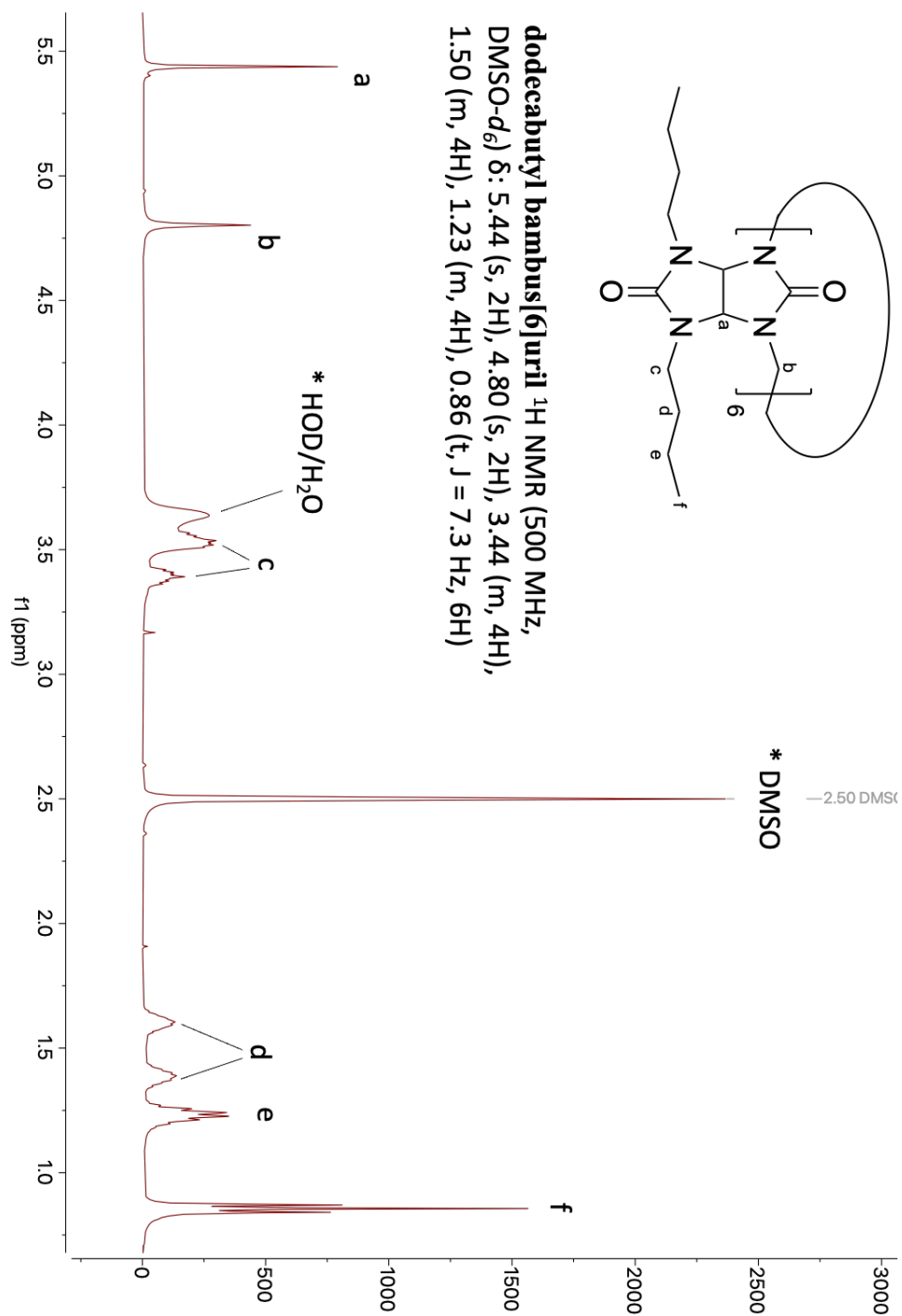


Figure B3. Dodeca-n-butyl bambus[6]uril ^1H NMR for purposes of structure confirmation

Dodeca-n-butyl bambus[6]uril ^{13}C NMR (Figure B4)

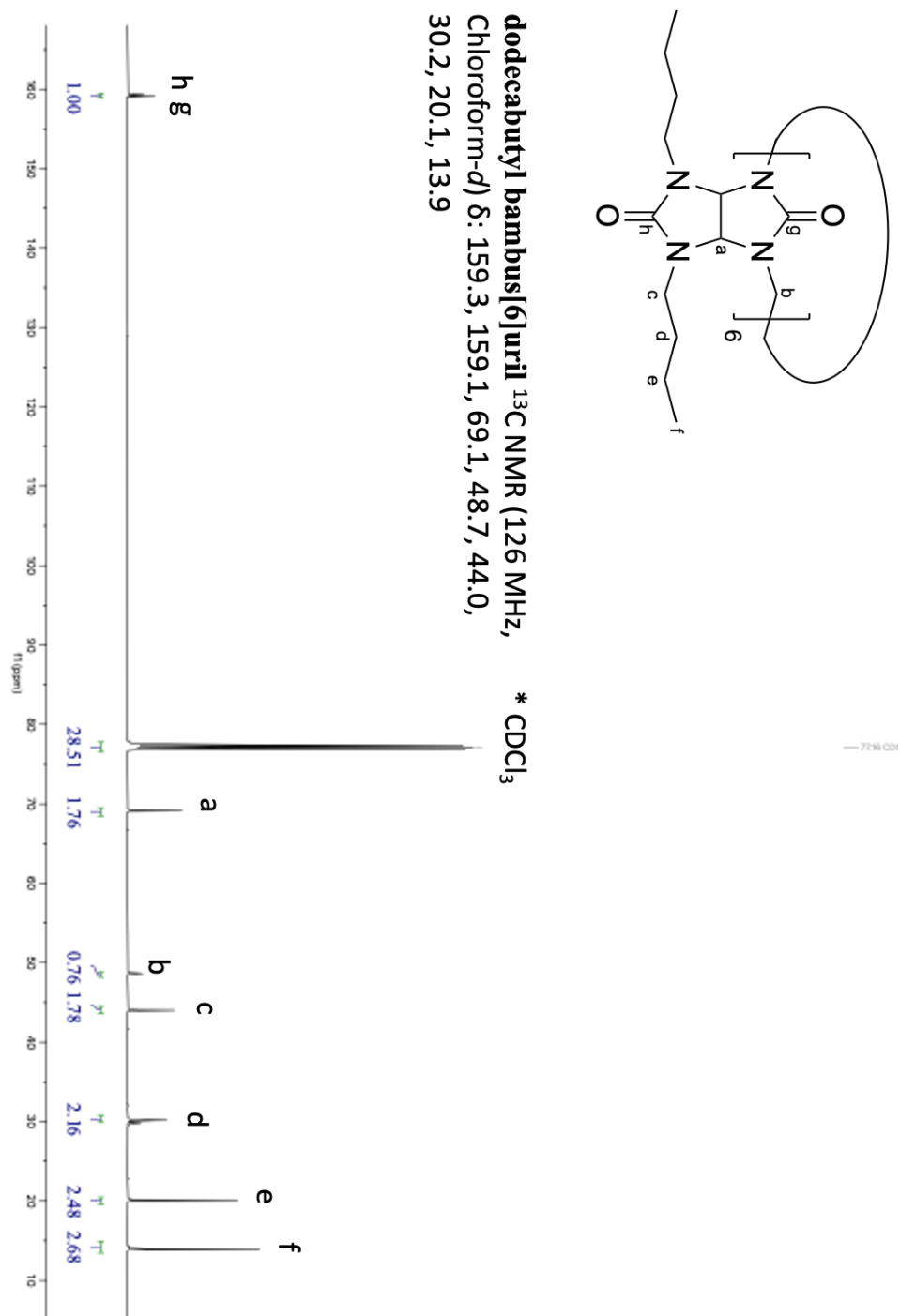


Figure B4. Dodeca-n-butyl bambus[6]uril ^{13}C NMR for purposes of structure confirmation

Dodeca-*n*-butyl bambus[6]uril Crystal Structure (Figure B5)

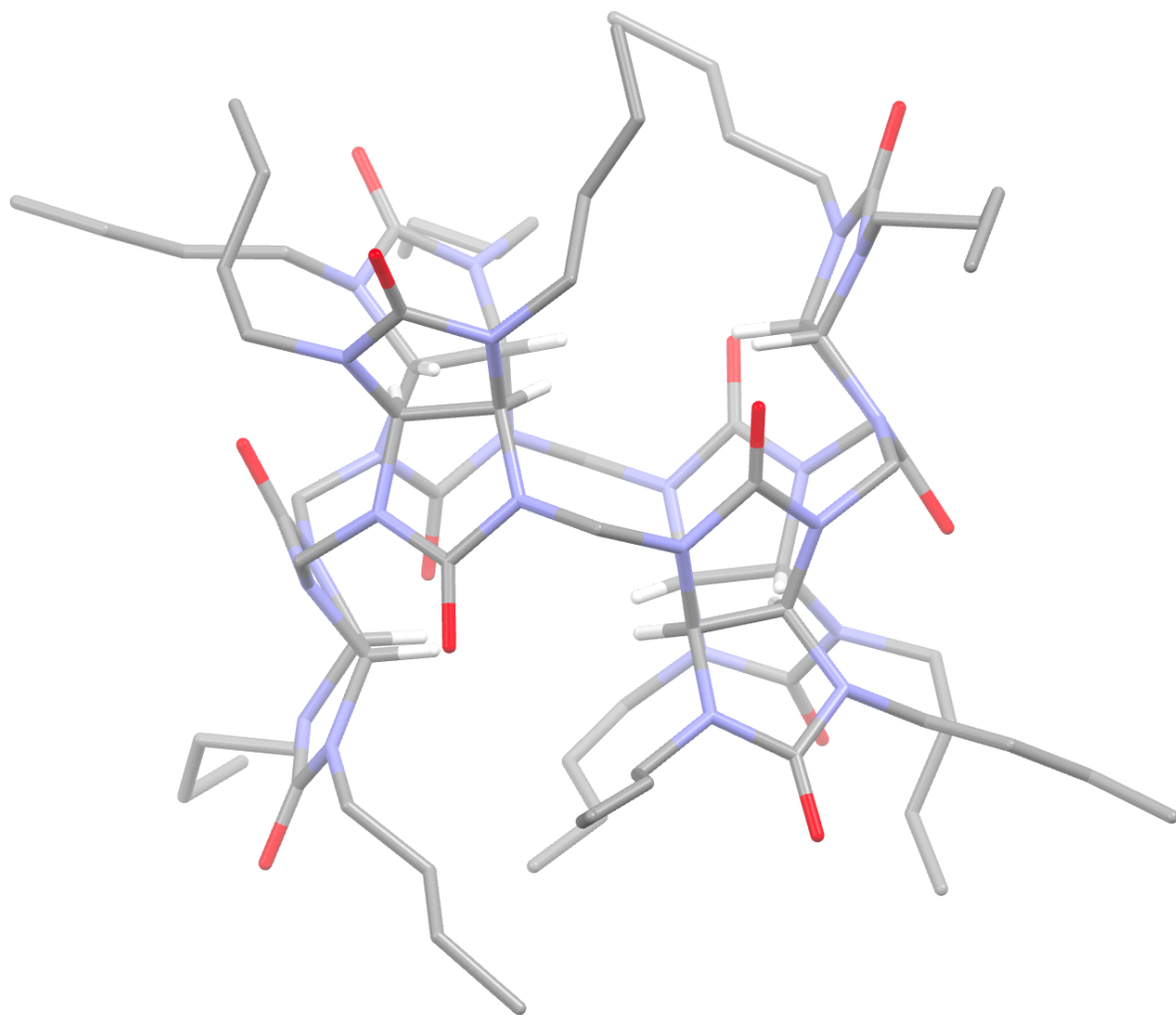


Figure B5. Dodeca-*n*-butyl bambus[6]uril Crystal Structure (side view, C-H hydrogen bond donors depicted, all other hydrogens omitted for clarity)

Dodeca-n-butyl bambus[6]uril Crystal Structure (Figure B6)

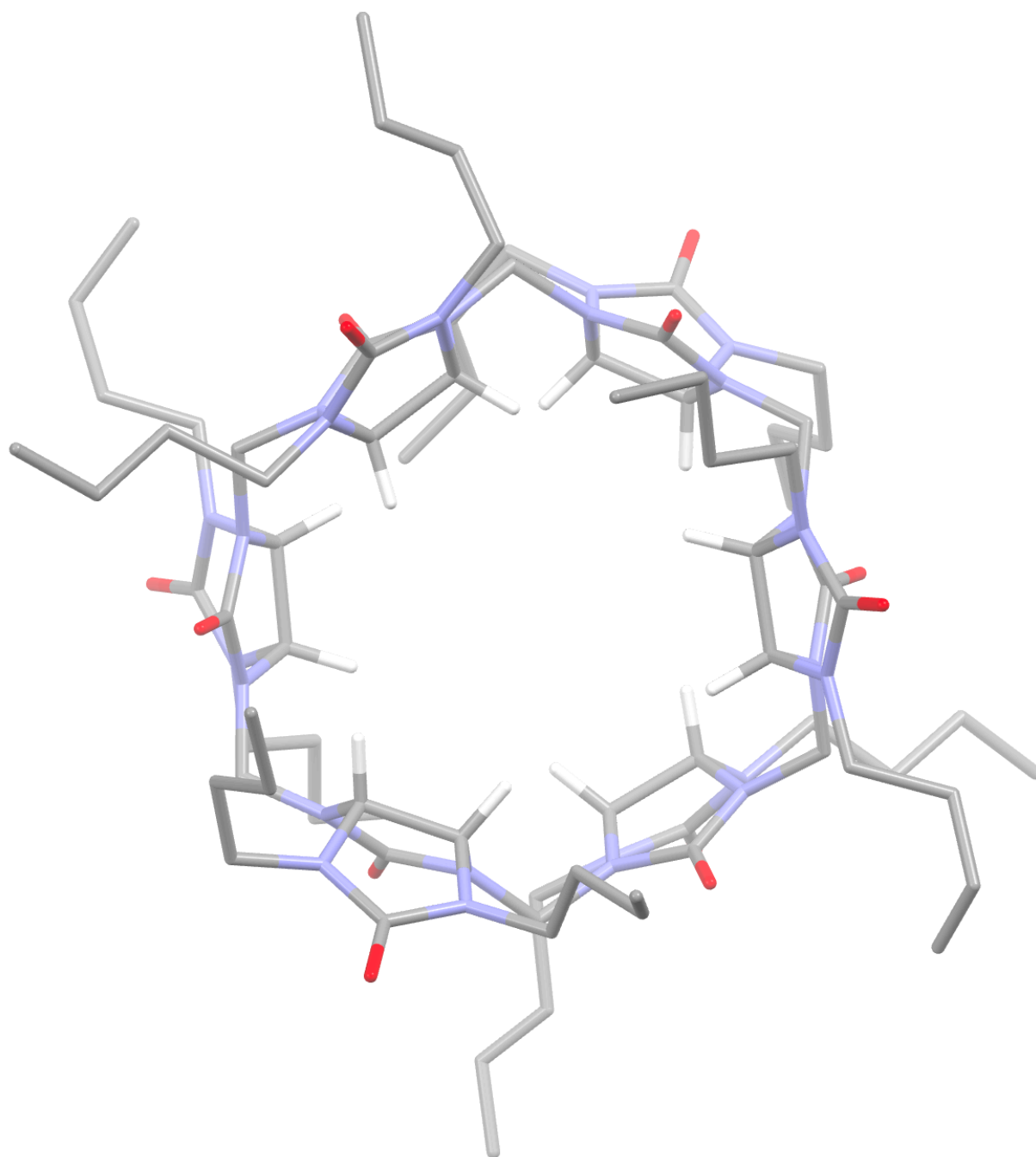


Figure B6. Dodeca-n-butyl bambus[6]uril Crystal Structure (top view, C-H hydrogen bond donors depicted, all other hydrogens omitted for clarity)

X-ray Crystallography.

Diffraction intensities for **DWJ334** were collected at 173 K on a Bruker Apex2 CCD diffractometer using a *Incoatec* Cu $I\mu S$ source, CuK α radiation, 1.54178 Å. Space group was determined based on systematic absences. Absorption correction was applied by SADABS.¹ Structure was solved by direct methods and Fourier techniques and refined on F^2 using full matrix least-squares procedures. All non-H atoms were refined with anisotropic thermal parameters. H atoms were refined in calculated positions in a rigid group model. X-ray diffraction at high angles was very weak due to the high disorder of solvent molecules and terminal $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ groups inside the structure. Even by using a strong *Incoatec* Cu $I\mu S$ source it was possible to collect diffraction data only up to $2\theta_{\text{max}} = 99.83^\circ$. The data collected provide appropriate number of measured reflections per refined parameters, 9438 per 1028. Thermal parameters for some of the terminal $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ groups are significant elongations. Solvent molecules fill out cavities in the neutral main molecule and in the packing and are highly disordered. All these solvent molecules were treated by SQUEEZE² and not included into the formula of the compound given in the CIF file. Correction of the X-ray diffraction by SQUEEZE was 304 electron/cell. The RIGU option was used in the final refinement. The structure was determined inside space group $P2_1$, but absolute configuration was not determined. The structure of **DWJ334** has not been precisely determined but it provides clear chemical results discussed in the paper. All calculations were performed by the Bruker SHELXL-2014/7 package.³

Crystallographic Data for DWJ334: $\text{C}_{78}\text{H}_{132}\text{N}_{24}\text{O}_{12}$, $M = 1598.07$, $0.16 \times 0.16 \times 0.07$ mm, $T = 173(2)$ K, Monoclinic, space group $P2_1$, $a = 13.0608(10)$ Å, $b = 24.6448(2)$ Å, $c = 15.6448(13)$ Å, $\beta = 102.704(3)^\circ$, $V = 4916.1(7)$ Å³, $Z = 2$, $D_c = 1.080$ Mg/m³, $\mu(\text{Cu}) = 0.606$ mm⁻¹, $F(000) = 1728$,

$2\theta_{\max} = 99.83^\circ$, 20913 reflections, 9438 independent reflections [$R_{\text{int}} = 0.0328$], $R1 = 0.0754$, $wR2 = 0.2085$ and $\text{GOF} = 1.009$ for 9438 reflections (1028 parameters) with $I > 2\sigma(I)$, $R1 = 0.0860$, $wR2 = 0.2220$ and $\text{GOF} = 1.035$ for all reflections, max/min residual electron density $+0.541/-0.295$ $\text{e}\text{\AA}^{-3}$.

ChemFET Sensor and Construction

Silicon nitride-gated field effect transistors (FETs) were purchased from Winsense (<http://www.winsense.co.th>, WIPS-C) and cleaned with ethanol and soaked in H_2O_2 for 10 minutes prior to functionalization. Polyvinyl chloride (PVC), 2-nitrophenyl octyl ether (NPOE), and Tetraoctylammonium Nitrate (TOAN) were obtained from Fisher Scientific and TCI Chemicals. All receptor-containing sensors contained 65 weight percent PVC, 32 weight percent NPOE, 2 weight percent TOAN, and 1 weight percent bambusuril receptor. Control sensors membrane composition were 66 weight percent PVC, 32 weight percent NPOE, and 2 weight percent TOAN. Chemically selective membranes were deposited onto the FET surface by manual drop-casting. Four aliquots of $1.6 \mu\text{L}$ were applied at 30-minute increments before being placed in an oven at 60°C for at least 4 hours, yielding an approximate film thickness of $50 \mu\text{m}$.

ChemFET Reference Electrode (RE) Construction

Ag/Ag₂S reference electrodes (REs) were used for hydrosulfide testing, and Ag/AgCl Res were used in all other potentiometric experiments. All REs in this report were made in-house following previously-reported procedures. (T. J. Sherbow, G. M. Kuhl, G. A. Lindquist, J. D. Levine, M. D. Pluth, D. W. Johnson and S. A. Fontenot, *Sensing and Bio-Sensing Research*, 2021, **31**, 100394.)

ChemFET Sensor and Reference Electrode (RE) Operation

The ChemFETs were driven by a benchtop power source. In operation, the drain voltage (V_{ds}) is held at 617.5 mV and the drain current (I_{ds}) at 100 mA. The external RE is held at ground, and the voltage between ground and the source (V_{gs}) terminal changes to maintain the values of V_{ds} and I_{ds} . V_{gs} is recorded as the measurement signal. NI-DAQ 6009 at a rate of 1 kHz was used for data acquisition paired with a custom Labview program for collection. All potentiometric tests were recorded for 300 seconds, and comprised of four identically-constructed ChemFETs. The four ChemFET sensors were run through a series of 12 solutions of 0.1M, 0.05M, 0.01M, 0.005M, 0.001M, 0.0005M, 0.0001M, 0.00005M, 0.00001M, 0.000005M, 0.000001M, and 0.0000005M analyte, each with constant 50 mM PIPES.

Each data point consisted of four identically-constructed ChemFET sensors run through 12 solutions, in triplicate, of alternating order (low to high concentration, then high to low concentration, then low to high concentration).

Counter-cation analysis

Initial ChemFET screening was accomplished via identical sensor membrane formulation as the rest of the analysis presented in this paper (dodeca-n-butyl bambus[6]uril, tetraoctyl ammonium nitrate, polyvinyl chloride, and nitrophenyl octyl ether). This screening evaluation was accomplished via 5 solutions of constant 50mM PIPES and varying analyte concentration. To study the effect of the counter cation, the anion was kept constant and a number of counter cations evaluated to study impact on two metrics: sensitivity and detection limit. The four counter cations were selected to evaluate impact of differing size (cesium vs lithium), shape (spherical cations vs ammonium), and charge (Ca^{2+} vs NH_4^+ , Cs^+ , and Li^+) (Results in Table B1).

Salt	Sensitivity ± 2 standard deviations	Detection Limit (mM) $\pm$ Confidence interval at 2 standard deviations
CaCl₂	60 $\pm$ 12	6 $\pm$ 2
NH₄Cl	50 $\pm$ 8	6 $\pm$ 1
CsCl	50 $\pm$ 6	5 $\pm$ 2
LiCl	40 $\pm$ 2	3 $\pm$ 1

Table B2. Initial counter cation screening. This quick analysis indicated potential impact of counter cation on sensitivity and detection limit of chloride. The screening comprised of four sensors containing dodeca-n-butyl bambus[6]uril evaluated through five series of salt solutions in order of magnitude concentration intervals. Results of this quick analysis prompted a follow-on more robust, in-depth study on any statistically-significant impacts of counter cation on anion measurement by ChemFET.

Initial screening results suggested possible differences in detection limits between calcium, ammonium, cesium, and lithium. Calcium and lithium represented the extreme differences, so a more in-depth characterization was accomplished of the full 12 solutions of constant 50 mM PIPES and varying concentration of these two chloride salts.

The full run of 12 solutions comprised 0.1M, 0.05M, 0.01M, 0.005M, 0.001M, 0.0005M, 0.0001M, 0.00005M, 0.00001M, 0.000005M, 0.000001M, and 0.0000005M analyte, each with constant 50 mM PIPES.

Upon running four dodeca-n-butyl bambus[6]uril sensors through the full 12-series of LiCl and CaCl₂ solutions (in triplicate), any suspected differences in figures of merit caused by changing counter-cations disappeared. No statistically significant impacts to sensitivity or detection limit were observed by changing counter cation of the chloride salt (Results in Table B2).

Salt	Sensitivity ± 2 standard deviations)	Detection Limit (mM) ± Confidence interval at 2 standard deviations
CaCl ₂	43 ± 3.2	3.2 ± 0.97
LiCl	40 ± 4.3	2.6 ± 0.59

Table B2. Comprehensive counter cation analysis. This robust analysis focused on the two counter cations observed during screening to have the most differing figures of merit (sensitivity and detection limit). As indicated by the results in the table, upon a more in-depth evaluation there were no statistically-significant observed differences to ChemFET figures of merit by changing counter-cations.

Appendix B References:

1. G. M. Sheldrick, *Bruker/Siemens Area Detector Absorption Correction Program*, Bruker AXS, Madison, WI, 1998.
2. P. Van der Sluis, A. L. Spek *Acta Cryst., Sect. A.* 1990, **A46**, 194-201.
3. G. M. Sheldrick, *Acta Cryst.* 2015, **C71**, 3-8.

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 3: EVALUATING IMPACTS OF BAMBUSURIL POCKET SIZE AND STERICS ON ANION BINDING TRENDS USING CHEMFET SENSORS

Dodecabenzyl bambus[6]uril ^1H NMR (Figure C1).

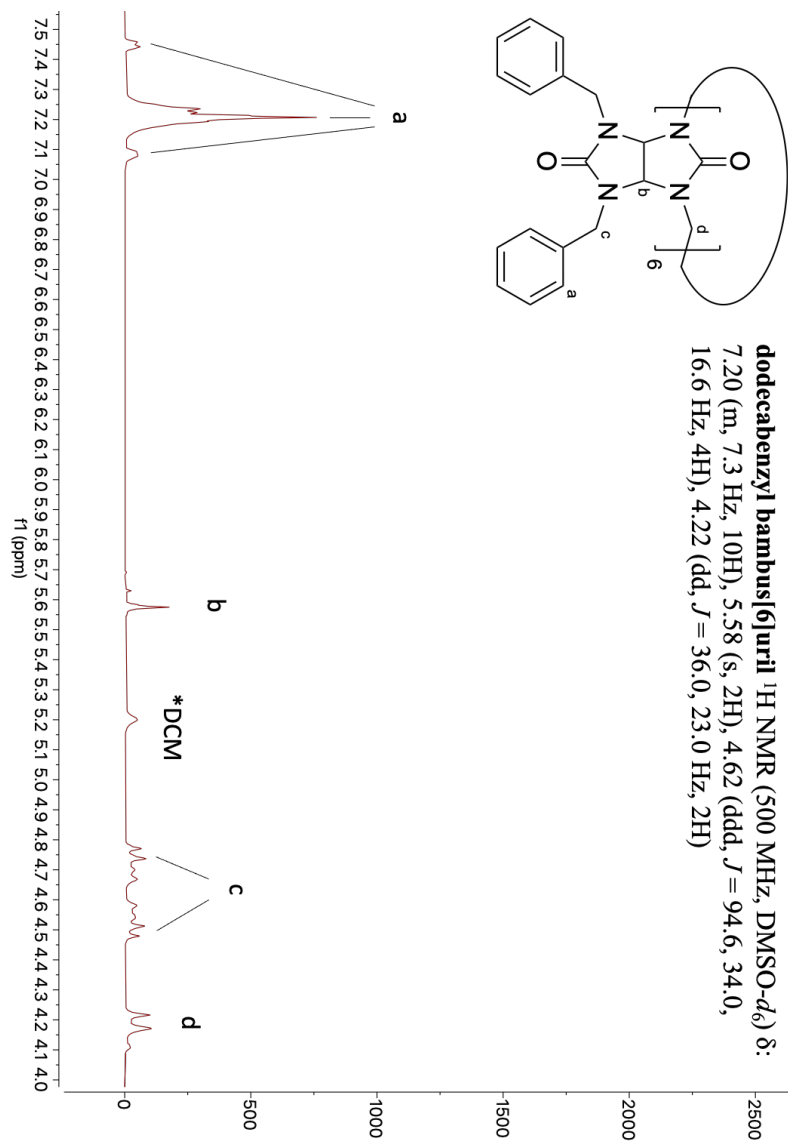


Figure C1. Dodecabenzyl bambus[6]uril was synthesized following literature,¹ with NMR spectra used for confirmation of synthesis product.

Dodeca-n-butyl Bambus[6]uril Characterization

All characterization of dodeca-n-butyl bambus[6]uril was reported in the electronic supplementary information (ESI) of a previous publication: DOI 10.1039/D3SC03616B

ChemFET Sensor and RE Construction

All sensor and reference construction, and all sensor data analysis was accomplished following previously-reported procedures and chapter 1.²⁻⁵

Appendix C References:

- 1 V. Havel, J. Svec, M. Wimmerova, M. Dusek, M. Pojarova and V. Sindelar, *Org. Lett.*, 2011, **13**, 4000–4003.
- 2 T. J. Sherbow, G. M. Kuhl, G. A. Lindquist, J. D. Levine, M. D. Pluth, D. W. Johnson and S. A. Fontenot, *Sensing and Bio-Sensing Research*, 2021, **31**, 100394.
- 3 D. H. Banning, G. M. Kuhl, S. A. Fontenot, and D. W. Johnson, *Supramol. Chem.*, 2024, DOI: 10.1080/10610278.2024.2353565
- 4 G. M. Kuhl, D. H. Banning, H. A. Fargher, W. A. Davis, M. M. Howell, L. Zakharov, M. D. Pluth and D. W. Johnson, *Chem. Sci.*, 2023, **14**, 10273–10279.
- 5 G. M. Kuhl, D. T. Seidenkranz, M. D. Pluth, D. W. Johnson and S. A. Fontenot, *Sensing and Bio-Sensing Research*, 2021, **31**, 100397.

APPENDIX D

SUPPORTING INFORMATION FOR CHAPTER IV: CHARACTERIZING THE ANION AFFINITY PROFILE OF COPPER TRIAZOLATE METAL ORGANIC FRAMEWORK USING CHEMFET SENSORS

Experimental Methods

Materials

All commercial chemicals were used as received unless stated otherwise. 1H-1,2,3-triazole (98+%, TCI), *N,N*-dimethylformamide (DMF, ACS grade, Fisher Scientific), methanol (MeOH, HPLC grade, Oakwood Chemical), cupric (II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, Mallinckrodt Chemical Works). Solvothermal syntheses were conducted in 20-mL scintillation vials with stir bars heated in an aluminum dry bath at 120°C for 2 hours. K_2SO_4 , KNO_3 , and KBr were obtained from Mallinckrodt; KI from EMD; KCl from Acros; KClO_4 from Oakwood; and KF , PVC, NPOE, TOAN, and PIPES were obtained from Aldrich. All were used without further purification. ChemFETs were purchased from Winsense™ (WIPS-C ChemFETs, www.winsense.co.th).

Characterization

Sample purity was verified by powder X-Ray diffraction (PXRD) with a Bruker D2 Phaser benchtop diffractometer. Scans are collected between 5 and 35 degrees two theta, with an increment of 0.02 degrees. A variable detector opening mode was used in conjunction with a low knife edge height of 1 mm and a divergence slit of 0.6 mm to minimize air scattering at low angles. Scanning electron microscopy (SEM) images were collected using a Thermo Scientific Apreo 2 SEM with 5.0-10kV energy and 25 pA-0.6 nA current. SEM samples were prepared by

drop-casting particle dispersions in MeOH outside of N₂ atmosphere onto silicon substrates and drying under a flow of N₂. Samples were left to dry for 24 hours before imaging. The same procedure was done for the PVC/MOF composite before SEM imaging. Zeta potential (ZP) and dynamic light scattering (DLS) titrations were done using a dip cell and a Malvern Zetasizer Nano ZS and analysed using Zetasizer software. All ZP and DLS measurements were done in DMF. Salt titrations were done from stock solutions of all salts in DMF. KF was not done, due to insolubility in DMF.

ChemFET

Potassium was used as counter cation for all anions evaluated to ensure attribution of affinity trends to anion identity alone. ChemFET sensor setup per previous publications¹⁻⁵ Dropcast solution was 1 wt % Cu(TA)₂ (1 mg) in 1 mL DMF, 2 wt % tetraoctyl ammonium nitrate (TOAN) (2 mg) and 97 wt % PVC/NPOE polymer (65 mg polyvinyl chloride (PVC), 32 mg ortho-nitrophenyl octyl ether (NPOE)) in 0.75 mL THF. Dropcast applied to ChemFET sensor surface in 4x 1.6 µL drops. Drops were applied at 30-minute intervals, with sensors placed in 80°C oven between drops, and then placed in oven overnight to facilitate complete solvent removal. Sensor membrane application accomplished per previously published methods paper (DOI: 10.1080/10610278.2024.2353565) Evaluation per previously published methods paper, comprising 4x sensors run through 12 analyte solutions (0.100 M, 50.0 mM, 10.0 mM, 5.00 mM, 1.00 mM, 500, 100 µM, 50.0 µM, 10.0 µM, 5.00 µM, 1.00 µM, and 0.500 µM analyte, each with constant 50.0 mM non-interfering PIPES buffer to ensure minimum ionic strength at low concentrations) in triplicate (low conc. to high, high to low, low to high), with results averaged to determine detection limits. Detection limits calculated per previously published methods paper,

determined by the point at which increasing analyte concentration produces measurable change in response vs the background.

MOF Synthesis & Characterization

Cu(TA)₂ synthesis

An aluminum dry bath was preheated to 120°C. Meanwhile, 0.015g, 0.06mmol of Cupric (II) nitrate trihydrate was dissolved in 6mL of DMF. Sonication was used to assist the dissolution if necessary. Then, 140uL, 2.4mmol, was added to the copper-DMF solution under stirring, Vial was capped and placed in the dry bath with stirring for 2 hours. After, product was collected by centrifugation. The blue product was resuspended in DMF and centrifuged again to wash away unreacted precursors. This was repeated three times, once more with DMF and twice more with MeOH. Product was dried on a Schlenk line and saved until further use.

