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A highly selective fluorescent probe based on Michael addition for fast detection of hydrogen sulfide

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ABSTRACT: A new 4-hydroxy-1,8-naphthalimide-based compound (probe 1) has been designed and synthesized. The colorimetric and fluorescent properties of probe 1 towards hydrogen sulfide (H_2S) were investigated in detail. The results show that the probe 1 could selectively and sensitively recognize H_2S rather than other reactive sulfur species. The reaction mechanism of this probe is an intramolecular cyclization caused by the Michael addition of H_2S to give 4-hydroxy-1,8-naphthalimide. The intramolecular charge transfer of 4-hydroxy-1,8-naphthalimide is significant. Probe 1 quickly responded to H_2S and showed a 75-fold fluorescence enhancement in 5 minutes. Moreover, probe 1 could detect H_2S quantitatively with a detection limit as low as 0.23 μM.

Keywords: 1,8-Naphthalimide, Fluorescent probe, Hydrogen sulfide, Michael addition.

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1. Introduction

Hydrogen sulfide (H_2S) is a colorless gas with a typical rotten egg odor. It has long been considered chemical pollutants with toxicity. Exposing in the H_2S will irritate respiratory system[1] and excessive inhalation will make people lose consciousness, cardiac arrest and even death[2-4]. In addition, the excess H_2S in aerobic environment could have a negative effect on the air and water[5], which could seriously corrode metal pipes[6] and other serious damages[7]. However, recent reports show that H_2S is the third gaseous compound endogenously produced in vivo after carbon monoxide (CO) and nitric oxide (NO) [8-10]. It plays important roles in many biological processes, such as it can influence the proliferation and apoptosis of human uterine leiomyoma cells[11], protect the lung injury secondary to traumatic hemorrhagic shock[12], slow down the calcification of human umbilical vein smooth muscle cells[13,14], regulate the myocardial contractile force[15,16] and promote liver cancer cells proliferation/anti-apoptosis effects[17]. Therefore, an efficient method for sensitively and selectively probing H_2S is urgently required.

Currently, among the major methods for H_2S detection, the fluorescent probe has attracted more and more attention and become an active field because of its advantages of high selectivity and sensitivity. A number of fluorescent probes for H_2S have been developed mainly based on specific reactions between H_2S and the probes. These reactions includes H_2S reductive reactions[18-20], H_2S nucleophilic reactions[21-23], and copper sulfide precipitation reaction[24-26]. Among these, the probes based on Michael addition reactions are attractive, because Michael acceptors are able to react with H_2S and promote the intramolecular cyclization to release the fluorophore, whereas the reactions between Michael acceptors and biological thiols were reversible and therefore should not consume the probes[27]. Based on this

strategy, Y. Qian's group and several other groups presented some probes containing Michael acceptors for the detection of H_2S[28-31], but there are many aspects left to be improved, such as narrow pH range, long reaction time and complex preparation. Therefore, the development new probes for H_2S detection that are selective, sensitive, rapid, and applicable to the environmental and biological sensing are still in great demand.

In this work, we report a new fluorescence probe based on fluorophore, 4-hydroxy-1,8-naphthalimide, because of its high photostability, large Stokes' shift and ratiometric detection capability[32-35]. Michael acceptors, 2-(2-cyano-3-methoxy-3-oxoprop-1-enyl)-benzoic acid was chosen as H_2S accepter. The probe has high selectivity and good sensitivity to H_2S and meets the requirements of practical applications.

2. Experimental

2.1. Materials

4-Bromo-1,8-naphthalimide, EDC (1-(3-dimethylamino-propyl)-3-ethylcarbodiimide Hydrochloride), DMAP (4-dimethyl-aminopyridine), Methanol, Sodium metal, Hydrogen bromide and other chemicals were purchased from Sigma-Aldrich or Aldrich and were used as received. Solvents were dried according to standard procedures. $Na_2S{\cdot}9H_2O$ as the H_2S donor was analytical grade and purchased from Sigma-Aldrich [36]. All other reactive sulfur species, including cysteine (Cys), homocysteine (Hcy), glutathione (GSH), Na_2SO_3 and $Na_2S_2O_3$ were analytical grade. Probe 1 was synthesized using a modification of a literature method [27,37,38].

