A chemosensor for dihydrogenphosphate based on an oxoazamacrocycle possessing three thiourea arms

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We report a new H-bond macrocyclic chromogenic chemosensor in organic media, H$_4$L, which displayed drastic changes in the UV-Vis spectra revealing selectivity for dihydrogenphosphate over other inorganic anions, such as acetate or fluoride. The X-ray crystal structures of the [H$_4$L $\cdot$ NO$_3$](CH$_3$CN)$_4$ and [H$_4$L $\cdot$ CF$_3$CO$_2$](CH$_3$CN)$_2$ salt complexes are also reported.

Introduction

The molecular recognition and sensing of anionic analytes is an area of increasing research activity, mainly because of the importance of these species in many biological and chemical processes. Sensing of phosphates and their derivatives is of special importance as they compose the backbone of DNA and RNA and also play crucial roles in signal transduction and energy storage in biological systems. Even though a number of sensors for phosphate anions have been reported, there is still a need for simple receptors with improved optical response and selectivity.

Most chemosensors for inorganic anions that work in organic media are neutral receptors equipped with NH recognition units, such as amines, thioureas, amides, sulfonamides and pyroles. The NH groups of these devices usually act as H-bond donors, and the anion as an H-bond acceptor. The more acidic the donor, or the more basic the acceptor, the stronger the hydrogen bonding interaction. In a limiting situation, exceptionally acidic donors may be deprotonated on reaction with strongly basic acceptors. For fluoride anions the deprotonation process is also favoured due to the formation of the highly stable [HF$_2$]$^-$ self-complex. Acetate and phosphate anions can also promote the formation of [HX$_3$]$^-$ dimers, but their stability is lower. This explains why most of this class of chemosensors display greater response along the series F$^-$ $>$ CH$_2$CO$_2$$^-$ $>$ H$_2$PO$_4$$^-$ although the selectivity between F$^-$ and CH$_2$CO$_2$$^-$ is often poor. In this context, we have recently demonstrated how selectivity for fluoride can be enhanced by using an imine group as an intramolecular H-bond modulator. However, examples of H-bond receptors that show selectivity to dihydrogenphosphate against acetate or fluoride are very scarce. We present a new thiourea-based oxo-azamacroyclic chemosensor (Scheme 1, H$_4$L) which allows naked-eye detection of fluoride, acetate and dihydrogenphosphate anions in MeCN solution. This receptor shows high sensitivity for F$^-$, CH$_2$CO$_2$$^-$ and H$_2$PO$_4$$^-$ and, surprisingly, displays selectivity towards H$_3$PO$_4$$^-$ against the other two anions. The X-ray crystal structures of the [H$_4$L $\cdot$ NO$_3$](CH$_3$CN)$_4$ and [H$_4$L $\cdot$ CF$_3$CO$_2$](CH$_3$CN)$_2$ salt complexes are also discussed.

Experimental section

General information

Chemicals and solvents of the highest commercial grade available were used as received. Oxaazamacrocycle 1 was synthesized as described in the literature. High-Performance Liquid Chromatography (HPLC) was made using an Agilent 1100 series LC/MSD instrument. Analytical HPLC was run using a Phenomenex Luna C18 (250 x 4.60 mm) analytical reverse phase column. Retention Time (RT) for the ligand of 27.2 minutes and a m/z ratio of 925.1 corresponding to the quasy-molecular ion [M+H]$^+$. For the purification in the semi-preparative scale, we used a gradient from 50-85 % of solvent B for 30 minutes at a flow of 3 mL/min using a Waters Picogradient 2D where solvent A was 0.1%Trifluoroacetic acid. A: water with 0.1 % Trifluoroacetic acid; B: Acetonitrile with 0.1 % Trifluoroacetic acid.

