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A Boron-dipyrromethene-Based Fluorescent Probe for Colorimetric and Ratiometric Detection of Sulfite

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Supporting Information

ABSTRACT: BODIPY-Le, a colorimetric and ratiometric fluorescent probe based on boron-dipyrromethene for selective detection sulfite ion, was investigated. Boron-dipyrromethene levulinyl ester (BODIPY-Le) is composed of an indole-based BODIPY dye and the levulinyl protective group, which could be easily and selectively deprotected by sulfites. As a result, the absorption and emission spectra show a dramatic red shift, and the development of a colorimetric and ratiometric fluorescent sulfite probe could be achieved. Besides, BODIPY-Le also exhibited prominent turn-on or turn-off type fluorogenic signaling toward sulfite ions once excited at 510 and 620 nm, respectively.

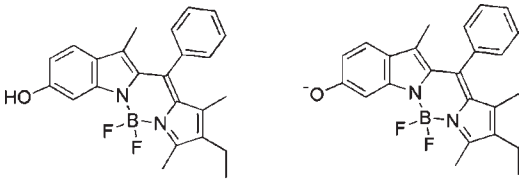
KEYWORDS: Boron-dipyrromethene, ratiometric fluorescent probe, sulfite

INTRODUCTION

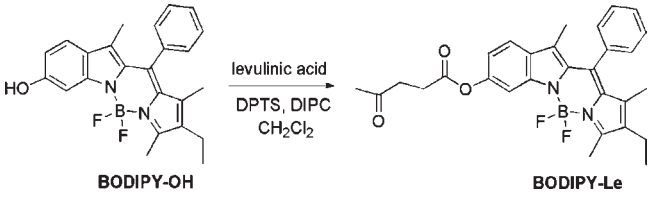
Sulfites are commonly used to preserve many processed foods and beverages from oxidation, browning, and microbial reactions.[1] However, sulfites are toxic in high doses and are well-known to cause asthma and other allergic reactions in persons who are hypersensitive to them.[2−4] Hence, the Joint FAO/WHO Expert Committee on Food Additives has issued that an acceptable daily intake should be lower than 0.7 mg/kg of body weight.[5] For these reasons, the development of methods that can detect this anion has become a topical objective.[6−8] Several traditional methods have been applied to sense sulfite, such as electrochemistry and chromatography.[9−11]

As an alternative, developments of sensors based on anion-induced changes in fluorescence are particularly attractive because of the simplicity and high spatial and temporal resolution of fluorescence.[12−19] The construction of small molecule fluorescent probes to detect anions based on an increase or decrease of the emission intensity is relatively easy. However, as the change in fluorescence intensity is the only detection signal, factors such as the probe concentration, instrumental efficiency, and environmental conditions can interfere with the signal output.[20−23] It is therefore desirable to eliminate the effects of these factors. Ratiometric sensors that exhibit a spectral shift upon binding to anions can eliminate most or all ambiguities by self-calibration of two emission bands. Therefore, we report a judiciously designed fluorescent probe based on boron-dipyrromethene dye in this work, which displays a ratiometric response to sulfite through a specific chemical transformation. The chemical transformation-based "sensing" promoted by analyte has been demonstrated to be a new approach on the venerable field of analyte-specific qualitative analysis.[24,25] The search of specific reaction promoted by sulfite is crucial. It has been established that levulinate-protected phenol moieties could be easily and selectively deprotected by sulfites under mild and neutral conditions.[6,26,27] On the basis of this platform, compound

Chart 1. Structure of BODIPY-OH, BODIPY-O$^-$, and Their Photophysical Properties in H_2O/DMSO Solution (1:1)



Scheme 1. Synthesis of BODIPY-Le



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BODIPY-Le was designed as the ratiometric fluorescent probe for sulfite. BODIPY-Le is composed of an indole-based BODIPY dye and the levulinyl protective group. The indole-based BODIPY was chosen as a signaling unit due to the excellent characteristics of BODIPY-OH and its derivatives (Chart 1), such as strong emission of wavelengths over 500 nm and intense fluorescence quantum yields of BODIPY-OH, BODIPY-O^-, and BODIPY-OCOR, which have been used to construct a benzenethiols probe.[28,29] The levulinate protective group could be deprotected by sulfite to give BODIPY-O^-. In other words, the ester group was transformed into O^- unit, and the electron-donating ability was regulated in the deprotection process. As O^- is a stronger electron-donating group, the absorption and emission of BODIPY-O^- show dramatic redshifts relative to that of BODIPY-Le. As a result, the development of a colorimetric and ratiometric sulfite probe could be achieved.

