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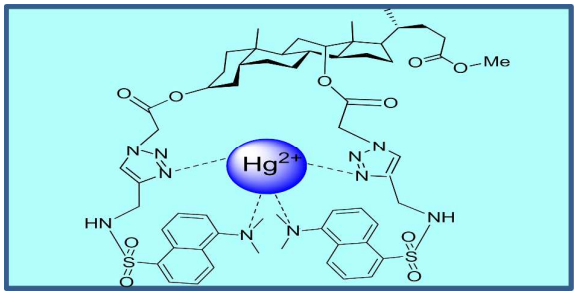
Table of Contents Entry



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ARTICLE TYPE

A novel dansyl-appended bile acid receptor for preferential recognition of Hg^{2+}

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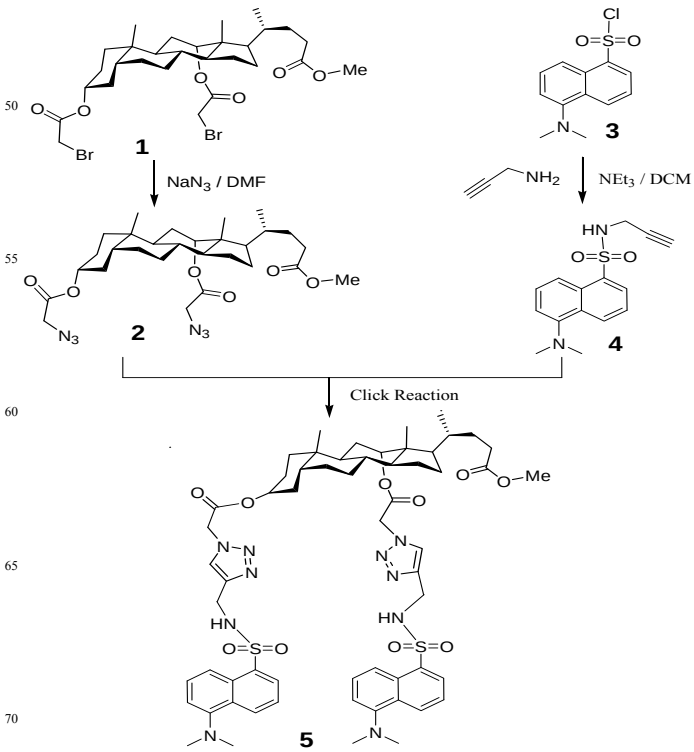
We have designed and synthesized a novel dansyl appended bile acid chemosensor using click chemistry. The chemosensor shows selective and efficient recognition of Hg^{2+} ions by forming a 1:1 complex with Hg^{2+} with a binding constant of 3.3×10^4 M^{-1}. The limit of detection for Hg^{2+} was estimated to be ~2μM.

Bile acids and their derivatives have been a subject of considerable attention to the scientific community due to their potential applications in supramolecular chemistry.[1] Their unique structural features, such as, slightly curved shape, rigidity, amphiphilicity, and availability of hydroxyl groups for further derivatization, render them useful for development of variety of chemosensors for the recognition of various guests.[2] Recently, 1,2,3-triazole based bile acid frameworks utilizing click chemistry for ionic recognition have gained importance because of their geometry and coordination properties with anions and metal ions.[3]

Since fluorescence-based methods for recognition offer high sensitivity coupled with use of simple instrumentation,[4] considerable efforts have been invested in designing fluorescent chemosensors for detection of heavy metal ions,[5] specially mercury. Although mercury and its salts have high toxicity, they are widely used in industry and hence are widespread in the environment. The exposure to mercury even at low levels leads to various health problems, especially neurological disorders.[6-8] Most of the reported chemosensors for Hg^{2+} display low selectivity and sensitivity; many suffer from interference from other metals ions such as Cu^{2+}, Cd^{2+} and Pb^{2+}.[9-11] There is always a need to design and develop better performing sensors for Hg^{2+}.

In this *Communication*, we report a novel bile acid-based fluorescent receptor **5** in which two dansyl moieties are conjugated by a triazole group acting as a linker as well as binding site for heavy metal ions. Dansyl group, when covalently bound to a host molecule, offers many attractive and pertinent features due partly to (i) its strong fluorescence (absorption bands in the near-uv and intense fluorescence in the visible region), (ii) relatively long emission wavelength, and (iii) its solvatochromic nature (i.e., sensitivity to the polarity of the medium owing to the presence of twisted intramolecular charge transfer (TICT) excited-state.[12] Although dansyl-appended cholic acid derivatives have been used to investigate protein binding and aggregation of