FAB mass spectrometry (FAB-MS) was performed on a Micromass Autospec spectrometer employing m-nitrobenzyl alcohol as matrix. Electrospray ionization mass spectrometry (ESI-MS) was performed on an Agilent 1100 Series LC/MSD instrument in positive scan mode using direct injection. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer. NMR spectra were recorded on a Bruker AMX-500 spectrometer, using deuterated CD$_3$CN as solvent. UV-vis spectra were performed on a JASCO V-630 spectrophotometer equipped with a Peltier thermostat. All the UV-vis titrations experiments were performed on 2 mL samples of solutions of the receptor (20 µM) in CH$_3$CN, by addition of CH$_3$CN stock solutions of appropriate anion in the form of tetrabutylammonium salts. The UV/Vis titration data were fitted using the SPECTFIT/32 and the HYPERCHEM programmes.
Crystal structure determinations were performed at low temperature (100K) with a Bruker APEXII CCD diffractometer, using graphite-monochromated Mo-Kα radiation from a fine focus sealed tube source. All Computing data and reduction was made with the APPEX II software. Empirical absorption corrections were also applied. The structures were solved by direct methods using SHELX-97 and finally refined by full-matrix, least-squares based on F^2 by SHELXL. All non hydrogen atoms were anisotropically refined and redefined using a riding model, except the hydrogen atoms of N-H groups involved in H-Bonds that were located in a difference map and its position refined isotropically [Uiso(H) = 1.2Ueq(O)].

Receptor H3L synthesis (Scheme 1): A solution of 4-nitrophenylisothiocyanate (0.5 g, 3 mmol) in dry CH2Cl2 (25 mL) was added dropwise to a refluxing solution of the oxaazamacrocycle 1 (0.38 g, 1 mmol) in the same solvent (25 mL). The resulting solution was refluxed with magnetic stirring for 24h, and then evaporated to dryness under reduced pressure. The solid residue was dissolved in CHCl3 and extracted with deionized water. The organic phase was dried with MgSO4, filtered and concentrated to dryness under reduced pressure to give the desired pure product, confirmed by ESI+MS (see ESI).

Yield: 65%. Anal. Found: C, 53.5; H, 5.1; N, 14.5; S, 10.2; Calc. for C34H24Ni4O6S4: C, 53.7; H, 5.0; N, 14.6; S, 10.0. Mass spectrometry (FAB): m/z = 925 [H3L + H]+. Mass spectrometry (ESI): m/z = 925.3 [H3L + H]+. 1H NMR (500 MHz, CD3CN, 25 ºC, ppm): 8.86 (s), 8.03 (m, 6H), 7.61 (d, 2H), 7.49 (d, 2H), 7.29 (t, 2H), 7.24 (d, 2H), 7.02 (m, 2H), 5.14 (s, 4H), 4.38 (s, 4H), 3.96 (s, 4H), 3.66 (s, 2H), 2.91 (s, 2H), 7.49 (d, 4H), 7.29 (t, 2H), 7.24 (d, 2H), 7.02 (m, 4H), 5.14 (s, 4H), 4.38 (s, 4H), 3.96 (s, 4H), 3.66 (s, 2H), 2.91 (s, 4H), 2.85 (t, 2H). λ_{max} (ε, CH3CN) = 345 (36250 M^{-1} cm^{-1}) nm.

Results and discussion

Synthesis of H3L

Oxaazamacrocycle 1 was prepared following a previously reported method. Reaction of primary and secondary amines of 1 with 4-nitrophenylisothiocyanate in dry CH2Cl2 resulted in free receptor H3L (Scheme 1). This compound was purified by semi-preparative HPLC and characterized by a variety of techniques, including FAB and ESI mass spectrometry, UV/Vis and 1H NMR spectroscopy and elemental analysis (see ESI).

H3L is composed of a 17-membered oxaazamacrocycle skeleton equipped with three thiourea arms, two of them directly attached to the macrocycle body, with which they share a nitrogen atom, and the other one linked to the remaining nitrogen atom of the macrocycle through an alkyl spacer.