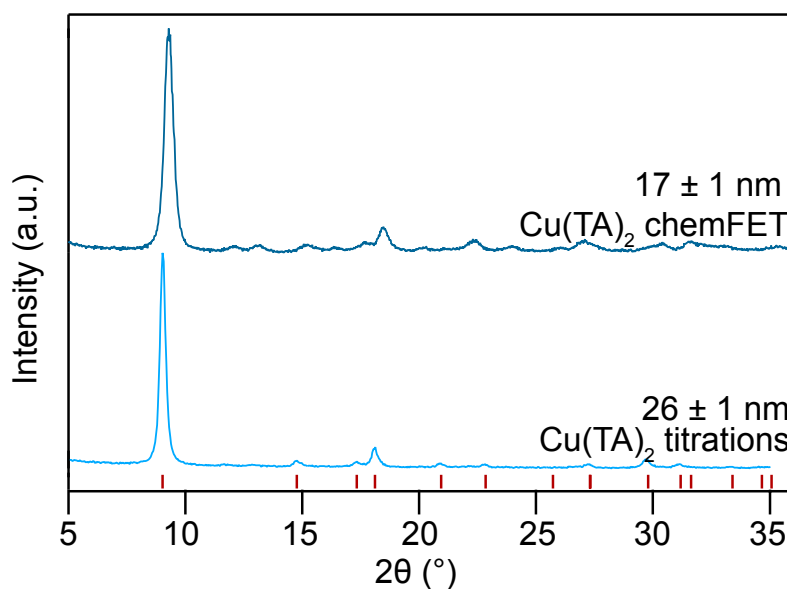


Figure D1: PXRD patterns of particles used for chemFET sensors and zeta potential titrations. Scherrer sizes are included with standard deviations. Simulated reflections are indicated with tick marks.⁶ Peaks between 10° and 15° are due to Jahn-Teller distortions present in dried particles.

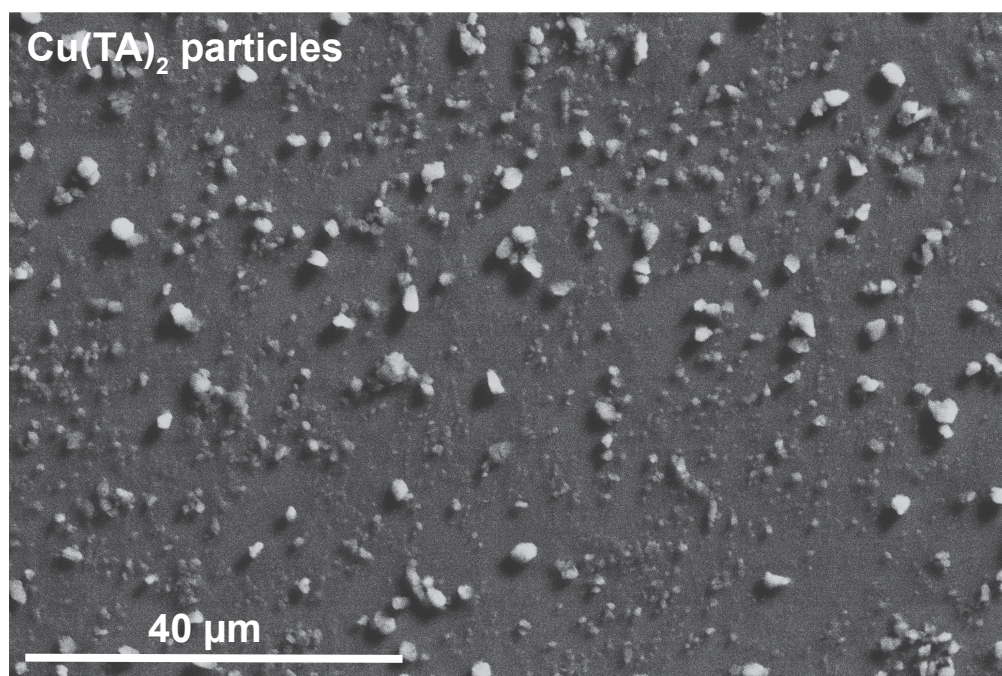


Figure D2: SEM of Cu(TA)₂ particles used for both titration and chemFET experiments.

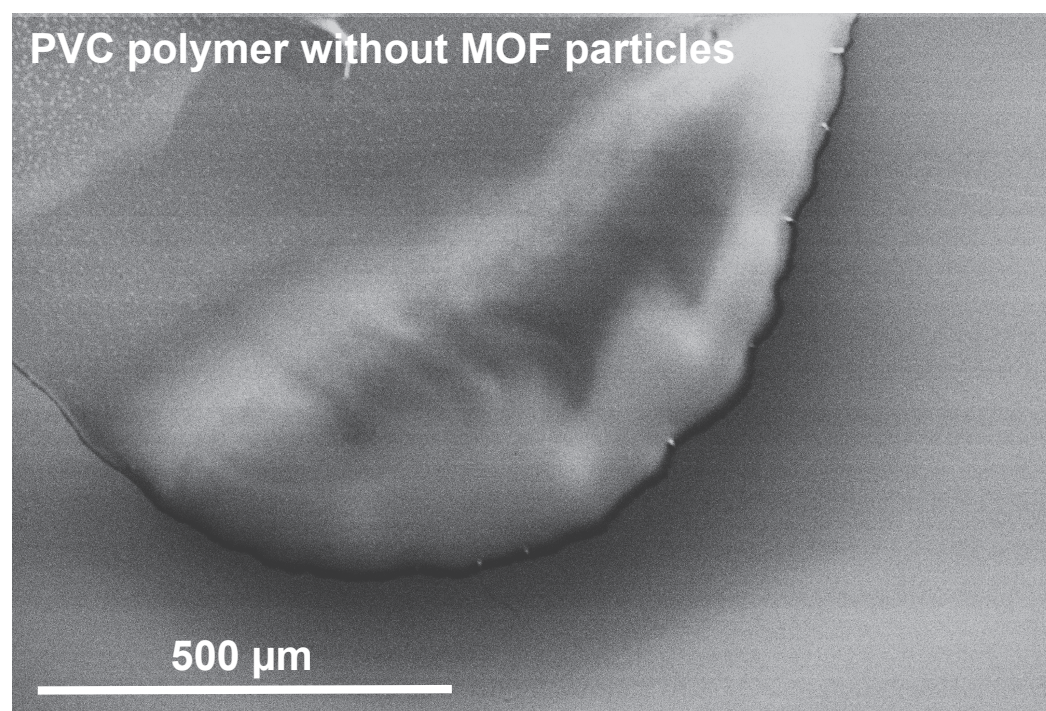


Figure D3: SEM of PVC polymer without MOF particles.

Zeta Potential

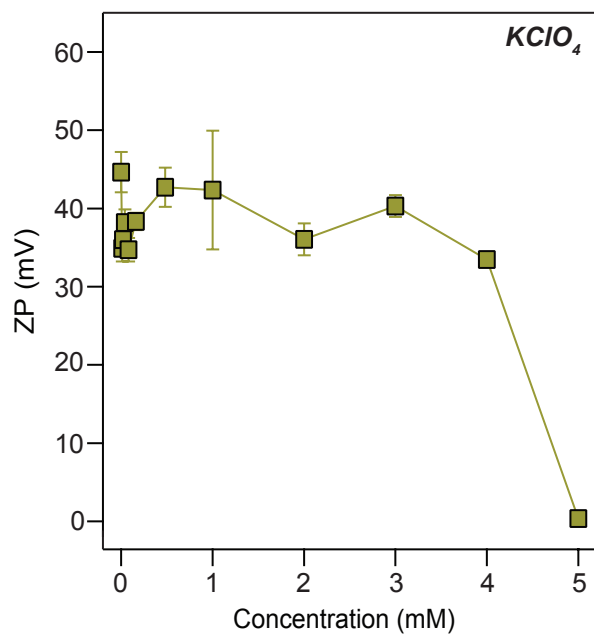


Figure D4: Titrations of $KClO_4$ into 3mg/mL suspension of $Cu(TA)_2$ particles in DMF.

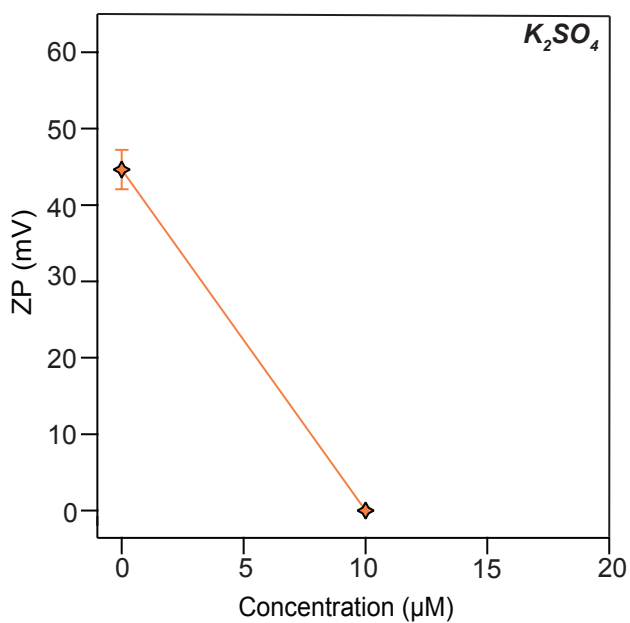


Figure D5: Titrations of K_2SO_4 into 3mg/mL suspension of $Cu(TA)_2$ particles in DMF.

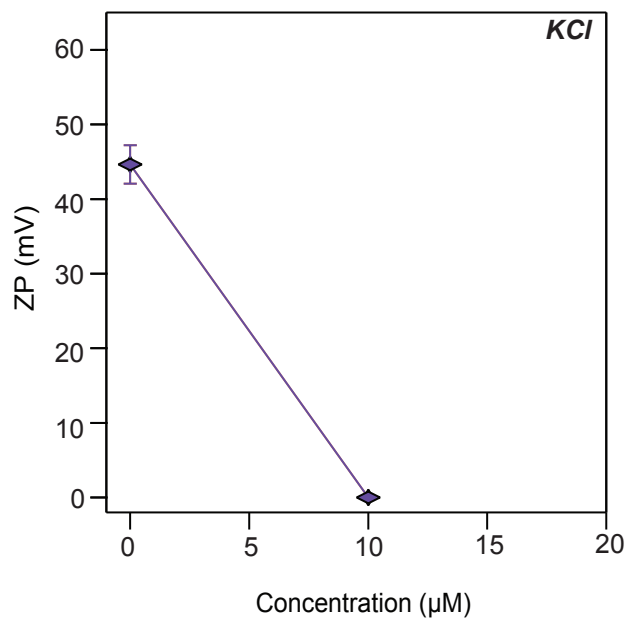


Figure D6: Titrations of KCl into 3mg/mL suspension of Cu(TA)₂ particles in DMF.

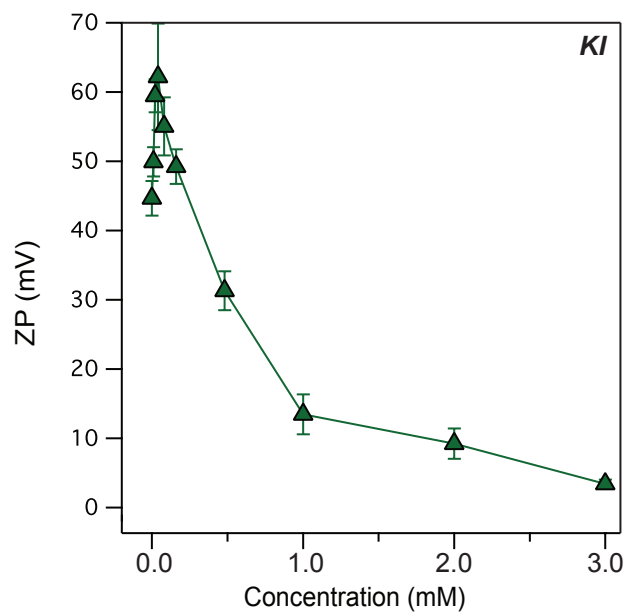


Figure D7: Titrations of KI into 3mg/mL suspension of Cu(TA)₂ particles in DMF.

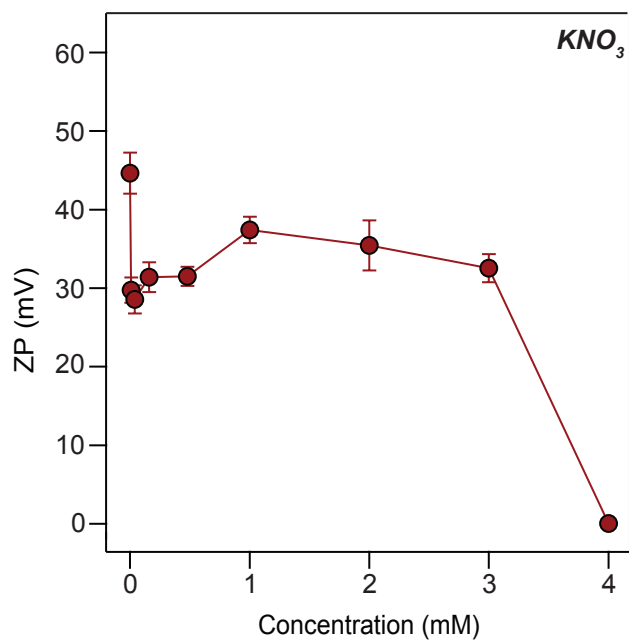


Figure D8: Titrations of KNO_3 into 3mg/mL suspension of $Cu(TA)_2$ particles in DMF.

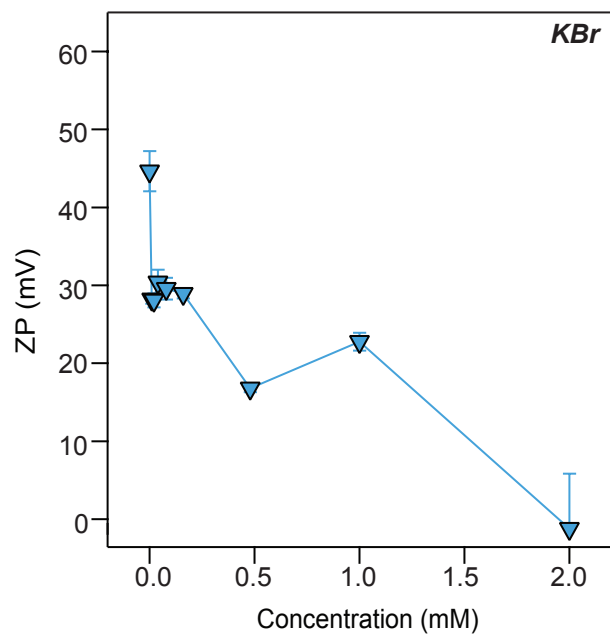


Figure D9: Titrations of KBr into 3mg/mL suspension of $Cu(TA)_2$ particles in DMF.

<i>Salt, Concentration (mM)</i>	DLS Size	Zeta Potential (mV)
<i>None, 0 (CuTA in DMF)</i>	85.93 ± 1.22	44.64 ± 2.6
<i>KI, 0.01</i>	95.02 ± 0.3	49.92 ± 2.1
<i>KI, 0.02</i>	92.34 ± 0.62	59.46 ± 2.4
<i>KI, 0.04</i>	89.06 ± 1.46	62.18 ± 7.7
<i>KI, 0.08</i>	88.49 ± 0.90	55.02 ± 4.2
<i>KI, 0.16</i>	91.49 ± 0.37	49.24 ± 2.6
<i>KI, 0.48</i>	94.62 ± 0.25	31.3 ± 2.8
<i>KI, 1.0</i>	91.79 ± 1.59	13.46 ± 2.9
<i>KI, 2.0</i>	119.33 ± 3.77	9.23 ± 2.2
<i>KI, 3.0</i>	2139 ± 501.73	3.414 ± 0.7
<i>KNO₃, 0.01</i>	86.47 ± 0.46	29.76 ± 1.6
<i>KNO₃, 0.04</i>	112.30 ± 1.06	28.58 ± 1.8
<i>KNO₃, 0.16</i>	92.2 ± 0.45	31.38 ± 1.9
<i>KNO₃, 0.48</i>	98.65 ± 0.48	31.5 ± 1.2
<i>KNO₃, 1.0</i>	83.96 ± 1.04	37.42 ± 1.74
<i>KNO₃, 2.0</i>	90.00 ± 0.77	35.42 ± 3.2
<i>KNO₃, 3.0</i>	95.97 ± 0.35	32.54 ± 1.8
<i>KNO₃, 4.0</i>	86.86 ± 1.09	0.052 ± 0.1
<i>KClO₄, 0.01</i>	85.81 ± 0.3	34.9 ± 1.7
<i>KClO₄, 0.02</i>	86.30 ± 2.2	36.02 ± 1.6
<i>KClO₄, 0.04</i>	87.33 ± 0.5	38.18 ± 1.7
<i>KClO₄, 0.08</i>	89.83 ± 0.52	34.72 ± 1.5
<i>KClO₄, 0.16</i>	90.10 ± 0.6	38.34 ± 1.1
<i>KClO₄, 0.48</i>	90.86 ± 0.3	42.72 ± 2.5
<i>KClO₄, 1.0</i>	85.82 ± 0.5	42.34 ± 7.6
<i>KClO₄, 2.0</i>	95.26 ± 0.4	36.04 ± 2.0
<i>KClO₄, 3.0</i>	97.1 ± 0.1	30.32 ± 1.5
<i>KClO₄, 4.0</i>	84.92 ± 0.3	33.48 ± 0.7
<i>KClO₄, 5.0</i>	88.11 ± 0.8	0.346 ± 0.3
<i>KBr, 0.01</i>	105.37 ± 1.5	28.2 ± 0.6
<i>KBr, 0.02</i>	111.83 ± 1	28.06 ± 0.9
<i>KBr, 0.04</i>	86.07 ± 0.03	30.38 ± 1.6
<i>KBr, 0.08</i>	97.35 ± 0.93	29.56 ± 1.4
<i>KBr, 0.16</i>	109.1 ± 0.2	28.92 ± 0.6
<i>KBr, 0.48</i>	104.83 ± 2.72	16.78 ± 0.5
<i>KBr, 1.0</i>	175.97 ± 21.19	22.76 ± 1.2
<i>KBr, 2.0</i>	2454.33 ± 448.41	-1.19 ± 7
<i>K₂SO₄, 0.01</i>	86.29 ± 0.08	-0.007 ± 0.8
<i>KCl, 0.01</i>	88.14 ± 0.59	-0.05 ± 0.08

Table D1: Dynamic light scattering (DLS) data and zeta potential data with standard deviations for each salt titration.

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APPENDIX E

SUPPORTING INFORMATION FOR CHAPTER V: OPTIMIZATION OF F-AL13 CLUSTER SYNTHESIS USING HISTORICAL DATA AND DESIGN OF EXPERIMENTS

Experimental Details

General Information

Dynamic light scattering (DLS) was performed on a Wyatt Technologies Mobius DLS-PALS using standard cumulant analysis methods. Samples were passed through a 0.2 micron PTFE filter after five minutes of sonication immediately prior to analysis. The filtrate was transferred to a quartz cuvette for analysis using an incident laser wavelength of 532 nm at 20 °C. Dynamics Software 7.6.0.48 was used to average 10 measurements with an acquisition time of 10 seconds. This process was performed in triplicate for each sample. Sizes and polydispersities presented here-in are an average of all runs.

Optimized Reaction Conditions

Synthetic procedures followed previously published literature methods.^{1,2} DOE optimization resulted in new conditions requiring minute changes to the synthesis. Validation run 1 called for a concentration of 0.2 M [Al] and a starting temperature of 25 °C, and validation run 2 used a concentration of 0.08 M [Al] and a starting temperature of 40 °C. The pH of each reaction was held at 3.2, which required minute additions of 10% HNO₃ as the reactions progressed. Each reaction was run for 24 hours at 40 °C, with aliquots being removed for DLS and analyzed within 10 minutes of reaction completion.

Synthetic References

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Design of Experiments (DOE) Supplemental Information

Inputs			Outputs	
pH	Temperature	Concentration	PD%	Particle Size (nm)
3.9	22	0.1004	0.238	2.1
3.1	40	0.1004	0.571	2.7
3.1	40	0.1004	0.19	2.1
3.1	40	0.1004	0.169	1.7
3.1	40	0.1004	0.192	1.7
3.2	40	0.1004	0.242	2.4
3.2	40	0.1004	0.237	2.2
3.2	40	0.1004	0.571	2.5
3.2	40	0.0769	0.571	5
3.2	40	0.0769	0.205	1.8
3.2	40	0.0769	0.138	1.7
3.2	40	0.0769	0.125	1.8
3	40	0.0767	0.37	1.8
3	40	0.0767	0.2	1.2
3	40	0.0767	0.149	1.2
3.2	22	0.1443	0.374	2.7
3.2	22	0.1443	0.267	2.7
3.2	22	0.1864	0.219	2
3.2	22	0.1864	0.225	2.2
3.2	22	0.0769	0.27	3.6
3.9	22	0.1004	0.232	1.8
3.9	22	0.1004	0.232	1.9
3.9	22	0.1004	0.18	1.8
3.9	22	0.1004	0.227	1.7
3.9	22	0.1004	0.184	1.7

Table E1. Input data used for the model. Over 3000 data points were analyzed, and only the reaction conditions resulting in the desired product were used to build the model.

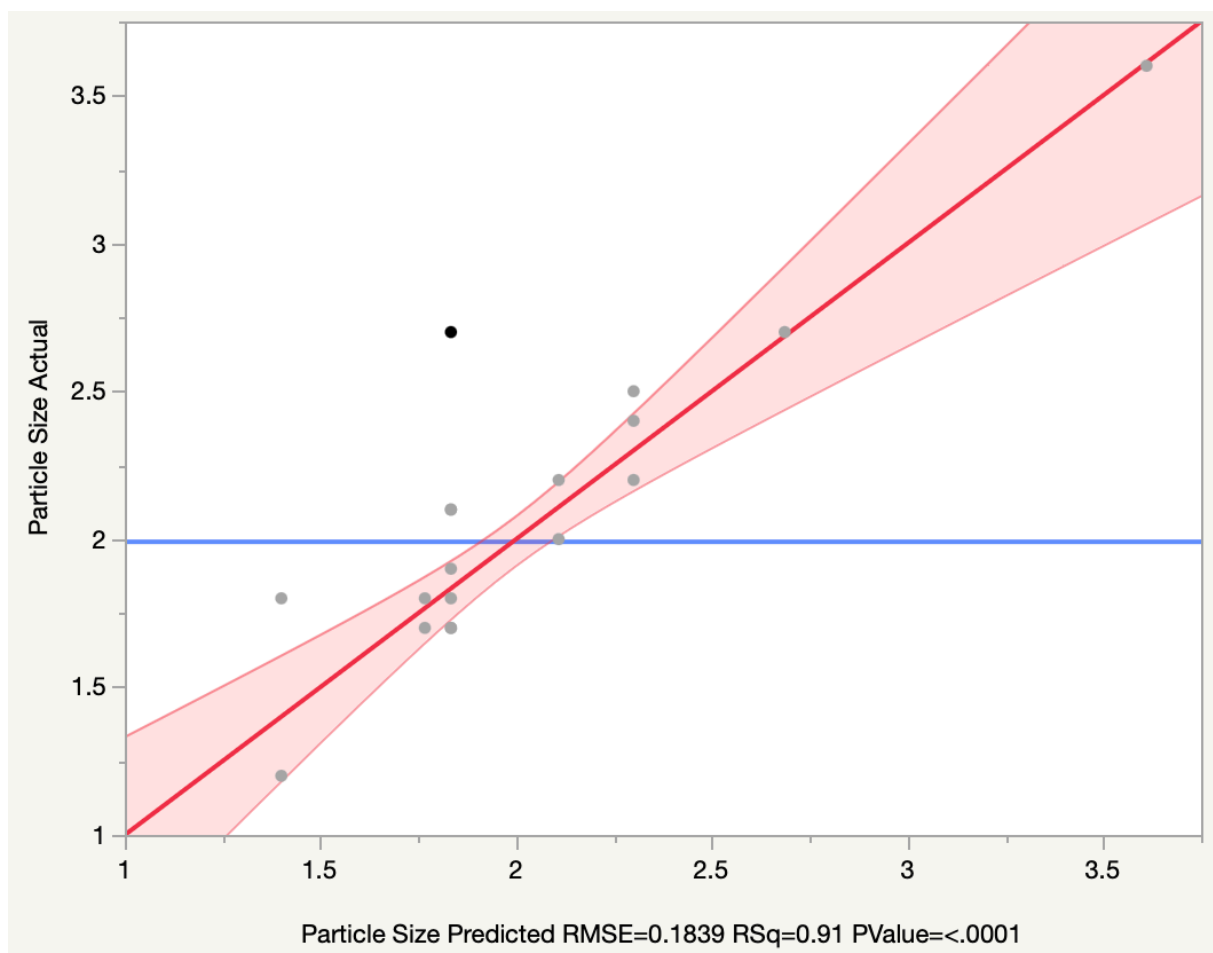


Figure E1. Actual by Predicted Plot. If the red uncertainty area covers the blue line, the model displays no correlation to the output data. Reported R squared of 0.91 indicates close fit of observed data to the model.

Design of Experiments analysis conducted using JMP software version 16.0.0. Historical data was compiled, and then the data points incorporated into JMP DOE platform (Table S1).

The data was then analyzed by “best fit” to include all main and two-factor interactions. The information was then used to determine p-values to quantitatively rank the main factor and two-factor interactions influencing the output parameters (Table 1). The model was then used to predictively outline conditions for optimal desired outputs, in this case polydispersity index (minimize) and particle size (minimize). The pH slider was set to 3.2 to ensure $f\text{-Al}_{13}$ formation, and then the other sliders for concentration and temperature moved accordingly to create the

desired outputs (Figures S1-3). This outlined two sets of optimization conditions at pH 3.2, titled Run 1 and Run 2 (Table 3).

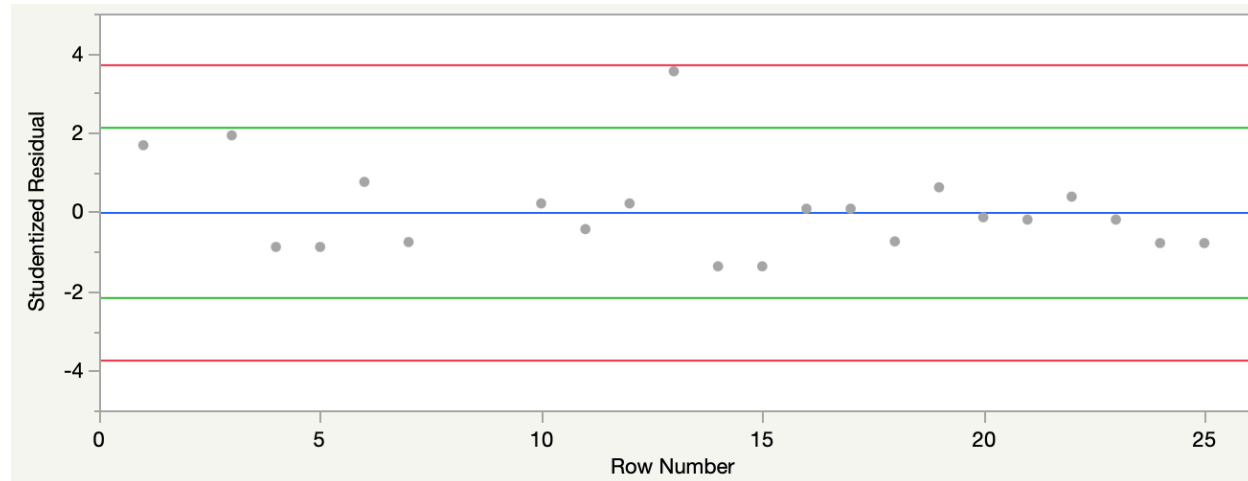


Figure E2. Externally Studentized Residuals. Displayed with 95% simultaneous Bonferroni limits in red, individual limits in green.

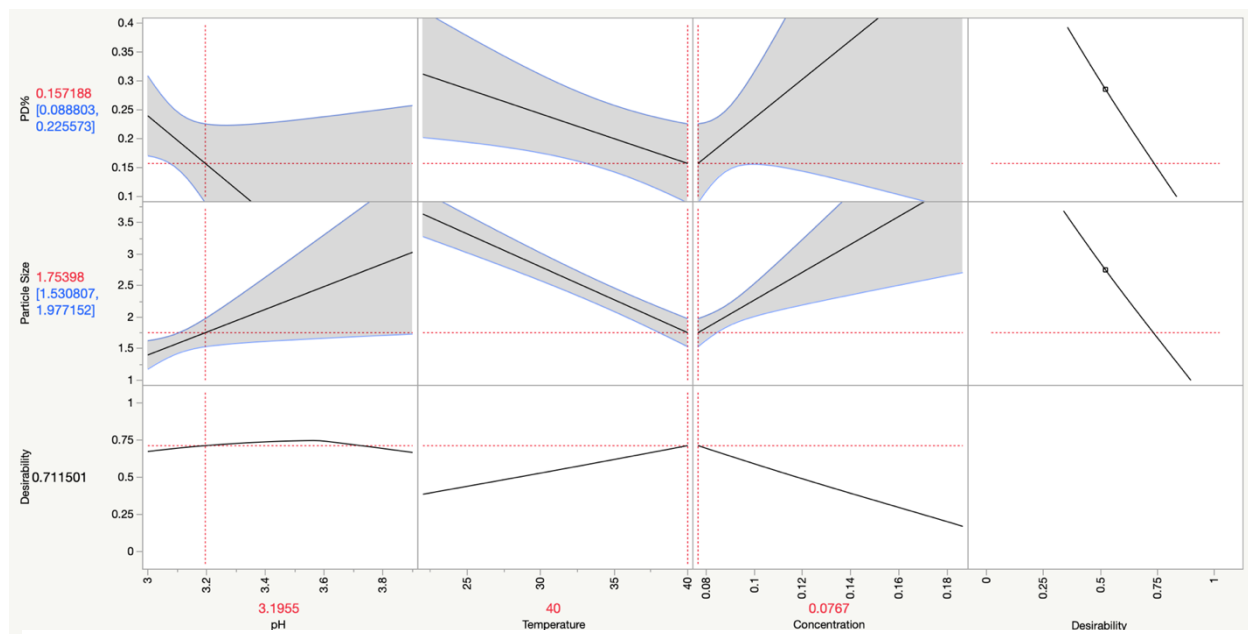


Figure E3. Illustration of Predictive Model. As seen, the pH slider was set to 3.2 to ensure desired small particle product synthesized. Image is taken of Run 2, which resulted in the greatest observed improvement of desired properties.

APPENDIX F

THE DUAL ROLE OF UREA AS A NEW MOTIF FOR HYDROGEN BOND ENHANCED HALOGEN BONDING IN ANION RECOGNITION

From: S. Moaven, H. J. Bates, T. de Faria, J. A. Lohrman, D. H. Banning, A. E. Wagner, M. D. Pluth*, M. M. Haley* and Darren W. Johnson* *Manuscript in Prep.*

Note: this draft manuscript is still a work in-progress. Dr. Hannah Bates, Dr. Thaís de Faria, and Dr. Jessica Lohrman initially designed the receptor. Synthesis was planned and carried out by myself, Dr. Shiva Moaven, Dr. Hannah Bates, Dr. Thaís de Faria, Dr. Jessica Lohrman, and Allyson Wagner. Characterization of binding constants via ^1H NMR titrations were carried out by myself, Dr. Shiva Moaven, and Allyson Wagner.

Abstract

Urea can be used as a functional group to form hydrogen bond-enhanced halogen bond (HBeXB) donor compounds. Here we report the synthesis of a new HBeXB donor anion receptor containing a urea functional group in which one urea hydrogen atom contributes to the formation of the HBeXB donor and the other, functions cooperatively with the HBeXB donor to bind anions.

Overview

The formation of a halogen bond (XB) donor by reversible intramolecular hydrogen bonding (HB) close to a halogen atom is known as hydrogen bond enhanced halogen bonding (HBeXB).^{1–3} This emerging supramolecular motif has also been recognized in assembly and recognition strategies in biological systems.⁴ More recently, synthetic models using amino ($-\text{NH}_2$) and amide ($-\text{CONHR}$) functional groups (see Fig. 1 (a)) were shown to be useful in forming HBeXB interaction motifs and were used in both anion recognition, catalysis, and foldamers.^{3,5–11}

The urea functional group is another strong HB donor moiety, which has shown wide applicability in anion recognition by many groups including ours.^{12–18} This hydrogen bond donor group can also be used to form an HBeXB donor compound in anion recognition. We have designed a new anion receptor with a urea functional group that uses one of the urea HB donors to form the HBeXB donor (red circle in Fig. 1 (b)) and the second HB donor (blue circle in Fig. 1 (b)) to cooperatively bind the anion.

To assess the ability of receptor **1I** to interact with anions via cooperative HB and HBeXB interactions, we also prepared control compounds **1Br** and **1H**. Compound **1Br** should be a weaker HBeXB donor than the target **1I** and have lower association constants (K_a) and binding energies ($\Delta E_{\text{bind.}}$) with anions.^{19,20} The conformation of the receptors will also greatly influence the HBeXB donor compounds **1I** and **1Br**, with the **1X** conformation resulting in cooperative HB-HBeXB binding. Whereas the **1X'** conformation will only form three HB donor sites (Fig. 1 (b)). To differentiate between these interactions, we also prepared **1H**, which lacks any halogen substitution, as a second control compound to investigate the magnitude of the magnitude of the HB interactions alone. Comparison of the K_a and $\Delta E_{\text{bind.}}$ values of **1H** with the HBeXB donor compounds will allow for better differentiation of the HBeXB and HB contributions to anion binding.

