Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 8683–8686

Fluorescent sensing of pyrophosphate and ATP in 100% aqueous solution using a fluorescein derivative and Mn^{2+}

K. M. K. Swamy,[a,b,†] Soo Kyung Kwon,[a,†] Ha Na Lee,[a] S. M. Shantha Kumar,[b] Jong Seung Kim[c] and Juyoung Yoon[a,*]

[a]*Division of Nano Sciences (BK21) and Department of Chemistry, Ewha Womans University, Seoul 120-750, Republic of Korea*
[b]*Department of Pharmaceutical Chemistry, V.L. College of Pharmacy, Raichur 584 103, India*
[c]*Department of Chemistry, Korea University, Seoul 136-701, Republic of Korea*

Received 3 September 2007; revised 29 September 2007; accepted 4 October 2007
Available online 6 October 2007

Abstract—A new fluorescein derivative has been synthesized for the detection of pyrophosphate (PPi) and ATP in 100% aqueous solution. Chemosensor **1** in the presence of Mn^{2+} (2.5 equiv) displayed selective fluorescent enhancements with PPi and ATP at pH 7.4. among the anions examined. The association constant of **1** in the presence of Mn^{2+} with PPi and ATP was calculated as 4.2×10^4 and 3.5×10^4 M^{-1}.

Anions play a fundamental role in a wide range of chemical and biological processes, and numerous efforts have been devoted to the development of abiotic receptors for anionic species.[1] Anion-selective fluorescent sensors[1] have attracted growing attention. Especially, adenosine 5′-triphosphate (ATP) is known to be the universal energy currency in all of the biological systems and has been a significant target for the design of molecular receptors. On the other hand, the detection of PPi is considered to be an important issue in cancer research.[2] Furthermore, patients with calcium pyrophosphate dehydrate (CPPD) crystals and chondrocalcinosis have been shown to have high synovial fluid PPi level.[3]

In this regard, the detection of PPi[4,5] and ATP[6] has been the main focus of the efforts of several research groups. However, fluorescent chemosensors for PPi working in aqueous solution are still rare.[4]

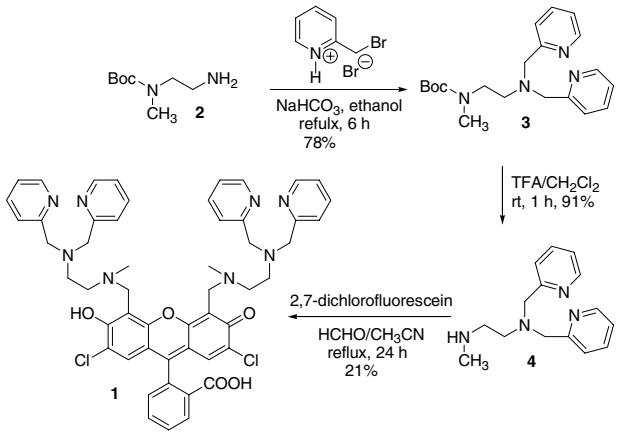
Herein, we synthesized a new fluorescein derivative bearing two (2-aminoethyl)bis(2-pyridylmethyl)amine groups for the detection of metal ions and the solution of **1** and Mn^{2+} (2.5 equiv) was further utilized as PPi and ATP selective fluorescent sensors in 100% aqueous solution. As far as we are aware of, this is the first example of fluorescent chemosensor for PPi or ATP, which utilizes Mn^{2+} as a recognition site. Among the ATP, ADP, and AMP series, this system displays selectivity for ATP over ADP and AMP.

A mixture of *N*-(2-aminoethyl)-*N*-methylcarbamic acid *t*-butyl ester **2** and 2-pyridylmethyl bromide in ethanol gave Boc-protected ligand **3**[7] in 78% yield, which was then treated with trifluoroacetic acid (TFA) to remove the Boc group and ligand **4**[8] was obtained in 91% yield (Scheme 1). Compound **1**[9] was synthesized using the Mannich reaction between 2′,7′-dichlorofluorescein and the iminium ion condensation of product of formaldehyde and ligand **4** with a 21% yield.

