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A ratiometric "two-in-one" fluorescent chemodosimeter for fluoride and hydrogen sulfide

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ABSTRACT

Styryl-BODIPY containing 2,4-dinitrobenzenesulfonyl, DNBS, and trihexylsilylacetylene units, THS, shows fast and high differential responses for fluoride and hydrogen sulfide sensing. The chemodosimeter can sense F^- expressed by a large 60 nm blue-shift of the fluorescence band as well as an emission intensity enhancement with a lower detection limit of 14.5 nM, while it can also sense HS^- by fluorescence in a quasi-OFF-ON manner with a 10-nm red-shift of the luminescence band. The fluorescence of the probe is stable and pH-independent in the physiological pH range. To the best of our knowledge, this is the first report on the single fluorescent molecule sensing both F^- and HS^- each with distinct spectroscopic responses. Finally, the probe has been used successfully for bioimaging the hydrogen sulfide in living cells. This "two-in-one" design strategy will inspire more multimodal agents for various applications.

1. Introduction

Fluorescent probes are widely used as valuable molecular tools for the detection of neutral and ionic species owing to their high sensitivity, selectivity, and ease of operation. Large libraries of chemosensors for single ions are generally designed [1], but a single fluorescent probes capable of the simultaneous detection of multiple targets are still rare [2–5]. Bifunctional probes are of fundamental importance because they can differentiate and detect analytes with distinct spectroscopic responses by overcoming difficulties such as cross-talk and a large invasive effect [6]. Furthermore, multimodal sensors, imaging agents and theranostic agents are emerging owing to the advantages of realization of multi-purposes in one agent or one test. Based on this background, we are devoted to design "two-in-one" or "more-in-one" sensors. However, only a few examples of bifunctional sensors have been reported so far in order to detect anions. The main challenge that electrons in anions are more diffused than those of cations, leading to weak electrostatic interactions between anions and receptor molecules [7] and thus making recognition of anions is more difficult.

Fluoride is of great importance in areas as diverse as the analysis of drinking water, treatment of osteoporosis and dental care [8]. However, high concentrations of the fluoride are hazardous and can lead to fluorosis or cancer [9]. Hydrogen sulfide, H_2S, is a gas molecule that contributes to various physiological processes, including ischemia reperfusion injury, vasodilation, oxygen sensing, apoptosis, angiogenesis, insulin signaling, inflammation, and neuromodulation [10]. Concurrently, abnormal H_2S levels are associated with diseases such as Alzheimer's disease, Down's syndrome, diabetes and liver cirrhosis [11]. Therefore, highly sensitive and selective detection of fluoride and hydrogen sulfide is crucial and has attracted a wide research interest. A commonly used substitute for H_2S is NaHS since in physiological medium of pH 7.4 most endogenous H_2S undergoes dissociation (i.e. $H_2S \rightarrow HS^- + H^+$) [12]. Even though individual reports for either F^- or H_2S are common [13–18], there is no report for ratiometric and colorimetric sensing of F^- and HS^- with distinct emission signals. Molecular sensors exhibiting long wavelength absorption and emission bands, sufficiently soluble in water, and exhibiting good cell membrane permeability are highly desirable as they are suitable for biological imaging in living cells. We recently reported a fluorescent THS (trihexylsilylacetylene)-functionalized boron-dipyrromethene, BODIPY **1** (Scheme 1) for F^- sensing [19]. Upon additions of F^-, this probe formed **2** exhibiting a distinct green emission, but the fluorescence band showed only a modest blue-shift from 571 to 554 nm.

