ORGANIC LETTERS

2010
Vol. 12, No. 7
1468−1471

Selective Perborate Signaling by Deprotection of Fluorescein and Resorufin Acetates

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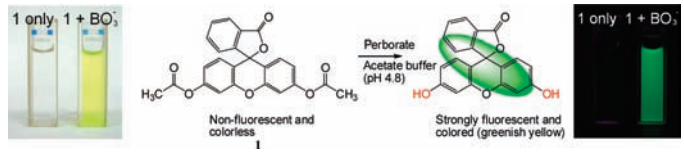
Received January 18, 2010

ABSTRACT



The acetate derivatives of fluorescein and resorufin exhibited a prominent turn-on type signaling behavior toward BO_3^- ions over other common anions. Signaling is based on the selective deprotection of acetate groups by perborate, which resulted in significant chromogenic and fluorogenic signaling. Compound 1 also exhibited a pronounced perborate selectivity over other commonly used oxidants in 90% aqueous acetonitrile solution.

Selective chemosensing and visualization of reactive oxygen species is an important area of research in light of their roles in biological and physiological processes.[1] Among many important oxidants, hydrogen peroxide, superoxide, hypochlorite, and peroxynitrite have attracted considerable research interest.[2] Perborates are widely used in our daily life, such as bleaches, cosmetics, medicinal formulations, and detergents.[3] They are cheap and are used mainly as active oxygen sources for these commodities.[4] On the other hand, the versatility of sodium perborate in the oxidation of various functional groups[5] such as thiols, sulfides, amines, olefins, and organoboranes has been highlighted as a green chemical alternative to hydrogen peroxide.[6] Because sodium perborate was found to be a direct-acting in vitro mutagen,[7] close monitoring of its usage is very important. However, perborate risk has not attracted much interest so far when compared with other widely used oxidants, except for a few reports about the toxicity of perborate or other boron species on human health and environment.[8] Furthermore, despite their wide usage, selective and convenient chemosignaling systems for perborate have not yet been reported.[9]

Signaling by the selective chemical transformation of chemodosimeters or chemical probes has been uniquely employed for the construction of many sophisticated signaling systems,[10] such as those for hydrogen peroxide,[11]

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10.1021/ol100126c
Published on Web 03/11/2010

fluoride,[12] cyanide,[13] sulfide,[14] phosphate,[15] Cu^{2+},[16] and Hg^{2+} ions.[17] Recently, Bandgar et al. reported a facile and selective deprotection of aryl acetates using sodium perborate under mild conditions.[18] We attempted to exploit this finding for the design of a perborate-selective probe system.[19] For instance, the conversion of colorless and nonfluorescent fluorescein acetate[20] into fluorescein would exhibit pronounced chromogenic and off−on type fluorescent signaling behavior.

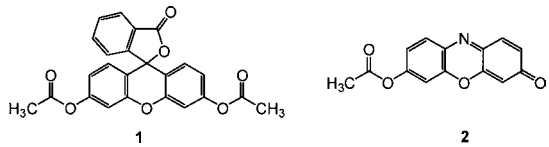

Figure 1. Perborate-selective chemical probes.

Fluorescein diacetate **1** was chosen as a signaling reporter for perborate detection (Figure 1), analogous to the signaling system for hydrogen peroxide by boronate deprotection to give fluorescein.[21] Resorufin, which exhibits very desirable chromogenic and fluorogenic signaling behaviors,[22] was also tested as an acetate **2** to determine the general applicability of acetate derivatives of other functional dyes as a perborate signaling system.

