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# Solvent effect and amine interference on colorimetric changes of azobenzene-conjugated dithiaazadioxo crown ether mercury sensor



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# Introduction

# Highly sensitive detection of mercury ion (Hg<sup>2+</sup>) is of great importance since diverse mercury compounds are emitted from many industries to surrounding milieu as byproducts or wastes, thereby being an environmentally threatening agent for the public health even in extremely low concentrations. Due to its highly toxic nature toward human body, mercury contents of foods and water are strictly regulated in most countries; it is reported to be related with many health problems in the central nerve systems, kidney, and endocrine systems.<sup>1</sup> Therefore, sensitive and costeffective detection methods to monitor the concentrations of Hg<sup>2+</sup> ion have been highly demanding and have attracted much research efforts, which might eventually enable ordinary consumers to measure the content of the hazardous ions. Spurred by these demands, colorimetric mercury sensors have been enthusiastically pursued, and many specialized organic ligands were devised, showing some diversities in terms of specific affinity for mercury ions, cost-effectiveness, and accessibility of synthetic routes. Aside from many organic ligands, oligonucleotides tethered on gold nanoparticles<sup>3</sup> or electrochemical detections<sup>4</sup> have provided

ABSTRACT

The colorimetric behavior of an azobenzene-based dye containing dithiaazadioxo ring for Hg<sup>2+</sup>-detection was investigated in diverse solvents. The mercury specific ligand shows hypsochromic changes in pure and aqueous MeCN upon Hg<sup>2+</sup>-binding while strong bathochromic shift was observed in chloroform. In both solvent systems, dithiaazadioxo ring is revealed to be indispensable to the selective detection of mercury ion, and several binding schemes in different solvents were proposed on the selective Hg<sup>2+</sup> binding. Grafted on cationic polymer (PEI) through amide coupling, it was observed that the ligand basically maintains the Hg<sup>2+</sup> detection capability, but substantially hindered by amine moieties in PEI.

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alternative routes for mercury detection with high sensitivity. Most of fluorogenic or colorimetric dyes largely consist of mercury-binding sites including azathiacrown,<sup>5</sup> dithiacarbamate,<sup>6</sup> or thiooxaaza macrocycles<sup>7</sup> and chromogenic centers like squarine,<sup>8</sup> azobenzene,<sup>5c,9</sup> or rhodamine moieties.<sup>10</sup> An excellent review Letter can be found elsewhere, which is helpful for systematic understanding of recent developments in optical metal ion detections.<sup>2</sup> Herein, a synthesis of azobenzene-based and colorimetric mercury sensor is reported and its distinct color changes on solvent variation are investigated; dithiaazadioxo crown ether, as a mercury capture ligand, is linked to the core chromophore. The sensing molecules are also attempted to covalently tether onto a polymer backbone, which might enable one to reuse the colorimetric indicator on polymer resins. Upon immobilization, the colorimetric indicator showed substantially different behaviors and the effects of polymer grafting are discussed (see Fig. 1).

#### **Results and discussion**

# Dye design and synthesis for Hg<sup>2+</sup> detection

As shown in Scheme 1, initial *N*-phenyl compound 1 was converted into dithiaazadioxo crown ether 2 through the coupling reaction between the dimesylate and corresponding dithiol, which

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Figure 1. The absorbance spectra and optical images of various metal ions (100 µM) with L3 (50 µM) (a) in a mixed solvent of acetonitrile/water (1:1, v/v), (b) in neat acetonitrile, and (c) in chloroform.



Figure 2. The absorbance spectra and optical images of L3 (50 µM) upon the gradual addition of Hg(ClO<sub>4</sub>)<sub>2</sub> (a) in acetonitrile/water (4:1, v/v), (b) in neat acetonitrile, and (c) in chloroform.



**Scheme 1.** A schematic cartoon depicting the synthesis of dithiaazadioxo crown ether, subsequent conjugation with 4-aminobenzoic acid as a mercury sensor, and further conjugation with ethanolamine via amide formation.

was followed by the in-situ intramolecular ring-closure reaction as described in the literature; the overall yield was ~28%.<sup>5d</sup> In the following synthesis of azobenzene chromophore, diazonium salt that had been prepared in advance was coupled to the compound **2**,<sup>5c</sup> yielding the final carboxylated Hg<sup>2+</sup>-binding chromophore **L3** (~65% yield). The major difference with previous reports from other groups is the fact that the current chromophore bears a carboxylate group in the *para*-position instead of nitro group, enabling it to be tethered onto solid or polymeric substrates as a platform for advanced mercury sensor in the future.

