

ORIGINAL ARTICLE

Syntheses and Ion Selectivities of Dimeric Rhodamine 6G Chemosensors

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Abstract

Novel rhodamine 6G fluorescent chemosensors **1** and **2** for the detection of transition metal cations were synthesized through the condensation of rhodamine 6G ethylenediamine with each of 2-hydroxy-1-naphthaldehyde and 2,6-pyridinedicarbaldehyde, respectively. **1** and **2** were characterized using ¹³C NMR, ¹H NMR and mass spectroscopy. Fluorometric and colorimetric measurements involving various metal ions revealed the ring opening of the rhodamine 6G spirocycle framework. In the absence of metal cations, **2** was colorless and non-fluorescent, whereas the addition of metal cations (Hg²⁺ and others) changed the color to pink, accompanied by the appearance of an orange fluorescence. The chemosensors exhibited high selectivity for Hg²⁺ over other divalent first-row transition metals. The complexes of Hg²⁺ with **1** and **2** were successfully isolated. A huge enhancement in the fluorescence for both one- and two-photon excitations makes these compounds suitable candidates to be used for fluorescent labeling of biological systems.

Key words : Rhodamine 6G, Fluorescent, Colorimetric, Chemosensor, Mercury

1. Introduction

During the past few years, significant advances have been made in supramolecular chemistry owing to its role in the understanding of molecular processes occurring in synthetic chemistry, medical chemistry, catalysis, activation, transport and separation phenomena. The development of sensitive signaling systems for the determination and visualization of important chemical species is relevant in light of the recent spurt in interest in the fields of chemosensing and molecular imaging (Chang et al., 2012; Chang and Kim, 1994; Huang et al., 2005; Huang et al., 2005).

A large number of chemosensors with either

unique spectroscopic or electrochemical responses have been devised (De silva et al., 1997).

Recently, several interesting rhodamines have been used in various chemical and biological applications (Johnsson and Johnsson, 2007). In addition, efficient visualization systems for the molecular imaging of cells have also been developed (Yang et al., 2005).

Selective detection and recognition of transition metal ions have attracted increasing attention in supramolecular chemistry because of their importance in chemical, biological, and environmental processes (Valeurand and Leray, 2000). In particular, numerous research groups have developed superior fluorescent chemosensors for the detection and recognition of

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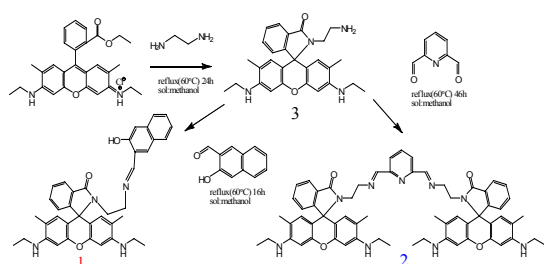
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Cu^{2+} and highly toxic Hg^{2+} ions (Lee et. al., 2008; Renzoni et. al., 1998). Hg^{2+} can be converted into methylmercury by bacteria, which can affect human health via marine food (Boening, 2000). Methylmercury can cause mercurial poisoning, which can lead to disorders of the central nervous system such as Minamata disease (Myers et. al., 2004).

On the other hand, an overdose of copper in body tissues could cause symptoms of the Wilson's disease (Kumar, 2006). Therefore, detection of copper and toxic mercury ions in living systems is extremely important.

The structure of the chemosensors and the accompanying ring-opening process was characterized using NMR and FAB (Fast Atom Bombardment)-mass spectroscopy. The colorimetric behavior has been examined using a highly selective chemodosimeter (Yang et. al., 2005).



Scheme 1. Syntheses of compounds **1** and **2**.

Rhodamine 6G has been used as a fluorescent chemosensor for Hg^{2+} (Zheng et. al., 2006). A bis-rhodamine-based chemosensor for transition metals (Xiang and Tong, 2006), a rhodamine derivative for Cu^{2+} (Xiang et. al., 2006) and a new rhodamine derivative showing highly selectivity for Hg^{2+} were recently reported (Wu et. al., 2007).

Mercury contamination occurs through oceanic and volcanic emission, gold mining (Malm, 1998), solid waste incineration, etc. Owing to the high toxicity of mercury, considerable attention has been devoted to the development of new fluorescent chemosensors

for the detection of mercury and its salts with high selectivity.

