Development and Applications of Fluorogenic Probes for Mercury(II) Based on Vinyl Ether Oxymercuration

Shin Ando and Kazunori Koide*

Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, Pennsylvania 15260, United States

S *Supporting Information*

ABSTRACT: Mercury is a major threat to the environment and to human health. It is highly desirable to develop a user-friendly kit for on-site mercury detection. Such a method must be able to detect mercury below the threshold levels for drinking water, 1−2 ppb. We developed a fluorescence method based on the oxymercuration of vinyl ethers to detect mercury in dental and environmental samples. Chloride ions interfered with the oxymercuration reaction, but the addition of $AgNO_3$ solved this problem. Fine electronic and structural tuning led to the development of a more responsive probe that was less sensitive to chloride ion interference. This second-generation probe could detect 1 ppb mercury ions in water.

■ INTRODUCTION

Mercury is one of the most toxic metals, as seen in such unfortunate incidents as Minamata disease[1] and mercury poisoning in Iraq.[2] As these cases showed, methylmercury is extremely toxic and damages the central nervous system.[3] The tragedy of Professor Karen Wetterhahn, who was studying organic mercury compounds,[4] led to a moratorium on the use of these highly toxic and skin-permeable organic mercury compounds at many American institutions, including ours.[5] Inorganic mercury compounds exhibit severe effects on the human heart, kidney, stomach, and genes.[6] Despite the indisputable toxicity of mercury, it remains unclear whether there is a direct link between mercury and autism.[7] Nonetheless, pregnant women are advised to avoid mercury-rich diets such as shark and tuna as a precaution.[8]

Mercury metal is volatile and can travel far in the air. It is then deposited on land or in water and oxidized to mercury(II).[9] Oxidized inorganic mercury(II) may be converted to organic mercury by microorganisms.[10] Mercury in the environment originates not only from geological events (e.g., volcano emissions[11]) but also from modern human activities. For example, the wastewater from dental offices is a major source of mercury pollution, partially because dentists are exempt from federal regulations on waste.[12] Coal-fired power plants generate substantial amounts of mercury, which is monitored by the United States Environmental Protection Agency (US EPA).[13] In addition, the combination of human activities and geological samples such as the recent oil spill in the Gulf of Mexico has raised great concern among the general public.[14]

Mercury is generally quantified by cold vapor atomic absorption spectroscopy[15] or inductively coupled plasma mass spectroscopy.[16] Although these methods are quantitative and powerful, the analyses require large and expensive instruments, highly trained personnel, and tedious maintenance. Mostly because of the size of the instruments, mercury-contaminated samples are generally analyzed off-site. The off-site analysis format retards the remediation of wastewater[17] in coal-fired power plants and makes it essentially impractical to monitor dental offices and their surroundings.[12] It is also important to note that these expensive and sophisticated analytical techniques cannot be employed in developing countries in which mercury pollution is severe and can be found even in tap water.[18]

Optical methods are more amenable to the on-site analysis of mercury with fewer resources. Therefore, numerous fluorescent chemosensors and chemodosimeters have been reported in the literature.[19−22] These methods may facilitate mercury analyses, even if they are only semiquantitative. Because mercury is a soft Lewis acid, a vast majority of the chemosensors and chemodosimeters for mercury contain sulfur atom(s) that can tightly coordinate the metal as part of off-on fluorescence switches.[19,21] Some of the chemosensors and chemodosimeters were successfully applied to real world "dirty" samples and in biological settings with low concentrations of mercury ions.[19,22]

As the protocol of the US EPA indicates,[23] mercury-containing environmental samples are pretreated with harsh oxidants such as $Cl-Br$[23] and H_2O_2[24] to transform various forms of organic and sulfur-bound (e.g., cysteine-bound) mercury species[25] to sulfur-free inorganic mercury(II). Therefore, we felt that chemodosimeters and chemosensors for mercury in environmental and biological fluid samples might need to be compatible with oxidants. Although others have successfully demonstrated the utility of their sulfur-based indicators, we turned our attention to the π electrophilicity of mercury ions and developed a chemodosimeter based on the oxymercuration of an alkyne.[26] This chemodosimeter was found to be resistant to strong oxidants such as H_2O_2 and N-chlorosuccinimide (NCS). This method enabled us to detect mercury from fish and dental samples. However, the oxymercuration reaction needed to be heated to 90 °C. Moreover, we were not able to detect mercury ions below 4 ppb. The ability to detect mercury ions below this level is critically important, because the limit of mercury concentration in drinking water is 2 ppb in the United States.[27] We hypothesized that a more electron rich π bond might be more reactive toward mercury ions, allowing for mercury detection at a lower temperature and at lower mercury concentrations. Herein, we report user-friendly and more sensitive

Scheme 1. Two-Step Sequence To Cleave an Allyl Ether (a), Platform for a Fluorescence Off-On Switch (b), and Preparation of 3 and Its Reaction with HgCl$_2$ To Form 1 (c)
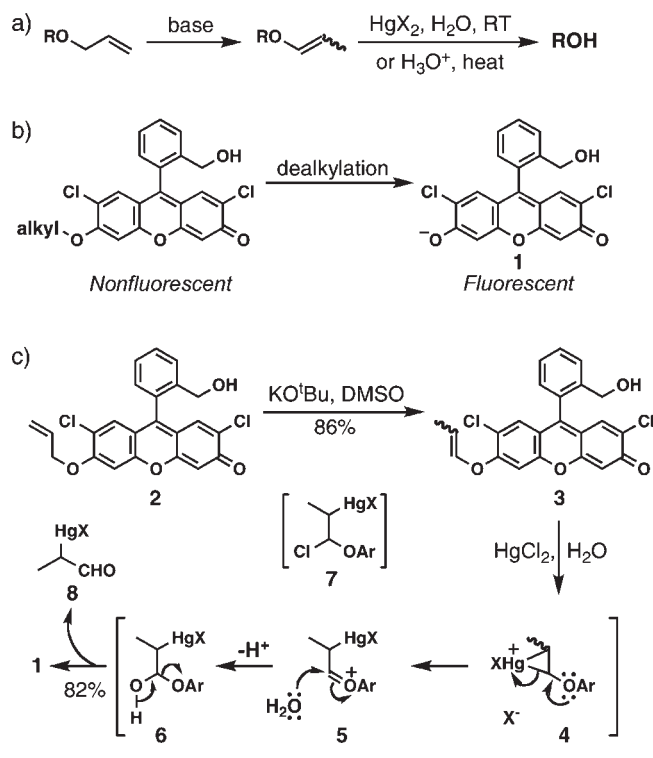
new fluorescent chemodosimeters that react with mercury ions at ambient temperature. The second-generation chemodosimeter in this study was found to be particularly powerful, detecting mercury ions at a 1 ppb level.

■ RESULTS AND DISCUSSION

Design and Synthesis of Probe 3. An allyl ether is used as a protecting group and can be removed via a base-catalyzed olefin migration followed by either an acid-catalyzed hydrolysis at elevated temperature[28] or mercury-promoted hydrolysis (Scheme 1a).[29] Because the latter can be employed at ambient temperature, we hypothesized that this transformation might be used as a means to selectively convert a nonfluorescent molecule to a fluorescent molecule with mercury ions.

We previously demonstrated the conversion shown in Scheme 1b as a general platform for the development of fluorescence methods.[26,30,31] In order to couple this platform with the chemistry shown in Scheme 1a, allyl ether 2[30,32,33] was treated with KO^{t}Bu in DMSO[34] to form vinyl ether 3 in 86% yield as an inseparable mixture of cis and trans isomers (Scheme 1c). The purity of 3 was ensured by HPLC analysis (Figure S2, Supporting Information). The absorption and emission spectra of 3 are shown in Figure S1 (Supporting Information).

Reactivity of Probe 3 with Metal Ions. Treatment of 3 with HgCl$_2$ (1.0 equiv) at 25 °C afforded 1 in 82% isolated yield, indicating that this transformation could be used to develop a fluorescence method for Hg(II) (possibly HgCl$^+$, because HgCl$_2$ is mostly dissociated into HgCl$^+$ and Cl$^-$)[35] at a more convenient temperature. Although vinyl ethers can be hydrolyzed under acidic

conditions in a refluxing acetone−water mixture,[28] such hydrolysis did not occur when vinyl ether 3 was incubated in a pH 4 buffer at 80 °C, ensuring its stability during storage.

Next, we optimized conditions for a Hg(II)-promoted hydrolysis of vinyl ether 3 to form 1[33] in various buffers (for details, see Table S1 in the Supporting Information) at 25 °C. The ratio of Hg(II)-promoted and Hg(II)-free hydrolysis—the latter was negligible—was optimal at pH 3 (Figure 1a). However, the pH 3 reaction media was more difficult to neutralize than the pH 4 media for fluorescence measurement in a high-throughput manner. Therefore, pH 4 was chosen for the remaining part of this study.

In a pH 4 buffer, we subjected vinyl ether 3 to various metal ions at 5 μM, demonstrating that the conversion of 3 to 1 was most efficiently promoted by Hg(II) (Figure 1b and Table S2 in the Supporting Information). A slight signal increase was observed in the presence of Pt(II).

The conversion of 3 to 1 in the presence of Hg(II) (0.30 μM) in a pH 4 buffer showed ∼40% completion after 1 h, and the reaction continued to proceed (Figure 1c). The 1 h duration was considered an optimal balance between sensitivity and convenience. The fluorescence signals generated by the conversion of 3 to 1 was [Hg(II)] dependent with a distinct linear signal beginning as low as 4 ppb (=20 nM) Hg(II) (● in Figure 1d; Figure S3a and Table S3 in the Supporting Information).

Oxidative pretreatment of environmental samples with Br−Cl is a standard procedure by the US EPA. We found that NCS could also oxidatively disrupt the Hg−S bond.[26] Because NCS can react with olefins, we were concerned about the stability of vinyl ether 3 toward NCS. However, this probe was stable against NCS while remaining responsive to Hg(II) (○ in Figure 1d and Table S3 in the Supporting Information). These data indicate that probe 3 could be used to detect Hg(II) in sulfur-containing samples in the presence of NCS.

