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Sensing Performance Enhancement via Acetate-Mediated N-Acylation of Thiourea Derivatives: A Novel Fluorescent Turn-On Hg^{2+} Chemodosimeter

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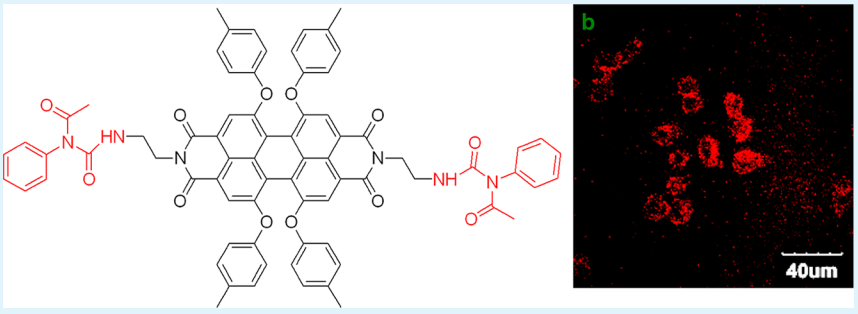
Supporting Information



ABSTRACT: A Hg^{2+} chemodosimeter **P3** derived from a perylenebisimide scaffold and thiourea fragments was systematically studied with focus on the photophysical, chemodosimetric mechanistic, as well as fluorogenic behaviors toward various metal cations for the sake of improving selectivity to Hg^{2+}. As demonstrated, Hg^{2+} can promote a stepwise desulfurization and N-acylation of **P3** with the help of an acetate anion (OAc^-), resulting in an N-acylated urea derivative. Interestingly, OAc^- has the effect of improving the selectivity of **P3** to Hg^{2+} among other metal ions; that is, in an acetone/Britton−Robinson buffer (9:1, v/v; pH 7.0) upon excitation at 540 nm, the relative fluorescence intensity is increased linearly with increasing concentration of Hg^{2+} in the range of 2.5−20 μM with a detection limit of 0.6 μM, whereas the fluorescence intensity of **P3** to other metal ions, including Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} Ag^+, Cd^{2+}, Pb^{2+}, and Cu^{2+}, is negligible. The fluorescent bioimaging of chemodosimeter **P3** to detect Hg^{2+} in living cells was also reported.

KEYWORDS: *mercury, perylenebisimides, fluorescence, chemodosimeter, thiourea, N-acylation*

INTRODUCTION

Mercury, as a highly toxic and dangerous element, has received considerable attention because it can be converted by bacteria in the environment and subsequently bioaccumulates through the food chain.[1−3] Designing selective and sensitive fluorescent sensors[4] for mercury (Hg^{2+}) species in biological samples has become an active research. Among many widely used signaling approaches, chemodosimeters are particularly attractive because of their highly selective behavior, with a unique spectroscopic change resulting from a specific chemical reaction with analyte. Until now, a variety of fluorogenic chemodosimeters[5] for Hg^{2+} have been developed with some successful applications for cell imaging and test kits of Hg^{2+}, such as spirolactam ring opening,[6] cyclic oxadiazolation,[7] and mercuration.[8] However, these previously reported Hg^{2+} chemodosimeters suffer from disturbance of thiophilic metal ions such as Ag^+,[9,10] Cu^{2+},[7,11] and Cd^{2+}.[12]

It should be pointed out that, although much attention has been paid to the design in chemodosimeters for monitoring Hg^{2+}, there are a few reports discussing the effect of anions on Hg^{2+} selectivity.[13] Recently, our group has developed a selective near-infrared (NIR) fluorescent Hg^{2+} chemodosimeter based upon Hg^{2+}-promoted intramolecular cyclic guanylation.[14] Herein we present a chemodosimeter *N*,*N*′-bis(1-phenyl-thiouredoethyl)-1,6,7,12-tetrakis(4-methylphenoxy)perylene-3,4,9,10-tetracarboxylic diimide (**P3**) derived from a perylene-bisimide (PBI) scaffold and thiourea fragments for the sake of improving selectivity to Hg^{2+} (Figure 1). Notably, Hg^{2+} can promote a stepwise desulfurization and N-acylation of 1,3-disubstituted thiourea derivatives with the help of an acetate anion (OAc^-), resulting in N-acylated urea derivatives. With the investigation of various anion effects, **P3** shows high sensitivity and selectivity to Hg^{2+} with "turn-on" fluorescence in an acetate media. To the best of our knowledge, there is still no study on the advantage of anions for improving the selectivity of Hg^{2+}.

