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Fluorescent dye containing phenol-pyridyl for selective detection of aluminum ions

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ABSTRACT

A phenol-pyridylimine probe was utilized as an optical sensor to quantify the presence of aluminum ions using a turn-on fluorescence enhancement approach. The high sensitivity was the result of FRET amplification of the receptor subunit fluorescence emission. The complex stability constant (K_s) for the stoichiometric 1:1 complex of the sensor with aluminum ions was obtained by fluorimetric titration. Remarkably, fluorescence output was not significantly affected by other trivalent cations, particularly Ga^{3+} and In^{3+}.

Aluminum is the third most abundant metallic element (about 8% by weight) on Earth, and its soluble form is highly toxic to plant growth [1]. A trivalent cation of aluminum is found in most animal and plant tissues as well as in natural waters everywhere. People are exposed to aluminum due to its widespread use in food additives, aluminum-based pharmaceuticals, and cooking utensils. If a significant load of aluminum ions exceeds the body's excretory capacity, the excess is deposited in various tissues, including bone, muscle, heart, spleen, liver, and brain. Aluminum ion accumulation in tissues and organs results in dysfunction and toxicity [2]. The iron binding protein is the main carrier for Al^{3+} in plasma and Al^{3+} can enter the brain and reach the placenta and fetus. Aluminum concentrations in the brain should be maintained at <2 μg/g [3]. Aluminum has been explored as a possible toxic agent involved in the development of Alzheimer's disease, as aluminum ions induce oxidative stress within brain tissue. Al^{3+} has been implicated as a causative factor in Alzheimer's disease and has been associated with damage to the central nervous system in humans [4–7]. However, it is still unclear whether aluminum ions play any part in Alzheimer's disease. Some scientists believe that breathing aluminum dust may cause health problems, as it may cause aluminosis, and unusual concentrations of aluminum ions have been found in the brains of patients with Alzheimer's disease.

Fluorescent chemosensors have attracted significant interest for use in medical, medicinal, and environmental research. Until recently, some fluorescent chemosensors based on Schiff bases [8–10], triazoles [11,12], triazole-pyridyl [13–15], calixarene [16,17], and secondary/tertiary amines [18,19], have been developed for detecting Al^{3+}. Most fluorescent Al^{3+} sensors have binding environments containing imine nitrogen and/or oxygen which create a hard-base environment for the hard-acid Al^{3+}. We have reported on a fluorescent chemosensor containing a salicylimine moiety for selective aluminum ion sensing [8]. Here, we developed a new phenol/pyridyl-based chemosensor that exhibited high selectivity for Al^{3+}. This chemosensor provided high sensitivity derived from the turn-on fluorescence changes upon titration with Al^{3+} and good selectivity even relative to gallium and indium ions.

The fluorophore CPAP was synthesized by coupling 2-hydroxyphenyl-2-pyridylmethylamine and 4-chloro-3-nitrocoumarin with a 71% yield in acetonitrile (Scheme 1). The precursor 2-hydroxyphenyl-2-pyridylmethylamine was prepared by consecutive reduction of the imine compound produced from reacting 2-aminomethylpyridine and 2-hydroxy benzaldehyde in methanol.

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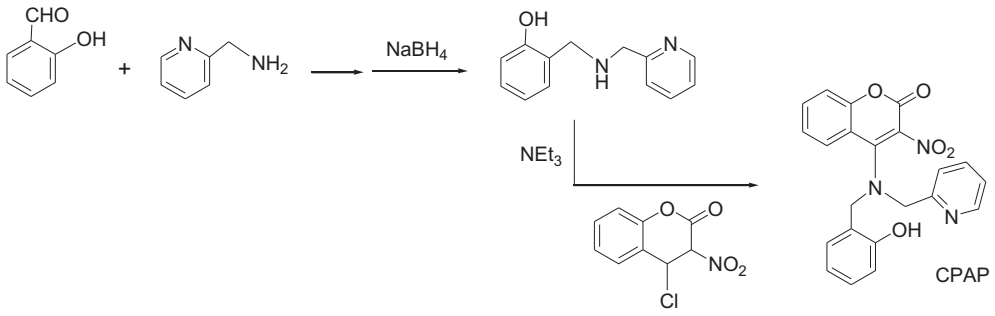


Scheme 1. Synthetic CPAP procedure.

