

A “Reactive” Ratiometric Fluorescent Probe for Mercury Species

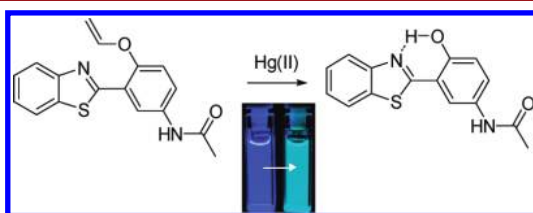
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ABSTRACT



A ratiometric fluorescent probe for mercury species is developed based on the metal-promoted hydrolysis of a vinyl ether derivative of 2-(benzothiazol-2-yl)phenol in a buffer solution. The probe responds selectively to mercury species over various other metal ions with a marked fluorescence change from blue to cyan through the excited state intramolecular proton transfer (ESIPT) process. The fluorescence titration is complete with 0.5 equiv of HgCl_2 , which indicates that the probe also responds to organomercury species, R^nHgCl .

The widespread contamination of pollutants such as highly poisonous mercury species could jeopardize our ecosystem, imposing a great threat to human health. In particular, organomercury species (typically methylmercury, CH_3HgX , $\text{X} = \text{Cl}, \text{AcO}^-$, etc.) are much more virulent than inorganic mercury(II), as they readily accumulate in various organs as well as cross the blood–brain barrier to cause damage in the central nervous system.^{1,2} Ingestion of methylmercury-contaminated fish and grain triggered the epidemics in Japan and in Iraq, respectively.³ Therefore, there is an impetus to develop versatile analytical tools for the detection and quantification of mercury species including methylmercury. Among the existing analytical methods, those based on fluorometry attract considerable attention owing to their favorable features of operational simplicity and cost-effectiveness, in addition to the high sensitivity and selectivity. A number of fluorescent molecular probes based on the coordination of

heteroatom-based ligands to mercury(II) ions have been reported in recent years, enabling easy detection of mercury ions with high sensitivity.⁴ These fluorescent probes, however, suffer from incomplete selectivity over the competing metal ions and, in most cases, show a lack of sensitivity toward organomercury species. To overcome these limitations, recently we devised a novel reaction-based sensing approach that does not involve the metal ion coordination by heteroatom-based ligands but relies on a chemical reaction specific to the mercury species.⁵ We have found that the “reactive” fluorescent probe based on the

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(6) Koide and co-workers later reported a work of mercury sensing with exactly the same probe as ours (ref 5a): Ando, S.; Koide, K. *J. Am. Chem. Soc.* **2011**, *133*, 2556.

hydrolysis of an aryl vinyl ether promoted by mercury species,⁶ so-called the oxymercuration reaction, can provide two notable features that overcome the existing challenges: the reaction is specific toward mercury(II) ions; more importantly, the reaction also proceeds by methylmercury species. The reactive probe also emits “turn-on” fluorescence toward the mercury species.

Our continuing efforts to develop versatile molecular probes with useful features for bioimaging, environmental monitoring, or ready quantification of mercury species led us to investigate a ratiometric version of the vinyl ether probe. Such a ratiometric fluorescent probe would offer an advantage over the intensity-based probes such as less sensitivity to the errors associated with the probe concentration, photobleaching, instrument's sensitivity, and environmental effects.⁷ Various strategies have been adopted for the design of ratiometric fluorescent probes.⁸ A handful of ratiometric fluorescent probes for mercury species, however, have been reported so far.⁹ As our approach of the vinyl ether hydrolysis by mercury ions has proven to be effective,^{5a} a ratiometric fluorescent probe based on the hydrolysis reaction would provide us with a valuable tool for the detection of the toxic species, with an added improvement over the potential errors. Reported here are preliminary results from our efforts in this endeavor.

2-(Benzothiazol-2-yl)phenol and its derivatives, upon irradiation, generate the excited-state intramolecular proton transfer (ESIPT) tautomers (the keto forms), which fluoresce more strongly and at longer wavelength compared to the phenol forms. Recently *O*-functionalized 2-(benzothiazol-2-yl)phenols were developed as the ratiometric fluorescent probes for anions such as F[−] and phosphatases.^{8d–g} In these examples, the *O*-functionalized compounds are converted to the starting phenols by analyte-promoted/-catalyzed reactions, with the conversions offering ratiometric responses because 2-(benzothiazol-2-yl)phenols and their *O*-functionalized derivatives fluoresce at different wavelengths.

