

Selective Ratiometric Fluorescence Detection of Acetate Based on a Novel Schiff Base Derivative

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Abstract A ratiometric fluorescence and colorimetric probe based on 5,5'-methylene-bis-salicylaldehyde-p-nitrophenylhydrazone (1), which was characterized by ^1H NMR, mass spectrometer and elemental analysis, was prepared through Schiff base reaction. The probe exhibited visible color changes from yellow to purple upon interacting with acetate ion. Particularly, the compound showed ratiometric fluorescence response to acetate with significant blue shift about 150 nm from 410 nm to 560 nm. Thence, the probe 1 was a selective ratiometric fluorescence probe for acetate ion in DMSO solution.

Keywords Anion probe · Colorimetric · Ratiometric · Fluorescence · Schiff base

Introduction

The selective recognition and sensing of anions is an important research topic due to their importance in chemistry and biotechnology [1–3]. Among various anions, acetate (AcO^-) has attracted the interest of chemists due to that acetate is a critical component of numerous metabolic processes [4] and the rate of AcO^- production and oxidation has been frequently used as an indicator of organic decomposition in marine sediments [5]. Thus,

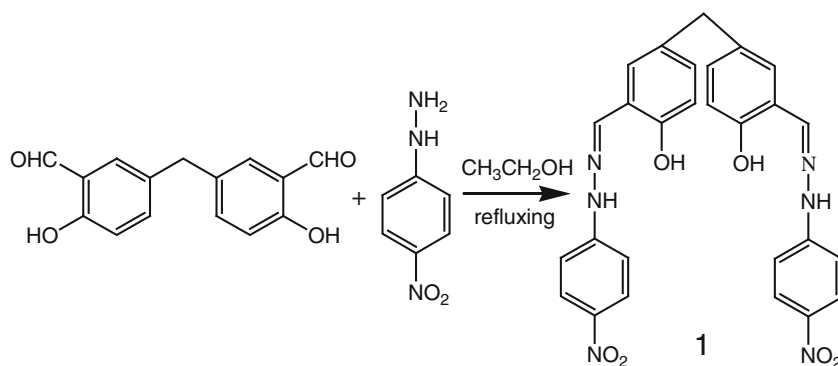
detecting trace amounts of AcO^- is important not only for biological process, but also for environmental applications. One of the tools for detecting AcO^- is to utilize a fluorescent chemosensor [6, 7]. However, most artificial fluorescent chemosensors give rise to quenching or enhancement of signals upon AcO^- binding [6–8]. Only a few examples were reported to be ratiometric fluorescence sensors toward acetate ion [9]. The ratiometric fluorescence signaling, which involves the measurement of changes in the ratio of the fluorescence intensities at two different wavelengths, is superior to the conventional method of monitoring the fluorescence intensity at a single wavelength [10]. Unfortunately, the ratiometric fluorescence sensors for anions were rarely reported in the literature. Accordingly, development of ratiometric fluorescence probe for acetate is very necessary.

To date, a variety of signaling mechanisms have been proposed and utilized for optical detection of different species, including photo-induced electron transfer (PET) [11, 12], photo-induced charge transfer (PCT) [13, 14], excimer/excplex formation [15], and excited-state intra-/intermolecular proton transfer (ESIPT) [16]. However, two signaling mechanisms used frequently in development of fluorescence anion sensor are PET and PCT. In general, the hydrogen-bonded complex with anion activated the PET process more efficiently and showed up a greater fluorescence quenching. Accordingly, fluorescence of the anion PET chemosensor is 'switch-off' rather than 'switch-on' upon ion sensing unlike most PET sensors for cations [17–19]. In addition, fluorescence anion sensor based PCT contains at least two parts: the fluorophore and the anion binding site. Furthermore, the anion binding site as an electron-donating group is conjugated to a fluorophore group with an electron-withdrawing unit. When the host-guest system forms, it undergoes photo-induced charge

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Scheme 1 Synthesis route for the compound 1



transfer (PCT) from the donor to the acceptor upon excitation by light. The consequent change in dipole moment results in ratiometric fluorescence change [20].

