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Nanomolar Hg(II) Detection Using Nile Blue Chemodosimeter in Biological Media

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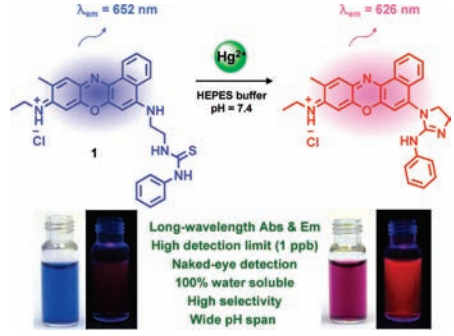
ABSTRACT



A Nile blue-based chemodosimeter (1) was newly synthesized, and its application for detection of the Hg^{2+} ion in 100% aqueous solution was demonstrated. Upon its addition into aqueous Hg^{2+} ion solution, it exhibited a considerable blue-shift in its absorption and emission spectra, driven by a desulfurization reaction. Detection at an emission of 652 nm was extremely sensitive (less than 1.0 ppb), even in biological media such as blood plasma and albumin.

Mercury has been known to be a toxic and dangerous element to human beings when ingested or inhaled.[1,2] Thus, methods for selective and sensitive detection of mercury species in biological samples have received a great deal of attention lately.

As a potential sensing system, the chemodosimeter has garnered recent attention due to its ability to selectively perform a chemical reaction with a specific metal ion to give a unique spectroscopic change.[3] Indeed, many fluorescent chemodosimeters for Hg^{2+} ion-selective detection have been designed on the basis of the mercury-desulfurization reaction.[4] Although these efforts have improved mercury chemodosimeters regarding metal ion selectivity, long-range excitation and emission wavelengths, and high quantum yield,

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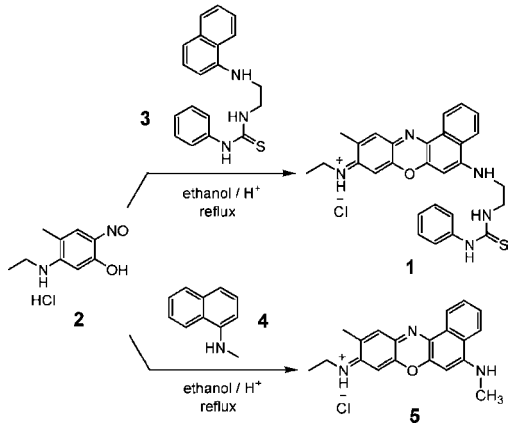
their application toward biological samples are hampered by poor solubility in water and modest sensitivities.

With such problems at the forefront, we have focused on the synthesis of a new derivative of Nile blue (**1**) and investigated its chemodosimetric properties that can provide sensitive measurement of the Hg^{2+} ion in a 100% aqueous environment. Compound **1** absorbs and emits lights at 630 and 652 nm, respectively, with a high quantum yield. In addition, and more importantly, its chloride salt shows an excellent water-solubility and intense fluorescence, even in aqueous media.[5] Its reaction with the Hg^{2+} ion induces a desulfurization-based cyclization followed by an absorption and emission shift of **1**. As such, the Nile blue derivative (**1**) is a good candidate for the Hg(II) chemodosimeter. For demonstration of its application in biological samples, various experiments using BSA (bovine serum albumin) and human serum were implemented and discussed.

As depicted in Scheme 1, Nile blue derivative (**1**) was prepared in ~90% yield from the reaction of 5-(ethylamino)-4-methyl-2-nitrosophenol (**2**) with naphthalene derivative (**3**) in ethanol/H^+ solution. To gain insight into the role of the *N*-phenylthiourea unit in the Hg^{2+} chemodosimetric detection, a reference compound (**5**) lacking the dosimetric portion was synthesized in high yield (90%) from the reaction of **2** with **4**. The identities of all synthetic compounds were fullly confirmed by ^1H NMR, ^{13}C NMR, and FAB-MS in Figures S10−S18 (Supporting Information).

