DOI: 10.1002/ange.200804714

Chemosensors

A Selective Fluoroionophore Based on BODIPY-functionalized Magnetic Silica Nanoparticles: Removal of Pb^{2+} from Human Blood**

Hye Young Lee, Doo Ri Bae, Ji Chan Park, Hyunjoon Song, Won Seok Han, and Jong Hwa Jung**

Since heavy metal ions can cause severe risks for human health and the environment, methods for the facile preparation of fluorescence receptors with high selectivity and sensitivity for heavy metal ions have received much attention.[1] Among the known heavy metal ions, Pb^{2+} is one of the most dangerous, causing adverse health effects from lead exposure, particularly in children.[2] A variety of symptoms have been attributed to lead poisoning, including abdominal pain and vomiting. The accumulation of Pb^{2+} in the body leads to many serious human afflictions, including muscle paralysis, mental confusion, memory loss, and anemia, suggesting that Pb^{2+} affects multiple targets in vivo.[3] Plausible molecular targets of lead include calcium- and zinc-binding proteins which control cell signaling and gene expression, respectively.[4] Thus, it is important to develop a safe and effective procedure to detect and remove lead from the body after toxic lead contamination.[5]

In the last few years many approaches, including chemical precipitation, membrane filtration, ion exchange, and liquid extraction, have been employed for the removal or recovery of heavy metal ions from aqueous phases. However, no practical means for removal have yet been developed. Despite its own limitation for in vivo use, the magnetically assisted chemical separation process, which utilizes magnetic nanoparticles functionalized with a fluorescence receptor, offers a promising approach for simple and efficient recovery of heavy metal ions for biological, toxicological, and environmental use.[6] The magnetic nanoparticles having appended receptors have some important advantages as a solid chemosensor and adsorbent in heterogeneous solid-liquid phases. First, such nanoparticles are readily synthesized by sol-gel condensation, a versatile technique that allows chemical functionalities. Second, the receptors immobilized on an inorganic support can remove the guest molecules (toxic metal ions and anions) from the pollutant solution. Third, the magnetic nanoparticles can be easily isolated from pollutants by using a small magnet and then repeatedly utilized with suitable treatment. In particular, the magnetic nanoparticles can also provide efficient binding to the guest molecules because their high surface-to-volume ratio simply offers more contact area.

Xu and co-workers recently reported the use of bisphosphate-modified magnetic nanoparticles to remove radioactive metal toxins (e.g., UO_2^{2+}) with high efficiency from blood.[6c] In their study, they found that the designed magnetic nanoparticles can remove 69% of the initial 100 ppm UO_2^{2+} from blood. They also suggested that these functionalized, biocompatible magnetic nanoparticles can act as useful and effective agents for selective and rapid removal of radioactive metal toxins in vivo. This result prompted us to carry out the corresponding reactions because the fabrication of new biocompatible magnetic nanoparticles might be possible by introducing the modified fluorescence receptor for probing metal toxins, as well as effective separation of metal toxins from blood. In this work, a new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivative (**2**) was selected as a signal-transducing unit because it absorbs and emits in the visible region with high excitation coefficients, high fluorescent quantum yields, and high photostability.[7] When the fluorescence receptor **2** is combined with the magnetic property, the new class of BODIPY-functionalized magnetic silica nanoparticles can therefore be an ideal candidate for the removal or recovery of Pb^{2+} with high efficiency from water or human blood.

Although a few dual signaling and remediation systems for detection and adsorption of metal ions from water or common solvents are now known, to best of our knowledge, only one case of a fluorescence receptor immobilized on magnetic nanoparticles for the removal of a metal toxin from blood has been reported by Xu and co-workers.[6c] Furthermore, there have been no reports of a fluorescence receptor being immobilized on magnetic nanoparticles for the removal of Pb^{2+} from blood. Herein, we report the synthesis of **1** and its uses for the detection and removal of Pb^{2+} in both water and human blood.

Compound **3** was synthesized as described previously.[8] Nickel nanoparticles were prepared by a modified polyol process.[9] The particles were then coated with silica shells by using the Stöber method using tetraethyl orthosilicate (TEOS) as a silica source in water to yield $Ni@SiO_2$ core/shell particles,[10] as detailed in the Supporting Information.