2.2 Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. ^{1}H NMR and ^{13}C NMR spectra were collected on a Bruker Avance DRX 300 MHz nuclear magnetic resonance spectrometer (Fällanden, Switzerland). Chemical shifts were reported relative to $CDCl_3$ (δ7.26) for ^{1}H NMR and $CDCl_3$ (δ 77.0) for ^{13}C NMR. UV absorption spectra were recorded on a Shimadzu UV-265 spectrophotometer (Tokyo, Japan). Fluorescence spectra were taken on a Hitachi F4500 spectrofluorometer (Tokyo, Japan). A PO-120 quartz cell (10 mm) was purchased from ShanhaiHuamei Experiment Instrument Plants, China. Fluorescence measurements were carried out with a slit width of 10 nm (λex=310 nm).

2.3. Preparation and characterization of probe

Synthesis of probe (1) is summarized in Scheme 1. N-Butyl-4-hydroxy-1,8-naphthalimide (2) and 2-(2-cyano-3-methoxy-3-oxoprop-1-enyl)-benzoic acid (5) were synthesized following the literature methods.

Dry CH_2Cl_2 (10 mL) was added to a mixture of N-butyl-4-Hydroxy-1,8-naphthalimide 2 (54mg, 0.2 mmol), 2-(2-cyano-3-methoxy-3-oxoprop-1-enyl)-benzoic acid (46 mg, 0.2 mmol) , EDC (38 mg, 0.2 mmol), and DMAP (2.5 mg, 0.02 mmol) in a 25 mL round-bottomed flask at room temperature. The mixture was stirred for 14h. Solvents were removed under reduced pressure and the crude material was purified by column chromatography using CH_2Cl_2 /ethyl acetate (10:0.5, v/v) to afford the desired probe as a white solid (25 mg, 30%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.58-8.68 (m, 3H), 8.26-8.29 (t, 1H, J = 8.14 Hz), 7.78-7.96 (m, 4H), 7.66-7.69 (t, 1H, J=7.24 Hz), 4.18-4.23 (t, 2H, J = 4.54 Hz), 1.68-1.76 (m, 2H), 1.42-1.47 (m, 2H), 0.97-1.02 (t, 3H, J = 1.57); ^{13}C NMR (100 MHz, CDCl3) δ 164.4, 163.8, 163.6, 162.1, 156.7, 151.4, 135.4, 134.8, 132.0,

131.9, 130.5, 127.8, 127.7, 127.6, 125.4, 119.9, 107.6, 53.8, 40.6, 30.4, 20.6, 14.1. (Fig.S3,S4)

< Insert Scheme 1 >

2.4. General UV–vis and fluorescence spectra measurements

The stock solutions of the probe 1 (2.5 mM) were prepared in acetonitrile. And phosphate buffer saline (PBS buffer) was prepared in deionized water, while $Na_2S·9H_2O$, cysteine (Cys), glutathione (GSH), homocysteine (Hcy), Na_2SO_3 and other solutions were prepared in PBS buffer. Cetrimonium bromide (CTAB) solution was prepared in deionized water. In this experiment, test solutions were prepared by placing 16 μL of the probe stock solution into the solution of 4 mL acetonitrile: buffer (1:1 v/v, PBS buffer, pH 7.4). The requisite volume of $Na_2S·9H_2O$ were added into the solutions gradually by using a pipette. 3mL of the solution was transferred into a 1cm quartz cell and fluorescence spectra were measured after addition of analytes for 5 min. UV-Vis and fluorescence spectra were obtained in acetonitrile: buffer (1:1 v/v, PBS buffer, pH 7.4) solutions.

