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A novel chromo- and fluorogenic dual responding $H_2PO_4^-$ receptor based on an azo derivative

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ABSTRACT

A simple, novel anion receptor based on 4-hydroxy-3-(*N*-phenyl-thiourea-*N*′-nitrilo-methylidynyl)-azobenzene with hybrid –OH and thiourea binding sites was synthesized and characterized. Anion binding character was determined using visual inspection, UV–vis, fluorescence and ^{1}H NMR analyses. The addition of F^-, AcO^- and $H_2PO_4^-$ resulted in a marked red shift of the charge-transfer absorbance band ($\Delta\lambda = 140$ nm, from 340 nm to 480 nm) accompanied by a color change from light yellow to orange.

1. Introduction

Owing to the ubiquity of anions and their importance as agricultural fertilizers and industrial raw materials [1], the development of sensors and receptors for anions is still receiving considerable attention [2–4]. In particular, phosphates are biologically relevant anions that commonly occur; phosphorylated species play critical roles in a variety of fundamental processes such as genetic information storage, energy transduction, signal processing, and membrane transport [5]. In addition, phosphates can be found in many chemotherapeutic and antiviral drugs [6–8]. It is for sure that phosphate originating from the over use of agricultural fertilizers can also lead to eutrophication in inland waterways [9]. Accordingly, more and more attention has been received on the development of selective receptors for phosphate ions and phosphorylated biomolecules [10–13].

In many cases, a colorimetric and fluorescent receptor for special anionic species is of particular interest owing to its simplicity and high sensitivity [14,15]. In particular, colorimetric-based sensing is especially attractive, as it allows naked-eye detection of the analyte without resort to any expensive equipment [16,17]. In general, these chemosensors are constructed according to the receptor–chromophore general binomial, which involves the binding of a special anion substrate with receptor sites and a chromophore responsible for translating the receptor–anion association into an optical signal [18–20]. The color variation will occur when a charge-transfer complex is formed [21]. 'Naked-eye' detection of anions based on tautomeric azophenol-containing receptors is rarely reported in the literature, although such cation receptors which undergo azophenol to quinone-hydrazone tautomerization upon presence of cations, have been successfully shown [22,23]. Hence, there is a need for the development of colorimetric anion receptor with anion-induced azophenol to quinone-hydrazone tautomerization as signaling mechanism.

By exploiting the optical properties of an azo group, many functional molecules have been reported in the literatures [24,25]. Thiourea is among the most frequently used fragments to design neutral receptors for the selective recognition of anions as it is able to strongly bind anions using directional hydrogen bonding interactions even in the aqueous solution [26–28]. We reasoned that a novel colorimetric anion receptor would be obtained through coupling the thiourea group as recognition site with an azophenol chromophore as a signal group. We further anticipated that compound **1** could display azophenol to quinone-hydrazone tautomerization, stimulated by anionic species in solution. Just as expected, the remarkable color change from light yellow to orange was seen during the spectral titration process.

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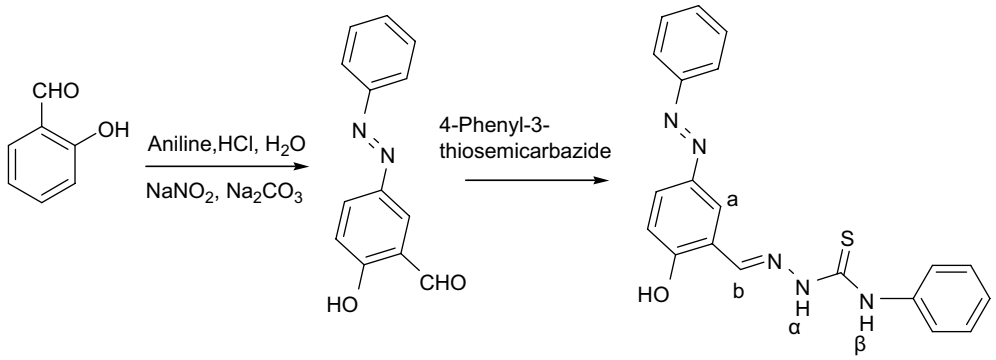


Scheme 1. Synthesis of receptor **1**.

