Tetrahedron Letters 53 (2012) 2001–2004

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Both visual and ratiometric fluorescent sensor for Zn^{2+} based on spirobenzopyran platform

Jian-Fa Zhu, 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 28 December 2011
Revised 27 January 2012
Accepted 7 February 2012
Available online 14 February 2012

Keywords:
Ratiometric
Fluorescent
Zn^{2+} chemosensor
Spirobenzopyran

ABSTRACT

A ratiometric fluorescent Zn^{2+} chemosensor, **SPQH**, based on spirobenzopyran platform, was synthesized. In aqueous HEPES 7.4 buffer solution, upon chelation with Zn(II), **SPQH** demonstrates high selectivity and subnanomolar sensitivity for zinc ion with 36-fold enhancement in the NIR fluorescence output.

Design and synthesis of fluorescent chemosensors with desirable properties is an active field of supramolecular chemistry owing to their potential applications in cell physiology, analytical, environmental, and biological chemistry.[1] Behind iron, zinc is the second most abundant d-block metal ion in the human body, and it plays diverse roles in biological processes.[2–5] However, physiological roles of Zn^{2+} in the human body are still far from well understood.[6]

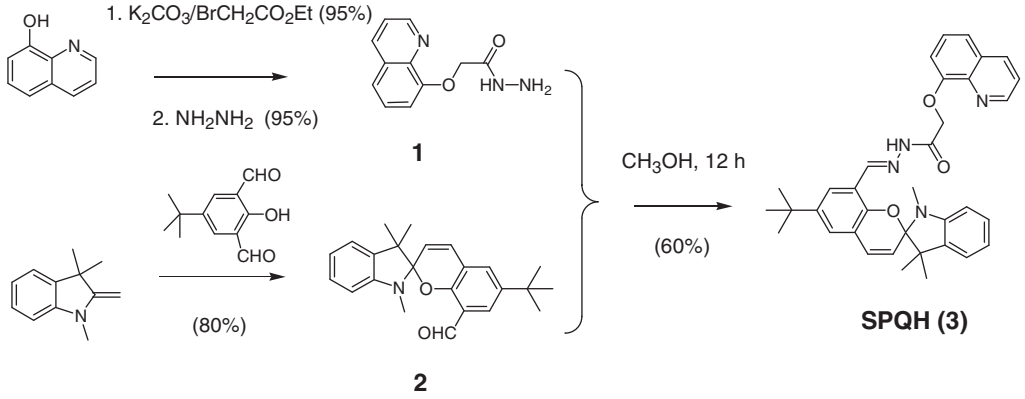
In particular, studies on various aspects of zinc in neurobiology have recently emerged as a multi-disciplinary cutting edge research field.[7] The significance of zinc hemeostasis to neuro-physiology and neuropathology has attracted much interest in devising new ways to detect Zn^{2+} in biological samples. Owing to their high sensitivity, fluorescent chemosensors have been extensively developed and recognized as one of the viable means for detecting micromolar or subnanomolar concentration of Zn^{2+}.[8] Among these recent publications of Zn^{2+}chemosensors, some Zn^{2+} chemosensors suffered from the cross interference of Cd^{2+} and/or Cu^{2+}.[8e,f,k–m] It is noteworthy that Lippard and coworkers have pioneered in this field by utilizing fluorescein as the fluorophoric platform for developing an array of Zn^{2+} chemosensors, allowing quantitative measurements of intracellular changes in zinc metal ion concentration.[8l] Ratiometric sensor in which the ratio between two emission intensities can be used to evaluate the analyte concentration and provide a built-in correction for environmental effects is a widely sought after sensing probe.[9] To date, however, relatively few small-molecule ratiometric Zn^{2+} probes are available.[8f,g,10] Additionally, to eliminate the autofluorescence phenomenon in cell environment sensors capable of exhibiting near infrared (NIR) emissive output signal are intensively sought after. To our knowledge, only one ratiometric NIR Zn^{2+} sensor is known, no biological applications of this probe were or are reported as of yet.[11] Recently, exploiting the unique optical properties of multifunctional spiropyran derivatives,[12] we have developed a water soluble ratiometric probe for Cu^{2+} and Zn^{2+}, respectively.[13]

