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**PAPER**

# 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, $\mathbf{H_3L}$, which displayed drastic changes in its UV–vis spectra revealing selectivity for dihydrogenphosphate over other inorganic anions, such as acetate or fluoride. The X-ray crystal structures of the $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ and $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$ 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.[1] 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.[2] Even though a number of chemosensors for phosphate anions have been reported,[1,3] 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 ureas,[4] thioureas,[5] amides,[6] sulfonamides[7] and pyrroles.[8] The NH groups of these devices usually act as H-bond donors, and the anion as an H-bond acceptor.[9] 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, such as fluoride, acetate or dihydrogenphosphate, whose basicity varies in the series $F^- > CH_3CO_2^- \geq H_2PO_4^-$.[10] For fluoride anions, the deprotonation process is also favoured due to the formation of the highly stable $[HF_2]^-$ self-complex.[11] Acetate and dihydrogenphosphate anions can also promote the formation of $[HX_2]^-$ dimers, but their stability is lower.[12] This explains why most of this class of chemosensors display greater response along the series $F^- > CH_3CO_2^- \geq H_2PO_4^-$,[13] although the selectivity is often poor.[14] 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.[15] However, examples of H-bond receptors that show selectivity for dihydrogenphosphate over acetate or fluoride are very scarce.[12b,16]

We present a new thiourea-based oxoazamacrocyclic chemosensor (Scheme 1, $\mathbf{H_3L}$) which allows naked-eye detection of fluoride, acetate and dihydrogenphosphate anions in MeCN solution. This receptor shows high sensitivity for $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$ and, surprisingly, displays selectivity towards $H_2PO_4^-$ over the other two anions. The X-ray crystal structures of the $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ and $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$ salt complexes are also discussed.

## Experimental section

### General information

Chemicals and solvents of the highest commercial grade available were used as received. Oxoazamacrocycle **1** was synthesized as described in the literature.[17]

High-Performance Liquid Chromatography (HPLC) was carried out using an Agilent 1100 series LC/MSD instrument. Analytical HPLC was run using a Phenomenex Luna C18 (250 × 4.60 mm) analytical reverse phase column using an Agilent 1100 HPLC equipped with a Mass Spectrometry detector Agilent LC/MSD VL. The purification of $\mathbf{H_3L}$ was

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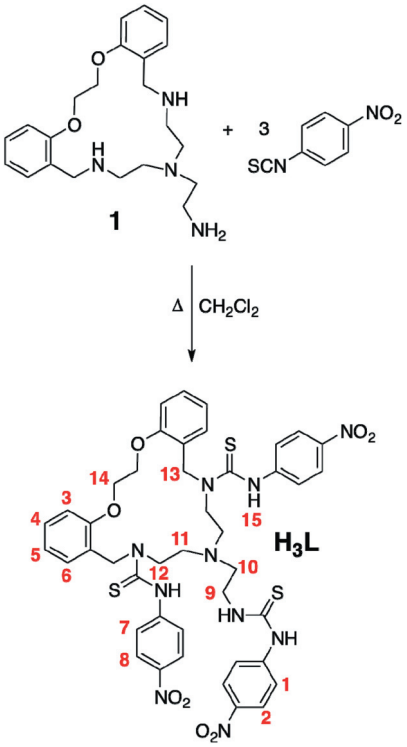
† Electronic supplementary information (ESI) available: Crystallographic data for $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ and $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$. Selected bond distances and angles for $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ and $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$. $^1$H NMR spectra of $\mathbf{H_3L}$. $^1$H NMR titrations of $\mathbf{H_3L}$ with $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$. Spectrophotometric titrations of $\mathbf{H_3L}$ with $OH^-$, $F^-$, $CH_3CO_2^-$ and $NO_3^-$. Fit of the equilibrium constants calculated for $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$. CCDC 833635 (nitrate salt) and 863706 (TFA salt). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25498k




