Inorganic Chemistry

Article

pubs.acs.org/IC

A Fluorescent Chemosensor for Hg^{2+} and Cd^{2+} Ions in Aqueous Medium under Physiological pH and Its Applications in Imaging Living Cells

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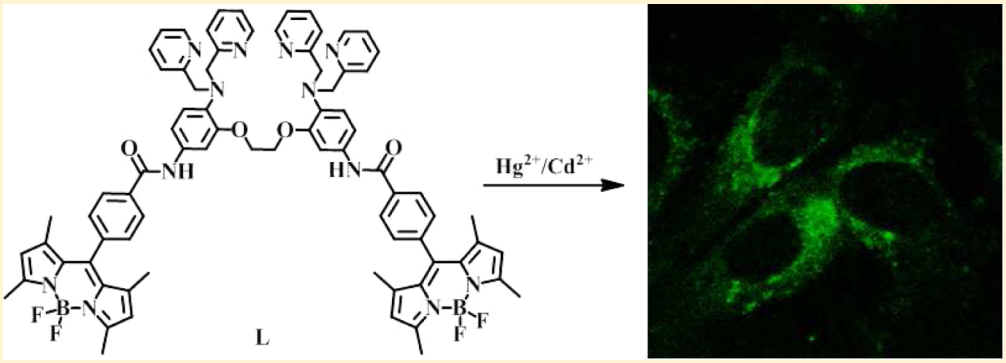
Supporting Information



ABSTRACT: A new BODIPY derivative with 2,2′-(ethane-1,2-diylbis(oxy))bis(*N*,*N*-bis(pyridine-2-ylmethyl)aniline unit as the metal receptor has been designed and synthesized. The dye selectively detects either Cd^{2+} or Hg^{2+} ions in the presence of hosts of other biologically important and environmentally relevant metal ions in aqueous medium at physiological pH. Binding of metal ions causes a change in the emission behavior of the dye from weakly fluorescent to highly fluorescent. Confocal microscopic experiments validate that the dye can be used to identify changes in either Hg^{2+} or Cd^{2+} levels in living cells.

INTRODUCTION

The selective detection of heavy and toxic metal ions in the background of biologically relevant metal ions is important with respect to human health and the environment.[1] Among the heavy metal ions, Hg^{2+} is extremely toxic even at very low concentration. Some microorganisms, particularly sulfate-reducing bacteria,[2] generate methyl mercury, a potent neurotoxin, from other forms of mercury. Methyl mercury causes serious health problems by damaging the central nervous and endocrine systems, leading to cognitive and motion disorders.[3] Besides, the multiple channels of spreading mercury through air, food, water, etc. is of serious concern because it persists in the environment and subsequently accumulates through the food chain.[4] On the other hand, cadmium, which is extensively used in several industries and also in agriculture, has been considered as a carcinogen and highly toxic heavy metal.[5] Increasing exposure of this metal can cause decalcification, kidney problems, anemia, and hypertension besides substitution of Zn^{2+} from several enzymes.[6] Long-term exposure of Cd^{2+} can cause prostrate, lung, breast, or endometrial cancer.[7] Therefore, development of fluorescence turn-on sensors for the selective and sensitive determination of Cd^{2+} and Hg^{2+} ion in aqueous medium is of crucial importance. Because of its easy operational techniques, low cost, real-time response, and high selectivity as well as sensitivity, fluorescence turn-on is regarded as a promising approach for Cd^{2+} and Hg^{2+} detection.[8]

Therefore, considerable efforts have been devoted to develop fluorescence probes for bivalent cadmium[9] and mercury ions.[10] However, such systems mostly work in nonaqueous medium, and fluorescence enhancement in the majority of cases is rather small and usually suffers from a high background. Only a few of them are amenable to measurement in aqueous medium and sensitive enough for live cell imaging as well as environmental detection.[11]

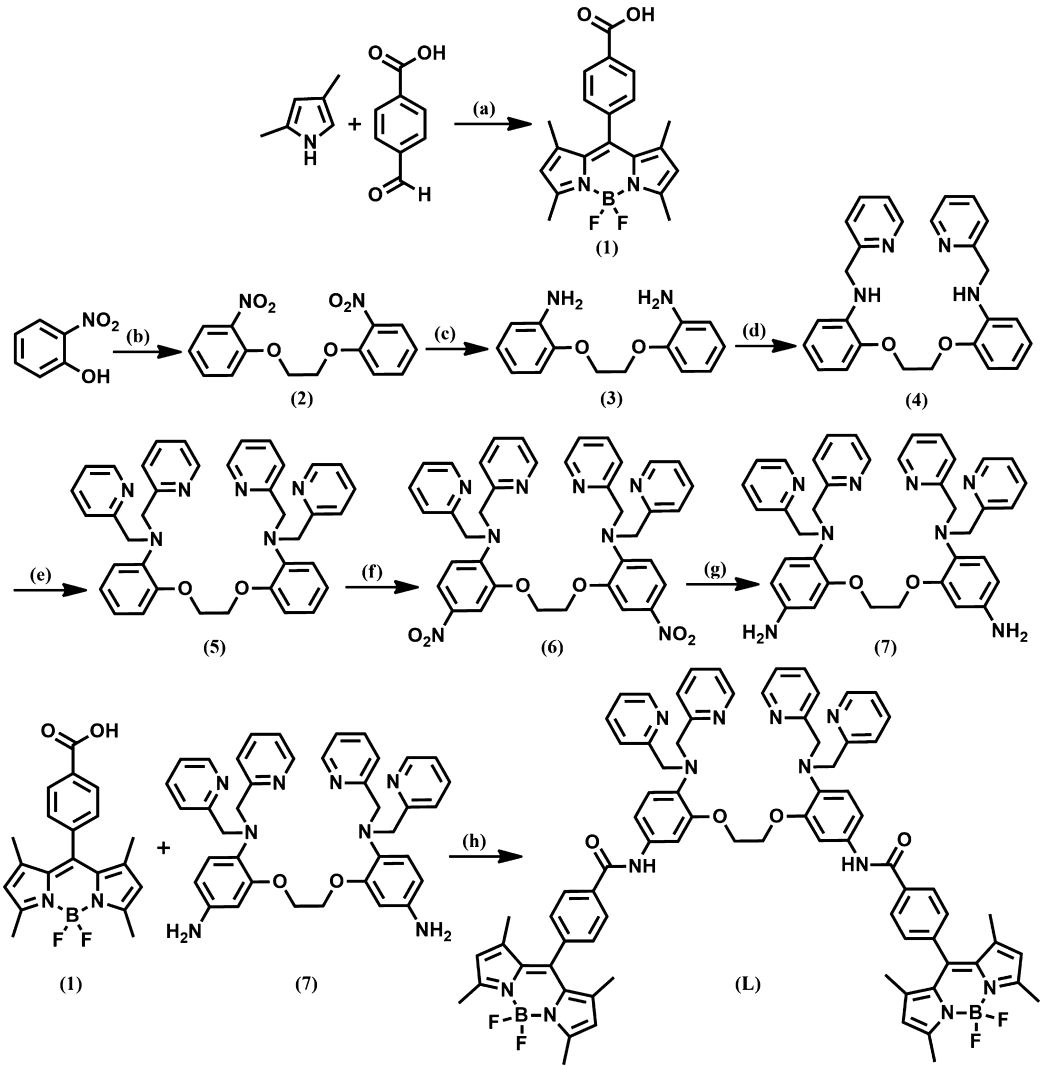
Recently, Chang and co-workers reported[12] a fluorescent sensor for selective detection of Hg^{2+} and Cd^{2+} ions in aqueous CH_3CN medium. Variation of the percentage of water in the solution was found to alter the signaling event of Hg^{2+} and Cd^{2+}. A selective signaling behavior toward Hg^{2+} ion was observed when water content was 50%. However, lack of solubility of the dye in pure water makes it unsuitable for environmental and cellular applications. Therefore, fluorescent chemosensors for both Cd^{2+} and Hg^{2+} ions that are capable of differentiating the two are scarcely reported.

