ORGANIC LETTERS

2011
Vol. 13, No. 20
5532–5535

Toward the Development of the Direct and Selective Detection of Nitrates by a Bioinspired Mo−Cu System

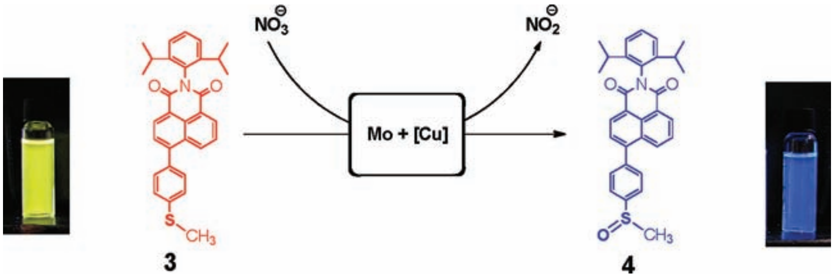
Hanit Marom, Yanay Popowski, Svetlana Antonov, and Michael Gozin*

Department of Chemistry, Raymond and Beverly Sackler Faculty of Exact Science,
Tel-Aviv University, Tel Aviv 69978, Israel

cogozin@mgchem.tau.ac.il

Received August 21, 2011

ABSTRACT



The development of a new platform for the direct and selective detection of nitrates is described. Two thioether-based chemosensors and the corresponding sulfoxides and sulfones were prepared, and their photophysical properties were evaluated. Upon selective sulfoxidation of these thioethers with nitrates via an oxygen-transfer reaction promoted by a bioinspired Mo−Cu system, significant fluorescence shifts were measured. A selective response of these systems, discriminating between nitrate salts and H_2O_2, was also shown.

Nitrates are the world's most widespread groundwater contaminant, mainly resulting from crop fertilization, livestock wastes, and organic wastes (nitrates that are formed from organic nitrogen-containing compounds found in manure).[1] Excessive consumption of nitrates can lead to a fatal medical condition called methemoglobinemia and other problems, such as spontaneous abortion and birth defects in the central nervous system.[2]

A variety of analytical methods have been developed for the determination of nitrates. Most of them are based on spectrophotometry,[3] ion chromatography,[4] flow-injection analysis,[5] electrochemistry,[6] and capillary electrophoresis.[7] Spectroscopic methods are the most commonly used because of their low detection limits and convenient sample-preparation protocols. A broad range of spectroscopic techniques (in many cases in conjunction with suitable chemical reactions) are applicable, including UV−vis,[8] chemoluminescence,[9] fluorescence,[10] IR,[11] Raman,[12] and molecular-cavity emission spectroscopies.[13] However, these

---

(1) Johnson, C. J.; Kross, B. C. *Am. J. Ind. Med.* **1990**, *18*, 449.

(2) Manassaram, D. M.; Backer, L. C.; Moll, D. M. *Environ. Health Perspect*. **2006**, *114*, 320.

(3) (a) Zhag, M.; Yuan, D. X.; Chen, G. H.; Li, Q. L.; Zhang, Z.; Liang, Y. *Microchim. Acta* **2008**, *160*, 461. (b) López Pasquali, C. E.; Gallego-Picó, A.; Fernández Hernando, P.; Velasco, M.; Durand Alegría, J. S. *Microchem. J.* **2010**, *94*, 79.

(4) (a) Abha, C.; Anil, K. B.; Gupta, V. K. *Talanta* **2001**, *55*, 789. (b) Tirumalesh, K. *Talanta* **2008**, *74*, 1428.

(5) Gamboa, J. C. M.; Peña, R. C.; Paixăo, T. R. L. C.; Bertotti, M. *Talanta* **2009**, *80*, 581.

(6) (a) Li, H. L.; Chambers, J. Q.; Hobbs, D. T. *J. Appl. Electrochem.* **1988**, *18*, 454. (b) Moorcroft, M. J.; Nei, L.; Davis, J.; Compton, R. G. *Anal. Lett*. **2000**, *33*, 3127. (c) Mori, V.; Bertotti, M. *Anal. Lett*. **1999**, *32*, 25. (d) Bouamrane, F.; Tadjeddine, A.; Butler, J. E.; Tenne, R.; Levy-Clement, C. *J. Electroanal. Chem*. **1996**, *405*, 95. (e) Reuben, C.; Galun, E.; Cohen, H.; Tenne, R.; Kalish, R.; Muraki, Y.; Hashimoto, K.; Fujishima, A.; Butler, J. M.; Levy-Clement, C. *J. Electroanal. Chem*. **1995**, *396*, 233.

