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Enhancing the Stokes' shift of BODIPY dyes via through bond energy transfer and its application for Fe^{3+}-detection in live cell imaging

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I. Experimental details

I.1 Materials and methods

Unless otherwise noted, all reagents or solvents were obtained from commercial suppliers and used without further purification. All air and moisture sensitive reactions were carried out under an argon atmosphere. Dry CH_2Cl_2 was obtained by refluxing and distilling over CaH_2 under nitrogen. Dry THF was distilled from sodium/benzophenone. Triethylamine was obtained by simple distillation. The ^1H NMR and ^{13}C NMR spectra were measured on Bruker 500 MHz or Bruker 300 MHz spectrometer. The measurements for ^1H NMR and ^{13}C NMR were performed at 300 MHz (DPX-300) or 500 MHz (DRX-500), and 75 MHz (DPX-300) or 125 MHz (DRX-500), respectively. Mass spectra were measured on a Bruker Daltonics Autoflex II^{TM} MALDI TOF MS spectrometer. Fluorescence spectral measurements were carried out by using an Hitachi F-4600 fluorescence spectrophotometer. Electronic absorption spectra were recorded with Shimadzu UV-2550 spectrophotometers. Fluorescence lifetimes were determined by Edinburgh instrument laser impulse fluorometer with picosecond time resolution. Elemental analyses for C, H, and N were performed on a Vario MICRO elemental analyzer.

I.2 Quantum Yields determination

Fluorescence and absorption measurements were performed on Hitachi F-4600 fluorescence spectrophotometer and Shimadzu UV-2550 spectrophotometer. The slit width was 2.5 nm for excitation and 5.0 nm for emission. **S1**Determined the BODIPY segment of **1** fluorescent, Zinc phthalocyanine in 1% pyridine in toluene ($\Phi_f = 0.30$) were used as the standard for the fluorescent quantum yield calculation according to the absorption of the test sample, and the emission spectra area of the measured compound. **S1**Determined the bodipy segment of **2** fluorescent, rhodamine 6G in EtOH ($\Phi_f = 0.88$) were used as the standard for the fluorescent quantum yield calculation according to the absorption of the test sample, and the emission spectra area of the measured compound were obtained from 500 to 650 nm. **S1**Determined the 2-(thiophen-2-yl)quinoline segment fluorescent, Quinine sulfate ($\Phi_f = 0.55$ in 0.1M H_2SO_4) were used as the standard for the fluorescent quantum yield calculation according to the absorption of the test sample, and the emission spectra area of the measured compound were obtained from 345 to 450 nm. The spectroscopic grade solvents and a 1 cm quartz cuvette were used. Dilute solutions (10^{-6} M) were used to minimize the reabsorption effects. The quantum yield of fluorescence were measured three times for each dye and averaged. Quantum yields were determined using the following equation:

$$Yu=Ys*Fu/Fs*As/Au*Gu^2/Gs^2$$

Ys is the reported quantum yield of the standard rhodamine 6G. Fu is the area under the emission spectra of the measured compound. Fs is the area under the emission spectra of the srandard. As is the absorbance at the excitation wavelength of the measured compound. Au is the absorbance at the excitation wavelength of the standard. Gu is the refractive index of the solvent used. Gs is the refractive index of the solvent of the standard. Molar extinction coefficients were obtained from the slope of a graph of absorbance vs concentration for each dye with five different concentrations (10^{-6} M).

I.3 X−Ray structure determination

The X-ray diffraction data were collected on a Bruker Smart CCD Apex DUO diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) using the ω-2θ scan mode. The data were corrected for Lorenz and polarization effects. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELXTL-2000. All calculations and molecular graphics were carried out on a computer using the SHELX-2000 program package and Diamond. A red block-like crystal of the approximate dimensions 0.28×0.24×0.22 mm^3 was measured. Monoclinic, space group P2(1)/c, a = 16.0905(14) Å, b = 10.5629(9) Å, c = 13.9165(12) Å, β= 106.691(2)°, V = 1519.3(2) $Å^3$, Z = 4, F(000) = 952, ρ = 1.341 Mgm^{-3}, $2\theta_{max} = 56.62°$, $R_1 = 0.0403$, $R_w2 = 0.1036$, GOF = 1.044, residual electron density between 0.349 and -0.250 $eÅ^{-3}$. CCDC 856179 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

I.4 Cell culture and fluorescence bioimaging

The Michigan Cancer Foundation -7 (MCF-7) cell line was provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). Cells were grown in high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO_2. Cells (5×10^8/L) were plated on 14 mm glass coverslips and allowed to adhere for 24 h. Experiments to assess Fe^{3+} uptake were performed over 1 h in the same medium supplemented with 50 μM $FeCl_3$.

