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Supporting information for

Long Wavelength Red Fluorescent Dyes from 3,5-Diiodo-BODIPYs

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General: Reagents were purchased as reagent-grade and used without further purification unless otherwise stated. Solvents were used as received from commercial suppliers unless noted otherwise. THF was freshly distilled from sodium benzophenone ketyl. All reactions were performed in oven-dried or flame-dried glassware unless otherwise stated, and were monitored by TLC using 0.25 mm silica gel plates with UV indicator (60F-254). ^{1}H- and ^{13}C-NMR are obtained on an AV-300 Bruker (or an AV-400 Bruker) spectrometer at 298 K. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ 7.26 (^{1}H) and 77 ppm (^{13}C). HRMS spectra were obtained on the MALDI-TOF (LDI-1700) at the Changchun Institute of Applied Chemistry, the matrix used for the MALDI analyses was 2',4',6'-trihydroxyacetophenone.

Fluorometric analysis: UV-visible absorption spectra were recorded on a Hitachi U-3010 Spectrophotometer (190-1100 nm scan range). Fluorescence emission spectra were recorded on a Hitachi F-4600 FL Spectrophotometer. The slit widths were 2.5 nm and 5.0 nm for excitation and emission respectively. Fluorescent quantum yields were calculated using a secondary standard method[1] by comparing the areas under the corrected emission spectrum of the test sample in various solvent with that of the standard. Fluorescein[1] in 0.1 N NaOH aqueous solution ($\Phi = 0.90$) and methylene blue[2] in MeOH ($\Phi = 0.03$) were used as the standard for the fluorescent quantum yield calculation according to the absorption of the test sample. Non-degassed, spectroscopic grade solvents and a 10 mm quartz cuvette were used. Dilute solutions ($0.01<A<0.05$) were used to minimize the reabsorption effects. Quantum yields were determined using the following equation[3]:

$$\Phi_X = \Phi_S (I_X/I_S) (A_S/A_X) (\eta_X/\eta_S)^2$$

Where Φ_S stands for the reported quantum yield of the standard, I stands for the integrated emission spectra, A stands for the absorbance at the excitation wavelength and η stands for the refractive index of the solvent being used ($\eta = 1$ when the same solvent was used for both the test sample and the standard). X subscript stands for the test sample, and S subscript stands for the

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standard. Solvents have little effect on the photophysical properties of the resultant BODIPYs as shown in Table S1.

Table S1. Photophysical properties of BODIPY **1**, **4a-c, 6** and **7** in hexane, methanol and DMSO**.**

BODIPYs	solvent	λmax (nm)	lgε	λem (nm)	Φ[a]	Stokes Shift (nm)
1	hexane	556	4.87	575	0.07	19
	methanol	558	4.92	575	0.08	17
	DMSO	557	4.83	580	0.07	23
4a	hexane	658	4.72	669	0.18	11
	methanol	660	4.78	680	0.16	20
	DMSO	671	4.68	683	0.17	12
4b	hexane	629	4.78	641	0.39	12
	methanol	626	4.67	642	0.36	16
	DMSO	627	4.71	654	0.36	27
4c	hexane	644	4.87	661	0.32	17
	methanol	642	4.82	660	0.32	18
	DMSO	647	4.70	673	0.28	26
6	hexane	554	4.60	572	0.05	18
	methanol	558	4.62	571	0.05	13
	DMSO	555	4.57	577	0.05	22
7	hexane	664	4.40	675	0.12	11
	methanol	664	4.33	676	0.12	12
	DMSO	675	4.61	689	0.12	14

For BODIPY **1** and **6**, the fluorescence quantum yields were calculated using fluorescein[1] in 0.1 N NaOH aqueous solution (Φ = 0.90) as the standard; for BODIPY **4a-c** and **7**, methylene blue[2] in MeOH (Φ = 0.03) was used as the standard.

Cell Culture. All tissue culture media and reagents were obtained from Invitrogen. Human HEp2 cells were obtained from the ATCC and maintained in a 50:50 mixture of DMEM: Advanced MEM (Gibco) supplemented with 5% FBS (Gibco). The cells were subcultured biweekly to maintain subconfluent stocks. All the solutions of BODIPY **7** were filter sterilized using a 0.22 mm Pal syringe filter.

Microscopy. HEp2 cells were placed on Lab Tek II chamber coverslips and incubated for 48 h prior to exposure to BODIPY **7**. The stock solutions of BODIPY **7** were first prepared in DMSO to a concentration of 10 mM and were diluted to a working concentration of 1 μM in medium. The cells were then loaded with BODIPY **7** and were incubated for 1 h. Organelle tracers were obtained from Molecular Probes and incubated concurrently with conjugate for 30 min to 1h prior

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to microscopy. Mitochondria were visualized using MitoTracker Green FM at 250 nM, lysosomes with LysoSensor Green at 50 nM. Endoplasmic Reticulum (ER) with ER Tracker Blue/White at 100 nM and Golgi complex with BODIPY FL C5-ceramide at 50 nM. The cells were then washed three times with PBS (pH 7.4) buffer. The cells were viewed using a Leica DMRXA microscope with 40x NA 0.8 dip objective lens and DAPI, FITC and Cy5 filter cubes.

