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# A Colorimetric Ligand for Mercuric Ion

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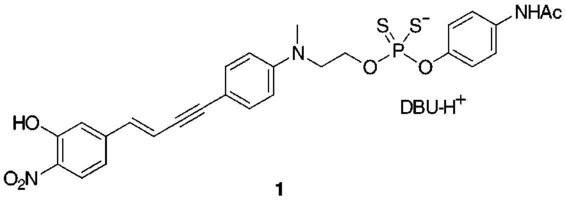
## ABSTRACT



**1**

**Practical materials are needed to expedite the detection and screening of heavy metals. This Letter describes the synthesis of a new phosphorodithioate-based ligand 1 that specifically communicates a color change when exposed to mercuric ion. The origin of this recognition arises through a charge transfer and solubility change. This ligand now extends a new means to visually screen and spectroscopically quantify mercuric ion.**

Human health risks associated with the increased release of toxic metals over the last century have drawn attention to their vectors of presentation.[1] This concern has gained an added dimension due to the fact that elemental or ionic mercury is converted by aquatic organisms to methylmercury, which subsequently bioaccumulates through the food chain.[2] Recent synthetic activity has focused on tailoring a $Hg^{2+}$-specific sensor. The resulting materials typically contain a platform for ion recognition as well as an optical transducer to measure the binding event. In this context, fluorescence has been used extensively.[3−5] Even though these probes are very sensitive and show excellent selectivity for $Hg^{2+}$, the extent of their utility and ultimate throughput can be limited by the need for instrumentation.

Materials that change their color upon recognition of macromolecules are widely accepted for fingerprinting[6] or conducting pregnancy tests at home.[7] Clearly, the throughput of this testing was profoundly advanced by the elimination of instrumental requirements. We sought to extend a visual test for mercuric ion. Prior to our investigation, dyes based on thiocarbazone moieties, such as dithizone and $\beta$-naphthylthiocarbazone, were known to respond colorimetrically to $Hg^{2+}$.[8] However, the reliability of these systems was limited by a complex equilibrium which presented an array of differently colored complexes. We now demonstrate how to circumvent this problem through perturbing such equilibria by selective precipitation.

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(2) (a) Nendza, M.; Herbst, T.; Kussatz, C.; Gies, A. *Chemosphere* **1997**, *35*, 1875. (b) Boudou, A.; Ribeyre, F. *Metal Ions Biol. Systems* **1997**, *34*, 289. (c) Clarkson, T. W. *Am. J. Clin. Nutrit.* **1995**, *61*, 682S.

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(5) For fluorescent sensors that monitor metal binding in general, see: (a) Walkup, G. K.; Imperiali, B. *J. Org. Chem.* **1998**, *63*, 6727. (b) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443. (c) Yoon, J. Y.; Ohler, N. E.; Vance, D. H.; Aumiller, W. D.; Czarnik, A. W. *Tetrahedron Lett.* **1997**, *38*, 3845. (d) Kimura, M.; Horai, T.; Hanabusa, K.; Shirai, H. *Adv. Mater.* **1998**, *10*, 459. (e) Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283. (g) Desantis, G.; Fabbrizzi, L.; Licchelli, M.; Mangano, C.; Sacchi, D.; Sardone, N. *Inorg. Chim. Acta* **1997**, *257*, 69.

(6) Hauze, D.; Joullié, M. M. *Tetrahedron* **1997**, *53*, 4239.

(7) (a) Bastian, L. A.; Piscitelli, J. T. *JAMA* **1998**, *279*, 1264. (b) Bose, R. J. *Assist. Reproduct. Gen.* **1997**, *14*, 497. (c) Alfthan, H.; Stenman, U. H. *Cell. Endocrinol.* **1996**, *125*, 107.