Immediately before the experiments, cells were washed with PBS buffer and then incubated with 10 μm **2** in PBS for 30 min at 37 °C. Cell imaging was then carried out after washing the cells with PBS. Confocal fluorescence imaging was performed with a Zeiss LSM 710 laser scanning microscope and a 63×oil-immersion objective lens. Cells incubated with **2** were excited at 405 nm and 488 nm using a multi-line argon laser.

I.5 Synthesis section

2-(thiophen-2-yl)quinoline (4) was synthsised by reference[S2]. White solid; 84% yield. ^{1}H NMR($CDCl_3$, 300 MHz): 8.15-8.09 (m, 2H), 7.81-7.75 (m, 3H), 7.70 (t, 1H, J = 3.0 Hz), 7.50-7.47 (m, 2H), 7.16 (t, 1H, J = 3.0 Hz).

5-(quinolin-2-yl)thiophene-2-carbaldehyde (3) The 2-(thiophen-2-yl)quinoline (4) (4.6 g, 21.75 mmol) was dissolved in dry THF(50 mL) under an argon atmosphere. The n-butyl lithium (14.95 mL, 23.93 mmol) was slowly added and the mixture solution was stirred for 30 min at -78 °C. Dry N,N-dimethylformamide(1.59 mL) was added and stirred for an additional 12 h at ambient temperature. The reaction mixture was washed twice with 50 mL water and 50 mL brine, dried over the anhydrous magnesium sulfate, and concentrated at reduced pressure. The crude product was recrystallized with ethyl acetate.

Compound **3**: yellowy solid; 59.05% yield; m.p.: 143 °C. ^{1}H NMR($CDCl_3$, 300 MHz): 9.96 (s, 1H), 8.22 (d, 1H, J = 3.0 Hz), 8.14 (d, 1H, J = 3.0 Hz), 7.85-7.73 (m, 4H), 7.75 (d, 1H, J = 6.0 Hz), 7.56 (d, 1H, J = 6.0 Hz). ^{13}C NMR($CDCl_3$, 75 MHz): 117.7, 126.1, 127.1, 127.6, 127.8, 129.6, 130.3, 136.8, 137.0, 144.8, 148.1, 150.8, 154.3, 183.2. EI-TOF MS: m/z: 239.9 (M^+).

8[5-(quinolin-2-yl)thiophene]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (2) The 2,4-dimethl-pyrrole (480 mg, 5.02 mmol) and 5-(quinolin-2-yl)thiophene-2-carbaldehyde (3) (600 mg, 2.52 mmol) were dissolved in dry CH_2Cl_2 (100 mL) under an argon atmosphere. One drop of trifluoroacetic acid (TFA) was added, and the solution was stirred for 10 h at ambient temperature in the dark (until TLC indicated complete consumption of the aldehyde).The 2,3-dichloro-5,6-dicyanoquinone (DDQ, 560 mg, 2.52 mmol) was added, and the mixture was stirred for an additional 3 h. The reaction mixture was then treated with triethylamine (6 mL) for 5 min. Boron trifluoride etherate (6 mL) was added and the mixture was stirred for another 40 min. The dark brown reaction mixture was washed twice with 50 mL water and 50 mL brine, dried over Na_2SO_4, and concentrated at reduced pressure. The crude product were purified by silica-gel column chromatography (dichloromethane/ petroleum ether:1/2) and recrystallized with dichloromethane/hexane.

Compound **2**: red solid; 65% yield; m.p. > 250 °C; ^{1}H NMR($CDCl_3$, 500 MHz): 8.19 (d, 1H, J = 10 Hz), 8.08 (d, 1H, J = 10.0 Hz), 7.81 (m, 2H), 7.76 (d, 1H, J = 3.5 Hz), 7.71 (t, 1H, J = 5.0 Hz), 7.51 (t, 1H, J = 5.0 Hz), 7.04 (d, 1H, J = 5.0 Hz), 6.02 (s, 2H), 2.57 (s, 6H), 1.76 (s, 6H); ^{13}C NMR($CDCl_3$, 125 MHz): 156.3, 151.6, 148.1, 147.7, 143.5, 137.7, 136.8, 133.7, 132.3, 130.1, 129.4, 128.9, 127.5, 126.5, 125.8, 121.6, 117.3, 14.7, 14.0. MALDI-TOF MS: m/z: 457.4(M^+). Anal. Calcd for C26H22BF2N3S; C, 68.28; H, 4.85; N, 9.19; found C,68.23; H,4.91; N,9.16.

