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# Ratiometric Sensing of $Hg^{2+}$ Based on the Calix[4]arene of *Partial Cone* Conformation Possessing a Dansyl Moiety

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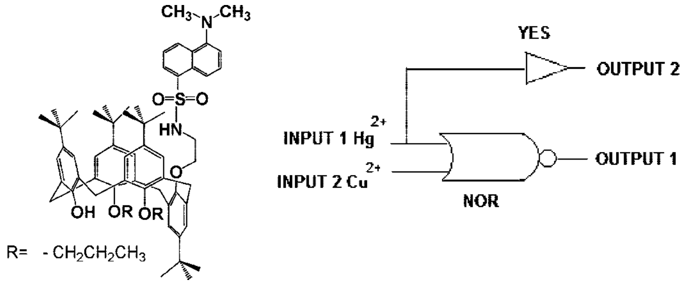
## ABSTRACT



**A new fluorescent chemosensor based on the calix[4]arene of *partial cone* conformation possessing a dansyl moiety has been synthesized. The chemosensor demonstrates selective optical recognition of $Hg^{2+}$ and $Cu^{2+}$ in two contrasting modes. The receptor exhibited ratiometric sensing of $Hg^{2+}$ and "ON−OFF" type of fluorescence behavior in the presence of $Cu^{2+}$. The compound behaves as a fluorescent molecular switch upon chemical inputs of $Hg^{2+}$ and $Cu^{2+}$ ions.**

Among heavy and soft metal ions, mercury is considered as a highly toxic element, and its contamination is widespread which arises from a variety of natural and anthropogenic sources[1] including oceanic and volcanic emission,[1,2] gold mining,[3] solid waste incineration, and combustion of fossil fuels.[4] The exposure to mercury even at very low concentration leads to digestive, kidney, and especially neuorological diseases.[5] The Environmental Protection Agency (EPA) standard for the maximum allowable level of inorganic Hg(II) in drinking water is 2 ppm.[6] Keeping in view the roles played by mercury in day to day life, the development of techniques for mercury hazard assessment and mercury pollution management is in great demand. Fluorescence signaling is one of the first choices due to its high detection sensitivity and simplicity which translate molecular recognition into tangible fluorescence signals.[7] Thus, designing fluorescent sensors for mercury[8] has drawn worldwide attention. Recently, fluorescent chemosensors based on the calix[4]arene scaffold are coming up as good candidates for the detection of various metal ions.[9] However, there are only a few examples of calix[4]arene-based chemosensors for the detecion of heavy and soft metal ions[10] in particular for $Hg^{2+}$.[11]

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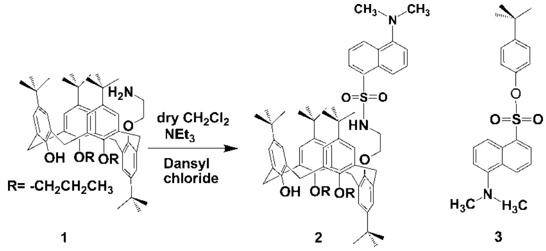
10.1021/ol801984y CCC: $40.75

Our research involves the design, synthesis, and evaluation of calix[4]arene- and thiacalix[4]arene-based receptors selective for soft metal ions.[12] We reported[13] recently a new ratiometric fluorescent sensor for copper based on a thiacalix[4]arene of 1,3-*alternate* conformation possessing two pyrene moieties. The compound behaves as an INHIBIT logic gate with NOT and YES logic functions in the presence of $Cu^{2+}$ and $K^+$ as chemical inputs. Now we have prepared a new ratiometric chemosensor for $Hg^{2+}$ based on a calix[4]arene of *partial cone* (*paco*) conformation possessing a dansyl moiety. In the presence of $Hg^{2+}$ ions, the receptor **2** undergoes fluorescence quenching at 502 nm and shows the formation of two new blue-shifted bands at 412 and 435 nm. The compound behaves as a fluorescent molecular switch upon chemical inputs of $Hg^{2+}$ and $Cu^{2+}$. To the best of our knowledge, this is the first report where a calix[4]arene with one dansyl moiety in *paco* conformation has been used as a receptor for molecular switching with dual outputs.