Herein, we synthesized two new rhodamine derivatives **1** and **2** bearing urea groups (**Scheme 1**). Compound **3** was synthesized using a one-step condensation of rhodamine 6G with ethylenediamine. Compounds **1** and **2** were then obtained by reacting **3** with either 2-hydroxy-1-naphthaldehyde or 2,6-pyridinedicarbaldehyde, respectively. Among the various metal ions, chemosensor **2** displayed highly selective colorimetric and fluorescent changes with respect to Hg^{2+} (**Fig. 1-2**). Hg^{2+} -complexes of **1** and **2** revealed high fluorescence quantum yields. The sensing mechanism of **1** and **2** is based on a change in the structure from the spirocyclic to the ring-open form or vice versa. With the addition of Hg^{2+} , the formation of the ring-open state occurred, which gave rise to a sensitive dual chromogenic and fluorogenic observation (OFF-ON).



Fig. 1. Colorimetric change in the color of 1×10^{-5} M solution of **1** (above), **2** (below) with 8 equivalents of the metal ion.

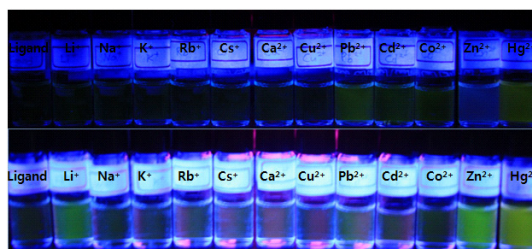


Fig. 2. Fluorometric change in the color of 1×10^{-5} M solution of **1** (above) and **2** (below) with 8 equivalents of the metal ion.

2. Experimental

2.1. General methods

A spectrometer at the Korea Basic Science Institute (Daegu, Korea) was used. ^1H - and ^{13}C -NMR spectra were obtained using 300 MHz Varian Mercury NMR Spectrometer, with tetramethylsilane (TMS) as an internal standard and CDCl_3 as solvent. The chemical shift was recorded in ppm (multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet). Fourier transform infrared (FT-IR) spectra were recorded using a Mattson Instrument Genesis II spectrometer. The mass spectrum was measured using a spectrometer. The absorption spectra were obtained on a Hewlett Packard UV-visible spectrophotometer UV-8453.

Materials: Rhodamine 6G and reagents were purchased from Aldrich and used as received. The solvents were of analytical grade, purchased from DC Chemical Co. Ltd., Korea, and used without further purification. Spectral grade solvents were obtained from Junsei Chemical Co. Ltd., Japan.

2.2. Synthesis of compound 3

Rhodamine 6G (1 g, 2.09 mmol) was dissolved in 25 mL methanol followed by the addition of ethylenediamine (0.67 mL, 10.05 mmol). The reaction mixture was refluxed for 24 h until the color was dark orange and then, cooled down to room temperature. The solvent was evaporated in vacuo followed by the addition of CH_2Cl_2 (100 mL) and water (200 mL) to separate the organic layer. The CH_2Cl_2 layer was washed twice with water and dried over anhydrous MgSO_4 . After filtering out MgSO_4 , evaporation of the solvent in vacuo yielded 0.8 g of **3** in 85% yield; m.p.: 255–260°C; ^1H NMR (300 MHz, CDCl_3) δ 7.94 (m, 1H), 7.47 (m, 2H), 7.10 (m, 1H), 6.36 (s, 2H), 6.21 (s, 2H), 3.19 (m, 6H), 2.38 (t, 2H), 1.81 (s, 6H), 1.30 (t, 6H).

2.3. Synthesis of compound 1

Compound **3** (45.6 mg, 0.1 mmol) was dissolved

in 10 mL methanol followed by the addition of 2-hydroxy-1-naphthaldehyde (17.2 mg, 0.1 mmol). The reaction mixture was refluxed for 16 h until the color was light orange, and then cooled down to room temperature. The solvent was evaporated in vacuo yielding 39 mg of **1** in 82% yield. m.p.: 264–267 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.44 (s, 1H), 7.91 (m, 1H), 7.78 (m, 1H), 7.60 (m, 2H), 7.46 (m, 2H), 7.21 (m, 2H), 7.09 (m, 1H), 6.82 (m, 1H), 6.36 (s, 2H), 6.21 (s, 2H), 3.41 (m, 4H), 3.19 (m, 4H), 1.81 (s, 6H), 1.30 (t, 6H); ^{13}C NMR (300MHz, CDCl_3) δ 176.04, 168.42, 158.23, 153.40, 151.68, 147.52, 136.98, 133.70, 132.74, 130.85, 129.04, 128.23, 128.17, 127.75, 126.05, 124.88, 123.88, 122.89, 122.56, 118.10, 117.98, 106.73, 105.45, 96.47, 65.09, 50.39, 40.76, 38.26, 16.65, 14.67. FAB MS $m/z = 611.30[\text{M}+\text{H}]^+$, calc. for $\text{C}_{39}\text{H}_{38}\text{N}_4\text{O}_3 = 610.74$.

