JOC Note

New BODIPY Derivatives as OFF−ON Fluorescent Chemosensor and Fluorescent Chemodosimeter for Cu^{2+}: Cooperative Selectivity Enhancement toward Cu^{2+}

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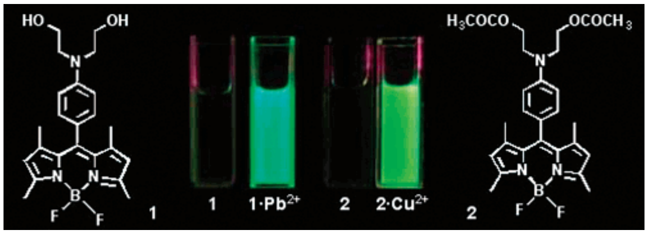

New 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives (**1** and **2**) were synthesized as an "off−on" fluorescent chemosensor and fluorescent chemodosimeter for Cu^{2+} and Pb^{2+}. Compound **1** displayed selective and large chelation enhanced fluorescence effects with Pb^{2+} and Cu^{2+} among the metal ions examined. On the other hand, compound **2**, a fluorescent chemodosimeter, effectively recognized Cu^{2+} via a selective hydrolysis of the acetyl group.

Sensors based on the ion-induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[1] Over the past few years, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives have been utilized as useful fluorophores because of their advantages, such as high excitation coefficients, high fluorescence quantum yields, and high stability against light and chemical reactions.[2] Recently, the fluorescent chemosensors based on the BODIPY[3] have been applied as a Ag^+ sensor,[3l] Na^+ sensor,[3k] NO sensor,[3h] Hg^{2+} sensor,[3a,3g] Zn^{2+} sensor,[3c,3e] Ca^{2+} sensor,[3f] F^- sensor,[3i] and pH sensor.[3j]

Pb^{2+} is one of the important targets because of the adverse health effects of lead exposure, particularly in children.[4] The design of a Cu^{2+} sensor has also received intensive attraction since this metal ion is a significant environmental pollutant and an essential trace element in biological system.[5] However, Cu^{2+} and Pb^{2+} are known as inherent quenching metal ions,[1d,6] and therefore, relatively few reports are available for "off−on"-type fluorescent chemosensors, which can selectively recognize Cu^{2+}[7] and Pb^{2+} ions.[8]

We report herein utilizing new BODIPY derivatives (**1** and **2**) as an "off−on" fluorescent chemosensor and fluorescent chemodosimeter for Cu^{2+} and Pb^{2+}. Compound **1** displayed selective and large chelation enhanced fluorescence (CHEF) effects with Pb^{2+} and Cu^{2+} among the metal ions examined. On the other hand, compound **2**, a fluorescent chemodosimeter, effectively recognized Cu^{2+} via a selective hydrolysis of acetyl group.

The direct synthesis of the target **1** based on the reported route[3j] using 4-*N*,*N*-bis-(2-hydroxyethyl)aminobenzaldehyde and 2,4-dimethylpyrrole failed due to the poor solubility of the aldehyde in methylene chloride. Therefore, the hydroxyl groups on the aldehyde were then first acetylated to improve the solubility. Although the monoacetylated product was also soluble enough in condensation condition, the bisacetylated derivative was preferential, considering the ease of the separation of **2** (Scheme 1). In the X-ray structure of **2** shown in Figure 1 (see S-Figure 1 in the Supporting Information for **1**), a *C*2 axis passes through the B1, C7, C8, and C11 atoms in **2**. The phenyl and pyrrole rings are planar, with an average deviation of 0.0033 and 0.0102 Å, respectively. Similarly, the phenyl and pyrrole

