Supporting Information for

A Benzofuran-[*b*]-Fused BODIPY Trimer Enabled by dual TBET and PET Mechanisms for High-Performance Two-Photon Fluorescence Imaging

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Contents:

1. General information

Reagents and solvents were used as received from commercial suppliers (Energy Chemicals, Shanghai, China; Leyan, Shanghai, China) unless noted otherwise. All reactions were performed in oven-dried or flame-dried glassware unless stated otherwise and were monitored by TLC using 0.25 mm silica gel plates with UV indicator (60F-254). ^{1}H and ^{13}C NMR were recorded on a 400 MHz NMR spectrometer at room temperature. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77 ppm for ^{13}C) to internal TMS. High-resolution mass spectra (HRMS) were obtained using ESI-TOF in positive mode.

Absorption and emission measurements.

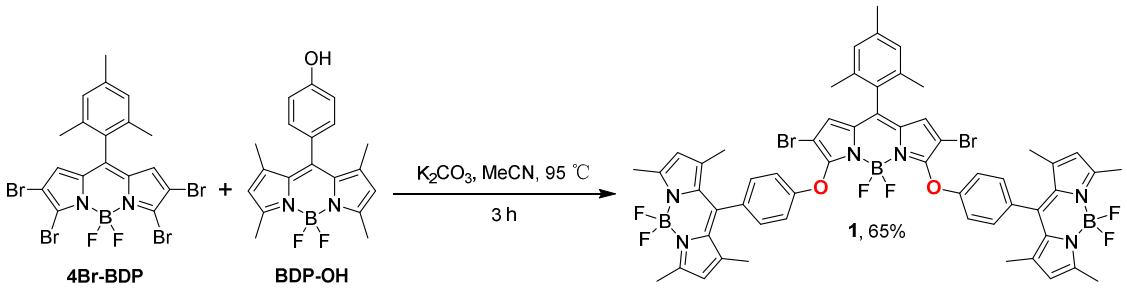
UV-visible absorption and fluorescence emission spectra were recorded on commercial spectrophotometers (Shimadzu UV-2450 and Edinburgh FS5 spectrometers). All measurements were made at 25 ℃, using 5×10 mm cuvettes. Non-degassed, spectroscopic grade solvents and a 10 mm quartz cuvette were used. Absolute fluorescence quantum efficiencies of BODIPY derivatives were measured by absolute PL quantum yield spectrometer (Hamamatsu, C11347) in integrating sphere, using equation 1 given below[1]:

$$\Phi_F = \frac{N_{em}}{N_{abs}} = \frac{\alpha \int \frac{\lambda}{hc} I_{em}(\lambda)\, d\lambda}{\alpha \int \frac{\lambda}{hc} [I_{em}(\lambda) - I'_{ex}(\lambda)]\, d\lambda} \quad \text{.....(1)}$$

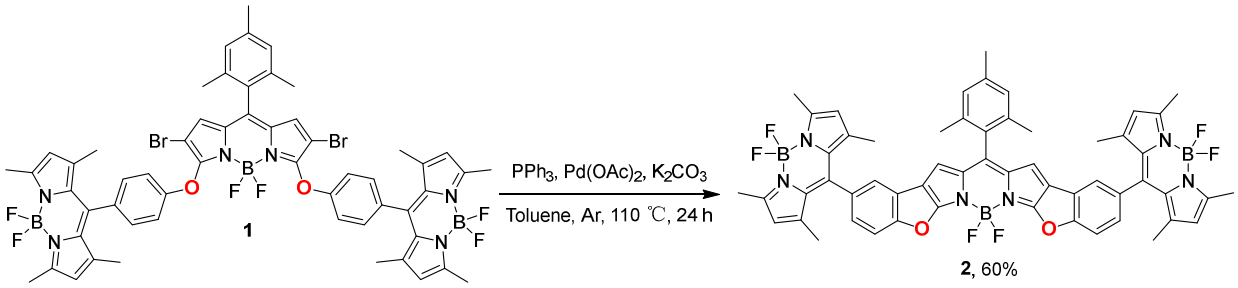
where N_{em} and N_{abs} are the numbers of emitted and absorbed photons, respectively, α is the calibration factor for the measurement setup, λ is the wavelength, h is the Plank's constant, c is the speed of light, $I_{em}(\lambda)$ is the emission intensity at λ, $I_{ex}(\lambda)$ and $I'_{ex}(\lambda)$ are the intensities of the excitation laser beam with λ in the absence and presence of the sample, respectively. The measured Φ_F value is independent of sharp and thickness of sample and power of excitation laser.

2. Synthesis and characterization

BODIPYs **D-BDP, 1-A, 2-A, 4Br-BDP, and BDP-OH** were synthesized according to literatures[2].



Synthesis of 1. To **4Br-BDP** (62 mg, 0.1 mmol) in CH_3CN (5 mL) was added **BDP-OH** (102 mg, 0.3 mmol, 3 equivalent) and K_2CO_3(41 mg, 0.3 mmol, 3 equivalent). The mixture was stirred under 95 ℃ for 3 h. After cooling down to room temperature, the reaction mixture was filtrated to remove excess amounts of K_2CO_3. The organic layer was evaporated to dryness under vacuum and the crude product was purified through column chromatography on silica using petroleum ether/dichloromethane (1:1, v/v) as eluent, from which the desired products trimer **1** (68 mg, 60%) was obtained as red solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 8H), 6.99 (s, 2H), 6.73 (s, 2H), 5.98 (s, 4H), 2.55 (s, 12H), 2.37 (s, 3H), 2.20 (s, 6H), 1.44 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 155.8, 155.7, 155.3, 143.1, 142.9, 140.5, 139.6, 136.7, 131.5, 131.1, 129.7, 128.5, 127.9, 127.5, 121.4, 119.1, 95.7, 21.2, 20.1, 14.9, 14.6 ppm. HRMS (ESI) m/z calcd for $C_{56}H_{49}B_3Br_2F_6N_6O_2Na^+$ $(M+Na)^+$ 1167.2339, found 1167.2355.



Synthesis of 2. To a stirred solution of trimer **1** (20 mg, 0.0175 mmol) in toluene (2 mL), $Pd(OAc)_2$ (0.9 mg, 0.0053 mmol, 0.3 equivalent), triphenylphosphine (2.8 mg, 0.0105 mmol, 0.6 equivalent) and K_2CO_3 (7.3 mg, 0.0525 mmol, 3 equivalent) were added. The resulting suspension was stirred under argon at 110 °C for 24 h. Upon the completion of the reaction, the reaction mixture was poured into dichloromethane (30 mL) and washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residual was purified by chromatography on silica using petroleum ether/dichloromethane (1:1, v/v) as eluent, from which the desired products trimer **2** (11.2 mg, 65%) was obtained as red solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 1.7 Hz, 2H), 7.27 (d, J = 1.7 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H), 7.01 (s, 2H), 6.68 (s, 2H), 5.99 (s, 4H), 2.56 (s, 12H), 2.39 (s, 3H), 2.22 (s, 6H), 1.37 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 160.9, 155.9, 146.5, 143.0, 140.5, 139.3, 136.9, 134.9, 131.6, 128.5, 126.3, 122.3, 121.4, 121.3, 117.9, 117.6, 113.9, 21.2, 20.0, 14.7 ppm. HRMS (ESI) m/z calcd for $C_{56}H_{47}B_3F_6N_6O_2Na^+$ $(M+Na)^+$ 1005.3836, found 1005.3832.

3. Crystal data

X-ray structure analysis.

Crystal of **1** (CCDC 2384326) and **2** (CCDC 2384327) suitable for X-ray analysis was obtained from the slow diffusion of petroleum ether into their concentrated dichloromethane solution. The vial containing this solution was placed, loosely capped, to promote the crystallization. A suitable crystal was chosen and mounted on a glass fiber using grease. Data were collected using a diffractometer equipped with a graphite crystal monochromator situated in the incident beam for data collection at room temperature. Cell parameters were retrieved using SMART[3] software and refined using SAINT on all observed reflections. The determination of unit cell parameters and data collections were performed with Mo *Kα* radiation (λ) at 0.71073 Å. Data reduction was performed using the SAINT software,[4] which corrects for Lp and decay. The structure was solved by the direct method using the SHELXS-97[2] program and refined by least squares method on F^2, SHELXL-2018/3,[5] incorporated in SHELXTL V5.10.[6] These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

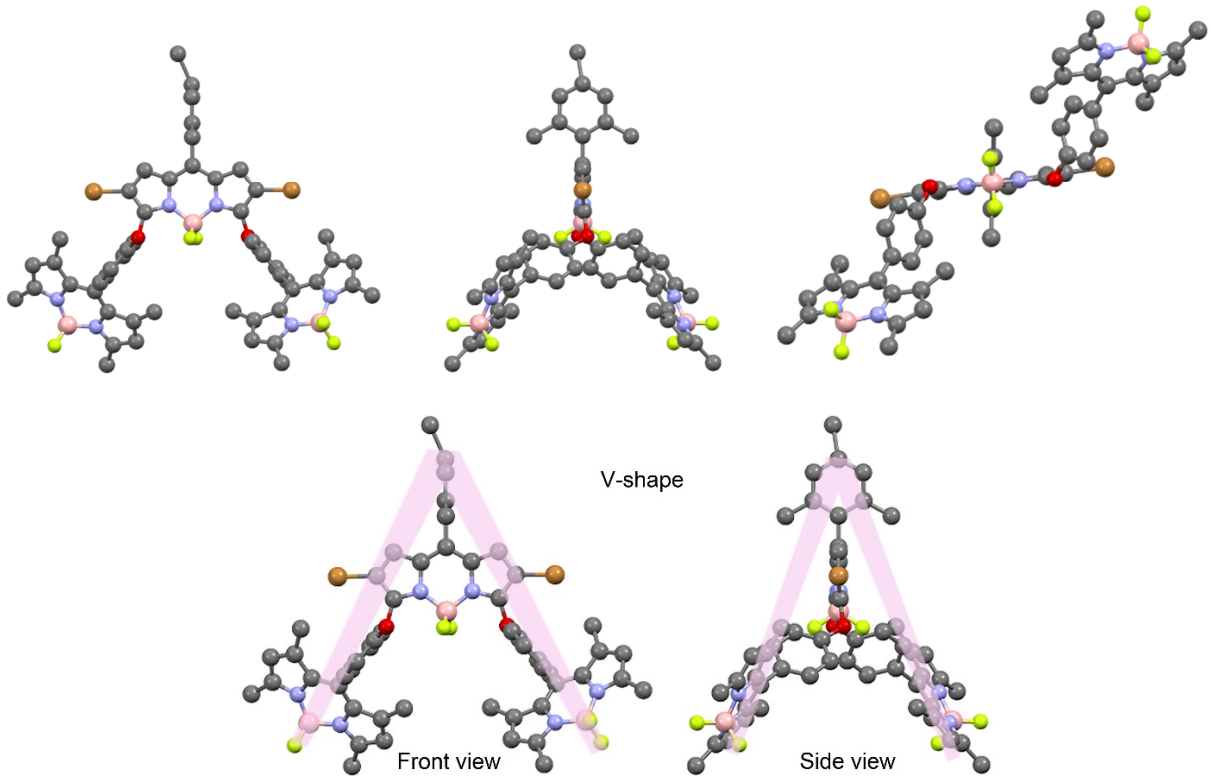


Figure S1: X-ray structures of **1**: C, gray; N, purple; B, pink; O, red; F, yellow; Br, Orange. Hydrogen atoms were removed for clarity.

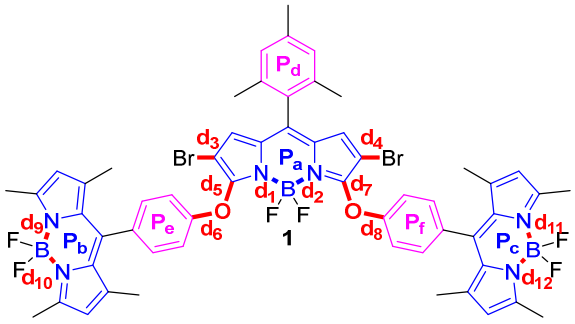
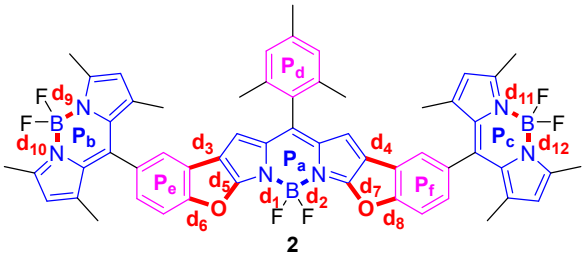


Table S1. Selected bond lengths [Å] and dihedral angles [deg] of **1** obtained from X-ray crystallography.

