

# A colorimetric sensor for the recognition of biologically important anions

Weiwei Huang · Xudong Yu · Hai Lin ·  
Huakuan Lin

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**Abstract** A colorimetric anion sensor **1** based on 3-phthaloyl-*N*-4-nitrophenylhydrozone was synthesized and characterized. The binding ability evaluated by UV–vis experiment reveals that **1** can selectively recognize fluoride. Further insights into the nature of interactions between sensor **1** and anions were investigated by H NMR titrations experiments. In addition, the color changes induced by fluoride can provide a way of detection by ‘naked-eye’.

**Keywords** Anion recognition · Fluoride · Acetate · Sensor

## Introduction

The design and synthesis of efficient sensors that can recognize anions selectively through electrochemical or optical responses is an increasingly important field in supramolecular chemistry in recent years [1–5]. Colorimetric sensor is more noticeable for it can detect anions by naked eye with reliable calibration stability. Anions such as fluoride, chloride, bromide, iodide, dihydrogenphosphate

and acetate play important roles in biological, industrial, and environmental process [6–8]. For example, fluoride is essential in a variety of biological processes such as preventing children’s dental caries and treating osteoporosis [9]. Acetate plays an important role in enzymes and antibodies [10], because carboxylates are critical components in numerous metabolic processes [11]. Thus, there is a need to develop receptors which can selectively recognize biologically important fluoride ion or acetate ion.

It is well-known that amides [7], (thio)ureas [12], and ammonium [13] derivatives are particularly effective in the reaction with anions through the formation of hydrogen bond between the active N–H and anions. Furthermore, with the strong inducement of the anions, proton transfer often takes place in the host–anions reaction [14]. In this paper, we prepared a highly selective sensor for fluoride ion with dramatic color changes from colorless to red. On the contrary, no detectable color changes were observed when adding  $\text{AcO}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$  and  $\text{I}^-$  ions, even in a large amount to the solution. These results suggest that sensor **1** can distinguish  $\text{F}^-$  from many other anions.

## Experimental

### Materials

All reagents for synthesis obtained commercially 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  $\text{CaH}_2$  and then distilled in reduced pressure.

W. Huang · X. Yu · H. Lin (✉)  
Department of Chemistry, Nankai University, Tianjin 300071,  
People’s Republic of China  
e-mail: hklin@nankai.edu.cn

H. Lin  
Key Laboratory of Functional Polymer Materials  
of Ministry of Education, Nankai University, Tianjin 300071,  
People’s Republic of China

## General method

<sup>1</sup>H NMR spectra were recorded on a Varian UNITY Plus-400 MHz Spectrometer at the Key Laboratory of Functional Polymer Materials of Ministry of Education, Nankai University. UV–vis spectroscopy titrations were performed on a Shimadzu UV2450 Spectrophotometer at 298 K. Elemental analysis for C, H, and N were carried out on a PerkinElmer 240C element analyzer at the Institute of Elemento-Organic Chemistry, Nankai University.

A series of DMSO solutions having same host concentration and different anion concentrations were prepared, respectively. The affinity constants  $K_s$  were obtained by the determination of absorption of the series of solutions and analysis of obtained absorption values with non-linear least square calculation method for data fitting.

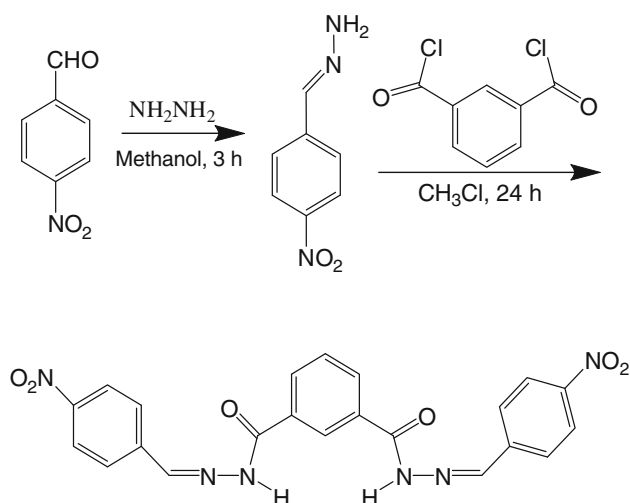
## Synthesis

### Preparation of *m*-phthaloyl chloride (Scheme 1)

0.33 g (2.0 mmol) *m*-phthalic acid, 5 mL SOCl<sub>2</sub> chloride (60 mmol), 3 drops of DMF were refluxed in CHCl<sub>3</sub> for 8 h. Then SOCl<sub>2</sub> and CHCl<sub>3</sub> were evaporated and the residue were dried in vacuum, faint yellow solid was acquired, m.p. 41–42 °C. It is directly used for further reaction without purification.

