

Research Article

Synthesis of Novel Fluorescent Sensors Based on Naphthalimide Fluorophores for the Highly Selective Hg^{2+} -Sensing

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With an aim to develop the new sensors for optical detection of Hg^{2+} ions, two novel fluorometric sensors were designed and successfully prepared using 2-(3-(2-aminoethylsulfanyl)propylsulfanyl)ethanamine and one or two *N*-methylnaphthalimide moieties (**1** and **2**). Sensor **1** was obtained via *N*-alkylation, *N*-imidation and a one-pot nucleophilic aromatic substitution, and *N*-formylation of the amine, while sensor **2** was prepared via *N*-alkylation, *N*-imidation, and nucleophilic aromatic substitution. The characterization, including ^1H NMR, ^{13}C NMR, and mass spectrometry, was then performed for **1** and **2**. The Hg^{2+} -binding behaviors of the sensors were investigated in terms of sensitivity and selectivity by fluorescence spectroscopy. Sensor **1** especially provided the reversible and highly Hg^{2+} -selective ON-OFF fluorescence behavior by discriminating various interfering ions such as Pb^{2+} , Co^{2+} , Cd^{2+} , Mn^{2+} , Fe^{2+} , K^+ , Na^+ , and in particular Cu^{2+} and Ag^+ with a detection limit of 22 ppb toward Hg^{2+} ions.

1. Introduction

Mercury is recognized as a toxic heavy metal as well as the hazardous and poisonous pollutants, which can be accumulated and persisted in the environment: soil, water, and air [1–5]. Mercury is considered as a neurotoxin which can lead to many serious human health problems such as permanent central nervous system damage, DNA damage, and mitosis impairment [6–8]. In the marine system, inorganic mercury is changed to methylmercury via bacteria systems. This methylmercury can then go through biological membranes and finally enter into the food chain [2–4, 8]. Fluorescence spectroscopy is an alternative method for the detection of many analytes, which presents several promising approaches and can be very useful in the qualitative and quantitative detection, such as the determination of small molecules [9], proton [10], anions [11], or cations, including Hg^{2+} ions [8, 12–26]. The method is relatively inexpensive,

nondestructive to samples, sensitive, and selective and allows prompt determination, which can be utilized as a tool for real time tracking of Hg^{2+} [8, 26].

Although there have been many fluorescence Hg^{2+} -chemosensors designed by utilizing multidentate nitrogen, oxygen, and thioether-containing ionophores [8, 26], such as cyclen [12, 13], hydroxyquinoline [14], azine [17, 18], cyclams [19–21], calixarenes [22–24], and diazatetrathia crown ethers [25], many of these sensors still have some limitations in production and practical usages. This may be because of the relatively high cost of commercial ionophores, low selectivity, or cross sensitivity toward competitive cations such as silver ions, copper ions, and lead ions, as a result from their similarity in chemical behaviors to Hg^{2+} [14, 17, 18, 21, 22, 27–29]. Moreover, some sensors exhibit the absorptions and fluorescent emissions in the UV region, so their optical signals are in the same ranges of wavelength absorbed by matrix interference in biological samples and glass optical

systems [26]. This can lead to the low sensor signals and more limitations on further uses of these sensors in portable and inexpensive testing devices [26].

In most fluorescent sensor systems, an ionophore is responsible for selective binding of a target ion, whereas the sensitivity is normally controlled by a selected fluorophore. Here, our strategy is to introduce an acyclic ionophore which contained nitrogen and sulfur atoms as a receptor site for Hg^{2+} . Unlike preorganized ionophores such as cyclen, cyclam, and calixarenes, acyclic ionophore can offer a high degree of freedom for self-assembly to Hg^{2+} . This is due to some electrostatic interactions between ions coordinated with donor atoms of acyclic ionophore. As a result, the selectivity of these sensor systems is fairly high. Moreover, since the naphthalimide fluorophore has strong fluorescence, high absorption coefficient, high quantum yield, and large Stokes shift, it was used as the signaling portion of the sensors here [8, 15, 30–32]. In addition, we expected that the modification of naphthalimide at the 4-position by an amine group could induce a bathochromic shift of absorption and fluorescence spectra into visible region, which will be beneficial for further fabrication of economical devices.

In this study, the designed sensors, **1** and **2**, composed of 2-(3-(2-aminoethylsulfanyl)propylsulfanyl)ethanamine and one or two *N*-methyl-naphthalimide moieties were prepared. Their Hg^{2+} sensing behaviors were examined under various conditions, including foreign ion interference. These sensors should function in visible region with high sensitivity and selectivity toward Hg^{2+} .

2. Experimental Section

2.1. Materials. The reagents and solvents were purchased from Fluka Chemical Corporation while the metal salts (perchlorate salts) were purchased from Strem chemicals, Inc. All reagents, solvents, and metal salts were used as received.

2.2. Methods. NMR spectra in CDCl_3 solutions were recorded using Bruker Avance 300 spectrometer operating at 75 MHz for ^{13}C and 300 MHz for ^1H . Fluorescence spectra were measured using Perkin Elmer Luminescence spectrometer LS 50B. The emission slit widths used in fluorescence measurements were 5.0 nm with a scan rate of 300 nm/min. ThermoElectron LCQ-DECA-XP, ESI-Ion trap mass spectrometer, was used to record mass spectra of the synthetic compounds. Discovery Studio 5.5 program package was employed to perform the molecular modeling studies.