Parameters	**1**
B-N bond length (Å)	d_1=d_2=1.5502(57) d_9=d_{11}=1.5253(65) d_{10}=d_{12}=1.5468(64)
C-O bond length (Å)	d_5=d_7=1.3517(62) d_6=d_8=1.3759(44)
C-O-C angle	117.306(393)
C-Br bond length (Å)	d_3=d_4=1.8692(52)
Dihedral angles between P_a and P_b (deg)	38.037(79)
Dihedral angles between P_a and P_c (deg)	38.037(79)
Dihedral angles between P_a and P_d (deg)	80.838(330)
Dihedral angles between P_b and P_e (deg)	84.245(247)
Dihedral angles between P_c and P_f (deg)	84.245(247)

Table S2. Crystal data and structure refinements for **1** obtained from crystallography.

Crystal data	**1**
CCDC number	2384326
Empirical formula	$C_{56}H_{49}B_3Br_2F_6N_6O_2$
Formula weight	1144.26
Temperature/K	293.15
Crystal system	monoclinic
Space group	C2/c
a/Å	28.42(8)
b/Å	13.14(4)
c/Å	16.94(4)
α/°	90
β/°	101.25(5)
γ/°	90
Volume/Å^3	6204(29)
Z	4
ρ_{calc} g/cm^3	1.225
μ/mm^{-1}	1.366
F(000)	2328.0
Crystal size/mm^3	0.19 × 0.18 × 0.15
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	2.922 to 55.074
Index ranges	-34 ≤ h ≤ 36, -17 ≤ k ≤ 17, -21 ≤ l ≤ 21
Reflections collected	26937
Independent reflections	7094 [R_{int} = 0.1275, R_{sigma} = 0.0983]
Data/restraints/para meters	7094/39/342
Goodness-of-fit on F^2	0.970
Final R indexes [I>=2σ (I)]	R_1 = 0.0607, wR_2 = 0.1794
Final R indexes [all data]	R_1 = 0.1322, wR_2 = 0.2102
Largest diff. peak/hole / e Å^{-3}	0.36/-0.45



2

Table S3. Selected bond lengths [Å] and dihedral angles [deg] of **2** obtained from X-ray crystallography.

Parameters	**2**
B-N bond length (Å)	d_1 =1.5510(46); d_2 =1.5559(43) d_9 =1.5418(50); d_{10} =1.5318(49) d_{11} =1.5241(47); d_{12} =1.5252(56)
C-C bond length (Å)	d_3 =1.4534(40); d_4 =1.4437(42)
C-O bond length (Å)	d_5=1.3513(40); d_6=1.4217(39) d_7=1.3613(40); d_8=1.466(35)
C-O-C angle	102.246(237); 102.383(259)
Dihedral angles between P_a and P_b (deg)	79.667(74)
Dihedral angles between P_a and P_c (deg)	85.343(81)
Dihedral angles between P_a and P_d (deg)	87.205(112)
Dihedral angles between P_b and P_e (deg)	76.137(218)
Dihedral angles between P_c and P_f (deg)	78.973(199)

Table S4. Crystal data and structure refinements for **2** obtained from crystallography.

Crystal data	**2**
CCDC number	2384327
Empirical formula	$C_{56}H_{47}B_3F_6N_6O_2$
Formula weight	982.42
Temperature/K	273.15
Crystal system	triclinic
Space group	P-1
a/Å	11.5461(6)
b/Å	17.9390(9)
c/Å	26.5942(13)
α/°	79.276(3)
β/°	85.459(3)
γ/°	89.219(3)
Volume/Å^3	5395.1(5)
Z	4
ρ_{calc} g/cm^3	1.209
μ/mm^{-1}	0.728
F(000)	2040.0
Crystal size/mm^3	0.18 × 0.16 × 0.15
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	5.014 to 127.37
Index ranges	$-13 \le h \le 13$, $-19 \le k \le 20$, $-30 \le l \le 30$
Reflections collected	107648
Independent reflections	17663 [R_{int} = 0.4218, R_{sigma} = 0.1781]
Data/restraints/para meters	17663/0/1337
Goodness-of-fit on F^2	1.088
Final R indexes [I>=2σ (I)]	R_1 = 0.1142, wR_2 = 0.2820
Final R indexes [all data]	R_1 = 0.1350, wR_2 = 0.3104
Largest diff. peak/hole / e Å^{-3}	0.40/-0.60

4. Photophysical properties

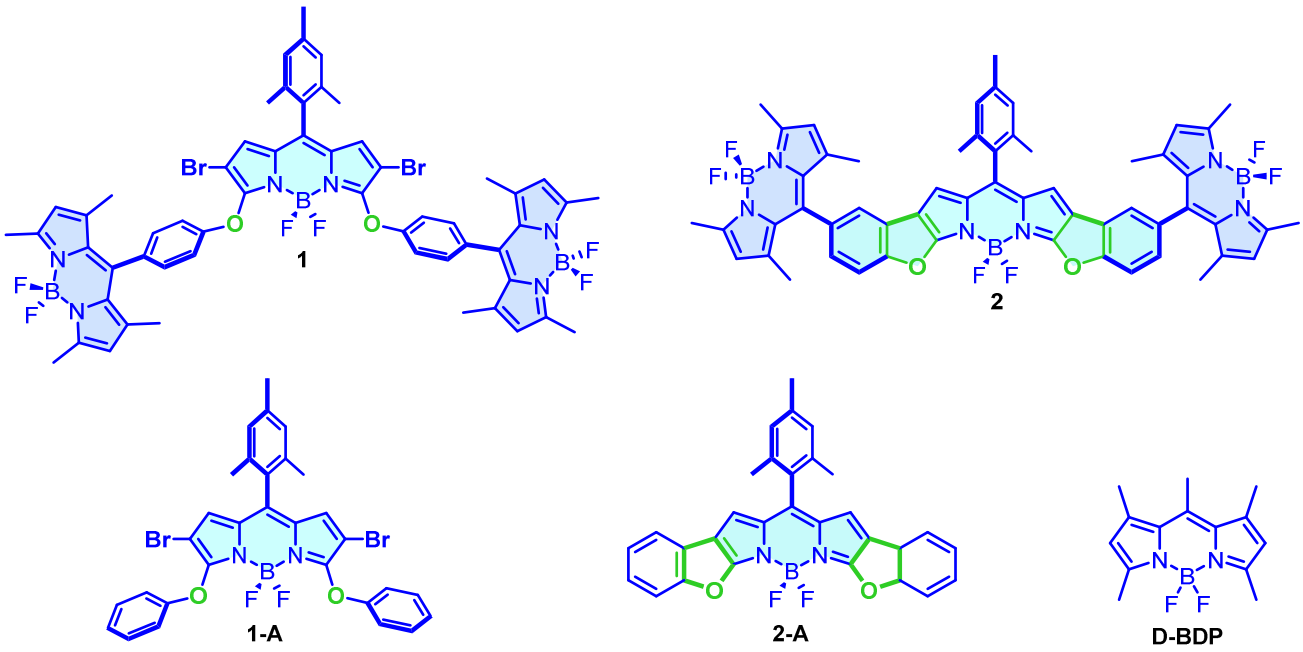


Table S5. Photophysical properties of all BODIPYs in different solvents at room temperature.

dyes	solvent	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)	ε (M^{-1} cm^{-1})	(pseudo-)Stokes shift (cm^{-1})	Φ_{FL}[b]
D-BDP	*n*-Hexane	497	513	64100	630	0.953
	Toluene	499	515	63000	620	0.944
	DCM	496	513	65700	670	0.946
	THF	495	514	77600	750	0.924
	MeCN	491	507	49600	640	0.881
	MeOH	492	509	62000	680	0.900
1-A	*n*-Hexane	544	558	86300	460	0.503
	Toluene	548	567	83200	610	0.471
	DCM	544	565	79400	680	0.447
	THF	543	563	79200	650	0.421
	MeCN	539	560	69400	690	0.411
	MeOH	541	558	74700	560	0.404
2-A	*n*-Hexane	584	598	188800	400	0.772
	Toluene	591	607	175000	450	0.749
	DCM	587	604	162600	480	0.761
	THF	584	602	163400	510	0.742
	MeCN	581	601	147600	570	0.723
	MeOH	580	600	172200	580	0.665

1	*n*-Hexane	502, 546	556	155500, 87200	1940[b]	0.360
	Toluene	504, 550	562	151800, 84600	2050[b]	0.008
	DCM	502, 548	560	152600, 83200	2060[b]	0.006
	THF	502, 546	558	154100, 77500	2000[b]	0.008
	MeCN	498, 542	556	144900, 71800	2100[b]	0.005
	MeOH	498, 542	556	145800, 71900	2100[b]	0.006
2	*n*-Hexane	502, 588	602	133700,182100	3310[b]	0.931
	Toluene	504, 594	609	139900,169500	3420[b]	0.934
	DCM	502, 590	606	132100,156700	3420[b]	0.012
	THF	502, 588	604	141400,160800	3360[b]	0.009
	MeCN	498, 584	601	130200,142900	3440[b]	0.006
	MeOH	498, 584	603	138300,161400	3500[b]	0.003

[a] The pseudo-Stokes shift of **1** and **2** were computed by $\lambda_{em(accept)}$-$\lambda_{abs(donor)}$. [b] Fluorescence quantum yields were measured by Hammatsu C11347 using integrating sphere. The standard errors are less than 5%.

Table S6. Fluorescent lifetimes, radiative decay rate constants (k_r), and nonradiative decay rate constants (k_{nr}) of **1-A**, **2-A**, **1** and **2** in Hexane or MeCN at room temperature.

Parameter	Solvent	**1-A**	**2-A**	**1**	**2**
Φ_{FL}[a]	Hexane	0.503	0.772	0.360	0.931
	MeCN	0.413	0.723	0.005	0.006
τ/ns[b]	Hexane	4.04	4.97	4.10	3.85
	MeCN	3.81	5.05	0.05	0.03
$k_r/\times10^8$ s^{-1}[c]	Hexane	1.24	1.55	0.85	2.42
	MeCN	1.08	1.43	1.00	2.00
$k_{nr}/\times10^8$ s^{-1}[d]	Hexane	1.24	0.46	1.59	0.18
	MeCN	1.55	0.55	199.00	331.33

[a]Fluorescence quantum yields were measured by Hammatsu C11347 using integrating sphere. The standard errors are less than 5%. [b]τ = lifetime of different solution. [c]$k_r = \Phi/\tau$. [d]$k_{nr} = k_r * (1/\Phi - 1)$.

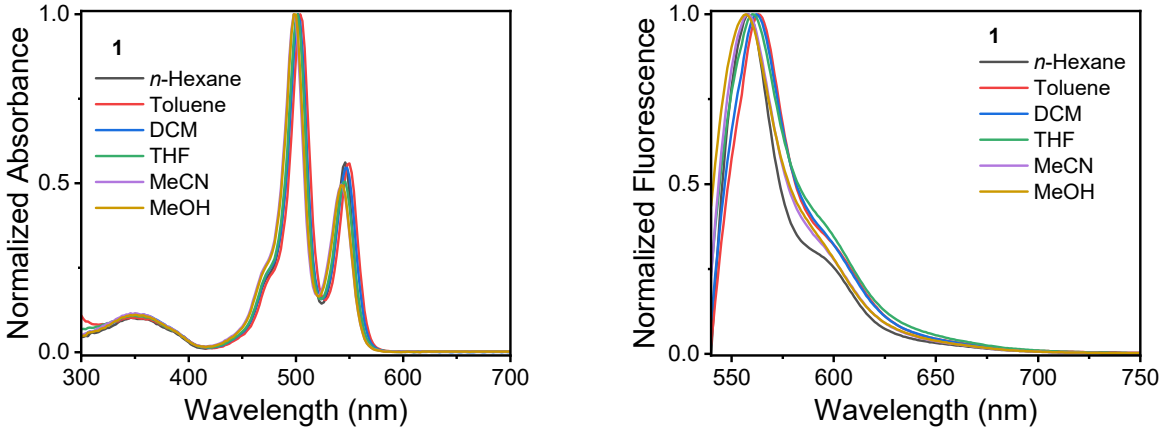


Figure S2: Normalized absorption (left) and emission (right) spectra of compound **1** (10 μM) recorded in different solvents. Excited at 470 nm.

