8,8’-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis-(methylene)diquinolin-7-ol (TDBQ) was synthesized and proved to recognize Hg$^{2+}$ via reducing Hg$^{2+}$ to Hg$^{+}$, forming a unique Hg$^{2+}$/TDBQ complex.

Mercury is considered to be one of the most serious environmental and health threats. The long atmosphere residence span of Hg0 vapour and its oxidized inorganic Hg$^{2+}$ form provide facile pathways to contaminate vast amounts of water and soil. To date, intensive efforts have been made to develop molecular sensing systems for the mercury ion. Most of the detection methods have one or more limitations in terms of actual applicability, including interference from other metal ions, slow response to Hg$^{2+}$, incompatibility with aqueous solution, low selectivity and fluorescence quenching upon Hg$^{2+}$ coordination via enhanced spin-orbit coupling, energy or electron transfer. To date, the fluorescence technique offers a promising approach for simple and rapid tracking of mercury ions.

Herein, we present the facile synthesis of a novel mercury ion chemosensor based on diaza-18-crown-6 appended with dual 7-hydroxyquinoline (7HQ) groups, forming 8,8’-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis-(methylene)-diquinolin-7-ol (TDBQ) via a Mannich type of condensation. In this approach, diaza-crown ethers are especially focused on. Upon binding, the positive charge density of the metal ion leads to a reduction in electron density on the coordinating aza nitrogen atom and thereby may cause changes in the optical properties. Moreover, metal ion complexation abilities and selectivities of diaza-crown can be greatly improved when the ligating, proton-ionizable groups are attached to the crown ethers. As a strategic design, the phenolic groups and/or the pyridyl nitrogen in 7HQ may act as a sidearm to enhance the complexing ability for selected metal ions. Also, the double armed diaza-crowns in TDBQ may serve as a pseudo-cryptand, which renders additional binding capability. This viewpoint has been supported by an elegant work recently published by Farruggia et al., in which they applied two 8-hydroxyquinoline bearing a diaza-18-crown-6 derivative binding site as a fluorescent...
As for the signal transduction, 7HQ is selected mainly based on two characteristics. First, 7HQ is water soluble and is a bifunctional probe with derivatives comparable to those in many biological molecules, including DNA base units. Second, due to the unique proton tautomerization, 7HQ in aqueous solutions, depending on pH, consists of four protropic equilibrium species: a normal molecule (N), a cation (C), a anion (A), and a zwitterion (Z) (see Scheme 1). Upon metal ion binding, we then expect significant spectral variation suited for signal differentiation.

As depicted in Fig. 1 (line (a)), TDBQ in H2O (pH = 7) revealed lower lying absorption bands at ~330 and ~410 nm, the results of which are reminiscent of normal (N) and zwitterion (Z) species of 7HQ, respectively, assigned in neutral water. Based on the spectral convolution, Mason et al. deduced the equilibrium molar percentages of N and Z species in 7HQ to be ~67% and 30%, while the sum of cation (C) and anion (A) species was estimated to be as small as ~3% in neutral water. Upon adding Hg2+ the absorbance of both N (330 nm) and Z (410 nm) tends to decrease, accompanied by a gradual increase of the anion species (A) at 358 nm. Throughout the titration, the appearance of isosbestic points at 383, 327, 315 nm was observed.

The absorption spectral changes suggest that equilibrium greatly shifts from N and Z species to A in the presence of Hg2+. Moreover, the sigmoid plot for e.g. 358 nm absorbance versus Hg2+ concentration (see inset of Fig. 1) leads to a proposal of TDBQ complexation requiring a cooperative addition of two Hg2+ ions. Firm support of this was provided by the Job’s plot analysis, in which increases of absorbance at e.g. 358 nm were plotted against molar fractions of TDBQ and Hg2+ under the condition of a constant total concentration. As such, the concentration of TDBQ-Hg2+ reached a turning point when the molar fraction of [Hg2+]/([Hg2+] + [TDBQ]) was about 0.6 (Fig. 2A), indicating a stoichiometry of 2(Hg2+): 1(TDBQ) for the association.