In this paper, we reported a ratiometric fluorescence and colorimetric anion probe 1 based on a simple Schiff base derivative. In DMSO solution, the probe 1 exhibited photo-induced charge transfer (PCT) processes upon binding anions in the excited state. As a result, the chemosensor 1 showed ratiometric fluorescence responses towards anions tested with strong basicity such as AcO^- and F^- ions.

Experimental

Apparatus and Chemicals

^1H NMR spectra were obtained on a Varian UNITY Plus-300 MHz Spectrometer. ESI-MS was performed with a MARINER apparatus. C, H, N elemental analyses were made on an elemental vario EL. UV-vis spectra were recorded on a TU-1810 Spectrophotometer made by Beijing Puxi Tongyong apparatus company with quartz cuvette (path length = 1 nm), and fluorescence spectra were recorded on a

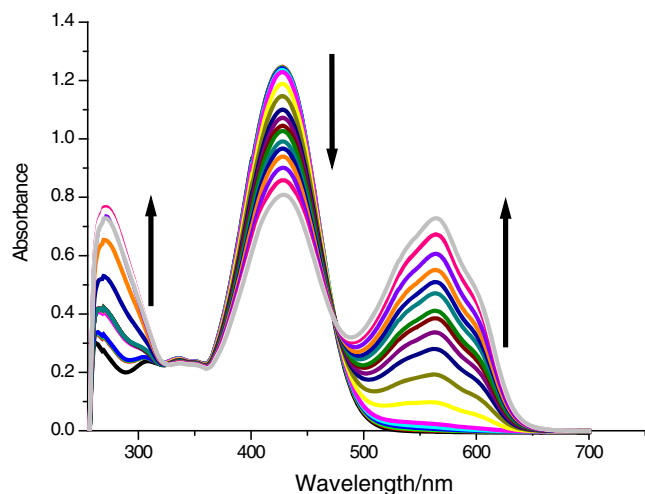


Fig. 1 UV-vis spectral changes of the probe 1 (2×10^{-5} M) in absence and presence of acetate ion in DMSO

F96 Spectrophotometer made by Shanghai Lengguang Technology Co.,LTD.. The width of the slits is 5 nm.

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

Synthesis of 5,5'-methylene-bis-salicylaldehyde-p-nitrophenylhydrazone (1)

The synthesis route of probe 1 was shown in Scheme 1. 5,5'-methylene-bis-salicylaldehyde [1] (0.256 g, 1 mmol) in ethanol (10 ml) was added dropwise a solution of p-nitrophenylhydrazine (0.306 g, 2 mmol) in ethanol (25 ml). Then the mixture was heated to reflux under magnetic stirring for 2 h. During the reaction a yellow precipitate appeared which was collected by filtration, washed with ethanol and the pure product (0.391 g) was obtained. Yield = 74%. ^1H NMR (DMSO- d_6 , 300 MHz) δ : 11.28 (s, 2H, -OH), 9.98 (s, 2H, -NH), 8.34 (s, 2H, Ar-H), 8.11 (d, $J=5.4$ Hz, 4H, Ar-H), 7.63 (d, $J=0.6$ Hz, 2H, Ar-H), 7.09 (t, $J=8.4$ Hz, 6H, Ar-H), 6.84 (d, $J=4.8$ Hz, 2H, Ar-H), 3.84 (s, 2H, $-\text{CH}_2-$); ESI-MS: m/z calcd. for $\text{C}_{27}\text{H}_{22}\text{N}_6\text{O}_6$ [M] $^-$: 526.16, found: 525.10; elemental analysis calcd. For $\text{C}_{27}\text{H}_{22}\text{N}_6\text{O}_6$: C, 61.16; H, 4.82, N, 12.84; found: C, 61.39; H, 4.89; N, 12.48.

