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Selective Binding and Extraction of Aqueous Dihydrogen Phosphate Solutions via Three-armed Thiourea Receptors

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Abstract: A series of neutral anion receptors with one to three thiourea arms were synthesized and their binding to tetrabutylammonium chloride, acetate, and dihydrogen phosphate salts in aqueous DMSO mixtures were examined. The three-armed thiourea host was found to strongly and selectively bind $H_2PO_4^-$ even in DMSO solutions containing up to 30% water. This enabled the dihydrogen phosphate salt to be extracted from water into chloroform in its dibasic form despite the high heat of hydration of HPO_4^{2-}.

Introduction

Phosphate-containing anions are essential in living organisms as they are present in energy and genetic information storage molecules (i.e., ATP, and DNA and RNA, respectively) as well as cell membrane phospholipid bilayers and bones. They also take part in cell signaling and serve as a buffer in serum and urine[1-5]. In humans, excessive serum phosphate is removed by the kidneys but many people have abnormal levels indicative of health problems that require treatment[6]. For example, both chronic kidney disease and hyperphosphatemia (a condition corresponding to elevated phosphate levels in blood) require phosphate concentrations to be lowered[7]. Several drugs such as Sevelamer are currently on the market for this purpose, but they are slow acting and typically plateau at phosphate levels above the recommended 5.5 mg/dL amount for patients with the end stage chronic kidney disease[8-11].

In agriculture inorganic phosphate in its different forms (i.e., $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) plays a critical role as more than 30,000,000 tons of phosphate–containing fertilizers are used annually. Phosphate is also commonly found in detergents, and although some countries have banned it for this purpose, the resulting overall world-wide phosphate pollution causes eutrophication of natural water sources (i.e., the ecosystem response to excess nutrients). This leads to green and blue algal blooms with toxin-producing cyanobacteria strains that threaten aquatic life and drinking water safety[12]. In the United States at least one-third of the larger lakes $\geq$ 10 acres contain cyanobacteria, and the situation is worse in many other countries[13]. Detection and extraction of phosphate from bio- and ecosystems, consequently is of critical importance[14-19]. Due to the high hydration energies and low basicities of phosphate anions this is a difficult task that is compounded by the need for high selectivity over commonly found anions such as chloride and acetate which are more lipophilic and basic, respectively[20].

Different types of interactions have been used in developing anion receptors, but the most promising are the more directional ones such as hydrogen bonding since they allow one to adjust the binding pocket of the receptor to the size and shape of the target of interest[12]. Given our previous work on hydrogen bond catalysts with a 1,3,5-triphenylbenzene core and the molecular recognition studies of Tobe *et al.*, Choi *et al.* and others[21-27], we decided to explore a series of neutral thioureas as potential phosphate anion receptors. Our results on mono-, bis- and tris(thioureas) (Fig. 1) are reported herein[28]. All three side chains are found to play an important role in the tris(thiourea), and this compound is a strong enough binder that it can be used to extract dibasic phosphate out of water into chloroform.

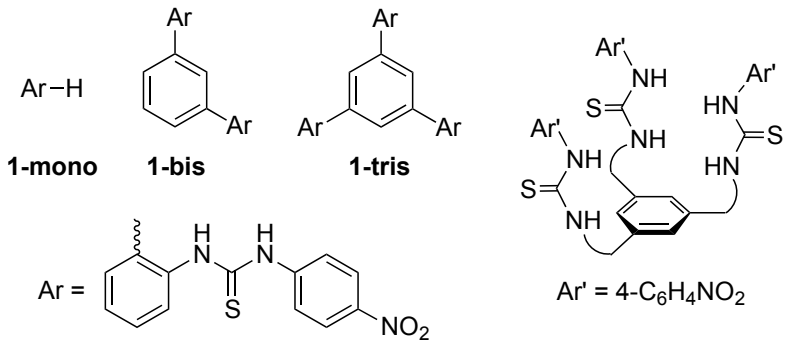


Fig. 1. Anion receptors with 1–3 thiourea arms and a schematic representation of the syn all NH trans conformation of **1-tris** in which curved lines are used for the peripheral aromatic rings.

