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A Highly Selective Fluorescent Chemosensor for Pb^{2+}

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Abstract: A new fluorescent sensor based on rhodamine B for Pb^{2+} was synthesized. The new fluorescent sensor showed an extreme selectivity for Pb^{2+} over other metal ions examined in acetonitrile. Upon the addition of Pb^{2+}, an overall emission change of 100-fold was observed, and the selectivity was calculated to be 200 times that of Zn^{2+}. The signal transduction occurs via of reversible CHEF (chelation-enhanced fluorescence) with this inherent quenching metal ion.

Introduction

The selective binding of chemical species upon molecular recognition can lead to large perturbations in the host environment, particularly when the guest is ionic. Since fluoroionophores can provide chemical information on the ion concentrations, they are important subjects in metal ion analysis.[1]

Among the metal ions, Pb^{2+} is one of the important targets because of the adverse health effects of lead exposure, particularly in children.[2] Even though considerable efforts have been devoted to developing fluorescent chemosensors for various metal ions over the last few decades,[1] there have been relatively few reports on Pb^{2+}-selective fluorescent chemosensors. Lu et al. utilized catalytic DNAs as a unique class of biosensors for Pb^{2+}.[3] Ma and co-workers reported a fluorescent probe based on the Pb^{2+}-catalyzed hydrolysis of phosphodiester.[4] Even though the above results were excellent examples of Pb^{2+}-selective fluorescent sensors, the probes were hydrolyzed by Pb^{2+} in both cases, and these are irreversible processes. For a real-time fluorescent sensor, Chen et al. used a ketoaminocoumarine derivative bearing a azacrown binding site as a new ratiometric fluorescent sensor for Pb^{2+}.[5] Godwin et al. also reported a new ratiometric fluorescent sensor for Pb^{2+} based on the dansyl-tetrapeptide framework.[6] On the other hand, Czarnik and co-workers reported a *N*-methyl-9-anthrylthiohydroxamic acid as a new fluorescent chemosensor for Pb^{2+}.[7] In addition, some of fluorophore-appended macrocycles have been reported.[8]

Rhodamine dyes have been used extensively for conjugation with biomolecules, owing to their excellent fluorescence properties. A few rhodamine B derivatives have also been used as fluorescent chemosensors for metal ions.[9,10] Czarnik et al. reported that rhodamine B hydrazide could be used as a fluorescent chemodosimeter for Cu^{2+}.[9]

We report a new fluorescent sensor **1** based on rhodamine B for Pb^{2+}. The structure of compound **1** was confirmed by X-ray crystallography in addition to NMR and mass data. The new fluorescent sensor **1** displayed an extreme selectivity for Pb^{2+} in acetonitrile compared with other metal ions examined. The signal transduction occurs via of reversible CHEF (chelation-enhanced fluorescence) with this inherent quenching metal ion. ^{1}H NMR, ^{13}C NMR, IR, and the electrospary ionization (ESI) mass data were used to explain the binding mode of **1** with metal ions.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification.

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Flash chromatography was carried out on silica gel 60 (230−400 mesh ASTM; Merck). Thin-layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with a thickness of 1 mm.

Melting points were measured using a Buchi 530 melting point apparatus, and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 or Varian 500. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/vis Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu). Electrospray ionization mass (ESI-MS) spectra were performed on a Thermo Finnigan (San Jose, CA) LCQ Advantage MAX quadrupole ion trap instrument with samples of metal complexes.

