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One-Step Click Engineering Considerably Ameliorates the Practicality of an Unqualified Rhodamine Probe

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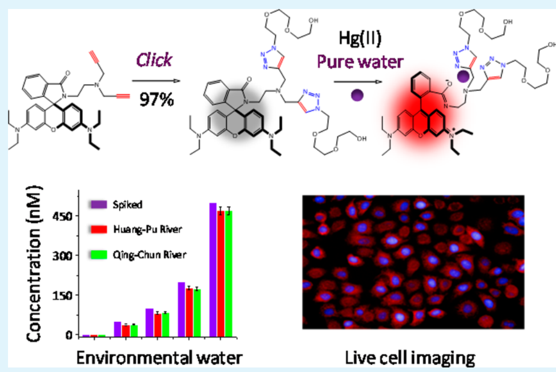
Supporting Information

ABSTRACT: This study describes the exploitation of click chemistry in the one-step molecular engineering of an unqualified rhodamine probe, leading to its considerable functional enhancement in terms of water solubility, ion selectivity, and usefulness in detecting biological and environmental samples. A dipropargyl rhodamine dye previously identified as an unselective and poorly water-soluble mercury(II) probe was used to couple with an azido polyethylene glycol (PEG) by the Cu(I)-catalyzed azide−alkyne 1,3-dipolar cycloaddition click reaction in almost quantitative yield. The simple click-engineered rhodamine probe shows, remarkably, better water solubility and mercury(II) selectivity comparing to the raw counterpart, and can be used to sensitively image mercury ions internalized by live cells and to accurately quantify the ion spiked in river water specimens. This study provides insights into the simple functional improvement of unqualified molecular dye probes via the efficient "click engineering".



KEYWORDS: *click chemistry, rhodamine, probe, triazole, PEG, cell imaging*

INTRODUCTION

Full-aqueous-medium detection of a unique chemical species using a simply constructed chemical probe represents a big challenge that chemists and biologists encounter. Many elegant strategies have been developed recently that exploit polymerization, nanoparticle and material cofunctionalization for enhancing the water solubility, and functionality of an unsatisfactory chemosensor.[1−15] However, the expedient construction of small-molecule chemosensors competent for detection of biological and environmental samples is still the most practical choice because of the conciseness in preparation and manipulation and the relatively low cost for scale-up production. Moreover, synthetic small-molecule dyes are structurally unified, facilitating the standard detection of a species with high reproducibility.

Mercury(II) is highly toxic to the human body, which may be lethal at a low dosage. A number of small-molecule mercury(II) chemo-sensors based on fluorescence (FL) organic dyes have been developed, but the majority of which requires the blending of a certain portion of organic solvents for detection and/or have unsatisfactory ion selectivity.[16−28] Furthermore, some selective reaction-based mercury(II) probes suffer from long synthetic routes and low overall yields.[21,25] All these drawbacks hamper their practicality for use in monitoring real samples.

To address the said issues, we exemplify here a one-step click-chemistry-based[29] molecular engineering approach that effectively enhances the property of an unqualified rhodamine-based mercury(II) probe with low selectivity and solubility. A previously developed unselective dipropargyl rhodamine dye (**1**)[30] was coupled with a water-soluble and biocompatible azido polyethylene glycol (PEG) tail by the Cu(I)-catalyzed azide−alkyne 1,3-dipolar cycloaddition (CuAAC) click reaction (Scheme 1).[31,32] The as-formed bis-triazolyl PEG rhodamine derivative showed excellent selectivity toward mercury(II) in pure water, and was applicable for the imaging of mercury ions internalized by a variety of live cells and quantification of the ion spiked in river water samples. This proof-of-concept study might provide insights into the improvement of unqualified small-molecule chemo-sensors by the simple 'click molecular engineering' strategy.

EXPERIMENTAL SECTION

Compounds **1**,[30] **2**,[42] and **3**[44] have been synthesized according to literature procedures.