Results and discussion

Mono(thiourea) **1-mono** was synthesized by reacting aniline with *p*-nitrophenylisothio-cyanate (**2**). The bis(thiourea) derivative **1-bis** was prepared from the previously reported diamine **3-bis** as indicated in Fig. 2, and tris(thiourea) **1-tris** was generated in the same manner only starting with 1,3,5-tribromobenzene[29-31].

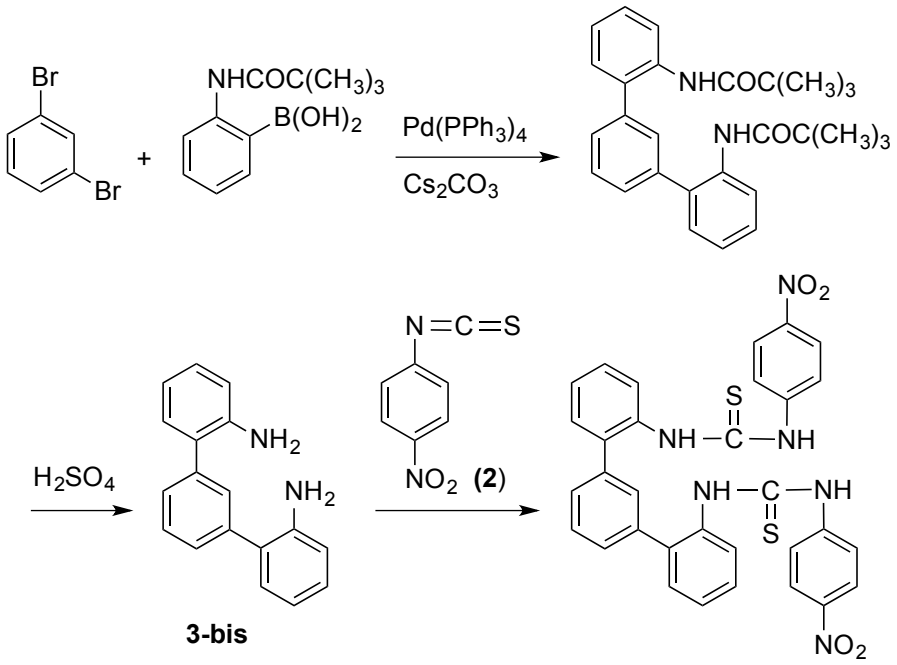


Fig. 2. Synthetic scheme for the preparation of **1-bis**

Anion binding investigations of neutral receptors are typically carried out in non-polar or weak hydrogen bond accepting solvents such as chloroform and acetonitrile. Dimethylsulfoxide (DMSO) provides a much less favorable environment for such studies because of its high dielectric constant and strong hydrogen bond acceptor ability. Aqueous DMSO mixtures are even more inhospitable, but **1-tris** was found to strongly bind tetrabutylammonium dihydrogen phosphate in a 99.5 to 0.5 (v/v) DMSO to H_2O mixture. Given that the UV-visible absorbance changes were nearly linearly dependent upon the added concentration of the dihydrogen phosphate salt up to 1 equivalent, the association constant K is $\geq$ ~5 x 10^6 M^{-1} and too large to accurately measure by this method. Under the same conditions tetrabutylammonium acetate (TBAOAc) deprotonates **1-tris**. This was revealed by UV-visible spectroscopy in that the addition of 1 equivalent of TBAOAc or TBAOH to the tris(thiourea) led to the same spectral changes, and they are distinct from those arising from dihydrogen diphosphate (Fig. S1). Infrared

spectroscopy supports this conclusion in that the reaction of **1-tris** with TBAOAc leads to the appearance of the carbonyl stretch of acetic acid at 1714 cm^{-1} (Fig. S2). These results are also consistent with the known pK_a value of 12.3 for acetic acid in DMSO and estimates of ~4 for phosphoric acid[32] and ≤ ~8 for **1-mono**, **1-bis**, and **1-tris** (Fig. S3).