Failed Application of 3 to Detect Hg and Revision of Reaction Conditions. We proceeded to apply the above method for the detection of Hg(II) in actual "dirty" samples in order to evaluate the utility of the method. Known amounts of Hg(II) were spiked into river water that contained <1 ppb total mercury.[36] The resulting solutions, after the pH was adjusted to 4, were treated with 3 in an attempt to convert 3 to 1, but to no avail (Figure 3a, left). This failure prompted us to revisit the generality of the method. Rather than testing each metal separately, we examined mixtures of HgCl$_2$ (2.5 μM) with various inorganic reagents (25 μM). Figure 2a (white bars) shows that most reagents interfered with the conversion of 3 to 1. It appeared that this interference was not caused by metals but by chloride ions. We compared the effect of NaCl and NaNO$_3$ to verify the effect of chloride ions. Treatment of compound 3 (1.0 μM) with HgCl$_2$ (2.5 μM) and excess NaCl (1.0 mM) in a pH 4 buffer showed no fluorescence increase (Figure 2b). Under similar reaction conditions, we only observed the starting material 3 by ^{1}H NMR spectroscopy (Figure S18, Supporting Information). In contrast, the presence of NaNO$_3$ did not impact the conversion of 3 to 1. We concluded that chloride ions interfered with our fluorescence method. This may also account for the failure of our earlier river water study and limit the applications of 3 in biological systems.

We asked how chloride ions interfered with the Hg(II)-promoted conversion of 3 to 1. The formation of 7 from the electrophilic species 5 (Scheme 1c) could be ruled out by the aforementioned NMR analysis. It is possible that the equilibrium shifted from more reactive HgX$^+$ (X = Cl, phosphate, etc.) to less reactive HgXCl. This working hypothesis could account for the
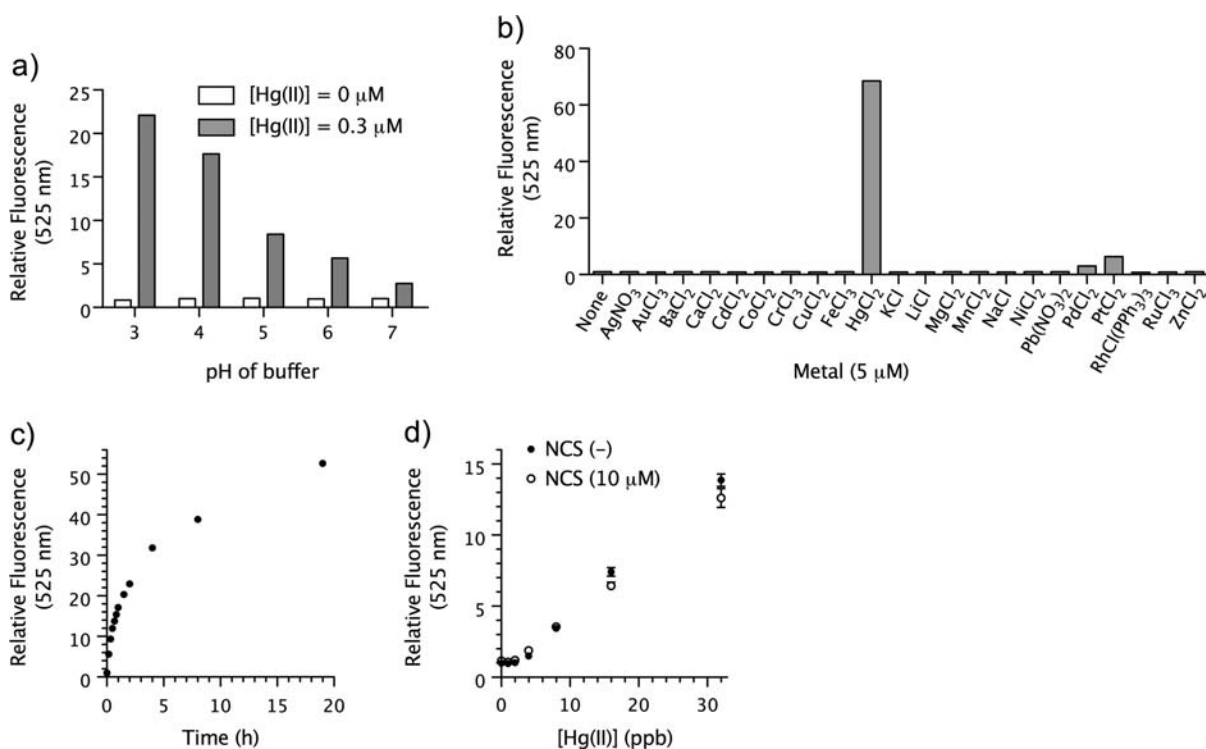
Figure 1. Conversion of **3** to **1** at 25 °C. [**3**] = 1.0 μM for all of the experiments. Except for (c), all of the reactions were performed for 1 h. Fluorescence intensities were measured after addition of 1.23 M phosphate pH 7 buffer. (a) pH dependence. (b) Metal selectivity in a 50 mM phthalate pH 4 buffer. Metal reagents: AgNO$_3$, AuCl$_3$, BaCl$_2$, CaCl$_2$, CdCl$_2$·2.5H$_2$O, CoCl$_2$, CrCl$_3$, CuCl$_2$·2H$_2$O, FeCl$_3$, HgCl$_2$, KCl, LiCl, MgCl$_2$, MnCl$_2$·4H$_2$O, NaCl, NiCl$_2$, Pb(NO$_3$)$_2$, Pd(NO$_3$)$_2$, PtCl$_2$, Rh(PPh$_3$)$_3$, RuCl$_3$, and ZnCl$_2$. (c) Time course of the oxymercuration reaction: [Hg(II)] = 0.30 μM, 50 mM phthalate pH 4 buffer. (d) Correlation between fluorescence intensities and [Hg(II)] in the presence and absence of N-chlorosuccinimide (NCS). The experiments were performed in a 50 mM phthalate pH 4 buffer in triplicate. The graph shows the mean values and standard deviations.
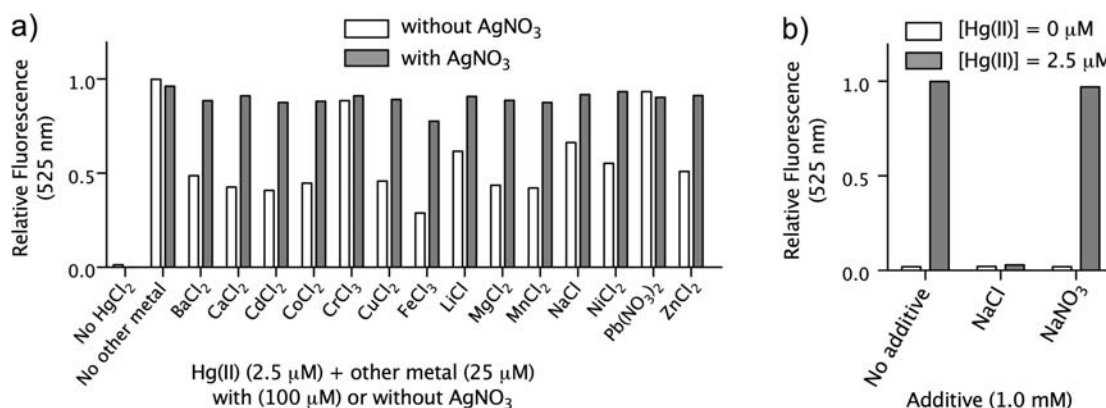
Figure 2. Compatibility of our method to detect Hg(II) in the presence of inorganic ions: (a) interference by inorganic materials ([HgCl$_2$] = 2.5 μM, [reagent shown] = 25 μM); (b) interference by NaCl but not by NaNO$_3$. These experiments were performed in pH 4 buffer.

noninterference of the mixture of HgCl$_2$ and AgNO$_3$ (not shown). These results prompted us to hypothesize that the addition of excess AgNO$_3$ to the mixtures of HgCl$_2$ and MCl$_n$ (M = Li, Na, etc.) might facilitate the conversion of HgCl$_2$ (XHg—Cl bond 24 kcal/mol[37]) to HgCl$^+$ by virtue of the formation of poorly water soluble AgCl[38] (Ag—Cl bond 71.7 kcal/mol[37]). In effect, the combination of HgCl$_2$ (2.5 μM), AgNO$_3$ (100 μM), and MCl$_n$ (25 μM) generated a nearly uniform fluorescence signal (Figure 2a, gray bars). Thus, the addition of AgNO$_3$ was found to afford a more general fluorometric detection method for Hg(II) in the presence of various inorganic molecules.