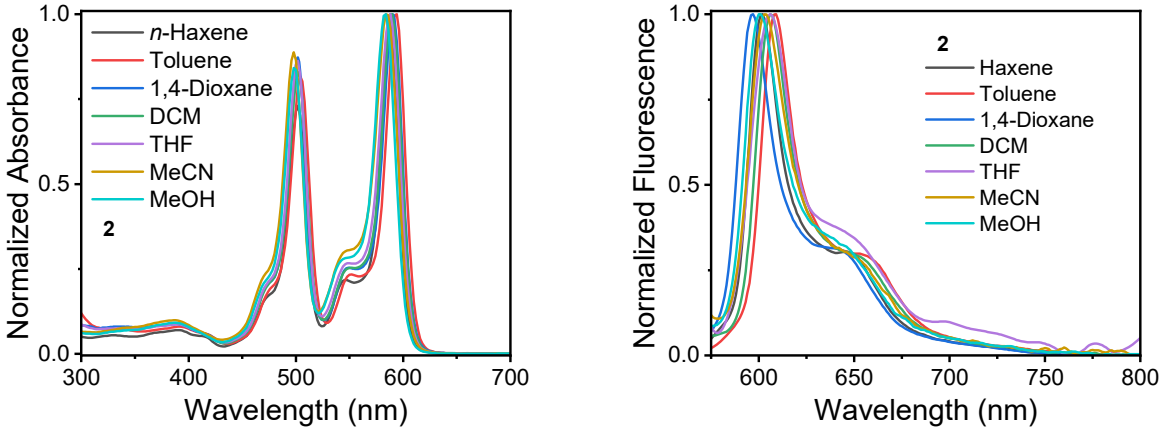


Figure S3: Normalized absorption (left) and emission (right) spectra of compound **2** (10 μM) recorded in different solvents. Excited at 470 nm.

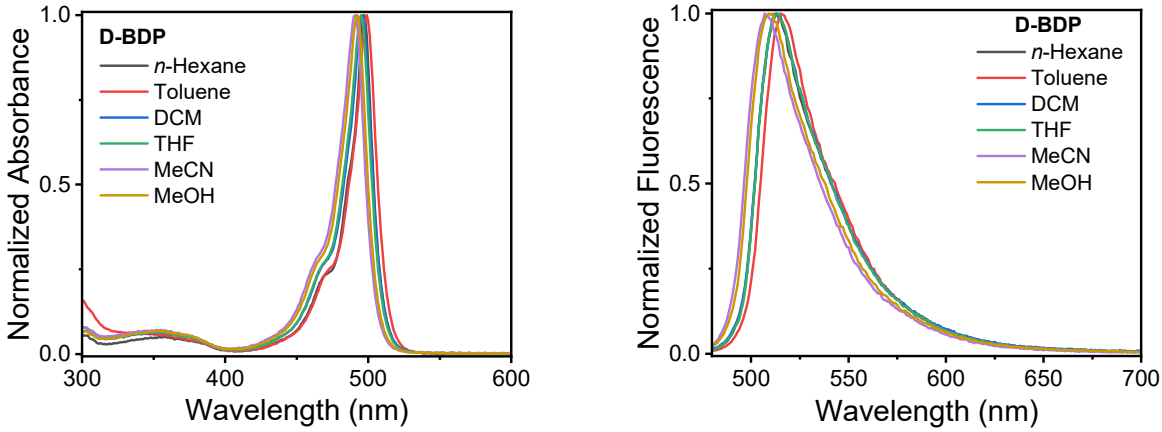


Figure S4: Normalized absorption (left) and emission (right) spectra of compound **D-BDP** (10 μM) recorded in different solvents. Excited at 470 nm.

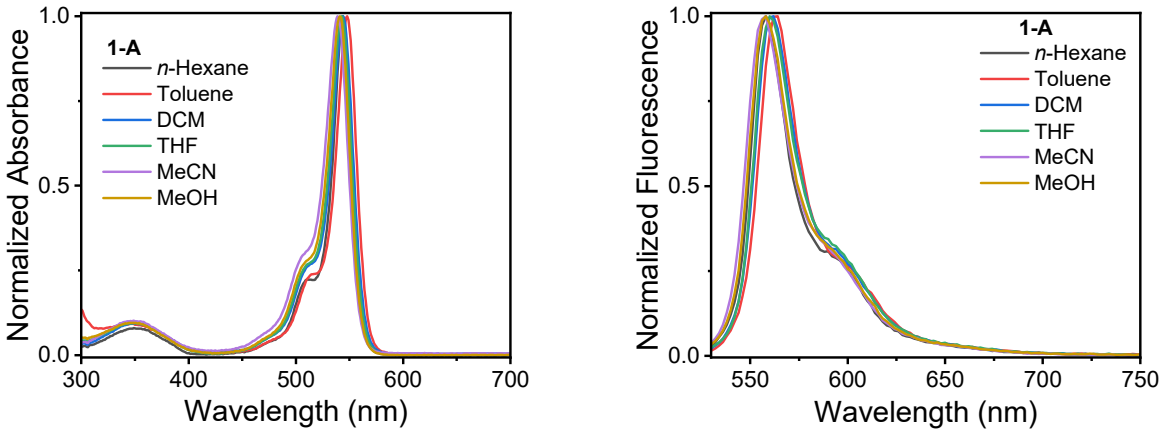


Figure S5: Normalized absorption (left) and emission (right) spectra of compound **1-A** (10 μM) recorded in different solvents. Excited at 520 nm.

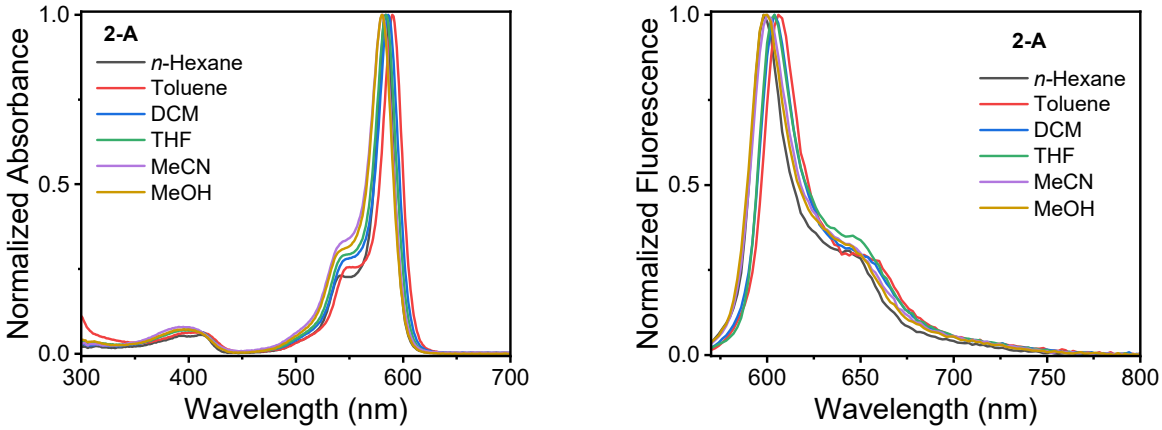


Figure S6: Normalized absorption (left) and emission (right) spectra of compound **2-A** (10 μM) recorded in different solvents. Excited at 560 nm.

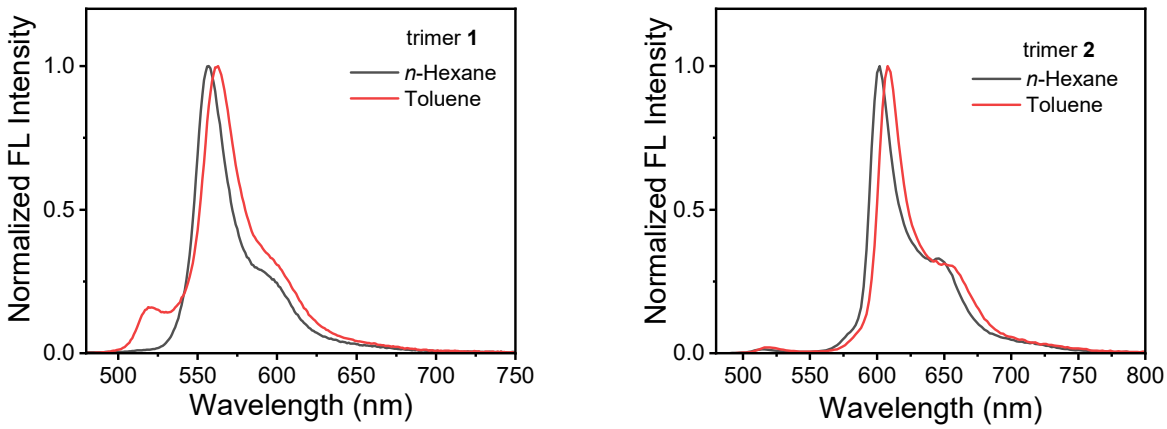


Figure S7: Fluorescence emission spectra of BODIPY trimers **1** (left, 10 μM) and **2** (right, 10 μM) in different solvents. Excited at 470 nm.

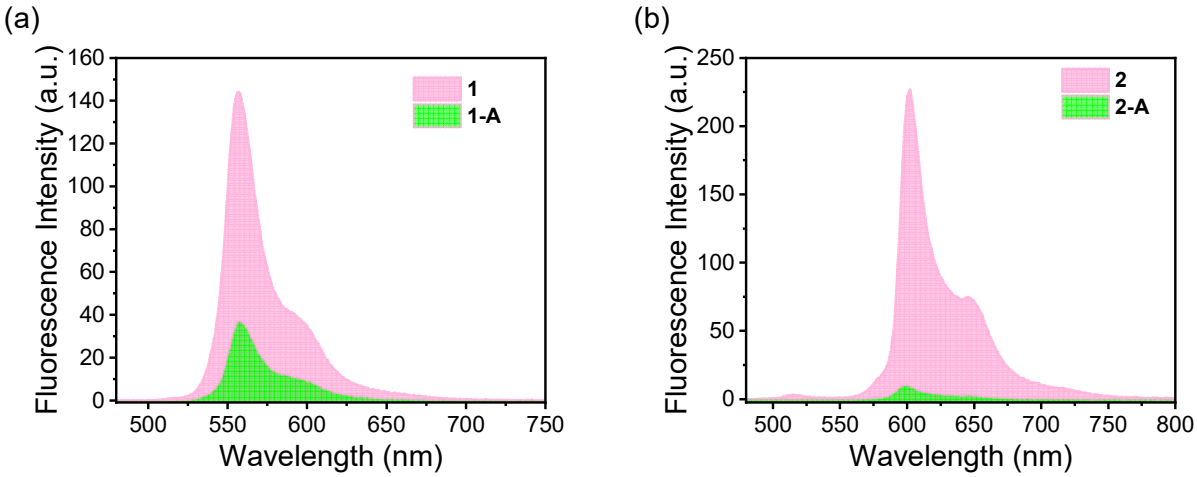


Figure S8: Fluorescence emission spectra of **1**/**1-A** (a, 10 μM, FEF = 3.9) and **2**/**2-A** (b, 10 μM, FEF = 22.4) in different solvents. Excited at 470 nm.

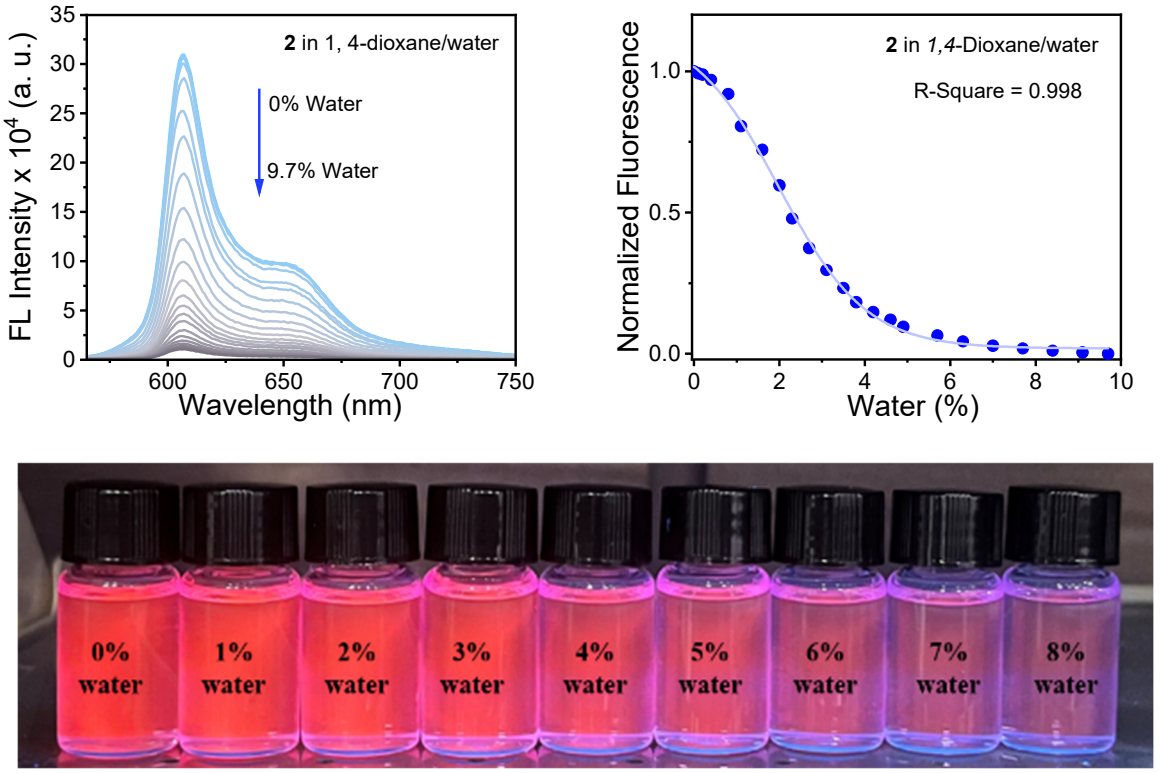


Figure S9: Emission (left) spectra and curve fitting spectra (right) of compound **2** (10 μM) recorded in 1,4-Dioxane with different concentrate of water. Excited at 470 nm.