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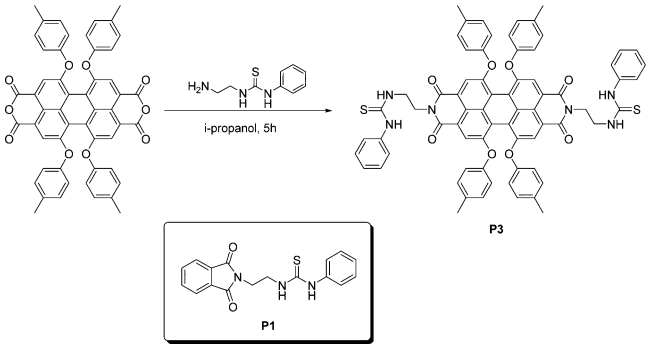


Figure 1. Synthetic route of **P3** and reference compound **P1**.

EXPERIMENTAL SECTION

Materials. 1,6,7,12-Tetrakis(4-methylphenoxy)perylene-3,4,9,10-tetracarboxylic dianhydride and 1-(2-aminoethyl)-3-phenylthiourea were prepared by established literature procedures.[16b,c] All other reagents and solvents were purchased from commercial sources and were of analytical grade.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer with tetramethylsilane as the internal standard, operating at 400 and 100 MHz, respectively. High-resolution mass spectrometry (HRMS) spectra were recorded with a Waters electrospray ionization (ESI) mass spectroscopy. The UV−vis spectra were obtained by using a Varian Cary 500 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescent spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. The slit width was 5 nm for both excitation and emission. Thin-layer chromatography (TLC) analyses were performed on silica gel plates, and flash column chromatography was conducted using silica gel column packages purchased from Qingdao Haiyang Chemical Co., Ltd. (China).

Synthesis of *N*-(3-Phenylthioureidoethyl)phthalimide (P1). A mixture of phthalic anhydride (148.0 mg, 1.0 mmol), 1-(2-aminoethyl)-3-phenylthiourea (195.0 mg, 1.0 mmol), and triethylamine (TEA; 0.3 mL) in isopropyl alcohol (5 mL) was refluxed for 5 h under argon. The resulting solution was concentrated in vacuo, and the residue was subjected to flash column chromatography on silica gel using dichloromethane as the eluent. The obtained product was precipitated with hexane to give **P1** as colorless needle crystals (230.0 mg, 70%). Mp: 147.5−148.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.83 (m, 2H, phthalene−H), 7.73 (m, 2H, phthalene−H), 7.60 (s, 1H, Ph−N*H*), 7.43 (t, J = 7.6 Hz, 2H, P−H), 7.33 (t, J = 7.6 Hz, 1H, Ph−H), 7.21 (d, J = 7.6 Hz, 2H, Ph−H), 6.41 (t, J = 4.4 Hz, 1H, $-CH_2NH$), 3.96 (m, 2H, $-CH_2CH_2NH$), 3.92 (m, 2H, $-CH_2NH$). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 181.46, 168.41, 135.68, 134.16, 131.94, 130.15, 127.65, 125.87, 123.41, 44.83, 37.11. HRMS (TOF-ESI$^+$, m/z). Calcd for $C_{17}H_{16}N_3O_2S$: 326.0963 ([M + H$^+$]). Found: 326.0965.