Thus, the reaction of 5,11,17,23,-tetra-*tert*-butyl-25-*anti*-(2-aminoethoxy)-27-hydroxy-26,28-dipropoxy calix[4]arene **1** of *paco* conformation with 1.1 mol equiv of dansyl chloride in the presence of 1.1 mol equiv of triethyl amine in dry dichloromethane afforded podand **2** in 30% yield (Scheme 1). The structure of compound **2** was confirmed from its spectroscopic and analytical data. The $^1H$ NMR spectrum of compound **2** showed three singlets (18H, 9H, 9H) for *tert*-butyl protons, two multiplets for $OCH_2$ protons (2H and 4H), one triplet for $NCH_2$ protons, one singlet for $NCH_3$ protons (6H), one multiplet for $CH_2$ protons (4H), one triplet for $CH_3$ protons (6H), four doublets for $CH_2$, two singlets (1H) and two doublets (1H) for aromatic protons of calix[4]arene moiety, four doublets for dansyl protons (6H), a sharp singlet for one phenolic proton, and one broad signal for the NH proton (see Supporting Information S4 and S14). This NMR data suggest that compound **2** is in *paco* conformation. The X-ray structure (see Supporting Information S6) confirms the above observation and further shows that there is an intramolecular hydrogen bond formation between the phenolic OH group and the oxygen atom of the propyl chain on the adjacent benzene ring (see Supporting Information S7), which is believed to preclude through annulus rotation of the phenolic group, and hence compound **2** stays in the *paco* conformation in solution. We have also synthesized a simple reference compound **3** (for details, see Supporting Information S5 and S20) from *p*-*tert*-butylphenol and dansyl chloride.

**Scheme 1**



The binding behavior of compound **2** toward different cations ($Cu^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Ag^+$, $K^+$, $Na^+$, and $Li^+$) was investigated by UV−vis absorption and fluorescence spectroscopy. The absorption spectrum of **2** is characterized by the presence of a typical dansyl absorption band at $\lambda = 340$ nm in THF/$H_2O$ (9:1) which undergoes a blue shift of 55 nm as mercury ions were added (Figure 1). This indicates that the mercury ions are interacting with the receptor **2**. The addition of increasing amounts of $Hg^{2+}$ from 5 to 50 $\mu$M (0.1−10 equiv) resulted in a decrease in absorption at 340 nm and an increase of a new blue-shifted band at $\lambda = 286$ nm with an isosbestic point at 311 nm (Figure 1). This new blue-shifted band at 286 nm is a characteristic band[14] for the protonated form of the dimethylamino group of the dansyl moiety. Thus, the spectral changes in Figure 1 are due to protonation of the dimethylamino group, which causes a significant decrease in the charge density on the nitrogen atom of the dimethylamino group. This decreases the charge-transfer interactions between electron-rich and electron-deficient moieties resulting in a band shift.

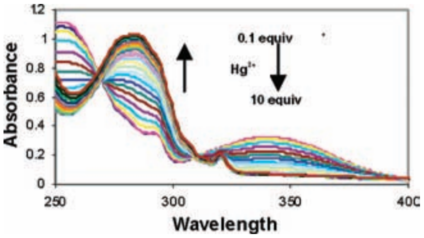


**Figure 1.** UV−visible spectra of **2** ($5 \times 10^{-5}$ M) in the presence of $Hg^{2+}$ ions (0.1−10 equiv) in THF/$H_2O$ (9:1, v/v) buffered with HEPES, pH = 7.0.