2.3. Syntheses

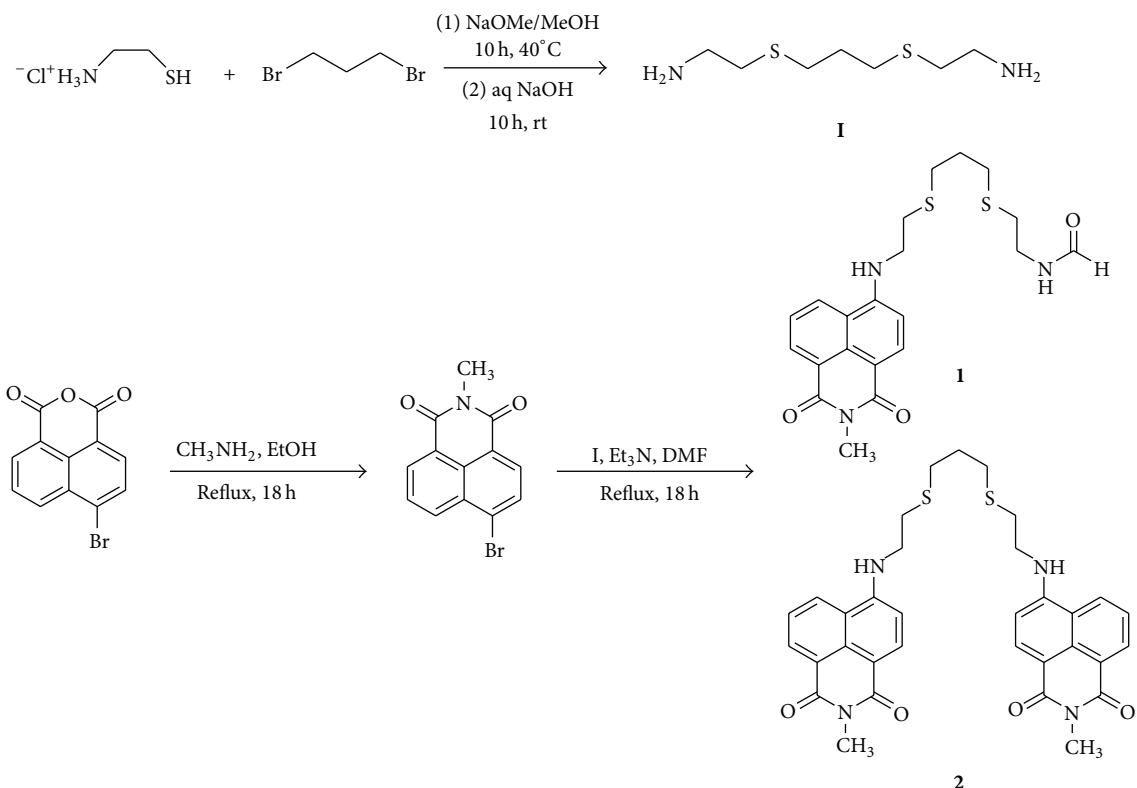
2.3.1. 2-[3-(2-Aminoethylsulfanyl)propylsulfanyl]ethanamine. This compound was prepared using the same procedure as in reference [33]. Its synthetic pathway is presented in Scheme 1.

2.3.2. 4-Bromo-*N*-methyl-naphthalimide. 0.20 g of 4-bromo-1,8-naphthalic anhydride (0.72 mmol) was dissolved in dried ethanol (8 mL), followed by the addition of methylamine

(0.4 mL). After the solution was refluxed under an argon atmosphere for 18 h, the solution was filtered and its solvent was removed under rotary evaporator. 30 mL of dichloromethane (CH_2Cl_2) was added; then three times of extraction were done using 30 mL of deionized water each. The CH_2Cl_2 portion was dried over anhydrous sodium sulfate before it was removed by rotary evaporator. Finally, yellow powder of 4-bromo-*N*-methyl-naphthalimide was obtained and purified by preparative thin layer chromatography (TLC) (CH_2Cl_2 , $R_f = 0.24$), 41%. ^1H NMR (300 MHz, CDCl_3): δ 3.57 (s, 3H), 7.85 (t, $J = 8.1$ Hz, 1H), 8.04 (d, $J = 8.1$ Hz, 1H), 8.42 (d, $J = 7.8$ Hz, 1H), 8.57 (d, $J = 8.4$ Hz, 1H), 8.66 (d, $J = 7.2$ Hz, 1H) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ 27.0 (CH_3), 121.8 (C), 122.7 (C), 127.9 (CH), 128.3 (C), 130.1 (C), 130.3 (C), 130.9 (CH), 131.5 (CH), 133.5 (2CH), 163.6 (2C=O) ppm. HRMS (ESI) calcd for $\text{C}_{13}\text{H}_9\text{BrNO}_2^+ (\text{M}+\text{H})^+$ 289.9817, found 289.9736.

