Novel dansyl-appended calix[4]arene frameworks: fluorescence properties and mercury sensing†

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Covalently-attached fluorophores may impart enhanced chemosensing capabilities to calixarene frameworks. Synthesis and characterization of six novel dansyl-appended calix[4]arenes, namely, **H/Dan$_4$**, **NO$_2$/Dan$_4$**, **H/(OH)$_2$Dan$_2$**, **H/(Ester)$_2$(Dan)$_2$**, **t-Bu/(OH)$_2$Dan$_2$**, and **t-Bu/(Ester)$_2$Dan$_2$**, containing two or four dansyl moieties are reported. Among these, fluorescence intensity of **NO$_2$/Dan$_4$** is observed to decrease significantly in the presence Hg^{2+} in the solution. Based on the decrease in fluorescence, a limit of detection for Hg^{2+} of 20 ppb is obtained. **NO$_2$/Dan$_4$** as a chemosensing agent for Hg^{2+} shows excellent selectivity and adequate reversibility. Complexation of **NO$_2$/Dan$_4$** with Hg^{2+} is investigated using fluorescence spectroscopy and is observed to be 2:1. The formation constant of $(NO_2/Dan_4)_2Hg^{2+}$ is estimated to be $5.2(\pm 0.8) \times 10^{10}$ M^{-2} at ambient conditions. These observations are traced to the fact that while all other dansyl-appended calix[4]arenes show cone conformation in the solution, **NO$_2$/Dan$_4$** is in the 1,3-alternate conformation. Stokes shift *versus* solvent orientational polarizability for **NO$_2$/Dan$_4$** also indicates the difference in the ground- to excited-state dipole moment of this compound to be the maximum among all six, rendering it most sensitive to its environment. Fluorescence emission of **NO$_2$/Dan$_4$** in nonpolar chloroform, polar-aprotic acetonitrile, and polar-protic ethanol is observed to be different than that of the rest of the dansyl-appended compounds as well.

Introduction

Calix[4]arenes and their derivatives have been of continuous interest to the scientific community due to their widespread applications; the most important perhaps being their behavior as unique and versatile hosts.[1–6] A variety of guests with differing size, shapes, properties, and characteristics have been recognized by different calix[4]arenes and their derivatives.[1] It is established that the versatility of these hosts as far as either the recognition of more types and classes of guests is concerned or increasing the effectiveness of overall recognition process for a particular guest is of primary interest can be expanded immensely due to enormous derivatization possibilities of calixarene molecular frameworks. In this context, facile derivatization at the lower and/or the upper rim of a calixarene molecular framework may result in improved efficiency as well as versatility toward guest recognition.[1] Due to this, calixarenes routinely serve as an ideal platform for the development of a variety of chemosensors for the recognition of cations, anions, and small organic molecules.[2,3,5]

Fluorescence-based investigations of complexation and sensing involving calix[4]arenes and related compounds are routine due to inherently high sensitivity associated with fluorescence-based techniques.[7,8] Reduction or enhancement in steady-state fluorescence intensity or anisotropy, shifts in fluorescence band maxima, appearance and/or disappearance of fluorescence bands, changes in the excited-state intensity or anisotropy decay kinetics, among others, may form the basis of the recognition event. In general, one of two approaches is used in such investigations; either the calix[4]arene-based host is suitably modified by covalent attachment of a judiciously selected fluorophoric moiety (or moieties), or the fluorescence from the guest and/or some external analyte becomes the basis of analysis.[2,7] Needless to say, researchers are actively involved in exploring both these avenues to come up with calixarene-based effective and versatile chemosensors. However, the former of the two approaches has garnered more attention from organic chemists perhaps due to its well-documented effectiveness and more potential applications. Inadequate fluorescence from most guests of interest and unavailability of suitable externally-added fluorophores are also to blame for this bias. Further, due to the availability of a variety of derivatization strategies associated with the calixarene molecular framework, the design and development of novel fluorophore-appended calixarene-based fluorescence chemosensors with enhanced sensitivity and increased selectivity is both exciting and useful. Due to enormous possibilities, the development of fluorescent molecular sensors based on calixarenes is a challenge, as they can offer high sensitivity and potential applications in the field of analytical, pharmaceutical, and biological chemistry.[6,9–12]

Several common and popular fluorophoric moieties have been utilized to design and synthesize fluorophore-appended calixarene-based fluorescence chemosensors in the past.[13] Among others, dansylated calixarenes (fluorogenic calixarenes with dansyl/dansyl-appended calixarenes) have received ever-increasing (albeit limited) attention for the recognition and sensing of heavy, transition, and alkali metals, such as, Hg^{2+}, Pb^{2+}, Cd^{2+},

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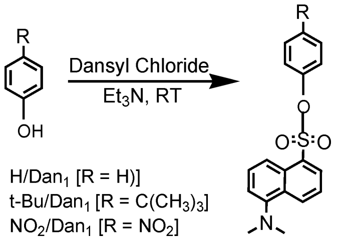
Cr^{3+}, Cu^{2+}, Na^{+}, *etc.*[5,11–13] The dansyl group, when covalently bound to a host molecule, offers many attractive and pertinent features due partly to (i) its strong fluorescence (absorption bands in the near-UV and intense fluorescence in the visible region),[14] (ii) relatively long emission wavelength,[5a] and (iii) its solvatochromic nature (*i.e.*, sensitivity to the polarity of the medium owing to the presence of a twisted intramolecular charge-transfer (TICT) excited state).[15–17]

In recent years considerable effort has been devoted to the selective and efficient detection of heavy metal ions, especially Hg^{2+}, as mercury and its salts have high toxicity, and they are widely used in industry and hence are widespread in the environment.[11–13] As a result, there has been much effort (and numerous reports) on the design and development of fluorescent chemosensors for detection of Hg^{2+}.[11,12] However, very few offer acceptable sensitivity, adequate selectivity and long-term reversibility for potential applications in biological and environmental monitoring. In this paper, we report the synthesis of a series of novel dansyl-appended fluorescent calix[4]arenes containing two or four covalently attached dansyl moieties at the lower rim of the calix[4]arene molecular framework possessing other functionalities at various positions (Scheme 1). The potential of these dansyl-appended calix[4]arenes as fluorescence chemosensors for metal ions is also explored and presented. Spectroscopic properties relevant to their behavior as fluorescence chemosensing agents for metal ions are used to explain such behavior of these novel compounds. In this context, the fluorescence spectroscopic behavior of these two or four dansyl-appended calix[4]arenes is compared with those of mono-dansylated reference compounds (Scheme 2). One of our novel dansyl-appended calix[4]arenes, **NO_2/Dan$_4$** (Scheme 1), is observed to offer very high sensitivity, adequate selectivity, and acceptable reversibility toward sensing Hg^{2+} in solution.