In this work, a fluorescent sensor having BODIPY fluorophore as the signaling unit is described based on the

Received: January 19, 2015
Published: April 9, 2015

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929−3936

Scheme 1. Synthetic Scheme for the Sensor, L[a]



[a]Reagents and conditions: (a) (i) TFA, (ii) DDQ, (iii) NEt_3, $BF_3{\cdot}OEt_2$; (b) 1,2-dibromoethane, K_2CO_3, DMF, 100 °C, 6 h; (c) 10% Pd/C, EtOAc, $NH_2{-}NH_2{\cdot}H_2O$, reflux, 3 h; (d) (i) pyridine-2-aldehyde, CH_3OH, reflux, 12 h; (ii) $NaBH_4$, 6 h, rt; (e) pyridine-2-aldehyde, 1,2-DCE, $NaB(OAc)_3H$; (f) glacial AcOH, concentrated HNO_3, concentrated H_2SO_4, 30 min, rt; (g) 10% Pd/C, CH_3OH, $NH_2{-}NH_2{\cdot}H_2O$, reflux, 2 h; (h) DCC, HOBt, DMF, 12 h, 60 °C.

photoinduced electron transfer (PET) mechanism. This dye works in aqueous medium at physiological pH for both Cd^{2+} and Hg^{2+} ions and hence can be used to detect either ion in case of poisoning. Compounds having di-2-pyridylamine unit as the metal binding site have been utilized for detection of various metal ions [e.g., Cu(II), Cd(II), Zn(II)] reported recently.[13] The BODIPY core boradiazaindacene has become the fluorophore of choice in designing many fluorescent sensors[14] because of their exceptional properties as fluorophores, such as high emission quantum yield, resistance to photobleaching, insensitivity to solvent polarity, and change of pH. Moreover, this core is in high demand due to its extraordinarily rich chemistry and versatile usefulness in solar cell applications and photodynamic therapy.[15] Herein, we report a probe consisting of a multidonor binding site, 2,2′-(ethane-1,2-diylbis(oxy))bis(*N*,*N*-bis(pyridine-2-ylmethyl)-aniline, which is covalently connected through aromatic amides with two BODIPY fluorophores that selectively detects both Hg^{2+} and Cd^{2+} ions. The receptor has been designed to effectively wrap around a metal ion and, at the same time, make the dye water-soluble for its operation in aqueous environment. It works in aqueous medium at physiological pH and, hence, can be used in live cell imaging studies with detection limit in the nM region for both Cd^{2+} and Hg^{2+} ions.

EXPERIMENTAL SECTION

Materials. Reagent grade 2,4-dimethylpyrrole, 4-formylbenzoic acid, 2-nitrophenol, 1,2-dibromoethane, pyridine-2-aldehyde, $NaB(OAc)_3H$, DCE, triethylamine, $BF_3{\cdot}Et_2O$, $POCl_3$, 10% palladium on carbon, and all metal perchlorate salts were purchased from commercial suppliers (Aldrich, USA and S. D. Fine Chemicals, India) and used as received without further purification. All the solvents were bought from commercial suppliers and were purified prior to use following standard literature methods. Chromatographic separations were done by column chromatography using silica gel (100−200 mesh) and basic alumina obtained from S. D. Fine Chemicals.

Caution! *Perchlorate salts are potentially explosive (especially if they are dry) and should be handled with extreme care. We have used it in small quantities at a time and not encountered any problem during this work.*

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929−3936

Analysis and Measurements. Various spectroscopic techniques were employed to characterize the newly synthesized compounds. Both ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of the compounds were recorded on a JEOL spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard. The ESI-mass data were obtained in methanol from a Waters Q-Tof Premier mass spectrometer. UV−visible spectra were recorded on a Shimadzu 2450 UV−vis spectrophotometer in aqueous buffer solution at 298 K. Steady-state fluorescence spectra were obtained using a PerkinElmer LS 50B luminescence spectrometer at 298 K. The excitation wavelength was 460 nm, and the spectra were recorded in the range 480−650 nm. Fluorescence quantum yields were determined by comparing the corrected spectra with that of pure Rhodamine B in ethanol[16] taking the total area under the curve and using eq i

$$\Phi_S = \Phi_R(F_S A_R / F_R A_S)(\eta_S/\eta_R)^2 \quad \text{(i)}$$

where Φ stands for quantum yield, F stands for area under the curve, A stands for absorbance value, and η stands for the refractive index value. The subscript "R" indicates the value of the parameter for reference (i.e., Rhodamine-B), and "S" subscript indicates value of the parameter for the sample.