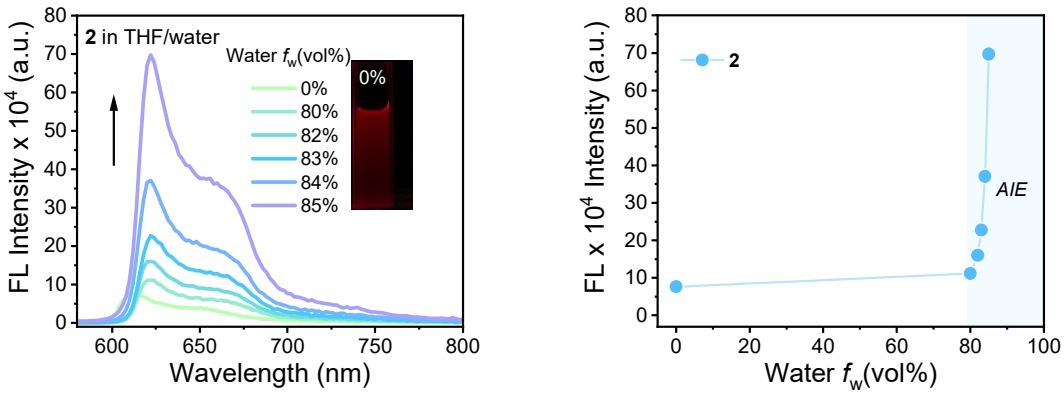


Figure S10: Left: Fluorescence spectra of **2** (40 μM, excited at 470 nm) in THF/H_2O with varying water content (f_w). Right: Plot of the fluorescent intensity integration area versus different water fractions of **2**.

Calculations of two-photon cross section

The two-photon-induced fluorescent spectra of **2** were determined with the same excitation wavelength (from 700 to 1050 nm, every 10 nm) using fluorescein as the reference by femtosecond two-photon excited fluorescence (TPEF) technique. The Two-photon cross section was calculated by using the below equation according to previous reports.[7]

$$\delta_2 = \frac{\delta_1 \Phi_1 (F_2 c_1)}{\Phi_2 (F_1 c_2)} \qquad \text{Eq. S2}$$

In which the subscripts 1 and 2 refer to the reference of fluorescein and our probe **2**, respectively; F is the integration of the fluorescent spectra; Φ is the fluorescence quantum yield (Φ = 0.95 for fluorescein, NaOH solution, pH =11; Φ = 0.93 for **2** in toluene solution); c is the concentration of fluorescein (100 μM) and our sample (100 μM); and δ_1 is the TPA cross-section of the reference of fluorescein according to previous reports.[8]

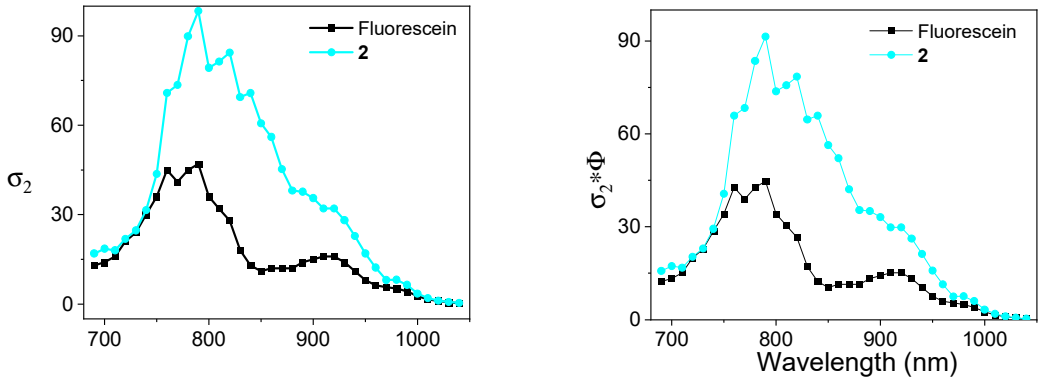


Figure S11. (Activity) two-photon cross section fluorescence spectra of **2** (100 μM, in toluene) or fluorescence (100 μM, NaOH solution, pH =11) at different excitation wavelength.

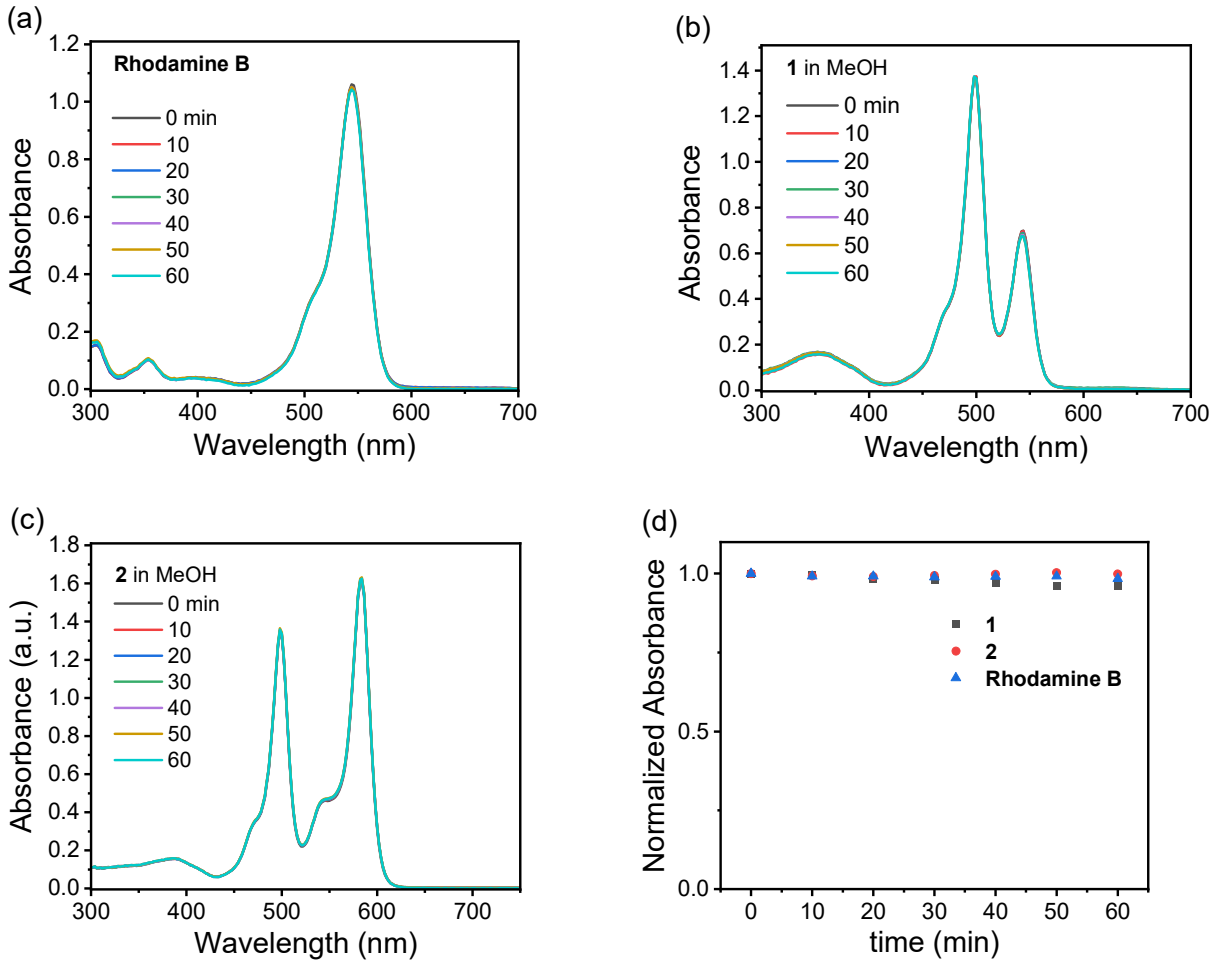


Figure S12: Absorbance change of **Rhodamine B** (a, 10 μM), trimer **1** (b, 10 μM), trimer **2** (c, 10 μM) in methanol under strong continuous irradiation with a 50 W white LED lamp over 60 min. (d) Comparison of the photostability of BODIPY trimers **1**, **2** and **Rhodamine B** in methanol under continuous irradiation with a 50 W white LED lamp over 60 min.

5. Electrochemical properties

Electrochemical measurements.

Cyclic voltammograms were obtained on a CH Instruments electrochemical workstation (CHI 610E, USA). Cyclic voltammograms of 4.0 mM BODIPY trimers **1** and **2** were measured in dichloromethane solution, containing 0.1 M $TBAPF_6$ as the supporting electrolyte, glassy carbon electrode as a working electrode, Pt wire as a counter electrode, and saturated calomel electrode (SCE) as reference electrode at 100 $mV \cdot s^{-1}$ of scanning rate at room temperature. Before each experiment, the working electrode was polished on a felt pad with 0.3 μm alumina (Buehler, Ltd., Lake Bluff, IL) and sonicated in Milli-Q deionized water and then in ethanol. The counter and reference electrodes were cleaned by rinsing and sonicating in water, and ethanol.

Table S7: Electrochemical data acquired at 100 mV s^{-1}, and HOMO-LUMO Gaps determined from spectroscopy of **1** and **2**.

dyes	$E_{1/2}^{red}$ (V)	E_{red}^{onset} (V)	$E_{1/2}^{ox}$ (V)	E_{ox}^{onset} (V)	LUMO (eV)	HOMO (eV)	E_g^e (eV)	E_g^o (eV)
1	-1.32, -0.81	-0.78	1.16, 1.41	1.12	-3.62	-5.52	1.90	2.18
2	-1.33, -0.89	-0.85	1.16, 1.33	1.12	-3.55	-5.52	1.97	2.04

[a] $E_{1/2}^{red}$ = reversible reduction peak potentials; E_p^{red} = irreversible reduction peak potentials; E_{red}^{onset} = the onset reduction potentials; $E_{1/2}^{ox}$ = reversible oxidation peak potentials; E_{ox}^{onset} = the onset oxidation potentials; E_{LUMO} = -e(E_{red}^{onset} + 4.4); E_{HOMO} = -e(E_{ox}^{onset} + 4.4); E_g^e = bandgap, obtained from the intercept of the electrochemical data; $E_g^e = E_{LUMO} - E_{HOMO}$; E_g^o = bandgap, obtained from the intercept of the absorption spectra.

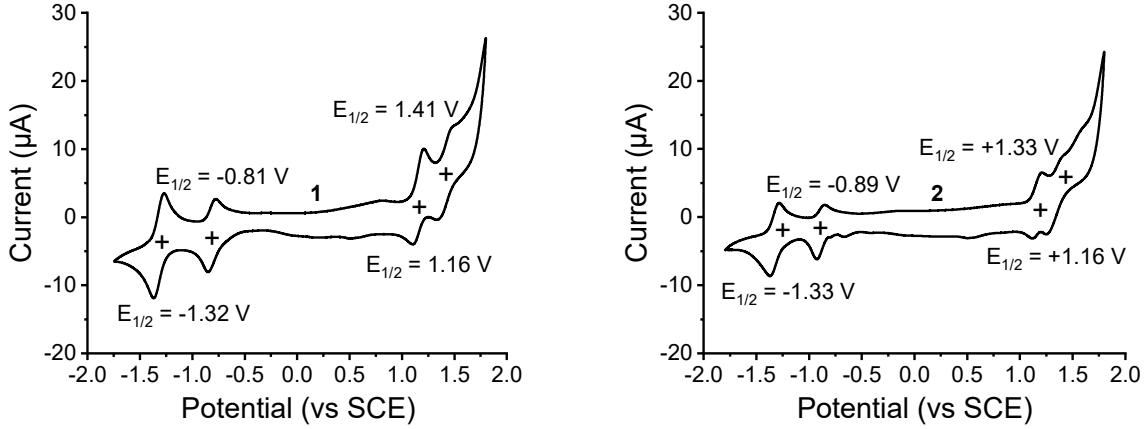


Figure S13: Cyclic voltammograms of 4.0 mM **1** (left) and **2** (right) measured in dichloromethane solution containing 0.1 M $TBAPF_6$ as the supporting electrolyte at room temperature. Glassy carbon electrode as a working electrode, and the scan rate at 100 mV s^{-1}.

