A colorimetric and fluorescent merocyanine-based probe for biological thiols†

Shu-Ping Wang, Wu-Jian Deng, Dan Sun, Min Yan, Hong Zheng* and Jin-Gou Xu

Received 19th May 2009, Accepted 9th July 2009
First published as an Advance Article on the web 10th August 2009
DOI: 10.1039/b909760k

A new "dual-mode" chromogenic and fluorescent turn-on probe (**2**) for the selective sensing of biological thiols is reported. In MeOH–H_2O cosolvent at physiological pH 7.40 (MeOH–H_2O = 3:7), biological thiols cleave the 2,4-dinitrobenzenesulfonyl group to release the chromo- and fluorophore merocyanine (**3**).

Introduction

Recently, particular interest has been focused on the chemosensing of sulfhydryl-containing amino acids and peptides, as they play many crucial roles in biological systems.[1–3] For example, the thiol group in cysteine (Cys) residues is involved in the three-dimensional structures of proteins through disulfide bond formation, and plasma levels of glutathione (GSH), cysteine (Cys), and homocysteine (Hcy) have been linked to various human diseases.[4] Consequently, selective detection and quantification of the biological thiols is of growing importance.

Classical determination of biological thiols in a chemical system is generally accomplished by high-performance liquid chromatography combined with Ellman's reagent;[5] this, however, is inconvenient and requires expensive equipment. Selective optical sensing can overcome the drawbacks of HPLC owing to the use of "low-tech" spectroscopic instrumentation to assay relevant chemical species in biological and environmental processes. Therefore, several colorimetric or fluorescent chemosensors for thiol-containing amino acids have been developed.[6] The signal export of these probes clearly only relates to their fluorescence changes or UV band shifts upon thiol species introduction. A probe with a dual-mode sensing behaviors, *i.e.*, a color change together with fluorescence enhancement, would combine the sensitivity of fluorescence with the convenience and aesthetic appeal of a colorimetric assay, but to date, dual-mode probes for thiol-containing amino acids have been rather rare.[7] Therefore, there is still plenty of room to devise selective and sensitive chemosensors for thiol-containing amino acids that show a useful color change and "turn-on" type of fluorescence and absorbance response under physiological conditions.

In this context, we have designed a merocyanine-containing chemodosimeter responsible for the thiol-mediated nucleophilic reaction which involves a cleavage event leading to irreversible and unique color changes. Merocyanine dyes are conjugate π-electron systems with "push–pull" substituent pairs. Such molecules show strong intramolecular charge transfer (ICT),[8] and so the merocyanine unit is very useful because both its color and fluorescence changes have a significant response to the electronic effect of the substrate in the event of the thiol attack. We can envision that masking the merocyanine chromophore by an electron-withdrawing group such as the dinitrobenzenesulfonyl group (DNS) would significantly change its ICT process. Reaction with this protected cyanine dye would then result in dual-mode signals (color change and fluorescence variation) that would be convenient and sensitive enough for practical utilization.

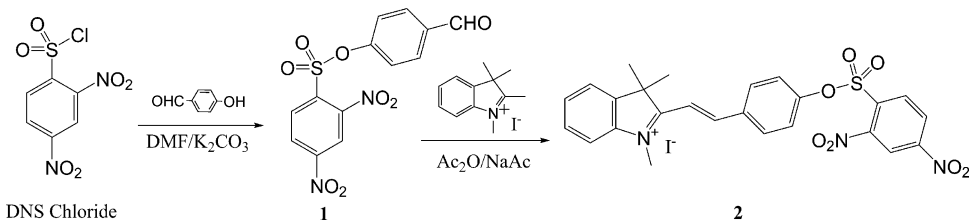
Therefore, a merocyanine chromophore, **3**, was first appended to the DNS group to gain the target probe **2** (Scheme 1), which "switches off" the color and fluorescence of the dye. Then the DNS group can be removed by a well-known thiol-mediated cleavage by an S_NAr process (Scheme 2),[6d,7a] liberating the free merocyanine chromophore. This leads to an obvious visual color change as well as an "off–on" type of fluorescent sensing. To the best of our knowledge, this is the first report that demonstrates the use of merocyanine dyes for the detection of biological species.