Detection of Hg(II) in River Water. With this improvement, we attempted to detect mercury species in environmental samples again. In wastewater, the permitted discharge limits for total mercury may be 5 ppb[39] or 10 ppb.[40] Thus, as a proof-of-concept experiment,[41] we proceeded to apply our method, which involved the use of AgNO$_3$ for the detection of spiked Hg(II) (0−256 ppb) in river water (Figure 3a,b). Figure 3a shows that, unlike the previously failed case (left), the addition of AgNO$_3$ improves the signal recovery to nearly 100%. The standard curve in Figure 3b (also Table S4, Supporting Information) indicates that compound **3** could detect Hg(II) at ∼8 ppb in river water.
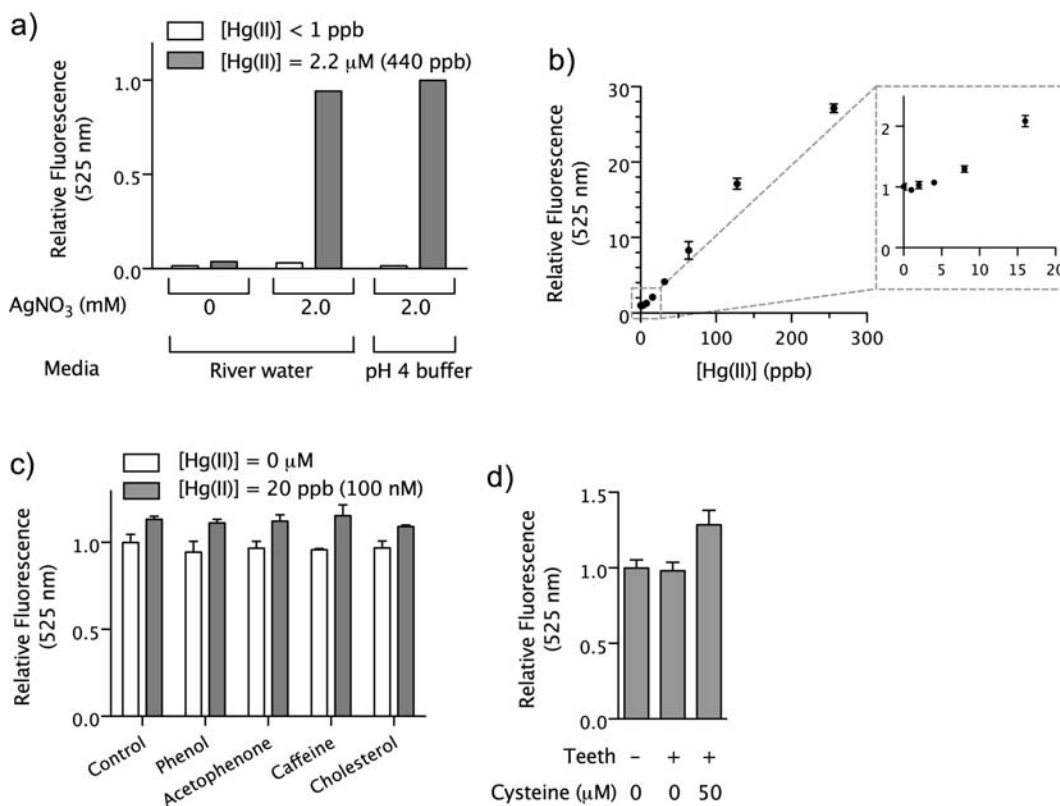
Figure 3. Applications of probe **3** for the detection of mercury species in environmental and dental samples. All detection was performed after adjustment of pH of samples to pH 4. (a) Comparison of river water and commercial pH 4 buffer ($[HgCl_2] = 2.2 \mu M$ (440 ppb)). In the absence of AgNO$_3$, Hg(II) cannot be detected by the method (left). In the presence of AgNO$_3$, Hg(II) can be detected (middle). (b) Mercury detection in river water ($[HgCl_2] = 0-256$ ppb, $[AgNO_3] = 2.0$ mM). (c) Mercury detection in river water in the presence of organic compound ($[AgNO_3] = 2.0$ mM, [compound] = 100 ppb). (d) Dental samples ([NCS] = 500 μM).

We proceeded to determine whether the method based on the oxymercuration of vinyl ether **3** could be compatible with organic contaminants in environmental samples. We applied our method in the presence of various typical organic contaminants in wastewater[42] to assess the robustness of **3** with other functional groups (Figure 3c and Table S5, Supporting Information). It was found that this probe could detect Hg(II) even if the reaction solution was contaminated with organic compounds, including an alkene.[43] This may not be surprising, because the olefin of compound **3** is more electron rich and thus more reactive toward Hg(II) than most alkenes.

Detection of Hg(II) in Dental Samples. In addition to environmental samples, dental samples were examined to broaden the applications of the fluorescence method. We hypothesized that cysteine from food might facilitate the dissolution of Hg from amalgam-filled teeth. Thus, the previously used two teeth[26] were stirred in a cysteine solution. After the teeth were removed, the resulting solution was treated with NCS to oxidize the thiol and Hg-bound sulfur atoms before the addition of **3**. Figure 3d and Table S6 (Supporting Information) show that we were able to detect, although unable to quantify,[44] leached mercury from dental samples. The difference between bars 2 and 3 (3.63×10^4 in the raw data; see page S17 in the Supporting Information) was greater than 3 times the standard deviation of bar 2 (6.85×10^3), enabling us to detect Hg(II) with a confidence level of over 90%.[44] Additional studies of dental samples are needed to validate the utility of the method in

Scheme 2. Preparation of 12 and Its Reaction with HgCl$_2$ To Form 13
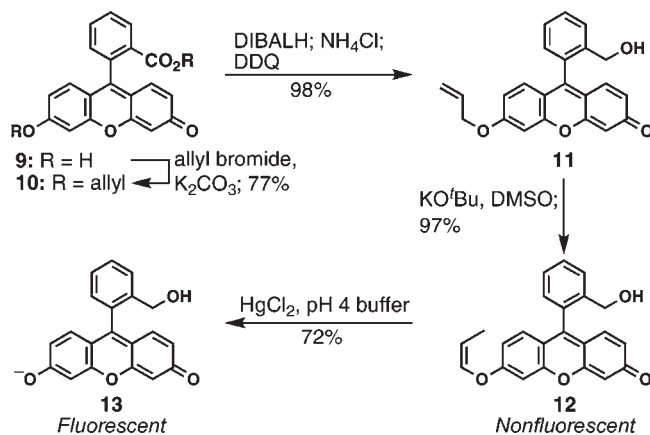
dentistry. Nonetheless, this result may warrant further studies on how sulfur-rich food may dissolve mercury amalgams.

It should be noted that, during this work, Ahn et al. reported a very similar compound for Hg detection.[45] There are notable differences: **3** cannot be contaminated with heavy metals, it reacts with Hg(II) in a 1:1 stoichiometry, and we discovered anion interference and a solution to this interference.

Design, Preparation, and Characterization of 12 and 13. While developing a protocol to circumvent interference by
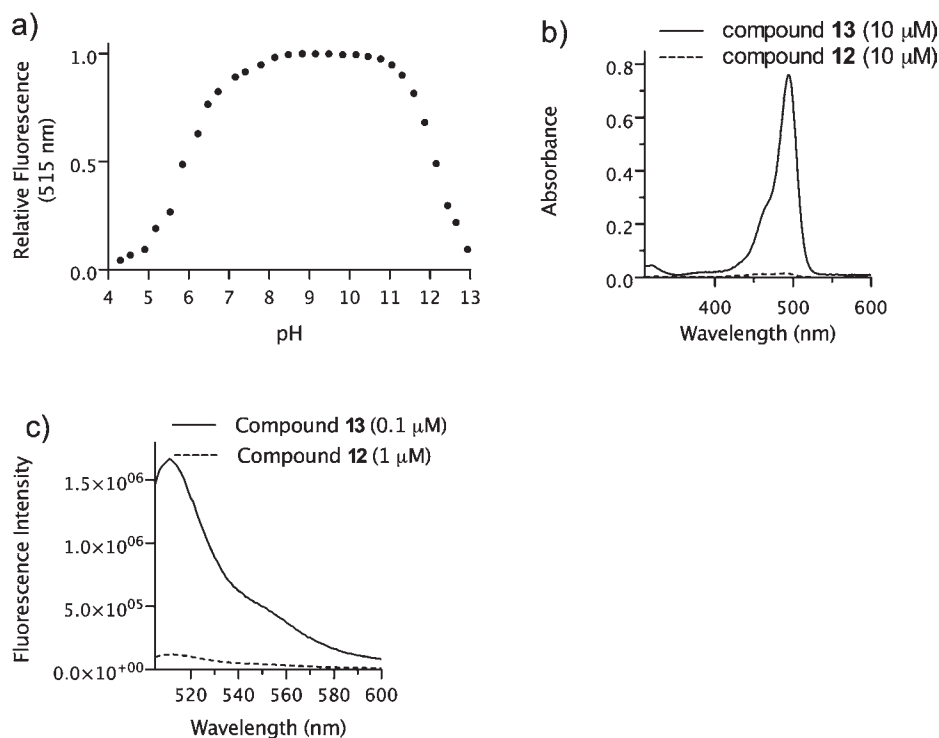
Figure 4. (a) pH titration of compound **13**. The fluorescence intensity at 515 nm was monitored at ∼0.3 pH intervals. (b) UV−vis absorption spectra of **12** and **13** in 1% DMSO/pH 8 buffer. (c) Emission spectra of **12** and **13** in 0.1% DMSO/pH 8 buffer.

chloride ions, we realized that the chlorine atoms that were built into **3** might interfere, although organic and inorganic chlorides are not the same. Further consideration on removing the chlorides from **3** suggested that the vinyl ether of **12** (Scheme 2) might be more reactive toward Hg(II), due to the lack of electron-withdrawing chloride groups. Our minor concern was related to the inferiority of fluorescein over 2′,7′-dichlorofluorescein due to the higher pK_a (6.4) of fluorescein compared to that of 2′,7′-dichlorofluorescein (4.3; the pK_a of compound **1** is 4.3).[33] Nonetheless, we proceeded to synthesize compound **12** as shown in Scheme 2. This time, the olefin migration of the allyl ether **11** was highly stereoselective, only giving the cis product **12**. Compound **12** also reacted with HgCl$_2$ smoothly at 25 °C and gave the new fluorescent compound **13** in 72% isolated yield. The linear correlation was confirmed between the concentration of **13** and the fluorescence signals (Figure S4, Supporting Information).

The pK_a of the phenolic hydroxy group of compound **13** was 6.0 (Figure 4a and Figure S5, Supporting Information), noticeably lower than that of fluorescein (6.4). This was unexpected, because the conversion of the stronger electron-withdrawing carboxy group of fluorescein to the weaker hydroxymethyl group should decrease the acidity of the phenol hydroxy group. We postulate that the carboxylate anion of fluorescein might destabilize the phenoxy anion intramolecularly. Although the reason is not yet clear, the fine tuning of the acidity of phenol in fluorescein derivatives is a fruitful endeavor to develop new assay methods.[46]

The UV−vis absorption spectra of **12** and **13** are shown in Figure 4b. Compound **12** showed no absorption peak at ∼490 nm, but compound **13** did. The fluorescence emission spectra of **12** and **13** indicated that the signal of **12** was 140 times lower than that of **13** in a pH 8 buffer at 515 nm (λ_{max}) at the same concentration (Figure 4c; see also Figure S1). This is consistent with the platform depicted in Scheme 1b.