2.3.3. Sensor 1. 4-Bromo-*N*-methyl-naphthalimide (0.102 g, 0.35 mmol) was reacted with 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine (0.017 g, 0.08 mmol) in *N,N*-dimethylformamide (7 mL), followed by the addition of 0.24 mL of triethylamine under an argon atmosphere. After the solution was refluxed for 18 h, its solvent was removed by rotary evaporator. 20 mL of CH_2Cl_2 was added; then three times of extraction were done using 20 mL of deionized water each. The CH_2Cl_2 portion was dried over anhydrous sodium sulfate before it was removed by rotary evaporator. Finally, to obtain yellow powder of sensor **1**, the residue was purified by preparative TLC (CH_2Cl_2 : MeOH 97:3, $R_f = 0.12$) to yield 18.3 mg of **1** as a yellow powder, 53%. ^1H NMR (300 MHz, CDCl_3): δ 1.74–1.96 (m, 2H), 2.63–2.72 (m, 6H), 2.98 (t, $J = 6.3$ Hz, 2H), 3.49 (t, $J = 3.6$ Hz, 2H), 3.53 (s, 3H), 3.61 (q, $J = 5.7$ Hz, 2H), 6.72 (d, $J = 8.4$ Hz, 1H), 7.64 (t, $J = 8.1$ Hz, 1H), 8.19 (d, $J = 7.5$ Hz, 1H), 8.20 (s, 1H), 8.46 (d, $J = 8.1$ Hz, 1H), 8.59 (d, $J = 7.2$ Hz, 1H) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ 26.8 (CH_2), 28.9 (CH_2), 30.1 (CH_2), 30.3 (CH_2), 30.9 (CH_3), 31.5 (CH_2), 37.2 (CH_2), 41.7 (CH_2), 104.5 (CH), 110.8 (C), 120.5 (C), 123.0 (C), 124.9 (CH), 126.1 (CH), 129.7 (C), 132.2 (CH), 134.3 (CH), 149.0 (C=O), 161.2 (C), 164.4 (C=O), 164.9 (C=O) ppm. The calculated HRMS (ESI) for $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_3\text{S}_2\text{Na}^+ (\text{M}+\text{Na})^+$ is 454.1230 while the obtained HRMS (ESI) is 454.1239.

2.3.4. Sensor 2. Using similar procedure to sensor **1**, sensor **2** was obtained as yellow oil with the yield of 6% (2.9 mg), (CH_2Cl_2 : MeOH = 95:5, $R_f = 0.49$). ^1H NMR (300 MHz, CDCl_3): δ 1.88–1.98 (m, 2H), 2.70 (t, $J = 6.9$ Hz, 4H), 2.95 (t, $J = 6.3$ Hz, 4H), 3.52 (s, 6H), 3.54–3.60 (m, 4H), 6.68 (d, $J = 8.7$ Hz, 2H), 7.62 (t, $J = 7.8$ Hz, 2H), 8.10 (d, $J = 8.4$ Hz, 2H), 8.44 (d, $J = 8.4$ Hz, 2H), 8.57 (d, $J = 7.5$ Hz, 2H) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ 26.4 (CH_2), 29.5 (2 CH_2), 29.9 (2 CH_2), 42.9 (2 CH_2), 54.9 (2 CH_3), 103.7 (2CH), 108.0 (2C), 120.1 (2C), 121.8 (2C), 124.3 (2CH), 128.3 (2CH), 129.2 (2C), 130.5 (2CH), 134.0 (2CH), 150.1 (2C), 163.1 (2C=O), 164.0 (2C=O) ppm. The calculated HRMS (ESI) for $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_3\text{S}_2\text{Na}^+ (\text{M}+\text{Na})^+$ is 635.1757 while the obtained HRMS (ESI) is 635.1692.

SCHEME 1: Syntheses of fluorescence sensors **1** and **2**.

2.4. Binding Studies. For **1** and **2**, their binding studies to metal ions were done in acetonitrile (CH_3CN) and CH_2Cl_2 , respectively. By dissolving selected amount of salt in water, 1.0×10^{-2} M of perchlorate salt solutions was prepared. The fluorescence titration was carried out with solutions of 1.5×10^{-7} and 8.2×10^{-7} M for **1** and **2**, respectively, and the excitation wavelengths (λ_{ex}) were 426 nm for **1** and 420 nm for **2**. Fluorescence spectra were collected at a fixed wavelength range of 440–620 nm.

3. Results and Discussion

3.1. Design and Preparation of 1 and 2. 2-(3-(2-Aminoethylsulfanyl)propylsulfanyl)ethanamine, which has two atoms of S and N for the selective binding sites of Hg^{2+} , was utilized as an acyclic host [5, 8]. The selective ion binding was expected to occur via the self-assembly of the acyclic sensors and Hg^{2+} as there are favorable ion-dipole interactions of the ions coordinated with the donor atoms [5, 8]. As a result, there were emission changes of the *N*-methylnaphthalimide fluorophores. Moreover, the acyclic host was expected to provide a high degree of freedom and consequently correct directional binding to Hg^{2+} compared to rigid ionophores, such as cyclens, cyclams, and diazatetrathia crown ethers.

Sensor **1** was synthesized by *N*-alkylation, *N*-imidation, one-pot nucleophilic aromatic substitution, and *N*-formylation of the amine [34]. Sensor **2** was prepared

via *N*-alkylation, *N*-imidation, and nucleophilic aromatic substitution.