In the Table S1 of the Supporting information, several azobenzene dyes were listed as the chromophore for  $Hg^{2+}$  detection, and more fluorogenic or colorimetric dyes employing the dithiaazadioxo ring were also provided. The dyes adopting azobenzenes are mostly colorimetric types and their studies were performed in organic solvents like MeCN or methanol because of the low solubility in aqueous solution. In the meantime, some controversial observations are noteworthy in those dves with the dithiaazadioxo rings as Hg<sup>2+</sup> capture ligand, which motivated us to investigate them in detail. For instance, an anthracene-based dye (D4 in Supporting information) showed preferential selectivity for Ag<sup>+</sup> ions instead of Hg<sup>2+</sup> ions,<sup>11</sup> or some dyes with the same ring showed additional responses for other metal ions like Cu<sup>2+</sup> or Fe<sup>2+</sup> in conjunction with  $Hg^{2+}$  ions while others did not.<sup>9,12</sup> Moreover, similar fluorogenic dyes containing the same tetrathiaaza crown ether (NS<sub>4</sub> ring) demonstrated different selectivity for heavy metal ions. For instance, NS<sub>4</sub> ring with a xanthene derivative showed a preference for Hg<sup>2+</sup> ions<sup>5b</sup> while the same ring with a naphthalimide chromophore preferred to interact with Ag<sup>+</sup> ions.<sup>5e</sup>

### Solvatochromic observations upon Hg<sup>2+</sup> addition

In the first place, the colorimetric changes of the **L3**, responding to diverse metal ions, were examined by dissolving it (50  $\mu$ M) in excessive metal solutions (~2 equiv). The internal charge transfer (ICT) band of **L3** centered at 450 nm shifted to 350 nm only for Hg<sup>2+</sup> ion in aqueous MeCN solution, which yields apparently losing the typical yellow color of **L3** upon binding with Hg<sup>2+</sup> ions. The hypsochromic shift is largely coincident with previous works employing azobenzene chromophores,<sup>5c</sup> and is mostly attributed to the mitigation of electron donating ability with the amine moiety in the dithiaazadioxo ring. As for Ag<sup>+</sup> and Cu<sup>2+</sup> ions, the band intensity slightly diminished, suggesting some interactions with the ligand. In fact, it was previously reported that anthracenebased fluorometric dye (D4) containing the same dithiaazadioxo ring showed highly selective fluorescence toward Ag<sup>+</sup> ion, but not to  $Hg^{2+}$  ions. However, the ICT band of **L3** that had already shifted by chelation of  $Hg^{2+}$  ion (at 350 nm) did not restore the original spectrum (toward 450 nm) in the addition of  $Ag^+$  or  $Cu^{2+}$  ions, indicating preferential binding with  $Hg^{2+}$  over  $Ag^+$  or  $Cu^{2+}$  ions. These controversial observations might be attributed to a new combination of the dithiaazadioxo ring with azobenzene instead of anthracene dye, and suggests some co-operative interaction of  $Hg^{2+}$  ion with the diazo group.<sup>5c,13</sup>

On the other hand, when the working solvent was changed into MeCN only, the overall trends were maintained except two minor changes: First, the hypsochromic shift in response to Hg<sup>2+</sup> ion was widened into a band at 330 nm ( $\Delta v \sim 120$  nm), indicative of changes in push-pull dipole in the absence of water. Second, Cu<sup>2+</sup> ions additionally developed comparable bathochromic bands as well as the small hypsochromic changes mentioned above; similarly Pb<sup>2+</sup> solution also exhibited small bathochromic shoulder band. Although the hypsochromic band height was similar with the bathochromic one in intensity for Cu<sup>2+</sup> ions, the apparent color change in the visible range seems dominated by the bathochromic change (i.e., pink color). Nevertheless, L3 still demonstrated preferential binding with Hg<sup>2+</sup> ions in pure MeCN, even in the presence of other metal ions as observed in MeCN/H<sub>2</sub>O mixture solvent (data not shown).

