ORGANIC LETTERS

2010
Vol. 12, No. 24
5624−5627

Chromogenic and Fluorogenic Signaling of Sulfite by Selective Deprotection of Resorufin Levulinate

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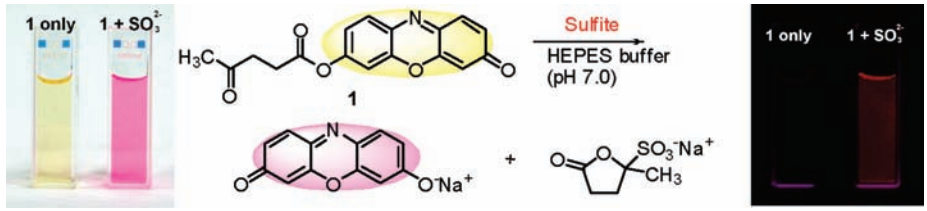
Received September 24, 2010

ABSTRACT



A new sulfite-selective probe system based on resorufin was investigated. Levulinate of resorufin exhibited a prominent chromogenic and turn-on type fluorogenic signaling toward sulfite ions in aqueous media based on the selective deprotection of the levulinate group. The sulfite-selective signaling was possible in the presence of commonly encountered anions.

Sulfites are widely used as preservatives in food and beverages,[1] and the development of analytical methods for the determination of sulfite levels is important for consumer safety.[2] Sulfites are known to be associated with allergic reaction and food intolerance symptoms. The most frequent sulfite-induced symptoms are of the asthmatic and allergic type, such as difficulty in breathing, wheezing, and hives, as well as gastrointestinal distress.[3] Sulfites are potentially toxic, and the acceptable daily intake is strictly regulated as 0.7 mg/kg of body weight.[4]

Hence, the development of convenient methods for sulfite analysis is important for food safety and quality control.[5] Sulfites in food and beverages are determined by conventional methods, such as titrimetry,[6] chromatography,[7] electrochemistry,[8] capillary electrophoresis,[9] and flow injection analysis.[10] However, conventional methods for sulfite analyses usually require troublesome sample pretreatment and reagent preparation and are either time-consuming or require complicated instruments unsuited for routine analysis.[11] For this reason, more convenient tools, such as optical sensors[12] and chromoreactands,[13] have attracted much research interest.

Signaling by selective chemical transformation of chemodosimeters or chemical probes has been uniquely employed for the construction of many sophisticated signaling systems.[14] Representative examples of this approach are Cu^{2+} signaling by the hydrolysis of rhodamine hydrazide[15] and hydrogen peroxide visualization by boronate deprotection of

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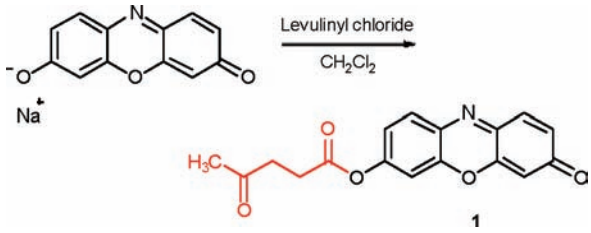
10.1021/ol102298b
Published on Web 11/17/2010

fluorescein and resorufin.[16] Other successfully designed systems are probes for the signaling of fluoride,[17] cyanide,[18] sulfide,[19] phosphate,[20] Cu^{2+},[21] and Hg^{2+} ions.[22]

The levulinyl group is frequently used as a protection tool for the hydroxyl group in nucleotides, peptides, and sugars.[23,24] Ono et al. have reported that levulinate-protected phenol moieties could be easily and selectively deprotected by sulfites under mild and neutral conditions.[25] On the basis of this report, we attempted to construct a novel sulfite-selective probe, which yielded naked-eye detectable chromogenic and fluorogenic signaling. The resorufin fluorophore was chosen as a signaling handle for this purpose as in other signaling systems for the detection of fluoride,[17] DNA hybridization,[26] and hydrolase.[27] Levulinate of resorufin was prepared by reaction of resorufin sodium salt with levulinyl chloride in good yield (75%) (Scheme 1).