The modulation in the electron-donating capabilities of the nitrogen atom of the dimethylamino group in the presence and in the absence of mercury directly influences the intramolecular charge transfer (ICT) from the nitrogen atom of the dimethylamino group to the electron-deficient naphthalene moiety. In the absence of mercury, ICT is efficient, while in the presence of mercury, the extent of ICT from the nitrogen atom of the dimethylamino group to the naphthalene is decreased. Similar results were obtained when relatively strong acid trifluoroacetic acid (TFA) was specif-

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ically employed which confirms the protonation of the dimethylamino group in the dansyl moiety (see Supporting Information S8).

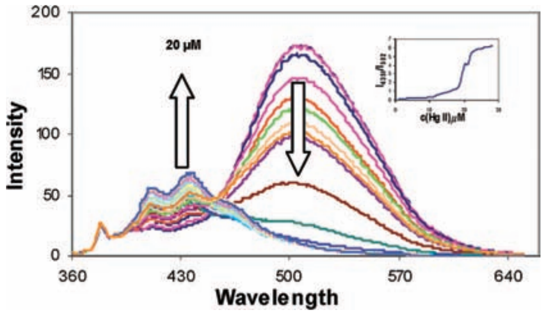

**Figure 2.** Fluorescence spectra of **2** (1 $\mu$M) in response to the presence of $Hg^{2+}$ ions (20 $\mu$M) in $THF/H_2O$ (9:1, v/v) buffered with HEPES, pH = 7.0; $\lambda_{ex} = 338$ nm. Inset: Ratiometric calibration curve $I_{435}/I_{502}$ as a function of $Hg^{2+}$ ion concentration.

In the fluorescence spectrum, compound **2** exhibits a characteristic emission band[11] at 502 nm when excited at 338 nm (Figure 2). Upon addition of small amounts of $Hg^{2+}$ (0.1−20 $\mu$M) to the solution of compound **2**, a significant decrease in the 502 nm emission band with new blue-shifted emission bands centered at 435 and 412 nm which were attributed to the $\mathbf{2}\cdot Hg^{2+}$ complex was observed with an isoemission point at 458 nm (Figure 2). The inset in Figure 2 shows the dependence of intensity ratios of emission at 435 nm to that at 502 nm ($I_{435}/I_{502}$) on $Hg^{2+}$, which indicates the formation of a complex between **2** and $Hg^{2+}$. These new blue-shifted emission bands centered at 435 nm and at 412 nm were also formed when the fluorescence titrations were carried out with trifluoroacetic acid; however, there was no change in the emission band at 502 nm (see Supporting Information S9) indicating that the new bands are formed due to the protonation of the dansyl moiety. On the basis of these results, we propose that the spectral changes in Figure 2 are due to the protonation of the dansyl moiety as a result of proton transfer from the phenolic hydroxyl group (see Supporting Information S10) in the presence of mercury perchlorate. The deprotonation of a phenolic OH group observed in the present investigation has been previously reported by Kim[15] in the presence of lead and indium perchlorates.

Under the same conditions as used above for $Hg^{2+}$, we also tested the fluorescence response of **2** to other metal ions such as $Li^+$, $Na^+$, $K^+$, $Ni^+$, $Zn^{2+}$, $Cd^{2+}$, $Ag^+$, $Cu^{2+}$, and $Pb^{2+}$, besides $Hg^{2+}$, and as shown in Figure 3a, no significant fluorescence change of **2** occurred in the presence (0.1−20 $\mu$M) of these metal ions except copper where fluorescence quenching takes place on addition of 8 $\mu$M of copper but no new bands are formed (see Supporting Information S11).

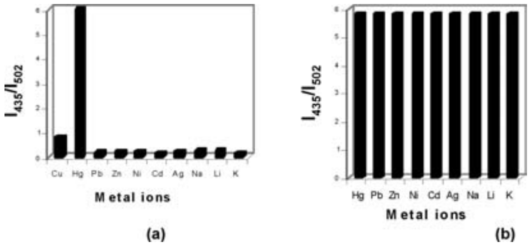

**Figure 3.** (a) Ratiometric ($I_{435}/I_{502}$) selectivity of **2** (1 $\mu$M) upon addition of different cations (20 $\mu$M). (b) Ratiometric fluorescent response of sensor **2** (1 $\mu$M) to $Hg^{2+}$ (20 $\mu$M) over the selected metal ions (20 $\mu$M).

