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A new rhodamine-based fluorescent chemosensor for transition metal cations synthesized by one-step facile condensation

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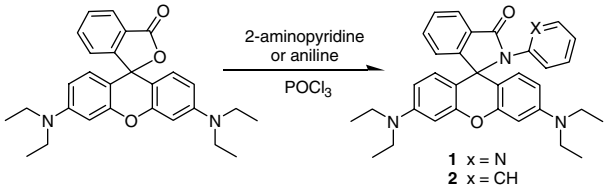
Abstract—A new rhodamine-based fluorescent chemosensor (**1**) for transition metal cations was synthesized by one-step facile condensation of rhodamine B and 2-aminopyridine. Without metal cations, **1** is colorless and nonfluorescent, whereas addition of metal cations (Fe^{3+}, Hg^{2+}, Pb^{2+}, and Fe^{2+}) leads to an obvious color change to pink and an appearance of orange fluorescence.

Design of artificial receptors for monitoring biologically and environmentally important ionic species in solution, especially heavy and transition metal (HTM) cations, is currently of great importance.[1] Much attention has been paid to the design of fluorescent chemosensors, because they allow nondestructive and quick detection of ionic species by a simple fluorescent enhancement (turn-on) or quenching (turn-off) response.[2] Many HTM cations, however, usually act as a fluorescent quencher; therefore, most of classical and already-reported chemosensors show turn-off response to HTM cations.[3] Such turn-off response is less sensitive than the turn-on response because of low signal-to-noise ratio.[2d] The design of turn-on type fluorescent chemosensors for HTM cations is, therefore, necessary for practical applications.[4]

Rhodamine is a molecule used extensively as a fluorescent labeling reagent and a dye laser source because of its excellent photophysical properties, such as long wavelength absorption and emission, high fluorescence quantum yield, large extinction coefficient, and high stability against light.[5] Recently, rhodamine-based fluorescent chemosensors for metal cations have received increasing interest.[6] The on/off fluorescence switching of these chemosensors is based on structure change of the rhodamine moiety between spirocyclic and open-ring forms. The spirocyclic (closed-ring) form is basically colorless and nonfluorescent (turn-off). In contrast, addition of metal cation allows the spirocycle to be opened (formation of open-ring form) via coordination with metal cation.[6] This allows appearance of a pink color and an orange fluorescence (turn-on). On the other hand, rhodamine-based fluorescent chemodosimeters have also been studied;[7] however, they produce the open-ring form by a metal-catalyzed irreversible chemical reaction.[7] The design of reusable fluorescent chemosensors is necessary for practical detection of HTM cations. However, to the best of our knowledge, there are only five reports of rhodamine-based fluorescent chemosensor.[6] These sensors require two step procedures for synthesis and their overall yields are relatively low (18–57%). New rhodamine-based fluorescent chemosensor synthesized with simple one-step procedure is therefore necessary.

Herein, we report a new rhodamine-based fluorescent chemosensor (**1**) containing a pyridine moiety, which is synthesized by one-step facile condensation (Scheme 1). We envisaged that the carbonyl oxygen and the pyridine nitrogen of **1** cooperatively coordinate with metal



Scheme 1. Synthesis of **1** and control compound **2**.

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cations, like *N*-(2-pyridyl)-acetamide,[8] by a six-membered chelate ring formation, which will result in the spirocycle opening of **1** and, hence, act as a new turn-on fluorescent sensor. We report here the detailed spirocycle opening mechanism of **1** by a six-membered chelate ring formation, enabling turn-on detection of HTM cations. This is the first rhodamine-based fluorescent chemosensor forming a six-membered chelate ring, whereas the early-reported sensors form five- or seven-membered ring.[6]

Probe **1** was synthesized by one-step condensation of rhodamine B and 2-aminopyridine with a catalytic amount of $POCl_3$ at 70 °C for 30 min (Scheme 1). Washing the resultant by an aqueous NaOH solution followed by recrystallization from acetone gave **1** with 60% yield. Control compound **2** was obtained in a similar manner followed by an additional purification by a silica gel column chromatograph ($CHCl_3/CH_3OH = 30/1$ v/v) with 20% yield. These were confirmed by ^1H and ^{13}C NMR and FAB-MS.[9,10] Absorption and fluorescence measurements were performed with respective cations as perchlorate (Li^+, Na^+, K^+, Fe^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) or nitrate (Fe^{3+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Ag^+) salts.

