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A selective fluorescent sensor for Pb(II) in water

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Abstract—A new fluorescent sensor (**1**) containing bis(2-pyridylmethyl)amine group as a binding moiety for Pb^{2+} was developed. Compound **1** shows selective response to Pb^{2+} over other metal ions in pH 7.0 HEPES buffer solution. The fluorescence intensity enhancement was ascribed to the complex formation between Pb^{2+} and **1** which blocked the photo-induced electron transfer process.

A wide variety of symptoms such as digestive, neurologic, cardiac diseases, and mental retardation have been attributed to lead poisoning.[1] Thus, we are challenged to develop the analysis of trace amounts of lead in water. Currently, lead levels are determined by the atomic absorption spectrophotometer and the ICP emission spectrophotometer.[2] However, fluorescence methods will provide many advantages such as high sensitivity, convenience, rapid on-site evaluation and low cost. Although many effective fluorescent sensors have been successfully developed for sensing alkali and alkaline earth cations,[3] there are a few examples of sensors for heavy metal ions.[4] Many heavy metal ions are known as fluorescence quenchers via enhanced spin–orbital coupling,[5] energy or electron transfer.[6] Therefore, the construction of a turn-on fluorescent sensor that is selective and sensitive to lead ions over other heavy metal ions has attracted much attention. Recently, considerable efforts have been undertaken to develop fluorescent sensors for lead ions,[1,7] however, most of them can only work in organic media.[1b,7b,d] A few examples can be performed in aqueous media by the aid of surfactants,[7a,e,i] multi-peptide-based self-assembled biomolecular sensor[7f] or nano-material such as gold nanoparticles assembled by DNAzymes.[8] Since bis(hydroxyldistyryl)pyrazine (**2**) is an excellent fluorophore which emits green fluorescence,[9] and the bis-(2-pyridylmethyl)amine (Dpa) group provides a good binding site for lead ions,[10] we were able to develop a single agent fluorescent sensor (**1**) for lead ions by the combination of these two moieties. Compound **1** containing four bis(2-pyridylmethyl)amine (Dpa) groups exhibits a highly selective and sensitive response to lead ions over other heavy metal ions in pH 7.0 aqueous media, and the emission intensity enhances with respect to the metal ion-free state. Compounds **2** and **3** were used as control compounds.

Compounds **1**–**3** were prepared by Knoevenagel condensation reactions between 2,5-di(diethyoxyphosphorylmethyl)pyrazine or 5-methylpyrazin-2-yl-methylphosphonic acid diethyl ester and benzaldehyde derivatives as depicted in Scheme 1. All compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, and mass spectral data.[11] Binding abilities of **1** toward various metal ions (perchlorate salts) were studied in an aqueous solution of 10 mM HEPES buffer (pH 7.0, HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) at 25 °C.

Figure 1 shows the fluorescence titration of **1** with Pb^{2+}. Addition of a 20-fold Pb^{2+} results in a 35 times enhancement of fluorescence intensity with respect to the metal ion-free state. A linear response as a function of Pb^{2+} concentration was obtained ranging between 1.9×10^{-7} and 6.0×10^{-6} M (shown in inset of Fig. 1). The regression equation is: $I_F = 2.4188 + 4.7728 \times 10^7 \times C_{Pb^{2+}}$ (M). The detection limit, calculated as three times of the standard deviation of the background noise, was found to be 1.9×10^{-8} M (3.9 μg L^{-1}) which is lower than the maximal permitted amount of lead ion (10 μg L^{-1}) in drinking water.[1a] The method is highly

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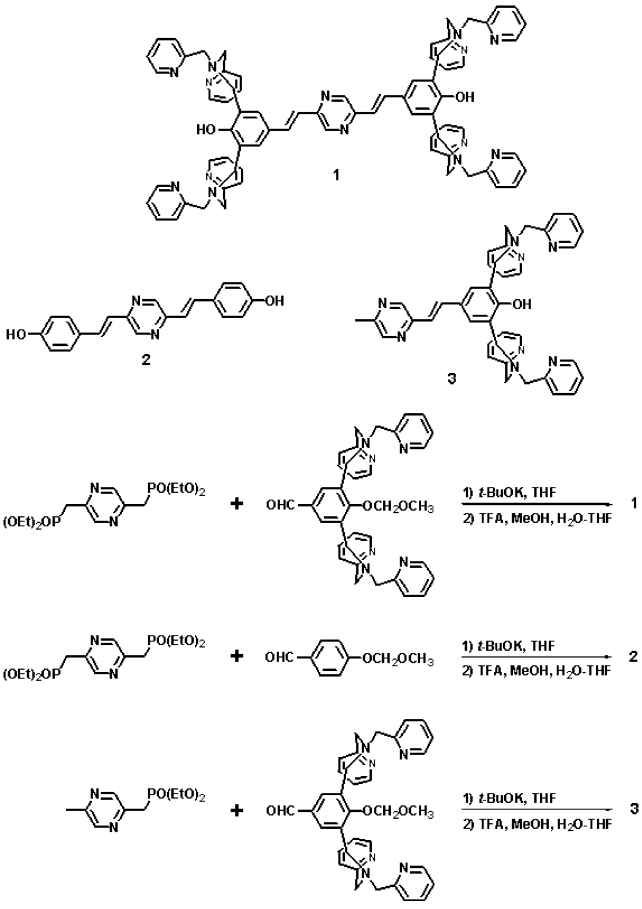