2. Experimental

2.1. Apparatus

^{1}H NMR spectra were obtained on a Varian UNITY Plus-400 MHz spectrometer. ESI-MS was performed with a MARINER apparatus. C, H, N elemental analyses were made on an Elementar Vario EL. UV–vis spectra were recorded on a Shimadzu UV2450 spectrophotometer with quartz cuvette (path length = 1 cm) and fluorescent spectra were recorded on a Shimadzu RF-5301PC spectrophotometer at 298.2 ± 0.1 K and the width of the slits used is 5 nm.

2.2. Chemicals

All reagents obtained commercially for synthesis were used without further purification. In the titration experiments, all the anions were added in the form of tetrabutylammonium (TBA) salts, which were purchased from Sigma–Aldrich Chemical, stored in a vacuum desiccator containing self-indicating silica and dried fully before using. DMSO was dried with CaH_2 and then distilled under reduced pressure.

2.3. General method

All titration experiments were carried out at 298.2 K, unless otherwise mentioned. UV–vis spectra were measured using a UV–vis spectrophotometer, UV2450 (Shimadzu Corp., Kyoto, Japan). A 2.0×10^{-5} M solution of compound **1** in dried DMSO and solutions of 0.10 M tetrabutylammonium (TBA) salts of the respective anions in dried DMSO were prepared and stored under the dry atmosphere. These solutions were used for all spectroscopic studies after appropriate dilution. Then, given amount of the solution of **1** was added to the quartz cuvette and the increased amount of anions tested (0.1 M in DMSO-d_6) was added to the above-mentioned solution, whose absorbance/emission spectra was tested immediately. Affinity constants of receptor **1** for anionic species were determined by non-linear fitting analyses program ORIGIN according to the equation reported by B. Valeur, 1:1 host–guest complexation [29]. The fluorescence quantum yields, ϕ_f, were estimated using the integrated emission intensity of fluorescein (0.85) in 0.1 M NaOH aq. as a standard via the follwing equation:

$$\phi_f = \phi'_f\left(I_{sample}/I_{std}\right)\left(A_{std}/A_{sample}\right)\left(\eta^2_{sample}/\eta^2_{std}\right)$$

where ϕ'_f is the quantum yield for the fluorescein (0.85) in 0.1 M NaOH aq. used as a standard [30]; I_{sample} and I_{std} are the integrated emission intensities; A_{sample} and A_{std} are the absorbances at the excitation wavelength, and η^2_{sample} and η^2_{std} are the respective refractive indices.

^{1}H NMR titration experiments were carried out in DMSO-d_6 solution (TMS is used as an internal standard). A 1.0×10^{-2} M solution of compound **1** in DMSO-d_6 was prepared. Then, the increased amount of fluoride anion (1.0 M in DMSO-d_6) was added to the above-mentioned solution and ^{1}H NMR of the host–guest system was tested.

2.4. Synthesis

2.4.1. 5-Phenylazo-salicylaldehyde (*2*)

The synthesis route of compounds **1** and **2** is demonstrated in Scheme 1. 5-Phenylazo-salicylaldehyde was prepared according to the literature reported [31]. To a solution of 5 ml aniline (0.05 mol) in a small quantity of water was added slowly 6 ml of 37% HCl at 0–5 °C when stirring. Then, 20 ml of 20% $NaNO_2$ was added to the above-mentioned mixture and the resulting solution was stirred for 1 h to give a bright yellow solution. Five milliliters of salicylaldehyde (0.05 mol) was dissolved in the solution of $NaCO_3$ (18 g $NaCO_3$ + 150 mol H_2O). Then the solution of salicylaldehyde was added dropwise to the bright yellow solution for 1 h. After stirring for 4 h, the reaction mixture was neutralized with HCl. The brown crude solid was filtered and recrystallized from ethanol to afford a pure product. Mp: 120 °C.

