Supporting Information

Novel nonplanar and rigid fluorophores with intensive emission in water and the application in two-photon imaging of live cells

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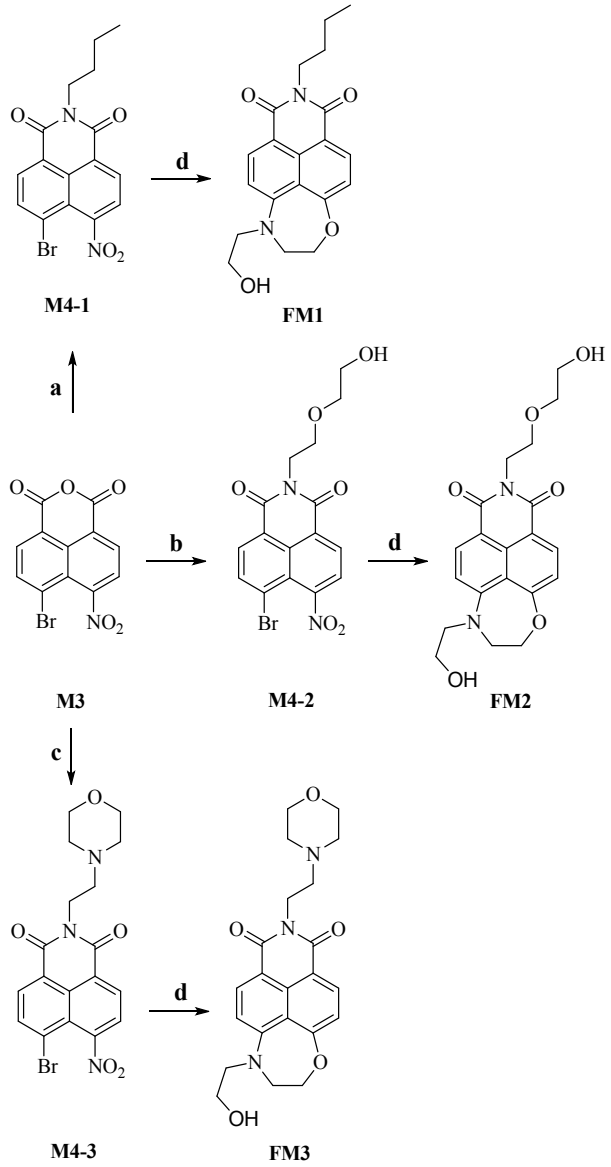
Contents

1. Materials and Instruments

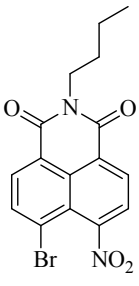
All solvents (AR, analytical reagent grade) are commercially available and were used without further purification. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and DMSO-d_6; TMS as internal standard at 25 ^{o}C on a Bruker AV-400 spectrometer. pH titration was carried out by using a pH-Meter PB-10. All reactions were monitored by thin-layer chromatography (TLC) using UV-light (254 nm) and Flu-light (365nm). Mass spectra Electrospray ionization (ESI) mass spectrometry was carried out in a HP 1100 LC-MS spectrometer. Deionized water was prepared with a Millipore Milli-Q A10 super-water system. Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.) was used for column chromatography. All reagents were obtained commercially and used without further purification unless stated otherwise.

Bioassay: MCF-7 (human breast carcinoma) cells were obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS). Fluorescence imaging was acquired on an Olympus FV1000 confocal microscope.

2. Synthesis and characterization



Scheme S1. Synthetic routes toward compound **FM1**, **FM2**, and **FM3**. (a) n-butylamine, ethanol, reflux, 2 h; (b) 2-(2-aminoethoxy)ethanol, ethanol, reflux, 2 h; (c) 2-morpholinoethanamine, ethanol, reflux, 2 h; (d) diethanolamine, $CH_3OCH_2CH_2OH$, reflux, 7 h.

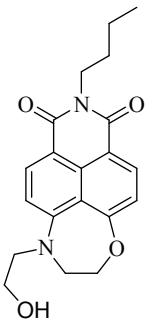


6-Bromo-2-butyl-7-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (M4-1)[1]

To a solution of compound **M3** (100 mg, 0.31 mmol) in ethanol (10 mL) was added n-butylamine (23 mg, 0.31 mmol) dissolved in 5 mL ethanol dropwise under 50 °C. Then the reaction was stirred under reflux for 2 h. After that, the mixture was evaporated in vacuum to get residue, which was purified successively by silica gel column chromatography (DCM/MeOH = 200/1, v/v) and recrystallization in ethanol to give pale yellow solid **M4-1** (51 mg, yield: 49%). Melting point: 175.4-176.2 °C.

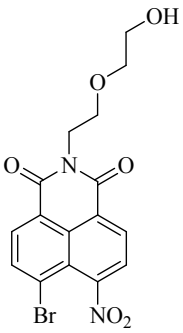
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (d, J = 8.0 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.40 (d, J = 6.8 Hz, 1H), 8.39 (d, J = 7.6 Hz, 1H), 4.03 (t, J = 7.6 Hz, 2H), 1.66-1.59 (m, 2H), 1.41-1.32 (m, 2H), 0.92 (t, J = 7.6 Hz, 3H). HRMS (EI) calcd for $C_{16}H_{13}N_2O_4Br$ $[M]^+$: 378.0038; found: 378.0042.

8-Butyl-4-(2-hydroxyethyl)-3,4-dihydropyrido[3',4',5':4,5]naphtho[1,8-ef][1,4]oxazepine-7,9(2H,8H)-dione (FM1)



Compound **M4-1** (377 mg, 1.00 mmol) and diethanolamine (1.70 mL, 17.7 mmol) were mixed in 3.5 mL 2-methoxyethanol and refluxed for 7 h. The resulting reaction was cooled down to room temperature and extracted with dichloromethane (30 mL × 3). The organic layers were collected, washed with saturated brine (30 mL) and dried with anhydrous Na_2SO_4 and evaporated under vacuum to give residue. The remaining residue was separated by silica gel column chromatography (DCM/MeOH = 50/1, v/v) to yield orange solid **FM1** (103 mg, yield: 29%). Melting point: 175.4-175.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, J = 6.4 Hz, 1H), 8.21 (d, J = 7.2 Hz, 1H), 7.10 (d, J = 6.4 Hz, 1H), 7.02 (d, J = 7.2 Hz, 1H), 4.96 (t, J = 4.0 Hz, 1H), 4.64 (t, J = 3.8 Hz, 2H), 3.99 (t, J = 6.0 Hz, 2H), 3.85 (t, J = 3.8 Hz, 2H), 3.78-3.74 (m, 4H), 1.60-1.54 (m, 2H), 1.36-1.28 (m, 2H), 0.91 (t, J = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.1, 162.8, 161.6, 153.7, 132.9, 132.9, 115.8, 115.2, 114.7, 109.4, 108.4, 73.8, 57.7, 55.3, 54.4, 38.8, 29.7, 19.8, 13.7. HRMS (ESI) calcd for $C_{20}H_{22}N_2O_4$ $[M+H]^+$: 355.1658; found: 355.1653. $C_{20}H_{22}N_2O_4$ (354.40) calcd (%): C 67.78, H 6.26, N 7.90; found: C 67.73, H 6.15, N 7.92.

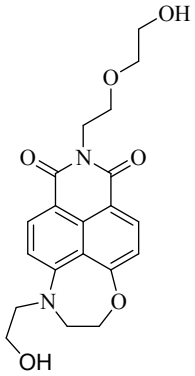
6-Bromo-2-(2-(2-hydroxyethoxy)ethyl)-7-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (M4-2)[2]



To a solution of compound **M3** (2.00 g, 6.2 mmol) in ethanol (50 mL) was added 2-(2-aminoethoxy)ethanol (616 μL, 6.2 mmol) dissolved in 15 mL ethanol dropwise under 50 °C. Then the reaction was stirred under reflux for 2 h. After that, the mixture was evaporated in vacuum to get residue, which was purified by silica gel column chromatography (DCM/MeOH = 200/1, v/v) to give pale yellow solid **M4-2** (835 mg, yield: 33%). Melting point: 176.4-176.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (d, J = 4.0 Hz, 1H), 8.52 (d, J = 4.0 Hz, 1H), 8.21 (d, J = 4.0 Hz, 1H), 7.93 (d, J = 4.0 Hz, 1H), 4.44 (t, J = 5.6 Hz, 2H), 3.86 (t, J = 5.6 Hz, 2H), 3.67-3.69 (m, 2H), 3.63-3.65 (m, 2H), 2.08 (s, 1H). HRMS (ESI) calcd for $C_{16}H_{14}N_2O_6Br$ $[M+H]^+$: 409.0035; found: 409.0029.

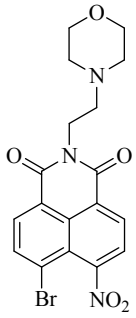
8-(2-(2-Hydroxyethoxy)ethyl)-4-(2-hydroxyethyl)-3,4-dihydropyrido[3',4',5':4,5]naphtho[1,8-ef][1,4]oxazepine-7,9(2H,8H)-dione (FM2)

Compound **M4-2** (200 mg, 0.488 mmol) and diethanolamine (472 μL, 4.88 mmol) were mixed in 3.5 mL of 2-methoxyethanol and refluxed for 7 h. The resulting reaction was cooled down to room temperature and extracted with dichloromethane (30 mL × 3). The organic layers were collected, washed with saturated brine (30 mL) and dried with anhydrous Na_2SO_4 and



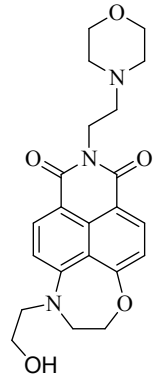
evaporated under vacuum to give residue. The remaining residue was separated by silica gel column chromatography (DCM/MeOH = 100/1, v/v) to yield orange solid **FM2** (102 mg, yield: 54%). Melting point: 108.4-109.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 4.65 (t, J = 5.0 Hz, 2H), 4.41 (t, J = 5.6 Hz, 2H), 4.02 (t, J = 5.2 Hz, 2H), 3.81-3.85 (m, 6H), 3.67-3.69 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD): δ 164.7, 164.6, 161.8, 153.9, 133.8, 133.7, 133.3, 116.6, 115.9, 115.8, 110.8, 108.6, 73.7, 72.4, 68.6, 61.4, 58.8, 55.6, 55.2, 39.4. HRMS (ESI) calcd for $C_{20}H_{23}N_2O_6$ $[M+H]^+$: 387.1556; found: 387.1547.