Synthesis of *N*-(3-Formyl-3-phenylthioureidoethyl)-phthalimide (P2). A mixture of **P1** (80.0 mg, 0.25 mmol) and $Hg(OAc)_2$ (10.0 mg, 0.31 mmol) in acetone (5 mL) was stirred for 15 min at room temperature (monitored by TLC). The resulting solution was filtered and concentrated in vacuo, and the resulting oil was subjected to flash column chromatography on silica gel using dichloromethane/methanol (100:1) as the eluent, giving **P2** as a colorless solid (50.0 mg, 57%). Mp: 113.5−114.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.26 (t, J = 5.6 Hz, 1H, $-CH_2NH$), 7.86 (m, 2H, phthalene−H), 7.71 (m, 2H, phthalene−H), 7.38−7.44 (m, 3H, Ph−H), 7.21 (d, J = 7.6 Hz, 2H, Ph−H), 3.94 (t, J = 5.6 Hz, 2H, $-CH_2CH_2NH$), 3.60 (q, J = 5.6 Hz, 2H, $-CH_2NH$). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 174.35, 168.38, 155.07, 139.20, 133.95, 132.10, 129.56, 128.98, 128.77, 123.34, 39.45, 37.45, 26.28. HRMS (TOF-ESI$^+$, m/z). Calcd for $C_{19}H_{18}N_2O_4$: 352.1297 ([M + H$^+$]). Found: 352.1297.

Synthesis of *N,N′*-Bis(1-phenylthiouredoethyl)-1,6,7,12-tetrakis(4-methylphenoxy)perylene-3,4,9,10-tetracarboxylic Diimide (P3). A mixture of 1,6,7,12-tetrakis(4-methylphenoxy)-perylene-3,4,9,10-tetracarboxylic dianhydride (100.0 mg, 0.12 mmol), 1-(2-aminoethyl)-3-phenylthiourea (100.0 mg, 0.5 mmol), and TEA (0.2 mL) in isopropyl alcohol (5 mL) was refluxed under argon for 5 h. After cooling to room temperature, the reaction mixture was acidified with 1 M HCl (150 mL) and then extracted with dichloromethane (3 × 70 mL). The collected organic phase was washed with water (3 × 70 mL) and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel (300−400 mesh). The column was eluted first with dichloromethane to separate the side products and subsequently with a solvent mixture [dichloromethane/methanol (80:1, v/v)]. A subsequent precipitation from a mixture of hexane and dichloromethane (30:1, v/v) gave pure product **P3**, collected as a dark-purplish solid (58.0 mg, 40%). Mp: >400 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.06 (s, 4H, perylene−H), 7.52 (s, 2H, Ph−NH), 7.20 (t, J = 7.6 Hz, 4H, Ph−H), 7.12 (d, J = 8.4 Hz, 8H, Ph−H), 7.08 (m, J = 8.4 Hz, 6H, Ph−H), 6.88 (d, J = 8.4 Hz, 8H, Ph−H), 6.65 (t, J = 8.0 Hz, 2H, $-CH_2NH$), 4.34 (t, J = 8.0 Hz, 4H, $-CH_2CH_2NH$), 3.95 (q, J = 8.0 Hz, 8H, $-CH_2NH$), 2.37 (s, 12H, $-CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 181.35, 163.79, 156.43, 152.86, 135.58, 134.53, 132.75, 130.62, 129.93, 127.47, 126.12, 121.96, 120.42, 120.21, 119.57, 119.26, 45.62, 38.84, 20.83. HRMS (TOF-ESI$^+$, m/z). Calcd for $C_{70}H_{55}N_6O_8S_2$: 1171.3523 ([M + H$^+$]). Found: 1171.3518.

RESULTS AND DISCUSSION

Design and Synthesis. The chromophore of PBI has a unique combination of chemical stability, visible excited state, and long-wavelength emission with high fluorescence quantum yield.[17] Inspired by a desulfurization reaction promoted by Hg^{2+} as a thiophile[15] and the reactions of carbodiimides with carboxylic acids,[16a] **P3** was synthesized by the condensation of 1,6,7,12-tetrakis(4-methylphenoxy)perylene-3,4:9,10-tetracarboxylic dianhydride with 1-(2-aminoethyl)-3-phenylthiourea in 40% yield (Figure 1). **P1**, as a reference without a fluorophore, was also prepared in a similar way with a yield of 70%. All of these compounds were fully characterized by ^{1}H and ^{13}C NMR, IR, and HRMS in the Experimental Section.