Reactivity of 12 with Metal Ions. Solutions of the vinyl ether **12** were treated with Hg(II) (0.3 μM = 60 ppb) in pH 4, 5, 6, and 7 buffer (Figure 5a). Similarly to compound **3**, compound **12** was more reactive at a lower pH. The reactivity of **12** toward Hg(II) was very high (Figure 5b). The only other metal that reacted with **12** was Pd(II) (cf. Figure 1b). In the presence of AgNO$_3$, none of the coexisting metal reagents interfered with the Hg(II)-promoted conversion of **12** to **13** (Figure 5c). It was found that the fluorescence method using **12** allowed for the detection of 1 ppb of Hg(II) (Figure 5d and Table S7, Supporting Information). Figure 5e indicates that chloride, bromide, and iodide ions interfered with the Hg(II)-promoted conversion of **12** to **13** but nitrate and sulfate ions did not. The interference by chloride ions was less severe for compound **12** than for compound **3** (Figure S6, Supporting Information). The halide-ion-mediated interference could be overcome by the addition of AgNO$_3$ (Figure 5c).

Next, we compared the new chemodosimeter **12** with **3** and **14**.[26] As Figure 6a shows, compound **12** was far more reactive than **14** toward Hg(II) in a pH 4 buffer at 25 °C. For example, after 10 min the fluorescence increase with **12** was 242 times greater than that with **14**. The reaction with **12** was >60% complete after 1 h. The initial rate was about 12 times greater for **12** relative to **3** (Figure 6b). Additionally, compound **12** was more reactive than compound **3** at pH 7 (Figure 6c), implying potential biological applications.

Application with River Water. We applied chemodosimeter **12** for detection of Hg(II) spiked in river water again. Chemodosimeter **12** was able to detect 1 ppb of Hg in river water (Figure 7 and Table S8, Supporting Information). The heightened sensitivity of **12** in determining [Hg(II)] in such a complex media as natural water indicates its robustness and potential for facile on-site monitoring of water safety. When our method
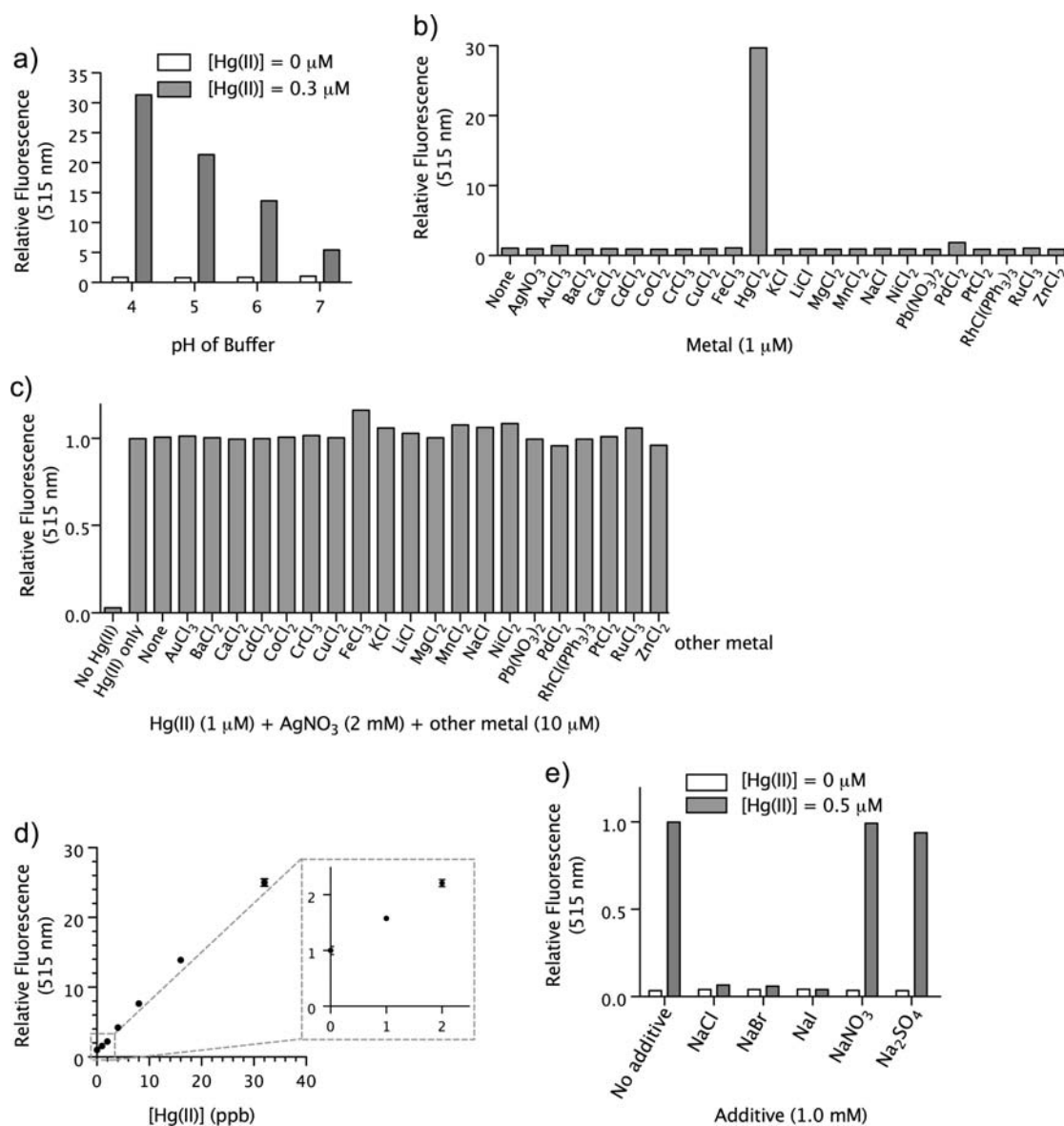
Figure 5. Comparison of reaction conditions for the conversion of **12** to **13**. (a) pH dependence. Fluorescence intensities were measured after addition of 1.3 M pH 7 buffer and 500 mM pH 10 buffer. (b) Metal selectivity. All the metals were tested at 5 μM in pH 4 buffer. (c) Hg detection in the presence of various metal ions. All of the reactions were performed in pH 4 buffer. Metal reagents: $AgNO_3$, $AuCl_3$, $BaCl_2$, $CaCl_2$, $CdCl_2 \cdot 2.5H_2O$, $CoCl_2$, $CrCl_3$, $CuCl_2 \cdot 2H_2O$, $FeCl_3$, $HgCl_2$, KCl, LiCl, $MgCl_2$, $MnCl_2 \cdot 4H_2O$, NaCl, $NiCl_2$, $Pb(NO_3)_2$, $Pd(NO_3)_2$, $PtCl_2$, $Rh(PPh_3)_3$, $RuCl_3$, and $ZnCl_2$. (d) Correlation between fluorescence intensities at 515 nm and [Hg(II)]. The experiments were performed at pH 4 in triplicate. (e) Effect of anion. All detection was performed in pH 4 buffer.

was applied to the analysis of two wastewater samples from a coal-fired power plant, we found compound **12** to be a qualitative indicator of Hg(II) (Supporting Information). Although we were unable to quantitatively assess Hg(II) concentrations in these more complex samples, we were able to qualitatively discriminate between concentration values as low as 2 ppb and <500 ppt. Further studies are needed to determine the scope and limitations of the use of **12** with additional complex samples.

contaminants. The method with **3** was effective in the detection of Hg(II) in river water and dental samples. Further structural fine tuning led to the development of the vinyl ether **12** (Scheme 3). Compound **12** could react with Hg(II) after the removal of chloride ions with $AgNO_3$. This compound was 242 times more reactive than the previously reported alkyne **14** toward Hg(II) at ambient temperature and could be used to detect 1 ppb Hg(II). Further evaluations of this method with additional real-world samples are underway in our laboratory.

■ CONCLUSIONS

In summary, we have developed a sensitive and selective fluorometric method to detect mercury species at ambient temperature in the presence of various organic, inorganic, and anionic

■ EXPERIMENTAL SECTION

Synthetic Materials and Methods. All of the reactions in Schemes 1c and 2 were carried out with commercial-grade reagents
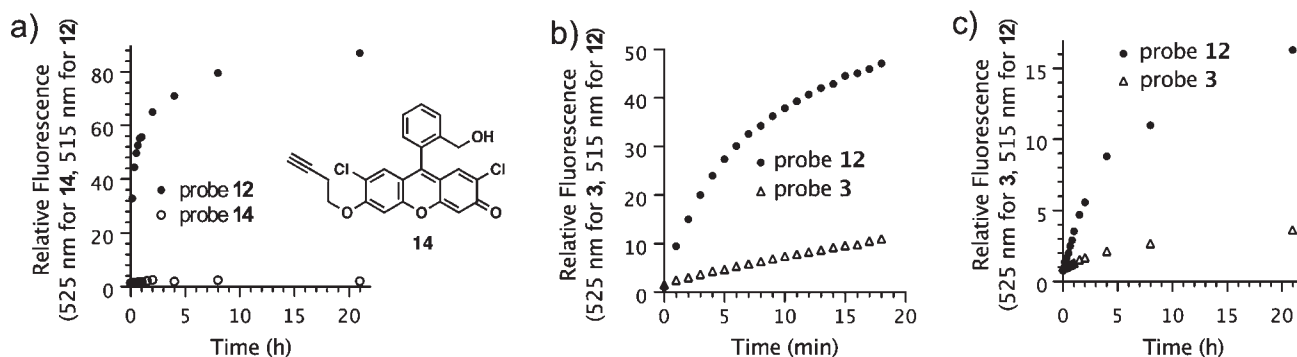
Figure 6. Comparisons of reactivity between probes developed by our group ([probe] = 1.0 μM, [Hg(II)] = 0.3 μM). (a) Time course of the oxymercuration reaction at 25 °C in 50 mM phthalate pH 4.0 buffer. The comparison between probes **12** and **14** is shown. (b) Time course of the oxymercuration reaction at 25 °C in 50 mM phthalate pH 4.0 buffer. The comparison between probes **3** and **12** is shown. To focus on their initial rate, fluorescence measurements were carried out every 1 min for 18 min. (c) Time course of the oxymercuration reaction in 50 mM phosphate pH 7 buffer. The comparison between probes **3** and **12** is shown.
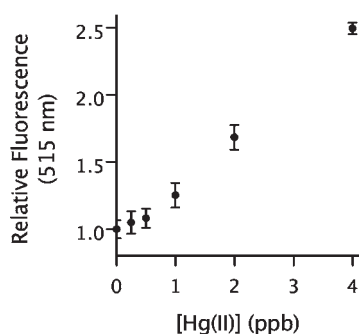

Figure 7. Application of **12** for the detection of Hg(II) in river water ([**12**] = 1.0 μM; [Hg(II)] = 0, 0.25, 0.5, 1, 2, and 4 ppb; [AgNO$_3$] = 2.0 mM; 0.5% DMSO in 50 mM phthalate pH 4 water; 1 h; 25 °C). Titration of Hg(II) in river water was performed.

without further purification. DMF was used after distillation from silica gel. CH$_2$Cl$_2$ was used after distillation from CaH$_2$. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light (254 nm) for visualization or phosphomolybdic acid in ethanol as developing agents and heat for visualization. TSI silica gel (230−400 mesh) was used for flash chromatography. NMR spectra were recorded on AM300 or AM400 (Bruker) instruments and calibrated using a solvent peak as an internal reference. The following abbreviations are used to indicate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent. High-resolution mass spectra were obtained using EBE geometry.