Experimental

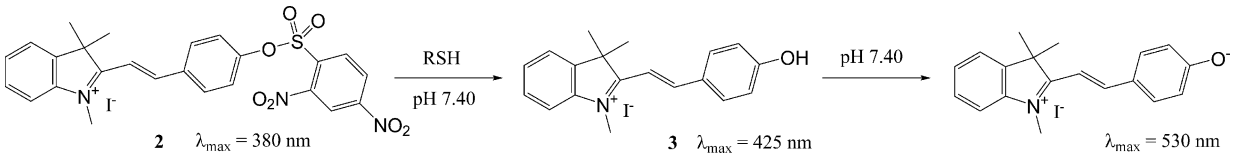
NMR spectra in DMSO-d_6 were recorded on Bruker AV400 NMR spectrophotometer. Mass spectra were measured with a JEOL JMS-T100LC mass spectrometer (ESI+). Uncorrected fluorescence emission spectra were conducted on a Hitachi F-4500 luminescence spectrometer. Absorption spectra were determined on a Beckman DU-7400 spectrophotometer. Doubly distilled

Department of Chemistry and The Key Laboratory of Analytical Sciences of MOE, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, P. R. China. E-mail: hzheng@xmu.edu.cn

† Electronic supplementary information (ESI) available: UV–vis spectra of **2** with Hcy, GSH and fluorescence spectra of **2** with Cys and Hcy, and NMR spectra of compounds **1–3**. See DOI: 10.1039/b909760k



Scheme 1 Synthesis of **2**.



Scheme 2 Cleavage of **2** with thiols.

water was used throughout the experiments. All the materials for synthesis were purchased from commercial suppliers and used without further purification. Reactions were monitored by TLC with visual observation of the dye spots.

Synthesis of 2,4-dinitrophenyl 4-formylphenyl sulfate (1)

A mixture of 4-hydroxybenzaldehyde (1.0 g, 3.75 mmol), 2, 4-dinitrobenzenesulfonyl chloride (DNSCl, 0.458 g, 3.75 mmol) and K_2CO_3 (0.62 g, 4.49 mmol) in dry acetonitrile (12 mL) was stirred at room temperature for 5.0 hours. The solvent was removed under reduced pressure, the residue was added with saturated Na_2CO_3 solution, and the product extracted with ethyl acetate, washed with brine and dried over anhydrous $MgSO_4$. Evaporation of the solvents under reduced pressure yielded 0.82 g (60% yield) of crude product, which was used in the next step without further purification.

^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 10.01 (s, 1H, -CHO), 9.13 (s, 1H, ArH), 8.61 (d, J = 8.70, 1H, ArH), 8.29 (d, J = 8.68, 1H, ArH), 8.00 (d, J = 7.89, 2H, ArH), 7.44 (d, J = 7.95, 2H, ArH). ^{13}C NMR: (100 MHz, DMSO-d_6, ppm) δ 191.87, 152.15, 151.55, 148.02, 135.48, 133.56, 131.74, 130.46, 127.53, 122.84, 121.11. ESI MS, m/z: 352.3 (**1**).$^+$

Synthesis of (*E*)-2-(4-(2,4-dinitrophenylsulfonyloxy)styryl)-1,3,3-trimethyl-3*H*-indolium iodide (2)

Compound **1** (0.439 g, 1.19 mmol) was dissolved in 10 mL of acetic anhydride, then 1,2,3,3-tetramethyl-3*H*-indolium iodide (0.2994 g, 0.0994 mmol) and sodium acetate (0.0816 g, 0.0994 mmol) was added. Stirring was continued at room temperature for 12 hours, then 20 mL of diethyl ether was added to the solution, and a brown precipitate was obtained. The precipitate was filtered and washed with redistilled water and cold acetonitrile for three times, respectively. Drying the precipitate in vacuum afforded 0.38 g of yellow product (50.5%).

^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 9.15 (d, J = 2.0 Hz, 1H), 8.63 (dd, J = 8.8 Hz, 2.4, 1H), 8.40 (d, J = 16.4, 1H, -CH=CH-), 8.31–8.29 (m, 3H), 7.95–7.89 (m, 2H), 7.70 (d, J = 16.4 Hz, 1H, -CH=CH-), 7.67–7.64 (m, 2H), 7.430 (d, J = 8.8 Hz, 2H), 4.17 (s, 3H, -CH_3), 1.78 (s, 6H, -CH_3). ^{13}C NMR: (100 MHz, DMSO-d_6, ppm) δ 181.72, 151.47, 150.90, 150.26, 148.04, 143.68, 141.68, 134.34, 133.57, 132.33, 130.50, 129.67, 128.94, 127.51, 122.78, 121.11, 115.44, 114.70, 52.33, 34.87, 24.92. ESI MS, m/z: 508.3 (**2** – I)$^+$.

