Tetrahedron Letters 51 (2010) 3550–3554

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A FRET fluorescent chemosensor SPAQ for Zn^{2+} based on a dyad bearing spiropyran and 8-aminoquinoline unit

Jian-Fa Zhu, Han Yuan, Wing-Hong Chan *, Albert W. M. Lee

Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 19 March 2010
Revised 27 April 2010
Accepted 29 April 2010
Available online 4 May 2010

ABSTRACT

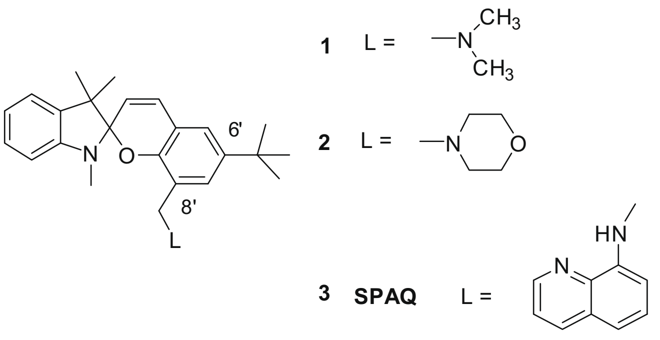
A novel FRET fluorescent sensor **SPAQ** containing 8-aminoquinoline (donor) and spiropyran derivative (acceptor) was designed and synthesized for detecting Zn^{2+}. The probe successfully exhibited a fluorescence turn on and ratiometric response for Zn^{2+} in ethanol solution with high selectivity. Upon excitation at 370 nm, the modulation of the emission intensity of **SPAQ** at 645 and 470 nm were achieved in the presence of Zn^{2+} by fluorescent resonance energy transfer (FRET) and chelation enhanced fluorescence (CHEF) effects.

© 2010 Elsevier Ltd. All rights reserved.

The design and synthesis of metal chemosensors with high selectivity and sensitivity is an active field of supramolecular chemistry.[1] Especially of considerable importance are sensing probes targeting heavy and/or transition metal (HTM) ions, such as zinc cations.[2] Despite having many commercial Zn^{2+} sensors, chemists have continued their efforts in developing new sensing devices to improve their sensitivity, selectivity, and application scope.[3] Due to the intrinsic sensitivity of fluorescent turn-on sensors, such a design has been drawn much attention in the sensor community. Most of the currently reported Zn^{2+} fluorescent turn-on sensors have operated on chelation enhanced fluorescence (CHEF) effect or inhibition of photo-induced electron transfer (PET) quenching pathway as a result of the metal binding event.[3a,c] Recently, a number of ratiometric fluorescent Zn^{2+} sensors, which allow the quantitation of Zn^{2+} by measuring the ratio of fluorescence intensities at two different wavelengths upon metal binding, have been reported.[3d,g,4] A ratiometric detection not only can increase the selectivity and sensitivity of a measurement, but also can eliminate most of the possible variability due to differences in instrumental efficiency and content of the effective dye.[5] Therefore, there is a huge demand and potential for exploring novel fluorophores for ratiometic Zn^{2+} sensing.

Spiropyrans (SPs) are photochromic compounds that can isomerize in response to UV–Vis irradiation as a result of a reversible heterolytic cleavage of the spiro C–O bond followed by cis–trans isomerization which generates a metastable merocyanine (ME).[6] By appending an additional receptive site in position C8′ on the benzopyran moiety, the isomerization of SP to ME could be modulated by specific metal ions. The metal ion mediated ring opening of SP to ME is characterized by its color change from colorless to red, recognized readily by the naked-eyes. Spiropyran-based chemosensors for alkali, alkaline earth, and lanthanides metals have been reported.[7] We have found that by superseding the commonly used nitro-group in C6′-position with a *tert*-butyl group, a more photostationary closed SP form can be resulted. Such a photostationary SP derivative is an ideal molecular platform for the design of molecular/ion optical sensing probes. In this context, we have developed novel spiropyran-based chemosensors **1** and **2** for the colorimetric and fluorescent detection of Cu^{2+} (Scheme 1).[8] To continue our interests in the sensor development, herein, a new fluorogenic sensor **SPAQ** (**3**), capable of illustrating Zn^{2+}—triggered fluorescence resonance energy transfer (FRET) changes has been synthesized. Based on the FRET changes, **SPAQ** bearing spiropyran–aminoquinoline moieties can respond selectively to zinc ion in ethanol solution to give ratiometric fluorescence output signals.[9]