Receptors **1I**, **1Br**, and **1H** were prepared from the common intermediate **3**, which was prepared in three steps from the parent aniline (Scheme 1). Sonogashira cross-coupling reaction between **3** and the desired pyridine generated **2H** and **2Br**. Installation of urea was accomplished by further reaction with 4-(trifluoromethyl)phenyl isocyanate to generate **1H** and **1Br**. Compound **2Br** could also be iodinated using a copper catalysed halogen exchange reaction to generate **2I**, which was further functionalized with 4-(trifluoromethyl)phenyl isocyanate to provide receptor **1I**.

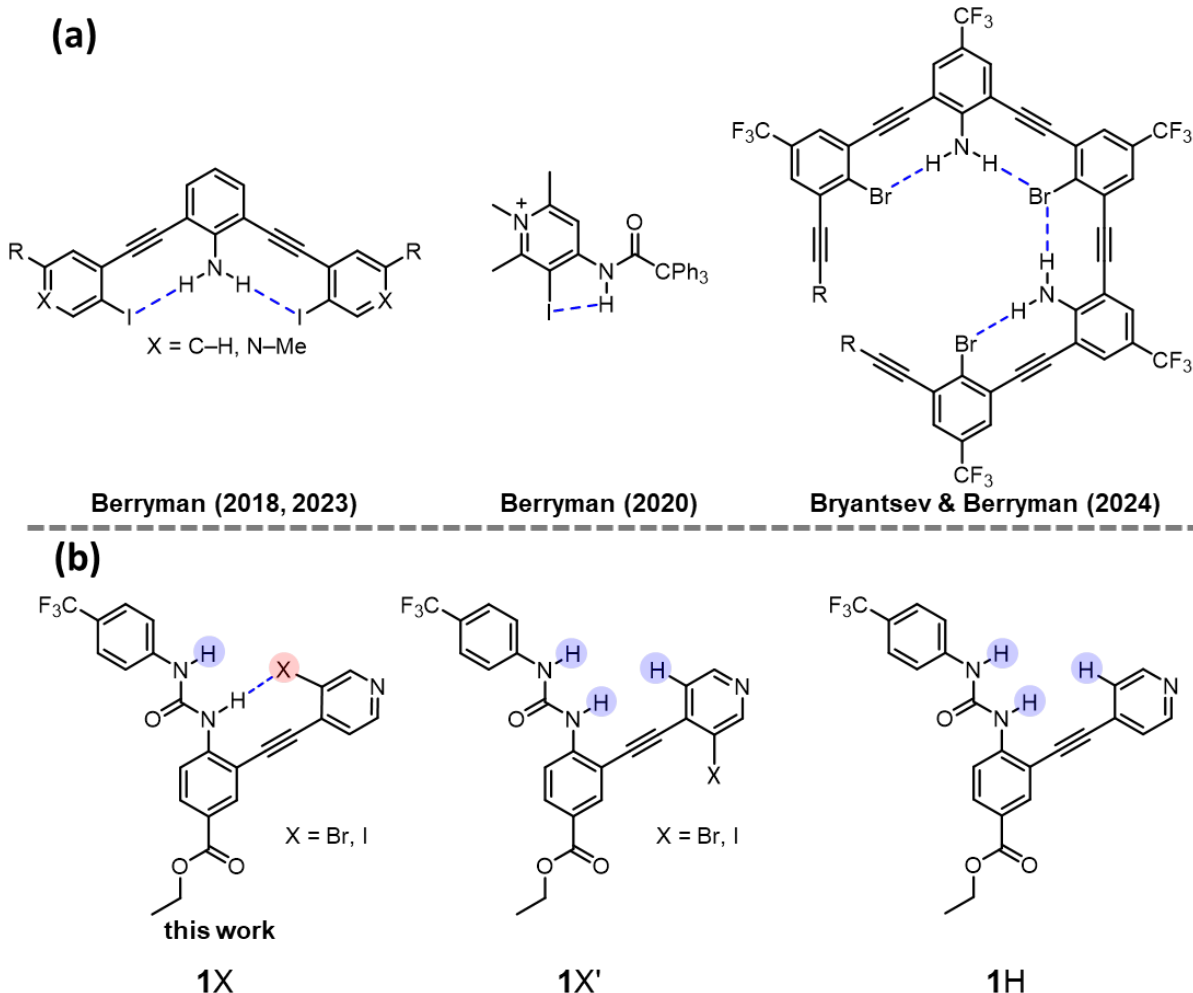
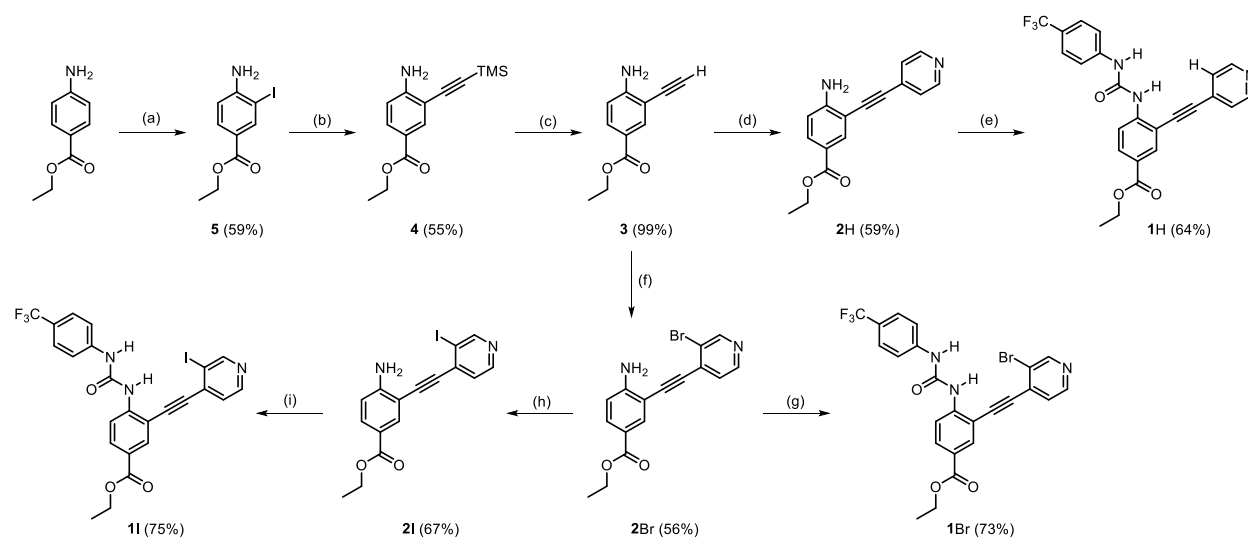


Figure F1. (a) Examples of HBeXB compounds used in anion recognition, organocatalysis, and foldamers. (b) Schematic representation of the urea-based compound prepared in this work (**1X**) and different conformations of the anion receptors **1X** and **1X'** (for **1Br** and **1I**) and **1H**. HB and HBeXB donors are indicated by blue and red circles respectively.

To differentiate between the ability of the three receptors (**1H**, **1Br**, **1I**) to bind to different halides, Density Functional Theory (DFT) was used (see ESI for details). These calculations reveal that the binding pocket of the receptors is not large enough to completely accommodate Cl^- , Br^- , or I^- . The most stable binding conformation of the receptors with different halide anions was calculated by running geometry optimization on the optimized structure of each receptor and corresponding anion to reach its minimized energy.



Scheme F1 Synthesis of HBeXB anion receptors **1I** and **1Br**, and the all HB donor control receptor **1H**; reagents and conditions: (a) 1 equiv. KIO_4 , 2 equiv. NaCl , 1 equiv. KI , $\text{H}_2\text{O} : \text{AcOH}$ (1 : 10), 23 °C, 12 hours; (b) 1.1 equiv. TMSA , 4.9 mol% $(\text{PPh}_3)_4\text{PdCl}_2$, 5.1 mol% CuI , triethylamine, N_2 , 21 °C, 18 hours; (c) 2 equiv. KF , MeOH ; (d) 1 equiv. 4-iodopyridine, 4.9 mol% $(\text{PPh}_3)_4\text{PdCl}_2$, 5.1 mol% CuI , triethylamine, N_2 , 21 °C to 50 °C, 18 hours; (e) 1 equiv. 4-(trifluoromethyl)phenyl isocyanate, toluene, N_2 , 0 °C to 50 °C, 12 hours; (f) 1 equiv. 3-bromo-4-iodopyridine, 4.9 mol% $(\text{PPh}_3)_4\text{PdCl}_2$, 5.1 mol% CuI , triethylamine, N_2 , 21 °C to 50 °C, 18 hours; (g) 1 equiv. 4-(trifluoromethyl)phenyl isocyanate, toluene, N_2 , 0 °C to 50 °C, 12 hours; (h) 2 equiv., NaI , 10 mol% CuI , 20 mol% N,N' -DMEDA, 1,4-dioxane, N_2 , 110 °C; (i) 1 equiv. 4-(trifluoromethyl)phenyl isocyanate, toluene, N_2 , 0 °C to 50 °C, 12 hours.

Electrostatic Potential Mapping (ESP) and surface analysis were used to measure the maximum potential ($V_{\text{S,max}}$) of the HB and HBeXB sites of the receptors. To get the $V_{\text{S,max}}$ values that would be more informative on the receptors' interactions with anions, the frozen monomer of each receptor in its optimized binding conformation with Cl^- was used (see Figures F2 in ESI) to map the ESPs and calculate the $V_{\text{S,max}}$ values (at 0.001 a.u.). For all-HB donor, compound **1H**, two $V_{\text{S,max}}$ values were measured. The stronger $V_{\text{S,max}}$ occurs between the two protons of the urea motif with 294 kJ/mol potential and the weaker $V_{\text{S,max}}$ was calculated to have a potential value of 164 kJ/mol on the surface of the hydrogen atom on the 3-position of the pyridyl ring close to the urea motif (Fig. F2 (a), left). The measured $V_{\text{S,max}}$ for compound **1I** in its optimized binding conformation with Cl^- is 242 kJ/mol on the overlapping surface of the urea hydrogen atom (closer

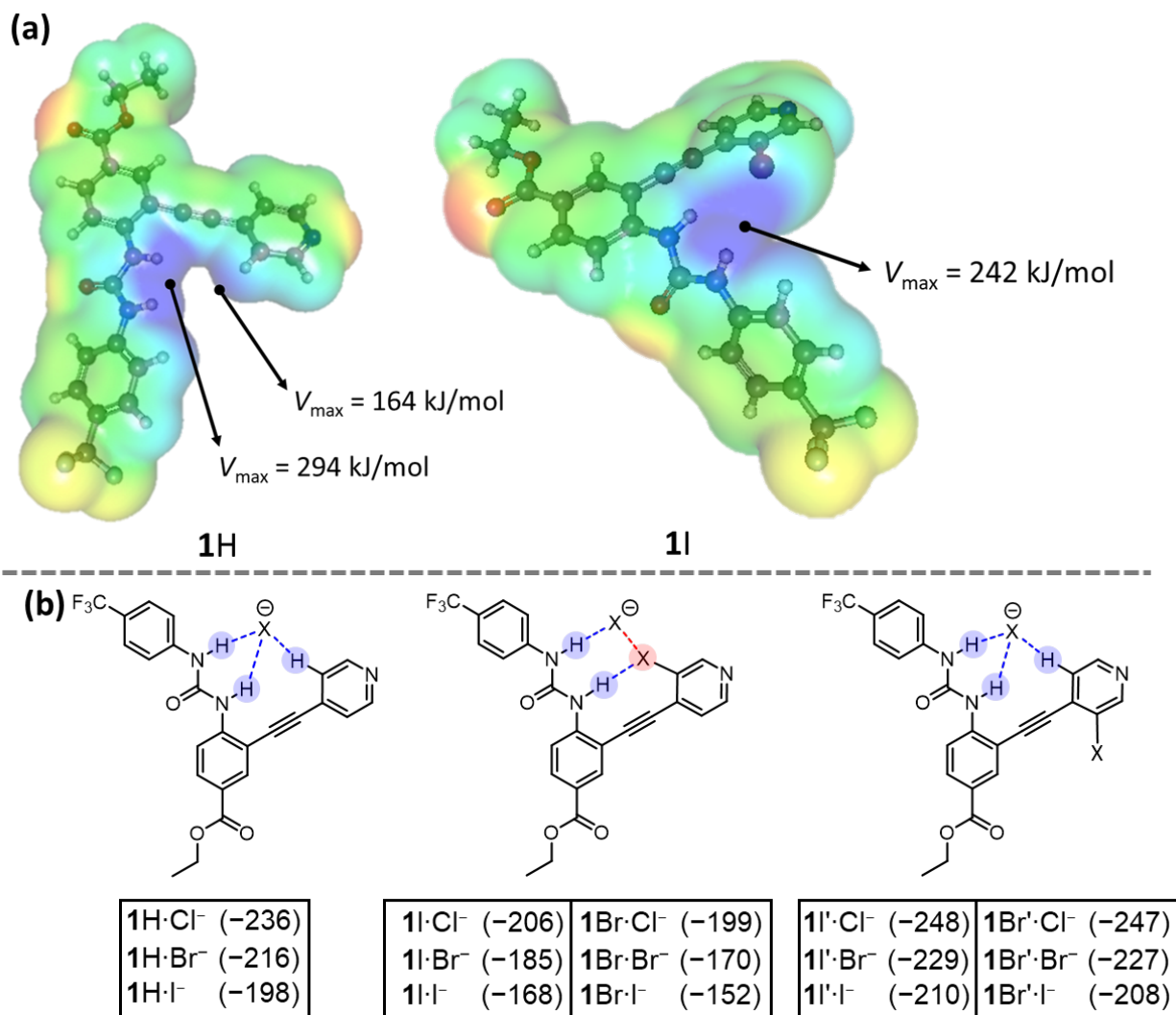


Figure F2. (a) ESPs of the 1H and 1I anions receptors in their binding conformations. Maximum potentials are indicated ($V_{S,max}$) in kJ/mol. (b) schematic representation of 1H and 1I/Br in the XB/HB and 1I'/Br' in all HB conformation. The binding energy of each receptor with the corresponding halide ion is shown in parentheses (kJ/mol)

to the *p*-CF₃-phenyl group) and the iodine atom (Fig. 2(a), right). These ESP studies for 1H and 1I suggest that the 1H receptor will be a stronger anion binder compared to 1I due to higher $V_{S,max}$ value, and the presence of a second $V_{S,max}$ on the pyridyl ring directed into the binding pocket.

DFT calculations were used to calculate ΔE_{bind} values for 1H, 1I, and 1Br in their two different binding conformations (Fig 2 (b)). All ΔE_{bind} values were calculated based on the energy generated

after the formation of the anion receptor complex subtracted from the energy of the most stable conformation of the receptor and anion. these values were corrected by basis set superposition error (ΔE_{BSSE}) and dispersion correction ($\Delta E_{\text{disp.}}$) methods ($\Delta E_{\text{bind.}} = (E_{\text{complex}} - E_{\text{recep.}} - E_{\text{anion}}) + \Delta E_{\text{BSSE}} + \Delta E_{\text{disp.}}$). The $\Delta E_{\text{bind.}}$ values for the **1H** receptor were higher compared to both **1Br** (37 kJ/mol for Cl^- and 46 kJ/mol for Br^- and I^-) and **1I** (almost 30 kJ/mol for all anions) compounds which correlate well with the ESP mapping values. As would be expected for the HBeXB donor compound **1I** shows higher affinity towards the halide ions compared to its lighter analogue **1Br**, with a higher binding energy of 7 kJ/mol for Cl^- and 15 kJ/mol for Br^- and I^- . As mentioned earlier, the HBeXB donor compounds can interact with the anions in two possible conformations (Fig. 1 and Fig. 2 (b)). It would be expected that all-HB donor conformation (**1Br'** and **1I'**) would have more favorable $\Delta E_{\text{bind.}}$ compared to the conformation featuring both HB and HBeXB donors (**1Br** and **1I**). Binding energy calculations support this as well, where the calculated $\Delta E_{\text{bind.}}$ for **1Br'** and **1I'** with different halides were higher compared to the cooperative HB-HBeXB donor compounds. the $\Delta E_{\text{bind.}}$ values for the **1X'·X⁻** was slightly higher (between 10-12 kJ/mol) than their **1H·X⁻** counterparts due to the induction and dispersion effects of the halo-substituent on the pyridyl rings.^{16,21}

Results and Discussion

To measure the binding affinity of **1I** and the control compounds **1Br** and **1H**, solution-state ^1H NMR spectroscopy was employed. Titrations with tetrabutylammonium chloride (TBACl) with all receptors followed the anticipated trend from energy decomposition analyses where **1H** shows the highest association constant of 4900 M^{-1} and **1I** and **1Br** have binding affinities of 3700 M^{-1} and 2100 M^{-1} , respectively. The difference between the measured association constants for **1I** and

Receptor	K_a (M^{-1})	Receptor	K_a (M^{-1})	Receptor	K_a (M^{-1})
1I ·Cl ⁻	3700 ± (180)	1Br ·Cl ⁻	2100 ± (150)	1H ·Cl ⁻	4900 ± (130)
1I ·Br ⁻	760 ± (70)	1Br ·Br ⁻	660 ± (10)	1H ·Br ⁻	620 ± (10)
1I ·I ⁻	100 ± (10)	1Br ·I ⁻	80 ± (5)	1H ·I ⁻	90 ± (5)

Table F1 Binding affinities (K_a) for TBA salts of Cl⁻, Br⁻, and I⁻ with **1I** and control compounds **1Br**, **1H** in 10% MeCN-*d*₃ solution of CDCl₃. The error values in parentheses are the standard deviation of three titrations for each anion.

1Br in the presence of TBACl suggests that both compounds mainly interact with the chloride anions via one HBeXB and one HB interaction.

The measured binding affinities for both HBeXB donors (**1I** and **1Br**) with the Br⁻ and I⁻ anions were quite similar and no trend across the receptors was observed for the heavier halides compared to that observed in the Cl⁻ binding series. This suggests that due to the small pocket size of the HBeXB donor receptors in **1I** and **1Br**, the pyridyl ring will undergo a rotation to bind to Br⁻ and I⁻ in the all-HB binding mode (i.e. the **1I'** and **1Br'** conformations). This was further confirmed by measuring the binding affinity of the **1H** receptor, where only HB donor moieties are present on the receptor, which showed similar K_a values to the ones measured for the HBeXB donors.

Conclusion

In summary, this work presents the applicability of the urea functional group to induce the formation of a hydrogen bond enhanced halogen bond donor. We showed that one urea N–H group (closer to the halogen atom) can participate in the activation of the HBeXB donor and the second urea N–H group will cooperatively, with the XB donor, bind to the chloride anion for both HBeXB donors. This trend did not continue with the larger halides, where conformations that supported a larger binding pocket featuring only hydrogen bonds dominated. The

combination of DFT calculations and solution NMR titrations supported the conclusion that the HB-HBeXB donor pocket size is not large enough to accommodate anions larger than chloride in this receptor series; the HBeXB served a key structural role in the binding of the chloride only.

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SUPPORTING INFORMATION FOR APPENDIX F

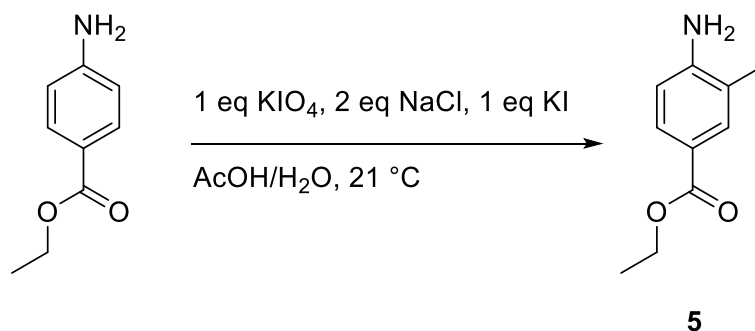
Experimental Details

General Methods

All starting materials, Ethyl-4-aminobenzoate (Aldrich, 98%), potassium iodide (ACS grade, Fisher), potassium periodate (Aldrich, 96%), sodium chloride (ACS grade, Fisher), magnesium sulfate (ACS grade, Fisher), bis(triphenylphosphine)palladium chloride (TCI, 99%), copper(I) iodide (Sigma-Aldrich, 99%), trimethylsilyl acetylene (TCI, 99%), 4-iodopyridine (TCI, 98%), 3-bromo-4-iodopyridine (Ambeed, 96%), *N,N'*-dimethyl ethylene diamine (TCI, 99%), *p*-trifluoromethyl phenyl isocyanate (TCI, 99%) were used as purchased. Anhydrous 1,4-dioxane and toluene were obtained by passing HPLC-grade solvents over a bed of activated molecular sieves in a commercial (Pure Process Technology) solvent purification system (SPS). Anhydrous triethylamine was prepared by stirring (ACS grade, Fisher) triethylamine over potassium hydroxide (ACS grade, Fisher) at 50 °C for 24 hours and distillation under nitrogen gas. The distilled triethylamine was degassed using three freeze-pump-thaw cycles and stored inside a Schlenk flask under a blanket of nitrogen. Deuterated solvents were purchased from Cambridge Isotopes Laboratory. All NMR spectra were collected by a Varian Inova or Bruker Avance-III HD 500 MHz NMR spectrometer. All IR spectra were obtained using a Nicolet 6700 FT-IR spectrometer equipped with an ATR accessory.

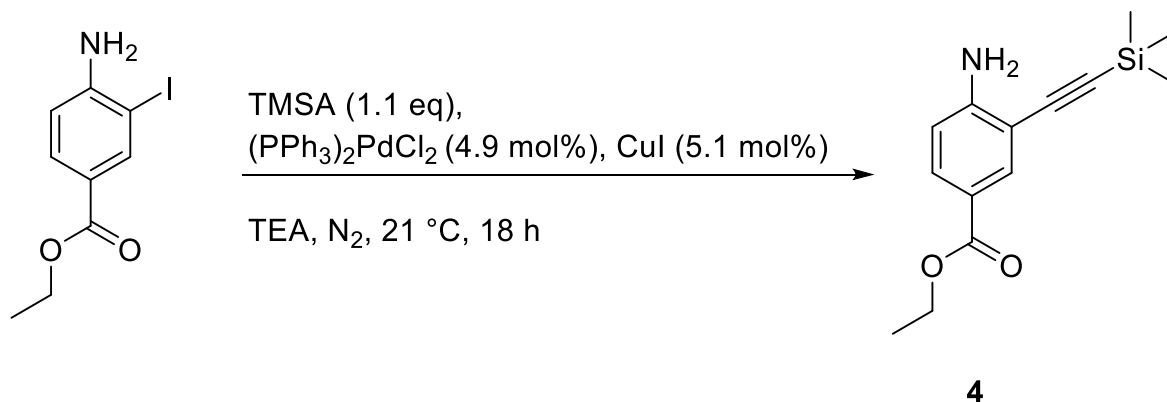
Synthesis

Synthesis of Compound **5** (Scheme S1)



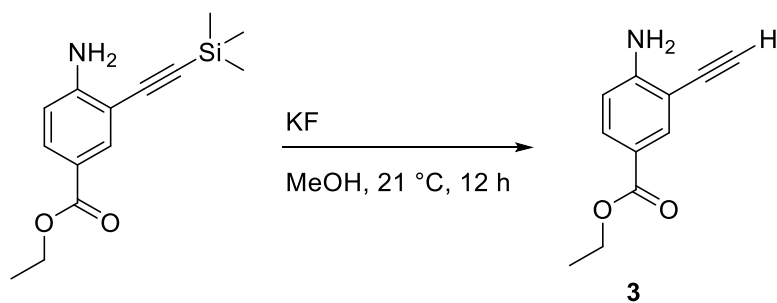
Compound **5** was prepared following a reported procedure.¹ Ethyl-4-aminobenzoate (10.00 g, 60.54 mmol), potassium iodide (10.05 g, 60.54 g), potassium periodate (13.92 g, 60.54 mmol), and sodium chloride (7.08 g, 121.08 mmol) were sparged with nitrogen gas in a 500 mL round bottom flask with a septum cap for 10 minutes. Throughout the septum 120 mL of glacial acetic acid and 13 mL of water were added. This mixture was stirred at 21 °C for 18 hours. The reaction mixture was filtered through filter paper via vacuum filtration. Filtrate was diluted with 150 mL of ethyl acetate and washed with 200 mL solution of saturated sodium chloride twice, then it was washed with 200 mL solution of thiosulfate twice and finally washed with 200 mL of saturated bicarbonate twice. The organic layer was dried over MgSO₄ and filtered through filter paper and then all volatiles were evaporated under reduced pressure to give a pale orange crude product. This crude product was recrystallized with ethanol to yield 10.32 g (59%) of colorless crystals. mp = 80–82 °C. ¹H NMR (500 MHz, chloroform-*d*, δ): 1.36 (t, *J* = 7.1 Hz, 3H), 4.32 (q, *J* = 7.2 Hz, 2H), 4.51 (s, 2H), 6.70 (d, *J* = 8.8 Hz, 1H), 7.82 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.33 (dd, *J* = 1.9 Hz, 1H). ¹³C{¹H} NMR (126 MHz, chloroform-*d*, δ): 14.44, 60.71, 82.16, 113.14, 121.34, 140.93, 150.80, 165.39. FTIR (ATR, cm⁻¹) 3454, 3362 (ν_{N-H}, m); 3066, 2980, 2903 (ν_{C-H}, w); 1685 (ν_{C=O}, s); 1610 (ν_{C=C}, s), 1590 (δ_{N-H}, s), 1245 (ν_{C-O}, s).

Synthesis of Compound **4** (Scheme S2)



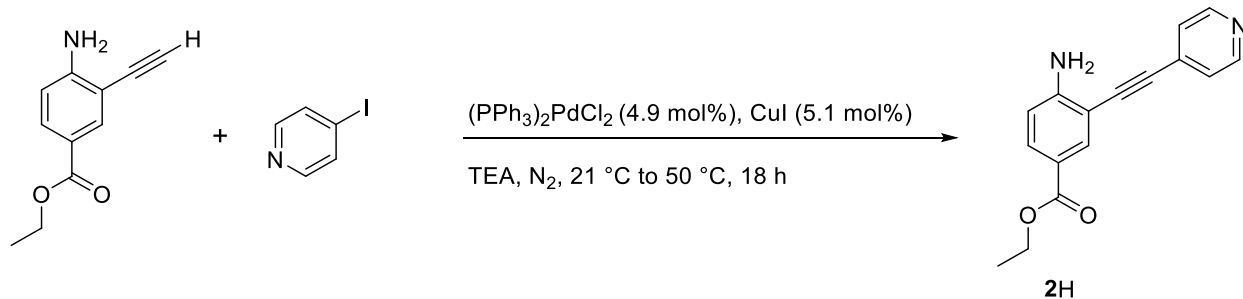
Compound **4** was prepared following a reported method with slight modifications.² Compound **5** (5.822 g, 19.99 mmol), bis(triphenylphosphine)palladium chloride (0.672 g, 0.96 mmol), and copper(I) iodide (0.194 g, 1.02 mmol) were transferred into a 100 mL oven-dried two necked flask. All solids were evacuated and refilled with nitrogen gas three times. Then 150 mL of dry and deoxygenated triethylamine was added to the flask through a septum followed with the addition of 3.13 mL trimethylsilyl acetylene (22.00 mmol). The mixture was allowed to stir at 21 °C for 18 hours. After 18 hours, the solution mixture was vacuum filtered through a bed to silica (2 cm) and rinsed with 200 mL of DCM. All volatiles were evaporated under vacuum to yield brown wax. This wax was triturated in hexanes to give a beige solid. Yield 2.85 g (55%). mp = 106–110 °C. ^1H NMR (500 MHz, chloroform-*d*, δ): 1.36 (t, J = 7.1 Hz, 3H), 4.31 (q, J = 7.1 Hz, 2H), 4.65 (s, 2H), 6.65 (d, J = 8.5 Hz, 1H), 7.79 (dd, J = 8.5, 2.0 Hz, 1H), 8.00 (dd, J = 2.0 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, chloroform-*d*, δ): 0.19, 14.53, 60.61, 100.57, 100.75, 107.11, 113.25, 119.73, 131.75, 134.62, 151.94, 166.21. FTIR (ATR, cm^{-1}) 3461, 3357 ($\nu_{\text{N-H}}$, m); 3206, 2976, 2901 ($\nu_{\text{C-H}}$, w); 2125 ($\nu_{\text{C}\equiv\text{C}}$, m); 1682 ($\nu_{\text{C=O}}$, s); 1620 ($\nu_{\text{C=C}}$, s), 1592 ($\delta_{\text{N-H}}$, s), 1243 ($\nu_{\text{C-O}}$, s).

Preparation of Compound **3** (Scheme S3)



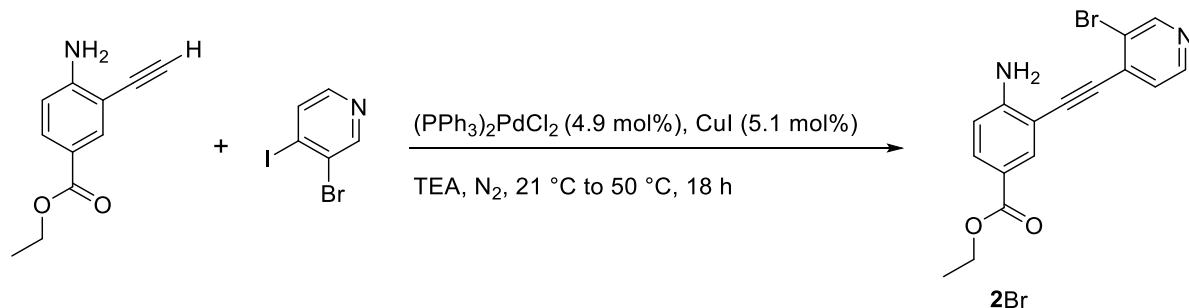
Compound **3** was prepared following a previously published method.³ Compound **4** (3.137 g, 12.00 mmol), was dissolved in 40 mL of methanol. To the stirring solution of this compound was added 1.394 g (24.00 mmol) of anhydrous potassium fluoride and stirred at 21 °C. After 12 hours of stirring the suspension was added to 100 mL of saturated sodium chloride solution and extracted with 150 mL of diethyl ether. The organic layer was dried over MgSO₄ and all volatiles were evaporated under reduced pressure. A light brown solid was recovered with 2.242 g mass (99% yield). Mp = 68–70 °C. ¹H NMR (500 MHz, chloroform-*d*, δ): 1.36 (t, *J* = 7.1 Hz, 3H), 3.40 (s, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.66 (s, 2H), 6.67 (d, *J* = 8.6 Hz, 1H), 7.83 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.04 (d, *J* = 2.0 Hz, 1H). ¹³C {¹H} NMR (125.8 MHz, chloroform-*d*, δ): 14.53, 60.67, 79.72, 83.05, 105.90, 113.39, 119.85, 131.99, 135.00, 152.20, 166.12. FTIR (ATR, cm⁻¹) 3473, 3359 (ν_{N-H}, m); 3279, 2979 (ν_{C-H}, m-w); 2092 (ν_{C≡C}, w); 1687 (ν_{C=O}, s); 1623 (ν_{C=C}, s), 1593 (δ_{N-H}, s), 1247 (ν_{C-O}, s).

Preparation of Compound 2H (Scheme S4)



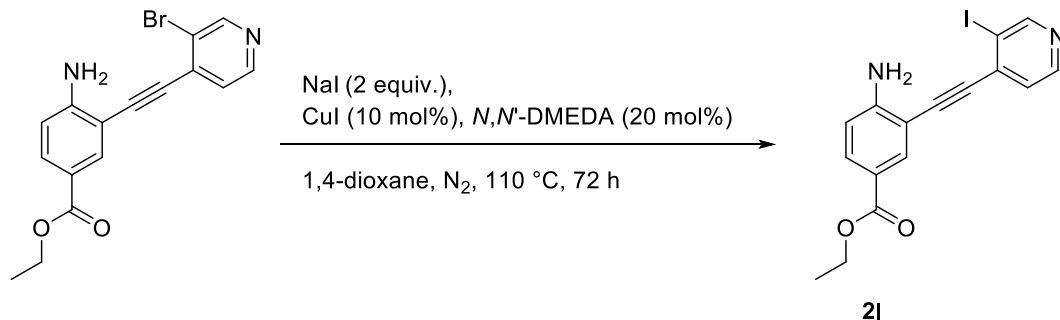
Compound **2H** was prepared following the same procedure as compound **4**. Compound **3** (0.378 g, 2.00 mmol), 4-iodopyridine (0.410 g, 2.00 mmol), bis(triphenylphosphine)palladium chloride (0.098 g, 0.098 mmol), and copper(I) iodide (0.020 g, 0.102 mmol) were transferred into a 100 mL oven-dried two necked flask. All solids were evacuated and refilled with nitrogen gas three times. Then 30 mL of dry and deoxygenated triethylamine was added to the flask through a septum. The temperature of the reaction mixture was increased to 50 °C and allowed to stir for 18 hours. After 18 hours, the solution mixture was vacuum filtered through a bed to Celite (1 cm) and rinsed with 50 mL of DCM. All volatiles were evaporated off under vacuum to yield the crude product as a brown wax. The pure product was obtained via column chromatography. Impurities were eluted with a DCM and Hexanes (7 : 3) solvent mixture and the pure product was flushed out with ethyl acetate. Evaporation of ethyl acetate gave a beige solid. Yield 0.317 g (59%). Mp = 160–162 °C. ¹H NMR (500 MHz, benzene-*d*₆, δ): 1.03 (t, *J* = 7.1 Hz, 3H), 3.88 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 6.11 (d, *J* = 8.7 Hz, 1H), 6.84 (dd, *J* = 8.5, 2.0 Hz, 2H), 8.05 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.43 (dd, *J* = 4.4, 1.7 Hz, 2H), 8.45 (d, *J* = 2.0 Hz, 1H). ¹³C{¹H} NMR (126 MHz, benzene-*d*₆, δ): 14.40, 60.55, 89.76, 92.83, 106.11, 113.74, 120.74, 120.75, 130.68, 132.64, 135.19, 150.36, 151.95, 165.56. FTIR (ATR, cm⁻¹) 3429, 3337 (ν_{N-H}, m); 3201, 3042, 2975 (ν_{C-H}, m-w); 2208 (ν_{C≡C}, w); 1686 (ν_{C=O}, s); 1623 (ν_{C=C}, m), 1590 (δ_{N-H}, s), 1246 (ν_{C-O}, s).