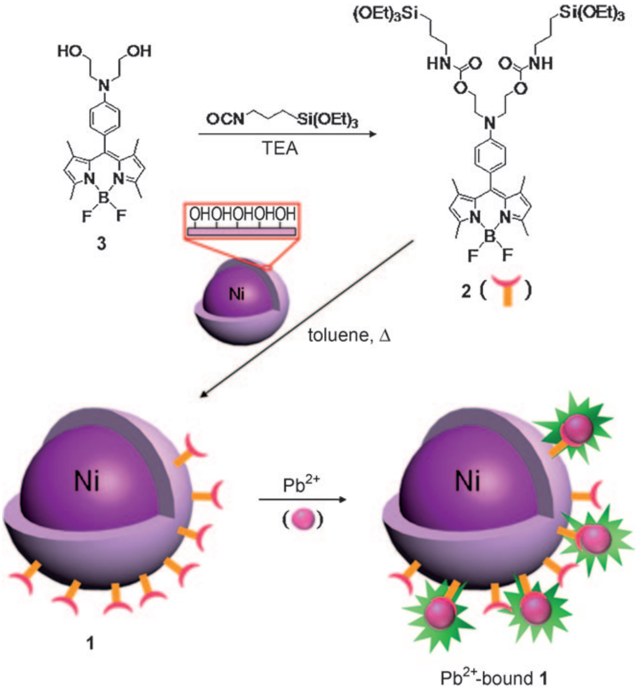
---

[*] H. Y. Lee, D. R. Bae, Dr. W. S. Han, Prof. J. H. Jung
Department of Chemistry and Research Institute of Natural
Sciences and Environmental Biotechnology National Core Research
Center, Gyeongsang National University, Jinju 660-701 (Korea)
Fax: (+82) 55-758-6027
E-mail: wshan@gnu.ac.kr
jonghwa@gnu.ac.kr
J. C. Park, Prof. H. Song
Department of Chemistry and School of Molecular Science
Korea Advanced Institute of Science and Technology
Daejeon 305-701 (Korea)

[**] This work was supported by a grant from the EB-NCRC (grant no. R15-2003-012-01001-0) and (grant no. R01-2007-000-20299-0) supported by the KSEF/MOST of Korea. BODIPY = 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804714.

Receptor **2** was easily prepared by treating **3** with 3-(triethoxysilyl)propyl isocyanate in the presence of triethylamine (Scheme 1). The Ni@SiO$_2$ core/shell particles were then reacted with **2** in toluene under vigorous stirring overnight to covalently link **2** to the surface of the Ni nanoparticles by the sol-gel reaction. The synthetic **1** was well characterized by transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), FTIR spectroscopy, time-of-flight second ion mass spectroscopy (TOF-SIMS), and fluorophotometry methods.



Scheme 1. Synthesis of BODIPY-functionalized magnetic silica nanoparticles **1**.

The TEM image of Pb^{2+}-bound **1** revealed a spherical structure with a narrow size distribution (30–40 nm) which remained as crystalline nanocrystals (Figure 1 a). The spheres of Pb^{2+}-bound **1** were also directly observed in solution state by CLSM methods, which showed slight aggregation as the result of surface modification by the attachment of **2** in comparison to the as-prepared Ni@SiO$_2$ core/shell particles (Figure 1 b). For additional proof of the new bond formation, we took IR and TOF-SIMS spectra of **1**. In the IR spectrum of **1**, the bands attributed to the Ni@SiO$_2$ core/shell particles themselves were observed, as well as strong new bands at 1608, 1541, 1402, 1301, and 1186 cm^{-1} appeared, which originated from the receptor **2**, indicating that **2** resided on the Ni@SiO$_2$ core/shell particles (see the Supporting Information, Figure S1). The TOF-SIMS spectrum of **1** displayed the fragments of **2** ($m/z = 396$, 427, 582), giving us firm evidence that **2** was covalently anchored onto the surface of the Ni@SiO$_2$ core/shell particles (see the Supporting Information, Figure S2).