We designed vinyl ethers **1–6** as potential ratiometric probes for mercury species (Figure 1). These vinyl ethers are expected to undergo the mercury-promoted oxymercuration followed by hydrolysis to generate the corresponding 2-(benzothiazol-2-yl)phenols. We have

evaluated vinyl ethers **1–6** with respect to their reactivity toward HgCl₂, their solubility in aqueous media, and their photophysical properties. The results can be summarized as follows: The hydrolysis reaction becomes faster when electron-donating groups are substituted at the phenol ring, in particular at the *para* position with respect to the hydroxyl group; otherwise, the hydrolysis becomes slower, and thus the corresponding vinyl ethers are not suitable for the purpose of sensing. For example, vinyl ethers **1–3** reacted faster with HgCl₂ than the *meta* derivatives **4** and **5** as well as the nonsubstituted analogue **6**. Also, among the *para* analogues, vinyl ether **1** undergoes the hydrolysis reaction faster than **2** and **3**; the hydrolysis is complete within 30 min in the case of **1** (10 μM, 2 equiv with respect to [HgCl₂]); however, it takes more than 1 h in the cases of **2** and **3** (Figures S1–S5). In addition, the vinyl ethers substituted with the –NHCOPh or –NHCOCF₃ group, for example, **2** and **3**, required 20–30% of CH₃CN (by volume) to dissolve them in water, whereas only ~1% of CH₃CN is sufficient to dissolve **1** in a phosphate buffer solution.

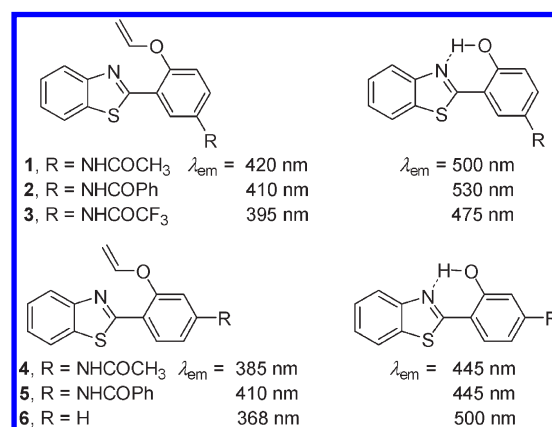
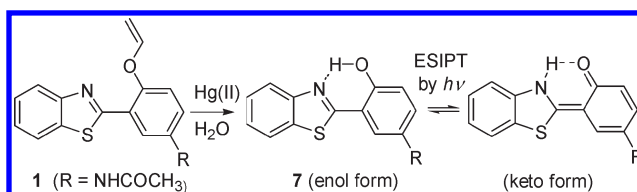


Figure 1. Vinyl ethers **1–6** and their devinylated products evaluated. The maximum emission wavelengths were obtained by excitation at the corresponding maximum absorbance wavelengths.

The emission data for the vinyl ethers and the corresponding phenols also show that the emission wavelengths are affected by the substituents. From these results, vinyl ether **1** was chosen as an optimal probe for mercury species as it shows the desired reactivity, solubility, and photophysical properties compared with the others. The synthesis and

Scheme 1. Hydrolysis of Probe **1** by Mercury Species



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characterization of vinyl ethers **1**–**6** are given in the Supporting Information.

Probe **1** showed only blue fluorescence as the hydroxyl group was vinylated, whereas it readily underwent HgCl_2 -promoted hydrolysis and consequently emitted cyan fluorescence responsible for the keto form generated through the ESIPT (Scheme 1). The changes in the UV/vis absorption and fluorescence spectra depending on the reaction time were recorded for a 2:1 mixture of **1** ($10\ \mu\text{M}$) and HgCl_2 in a phosphate buffer solution (PBS: phosphate buffered saline, pH 7.4) containing $\sim 1\%$ CH_3CN by volume at room temperature (Figure 2). Both the absorption and emission spectra showed gradual changes as the vinyl ether underwent mercury ion-promoted hydrolysis to generate phenol **7**. The absorption maximum at 295 nm gradually decreased while the absorption at 330 nm increased. When the mixture was excited at the isosbestic point (318 nm) of the absorption spectra, the emission peak at 420 nm was much higher than that at 500 nm (Figure S1). Thus, a better ratiometric feature in the spectra was obtained at an excitation wavelength of 365 nm.