Cell Culture and Imaging. HeLa cell line was cultured in Dulbeco's modified Eagle's medium (DMEM, WelGene) supplemented with 10% FBS (fetal bovine serum, WelGene), 1% penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells were grown at 37 °C under humidified atmosphere in 5% CO_2. Before imaging, the cells were placed for 48 h on Delta T dishes (Bioptechs). Cells were incubated with 5 μM probe (1% DMSO buffer solution) for 30 min, and probes not taken up by the cells were removed by washing three times with PBS solution. Aqueous solutions of Hg^{2+} and Cd^{2+} were treated for an additional 30 min. Cell images were obtained with a confocal microscope (Leica TCS SP2 model) fitted with a 100× oil lens (numerical aperture = 1.30). Fluorescence images were collected at 480−650 nm range following excitation at 458 nm. Internal photomultiplier tubes (PMTs) were used to collect the signals in 8-bit unsigned 512 × 512 pixels at 400 Hz scan speed.

Synthesis. The synthesis of **L** can be achieved in multisteps as outlined in Scheme 1.

Synthesis of ***1****.* The BODIPY dye **1**, [(8-(4-carboxyphenyl)-1,3,7,9-tetramethyl-BODIPY)], was synthesized following the reported procedure.[17] 4-Formylbenzoic acid (1.6 g, 10.66 mmol) and 2,4-dimethylpyrrole (2.2 mL, 21.37 mmol) were added to freshly purified CH_2Cl_2 (300 mL) purged with N_2, a few drops of TFA were added to the mixture, and stirring was continued for 8 h at room temperature. After TLC showing complete consumption of pyrrole, DDQ was added to the reaction mixture and stirring was continued for another 4 h. The resultant mixture was cooled to 5 °C and treated with triethylamine (10 mL) followed by boron trifluoride etherate (10 mL). After 3 h of stirring, the resulting solution was poured into water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, and the solvent was evaporated completely in a rotary evaporator. The crude product was purified by column chromatography using silica gel with hexane:ethyl acetate (9:1 v/v) as the eluent. Evaporation of the solvent afforded about 0.98 g of the desired product as a red solid. Yield ∼25%. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS) δ: 1.29 (s, 6H), 2.42 (s, 6H), 6.16 (s, 2H), 7.49 (d, 2H, J = 8.3 Hz), 8.06 (d, 2H, J = 8.3 Hz). ESI-mass (m/z): calcd 367.1430 $[M - H^+]^+$, found 367.1423.

Synthesis of ***2****.* To a preheated solution of 1,2-dibromoethane (6 g, 31.94 mmol) and K_2CO_3 (9.5 g, 68.84 mmol) in 100 mL of anhydrous DMF was added a solution of 2-nitrophenol (9.5 g, 68.34 mmol) in 40 mL of acetonitrile over a period of 15 min. The reaction mixture was then heated to reflux for 6 h. After the nitrophenol was completely consumed, the solvent was distilled off and the residue was stirred in cold water, whereupon a solid substance precipitated out. It was collected by filtration, washed several times with ice-cold water, and recrystallized from methanol to obtain a light yellow solid. Yield ∼78%. Mp: 157 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 4.53 (s, 4H), 7.07 (t, 2H, J = 8.3 Hz), 7.23 (d, 2H, J = 8.3 Hz), 7.56 (t, 2H, J = 7.4 Hz), 7.83 (dd, 2H, J = 1.7 and 8 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 68.72, 115.91, 121.45, 125.70, 134.42, 151.96. Anal. Calcd for $C_{14}H_{12}N_2O_6$: C, 55.27; H, 3.98; N, 9.21%. Found: C, 55.39; H, 4.05; N, 9.11%.

Synthesis of ***3****.* To a solution of **2** (15 g, 49.34 mmol) in 200 mL of ethyl acetate was added 10% Pd/C (1.5 g), and the reaction mixture was heated under N_2 atmosphere. To this solution was added dropwise 15 mL of $N_2H_4{\cdot}H_2O$ over 30 min. Once the addition was complete, the mixture was heated to reflux for 3 h. It was then filtered under hot condition and washed with hot methanol. The combined filtrate upon evaporation gave a solid, which was recrystallized from methanol to obtain about 10.9 g of white crystalline solid. Yield ∼90%. Mp: 132 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 3.67 (br, s, 4H), 4.36 (s, 4H), 6.72−6.74 (m, 4H), 6.82−6.87 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 67.52, 112.56, 115.46, 118.48, 122.00, 136.89, 146.33. Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47%. Found: C, 68.94; H, 6.87; N, 11.36%.

Synthesis of ***4****.* Pyridine-2-aldehyde (11.70 mL, 122.81 mmol) was added to a solution of **3** (10 g, 40.93 mmol) in 150 mL of methanol, and the solution was heated to reflux for 12 h. After the reaction mixture was cooled to room temperature, excess $NaBH_4$ was added portionwise with stirring. After stirring for about 6 h at room temperature, the solvent was evaporated and the residue dissolved in dichloromethane. The organic layer was washed several times with water and then dried over anhydrous Na_2SO_4. Finally it was completely evaporated off to obtain a brown colored solid. The solid was subjected to column chromatography using basic alumina with dichloromethane:hexane (3:2 v/v) as the eluent to obtain compound **4** as a light yellow crystalline solid. Yield ∼70%. Mp: 135 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 4.41 (s, 4H), 4.47 (s, 4H), 6.48 (d, 2H, J = 8 Hz), 6.65 (t, 2H, J = 8 Hz), 6.80 (t, 2H, J = 7.4 Hz), 6.87 (d, 2H, J = 7.4 Hz), 7.08 (t, 2H, J = 6.8 Hz), 7.20 (d, 2H, J = 8 Hz), 7.47 (t, 2H, J = 7.4 Hz), 8.50 (d, 2H, J = 4.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 46.26, 67.64, 110.70, 111.79, 116.71, 121.17, 121.99, 122.17, 136.71, 138.53, 145.97, 149.29, 159.3. ESI-mass (m/z): calcd 427.2134 $[M + H^+]^+$, found 427.2131 (100%). Anal. Calcd for $C_{26}H_{26}N_4O_2$: C, 73.22; H, 6.14; N, 13.14%. Found: C, 73.14; H, 6.31; N, 13.07%.