Anion binding studies

The interaction of H3L with a variety of inorganic anions was studied by UV/Vis titrations, which were performed in MeCN by addition of a standard solution of the corresponding tetraalkylammonium salt of the corresponding anion to a 20 µM solution of the receptor.

Figure 1 displays the family of absorption spectra obtained during titration of H3L with dihydrogenphosphate. The
absorption spectrum of $\text{H}_2\text{L}$ in acetonitrile has one band with a maximum at 345 nm, assigned to the charge transfer transition from thiourea to nitrobenzene. Titration with $\text{H}_3\text{PO}_4^-$ resulted in an initial red shift of the band at 345 nm and the formation of a new intense CT band at 470 nm, with an isosbestic point at 380 nm. A titration profile, obtained by plotting the molar absorbance at 470 nm vs. the concentration of $\text{H}_3\text{PO}_4^-$ in the media, is shown in the inset. This new band at 470 nm matches well with the absorption band generated when $\text{H}_3\text{L}$ reacts with tetrabutylammonium hydroxide, and can be assigned to the deprotonated receptor $\text{L}^-$ (see ESI). Moreover, the addition of dihydrogenphosphate or hydroxide induced a change in the color of the solution from pale to bright yellow (see ESI).

Analogous titration experiments were also carried out with $\text{F}^-$, $\text{CH}_3\text{CO}_2^-$, $\text{HSO}_4^-$, $\text{Cl}^-$, $\text{Br}^-$, and $\text{NO}_3^-$ (see ESI). We observed that only $\text{CH}_3\text{CO}_2^-$ and $\text{F}^-$ were able to deprotonate $\text{H}_3\text{L}$ in a similar way to $\text{H}_3\text{PO}_4^-$. However, spectral modifications on titration with $\text{HSO}_4^-$, $\text{Cl}^-$, $\text{Br}^-$, and $\text{NO}_3^-$ were very moderate, even after the addition of a large excess of anions, suggesting that only H-bonding takes places but not deprotonation.

The initial red shift of the bands at 345 nm suggests that the receptor $\text{H}_3\text{L}$ forms H-bonded adducts with $\text{H}_3\text{PO}_4^-$, $\text{CH}_3\text{CO}_2^-$ and $\text{F}^-$, when the anion concentration in the media is low.\(^{40,21}\)

In order to gain some insight about these processes, we decided to carry out further UV-vis titration experiments in the range between 0-10 eq. of the corresponding anion (see ESI). In the three experiments, the 345 nm band of $\text{H}_3\text{L}$ undergoes a bathochromic shift after the addition of the corresponding anion. In the case of $\text{F}^-$ and $\text{CH}_3\text{CO}_2^-$, the formation of the 470 nm band is observed even at very low concentration, indicating that the deprotonation processes are competing with the adduct formation. However, the formation of this new band is not observed during the titration experiment with $\text{H}_3\text{PO}_4^-$. These results suggest that the H-bonded adduct formation is favoured for dihydrogenphosphate with respect to fluoride or acetate.

**NMR studies**

![Figure 2](image)

**Figure 2.** $^1\text{H}$ NMR spectra taken over the course of the titration of a CD$_3$CN solution of $\text{H}_2\text{L}$ (0.6 mM) with a standard solution of $[\text{NBu}_4]\text{H}_3\text{PO}_4$.

The $^1\text{H}$ NMR spectra in CD$_3$CN of $\text{H}_2\text{L}$ show drastic changes upon addition of dihydrogenphosphate anions (Figure 2). The thioamide signal (8.86 ppm for free $\text{H}_2\text{L}$) rapidly shifts downfield when the concentration of anion is low, but it disappears in an excess of $\text{H}_3\text{PO}_4^-$. On the other hand, the nitrobenzene proton signals (8.03, 7.61 and 7.49 ppm) shift appreciably when anions are added, whereas the phenol protons (7.29, 7.24, 7.02 ppm) move very little. The other signals change little during the titration experiment. This NMR behaviour is very similar to those observed in the case of fluoride and acetate anions (see ESI).