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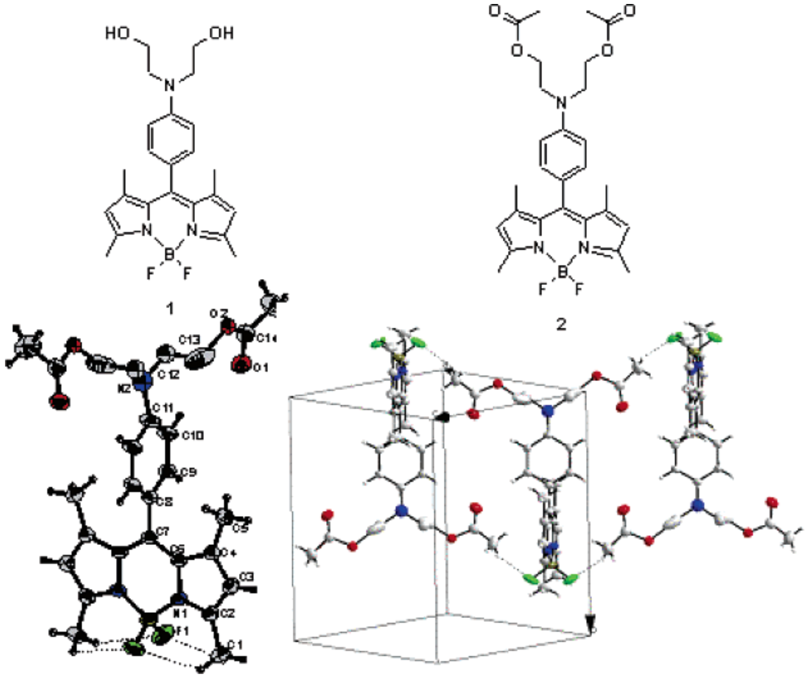
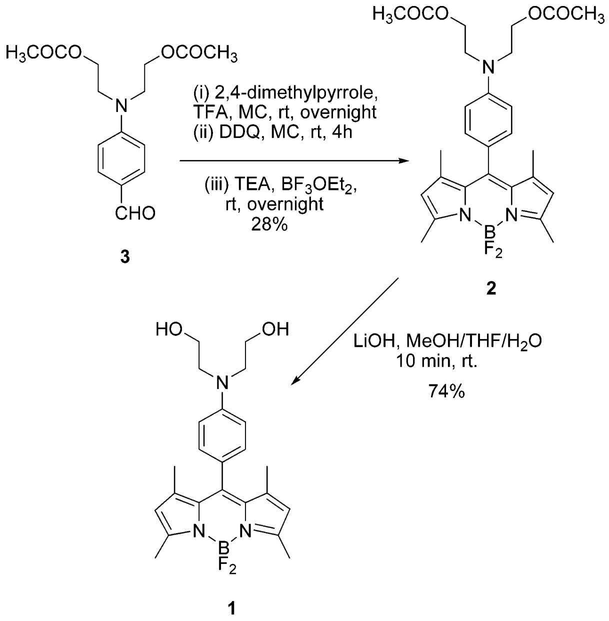


FIGURE 1. Structures of **1** and **2** and the X-ray crystal structure of **2**.

SCHEME 1. Syntheses of Compounds 1 and 2



rings in **1** are also planar, with an average deviation of 0.0070 and 0.0540 Å, respectively. The dihedral angle between the phenyl and pyrrolyl rings (86.5°) in **2** is bigger than that in **1** (74.6°), which may be caused by different interaction of hydrogen bonds. The two kinds of intramolecular hydrogen bonds (C1−H1A···F1 and C1−H1C···F1) between F atoms and methyl groups (3, 3′) in **2** (S-Table 1, 2) are much weaker than those in **1** (C1−H1A···F1 and C12−H12A···F2). By intermolecular hydrogen bonds, the molecules of **2** (C15−H15C···F1) are linked into a complex sheet (Figure 1).

Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Cs^+, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, and Zn^{2+} ions were used to evaluate the metal ion binding properties of **1** and **2**. All fluorescent studies were conducted in acetonitrile and using a 1 μM concentration of compounds. The fluorescence emission changes of **1** and **2** upon the addition of various metal ions (100 equiv) are illustrated in Figures 2 and 3, respectively. The fluorescence spectra were obtained by excitation into the BODIPY fluorophore at 504 nm, and both the excitation and emission slits were 1.5 or 3 nm. As shown in Figure 2, compound **1** displayed a large CHEF effect only with Pb^{2+} (also see S-Figure 2), even though there were relatively small CHEF effects with Zn^{2+} and Cu^{2+}. Since the compound itself did not exhibit any fluorescence, the large CHEF effects with Pb^{2+} can be explained by the blocking of the photoinduced electron transfer (PET) process.[1d] On the other hand, **2** showed a highly selective CHEF effect only with Cu^{2+} among the metal ions examined (Figure 3). As shown in the Supporting Information (S-Figure 3), the fluorescence emission intensity of **2** reached its maximum when 5 equiv of Cu^{2+} was added, and a gradual decrease in its emission intensity as well