1 (RBDPA-1). A solution of rhodamine B base (1.0 g, 2.3 mmol) in 1,2 dichloromethane (12 mL) was stirred, and phosphorus oxychloride (0.6 mL) was added dropwise over 5 min. The solution was refluxed for 4 h. The reaction mixture was cooled and evaporated in vacuo to give rhodamine B acid chloride, which was not purified but confirmed with the reported ^{1}H NMR.[9]

The crude acid chloride was dissolved in acetonitrile (125 mL) and added dropwise over 5 h to a solution of (2-aminoethyl)bis(2-pyridylmethyl)amine[11] (1.1 g, 4.6 mmol) in acetonitrile (50 mL) at room temperature. The reaction mixture was then refluxed for 1 h. After the solvent was evaporated under reduced pressure, the crude product was purified by column chromatography ($CHCl_3$:MeOH = 9:1, v/v) to give the 643 mg of **1** (yield; 42%): mp 56−59 °C.; ^{1}H NMR ($CDCl_3$) δ 8.44 (d, 2H, J = 4.8 Hz), 7.87 (t, 1H, J = 2.9 Hz), 7.57 (t, 1H, J = 7.7 Hz), 7.41 (m, 4H), 7.06 (m, 3H), 6.35 (m, 4H), 6.20 (dd, 2H, J = 8.8 and 2.6 Hz), 3.69 (s, 4H), 3.34 (m, 10H), 2.38 (t, 2H, J = 7.9 Hz), 1.16 (t, 2H, J = 7.0 Hz);^{13}C NMR ($CDCl_3$) δ 168.4, 159.3, 153.9, 153.6, 149.8, 137.5, 132.7, 131.6, 129.2, 128.4, 126.3, 124.2, 123.4, 123.1, 122.3, 108.6, 108.4, 105.8, 98.3, 65.3, 60.1, 52.6, 44.8, 38.6, 14.5; HRMS (FAB) m/z = 667.3755 $(M + H)^+$, calcd for $C_{42}H_{47}N_6O_2$ = 667.3761.

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, excitation wavelength was 510 nm. Both excitation and emission slit widths were 5 nm.

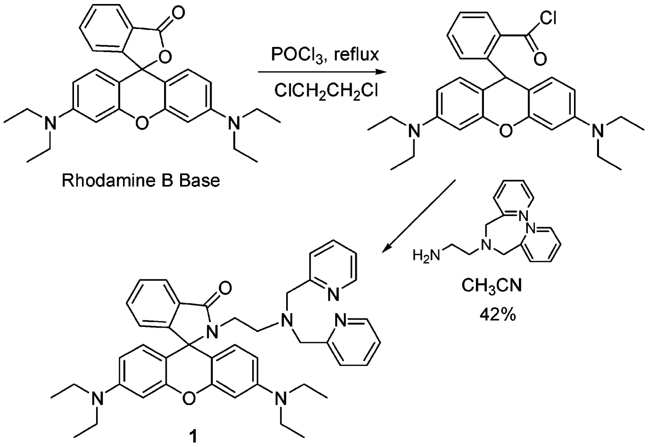
Electrospray Ionization Mass (ESI-MS). The electrospray ionization mass (ESI-MS) spectrum of **1** was obtained by direct introduction of the solution of samples (10 μM) in CH_3CN into the source at 25 μL/min using a syringe pump. The spray voltage of the spectrometer was set at 5 kV, and the capillary temperature, at 150 °C.

X-ray Data. All the X-ray data were collected on a SMART APEX CCD equipped with a Mo X-ray tube at the ambient temperature. Crystal data of **1**: *monoclinic*, $P2_1$ (No. 4), $Z = 2$, $a = 11.896(3)$ Å, $b = 11.812(3)$ Å, and $c = 12.988(3)$ Å, $\beta = 90.592(4)°$, $V = 1824.8(7)$ Å^3, $\mu = 0.076$ mm^{-1}, $d_{calc} = 1.214$ g/cm^3, R1 = 5.81% and wR2 = 11.62% for 1946 unique reflections and 451 variables. The structure solution and refinement of the data were handled with the SHELXS-86 and SHELXL-97 programs.

Results and Discussion

(2-Aminoethyl)bis(2-pyridylmethyl)amine was synthesized according to the published procedure.[11] **1** was synthesized by treating rhodamine B with $POCl_3$,[4a] which was followed without purification by (2-aminoethyl)bis(2-pyridylmethyl)amine. After column chromatography using $CHCl_3$:MeOH (9:1, v/v), **1** was obtained in a 42% yield (Scheme 1).

