ORGANIC LETTERS

2012
Vol. 14, No. 16
4198–4201

Ring Expansion of Spiro-thiolactam in Rhodamine Scaffold: Switching the Recognition Preference by Adding One Atom

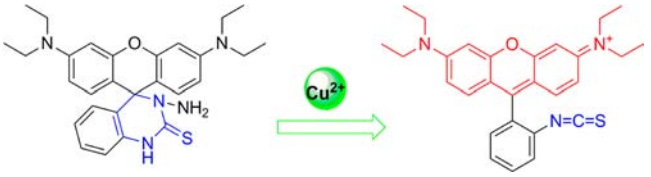
Cong Wu, Qing-Na Bian, Bian-Guo Zhang, Xin Cai, Shu-Dong Zhang, Hong Zheng,* Shi-Yao Yang, and Yun-Bao Jiang

Department of Chemistry, College of Chemistry and Chemical Engineering, and the MOE Key Laboratory of Analytical Sciences, Xiamen University, Xiamen 361005, China

hzheng@xmu.edu.cn

Received July 6, 2012

ABSTRACT



A new rhodamine spiro scaffold with a six-membered reactive ring was developed by inserting a nitrogen atom in the known probe rhodamine B spiro thiohydrazide, which switched the recognition preference of the probe from Hg^{2+} to Cu^{2+}. This probe is shown to be an efficient "turn-on" fluorescent chemodosimeter for Cu^{2+} in a neutral aqueous medium. Mechanism studies suggested that the probe opened its spiro-ring by a Cu^{2+}-induced transformation of the cyclic thiosemicarbazide moiety to an isothiocyanate group.

The development of fluorescent molecular sensors for metal ions, especially for cations of environmental or biological interest, has always been of particular importance. The work usually involves the design and synthesis of molecules containing binding sites and a signaling subunit that is able to undergo selective changes in fluorescence emission intensity upon ligand binding. Rhodamines are dyes extensively employed in the study of complex biological systems as molecular probes because of their high absorption coefficients, high fluorescence quantum yields, and long-wavelength absorptions and emissions.[1] Among the rhodamines with a spirocyclic form, the rhodamine spirolactam scaffold, which undergoes the unique conformational transformation from the spirolactam (colorless and nonfluorescent) to an open-ring structure (colored and fluorescent), has been applied in the design of numerous chemosensors in recent years, and these new chemosensors have recently been discussed in excellent review articles.[2] In this spirolactam scaffold, the sensing event occurs in its reactive five-membered lactam moiety. Obviously, the favorable properties of opening the spiro-ring of the rhodamine scaffold still present ample opportunities for the design of new fluorescent probes.

On the other hand, taking into account that six-membered rings are stable and are the most frequently encountered structure in organic compounds, we wonder if we could expand this five-membered reactive lactam to a six-membered cyclic structure, what would happen in this new spiro scaffold. Therefore, in the present paper, we designed and synthesized a new type of rhodamine spiro-ring-based chemosensor that contained such a six-membered reactive ring. By careful comparison of the structure of thioamide with that of thiourea (Scheme 1), the latter can be regarded as a "thioamide" group with an added nitrogen atom. Therefore, in a five-membered spiro-thiolactam scaffold of known chemosensor **2**,[3] we added a nitrogen atom in proximity to the "thiolactam" group affording a new

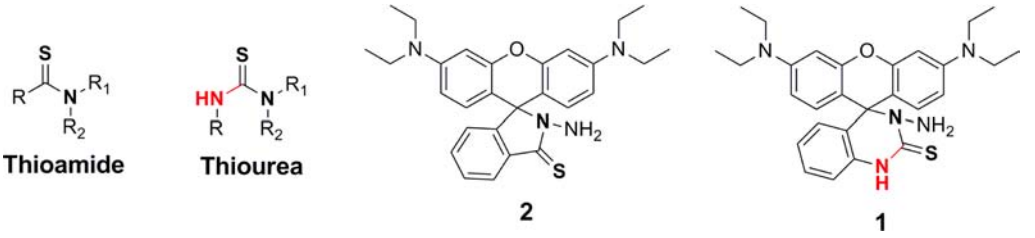
(1) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes, Inc.: Eugene, OR 97402, and examples therein.