Spectral Characterizations. As shown in Figure 2a, three peaks at 445, 537, and 576 nm are observed in the absorption spectra of **P3**, which is typical for PBIs with four substituents at the bay region. It is suggestive that the incorporation of thiourea groups at imide nitrogen atoms does not have an effect on the ground state of PBI.[18] Similarly, the UV−vis absorption spectra of **P3** show almost negligible changes in the presence of a variety of metal ions (including Hg^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Na^+, K^+, Cu^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, and Ag^{2+}) in the buffer solution [acetone/3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer, 90:10, v/v; pH 7.0; Figure 2b].

The response of **P3** to Hg^{2+} was further investigated by fluorescence detection. Figure 3a shows the fluorescence response of **P3** upon the addition of various mercury salts such as $Hg(ClO_4)_2$, $Hg(NO_3)_2$, $HgCl_2$, and $Hg(OAc)_2$ in an acetone/MOPS buffer (90:10, v/v; pH 7.0). Unexpectedly, only $Hg(OAc)_2$ with **P3** showed enhancement response with respect to other mercury salts in emission spectra. Interestingly, if the buffer medium of **P3** was changed with the titration of Hg^{2+} to an acetate medium (acetone/BR buffer, 90:10, v/v; pH 7.0), all of these mercury salts displayed similar enhancements in the fluorescence intensity (Figure 3b). The unexpected fluorescent behavior indicates that the specific acetate anion might play an important role in the Hg^{2+}-promoted chemodosimetric approach of **P3**.

Mechanism: NMR, Fourier Transform Infrared (FT-IR), and HRMS Analysis. To further gain insight into the

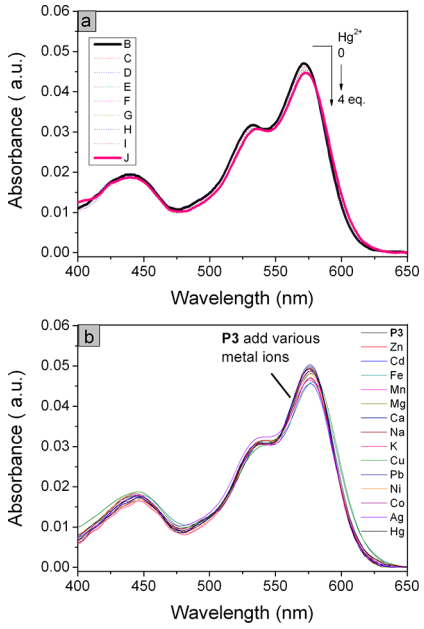


Figure 2. Absorption spectra of **P3** (2 μM): (a) in the presence of Hg^{2+} (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 3.0, and 4 equiv) in an acetone/BR buffer (9:1, v/v; pH 7.0); (b) in the presence of various metal ions (4 μM) in an acetone/MOPS buffer (9:1, v/v; pH 7.0).