Intriguingly, another dramatic change was observed when the working solvent was further changed into CHCl<sub>3</sub>. The ICT band at 450 nm substantially red-shifted into ~550 nm for Hg<sup>2+</sup> ions, suggesting that Hg<sup>2+</sup> ion is differently interacting with the L3 in CHCl<sub>3</sub> since the changing direction in the ICT band is guite opposite. Additionally, small decrease in intensity was observed as for Pb<sup>2+</sup> ions, which had shown a bathochromic shoulder band with L3 in MeCN. Resultantly, only in response to Hg<sup>2+</sup> ions, the color of L3 turned to bright pink instead of being colorless as observed in MeCN or MeCN/H<sub>2</sub>O solvents. As noticed above, Cu<sup>2+</sup> ions in pure MeCN exhibited a similar color change, albeit to less extent. The pink color indicates that a similar binding scheme observed in Hg<sup>2+</sup> ions in CHCl<sub>3</sub> might partially contribute to Cu<sup>2+</sup>-L3 interactions in pure MeCN. At this stage, it seems noteworthy that the bright pink color of L3 is usually observed in acidic aqueous solution (lower than pH 1), which is well known to be related with the protonation of diazo group in the push-pull structure of ICT chromophore (carboxylic acid as electron withdrawing group).<sup>13,14</sup> Therefore, the bathochromic shift in CHCl<sub>3</sub> is tentatively attributed



Figure 3. <sup>1</sup>H NMR spectra of L3 (1 mM) in CDCl<sub>3</sub> solution in the presence of different amounts of  $Hg(ClO_4)_2$ .

to diazo chelation of Hg<sup>2+</sup> ion as is depicted in Figure 6. The bathochromic shift from protonation or mercury chelation of diazo group could be elaborated by enlarging ICT dipole moment through fortifying acceptor capability of the push-pull dipole scheme via diazo chelation.<sup>13</sup> In fact, azobenzene groups grafted on silica matrix were reported to have an affinity toward Hg<sup>2+</sup> ion co-operatively and illustrated bathochromic changes.<sup>15</sup> However, the bright pink color of **L3** quickly disappeared by addition of aqueous organic solvents like methanol or MeCN, suggesting that the diazo chelation of Hg<sup>2+</sup> occurs only in the absence of water (data not shown). Overall, all these solvatochromic observations with different solvents suggest that the binding between Hg<sup>2+</sup> ions and L3 is strongly dependent on the natures of solvents. Moreover, to our best knowledge on Hg<sup>2+</sup>-chelating dyes, these quite distinct color changes (colorless in MeCN vs bright pink in CHCl<sub>3</sub>) toward  $Hg^{2+}$  binding have never been observed (see Fig. 1).

# UV/vis titrations upon Hg<sup>2+</sup> addition

In order to evaluate the Hg<sup>2+</sup>-binding ability of **L3** in detail, titrations with various concentrations of Hg<sup>2+</sup> ion were attempted in a fixed concentration of **L3** (50  $\mu$ M). As shown in Figure 2a and b, Hg<sup>2+</sup> ions were observed to interact with **L3** through a one to one correspondence in MeCN solution (aqueous or pure), which was well coincident with previous studies adopting dithiaazadioxobased chromophores.<sup>5d,13</sup> On the other hand, when **L3** was titrated



**Figure 4.** <sup>1</sup>H NMR spectra of **L3** (1.0 mM) (a) in  $CD_3CN/D_2O$  solution and (b) in  $CD_3CN$  by gradual addition of  $Hg(ClO_4)_2$ .

in CHCl<sub>3</sub> as shown in Figure 2c, the interaction ratio changed to 2:1 correspondence, indicating that two molecules of L3 are interacting with a single Hg<sup>2+</sup> ion. Considering the alleged participation of diazo group in binding with Hg<sup>2+</sup> in CHCl<sub>3</sub>, a new binding scheme in CHCl<sub>3</sub> should be proposed for Hg<sup>2+</sup>–L3 interaction where a single Hg<sup>2+</sup> ion is sandwiched between two diazobenzene groups of the two L3 ligands. At this moment, dithiaazadioxo ring appears not to bind with Hg<sup>2+</sup> ions in CHCl<sub>3</sub>, while the same ring captures Hg<sup>2+</sup> ion in MeCN or MeCN/H<sub>2</sub>O solution, which is intriguing in that the dithiaazadioxo ring that was known for a strong mercury chelator is unable to bind mercury in CHCl<sub>3</sub>.

