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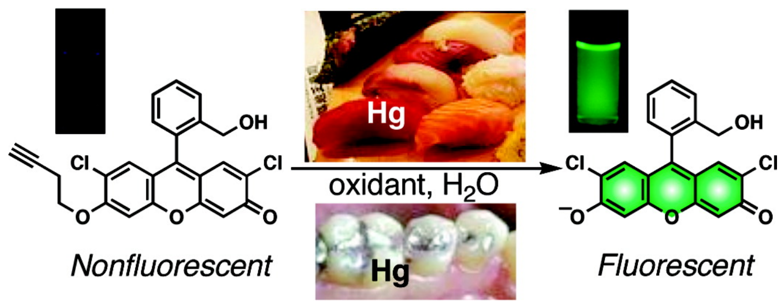

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Oxidation-Resistant Fluorogenic Probe for Mercury Based on Alkyne Oxymercuration

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Recent years have witnessed the development of many fluorescence methods to detect mercury ion[1] because mercury continues to be a major environmental and health concern. While some probes for Hg^{2+} are based on the coordination of multiple nitrogen atoms with the metal ion, the majority of probes for Hg^{2+} are based on extremely strong Hg−S binding. Despite the development of these probes, applications with real-life samples are rare.[2] Potential drawbacks of these probes may be 3-fold: (1) undesired air-oxidation of amines and particularly sulfides during long-term storage at ambient temperature; (2) undesired oxidation of these functional groups by oxidizing agents (e.g., Cl−Br,[3] H_2O_2[4]) that are used to convert $MeHg^+$ to Hg^{2+}; and (3) possible lack of mercury detection in sulfur-rich environments where mercury is abundant.[5] Here we describe a new methodology for mercury detection based on the reactivity of Hg^{2+} with alkynes. This method addresses the aforementioned three concerns and could be applied to detection of mercury in biological samples such as dental and fish samples.

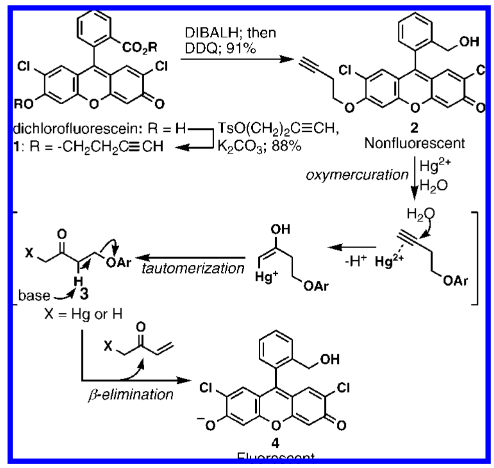
We hypothesized that if Hg^{2+} promotes the cleavage of the fluorescence-masking alkyl group from **2** to afford **4** (Scheme 1), such a system could be used for Hg^{2+} detection. It is known that Hg^{2+} catalyzes hydration of alkynes to form the corresponding ketones,[6] and rigorous kinetic studies were performed for this transformation,[7] although the detailed mechanism is not well understood.[6] On the basis of this chemistry in combination with a β-elimination process (**3** to **4**), we have designed and synthesized compound **2** in two steps from commercially available 2′,7′-dichlorofluorescein in 80% yield for the two steps. The fluorescence of **2** was found to be 219 times weaker than that of **4** (Supporting Information, Figure S1 and Table S1).

Acids promote the turnover frequency of the mercury-catalyzed hydration but may also convert **2** to **4** directly through ether cleavage. To suppress the acid-catalyzed ether cleavage in the absence of Hg^{2+}, the conversion of **2** to **4** was carried out by heating **2** and Hg^{2+} (1 equiv) in pH 7 buffer at 90 °C; although compound **3** could not be isolated presumably because the elimination step is faster than oxymercuration, compound **4** was isolated in 61% yield,[8] and methyl vinyl ketone was detected by HPLC as an indirect evidence for the intermediacy of **3** (Figure S2).[9] Further analyses of the conversion of **2** to **4** are shown in Figures S3 and S4.

It was found that for a low ppb range, good sensitivity was obtained with a low concentration of **2** (0.1 μM) in pure water (Figure 1a; signal-to-background (S/B) ratio = 3 at 8 ppb Hg^{2+}) or in pH 7 buffer (Figure S5) both at 90 °C. For environmentally relevant, higher mercury concentrations, a better correlation between [Hg^{2+}] and fluorescence intensity was obtained when [**2**] was 1 μM (Figure 1b). A kinetic experiment (Figure 1c) showed that 1 h incubation gave a near-optimal S/B ratio.

To determine the metal specificity, we subjected **2** (1 μM) to a mixture of Hg^{2+} (2.5 μM = 0.5 ppm) and each of the metals (25 μM) as shown in Figure 1d. The deviations from other metals'

Scheme 1. Preparation of Probe **2** and its Oxymercuration-Elimination to Form **4**



interference are less than 8% in the coexisting metal experiment, strongly indicating that this method can be used in metal mixtures for mercury detection. Although a mixture of Hg^{2+} and Pb^{2+} gave a slightly stronger signal, Pb^{2+} by itself did not enhance fluorescence (Figure S6). As shown in Figure 1e, even in the presence of cysteine (10 μM), which is known to form stable complexes with mercury,[10] probe **2** was responsive to mercury by virtue of the strong oxidant NCS (100 μM).[11] This example shows one of the advantages of this probe, that is, being resistant to oxidation, and indicates that this fluorescence method may be used in combination with mercury extraction from solid materials, including fish[12] with cysteine,[13] as a streamlined extraction-analysis procedure.