### Preparation of 4-nitrophenylhydrozone

4-Nitrobenzaldehyde (0.33 mmol) in 20 mL methanol was added dropwise to rapidly stirring anhydrous hydrazine (2.0 mmol, 64 g). After stirring, the mixture was refluxed for 3 h. After cooling, the acquired yellow precipitate was



**Scheme 1** General synthetic routes to the target sensor **1**

washed with saturated sodium chloride solution for three times and then recrystallized by ethanol. <sup>1</sup>H NMR (400 Hz, CDCl<sub>3</sub>); 5.88 (2H, s), 7.27 (1H, s), 7.67 (2H, d,  $J = 8$  Hz), 7.74 (1H, s), 8.20 (2H, d,  $J = 8$  Hz).

### Preparation of 1,3-phthaloyl-*N*-4-nitrophenylhydrozone

A solution of 4-nitrophenylhydrozone (0.5 mmol) in dry CHCl<sub>3</sub> was added dropwise to the mixture of *m*-phthaloyl chloride (0.5 mmol), 1 mL triethylamine and 20 mL CHCl<sub>3</sub> over a period of 10 min. After continuous stirring for about 24 h, the mixture was filtered and the precipitate was recrystallized by CHCl<sub>3</sub>. <sup>1</sup>H NMR (400 Hz, DMSO-*d*<sub>6</sub>): 7.75 (1H, t), 8.01–8.04 (4H, m), 8.17 (2H, d,  $J = 7.2$  Hz), 8.31–8.36 (4H, m), 8.53 (1H, s), 8.62 (2H, d,  $J = 7.2$  Hz), 12.37 (2H, s). Elemental analysis calcd. for C<sub>22</sub>H<sub>16</sub>N<sub>6</sub>O<sub>6</sub>: C, 57.39; N, 18.25; H 3.5. Found: C, 57.42; N, 18.24; H, 3.5.

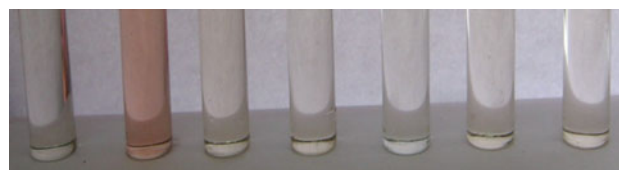
## Results and discussion

### Colorimetric experiments

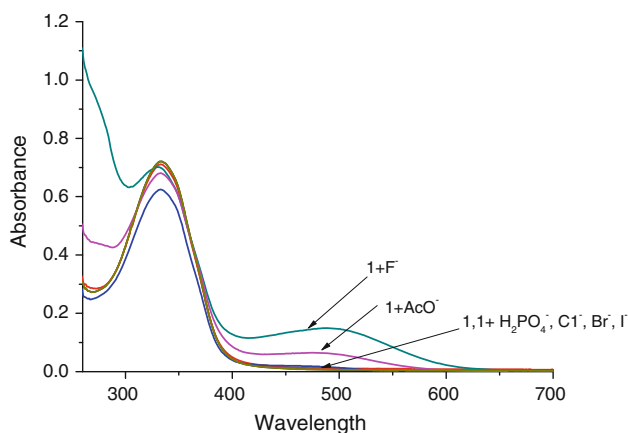
In the naked-eye colorimetric experiments (see Fig. 1), sensor **1** underwent a dramatic color change from almost colorless to red upon the addition of F<sup>−</sup>, while there were no noticeable color changes when adding other anions such as AcO<sup>−</sup>, H<sub>2</sub>PO<sub>4</sub><sup>−</sup>, Cl<sup>−</sup>, Br<sup>−</sup>, I<sup>−</sup>. It clearly suggested that **1** showed the selective recognition for F<sup>−</sup>.

