



A Fluorescent coumarinylalkyne probe for the selective detection of mercury(II) ion in water

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ABSTRACT

A coumarin-based alkyne was developed as a fluorescent chemodosimeter for the selective detection of mercury ion. It showed a high selectivity and sensitivity to mercury(II) ion over other metal ions in water.

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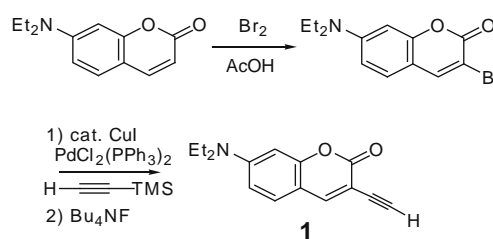
Fluorescent sensors for heavy metals such as mercury, cadmium and lead are of growing interest due to their bioaccumulation, bio-magnification, and persistence in the environment. Especially mercury ion detection by optical probes is a rapidly growing area.¹ Bacteria-mediated organomercury accumulates in higher organisms and has been implicated as a cause of prenatal brain damage,² various cognitive and motion disorders,³ or Minamata disease.⁴ Therefore, it is of considerable importance to sense the lethal mercury ion with a cost-effective, facile, and biologically applicable detection method.

Over the past years, researchers have tried to develop efficient, selective, and sensitive mercury ion probes based on fluorescent small molecules.⁵ Most of them, however, were operating in an excess or at least an equivalent amount of mercury ion.⁶ To the best of our knowledge, there are only a few fluorescent probes known, which modulate the fluorescence intensities significantly in the presence of catalytic or substoichiometric amounts of mercuric ions.⁷ Koide and co-workers recently developed a fluorescent chemodosimeter for mercuric ion using a fluorescein alkyne.^{7c} Herein, we report a luminescent probe operating in the presence of substoichiometric amounts of mercuric ions based on a fluorescent coumarin-derived alkyne.

It is well known as Kucherov reaction that alkynes reacts with water in the presence of catalytic amount of mercury(II) ion to give an enol, which tautomerizes to ketone.^{7,8} To visually sense a mercuric ion in water, a coumarin-derived alkyne (**1**) was prepared as a fluorescent chemodosimeter according to the modified literature procedure (Scheme 1).⁹ It was expected that the coumarin unit would operate as a signaling unit whereas the acetylene as a reaction unit. Treatment of 7-diethylamino-coumarin with bromine in acetic acid gave rise to the 3-bromo-7-diethylamino-coumarin. An acetylene functional group of **1** was introduced through the Sonogashira reaction of 3-bromo-7-diethylaminocoumarin with tri-

methylsilylacetylene in the presence of catalytic amount of PdCl₂(PPh₃)₂ and CuI. Desilylation of the intermediate with tetrabutyl-ammonium fluoride afforded the desired product **1** as a yellow solid in 28% yield over 3 steps.

Chemical reaction of **1** with water in the presence of mercuric ion was monitored by ¹H NMR spectroscopy. Upon the addition of aqueous mercury(II) ion to **1** in DMSO-*d*₆, the acetylenic proton



Scheme 1. The synthesis of probe **1**.

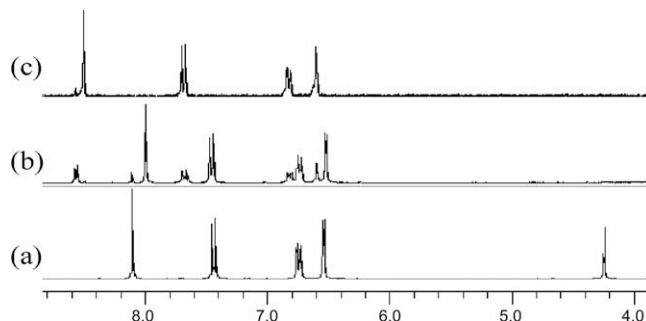


Figure 1. Partial ¹H NMR spectra of **1** (20 mM in DMSO-*d*₆/D₂O) upon the addition of HgCl₂ (5.0 equiv) at 25 °C. (a) **1**, (b) after 6.8 min, (c) after 1.4 h, treated with NaOH (10 equiv).