Preparation of Compound 3. KO^{t}Bu (11 mg, 0.10 mmol) was added to a solution of compound **2**[30] (21 mg, 50 μmol) in DMSO (1.0 mL) at 25 °C under a nitrogen atmosphere, and the resulting solution was heated in a 90 °C oil bath for 12 h. The reaction mixture was then poured onto ice-cold H$_2$O (5.0 mL), and the resulting mixture was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (5 → 20% EtOAc in hexanes) on silica gel (10 mL) to afford compound **3** (18 mg, 86%, mixture of cis/trans (2:1)) as an orange solid. Data for **3**: mp 146−155 °C; R_f = 0.31 (30% EtOAc in hexanes); IR (KBr pellet) ν_{max} 3368 (broad, O−H), 2921, 2859, 1606 (C=O), 1482, 1435, 1411, 1269, 1175, 1034, 874, 725 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6, 293 K, Figure S7) δ 7.52−7.39 (m, 2H, Ar),
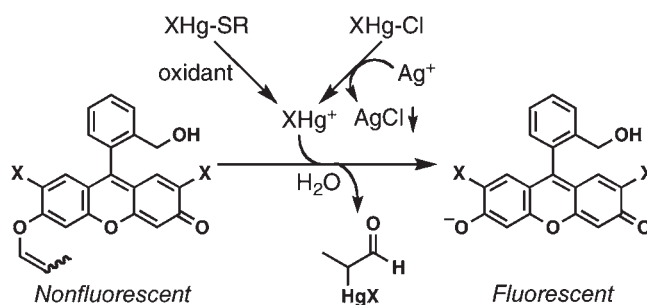
7.34−7.27 (m, 1H, Ar), 7.05 (s, 0.67H, Ar (cis isomer)), 7.03 (s, 0.33H, Ar (trans isomer)), 6.99−6.97 (m, 2H, Ar), 6.91−6.88 (m, 1H, Ar), 6.87 (s, 1H, Ar), 6.68 (dq, J = 12.0, 1.8 Hz, 0.33H, CH$_3$CH=CHOAr (trans isomer)), 6.65 (dq, J = 6.6 (a similar coupling constant was observed in a related compound[47], 1.8 Hz, 0.67H, CH$_3$CH=CHOAr (cis isomer)), 5.51 (dq, J = 12.0, 6.6 Hz, 0.33H, CH$_3$CH=CHOAr (trans isomer)), 5.42 (s, 2H, ArCH$_2$OH), 5.13 (dq, J = 6.6,[47] 6.6 Hz, 0.67H, CH$_3$CH=CHOAr (cis isomer)), 1.76−1.66 (m, 3H, CH$_3$CH=CHOAr) ppm; ^{13}C NMR (75 MHz, acetone-d_6, 293 K, Figure S8) δ 154.6, 154.2, 154.0, 150.44, 150.38, 145.8, 142.1, 140.9, 139.7, 130.7, 130.4, 129.5, 124.1, 122.3, 121.0, 118.8, 118.4, 118.3, 117.2, 111.7, 110.7, 104.7, 104.5, 104.2, 83.6, 73.4, 12.3, 9.6 ppm; HRMS (EI+) m/z calcd for C$_{23}$H$_{16}$O$_4$Cl$_2$ [M]$^+$ 426.0426, found 426.0425.

Conversion of Compound 3 to Compound 1. HgCl$_2$ (14 mg, 50 μmol) was added to a solution of compound **3** (21 mg, 50 μmol) in a 1/9 DMSO/50 mM phthalate pH 4 buffer (20 mL) mixture. The resulting mixture was stirred at 25 °C for 1 h and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (10 → 70% EtOAc in hexanes) on silica gel (10 mL) to afford compound **1** (16 mg, 82%) as an orange solid. The spectroscopic data of compound **1** were consistent with the literature.[30] Compound **8** was isolated from other fractions. R_f = 0.66 (50% EtOAc in hexanes). ^{1}H NMR (300 MHz, acetone-d_6, 293 K, Figure S9): δ 9.63 (d, J = 1.2 Hz, 1H, CHO), 3.66 (qd, J = 6.9, 1.2 Hz, 1H, OHC−CHHgX), 1.53 (d, J = 6.9 Hz, 3H, CH$_3$) ppm. Due to the extreme toxicity of organomercury species, we discarded this material in collaboration with the Environmental Health and Safety office at the University of Pittsburgh (http://www.ehs.pitt.edu) without obtaining further spectroscopic data.

Preparation of Compound 10. K_2CO_3 (4.15 g, 30.0 mmol) was added to a solution of fluorescein (3.32 g, 10.0 mmol) in DMF (20 mL) at 25 °C under a nitrogen atmosphere, followed by allyl bromide (2.60 mL, 30 mmol). After it was stirred for 48 h at 25 °C, the reaction mixture was poured onto H_2O (500 mL). The resulting mixture was then extracted with EtOAc (3 × 200 mL), and the combined extracts were washed with H_2O (3 × 500 mL) and brine (500 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was recrystallized from hexanes/EtOAc to afford compound 10 (3.17 g, 77%) as an orange solid. Data for 10: mp 153−155 °C; R_f = 0.34 (60% EtOAc in hexanes); IR (KBr pellet) ν_{max} 3054, 2986, 2932, 1727 (C=O), 1643 (C=O), 1595, 1517, 1481, 1256, 1211, 1106, 855, 759 cm^{-1}; ^1H NMR (300 MHz, CDCl$_3$, 293 K, Figure S10) δ 8.27 (dd, J = 7.5, 1.5 Hz, 1H, Ar), 7.75 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, Ar), 7.68 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, Ar), 7.32 (dd, J = 7.5, 1.5 Hz, 1H, Ar), 6.96 (d, J = 2.4 Hz, 1H, Ar), 6.90 (d, J = 9.0 Hz, 1H, Ar), 6.87 (d, J = 9.6 Hz, 1H, Ar), 6.77 (dd, J = 9.0, 2.4 Hz, 1H, Ar), 6.56 (dd, J = 9.6, 1.8 Hz, 1H, Ar), 6.47 (d, J = 1.8 Hz, 1H, Ar), 6.07 (dddd, J = 17.4, 10.5, 5.4, 5.4 Hz, 1H, CH$_2$=CHCH$_2$OAr), 5.60 (dddd, J = 17.4, 10.2, 6.0, 6.0 Hz, 1H, ArCO$_2$CH$_2$CH=CH$_2$), 5.46 (dd, J = 17.4, 1.2 Hz, 1H, H_{trans}CH=CHCH$_2$OAr), 5.37 (dd, J = 10.5, 1.2 Hz, 1H, HCH_{cis}=CHCH$_2$OAr), 5.14−5.08 (m, 2H, ArCO$_2$CH$_2$CH=CH_2), 4.66 (ddd, J = 5.4, 1.2, 1.2 Hz, 2H, CH$_2$=CHCH_2OAr), 4.48 (dddd, J = 12.9, 6.0, 1.2, 1.2 Hz, 1H, ArCO$_2$CH_aHCH=CH$_2$), 4.46 (dddd, J = 12.9, 6.0, 1.2, 1.2 Hz, 1H, ArCO$_2$CHH_bCH=CH$_2$) ppm; ^{13}C NMR (75 MHz, CDCl$_3$, 293 K, Figure S11) δ 185.8, 165.2, 163.1, 159.1, 154.3, 150.2, 134.5, 132.9, 132.0, 131.4, 131.1, 130.67, 130.65, 130.4, 130.1, 129.9, 129.1, 119.3, 118.9, 117.9, 115.1, 113.9, 105.9, 101.3, 69.6, 66.2 ppm; HRMS (ESI+) m/z calcd for $C_{26}H_{21}O_5$ [M]$^+$ 413.1389, found 413.1373.

Preparation of Compound 11. A solution of DIBALH (9.6 mL, 1.0 M in CH$_2$Cl$_2$) was added dropwise to a solution of compound 10 (0.83 g, 2.0 mmol) in CH$_2$Cl$_2$ (7.0 mL) over 15 min at −78 °C under a nitrogen atmosphere. The resulting solution was stirred at the same temperature for 10 min and then warmed to 25 °C. After stirring at the same temperature for 2 h, Et$_2$O (5.0 mL) was added to the resulting solution at 0 °C with stirring, and then saturated aqueous NH$_4$Cl (3.5 mL) was added dropwise to the mixture at the same temperature. This mixture was warmed to 25 °C again and stirred for 1 h. The reaction mixture was then diluted with Et$_2$O (5.0 mL), and DDQ (0.45 g, 2.2 mmol) was added slowly to this mixture at 0 °C. After being stirred for 1 h at 25 °C, the mixture was filtered through a pad of Celite, and the pad was rinsed with EtOAc. The filtrate was dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (10 → 30% EtOAc in hexanes) on silica gel (180 mL) to afford compound 11 (701 mg, 98%) as a pale yellow solid. Data for 11: mp 144−146 °C; R_f = 0.32 (30% EtOAc in hexanes); IR (KBr pellet) ν_{max} 3228 (broad, O−H), 2921, 2852, 1616 (C=O), 1501, 1517, 1456, 1430, 1335, 1224, 1180, 1003, 841, 759 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6, 293 K, Figure S12) δ 7.44 (d, J = 7.5 Hz, 1H, Ar), 7.37 (dd, J = 7.5, 7.5 Hz, 1H, Ar), 7.26 (dd, J = 7.5, 7.5 Hz, 1H, Ar), 6.89 (d, J = 8.7 Hz, 1H, Ar), 6.84 (d, J = 7.5 Hz, 1H, Ar), 6.83 (d, J = 8.7 Hz, 1H, Ar), 6.76 (d, J = 2.4 Hz, 1H, Ar), 6.67 (d, J = 2.4 Hz, 1H, Ar), 6.66 (dd, J = 8.7, 2.4 Hz, 1H, Ar), 6.58 (dd, J = 8.7, 2.4 Hz, 1H, Ar), 6.07 (dddd, J = 17.4, 10.5, 5.4, 5.4 Hz, 1H, CH$_2$=CHCH$_2$OAr), 5.42 (dd, J = 17.4, 1.2 Hz, 1H, H_{trans}CH=CHCH$_2$OAr), 5.28 (s, 2H, ArCH_2OH), 5.24 (dd, J = 10.5, 1.2 Hz, 1H, HCH_{cis}=CHCH$_2$OAr), 4.61 (ddd, J = 5.4, 1.2, 1.2 Hz, 2H, CH$_2$=CHCH_2OAr) ppm; ^{13}C NMR (75 MHz, acetone-d_6, 293 K, Figure S13) δ 160.2, 159.0, 152.2, 152.1, 146.7, 140.1, 134.5, 131.0, 130.9, 129.1, 128.8, 124.3, 121.9, 118.9, 117.74, 117.67, 112.7, 112.3, 107.8, 101.9, 84.1, 72.7, 69.5 ppm; HRMS (ESI+) m/z calcd for $C_{23}H_{18}O_4Na$ [M + Na]$^+$ 381.1103, found 381.1072.