A direct, firm evidence of the stoichiometry of complexation perhaps relies on the structural identification. In this approach, single crystal was successfully

![Scheme 1.](image-url)
obtained from a slow diffusion of MeOH into a water solution of either Hg\(^{2+}\) free TDBQ or a 1:2 mixture of TDBQ and Hg(ClO\(_4\))\(_2\) • xH\(_2\)O. As shown in Fig. 3(a), the molecular structure of TDBQ reveals a pocket-like geometric conformation due to the hydrogen bond interaction between the hydroxyl group (O(11)-H(11) of 7HQ and nitrogen atom (N(1)) of the macrocycle with an O(11)--N(1) distance of 2.683 Å. Upon addition of Hg\(^{2+}\), TDBQ also forms a pocket-like geometric conformation (Fig. 3(b)), in which two mercury cations are trapped by the macrocycle, mainly due to the coordination of mercury ions (Hg(1) and Hg(2)) with two nitrogen atoms (N(1) and N(2)), two oxygen atoms (O(1) and O(3)) of the macrocycle and nitrogen atoms (N(3), N(4)) of 7HQ. In a qualitative manner, the motion of TDBQ to recognize mercury ion resembles the Sensitive Plant. In other words, TDBQ is able to pinch and form a cavity suitable for accommodating dual mercury ions.

As depicted in Fig. 3(b), the nitrogen of 7HQ also donates a lone-pair electron to the mercury ion, facilitating the complex formation. Surprisingly, the Hg(1)-Hg(2) distance of 2.5207(3) Å is significantly shorter than Hg-Hg distances of 2.7362(6), 2.6881(4), and 2.5469(8) Å reported in [Pt(Hg\(_2\))(P\(_3\)phen)](PF\(_6\))\(_2\), [Pd(Hg\(_2\))(P\(_3\)phen)](PF\(_6\))\(_2\), and [Hg\(_2\)(TLA)](ClO\(_4\))\(_2\), respectively;\(^{11(a),(b)}\) however, it is comparable with that of 2.5358(8) Å in [Hg\(_2\)(C\(_6\)H\(_4\)N\(_6\)Se\(_2\))]\(\cdot\)(PF\(_6\))\(_2\).\(^{11(d)}\) It is also noteworthy that the bond length for Hg\(^{2+}\) is 2.524(2) Å in water.\(^{11(d)}\) The result indicates the sum of charges for two mercury ions to be +2 in the crystal.

To provide another evidence of the charge of mercury ion in the solid crystal, XANES measurement was performed at Hg LIII-edge of the corresponding crystal. For comparison, spectra of HgCl\(_2\) and Hg\(_2\)Cl\(_2\) are also shown in Fig. 4. According to the electric-dipole selection rules, the spectral region from 12280-12295 eV is assigned to the transition of 2p\(_{3/2}\) → 6s,\(^{12}\) while those of > 12295 eV are attributed to the transitions of 2p\(_{3/2}\) → 6d. Hg\(^{2+}\) exhibits an obvious peak at 12285 eV, which apparently lacks at the same energy for Hg\(^+\). This is due to the fact that Hg\(^{2+}\) has a higher density of the empty 6s electronic state than that of Hg\(^+\). The XANES spectrum of the prepared TDBQ-mercury crystal resembles that of the reference Hg\(^+\), indicating that the oxidation state of the crystal is best described as Hg\(^+\).

The reduction of Hg\(^{2+}\) has been observed in the structural characterization of a Hg\(_2\)\(^{2+}\) complex containing a tripodal nitrogen ligand.\(^{13}\) It is plausible that Hg\(^{2+}\)
would be reduced to Hg⁺ under the tripodal nitrogen ligands, i.e. diazacyclooctadecane in TDBQ, containing solution. The reduction of Hg²⁺ may be facilitated by Hg(I)-Hg(I) bonding formation in combination with further stabilization by diazacyclooctadecane complexation, forming a stable Hg₂²⁺/TDBQ complex. This may lead to a positive reduction potential, which is large enough to have the overall negative changes of ΔG upon coupling with e.g. oxidation of water. Nevertheless, full decipherment of the actual redox mechanism is still pending resolution.

