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Naphthalimide Appended Rhodamine Derivative: Through Bond Energy Transfer for Sensing of Hg^{2+} Ions

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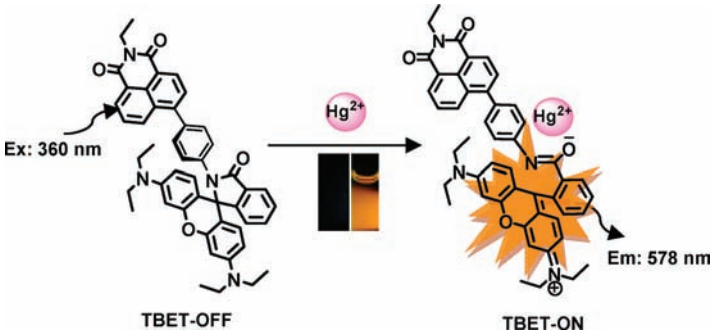
ABSTRACT



A naphthalimide appended rhodamine based fluorescent chemosensor '1' is synthesized which undergoes through bond energy transfer in the presence of Hg^{2+} ions in mixed aqueous media.

Mercury is one of the most significant cations among various heavy and soft cations because of its toxic effects.[1] Mercury contamination occurs through a variety of natural and anthropogenic sources including oceanic and volcanic emissions, gold mining, and combustion of fossil fuels.[2] The biological targets and toxicity profile of mercury species depend on their chemical composition.[3] The exposure to mercury, even at very low concentration, leads to digestive, kidney, and especially neurological diseases[4] as mercury can easily pass through the biological membranes. Thus, keeping in view the role played by mercury in day-to-day life, simple and rapid sensing of mercury[5] in biological and environmental systems is very important. Fluorescence signaling is one of the first choices due to its high detection sensitivity and simplicity which translates molecular recognition into tangible fluorescence signals.[6] In most of the fluorescent sensors the cation binding involves photophysical changes such as photoinduced electron transfer (PET),[7] photoinduced charge transfer (PCT),[8] formation of monomer/excimer,[9] and energy transfer,[10] and more recently fluorescence resonance energy transfer (FRET) where the excitation energy

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from one fluorophore, the donor, is transferred to another fluorophore, the acceptor, without emission of a photon.[11] However, there is a problem in many biochemical experiments which involve irradiation of different fluorescent labels with a single excitation source. The dye which is to emit at a longer wavelength absorbs at the excitation source less effectively and hence results in loss in fluorescence intensity. This is an important issue in those cases where detection of low levels of fluorescence is involved. Fluorescence resonance energy transfer (FRET) provides a solution to some extent. However, the number of FRET based systems is less, as these systems require that donor emission must overlap with the acceptor absorption.[11] On the other hand, through bond energy transfer (TBET) is theoretically not subjected to the requirement of spectral overlap between the donor emission and acceptor absorption and is expected to have large Stokes shifts and emission shifts.[12] These spectral benefits are very important for the use of fluorescent dyes in chemistry, biology, medicine, and material science. In TBET systems the donor and acceptor are joined by a conjugated spacer which prevents them from becoming flat and conjugated. These types of systems absorb at a wavelength characteristic of a donor then emit via a receptor. Recently, Burgees et al.[12a,b] have developed excellent (TBET) systems based on rhodamine and fluorescein for use in biotechnology, but no such systems for fluorogenic sensing of metal ions have been developed so far.

In the present investigation, we have designed and synthesized a naphthalimide appended rhodamine based chemosensor where through bond energy transfer has been used for the selective sensing of Hg^{2+} ions in mixed aqueous media. The attachment of a naphthalimide moiety with rhodamine through a conjugated spacer like benzene exhibits the phenomenon of through bond energy transfer in the presence of mercury ions. To the best of our knowledge, this is the first report where a TBET is observed between naphthalimide and rhodamine moieties in the presence of Hg^{2+} ions.

