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A Selective and Sensitive Phenanthroimidazole-Based "Reactive" Ratiometric Sensor for Recognition of Hg^{2+} Ions in Aqueous Solution

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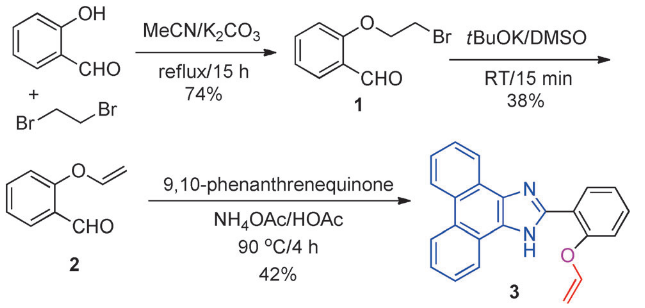
Based on specific reactivity of the vinyloxy group to Hg^{2+} ions, a new ratiometric phenanthroimidazole-based fluorescent sensor, 2-(2-(vinyloxy)phenyl)-1*H*-phenanthro[9,10-*d*]imidazole, is prepared. The selectivity of the probe toward Hg^{2+} ions is very high, and shows minimal interference from other commonly coexistent metal ions. The reactive stoichiometry of the probe with Hg^{2+} ion is 2:1. Its detection limit is evaluated and Hg^{2+} ions can be detected down to a lower limit of 2.5×10^{-8} M. Moreover, the Hg^{2+}-promoted selective hydrolysis of a vinyloxy group of the probe is further confirmed by ^{1}H NMR spectroscopy and mass spectrometry.



Scheme 1. Synthetic approach for sensor **3**. DMSO = dimethyl sulfoxide.

Mercury is one of the most dangerous and toxic environmental pollutants to human beings. Even at a low concentration the Hg^{2+} ion can bioaccumulated through the food chain and cause damage to the central nervous system and other organs by effecting the blood–brain barrier.[1,2] Accordingly, development of selective and sensitive chemosensors for the detection of Hg^{2+} ions over other heavy- and transition-metal (HTM) ions has received much attention.[3] To overcome the incomplete selectivity for Hg^{2+} ions over other competing metal ions, development of more accurate and sensitive ratiometric fluorescent probes based on the specific recognition of Hg^{2+} ion is highly desirable. Many attractive sensors for the detection of Hg^{2+} ions have been reported that depend on a chemical reaction specific to the mercury species.[4] Recently, a few phenanthroimidazole-based fluorescent probes were used for the recognition of Mg^{2+},[5a] Cu^{2+},[5b] ClO^-,[5c] cysteine and homocysteine,[5d] Cu^{2+} and Cd^{2+},[5e] and H_2S. [5f] As part of our ongoing research program on developing selective and sensitive chemosensors for the detection of Hg^{2+} ions,[6] herein we have designed and prepared a new ratiometric phenanthroimidazole-based fluorescent sensor **3** (Scheme 1) by introduction of the vinyl group into the fluorophore. Hereby the specific reactivity of Hg^{2+} ions with the vinyloxy unit is exploited.

As shown in the Scheme 1, 2-hydroxybenzaldehyde was treated with 1,2-dibromoethane and gave 2-(2-bromoethoxy)-benzaldehyde (**1**) in a 74% yield. Then in the presence of *t*BuOK, 2-vinyloxybenzaldehyde (**2**) was obtained in a 38% yield. Finally, compound **2** was treated with 9,10-phenanthrenequinone and afforded **3**, which was characterized by ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry. As shown in Figure 1, the UV/Vis spectrum of **3** showed a strong band at 388 nm (excitation = 310 nm). When Hg^{2+} was added to a solution of **3**, interestingly, although the fluorescence intensity at 388 nm decreased, an increase in the fluorescence intensity having a red-shift at 445 nm was observed. Whereas other metal ions tested, such as Fe^{3+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, K^+, Cs^+, and Ag^+, showed no clear enhancement or decrease in the fluorescence intensity.

