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Electroactive Anion Receptor with High Affinity for Arsenate
Samuel I. Etkind, Douglas A. Vander Griend, and Timothy M. Swager*

ABSTRACT: Herein, we present the synthesis and characterization of a macrocyclic polyamide cage that incorporates redox-active 1,4-dithiin units. UV/vis titration experiments with eight anions in acetonitrile revealed high affinity for H$_2$AsO$_4^-$ (log $\beta_2 = 10.4^{+0.4}_{-0.4}$) and HCO$_3^-$ (log $K_{1:1} = 8.3^{+0.3}_{-0.4}$) over other common anionic guests, such as Cl$^-$ (log $K_{1:1} = 3.20^{+0.02}_{-0.02}$), HSO$_4^-$ (log $K_{1:1} = 3.57^{+0.03}_{-0.03}$), and H$_2$PO$_4^-$ (log $K_{1:1} = 4.24^{+0.05}_{-0.04}$), by the selective formation of HG complexes. The recognition of arsenate over phosphate is rare among both proteins and synthetic receptors, and though the origin of selectivity is not known, exploiting the difference in the binding stoichiometry represents an underexplored avenue toward developing receptors that can differentiate between the two anions. Additional analysis by $^1$H NMR in 1:3 CD$_2$Cl$_2$/MeCN-d$_3$ found a strong dependence of anion binding stoichiometry with the solvent employed. Finally, titration experiments with cyclic voltammetry provided varying and complex responses for each anion tested, though reaction between the anion and receptors was observed in most cases. These results implicate 1,4-dithiins as interesting recognition moieties in the construction of supramolecular receptors.

INTRODUCTION
Simple anions can often act as potent environmental pollutants. For example, nitrate and phosphate leached from fertilizers can lead to algal blooms, depriving aquatic ecosystems of oxygen needed to sustain animal life.$^5$ The increasing levels of atmospheric CO$_2$ is coupled with an increase in oceanic bicarbonate concentration and therefore ocean acidity, resulting in the degradation of CaCO$_3$, a protective layer for many organisms.$^6$ Millions in Bangladesh are at risk of arsenic poisoning, which could be mitigated by the environmental monitoring of arsenate levels.$^7$ Typically, the accurate quantification and detection of anions at environmentally relevant concentrations require the use of costly analytical methods, such as high-performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS).$^4$–$^7$ These methods, however, could be potentially supplanted by cost-effective techniques that use supramolecular receptors to aid in the detection of anionic species.

To apply anion receptors to the detection of anions in complex mixtures, researchers utilize a variety of methods, such as changes in fluorescence,$^8$–$^{11}$ absorbance (i.e., colorimetric sensors),$^{12}$–$^{14}$ or NMR resonance$^{15}$–$^{19}$ in the presence of guests. More recently, there have been efforts by a number of groups to employ electrochemical methodology toward the detection of anions via chemiresistive,$^{20}$ potentiometric,$^{21}$–$^{22}$ capacitive,$^{23}$ or redox-based sensing,$^{24}$ due to their potential application in the development of cost-effective devices. We were particularly drawn to the development of redox-active receptors, as the effect of transiently creating cationic species has the potential to effectively bind anions in competitive media, such as water.$^{25}$

Electroactive anion receptors have been well described.$^{25}$ Most designs involve appending a redox transducer, most commonly ferrocene,$^{26}$–$^{28}$ to a known anion receptor; however, other strategies have been employed, such as the use of electroactive ureas$^{29}$ and mixed-valence systems.$^{30}$ The change in reduction potential upon introduction of an anionic guest is proposed to proceed via a variety of mechanisms, including through-space interactions between the redox-active moiety and anion, as well as conformational changes of the redox transducer upon anion complexation, which result in the guest binding to the oxidized host with a greater affinity than
to the nonoxidized host. The reduction potential of the host−guest complexes may differ depending on the included guest. Additionally, the observation of "two-wave" behavior, in which the host and guest are in slow equilibria, could potentially be useful in differentiating between multiple anionic species.

We hypothesized that the modification of an existing anion receptor with a carefully chosen redox transducer that is in close proximity to the binding site could give large shifts in the reduction potential of the host−guest complex upon oxidation. The modification of known supramolecular receptor scaffolds, such as in the introduction of halogen bonding, altering distal functionalities to bias host−guest interactions, or systematically altering the size and electronic biases of the binding site, has been demonstrated to be an effective approach toward designing receptors with high affinity toward various anions, in addition to giving novel insights in structure−property relationships. Additionally, we recognized 1,4-dithiin, a sulfur-rich heterocycle that exhibits a well-behaved single-electron oxidation that is coupled with a geometric change from bent to planar, as largely overlooked in supramolecular scaffolds. Herein, we present our work on the incorporation of 1,4-dithiin moieties into Receptor 1 to form Receptor 3, which was previously reported as a nitrate-selective receptor by Anslyn and co-workers (Figure 1). Notably, Kim, Sessler, and co-workers have demonstrated the effectiveness of systematic alterations to Anslyn’s Receptor 1 in the development of pyrrole- and imine-containing Receptor 2, which shows a stronger preference for tetrahedral anions via the incorporation of hydrogen-bond-accepting moieties.