8[5-(quinolin-2-yl)thiophene]-4,4-difluoro-1,3,5,7-tetra(4-methoxystyry)-4-bora-3a,4a-diaza-s-indacene (1)[S3] Compound **2** (0.22 mmol, 100 mg) and methoxy benzaldehyde (1.12 mmol, 152 mg) were added to a 100 mL round bottomed flask containing 50 mL toluene, and then piperidine (0.4 mL) and acetic acid (0.4 mL) were added to this solution. The mixture was heated under reflux by using a Dean Stark trap and reaction was monitored by TLC in solvent CH_2Cl_2. When all the starting material had been disappeared, the mixture were cooled to room temperature and concentrated at reduced pressure. The dark brown reaction mixture was washed with 100 mL water and extracted twice with 100 mL chloroform., dried over Na_2SO_4, and concentrated at reduced pressure. The crude products were purified by silica-gel column chromatography using CH_2Cl_2 as the eluant.

Compound **1**: black solid; 10%; m.p. > 250 °C; ^{1}H NMR($CDCl_3$, 500 MHz): 8.29 (d, 1H, J = 10.0 Hz), 8.12 (d, 1H, J = 10.0 Hz) 7.95 (m, 2H), 7.87 (d, 1H, J = 10.0 Hz), 7.73 (t, 1H, J = 10.0 Hz), 7.68 (d, 2H, J = 15.0 Hz), 7.65 (d, 4H, J = 10.0 Hz), 7.55 (t, 1H, J = 10.0 Hz), 7.39 (d, 2H, J = 15.0 Hz), 7.11 (s, 2H), 7.06 (d, 4H, J = 10.0 Hz), 6.97 (d, 4H, J = 5.0 Hz), 6.94 (d, 2H, J = 15.0 Hz), 6.51 (d, 4H, J = 10.0 Hz), 6.21 (d, 2H, J = 15.0 Hz), 3.88 (s, 6H), 3.61 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz):160.6, 159.5, 153.8, 151.3, 148.7, 148.6, 148.4, 142.4, 138.8, 137.0, 136.6, 134.6, 131.7, 131.6, 130.2, 130.0, 129.7, 129.6, 129.3, 128.0, 127.6, 127.5, 126.7, 126.2, 119.4 117.3, 117.2, 114.4, 113.9, 111.2, 55.4, 55.1. MALDI-TOF MS: m/z: 929.5(M^+).

II Supplementary data

II.1

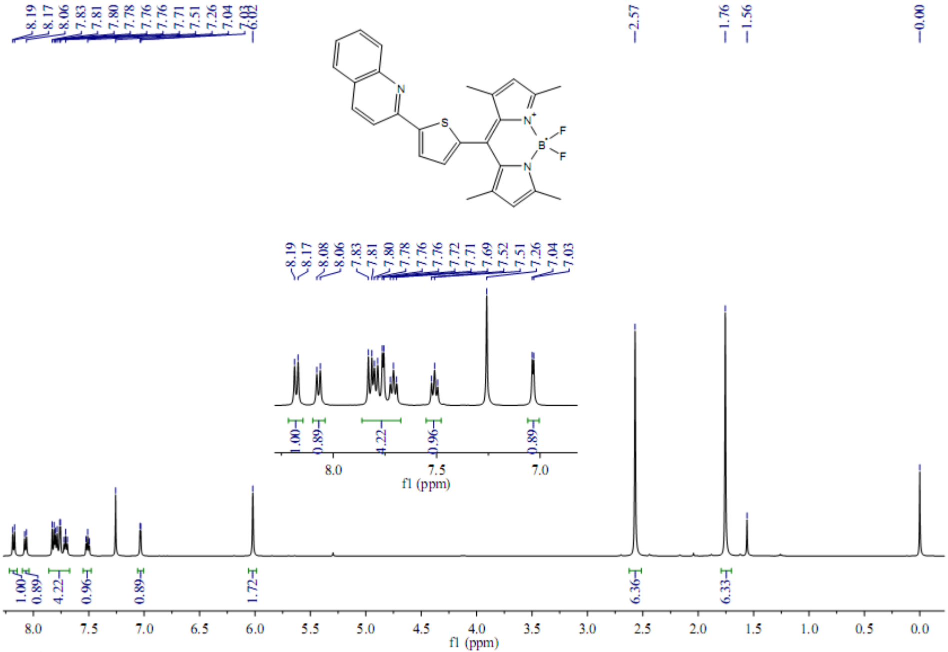

Figure S1. ^{1}H NMR spectrum of **2**.