6. Theoretical calculations

The ground state geometry was optimized by using DFT method at B3LYP/6-31G(d) level. The same method was used for vibrational analysis to verify that the optimized structures correspond to local minima on the energy surface. The calculated molecules in dichloromethane were done using the Self-Consistent Reaction Field (SCRF) method and Polarizable Continuum Model (PCM). All of the calculations were carried out by the methods implemented in Gaussian 09 package. [9]

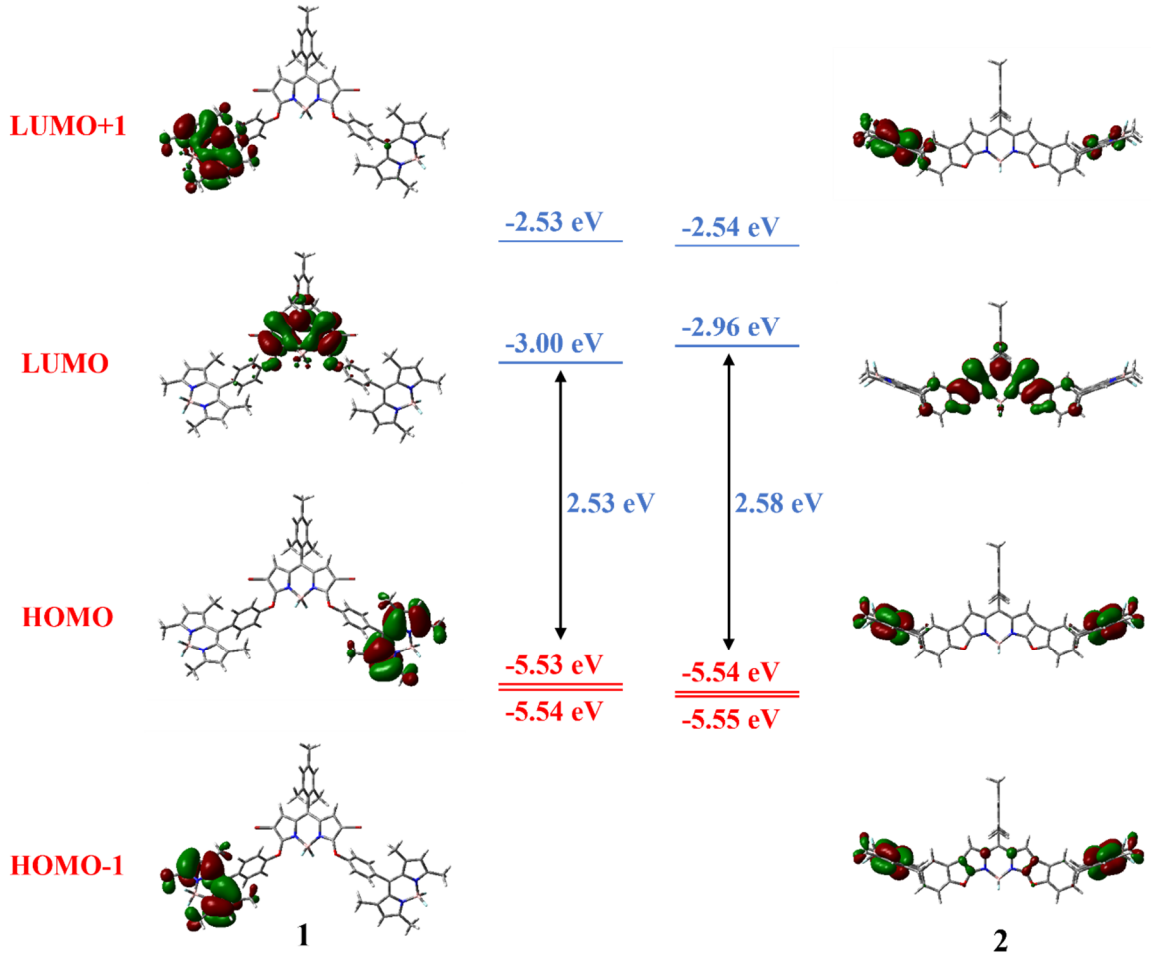


Figure S14. HOMO and LUMO energy levels for **1** and **2** obtained from DFT calculations. (B3LYP/6-31 G(d) level).

DFT optimized coordinates for **1-A** optimized S_0 state Geometry by B3LYP/6-31G(d).

F	-3.36295513	2.13158211	-2.48456648
F	-2.55616513	2.42681111	-0.36107348
N	-4.87106113	1.74595211	-0.62870148
N	-3.00680113	0.16626011	-1.10163748
C	-5.62844813	0.65453611	-0.16316948
C	-6.90895413	2.48861411	0.01549052
C	-5.63802213	2.83618111	-0.52084748

C	-5.11143613	-0.63812089	-0.14582048
C	-6.90568013	1.12811111	0.24004452
H	-7.70530813	0.52237211	0.64071752
C	-3.80448913	-0.87213589	-0.61163048
C	-3.06801813	-2.07503389	-0.67418348
C	-1.82400913	-0.36656989	-1.45702248
C	-1.82593713	-1.75777289	-1.20765948
B	-3.39727113	1.68621211	-1.17568248
H	-3.42026113	-3.05054489	-0.37217148
C	-5.93621113	-1.77669089	0.36694552
C	-6.74692713	-2.50941489	-0.52611848
C	-5.89376513	-2.11109389	1.73723652
C	-7.50534713	-3.57222289	-0.02444848
C	-6.66985713	-3.18324989	2.18986752
C	-7.47914013	-3.92894489	1.32731552
H	-8.13388313	-4.13514189	-0.71118848
H	-6.63986113	-3.44044389	3.24651452
C	-8.28346613	-5.10217989	1.83638852
H	-9.19745613	-5.24495689	1.25065752
H	-7.70628213	-6.03402689	1.77035752
H	-8.56556713	-4.96896789	2.88587652
C	-6.81383013	-2.16541489	-1.99768648
H	-7.18312313	-1.14573189	-2.15846648
H	-5.82742613	-2.22476489	-2.47215248
H	-7.48222713	-2.85139789	-2.52545748
C	-5.03643613	-1.33690189	2.71393252
H	-3.97919813	-1.35410289	2.42481752
H	-5.33493613	-0.28339089	2.76730352
H	-5.11701213	-1.75956989	3.71929552
O	-5.19444713	4.03406411	-0.88439848
O	-0.88968913	0.40315211	-2.04873048
C	0.42006987	0.38416311	-1.56925348
C	0.70800987	0.25896511	-0.21138248
C	1.42971087	0.55737511	-2.51245548
C	2.04075987	0.26683611	0.19761552
H	-0.09198913	0.15506011	0.51406652
C	2.75725087	0.57058811	-2.08904848
C	3.07718187	0.41207811	-0.73357448
H	2.27510987	0.15735011	1.25234552
H	3.55092587	0.69196611	-2.82022448
H	1.17080787	0.66445911	-3.56052348
Br	-0.41253013	-2.94159489	-1.58531548
Br	-8.26240213	3.76053611	0.31551652
C	-6.22558636	4.99722021	-0.65205773

C	-7.14669484	5.28945516	-1.65835258
C	-6.31059475	5.64445047	0.58058768
C	-8.15214224	6.22921606	-1.43219621
H	-7.07893886	4.77958205	-2.63029925
C	-7.31688709	6.58380237	0.80731134
H	-5.58480804	5.41411360	1.37387678
C	-8.23752198	6.87636524	-0.19885733
H	-8.87782519	6.46007040	-2.22553538
H	-7.38391566	7.09365653	1.77943794
H	-9.03040287	7.61725323	-0.02075282
H	4.09850587	0.40215920	-0.41467181

SCF done: -6785.123122 a.u.
No imaginary Frequency.

DFT optimized coordinates for **2-A** optimized S_0 state Geometry by B3LYP/6-31G(d).

F	4.13935708	-3.05799661	-1.14498410
F	4.13884608	-3.06112961	1.13906590
N	5.38001308	-1.31936661	-0.00033510
N	2.90595308	-1.31413161	-0.00085910
C	5.36034108	0.10032239	-0.00053010
C	7.53349308	-0.51405761	0.00065690
C	6.67188208	-1.63259361	0.00035790
C	4.14975908	0.79962539	-0.00092710
C	6.69682708	0.59758739	0.00007390
H	6.95840808	1.64617639	0.00011490
C	2.93134308	0.10441539	-0.00102510
C	1.60145208	0.60865139	-0.00090010
C	1.61197608	-1.62190261	-0.00061110
C	0.75760708	-0.50173961	-0.00060610
B	4.14094108	-2.27329161	-0.00185910
H	1.34441408	1.65828539	-0.00092310
C	4.15182508	2.29722539	-0.00113410
C	4.15479108	2.99644039	-1.22677910
C	4.15483508	2.99682539	1.22427890
C	4.15970408	4.39497039	-1.20063810
C	4.15972908	4.39535639	1.19769190
C	4.15871608	5.11426239	-0.00158410
H	4.16567508	4.93518639	-2.14499510
H	4.16569608	4.93587339	2.14187490
C	4.13226908	6.62487539	-0.00184710
H	4.62724708	7.03368839	-0.88882810
H	3.10063108	7.00110039	-0.00281710
H	4.62571708	7.03397339	0.88584890
C	4.15753308	2.26804939	-2.55255610

H	5.04134308	1.62851539	-2.66031410
H	3.28017508	1.61946939	-2.65853810
H	4.15310108	2.97797339	-3.38441310
C	4.15766908	2.26886839	2.55029590
H	3.28101708	1.61930239	2.65601490
H	5.04217308	1.63040239	2.65874590
H	4.15198308	2.97905739	3.38191890
O	7.29655008	-2.82176061	0.00075590
O	0.98005008	-2.81104861	-0.00039710
C	8.66479308	-2.48501761	0.00140590
C	8.86648008	-1.08390061	0.00137990
C	9.68778808	-3.41180961	0.00200090
C	10.17866808	-0.60069061	0.00198790
C	10.99253408	-2.90078461	0.00260090
H	9.48600408	-4.47745861	0.00199590
C	11.23155308	-1.51937461	0.00259390
H	10.37421608	0.46717039	0.00198690
H	11.83028708	-3.59141861	0.00307890
C	-0.38103492	-2.46830161	-0.00018910
C	-1.41265992	-3.38526161	0.00012190
C	-0.57668792	-1.06562761	-0.00029910
C	-2.71349792	-2.86799661	0.00029490
H	-1.22290092	-4.45295761	0.00022090
C	-1.88456292	-0.57445961	-0.00011310
C	-2.95270792	-1.48217661	0.00017490
H	-3.55597792	-3.55244561	0.00053390
H	-2.07447892	0.49428339	-0.00020410
H	12.25496008	-1.15619761	0.00306690
H	-3.95866222	-1.11754540	0.00030454

SCF done: -1640.522454 a.u.
No imaginary Frequency.

DFT optimized coordinates for **D-BDP-H** optimized S_0 state Geometry by B3LYP/6-31G(d).

C	-2.31323473	0.13059701	-0.03028190
C	-1.66569673	0.36243501	-1.24647090
C	-1.66694173	0.36207601	1.18663310
C	-2.10803673	0.20939201	2.53667810
C	-1.03573873	0.59262301	3.33163210
C	0.02797427	0.96769701	2.49142010
C	-2.10545373	0.21022701	-2.59700790
C	-1.03236373	0.59372301	-3.39076190
C	0.03049427	0.96854901	-2.54936290
N	-0.35501273	0.82806201	1.21064410
N	-0.35375473	0.82845601	-1.26900790

B	0.52155427	1.12724001	-0.02869190
H	-1.00980073	0.60494801	4.41391610
H	-1.00539273	0.60652001	-4.47301390
F	1.64877527	0.30646801	-0.02825690
F	0.91177327	2.46556701	-0.02826890
C	-3.42792673	-0.25618099	-3.10743890
H	-3.66718873	-1.26666899	-2.76025490
H	-4.24564473	0.39152401	-2.77423790
H	-3.42146373	-0.26264599	-4.20083190
C	1.38267527	1.45046401	-2.93253690
H	2.15605127	0.79915601	-2.51272490
H	1.48576127	1.47513201	-4.01913790
H	1.56111127	2.45379501	-2.53225690
C	-3.43100473	-0.25726599	3.04559510
H	-4.24832773	0.39089101	2.71228410
H	-3.67015373	-1.26739699	2.69731410
H	-3.42546973	-0.26467499	4.13898710
C	1.37978227	1.44945201	2.87610910
H	1.48175327	1.47382001	3.96282210
H	2.15356327	0.79822201	2.45691910
H	1.55867627	2.45287901	2.47627410
H	-3.32059364	-0.23013519	-0.03084976

SCF done: -838.671539 a.u.
No imaginary Frequency.