On the other hand, a commercially obtained diazo-compound, without any specific mercury binder, did not show any mercury selectivity, nor did any spectral changes over other metal ions. This suggests the participation of dithiaazadioxo ring upon discriminating  $Hg^{2+}$  ion in CHCl<sub>3</sub> through an unknown mechanism. Interestingly, the involvement of two ligands against a single  $Hg^{2+}$  ion was already observed with a similar azobenzene dye with L3 along with bathochromic shift, but in a different solution.<sup>9</sup> Although any specific scheme was not presented for the interaction with  $Hg^{2+}$  ions, we assume that the same diazo chelation might be applied to the report. In the next section, the proposed binding schemes in different solvents were further investigated by NMR observations during the  $Hg^{2+}$ -titrations.

# NMR titrations upon Hg<sup>2+</sup> addition

In NMR spectra of **L3** in CDCl<sub>3</sub>, protons in the core benzene ring (near the diazo group, 6–8th protons) gradually decreased and broadened as the content of  $Hg^{2+}$  ion increased (see Fig. 3). Among the aliphatic protons in the dithiaazadioxo rings, only protons next to the amine group (5th protons) responded to the  $Hg^{2+}$  ions, indeed indicating the diazo chelation of  $Hg^{2+}$  ions that was proposed above.

Recalling the stoichiometry of **L3** to  $Hg^{2+}$  ion (2:1) in CHCl<sub>3</sub>, a chelation scheme of **L3** could be presented where two diazogroups are interacting with a single  $Hg^{2+}$  ion, but is relatively leaning toward the dithiaazadioxo ring as will be seen below (Fig. 6a). The diazo chelation of **L3** was further confirmed in a control experiment where only the dithiaazadioxo ring (compound **2**) was titrated with  $Hg^{2+}$  and monitored by NMR in CDCl<sub>3</sub>. In this titration, no apparent change was observed in NMR spectra, suggesting diazo-chelation in **L3** is the dominant interaction in CHCl<sub>3</sub> solvent (see Supporting information, Fig. S5).

On the other hand, the spectral shifts of the protons of **L3** in d-MeCN/D<sub>2</sub>O are quite different compared to the changes in CHCl<sub>3</sub> (Fig. 4a). Instead of band broadening, new bands (black arrow) emerged and original bands diminished as the titration proceeded. These NMR spectra seem to definitely show that the Hg<sup>2+</sup> binding modes in two solvents (CHCl<sub>3</sub> vs MeCN/H<sub>2</sub>O) are different and that the dithiaazadioxo rings are dominantly involved in MeCN/H<sub>2</sub>O. In detail, the bands of protons near two sulfur atoms in the dithiaazadioxo ring (3rd and 4th protons) quickly diminished in intensity and shifted down-field, while other bands in the ring were substantially broadened and new bands developed simultaneously; the individual identifications for the newly developed bands were difficult, but were tentatively assigned at our best.

In addition, protons in the aromatic backbone of **L3** also appear to be affected by the addition of  $Hg^{2+}$  ions so that a new band for 6th protons developed down-field while the original one disappears, and other aromatic protons (7–8th) developed up-field; original peak intensities of all the protons in the aromatic backbone diminished and new bands were developed (albeit also broadened), which are different from observations in CDCl<sub>3</sub> (i.e., band broadening without position shifts). These drastic changes in protons of the dithiaazadioxo ring suggest that the dithiaazadioxo cyclic ring plays the expected and major role in capturing  $Hg^{2+}$  ions as designed. The growth of new bands might indicate stronger binding with  $Hg^{2+}$  ions while the band broadening observed in CHCl<sub>3</sub> shows weaker or dynamic interactions. Since similar changes in the NMR titration of compound **2** (without diazo group) were observed in the same solvent system (*d*-MeCN/D<sub>2</sub>O), the major role of the dithiaazadioxo ring and changes in aromatic backbone could be reasonably supported (see Supporting information, Fig. S6).

The band shifts of the dithiaazadioxo ring were more clearly observed in pure *d*-MeCN solvent since the interfering broad and intensive HOD band at ~3 ppm is absent in *d*-MeCN only (Fig. 4b). In this titration, only 1st protons in the dithiaazadioxo ring maintained the characteristic sharp peak, albeit in a shifted position, while other protons (2nd–5th) were both broadened and shifted, implicating that Hg<sup>2+</sup> ion is a little bit biased toward the amine atom. Besides the dithiaazadioxo ring protons, protons in the aromatic diazo group also suffered changes in the positions; 6th proton most drastically shifted down-field while the other bands mildly moved to down-field. Compared with the band shift in *d*-MeCN/D<sub>2</sub>O, the band shifts in the aromatic group (7–9th protons) were reverted into down-field shifting. These observations suggest that hydration layer of Hg<sup>2+</sup> or **L3** seems to affect the binding in *d*-MeCN/D<sub>2</sub>O compared to pure *d*-MeCN.