Figure 1b shows a change in the absorption spectra of **1** (10 μM) dissolved in acetonitrile with an amount of Fe^{3+}. The free probe **1** scarcely shows absorption at 400–650 nm, indicating that **1** exists as a spirocyclic form.[6,7] This is confirmed by a distinctive spirocycle carbon shift at 66.64 ppm in ^{13}C NMR spectrum of **1** (Fig. S2).[6] With <0.25 equiv of Fe^{3+}, absorption still scarcely appears. In contrast, with >0.25 equiv of Fe^{3+}, a distinctive absorption centered at 558 nm appears and the absorbance increases drastically, along with a clear color change from colorless to pink (Fig. 1a). Absorbance titration shows a typical sigmoidal curve (Fig. 1b, inset); saturation of the absorbance increase at >0.5 equiv of Fe^{3+} implies a 1:2 stoichiometry for coordination between Fe^{3+} and **1**. This is confirmed by the Job's plot (Fig. 1c). As shown in Figure 2, addition of Hg^{2+}, Pb^{2+}, Fe^{2+}, and Zn^{2+} cations (0.5 equiv) shows similar absorption spectra, whose intensity increase also shows sigmoidal curve (Fig. S7). In contrast, 10 equiv of others cations (Li^+, Na^+, K^+, Cu^{2+}, Cd^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Ag^+) show almost no increase in absorbance. These imply that **1** allows a naked-eye detection for Fe^{3+}, Hg^{2+}, Pb^{2+}, and Fe^{2+} cations.

As shown in Figure 3, **1** dissolved in acetonitrile (1 μM) is nonfluorescent ($\lambda_{ex} = 510$ nm). Addition of <2.5 equiv of Fe^{3+} still does not show fluorescence. However, with >2.5 equiv of Fe^{3+}, a distinctive emission centered at 580 nm appears and the intensity increases drastically upon addition of >3 equiv of Fe^{3+} (Fig. 3b, inset). Similar fluorescence enhancement is observed with Hg^{2+}, Pb^{2+}, Fe^{2+}, and Zn^{2+} cations (Figs. 4 and S8): the respective emission enhancements upon addition of 5 equiv of cations are 627-fold (Fe^{3+}), 602-fold (Hg^{2+}), 547-fold (Pb^{2+}), 438-fold (Fe^{2+}), 134-fold (Zn^{2+}). Probe **1**, therefore, acts as a potential turn-on fluorescent