6-Bromo-2-(2-morpholinoethyl)-7-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (M4-3)

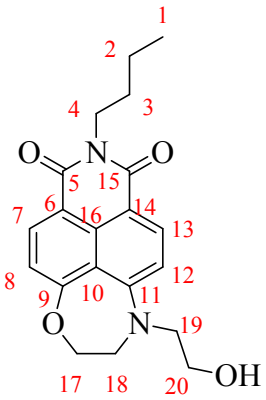


To a solution of compound **M3** (2.00 g, 6.21 mmol) in ethanol (50 mL) was added 2-morpholinoethanamine (808 mg, 6.21 mmol) dissolved in 15 mL ethanol dropwise under 50 °C. Then the reaction was stirred under reflux for 2 h. After that, the mixture was evaporated in vacuum to get residue, which was purified by silica gel column chromatography (DCM/MeOH = 200/1, v/v) to give pale yellow solid **M4-3** (1.05 g, yield: 39%). Melting point: 200.7-201.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (d, J = 7.6 Hz, 1H), 8.51 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 4.33 (t, J = 6.2 Hz, 2H), 3.65 (br, 4H), 2.70 (t, J = 6.2 Hz, 2H), 2.57 (br, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 162.1, 151.3, 136.0, 132.3, 131.2, 130.6, 125.7, 124.2, 123.6, 122.4, 121.2, 67.0, 55.9, 53.8, 37.7. HRMS (ESI) calcd for $C_{18}H_{17}N_3O_5Br$ $[M+H]^+$: 434.0352; found: 434.0344.

4-(2-Hydroxyethyl)-8-(2-morpholinoethyl)-3,4-dihydropyrido[3',4',5':4,5]naphtho[1,8-ef][1,4]oxazepine-7,9(2H,8H)-dione (FM3)



Compound **M4-3** (100 mg, 0.230 mmol) and diethanolamine (221 μL, 2.30 mmol) were mixed in 2.5 mL 2-methoxyethanol and refluxed for 7 h. The resulting reaction was cooled down to room temperature and extracted with dichloromethane (20 mL × 3). The organic layers were collected, washed with saturated brine (20 mL) and dried with anhydrous Na_2SO_4 and evaporated under vacuum to give residue. The remaining residue was separated by silica gel column chromatography (DCM/MeOH = 100/1, v/v) to yield orange solid **FM3** (56 mg, yield: 59%). Melting point: 213.2-215.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 8.8 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 4.98 (br, 1H), 4.65 (t, J = 4.6 Hz, 2H), 4.13 (t, J = 7.0 Hz, 2H), 3.85 (t, J = 4.6 Hz, 2H), 3.75 (br, 4H), 3.53 (br, 4H), 2.54-2.51 (m, 2H), 2.45 (bs, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.1, 162.8, 161.6, 153.8, 132.9, 115.8, 115.1, 114.6, 109.3, 108.4, 73.8, 66.2, 57.7, 55.7, 55.3, 54.4, 53.4, 36.3. HRMS (ESI) calcd for $C_{22}H_{26}N_3O_5$ $[M+H]^+$ 412.1872; found: 412.1881.



FM1

Table S1. 1D and 2D NMR data of compound **FM1** in DMSO-d_6

No.	$^{1}H^{a}$ δ_H, multi. (*J* /Hz)	$^{13}C^{b}$ δ_C	COSY	HMQC	HMBC	DEPT 135	DEPT 90
1	0.91, 3H, t (6.0)	13.7	H_2	C_1	$C_{1\text{-}3}$	+	
2	1.28-1.36, 2H, m	19.8	H_1,H_3	C_2	$C_{1\text{-}4}$	—	
3	1.54-1.60, 2H, m	29.7	H_2,H_4	C_3	$C_{1\text{-}4}$	—	
4	3.99, 2H , t (6.0)	38.8	H_3	C_4	C_2, C_3 C_5/C_{15}	—	
5		163.1					
6		114.7					
7	8.33, 1H, d (6.4)	132.9	H_8	C_7	C_7， C_9	+	+
8	7.10, 1H, d (6.4)	115.8	H_7	C_8	C_9， C_{16}	+	+
9		161.6					
10		109.4					
11		153.7					
12	7.02, 1H, d (7.2)	108.4	H_{13}	C_{12}	C_{10}， C_{14}	+	+
13	8.21, 1H, d (7.2)	132.9	H_{12}	C_{13}	$C_{11\text{-}13}$， C_{15}	+	+
14		114.7					
15		162.8					
16		115.2					
17	4.64, 2H, t (3.8)	73.8	H_{18}	C_{17}	C_9	—	
18	3.85, 2H, t (3.8)	54.4	H_{17}	C_{18}	C_{11}	—	
19	3.74-3.78, 4H, m	57.7		C_{19}	C_{11}， C_{20}	—	
20		55.3	OH	C_{20}	C_{19}	—	
OH	4.96, 1H, t (4.0)		H_{20}				

[a] recorded at 400 MHz, [b] recorded at 100 MHz

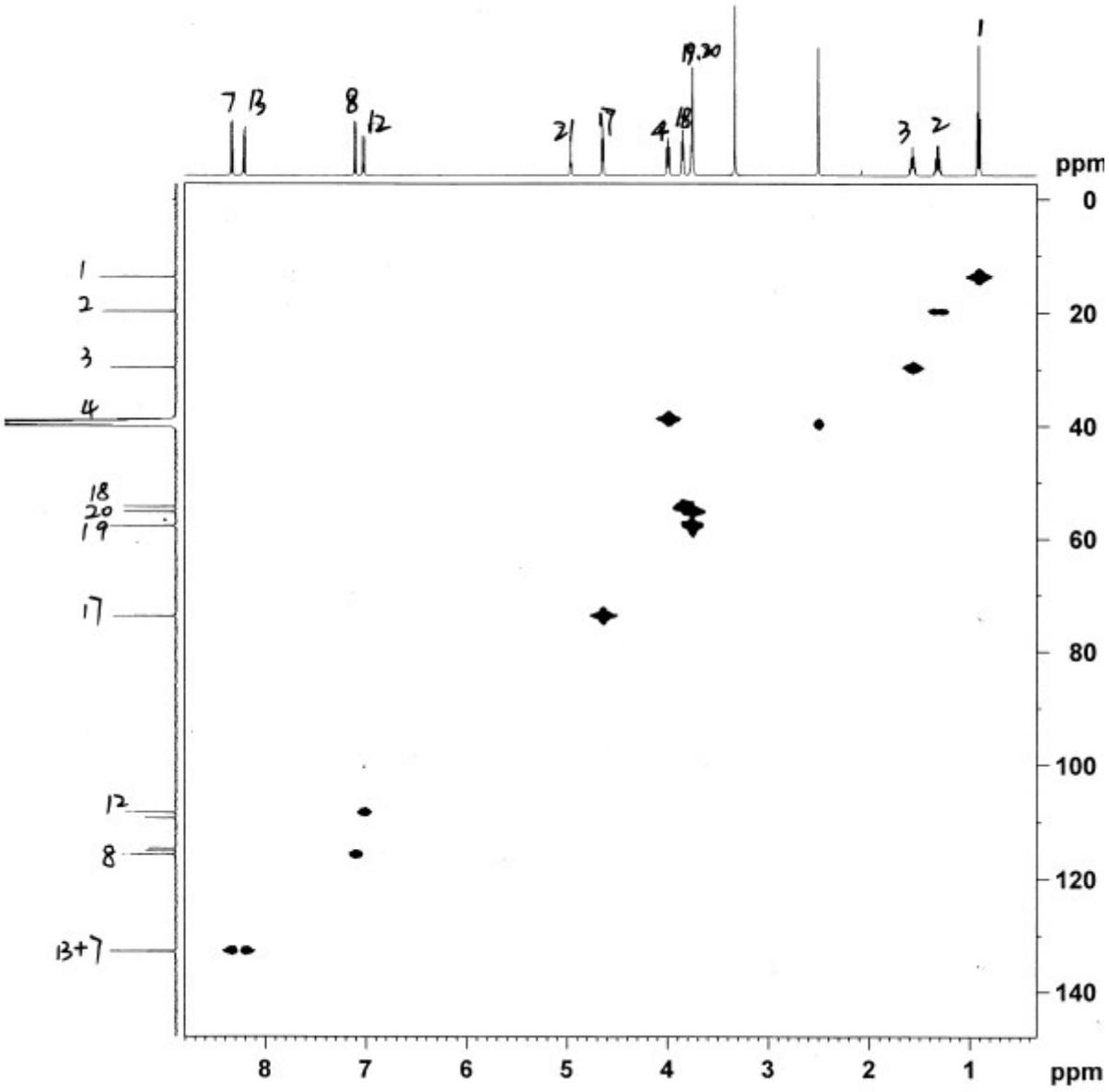


Fig. S1. HMQC spectrum of **FM1** (DMSO-d_6)