Scheme 1. Synthesis of **1**



A single crystal of **1** was grown from a $CHCl_3/CH_3CN$ solution and was characterized using X-ray crystallography (Figure 1). The crystal structure clearly represents the unique spirolactam-ring formation. Even though the crystal and molecular structures of several fluoran-based color formers by single-crystal X-ray diffraction analysis have been reported,[12] as far as we are aware, the X-ray structure of the rhodamine B derivative bearing the lactam moiety has not been reported.

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+} ions were used to evaluate the metal ion binding properties of **1**. Stock solutions (1 mM) of the perchlorate salts of the metal ions in acetonitrile were prepared. A stock solution of the host (0.1 mM) in acetonitrile was also prepared. The test solutions were prepared by placing 4−40 μL of the probe stock solution into a test tube, adding the appropriate aliquot of each metal stock, and diluting the solution to 4 mL with acetonitrile. For all the measurements, excitation wavelength was 510 nm, and both the excitation and emission slit widths were 5 nm.

All titration studies were conducted using a 1 μM concentration of **1**. Using these metal ions (100 equiv), **1** showed a large CHEF effect *only* with Pb^{2+}, even though there was a relatively small CHEQ effect with Cu^{2+} and Zn^{2+} (Figure 2). Figure 3 shows the fluorescent emission changes of **1** upon the addition of Pb^{2+} ions. As shown in Figures 2 and 3, the addition of Pb^{2+} and Zn^{2+} caused a significant red shift (~30 nm). From the fluorescent titrations, the association constants for Pb^{2+} and Zn^{2+} were calculated to be 195000 and 900 M^{-1}, respectively (errors <10%).[13] The selectivity for Pb^{2+} over Zn^{2+} was more than over 200 times. An overall emission change of 100-fold was observed for Pb^{2+}. Furthermore, there was no significant change in the association constant for Pb^{2+} when excess Zn^{2+} (0.1 mM) was present. On the other hand, upon the addition of Cu^{2+}, **1** displayed a CHEF effect without a red shift. As shown in Figure 2, Li^+ also caused a similar but smaller fluorescence change to Cu^{2+}.

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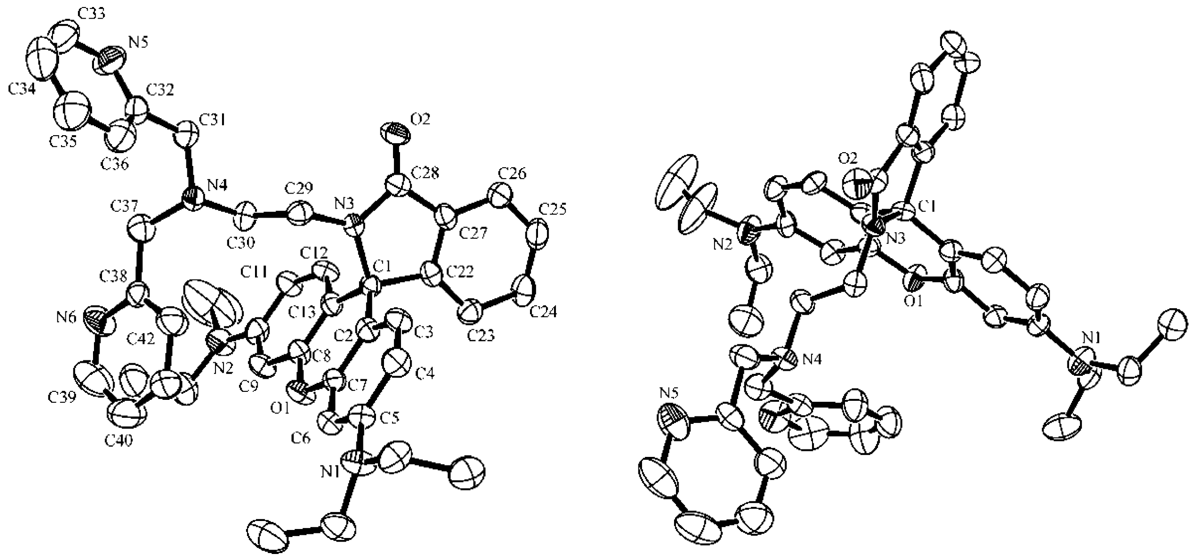

Figure 1. Side and top view of X-ray crystal structure of **1**.

