Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 1261–1265
Tetrahedron Letters

New acridine derivatives bearing immobilized azacrown or azathiacrown ligand as fluorescent chemosensors for Hg^{2+} and Cd^{2+}

Han Na Lee [a,†], Ha Na Kim [a,†], K. M. K. Swamy [a,b], Min Sun Park [a], Jimi Kim [c], Hyunsook Lee [c], Keun-Hyeung Lee [d], Sungsu Park [a], Juyoung Yoon [a,*]

[a] *Division of Nano Science 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 Biological Sciences, Research Center for Functional Cellulomics, College of Natural Sciences, Seoul National University, Seoul 151-742, Republic of Korea*
[d] *Department of Chemistry, Inha University, Incheon 402-751, Republic of Korea*

Received 28 October 2007; revised 21 November 2007; accepted 27 November 2007
Available online 3 December 2007

Abstract

Two new acridine derivatives bearing azacrown or azathiacrown ligand were synthesized as fluorescent chemosensors for Hg^{2+} and Cd^{2+} in aqueous solution. Compounds **1** and **2** displayed selective CHEF (chelation enhanced fluorescence) effects with Hg^{2+} or Cd^{2+} among the metal ions examined. The practical use of these probes was demonstrated by their applications to the detection of Hg^{2+} and Cd^{2+} ions in mammalian cells.

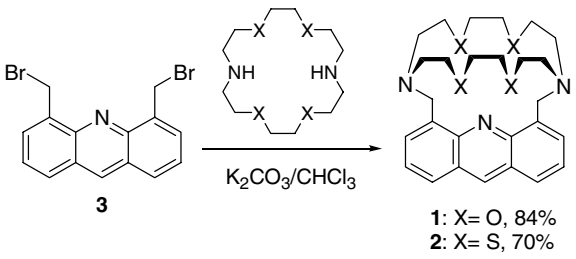
Detection of metal ions with high specificity under physiologically relevant conditions is an important aspect in the design of fluorescent chemosensors for biological and environmental applications.[1] In particular, Hg^{2+} and Cd^{2+} are the environmentally important metal ions due to their detrimental effects on human health. Mercury contamination through oceanic and volcanic emission,[2] gold mining,[3] or solid waste incineration has been an important issue because of its severe immunotoxic, genotoxic, and neurotoxic effects. Chronic cadmium exposure can cause renal dysfunction and increased incidence of certain forms of cancer.[4] Accordingly, considerable attention has been devoted to the development of new fluorescent chemosensors for the detection of Hg^{2+} or Cd^{2+} with sufficient selectivity. However, there are relatively few examples available, which display fluorescence enhancement upon the addition of Hg^{2+} or Cd^{2+} in aqueous solution.[5,6]

Herein, we report two new acridine derivatives (**1** and **2**) bearing azacrown or azathiacrown ligand as fluorescent chemosensors for Hg^{2+} and Cd^{2+} at pH 7.4. For these compounds, either azacrown or azathiacrown ligand was coupled to the 4- and 5-position of acridine, in which a relatively rigid binding pocket is generated for metal ions. Compounds **1** and **2** displayed large CHEF (chelation enhanced fluorescence) effects only with Hg^{2+} and Cd^{2+} among the metal ions examined. Interestingly, compound **1** displayed a large CHEF effect with Hg^{2+}, on the other hand, compound **2** displayed a large CHEF effect with Cd^{2+}. Cooperative binding from an immobilized ligand and nitrogen on acridine may provide such selectivity. The practical use of these probes was demonstrated by their application to the detection of Hg^{2+} and Cd^{2+} ions in mammalian cells.

4,5-Bis(bromomethyl)acridine **3** was prepared following the published procedure.[7] Treatment of 4,5-bis(bromomethyl)acridine **3** with 4,13-diaza-18-crown 6-ether or 1,10-diaza-4,7,14,17-tetrathiacyclooctadecane in anhydrous chloroform in the presence of K_2CO_3 followed by purification

* Corresponding author. Tel.: +82 2 3277 2400; fax: +82 2 3277 2384.
E-mail address: jyoon@ewha.ac.kr (J. Yoon).
† Contributed equally to this work.

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.11.158



Scheme 1. Synthesis of fluorescent chemosensor **1** and **2**.

on a basic alumina column using CH_2Cl_2–MeOH (99:1, v/v) as eluent gave compound **1**[8] and **2**[9] in 84 % and 70% yields, respectively. Both of these compounds were fully characterized by ^1H and ^{13}C NMR as well as high resolution mass spectroscopy. The characterization data are presented in the Supplementary data Scheme 1.

The perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Fe^{3+}, 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 compounds **1** and **2**. Figures 1 and 2 explain the fluorescent emission changes of **1** (3 μM) and **2** (3 μM or 2 μM), respectively, upon the addition of various metal ions in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v). In the case of Pb^{2+}, pH 7.4 was adjusted without using buffer solution due to the precipitation problem of Pb^{2+} in HEPES solution. Azacrown derivative **1** displayed a large CHEF effect with Hg^{2+}, and a relatively smaller CHEF effect was observed with Cd^{2+} (Fig. 1). On the other hand, azathiacrown derivative **2** displayed a large CHEF effect with Cd^{2+}, and a relatively smaller CHEF effect with Hg^{2+} (Fig. 2). There were no significant changes when other metal ions were added to compounds **1** and **2**. The CHEF effects can be explained by two reasons. The nitrogen on the acridine moiety can participate in the binding with Hg^{2+} or Cd^{2+}, which can induce the fluorescent

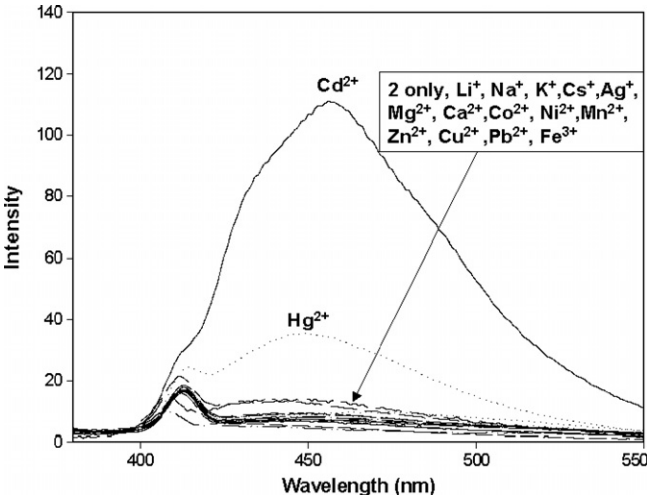


Fig. 2. Fluorescent changes of compound **2** (3 μM) upon the addition of various metal ions (100 equiv) in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v) (excitation at 356 nm).

increase. The fluorescence enhancement due to the interaction between metal ions and nitrogen on acridine[10] or the hydrogen bonding with a nitrogen on acridine[11] were also reported. The CHEF effect with these metal ions can also be explained by the blocking of the PET (photo-induced electron transfer) process from the benzylic nitrogens. The binding of an amine group with metal ions in fluorophore-amine conjugates is reported to eliminate photoinduced electron transfer (PET).[1d] Therefore, the CHEF effects are expected to be observed in these systems.

For compound **1**, Hg^{2+} displayed a larger CHEF effect than Cd^{2+} when 100 equiv of each metal ion were added. When excess Cd^{2+} was added to the solution of **1**, a similar large CHEF effect was also observed (S-Fig. 1). From the fluorescent titration experiments, the association constants of compound **1** with Hg^{2+} (Fig. 3) and Cd^{2+} (S-Fig. 1) were

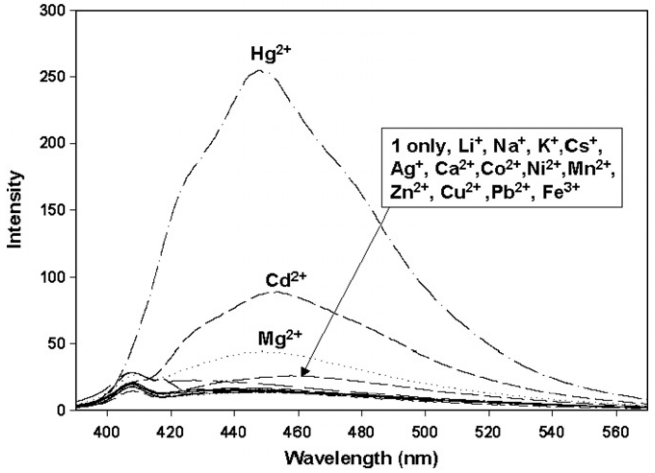


Fig. 1. Fluorescent changes of compound **1** (3 μM) upon the addition of various metal ions (100 equiv) in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v) (excitation at 356 nm).