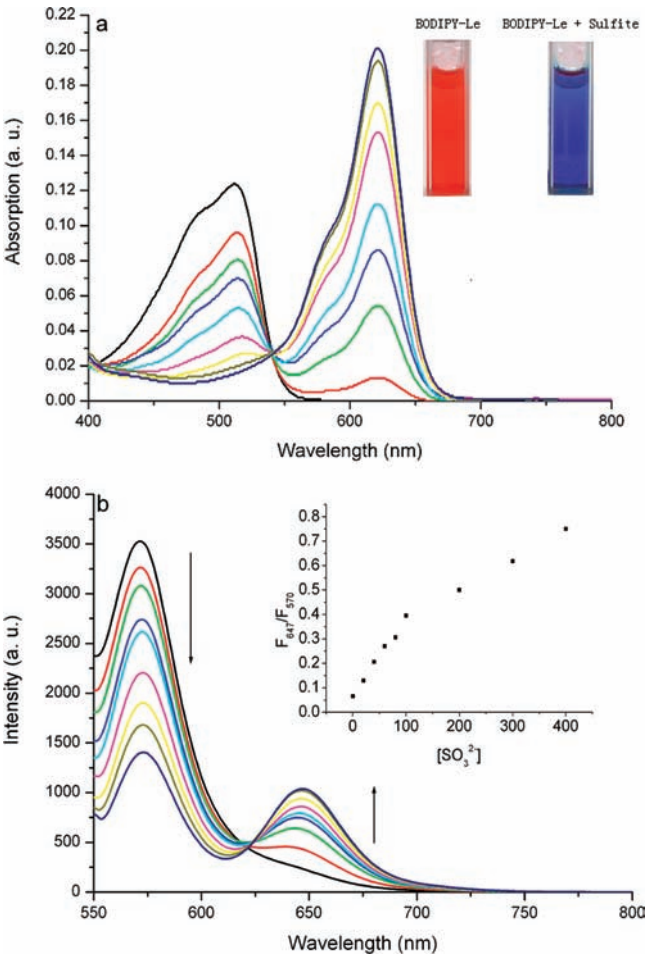


Figure 1. (a) Absorption spectra of BODIPY-Le (5×10^{-6} M) in the presence of different concentrations of sodium sulfite (0, 20, 40, 60, 80, 100, 200, 300, and 400 equiv) in H_2O/DMSO solution (1:1). Insets: Color changes of BODIPY-Le upon additions of sodium sulfite (100 equiv). (b) Fluorescence spectra of BODIPY-Le (λ_{ex} = 538 nm) in the presence of different concentrations of sodium sulfite (20, 40, 60, 80, 100, 140, 200, 300, and 400 equiv). Inset: Ratiometric calibration curve of F_{647}/F_{570} as a function of sulfite concentration. Each measurement was performed after 20 min of mixing.

