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A LONG-WAVELENGTH EMISSION FLUORESCENT PROBE BASED ON TCF DERIVATIVES FOR HIGH-SENSITIVITY DETECTION OF Hg²⁺

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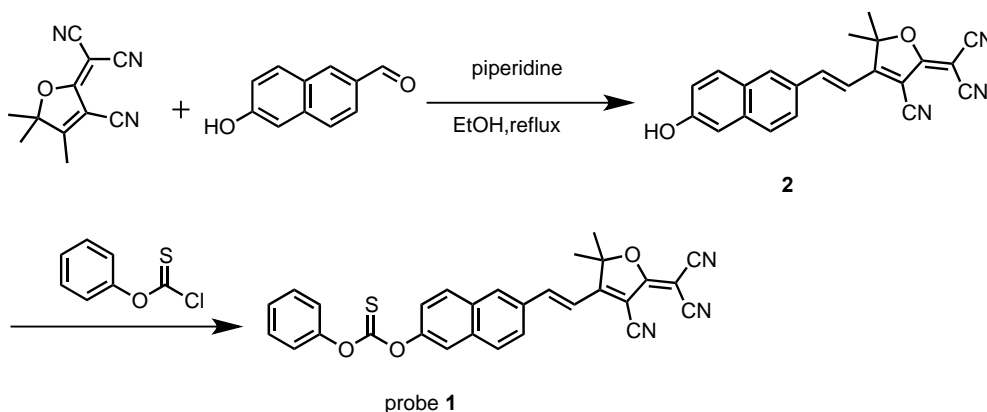
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Abstract – A new fluorescent probe **1** that derived from 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) has been developed. Probe **1** displays selective recognition toward Hg²⁺ in MeOH/Tris (1:1, v/v, Tris 20 mM, pH = 7.4) solution through fluorescence turn on behavior. The Hg²⁺ sensing mechanism underwent Hg²⁺-triggered removal of thiocarbonyl moiety of probe **1**, which resulting in releasing of the highly emissive compound **2**. The Hg²⁺ recognition process holds some advantages including rapid response (<3 min), low detection limit (1.06 nM), and a large Stokes shift (160 nm). Cell imaging studies showed that probe **1** has membrane permeability and low toxicity to cells, and can be used to detect Hg²⁺ in living cells.

Mercury is a highly toxic non-biological element that naturally exists in the environment.^{1,2} At present, mercury is mainly used in the synthesis of chemicals and the manufacture of electronic or electrical products,³ lead to the emission of mercury-containing pollutants.⁴ Even at ppm levels of mercury accumulation,⁵ it will also cause serious harm to the human brain,^{6,7} heart, lung, kidney,⁸ central nervous system and immune system.^{9,10} Therefore, the highly sensitive and specific detection of mercury ion (Hg²⁺) is of great significance to the environment and human health.^{11,12}

The conventional methods for measuring Hg²⁺ include atomic absorption spectrometry,^{13,14} high performance liquid chromatography, electrochemical methods, and so on.^{15,16} Although these methods are highly selective, they are time-consuming and labor-intensive, and usually require expensive equipment, making them difficult to detect Hg²⁺ in real time.^{17,18} Compared with the aforementioned methods,