**Scheme 1** Synthesis and hydrogen labelling scheme of receptor $H_3L$.

performed on a Phenomenex Luna C18 (250 × 10 mm) semi-preparative reverse phase column. Standard conditions for analytical RP-HPLC consisted of an isocratic regime during the first 5 min, followed by a linear gradient from 15 to 95% of solvent B for 30 min at a flow of 1 mL $min^{-1}$ with a Retention Time (RT) for the ligand of 27.2 min and a *m/z* ratio of 925.1 corresponding to the quasi-molecular ion $[M + H]^+$. For the purification in the semi-preparative scale, we used a gradient from 50–85% of solvent B for 30 min at a flow of 3 mL $min^{-1}$, in this conditions the RT reduced to 19.0 min. 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 $CD_3CN$ 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 $CH_3CN$, by addition of $CH_3CN$ 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 (100 K) 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.[18] The structures were solved by direct methods using SIR-97,[19] and finally refined by full-matrix, least-squares based on $F^2$ by SHELXL.[20] All non hydrogen atoms were anisotropically refined and the hydrogen atoms positions geometrically calculated and refined using a riding model, except the hydrogen atoms of N–H groups involved in H-Bonds that were located in a difference map and their position refined isotropically [Uiso(H) = 1.2Ueq(O)].

### Synthesis

**Synthesis of receptor $H_3L$ (Scheme 1).** A solution of 4-nitrophenylisothiocyanate (0.5 g, 3 mmol) in dry $CH_2Cl_2$ (25 mL) was added dropwise to a refluxing solution of the oxoazamacrocycle **1** (0.38 g, 1 mmol) in the same solvent (25 mL). The resulting solution was refluxed with magnetic stirring for 24 h, and then evaporated to dryness under reduced pressure. The solid residue was dissolved in $CHCl_3$ and extracted with deionized water. The organic phase was dried with $MgSO_4$, filtered and concentrated to dryness in a rotatory evaporator. The yellow solid residue was purified by HPLC using MeOH–$H_2O$ 0.1% TFA as eluent. Collected fractions were partly concentrated under reduced pressure at 30 °C to eliminate the MeOH, then frozen and freeze dried, using a *Thermo ModulyoD* drier from *Thermo Scientific*, to give the desired product as a TFA salt. This salt was dissolved in water, neutralized with NaOH 1M and extracted with dichloromethane. The organic phases were dried ($MgSO_4$), 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 $C_{43}H_{44}N_{10}O_8S_3{\cdot}2H_2O$: C, 53.7; H, 5.0; N, 14.6; S, 10.0 Mass spectrometry (FAB): *m/z* = 925 $[H_3L + H]^+$. Mass spectrometry (ESI): *m/z* = 925.3 $[H_3L + H]^+$. $^1H$ NMR (500 MHz, $CD_3CN$, 25 °C, ppm): 8.86 (hydrogen 15 in Scheme 1, bs), 8.03 (*2* + *8*, m, 6H), 7.61 (*1*, d, 2H), 7.49 (*7*, d, 4H), 7.27 (*5* + *6*, m, 4H), 7.02 (*4* + *3*, m, 4H), 5.14 (*14*, bm, 4H), 4.38 (*13*, s, 4H), 3.96 (*12*, bm, 4H), 3.66 (*9*, bm, 2H), 2.88 (*10* + *11*, m, 6H). $\lambda_{max}$ ($\varepsilon$, $CH_3CN$) = 345 (36 250 $M^{-1}$ $cm^{-1}$) nm.

## Results and discussion

### Synthesis of $H_3L$

Oxoazamacrocycle **1** was prepared following a previously reported method.[20] Reaction of primary and secondary amines of **1** with 4-nitrophenylisothiocyanate in dry $CH_2Cl_2$ resulted in free receptor $H_3L$ (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†).

$H_3L$ is composed of a 17-membered oxoazamacrocycle 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.

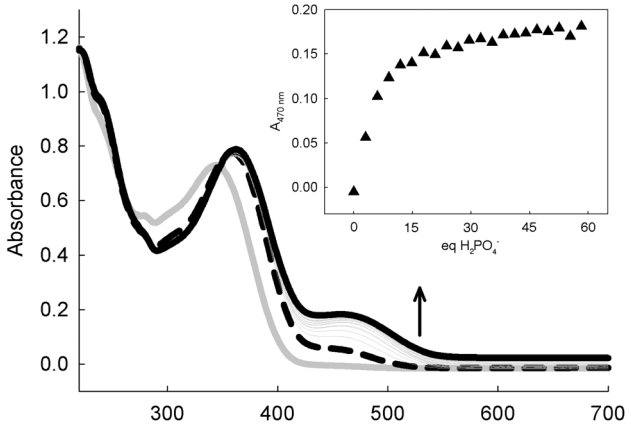



**Fig. 1** Spectrophotometric titration of a $CH_3CN$ solution 20 μM in $\mathbf{H_3L}$ with a standard solution of dihydrogenphosphate ions. Inset: absorbance at 470 nm *vs.* concentration of dihydrogenphosphate ions.