Additional aqueous DMSO mixtures were investigated with increasing amounts of water given the strong binding observed in 99.5% : 0.5% (v/v) DMSO/H_2O. As expected, the association constants of **1-tris** for tetrabutylammonium dihydrogen phosphate decrease with increasing water content (Table 1). In each mixture that was examined $K \geq 10^3$ M^{-1} and interestingly over the 5 – 30% water range, the logarithm of K is linearly correlated with the water percentage. In the higher water content mixtures (i.e., ≥ 25% H_2O), acetate and hydroxide ions led to different UV-vis spectral changes (Fig. S4) and the resulting data could be fit to 1:1 binding isotherms. This suggests that the relative acidities of **1-tris** and HOAc change with the water content and that the latter compound is less acidic when there is little water present, the two species are similar in acidity when there is 12.5 – 20% water, and acetic acid is more acidic than **1-tris** in ≥ 25% aqueous DMSO mixtures.

Table 1. Association constants for 1:1 binding of **1-tris** to tetrabutylammonium salts of dihydrogen phosphate and acetate in DMSO–H_2O mixtures.

%H_2O	K (M^{-1})[a]		%H_2O	K (M^{-1})	
	$H_2PO_4^-$	OAc^-		$H_2PO_4^-$	OAc^-
0.5	≥ 5 x 10^6	P.T.	20	1.5 x 10^4	
5	2.3 x 10^5	P.T.	25	4.0 x 10^3	35
12.5	7.5 x 10^4	P.T.	30	1.4 x 10^3	13

[a]P.T. = proton transfer.

In wet DMSO with 25% H_2O 1 : 1 binding constants of **1-tris** with the tetrabutylammonium salts of dihydrogen phosphate, acetate, and chloride were measured. Association constants of 4.0 x 10^3, 35 and 1 M^{-1}, respectively were obtained. This makes **1-tris** the strongest and most selective neutral receptor for dihydrogen phosphate reported to date. The importance of all three thiourea arms in binding the acetate and dihydrogen phosphate salts were also determined by exploring the corresponding mono and bis(thioureas); chloride was not examined in this way since the tris(thiourea) derivative does not effectively coordinate with it. Acetate anion binds three times more strongly with **1-tris** than **1-mono** (i.e., K = 12 vs 35 M^{-1}, respectively) which corresponds to the statistical ratio for association when only one thiourea arm is used for binding. For dihydrogen phosphate, $K_{\mathbf{1\text{-}bis}}/K_{\mathbf{1\text{-}mono}}$ = 28/12 or ~2 and this indicates that one arm is used for these two receptors as well. In contrast, the tris derivative has a $H_2PO_4^-$ association constant that is more than 100-fold larger than the statistical value of 36 M^{-1} for binding with one arm. All three arms, consequently, are used in a cooperative fashion in the 1:1 complex of **1-tris** with $H_2PO_4^-$. In accord with this deduction, a B3LYP/6-31+G(d,p) optimized structure for this species was located (Fig. 3) in which each thiourea arm participates in two NH ••• O hydrogen bonds.

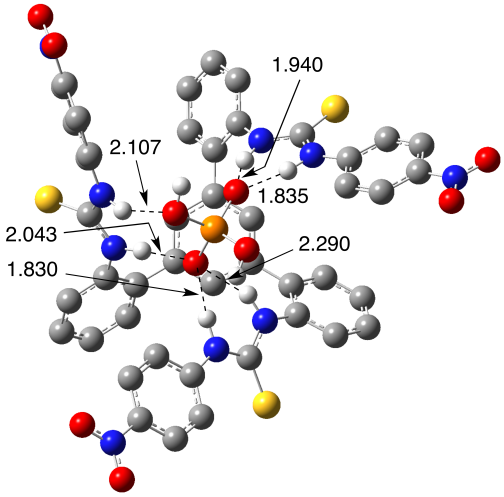
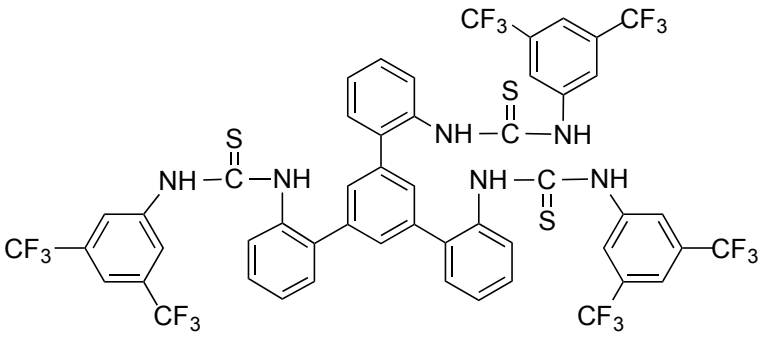


Fig. 3. An optimized B3LYP/6-31+G(d,p) structure for the 1:1 complex between **1-tris** and $H_2PO_4^-$ with six NH ••• O hydrogen bonds ranging in length from 1.835 – 2.290 Å; CH hydrogens omitted for clarity.