To achieve the required selectivity towards a specific metal ion, it is necessary to complement the optical and binding properties of the spiropyran backbone with an appropriate multi-ligating group such that optical signal changes can be induced in response to the metal recognition event. Commercially available 8-aminoquinoline



Scheme 1. Structures of spiropyran-based fluorescent sensors.

---

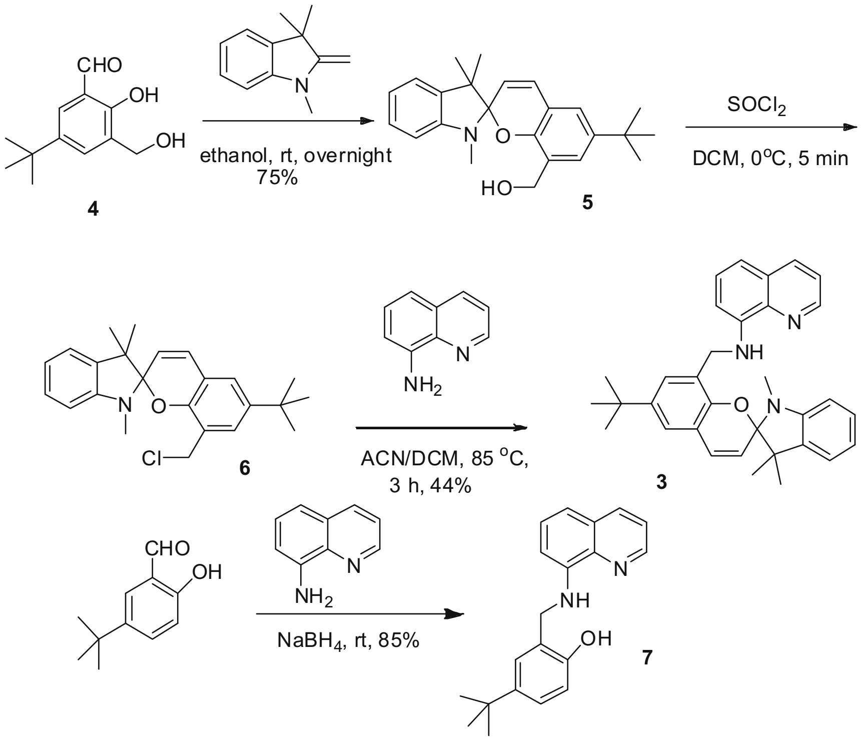
* Corresponding author. Tel.: +825 34117076; fax: +825 34117348.
E-mail address: whchan@hkbu.edu.hk (W.-H. Chan).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.04.127

is a judicious choice to be appended on the spiropyran scaffold for the design of fluorescent Zn^{2+} sensor. After conjugating 8-aminoquinoline to spiropyran backbone via a CH_2 spacer at the C8′ carbon, the resultant sensor **3, SPAQ** on one hand would possess a tridentate 'ONN' binding site for Zn^{2+} and on the other hand would provide opportunity to construct a FRET relay between the two fluorophoric subunits present in the system. To assemble the sensing probe, the requisite spiropyran precursor **6** was prepared from 2-hydroxy-3-hydroxymethyl-5-*tert*-butylbenzaldehyde (**4**) in two synthetic operations as shown in Scheme 2.[10] Condensation reaction between **4** and *N*-methyl-2,3,3-trimethylindolenine in ethanol gave good yield of spiropyran derivative **5** which underwent a fast nucleophilic substitution reaction with thionyl chloride to afford the corresponding chloride **6**. The crude labile **6** without purification was allowed to conjugate with 8-aminoquinoline to give rise to multifunctional spiropyran-based fluorescent sensor **SPAQ** (**3**) in 44% yield.[11] On the other hand, controlled compound **7**, without the spiropyran subunit, bearing the same tridentate 'ONN' binding site as the sensor was synthesized by reductive amination of 4-*t*-butylsalicyaldehyde with 8-aminoquinoline in the presence of sodium borohydride.