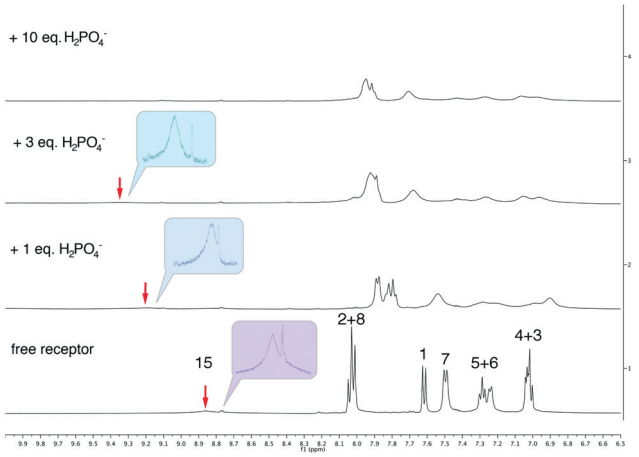



**Fig. 2** $^1$H NMR spectra taken over the course of the titration of a $CD_3CN$ solution of $\mathbf{H_3L}$ (0.6 mM) with a standard $CD_3CN$ solution of $[NBu_4]H_2PO_4$.

### Anion binding studies

The interaction of $\mathbf{H_3L}$ 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.

Fig. 1 displays the family of absorption spectra obtained during titration of $\mathbf{H_3L}$ with dihydrogenphosphate. The absorption spectrum of $\mathbf{H_3L}$ in acetonitrile has one band with a maximum at 345 nm, assigned to the charge transfer transition from thiourea to nitrobenzene. Titration with $H_2PO_4^-$ 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 $H_2PO_4^-$ in the media, is shown in the inset. This new band at 470 nm matches well with the absorption band generated when $\mathbf{H_3L}$ reacts with tetrabutylammonium hydroxide, and can be assigned to the deprotonated receptor $\mathbf{L^{3-}}$ (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 $F^-$, $CH_3CO_2^-$, $HSO_4^-$, $Cl^-$, $Br^-$, $I^-$ and $NO_3^-$ (see ESI†). We observed that only $CH_3CO_2^-$ and $F^-$ were able to deprotonate $\mathbf{H_3L}$ in a similar way to $H_2PO_4^-$. However, spectral modifications on titration with $HSO_4^-$, $Cl^-$, $Br^-$, $I^-$ and $NO_3^-$ were very moderate, even after the addition of a large excess of anions. It has to be noted that detection of acetate, fluoride or dihydrogenphosphate by $\mathbf{H_3L}$ does not suffer interferences from the other anions used in this study. On the contrary, the receptor remains sensitive to the addition of $F^-$ or $CH_3CO_2^-$ in the presence of $H_2PO_4^-$.

The initial red shift of the bands at 345 nm suggests that the receptor $\mathbf{H_3L}$ forms H-bonded adducts with $H_2PO_4^-$, $CH_3CO_2^-$ and $F^-$, when the anion concentration in the media is low.[4b,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 $\mathbf{H_3L}$ undergoes a bathochromic shift after the addition of the corresponding anion. In the case of $F^-$ and $CH_3CO_2^-$, the formation of the 470 nm band is observed even at very low concentration, indicating that the deprotonation process is always competing with the adduct formation. However, the formation of this new band is not observed during the titration experiment with $H_2PO_4^-$.

A linear response was observed when the detection was carried out for $H_2PO_4^-$ concentration in the range of 5–50 ppm (see ESI†). The limit of detection (LOD) of the method, defined as the concentration equivalent to a signal of blank plus five times the standard deviation of the blank, is calculated to be 37.6 ppm.

### NMR studies

The $^1$H NMR spectra of $\mathbf{H_3L}$ in $CD_3CN$ (see ESI†) show drastic changes upon addition of dihydrogenphosphate anions (Fig. 2). The thioamide signal (8.86 ppm for free $\mathbf{H_3L}$, hydrogen *15* in Scheme 1) rapidly shifts downfield when the concentration of anion is low, but it disappears in an excess of $H_2PO_4^-$. On the other hand, the nitrobenzene proton signals [8.03 (*2* + *8*), 7.61 (*1*) and 7.49 (*7*) ppm] shift appreciably when anions are added, whereas the phenol protons [7.27 (*5* + *6*), 7.02 (*4* + *3*) ppm] move very little. The aliphatic signals change little during the titration experiment. This NMR behaviour is very similar to that 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 $^1$H NMR spectrum of the free receptor and, unfortunately, we have not been able to perform $^{13}$C NMR experiments with receptor $\mathbf{H_3L}$ in $CD_3CN$ due to solubility problems. All these drawbacks prevent us from determining exactly which thioamide groups are involved in the adduct formation.