MATERIALS AND METHODS

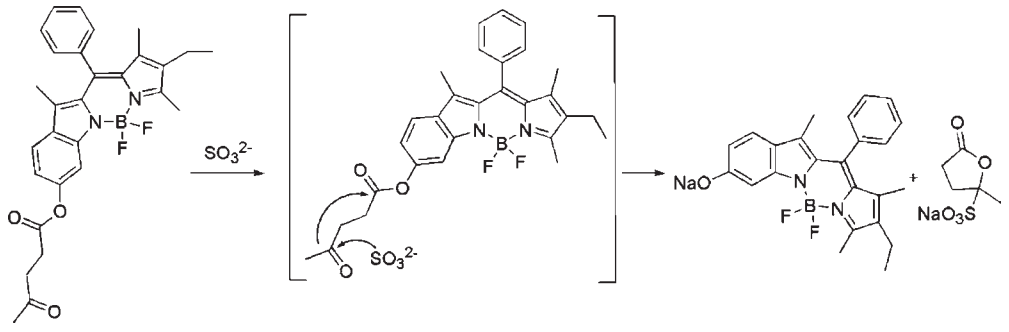
General Methods. All chemical reagents and solvents for synthesis were purchased from commercial suppliers and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard) at room temperature. The analytical high-performance liquid chromatography (HPLC) was performed on Waters 600E HPLC system. Mass spectra were measured on a HP 1100 LC-MS spectrometer. UV−vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian CARY Eclipse Fluorescence spectrophotometer. Spectral-grade solvents were used for measurements of UV−vis absorption and fluorescence.

Preparation of BODIPY-Le. A mixture of BODIPY-OH (404 mg, 1.0 mmol), levulinic acid (119 mg, 1.03 mmol), DPTS (810 mg, 2.6 mmol), and DIPC (327 mg, 2.6 mmol) in CH_2Cl_2 was refluxed overnight. The solvent was removed in vacuo, and the residual solid was purified by flash chromatography (silica gel) to afford 426 mg (85%) of BODIPY-Le. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58−7.52 (m, 3H), 7.46−7.38 (m, 2H), 7.34 (dd, J = 6.4, 2.7 Hz, 2H), 6.77 (dd, J = 8.8, 2.0 Hz, 1H), 2.92−2.82 (m, 4H), 2.70 (s, 3H), 2.37 (q, J = 7.6 Hz, 2H), 2.24 (s, 3H), 1.60 (s, 3H), 1.40 (s, 3H), 1.04 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 206.37, 171.27, 164.83, 151.35, 144.48, 142.69, 142.04, 137.84, 136.73, 134.95, 133.66, 130.80, 129.34, 128.10, 121.55, 115.53, 106.96, 38.00, 29.91, 28.22, 17.16, 14.09, 13.60, 12.28, 11.13. HRMS (ESI-TOF) m/z calcd for $C_{29}H_{28}BF_2N_2O_3$ $[M - H]^-$, 501.2161; found, 501.2173.

RESULTS AND DISCUSSION

BODIPY-OH was prepared according to literature method.[28,29] BODIPY-Le was readily synthesized in one step by reaction of BODIPY-OH with levulinic acid in the presence of *N*,*N*′-diisopropylcarbodiimide (DIPC) and *p*-(dimethylamino)-pyridinium *p*-toluenesulfonate (DPTS) in good yield (Scheme 1).