II.2

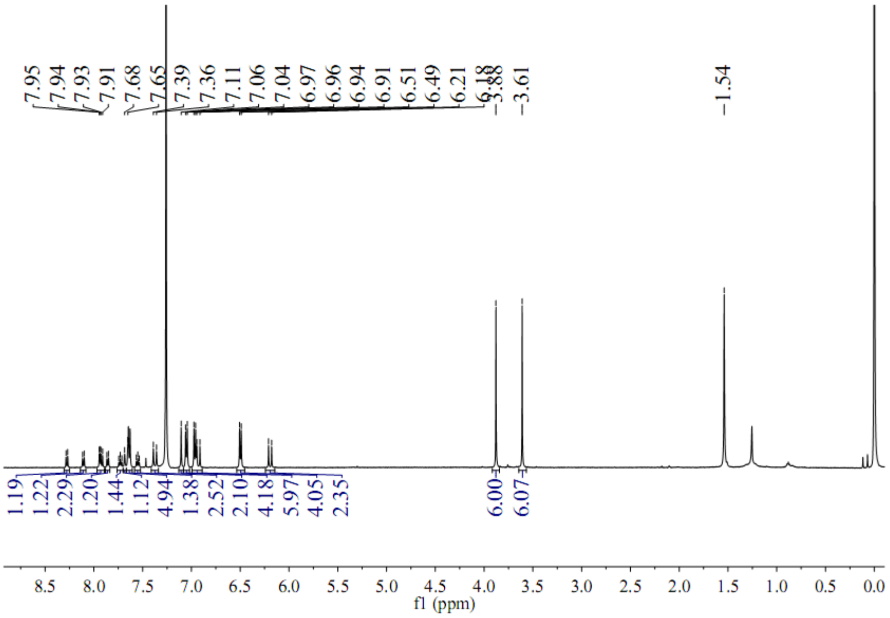

Figure S2. ^{1}H NMR spectrum of **1**.

II.3

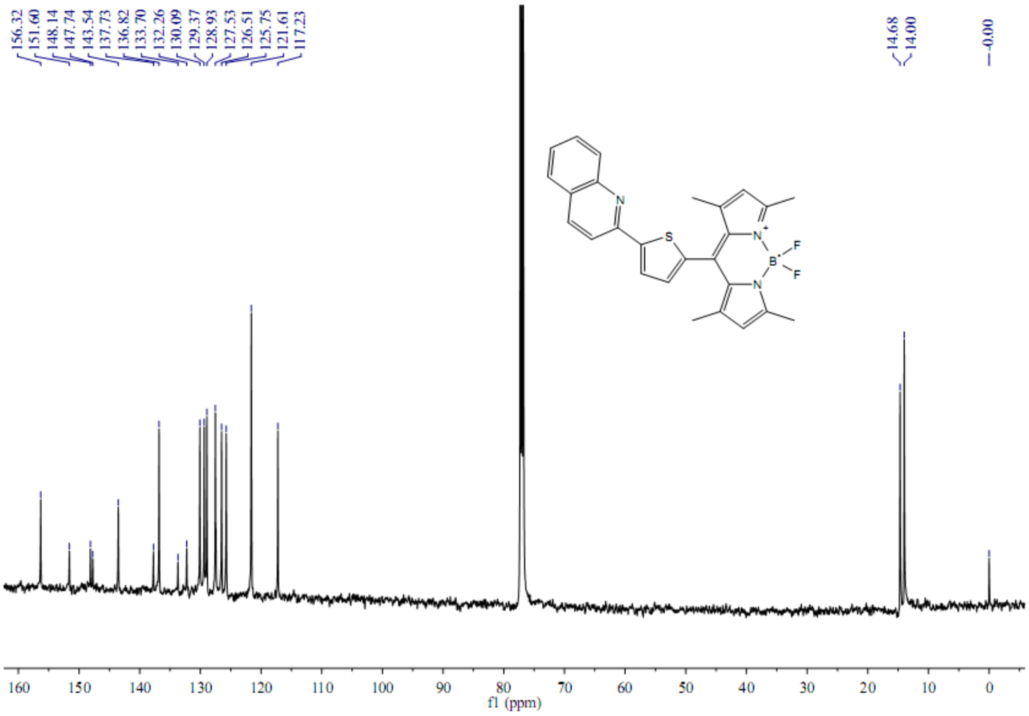

Figure S3. ^{13}C NMR spectrum of **2**.

