

Hg²⁺-Induced hydrolysis-based selective fluorescent chemodosimeter

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This paper is dedicated with respect and affection to Professor Richard A. Bartsch, an inspiring teacher and mentor, for his contributions in the field of organic chemistry, as well as his 70th birthday celebration

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Abstract

A new chemodosimeter-based approach to the detection of Hg(II) ions is described, involving the analyte-induced fluorescence enhancement of iminopyrene derivatives. The chemical and photo-physical properties of the chemodosimetric irreversible reaction between **1** and the Hg(II) ion were studied in a solution of CH₃CN/H₂O using ¹H-NMR, UV/Vis, and fluorescence spectroscopies. Among the iminopyrenes **1-3**, compound **1** shows a selective change in UV/Vis absorption, fluorescence emission, and color towards the Hg(II) cation over other cations in CH₃CN:H₂O (9:1).

Keywords: Mercuric ion, imine, pyrene, hydrolysis, chemodosimeter

Introduction

Recently, much attention has been paid to the use of chemodosimeters as chemical sensors through a specific irreversible chemical reaction between dosimetric molecules and target species, leading to a fluorescent/color change in the receptor.^{1,2} One of the equally attractive approaches in this field involves the use of highly selective reactions (usually irreversible) induced by target analytes, in which an accumulative effect is directly related to analyte concentration.

Chemodosimeters directed toward detection and measurement of Hg(II) are of particular interest as mercury is the third most frequently found and second most common toxic heavy metal

in the list of the Agency for Toxic Substances and Disease Registry (ATSDR) of the Department of Health and Human Services in the United States.^{3,4} Mercury contamination is widespread and arises from a variety of natural sources.⁵ Once introduced into the marine environment, bacteria convert inorganic Hg^{2+} ions into methylmercury, which is neurotoxic and has been implicated as a cause of mercury pollution related to serious irreversible neurological damage.⁶ Accordingly, it is exigent to provide analytical methods for the sensitive and selective determination of the mercury ion. In addition, several chemodosimeters have been designed to adopt mercury-promoted desulfurization to give irreversible chemical events between thioamide derivatives and Hg^{2+} ions.⁷ Desulfurization of the thioacetal group by Hg^{2+} ions has also been developed as a mercury chemodosimetric probe.⁸

Imine (Schiff base) ligands are considered 'privileged ligands' as they are easily prepared from the condensation of aldehydes and amines. It is known that Schiff base ligands are able to coordinate many different metal ions⁹ and stabilize them in various oxidation states. The Schiff base complexes have been also used in catalytic reactions¹⁰ and as models for biological systems.¹¹ Regarding a chemodosimetric irreversible reaction; thus far, Fe^{3+} and Cu^{2+} ions have been known to promote hydrolysis of Schiff bases.¹²⁻¹⁴ However, in the sensing context, the Hg^{2+} -promoted chemodosimetric hydrolysis of Schiff base described herein has never been reported to date.

Results and Discussion

A new pyrene-based fluorescent sensor **1**¹⁵ is described in Chart 1 that exhibits a unique fluorescence change in the presence of the Hg^{2+} ion and high selectivity over other metal cations. To establish a mechanism for these fluorescence changes upon addition of Hg^{2+} , two additional analogues **2**¹⁶ and **3** were prepared and their fluorescent properties examined. The structures of **1** - **3** were confirmed by ¹H-NMR, ¹³C-NMR, and FAB-MS.

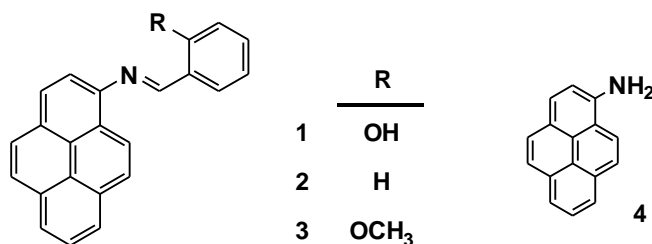


Chart 1. Iminopyrene derivatives **1–3** and 1-aminopyrene **4**.