fluorescence technology has attracted much attention due to its simple operation, fast response speed, high selectivity and sensitivity.^{19,20} Therefore, a large number of researchers have developed small molecule fluorescent probes for the detection of mercury ions.^{21,22} So far, many fluorescent probes based on formation of Hg^{2+} -ligand complex or Hg^{2+} -triggered reactions have been developed.²³ Among these methods, fluorescent probes based on ligand complexation usually show low selectivity.^{24,25} Due to interference from other metal ions, the reaction-based Hg^{2+} fluorescent probes have attracted considerable attention due to the high selectivity, simplicity, and unique spectra changes.^{26,27} Due to the highly thiophilic nature of Hg^{2+} , the reaction type probes that have been developed mainly include the ring-opening of the spironolactone, Hg^{2+} promoted cycloaddition of thiourea substrates, desulfurization of thioacetal substrates and thiocarbonyl,^{28,29} etc. It is worth noting that only a few Hg^{2+} -specific fluorescent probes exhibit both long-wavelength emission and a large Stokes shift.^{30,31} Therefore, it is still attractive to develop Hg^{2+} -specific fluorescent probes with long-wavelength emission and a large Stokes shift.^{32,33} 2-Dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) possesses strong electron withdrawing ability, it usually acts as electron acceptor in the D- π -A conjugate system,³⁴ thus it has been widely used to construct long-wavelength emission fluorescent probes. Based on this idea, we herein designed and synthesized a TCF-derivatized fluorescence probe **1** (Scheme 1). The hydroxy group in compound **2** acts as an electron donor, formed D- π -A conjugated structure with TCF, the thiocarbonate moiety acts as both a quenching group and a recognition group, it can react selectively toward Hg^{2+} , leading to removal of thiocarbonyl and releasing of compound **2**, thus realizing Hg^{2+} -triggered fluorescence turn on response through restoration of intramolecular charge transfer (ICT).



Scheme 1. Synthesis of the probe **1**

Optical response of probe **1** to Hg^{2+}

We firstly studied the fluorescence response of probe **1** to different concentrations of Hg^{2+} in MeOH/Tris (1:1, v/v, Tris 20 mM, pH = 7.4) solution. As shown in Figure 1, probe **1** solution shows weak fluorescence intensity at 550 nm, upon gradual addition of Hg^{2+} , a gradually increasing fluorescence

emission centered at 625 nm can be observed. When the added Hg^{2+} reached 30 μM , the fluorescence intensity tends to be stable. This may be due to the Hg^{2+} -triggered removal of the thiocarbonyl group, and the release of hydroxy group leading to enhanced ICT effects. These results prove that probe **1** has good sensing properties for Hg^{2+} . According to the titration curve, a satisfactory linear relationship is obtained between the fluorescence intensity ratios at 625 nm (F/F_0 relative intensity divided by the fluorescence intensity in the absence of metal ions) and the Hg^{2+} (0–30 μM) concentration (Figure 2). The detection limit of probe **1** for Hg^{2+} was then calculated to be 1.06 nM based on the equation $\text{LOD} = 3\sigma/\rho$ (σ is the standard deviation of the blank solution; ρ is the slope of the calibration curve). At the same time, the time-dependent fluorescence intensity ratios (F/F_0) change of probe **1** to Hg^{2+} (30 μM) was recorded, the response of probe **1** to Hg^{2+} can be completed within 3 minutes (Figure 3), indicating that probe **1** is capable of detecting Hg^{2+} in real time.

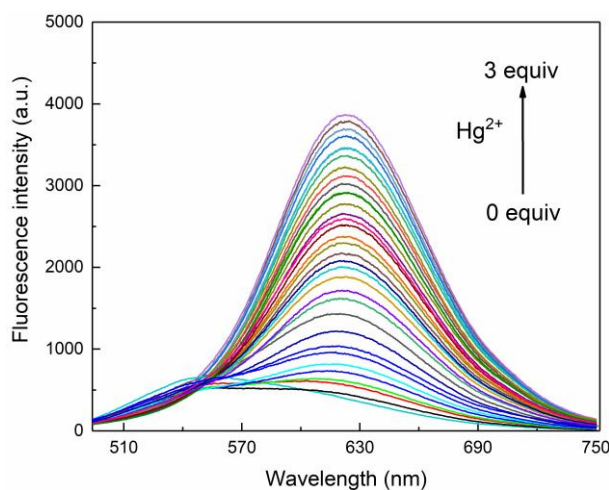


Figure 1. Changes of fluorescence spectra of probe **1** (10 μM) in MeOH/Tris (1:1, v/v, Tris 20 mM, pH = 7.4) solution with gradual addition of Hg^{2+} (0–30 μM). $\lambda_{\text{ex}} = 465 \text{ nm}$