Results and Discussion

UV-vis Spectral Titrations and Naked-eye Experiments

The anion-binding properties of compound 1 were investigated by UV-vis spectroscopy in dry DMSO solution. Figure 1 shows the changes in the absorption spectrum of 1 as a function of AcO^- concentration at room temperature. The free 1 showed a main absorbance band centered at 427 nm, which could be assigned to the

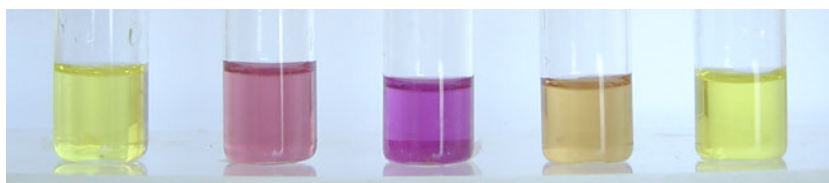


Fig. 2 Color changes of the probe 1 (2×10^{-5} M) in absence and presence of 30 equiv various anions tested (from left to right: 1 only; 1 + F^- ; 1 + AcO^- ; 1 + $H_2PO_4^-$; 1 + Cl^- , Br^- or I^-) in DMSO, respectively

intramolecular charge transfer (ICT) nature of *p*-nitrobenzene substituent [21]. There were gradual increase in 272 nm and decrease in 427 nm upon addition of acetate ions. Synchronously, a new red-shifted absorbance band at 565 nm (about 138 nm red shift) was observed, which responsible for naked-eye detection of acetate with color changes from yellow to deep purple (Fig. 2). The probe 1 demonstrated color changes from yellow to light purple and brown upon exposure to fluoride and dihydrogen phosphate, respectively. However, no visible color changes were observed when the other anions tested were added. The new band and color changes resulted from the host bond by anion and deprotonation of anion binding sites, which could be further validated by 1H NMR titrations of 1 with fluoride ion. In addition, there were two well-defined spectral isobestic points at 318 nm and 473 nm, respectively, suggesting the formation of a 1/ AcO^- complex.

In comparison to acetate ion, a similar optical response is observed when the probe is treated with F^- and $H_2PO_4^-$ (see Figure 3). However, addition of the other anions with weak basicity such as Cl^- , Br^- and I^- did not result in any obvious spectral changes and color changes, which were demonstrated in Figs. 2 and 3. The results indicated that the probe 1 could selective and naked-eye detection anions such as AcO^- , F^- and $H_2PO_4^-$ with strong basicity from the anions such as Cl^- , Br^- and I^- with weak basicity.

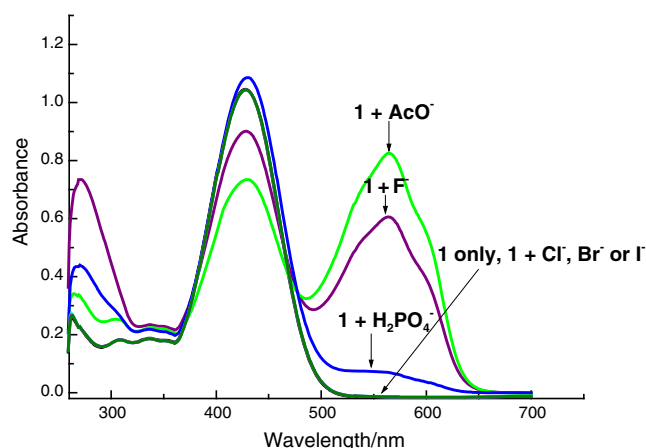


Fig. 3 UV-vis spectral changes of the probe 1 (2×10^{-5} M) in absence and presence of 30 equiv various anions tested in DMSO

Fluorescence Titrations

Next, fluorescence responses to various anions of 1 (2×10^{-5} M) were studied in DMSO solution. When a solution of 1 was excited at 365 nm, the compound 1 exhibited main emission around 560 nm with shoulder emission around 410 nm. Just as Fig. 4 showed, the presence of acetate ion induced disappearance of emission at 560 nm and appearance of fluorescence enhancement at 410 nm (a 150-nm emission band shift), which was rarely seen in the ever-reported anion probes. Accordingly, ratiometric fluorescence detection of AcO^- could be achieved in virtue of the probe 1. Such ratiometric changes of fluorescence could be rationalized on basis of photo-induced charge transfer (PCT) mechanism [20]. In compound 1, the anion binding site ($-OH$, electron-donating group) is conjugated to the fluorophore group (5,5'-methylene-bis-salicylaldehyde). When 1 interacted with acetate, the consequent changes in dipole moment were obtained and therefore ratiometric fluorescence changes were observed. Importantly, the compound 1 could act as a high selective ratiometric fluorescence probe for acetate ion, which could be seen in Fig. 5.