II.4

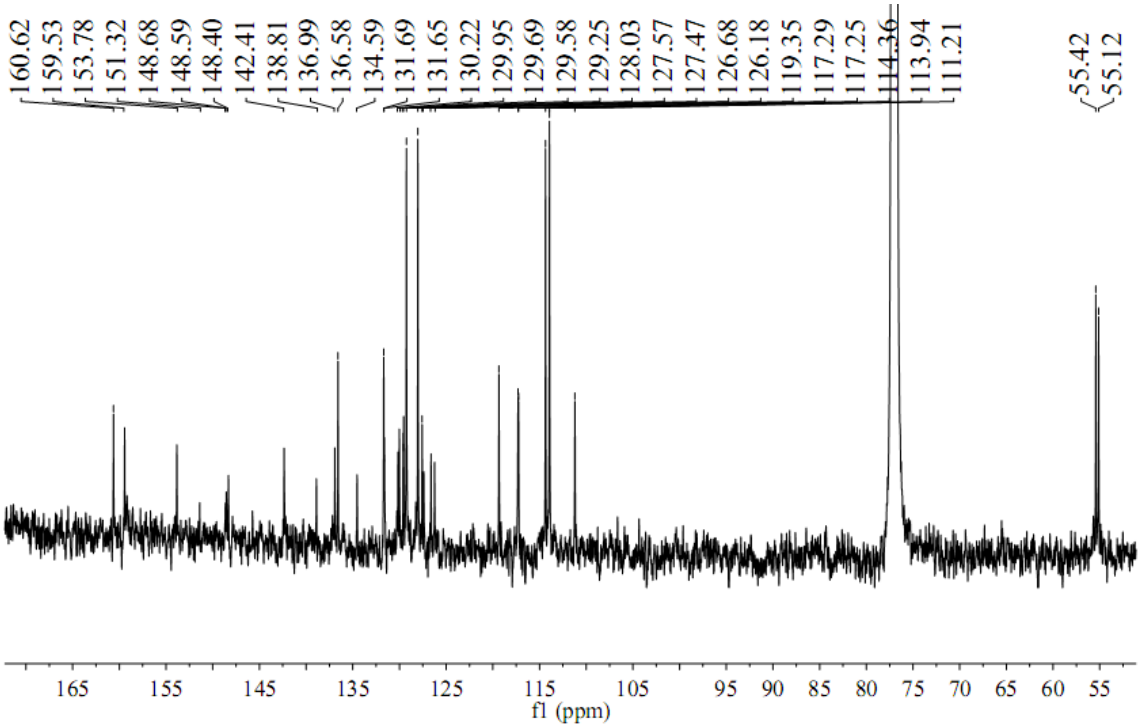

Figure S4. ^{13}C NMR spectrum of **1**.

II.5

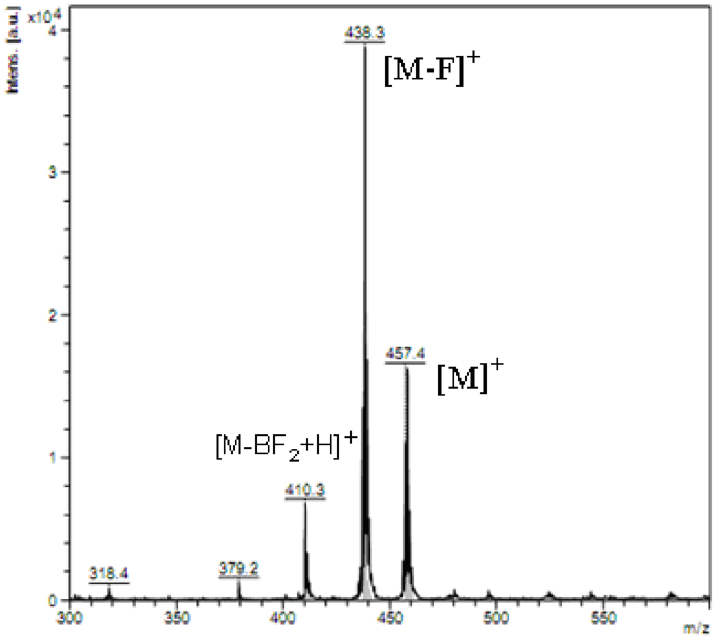

Figure S5. MALDI-TOF –MS spectrum of **2**.