Preparation of Compound 2Br (Scheme S5)



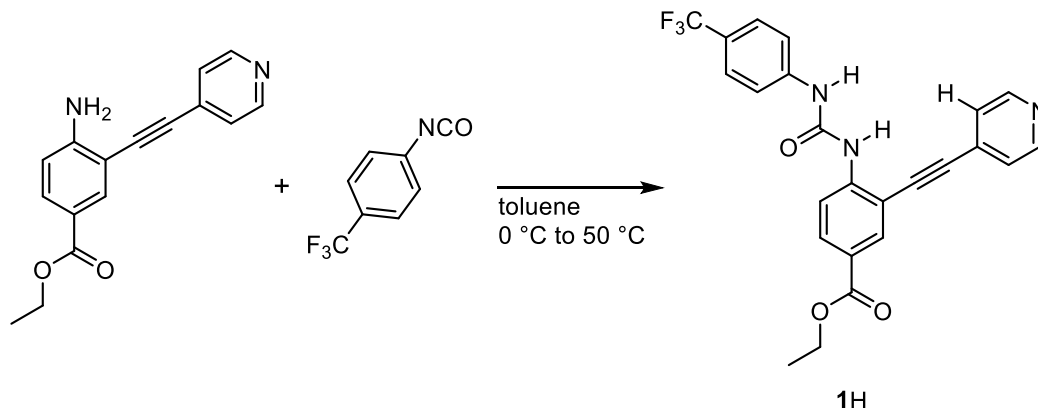
Compound **2Br** was prepared following the same procedure as compound **4**. Compound **3** (0.946 g, 5.00 mmol), 3-bromo-4-iodopyridine (1.420 g, 5.00 mmol), bis(triphenylphosphine)palladium chloride (0.172 g, 0.245 mmol), and copper(I) iodide (0.049 g, 0.255 mmol) were transferred into a 100 mL oven-dried two necked flask. All solids were evacuated and refilled with nitrogen gas three times. Then 50 mL of dry and deoxygenated triethylamine was added to the flask through a septum. The temperature of the reaction mixture was increased to 50 °C and allowed to stir for 18 hours. After 18 hours, the solution mixture was vacuum filtered through a bed to Celite (2 cm) and rinsed with 200 mL of DCM. All volatiles were evaporated off under vacuum to yield the crude product as orange wax. The pure product was obtained via column chromatography. Impurities were eluted with a DCM and Hexanes (7 : 3) solvent mixture and pure product was flushed out with ethyl acetate. Evaporation of ethyl acetate gave a yellow solid. Yield 0.960 g (56%). Mp = 110–112 °C. ¹H NMR (500 MHz, benzene-*d*₆, δ): 1.04 (t, *J* = 7.2 Hz, 3H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.19 (s, 2H), 6.09 (d, *J* = 8.7 Hz, 1H), 6.70 (d, *J* = 4.9 Hz, 1H), 8.06 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.11 (d, *J* = 4.5 Hz, 1H), 8.46 (d, *J* = 2.0 Hz, 1H), 8.67 (s, 1H). ¹³C{¹H} NMR (126 MHz, benzene-*d*₆, δ): 14.41, 60.57, 91.84, 95.11, 105.40, 113.75, 120.57, 122.49, 126.25, 132.51, 133.12, 135.13, 148.29, 151.91, 152.72, 165.50. FTIR (ATR, cm⁻¹) 3427, 3337 (ν_{N-H}, m-s); 3178, 3012, 2973, 2937 (ν_{C-H}, w); 2203 (ν_{C≡C}, s); 1670 (ν_{C=O}, s); 1608 (ν_{C=C}, s), 1575, 1562 (δ_{N-H}, s), 1254 (ν_{C-O}, s).

Preparation of Compound 2I (Scheme S6)



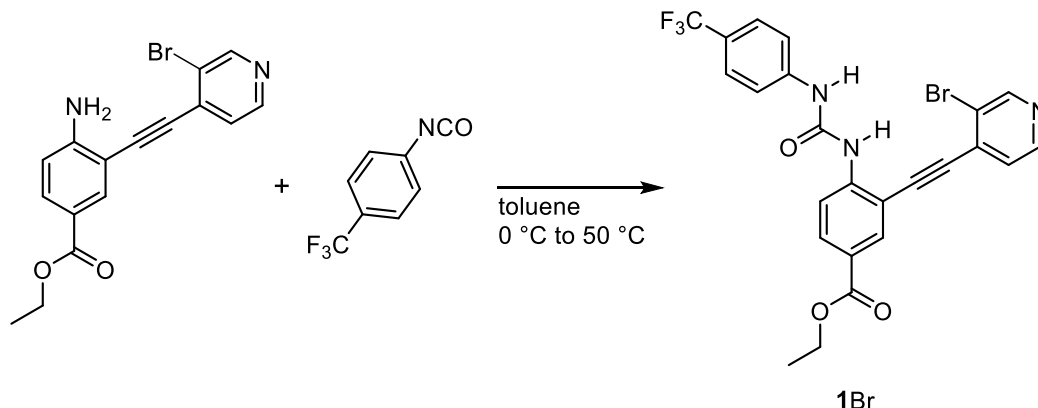
Compound **2I** was prepared following a reported procedure. All solid reagents **2Br** (1.337 g, 3.87 mmol), sodium iodide (1.151 g, 7.68 mmol), and copper(I) iodide (0.0750 g, 3.94 mmol) were added to a 25 mL three-necked flask equipped with a reflux condenser. All solids were evacuated and refilled with nitrogen gas for three cycles. Then, 15 mL of dry and degassed 1,4-dioxane was added to the solids followed by the addition of 85 μL (0.790 mmol) of *N,N'*-dimethyl ethylene diamine. The mixture was stirred vigorously for 72 hours at 110 $^\circ\text{C}$. After this time, the mixture was added to a 10% aqueous solution of ammonium hydroxide. The final product was extracted from the aqueous solution three times with 30 mL of DCM. The DCM solution was dried with MgSO_4 and, after filtration, dried under reduced pressure to give a brown solid. This solid was crystallized with a mixture of DCM and hexanes (5 to 1) to give light yellow crystals. Yield 1.020 g (67%). mp = 118–120 $^\circ\text{C}$. ^1H NMR (500 MHz, chloroform-*d*, δ): 1.39 (t, J = 7.1 Hz, 3H), 4.35 (q, J = 7.2 Hz, 2H), 4.97 (s, 2H), 6.73 (d, J = 8.5 Hz, 1H), 7.89 (dd, J = 8.5, 2.0 Hz, 1H), 8.14 (d, J = 2.0 Hz, 1H), 8.54 (d, J = 5.0 Hz, 1H), 8.98 (d, J = 0.6 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, chloroform-*d*, δ): 14.56, 60.80, 94.17, 94.69, 98.77, 105.27, 113.73, 120.07, 126.32, 132.92, 135.13, 137.92, 148.63, 152.47, 157.02, 165.99. FTIR (ATR, cm^{-1}) 3468, 3334 ($\nu_{\text{N-H}}$, m); 3181, 2979, 2923 ($\nu_{\text{C-H}}$, w); 2203 ($\nu_{\text{C}\equiv\text{C}}$, m); 1670 ($\nu_{\text{C=O}}$, s); 1610 ($\nu_{\text{C=C}}$, s), 1565, 1562 ($\delta_{\text{N-H}}$, s), 1254 ($\nu_{\text{C-O}}$, s).

Preparation of Compound 1H (Scheme S7)



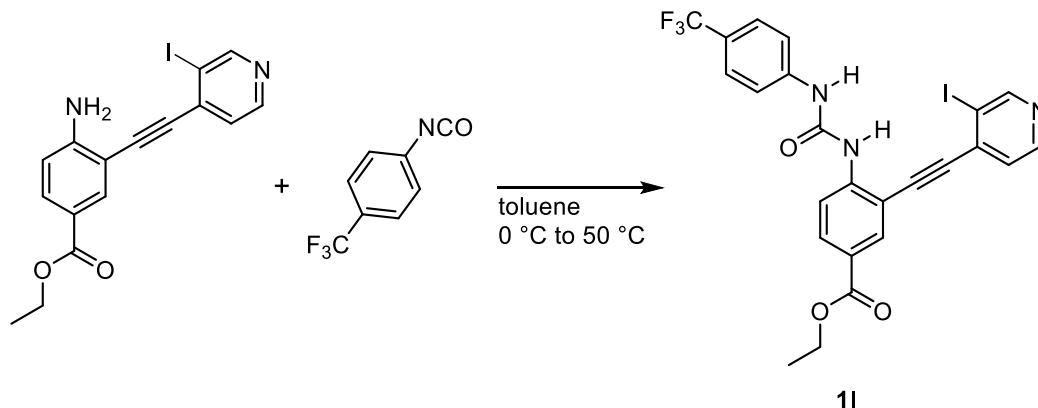
The preparation for compound **1H** was adapted from a previous report. The solid **2H** compound (0.266 g, 0.999 mmol) was added to a 100 mL two-necked flask. The flask was evacuated and refilled with nitrogen gas for three cycles. Then, 30 mL of dry and degassed toluene was added to the flask. The solution was cooled down to 0 °C, followed by the addition of 160 μ L (1.12 mmol) of *p*-trifluoromethyl phenyl isocyanate. The solution was allowed to warm up to 22 °C and then to 50 °C. After 12 hours of stirring at 50 °C, the mixture was cooled down to 22 °C and all volatiles were evaporated under reduced pressure. The crude product was recrystallized in acetone to give a beige powder. Yield 0.285 g (64%). mp = 232 °C (decomposed). ^1H NMR (500 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 1.34 (t, J = 7.1 Hz, 3H), 4.31 (q, J = 7.2 Hz, 2H), 7.45 (d, J = 5.0 Hz, 2H), 7.49 (d, J = 8.4, 2H), 7.56 (d, J = 8.1, 2H), 7.64 (s, 1 H), 7.97 (d, J = 8.5 Hz, 1H), 8.13 (s, 1H), 8.36 (d, J = 8.7 Hz, 1H), 8.61 (d, J = 4.2 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 13.57, 60.27, 87.77, 92.69, 109.70, 117.89, 117.94, 117.99, 123.77, 125.04, 125.50, 131.02, 133.47, 141.78, 143.48, 149.29, 150.86, 153.60, 164.51. FTIR (ATR, cm^{-1}) 3325, 3198 ($\nu_{\text{N-H}}$, m); 3049, 2995 ($\nu_{\text{C-H}}$, w); 2225 ($\nu_{\text{C}\equiv\text{C}}$, m); 1712 ($\nu_{\text{NC=O}}$, s); 11710 ($\nu_{\text{OC=O}}$, s); 1584 ($\nu_{\text{C=C}}$, s).

Preparation of Compound 1Br (Scheme S8)



The preparation for compound **1Br** was adapted from a previous report. The solid **2Br** compound (0.345 g, 0.999 mmol) was added to a 100 mL two-necked flask. The flask was evacuated and refilled with nitrogen gas for three cycles. Then, 30 mL of dry and degassed toluene was added to the flask. The solution was cooled down to 0 °C, followed by the addition of 160 μ L (1.12 mmol) of *p*-trifluoromethyl phenyl isocyanate. The solution was allowed to warm up to 22 °C and then to 50 °C. After 12 hours of stirring at 50 °C, the mixture was cooled down to 22 °C and all volatiles were evaporated under reduced pressure. The crude product was recrystallized in acetone to give a white powder. Yield 0.389 g (73%). mp = 224 °C (decomposed). ^1H NMR (500 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 1.34 (t, J = 7.1 Hz, 3H), 4.32 (q, J = 7.0 Hz, 2H), 7.47 (m, 1H), 7.50 (d, J = 9.6, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.72 (s, 1H), 7.89 (s, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.18 (s, 1H), 8.35 (d, J = 9 Hz, 1H), 8.54 (d, J = 4.5 Hz, 1H), 8.98 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 13.86, 60.71, 91.84, 95.18, 99.08, 109.61, 118.12, 118.28, 124.07, 125.07, 125.75, 126.28, 131.71, 133.60, 136.49, 141.79, 143.79, 148.06, 151.09, 156.49, 164.90. FTIR (ATR, cm^{-1}) 3328, 3292 ($\nu_{\text{N-H}}$, w); 3051, 2996, 2953 ($\nu_{\text{C-H}}$, w); 2222 ($\nu_{\text{C}\equiv\text{C}}$, m); 1724 ($\nu_{\text{NC=O}}$, s); 1709 ($\nu_{\text{OC=O}}$, s); 1583 ($\nu_{\text{C=C}}$, s).

Preparation of Compound 1I (Scheme S9)



The preparation for compound 1I was adapted from a previous report. The solid 2I compound (0.784 g, 2.00 mmol) was added to a 100 mL two-necked flask. The flask was evacuated and refilled with nitrogen gas for three cycles. Then, 30 mL of dry and degassed toluene was added to the flask. The solution was cooled down to 0 °C, followed by the addition of 310 μ L (2.17 mmol) of *p*-trifluoromethyl phenyl isocyanate. The solution was allowed to warm up to 22 °C and then to 50 °C. After 12 hours of stirring at 50 °C, the mixture was cooled down to 22 °C and all volatiles were evaporated under reduced pressure. The crude product was recrystallized in acetone to give a white powder. Yield 0.872 g (75%). mp = 230 °C (decomposed). ^1H NMR (500 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 1.34 (t, J = 7.1 Hz, 3H), 4.32 (q, J = 7.0 Hz, 2H), 7.47 (m, 1H), 7.50 (d, J = 9.6, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.72 (s, 1H), 7.89 (s, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.18 (s, 1H), 8.35 (d, J = 9 Hz, 1H), 8.54 (d, J = 4.5 Hz, 1H), 8.98 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.8 MHz, 10%MeCN-*d*3 in chloroform-*d*, δ): 13.86, 60.71, 91.84, 95.18, 99.08, 109.61, 118.12, 118.28, 124.07, 125.07, 125.75, 126.28, 131.71, 133.60, 136.49, 141.79, 143.79, 148.06, 151.09, 156.49, 164.90. FTIR (ATR, cm^{-1}) 3329, 3291 ($\nu_{\text{N-H}}$, w); 3050, 2938 ($\nu_{\text{C-H}}$, w); 2212 ($\nu_{\text{C}\equiv\text{C}}$, m); 1724 ($\nu_{\text{NC=O}}$, s); 1699 ($\nu_{\text{OC=O}}$, s); 1583 ($\nu_{\text{C=C}}$, s).

Spectroscopy

NMR Spectra of the synthesized compounds

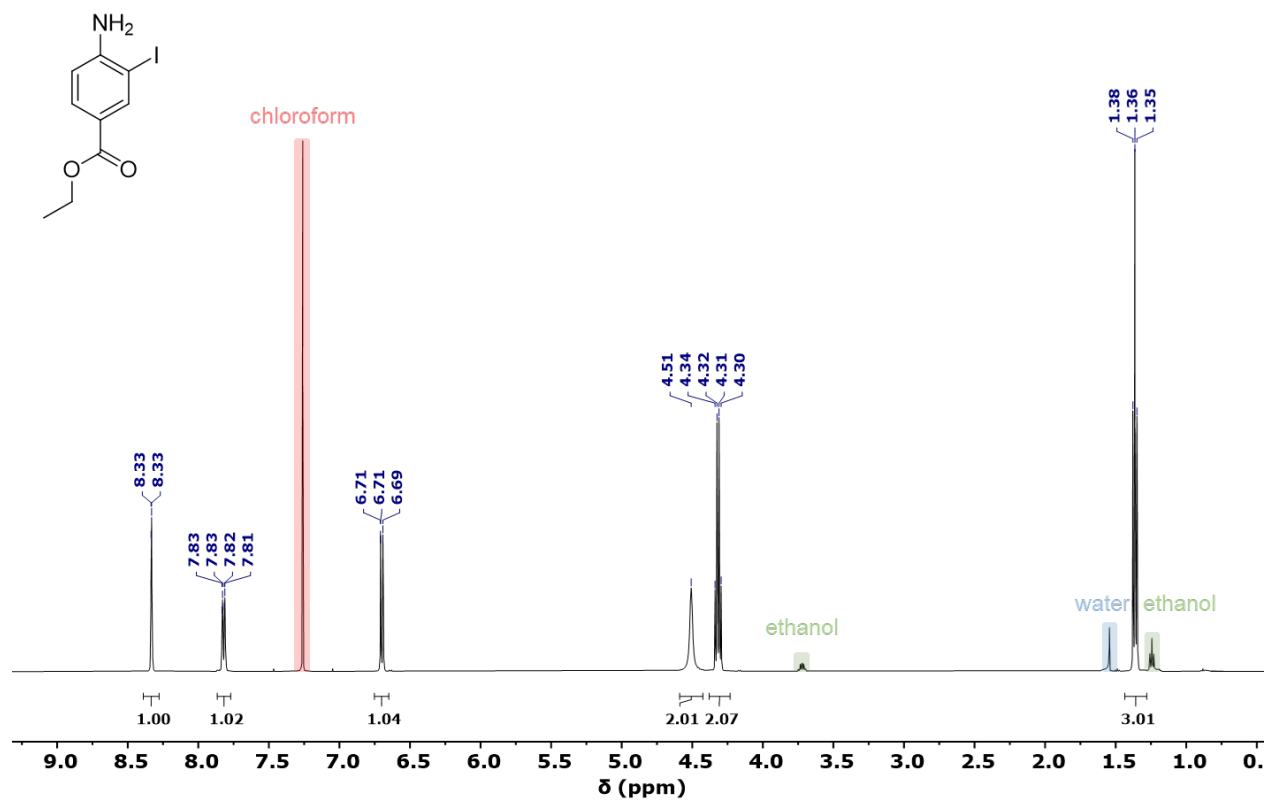


Figure S2. ¹H NMR of **5** (500 MHz, CDCl₃, 298 K).

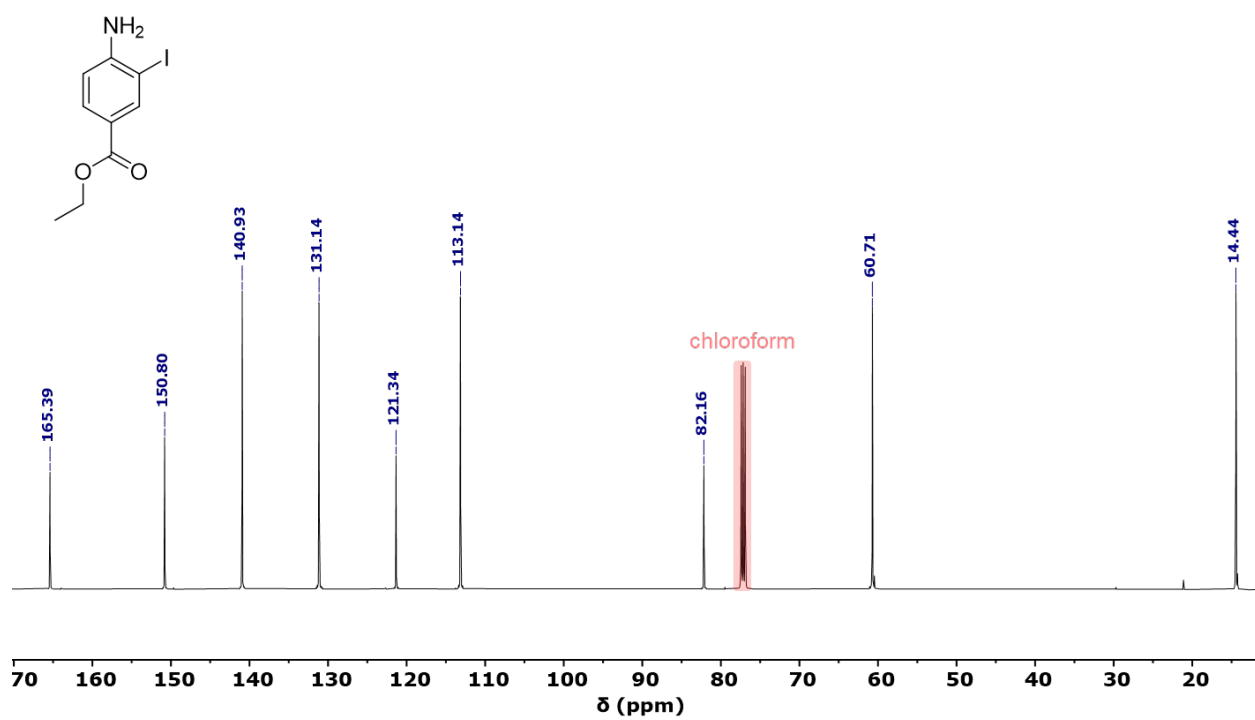


Figure S3. ¹³C NMR of **5** (125.8 MHz, CDCl₃, 298 K).

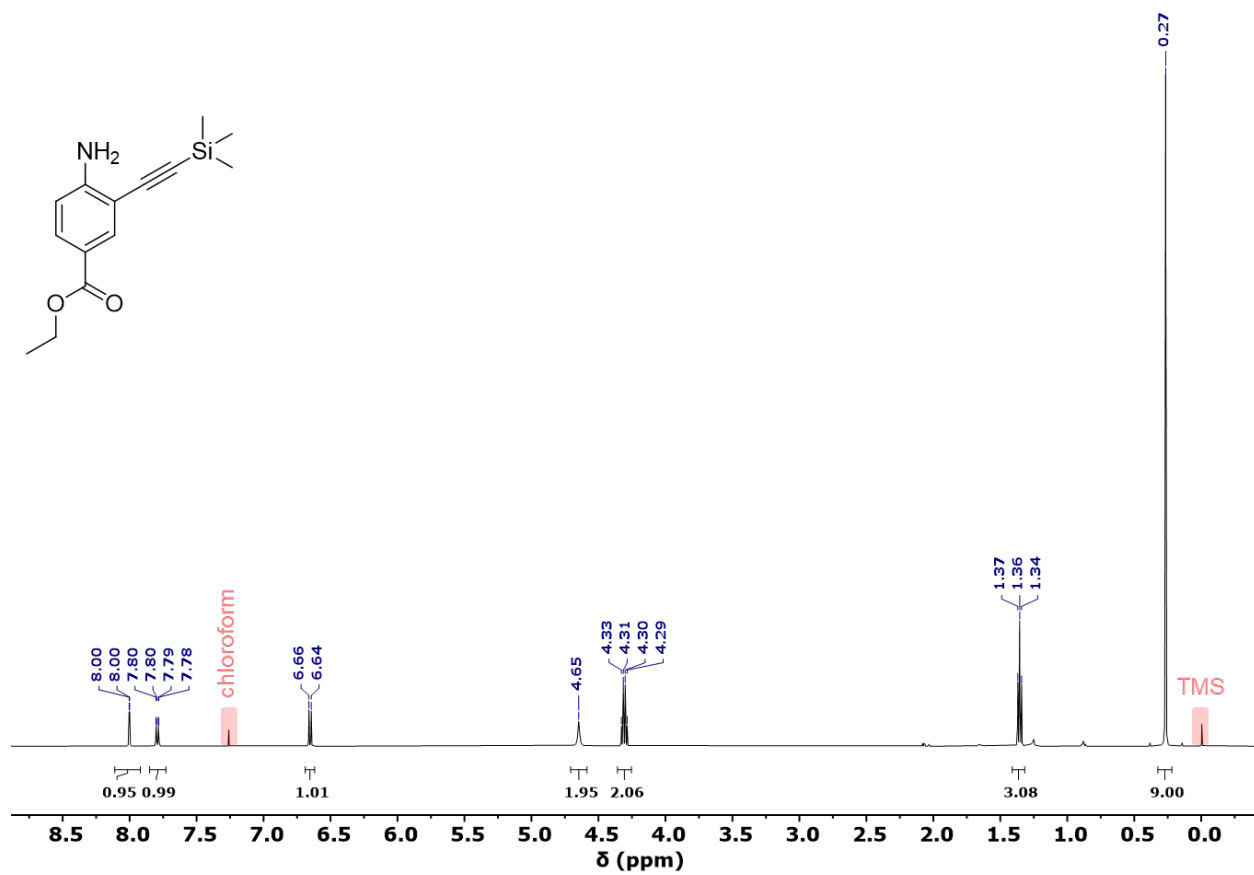


Figure S4. ¹H NMR of 4 (500 MHz, CDCl₃, 298 K).

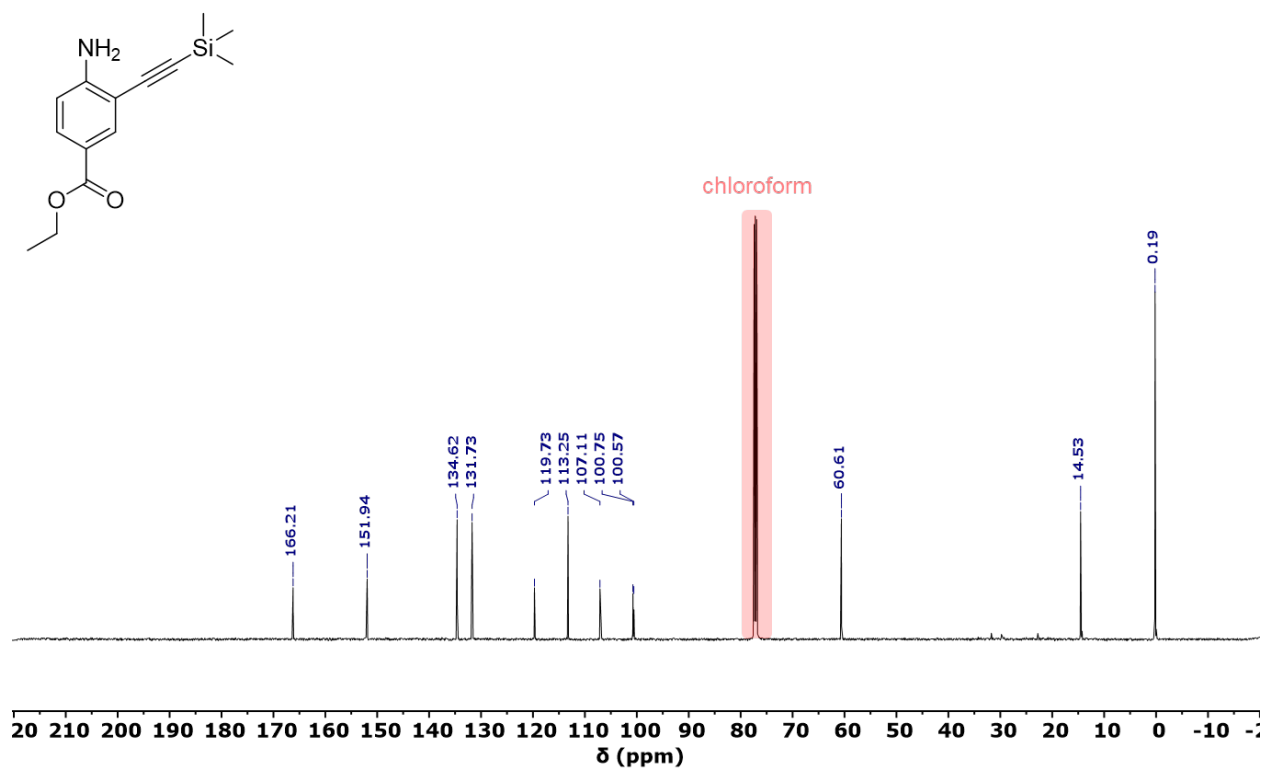


Figure S5. ^{13}C NMR of 4 (125.8 MHz, CDCl_3 , 298 K).

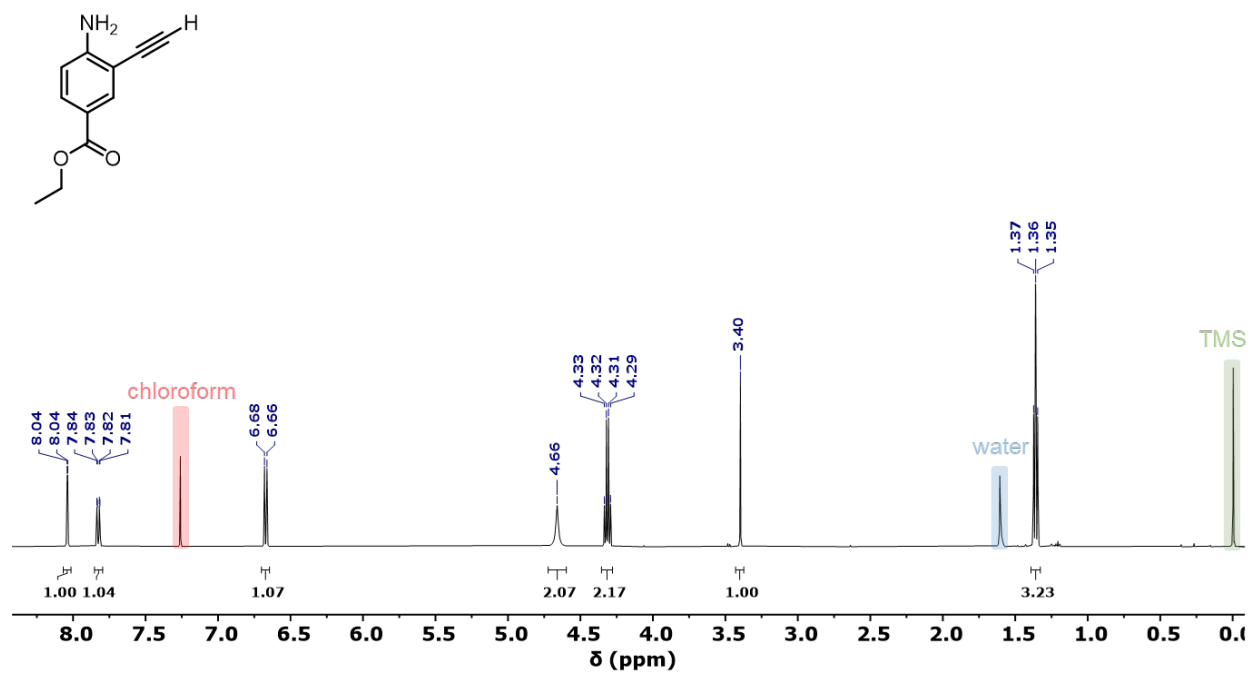


Figure S6. ¹H NMR of 3 (500 MHz, CDCl₃, 298 K).