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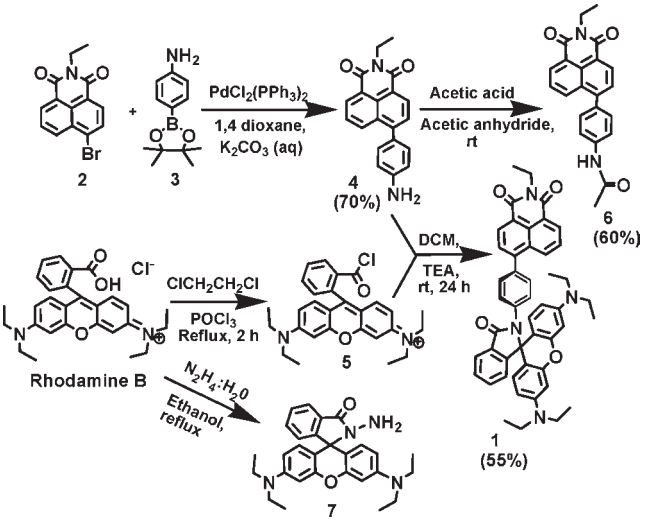
Suzuki−Miyaura cross coupling of boronic ester **3**[13] with **2**[14] catalyzed by Pd(II) furnished compound **4** (Scheme 1) in 70% yield (Supporting Information pp S5−S8, S23−S25). The reaction of compound **4** with rhodamine acid chloride **5** formed from the reaction of rhodamine and phosphorus oxychloride gave the desired compound **1** in 55% yields. The structure of compound **1** was confirmed from its spectroscopic and analytical data (Supporting Information pp S16−S18).

Scheme 1. Synthesis of **1**



The binding behavior of compound **1** was studied toward different metal cations (Hg^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, Co^{2+}, Mg^{2+}, Li^{+}, Na^{+}, and K^{+}) as their perchlorate salts by UV−vis and fluorescence spectroscopy. The absorption spectrum of compound **1** in THF/H_2O (9.5: 0.5, v/v) shows two absorption bands at 320 and 363 nm (Figure 1) due to the naphthalimide moiety, but there was no band corresponding to the rhodamine moiety. However, on addition of Hg^{2+} ions

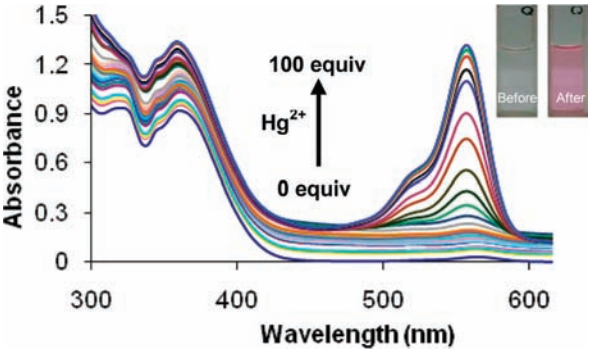


Figure 1. UV−vis spectra of **1** (5 μM) in THF/H_2O (9.5:0.5, v/v) buffered with HEPES, in the presence of Hg^{2+} ions (100 equiv). Inset showing the change in color before and after the addition of Hg^{2+} ions.

(0−100 equiv), the intensity of the band at 363 nm increases and a new absorption band appears at 565 nm along with a color change from colorless to pink. The formation of a new band at 565 nm is due to the opening of the spirolactam ring of the rhodamine moiety. Thus, in the presence of mercury ions, compound **1** shows an absorption spectrum characteristic of both the donor and acceptor components. Under the same conditions as those used above for compound **1**, we also carried out UV−vis studies of model compound **6** (naphthalimide donor; Supporting Information pp S5−S8, S26−S28) and rhodamine acceptor **7**[15] (Supporting Information pp S5−S8) with Hg^{2+} ions independently and found that the combined behavior was the same (Supporting Information p S15) as was observed with compound **1** in which two moieties are attached to each other through a conjugated spacer. This indicates that naphthalimide and rhodamine moieties in compound **1** are interacting with Hg^{2+} ions independent of each other. In other words, there are no electronic interactions between these in the ground state in the presence of Hg^{2+} ions, and thus compound **1** behaves like a cassette[12] and not as a planar totally conjugated dye.

The fluorescence spectrum of compound **1** in THF/H_2O (9.5:0.5, v/v), in the absence of mercury ions, exhibited a very weak emission at 472 nm attributed to the naphthalimide moiety when excited at 360 nm (Figure 2). The weak

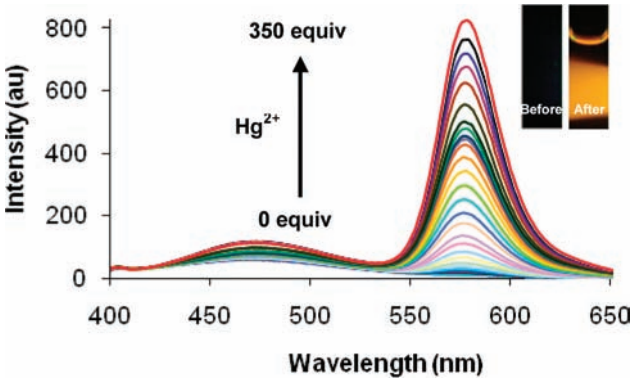


Figure 2. Fluorescence spectra of **1** (5 μM) in response to the presence of Hg^{2+} ions (350 equiv) in THF/H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm. Inset showing the fluorescence before and after the addition of Hg^{2+} ions.

