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Pyrene Excimer-Based Calix[4]arene FRET Chemosensor for Mercury(II)

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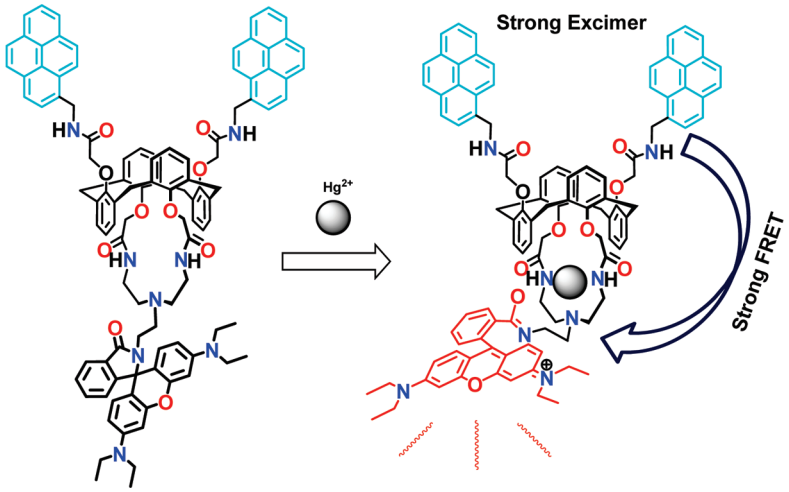

A novel calix[4]arene derivative locked in the 1,3-alternate conformation (**2**) bearing two pyrene and rhodamine fluorophores was synthesized as a selective sensor for the Hg^{2+} ion. The sensoring is based on FRET from pyrene excimer emissions to ring-opened rhodamine absorption upon complexation of the Hg^{2+} ion. Addition of Hg^{2+} to a mixed solution of **2** gave significantly enhanced fluorescence at ~576 nm via FRET with excitation at 343 nm. We also found that the pyrene excimer emissions formed by the intramolecular $\pi-\pi$ interactions are more effective in obtaining strong FRET bands than those by intermolecular $\pi-\pi$ interactions.

Introduction

Mercury undergoes long-range transport in the environment among various media such as air, soil, and water by deposition from anthropogenic releases. However, elemental and ionic mercury can be converted into methylmercury by bacterial or chemical action; methylmercury is highly toxic and causes mercury poisoning due to its special properties, such as migration through cell membranes and bioaccumulation within living tissues.[1]

To detect environmentally and biologically hazardous mercury, a fluorogenic mercury chemosensor based on artificial receptors is viewed as an efficient method because of several advantages over other analytical methods with respect to high sensitivity, specificity, and real-time monitoring with fast response times.[2,3] To fulfill efficient detection of targeted molecules with a chemosensor, it should be pursued in accordance with literature precedence that the sensing ability of the chemosensor is dependent upon the interaction between the recognizing unit (ionophore) responsible for selectively binding the ions and the fluorogenic unit (fluorophore) for signal transduction, whether by fluorescence enhancement or inhibition.[4]

Calix[4]arene has been widely exploited as the basic molecular framework for many fluorescent chemosensors in the

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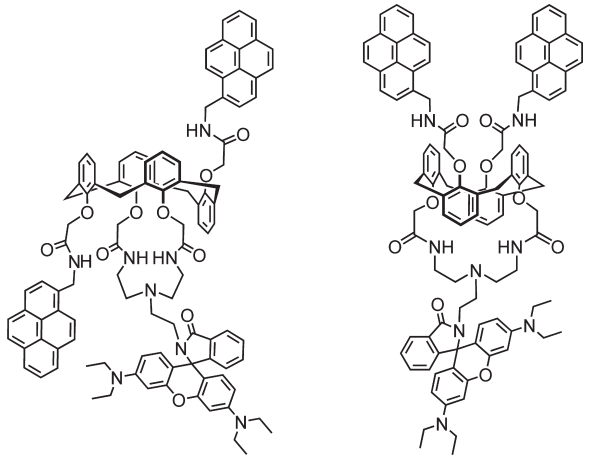
construction of selective binding sites given its structural rigidity, various conformations, and facile introduction of fluorophores.[5] In particular, calix[4]arene derivatives bearing two pyrene moieties as fluorogenic units have attracted considerable interest because they form an efficient excimer signal by the strong intramolecular $\pi-\pi$ interactions between the two pyrenes.[6] For these reasons, until now, a variety of calix[4]arene-based chemosensors with pyrene units have been reported for the detection of organic or inorganic species.[4,5,7]

Recently, fluorogenic research with FRET systems has actively progressed in the region of supramolecular chemistry given its potentially practical benefits in cell physiology and optical therapy, as well as selective and sensitive sensing toward targeted molecules or ionic species.[8,9] FRET arises from an excited-state energy interaction between two fluorophores in which an excited donor transfers energy to an acceptor without photoemission. Accordingly, it is strongly influenced by the distance between donor and acceptor, the extent of spectral overlap between donor emission and acceptor absorption spectra, and the relative orientation of the donor emission dipole moment and acceptor absorption moment.[4,10]

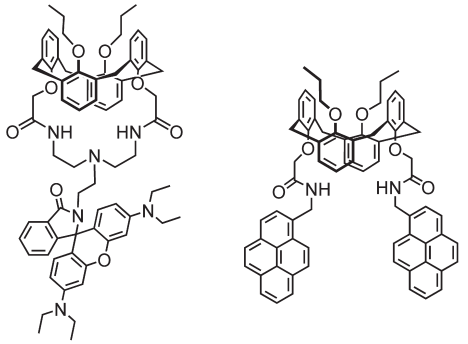
The rhodamine moiety has been used widely in the field of chemosensors, especially as a chemodosimeter, given its fluorescence OFF−ON behavior that results from its particular structural properties. In general, spirolactam formation of rhodamine derivatives is nonfluorescent, whereas its ring-opened amide system gives rise to a strong fluorescence emission.[11] Furthermore, the rhodamine fluorophore exhibits a longer wavelength emission (over 550 nm), often serving as a sensing group for the analyte to avoid the influence of background fluorescence (below 500 nm).[12]