Synthesis of KB4. To a solution of **1** (100 mg, 0.18 mmol) and **2** (95 mg, 0.54 mmol) in CH_2Cl_2/H_2O (5 mL/5 mL) were added sodium ascorbate (282 mg, 1.42 mmol) and $CuSO_4{\cdot}5H_2O$ (266 mg, 1.07 mmol), and the mixture was stirred overnight at room temperature (r.t.). The resulting mixture was directly purified by

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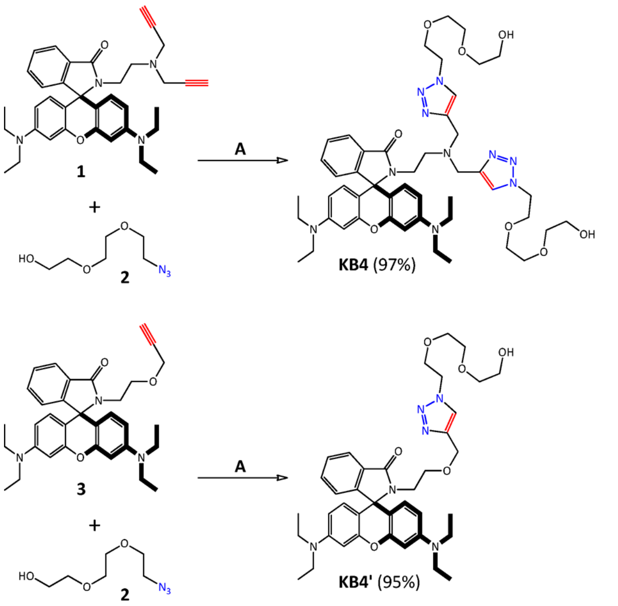
Scheme 1. Synthesis of KB4 and KB4′ by the One-Step Click Engineering[a]



[a]Reagents and conditions: (A) $CuSO_4 \cdot 5H_2O$, sodium ascorbate in CH_2Cl_2/H_2O (1:1, v/v).

column chromatography on silica gel (DCM/MeOH = 30:1, v/v) to afford **KB4** (157 mg, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (t, J = 6.8 Hz, 12H), 2.27 (s, 2H), 3.32–3.38 (m, 8H), 3.58–3.63 (m, 16H), 3.74 (t, J = 4.0 Hz, 4H), 3.90 (t, J = 4.0 Hz, 4H), 4.55 (t, J = 4.0 Hz, 4H), 6.26–6.28 (m, 2H), 6.39–6.43 (m, 4H), 7.11 (t, J = 3.2 Hz, 1H), 7.44–7.47 (m, 2H), 7.89–7.91 (m, 1H), 7.97 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.6, 29.7, 44.4, 50.1, 50.1, 61.5, 65.2, 69.4, 70.3, 70.5, 72.6, 77.3, 97.8, 105.2, 105.3, 108.1, 122.6, 123.9, 124.9, 125.1, 128.1, 128.7, 131.0, 132.5, 148.8, 153.3, 153.7. HR-ESI-MS m/z: $[M + H]^+$ calcd. for 911.5143, found 911.5154.

Synthesis of KB4′. To a solution of **3** (100 mg, 0.19 mmol) and **2** (95 mg, 0.54 mmol) in CH_2Cl_2/H_2O (5 mL/5 mL) were added sodium ascorbate (287 mg, 1.45 mmol) and $CuSO_4 \cdot 5H_2O$ (268 mg, 1.08 mmol), and the mixture was stirred overnight at r.t. The resulting mixture was directly purified by column chromatography on silica gel (DCM/MeOH = 50:1, v/v) to afford **KB4′** (124 mg, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (t, J = 6.8 Hz, 12H), 2.22 (s, 1H), 3.19 (t, J = 6.0 Hz, 2H), 3.34–3.40 (m, 10H), 3.58–3.62 (m, 6H), 3.77 (t, J = 4.0 Hz, 2H), 3.86 (t, J = 4.8 Hz, 2H), 4.40 (s, 2H), 4.53 (t, J = 4.8 Hz, 2H), 6.28–6.45 (m, 6H), 7.07–7.09 (m, 1H), 7.43–7.45 (m, 2H), 7.86–7.92 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.6, 29.7, 44.4, 50.2, 50.6, 61.6, 64.4, 69.3, 70.2, 70.5, 72.5, 77.3, 97.7, 105.3, 105.4, 108.1, 122.8, 123.8, 128.0, 128.2, 128.7, 129.0, 130.9, 132.5, 148.8, 153.3, 153.7. HR-ESI-MS m/z: $[M + H]^+$ calcd. For 721.3690, found 721.3650.