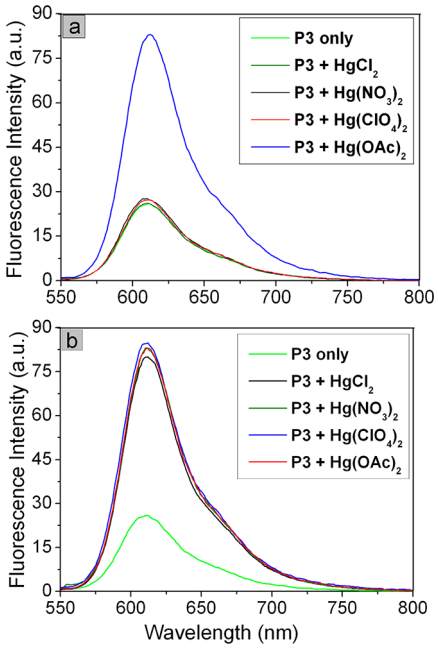
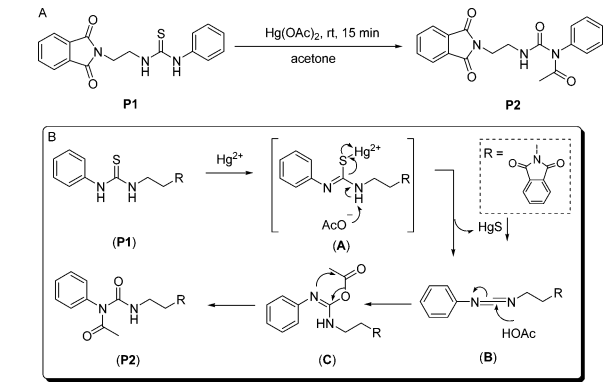


Figure 3. Fluorescence spectra of **P3** (2 μM) with various kinds of mercury salts (a) in an acetone/MOPS buffer and (b) in an acetone/BR buffer (9:1, v/v; pH 7.0) upon excitation at 540 nm.

mechanism of an acetate anion, **P1** without a PBI fluorophore was studied as the reference. Notably, we found that only a single regioisomer **P2** (57% yield) was isolated from the reaction of asymmetrical **P1** with 1 mol equiv of $Hg(OAc)_2$ in acetone at room temperature (Scheme 1A). The fact that aliphatic thiourea does not undergo N-acylation may result from the substantial basic character of an aliphatic amine.[19]

To confirm the above N-acylation process, the titration of ^{1}H NMR signals of **P1** with $Hg(OAc)_2$ was investigated in Figure 4. Two N*H* protons in the thiourea unit and two CH_2 protons

Scheme 1. Preparation of N-Acylated Urea P2 from Thiourea P1 (A) and Its Proposed Mechanism (B)



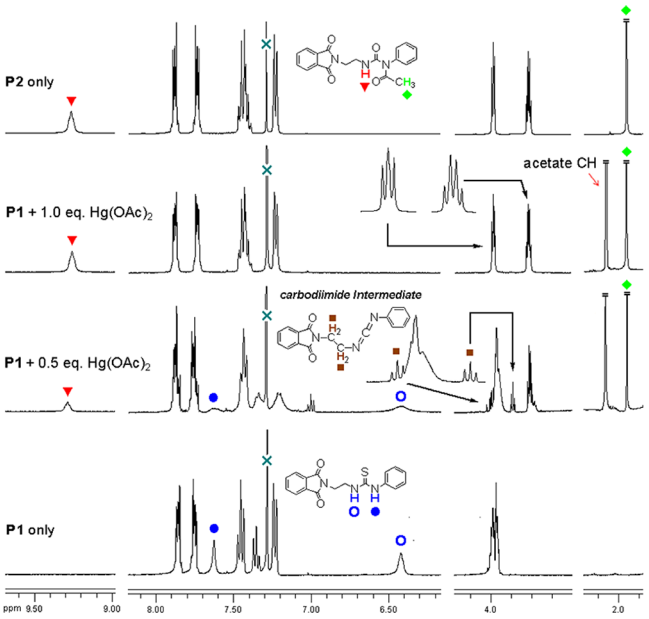


Figure 4. ^{1}H NMR spectra of **P2** and **P1** in $CDCl_3$ upon the addition of 0, 0.5, and 1.0 equiv of $Hg(OAc)_2$. Note that the single peak located at 2.10 ppm corresponds to an acetate anion from $Hg(OAc)_2$.