Scheme 1. Synthetic Routes to **1** and **5**



A stock solution of **1** was prepared in 100% distilled water, and all photochemical experiments were carried out in 10 mM HEPES buffer at pH 7.4 without any organic solvent. Parts a and b of Figure 1 show absorption and fluorescence spectra of **1** upon addition of 5.0 equiv of various metal ions. Compound **1** revealed two absorption maxima at 630 nm ($\varepsilon = 1.9 \times 10^4\ M^{-1}\ cm^{-1}$) and 592 nm ($\varepsilon = 1.6 \times 10^4\ M^{-1}\ cm^{-1}$). Upon addition of the Hg^{2+} ion, the maxima were shifted to 583 nm ($\varepsilon = 1.2 \times 10^4\ M^{-1}\ cm^{-1}$) and 546 nm ($\varepsilon = 1.6 \times 10^4\ M^{-1}\ cm^{-1}$), respectively (Figure 1a). In the fluorescence spectra of **1**, an obvious blue shift from 652 nm ($\Phi_F = 0.20$) to 626 nm ($\Phi_F = 0.35$), with an intensity increase, was observed only in the presence of Hg^{2+} ions (Figure 1b).[6] Figure S1 (Supporting Information) gives detailed UV/vis and fluorescence changes of **1** as a function of [Hg^{2+}]. Upon increasing [Hg^{2+}], the absorption bands of **1** decreased gradually, and new absorption bands attributable to the Hg^{2+}-induced cyclization concomitantly increased (Figure S1a, Supporting Information). Similar results are also observed in the fluorescence titration spectra (Figure S1b, Supporting Information). For further photophysical studies, fluorescence experiments of **1** were performed with an excitation at 610 nm (Figure S2, Supporting Information). In addition, the reaction responsible for these changes reaches completion within the time frame (<1 min) of these measurements.

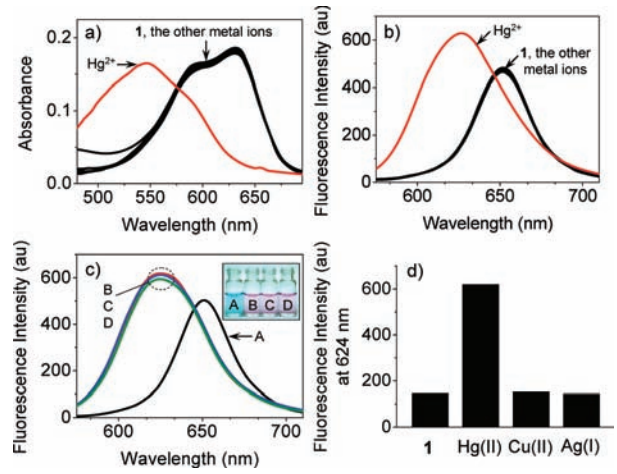


Figure 1. (a) Absorption and (b) fluorescence spectra of **1** (10.0 and 5.0 μM, respectively) upon addition of Cl^- salts of K^+, Na^+, Hg^{2+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, and Fe^{2+} (5.0 equiv, respectively) in HEPES (pH = 7.4) buffer at room temperature, $\lambda_{ex} = 540$ nm. (c) Metal ion selectivity of **1** in the presence of various metal cations: (A) **1**; (B) **1** + Hg^{2+} (25 μM); (C) **1** + Hg^{2+} (25.0 μM) + other metal ions ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, 1.0 mM, respectively); (D) **1** + Hg^{2+} (25.0 μM) + other metal ions ($Zn^{2+} + Cd^{2+} + Co^{2+} + Fe^{2+} + Ba^{2+} + Ni^{2+}$, 25.0 μM, respectively). Inset: color changes of A−D. (d) Fluorescence response of **1** at 624 nm in the presence of thiophilic metal cations.

The results of cation-competitive experiments are depicted in Figure 1c. We found that the selectivity and sensitivity of **1** toward Hg^{2+} are not influenced by biologically active metal ions such as highly concentrated Na^+, K^+, Ca^{2+}, and Mg^{2+} (1.0 mM, 200 equiv). Compound **1** retains a high Hg^{2+}-selective chemodosimetric response, even under polluted conditions containing heavy- and transition-metal cations such as Zn^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Ba^{2+}, and Ni^{2+} (25.0 μM of each). Moreover, **1** can detect Hg^{2+} in the "naked-eye"

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manner, even in the presence of other miscellaneous cations (see Figure 3c inset). For the Hg^{2+}-induced desulfurization chemodosimetric mechanism, the reactivity of **1** toward other thiophilic metal cations (Cu^{2+} and Ag^{+}) was also investigated by measuring fluorescence intensities centered at 624 nm; no fluorescence changes occurred in **1** (Figure 1d).

