Monatsh Chem (2010) 141:615–620
DOI 10.1007/s00706-010-0311-7

ORIGINAL PAPER

An unprecedented rhodamine-based fluorescent and colorimetric chemosensor for Fe^{3+} in aqueous media

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Received: 30 July 2009 / Accepted: 18 March 2010 / Published online: 16 April 2010

Abstract A novel rhodamine-based chemosensor has been designed and synthesized. The chemosensor, a colorless and nonfluorescent compound, exhibited remarkable fluorescent and colorimetric response toward Fe^{3+} in aqueous media with high selectivity and sensitivity, and no significant response toward other metal ions such as Hg^{2+}, Ag^{+}, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Ce^{3+}, Mg^{2+}, K^{+}, and Na^{+}.

Keywords Fluorescence · Colorimetric · Chemosensor · Rhodamine B spirolactam

Introduction

In recent years, considerable efforts have been devoted to development of artificial fluorescent receptors for detection of biologically and environmentally important ionic species, especially heavy transition-metal (HTM) cations [1–6]. Among them, the Fe^{3+} ion plays vital roles in many biological processes, and deficiencies or excesses of this ion are toxic or can lead to a variety of diseases [7–9]. For instance, Fe^{3+} provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain.

It is well known that Fe^{3+} acts as a fluorescence quencher due to its paramagnetic nature, and in general the fluorescence turn-off response leads to less sensitivity than the turn-on response because of the low signal-to-noise ratio. Interestingly, relatively few examples of fluorescent chemosensors that selectively identify iron cations with amplified fluorescence have been documented [10–14]. Thus, there is an urgent need to develop selective fluorescence turn-on chemosensors for Fe^{3+}.

Rhodamines are an important class of fluorogenic and chromogenic probes and are ideal platforms for development of fluorescent chemosensors for specific HTM cations, due to their unique properties such as long-wavelength emission, high fluorescence quantum yield, and large molar extinction coefficient. They are generally nonfluorescent and colorless, whereas metal-induced ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and pink color [15–20].

Herein, we report the synthesis and the sensing properties of a new derivative of rhodamine-based fluorescent and colorimetric chemosensor **1**, which exhibited high selectivity and sensitivity toward Fe^{3+} in aqueous media (Scheme 1).

Results and discussion

Recently, Kim and coworkers [21] reported a new rhodamine 6G derivative **2**, as a fluorescent and colorimetric chemodosimeter in aqueous solution with broad pH span and high selectivity towards Hg^{2+} through a guanylation reaction. Based on this irreversible desulfurization reaction, we envisioned that the introduction of an additional

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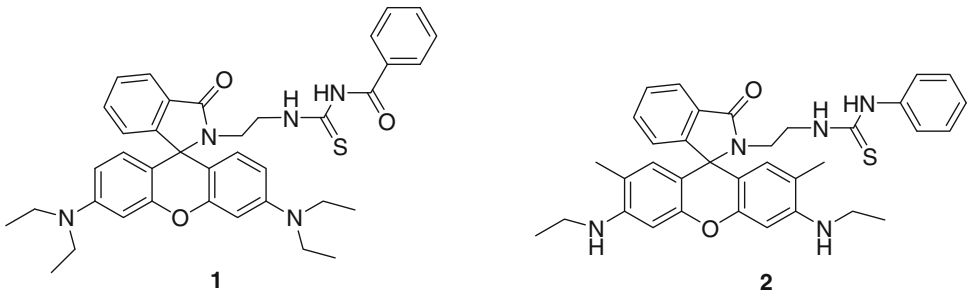
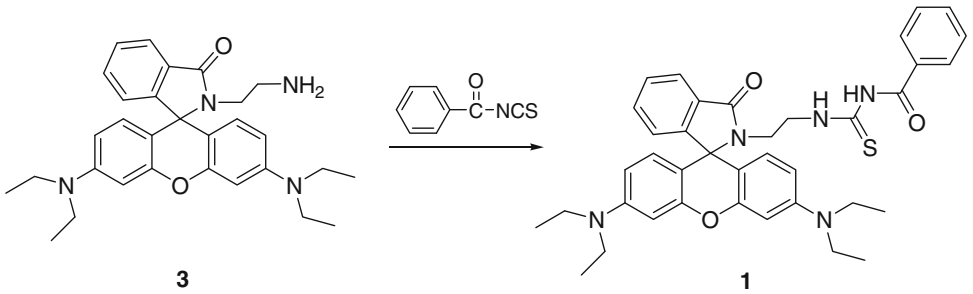
Scheme 1