in $-CH_2CH_2-$ unit of **P1** show chemical shifts at 7.60, 6.41, 3.96, and 3.90 ppm, respectively. Upon an increase in the amount of $Hg(OAc)_2$, both of the N*H* and CH_2 protons of **P1** disappeared, whereas new signals form at 9.26 (triplets), 3.94 (triplets), 3.60 (quartets), and 1.90 (singlet) ppm, which can be attributed to the N*H* proton, two CH_2 protons in the $-CH_2CH_2-$ unit, and the methyl proton of **P2**, respectively. Interestingly, the addition of $Hg(OAc)_2$ (0.5 equiv) promotes two small but clear triplets at 4.00 and 3.78 ppm for the ethyl CH_2 protons. Notably, no quartet splitting for vicinal C*H*−N*H* coupling is observed in the range of 3.7−4.1 ppm. Therefore, these small triplet signals might be ascribed to the ethyl CH_2 of the carbodiimide intermediate, which can be quickly developed from thioureas in the presence of $Hg(OAc)_2$.[15] Consistently, the ^{13}C NMR titration of **P1** with the treatment of $Hg(OAc)_2$ shows that the disappearance at the chemical shift of 182 ppm, along with the appearance at that of 154 and 26 ppm, can be assigned to the formation of N-acetylated urea derivative **P2** (Figure 5).

The titration process of $Hg(OAc)_2$ to **P1** was also examined by mass spectrometry. As shown in Figure S1 in the Supporting

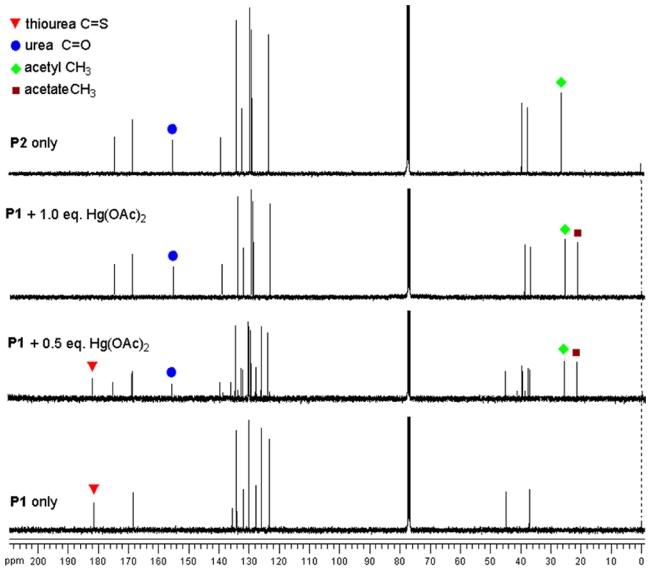


Figure 5. ^{13}C NMR spectra of **P1**, **P1** with 0.5 equiv of $Hg(OAc)_2$, **P1** with 1.0 equiv of $Hg(OAc)_2$, and **P2** ($CDCl_3$, 100 MHz). Note that the peak located at 22 ppm corresponds to an acetate anion from $Hg(OAc)_2$.

Information, the HRMS spectrum of **P1** treated with 0.9 equiv of $Hg(OAc)_2$ shows three distinct peaks at m/z 326.0961, 292.1086, and 352.1297, corresponding to $[\mathbf{P1} + H]^+$, carbodiimide intermediate $[\mathbf{B} + H]^+$, and $[\mathbf{P2} + H]^+$, respectively. The typical carbonyl stretching at 1770 cm^{-1} in FT-IR spectra (Figure S2 in the Supporting Information) can further verify the formation of **P2** with an *N*-acetyl group, indicating that the thiourea group changes into *N*-acylurea via a carbodiimide intermediate. This strong evidence implies that the addition of $Hg(OAc)_2$ to **P1** can lead to a novel chemodosimetric approach rather than the traditional Hg^{2+}-promoted intramolecular cyclic guanylation.