Fitting the changes in the UV−visible spectra of compound **2** with mercury ions, using the nonlinear regression analysis program SPECFIT,[16] gave a good fit and demonstrated that 1:1 stoichiometry (host:guest) was the most stable species in the solution with a binding constant log $\beta_1 = 5.94$. The method of continuous variation (Job's plot) was also used to prove the 1:1 stoichiometry (host:guest) (see Supporting Information S12). To test the practical applicability of compound **2** as a $Hg^{2+}$-selective fluorescence sensor, competitive experiments were carried out in the presence of $Hg^{2+}$ at 20 $\mu$M mixed with $Li^+$, $Na^+$, $K^+$, $Ni^+$, $Zn^{2+}$, $Cd^{2+}$, $Ag^+$, and $Pb^{2+}$ at 20 $\mu$M, and as shown in Figure 3b, no significant variation in the intensity ratio ($I_{435}/I_{502}$) was found by comparison with and without the other metal ions besides $Hg^{2+}$. This means that compound **2** has a high selectivity for $Hg^{2+}$ ions. The detection limit[17] of **2** as a fluorescent sensor for the analysis of $Hg^{2+}$ was determined from a plot of fluorescence intensity as a function of the concentration of the added metal ions. It was found that **2** has a detection limit of $1 \times 10^{-7}$ mol/L for $Hg^{2+}$ which is sufficiently low for the detection of the submillimolar concentration range of $Hg^{2+}$ ions found in many chemical systems.

To elucidate the binding mode of receptor **2** with mercury, the $^1H$ NMR spectrum of its complex with mercury perchlorate was also recorded. The proton of the phenolic hydroxyl group disappeared, while a significant downfield shift of 0.49 ppm is observed for the protons of the $CH_3{-}N{-}CH_3$ group in the mercury complex (see Supporting Information S16 and S17), which indicates that the lone pair of electrons on the nitrogen atom is protonated. The protons of the aromatic ring bearing a hydroxyl group undergo an upfield shift by 0.21 ppm, while protons of the other rings undergo an upfield shift by 0.11, 0.10, and 0.03 ppm, respectively. This upfield shift is due to the fact that after deprotonation of the phenolic hydoxy group the electron density in the aromatic ring increases which results in an upfield shift of the protons. Thus, from this NMR study, we may conclude that mercury is interacting with receptor **2** as supported by UV and fluorescence studies. The binding behavior of reference compound **3** was also studied under the same conditions as used above for compound **2**, and it was observed that no significant fluorescence change occurred on adding 20 $\mu$M of $Hg^{2+}$ ions (see Supporting Information S13) which indicates the role of the calix[4]arene framework of receptor **2** in detecting mercury ions. Further,

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we believe that the *paco* conformation of the receptor might be responsible for the selectivity toward $Hg^{2+}$ ions since in this conformation the phenolic hydroxyl group is involved in hydrogen bonding with the oxygen atom on the adjacent alkyl group, and on adding mercury, this hydroxyl group gets deprotonated and the mercury coordinated to the ionized phenoxide group. This type of hydrogen bonding may not be possible in the case of 1,3-*alternate* conformation but may be possible in the case of 1,2-*alternate* or *cone* conformation. Work is in progress to synthesize these derivatives in our laboratory.