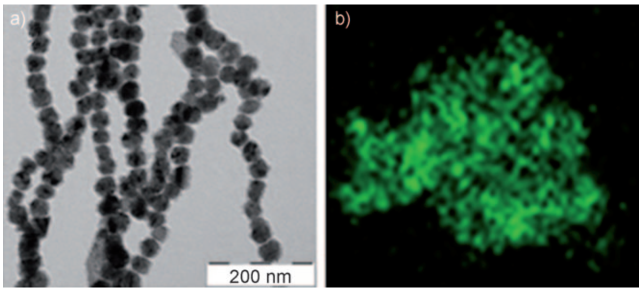


Figure 1. a) TEM and b) CLSM images ($\lambda_{ex} = 505$ nm) of Pb^{2+}-bound **1** prepared in water.

Spectroscopic measurements with **1** were performed in 0.2 M 3-(*N*-morpholine)propanesulfonic acid (MOPS), buffer pH 7. The absorption spectrum of free **1** showed a single visible absorption band at 505 nm ($\varepsilon = 6.50 \times 10^4$ M^{-1}cm^{-1}) and a corresponding emission maximum at 510 nm. As expected, **1** is virtually nonfluorescent in its apo state ($\Phi < 0.003$), which resulted from the efficient photoinduced electron transfer (PET)[11] quenching of the fluorophore by the lone pair of electrons on the nitrogen atom in the benzoyl moiety. Upon the addition of increasing amounts of Pb^{2+}, **1** showed a large CHEF (chelation-enhanced fluorescence) effect in the emission spectra, resulting from the blocking of the PET process. An overall emission change of approximately 8-fold ($\Phi = 0.019$, Figure 2) at the emission maximum ($\lambda_{em} = 510$ nm) was observed for Pb^{2+}. The absorption spectrum of Pb^{2+}-bound **1** showed a single visible absorption band at 505 nm ($\varepsilon = 6.27 \times 10^4$ M^{-1}cm^{-1}). Interestingly, the increase in the fluorescence intensity of **1** upon exposure to Pb^{2+} was reversible with the addition of a strong base (0.01 N NaOH) to the acidic solution (0.01 N HCl) (Figure 3). In addition, the fluorescence change was reproducible over several cycles of detection/separation. The Job's plot using the fluorescence changes indicated 1:1 binding for **1** with Pb^{2+} (see the Supporting Information, Figure S3). From the fluorescence

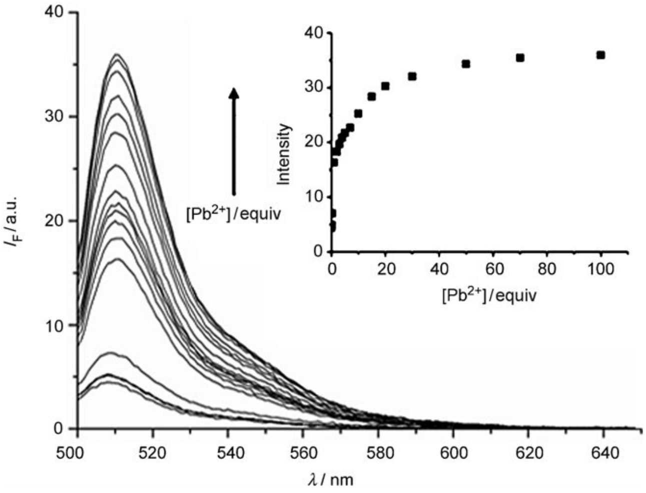


Figure 2. Fluorescence titrations of **1** (5.2×10^{-6} M) upon addition of increasing amounts of Pb^{2+} (0, 0.1, 0.2, 0.3, 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 50, 70, and 100 equiv) in water. The pH value was adjusted by using 0.2 M MOPS, buffer pH 7. Excitation was at 505 nm, and emission was monitored at 510 nm.