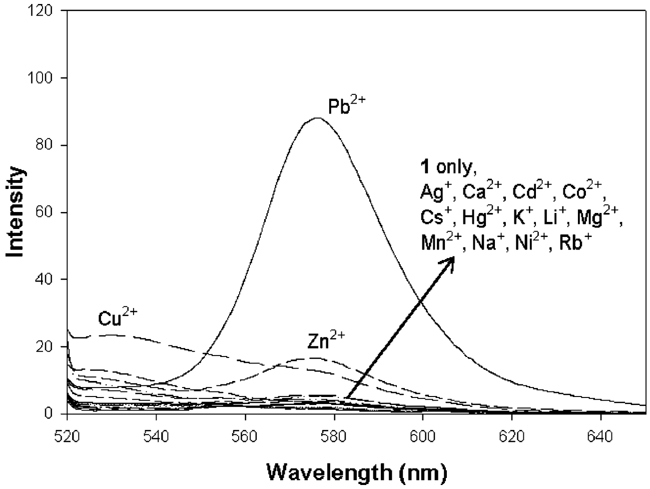

Figure 2. Fluorescent emission changes of **1** (1 μM) upon addition of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Rb^+, and Zn^{2+} (100 equiv) in acetonitrile (excitation at 510 nm).

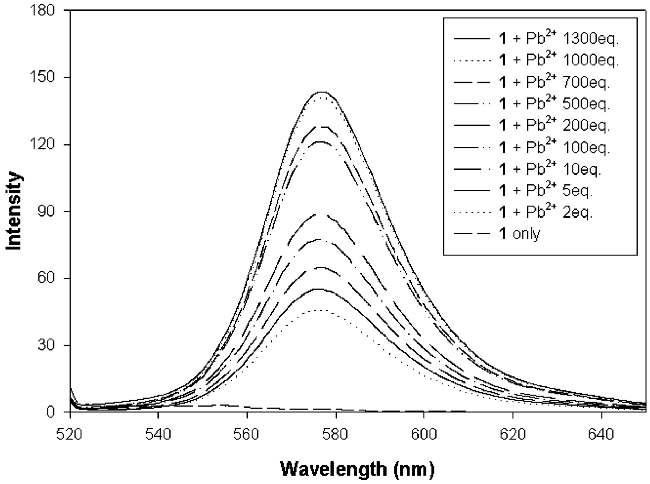

Figure 3. Fluorescent titrations of **1** (1 μM) upon addition of Pb^{2+} in acetonitrile (excitation at 510 nm).

There was also a large enhancement (~120-fold) in the UV absorption ($\lambda_{max} = 553$ nm) of **1** upon the addition of Pb^{2+}, as shown in Figure 4. The fluorescence quantum yield was calculated as 52% by using perylene as a reference.[14] **1** displayed a similar UV enhancement (~20-fold) when Zn^{2+} was added to acetonitrile. On the other hand, when Cu^{2+} was added, the **1** did not display a new peak around 550 nm, and only broad absorption increase was observed around 450 nm (see Supporting Information).

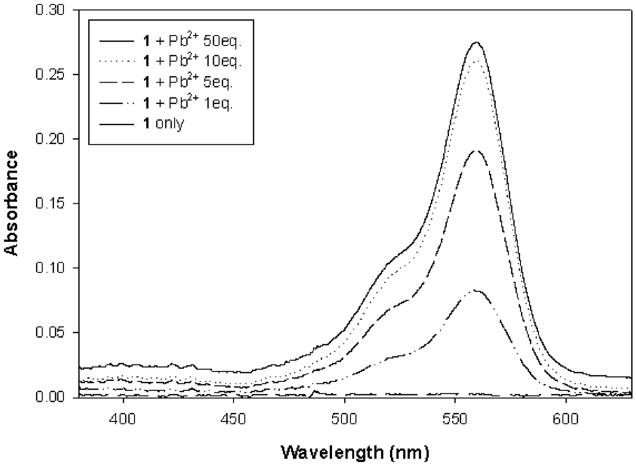

Figure 4. UV absorption changes of **1** (0.1 mM) upon addition of Pb^{2+} in acetonitrile.

