Tetrahedron Letters 53 (2012) 804–807

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

A novel calixsalen macrocycle: metal sensing behavior for Zn^{2+} and intracellular imaging application

Yong Fu [a], Zhitao Xing [a], Chengcheng Zhu [a], Hongwei Yang [a], Weijiang He [a], Chengjian Zhu [a,b,*], Yixiang Cheng [a,c,*]

[a] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China
[b] State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China
[c] Key Laboratory of Mesoscopic Chemistry of MOE, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

ARTICLE INFO

Article history:
Received 12 October 2011
Revised 25 November 2011
Accepted 2 December 2011
Available online 8 December 2011

Keywords:
Calixsalen
Fluorescence
Recognition
Living cells

ABSTRACT

The chiral [3+3] macrocycle **1** which displays a calixarene-like crystal structure has been synthesized. The UV/Vis and fluorescence spectral studies show that **1** and Zn^{2+} have a 1:3 complex stoichiometry. It exhibits high selectivity toward Zn^{2+}, but no significant responses toward other competitive cations. The intracellular imaging ability has been tested in HeLa cells using a confocal microscope.

Introduction

The design and synthesis of artificial receptors for metal ions are of considerable importance in supermolecular chemistry due to their fundamental role in medical, environmental, and biological applications.[1] Especially, as the second most abundant transition metal ions in the human body, zinc(II) ion plays an essential role in cellular metabolism, gene expression, apoptosis, and neurotransmission.[2] Deregulation of zinc(II) homeostasis would be associated with a number of diseases, ranging from Alzheimer's disease[3] to prostate cancer[4] and diabetes.[5] Due to the importance of Zn^{2+} in numerous biological systems, there is a great emphasis placed on the development of chemosensors to determine Zn^{2+}. Given that the closed-shell $3d^{10}$ electronic configuration of Zn(II) renders it unable to give any spectroscopic or magnetic signals, fluorescence or UV/Vis spectroscopy is best suitable for Zn^{2+} detection in biological contexts or living systems. In recent years, much attention has been paid to the design of fluorescent probes for the detection of zinc ion so as to understand their biological roles.[6]

A number of fluorescence sensors containing quinoline,[7] fluorescein,[8] and peptides[9] for Zn^{2+} detection have been reported in previous literatures, but most of them are based on small molecules. Nowadays, numerous heteroanalogues as well as homologues of calixarenes exhibiting vase-like structures are known.[10] These compounds have been studied extensively for their applications in molecular recognition, host–guest chemistry, supermolecular structures, material chemistry, and catalysis. Among these macrocycles, especially, salen-based macrocycle molecules derived from diamines and hydroxydialdehydes possess the advantage that they can be obtained in different ring sizes and can be further functionalized at well-defined molecular level.[11] Although these salen-based macrocycles named 'calixsalens' have been widely used to synthesize mono-, di-, tri-, and tetranuclear macrocycle complexes, the calixsalen macrocycles have not been utilized for the fluorescence sensors.

In this Letter, we report a novel chiral [3+3] macrocycle **1** used as fluorescence probes specific for high selectivity to Zn^{2+} and the intracellular imaging ability in HeLa cells. To the best of our knowledge, this is the first calixsalen-type macrocycle sensor with turn-on fluorescence, high selectivity, and rapid response to zinc ion.

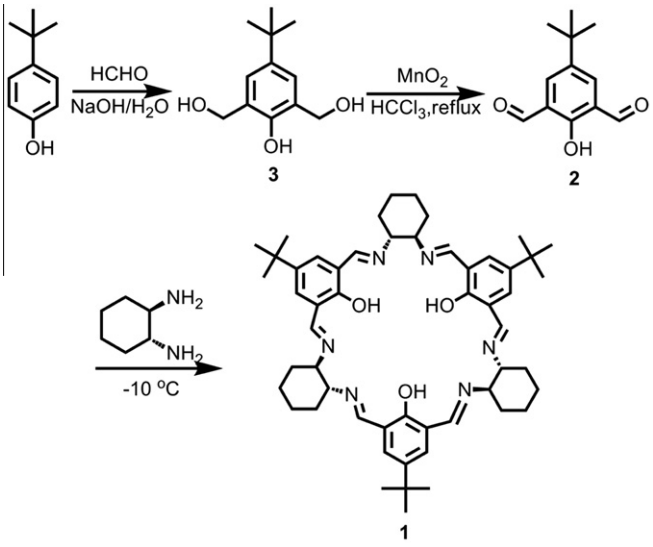
Results and discussion

Synthesis and feature of the chiral [3+3] macrocycle 1

The synthesis procedure of sensor **1** is shown in Scheme 1. Firstly, compound **2** is synthesized according to the literature.[12] Then, macrocycle **1** was prepared from *trans*-(1*R*,2*R*)-1,2-cyclohexanediamine and compound **2** in 99% yield by Schiff-base formation via nucleophilic addition–elimination reaction.[13] In this Letter we easily obtained the pure ligand [3+3] hexanuclear macrocycle **1**