On the above basis, thermodynamic parameter such as the association constant of the complexation can be deduced via the, relationship of absorbance, $A_{ab}$ versus added Hg²⁺ concentration ($C_g$) expressed in eq. 1

$$\frac{C_g}{A_{ab} - \varepsilon_{TDBQ} C_g} = \left( \frac{1}{C_g} \right) \frac{1}{\varepsilon_{TDBQ} - \varepsilon_{TDBQ}}$$

where $C_g$ denotes the initially prepared concentration of TDBQ. $\varepsilon_{TDBQ}$ (~ $2.1 \times 10^4$ M⁻¹ cm⁻¹) is the molar extinction coefficient of TDBQ (A) at peak wavelength of 358 nm. As a result, a straight line plot of $C_g/(A_{ab} - \varepsilon_{TDBQ} C_g)$ versus TDBQ/[Hg²⁺] (see Fig. 2B) supports the proposal of Hg₂²⁺ for the complexation. The association constant $K_a$ was then deduced to be $4.47 \times 10^6$ M⁻² from the quotient of intercept divided by the slope.

As for the fluorescence titration, the excitation of Hg²⁺ free TDBQ at e.g. 358 nm leads to a zwitterion-like (Z) emission maximized at ~517 nm. Upon addition of [Hg²⁺], the 358 nm excitation, which is the peak wavelength of the TDBQ anion species incorporating Hg₂²⁺, rendered an increase of the emission band with peak wavelength at 527 nm (see Fig. 5). The lack of 7HQ-anion-like 450 nm emission¹⁰,¹¹ leads us to propose that the Hg₂²⁺ incorporated TDBQ anion species undergoes excited-state charge transfer from phenolic (HOMO) to the pyridyl (LUMO) moiety,¹⁴ resulting in strengthening of the pyridyl-Hg₂²⁺ dative bond. The net result resembles a zwitterion-like structure (see Scheme 1) that gives rise to the 527 nm emission similar to that of the Z form of 7HQ.¹⁴ Note the emission intensity increases by ~one order of magnitude during the titration (see Fig. 5). Based on emission spectra, the reversibility of the recognition process was performed by adding mercury chelating agents, sodium-2,3-dimercapto-1-propanesulfonate (DMPS). As shown in Fig. 5, after addition of 1 mM DMPS into the solution containing TDBQ and mercury ion, the zwitterion (Z) emission intensity decrease by ~5 folds. Subsequently,
adding 1.5 mM Hg(ClO$_4$)$_2$ leads to the increase of the emission back to ~80% of the original intensity, revealing its good reversibility.

To probe the Hg$_2$$^{2+}$/TDBQ complex stability as a function of pH, the absorption spectrum of a solution containing TDBQ and Hg$_{2}$$^{2+}$ in equimolar amounts (1.5 μM) at various pH values was constructed. For comparison, acid-base absorption titration of TDBQ was also performed. The results of TDBQ showed that pK$_a$ values for the deprotonation of the nitrogen atom and of the hydroxyl groups in H$_2$O were 5.5 and 9.2, respectively. For Hg$_{2}$$^{2+}$/TDBQ, the second deprotonation process (pK$_a$ ~8.2) shifted to lower pH values than the first one. This change in pK$_a$ was presumably caused by chelation of mercury ion with the phenolic oxygen. Deprotonation of both phenol groups on TDBQ associated with Hg$_{2}$$^{2+}$ yielded a neutral complex. This neutral complex with Hg$_{2}$$^{2+}$ was formed at pH values higher than 7. Similar results have been reported in the structural characterization of diaza-18-crown-6 appended with two 5-chloro-8-hydroxyquinoline-7-yl groups, the effective chemosensors for Mg$_{2}$$^{2+}$ or Hg$_{2}$$^{2+}$.

Finally, competitive experiments have been performed and the results are depicted in Fig. 6. In this experiment, 1 μM solution of TDBQ was tested in the presence of other metal cations, including alkali, alkaline earth metals and certain heavy metals. As shown by the enhancement of emission intensity after adding equal molar Hg$_{2}$$^{2+}$ with respect to the competing metal ions (Fig. 6), TDBQ seems to retain moderate selectivity for Hg$_{2}$$^{2+}$ in the presence of a range of competing ions found in environmental and biological settings.

In conclusion, TDBQ achieves Hg$_{2}$$^{2+}$ recognition via the reduction of Hg$_{2}$$^{2+}$, forming a Hg$_{2}$$^{2+}$/TDBQ complex. In this approach, two 7HQ moieties not only act as dual sidearms attached to diaza-18-crown-6, forming pseudo-cryptand that greatly enhances the binding strength, but also serve as a signal transducer. Single crystals of TDBQ-mercury complex have unambiguously resolved a dinuclear (Hg) center with Hg$_{2}$$^{2+}$ formation that is unique among numerous approaches for sensing mercury ion.


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