3. Results and discussion

3.1 Spectroscopic properties of probe 1

The spectroscopic properties of probe 1 were tested in MeCN/buffer (1:1, 50mM PBS buffer, pH=7.4) solution. As shown in Fig.1, the probe 1 displayed an absorption band (280-340 nm) and the maximum absorption was about 310nm ($\varepsilon=4.2\times10^4$ M^{-1} cm^{-1}). The fluorescence spectra were also detected and shown in Fig.1, the probe 1

showed a weak fluorescence band and the emission maximum at about 450 nm. The fluorescence quantum yield was 0.032, due to photoinduced electron transfer (PET) effect caused by carbon-carbon double bond in the a,ß -unsaturated ketone moiety[39].

To examine the response of probe 1 for H_2S, probe 1 was treated with 100 μM H_2S, and the signals were recorded after mixing for 5 min. As shown in Fig.1, the absorption band was decreased and a new band at 450nm ($\varepsilon=3.7\times10^4$ M^{-1} cm^{-1}) appeared. Meanwhile, the color of the solution changed from colorless to yellow, which was clearly recognized by direct visual observation. The fluorescence emission at 450nm was decreased and a new strong fluorescence emission at 550nm was appeared. We also noted that the fluorescence of the solution changed from weak blue to yellow-green under illumination with a 365 nm UV lamp. Clearly, the results indicated that H_2S could be detected through both fluorescence and colorimetric methods by probe 1.

<insert Fig.1>

3.2 Fluorescence responses of probe 1 for H_2S

Preliminary examination has revealed that H_2S could turn on the fluorescence of probe 1 at 550 nm. Thus fluorescence spectra of probe 1 upon titration with H_2S were detected in MeCN/buffer (1:1, 50mM PBS buffer, pH=7.4, 1mM CTAB) solution. According to the previous work [40, 41], the purpose of CTAB may be attributed to 1) CTAB can increase the solubility of the probe in aqueous buffers, and 2) CTAB is a cationic surfactant, which may facilitate the reaction between sulfide anion and the probe. From Fig.2, we can found that the probe 1(10 μM) displayed an emission at 450 nm in the absence of H_2S, with increasing concentration of H_2S (0-30 μM) to the solution of probe **1**, a fluorescence emission at 550 nm was observed and enhanced

gradually, meanwhile, the fluorescence emission at 450nm decreased. The fluorescence emissions displayed about 75-fold fluorescence enhancement and minimal changes could be observed when the concentration of H_2S went beyond 20μM. The changes in fluorescence intensity should be attributed to the conjugate addition of H_2S to carbon-carbon double bond, which blocked the PET process.

A good linear relationship (R=0.98) was found between the fluorescence intensity at 550 nm and the concentration of H_2S from 0 to 20 μM (insert Fig.2). The detection limit for H_2S, based on the definition by IUPAC (CDL=3Sb/m) was calculated to be 0.23 μM. Probe **1** shows a high sensitivity towards H_2S which is comparable to other reported H_2S probes [27,29,42,43,44] and can be applied to quantitative detection of H_2S.

<insert Fig.2>

3.3 Selectivity over biothiols

To examine the selectivity of probe 1 toward H_2S, probe 1(10 μM) was treated with different reactive sulfur species, including Cys, GSH, Hcy, SO_3^{2-} , $S_2O_3^{2-}$ (all at 100 μM) in MeCN/buffer (1:1, 50mM PBS buffer, pH=7.4, 1mM CTAB), and fluorescence signals were recorded after mixing for 5 min. The fluorescence intensity at 550nm of probe 1 showed 75-fold fluorescence enhancement upon reaction with H_2S, whereas little fluorescence intensity changes were observed in the presence of other reactive sulfur species (Cys, GSH, Hcy, SO_3^{2-} and $S_2O_3^{2-}$), an error of ±10.0% in the relative fluorescence intensity was considered to be tolerable. Moreover, when reactive sulfur species, including SO_3^{2-}, $S_2O_3^{2-}$, GSH, Cys and Hcy, co-existed with H_2S, we observed almost the same fluorescence enhancement as that only treated by

100 mM H_2S. These results clearly demonstrated the probe 1 has the excellent selectivity even when H_2S co-existed with reactive sulfur species.