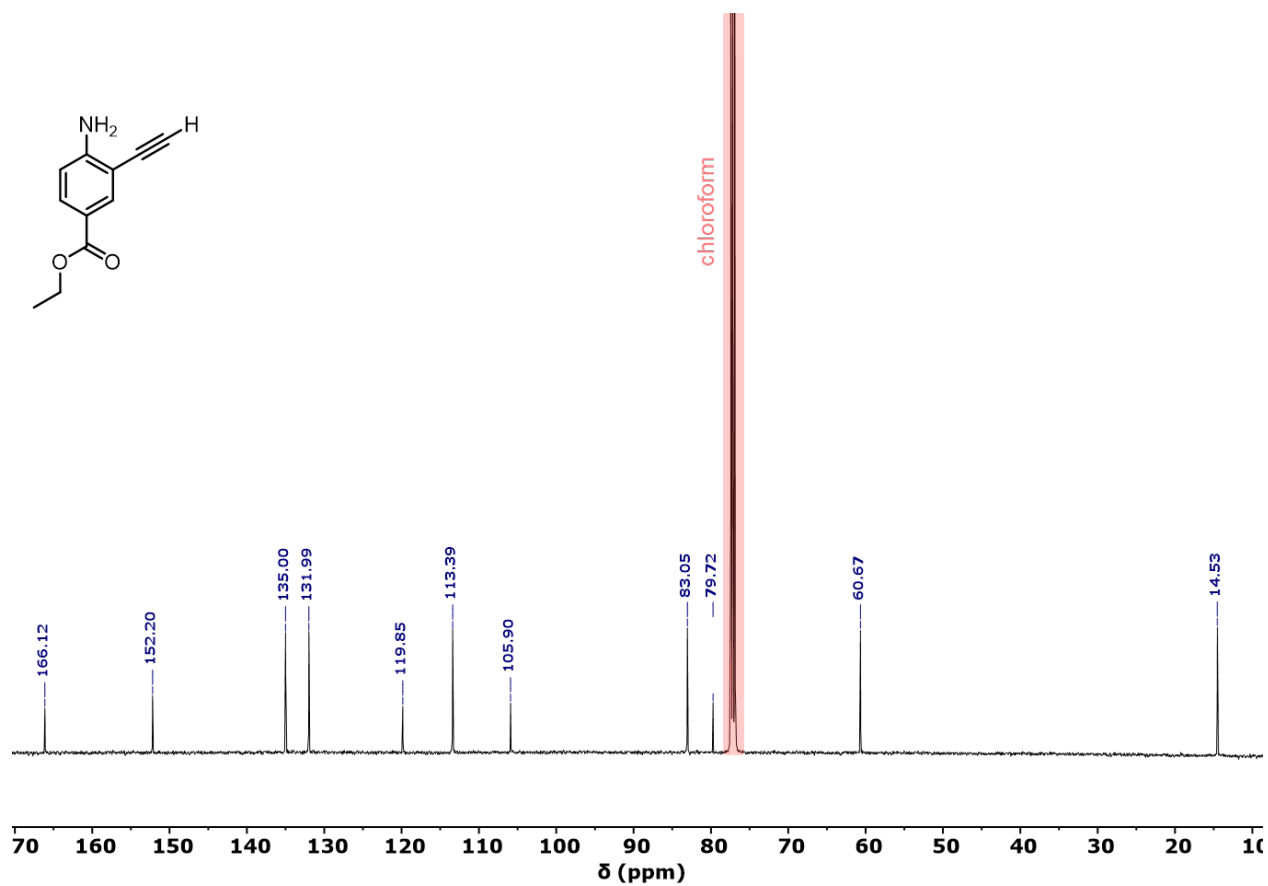


Figure S7. $^{13}\text{C}\{^1\text{H}\}$ NMR of **3** (125.8 MHz, CDCl_3 , 298 K).

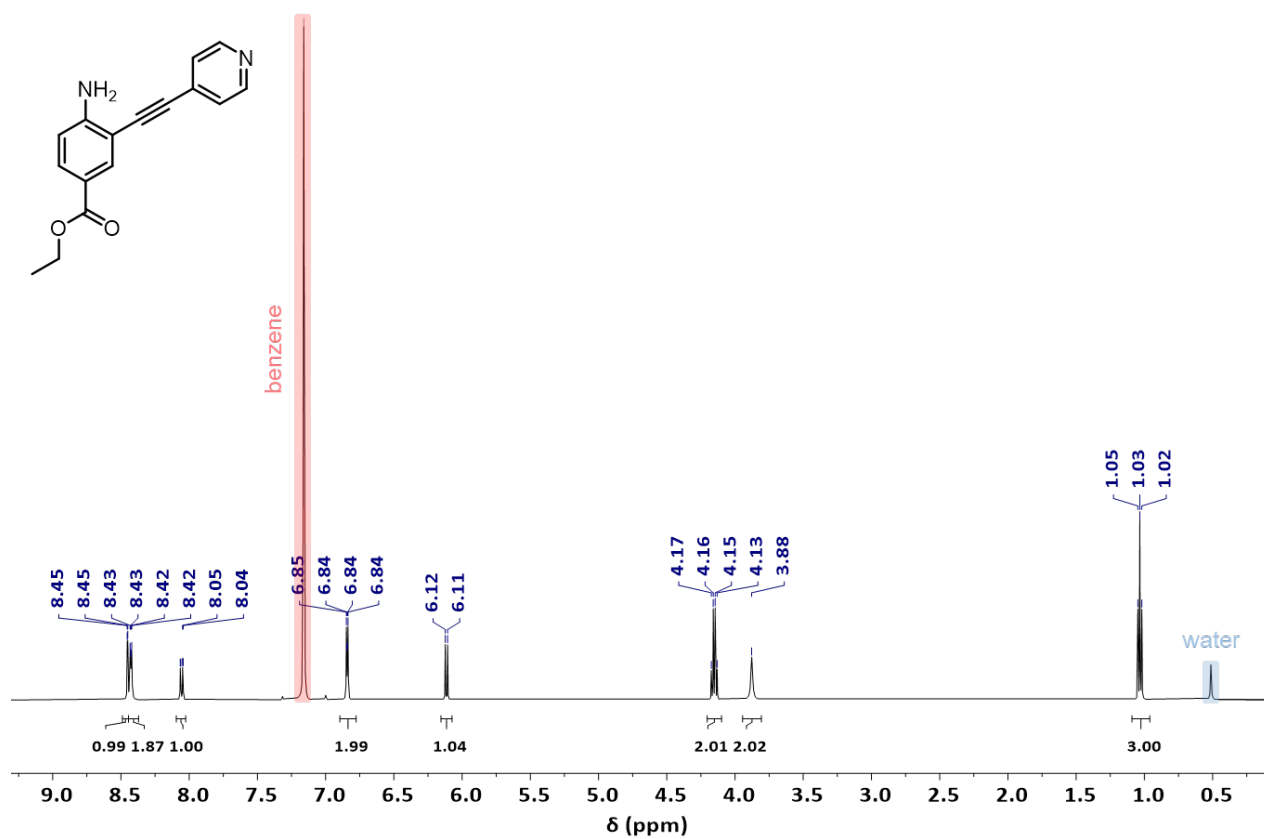


Figure S8. ¹H NMR of 2H (500 MHz, C₆D₆, 298 K).

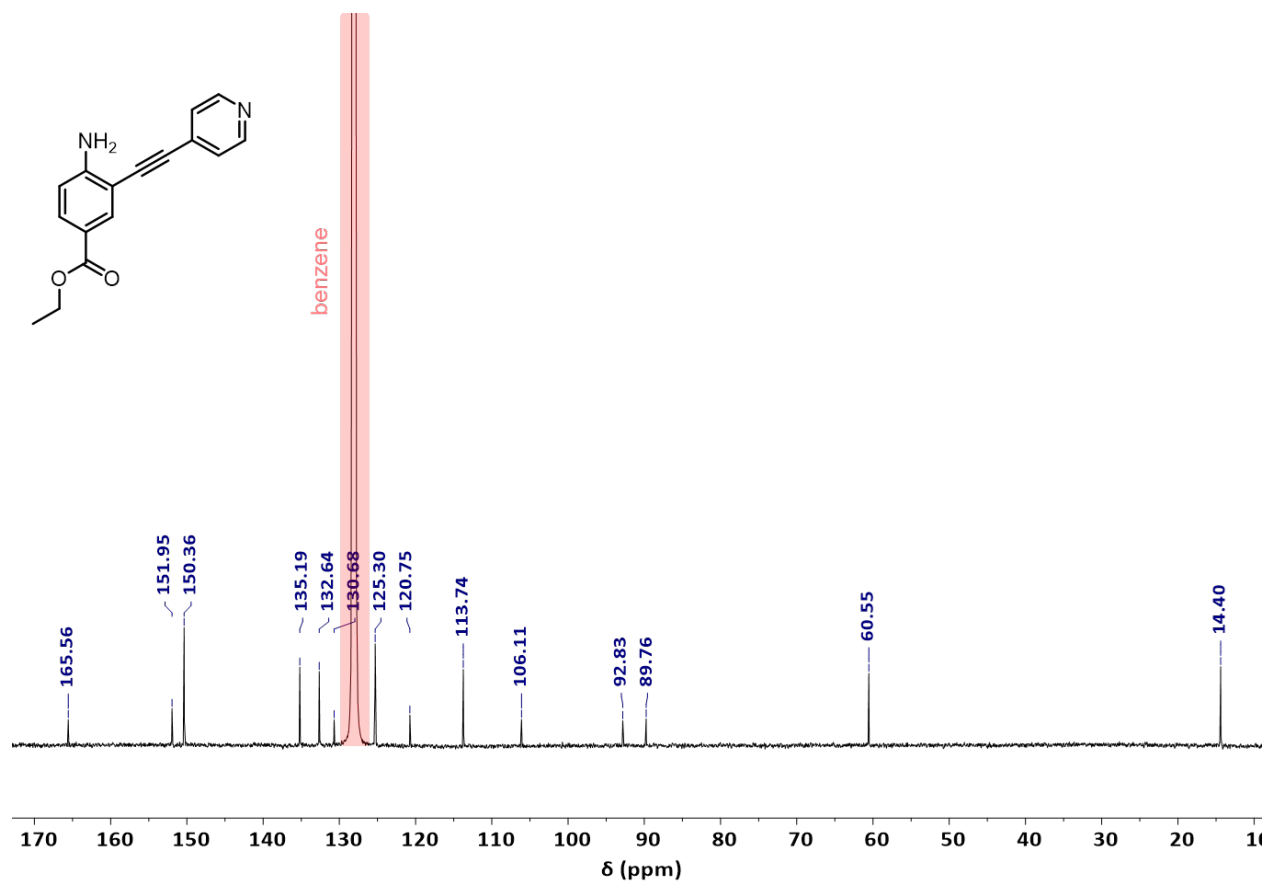


Figure S9. ^{13}C NMR of 2H (125.8 MHz, C_6D_6 , 298 K).

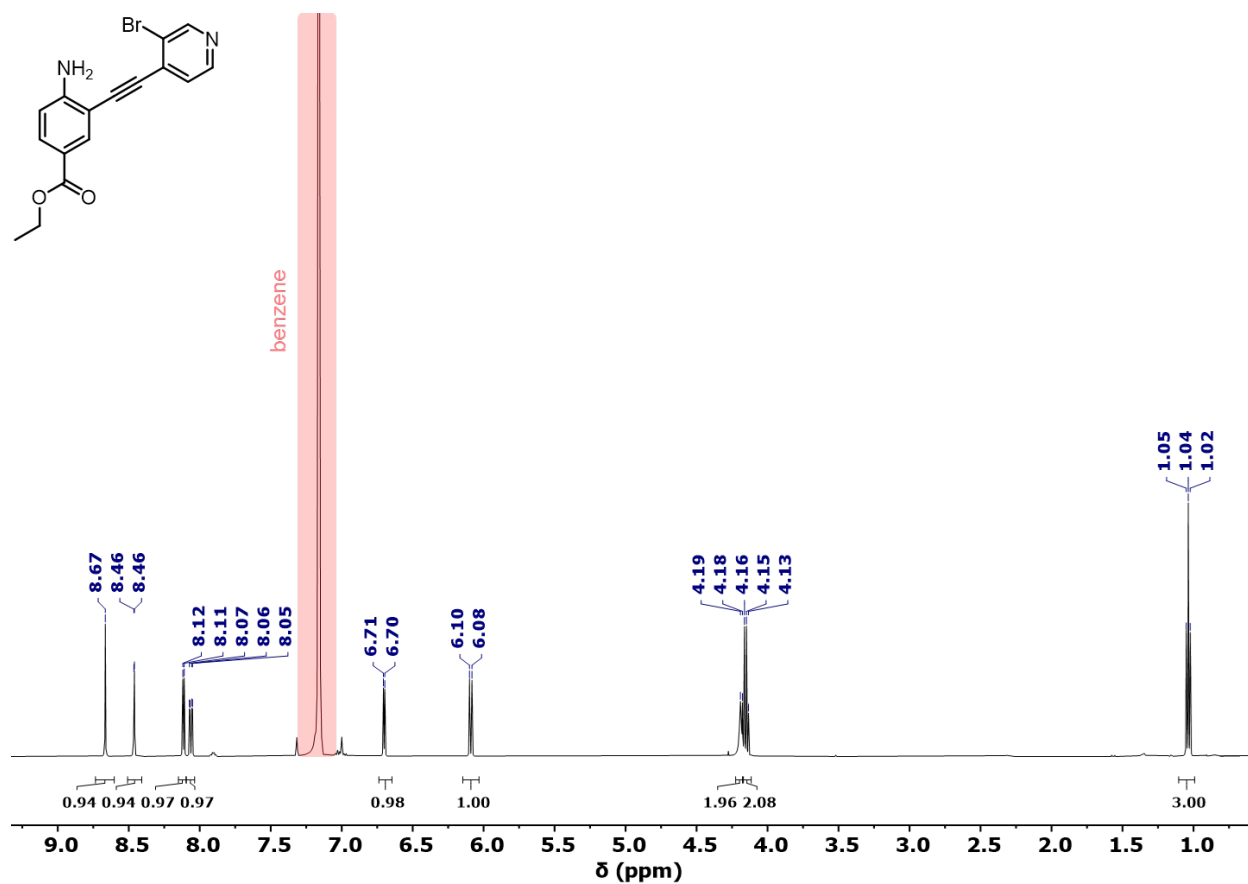


Figure S10. ¹H NMR of 2Br (500 MHz, C₆D₆, 298 K).

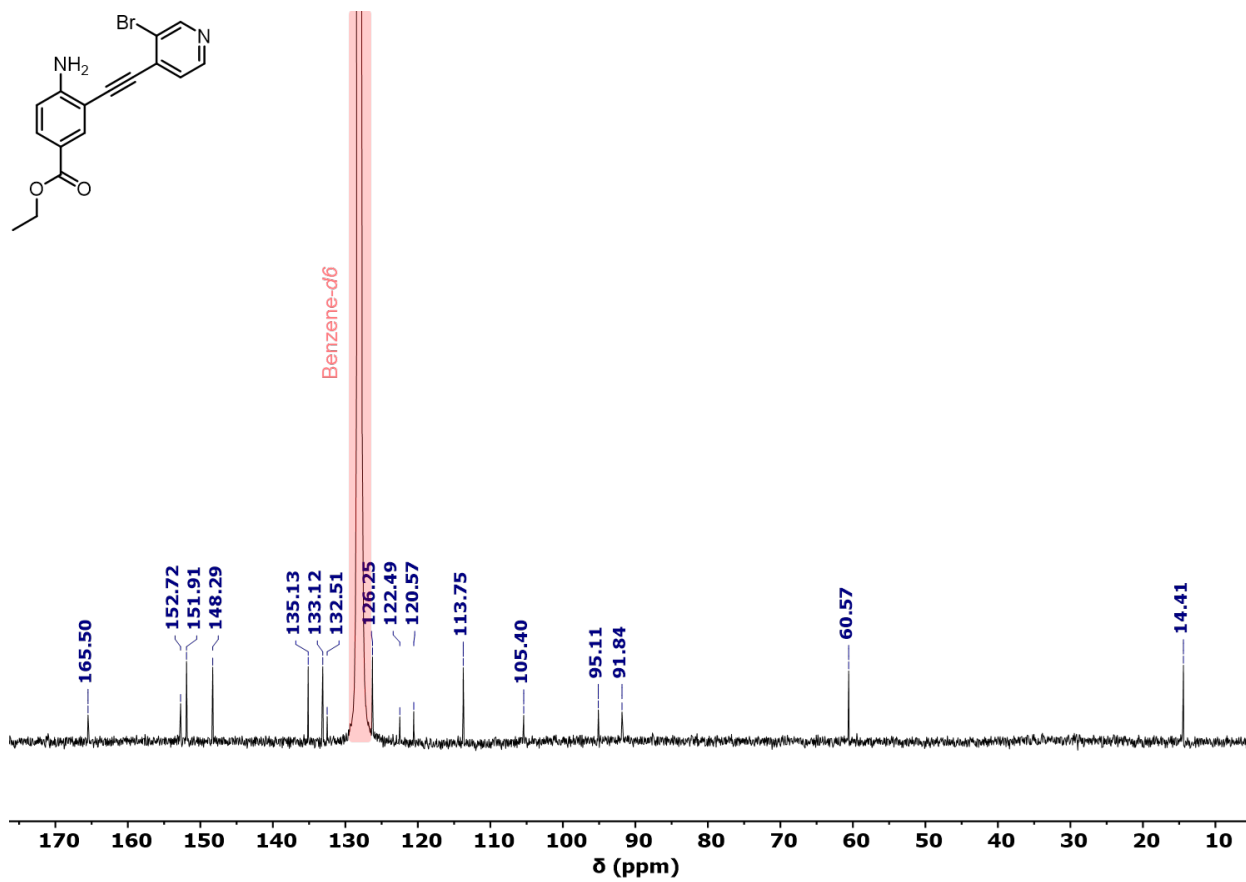


Figure S11. ¹³C NMR of 2Br (125.8 MHz, C₆D₆, 298 K).

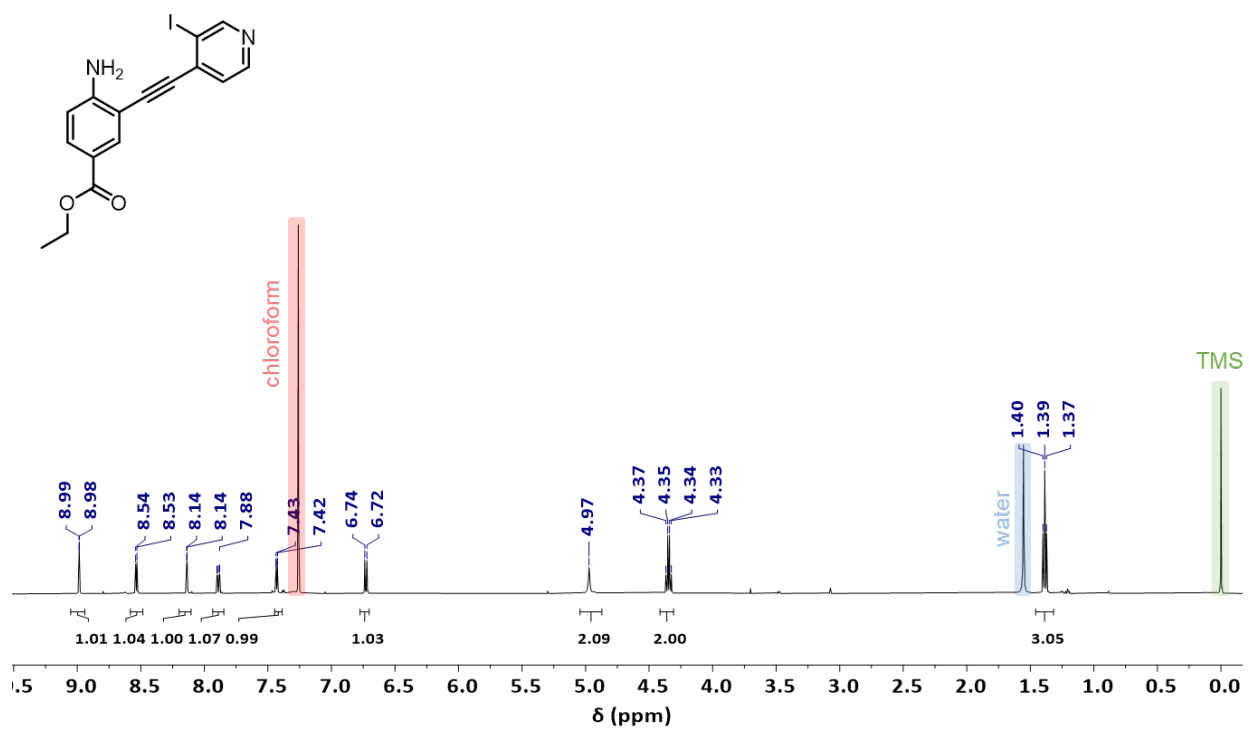


Figure S12. ¹H NMR of 2I (500 MHz, CDCl₃, 298 K).

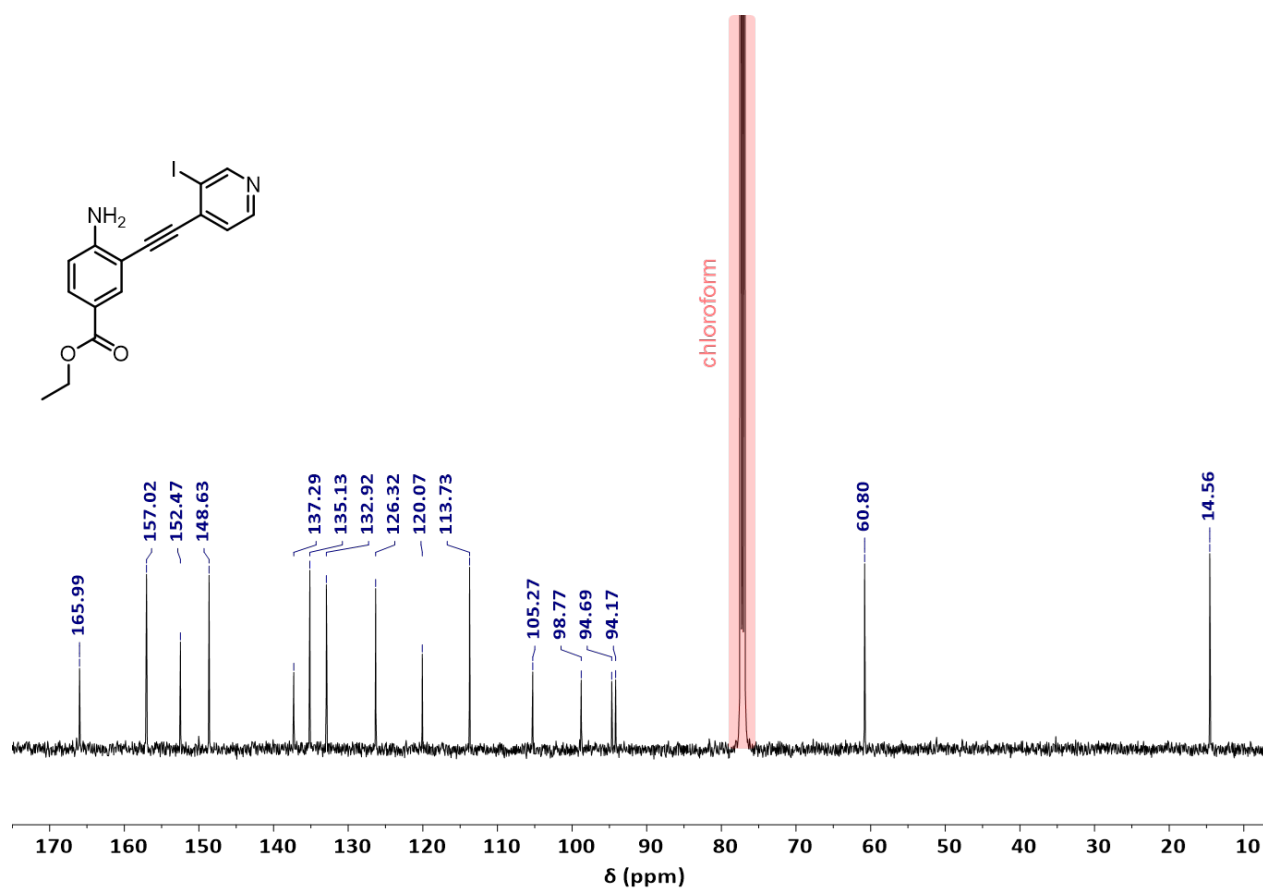


Figure S13. ¹³C NMR of 2I (125.8 MHz, CDCl₃, 298 K).

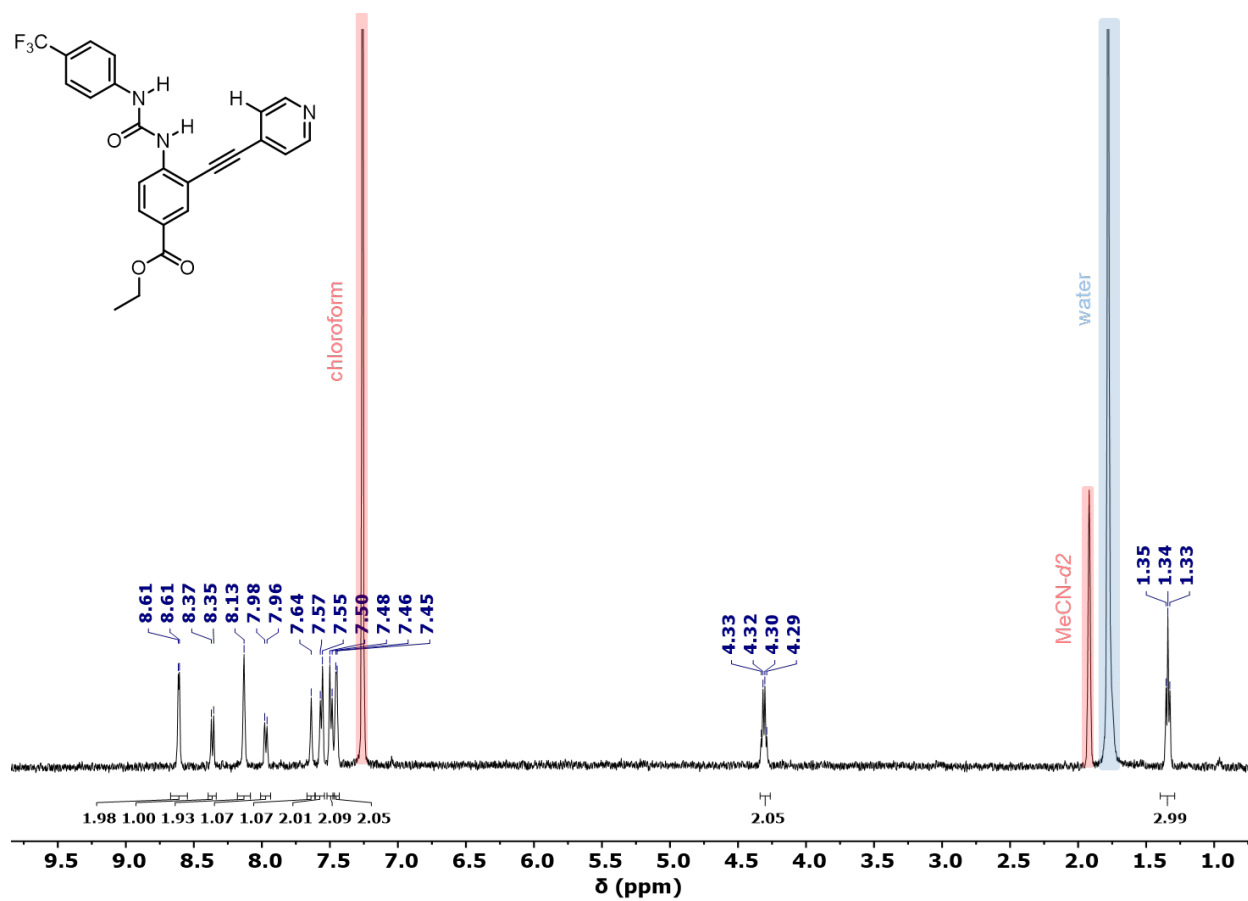


Figure S14. ^1H NMR of **1H** (500 MHz, 10% MeCN- d_3 10% acetone- d_6 in CDCl_3 , 298 K).

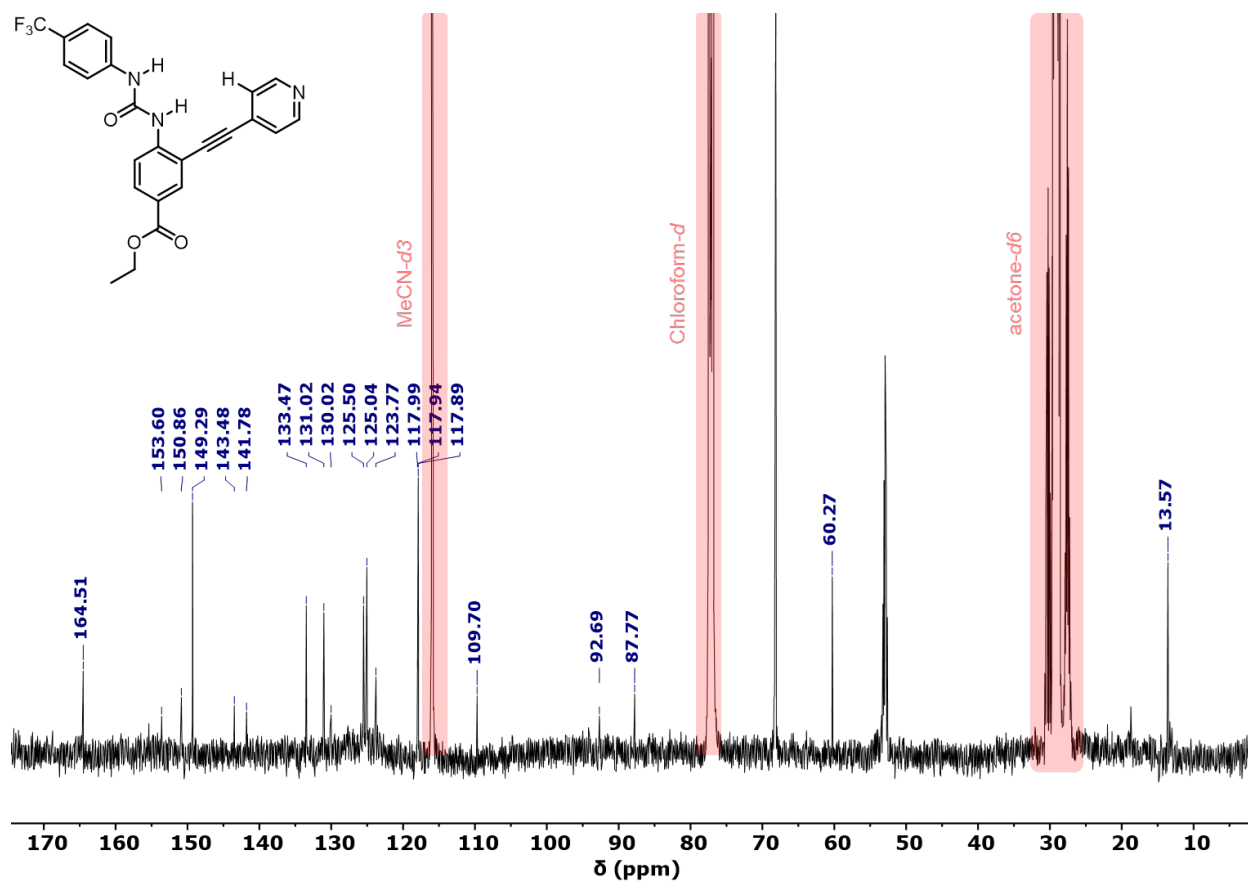


Figure S15. ¹³C NMR of **1H** (125.8 MHz, 10% MeCN-d₃ 10% acetone-d₆ in CDCl₃, 298 K).

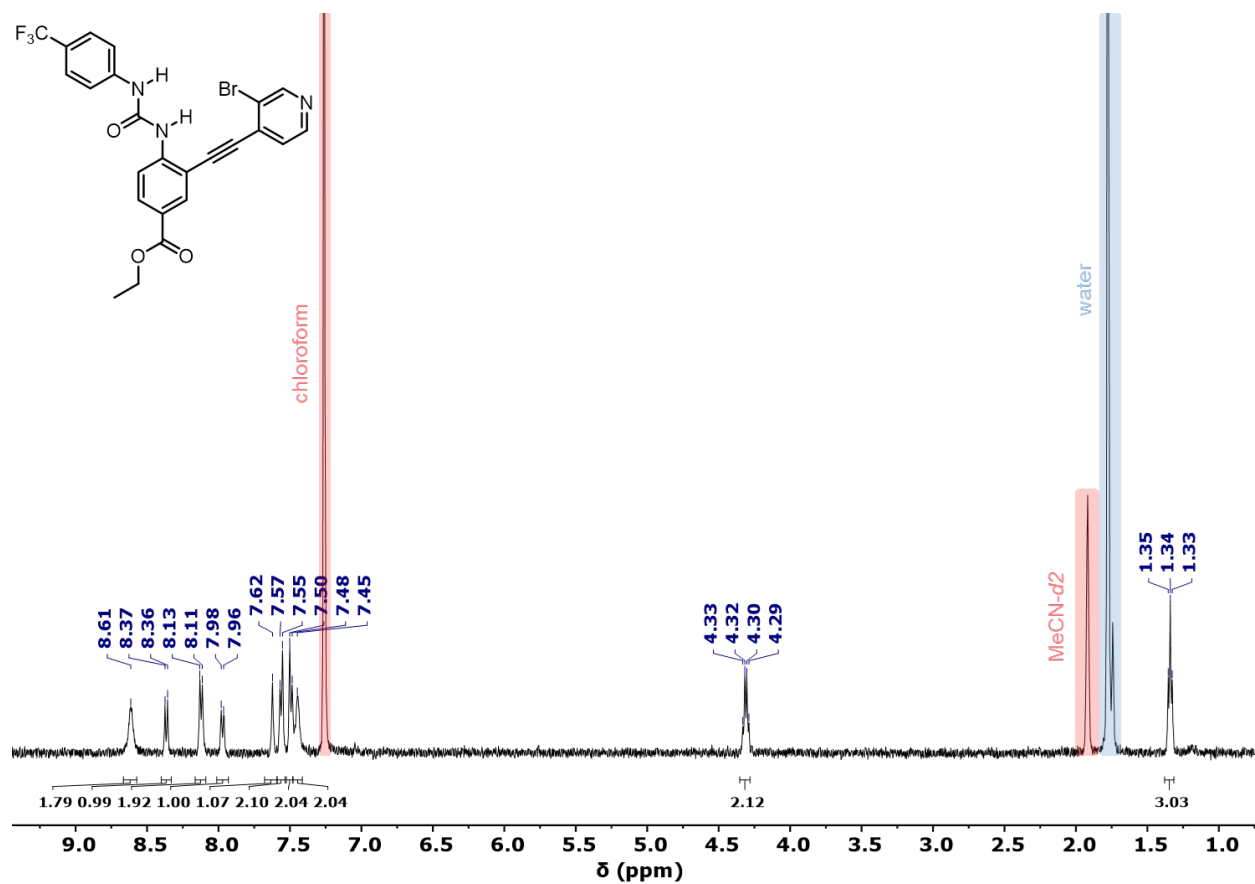


Figure S16. ¹H NMR of 1Br (500 MHz, 10% MeCN-d₃ in CDCl₃, 298 K).