Synthesis of ***5****.* To a solution of **4** (10 g, 23.45 mmol) in 200 mL of dichloroethane (DCE) at room temperature was added pyridine-2-aldehyde (6.7 mL, 70.31 mmol). After 1 h stirring, $NaB(OAc)_3H$ (14.90 g, 70.31 mmol) and an additional 50 mL of DCE were added. After overnight stirring, the solvent was removed under reduced pressure, 100 mL water was added, and the pH was adjusted between 8 and 9 with saturated bicarbonate solution. The product was extracted with chloroform (60 mL × 3). The combined organic fractions were dried over anhydrous Na_2SO_4, and the solvent was removed. The product was purified on basic alumina with ethyl acetate:hexane (1:1 v/v) as the eluent affording the desired compound as a white crystalline solid. Yield ∼60%. Mp: 148 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 4.09 (s, 4H), 4.48 (s, 8H), 6.66 (dd, 2H, J = 1.5 and 8 Hz), 6.78 (t, 2H, J = 9.1 Hz), 6.82−6.86 (m, 10H), 6.94 (dd, 2H, J = 1.2 and 8 Hz), 7.67 (t, 4H, J = 4.6 Hz), 8.37 (t, 4H, J = 3.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 58.95, 66.23, 111.83, 119.93, 120.98, 121.78, 122.16, 136.49, 139.39, 148.79, 151.38, 159.77. ESI-mass (m/z): calcd 609.2978 $[M + H^+]^+$, found 609.2955 (100%). Anal. Calcd for $C_{38}H_{36}N_6O_2$: C, 74.98; H, 5.96; N, 13.81%. Found: C, 75.16; H, 6.09; N, 13.69%.

Synthesis of ***6****.* To a solution of **5** (1 g, 16.43 mmol) in 3 mL of glacial acetic acid was added concentrated HNO_3 (250 μL) followed by concentrated H_2SO_4 (250 μL). The resulting brown reaction mixture was stirred at room temperature for 30 min. Then it was quenched carefully with saturated $NaHCO_3$ solution and extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to a mustard yellow residue. Trituration of this residue with methanol:ethyl acetate (1:1, v/v) afforded **6** as a yellow solid. Yield ∼70%. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 3.59 (s, 4H), 4.72 (s, 8H), 6.81(d, 2H, J = 9.1 Hz), 7.08 (t, 4H, J = 6.3 Hz), 7.22 (d, 2H, J = 2.3 Hz), 7.38(d, 4H, J = 8.1 Hz), 7.52 (t, 4H, J = 8 Hz), 7.72 (dd, 2H, J = 2.3 and 9.1 Hz), 8.44 (d, 4H, J

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929−3936

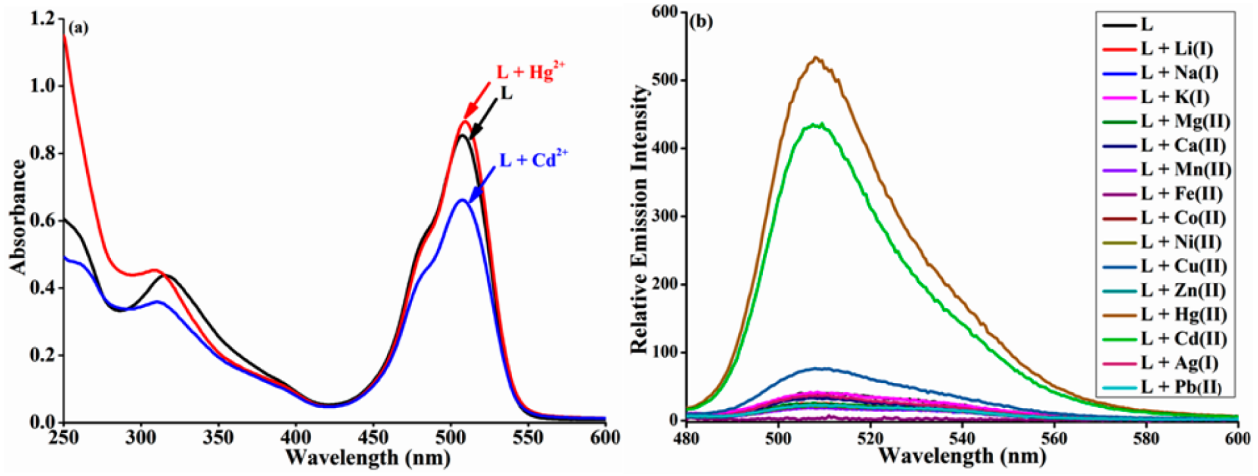


Figure 1. (a) Absorption spectral features of **L** in the presence of Cd^{2+} and Hg^{2+} ions. (b) Emission spectra of **L** in the presence of 5 equiv of different ionic inputs in aqueous medium (containing 20% DMSO) at pH 7.4 (HEPES buffer, 20 mM). Excitation wavelength = 460 nm. Slit: 5/5 nm. [**L**] = 10 μM.

= 4.6). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 58.18, 65.99, 107.42, 116.29, 118.42, 121.18, 122.20, 136.71, 140.05, 146.25, 148.25, 149.64, 158.25. ESI-mass (m/z): calcd 699.2679 $[M + H^+]^+$, found 699.2687. Anal. Calcd for $C_{38}H_{34}N_8O_6$: C, 65.32; H, 4.90; N, 16.04%. Found: C, 65.24; H, 4.83; N, 15.95%.