**RESULTS AND DISCUSSION**

**Synthesis.** The preparation of Receptor 3 was carried out according to Scheme 1 (see Section S1.1 (Supporting Information) for preparation of starting materials). The treatment of ethyl acetoacetate (4) with disulfur dichloride in hexane furnishes thioether 5. Initial efforts involved the direct conversion of 5 to dithiin 7 via a variety of thionating reagents, such as Lawesson’s reagent, Curphey’s reagent, and P₄S₁₀, but such attempts resulted in the formation of complex mixtures. Instead, 5 can be treated with sodium hydride and Ts₂O to yield vinyl tosylate 6 as a mixture of isomers. Treatment of the crude mixture with sodium sulfide gives dithiin 7, which can then be hydrolyzed to give diacid 8. This serves as a highly efficient methodology to form 1,4-dithiin rings, the synthetic literature of which has been limited in...
scope. In addition, gram-scale synthesis of diacid 8 was enabled via this synthetic route. Conversion to diacyl chloride 9 can be accomplished by oxalyl chloride under standard conditions.

We initially attempted the direct cyclization of acyl chloride 9 with triamine 10 to form Receptor 3; however, this resulted primarily in the formation of oligomeric species. A more robust approach involves molecule 13, which can be synthesized starting with the preparation of protected amine 11, followed by coupling with acyl chloride 9 to form 12 and deprotection under acidic conditions. Use of HCl in dioxane as a deprotecting agent was crucial as the use of trifluoroacetic acid resulted in the formation of unidentified side products. Slow addition of acyl chloride 9 to a dilute solution of 13 in chloroform with gentle heating provided Receptor 3 in a 20% yield (Section S4, Supporting Information). The structure was confirmed by NMR, high-resolution mass spectrometry (HRMS), and X-ray crystallography (Section S6, Supporting Information). Notably, the X-ray structure shows all amide N–H bonds oriented toward the cavity of the receptor, potentially preorganizing the scaffold toward interacting with anions (Figure 2).

**Binding Characterization.** Association constants of Receptor 3 in acetonitrile with various tetrabutylammonium or tetaethylammonium salts were determined via UV/vis titrations, using SIVVU for data analysis (Table 1 and Section S2, Supporting Information). We chose a list of environmentally and biologically relevant monoanionic guests to probe the binding behavior of Receptor 3. Asymmetric errors on the binding constant terms were calculated by bootstrapping each data set column-wise 1000 times. The errors of at least three separate titrations were then combined according to model 2 of Barlow’s paper and were reported as 95% confidence intervals. The absorbance of all species, including the host, guest, and complexes thereof, was refined as part of our data analysis. As a result of the low concentration used for titration experiments (<35 μM) and the polar solvent employed, ion-pairing interactions were ignored. Binding stoichiometries were assessed by applying various binding models in the formation of HG and HG₂ species to each experiment and selecting the binding stoichiometry that gave the lowest error, as is currently considered best practices. Additionally, when measuring the stability constants of select anions, such as H₃AsO₄⁻, H₂PO₄⁻, HSO₄⁻, and NO₃⁻, titrations were performed at least two concentrations between 5 and 35 μM to give further support to the assigned binding stoichiometry and determined association constant.

Cl⁻ was bound with low affinity, a useful feature given its ubiquity in environmental and biological settings. NO₃⁻, HSO₄⁻, and CN⁻ were bound with moderate affinity, followed by H₂PO₄⁻ and OAc⁻ with stronger binding affinity. Additionally, when measuring the stability constants of Receptor 3 with HCO₃⁻, binding isotherms were produced that could not be accurately fit to a 1:1 binding model, and with H₂AsO₄⁻, the application of a 1:1 binding model provided fits that were associated with higher error than our other anion binding experiments. Instead, it was found that the binding of H₂AsO₄⁻ and HCO₃⁻ could be more accurately fit with a 1:2 host/guest binding mode in accordance with the following equations

$$H + G \rightleftharpoons HG \quad K_{1,1}$$

$$HG + G \rightleftharpoons HG_2 \quad K_{1,2}$$

$$H + 2G \rightleftharpoons HG_2 \quad \beta_2$$

From the $K_{1,1}$ and $K_{1,2}$ values, the binding cooperativity can be assessed by calculation of the interactivity parameter $\alpha$ (Table 1). Interestingly, while HCO₃⁻ demonstrates negative

![Figure 2. Side (left) and top (right) views of the crystal structure of Receptor 3 with CH₂Cl₂ as a solvate. C–H hydrogens are omitted for clarity.](image)

**Figure 2.** Side (left) and top (right) views of the crystal structure of Receptor 3 with CH₂Cl₂ as a solvate. C–H hydrogens are omitted for clarity.

<table>
<thead>
<tr>
<th>anion</th>
<th>log $K_{1,1}$</th>
<th>log $K_{1,2}$</th>
<th>log $\beta_2$</th>
<th>$\Delta G_{K_{1,1}}$</th>
<th>$\Delta G_{K_{1,2}}$</th>
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<td>10.4±0.4</td>
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<tr>
<td>HCO₃⁻</td>
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<td>3.53±0.05</td>
<td>8.3±0.05</td>
<td>−26.1±0.7</td>
<td>−19.4±0.3</td>
<td>−45.4±2</td>
<td>0.25</td>
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<td>OAc⁻</td>
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<td>−d</td>
<td>−d</td>
<td>−28.7±0.5</td>
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<td>−d</td>
<td>−d</td>
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<tr>
<td>H₂PO₄⁻</td>
<td>4.24±0.05</td>
<td>−d</td>
<td>−d</td>
<td>−23.3±2.1</td>
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<td>−d</td>
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<tr>
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<td>−d</td>
<td>−20.3±2.2</td>
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<tr>
<td>HSO₄⁻</td>
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<td>NO₃⁻</td>
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<td>Cl⁻</td>
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<td>−d</td>
<td>−17.6±0.1</td>
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</tbody>
</table>

**Table 1. Stability Constants of 3 with Various Anions**

49 Errors are given from at least three experiments with asymmetric errors at 95% confidence intervals. Interaction parameter, defined as $\alpha = 4K_{1,1}/K_{1,2}$

50 Tetraethylammonium bicarbonate was employed. Not applicable.
cooperativity ($\alpha = 0.25$), $\text{H}_2\text{AsO}_4^-$ demonstrates positive cooperativity ($\alpha = 9$), indicating that the binding of one anionic species makes the binding of the second more favorable. Such binding stoichiometry may be indicative of the formation of anti-electrostatic hydrogen bonds,\textsuperscript{54−58} however, we have been able to obtain neither solution- nor solid-state evidence for the exact binding mode of these two anions.

