Electronic Supplementary Information (ESI)

Co-self-assembled Nanoaggregates of BODIPY Amphiphiles for Dual Colour Imaging of Live Cells

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1. General Methods

Chemicals and Reagents: All the chemicals used were analytical grade and were used without further purification unless otherwise stated. Ethyl magnesium bromide, iodomethane, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 1-dimethylamino-2-propyne, 2,4-Dimethylpyrrole, $BF_3{\cdot}Et_2O$, and trifluoroacetic acid (TFA) were purchased from commercial source. Dichloromethane (DCM) and triethylamine were distilled over CaH_2 and stored under Ar. ER-Tracker Red and Hoechst 33342 were purchased from Invitogen (USA). Silica gel (200-300 mesh) was used for column chromatography.

NMR spectroscopy: ^1H and ^{13}C NMR spectra were recorded at 300 K on a Bruker Avance (400 MHz or 500MHz) spectrometer using $CDCl_3$ or CD_3OD as solvent and tetramethylsilane (TMS) as internal standard. Multiplicities for proton signals are abbreviated as *s*, *t*, and *m* for singlet, triplet, and multiplet, respectively.

Mass spectrometry: ESI mass spectra were measured with a Bruker Daltonics micrOTOF-QII LC-MS system.

UV/Vis spectroscopy: UV/Vis absorption spectra were recorded on an Agilent Technologies Cary 300 UV/Vis spectrophotometer. The solvents for spectroscopic studies were spectroscopic grade and used as received. The spectra were recorded in quartz glass cuvettes and the extinction coefficients ε were calculated according to Lambert-Beer's law.

Fluorescence spectroscopy: Fluorescence spectroscopic studies were performed under ambient conditions on a FluoroLog-3 spectrofluorometer. The fluorescence quantum yields of monomeric **1** and **2** were determined using following equation according to the literature[1]: $\Phi_{F(x)} = (A_s/A_x)(F_x/F_s)(n_x/n_s)^2\Phi_{F(s)}$, where $\Phi_{F(x)}$ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission spectrum, and n is the refractive index of the solvents used. Subscripts "s" and "x" refer to the standard and to the unknown, respectively. Fluorescein in 0.1 M NaOH solution ($\Phi_{F(s)} = 0.92$) or Rhodamine B in EtOH ($\Phi_{F(s)} = 0.50$) was used as reference. The calculation performed by using above equation resulted in a quantum yield of 0.17 for **1** and 0.23 for **2** in DMSO, respectively.

Confocal laser scanning microscopy: Confocal fluorescence images were recorded on a Zeiss LSM710 confocal laser scanning microscope (CLSM) (Jena, Germany). The excitation was performed with Ar lase at wavelengths of 488 nm, 543 nm, 594 nm, and the emission was monitored at 500-550 nm, 550-600 nm or 600-650 nm respectively.

Dynamic light scattering (DLS): DLS measurements were performed at 25 °C on a Delsa Nano C Particle Analyzer, Beckman Coulter, using a 25 mW helium-neon laser (632.8 nm).

Electron microscopy: Transmission electron microscopy (TEM) measurements were performed on a Tecnai G^2 20 S-TWIN Transmission Electron Microscope, operating at an acceleration voltage of 200 kV. For the observation of aggregates, a drop of sample (0.1-1.0 mg/mL) was placed on 400-mesh formvar copper grids coated with carbon. About 2 min after the deposition, the grid was tapped with filter paper to remove surface water. Negative staining was performed by addition of a drop of uranyl acetate aqueous solution (0.5 %) onto the copper grid. After 1 min, the surface liquid on the grid was removed by tapping with filter paper. For scanning electron microscopy (SEM), the dye co-aggregates suspension was evaporated on silica wafer, sputtered with an Au layer (5 nm), and examined in a JEOL S-4800 field-emission scanning electron microscope.

Preparation of co-aggregates: Solvent-switch methods[2] were used to prepare the co-aggregates of **1** and **2**. For typical solvent-switch preparation, a mixture of dye **1** and **2** (total weight of 1 mg) was dissolved in DMSO (10 μL), and then deionized water (1-5 mL) was slowly injected into the above solution.

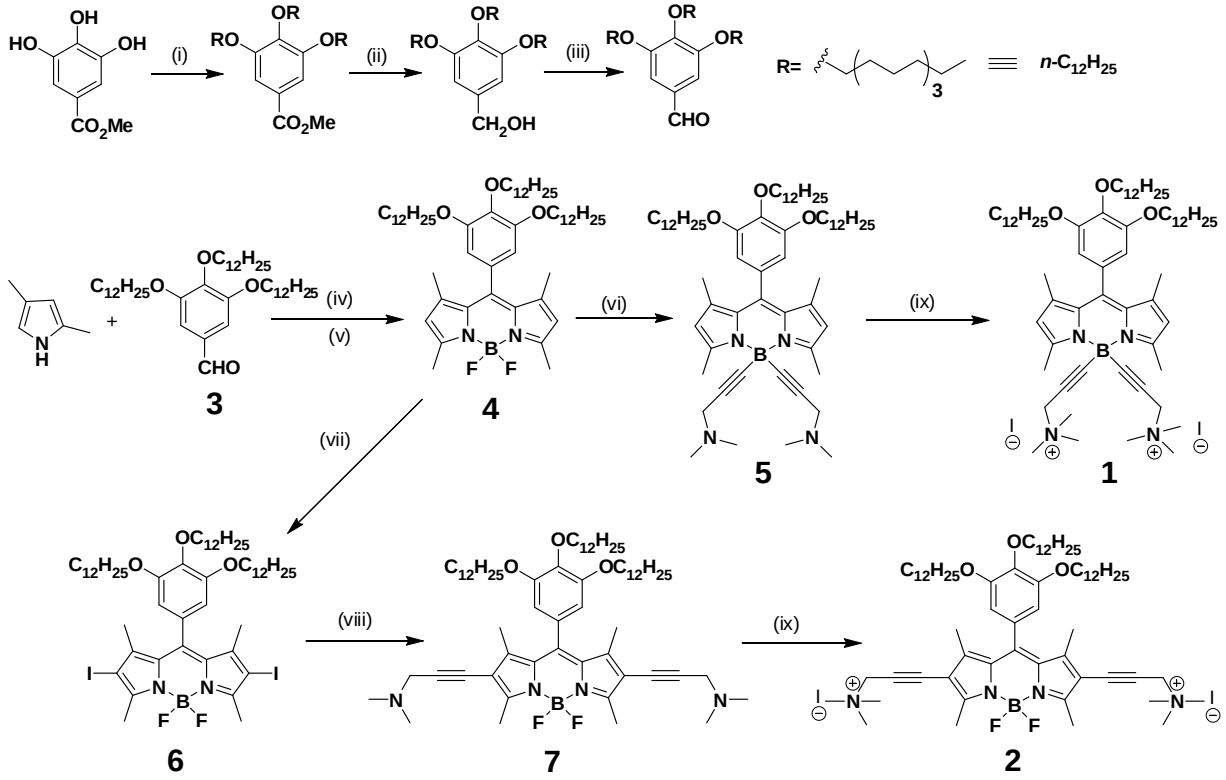
Atomic force microscopy (AFM): Tapping mode AFM studies were performed on a Nanoscope IIID AFM instrument (Veeco Metrology, USA) under ambient conditions. Commercial silicon tips with a nominal spring constant of 40 N/m and resonance frequency of 300 kHz were used in all of the experiments. The vesicle solution was allowed to incubate on mica at room temperature for 20 min.

Cell incubation and imaging: HeLa cells (human cervical carcinoma cells) were cultured in DEME medium supplemented with 10% fetal bovine serum at 37 ºC in an atmosphere containing 5% CO_2. For live cell imaging, HeLa cells were first seeded at a density of 2×10^5 cells per dish in a confocal microscope dish. Dye vesicles suspensions (50 μL, [**1**]/[**2**]= 1/5, [**2**]= 2×10^{-4} M) were injected into the dish containing 1 mL serum free medium. After incubation at 37 ^oC for 2 h, the cells were imaged using a Zeiss LSM710 confocal laser scanning microscope with a 100×objective lens. For co-localization analysis, commercial organelle specific dyes were used. The HeLa cells were first stained with dye vesicles as described above, then incubated with ER-Tracker Red (1μM, for ER co-localization) for 1 h and with Hoechst 33342 (10 μg/mL) for 10 min, and then washed with PBS three times. After replacement of medium, cells were imaged using CLSM with a 60×objective lens or a 100×objective lens. For quantity analysis, background was subtracted from images prior to

analysis.

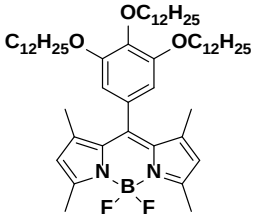
Cell Cytotoxicity Assay: HeLa cells were seeded at a density of 1×10^4 cells/well in a 96-well plate. After 24 h incubation, HeLa cells were treated with the above four dyes at serial concentrations (5, 10, 20, 40 μM) for 24 h. Then the medium was removed and cells were washed with PBS twice. Cell viability was determined by cell-counting kit-8 (CCK-8) assay, and data were expressed as mean ± standard deviation (SD) for at least six independent experiments.

2. Synthesis and characterization of dyes 1 and 2



Scheme S1 Synthesis of BODIPY dyes **1** and **2**. Reagents and conditions: (i) 1-bromododecane, K_2CO_3, DMF, 80 °C, 4 h; (ii) $LiAlH_4$, THF; (iii) Pyridinium-chlorocromate (PCC), CH_2Cl_2, reflux 4 h; (iv) CF_3COOH, CH_2Cl_2, DDQ, rt, 2 h; (v) $BF_3{\cdot}Et_2O$, triethylamine, rt; (vi) 1-dimethylamino-2-propyne, EtMgBr, THF, 60 °C, 12 h; (vii) I_2, HIO_3, EtOH, H_2O, 60 °C, 3 h; (viii) 1-dimethylamino-2-propyne, $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, 50 °C, 12 h; (ix) CH_3I, diethyl ether, 35 °C, 24 h.

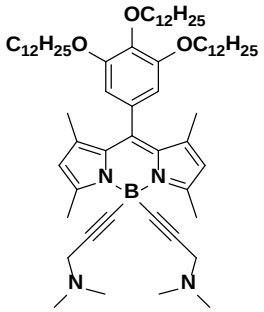
4,4-difluoro-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (4)



1,2,3-Tris(dodecyloxy)-5-benzaldehyde (compound **3**)[3] (1.3 g, 2.0 mmol) and 2,4-dimethypyrrole (0.4 mL, 4.0 mmol) were dissolved in dry CH_2Cl_2 (30 mL) under Ar. After stirring for 15 min, 0.036 mL TFA was added and the reaction was continued at room temperature for about 2 h. After the complete consumption of starting compound **3** (monitored by TLC), DDQ (8.5 mmol, 2.0 g) was

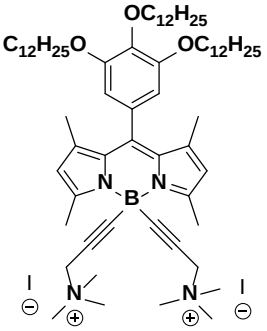
added into the reaction mixture. After stirring for 2 h, triethylamine (10.0 mL, 57 mmol) and $BF_3{\cdot}Et_2O$ (10.0 mL, 79 mmol) were added subsequently. The reaction mixture was stirred at room temperature for 10 h. Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2/*n*-Hexane = 1/1, v/v) to give an orange-colored viscous substance (0.53 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$, 300K, TMS): δ = 6.47 (*s*, 2H), 5.99 (*s*, 2H), 3.96 (*qd*, *J* = 19.54), 2.55 (*s*, 6H), 1.87-1.70 (*m*, 6H), 1.53 (*s*, 6H), 1.49-1.39 (*m*, 6H), 1.26 (*m*, 48H), 0.89 (*m*, 9H).