The metal mediated interconversion of the colorless functionalized SP to the red colored ME is well known to be solvent dependent. Thus, the metal binding property of sensor **3** was initially investigated in acetonitrile and ethanol solution separately. The formation of red colored ME derivative was observed readily by adding 1 equiv of a variety of different transition metal ions to the colorless ACN solution of **SPAQ**. In contrast, in ethanol solution of the probe, among a total of 13 metal cations being studied (vide infra), only Cu^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} can immediately turn the color of the solution from colorless to red (Fig. S1, ESI). To evaluate the viability of using **SPAQ** for detecting Zn^{2+} in ethanol solution, its signal response toward Zn^{2+} was recorded in both absorption and emission spectra. As shown in Figure 1, two broad absorption peaks centered at 380 and 540 nm emerged when **SPAQ** was titrated with increasing concentration of Zn^{2+}. On the basis of non-linear fitting, the binding constant was found to be $7.2 \times 10^4\ M^{-1}$. Moreover, a Job's plot, which exhibits a maximum at 0.5 mole fraction of Zn^{2+}, indicates that a 1:1 complex is formed (Fig. S2, ESI).

On the other hand, when excited at 520 nm, upon addition of 5 equiv of Zn^{2+} to **SPAQ** solution, ca. 170-fold increase in fluorescence intensity of **SPAQ** at 645 nm was observed. The emergence of this fluorescent turn-on signal as a result of the formation of sensor–Zn^{2+} complex can be rationalized as a combination of internal charge transfer (ICT) and chelation enhanced fluorescence (CHEF).[12] Other metal ions being studied was unable to turn on the fluorescent signal of the sensor (Fig. S3, ESI), apparently, **SPAQ** is well qualified as a fluorescent turn-on chemosensor for detecting Zn^{2+}. To unravel whether the metal binding event will affect the photophysical properties of the 8-aminoquinoline moiety, the fluorescent signal response of the controlled compound **7** toward Zn^{2+} was recorded with excitation at 370 nm. Upon the addition of 10 equiv of Zn^{2+}, the emission peak at 470 nm was reduced only by about 10% (Fig. S4, ESI). When excited at 370 nm, the emission spectrum of **SPAQ** overlaps significantly with the long wave absorption peak of **SPAQ**–Zn^{2+} (Fig. S5, ESI). To explore the possibility of developing a ratiometric Zn^{2+} sensor, we examined whether fluorescence energy transfer (FRET) occurs in our sensing dyad by exciting the quinoline moiety at 370 nm. As shown in Figure 2, upon increasing the concentration of Zn^{2+}, the long wave fluorescent peak at 645 nm of the probe enhances with concomitant reduction of its short wave fluorescent peak at 470 nm. A distinctive isoemissive peak at 570 nm is evident. When 5 equiv of Zn^{2+} was added, the ratio of the emission intensity at 645 and 470 nm ($I_{645\ nm}/I_{470\ nm}$) increased from 0.05 (free **SPAQ**) to 3.26 (Zn^{2+}/**SPAQ**—complex) (inset in Fig. 2). Additionally, the extent of fluorescence quenching of **SPAQ** caused by Zn^{2+} at 470 nm was much greater than that of the controlled compound. Conceivably, in addition to the Inner Filter Effect exhibited by the merocyanine moiety of **SPAQ**, a fairly efficient FRET appeared to take place. Significantly, the validity of **SPAQ** as a ratiometric fluorescent chemosensor for Zn^{2+} can be established.



Scheme 2. Synthesis of sensor **SPAQ** (**3**).