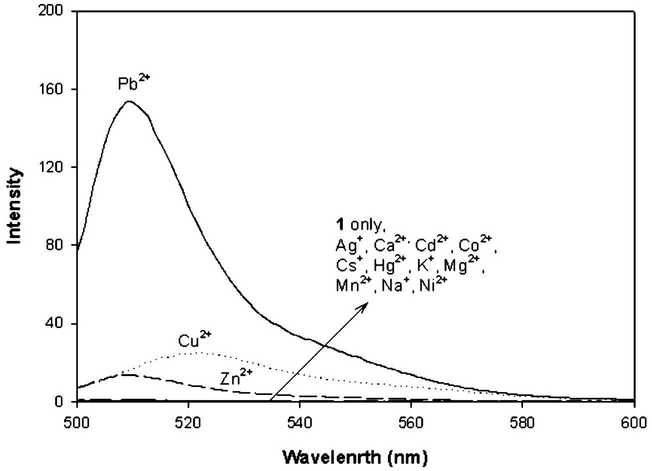


FIGURE 2. Fluorescent emission changes of 1 (1 μM) upon addition of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, and Zn^{2+} (100 equiv) in acetonitrile (excitation at 504 nm).

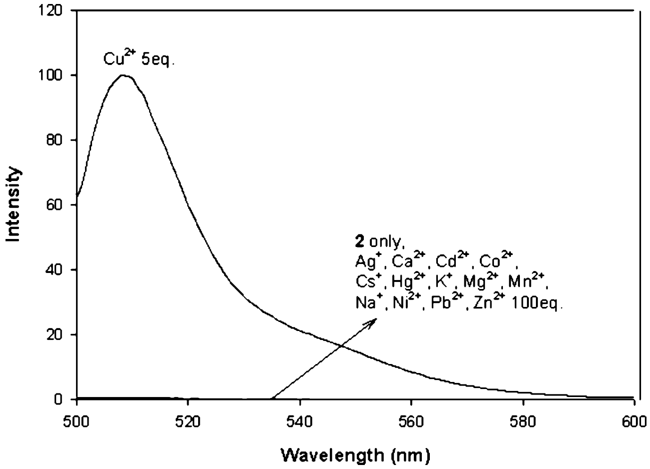


FIGURE 3. Fluorescent emission changes of **2** (1 μM) upon addition of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, and Zn^{2+} in acetonitrile (containing 0.5% water) (excitation at 504 nm).

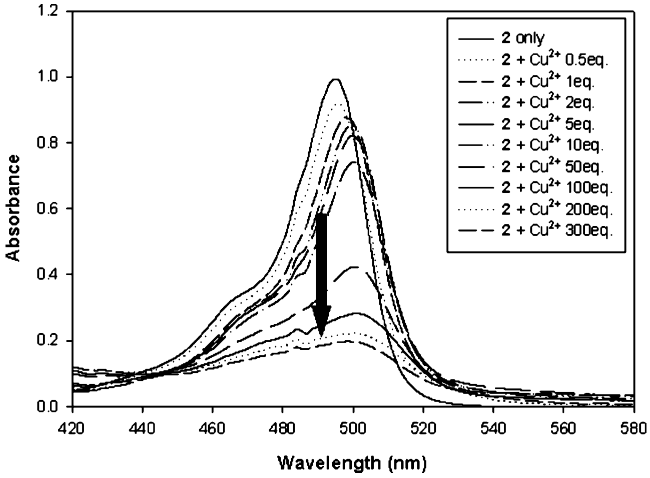


FIGURE 4. UV titrations of **2** (15 μM) upon addition of Cu^{2+} in acetonitrile.

as a red-shift (~9 nm) were observed as the concentration of Cu^{2+} increased. A similar red shift and an absorbance decrease of **2** were also observed in its UV spectra (Figure 4). A ratiometric change in UV spectra was observed upon the addition of Cu^{2+} in acetonitrile. Compound **1** displayed similar fluorescent titration spectra (S-Figure 4) and UV spectra with Cu^{2+}. The large CHEF effects with relatively small equivalents of Cu^{2+} can be explained in the same way described for Pb^{2+}. On the other hand, it is reported[1d] that when Cu^{2+} binds tightly to the host compound, intracomplex quenching takes place (via energy or electron transfer[9]). From the fluorescence titration experiments, the association constants of **1** with Pb^{2+} and Zn^{2+} were observed to be 8800 and 510 M^{-1}, respectively (errors < 10%).[10] The selectivity for Pb^{2+} over Zn^{2+} was more than 15 times. The association constant of **1** with Cu^{2+} was calculated as 55 000 M^{-1} from the fluorescent titration experiment (error < 10%) in the presence of 0.5% water, which was further confirmed in the case of compound **2**.[10] In the presence of 0.5% water, fluorescent emission intensities of **1** or **2** were decreased (~50%) compared to those in acetonitrile. The job plots using the fluorescence changes indicated 1:1 binding for **1** with Pb^{2+} and Cu^{2+}. The relative quantum yields of fluorescence in acetonitrile were calculated as 0.4 [**1** with Pb^{2+} (600 equiv)] and 0.11 [**1** with Cu^{2+} (10 equiv)], respectively, in that of fluorescein (0.85) in 0.1 M NaOH.[3h]