4,4-bis-(3-dimethylamino-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a, 4a-diaza-s-indacene (5)



To a solution of 1-dimethylamino-2-propyne (0.14 g, 2.0 mmol) in dry THF (5 mL) under Ar in a flask, was added EtMgBr (1.0 M in THF, 1.7 mL) and the mixture was stirred at 60 °C for 2 h. Compound **4** (0.70 g, 0.79 mmol) was dissolved in a separate flask in dry THF (3 mL) under Ar, and then the solution was transferred via a cannula to the flask containing the Grignard reagent. The mixture was stirred at 60 °C for 2 h and then water (3 mL) was added. The mixture was extracted with CH_2Cl_2 (20 mL × 3). After the solvent was evaporated under reduced pressure, the residue was purified by column chromatography (silica gel, CH_2Cl_2 first, then CH_2Cl_2/MeOH = 3/1, v/v) to give a dark-orange solid (0.60 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$, 300K, TMS): δ = 6.50 (*s*, 2H), 6.01 (*s*, 2H), 4.02 (*d*, *J* = 6.36 Hz, 2H), 3.91 (*t*, *J* = 6.53 Hz, 4H), 3.22 (*s*, 4H), 2.78 (*s*, 6H), 2.30 (*s*, 12H), 1.77 (*dd*, *J* = 14.22, 7.01 Hz, 6H), 1.52 (*s*, 6H), 1.48-1.39 (*m*, 6H), 1.25 (*m*, 48H), 0.88 (*m*, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 154.79, 154.14, 142.12, 141.65, 138.57, 129.88, 129.60, 121.55, 106.48, 86.60, 73.77, 69.48, 48.60, 43.30, 32.05-32.03, 29.87-29.72, 29.50-29.41, 26.24, 26.11, 22.81, 22.81-22.80, 16.27, 14.57, 14.24. MS (ESI, positive mode): calculated for $C_{65}H_{107}BN_4O_3$ 1002.84, found: m/z $[M+H]^+$1003.86.

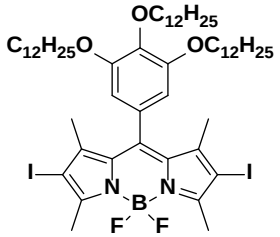
4,4-bis-(3-trimethylaminoiodine-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-b ora-3a,4a-diaza-s-indacene (1)



Compound **5** (0.10 g) was stirred with iodomethane (3-5 equiv.) in refluxing dry diethyl ether for 24 h. After cooling to room temperature, the solid precipitation in the reaction mixture was isolated by filtration, and then slowly precipitated again from CH_2Cl_2/Et_2O to give an orange colored powder (0.12 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$, 300K, TMS): δ = 6.50 (*s*, 2H), 6.12 (*s*, 2H), 4.71 (*s*, 4H), 4.02 (*s*, 2H), 3.94 (*t*, *J* = 5.70 Hz, 4H), 3.53 (*s*, 18H), 2.72 (*s*, 6H), 1.80-1.75 (*m*, 6H), 1.57 (*s*, 6H), 1.48-1.44 (*m*, 6H), 1.25 (*m*, 48H), 0.92-0.84 (*m*, 9H). ^{13}C NMR (126 MHz, CD_3OD): δ = 156.21,

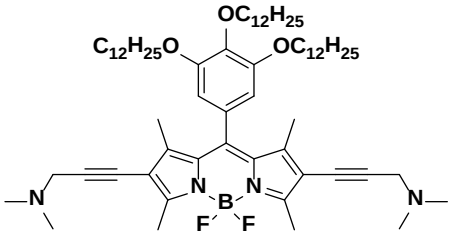
144.18, 143.71, 140.68, 133.16, 131.12, 129.03, 122.96, 111.12, 84.32, 66.87, 58.51, 53.37, 21.43, 16.72, 14.88. HRMS (ESI, positive mode): calculated for $C_{67}H_{113}BN_4O_3^{2+}$ 1032.8895, found: m/z $[M]^{2+}$ 516.4491. Elemental analysis: Anal. calcd for $C_{67}H_{113}BN_4O_3I_2$: C 62.51%, H 8.85%, N 4.35%; found: C 62.41%, H 8.81%, N 4.19%. UV/Vis (DMSO): $\lambda_{max}(\varepsilon)$ = 365 (6120 mol^{-1} L cm^{-1}), 471 (17400 mol^{-1} L cm^{-1}), 501 (89300 mol^{-1} L cm^{-1}). Fluorescence (DMSO): λ_{max} = 510 nm; quantum yield: Φ_{fl} = 0.17.

4,4-difluoro-2,6-diiodo-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (6)



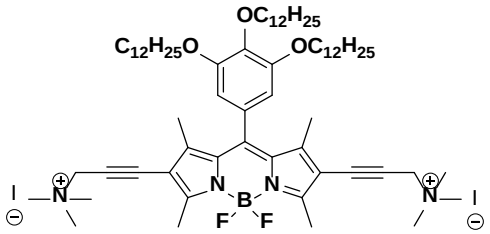
Compound **4** (0.44 g, 0.5 mmol) and Iodine (0.13 g, 0.5 mmol) were dissolved in ethanol (40 mL). 0.5 mL aqueous solution containing HIO_3 (0.19 g, 1.04 mmol) was added into above mixture dropwisely. The reaction was continued for about 3 h under 60 °C until complete consumption of the starting material (observed by TLC, CH_2Cl_2/*n*-Hexane = 4/1, v/v). Saturated NaS_2O_3 solution was added and the mixture was extracted with CH_2Cl_2 twice. The combined organic phase was dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/*n*-Hexane = 8/1, v/v) to give dark red solid (0.43 g, 76 %). ^{1}H NMR (400 MHz, $CDCl_3$, 300K, TMS) : δ = 6.42 (*s*, 2H), 4.03 (*dd*, *J* = 9.08, 3.37 Hz, 2H), 3.91 (*t*, *J* = 5.79 Hz, 4H), 2.64 (*s*, 6H), 1.77 (*dd*, *J* = 13.82, 6.88 Hz, 6H),1.54 (*s*, 6H), 1.47 (*dd*, *J* = 32.31, 6.65 Hz, 6H), 1.26 (*m*, 48H), 0.87 (*m*, 9H).

4,4-difluoro-2,6-bis-(3-dimethylamino-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (7)



To a degassed solution of compound **6** (0.20 g, 0.18 mmol) in benzene (2 mL), were added triethylamine (2 mL), $[Pd(PPh_3)_2Cl_2]$ (0.008 g, 0.012 mmol), CuI (0.003 g, 0.012 mmol), and 1-dimethylamino-2-propyne (0.034 g, 0.49 mmol). The mixture was stirred at 50 °C over night. The mixture was diluted with CH_2Cl_2. After evaporating the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1, v/v) to give a red solid (0.16 g, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$, 300K, TMS): δ = 6.43 (*s*, 2H), 4.02 (*t*, *J*=6.50 Hz, 2H), 3.90 (*t*, *J* = 6.50 Hz, 4H), 3.51 (*s*, 4H), 2.63 (*s*, 6H), 2.33 (*s*, 12H), 1.82-1.74 (*m*, 6H), 1.61 (*s*, 6H),1.48-1.39 (*s*, 6H), 1.26 (*m*, 48H), 0.88 (*m*, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 158.28, 154.37, 144.31, 142.64, 138.97, 131.04, 129.14, 116.14, 106.25, 91.41, 73.88, 69.59, 48.91, 44.20, 32.06, 30.42, 29.89-29.75, 29.54-29.44, 26.28-26.16, 22.83, 14.26, 13.75, 13.41. MS (ESI, positive mode): calculated for $C_{65}H_{105}BF_2N_4O_3$ 1038.82, found: m/z $[M+2H]^{2+}$ 520.42.

4,4-difluoro-2,6-bis-(3-trimethylaminoiodine-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (2)



Compound **6** (0.13 g) were stirred with iodomethane (10 equiv., 0.18 g, 1.3 mmol) in refluxing dry diethyl ether until no more solid appeared (ca. 24 h). After cooling to room temperature, the precipitation was isolated by filtration, and then slowly precipitated again from MeOH/Et_2O to give a dark red powder (0.10 g, 61%). ^{1}H NMR (400 MHz, CD_3OD, 300K, TMS): δ = 6.64 (*s*, 2H), 4.63 (*s*, 4H), 4.00 (*td*, *J* = 17.87, 6.20 Hz, 6H), 3.26 (*s*, 18H), 2.64 (*s*, 6H), 1.83-1.74 (*m*, 6H), 1.72 (*s*, 6H), 1.52 (*dd*, *J* = 14.34, 7.26 Hz, 6H), 1.37-1.22 (*m*, 48H), 0.90 (*m*, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 160.33, 155.92, 147.98, 146.23, 140.18, 132.48, 129.91, 114.84, 107.21, 85.15, 74.82, 73.87, 70.54, 64.43, 58.29 , 53.35, 33.11, 31.41, 30.93-30.51, 27.35-27.31, 23.76, 14.47, 13.79. HRMS (ESI, positive mode): calculated for $C_{67}H_{111}BF_2N_4O_3^{2+}$1068.8706, found: m/z $[M]^{2+}$534.4395. Elemental analysis: Anal. calcd for $C_{67}H_{111}BF_2N_4O_3I_2$: C 60.81%, H 8.46%, N 4.23%; found: C 60.66%, H 8.44%, N 3.94%. UV/Vis (DMSO): $\lambda_{max}(\varepsilon)$ = 388 (8770 mol^{-1} L cm^{-1}), 507 (24000 mol^{-1} L cm^{-1}), 538 (75400 mol^{-1} L cm^{-1}). Fluorescence (DMSO): λ_{max} = 558 nm; quantum yield: Φ_{fl} = 0.23.

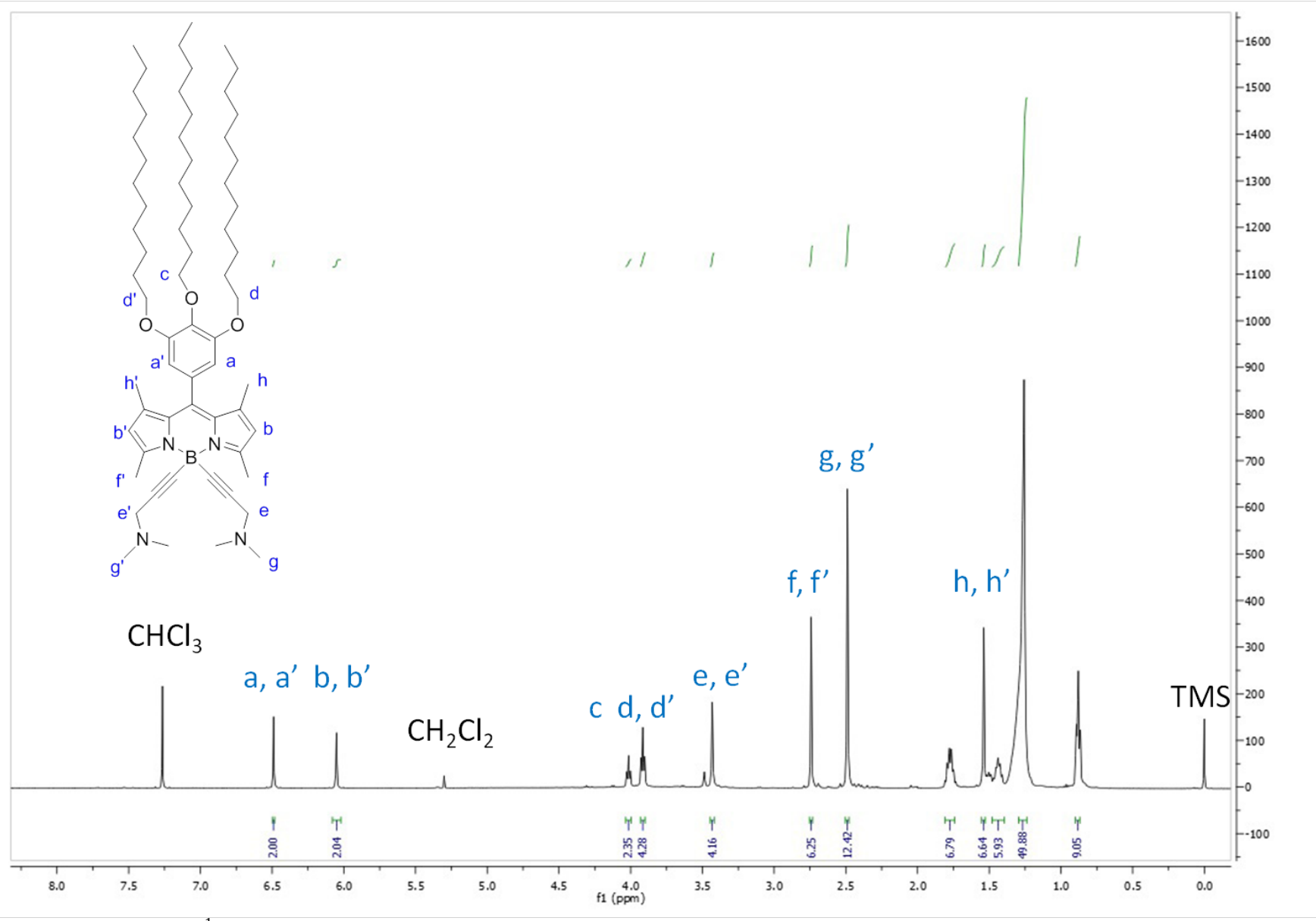


Fig. S1 ^{1}H NMR spectrum with corresponding assignments and chemical structure of compound **5** in $CDCl_3$.