The suggested mechanism for N-acylation involving the intermediate of carbodiimide is proposed (Scheme 1B). **P1** reacts with $Hg(OAc)_2$ to produce intermediate **A**, which with expulsion of HgS will produce carbodiimide **B**. The *O*-acyl → *N*-acyl migration[16] of **C** followed by an acetate anion attack gives *N*-acylurea derivatives (**P2**). Fortunately, the HRMS spectrum of **P3** with 2.0 equiv of $Hg(OAc)_2$ also exhibits a major signal at 1223.4196, corresponding to the N-acylation product **P4** (Figure S3 in the Supporting Information), consistent with the above mechanism analysis. Obviously, the observed fluorescence enhancement upon Hg^{2+} can be ascribed to the resulting higher fluorescent quantum yield of **P4** than that of **P3**.

Detection Limit and Selectivity to Hg^{2+}. On the basis of the above N-acylation mechanism of the resulting thiourea derivatives, the acetone/BR buffer (90:10, v/v; pH 7.0) was chosen as both the acetate and neutral medium supplier in the following analyte detection. In Figure 6a, the fluorescence intensity of **P3** titrated with Hg^{2+} at 611 nm is well proportional to the amount of Hg^{2+} until the stoichiometric point is reached, with the quantum yield changing from 0.34 to 0.86, which was determined in reference to *N*,*N*′-di-2,6-dibutyl-1,6,7,12-tetrakis(4-*tert*-butylphenoxy)-3,4,9,10-perylenetetracarboxylic diimide (F = 0.66, in CH_2Cl_2).[20] Here, the sensing mechanism is just dependent upon the different quantum yield from **P3** to **P4** with fluorescent enhancement. When the

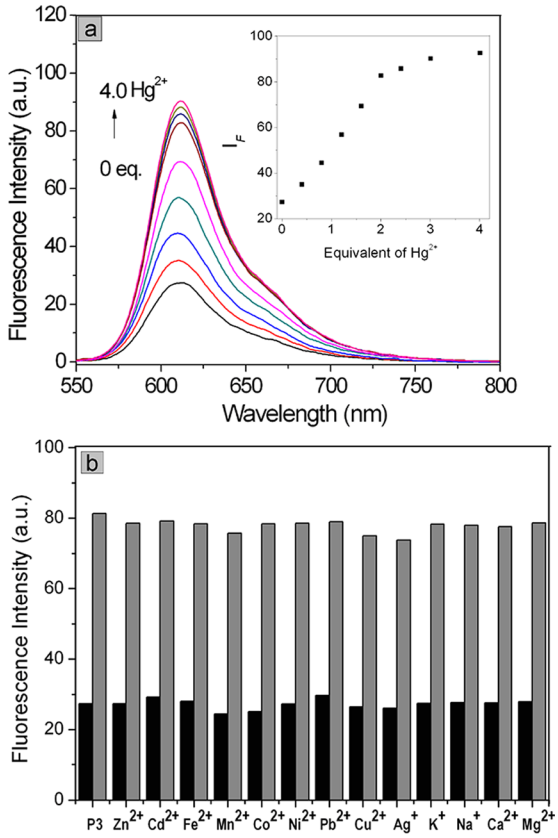


Figure 6. (a) Fluorescence spectra of **P3** (2 μM) in the presence of $Hg(ClO_4)_2$ (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 3.0, and 4 equiv) in an acetone/BR buffer (9:1, v/v; pH 7.0) upon excitation at 540 nm. Inset: Fluorescence curve as a function of the Hg^{2+} equivalents. (b) Fluorescent selectivity of **P3** toward Hg^{2+} over miscellaneous competitive metal ions in an acetone/BR buffer (9:1, v/v; pH 7.0) upon excitation at 540 nm. Black bars represent the addition of the intensity of **P3** in the presence of 2.0 equiv of Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} Ag^+, Cd^{2+}, Pb^{2+}, and Cu^{2+}. Gray bars represent the subsequent addition of 2.0 equiv of Hg^{2+} to the solution.

fluorescence intensity changes of **P3** at 611 nm are plotted as a function of the Hg^{2+} concentration, chemodosimeter **P3** responds to Hg^{2+} in 1:2 stoichiometry. In addition, the detection limit of **P3** for Hg^{2+} is about 0.6 μM, when the sensor is employed at 10 μM (Figure S4 in the Supporting Information).

