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Simple Ratiometric Fluorophore for the Selective Detection of Mercury through Hg(II)-Mediated Oxazole Formation

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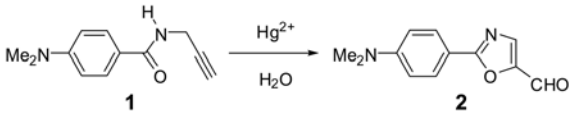
A simple propargylamide-fuctionalized chemodosimeter was prepared for the ratiometric fluorescence detection of mercuric ions in HEPES buffer. The chemodosimeter exhibited Hg^{2+}-induced propargyl amide-to-oxazole transformation, with a significant accompanying ratiometric change in fluorescence. It afforded high selectivity for mercuric ion detection without any competitive inhibition by common alkali, alkaline earth, or other transition metal ions. The probe showed a 17×10^{-6} M detection limit for Hg^{2+} ions and potential applicability for detecting aqueous Hg^{2+} ions.

Key Words : Chemodosimeter, Fluorophore, Mercury, Oxazole, Ratiometric

Introduction

As one of the heavy metals, mercury ion can severely inhibit organisms' normal functioning.[1] Its accumulation in various areas of the human body[2] can cause diseases in the digestive, the cardiovascular, and notably the neurological systems.[3] In spite of stringent regulations designed to reduce the industrial use of mercury, it remains an important pollutant[4] that affects natural and human environments.[5] Although several fluorescent probes have been developed that show changes of fluorescence intensity upon binding with mercury ions,[6-8] their accurate determination of mercury ion concentrations is vulnerable to their environment. Therefore, the development of a ratiometric probe for mercury detection that exhibits fluorescence that is insusceptible to environmental changes would be beneficial. This work reports a simple ratiometric probe for the detection of mercuric ions through a mercury(II)-mediated transformation of propargylamide to oxazole, which led to highly selective ratiometric responses to mercuric ions in HEPES buffer.[9]

Kuscheroff reactions of alkynes with water in the presence of mercury(II) ions afford ketones.[10] Several researchers including the Koide's group employed such alkyne-to-ketone conversion in the sensing of mercury ions.[11] Recently our group also reported an alkyne-based chemodosimeter for mercuric ions that used the alkynophilicity of Hg(II) ions, in which fluorescence was turned off in the presence of Hg(II).[12] To increase fluorescence in a probe and to induce the ratiometric response, an alkyne-tethered simple benzamide (**1**) was designed (Fig. 1). Hg(II) ions were expected to activate the alkyne and to induce a cyclization reaction to afford an oxazole ring, a similar reaction to one reported involving gold ions.[13] Therefore, **1** was prepared here from 4-*N*,*N*-dimethylaminobenzoic acid by the modification of a reported procedure.[14]



1 **2**

Figure 1. Hg(II)-mediated transformation of a propargylamide (**1**) into an oxazole (**2**).

Experimental Section

General. 4-Dimethylaminobenzoic acid, oxalyl chloride, and propargylamine hydrochloride were from Aldrich Chemical Co. and used without further purification. Spectroscopic grade solvents for UV-vis and fluorescence spectroscopy were from Dae Jung Co. NMR measurements were performed on Bruker Avance-300 (300 MHz) or Varian-200 (200 MHz) spectrometers using dimethyl sulfoxide (DMSO-d_6) or D_2O solvents. All peaks are given as δ in ppm relative to the residual solvent peaks as a reference; they correspond to signals from compounds' nondeuteriated components. UV-vis spectra were recorded with an Agilent 8453 spectrometer. Fluorescence spectra were measured on a Jasco FP-6500 spectrophotometer. Mass spectra were recorded on a G6401A MS-spectrometer. TLC analyses were performed on silica gel plates and flash chromatography was conducted using silica gel column packages from Merck.