Synthesis of ***7****.* To a solution of 6 (1.4 g, 2 mmol) in 50 mL of methanol was added 10% Pd/C (100 mg), and the reaction mixture was heated under N_2 atmosphere. To this solution was added dropwise 5 mL of $N_2H_4{\cdot}H_2O$ over 10 min. The mixture was heated to reflux for 2 h. It was filtered under hot condition and washed with hot methanol. The combined filtrate was evaporated to give 0.8 g of a light yellow crystalline solid, which was used as such for the next step without further purification. Yield ~84%. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 2.94 (br, s, 4H), 4.18 (s, 4H), 4.38 (s, 8H), 6.13 (m, 4H), 6.78 (d, 2H, J = 4 Hz), 6.92 (m, 4H), 7.04 (t, 4H, J = 7.4 Hz), 7.65 (d, 4H, J = 7.4 Hz), 8.39 (d, 4H, J = 4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 59.56, 66.48, 100.92, 107.05, 121.73, 122.32, 122.60, 131.53, 136.53, 142.50, 148.58, 153.17, 160.07. ESI-mass (m/z): calcd 639.3196 $[M + H^+]^+$, found 639.3195 (15%), 661.3014 (60%) $[M + Na]^+$, 320.1577 (100%) $[M/2 + H^+]^+$. Anal. Calcd for $C_{38}H_{38}N_8O_2$: C, 71.45; H, 6.00; N, 17.54%. Found: C, 71.39; H, 5.94; N, 17.48%.

Synthesis of ***L****.* To a solution of compound **1** (0.45 g, 1.22 mmol) in 20 mL of anhydrous DMF was added DCC (0.62 g, 3 mmol) followed by HOBt (0.47 g, 3.48 mmol) with stirring at room temperature for 1 h. The diamine **7** (0.3 g, 0.47 mmol) was added to it, and the reaction mixture was heated to 60 °C and continued for another 12 h. The precipitated DCU was removed by filtration, and the filtrate was evaporated to dryness. The resulting residue was subjected to column chromatography using basic alumina with DCM:MeOH (1:1, v/v) as the eluent. The desired product was obtained as a dark red solid. Yield ~40%. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 1.32 (s, 12H), 2.53 (s, 12H), 4.16 (s, 4H), 4.46 (s, 8H), 5.96 (s, 4H), 6.92 (d, 2H, J = 8.7 Hz), 6.95–6.98 (m, 4H), 7.03 (s, 2H), 7.24–7.27 (m, 4H), 7.35 (t, 4H, J = 8.2 Hz), 7.54 (d, 4H, J = 7.8 Hz), 8.01 (d, 4H, J = 8.2 Hz), 8.38 (d, 4H, J = 4.1 Hz), 8.52 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, $Si(CH_3)_4$) δ: 14.67, 14.72, 59.00, 66.97, 105.99, 112.78, 120.94, 121.62, 121.99, 122.48, 128.08, 128.61, 131.08, 133.27, 135.61, 136.32, 136.63, 138.60, 140.19, 142.94, 148.79, 151.87, 156.10, 159.44, 164.90. ESI-mass (m/z): calcd 1339.60 $[M + H^+]^+$, found 1339.6019 (15%), 670.2922 (100%) $[M/2 + H^+]^+$. Anal. Calcd for $C_{78}H_{72}B_2F_4N_{12}O_4$: C, 69.96; H, 5.42; N, 12.55%. Found: C, 70.02; H, 5.57; N, 12.48%.

RESULTS AND DISCUSSION

The chemical structures of the probe **L** and all its precursors are confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy and ESI-mass spectrometry besides elemental analysis. Compound **1** can be obtained in a three-step procedure via condensation of 2,4-dimethylpyrrole with 4-formylbenzoic acid followed by oxidation with DDQ and then boron insertion with $BF_3{\cdot}Et_2O$. The overall yield is 25% for the three steps. Synthesis of the receptor, 2,2′-(ethane-1,2-diylbis(oxy))bis(*N*,*N*-bis-(pyridine-2-ylmethyl)aniline unit, is realized in six steps. Addition of 1,2-dibromoethane to a solution of *o*-nitrophenol in the presence of K_2CO_3 affords dinitro compound **2** in 78% yield. Reduction of **2** in the presence of hydrazine hydrate in ethyl acetate with 10% Pd/C as catalyst gives the diamine compound **3**. Condensation of **3** with pyridine-2-aldehyde followed by reduction with $NaBH_4$ makes **4** in 70% yield. Further condensation of **4** with pyridine-2-aldehyde in the presence of $NaB(OAc)_3H$ in DCE affords **5** in 60% yield. Subsequent nitration of **5** with concentrated HNO_3 and concentrated H_2SO_4 in the volume ratio of 1:1 at room temperature generates **6** in 70% yield, which is further reduced to the amine component **7** in the presence of hydrazine hydrate in methanol with 10% Pd/C catalyst in 84% yield. The coupling of **1** with **7** in refluxing DMF in the presence of DCC and HOBt affords **L** as a dark red solid in 40% yield after workup and purification. The dye is readily soluble in common organic solvents as well as in water.