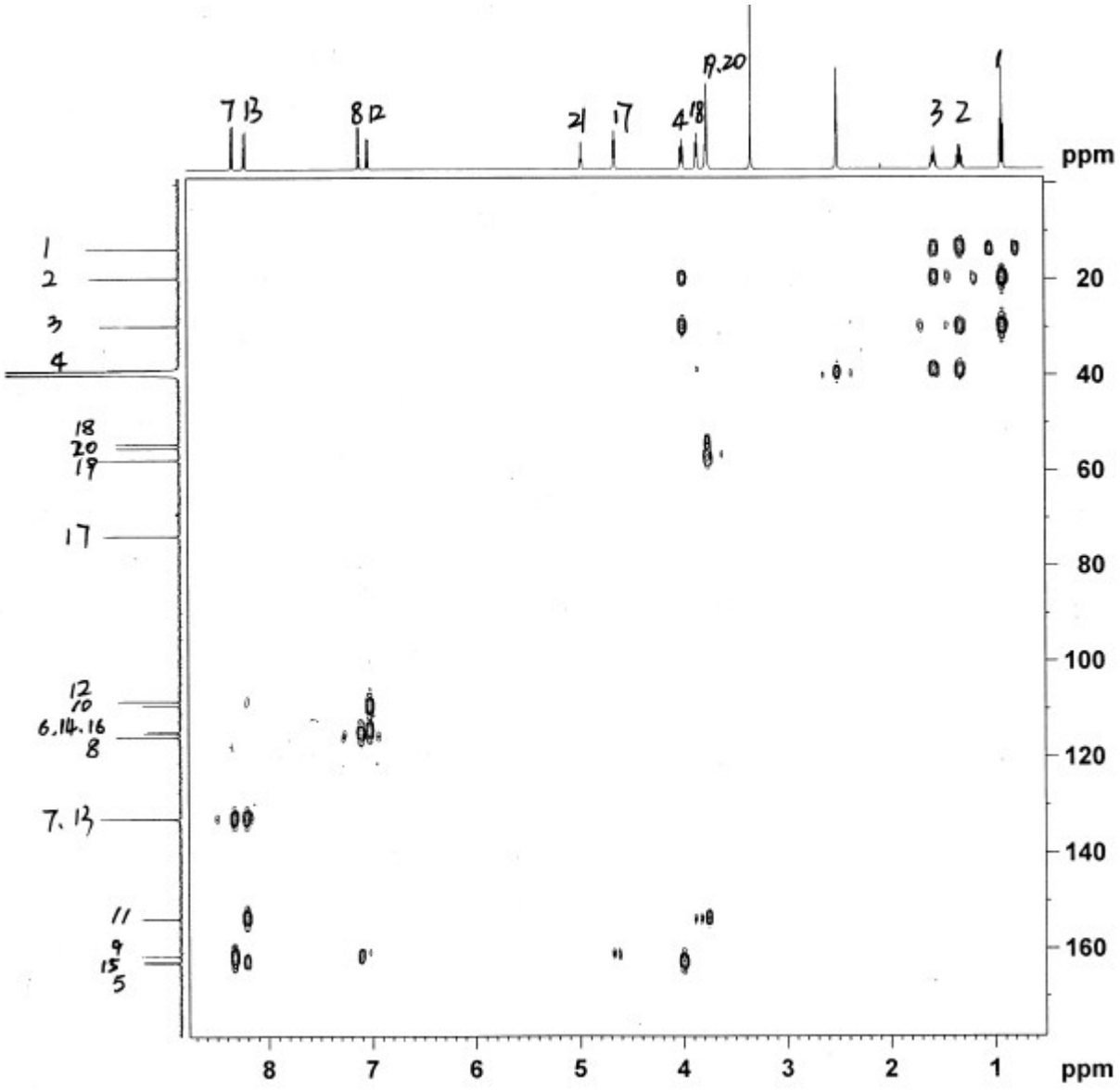


Fig. S2. HMBC spectrum of **FM1** (DMSO-d_6)

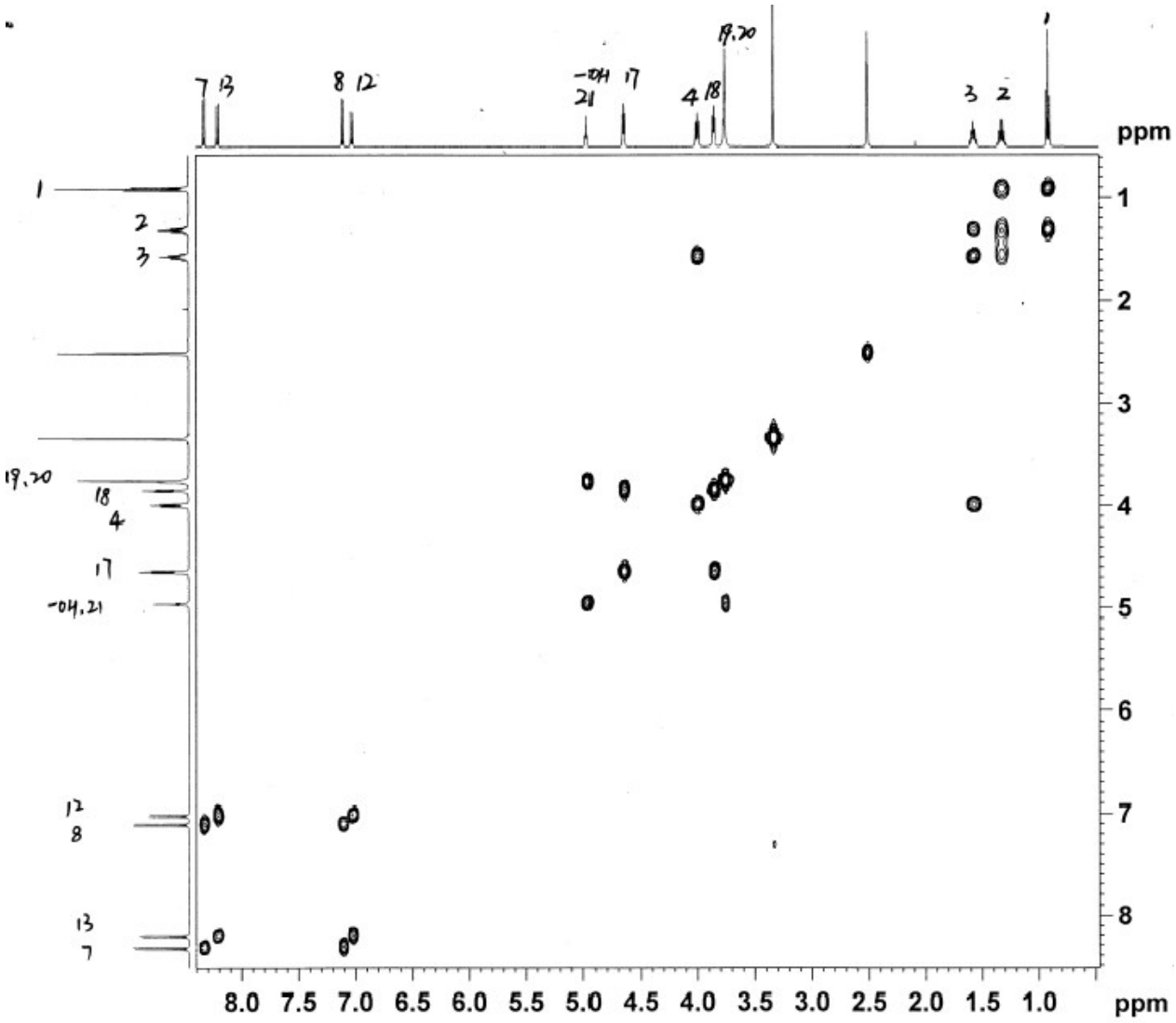


Fig. S3. ^{1}H-^{1}H COSY spectrum of **FM1** (DMSO-d_6)

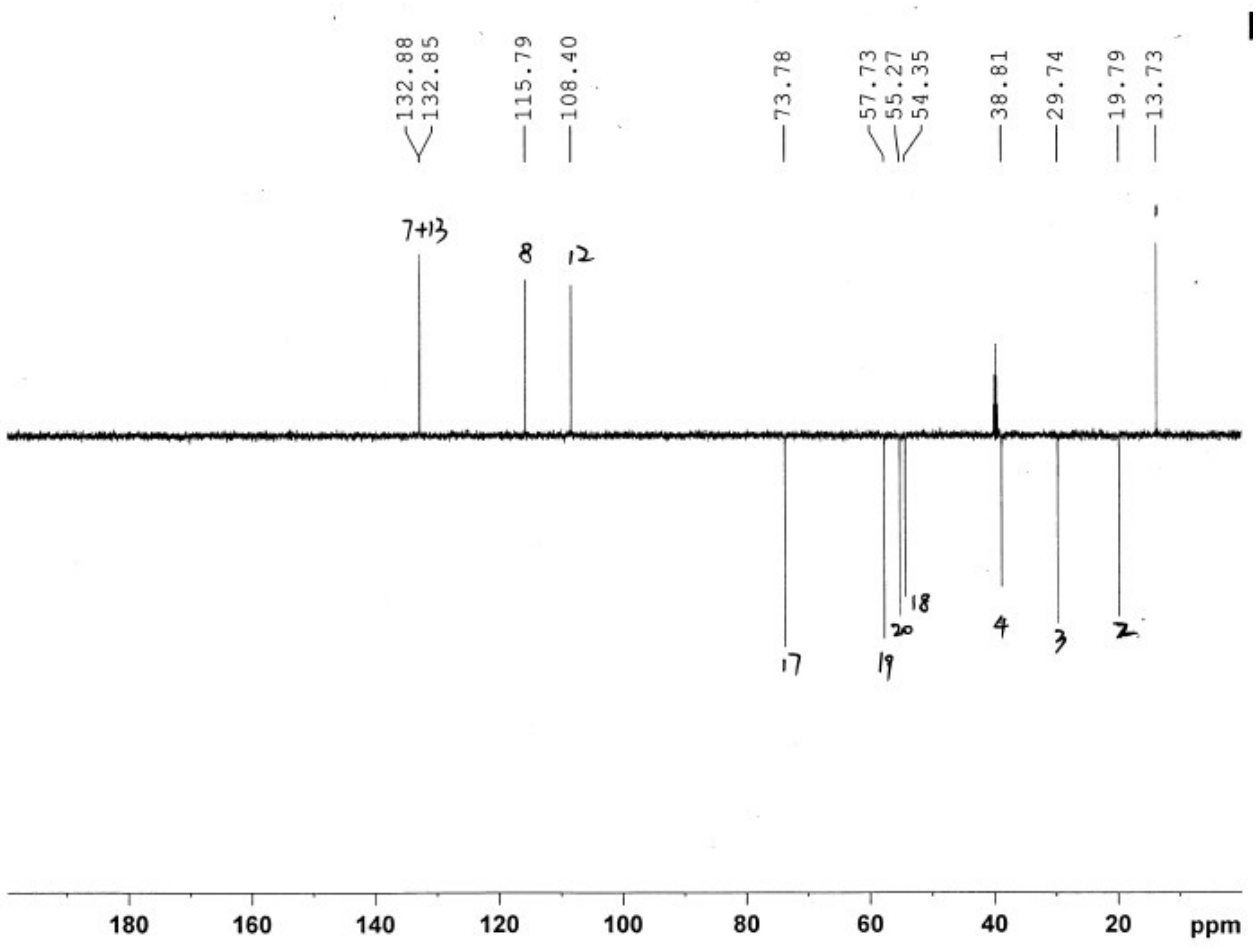


Fig. S4. DEPT 135 spectrum of **FM1** (DMSO-d_6)