Preparation of 1. 4-Dimethylaminobenzoic acid (825 mg, 5.0 mmol) and oxalyl chloride (2 M, 3.5 mL, 7.0 mmol) were dissolved in 20 mL CH_2Cl_2 under N_2 and stirred at room temperature overnight. The solvent was then removed under reduced pressure and replaced with 15 mL CH_2Cl_2. The resulting mixture was added dropwise to a mixture of propargylamine hydrochloride (686 mg, 7.5 mmol) and TEA (1.5 g, 15 mmol) in 20 mL CH_2Cl_2 under N_2 at 0 °C and stirred overnight at room temperature. Volatiles were then evaporated under reduced pressure and the residue was purified by recrystalization in CH_2Cl_2, affording **1** as a white solid (462 mg, yield 45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (t, ^3J = 8.1, 1H), 7.72 (d, ^3J = 12.9, 2H), 6.70 (d, ^3J = 12.9, 2H), 4.01 (dd, ^3J = 8.1, ^4J = 3.6, 2H), 3.08 (t, ^4J = 3.9,

1H), 2.97 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.3, 152.6, 151.7, 129.1, 120.8, 111.2, 82.4, 72.9, 28.7. HRMS (*m*-NBA, FAB$^+$) *m/z* obsd 203.1186 ([M+H]$^+$, calcd 203.1184 for $C_{12}H_{15}N_2O$).

Preparation of 2. 4-(Dimethylamino)-*N*-(prop-2-ynyl)-benzamide (100 mg, 0.5 mmol) and mercuric chloride (203 mg, 0.75 mmol) were dissolved in 10 mL THF and stirred at room temperature overnight. The solvent was then removed under reduced pressure and the residue was purified by column chromatography using CH_2Cl_2/MeOH (v/v 10:1, R_f = 0.65) to afford **2** as a yellow solid (19 mg, yield 18%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.25 (s, 1H), 7.90 (d, ^3J = 13.5, 2H), 6.84 (d, ^3J = 13.8, 2H), 3.04 (s, 6H). HRMS (*m*-NBA, FAB$^+$) *m/z* 217.0979 ([M+H]$^+$, calcd 217.0977 for $C_{12}H_{13}N_2O_2$).

General UV-vis and Fluorescence Spectral Measurements. Since the chemosensor was not fully soluble in pure water, a minimal amount of DMF was used to aid solubility. A stock solution of **1** (10 mM) was prepared in DMF. UV-vis and fluorescence spectra were obtained in 0.1 M HEPES buffer at pH 7.4. Sample solutions for these measurements were obtained by mixing an appropriate amount of the stock solution of **1** (10 mM in DMF) with an aqueous stock solution containing metal chlorides (20 mM) and then diluting this mixture with buffer. An aqueous solution of the desired concentrations of **1**, metal ions, and buffer resulted. Fluorescence measurements employed slit widths of 3 nm/3 nm.

Binding Stoichiometry Determination. The binding stoichiometry of **1** with Hg^{2+} ions was determined using Job's plot.[15] For which, a series of solutions with varying mole fractions of metal ions were prepared with constant total concentrations of **1** and Hg^{2+} ions (200 μM). Fluorescence emissions were measured at 322-700 nm for each sample by exciting at 312 nm. Fluorescence intensity at 445 nm was plotted against the mole fraction of final analyte solution.

Results and Discussion

Oxazole compound **2** was prepared in moderate yield by the Kuscheroff reaction of 2 equivalents of mercury chloride with **1**, which was prepared by the Schotten-Baumann reaction of propargylamine with (dimethylamino)benzoyl chloride (Scheme 1). The chemosensing behavior of **1** was investigated by UV-vis and fluorescence measurements.

^{1}H NMR spectra were recorded before and after the addition of the mercury chloride to **1** (Fig. 2).

After the reaction was complete, the product was characterized by ^{1}H NMR spectroscopy. Cyclization resulted in new aldehyde (CHO) and oxazole (H^c) protons at 9.62 and 8.19 ppm, respectively; the signals from allyl (H^a, 4.02 ppm) and alkyne (H^b, 3.08 ppm) protons disappeared, as did the broad amide NH proton signal.