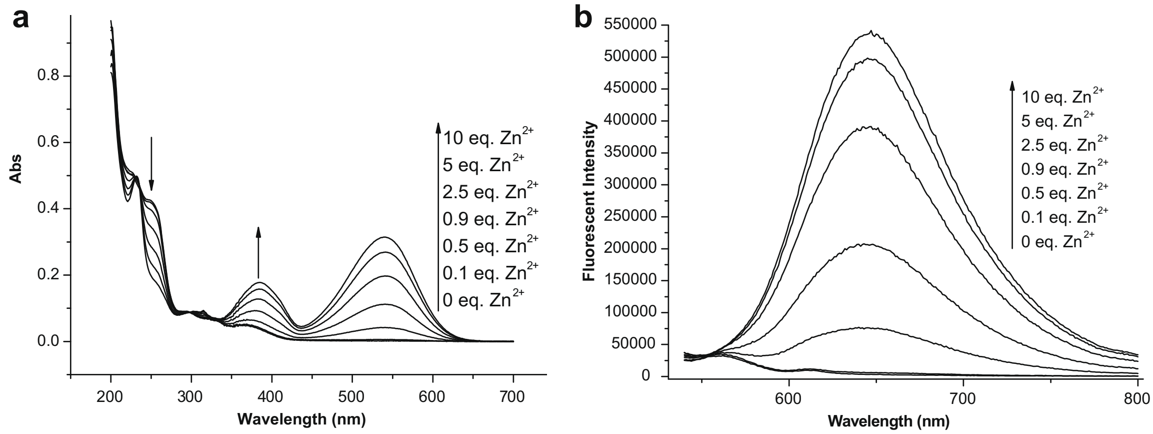


Figure 1. (a) UV–Vis spectra of **SPAQ** (20 μM) upon the titration of Zn^{2+} (0–10 equiv) in ethanol solution; (b) fluorescence emission spectra (λ_{ex} = 520 nm) of **SPAQ** (10 μM) upon the titration of Zn^{2+} (0–10 equiv) in ethanol solution.

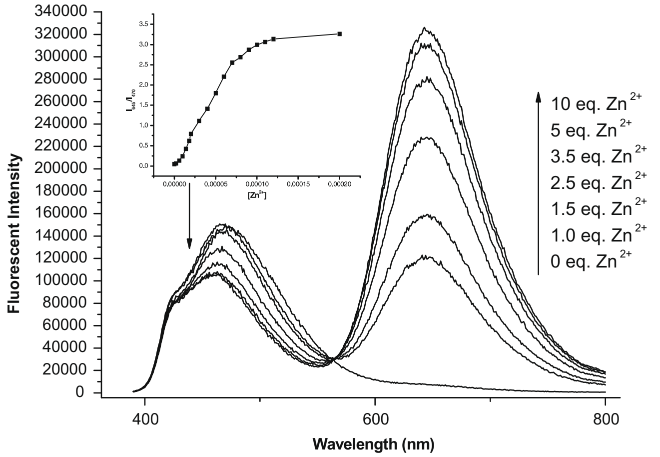


Figure 2. Fluorescence emission spectra (λ_{ex} = 370 nm) of **SPAQ** (20 μM) upon the titration of Zn^{2+} (0–10 equiv) in ethanol solution. (Inset) Ratiometric calibration curve $I_{645\ nm}/I_{470\ nm}$ as a function of Zn^{2+} concentration.