The chromogenic signaling behavior of fluorescein derivative **1** was investigated in aqueous 10% acetonitrile solution (H_2O:CH_3CN = 90:10, v/v) buffered at pH 4.8 with acetate buffer (10 mM), where most pronounced signaling behavior was observed (Figures S1 and S2, SI). Diacetate **1** revealed almost no UV−vis absorption above 400 nm. Upon treatment with 100 equiv of perborate, a strong absorption band centered at 491 nm developed (Figure 2). Concomitantly, a prominent greenish-yellow color characteristic of fluorescein was observed. The increase in absorbance was quite large, as has been reported in other fluorescein-based signaling systems. Other common anions were almost nonresponsive (Figure S3, SI).

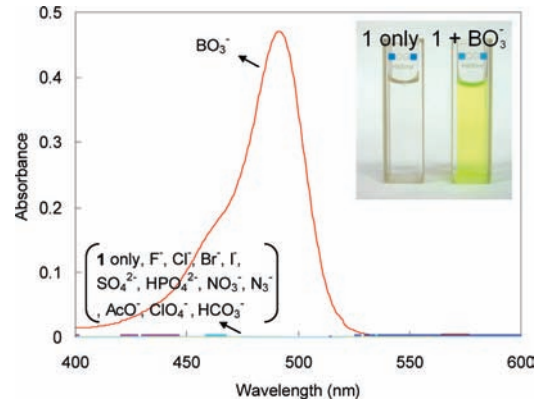

Figure 2. UV−vis spectra of **1** in the presence of common physiologically and environmentally relevant anions. [**1**] = 5.0×10^{-6} M, $[A^{n-}] = 5.0 \times 10^{-4}$ M in acetate-buffered H_2O (pH 4.8, 10 mM)−CH_3CN (90:10, v/v).

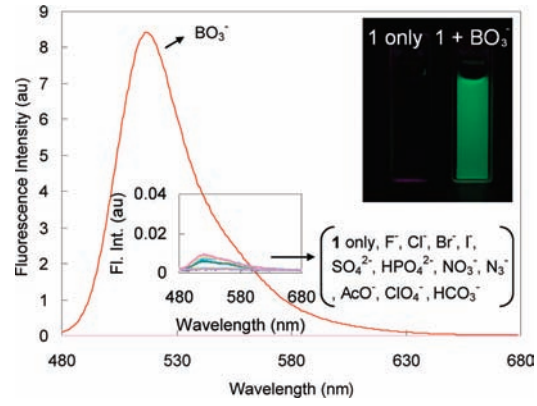

Figure 3. Fluorescence spectra of **1** in the presence of common anions. [**1**] = 5.0×10^{-6} M, $[A^{n-}] = 5.0 \times 10^{-4}$ M in acetate-buffered H_2O (pH 4.8, 10 mM)−CH_3CN (90:10, v/v). λ_{ex} was 470 nm to obtain a full view of the fluorescence spectra.

Next, the fluorogenic signaling behavior of **1** toward perborate ions was investigated. Compound **1** showed almost no emission above 470 nm. However, upon treatment with 100 equiv of perborate, an intense fluorescence was observed at 517 nm with a dramatic color change from dark to green under illumination with a UV lamp (Figure 3). Other common anions were almost nonresponsive (Figure S4, SI). Under the present experimental condition, perborate signaling was relatively fast, being completed within 5 min after sample preparation (Figure S5, SI). However, with lower

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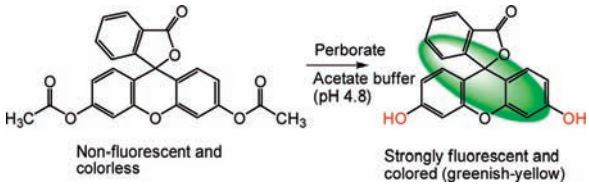
amounts of perborate, the signaling became more sluggish. Therfore, all the measurements were performed after 1 h of sample preparation, where the signaling was relatively stabilized.