<insert Fig.3>

3.4 pH effects and time-dependence in the detection process of H_2S

The pH effects on fluorescence signals of probe 1 have been investigated. As shown in Fig. 4, the fluorescence of probe 1 appeared to be stable. In the presence of H_2S, a large emission enhancement at 550nm is observed over a wide pH range of 4-8. It is known that H_2S is a weak diprotic acid, in aqueous state under different pH, there is an equilibrium distribution of three species including H_2S, HS^- , and S^{2-}. The Ka_2 of H_2S is 1.00×10^{-14}, so there shouldn't be much S^{2-} at most pHs employed, the major form of H_2S in pH=4~8 exists as HS^- and H_2S, the ratio of $[HS^-]$:$[H_2S]$ was calculated based on $Ka_1 = 9.55 \times 10^{-8}$ of H_2S and list in Table 1. From the Table 1, we can find that H_2S is over 1000 times greater in concentration than HS^- at pH 4.00, as the pH decreases the equilibrium shifts toward fully protonated form, $[HS^-]$:$[H_2S]$ =2.39:1.00 at pH 7.4. At pH 8, $[HS^-]$:$[H_2S]$ =9.55:1.00 and the fluorescence intensity at 550nm plummeted. Such findings showed that H_2S is in fact the donor analyte and probe 1 could be used in biological applications and environmental analysis.

<insert Fig.4 and Table 1>

We also carried out time-dependent fluorescence changes of probe 1 to H_2S at room temperature. As depicted in Fig. 5, the fluorescence intensity at 550 nm increased dramatically when H_2S was added in the solution and the fluorescence intensity were able to reach a maximum in less than 5 min. It showed that the reaction between probe 1 and H_2S was completed within 5 min in PBS buffer at room temperature.

<insert Fig.5>

3.5 Proposed mechanism

Some references reported that simple thiols reacted readily with some Michael acceptors at physiological pH[27,45], so Michael acceptor, 2-(2-cyano-3-methoxy-3-oxoprop-1-enyl)-benzoic acid, was be used as H_2S receptors in probe 1. As shown in scheme 2, probe 1 can react with H_2S to form a free SH containing intermediate A2, the SH group should undergo a spontaneous cyclization to form by-product A3 and release the fluorophore A4, 4-hydroxy-1,8-naphthalimide. It shows long-wavelength fluorescence emission due to the stronger electron donating ability of hydroxyl group. The hypothesis was confirmed by the reaction between probe 1(0.1 mmol) and $Na_2S{\cdot}9H_2O$ (1.0 mmol) in CH3CN: PBS buffer (1:9) solution (20mL). The cyclization product A3 was isolated and confirmed by ^{1}HNMR and ^{13}CNMR (Fig.S5, Fig.S6). Biothiols (RSH) such as Cys, Hcy and GSH could react with probe1 in an analogous fashion to yield B1, which does not contain sulfhydryl group. Therefore, B1 cannot undergo an intramolecular nucleophilic attack. Clearly, probe 1 can be used to selectively trap H_2S and they are hardly consumed by thiols.

<insert Scheme 2>

4. Conclusions

In summary, we reported the development of a new 4-hydroxy-1,8-naphthalimide-based fluorescence off–on probe for H_2S detection. The probe exhibited good selectivity and sensitivity toward H_2S even in the presence of biothiols. The fluorescence emission displayed 75-fold enhancement for H_2S in MeCN/buffer

(1:1, 50mM PBS buffer, pH=7.4) solution within 5min. Moreover, the detection mechanism of probe to H_2S was proposed, after H_2S added, the new emission enhanced was caused by H_2S-mediated Michael addition followed by intramolecular cyclization reaction.