Fluorescence Spectroscopy. Stock solution of **KB4** (5 mM) was prepared in deionized water. Stock solutions of 5 mM of $LiClO_4$, $Cd(ClO_4)_2$, $Cr(ClO_4)_3$, $Zn(ClO_4)_2$, $Ba(ClO_4)_2$, $Mg(ClO_4)_2$, $KClO_4$, $NaClO_4$, $Mn(ClO_4)_2$, $Fe(ClO_4)_3$, $Ca(ClO_4)_2$, $Ni(ClO_4)_2$, $Hg(ClO_4)_2$, $AgClO_4$, $CuClO_4$, and $Cu(ClO_4)_2$ were prepared in deionized water. The fluorescence measurements were carried out with a path length of 10 mm and an excitation wavelength at 530 nm by scanning the spectra between 540 and 750 nm. The bandwidth for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded in aqueous solution at r.t.

Determination of the Spiked Hg(II) Concentration in River Water Samples. Water samples were collected from Huang-Pu River of Shanghai and Qing-Chun River of East China University of Science and Technology. The samples were passed through a microfiltration membrane before use. Prior to detection, the FL of **KB4** (5 μM) in the presence of various concentrations of mercury(II) was quantified, and the corresponding plot was prepared as the standard curve (see Figure 3a). Then the samples spiked with different concentrations of mercury(II) were quantified by fitting to the standard curve shown in Figure 3a.

Cell Imaging Assay. Cells were cultured in DMEM supplemented with 10% FBS. For the imaging experiments, cells (1.5×10^4/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. After pretreatment with or without 600 μM of $Hg(ClO_4)_2$ in 10 mM HEPES for 30 min (followed by three rinses by HEPES), the cells were incubated with 20 μM of **KB4** in 10 mM HEPES for another 15 min. Then the cells on the microplate were rinsed in warm HEPES and fixed by 4% paraformaldehyde in HEPES for 15 min at r.t.. After three rinses in HEPES (5 min each time), the fluorescence was detected and photographed with an Operetta high content imaging system (Perkinelmer, US).

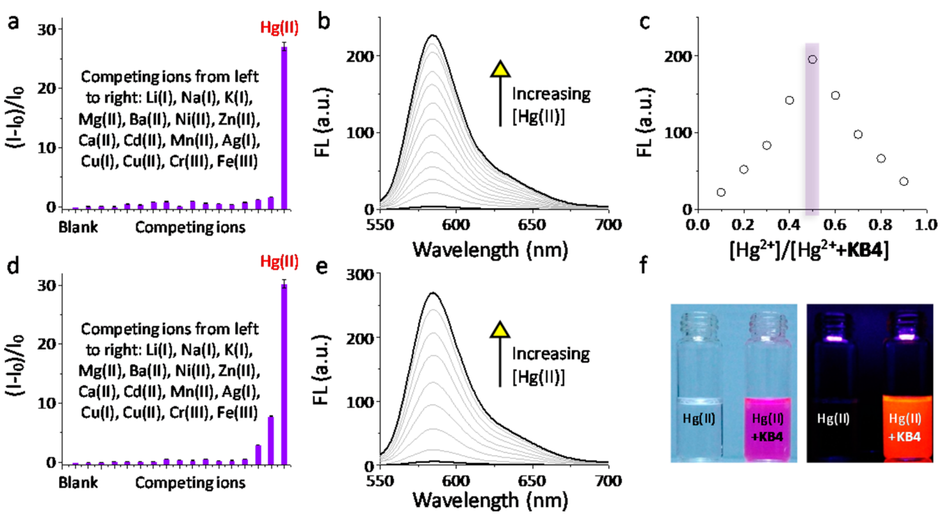


Figure 1. Selectivity of (a) **KB4** (5 μM in pure water) and (d) **KB4′** (5 μM in pure water) in the presence of Hg(II) and competing ions (50 μM); Fluorescence spectra of (b) **KB4** (5 μM in pure water) and (e) **KB4′** (5 μM in pure water) in the presence of increasing Hg(II) (0–6 μM for **KB4** and 0–8 μM for **KB4′**, λ_{ex} = 530 nm). (c) Job plot analysis of **KB4** in complex with Hg(II). (f) Colorimetric (left) and fluorescence (right) change of **KB4** with Hg(II). The original fluorescence spectra of panels a and d are shown in Figure S1a and d (Supporting Information), respectively.