First, we studied CPAP absorbance changes following treatment with aluminum ions. CPAP had two absorption bands at 268 and 316 nm (Fig. 1a). Al^{3+} Treatment caused a progressive decrease in the 268 and 316 nm bands, and two new absorption bands appeared at 259 and 381 nm. Absorbance at 316 nm continued to decrease, and the 381 nm band increased with an increase in Al^{3+} concentrations up to 1 equiv (Fig. 1b). Three isosbestic points were observed at 264, 290, and 345 nm, indicating that only one product was generated from CPAP upon binding to Al^{3+}. CPAP had a quantum yield of 0.095.

Fluorescence emission was examined following treatment with various metal ions in methanol (Fig. 2a). CPAP alone did not show any significant emission upon excitation at 353 nm. However, Al^{3+} treatment resulted in a large increase in intensity at a wavelength of 420 nm. In contrast, no fluorescence enhancement was observed after adding other mono-, di-, and tri-valent metal ions including Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Cr^{3+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, In^{3+}, and Fe^{2+}. Only, adding Ga^{3+} to CPAP produced some emission but with much reduced intensity. Al^{3+} selectivity with 10 μM CPAP was evaluated by testing sensor response to other metal ions at a concentration of 200 μM (Fig. 2b). Only the Al^{3+} solution produced marked fluorescence enhancement relative to that of control ions. The intensity ratio (I_{Al}/I_{Ga}) of 25 with CPAP was remarkably high relative to 2.1 and 3.4 reported with chemosensors based on salicylimine and naphthol/quinoline,

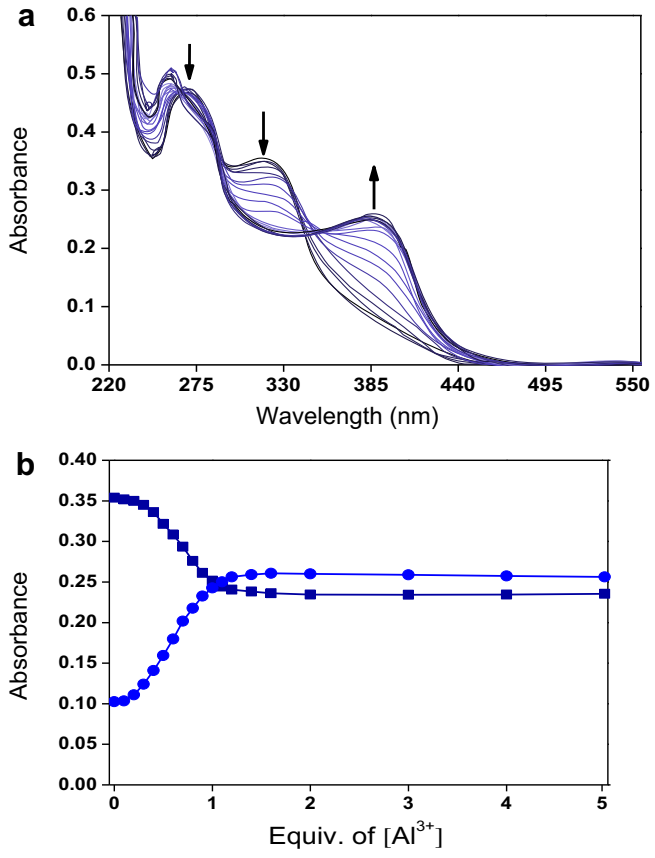


Fig. 1. (a) UV–visible spectral changes in CPAP (40 μM) after adding aluminum ions (up to 5 equiv) in methanol. (b) Graph of the absorbance at 316 (■, squares) and 381 nm (●, circles) as a function of Al^{3+} concentration.