The spectral changes of **1** (20.0 μM) upon addition of various perchlorate salts (10 eq) of Li^+ , Na^+ , K^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Rb^{2+} , Sr^{2+} , Cd^{2+} , Ba^{2+} , Hg^{2+} , Pb^{2+} , and Fe^{3+} (total of 18) were screened by UV-visible and fluorescence spectroscopy. In Figure 1a, a pale

yellow solution of **1** in CH₃CN:H₂O (9:1 v/v) displayed an intensive absorption band at 380 nm ($\epsilon = 3.9 \cdot 10^4 \text{ M}^{-1}\text{cm}^{-1}$). Introduction of Hg²⁺ ions to a solution of **1** induced a UV/Vis absorption band of **1** to a distinct blue-shift, whereas no significant spectral changes were observed upon addition of other metal ions. Figure 1b shows a spectral variation of **1** upon the gradual addition of Hg(ClO₄)₂ to 20.0 μM of **1** in a solution of CH₃CN:H₂O (9:1 v/v). In a function of [Hg²⁺], a new absorption band centered at 340 nm ($\epsilon = 3.7 \cdot 10^4 \text{ M}^{-1}\text{cm}^{-1}$) lead to a distinct color change from yellow to colorless. A sharp isosbestic point at 350 nm in a ratiometric manner was then observed, indicating conversion of **1** into a new species upon addition of the Hg²⁺ in a fashion of a 1:1 stoichiometric reaction. The new band formation in a short period at 340 nm corresponded to that of **4**, implicating that hydrolysis of **1** by Hg²⁺ ions afforded **4** as shown in Scheme 1. Therefore, at this stage, it is noteworthy that **1** showed a very high selectivity for the Hg²⁺ ion over other metal cations during chemodosimetric hydrolysis, to yield the 1-aminopyrene **4** along with a distinctive color change.

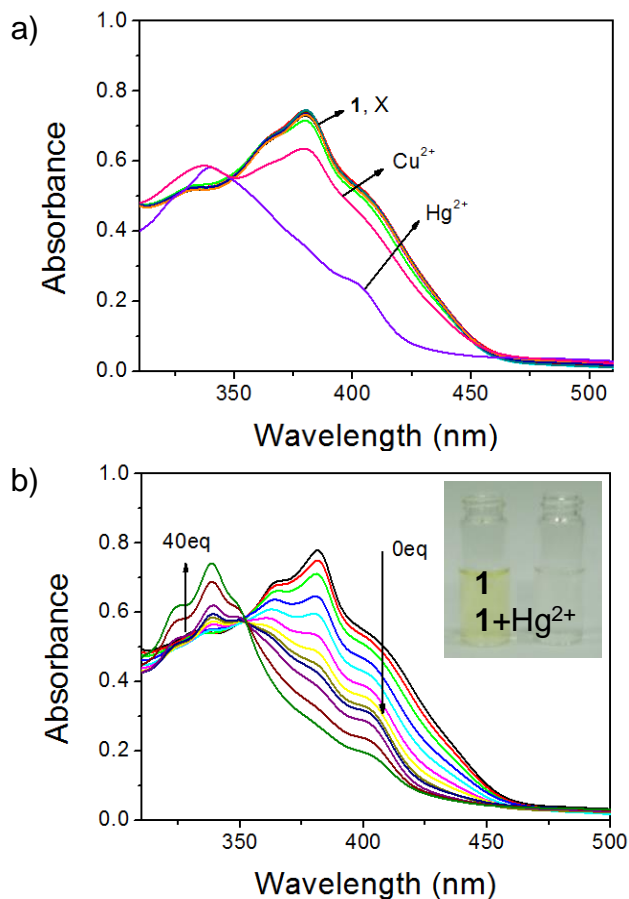
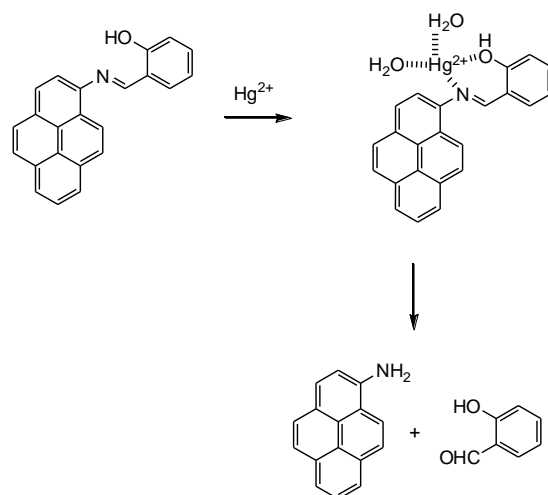


