Synthesis and fluorescent properties of a novel dansyl-based fluorescent probe for Hg^{2+}

Xiao Zuo-An*, Zhan Dan and Yang Xiao

College of Chemical Engineering and Food Science, Hubei University of Arts and Science, Xiangyang 441053, P.R. China

A novel turn-off fluorescent probe containing a dansyl fluorophore has been synthesised. Its recognition properties towards various metal ions have been studied by fluorescence spectrometry. The compound showed a high sensitivity and selectivity to Hg^{2+} ion and a complexation ratio towards Hg^{2+} of 2 : 1. Its fluorescence intensity varied almost linearly *versus* the concentration of Hg^{2+} (0.8–8.4 μmol L^{-1}), and the detection limit of Hg^{2+} was estimated to be 0.88 μmol L^{-1}.

Keywords: dansyl, fluorescent probe, Hg^{2+}, selectivity

The selective and sensitive detection of transition and heavy metal ions is very important for medical diagnostics, environmental, and biological applications.[1,2] Hg^{2+} is considered the most toxic heavy metal ion and its pollution has attracted much attention. It is commonly spread into the environment by anthropogenic and industrial releases and is converted into toxic methylmercury by bacterial and chemical actions. The bioaccumulation of such toxic material in the human body *via* the food chain can lead to digestive, kidney and neurological diseases even at very low concentration.[3,4] The Environmental Protection Agency has set the maximum allowable level of inorganic mercury in drinking water at 2 ppb.[5] Therefore, the development of convenient and rapid methods for detection of Hg^{2+} has attracted considerable interest. Consequently, great efforts have been made to design efficient fluorescent probes to detect Hg^{2+} either by fluorescence enhancement or quenching and colour changes.[6–9] However, only a few examples of Hg^{2+}-selective fluorescent probes have been found to be effective.[10–13] Therefore, designing a highly selective sensor for Hg^{2+} remains a challenge. We now report a novel and selective fluorescent probe for Hg^{2+}.

The dansyl group has often been chosen to be incorporated into fluorescent probes due to its good fluorescent properties.[14,15] We have designed (Scheme 1) a dansyl-based fluorescent probe **4**, in which the dansyl group is linked to a chelating moiety [bis(2-(ethylthio)ethyl)amine, **3**]. Scheme 1 shows how the intermediates **2** and **3** were prepared according to the literature method.[16,17] The reaction of dansyl chloride with 2 equiv. of **3** in acetone afforded **4** in 76% yield. Compound **4** was fully characterised by ^1H NMR, ^{13}C NMR, IR, MS and elemental analysis, and its response to Hg^{2+} was studied through fluorescent spectrometry. The primary test showed that **4** exhibited highly selective fluorescence quenching toward Hg^{2+} in a aqueous EtOH solution.

The maximum excitation (λ_{ex}) and emission (λ_{em}) wavelengths of **4** are 368 and 552 nm, respectively. The fluorescence response of **4** to various metal ions (K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+} and Hg^{2+}) is illustrated in Fig. 1. The results show that Hg^{2+} produced significant quenching in the fluorescent emission of **4**, while other metal ions did not exhibit significant changes under identical conditions, except that Cu^{2+} showed relatively

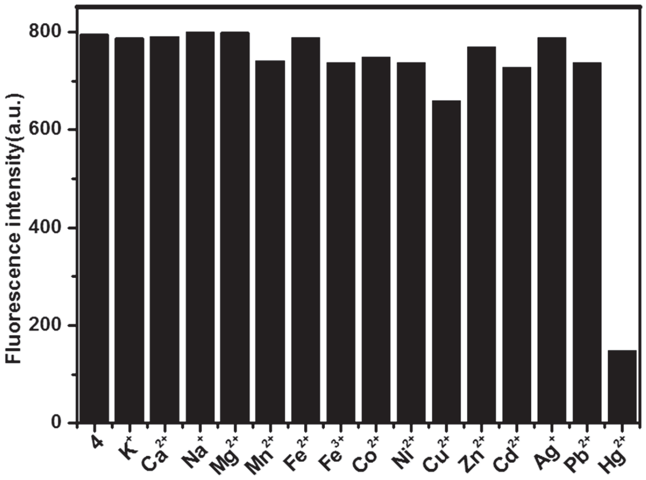
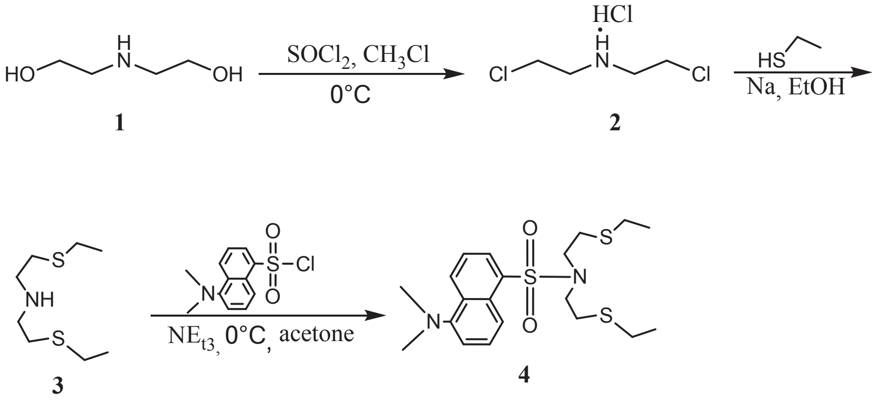