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■ RESULTS AND DISCUSSION

A dipropargyl rhodamine derivative **1** synthesized[30] previously has been reported to be a poorly water-soluble and unselective fluorogenic probe toward Pd(II), Au(III), Cu(II),[33] and Hg(II).[30] Here, we use the CuAAC click reaction to simply couple **1** with a water-soluble PEG tail to enhance the water solubility. In addition, since the as-formed bis-triazolyl motif is envisioned to chelate more strongly and selectively a metal ion,[34−41] a monotriazolyl PEG rhodamine derivative was synthesized for analyzing the structure activity relationship.

As shown in Scheme 1, CuAAC coupling of **1** with azido PEG **2**[42] gave effectively the bis-triazolyl PEG rhodamine **KB4** in one-step with almost quantitative yield. Likewise, the monotriazolyl counterpart **KB4′**[43] was afforded by CuAAC of a monopropargyl rhodamine **3**[44] with **2** in high yield.

With the compounds in hand, we primarily tested their metal ion sensitivity in pure water. To our delight, both bis-triazolyl and monotriazolyl rhodamine derivatives showed sharp FL enhancement in the presence of micromolar Hg(II) in full aqueous medium (Figure 2). With a range of competing ions, while the monotriazole **KB4′** showed weak FL response to Cr(II) and Fe(III) (Figure 2d), the FL of the bis-triazole **KB4** was hardly fluctuated (Figure 2a). Additionally, the presence of Ag(I) and Cu(I) similar to Hg(II) did not cause FL change of both probes.

We then determined that both **KB4** and **KB4′** showed concentration-dependent FL increase with increasing Hg(II) (Figure 1b for **KB4** and Figure 1e for **KB4′**), producing broad linear ranges (Supporting Information Figure S1c for **KB4** and Supporting Information Figure S1f for **KB4′**). Meanwhile, increase of Hg(II) led to the gradual increase of absorbance of both **KB4** (Supporting Information Figure S1b) and **KB4′** (Supporting Information Figure S1e). This is in agreement with the substantial color change of the bis-triazolyl probe with Hg(II) in pure water solution observed by the naked-eye (Figure 1f, left). The evident FL change of **KB4** upon excitation by a portable UV lamp in the presence of the ion is shown in Figure 1f. Moreover, while the presence of a range of anions did not cause FL enhancement of **KB4** (Supporting Information Figure S3a), addition of S^{2-} quenched the FL of the **KB4**−Hg(II) mixture. The latter observation suggests that the detection process is reversible (Supporting Information Figure S3b).

The above observations suggest that the click engineering of the weak dipropargyl probe **1** greatly enhanced its water solubility and selectivity for mercury(II) ion in aqueous medium. The bis-triazole **KB4** showed better Hg(II)-sensitivity than the monotriazole **KB4′**, which was reflected, additionally, by the stronger association constant (**KB4** = 1.2×10^5 M^{-1} vs **KB4′** = 5.8×10^4 M^{-1}) and lower limit of detection (**KB4** = 4.6 ppb vs **KB4′** = 11.8 ppb) of the former. The different binding strength and selectivity between the two analogous probes suggest that the bis-triazolyl motif formed upon a dye platform might serve as a stronger metal ion chelating site than the monotriazolyl counterpart.

The conventional technique for Hg(II) detection mainly lies on the cold vapor atomic absorption spectrometry (limit of detection: 0.1 ppb). Comparison with **KB4**, this method has some substantial disadvantages involving the high cost, long detection time and complex detection procedures. The sensing property of **KB4** was also compared thoroughly with some literature Hg(II) probes in terms of solubility and limit of detection (Supporting Information Table S1). Obviously, while the majority of the former probes suffer from solubility issues, the sensitivity of those with the ability to detect Hg(II) in full aqueous media was lower than that of **KB4**. Notably, the limit of detection of **KB4** was among the closest to the regulated level of Hg(II) in drinking water (2 ppb).