* Corresponding authors. Tel.: +86 25 83594886; fax: +86 25 83317761 (C.Z.).
E-mail addresses: cjzhu@nju.edu.cna (C. Zhu), yxcheng@nju.edu.cn (Y. Cheng).

0040-4039/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2011.12.005



Scheme 1. Synthesis procedures of sensor **1**.

under −10 °C in high yield without further purification. The single crystal X-ray structure of **1** shows the exact structure for calixarenes and the 27-membered macrocycle (Fig. 1). The *C*3-symmetric neutral macrocycle contains three chiral-diamino backbones arranged in the form of an equilateral triangle, and the N_2O cavities are proximal to each other. The large size of these sub-cavities appears suitable for metal complexation as the two N and two O atoms provide appropriate coordination sites.

Absorption study

Figure 2 shows the spectral variation of **1** upon the gradual addition of $Zn(NO_3)_2$. The absorption spectrum of free sensor **1** exhibits two bands centered at 238 and 343 nm at room temperature in THF, which are associated with n–π* transition of C=N and π–π* transitions of benzene rings, respectively. To investigate the binding property of **1** toward Zn^{2+}, we measured the UV/Vis spectra of **1** (1×10^{-5} M) in the presence of various concentrations of

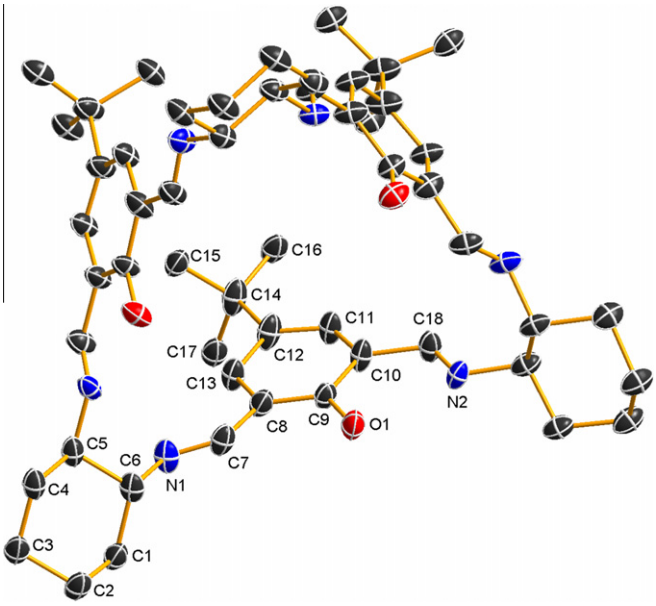


Figure 1. Single-crystal structure of sensor **1**. Hydrogen atoms and lattice solvent molecules have been omitted for clarity.

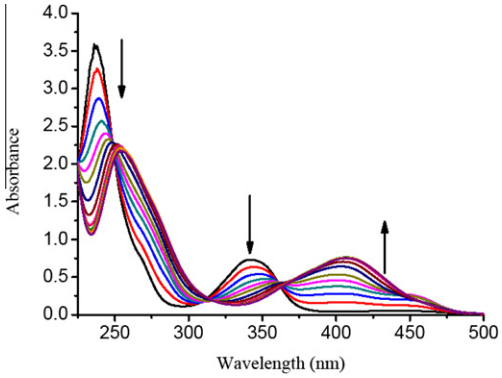


Figure 2. UV/Vis spectra of **1** (1×10^{-5} M) in THF with increasing amounts of $Zn(NO_3)_2$ (0–3.0 equiv).

Zn^{2+} (0–3.0 × 10^{-5} M), as shown in Figure 2. Upon the addition of increasing concentration of Zn^{2+} ion, the absorbance of **1** at 238 and 343 nm gradually decreases. Moreover, a new absorption band appears at 406 nm, and its absorbance gradually increases with the addition of Zn^{2+}. This absorption peak is likely due to the coordination of **1** and Zn^{2+}. The changes that occur in the UV/Vis spectra arise from the coordination of Zn^{2+} to the C=N sites, which increase its coplanarity of the conjugated system.