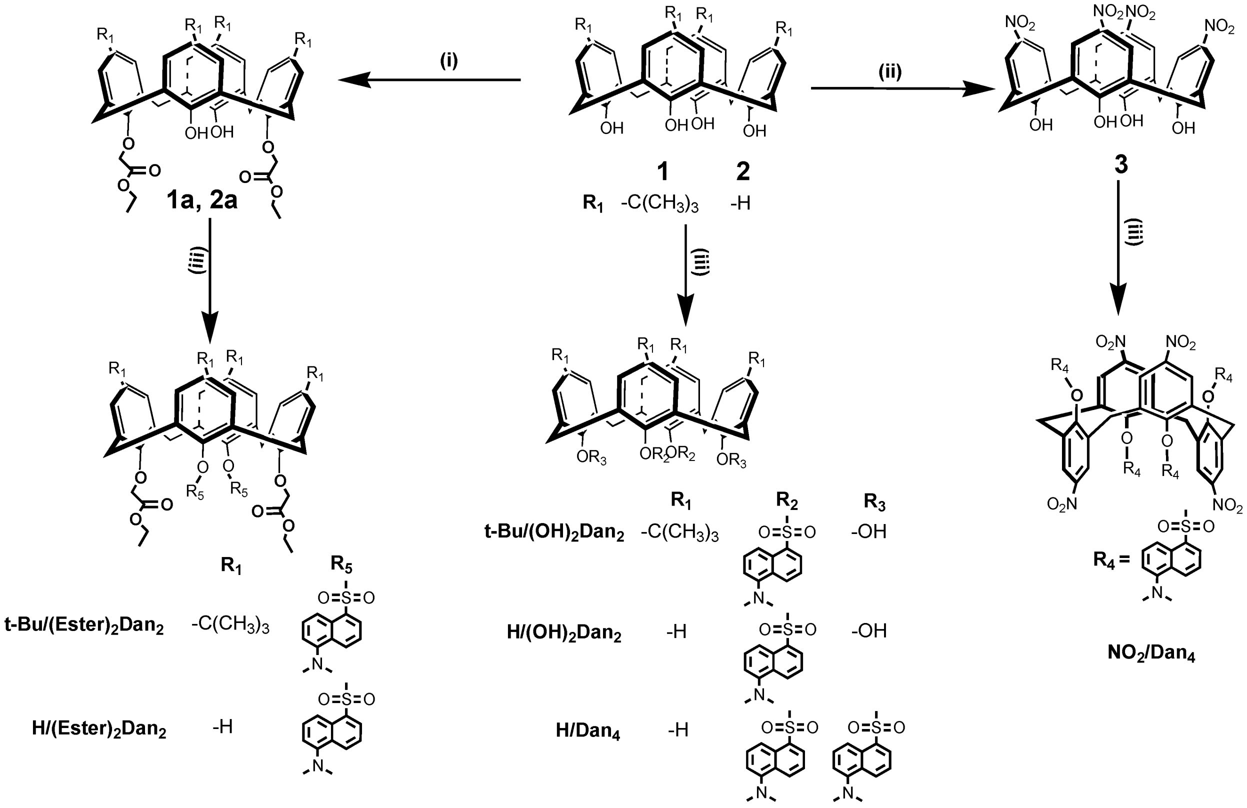


Scheme 2 Synthesis of reference compounds, **H/Dan$_1$**, **t-Bu/Dan$_1$**, and **NO_2/Dan$_1$**.

Results and discussion

Synthesis and characterization of novel dansyl-appended compounds

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrahydroxy-calix[4]-arene (**1**) and 25,26,27,28-tetra-hydroxy-calix[4]arene (**2**) were synthesized according to the reported literature procedure.[18] 5,11,17,23-Tetra-*tert*-butyl-25,27-di(ethoxycarbonyl-methoxy)-26,28-dihydroxy-calix[4]arene (**1a**) and 25,27-di(ethoxycarbonyl-methoxy)-26,28-dihydroxy-calix[4]arene (**2a**) were synthesized by refluxing the corresponding calix[4]arenes (**1** and **2**, respectively) with ethyl bromoacetate in the presence of K_2CO_3 in acetonitrile for 15 hours.[18]



Scheme 1 Synthesis of novel dansyl-appended calix[4]arenes. *Reagents and conditions*: **(i)** ethyl bromoacetate, K_2CO_3, reflux; **(ii)** 100% HNO_3, glacial acetic acid, stirring, ice-bath; **(iii)** dansyl chloride, stirring, Et_3N.

Compound **1** was converted to its tetra-nitro derivative (**3**) *via ipso*-nitration.[19a,b] The reaction of compounds **2** and **3** with equimolar amounts per hydroxyl group of the reagents (1:4:4; OH:dansyl chloride:Et_3N) resulted in tetra-dansylated derivatives: 25,26,27,28-tetra(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))calix[4]arene (**H/Dan$_4$**) and 5,11,17,23-tetra-nitro-25,26,27-tetra(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))calix[4]-arene (**NO_2/Dan$_4$**), respectively (Scheme 1). Corresponding reactions of **1**, **2**, **1a**, and **2a** with dansyl chloride in the presence of triethylamine (Et_3N) as base (1:2:2 molar ratio) resulted in di-dansylated calix[4]arene derivatives: 5,11,17,23-tetra-*tert*-butyl-25,27-*bis*(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))-26,28-dihydroxycalix[4]arene (**t-Bu/(OH)$_2$Dan$_2$**), 25,27-*bis*(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))-26,28-dihydroxycalix[4]-arene (**H/(OH)$_2$Dan$_2$**), 5,11,17,23-tetra-*tert*-butyl-25,27-di(ethoxycarbonyl-methoxy)-26,28-*bis*(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))calix[4]arene (**t-Bu/(Ester)$_2$Dan$_2$**), and 25,27-di (ethoxycarbonyl-methoxy)-26,28-*bis*(*N*-(5-dimethylaminonaphthalene-1-sulfonyl))calix[4]arene (**H/(Ester)$_2$Dan$_2$**), respectively (Scheme 1). It is interesting to note that when **1** was reacted with four equivalents of dansyl chloride and triethylamine, instead of the expected tetra-dansylated product, a di-dansylated derivative of *tert*-butyl calix[4]arene was obtained. Possible steric hindrance due to the presence of four each of *tert*-butyl and dansyl groups in close proximity may explain this outcome. To compare the spectroscopic properties of our dansylated calix[4]arenes, model compounds **t-Bu/Dan$_1$**, **H/Dan$_1$**,[14] and **NO_2/Dan$_1$** were synthesized by the base-catalyzed acylation of *p*-*tert*-butylphenol, phenol, and *p*-nitrophenol, respectively, with dansyl chloride and triethylamine (Scheme 2). These model compounds were further purified by column chromatography and re-crystallized from $CHCl_3/CH_3OH$.

The structures of novel dansylated calix[4]arene derivatives [**H/Dan$_4$**, **NO_2/Dan$_4$**, **t-Bu/(OH)$_2$Dan$_2$**, **H/(OH)$_2$Dan$_2$**, **t-Bu/(Ester)$_2$Dan$_2$**, and **H/(Ester)$_2$Dan$_2$**] and model compounds [**t-Bu/Dan$_1$**, **H/Dan$_1$**,[14] and **NO_2/Dan$_1$**] were established by the analysis of their ^1H and ^{13}C NMR spectra as well as MALDI-TOF MS analysis. The ^1H NMR spectra of **t-Bu/(OH)$_2$Dan$_2$**, **H/(OH)$_2$Dan$_2$**, **H/Dan$_4$**, **t-Bu/(Ester)$_2$Dan$_2$**, **H/(Ester)$_2$Dan$_2$** show two sets of doublets for bridging methylene protons. The presence of a pair of doublets for $ArCH_2Ar$ protons in the ^1H NMR spectra of synthesized dansylated calix[4]arene derivatives (except **NO_2/Dan$_4$**) and only one signal for methylene carbon in the range of 29–32 ppm in their ^{13}C NMR spectra suggested that these dansylated calix[4]arene derivatives were in a symmetrical cone conformation in solution.[19c] For example, the ^1H NMR spectrum of **t-Bu/(OH)$_2$Dan$_2$** shows two pairs of doublets for the axial and equatorial protons at 2.92 and 3.85 ppm, respectively (Fig. 1), and one distinct signal at 31.5 ppm for the methylene carbon in ^{13}C NMR spectrum (ESI: Fig. S1). The–$N(CH_3)_2$ signals appeared at 2.82 ppm in ^1H NMR and 45.3 ppm in ^{13}C NMR spectrum. The *tert*-butyl group was represented by two singlets at 1.17 and 0.74 ppm in ^1H NMR spectrum and at 32.4 ppm in C^{13} NMR spectrum. All aromatic protons for dansyl group as well as *p*-*tert*-butyl-calix[4]arene appeared in the range of 8.59 to 6.52 ppm. The signal for the -OH proton appeared at 1.49 ppm, which is exchangeable with D_2O. In contrast, the ^1H NMR spectrum of **NO_2/Dan$_4$** shows a singlet at 3.37 ppm for the methylene bridged $ArCH_2Ar$ protons instead of two pairs of doublets as observed in the case of other dansylated calix[4]arene derivatives (Fig. 2). Appearance of a singlet for methylene bridged protons indicates that **NO_2/Dan$_4$** is present in the 1,3-alternate conformation,[12,19d,e] which is further confirmed by the appearance of one signal at 34.3 ppm in ^{13}C NMR spectrum (ESI: Fig. S2).