To continue our quest for novel fluorescence Zn^{2+} probe, we have defined the attributes of such probes as: (1) it should be readily accessible synthetically; (2) it should be operative in aqueous pH 7.4 buffer solution; (3) it must be a sensitive and selective probe; (4) it should display fluorescence ratiometric response to Zn^{2+} and (5) the output signal of the probe ideally can be in the NIR region (i.e., >650 nm). To this end, here, by appending an elaborated metal binding motif onto the spirobenzopyran molecular platform, we demonstrate that a NIR emission ratiometric Zn^{2+} probe, a spirobenzopyran quinolone hydrazine derivative, **SPQH** (**3**) is able to detect the subnanomolar concentration of Zn^{2+} in aqueous pH 7.4 HEPES buffer solution.

We and others have shown that the novel design of spirobenzopyran-based specific metal binding optical probes can be realized by incorporating metal ligating group on the C-8 position of the spirobenzopyran scaffold.[12b,c,13,14] Among metal ion chelates, di(2-picolyl)amine (DPA) ligand was widely chosen for binding Zn^{2+}. Because the DPA moiety is well known for its binding affinity to Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+},[8e,f,i–n,15] we deliberately avoided the use of this ligand in the design of the probe. On this background and our recent work,[8g,13b] as shown in Scheme 1, a 8-hydroxyquinoline-based multi-dentate ligand 2-(8-quinolinoxy)ethanohydrazide (**1**) was prepared and its subsequent condensation with 8-carboaldehyde spirobenzopyran derivative **2**

* Corresponding author. Tel.: +852 34117076; fax: +852 34117348.
E-mail address: whchan@hkbu.edu.hk (W.-H. Chan).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2012.02.031



Scheme 1. Synthesis of **SPQH** (**3**).

Figure 1. Naked eye detection of Zn^{2+}, Cu^{2+}. (a) Color changes of 10 μM **SPQH** upon the addition of 0, 500 nM, 1 μM, 5 μM, 10 μM zinc ion in buffer solution (50 mM, HEPES, 40% methanol, pH 7.4) (b) Color changes of 10 μM **SPQH** upon the addition of 0, 500 nM, 1, 5, 10 μM copper ion in buffer solution (50 mM, HEPES, 40% methanol, pH 7.4).

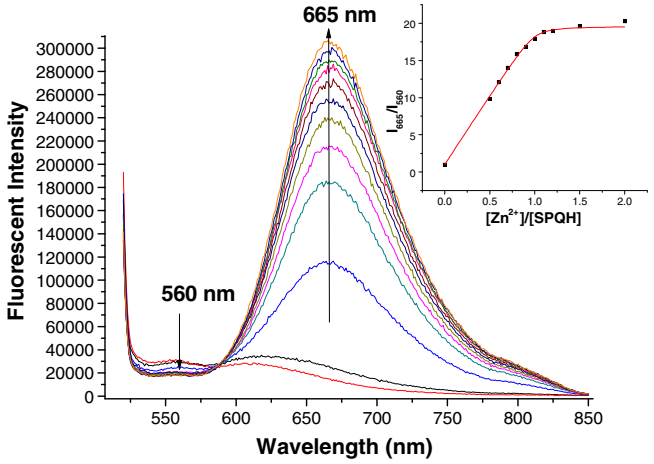


Figure 2. Emission spectra of **SPQH** (10 μM) at increasing concentration of Zn^{2+} (0, 0.1, 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.5, 2.0 equiv) (50 mM HEPES buffer, 40% ethanol, pH 7.4). Inset: emission ratio change of $I_{665\ nm}/I_{560\ nm}$ with increasing concentration of Zn^{2+}. Excitation wavelength was 510 nm with 10 nm slit width.