1H NMR Titrations

To shed light on the nature of the interactions between the probe 1 and the anions, 1H NMR spectral changes upon

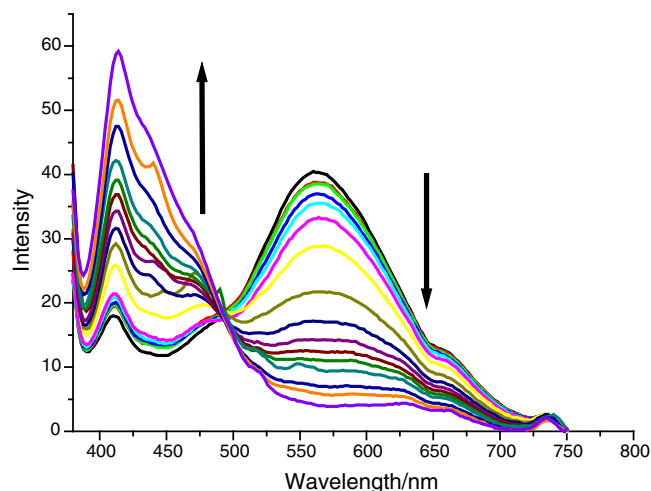


Fig. 4 Fluorescence changes of the probe 1 (2×10^{-5} M) in absence and presence of acetate ion in DMSO ($\lambda_{ex}=365$ nm)

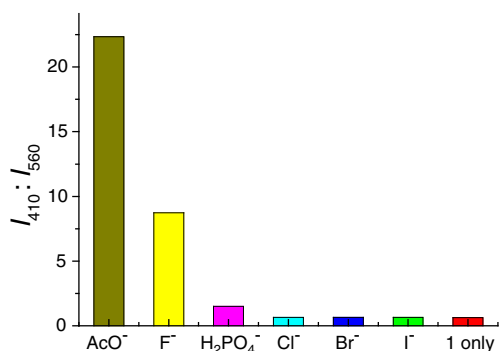


Fig. 5 Ratiometric fluorescence changes (I_{410}/I_{560}) of the probe 1 (2×10^{-5} M) in absence and presence of 30 equiv various anions tested in DMSO

addition of AcO^- to the $\text{DMSO-}d_6$ solution of 1 (1×10^{-2} M) were investigated. Figure 6 showed ^1H NMR spectral changes of the receptor 1 (0.01 M) in $\text{DMSO-}d_6$ in the absence and presence of different equiv of acetate ions. Chemical shifts of the compound resulting from the formation of hydrogen bond between the binding sites and the anion can be attributed to two effects [22]: (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. Obviously, it was found that the proton signals (δ : 11.28 and 9.98) underwent a slim downfield shift and were broaden (Fig. 6), with increasing AcO^- concentration from 0 equiv to 2 equiv indicating the formation of a host–guest hydrogen-bonding complex [30]. The phenyl protons especially for the protons (7.09 and 6.84 ppm) clearly

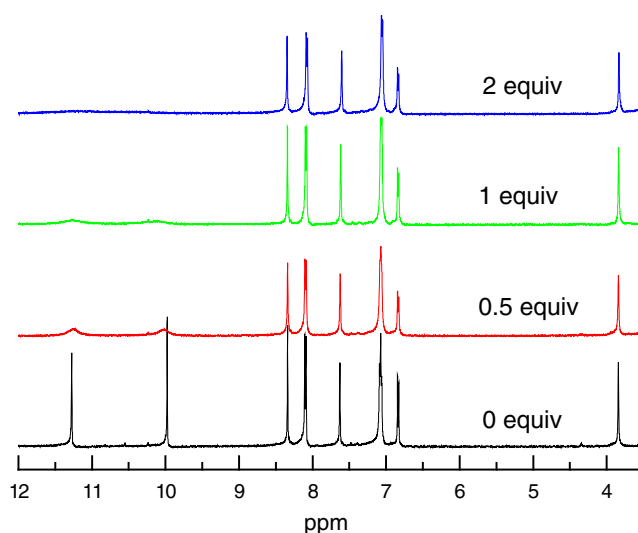


Fig. 6 ^1H NMR titrations of the probe 1 (1.0×10^{-2} M, $\text{DMSO-}d_6$) with different equiv of acetate