To examine the sensitivity of **1** for Hg^{2+} sensing, its detection limit is also depicted in Figure 2. The fluorescence

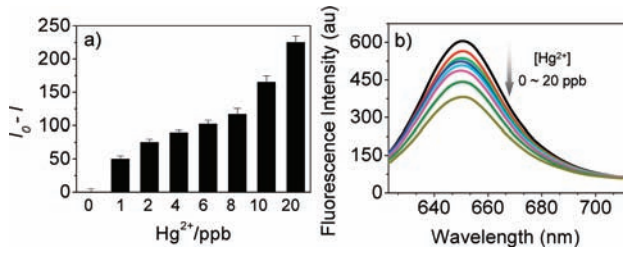


Figure 2. (a) Fluorescence responses (at 652 nm) and (b) spectral changes of **1** (5.0 μM) to Hg^{2+} ions (0−20.0 ppb) in HEPES buffer (pH = 7.4) at room temperature, λ_{ex} = 610 nm. Error bars represent sd, n = 4.

titration profile of **1** versus [Hg^{2+}] clearly demonstrates that **1** can respond up to ~1.0 ppb level of the aqueous Hg^{2+} ion, indicating that the detection limit would be well below the limited level of Hg^{2+} ion in drinking water regulated by the U.S. EPA (2.0 ppb).[7]

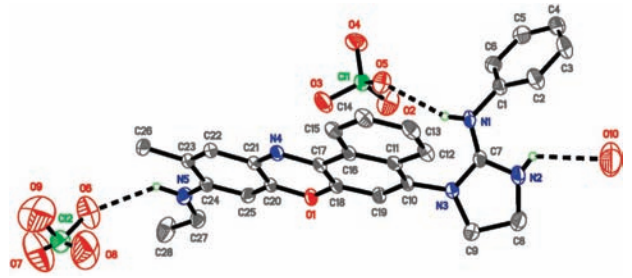
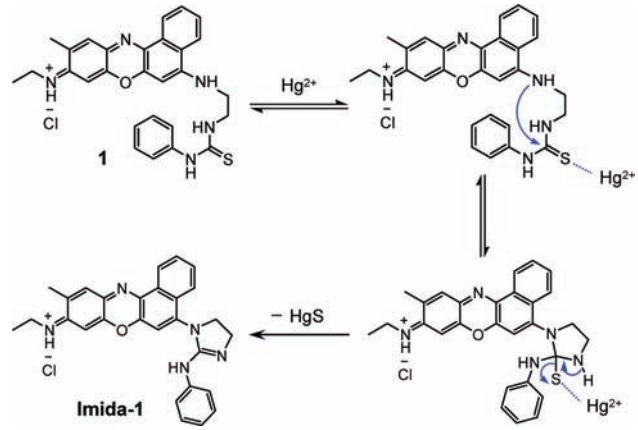


Figure 3. X-ray crystal structure of [**Imida-1**H]$(ClO_4)_2$·(H_2O). Displacement ellipsoids are scaled to a 6.0% probability level. Most of the hydrogen atoms have been removed for clarity.

To obtain insight into the dosimetric portion in **1** responsible for Hg^{2+} ion selectivity, **5**, lacking the *N*-phenylthiourea unit, was investigated as a reference. No changes in either the absorption or emission spectra of **5** upon addition of various metal ions, including the Hg^{2+} ion, were noted, implicating that the *N*-phenylthiourea group of **1** plays an important role in controlling the dosimetric event for Hg^{2+} (Figure S3, Supporting Information). In addition, spectral changes using **3**, having a naphthalene unit, were tested in the presence of various metal ions. Like **1**, **3** shows a blue-shift of its absorption and fluorescence spectra upon addition of the Hg^{2+} ion, attributable to Hg^{2+}-induced desulfurization to give an imidazoline product (**Imida-3**) (Figure S4, Supporting Information). The identity of **Imida-3** was confirmed by FAB-MS, showing peaks at m/z 287.6 [**Imida-3**]$^+$ and 288.7 (base peak, [**Imida-3**+H]$^+$) (Figure S5, Supporting Information).

From the above observations, it is certain that the thiophilic Hg^{2+} induces a cyclization of **1** followed by desulfurization to give the imidazoline derivative (**Imida-1**),[4,8] which suppresses the intramolecular charge transfer of **1** to produce significant absorption and emission spectral changes (Scheme 2).[9]

Scheme 2. Chemodosimetric Reaction Mechanism of **1** upon Hg^{2+} Ion Addition



Moreover, we obtained a crystal structure of **Imida-1** (Figure 3).[10] The N−H hydrogen atoms are involved in the N−H···O (ClO_4^- or H_2O) hydrogen bonding. The phenyl (C1−C6) and imidazoline (N2, N3, and C7−C9) moieties are significantly twisted from the molecular plane (O1, N4, and C10−C25) with the dihedral angles of 76.5(1)° and 66.5(1)°, respectively. Additionally, the crystal of **Imida-1** was dissolved in water to compare its absorption and fluorescence spectra with those of **1** + Hg^{2+} ion. We then found that the absorption and fluorescence spectra of **Imida-1** and **1** + Hg^{2+} are identical, which indicated that Hg^{2+} induced desulfurization to afford **Imida-1** (Figure S6, Supporting Information).