(2) (a) Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465–1472. (b) Beija, M.; Afonso, C. A. M.; Martinho, J. M. G. *Chem. Soc. Rev.* **2009**, *38*, 2410–2433. (c) Chen, X. Q.; Pradhan, T.; Wang, F.; Kim, J. S.; Yoon, J. Y. *Chem. Rev.* **2012**, *112*, 1910–1956. (d) Yang, Y. M.; Zhao, Q.; Feng, W.; Li, F. Y. *Chem. Rev.* **2012**dx.doi.org/10.1021/cr2004103.

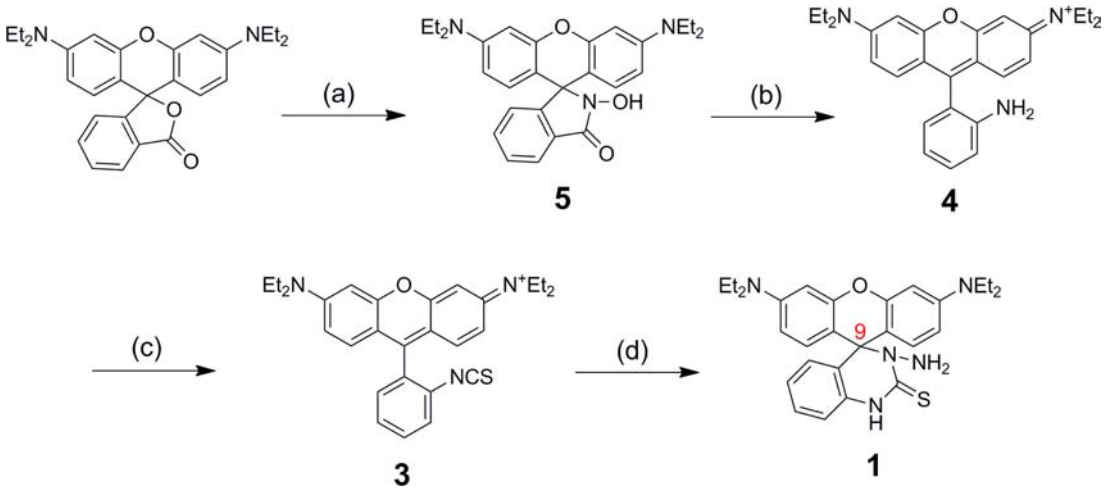
(3) Zheng, H.; Qian, Z. H.; Xu, L.; Yuan, F. F.; Lan, L. D.; Xu, J. G. *Org. Lett.* **2006**, *8*, 859–861.

10.1021/ol3018598
Published on Web 08/07/2012

Scheme 1. Structures of Spiro-thioamide and Spiro-thiourea of Rhodamine Scaffold



Scheme 2. Synthetic Route of **1**[a]



[a] (a) $POCl_3$, reflux; then NH_2OH in CH_3CN, rt; (b) TsCl, Et_3N, CH_3CN, rt; (c) $CSCl_2$, NaOH, CH_3CN; (d) NH_2NH_2, CH_3CN, rt.

probe **1**. It is noteworthy that this design concept has not been previously employed in the construction of spirocyclic rhodamine-based fluorescent probes, and herein, we present study results of the synthesis, absorption, and fluorescence response as well as the sensing mechanism regarding the new probe.

The synthetic procedures of **1** are given in Scheme 2; probe **1** was synthesized from rhodamine B by a four-step procedure with a total yield of 40%, and the structure of probe **1** was confirmed by ^1H NMR, ^{13}C NMR, and ESI-MS spectroscopy (Figures S4–S6). In addition, the peak of the 9-carbon in probe **1** at 65.45 ppm in the ^{13}C NMR spectrum supports the presence of the spiro-ring structure (Figure S5).[4]

Since probe **2** is a known chemosensor for Hg^{2+},[3] we then preliminarily detected the fluorescent spectroscopic characteristics of probe **1** in sensing applications. Although the fluorescence intensity of probe **1** increased slightly at pH values below 5.0, it was almost nonfluorescent when the pH was above 5.0 and found to only display a strong fluorescent response toward Cu^{2+} in neutral solution (Figure S7). Therefore, an optimized buffer solution of 3,3-dimethylglutaric acid/NaOH (10 mM in water/acetonitrile solution, 80: 20, v/v) at pH 7.0 was selected as experimental media for further spectral investigation.