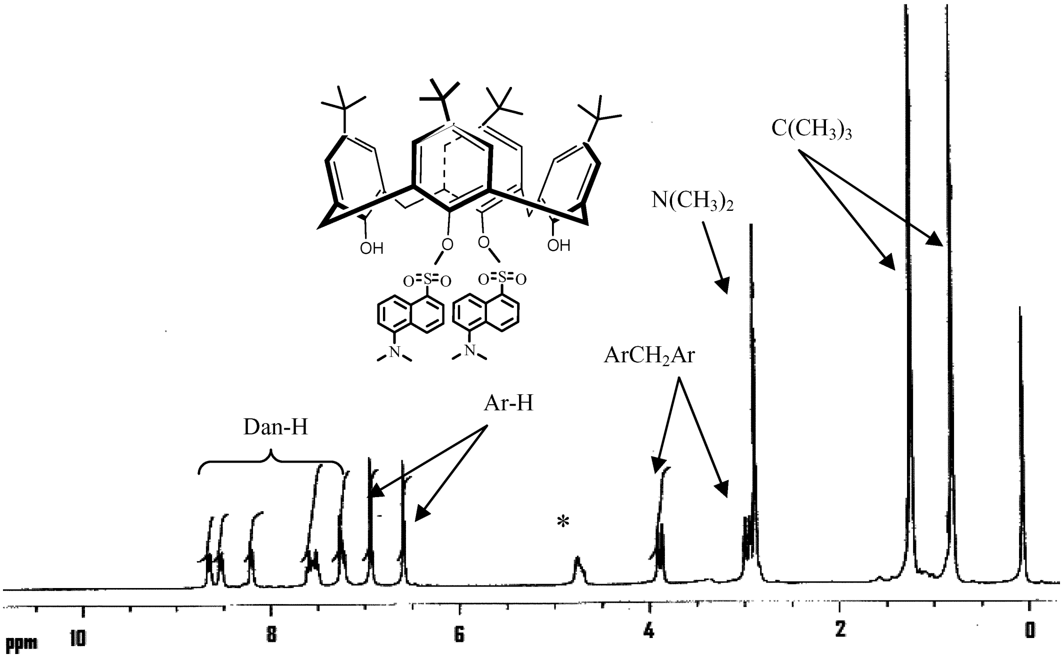


Fig. 1 Molecular structure and ^1H NMR spectrum of **t-Bu/(OH)$_2$Dan$_2$** at 25 °C after D_2O exchange. The peak labeled * represents residual D_2O after exchange.

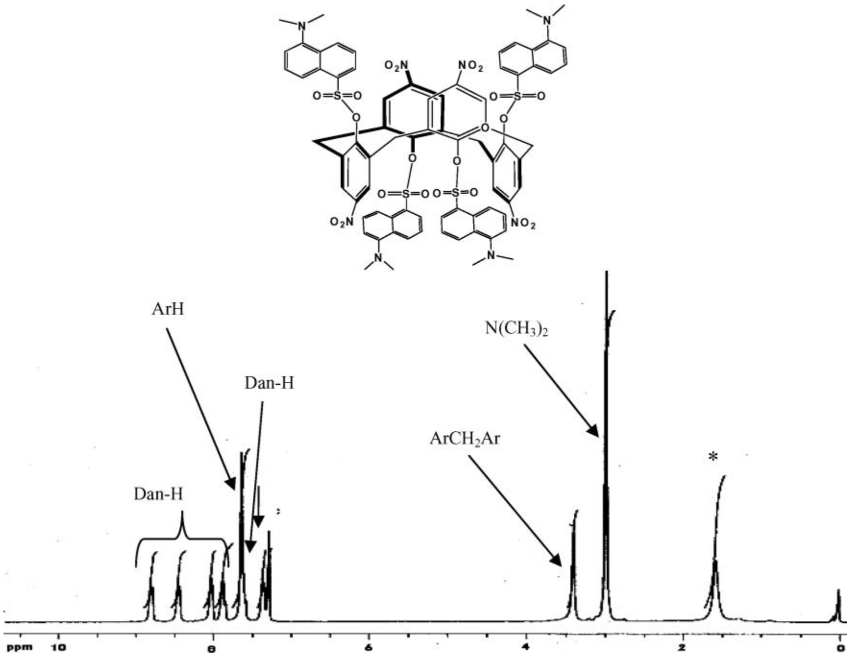


Fig. 2 Molecular structure and ^{1}H NMR spectrum of **NO_2/Dan$_4$** at 25 °C in $CDCl_3$ (* represents signal due to residual solvent).

These δ values from NMR results are in good agreement with those reported for other 1,3-alternate compounds.[12b,19d,e]

Temperature-dependent ^{1}H NMR was also carried out on **NO_2/Dan$_4$** in $CDCl_3$ as **3**, the parent compound of **NO_2/Dan$_4$**, shows a pair of doublet at 23 °C which start to coalesce into a singlet at temperature above 23 °C.[19a] From the low-temperature ^{1}H NMR spectrum it is clearly confirmed that the singlet for $ArCH_2Ar$ of **NO_2/Dan$_4$** remains as a singlet when the temperature was decreased from +48 °C to −25 °C (ESI: Fig. S3 and **S4**), which is a characteristic of the 1,3-alternate conformation.[19d,e] Existence of **NO_2/Dan$_4$** in the 1,3-alternate instead of the cone conformation may be explained in part on the basis of the steric hindrance due to the presence of four nitro and four dansyl groups; the weak interaction between -NO_2 and -$N(CH_3)_2$ of the dansyl moiety due to electron-withdrawing nature of NO_2 group may contribute to this as well. The different conformation of **NO_2/Dan$_4$** as compared to our other dansyl-appended calix[4]arenes may result in its unique sensing behavior (*vide infra*).

Dansyl-appended calix[4]arenes as fluorescence chemosensors

Due to the solvatochromic nature of the dansyl-based fluorophores[20,21] and well-documented host nature of the calix[4]arene framework[11] to which the dansyl moieties are attached, we decided to explore the potential of our novel dansyl-appended calix[4]arenes as possible fluorescence chemosensing agents for metal ion sensing. For this purpose, dilute solutions of dansyl-appended calix[4]arenes (~10 μM) in acetonitrile (ACN) were excited at an optimum excitation wavelength of 351 nm;[20] the resulting fluorescence was subsequently measured in the presence of varying concentration of metal ions.

Interestingly, as shown in Fig. 3, our investigation indicated a significant decrease in fluorescence intensity of **NO_2/Dan$_4$** upon addition of Hg^{2+}; a 74% reduction in fluorescence intensity was observed upon addition of 8 μM Hg^{2+}. Clearly, **NO_2/Dan$_4$** shows tremendous sensing/recognition ability toward Hg^{2+}. If the reduction in **NO_2/Dan$_4$** fluorescence intensity (*i.e.*, $\Delta F = F_0 - F$, where F_0 and F are the fluorescence intensities in the absence and presence of Hg^{2+}, respectively) as up to 8 μM Hg^{2+} is added to the host solution is assumed to vary linearly with [Hg^{2+}] (inset Fig. 3), limit of detection (LOD) for Hg^{2+} can be calculated for **NO_2/Dan$_4$** as fluorescence chemosensing agent using calibration sensitivity (m) of ΔF *versus* [Hg^{2+}] in the aforementioned range.[21] For the

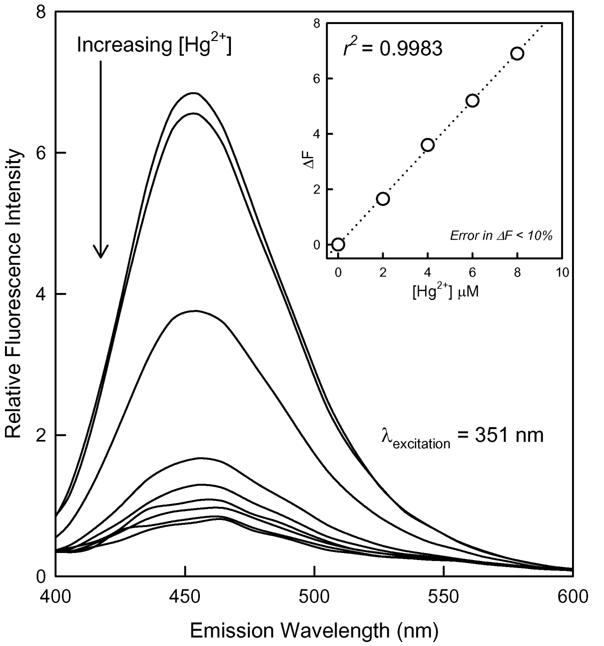


Fig. 3 Quenching of **NO_2/Dan$_4$** fluorescence intensity in the presence of Hg^{2+} in acetonitrile at ambient conditions ([**NO_2/Dan$_4$**] = 1×10^{-5} M, $\lambda_{excitation}$ = 351 nm). Inset shows change in fluorescence (ΔF) upon addition of Hg^{2+} for $0 \leq [Hg^{2+}] \leq 8$ μM.

purpose of LOD calculation, minimum change in the signal (ΔF_{min}) due to the presence of Hg^{2+} was taken to be $3{\times}s_0$, where s_0 is the standard deviation of F_0 for twelve replicate measurements.[22] Thus, the LOD is calculated using $LOD_{[Hg^{2+}]} = \frac{3 \times s_0}{m}$, and it is observed to be ~0.1 μM (or ~20 ppb) Hg^{2+}. Such low LOD clearly demonstrates the high sensitivity of **NO_2/Dan$_4$** toward Hg^{2+}.