Scheme 2



carbonyl group in **2** could have an activating effect on the guanylation reaction [22–25]. Therefore, compound **1** bearing a benzoyl group was designed to detect Hg^{2+} in aqueous media in order to obtain pronounced fluorescent emission and high selectivity compared with other HTMs. Contrary to our expectation, **1** showed almost no detectable binding affinity toward Hg^{2+}, instead behaving as a turn-on chemosensor for Fe^{3+} with high selectivity and sensitivity.

*Synthesis of chemosensor **1***

Chemosensor **1** was readily prepared from rhodamine B through a two-step protocol. According to Scheme 2, reaction of rhodamine B and ethylenediamine (EDA) in ethanol gave **3** [26], which on treatment with benzoyl isothiocyanate in acetone under reflux afforded **1** in 51% yield. Compound **1** was confirmed by spectroscopic and analytical data, which are in good agreement with the presented structure.

Spectral studies

Time evolution of the response of **1** (10 μM) in the presence of 20 equivalents of Fe^{3+} in methanol–water (9:1, v/v) was first studied. As shown in Fig. 1, the recognition interaction was completed at 40 min after addition of Fe^{3+}. Thus, further optical spectral data in this work were recorded at 40 min after addition of ionic species.

The fluorescence response of **1** (10 μM) toward various metal ions (Hg^{2+}, Ag^{+}, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Ce^{3+}, Mg^{2+}, K^{+}, and Na^{+}) in methanol–water (9/1, v/v) was then

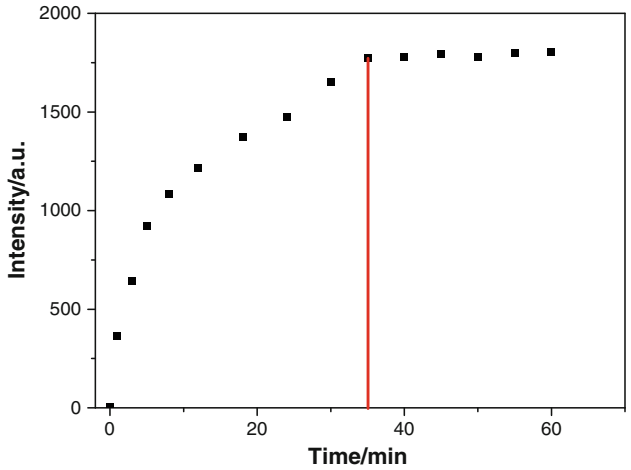


Fig. 1 Time course of response of **1** to Fe^{3+} (10 μM **1** and 0.2 mM Fe^{3+}) in methanol–water (9:1, v/v). Excitation wavelength was 530 nm

investigated, and the results are shown in Fig. 2a. Chemosensor **1**, without any metal ion, is colorless and has a weak fluorescent emission at 567 nm when excited at 530 nm. Upon addition of 20 equivalents Fe^{3+} to a solution of **1**, a red-shift and strong fluorescence peak at 580 nm with 92-fold fluorescence enhancement were observed. At the same time, under identical conditions, none of the other tested metal ions led to considerable fluorescence increase. Hence, in order to verify the high selectivity of **1** toward Fe^{3+}, competition experiments in the presence of potentially competitive metal ions were also conducted. Figure 2b shows that, except for Fe^{3+}, other metal ions (100 equiv to **1**) did not produce any noticeable increase in