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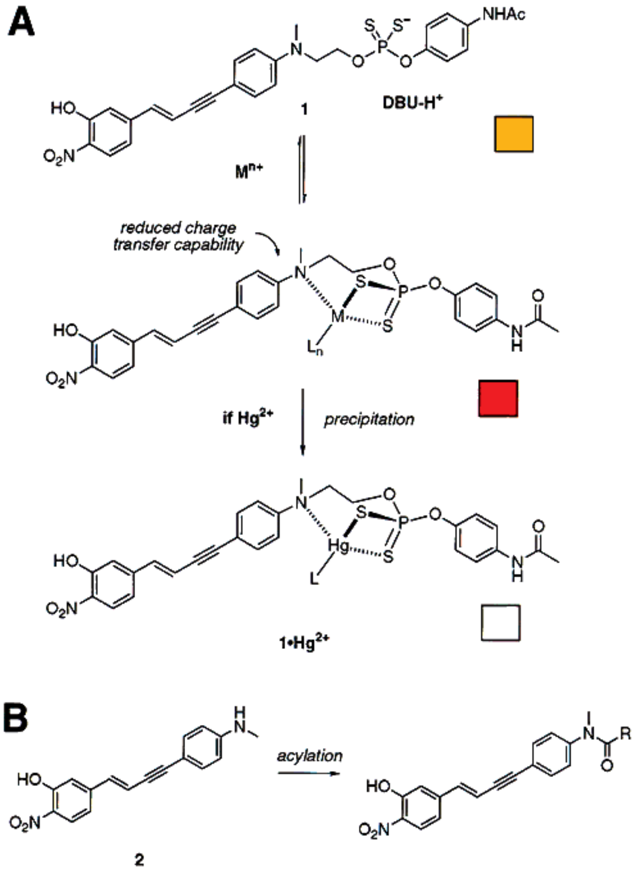
The design was based on monitoring the regulation of a dye's charge transfer upon binding of Hg$^{2+}$ to a pendant sulfur-containing ligand. Chemosensor **1** contains both a soft metal binding site, a phosphorodithioate,[9] and an adjacent amine, which is intimately involved in charge transfer (Scheme 1).[10] We envisioned that the binding of metal ion to the phosphorodithioate would alter the electron-donating capability of the terminal nitrogen in **1** either through ligand participation or environmental alteration (Scheme 1A), ultimately diverting the charge transfer and hypsochromically shifting the absorption maximum.

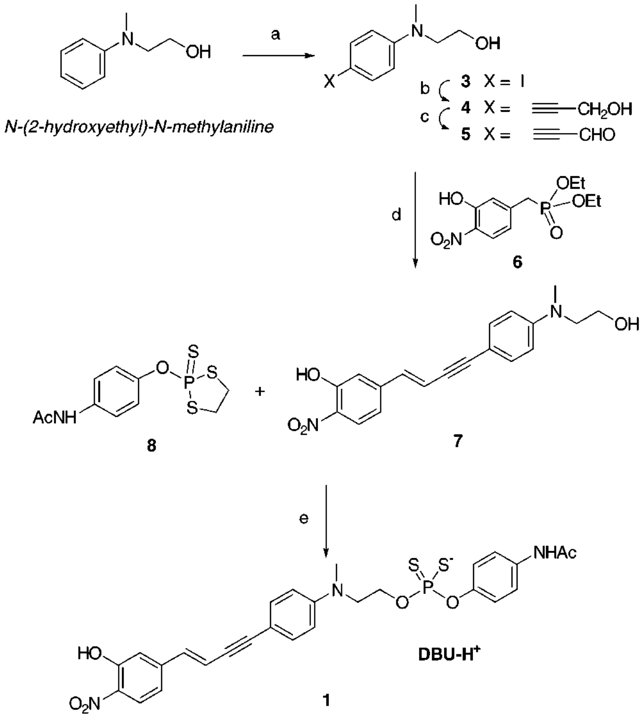
**Scheme 1**[a]



[a] **(A)** Indicator **1** senses mercury through subsequent regulation of its ability to undergo charge transfer. Most metal ions exist in equilibrium between metal-bound and free states of **1**. Certain metals, such as mercury, form strong complexes that subsequently precipitate, leaving the aqueous phase colorless (bleached). The apparent color of the solution is provided next its chemical equivalent. Precipitated complexes leave the solution colorless. **(B)** Acylation of amine **2** diminishes its intramolecular charge transfer. This acylation was accompanied by a shift in the absorption maximum from 425 to 398 nm in methylene chloride.

Examination of this approach began with the synthesis of **7**, an immediate precursor to **1** (Scheme 2). We opted for this route, as **7** can act as a cassette wherein other functionality may be readily inserted for the purpose of examining other metal−ligand interactions. The host chromophore **7** was synthesized in four steps from *N*-2-hydroxyethyl-*N*-methylaniline. Iodination in the *para*-position, Heck coupling with propargyl alcohol, and oxidation with $MnO_2$ led to aldehyde **5**, which was subsequently condensed with diethyl 2-hydroxy-4-nitrobenzylphosphonate (**6**) using a Wadsworth−Horner−Emmons reaction. Final conversion to **1** was achieved by phosphorylation of **7** with 2-phenoxy-2-thio-1,3,2-dithiophospholane **8**[11] in the presence of DBU.