1, optimized S_0 state Geometry.

B	-1.62964200	-0.00207200	0.26585300
C	-1.00299600	-2.57544900	0.00582300
C	-2.34847400	-3.15616400	-0.05369900
C	-3.43830500	-2.64881300	0.67804000
C	-4.67919300	-3.27726500	0.61286600
C	-4.86188800	-4.41294400	-0.17859800
C	-3.78721900	-4.92632300	-0.90821200
C	-2.54236000	-4.30845200	-0.84180700
C	0.19675700	-3.33072100	-0.05093000
C	1.27690900	-2.46660300	0.06880900
C	0.69869100	-1.15067400	0.16294500
F	-2.18126600	0.01125000	1.55815400
F	-2.60323300	-0.01328000	-0.70546000
N	-0.69254900	-1.25622900	0.13865600
N	1.35453800	-0.00264400	0.14506800
C	-1.00746400	2.56735400	-0.02984200
C	-2.35443400	3.14653800	-0.08544100
C	-3.43689200	2.65084200	0.66490200

C	-4.67854300	3.27829400	0.60278900
C	-4.86975000	4.40132500	-0.20436900
C	-3.80271800	4.90266300	-0.95326200
C	-2.55699200	4.28620400	-0.88985100
C	0.19023500	3.32161500	-0.12453800
C	1.27435000	2.45857000	-0.04297800
C	0.69924000	1.14417400	0.09251900
N	-0.69303300	1.24980000	0.10822900
H	-3.30355900	-1.77879500	1.30722400
H	-5.50897900	-2.87706800	1.18894800
H	-3.29609600	1.79006900	1.30517400
H	-5.50221100	2.88687800	1.19352300
C	2.69523400	2.81837900	-0.07887000
C	3.09600000	4.10879500	0.31982400
C	3.68506300	1.92077600	-0.52431000
C	4.43351300	4.49058700	0.27310500
H	2.35255700	4.80700500	0.69334500
C	5.02284900	2.30760300	-0.56916300
H	3.39707400	0.92452000	-0.83652500
C	5.40450200	3.59112600	-0.17366500
H	4.71938700	5.48929600	0.59230400
H	5.77032200	1.60193400	-0.92199300
H	6.44909500	3.88831000	-0.21149200
C	2.69918000	-2.81580100	0.08160900
C	3.13506700	-4.00103800	-0.54230700
C	3.65687100	-2.00949500	0.72696000
C	4.47738500	-4.36807500	-0.52307100
H	2.41518900	-4.62387000	-1.06554800
C	4.99984000	-2.38011500	0.74268500
H	3.33959400	-1.09706400	1.21736100
C	5.41681200	-3.55870300	0.12065100
H	4.79242500	-5.28371200	-1.01638100
H	5.72278200	-1.74751900	1.25083200
H	6.46499100	-3.84499500	0.13663400
H	0.22508100	4.39568800	-0.23669400
H	0.23398300	-4.40981900	-0.09402900
H	-1.71620400	-4.69954600	-1.42804400
H	-3.91972900	-5.80531200	-1.53306000
H	-5.83455900	-4.89508100	-0.22698800
H	-5.84292100	4.88270000	-0.25032400
H	-3.94175000	5.77121800	-1.59115300
H	-1.73808700	4.66804700	-1.49198000

SCF done: -8460.06170261 Hartree

No imaginary Frequency.

2, optimized S_0 state Geometry.

F	0.00043500	-2.86789000	0.80724200
F	0.00069700	-2.61809400	-1.46347600
F	-12.49022900	-0.43824200	0.05412300
O	3.16061000	-2.50833300	-0.21330800
F	-11.69592100	1.70222000	0.20829300
F	11.69604500	1.70377600	0.20931500
F	12.49109400	-0.43618900	0.05208400
O	-3.15966100	-2.50882800	-0.21360400
N	-10.48003900	0.12817800	-1.17351900
N	10.45380700	-0.06192400	1.30759400
N	-10.45218000	-0.06165800	1.30772900
N	1.23770000	-1.01135700	-0.13733300
N	-1.23692700	-1.01158300	-0.13761100
N	10.47979000	0.13091800	-1.17344600
C	-9.13577200	-0.53899600	1.23500700
C	-9.16514600	-0.35815100	-1.20410100
C	9.13738500	-0.53930700	1.23526600
C	2.54697600	0.90418300	0.00961300
H	2.80566300	1.95063500	0.08620100
C	2.53036600	-1.32163700	-0.13997500
C	4.52297500	-2.16951200	-0.17032100
C	0.00022600	1.10089400	-0.00261300
C	-0.00048900	2.59584100	0.08351200
C	4.72055100	-0.77111400	-0.07023900
C	-7.09470100	-1.19235000	-0.06507700
C	7.09547900	-1.19147500	-0.06414800
C	-1.21414100	0.40448500	-0.04351600
C	6.02852800	-0.28363200	-0.01656500
H	6.21939400	0.78212500	0.06057100
C	3.38729100	-0.20504600	-0.05185400
C	1.21470000	0.40467100	-0.04263500
C	-10.88133400	0.36626400	-2.44041300
C	8.50261200	-0.68447900	-0.00935100
C	6.85437000	-2.57322600	-0.16540300
H	7.69586500	-3.25788900	-0.20236000
C	-8.50188200	-0.68553400	-0.00991100
C	-8.68864400	-0.79738000	2.57410300
C	-2.54649300	0.90386800	0.00785400
H	-2.80532800	1.95031600	0.08385700
C	-6.02784500	-0.28437200	-0.01801800
H	-6.21882300	0.78139000	0.05878400
C	-4.71982500	-0.77172100	-0.07160000

C	-3.38663800	-0.20551400	-0.05309300
C	-2.52956200	-1.32203400	-0.14049300
C	-0.00368200	3.22672400	1.34523300
C	8.69126800	-0.79933200	2.57438400
C	-4.52207900	-2.17015000	-0.17103200
C	-6.85344200	-2.57410900	-0.16586700
H	-7.69485400	-3.25889300	-0.20244400
C	-8.75152800	-0.41737400	-2.57702700
C	10.82663900	-0.01990400	2.60444100
C	5.55306900	-3.08666900	-0.21988300
H	5.36218700	-4.15131400	-0.29737100
C	-12.23542100	0.89991900	-2.77776100
H	-13.01920400	0.25705100	-2.36407000
H	-12.36014500	0.96473300	-3.86115700
H	-12.37952300	1.89564200	-2.34377700
C	9.16497800	-0.35564200	-1.20365400
C	-10.82404600	-0.01805700	2.60481600
C	-9.83417500	0.03739800	-3.32287500
H	-9.87711300	0.12698200	-4.40088700
C	-9.75484200	-0.46665300	3.40424400
H	-9.77226200	-0.53739400	4.48436100
C	-5.55207800	-3.08743300	-0.22020500
H	-5.36109600	-4.15209400	-0.29720300
C	-0.00010400	5.40845900	0.23731000
C	-0.00208200	4.62498200	1.39515300
H	-0.00126600	5.11269400	2.36760700
C	9.75806700	-0.46956100	3.40412700
H	9.77629800	-0.54159400	4.48414500
C	7.36855700	-1.31652200	3.05820200
H	6.54176600	-0.65378300	2.78101900
H	7.38286800	-1.40123900	4.14892200
H	7.13511800	-2.30296000	2.64407400
C	0.00603600	3.36133700	-1.10238400
C	0.00765400	4.75590300	-0.99969600
H	0.01621600	5.34690200	-1.91310200
C	7.44490400	-0.86208200	-3.16409700
H	7.21873800	-1.90505600	-2.91786200
H	7.48205500	-0.77247000	-4.25388800
H	6.60246000	-0.26252700	-2.80329700
C	-7.36555700	-1.31394200	3.05756900
H	-7.13184500	-2.30036900	2.64356900
H	-7.37944200	-1.39840200	4.14831600
H	-6.53914800	-0.65091200	2.77995800
B	-11.33581800	0.35345400	0.10135900

C	8.75046500	-0.41351600	-2.57637100
C	12.17676800	0.44192900	3.04693300
H	12.96259900	-0.18476300	2.61160000
H	12.25408300	0.40468000	4.13599800
H	12.36494900	1.46675700	2.70977600
C	10.88014900	0.37054000	-2.44035100
B	0.00047900	-1.96301600	-0.24173900
C	-12.17381200	0.44440800	3.04773700
H	-12.36131200	1.46961400	2.71129200
H	-12.25104400	0.40654500	4.13678600
H	-12.96009400	-0.18142800	2.61203000
C	-7.44623700	-0.86627500	-3.16508200
H	-6.60349000	-0.26713100	-2.80437700
H	-7.48358600	-0.77656300	-4.25485700
H	-7.22043800	-1.90939200	-2.91902300
B	11.33639400	0.35499200	0.10106500
C	-0.00472300	2.42737900	2.62943200
H	-0.88519000	1.77816100	2.70014300
H	-0.00629700	3.09138600	3.49831400
H	0.87618000	1.77899800	2.70251800
C	12.23379800	0.90512200	-2.77798100
H	12.37697900	1.90130700	-2.34471000
H	12.35851600	0.96935600	-3.86141800
H	13.01808700	0.26324400	-2.36377100
C	-0.03051400	6.91679700	0.31805100
H	-1.06120500	7.29294100	0.27371800
H	0.51604300	7.37332700	-0.51384500
H	0.40722200	7.27648600	1.25479500
C	9.83249100	0.04235600	-3.32245600
H	9.87473900	0.13295400	-4.40041100
C	0.01488200	2.70660400	-2.46603000
H	0.89895800	2.07338400	-2.60460300
H	0.01467600	3.46082900	-3.25791700
H	-0.86229700	2.06545400	-2.61171400

SCF done: -3315.45751402 Hartree

No imaginary Frequency.

7. Cellular studies

Cell culture

HeLa cells (human cervical cancer cells) were cultured in culture media (RPMI-1640, supplemented with 10% FBS and 1% penicillin/streptomycin solution) at 37 ºC in an atmosphere of 5% CO_2 and 95% humidified atmosphere for 24 h.

Cell incubation and colocalization imaging

A total of 30000 HeLa cells were seeded into a glass bottom dish and were cultured in culture media (RPMI-1640, supplemented with 10% FBS) at 37 °C in an atmosphere of 5% CO_2 and 95% humidified atmosphere for 24 h. HeLa cells were stained with **2** (0.5 μM) and **Lipi-Blue** (0.5 μM) at 37 ºC in an atmosphere of 5% CO_2 for 5 h. Finally, the morphologies of the HeLa cells were observed using a confocal fluorescence microscope (Leica Microsystems SP8 MP, excitation at 405 and 488 nm for **Lipi-Blue**, and **2**, respectively).

Cytotoxicity

The cytotoxicity of the **2** was evaluated on HeLa cells. These cells were seeded into 96-well plates with a density of 5000 cells per well and cultured overnight. Then, solutions of **2** with a serious of different concentrations were added and incubated with cells for 24 h. Every experiment was performed for at least three times. The working solutions were then removed, and the cells were washed with PBS buffer for two times. A total of 10 μL of CCK-8 (Cell Counting Kit-8, BIOMIKY) was added into each well, and the cells were further incubated at 37 ºC for 2 h. Then the plate was shaken for 5 min (protect from light), and the absorbance at 450 nm was measured with a microplate reader (Multiskan Sky).