II.6

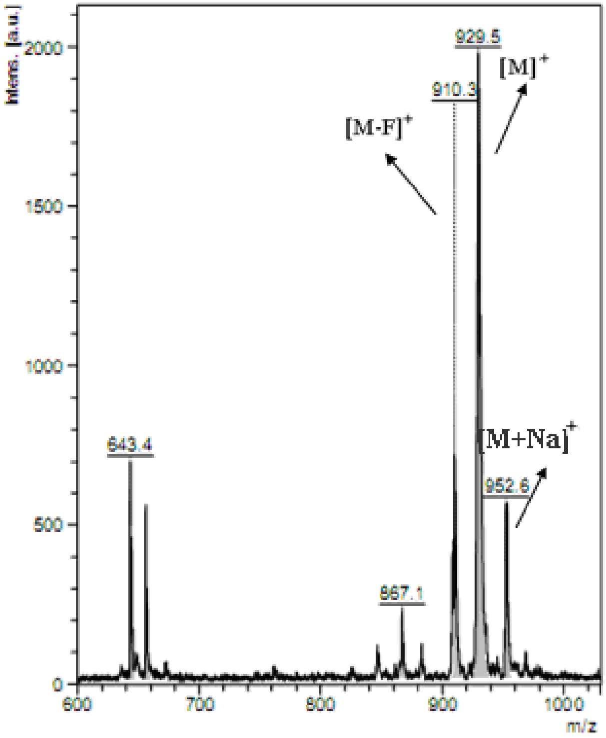

Figure S6. MALDI-TOF –MS spectrum of **1**.

II.7

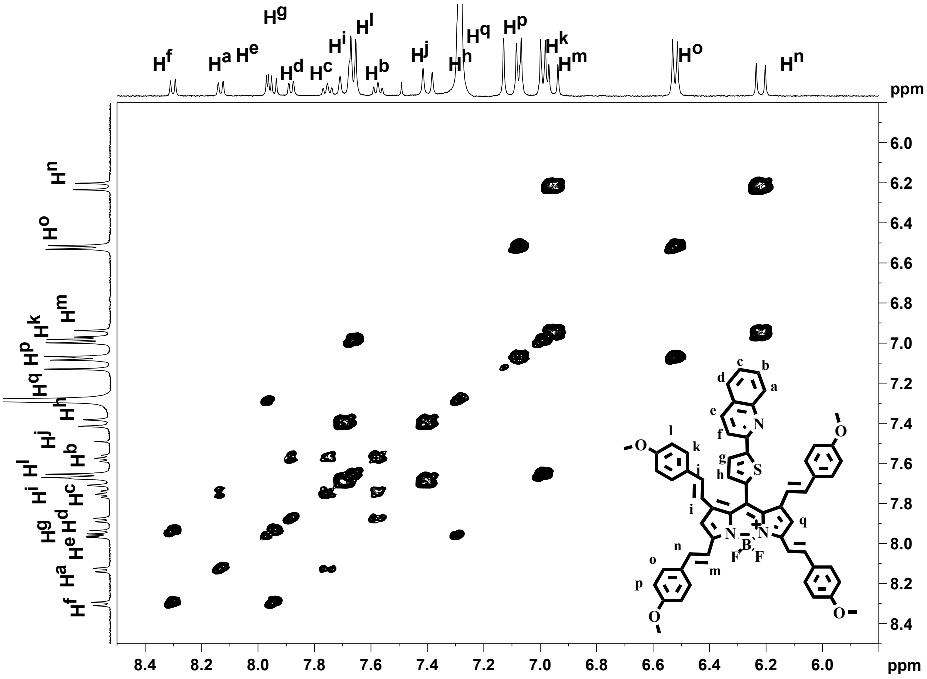

Figure S7. ^{1}H-H COSY spectrum of **1**.

II.8

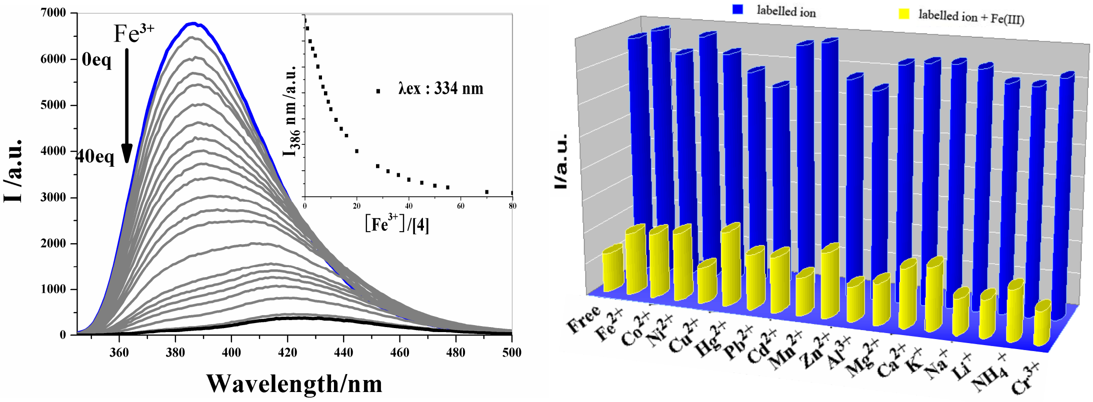

Figure S8. a) Changes in the fluorescence spectrum of compound **4** (10 μM in CH_3OH:H_2O=1:1) as the Fe^{3+} concentration is increased. The emission intensity at 386 nm changes with an excitation at 334 nm as a function of Fe^{3+} concentration. b) Blue bars denote intensity ratios for fluorescence after addition of the labelled ions by exciting wavelength at 334 nm. Yellow bars denote ratios upon the subsequent addition of Fe^{3+}.