Scheme 1. Synthesis of a Sulfite-Selective Probe **1**



First, the chromogenic signaling behavior of resorufin levulinate **1** was investigated in aqueous solution containing a minimal amount of acetonitrile as a solubilizer (H_2O:CH_3CN = 98:2, v/v) at pH 7.0 (HEPES buffer, 10 mM). Levulinate **1** revealed moderate UV−vis absorptions at 359 and 456 nm. Upon interaction with 100 equiv of sodium sulfite, a strong absorption band centered at 571 nm was developed (Figure 1). Concomitantly, a prominent pink color, which is a characteristic of resorufin, developed that allowed colorimetric detection of sulfite by the naked eye. The change in absorption profile was quite large, as has been reported in other resorufin-based signaling systems via the deprotection to resorufin. With sulfite, the absorbance ratio A_{571}/A_{359} at the two characteristic wavelengths of 571 and 359 nm increased over 320-fold. Other common anions were relatively nonresponsive, and A_{571}/A_{359} values varied in a limited range between 0.76 (for iodide) and 1.32 (for hydrogen phosphate) (Figure S1, Supporting Information).

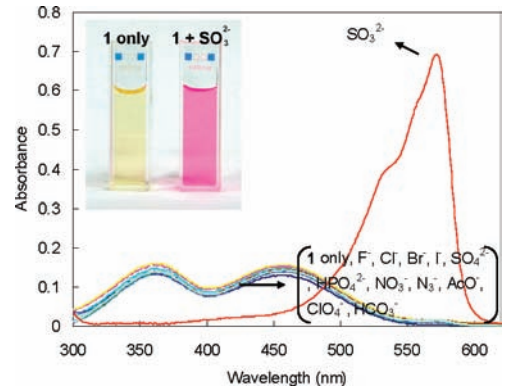


Figure 1. UV−vis spectra of probe **1** in the presence of common anions. [**1**] = 1.0×10^{-5} M, [A^{n-}] = 1.0×10^{-3} M. In HEPES buffered (pH 7.0, 10 mM) H_2O−CH_3CN (98:2, v/v), measured after 20 min of each mixing.

Next, the fluorogenic signaling behavior of **1** toward sulfite was measured. Levulinate **1** showed a weak emission at 584 nm. However, upon treatment with 100 equiv of sulfite, intense emission appeared at 588 nm (Figure 2). The fluorescence enhancement factor I/I_o observed at 588 nm was large (57-fold), and the solution revealed a dramatic color change from dark to deep pink under illumination with a UV lamp. Other common anions were relatively nonresponsive, and I/I_o at 588 nm varied in a limited range between

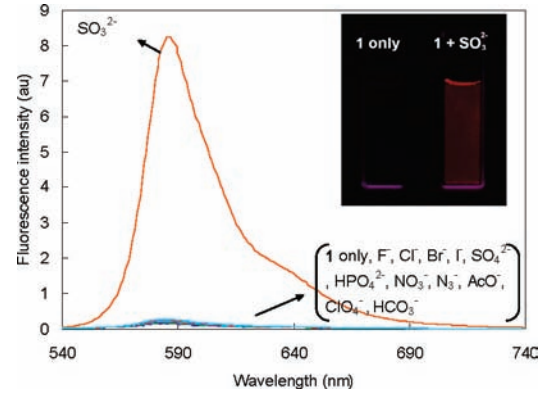


Figure 2. Fluorescence spectra of probe **1** in the presence of common anions. [**1**] = 5.0×10^{-6} M, [A^{n-}] = 5.0×10^{-4} M. In HEPES buffered (pH 7.0, 10 mM) H_2O−CH_3CN (98:2, v/v), measured after 20 min of each mixing. λ_{ex} = 487 nm.

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1.08 (for fluoride) and 1.88 (for perchlorate) (Figure S2, Supporting Information).

The chromogenic and fluorogenic signaling are due to sulfite-induced selective deprotection of resorufin levulinate **1** (Scheme 2). The cleavage of levulinate was effected by

Scheme 2. Sulfite-Selective Signaling Mechanism

initial attack of sulfite to the carbonyl carbon at the 4-position of levulinate with the formation of a tetrahedral intermediate and subsequent intramolecular cyclization leading to cleavage of the ester function.[25] Thus generated resorufin exhibited its characteristic chromogenic and fluorogenic signaling behaviors.

The suggested sulfite-induced transformation was evidenced by NMR, UV−vis, and fluorescence measurements. The ^{1}H NMR spectrum of **1** in the presence of 20 equiv of sulfite was almost identical to that of resorufin with additional residual peaks of sulfonate byproduct around 2.1, 2.6−2.7, and 3.0−3.2 ppm (Figure 3). The UV−vis and fluorescence