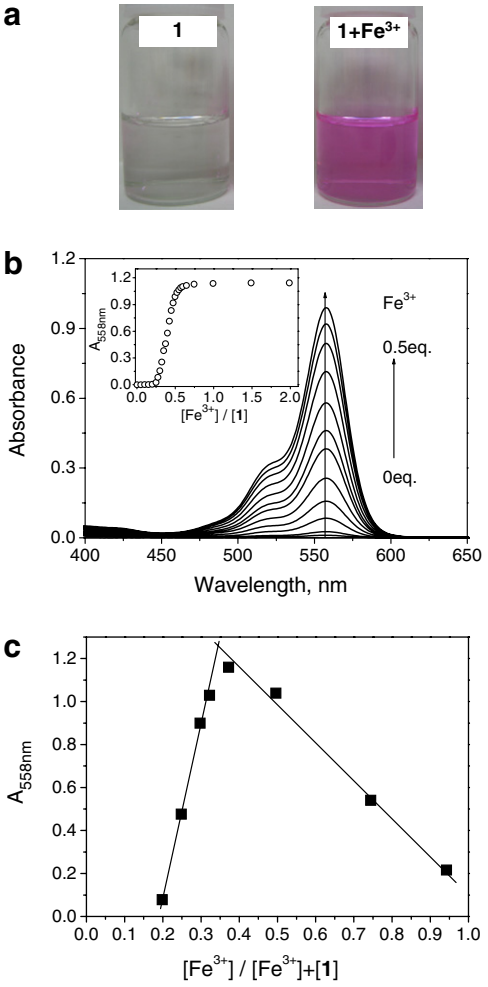


Figure 1. Changes in (a) color and (b) absorption spectra of **1** (10 μM) upon addition of Fe^{3+} in acetonitrile (the inset shows the change in absorbance at 558 nm). (c) Job's plot of Fe^{3+} versus **1** ($[Fe^{3+}] + [1] = 20$ μM).

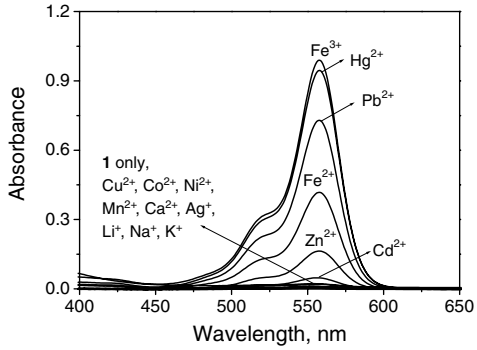


Figure 2. Absorption spectra of **1** (10 μM) obtained with 0.5 equiv of Fe^{3+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Zn^{2+}, and 10 equiv of Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+}, Ag^+, Li^+, Na^+, K^+.

chemosensor for these HTM cations. The fluorescence quantum yields of **1** with 5 equiv of Fe^{3+}, Hg^{2+}, and Pb^{2+} are estimated to be 0.20, 0.20 and 0.19, respectively, using rhodamine B as a standard ($\Phi = 0.69$).[11] In contrast, as shown in Figure 4, almost no emission

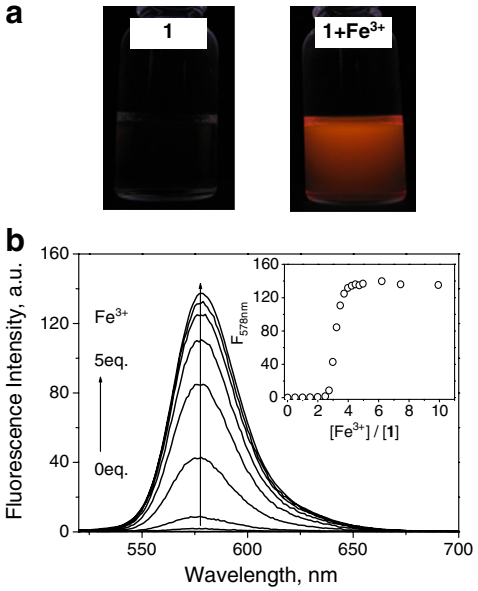


Figure 3. Changes in (a) fluorescence color and (b) spectra of **1** (1 μM) upon addition of Fe^{3+} in acetonitrile (the inset shows the change in the fluorescence intensity monitored at 578 nm).

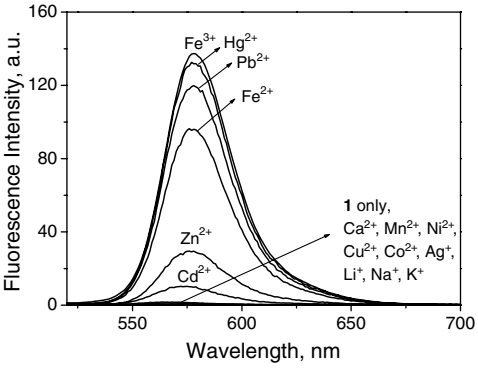


Figure 4. Fluorescence spectra of **1** (1 μM) obtained with 5 equiv of Fe^{3+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Zn^{2+}, and 100 equiv of Cd^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Ag^{+}, Li^{+}, Na^{+}, K^{+}.

enhancement is observed even upon addition of 100 equiv of other cations, except for Cd^{2+} (emission enhancement with 5 and 100 equiv of Cd^{2+} is 6- and 45-fold, respectively).