Next, we investigated the change in fluorescence intensity of **NO_2/Dan$_4$** in the presence of other metal ions besides Hg^{2+} under identical conditions as above (Fig. 4). Although, as discussed earlier, the fluorescence of **NO_2/Dan$_4$** was severely decreased by Hg^{2+}, interestingly, no significant change in **NO_2/Dan$_4$** fluorescence intensity was observed in the presence of 8 μM Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, CO^{2+}, Pb^{2+}, Cr^{3+}, Cd^{2+}, Na^+, Li^+, and K^+, respectively. These results clearly imply the high selectivity of **NO_2/Dan$_4$** toward Hg^{2+} recognition. Selectivity coefficients, $k_{M^{n+},Hg^{2+}} = \frac{m_{M^{n+}}}{m_{Hg^{2+}}} = \frac{\Delta F_{M^{n+}}}{\Delta F_{Hg^{2+}}}$, were calculated for all metal ions investigated for possible interference.[23] Almost no change in the fluorescence intensity of **NO_2/Dan$_4$** is observed in the presence of Fe^{2+}, Pb^{2+}, and K^+, respectively (Fig. 4), suggesting no interference from these metal ions (*i.e.*, $k_{M^{n+},Hg^{2+}} = 0$). Values of $k_{M^{n+},Hg^{2+}}$ for other metal ions are calculated and plotted in Fig. 4 (inset). A careful observation of this data reveals $k_{Mn^+,Hg^{2+}}$ for most metal ions to be small enough to pose any significant interference with the detection of Hg^{2+}. We also carried out interference studies by measuring reduction in fluorescence signal of **NO_2/Dan$_4$** by Hg^{2+} in the presence of other metal cations (ESI: Fig. S5). The data implies that even in the presence of other metal cations, the efficiency of Hg^{2+} in quenching **NO_2/Dan$_4$** fluorescence is significant, and in many cases, even similar to that observed in the absence of any other metal cation. These results imply significantly high selectivity associated with host **NO_2/Dan$_4$** toward the sensing of Hg^{2+}. It is important to mention that under identical conditions fluorescence response of all of our other dansyl-appended calix[4]arenes as well as the model compounds are not affected to the same extent as Hg^{2+} is added to the solution. As shown in Fig. 5, the % reduction in fluorescence intensity upon addition of 8 μM Hg^{2+} is observed to be insignificant for **t-Bu/(OH)$_2$Dan$_2$** and **t-Bu/(Ester)$_2$Dan$_2$**, but were found to be *ca.* 38%, 31%, and 20% for **H/Dan$_4$**, **H/(OH)$_2$Dan$_2$**, and **H/(Ester)$_2$Dan$_2$**, respectively. Though the% reduction in fluorescence intensities of **H/Dan$_4$**, **H/(OH)$_2$Dan$_2$**, and **H/(Ester)$_2$Dan$_2$** upon addition of 8 μM Hg^{2+}, respectively, may not be deemed insignificant, it is certainly not as dramatic as that of **NO_2/Dan$_4$**. It is clear that fluorescence of NO_2-substituted dansyl-appended calix[4]arene has the highest sensitivity to the presence of Hg^{2+} followed by a much lower sensitivity to the unsubstituted and almost no sensitivity to the *tert*-butyl-substituted dansyl-appended calix[4]arenes. At this point, we would like to propose the conformational dissimilarity of **NO_2/Dan$_4$** (it shows a 1,3-alternate conformation, *vide supra*) with that of our other dansyl-appended calix[4]arenes (they exist in cone conformation) to be the reason for this unique behavior. This is further supported by a previous literature report implying that calixarene derivatives in the 1,3-alternate conformation show superior metal binding capabilities as compared to their cone counterparts.[12b]

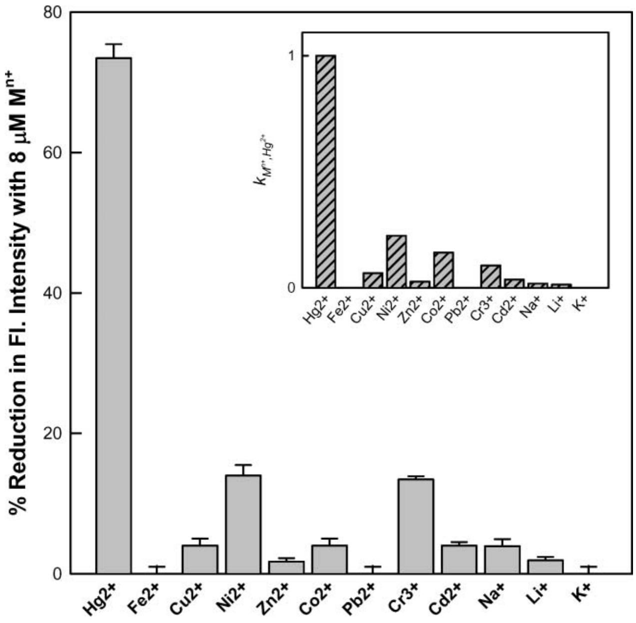


Fig. 4 Percentage reduction in the fluorescence intensity of **NO_2/Dan$_4$** (1×10^{-5} M) in the presence of 8 μM M^{n+} in acetonitrile at ambient conditions ($\lambda_{excitation}$ = 351 nm). Inset shows selectivity coefficients $k_{M^{n+},Hg^{2+}} = \frac{m_{M^{n+}}}{m_{Hg^{2+}}} = \frac{\Delta F_{M^{n+}}}{\Delta F_{Hg^{2+}}}$ for **NO_2/Dan$_4$**.

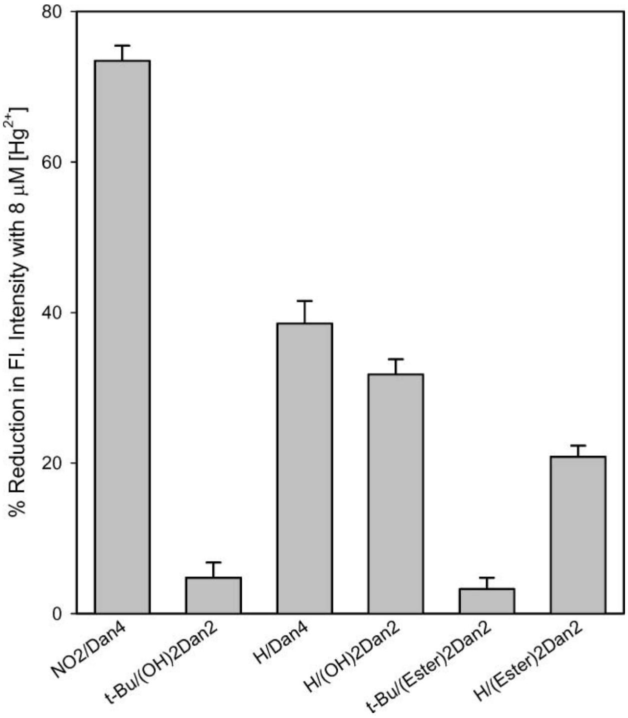


Fig. 5 Percentage reduction in the fluorescence intensity of **NO_2/Dan$_4$**, **t-Bu/(OH)$_2$Dan$_2$**, **H/Dan$_4$**, **H/(OH)$_2$Dan$_2$**, **t-Bu/(Ester)$_2$Dan$_2$**, and **H/(Ester)$_2$Dan$_2$** in the presence of 8 μM Hg^{2+} in acetonitrile at ambient conditions ($\lambda_{excitation}$ = 351 nm).

Reversibility is universally recognized as one of the most desired properties of a modern day chemosensor.[20] To further explore the properties of **NO_2/Dan$_4$** as a chemosensing agent for Hg^{2+}, we carried out a set of experiments to determine the reversibility of this system. For this purpose, we used ethylenediaminetetraacetic acid (EDTA), an external additive, as the formation constant of EDTA:Hg^{2+} complex is very high ($K_f = 6.3 \times 10^{21}$ at 20 °C).[24] We observed that the decrease in fluorescence intensity of **NO$_2$/Dan$_4$** due to the addition of Hg^{2+} was completely recovered by the addition of EDTA of the same concentration as Hg^{2+} (data not shown). Further addition of Hg^{2+} again reduced the fluorescence intensity of **NO$_2$/Dan$_4$** in the solution. This cycle was repeated several times with the same final outcome. This clearly establishes the reversible nature of our chemosensing agent. In summary, we have demonstrated the sensing capability of our dansyl-appended calix[4]arene molecular framework, **NO$_2$/Dan$_4$**, for Hg^{2+} to be highly sensitive, adequately selective and reversible.