2.4.2. 4-Hydroxy-3-(N-phenyl-thiourea-N′-nitrilo-methylidynyl)-azobenzene (*1*)

To a solution of 3-phenylazo-salicylaldehyde (0.226 g, 1 mmol) and a catalytic amount of acetic acid in ethanol was added

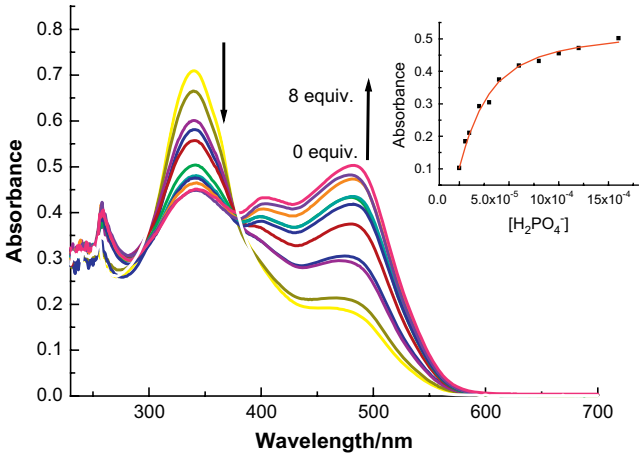


Fig. 1. Evolution of UV–vis spectrum of receptor **1** (2.0×10^{-5} M in DMSO) during the titration with tetrabutylammonium (TBA) $H_2PO_4^-$. Inset: absorbance at 480 nm vs. the concentration of $H_2PO_4^-$ added.

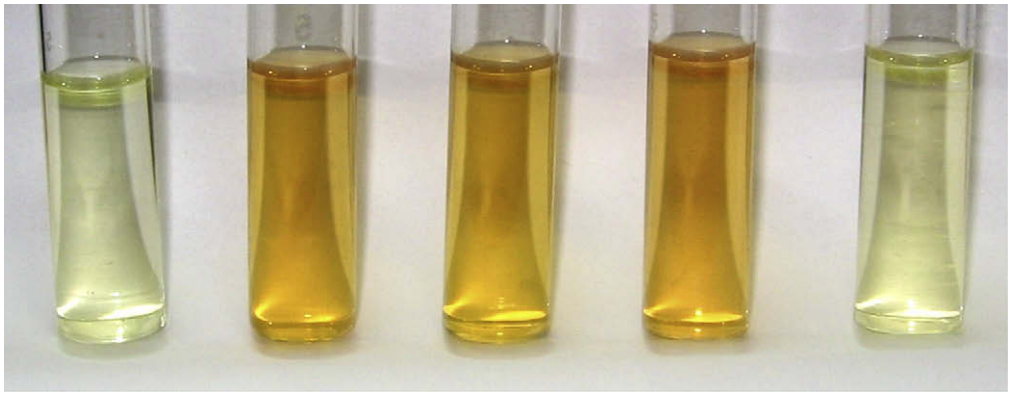


Fig. 2. Color changes observed in receptor **1** in DMSO (2.0×10^{-5} M) in the presence of 10 equiv. of anions as TBA salts (from left to right: **1** only, **1** + $H_2PO_4^-$, **1** + AcO^-, **1** + F^-, **1** + Cl^-, Br^- and I^-).