Figure 5 shows the partial ^{1}H NMR spectra of **1** upon the addition of $Zn(ClO_4)_2$ (2 equiv). The NMR peaks were assigned on the basis of the ^{1}H$-$^{1}H COSY spectrum (see Supporting Information).

For example, upon the addition of 2 equiv of Zn^{2+}, the H_a and H_c on the pyridine ring in **1** showed relatively large downfield shifts (H_a, $\Delta\delta = 0.44$ ppm; H_c, $\Delta\delta = 0.54$ ppm).

The carbonyl carbon peak in the ^{13}C NMR spectra moved from 167.5 to 171.5 ppm in CD_3CN:$CDCl_3$ (9:1, v/v) when 1.2 equiv of $Zn(ClO_4)_2$ was added (see Supporting Information). The cleavage of the lactone ring in the fluoran-based dye was reported and explained on the basis of the changes in the ^{13}C NMR spectrum.[10a,14] In the case of the fluoran-based dye, the spiro carbon in the lactone ring moiety appeared at approximately δ 75$-$85 ppm and moved to δ ~160 ppm in the

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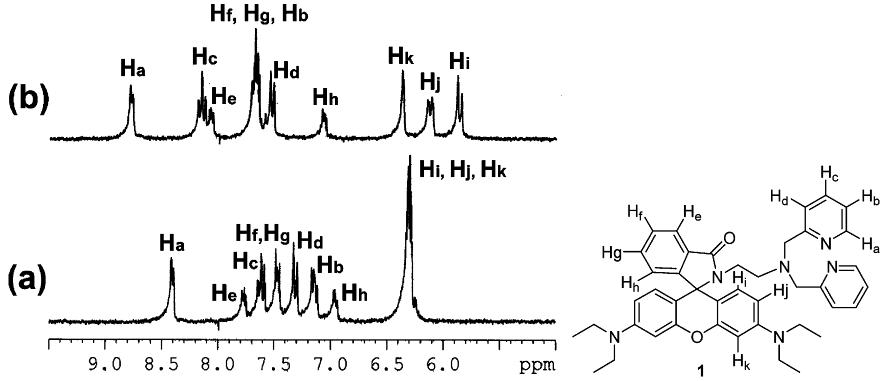

Figure 5. Partial ^{1}H NMR (250 MHz) of **1** (2 mM) in CD_3CN. (a) **1** only; (b) **1** + 2 equiv of zinc(II) perchlorate.