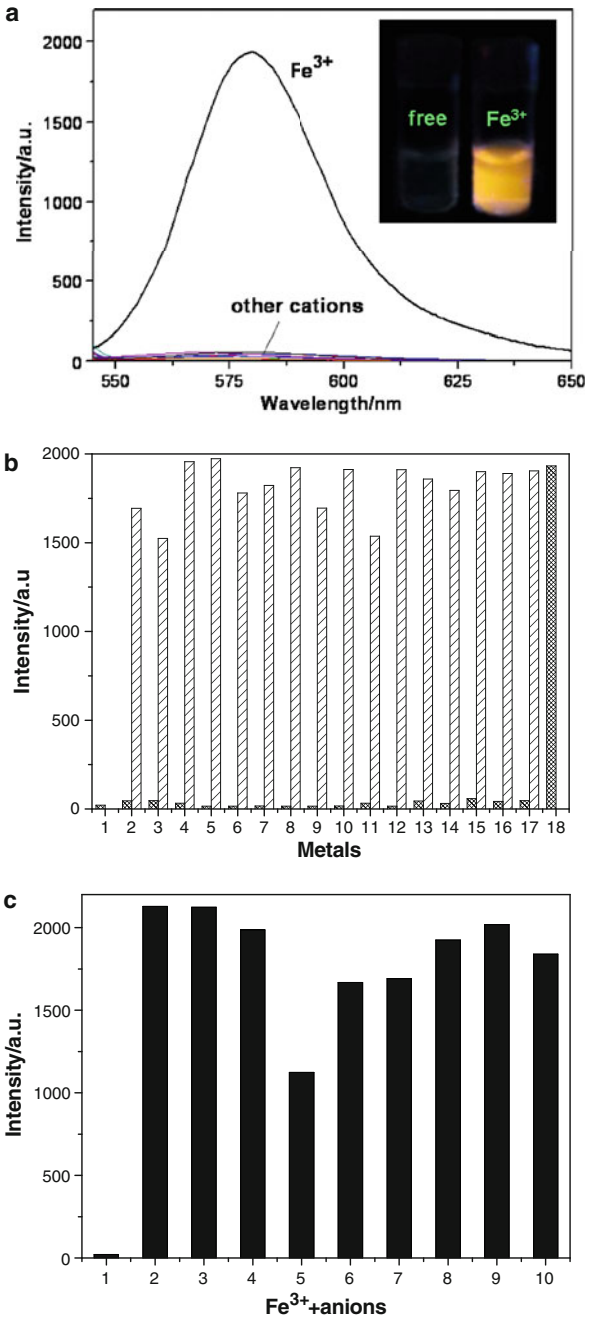


Fig. 2 **a** Fluorescent emission changes of **1** (10 μM) upon addition of various metal ions (20 equiv) in methanol–water (9:1, v/v). Excitation wavelength was 530 nm. **b** Fluorescence responses of **1** (10 μM) to various metal ions in methanol–water (9:1, v/v) at 580 nm. *Black bars* represent the addition of an excess of the appropriate metal ion (1.0 mM for all other cations and 0.2 mM for Fe^{3+}) to a 10 μM solution of **1**. *White bars* represent the addition of Fe^{3+} (0.2 mM) to the solutions containing **1** and 100 equiv. of the cations studied. *1* none; *2* Hg^{2+}; *3* Ag^{+}; *4* Pb^{2+}; *5* Sr^{2+}; *6* K^{+}; *7* Ba^{2+}; *8* Cd^{2+}; *9* Ni^{2+}; *10* Co^{2+}; *11* Fe^{2+}; *12* Mn^{2+}; *13* Cu^{2+}; *14* Zn^{2+}; *15* Ce^{3+}; *16* Mg^{2+}; *17*. Na^{+}; *18* Fe^{3+}. **c** Fluorescence responses of **1** (10 μM) containing 0.2 mM Fe^{3+} to the selected anions (0.2 mM) in methanol–water (9:1, v/v) at 580 nm. *1* none; *2* $FeCl_3$; *3* Br^{-}; *4* NO_3^{-}; *5* CO_3^{2-}; *6* HCO_3^{-}; *7* CH_3COO^{-}; *8* $H_2PO_4^{-}$; *9* ClO_4^{-}; *10* SO_4^{2-} (excitation slit 2.5 nm; emission slit 5.0 nm)

fluorescence intensity. Nevertheless, upon addition of Fe^{3+} (20 equiv to **1**) to the solution containing **1** and competitive cations, a significant increase in fluorescence intensity was observed. These results indicate that the recognition of Fe^{3+} by **1** is not significantly influenced by other coexisting cations and therefore that **1** exhibits very high selectivity toward Fe^{3+}. Furthermore, the behavior of chemosensor **1**–Fe^{3+} in the presence of various anions such as Cl^{-}, Br^{-}, NO_3^{-}, CO_3^{2-}, HCO_3^{-}, CH_3COO^{-}, $H_2PO_4^{-}$, ClO_4^{-}, and SO_4^{2-} in methanol–water (9/1, v/v) was investigated. As shown in Fig. 2c, no significant variation in fluorescence intensity was found, except for 47% quenching by CO_3^{2-} and 26% quenching by HCO_3^{-} and CH_3COO^{-}. Due to their competitive affinity with Fe^{3+}, the spirocyclic form of **1** is reproduced in the mixture, thus CO_3^{2-}, HCO_3^{-}, and CH_3COO^{-} can cause some interferences for chemosensor **1**–Fe^{3+} in the proposed method.