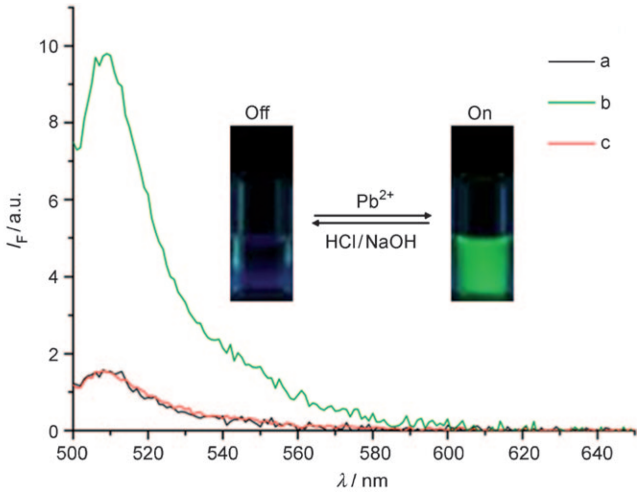


Figure 3. Fluorescence spectra of **1** without (a) and with (b) Pb^{2+}, and after treatment with HCl/NaOH in water (c). The inset is a photograph of **1** without (left) and with (right) Pb^{2+} in water. The pH value was adjusted by using 0.2 M MOPS, buffer pH 7.

titrations, the association constant (K) for Pb^{2+} coordination to **1** was calculated to be $1.05 \times 10^5\,\text{M}^{-1}$ ($\log K = 5.02$).[12] The addition of 15 ppb Pb^{2+}, which is the maximum limit (provided by the United States Environmental Protection Agency) for the allowable level of lead in drinking water, to a 0.1 μM solution of **1** resulted in a 10.5 % increase in the fluorescence intensity (see the Supporting Information, Figure S4).[5a] Spectroscopic measurements using **2** were performed in an acetonitrile solution to provide a comparison. Receptor **2** also showed a single visible absorption band at 497 nm ($\varepsilon = 8.30 \times 10^4\,\text{M}^{-1}\text{cm}^{-1}$) and a corresponding emission maximum at 510 nm ($\Phi = 0.067$). Upon the addition of Pb^{2+}, the fluorescence intensity of **2** increased by approximately 3-fold ($\Phi = 0.189$, see the Supporting Information, Figure S5) with the same absorption maximum ($\lambda_{abs} = 497$ nm, $\varepsilon = 8.06 \times 10^4\,\text{M}^{-1}\text{cm}^{-1}$) and emission maximum ($\lambda_{em} = 510$ nm) as the apo form. It should be mentioned that Yoon and co-workers reported compound **3** as a fluorescence chemosensor for Pb^{2+}.[8] From the fluorescence titrations, the association constant for **3** with Pb^{2+} was observed to be $8.8 \times 10^3\,\text{M}^{-1}$. The selectivity of **1** for Pb^{2+} is approximately 12-fold higher than that for **3**, because of the preorganization of **2** on the surface of the nanoparticle.[6e] In general, the preorganization of receptors on the surface reduces their conformational flexibility and increases their effective concentration. In this organic-inorganic hybrid material **1**, chemical detection of Pb^{2+} is facilitated by the synergistic effects of multiple noncovalent interactions.

To additionally evaluate the utility of **1** as an ion-selective fluorescence probe for Pb^{2+}, the competition-based fluorescence emission changes of **1**, upon addition of various biologically and environmentally relevant metal ions such as Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Hg^{2+}, were investigated in aqueous solution (Figure 4). Compound **1** displayed a large CHEF effect only with Pb^{2+}. Minimal or no changes were observed with the other metal ions, even though there was a relatively small quenching effect with Cu^{2+} (Figures 4a and c). When Cu^{2+} binds tightly to the host compound, intracomplex quenching has been reported (via energy or electron transfer).[13] Also, the fluorescence intensities of Pb^{2+}-bound **1** are unchanged in the presence of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Hg^{2+}, indicating that **1** is very promising as a selective adsorbent for the separation of Pb^{2+} in vivo (Figure 2 and Figure 4b). We also probed the binding abilities of **2** for metal ions on the basis of fluorescence changes upon the addition of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, and Pb^{2+} in an