dropwise a solution of 1-amido-3-phenylthiourea (0.167 g, 1 mmol) in ethanol. Then the mixture was heated to boil under magnetic stirring for 2 h. During the reaction a yellow precipitate appeared which was collected by filtration, washed with ethanol and dried in vacuo. Desired solid (0.300 g) was obtained. Yield = 80%. ^{1}H NMR (DMSO-d_6): δ 11.86 (1H, s, OH), 10.90 (1H, s, NH_α), 10.24 (1H, s, NH_β), 8.75 (1H, s, phH), 8.57 (1H, s, phH), 7.82 (3H, d, J = 7.2, phH), 7.54 (5H, d, J = 7.6, phH), 7.38 (2H, t, phH), 7.22 (1H, d, J = 7.2, H_a), 7.08 (1H, d, J = 8.8, H_b); ESI-mass: m/z calcd for $C_{20}H_{17}N_5SO$ $[M]^-$: 375.12, found: 374.34 $[M-H]^-$. Elemental analysis calcd for $C_{20}H_{17}N_5SO$: C, 63.98; H, 4.56; N, 18.65. Found: C, 63.85; H, 4.23; N, 18.69.

3. Results and discussion

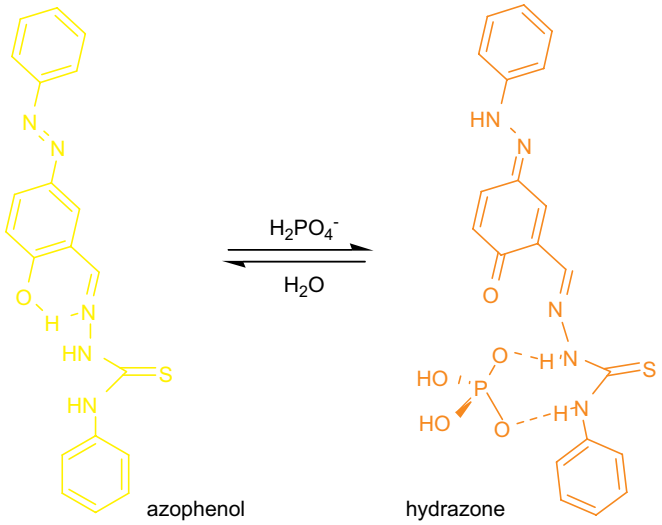
3.1. UV–vis spectral titrations and colorimetric signaling

UV–vis titrations were carried out in DMSO at a concentration of 2.0×10^{-5} M with the addition of tetrabutylammonium $H_2PO_4^-$; the spectra are shown in Fig. 1. Compound **1** exhibited strong absorption at 340 nm with a shoulder at 470 nm, which was assigned to the charge transfer of the azo moiety. As the concentration of $H_2PO_4^-$ was increased, the absorption intensity at 340 nm gradually decreased and a new absorption peak appeared at 480 nm, which was accompanied by a visual color change from light yellow to orange (Fig. 2). The results can be rationalized on the basis of the anion-induced tautomeric equilibrium between azophenol and quinone-hydrazone in DMSO [22,23,32]. Prior to coordination with $H_2PO_4^-$, the azophenol isomer of compound **1** dominates in solution, to which can be attributed the observed, strong absorption centered at 340 nm. Once compound **1** has bonded with $H_2PO_4^-$, deprotonation of the –OH group occurs, which will reinforce the formation of hydrazone via azophenol to quinone-hydrazone tautomerization (Scheme 2). This will result in both visual and spectral changes observed. However, the presence of protic solvent such as H_2O, which will compete with anions for binding sites and, which will, therefore, disturb the H-bond interactions between the host and the anionic guest, will lead to a reversal of the visual color change and the spectral change. Similar effects were observed in the UV–vis spectra of **1** upon the addition of F^- and AcO^- ions (Fig. 3). In the case of weak basic ions such as Cl^-, Br^- and I^-, the spectral changes were too small to calculate the corresponding association constants. In addition, the presence of a single, well-defined isosbestic point indicated that both **1** and **1**–anion complex coexist. The titration profile at 480 nm (Fig. 1) supports the formation of a 1:1 stoichiometric, host–guest species with an association constant K_{ass} of 56 079 ± 880 M^{-1} (obtained through non-linear fitting analyses of the titration curves).