Stoichiometry and efficiency of NO$_2$/Dan$_4$: Hg^{2+} interaction

Fluorescence data of **NO$_2$/Dan$_4$** in the presence of varying [Hg^{2+}] (at higher concentration regime) was further analyzed to obtain key information concerning the interaction between the host and the guest. A detailed Stern–Volmer analysis[2,20] of the fluorescence data ruled out the possibility of dynamic or static quenching or a combination of both ($\frac{F_0}{F}$ *versus* $[Hg^{2+}]$ as well as $\frac{\frac{F_0}{F}-1}{[Hg^{2+}]}$ *versus* $[Hg^{2+}]$ are observed to be far from linear) suggesting interaction or complexation between **NO$_2$/Dan$_4$** and $[Hg^{2+}]$ to be not of the 1:1 type. A plot of $\frac{F_0-F}{F}$ *versus* the mole fraction of the host (*i.e.*, x_{NO_2/Dan_4}) (please refer to Fig. 6) clearly shows the stoichiometry of the complexation to be NO$_2$/Dan$_4$:[Hg^{2+}] = 2:1. Based on our data, formation of the complex of the type (NO$_2$/Dan$_4$)$_2$Hg^{2+} may be proposed as the result of the recognition of Hg^{2+} by **NO$_2$/Dan$_4$**. Based on the equilibrium

$$2\ NO_2/Dan_4 + Hg^{2+} \rightleftharpoons (NO_2/Dan_4)_2Hg^{2+}$$

where

$$K_{eq} = K_f = \frac{[(NO_2/Dan_4)_2Hg^{2+}]_{eq}}{[NO_2/Dan_4]^2_{eq}[Hg^{2+}]_{eq}} \qquad (1)$$

a plot of $\frac{1}{F^2}$ *versus* $\frac{[Hg^{2+}]_0}{\Delta F}$ is observed to show good linear behavior (refer to inset Fig. 6 and ESI), where ΔF and F are the decrease in fluorescence intensity and the fluorescence intensity at the wavelength of analysis, respectively, and $[Hg^{2+}]_0$ is the analytical concentration of Hg^{2+} in the solution. Using the values of the slope and the *y*-intercept along with independently obtained relationship between $[NO_2/\mathrm{Dan}_4]$ and F, the K_{eq} (or the K_f) for the complexation of **NO$_2$/Dan$_4$** with Hg^{2+} is calculated to be 5.2(±0.8) × 10^{10} M^{-2} at ambient conditions (see ESI for details). A K_{eq} (or K_f) = 5.2(±0.8) × 10^{10} M^{-2} represents a fairly efficient complexation between **NO$_2$/Dan$_4$** and Hg^{2+} that manifests itself in a favorable LOD for Hg^{2+} by this novel host (*vide supra*).

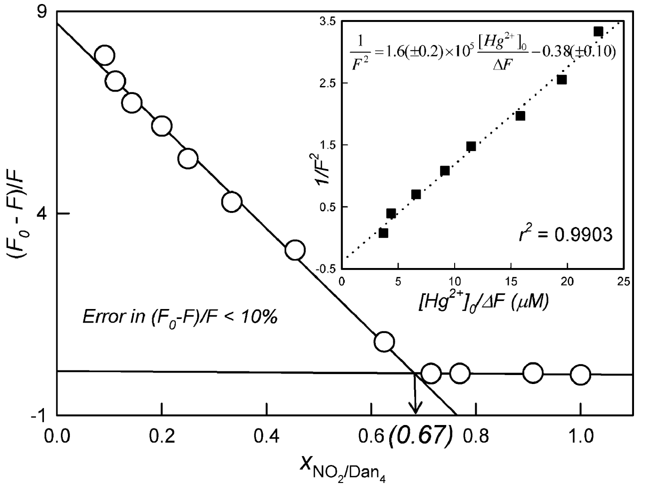


Fig. 6 $\frac{F_0-F}{F}$ *versus* x_{NO_2/Dan_4} from fluorescence reduction of **NO$_2$/Dan$_4$** in the presence of Hg^{2+} ($\lambda_{excitation}$ = 351 nm) clearly showing the stoichiometry of the complexation to be 2 **NO$_2$/Dan$_4$** per Hg^{2+} at ambient conditions. Inset shows the plot of $\frac{1}{F^2}$ *versus* $\frac{[Hg^{2+}]_0}{\Delta F}$, where $[Hg^{2+}]_0$ is the analytical concentration of Hg^{2+} in the solution (for detail, see ESI).

Fluorescence of dansyl-appended compounds: Stokes shifts

The Stokes shift (SS), difference between the electronic excitation and emission maxima in cm^{-1}, for a solvatochromic fluorescence probe is observed to depend empirically upon the dielectric constant (*ε*) and the refractive index (*n*) of the cybotactic region through the Lippert-Mataga equation[21]

$$SS = \frac{\Delta f}{hca^3}(\mu_E - \mu_G)^2 + \text{constant} \qquad (2)$$

where *h* is Planck's constant, *c* is speed of light, *a* is the cavity radius swept out by the fluorophore, and μ_E and μ_G are the fluorophore excited-state dipole moment and fluorophore ground-state dipole moment, respectively. The term Δ*f* is empirical in nature and is called solvent orientational polarizability:

$$\Delta f = \left[\frac{\varepsilon - 1}{2\varepsilon + 1}\right] - \left[\frac{n^2 - 1}{2n^2 + 1}\right] \qquad (3)$$

In order to assess the dansyl cybotactic region in different compounds, we collected emission and excitation maxima of all our dansyl-appended compounds in seven different solvents: tetrahydrofuran (THF), ethanol (EtOH), acetic acid, toluene, CHCl$_3$, dichloromethane (DCM), and acetonitrile (ACN) at ambient conditions. Stokes shifts are calculated from the fluorescence spectra and are presented in Fig. 7. While for single dansyl-containing model compounds it may be assumed that the fluorescence behavior of dansyl depends on the solvent only, for dansyl-appended compounds it may not be so. The molecular architecture around dansyl moiety may have significant affect on its fluorescence behavior. A careful examination of Fig. 7 reveals several interesting outcomes. It is convenient to note that average SS of all Dan$_4$ compounds is observed to be lower than those for Dan$_2$ and Dan$_1$ compounds (8530 ± 295 cm^{-1} *versus* 9042 ± 537 cm^{-1} *versus* 9013 ± 611 cm^{-1}). Clearly, while the presence of two dansyl moieties on a calix[4]arene framework does not alter the dipolarity of the dansyl cybotactic region in comparison to that of mono-dansylated compounds, introduction of two more dansyl reduces the dipolarity of the dansyl microenvironment. This may suggest the presence of interaction between the dansyl moieties when four of them are crowding the one calix[4]arene framework. Further, in general, the average SS of the compounds are in agreement with the dipolarity of the solvents. Specifically, the maximum average SS decreases in the order: acetic acid > ACN > THF > EtOH > DCM > $CHCl_3$ > toluene. In model compounds, except in toluene, the decreasing order of the SS is **t-Bu/Dan$_1$** > **NO_2/Dan$_1$** > **H/Dan$_1$**. This must be due to the nature of the substituents on the model compound framework (*i.e.*, t-Bu *versus* NO_2 *versus* H). If the cavity radius swept by dansyl is considered similar for all three, the difference ($\mu_E - \mu_G$) appears to be maximum for **t-Bu/Dan$_1$**. However, the fact that a similar trend is not observed for multi dansyl-appended calix[4]arenes with different functionalities (see Fig. 7) further indicates interaction of dansyl moiety with other dansyl moiety (or moieties) and/or with the molecular architecture around the dansyl.

For each compound, SS in different solvents is plotted against Δf of the solvent (eqn 3). Fair-to-good linear regression coefficients indicate compliance with Lippert-Mataga empirical relationship (eqn 2). The most important information is afforded by the slope of the Lippert-Mataga equation (inset Fig. 7). To our convenience, the slope for **NO_2/Dan$_4$** is observed to be significantly higher than those for all other compounds. This provides an explanation and lends support to our chemosensing results (*vide supra*), which indicated the better sensitivity of **NO_2/Dan$_4$** fluorescence in the presence of Hg^{2+} as compared to our other dansyl-appended compounds. The slope of the Lippert-Mataga equation is $\frac{(\mu_E - \mu_G)^2}{hca^3}$. Considering similar cavity radius (a) for dansyl moiety in all compounds investigated, a maximum ($\mu_E - \mu_G$) for **NO_2/Dan$_4$** indicates the difference in the transition dipole moment from ground to excited electronic state to be maximum for this compound. In general, the larger the ($\mu_E - \mu_G$), the more sensitive the fluorophore (or fluorophoric moiety) is toward its immediate microenvironment. We may propose that a relatively large difference in the ground to excited state dipole moment imparts the unique chemosensing behavior to **NO_2/Dan$_4$**.