# Colorimetric behavior upon polymer immobilization

Although the current **L3** has shown very interesting features upon Hg<sup>2+</sup>-binding in organic solvents like CHCl<sub>3</sub> and MeCN, or in aqueous mixtures, most frequent test samples for Hg<sup>2+</sup> ions might be an aqueous solution from food, tap water, or river in the neighborhood. Therefore, most colorimetric sensors for metal ions may be preferable to be designed for water sample. In this regard, several attempts were made to immobilize the mercury chelating ligand onto silica matrix and demonstrated good performances.<sup>15</sup> Instead, we attempted to tether the colorimetric Hg<sup>2+</sup> sensors onto a polymer backbone, which enables **L3** to dissolve easily in aqueous solution and thereby to widen the applicability of the diazo compound, for instance, by layer-by-layer deposition protocol. In Figure 5a, titration curves of **L3** tethered on cationic polymer PEI are shown; roughly 1.0% among the primary amines present in PEI (M.W. 25,000) was decorated with **L3** and the



**Figure 5.** UV/vis spectra of the **L3** (~10  $\mu$ M) conjugated on cationic polymer PEI upon gradual addition of Hg(ClO<sub>4</sub>)<sub>2</sub> in water; (a) **PEI**<sub>1.0</sub>-**L3** (b) **PEI**<sub>65</sub>-**L3**. (c) UV/vis spectra of the **L4** (50  $\mu$ M) on the addition of Hg<sup>2+</sup> ions in aqueous MeCN solution and (d) UV/vis spectral changes of **L3** with one equivalent of Hg<sup>2+</sup> restoring original band upon the addition of free amine molecules.

concentration of the grafted L3 was adjusted to be 10 µM (PEI1.0-L3). Unlike in the state of free L3, PEI1.0-L3 showed substantially decreased sensitivity; for full spectral changes, twenty equivalents or more Hg<sup>2+</sup> ions were needed. In order to see the effect of tagging ratio, it was attempted that more primary amines (25% and 65%) in polymeric PEI were decorated with L3 (PEI<sub>25</sub>-L3 and PEI<sub>65</sub>-L3, respectively). Interestingly, although PEI<sub>25</sub>-L3 demonstrated improved sensitivity to some extent, the responses toward Hg<sup>2+</sup> ions still lagged, indicating that PEI grafting still interfering to the Hg<sup>2+</sup> ion capture. Further increase in L3 grafting ratio up to 65% showed similar responses with PEI25-L3 and still failed to revamp the sensitivity of free L3 ligand, as shown in Figure 5b. With **PEI<sub>65</sub>-L3**, about ten equivalents of Hg<sup>2+</sup> ions ( $\sim$ 100  $\mu$ M) were needed to saturate the spectral changes of the tethered L3  $(\sim 10 \text{ }\mu\text{M})$ . These observations revealed that simply high dose of L3 in cationic PEI cannot improve its binding affinity toward Hg<sup>2+</sup> ions.

In order to clarify the grafting effect, L3 was conjugated with a simple amine like ethanolamine (L4), which is also converting the carboxylic group into an amide one and might affect the push-pull dipole of azobenzene in the same way as in PEI grafting. Intriguingly, L4 demonstrated almost the same responses as in L3 on Hg<sup>2+</sup> additions without decreasing the sensitivity, but **L4** showed two differences as shown in Figure 5c. First, the Hg<sup>2+</sup>-titration of L4 in aqueous MeCN solution revealed very similar responses with L3 (i.e., hypsochrmic changes and one to one correspondence to  $Hg^{2+}$  ions) except one aspect; the hypsochromic changes of L4 in aqueous MeCN are widened (~330 nm) while L3 moved less in the same MeCN/H<sub>2</sub>O solvent (~350 nm). The larger hypsochromic shifts were also observed with PEI<sub>x</sub>-L3 in aqueous solution and with L3 in pure MeCN. Second, the titration of the synthesized L4 with Hg<sup>2+</sup> ion in CHCl<sub>3</sub> demonstrated the same pink color change (bathochromic shifts) and the same spectral features as in L3, but L4 showed 1:1 reaction ratio, suggesting different behaviors of L4 from L3. Since the same bathochromic shift of ICT band was observed in L4, it seems plausible to assume the same diazo chelation with  $Hg^{2+}$  ions in CHCl<sub>3</sub>, but the observed stoichiometry (L4 to  $Hg^{2+}$ , 1:1) seems to require further elaboration. Considering the only difference between L3 and L4, the distinct stoichiometry in CHCl<sub>3</sub> and widened hypsochromic band shift in aqueous MeCN could be plausibly attributed to loss of acidic proton from carboxvlic acid, owing to the amide conjugation.<sup>14</sup>