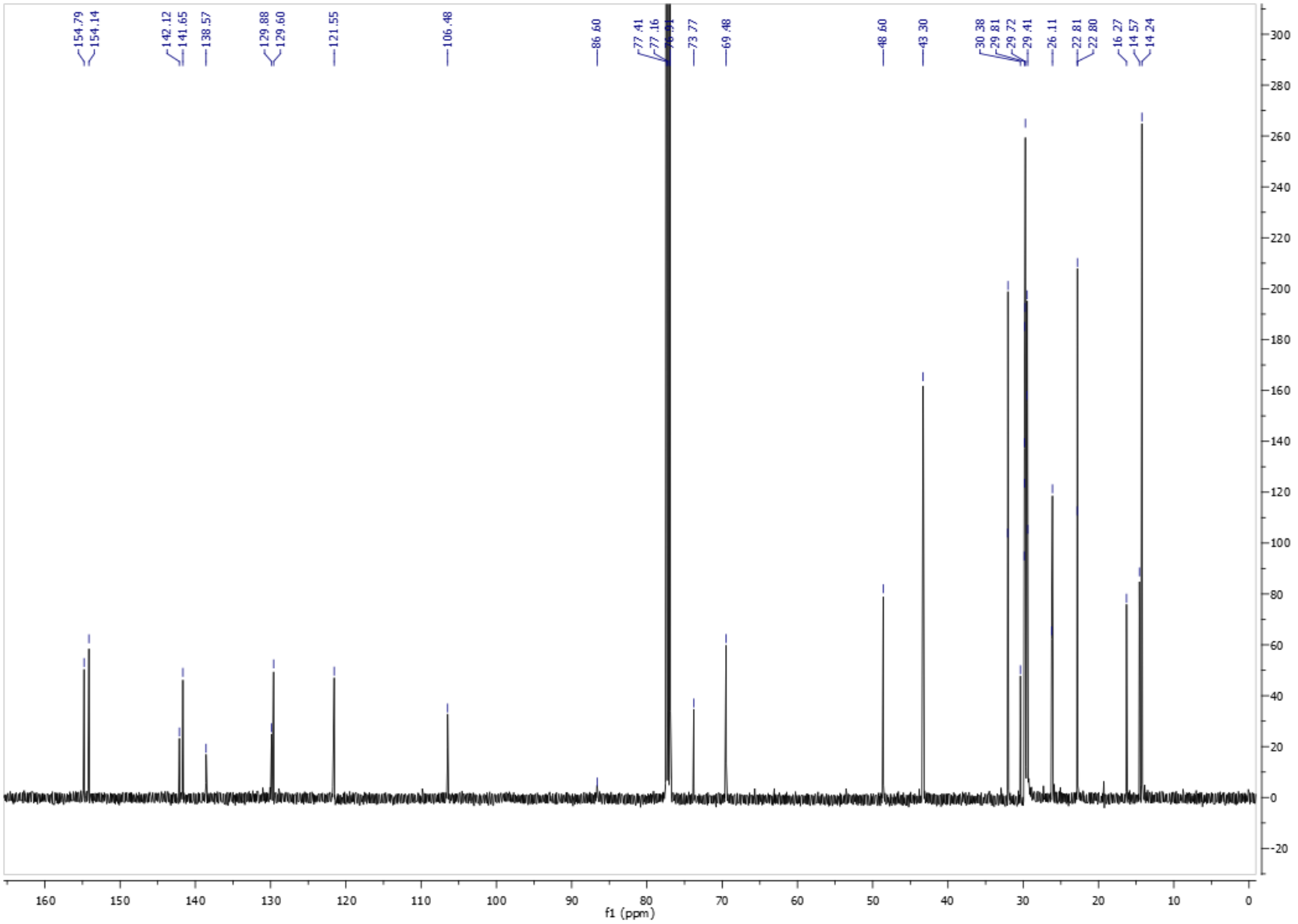


Fig. S2 ^{13}C NMR spectrum of compound **5** in $CDCl_3$.

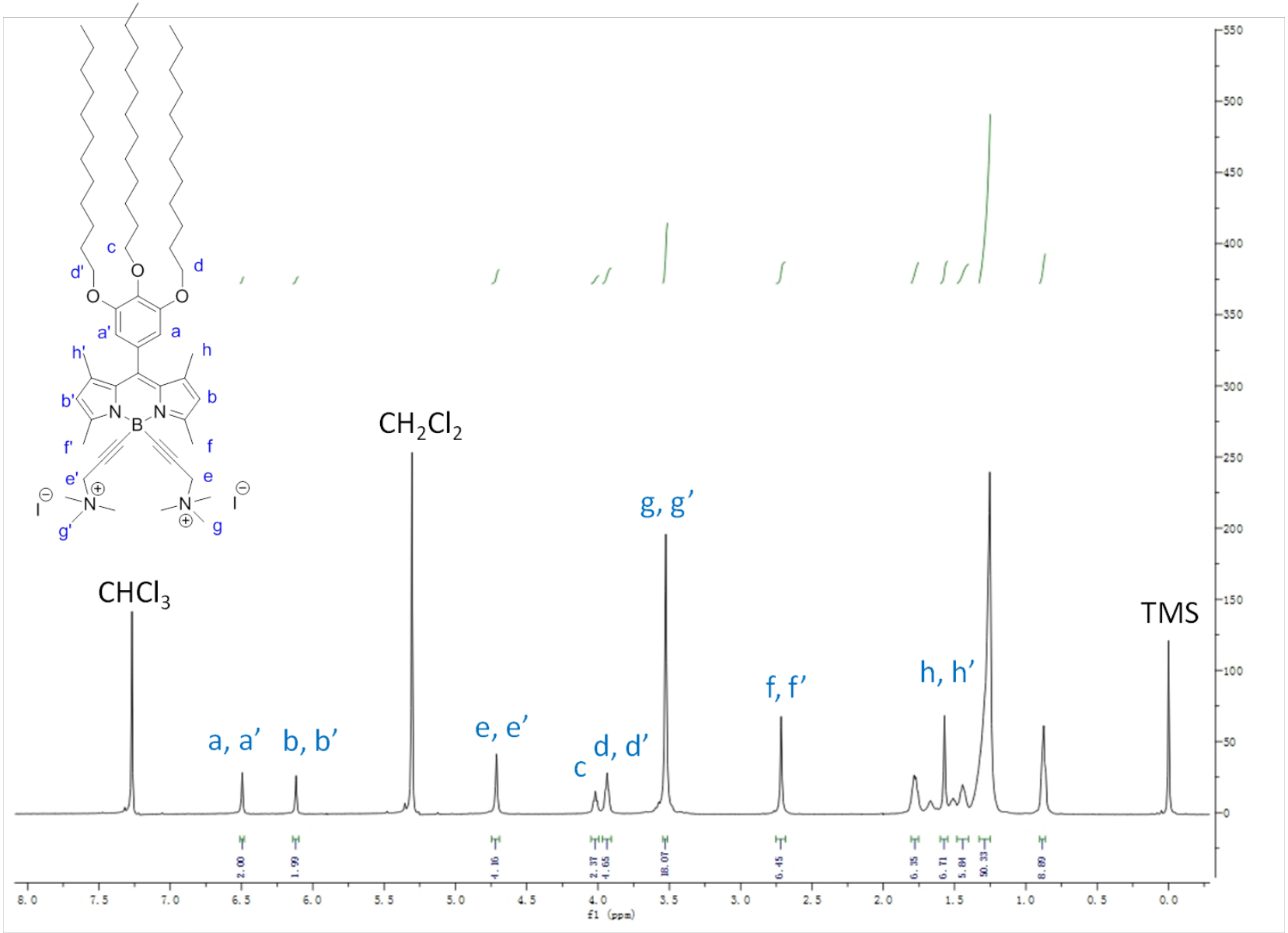


Fig. S3 ^{1}H NMR spectrum with corresponding assignments and chemical structure of BODIPY **1** in $CDCl_3$.

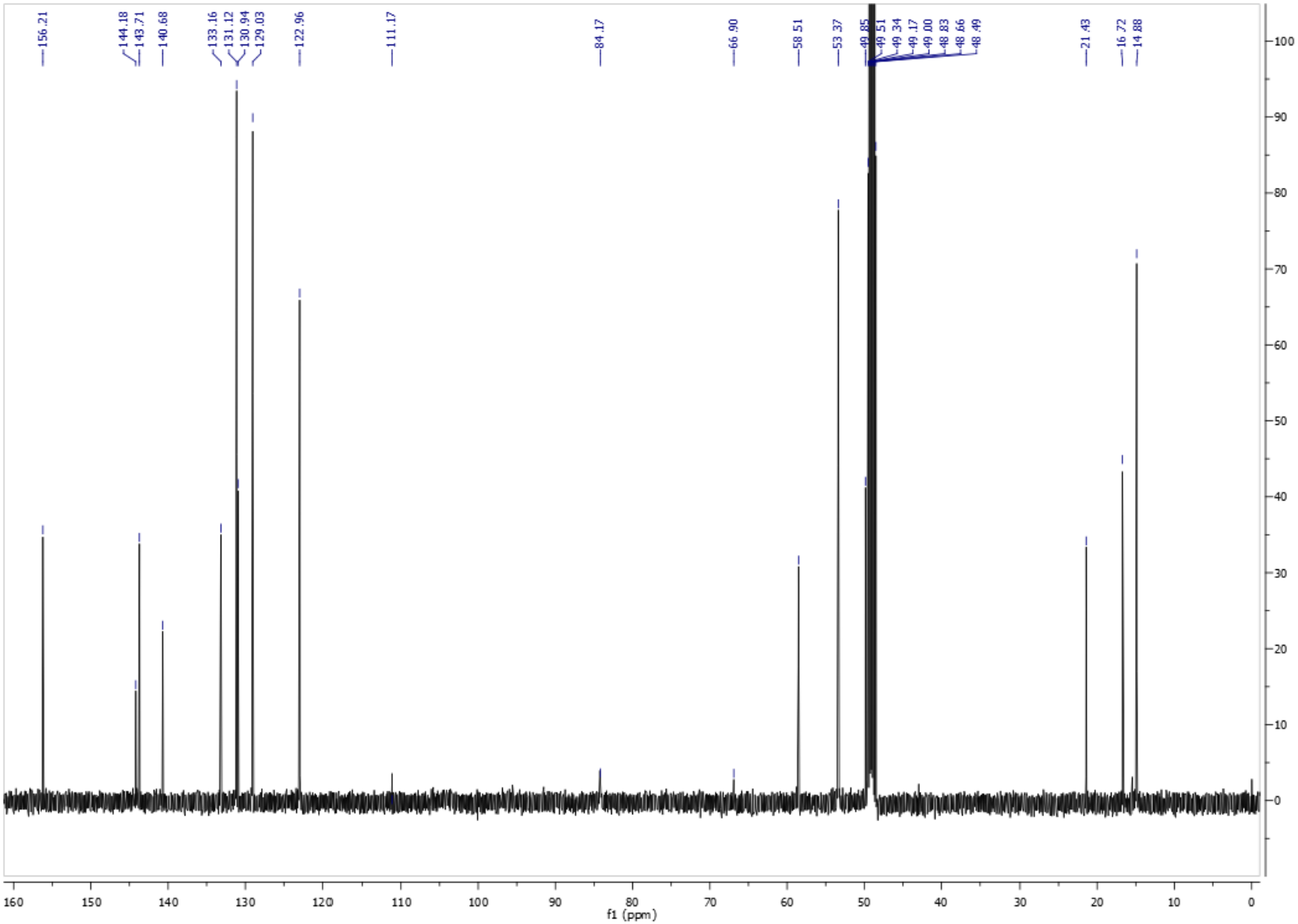


Fig. S4 ^{13}C NMR spectrum of BODIPY **1** in CD_3OD.