The ligand-ion binding mode was further scrutinized by the Job plot and ^{1}H NMR titration analyses. The Job plotting shown in Figure 1c suggests that **KB4** forms a 1:1 complex with Hg(II) as the ratio of [Hg(II)] to [Hg(II) + **KB4**] points to 0.5. Then, we resorted to ^{1}H NMR titration for gaining better insights into the coordination manner. As shown in Figure 2a,

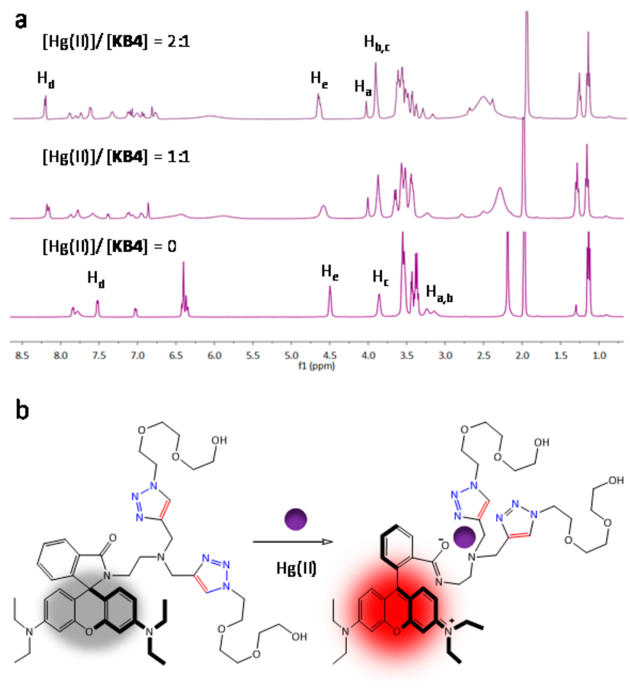

Figure 2. (a) ^{1}H NMR titration of **KB4** with increasing Hg(II) measured in CD_3CN and (b) the proposed ligand−ion coordination mode.

addition of 1 equiv. Hg(II) led to peak shift of the methylene protons adjacent to the tertiary amine, the triazole protons as well as the neighboring PEG methylene protons of triazole, whereas increase of the Hg(II) concentration to 2 equiv. did not cause further peak shifting.

On the basis of the above results, we attempted to propose the plausible ligand-ion binding motif (Figure 2b). The tertiary amine nitrogen, two separate nitrogen atoms of the two triazole rings and the carbonyl oxygen of rhodamine together chelate a mercury ion, leading to the opening of the spirolactam ring, thereby releasing the FL of rhodamine.[19] The stable bis-triazole coparticipated complexation mode might account for the enhanced Hg(II) sensitivity and selectivity of **KB4**.

Next, we interrogated the ability of **KB4** to probe Hg(II) in real samples. We used river water specimens collected from the Huang-Pu river of shanghai and Qing-Chun river of East China University of Science and Technology. These specimens were spiked with Hg(II) of different concentrations (50, 100, and 200, and 500 nM). Then the ion concentrations spiked were measured by the probe according to the linear plotting shown in Figure 3a. As shown in Figure 3b, the recovered Hg(II) concentration of each measuring point (red or green) is in

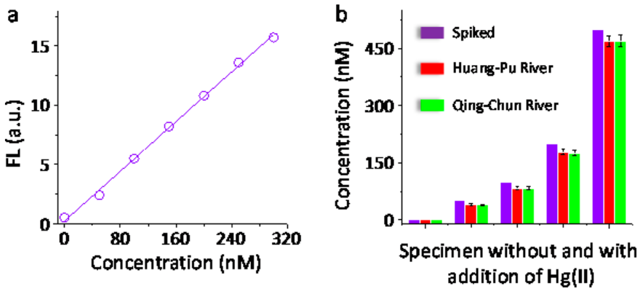

Figure 3. (a) Plotting the fluorescence intensity (FL) of **KB4** as a function of Hg(II) concentration and (b) spiked and recovered Hg(II) concentration in Huang-Pu River and Qing-Chun River determined by the low-concentration linear plotting.

good agreement with the originally spiked concentration (violet), suggesting the potential of **KB4** for monitoring trace Hg(II) ions in environmental water.