Fluorescence of dansyl-appended compounds: relative fluorescence intensities

The reason for the solvatochromism of dansyl and similar fluorophores can be traced to at least two major reasons. The first reason is the change in fluorophore's dipole moment on optical excitation as a result of general solvent effect depending on static dielectric constant and refractive index of the solvent milieu according to Lippert-Mataga equation (*vide supra*).[20] A second contribution to their solvatochromism arises from the fact that they possess two excited states; locally excited (LE) and twisted intramolecular charge transfer (TICT). On excitation, the less polar, semi-planar state forms initially, which is very similar in structure to the ground state, giving rise to structured fluorescence spectrum. This LE state can undergo intramolecular electron/charge transfer from the donor site -$N(CH_3)_2$ to the acceptor site -SO_2- with a corresponding twist of the donor residue about the bond that connects it to the acceptor. Subsequently, in the TICT state, the donor and acceptor are orthogonal to one another, and this state is much more polar in comparison to the LE state. The fluorescence corresponding to this TICT state is usually structure-less and is shifted bathochromically with respect to the LE state. The propensity to form the TICT state and thus shift the spectrum is influenced by the solvent dielectric properties which help to mediate charge transfer *and* the ability of the donor residue to rotate within the fluorophore. The ability of the donor

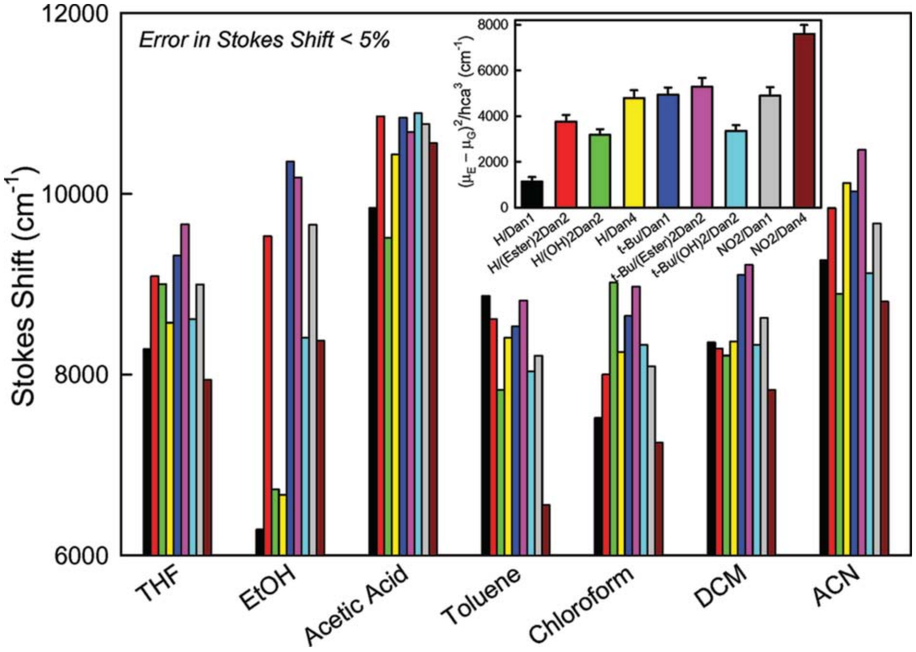


Fig. 7 Stokes shifts of novel dansyl-appended calix[4]arenes in various solvents at ambient conditions. Inset shows $\frac{(\mu_E - \mu_G)^2}{hca^3}$ (*i.e.*, the slope of the Stokes shifts *versus* orientational polarizability of solvents plot according to eqns 2 and 3) for six novel dansyl-appended calix[4]arenes as well as three reference compounds. The color of the bar corresponding to a compound in the inset represents the Stokes shift of the same compound in each of the solvents.

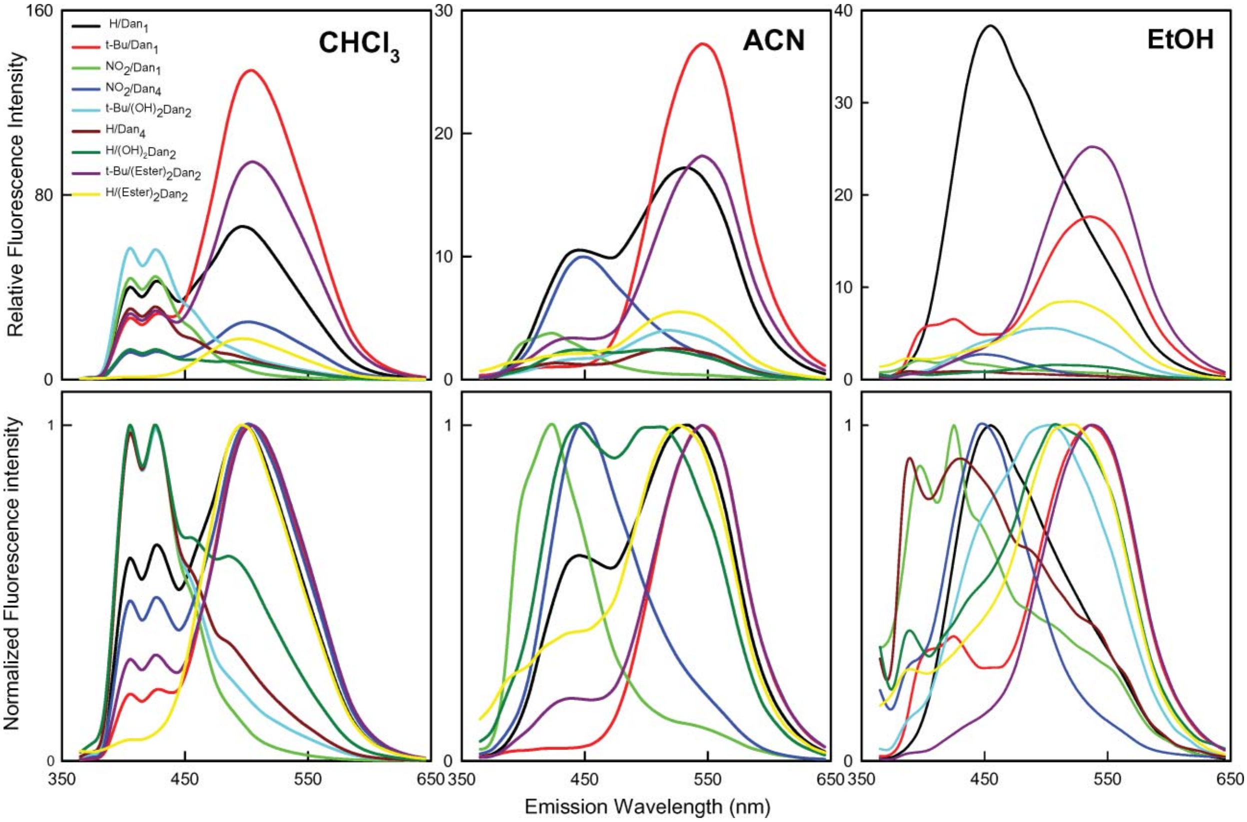


Fig. 8 Fluorescence spectra of six novel dansyl-appended calix[4]arenes and three reference compounds in nonpolar chloroform (left panels), polar-aprotic acetonitrile (middle panels), and polar-protic ethanol (right panels) at ambient conditions ($\lambda_{\text{excitation}} = 351$ nm). Relative and normalized fluorescence intensities are represented on the *y*-axis in the upper and lower panels, respectively.

to rotate depends, in part, on any physical impediments to donor rotation.[21]