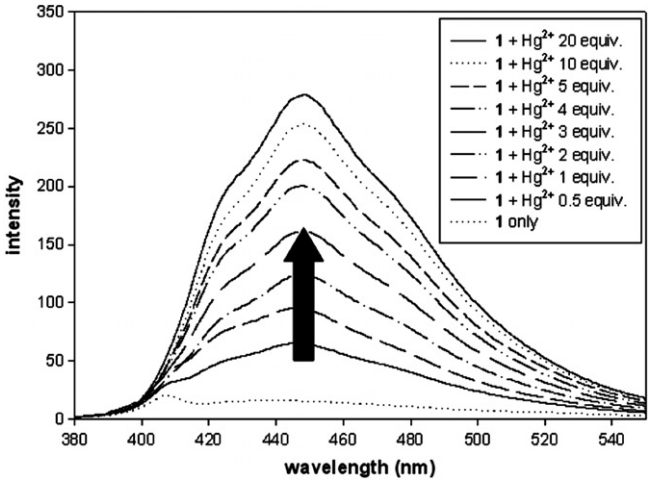


Fig. 3. Fluorescent titrations of compound **1** (3 μM) with Hg^{2+} in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v) (excitation at 356 nm).

calculated to be 1.18×10^5 and $4.48 \times 10^3\ M^{-1}$ {errors <10%, 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v)}, respectively.[12] On the other hand, for compound **2**, Cd^{2+} displayed a larger CHEF effect than that of Hg^{2+} when 100 equiv of each metal ion was added. In a similar way, the association constants of compound **2** with Hg^{2+} (Fig. 4) and Cd^{2+} (Fig. 5) were calculated to be $>10^7$ and $3.28 \times 10^4\ M^{-1}$ {errors <10%, 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v)}, respectively.[10] The job plots show 1:1 binding of these hosts with either Hg^{2+} or Cd^{2+}. Compound **2** displayed an extremely high association constant with Hg^{2+}, which was also preserved in the presence of excess other metal ions (50 equiv of Cd^{2+} as well as 100 equiv of Ca^{2+}, Mg^{2+} and Zn^{2+}). Even though the association constant of **2** with Hg^{2+} is about >500 times larger than that with Cd^{2+}, the CHEF effect with Hg^{2+} (~6 times) is much smaller than that with Cd^{2+} (~12 times). The lack of an X-ray crystal structure of the complex and the broadness of ^1H NMR peaks in the aliphatic region prohibit a clear explanation for this smaller CHEF effect with Hg^{2+}. The smaller CHEF effect may be attributed to the fact that a PET quenching from benzylic nitrogens is still available in **2**-Hg^{2+}. This difference may come from stronger affinity of sulfur in **2** towards Hg^{2+} compared with that of oxygen in **1** and a relatively larger cavity size of azathiacrown. Hg^{2+} can possibly coordinate to central nitrogen and four sulfur atoms. Figure 6 displays the ^1H NMR changes of **2** in aromatic region when Hg^{2+} was added in DMSO-d_6. All the peaks of acridine moiety moved downfield, for example, H-9 moved from 9.09 ppm to 9.49 ppm when 2 equiv of Hg^{2+} was added.

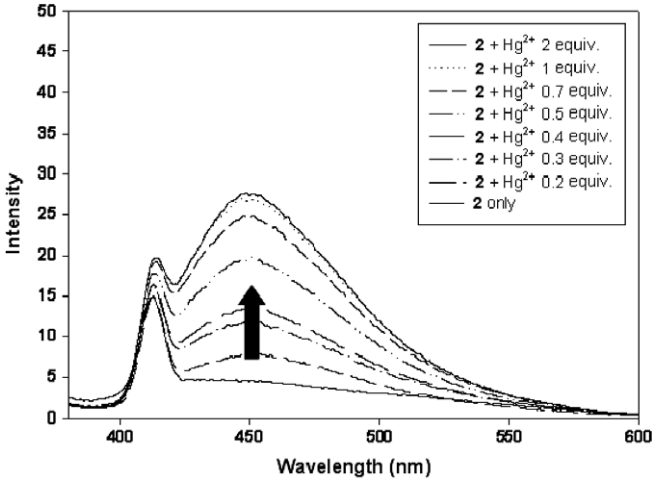


Fig. 4. Fluorescent titrations of compound **2** (3 μM) with Cd^{2+} in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v) (excitation at 356 nm).