Given the strength and selectivity of the binding for dihydrogen phosphate displayed by **1-tris**, we decided to examine if this compound can be used to extract $H_2PO_4^-$ out of water and into an immiscible organic layer. Chloroform was chosen for this purpose, but since **1-tris** is not very soluble in this medium an analog in which the *p*-nitrophenyl ring was replaced by a 3,5-bis(trifluoromethyl)phenyl substituent was employed (Fig. 4). This tris(thiourea) (**4-tris**) has a



4-tris

Fig. 4. Anion receptor **4-tris**.

similar affinity as **1-tris** for tetrabutylammonium dihydrogen phosphate in 0.5% and 25% aqueous DMSO solutions where its measured binding constants are 7.7 x 10^5 and 1.0 x 10^4 M^{-1}, respectively. Consequently, a chloroform-*d* solution of **4-tris** was treated with aqueous tetrabutylammonium dihydrogen phosphate and a proton nuclear magnetic resonance (^{1}H NMR) spectrum of the organic layer revealed the presence of the tetrabutylammonium group. It was not observed when **4-tris** was absent indicating that extraction of a phosphorous-containing anion salt is taking place. To identify its structure, ^{31}P NMR spectra were examined. A broad singlet at 4.0 δ was observed for the extract in the chloroform–*d* solution whereas control experiments revealed that a mixture of **4-tris** and tetrabutylammonium dihydrogen phosphate gives rise to an

upfield signal at 0.5 δ and the salt itself has a signal at 2.2 δ. These findings indicate that dihydrogen phosphate is not the phosphorous-containing counterion being extracted into the chloroform–*d* layer. A mixture of **4-tris**, tetrabutylammonium dihydrogen phosphate, and tetrabutylammonium hydroxide, however, has a resonance at 4.5 δ which is close to what was observed in the extraction experiment. This suggests that tetrabutylammonium hydrogen phosphate (i.e., dibasic phosphate $(Bu_4N^+)_2HPO_4^{2-}$) is the salt being removed from the water layer.

To test this conclusion, the ^1H and ^{31}P NMR spectra were integrated in the presence of internal standards and the tetrabutylammonium to phosphorous ratio was found to be 2 : 1 as required for the dibasic phosphate salt. The stoichiometry for this extraction process requires an equivalent of phosphoric acid to be produced in the water layer, and consistent with this requirement the pH of the aqueous layer was found to decrease. These observations taken together indicate that **4-tris** is able to transfer $(Bu_4N^+)_2HPO_4^{2-}$ from water into chloroform. As a result, it is not surprising that the extraction process is more efficient for hydrogen phosphate than dihydrogen phosphate (Table 2). Concentrations as low as 100 μM of dibasic phosphate were found to bind 10% of the receptor in the organic layer (entry 4). This concentration is 2.5 fold less than the typical lower limit for phosphate concentrations in blood (2.4 mg/dL)[33] and 30 times lower than intestinal phosphate levels in patients with renal failure[34]. Compounds of this sort, consequently, are promising leads for developing analytical methods to measure serum phosphate levels and drug candidates for treating hyperphosphatemia[35]

Table 2. Extraction of aqueous tetrabutylammonium salts of mono and dibasic phosphate into chloroform-*d* with 2 equivalents of a 2 mM solution of **4-tris**.[a]

Entry	[phosphate salt], mM	% Bu_4N^+ extracted	
		$(Bu_4N^+)H_2PO_4^-$	$(Bu_4N^+)_2HPO_4^{2-}$
1	10	55%	87%
2	1.0	30%	51%
3	0.33	nd	38%
4	0.10	nd	20%

[a] nd = not determined.