In our previous works, a calix[4]arene was reported that included one 2,3-naphthocrown-6 and two coumarin amide units in a partial-cone conformation that acted as colorimetric and fluorometric sensors for F^- and Cs^+ ions via a FRET system from the naphthalene emission to the coumarin absorption.[13] We have also made efforts toward a pyrenyl excimer-induced FRET system, preliminary to this work, using a rhodamine group as an acceptor. Unfortunately, it did not exhibit effective FRET behavior due to very weak pyrene excimer emissions.[14] Li and co-workers reported a fluorescent probe possessing both BODIPY and rhodamine moieties for selective detection of Hg^{2+} and Ba^{2+} ions by a logic gate system.[15] Nevertheless, reports on FRET-based ion sensors with calix[4]arene remain very rare.

Keeping the aforementioned research in mind, we herein report not only the synthesis of a new pyrene excimer-based calix[4]arene FRET chemosensor (**2**) involving two pyrenyl and one rhodamine substituent, but also its fluorometric properties and sensing abilities toward Hg^{2+} with respect to FRET changes. Above all, the correlation between the pyrene excimer emission and FRET behavior, using **1** and **2**, is proven, which exhibits different intensities of the pyrene excimer emissions that occur by two separate molecular interactions.



1 **2**



3 **4**

Results and Discussion

Two calix[4]arene derivatives (**1** and **2**), both possessing bisamidopyrenyl and rhodamine moieties in the partial-cone and 1,3-alternate conformations, respectively, were prepared

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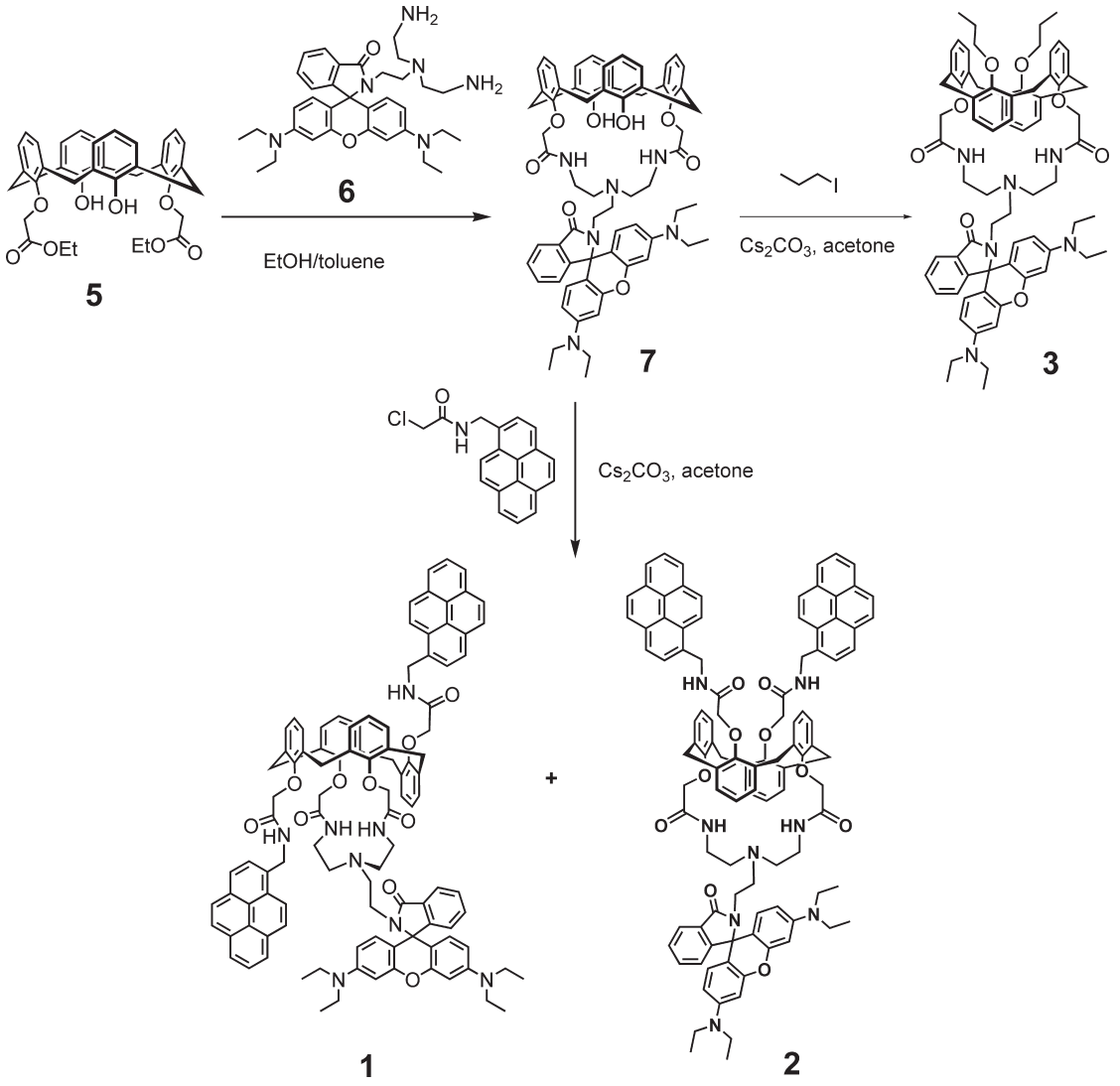
SCHEME 1. Synthetic Routes of Compounds 1−3 and 7



by the synthetic routes depicted in Scheme 1. Cone-**7** was synthesized by the reaction of calix[4]arene diethyl ester **5** and tren-appended rhodamine **6** (tren = N^1,N^1-bis(2-aminoethyl)-ethane-1,2-diamine) in toluene/ethanol (1:1) under reflux conditions to give a white solid. To prepare **1** and **2**, **7** was reacted with 2.1 equiv of *N*-(1-pyrenylmethyl)chloroacetamide and excess Cs_2CO_3 in refluxing acetone. Each conformation was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Synthesis of 1, 3-alternate **3** was achieved by alkylation of **7** with 1-iodopropane in the presence of Cs_2CO_3 as a base.