(7) Bord, N.; Cretier, G.; Rocca, J. L.; Bailly, C.; Souchez, J. P. *J. Chromatogr., A* **2005**, *1100*, 223.

(8) (a) Stanley, M. A.; Maxwell, J.; Forrestal, M.; Doherty, A. P.; MacCraith, B. D.; Diamond, D.; Vos, J. G. *Anal. Chim. Acta* **1994**, *299*, 8. (b) Takeda, K.; Fujiwara, K. *Anal. Chim. Acta* **1993**, *276*, 25.

(9) (a) Yang, F.; Troncy, E.; Francoeur, M.; Vinet, B.; Vina, P.; Czaika, G.; Blaise, G. *Clin. Chem*. **1997**, *43*, 657. (b) Aoki, T.; Fukuda, S.; Hosoi, Y.; Mukai, H. *Anal. Chim. Acta* **1997**, *349*, 11. (c) Yoshizumi, K.; Aoki, K. *Anal. Chem*. **1985**, *57*, 737. (d) He, Z. K.; Fuhrmann, B.; Spohn, U. *Fresenius' J. Anal. Chem*. **2000**, *367*, 264.

(10) (a) Ohta, T.; Arai, Y.; Takitani, S. *J. Pharm. Sci*. **1987**, *76*, 531. (b) Lee, S. H.; Field, L. R. *Anal. Chem*. **1984**, *56*, 2647. (c) Huang, Z.; Korenaga, T.; Helaleh, M. I. H. *Mikrochim. Acta* **2000**, *134*, 179. (d) Buldt, A.; Karst, U. *Anal. Chem*. **1999**, *71*, 3003. (e) Geetha, K.; Balasubramanian, N. *Anal. Lett*. **2000**, *33*, 1869.

(11) Jiao, G.; Lips, S. H. *J. Plant Nutr*. **2000**, *23*, 79.

(12) Aker, P. M.; Zhang, J.; Nichols, W. *J. Chem. Phys*. **1999**, *110*, 2202.

(13) (a) Celik, A.; Henden, E. *Analyst* **1989**, *114*, 563. (b) Al-Zamil, I. Z.; Townshend, A. *Anal. Chim. Acta* **1982**, *142*, 151.

10.1021/ol2022627

chemical reactions for the detection of nitrate (including the most popular, the Griess assay[14]) are indirect and rely on the detection of the more reactive nitrite, which is produced by chemical reduction of nitrate.[15] The concentration of the latter anion is analyzed by measurements of concentration of compounds formed in a reaction between suitable chromogenic or fluorogenic precursors and nitrite.

More recent chemical detection methodologies utilize the reaction of nitrite with 4-(*N*-methylhydrazino)-7-nitro-2,1,3-benzooxadiazole (MNBDH),[16] 2,3-diaminonaphthalene,[17] or functionalized gold nanoparticles.[18] To the best of our knowledge, no direct detection of nitrates by colorimetric or fluorometric methods has been reported in the literature to date, and the development of chemical systems for the direct detection of nitrates still presents a significant challenge. Reliable technologies for simple-to-use chemical sensors could be very important for the monitoring quality of water sources, soils, and foods. No less important is the issue of field detection of nitrate-containing explosives.[19]

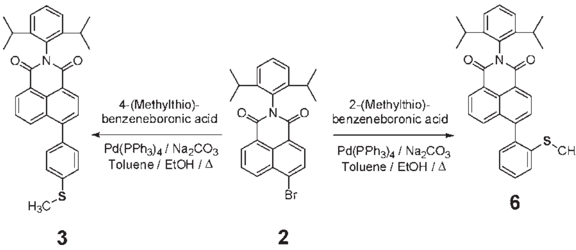
In our previous work, we reported the selective oxidation of thioethers to the corresponding sulfoxides through the oxygen-atom-transfer (OAT) reaction with the use of a bioinspired molybdenum−copper catalytic system (see Scheme 2) and nitrate salts as oxidants.[20] With the use of a thioether-based chemosensor[21] in the latter chemical transformation, one can envision a novel platform for the development of a *direct* nitrate detection system.