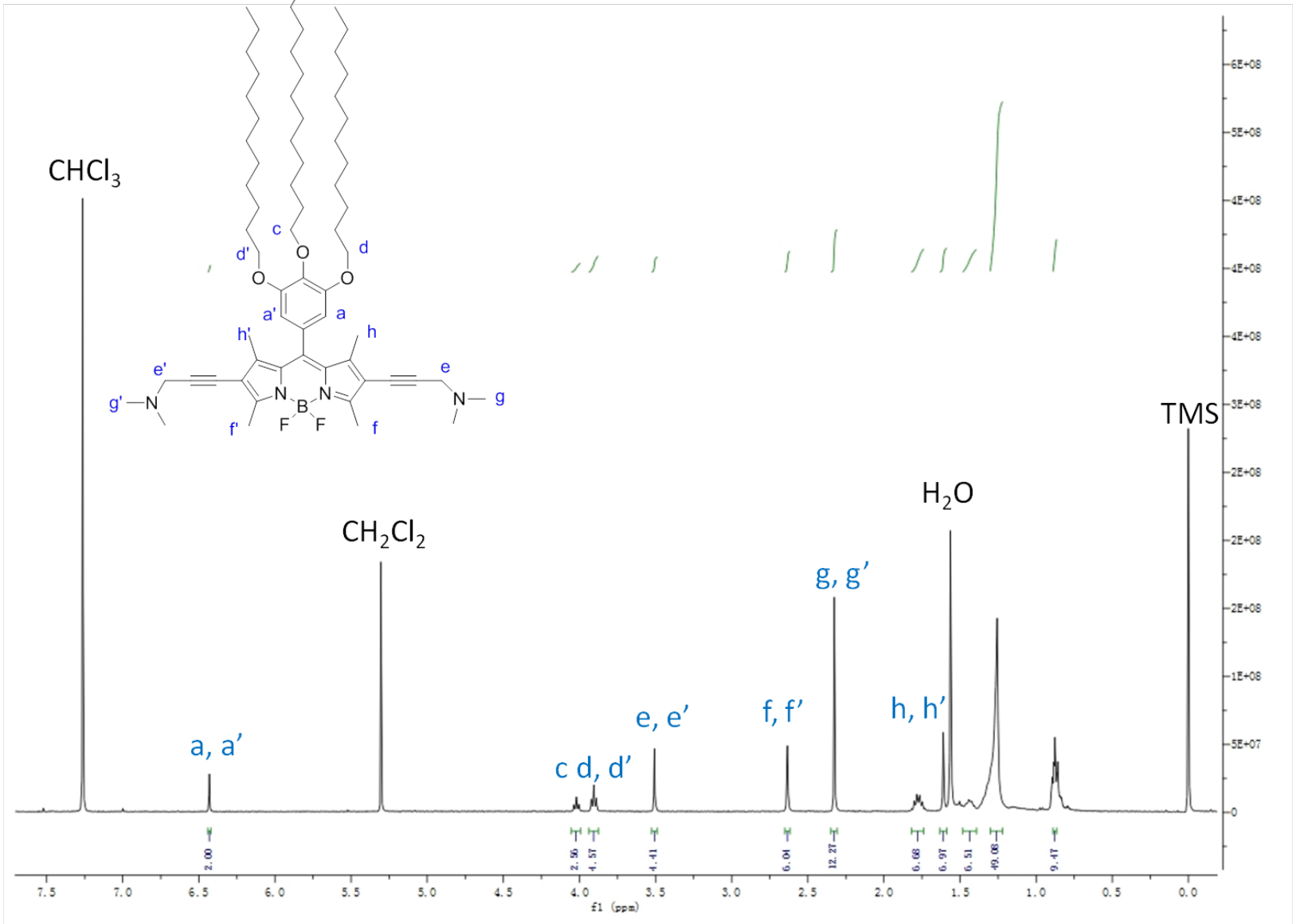


Fig. S5 ^{1}H NMR spectrum with corresponding assignments and chemical structure of compound **7** in $CDCl_3$.

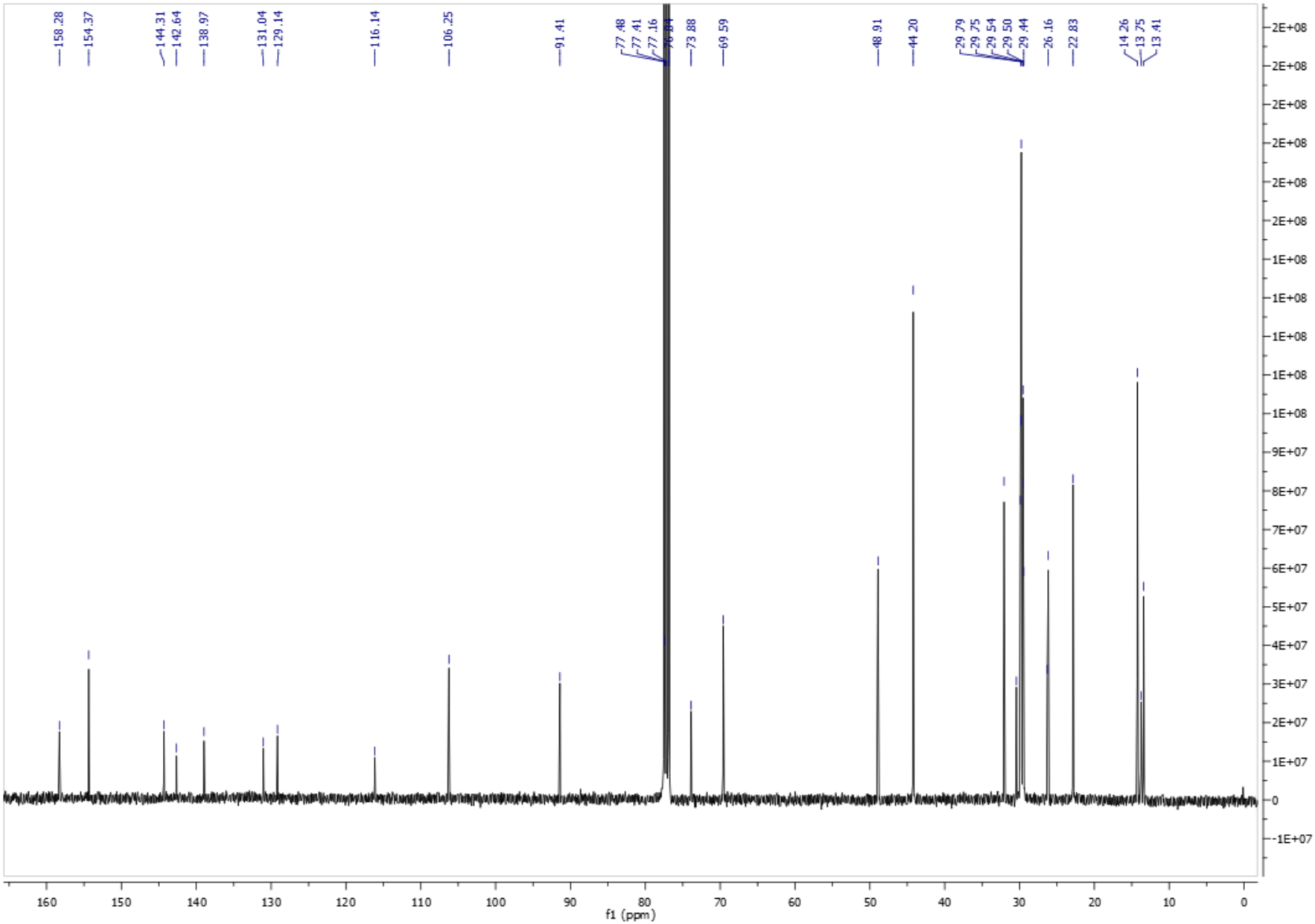


Fig. S6 ^{13}C NMR spectrum of compound **7** in $CDCl_3$.

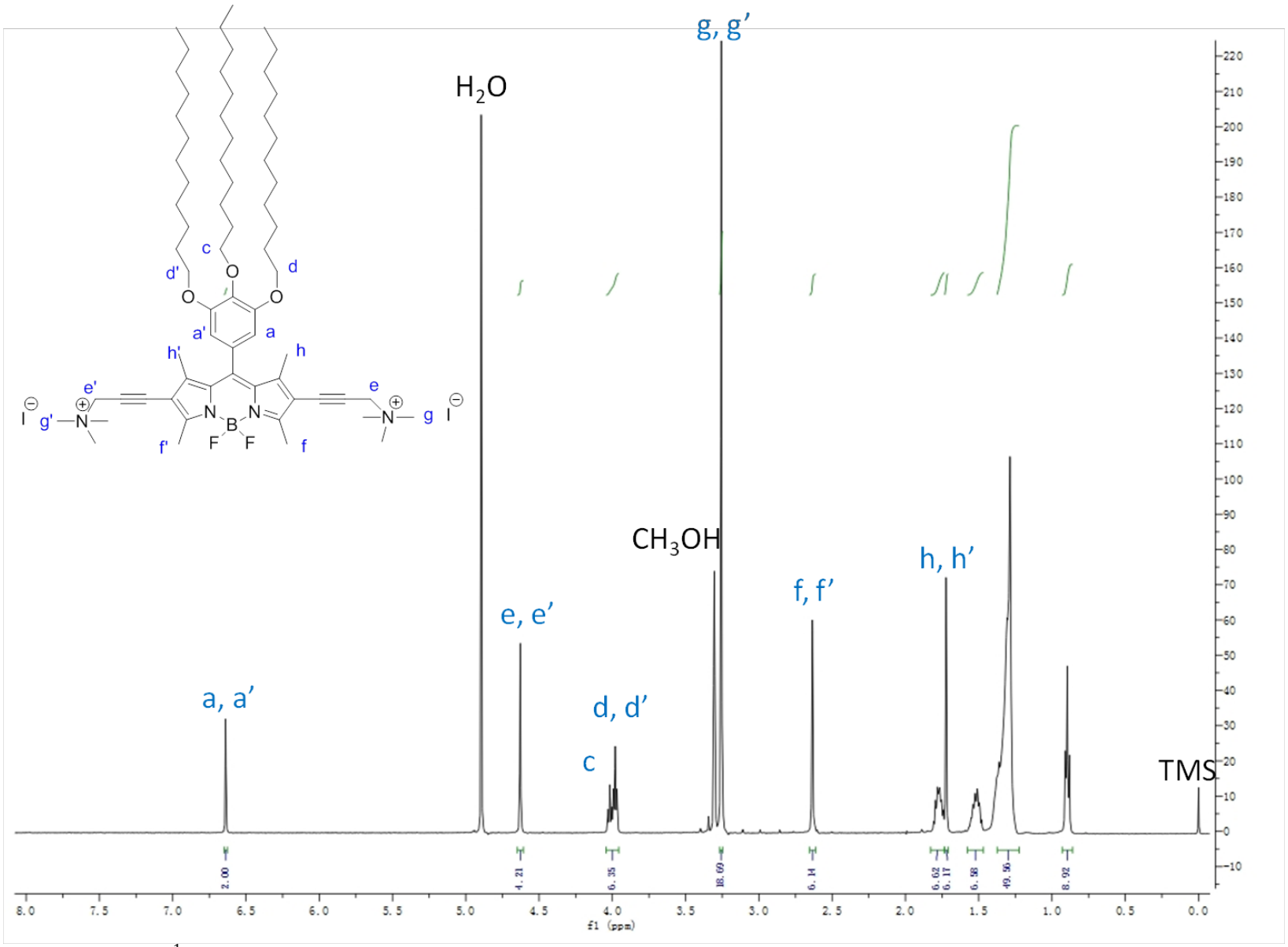


Fig. S7 ^{1}H NMR spectrum with corresponding assignments and chemical structure of BODIPY **2** in CD_3OD.