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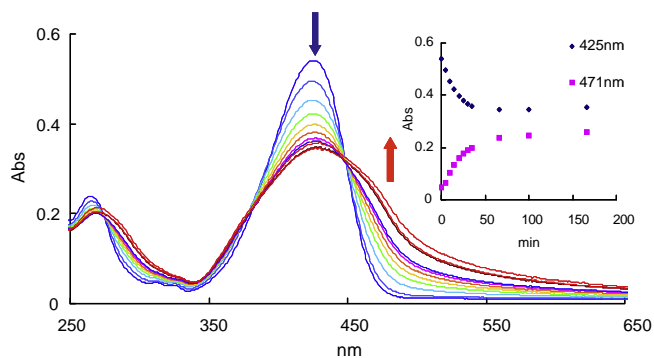


Figure 2. Time-dependent UV-vis spectral change upon the addition of 0.5 equiv of Hg^{2+} to **1** (30 μM , pH 7.4 PBS buffer).

at 4.24 ppm disappeared while another simple set of aromatic protons were appearing (Fig. 1), which was consistent with the 3-acyl-7-diethylamino-coumarin (Ref) (Fig. S1 in Supplementary data). The chemical reaction was also confirmed by UV-vis spectroscopy (Fig. 2). Absorbance of **1** at λ 425 nm decreased while the absorbance at λ 471 nm was increasing with a pseudoisobestic point at λ 450 nm upon the addition of mercury(II) ion in PBS buffer (pH 7.4). A bathochromic shift was observed as the acetylene functionality was converted to the more electron-withdrawing carbonyl group. The chemical conversion is so fast that the reaction is complete within 1 h at 25 °C under a high dilution condition of reactants and substoichiometric amount of Hg^{2+} ($[\mathbf{1}] = 30 \mu\text{M}$, $\text{Hg}(\text{OAc})_2 = 0.5$ equiv, Fig. 2 inset).

The fluorescence intensity of **1** at λ_{em} 488 nm decreased completely upon the addition of 0.5 equiv of Hg^{2+} ion to **1** (30 μM) in water (PBS buffer at pH 7.4),¹⁰ whereas no significant changes were observed in the presence of other metal ions in excess such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Al^{3+} , Mn^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , and Ag^+ ions (5.0 equiv each). In contrast to Hg^{2+} ion, other heavy metal ions such as Pb^{2+} and Cd^{2+} ions do not induce any detectable fluorescence changes either (Fig. 3). Selectivity factor, defined as F_0/F , for mercuric ion was calculated to be more than 13 while the factor for other metals was less than 2.0, which are comparable to probe **1**. Of note is the observed selectivity for Hg^{2+} over Cu^{2+} and Pb^{2+} , major competitors in real-life field samples.

Competitive experiments in the presence of excess of other metals (5.0 equiv) also corroborated the mercuric ion (0.5 equiv) selectivity of **1** (Fig. S2).

The fluorescence response of the chemodosimeter was also detectable by the naked eye using a UV lamp (λ_{ex} 365 nm,

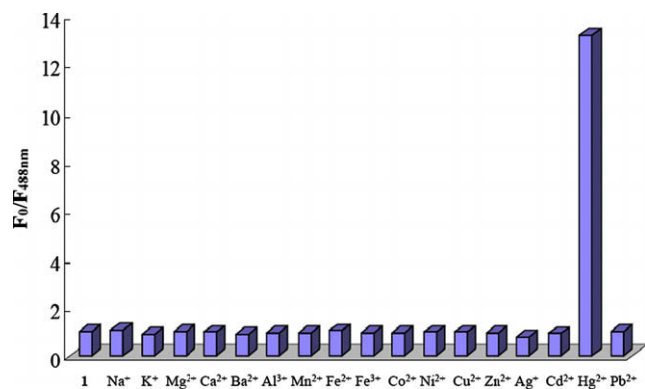


Figure 3. Metal ion selectivity of **1** (30 μM , PBS pH 7.4) in the presence of various metals (each in 5.0 equiv except 0.5 equiv of Hg^{2+}), where λ_{ex} at 425 nm and λ_{em} at 488 nm.

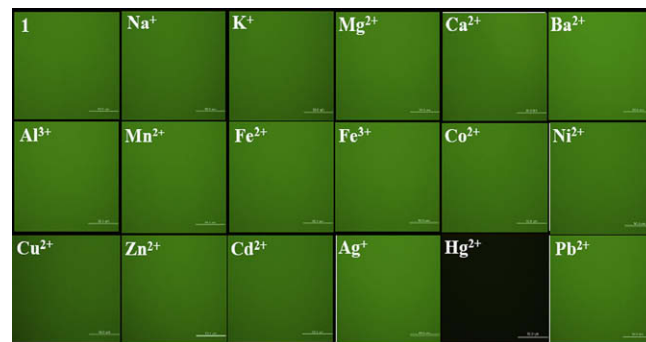


Figure 4. Fluorescence microscopic images of **1** (30 μM , PBS pH 7.4) in the presence of various metals (each in 5.0 equiv except 0.5 equiv of Hg^{2+}). Scale bar 50 μm .