Photophysical Properties. Spectroscopic measurements of **L** are investigated in aqueous medium at physiological pH (20 mM HEPES buffer, pH = 7.4) under ambient conditions. The absorption spectrum of **L** is typical of a meso-substituted BODIPY derivative showing a strong band centered at 504 nm with a prominent shoulder at 482 nm (Figure 1a).[17] Addition of a perchlorate salt of a biologically relevant alkali, alkaline earth, transition, or toxic heavy metal ion such as Ag^+ or Pb^{2+} does not give any discernible change in the absorption spectral profile of the dye. This suggests no interaction between the fluorophore and the added metal ion. However, in the presence of either Hg^{2+} or Cd^{2+} ion, prominent changes in the UV–vis spectra are observed (Figure 1a). The metal-free dye upon excitation at 460 nm gives only a weak fluorescence (Φ = 0.0054) due to highly efficient photoinduced electron transfer

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929–3936

(PET) from the receptor to the fluorophore. This emission profile does not change to any noticeable extent in terms of both intensity and peak positions in the presence of alkali, alkaline earth, transition, Ag^+, or Pb^{2+} metal ion. However, addition of the perchlorate salt of either Hg^{2+} or Cd^{2+} elicits a significant enhancement of the fluorescence intensity (~30-fold for Hg^{2+} ion and 25-fold for Cd^{2+} ion) with a maximum at 511 nm for Cd^{2+} and 509 nm for Hg^{2+} ions (Figure 1b). The turn-on fluorescence response is found to be reversible as addition of an aqueous solution of EDTA to the **L**-Hg^{2+} solution or an aqueous solution of TPEN to the **L**-Cd^{2+} solution changes the fluorescence output to the level of metal-free **L**.

Once **L** was found to exhibit fluorescence enhancement in the presence of both Hg^{2+} and Cd^{2+} ions, its selectivity profile was checked through competition experiments. The dye **L** (10 μM) was first mixed with ~100 equiv of metal ions of interest that include alkali and alkaline-earth metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}), transition metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}), and heavy metal ions (Ag^+, Pb^{2+}), followed by addition of 5 equiv of either Cd^{2+} or Hg^{2+} ion. The emission responses of the dye toward Cd^{2+} and Hg^{2+} ion remain unaltered when other metal ions are present in excess with the exception of Cu^{2+}, which reduces the enhancement by about 20%. We assume that Cu^{2+} has affinity of binding with the picolyl amine units when it is present in large excess causing the slight reduction in the emission. It is well-known that di-2-pyridylamine is a commonly used ligand scaffold in various turn-on Zn^{2+} selective fluorescence probes.[13h] Here, under the present experimental conditions the binding affinity of Zn^{2+} is very poor. It is found that Zn(II) forms a 1:1 complex with **L** as is evident from the appearance of a peak at 701.2550 (20%) in the ESI mass spectrum (Figure S21 in the Supporting Information). However, presence of 5 equiv of Zn^{2+} has no effect on the emission intensity of **L**. Even when ~1000 equiv of metal ion is added, the change in emission intensity of **L** is found to be affected insignificantly (Figure S22 in the Supporting Information). This may be attributed to higher solvation energy of the Zn^{2+} ion in water.

Therefore, the dye **L** can serve as a selective fluorescent chemosensor for both Hg^{2+} and Cd^{2+} ions in the presence of other competing metal ions (Figures S23 and S24 in the Supporting Information) at physiological pH. Once the selectivity of the dye toward Hg^{2+} and Cd^{2+} ions is found, the binding stoichiometry is evaluated from the Job's plot experiments.[18] The Job's plot experiments give a 1:1 binding stoichiometry for both Hg^{2+} and Cd^{2+} ions with **L** (Figures S25 and S26 in the Supporting Information).

The binding stoichiometry is further studied by electron spray ionization (ESI) mass spectral studies of both Cd^{2+} and Hg^{2+} complexes of **L**. The appearance of a peak at 726.2476 (100%) for the Cd(II) complex and that of a peak at 770.2783 (100%) for the Hg(II) complex with the dye **L** support 1:1 stoichiometry (Figures S27 and S28 in the Supporting Information).

For practical applications, DMSO–water ratio was optimized. For better sensitivity, we carried out all the experiments in 20% DMSO–water medium (Figures S29 and S30 in the Supporting Information). It should be noted that emission does occur at lower concentration of DMSO as well and the cell imaging studies were carried out in 1% DMSO in aqueous buffer solution.

The association constant[19] values were evaluated from the emission titration experiments. The emission titration data afforded the association constant values as 1.8×10^5 M^{-1} for Hg^{2+} and 3.77×10^4 M^{-1} for Cd^{2+} ions (Figure 2 and Figure 3).

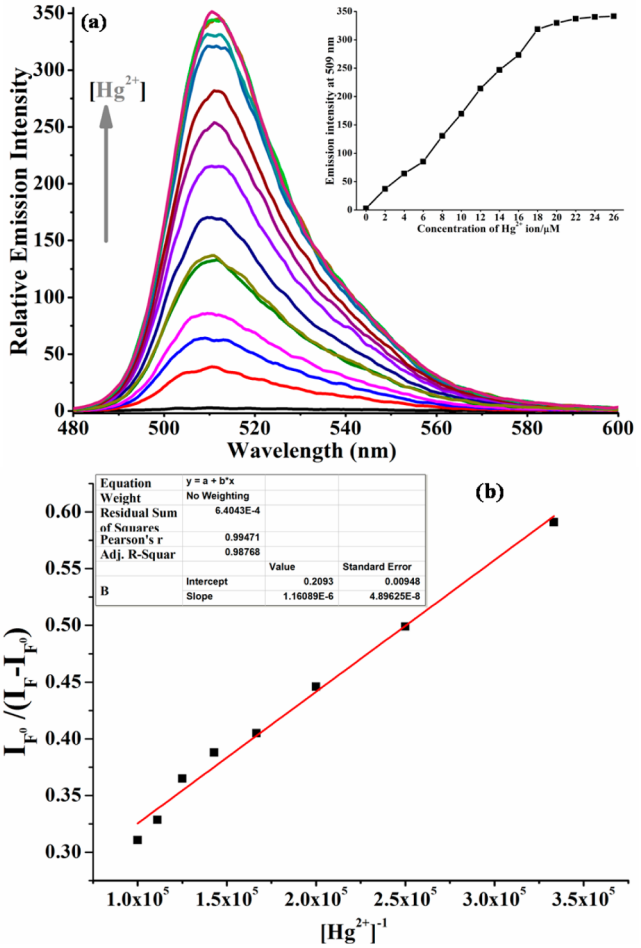


Figure 2. (a) Emission titration of **L** with different amounts of Hg^{2+} ion in aqueous medium (containing 20% DMSO) at pH 7.4 (HEPES buffer, 20 mM). Arrow indicates the trend in increasing Hg^{2+} ion concentration. Inset: The plot of emission intensity at 509 nm as a function of [Hg^{2+}]. [**L**] = 20 μM. λ_{exc} = 460 nm. Slit: 5/2.5 nm. (b) Binding constant plot of **L** for Hg^{2+} ion.