2.4. Synthesis of compound 2

Compound **3** (91.2 mg, 0.2 mmol) was dissolved in 15 mL methanol followed by the addition of 2,6-pyridinedicarbaldehyde (13.5 mg, 0.1 mmol). The reaction mixture was refluxed for 46 h until the color changed to dark orange, and then cooled down to room temperature. The solvent was evaporated in vacuo yielding 40 mg of **2** in 86% yield. m.p.: 215–217 °C; ^1H NMR (300MHz, CDCl_3) δ 8.02 (s, 2H), 7.92 (m, 2H), 7.76 (d, 2H), 7.67 (t, 1H), 7.43 (t, 4H), 7.03 (d, 2H), 6.32 (s, 4H), 6.25 (s, 4H), 3.46 (t, 4H), 3.17 (m, 12H), 1.87 (s, 12H), 1.81 (t, 12H); ^{13}C NMR (300MHz, CDCl_3) δ 168.65, 162.89, 154.19, 154.09, 151.89, 147.60, 137.08, 132.73, 131.20, 128.77, 128.22, 124.04, 123.02, 122.42, 118.12, 106.12, 96.75, 65.23, 59.10, 41.12, 38.56, 16.97, 14.98. FAB MS $m/z = 1012.52[\text{M}+\text{H}]^+$, calc. for $\text{C}_{63}\text{H}_{65}\text{N}_9\text{O}_4 = 1012.25$.

3. Results and discussion

The intermediate **3** was efficiently synthesized from rhodamine 6G in a relatively good yield. Compound **3** was a valuable intermediate as it could either act as a tagging material or could be used for the introduction of various functional groups.

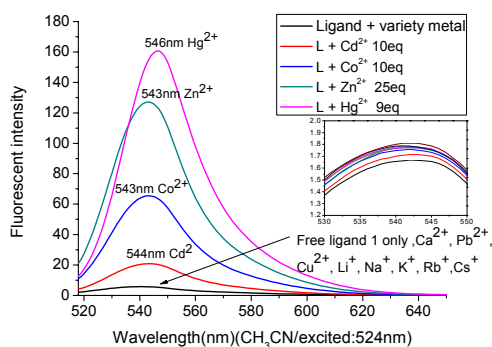


Fig. 3. Fluorescent emission spectra of **1** (0.1 μM) obtained upon addition of various metal ions (9 equiv.) in CH₃CN (excitation at 524 nm) (excitation and emission slit: 5).

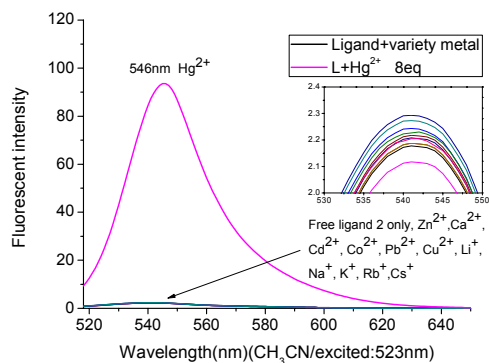


Fig. 4. Fluorescent emission spectra of **2** (0.1 μM) upon addition of various metal ions (8 equiv.) in CH₃CN (excitation at 523 nm) (excitation and emission slit: 2.5).

Treatment of **3** with 2-hydroxy-1-naphthaldehyde and 2,6-pyridinedicarbaldehyde gave **1** and **2** in 82% and 86% yields, respectively.