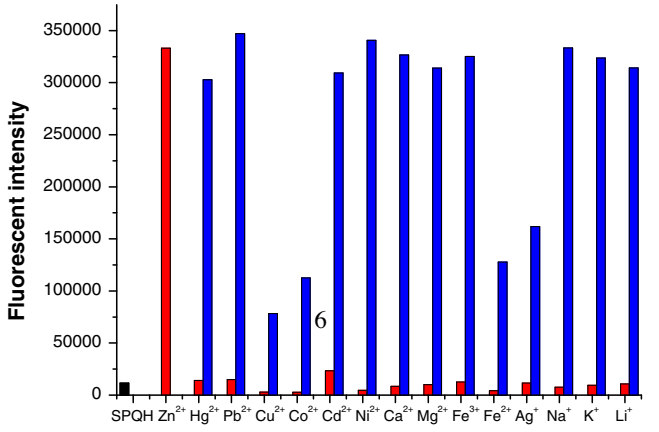


Figure 3. Metal ion selectivity profiles of **SPQH** (10 μM) in the presence of various metal ions in buffer solution (50 mM, HEPES, 40% methanol, pH 7.4): (a) (black bar) fluorescence intensity at 665 nm of **SPQH** as the control; (b) (red bars) fluorescence intensity at 665 nm of the probe in interacting with 5 equiv of Zn^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Fe^{2+}, Ag^{+}, Na^{+}, K^{+}, Li^{+}; (c) (blue bars) fluorescence intensity of the probe in the mixture containing separately 5 equiv of Ag^{+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Li^{+}; 500 equiv of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Fe^{2+} in the presence of 5 equiv of zinc ions.

afforded **SPQH** in a 60% yield in a convergent manner (Supplementary data). **SPQH** was fully characterized by NMR and HRMS method (Figs. S1–S3).[16,17]

In aqueous ethanol solution (50 mM HEPES buffer, 40% ethanol, pH 7.4), only Zn^{2+} and Cu^{2+} can mediate the ring opening of colorless spiropyran form of **SPQH** triggering its formation of the pink colored metal chelated merocyanine complex. As shown in Figure 1, naked-eye selective and sensitive detection of these two ions can be actualized. The absorption spectra of **SPQH** and Zn^{2+}–**SPQH** (Fig. S4)[16] show a dramatic change from the visible transparent pattern of the apo-ligand to the emergence of a broad peak at 550 nm of the metal–merocyanine complex. Additionally, the

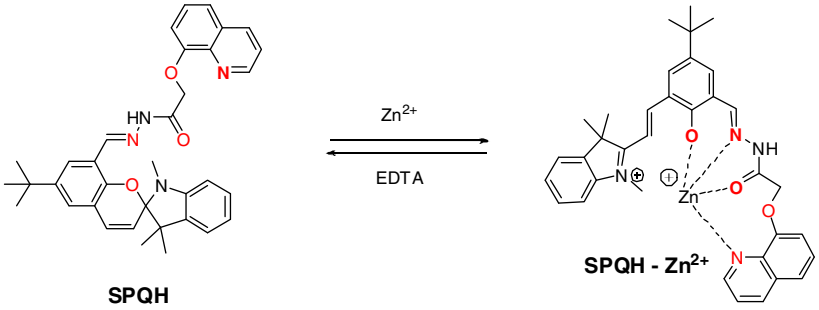


Figure 4. Possible binding model of **SPQH**–Zn^{2+}.

brightness of the metal complex at 550 nm was found to be 215.6 M^{-1} cm^{-1} (0.0069 × 31335 M^{-1} cm^{-1}).