Then a detailed study on the recognition characteristics of probe **1** toward Cu^{2+} was carried out. The absorption spectra of probe **1** in the presence of varying Cu^{2+} concentrations were recorded first. As shown in Figure 1, the solution of probe **1** alone (1.0×10^{-5} M) exhibits no absorption peak above 400 nm, consistent with the fact that the solution is colorless, which also indicates that the spiro-form of probe **1** predominates under the detected aqueous conditions. Upon addition of Cu^{2+}, the probe **1** solution showed a new maximum absorption wavelength at 570 nm, which can be ascribed to the delocalized xanthene moiety of rhodamine. In addition, the absorption behavior changes the color of the resultant solution from colorless into magenta, allowing "naked-eye" detection (Figure S8).

The reaction of probe **1** with Cu^{2+} also produced noticeable changes in the fluorescence emission. A solution of probe **1** was nonfluorescent, but the addition of Cu^{2+} caused the development of a strong fluorescence peak at 597 nm; a fluorescent photo image also proves the change in fluorescence (Figure S9). The fluorescence titration

(4) Anthoni, U.; Christophersen, C.; Nielsen, P. H.; Piischl, A.; Schaumburg, K. *Struct. Chem.* **1995**, *6*, 161–165.

curve revealed that the fluorescence intensity at 597 nm increased linearly with increasing concentrations of Cu^{2+} between 0.10 and 10.0 μM (R^2 = 0.9969) (Figure S10) and further smoothly increased until a maximum was reached up to 20.0 μM Cu^{2+}, where an approximately 400-fold increase in fluorescence emission was observed (Figure 2). Given these titration results, the detectable linear concentration range is acceptable based on the US EPA limit (20 μM) for the detection of Cu^{2+} in drinking water.

Further, the time-dependence of probe **1** fluorescence was also evaluated in the presence of Cu^{2+} ions. In this set

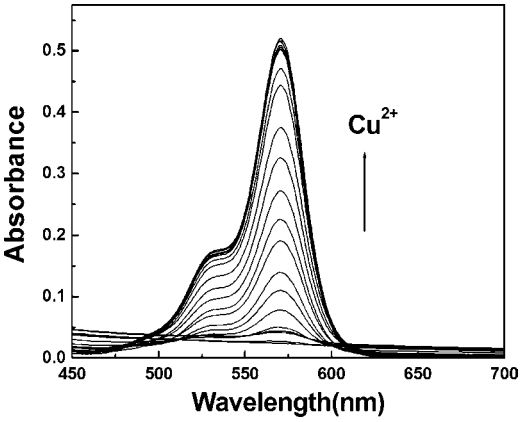


Figure 1. Absorption spectra of probe **1** (10 μM) at pH 7.0 of 0.01 M 3,3-dimethylglutaric acid/NaOH buffer solution (H_2O/CH_3CN = 80/20, v/v).

of experiments, several concentrations of Cu^{2+} ions were tested with a fixed concentration of probe **1**. The results showed that, upon the reaction of probe **1** with Cu^{2+}, the fluorescence of all the tested solutions remarkably increased to their maximum value within the first 5 min. No changes in fluorescence were detected in the absence of Cu^{2+} (Figure 3).

Metal ions, such as alkali or alkaline-earth metal ions, and some transition metal ions were used to analyze the selectivity of probe **1** (10 μM). The fluorescence spectra were recorded 5 min after the addition of 1.0 equiv of each of these metal ions. The results showed that the fluorescence intensity at 597 nm was dramatically increased only by addition of the Cu^{2+} ion, whereas the other tested metal ions did not induce any significant fluorescence changes (Figure 4). In addition, a competitive experiment revealed that all of the tested foreign metal ions, except Ag^+, 1.0 equiv. of which relative to Cu^{2+} can limit the turn-on response of **1** to Cu^{2+} to a *ca.* 60% extent, had minor or no interference with the fluorescent response to Cu^{2+} (Figure S11). Therefore, these results suggest that probe **1** displays high selectivity toward Cu^{2+} in water at neutral pH.