The structures of **1**−**3** and **7** were characterized by ^{1}H NMR, ^{13}C NMR, FAB-MS, and MALDI-TOF MS. The spirolactam form of the rhodamine unit in **1**−**3** was confirmed by the presence of a peak at ~65 ppm in the ^{13}C NMR spectra.[16] Furthermore, the ^{1}H NMR spectrum of **1**, showing the three sets of doublet peaks at 4.44, 3.92, and 3.09 ppm for eight hydrogen atoms, and double sets of ^{13}C NMR peaks at 38.12 and 30.94 ppm on the Ar*CH*$_2$Ar moiety, respectively, was consistent with the partial-cone conformation of calix[4]arene, whereas **2** displayed a typical ^{1}H NMR spectrum of a calix[4]arene bridge moiety in the 1,3-alternate conformation at 3.47 ppm as a singlet peak and ^{13}C NMR at 37.13 ppm, respectively.[17]

The binding abilities of **1** and **2** were first probed based on UV/vis spectra changes in their mixed organic solutions produced by addition of the perchlorate salt of various metal ions, such as Hg^{2+}, Pb^{2+}, Na^+, K^+, Cs^+, Rb^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Al^{3+}, and In^{3+}, as illustrated in Figure 1. Upon addition of 10 equiv of cations to a solution of **2**, Hg^{2+} and Pb^{2+} lead to the appearance of a new absorption band centered at 559 nm. Figure 1 reveals that complexation of **2** with Hg^{2+} resulted in a relatively larger absorption peak, as well as strong color changes compared to that of Pb^{2+}. The visual color change from colorless to pink was indicative of a typical ring-opened spirolactam moiety of rhodamine toward Hg^{2+}.[18] Compound **1** also showed similar

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UV−vis spectral patterns and visual changes toward Hg^{2+} and Pb^{2+}, but smaller absorbance intensities compared with that of **2** as depicted in Figure S13 in the Supporting Information.

To compare the intensity of the pyrene excimer emission of each ligand (**1**−**3**) without the metal ion, a fluorescence experiment was conducted in the mixed solvent system under the excitation region of pyrene. As shown in Figure 2a, **2** exhibited a strong pyrenyl excimer emission at ~460 nm and weak monomer emissions at 375 and 395 nm, suggesting that the two pyrenyl moieties present on the calix[4]arene skeleton formed a strong intramolecular $\pi-\pi$ interaction between an excited pyrene and ground-state pyrene under UV/vis irradiation. Nevertheless, **1** displayed both a strong monomer peak induced by the monopyrenyl moiety located at the partial-cone position of the calix[4]arene and a slightly larger excimer emission peak, resulting from the intermolecular $\pi-\pi$ interaction between the pyrene and another pyrene bound to the neighboring calix[4]arene.[19] In the case of **3**, it does not give the fluorescence because of the absence of any fluorophore capable of excitation under these conditions. From the results of the pyrene excimer emission, the probability of the FRET formation with two calix[4]arene derivatives (**3** and **4**), involving the respective bispyrenylamido and rhodamine groups, could be estimated. As expected, the spectral results of **4** and **3**·Hg^{2+} exhibited the proper spectral overlap capable of giving rise to a FRET signal of ring-opened rhodamine when the pyrenyl moiety was excited (Figure 2b).

Figure 3a demonstrates the detailed fluorescence changes of **2** (10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v) as a function of Hg^{2+} concentration when excited at 343 nm. Upon gradual addition of Hg^{2+} to a solution of **2**, the increased emission of the ring-opened rhodamine at 576 nm was observed with a concomitantly declining pyrenyl excimer emission at 470 nm with an isosbestic point centered at 550 nm. This ratiometric fluorescence change indicated that an energy transfer from the pyrene excimer to rhodamine was triggered by Hg^{2+} ions.

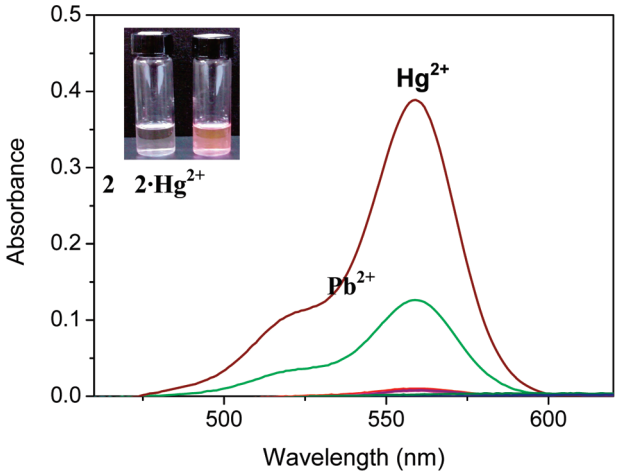

FIGURE 1. UV/vis spectra and visual change of **2** (10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v) in the presence of various metal ions, including Hg^{2+}, Pb^{2+}, Na^+, K^+, Cs^+, Rb^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Al^{3+}, and In^{3+} (10 equiv, respectively, as the perchlorate).