II.9

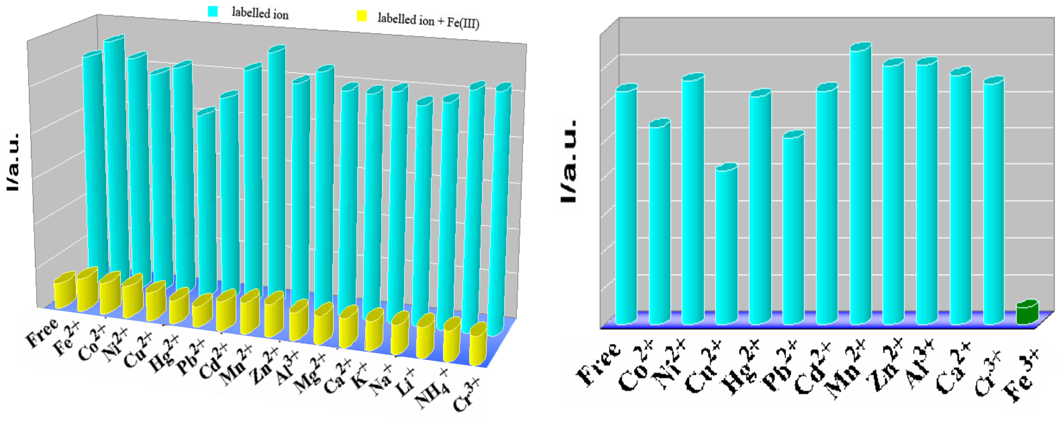

Figure S9. (Left) Blue bars denote intensity ratios for fluorescence of **2** after addition of the labelled ions by exciting wavelength at 334 nm. Yellow bars denote ratios upon the subsequent addition Fe^{3+}. (Right) Fuorescence intensity of **1** observed at 732 nm upon addition of various metal ions.

II.10

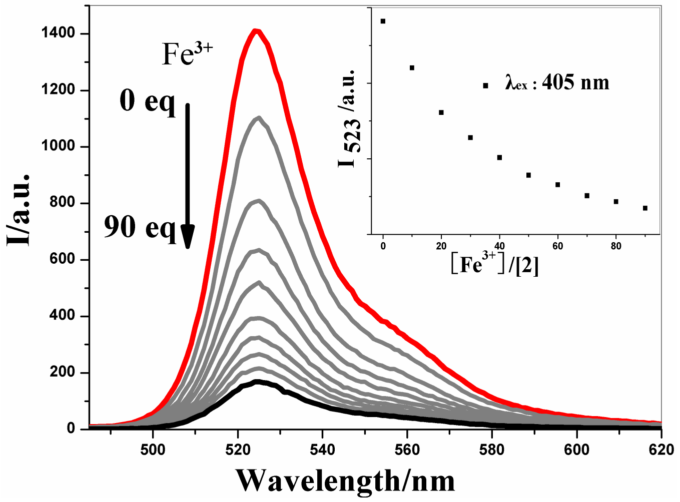

Figure S10. Changes in the fluorescence spectrum of compound **2** (10 μM in $CH_3OH:H_2O=4:1$) as the Fe^{3+} concentration is increased. Insert: the emission intensity at 523 nm changes with an excitation at 334 nm as a function of Fe^{3+} concentration.