fluorescence emission of receptor **1** is due to the photoinduced electron transfer (PET) from nitrogen atom of the spirolactam ring to the photoexcited naphthalimide moiety. Upon addition of Hg^{2+} ions (0−10 equiv) to the solution of **1** in mixed aqueous media (THF/H_2O, 9.5:0.5) an emission band characteristic of the acceptor component appears at 578 nm. This fluorescence enhancement at 578 nm is attributed to the opening of the spirolactam ring of rhodamine to an amide form (Figure 3). The mode of energy transfer in receptor **1** is a very fast mechanism

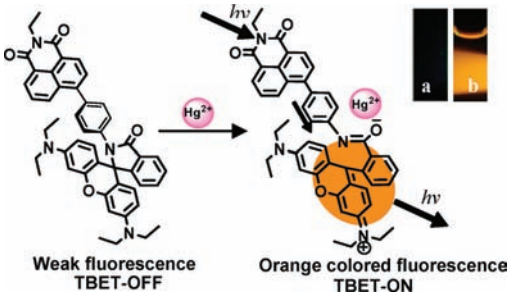


Figure 3. Hg^{2+} induced TBET OFF−ON. Inset showing the fluorescence of ring opened rhodamine B (a) and receptor **1** (b).

operating through bonds, i.e., via the congugated linker which allows energy transfer from donor to acceptor through bonds. However, the energy transfer was not 100% because some of the flourescence leaks from the naphthalimide donor rather being transferred to the acceptor. Under the same conditions as those used above for compound **1** we also carried out fluorescence studies of an equimolar mixture of naphthalimide donor **6** and rhodamine acceptor (ring opened form of rhodamine B) and found that no visible quenching of **6** and no enhancement in the fluorescence emission of the rhodamine acceptor was observed when the mixture was excited at the naphthalimide absorption band, i.e., at 360 nm (Supporting Information p S13), which clearly indicates that there is no intermolecular energy transfer between the naphthalimide donor and rhodamine acceptor in the mixture. Thus, the advantage of the TBET system for energy transfer is obvious. Further, for practical applications it is very important that the fluorescence intensity of the acceptor in the cassette is greater than that of the acceptor without the donor when it is excited at the donor absorption wavelength. The fluorescence enhancement factor for compound **1** is 407-fold compared to the ring opened form of rhodamine B when excited at 360 nm (Supporting Information p S14). This enhancement factor is far higher when compared with other FRET based systems.[16] Moreover the fluorescence of compound **1** is much brighter than that of the ring opened form of rhodamine B (inset of Figure 3).

We also tested the fluorescence response of **1** to other metal ions such as Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, Co^{2+}, Mg^{2+}, Li^{+}, Na^{+}, and K^{+}, in mixed aqueous media (THF/H_2O; 9.5:0.5); however, no significant variation in the fluorescence spectra of **1** (Figure 4A) was observed with any other metal ion except Fe^{2+} and Fe^{3+} (Supporting Information p S10) which also induce similar fluorescence emission but to a small extent. To check the practical ability of compound **1** as a Hg^{2+} selective fluorescent sensor, we carried out competitive experiments in the presence of Hg^{2+} at 350 equiv mixed

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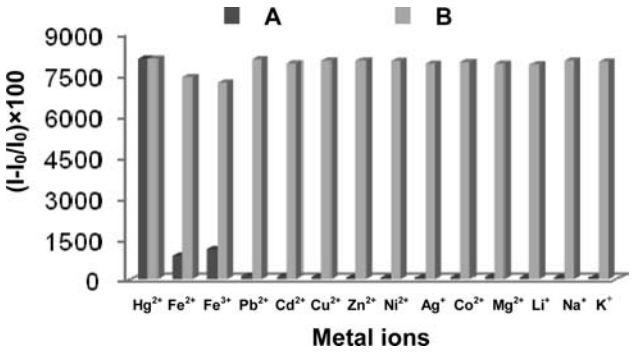