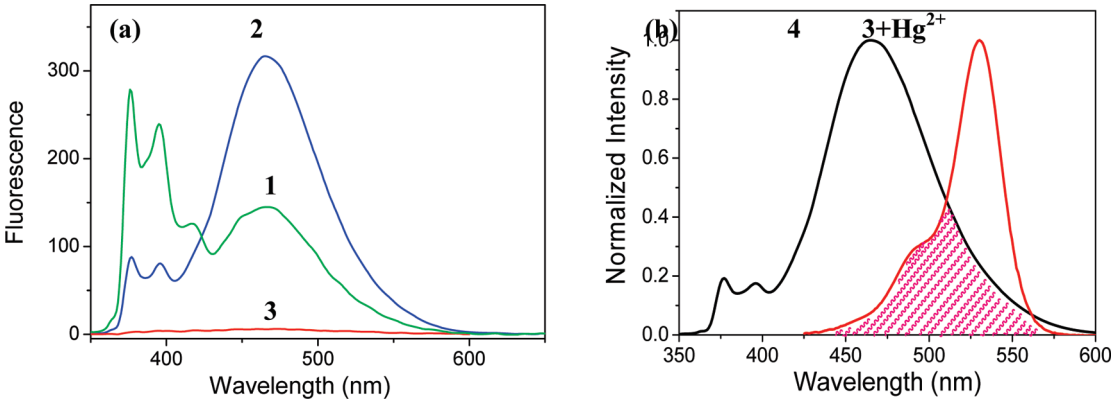

FIGURE 2. (a) Fluorescence spectra of **1**−**4** (10.0 μM) without metal ion and (b) spectral overlap between pyrenyl emission (black) of **4** with an excitation at 343 nm and ring-opened rhodamine absorption (red) of **3**·Hg^{2+} in $CHCl_3/CH_3CN$ (50:50, v/v).

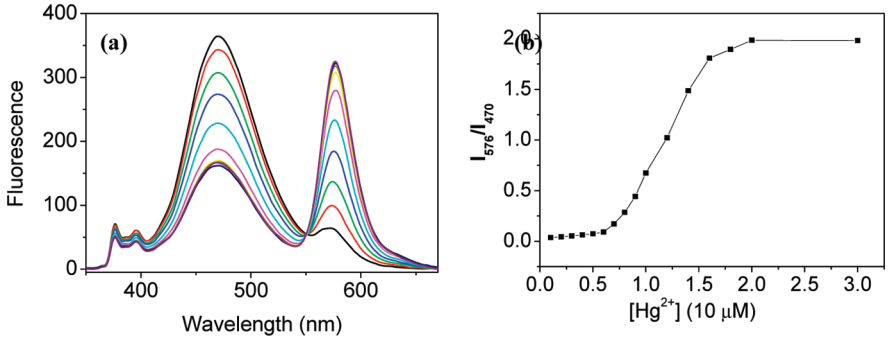

FIGURE 3. (a) Fluorescence titration spectra of **2** (10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v) upon addition of different concentrations of $Hg(ClO_4)_2$ (0.7, 0.8, 0.9, 1, 1.2, 1.4, 1.6, 1.8, 2.0, 3.0 equiv) and (b) the corresponding plot of ratiometric fluorescence intensity ratios (I_{576}/I_{470}) versus Hg^{2+} concentration (0.1−3.0 equiv, λ_{ex} = 343 nm).

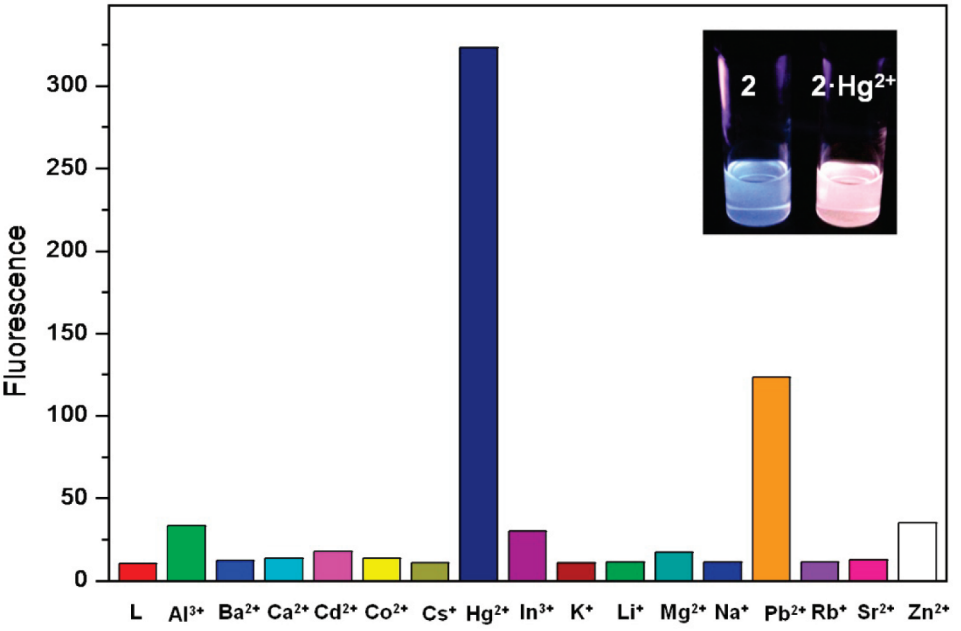

FIGURE 4. Bar profiles of fluorescence intensity for **2** (10.0 μM) at 576 nm in $CHCl_3/CH_3CN$ (50:50, v/v) upon addition of 10 equiv of various metal ions as perchlorates with an excitation at 343 nm. Fluorescence photograph of **2** and **2**·Hg^{2+} under UV light (inset).