Here we report a new strategy for the selective detection of nitrate salts by fluorescence spectroscopy. Moreover, our methodology allows the construction of chemosensors capable of discriminating between nitrates and other oxidants such as peroxides. We designed, synthesized, and evaluated the performance of compound **3** as a nitrate chemosensor (Scheme 1). The structure of **3** is based on the frame of the 1,8-naphthalimide fluorophore, as 1,8-naphthalimide derivatives are conveniently accessible and their spectral properties can be readily fine-tuned. Two possible functionalization sites are available on the 1,8-naphthalimide frame: the naphthalic aromatic moiety and the imide moiety. Certain derivatives substituted at the aromatic C-4 position, have an extended π-conjugated system. These compounds frequently exhibit a bathochromic shift in their absorption and fluorescence spectra (vs the parent chromophore), especially in cases of electron-donating substituents.[22]

The preparation of **3** included two synthetic steps (Scheme 1). 6-Bromobenzo[*de*]isochromene-1,3-dione (**1**) was treated with 2,6-diisopropylaniline in a mixture of *N*-methylpyrrolidine (NMP) and acetic acid at 120 °C to produce compound **2** in 90% yield. The yield could be further improved to quantitative by using an excess of 2,6-diisopropylaniline and conducting the reaction for a prolonged period of time. The synthesis of **3** was completed in 87% yield by Suzuki coupling of **2** with 4-(methylthio)-benzeneboronic acid in a mixture of toluene and ethanol in the presence of $Pd(PPh_3)_4$ catalyst under an inert atmosphere.

Prior to testing the performance of chemosensor **3**, we prepared the corresponding sulfoxide **4** by oxidation of **3** with 1 equiv of H_2O_2. The absorbance and fluorescence spectra of **4** were measured, revealing a strong absorption peak at $\lambda_{max} = 350$ nm (vs $\lambda_{max} = 370$ nm for **3**) and a high-intensity emission peak at $\lambda_{max} = 425$ nm (vs $\lambda_{max} = 540$ nm for **3**), thus strongly indicating the potential of **3** to function as a chemosensor in our system [see the Supporting Information (SI) and Figure 1]. Optimization of the response of chemosensor **3** required the determination of the concentration at which compound **4** would give the strongest fluorescence signal. By preparing and measuring the fluorescence of a series of CH_3CN solutions containing compound **4** (at concentrations between 6.7×10^{-9} and 6.7×10^{-4} M), we found that the strongest fluorescence was observed at approximately 3×10^{-5} M. At higher concentrations, reduction of the fluorescence signal intensity could be explained by self-quenching.

Scheme 1. Preparation of **3** and **6**



The linear range of the fluorescence response of chemosensor **3** (the range over which the correlation between the concentration and the fluorescence intensity of **4** is linear) was found to be 5.5×10^{-7} to 8.3×10^{-6} M; thus, the

---

(14) (a) Ahmed, M. J.; Stalikas, C. D.; Tzouwara-Karayanni, S. M.; Karayannis, M. I. *Talanta* **1996**, *43*, 1009. (b) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131. (c) Pratt, P. F.; Nithipatikom, K.; Campbell, W. B. *Anal. Biochem.* **1995**, *231*, 383.

(15) (a) Jannasch, H. W.; Johnson, K. S.; Sakamoto, C. M. *Anal. Chem.* **1994**, *66*, 3352. (b) Daniel, A.; Birot, D.; Lahaitre, M.; Poncin, J. *Anal. Chim. Acta* **1995**, *308*, 413. (c) Nakashima, S.; Yagi, M.; Zenki, M.; Takahashi, A.; Tôei, K. *Fresenius' J. Anal. Chem.* **1984**, *319*, 506.

(16) Buldt, A.; Karst, U. *Anal. Chem.* **1999**, *71*, 3003.

(17) Nussler, A. K.; Glanemann, M.; Schirmeier, A.; Liu, L.; Nuessler, N. C. *Nat. Protoc.* **2006**, *1*, 2223.