CD spectra

Figure 3 shows the CD spectra of **1** in the absence and presence of 3.0 equiv Zn^{2+} in THF. According to Figure 3, CD spectra of 1–Zn^{2+} appear great changes compared to free sensor **1**. The CD signal intensity gradually reduces by 30% and 42%, and the long wavelength CD effect of metal-chelated **1** appears the gradual red shift from 231 to 244 nm, 275 to 282 nm, respectively. The intensity at 357 nm appears slight changes, only red shift from 357 to 393 nm. The result demonstrates that the CD spectra of **1** show high sensitivity to Zn^{2+}. The obvious CD effect changes also indicate that Zn^{2+} can coordinate with **1** and produce an induced circular dichroism (ICD) in the absorption regions of **1**.

Fluorescent spectra and titration

The fluorescence titration of **1** with Zn^{2+} is performed. As shown in Figure 4, **1** displays a weak and broad emission band situated at 554 nm due to the extended π-electronic structure. The fluorescence intensities of **1** show gradual enhancement as high as 68-fold upon the concentration molar ratio addition of Zn^{2+} from 0 to 3.0 equiv. The nonlinear fitting of the titration curve also assumes a 1:3 stoichiometry for the 1–Zn^{2+} complex (Fig. S2, electronic

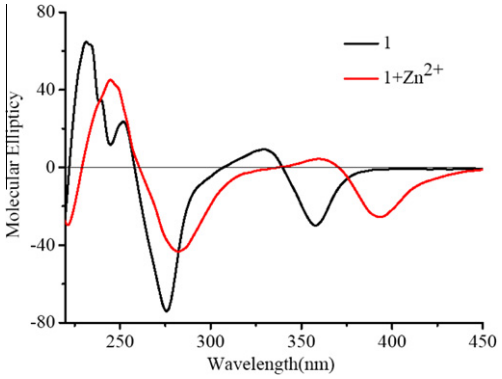


Figure 3. CD spectra of **1** (1×10^{-5} M) in the absence and presence of 3.0 equiv Zn^{2+} in THF solution.

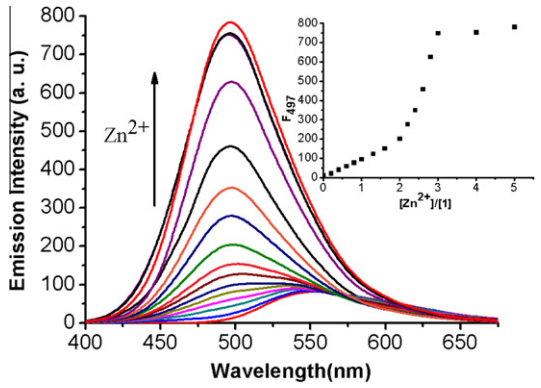


Figure 4. Fluorescence spectra of **1** (1×10^{-5} M) in THF with increasing amounts of Zn^{2+} (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.3, 1.6, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 4.0, 5.0 equiv) (λ_{ex} = 343 nm). Inset: the fluorescence intensity at 497 nm of **1** as a function of Zn^{2+} concentration.

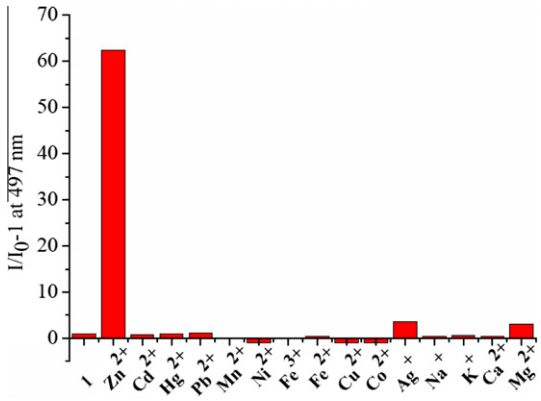


Figure 5. Emission change at 497 nm of **1** (1×10^{-5} M) in THF induced by different metal cations, λ_{ex} = 343 nm. The concentration of Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Co^{2+}, and Ag^{+} is 3×10^{-5} M, while that of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} is 1×10^{-3} M, respectively.

Supplementary data). The quantum yield of Zn^{2+}/**1** complex is ~0.134.[14] A competitive binding experiment gave an estimated K_d of 1.44×10^{-14} M for Zn^{2+}/**1** complex.[15] Moreover, the maximum emission wavelength of **1** is remarkably blue shifted from 554 to 497 nm along with a dramatic enhancement of fluorescence intensity when it is excited at 343 nm. The obvious fluorescent enhancement can be attributed to the ICT (intramolecular charge transfer) process of the Zn^{2+} coordination according to the correlative review.[16] Zn^{2+}-coordination to **1** should also enhance the photo-induced charge transfer (PCT) effect, trigger the emission change, and increase the emission intensity.