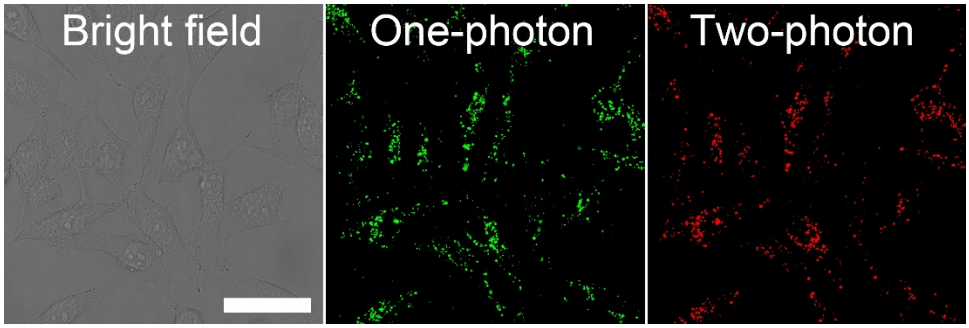


Figure S15: Two-photon fluorescence images of **2** (5 μM) in HeLa cells incubated for 10 h. Green channel: single photon images. Red channel: two-photon images. Scale bar: 50 μm. One-photon: λ_{ex}: 488 nm; λ_{em} :580~680 nm. Two-photon: λ_{ex}: 790; λ_{em} :580~680 nm.

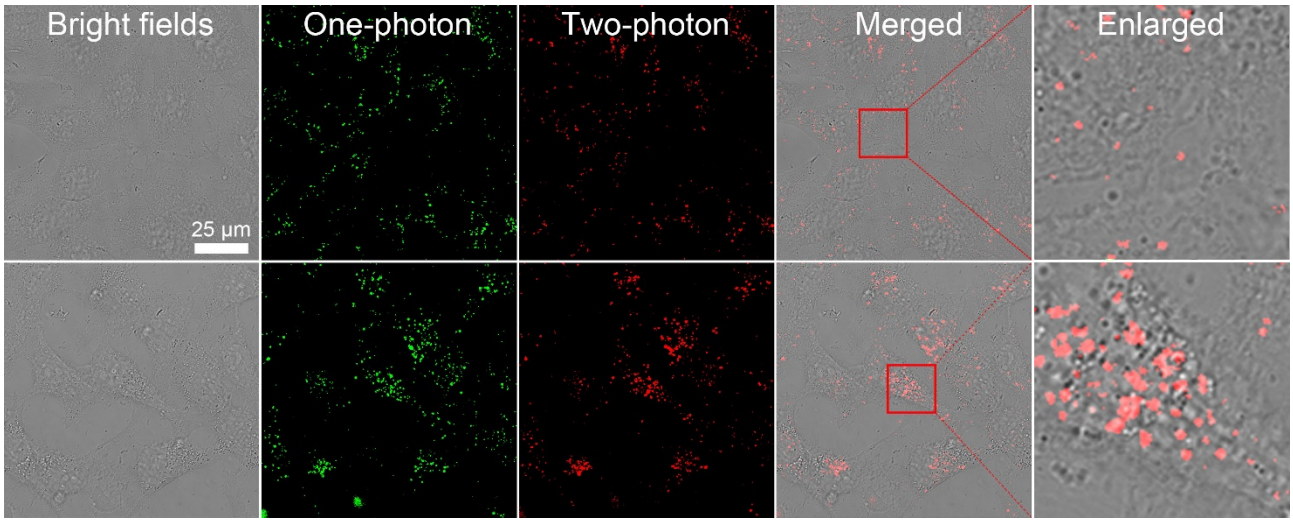


Figure S16: HeLa cells were pretreated with oleic acids (100 μM) for 4 h or not then labeled with **2** (5 μM). Green channel: single photon images. Red channel: two-photon images. Scale bar: 25 μm. One-photon: λ_{ex}: 488 nm; λ_{em} :580~680 nm. Two-photon: λ_{ex}: 790; λ_{em} :580~680 nm.

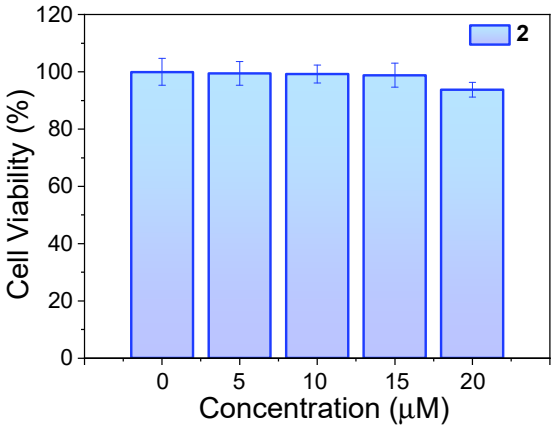
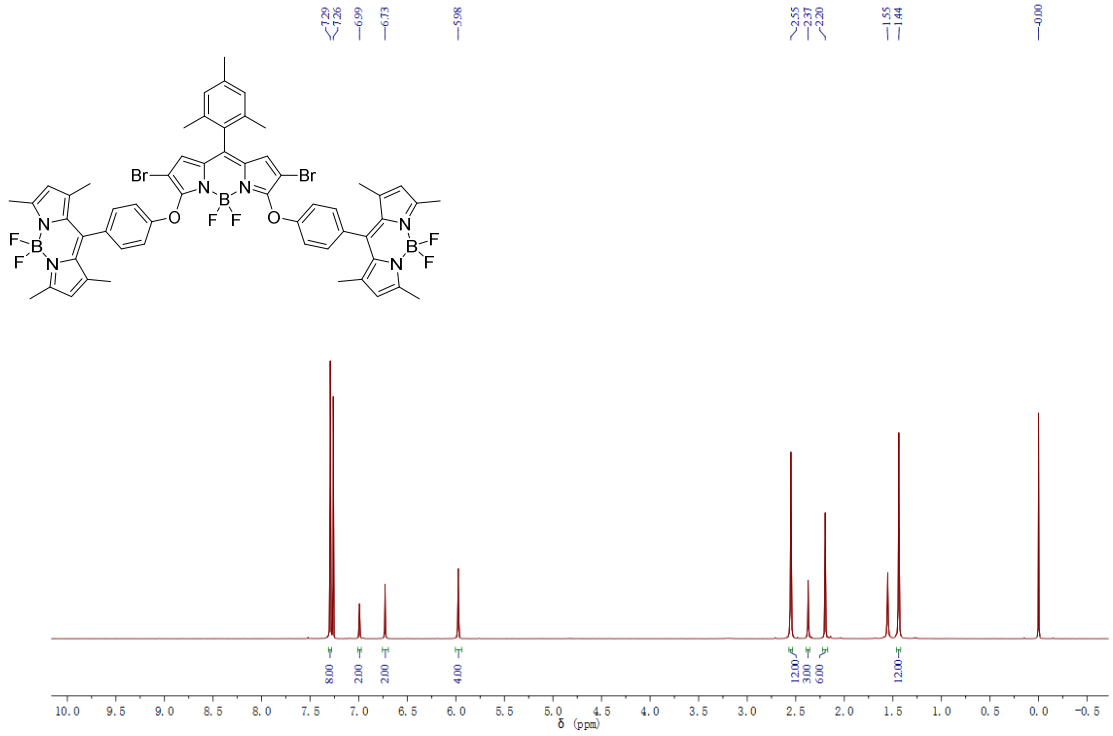
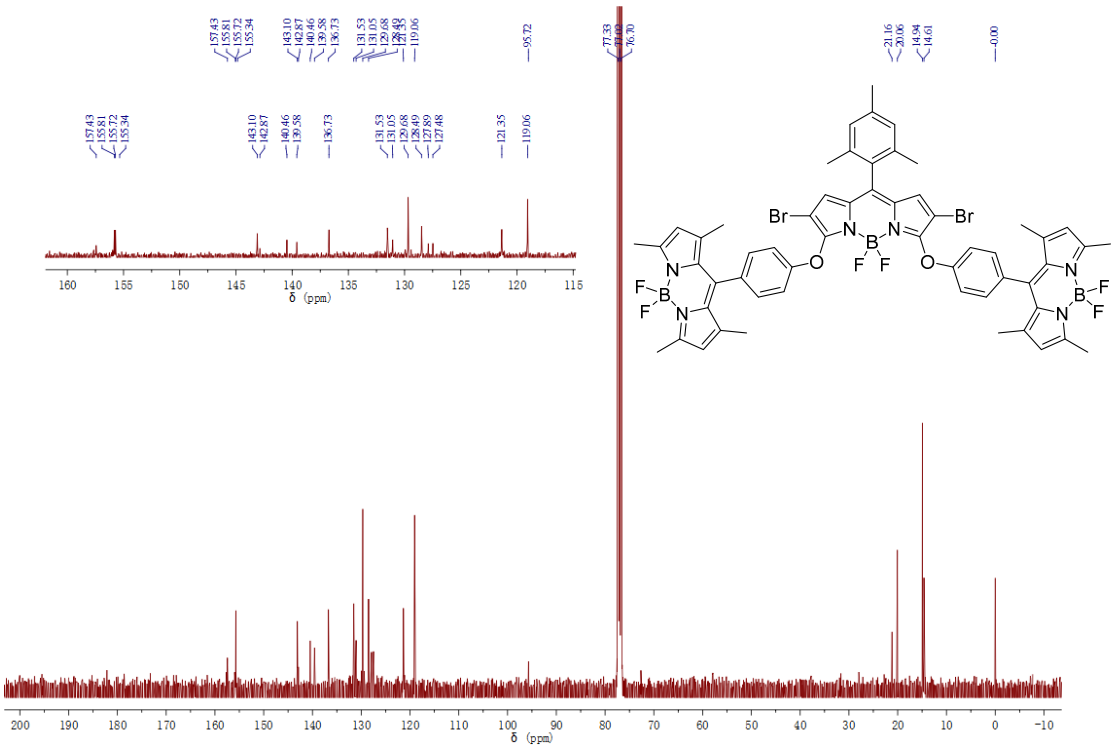


Figure S17: Cytotoxicity of HeLa cells treated with different concentrations of **2** for 24 h as demonstrated by CCK-8 assay.

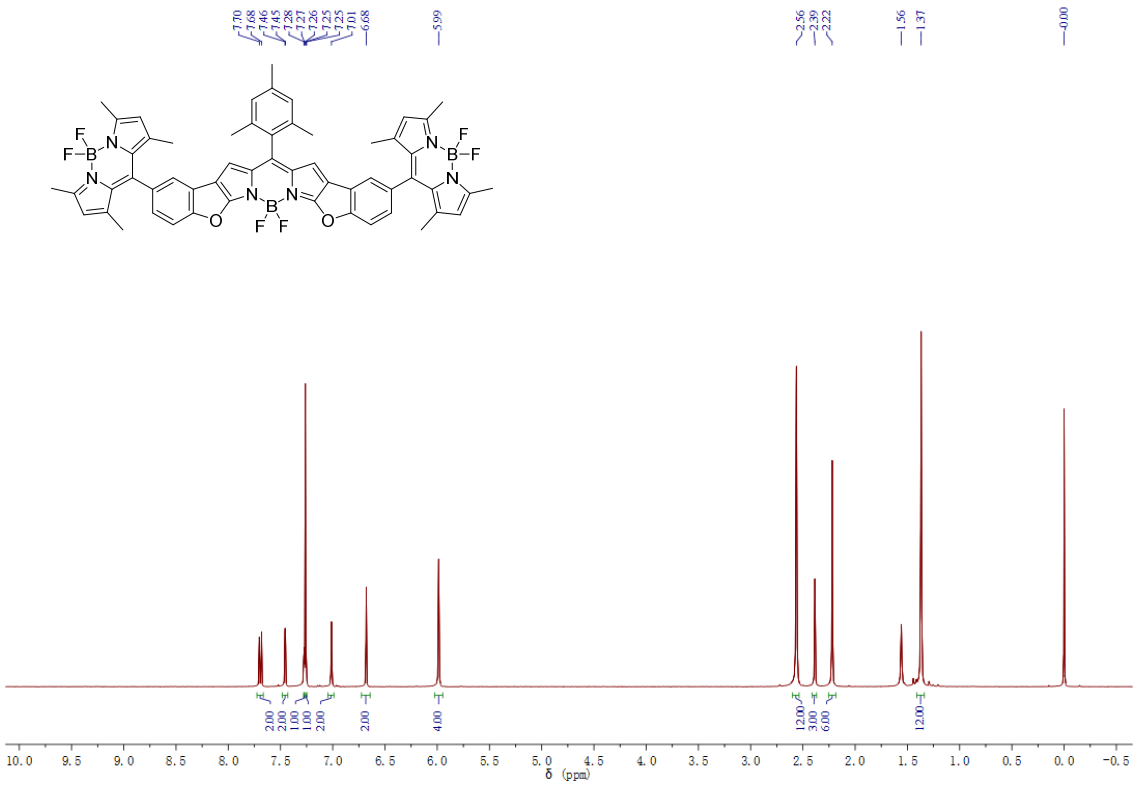
8. ^{1}H, ^{13}C NMR and HRMS spectra for all new compounds