In the subsequent NMR titration, L4 showed similar changes in d-MeCN with L3, fortifying that similar binding scheme could be applied (see Supporting information, Fig. S7); 1st protons drastically shifted down-field with sharp characteristics while other protons in the dithiaazadioxo ring were shifted and broadened. While additional aliphatic protons from ethanolamine showed no changes indicating less involvement upon Hg<sup>2+</sup> binding, 6th protons in aromatic backbone showed largest shift and other protons in azobenzene chromophore suffered minor changes. Upon the titration in CDCl<sub>3</sub> solvent, no apparent change was observed among protons in the dithiaazadioxo ring except 5th protons as observed in L3. Overall, we could not find any significant changes from NMR titration, which suggests a similar binding scheme on L3 and L4. Therefore, a similar but slightly different binding scheme was proposed for L4 in CHCl<sub>3</sub> as is shown in Figure 6c. In spite of the detailed investigation on L4, we largely failed to account for the origin of the fouled sensitivity in the PEI conjugation of **L3**.

As such, another control experiment was performed in which primary amine molecules were simply added into  $Hg^{2+}$ -saturated L3 solution (MeCN/H<sub>2</sub>O) in advance. As shown in Figure 5d, once shifted ICT band of L3 (~350 nm) gradually restored the original spectrum by the incremental addition of ethanolamine, revealing that free amine groups are preventing L3 (or the dithiaazadioxo



**Figure 6.** Proposed binding schemes of **L3** with  $Hg^{2+}(a)$  in  $CHCl_3$ , (b) in MeCN, and for **L4** with  $Hg^{2+}$  ions, (c) in  $CHCl_3$  and (d) in MeCN.

ring) from binding with Hg<sup>2+</sup> ions. Although it is hard to pin-point the origin of this lagging, it seems that free amine molecules might intercept the added Hg<sup>2+</sup> ions as an alternative chelator or might co-ordinate with the dithiaazadioxo rings preferentially. In either condition, the polymeric nature of PEI seems to exacerbate the binding efficiencies of grafted **L3**. Moreover, it is noteworthy that secondary and tertiary amines (not to mention primary amines) are still massively present even in **PEI<sub>65</sub>-L3**. This is quite an interesting observation since the amine-hindering is unprecedented in the colorimetric mercury detection, while interferences from other metal ions or anions have been intensively investigated, hitherto.<sup>7,9</sup> In addition, this finding might have technical implications in treating biological samples with these synthetic dyes when evaluating mercury level through colorimetric methods.

Combining the overall NMR titration data and UV/vis spectra upon gradual addition of Hg<sup>2+</sup> ions, two major binding schemes were proposed for L3 in MeCN/H2O and CHCl3 solvents as shown in Figure 6. In  $CHCl_3$ , it seems that a single  $Hg^{2+}$  ion resides between two azobenzenes of two L3 ligands (Fig. 6a), while a single Hg<sup>2+</sup> ion does in a dithiaazadioxo ring of L3 in MeCN (pure or aqueous, Fig. 6b). Although the NMR shifts of aromatic protons in pure MeCN were slightly different from those in aqueous MeCN, the proposed binding scheme was tentatively maintained due to the drastic changes of the dithiaazadioxo ring in NMR titrations and similar UV/vis spectral changes in two solvent systems. As noticed before, quite different stoichiometry between L3 and L4 in CHCl<sub>3</sub> is ascribed to the loss of the acidic proton in **L3**, so that two Hg<sup>2+</sup> ions are sandwiched between two L4 as shown in Figure 6c. In aqueous MeCN, L4 is assumed to have the same binding scheme with L3 (Fig. 6d), along with the absence of protonation at the diazo group

that widens the hypsochromic shift in UV/vis spectra as shown above.