Fig. S3) or a fluorescence microscope (λ_{ex} band 450–480 nm, λ_{em} 500 nm). The obvious fluorescence decrease was prominently observed only for mercury ion. Most other metal ions did not show any significant fluorescence changes but almost the same intensity as **1** itself (Fig. 4).

The conversion of an acetylene to ketone is well known to proceed in the presence of catalytic amounts of mercury ions in water.⁸ Motivated by the catalytic role of mercuric ions in the reaction, we tried to determine the limit of mercury ion concentration enough to cause a detectable fluorescence change of **1**. The concentration of Hg^{2+} ion was decreased from 5.0 μM to 1.0 nM. As low as 1.2 μM of mercuric ions, $[\text{Hg}^{2+}]/[\mathbf{1}] = 0.6$, has completely quenched the fluorescence of **1** (2.0 μM) in water (PBS pH 7.4) (Fig. 5). According to the sensitivity curve, less than 0.24 ppm of mercuric ions is enough to monitor the complete quenching of the fluorescence of **1**. This indicates probe **1** can be utilized not only to the qualitative analysis of Hg^{2+} ions using a UV lamp or a fluorescence microscope but also to the quantitative analysis of micromolar concentration of Hg^{2+} ions in water to enable an application for assaying fish for safe human consumption.¹¹

From the observed results, we propose the fluorescence sensing mechanism of **1**, which is specific to mercury(II) ion. Mercuric ion catalyzes the conversion reaction from an alkyne to ketone in water (Fig. 6). Mercury(II)-promoted hydration of **1** through an enol-keto tautomerization may lead to the stable keto product (Ref). The fluorescence intensity of **1** decrease as the reaction proceeds due to the possible transition of the coumarin HOMO electron to the carbonyl orbital in LUMO upon the excitation of coumarin chromophore.¹²

In summary, we have developed a novel fluorescent chemodosimeter, based on a fluorescent coumarin-derived alkyne. The alkyne probe reacted with water in the presence of mercuric ion and displayed a sensitive fluorescence response to substoichiomet-

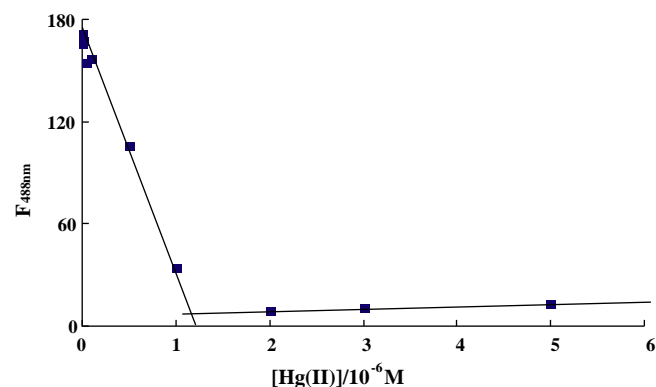


Figure 5. Mercuric ion sensitivity of **1** (2.0 μM , PBS pH 7.4).

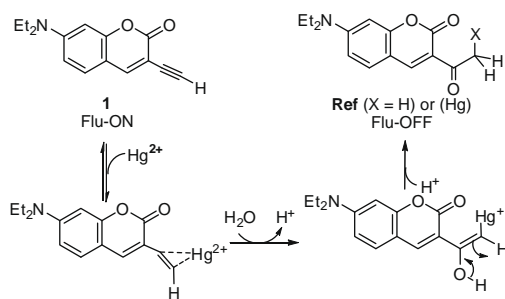


Figure 6. A proposed mechanism.

ric amounts of mercury(II) ions in a biological condition (PBS buffer at pH 7.4).

Acknowledgments

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Supplementary data

Supplementary data (experimental procedure and selected spectral data (NMR, MS, fluorescence spectra) for **1**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.017.

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- Though the mercury-mediated hydration of an alkyne is well known to occur in an acidic solution, the fluorescence intensity of **1** was very weak in the acidic condition and its change was negligible upon the addition of $\text{Hg}(\text{II})$ to **1**. Significant fluorescence changes could be observed at the pH range between 7 and 9 (Fig. S4).
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