Synthesis of 3 by reaction of 2 with thioglycolic acid

To a stirred solution of **2** (50 mg, 0.18 mmol) in MeOH (2.0 mL) were added $HSCH_2CO_2H$ (21 mg, 0.23 mmol) and Et_3N (35 mg, 0.36 mmol). Stirring was continued at room temperature for 2–3 h. Concentrated HCl (0.3 mL) was added to acidify the solution, and then the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel using MeOH/$CHCl_3$ as the mobile phase to give free dye **3** (26.5 mg, 53%).

^{1}H NMR (d_6-DMSO, 400 MHz) δ 1.770 (s, 6H), 4.081 (s, 3H), 6.96 (d, J = 8.8 Hz, 2H), 7.463 (d, J = 16.4 Hz, 1H), 7.559–7.634 (m, 2H), 7.827–7.855 (m, 2H), 8.123 (d, J = 8.4 Hz, 2H), 8.363 (d, J = 16 Hz, 1H), 10.822 (s, 1H). ^{13}C NMR (d_6-DMSO, 100 MHz) δ 26.143 34.522, 52.196, 109.789, 115.134, 116.901, 123.261, 126.477, 129.223, 129.324, 134.060, 142.346, 143.665, 154.215, 163.699, 181.871. ESI MS, m/z: 278.2 (**3** – I)$^+$.

General procedure for spectroscopic measurements

A 1.0×10^{-3} M stock solution of compound **2** was prepared in MeOH. To 10-mL glass tubes containing 1.0 mL of 100 mM phosphate buffer (pH 7.40) and different amounts of amino acids, were added redistilled water to achieve a volume of 7.0 mL, then 3.0 mL of MeOH. The resulting solution were mixed thoroughly. Then 0.10 mL of 1.0×10^{-3} M stock solution of compound **2** was added directly with a micropipette, the solutions were mixed again, and the absorption and fluorescence sensing of aminoacids were run after 15 minutes.

Results and discussion

Compound **2** was synthesized easily by a two-step reaction (Scheme 1). The intermediate compound **1** was synthesized from 4-hydroxybenzaldehyde with DNS-Cl, and **2** was prepared by coupling 1,2,3,3-tetramethyl-3*H*-indolium iodide with **1** in 50.5% yield.

For fully dissolved probe **2** in aqueous solutions, an methanol–water (3:7, v/v) with pH 7.40 phosphate buffer solution (PBS) was optimal for the spectroscopic investigations. The reaction of **2** with Cys was first studied by UV-Vis spectroscopic titration. The spectral changes during the titration of Cys are shown in Fig. 1.

Compound **2** exhibits an absorption maximum at 380 nm (ε ≈ 21000 M^{-1} cm^{-1}), which is responsible for the slight yellow color of the solution. In the presence of Cys, the absorbance at 380 nm decreased dramatically while a new band at 530 nm developed with the absorbance increasing linearly with increasing the concentration of Cys from 1.0×10^{-7} M to 8.0×10^{-5} M (linear correlation coefficient of 0.9976) (Fig. 1), the relatively large molar absorptivity at 530 nm (ε ≈ 78000 M^{-1} cm^{-1}) may be responsible for this high spectrophotometric detective sensitivity. Such a huge red-shift of 150 nm in absorption behavior changes the color of the resultant solution from slight yellow into orange, allowing "naked-eye" detection. Similar experimental phenomena were also observed when we added Hcy or GSH into the solution of **2** (Fig. S1 and S2†).