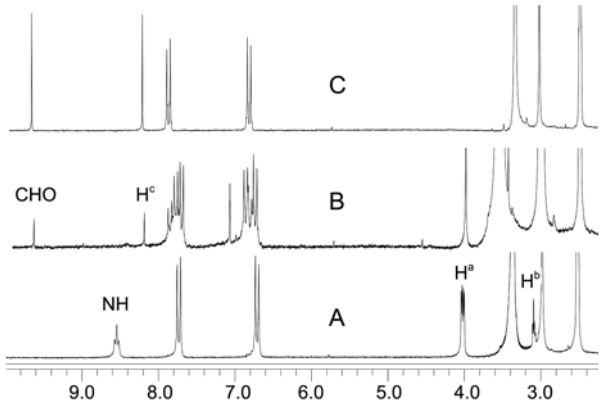


Figure 2. Partial ^{1}H NMR spectra of **1** (20 mM) upon the addition of 2 equiv $HgCl_2$ in DMSO-d_6. (a) **1**, (b) **1** + Hg^{2+}, (c) **2**.

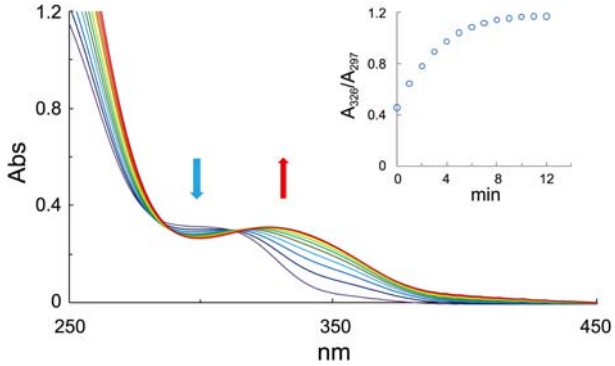
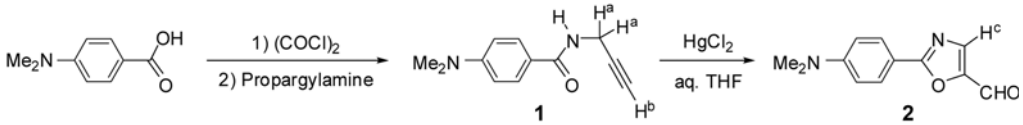


Figure 3. Time-dependent UV-vis spectra of **1** (20 μM) in HEPES buffer (0.1 M, pH 7.4). Inset: its kinetics.

Time-dependent UV-vis and fluorescence spectra of **1** exhibited ratiometric changes when **1** (20 μM) was treated with Hg(II) (5.0 mM) in HEPES buffer (0.10 M, pH 7.4). The absorbance of **1** around 297 nm decreased and that at 326 nm gradually increased to afford an apparent isosbestic point at 312 nm. The transformation of **1** to **2** was complete within 12 min at 25°C, $k = 1.8 \times 10^{-1}$ M^{-1}s^{-1} (Fig. 3).

The fluorescence spectrum of **1** in HEPES buffer (0.1 M, pH 7.4) displayed moderate fluorescence intensity at 380 nm. Fluorescence spectra were not changed significantly in the presence of representative alkali (Na^+, K^+), alkaline earth (Ca^{2+}), or other transition metal (Fe^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Pd^{2+}) ions (Fig. 4(b)).[16] However, the addition of Hg^{2+} ions considerably increased fluorescence intensity and red-shifted the signal from 380 to 445 nm (Fig. 4(a)).



Scheme 1. Synthesis of **1** and its Hg(II)-mediated cyclization.