Scheme 2. Proposed Mechanism of the Reaction of BODIPY-Le with Sulfite



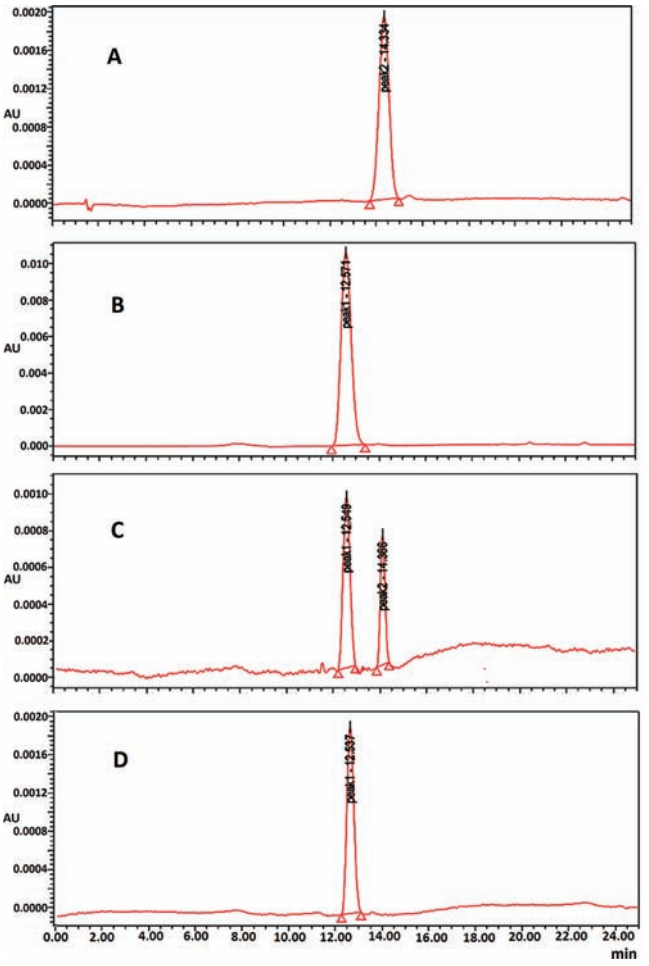


Figure 2. The HPLC profile of (A) BODIPY-Le; (B) BODIPY-OH in presence of 100 equiv of sodium sulfite; (C) the mixture of 100 equiv of sodium sulfite and BODIPY-Le, measured after 10 min of mixing; and (D) the mixture of 100 equiv of sodium sulfite and BODIPY-Le, measured after 30 min of mixing. Conditions: columnm Waters SunFire C18 5 μm, 4.6 mm × 150 mm column; solvents, CH_3CN/H_2O; gradient, 70−95% CH_3CN (0−15 min), 95% CH_3CN (15−25 min); and detection wavelength, 540 nm.

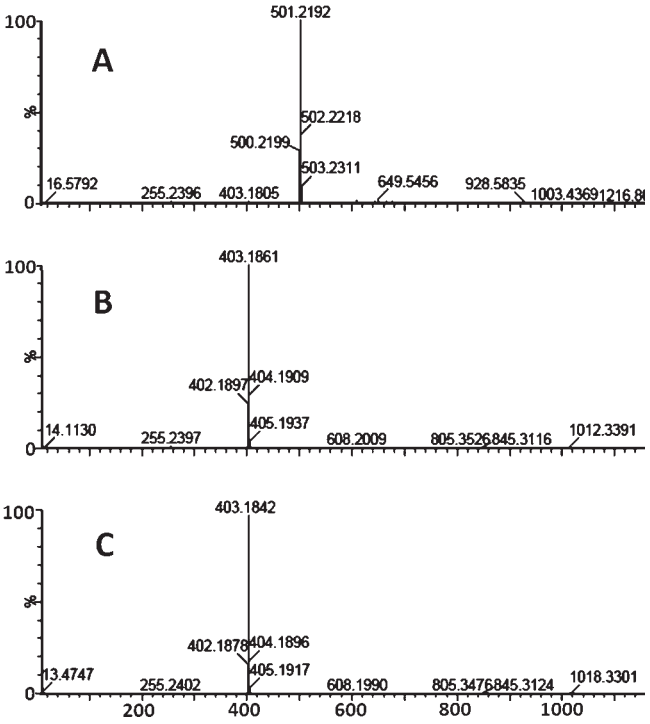


Figure 3. The ESI-MS spectra (negative) of (A) BODIPY-Le; (B) BODIPY-OH in the presence of 100 equiv of sodium sulfite; and (C) the mixture of 100 equiv of sodium sulfite and BODIPY-Le, measured after 30 min of mixing.

The sensory response of probe BODIPY-Le is exemplified by its reaction with sulfite (Figure 1). The optical features of BODIPY-Le are characteristic of the BODIPY platform. The main absorption band of BODIPY-Le, attributed to the 0−0 vibrational band of a strong S_0-S_1 transition, is centered at 510 nm in H_2O/DMSO solution (1:1). Upon gradual addition of sodium sulfite to a solution of BODIPY-Le in H_2O/DMSO solution (1:1), a decrease in the absorption band at 510 nm and a concomitant increase of a new band at 620 nm were observed, with a distinct isosbestic point at 538 nm. Concomitantly, the color of the solution turned from orange to blue. Notably, upon addition of SO_3^{2-}, a pronounced enhancement at 620 nm was noted after 5 min, indicative of a fast reaction of BODIPY-Le with SO_3^{2-} (Figure S5 in the Supporting Information). The absorption band at 620 nm is the characteristic absorption of BODIPY-O^-, which is red-shifted by 110 nm as compared to that of BODIPY-Le. Moreover, the ratio of the absorbance at 620 and 510 nm increased over 500-fold. This indicates the capability of BODIPY-Le for detecting sulfite by absorption ratiometry.