Upon addition of ethylenediamine to a solution containing **1** with Fe^{3+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, or Zn^{2+}, both pink color and orange fluorescence immediately disappear. This indicates a reversible coordination of **1** with metal cations and rules out the occurrence of an irreversible chemical reaction.[7] Considering the behaviors of fluorescence and absorption spectra, the turn-on response of **1** to HTM cations may be explained by the spirocycle open–close mechanism, as is also the case for rhodamine-based chemosensors:[6] the free probe **1** is the spirocyclic form, which is colorless and nonfluorescent, whereas the coordination of the amide carbonyl oxygen and the pyridine nitrogen to cations[8] leads to the spirocycle opening, resulting in an appearance of visible absorption and fluorescence.[6]

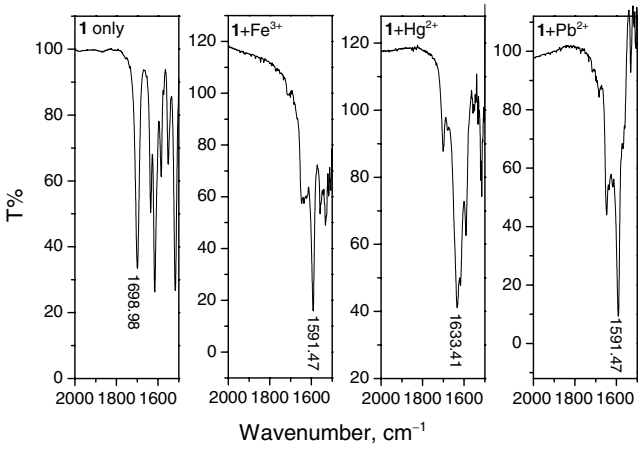


Figure 5. Infrared spectra of **1** (25 mM) measured in acetonitrile with or without 0.5 equiv of Fe^{3+}, Hg^{2+}, and Pb^{2+}, respectively.

To confirm the binding mechanism, IR spectrum of **1** (25 mM) was measured in acetonitrile (Figs. 5 and S9). The amide carbonyl absorption of **1** at 1698.98 cm^{-1} drastically shifts to lower frequency upon addition of 0.5 equiv of Fe^{3+} (1591.47 cm^{-1}), Hg^{2+} (1633.41 cm^{-1}), Pb^{2+} (1591.47 cm^{-1}), Fe^{2+} (1590.99 cm^{-1}), Zn^{2+} (1590.98 cm^{-1}), and Cd^{2+} (1634.38 cm^{-1}). These indicate that the amide carbonyl O of **1** is actually involved in the coordination with metal cations.[6a,8] ^1H NMR titration (CD_3CN, 303 K) was also performed for further confirmation. Most of the aromatic protons of **1** (5 mM) become broader and shift to downfield upon addition of 0.5 equiv of Fe^{3+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, and Zn^{2+} (Fig. S10). Without metal cations, $-CH_2CH_3$ and $-CH_2CH_3$ protons of **1** appear at 1.09 and 3.31 ppm, respectively. Upon addition of metal cations, new protons appear at upperfield along with the disappearance of the original protons (Fig. S11): Fe^{3+} (1.22 and 3.57 ppm), Hg^{2+} (1.16 and 3.48 ppm), Pb^{2+} (1.23 and 3.59 ppm), Fe^{2+} (1.23 and 3.59 ppm), and Zn^{2+} (1.21 and 3.54 ppm). This clearly suggests the formation of open-ring form of **1** via the metal cation coordination.[7b] Upon addition of 2 equiv of Cd^{2+} (Fig. S11), only weak protons appear at 1.22 and 3.57 ppm. This is indicative of weak binding of **1** with Cd^{2+}, which is consistent with weak absorption and fluorescence response to Cd^{2+} (Figs. 2 and 4).