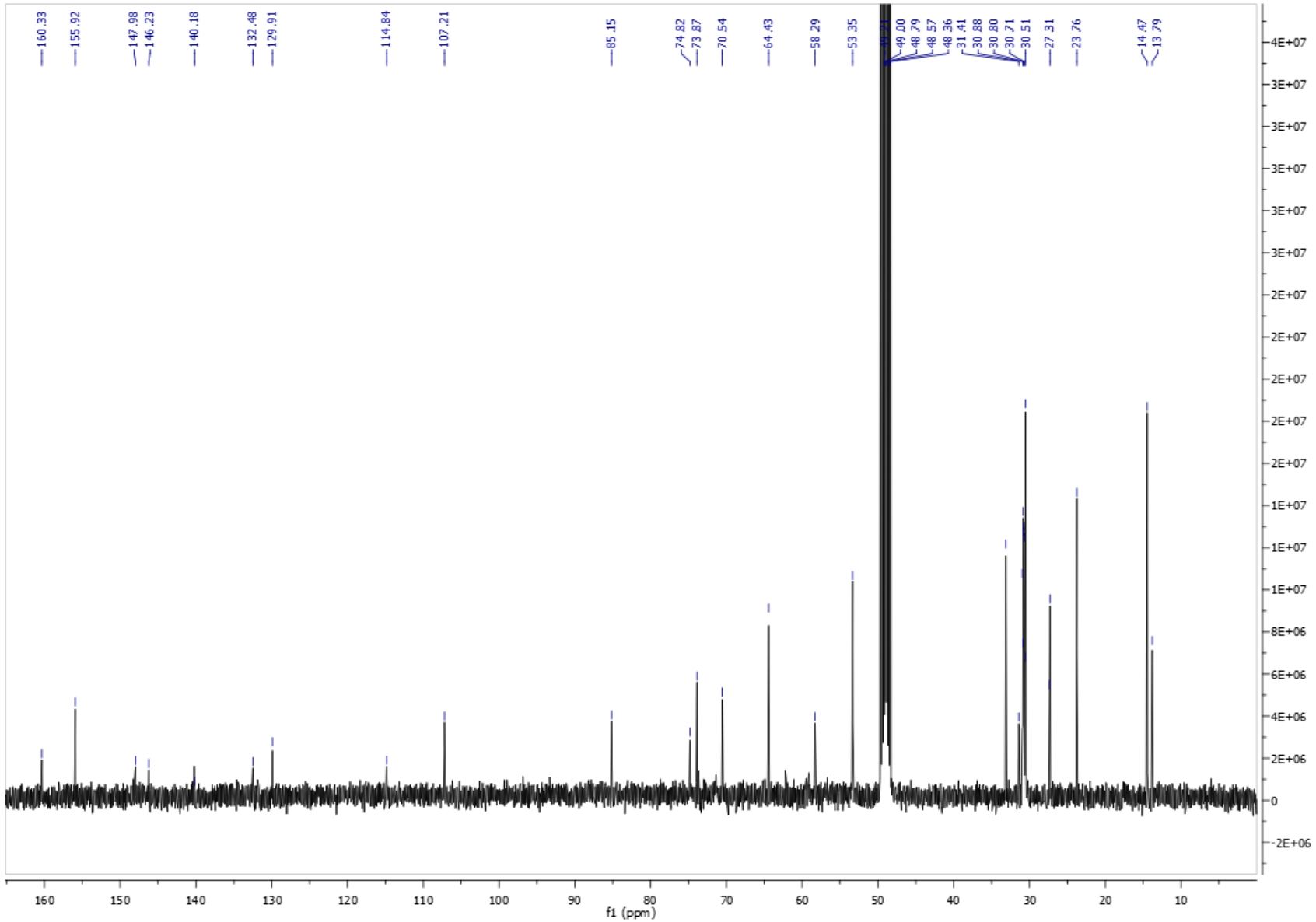


Fig. S8 ^{13}C NMR spectrum of BODIPY **2** in CD_3OD.

3. Aggregation studies

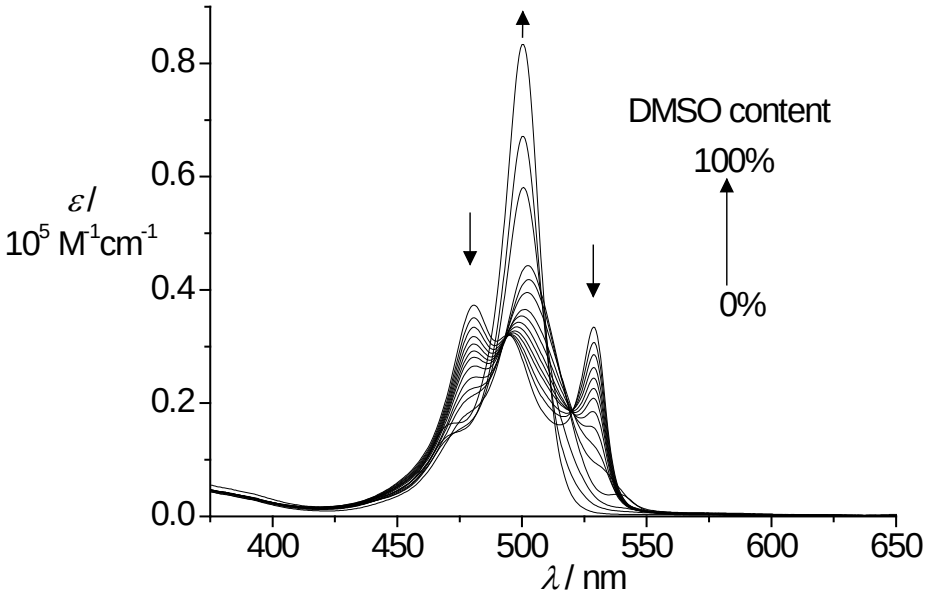


Fig. S9 UV/Vis absorption spectra of **1** (1.0×10^{-5} M) in DMSO-containing water with increasing DMSO content (0% to 100%, v/v).

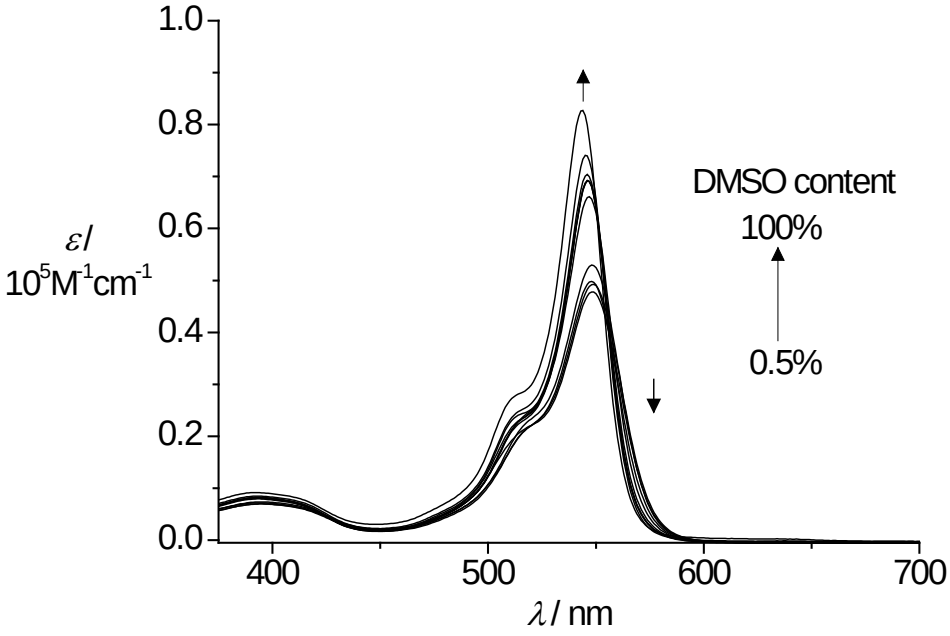


Fig. S10 UV/Vis absorption spectra of **2** (1.0×10^{-5} M) in DMSO-containing water with increasing DMSO content (0.5% to 100%, v/v).

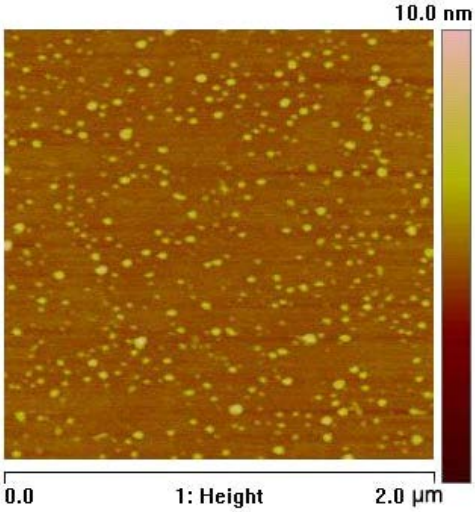


Fig. S11 Tapping mode atomic force microscopy height images of co-aggregates of **1** and **2** ([**1**]/[**2**] = 1/5, [**2**] = 0.2 mg/mL) on mica.

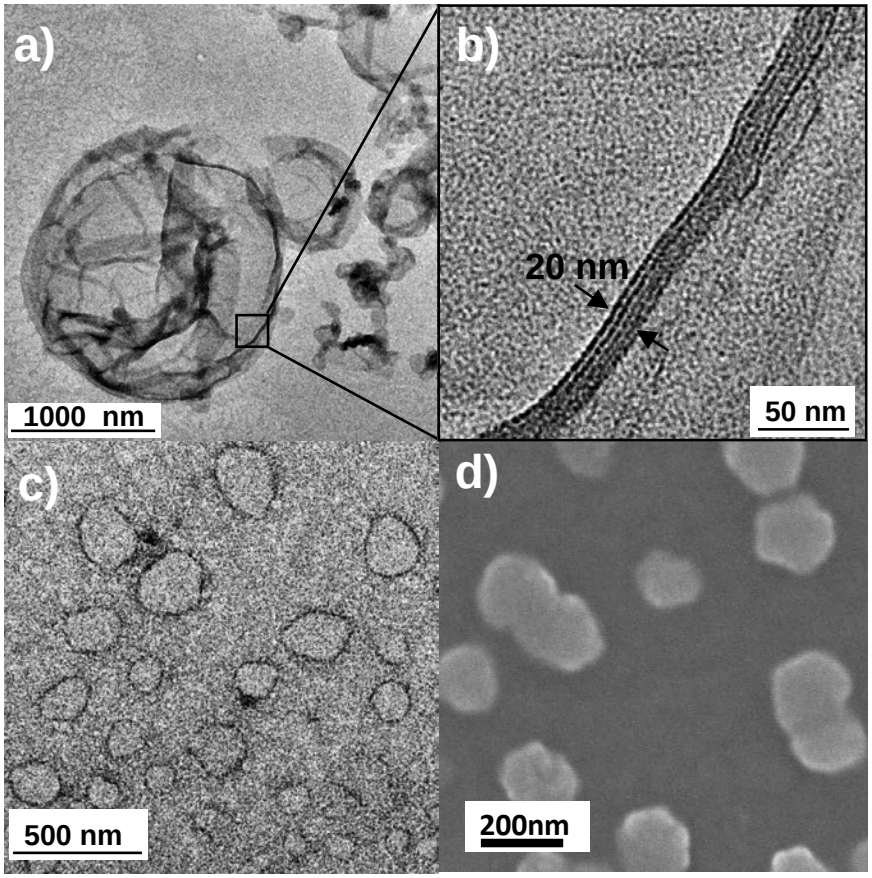


Fig. S12 (a) TEM images of co-assemblies of dye **1** and **2** prepared in DMSO-containing water (0.5% DMSO, v/v), [**1**]/[**2**] = 1/1, [**2**] = 0.5 mg/mL; (b) Local enlargement of image (a); (c) TEM images of co-assemblies of dye **1** and **2** prepared in DMSO-containing water (0.5% DMSO, v/v) [**1**]/[**2**] = 1/3, [**2**] = 0.5 mg/mL; (d) SEM images of co-aggregates of **1** and **2**, [**1**]/[**2**] = 1/5, [**2**] = 0.2 mg/mL.