The binding characteristics of **SPQH** toward Zn^{2+} in aqueous HEPES buffer solutions were examined by fluorescent titrations. Figure 2 revealed that **SPQH** can be developed as a ratiometric sensor for Zn^{2+}. Upon gradual addition of Zn^{2+} into 10 μM **SPQH** measuring solution, excited with 510 nm, the relatively weak emission peak of the sensor at 560 nm decreased with the concomitant formation of new peak at 665 nm. A large Stokes shift of 110 nm, which is rarely observed in the sensor, is apparent. On further addition of the Zn^{2+} probe, the ratiometric change of the fluorescence spectra became evident with a clear isoemission point at 585 nm. The observed bathochromatic shift of the probe after binding with Zn^{2+} is conceivably induced by intramolecular charge transfer (ICT).[18] The emission ratio of $I_{665\ nm}/I_{560\ nm}$ records a 36-fold signal enhancement and remained at a plateau in the presence of more than 1 equiv of Zn^{2+} (inset, Fig. 2). The Job's plot confirms the 1:1 binding stoichiometry between **3** and Zn^{2+}, consistent with the fluorescent titration results (Fig. S5).[16] On the basis of non-linear fitting of the titration curve of 1:1 binding model, the dissociation constant of Zn^{2+}–**SPQH** was computed to be (1.34 ± 0.70) × 10^{-7} M (R^2 = 0.9888), indicating that the probe can detect Zn^{2+} in the subnanomolar range.

The fluorescence of the metal–ligand complex attains a constant value in the biological relevant pH range (i.e., 6.5–8.0, Fig. S6).[16] To define the application scope of the probe in metal sensing, interference studies were undertaken. Figure 3 shows that all biological relevant cations (i.e., Na^+, K^+, Ca^{2+}, and Mg^{2+}) even in 500 equiv excess are non-responsive to the probe. Among common transition metals, moderate quenching on the fluorescence of the probe was caused only by Ag^+, Co^{2+}, Fe^{2+}, and Cu^{2+}.

To evaluate the binding mode of **SPQH**–Zn^{2+} complex, detailed ^1H NMR titration was carried out. Upon gradual addition of Zn^{2+} to the CD_3OD-d_4/D_2O (8:2) solution of **SPQH**, consistent with metal-induced transformation of spiropyran to Zn^{2+} merocyanine complex, the pyranyl protons (at δ 6.63 and 6.85) and N–CH_3 resonate at 2.78 recorded a substantial downfield shift. As a result of metal chelation, all protons in the vicinity of the ligating groups (i.e., phenoxy-oxygen, imino-nitrogen, carboxy-oxygen, pridinyl-*N*) underwent different extent of shift. Further addition of Zn^{2+} with up to 1 equiv to the probe, most of the proton resonates of the complex became unchanged (Fig. S7).[16] The chemical induced shift (CIS) of selected protons of the probe as the function of [Zn^{2+}]/[**SPQH**] shed light on the mode of interaction (Fig. S8).[16] Importantly, all these observations suggested the direct interaction between Zn^{2+} and the ligating groups of the probe. Convincing evidence on the 1:1 binding mode of the complex of **SPQH** and Zn^{2+} was obtained by the MALDI-TOF HRMS spectroscopic method. When the complex was subjected to mass spectral measurement, a clear peak of m/z 723.1563 corresponding to [M + Zn^{2+} + ClO_4^-]$^+$ was observed (Fig. S9).[16] On the basis of the combined spectroscopic information, the binding mode of **SPQH**–Zn^{2+} is proposed in Figure 4. Finally, the reversibility of binding between the probe and Zn^{2+} was established by successive treatment with EDTA and Zn^{2+} (Fig. S10).[16]

In summary, we developed a novel spirobenzopyran-based ratiometric NIR Zn^{2+} chemosensor **SPQH** that effectively and selectively recognizes Zn^{2+} under aqueous conditions. On the basis of the low dissociation constant of **SPQH**–Zn^{2+} complex, the probe can detect zinc ion in the subnanomolar concentration range. Due to the visible excitation, great fluorescence enhancement of the emission at the NIR region and the large Stokes shift, the probe will be useful for chemical and biochemical researches.

Acknowledgements

The financial supports from the Research Grant Council of Hong Kong (HKBU 200407) and Hong Kong Baptist University (FRGI/10-11/026) are acknowledged.