### UV–vis titrations

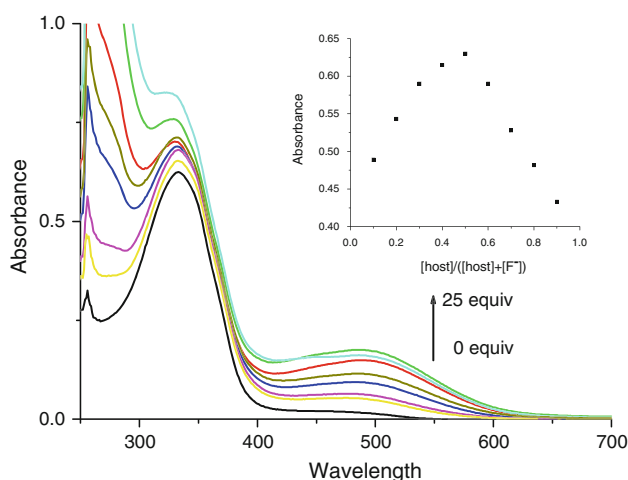
In order to study the binding selectivity of sensor **1**, UV–vis experiments of sensor **1** with different anions in the form of tetrabutylammonium salts were performed in DMSO at 298.2 ± 0.1 K. Obviously spectra changes were observed after adding F<sup>−</sup> to the solution (see Fig. 2). However, no detectable optical responses were observed when adding AcO<sup>−</sup>, H<sub>2</sub>PO<sub>4</sub><sup>−</sup>, Cl<sup>−</sup>, Br<sup>−</sup> and I<sup>−</sup> ions, even in a large amount to the solution. These results suggest that sensor **1** can distinguish F<sup>−</sup> from many other anions.



**Fig. 1** Color changes observed for sensor **1** in DMSO solution ( $2 \times 10^{-5}$  M) upon addition anions as TBA salts: from left to right: **1** only, **1** + F<sup>−</sup>, **1** + AcO<sup>−</sup>, **1** + H<sub>2</sub>PO<sub>4</sub><sup>−</sup>, **1** + Cl<sup>−</sup>, **1** + Br<sup>−</sup> and **1** + I<sup>−</sup>



**Fig. 2** UV-vis spectra of **1** ( $2 \times 10^{-5}$  M) in the presence of 10 equiv. of  $F^-$ , or  $AcO^-$ ,  $H_2PO_4^-$ ,  $Cl^-$ ,  $Br^-$  and  $I^-$

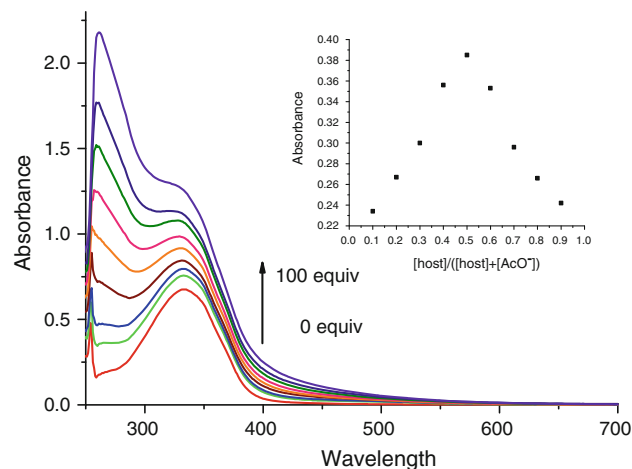


**Fig. 3** Evolution of the UV-vis spectrum of sensor **1** ( $2.0 \times 10^{-5}$  M) during the titration with  $F^-$  in DMSO. Inset Job's plot for complexation of sensor **1** with  $F^-$  determined by UV-vis in DMSO,  $[1] + [anion] = 2.0 \times 10^{-3}$  M