3.2. Sensitivity Studies. Absorption and fluorescence measurements were performed to explore the spectroscopic and photophysical properties of the designed sensors. As shown in Figure 1, the absorption and emission spectra of solution **1** were in the visible region ($\lambda_{\text{ex}} = 426$ nm, $\lambda_{\text{em}} = 512$ nm) and there was a large Stokes shift approximately 86 nm in solution of **1**. It should be noted that the modification of 2-(3-(2-aminoethylsulfanyl)propylsulfanyl)ethanamine at the 4-position of the naphthalimide fluorophore can induce the absorption and the emission spectra to the red region compared to the reported sensors [8, 35], modifying naphthalimide fluorophore at *O*-position which exhibited absorption and fluorescent emissions in the UV region ($\lambda_{\text{ex}}, \lambda_{\text{em}} < 400$ nm).

The sensitivity studies of **1** and **2** were performed by adding Hg^{2+} with different concentrations to the solutions of the designed sensors; then observe their emission responses. The sensors showed high Hg^{2+} -sensitivity justified from the emission of the modified *N*-methylnaphthalimide fluorophores centered at about 512 nm and 492 nm for **1** and **2**, respectively. The fluorescence spectra of sensors **1** and **2** in the absence and presence of various concentrations of Hg^{2+} were illustrated in Figures 2 and 3, respectively.

The fluorescence signal of the sensor reached the maximum when there was no Hg^{2+} ion. On the other hand, the

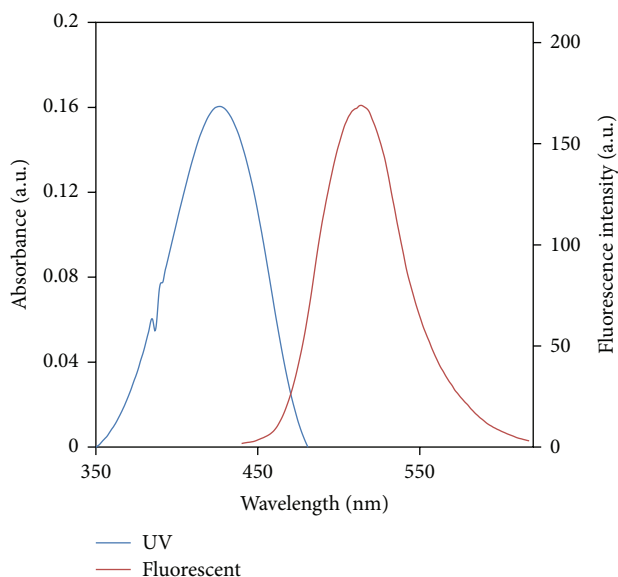


FIGURE 1: Absorption and fluorescence spectra of **1** ($0.92\ \mu\text{M}$) in acetonitrile.

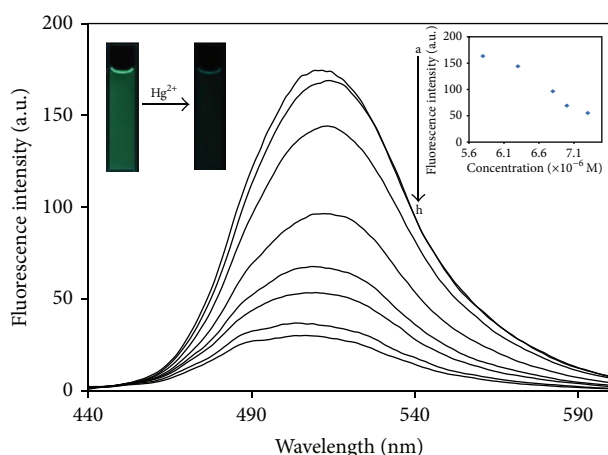


FIGURE 2: Fluorescence spectra ($\lambda_{\text{ex}}\ 426\ \text{nm}$) of **1** ($0.15\ \mu\text{M}$) in CH_3CN as a function of $[\text{Hg}^{2+}]$. a: $0\ \text{M}$, b: $4.6\ \mu\text{M}$, c: $6.3\ \mu\text{M}$, d: $6.8\ \mu\text{M}$, e: $7.0\ \mu\text{M}$, f: $7.9\ \mu\text{M}$, g: $10\ \mu\text{M}$, and h: $19\ \mu\text{M}$. (The inset shows intensity of sensor **1** versus concentration of Hg^{2+} .)

signal was decreased (“turn off”) when there was an increase in Hg^{2+} concentration. As can be seen in Figure 2, when an ion complexation was operative, there was an “ON-OFF” switching mechanism, responding to Hg^{2+} ion complexation, observed in the fluorescence of **1**. Moreover, nonlinear curve fitting of the changes in fluorescence titration results was used to calculate the association constant, K_{assoc} [8, 33, 36–40]. In this case, K_{assoc} was $3.7 \times 10^7\ \text{M}^{-1}$ for 1:1 complex formation of **1**- Hg^{2+} , which also agreed well with molecular modeling shown in Figure 4 as well as Job’s plot analysis shown in Figure 5. Using coumarin 6 as a reference with a fluorescence quantum yield (Φ_f) of 0.78 in ethanol [41], the Φ_f of **1** was also observed with the value of 0.14.