Fluorescence spectroscopy was used to assess the ability of BODIPY-Le to detect sulfite by ratiometric fluorescence measurement. Figure 1b displayed the emission spectra change of BODIPY-Le upon addition of an increasing amount of sodium sulfite. Free BODIPY-Le showed strong fluorescence at about 553 nm, and no visible variations was observed in the assay condition, suggesting that BODIPY-Le was stable and not converted to BODIPY-O^-. However, a remarkable fluorescence change was observed upon addition of sodium sulfite with the excitation wavelength at the isosbestic point. At a lower sulfite concentration (<20 equiv), the emission maximum at 553 nm was red-shifted to 570 nm (Figure S1 in the Supporting Information). With further increasing sulfite concentration, the emission intensity at 570 nm decreased gradually with the simultaneous appearance of a new red-shifted emission band at 647 nm. It is worth noting that the ratios of the emission intensities at 647 and 570 nm (F_{647}/F_{570}) showed a 12-fold ratiometric enhancement (Figure 1b, inset), which indicates the capability of BODIPY-Le for detecting sulfite by fluorescence ratiometry. Free BODIPY-Le has no characteristic emission band at 647 nm; however, introduction of sulfite elicited a dramatic increase in the emission around 647 nm with the excitation wavelength at 620 nm. This result is indicative of an off-on switch triggered by sulfite ion (Figure S2 in the Supporting Information). When the emission intensity change ($I_{min} - I/I_{min} - I_{max}$) of BODIPY-Le at 647 nm was plotted against SO_3^{2-} concentration, a calibration curve (Figure S4 in the Supporting Information) revealing a linear relationship (R value = 0.98) in the SO_3^{2-} concentration range of 0−2 mM was obtained. The detection limit of BODIPY-Le toward SO_3^{2-} was determined as 5.8×10^{-5} M under the experimental conditions.