Syntheses and Characterizations of Compounds

BODIPY **1** was synthesized from the readily available dipyrromethane **3**[4] in a one-pot, four-step process via the catalytic hydrogenation, decarboxlative iodination, DDQ oxidation, and the complexation with $BF_3{\cdot}OEt_2$. The detailed synthesis was described below: To a 100 mL round bottom flask was added Pd/C (644 mg, 0.6 mmol). After freez-thaw for three times, 15 mL freshly distilled THF was added, and hydrogen gas was applied through a balloon. It was stirring for 20-30 min to active the Pd/C catalyst before adding dipyrromethane **3** (1.84 g, 3.0 mmol) in THF (25 mL) through syringe. It was stirred in the hydrogen atmosphere in dark at room temperature for 6 h. After removal of the Pd/C catalyst through filtration, organic solvent was removed under vacuum. The residue was dissolved in 2 M NH_4OH (300 mL), and acidified with AcOH to reach pH 5. The resultant precipitate was collected through filtration to give 1,9-biscarboxylic acid dipyrromethane (1.25 g, 96% yield) as greyish solid, which was suspended in H_2O (95 mL)/MeOH (30 mL) to from a slurry. $NaHCO_3$ (1.40 g, 16.0 mmol) was added and sonicated to form clear pink solution. To this solution was slowly added iodine (1.20 g, 4.6 mmol) in MeOH (65 mL) at room temperature, and the mixture turned to brown during the course of addition. After vigorous stirring at room temperature, the mixture was placed into an ice-bath and stirred at 0 ^oC for an additional 1.5 h. The resultant precipitate was filtered, washed with H_2O, saturated aqueous $NaHCO_3$, H_2O and hexanes before drying in vacuum for 24 h to give the diiododipyrromethane (1.38 g, 80% yield) as brown solid, which was directly dissolved in DCM (110 mL), cooled to 0 ^oC, and

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DDQ (500 mg, 2.2 mmol) was added in one portion. After 1 min, the mixture was poured into water and extracted with DCM. Organic layers were combined and concetrated in the vacumn. After column chromatography, dipyrromethene **2**[4] was obtained in 60% yeild (824 mg). Its characterization was in agreement with literature results[4]. To dipyrromethene **2** in toluene (100 mL) was added triethylamine (2 mL) and BF_3•OEt_2 (2 mL). The mixture was stirred at 50 ℃ for 1.5 h. After work-up and purification using column chromatography (silica gel, DCM:Hexane = 2:1, v/v), BODIPY **1** was obtained as reddish-brown powder in 90% yeild (792 mg, and 41% overall yield for four steps from **3**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (s, 1H), 3.65 (s, 6H), 3.96 (t, J = 7.3 Hz, 4H), 2.56 (t, J = 7.3 Hz, 4H), 2.01 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.5, 140.1, 136.5, 133.2, 119.7, 109.2, 51.9, 34.5, 20.1, 12.4. HRMS (negative mode) Calcd. for $C_{19}H_{20}BF_2I_2N_2O_4$ $[M-H]^-$ 642.9579, found 642.9573. HRMS (postive mode) Calcd. for $C_{19}H_{21}BF_2I_2N_2O_4$ $[M]^+$ 643.9646, found 624.9645.

BODIPY **6** was synthesized as a reddish-brown powder in 33% overall yield (566 mg) for four steps from dipyrromethane **5**[6] (1.60 g, 3.0 mmol) using the procedure[5] described for BODIPY **1**. ^{1}H NMR (300 M Hz, $CDCl_3$): δ 6.94 (s, 1H), 3.64 (s, 3H), 2.92-2.87 (m, 2H), 2.52-2.47 (m, 2H), 2.20 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 139.4, 138.6, 137.4, 136.0, 133.5, 132.6, 119.0, 110.3, 107.3, 47.2, 34.6, 22.4, 12.3, 10.4, 8.8. HRMS Calcd. for $C_{16}H_{17}BFI_2N_2O_2$ $[M-F]^+$: 552.9368, found 552.9450.

