A "turn-on" coumarin-based fluorescent sensor with high selectivity for mercury ions in aqueous media

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Supporting Information Available:

I) Experimental section

II) Spectral Data (^{1}H, ^{13}C)

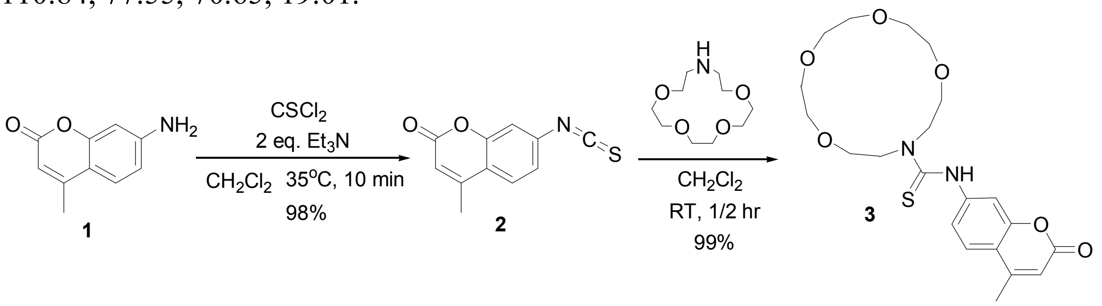
III) Fluorescence Spectra for **3** in the free and Hg^{2+}-bound form

I) Experimental Section

General Procedures: All reactions were carried out under anhydrous conditions in dry solvents, using argon or nitrogen in flame-dried glassware. Reactions were monitored by thin-layer chromatography (TLC) using silica gel plates from Merck (60F254), which were visualized under a UV-Vis Lamp (254 and 366 nm, respectively) or with a 7% ethanolic solution of phosphomolybdic acid. Flash column chromatography was performed in silica gel 60 from Merck (230-400 mesh). NMR spectra were taken on an AMX500 Bruker FT-NMR or a MSL300 Bruker FT-NMR spectrometer; proton chemical shifts are reported in ppm relative to tetramethylsilane. Fluorescence spectra were recorded on an Aminco Bowman spectrofluorimeter (Spectronics Co., USA). Mass spectrometric experiments were performed using the DART-TOF-MS (JEOL: JMS T100TD) technique.[1]

4-methyl-7-isothiocyanatecoumarin 2: To a solution of 7-amino-4-methylcoumarin (**1**, 245 mg, 1.4 mmol) in 14 ml CH_2Cl_2 was added triethylamine (390 μl, 2.8 mmol) and the solution was stirred at 35° C for 10.0 min. After cooling the system to room temperature, thiophosgene (320 μl, 4.2 mmol) was added dropwise and the mixture was stirred at 35° C for 20.0 min. The reaction was completed at the end of 20.0 min, as indicated by TLC (20% acetone in toluene). Excess of $CSCl_2$ was removed by distillation in high vacuum. The orange precipitate was dissolved in 10 ml CH_2Cl_2 filtered through silica gel for the removal of triethylamine hydrochloride, washed with 3 ml dichloromethane and dried under high vacuum to yield 301 mg (98%) of **2** as a light orange solid. ^1H NMR (500 MHz, $CDCl_3$): 7.54 (d, J=5.5, 1H), 7.23 (s, 1H), 7.11 (d, J=5,5 1H), 7.08 (s, 1H), 2.40 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): 160.32, 154.43, 151.98, 139.09, 134.87, 126.36, 122.43, 119.39, 115.65, 114.18, 19.27.

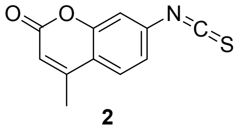
Cyclam-coumarin thiocarbamide-linked probe 3: To a solution of isothiocyanate **2** (132 mg, 0.608 mmol) in 5 ml CH_2Cl_2 was added 1-aza-15-crown-5 (133 mg, 0.608 mmol) and the solution was stirred at room temperature for 30.0 min. After the completion of the reaction, as indicated by TLC (20% acetone in toluene), 3 ml of MeOH were added to the flask and the volume of the solution was reduced (*in vacuo)* to 0.5 ml. Formation of a white precipitate was immediately observed. The precipitate was filtered, washed with cold methanol and dried in high vacuum to yield 263 mg (99%) of **3**. ^1H NMR (500 MHz, $CDCl_3$): 9.94 (s, 1H), 7.59 (dd, J_1=9.0 Hz, J_2=1.0 Hz, 1H), 7.48 (d, J=9.0 Hz, 1H), 7.32 (d, J=1.0 Hz, 1H), 6.16 (s, 1H), 4.10-3.80 (m, 8H), 3.74-3.65 (m, 8H), 3.62-3.58 (m, 4H); 2.38 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): 183.04, 161.64, 154.28, 152.88, 144.66, 124.51, 120.41, 116.24, 113.51, 110.84, 77.55, 70.65, 19.01.



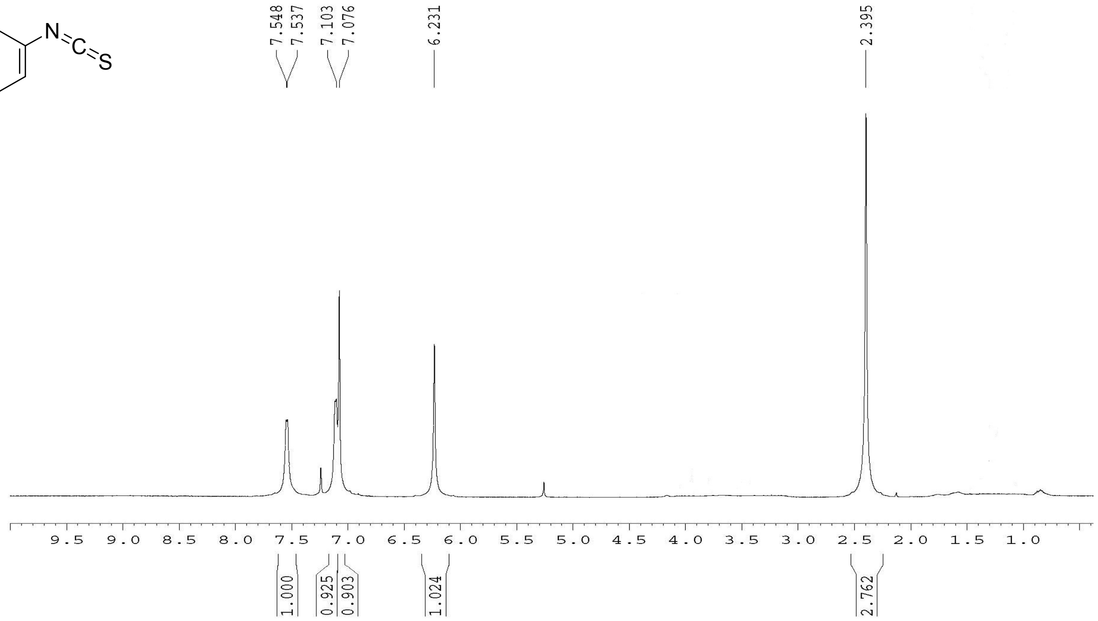
Scheme 1. Synthesis of indicator **3**.

II) Spectral Data for Compounds 2 and 3.

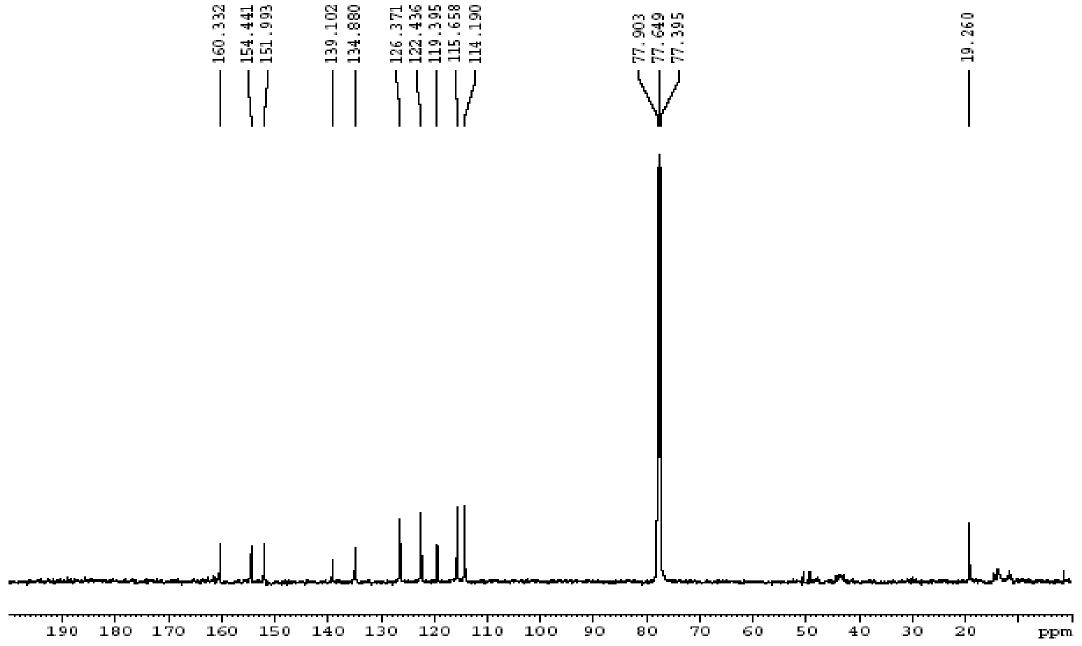
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound 2



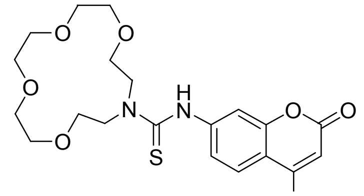
2

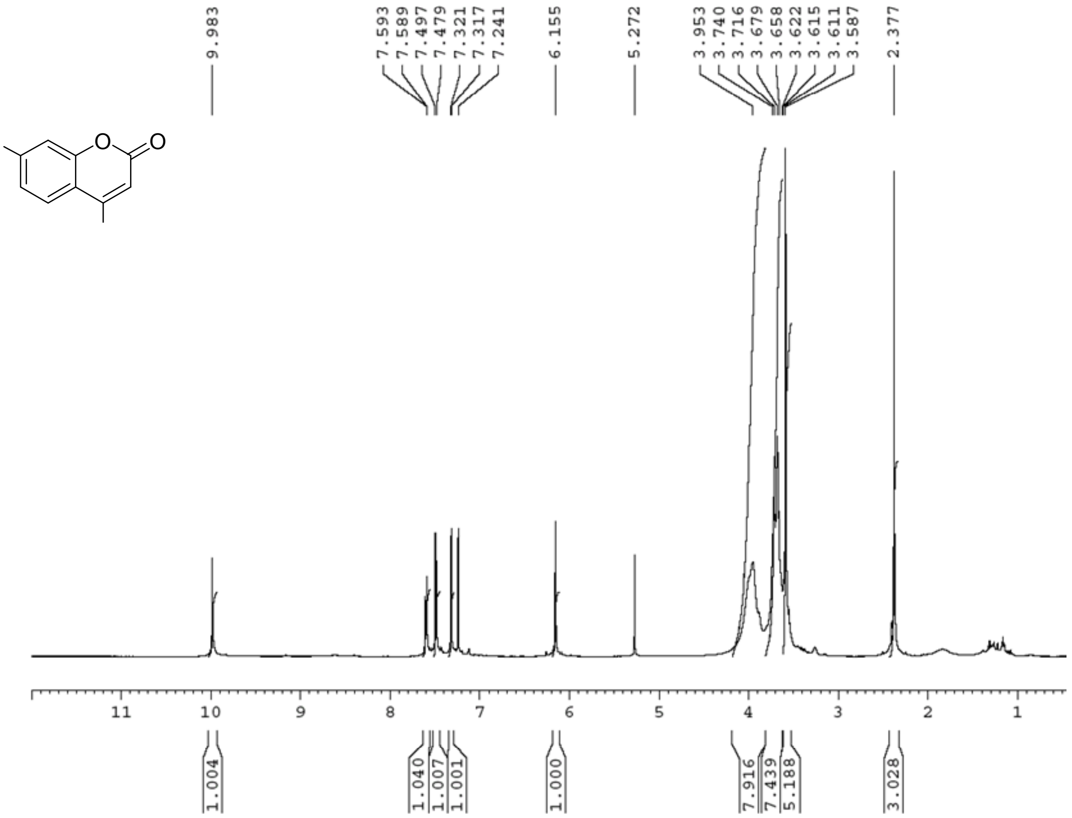


^{13}C NMR spectrum (500 MHz, $CDCl_3$) of compound 2



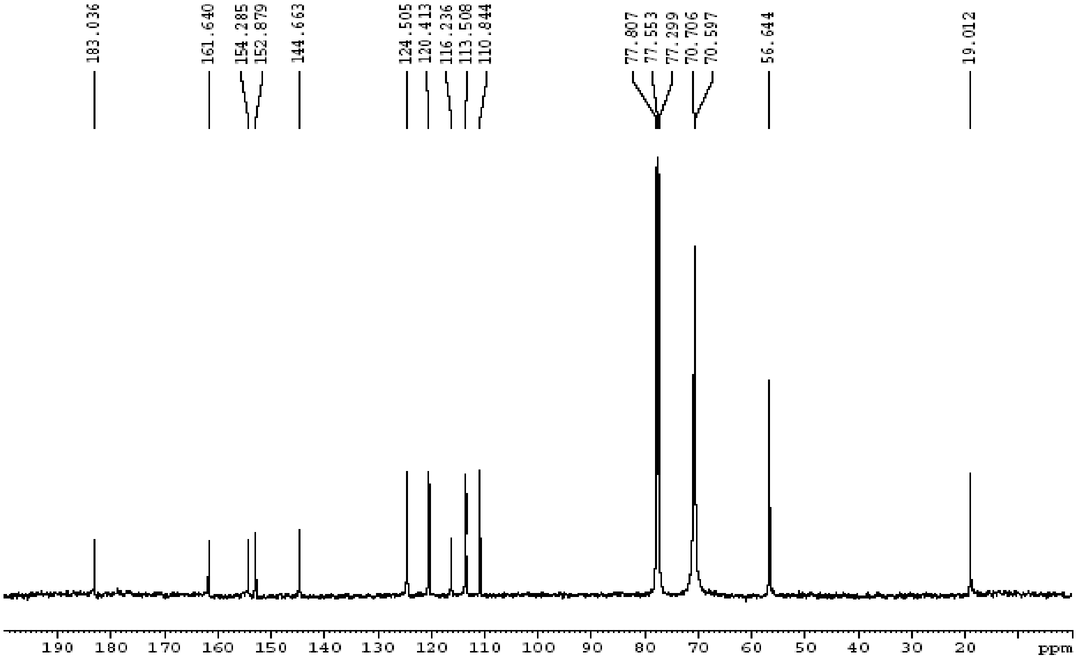
^{1}H NMR spectrum (500 MHz, CDCl$_3$) of compound 3



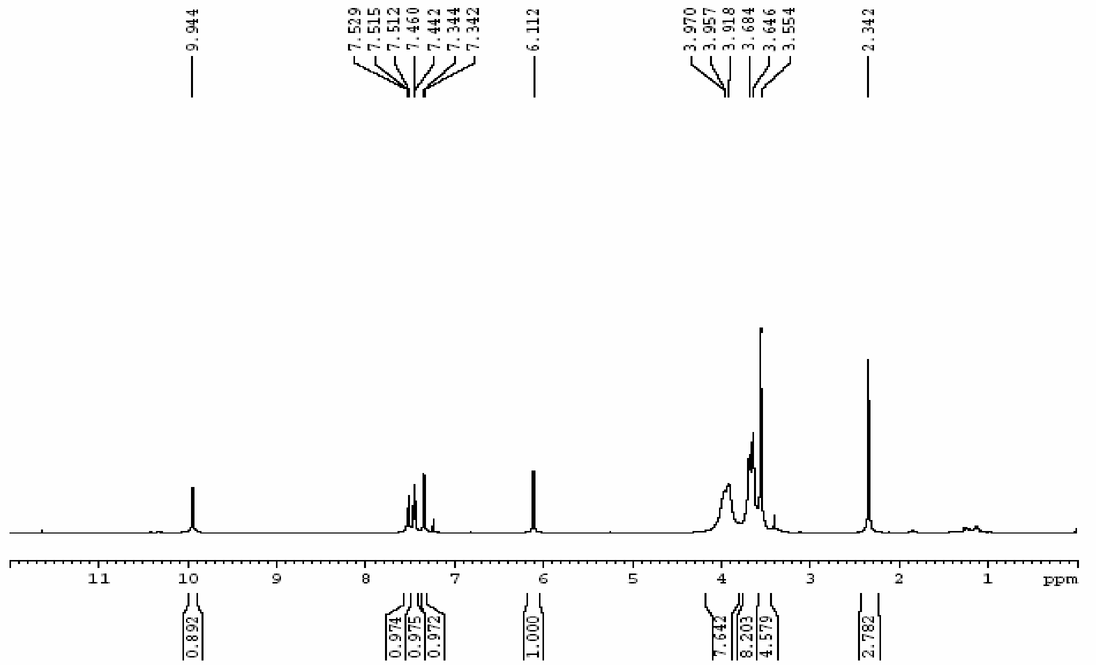


Peak at 5.27 ppm from trace of CH_2Cl_2

^{13}C NMR spectrum (500 MHz, CDCl$_3$) of compound 3



Clean H NMR of compound 3



III) Fluorescence Spectra of Compound 3

Preparation of indicators solutions

A stock indicator solution was prepared in DMSO (≥99.5%) at 10 mM concentration. An aliquot of this stock solution was then added to nanopure water, to make a final indicator concentration of 10 μM. Mercury-containing solutions were prepared by adding Hg^{2+} (0.01 M $HgCl_2$ solutions prepared in nanopure water) to the final indicator solution, to give solutions with Hg^{2+} concentrations ranging from 500 nM to 80 μM. The sensor does not respond to lower concentrations, and addition of higher concentrations than the maximum one (80 μM), resulted in fluorescence quenching.

The ion competition study was performed by adding an aliquot of the stock indicator solution in nanopure water to make a final indicator concentration of 10 μM. Stock solutions of 0.01 M in metal ions were prepared in nanopure water from the corresponding chlorides. Aliquots of these solutions were added to the indicator solution to make a final metal concentration of 60 μM, and fluorescence emission of each sample was measured.

Fluorescence studies of indicator 3 in the presence of increasing concentrations of Hg^{2+} ions

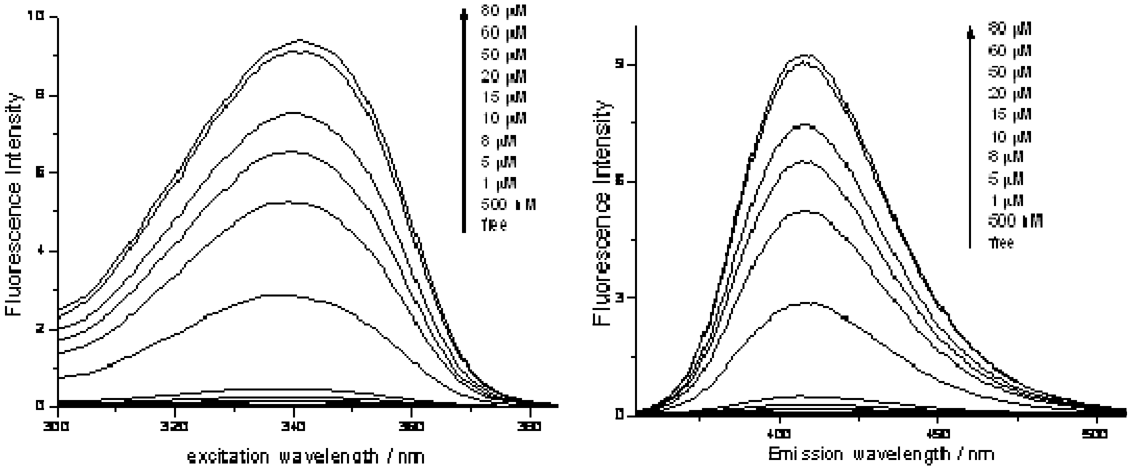


Fig. 1. Excitation and emission spectra of 10 μM of dye **3** in solutions of increasing Hg^{2+} concentrations. The excitation and emission wavelengths were set at 341 and 407 nm, respectively.

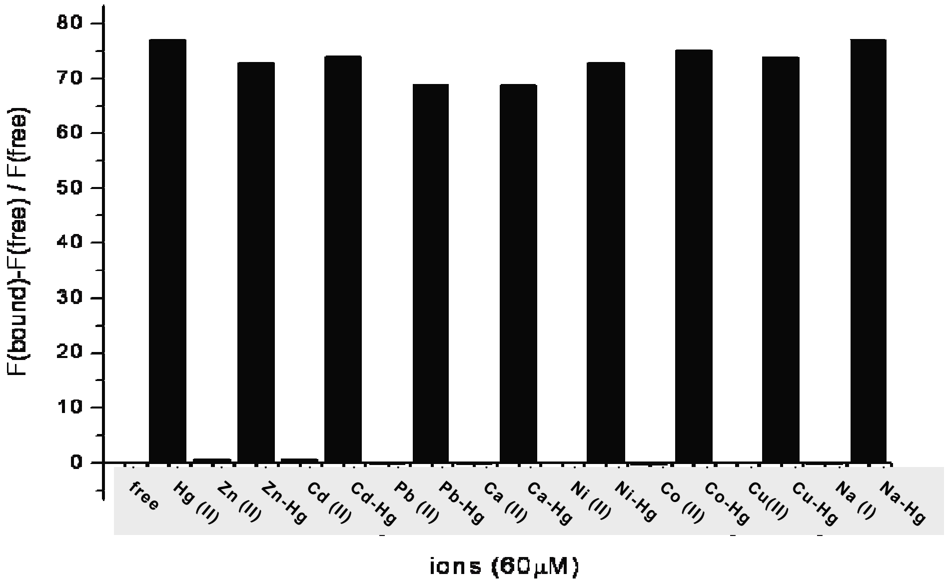


Fig. 2. Ion competition study for dye **3.** First measurement (free): ratio for free probe (zero). Second measurement (Hg(II)): increase of fluorescence ratio $[F_{(407)}-F^{o}_{(407)}]/F^{o}_{(407)}$ in the presence of 60 μM Hg^{2+}. Third measurement (Zn(II)): fluorescence ratio in the presence of 60 μM Zn^{2+}. Forth measurement (Zn-Hg): increase of fluorescence ratio in the presence of both 60 μM Zn^{2+} and 60 μM Hg^{2+}.. Likewise, the rest of the measurement sets indicate the fluorescence ratio of the dye in the presence of 60 μM of metal ions (Cd(II), Pb(II), etc.) vs. the ratio after addition of 1 equiv. of Hg^{2+} to the sample (Cd-Hg, Pb-Hg, etc).

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[1] Morlock, G. G.; Ueda, Y., *J. Chromatogr. A*, 1143, 2007, 243.