Acknowledgments

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Captions

Scheme 1. The synthesis route of probe 1.

Scheme 2. Proposed detection mechanism of probe 1 against H_2S.

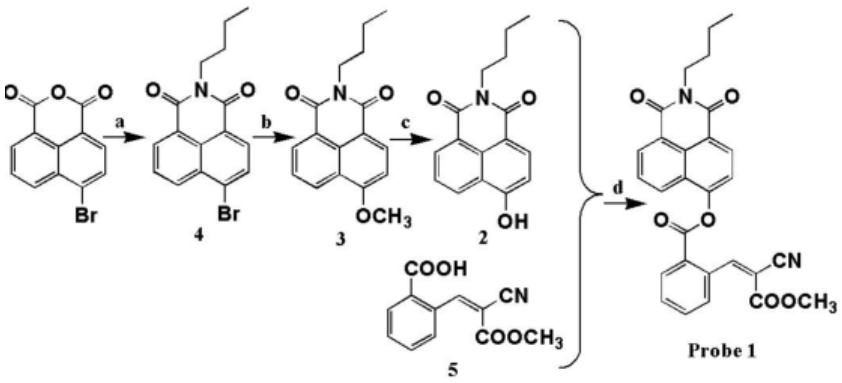
Fig.1 The UV-Vis and fluorescence spectra of probe 1 (10 μM) in MeCN/buffer (1:1, 50 mM PBS buffer, pH=7.4, 1 mM CTAB) solution without and with addition of Na_2S (100 uM) for 5 min at room temperature, λex=310 nm. Inset: the color changes of probe without and with addition of Na_2S. left: under ambient lighting, right: under UV irradiation.

Fig.2 The emission spectra of probe 1 (10 μM) in MeCN/buffer (1:1, 50 mM PBS buffer, pH=7.4, 1 mM CTAB) solution with different concentrations of Na_2S (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 μM) for 5 min at room temperature, λex=310 nm. Insert is the concentration of Na_2S dependence of the intensity of fluorescence emission (F_{550}).

Fig.3 The fluorescence intensity of probe 1 (10 μM) in MeCN/buffer (1:1, 50 mM PBS buffer, pH=7.4, 1 mM CTAB) solution in the presence of various reactive sulfur species: (1) control; (2) 100μM $Na_2S_2O_3$; (3) 100μM Na_2SO_3; (4) 100μM GSH; (5) 100μM Hcy; (6) 100μM Cys; (7) 100μM Na_2S; (8) 100μM Na_2S+100μM $Na_2S_2O_3$; (9) 100μM Na_2S+100μM Na_2SO_3; (10) 100μM Na_2S+100μM GSH; (11) 100μM Na_2S+100μM Hcy; (12) 100μM Na_2S+100μM Cys. λex=310 nm. Each spectrum was acquired 5 min after various analytes addition at 25 ^oC.

Fig.4 pH-dependent fluorescence intensity (550nm) changes of probe 1 (10 μM) in the presence of Na_2S (100 μM).

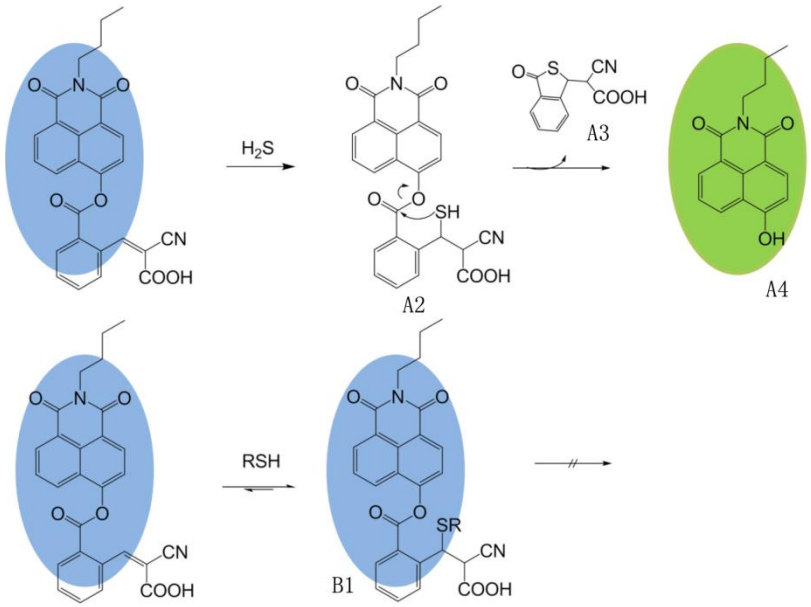
Fig.5 Time-dependent fluorescence response of the probe 1 (10 uM) to Na_2S (100 uM) in MeCN/buffer (1:1, 50 mM PBS buffer, pH=7.4, 1 mM CTAB) solution.