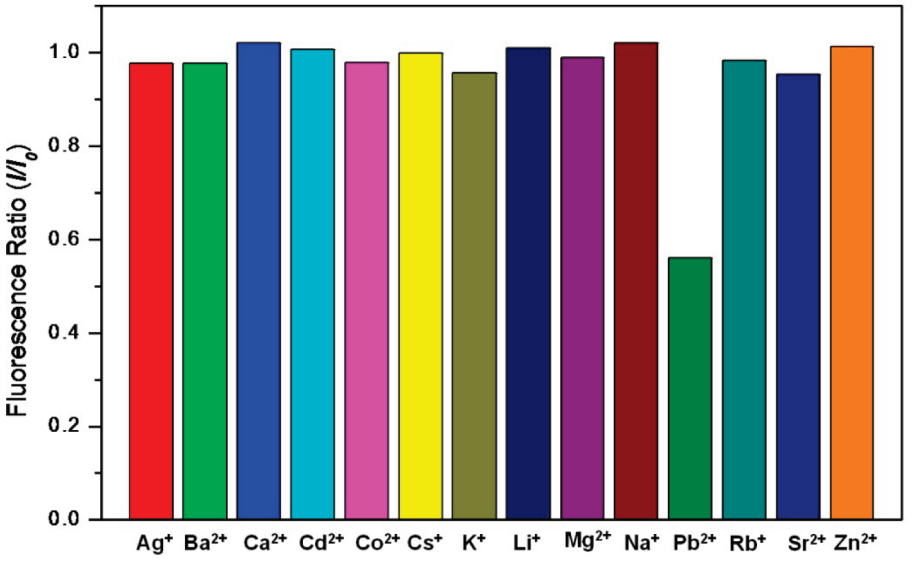

FIGURE 5. Competition experiment of **2** (10.0 μM) at 576 nm with 10 equiv of various coexisting metal cations in $CHCl_3/CH_3CN$ (50:50, v/v) in the presence of Hg^{2+} (3 equiv). I_0 and I denote the fluorescence intensity of **2**·Hg^{2+} and various metal ions in the presence of **2**·Hg^{2+}.

Moreover, the plot of fluorescence intensity ratios at I_{576}/I_{470} (energy acceptor/excited-state energy donor) versus Hg^{2+} concentration showed that a ratiometric fluorescence change commenced with 0.5 equiv of Hg^{2+} capable of estimating a detection limit near 5.0 μM of **2** toward Hg^{2+} using FRET, as depicted in Figure 3b.

The selective fluorometric response of **2** over other metal ions, including Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, Hg^{2+}, In^{3+}, K^{+}, Li^{+}, Mg^{2+}, Na^{+}, Pb^{2+}, Rb^{+}, Sr^{2+}, and Zn^{2+} as perchlorate salts, was also studied. Figure 4 indicates that **2** detected Hg^{2+} with high selectivity based on the FRET system. Although Pb^{2+} also induced a similar appearance of fluorescence increase, the intensity was quite lower than that of Hg^{2+}. Little effect was also observed in the case of other cations such as Al^{3+}, In^{3+}, and Zn^{2+}.

Competition experiments were conducted to confirm a potential applicability of **2** for the selective detection of Hg^{2+} ion in $CHCl_3/CH_3CN$ (50:50, v/v) in the presence of 10 equiv of various metal ions. Marginal fluorescence changes were seen in most of the cations as shown in Figure 5. However, addition of Pb^{2+} to a solution of **2**·Hg^{2+} remarkably reduced FRET fluorescence intensity. This finding can be interpreted by Pb^{2+} complexation to two amides, thus serving to diminish the excimer signal through reverse PET (photoinduced energy transfer) or a heavy-metal effect.[6] Nevertheless, these results

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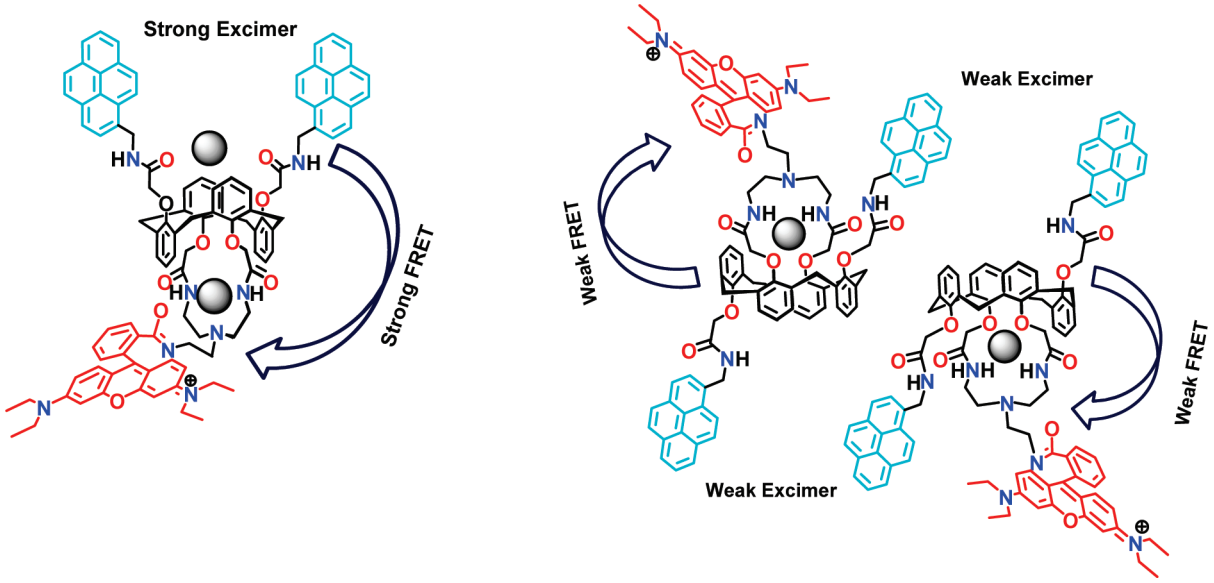
SCHEME 2. Expected FRET System of 1 (Right) and 2 (Left) for Hg^{2+} (Filled Gray Circle)