Figure 3 shows the fluorescence titration of **1** (10 μM) with Fe^{3+} under excitation at $\lambda_{ex} = 530$ nm in methanol–water (9:1, v/v). Incremental addition of Fe^{3+} resulted in gradual increase of the fluorescence intensity with emission peak at 580 nm, reaching saturation at addition of 150 equivalents. Nonlinear least-squares fitting of the titration profiles based on the 1:1 binding model strongly support the 1:1 stoichiometry of **1** and Fe^{3+}, and the binding constant was calculated to be 4.27×10^5 [27]. The equation used was:

$$Y = Y_0 + \frac{Y_{\lim} - Y_0}{2}\left\{1 + \frac{C_M}{C_L} + \frac{1}{K_s C_L} - \left[\left(1 + \frac{C_M}{C_L} + \frac{1}{K_s C_L}\right)^2 - 4\frac{C_M}{C_L}\right]^{\frac{1}{2}}\right\},$$

where Y is the recorded fluorescent intensity, Y_0 is the start value without addition of Fe^{3+}, $Y_{\lim}$ is the limiting value,

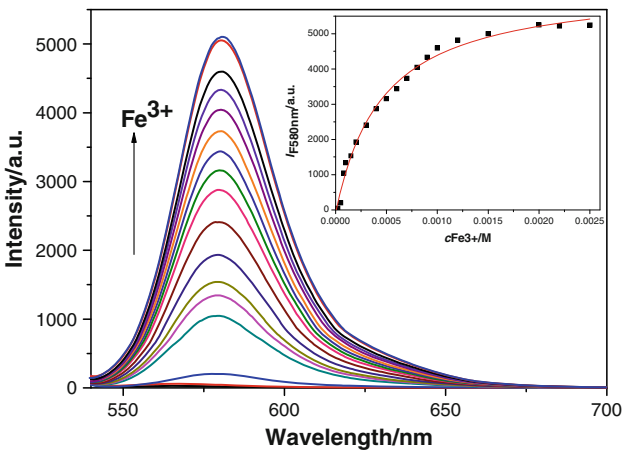


Fig. 3 Fluorescence titration spectra of **1** (10 μM) in methanol–water (9:1, v/v) upon gradual addition of Fe^{3+} ($\lambda_{ex} = 530$ nm). Inset: changes in fluorescence intensity at 580 nm against concentration of Fe^{3+}. Excitation wavelength was 530 nm (excitation slit 2.5 nm; emission slit 5.0 nm)

C_M is the Fe^{3+} concentration, and C_L is the sensor concentration.

Furthermore, upon addition of an increasing amount of Fe^{3+} ions to **1**, as shown in Fig. 4, a new absorption band centered at 557 nm appeared with increasing absorbance, which corresponds to the ring-opening process of the spirolactam moiety in **1**. The binding stoichiometry of **1** and Fe^{3+} was further proved by a Job plot according to the continuous variations with a total concentration of $[Fe^{3+}] + [\mathbf{1}]$ of 200 μM (Fig. 5). The absorbance approached a maximum when the molar ratio of Fe^{3+} was 0.5, indicating a 1:1 stoichiometry for the **1**–Fe^{3+} complex.

We also investigated the reversibility of the **1**–Fe^{3+} binding by a simple titration methodology with EDA. Accordingly, titration of **1**–Fe^{3+} with EDA led to significant decrease of absorbance and fluorescence intensity. At the end point, the solution returned to its original colorless state, which reveals that the response of **1** to Fe^{3+} is reversible rather than a cation-catalyzed reaction. This colorless solution was further confirmed as compound **1** by thin-layer chromatography (TLC) and mass spectrometry (MS) analysis. For practical applicability, the effect of pH on the absorbance of **1** in both the presence and absence of Fe^{3+} in methanol–water (9:1, v/v) was evaluated. Figure 6 clearly indicates that the chemosensor **1**, which has weak absorbance, can detect Fe^{3+} ions in the pH range between 4 and 7 because of its remarkable increase of absorbance on addition of Fe^{3+} ions.