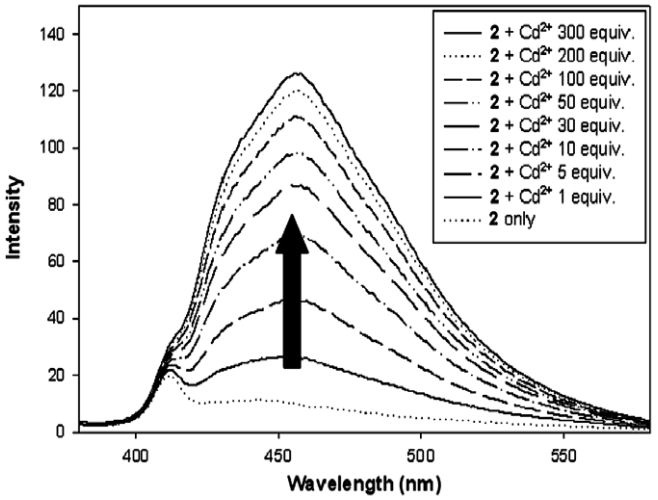


Fig. 5. Fluorescent titrations of compound **2** (3 μM) with Hg^{2+} in 0.1 M HEPES (pH 7.4)-DMSO (95:5, v/v) (excitation at 356 nm).

The ability of compounds **1** and **2** to detect Cd^{2+} and Hg^{2+} in mammalian cells was also studied (Fig. 7). Human keratinocyte cell line HaCaT and human colon cancer cells (HCT-116) were cultured in RPMI1640 and human neuroblastoma cell line SK-N-SH was cultured in MEM, which were supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% heat-inactivated fetal bovine serum. The cells were treated with 50 μM of **1** and **2** for 3 h and washed three times with PBS. Then the cells were incubated with 50–200 μM Hg^{2+} or Cd^{2+} for 1 h. The cell cultures were washed with PBS to remove the remaining Cd^{2+} and Hg^{2+}. As shown in Figure 7, there was no fluorescence in cells treated with only sensor. When cells were incubated with Hg^{2+} or Cd^{2+} after adding sensors, fluorescence enhancement was observed not only in the normal cell lines but also in the cancer cell line. The sensor **1** displayed a large fluorescent enhancement with Hg^{2+}. On the other hand, sensor **2** displayed a significant fluorescent enhancement with Cd^{2+}. Even though the binding of **2** with Hg^{2+} is much better than that with Cd^{2+}, we observed a small fluorescent change in the cell due to the relatively smaller optical change with Hg^{2+}.

In conclusion, we report two new acridine derivatives bearing immobilized azacrown or azathiacrown ligand as

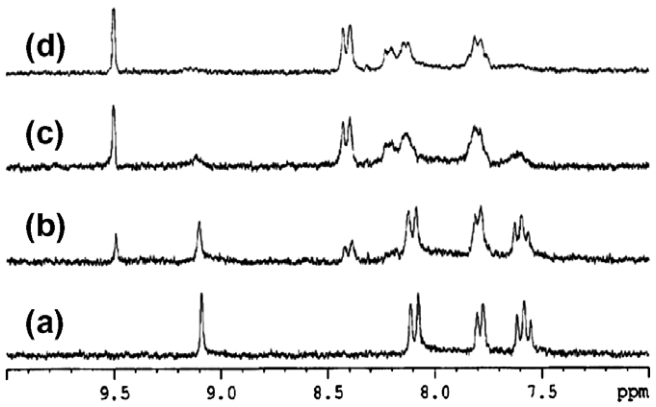


Fig. 6. Partial ^1H NMR (250 MHz) spectra of compound **2** (3 mM) with Hg^{2+} in DMSO-d_6: (a) **2** only; (b) **2** + Hg^{2+} (0.4 equiv); (c) **2** + Hg^{2+} (1 equiv); (d) **2** + Hg^{2+} (2 equiv).