(18) Weston, L. D.; Han, M. S.; Jae-Seung, L.; Mirkin, C. A. *J. Am. Chem. Soc.* **2009**, *131*, 6362.

(19) (a) Germain, M. E.; Knapp, M. J. *Chem. Soc. Rev.* **2009**, *38*, 2543. (b) Smith, R. G.; D'Souza, N.; Nicklin, S. *Analyst* **2008**, *133*, 571. (c) Almog, J.; Burda, G.; Shloosh, Y.; Abramovich-Bar, S.; Wolf, E.; Tamiri, T. *J. Forensic Sci.* **2007**, *52*, 1284.

(20) Marom, H.; Antonov, S.; Popowski, Y.; Gozin, M. *J. Org. Chem.* **2011**, *76*, 5240.

(21) (a) Malashikhin, S.; Finney, N. S. *J. Am. Chem. Soc.* **2008**, *130*, 12846. (b) Dane, E. L.; King, S. B.; Swager, T. M. *J. Am. Chem. Soc.* **2010**, *132*, 7758. (c) Jenks, W. S.; Gregory, D. D.; Guo, Y.; Lee, W.; Tetzlaff, T. In *Organic Photochemistry*; Ramamurthy, V., Schanze, K. S., Eds.; Marcel Dekker: New York, 1997; Vol. 1, pp 1−56.

(22) (a) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551. (b) Jian-Xin, Y.; Xin-Liang, W.; Xue-Mei, W.; Long-He, X. *Dyes Pigm.* **2005**, *66*, 83. (c) Duke, R. M.; Veale, E. B.; Pfeffer, F. M.; Krugerc, P. E.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2010**, *39*, 3936.

amount of nitrate could be determined quantitatively over this range.

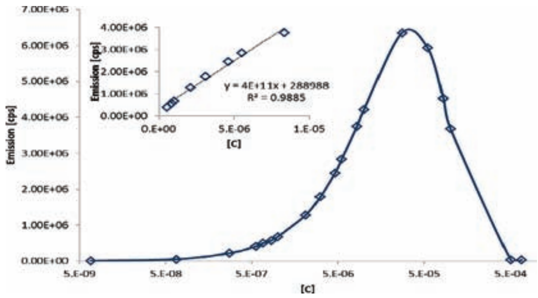
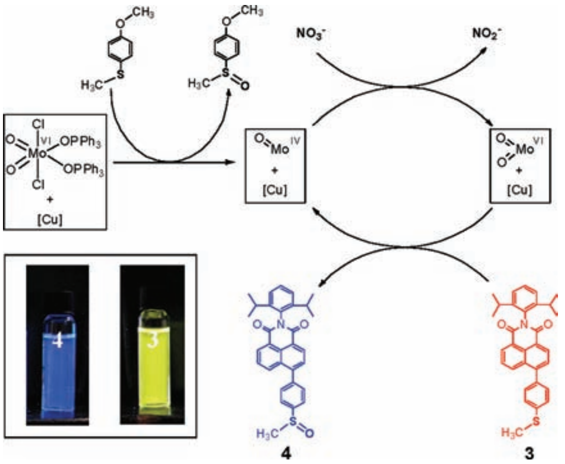


Figure 1. Determination of the optimal concentration of **4** based on fluorescence spectroscopy; inset: a correlation between the concentration of **4** and the intensity of the corresponding fluorescence.

In the previously reported Mo−Cu catalytic system for the sulfoxidation of thioethers, the first step of the OAT catalytic cycle is the oxidation of the thioether substrate.[20] These conditions would be unsuitable for the purpose of nitrate detection, as such an approach would generate a false-positive response (1 equiv of the catalyst would lead to the oxidation of 1 equiv of chemosensor **3** *without the presence of nitrate*). Therefore, we needed to invert the starting point of the reaction by converting the dioxo Mo^{VI} complex to the reduced mono-oxo Mo^{IV} complex so that the conversion of nitrate to nitrite would initiate the detection process. Our solution to this problem was the addition of an equimolar amount (with respect to the dioxo Mo^{VI} complex) of 4-methoxythioanisole (4MTA) "reducing agent" prior to the addition of chemosensor **3** to the detection mixture (Scheme 2). Also, it was necessary to use $CuCl_2$ instead of $Cu(NO_3)_2$ as a source of the Cu copromoter, as nitrate ions are the target analyte.