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+}, and Zn^{2+} ions were used to evaluate the metal ion binding properties of compound **1** in 100% aqueous solution. The fluorescence spectra were obtained by excitation of the fluorescein fluorophore at 505 nm. Both the excitation and emission slits were 1.5 nm. Among these metal ions (2 equiv), compound **1** showed large chelation enhanced fluorescence quenching (CHEQ) effects with Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Ag^+, and Mn^{2+} at pH 7.4 (Fig. 1). As shown, in Figure 1, compound **1** displayed

* Corresponding author. Tel.: +82 2 3277 2400; fax: +82 2 3277 2384; e-mail: jyoon@ewha.ac.kr
† Contributed equally to this work.

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.10.022



Scheme 1. Synthesis of compound **1**.

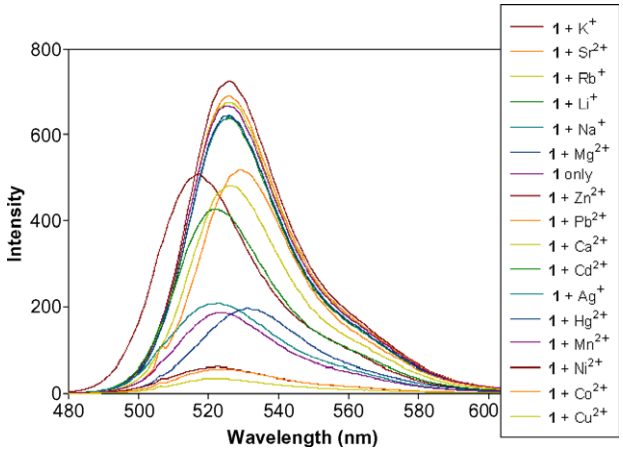


Figure 1. Fluorescent emission changes of **1** (3 μM) upon addition of various metal ions (2 equiv) at pH 7.4 (0.02 M HEPES) (excitation at 505 nm, excitation and emission slit: 1.5 nm).

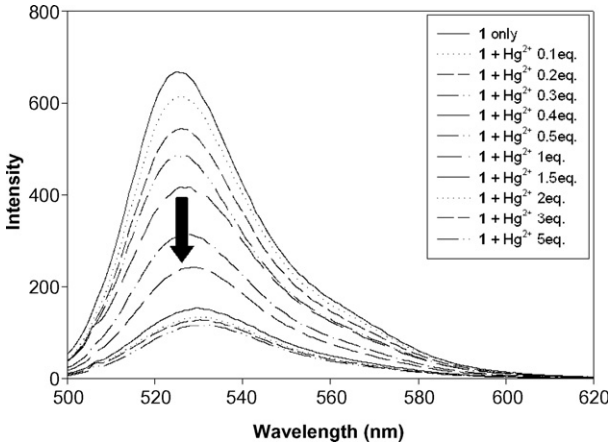


Figure 2. Fluorescent titrations of **1** (3 μM) with Hg^{2+} at pH 7.4 (0.02 M HEPES) (excitation at 505 nm, excitation and emission slit: 1.5 nm).

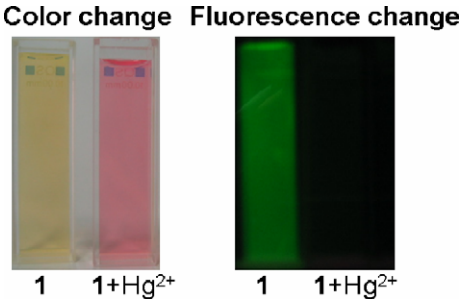


Figure 3. Fluorescent titrations of **1** (3 μM) with Hg^{2+} at pH 7.4 (0.2 M HEPES) (excitation at 505 nm, excitation and emission slit: 1.5 nm).

tight binding with these metal ions since the addition of only 2 equiv of metal ions induced such large fluorescent changes, which means (2-aminoethyl)bis(2-pyridylmethyl)amine ligand along with phenolic oxygen can provide an excellent binding site for metal ions. Figure 2 explains the fluorescent titrations of **1** with Hg^{2+}. 1:2 stoichiometry was also confirmed by the Job plot using the fluorescence changes. The association constant for **1** with Hg^{2+} was calculated as $6.3 \times 10^7\ M^{-2}$.[10] Colorimetric changes as well fluorescent changes upon the addition of Hg^{2+} are shown in Figure 3.