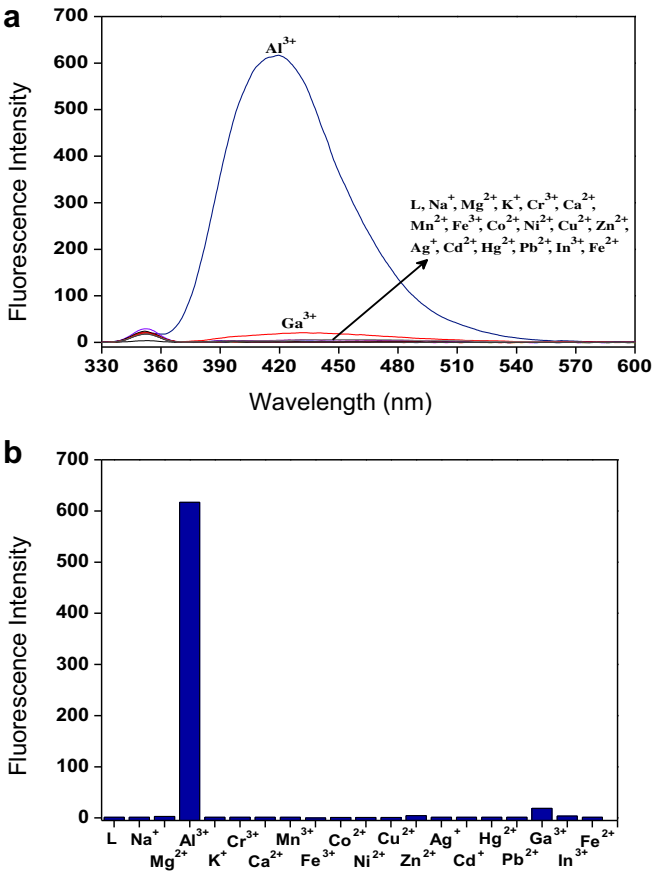


Fig. 2. (a) Fluorescence spectra of CPAP (10 μM) after adding metal salts (20 equiv) of Li^{+}, Na^{+}, K^{+}, Ag^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+}, and In^{3+} in MeOH. The excitation wavelength was 353 nm. (b) Selectivity of the analysis for metal ions with CPAP. The concentration of all metal ions was 200 μM. Left to right: CPAP alone, Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Cr^{3+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Ga^{3+}, In^{3+}, and Fe^{2+}. L indicates CPAP.

respectively [8,9]. In another report, a probe containing pyrene exhibited an intensity ratio of 4.0 [19].

When a 10 μM CPAP solution was titrated with Al^{3+}, fluorescence intensity at 420 nm increased steeply up to 10 equiv and then showed almost no significant increase up to a concentration of 40 equiv (Fig. 3). However, the CPAP chemosensor developed in this study showed spectral changes from 0 to 10 equiv Al^{3+}, and Al^{3+} could be detected down to 0.5 μM based on the 3α/slope when 10 μM CPAP was employed. A Job plot obtained based on emission data referred to 1:1 stoichiometric complexation of CPAP with Al^{3+} (Supporting Information Fig. S1). According to the above titration fluorimetry, the stability constant (K_s) of CPAP-Al^{3+} was calculated by using the nonlinear least-squares analysis. The K_s value turned out 3.3×10^4 which was within the range $10^3 \sim 10^9$ of those reported for Al^{3+}-binding chemosensors.[9,11,13,15,18] The need of more equiv Al^{3+} to reach a saturation point resulted from the low CPAP concentration used in fluorescence titration experiments relative to UV–vis and NMR.

Fig. 4 shows CPAP fluorescence intensity with Al^{3+} in the presence of various competing metal ions. Compared to CPAP intensity obtained with 1 equiv Al^{3+}, the presence of other metal ions resulted in no interference for detecting Al^{3+}, except Fe^{3+}. No significant fluorescence signal interference was observed, even in the case of In^{3+} and Ga^{3+}. Thus, CPAP appeared to be a selective fluorescent sensor for detecting Al^{3+} in the presence of competing metal ions.