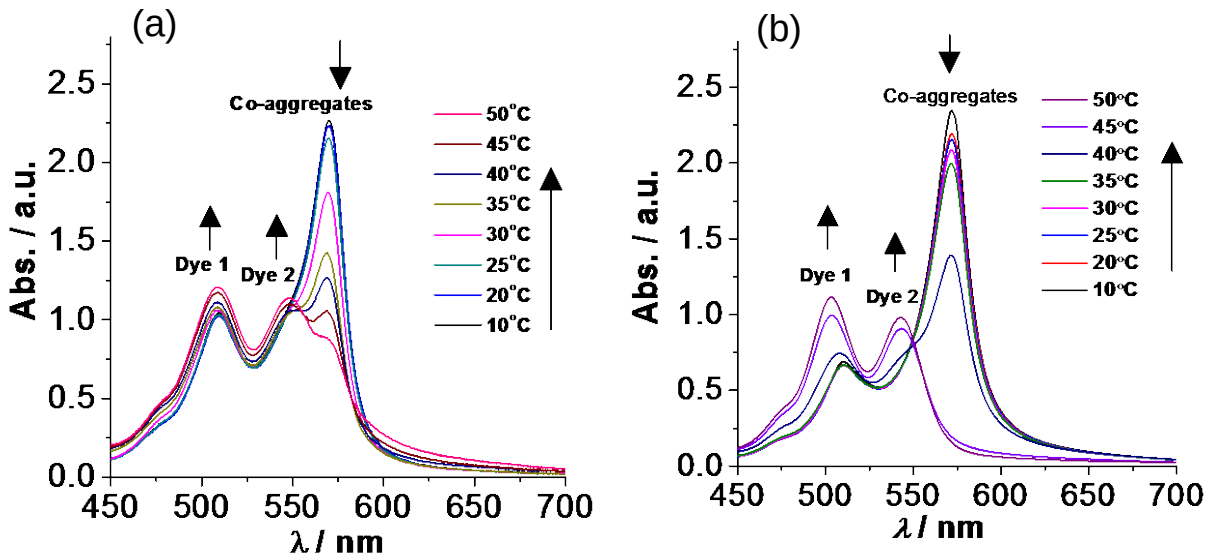


Fig. S13 Temperature-dependent UV/Vis absorption spectra of **1**/**2** mixture in molar ratio of 1:1 ([**1**] = [**2**] = 2.5 × 10^{-4} M) in (a) serum-free medium (containing 0.5% DMSO, v/v) and (b) serum-containing medium (containing 10% fetal bovine serum and 0.5% DMSO, v/v). Arrows indicate spectra changes upon increasing concentrations or temperature.

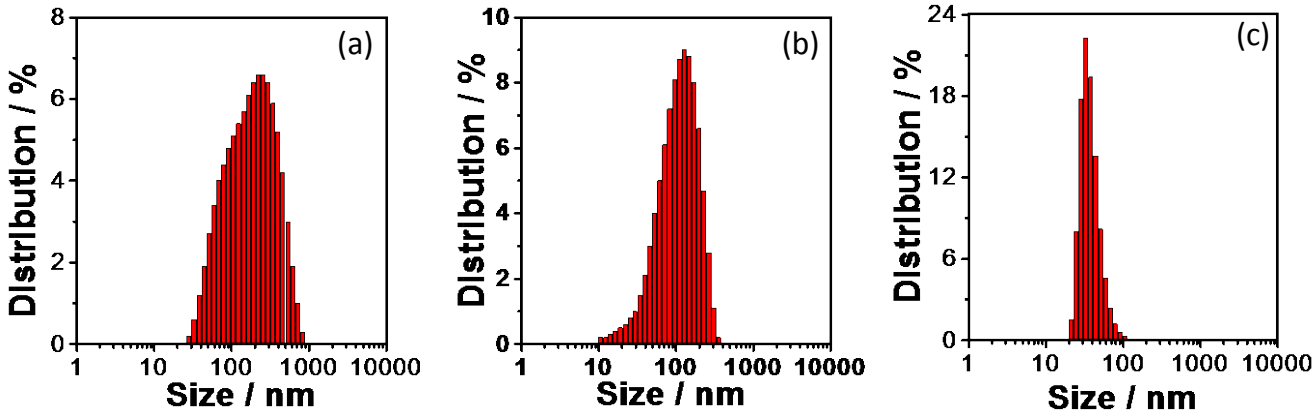


Fig. S14 Size distribution of the co-aggregates of **1** and **2** by DLS measurements in DMSO-containing water (0.5% DMSO, v/v): (a) [**1**]/[**2**] = 1/1, [**2**]= 0.5 g/mL; (b) [**1**]/[**2**] = 1/3, [**2**]= 0.5 g/mL; (c) [**1**]/[**2**] = 1/8, [**2**] = 0.2 mg/mL.

Estimation of critical aggregation concentrations for dyes 1 and 2

The concentration dependent UV/Vis spectra of dyes **1**, **2**, and their co-assemblies were recorded in pure water or DMSO-containing water (0.5%, v/v). Note that dye **2** is not soluble in pure water. The apparent molar absorption coefficients were fitted by nonlinear least-square regression analysis to a simple isodesmic model.[4] The fraction of aggregated molecules α_{agg} was plotted versus the total concentration of the dye. The critical aggregation concentrations (the onset values for the fitting curve) of dyes **1**, **2**, and 1:1 co-assemblies were estimated as 7×10^{-7} M, 2×10^{-7} M, and 3×10^{-7} M, respectively.

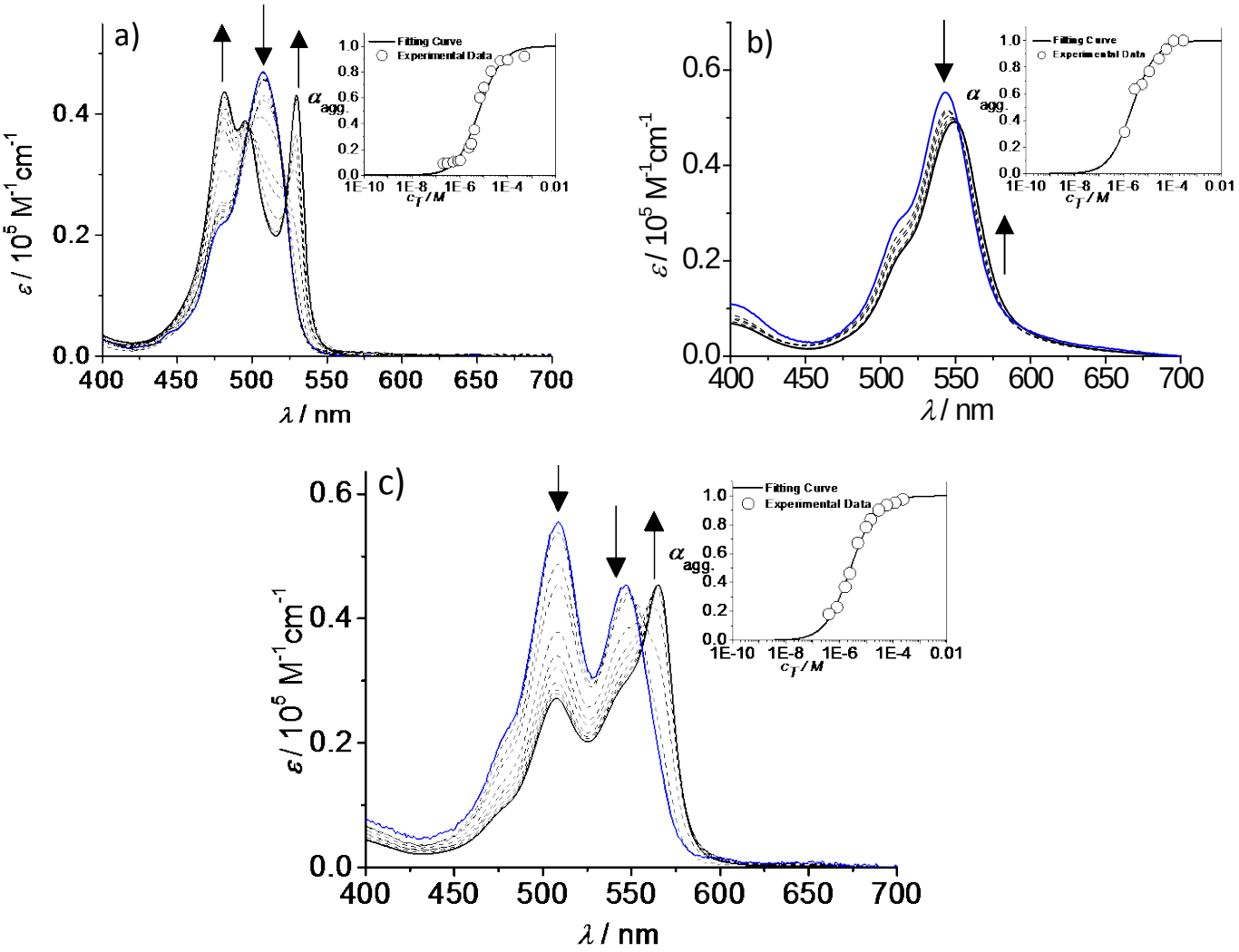


Fig. S15 a) Concentration-dependent UV/Vis absorption spectra of a) dye **1** in water with concentration increasing from 2.0×10^{-7} to 5.0×10^{-4} M, b) dye **2** in DMSO-containing water (0.5%, v/v) with concentration increasing from 1.1×10^{-6} to 2.8×10^{-4} M, and c) **1**/**2** mixture in molar ratio of 1:1 in DMSO-containing water (0.5%, v/v) with concentration increasing from 4.0×10^{-7} to 2.3×10^{-4} M.

Estimation of critical packing parameters (P_c) for dyes 1 and 2

According to Israelachvili,[5] P_c can be estimated from the equation (1),

$$P_c = \frac{v}{a_0 \times l_c} \qquad (1)$$

where v is the volume of an individual molecule, a_0 stands for the optimal area occupied by the hydrophilic part, and l_c stands for the length of the molecule. For dye **1** and **2**, $a_0 = 2 \times 0.47$ nm^2 (for one quaternary ammonium groups,[6] $a_0 = 0.47$ nm^2). For dye **1**, $l_c = 2.5$ nm and for dye **2**, $l_c = 2.3$ nm (based on molecular modeling). The volume of dye **1** and **2** v can be considered as the sum of two parts, *i.e.* the three dodecyl chains (v_1) and the rest of the molecule (v_2). The value of v_1 can be obtained from equation (2),[6]

$$v_1 = 3 \times (27.4 + 26.9n) \times 10^{-3} nm^3 \qquad (2)$$

where n is the number of carbon atoms. For $n = 12$, v_1=1.05 nm^3 can be calculated.

The value of v_2 can be estimated by $v_2 = m / \rho$, where m is the mass of this part and ρ is the density obtained from the crystallographic data of related BODIPYs. Accordingly, v_2 = (1.29 × 10^{-21} g) / (1.4 g/cm^3) = 0.92 nm^3.