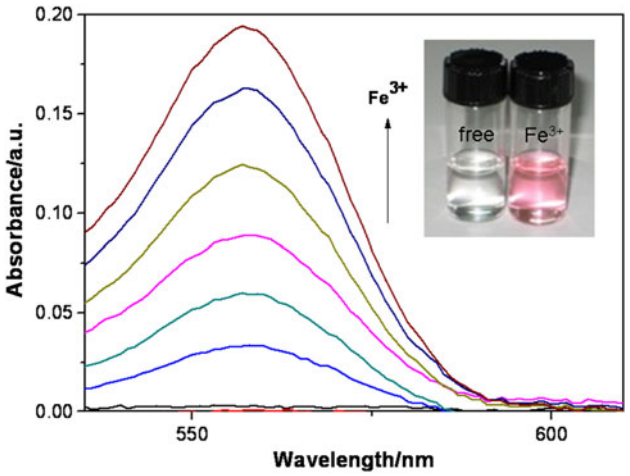


Fig. 4 UV–Vis titration spectra of **1** (10 μM) in methanol–water (9:1, v/v) upon gradual addition of Fe^{3+}

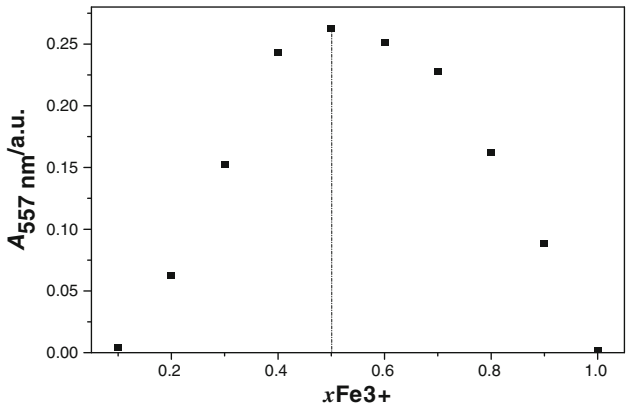


Fig. 5 Job plot according to the method of continuous variations, indicating the 1:1 stoichiometry of **1** and Fe^{3+}. The total concentration of ($[Fe^{3+}] + [\mathbf{1}]$) was 200 μM

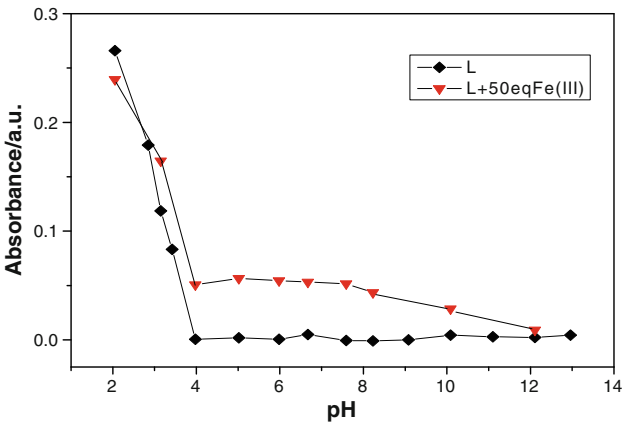
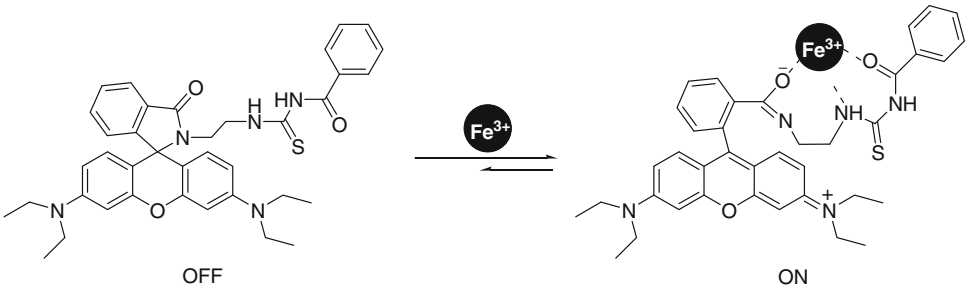