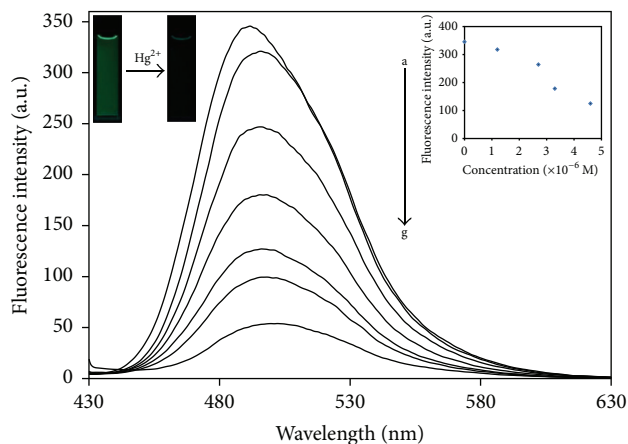


FIGURE 3: Fluorescence spectra ($\lambda_{\text{ex}}\ 420\ \text{nm}$) of **2** ($0.82\ \mu\text{M}$) in CH_2Cl_2 as a function of $[\text{Hg}^{2+}]$. a: $0\ \text{M}$, b: $1.2\ \mu\text{M}$, c: $2.7\ \mu\text{M}$, d: $3.3\ \mu\text{M}$, e: $4.6\ \mu\text{M}$, f: $24.3\ \mu\text{M}$, and g: $79.6\ \mu\text{M}$. (The inset shows intensity of sensor **2** versus concentration of Hg^{2+} .)

According to the plot of fluorescence intensity as a function of the Hg^{2+} concentrations, the detection limit of **1** for the determination of Hg^{2+} was calculated and found to be $1.1 \times 10^{-7}\ \text{M}$ or 22 ppb for Hg^{2+} . Compared with the previously reported Hg^{2+} -fluorescence sensors, the value of detection limit of **1** here is lower or in the same range [8, 14, 19–22, 28, 29, 32, 42–45] and it is low enough to detect mercury levels in environmental sources and dietary [46, 47].

For sensor **2**, quantitative binding affinities of **2** with Hg^{2+} were also investigated. As can be seen in Figure 3, sensor **2** provided fluorescence quenching upon Hg^{2+} binding. However, in comparison to sensor **1**, sensor **2** was an inferior sensor in term of sensitivity. Moreover, the detection limit for the analysis of Hg^{2+} with the value of $2.94 \times 10^{-7}\ \text{M}$ or 59 ppb toward Hg^{2+} was observed for sensor **2**. The fluorescence quantum yield (Φ_f) of **2** was obtained with the value of 0.33. In addition, K_{assoc} for **2** was $1.1 \times 10^{11}\ \text{M}^{-2}$, suggesting that it should be 1:2 complex formation of sensor **2**- Hg^{2+} .

3.3. Sensor Binding Modes. The Discovery Studio 5.5 program was used to model sensors **1** and **2** as well as the binding conformations of sensors **1** and **2** upon binding Hg^{2+} . From the X-ray crystal structure of *N,N'*-(3,7-diazanonylene)-bis-naphthalimide (PDB 1CX3), the initial structures of **1** and **2** were modified and optimized using CHARMM force field. The low energy configurations of the sensors **1** and **2** were further investigated by molecular dynamics (MD) simulations in the implicit solvent model (in acetonitrile and dichloromethane) at the constant temperature at 300 K for 1000 ps with a time step of 1 fs under NVT ensemble. The distance-dependent dielectrics for acetonitrile and dichloromethane were 37.5 and 8.93, respectively. Moreover, using the density functional theory with local density approximation (LDA) of local functional PWC with implicit distance-dependent dielectrics and the energy of sensors,

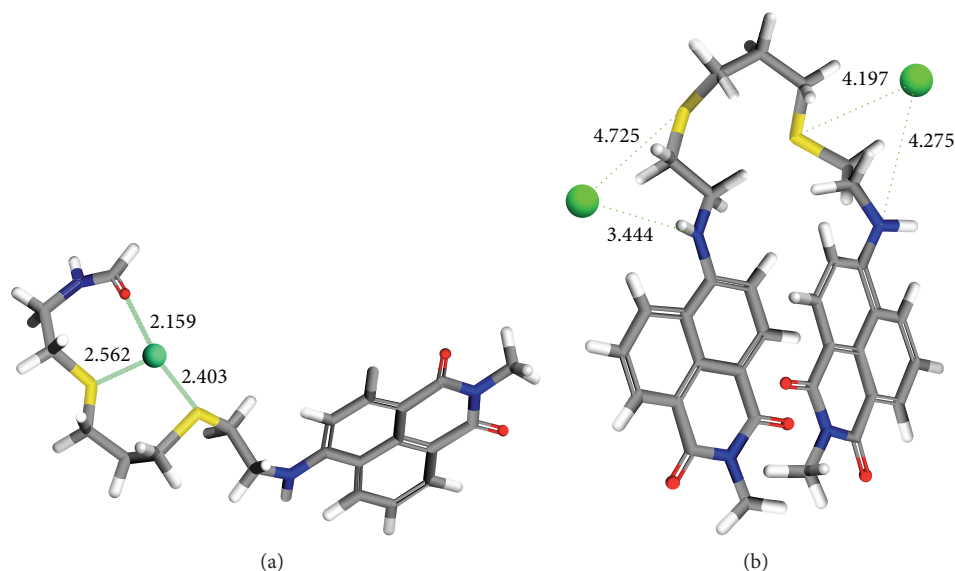


FIGURE 4: Optimized structures of (a) 1- Hg^{2+} and (b) 2- Hg^{2+} complexes from MD with local density approximation (LDA) of local functional PWC. (The distances between Hg^{2+} and donor atoms are indicated in Å.)