In order to explore the dye **L** as a real sensor, the detection limit of the dye for both Hg^{2+} and Cd^{2+} ions was determined.[20] A 38 nM detection limit for aqueous Hg^{2+} and a 77 nM detection limit for aqueous Cd^{2+} are obtained (Figures S31 and S32 in the Supporting Information).

The effect of pH on the emission responses of the present system was evaluated to tackle various pH values of environmental samples. The emission intensity against the pH value in the absence and presence of both Cd^{2+} and Hg^{2+} ions were measured. The metal-free dye does not show any significant emission over a wide pH range of 4 to 13 (Figure 4). However, the emission intensity of **L**-Cd^{2+} increases dramatically upon increasing the pH from 4 to 7 reflecting the competition between the proton and the cadmium ion for the N lone pair (Figure 4). Although no discernible change in the fluorescence spectrum can be observed in the pH range 7–10, the emission quantum yield decreases under alkaline conditions due to formation of metal hydroxide. The effect of pH on the emission response of **L** in the presence of Hg^{2+} ion was also

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929–3936

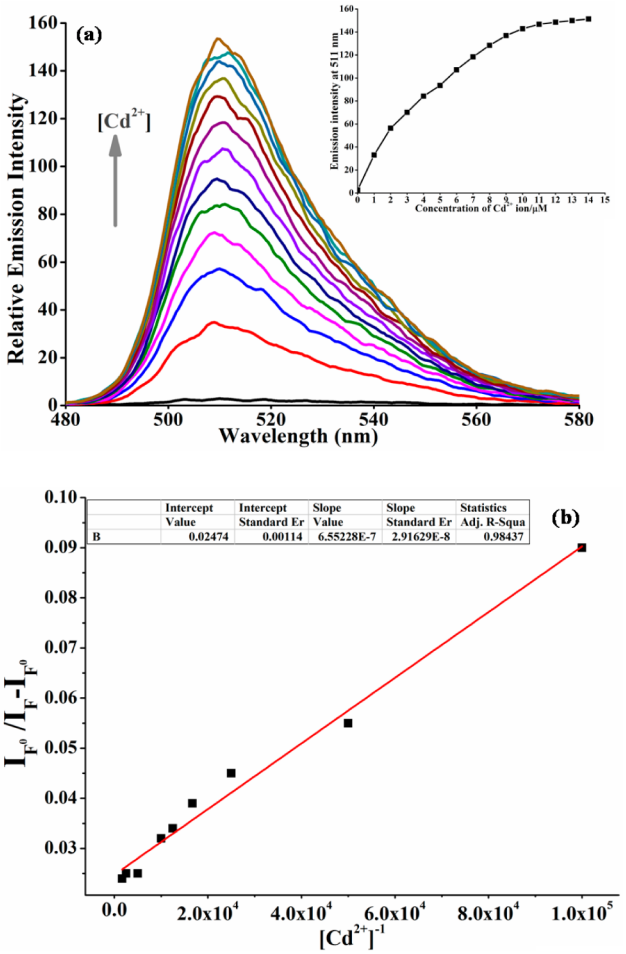


Figure 3. (a) Emission titration of **L** with different amount of Cd^{2+} ion in aqueous medium (containing 20% DMSO) at pH 7.4 (HEPES buffer, 20 mM). Arrow indicates the trend in increasing Cd^{2+} ion concentration. Inset: The plot of emission intensity at 511 nm as a function of $[Hg^{2+}]$. [**L**] = 10 μM. λ_{exc} = 460 nm. Slit: 5/2.5 nm. (b) Binding constant plot of **L** for Cd^{2+} ion.

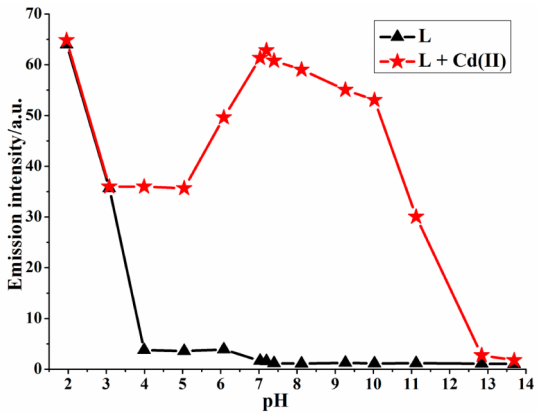


Figure 4. Emission intensities of **L** and **L** + Cd^{2+} at various pH values in aqueous medium (containing 20% DMSO). Excitation wavelength = 460 nm. Slit: 2.5/2.5 nm. [**L**] = 1 μM, $[Cd^{2+}]$ = 5 μM.

investigated as shown in Figure 5. It exhibits significant change in emission response over pH range from 4 to 8.

^{1}H NMR Titrations. To probe the binding of either metal ion to the receptor, ^{1}H NMR titration experiments in DMSO-

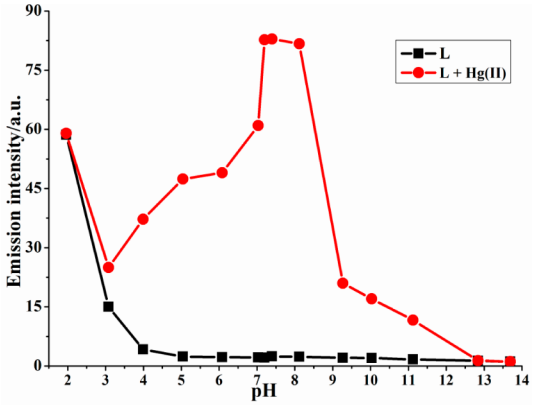


Figure 5. Emission intensities of **L** and **L** + Hg^{2+} at various pH values in aqueous medium (containing 20% DMSO). Excitation wavelength = 460 nm. Slit: 2.5/2.5 nm. [**L**] = 1 μM, $[Hg^{2+}]$ = 5 μM.