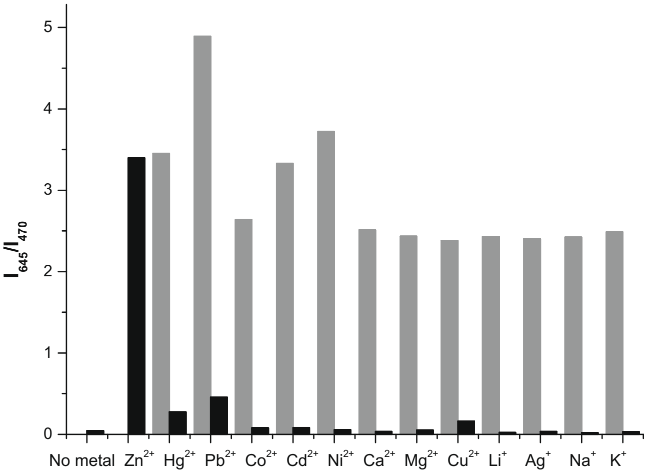


Figure 3. Metal ion selectivity profiles of **SPAQ** (20 μM) in the presence of various metals ions in ethanol solution (λ_{ex} = 370 nm): (black bars) fluorescence emission intensity ratio of 645 nm to 470 nm in the presence of 5 equiv of Zn^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Li^{+}, Ag^{+}, Na^{+} and K^{+}; (grey bars) fluorescence emission intensity ratio of 645–470 nm in the presence of 5 equiv of Zn^{2+}, followed by adding 5 equiv of Hg^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Li^{+}, Ag^{+}, Na^{+} and K^{+}.

The selectivities of **SPAQ** to various metal ions were examined systematically. Among 13 metal ions being studied, upon excitation at 370 nm, only Zn^{2+} induced a dramatic fluorescence change of **SPAQ** with respect to the ratio of the emission intensity at 645 and 470 nm and a much less variation (i.e., <0.5) was obtained by interacting the probe with Pb^{2+} and Hg^{2+} separately. Common competing metal ions in Zn^{2+} determination such as Mg^{2+}, Ca^{2+} and Cd^{2+} did not respond to **SPAQ**. The titration of sensor **SPAQ** with Zn^{2+} in the presence of potential competing metal ions was further determined. The results shown (red bar, Fig. 3) revealed that except Pb^{2+} all other common cations did not significantly change the effect by the existence of 5 equiv of Zn^{2+}.

Furthermore, the binding mode of the complex was studied by mass and ^1H NMR spectroscopic method. Convincing evidence on the 1:1 binding mode of the complex of sensor **SPAQ** and Zn^{2+} was obtained by the observation of a peak of [**SPAQ**-Zn-ClO_4]$^+$ of *m*/*z* 652.2 from FAB-MS method (Fig. S6, ESI).

The poor solubility of zinc salts in ethanol forced us to conduct the NMR binding experiments in CD_3CN-d_3. By using 2D ^1H NMR techniques (i.e., ^1H–1H COSY and ^1H–1H NOESY), we are able to assign all individual proton signals of **SPAQ** and **SPAQ**–Zn^{2+} complex (Fig. S7, ESI). Upon addition of 1 equiv of Zn^{2+} to the CD_3CN-d_3 solution of **SPAQ**, all aromatic protons underwent a different extent of downfield shift (Fig. 4). Particularly, a huge downfield shift of the H_{15} proton from 6.57 to 7.59 was observed as a result of changing the closed SP form of the sensor to the opened metal complexed ME form. In consistent with the ring opening isomerization of the spiropyran moiety triggered by Zn^{2+} ion, the two doublets at δ 5.74 and 6.93 ascribed to the vinyl protons of the dihydropyran ring of **SPAQ** almost coalesced as a multipet at 7.61 and a doublet at 8.78 which was assigned to the two vinyl protons of the opened metal complex (i.e., H_{11} and H_{12}). A large vinyl coupling constant of 15.9 Hz observable for H_{12} confirmed the trans double bond character of the metal complex. A notable downfield shift of the *N*-methyl group signal from 2.71 to 3.91 was observed which corroborated with the formation of a pyrrolidium ring. The 'ONN' binding mode of the sensor to metal as proposed in Figure 5 can be rationalized by observing the shift of the crucial protons. For instance, after coordinating with Zn^{2+}, the broad singlet assigned to the N–H (6.45 ppm) experienced a large upfield shift to 5.14 whereas the CH_2 adjacent to the amino group (4.24 ppm) split as a doublet of triplets, presumably due to the restriction of bond rotation as a result of complex formation. The resonates at 6.57 and 7.00 ascribed to the proton *ortho*-(H_6) and *para*-(H_4) to the amino group underwent a large downfield shift