**Scheme 2.** Synthesis of Chemosensor **1**[a]



[a] (a) $I_2$, $NaHCO_3$, $CH_2Cl_2$, $H_2O$, 0 °C, 92%; (b) propargyl alcohol, $Cl_2Pd(PPh_3)_2$, CuI, $Et_3N$, THF, rt, 18 h, 93%; (c) $MnO_2$, $CH_2Cl_2$, rt, 6 h, 89%; (d) add NaHMDS in THF to **6** in DMF, 0 °C to rt, 1 h; add **5** in THF, −20 °C to rt, 8 h, 74%; (e) DBU, $CH_3CN$, rt, 4 h, 24%. Unreacted **2** (55%) from the final step was collected and recycled.

Given their high degree of homology, compounds **1** and **7** could not be distinguished by their absorption of visible light. However, this was not the case when metal was added. Addition of metal salts to **1** resulted in significant reduction and hypsochromic shift of the absorption between 415 and 450 nm. Dissociation constants ($K_D$'s) were calculated by comparing the decrease in absorption at 415 nm relative to that at 250 nm over a series of metal concentrations ranging from 0 to 35 equiv of metal nitrate ($Cd^{2+}$, $Eu^{3+}$, $Gd^{3+}$, $Hg^{2+}$, $In^{3+}$, $La^{3+}$, $Pb^{2+}$, $Tb^{3+}$, $Co^{2+}$, $Zn^{2+}$, $K^+$) (Table 1).[12] Weak binding metals ($Co^{2+}$, $Zn^{2+}$, $K^+$) required up to 150 equiv to alter the absorption of **1**. The latter effect was independent

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[9] For metal binding to phosphorodithioates, see: Hayashi, K.; Sasaki, Y.; Tagashira, S.; Yanagidani, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1255.

[10] Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.

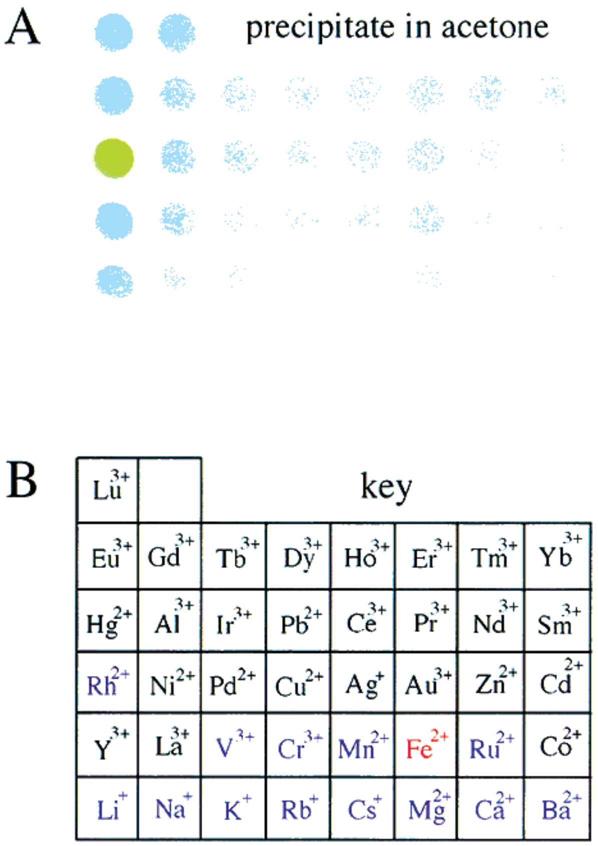
[11] Brümmer, O.; Gao, C.; Mao, S.; Weiner, D. P.; Janda, K. D. *Lett. Pept. Sci.* **1999**, in press. For the synthesis of phosphorodithioates, see also: Martin, S. F.; Wagman, A. S. *J. Org. Chem.* **1996**, *61*, 8016.