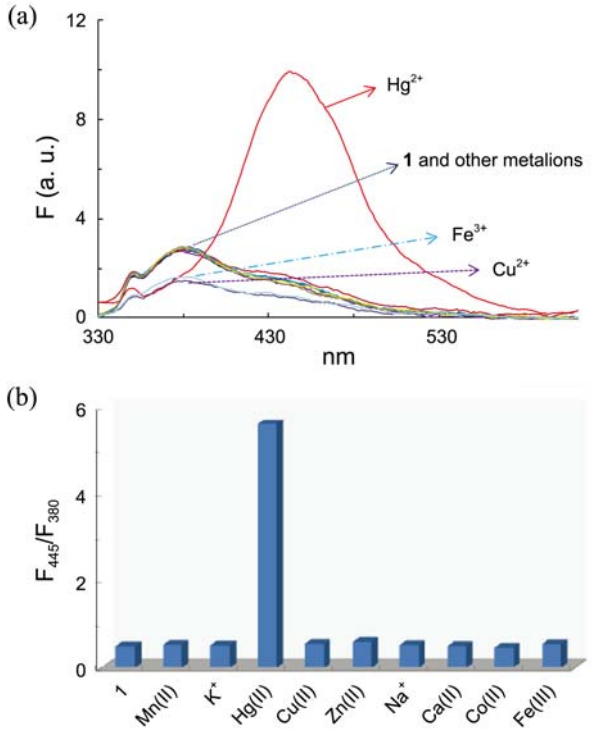


Figure 4. (a) Fluorescence spectra of **1** (20 μM) in HEPES buffer (0.1 M, pH 7.4) upon the addition of various metal ions. (250 equiv, excitation at 312 nm). (b) Its ratiometric fluorescence intensity (F_{445}/F_{380}).

Opposite to Hg(II), Cu(II) and Fe(III) ions showed a hypochromic effect on the fluorescence intensity of **1**. The F_{445}/F_{380} fluorescence intensity ratios (Fig. 4(b)) clearly demonstrate the prominent selectivity of **1** towards Hg(II) ions.

The fluorescence of **1** (20 μM) for Hg^{2+} signaling was quantitatively investigated through F_{445}/F_{380} ratio measurements (Fig. 5) during titration with $HgCl_2$ in HEPES buffer (0.1 M, pH 7.4). With increasing concentrations of Hg^{2+} ions, the ratiometric fluorescence intensities of **1** increased significantly before reaching a plateau. The linear range of increasing signal intensity showed a standard deviation (σ = 0.15) of **1** in the absence of Hg^{2+} ions, its slope (m = 0.53, n = 3) to afford the limit of detection of Hg(II) ions of 17 μM (0.85 equiv. Hg^{2+} ions) at $3\sigma/m$ (Fig. 5, inset).

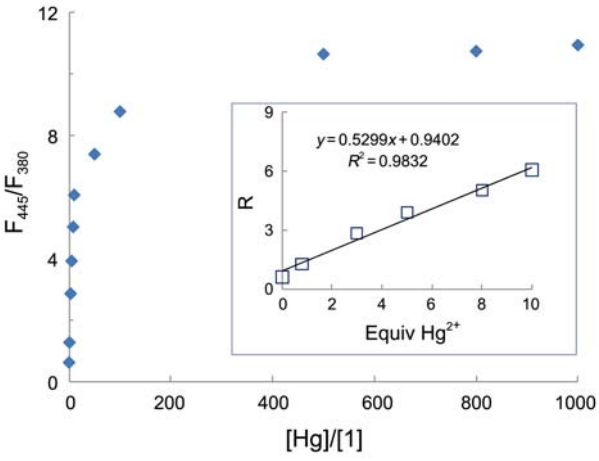


Figure 5. Titration graph of **1** with Hg^{2+} ions. [**1**] = 20 μM in HEPES buffer, λ_{ex} 312 nm.