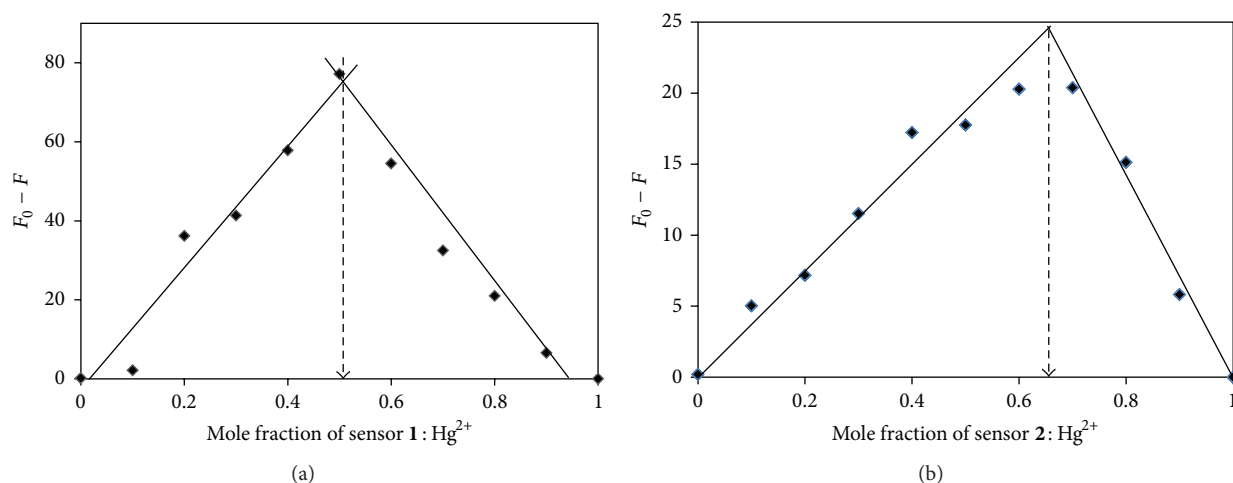


FIGURE 5: Job's plots for (a) 1 with Hg^{2+} in CH_3CN and (b) 2 with Hg^{2+} in CH_2Cl_2 . The total concentrations of [Sensors] + [Hg^{2+}] are $1\ \mu\text{M}$.

Hg^{2+} and complex were calculated. The complexation energies of the host-guest structure were obtained from the energy of complex minus energy of sensor minus energy of Hg^{2+} [8, 48].

The results of MD simulations show that ion recognitions of the sensors created the self-assembly of sensor 1 with Hg^{2+} ion and sensor 2 with two Hg^{2+} ions. The sensor- Hg^{2+} recognitions occurred because electrostatic interactions of Hg^{2+} coordinated with the S and N atoms of the sensor are favored, which resulted in the formation of helical wrapping-like structures. Figure 4 shows the distances between Hg^{2+} and donor atoms, indicating the binding sites of Hg^{2+} bound to the sensors. The lowest calculated complexation energies of 1- Hg^{2+} and 2- 2Hg^{2+} were -212.2 and -137.4 kcal/mol, respectively. These energies suggested the stability of the

complexes for a 1:1 complex formation of 1: Hg^{2+} and a 1:2 complex formation of 2: 2Hg^{2+} , respectively. These complex formations also agreed well with Job's plot experiments shown in Figure 5. The Job's plot analysis with respect to 512 nm (for 1) and 492 nm (for 2) showed maximum fluorescence signals at the mole fraction of 0.5 and 0.67 which can be attributed to the existence of 1:1 and 1:2 stoichiometry.

3.4. Selectivity Studies. The fluorescence spectra of the sensors before and after the addition of perchlorate salts of Hg^{2+} , Co^{2+} , Ag^+ , Cd^{2+} , Mn^{2+} , K^+ , Pb^{2+} , Na^+ , Fe^{2+} , Ca^{2+} , Mg^{2+} , and Cu^{2+} were observed and compared. The results show that a quenching in fluorescence appeared clearly after the addition of Hg^{2+} into the solution as shown in Figure 6. The selectivity of 1 to Hg^{2+} is also the highest compared to other cations.