At this point, we decided to study the $H_2PO_4^-$ binding by $^{31}$P NMR spectroscopy (Fig. 3). The spectrum of $NaH_2PO_4$ in $CD_3CN$ solution (10 mM) shows a unique signal at −3.6 ppm. An aliquot containing 0.5 eq of the receptor $\mathbf{H_3L}$ was added to this solution. The new $^{31}$P spectrum shows a dramatic increase in the chemical shift of the phosphate signal (+4.5 ppm), which

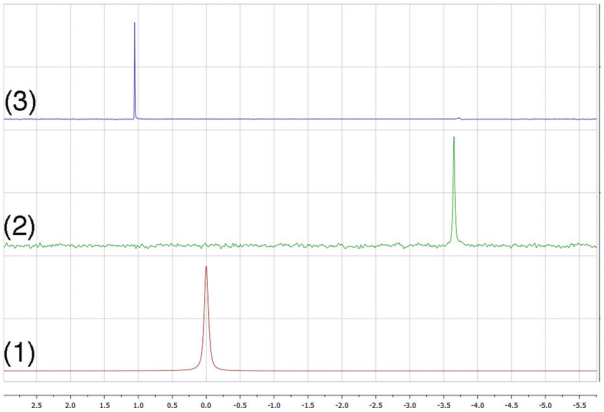



**Fig. 3** $^{31}P$ NMR spectra of $CD_3CN$ solutions of (1) $H_3PO_4$ (10 mM), (2) $NaH_2PO_4$ (10 mM) and (3) **$H_3L$** + 2 eq. $NaH_2PO_4$ (10 mM).

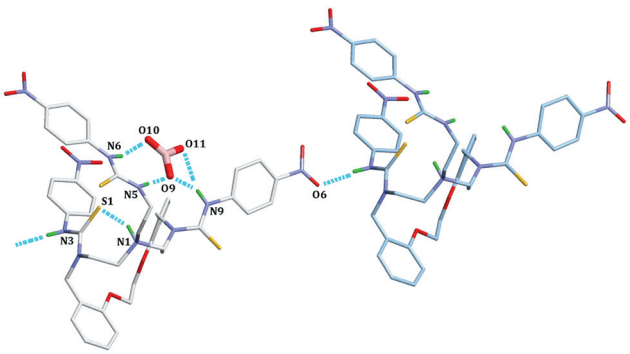



**Fig. 4** Stick representation of part of the crystal cell of the salt complex $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$, showing the inter and intramolecular H-bonds established.

can be ascribed to a deshielding effect due to the adduct formation,[22] as it cannot be ascribed to the formation of $H_3PO_4$.

Taking into account all these observations, we suggest that the receptor **$H_3L$** forms H-bonded adducts at low concentrations of dihydrogenphosphate anion in acetonitrile solution, and that the excess of $H_2PO_4^-$ causes the deprotonation of the thiourea groups of **$H_3L$**.

### X-Ray studies

Attempts were made to obtain crystals suitable for X-ray diffraction studies for all the H-bonded complexes of **$H_3L$** investigated in solution. As a general procedure, a $CH_3CN$ solution containing **$H_3L$** plus an excess of the selected anion was allowed to slowly evaporate at room temperature. At first, we used their tetrabutylammonium salts as the 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.† Fig. 4 and 5 exhibit the stick representations of part of their crystal cells.

The colourless crystalline product resulting from the crystalization of **$H_3L$** with $HNO_3$ consists of the protonated receptor (the

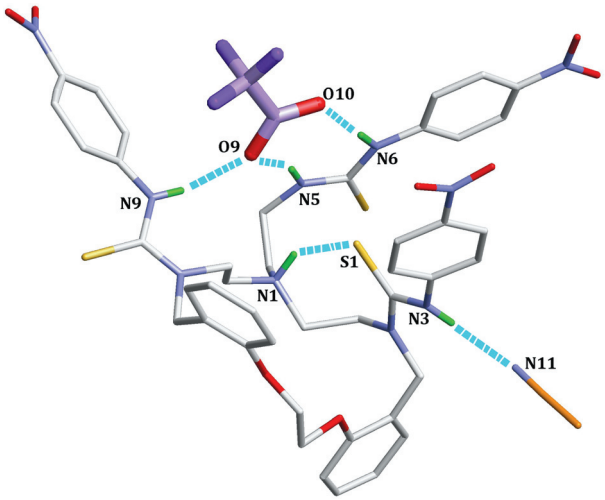



**Fig. 5** Stick representation of part of the crystal cell of the salt complex $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$, showing the inter and intramolecular H-bonds established.