The critical packing parameter of dye **1** can be calculated as:

$$P_c = \frac{v}{a_0 \times l_c} = \frac{1.05 + 0.92}{0.94 \times 2.5} = 0.84$$

And for dye **2**:

$$P_c = \frac{v}{a_0 \times l_c} = \frac{1.05 + 0.92}{0.94 \times 2.3} = 0.91$$

4. Cell imaging studies

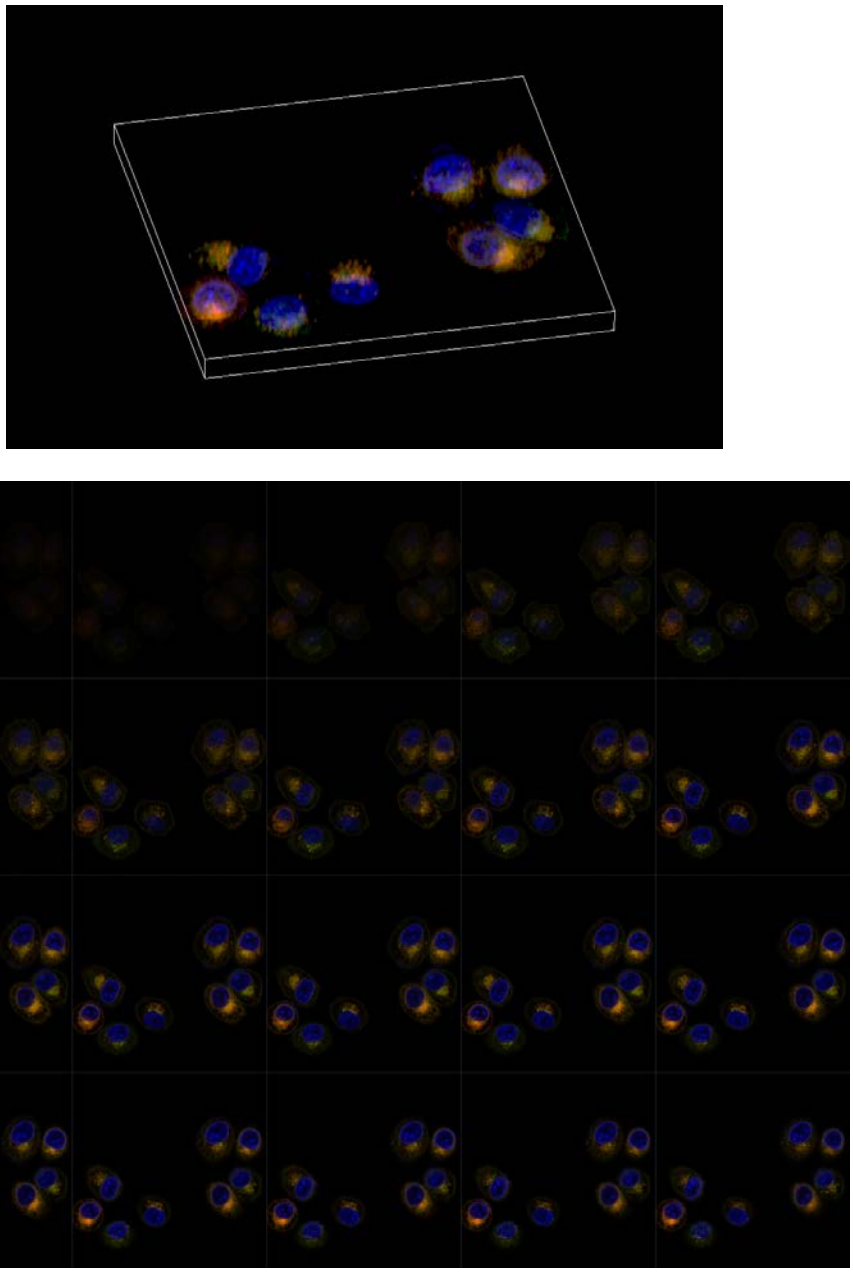


Fig. S16 *Z*-stack confocal images obtained from HeLa cells using dye vesicles as imaging probes and emission was collected in the range of λ_{em} = 525 ± 25 nm (λ_{ex} = 488 nm) and λ_{em} = 575 ± 25 nm (λ_{ex} = 543 nm).

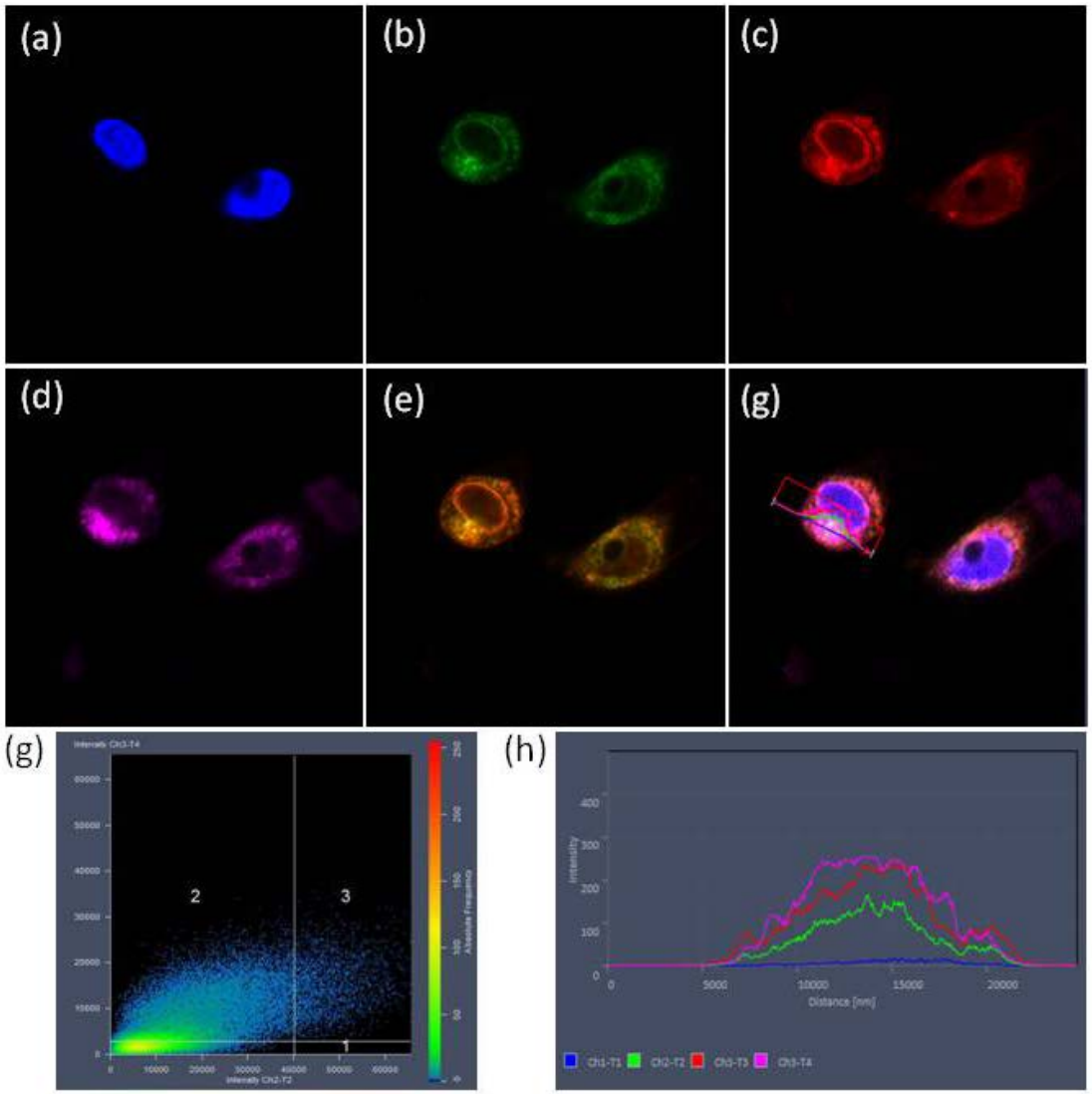


Fig. S17 Confocal microscope images of HeLa cells after incubation with the BODIPY co-aggregates ([1]/[2]=1/5), Lyso Tracker Red and Hoechst 33342. (a) Image for Hoechst. (b) Image for co-aggregates (channel 1, λex = 488 nm, λem = 525 ± 25 nm). (c) Image for co-aggregates (channel 2, λex = 543 nm, λem =575 ± 25 nm). (d) Image for Lyso Tracker Red (channel 3, λex = 647 nm, λem = 668 nm). (e) Merged image of channels 1 and 2. (f) Merged images. (g) Correlation plot of BODIPYs and Lyso Tracker Red emission intensities. Overlap coefficient is 0.76. (h) Normalized intensity profile of ROIs across Hela cells in image.

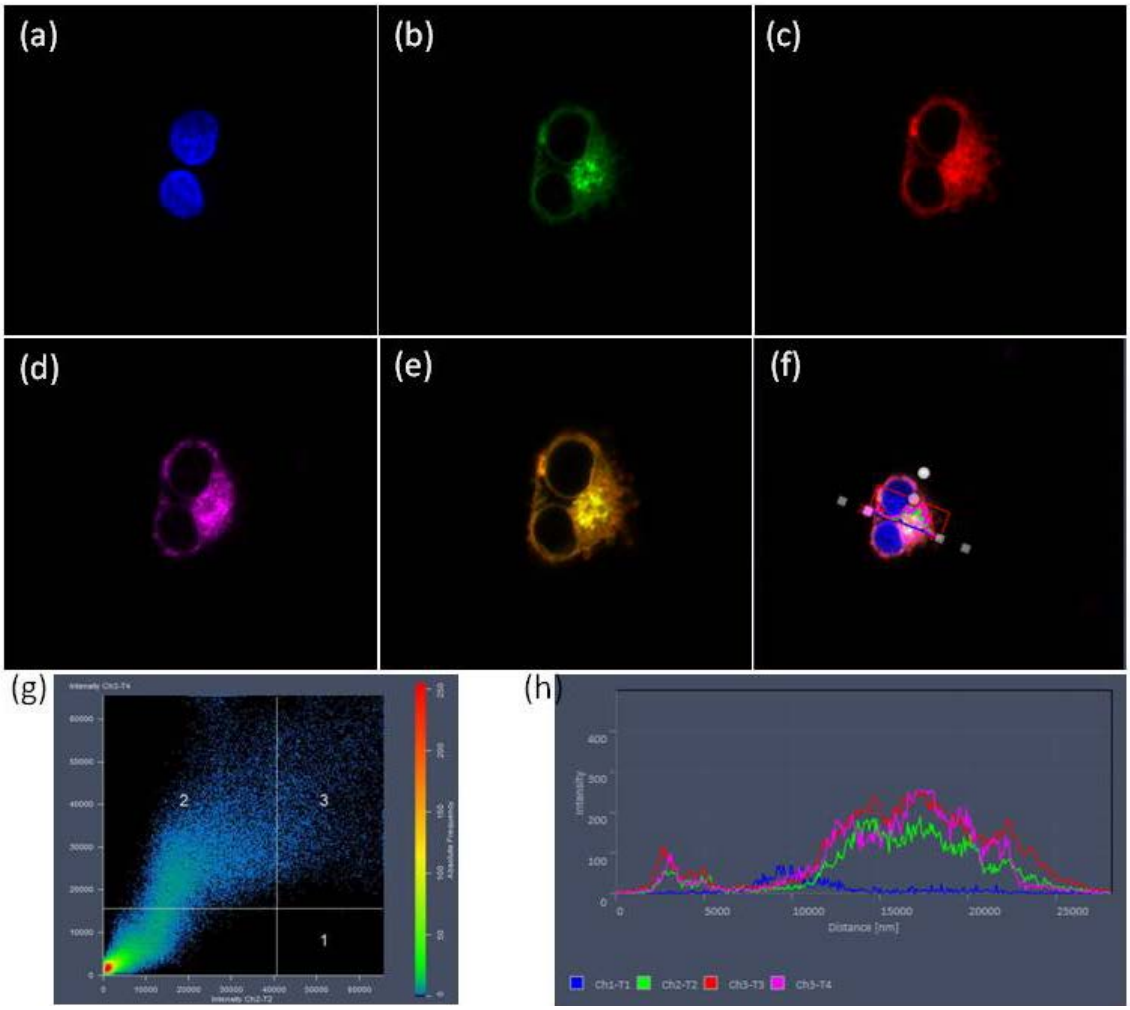


Fig. S18 Confocal microscope images of HeLa cells after incubation with the BODIPY co-aggregates ([**1**]/[**2**]=1/5), Mito Tracker Red and Hoechst 33342. (a) Image for Hoechst. (b) Image for co-aggregates (channel 1, λ_{ex} = 488 nm, λ_{em} = 525 ± 25 nm). (c) Image for co-aggregates (channel 2, λ_{ex} = 543 nm, λ_{em} =575 ± 25 nm). (d) Image for Mito Tracker Red (channel 3, λ_{ex} = 647 nm, λ_{em} = 668 nm). (e) Merged image of channels 1 and 2. (f) Merged images. (g) Correlation plot of BODIPYs and Mito Tracker Red emission intensities. Overlap coefficient is 0.68. (h) Normalized intensity profile of ROIs across Hela cells in image.