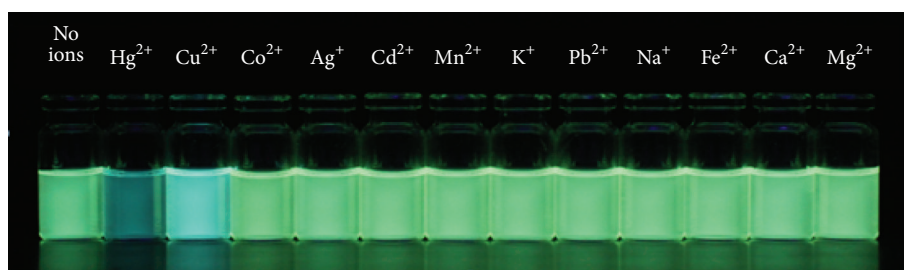
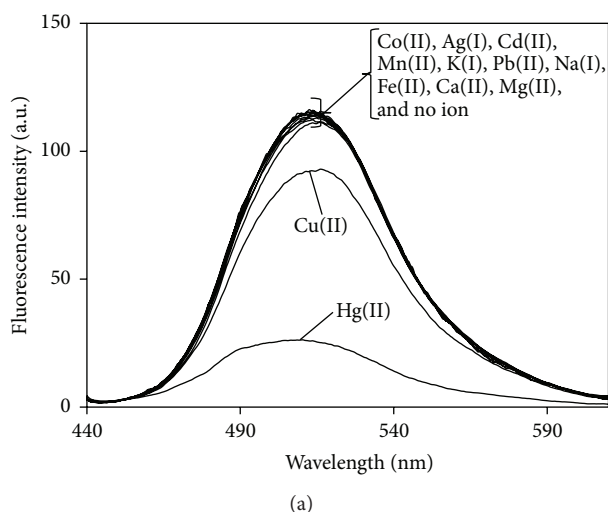


FIGURE 6: (a) Fluorescence spectra of **1** ($\lambda_{\text{ex}} = 426 \text{ nm}$, $0.15 \mu\text{M}$) with addition of perchlorate salts of Hg^{2+} , Co^{2+} , Ag^+ , Cd^{2+} , Mn^{2+} , K^+ , Pb^{2+} , Na^+ , Fe^{2+} , Ca^{2+} , Mg^{2+} , and Cu^{2+} , ($123 \mu\text{M}$). (b) The luminescence under a UV of **1** ($2.3 \mu\text{M}$) in the presence and absence of Hg^{2+} , Cu^{2+} , Co^{2+} , Ag^+ , Cd^{2+} , Mn^{2+} , K^+ , Pb^{2+} , Na^+ , Fe^{2+} , Ca^{2+} , and Mg^{2+} ($3.0 \mu\text{M}$).

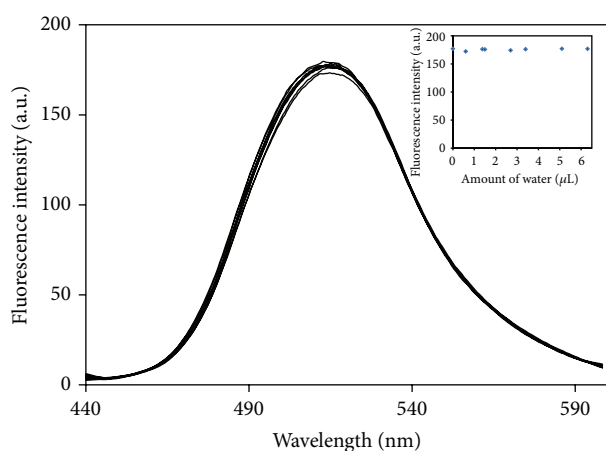


FIGURE 7: Fluorescence spectra ($\lambda_{\text{ex}} 426 \text{ nm}$) of **1** ($0.15 \mu\text{M}$) in acetonitrile as a function of water. $0 \mu\text{L}$, $0.6 \mu\text{L}$, $1.38 \mu\text{L}$, $1.5 \mu\text{L}$, $2.7 \mu\text{L}$, $3.4 \mu\text{L}$, $5.1 \mu\text{L}$, and $6.3 \mu\text{L}$.

Moreover, the quenching of the fluorescence signal cannot be affected by the addition of water ($0\text{--}6.3 \mu\text{L}$) into the solution of sensor as illustrated in Figure 7.

For the competitive studies of **1**, the competitive fluorescence signaling behaviors of **1** with Hg^{2+} in the presence

of 10 equivalents of the other cations as background were investigated at 512 nm and the results were shown in Figure 8. I_0 was the initial fluorescence emission signal, while I_F was the fluorescence emission $0.15 \mu\text{M}$ of **1** observed when there were also competitive background cations in the solution ($90 \mu\text{M}$ each of Cu^{2+} , Co^{2+} , Ag^+ , Cd^{2+} , Mn^{2+} , K^+ , Pb^{2+} , Na^+ , Fe^{2+} , Ca^{2+} , and Mg^{2+}) and Hg^{2+} ($9 \mu\text{M}$). When I_F was the fluorescence intensity of **1** in the presence of Hg^{2+} ions only, I_F/I_0 was equal to 0.21 and was used as a reference. In these competitive studies, I_F/I_0 values of **1** were 0.20–0.35 which indicated that Hg^{2+} -induced fluorescence quenching in the background interfering ions was relatively consistent. In addition, the sensing ability of **1** represented the selectivity for Hg^{2+} in the presence of Ag^+ , Cu^{2+} , and Pb^{2+} , which were important cation competitors.