4a: To a 50 mL Schlenk flask were added BODIPY **1** (64 mg, 0.1 mmol), $Pd(PPh_3)Cl_2$ (11 mg), CuI (6.4 mg) and 5 mL freshly distilled THF. After freeze-thaw for three times, Et_3N (0.2 mL) and 2-ethylenethiophene (108 uL dissolved in 0.5 mL THF) were added into the mixture through syringe. It was reacted at 65 ^{o}C for 2 h, cooled to room temperature, filtrated through a Celite cake, and washed with DCM (20 mL × 3). Organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. After column separation (hexane/DCM/EtOAc = 5/1/1, v/v/v), the desired **4a** was obtained as a

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dark-blue solid in 67% yield (43 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48-7.41 (m, 2H), 7.38-7.25 (m, 2H), 7.19 (s, 1H), 7.03-7.00 (m, 2H), 3.61 (s, 6H), 2.88 (brs, 4H), 2.49 (brs, 4H), 2.19 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 142.8, 141.8, 139.6, 137.3, 136.1, 135.4, 134.2, 133.5, 131.7, 129.7, 128.9, 128.2, 128.1, 127.7, 127.4, 126.8, 119.2, 118.8, 117.7, 98.5, 86.5, 51.9, 35.0, 34.7, 19.9, 19.5, 12.0, 9.7. HRMS (negative mode) Calcd. for $C_{31}H_{28}BF_2N_2O_4S_2$ $[M+H]^+$ 605.1557, found 605.1569.

4b: By reacting BODIPY **1** (64 mg, 0.1 mmol) with 3-ethylenethiophene (108 uL dissolved in 0.5 mL THF) under the same reaction condition as described above, BODIPY **4b** was obtained as a dark blue solid in 66% yield (42 mg) after column separation ((hexane/DCM/EtOAc = 5/1/1，v/v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (s, 2H), 7.32-7.28 (m, 4H), 7.17 (s, 1H), 3.69 (s, 6H), 3.07 (t, J = 7.2 Hz, 4H), 2.61 (t, J = 7.0 Hz, 4H), 2.16 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 139.6, 137.6, 135.1, 131.5, 131.1, 130.2, 125.5, 121.6, 119.3, 100.2, 82.1, 51.8, 34.7, 19.9, 9.7. HRMS (negative mode) Calcd. for $C_{31}H_{27}BF_2N_2O_4S_2$ $[M+H]^+$ 604.1479, found 604.1482.

4c: By reacting BODIPY **1** (64 mg, 0.1 mmol) with 1-ethynyl-4-methoxybenzene (132 uL dissolved in 0.5 mL THF) under the same reaction condition as described above, BODIPY **4c** was obtained as dark blue solid in 67% yield (44 mg) after column separation ((hexane/DCM/EtOAc = 5/1/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J = 7.1 Hz, 4H), 7.11 (s, 1H), 6.93 (d, J = 7.2 Hz, 4H), 3.86 (s, 6H), 3.69 (s, 6H), 2.96 (brs, 4H), 2.58 (brs, 4H), 2.16 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 160.6, 139.2, 137.7, 135.0, 134.0, 133.1, 131.0, 118.4, 114.6, 114.0, 105.6, 81.9, 55.3, 51.8, 34.7, 19.8, 9.7. HRMS Calcd. for $C_{37}H_{35}BF_2N_2O_6$ $[M]^+$ 652.2551, found 652.2550; HRMS Calcd. for $C_{37}H_{35}BFN_2O_6$ $[M-F]^+$ 633.2567, found 633.2560.

BODIPY **7** was obtained using the same procedure described above for **4a**, by reacting BODIPY **6** (57 mg, 0.1 mmol) with 2-ethylenethiophene (108 uL dissolved in 0.5 mL THF) in 5 mL freshly distilled THF at the presence of Et_3N (0.2 mL) in a 50 mL Schlenk flask. $Pd(PPh_3)Cl_2$ (11 mg)

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and CuI (6.4 mg) was used as the catalyst. It took 2 h for this reaction to reach completion at 65 ^{o}C. After work-up and column separation (hexane/DCM/EtOAc = 5/1/1, v/v/v), BODIPY **7** was obtained as dark-blue solid in 72% yield (39 mg). mp > 260 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 4.8 Hz, 1H), 7.32 (t, J = 5.4 Hz, 2H), 7.05 (t, J = 3.0 Hz, 2H), 6.97 (s, 1H), 3.67 (s, 3H), 2.95 (t, J = 7.9 Hz, 2H), 2.54 (t, J = 7.7 Hz, 2H), 2.25 (s, 3H), 2.23 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.1, 152.0, 150.3, 143.5, 139.3, 138.2, 135.9, 134.3, 131.2, 130.1, 129.4, 128.3, 127.1, 119.6, 116.5, 68.4, 52.1, 35.5, 19.8, 12.3, 12.2, 9.8. ESI-MS Calcd for $C_{28}H_{23}BF_2N_2O_2S_2$ $[M]^+$: 532.43, found 532.38.

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