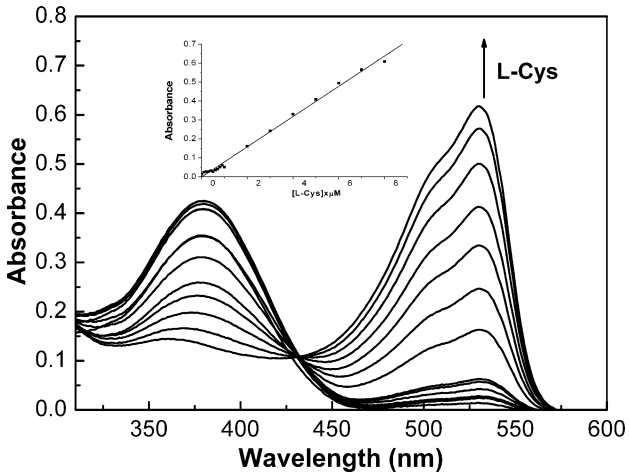


Fig. 1 UV–Vis spectra of **2** in pH 7.40 of phosphate buffer solution (MeOH–H_2O = 3:7, v/v) in the presence of Cys. Inset: plot of absorbance at 530 nm *vs.* concentration of Cys. [**2**] = 2.0×10^{-5} M. [Cys] = 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 μM, respectively.

We noticed that the final absorption spectra of the reaction system composed of Cys and **2**, and that of free merocyanine **3** in the same buffer solution, were essentially identical, indicating the release of **3** in the reaction (Fig. S3). Meanwhile, in the titration traces, a clear isosbestic point at 436 nm was observed, which was attributed to the formation of the **3** in equilibrium with **2**. Moreover, the validity of the proposed product was further confirmed by the synthesis of the free dye **3** by reaction of **2** with thioglycolic acid. These facts supported the proposed reaction shown in Scheme 2.

Since the design of **2** is based on a classical donor–π-acceptor architecture, this obvious red shift of absorption can be explained by the thiol-mediated cleavage of the electron-withdrawing sulfonyl group from **2**, releasing an ionized hydroxyl group of **3** (Scheme 2). This increases the ICT character of merocyanine chromophore, resulting in a large bathochromic shift in its absorption spectrum.

An important feature of compound **2** is its high selectivity toward the analyte over other competitive species. Fig. 2 shows a representative chromogenic behavior of **2** toward a variety of tested aminoacids. Only thiol-containing amino acids such as Cys, Hcy and GSH led to a prominent decrease in the absorbance at 380 nm and the development of a new absorption band at 530 nm. A minor change in the absorbance of **2** was found upon addition of Gly, Ser, Asp, Met, Asn, Lys, Tyr, Trp, Arg, and His, confirming **2** as a highly selective colorimetric chemodosimeter for thiol-containing amino acids. Furthermore, as shown in Fig. 3, **2** also displayed in its color response an excellent selectivity to Cys, Hcy and GSH.

We also noticed that the reaction of **2** with GSH produced an "off–on" type of fluorescence emission at 553 nm with maximum excitation at 490 nm. Therefore, a fluorescence titration of GSH was conducted (Fig. 4). As shown in Fig. 4, **2** alone showed minimized fluorescence at 553 nm, addition of GSH induced prominent fluorescence enhancement. The fluorescence intensity of **2** solution at 553 nm increases linearly with the increasing concentration of GSH in the range of 1×10^{-7} mol/L – 5×10^{-6} mol/L (R = 0.9959) with a detection limit of about 2.4×10^{-8} mol/L. The similar experimental phenomena were

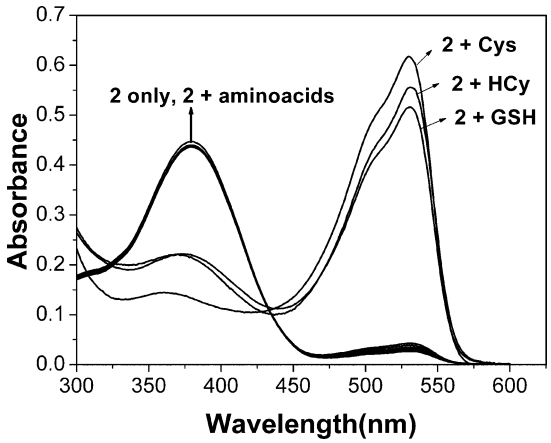


Fig. 2 Absorbance spectra of **2** in pH 7.40 of phosphate buffer solution (MeOH–H_2O = 3:7, v/v) in the presence of 0.8 equiv. of Hcy, 0.4 equiv. of Cys, GSH and other amino acids. Amino acids containing: Gly, Ser, Asp, Met, Asn, Lys, Tyr, Trp, Arg, and His. [**2**] = 2.0×10^{-5} M.