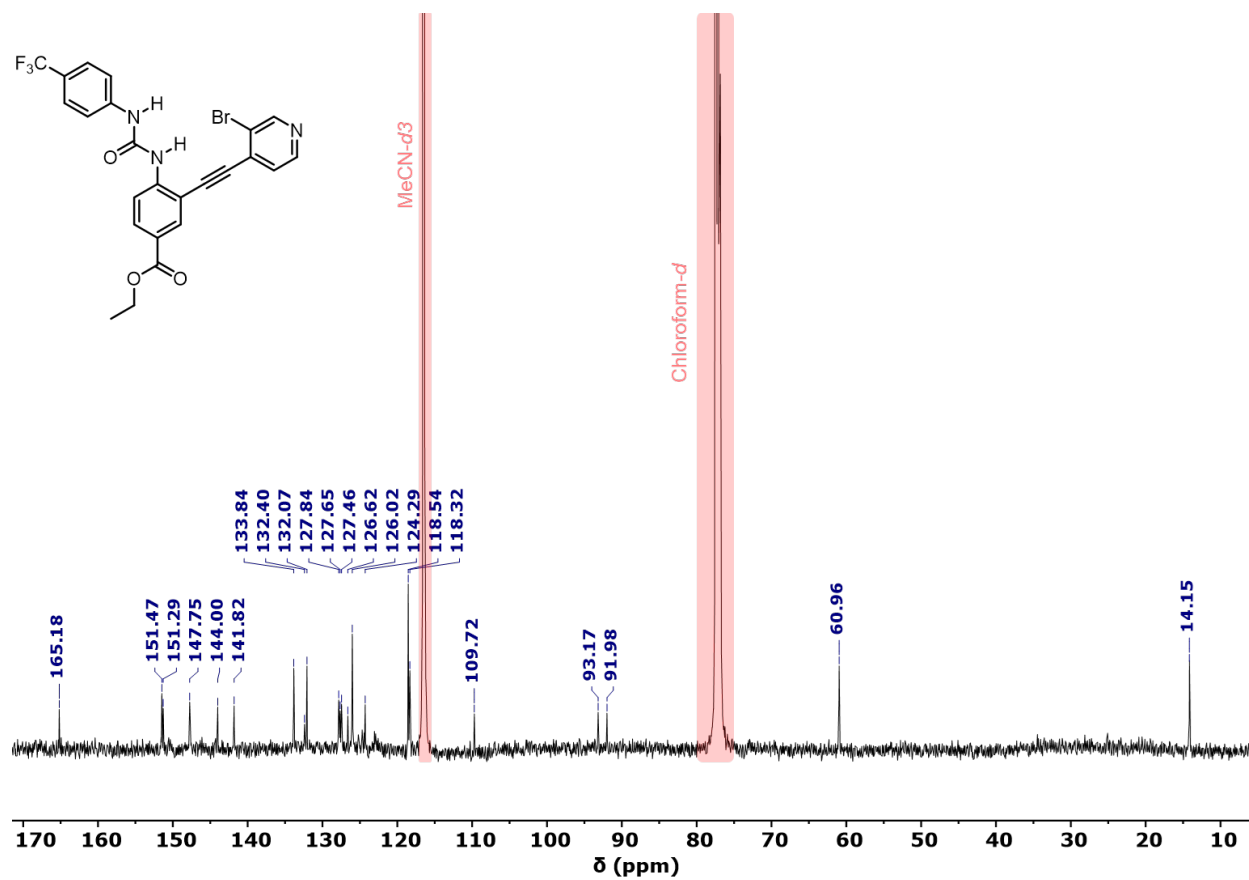


Figure S17. $^{13}\text{C}\{^1\text{H}\}$ NMR of **1Br** (125.8 MHz, 10% MeCN- d_3 in CDCl_3 , 298 K).

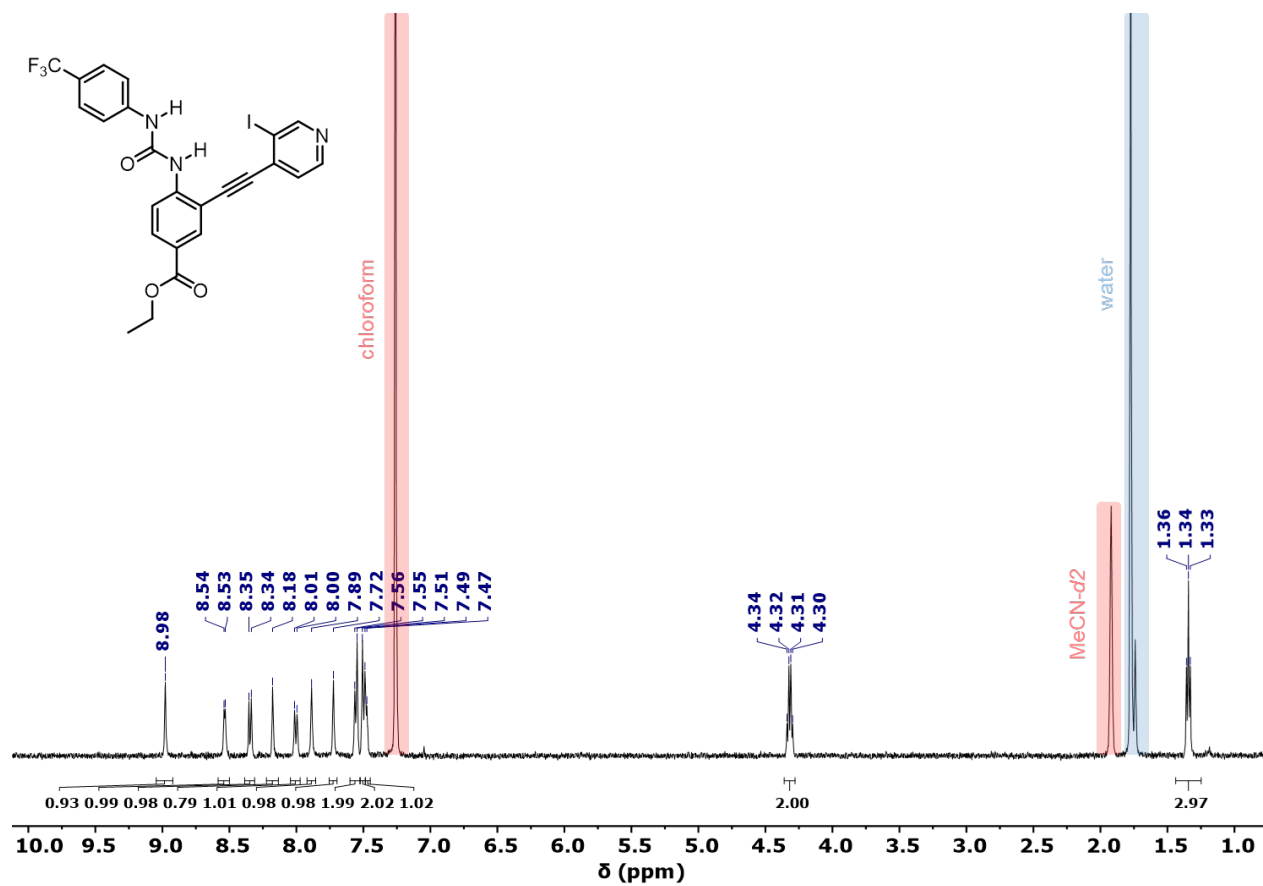


Figure S18. ^1H NMR of **11** (500 MHz, 10% MeCN- d_3 in CDCl_3 , 298 K).

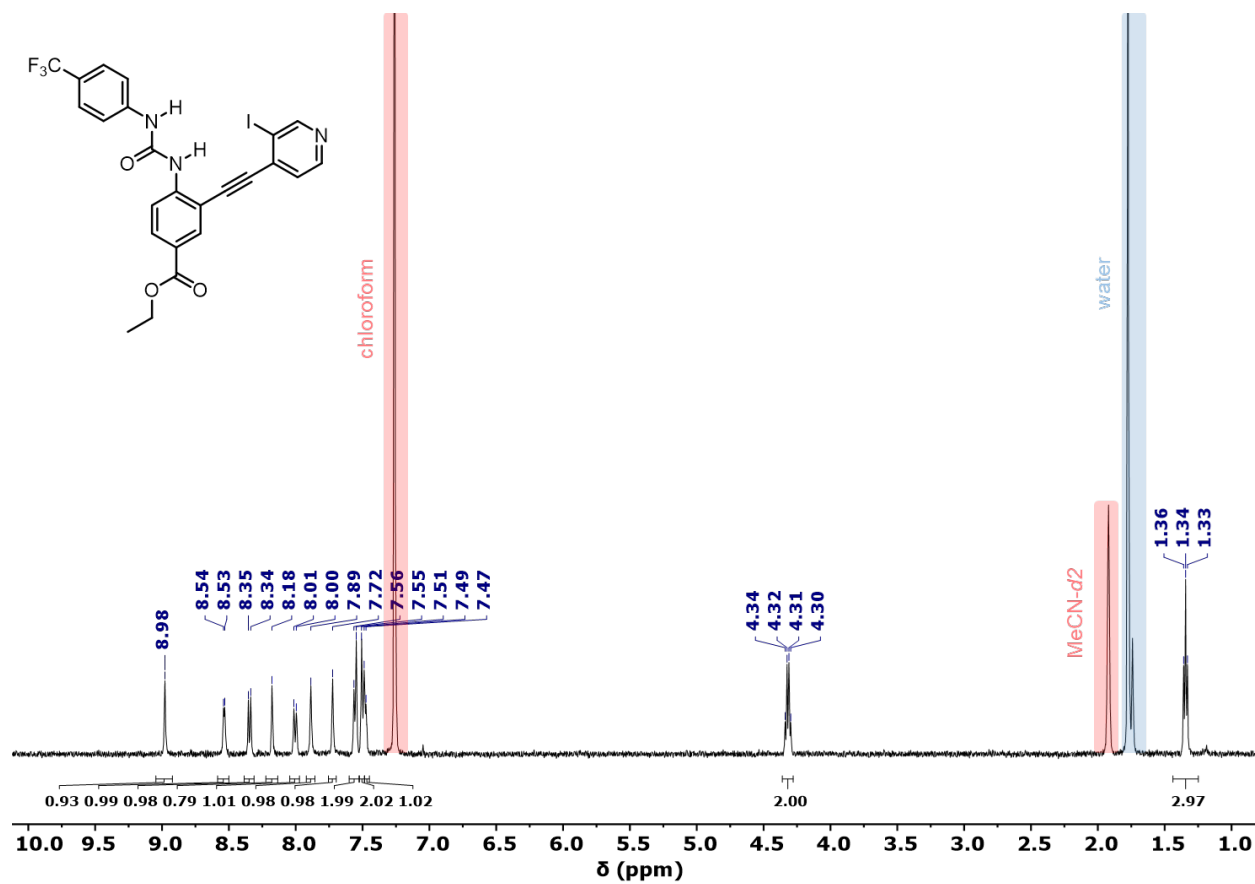


Figure S19. ¹³C{¹H} NMR of **11** (125.8 MHz, 10% MeCN-d₃ in CDCl₃, 298 K).

¹H NMR titrations

All NMR titrations were collected at 25 °C on a Varian Inova-500 instrument (¹H: 500 MHz). All chemical shifts (δ) are reported in ppm relative to residue CHD₂CN (¹H: 1.940 ppm). Before titration with each guest compound (TBACl, TBABr, TBAI) a stock solution of the receptor (After ensuring the receptor was completely dissolved in the solution using ultrasonic waves) was prepared in a 10% MeCN-*d*₃ solution of CDCl₃ (ranging between 1.2 mM – 1.5 mM). The guest complex was dissolved in 2.0 mL of the host solution to maintain a constant host concentration. The guest samples were transferred into septa-sealed vials. The host solution was transferred into three separate NMR tubes (500 μL each). After collecting the ¹H NMR spectrum of the host solution at 25 °C, an aliquot of the TBAX solution was added to the NMR tube using gas-tight syringes, and the ¹H NMR spectrum was collected after each addition. The association constants (*K*_a) were determined using the Nedler-Mead method in the BindFit program for a 1:1 model. Titrations with each guest complex were performed in triplicate and the average of the resulting *K*_a values was reported with 10% error.

Representative ^1H NMR Titration Data

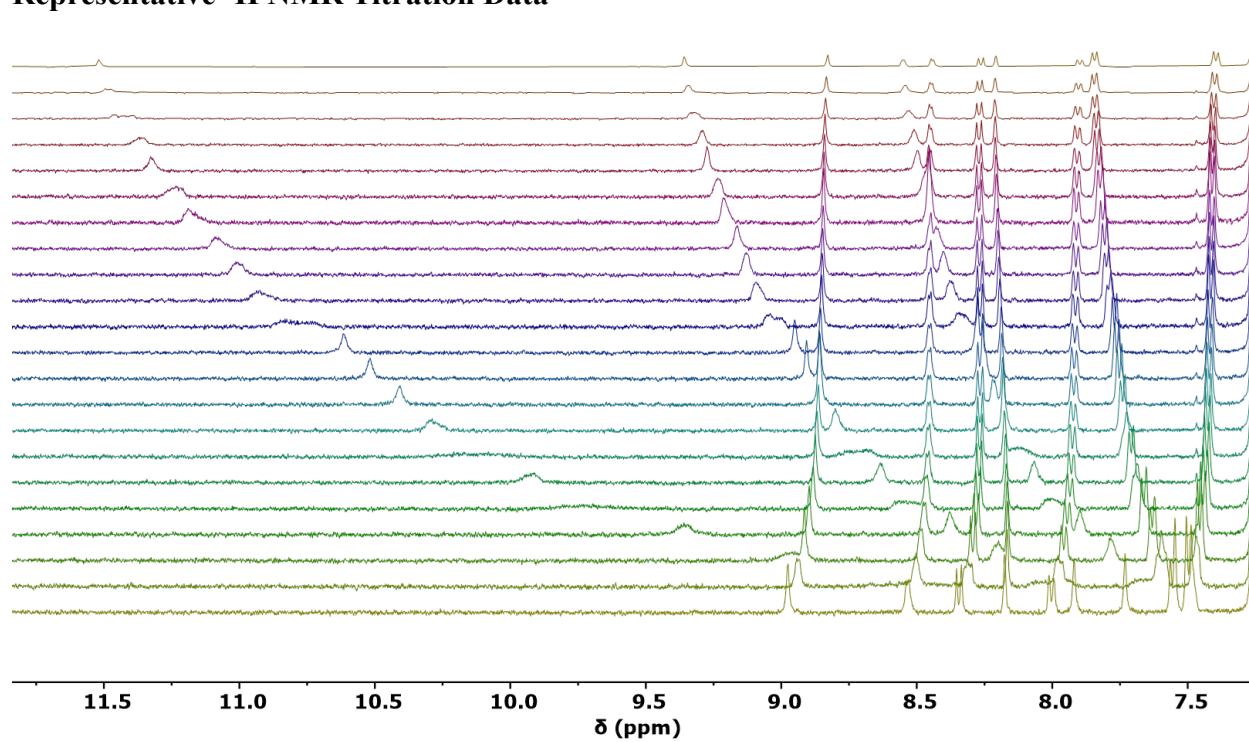


Figure S20. Superimposed titration data of II with Cl^- .

Table S3. Representative titration data of **II** with Cl^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0	0.00135	0.000	7.9193	7.7312	8.9761
2	2	0.16	0.00135	0.000211	8.5026	7.9622	8.9387
3	4	0.31	0.00135	0.000421	8.971	8.2015	8.915
4	6	0.46	0.00135	0.000627	9.3589	8.3775	8.8971
5	8	0.62	0.00135	0.000833	9.7277	8.5459	8.8828
6	10	0.77	0.00135	0.00104	9.9246	8.6339	8.8747
7	12	0.92	0.00135	0.00124	10.1168	8.7116	8.8686
8	14	1.07	0.00135	0.00144	10.2886	8.7995	8.8637
9	16	1.21	0.00135	0.00164	10.4091	8.8589	8.8589
10	18	1.36	0.00135	0.00184	10.5184	8.9066	8.8577
11	20	1.51	0.00135	0.00203	10.6144	8.9501	8.8549
12	25	1.87	0.00135	0.00252	10.8049	9.0352	8.8509
13	30	2.22	0.00135	0.00299	10.927	9.0909	8.8486
14	35	2.56	0.00135	0.00346	11.0061	9.129	8.8471
15	40	2.90	0.00135	0.00392	11.0825	9.1629	8.8452
16	50	3.56	0.00135	0.00481	11.1852	9.2093	8.8438
17	60	4.20	0.00135	0.00567	11.2351	9.2333	8.8422
18	80	5.40	0.00135	0.00730	11.3237	9.2737	8.8408
19	100	6.53	0.00135	0.00881	11.366	9.2918	8.8388
20	150	9.04	0.00135	0.0122	11.4289	9.324	8.8367
21	200	11.19	0.00135	0.0151	11.4817	9.3433	8.8336
22	300	14.69	0.00135	0.0198	11.5182	9.3576	8.8286

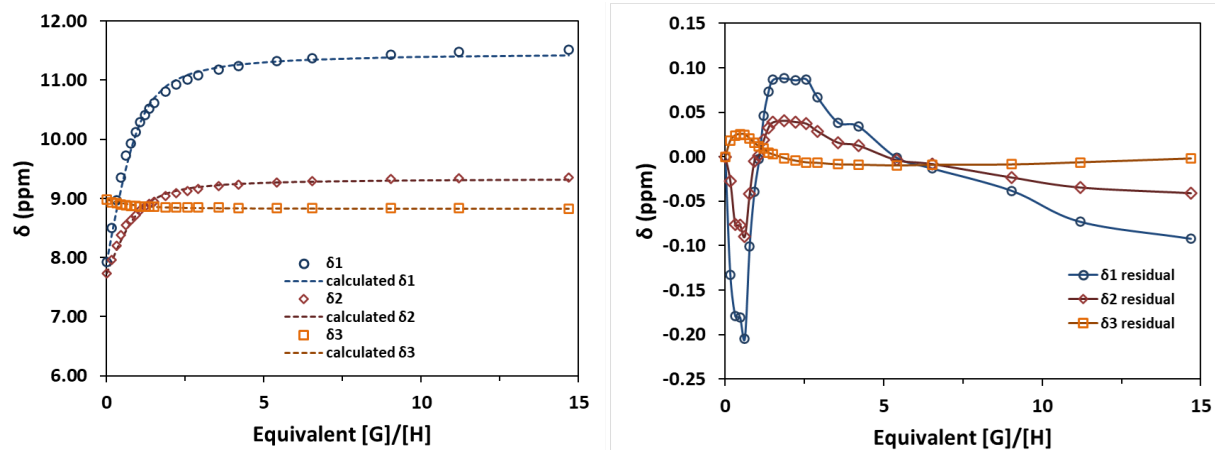


Figure S21. Representative binding isotherm and residuals for **II** titration with Cl^- in 10% MeCN- d_3 solution of CDCl_3 .

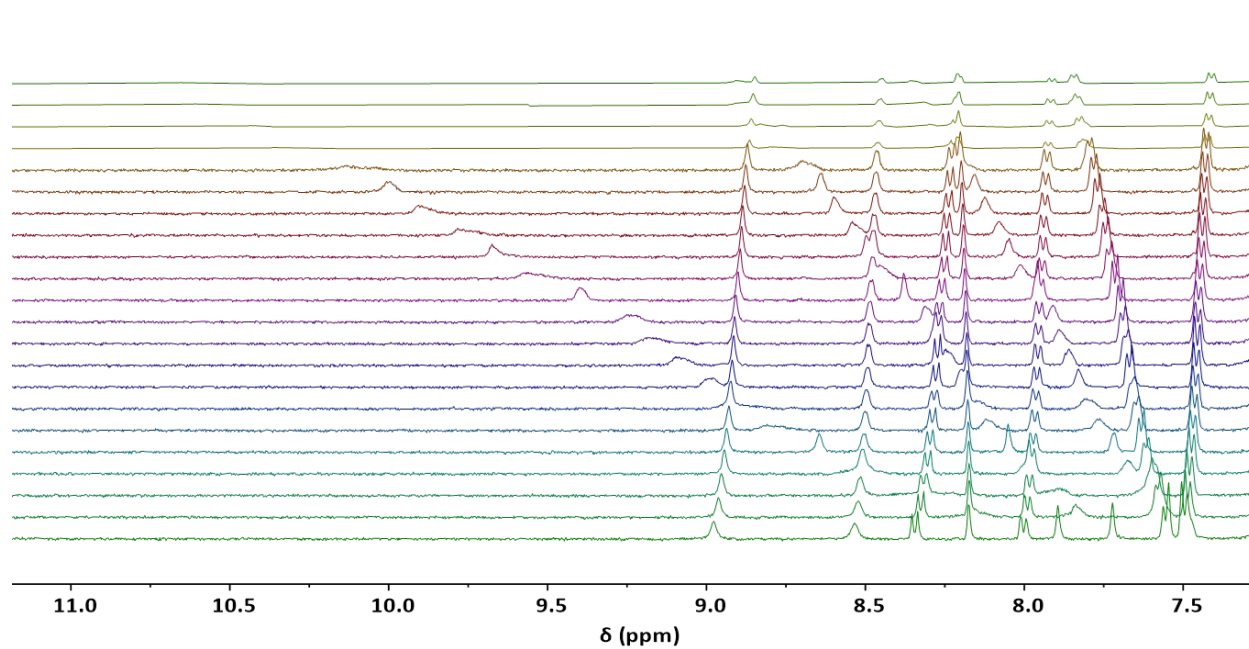


Figure S22. Superimposed titration data of **II** with Br^- .

Table S4. Representative titration data of **II** with Br^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0.00	0.00135	0.000000	7.894	7.7222	8.9774
2	2	0.15	0.00135	0.000198	8.1538	7.8358	8.9621
3	4	0.29	0.00135	0.000395	8.2983	7.8966	8.9529
4	6	0.44	0.00135	0.000590	8.5088	7.9948	8.9435
5	8	0.58	0.00135	0.000784	8.6449	8.0505	8.9365
6	10	0.72	0.00135	0.000976	8.7912	8.1144	8.9298
7	12	0.86	0.00135	0.00117	8.8846	8.1472	8.9247
8	14	1.00	0.00135	0.00136	8.9867	8.199	8.9191
9	16	1.14	0.00135	0.00154	9.0817	8.2404	8.9147
10	18	1.28	0.00135	0.00173	9.1674	8.2793	8.9117
11	20	1.42	0.00135	0.00191	9.2363	8.3086	8.9081
12	25	1.76	0.00135	0.00237	9.395	8.3781	8.9011
13	30	2.09	0.00135	0.00282	9.5473	8.448	8.8943
14	35	2.41	0.00135	0.00326	9.6697	8.498	8.8894
15	40	2.73	0.00135	0.00369	9.757	8.5348	8.8861
16	50	3.35	0.00135	0.00453	9.8942	8.5954	8.88
17	60	3.95	0.00135	0.00533	10.0001	8.6401	8.8762
18	80	5.09	0.00135	0.00687	10.1112	8.6906	8.8711
19	100	6.15	0.00135	0.00830	10.2887	8.7761	8.8647
20	150	8.51	0.00135	0.01149	10.4267	8.7947	8.859
21	200	10.54	0.00135	0.01423	10.5462	8.8954	8.8531
22	300	13.83	0.00135	0.0187	10.6166	8.9072	8.848

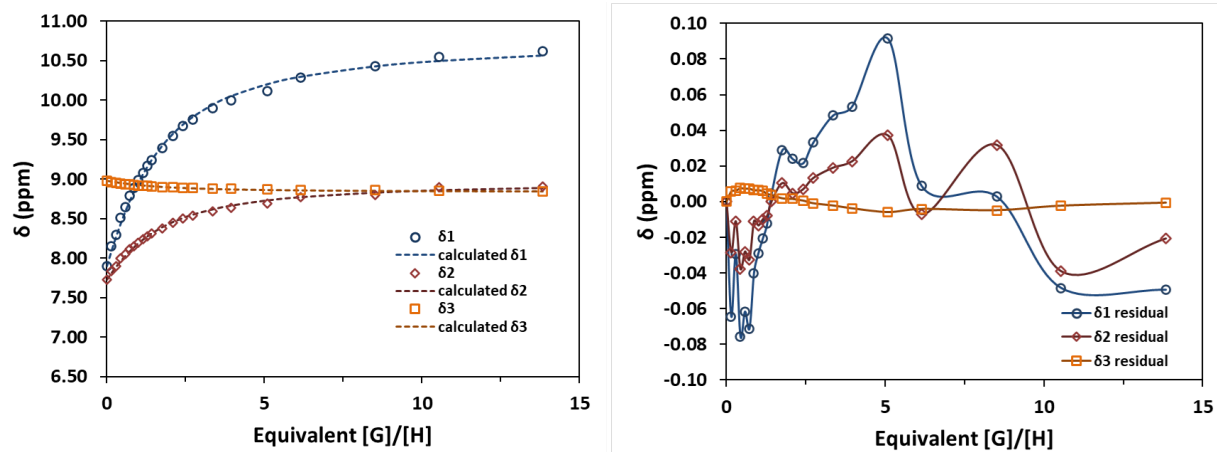


Figure S23. Representative binding isotherm and residuals for **1I** titration with Br^- in 10% MeCN- d_3 solution of CDCl_3 .

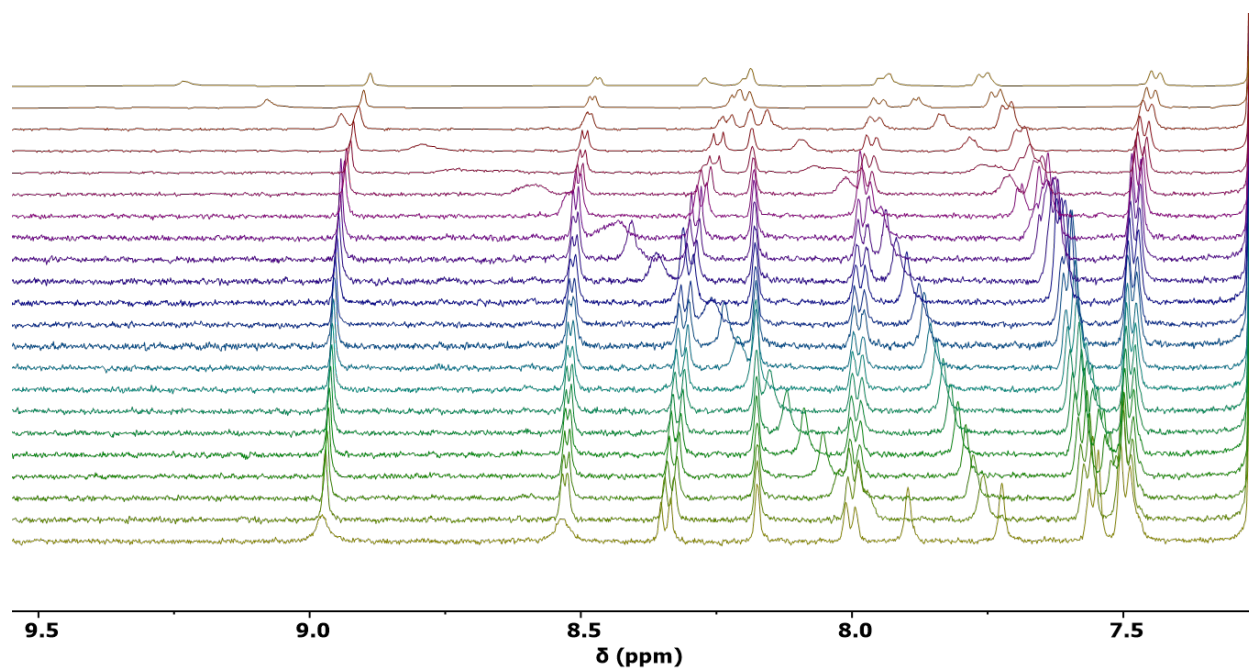


Figure S24. Superimposed titration data of **1I** with I^- .

Table S5. Representative titration data of **II** with I^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0.00	0.00145	0.000000	7.897	7.7239	8.9772
2	2	0.16	0.00145	0.000234	7.979	7.7584	8.9713
3	4	0.32	0.00145	0.000465	8.0235	7.7763	8.9678
4	6	0.48	0.00145	0.000695	8.0535	7.7905	8.9656
5	8	0.64	0.00145	0.000923	8.0884	7.8052	8.964
6	10	0.79	0.00145	0.00115	8.1208	7.8186	8.9617
7	12	0.95	0.00145	0.00137	8.1521	7.8318	8.9597
8	14	1.10	0.00145	0.00160	8.1778	7.844	8.9582
9	16	1.25	0.00145	0.00182	8.2098	7.8555	8.956
10	18	1.40	0.00145	0.00204	8.2348	7.8664	8.9548
11	20	1.55	0.00145	0.00225	8.2591	7.8767	8.9532
12	25	1.92	0.00145	0.00279	8.2924	7.8989	8.9502
13	30	2.29	0.00145	0.00332	8.3602	7.9178	8.9465
14	35	2.64	0.00145	0.00383	8.4069	7.9375	8.9442
15	40	2.99	0.00145	0.00434	8.4355	7.948	8.9423
16	50	3.67	0.00145	0.00533	8.5247	7.9665	8.9362
17	60	4.33	0.00145	0.00628	8.5882	8.0121	8.9319
18	80	5.58	0.00145	0.00808	8.7189	8.0586	8.9247
19	100	6.74	0.00145	0.00977	8.7883	8.0933	8.9193
20	150	9.33	0.00145	0.0135	8.9416	8.1877	8.9103
21	200	11.55	0.00145	0.0167	9.0776	8.2223	8.9006
22	300	15.16	0.00145	0.0220	9.2299	8.2711	8.8883

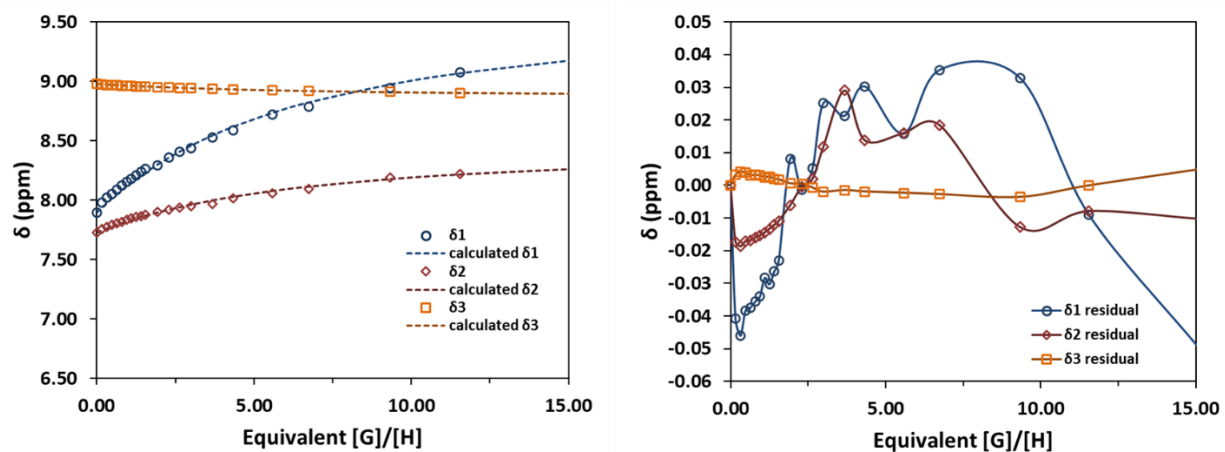


Figure S25. Representative binding isotherm and residuals for **1I** titration with I^- in 10% MeCN- d_3 solution of $CDCl_3$.