a) n-BuNH2, EtOH, reflux,89%; b) CH3ONa/CH3OH, reflux, 89%;

c) HBr, reflex, 65%; d) EDC, DMAP, 52%.

Scheme 1



Scheme 2

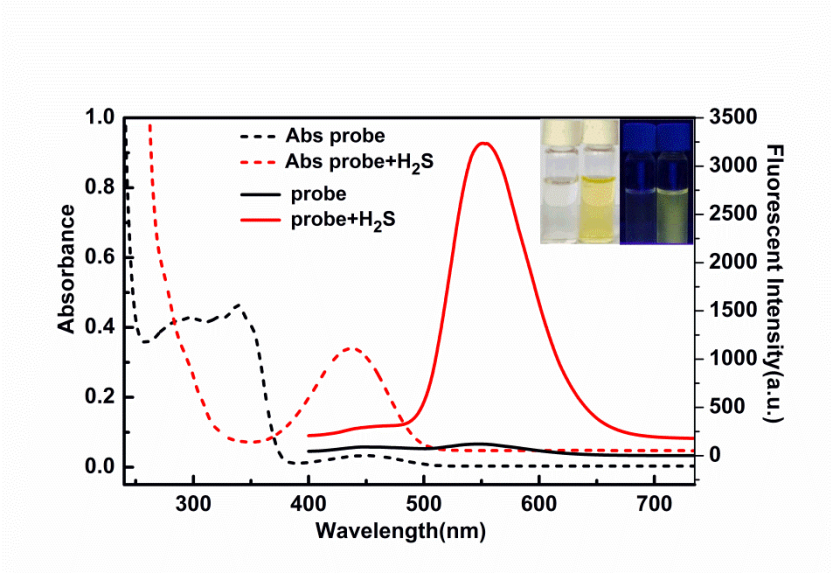

Abs probe
Abs probe+H2S
probe
probe+H2S
Absorbance
Fluorescent Intensity(a.u.)
Wavelength(nm)
1.0
0.8
0.6
0.4
0.2
0.0
3500
3000
2500
2000
1500
1000
500
0
300
400
500
600
700