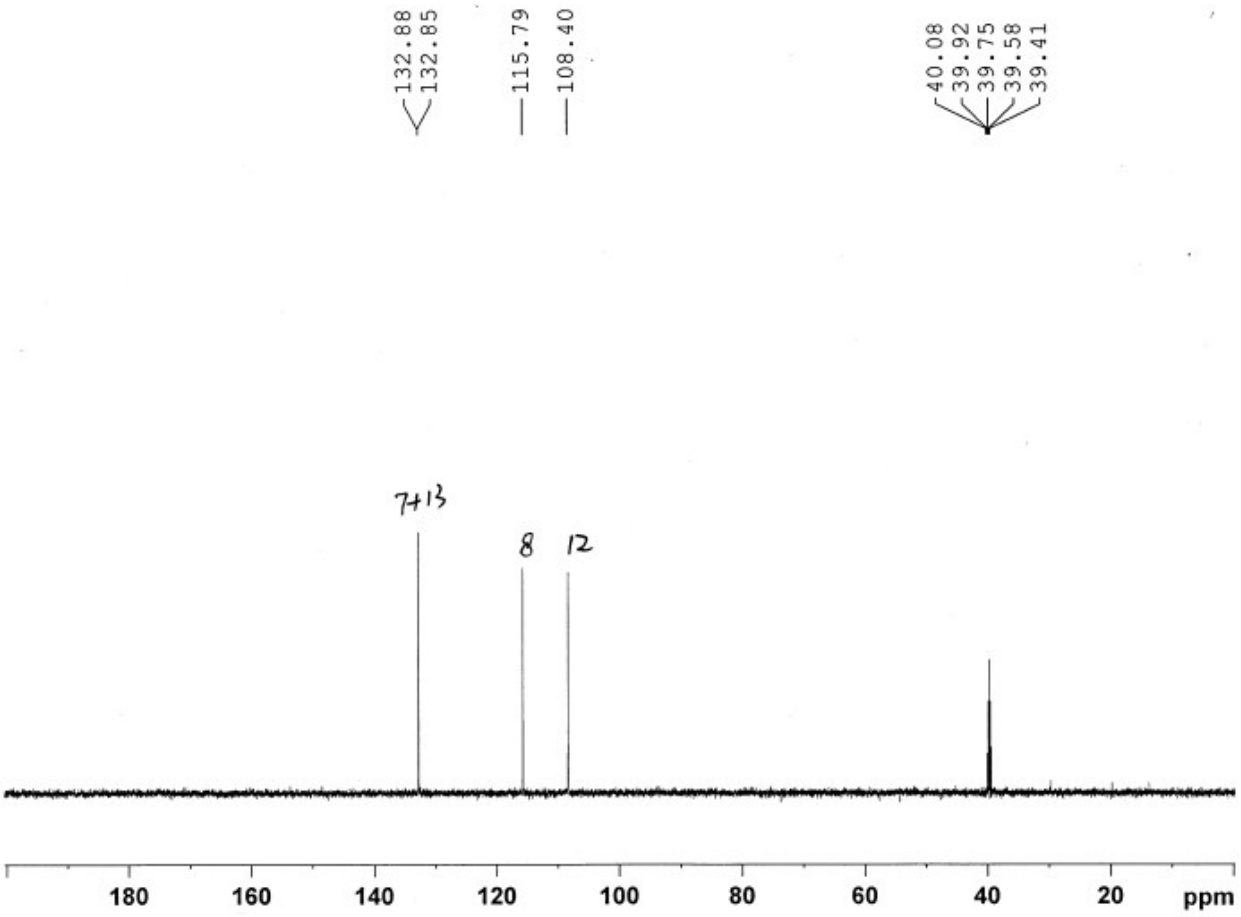


Fig. S5. DEPT 90 spectrum of **FM1** (DMSO-d_6)

3. X-ray crystal structure determination of FM2

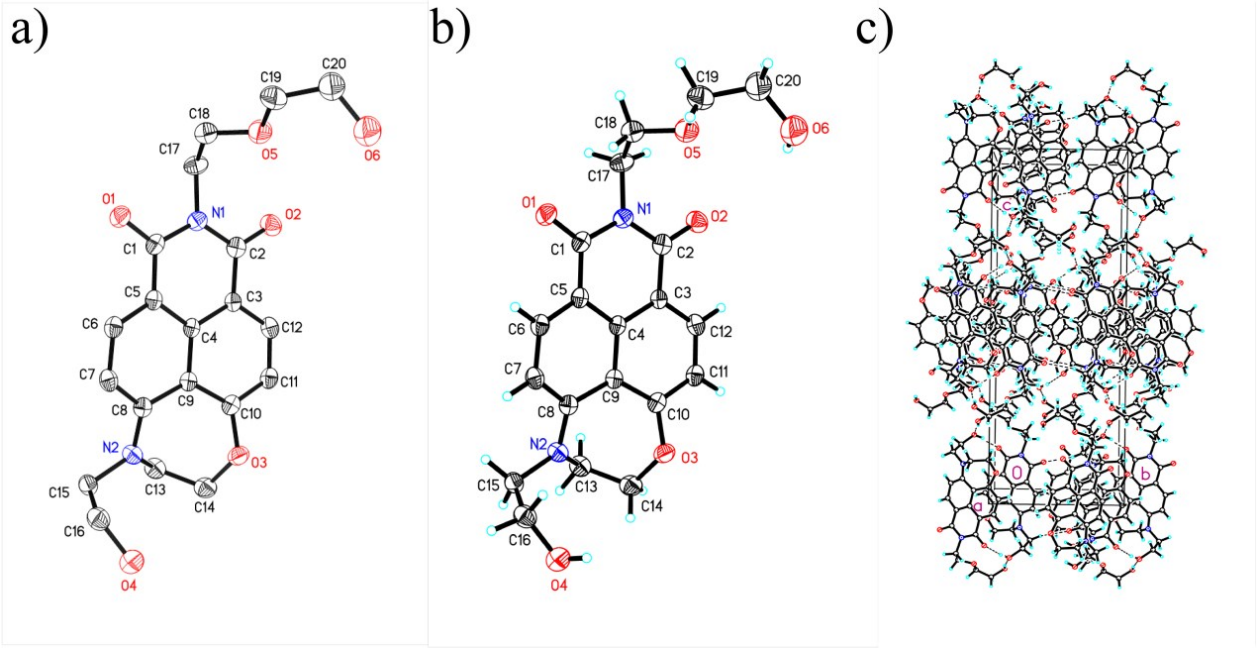


Fig. S6. Crystal structure of **FM2**. a) Thermal ellipsoid drawing of top view at 30% probability level, those unlabeled balls represent hydrogen atoms. b) Thermal ellipsoid drawing of top view at 30% probability level without hydrogen atoms. c) Packing structure along b-axis. Therein colour coding is as follows: C (gray), H (green), O (red), N (blue).

Table S2. Crystal data and structure refinement for **FM2**.

Empirical formula	$C_{20}\,H_{22}\,N_2\,O_6$	
Formula weight	386.39	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P b c a	
Unit cell dimensions	a = 11.177(4) Å	a= 90°.
	b = 10.727(4) Å	b= 90°.
	c = 29.751(11) Å	g = 90°.
Volume	3567(2) $Å^3$	
Z	8	
Density (calculated)	1.439 Mg/m^3	
Absorption coefficient	0.107 mm^{-1}	
F(000)	1632	
Crystal size	0.160 x 0.110 x 0.060 mm^3	
Theta range for data collection	2.279 to 24.998°.	
Index ranges	-13<=h<=13, -12<=k<=10, -35<=l<=32	
Reflections collected	18539	
Independent reflections	3143 [R(int) = 0.1857]	
Completeness to theta = 25.242°	97.4 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6098	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3143 / 0 / 256	
Goodness-of-fit on F^2	1.018	
Final R indices [I>2sigma(I)]	R1 = 0.0687, wR2 = 0.1290	
R indices (all data)	R1 = 0.1455, wR2 = 0.1587	
Extinction coefficient	0.0042(14)	
Largest diff. peak and hole	0.335 and -0.238 $e.Å^{-3}$	

4. Photophysical properties

4.1 Absorption and emission spectra

Absorption and emission spectra were performed at identical condition (25 ºC) with a Varian Cary 100 spectrometer and a Varian Cary Eclipse fluorescence spectrometer, respectively.

4.2 Fluorescence quantum yield

Fluorescein was utilized as a standard sample with known quantum yield (Φ_s = 0.79) in 0.1 M NaOH aqueous solution[3]. The relative fluorescence quantum yields were calculated according to the following equation[432]

$$\Phi_x = \Phi_s \, (Grad_x/Grad_s) \, (n_x^2/n_s^2)$$

Where the subscripts x and s represent test and standard respectively, Φ means fluorescence quantum yield, *Grad* denotes the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and *n* is the refractive index of the selected solvent.

All the final compounds **FM0**, **FM1**, **FM2**, and **FM3** were prepared into 1 mM DMSO stock solution for test. And Fluorescence quantum yields of each compound were measured in various solvents including deionized water, PBS buffer (0.01 M, pH = 7.4), Tris-HCl buffer (0.1 M, pH = 7.4), analytically pure ethanol, acetonitrile, tetrahydrofuran (**Table S3**). Fluorescence testing slit: 5/2.5 nm