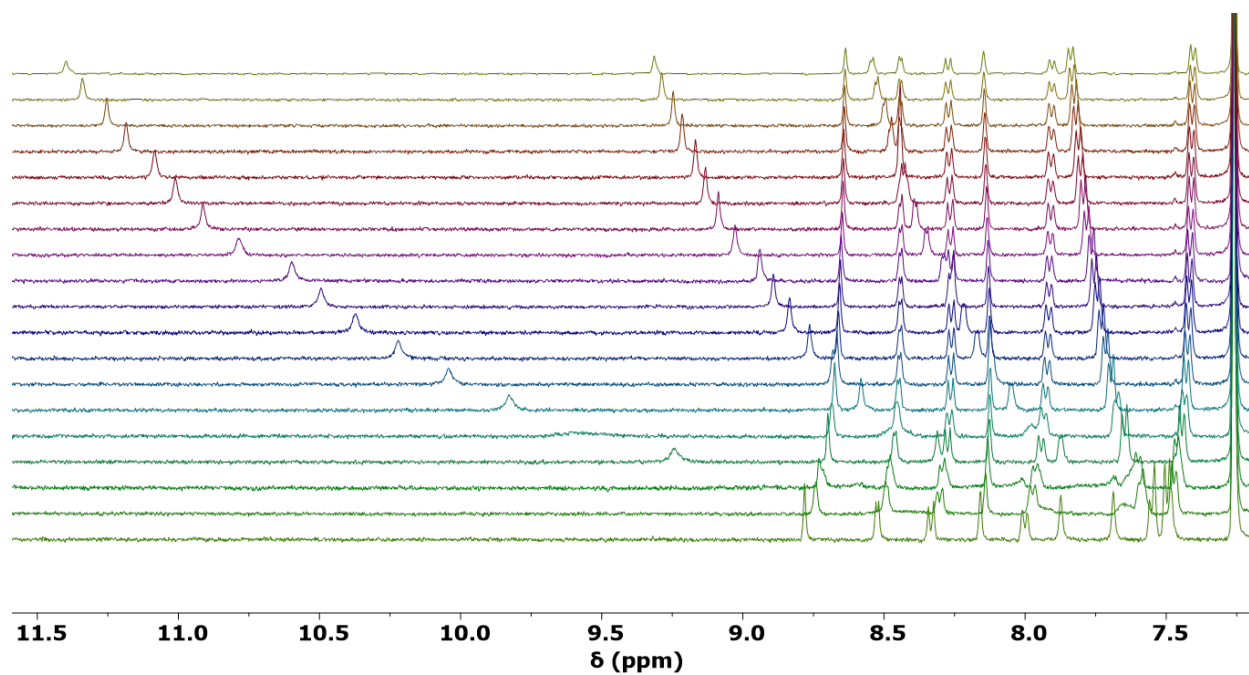


Figure S26. Superimposed titration data of **1Br** with Cl^- .

Table S6. Representative titration data of **1Br** with Cl^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	δ1 (ppm)	δ2 (ppm)	δ3 (ppm)
1	0	0	0.00120	0.000000	7.8773	7.6873	8.7811
2	2	0.19	0.00120	0.000230	8.4283	7.9254	8.7419
3	4	0.38	0.00120	0.000458	8.7153	8.1327	8.7296
4	6	0.57	0.00120	0.000685	9.2419	8.3108	8.6986
5	8	0.76	0.00120	0.000909	9.5716	8.4534	8.6814
6	10	0.94	0.00120	0.00113	9.8271	8.5812	8.6744
7	12	1.13	0.00120	0.00135	10.0426	8.6818	8.667
8	14	1.31	0.00120	0.00157	10.2209	8.7637	8.6612
9	16	1.49	0.00120	0.00179	10.3729	8.8346	8.6573
10	18	1.67	0.00120	0.00201	10.4947	8.8918	8.6543
11	20	1.85	0.00120	0.00222	10.5971	8.9401	8.6517
12	25	2.29	0.00120	0.00275	10.7851	9.0273	8.6476
13	30	2.72	0.00120	0.00327	10.9126	9.0869	8.6445
14	35	3.15	0.00120	0.00378	11.0106	9.1329	8.6436
15	40	3.56	0.00120	0.00428	11.0841	9.1678	8.6422
16	50	4.38	0.00120	0.00525	11.1852	9.2147	8.6403
17	60	5.16	0.00120	0.00619	11.2538	9.2468	8.6392
18	80	6.64	0.00120	0.00797	11.3394	9.2873	8.6375
19	100	8.02	0.00120	0.00962	11.3974	9.314	8.6362

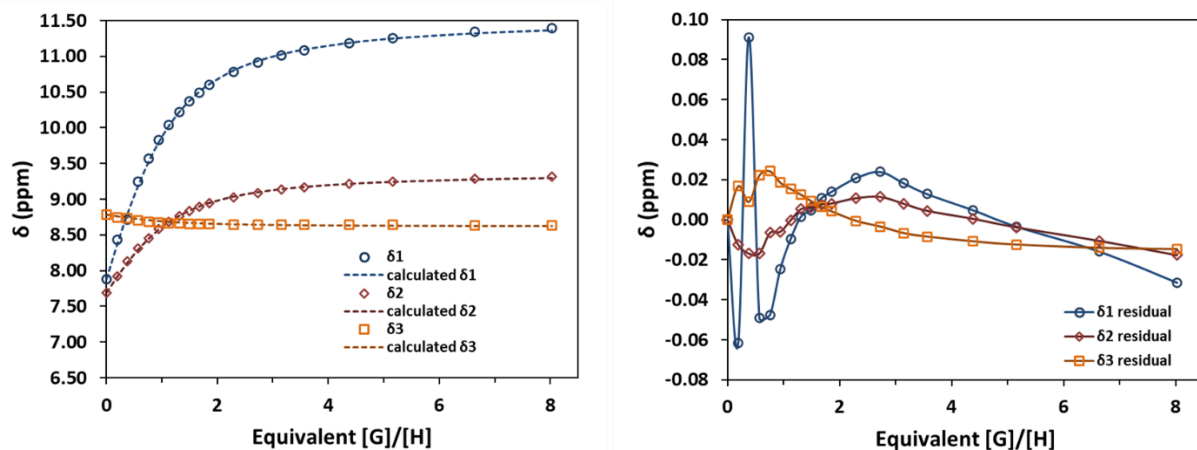


Figure S27. Representative binding isotherm and residuals for 1Br titration with Cl^- in 10% MeCN- d_3 solution of CDCl_3 .

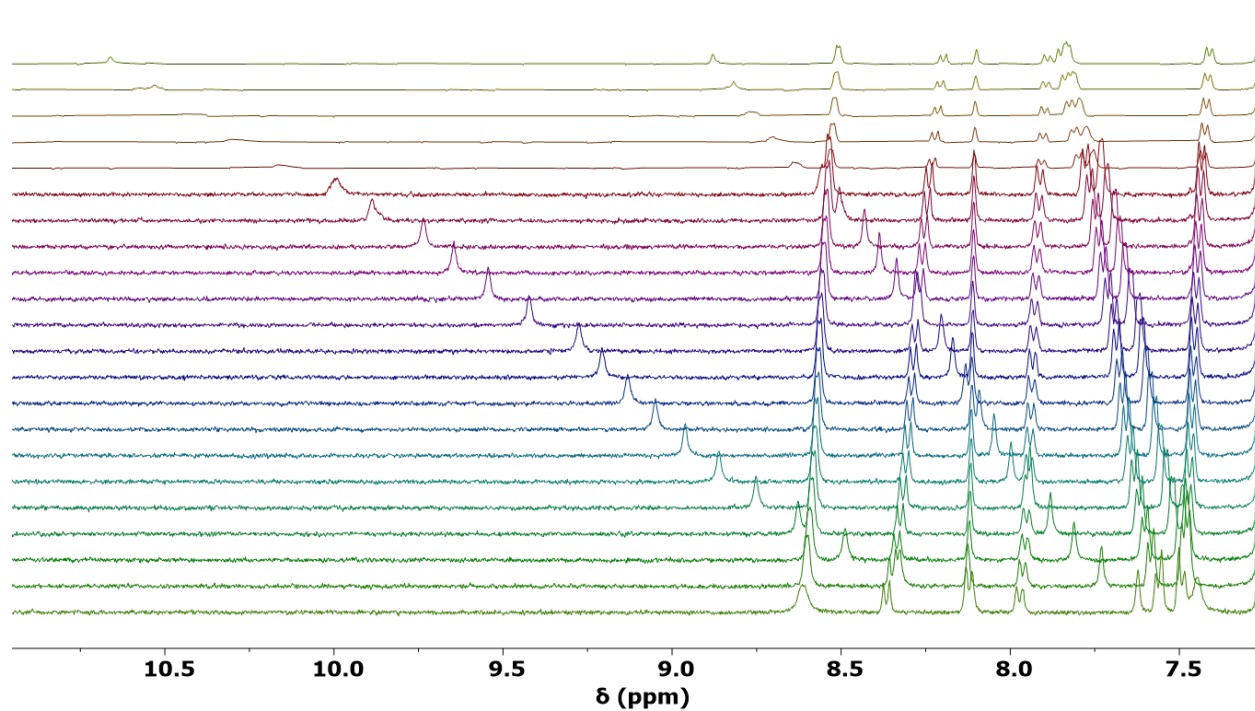


Figure S28. Superimposed titration data of 1Br with Br^- .

Table S7. Representative titration data of **1Br** with Br^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0.00	0.00128	0.000000	7.8805	7.6909	8.7824
2	2	0.15	0.00128	0.000189	8.1207	7.7987	8.7681
3	4	0.29	0.00128	0.000376	8.3015	7.8801	8.7568
4	6	0.44	0.00128	0.000562	8.4591	7.9497	8.7488
5	8	0.58	0.00128	0.000746	8.5973	8.0113	8.7414
6	10	0.73	0.00128	0.000929	8.7182	8.0657	8.7356
7	12	0.87	0.00128	0.00111	8.8278	8.1148	8.7304
8	14	1.01	0.00128	0.00129	8.926	8.1527	8.7251
9	16	1.15	0.00128	0.00147	9.0156	8.1986	8.721
10	18	1.29	0.00128	0.00165	9.0993	8.2363	8.717
11	20	1.42	0.00128	0.00182	9.1734	8.2694	8.7136
12	25	1.76	0.00128	0.00226	9.3349	8.3419	8.7061
13	30	2.10	0.00128	0.00268	9.4651	8.4007	8.7004
14	35	2.42	0.00128	0.00310	9.5782	8.4513	8.6954
15	40	2.74	0.00128	0.00351	9.6716	8.4928	8.6915
16	50	3.37	0.00128	0.00431	9.8175	8.5579	8.6852
17	60	3.97	0.00128	0.00508	9.9313	8.6095	8.6808
18	80	5.11	0.00128	0.00654	10.0982	8.6842	8.6736
19	100	6.17	0.00128	0.00790	10.2101	8.7338	8.668
20	150	8.54	0.00128	0.0109	10.4063	8.8213	8.6596
21	200	10.58	0.00128	0.0135	10.481	8.8548	8.656

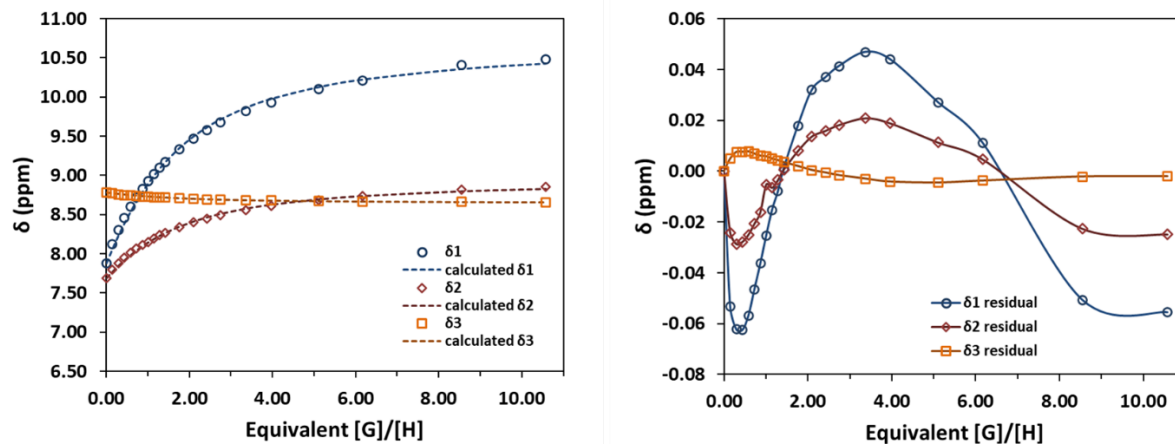


Figure S29. Representative binding isotherm and residuals for 1Br titration with Br^- in 10% MeCN- d_3 solution of CDCl_3 .

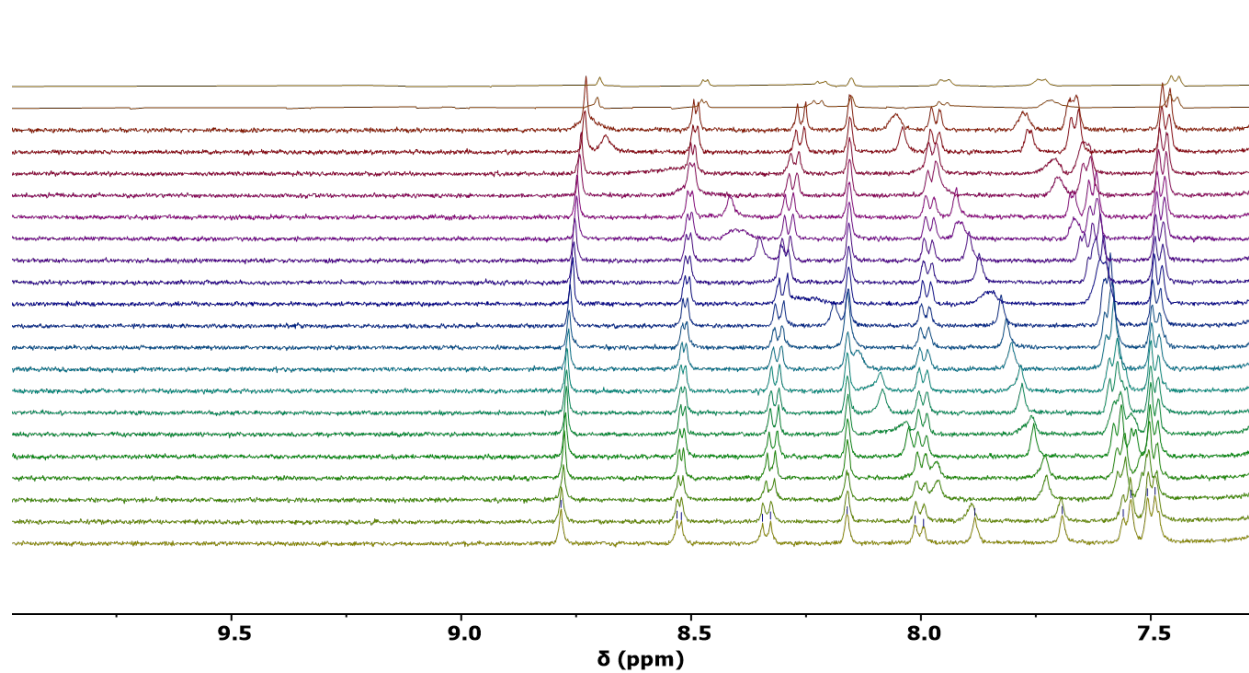


Figure S30. Superimposed titration data of 1Br with I^- .

Table S8. Representative titration data of **1Br** with I^- .

	V guest (μ L)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0.00	0.00150	0.00000	7.6925	7.8827	8.7831
2	2	0.16	0.00150	0.00024	7.6958	7.8905	8.7817
3	4	0.32	0.00150	0.00049	7.7278	7.9642	8.7778
4	6	0.48	0.00150	0.00073	7.7306	7.9668	8.7761
5	8	0.64	0.00150	0.00097	7.7544	8.0267	8.7733
6	10	0.80	0.00150	0.00120	7.7596	8.0373	8.7713
7	12	0.96	0.00150	0.00144	7.7803	8.0836	8.7708
8	14	1.11	0.00150	0.00167	7.7832	8.0887	8.7701
9	16	1.27	0.00150	0.00190	7.8033	8.1374	8.7672
10	18	1.42	0.00150	0.00213	7.8139	8.1599	8.7654
11	20	1.57	0.00150	0.00236	7.8254	8.1888	8.7632
12	25	1.95	0.00150	0.00292	7.8521	8.2914	8.7583
13	30	2.31	0.00150	0.00347	7.8732	8.3036	8.7554
14	35	2.67	0.00150	0.00401	7.8955	8.3506	8.753
15	40	3.03	0.00150	0.00454	7.9163	8.3983	8.749
16	45	3.38	0.00150	0.00506	7.9235	8.415	8.7482
17	55	4.05	0.00150	0.00608	7.9667	8.5003	8.7423
18	65	4.70	0.00150	0.00705	7.9833	8.5013	8.7397
19	85	5.94	0.00150	0.00891	8.0399	8.6857	8.7315
20	100	6.81	0.00150	0.01022	8.0552	8.7288	8.7288
21	200	11.68	0.00150	0.01752	8.1509	9.0946	8.7041
22	300	15.33	0.00150	0.02300	8.2623	9.2028	8.6985
23	400	18.17	0.00150	0.02725	8.3277	9.3718	8.6863

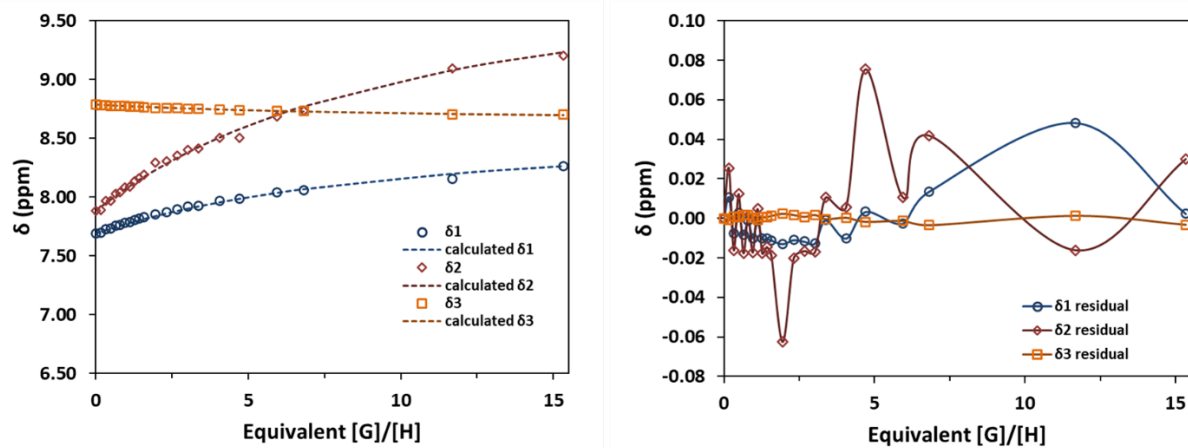


Figure S31. Representative binding isotherm and residuals for 1Br titration with I^- in 10% MeCN- d_3 solution of $CDCl_3$.

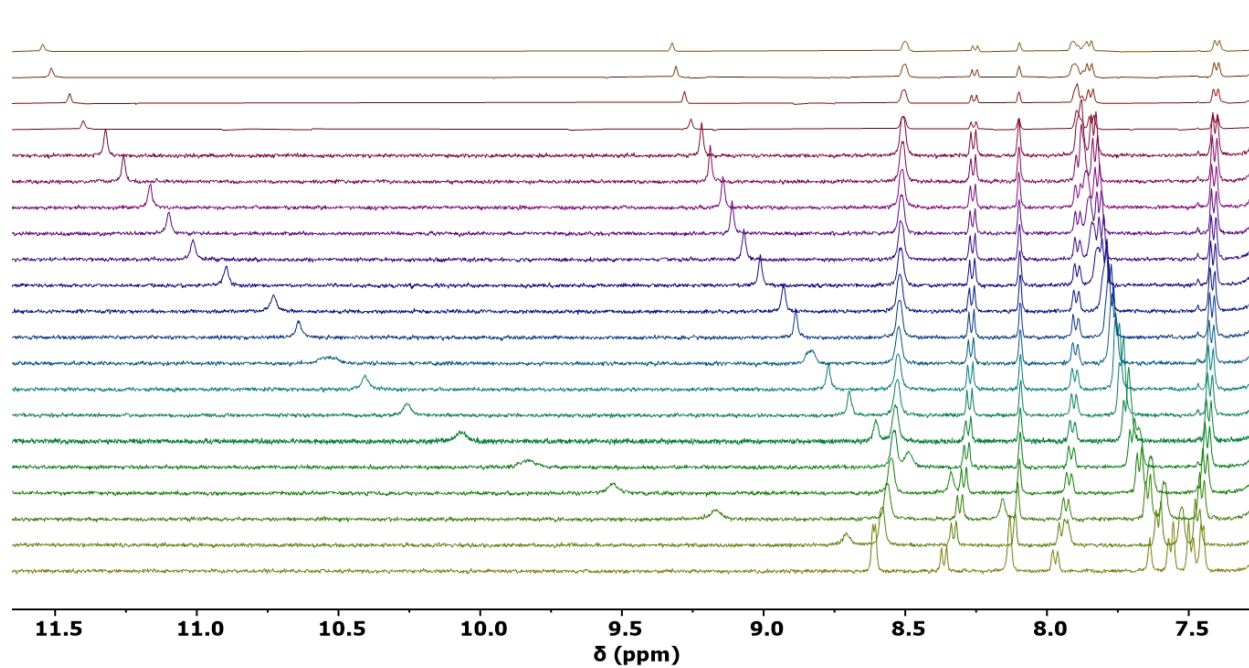


Figure S32. Superimposed titration data of $1H$ with Cl^- .

Table S9. Representative titration data of **1H** with Cl^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0	0.00128	0.000000	8.1317	7.6372	8.6099
2	2	0.15	0.00128	0.000189	8.7079	7.9574	8.5819
3	4	0.29	0.00128	0.000376	9.1682	8.1566	8.5634
4	6	0.44	0.00128	0.000561	9.5327	8.3384	8.5496
5	8	0.58	0.00128	0.000745	9.8336	8.4878	8.5401
6	10	0.73	0.00128	0.000928	10.0665	8.6047	8.5347
7	12	0.87	0.00128	0.00111	10.2583	8.6973	8.5287
8	14	1.01	0.00128	0.00129	10.4066	8.7708	8.5254
9	16	1.15	0.00128	0.00147	10.5348	8.8328	8.5235
10	18	1.29	0.00128	0.00164	10.6406	8.8867	8.5206
11	20	1.42	0.00128	0.00182	10.7288	8.9297	8.5192
12	25	1.76	0.00128	0.00225	10.8969	9.0119	8.5164
13	30	2.09	0.00128	0.00268	11.0135	9.0694	8.5145
14	35	2.42	0.00128	0.00309	11.0989	9.111	8.5133
15	40	2.74	0.00128	0.00350	11.1648	9.1431	8.5123
16	50	3.36	0.00128	0.00430	11.2587	9.1885	8.5076
17	60	3.96	0.00128	0.00507	11.322	9.2187	8.5087
18	80	5.10	0.00128	0.00653	11.4006	9.2566	8.507
19	100	6.16	0.00128	0.00789	11.4489	9.2792	8.5069
20	150	8.54	0.00128	0.0109	11.5134	9.3095	8.503
21	200	10.57	0.00128	0.0135	11.5437	9.3229	8.5009

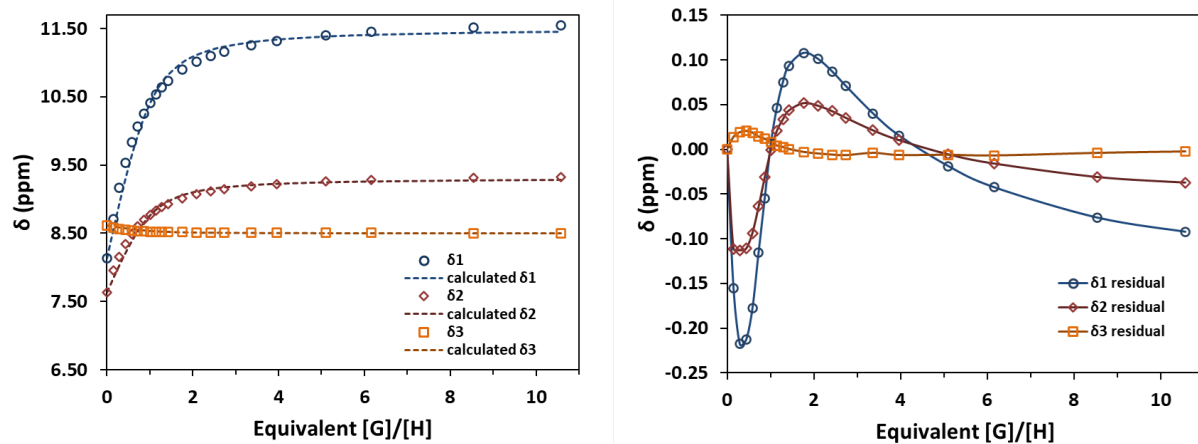


Figure S33. Representative binding isotherm and residuals for $1H$ titration with Cl^- in 10% MeCN- d_3 solution of CCl_3 .

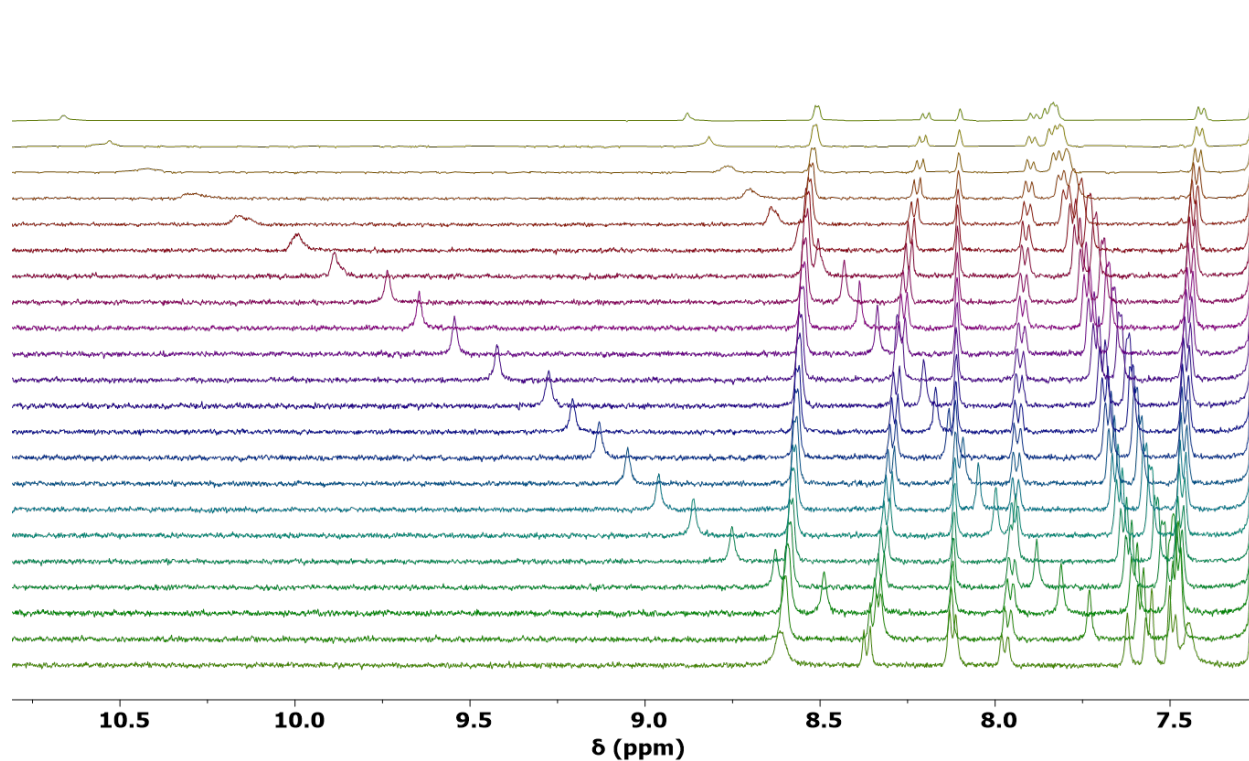


Figure S34. Superimposed titration data of $1H$ with Br^- .

Table S10. Representative titration data of **1H** with Br^- .

	V guest (μL)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0	0.00132	0.000000	8.1306	7.6223	8.6133
2	2	0.14	0.00132	0.000185	8.3262	7.7304	8.6015
3	4	0.28	0.00132	0.000368	8.4884	7.8112	8.5931
4	6	0.42	0.00132	0.000549	8.6273	7.881	8.587
5	8	0.55	0.00132	0.000730	8.7516	7.9417	8.5818
6	10	0.69	0.00132	0.000909	8.8619	7.9979	8.5775
7	12	0.82	0.00132	0.00108	8.9611	8.0476	8.5737
8	14	0.96	0.00132	0.00126	9.0497	8.0916	8.5696
9	16	1.09	0.00132	0.00144	9.1311	8.1326	8.5673
10	18	1.22	0.00132	0.00161	9.207	8.1699	8.5637
11	20	1.35	0.00132	0.00178	9.2754	8.2035	8.5603
12	25	1.67	0.00132	0.00221	9.4225	8.2654	8.5569
13	30	1.99	0.00132	0.00262	9.5439	8.3362	8.5509
14	35	2.30	0.00132	0.00303	9.6453	8.3867	8.5453
15	40	2.60	0.00132	0.00343	9.7358	8.4309	8.5444
16	50	3.19	0.00132	0.00421	9.8869	8.5051	8.5385
17	60	3.76	0.00132	0.00496	9.9946	8.5598	8.5355
18	80	4.84	0.00132	0.00639	10.1548	8.6351	8.5294
19	100	5.85	0.00132	0.00772	10.288	8.6995	8.5241
20	150	8.10	0.00132	0.0107	10.4203	8.7641	8.5187
21	200	10.03	0.00132	0.0132	10.5309	8.8178	8.5144

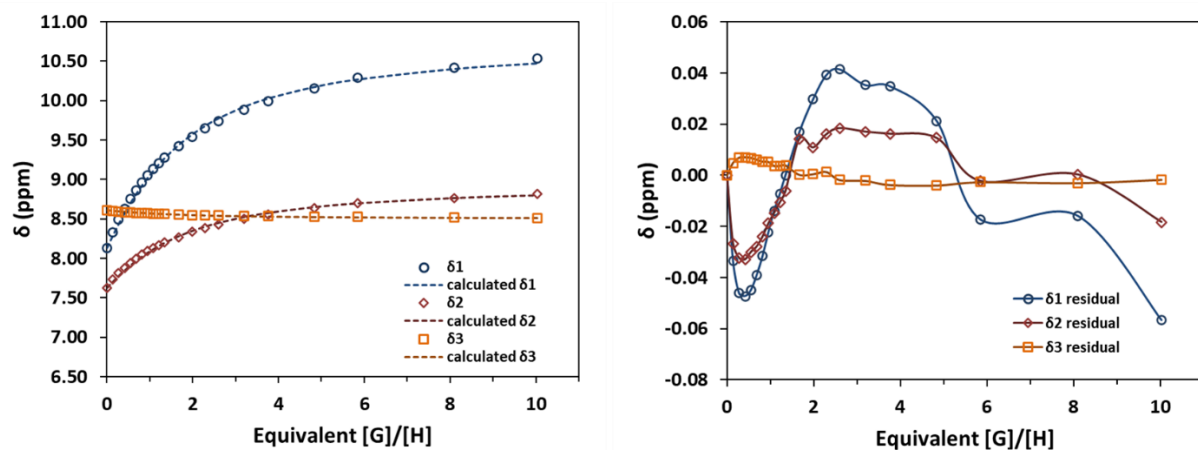


Figure S35. Representative binding isotherm and residuals for $1H$ titration with Br^- in 10% MeCN- d_3 solution of $CDCl_3$.

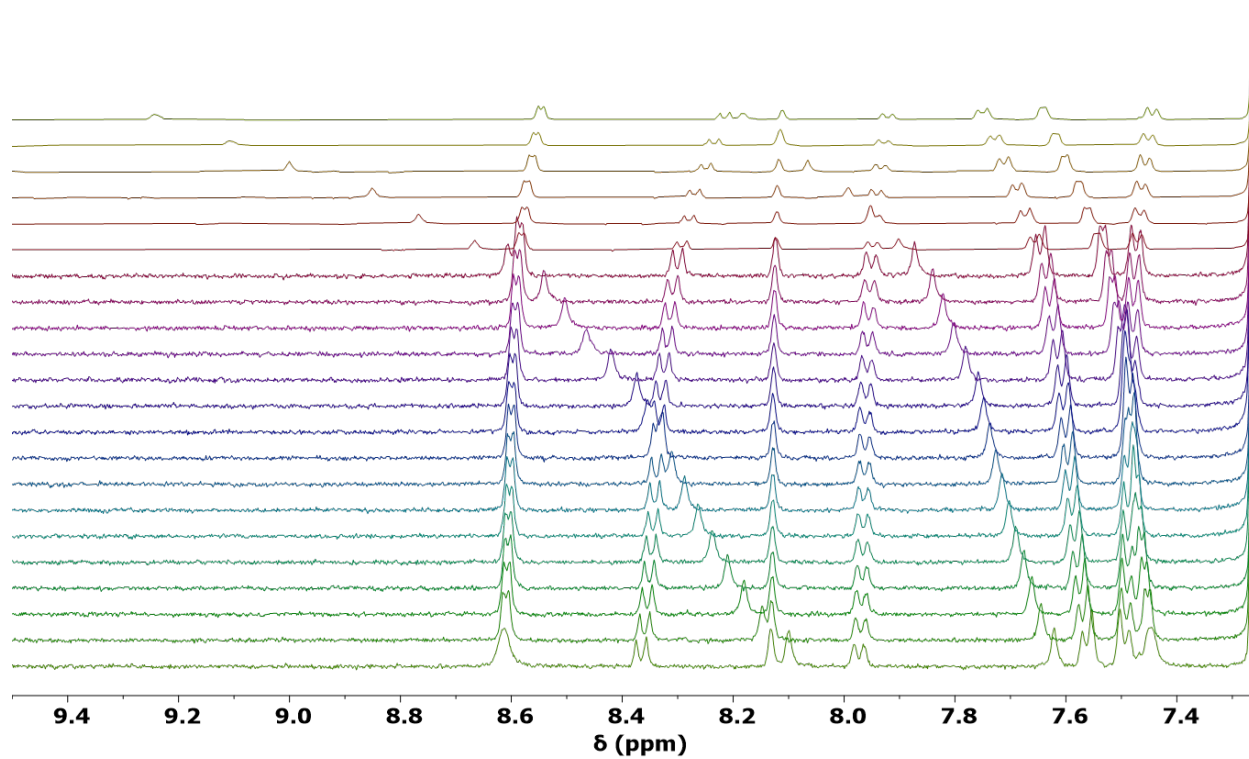


Figure S36. Superimposed titration data of $1H$ with I^- .