Scheme 1. Perborate Signaling Mechanism



The mechanism for perborate signaling is via the selective perborate-induced deprotection of fluorescein diacetate **1** (Scheme 1), as evidenced by NMR, HPLC, UV−vis, and fluorescence measurements. With ^{1}H NMR spectroscopy, the progress of the reaction between **1** and perborate could be easily followed (Figure S6, SI). Upon interaction with 5 equiv of perborate ions, the ^{1}H NMR spectrum of the **1**-BO_3^- system in deuterated acetate buffered DMSO-d_6 revealed mainly **1** and monodeprotected fluorescein monoacetate after 5 min of sample preparation. After 1 h, the **1**-BO_3^- system subsequently transformed into a mixture consisting mainly of fluorescein monoacetate and fully deprotected fluorescein. HPLC measurements also clearly revealed the consecutive reaction mode of **1**; the **1**-BO_3^- system initially yields monodeprotected fluorescein monoacetate, which was subsequently converted to fully deprotected fluorescein (Figure S7, SI). The UV−vis and fluorescence spectra of the **1**-BO_3^- system, obtained by mixing **1** (5.0×10^{-6} M) with 20 equiv of $NaBO_3$, were indistinguishable from those of fluorescein (Figures S8 and S9, SI).

The selective signaling is peculiar to perborate and other boronic species did not induce any signaling of **1** (Figure S10, SI). Upon reaction with acetate **1**, perborate is converted to boric acid.[8c] Because the signaling is not a catalytic process but a bimolecular reaction between BO_3^- and **1**, the effects of using higher concentrations of **1** on the signaling selectivity were studied. The selectivity of **1** toward perborate was not affected when using a higher concentration up to 5.0×10^{-5} M.

The quantitative analytical behavior of **1** for the analysis of perborate was examined by fluorescence titration (Figure 4). As the amount of perborate was increased up to 10 equiv, the fluorescence of **1** steadily increased with a slight red shift ($\Delta\lambda_{max} = 3$ nm). From this titration, the detection limit of perborates in aqueous 10% acetonitrile solution by **1** was estimated to be 2.2×10^{-5} M.

The practical applicability of perborate signaling by **1** was ascertained by competition experiments with commonly encountered anions (Figure S11, SI). The fluorescence response of **1** toward perborate was not significantly affected by the presence of 100 equiv of coexisting anions. The interference from other anions expressed as the ratio $I_{\mathbf{1}+\text{Perborate}+\text{Anion}}/I_{\mathbf{1}+\text{Perborate}}$ at 517 nm varied in a limited range from 0.98 (ClO_4^-) to 1.12 (N_3^-), which suggests that the interference is negligible. Furthermore, the selective perborate signaling behavior of **1** could be readily applied for simulated samples of laundry detergent and tooth whitening solution (Figure S12, SI).

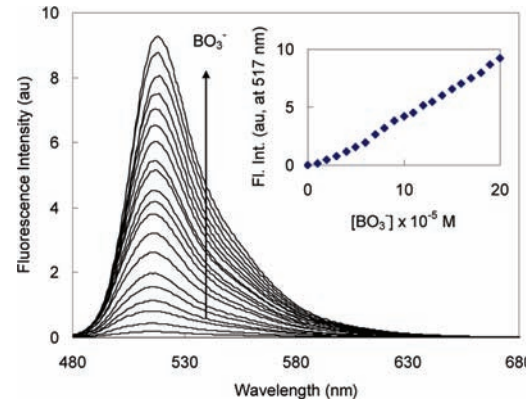


Figure 4. Titration of **1** with perborate in aqueous 10% acetonitrile. [**1**] = 2.0×10^{-5} M, pH 4.8 (acetate-buffered), $\lambda_{ex} = 470$ nm.