Scheme1. Synthesis of receptor **5** *via* click reaction (10 mol% $CuSO_4.5H_2O$, 20 mol% sodium ascorbate, H_2O/*t*-BuOH).

bile acids,[13] this is the first study where a host is synthesized by covalently attaching two dansyl moieties to bile acid via 1,2,3-triazole using click chemistry and is investigated for its potential as a fluorescence-based chemosensor for metal ion detection. Receptor **5** is found to effectively recognize mercury ion amongst lithium, sodium, magnesium, zinc, lead, manganese, copper, cobalt, and cadmium via fluorescence quenching mechanism. Receptor **5** was obtained in 80% yield through a series of reactions presented in Scheme 1.[3,14] After synthesis, its molecular structure was confirmed by $^1H/^{13}C$ NMR spectroscopy as well as

ESI-TOF MS analysis (Supplementary Information, Fig. S1 and S2).

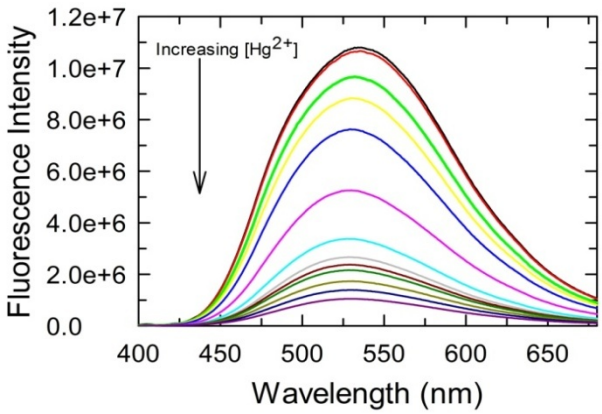


Figure 1. Quenching of fluorescence intensity of receptor **5** (25 μM) in the presence of Hg^{2+} in $CHCl_3$:MeOH (7:3, v/v) ($\lambda_{excitation}$ = 351 nm).

To explore Hg^{2+} recognition ability of the receptor, its dilute solution (25 μM) in $CHCl_3$:MeOH (7:3, v/v) was excited at an optimum wavelength of 351 nm,[12,15] the resulting fluorescence (maxima at 536 nm, characteristic of dansyl fluorescence) was subsequently measured in the presence of varying concentration of metal ions. Interestingly, as shown in Fig. 1, a significant quenching of the fluorescence emission intensity along with ~10 nm hypsochromic shift was observed in the presence of Hg^{2+} (fluorescence intensity of receptor **5** decreases by 76% in the presence of 100 μM Hg^{2+}). Clearly, receptor **5** shows excellent recognition ability towards Hg^{2+}.

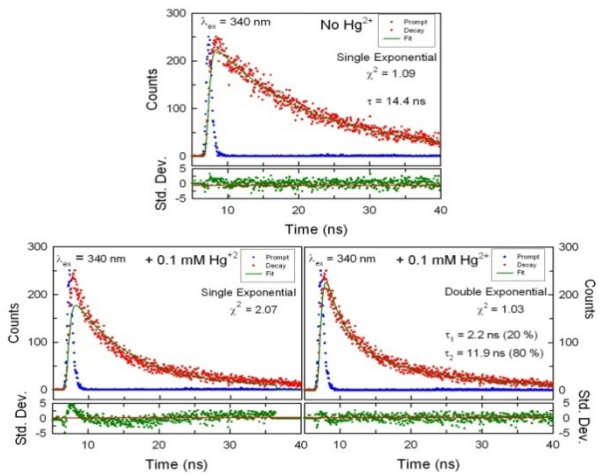


Figure 2. Excited-state intensity decay curves of receptor **5** (25 μM) in the absence (upper panel) and presence (lower panel) of 100 μM Hg^{2+} in $CHCl_3$:MeOH (7:3, v/v).

This is supported by time-resolved fluorescence measurements. A 340 nm LED is used as excitation source and excited-state intensity decay data of the receptor is collected in the absence and

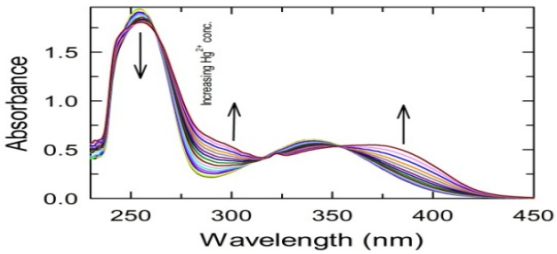


Figure 3. Change in UV-Vis spectra of receptor **5** (100 μM) upon addition of Hg^{2+} in $CHCl_3$:MeOH (7:3, v/v).