SCHEME 2. Proposed Mechanism of Recognition/Reactivity of Compound 2 toward Cu^{2+}

Unlike that of **1**, the recognition of $\mathbf{2}\cdot Cu^{2+}$ was an irreversible process because the solution of $\mathbf{2}\cdot Cu^{2+}$ after 10 min of mixing appeared in the same position on TLC as that of **1**. Furthermore, a chloroform and EDTA aqueous solution was added to the $\mathbf{2}\cdot Cu^{2+}$, and the ^1H NMR of the extracted product was found to be identical to that of **1**. In the ESI mass spectrum of compound **2** upon the addition of $Cu(ClO_4)_2$, a peak at *m/z* 245.5 (calculated value = 245.4) corresponding to $[\mathbf{1} + Cu]^{2+}$ was observed (S-Figure 6). On the other hand, the solution of $\mathbf{2}\cdot Zn^{2+}$ and $\mathbf{2}\cdot Pb^{2+}$ displayed no changes on TLC even after 24 h, which was also confirmed by fluorescence study. Chemodosimeters are devices that utilize abiotic receptors to achieve analyte recognition with concomitant irreversible transduction of a human-observable signal. As shown in Scheme 2, possibly a small amount of water coming from copper perchlorate hydrate as well as tightly bound Cu^{2+} in the binding site may promote hydrolysis of acetyl groups in **2**. A selective Cu^{2+} recognition/reactivity of compound **2** demonstrated that this compound has a potential to be utilized as a selective fluorescent chemodosimeter for Cu^{2+}. In the presence of 2 equiv of Cu^{2+}, the conversion from **2** to **1** was effectively complete within 4 min in acetonitrile (S-Figure 5).

In conclusion, the new fluorescent sensor **1** displayed selective large CHEF effects with Pb^{2+} and Cu^{2+}. The selectivity for Pb^{2+} was calculated to be 15 times that of Zn^{2+}. On the other hand, the fluorescent chemodosimeter **2** showed an extreme selectivity for Cu^{2+} over the other metal ions examined. A highly selective Cu^{2+} recognition/reactivity of fluorescent chemodosimeter **2** demonstrated that this approach can cooperatively enhance and control a selectivity toward metal ions.

Experimental Section

4-*N*,*N*-Bis-(2-acetoxyethyl)aminobenzaldehyde (3).[11] Acetic anhydride (0.227 mL, 2.4 mmol) was added to a solution of 4-*N*,*N*-bis-(2-hydroxyethyl)aminobenzaldehyde (418.5 mg, 2 mmol), DCC

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[11] Same compound was synthesized by the formylation reaction. Yin, D.-D.; Ren, Y.; Zhai, J.-F.; Qiu, L.; Shen, Y.-Q. *Huaxue Xuebao* **2004**, *62*, 518–522.

(515.8 mg, 2.5 mmol), and DMAP (25 mg, 0.2 mmol) in anhydrous THF (40 mL) at room temperature under nitrogen atmosphere. After 24 h, the reaction mixture was passed through a short silica gel column (~10 g) and rinsed with ethyl acetate (50 mL). The eluent was concentrated to dry and was redissolved by a mixture of ethyl acetate/hexane (25:25 mL). The suspension was filtered through a cotton plug and concentrated again. The crude residue was purified by silica gel chromatography (ethyl acetate/hexane = 1:3 to 2:1) to afford a colorless oil (539.7 mg, yield 92%), which could be directly used for further condensation. ^{1}H NMR ($CDCl_3$): δ 2.02 (s, 6H), 3.68 (t, 4H, $J = 6.2$ Hz), 4.23 (t, 4H, $J = 6.2$ Hz), 6.66 (d, 2H, $J = 8.6$ Hz), 7.70 (d, 2H, $J = 8.9$ Hz). MS (FAB) *m*/*z* = 294.3 $(M + H)^+$.