Fig.1

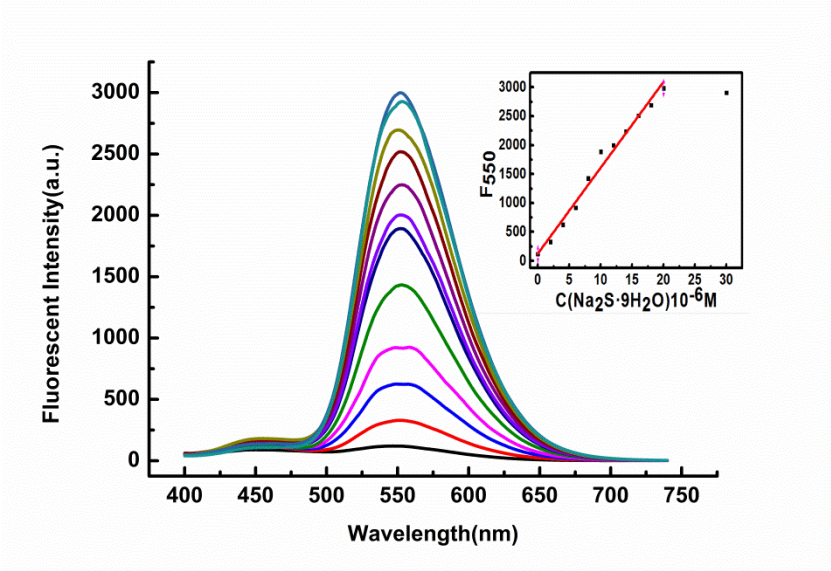

Fluorescent Intensity(a.u.)
3000
2500
2000
1500
1000
500
0
400
450
500
550
600
650
700
750
Wavelength(nm)
F550
C(Na2S·9H2O)10-6M
0
5
10
15
20
25
30

Fig.2

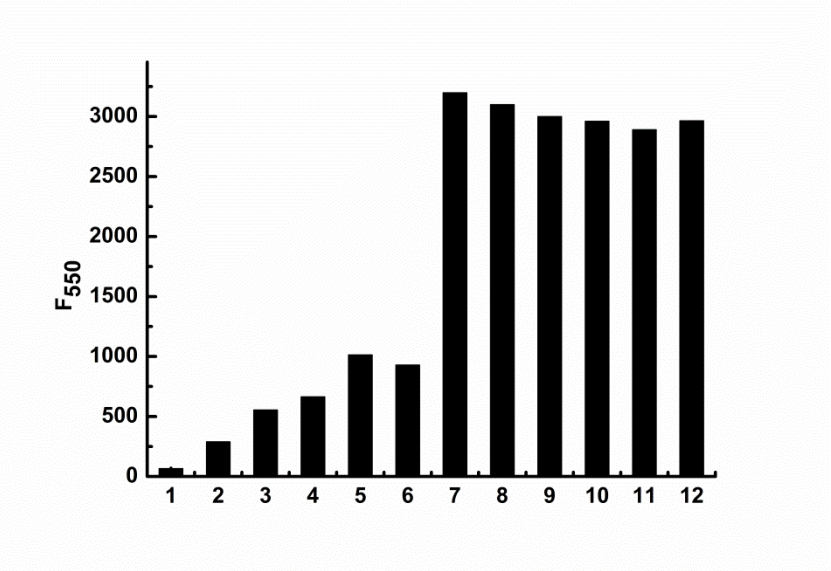


Fig.3

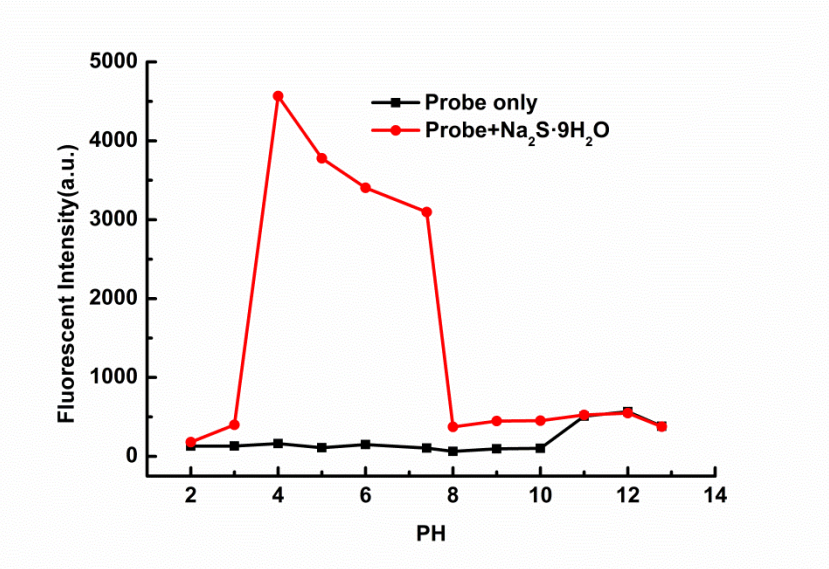

Probe only
Probe+Na2S·9H2O
Fluorescent Intensity(a.u.)
PH
5000
4000
3000
2000
1000
0
2
4
6
8
10
12
14