In summary, the colorimetric behavior of azobenzene-based chromophore containing the dithiaazadioxo ring for Hg<sup>2+</sup>-detection was investigated in various solvent systems. The Hg<sup>2+</sup>-specific ligand showed hypsochromic changes in pure and aqueous MeCN upon Hg<sup>2+</sup> binding while a strong bathochromic shift was observed in CHCl<sub>3</sub>. For the bathochromic shift, the absence of water was essential and these observations were unprecedented in previous studies employing dithiaazadioxo crown ether for a specific ligand of mercury ion. In both solvent systems, dithiaazadioxo ring is revealed to be indispensable to the selective detection of mercury ion. Guided by these observations, a few binding schemes in different solvents were proposed. Grafted on a cationic polymer (i.e., PEI) through amide coupling, it was observed that **L3** largely maintained the Hg<sup>2+</sup> detection capability, but was substantially hindered by amine moieties in PEI.

### **Experimentals**

# Materials

*N*-Phenyldiethanolamine, methanesulfonyl chloride, 3,6-dioxa-1,8-octanedithiol, 4-aminobenzoic acid, ethanolamine, and deuterium oxide were purchased from Sigma–Aldrich. Chloroform-*d*, acetonitrile- $d_3$ , and dimethylsulfoxide- $d_6$  were purchased from Cambridge isotope laboratories (CIL). <sup>1</sup>H NMR spectra were measured with a Varian HFT 80 spectrometer or with WH300, and UV/vis spectra were obtained from Perkin Elmer VT 3000 spectrophotometer.

# (Phenylazanediyl)bis(ethane-2,1-diyl)dimethanesulfonate (1)

*N*-Phenyldiethanolamine (10.00 g, 55.18 mmol) and triethylamine (22.33 g, 220 mmol) were dissolved in 400 mL of CHCl<sub>3</sub> at 0 °C, and then methanesulfonyl chloride (13.91 g, 120 mmol) was added dropwise. After stirring for 1 h at 0 °C, the reaction mixture was washed with ice water, 5% HCl, saturated NaHCO<sub>3</sub>, and 5% brine sequentially. The organic layer was combined and dried over anhydrous MgSO<sub>4</sub>, filtered, and evaporated in vacuo to give **1** as a solid (18.54 g, 99.6% yield).

# 1,4-Dioxa-7,13-dithia-10-phenyl-10-azacyclopentadecane (2)

3,6-Dioxa-1,8-octanedithiol (9.00 g, 49.37 mmol) was dissolved in 300 mL of dry MeCN, followed by addition of K<sub>2</sub>CO<sub>3</sub> (15.00 g, 108.53 mmol). This suspension was stirred and heated up to 80 °C. Then, **1** (18.54 g, 55.18 mmol) dissolved in 150 mL dry MeCN, was added dropwise for 5 h. After another 12 h, the reaction mixture was cooled down to room temperature, white precipitate was filtered off, and the solvent evaporated in vacuo. The crude residue was chromatographed on silica gel with CHCl<sub>3</sub>, yielding **2** as a white crystalline solid (1.56 g, 28.4%,  $R_f$  = 0.84 with CHCl<sub>3</sub>/ MeOH mixture (9:1)). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.74–2.77 (t, 4H, -CH<sub>2</sub>S), 2.87–2.91 (t, 4H, -CH<sub>2</sub>S), 3.60–3.65 (m, 8H, – CH<sub>2</sub>N, -CH<sub>2</sub>O), 3.78–3.82 (t, 4H, -CH<sub>2</sub>O), 6.63–6.70 (m, 3H, ArH), 7.19–7.24 (t, 2H, ArH).