Experimental

1,3,5-tris(2-Pivaloylaminophenyl)benzene (5). A round-bottomed flask was charged with 2-pivaloylaminophenylboronic acid (2.66 g, 12.0 mmol)[29], 1,3,5-tribromobenzene (945 mg, 3.0 mmol), cesium carbonate (9.78 g, 30 mmol), 1,2-dimethoxyethane (15 mL) and water (15 mL). Nitrogen was bubbled through the two layered solution with stirring for 10 min to remove oxygen from the system. Tetrakis(triphenylphosphine)palladium(0) (347 mg, 0.30 mmol) was then added and the reaction mixture was refluxed with vigorous stirring for 13 h under nitrogen. Upon cooling to room temperature ethyl acetate (50 mL) and water (20 mL) were added and the two layers were separated. The aqueous solution was extracted with EtOAc (20 ml) and the combined organic material was washed with brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue via medium pressure liquid chromatography (MPLC) on silica gel with ethyl acetate : hexanes (15 : 85 to 67 : 33) afforded 1.47 g (81%) of **5** (1,3,5-(1,2-$(CH_3)_3CCONHC_6H_4)_3C_6H_3$) as a white solid ($R_f$ = 0.19 (EtOAc : hexanes, 33 : 67)).

^{1}H NMR (500 MHz, acetone-d_6) δ 8.01 (br s, 3H), 7.96 (d, J = 8.0 Hz, 3H), 7.49 (s, 3H), 7.41 (dd, J = 1.5 and 7.5 Hz, 3H), 7.38 (dt, J = 1.5 and 8.0 Hz, 3H), 7.26 (dt, J = 1.0 and 7.5 Hz, 3H), 1.04 (s, 9H). ^{13}C NMR (75 MHz, acetone-d_6) δ 177.1, 141.2, 137.0, 135.6, 131.1, 130.5, 129.6, 126.2, 125.4, 40.5, 28.2. HRMS-ESI: calcd for $C_{39}H_{45}N_3O_3Na$ $(M + Na)^+$ 626.3359, found 626.3365.

1,3,5-tris(2-Aminophenyl)benzene (3-tris). A round-bottomed flask equipped with a condenser was charged with tris-amide **5** (1.44 g, 2.39 mmol) and 65% aqueous sulfuric acid (prepared by mixing 10 mL of concentrated H_2SO_4 and 10 mL of water). The resulting mixture was heated at 125 °C under nitrogen for 17 h. It was then allowed to cool to room temperature, poured into water (20 mL), and the aqueous solution was made basic (i.e., pH = 9) with *ca.* 200 mL of 30% aqueous ammonia. The resulting suspension was extracted with EtOAc (2 x 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford 827 mg (99%) of **3-tris** as a pale yellow powder (R_f = 0.26 (EtOAc : hexanes, 67 : 33)). Its spectral data were consistent with those that have been previously reported[30].

1,3,5-tris(2-(3-(4-Nitrophenyl)thioureido)phenyl)benzene (1-tris). To a mixture of **3-tris** (70 mg, 0.20 mmol) and 4-nitrophenylisothiocyanate (**2**, 130 mg, 0.72 mmol) was added dry THF (0.5 mL) under nitrogen. The solution was stirred for 16 h and concentrated under reduced pressure. Purification of the residue via MPLC on silica gel with ethyl acetate : hexanes (12 : 78 to 100 : 0) afforded 137 mg (77%) of **1-tris** as a yellow powder. ^{1}H NMR (500 MHz, acetone-d_6) δ 9.38 (br s, 6H), 7.98 (d, J = 8.5 Hz, 6H), 7.59 (s, 3H), 7.58 (d, J = 8.5 Hz, 6H), 7.50 – 7.43 (m, 9H), 7.34 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ 181.7, 146.9, 144.9, 140.6, 139.9 136.4, 132.0, 130.3, 130.1, 129.7, 129.2, 125.0, 124.5. HRMS-ESI: calcd for $C_{45}H_{33}N_9O_6S_3Na$ $(M + Na)^+$ 914.1608, found 914.1618.

