Electronic Supporting Information

Novel cationic *meso*-CF_3 BODIPY-based AIE fluorescent rotors for imaging viscosity in mitochondria

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Experimental section

1. General

Nitrogen protection was used for all the reactions. The spectroscopic measurements were performed in deionized water. All the chromatographic purifications were through silica gel (Qingdao Haiyang, 300-400 mesh). Solvents and reagents with analytical or reagent grade were used with received for the measurements and reactions. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded with a Bruker AVANCE III 400 or 600 spectrometer (^{1}H, 400 or 600; ^{13}C, 126 MHz) in $CDCl_3$ or DMSO-d_6, which were referenced internally using the residual solvent (^{1}H: δ 7.26 for $CDCl_3$ and 2.50 for DMSO-d_6) or solvent (^{13}C: δ 77.16 for $CDCl_3$) resonances relative to $SiMe_4$. Electrospray ionization (ESI) mass data was recorded on an AB SCIEX TripleTOF 4600 mass spectrometer for all the new compounds. The electronic absorption and fluorescence spectra were performed on a UV-2550 spectrophotometer (Shimadzu, Japan) and a F-4500 spectrofluorometer (Hitachi, Japan), respectively. The corresponding fluorescence quantum yields (**Φ_F**) were calculated using the equation [S1]: $\Phi_{F(sample)} = (F_{sample}/F_{ref})$ x (A_{ref}/A_{sample}) x (n_{sample}^2/n_{ref}^2) x $\Phi_{F(ref)}$, where *F*, A and n stand for the integrated area, the absorbance at the excitation wavelength and the corresponding refractive index of solvents. The subscripts sample and ref stand for unknown and the standard samples, respectively. Zinc phthalocyanines (ZnPC) in N,N-dimethylformanide (DMF) was used as the reference [$\Phi_{F(ref)} = 0.28$] [S2]. Compound **6** was prepared according to the reported method with slight changes [S3].

2. Synthesis

2.1. Synthesis of monostyryl BODIPY **5**

A mixture of BODIPY **1** (50 mg, 158 μmol), benzaldehyde **4** (23.6 mg, 158 μmol), piperidine (0.20 mL) and acetic acid (0.20 mL) in toluene (20 mL) was heated under 87 °C for 1 h. After cooling, the obtained mixture was further added with petroleum ether and then directly purified by column chromatography using CH_2Cl_2/petroleum ether (from 1:3 to 2:3, v/v) as the eluent to give a dark blue solid (30 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53-7.48 (m, 3 H, ArH and C=CH), 7.34 (m, 1 H, J = 16.0 Hz, C=CH), 6.80 (s, 1 H, pyrrole-H), 6.67 (d, J = 8.8 Hz, 2 H, ArH), 6.10 (s, 1 H, pyrrole-H), 3.05 (s, 6 H, NCH_3), 2.55 (s, 3 H, CH_3), 2.34 (d, J = 3.2 Hz, 3 H, CH_3), 2.29 (d, J = 3.2 Hz, 3 H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ 158.0, 153.8, 151.8, 142.7, 141.6, 139.3, 134.8, 130.3, 124.2, 122.8, 121.7, 113.9, 112.1, 40.4, 16.2, 15.8, 15.0. HRMS (ESI): m/z calcd for $C_{23}H_{23}BF_5N_3$ $[M + H]^+$: 448.1978; found: 448.1982.

2.2. Synthesis of distyryl BODIPY **6**

Compound **6** was prepared according to the reported method with slight changes [S3]. A mixture of BODIPY **1** (50 mg, 158 μmol), benzaldehyde **4** (70.8 mg, 474 μmol), piperidine (0.20 mL) and acetic acid (0.20 mL) in toluene (20 mL) was heated under 80 °C for 1 h. After cooling, the obtained mixture was further added with petroleum ether and then directly purified by column chromatography using

CH_2Cl_2/petroleum ether (from 1:3 to 1:1, v/v) as the eluent to give a metallic green solid (50 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59-7.53 (m, 6 H, ArH and C=CH), 7.27 (m, 1 H, C=CH, overlapped with the residual of $CDCl_3$), 6.78 (s, 2 H, pyrrole-H), 6.70 (d, *J* = 8.8 Hz, 4 H, ArH), 3.05 (s, 12 H, NCH_3), 2.34 (s, 6 H, CH_3).