For further clarification of the coordination behavior, ^1H NMR titration of **1** was carried out with Cd^{2+} (Fig. 6).[12] Upon Cd^{2+} addition, aromatic protons (H_b, H_c, and H_d) on the pyridine moiety of **1** display continuous downfield shift ($\Delta\delta = 0.14$, 0.05, and 0.13 ppm, with 2 equiv of Cd^{2+}), as is generally observed for coordination of pyridine-based ligands with metal cations.[6a,8,13–16] This is due to the decrease in electron density of the pyridine moiety upon coordination.[8c,16] This indicates that the pyridine N of **1** is involved in the metal cation coordination. The participation of the pyridine N to the coordination is confirmed with a

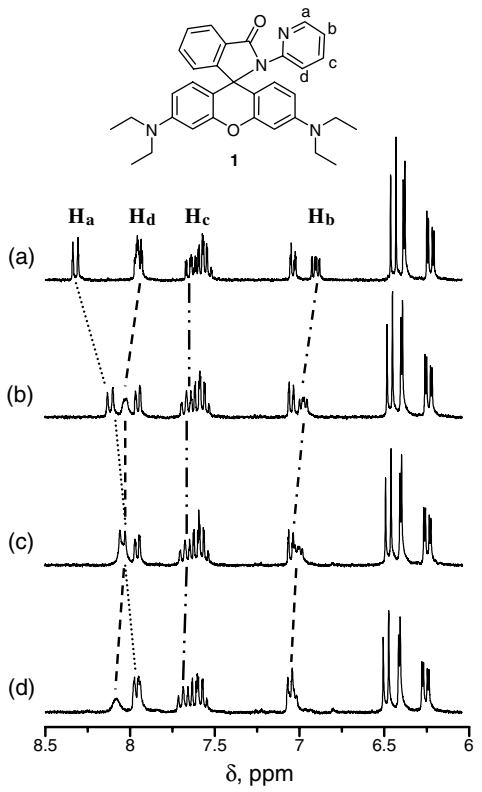
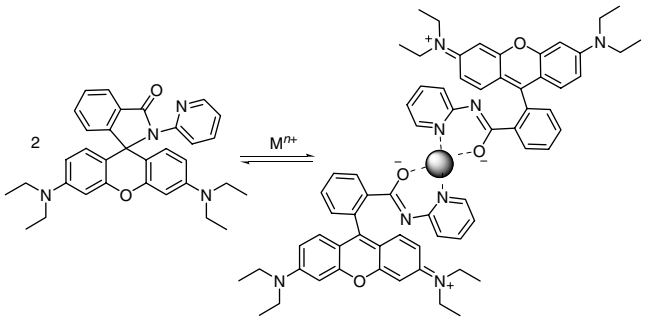


Figure 6. Partial ^{1}H NMR (270 MHz) spectra of **1** (5 mM) measured in CD_3CN (a) without metal cations and with (b) 0.5 equiv, (c) 1.0 equiv, (d) 2.0 equiv of Cd^{2+}.

control compound **2**, which contains a benzene moiety in place of pyridine. **2** shows only small changes in absorption and fluorescence spectra upon Fe^{3+} addition (Figs. S12 and S13). This means that, as expected, **2** scarcely produces the open-ring form due to the lack of the pyridine N binding site. These findings clearly indicate that the pyridine N of **1** is actually involved in the cooperative coordination to metal cations.

Considering the Job's plot result (Fig. 1c) and the above experimental evidence, coordination of metal cation with **1** forms a 1:2 complex (Scheme 2), where the two binding sites (amide carbonyl O and pyridine N) within each of **1** form a six-membered chelate ring with cation. The proposed structure is reinforced by ^{1}H NMR titration with Cd^{2+}. As shown in Figure 6, H_a on the pyridine ring of **1** shows continuous upperfield shift ($\Delta\delta = 0.37$ ppm, with 2 equiv of Cd^{2+}), while the other protons (H_b, H_c, and H_d) show downfield shift. This means that H_a proton is shielded by metal coordination, while the others are not.[8b,c,15,16] This is due to the anisotropic effect by ring currents from adjacent π electrons on another **1** moiety (Scheme 2) within the complex.[15,16] Such upperfield H_a proton shift (0.11–1.56 ppm) by metal coordination is also observed for several pyridine-containing ligands forming similar 1:2 complex,[16] where the orientation of the ligands sterically affects each other by metal coordination. This leads to anisotropic effect from the other pyridine ligand, resulting in upperfield proton shift. The upperfield H_a proton shift of **1** (Fig. 6), therefore, supports the formation of 1:2 complex with sterically hindered two **1** ligands (Scheme 2).