Fig. 1 The effect of metal ions (10 μM) on the fluorescent properties of probe **4** (10 μM) in 100 mM Tris-HCl buffer (pH 7.4) (H_2O : EtOH = 9 : 1, v/v) with an excitation at 368 nm. The samples were incubated for 10 min at room temperature prior to measurements.



Scheme 1 Synthesis of fluorescent probe **4**.

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* Correspondent. E-mail: blueice8250@aliyun.com

small fluorescence intensity changes. To further estimate the selectivity for Hg^{2+} over other metal ions, competition experiments of Hg^{2+} mixed with other metal ions were carried out by fluorescence spectra and the results are shown in Fig. 2. The fluorescence intensity of **4** (10 µmol L^{-1}) in the presence of 1 equiv. of Hg^{2+} was almost unaffected by the addition of 10 equiv. of competing metal ions. Thus it can be concluded that **4** has a high selectivity for recognition of Hg^{2+}.

The sensitivity of the fluorescence emission response of **4** towards Hg^{2+} was also examined by fluorescent titration (Fig. 3). The fluorescence intensity of **4** was decreased continually upon addition of Hg^{2+}. When the concentration of Hg^{2+} increased to 3 equiv., the fluorescence of **4** was nearly quenched. At a very high concentration of Hg^{2+}, the fluorescence of **4** was completely quenched. This clearly indicates that the Hg^{2+}–**4** complex is non-fluorescent in nature. Moreover, as depicted in the inset in Fig. 3, an obvious fluorescence change from bright yellow to dark can be observed under irradiation at 365 nm after the addition of 3 equiv. of Hg^{2+} to the solution of **4**. It was found that a linear regression curve (linear correlation coefficient $R^2 = 0.9947$) of the Hg^{2+}–**4** complex fitted the relationship between the fluorescence of **4** and the concentration of Hg^{2+} (0.8–8.4 µmol L^{-1}) (Fig. 4). The detection limit of **4** to Hg^{2+} was determined to be 0.88 µmol L^{-1} according to the calculation method reported in the literature.[18] The above results indicate a high sensitivity of **4** to Hg^{2+}.

To determine the stoichiometry of **4** and Hg^{2+} in the complex, Job's plot method was employed using the emission changes at 552 nm as a function of the molar fraction of **4**. As shown in Fig. 5, the maximum point was observed at the mole fraction between 0.6 and 0.7, indicating that Hg^{2+} forms a 1 : 2 complex with **4**.

Based on above experiments and a recent report, [19] a possible complexation mechanism seemed to be reasonable for the binding site of **4** with Hg^{2+}. In the chelating moiety of **4**, there are two sulfur atoms and one nitrogen atom, which probably form a suitable binding site for Hg^{2+}. When Hg^{2+} is added, the sulfur and nitrogen atoms donate their lone pair of electrons to the empty orbital of Hg^{2+}. The capture of Hg^{2+} causes the electron or energy transfer between the chelating unit and the dansyl group, thus resulting in the fluorescence quenching of the dansyl group. Therefore we propose that the reaction of **4** with Hg^{2+} would yield the 1 : 2 complex shown in Scheme 2.

In summary, we have synthesised a novel dansyl-based fluorescent probe **4**. A fluorescent study has shown that **4** is selective and sensitive to Hg^{2+}, and it formed a 2 : 1 complex with Hg^{2+}. The detection limit of **4** to Hg^{2+} was determined to be 0.88 µmol L^{-1}.