Fig. 3 The color change of **2** in pH 7.40 of phosphate buffer solution (MeOH–H_2O = 3:7, v/v) in the presence of 10 equiv. of certain amino acids. From left to right: none, Tyr, Glu, Met, Lys, His, Cys, Hcy and GSH. [**2**] = 2.0×10^{-5} M.

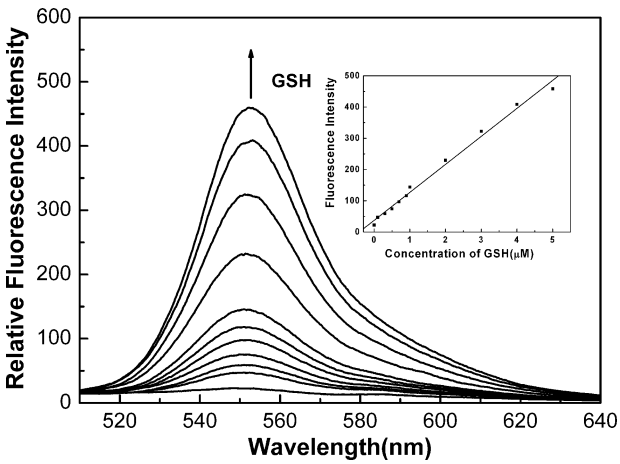


Fig. 4 Fluorescence spectra of **2** in pH 7.40 of phosphate buffer(0.01 M) solution (MeOH–H_2O = 3:7, v/v) in the presence of GSH with an exitation at 490 nm. Inset: fluorescence intensity at 553 nm *vs* GSH concentration. [**2**] = 1.0×10^{-5} M, [GSH] = 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 2.0, 3.0, 4.0 and 5.0 μM, respectively.

also observed when added Cys or Hcy into the solution of **2** (Fig. S4 ~ S5).

The changes in the fluorescence properties of **2** solution caused by different amino acids, including Gly, Ser, Asp, Met, Asn, Lys, Tyr, Trp, Arg, and His, were also measured, and they induced no observable fluorescence enhancement, whereas the fluorescence

enhanced markedly with the addition of Cys, Hcy and GSH. These results show that compound **2** is remarkably selective for thiol-containing amino acids (Fig. 5).

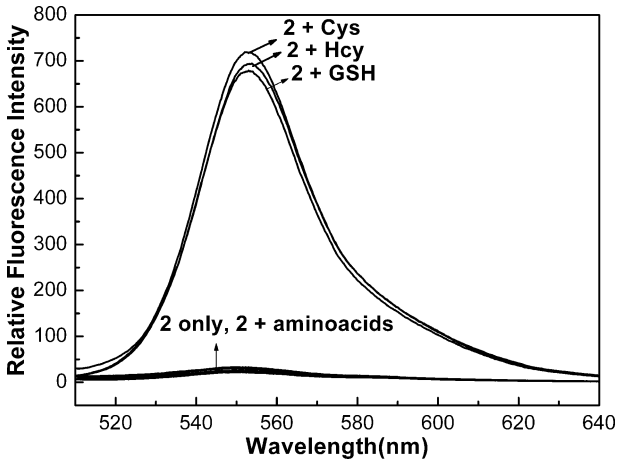


Fig. 5 Fluorescence response of **2** in pH 7.40 of phosphate buffer solution (MeOH–H_2O = 3:7, v/v) in the presence of 1.0 equiv. of Cys, Hcy, GSH and different amino acids. Amino acids containing: Gly, Ser, Asp, Met, Asn, Lys, Tyr, Trp, Arg, and His. [**2**] = 1.0×10^{-5} M.

Conclusions

In conclusion, we have developed a rapid and simple method for recognition and quantification of biological thiols by using a merocyanine-based chemodosimeter that exhibits a dual-mode of optical signal output. The recognition of biological thiols gave obvious color changes from light yellow to orange, which was clearly visible to the naked eye, while it also showed a "turn-on" type of absorption and fluorescence response. Furthermore, both the colorimetric and fluorescent response exhibit excellent selectivity and sensitivity toward sulfhydryl-containing amino acids.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20675067, No. 20835005) and NCET of Fujian Province of China.