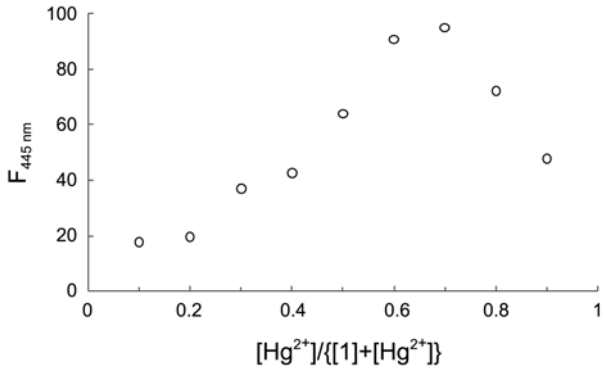


Figure 6. Job's plot of **1**-Hg^{2+}, where the total concentrations were maintained as [**1**] + [Hg^{2+}] = 200 μM in HEPES buffer (0.1 M, pH 7.4).

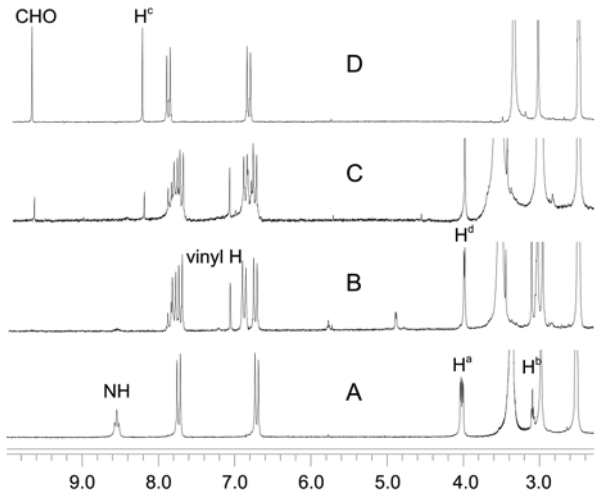


Figure 7. Time-dependent ^{1}H NMR spectra of **1** upon addition of 2 equiv $HgCl_2$ and **2** in DMSO-d_6/D_2O (20:1, v/v). (A) **1**, (B) **1** + 2 equiv Hg(II), 15 min, (C) **1** + 2 equiv Hg(II), 1 d, and (D) **2**.

To determine the reaction stoichiometry between **1** and Hg(II), a Job's plot was carried out under the concentration

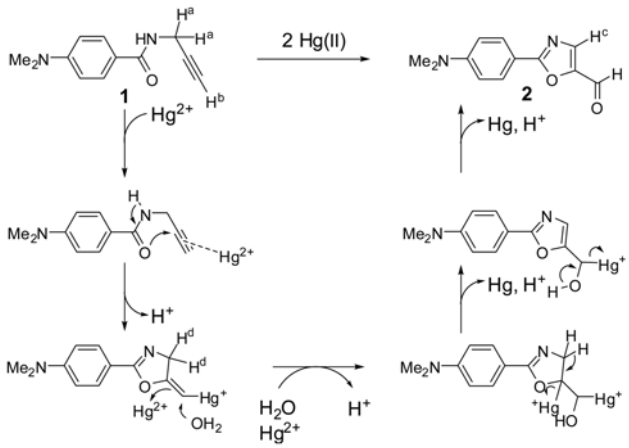


Figure 8. A proposed mechanism.

condition of [**1**] + [Hg^{2+}] = 200 μM in HEPES buffer. The plot indicates that **1** reacted with 2 equivalents of Hg(II) ions, showing that the 1:2 complex stoichiometry was maintained (Fig. 6).

To explore the reaction mechanism, time-dependent NMR analysis was conducted (Fig. 7). **1** showed rapid formation of vinyl mercurate in the presence of 2 equivalents of $HgCl_2$ owing to activation of alkyne by a Hg(II) ion, which subsequently reacted with water and a second Hg(II) ion to afford a vicinal dimercurate species, similar to the gold ion-mediated reaction.[13b,c] Slow reduction of the organomercurate likely led to a stable aromatic oxazole compound, **2**, with an aldehyde functional group (Fig. 8).