In order to address this drawback, we now report chemosensor **3** (Scheme 2), a new bifunctional probe capable of sensing both F^- and HS^- with distinct and well-separated fluorescence signals in a fast

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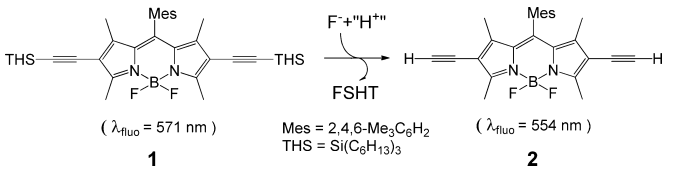


Scheme 1. Reaction of sensor **1** with F^- anion (in acetone; $t < 5$ min).

manner and excellent detection limits. This unique sensor is composed of three key functional moieties: (1) a strongly red-emitting styryl-containing BODIPY, (2) a THS group extremely sensitive to F^- and (3) a DNBS (2,4-dinitrobenzenesulfonyl) unit as an electron acceptor known to quench fluorescence by electron transfer [20–22]. This "two-in-one" tailoring strategy should be valuable for the design of future multimodal agents.

2. Experimental

2.1. Apparatus and reagents

The NMR spectra were measured in appropriate deuterated solvents on a JEOL EX270 or a Varian Inova 400 MHz FT-NMR spectrometer, using $SiMe_4$ as an internal standard. The fluorescence spectra were measured using a Perkin–Elmer LS-55 spectrofluorophotometer. A UNICO 4802 UV–vis double-beam spectrophotometer was used to measure absorption spectra. The ESI mass spectra were measured on a Finnigan LCQ advantage mass spectrometer. Matrix-assisted laser desorption ionization time-of-flight mass spectra were measured on a Voyager-DESTR MALDI-TOF mass spectrometer. Absolute quantum yield were measured in acetone on Edinburgh Instruments (FLS920).

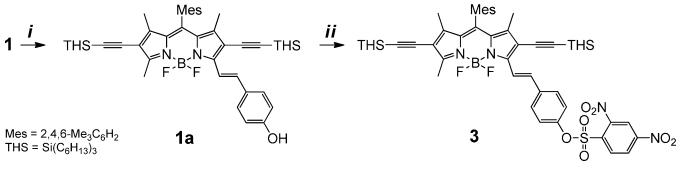
All reagents and chemicals, unless stated otherwise, were purchased from commercial suppliers and used without further purification. Solvents were dried and distilled from drying agents under an inert atmosphere prior to use. Twice-distilled water was used throughout all experiments.

2.2. Synthesis of *1*

1 was synthesized according to a reported procedure [19]. **1** (5.1 g, 74% yield) was red oily solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (s, 2H), 2.64 (s, 6H), 2.34 (s, 3H), 2.05 (s, 6H), 1.46 (s, 6H), 1.30 (m, 48H), 0.86 (m, 18H), 0.62 (m, 12H). MS: 979 (M^+).

2.3. Synthesis of *1a*

1 (476.2 mg, 0.50 mmol), 4-hydroxybenzaldehyde (61.1 mg, 0.50 mmol), toluene (20 mL), piperidine (2 mL) and glacial acetic acid (2 mL) were added to a 50 mL round-bottomed flask equipped with a Dean–Stark trap. The reaction mixture was refluxed for 12 h. The solvent was removed under reduced pressure. The resulting residue was purified by column chromatography (ethyl acetate:petroleum = 1:4) to afford **1a** (12.6 mg, 24.0%) as red oily solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (d, J = 16.2 Hz, 2H), 7.60 (dd, J_1 = 16.2 Hz, J_2 = 8.2, 2H), 7.02 (s, 2H), 6.90 (d, J = 8.2 Hz, 2H), 2.73 (s, 3H), 2.40 (s, 6H), 2.12 (s, 9H), 1.35 (m, 48H), 0.92 (m, 18H), 0.70 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.4, 157.1, 153.0, 146.1, 143.4, 141.3, 139.1, 135.2, 131.4, 131.0, 130.7, 130.0, 129.6, 129.3, 116.7, 116.4, 116.0, 102.4, 100.2, 98.6, 33.5, 31.8, 24.2, 23.3, 22.8, 21.5, 19.8, 15.3, 14.4, 13.6, 12.6; HRMS (MALDI-TOF): m/z calcd for $C_{69}H_{105}BF_2N_2OSi_2$ $[M]^+$: 1082.7827; found 1082.9008.