Recently, there has been a lot of activity in the development of supramolecular systems behaving as molecular logic gates.[18,19] In particular, a system consisting of chemically encoded information as input and fluorescent signal as output has attracted a lot of interest. Thus, various molecular systems showing AND, OR, NOR, INHIBIT, XOR, YES, NOT, and XNOR logic gates have been reported[17,18] mostly with only one output mode. However, recent interest is focused on integrated systems involving multiple fluorescent output modes;[20] as with multiple output modes, molecular systems forming basic logic gates can be programmed into a single molecular switch. These molecular switches can be used in complex circuits in design and development of molecular electronic and photonic devices for information, processing, sensing, and computation. However, there are only a few reports of such logic circuits. Since there has not been any report in which the calixarene of *paco* conformation containing a dansyl unit has been used as molecular logic gates with $Hg^{2+}$ and $Cu^{2+}$ as inputs, we utilized our system as a NOR logic gate with a YES logic function. The NOR gate has a high value in electronics, since multiple copies of this gate can be wired up to emulate all the other logic types.

Addition of 8 $\mu$M $Cu^{2+}$ to the solution of receptor **2** quenches the flourescence intensity of the receptor at 502 nm. This remarkable fluorescence quenching induced by $Cu^{2+}$ is ascribed to reverse PET from the naphthalene unit to the nitrogen atom of which the electron density is diminished by metal complexation. Addition of $Hg^{2+}$ to the **2**•$Cu^{2+}$ complex completely quenched the emission at 502 nm with formation of new blue-shifted bands at 412 and 435 nm (Figure 4a). Thus, from the above fluorescent behavior of **2**, following a truth table (Table 1) and logic circuit, Figure 4b is constructed. In the absence of any chemical input, receptor **2** shows a chracteristic band of dansyl at 502 nm, i.e., output 1. By operation by $Hg^{2+}$ to receptor **2**, output 1 becomes “0”, and a new blue-shifted band appears at 435 nm which is considerd as output 2; hence, the fluorescence becomes on and the output 2 is “1” (Figure 4a). In the prescence of $Cu^{2+}$, the band at 502 nm is completely quenched, hence the output 1 becomes “0” (Figure. 4a). In the simultaneous presence of both the metal ions, the output 1 becomes “0” and output 2 becomes “1” due to the formation of the more stable $Hg^{2+}$ complex (Figure 4a). The truth table value for output 1 forms a NOR logic gate, and the truth table values for output 2 form a YES logic gate. The combination of these intrinsic properties with selectivity of actions by different chemical inputs allows its implementation to design a complex molecular switch as shown in Figure 4b.

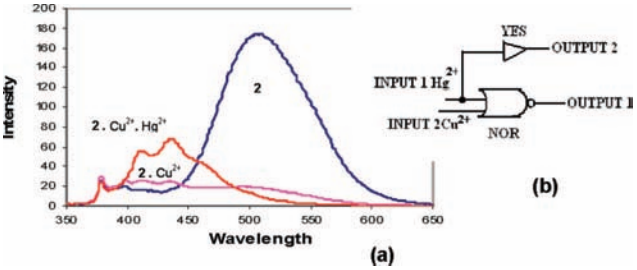

**Figure 4.** (a) Addition of 8 $\mu$M $Cu^{2+}$ to a solution of **2** (1 $\mu$M) and further addtion of 20 $\mu$M $Hg^{2+}$. (b) Combinatorial logic circuit of a dual output molecular switch.

**Table 1.** Truth Table

| entry | input 1<br>$Hg^{2+}$ | input 2<br>$Cu^{2+}$ | output 1<br>502 nm | output 2<br>435 nm |
|---|---|---|---|---|
| 1 | 0 | 0 | 1 | 0 |
| 2 | 1 | 0 | 0 | 1 |
| 3 | 0 | 1 | 0 | 0 |
| 4 | 1 | 1 | 0 | 1 |

Thus, we have synthesized a new chemosensor based on a calixarene of *paco* conformation possessing a dansyl moiety which has selectivity for $Hg^{2+}$ and $Cu^{2+}$ but in contrasting modes. In addition, a dual output molecular switch is designed when the system is operated by inputs of $Hg^{2+}$ and $Cu^{2+}$. The concept presented here contributes to the construction of a more miniaturized and integrated molecular level device with multiple functions.

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**Supporting Information Available:** Experimental details and spectroscopic data of compounds **2** and **3** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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