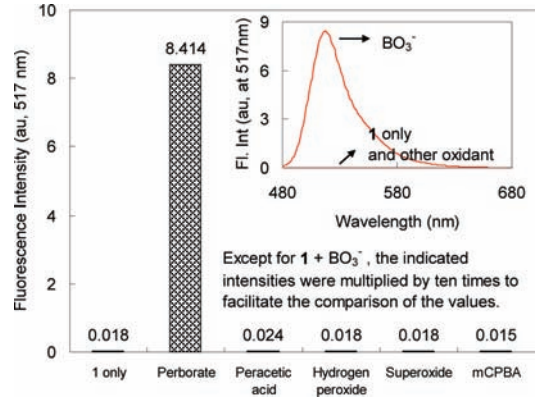


Figure 5. Changes in fluorescence intensity of **1** at 517 nm in the presence of common oxidants. [**1**] = 5.0×10^{-6} M, [oxidant] = 5.0×10^{-4} M in acetate-buffered H_2O (pH 4.8, 10 mM)−CH_3CN (90:10, v/v). $\lambda_{ex} = 470$ nm.

One additional interesting observation was that the diacetate **1** had a pronounced selectivity for perborate over other common oxidants. In the presence of 100 equiv of perborate, superoxide, *m*-CPBA, peracetic acid, or hydrogen peroxide, only perborate induced a significant response to **1** (Figure 5). In absorption spectra, a prominent selectivity of **1** for perborate over other oxidants was also observed (Figure S13, SI).

Next, the chemosignaling ability of resorufin acetate **2** was tested. Compound **2** also exhibited a selective chromogenic and fluorogenic signaling behavior toward perborate ions, similar to that of **1**. Upon interaction with perborate, the absorption spectrum of **2** changed dramatically with a concomitant color change from light amber to pink (Figure

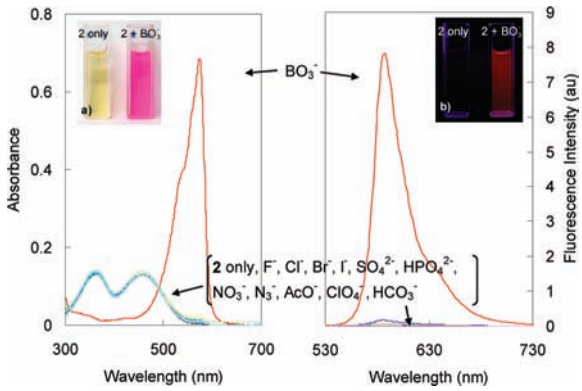


Figure 6. (a) UV−vis and (b) fluorescence spectra of **2** in the presence of common physiologically and environmentally relevant anions. [**2**] = 5.0 × 10^{-6} M, [A^{n-}] = 5.0 × 10^{-4} M in acetate-buffered H_2O (pH 4.8, 10 mM)−CH_3CN (90:10, v/v). λ_{ex} = 487 nm.

6a). The absorption bands of **2** at 362 and 453 nm disappeared and a new intense band at 572 nm appeared; the ratio of absorbances at 572 and 453 nm (A_{572}/A_{453}) changed over 520-fold (Figure S14, SI). On the other hand, the ratio for all of the other surveyed anions varied in a limited range between 0.58-fold (I^-) and 3.98-fold (HCO_3^-). Paralleling the UV−vis responses, changes in fluorescence were also significant (Figure S15, SI), and the solution color changed from dark to red under UV illumination (Figure 6b). These observations clearly demonstrate that the acetate derivatives of fluorescein and resorufin, representing typical signaling molecules, could function as perborate-selective chemical probes.

In summary, we developed a new convenient and selective chemosignaling system derived from two typical fluorophores of fluorescein and resorufin for perborate ions in an aqueous environment. The acetate derivatives in the presence of perborate ions revealed a selective turn-on type chromogenic and fluorogenic signaling behavior based on the selective and efficient cleavage of acetate groups by perborate ions. The acetate derivatives also exhibited pronounced perborate selectivity over other commonly employed oxidants.

Acknowledgment. M.G.C. gratefully acknowledges the support from the Seoul Science Fellowship Program of Seoul City (2009).

Supporting Information Available: Experimental details, NMR spectra, HPLC, UV−vis, and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL100126C