Supplementary Data

For

A BODIPY-based reactive probe for ratiometric fluorescence sensing of mercury Ions

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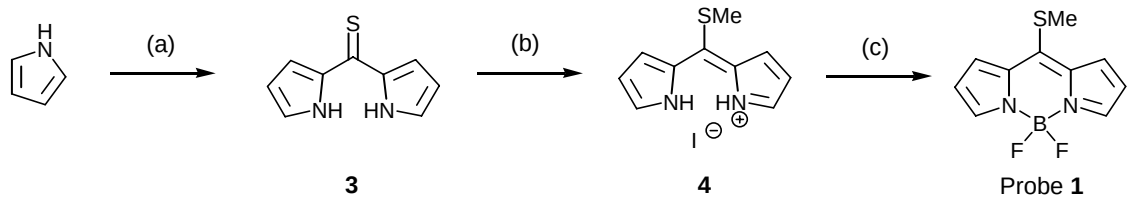
CONTENT

General Methods:

^{1}H and ^{13}C NMR spectra were measured with a Bruker DPX-300. UV absorption spectra were obtained using a HP8453 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Photon Technical International Fluorescence system. Quantum mechanical calculations at the DFT level were reported by Wavefunction, Inc. Spartan '08 (Linux version) program. Commercially available reagents were used without further purification. Anhydrous solvents for organic synthesis were prepared by solvent purification tower. Thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 glass plates. pH-dependent fluorescence experiments and detection limit experiments were recorded by fluorescence Victor multi-label counter with excitation wavelength at 355nm and detection wavelength at 460nm.

Synthesis of probe 1:

Reference: Goud, T, V.; Tutar, A.; Biellmann, J. *Tetrahedron*. **2006**, *62*, 5084-5091.



3 **4** Probe **1**

Scheme 1. (a) (i) Thiophosgene (0.48 equiv.), Toluene, Et_2O, 25 °C, 10 min. (ii) 10% MeOH in water, 25 °C, 30 min. (b) CH_3I (18 equiv.), DCM, 25 °C, 21 h. (c) Et_3N (5.3 equiv.), $BF_3{\cdot}Et_2O$ (4.9 equiv.), dichloromethane, 25 °C, 24 min.

Bis-(1*H*-pyrrol-2-yl)-methione (3): A solution of pyrrole (4.06 g, 4.2 mL, 59.4 mmol) in dry ether (90 mL) under argon atmosphere was added dropwise to a vigorously stirred solution of thiophosgene (3.45 g, 2.3 mL, 30.0 mmol) in dry toluene (80 mL) at 0 °C. After 10 min, aqueous methanol (10% methanol) (*ca.* 100 mL) was added and the mixture stirred for further 30 min at room temperature. The solvents were removed in vacuo and the residue was chromatographed on neutral alumina (column diameter = 4 cm, height of neutral alumina = *ca* 20 cm, eluent: hexane/dichloromethane = 1/3) The pure compound fraction was collected, which, after removal of the solvents in vacuo, yielded the thioketone **3** as a crystalline dark-red solid (3.86 g, 76%). ^{1}H NMR ($CDCl_3$, 300 MHz, 293K): δ 6.40 (2H, m), 7.04 (2H, m), 7.19 (2H, m), 9.76 (2H, s); ^{13}C NMR ($CDCl_3$, 75 MHz, 293K): δ 112.5, 114.8, 127.7, 138.4, 193.2.

2-[Methylsulfanyl-(1H-pyrrol-2-yl)-methylene]-2H-pyrrolium iodide (4) : To a solution of compound **3** (1.10 g, 6.24 mmol) in anhydrous dichloromethane (18 mL) under argon atmosphere was added methyl iodide (16.0 g, 7.0 ml, 112 mmol) at room temperature. The reaction mixture was stirred for 21 h (TLC monitoring). Solvent was removed under reduced pressure to obtain a brownish and gummy solid (2.04 g). The compound **4** was used for the next reaction without further purification.

8-(Thiomethyl)4,4-difluoro-4-bora-3a,4a-diaza-sindacene (Probe 1): To a solution of compound **4** (2.04 g) in anhydrous dichloromethane (45 mL) under argon atmosphere at room temperature was added triethylamine (3.34 g, 4.6 mL, 33.0 mmol). After being stirred for 30 min, $BF_3{\cdot}Et_2O$ (4.37 g, 3.8 mL, 30.8 mmol) was added to the mixture. The mixture was stirred for 24 h at room temperature. Solvent was evaporated under vacuum, and the residue was chromatographed on silica gel (column diameter = 3 cm,

height of silica gel = *ca* 20 cm, eluent: hexane/dichloromethane = 10/1 → 1/1 → 1/2) to yield probe **1** as a crystalline dark red solid (404 mg, 26% from two steps). ^{1}H NMR ($CDCl_3$, 300 MHz, 293K): δ 7.80 (2H, m), 7.26-7.42 (2H, m), 6.50-6.51 (2H, m), 2.91 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz, 293K): δ 154.0, 140.8, 133.4, 127.3, 117.6, 20.0.

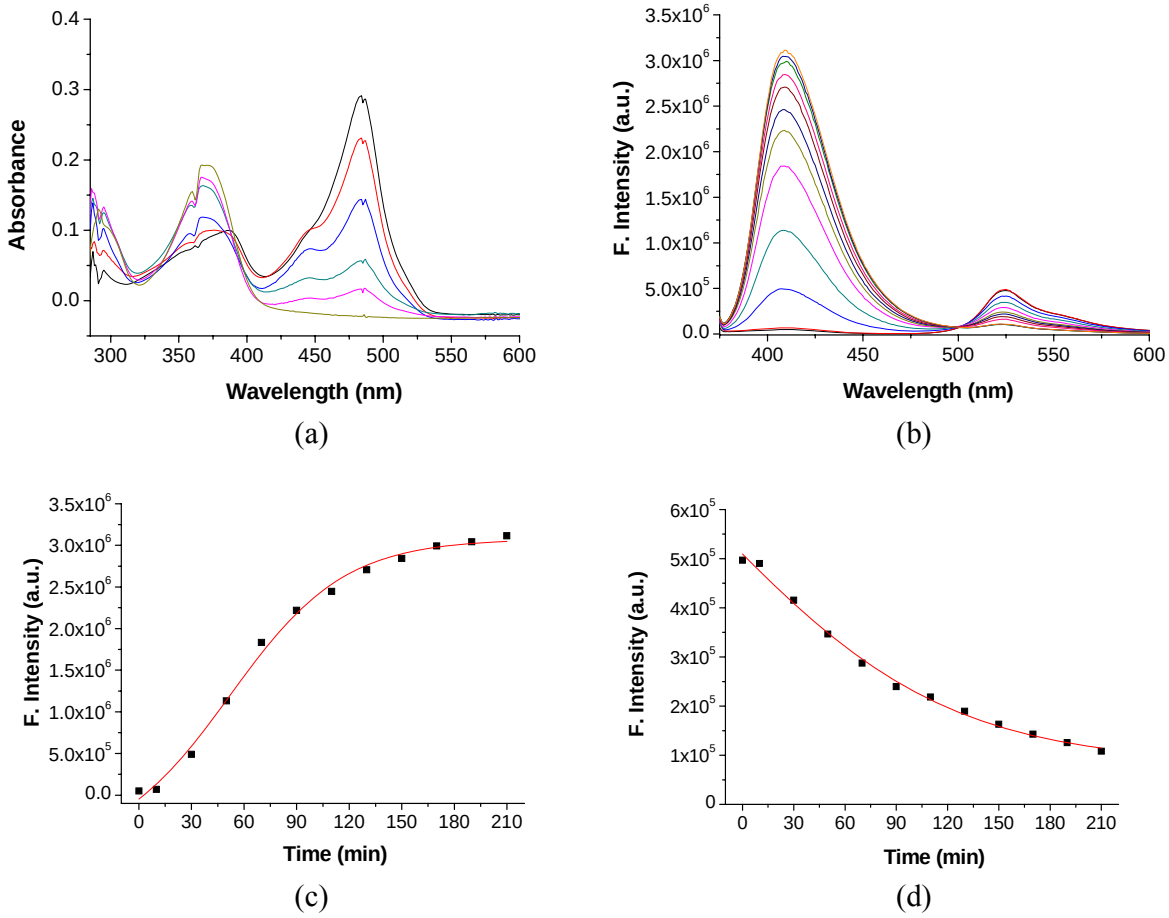
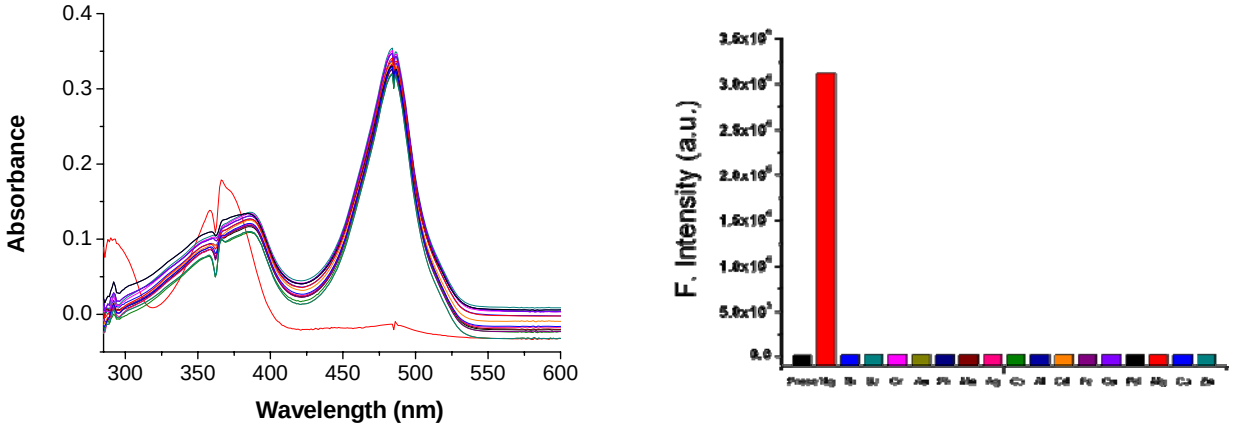
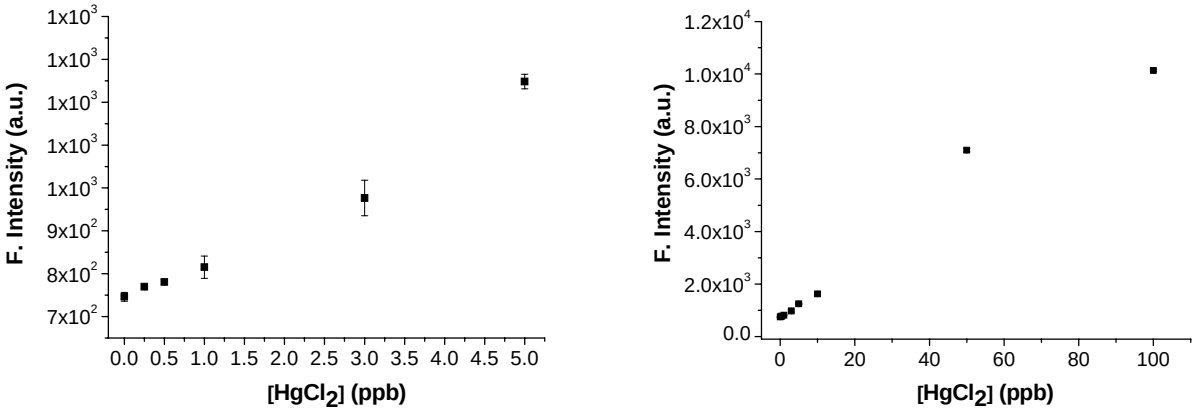


Figure S1. Time-dependent absorbance and fluorescence emission spectra of probe **1** (10uM) upon addition of $HgCl_2$ (1:1eq) in the 10% CH_3CN/HEPES buffer (10 mM, pH 7.4). (a) Absorbance spectra: each spectrum was recorded after 0, 30, 50, 70, 90, and 110 min. (b) Fluorescence emission spectra: each spectrum was recorded after 0, 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, and 210 min. Excitation wavelength = 370 nm. (c) A plot of fluorescence intensity that is estimated as the peak height at 409 nm. (d) A plot of fluorescence intensity that is estimated as the peak height at 525 nm.



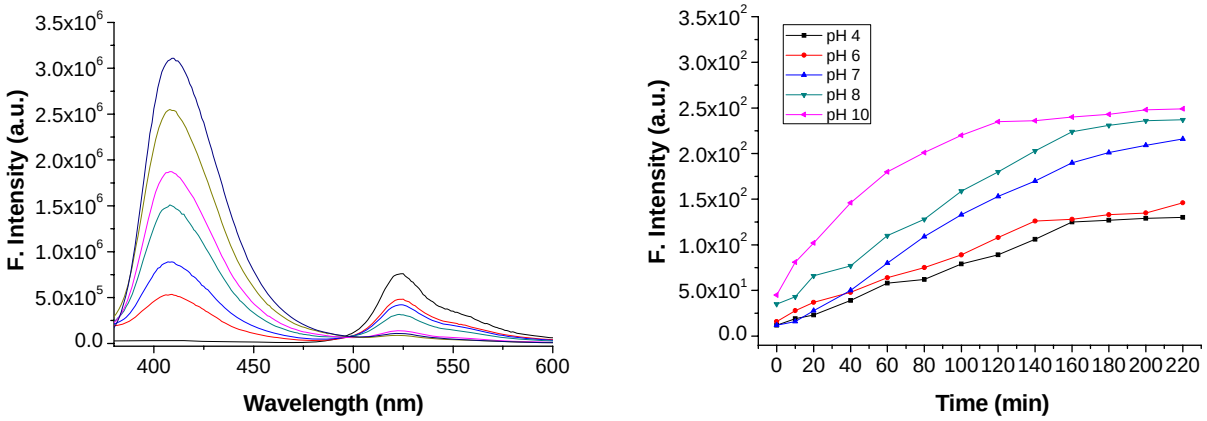
(a) (b)

Figure S2. Absorbance and fluorescence emission data for a 1:1 mixture of probe **1** (10 μM) and each of the various metal ions (Hg^{2+}, Ni^{2+}, Ba^{2+}, Cr^{2+}, Au^{3+}, Pb^{2+}, Mn^{2+}, Ag^{+}, Co^{2+}, Al^{3+}, Cd^{2+}, Fe^{3+}, Cu^{2+}, Pd^{2+}, Mg^{2+}, Ca+, and Zn^{2+}; as the chloride salts) in the 10% Ch_3CN/HEPES buffer (10 mM, pH 7.4), acquired after 180 min: (a) absorbance spectra; (b) fluorescence intensity at 409 nm (excitation wavelength = 370 nm).



(a) (b)

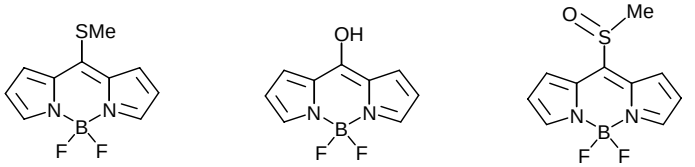
Figure S3. A plot of fluorescence intensity depending on the concentration of $HgCl_2$ in the range from 0.25 ppb to 100 ppb. Each measurement was done after 3 h of mixing for a probe **1** and $HgCl_2$ in the HEPES buffer (10 mM, pH 7.4) containing 10% CH_3CN. Fluorescence intensity was recorded by Victor multi-label counter with excitation wavelength at 355 nm and detection wavelength at 460 nm: (a) a region between 0 ppb to 5 ppb [$HgCl_2$]; (b) a region between 0 ppb to 100 ppb [$HgCl_2$].



(a) (b)

Figure S4. (a) Fluorescence spectra of probe **1** (10 μM) in the presence of different concentration of $HgCl_2$ (0, 0.5, 1, 3, 5, 10, 20 equiv.) in the 10% CH_3CN/HEPES buffer (10 mM, pH 7.4), acquired after 60 min (black line: 0 equiv.; indigo line: 20 equiv.). (b) Time-dependent fluorescence intensity for a 1:1 mixture of probe **1** (10 μM) and $HgCl_2$ at the various pHs (pH 4, 6, 7, 8, 10) by Victor multi-label counter with excitation wavelength at 355 nm and detection wavelength at 460 nm.

Table S1. Quantum mechanical calculation data for probe **1**, product **2** and sulfoxo-BODIPY **6**.



Probe **1** Product **2** Sulfoxo-BODIPY **6**

	1st Ex. Energy (au)	1st Ex. Energy (eV)	E LUMO (eV)	E HOMO (eV)	Δ E(eV)	λ nm
1	0.11637	3.1666	-2.9630	-5.9915	3.0284	391.27
2	0.13440	3.6571	-2.5483	-6.0319	3.4836	338.79
6	0.10885	2.9618	-3.1657	-6.1718	3.0062	418.33

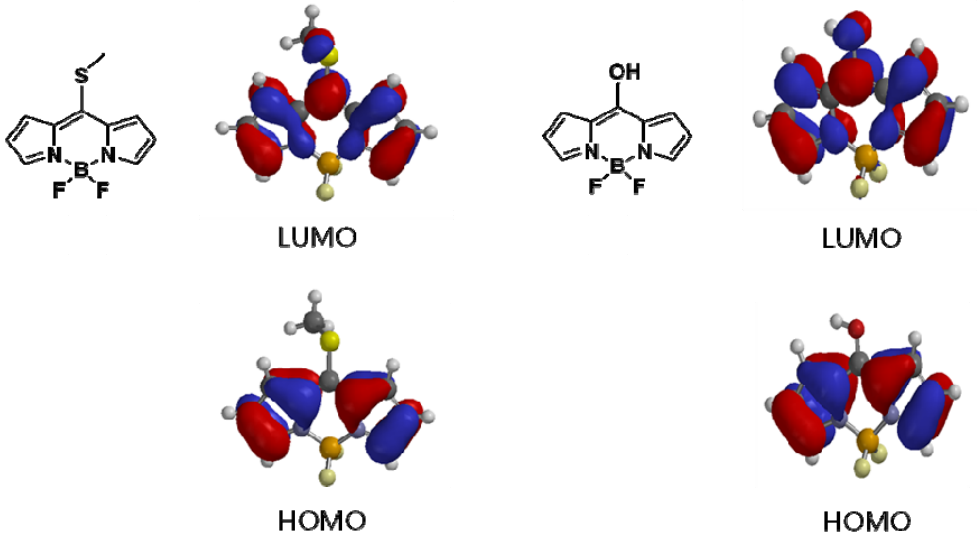


Figure S5. Electronic density of the HOMO and LUMO states of probe **1** and product **2** obtained by DFT quantum mechanical calculations.

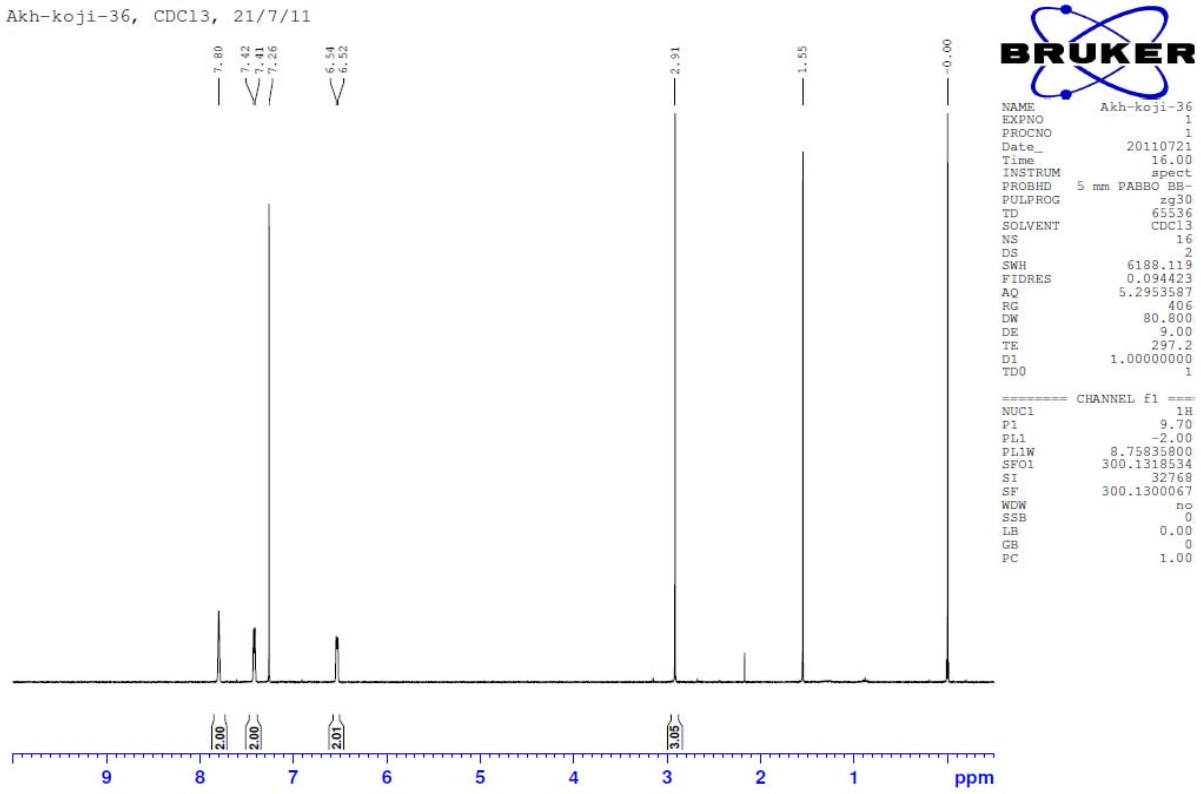
Table S2. Fluorescence titrations under different conditions.

	Solvent	Hg^{2+} (equiv.)	Result [c]
Condition 1	CH_3CN	0	No ratiometric behavior
Condition 2	HEPES buffer [a]	0	No ratiometric behavior
Condition 3	CH_3CN	1	No ratiometric behavior
Condition 4	HEPES buffer	1	Ratiometric behavior[b]
Condition 5	CH_3CN-HEPES buffer (1:1)	1	Ratiometric behavior

[a] HEPES buffer (pH 7.4, 10 mM). [b] Ratiometric result under UV radiation. [c] General conditions: 50 μM probe **1**, at 28 °C, for 6 h, under argon atmosphere.

^{1}H NMR data

8-(Thiomethyl)-4,4-difluoro-4-bora-3a,4a-diaza-sindacene (Probe 1).



8-(Hydroxy)-4,4-difluoro-4-bora-3a,4a-diaza-sindacene (product 2)