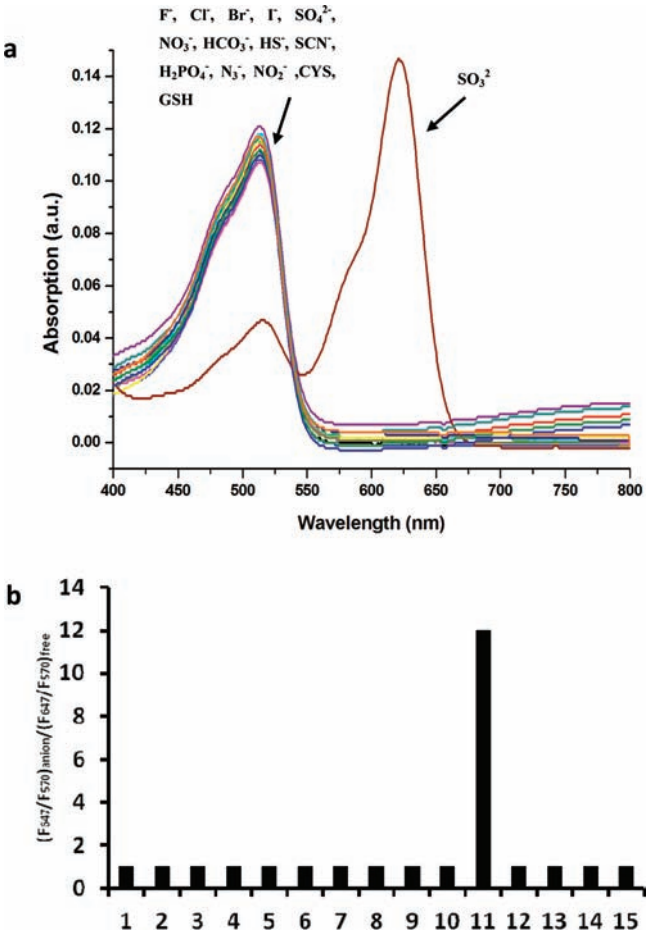


Figure 4. (a) Absorption spectra of BODIPY-Le in the absence and presence of 100 equiv of various anions. (b) Fluorescence ratiometric response of BODIPY-Le in the absence and presence of 100 equiv of various anions in H_2O/DMSO solution (1:1): 0, free; 1, F^-; 2, Cl^-; 3, Br^-; 4, I^-; 5, SO_4^{2-}; 6, NO_3^-; 7, HCO_3^-; 8, HS^-; 9, N_3^-; 10, NO_2^-; 11, SO_3^{2-}; 12, SCN^-; 13, H_2PO^{4-}; 14, CYS; and 15, GSH. Each measurement was performed after 20 min of mixing.

The UV−vis and fluorescence spectra of the BODIPY-Le-sulfite system, obtained by interaction of BODIPY-Le with 400 equiv of sulfite, are characteristics of BODIPY-O^-. Therefore, the sensing mechanism can be proposed as the sulfite-induced selective deprotection of BODIPY-Le to BODIPY-O^- (Scheme 2).

To validate that the sensing response of the probe to sulfite is indeed due to the conversion of BODIPY-Le to BODIPY-O^-, HPLC and mass analysis were further performed to confirm the production of BODIPY-O^- in the reaction. As shown in Figure 2, the retention times of standard BODIPY-Le and BODIPY-OH + Na_2SO_3 (100 equiv) were 14.3 and 12.5 min, respectively. In the absence of sulfite, BODIPY-Le gave a peak at 14.3 min. However, upon addition of sulfite, the peak at 14.3 min decreased in intensity until it disappeared after 30 min; meanwhile, a new peak with the retention time at 12.5 min appeared, due to the formation of BODIPY-O^-. Similarly, the ESI mass spectrum (negative) of the standard of BODIPY-Le in the absence and presence of sodium sulfite gave peaks of $[M - 1]^-$ at m/z = 501.21 and 403.18, respectively, identical to that of BODIPY-Le and BODIPY-O^- (Figure 3). All of these studies confirm that BODIPY-Le reacted with sulfite to form BODIPY-O^-.

We then evaluated the selectivity of BODIPY-Le toward sulfite over relevant anions by measuring the changes in the optical spectra upon addition of excess amount of various anions. The unique absorption change with the appearance of the characteristic absorption of BODIPY-O^- was observed only by the addition of sulfite, which can be ascribed to the sulfite-promoted cleavage of levulinate. On the other hand, no change in the UV spectra was noted with other anions such as F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, HCO_3^-, HS^-, SCN^-, $H_2PO_4^-$, N_3^-, NO_2^-, cysteine (CYS), and glutathione (GSH) (Figure 4a).

The fluorescence response of BODIPY-Le with various anions and its selectivity for sulfite are shown in Figure 4b and Figure S3 in the Supporting Information. The fluorescence change was not observed upon addition of 100 equiv of F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, HCO_3^-, HS^-, SCN^-, $H_2PO_4^-$, N_3^-, NO_2^-, CYS, and GSH. Only when sulfite was added, the unique fluorescent characteristic of BODIPY-O^- was detected. Furthermore, the unique absorbance and fluorescence bands resulting from the addition of the SO_3^{2-} ion were not influenced by the subsequent addition of other anions. These results indicate the excellent selectivity of BODIPY-Le toward the sulfite over the other competitive anions.

In summary, we have developed a novel boron-dipyrromethene-based fluorescent sensor for sulfite. It displays a 110 nm red shift of the absorption and a dramatic color change from orange to blue, as well as emission ratiometric change upon addition of sulfite. The sulfite-promoted deprotection of levulinate liberating BODIPY-O^- is responsible for the colorimetric and ratiometric detection. The developed probe features a dual spectroscopic ratiometric signal; it could be favorable for applications in environmental settings.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. NMR and fluorescence spectra for all samples described in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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