References

1 Z. A. Wood, E. Schröder, J. R. Harris and L. B. Poole, *Trends Biochem. Sci.*, 2003, **28**, 32.
2 (*a*) S. Y. Zhang, C.-N. Ong and H.-M. Shen, *Cancer Lett.*, 2004, **208**, 143; (*b*) J. B. Schulz, J. Lindenau, J. Seyfried and J. Dichgans, *Eur. J. Biochem.*, 2000, **267**, 4904.
3 R. O. Ball, G. Courtney-Martin and P. B. Pencharz, *J. Nutr.*, 2006, **136**, 1682.
4 (*a*) F. J. T. Staal, S. W. Ela, M. Roederer, M. T. Ansderson and L. A. Herzenberg, *Lancet*, 1992, **339**, 909–913; (*b*) K. S. McCully, *Nat. Med.*, 1996, **2**, 386–389; (*c*) M. T. Heafield, S. Fearn, G. B. Steventon, R. H. Waring, A. C. Williams and S. G. Sturman, *Neurosci. Lett.*, 1990, **110**, 216; (*d*) K. S. McCully and B. D. Ragsdale, *Am. J. Pathol.*, 1970, **61**, 1; (*e*) O. Nygard, J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad and S. E. Vollset, *N. Engl. J. Med.*, 1997, **337**, 230; (*f*) D. W. Jacobsen, *Clin. Chem.*, 1998, **44**, 1833.
5 (*a*) G. Chwatko and E. Bald, *Talanta*, 2000, **52**, 509; (*b*) Y. V. Tcherkas and A. D. Denisenko, *J. Chromatogr. A.*, 2001, **913**, 309; (*c*) O. Nekrassova, N. S. Lawrence and R. G. Compton, *Talanta*, 2003, **60**, 1085; (*d*) H. Refsum, A. D. Smith, P. M. Ueland, E. Nexo, R. Clarke, J. McPartlin, C. Johnston, F. Engbaek, J. Schneede, C. McPartlin and J. M. Scott, *Clin. Chem.*, 2004, **50**, 3.
6 (*a*) D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. V. Ros-Lis, A. Benito and J. Soto, *J. Am. Chem. Soc.*, 2003, **125**, 9000; (*b*) O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian and R. M. Strongin, *J. Am. Chem. Soc.*, 2004, **126**, 438–439; (*c*) W. H. Wang, J. O. Escobedo, C. M. Lawrence and R. M. Strongin, *J. Am. Chem. Soc.*, 2004, **126**, 3400–3401; (*d*) W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949–15958; (*e*) H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki and N. Itoh, *Angew. Chem., Int. Ed.*, 2005, **44**, 2922–2925; (*f*) M. S. Han and D. H. Kim, *Tetrahedron*, 2004, **60**, 11251–11257; (*g*) T. Matsumoto, Y. Urano, T. Shoda, H. Kojima and T. Nagano, *Org. Lett.*, 2007, **9**, 3375–3377; (*h*) N. Shao, J. Y. Jin, S. M. Cheung, R. H. Yang, W. H. Chan and T. Mo, *Angew. Chem., Int. Ed.*, 2006, **45**, 4944–4948; (*i*) C. F. Chow, B. K. W. Chiu, M. H. W. Lam and W. Y. Wong, *J. Am. Chem. Soc.*, 2003, **125**, 7802–7803; (*j*) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666–11667; (*k*) A. Shibata, K. Furukawa, H. Abe, S. Tsunedab and Y. Ito, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2246–2249; (*l*) F. Tanaka, N. Mase and C. F. Barbas III, *Chem. Commun.*, 2004, 1762; (*m*) K.-S. Lee, T.-K. Kim, J. H. Lee, H.-J. Kim and J.-I. Hong, *Chem. Commun.*, 2008, 6173; (*n*) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2008, **47**, 7883; (*o*) M. M. Pires and J. Chmielewski, *Org. Lett.*, 2008, **10**, 837–840.
7 (*a*) J. Bouffard, Y. Kim, T. M. Swager, R. Weissleder and S. A. Hilderbrand, *Org. Lett.*, 2008, **10**, 37–40; (*b*) M. Zhang, M. X. Yu, F. Y. Li, M. W. Zhu, M. Y. Li, Y. H. Gao, L. Li, Z. Q. Liu, J. Zhang, D. Q. Zhang, T. Yi and C. H. Huang, *J. Am. Chem. Soc.*, 2007, **129**, 10322.
8 A. P. De Silva, H. N. Q. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.