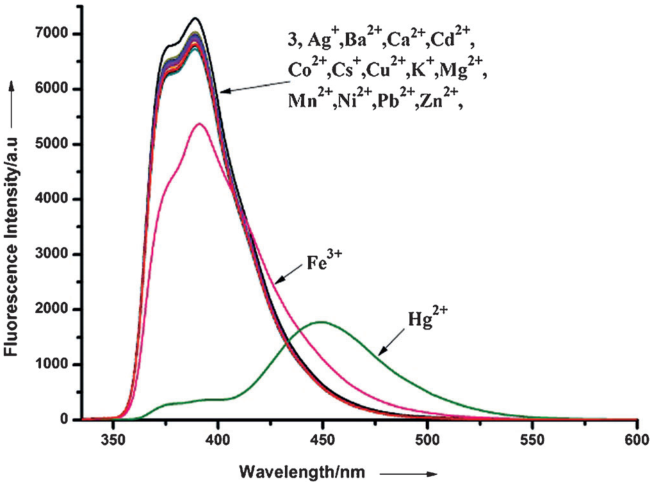


Figure 1. Variation of the fluorescence intensity of **3** (5×10^{-6} M) in CH_3CN-HEPES (1/1, v/v; 0.02 M, pH 7.0) at 293 K in the presence of 10.0 equivalents of the respective metal ions; the excitation wavelength for Hg^{2+} was 330 nm, and excitation wavelength for other ions was 310 nm.

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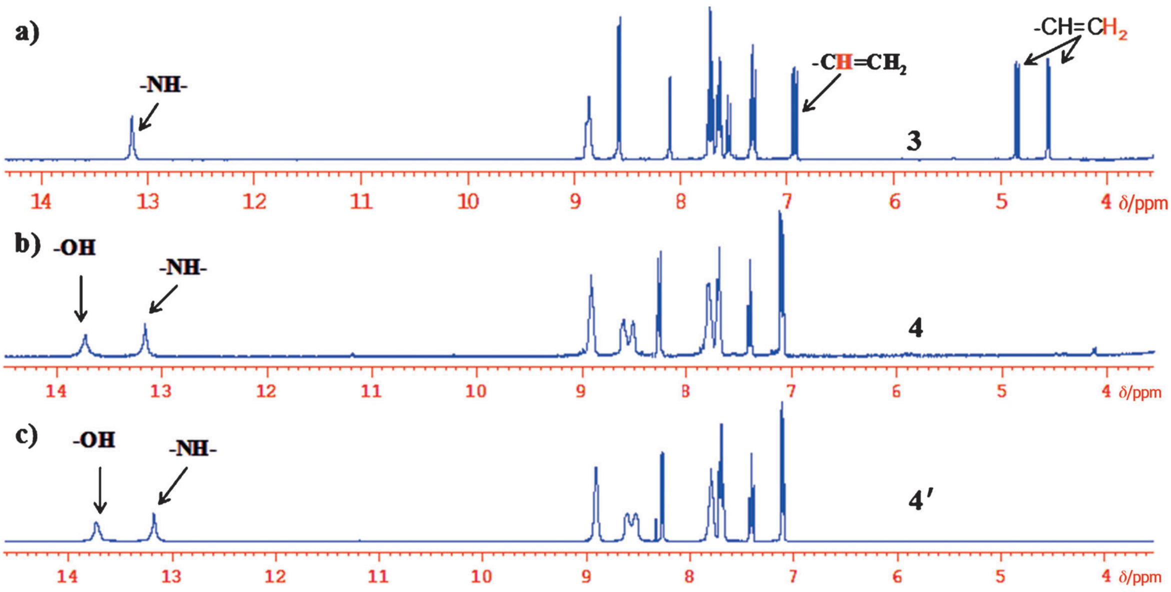
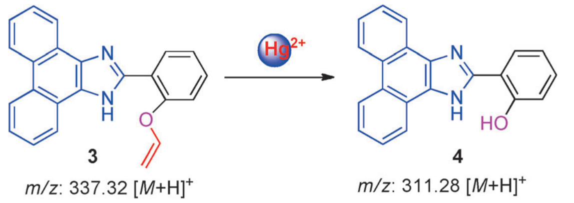


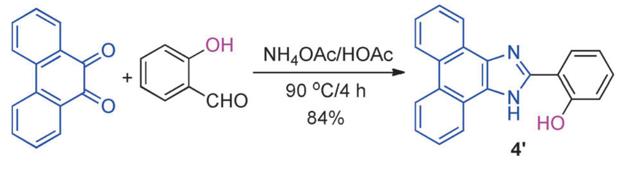
Figure 2. Partial ^{1}HNMR spectra of **3** (a), **4** (b) and **4′** (c) in [D$_6$]DMSO.