We were especially surprised to discover that the 1:2 binding behavior in the case of $\text{H}_2\text{AsO}_4^-$ does not hold for $\text{H}_2\text{PO}_4^-$, given their similar sizes and properties.\textsuperscript{59} We closely scrutinized this behavior by subjecting several arsenate and phosphate titrations to both 1:1 and 1:2 binding models to assess the best fit. The absorbance and accompanying fits from 1:1 and 1:2 binding models at 292 nm are shown in Figure 3. We found analysis of the residuals to be particularly useful in the determination of the binding stoichiometry, and such analysis has been demonstrated to be significantly more informative and robust than Job’s plot analysis.\textsuperscript{60,61} As shown in Figure 3a,b, the application of a 1:1 binding model for $\text{H}_2\text{AsO}_4^-$ results in residuals that trace a sinusoidal curve, which has been demonstrated to be indicative of an additional binding event.\textsuperscript{60} Additionally, a global fit of this experiment results in extinction coefficients for the HG complex of 0 at certain wavelengths, a nonrealistic value, and large absorbance of the guest, an artifact not observed in the absence of the host (Figure S81). Removing guest absorbance from the binding model does not improve the resulting fit. Application of a 1:2 binding model, instead, provides significantly reduced residuals and realistic extinction coefficients for all species involved (H, G, HG, and HG\textsubscript{2}) (Figure S83). Application of the same process to titration experiments with $\text{H}_2\text{PO}_4^-$ provides similar residuals for both the 1:1 and 1:2 binding models, which is indicative that a 1:2 binding model is not necessary to account for the change in absorbance (Figure 3c,d). Thus, evidence from UV/vis titration experiments indicates that phosphate and arsenate undergo different binding events with Receptor 3 in acetonitrile (see Section S2 (Supporting Information) for global fits of all titrations and three-dimensional (3D) plots of residuals). Indeed, strong selectivity between arsenate and phosphate is rare in both proteins and synthetic receptors, and only a few reports are present in the literature.\textsuperscript{59,61−63}

We sought to further probe the difference in binding behavior between $\text{H}_2\text{AsO}_4^-$ and $\text{H}_2\text{PO}_4^-$ by electrospray ionization mass spectrometry (ESI-MS) studies in acetonitrile. Although ESI-MS studies are, by themselves, insufficient in determining the stoichiometry of a host–guest interaction, results of such experiments can serve as supporting evidence for the observed binding stoichiometry from solution-phase experiments.\textsuperscript{64,65} While experiments with TBAH$_2$PO$_4$ only revealed evidence of a 1:1 complex (Section S3.2, Supporting Information), similar experiments with TBAH$_2$AsO$_4$ revealed a mass corresponding to [M + NBu$_4$ + H$_2$As$_2$O$_7$]$,^-$  which could provide additional evidence of the formation of a 1:2 complex (Section S3.1 and Figure 4). The preparative formation of pyroarsenate salts requires temperatures in an excess of 150 °C, typically for several hours, and they degrade rapidly in the presence of water.\textsuperscript{66} Given that the ESI source was held at 100 °C, it could be possible that the guest induces dehydration of $\text{H}_2\text{AsO}_4^-$ at elevated temperatures or in the gas phase, due either to hydrogen bonding or by simply holding two guest molecules in close proximity to one another. The formation of pyroarsenate induced by Receptor 3 in solution, however, could potentially explain the difference in binding energies between $\text{H}_2\text{PO}_4^-$ and $\text{H}_2\text{AsO}_4^-$, as computational studies indicate that the formation of pyroarsenate is ∼20 kJ/mol less unfavorable than the formation of pyrophosphate.\textsuperscript{67} Solution-phase evidence to confirm or refute pyroarsenate formation, however, is currently elusive, and we are actively investigating the possibility of this phenomenon.

We also endeavored to further probe the binding interactions with all anions by $^1$H NMR titration, as observation of similar binding interactions at different concentrations can serve as further evidence for assigned binding behavior (Section S5, Supporting Information).\textsuperscript{55} As a result of aggregation and solubility issues observed in pure MeCN-$d_4$ at concentrations greater than 0.25 mM, a mixed-solvent system, 25% CD$_2$Cl$_2$ in MeCN-$d_4$, was employed. A representative titration experiment with HCO$_3^-$ is shown in Figure 5. In all cases, the resonances corresponding to the amide protons shift downfield with increasing equivalents of