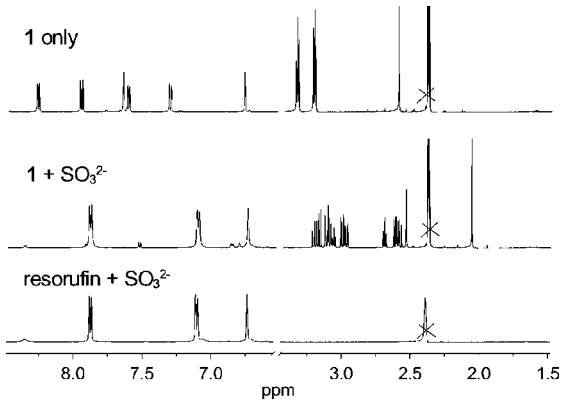

Figure 3. Partial ^{1}H NMR spectra of **1** only, **1** + sulfite, and resorufin + sulfite. [**1**] = [resorufin] = 5.0×10^{-3} M, [Na_2SO_3] = 1.0×10^{-1} M. In D_2O−CD_3CN (50:50, v/v).

spectra of the **1**−sulfite system, obtained by the interaction of **1** (1.0×10^{-5} M) with 100 equiv of sulfite, were almost identical to those of resorufin itself.

Quantitative analytical behavior of **1** for the analysis of sulfite was investigated by UV−vis measurement using a series of solutions having different amounts of analyte. As the concentration of sulfite increased, the absorbance at 571 nm grew at increasing rates (Figure S4, Supporting Information). In spectra taken 20 min after addition of sulfite, the absorbance increased steadily to about 20 equiv of sulfite (Figure 4). From this concentration-dependent signaling

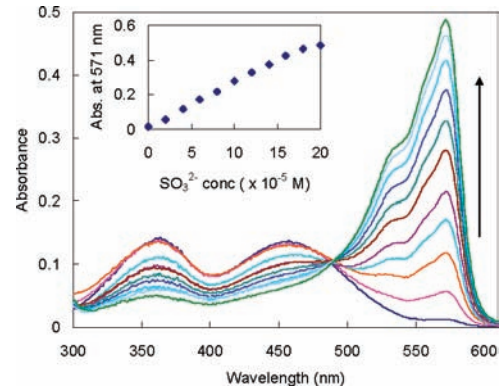

Figure 4. Concentration-dependent chromogenic signaling of sulfite by probe **1**. [**1**] = 1.0×10^{-5} M. In HEPES buffered (pH 7.0, 10 mM) H_2O−CH_3CN (98:2, v/v), measured after 20 min of each mixing.

behavior, the detection limit[28] of **1** for the analysis of sulfite was estimated as 4.9×10^{-5} M (4.0 ppm) in aqueous 2% acetonitrile solution.

Practical applicability of the sulfite signaling by **1** was ascertained by competition experiments with commonly encountered anions. The signaling of **1** toward sulfite was not affected by the presence of 5 equiv of coexisting representative anions, and the interference from other anions expressed as the ratio $A_{\mathbf{1}+\text{Sulfite}+\text{Anion}}/A_{\mathbf{1}+\text{Sulfite}}$ at 571 nm varied in a limited range from 0.94 for iodide to 1.04 for fluoride (Figure 5 and Figure S3, Supporting Information). These observations imply that the designed levulinate **1** could be used as a selective and efficient signaling probe for the sulfite ions in aqueous environment.

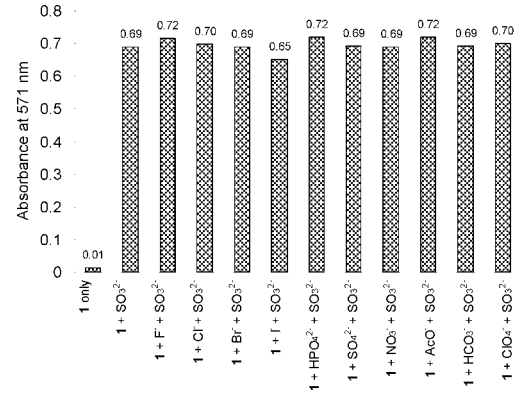

Figure 5. Competition experiments for a **1**−sulfite system in the presence of coexisting anions. [**1**] = 1.0×10^{-5} M. [Sulfite] = 2.0×10^{-4} M. [A^{n-}] = 1.0×10^{-3} M. In HEPES buffered (pH 7.0, 10 mM) H_2O−CH_3CN (98:2, v/v), measured after 20 min of each mixing.

In summary, we have devised a new sulfite-selective probe by utilizing the sulfite-selective deprotection of levulinate. With the representative signaling moiety of resorufin, a pronounced sulfite-selective chromogenic and fluorogenic signaling system was realized. The developed system could be used as a convenient and practical signaling tool for the optical determination of sulfites in routine chemical analytes in aqueous environment.

Acknowledgment. This work was supported by a fund from the Korea Research Foundation of the Korean Government (2010-0008631) and Seoul Science Fellowship (MGC) in 2010.

Supporting Information Available: Experimental details, NMR spectra, and additional chemosignaling behavior of **1** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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