1,3-bis(2-(3-(4-Nitrophenyl)thioureido)phenyl)benzene (1-bis). To a mixture of 1,3-bis(2-aminophenyl)benzene (**3-bis**, 52 mg, 0.20 mmol)[29] and 4-nitrophenylisothiocyanate (79 mg, 0.44 mmol) was added dry THF (0.5 mL) under nitrogen. The solution was stirred for 22 h and concentrated under reduced pressure. Medium pressure liquid chromatography on silica gel of the residue with ethyl acetate : hexanes (12 : 78 to 100 : 0) afforded 124 mg (100%) of **1-bis** as a yellow powder (R_f = 0.24 (EtOAc : hexanes, 50 : 50)). ^{1}H NMR (500 MHz, acetone-d_6) δ 9.36 (br s, 2H), 9.25 (br s, 2H), 8.04 (d, J = 9.5 Hz, 4H), 7.61 (d, J = 8.5 Hz, 4H), 7.59 (s, 1H), 7.52 (d, J = 7.5 Hz, 2H), 7.49 (app s, 3H), 7.44 (d, J = 9.0 Hz, 2H), 7.43 (t, J = 9.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, acetone-d_6) δ 181.8, 147.1, 144.8, 140.4, 140.0, 136.5, 132.1, 130.6, 130.2, 130.0, 129.7, 129.3, 129.0, 125.0, 124.2. HRMS-ESI: calcd for $C_{32}H_{24}N_6O_4S_2Na$ $(M + Na)^+$ 643.1193, found 643.1195.

1-(4-Nitrophenyl)-3-phenylthiourea (1-mono). To a mixture of aniline (91 μL, 1.0 mmol) and 4-nitrophenylisothiocyanate (180 mg, 1.0 mmol) was added dry THF (1.0 mL) under nitrogen. The solution was stirred for 5 h, concentrated under reduced pressure, and the residue was recrystallized from toluene to afford 189 mg (69%) of **1-mono** as a yellow powder. Its spectral data were consistent with those that have been previously reported[31].

1,3,5-tris(2-(3-(3,5-Trifluoromethylphenyl)thioureido)phenyl)benzene (4-tris). To a solution of tris-aniline **3-tris** (176 mg, 0.50 mmol) in dry THF (1.0 mL) was added 3,5-trifluoromethylphenylisothiocyanate (0.33 mL, 1.8 mmol) under nitrogen. The resulting solution was stirred for 20 h and then concentrated under reduced pressure. Medium pressure liquid chromatography on silica gel of the residue with ethyl acetate : dichloromethane (0 : 100 to 5 : 95) afforded 170 mg (29%) of **4-tris** as a white powder (R_f = 0.47 (EtOAc : DCM, 5 : 95)). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (br s, 3H), 7.71 (br s, 3H), 7.66 (s, 6H), 7.56 (s, 3H), 7.51 –

7.42 (m, 12H), 7.36 (d, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.1, 139.3, 138.7, 137.5, 133.0, 131.8 (q, J_{C-F} = 34 Hz), 131.1, 130.0, 129.5, 128.7, 128.3, 124.5, 122.7 (q, J_{C-F} = 271 Hz), 119.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ -63.5. HRMS-ESI: calcd for $C_{51}H_{30}F_{18}N_6S_3Na$ $(M + Na)^+$ 1187.1299, found 1187.1263.

Binding constant determinations

Dilute non-aggregating solutions of a receptor S were titrated with a given anion X^- via a solution of its tetrabutylammonium salt and were monitored by UV-vis spectroscopy. Unless otherwise stated, UV absorption spectra were recorded three times for each solution from 250-600 nm and average values at each wavelength were used after correcting for the absorption of the solvent. The wavelength with the maximum change in the concentration corrected absorption (A_{cor} = $A \cdot C_0/C$, where C and C_0 are the current and the initial receptor concentrations) was determined and then used for further analysis. Absorptions were plotted versus anion concentrations and the binding constants were determined by an iterative non-linear least squares curve fitting routine implemented in Excel using the equations below[36] where K = the binding constant for 1:1 complex formation, $[S]$ = free receptor concentration, $[S]_0$ = total receptor concentration, $[SX]$ = bound receptor concentration, $[X]_0$ = total anion concentration, A = absorption of the solution, A_0 = absorption of the initial solution without any added anion, A_{max} = absorption of the solution if all the receptor is bound (infinite anion excess at the initial receptor concentration).