To verify the nature of the fluorescence response of probe **1** to Cu^{2+}, an additional experiment in which 100 equiv of EDTA were added to a **1**−Cu^{2+} solution was carried out and revealed that EDTA could not eliminate either the fluorescence or the absorption of the **1**−Cu^{2+}

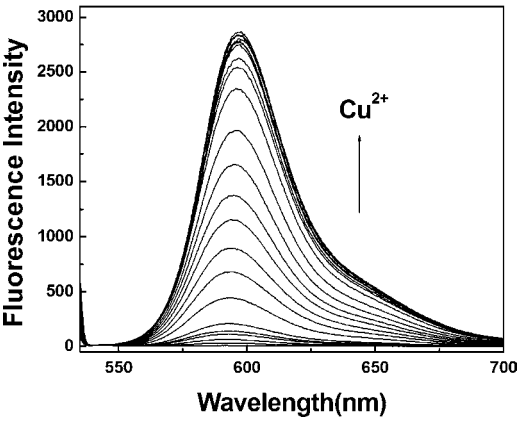


Figure 2. Fluorescence titration of probe **1** (10 μM) at pH 7.0 of 0.01 M 3,3-dimethylglutaric acid/NaOH buffer solution (H_2O/CH_3CN = 80/20, v/v) in the presence of different amounts of Cu^{2+}. Excitation was performed at 530 nm, slit = 2.5/5 nm.

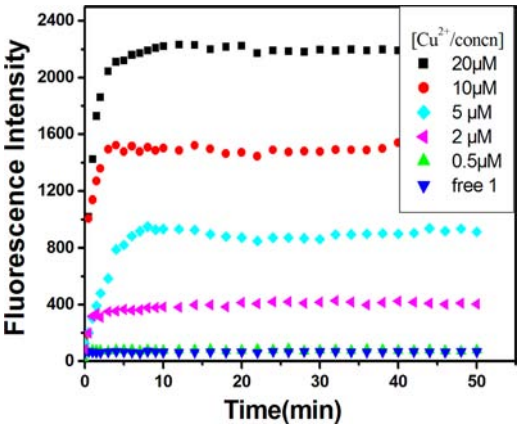


Figure 3. Kinetics of probe **1** reacted with various concentrations of Cu^{2+} at pH 7.0 of 0.01 M 3,3-dimethylglutaric acid/NaOH buffer solution (H_2O/CH_3CN = 80/20, v/v). [**1**] = 10 μM; $\lambda_{ex}/\lambda_{em}$ = 530 nm/597 nm.

solution (Figure S12). This phenomenon suggested that **1**−Cu^{2+} might have undergone some chemical reaction, such that probe **1** has great potential for use as a chemodosimeter for Cu^{2+} that exhibits a fluorescence enhancement response in water. To understand the product produced by the combination of probe **1** and Cu^{2+}, we carried out ESI-MS measurements for a **1**−Cu^{2+} solution, and the resultant solution gave an intense peak at m/z 455.9, exactly equal to that of compound **3** (Figure S13). To further verify the identity of the final product, we treated probe **1** with 3.0 equiv of copper nitrate in acetonitrile, and ^1H, ^{13}C NMR and HR-MS spectra (Figures S14−S16) of the isolated fluorescent product confirm that the final product is indeed compound **3** (ϕ_f = 0.079).[8] Furthermore, according to the synthesis route of probe **1** (Scheme 2), the

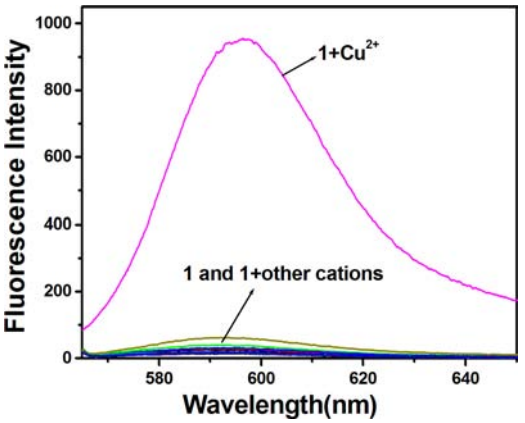


Figure 4. Fluorescence responses of **1** (10 μM) with 1.0 equiv of metal ions at pH 7.0 of 0.01 M 3,3-dimethylglutaric acid/NaOH buffer solution (H_2O/CH_3CN = 80/20, v/v). Metal ions include K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Fe^{3+}, Ag^+, and Mn^{2+}. λ_{ex} = 560 nm.

absorption and fluorescence behaviors of the experiments of regenerating probe **1** *in situ* by adding hydrazine to a **1**−Cu^{2+} solution also supported this result (Supporting Information, Figure S17). Therefore, the spectral changes in sensing events could be due to the Cu^{2+}-induced transformation of a cyclic thiosemicarbazide moiety to an isothiocyanate group in probe **1** to open its spiro-ring.