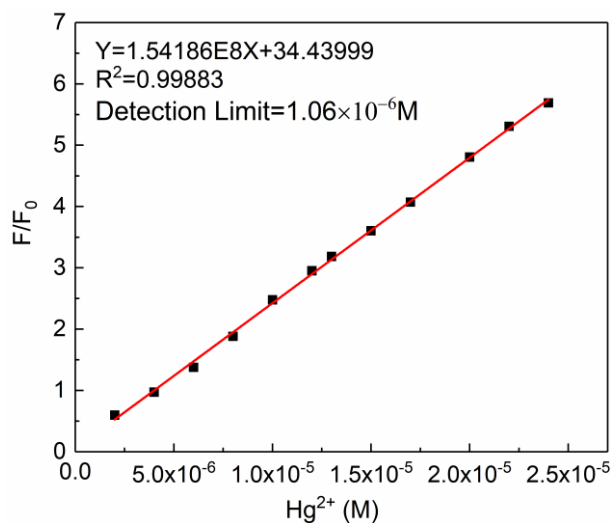


Figure 2. Linear relationship between fluorescence intensity ratios (F/F_0) and Hg^{2+} concentration (0–30 μM)

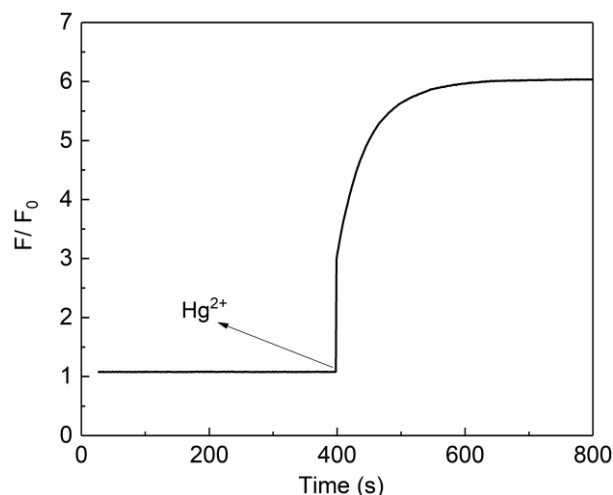


Figure 3. Time-dependent fluorescence intensity ratios (F/F_0) changes of probe **1** in the absence and presence of Hg^{2+} ($30 \mu\text{M}$)

In order to verify the selectivity of probe **1** to Hg^{2+} , we detected the changes of the fluorescence spectra of probe **1** in the presence of different metal ions, including Cu^{2+} , Co^{2+} , Zn^{2+} , Cd^{2+} , Cr^{3+} , Fe^{3+} , Sr^{2+} , Na^+ , Hg^{2+} , Ag^+ , Pb^{2+} , Mn^{2+} , Al^{3+} , K^+ , Ni^{2+} , Ba^{2+} , Mg^{2+} , Fe^{2+} and Ca^{2+} (3 equiv. of each) (Figure 4). It can be seen that the fluorescence intensity of probe **1** increased significantly at 625 nm on addition of Hg^{2+} and Ag^+ , however, no significant fluorescence enhancement was observed with the addition of other metal ions. Since Ag^+ and Cl^- can readily form insoluble precipitate AgCl , the interference from Ag^+ can be effectively shielded by introducing an appropriate concentration of NaCl (Figure S1). We also compared the UV-vis absorption spectra of probe **1**, probe+ Ag^+ and probe+ Hg^{2+} (Figure S2), the observed large overlap of the absorption spectra indicate that probe **1** is limited in detecting Hg^{2+} by UV-vis absorption spectra. These results show that probe **1** has applicability for fluorescence detection of Hg^{2+} in biological and environmental analysis.