Conclusion

A ratiometric chemodosimeter for toxic Hg(II) ions was designed using an alkyne-functionalized simple benzene derivative. Hg(II)-mediated cyclization resulted in highly selective and ratiometric fluorescence responses toward the Hg(II) ions over other metal ions. The sensor showed a detection limit of 17 μM aqueous mercury ions.

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References

1. (a) Huang, C.-C.; Chang, H. T. *Anal Chem.* **2006**, *78*, 8332-8338. (b) Zheng, W.; Aschner, M.; Ghersi-Egea, J.-F. *Toxicol. Appl. Pharmacol.* **2003**, *192*, 1-11. (c) Hoyle, I.; Handy, R. D. *Aquat Toxicol.* **2005**, *72*, 147-159.
2. (a) Vupputuri, S.; Longnecker, M. P.; Daniels, J. L.; Guo, X.; Sandler, D. P. *Environ. Res.* **2005**, *97*, 194-199. (b) Baughman, T. A. *Environ. Health Perspect.* **2006**, *114*, 147-152. (c) Mutter, J.; Naumann, J.; Schneider, R.; Walach, H.; Haley, B. *Neuroendocrinol Lett.* **2005**, *26*, 439-446. (d) Zalups, R. K. *Pharmacol Rev.* **2000**, *52*, 113-143.
3. (a) Onyido, I.; Norris, A. R.; Buncel, E. *Chem. Rev.* **2004**, *104*, 5911-5929. (b) Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 543-566. (c) Matsushita, M.; Meijler, M. M.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Org. Lett.* **2005**, *7*, 4943-4946.
4. (a) Regulatory Impact Analysis of the Clean Air Mercury Rule EPA-452/R-05-003; U.S. Environmental Protection Agency: Research Triangle Park, NC, **2005**. (b) Yoon, S.; Miller, E. W.; He, Q.; Do, P. H.; Chang, C. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 6658-6661.
5. (a) Renzoni, A.; Zino, F.; Franchi, E. *Environ. Res.* **1998**, *77*, 68-72. (b) Malm, O. *Environ. Res.* **1998**, *77*, 73-78.
6. (a) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J Am. Chem. Soc*. **2006**, *128*, 10-11. (b) Wang, J.; Qian, X.; Cui, J. *J. Org. Chem.* **2006**, *71*, 4308-4311. (c) Yang, R. H.; Chan, W. H.; Lee, A. W.; Xia, P. F.; Zhang, H. K.; Li, K. *J. Am. Chem. Soc.* **2003**, *125*, 2884-2885. (d) Caballero, A.; Martinez, R.; Lloveras, V.; Ratera, I.; Vidal-Gancedo, J.; Wurst, K.; Tarraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666-15667. (e) Nolan, E. M.; Jaworski, J.; Okamoto, K.; Hayashi, Y.; Sheng, M.; Lippard, S. J. *J. Am. Chem. Soc*. **2005**, *127*, 16812-16823. (f) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc*. **2004**, *126*, 2272-2273. (g) He, Q.; Miller, E. W.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc*. **2006**, *128*, 9316-9317. (h) Liu, J.; Lu, Y. *J. Am. Chem. Soc*. **2005**, *127*, 12677-12683. (i) Yang, Y. K.; Yook, K. J.; Tae, J. *J. Am. Chem. Soc*. **2005**, *127*, 16760-16761. (j) Ono, A.; Togashi, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 4300-4302. (k) Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc*. **2006**, *128*, 9988-9989. (l) Chen, X.; Nam, S.-W.; Jou, M. J.; Kim, Y.; Kim, S.-J.; Park, S.; Yoon, J. *Org. Lett*. **2008**, *10*, 5235-5238.