![Figure 3. Comparison of 1:1 and 1:2 fits of Receptor 3 at (a) 28.7 μM after the addition of 5.08 equiv of TBAH$_2$AsO$_4$ with (b) the resulting residuals from the application of 1:1 and 1:2 binding models and (c) 30.0 μM after the addition of 15.5 equiv of TBAH$_2$PO$_4$ with (d) the resulting residuals from the application of 1:1 and 1:2 binding models. Absorbance data from 275 to 500 nm were used to determine the association constant and absorbance of all species in solution. Full binding models for these experiments are available in the Supporting Information in Figures S81–S84 and S65–S68 for TBAH$_2$AsO$_4$ and TBAH$_2$PO$_4$, respectively.](https://dx.doi.org/10.1021/acs.joc.0c01206)
anion, which is indicative of hydrogen-bonding interactions. Interestingly, titration with TBAH₂AsO₄ resulted in exceptionally broad receptor peaks, which may be a result of the inclusion of quadrupolar nuclei (Figure S110). This may implicate Receptor 3 as a probe for the presence of arsenate by ¹H NMR. Due to the decreased solvent polarity, similar, but slightly higher, association constants were obtained for HSO₄⁻ (Figure S112) and NO₃⁻ (Figure S113). For experiments with HCO₃⁻, different values were obtained for K₁:1 and K₁:2 than from UV/vis titration experiments, but data analysis indicated the same binding stoichiometry and similar value for β₂.

In the case of Cl⁻, a small K₁:2 is observed (log K₁:2 ~ 1.0) that may arise due to ion-pairing equilibria from both the higher concentration and less polar solvent system employed (Figure S114). ¹H NMR titrations with OAc⁻ provided a lower association constant, but such activity is unsurprising given that OAc⁻ has a K₁:1 beyond the detection limit for ¹H NMR experiments (Figure S108).³⁵ For H₂PO₄⁻, ¹H NMR titration revealed a K₁:1 that is greater than that measured in our UV/vis titration experiments (log K₁:1 ~ 4.6), as well as a relatively small K₁:2 (log K₁:2 ~ 3.4, log β₂ ~ 8.1) (Figure S109). As evidenced by our experiments with arsenate and bicarbonate, the size of the binding site does not preclude the inclusion of two anionic species, and it may be that the slightly less polar solvent system induces further burying of the hydrophilic surface area of H₂PO₄⁻, forming higher-order complexes. Additionally, switching of the binding stoichiometry is not uncommon upon changing the solvent polarity.⁶⁴,⁶⁸,⁶⁹ Finally, ¹H NMR titrations with CN⁻ were inconsistent with our UV/vis experiments, but we also observed a change in solution color (colorless to orange), which may indicate some reaction of cyanide with the receptor, complicating the binding model (Figure S111). Such behavior was not observed at lower concentrations in the case of the UV/vis experiment. See Section S5.1 (Supporting Information) for further discussion on the ¹H NMR experiments.

The overall affinities of Receptor 3 with the anions surveyed are surprisingly high. Although Receptor 1 demonstrates relatively high affinity for NO₃⁻ and OAc⁻ (log K₁:1 ~ 2.5 and 2.9 in 25% CD₂Cl₂ in MeCN-d₃), the overall anion affinity is significantly decreased compared to that of Receptor 3.⁴³ We speculate that this may be due to repulsive interactions between the pyridine nitrogen and anionic guest. The sulfur atom, on the other hand, is bent out of the plane of the amide hydrogens, preventing such repulsive interactions, as observed from the crystal structure and density functional theory (DFT)-calculated geometry (Figures 2 and S169). Additionally, modeling of Receptor 3 with either one or two molecules of H₂AsO₄⁻ or H₂As₂O₇²⁻ indicated the formation of C–H hydrogen-bonding interactions with the methyl groups on the 1,4-dithiin rings, which may enhance interactions with guests (Figures S170–S172, Section S11, Supporting Information). This increase in affinity is advantageous in that it could help to overcome the large solvation energy of anions, allowing binding in more polar solvents.

It is important to note, however, that Receptor 1 was not subjected to titration experiments with H₂AsO₄⁻, and its binding selectivity for H₂PO₄⁻ over H₂AsO₄⁻ is unknown. Most receptors that have been found to complex phosphate, in fact, have not been tested with arsenate. Due to the environmental and biological relevance of arsenate, we believe that this anion should become a more standard guest in the

Figure 4. ESI-MS of Receptor 3 (95 μM) in the presence of 2 equiv of TBAH₂AsO₄ in MeCN. The attached table shows the observed masses and their assigned adducts.

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<thead>
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<th>adduct</th>
<th>m/z (Calc.)</th>
<th>m/z (Obs.)</th>
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<tr>
<td>[M+H₂AsO₄]⁻</td>
<td>1227.2543</td>
<td>1227.2572</td>
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<td>1592.4454</td>
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Figure 5. (a) ¹H NMR titration of Receptor 3 (1.02 mM) with TEAHC₂O in 25% CDCl₃ in MeCN-d₃ (the arrow indicates the resonance associated with the amide proton), (b) assignment of protons in Receptor 3, and (c) derived changes in the chemical shift for nonamide hydrogens.

https://dx.doi.org/10.1021/acs.joc.0c01206
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characterization of supramolecular receptors to help establish trends in arsenate−phosphate discrimination.