The 1:1 binding stoichiometry of CPAP and Al^{3+} was examined by ^1H NMR in CD_3OD. Adding 1 equiv of Al^{3+} to a solution of the receptor resulted in different peak profiles (Fig. 5). Adduct formation between Al^{3+} and CPAP was evident from the dramatic changes in the chemical shifts for both the phenol and pyridyl protons of CPAP. The proton signals of the phenol group at 6.6–7.3 ppm were shifted to 6.5–6.8 ppm. At the same time, the pyridyl moiety proton signals at 7.4–8.5 ppm were somewhat shifted in a similar region. Coumarin aromatic protons at 7.3–7.6 ppm were also shifted slightly. The two CPAP methylene protons split up into two doublets upon binding to Al^{3+}. Such a splitting of methylene protons in pyridyl methyl moieties was observed when a chemosensor was coordinated to metal ions [20]. In the CPAP-Al^{3+} coordination complex, two nitrogens and an oxygen of CPAP could bind with Al^{3+} and the remaining three binding sites (X) of the Al^{3+}-CPAP complex may be occupied by nitrate anions or methanol (Scheme 2). The coordination of nitrate or perchlorate ions was proposed when chemosensors were bound to Al^{3+} with a 1:1 stoichiometry in organic solvents [8,13,15].

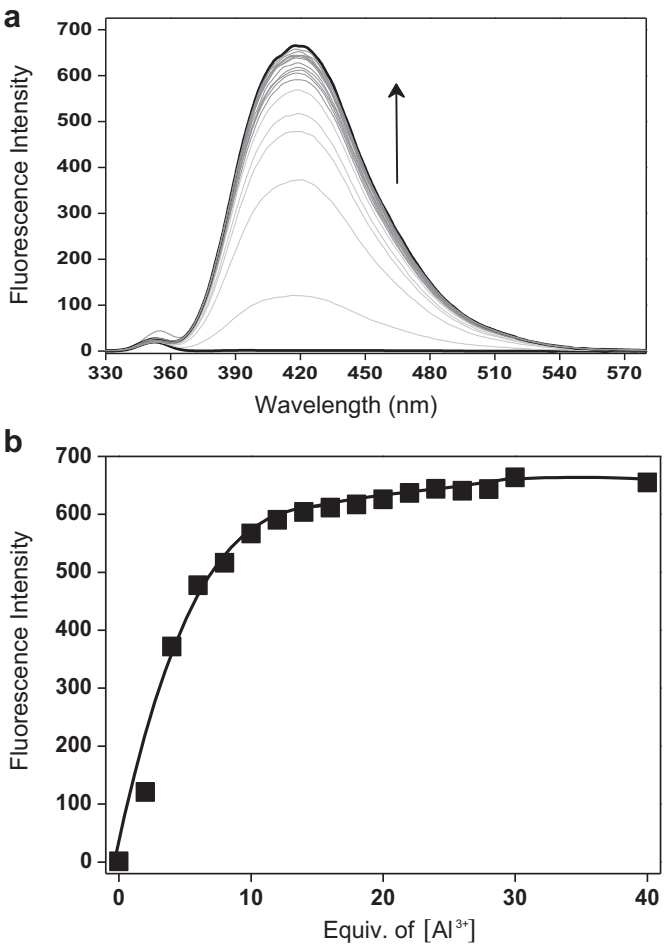


Fig. 3. (a) Fluorescence spectra of CPAP (10 μM) with different concentrations of Al^{3+} in MeOH. (b) Graph of the fluorescence intensity at 420 nm as a function of Al^{3+} concentration.

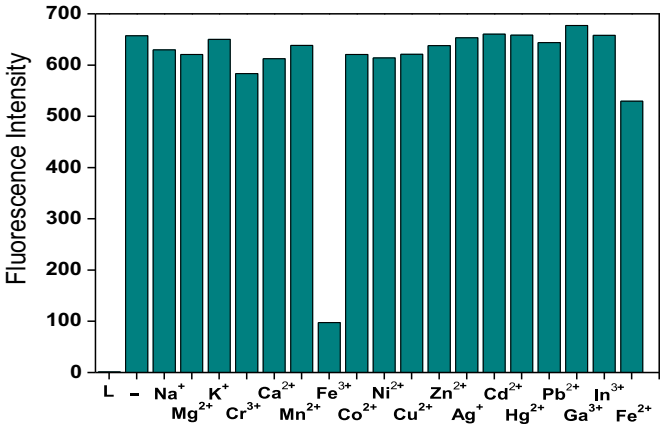


Fig. 4. Relative fluorescence of CPAP alone and its complexation with Al^{3+} in the presence of various metal ions. The CPAP response was included as a control (labeled as L). Conditions: 10.0 μM CPAP, 1 equiv metal ions with 1 equiv of Al^{3+} ($E_\lambda = 353$ nm).