7. (a) Liu, C.-W.; Huang, C.-C.; Chang, H.-T. *Anal. Chem.* **2009**, *81*, 2383-2387. (b) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2006**, *128*, 14474-14475. (c) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270-14271. (d) Nolan, E. M.; Racine, M. E.; Lippard, S. J. *Inorg. Chem*. **2006**, *45*, 2742-2749. (e) Meng, X.-M.; Liu, L.; Hu, H.-Y.; Zhu, M.-Z.; Wang, M.-X.; Shi, J.; Guo, Q.-X. *Tetrahedron Lett.* **2006**, *47*, 7961-7964. (f) Wang, J.; Qian, X. *Chem. Commun.* **2006**, 109-111. (g) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc*. **2006**, *128*, 14474-14475. (h) Descalzo, A. B.; Martínez-Máñez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc*. **2003**, *125*, 3418-3419. (i) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S.-K. *Org. Lett.* **2006**, *8*, 371-374. (j) Choi, M. J.; Kim, M. Y.; Chang, S.-K. *Chem. Commun.* **2001**, 1664-1665.
8. (a) Dickerson, T. J.; Reed, N. N.; LaClair, J. J.; Janda, K. D. *J. Am. Chem. Soc.* **2004**, *126*, 16582-16586. (b) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc*. **2003**, *125*, 14270-14271. (c) Matsushita, M.; Meijler, M. M.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Org. Lett.* **2005**, *7*, 4943-4946.
9. (a) Wang, Z.; Zhang, D. Q.; Zhu, D. B. *Anal. Chim. Acta.* **2005**, *549*, 10-13. (b) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc*. **2007**, *129*, 5910-5918. (c) Zhang, X. L.; Xiao, Y.; Qian, X. H. *Angew. Chem. Int. Ed*. **2008**, *47*, 8025-8029. (d) Suresh, M.; Mishra, S.; Mishra, S. K.; Suresh, E.; Mandal, A. K.; Shrivastav, A.; Das, A. *Org. Lett.* **2009**, *11*, 2740-2743.
10. Kutscheroff, M. G. *Chem. Ber.* **1909**, *42*, 2759-2762.
11. (a) Budde, W. L.; Dessy, R. E. *J. Am. Chem. Soc.* **1963**, *85*, 3964-3970. (b) Song, F.; Watanabe, S.; Floreancig, P. E.; Koide, K. *J. Am. Chem. Soc*. **2008**, *130*, 16460-16461. (c) Dong, M.; Wang, Y.-W.; Peng, Y. *Org. Lett.* **2010**, *12*, 5310-5313. (d) Lee, H. Y.; Jo, J.; Park, H.; Lee, D. *Chem. Commun*. **2011**, *47*, 5515-5517. (e) Lee, H.; Kim, H.-J. *Tetrahedron Lett*. **2011**, *52*, 4775-4778.
12. Lee, D.-N.; Kim, G.-J.; Kim, H.-J. *Tetrahedron Lett*. **2009**, *50*, 4766-4768.
13. (a) Yang, Y.-K.; Lee, S.; Tae, J. *Org. Lett.* **2009**, *11*, 5610-5613. (b) Jou, M. J.; Chen, X.; Swamy, K. M. K.; Kim, H. N.; Kim, H.-J.; Lee, S.-g.; Yoon, J. *Chem. Commun.* **2009**, 7218-7220. (c) Egorova, O. A.; Seo, H.; Chatterjee, A.; Ahn, K. H. *Org. Lett*. **2010**, *12*, 401-403. (d) Do, J. H.; Kim, H. N.; Yoon, J.; Kim, J. S.; Kim, H.-J. *Org. Lett*. **2010**, *12*, 932-934.
14. Kim, H.-J.; Kim, Y.-H.; Hong, J.-I. *Tetrahedron Lett*. **2001**, *42*, 5049-5052.
15. Connors, K. A., *Binding Constants*; Wiley: New York, 1987.
16. Au(III) ion was unstable in HEPES buffer and it did not induce any significant fluorescence enhancement of **1**. Actually, we only observed a dark Au precipitates upon the addition of Au(III) in HEPES.