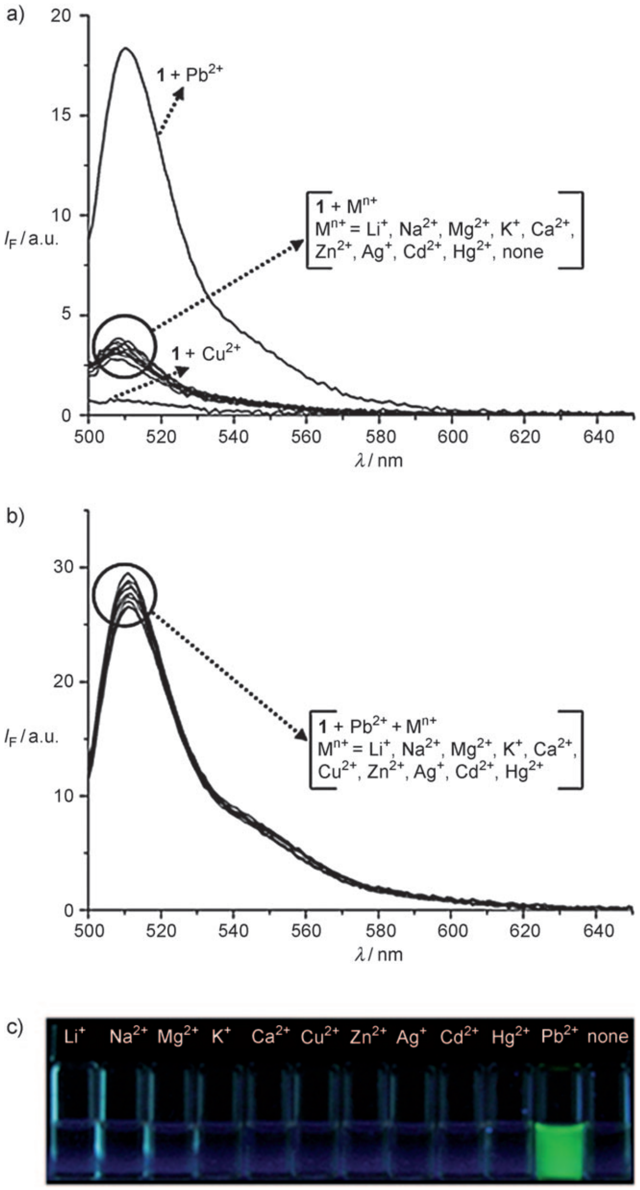


Figure 4. a) Fluorescence responses of **1** (3.9×10^{-5} M) upon addition of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, and Pb^{2+} (20 equiv) in water. b) Fluorescence responses of **1** (8.9×10^{-5} M) upon addition of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Hg^{2+} (20 equiv), and subsequent addition of Pb^{2+} (20 equiv) in water. c) Visual fluorescence changes for the same samples used in (a). For all measurements, the pH value was adjusted by using 0.2 M MOPS, buffer pH 7. Excitation is at 505 nm, and emission is monitored at 510 nm.

acetonitrile solution (see the Supporting Information, Figure S6). As expected, the selectivity of **2** for metal ions was almost the same as that of **1**. In the case of **2**+Pb^{2+}, the fluorescence change is ascribed to the complexation of a Pb^{2+} ion which induces a conformational change of two carbonyl groups, giving a $C{=}O\cdots Pb^{2+}$ coordination mode, resulting in an enhanced emission intensity (see the Supporting Information, Figure S7).[14] To understand the selective binding property of **2** in the presence of Pb^{2+}, we measured IR spectra of **2** upon the addition of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, and Pb^{2+}. The characteristic peak of the C=O groups in **2** at 1693 cm^{-1} shifted to 1678 cm^{-1} when it formed a complex with Pb^{2+}, whereas no shifts in IR spectra were observed with the other metal ions, giving us firm evidence that Pb^{2+} was coordinated to the oxygen atoms in the carbonyl groups (see the Supporting Information, Figure S8). Meanwhile, Yoon and co-workers probed the binding abilities of **3** upon addition of various metal ions in an acetonitrile solution.[8] Compound **3** also displayed a large CHEF effect with Pb^{2+}, whereas it showed a small CHEF effect with Zn^+ and Cu^{2+}. This difference suggests that the carbonyl groups of **2** play an important role in the selectivity for Pb^{2+} over other competing metal ions.

The above results encouraged us to test the removal of Pb^{2+} from human blood. The overall procedure for using **1** to remove Pb^{2+} from human blood is illustrated in Figure 5a.