Scheme 2. Synthesis of **3**. (i) 4-Hydroxybenzaldehyde/piperidine/PhMe. (ii) 2,4-Dinitrobenzenesulfonylchloride/Et_3N/CH_2Cl_2.

2.4. Synthesis of *3*

1a (54.3 mg, 0.050 mmol) was dissolved in CH_2Cl_2 (10 mL). Triethylamine (0.1 mL) was added to the solution, and the mixture was stirred for 5 min. A solution of 2,4-dinitrobenzeneslfonylchloride (66.7 mg, 0.25 mmol) in CH_2Cl_2 (2 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 3 h at 50 °C. After removing the solvents by evaporation, the resulting crude mixture was separated by column chromatography (CH_2Cl_2:petroleum = 1:2) to afford **3** (21.2 mg, 32.0%) as purple oily solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 8.56 (d, J = 8.7 Hz, 2H), 8.32 (d, J = 8.2 Hz, 1H), 8.22 (d, J = 8.6 Hz, 1H), 7.62 (m, 2H), 7.25 (d, J = 4.3 Hz, 2H), 7.03 (s, 2H), 2.71 (s, 3H), 2.41 (s, 6H), 2.12 (s, 9H), 1.34 (m, 48H), 0.90 (m, 18H), 0.69 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3, 151.1, 150.6, 149.2, 145.3, 142.4, 139.4, 137.5, 135.0, 134.3, 133.7, 131.5, 129.4, 126.7, 122.5, 120.5, 119.3, 117.4, 113.5, 102.6, 101.1, 100.1, 98.0, 33.4, 31.8, 30.0, 24.2, 23.3, 22.8, 21.5, 19.7, 15.3, 14.4, 13.6, 12.8;. HRMS (MALDI-TOF): m/z calcd for $C_{75}H_{107}BF_2N_4O_7SSi_2$ $[M]^+$: 1312.7460; found 1312.4034.

2.5. Synthesis of *4a*

4a was synthesized the same way to the reported procedure [23]. **4a** (20.8 mg, 18.4% yield) was red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 11.6 Hz, 2H), 7.48 (d, J = 10.4 Hz, 1H), 6.97 (d, J = 12.0 Hz, 4H), 6.84 (d, J = 11.2 Hz, 1H), 6.57 (s, H), 5.99 (s, H), 2.60 (s, 3H), 2.34 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H). MS: 469.6 (M^+).

2.6. Synthesis of *4*

4 was synthesized the same way to the reported procedure [23]. **4** (15.6 mg, 25.0% yield) was red oily solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1H), 8.48 (m, 4H), 8.25 (d, J = 11.6 Hz, 2H), 7.54 (d, J = 11.2 Hz, 2H), 7.18 (d, J = 12.0 Hz, 1H), 6.97 (s, 1H), 6.56 (s, 1H), 6.04 (s, 1H), 2.59 (s, 3H), 2.35 (s, 3H), 2.10 (s, 6H), 1.43 (s, 3H), 1.42 (s, 3H). MS: 700.8 (M^+).

2.7. Preparation of the test solutions

The stock solution of **3** was prepared at 1.0 mM in acetone. The solutions of various testing species were prepared from NaHS, NaCl, KBr, NaI, CH_3COONa, Na_2CO_3, $Na_2S_2O_3$, $NaNO_2$, $NaHCO_3$, Na_2SO_3, Na_2HPO_4, KSCN, H_2O_2, NaClO, ascorbic acid, cysteine, glutathione in the twice-distilled water, and tetrabutylammonium fluoride in tetrahydrofuran.

2.8. Cytotoxicity assays

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of **3** (DMSO). The final concentrations of the probe were kept from 0 to 50 μM. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h, followed by MTT (methyl thiazole tetrazolium) assays.