To further validate the practicality of probe **1** for Hg^{2+} detection, competition experiments were performed (Figure 5). In the presence of tested metal ions (except Ag^+), the fluorescence intensity of probe **1** remained almost unchanged. When Hg^{2+} ($30 \mu\text{M}$) was subsequently added, the fluorescence intensity ratios (F/F_0) increased significantly at 625 nm. These results demonstrate that other coexisting metal ions (except for Ag^+) exert no obvious interference on Hg^{2+} recognition. We also examined the effects of pH on Hg^{2+} recognition by probe **1** (Figure 6). The fluorescence intensity ratios (F/F_0) of probe **1**+ Hg^{2+} (3 equiv.) increased significantly between pH 5.0 and 9.0. The results show that probe **1** can detect Hg^{2+} in a wide pH range, especially at near-neutral conditions.

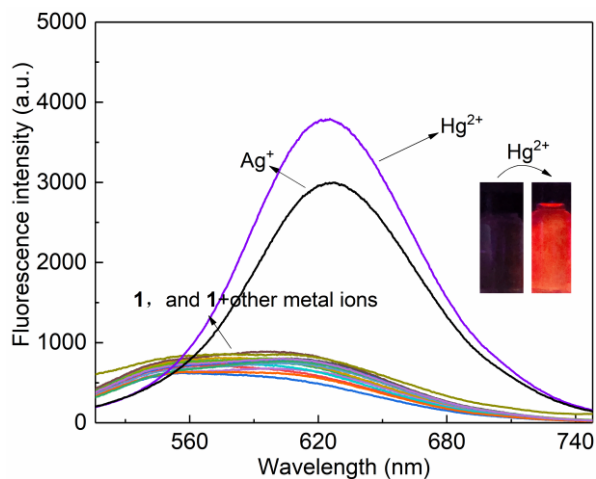


Figure 4. Fluorescence spectra changes of probe **1** (10 μM) in the presence of various a metal ions (30 μM) in MeOH/Tris (1:1, v/v, Tris 20 mM, pH = 7.4) solution. $\lambda_{\text{ex}} = 465$ nm. Inset: Fluorescence photographs of the probe solution before and after adding Hg^{2+} .

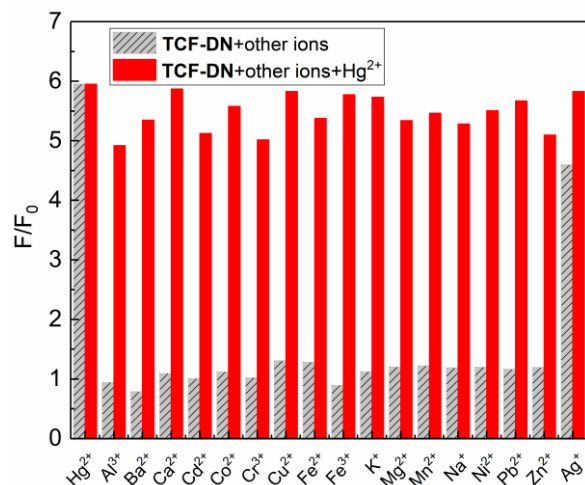


Figure 5. Changes in fluorescence intensity ratios (F/F_0) of probe **1** (10 μM) upon addition of different metal ions (30 μM) and followed by further addition of Hg^{2+} (30 μM). $\lambda_{\text{em}} = 625$ nm

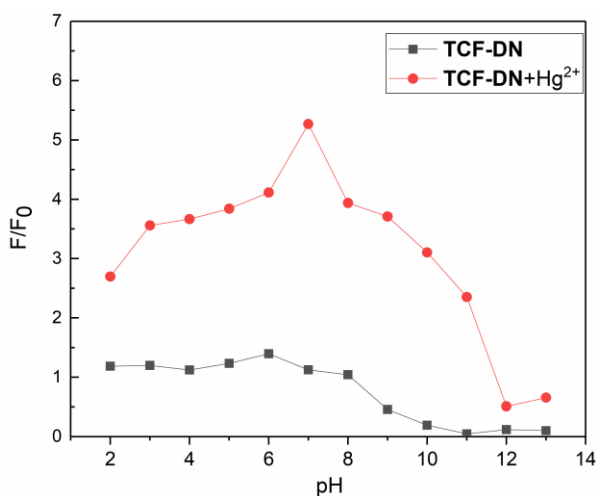


Figure 6. Fluorescence intensity ratios (F/F_0) changes of probe **1** in the presence and absence of Hg^{2+} at different pH conditions