Fluorescence titration of 1 with different metal cations is also investigated (Fig. 5). Other different kinds of metal ions (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ag^{+}, Cd^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}) which probably interfere with the detecting result is performed under the similar conditions. As depicted in Figure S1 (ESI*), the addition of 3.0 equiv of Fe^{3+}, Hg^{2+}, and Pb^{2+} shows slight changes to the emission band of 1 at 497 nm. But Cu^{2+}, Co^{2+}, and Ni^{2+} cause a little bit quenching of the fluorescence intensity. Ag^{+} shows gradual enhancement as high as 3.6-fold at 539 nm. On the other hand, the presence of 100 equiv of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} do not interfere with its fluorescent response to Zn^{2+}.

Cellular experiment

The independent emission and the specific Zn^{2+}-amplified emission which is not interfered by the cell abundance of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}, suggest that **1** may be a candidate for intracellular Zn^{2+} imaging. The intracellular Zn^{2+} imaging behavior of macrocycle **1** on HeLa cells is studied with a laser scanning confocal microscope. After incubation with **1** solution (10 μM in PBS, DMSO/water 1:9, v/v) at 25 °C for 20 min, the HeLa cells display very faint intracellular fluorescence (Fig. 6). However, HeLa cells exhibit intensive fluorescence when exogenous Zn^{2+} is introduced into the cells via incubation with $ZnSO_4$/pyrithione solution. Moreover, the intensive fluorescence is deeply depressed by scavenging Zn^{2+} from the cells with the cell permeable metal chelator, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). These results indicate that **1** is an effective intracellular Zn^{2+} imaging agent with cell permeability. It also implies that macrocycle **1** could be an effective model fluorophore to construct ratiometric sensors for Zn^{2+}.

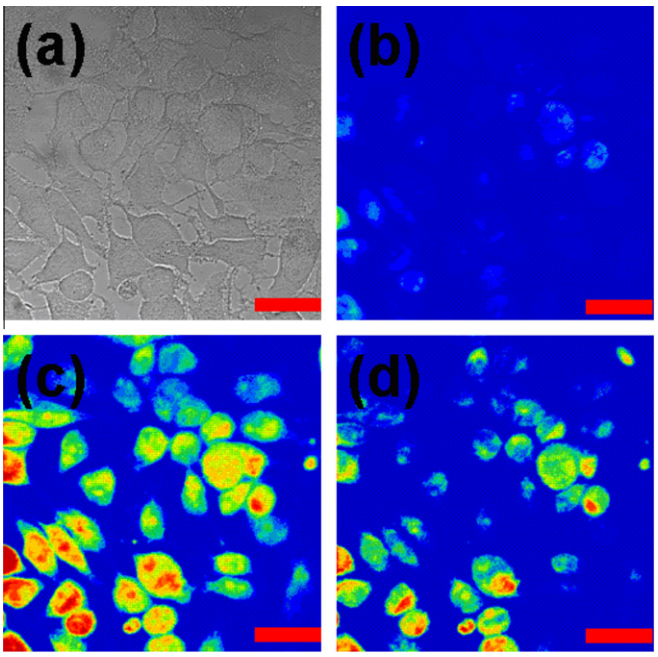


Figure 6. Confocal fluorescence imaging of HeLa cells: (a) brightfield transmission image of cells labeled with **1** (10 μM, PBS solution containing 10% DMSO) at 25 °C for 20 min; (b) fluorescence image of (a); (c) fluorescence image after incubation with 5 μM $ZnSO_4$/pyrithione (1:1) solution followed by rinse with 10 μM sensor **1** solution; (d) fluorescence image of HeLa cells in (c) followed by further incubation with 50 μM TPEN solution for 20 min. λ_{ex} = 488 nm. The transformation from light blue to brown denotes the emission enhancement, Bar = 20 μm.

Conclusions

In summary, a novel calixarene-like chiral [3+3] macrocycle **1** is synthesized, which is an excellent fluorescent sensor for the detection of Zn^{2+}. The UV/Vis and fluorescence spectral studies show that **1** and Zn^{2+} have a 1:3 complex stoichiometry. It produces a remarkably high selectivity toward Zn^{2+} ions over other competitive cations, such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^{+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}. Most importantly, the large Stokes shift and cell imaging ability of macrocycle **1** indicate that the novel calixarene-like structure could be a suitable platform to construct a novel sensor for specific cation imaging in biological systems.