d_6:CD_3CN (1:1, v/v) are carried out. In the spectrum of **L**, the signal for NH protons appears at 11.41 ppm. Addition of either Hg^{2+} or Cd^{2+} ions leads to no significant shifts of the amide protons, indicating its nonparticipating nature in the binding of either metal ion. Instead, significant chemical shifts of the protons of the pyridyl units are observed in the presence of Hg^{2+} or Cd^{2+} ion. The chemical shifts of the protons are shown in Figures S33 and S34 in the Supporting Information. The chemical shifts of the protons are found to be significant up to the addition of 1 equiv of Hg^{2+} or Cd^{2+} ion. Addition of an excess of either metal ion does not result in any further effect on the splitting pattern or chemical shifts of either aromatic or aliphatic protons, indicating that the sensor forms 1:1 complexes with these metal ions. The significant change in the resonance for the picolylamine protons along with that of the protons adjacent to the ether group suggests that the metal ion shows strong binding interactions with the 2,2′-(ethane-1,2-diylbis(oxy))bis(*N*,*N*-bis(pyridine-2-ylmethyl)aniline unit.

Cell Imaging Study. The ability of a biosensor in tracking guests in the living cell is of great importance.[21] Therefore, experiments were performed to probe the ability of **L** to track either Hg^{2+} or Cd^{2+} in living cells through confocal microscopy. HeLa cells incubated with 5 μM **L** for up to 30 min at 37 °C show negligible intracellular fluorescence (Figure 6A, control), while **L** stained cells exposed to different concentrations (3 μM,

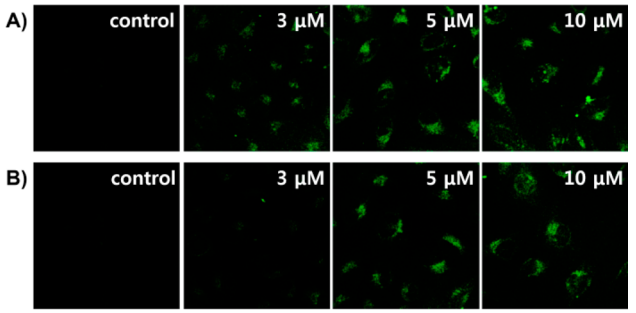


Figure 6. Fluorescence image of HeLa cells upon treatment with **L** (5 μM) and metal ions. (A) Fluorescence change upon the addition of different concentrations of Hg^{2+} ions. (B) Fluorescence change upon the addition of various concentrations of Cd^{2+} ions. Cells were incubated at 37 °C under humidified atmosphere 5% CO_2. The excitation wavelength was 458 nm, and images were collected at 480–650 nm.

DOI: 10.1021/acs.inorgchem.5b00106
Inorg. Chem. 2015, 54, 3929–3936

5 μM, and 10 μM) of Hg^{2+} for 30 min at 37 °C display intracellular fluorescence that enhances with increasing metal ion concentration (Figure 6A). Likewise, when the HeLa cells incubated with 5 μM **L** are treated with different concentrations of Cd^{2+} ion (Figure 6B), the intracellular fluorescence is found to increase with the concentration of the metal ion.

Treatment of cells loaded with **L** (5 μM) and Hg^{2+} (10 μM) with the metal chelator EDTA (250 μM) for 30 min at 37 °C and that of cells loaded with **L** (5 μM) and Cd^{2+} (10 μM) with the heavy metal chelator TPEN (250 μM) for 30 min at 37 °C reverse the observed fluorescence intensities (Figures 7c and 7d). These data prove that **L** can respond toward the changing in intracellular Hg^{2+} and Cd^{2+} levels in living cells.

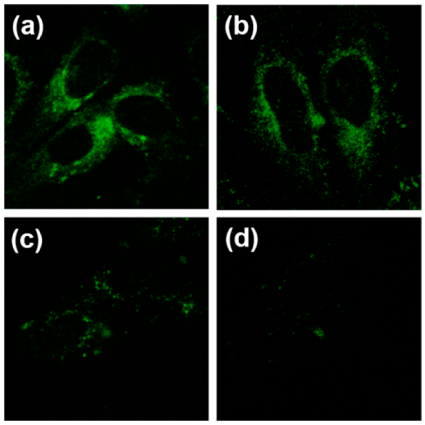


Figure 7. Confocal microscopy images of HeLa cells treated with 5 μM **L**. (a) Cd^{2+} (10 μM), (b) Hg^{2+} (10 μM), (c) Cd^{2+} (10 μM) and TPEN (250 μM), and (d) Hg^{2+} (10 μM) and EDTA (250 μM) were added, respectively. Cells were incubated at 37 °C under humidified atmosphere 5% CO_2. The excitation wavelength was 458 nm, and images were collected at 480−650 nm.

CONCLUSION

To conclude, we have described here the synthesis, photophysical properties, and cellular applications of a PET based sensor where two BODIPY derivatives are covalently linked with an ionophore, 2,2′-(ethane-1,2-diylbis(oxy))bis(*N*,*N*-bis(pyridine-2-ylmethyl)aniline. The dye features visible excitation and emission profiles and selective turn-on fluorescence response to either Hg^{2+} or Cd^{2+} ion in the presence of a large excess of biologically relevant metal ions in aqueous environment at physiological pH. Because of its usability in aqueous medium at physiological pH, it can be used for tracking Hg^{2+} or Cd^{2+} through live cell imaging studies.

ASSOCIATED CONTENT

Supporting Information

The ^1H and ^{13}C NMR spectra, ESI-mass spectra, and additional spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Department of Science and Technology, New Delhi, India (No. SERB/CHM/20130239 to P.B.K.), and CRI project (No. 2009-0081566 to J.S.K.) from the National Research Foundation of the Ministry of Science, ICT & Future Planning in Korea. A senior research fellowship from the CSIR, New Delhi, to S.B.M. is gratefully acknowledged.

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DOI: 10.1021/acs.inorgchem.5b00106
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