We envisaged that the above results may be due to selective reactivity of the vinyloxy group of **3** in the presence of Hg^{2+} ions. To testify this idea, the product **4** was separated from a mixture of **3** and Hg^{2+} ions (see the Supporting Information). Solid evidence for the proposed reaction came from comparing the ESI-MS spectra of **3** and **4**. As shown in Scheme 2, the signal at *m/z* 337.32 corresponds to **3** ([*M*+H]$^+$), whereas the signal at *m/z* 311.28 corresponds to **4** ([*M*+H]$^+$). Meanwhile, we directly prepared **4′** as a control by treatment of 9,10-phenanthrenequinone with 2-hrdroxybenzaldehyde (Scheme 3).



Scheme 2. The selective cleavage of probe **3** to form **4** in the presence of Hg^{2+} ions.

Evidence for the Hg^{2+}-promoted hydrolysis reaction was further supported by comparing ^{1}H NMR spectra of **3**, **4**, and **4′**. As shown in the partial ^{1}H NMR spectrum of Figure 2a, the chemical shifts corresponding to the -CH═CH$_2$ protons of **3** appear at $\delta = 4.77$ and 5.15 ppm; upon addition of Hg^{2+} ions, the signal for these protons disappear while a new signal corresponding to the OH proton of **4** appears at $\delta = 13.72$ ppm (Figure 2b). Moreover, the ^{1}H NMR spectrum of **4** is identical to that of the control **4′** (Figure 2c). Therefore, **4** and **4′** are the same compound. Based on the above results, a proposed sensing mechanism is described in Scheme 4.



Scheme 3. Preparation of **4′** as the control.

The ratios of fluorescent intensity at 445 and 388 nm (I_{445}/I_{388}) of sensor **3** in the presence of Hg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, K$^+$, Cs$^+$, and Ag$^+$ in a solution of CH_3CN-HEPES (1/1 v/v; 0.02 м, pH 7.0) are shown in Figure 3. Notably, a drastic increase of I_{445}/I_{388} value from 0.11 to 4.21 was observed only when Hg^{2+} was added to the solution of **3**. This increase in intensity was accompanied by a resonance color change from purple/blue to bright blue

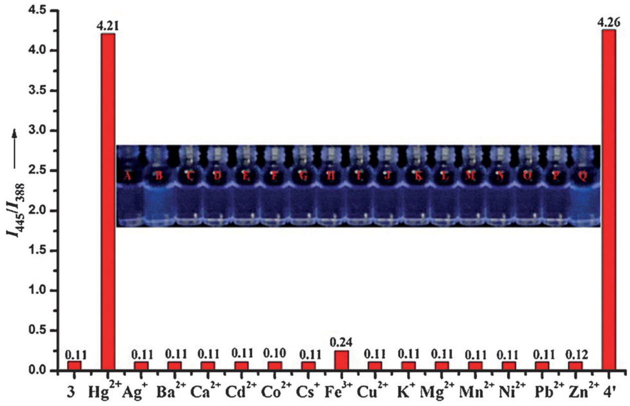
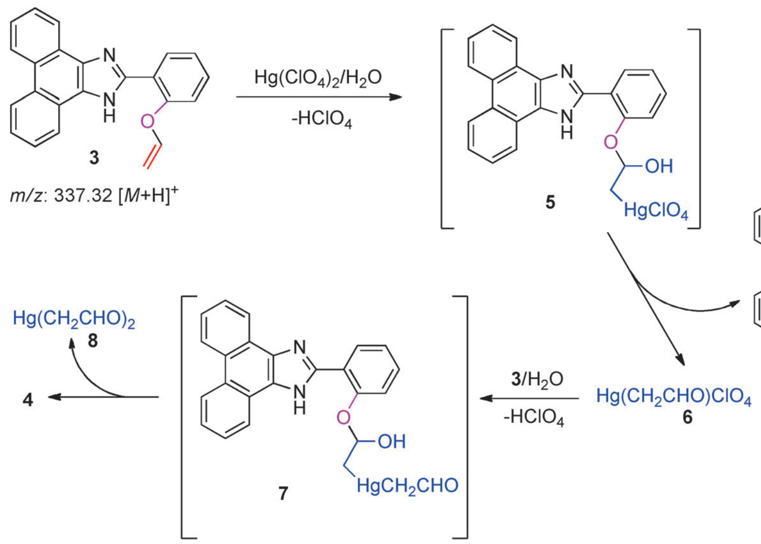