Scheme 2. Proposed binding structure of **1** to metal cation.

In conclusion, we have synthesized a new rhodamine-based fluorescent chemosensor, **1**, by one-step condensation. **1** exhibits a strong fluorescence enhancement upon addition of Fe^{3+}, Hg^{2+}, Pb^{2+}, and Fe^{2+} while showing almost no response to other cations. **1** may therefore be applicable as a rhodamine-based turn-on type fluorescent chemosensor. The obtained findings also indicate that various rhodamine-based chemosensors for HTM cations may easily be made by incorporating various ligand groups. The works are in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data (absorption, fluorescence, IR, ^{1}H and ^{13}C NMR spectra of **1** and **2** measured with and without metal cations) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.171.

References and notes

1. (a) Amendola, V.; Fabbrizzi, L.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Parodi, L.; Poggi, A. *Coord. Chem. Rev.* **1999**, *190–192*, 649–669; (b) Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *205*, 59–83; (c) Rurak, K. *Spectrochim. Acta A* **2001**, *57*, 2161–2195.
2. (a) *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington DC, 1993; (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566; (c) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40; (d) Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116–127.

3. For Fe^{3+}: (a) Palanché, T.; Marmolle, F.; Abdallah, M. A.; Shanzer, A.; Albrecht-Gary, A.-M. *J. Biol. Inorg. Chem.* **1999**, *4*, 188–198; (b) Liu, J.-M.; Zheng, Q.-Y.; Yang, J.-L.; Chen, C.-F.; Huang, Z.-T. *Tetrahedron Lett.* **2002**, *43*, 9209–9212; (c) Wolf, C.; Mei, X.; Rokadia, H. K. *Tetrahedron Lett.* **2004**, *45*, 7867–7871; (d) Ma, Y.; Luo, W.; Quinn, P. J.; Liu, Z.; Hider, R. C. *J. Med. Chem.* **2004**, *47*, 6349–6362; (e) Tumambac, G. E.; Rosencrance, C. M.; Wolf, C. *Tetrahedron* **2004**, *60*, 11293–11297; (f) Kikkeri, R.; Traboulsi, H.; Humbert, N.; Gumienna-Kontecka, E.; Arad-Yellin, R.; Melman, G.; Elhabiri, M.; Albrecht-Gary, A.-M.; Shanzer, A. *Inorg. Chem.* **2007**, *46*, 2485–2497; For Hg^{2+}: (a) Moon, S. Y.; Cha, N. R.; Kim, Y. H.; Chang, S.-K. *J. Org. Chem.* **2004**, *69*, 181–183; (b) Moon, S.-Y.; Youn, N. J.; Park, S. M.; Chang, S.-K. *J. Org. Chem.* **2005**, *70*, 2394–2397; (c) Yu, Y.; Lin, L.-R.; Yang, K.-B.; Zhong, X.; Huang, R.-B.; Zheng, L.-S. *Talanta* **2006**, *69*, 103–106; (d) Ha-Thi, M.-H.; Penhoat, M.; Michelet, V.; Leray, I. *Org. Lett.* **2007**, *9*, 1133–1136; For Pb^{2+}: (a) Kim, S. K.; Lee, S. H.; Lee, J. Y.; Lee, J. Y.; Bartsch, R. A.; Kim, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16499–16506; (b) Kim, I.-B.; Dunkhorst, A.; Gilbert, J.; Bunz, U. H. F. *Macromolecules* **2005**, *38*, 4560–4562; (c) Kavallieratos, K.; Rosenberg, J. M.; Chen, W.-Z.; Ren, T. *J. Am. Chem. Soc.* **2005**, *127*, 6514–6515.
4. For Fe^{3+}: (a) Hua, J.; Wang, Y.-G. *Chem. Lett.* **2005**, *34*, 98–99; (b) Bricks, J. L.; Kovalchuk, A.; Trieflinger, C.; Nofz, M.; Buschel, M.; Tolmachev, A. I.; Daub, J.; Rurack, K. *J. Am. Chem. Soc.* **2005**, *127*, 13522–13529; For Hg^{2+}: (a) Descalzo, A.; Martínez-Mañez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418–3419; (b) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270–14271; (c) Guo, X.-F.; Qian, X.-H.; Jia, L.-H. *J. Am. Chem. Soc.* **2004**, *126*, 2272–2273; (d) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300–4302; (e) Zhang, H.; Han, L.-F.; Zachariasse, K. A.; Jiang, Y.-B. *Org. Lett.* **2005**, *7*, 4217–4220; (f) Caballero, A.; Martínez, R.; Lloveras, V.; Ratera, I.; Vidal-Gancedo, J.; Wurst, K.; Tárraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666–15667; (g) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030–16031; (h) Wang, J.-B.; Qian, X.-H. *Org. Lett.* **2006**, *8*, 3721–3724; (i) Ou, S.-J.; Lin, Z.-H.; Duan, C.-Y.; Zhang, H.-T.; Bai, Z.-P. *Chem. Commun.* **2006**, 4392–4394; (j) Meng, X.-M.; Liu, L.; Hu, H.-Y.; Zhu, M.-Z.; Wang, M.-X.; Shi, J.; Guo, Q.-X. *Tetrahedron Lett.* **2006**, *47*, 7961–7964; (k) Avirah, R. R.; Jyothish, K.; Ramaiah, D. *Org. Lett.* **2007**, *9*, 121–124; For Pb^{2+}: (a) Deo, S.; Godwin, H. A. *J. Am. Chem. Soc.* **2000**, *122*, 174–175; (b) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466–10467; (c) Chen, C.-T.; Huang, W.-P. *J. Am. Chem. Soc.* **2002**, *124*, 6246–6247; (d) Sun, M.; Shangguan, D.-H.; Ma, H.-M.; Nie, L.-H.; Li, X.-H.; Liu, G.-Q.; Thiemann, W. *Biopolymers* **2003**, *72*, 413–420; (e) Wu, F.-Y.; Bae, S. W.; Hong, J.-I. *Tetrahedron Lett.* **2006**, *47*, 8851–8854.
5. (a) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006; pp 67–69; (b) Valdes-Aguilera, O.; Neckers, D. C. *Acc. Chem. Res.* **1989**, *22*, 171–177.
6. (a) Kwon, J. Y.; Jang, Y. J.; Lee, Y. J.; Kim, K. M.; Seo, M. S.; Nam, W.; Yoon, J. *J. Am. Chem. Soc.* **2005**, *127*, 10107–10111; (b) Xiang, Y.; Tong, A.-J. *Org. Lett.* **2006**, *8*, 1549–1552; (c) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859–861; (d) Xiang, Y.; Tong, A.-J.; Jin, P.-Y.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863–2866; (e) Zhang, M.; Gao, Y.-H.; Li, M.-Y.; Yu, M.-X.; Li, F.-Y.; Li, L.; Zhu, M.-W.; Zhang, J.-P.; Yi, T.; Huang, C.-H. *Tetrahedron Lett.* **2007**, *48*, 3709–3712.
7. (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387; (b) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760–16761; (c) Ko, S.-K.; Yang, Y.-K.; Tae, J.; Shin, I. *J. Am. Chem. Soc.* **2006**, *128*, 14150–14155; (d) Wu, J.-S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org. Lett.* **2007**, *9*, 907–910; (e) Xiang, Y.; Mei, L.; Li, N.; Tong, A.-J. *Anal. Chim. Acta* **2007**, *581*, 132–136.