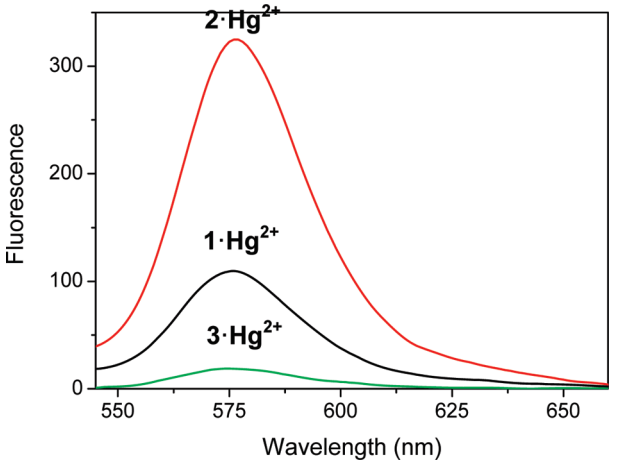


FIGURE 6. FRET intensity of $\mathbf{L}\cdot Hg^{2+}$ ($\mathbf{L}$ = **1**–**3**, 10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v) upon addition of $Hg(ClO_4)_2$ (3.0 equiv) with excitation at 343 nm.

suggest that **2** can be used as a potential Hg^{2+}-selective chemosensor.

To gain insight into the role of the calix[4]arene framework on the FRET effects in **2**, its analogue **1** was employed and compared to the FRET efficiency with Hg^{2+}. As expected, **2** displayed a 3-fold enhanced fluorescence over that of **1** in the presence of 3 equiv of Hg^{2+} ion, as shown in Figure 6. This is because the calix[4]arene framework of **2** furnished the geometrical advantage of two pyrenyl substituents to be on the same side of each other, producing stronger pyrene excimer energy. However, **1** showed weak FRET because two pyrenyl substituents were too remote to interact strongly with each other (Figure 6 and Scheme 2).

To determine the stoichiometry of the $\mathbf{2}\cdot Hg^{2+}$ complex, a UV/vis titration experiment was performed with **2** (10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v). The absorption peak at 559 nm was gradually increased upon addition Hg^{2+}. A Job's plot analysis exhibited a maximum at a 0.66 mol fraction of Hg^{2+} (inset of Figure S15 in the Supporting Information), indicating a 1:2 complexation between **2** and Hg^{2+}. Presumably, the complexation mechanism of $\mathbf{2}\cdot Hg^{2+}$ can be deduced as two Hg^{2+} ions interact with the tren-appended spirolactam resulting in the ring-opening of the rhodamine unit and bispyrenylamido moieties present on the same side of the calix[4]arene core to produce the FRET-ON signal.

Further investigation to provide evidence on the complexation behavior was also carried out with proton NMR study of the $\mathbf{2}\cdot Hg^{2+}$ complex in a mixed solvent system ($CDCl_3/CD_3CN$, 50:50, v/v). Due to the different solubility of the ligand and metal ion in the organic solvent, the NMR peaks of the $\mathbf{2}\cdot Hg^{2+}$ complex were so broad that the peak changes could not be clearly assigned. Nevertheless, metal ion-induced chemical shifts of the specific peaks related to the complexation were assignable for the ^1H NMR spectrum. As shown in Figure S16 in the Supporting Information, two methylene protons of the tren moiety (H_a and H_b) were obviously shifted downfield, implying that the Hg^{2+} ion was coordinated to the nitrogen atoms of the azacrown portion. Moreover, phenyl proton peaks of the calix[4]arene core appended to the para and meta positions underwent downfield shifts as a result of the cation–π interactions that formed between the two benzene rings and metal ion.[20] To elucidate the two factors of metal binding, such as π–metal ion complexation and coordination behavior of the tren moiety, a more detailed investigation of the proton NMR study was performed with **3** and **4**. As shown in Figures S17 and S18 in the Supporting Information, in the case of $\mathbf{3}\cdot Hg^{2+}$, the proton NMR spectrum of the methylene (H_a and H_b) present in the tren moiety was shifted downfield, which is similar with the complexation behavior of $\mathbf{2}\cdot Hg^{2+}$. Furthermore, the partial proton NMR spectroscopy of **4** and $\mathbf{4}\cdot Hg^{2+}$ showed the plain π–metal ion interactions between the para-positioned proton of the benzene ring and the Hg^{2+} ion.

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Conclusions

We synthesized a novel calix[4]arene-appended FRET chemosensor (**2**) based on pyrene excimer emission and rhodamine ring-opened absorption for selective detection of the Hg^{2+} ion. Photophysical studies of the pyrene excimer and rhodamine fluorophores revealed metal ion-induced FRET OFF → ON behavior. Complexation of the Hg^{2+} ion with **2** induced a ring-opening of the rhodamine moiety to give a specific color change, as well as fluorescence enhancement at ~576 nm with an excitation at 343 nm. Interestingly, it was found that the pyrene excimer formed by the intramolecular $\pi-\pi$ interaction exhibited a stronger FRET band compared to that formed by intermolecular $\pi-\pi$ interactions.

Experimental Section

Calix[4]arene diethyl ester **5**,[21] tren-appended rhodamine **6**,[18c] and 1,3-alternate 25,27-bis(ethyl acetoethoxy)-26,28-dipropyloxycalix[4]arene **7**[6] were prepared according to literature procedures.