Acknowledgments

We are very grateful for the support of this work from the National Natural Science Foundation of China (Nos. 20832001, 20972065, 21074054) and the National Basic Research Program of China (Nos. 2007CB925103, 2010CB923303) for their financial

support. The Major Scientific and Technological Special Project (No. 2009ZX09103-081) is also acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.005.

References and notes

1. (a) Silva, A. P. D.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademache, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566; (b) Amendola, V.; Fabbrizzi, L.; Forti, F.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Poggi, A.; Sacchi, D.; Taglieti, A. *Coord. Chem. Rev.* **2006**, *250*, 273–299.
2. (a) Berg, J. M.; Shi, Y. *Science* **1996**, *271*, 1081–1085; (b) O'Halloran, T. V. *Science* **1993**, *261*, 715–724; (c) Williams, R. J. P.; Fraústo da Silva, J. J. R. *Coord. Chem. Rev.* **2000**, *200–202*, 247–348; (d) Coleman, J. E. *Annu. Rev. Biochem.* **1992**, *61*, 897–946.
3. Sorensen, M. B.; Stoltenberg, M.; Juh, l. S.; Danscher, G.; Ernst, E. *Prostate* **1997**, *31*, 125–130.
4. Chausmer, A. B. *J. Am. Coll. Nutr.* **1998**, *17*, 109–115.
5. Ciglenečki, I.; Bura-Nakić, E.; Inzelt, G. *Electroanalysis* **2007**, *19*, 1437–1445.
6. (a) Kimura, E.; Kioke, T. *Chem. Soc. Rev.* **1998**, *27*, 179–184; (b) Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517–1549; (c) Jiang, P.; Guo, Z. J. *Coord. Chem. Rev.* **2004**, *248*, 205–229; (d) Lim, N. C.; Freake, H. C.; Bruckner, C. *Chem. Eur. J.* **2005**, *11*, 38–49.
7. (a) Jiang, P. J.; Chen, L. Z.; Lin, J.; Liu, Q.; Ding, J.; Gao, X.; Guo, Z. J. *Chem. Commun.* **2002**, 1424–1425; (b) Wang, H.; Gan, Q.; Wang, X.; Xue, L.; Liu, S.; Jiang, H. *Org. Lett.* **2007**, *9*, 4995–4998; (c) Zhang, Y.; Guo, X.; Si, W.; Jia, L.; Qian, X. *Org. Lett.* **2008**, *10*, 473–476.
8. (a) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831–7841; (b) Woodroofe, C. C.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 11458–11459.
9. (a) Shults, M. D.; Pearce, D. A.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 10591–10597; (b) Joshi, B. P.; Cho, W. M.; Kim, J.; Juyoung, Yoonc; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6425–6429.
10. (a) Jiang, J.; MacLachlan, M. J. *Org. Lett.* **2010**, *12*, 1020–1023; (b) Zhao, D.; Moore, J. S. *Chem. Commun.* **2003**, 807–818; (c) Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004**, *104*, 2723–2750; (d) van Veggel, F. C. J. M.; Verboom, W.; Reinhoudt, D. N. *Chem. Rev.* **1994**, *94*, 279–299; (e) Atwood, J. L.; Barbour, L. J.; Hardie, M. J.; Raston, C. L. *Coord. Chem. Rev.* **2001**, *222*, 3–32; (f) Gao, J.; Zingaro, R. A.; Reibenspies, J. H.; Martell, A. E. *Org. Lett.* **2004**, *6*, 2453–2455.
11. Paluch, M.; Lisowski, J.; Lis, T. *Dalton. Trans.* **2006**, 381–388.
12. Huang, W.; Gou, S. H.; Hu, D. H.; Meng, Q. J. *Synth. Commun.* **2000**, *30*, 1555–1561.
13. (a) Korupoju, S. R.; Zacharias, P. S. *Chem. Commun.* **1998**, 1267–1268; (b) Gao, J.; Reibenspies, J. H.; Zingaro, R. A.; Woolley, F. R.; Martell, A. E.; Clearfield, A. *Inorg. Chem.* **2005**, *44*, 232–241.
14. Crosby, G. A.; Demas, J. N. *J. Phys. Chem.* **1971**, *75*, 991–1024.
15. Cielen, E.; Stobiecka, A.; Tahri, A.; Hoornaert, G. J.; De Schryver, F. C.; Gallay, J.; Vincent, M.; Boens, N. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1197–1206.
16. (a) Bourson, J.; Valeur, B. *J. Phys. Chem.* **1989**, *93*, 3871–3876; (b) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40; (c) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588.