4.3 pH titration

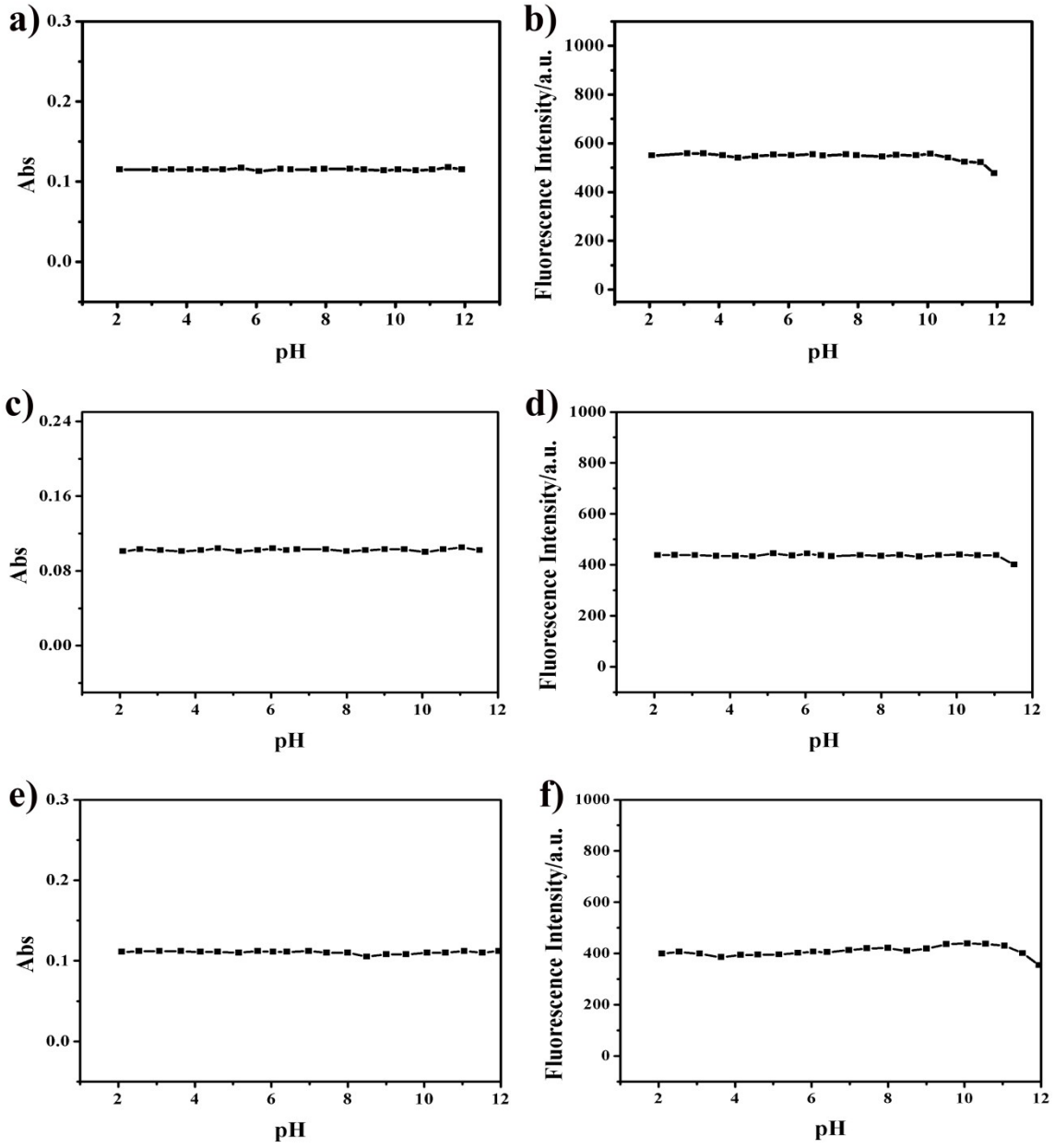


Fig. S7. Absorption (a, c, e) and emission (b, d, f) changes of **FM1, FM2, FM3** (5 μM) as a function of pH values ranging from 2 to 12 in Water (0.5% DMSO). Excitation wavelength: 470 nm, slit: 5/2.5 nm.

Table S3. Absorption and emission properties of **FM0**, **FM1**, **FM2**, and **FM3** in selected solvents.[a]

compounds	Solvent	λ^{max}_{abs} (nm)	λ^{max}_{em}[c] (nm)	Δλ (nm)	ε ($\times10^4$ $M^{-1}cm^{-1}$)	Φ[d]	Brightness[e] ($\times10^4$ $M^{-1}cm^{-1}$)
FM0[b]	CH_3CN	433	520	87	1.47	0.82	1.21
	EtOH	442	530	88	1.50	0.86	1.29
	THF	429	501	72	1.42	0.86	1.22
	water	455	542	87	1.11	**0.082**	**0.09**
	PBS	456	542	86	1.07	0.083	0.09
	Tris-HCl	460	548	88	1.12	0.073	0.08
FM1	CH_3CN	442	500	58	1.99	0.86	1.71
	EtOH	450	502	52	1.94	0.97	1.88
	THF	437	488	51	1.98	0.93	1.84
	water	469	525	56	2.44	**0.62**	**1.51**
	PBS	467	525	58	2.18	0.58	1.26
	Tris-HCl	469	525	56	2.27	0.59	1.34
FM2	CH_3CN	443	501	58	1.90	0.85	1.62
	EtOH	450	505	55	1.68	0.92	1.55
	THF	440	491	51	1.74	0.91	1.58
	water	467	527	60	2.05	**0.66**	**1.35**
	PBS	468	525	57	2.00	0.64	1.28
	Tris-HCl	468	525	57	2.36	0.65	1.53
FM3	CH_3CN	441	498	57	1.79	0.92	1.65
	EtOH	453	502	49	1.98	0.99	1.96
	THF	437	486	49	1.78	0.96	1.71
	water	469	526	57	2.18	**0.60**	**1.31**
	PBS	467	528	61	2.11	0.62	1.31
	Tris-HCl	469	525	56	2.06	0.60	1.24

[a] Stokes shifts, Δλ; molar extinction coefficient at longest wavelength transition, ε; fluorescence quantum yields, Φ.

[b] Control compound: N-butyl-4-butylamine-1,8-naphthalimide, usually used as reference ($\Phi_s = 0.81$ in alcohol).

[c] Excited at maximum absorption wavelength.

[d] Determined relative to fluorescein ($\Phi_f = 0.79$ in 0.1 M sodium hydroxide aqueous solution).

[e] Brightness was the product of the molar extinction coefficient at λ^{max}_{abs} and the corresponding fluorescence quantum yield.

4.4 Photostability [5]

The photostability of **FM0**, **FM1**, **FM2**, and **FM3**, fluorescein and BODIPY were measured under irradiation of a 150 W Incandescent Lamp for 200 min. **FM0**: 10% DMSO (v/v) in water, exited at 455 nm, slit: 5/5 nm; **FM1**, **FM2**, and **FM3**: 0.5% DMSO (v/v) in water, exited at 470 nm, slit: 5/2.5 nm; fluorescein: 0.1M sodium hydroxide solution, exited at 480 nm, slit: 5/2.5 nm; BODIPY: 10% DMSO (v/v) in water, excited at 480 nm, slit: 5/2.5 nm. These six samples were 10 cm from the light source, between which was placed a transparent capacity full of saturated potassium nitrate solution. Therein, saturated salt solution was utilized to avoid influence from strong heat of lamp.

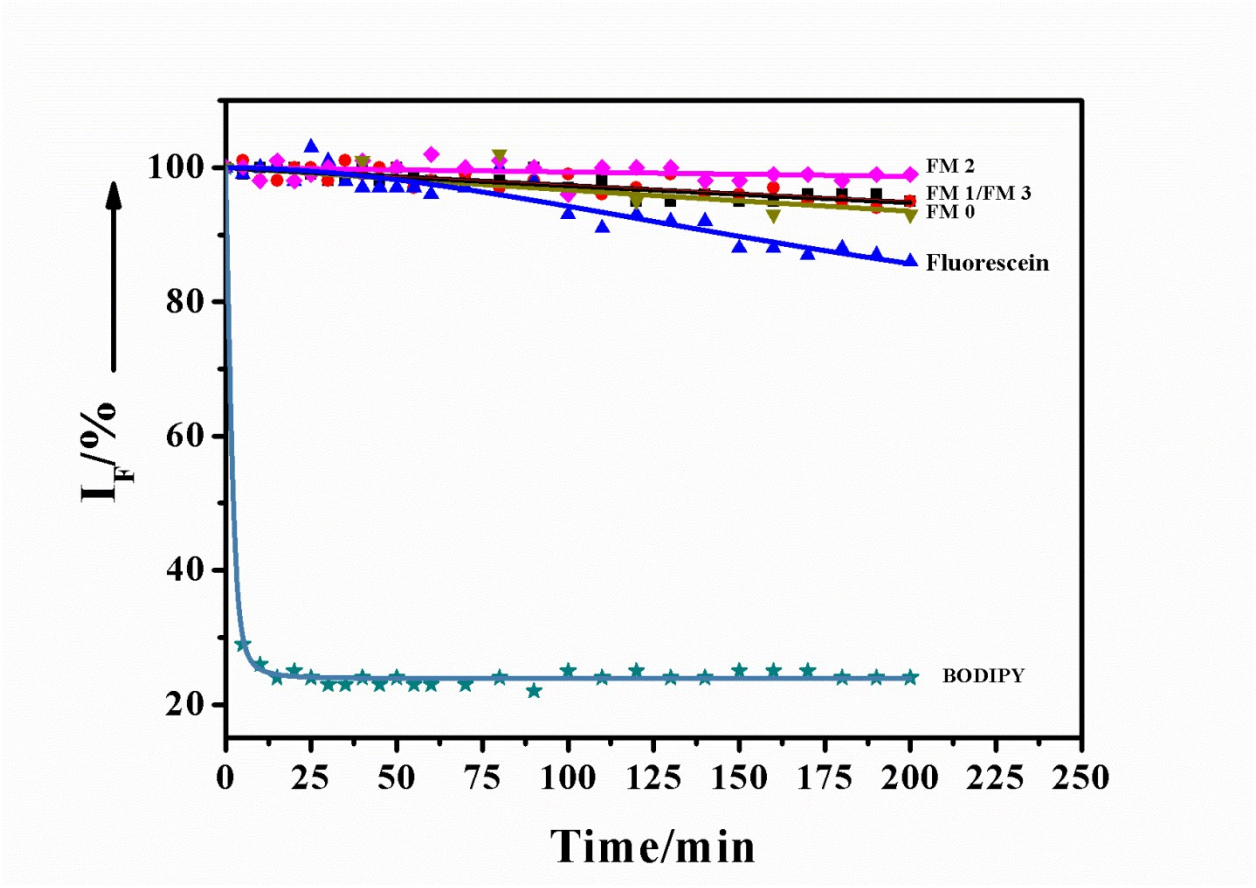


Fig. S8. Photobleaching curves for **FM0** (dark yellow), **FM1** (black), **FM2** (pink), **FM3** (red), Fluorescein (blue), BODIPY (cyan). The plot was obtained by using a 150 W Incandescent Lamp in water (10% DMSO, v/v) for **FM0** and BODIPY, water (0.5% DMSO, v/v) for **FM1**, **FM2**, and **FM3**, 0.1 M sodium hydroxide solution for fluorescein. BODIPY: 10- (4-carboxyphenyl) -5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo [1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide.

5. Cell imaging

5.1 Two-photon action cross section

TPA spectra were measured through a femtosecond two-photon-excited fluorescence (TPEF) technique[6].

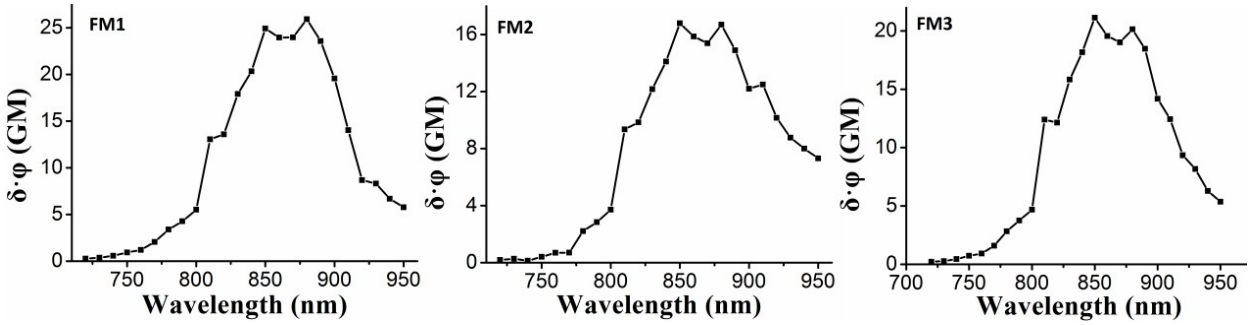


Fig. S9. Two-photon action (δ·φ) spectra of compounds **FM1**, **FM2**, and **FM3** in EtOH.