The perchlorate salts of Hg²⁺, Ca²⁺, Cd²⁺, Co²⁺, Pb²⁺, Cu²⁺, Cs⁺, K⁺, Li⁺, Na⁺, Rb⁺, and Zn²⁺ ions were used to evaluate the metal ion-binding properties of **1**

and **2** in acetonitrile. The fluorescence spectra were obtained by excitation at either 524 nm or 523 nm. Both the excitation and emission slits were either 5 nm or 2.5 nm. Among these metal ions (25 equiv.), **1** showed a large chelation-enhanced fluorescence (CHEF) effect with Hg²⁺ and Zn²⁺, while it showed a relatively smaller CHEF effect with Co²⁺ and Cd²⁺ (**Fig. 3**).

On the other hand, **2** displayed a highly selective CHEF effect with Hg²⁺ even though there was a smaller CHEF effect with Zn²⁺ (**Fig. 4**). An overall emission change of over 8-fold was observed for Hg²⁺. There was also a large enhancement (~9-fold) in the UV absorption (λ_{max} = 523 nm) of **2** upon the addition of Hg²⁺.

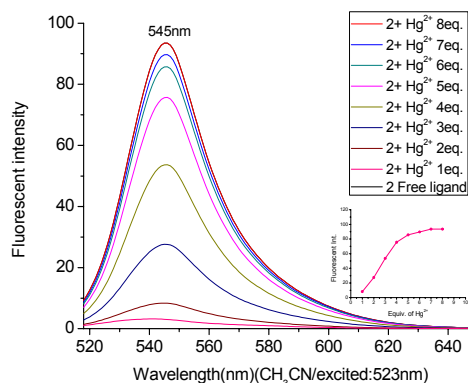


Fig. 5. Fluorescent titrations of **2** (0.1 μM) with Hg²⁺ in CH₃CN (excitation at 523 nm) (excitation and emission slit: 2.5).

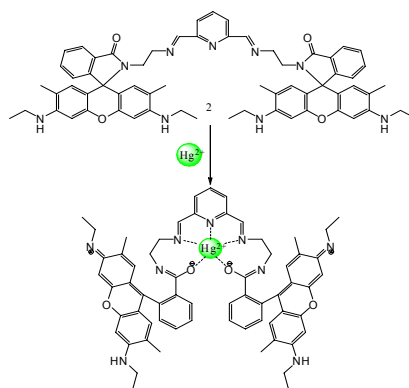


Fig. 6. Mechanism of change of fluorescence upon the addition of Hg²⁺.

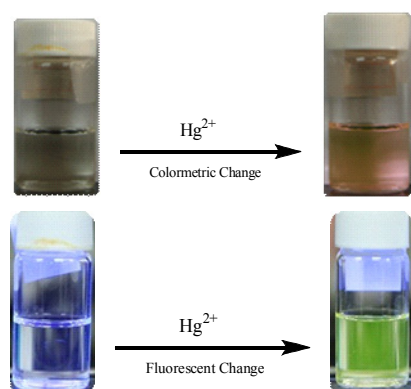


Fig. 7. Colorimetric and fluorescent changes of **2** (0.1 μ M) with Hg^{2+} (8 equiv.) in CH_3CN .

The stoichiometry (1:1) was also confirmed through the Job plot based on the changes in fluorescence.

Figure 5 displays the fluorescence turn-on response of **2** towards Hg^{2+} ions. The addition of 8.0 equiv. of Hg^{2+} triggered a 40-fold increase in integrated emission. The proposed mechanism of this change in fluorescence is explained in **Fig. 6**. Upon the addition of Hg^{2+} to a colorless solution of **2**, both red color and the fluorescent characteristics of rhodamine 6G appeared (**Fig. 7**). The large enhancement in fluorescence as well as the colorimetric change could be attributed to the spirolactam ring opening, which was induced by the complexation of Hg^{2+} . Two carbonyl oxygens as well as three nitrogens provided an appropriate binding pocket for Hg^{2+} as shown in **Fig. 6**. Compound **1** displayed a smaller but similar fluorescent enhancement with Hg^{2+} in acetonitrile compared to **2**.

4. Conclusion

In summary, we have demonstrated rhodamine-based mercury ion sensitive sensors. Two new rhodamine derivatives bearing urea groups were synthesized using a two-step procedure. The dimeric rhodamine derivative **2** displayed a highly selective fluorescent enhancement and colorimetric change with respect to Hg^{2+} . In addition, an aminoethyl rhodamine **3** was

synthesized in good yield from rhodamine 6G. Compound **3** is a useful intermediate that could act as a tagging material for various biological purposes. The advantages of sensor **2** include high sensitivity and rapid response.

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