**Electrochemical Characterization.** 1,4-Dithiins, similar to thianthrenes, are known to undergo a reversible, single-electron oxidation under anhydrous conditions. As a result of the proximity of the redox-active moiety to the binding site, as well as the planarization of 1,4-dithiin moieties upon single-electron oxidation, we hypothesized that the inclusion of different anions could give rise to different electrochemical responses, such as varying cathodic perturbations in the observed $E^{1/2}$. In a solution of 0.1 M TBAPF$_6$ in MeCN, the macrocycle undergoes a single quasi-reversible oxidation event (Figures 6a and S124). Although Receptor 3 has limited solubility in pure acetonitrile, the addition of TBAPF$_6$ allows for effective dissolution. In comparison with model compound S$_5$, both are oxidized at similar potentials ($3, E^{1/2} = 0.65$ V vs Fc/Fc$^+$, S$_5, E^{1/2} = 0.71$ V vs Fc/Fc$^+$; Figure S125), but the oxidation of Receptor 3 is kinetically slower, as indicated by the larger shift in peak potential with the scan rate. To determine the electron-transfer number for the oxidation of Receptor 3, we performed chronocoulometry and cyclic voltammetry with a microelectrode. Analysis of the data as established by Amatore and co-workers indicated that the oxidation event involved a degenerate or near-degenerate transfer of three electrons (Section S7.2, Supporting Information). Formation of a tricationic complex would foster stronger interactions with guests via electrostatic interactions.

Figure 6. Cyclic voltammograms of Receptor 3 with (a) no additive and varying equivalents of (b) TBAH$_2$AsO$_4$, (c) TEAHCO$_3$, (d) TBAOAc, (e) TBAH$_2$PO$_4$, (f) TBACN, (g) TBAH$_2$SO$_4$, and (h) TBANO$_3$. All measurements were performed in MeCN with 0.1 M TBAPF$_6$ platinum working (0.031 cm$^2$) and counter electrodes, and Ag wire pseudoreference electrode at 100 mV/s and were referenced to the Fc/Fc$^+$ redox couple.
attraction, further enhancing the affinity of the oxidized complex with each anion.

Next, the electrochemical behavior of Receptor 3 in the presence of various anions was surveyed (Figure 6b–h). Due to the relatively low oxidation potential of chloride (E1/2 ≈ 0.52 V vs Fc/Fc+; Figure S132), it was excluded from this study. Receptor 3 exhibits complex oxidation behavior for each anionic guest, which we believe results from unique binding behavior of the oxidized receptor, changes in the E1/2 and electron-transfer kinetics upon complexation, and subsequent reaction of the anion with Receptor 3 upon oxidation. Upon addition of NO3−, the oxidation wave shifts to less positive potentials and becomes chemically irreversible (Figure 6h). This can be rationalized by the oxidation of the tri-radical cation by nitrate, a known behavior in thiairenne radical cations.71 The addition of H3PO4−, H2AsO4−, HCO3−, CN−, and OAc− gave irreversible redox couples as well, but analysis of subsequent scans demonstrated varying degrees of precipitation on the electrode surface (Figure 6b–f and Section S7.4). In the case of H3PO4− (Figure S135), HCO3− (Figure S145), and CN− (Figure S139), the acquisition of subsequent scans by cyclic voltammetry resulted in little signal, indicating the formation of an insulating film on the electrode surface. Subsequent scans, however, in the case of NO3− (Figure S141), H2AsO4− (Figure S137), and OAc− (Figure S143) gave similar voltammograms but with lower current, indicating that the fidelity of the electrode surface is maintained, but that Receptor 3 proximate to the electrode surface was consumed due to an irreversible chemical reaction with the anionic guest. Finally, while HSO4− exhibited a moderate association constant with the neutral receptor as determined by UV/vis titrations, we found that the addition of bisulfate caused dramatic changes in the shape of both the oxidation and reduction waves while maintaining chemical reversibility (Figures 6g and S133). Interestingly, in the case of NO3−, “two-wave” behavior is observed (Figure 6h). Such behavior is indicative of slow-exchange interactions and is potentially useful in the differentiation of anions in a similar fashion that has been described for slow host–guest exchange in NMR studies.17 In other cases, such as H2AsO4−, H3PO4−, OAc−, HCO3−, and CN−, as many as three distinct oxidation waves can be observed after the addition of various equivalents of the anion (Figure 6b–f). Due to the irreversible nature of these redox couples, exact analysis of the oxidation event is difficult, and we abandoned further electrochemical experiments due to the high reactivity of Receptor 3 in its oxidized form. Additionally, more complex mechanisms, such as the loss of degeneracy in the oxidation of Receptor 3 or the formation of additional electroactive materials, may be at play, further complicating the response.

The results of the electrochemical anion titrations are summarized in Table 2. Although the magnitude of the change in peak potential is similar to the more common strategy of appending receptors with well-known redox transducers, such as ferrocene, changes in the peak shape and degree of chemical reversibility vary widely based on the identity of the anionic guest. In our case, we also believe that the conformational activity of the electroactive moiety may participate in enhancing the response toward anions. Geometry of the triply oxidized host, as generated from DFT calculations, indicates that the planarization of the 1,4-dithiin moieties generates a cationic binding site of unique size as compared to the neutral receptor, which may aid in enhancing the change in association constant with the oxidized receptor (Figure S173, Supporting Information). Interestingly, we found that Receptor 3 exhibits a moderate association constant to the supporting electrolyte, TBAPF6 (log Kd ≈ 3.7; Figures S130 and S131, Supporting Information). In spite of this, the addition of other anionic guests, even those with comparable Kd, gave altered voltammograms. This may indicate that binding occurs almost solely with the oxidized host under the conditions for electrochemical measurements. Finally, we found that after the addition of 5 equiv of TBAHSO4 with a shift in E1/2 of 126 mV and a three-electron process, a binding enhancement factor (BEF) of 2.5 × 106 could be estimated.31 Moreover, larger perturbations in the peak potential of oxidation were observed with other anions, but the lack of return waves precluded estimation of the BEFs. Although the use of 1,4-dithiins as redox transducers may be too sensitive for practical applications in the presence of nucleophilic species, the incorporation of multiple redox-active moieties adjacent to the binding site could give molecules with sufficiently high ion affinities when oxidized to effectively bind to anions in polar media.