The association constants (K_{ass}, M^{-1}) of **1** with anions in DMSO at 298.2 ± 0.1 K are shown in Table 1. The selectivity trend of binding affinity of **1** for anions followed the following order: $H_2PO_4^- > AcO^- > F^- > \sim Cl^- \sim Br^- \sim I^-$. It is apparent that the selectivity for specific anions can be rationalized on the basis of the anion's basicity and the spacial interactions between the host and the anionic guests. However, multiple hydrogen-bond interactions are also necessary in high-affinity anion binding sites [33]. As expected from their basicity, $H_2PO_4^-$, AcO^- and F^- will bind more strongly than the other anions studied; in addition, the tetrahedral configuration of $H_2PO_4^-$ ion may well match **1** in terms of shape and could form multiple hydrogen bonding interactions with **1** (Scheme 2). Consequently, $H_2PO_4^-$ ion can be selectively recognized from other anions based on its association constant. Also, the association constants of the host **1** with $H_2PO_4^-$ ion was larger than that of the receptor reported by Gunnlaugsson [34] only with thiourea moiety as binding sites. The explanation is likely that **1** with –OH and thiourea moieties could interact with $H_2PO_4^-$ ion through multiple hydrogen bonding.

3.2. Fluorescent responses of receptor 1 toward anions

The fluorescent spectral properties of host **1** ($c = 2.0 \times 10^{-5}$ M) were determined in DMSO solvent which shows a very weak fluorescence ($\phi_f = 0.14$) at 333 nm with a shoulder at 307 nm (λ_{exc} = 279 nm). The fluorescent emission of receptor **1** did not originate from the azobenzene unit, as it is well known that the



Scheme 2. Proposed binding mode of receptor **1** with $H_2PO_4^-$ ion in solution.

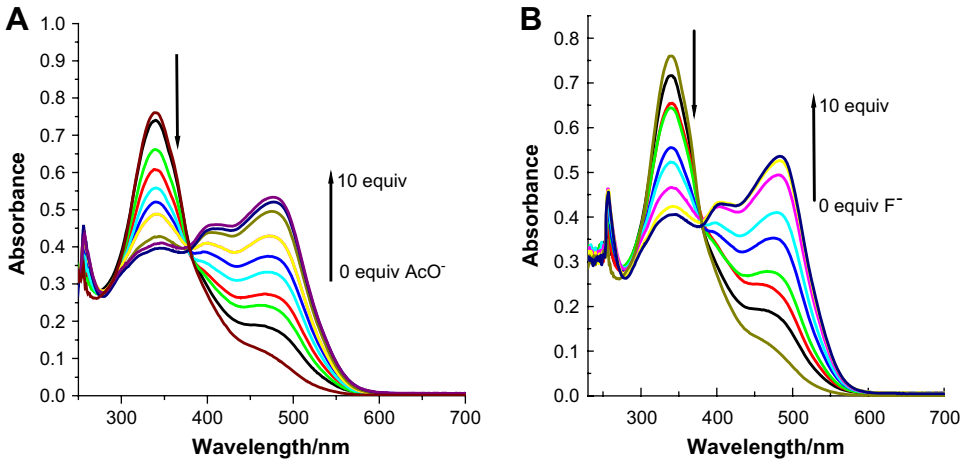


Fig. 3. Evolution of UV–vis spectrum of receptor **1** (2.0×10^{-5} M in DMSO) during the titration with (A) AcO^- and (B) F^-.