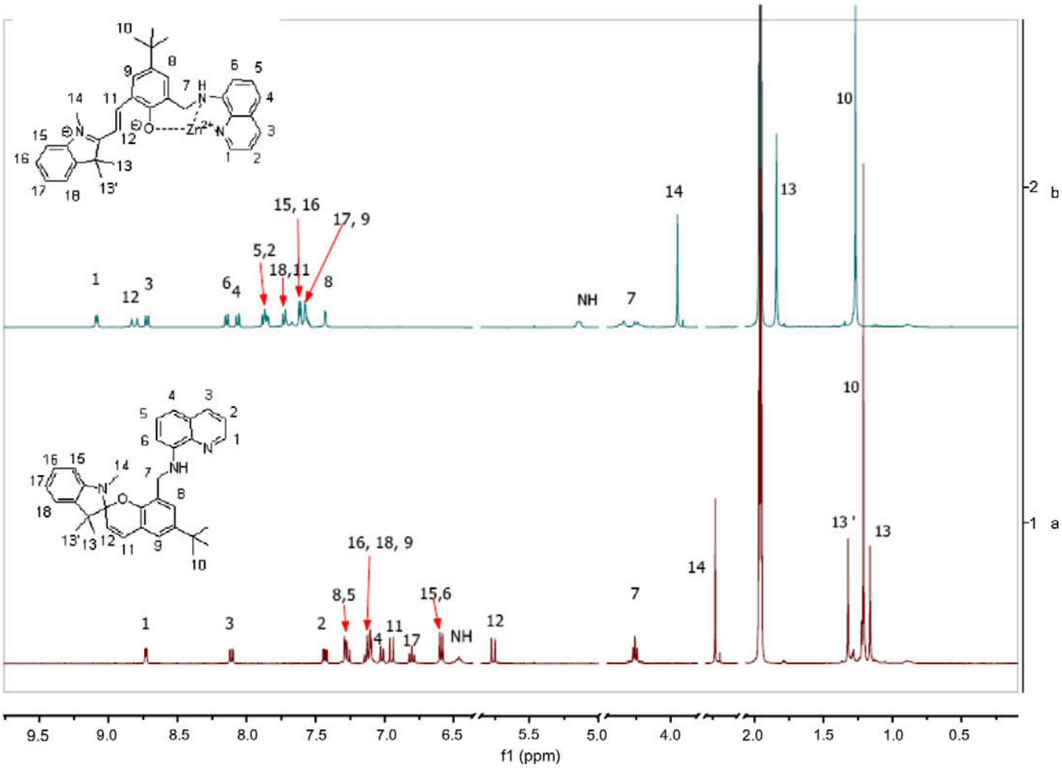


Figure 4. Partial ^{1}H NMR spectra (400 MHz) of **SPAQ** (20 mM) in CD_3CN-d_3: (a) free **SPAQ**; (b) **SPAQ** + 1 equiv of Zn^{2+}.

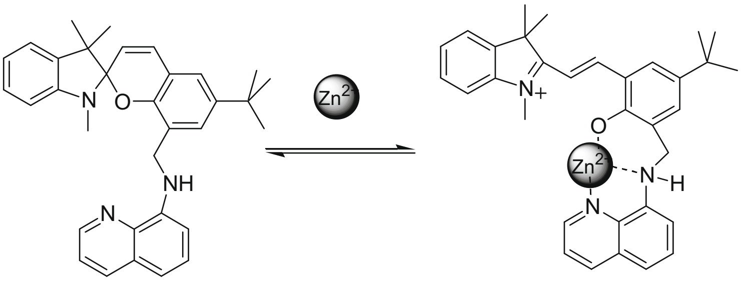


Figure 5. Binding model of **SPAQ** and Zn^{2+}.

to 8.12, 8.04, respectively. The binding of Zn^{2+} by the quinoline N promoted a downfield shift for all aromatic protons in the heterocyclic ring (i.e., H_1, H_2 and H_3). Interestingly, the H_8 and H_9 of phenoxy ring underwent a relatively small downfield shift (i.e., less than 0.3 ppm), indicating that the binding of electron rich phenoxy group with the metal only slightly reduce the electron density of the aromatic ring. Further addition of Zn^{2+} into the 1:1 Zn^{2+}–**SPAQ** solution did not induce any additional change in its NMR spectrum (Fig. S8, ESI). Thus, the 1:1 binding mode is further substantiated.