5.2 General methods

MCF-7 (human breast carcinoma) cells were obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS). Fluorescent imaging was acquired on an Olympus FV1000 confocal microscope.

5.3 Fluorescence imaging

The cells were cultured in Dulbecoo's modified Eagle's medium (DMEM) medium supplemented with 10% FBS (fetal bovine serum) and Penicillin-Streptomycin at 37°C in 95% air with 5% CO_2. Cells cultured in glass bottom dishes for 24 h and stained with **FM0** (2 μM, 20 min), **FM1** (1 μM, 10 min), **FM2** (2 μM, 20 min) or **FM3** (2 μM, 20 min). And then cells were washed with PBS for three times. **FM3** stained cells were incubated for more 20 min.

Table S4. The laser intensities and gain settings of compounds **FM0, FM1, FM2** and **FM3** in cell imaging experiment

compound	laser intensities		gain settings	
	OPM	TPM	OPM	TPM
FM0	2%	9%	600 V	700 V
FM1	5%	12%	600 V	700 V
FM2	30%	36%	700 V	700 V
FM3	16%	20%	650 V	680 V

5.4 MTT assay

MCF-7 cells in exponential phase of growth were used to assess the cytotoxic effect of **FM1**, **FM2**, and **FM3**. 3×10^3 cells/well (100 μL) were seeded into 96-well plates and allowed to grow for 24 h prior to treatment with **FM1**, **FM2**, and **FM3**. **FM1**, **FM2**, and **FM3** at different concentrations (0, 1, 2, 10, 20 μM, dispersed in 100 μL medium) were added into wells (final concentration 0, 0.5, 1, 5, 10 μM) and incubated for 24 h. MTT is added (final concentration 0.5 mg/mL) and incubate for more 4 h. The medium was removed and 200 μL DMSO was added to dissolve the formazan crystals. Optical densities were measured at 490 nm by a Thermo Scientific Multiskan FC spectrophotometer.

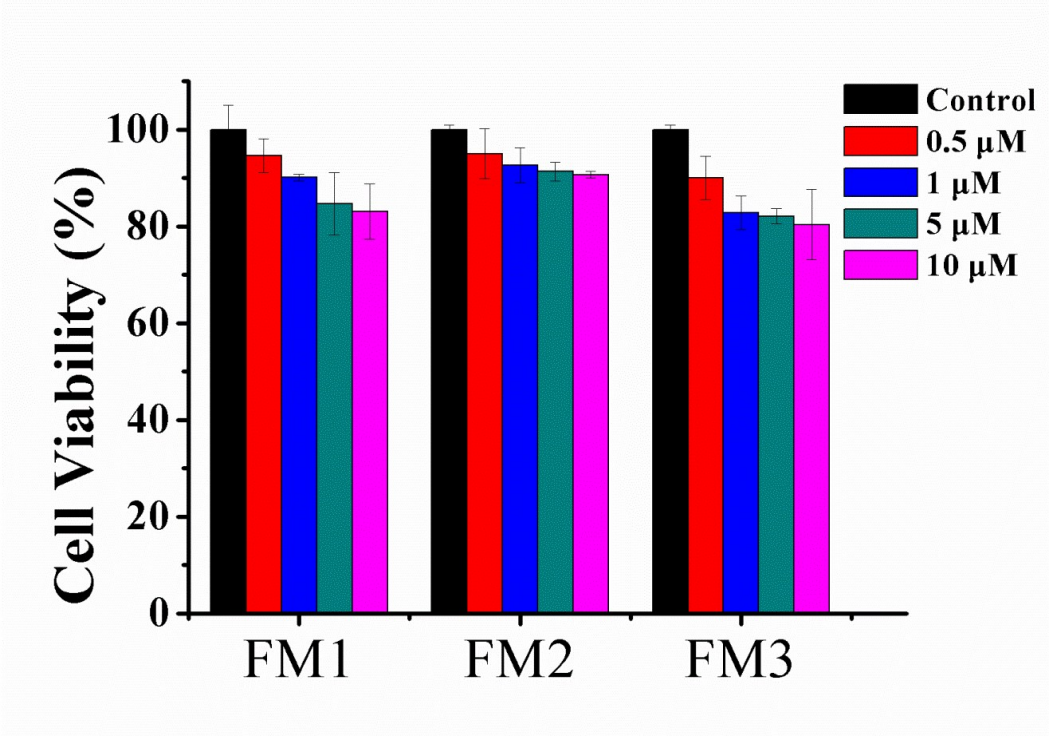


Fig. S10. Cell viability of compounds **FM1**, **FM2**, and **FM3** at different concentrations for 24 h.

6. NMR and HRMS spectra

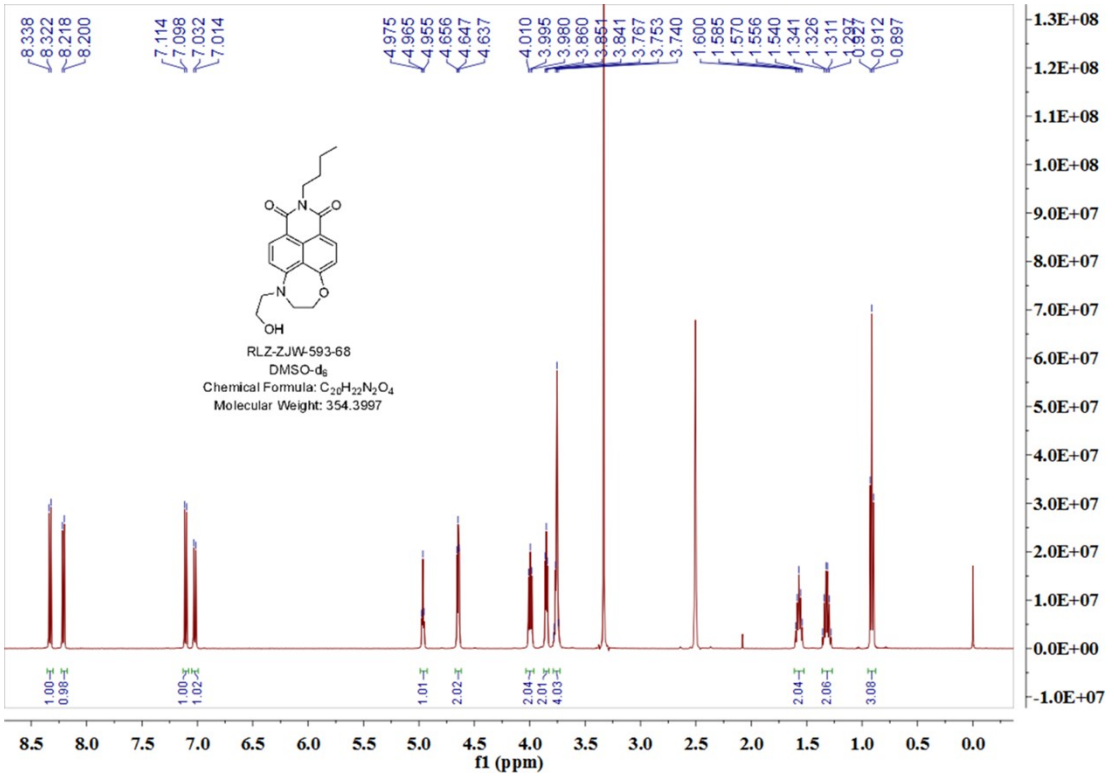


Fig. S11. [1]H NMR spectrum of **FM1** (DMSO-d_6, 400 MHz)

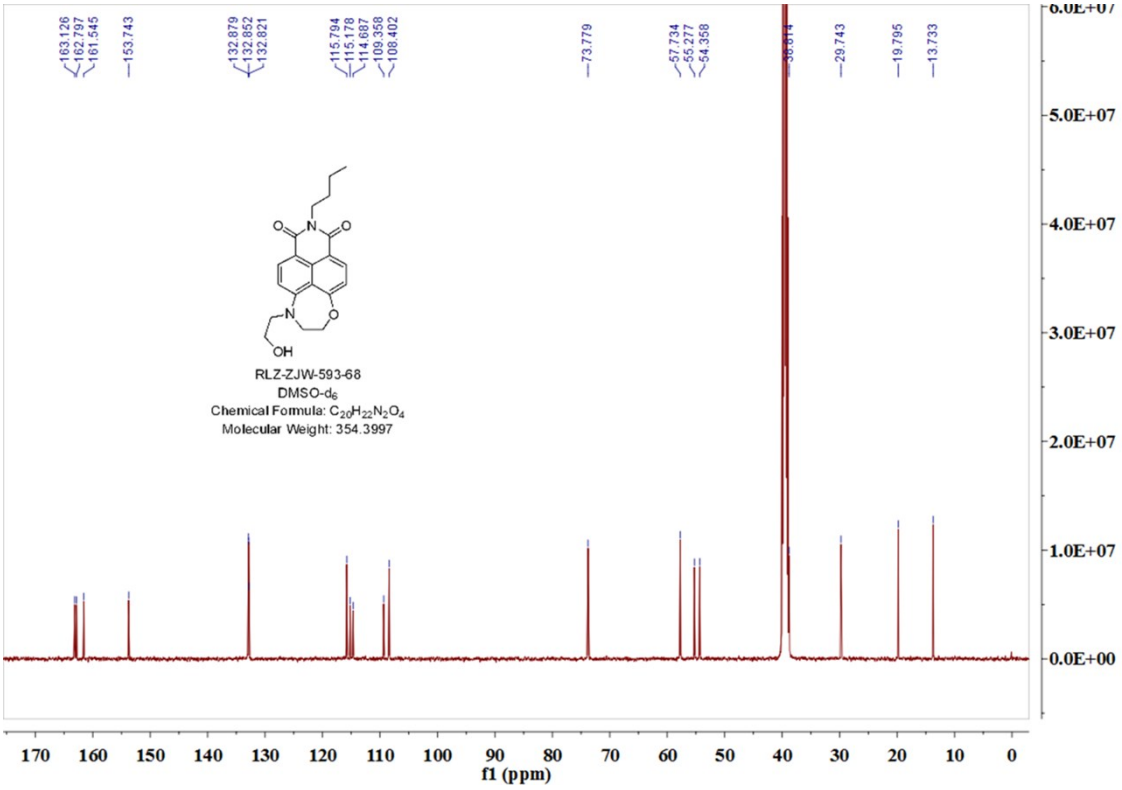


Fig. S12. [13]C NMR spectrum of **FM1** (DMSO-d_6, 100 MHz)