Preparation of Compound 12. KO^{t}Bu (56 mg, 0.44 mmol) was added to a solution of compound 11 (72 mg, 0.20 mmol) in DMSO (1.7 mL) at 25 °C under a nitrogen atmosphere, and the resulting solution

was heated in a 50 °C oil bath for 1 h. The reaction mixture was then poured onto ice-cold H_2O (10 mL), and the resulting mixture was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (30 mL), dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (10 → 30% EtOAc in hexanes) on silica gel (10 mL) to afford compound 12 (70 mg, 97%) as an orange solid. Data for 12: mp 96−98 °C; R_f = 0.30 (40% EtOAc in hexanes); IR (KBr pellet) ν_{max} 3304 (broad, O−H), 3045, 2921, 2858, 1610 (C=O), 1497, 1459, 1426, 1270, 1177, 1109, 1023, 846, 804, 725 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6, 293 K, Figure S14) δ 7.47 (d, J = 7.6 Hz, 1H, Ar), 7.39 (dd, J = 7.6, 7.6 Hz, 1H, Ar), 7.28 (dd, J = 7.6, 7.6 Hz, 1H, Ar), 6.96 (d, J = 8.8 Hz, 1H, Ar), 6.84 (d, J = 8.8 Hz, 1H, Ar), 6.83 (d, 6.82 J = 7.6 Hz, 1H, Ar), (d, J = 2.4 Hz, 1H, Ar), 6.75 (dd, J = 8.8 2.4 Hz, 1H, Ar), 6.67 (d, J = 2.4 Hz, 1H, Ar), 6.60−6.55 (m, 2H, Ar and CH$_3$CH=CHOAr), 5.30 (s, 2H, ArCH_2OH), 4.96 (dq, J = 6.8, 6.8 Hz, 1H, CH$_3$CH=CHOAr), 1.68 (dd, J = 6.8, 1.6 Hz, 3H, CH_3CH=CHOAr) ppm; ^{13}C NMR (100 MHz, acetone-d_6, 293 K, Figure S15) δ 159.2, 158.7, 152.12, 152.07, 146.7, 141.4, 140.0, 131.2, 131.0, 129.1, 128.9, 124.3, 121.9, 120.7, 117.6, 112.9, 112.7, 108.7, 103.5, 102.8, 84.0, 72.9, 9.6 ppm; HRMS (ESI+) m/z calcd for $C_{23}H_{18}O_4Na$ [M + Na]$^+$ 381.1103, found 381.1083.

Conversion of Compound 12 to Compound 13. HgCl$_2$ (18 mg, 67 μmol) was added to a solution of compound 12 (24 mg, 67 μmol) in a 1/9 DMSO/pH 4 buffer (26 mL) mixture. The resulting mixture was stirred at 25 °C for 1 h and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (10 → 70% EtOAc in hexanes) on silica gel (10 mL) to afford compound 13 (15 mg, 72%) as an orange solid. Data for 13: mp >200 °C; R_f = 0.32 (50% EtOAc in hexanes); IR (KBr pellet) ν_{max} 3430 (broad, O−H), 2869, 2600, 1602 (C=O), 1455, 1379, 1309, 1241, 1207, 1117, 860, 757 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6, 293 K, Figure S16) δ 7.45 (d, J = 7.5 Hz, 1H, Ar), 7.34 (dd, J = 7.5, 7.5 Hz, 1H, Ar), 7.27 (dd, J = 7.5, 7.5 Hz, 1H, Ar), 6.83 (d, J = 7.5 Hz, 1H, Ar) 6.81 (d, J = 8.4 Hz, 2H, Ar), 6.64 (d, J = 2.4 Hz, 2H, Ar), 6.56 (dd, J = 8.4, 2.4 Hz, 2H, Ar), 5.26 (s, 2H, ArCH_2OH) ppm; ^{13}C NMR (75 MHz, acetone-d_6, 293 K, Figure S17) δ 159.0, 152.3, 146.9, 140.2, 131.0, 129.1, 128.8, 124.4, 121.8, 117.9, 112.6, 102.8, 84.2, 72.6 ppm; HRMS (EI+) m/z calcd for $C_{20}H_{15}O_4$ [M]$^+$ 319.0970, found 319.0999.

Sample Preparations. Mercury standard solution (20 ppm in 5% HNO$_3$) was purchased from the RICCA Chemical Co. (Arlington, TX) and used as received. The other mercury standard solution (10 000 ppm in 2% HNO$_3$) was purchased from Ultra Scientific (North Kingstown, Rhode Island) and used as received. Omni*Trace* Ultra High Purity Acid HNO$_3$ (Hg < 10 ppt) was purchased from EMD (item number NX0408, lot number 48157) and used as received. ARISTAR ULTRA water was purchased from VWR (catalog number 7732-18-5) and used as received. AgNO$_3$ was purchased from EMD and used as received. River water was collected from the Allegheny River on Jan 29, 2008. The pH 3 buffer solution was made from a commercial pH 4 buffer (50 mM) and a pure HNO$_3$ solution.

Metal Solutions (1.0 mM). AuCl$_3$, BaCl$_2$, NiCl$_2$, CrCl$_3$, Pb-(NO$_3$)$_2$, NaCl, MnCl$_2$·4H$_2$O, MgCl$_2$, CoCl$_2$, HgCl$_2$, AgNO$_3$, ZnCl$_2$, LiCl, CuCl$_2$·2H$_2$O, and CaCl$_2$ were dissolved in H$_2$O. FeCl$_3$, CdCl$_2$·2.5H$_2$O, KCl, Rh(PPh$_3$)$_3$, and RuCl$_3$ were dissolved in MeOH. PtCl$_2$ was dissolved in MeOH/acetone (1/1). Pd standard solution (High-Purity Standards, Cat. No. 100038-1) was diluted with 1% HNO$_3$. The resulting solution was used as the Pd solution.

Fluorescence Measurement. All samples were incubated at 25 °C, and the pH values of the solutions were adjusted to an appropriate pH range (pH >5 for 1, pH >8 for 13) by the addition of 1.23 M phosphate pH 7 buffer (for 1, 4.0% of the total volume of a reaction solution) or a 1:5 mixture of 1.23 M phosphate pH 7 buffer and 500 mM borate

2563

dx.doi.org/10.1021/ja108028m |*J. Am. Chem. Soc.* 2011, 133, 2556−2566

pH 10 buffer (for **13**, 24% of the total volume of a reaction solution). The resulting samples were vortexed for 5 s prior to fluorescence measurement. Fluorescence spectra were recorded in a 1×1 cm disposable cuvette (VWR; catalog number 58017-880) on a Jobin Yvon Fluoro-Max-3 spectrometer under the control of a Windows-based PC running FluorEssence software. The samples were excited at 497 nm, and the emission intensities were collected at 523 nm (for **1**) or 515 nm (for **13**). All spectra were corrected for emission intensity using the manufacturer-supplied photomultiplier curves.

pH Dependence of Hg Detection (Figures 1a and 5a). *Reaction conditions*: $[Hg(II)] = 0.30\ \mu M$, $[\mathbf{3}$ or $\mathbf{12}] = 1.0\ \mu M$, 0.05% DMSO in buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 100 μM Hg standard solution (15 μL each) was added to a pH 3, 4, 5, 6, or 7 buffer (4.98 mL each). A 1.0 mM solution of **3** or **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to each of these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization. Note: for the pH 3 sample, an additional 1.0 mL of 500 mM borate pH 10 buffer was required to obtain a pH >5 solution.

Metal Selectivity (Figures 1b and 5b). *Reaction conditions*: [metal] = 5.0 μM, $[\mathbf{3}$ or $\mathbf{12}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: each of the 1.0 mM solutions of metal reagents in appropriate solvents (25 μL, see the Supporting Information for details) was added to a 50 mM phthalate pH 4 buffer (4.97 mL). A 1.0 mM solution of **3** or **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Time Course of the Oxymercuration Reaction (Figures 1c and 6). *Reaction conditions*: $[Hg(II)] = 0.30\ \mu M$, $[\mathbf{3}, \mathbf{12},$ or $\mathbf{14}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer or a 50 mM pH 7 phosphate buffer (20 mL), 25 °C. *Protocol*: a 100 μM Hg standard solution (60 μL) was added to a 50 mM phthalate pH 4 buffer (Figures 1c and 6a,b) or a 50 mM phosphate pH 7 buffer (Figure 6c) (20 mL). A 1.0 mM solution of **3**, **12**, or **14** in 1/1 DMSO/50 mM phosphate pH 8 buffer (20 μL) was added to each of the solutions, and the resulting reaction solutions were shaken for 3 s and incubated at 25 °C before fluorescence measurement. A fraction (1.5 mL) of each of these reaction solutions was taken for the measurement at 10, 20, 30, 40, 50, 60, 90, 120, 240, 480, and 1140 min. For Figure 6b, this measurement was carried out every 1 min for 18 min with 1.0 mL of the reaction solution. See Fluorescence Measurement for sample neutralization.