An important feature of chemodosimeters is high selectivity toward analyte over other competitive species. Subsequently, the response of **P3** to various metal ions (in the form of perchlorate salts) was investigated in an acetone/BR buffer (9:1, v/v; pH 7.0). In Figure S5 in the Supporting Information, the fluorescence emission of **P3** demonstrates that only Hg^{2+} causes a distinct enhancement within 20 min. In sharp contrast, other cations such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} have little effect on the fluorescence emission spectra of **P3**. Notably, the traditional thiophilic metal ions, such as Ag^+, Cd^{2+}, Pb^{2+}, and Cu^{2+}, also showed little disturbance on the fluorescence enhancement or quench. Furthermore, the increases in the fluorescence intensity resulting from the addition of Hg^{2+} were not influenced by the subsequent addition of miscellaneous cations, as depicted in Figure 6b. Impressively, the sensing performance of selectivity can be enhanced via acetate-mediated N-acylation of the thiourea derivative. **P3** shows high selectivity to Hg^{2+} over

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other competitive cations in the aqueous acetone/BR buffer medium.

Application in Cell Bioimaging. For the fluorescence sensing of Hg^{2+}, the emission peak of **P3** is centered at a long wavelength of 611 nm, indicating its potential application in biological imaging. Therefore, we employed **P3** to image low concentrations of Hg^{2+} in Hela cells with the use of a confocal laser scanning microscope. In the control experiment, staining of the Hela cells with 10 μM **P3** for 20 min at 25 °C led to weak intracellular fluorescence (Figure 7). In contrast, an obvious increase in fluorescence from the intracellular area was observed when the cells were treated with 10 μM **P3** in the growth medium for 20 min at 25 °C and then with 20 μM $Hg(OAc)_2$ for 30 min (Figure 7b). Further bright-field measurements confirmed that the cells treated with **P3** were viable throughout the imaging experiments (Figure 7c). The overlay of fluorescence and bright-field images revealed that the fluorescence signals are localized in the full area of the cell, indicating a cellular distribution of Hg^{2+} and good cell-membrane permeability of **P3** (Figure 7d). These results demonstrate the practical applicability of **P3** for imaging of Hg^{2+} in living cells.

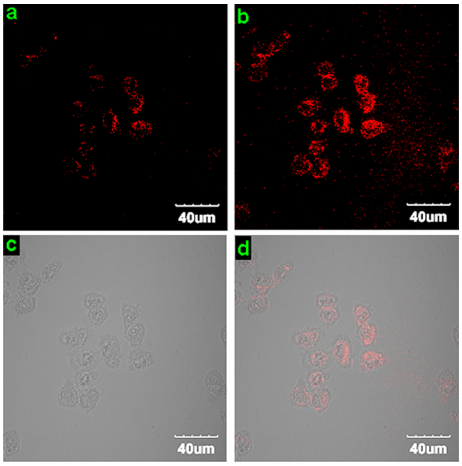

Figure 7. Confocal fluorescence and bright-field images of Hela cells: (a) cells incubated with 10 μM **P3** for 20 min at 25 °C; (b) cells supplemented with 20 μM $Hg(OAc)_2$ in the growth medium for 30 min at 25 °C; (c) bright-field image of cells shown in panel b; (d) overlay of panels b and c (λ_{ex} = 540 nm).

CONCLUSIONS

In summary, a novel "turn-on" fluorescent chemodosimeter **P3** was developed by utilizing irreversible stepwise desulfurization and N-acetylation. **P3** shows high selectivity toward Hg^{2+} ions over miscellaneous competitive cations, which was also successfully applied in an acetone/BR buffer solution and for the imaging of Hg^{2+} in living cells. Notably, the sensing performance of Hg^{2+} chemodosimeter was enhanced via acetate-mediated N-acylation of thiourea derivatives. This may pave a novel way to the development of novel Hg^{2+} chemodosimeters.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR, HRMS, IR spectra, and optical properties of **P1**−**P3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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