Fig. 6 Effect of pH on the absorbance of **1** (10 μM) in methanol–water (9:1, v/v) in absence and presence of Fe^{3+} at 557 nm

Binding mechanism

The possible binding mechanism of **1** with Fe^{3+} that led to the absorbance and fluorescence changes is shown in Scheme 3. According to the proposed mechanism of some rhodamine-based chemosensors reported so far, it may be that Fe^{3+} coordinates with the corresponding atom of **1** and induces ring-opening of the spirolactam. However, in recently reported rhodamine-based chemodosimeters with high selectivity and sensitivity toward Hg^{2+} [16, 18–26], the desulfurization guanylation mechanism is usually considered to be main reason for the recognition event. Nevertheless, during the spirolactam ring-opening and guanylation processes, the metal-induced spirolactam ring-opening is the first and critical step, which leads to significant absorbance and fluorescence enhancement, as they are the most important signals for the recognition event. Although chemosensor **1** has the potential model structure for the Hg^{2+}-promoted guanylation reaction, it exhibits higher binding affinity toward Fe^{3+} than other metal ions and leads to the easily detectable ring-opening process. This result may be attributed to the addition of a more electron-withdrawing carbonyl group to the thiourea moiety of **1**. The electron density on the sulfur atom of the

Scheme 3



thiourea group may decrease and lower the mercury affinity, and thus the relatively harder Fe^{3+} can easily bind with **1** according to the proposed mechanism depicted in Scheme 3.

Conclusions

We report herein a new simple rhodamine-based turn-on chemosensor **1** which displays high selectivity toward Fe^{3+} with no significant response to other metal ions. The Fe^{3+}-induced ring-opening of the rhodamine spirolactam moiety of **1** leads to dramatic absorption and fluorescent enhancement. The binding of **1** and Fe^{3+} is reversible with 1:1 binding stoichiometry.

Experimental

Compound **3** was prepared according to a literature procedure [26]. Acetone was dried over 4-Å molecular sieves prior to use. All other chemicals were purchased directly from commercial suppliers. Column chromatography was performed on silica gel (100–200 mesh). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 300 MHz NMR spectrometer with tetramethylsilane (TMS) as internal standard. High-resolution mass spectrometry (HRMS) was carried out on a UPLC/Q Tof mass spectrometer. Ultraviolet (UV) spectra were measured on a SP-1900 spectrophotometer. Fluorescence spectra were obtained with a Hitachi F-4500 FL spectrophotometer at room temperature for aerated solutions.

N-[[[2-[3′,6′-Bis(diethylamino)-3-oxospiro[1H-isoindole-1 , 9 ′-[9H]xanthen]-2(3H)-yl]ethyl]amino]thioxomethyl]-benzamide (**1**, $C_{38}H_{41}N_5O_3S$)

Compound **3** (0.6 g, 1.24 mmol) and 0.202 g benzoyl isothiocyanate (1.24 mmol) were dissolved in 30 cm^3 acetone and refluxed for 2 h. The solvent was evaporated under reduced pressure, and the crude solid was purified by silica-gel column chromatography with dichloromethane and ethylacetate (10:1) as eluents to give 0.43 g (51%) **1**. M.p.: 199–201 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.84$ (s, 1H), 7.51 (m, 9H), 6.27 (m, 2H), 6.19 (m, 6H), 3.51 (t, 2H), 3.33 (dd, 8H), 1.26 (t, 1H), 1.16 (t, 12H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 167.83$, 153.97, 153.30, 152.97, 152.56, 152.24, 152.09, 151.85, 149.41, 148.88, 146.15, 145.59, 136.78, 136.23, 135.97, 134.93, 134.52, 133.24, 132.53, 128.96, 128.62, 128.02, 127.48, 123.82, 123.03, 108.34, 97.94, 44.73, 44.36, 38.52, 12.61 ppm; HRMS (ESI) calcd. for $C_{38}H_{41}N_5O_3S$ 647.8286, found 647.8288.

Acknowledgments We are grateful to the Foundation of the Educational Department of Liaoning Province for financial support (no. 2008T002).

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