Titration Curve (Figures 1d and 5d). *Reaction conditions*: $[Hg(II)] = 0, 1, 2, 4, 8, 16,$ and 32 ppb, $[NCS] = 10\ \mu M$ for NCS(+) samples and 0 μM for NCS(−) samples, $[\mathbf{3}$ or $\mathbf{12}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 0, 200, 400, 800, 1600, 3200, or 6400 ppb solution of HgCl$_2$ in 5% HNO$_3$ (25 μL) and a 2.0 mM solution of NCS in water (25 μL) were added to each of 50 mM phthalate pH 4 buffers (4.95 mL). A 1.0 mM solution of **3** or **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to each of these solutions, and the resulting solutions were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Hg Detection in the Presence of Other Metal (Figure 2a (White Bar)). *Reaction conditions*: $[Hg(II)] = 2.5\ \mu M$, [other metal] = 25 μM, $[\mathbf{3}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 100 μM Hg standard solution in 5% HNO$_3$ (125 μL) and each of the 1.0 mM metal solutions (125 μL; see the Supporting Information for details) were added to each of 50 mM phthalate pH 4 buffer (4.75 mL). Each of these solutions was treated with a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL). The resulting solutions were shaken for 3 s and

incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Effect of Chloride or Nitrate Ion (Figures 2b and S6 (Supporting Information)). *Reaction conditions*: $[Hg(II)] = 2.5\ \mu M$, $[Cl^-$ or $NO_3^-] = 1.0$ mM, $[\mathbf{3}$ or $\mathbf{12}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 1.0 mM HgCl$_2$ solution in 5% HNO$_3$ (12.5 μL) and a 100 mM NaCl or NaNO$_3$ solution in water (50 μL) were added to a 50 mM phthalate pH 4 buffer (4.93 mL). Each of these solutions was treated with a 1.0 mM solution of **3** or **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL). The resulting samples were shaken for 3 s and incubated at 25 °C for 15 min (Figure 2b) or 1 h (Figure 5c) before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Interference of Inorganic Materials with AgNO$_3$ (Figures 2a (Gray Bar) and 5c). *Reaction conditions for Figure 2a*: $[Hg(II)] = 2.5\ \mu M$, [other metal] = 25 μM, $[AgNO_3] = 100\ \mu M$, $[\mathbf{3}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 1.0 mM HgCl$_2$ solution in 5% HNO$_3$ (12.5 μL) and a 1.0 mM solution of another metal (125 μL) were added to a 50 mM phthalate pH 4 buffer (4.86 mL). These solutions were vortexed for 5 s, and then a 10 mM solution of AgNO$_3$ in ultrapure water (50 μL) was added to the resulting solutions. After vortexing for 5 s, a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Reaction conditions for Figure 5c: $[Hg(II)] = 1.0\ \mu M$, [other metal] = 10 μM, $[AgNO_3] = 2.0$ mM, $[\mathbf{12}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 1.0 mM HgCl$_2$ solution in 5% HNO$_3$ (5.0 μL) and a 1.0 mM solution of another metal (50 μL) were added to a 50 mM phthalate pH 4 buffer (4.84 mL). These solutions were vortexed for 5 s, and then a 100 mM solution of AgNO$_3$ in ultrapure water (100 μL) was added to the resulting solutions. After vortexing for 5 s, a 1.0 mM solution of **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Detection of Hg in River Water by Probe 3 (Figure 3a, b). *Reaction conditions for Figure 3a*: $[Hg(II)] = 440$ ppb (550 ppb = 2.75 μM in river before dilution), $[AgNO_3] = 2.0$ mM, $[\mathbf{3}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.09 mL), 25 °C, 1 h. *Protocol*: river water (3.98 mL) was spiked with a 440 μM solution of HgCl$_2$ in 5% HNO$_3$ (25 μL). This solution was treated with a 250 mM phthalate pH 4 buffer (1.0 mL) and then a 100 mM solution of AgNO$_3$ in ultrapure water (100 μL). After vortexing for 5 s, a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to this solution, and the resulting sample was shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Reaction conditions for Figure 3b: $[Hg(II)] = 0, 1, 2, 4, 8, 16, 32, 64, 128,$ and 256 ppb, $[AgNO_3] = 2.0$ mM, $[\mathbf{3}] = 1.0\ \mu M$, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.1 mL), 25 °C, 1 h. *Protocol*: a 0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, or 51.2 ppm solution of HgCl$_2$ in 5% HNO$_3$ (25 μL) was spiked into river water samples (4.0 mL each). Each of these solutions was treated with a 250 mM phthalate pH 4 buffer (1.0 mL each) and then a 100 mM solution of AgNO$_3$ in ultrapure water (100 μL each). After being shaken for 3 s, a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Hg Detection in the Presence of Organic Compound (Figure 3c). *Reaction conditions*: ($[Hg(II)] = 16$ ppb, [organic contaminant] = 80 ppb, $[AgNO_3] = 2$ mM, $[\mathbf{3}] = 1\ \mu M$, 1.3% DMSO in a 50

mM phthalate pH 4 buffer (1.30 mL), 25 °C, 1 h. *Protocol*: river water (1.0 mL) was spiked with a 10 μM HgCl₂ solution in 5% HNO₃ (10 μL) and a 10 ppm solution of an organic contaminant in DMSO (phenol, acetophenone, caffeine, or cholesterol, 10 μL). Each of these solutions contained 0.1 μM (20 ppb) Hg(II) and 100 ppb of the compound. Each of the resulting solutions was treated with a 250 mM phthalate pH 4 buffer (250 μL each) and then a 100 mM solution of AgNO₃ in ultrapure water (25 μL). After vortexing for 5 s, a 0.1 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (12.5 μL) was added to this solution, and the resulting sample was shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Detection of Hg from Dental Amalgam (Figure 3d). *Reaction conditions*: two teeth filled with mercury amalgam, [cysteine] = 50 μM, [NCS] = 500 μM, [**3**] = 1.0 μM, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (4.06 mL), 25 °C, 1 h. *Protocol*: two teeth filled with an amalgam were added to a 20 mM solution of cysteine in water (2.0 mL), and the resulting mixture was shaken (200 rpm) for 1 h at 37 °C.[26] A portion (5.0 μL) of the resulting solution was added to a 50 mM phthalate pH 4 buffer (2.0 mL). After being shaken for 3 s, a 20 mM solution of NCS in water (50 μL) and a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (2.0 μL) were added to the resulting solution. A negative control sample was prepared by adding two teeth filled with an amalgam into water (2.0 mL) and shaken (200 rpm) for 1 h at 37 °C. A portion (5.0 μL) of the resulting mixture was added to a 50 mM phthalate pH 4 buffer (2.0 mL). This mixture was then treated with a 20 mM solution of NCS in water (50 μL) and a 1.0 mM solution of **3** in 1/1 DMSO/50 mM phosphate pH 8 buffer (2.0 μL). The resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

pH Titration of Compound 13 (Figure 4a). An aqueous solution (100 mL) containing **13** (5 μM) and NaCl (1.0 M) was prepared. To half of this mixture solution (50 mL) was added a small magnetic stir bar in a vial with a pH electrode. The pH of the solution was changed by adding 0.1 or 1 N HCl solution dropwise with stirring, and the fluorescence spectrum was recorded at ~0.3 pH intervals. The other half of this mixture (50 mL) was titrated with 0.1 or 1 N NaOH solution. In order to calculate the pK_a, the pH dependence of fluorescence spectra were analyzed using the following equation: pH = pK_a − $\log[(F_{max} - F)/(F - F_{min})]$.[48]

Effect of Anion (Figure 5e). *Reaction conditions*: [Hg(II)] = 0.5 μM, [Cl⁻, Br⁻, I⁻, SO₄²⁻, or NO₃⁻] = 2.0 mM, [**12**] = 1.0 μM, 0.05% DMSO in a 50 mM phthalate pH 4 buffer (5.0 mL), 25 °C, 1 h. *Protocol*: a 0.5 mM HgCl₂ solution in 5% HNO₃ (5.0 μL) and a 100 mM solution of NaCl, NaBr, NaI, Na₂SO₄, or NaNO₃ in water (100 μL) were added to a 50 mM phthalate pH 4 buffer (4.90 mL). A 1.0 mM solution of **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (5.0 μL) was added to these solutions, and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

Detection of Hg Spiked in River Water by Probe 12 (Figure 7). *Reaction conditions*: [Hg(II)] = 0, 0.25, 0.5, 1, 2, and 4 ppb, [AgNO₃] = 2.0 mM, [**12**] = 1.0 μM, 0.5% DMSO in a 50 mM phthalate pH 4 buffer (1.02 mL), 25 °C, 1 h. *Protocol*: a 50 mM phthalate pH 4 buffer (20 mL) was dried under reduced pressure to obtain a solid in a test tube. River water (20 mL) was passed through a syringe filter and poured into the test tube to adjust the pH to 4. A 0, 62.5, 125, 250, 500, or 1000 ppb solution of HgCl₂ in 5% HNO₃ (4 μL) was spiked into each of the resulting river water samples (1.0 mL each). A 100 mM solution of AgNO₃ in ultrapure water (20 μL each) was added to each of these samples, and the resulting mixtures were vortexed for 5 s and centrifuged (1 min, 2000 rpm) to remove precipitates. A 0.1 mM solution of **12** in 1/1 DMSO/50 mM phosphate pH 8 buffer (10 μL) was added to each of these solutions,

and the resulting samples were shaken for 3 s and incubated at 25 °C for 1 h before fluorescence measurement. See Fluorescence Measurement for sample neutralization.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Text, tables, and figures giving experimental procedures for all fluorescence analyses and chemodosimeter preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*koide@pitt.edu

■ ACKNOWLEDGMENT

■ REFERENCES

(1) (a) Harada, M. *Crit. Rev. Toxicol.* **1995**, 25, 1–24. (b) Weiss, B. *Toxicol. Sci.* **2007**, 97, 223–225.