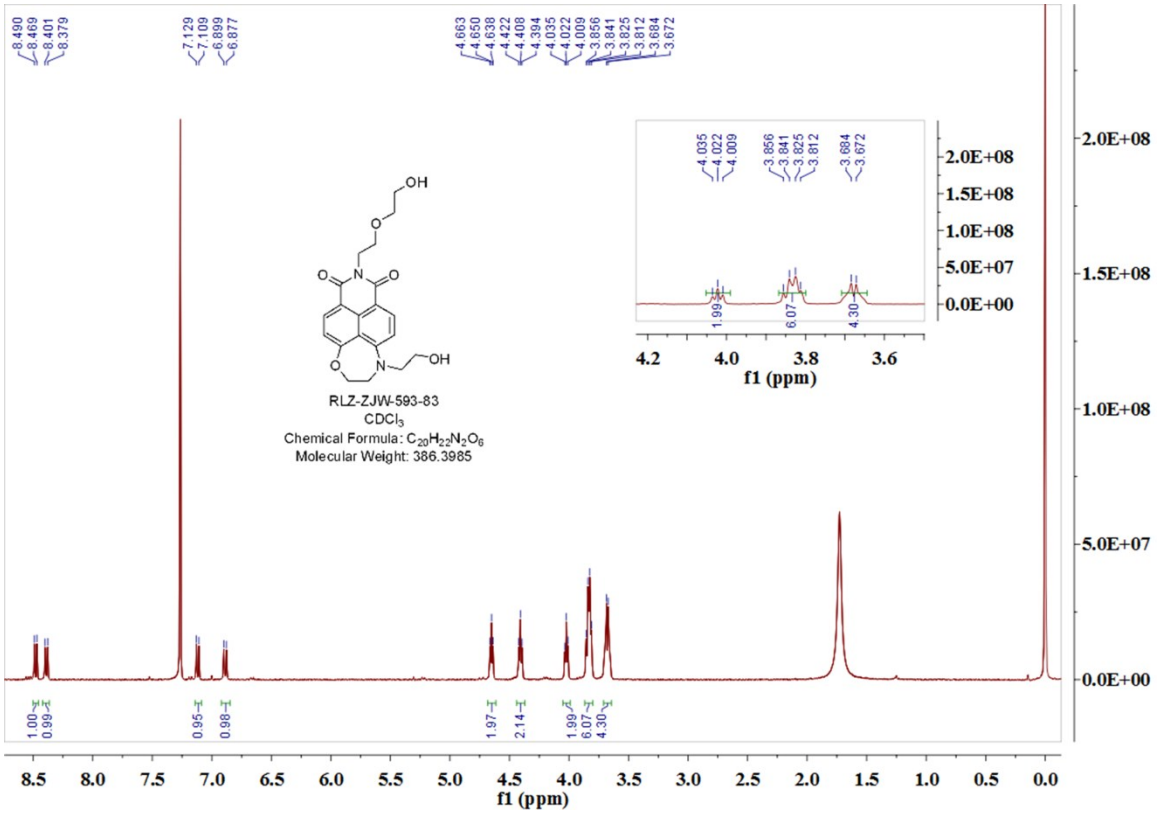


Fig. S13. ^{1}H NMR spectrum of **FM2** ($CDCl_3$, 400 MHz)

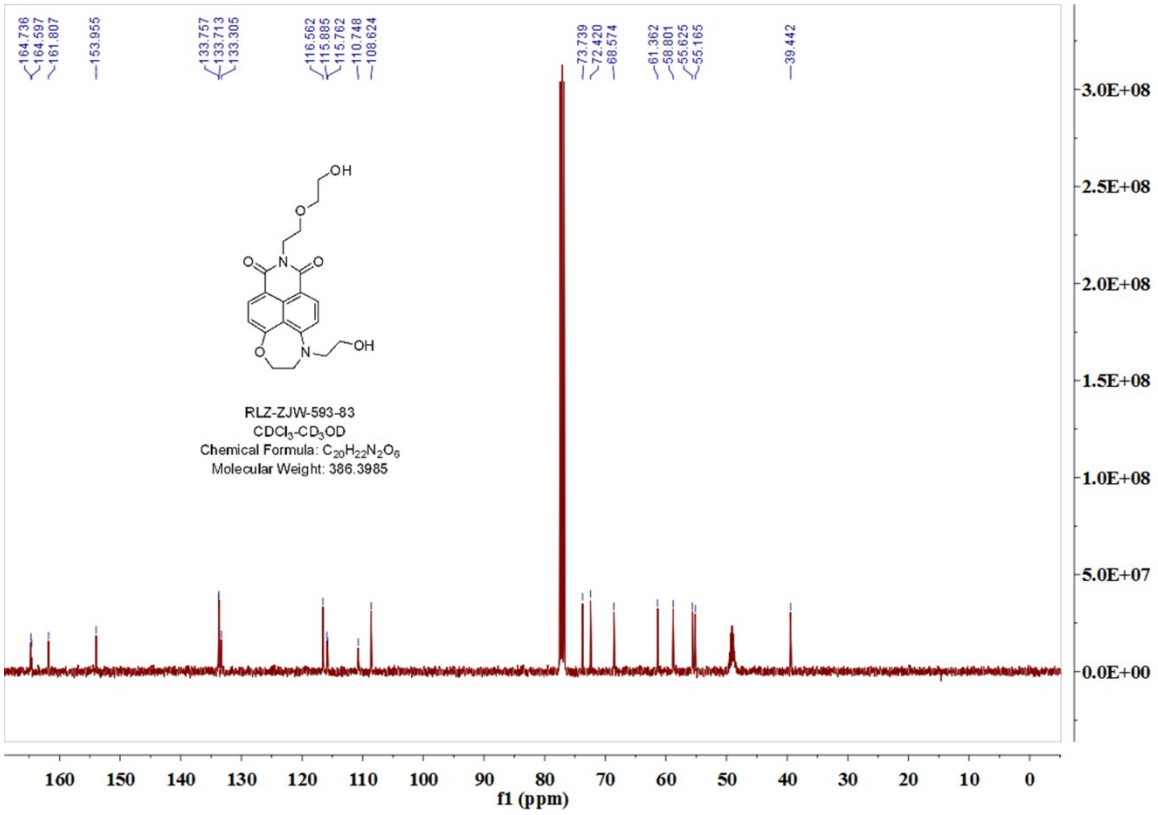


Fig. S14. ^{13}C NMR spectrum of **FM2** ($CDCl_3$-CD_3OD, 100 MHz)

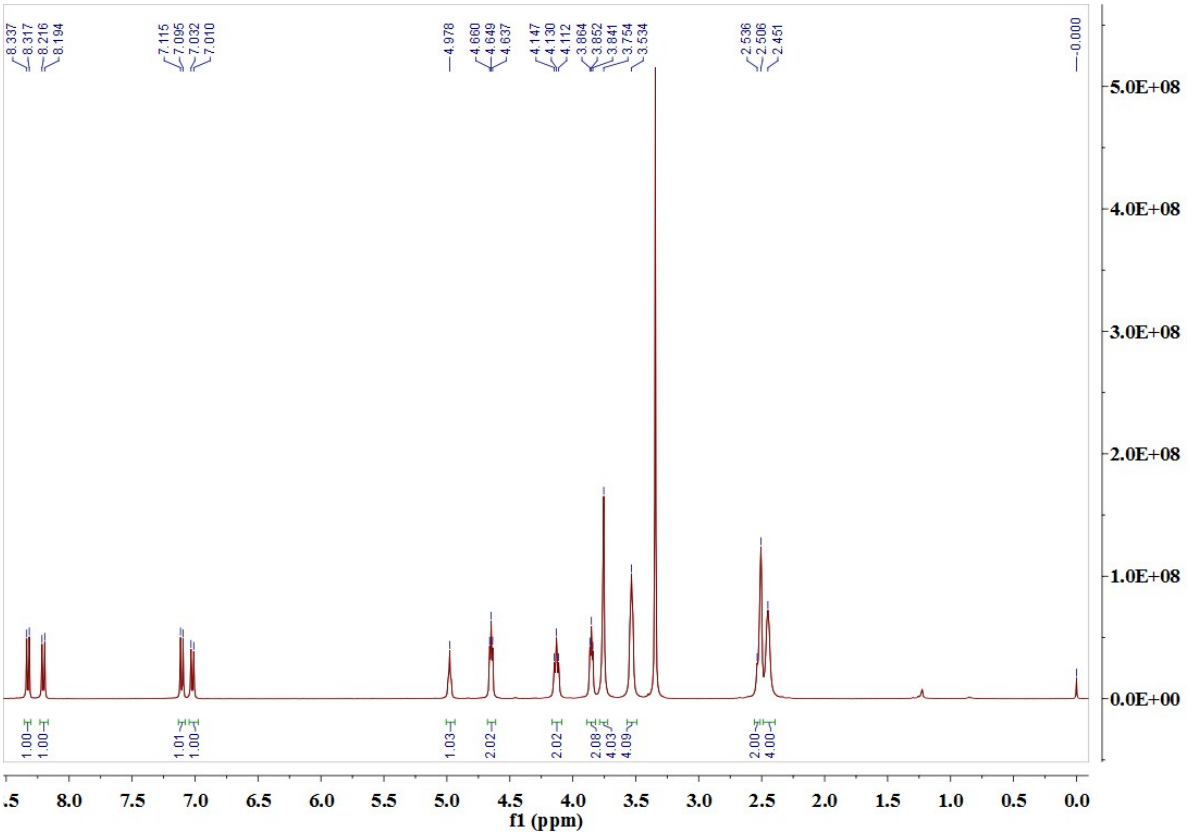


Fig. S15. ^{1}H NMR spectrum of **FM3** (DMSO-d_6, 400 MHz)