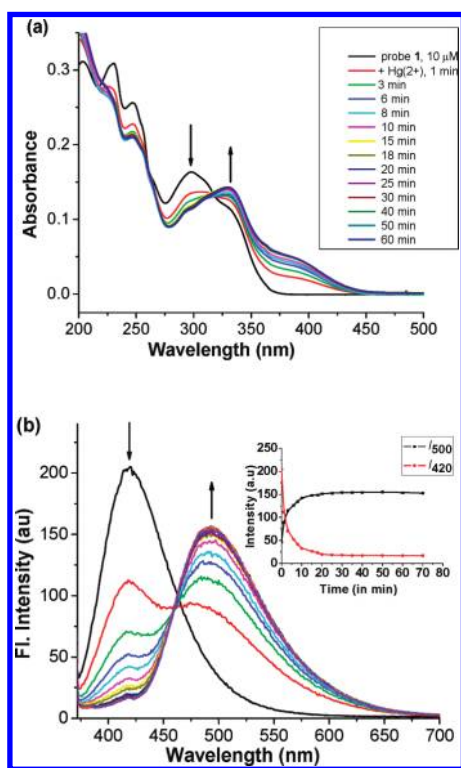


Figure 2. Time-dependent (a) UV/vis spectra and (b) fluorescence spectra measured for a 2:1 mixture of probe **1** ($10.0\ \mu\text{M}$) and HgCl_2 in PBS buffer (pH 7.4) containing $\sim 1\%$ CH_3CN . Inset: a plot of the fluorescence intensity change (based on the peak heights at the maxima) depending on time.

The mercury ion-promoted hydrolysis of probe **1** was complete within 30 min under the given conditions (Figure 2b). As known for other ESIPT compounds, phenol **7** itself shows a strong emission band originated from the

keto form (at 500 nm), whereas the emission from the enol form (at 420 nm) is very weak, as seen from the emission spectrum at the saturation stage (Figure 2b). The ratio of the two emission bands thus correlates with the mole ratio of the remaining probe **1** and the hydrolyzed product **7**.¹⁰

Next, we evaluated the fluorescence behavior of probe **1** toward other typical metal species, the results of which are summarized in Figure 3. The fluorescence data were obtained in the PBS buffer (pH = 7.4) for a 2:1 mixture of probe **1** ($10\ \mu\text{M}$) and each of the metal species. Probe **1** showed a distinct ratiometric behavior only toward Hg^{2+} species among the various metal ions examined. The probe solution, emitting blue fluorescence ($\lambda_{\text{max}} = 420\ \text{nm}$) in the absence of metal ions, showed only little or small changes in that peak upon addition of other metal ions (Cr^{2+} , Ca^{2+} , Mg^{2+} , Co^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Ba^{2+} , Cd^{2+} , Al^{3+} , Cu^{2+} , Fe^{3+} , Fe^{2+} , and Ag^{+}).

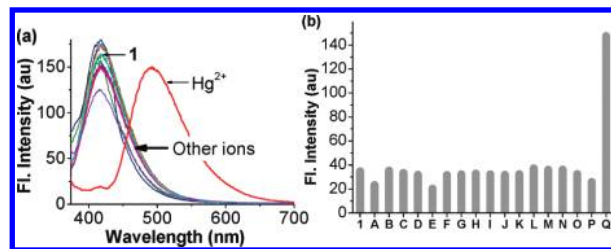


Figure 3. (a) Fluorescence changes observed after 1 h for a 2:1 mixture of **1** ($10\ \mu\text{M}$) and each of metal species (Mg^{2+} , Ca^{2+} , Ba^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Pb^{2+} , Ag^{+} , Al^{3+} , Fe^{3+} , Cd^{2+} , Cu^{2+} , Cr^{2+} , Mn^{2+} , Fe^{2+} , Au^{3+} , and Hg^{2+} ; as their chloride salts except for AgNO_3) in PBS buffer (pH 7.4) containing $\sim 1\%$ CH_3CN . (b) A bar graph showing the relative fluorescence intensity of the ions (from A to Q, which denote the ions in the same order as that in (a)) in comparison with probe **1** (the peak height at 500 nm).

In contrast, in the case of Hg^{2+} , the probe's emission at 420 nm disappeared while the emission from the keto form of **7** ($\lambda_{\text{max}} = 500\ \text{nm}$) appeared, indicating the hydrolysis occurs only in the presence of Hg^{2+} species. Also probe **1** showed the same behavior toward the Hg^{2+} species even in the presence of all the other metal ions, showing little fluorescence interference from these metal species (Figure S7). Thus, vinyl ether **1** is a ratiometric probe specific for Hg^{2+} species. The fluorescence titration of probe **1** with an increasing amount of HgCl_2 (from 0 to 1.0 equiv) showed a saturation behavior at 0.5 equiv (Figure 4a) in the PBS buffer (pH 7.4). The result indicates that the probe also reacts with the organomercury intermediate **8** generated in the oxymercuration process (Scheme 2).