Compounds 1 and 2. A mixture of **7** (0.30 g, 0.26 mmol), 2-chloro-*N*-(pyren-1-ylmethyl)acetamide (0.11 g, 0.37 mmol), and Cs_2CO_3 (0.18 g, 0.56 mmol) in dry acetone (40 mL) was refluxed for 3 d under a nitrogen atmosphere and evaporated under reduced pressure. Chromatography on silica gel with acetone/hexane (1:2) for **1** and acetone/hexane (1:1) for **2** gave 0.11 g of **1** (yield: 26%) and 0.22 g of **2** (yield: 52%) as a white solid, respectively. Compound **1**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24−7.72 (m, 18H, pyrene-*H*), 7.44−7.41 (m, 2H, rhodamine-*H*), 7.09−6.98 (m, 6H, Ar*H*, rhodamine-*H*), 6.90 (t, *J* = 7.38 Hz, 2H, Ar*H*), 6.81 (t, *J* = 4.89 Hz, 2H, N*H* amide), 6.66 (d, *J* = 7.11 Hz, 2H, Ar*H*), 6.38 (d, *J* = 2.07 Hz, 2H, rhodamine-*H*), 6.26−6.17 (m, 4H, rhodamine-*H*), 6.15 (d, *J* = 7.20 Hz, 2H, Ar*H*), 5.55 (t, *J* = 7.62 Hz, 2H, Ar*H*), 5.10 (t, *J* = 5.79 Hz, 1H, N*H* amide), 5.04 (d, *J* = 5.37 Hz, 1H, N*H* amide), 4.74 (d, *J* = 6.12 Hz, 2H, pyrene-CH_2), 4.44 (d, *J* = 15 Hz, 2H, ArCH_2Ar), 4.18 (s, 2H, OCH_2O), 3.97 (d, *J* = 15 Hz, 2H, ArCH_2Ar), 3.78 (s, 2H, pyrene-CH_2), 3.72 (d, *J* = 6.24 Hz, 2H, OCH_2CO), 3.29 (q, *J* = 7.05 Hz, 8H, CH_2CH$_3$), 3.09 (d, *J* = 13.71 Hz, 4H, ArCH_2Ar), 3.03 (br, 6H, CH_2CH$_2$), 2.34 (br, 6H, CH_2CH$_2$), 1.10 (t, *J* = 6.84 Hz, 12H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 30.9, 37.4, 38.1, 40.2, 41.4, 44.5, 52.6, 54.6, 65.0, 68.0, 71.32, 72.1, 98.2, 105.8, 108.1, 120.3, 122.7, 122.7, 123.0, 124.0, 124.2, 124.5, 124.8, 124.8, 124.9, 124.9, 125.1, 125.1, 124.5, 125.6, 125.7, 126.3, 126.3, 126.9, 127.5, 127.5, 127.6, 127.8, 127.9, 128.3, 128.4, 128.4, 128.9, 129.0, 129.1, 129.0, 129.7, 130.5, 130.6, 132.9, 131.0, 131.5, 131.9, 132.5, 132.9, 132.9, 134.0, 148.9, 152.5, 153.0, 153.2, 153.5, 153.8, 167.2, 167.6, 168.0, 168.4 ppm; mp > 300 °C; IR (deposit, cm^{-1}) 3414 (N−H), 3047 (aromatic C—H), 2968−2858 (aliphatic C—H), 1679 (C=O); HRMS (FAB) calcd for $C_{104}H_{97}N_8O_{10}$ [M$^+$] 1617.7360, found 1617.7321. Compound **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23−7.88 (m, 18H, pyrene-*H*), 7.47−7.45 (m, 2H, rhodamine-*H*), 7.25−7.23 (m, 1H, rhodamine-*H*), 7.13−7.11 (m, 1H, rhodamine-*H*), 6.78 (d, *J* = 7.48 Hz, 4H, Ar*H*), 6.71 (d, *J* = 7.6 Hz, 4H, Ar*H*), 6.47−6.43 (m, 4H, rhodamine-*H*), 6.40 (d, 2H, *J* = 8.90 Hz, Ar*H*), 6.23−6.20 (m, 2H, rhodamine-*H*), 6.02 (t, *J* = 7.48 Hz, 2H, Ar*H*), 5.86 (t, *J* = 4.48 Hz, 2H, N*H* amide), 5.18 (d, *J* = 5.68 Hz, 4H, pyrene-CH_2), 4.03 (s, 2H, OCH_2CO), 3.47 (s, 8H, ArCH_2Ar), 3.30 (q, *J* = 7.12 Hz, 8H, CH_2CH$_3$), 3.24−3.20 (br, 2H, CH_2CH$_2$), 3.12 (s, 4H, CH_2CH$_2$), 2.62 (br, 4H, CH_2CH$_2$), 2.50 (br, 2H, CH_2CH$_2$), 1.12 (t, *J* = 7.00 Hz, 12H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 37.1, 41.3, 44.6, 46.0, 65.1, 70.4, 70.5, 98.1, 105.9, 108.4, 122.9, 123.1, 124.2, 124.3, 124.7, 124.9, 125.1, 125.5, 125.5, 126.2, 127.0, 127.5, 127.7, 128.2, 128.5, 129.0, 129.1, 130.8, 131.0, 131.2, 131.4, 131.5, 131.7, 132.0, 132.7, 134.2, 134.6, 149.0, 153.0, 153.9, 154.1, 155.4, 167.6, 167.9, 168.6 ppm; mp > 300 °C; IR (deposit, cm^{-1}) 3410 (N—H), 3040 (aromatic C—H), 2958−2851 (aliphatic C—H), 1673 (C=O); HRMS (FAB) calcd for $C_{104}H_{97}N_8O_{10}$ [M$^+$] 1617.7360, found 1617.7306.