azobenzene unit does not exhibit luminescence [35], but resulted from the Schiff base unit [36]. As seen in Fig. 4, with the stepwise addition of $H_2PO_4^-$ to a solution of compound **1**, the fluorescence emission intensity of **1** at 333 nm increased gradually ($\phi_f = 0.20$). Two possible reasons for the enhanced fluorescence might be as follows: (1) inhibition of photoinduced electronic transfer (PET) [37] and (2) binding-induced rigidity of the host molecule [38,39]. Firstly, before coordination with $H_2PO_4^-$ ion, the nitrogen atoms of the imine of free **1** could form an intramolecular hydrogen bond with the hydrogen atom of –OH, which resulted in a photoinduced electron transfer, and the de-excitation of the resulting tautomer occurred mainly via a nonradiative pathway. These processes consequently led to the weak fluorescence of **1**. Once **1** was coordinated with $H_2PO_4^-$ ion, which induced deprotonation of the –OH, the electron-transfer process was forbidden. Therefore, an enhancement of the fluorescence emission of **1** was observed. Secondly, the configuration of free receptor **1** was flexible and could rotate freely. Upon complexation with $H_2PO_4^-$ ion, the host molecule **1** was rigidified, which gave birth to a large increase in emission intensity because of inhibiting vibrational and rotational relaxation modes of nonradiative decay. In addition, the addition of F^- and AcO^- resulted in similar fluorescent changes compared to that of $H_2PO_4^-$ and receptor **1** was found to be insensitive to the addition of a large excess of Cl^-, Br^- and I^- (see Fig. 5).

3.3. ^1H NMR titrations

To further elucidate the nature of the intermolecular interactions between anions and receptor **1**, as an example, ^1H NMR spectral changes upon addition of F^- as their tetrabutylammonium salts to the DMSO-d_6 solution of **1** (1×10^{-2} mol l^{-1}) were investigated. Two effects would be expected to result from the formation of hydrogen bond between the binding sites and the anion: (1) through-bond effects, which increase the electron density of the benzene ring and promote upfield shifts in ^1H NMR spectrum, and (2) through-space effects, which polarize C–H bond in proximity to hydrogen bond, create the partial positive charge on the proton and cause downfield shifts [40]. Obviously, from Fig. 6, the peak at 11.86 ppm, which was assigned to –OH, disappeared upon addition of 0.5 equiv. of F^- and at the same time a new peak at 16.10 ppm, which was the characteristic resonance of bifluoride $[HF_2]^-$, appeared. In addition, the signals of NH_α and NH_β (the peaks at 10.90 ppm and 10.24 ppm, respectively) broadened and exhibited a downfield shift indicating that NH_α and NH_β participated in hydrogen bonding interactions with F^-. The phenyl protons especially for the protons H_a (7.21 ppm) and H_b (7.08 ppm) clearly shifted upfield, indicative of the increase in the electron density of the phenyl ring owing to the through-bond effects. Thus, the results of ^1H NMR titration and spectrum titration implicated that the tautomeric equilibrium occurred during the anion recognition process. As mentioned above, the proposed anion recognition process in solution is shown in Scheme 2.

Table 1
Association constants (K_{ass}, M^{-1}) of receptor **1** with anions and the relative quantum yields of receptor **1** in the presence of 2.5 equiv. of anions tested in DMSO at 298.2 ± 0.1 K

Anions[a]	K_{ass}	R^[c]	ϕ_f[d]
$H_2PO_4^-$	$(5.60 \pm 0.88) \times 10^4$	0.990	0.20
AcO^-	$(1.37 \pm 0.22) \times 10^4$	0.994	0.19
F^-	$(4.90 \pm 1.00) \times 10^3$	0.992	0.17
Cl^-	ND[b]	–	–[e]
Br^-	ND	–	–
I^-	ND	–	–

[a] All anions were added in the form of tetra-*n*-butylammonium (TBA) salts.
[b] The association constant could not be determined.
[c] Correlation coefficient (R^) determined by non-linear fitting analyses.
[d] Quantum yield of fluorescence was determined using fluorescein (0.85) in 0.1 M NaOH aq. as a standard.
[e] Neglectable changes in the emission intensity induced by the anion.