It has to be noted that only one thioamide N-H signal has been observed in the $^1\text{H}$ NMR spectrum of the free receptor and, unfortunately, we have not been able to perform $^{31}\text{C}$ NMR experiments with receptor $\text{H}_3\text{L}$ in CD$_3$CN due to solubility problems. All these drawbacks prevent us from determining exactly which thioamide groups are involved in the adduct formation.

![Figure 3](image)

**Figure 3.** $^{31}\text{P}$ NMR spectra of CD$_3$CN solutions of 1) $\text{H}_3\text{PO}_4^-$ (10 mM), 2) NaH$_2$PO$_4$ (10 mM) and 3) $\text{H}_3\text{L}$ + 2 eq. NaH$_2$PO$_4$ (10 mM).

At this point, we decided to study the $\text{H}_3\text{PO}_4^-$ binding by $^{31}\text{P}$ NMR spectroscopy (Figure 3). The spectrum of NaH$_2$PO$_4$ in CD$_3$CN solution (10 mM) shows a unique signal at -3.6 ppm. An aliquot containing 0.5 eq. of the receptor $\text{H}_3\text{L}$ was added to this solution. The new $^{31}\text{P}$ spectrum shows a dramatic increase of the chemical shift in the phosphate signal (+4.5 ppm), which can be ascribed to a deshielding effect due to the adduct formation,\(^{22}\) as it cannot be ascribed to the formation of $\text{H}_2\text{PO}_4^-$.\(^{55}\)

Taking into account all these observations, we suggest that the receptor $\text{H}_3\text{L}$ forms H-bonded complexes at low concentrations of dihydrophosphate anion in acetonitrile solution, and that the excess of $\text{H}_3\text{PO}_4^-$ causes the deprotonation of the thiourea groups of $\text{H}_3\text{L}$.

**X-ray studies**

Attempts were made to obtain crystals suitable for X-ray diffraction studies for all the H-bonded complexes of $\text{H}_3\text{L}$ investigated in solution. As a general procedure, a CH$_3$CN solution containing $\text{H}_3\text{L}$ plus an excess of the selected anion was allowed to slowly evaporate at rt. At first, we used their tetrabutylammonium salts as anion source but, after several
failures, we decided to use the corresponding acids (1% of concentrated acid). Suitable crystals were obtained with this last method in the case of the nitrate and trifluoroacetate anions. The main crystallographic data and bond distances are listed in the ESI. Figures 4 and 5 exhibit the stick representations of part of its crystal cells.

**Figure 4.** Stick representation of part of the crystal cell of the salt complex [H₃L • NO₃(CH₃CN)]₆, showing the inter and intramolecular H-bonds established.

The colourless crystalline product resulted from the crystallization of H₃L with HNO₃ consist of the protonated receptor, a nitrate counterion and four molecules of acetonitrile: [H₃L • NO₃(CH₃CN)]₆. Two of the three thiourea arms of the receptor point their N-H fragments towards the NO₃⁻ ion. The only thiourea group equipped with two N-H units establishes with the nitrate a bifurcate interaction [H₅A ⋅ ⋅ ⋅ O9 1.98(3) Å; H₆A ⋅ ⋅ ⋅ O10 2.12(2) Å]. One of the two remain thiourea groups of H₃L interacts with one of the nitrate oxygens using its single N-H group [H₉A ⋅ ⋅ ⋅ O9 2.59(10) Å; H₉A ⋅ ⋅ ⋅ O11 2.41(4) Å], and the N-H group of the third thiourea arm is turned to the outside to allow an N-H ⋅ ⋅ ⋅ O interaction with one of the oxygen atoms of a nitro group of a neighbor H₃L molecule [H₃ ⋅ ⋅ ⋅ O6’ 2.10(2) Å; symmetry code: (i) x, y, z+1]. Then, two of the oxygen atoms of NO₃⁻ form H-bonds with the N-H groups of two of the thiourea arms of H₃L, whereas its third oxygen atom remains unbounded. The four molecules of acetonitrile present in the crystal cell of H₃L, which have been omitted for clarity in the figures, do not interact with the salt complex.