In conclusion, a phenol-pyridyl chemosensor is reported as an optical turn-on fluorescent probe for Al^{3+} ions. This probe enabled analysis of Al^{3+} ions with a sensitivity limit of 0.5 μM. In our assay, Al^{3+} selectively participated in complex formation with the receptor, which resulted in enhanced fluorescence. The chemosensor reported here exhibited the highest fluorescence intensity ratio between Al^{3+} and Ga^{3+}, relative to the values obtained in other reports. In further investigations, this chemosensor will be derivatized to improve sensitivity and to detect other metal ions in aqueous solution.

1. Experimental section

1.1. Materials and instrumentation

All solvents and reagents (analytical and spectroscopic grades) were obtained from Sigma–Aldrich (St. Louis, MO, USA) and used as received. The metal ion solutions were prepared with metal nitrate salts in methanol. NMR spectra were recorded on a Varian 400 spectrometer (Palo Alto, CA, USA). Chemical shifts (δ) are reported in ppm, relative to tetramethylsilane $Si(CH_3)_4$. Absorption spectra were recorded at 25 °C using a Perkin–Elmer model Lambda 2S UV/Vis spectrometer (Waltham, MA, USA). Emission spectra were recorded on a Perkin–Elmer LS45 fluorescence spectrometer.

1.2. Synthesis of CPAP

2-Aminomethyl pyridine (6 mmol, 624 μL) and 2-hydroxy benzaldehyde (5 mmol, 539 μL) were dissolved in methanol

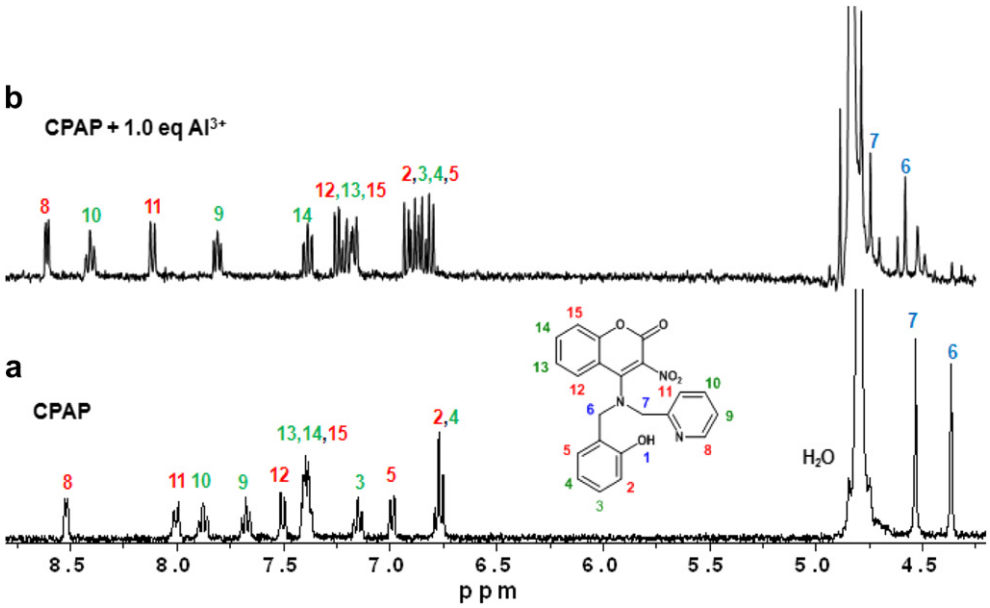
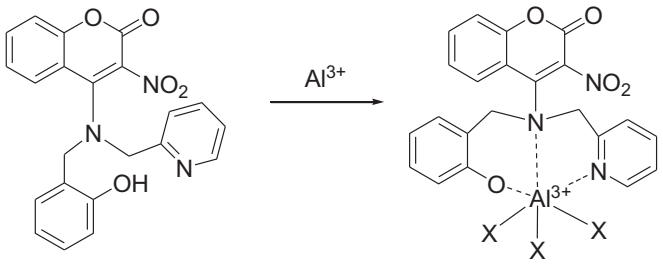


Fig. 5. CPAP ^{1}H NMR spectra with and without $Al(NO_3)_3$ in CD_3OD: (a) CPAP; (b) CPAP with 1 equiv of Al^{3+}.