Figure 3. Fluorescent intensity ratio (I_{445}/I_{388}) of **3** (5×10^{-6} м) in CH_3CN-HEPES (1/1, v/v; 0.02 м, pH 7.0) at 293 K in the presence of 10.0 equivalents of different metal ions; the excitation wavelength for Hg^{2+} was 330 nm, and excitation wavelength for other ions was 310 nm. Inset: Photograph of **3** in the presence of various metal ions. **A**: **3** (5×10^{-6} м); **Q**: **4** (5×10^{-6} м); **B–P**: **3**+Hg^{2+}, **3**+Ag$^+$, **3**+Ba^{2+}, **3**+Ca^{2+}, **3**+Cd^{2+}, **3**+Co^{2+}, **3**+Cs$^+$, **3**+Fe^{3+}, **3**+Cu^{2+}, **3**+K$^+$, **3**+Mg^{2+}, **3**+Mn^{2+}, **3**+Ni^{2+}, **3**+Pb^{2+}, and **3**+Zn^{2+} (5×10^{-5} м).



Scheme 4. The possible reaction mechanism of **3** for selective recognition of $Hg(ClO_4)_2$.

under illumination with UV light at 365 nm. In contrast other metal ions had negligible response. The I_{445}/I_{388} value of **4′** was equal to 4.26, and was used as the control. The reaction time profile of **3** (5×10^{-6} м) towards Hg^{2+} (2.5×10^{-6} м) in aqueous solution was examined (see Figure S1 in the Supporting Information), and the I_{445}/I_{388} value reached a maximum and remained steady after 20 min.

The fluorescent intensity of $3+Hg^{2+}$ remained almost constant when the pH value was maintained between 4.5 and 12 (Figure S2). The titration reaction curve of probe **3** toward Hg^{2+} ions is shown in Figure 4. Upon excitation at 330 nm, the free **3** displays a double emission band centered at 388 nm. On addition of Hg^{2+} ions, the fluorescent intensity of **3** at 388 nm significantly decreases with the simultaneous appearance of a new red-shift emission band at around 445 nm. The fluorescent intensity ratio at 445 and 388 nm (I_{445}/I_{338}) of **3** increases linearly at $1–2\times10^{-6}$ м of Hg^{2+} ions. Furthermore, the titration reaction curve shows a steady increase until a plateau is reached when 0.5 equivalents of Hg^{2+} ions are added.

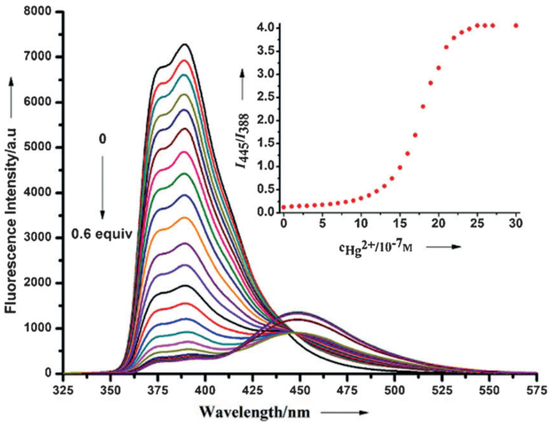


Figure 4. Fluorescence titration of **3** (5×10^{-6} м) in CH_3CN-HEPES (1/1, v/v; 0.02 м, pH 7.0) at 293 K with the concentration of Hg^{2+} ions ranging from 0 to 3×10^{-6} м; the excitation wavelength was 330 nm.