As we know, copper is an environmental pollutant when present at high concentrations; it is also a vital and the third most abundant trace element in humans and present at low levels in a variety of cells and tissues. Alterations of copper homeostasis in cells can lead to severe disorders.[5] Owing to these important roles of Cu^{2+}, many efforts have been made to develop fluorescent sensors specifically for Cu^{2+} ion detection with fluorescence enhancement.[6] Among them, a chemodosimetric approach for efficient and selective Cu^{2+} signaling is very attractive due to the cumulative and specific chemical reactions between the dosimeter molecule and analyte, which allows for highly selective and sensitive signaling. However, chemodosimeters detecting Cu^{2+} are quite seldom, primarily including Cu^{2+}-promoted hydrolysis of hydrazide derivatives,[7a−e] esters,[7f−h] and hydrazone[7i] as well as a Cu^+-catalyzed click reaction.[7j] To the best of our knowledge, there is still no example showing that Cu^{2+} could induce the transformation of a thiosemicarbazide moiety to an isothiocyanate group except the report of this work.

In conclusion, we have developed a probe based on a novel spiro-form of the rhodamine scaffold that contains a six-membered reactive ring. The experimental results clearly indicate that probe **1** can be used as a chemodosimeter for Cu^{2+} with good selectivity and high sensitivity. In addition, comparison of the structure of probe **1** to that of its five-membered cousin, probe **2**, which is a chemosensor for Hg^{2+} based on a chelation reaction, reveals that probe **1** can be regarded as a modified form of probe **2** that contains an additional nitrogen atom. Interestingly, the recognition preference and mechanism of probe **1** were thoroughly changed despite the small difference in structure (Figure S18). It is likely that the experimental results of this study will provide a new basis for the design of interesting spiro-rhodamine-based fluorescent chemosensors, and further studies including the design of new analogs of probe **1** as various chemosensors are underway.

Acknowledgment. This work was financially supported by the MOST of China (No. 2011CB910403), the National Natural Science Foundation of China (Nos. 20675067 and 20835005), and the NFFTBS (No. J1030415), which are gratefully acknowledged.

Supporting Information Available. Synthesis and characterization of **1**, **3**, and **4**. Some experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

(5) (a) Waggoner, D. J.; Bartnikas, T. B.; Gitlin, J. D. *Neurobiol. Disease* **1999**, *6*, 221–230. (b) Vulpe, C.; Levinson, B.; Whitney, S.; Packman, S.; Gitschier *J. Nat. Genet.* **1993**, *3*, 7–13. (c) Bull, P. C.; Thomas, G. R.; Rommens, J. M.; Forbes, J. R.; Cox, D. W. *Nat. Genet.* **1993**, *5*, 327–337. (d) Bruijn, L. I.; Miller, T. M.; Cleveland, D. W. *Annu. Rev. Neurosci.* **2004**, *27*, 723–749. (e) Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nat. Rev. Drug Discovery* **2004**, *3*, 205–214.