### CONCLUSIONS

In conclusion, we have synthesized and characterized an anion receptor with novel 1,4-dithiin moieties. The receptor shows high selectivity and affinity toward HCO3− and H2AsO4−, which were observed to bind in a 1:2 host/guest binding mode. Additionally, H2AsO4− was found to undergo different binding behavior with Receptor 3 than H3PO4−, an uncommon characteristic given the two anions’ similar size and properties. The origin of arsenate–phosphate discrimination, however, is currently unknown. Further analysis by cyclic voltammetry revealed unique responses upon the addition of anionic guests, but due to the high reactivity of Receptor 3 when oxidized, deconvolution of the response proved difficult in many cases. Due to the interest in the detection and binding of arsenate and the lack of effective strategies toward selective complexation, we hope this report will encourage other researchers to include this anion in their studies to help establish trends in arsenate–phosphate discrimination. Future experiments in our laboratory will involve the further synthesis and characterization of anion receptors with potential application in the monitoring of environmentally relevant anion contaminants.

### Table 2. Summary of Electrochemical Response toward Various Anions

<table>
<thead>
<tr>
<th>Anion</th>
<th>ΔE1/2 (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3AsO4−</td>
<td>−334a</td>
</tr>
<tr>
<td>HCO3−</td>
<td>−235</td>
</tr>
<tr>
<td>OAc−</td>
<td>−105</td>
</tr>
<tr>
<td>H3PO4−</td>
<td>−480</td>
</tr>
<tr>
<td>CN−</td>
<td>−142</td>
</tr>
<tr>
<td>HSO4−</td>
<td>−118</td>
</tr>
<tr>
<td>NO3−</td>
<td>−102</td>
</tr>
<tr>
<td>Cl−</td>
<td>N/D</td>
</tr>
</tbody>
</table>

aTetraethylammonium salts were employed. b Change in the peak potential of the oxidation event upon the addition of 5 equiv of anion. c Two oxidation events are observed. The more cathodic peak is reported here. d Tetraethylammonium bicarbonate was employed. e Not determined.
**EXPERIMENTAL SECTION**

**General Experimental Considerations.** All reagents were purchased from commercial sources and used without further purification unless otherwise specified. Tetrahydrofuran (THF) and dichloromethane (CH2Cl2) were purified using an Inert solvent purification system by passage through two alumina columns and were stored in a Schlenk flask over 4 Å molecular sieves under argon atmosphere. Tetrahydroammonium hexafluorophosphate for electrochemical analysis was recrystallized from EtOH three times and dried in a vacuum oven at 80 °C. All chemical manipulations were performed under an argon atmosphere in flame-dried glassware unless otherwise specified. The reported reaction temperatures refer to the temperature of external water or oil baths.

Chromatographic purifications were performed with a Biotage Isolera using Biotage SNAP Ultra columns with the specified solvent gradient. Typically, columns were performed with 1 column volume of the initial solvent gradient, followed by a linear gradient over 10 column volumes to the final solvent composition, followed by an additional 2 column volumes at the final solvent composition. NMR spectra were recorded on either a 400 MHz Bruker Avance-III HD Nanobay spectrometer, 500 MHz Bruker Avance Neo spectrometer, or 600 MHz Bruker Avance Neo spectrometer. Chemical shifts δ are reported in parts per million (ppm) downfield from tetramethylsilane by referencing to residual solvent signals.

**III HD Nanobay spectrometer, 500 MHz Bruker Avance Neo spectrometer, or 600 MHz Bruker Avance Neo spectrometer.**

**Titrations**

**All UV/vis titrations were carried out on an Agilent Cary-4000 UV/vis spectrometer, recording from 500 to 200 nm at intervals of 0.5 nm. Tetrahydroammonium salts were stored in a vacuum desiccator prior to titration. UV/vis titrations were carried out in HPLC-grade MeCN in 1 cm cuvettes at the specified host concentration. Host concentration was determined using the absorbance of Receptor 3 at 292 nm, which is linearly correlated with concentration (Figures S3, S4). Anionic guests were dissolved in a solution of the host to keep the host concentration constant over the course of the experiment.**

**Electrochemical Experiments.** Electrochemical experiments were performed in a nitrogen-filled glovebox (O2 < 20 ppm, H2O < 2 ppm) with a BioLogic SP-150 potentiostat. Platinum working electrodes (2 mm or 10 mm diameter) were polished using first suspensions of 1.0 μm, then 0.3 μm, then 0.05 μm alumina in water on a polishing pad followed by sonication in methanol to remove excess alumina and then chemical polishing by cycling the electrode in 1 M H2SO4 over the potential window of water until the resulting traces overlapped. The electrode was then rinsed with methanol and blown-dry with nitrogen before being transferred into the glovebox. A platinum wire was used as the counter electrode. A silver pseudoreference electrode was used, and all measurements were referenced to Fe/Ce (1 M TBPAPF6 as a supporting electrolyte). In the case that the oxidation was chemically irreversible, which is the case with all anions except for TBAHSO4, the first scan is reported. In the case of TBAHSO4, the third scan is reported, which is similar to the first two scans.

**ESI-MS Experiments.** Electrospray ionization (ESI) binding experiments were carried out on a JEOL Accu-TOF equipped with an FSI source. All ions were observed in negative mode, with the orifice and desolvating chambers held at 100 °C. The samples were run via direct infusion at the specified concentration at a rate of 1.0 mL/h using a syringe pump. All experiments were run, holding the host concentration at approximately 100 μM in acetonitrile.