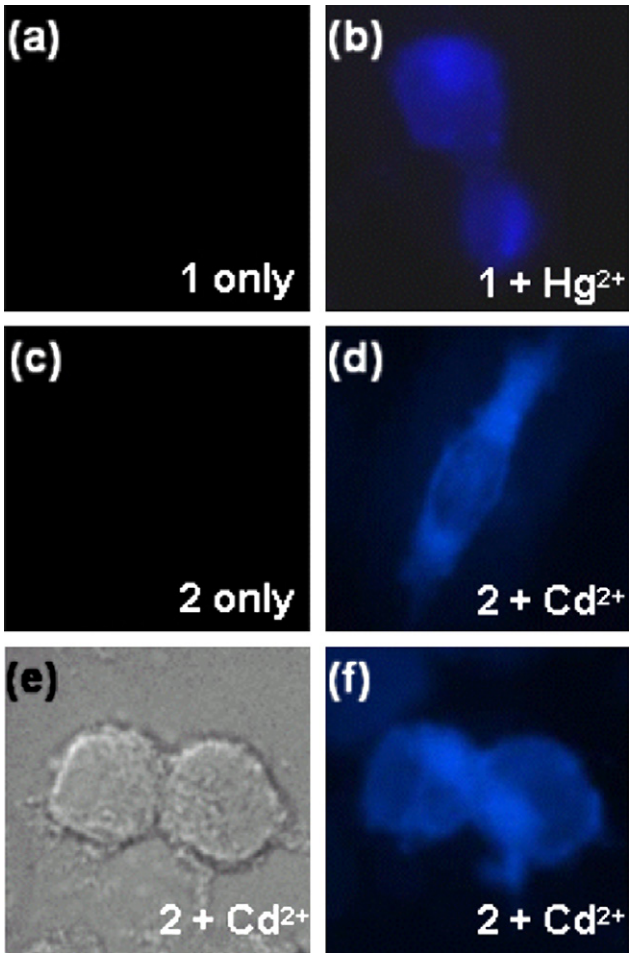


Fig. 7. (a) Image of sensor **1** only in HaCaT; (b) image of sensor **1** (50 μM) with Hg^{2+} (50 μM) in HaCaT; (c) image of sensor **2** only in SK-N-SH; (d) image of sensor **2** (50 μM) with Cd^{2+} (50 μM) in SK-N-SH; (e) bright-field image of sensor **2** (50 μM) with Cd^{2+} (50 μM) in human colon cancer cell line HCT-116; (f) fluorescence image of (e).

fluorescent chemosensors for Hg^{2+} and Cd^{2+} at pH 7.4. Compounds **1** and **2** displayed large CHEF effects with Hg^{2+} and Cd^{2+} among the metal ions examined. The association constants of compound **1** with Hg^{2+} and Cd^{2+} were calculated to be 1.18×10^5 and $4.48 \times 10^3\ M^{-1}$, on the other hand, the association constants of compound **2** with Hg^{2+} and Cd^{2+} were calculated to be $>10^8$ and $3.28 \times 10^4\ M^{-1}$, respectively. Cooperative binding from an immobilized ligand and nitrogen on acridine may provide such selectivity. Furthermore, we demonstrated their applications for the detection of Hg^{2+} and Cd^{2+} ions in mammalian cells. These new fluorescent sensors for toxic metal ions could be beneficial for biological and environmental applications.

Acknowledgments

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), Korea Ministry of Environment as 'The Eco-technopia 21 project' and BK21.

Supplementary data

The NMR spectra, fluorescence spectra. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.11.158.