Next, a salmon tissue was dissolved using Me_4NOH,[14] and the resulting solution was treated with **2** and NCS in pH 7 buffer (Figure 2a). This safe and HNO_3-free procedure produced a strong fluorescence signal, suggesting that this method could be used to monitor mercury concentrations in fish and potentially in other tissues.[15] Since 95% of mercury species exist as $MeHg^+$ in fish, and the mercury detection required NCS, it is reasonable to postulate that this method is capable of detecting this notoriously toxic mercury species after conversion to Hg^{2+}.

Major components of dental amalgam are mercury (50%) and silver (30−35%), thus raising concerns about leached mercury.[16] A fluorescent method that could be used outside of laboratories would be very useful in monitoring the quality of dental amalgam. A piece of Kimwipe soaked with saliva was pressed on an amalgam-filled tooth for 1 min, and the resulting Kimwipe was subjected to **2**. The fluorescence signal from this sample was significantly stronger than that from Kimwipe with saliva not pressed on a tooth (Figure 2b), showing that our method may be applied to the detection of leached mercury from dental amalgam. We also stirred a solution of cysteine with two amalgam-filled teeth in a flask at

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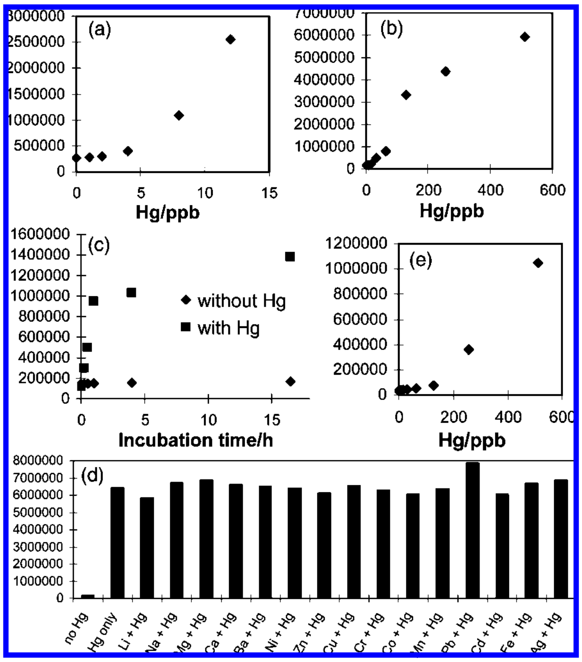

Figure 1. All reactions were performed in pH 7 phosphate buffer (except for panel a in pure water) at 90−100 °C for 1 h; *y*-axis, fluorescence intensity (au) at 523 nm. (a) Low [Hg^{2+}] detection. [**2**] = 0.1 μM. (b) High [Hg^{2+}] detection. [**2**] = 1.0 μM. (c) Kinetic study. [**2**] = 1.0 μM. [Hg^{2+}] = 0.2 μM (= 40 ppb). (d) Metal specificity. [**2**] = 1.0 μM. [Hg^{2+}] = 2.5 μM, [other metal] = 25 μM. Metal reagents: LiCl, NaCl, $MgCl_2$, $CaCl_2$, $BaCl_2$, $NiCl_2$, $ZnCl_2$, $CuCl_2$, $CrCl_3$, $CoCl_2$, $MnCl_2$, $Pb(NO_3)_2$, $CdCl_2$, $FeCl_3$, and $AgNO_3$. The average S/B ratio of the "Hg + metal" is 101% of that of "Hg only" with a standard deviation of 7.3%. (e) High [Hg^{2+}] detection in the presence of L-cysteine (10 μM). [**2**] = 0.10 μM, [NCS] = 100 μM.

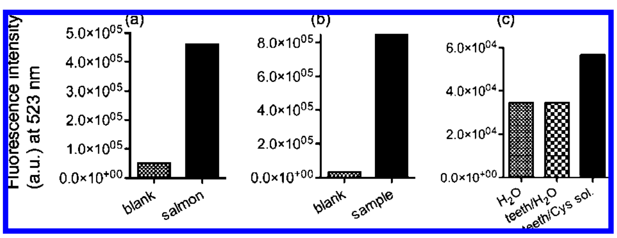

Figure 2. (a) Salmon tissue shows the presence of Hg. (b) "blank" = Kimwipe with saliva; "sample" = Kimwipe with saliva, which was pressed on a tooth filled with dental amalgam. (c) Rinsing teeth filled with dental amalgam using aqueous cysteine solution extracts Hg into solution.

35 °C for 1 h (to mimic eating sulfur-rich food) and treated the resulting solution with **2** and NCS. The presence of mercury in the solution was indicated as shown in Figure 2c, implying that mercury leaching from amalgam fillings caused by sulfur-rich food may be monitored by our method.

In summary, we have developed a sensitive and specific fluorogenic probe for mercury, and the probe is compatible with strong oxidants such as NCS. This compatibility is crucial because most mercury samples contain oxidants. In this work, the π-philicity of Hg^{2+} toward alkynes was used for the first time to develop fluorogenic probes for this metal.

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Supporting Information Available: Experimental procedures for all fluorescence analyses and compound preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

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