Recently, a few rhodamine B derivatives have also been used as fluorescent chemosensors for metal ions, in which the spirolactam (nonfluorescent) to ring-opened amide (fluorescent) process was utilized.[11] This ring-opening process can be also followed by the colorimetric change usually from light yellow to pink color. On the other hand, there have been only few reports in which the ring-opening processes of fluorescein derivatives were carefully examined.[4e,12] Based on the colorimetric changes, the following explanation can be proposed: At pH 7.4, compound **1** exists as a closed lactone form since the color of the solution was yellow. Upon the addition of Mn^{2+} or other metal ions such as Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, and Ag^{+}, the color of the solution turned pink, which can be attributed to the ring-opening process of lactone moiety.

We then examined the fluorescent changes toward various anions in the presence of metal ions. The fluorescent changes of **1** with PPi or ATP (10 equiv or 100 equiv) in the presence of Mn^{2+}, Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Ag^{+}, were examined at pH 7.4 (0.02 M HEPES). Among these metal ions, only Mn^{2+} gave a significant fluorescent enhancement with PPi/ATP. As shown in Figure 4, among the various anions such as PPi, ATP, ADP, AMP, $H_2PO_4^-$, HSO_4^-, and $CH_3CO_2^-$, only PPi and ATP induced significant fluorescent enhancements with this **1** and Mn^{2+} solution even though there was a relatively smaller fluorescent enhancement with ADP. From the fluorescent titrations, the association constant for PPi (Fig. 5), ATP (S-Fig. 1), and ADP (S-Fig. 2) were calculated as 4.2×10^4, 3.5×10^4, and $1.3 \times 10^4\ M^{-1}$, respectively (errors <15%).[10] On the other hand, AMP and $H_2PO_4^-$ did not induce any significant fluorescent change when large excess (100 equiv) of these anions were added. It is worth mentioning that discrimination between PPi and $H_2PO_4^-$ was excellent in 100% aqueous solution. In the presence of 50 equiv of $H_2PO_4^-$, a similar association constant was observed

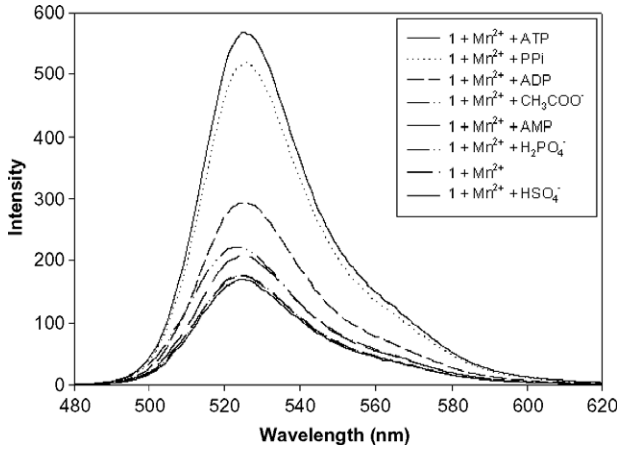


Figure 4. Fluorescent emission changes of **1** (3 μM) with Mn^{2+} (2.5 equiv) upon addition of PPi, ATP, ADP, AMP, CH_3CO^{2-}, $H_2PO_4^-$ and HSO_4^- (10 equiv) at pH 7.4 (0.02 M HEPES) (excitation at 505 nm, excitation and emission slit: 1.5 nm).

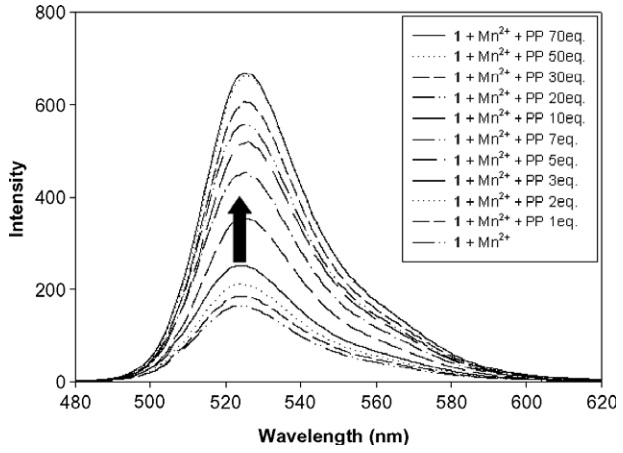


Figure 5. Fluorescent changes of **1** (3 μM) with pyrophosphate in the presence of 2.5 equiv of Mn^{2+} at pH 7.4 (0.02 M HEPES) (excitation at 505 nm, excitation and emission slit: 1.5 nm).