Compound 3. A mixture of **7** (0.20 g, 0.19 mmol), Cs_2CO_3 (0.18 g, 0.56 mmol), and 1-iodopropane (0.10 g, 0.59 mmol) in dry acetone (20 mL) was refluxed for 3 d under a nitrogen atmosphere. After cooling to room temperature, the solvent was removed under reduced pressure and purified with silica column chromatography with ethyl acetate/hexane (3:1) as the eluent to give 0.14 g of **3** as a white powder (yield: 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93−7.90 (m, 1H, rhodamine-*H*), 7.48−7.45 (m, 2H, rhodamine-*H*), 7.14−7.11 (m, 1H, rhodamine-*H*), 7.04 (d, *J* = 7.5 Hz, 4H, Ar*H*), 6.82−6.77 (m, 6H, rhodamine-*H*, Ar*H*), 6.51−6.41 (m, 6H, rhodamine-*H*, Ar*H*), 6.29−6.25 (m, 2H, rhodamine-*H*), 5.96 (t, 2H, *J* = 4.38 Hz, N*H* amide), 4.14 (s, 4H, OCH_2CO), 3.71 (d, *J* = 14.38 Hz, 4H, ArCH_2Ar), 3.55−3.49 (m, 6H, CH_2CH$_2$), 3.37−3.30 (m, 12H, CH_2CH$_2$, ArCH_2Ar), 2.63 (br, 4H, CH_2CH$_2$), 2.54−2.49 (br, 2H, CH_2CH$_2$), 1.64 (sextet, *J* = 7.62 Hz, 4H, CH$_2$CH_2CH$_3$), 1.28 (t, *J* = 7.14 Hz, 4H, CH_2CH$_2$CH$_3$), 1.17 (t, *J* = 6.81 Hz, 12H, CH$_2$CH_3), 0.98 (t, *J* = 7.35 Hz, 6H, CH$_2$CH$_2$CH_3) ppm; mp 280 °C; IR (deposit, cm^{-1}) 3408 (N—H), 3060 (aromatic C—H), 2980−2883 (aliphatic C—H), 1698 (C=O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.2, 12.6, 37.1, 44.4, 46.8, 52.4, 60.4, 64.9, 69.6, 72.9, 98.0, 105.7, 108.2, 121.6, 122.9, 123.9, 128.2, 128.9, 129.9, 130.9, 131.8, 132.4, 133.3, 134.0, 148.9, 153.0, 153.7, 154.5, 156.7, 167.5, 168.4 ppm; HRMS (FAB) calcd for $C_{72}H_{84}N_6O_8$ [M − H]$^+$ 1159.6270, found 1159.6272.

Compound 7. A mixture of **5** (1.0 g, 1.68 mmol) and **6** (0.93 g, 1.68 mmol) in mixed solvent (50 mL, toluene:ethanol = 1:1) was refluxed for 3 d under a nitrogen atmosphere and evaporated in vacuo. Chromatography on silica gel with acetone/CH_2Cl_2 (1:7) as the eluent gave 0.30 g (yield: 17%) of **7** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (t, *J* = 5.70 Hz, 2H, N*H* amide), 7.82−7.78 (m, 1H, rhodamine-*H*), 7.41−7.38 (m. 2H, rhodamine-*H*), 7.07 (d, *J* = 7.47 Hz, 4H, Ar*H*), 7.03−7.01 (m, 1H, rhodamine-*H*), 6.93 (s, 2H, ArO*H*), 6.80 (d, *J* = 7.08 Hz, 4H), 6.73−6.66 (m, 4H, Ar*H*), 6.42 (d, *J* = 2.22 Hz, 2H, rhodamine-*H*), 6.27−6.19 (m, 4H, rhodamine-*H*), 4.46 (s, 4H, OCH_2CO), 4.15 (d, *J* = 13.47 Hz, 4H, ArCH_2Ar), 3.38−3.29 (m, 16H, ArCH_2Ar, CH_2CH$_3$), 3.16−3.11 (m, 2H, CH_2CH$_2$), 2.53−2.51 (m, 4H, CH_2CH$_2$), 2.48−2.41 (m, 2H, CH_2CH$_2$), 1.16 (t, *J* = 7.05 Hz, 12H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 31.5, 38.0, 39.1, 44.6, 54.8, 65.0, 75.1, 98.2, 105.9, 105.9, 106.0, 108.3, 120.4, 122.6, 124.0, 126.2, 128.2, 129.1, 129.2, 129.2, 129.6, 131.8, 132.4, 148.9, 151.4, 152.6, 153.2, 153.8, 167.6, 168.3 ppm; mp 278 °C; IR (deposit, cm^{-1}) 3590−3396 (br, N—H, O—H), 3047 (aromatic C—H), 2972−2871 (aliphatic C—H), 1684 (C=O); HRMS (FAB) calcd for $C_{66}H_{70}N_6O_8$ [M + H]$^+$ 1075.5313, found 1075.5333.

UV−Visible and Fluorescence Studies. UV−visible and fluorescence spectra were recorded with a S-3100 spectrophotometer and a RF-5301PC spectrophotometer, respectively. Stock solutions of all compounds were prepared in $CH_3CN/CHCl_3$ (1:1). For all fluorescence spectral measurements, excitation was effected at 343 nm with excitation and emission slit widths at 3.0 nm. UV/visible and fluorescence titration experiments were performed starting with 10.0 μM solutions of **1**−**4** in $CH_3CN/CHCl_3$ (1:1), followed by varying the concentrations of the guest molecules in $CH_3CN/CHCl_3$ (1:1).

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Supporting Information Available: Additional experimental procedures, ^{1}H NMR titration spectra, and UV/vis and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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