To investigate the utility of **3** as an ion-selective fluorescent sensor for Hg^{2+} ions, cross-contamination tests were conducted in the presence of Hg^{2+} at a concentration of 2.5×10^{-6} м mixed with other metal ions such as Ni^{2+}, Ca^{2+}, Ba^{2+}, Cs^{+}, Fe^{3+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, K^{+}, Ag^{+}, Hg^{2+}, Co^{2+}, and Pb^{2+} at 5×10^{-5} м, respectively (Figure 5). This experiment demonstrated that although the concentration of these competing metal ions was 20-fold greater than that of Hg^{2+}, the selectivity of **4** towards Hg^{2+} was essentially unchanged in the presence of competitive ions.

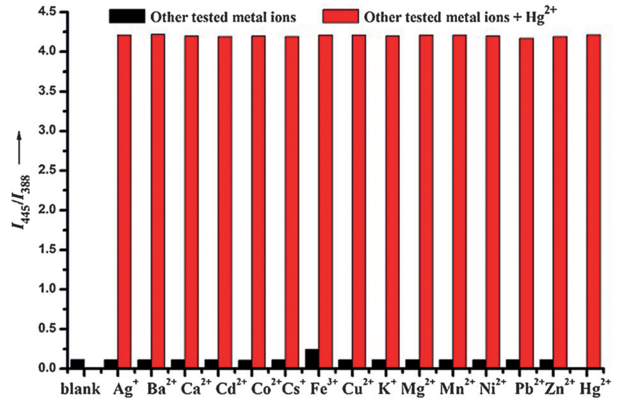


Figure 5. Results of the competition experiments for **3** (5×10^{-6} м) between Hg^{2+} and other metal ions in CH_3CN-HEPES (1/1, v/v; 0.02 м, pH 7.0) at 293 K; the concentration of Hg^{2+} ions was 2.5×10^{-6} м, and that of each competing metal ion was 5×10^{-5} м.

Finally, to examine the sensitivity of **3** towards Hg^{2+} ions, the detection limit was evaluated. As shown in Figure 6, the fluorescence titration profile of **3** (1×10^{-5} м) with Hg^{2+} ions indicates that Hg^{2+} ions can be detected down to 2.5×10^{-8} м, and the I_{445}/I_{338} value of **3** increases linearly with the concentration of Hg^{2+} ion $(0–5.0)\times10^{-7}$ м ($R^2=0.9944$).

In summary, we have developed a phenanthroimidazole-based "reactive" ratiometric fluorescent probe for selective and sensitive detection of Hg^{2+} ions in aqueous solution. The reactive stoichiometry of the probe with Hg^{2+} ion was 2:1. It was shown that the probe also responded to organomercury species. Its selectivity toward Hg^{2+} ions was very high, and little interference was detected for other commonly coexistent metal ions. Moreover, the Hg^{2+}-promoted selective hydrolysis of a vinyloxy group of **3** was further confirmed by ^1H NMR spectroscopy and MS spectrometery. The present probe could be used to determine Hg^{2+} ion concentrations in aqueous environments having a wide range of pH values.

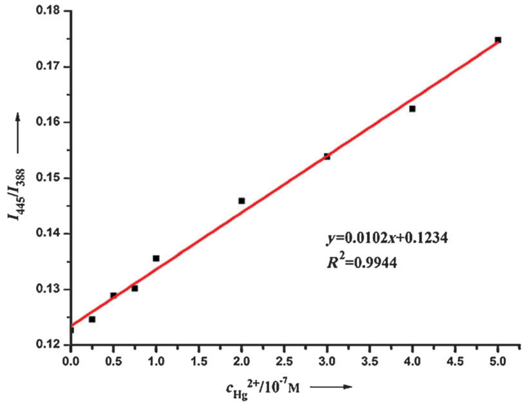


Figure 6. Fluorescence titration of **3** (1×10^{-5} M) with the concentration of Hg^{2+} ranging from 0 to 5×10^{-7} M in CH_3CN-HEPES (1/1, v/v, 0.02 M, pH 7.0) at 293 K; the excitation wavelength was 330 nm.