for **1** and Mn^{2+} solution with PPi. Figure 6 explains the colorimetric changes and fluorescent changes of **1** in the presence of Mn^{2+} with PPi at pH 7.4. Due to the lack of X-ray structure of the **1**-Mn^{2+}-PPi and NMR data, the exact reason for the color change as well as fluorescent changes of **1** and Mn^{2+} solution upon the addition of PPi is not quite clear at this moment. However, we believe that Mn^{2+} is not released from the DPA unit since small fluorescent quenching effects were observed when large excess of PPi (>150 equiv) was added and the addition of PPi to compound **1** only (in the absence of Mn^{2+}) did not induce any fluorescent change.

In conclusion, a new fluorescein derivative bearing two (2-aminoethyl)bis(2-pyridylmethyl)amine groups has been synthesized for the detection of PPi and ATP in 100% aqueous solution. The solution of **1** and Mn^{2+} was utilized as a fluorescent chemosensor for PPi and ATP for the first time. Chemosensor **1** in the presence of Mn^{2+} (2.5 equiv) displayed selective fluorescent enhancements with PPi and ATP at pH 7.4 among

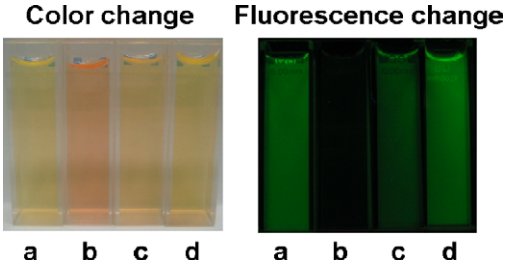


Figure 6. Fluorescent change and colorimetric change of **1** (15 μM) with pyrophosphate in the presence of 2.5 equiv of Mn^{2+} at pH 7.4 (0.02 M HEPES). (a; **1** only, b; **1** + Mn^{2+}, c; **1** + Mn^{2+} + PPi (10 equiv), d; **1** + Mn^{2+} + PPi (50 equiv)).

the anions examined. The association constant of **1** in the presence of Mn^{2+} with PPi was calculated as $4.2 \times 10^4\ M^{-1}$. Excellent selectivity for PPi over $H_2PO_4^-$ was observed in 100% aqueous solution. Among the ATP, ADP and AMP series, this system displays selectivity for ATP over ADP and AMP.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant (NRL) funded by the Korean government (MOST) (R04-2007-000-2007-0), the SRC program of the KOSEF (R11-2005-008-02001-0), Korea Ministry of Environment as 'The Eco-technopia 21 project' and BK21.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.022.

References and notes

1. (a) Kim, S. K.; Kim, H. N.; Xiaoru, Z.; Lee, H. N.; Lee, H. N.; Soh, J. H.; Swamy, K. M. K.; Yoon, J. *Supramol. Chem.* **2007**, *19*, 221; (b) Yoon, J.; Kim, S. K.; Singh, N. J.; Kim, K. S. *Chem. Soc. Rev.* **2006**, *35*, 355; (c) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551; (d) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094; (e) Martínez-Máñez, R.; Sancanón, F. *Chem. Rev.* **2003**, *103*, 4419; (f) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. A.; Huxley, T. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
2. Xu, S.; He, M.; Yu, H.; Cai, X.; Tan, X.; Lu, B.; Shu, B. A. *Anal. Biochem.* **2001**, *299*, 188.
3. (a) Doherty, M.; Becher, C.; Regan, M.; Jones, A.; Ledingham, J. *Ann. Rheum. Dis.* **1996**, *66*, 432; (b) Timms, A. E.; Zhang, Y.; Russell, R. G.; Brown, M. A. *Rheumatology* **2002**, *41*, 725.
4. (a) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S.-J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828; (b) Lee, H. N.; Swamy, K. M. K.; Kim, S. K.; Kwon, J.-Y.; Kim, Y.; Kim, S.-J.; Yoon, Y. J.; Yoon, J. *Org. Lett.* **2007**, *9*, 243; (c) Zhao, X.; Liu, Y.; Schanze, K. S. *Chem. Commun.* **2007**, 2914; (d) Cho, H. K.; Lee, D. H.;