(6) Selective fluorescent enhancement chemosensors for Cu^{2+}: (a) Ghosh, P.; Bharadwaj, P. K.; Roy, J.; Ghosh, S. *J. Am. Chem. Soc.* **1997**, *119*, 11903–1111909. (b) Ramachandram, B.; Samanta, A. *Chem. Commun.* **1997**, 1037–1038. (c) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968–969. (d) Zheng, Y. J.; Huo, Q.; Kele, P.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *Org. Lett.* **2001**, *3*, 3277–3280. (e) Kaur, S.; Kumar, S. *Chem. Commun.* **2002**, 2840–2841. (f) Xu, Z.; Xiao, Y.; Qian, X.; Cui, J.; Cui, D. *Org. Lett.* **2005**, *7*, 889–892. (g) Xu, Z.; Qian, X.; Cui, J. *Org. Lett.* **2005**, *7*, 3029–3032. (h) Wen, Z. C.; Yang, R.; He, H.; Jiang, Y. B. *Chem. Commun.* **2006**, 106. (i) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863–2866. (j) Singhal, N. K.; Ramanujam, B.; Mariappanadar, V.; Rao, C. P. *Org. Lett.* **2006**, *8*, 3525–3528. (k) Zhang, X.; Shiraishi, Y.; Hirai, T. *Org. Lett.* **2007**, *9*, 5039–5042. (l) Swamy, K. M. K.; Ko, S.-K.; Kwon, S. K.; Lee, H. N.; Mao, C.; Kim, J.-M.; Lee, K.-H.; Kim, J.; Shin, I.; Yoon, J. *Chem. Commun.* **2008**, 5915–5917. (m) Li, G.-K.; Xu, Z.-X.; Chen, C.-F.; Huang, Z.-T. *Chem. Commun.* **2008**, 1774–1776. (n) Jung, H. S.; Park, M.; Han, D. Y.; Kim, E.; Lee, C.; Ham, S.; Kim, J. S. *Org. Lett.* **2009**, *11*, 3378–3381. (o) Kim, H. J.; Hong, J.; Hong, A.; Ham, S.; Lee, J. H.; Kim, J. S. *Org. Lett.* **2008**, *10*, 1963–1966. (p) Lee, M. H.; Kim, H. J.; Yoon, S.; Park, N.; Kim, J. S. *Org. Lett.* **2008**, *10*, 213–216. (q) Zhou, Y.; Wang, F.; Kim, Y.; Kim, S.-J.; Yoon, J. *Org. Lett.* **2009**, *11*, 4442–4445. (r) Ballesteros, E; Moreno, D.; Gómez, T.; Rodríguez, T.; Rojo, J.; García-Valverde, M.; Torroba, T. *Org. Lett.* **2009**, *11*, 1269–1272. (s) Zhang, J. F.; Zhou, Y.; Yoon, J.; Kim, Y.; Kim, S. J.; Kim, J. S. *Org. Lett.* **2010**, *12*, 3852–3855. (t) Li, P.; Duan, X.; Chen, Z. Z.; Liu, Y.; Xie, T.; Fang, L. B.; Li, X. R.; Yin, M.; Tang, B. *Chem. Commun.* **2011**, *47*, 7755–7757.

(7) (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387. (b) Yu, M. X.; Shi, M.; Chen, Z. G.; Li, F. Y.; Li, X. X.; Gao, Y. H.; Xu, J.; Yang, H.; Zhou, Z. G.; Yi, T.; Huang, C. H. *Chem.—Eur. J.* **2008**, *14*, 6892–6900. (c) Liu, J. L.; Li, C. Y.; Li, F. Y. *J. Mater. Chem.* **2011**, *21*, 7175–7181. (d) Yuan, L.; Lin, W.; Chen, B.; Xie, Y. *Org. Lett.* **2012**, *14*, 432–435. (e) Kumar, M.; Kumar, N.; Bhalla, V.; Sharma, P. R.; Kaur, T. *Org. Lett.* **2012**, *14*, 406–409. (f) Kovacs, J.; Mokhir, A. *Inorg. Chem.* **2008**, *47*, 1880–1882. (g) Qi, X.; Jun, E. J.; Xu, L.; Kim, S.-J.; Hong, J. S. J.; Yoon, Y. J.; Yoon, J. *J. Org. Chem.* **2006**, *71*, 2881–2884. (h) Li, N.; Xiang, Y.; Tong, A. J. *Chem. Commun.* **2010**, *46*, 3363–3365. (i) Kim, M. H.; Jang, H. H.; Yi, S.; Chang, S.-K.; Han, M. S. *Chem. Commun.* **2009**, 4838–4840. (j) Xu, Q.; Lee, K. M.; Wang, F.; Yoon, J. *J. Mater. Chem.* **2011**, *21*, 15214–15217.

(8) In reference to rhodamine 6G (ϕf = 0.94 in EtOH) as a reference, see: Fischer, M.; Georges, J. *Chem. Phys. Lett.* **1996**, *260*, 115–116.

The authors declare no competing financial interest.