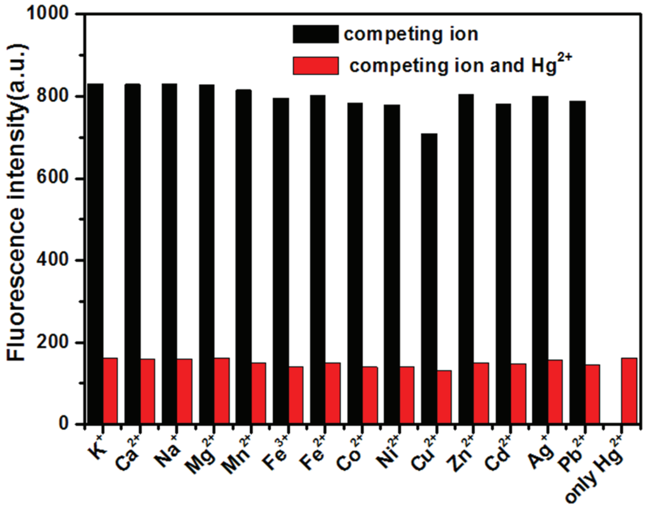


Fig. 2 The effect of Hg^{2+} on **4** (10 µmol L^{-1}) in the presence of different metal ions (100 µmol L^{-1}).

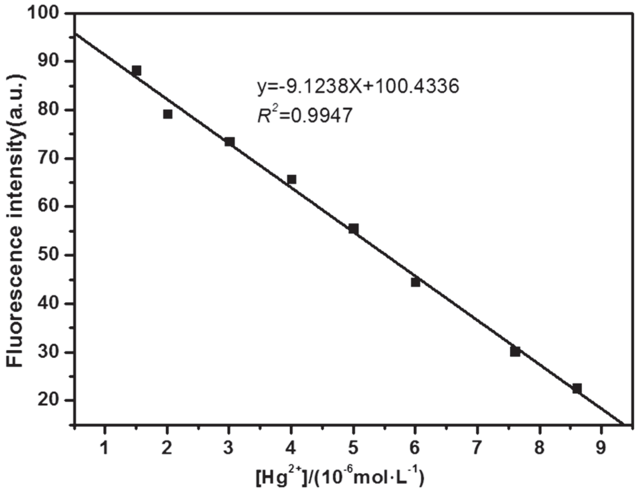


Fig. 4 Fluorescence intensity of **4** as a function of concentration of Hg^{2+} (0.8–8.4 µmol L^{-1}).

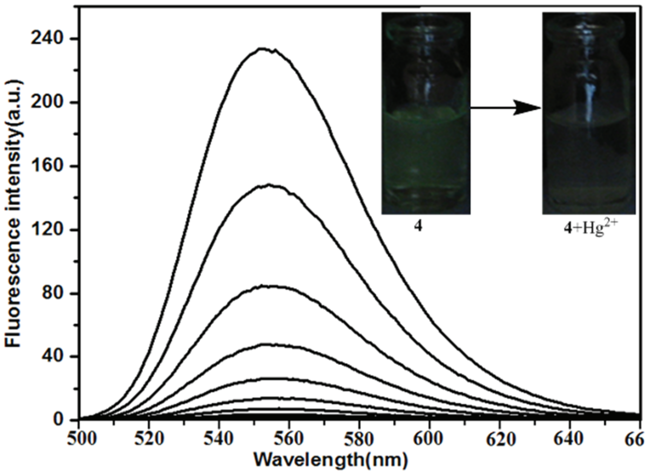


Fig. 3 The emission intensity of **4** (10 µmol L^{-1}) changes with increasing concentrations of Hg^{2+} (0–30 µmol L^{-1}), the inset shows photograph of **4** (10 µmol L^{-1}) in the absence and presence of Hg^{2+} under UV light (365 nm).