(2) (a) Amin-Zaki, L.; Elhassani, S.; Majeed, M. A.; Clarkson, T. W.; Doherty, R. A.; Greenwood, M. *Pediatrics* **1974**, 54, 587–595. (b) Bakir, F.; Damluji, S. F.; Amin-Zaki, L.; Murtadha, M.; Khalidi, A.; Al-Rawi, N. Y.; Tikriti, S.; Dhahir, H. I.; Clarkson, T. W.; Smith, J. C.; Doherty, R. A. *Science* **1973**, 181, 230–241.

(3) Atchison, W. D.; Hare, M. F. *FASEB J.* **1994**, 8, 622–629.

(4) Nierenberg, D. W.; Nordgren, R. E.; Chang, M. B.; Siegler, R. W.; Blayney, M. B.; Hochberg, F.; Toribara, T. Y.; Cernichiari, E.; Clarkson, T. *N. Engl. J. Med.* **1998**, 338, 1672–1676.

(5) To comply with a moratorium at our institution, this work does not involve the deliberate use of organic mercury compounds.

(6) Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. *Environ. Toxicol.* **2003**, 18, 149–175.

(7) (a) Bernard, S.; Enayati, A.; Redwood, L.; Roger, H.; Binstock, T. *Med. Hypotheses* **2001**, 56, 462–471. (b) Baker, J. P. *Am. J. Public Health* **2008**, 98, 244–253.

(8) (a) Davidson, P. W.; Myers, G. J.; Cox, C.; Axtell, C.; Shamlaye, C.; Sloane-Reeves, J.; Cernichiari, E.; Needham, L.; Choi, A.; Wang, Y.; Berlin, M.; Clarkson, T. W. *JAMA, J. Am. Med. Assoc.* **1998**, 280, 701–707. (b) Myers, G. J.; Davidson, P. W.; Cox, C.; Shamlaye, C. F.; Palumbo, D.; Cernichiari, E.; Sloane-Reeves, J.; Wilding, G. E.; Kost, J.; Huang, L.-S.; Clarkson, T. W. *Lancet* **2003**, 361, 1686–1692.

(9) (a) Sommar, J.; Gardfeldt, K.; Strömberg, D.; Feng, X. *Atmos. Environ.* **2001**, 35, 3049–3054. (b) Hall, B. *Water, Air, Soil Pollut.* **1995**, 80, 301–315.

(10) Hamdy, M. K.; Noyes, O. R. *Appl. Microbiol.* **1975**, 30, 424–432.

(11) Tomiyasu, T.; Okada, M.; Imura, R.; Sakamoto, H. *Sci. Total Environ.* **2003**, 304, 221–230.

(12) Erickson, B. E. *Chem. Eng. News* **2008**, 86, 47–49.

(13) United States Environmental Protection Agency, Clean air mercury rule, 40 CFR Parts 60, 63, 72, and 75, 2005.

(14) Solomon, G. M.; Janssen, S. *JAMA, J. Am. Med. Assoc.* **2010**, 304, 1118–1119.

(15) (a) Ghaedi, M.; Fathi, M. R.; Shokrollahi, A.; Shajarat, F. *Anal. Lett.* **2006**, 39, 1171–1185. (b) Taylor, A.; Branch, S.; Halls, D.; Patriarca, M.; White, M. *J. Anal. At. Spectrom.* **2002**, 17, 414–455.

(16) Beauchemin, D. *Anal. Chem.* **2008**, 80, 4455–4486.

(17) Hutchison, A. R.; Atwood, D. A. *J. Chem. Crystallogr.* **2003**, 33, 631–645.

(18) Thakur, J. S.; Prinja, S.; Singh, D.; Rajwanshi, A.; Prasad, R.; Parwana, H. K.; Kumar, R. *J. Epidemiol. Commun. Health* **2010**, *64*, 148–154.

(19) (a) Lee, M. H.; Lee, S. W.; Kim, S. H.; Kang, C.; Kim, J. S. *Org. Lett.* **2009**, *11*, 2101–2104. (b) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 5910–5918. (c) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030–16031. (d) Yoon, S.; Miller, E. W.; He, Q.; Do, P. H.; Chang, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 6658–6661.

(20) (a) Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443–3480. (b) Kumar, A.; Pandey, P. S. *Tetrahedron Lett.* **2009**, *50*, 5842–5845. (c) Lee, D. N.; Kim, G. J.; Kim, H. J. *Tetrahedron Lett.* **2009**, *50*, 4766–4768. (d) Loe-Mie, F.; Marchand, G.; Berthier, J.; Sarrut, N.; Pucheault, M.; Blanchard-Desce, M.; Vinet, F.; Vaultier, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 422–425. (e) Du, J. J.; Fan, J. L.; Peng, X. J.; Sun, P. P.; Wang, J. Y.; Li, H. L.; Sun, S. G. *Org. Lett.* **2010**, *12*, 476–479. (f) Fuertes, P.; Moreno, D.; Cuevas, J. V.; García-Valverde, M.; Torroba, T. *Chem. Asian J.* **2010**, *5*, 1692–1699. (g) Cho, Y. S.; Ahn, K. H. *Tetrahedron Lett.* **2010**, *51*, 3852–3854. (h) Hu, J.; Zhang, M.; Yu, L. B.; Ju, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4342–4345.

(21) (a) Zhang, J. F.; Kim, J. S. *Anal. Sci.* **2009**, *25*, 1271–1281. (b) Fan, J. L.; Guo, K. X.; Peng, X. J.; Du, J. J.; Wang, J. Y.; Sun, S. G.; Li, H. L. *Sensors Actuat. B-Chem.* **2009**, *142*, 191–196. (c) Kim, J. H.; Kim, H. J.; Kim, S. H.; Lee, J. H.; Do, J. H.; Kim, H. J.; Lee, J. H.; Kim, J. S. *Tetrahedron Lett.* **2009**, *50*, 5958–5961. (d) Atilgan, S.; Kutuk, I.; Ozdemir, T. *Tetrahedron Lett.* **2010**, *51*, 892–894. (e) Song, C. X.; Zhang, X. L.; Jia, C. Y.; Zhou, P.; Quan, X.; Duan, C. Y. *Talanta* **2010**, *81*, 643–649.

(22) Li, C. Y.; Zhang, X. B.; Qiao, L.; Zhao, Y.; He, C. M.; Huan, S. Y.; Lu, L. M.; Jian, L. X.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2009**, *81*, 9993–10001.

(23) United States Environmental Protection Agency, Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry, 2002.

(24) Morton, J.; Carolan, V. A.; Gardiner, P. H. E. *J. Anal. At. Spectrom.* **2002**, *17*, 377–381.

(25) Harris, H. H.; Pickering, I. J.; George, G. N. *Science* **2003**, *301*, 1203–1203.

(26) Song, F.; Watanabe, S.; Floreancig, P. E.; Koide, K. *J. Am. Chem. Soc.* **2008**, *130*, 16460–16461.

(27) United States Environmental Protection Agency, EPA 816-F-09-004, 2009.

(28) Gigg, J.; Gigg, R. *J. Chem. Soc. C* **1966**, 82–86.

(29) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903–1911.

(30) Song, F.; Garner, A. L.; Koide, K. *J. Am. Chem. Soc.* **2007**, *129*, 12354–12355.

(31) (a) Garner, A. L.; Koide, K. *Chem. Commun.* **2009**, 83–85. (b) Garner, A. L.; Koide, K. *Chem. Commun.* **2009**, 86–88. (c) Garner, A. L.; Song, F.; Koide, K. *J. Am. Chem. Soc.* **2009**, *130*, 5163–5171. (d) Garner, A. L.; St Croix, C. M.; Pitt, B. R.; Leikauf, G. D.; Ando, S.; Koide, K. *Nat. Chem.* **2009**, *1*, 316–321. (e) Song, F.; Carder, E. J.; Kohler, C. C.; Koide, K. *Chem. Eur. J.* **2010**, *16*, 13500–13508.

(32) Sparano, B. A.; Shahi, S. P.; Koide, K. *Org. Lett.* **2004**, *6*, 1947–1949.

(33) Koide, K.; Song, F.; de Groh, E. D.; Garner, A. L.; Mitchell, V. D.; Davidson, L. A.; Hukriede, N. A. *ChemBioChem* **2008**, *9*, 214–218.

(34) Prosser, T. J. *J. Am. Chem. Soc.* **1961**, *83*, 1701–1704.

(35) Morse, H. *Z. Phys. Chem.* **1902**, *41*, 709–734.

(36) Taken from the Allegheny River, Pittsburgh, PA. The mercury content was determined by cold vapor atomic absorption (Department of Environmental Protection, Bureau of Laboratories, Harrisburg, PA). We thank Dr. Taru Upadhyay for this analysis.

(37) Allen, T. L. *J. Chem. Phys.* **1957**, *26*, 1644–1647.

(38) Forbes, G. S.; Cole, H. I. *J. Am. Chem. Soc.* **1921**, *43*, 2492–2497.

(39) Zhang, F. S.; Nriagu, J. O.; Itoh, H. *Water Res.* **2005**, *39*, 389–395.

(40) Ritter, J. A.; Bibler, J. P. *Water Sci. Technol.* **1992**, *25*, 165–172.

(41) Actual mercury-contaminated discharged water samples could not be obtained from an environmental agency, presumably due to the safety for shipping.

(42) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211.

(43) Although the signals of samples with Hg(II) were lower than expected, it should be noted that organic compounds spiked with Hg(II) did not affect the reaction. A higher concentration of DMSO (1.3% in the reaction solutions; 26 times the concentration of the general method that we used in this study) seems to be the reason for these lower signals.

(44) MacDougall, D.; et al. *Anal. Chem.* **1980**, *52*, 2242–2249.

(45) Santra, M.; Ryu, D.; Chatterjee, A.; Ko, S. K.; Shin, I.; Ahn, K. H. *Chem. Commun.* **2009**, 2115–2117.

(46) Lavis, L. D.; Rutkoski, T. J.; Raines, R. T. *Anal. Chem.* **2007**, *79*, 6775–6782.

(47) Arata, M.; Miura, T.; Chiba, K. *Org. Lett.* **2007**, *9*, 4347–4350.

(48) Ei-Shishtawy, R. M.; Almeida, P. *Tetrahedron* **2006**, *62*, 7793–7798.