protonation site is the amine group N1), a nitrate counterion and four molecules of acetonitrile: $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ (Fig. 4). Two of the three thiourea arms of the receptor point their N–H fragments towards the $NO_3^-$ ion. The only thiourea group equipped with two N–H units establishes with the nitrate a bifurcate interaction [H5*A*···O9 1.98(3) Å; H6*A*···O10 2.12(2) Å]. One of the two remaining thiourea groups of **$H_3L$** interacts with one of the nitrate oxygens using its single N–H group [H9*A*···O9 2.59(10) Å; H9*A*···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 neighboring **$H_3L$** molecule [H3···O6$^{i}$ 2.10(2) Å; symmetry code: (i) *x*, *y*, *z* + 1]. Then, two of the oxygen atoms of $NO_3^-$ form H-bonds with the N–H groups of two of the thiourea arms of **$H_3L$**, whereas its third oxygen atom remains unbonded. The four molecules of acetonitrile present in the crystal cell of **$H_3L$**, which have been omitted for clarity in the figures, do not interact with the salt complex.

The crystalization of **$H_3L$** with TFA in acetonitrile was also successful. In this case the crystal cell consists of the protonated receptor, one $CF_3CO_2^-$ counterion and two acetonitrile molecules: $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$ (Fig. 5). 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 $CF_3CO_2^-$ [H5*N*···O9$^{ii}$ 2.02(4) Å; H6*N*···O10$^{ii}$ 2.11(4) Å; symmetry code: (ii) –*x* + 2, –*y* + 1, –*z* + 1]. Moreover, another thiourea group interacts with one of the oxygens of TFA using its single N–H group [H9*N*···O9$^{ii}$ 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 [H3*N*···N11$^{iii}$ 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 **$H_3L$**:acetate adduct, as $CH_3CO_2^-$ and $CF_3CO_2^-$ have almost identical structures.

### Equilibrium constants

The data collected suggest that changes in the UV–vis and NMR spectra of **$H_3L$** in MeCN in the presence of an excess of fluoride, acetate or dihydrogenphosphate anions are the consequence of the deprotonation of the chemosensor and the formation of the anionic specie $\mathbf{L^{3-}}$. Best fitting curves of the UV–vis titration data were obtained when assuming that the deprotonation process follows the acid–base reaction equilibrium below:[14b,15]

$$\mathbf{H_3L} + 3X^- = \mathbf{L^{3-}} + 3HX\ (\beta_D) \quad (1)$$

This equilibrium is progressively displaced to the right on addition of an excess of $X^-$ and the formation of $[HX_2]^-$ dimers:[23]

$$HX + X^- = HX_2^- \quad (2)$$

The overall equilibrium, with a stoichiometry of 1 : 6 for **$H_3L$**–X interactions, can be obtained by combining eqn (1) and (2):[14b,15]

$$\mathbf{H_3L} + 6X^- = \mathbf{L^{3-}} + 3HX_2^- \quad (3)$$

This two-step equilibrium can be applied to the deprotonation processes promoted by the anions $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$, as all of them can form $HX_2^-$ dimers.[11,12,14b,15,22] Unfortunately, we do not have experimental evidence of the formation of these dimer species in our system.

Interestingly, the value of log $\beta_D$ [eqn (1)] for $H_2PO_4^-$ [13.32(3)] is higher than those calculated for $F^-$ [11.95(12)] and $CH_3CO_2^-$ [11.94(1)] (see ESI†). The value of log $\beta_D$ for $OH^-$, which could be used as reference, is of 14.48(6). Since the basicity of these anions decreases in the series $F^- > CH_3CO_2^- \geq H_2PO_4^-$, the observed anomalous tendency in the value of $\beta_D$ for this system could be explained on the basis of the influence of the molecular properties of the H-bonded adduct formation on the sensing properties of **$H_3L$**.[19,21] As $\beta_D$ is a global deprotonation constant of a stepwise equilibrium which leads to the deprotonated form of the receptor, it is reasonable that the stability of the intermediates formed during this process could affect its final value. In this context, spectrophotometric studies in $CH_3CN$ solution showed that $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$ form 1 : 1 adducts with receptor **$H_3L$** at low concentration of the corresponding anion following the reaction equilibrium:

$$\mathbf{H_3L} + X^- = [\mathbf{H_3L} \cdots X]^-(K) \quad (4)$$

We have found that the value of log $K$ [eqn (4)] for $H_2PO_4^-$ [4.31(14)] is higher than that calculated for $F^-$ [2.21(4)] and only slightly higher than that founded for $CH_3CO_2^-$ [3.89(6), see ESI†).[24] This suggests that, at low concentrations, the tetrahedral $H_2PO_4^-$ ion forms a more stable H-bonded complex with **$H_3L$** than the spherical $F^-$ does,[25] and a complex more or less as stable than that with the triangular planar $CH_3CO_2^-$. Thus, it seems that the formation of the $[\mathbf{H_3L}\cdots H_2PO_4]^-$ and $[\mathbf{H_3L}\cdots CH_3CO_2]^-$ adducts can, in some grade, out-balance the higher basicity of the fluoride ion, as well as the higher stability of $[HF_2]^-$ dimer in comparison to $[H(H_2PO_4)_2]^-$ and $[H(CH_3CO_2)_2]^-$,[12b] but it can't explain by itself the difference in the values of log $\beta_D$ between $CH_3CO_2^-$ and $H_2PO_4$. Therefore, in the light of these data, it seems that anion basicity, instead of anion recognition, is the property determining anion sensing in this system.

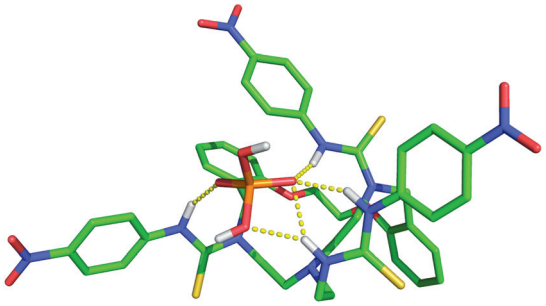


**Fig. 6** Optimized structure for the $[\mathbf{H_3L}\cdots H_2PO_4]^-$ adduct, as calculated with a semi-empirical method (AM1) with SPARTAN software, showing the hydrogen-bonding interactions of the dihydrogenphosphate anion with the three thiourea arms of the receptor.

### Molecular modelling studies

We carried out molecular modelling studies in order to gain some insight into the structural features of the $[\mathbf{H_3L}\cdots H_2PO_4]^-$ supramolecular adduct. Fig. 6 shows the structure of this adduct, as calculated by a semi-empirical method (AM1) by using the SPARTAN software. It shows that **$H_3L$** adopts a cone conformation reminiscent of those of calixarenes, with the NH donor groups facing the inside. Noticeably, the dihydrogenphosphate ion interacts with all the thioamide NH groups of the **$H_3L$** receptor. It has to be noted that examples of tripodal receptors such as **$H_3L$** suitable for accommodating phosphate anions have been previously reported.[26]

## Conclusions

In summary, we have developed a new colorimetric H-bond chemosensor based on the deprotonation of its three thiourea 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 deprotonation constants, calculated using the UV–vis titration data, for $H_2PO_4^-$ are two order of magnitude larger than those for $CH_3CO_2^-$ and $F^-$, making this receptor selective for this anion.

The UV–vis and $^1H$ and $^{31}P$ NMR titration experiments, as well as the X-ray crystal structures of the $[\mathbf{H_4L}\cdots NO_3]\cdot(CH_3CN)_4$ and $[\mathbf{H_4L}\cdots CF_3CO_2]\cdot(CH_3CN)_2$ salt complexes,[24] suggest that the receptor forms H-bonded complexes of the type $[\mathbf{H_3L}\cdots A]^-$ when the concentration of anion in the media is low (in the case of $F^-$, $CH_3CO_2^-$ and $H_2PO_4^-$), or at high concentrations of the other inorganic anions studied. From the equilibrium constants it seems that the receptor **$H_3L$** forms much more stable H-bonded intermediate complexes with $H_2PO_4^-$ or $CH_3CO_2^-$ than with $F^-$. The experimental and theoretical data collected suggest that anion basicity is the property determining anion sensing, although anion recognition is also influencing the deprotonation process.

We are currently modifying the structure of **$H_3L$** in order to modulate its affinity and selectivity.

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