Figure 1. (a) Absorption spectra of **1** (20.0 μM) upon respective addition of the ClO₄⁻ salt of: Li⁺; Na⁺; K⁺; Ag⁺; Mg²⁺; Ca²⁺; Fe²⁺; Co²⁺; Ni²⁺; Cu²⁺; Zn²⁺; Rb²⁺; Sr²⁺; Cd²⁺; Ba²⁺; Hg²⁺; Pb²⁺; Fe³⁺ (10 eq). (b) Absorption spectra of **1** (20.0 μM) at different concentrations of Hg²⁺ in 9:1 CH₃CN:H₂O.



Scheme 1. Proposed mechanism of Hg^{2+} -promoted imine hydrolysis of **1**.

To gain insight into the chemodosimetric Hg^{2+} selectivity in regards to fluorescence changes, the fluorescence spectra of **1** with the addition of various metal cations has also been taken. In general, in such an intramolecular H-bonding system, the electrons usually transfer from the nitrogen to the hydrogen atom, and as a consequence, the charge density on the N is remarkably reduced. We have, however, previously found from DFT calculations that the N atom of iminocoumarin, which has a coumarin fluorophore instead of pyrene in **1**, possessed a greater electron density than compounds without the -OH group, even though iminocoumarin has an intramolecular H-bonding between the N atom and the phenolic -OH group.¹⁵ From this result, we can assume this unusual negative charge allocation about the N of free **1** also lead to fluorescence quenching.¹⁵ Fluorescence intensity of **1**, however, markedly increased at 430 nm when the Hg^{2+} ion was added to a solution of $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1 v/v). The fluorescence enhancement at 430 nm was due to the chemodosimetric event of **1** that produced a strongly fluorescent 1-aminopyrene **4** in quantitative yield.

To prove the chemodosimetric reaction of **1** with Hg^{2+} , structural analysis of the reaction products were implemented by $^1\text{H-NMR}$ and FAB-MS spectroscopy. Upon addition of Hg^{2+} to a solution of **1**, the H-bonding proton (H_a) of **1** at 13.66 ppm disappeared with concomitant appearance of a new peak at 4.52 ppm, assignable to the corresponding amine proton of **4**. The mass spectrum of the purified product from the reaction was identical to that of **4** at 216.51 m/z , demonstrating that **1** was, indeed, transformed into **4** by Hg^{2+} ions (Figure 3b). These results conclusively suggest that irreversible hydrolysis of **1** with Hg^{2+} ion produce **4** in the presence of water. In addition, the formation of **4** as a result of Hg-promoted hydrolysis of the reagent **1** is also confirmed by the fluorescence and absorption spectra, as shown in Figure 4. The absorption spectra of **4** and **1**+ Hg^{2+} are nearly identical, indicating that hydrolysis of **1** by Hg^{2+} ions affords **4** within a short reaction time.

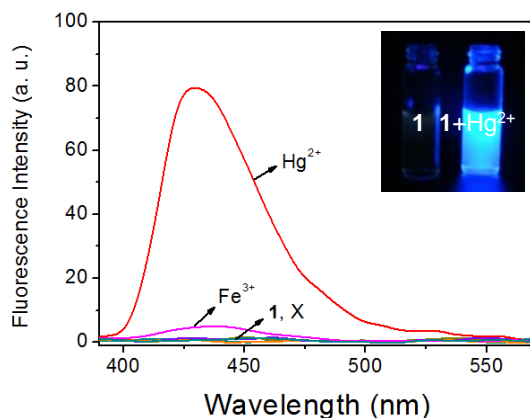


Figure 2. Fluorescence spectra of **1** (3.0 μM) upon addition of the ClO_4^- salt of: Li^+ ; Na^+ ; K^+ ; Ag^+ ; Mg^{2+} ; Ca^{2+} ; Fe^{2+} ; Co^{2+} ; Ni^{2+} ; Cu^{2+} ; Zn^{2+} ; Rb^{2+} ; Sr^{2+} ; Cd^{2+} ; Ba^{2+} ; Hg^{2+} ; Pb^{2+} ; Fe^{3+} (10 eq, respectively), with an excitation at 380 nm. Each spectrum was acquired 5 min after each cations addition.