We collected fluorescence emission spectra of our dansyl-appended calix[4]arenes in relatively nonpolar $CHCl_3$, polar-aprotic ACN, and polar-protic EtOH at ambient conditions (Fig. 8). Most importantly, in the fluorescence data collection the absorbance of the solutions of different compounds at the excitation wavelength are identical, such that the same number of photons of the given energy is used in the excitation process irrespective of the identity of the compound. Fig. 8 (left panels) presents fluorescence emission spectra of all the compounds in $CHCl_3$ at ambient conditions. While relative fluorescence is shown in the top panel, the bottom panel presents normalized fluorescence intensity with respect to the highest energy band for each of the compounds. Structured LE fluorescence from dansyl moiety occurs at lower wavelengths while the broad structure-less TICT band appears bathochromically shifted for all the compounds. It is convenient to note that, except for NO_2-containing dansylated compounds, the fluorescence intensity due to TICT is maximum for model compounds, **H/Dan$_1$** and **t-Bu/Dan$_1$**. It is easy to comprehend, as charge-transfer would be unhindered in these compounds. For calix[4]arene compounds appended with two or more dansyl moieties, reduction in the efficiency of TICT is represented in the reduced lower energy fluorescence from these compounds (*e.g.*, the TICT fluorescence intensity follows the trend **H/Dan$_1$** > **H/(Ester)$_2$Dan$_2$** > **H/(OH)$_2$Dan$_2$** ~ **H/Dan$_4$** and **t-Bu/Dan$_1$** > **t-Bu/(Ester)$_2$Dan$_2$** > **t-Bu/(OH)$_2$Dan$_2$**). Apparently, the presence of ester groups appears to help the TICT process. To our surprise, **NO$_2$/Dan$_4$** shows appreciable TICT fluorescence as compared to the model compound **NO$_2$/Dan$_1$**. This must be attributed to the electron-withdrawing capability of the NO_2 groups located on calix[4]arene framework. Exceptional behavior of **NO$_2$/Dan$_4$** is again demonstrated nonetheless.

The TICT is expected to be facilitated in ACN, a polar-aprotic solvent. It is clear from the fluorescence spectra of the compounds in ACN (Fig. 8, middle panels) that, in general, the structured higher energy LE fluorescence is replaced by broad and bathochromically shifted fluorescence bands in most compounds. Excited-state intramolecular interactions among dansyl groups are again clearly indicated by the decreased fluorescence from two or more dansyl-containing calix[4]arenes as compared to the model compounds. It is easy to comprehend that the similar order of TICT fluorescence is observed in ACN as that in $CHCl_3$; even the increased polarity of ACN could not enhance the TICT process in **NO$_2$/Dan$_1$**. It is clear again that TICT is restricted in NO_2-containing compounds which confirms our earlier results (*vide supra*). Fig. 8 (right panels) presents fluorescence of dansyl-appended compounds in EtOH at ambient conditions. In most cases, spectra have become broader as compared to the spectra in $CHCl_3$ and ACN. While the model compound **H/Dan$_1$** shows significant fluorescence; the fluorescence signal decreases considerably for two or more dansyl-containing calix[4]arenes, again indicating the possibility of excited-state energy transfer between the dansyl moieties within the calixarene molecular framework. Interestingly, NO_2-containing compounds show much reduced fluorescence in EtOH (top panel Fig. 8). We may conclude that fluorescence from dansyl-appended calix[4]arenes clearly demonstrate excited-state interaction between dansyl moieties, and further that the fluorescence from **NO_2/Dan$_1$** and **NO_2/Dan$_4$** show different trends from those shown by other compounds. These differences lend further support to the observation of selective chemosensing of Hg^{2+} by **NO_2/Dan$_4$**.

Conclusion

Among the six novel dansyl-appended calix[4]arene frameworks bearing two and four dansyl groups, fluorescence of one of the compounds, **NO_2/Dan$_4$**, is demonstrated to be remarkably sensitive to the presence of Hg^{2+} in the solution; the detection limit is estimated to be ~20 ppb. The compound shows high selectivity and adequate reversibility as a chemosensing agent for Hg^{2+}. The reason for this recognition behavior is proposed to be the 1,3-alternate conformation of **NO_2/Dan$_4$** as compared to the cone conformation of all other dansyl-appended calix[4]arenes. The stoichiometry of **NO_2/Dan$_4$** : Hg^{2+} complexation is observed to be 2:1 with a formation constant of $5.2(\pm 0.8) \times 10^{10}$ M^{-2} at ambient conditions. Stokes shift measurements of dansyl-appended calix[4]arenes indicate the **NO_2/Dan$_4$** cybotactic region to be the most sensitive, as the change in the dipole moment from ground state to excited state is found to be maximum for this compound. The fluorescence intensity of **NO_2/Dan$_4$** in chloroform, acetonitrile, and ethanol is observed to show different trends from other dansyl-appended calix[4]arenes. In conclusion, the synthesis, characterization, and interesting properties of novel dansyl-appended calix[4]arene molecular frameworks have been established for their potential utilization in various chemical applications.

Experimental

Materials and instrumentation

All the reagents used in the study were purchased from Sigma–Aldrich or Merck and were chemically pure. The solvents used were dried and distilled. HPLC grade solvents were used for fluorescence spectroscopic measurements. Column chromatography was performed on silica gel (60–120 mesh) obtained from Merck. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument using tetramethylsilane (TMS) at 0.00 as an internal standard. Low temperature ^1H NMR spectra were also recorded on the same instrument as mentioned above. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass spectrometer/Data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas while IR spectra were recorded on a Nicolet Protégé 460 spectrometer in KBr disks. Fluorescence spectra were acquired on a spectrofluorometer purchased from Horiba-Jobin Yvon, Inc. model FL 3–11 Fluorolog-3 modular spectrofluorometer with single Czerny-Turner grating excitation and emission monochromators having 450 W Xe arc lamp as the excitation source and PMT as the detector. A Perkin-Elmer Lambda bio-20 double beam spectrophotometer with variable bandwidth was used for acquisition of the uv-vis molecular absorbance. All the data were acquired using 1-cm^2 path length quartz cuvettes. Spectral response from appropriate blanks was subtracted before data analysis. All the measurements were taken in triplicate and averaged. Dilution correction was also carried out where necessary. Melting points were determined on an electrothermal melting point apparatus obtained from M/S Toshniwal and were uncorrected.

Synthesis of starting material

p-*Tert*-butylcalix[4]arene (**1**), calix[4]arene (**2**), 5,11,17,23-tetra(*p*-*tert*-butyl)-25,27-di(ethoxycarbonylmethoxy)-26,28-dihydroxy-calix[4]arene (**1a**), 25,27-di(ethoxycarbonylmethoxy)-26,28-dihydroxy-calix[4]arene (**2a**), and 5,11,17,23-tetranitro-25,26,27,28-tetrahydroxy-calix[4]arene (**3**) were synthesized as described previously.[18,19] The analytical data for compounds **1**, **2**, **1a**, **2a**, and **3** were found to be same as reported earlier.[18,19]

General procedure for the synthesis of dansylated calix[4]arene derivatives

To a solution of corresponding calix[4]arene (**1**, **2**, **1a**, **2a**, and **3**) in MeCN (20mL), Et_3N and dansyl chloride (equimolar to per hydroxyl group) were added under N_2 atmosphere with vigorous stirring. The reaction mixture was stirred at room temperature for 96 hours. In most of the cases a light yellow precipitate formed, that was filtered, redissolved in $CHCl_3$, and washed with water and extracted with $CHCl_3$. The organic layer was collected and evaporated to dryness under reduced pressure to yield dansylated calix[4]arene derivatives as yellow solid that was further purified by column chromatography and recrystallized from $CHCl_3$/CH_3OH.

Compound t-Bu/(OH)$_2$Dan$_2$. Pale yellow solid, yield: 66%, mp 231–233 °C; UV (λ_{max}, CH_3CN): 272, 355 nm. IR (KBr pellet, cm^{-1}): 3571, 1578, 1365, 1307, 1262, 1190, 1083. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.59 (2H, d, Dan-*H*), and 8.47 (2H, d, Dan-*H*), 8.14 (2H, d, Dan-*H*), 7.54 (4H, m, Dan-*H*), 7.14 (2H, d, Dan-*H*), 6.86 and 6.52 (8H, 2 s, Ar-*H*), 3.85 (d, 4H, ArCH_2Ar), 2.92 (d, 4H, ArCH_2Ar), 2.82 (s, 12H, N(CH_3)$_2$), 1.17, 0.74 (2 s, 36H, C(CH_3)$_3$), 1.49 (bs, 2H, OH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 151.3, 149.9, 149.0, 142.3, 133.0, 131.8, 129.9, 129.3, 127.9, 125.9, 125.0, 122.8, 119.9, 115.9, 45.3, 32.4, 31.5. HRMS (ESI-TOF) m/z: calcd. 1114.5200, found 1114.5498 [M$^+$].