The crystallization of H₃L with TFA in acetonitrile was also successful. In this case the crystal cell consists of the protonated receptor, one CF₃CO₂⁻ counterion and two acetonitrile molecules: [H₃L • CF₃CO₂⁻(CH₃CN)]₂. This crystal structure is very similar to that described above for nitrate. Only two of the three thiourea arms of the receptor interact with the TFA anion. The thiourea group equipped with two N-H units establishes a bifurcate interaction with the CF₃CO₂⁻ [H₅V ⋅ ⋅ ⋅ O9h 2.02(4) Å; H₆V ⋅ ⋅ ⋅ O10h 2.11(4) Å; symmetry code: (ii) −x+2, −y+z, −z+1]. Moreover, another thiourea group interacts with one of the oxygens of TFA using its single N-H group [H₉V ⋅ ⋅ ⋅ O9h 2.19(5) Å]. The N-H group of the remaining thiourea arm is turned to the outside of the receptor cavity to allow an N-H ⋅ ⋅ ⋅ N interaction with one acetonitrile molecule [H₃N ⋅ ⋅ ⋅ N11h 2.14(4) Å; symmetry code: (iii) −x+1, −y+1, −z+1]. We believe this crystal structure could be useful to speculate about the possible structural rearrangement of the H₃L:acetate adduct, as CH₃CO₂⁻ and CF₃CO₂⁻ have almost identical structures.

**Figure 5.** Stick representation of part of the crystal cell of the salt complex [H₃L • CF₃CO₂−(CH₃CN)]₂, showing the inter and intramolecular H-bonds established.

**Equilibrium constants**

The data collected suggest that changes in the UV/Vis and NMR spectra of H₃L in MeCN in the presence of an excess of fluoride, acetate or dihydrogenphosphate anions is the consequence of the deprotonation of the sensor and the formation of the anionic specie L⁻. Best fitting curves of the UV-Vis titration data were obtained when assuming that the deprotonation process follow the acid-base reaction equilibrium below:¹⁴b,¹⁵

\[
\text{H}_3\text{L} + 3\text{X}^- \leftrightarrow \text{L}^3^- + 3\text{HX} (\beta_0) \quad (1)
\]

This equilibrium is progressively displaced to the right on addition of an excess of X⁻ and the formation of [HX₃⁻] dimers:²³

\[
\text{HX} + \text{X}^- \leftrightarrow \text{HX}_2^- \quad (2)
\]

The overall equilibrium, with a stoichiometry of 1:6 for H₃L-X interactions, can be obtained combining eqn (1) and (2):¹⁴b,¹⁵

\[
\text{H}_3\text{L} + 6\text{X}^- \leftrightarrow \text{L}^3^- + 3\text{HX}_2^- \quad (3)
\]

This two-step equilibrium can be applied to the deprotonation processes promoted by the anions F⁻, CH₃CO₂⁻ and HPO₄²⁻, as all of them can form HX₂⁻ dimers.¹¹,¹²,¹³b,¹⁵,²²

Interestingly, the value of log β₀ (equation 1) for H₂PO₄⁻ [13.32(3)] is higher than those calculated for F⁻ [11.95(12)] and CH₃CO₂⁻ [11.94(11)] (see ESI).

Since the basicity of these anions decreases on the series F⁻ > CH₃CO₂⁻ > H₂PO₄⁻, the observed tendency in the value of β₀ for this system could be only explained on the basis of molecular properties of the receptor-anion interaction.¹⁹,²³ As
is a global deprotonation constant of a stepwise equilibrium which leads to the deprotonated form of the receptor, the stability of the intermediates formed during this process must affect its final value.