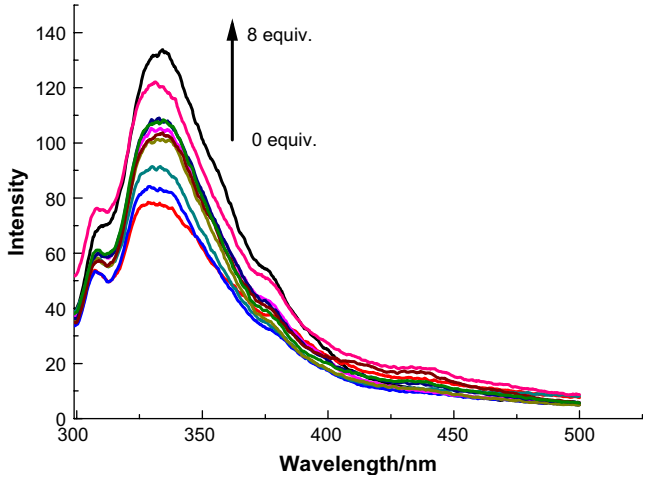


Fig. 4. Fluorescent titration of receptor **1** in DMSO (2.0×10^{-5} M) upon addition of $H_2PO_4^-$.

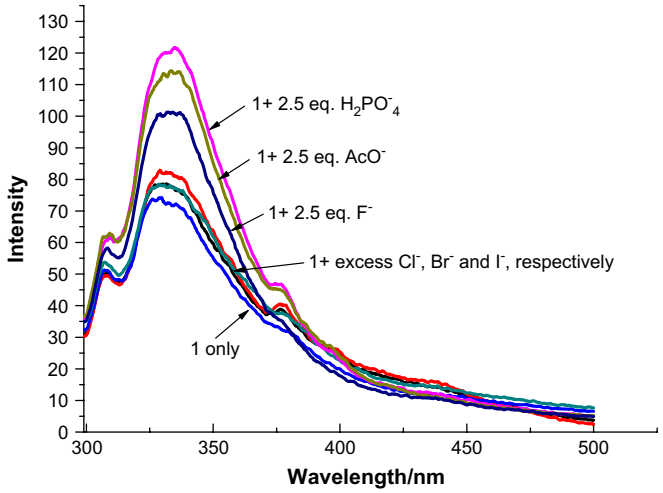


Fig. 5. Fluorescent changes of receptor **1** in DMSO (2.0×10^{-5} M) upon addition of different anions.

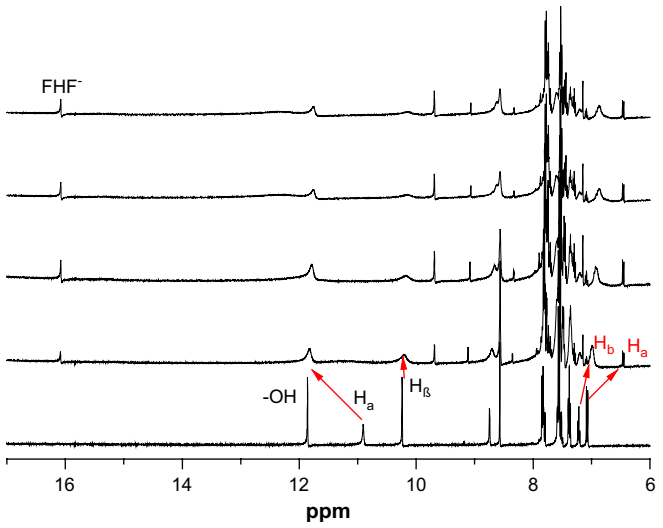


Fig. 6. ^{1}H NMR spectra of receptor **1** in DMSO-d_6 (1×10^{-2} M) upon addition of molar equiv. of F^- (from bottom to top: 0.5, 1.0, 1.5, 2.0).

4. Conclusions

In DMSO, the presence of anions such as $H_2PO_4^-$ gave birth to changes in the color of solution of **1** from light yellow to orange, as well as a red shift in the UV–vis spectrum and fluorescence intensity (OFF–ON). The findings demonstrated that **1** can bind strongly with anions and selectively distinguish $H_2PO_4^-$ from other anions according to the respective association constant. In particular, a tautomeric equilibrium occurred during the anion recognition process. The different electronic properties of the tautomer are responsible for the observed color and spectral changes.

Acknowledgments

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