In the selectivity studies of **2**, there are some fluorescence intensity changes at 492 nm promoted by some cations including Cu^{2+} and Ag^+ ; hence, in terms of the selectivity to Hg^{2+} , **2** was a poorer Hg^{2+} -fluorescence sensor compared to **1**. The lower selectivity of **2** might be due to inappropriate ions-binding sites and the steric effect from two bulky *N*-methylnaphthalimide groups upon Hg^{2+} -binding. Moreover, the decrease in Hg^{2+} -sensing sensitivity and selectivity may be due to the increase of another unit of

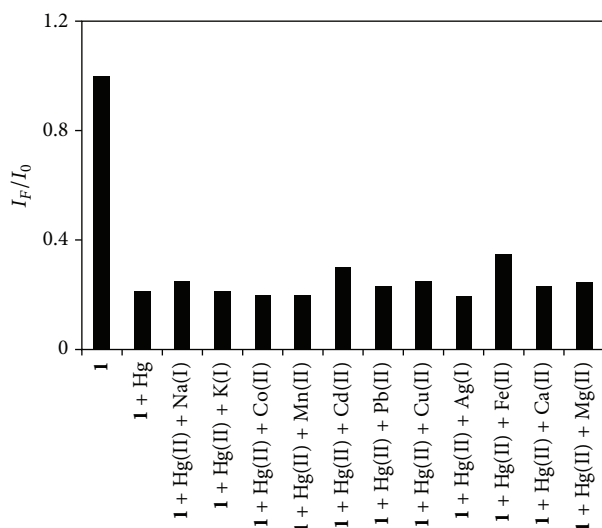


FIGURE 8: I_F/I_0 (where I_0 was the initial fluorescence emission signal and I_F was the fluorescence emission) in the 1- Hg^{2+} system containing $[1] = 0.15 \mu\text{M}$, $[\text{Hg}^{2+}] = 9 \mu\text{M}$ and $[\text{M}^{n+}] = 90 \mu\text{M}$ in CH_3CN .

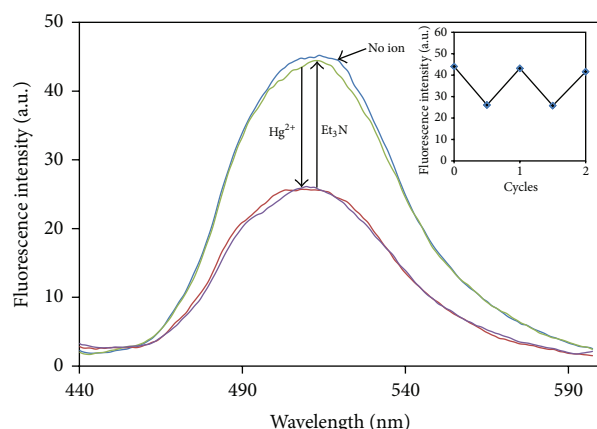


FIGURE 9: Fluorescence intensity before and after adding alternately $\text{Hg}^{2+}/\text{Et}_3\text{N}$ into the solution of **1** ($4.5 \mu\text{M}$), with the different concentrations: 0/0, 0.7/0, 0.7/1.4, 1.4/1.4, and 1.4/4.4.

the *N*-methylnaphthalimide moiety in the chemical structure of **2**, as compared to **1**.

3.5. Reversibility Studies. For sensing applications, the fluorescence sensor should have the reversibility features [49]. Herein, the reversibility studies of sensor **1** were carried out. Figure 9 represented the fluorescence intensity of **1** when Hg^{2+} and triethylamine (Et_3N) were added alternately into the solution of **1**.

In previous sensor applications, triethylamine (Et_3N), tetraethylammonium iodide (TEAI), and ethylenediaminetetraacetic acid (EDTA) had been utilized for reversibility studies [2, 49, 50]. In this study, Et_3N was chosen due to its high complex formation constant with Hg^{2+} ($6.31 \times 10^7 \text{ M}^{-2}$) [51] and its solubility in acetonitrile. Et_3N was expected to

be able to dissociate the **1**- Hg^{2+} complexes via the ligand exchange mechanism, as a result sensor **1** can be free again.

Herein, the fluorescence signal of sensor **1** was “turned off” at 512 nm when the Hg^{2+} concentration was added. By adding Et_3N into the solution containing **1** and Hg^{2+} , the fluorescence emission of **1** was “turned on,” suggesting the dissociation of the **1**- Hg^{2+} complex. On further alternate addition of Hg^{2+} ions and Et_3N , the fluorescence intensities of sensor **1** were “turned off” and “turned on” repeatedly. This result illustrates the reversible behavior of sensor **1** for sensing of Hg^{2+} ions.

4. Conclusion

Novel fluorescent sensors, **1** and **2**, for Hg^{2+} detection were successfully developed based on the modified *N*-methylnaphthalimide fluorophores using a conventional three-step synthesis. The modification of the amino group of the ionophore at 4-position of *N*-methylnaphthalimide could induce a bathochromic shift of absorption and the fluorescence spectra to the visible region. Moreover, sensor **1** has high sensitivity and selectivity toward Hg^{2+} over other interfering ions including Cu^{2+} , Ag^+ , and Pb^{2+} . Sensor **1** also shows reversible behavior which is important feature for applications. Hence, because of the synthetic simplicity, economic cost of material, and the good properties of these new sensors, the new sensor reported here offers great potential for the further fabrication of economical on-site tool for the detection of Hg^{2+} and commercial uses.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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