Figure 5. a) Illustration of the removal of Pb^{2+} from human blood. b) Photograph of a magnet attracting Pb^{2+}-bound **1** in water.

The test sample was prepared by the addition of **1** (10 mg) to 1 mL of human blood containing 100 ppb Pb^{2+}—the amount of lead in children's blood that the Centers for Disease Control and Prevention deems unsafe—which was then sonicated. A small magnet was then used to remove the Pb^{2+}-bound **1** from the human blood (Figure 5b). To determine the amount of Pb^{2+} separated by the magnetic silica nanoparticles, the amount of Pb^{2+} left in human blood was determined by inductively coupled plasma mass spectrometry (ICP-MS). The ICP-MS measurements indicated that only 4% residual Pb^{2+} remained in human blood, suggesting that the designed magnetic silica nanoparticles **1** removed 96% of Pb^{2+}. Additionally, we also tested the removal of Pb^{2+} from water using **1**. By using the ICP-MS measurements, we found that **1** (0.1 μM) can remove 97% of the initial 15 ppb Pb^{2+} from water. Thus, receptor **1** can be a potentially useful and effective for the selective separation and rapid removal of Pb^{2+} in vivo.

In summary, we have readily prepared BODIPY-functionalized magnetic silica nanoparticles **1**, a new type of synthetic fluorescence receptor for probing Pb^{2+} in vivo. The fluorescence receptor **1** exhibited a high affinity and high selectivity for Pb^{2+} over other competing metal ions in water, and successfully removed Pb^{2+} from human blood. These findings may lead to the development of a new type of tailor made biocompatible system built by immobilizing the appropriate fluorescence receptors onto the surface of novel magnetic nanomaterials for the detection, recovery, and removal of other heavy metal toxins from the human body.

Experimental Section

Experimental details are provided in the Supporting Information.

Received: September 26, 2008
Revised: December 10, 2008
Published online: January 7, 2009

Keywords: blood · chemosensors · fluorescence · lead · nanoparticles

---

[1] a) R. Krämer, *Angew. Chem.* **1998**, *110*, 804; *Angew. Chem. Int. Ed.* **1998**, *37*, 772; b) L. Fabbrizzi, A. Poggi, *Chem. Soc. Rev.* **1995**, *24*, 197.
[2] H. L. Needleman, *Human Lead Exposure*, CRC, Boca Raton, FL, **1992**.
[3] a) S. Araki, H. Sato, K. Yokoyama, K. Murata, *Am. J. Ind. Med.* **2000**, *37*, 193; b) N. Rifai, G. Cohen, M. Wolf, L. Cohen, C. Faser, J. Savory, L. DePalma, *Ther. Drug Monit.* **1993**, *15*, 71; c) D. A. Cory-Slechta, *Adv. Behav. Pharmacol.* **1984**, *4*, 211; d) C. Winder, N. G. Carmichael, P. D. Lewis, *Trends Neurosci.* **1982**, *5*, 207.
[4] E. S. Claudio, H. A. Godwin, J. S. Magyar, *Prog. Inorg. Chem.* **2003**, *51*, 1, and references therein.
[5] a) Q. He, E. W. Miller, A. P. Wong, C. J. Chang, *J. Am. Chem. Soc.* **2006**, *128*, 9316; b) J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam, J. Yoon, *J. Am. Chem. Soc.* **2005**, *127*, 10107; c) M. Sun, D. Shangguan, H. Ma, L. Nie, X. Li, S. Xiong, G. Liu, W. Thiemann, *Biopolymers* **2003**, *72*, 413; d) J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2003**, *125*, 6642; e) C.-T. Chen, W.-P. Huang, *J. Am. Chem. Soc.* **2002**, *124*, 6246; f) J. Li, Y. Lu, *J. Am. Chem. Soc.* **2000**, *122*, 10466; g) S. Deo, H. A. Godwin, *J. Am. Chem. Soc.* **2000**, *122*, 174; h) M.-Y. Chae, J. Yoon, A. W. Czarnik, *J. Mol. Recognit.* **1996**, *9*, 297.
[6] a) S. J. Lee, D. R. Bae, W. S. Han, S. S. Lee, J. H. Jung, *Eur. J. Inorg. Chem.* **2008**, 1559; b) J. V. Ros-Lis, R. Casasús, M. Comes, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. E. Haskouri, N. Garró, K. Rurack, *Chem. Eur. J.* **2008**, *14*, 8267; c) L. Wang, Z. Yang, J. Gao, K. Xu, H. Gu, B. Zhang, X. Zhang, B. Xu, *J. Am. Chem. Soc.* **2006**, *128*, 13358; d) H. W. Gu, K. M. Xu, C. J. Xu, B. Xu, *Chem. Commun.* **2006**, 941; e) A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann, K. Rurack, *Angew. Chem.* **2006**, *118*, 6068; *Angew. Chem. Int. Ed.* **2006**, *45*, 5924; f) S. I. Stoeva, F. W. Huo, J. S. Lee, C. A. Mirkin, *J. Am. Chem. Soc.* **2005**, *127*, 15362; g) P. Chen, B.