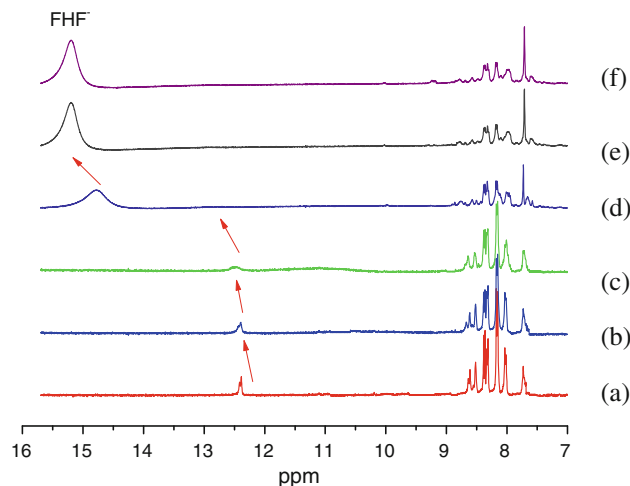
Then to explore more about the applicability of the sensor **1** for fluoride, Fig. 3 shows the UV-vis spectral changes of **1** during the titration with fluoride ions. The presence of fluoride resulted in an increasing of intensity of the absorbance at 495 nm gradually and the centered bands of sensor **1** at 255 nm developed, accompanied significant color changes from colorless to red. The presence of acetate ion has induced similar changes in UV-vis spectrum (see Fig. 4). However, other anions including  $Cl^-$ ,  $Br^-$ ,  $I^-$ ,  $H_2PO_4^-$  did not cause noticeable spectral changes or color changes, even in the presence of a large amount of them.

#### H NMR titrations

H NMR titrations carried out in  $DMSO-d_6$  give some creditable evidence for the complex system formed by



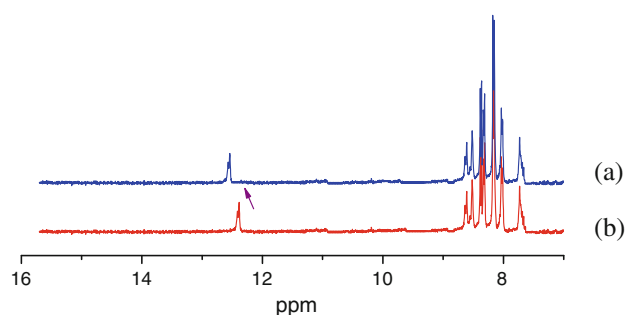
**Fig. 4** Evolution of the UV-vis spectrum of sensor **1** ( $2.0 \times 10^{-5}$  M) during the titration with  $AcO^-$  in DMSO. Inset Job's plot for complexation of sensor **1** with  $AcO^-$  determined by UV-vis in DMSO,  $[1] + [anion] = 2.0 \times 10^{-3}$  M



**Fig. 5** Partial  $^1H$  NMR spectra of **1** ( $1.0 \times 10^{-2}$  M) in  $DMSO-d_6$  upon the addition of  $F^-$ . (a) 0 equiv.; (b) 0.2 equiv.; (c) 1 equiv.; (d) 3 equiv.; (e) 5 equiv.

sensor **1** and anions. From Fig. 5, after the addition of fluoride anion, the signal of  $-NH$  at 12.36 ppm gradually broadened and disappeared, a new broad resonance at 15.10 ppm appeared clearly, which was ascribed to the  $FHF^-$  dimer [15, 16] and it indicated the deprotonation of  $H_a$  (see Fig. 5). H NMR titrations suggested that sensor **1** formed hydrogen bonds with  $F^-$  at first, then as increasing the concentration of  $F^-$ , the sensor **1** displayed the complete deprotonation eventually.

The interaction of sensor **1** with  $AcO^-$  was also studied (see Fig. 6). Upon the addition of 5 equiv. of  $AcO^-$ , the  $-NH$  had a down shift from 12.36 to 12.55 ppm, which indicated that a classic hydrogen bonding formed between **1** and  $AcO^-$ .



**Fig. 6** Partial  $^1\text{H}$  NMR spectra of **1** ( $1.0 \times 10^{-2}$  M) in  $\text{DMSO-}d_6$  upon the addition of  $\text{AcO}^-$ . (a) 0 equiv.; (b) 10 equiv.