# 4-((4-(1,4-Dioxa-7,13-dithia-10-azacyclopentadecan-10yl)phenyl)diazenyl)benzoic acid (L3)

Sodium nitrite (8.33 mg, 0.12 mmol) in 10 mL of water precooled at 0 °C was added dropwise into a solution of 4-aminobenzoic acid (16.48 mg, 0.12 mmol) in 20 mL of 6 M HCl at 0 °C and stirred for 20 min. The diazonium salt solution was added dropwise into a solution of **2** (40 mg, 0.12 mmol) in 50 mL of DMF at 0 °C. The solution was stirred for additional 6 h at 0 °C. Then, 20 mL of CHCl<sub>3</sub> and 10 mL of water were added. The organic layer was combined and dried over anhydrous MgSO<sub>4</sub> and filtered. After evaporation of solvent in vacuo, the crude residue was chromatographed on silica gel with CHCl<sub>3</sub>/MeOH mixture (9:1), yielding **L3** as an orange solid (37 mg, 65% yield,  $R_f$  = 0.42 with CHCl<sub>3</sub>/MeOH mixture, 9:1). Absorption maximum of the product is 450 nm. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.74–2.77 (t, 4H, –CH<sub>2</sub>S), 2.87–2.91 (t, 4H, –CH<sub>2</sub>S), 3.60–3.65 (m, 8H, –CH<sub>2</sub>N, –CH<sub>2</sub>O), 3.78–3.82 (t, 4H, – CH<sub>2</sub>O), 6.63–6.70 (d, 2H, ArH), 7.86–7.92 (d, 4H, ArH), 8.18–8.22 (d, 2H, ArH).

# Coupling of L3 with ethanolamine (L4)

**L3** (250.0 mg, 0.53 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 132.3 mg, 0.69 mmol), and *N*-hydroxysuccinimide (NHS, 79.4 mg, 0.69 mmol) were dissolved in methylene chloride/MeOH (10:1). After stirring at rt for 3 h, ethanolamine (48.3 mg, 0.79 mmol) in minimal amount of CHCl<sub>3</sub> was added. After stirring for another 12 h at rt, solvent was evaporated in vacuo. The crude residue was chromatographed on silica gel with CH<sub>2</sub>Cl<sub>2</sub>/MeOH mixture, 10:1, yielding **L4** as an orange solid (120.1 mg, 43.7% yield, *R*<sub>f</sub> = 0.4 with mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH, 10:1).

#### PEI<sub>25</sub>-L3

L3 (25.0 mg, 53.0  $\mu$ mol) in 10 mL of CHCl<sub>3</sub> was followed by the addition of dicyclohexylcarbodiimide (DCC, 10.9 mg, 58.3  $\mu$ mol) and NHS (6.0 mg, 58.3  $\mu$ mol). After stirring at rt for 12 h, 16.5 mg of polyethyleneimine (PEI, *M*w 25,000) was added to thus-formed NHS ester of L3 solution. After stirring for another 6 h, the reaction mixture was washed with water three times. The whole aqueous layer was dialyzed in dialysis bag (MWCO 7000) with water. After dialysis for 60 h, 100 ml of **PEI<sub>25</sub>-L3** aqueous solution (0.2 mg/ml) was obtained (49.3% yield).

# PEI<sub>65</sub>-L3

Typically, **L3** (122.0 mg, 0.26 mmol) in 10 ml of chloroform was followed by the addition of DCC (70.0 mg, 0.34 mmol) and NHS (40.0 mg, 0.35 mmol) in minimal amount of CHCl<sub>3</sub>. After stirring at rt for 12 h, PEI (*Mw* 25,000, 80.0 mg) dissolved in CHCl<sub>3</sub> was added to the solution of NHS ester of **L3**. After stirring for additional 6 h, the reaction mixture was washed with water three times through a separatory funnel. The whole aqueous layer was dialyzed in dialysis bag (MWCO 3500) with water. After dialysis for 72 h, 80 ml of aqueous **PEI<sub>65</sub>-L3** solution (0.55 mg/ml) was obtained.

#### NMR titration experiments

For the NMR titration studies in CDCl<sub>3</sub>, a stock solution of  $Hg(ClO_4)_2$  (5.0 mM) dissolved in CDCl<sub>3</sub> was added incrementally by using a micropipette (20–200 µL) to the **L3**, **L4**, and compound **2** (typically ~1.0 mM) dissolved in CDCl<sub>3</sub> (700 µL) in the NMR tube. For other solvent systems, previously dissolved  $Hg(ClO_4)_2$  in corresponding solvents was gradually added to **L3** or **L3** derivatives.

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#### Supplementary data

Supplementary data (additional NMR data for L3 and L4 syntheses, and  $Hg^{2+}$ -titrations of L4 and compound 2 in organic solvents, in which UV/vis titration curves of L4 in organic solvents and UV/

vis spectra of simple azobenzene responding to the addition of various metal ions) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013. 10.013. These data include MOL files and InChiKeys of the most important compounds described in this article.

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