Table S11. Representative titration data of *1H* with I^- .

	V guest (μ L)	Equiv. guest	[host] (M)	[guest] (M)	$\delta 1$ (ppm)	$\delta 2$ (ppm)	$\delta 3$ (ppm)
1	0	0	0.00119	0.000000	8.0998	7.6212	8.6133
2	2	0.19	0.00119	0.000222	8.1477	7.645	8.6102
3	4	0.37	0.00119	0.000443	8.1806	7.6615	8.6077
4	6	0.56	0.00119	0.000661	8.2102	7.6759	8.6055
5	8	0.74	0.00119	0.000878	8.2376	7.6901	8.6061
6	10	0.92	0.00119	0.00109	8.2629	7.7026	8.604
7	12	1.10	0.00119	0.00131	8.2877	7.7151	8.6024
8	14	1.28	0.00119	0.00152	8.3109	7.7263	8.6011
9	16	1.45	0.00119	0.00173	8.3275	7.7373	8.6004
10	18	1.63	0.00119	0.00194	8.3543	7.7483	8.5994
11	20	1.80	0.00119	0.00214	8.3739	7.7578	8.5973
12	25	2.23	0.00119	0.00266	8.4203	7.7806	8.5955
13	30	2.65	0.00119	0.00316	8.4643	7.8021	8.5932
14	35	3.07	0.00119	0.00365	8.5034	7.8216	8.591
15	40	3.47	0.00119	0.00413	8.5412	7.8405	8.5887
16	50	4.26	0.00119	0.00507	8.6071	7.8727	8.5854
17	60	5.02	0.00119	0.005975	8.6658	7.9018	8.5813
18	80	6.46	0.00119	0.00769	8.767	7.9524	8.5757
19	100	7.81	0.00119	0.00929	8.8507	7.9925	8.5719
20	150	10.82	0.00119	0.0129	9.0002	8.0655	8.5621
21	200	13.39	0.00119	0.0159	9.106	8.115	8.5554

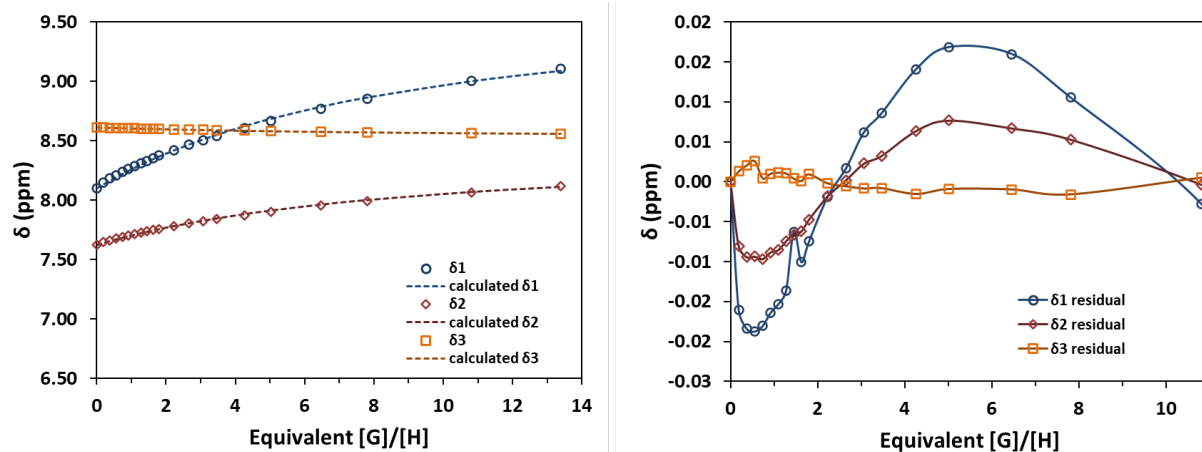


Figure S37. Representative binding isotherm and residuals for $1H$ titration with I^- in 10% MeCN- d_3 solution of $CDCl_3$.

S1.1 ATR FTIR

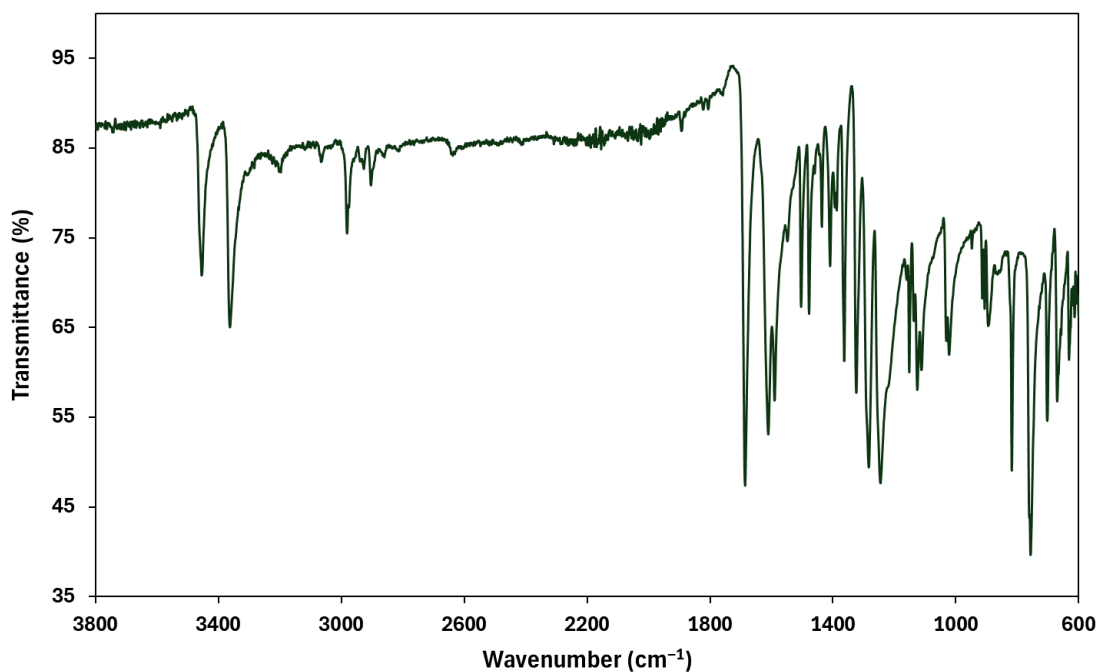


Figure S38. Di-ATR FTIR of **5**.

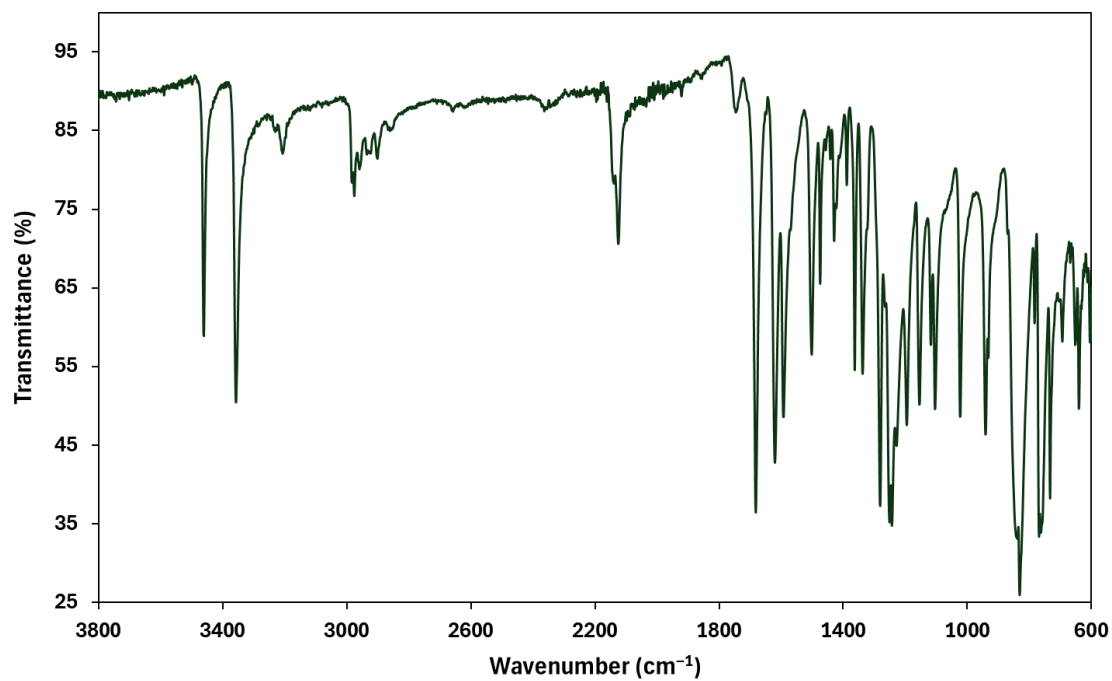


Figure S39. Di-ATR FTIR of 4.

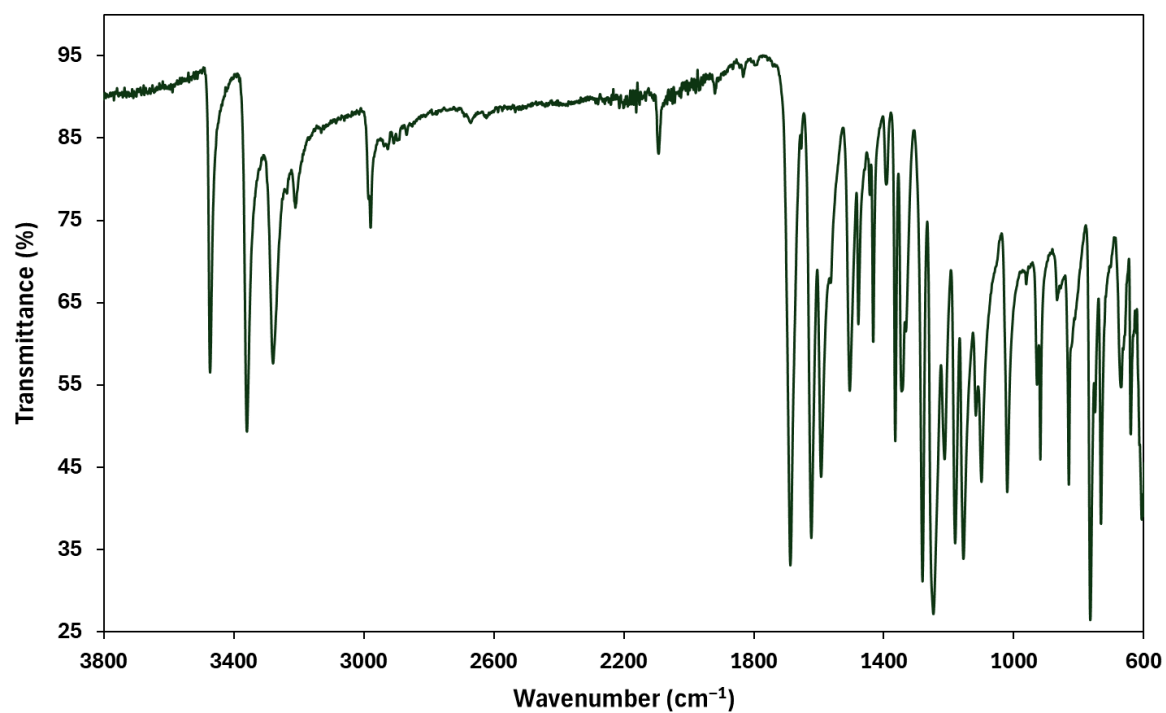


Figure S40. Di-ATR FTIR of 3.

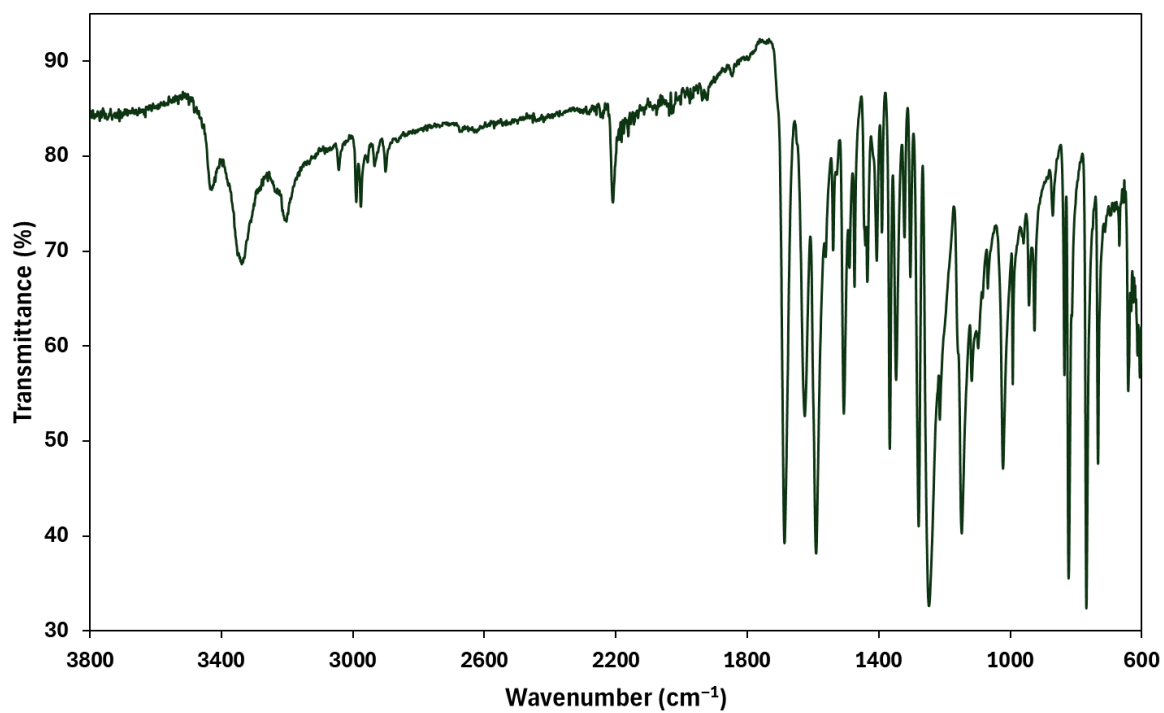


Figure S41. Di-ATR FTIR of 2H.

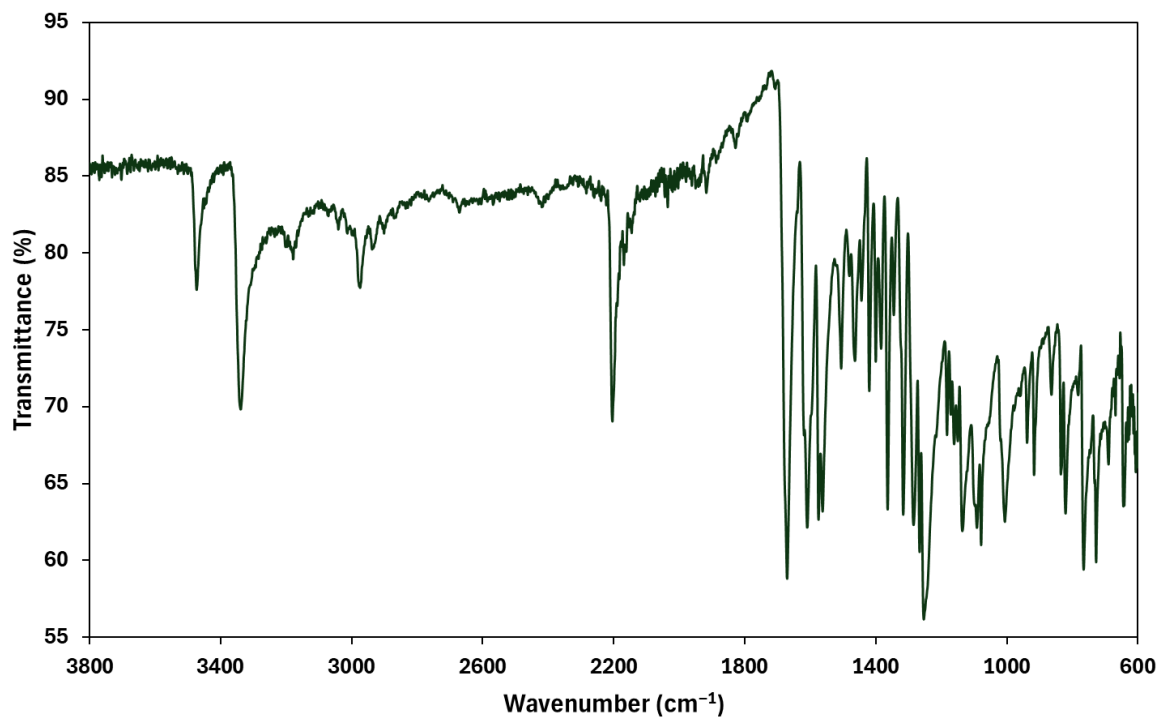


Figure S42. Di-ATR FTIR of 2Br.

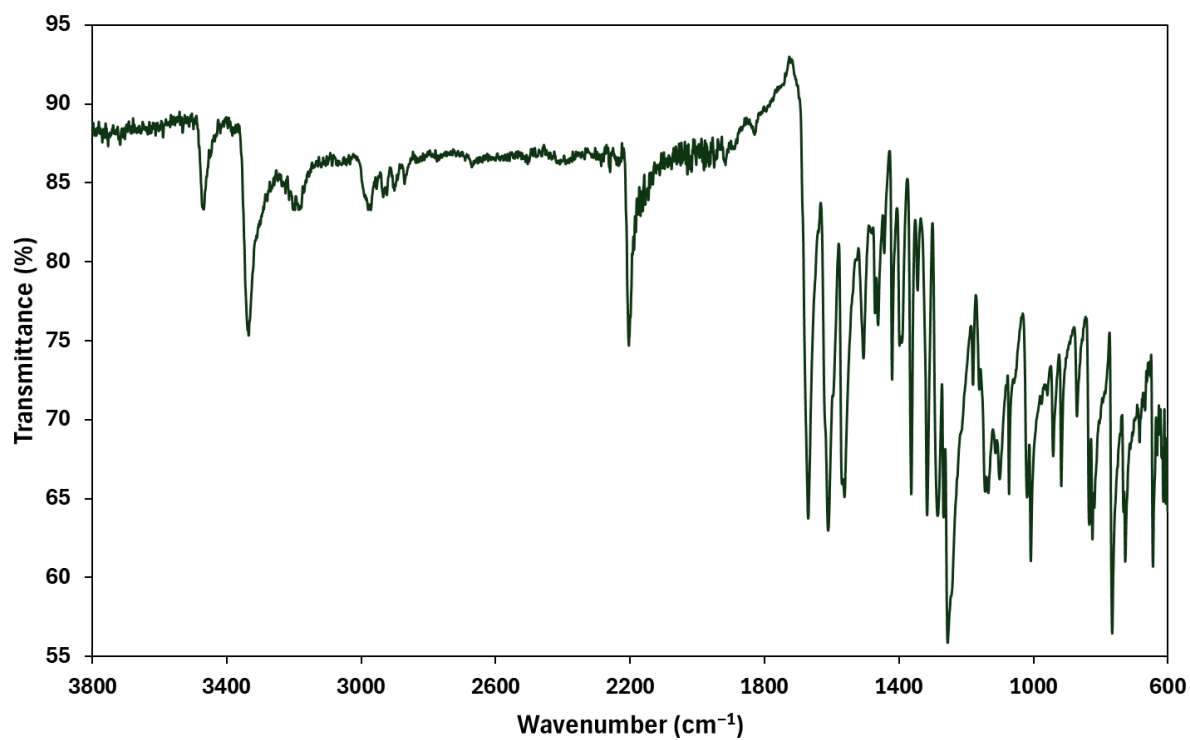


Figure S43. Di-ATR FTIR of 2I.

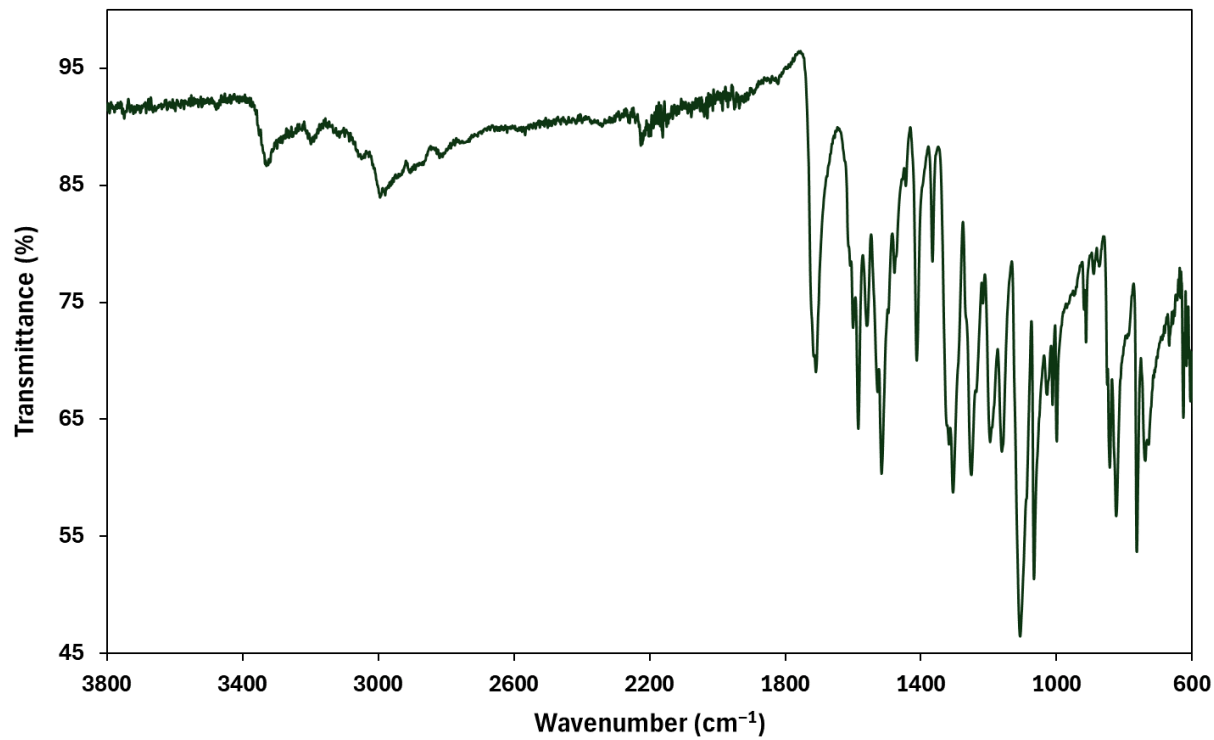


Figure S44. Di-ATR FTIR of 1H.

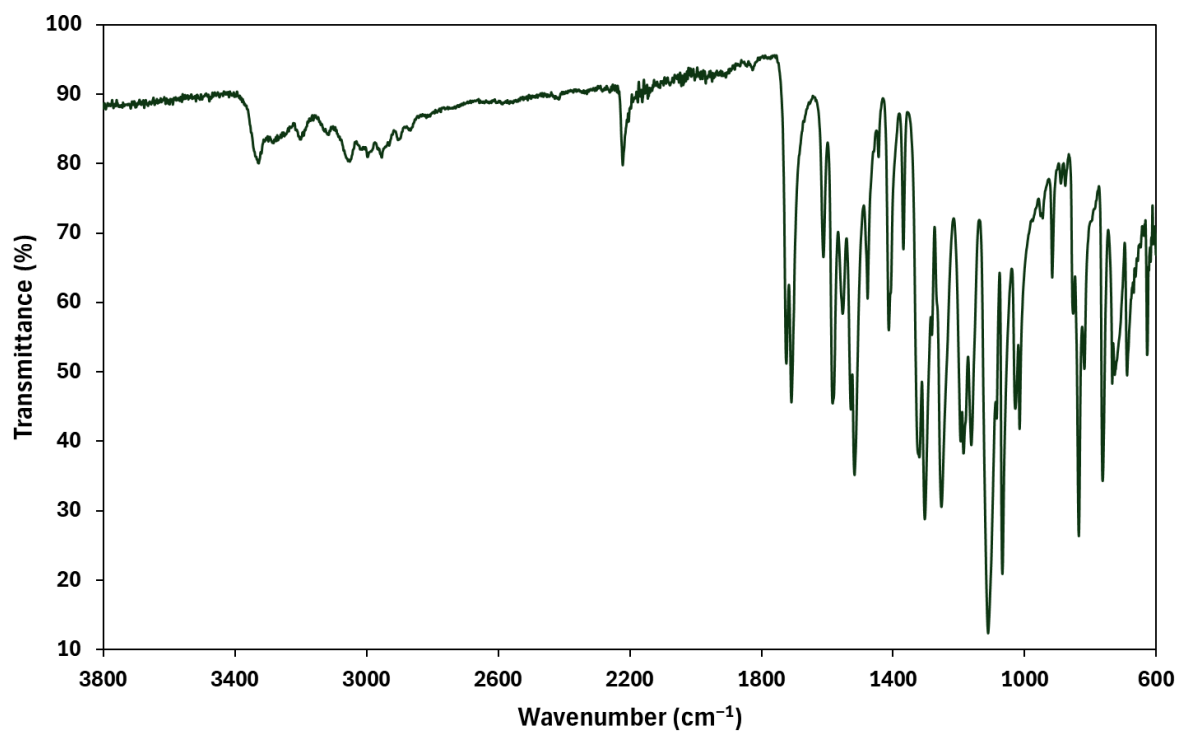


Figure S45. Di-ATR FTIR of 1Br.

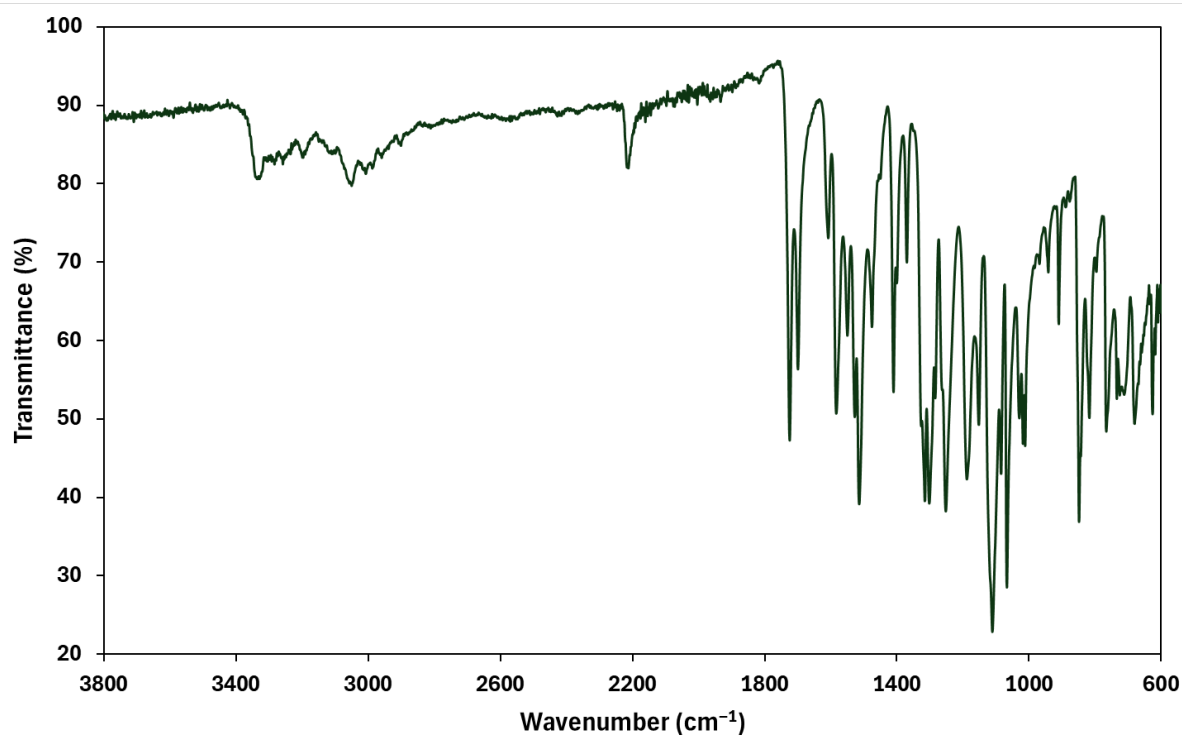


Figure S46. Di-ATR FTIR of 1I.

Computational Details

Calculations were performed using the ORCA 4.2.1 quantum chemistry program package from the development team at the Max Planck Institute for Bioinorganic Chemistry.⁴ All calculations were carried out using the Zero-Order Regular Approximation (ZORA).^{5,6} For geometry optimizations, frequencies, and thermochemistry the B97-D3⁷ functional and def2-TZVPP^{8,9} with SARC/J basis sets¹⁰ were used for hydrogen atoms and all other atoms respectively. Spin-restricted Kohn–Sham determinants¹¹ were chosen to describe the closed shell wavefunctions, employing the RI approximation¹² and the tight SCF convergence criteria provided by ORCA. The basis set superposition error (BSSE) was corrected using the Boys and Bernardi procedures.¹³ Molecular electrostatic potential (ESP) mapping was performed on the 0.001 isosurface of the electron density using the Multiwfn program.^{14,15} All visualizations of ESP maps were performed with Gabedit graphical interface software.¹⁶ The cartesian coordinates of the geometry optimized molecules and supramolecules are provided as .xyz files in a .zip file.

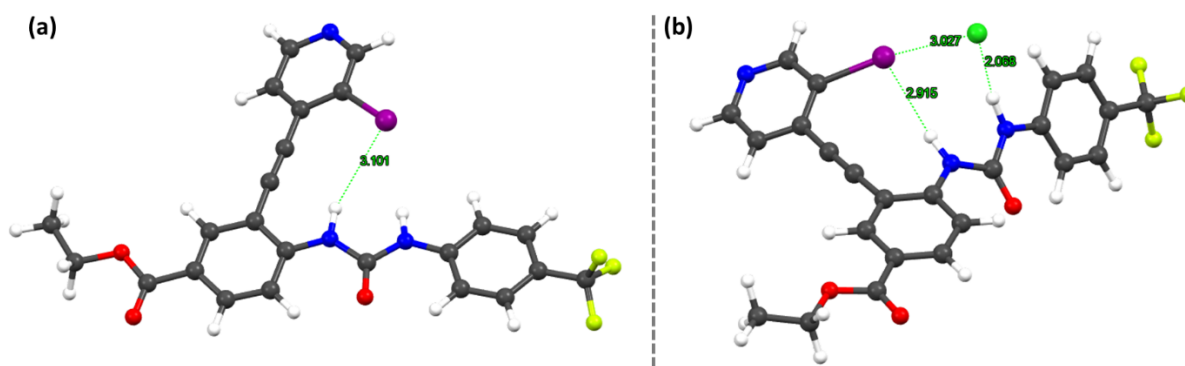


Figure S47. Geometry optimized models of (a) II and (b) II·Cl⁻. Color of atoms C, N, O, F, I, Cl, and H are black, blue, red, yellow, purple, green, and white, respectively. reported distances are in Å unit.

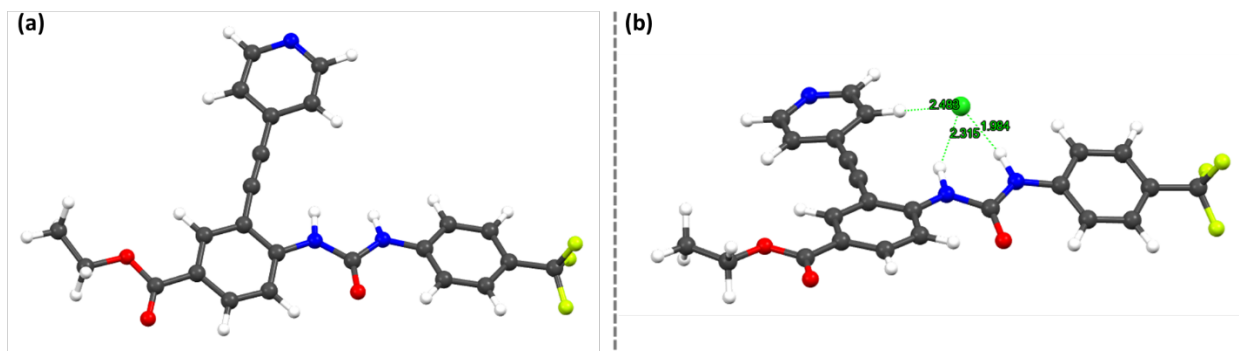


Figure S48. Geometry optimized models of (a) 1H and (b) 1H·Cl⁻. Color of atoms C, N, O, F, Cl, and H are black, blue, red, yellow, green, and white, respectively. reported distances are in Å unit.

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