4,4-Difluoro-8-[4-*N*,*N*-bis-(2-acetoxyethyl)aminophenyl]-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (2). 4-*N*,*N*-Bis-(2-acetoxyethyl)aminobenzaldehyde (293.3 mg, 1 mmol) and 2,4-dimethylpyrrole (190.3 mg, 2 mmol) were dissolved in anhydrous methylene chloride (30 mL) under argon atmosphere. One drop of trifluoroacetic acid was added, and the solution was stirred at room temperature overnight. A solution of 2,3-dichloro-5, 6-dicyano-1,4-benzoquinone (227.0 mg, 1 mmol) in anhydrous MC (40 mL) was added by syringe, and the stirring was continued for another 4 h. After the addition of triethylamine (6 mL), $BF_3 \cdot OEt_2$ (6 mL) was gradually added during 30 min in an ice−water bath followed by continuous stirring at room temperature overnight. The reaction solution was shaken with 5% aqueous sodium bicarbonate (100 mL), and the mixture was passed through a Celite pad and washed with MC (30 mL) to remove the black solid. The organic layer was washed with water and dried over anhydrous sodium sulfate. After concentration in vacuo, the residue was purified by flash chromatography (MC to MC/MeOH = 500:1) twice to afford an orange-red solid (144.2 mg, 0.282 mmol, yield 28.2%). The analytical sample was recrystallized from ethyl acetate/hexane to get a red crystal: mp 189 °C; ^{1}H NMR ($CDCl_3$): δ 1.48 (s, 6H), 2.08 (s, 6H), 2.55 (s, 6H), 3.65 (t, 4H, $J = 6.3$ Hz), 4.26 (t, 4H, $J = 6.2$ Hz), 5.97 (s, 2H), 6.82 (d, 2H, $J = 8.6$ Hz), 7.06 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.5, 14.7, 20.8, 49.5, 61.2, 112.0, 120.9, 122.8, 129.1, 132.0, 142.6, 143.1, 147.7, 154.8, 160.8, 170.9. HRMS: calcd M^+ for $C_{27}H_{32}BF_2N_3O_4$, 511.2454; found, 511.2457.

4,4-Difluoro-8-[4-*N*,*N*-bis-(2-hydroxyethyl)aminophenyl]-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1). 4,4-Difluoro-8-[4-*N*,*N*-bis-(2-acetoxyethyl)aminophenyl]-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (128 mg, 0.25 mmol) was dissolved in a mixture of THF (1 mL) and MeOH (4 mL). The solution was degassed for 10 min under nitrogen atmosphere. A solution of $LiOH \cdot H_2O$ (10.5 mg, 0.25 mmol) in water (1 mL, degassed for 10 min prior to use) was added dropwise during 20 min at ambient temperature under N_2. After 10 min (monitored by TLC, MC/MeOH = 10:1), the reaction mixture was concentrated and purified by silica gel chromatography (MC to MC/MeOH = 50:1) to afford a red solid (79.1 mg, 0.185 mmol, yield 74.1%). The analytical sample was recrystallized from ethyl acetate/hexane to get a red crystal: mp 200 °C; ^{1}H NMR ($CDCl_3$): δ 1.48 (s, 6H), 2.54 (s, 6H), 3.63 (t, 4H, $J = 4.4$ Hz), 3.64 (br, 2H), 3.89 (t, 4H, $J = 4.5$ Hz), 5.97 (s, 2H), 6.76 (d, 2H, $J = 8.5$ Hz), 7.04 (d, 2H, $J = 8.6$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.5, 14.7, 30.9, 55.6, 60.7, 112.8, 120.9, 123.0, 129.0, 132.0, 142.5, 143.1, 148.1, 154.9. HRMS: calcd M^+ for $C_{23}H_{28}BF_2N_3O_2$, 427.2243; found, 427.2235.

Preparation of Fluorometric Metal Ion Titration Solutions. Stock solutions (1 mM) of the perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Rb^+, and Zn^{2+} in acetonitrile were prepared. Stock solution of host (0.1 mM) was also prepared in acetonitrile. Test solutions were prepared by placing 4−40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 4 mL with acetonitrile.

For all measurements, the excitation wavelength was 504 nm. Both excitation and emission slit widths were either 1.5 or 3 nm.

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Supporting Information Available: General experimental section, fluorescence spectra, X-ray data, experimental procedures, and characterization data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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