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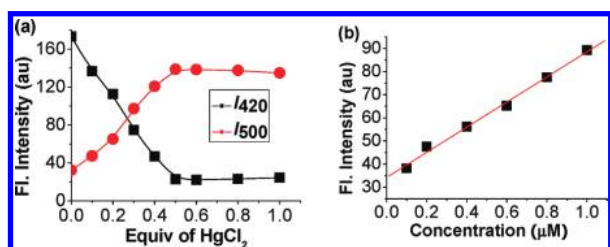


Figure 4. (a) Fluorescence intensity changes of probe **1** (10 μ M) with respect to the equivalents of HgCl_2 , expressed as the peak heights at 420 and 500 nm, respectively; taken after 30 min of each addition. (b) A plot of fluorescence intensity (the peak height at 500 nm) for a 2:1 mixture of probe **1** and HgCl_2 for the concentration range from 0.1 to 1.0 μ M, taken after 30 min of each addition. Both probe **1** and HgCl_2 were dissolved in PBS buffer (pH 7.4) containing $\sim 1\%$ CH_3CN . Excited at 365 nm.

A case of a fatal accident in handling organomercury in a laboratory^{11a} and other warnings^{11b} deterred us from carrying out further experiments with methylmercury species.¹² However, the 2:1 stoichiometry in the above fluorescence titration of **1** with HgCl_2 indicates that methylmercury, a typical organomercury species, may also be detected by the probe.

The hydrolysis reactivity of such vinyl ether compounds by organomercury species seems to be dependent on the aryl moiety of the vinyl ether compounds. A vinyl ether derived from a coumarin precursor developed by us previously¹³ showed reactivity only toward inorganic mercury and not toward organomercury species.

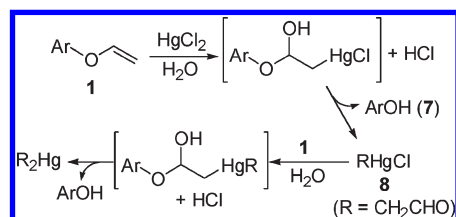
We investigated a set of fluorescence studies at different pH's from 4 to 9. In the absence of HgCl_2 , the fluorescence intensity of probe **1** at pH 4 remained nearly constant even after 24 h (Figure S8). As the pH of the solution increased from acidic to basic (pH = 9), the fluorescence change became slow (Figure S10). These observations support that the cleavage of the hemiacetal intermediate produced by the oxymercuration is facilitated by acid; hence, the overall hydrolysis rate becomes slow at higher pH. These observations corroborate our previous report.^{5a}

The fluorescence intensity of the probe solution gradually increased with the increasing concentration of mercury

(12) Warnings: Handling of mercury species has been carried out in a well-ventilated fume hood because diorganomercury species, R_2Hg , generates according to the reaction mechanism in Scheme 2, and all the resulting wastes including titrated solutions are carefully disposed following our institution's guidelines for toxic wastes.

(13) Cho, Y.-S.; Ahn, K. H. *Tetrahedron Lett.* **2010**, *51*, 3852.

Scheme 2. A Mechanism for the Hydrolysis of Probe **1** by HgCl_2



ions in the PBS buffer solution (Figures S6, S9). We obtained a linear relationship between the fluorescence intensity at 500 nm and the concentration of mercury ions (Figure 4b). From the data, a detection limit of 20 ppb was obtained, one order of magnitude lower in value in comparison with that of the previous fluorescein-based vinyl ether,^{5a} which precludes its application to detect mercury ions down to the very low concentration limit required for drinking water (2 ppb).¹⁴ For such a purpose, a further study is necessary to improve the fluorescence quantum yield of the fluorophore, 2-(benzothiazol-2-yl)phenol and probe **1** ($\Phi_F \approx 0.2$).¹⁵ The present probe may be used for other situations where the potential errors from detection conditions become a concern.

In summary, we have devised a ratiometric fluorescent probe for mercury species based on the metal-promoted hydrolysis of vinyl ether. The probe, an optimized vinyl ether derivative of 2-(benzothiazol-2-yl)phenol, only responds to mercury species and not to various other metal ions, showing a marked fluorescence change from blue to cyan (~ 80 nm bathochromic shift) through ESIP. The reaction stoichiometry indicates that the probe can also be used for the detection of methylmercury species.

Acknowledgment. This work was supported by grants from the EPB Center (R11-2008-052-01001).

Supporting Information Available. Experimental procedure, spectral data for all new compounds, and some optical spectra of probe **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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