Additional tests that preceded our nitrate detection experiments included reference measurements of the fluorescence signal of the final reaction mixture. This mixture contained the Mo−Cu promoting system, compounds **3** and **4** (at various ratios), tetrabutylammonium nitrate (TBAN), and tetrabutylammonium nitrite. We found that these mixtures did not affect the intensity of the fluorescence signal of compound **4** in comparison to the same measurements of **4** alone in CH_3CN.

Scheme 2. Detection Process



Following the control experiments, the nitrate analysis was carried out using a detection mixture that contained the Mo−Cu promoting system (0.7 mM), 4MTA (0.7 mM), and chemosensor **3** (2.1 mM) in CH_3CN. In a typical detection experiment in which TBAN was used as the target analyte (7 mM), we monitored the progress of the reaction (at 60 °C) by fluorescence spectroscopy. During the course of this reaction (the time of the complete conversion of **3** to **4** was about 5 h), 10 μL samples were withdrawn from the reaction mixture every 30 min, diluted 1000-fold (in order to avoid photomultiplier saturation and self-quenching), and analyzed. The results obtained (see Figure 2 and the SI) clearly showed the rise of a strong emission peak at λ_{max} = 425 nm (assigned to **4**), with the concomitant reduction of the original peak at λ_{max} = 540 nm (assigned to **3**). The corresponding changes in the color of the visible emission from yellow to blue are shown in Figure 2a.

The detection process was also tested on additional nitrate-containing analytes, including KNO_3 and urea nitrate. In both cases, results identical to those for TBAN were obtained, showing very good response of the detection system toward different nitrate salts. No response was obtained upon application of our detection methodology to various nitrite salts or to common explosives such as TNT and RDX, indicating high selectivity of the Mo−Cu/thioether system to the nature of the analyte.

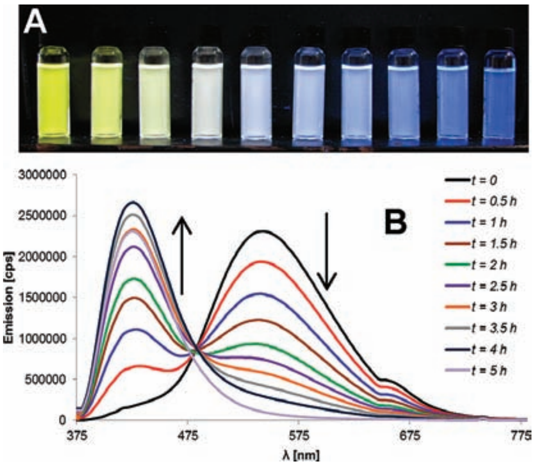


Figure 2. (a) Changes in the color of the fluorescence during the oxidation of **3** to **4**. (b) Changes in the fluorescence spectra during the oxidation of **3** to **4**, showing the concomitant rise in the fluorescence corresponding to **4** at 425 nm with the decay in the fluorescence at 540 nm corresponding to **3**.

In addition, the detection process was evaluated with H_2O_2 as another representative oxidant. Under the detection conditions developed for nitrate, upon reaction with H_2O_2, **3** was completely converted to the corresponding sulfone **5**, as was found by chromatography, mass spectroscopy, and NMR analyses. The latter compound exhibited a lower fluorescence intensity than the sulfoxide **4**, while the emission of **5** at λ_{max} = 411 nm closely overlapped the emission of **4** at λ_{max} = 425 nm (Figure 3).

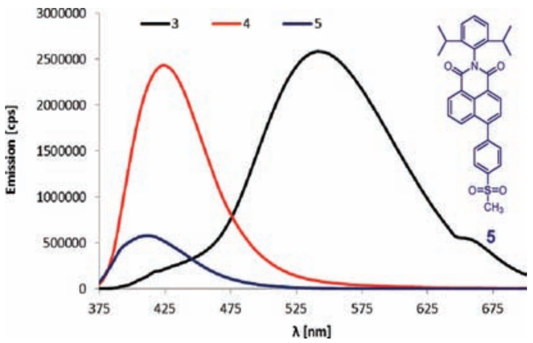


Figure 3. Fluorescence spectra of **3** (Φ_F = 0.26), **4** (Φ_F = 0.11), and **5**.