Figure 4. Fluorescence response of **1** (5 μM) to various cations (350 equiv) in THF/H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm. Bars represent the emission intensity ratio $(I - I_0/I_0) \times 100$ (I_0 = initial fluorescence intensity at 578 nm; I = final fluorescence intensity at 578 nm after the addition of Hg^{2+} ions). The black bars represent the addition of individual metal ions while the gray bars represent the change in the emission that occurs upon the subsequent addition of Hg^{2+} (350 equiv) to the above solution.

with Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, Co^{2+}, Mg^{2+}, Li^{+}, Na^{+}, and K^{+} (350 equiv each). As shown in Figure 4B no significant variation in the fluorescence emission was observed by comparison with or without the other metal ions. It was found that **1** has a detection limit of 2×10^{-6} mol L^{-1} for Hg^{2+} which is sufficiently low for the detection of the submillimolar concentration range of Hg^{2+} ions found in many chemical systems. The fluorescence quantum yield[17] (Φ_{fs}) of compound **1** in the free and Hg^{2+}-bound state was found to be 0.06 and 0.54 respectively. Fitting the changes in the fluorescence spectra of compound **1** with Hg^{2+} ions using the nonlinear regression analysis program SPECFIT[18] gave a good fit and demonstrated that a 1:1 stoichiometry (host/guest) was the most stable species in the solution with a binding constant $\log \beta_1 = 4.85$. The method of continuous variation (Job's plot) (Supporting Information p S9) was also used to prove the 1:1 stoichiometry.[19] We also carried out a reversibility experiment which proved that binding of Hg^{2+} ions to compound **1** was reversible. In the presence of KI, the iodide ions because of the strong affinity for Hg^{2+} ions form a complex with it, which results in the decomplexation of the receptor Hg^{2+} complex. On further addition of Hg^{2+} ions, the fluorescence intensity was revived again indicating the reversible behavior of the chemosensors **1** for the Hg^{2+} ions (Supporting Information p S12).

The potential biological application of the receptor was evaluated for *in vitro* detection of Hg^{2+} ions in prostate

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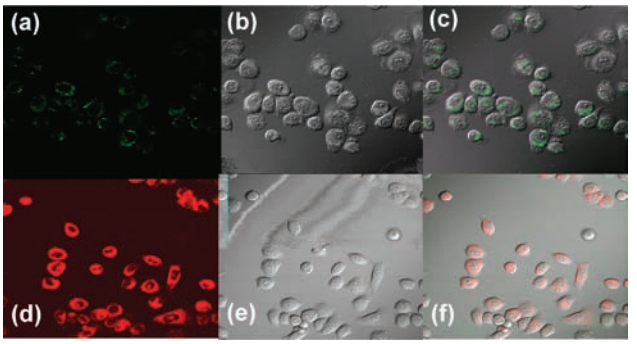

Figure 5. Fluorescence and brightfield images of PC3 cells lines. (a) Green fluorescence images of cells treated with probe **1** (1.0 μM) only for 20 min at 37 °C. (b) Brightfield images of (a). (c) Overlay image of (a) and (b). (d) Red fluorescence images of cells upon treatment with probe **1** (1.0 μM) and then $Hg(ClO_4)_2$ (10.0 μM) for 20 min. (e) Brightfield images of (d). (f) Overlay image of (d) and (e); λ_{ex} = 488 nm.

cancer (PC3) cell lines. The prostate cancer (PC3) cell lines were incubated with receptor **1** (1.0 μM in THF/H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0) in an RPMI-1640 medium for 20 min at 37 °C and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **1**. Microscope images showed a weak green intracellular fluorescence which indicated that compound **1** is cell permeable (Figure 5a). The cells were then treated with mercury perchlorate (10.0 μM) in the RPMI-1640 medium and incubated again for 20 min at 37 °C and washed with PBS buffer. After treatment with Hg^{2+} ions the color of the cells changed to red which clearly indicates the quenching of green emission with the appearance of red emission (Figure 5d). These results suggest that **1** is an effective intracellular Hg^{2+} imaging agent with the change in fluorescence emission from green to red, attributed to the working of the TBET phenomenon within the cells.

In conclusion, we synthesized naphthalimide appended rhodamine based fluorescent chemosensor **1** which showed through bond energy transfer in the presence of Hg^{2+} ions in mixed aqueous solution. Chemosensor **1** can also be used as a fluorescent probe for imaging Hg^{2+} ions in PC3 cell lines which will help in the understanding of biological processes at the molecular level.

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Supporting Information Available. Experimental data and synthetic details of compound **1** and **4** are given in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.