Scheme 1. Synthesis of compounds **1**–**3**. Detailed synthesis and spectral data are shown in Supplementary data.

promising for its applicability in the routine determination of trace lead ions in environmental and biological samples. The emission wavelength upon Pb^{2+} binding did not exhibit any detectable change, whilst the enhancement of emission intensity presumably resulted from the inhibition of the photo-induced intramolecular electron transfer (PET) process by metal ion complex formation.

Fluorescence quantum yields were measured to be 0.04 for **1**, 1.01 for **2**, and 0.11 for **3**, respectively, in anhydrous ethanol by using fluorescein as standard.[12] This

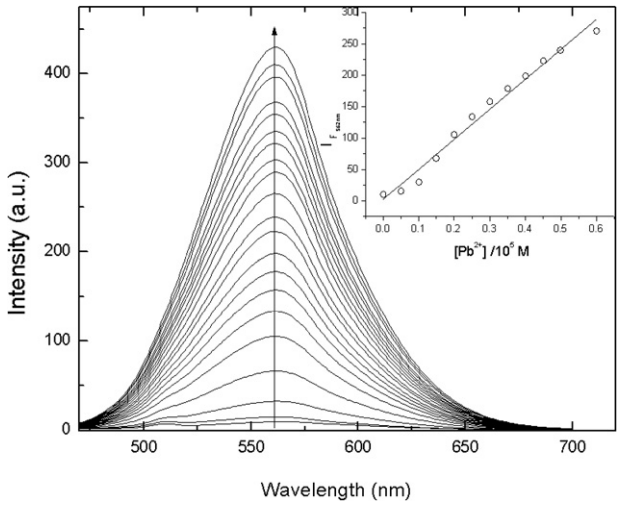

Figure 1. Fluorescence spectra changes of **1** (1.0×10^{-6} M) upon addition of increasing amount of Pb^{2+} in 10 mM HEPES buffer (pH 7.0, 1% MeCN (v/v)). Excitation wavelength was set at 435 nm. The arrow indicates the direction of concentration increase, $[Pb^{2+}] = 0$, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0×10^{-6} M. Inset shows linear relationship between fluorescence intensity and Pb^{2+}'s concentration.

indicates that before complexation, Dpa groups in **1** and **3** caused fluorescence quenching, due to the PET process by unpaired electrons of nitrogens in Dpa moieties. It is concluded that the lead ion complex (**1**-Pb^{2+}) formation blocks the PET process and results in an emission enhancement. UV–vis titration also exhibited a similar trend (Fig. S1). The maximum absorption wavelength of **1** is centered at 435 nm in pH 7.0 aqueous buffer solution. Upon addition of Pb^{2+} the absorbance at 435 nm increased, but the peak position did not change along with the peak-width narrowing. It means that binding interaction between **1** and lead ion results in conformation changes of **1**. Job's plot reveals a 1:4 complex (**1**:Pb^{2+}) formation (Fig. S2).

The selectivity of **1** over other metal ions was evaluated. The experimental results show that a 1000-fold excess of Li^+, Na^+, K^+, a 100-fold of Ag^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Cd^{2+}, Zn^{2+}, a 50-fold of Mn^{2+}, a 0.5-fold of Hg^{2+} existence (in each case, compared with Pb^{2+})

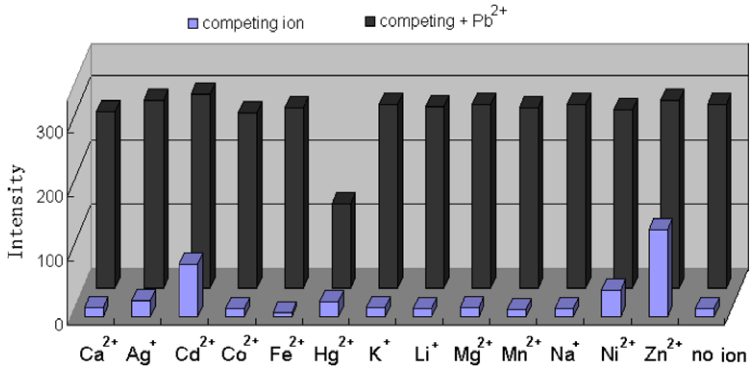

Figure 2. The fluorescence intensity profile of **1** (1.0×10^{-6} M) in 10 mM HEPES buffer (pH 7.0, 1% MeCN (v/v)) in the presence of selected metal ions (1.0×10^{-5} M). Excitation was monitored at 435 nm and emission was monitored at 562 nm.

results in less than ±10% fluorescence intensity changes of **1**-Pb^{2+}.[13] Zn^{2+} and Cd^{2+} show much weaker binding affinity to **1**. Thus the apparent fluorescence intensity does not change much upon addition of a 100-fold Zn^{2+} and Cd^{2+}. Also, the existence of a 1000-fold of anions such as Cl^-, Br^-, F^-, NO_3^-, ClO_4^-, CO_3^{2-} and SO_4^{2-} does not cause any interference. The interference of Cu^{2+} and Hg^{2+} could be eliminated by KCN in the pretreatment of the sample.