**Table 1.** Dissociation Constants, $K_D$, for the Interaction of **1** with Various Metals[a]

| metal | $K_D$ ($10^{-9}$ M) | metal | $K_D$ ($10^{-9}$ M) |
|---|---|---|---|
| $Cd^{2+}$ | 4200 | $In^{3+}$ | 12200 |
| $Co^{2+}$ | 92500 | $La^{3+}$ | 2300 |
| $Eu^{3+}$ | 2500 | $Pb^{2+}$ | 79.6 |
| $Gd^{3+}$ | 2600 | $Tb^{3+}$ | 2700 |
| $Hg^{2+}$ | 1.6 | | |

[a] As determined by examining the addition of 0.1−35 equiv of metal nitrate to 10.5 $\mu$M **1** in 35 mM HEPES in 30% acetonitrile/water at pH 7.0. The values presented were established by an average of four repetitions and were within 2% error

of metal and likely originated from adjustment of the media's ionic strength. Titration curves for several metals are provided in the Supporting Information.



| $Lu^{3+}$ | | | | | | | |
|---|---|---|---|---|---|---|---|
| $Eu^{3+}$ | $Gd^{3+}$ | $Tb^{3+}$ | $Dy^{3+}$ | $Ho^{3+}$ | $Er^{3+}$ | $Tm^{3+}$ | $Yb^{3+}$ |
| $Hg^{2+}$ | $Al^{3+}$ | $Ir^{3+}$ | $Pb^{2+}$ | $Ce^{3+}$ | $Pr^{3+}$ | $Nd^{3+}$ | $Sm^{3+}$ |
| $Rh^{2+}$ | $Ni^{2+}$ | $Pd^{2+}$ | $Cu^{2+}$ | $Ag^{+}$ | $Au^{3+}$ | $Zn^{2+}$ | $Cd^{2+}$ |
| $Y^{3+}$ | $La^{3+}$ | $V^{3+}$ | $Cr^{3+}$ | $Mn^{2+}$ | $Fe^{2+}$ | $Ru^{2+}$ | $Co^{2+}$ |
| $Li^{+}$ | $Na^{+}$ | $K^{+}$ | $Rb^{+}$ | $Cs^{+}$ | $Mg^{2+}$ | $Ca^{2+}$ | $Ba^{2+}$ |

**Figure 1.** Colorimetric response arising from the addition of metal salts to **1**. A total of 10 $\mu$L of a 200 $\mu$M stock of each metal was added to a distinct well in a 96-welled white Teflon plate loaded with 60 $\mu$L of a 35 $\mu$M acetonitrile solution of **1** and 130 $\mu$L of 50 mM HEPES (pH 7.0). The counterions of each metal are indicated by the color provided in the key: nitrates in black, chlorides in blue, and sulfates in red. Within seconds of addition, only the well containing mercury was clear, at which point the mother liquor was transferred to empty wells. **(A)** Yellow color appears upon redissolving the precipitate left in the original well containing $Hg^{2+}$ with acetone. **(B)** Key. Blank space indicates sample without metal.

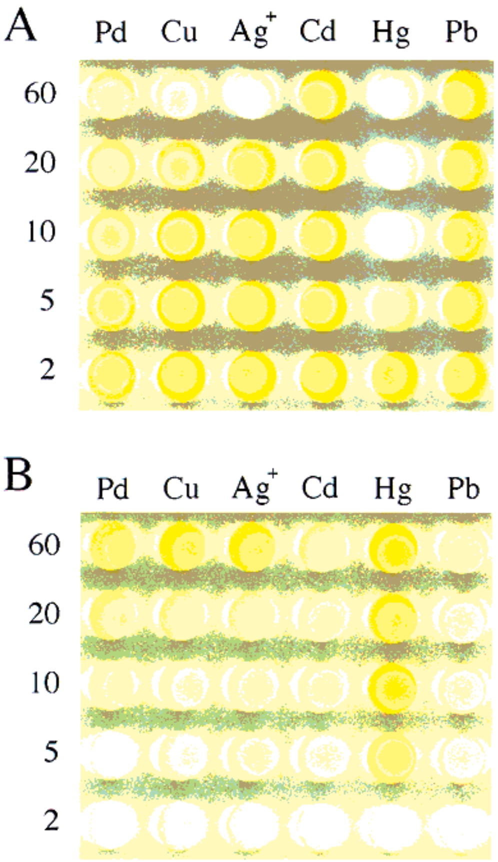


**Figure 2.** Interaction of **1** with metals after 30 min. The plate was loaded as described in Figure 1. The final metal concentration is provided in $\mu$M along the $y$-axis; all metals were added as their nitrate salts. **(A)** Solutions resulting after mother liquor was transferred to another well. The loss of color originates from precipitation of **1.** **(B)** Transfer of the remaining liquid to another well leaves behind the metal complex of **1** that provides a yellow color when dissolved in acetone. Note: cadmium and lead began to bleach these solutions only after 2 h.

Mercuric ion demonstrated the greatest affinity to **1**, thereby permitting detection of nanomolar quantities in the presence of millimolar levels of other common metal ions. The fact that absorption between 415 and 450 nm was *not* altered upon exposing 0.1 equiv of any metal to **7** or nonthiophilic metals to **1** indicated that complexation with the phosphorodithioate was an absolute requirement for this response (Scheme 1A).