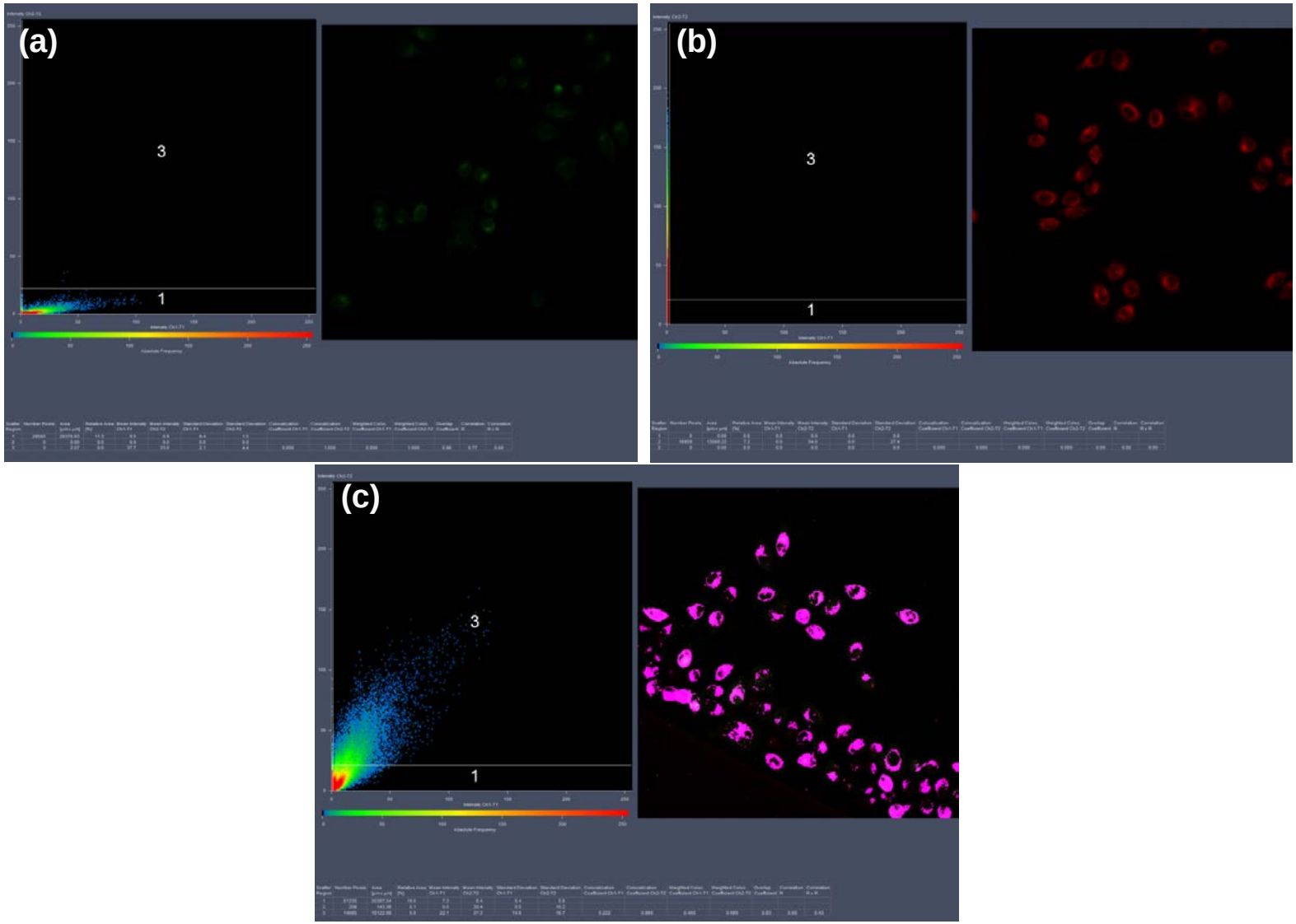


Fig. S19 (a) Image of HeLa cells acquired using dye vesicles ([**1**]/[**2**]=1/5) as imaging probes in simultaneous mode by CLSM (λ_{ex} = 488 nm, λ_{em} = 525 ± 25 nm) and its corresponding correlation plots with colocalization data and values. (b) Image of HeLa cells incubated with ER Tracker Red acquired in simultaneous mode by CLSM (λ_{ex} = 594 nm, λ_{em} = 625 ± 25 nm) and its corresponding correlation plots with colocalization data and values. (c) Image of HeLa cells acquired using both dye vesicles ([**1**]/[**2**]=1/5) and ER Tracker Red as imaging probes in simultaneous mode by CLSM (λ_{ex} = 488 nm, λ_{em} = 525 ± 25 nm; λ_{ex} = 594 nm, λ_{em} = 625 ± 25 nm), and its corresponding correlation plots with colocalization data and values.

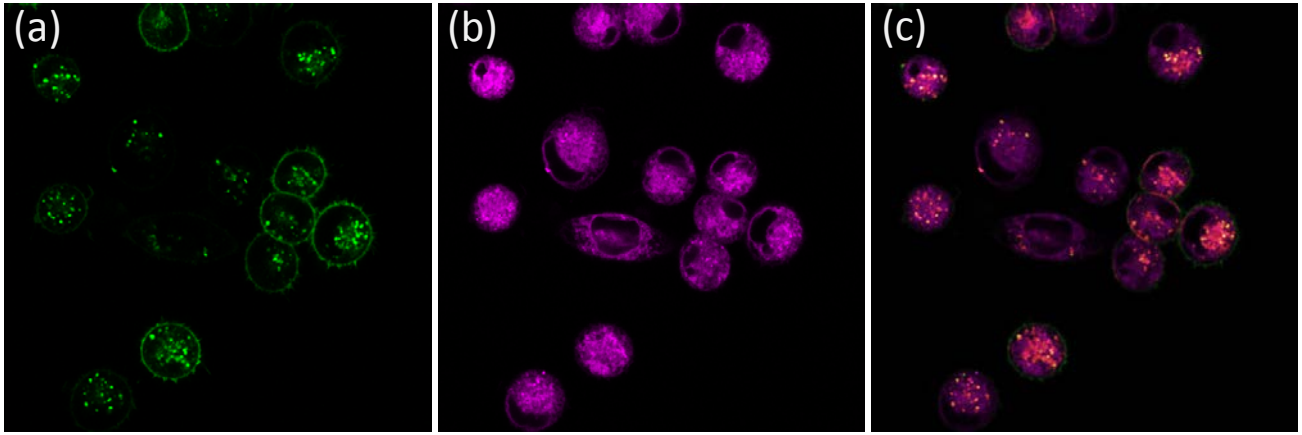


Fig. S20 Confocal microscope images of HeLa cells after incubation with the BODIPY dyes **1** and ER Tracker Red. (a) Image of HeLa cells cultured with dye **1** acquired in simultaneous mode by CLSM (λ_{ex} = 488 nm, λ_{em} = 525±25 nm). (b) Image of HeLa cells incubated with ER Tracker Red acquired in simultaneous mode by CLSM (λ_{ex} = 594 nm, λ_{em} = 625±25 nm). (c) Merged image of dye **1** and ER Tracker Red acquired in simultaneous mode by CLSM (λ_{ex} = 488 nm, λ_{em} = 525±25 nm; λ_{ex} = 594 nm, λ_{em} = 625±25 nm).

Table S1 The cell-imaging data using BODIPY **1** alone as probe.

Scatter Region	Number Pixels	Area [µm x µm]	Relative Area [%]	Mean Intensity Ch2-T2	Mean Intensity Ch2-T4	Standard Deviation Ch2-T2	Standard Deviation Ch2-T4	Colocalization Coefficient Ch2-T2	Colocalization Coefficient Ch2-T4	Weighted Coloc. Coefficient Ch2-T2	Weighted Coloc. Coefficient Ch2-T4	Overlap Coefficient	Correlation R	Correlation R x R
1	12769	221.77	1.2	30.9	13.4	15.5	7.4							
2	107720	1870.9	10.3	4.7	59	4.4	22.5							
3	23483	407.86	2.2	40.6	72.6	34.2	27.3	0.648	0.179	0.707	0.211	0.56	0.18	0.03

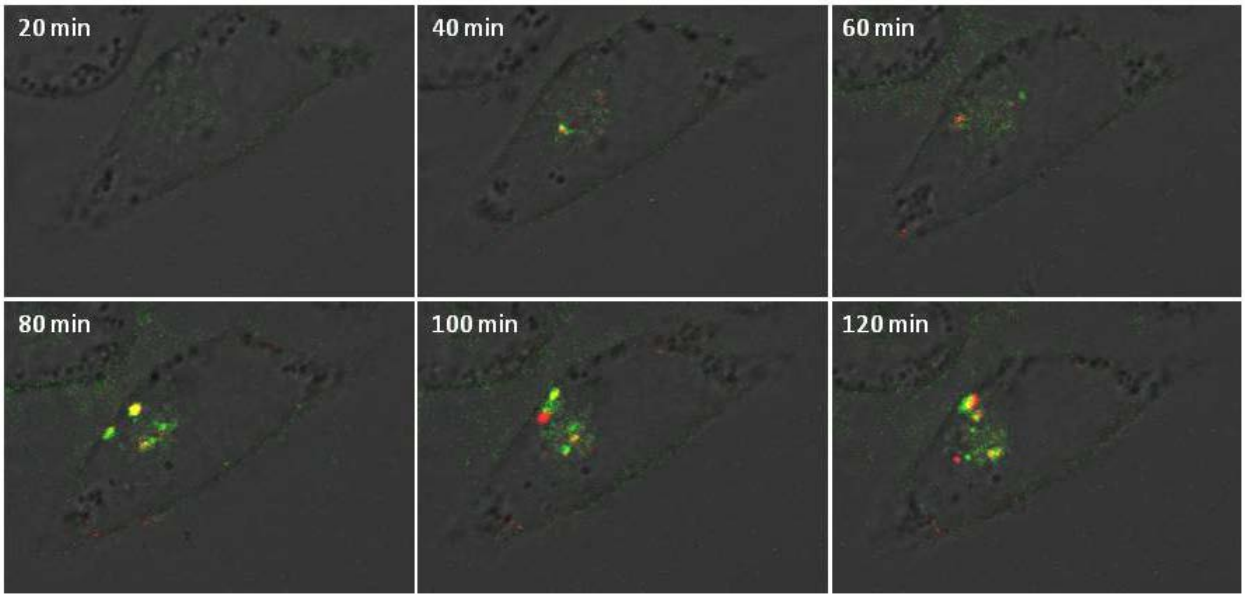


Fig. S21 The real-time confocal microscope images of HeLa cells. The cells were incubated with the BODIPY co-aggregates ([**1**]/[**2**]=1/5) in the 10% serum-contained medium.

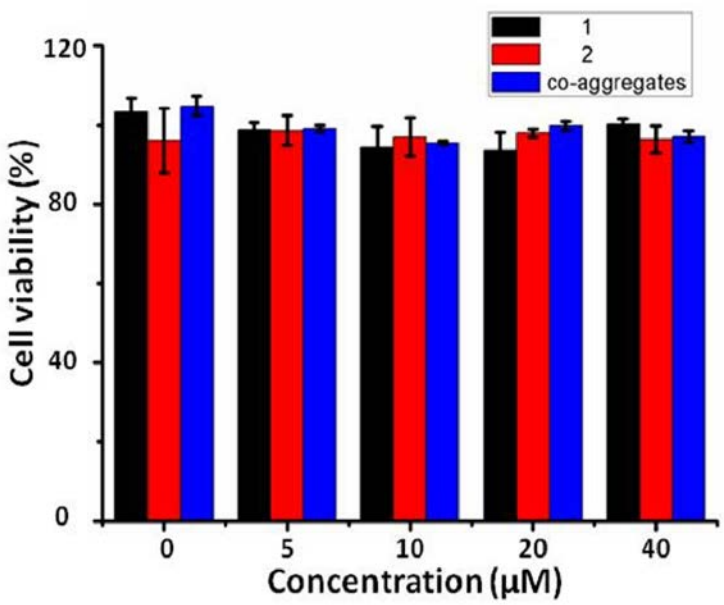


Fig. S22 Cytotoxicity comparisons of BODIPY dyes **1** and **2** at various concentrations (5 μM,10 μM, 20 μM, 40 μM) in living Hela cells for incubation of 24 h.

5. References

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6. E. Yu. Bryleva, N. A. Vodolazkaya, N. O. Mchedlov-Petrossyan, L.V. Samokhina and N.A. Matveevskaya, *Functional Materials*, 2006, **13**, 662.