References and notes

1. (a) *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993; (b) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302; (c) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1994**, *23*, 197; (d) 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; (e) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551; (f) Kim, J. S.; Quang, D. T. *Chem. Rev.* **2007**, *107*, 3780.
2. Renzoni, A.; Zino, F.; Franchi, E. *Environ. Res.* **1998**, *77*, 68.
3. Malm, O. *Environ. Res.* **1998**, *77*, 73.
4. *Cadmium in the Human Environment: Toxicity and Carcinogenicity*; Nordberg, G. F., Herber, R. F. M., Alessio, L., Eds.; Oxford University Press: Oxford, 1992.
5. (a) Wu, J. S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org. Lett.* **2007**, *9*, 907; (b) Soh, J. H.; Swamy, K. M. K.; Kim, S. K.; Kim, S.; Lee, S.-H.; Yoon, J. *Tetrahedron Lett.* **2007**, *48*, 5966; (c) Wang, J.; Qian, X. *Org. Lett.* **2006**, *8*, 3721; (d) Song, K. C.; Kim, J. S.; Park, S. M.; Chung, K.-C.; Ahn, S.; Chang, S.-K. *Org. Lett.* **2006**, *8*, 3413; (e) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859; (f) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S.-K. *Org. Lett.* **2006**, *8*, 371; (g) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760; (h) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030; (i) Mello, J. V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124; (j) Guo, X. G.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272; (K) For other references see Ref. 1e.
6. (a) Peng, X.; Du, J.; Fan, J.; Wu, Y.; Wang, J.; Zhao, J.; Xu, T. *J. Am. Chem. Soc.* **2007**, *129*, 1500; (b) Bronson, R. T.; Michaelis, D. J.; Lamb, R. J.; Husseini, G. A.; Farnsworth, P. B.; Linford, M. R.; Bradshaw, J. S.; Savage, P. B. *Org. Lett.* **2005**, *7*, 1105; (c) Youn, N. J.; Chang, S.-K. *Tetrahedron Lett.* **2005**, *46*, 125; (d) Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Org. Lett.* **2003**, *5*, 4065; (e) Kim, S. K.; Lee, J. H.; Yoon, J. *Bull. Korean Chem. Soc.* **2003**, *24*, 1032; (f) Costero, A. M.; Andreu, R.; Monrabal, E.; Martinez-Manez, R.; Sancenon, F.; Soto, J. *J. Chem. Soc., Dalton Trans.* **2002**, 1769; (g) Choi, M.; Kim, M.; Lee, K. D.; Han, K.-N.; Yoon, I.-A.; Chung, H.-J.; Yoon, J. *Org. Lett.* **2001**, *3*, 3455.
7. Chiron, J.; Galy, J.-P. *Synlett* **2003**, 2349.
8. *Compound* **1**. *Procedure A*: The solution of 4,13-diaza-18-crown 6-ether (0.36 g, 1.36 mmol) in chloroform (20 mL) followed by 4 equiv of anhydrous K_2CO_3 was added to the clear solution of 4,5-bisbromomethyl-acridine **3**[7] (0.5 g, 1.36 mmol) in anhydrous chloroform (40 mL). The reaction mixture was stirred at room temperature under nitrogen atmosphere until the disappearance of reactants (~48 h, TLC). After filtration of the solid, solvent was evaporated under vacuum. The gummy solid obtained was purified on basic alumina column using CH_2Cl_2 + MeOH (99:1) as eluent. The 0.54 g (84%) light yellowish gummy solid **1** was obtained after the evaporation of the solvent: ^1H NMR (250 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.87 (t, 4H, $J = 7.7$ Hz), 7.51 (t, 2H, $J = 7.2$ Hz), 4.71 (s, 4H), 3.28-3.71 (m, 16H), 3.09 (t, 8H, $J = 6.1$ Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 147.1, 139.5, 135.9, 130.7, 127.1, 126.3, 125.4, 70.4, 69.6, 55.1, 54.9; HRMS (FAB) $m/z = 466.2707$ $(M+H)^+$, calcd for $C_{27}H_{36}N_3O_4 = 466.2706$.

9. *Compound* **2**: Application of procedure A to 1.10-diaza-4,7, 14,17-tetrathiacyclooctadecane (0.44 g, 1.36 mmol) and 4,5-bis-bromomethyl-acridine **3** (0.5 g, 1.36 mmol) gave 0.53 g (70%) of **2** as an oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.89 (dd, 2H, $J = 8.5$ Hz and 1.2 Hz), 7.69 (d, 2H, $J = 6.8$ Hz), 7.45 (dd, 2H, $J = 8.3$ Hz and 6.8 Hz), 4.57 (s, 4H), 3.03 (t, 8H, $J = 6.9$ Hz), 2.74 (m, 8H), 2.46 (m, 8H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 147.2, 136.6, 134.7, 131.3, 127.9, 126.5, 125.3, 55.9, 53.2, 32.5, 29.4; HRMS (FAB) $m/z = 530.1786$ $(M+H)^+$, calcd for $C_{27}H_{36}N_3S_4 = 530.1792$.

10. Park, M. S.; Swamy, K. M. K.; Lee, Y. J.; Lee, H. N.; Jang, Y. J.; Moon, Y. H.; Yoon, J. *Tetrahedron Lett.* **2006**, *47*, 8129.
11. (a) Szarvas, S.; Majer, Z.; Huszthy, P.; Vermes, B.; Hollósi, M. *Enantiomer* **2002**, *7*, 241; (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.
12. (a) Conners, K. A. *Binding Constants*; Wiley: New York, 1987; (b) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom.