Supplementary Information

The emission enhancement of the NIR distyryl Bodipy dyes by the indrected $S_0 \rightarrow S_2$ excitation and its application towards Hg^{2+} probe †

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Table of contents

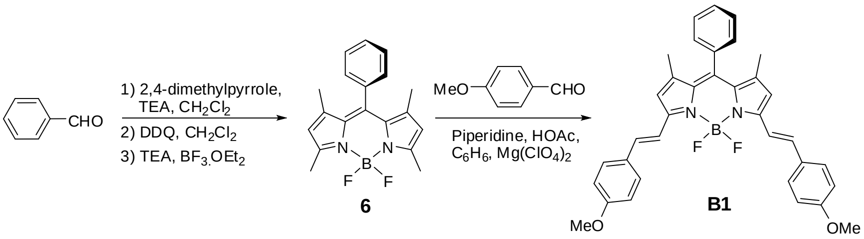
1. Materials and general methods

All reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods prior to use. Twice-distilled water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Qingdao Yuminyuan Chemicals GF254 silica gel coated plates. Flash chromatography (FC) was carried out using silica gel (200–300 mesh), obtained from the Qingdao Ocean Chemicals. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. Absorption spectra were taken on Agilent 8453 UV–vis spectroscopy system using a 1-cm quartz cell. Fluorescence spectra were taken on an Edinburgh Analytical Instruments (FL/FS 900).

2. Procedures of ions sensing: Deionized water was used throughout all experiments. Solutions of Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+} were prepared from their chloride salts; solutions of Cd^{2+}, Ag^+, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, and Hg^{2+} were prepared from their nitrate salts. A stock solution of **B2** (5 mM) was prepared in DMF–water (1:1, v/v). The stock solution of **B2** was then diluted to 5 μM with the CH_3CN–HEPES buffer solution (10 mM, pH = 7.4, 1:1, v/v). The Hg^{2+} stock solution of 10 mM was diluted to 1.0×10^{-3} M and 1.0×10^{-4} M with deionized water for spectra titration studies. In the titration experiments, a 2.0 mL solution of **B2** (5 μM) was poured into a quartz optical cell of 1 cm optical path length each time, and Hg^{2+} solution was added into the quartz optical cell gradually by using a micro-pipette.

3. Synthesis

*3.1 Synthesis of compound **B1***

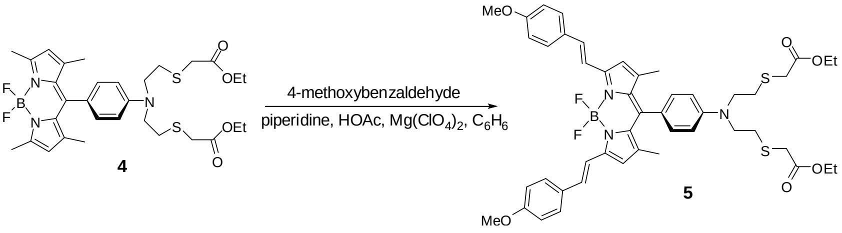


2,4-dimethylpyrrole (760 mg, 8.0 mmol) and benzaldehyde (0.42 g, 4.0 mmol) were dissolved in 400 ml absolute CH_2Cl_2 under N_2 atmosphere. One drop of TFA was added and the solution stirred at r.t. until TLC-control showed the complete consumption of benzaldehyde. At this point, a solution of tetrachlorobenzoquinone (TCBQ, 0.986g, 4.0 mmol) in 100 mL absolute CH_2Cl_2 was added, stirring was continued for 1 h followed by the addition of 6 mL of Et_3N and 6 mL of $BF_3{\cdot}OEt_2$ respectively. After stirring for 1h the reaction mixture was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel (CH_2Cl_2: petroleum ether = 4:1) to afford 0.73 g pure **6**. Yield: 56 %.

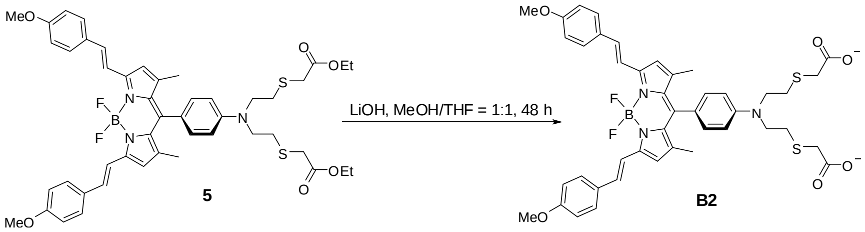
Compound **6** (0.32g, 1.0 mmol) and 4-Methoxybenzaldehyde (0.47 g, 3.5 mmol) were refluxed in a mixture of toluene (50 mL), glacial acetic acid (0.5 mL), piperidine (0.6 mL) and small amount of $Mg(ClO_4)_2$. Any water formed during the reaction was removed azeotropically by heating overnight in a Dean-Stark apparatus. Crude product concentrated under vacuum, then purified by silica gel column chromatography (CH_2Cl_2). The green colored fraction was collected and the solvent was removed under reduced pressure to yield the desired product **B1** (0.45 g, 80 %).

^{1}H NMR(300 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.54-7.57 (m, 5H), 7.45 (s, 3H), 7.15-7.29 (m, 5H), 6.90 (d, *J* = 8.4 Hz, 4H), 6.58 (s, 2H), 3.82 (s, 6H), 1.40 (s, 6H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 161.5, 153.8, 142.9, 139.2, 136.8, 136.4, 134.3, 130.7, 130.1, 130.0, 129.6, 118.6, 118.3, 115.4, 56.5, 15.7; HRMS calcd for $(M+Na)^+$ 583.2344, found 583.2344.

3.2 Synthesis of compound ***B2***



Compound 5: Compound **4** was synthesized according to the reported procedure.[S1] Compound **4** (0.63 g, 1.0 mmol) and 4-Methoxybenzaldehyde (0.47 g, 3.5 mmol) were refluxed in a mixture of toluene (50 mL), glacial acetic acid (0.5 mL), piperidine (0.6 mL) and small amount of $Mg(ClO_4)_2$. Water formed during the reaction was removed azeotropically by heating overnight in a Dean-Stark apparatus. Crude product concentrated under vacuum, then purified by silica gel column chromatography (CH_2Cl_2). The purple colored fraction was collected and the solvent was removed under reduced pressure to yield the desired product **5** (0.65 g, 75 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57-7.62 (m, 6H), 7.11-7.25 (m, 4H), 6.92 (d, *J* = 8.4 Hz, 4H), 6.85 (b, 2H), 6.60 (b, 2H), 4.20 (q, *J* = 7.2 Hz, 4H), 3.85 (s, 6H), 3.65 (b, 4H), 3.28 (s, 4H), 2.89 (b, 4H), 1.52 (s, 6H), 1.29 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4, 161.3, 153.4, 147.4, 142.8, 139.9, 136.5, 134.8, 134.2, 130.9, 130.7, 130.1, 118.4, 115.3, 113.8, 62.7, 56.5, 52.3, 34.8, 30.8, 30.4, 16.1, 15.3.



Compound B2: A solution of compound **5** (86 mg, 0.1 mmol) in a 1:1 THF/MeOH mixture (5 mL) under a nitrogen atmosphere was cooled to 0 ^{o}C and treated with 10 equiv. of lithium hydroxide. The resulting mixture was stirred for 2 days under nitrogen at which TLC analysis showed complete consumption of starting material. The solution was evaporated to dryness and the residue was directly used to prepare stock solutions in DMF–water (1:1, v/v) for further spectroscopic measurements. HRMS calcd for $(M)^{2-}$ 404.6294, found 404.6296.

4. Supplemental spectra data

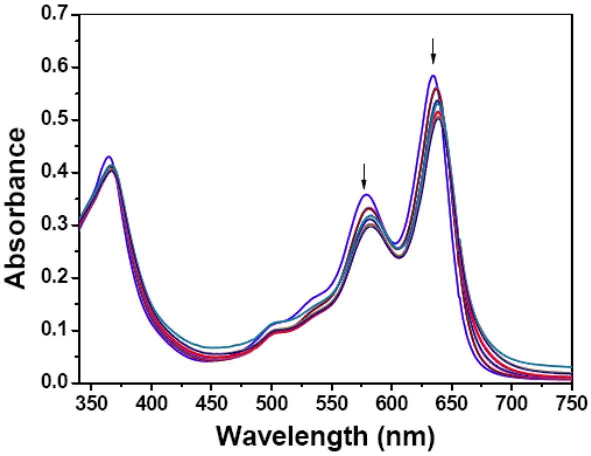


Fig. S1 Changes in absorption spectra of **B2** (5 μM) in CH_3CN–HEPES buffer solution (10 mM, pH = 7.4, 1:1, v/v) in the absence and presence of various amounts of Hg^{2+} ions.

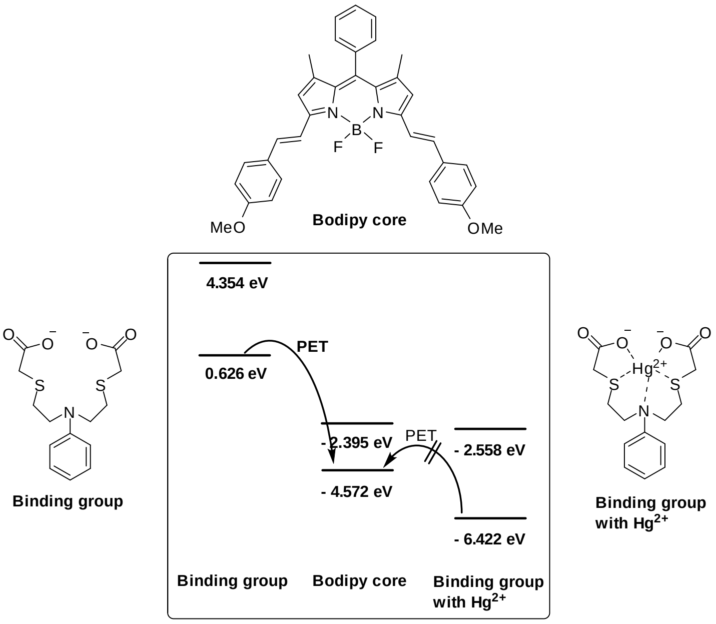


Fig. S2 Energy diagrams (in eV) of Bodipy core and binding group without and with Hg^{2+}, calculated at the B3LYP/6-31G(d) level of the Guassian 03 program.

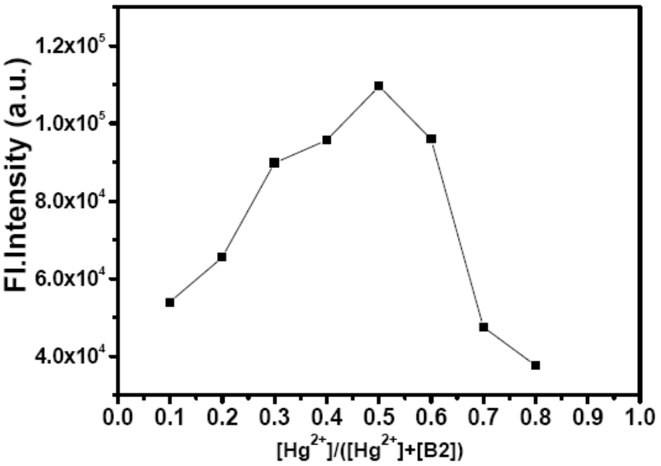


Fig. S3 Job's plots of the complexation between **B2** and Hg^{2+}. Total concentration of **B2** + Hg^{2+} was kept constant at 5 μM. Slit: 2 nm/2 nm.

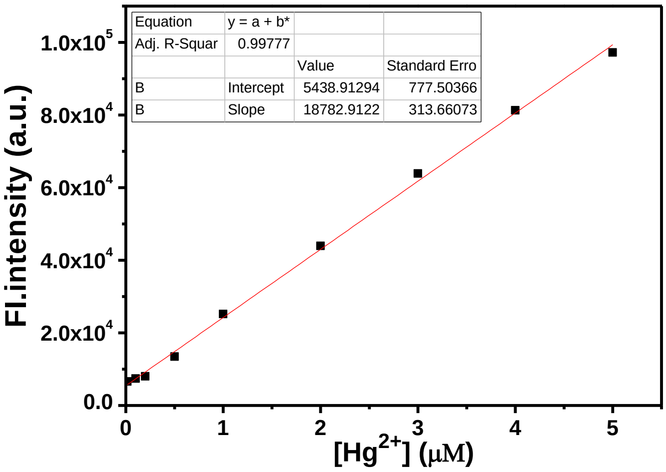


Fig. S4 Fluorescence response of **B2** (5 μM) to Hg^{2+}. Condition: CH_3CN–HEPES buffer solution (10 mM, pH = 7.4, 1:1, v/v); $\lambda_{ex}/\lambda_{em}$ = 370 /655 nm; Slit: 1 nm/1 nm.

5. Theoretical methods

All the calculations on **B1** were carried out with the Gaussian 03 program package[S2] by using density functional theory (DFT) and time-dependent DFT (TD-DFT): Becke's three-parameter functional[S3] combined with Lee, Yang, and Parr's correlation functional[S4] (B3LYP), along with the 6-31G(d) basis set, were used.

The optimized ground-state geometry of **B1** was showed as follows:

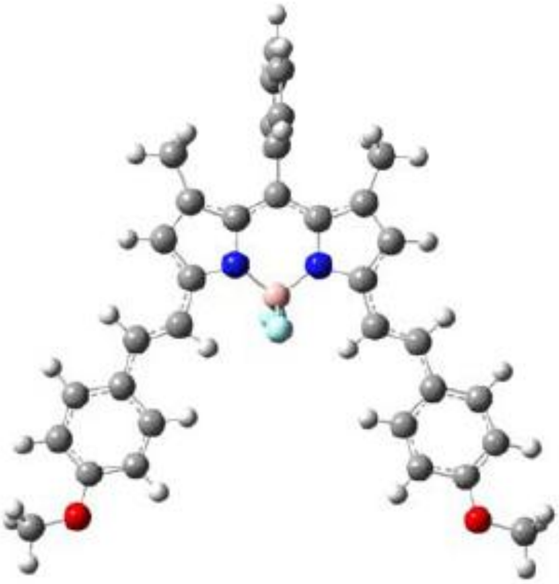


Table S1 The ground state geometry optimization of **B1**.

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	7.852233	-0.000057	-0.005918
2	6	0	7.153971	-0.000211	-1.214651
3	6	0	5.758309	-0.000221	-1.218114
4	6	0	5.044516	-0.000050	-0.011943
5	6	0	5.752877	0.000114	1.197507
6	6	0	7.148782	0.000100	1.199807
7	6	0	3.548894	-0.000037	-0.010824
8	6	0	2.856353	1.223377	-0.017944
9	6	0	2.856315	-1.223437	-0.018082
10	6	0	3.323923	-2.583994	-0.077420
11	6	0	2.193399	-3.378454	-0.100438
12	6	0	1.046383	-2.541757	-0.051410
13	7	0	1.462734	-1.247955	-0.005896
14	7	0	1.462769	1.247911	-0.005874
15	6	0	1.046430	2.541713	-0.051367
16	6	0	2.193459	3.378403	-0.100361
17	6	0	3.323970	2.583931	-0.077357
18	6	0	-0.346461	-2.909241	-0.061649
19	6	0	4.729109	-3.108019	-0.127180
20	6	0	-0.346406	2.909229	-0.061740
21	6	0	4.729168	3.107921	-0.127205
22	5	0	0.564556	-0.000001	0.261441
23	9	0	0.124482	0.000025	1.577524
24	9	0	-0.525878	-0.000021	-0.617228
25	6	0	-0.791417	-4.187837	0.005790
26	6	0	-2.178132	-4.632411	-0.011568
27	6	0	-2.459553	-6.007633	0.052209
28	6	0	-3.763353	-6.500575	0.038050
29	6	0	-4.835420	-5.604629	-0.041183
30	6	0	-4.579453	-4.223305	-0.104115
31	6	0	-3.280474	-3.750027	-0.089385
32	8	0	-6.149780	-5.962902	-0.062831
33	6	0	-6.472006	-7.344094	-0.000514
34	6	0	-0.791344	4.187814	0.005977
35	6	0	-2.178051	4.632420	-0.011524
36	6	0	-3.280396	3.750109	-0.090067
37	6	0	-4.579357	4.223435	-0.104936

38	6	0	-4.835302	5.604736	-0.041420
39	6	0	-3.763227	6.500612	0.038546
40	6	0	-2.459450	6.007622	0.052830
41	8	0	-6.149631	5.963075	-0.063261
42	6	0	-6.471782	7.344263	-0.000576
43	1	0	8.938184	-0.000058	-0.003603
44	1	0	7.695017	-0.000334	-2.156462
45	1	0	5.214655	-0.000333	-2.158420
46	1	0	5.204627	0.000236	2.135006
47	1	0	7.685640	0.000214	2.143988
48	1	0	2.181902	-4.457595	-0.165415
49	1	0	2.181968	4.457543	-0.165335
50	1	0	-1.046360	-2.086452	-0.139980
51	1	0	4.713425	-4.201330	-0.137124
52	1	0	5.321109	-2.785023	0.734076
53	1	0	5.264776	-2.769755	-1.019289
54	1	0	-1.046311	2.086472	-0.140367
55	1	0	4.713498	4.201229	-0.137535
56	1	0	5.264895	2.769301	-1.019141
57	1	0	5.321085	2.785250	0.734227
58	1	0	-0.058925	-4.989762	0.084530
59	1	0	-1.633957	-6.712205	0.114124
60	1	0	-3.929754	-7.569826	0.088772
61	1	0	-5.424185	-3.544965	-0.162900
62	1	0	-3.113410	-2.679035	-0.136233
63	1	0	-7.560888	-7.400595	-0.030173
64	1	0	-6.111567	-7.801545	0.929521
65	1	0	-6.058202	-7.894481	-0.854940
66	1	0	-0.058856	4.989704	0.085103
67	1	0	-3.113364	2.679132	-0.137379
68	1	0	-5.424092	3.545146	-0.164287
69	1	0	-3.929623	7.569842	0.089758
70	1	0	-1.633847	6.712136	0.115318
71	1	0	-7.560652	7.400851	-0.030506
72	1	0	-6.057695	7.894890	-0.854714
73	1	0	-6.111569	7.801394	0.929704

Number of Imaginary Frequency:0

SCF Done: E(RB+HF-LYP) =   -1837.09627962   A.U. after   6 cycles
             Convg   =   0.6887D-08   S**2   =   0.0000

Table S2 TD-DFT Calculation for the Oscillator Strengths.

TD-DFT Singlet Transition Energies (Eex) and Oscillator Strengths (f) for BDY. (HOMO = 147).

States	E_{ex} (nm)	Energy Level (eV)	*f*	Transition Weights Configuration coefficient	
1	613.7	2.02	1.1196	147 ->148	0.61300
2	445.5	2.78	0.3292	146 ->148 147 ->149	0.63866 0.27135
3	378.1	3.28	0.0017	144 ->148 145 ->148	0.53967 0.41204
4	368.3	3.37	0.0365	144 ->148 145 ->148 147 ->150	-0.40645 0.48913 -0.27690
5	360.6	3.44	1.5364	147 ->149 146 ->148	0.60897 -0.19182
6	354.6	3.50	0.0000	143 ->148	0.70204
7	345.4	3.59	0.0635	142 ->148	0.68150
8	336.3	3.69	0.0018	141 ->148 147 ->150	0.68354 -0.14296
9	325.5	3.81	0.0033	139 ->148 141 ->148 145 ->148 146 ->149 147 ->150	0.12572 0.15366 0.18622 0.20701 0.57023
10	316.8	3.91	0.0172	140 ->148 147 ->152 147 ->154	0.66850 -0.13475 0.11888
11	316.2	3.92	0.0035	139 ->148 147 ->150 147 ->153	0.65845 -0.10416 -0.17036
12	305.4	4.06	0.0000	147 ->151	0.70244
13	295.7	4.19	0.0167	138 ->148 147 ->152 147 ->154	-0.14272 0.58991 0.34153
14	292.6	4.24	0.0000	139 ->148 146 ->149 147 ->153	0.17667 0.39255 0.51664
15	291.4	4.25	0.0185	138 ->148 140 ->148 147 ->152 147 ->154	0.24320 -0.17464 -0.22702 0.56224
16	288.3	4.30	0.2348	138 ->148 146 ->150 147 ->152 147 ->154	0.58287 0.10893 0.24925 -0.17318
17	287.5	4.31	0.1093	146 ->149 147 ->150	0.49213 -0.10922

				147 ->153	-0.40556
18	266.1	4.66	0.0627	145 ->149	0.23499
				146 ->150	0.64079
19	257.8	4.81	0.0368	144 ->149	0.10007
				145 ->149	0.60844
				146 ->150	-0.14998
				147 ->155	0.19751
20	255.2	4.86	0.0060	137 ->148	0.66088
21	251.4	4.93	0.0052	144 ->149	0.66736
				147 ->155	-0.12375
22	247.1	5.02	0.0097	139 ->150	-0.16584
				140 ->149	0.31232
				145 ->153	0.15908
				146 ->152	0.42722
				146 ->154	-0.34665
				147 ->153	-0.12859
23	246.6	5.03	0.0017	139 ->149	0.32235
				140 ->150	-0.16859
				145 ->152	0.11921
				145 ->154	-0.10830
				146 ->153	0.54803
24	244.4	5.07	0.0000	146 ->151	0.70017
25	241.5	5.13	0.0574	142 ->149	0.65027
				144 ->150	0.17167
				146 ->154	-0.10369
26	238.6	5.20	0.0003	141 ->151	-0.12828
				143 ->149	0.68106
27	238.1	5.21	0.0160	145 ->150	0.36403
				146 ->152	0.41818
				146 ->154	0.40290
28	237.8	5.21	0.0209	135 ->148	0.13916
				142 ->149	-0.12854
				145 ->150	0.51194
				146 ->152	-0.20889
				146 ->154	-0.31638
				147 ->156	0.16812
29	236.7	5.24	0.0008	133 ->148	-0.12237
				135 ->148	0.66736
				145 ->150	-0.14343
30	231.7	5.35	0.0053	141 ->149	0.68606

6. References

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7. Cell culture and fluorescence imaging: The HeLa cell line was provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in RMPI 1640 medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5×106 cells per well and allowed to adhere for 12 hours. Fluorescence imaging was performed with by a Olympus FV1000 Laser Scanning Confocal Microscope (Japan). Before the experiments, cells were washed with PBS and then incubated with **B2** (10 μM) in PBS for 30 min at 37 °C. Experiments to assess Hg^{2+} uptake were performed in the same media supplemented with 20 μM $Hg(NO_3)_2$ for 30 min at 37 °C. Cell imaging was then carried out after washing cells with physiological saline.

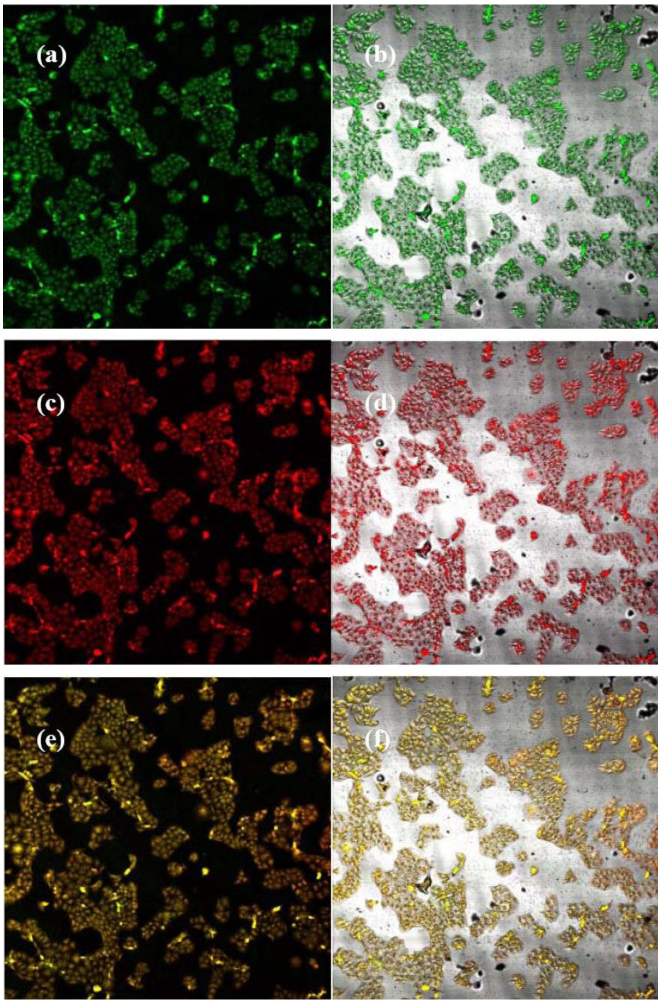

Fig. S5 (a–f) Dual-color cellular imaging photos. The HeLa cells are distinctively labeled by (a) H33258 dye (green) and (c) **B1**+Hg (red). (e) Superposition of the fluorescence images of (a) and (c). (b, d, f) The corresponding brightfield images.

8. NMR and MS spectra

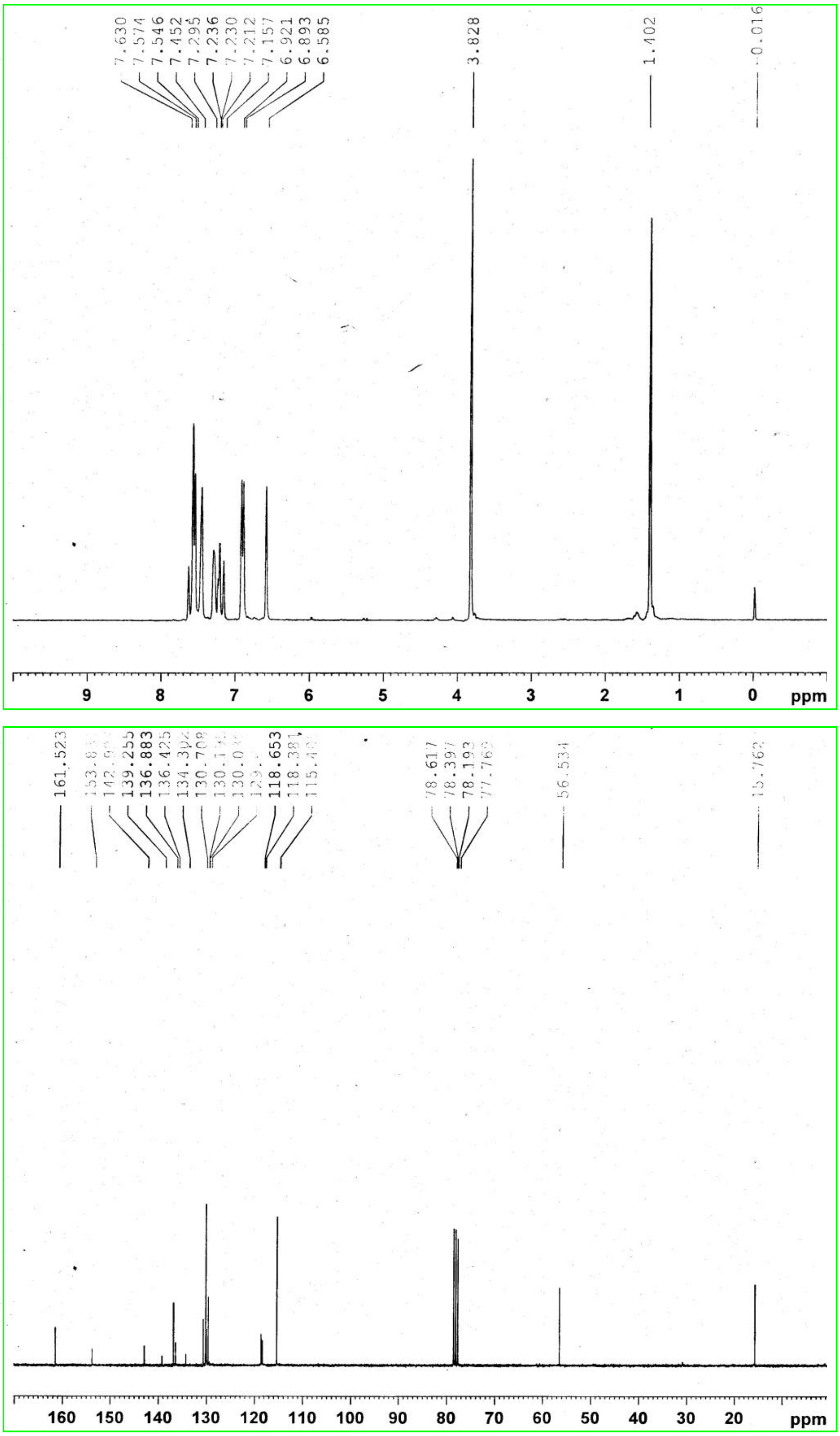

Fig. S6 ^{1}H NMR and ^{13}C NMR chart of **B1** ($CDCl_3$, 300MHz).

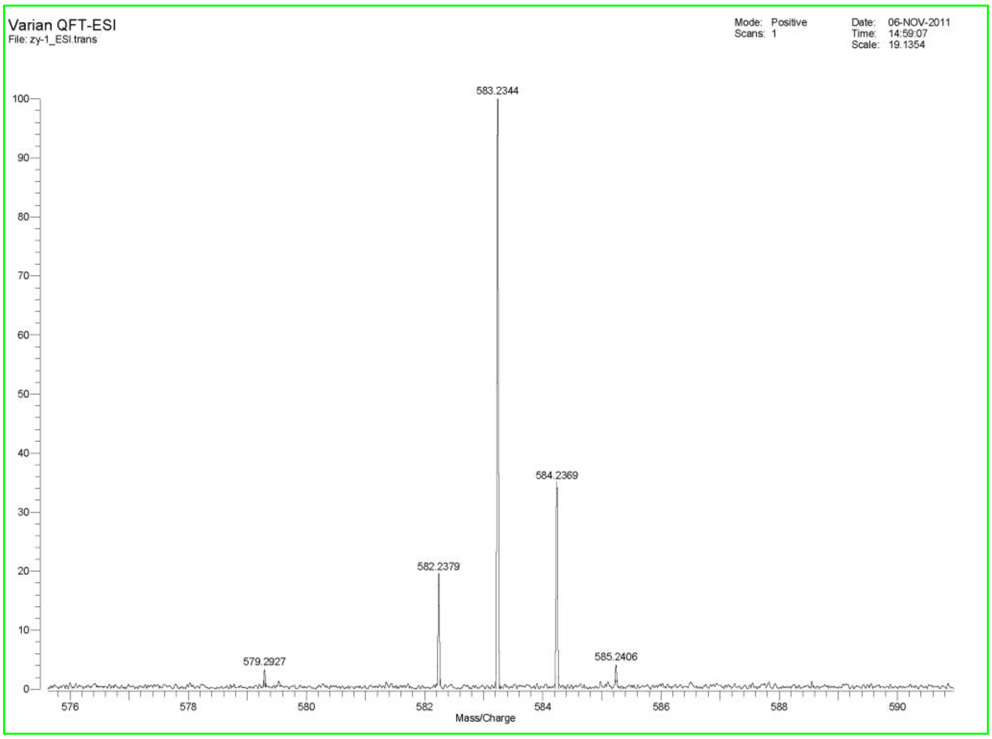

Fig. S6 HRMS chart of **B1**.

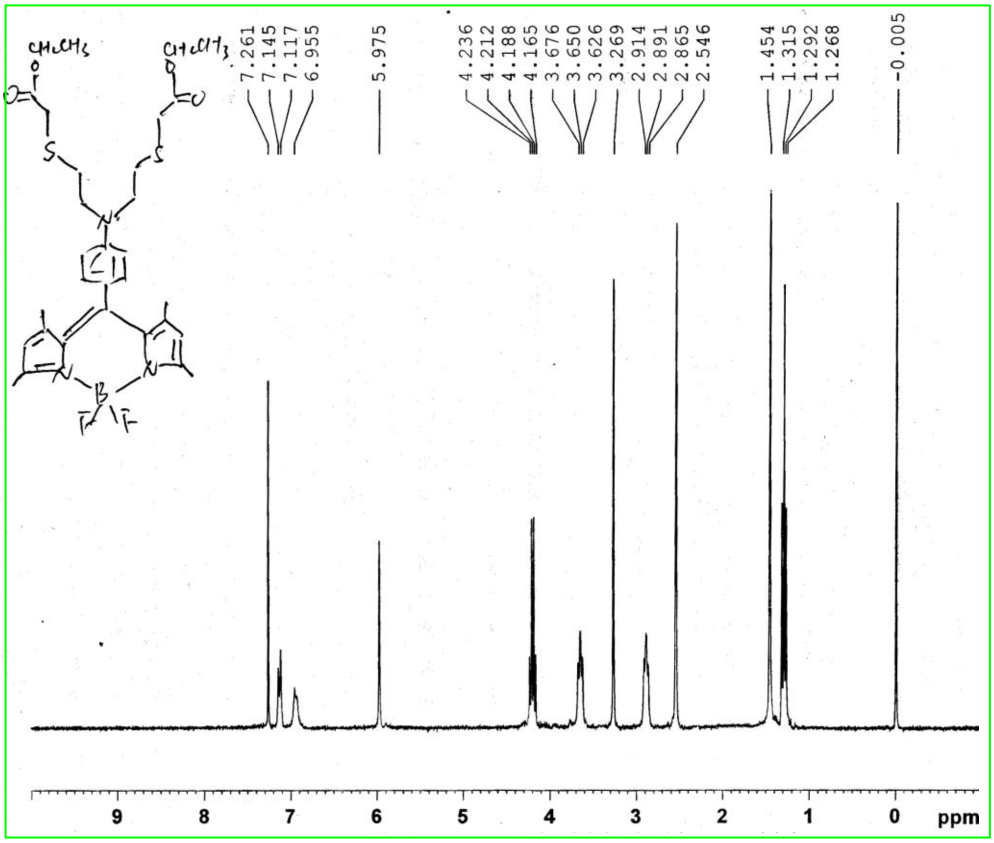

Fig. S7 ^{1}H NMR chart of **4** ($CDCl_3$, 300MHz).

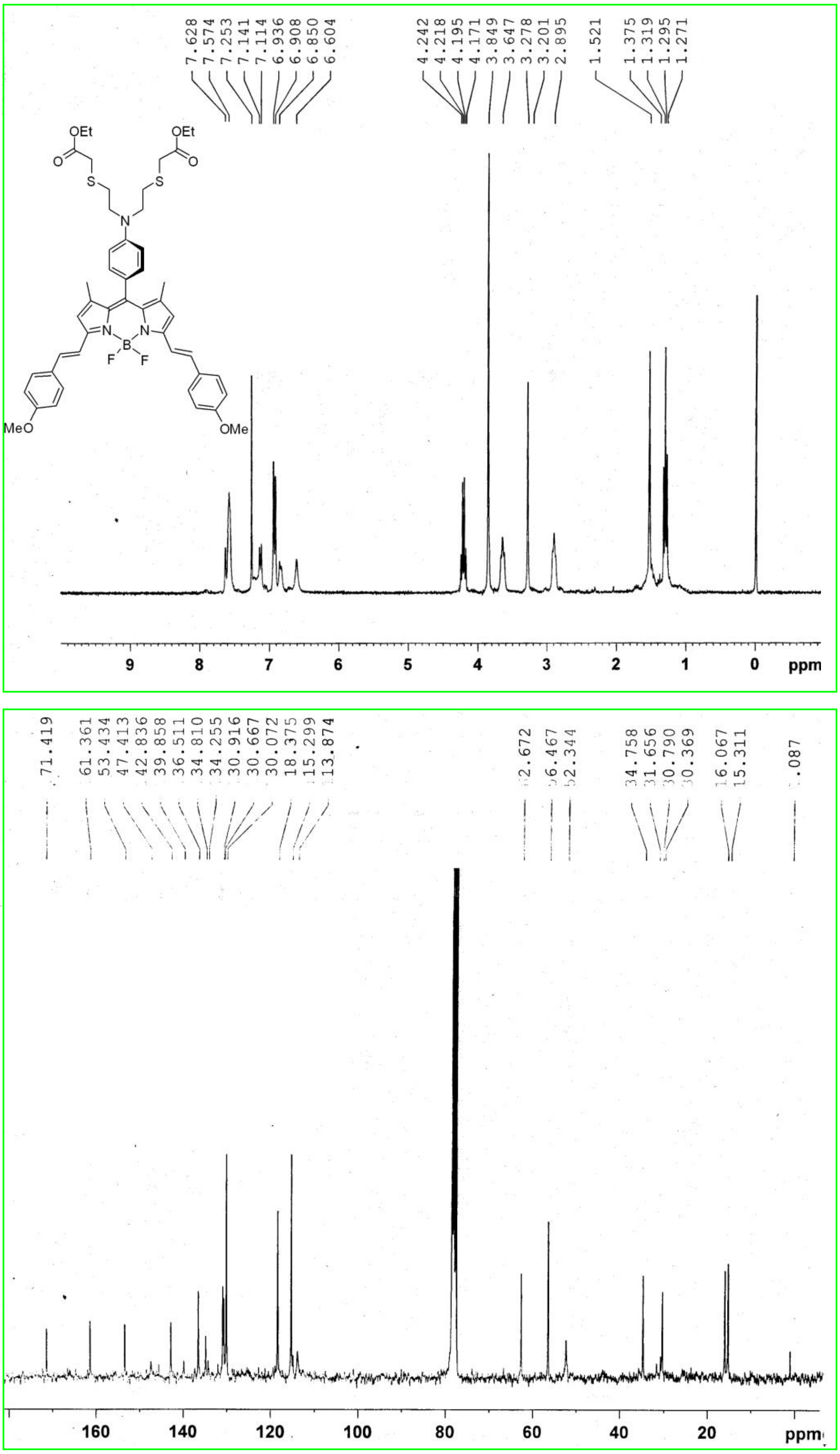

Fig. S8 ^{1}H NMR and ^{13}C NMR chart of **5** (CDCl$_3$, 300MHz).

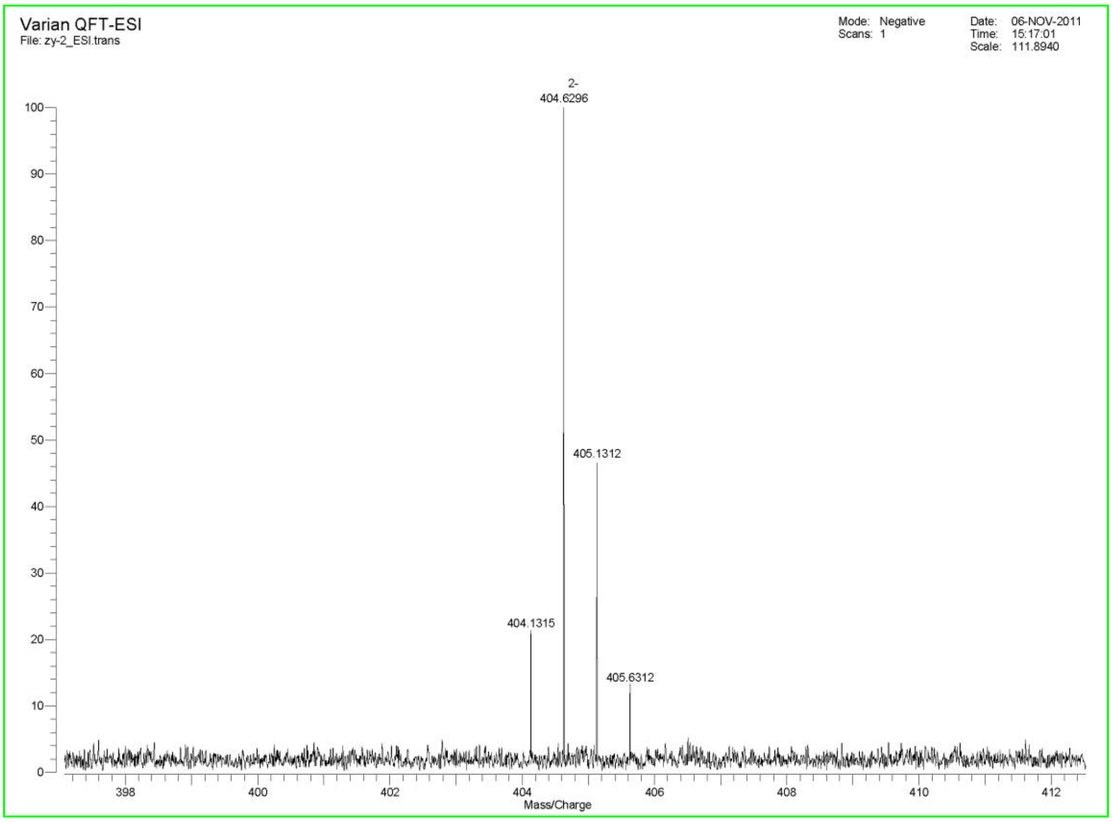

Fig. S9 HRMS chart of **B2**.