To address the possibility of interference from other chelating chemicals for the Hg^{2+} ion-mediated desulfurization of **1**, EDTA was added to the solution of **1** in the presence

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(10) Purple single crystals were grown from CH_3CN/H_2O. Details on crystal data such as intensity collection, refinement, selected bond lengths, and bond angles are given in Tables S1 and S2 (Supporting Information). The CCDC number is CCDC 706346.

of Hg^{2+} ions. The resulting blue-shifted absorption bands of **1** caused by Hg^{2+} show no absorption changes upon addition of highly concentrated EDTA (9.0 mM), demonstrating that unlike other chemosensors, chemodosimeter **1** proceeds an irreversible chemical reaction upon addition of the Hg^{2+} (Figure S7, Supporting Information).

Absorption and emission spectral changes of **1** as a function of pH were examined in aqueous solutions (Figure S8, Supporting Information). Figure 4 shows fluorescence

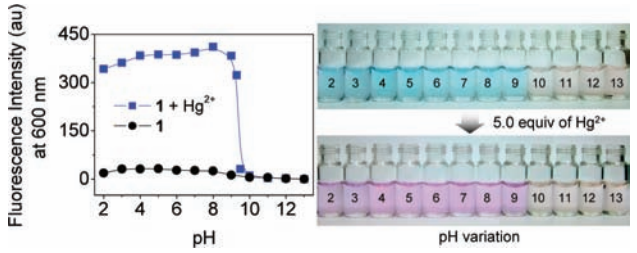

Figure 4. Variation in fluorescence intensity of **1** (5.0 μM) in aqueous solutions with and without Hg^{2+} (5.0 equiv), as a function of pH at λ_{ex} = 540 nm.

intensity changes at 600 nm within a pH range of 2−9, within which most biological samples can be tested. At pH over 9, deprotonation of the ammonium group in Nile blue occurs and leads to a fluorescence quenching.

For further practical usage, we have investigated **1** for detecting the Hg^{2+} ion in deproteinized blood plasma contaminated with 5.0 μM of Hg^{2+} ion. We then observed the emission band of **1** immediately blue-shifted, firmly indicating that **1** is capable of detecting Hg^{2+} ion in human blood samples at submicromolar concentrations (Figure 5).

Conversely, Hg^{2+} is well-known bind to albumin to form a Hg^{2+}−albumin complex.[11] However, even with highly concentrated bovine serum albumin (BSA) (0.1 mg/mL), the most abundant plasma protein in human blood serum, fluorescence of **1** also selectively changed in the presence of the Hg^{2+} ion (Figure S9, Supporting Information). These

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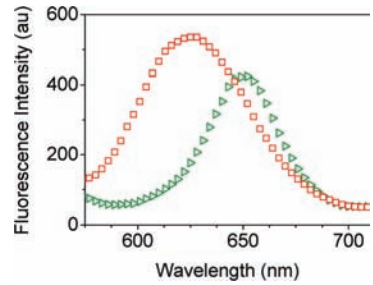

Figure 5. Fluorescence spectra of **1** (5.0 μM) in the presence (red) and absence (green) of Hg^{2+} (5.0 equiv) in 10.0% human blood plasma in HEPES (pH = 7.4) buffer, λ_{ex} = 540 nm.

results give a solid evidence that compound **1** is a potential chemodosimeter with selective and sensitive detection of the Hg^{2+} ion in biological samples.

In conclusion, we report the first synthesis and characterization of the chemodosimetric properties of a 100% water-soluble Nile blue derivative (**1**). It is a highly efficient colorimetric and fluorimetric sensor for Hg^{2+} ions within a pH range of 2−9 at room temperature. In particular, **1** exhibits a high selectivity toward Hg^{2+} and is not disrupted by the presence of other metal ions, with a response to the Hg^{2+} ions that is immediate and highly sensitive (less than 1.0 ppb). Furthermore, the selectivity and sensitivity of **1** toward the Hg^{2+} ion were also confirmed with blood plasma and albumin samples. Hence, synthesis of **1** and its selective and sensitive Hg^{2+} detection in aqueous systems can give a potential guideline in biological or environmental application-oriented chemodosimeter research.

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Supporting Information Available: Synthetic details, NMR copies, X-ray crystal data (CIF), and additional spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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