It has been known that Pb^{2+} targets both Ca^{2+}- and Zn^{2+}-binding sites in vivo[14] and accurate Pb^{2+} analysis is often disturbed by the presence of Cd^{2+}, Hg^{2+}, Fe^{2+}, and Mn^{2+}.[1b] Therefore, the selective sensing ability of **1** for Pb^{2+} over other metal ions such as Ca^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+} and Zn^{2+} is particularly important. The selectivity of **1** for Pb^{2+} over other metal ions was investigated by the competition experiments. Figure 2 shows the fluorescence intensity of **1** in the presence of 10-fold excess of selected metal ions. It is obvious that **1** has a highly selective response to Pb^{2+}.

Fluorescence titrations of **1**, with Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} in acetonitrile, cause emission increases along with blue shifts. The selectivity of **1** for Pb^{2+} over other metal ions is much better in an aqueous solution than in an organic solvent.

The fluorescence titration of **3** with Pb^{2+} was also carried out. Compound **3** exhibits smaller emission increases upon cation binding in pH 7.0 aqueous solution and Job's plot shows a 1:2 (**3**:Pb^{2+}) binding ratio. A linear relationship exists between the concentration of Pb^{2+} and its fluorescence intensity in the range of $0–8.8 \times 10^{-6}$ M with a calibration equation, $I = 28.9488 + 8.2607 \times 10^6 C_{Pb}{}^{2+}$ (M). The detection limit was determined to be 6.42×10^{-7} M. The lower sensitivity was ascribed to the fewer number of binding sites.

Similar fluorescence and absorption titration experiments were also performed on **2** in DMSO and acetonitrile. Neither spectra profile nor fluorescence intensity change was observed in the presence of metal ions such as Pb^{2+}, Zn^{2+}, and Cd^{2+}, because **2** does not combine with metal ions owing to the absence of binding sites.

In summary, a highly selective fluorescent sensor (**1**) for lead ions in aqueous solution was developed based on the PET mechanism. Compound **1** provides a sensitive (a detection limit of 3.9 μg L^{-1} in water) and single agent fluorescence method for the assay of Pb^{2+} in pH 7.0 aqueous solution.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.10.060.

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11. Compound **1**: ^{1}H NMR (500 MHz, acetone-d_6): δ 3.87 (s, 8H), 3.91 (s, 16), 7.17 (s, 2H), 7.20 (s, 2H), 7.22–7.24 (m, 8H), 7.59 (d, 8H, $J = 7.8$ Hz), 7.64 (s, 4H), 7.71–7.74 (m, 8H), 8.54 (d, 8H, $J = 4.6$ Hz), 8.60 (s, 2H); ^{13}C NMR (500 MHz, acetone-d_6): δ 55.328, 60.373, 122.341, 123.034, 123.849, 125.769, 128.100, 129.572, 129.796, 134.841,

137.459, 143.900, 149.851, 150.209, 160.313; FAB-MS: *m/e* calcd for $C_{72}H_{68}N_{14}O_2$ [M+H$^+$] 1161.5650, found [M+H$^+$] 1161.5516. Compound **2**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.81 (d, $J = 8.5$ Hz, 4H), 7.15 (d, $J = 16$ Hz, 2H), 7.52 (d, $J = 8.5$ Hz, 4H), 7.64 (d, $J = 16$ Hz, 2H), 8.65 (s, 2H), 9.76 (s, 2H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 104.091, 115.699, 121.179, 127.274, 128.782, 133.244, 142.827, 148.645, 158.274; HRMS (FAB): *m/e* calcd for $C_{20}H_{16}N_2O_2$ [M+H$^+$] 317.1212, found [M+H$^+$] 317.1290. Compound **3**: ^{1}H NMR (300 MHz, acetone-d_6): δ 2.51 (s, 3H), 3.88 (s, 4H), 3.92 (s, 8), 7.20(s, 1H), 7.21 (s, 1H), 7.22–7.25 (m, 4H), 7.60 (t, 4H, $J = 7.8$ Hz), 7.70–7.73 (m, 6H), 8.53–8.55 (m, 6H),^{13}C NMR (500 MHz, acetone-d_6): δ 21.339, 55.305, 60.355, 122.215, 123.006, 123.819, 125.691, 127.982, 129.459, 134.628, 137.423, 143.100, 144.792, 149.717, 149.836, 152.195, 158.006, 160.305; LRMS (FAB): *m/e* calcd for $C_{39}H_{38}N_8O$ [M+H$^+$] 635.3, found [M+H$^+$] 635.0.

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