presence of 100 μM Hg^{2+}. In the absence of Hg^{2+}, the excited-state intensity decay of receptor **5** was best fit to a single exponential decay model with lifetime of 14.4 ns (Fig. 2). This fluorescence lifetime is characteristic of dansyl moiety.[12] However, in the presence of 100 μM Hg^{2+}, the intensity decay data could not be fit to a single exponential decay model satisfactorily, instead a double exponential decay model with decay times of 2.2 and 11.9 ns were required to fit the data adequately. Presence of two decay times as well as gradual hypsochromic shift in the fluorescence band of receptor **5** in the presence of Hg^{2+} hint at possible formation of a weakly fluorescent complex between receptor **5** and Hg^{2+}. Detection limit (LOD) is obtained to be ~2 μM from linear calibration plot (0 - 25 μM Hg^{2+}) using departure of $3\times s_0$ in signal to be criterion for the detection, where s_0 represents standard deviation of fluorescence intensity in the absence of Hg^{2+} for 12 replicate measurements (SI, Fig S3).[16,17]

The mode of recognition of Hg^{2+} by receptor **5** was further studied by uv-vis molecular absorbance spectroscopy. In the absence of Hg^{2+}, it exhibited two absorbance peaks at 254 nm and 340 nm that could be assigned to triazole rings and dansyl moieties, respectively (Fig. 3). Gradual addition of Hg^{2+} to 100 μM solution of receptor **5** led to appearance of a new peak centered at 385 nm with two clearly present isobestic points at 317 nm and 354 nm. The uv-vis absorbance data clearly indicate that the complex forms between host **5** and guest Hg^{2+} and it exists in equilibrium with receptor **5** and Hg^{2+} within the solution. This adequately conforms with the fluorescence data as the complex formed between receptor **5** and Hg^{2+} is amply chromophoric albeit weakly fluorescent due to the presence of mercury as the heavy-atoms would promote intersystem crossing thus increasing the rates of non-radiative decay processes within the system.[12]

Selectivity is an important characteristic of a chemosensor as far as ion sensing is concerned. To assess the selectivity of receptor **5** towards Hg^{2+}, titration experiments were preformed with other metal ions besides Hg^{2+} with perchlorate as the counter ion under identical conditions. In the presence of other metal ions (Co^{2+}, Cu^{2+}, Mn^{2+}, Pb^{2+}, Mg^{2+}, Cd^{2+}, Na^+ and Li^+), the decrease in the fluorescence intensity of receptor **5** was insignificant in comparison to that Hg^{2+} ions (Fig. 4). It was found that Cu^{2+} and Co^{2+}, respectively, partly quench the fluorescence intensity of receptor **5** as they are well-known metal ion quenchers.[12] However, the quenching by these two ions was significantly less

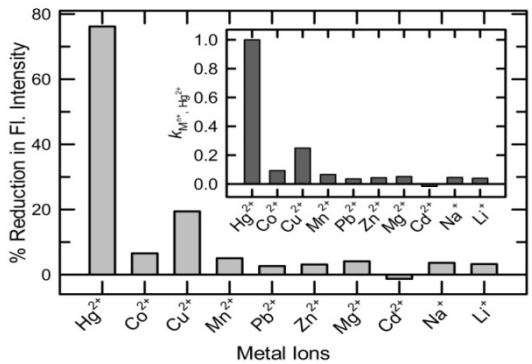


Figure 4. Percentage reduction in the fluorescence intensity of receptor **5** in the presence of 100 μM M^{n+} in $CHCl_3$:MeOH (7:3, v/v) ($\lambda_{excitation}$ = 351 nm). Inset shows selectivity coefficient, $k_{M^{n+},Hg^{2+}} = \frac{m_{M^{n+}}}{m_{Hg^{2+}}} = \frac{\Delta F_{M^{n+}}}{\Delta F_{Hg^{2+}}}$ for receptor **5**.