Experimental Section

Synthesis of 2-(2-bromoethoxy)benzaldehyde (1): To a stirred solution of 2-hydroxybenzaldehyde (1220 mg, 10.0 mmol) in dry CH_3CN (30 mL), 1,2-dibromoethane (9400 mg, 50.0 mmol) and anhydrous K_2CO_3 (2070 mg, 15.0 mmol) were added. The reaction mixture was heated at reflux for 15 h then cooled to RT. The mixture was filtered, and the filtrate was concentrated in vacuo and purified by column chromatograph on silica gel y using petroleum ether/ethyl acetate (8/1, v/v) as the eluent to afford **1** (1.694 g, 74% yield) as a white solid. M.p. 53–54 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=10.54$ (s, 1 H), 7.87–7.84 (m, 1 H), 7.57–7.53 (m, 1 H), 7.09–7.05 (m, 1 H), 6.97 (d, $J=8.4$ Hz, 1 H), 4.44 (t, $J=6.0$ Hz, 2 H), 3.73 ppm (t, $J=6.0$ Hz, 2 H); ESI-MS: m/z (%): 229.02 (37) $[M+H]^+$, 231.04 (33) $[M+H]^+$.

Synthesis of 2-vinyloxybenzaldehyde (2): To a solution of compound **1** (1145 mg, 5.0 mmol) in DMSO (20 mL) was added *t*BuOK (673 mg,6.0 mmol). The reaction mixture was stirred at RT for 15 min. Then the mixture was diluted with ethyl acetate (30 mL), and washed with ice water (3×20 mL) and brine (3×20 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by column chromatography on silica gel using petroleum ether/ethyl acetate (10/1, v/v) as the eluent to give **2** (281 mg, 38% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=10.45$ (s, 1 H), 7.89–7.86 (m, 1 H), 7.59–7.55 (m, 1 H), 7.19–7.16 (m, 1 H), 7.09 (d, $J=8.4$ Hz, 1 H), 6.73 (dd, $J=13.6$, 6.0 Hz, 1 H), 4.88 (dd, $J=13.6$, 2.0 Hz, 1 H), 4.61 ppm (dd, $J=6.0$, 2.0 Hz, 1 H); ESI-MS: m/z (%): 149.03 (22) $[M+H]^+$.

Synthesis of 2-(2-(vinyloxy)phenyl)-1*H*-phenanthro[9,10-*d*]imidazole (3): A mixture of 9, 10-phenanthroquinone (208 mg, 1.0 mmol), **2** (148 mg, 1.0 mmol), and ammonium acetate (1540 mg, 20 mmol) in glacial HOAc (10 mL) was heated at 90 °C for 4 h. The mixture was cooled to RT and filtered to collect the crude white solid, which was purified by preparative thin-layer chromatography using petroleum ether/ethyl acetate (5/1, v/v) as the eluent to afford **3** (141 mg, 42% yield) as a white solid. M.p. 198–200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=13.15$ (s, 1 H), 8.88 (t, $J=8.4$ Hz, 2 H), 8.59 (d, $J=0.8$ Hz, 1 H), 8.57 (d, $J=0.8$ Hz, 1 H), 8.11 (dd, $J=8.0$, 2.0 Hz, 1 H), 7.74 (t, $J=7.2$ Hz, 2 H), 7.65 (t, $J=7.6$ Hz, 2 H), 7.53–7.57 (m, 1 H), 7.30–7.36 (m, 2 H), 6.96 (dd, $J=13.6$, 6.0 Hz, 1 H), 4.86 (dd, $J=13.6$, 1.6 Hz, 1 H), 4.56 ppm (dd, $J=6.0$, 1.6 Hz, 1 H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=153.9$, 148.8, 146.6, 131.43, 131.40, 127.5, 125.6, 124.2, 121.6, 117.4, 96.7 ppm; ESI-MS: m/z (%): 337.32 (100) $[M+H]^+$.

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Keywords: fluorescence · mercury · phenanthroimidazoles · reactive probes · sensors

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