This system was advanced to provide a visual test. When examined at greater than 8 $\mu$M in **1** and 5 $\mu$M $Hg^{2+}$, the addition of mercuric ion resulted in a distinct color change from yellow to red. Observation of a comparable transition upon acylation of **2** suggested that this effect originated from reduction of charge transfer (Scheme 1B).[13] The red complex precipitated immediately, leaving the aqueous solution color-

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(12) For general metal ligand interactions, see: O'Sullivan, W. J.; Smithers, G. W. In *Methods in Enzymology*; Purich, D. L., Ed.; Academic Press: New York, 1979; pp 294−336.

(13) (a) La Clair, J. J. *J. Am. Chem. Soc.* **1997**, *119*, 7676. (b) La Clair, J. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 325.

less or bleached. HPLC, $^{31}P$ NMR, and mass spectral analysis indicated the formation of a 2:1 complex of **1** to $Hg^{2+}$ (see Supporting Information).[14] The precipitation of the **1**−$Hg^{2+}$ complex[15] and thereby removal of the metal ion from the solution now presents an irreversible color change.

The selectivity of this chemosensor was demonstrated by screening 42 different metal salts (Figure 1). Within a second after addition, only $Hg^{2+}$ produced an immediate red color and underwent precipitation, thereby satisfying the required selectivity. This observation was further verified by transferring the mother liquor to opaque white wells and by the reappearance of a yellow color upon dissolving the remaining precipitate with acetone (Figure 1A). Within 2 h, a similar response also occurred in wells containing $Ag^{+}$, $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, and $Pd^{2+}$. The relative visual sensitivity was determined to be $Hg^{2+} >>> Ag^{+}, Pd^{2+} >> Cu^{2+} > Pb^{2+} > Cd^{2+}$ (Figure 2). These data correlated with the dissociation constants ($K_D$) of these interactions (Table 1).

This analysis provided a confident visual signal when adding 5 $\mu$M mercuric ion to 8 $\mu$M **1** as judged by the observation of colored or bleached dye solutions (Figure 2). Further quantification of this approach was possible using a conventional spectrophotometer. Changes in absorption that are not distinguishable by the naked eye were apparent upon inspection at a single wavelength between 415 and 450 nm, extending the detection limit to $1 \pm 0.1$ $\mu$M $Hg^{2+}$.

The interaction of metals with the phosphorodithioate recognition group in ligand **1** can be effectively quantified through the spectral changes of a neighboring chromophore. Probe **1** now presents two dimensions of metal sensing. First, the interaction with eleven different metals ($Cd^{2+}$, $Eu^{3+}$, $Gd^{3+}$, $Hg^{2+}$, $In^{3+}$, $La^{3+}$, $Pb^{2+}$, $Tb^{3+}$, $Co^{2+}$, $Zn^{2+}$, $K^{+}$) could be quantified in terms of the dissociation constant, $K_D$, by using a conventional spectrophotometer. Second, reaction of **1** with $Hg^{2+}$ is accompanied by a color change and precipitation, providing an irreversible visual test. The material required for a million assays can be prepared in approximately 1 week from inexpensive materials, as a single visual analysis requires less than 150 ng of **1**. If desired, a microtiter plate reader may be used to extend this method for high-throughput screening and analysis.

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**Supporting Information Available:** Experimental procedures, spectroscopic characterization, metal titration curves, determination of the $K_D$'s, and details about colorimetric assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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[14] (a) Lawton, S. L. *Inorg. Chem.* **1971**, *10*, 328. (b) Burn, A. J.; Dewan, S. K.; Gosney, I.; McKendrick, K. G.; Warrens, C. P.; Wastle, J. P.; Watson, C. W. *J. Chem. Soc., Perkin Trans. 2* **1994**, 373.

[15] Metal ions also form a variety of higher order complexes in both metal and ligand with phosphorodithiolates. While spectroscopic and mass determinations indicate that the precipitation under the given conditions resulted in a 2:1 dye to metal complex, complete precipitation also occurs with far more metal per ligand. For reports describing the formation and precipitation of such complexes, see: (a) Drew, M. G. B.; Hobson, R. J.; Mumba, P. P. E. M.; Rice, D. A. *J. Chem. Soc., Dalton Trans.* **1987**, 1569. (b) Dakternieks, D. R.; Graddon, D. P. *Aust. J. Chem.* **1971**, *24*, 2077.