Further spectrophotometric studies in CH₃CN solution showed that F, CH₂CO₂⁻ and H₂PO₄⁻ form 1:1 adducts with receptor H₃L at low concentration of the corresponding anion:

\[
H₃L + X \leftrightarrow [H₃L \cdot X]^- (K)
\]

We have found that the value of log K (equation 4) for H₂PO₄⁻ [4.31(14)] is higher than those calculated for F [2.21(4)] and CH₂CO₂⁻ [3.89(6)] (see ESI).²⁵ This suggests that, at low concentrations of anion in the media, the tetrahedral H₂PO₄ ion forms a more stable H-bonded complex than the spherical F or the triangular planar CH₂CO₂ with receptor H₃L.²⁵ This adduct is capable of out-balancing the more basic contribution of fluoride and, to a lesser degree, acetate anions with respect to dihydrogenphosphate, it is also able to out-balance the higher stability of [HF₄]⁻ and [H(CH₂CO₂)₂]⁻ self-complexes in comparison to [H(H₂PO₄)₂]⁻.²⁶

At this point, we decided to carry out molecular modelling studies in order to gain some insight into the structural features of the [H₃L \cdot H₂PO₄] supramolecular adduct. Figure 6 shows the structure of this adduct, as calculated by a semiempirical method (AM1) by using the SPARTAN software. It shows that H₃L adopts a cone conformation reminiscent with those of calixarenes, with the NH donor groups facing the inside. Noticeably, the dihydrogenphosphate ion interacts with all the thioamide NH groups of the H₃L receptor. It has to be noted that examples of tripodal receptors such as H₃L suitable for accommodating phosphate anions have been previously reported.²⁶

![Figure 6](image)

Figure 6. Optimized structure for the [H₃L \cdot H₂PO₄] adduct, calculated with a semiempirical method (AM1) with SPARTAN software, showing the hydrogen-bonding interactions of the dihydrogenphosphate anion with the three thioarea arms of the receptor.

Conclusions

In summary, we have developed a new colorimetric H-bond sensor based on the deprotonation of its three thioarea donor fragments in the presence of basic inorganic anions such as fluoride, acetate or dihydrogenphosphate. The H-bond complexation/deprotonation mechanism has been demonstrated by spectrophotometric titrations and by NMR spectroscopy. The equilibrium constants, calculated using the UV-vis titration data, for H₂PO₄⁻ are two order of magnitude larger than those for CH₂CO₂⁻ and F, making this receptor selective for this anion.

The UV-vis and ¹H and ³¹P NMR titration experiments, as well as the X-ray crystal structures of the [H₃L \cdot NO₃](CH₃CN)₂ and [H₃L \cdot CF₃CO₂](CH₃CN)₂ salt complexes,²⁷ suggest that the receptor forms H-bonded complexes of the type [H₃L \cdot X] when the concentration of anion in the media is low (in the case of F, CH₂CO₂⁻ and H₂PO₄⁻), or at high concentrations of the other inorganic anions studied. From the equilibrium constants it seems that the receptor H₃L forms much more stable H-bonded intermediate complexes with the tetrahedral H₂PO₄ anion than with the spherical F or the triangular planar CH₂CO₂, which could explain this unusual behavior.

We are currently modifying the structure of H₃L in order to modulate its affinity and selectivity.

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⁴ Electronic supplementary information (ESI) available: Crystallographic data for [H₃L \cdot NO₃](CH₃CN)₂ and [H₃L \cdot CF₃CO₂](CH₃CN)₂. Selected bond distances and angles for [H₃L \cdot NO₃](CH₃CN)₂ and [H₃L \cdot CF₃CO₂](CH₃CN)₂. H¹ NMR spectra of H₃L. H¹ NMR titrations of H₃L with F, CH₂CO₂⁻ and H₂PO₄⁻. Spectrophotometric titrations of H₃L with OH⁻, F, CH₂CO₂⁻ and NO₃⁻. Fit of the equilibrium constants calculated for F⁻, CH₂CO₂⁻ and H₂PO₄⁻. CCDC reference numbers 833835 (nitrile salt) and 893706 (TFA salt).