These results provide a demonstration of the plausible selectivity of our detection methodology, which is potentially capable of distinguishing between nitrate salts and H_2O_2-containing formulations. Therefore, a redesign of **3** was required in order to accommodate the demands for significant and visually observable differences among the emission properties of the thioether chemosensor and its sulfoxide and sulfone derivatives. Most reported structural modifications of the C-4-substituted 1,8-naphthalimides have been carried out by replacing the attached substituent with another one having a different electronic nature. These substitutions typically result in significant changes in the photophysical properties of the fluorophore.[21] However, such an approach was found to be unsuitable in our case, since the presence of the thioether functional group is crucial to the detection chemistry. Our solution to this problem was to modify the structure of the fluorophore by altering the position of the thioether substituent on the aromatic ring attached to the naphthalimide frame. A precedent for a similar approach was reported by Huang, Lu, and coworkers.[23] An isomer of **3** that exhibited the required spectroscopic properties was found to be compound **6** (Figure 4).

Chemosensor **6** was prepared in 80% overall yield by a route similar to that for **3**, with the use of 2-(methylthio)-benzeneboronic acid instead of 4-(methylthio)benzeneboronic acid in the last synthesis step (Scheme 1). Remarkably, the nitrate-based oxidation of **6** to sulfoxide **7** revealed a significant red shift in the emission spectrum of the formed compound. The measured red shift was from λ_{max} = 425 nm (assigned to **6**) to λ_{max} = 492 nm (assigned to **7**), in contrast to the blue-shift response observed in the sulfoxidation of **3**. On the other hand, oxidation of **6** to sulfone **8** using H_2O_2 resulted in a major reduction in the fluorescence intensity and was accompanied by a blue shift of 30 nm to λ_{max} = 395 nm. It should be mentioned that under the reaction conditions in which nitrate was used as the oxidant, the formation of sulfoxide **7** proceeded at a lower rate than the formation of **4**.

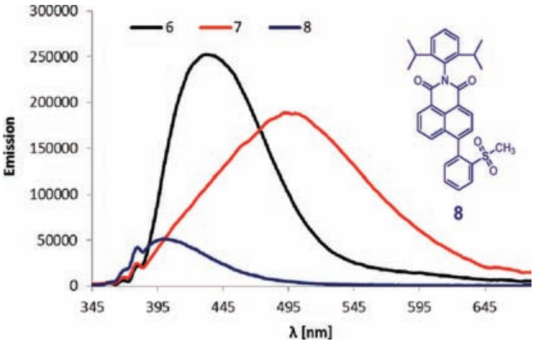


Figure 4. Fluorescence spectra of **6**, **7**, and **8**.

In conclusion, we have developed a new platform for the direct and selective detection of nitrate salts. In the present work, the first examples of thioether-based chemosensors, namely, compounds **3** and **6**, have been synthesized and evaluated in a bioinspired OAT detection process promoted by a Mo−Cu system. Sulfoxidation of **3** resulted in a large fluorescent hypsochromic shift (115 nm), while its isomer **6** was found to be capable of producing an unprecedented discriminating response upon its exposure to nitrate or H_2O_2. We are currently conducting further studies to improve the performance of our methodology via preparation of more efficient promoting systems and development of chemosensors and conditions for shorter reaction times.

Acknowledgment. We are grateful to Prof. A. Amirav (School of Chemistry, Tel Aviv University) for the Supersonic GC−MS analyses and to Dr. S. Hauptman and Dr. N. Tal (both from the School of Chemistry, Tel Aviv University) for the HRMS analyses. We thank Dr. S. Cohen (The Institute of Chemistry, The Hebrew University, Jerusalem) for the X-ray crystallography. We thank the Israel Ministry of Science and Technology, the Marian Gertner Institute for Medical Nanosystems, and Tel Aviv University for financial support.

Supporting Information Available. Experimental procedures; synthesis of substrates; and ^{1}H NMR, ^{13}C NMR, MS, FT-IR, UV−vis, and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

---

(23) Wang, Y.; Zhang, X.; Han, B.; Peng, J.; Hou, S.; Huang, Y.; Sun, H.; Xie, M.; Lu, Z. *Dyes Pigm.* **2010**, *86*, 190.