Detection mechanism of probe **1** to Hg^{2+}

The sensing mechanism of probe **1** for Hg^{2+} may owe to the strong thiophilic characteristic of Hg^{2+} , which results in releasing of compound **2** via the reaction of probe **1** with Hg^{2+} . To confirm this speculation, the HRMS spectrum of the reaction mixture of probe **1**+ Hg^{2+} was checked. The peaks appeared at $m/z = 370.1201$ and $m/z = 352.1094$ (Figure S3) can be assigned to the species of $[\mathbf{2}+\text{H}_2\text{O}-\text{H}]^-$ (calcd. $m/z = 370.1197$ $[\mathbf{2}-\text{H}]^-$ (calcd. $m/z = 352.1092$), respectively, which advocating the formation of compound **2** on addition of Hg^{2+} to probe **1** solution. We then separately conducted the reaction of probe **1** with Hg^{2+} , and the main product was isolated. The ^1H NMR spectra of the isolated product were then compared with those of probe **1** and compound **2** (Figure 7). Obviously, the ^1H NMR spectrum of the isolated product of probe **1**+ Hg^{2+} (Figure 7b) is almost identical with that of compound **2** (Figure 7c), proving that reaction of probe with Hg^{2+} indeed gives compound **2**. Based on these processes, the detection mechanism of probe **1** to Hg^{2+} can be explained in Scheme 2.

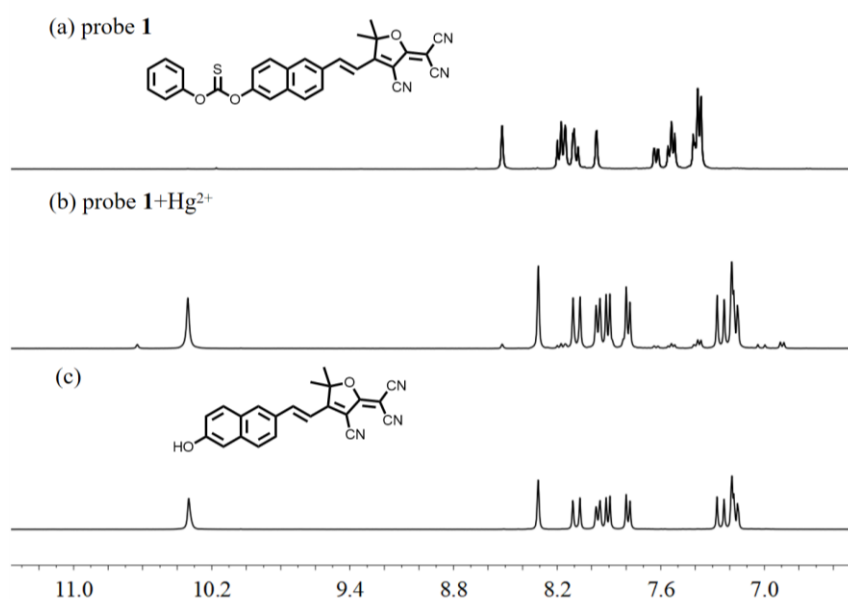
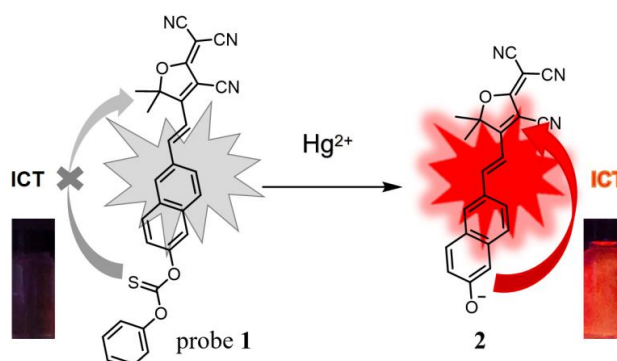