In conclusion, we have developed a new sensitive and selective fluorescent sensor **SPAQ** for detecting Zn^{2+}. Depending on the excitation wavelength, in response to Zn^{2+}, **SPAQ** can behave either as a fluorescent turn-on sensor or as a FRET ratiometric fluorescent sensor. The binding mode of the metal complex was established by combined UV–Vis, fluorescence and ^{1}H NMR spectroscopic method.

Acknowledgement

The work was supported by a grant from the Research Grant Council of Hong Kong (HKBU 200407).

Supplementary data

Supplementary data (UV–Vis spectra of **SPAQ** interacting with various metal ions, a Job's plot between **SPQA** and Zn^{2+}, fluorescence spectra of **SPAQ** and compound **7** upon the titration of Zn^{2+} and other metal ions, overlapping of the emission spectrum of **SPAQ** with the absorption spectrum of **SPAQ**–Zn^{2+}, FAB-MS spectrum of **SPAQ**–Zn^{2+}, ^{1}H–1H NOESY spectra of **SPAQ** and **SPAQ**–Zn^{2+}; ^{1}H NMR titration of **SPAQ** with Zn^{2+}; ^{1}H NMR, ^{13}C NMR and HRMS spectrum of **SPAQ**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.127.

References and notes

1. (a) For reviews, see: *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993; (b) 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; (c) Martinez-Manez, R.; Sancanon, F. *Chem. Rev.* **2003**, *103*, 4419; (d) Gunnlaugsson, T. A.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094.