**Computational Studies.** Theoretical calculations were carried out with the Spartan18 (1.4.4, Wave function Inc., Irvine CA) software packages on a computer operating with Windows 10 OS. Geometry optimizations were performed with density functional theory (DFT) calculations using the B3LYP functional with the 6-31G* basis set in the gas phase. Frequency calculations were performed to confirm that the structures were at a local energy minimum. Outputs were visualized with CYLview.

**Synthetic Procedures.**

**Diethyl 2,2'-Thiodis(3-oxobutanoate) (5).** Caution: This reaction produces stoichiometric amounts of HCl gas and must be performed in a fume hood or similarly well-ventilated area. The title compound was prepared according to a modified literature procedure. To a round-bottom flask open to the atmosphere, ethyl acetocetate (4 (10.0 mL, 42.4 equiv)), and hexanes (16 mL) were added. The mixture was then heated to 40 °C, upon which the solution became homogeneous. Disulfur dichloride (3.14 mL, 39.2 mmol, 1.0 equiv) was then added in one portion. After 1–2 min, the solution developed a yellow color and began to bubble vigorously. After the bubbling had subsided, the solution was allowed to react for a further 30 min, over which a solid precipitated. The reaction was then cooled in an ice bath, and the white solid was collected by vacuum filtration, washed first with hexanes and then water, and subsequently dried under vacuum at 50 °C to remove residual water. The solid was purified by recrystallization in acetone (25 mL) to give the target compound as colorless needles (4.59 g, 15.8 mmol, 40%).

**31P NMR (126 MHz, CDCl3) δ 6: [M+H] +.** The title compound was prepared according to a modified literature procedure. To a round-bottom flask containing sodium hydride (5.0 mL, 1.0 equiv) and hexanes (12 mL), was added. After conversion was complete, as judged by TLC (100 μM), the mixture became viscous. The reaction was then allowed to warm to room temperature. After conversion was complete, as judged by TLC, the reaction was quenched slowly with the addition of acetone (20 mL) and 1N HCl (20 mL). The reaction was concentrated under reduced pressure to a total volume of about 100 mL. The resulting mixture was then extracted with diethyl ether (3×31 mL) and the organic layers were combined, washed with saturated NaHCO3 (100 mL) and brine (100 mL), dried over Na2SO4, filtered, and concentrated under reduced pressure to give approximately 15 g of a red oil containing compound 6 as a mixture of isomers. The mixture was used without purification in the next step. The red oil was dissolved in EtOH (125 mL), and sodium sulfite nonhydrate (4.96 g, 20.7 mmol, 0.75 equiv) was added in one portion, resulting in the appearance of an orange solution and the precipitation of a solid. After 1.5 h, CH3Cl (125 mL) was added to further precipitate additional salts, and the mixture was filtered through a bed of celite, and the volatiles were removed under reduced pressure. The dark oil was then purified by flash chromatography (1 → 15% EtOAc in hexanes) to give the title compound as an orange oil.
that solidifies upon storage at 2–5 °C (5.08 g, 17.6 mmol, 64%). 1H NMR (400 MHz, CDCl3) δ 4.25 (q, J = 7.1 Hz, 4H), 2.41 (s, 6H), 1.32 (t, J = 7.2 Hz, 6H). 13C{1H} NMR (101 MHz, CDCl3) δ 162.9, 149.6, 122.3, 61.9, 22.7, 14.3. HRMS (DART-MS) m/z: [M + H]+ calcd for C24H41O2S2 429.2056; found 429.2055.

3.5-Dimethyl-1,4-dithiane-2,6-dicarboxylic Acid (8). Potassium hydride (23.0 mmol, 44.0 mmol, 94%) was added, and the vessel was subsequently evacuated and recharged with hydrogen five times. The reaction was purged with hydrogen several times until conversion was complete as observed by 1H NMR (typically 3 days). After the reaction was complete, the reaction mixture was filtered through a pad of celite. The filtrate was then concentrated under reduced pressure, giving the title compound as a pink solid (10.5 g, 42.1 mmol, >95%). 1H and 13C NMR spectra were consistent with those reported in the literature. 1H NMR (500 MHz, CDCl3) δ 3.87 (s, 6H), 2.82 (q, J = 7.5 Hz, 6H), 1.37 (s, 6H), 1.23 (t, J = 7.5 Hz, 9H). 13C{1H} NMR (126 MHz, CDCl3) δ 140.5, 137.6, 39.8, 22.7, 16.9.