Hong, J.-I. *Chem. Commun.* **2005**, 1690; (e) Jang, Y. J.; Jun, E. J.; Lee, Y. J.; Kim, Y. S.; Kim, J. S.; Yoon, J. *J. Org. Chem.* **2005**, *70*, 9603; (f) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777; (g) Aldakov, D.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2004**, *126*, 4752; (h) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920; (i) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397.

5. (a) Singh, N. J.; Jun, E. J.; Chellappan, K.; Thangadurai, D.; Chandran, R. P.; Hwang, I.-C.; Yoon, J.; Kim, K. S. *Org. Lett.* **2007**, *9*, 485; (b) Kim, S. K.; Singh, N. J.; Kwon, J.; Hwang, I.-C.; Park, S. J.; Kim, K. S.; Yoon, J. *Tetrahedron* **2006**, *62*, 6065; (c) Aldakov, D.; Anzenbacher, P., Jr. *Chem. Commun.* **2003**, 1394; (d) Gunnlaugsson, T.; Davis, A. P.; O'Brien, J. E.; Glynn, M. *Org. Lett.* **2002**, *4*, 2449; (e) Anzenbacher, P., Jr.; Jursíková, K.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 9350; (f) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463.
6. (a) Jose, D. A.; Mishra, S.; Ghosh, A.; Shrivastav, A.; Mishra, S.; Das, A. *Org. Lett.* **2007**, *9*, 1979; (b) Kanekiyo, Y.; Naganawa, R.; Tao, H. *Chem. Commun.* **2004**, 1006; (c) Kwon, J. Y.; Singh, N. J.; Kim, H.; Kim, S. K.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2004**, *126*, 8892; (d) McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 1114; (e) Sancenón, F.; Benito, A.; Lloris, J. M.; Martínez-Máñez, R.; Pardo, T.; Soto, J. *Helv. Chim. Acta* **2002**, *85*, 1505; (f) Ojida, A.; Park, S.-k.; Mito-oka, Y.; Hamachi, I. *Tetrahedron Lett.* **2002**, *43*, 6193; (g) Sancenón, F.; Descalzo, A. B.; Martínez-Máñez, R.; Miranda, M. A.; Soto, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2640; (h) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542.
7. *[2-(Bis-pyridin-2-ylmethylamino)ethyl-N-methyl]carbamic acid tert-butyl ester* (**3**): A mixture of *N*-(2-aminoethyl)-*N*-methylcarbamic acid *t*-butyl ester **2** (2 g, 11.48 mmol), sodium carbonate (5.37 g, 50.62 mmol), and 2-pyridylmethyl bromide hydro bromide (6.39 g, 25.25 mmol) in 125 mL of dry ethanol was refluxed for 6 h under argon and then the solvent was evaporated. The residue was dissolved in 80 mL of aqueous solution of sodium hydroxide (10%) and extracted with dichloromethane (3×100 mL). Then, the organic layer was dried over potassium carbonate and evaporated. The residue was chromatographed on aluminium oxide (CH_2Cl_2/MeOH = 98:2, v/v) to afford 3.2 g of [2-(bis-pyridin-2-ylmethylamino)ethyl-*N*-methyl]carbamic acid *tert*-butyl ester (**3**) (78%): ^{1}H NMR ($CDCl_3$) δ 8.52 (br s, 2 H), 7.64 (m, 4H), 7.16 (br s, 2H), 3.85 (s, 4H), 3.38 (m, 2H), 2.93, (m, 5H), 1.72 (s, 3H), 1.47 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 159.4, 155.6, 149.3, 122.7, 121.9, 79.3, 60.4, 51.9, 46.7, 34.5, 27.9; LRMS (FAB) $m/z = 357.2$ $(M+H)^+$, calcd for $C_{20}H_{29}N_4O_2 = 357.2$.