**Table 1** Affinity constants of sensor **1** with anions in DMSO at  $298.2 \pm 0.1$  K

Anions ( $\text{M}^{-1}$ )	$\text{F}^-$	$\text{AcO}^-$	$\text{H}_2\text{PO}_4^-$	$\text{Cl}^-$	$\text{Br}^-$	$\text{I}^-$
$\log K_{\text{ass}}$	$3.04 \pm 0.15$	$1.51 \pm 0.03$	ND	ND	ND	ND

ND cannot be determined

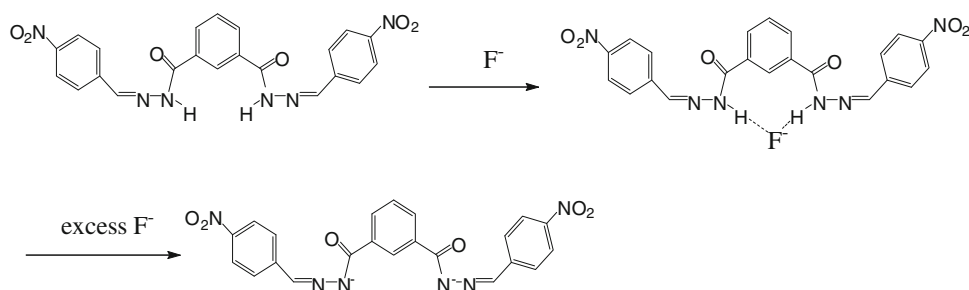
#### Determination of the binding constant and stoichiometry

Continuous variation method was used to determine the stoichiometric ratios of the host and anion guest [17]. Job's plot [18, 19] of sensor **1** and  $\text{F}^-$  in DMSO (see Fig. 3) showed the maxima was at a molar fraction of 0.5. This result indicated that the sensor **1** binded  $\text{F}^-$  anion guest with a 1:1 ratio. Similar results could also be obtained for  $\text{AcO}^-$  (see Fig. 4).

For a complex of 1:1 stoichiometry, the relation in Eq. 1 could be derived easily, where  $X$  is the absorption intensity, and  $C_{\text{H}}$  or  $C_{\text{G}}$  is the concentration of the host or the anion guest correspondingly [20].

$$X = X_0 + (X_{\text{lim}} - X_0) \left\{ C_{\text{H}} + C_{\text{G}} + 1/K_{\text{ass}} - [(C_{\text{H}} + C_{\text{G}} + 1/K_{\text{ass}})^2 - 4C_{\text{H}}C_{\text{G}}]^{1/2} \right\} / 2C_{\text{H}} \quad (1)$$

**Scheme 2** The proposed 1- $\text{F}^-$  binding mode



The affinity constants of sensor **1** for the studied anionic species are calculated and listed in the Table 1 below.

#### Discussion

As clearly shown in Table 1, the order of binding affinity of **1** with anions in DMSO is  $\text{F}^- > \text{AcO}^- \gg \text{H}_2\text{PO}_4^- \sim \text{Cl}^- \sim \text{Br}^- \sim \text{I}^-$ . The recognition ability of sensor **1** for  $\text{F}^-$  was the most remarkable. Two main reasons for preferring  $\text{F}^-$  were that fluoride has a relatively strong basicity and its shape is more complemented with two  $-\text{NH}$  of sensor **1** (Scheme 2).

According to the results from UV–vis titrations and H NMR titrations, the proposed mode for the host–guest bonding in solution was depicted in Scheme 2. In the structure, anions such as  $\text{F}^-$  and  $\text{AcO}^-$  combined sensor **1** via  $\text{N}-\text{H}\cdots$  anion hydrogen bonds.

In our UV–vis titration studies, we found when the concentration of sensor **1** is  $2 \times 10^{-5}$  M in DMSO, the limit of  $[\text{F}^-]$  detection (LOD) is  $1 \times 10^{-4}$  M before the host–guest binding reaction reaching an equilibrium. So we hope sensor **1** may be applied in detection of micro amounts of fluoride ion.

Compared with the previous research in literature [2], the sensor **1** showed significant color changes upon addition of fluoride ion. The process of sensing can literally be observed through the ‘naked-eye’ for the sharply color changes from colorless to red.

#### Conclusions

In summary, we have presented a colorimetric charge-neutral sensor **1**, which could recognize fluoride among the anions investigated. The whole process can be observed by the ‘naked-eye’, for its sharply color changes from colorless to red. This outstanding property is also bolstered by UV–vis and H NMR titrations experiments. It is expected to be applied for detection of biologically important anions in real life for its easy synthesis and highly selective sensing ability.

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