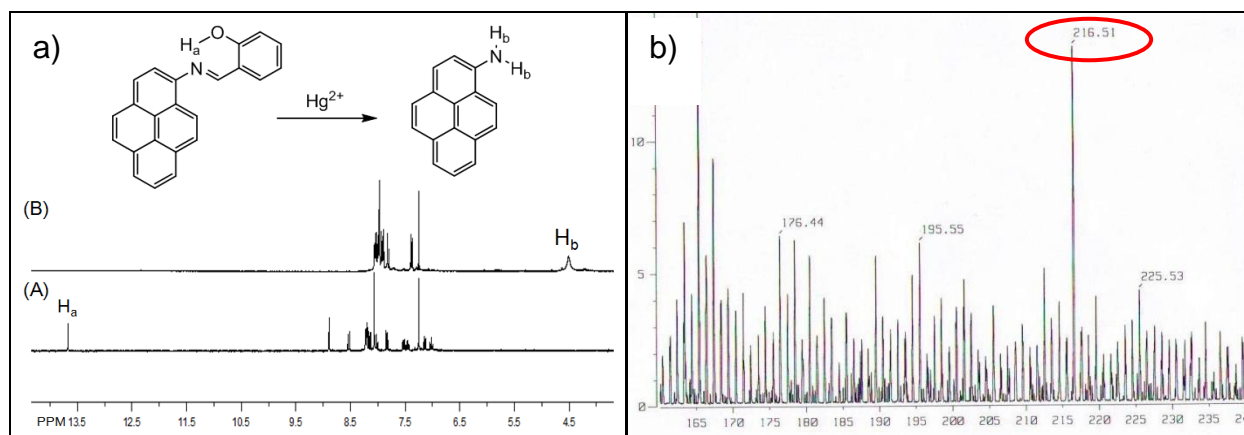


Figure 3. (a) ^1H -NMR spectral changes of **1** upon addition of Hg^{2+} : (A) **1** only; (B) **1**+ Hg^{2+} , in CDCl_3 . (b) FAB-Mass spectrum of **1**/ Hg^{2+} .

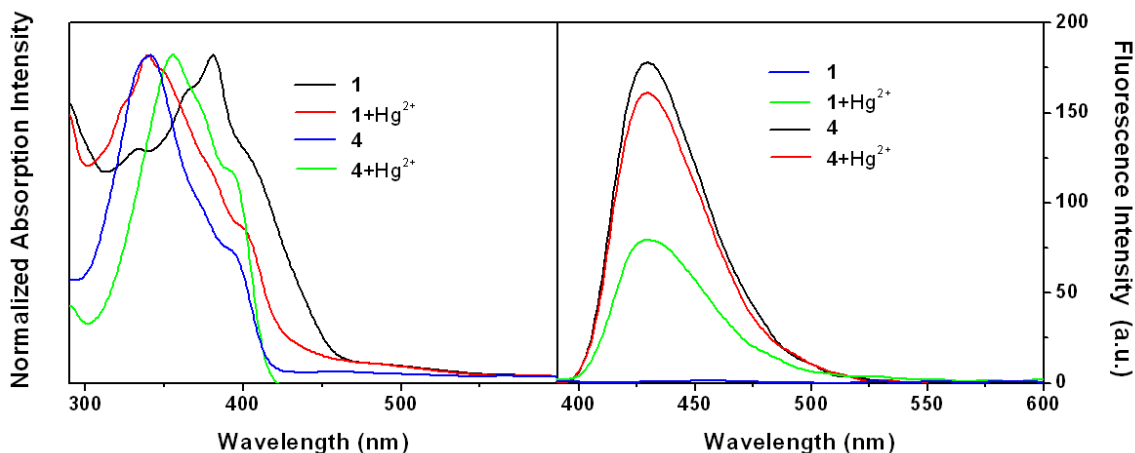


Figure 4. (left) Absorption spectra and (right) fluorescence spectra of **1** and **4** (20.0 and 3.0 μM) with/without Hg^{2+} (10 equiv, respectively) in 90:10 $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ with an excitation at 380 nm.