We also examined the imaging ability of **KB4** for Hg(II) ions internalized by live cells. Four different cancer cells derived from different human tissues were used (human cervix cancer Hela, human liver cancer Hep-G2, human colon cancer HCT116 and human lung cancer A549). The cells with or without pretreatment of mercury ions were incubated with the probe (20 μM), and then the FL was recorded. As shown in Figure 4, whereas the cells without pretreatment with Hg(II) did not show any FL, those with internalized Hg(II) showed strong FL of rhodamine upon incubation with **KB4**. Moreover, the bright field images showed that incubation of Hg(II) and the probe did not impact the viability of the cells during the FL imaging. This manifests the usefulness of the probe in imaging heavy metal ions in live cells.

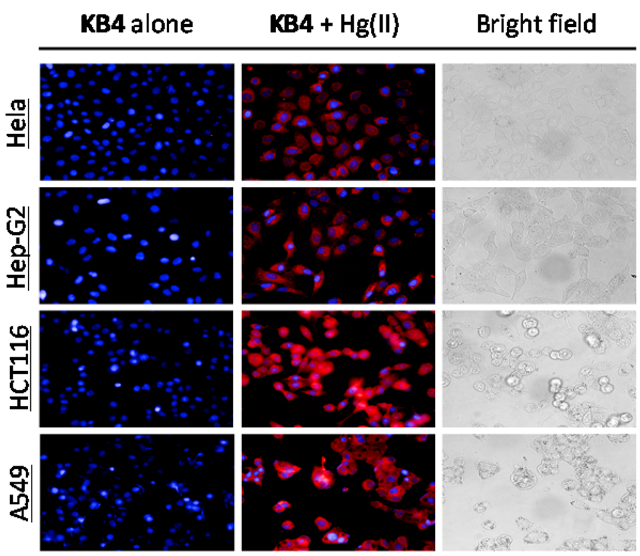

Figure 4. Fluorescence and bright field images of different cancer cells in the presence of **KB4** (20 μM) with or without preincubated Hg(II) (400 μM) (cell nuclei were stained by Hoechst).

Hela cells with good cellular morphology was then used for a kinetic study. The cells were pretreated with Hg(II) and then were with **KB4** with increasing incubation times (1−25 min). We observed that, while the cells showed gradually intensified FL from 1 to 15 min, further increase in incubation time did not lead to obvious FL increment. This suggests that the probe was probably internalized by the live cells in a time-dependent manner.

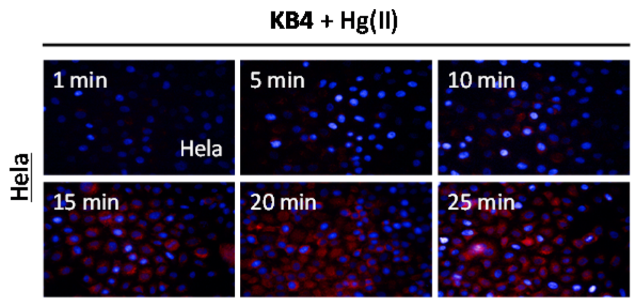

Figure 5. Fluorescence images of Hela cells in the presence of **KB4** (20 μM) and preincubated Hg(II) (600 μM) with increasing incubation time (cell nuclei were stained by Hoechst).

■ CONCLUSION

In summary, we demonstrated with this study that the click engineering strategy is effective in enhancing considerably the practicality of an unqualified chemosensor. By simply clicking a PEG tail to a weak dipropargyl rhodamine probe, the as-formed bis-triazolyl derivative showed much more improved water solubility and selectivity. The probe was also proven to be practical in sensitively monitoring trace heavy metal ions in real river water specimens and in imaging the ions internalized by live cells. Complementary to the many previous successful examples in building chemo-sensors for Hg(II),[45−50] the click strategy proposed here, because of its simplicity and powerfulness, might provide new insights into the concise functional improvement of unqualified molecular dye probes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Original NMR and MS spectra of new compounds, additional figures, and Table S1. 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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