2. Selected examples: (a) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831; (b) Jiang, P.; Chen, L.; Lin, J.; Liu, Q.; Ding, J.; Gao, X.; Guo, Z. *Chem. Commun.* **2002**, 1424; (c) Ohshima, A.; Momotake, A.; Arai, T. *Tetrahedron Lett.* **2004**, *45*, 9377.
3. Selected recent examples: (a) Parkesh, R.; Lee, T. C.; Gunnlaugsson, T. *Org. Biomol. Chem.* **2007**, *5*, 310; (b) Liu, Y.; Zhang, N.; Chen, Y.; Wang, L.-H. *Org. Lett.* **2007**, *9*, 315; (c) Wang, H.-H.; Gan, Q.; Wang, X.-J.; Xue, L.; Liu, S.-H.; Jiang, H. *Org. Lett.* **2007**, *9*, 4995; (d) Zhang, Y.; Guo, X.; Si, W.; Jia, L.; Qian, X. *Org. Lett.* **2008**, *10*, 473; (e) Xue, L.; Liu, C.; Jiang, H. *Org. Lett.* **2009**, *11*, 1655; (f) Wang, J.; Ha, C.-S. *Tetrahedron* **2009**, *65*, 6959; (g) Xu, Z.; Baek, K.-H.; Kim, H. N.; Cui, J.; Qian, X.; Spring, D. R.; Shin, I.; Yoon, J. *J. Am. Chem. Soc.* **2010**, *132*, 601.
4. (a) Huang, S.; Clark, R. J.; Zhu, L. *Org. Lett.* **2007**, *9*, 4999; (b) Liu, Z.; Zhang, C.; Li, Y.; Wu, Z.; Qian, F.; Yang, X.; He, W.; Gao, X.; Guo, Z. *Org. Lett.* **2009**, *11*, 795; (c) Joshi, J. P.; Park, J.; Lee, W. I.; Lee, K.-H. *Talanta* **2009**, *78*, 903.
5. *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.Probe Design and Chemical Sensing; Plenum: New York, 1994; Vol. 4,.
6. (a) Raymo, F. M.; Giordani, S. *J. Am. Chem. Soc.* **2001**, *123*, 4651; (b) Giodani, S.; Raymo, F. M. *Org. Lett.* **2003**, *5*, 3559; (c) Minkin, V. I. *Chem. Rev.* **2004**, *104*, 2751.
7. (a) Kimura, K.; Teranishi, T.; Yokoyama, M.; Yajima, S.; Miyake, S.; Sakamoto, H.; Tanaka, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 199; (b) Tanaka, M.; Makamura, M.; Salhin, M. A.; Ikeda, T.; Kamada, K.; Ando, H.; Shibutani, Y.; Kimura, K. *J. Org. Chem.* **2001**, *66*, 1533; (c) Ahmed, S. A.; Tanaka, M.; Ando, H.; Tawa, K.; Kimura, K. *Tetrahedron* **2004**, *60*, 6029; (d) Sakamoto, H.; Takagaki, H.; Nakamura, M.; Kimura, K. *Anal. Chem.* **2005**, *77*, 1999; (e) Kimura, K.; Uraumi, T.; Teranishi, T.; Yokoyama, M.; Sakamoto, H.; Okamoto, M.; Arakawa, R.; Moriguchi, H.; Miyaji, Y. *Angew. Chem., Int. Ed.* **1997**, *36*, 2452.
8. (a) Shao, N.; Zhang, Y.; Cheung, S.; Yang, R. H.; Chan, W. H.; Mo, T.; Li, K.; Liu, F. *Anal. Chem.* **2005**, *77*, 7294; (b) Shao, N.; Jin, J. Y.; Wang, H.; Zhang, Y.; Yang, R. H.; Chan, W. H. *Anal. Chem.* **2008**, *80*, 3466.
9. Recent examples of FRET-based fluorescence metal ion sensors: (a) Mokhir, A.; Kiel, A.; Herten, D.-P.; Kraemer, R. *Inorg. Chem.* **2005**, *44*, 5661; (b) Banthia, S.; Samanta, A. *J. Phys. Chem. B* **2006**, *110*, 6437; (c) Lee, S. Y.; Kim, H. J.; Wu, J.-S.; No, K.; Kim, J. S. *Tetrahedron Lett.* **2008**, *49*, 6141; (d) Jung, J. H.; Lee, M. H.; Kim, H. J.; Jung, H. S.; Lee, S. Y.; Shin, N. R.; No, K.; Kim, J. S. *Tetrahedron Lett.* **2009**, *50*, 2013.
10. Amato, M. E.; Ballistreri, F. P.; Pappalarda, A.; Sciotto, D.; Tomaselli, G. A.; Toscano, R. M. *Tetrahedron* **2007**, *63*, 9751.
11. Spectral data for **SPAQ**: Mp: 61–63 °C; ^{1}H NMR (400 MHz, CDCl$_3$): 8.68 (1H, dd, *J* = 4.2, 1.7 Hz), 8.01 (1H, dd, *J* = 8.3, 1.7 Hz), 7.33 (1H, dd, *J* = 8.2, 4.2 Hz), 7.23 (2H, m), 7.08 (1H, td, *J* = 7.6, 1.2 Hz), 7.00 (1H, dd, *J* = 7.3, 0.8 Hz), 6.98–6.94 (2H, m), 6.85 (1H, d, *J* = 10.2 Hz), 6.77 (1H, td, *J* = 7.5, 0.8 Hz), 6.49 (1H, d, *J* = 7.7 Hz), 6.43 (2H, m), 5.68 (1H, d, *J* = 10.2 Hz), 4.29 (2H, dd, *J* = 12.9, 6.1 Hz), 2.70 (3H, s), 1.32 (3H, s), 1.21 (9H, s), 1.18 (3H, s); ^{13}C NMR (100 MHz, CDCl$_3$): 149.52, 148.11, 146.59, 144.76, 142.28, 138.25, 136.76, 135.87, 129.81, 128.56, 127.79, 127.39, 126.34, 124.13, 122.43, 121.37, 121.22, 118.97, 118.84, 117.84, 113.53, 106.75, 105.13, 104.39, 51.35, 42.30, 34.01, 31.47, 29.01, 25.82, 20.44. HRMS (MALDI-TOF): calcd for [M+1]$^+$ of C$_{33}$H$_{35}$N$_3$O, 490.2853; found, 490.2870.
12. Aoki, S.; Kagata, D.; Shiro, M.; Takeda, K.; Kimura, E. *J. Am. Chem. Soc.* **2004**, *126*, 13377.