8. (a) Nonoyama, M.; Tomita, S.; Yamasaki, K. *Inorg. Chim. Acta* **1975**, *12*, 33–37; (b) Bould, J.; Brisdon, B. J. *Inorg. Chim. Acta* **1976**, *19*, 159–163; (c) Airoldi, C.; Dias, F. S.; de Sá, G. F.; Espínola, J. G. *J. Inorg. Nucl. Chem.* **1978**, *40*, 1537–1541.
9. Compound **1**: ^{1}H NMR (270 MHz, $CDCl_3$): δ (ppm) = 8.22 (pyridine, d, 1H, J = 5.4 Hz), 8.06–8.09 (pyridine, m, 1H), 7.98-8.01 (xanthene, m, 1H), 7.43–7.52 (pyridine and xanthene, m, 3H), 7.08–7.15 (xanthene, m, 1H), 6.75–6.79 (pyridine, m, 1H), 6.37–6.47 (xanthene, m, 4H), 6.12–6.16 (xanthene, m, 2H), 3.29 (CH_3CH_2, q, 8H, J = 8.1 Hz), 1.13 (CH_3CH_2, t, 12H, J = 6.7Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ (ppm) = 168.24, 153.81, 153.53, 150.74, 148.50, 147.21, 136.61, 133.25, 130.39, 127.92, 127.78, 124.30, 123.10, 118.91, 116.05, 108.87, 107.43, 98.03, 66.64, 44.35, 12.80. FAB-MS: Calcd for $C_{33}H_{34}N_4O_2$: 518.27. Found: m/z = 519.23 ($M+H^+$; 44%). ^{1}H, ^{13}C NMR and FAB-MS spectra are shown in Figures S1–S3.
10. Compound **2**: ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.98–8.01 (xanthene, m, 1H), 7.44–7.52 (xanthene, m, 2H), 7.06–7.16 (xanthene and phenyl, m, 4H), 6.78–6.81 (phenyl, m, 1H), 6.64 (xanthene, d, 2H, J = 8.1 Hz), 6.24–6.32 (xanthene, m, 4H), 3.31 (CH_3CH_2, q, 8H, J = 8.1 Hz), 1.14 (CH_3CH_2, t, 12H, J = 6.7 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ (ppm) = 167.42, 153.15, 153.11, 148.79, 136.77, 132.60, 131.11, 128.74, 128.32, 127.99, 127.13, 126.39, 123.94, 123.28, 108.35, 106.92, 98.17, 67.49, 44.43, 12.69. FAB-MS: Calcd for $C_{34}H_{35}N_3O_2$: 517.27. Found: m/z = 518.39 ($M+H^+$; 60%). ^{1}H, ^{13}C NMR and FAB-MS spectra are shown in Figures S4–S6.
11. (a) Parker, C. A.; Rees, W. T. *Analyst.* **1960**, *85*, 587–600; (b) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991–1024.
12. ^{1}H NMR spectrum of **1** changes drastically upon addition of Fe^{3+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, and Zn^{2+} due to their strong binding ability (Fig. S10). To track the continuous change in the spectrum, we chose Cd^{2+} as the titrant.
13. Soulti, K. D.; Troganis, A.; Papaioannou, A.; Kabanos, T. A.; Keramidas, A. D.; Deligiannakis, Y. G.; Raptopoulou, C. P.; Terzis1, A. *Inorg. Chem.* **1998**, *37*, 6785–6794.
14. Jaźwiński, J.; Duddeck, H. *Magn. Reson. Chem.* **2003**, *41*, 921–926.
15. Pazderski, L.; Szłyk, E.; Sitkowski, J.; Kamieński, B.; Kozerski, L.; Toušek, J.; Marek, R. *Magn. Reson. Chem.* **2006**, *44*, 163–170.
16. (a) Pazderski, L.; Toušek, J.; Sitkowski, J.; Kozerski, L.; Marek, R.; Szłyk, E. *Magn. Reson. Chem.* **2007**, *45*, 24–36; (b) Sahoo, H. S.; Chand, D. K.; Mahalakshmi, S.; Mir, M. H.; Raghunathan, R. *Tetrahedron Lett.* **2007**, *48*, 761–765; (c) Orellana, G.; Ibarra, C. A.; Santoro, J. *Inorg. Chem.* **1988**, *27*, 1025–1030.