To gain a better understanding of the hydrolysis of **1** in the presence of the Hg^{2+} ion in aqueous solution, fluorescence changes upon the addition of various metal perchlorates to non-aqueous MeCN solutions of **1** were next investigated. Fluorescence changes toward all metal ions tested, including Hg^{2+} in acetonitrile only, were rarely observed.

For practical applicability, the proper pH condition of this new chemodosimeter was also evaluated. For free **1**, the hydrolysis of imine took place easily at acidic condition ($\text{pH} < 4$) whereas on $\text{pH} > 4$ no significant hydrolysis was observed. In the presence of the Hg^{2+} ion, there was an obvious fluorescence OFF-ON change between $\text{pH} 4$ and 12 . It is thus noteworthy that chemodosimeter **1** can detect the Hg^{2+} ion in a wide pH span ($4\sim 12$) where reaction of **1** with Hg^{2+} ion induces a remarkable fluorescence Off-On.

To examine the role of the intramolecular H-bonding of **1** in the metal ion selectivity, **2** and **3** were also prepared. Upon addition of various metal ions to **2** and **3**, their observed fluorescence changes toward the Hg^{2+} ion were not as selective as **1**, implying that the H-bonding between the phenolic -OH and the imine N in **1** played a crucial role in the high selectivity for the Hg^{2+} ion over other cations (Figure 5). This is presumably due to the basicity of the ligand, where compound **1** was a weaker¹² base than **2** or **3**. The weaker the base, the slower the ligand responds to the Hg^{2+} ion, followed by hydrolysis, inducing rather higher selectivity toward the Hg^{2+} ion over other Lewis acid metal ions.

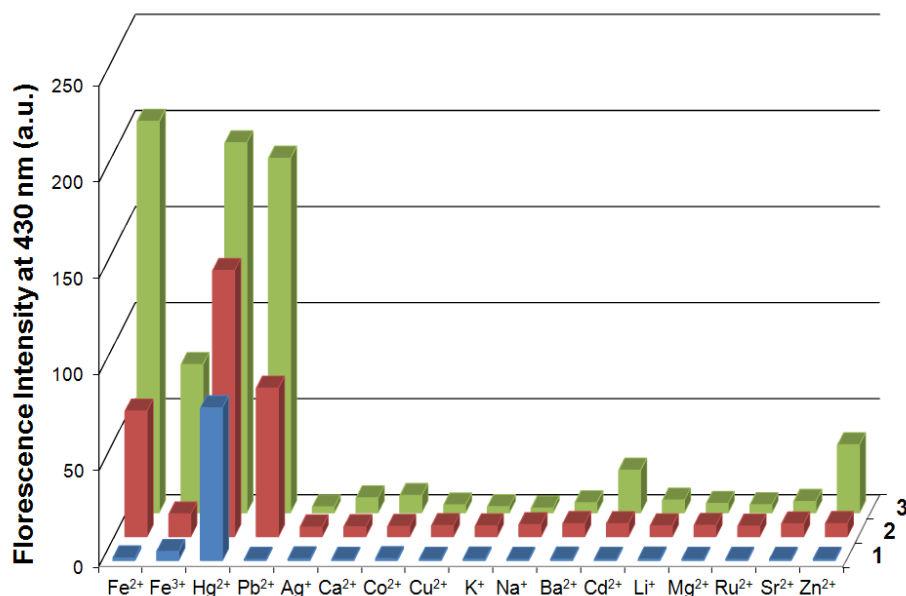


Figure 5. Fluorescence intensity at 430 nm of **1-3** (3.0 μM) upon addition of the ClO_4^- salt of: Li^+ ; Na^+ ; K^+ ; Ag^+ ; Mg^{2+} ; Ca^{2+} ; Fe^{2+} ; Co^{2+} ; Ni^{2+} ; Cu^{2+} ; Zn^{2+} ; Rb^{2+} ; Sr^{2+} ; Cd^{2+} ; Ba^{2+} ; Hg^{2+} ; Pb^{2+} Fe^{3+} (10 eq, respectively) in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1) with an excitation at 380 nm. Each spectrum was acquired 5 min after each cations addition.