Greenberg, S. Taghavi, C. Romano, D. van der Lelie, C. He, *Angew. Chem.* **2005**, *117*, 2775; *Angew. Chem. Int. Ed.* **2005**, *44*, 2715; h) K. B. Lee, S. Park, C. A. Mirkin, *Angew. Chem.* **2004**, *116*, 3110; *Angew. Chem. Int. Ed.* **2004**, *43*, 3048; i) C. J. Xu, K. M. Xu, H. W. Gu, H. Liu, R. K. Zheng, X. X. Zhang, Z. H. Guo, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 9938; j) C. J. Xu, K. M. Xu, H. W. Gu, X. F. Zhong, Z. H. Guo, R. K. Zheng, X. X. Zhang, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 3392; k) H. W. Gu, P. L. Ho, K. W. T. Tsang, C. W. Yu, B. Xu, *Chem. Commun.* **2003**, 1966; l) H. W. Gu, P. L. Ho, K. W. T. Tsang, C. Y. Yu, B. Xu, *J. Am. Chem. Soc.* **2003**, *125*, 15702; m) M. Lewin, N. Carlesso, C. H. Tung, X. W. Tang, D. Cory, D. T. Scadden, R. Weissleder, *Nat. Biotechnol.* **2000**, *18*, 410; n) E. Luboch, E. Wagner-Wysiecka, M. Fainerman-Melnikova, L. F. Lindoy, J. F. Biernat, *Supramol. Chem.* **2006**, *18*, 593; o) R. F. Fenton, L. F. Lindoy, R. C. Luckay, F. R. Turville, G. Wei, *Aust. J. Chem.* **2001**, *54*, 59.

[7] a) A. Loudet, K. Burgess, *Chem. Rev.* **2007**, *107*, 4891; b) R. P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, 9th ed., Molecular Probes, Eugene, **2002**.

[8] X. Qi, E. J. Jun, L. Xu, S.-J. Kim, J. S. J. Hong, Y. J. Yoon, J. Yoon, *J. Org. Chem.* **2006**, *71*, 2881.

[9] D. Seo, J. C. Park, H. Song, *J. Am. Chem. Soc.* **2006**, *128*, 14863.

[10] W. Stöber, A. Fink, E. J. Bohn, *J. Colloid Interface Sci.* **1968**, *26*, 62.

[11] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515.

[12] a) Association constants were obtained by using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1 LA, United Kingdom; b) K. A. Conners, *Binding Constants, The Measurement of Molecular Complex Stability*, Wiley, New York, **1987**.

[13] A. W. Varnes, R. B. Dodson, E. L. Wehry, *J. Am. Chem. Soc.* **1972**, *94*, 946.

[14] a) S. H. Lee, S. H. Kim, S. K. Kim, J. H. Jung, J. S. Kim, *J. Org. Chem.* **2005**, *70*, 9288; b) J. Y. Lee, S. K. Kim, J. H. Jung, J. S. Kim, *J. Org. Chem.* **2005**, *70*, 1463; c) S. K. Kim, S. H. Lee, J. Y. Lee, J. Y. Lee, R. A. Bartsch, J. S. Kim, *J. Am. Chem. Soc.* **2004**, *126*, 16499.