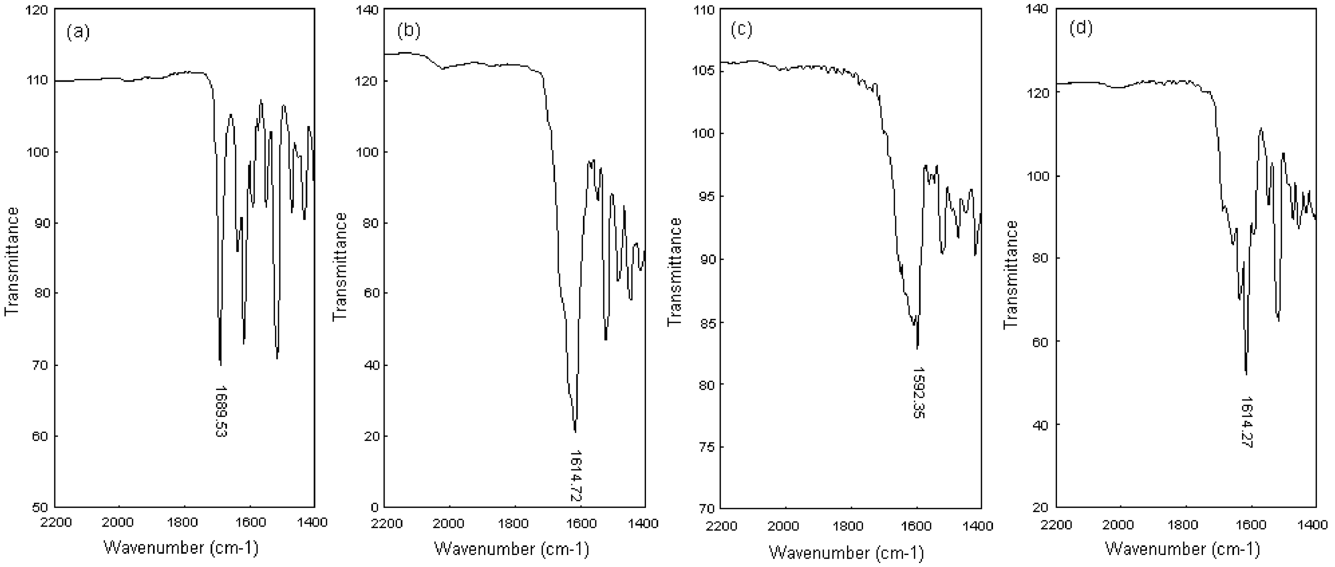

Figure 6. Infrared spectra of **1** (a), **1** with $Zn(ClO_4)_2$ (b), **1** with $Pb(ClO_4)_2$ (c), and **1** with $Cu(ClO_4)_2$ (d).

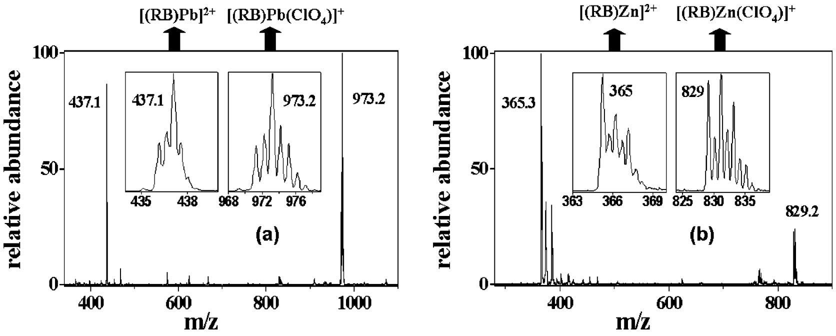

Figure 7. ESI mass spectra of **1** with $Pb(ClO_4)_2$ (a) and **1** with $Zn(ClO_4)_2$ (b).

lactone ring-opened structure.[10a,15] The spiro carbon in **1** appeared at δ 64.7 ppm in CD_3CN:$CDCl_3$ (9:1, v/v). When 1.2 equiv of $Zn(ClO_4)_2$ was added, the spiro carbon at δ 64.7 ppm disappeared and moved to δ 150 ppm. The addition of $Pb(ClO_4)_2$ caused a broadening of some peaks in the ^{1}H and ^{13}C NMR spectra. However, the disappearance of the spiro carbon at δ 64.7 ppm was also confirmed. On the other hand, when 2.0 equiv of $LiClO_4$ was added, the spiro carbon peak was clearly observed at δ 66.6 ppm, and the carbonyl carbon peak moved from 167.5 to 170.5 ppm.

Figure 6 shows the partial IR spectra of **1** (50 mM) with 1 equiv of $Zn(ClO_4)_2$, $Pb(ClO_4)_2$, and $Cu(ClO_4)_2$ in CH_3CN−$CHCl_3$ (4:1, v/v). The peak at 1689.53, which corresponds to the characteristic amide carbonyl absorption, was shifted to 1592.35 (with Zn^{2+}), 1614.72 (with Pb^{2+}), 1614.27 (with Cu^{2+}), and 1614.72 (with Li^+, see Supporting Information). These results also support that a strong binding participation of the carbonyl group occurs with metal ions.