Supplementary data

Supplementary data (synthesis, experimental details, ^1H, ^{13}C NMR and HRMS spectra of **SPQH**, naked-eye detection of Zn^{2+} and Cu^{2+} by **SPQH**, NMR titration data and HRMS of metal complex) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.031.

References and notes

1. (a)*Chemosensors of Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; NATO ASI Series C492, 1997; (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) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588; (d) Demchenko, A. P. *Introduction to Fluorescence Sensing*; Springer: New York, 2008.
2. (a) Berg, J. M.; Shi, Y. *Science* **1996**, *271*, 1081–1085; (b) Andrews, G. K. *Biometals* **2001**, *14*, 223–237; (c) Wu, Z. K.; Zhang, Y. F.; Ma, J. S.; Yang, G. D. *Inorg. Chem.* **2006**, *45*, 3140–3142.
3. Truong-Tran, A. Q.; Carter, J.; Ruffom, R. E.; Zalewski, P. D. *Biometals* **2001**, *14*, 315–330.
4. Falchuk, K. H. *Mol. Cell. Biochem.* **1998**, *188*, 41–48.
5. Frederickson, C. J.; Bush, A. I. *Biometals* **2001**, *14*, 353–366.
6. (a) Ngwendson, J. N.; Amiot, C. L.; Srivastava, D. K.; Banerjee, A. *Tetrahedron Lett.* **2006**, *47*, 2327–2330; (b) Roy, P.; Dhara, K.; Manassero, M.; Ratha, J.; Banerjee, P. *Inorg. Chem.* **2007**, *46*, 6405–6412.
7. (a) Ueno, S.; Ysukamoto, M.; Hirano, T.; Kikuchi, K.; Yamada, M. K.; Nishiyama, N.; Nagano, T.; Matsuki, N.; Ikegaya, Y. *J. Cell. Biol.* **2002**, *158*, 215–220; (b) Que, E. L.; Domaille, D. W.; Chang, C. J. Chem. Rev. 2008, 108, 1517–1549 and references therein.
8. (a) Ohshima, A.; Momotake, A.; Arai, T. *Tetrahedron Lett.* **2004**, *45*, 9377–9381; (b) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 12470–12476; (c) Royzen, M.; Durandin, A.; Young, V. G., Jr.; Geacintov, N. E.; Canary, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 3854–3855; (d) Liu, Y.; Zhang, N.; Chen, Y.; Wang, L-H. *Org. Lett.* **2007**, *9*, 315–318; (e) Wang, H.-H.; Gan, Q.; Wang, X.-J.; Xue, L.; Liu, S.-H.; Jiang, H. *Org. Lett.* **2007**, *9*, 4995–4998; (f) Huang, S.; Clark, R. J.; Zhu, L. *Org. Lett.* **2007**, *9*, 4999–5002; (g) Zhang, Y.; Guo, X.; Si, W.; Jia, L.; Qian, X. *Org. Lett.* **2008**, *10*, 473–476; (h) Yu, M.-M.; Li, Z.-

X.; Wei, L.-H.; Wei, D.-H.; Tang, M.-S. *Org. Lett.* **2008**, *10*, 5115–5118; (i) Xue, L.; Wang, H.-H.; Wang, X.-J.; Jiang, H. *Inorg. Chem.* **2008**, *47*, 4310–4318; (j) Liu, Z.; Zhang, C.; Li, Y.; Wu, Z.; Qian, F.; Yang, X.; He, W.; Gao, X.; Guo, Z. *Org. Lett.* **2009**, *11*, 795–798; (k) Xue, L.; Liu, C.; Jiang, H. *Org. Lett.* **2009**, *11*, 1655–1658; (l) Nolan, E. M.; Lippard, S. *J. Acct. Chem. Res.* **2009**, *42*, 193–203; (m) 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*, 601–610; (n) Zhou, X.; Yu, B.; Guo, Y.; Tang, X.; Zhang, H.; Liu, W. *Inorg. Chem.* **2010**, *49*, 4002–4007.