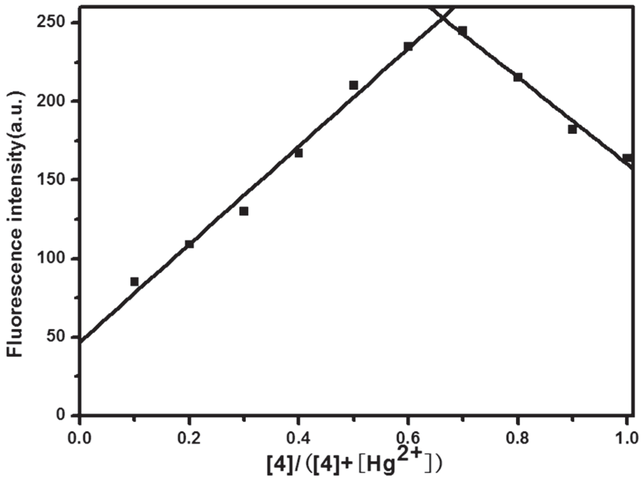
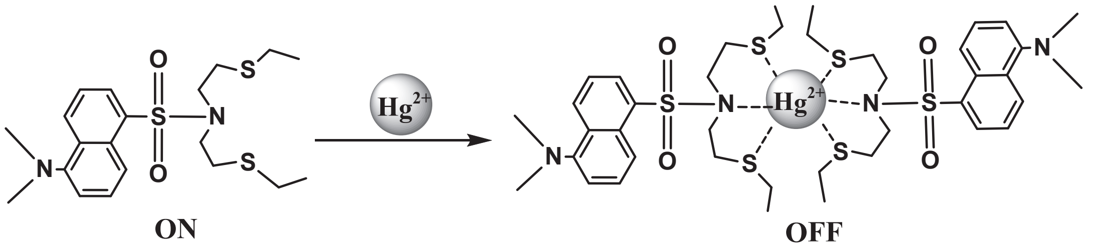


Fig. 5 Job's plot of **4** and Hg^{2+} ([**4**] + [Hg^{2+}] = 10 mmol L^{-1}).



Scheme 2 Proposed binding model of fluorescent probe **4** with Hg^{2+}.

Experimental

All reagents were purchased from commercial companies and directly used unless stated otherwise. Solvents were purified by standard methods. The ^1H NMR spectra were recorded on a Mercury Plus-400 spectrometer in $CDCl_3$. Mass spectra were obtained with a Finnigan Trace MS instrument using the EI method. The IR spectra were measured on a Perkin-Elmer Spectrum BX FT-IR instrument in tablets with potassium bromide. Elemental analyses were carried out on a Perkin-Elmer 2400 instrument. Fluorescence spectra were performed on a FluoroMax-P spectrofluorimeter.

5-(Dimethylamino)-N, N-bis(2-(ethylthio)ethyl)naphthalene-1-sulfonamide (**4**): The intermediate **3** (0.77 g, 0.4 mol) and triethylamine (0.40 g, 0.4 mol) were added to a solution of dansyl chloride (0.54 g, 0.2 mol) in acetone (100 mL). The mixture was stirred in an ice bath for 2 h. Then the ice bath was removed and the resulting yellow suspension was stirred at room temperature for another 3 h. The progress of the reaction was monitored by TLC until complete disappearance of dansyl chloride. After filtration and rotary evaporation, the residue was purified by flash chromatography (silica, petroleum ether/AcOEt, 5/1, v/v) to give a yellow compound **4**; yield: 76%. IR (KBr): 2948, 1542, 1476, 1422, 1380, 1248, 1152 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 1.28 (t, 6H, *J* 7.2 Hz, $-CH_3$), 2.45–2.58 (m, 8H, $-CH_2$), 2.88 (s, 6H, $-CH_3$), 3.52 (t, 4H, *J* 7.6 Hz, $-CH_2$), 7.22–7.62 (m, 3H, ArH), 8.22–8.65 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 152.4, 145.2, 1334.6, 129.2, 127.4, 126.8, 124.3, 123.2, 118.9, 116.8, 48.7, 45.8, 29.8, 27.2, 15.4. EI-MS: *m/z* 578. Anal. calcd for $C_{20}H_{30}N_2O_2S_3$:C, 56.30; H, 7.09; N, 6.57; found: C, 56.48; H, 7.28; N, 6.42%.

Fluorescence experiment studies:

A stock solution of **4** was prepared by dissolution in ethanol/water (8 : 2, v/v) containing 100 mmol L^{-1} Tris-HCl buffer (pH 7.4). Solutions of various metal ions (K^+**,** Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+}) were prepared in water (1 mmol L^{-1}). Fluorescence titration was performed on 3 mL solutions of **4** in a quartz cell, by adding different stock solutions of cations into the quartz cell portionwise using a microsyringe each time. After mixing well, the fluorescence spectrum of the solution was monitored.

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