In the ESI mass spectrum of **1**, a peak at *m*/*z* 973.2 (calculated value, 973.3) corresponding to $[\mathbf{1} + Pb(ClO_4)]^+$ was clearly observed upon the addition of lead(II) perchlorate (Figure 7). In addition, a peak at *m*/*z* 437.1 (calculated value, 437.2) corresponding to $[\mathbf{1} + Pb]^{2+}$ was observed. Upon the addition of zinc(II) perchlorate, two distinct peaks at *m*/*z* 829.2 and 365.3 were observed, which correspond to $[\mathbf{1} + Zn(ClO_4)]^+$ and [**1**

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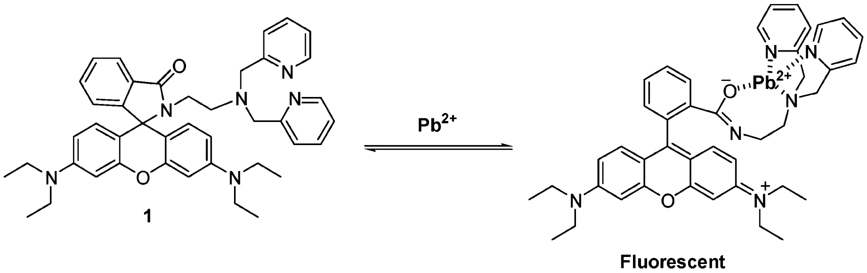
Scheme 2. Proposed Mechanism for the Fluorescent Changes of **1** upon the Addition of Pb^{2+}



$+ Zn]^{2+}$, respectively. In a similar way, two distinct peaks at *m/z* 828.1 and 364.5 were observed with Cu^{2+}, which correspond to [**1** + $Cu(ClO_4)]^+$ and [**1** + $Cu]^{2+}$, respectively (see Supporting Information). When cupper nitrate was added, a peak at *m/z* 791.2 (calculated value, 791.4) corresponding to [**1** + $Cu(NO_3)]^+$ was observed (see Supporting Information).

The proposed mechanism for these fluorescent changes is explained in Scheme 2. Upon the addition of Pb^{2+} to a colorless solution of**1** in acetonitrile, both a pink color and the fluorescence characteristics of rhodamine B appear. Because both disappear upon the addition of excess cyclen or ethylenediamine, it is believed that this process is reversible. Also, the ^1H NMR of the extracted product was identical to that of **1**. The ^{13}C NMR data also supports this spiro ring-opening mechanism. On the other hand, the addition of Cu^{2+} as well as some other metal ions caused relatively small CHEF effects without a red shift. This was also a reversible process since there was no change on TLC, and the characteristic fluorescent emission was recovered upon the addition of either cyclen or ethylenediamine. Furthermore, a chloroform and EDTA aqueous solution was added to the **1**·Cu^{2+}, and the ^1H NMR of the extracted product was identical to that of **1**. As shown in Figure 2, the addition of Cu^{2+} and Li^+ to **1** caused similar intensity changes. From the fact that the spiro carbon peak was still observed when 2.0 equiv of $LiClO_4$ was added, it may be possible that the spiro ring-opening mechanism did not occur upon the addition of Cu^{2+}. Since the compound itself did not exhibit any fluorescence, the small CHEF effects with Cu^{2+} and Li^+ can be also explained by the blocking of the PET (photoinduced electron transfer) process.

Conclusions

In conclusion, a new fluorescent sensor based on rhodamine B for Pb^{2+} was synthesized. The new fluorescent sensor displayed an extreme selectivity for Pb^{2+} over the other metal ions examined in acetonitrile. An overall emission change of 100-fold was observed upon the addition of Pb^{2+}, and the selectivity for Pb^{2+} was calculated to be 200 times that of Zn^{2+}. Variations in the ligands on the rhodamine B may provide various fluorescent chemosensors, which are selective for different metal ions.

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Supporting Information Available: NMR spectra, ESI mass spectra and X-ray crystallographic data (CIF) of **1** and its complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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