Compound H/(OH)$_2$Dan$_2$. Pale yellow solid, yield: 56%, mp 271–272 °C; UV (λ_{max}, CH_3CN): 272, 356 nm. IR (KBr pellet, cm^{-1}): 3549, 1577, 1365, 1311, 1243, 1184, 1063. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.67 (2H, d, Dan-*H*), and 8.65 (2H, d, Dan-*H*), 8.18 (2H, d, Dan-*H*), 7.67 (4H, m, Dan-*H*), 7.22 (2H, d, Dan-*H*), 6.93 (d, 4H, Ar-H), 6.64 (8H, m, Ar-*H*), 3.95 (d, 4H, ArCH_2Ar), 3.03 (d, 4H, ArCH_2Ar), 2.89 (s, 12H, N(CH_3)$_2$), 1.57 (bs, 2H, OH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 151.3, 149.9, 149.0, 142.3, 133.8, 132.1, 130.2, 129.4, 129.0, 128.6, 126.9, 122.9, 119.5, 116.0, 45.3, 31.5; HRMS (ESI-TOF) m/z: calcd. 891.2774, found 891.2812 [M$^+$].

Compound H/Dan$_4$. Pale yellow solid, yield: 70%, mp 260–262 °C; UV (λ_{max}, CH_3CN): 272, 339 nm. IR (KBr pellet, cm^{-1}): 1575, 1366, 1310, 1184, 1133, 1061. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.84 (4H, d, Dan-*H*), 8.81 (4H, d, Dan-*H*), 8.35 (4H, d, Dan-*H*), 7.84 and 7.71 (8H, t, Dan-*H*), 7.38 (4H, d, Dan-*H*), 7.09

(d, 4H, Ar-*H*), 6.83 (m, 8H, Ar-H), 4.10 (d, 4H, ArCH_2Ar), 3.19 (d, 4H, ArCH_2Ar), 3.06 (s, 24H, N(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 152.5, 151.5, 144.7, 133.9, 132.1, 130.2, 129.8, 129.4, 129.0, 128.6, 128.4, 126.9, 122.9, 119.6, 116, 45.4, 31.6 HRMS (ESI-TOF) m/z: calcd.1356.3717, found 1356.3727 [M$^+$].

Compound t-Bu/(Ester)$_2$Dan$_2$. Pale yellow solid, yield: 60%, mp 210–211 °C; UV (λ_{max}, CH_3CN): 272, 348 nm. IR (KBr pellet, cm^{-1}): 1761, 1581, 1362, 1304, 1184, 1122, 1065. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.62 (2H, d, Dan-*H*), 8.23 (4H, m, Dan-*H*), 7.55 (2H, t, Dan-*H*), 7.39 (2H, t, Dan-*H*), 7.12 (2H, d, Dan-*H*), 6.87 and 6.26 (8H, 2 s, Ar-*H*), 4.72 (s, OCH_2CO), 4.26 (q, 4H, OCH_2CH$_3$), 4.23 (d, 4H, ArCH_2Ar), 2.88 (s, 12H, N(CH_3)$_2$), 2.62 (d, 4H, ArCH_2Ar), 1.35 (t, 6H, CH_3), 1.23, 0.73 (2 s, 36H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 169.7, 153.0, 151.2, 147.6, 145.4, 142.6, 133.18, 132.3, 131.3, 129.4, 128.3, 125.9, 124.9, 123.2, 119.8, 115.1, 70.13, 60.5, 45.3, 32.3, 31.5, 14.2; HRMS (ESI-TOF) m/z: calcd 1309.5933, found 1309.5298 [M$^+$ + Na].

Compound H/(Ester)$_2$Dan$_2$. Pale yellow solid, yield: 45%, mp 222–224 °C; UV (λ_{max}, CH_3CN): 272, 347 nm. IR (KBr pellet, cm^{-1}): 1710, 1577, 1366, 1310, 1259, 1185, 1067. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.65 (2H, d, Dan-*H*), 8.25 (2H, d, Dan-*H*), 8.09 (2H, d, Dan-*H*), 7.53 (4H, m, Dan-*H*), 7.19 (2H, d, Dan-*H*), 6.74–6.27 (12H, m, ArH), 3.94–3.69 (8H, m, OCH_2CH$_3$ and OCH_2CO), 3.36 (d, 4H, ArCH_2Ar), 2.89 (s, 12H, N(CH_3)$_2$), 2.52 (d, 4H, ArCH_2Ar), 1.25 (t, 6H, CH_3), ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 169.4, 165.6,156.7, 151.4, 145.4, 135.9, 134.5, 132.3, 131.6, 130.8, 129.5, 128.7, 127.7, 125.3, 123.2, 122.7, 119.8, 115.4, 70.5, 60.8, 45.4, 31.9, 14.2; HRMS (ESI-TOF) m/z: calcd. 1062.7941, found 1062.7570 [M$^+$].

Compound NO$_2$/Dan$_4$. Pale yellow solid, yield: 40%, mp>232 °C (decomp.); UV (λ_{max}, CH_3CN): 272, 347 nm. IR (KBr pellet, cm^{-1}): 1579, 1529, 1345, 1261, 1189, 1094, 1026. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.78 (4H, d, Dan-*H*), 8.43 (4H, d, Dan-*H*), 8.01 (4H, d, Dan-*H*), 7.81 (4H, t, Dan-*H*), 7.56 (4H, t, Dan-*H*), 7.32(4H, d, Dan-H), 7.58 (8H, s, Ar-H), 3.37 (8H, s, ArCH_2Ar), 2.95 (s, 24H, N(CH_3)$_2$), ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 152.3, 149.8, 144.7, 134.9, 133.7, 130.9, 130.3, 125.0,122.9, 118.1, 116.6, 45.4, 34.2; HRMS [ESI-TOF] m/z: calcd.1538.3276, found 1538.3208 [M$^+$].

Compound t-Bu/Dan$_1$. Pale yellow solid, yield: 80%, mp 95–97 °C; UV (λ_{max}, CH_3CN): 272, 352 nm. IR (KBr pellet, cm^{-1}): 1575, 1368, 1302, 1263, 1150, 1065, 1020. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.54 (H, d, Dan-*H*), 8.51 (H, d, Dan-*H*), 8.04 (H, d, Dan-*H*), 7.61 (2H, t, Dan-*H*), 7.41 (2H, t, Dan-*H*), 7.10(H, d, Dan-H), 7.19(2H, d, Ar-H), 6.74 (2H, d, Ar-H), 2.84 (s, 6H, N(CH_3)$_2$), 1.15 (s, 9H, C(CH_3)$_3$).^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 151.4, 149.6, 147.0, 131.4, 130.7, 129.3, 126.0, 122.6 121.6, 119.2, 115.2, 45.1, 34.1; HRMS (ESI-TOF) m/z: calcd. 406.1453, found 406.1446 [M$^+$+ Na].

Compound H/Dan$_1$. Pale yellow solid, yield: 70%, mp 100–101 °C; UV (λ_{max}, CH_3CN): 272, 363 nm. IR (KBr pellet, cm^{-1}): 1577, 1365, 1310, 1265, 1143, 1065, 1020. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.57 (H, d, Dan-*H*), 8.38 (H, d, Dan-*H*), 8.21 (H, d, Dan-*H*), 7.56 (2H, t, Dan-*H*), 7.35 (2H, t, Dan-*H*), 7.09(H, d, Dan-H), 7.13 (3H, m, Ar-H), 6.72(2H, m, Ar-H), 2.77 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 152.3, 149.8, 144.7, 134.9, 133.7, 130.9, 130.3, 123.2, 122.6, 119.8, 116.2, 45.7; HRMS (ESI-TOF) m/z: calcd. 327.5991, found 327.5912 [M$^+$].

Compound NO$_2$/Dan$_1$. Pale yellow solid, yield: 85%, mp 98–100 °C; UV (λ_{max}, CH_3CN): 272, 356 nm. IR (KBr pellet, cm^{-1}): 1577, 1522, 1345, 1315, 1235, 1175, 1101, 1065. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 8.57 (H, d, Dan-*H*), 8.36 (H, d, Dan-*H*), 8.03 (2H, d, Ar-*H*), 7.65 (2H, t, Dan-*H*), 7.39 (2H, t, Dan-*H*), 7.10(2H, d, Dan-H), 7.19 (2H, d, Ar-H), 2.84 (s, 6H, N(CH_3)$_2$).^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 153.9, 152.1, 146.0, 132.6, 131.4, 129.4, 125.2,122.8, 118.8, 115.8, 45.3; HRMS (ESI-TOF) m/z: calcd. 373.5991, found 373.5901 [M$^+$].

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