Figure 7. Comparison of the ^1H NMR spectra of probe **1** (a), probe **1**+ Hg^{2+} (b) and compound **2** (c) in $\text{DMSO}-d_6$



Scheme 2. The sensing mechanism of probe **1** to Hg^{2+}

Hg²⁺ imaging in live cells

Due to the good sensing performance of probe **1** to Hg²⁺, we then explored its application to image Hg²⁺ in living cells. The cell toxicity experiments reveal that probe **1** has low toxicity to MCF-7 cells (Figure S4). Then, live cell imaging experiments were performed on MCF-7 cells. Firstly, MCF-7 cells were incubated with probe **1** (10 μ M) for 30 minutes at 37 $^{\circ}$ C, no fluorescence emission was observed in the red channel (Figure 8b). Then the MCF-7 cells pretreated with the probe were washed 3 times with PBS buffer, after further incubation with different concentrations of HgCl₂ (10, 50, and 100 μ M) for 30 minutes, obvious red fluorescence can be observed, and the fluorescence brightness increases with the increase of Hg²⁺ concentration (Figure 8b–k). These results indicate that probe **1** has good cell permeability and can image Hg²⁺ in MCF-7 cells.

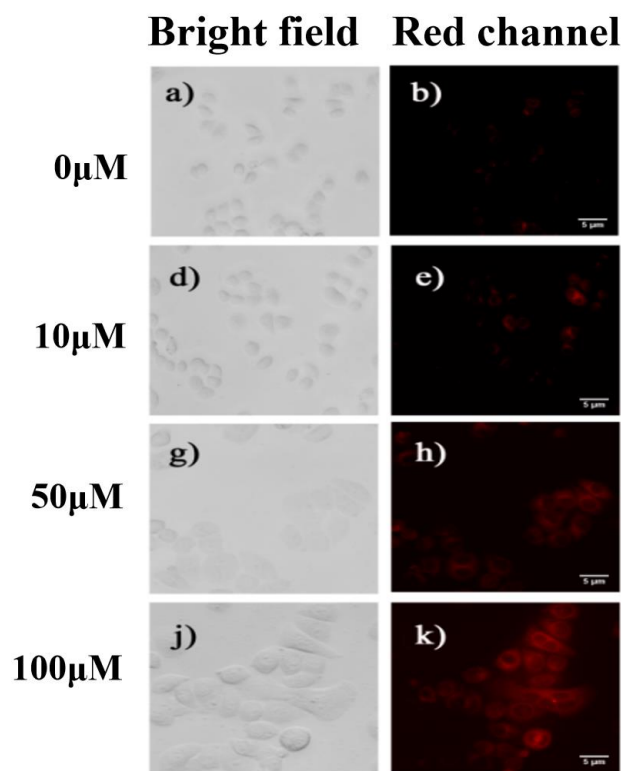


Figure 8. Bioimaging of MCF-7 cells incubated with probe **1** (10 μ M) and 0 μ M, 10 μ M, 50 μ M and 100 μ M Hg²⁺ ions respectively. Left column: Bright field. Right column: Red channel.

In conclusion, we have developed a new TCF-based fluorescent probe **1** for Hg²⁺ recognition in MeOH/Tris (1:1, v/v, Tris 20 mM, pH = 7.4) solution with long-wavelength emission and a large Stokes shift (160 nm). Probe **1** displays rapid response to Hg²⁺ (< 3 min) with a low detection limit (1.06 nM). The recognition mechanism is proved to undergo Hg²⁺-triggered releasing of the precursor compound **2**. Probe **1** has membrane permeability, and is applicable for fluorescence image of Hg²⁺ in living MCF-7 cells.

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SUPPORTING INFORMATION

Supplementary data (Experimental procedures and details, Characterization data for products, NMR spectra for all products) associated with this article can be found, in the online version, at URL: <https://www.heterocycles.jp/newlibrary/downloads/PDFsi/27267/102/10>.

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