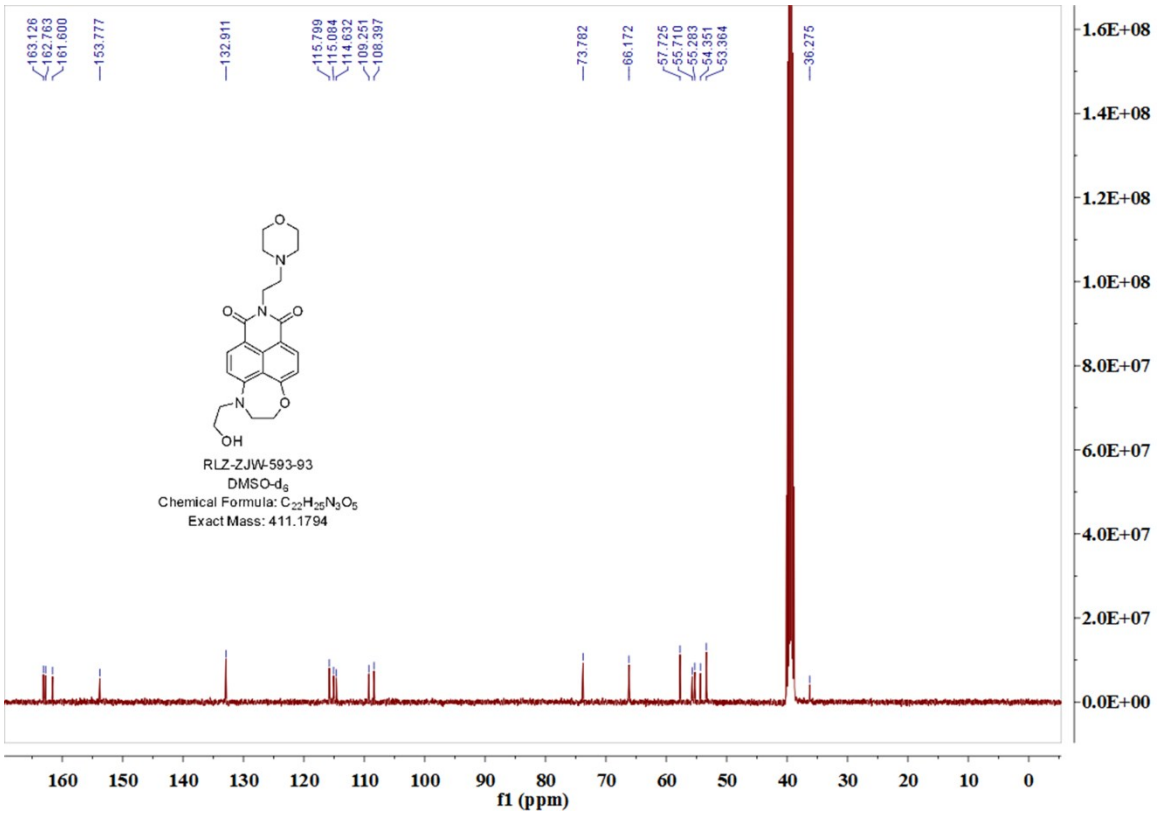
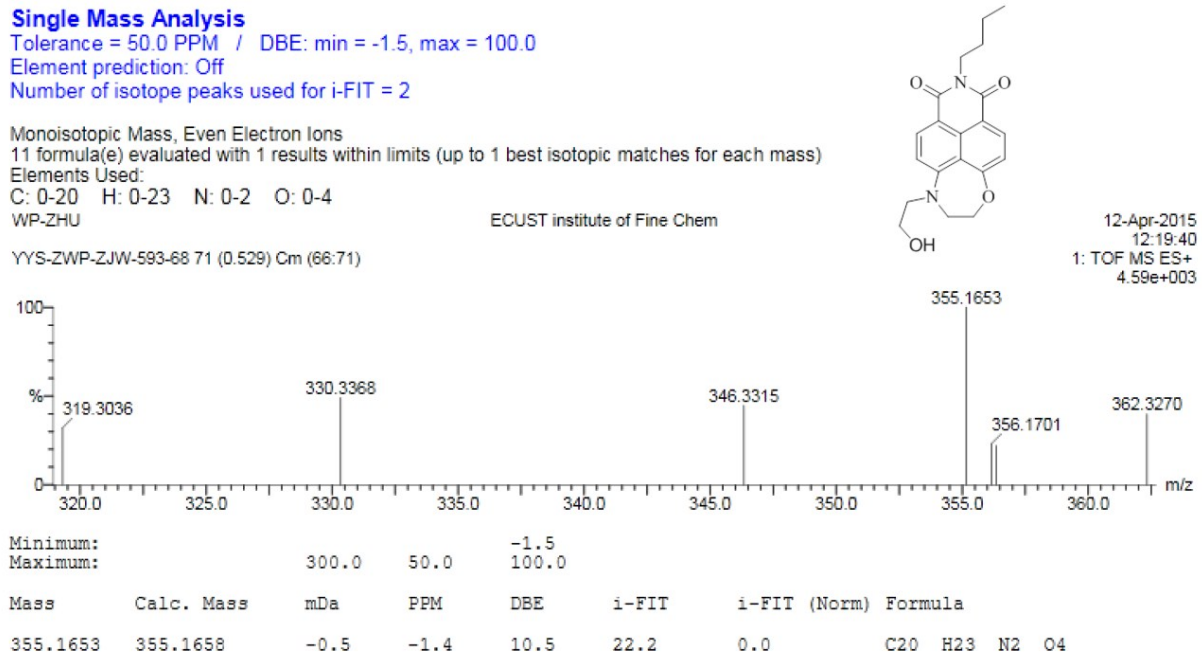
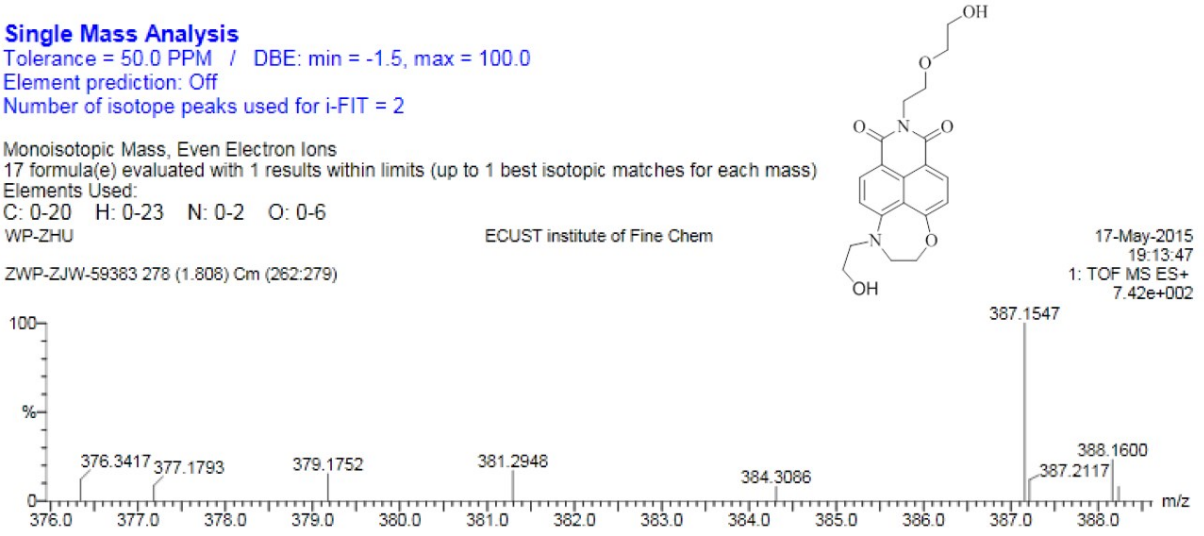


Fig. S16. ^{13}C NMR spectrum of **FM3** (DMSO-d_6, 100 MHz)



Minimum:		300.0	50.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
355.1653	355.1658	-0.5	-1.4	10.5	22.2	0.0	C20 H23 N2 O4

Fig. S17. ESI HRMS spectrum of **FM1**



Minimum:		300.0	50.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
387.1547	387.1556	-0.9	-2.3	10.5	24.2	0.0	C20 H23 N2 O6

Fig. S18. ESI HRMS spectrum of **FM2**

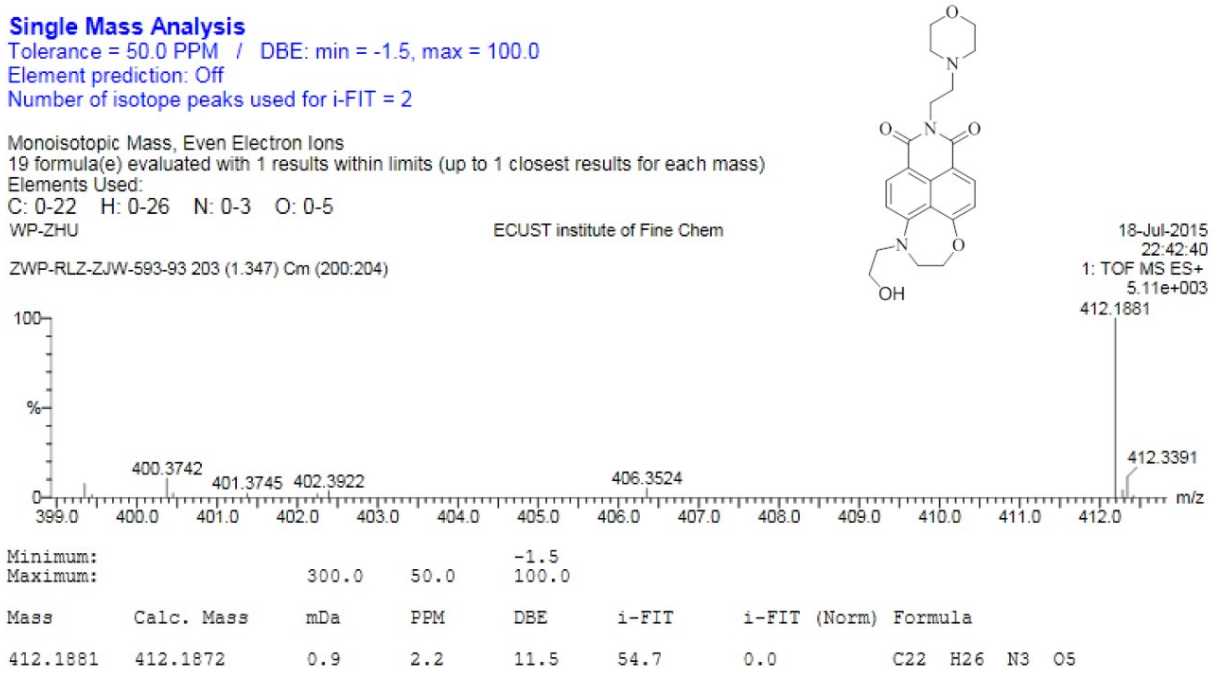


Fig. S19. ESI HRMS spectrum of **FM3**

7. References

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