II.11

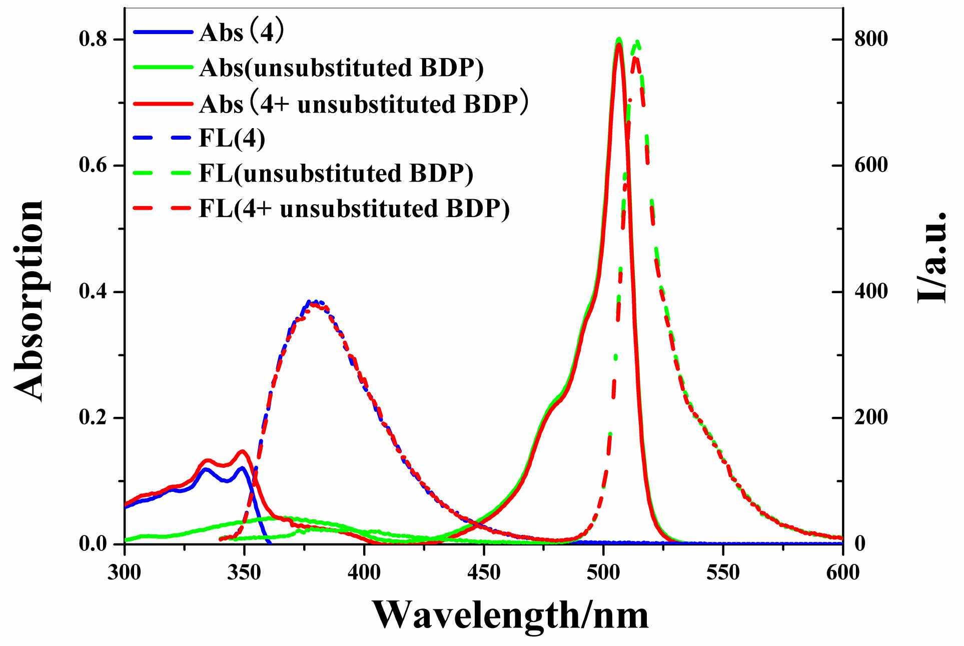

Figure S11. The absorption and emission spectra of **4**, *meso*-unsubstituted BODIPY and the mixture of **4** and *meso*-unsubstituted BODIPY in CH_2Cl_2 (10 μM, λ_{ex} = 334 nm).

II.12

Table S1. Spectroscopic and photophysical properties of **1**, **2** and **4** in various solvents at 298 K

compound	solvent	λ_{abs}^{max}(nm)/ $\log\varepsilon_{max}$	λ_{em}^{ex334}(nm)	$\lambda_{em}^{accptor}$(nm)	Φ_f^{ex334}	$\Phi_f^{accptor}$	ETE	τ(ns)
1	CH_2Cl_2	708/5.23	732	729	0.62	0.36	98%	4.51
	THF	705/5.26	725	723	0.58	0.40	99%	4.11
	CH_3CN	700/5.23	719	720	0.61	0.45	97%	4.31
	DMSO	716/5.22	736	735	0.54	0.57	99%	4.08
	Paraffin oil	707	722	720	0.66	0.50	99%	---
2	Hexane	513/4.74	522	517	0.048	0.038	97%	5.05
	CH_2Cl_2	514/4.77	525	524	0.066	0.054	99%	3.47
	THF	513/4.83	521	523	0.058	0.047	98%	3.44
	CH_3CN	509/4.84	520	520	0.040	0.034	99%	3.83
	CH_3OH	510/4.84	520	521	0.042	0.037	99%	4.21
	DMSO	514/4.43	527	525	0.123	0.109	99%	5.36
	Paraffin oil	515	524	524	0.731	0.695	91%	---
4	CH_2Cl_2	334/4.38, 348/4.38	386	---	0.09	---	---	---

Table S2. Crystal data and structure refinement for **2**.

Parameters name	**Data**	**Parameters name**	**Data**
Empirical formula	$C_{26}H_{22}BF_2N_3S$	Density (calc.) Mg/m^3	1.341
Formula weight	457.34	Absorption coefficient (mm$^{□1}$)	0.179
Crystal size (mm^3)	0.28×0.24×0.22	Temperature (K)	291(2)
Crystal system	Monoclinic	F(000)	952
Space group	*P2(1)/c*	Goodness□of□fit on F^{-2}	1.044
Z	4	Max. and min. absorption	0.9516 and 0.9617
a /Å	16.0905(14)	range for data collection (°)	1.93 to 28.37
b /Å	10.5629(9)	Index ranges	□17≤ h ≤21, 10≤ k ≤14, 18≤ l ≤15
c / Å	13.9165(12)	Total reflections	10594
α / (deg)	90	Independent reflections	5576 [$R_{(int)}$ = 0.1167]
□ / (deg)	106.691(2)	Parameters	302
γ / (deg)	90	Final R indices [I > 2]	R_1 = 0.0525 wR_2 = 0.1114
Volume / Å^3	2265.6(3)	R indices (all data)	R_1 = 0.0403 wR_2 = 0.1036

III. References

S1 J. Olmsted, *the Journal of Physical Chemistry*, 1979, **83**, 2581.

S2 Q. Zhao, C. Y. Jiang, M. Shi, F. Y. Li, T. Yi, Y. Cao, C. H. Huang, *Organometallics*, 2006, **25**, 3631.

S3 O. Buyukcakir, O. A. Bozdemir, S. Kolemen, S. Erbas, E. U. Akkaya, *Org. Lett.*, 2009, **11**, 4644.