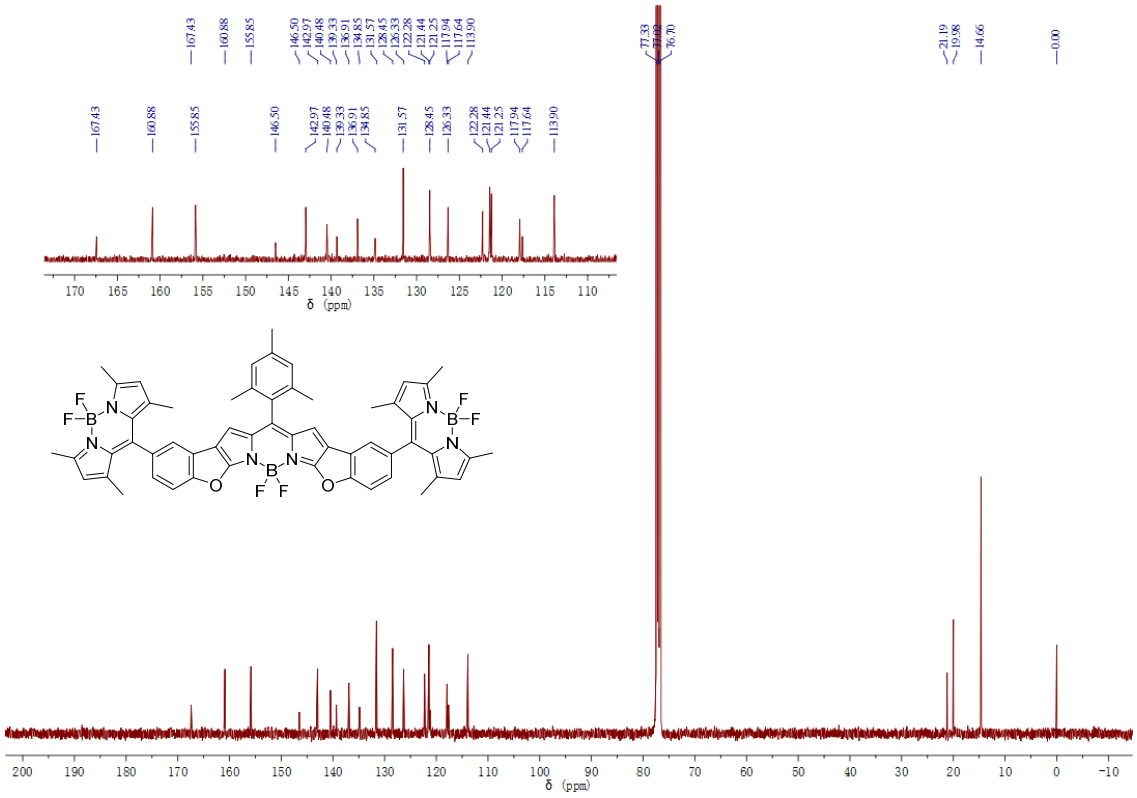
^{1}H NMR spectrum of **1** in $CDCl_3$ (400 MHz).



^{13}C NMR spectrum of **1** in $CDCl_3$ (100 MHz).

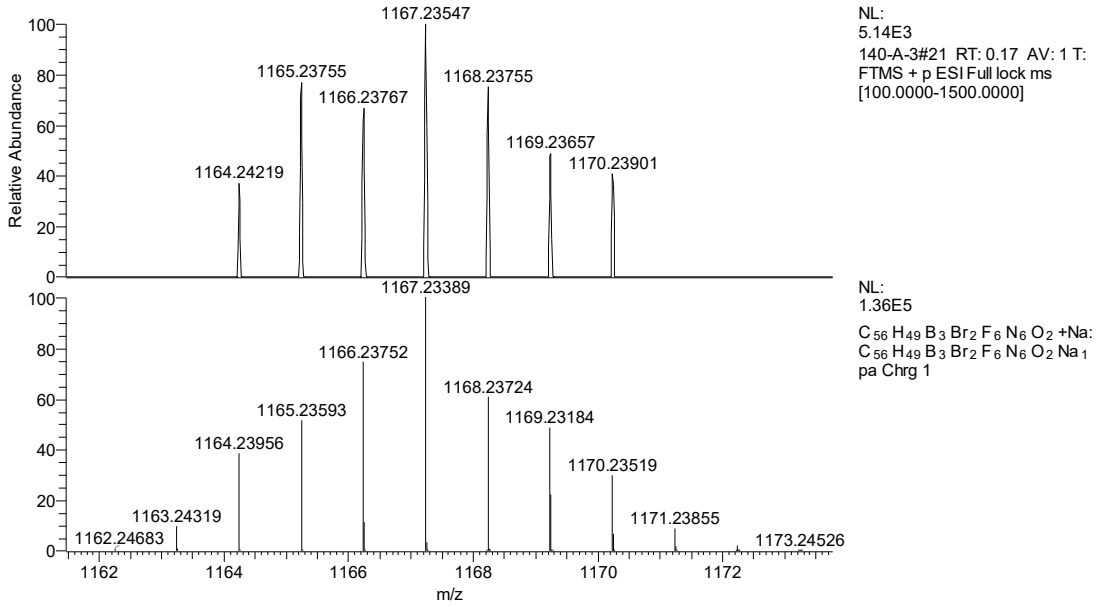


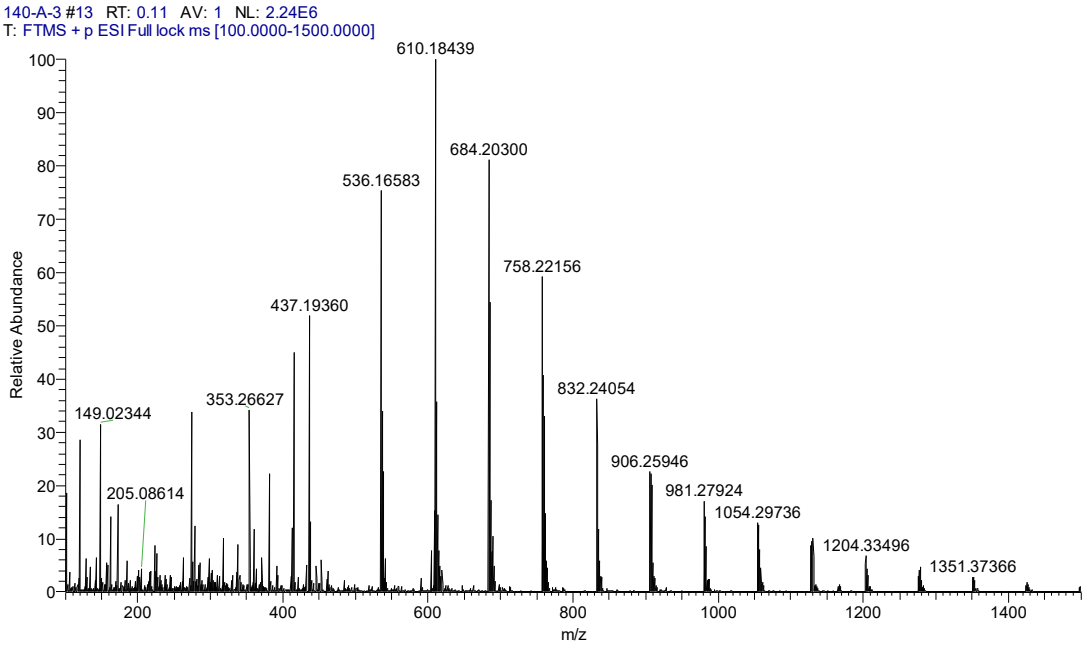
^{1}H NMR spectrum of **2** in $CDCl_3$ (400 MHz).



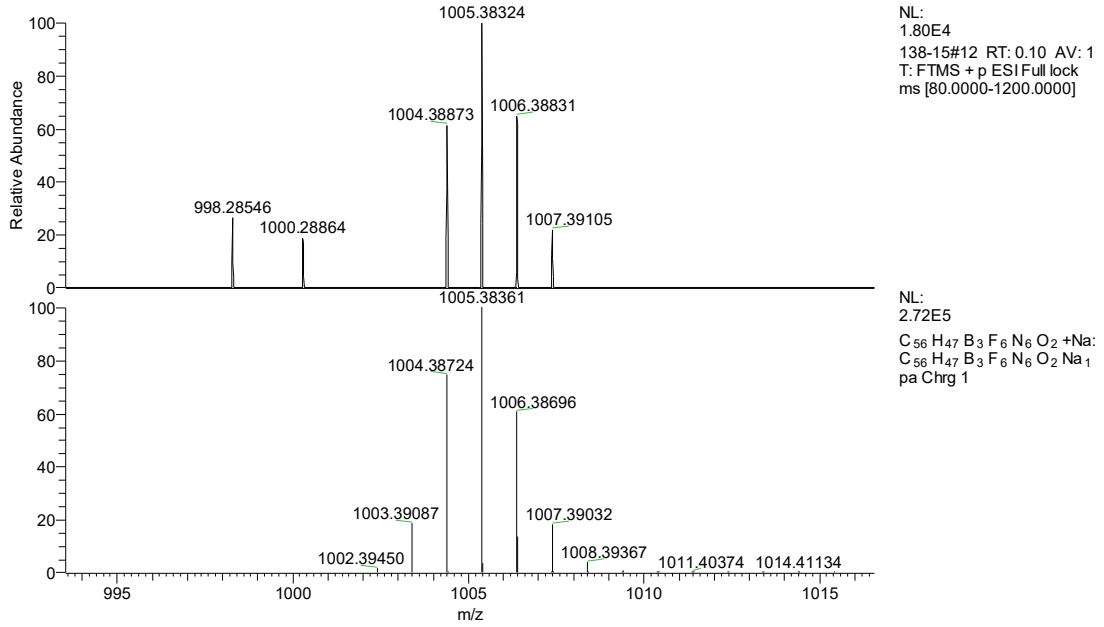
^{13}C NMR spectrum of **2** in $CDCl_3$ (100 MHz).

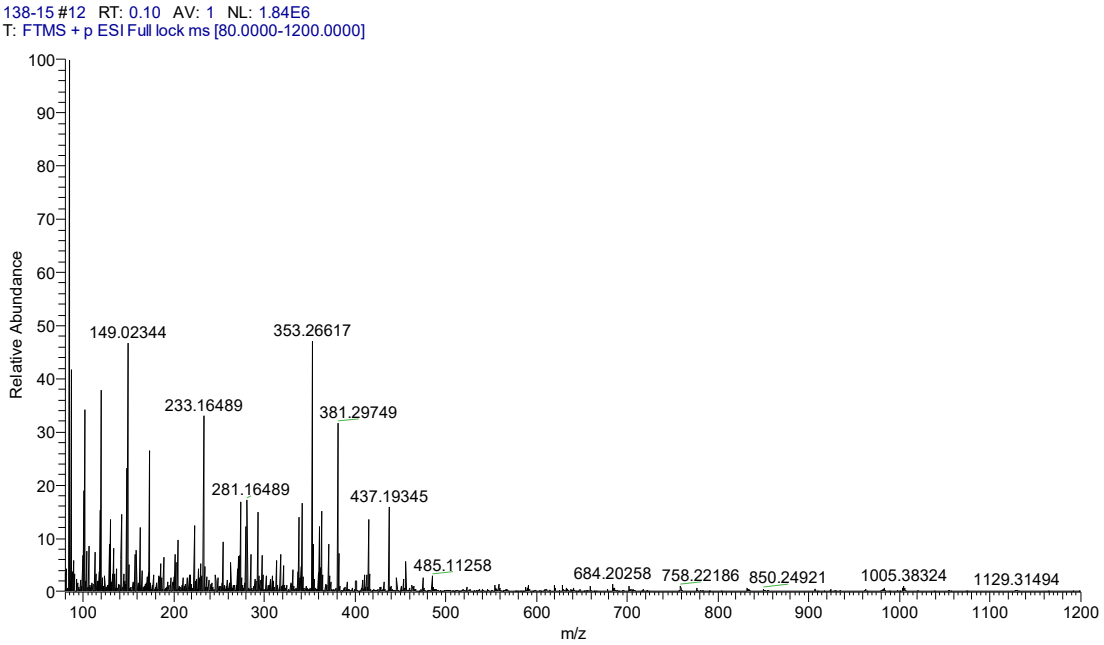
9. High resolution mass spectroscopes





HRMS for trimer **1**.





HRMS for trimer **2**.

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11. Author Contributions

H. Zuo carried out the synthesis, structural analysis and the steady state optical spectroscopy studies; Dr. X. Guo and Prof. S. Wang carried out data analysis and devised the project; L. Guo performed cell studies; Q. Wu carried out theoretical calculations; L. Wang and Z. Kang performed parts of synthesis and structural analysis; Prof. L. Jiao and Prof. E. Hao carried out data analysis, devised the project and co-wrote the manuscript.