8. *N′-Methyl-N,N-bis-pyridin-2-ylmethyl-ethane-1,2-diamine* (**4**): Trifluoroacetic acid (0.74 mL) was added dropwise to a solution of [2-(bis-pyridin-2-ylmethylamino)ethyl-*N*-methyl]carbamic acid *tert*-butyl ester **3** (3 g, 8.42 mmol) in 25 mL of dichloromethane at 0 °C and the mixture was stirred for 1 h at room temperature. After evaporation of the solvent, the residue was dissolved in an aqueous solution of sodium hydroxide (20 mL, 10%) and extracted with dichloromethane (4×25 mL). The collected organic layer was dried over potassium carbonate and evaporated to afford *N′*-methyl-*N*,*N*-bis-pyridin-2-ylmethyl-ethane-1,2-diamine **4** (2 g, 91%); ^{1}H NMR ($CDCl_3$) δ 8.52 (d, 2 H, $J = 4.9$ Hz), 7.63 (t, 2 H, $J = 13.5$ Hz), 7.43 (d, 2H, $J = 7.8$ Hz), 7.15 (t, 2H, $J = 4.9$ Hz), 3.89 (s, 4H), 2.60 (m, 4H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.7, 148.9, 136.7, 123.2, 122.6, 59.8, 51.2, 47.8, 33.1; LRMS (FAB) $m/z = 257.2$ $(M+H)^+$, calcd for $C_{15}H_{21}N_4 = 257.2$.
9. *9-(o-Carboxyphenyl)-2,7-dichloro-4,5-bis[N′-methyl-N,N-bis-pyridin-2-ylmethyl-ethane]-6-hydroxy-3-xanthenone* (**1**): 2,7-Dichlorofluoroscein (0.5 g, 1.25 mmol) and paraformaldehyde (0.11 g, 3.73 mmol) were combined in 20 mL of CH_3CN. *N′*-Methyl-*N*,*N*-bis-pyridin-2-ylmethyl-ethane-1,2-diamine **4** (1 g, 3.99 mmol) was added under stirring at room temperature followed with 10 mL of H_2O. The reaction mixture was refluxed for 24 h. The solvents were removed completely under vacuum and the residue was chromatographed over basic alumina using CH_2Cl_2/MeOH (95:5, v/v) as eluent. Evaporation of solvent gave a salmon pink solid (0.25 g, 21%): mp 65 °C, dec; ^{1}H NMR ($CDCl_3$) δ 8.50 (d, 4H, $J = 4.9$ Hz), 8.02 (d, 1H, $J = 5.6$ Hz), 7.61 (m, 10H), 7.14 (m, 5H), 6.62 (s, 2H), 3.91 (d, 4H, $J = 3.8$ Hz), 3.86 (s, 8H), 2.81 (m, 8H), 2.19 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 168.8, 158.7, 156.8, 148.9, 147.9, 136.6, 135.2, 130.2, 127.3, 125.5, 124.1, 123.6, 122.2, 117.3, 109.8, 109.1, 60.6, 54.7, 54.5, 50.9, 41.8; HRMS (FAB) $m/z = (M+H)^+$, calcd for $C_{52}H_{51}Cl_2N_8O_5 = 937.3359$.
10. (a) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom; (b) Conners, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.
11. (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386; (b) 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; (c) Xiang, Y.; Tong, A. *Org. Lett.* **2006**, *8*, 1549; (d) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863; (e) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859; (f) Wu, J. S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org. Lett.* **2007**, *9*, 907; (g) Soh, J. H.; Swamy, K. M. K.; Kim, S. K.; Kim, S.; Lee, S.-H.; Yoon, J. *Tetrahedron Lett.* **2007**, *48*, 5966.
12. (a) Margulies, D.; Meiman, G.; Shanzer, A. *J. Am. Chem. Soc.* **2006**, *128*, 4865; (b) Burdette, S. C.; Frederickson, C. J.; Bu, W.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 1778.