$$[SX] = \frac{K[S]_0 + K[X]_0 + 1 - \sqrt{(K[S]_0 + K[X]_0 + 1)^2 - 4K^2[S]_0[X]_0}}{2K}$$

$$A = A_0 \frac{[S]}{[S]_0} + A_{max} \frac{[SX]}{[S]_0}$$

Observed and calculated absorptions are given for each titration in the subsequent tables and figures. In cases where there is a large excess of the anion, $[X] \approx [X]_0$, and a plot of $\Delta A_{cor}/[X]$ versus ΔA_{cor} gives a straight line where the slope of the linear least squares fit of the data is the binding constant (K) and the intercept is $K \cdot \Delta A_{max}$ (eq. 1), where $\Delta A_{cor} = (A - A_0) \cdot C_0/C$, C and C_0 are the current and the initial receptor concentrations and $\Delta A_{max} = A_{max} - A_0$.

$$\frac{\Delta A_{cor}}{[X]} = -\Delta A_{cor} \cdot K + K \cdot \Delta A_{max} \quad (1)$$

Phosphate extraction from water into chloroform with 4-tris

A 10 mM solution of **4-tris** in chloroform (1.06 mL) and a 100 mM solution of tetrabutylammonium dihydrogen phosphate in water (0.30 mL) were placed in a 1 dram vial. The mixture was stirred vigorously for 30 minutes after which the organic layer was pipetted out and passed through a small piece of cotton. Diphenylmethane and triphenylphosphine were added as internal standards and integration of the ^{1}H NMR peaks at 3.99 ppm (the CH_2 of diphenylmethane) and 2.85 ppm (the CH_2's adjacent to nitrogen in the tetrabutylammonium ion) and the ^{31}P signals at -6.0 ppm (PPh_3) and 4.0 ppm (a broad singlet for the extracted species) gave the tetrabutylammonium cation and phosphorous concentrations in the organic phase (i.e., 11.8 mM and 5.1 mM, respectively). Similarly, a 2 mM solution of **4-tris** in chloroform (1.0 mL) was utilized to extract 0.5 equivalents of various volumes of aqueous solutions of tetrabutylammonium dihydrogen phosphate and tetrabutylammonium hydrogen phosphate. Extraction efficiencies were determined based on the amount of the tetrabutylammonium cation found in the organic phase as described above and are summarized in Table 2. The effects of complexation and deprotonation on the ^{31}P NMR chemical shift of dihydrogen phosphate was investigated by adding **4-tris** and/or tetrabutylammonium hydroxide to $Bu_4N^+H_2PO_4^-$ in $CDCl_3$.

Triphenylphosphine at -6.0 ppm was employed as a reference, and the resulting data are given in Table S20.

Computations

B3LYP[37,38] geometry optimizations were carried out with the 6-31+G(d,p) basis set using Gaussian 09[39] on work stations at the Minnesota Supercomputer Institute for Advanced Computational Research.

Conclusions

The first neutral receptor that binds dihydrogen phosphate strongly and selectively in the competitive environment of aqueous DMSO is reported. If chloroform is used as a solvent, extraction of phosphate in its dibasic form can be achieved from aqueous micromolar solutions. This indicates that this host and its derivatives are promising compounds for applications in therapeutics and medical and environmental analysis.

Acknowledgments

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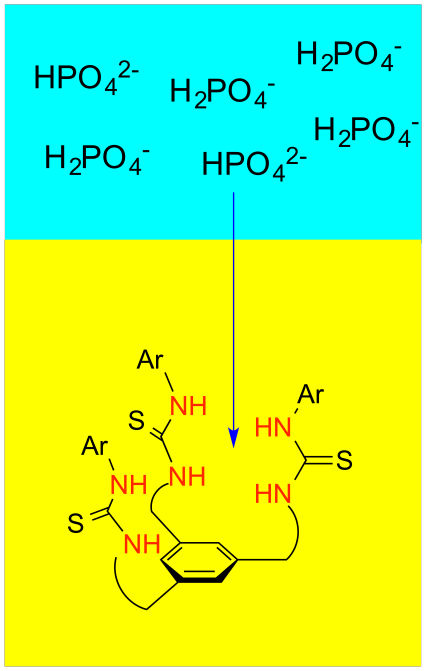
Table of Content Graphic



A three-armed thiourea host that strongly and selectively binds $H_2PO_4^-$ and extracts HPO_4^{2-} from water into chloroform.