(15 mL). After 1 h, $NaBH_4$ (5.1 mmol, 0.1929 g) was added, and the reaction solution was cooled in an ice bath. After the solution was stirred for 2 h, the solvent was removed under reduced pressure to produce a brown oil. The brown oil was dissolved in methylene chloride, and the solution was washed twice with water and then with saturated brine solution. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. The resulting oil (3.4 mmol, 0.7285 g), 4-chloro-3-nitro coumarin (3 mmol, 0.6768 g), and triethylamine (4 mmol, 560 μL) were dissolved in acetonitrile (15 mL). After stirring for 2 h, the solvent was evaporated. The yellow product was recrystallized from diethyl ether. Yield : 0.847 g (71%). IR(KBr) : 2990(br), 1721(s), 1598(s), 1546(s), 1514(m), 1488(w), 1404(m), 1278(w), 1153(m), 1099(s), 1050(s), 908(s), 756(s), 607(m), 529(m). ^{1}H NMR (methanol-d_4, 400 MHz) δ: 8.55 (d, 1H), 8.04 (d, 1H), 7.92 (t, 1H), 7.72 (t, 1H), 7.54 (d, 1H), 7.44 (m, 3H), 7.20 (t, 1H), 7.02 (d, 1H), 6.82 (m, 2H), 4.56 (s, 2H), 4.39 (s, 2H). Anal. calcd for $C_{22}H_{17}N_3O_5$ (403.39): C, 65.50; H, 4.25; N, 10.42%. Found: C, 65.23; H, 4.21; N, 10.75%. FAB MS m/z (M^+): calcd, 403.39; found, 403.39.

1.3. Fluorescence measurements

CPAP (6.06 mg, 0.015 mmol) was dissolved in methanol (3 mL), and 6 μL of the receptor (5 mM) was diluted in 2.994 mL methanol to make the final concentration of 10 μM. Then, $Al(NO_3)_3$ (75.03 mg, 0.2 mmol [20 mM]) was added to 3 mL of receptor solution (10 μM). After mixing, fluorescence spectra were obtained at room temperature.



Scheme 2. Proposed structure of a 1:1 complex of CPAP and Al^{3+}.

1.4. Job's plot

CPAP (6.06 mg, 0.015 mmol) was dissolved in methanol (3 mL). 24, 21.6, 19.2, 16.8, 14.4, 12, 9.6, 7.2, 4.8, and 2.4 μL of the receptor solution were taken and transferred to vials. Each vial was diluted with methanol to make a total volume of 2.976 mL. $Al(NO_3)_3$ (5.63 mg, 0.015 mmol) was dissolved in methanol (3 mL). The $Al(NO_3)_3$ solution were added to each diluted receptor solution. Each vial had a total volume of 3 mL. After voltexing the vials for a few minutes, UV–vis and fluorescence were taken at room temperature.

1.5. Competiton experiments with other metal ions

$M(NO_3)_3$ (M = Al, Cr, Fe, Ga, In, 0.2 mmol), $M(NO_3)_2$ (M = Mg, Ca, Mn, Co, Ni, Cu, Zn, Cd, Hg, Pb, 0.2 mmol) or MNO_3 (M = Na, K, Ag, 0.2 mmol) were dissolved in methanol (3 mL), respectively. Each metal solution (20 mM) was taken and added into 3 mL of receptor solution (10 μM) prepared above. After mixing them, fluorescence spectra were taken at room temperature. Then, 30 μL of Al solution (20 mM) was added into the mixed solution of each metal ion and receptor to 20 equivalents. After mixing them, fluorescene spectra were taken at room temperature. In the same method, 60 μL and 150 μL of the stock Al soluton (20 mM) were taken, respectively, and added into the mixed solution of each metal ion and receptor to make 40 and 100 equivalent, respectively. After mixing them, fluorescence spectra were taken at room temperature.

1.6. UV–vis measurements

$Al(NO_3)_3$ (5.63 mg, 0.015 mmol) was dissolved in methanol (3 mL). 0.6–12 μL of Al^{3+} solution (20 mM) was transferred to each receptor solution (40 μM) prepared above. After mixing them, UV absorption spectra were taken at room temperature.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2012.10.008.

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