Di-tert-butyl ((5-((Benzyloxy)carbonyl)amino)methyl)-2,4,6-triethyl-1,3-phenylene)bis(methylene)dicarbamate (54). The title compound was prepared according to a modified literature procedure. 1H NMR (400 MHz, 16.0 mmol, 1.0 equiv) and sodium hydride (960 mg, 24.0 mmol, 1.5 equiv) were added to a round-bottom flask and dissolved in dioxane (80 mL) and water (50 mL), and the solution was cooled to 0 °C. Benzylchloroforomate (3.87 mL, 27.2 mmol, 1.7 equiv) and di-tert-butyl dicarbonate (11.0 g, 50.5 mmol, 3.15 equiv) were dissolved in a minimal amount of dioxane to give a total volume of 20 mL and added over 4 h via a syringe pump. After the addition was complete, the reaction was allowed to come to room temperature and stirred for a further 2 h. The dioxane was then removed under reduced pressure, and the resulting aqueous layer was extracted with CH2Cl2 (3 × 50 mL). The organic layers were combined, washed with brine (100 mL), filtered, and concentrated under reduced pressure to give an orange solid. This solid was then chromatographed (5 × 20 mL) on silica gel to give the title compound as a white solid (2.42 g, 4.14 mmol, 26%). 1H and 13C NMR spectra were consistent with those reported in the literature. 1H NMR (500 MHz, CDCl3) δ 7.70–7.24 (m, 5H), 7.16 (t, J = 4.8 Hz, 1H), 6.53 (t, J = 4.8 Hz, 2H), 5.04 (s, 2H), 4.25 (d, J = 4.9 Hz, 2H), 2.72 (d, J = 4.9 Hz, 2H), 2.72–2.61 (m, 6H), 1.38 (s, 18H), 1.05 (s, J = 7.4 Hz, 9H). 13C{1H} NMR (126 MHz, CDCl3) δ 155.8, 155.2, 143.0, 142.7, 137.2, 132.2, 131.80, 128.3, 127.7, 127.6, 77.7, 65.3, 38.7, 38.1, 28.3, 22.5, 22.4, 16.2, 16.1. Di-tert-butyl (5-(Aminomethylene)-2,4,6-triethyl-1,3-phenylene)bis(methylene)dicarbamate (11). The title compound was prepared according to a modified literature procedure. A round-bottom flask was charged with 5% palladium on carbon (172 mg) and MeOH (40 mL). The reaction vessel was then evacuated and refilled with hydrogen five times. A solution of compound S4 (2.27 g, 3.89 mmol, 1.0 equiv) in MeOH (40 mL) was added, and the vessel was then evacuated and refilled with hydrogen five times. The reaction was then quenched with 1 M HCl (40 mL). After the reaction was complete, the reaction mixture was then concentrated under reduced pressure, giving the title compound as a white solid (1.75 g, 3.89 mmol, quant.). 1H and 13C NMR spectra were consistent with those reported in the literature. 1H NMR (500 MHz, CDCl3) δ 6.61 (t, J = 4.8 Hz, 2H), 4.17 (d, J = 4.8 Hz, 4H), 3.70 (s, 2H), 2.71 (q, J = 7.5 Hz, 4H), 2.64 (q, J = 7.5 Hz, 2H), 1.38 (s, 18H), 1.10 (t, J = 7.5 Hz, 6H), 1.06 (t, J = 7.3 Hz, 3H). 13C{1H} NMR (126 MHz, CDCl3) δ 155.2, 141.9, 141.8, 136.9, 132.0, 77.6, 38.2, 28.3, 22.4, 22.1, 16.5, 16.3.

Tetra-tet-butyl (((3,5-Dimethyl-1,4-dithiane-2,6-dicarboxylic acid)bis(azanediyl)bis(methylene)bis(2,4,6-triethylbenzene-5,1,3-triyldieterikato(methylene))tetrametachatramate (12). A round-bottom flask containing compound 11 (1.75 g, 3.89 mmol, 2.1 equiv) and triethylamine (0.65 mL, 4.6 mmol, 2.5 equiv) in CH2Cl2 (10 mL) was cooled to 0 °C. In a separate flask, diacetyl chloride 9 that was freshly prepared from diacid 8 (430 mg, 1.85 mmol, 1.0 equiv) was dissolved in CH2Cl2 (10 mL) and added dropwise to the solution of compound 11. After the addition was complete, the reaction was allowed to come to room temperature and was stirred overnight. The solution was then poured into 1 M HCl (40 mL), and the layers were separated. The aqueous layer was further extracted with CH2Cl2 (2 × 40 mL), and the organic layers were combined, washed with brine, dried over Na2SO4, filtered, and concentrated under reduced pressure to give an orange residue, which was purified by flash chromatography (0–3% MeOH in CH2Cl2) to give the title compound as an orange solid.
The compound was sonicated in PhMe (5 mL), and the PhMe was washed with acetone in PhMe, then 0 \( \times \) 1.15 (t, \( \text{J} = 7.4 \text{ Hz}, 6 \text{H} \)), 1.10 (t, \( \text{J} = 7.4 \text{ Hz}, 12 \text{H} \)).

\( \text{N}^2,\text{N}^2\)-Bis(3,5-bis(aminoethyl)-2,4,6-triethylbenzyl)-3,5-dimethyl-1,4-dithiine-2,6-dicarboxamide (13). Compound 12 (1.553 g, 1.42 mmol, 1.0 equiv) was dissolved in 4 M HCl in dioxane (12 mL), resulting in the formation of an orange precipitate. The reaction was stirred for 2 h, after which isopropanol was added to dissolve all materials, and the volatiles were removed under reduced pressure, giving the title compound as a light orange powder (1.18 g, 93%).

1H NMR (500 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 8.30 (t, \( \text{J} = 6.9 \text{ Hz}, 12 \text{H} \)), 2.66 (q, \( \text{J} = 7.5 \text{ Hz}, 12 \text{H} \)), 1.10 (t, \( \text{J} = 7.3 \text{ Hz}, 18 \text{H} \)).

13C{1H} NMR (126 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 164.0, 154.3, 144.8, 144.5, 133.7, 132.3, 125.1, 79.4, 39.4, 20.3, 23.6, 21.9, 16.6.

HRMS (Q-TOF-ESI-MS) \( m/z \): [M + H]^+ calc for \( \text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_2\text{S}_2 \) 495.1233; found 495.1210.

Notes
The authors declare no competing financial interest.

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REFERENCES