Conclusions

We herewith presented a series of iminopyrene as a Hg^{2+} ion selective chemodosimeter. Amongst tested iminopyrene **1-3**, compound **1** showed a selective change in UV absorption, fluorescence emission, and color towards the Hg^{2+} cation over other cations in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1). The fluorescence enhancement at 430 nm was due to the chemodosimetric event of **1** that produces a strongly fluorescent **4**. This obvious fluorescent change may allow probe **1** to be employed for fluorescent detection of Hg^{2+} ions in biological and environmental fields.

Experimental Section

General. Uncorrected melting points (MPs), Buchi 500. ^1H - and ^{13}C -NMR, Varian 400 MHz (δ in ppm from TMS, J in hertz). FAB MS mass spectra, JEOL-JMS-HX 110A/ 110A High Resolution Tandem Mass Spectrometry in Korea University (Korea). All the reactions were run under a nitrogen atmosphere. Silica gel (SiO_2 , Geduran 1.11567) was used for column chromatography. All reagents and solvents were of commercial grade and used without further

purification. Absorption spectra were recorded with a S-2000 UV-spectroscope and fluorescence spectra recorded with a RF-5301PC spectrofluorophotometer.

Absorption and fluorescence spectra. Stock solutions (1.00 mM) of the metal perchloride salts were prepared in CH₃CN. Stock solutions of **1** (0.3 mM) were prepared in CH₃CN. For all fluorescence spectra measurements, excitation was at 380 nm with excitation slit widths at 1.5 and 3.0 nm emissions. All UV/Vis experiments were performed using 20.0 μM of **1** in CH₃CN:H₂O (9:1, v/v). Fluorescence experiments were performed using 3.0 μM of **1** in CH₃CN:H₂O (9:1, v/v). The concentrations of the CH₃CN solutions were varied with a total volume fixed at 4.0 mL. After the mixture was shaken, the fluorescence emissions at 430 nm were recorded.

Compounds **1**¹⁵ and **2**¹⁶ were prepared by adaptation of reported procedures.

Compound (3). A portion of *o*-methoxybenzaldehyde (0.06 g, 0.46 mmol) and 1-aminopyrene (0.1 g, 0.46 mmol) were combined in hot absolute ethanol (10.0 mL) for 2 h to obtain a yellow solid of **3** (95.0 mg) in 61% yield. Mp: 100 - 102 °C. ¹H-NMR (CDCl₃, 400 MHz): δ 9.17 (s, NCH, 1H), 8.68-8.65 (d, 1H, ArH, *J* = 9.15 Hz), 8.47-8.44 (dd, 1H, ArH, *J* = 1.78 Hz, 7.99 Hz), 8.17-8.13 (m, 3H, ArH), 8.08-7.95 (m, 4H, ArH), 7.74-7.72 (d, 1H, ArH, *J* = 8.12 Hz), 7.51-7.47 (1H, ArH, *J* = 1.79 Hz, 8.13 Hz), 7.15-7.12 (t, 1H, ArH, *J* = 5.65 Hz), 7.01-6.99 (d, 1H, ArH, *J* = 8.39 Hz), 3.93 (s, 3H, OCH₃). ¹³C-NMR (100 MHz, CDCl₃): δ 158.8, 157.1, 146.8, 133.0, 131.7, 129.6, 128.0, 127.5, 127.1, 126.7, 126.2, 125.8, 125.5, 125.4, 125.3, 125.1, 125.0, 124.9, 123.7, 121.2, 116.0, 111.4, 55.8 ppm. FAB MS *m/z* (M⁺): calcd, 335.13; found, 336.14 (**3**+H⁺). IR (KBr pellet, cm⁻¹): 3049, 1604, 1475, 1252.

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