than that by Hg^{2+}. In contrast to the quenching of the fluorescence of receptor **5** by Hg^{2+}, the quenching in the presence of Cu^{2+} is purely dynamic or collisional in nature as the uv-vis absorbance spectra shows no change in the presence of Cu^{2+} clearly indicating absence of any complex formation between receptor **5** and Cu^{2+} (SI, Fig. S4: no new absorbance band or isobestic point(s) appear). Addition of Cd^{2+} resulted in a very slight enhancement of its fluorescence intensity (Fig. 4). Selectivity coefficient, $k_{M^{n+},Hg^{2+}} = \frac{m_{M^{n+}}}{m_{Hg^{2+}}} = \frac{\Delta F_{M^{n+}}}{\Delta F_{Hg^{2+}}}$, to assess possible interference was estimated and plotted for many metal ions (Fig. 4 inset). A careful observation of this data reveals $k_{M^{n+},Hg^{2+}}$ for most metal ions to be small enough to pose any significant interference with the detection of Hg^{2+} by receptor **5**. It is important to mention that the control experiments with dansyl chloride led to insignificant changes in the fluorescence intensity of the naked fluorophore (as opposed to quenching shown by receptor **5**) in the presence of Hg^{2+}. To further explore the extent of selectivity of the receptor towards Hg^{2+}, experiments were performed where 1 mM Hg^{2+} in combination with 1 mM competitive metal ions were present in the solution. The similar reduction in fluorescence intensity in the presence of several different metal ions (SI, Fig. S5) clearly highlights that all other metal ions have marginal effect on the fluorescence emission spectra of receptor **5**.

Fluorescence data of receptor **5** in the presence of varying [Hg^{2+}] was further analyzed to obtain key information regarding the mode of interaction between the host and the guest. For [Hg^{2+}] = 0 to 200 μM, the plot of [(F_0/F) –1] versus [Hg^{2+}] was found to be linear revealing the stoichiometry of the complex to be 1:1 (where F_0 and F are the fluorescence intensities in the absence and presence of [Hg^{2+}], respectively) (Fig. S7). The association equilibrium constant was estimated from the slope to be 3.3(±0.5)×10^4 M^{-1} implying adequate affinity of Hg^{2+} by receptor **5**.

^{1}H NMR titration experiments in $CDCl_3$:MeOD (7:3, v/v) were carried out to elucidate the binding mode of Hg^{2+} with receptor **5**.

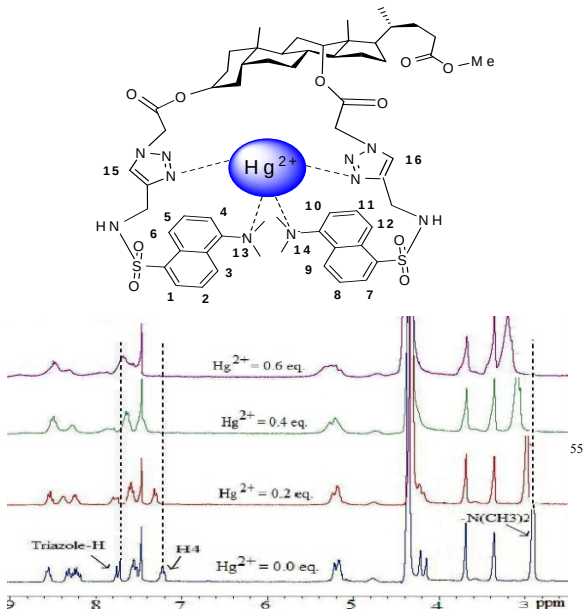


Figure 5. Partial ^{1}H NMR spectra (300 MHz, $CDCl_3$:CD_3OD, (7:3, v/v), δ in ppm) of receptor **5** showing chemical shift change in the presence of incremental addition of $Hg(ClO_4)_2$. H_2O ($CDCl_3$:CD_3OD, (7:3, v/v)).

The NMR spectra of receptor **5** in the presence of increasing equivalents of $Hg(ClO_4)_2$ resulted in deshielding and broadening of the signals of the protons of dansyl moieties as well as that of triazole units as compared to those of free receptor. The addition of 0.6 equivalent of Hg^{2+} led to a downfield shift of both triazole protons (H15 and H16) by 0.15 and 0.20 ppm, respectively. In the aromatic region, a significant downfield shift in H4 and H10 of dansyl moiety by 0.34 ppm, along with substantial deshielding of $N(CH_3)_2$ protons by 0.25 ppm (13 and 14) suggested close proximity of Hg^{2+} with $N(CH_3)_2$ groups of the dansyl framework. This highlights key role of both dansyl group and triazole unit in Hg^{2+} recognition. Based on ^{1}H NMR data, a tentative binding model depicting possible mode of complexation involving receptor **5** and Hg^{2+} is presented as a cartoon in Fig. 5 (the structures are not drawn according to size and shape). Similar mode of complexation involving $N(CH_3)_2$ groups of dansyl moietie have been reported earlier also in some dansyl-appended metal-ion-receptors.[18,19]

Conclusions

In conclusion, a novel-dansyl appended bile acid based fluorescent chemosensor for Hg^{2+} is presented. The chemosensor is found to possess good sensitivity and excellent selectivity towards Hg^{2+}. The recognition is *via* formation of a weakly fluorescent complex of 1:1 stoichiometry with adequate binding affinity.

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Notes and references

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