Fig.4

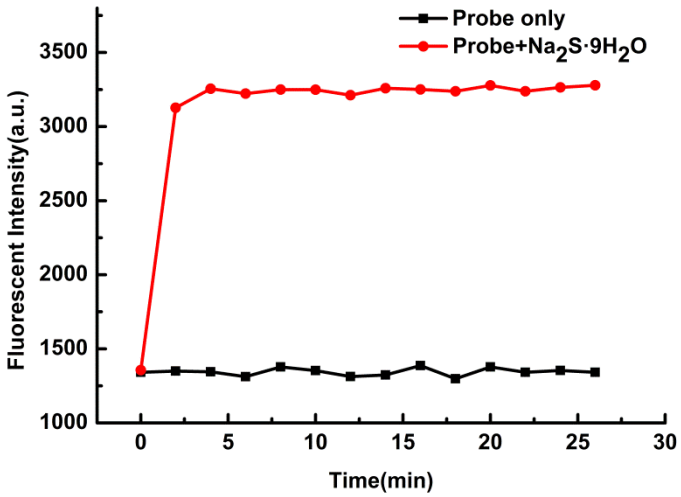
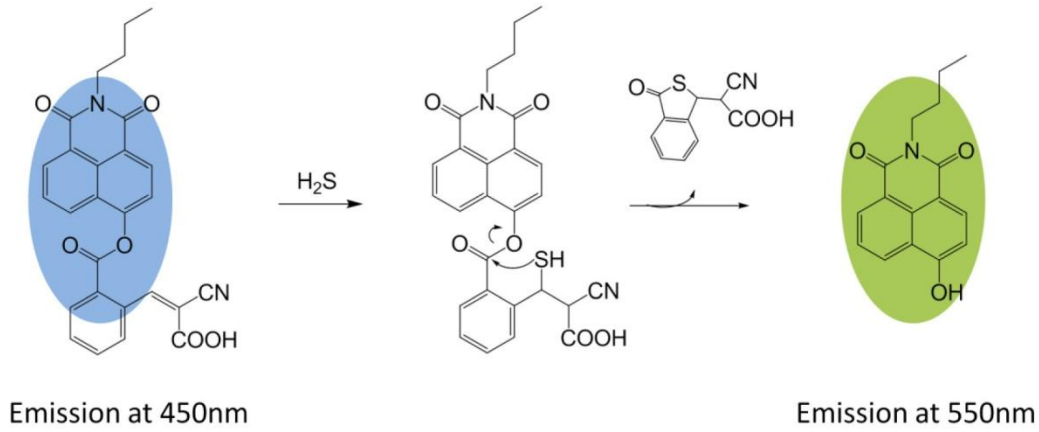

Probe only
Probe+Na2S·9H2O
Fluorescent Intensity(a.u.)
3500
3000
2500
2000
1500
1000
0
5
10
15
20
25
30
Time(min)

Fig.5

Table 1 The ratio of $[HS^-]$:$[H_2S]$ at different pH

pH=4	pH=5	pH=6	pH=7	pH=7.4	pH=8
9.55×10^{-4}:1	9.55×10^{-3}:1	9.55×10^{-2}:1	0.96:1.00	2.39:1.00	9.55:1.00



Graphical abstract

Highlights

- A new colorimetric and ratiometric fluorescence probe for hydrogen sulfide is developed.
- The use of Michael acceptor as the sensing group of H_2S shows high selectivity and sensitivity.
- Probe 1 for hydrogen sulfide detection is based on intramolecular charge transfer (ICT) mechanism.