9. (a) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440–3450; (b) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712–713.
10. Chang, C. J.; Lippard, S. J. *Metal Ions Life Sci.* **2006**, *1*, 281–320.
11. Kiyose, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 6548–6549.
12. (a) Shao, N.; Jin, J. Y.; Cheung, S. M.; Yang, R. H.; Chan, W. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4944–4948; (b) Sakata, T.; Jackson, D. K.; Mao, S.; Marriott, G. *J. Org. Chem.* **2008**, *73*, 227–233; (c) Tian, Z.; Wu, W.; Wan, W.; Li, A. D. Q. *J. Am. Chem. Soc.* **2009**, *131*, 4245–4252; (d) Shao, N.; Jin, H.; Wang, J.; Zeng, J.; Yang, R. H.; Chan, W. H. *J. Am. Chem. Soc.* **2010**, *132*, 725–736; (e) Shao, N.; Wang, H.; Gao, X.; Yang, R. H.; Chan, W. H. *Anal. Chem.* **2010**, *82*, 4628–4636.
13. (a) Shao, N.; Jin, J. Y.; Wang, H.; Zhang, Y.; Yang, R. H.; Chan, W. H. *Anal. Chem.* **2008**, *80*, 3466–3475; (b) Zhu, J.-F.; Yuan, H.; Chan, W.-H.; Lee, A. W. M. *Org. Biomol. Chem.* **2010**, *8*, 3957–3964.
14. (a) Tanaka, M.; Ikeda, T.; Xu, Q.; Ando, H.; Shibutani, Y.; Nakamura, M.; Sakamoto, H.; Yajima, S.; Kimura, K. *J. Org. Chem.* **2002**, *67*, 2223–2227; (b) Sakamoto, H.; Takagaki, H.; Nakamura, M.; Kimura, K. *Anal. Chem.* **2005**, *77*, 1999–2006; (c) Guo, Z.-Q.; Chen, W.-Q.; Duan, X.-M. *Org. Lett.* **2010**, *12*, 2202–2205.
15. Goldsmith, C. R.; Lippard, S. *J. Inorg. Chem.* **2006**, *45*, 555–561.
16. See Supplementary data.
17. **SPQH** (**3**) was isolated as yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.27 (s, 1H), 8.41 (s, 1H), 8.16 (m, 2H), 7.95 (d, J = 2.4 Hz, 1H), 7.54 (m, 2H), 7.43 (dd, J = 8.4, 4.0 Hz, 1H), 7.29 (dd, J = 7.2, 1.6 Hz, 1H), 7.20 (m, 2H), 7.11 (d, J = 2.8 Hz, 1H), 6.92–6.82 (m, 2H), 6.65 (d, J = 7.4 Hz, 1H), 5.74 (d, J = 10.4 Hz, 1H), 5.10–4.79 (m, 2H), 2.82 (s, 3H), 1.36 (s, 3H), 1.30 (s, 9H), 1.22 (s, 3H).; ^{13}C NMR (100 MHz, CDCl3) δ 164.7, 154.0, 151.0, 149.0, 148.3, 144.1, 143.0, 139.9, 136.9, 136.7, 129.8, 129.5, 127.7, 127.1, 126.4, 123.3, 122.1, 122.1, 121.7, 119.3, 119.1, 119.1, 118.5, 113.6, 106.9, 104.9, 70.7, 51.9, 34.2, 31.4, 29.0, 25.9, 20.4. HRMS (MALDI-TOF) for $C_{35}H_{36}N_4O_3$ calcd 561.2860 ($M+H^+$), found, 561.2854.
18. (a) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968–969; (b) Callan, J. F.; de Silva, A. P.; Ferguson, J.; Huxley, A. J. M.; O'Brien, A. M. *Tetrahedron* **2004**, *60*, 11125–11131; (d) Liu, Y.; Hau, H.; Zhang, H.-Y.; Yang, L.-X.; Jiang, W. *Org. Lett.* **2008**, *10*, 2873–2876.