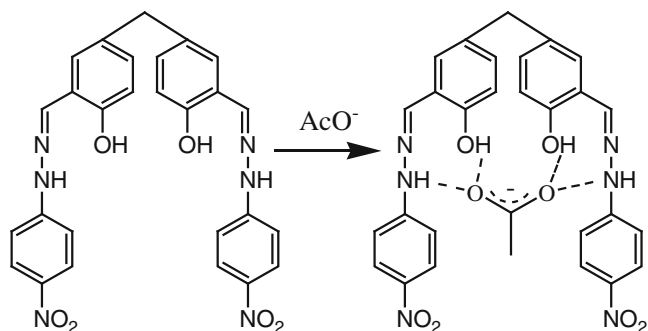
shifted upfield, indicative of the increase in the electron density of the phenyl ring owing to the through-bond effects. As mentioned above, the proposed binding mode in solution was demonstrated in Scheme 2.

Association Constants

Affinity constants of probe 1 for anionic species in DMSO , which were shown in Table 1, were determined by non-linear fitting analyses of the titration curves according to the Eq. 1 derived from the 1:1 host–guest complexation [23].

$$Y = Y_0 + \frac{(Y_{\text{lim}} - Y_0) \{c_H + c_G + 1/K_{\text{ass}} - [(c_H + c_G + 1/K_{\text{ass}})^2 - 4c_H c_G]^{1/2}\}}{2c_H} \quad (1)$$

where c_G and c_H are the concentration of guest and host, respectively and Y is the absorption of UV–vis or intensity of emission at certain concentration of host and guest. Y_0 is



Scheme 2 The proposed host–guest interaction mode in the solution

the absorption of UV–vis or intensity of emission of host only and Y_{lim} is the maximum absorption of UV–vis or intensity of fluorescence of host when guest was added. K_{ass} is the affinity constant of host–guest complexation.

Table 1 Association constants K_{ass} ($\text{mol}^{-1} \cdot \text{L}$) between receptor 1 and anions in DMSO

Anions	AcO^-	F^-	H_2PO_4^-	Cl^-	Br^-	I^-
K_{ass} ($\text{L} \cdot \text{mol}^{-1}$)	5,556 ^a	1,639	817	ND ^c	ND	ND
K_{ass} ($\text{L} \cdot \text{mol}^{-1}$)	12,500 ^b	8,333	3,333	ND	ND	ND

^a The Association constants were determined by the UV–vis spectra

^b The Association constants were determined by the fluorescence spectra

^c ND—The association constant could not be determined

Obviously, the trends of binding ability of the probe 1 with anions tested were $\text{AcO}^- > \text{F}^- > \text{H}_2\text{PO}_4^- \sim \text{Cl}^- \sim \text{Br}^- \sim \text{I}^-$. The selectivity for special analyte of the host molecule could be rationalized on the basis of not only the guest basicity but also shape complementarity between the host and the anionic guests [24]. Obviously seen from Scheme 2, the plane triangle acetate anion might be the fittest for the hydrogen atoms on binding sites of the probe among the anions. Interestingly, the association constants of the probe 1 with the same anion, which are determined by the UV–vis spectra and the fluorescence spectra, are very different. The acidity of the –OH moiety will be greatly enhanced upon excitation which has been proven in the literature [29]. As we all know, the stronger hydrogen bond interactions will form between the group with strong acidity and anions. Therefore, the association constants of the probe 1 with the same anion determined by the fluorescence spectra are bigger than those determined by the UV–vis spectra except fluoride ion.

Conclusion

In summary, a selective ratiometric fluorescence probe for acetate ion was designed and synthesized. In this probe, a hydrazone unit and a phenol unit are anion binding sites, and 5,5'-methylene-bis-salicylaldehyde group is a fluorophore. Presence of anions such as acetate resulted in ratiometric fluorescence changes of the probe with 150 nm blue shift from 410 nm to 560 nm. Such ratiometric fluorescence changes could be rationalized on basis of photo-induced charge transfer (PCT) mechanism.

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