*2.3. Synthesis of cationic monostyryl BODIPY **2***

A mixture of **4** (30 mg, 64.9 μmol) and iodomethane (1 mL) in N, N-dimethylformamide (3 mL) was heated under 80 °C for 2 h. The reaction process was monitored by thin-layer chromatography. After cooling, the obtained mixture was crystalized with ethyl ether and the residual was further dissolved in ethanol with further addition of ethyl ether or petroleum ether for several times to give a dark blue solid (15.0 mg, 38%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.04 (d, *J* = 8.4 Hz, 2 H, ArH), 7.83 (d, *J* = 9.0 Hz, 2 H, ArH), 7.80 (d, *J* = 16.2 Hz, 1 H, C=CH), 7.56 (d, *J* = 16.2 Hz, 1 H, C=CH), 7.26 (s, 1 H, pyrrole-H), 6.55 (s, 1 H, pyrrole-H), 3.62 (s, 9 H, NCH_3), 2.53 (s, 3 H, CH_3), 2.34 (s, 3 H, CH_3), 2.30 (s, 3 H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ 160.4, 153.8, 147.6, 146.1, 144.9, 144.8, 143.2, 141.7, 137.4, 137.1, 128.7, 125.9, 121.9, 121.5, 120.9, 119.9, 56.5, 15.6, 15.1. HRMS (ESI): m/z calcd for $C_{24}H_{26}BF_5N_3$ $[M]^+$: 462.2134; found: 462.2137.

*2.4. Synthesis of cationic distyryl BODIPY **3***

A mixture of **6** (30 mg, 49.3 μmol) and iodomethane (2 mL) in N, N-dimethylformamide (3 mL) was heated under 80 °C for 2 h. The reaction process was monitored by thin-layer chromatography. After cooling, the obtained mixture was crystalized with ethyl ether and the residual was further dissolved in ethanol with further addition of ethyl ether or petroleum ether for several times to give a dark green solid (19.0 mg, 44%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.04 (d, *J* = 9.0 Hz, 4 H, ArH), 7.90-7.86 (m, 6 H, ArH), 7.61 (d, *J* = 16.8 Hz, 4 H, ArH), 7.34 (s, 2 H, pyrrole-H), 3.64 (s, 18 H, NCH_3), 2.37 (s, 6 H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ 154.8, 148.0, 142.3, 138.4, 137.8, 133.9, 131.4, 129.3, 128.4, 122.9, 121.9, 120.3, 112.8, 56.9, 16.0. HRMS (ESI): m/z calcd for $C_{34}H_{38}BF_5N_4$ $[M]^{2+}$: 304.1550; found: 304.1555.

3. Preparation of stock solutions for spectroscopic measurements

BODIPYs **1-3** (2 mM) in N, N-dimethylformamide were prepared as the stock solutions for all the spectroscopic experiments. All the interfering agents, including the amino acids, metal ions, anions and oxidizing species, were prepared with concentration of 2 mM in aqueous solutions. Solutions of probe (10 μM) in different solvents or with different concentration of interfering species (100 μM) in phosphate buffer saline (abbreviated as PBS, 0.1 M PBS, pH 7.4) were prepared for use and stored in refrigerator for one night. And then all the UV-Vis and fluorescence

spectroscopic measurements were performed after the samples were kept for 30 minutes in room temperature.

For preparing different fractions of glycerol and water mixtures (20 mL), we firstly calculated the corresponding weight of glycerol and water in each mixture, and then prepared them on a balance. Finally, the probes in stock solution were added to the each mixture (3 mL) and shook up the mixtures. At 20 °C, water has a viscosity (η) of 1.00 cP and glycerol has a viscosity of 950.17 cP. Viscosity for the mixtures was calculated using the following equation [S4]:

$$\ln(\eta_{mix}) = \Sigma\, w_i \times \ln(\eta_i)$$

where η_{mix} is the viscosity of the mixture, η_i is the viscosity of each component, and w_i is the weighting factor ($0<w<1$) of each component.

4. General procedures of cell culture and MTT assay

SH-SY5Y cells were used with Dulbecco's modified Eagle's medium (DMEM) with high glucose, as the culture medium under the atomosphere of 5% CO_2 at 37 °C for 48 h. To evaluate the cytotoxicity of these two probes, MTT assay were used. SH-SY5Y cells were seeded onto a 96-well cell-culture plate with 1.5×10^4 cells/well for 24 h. Then, various concentrations of probe (0, 3.125, 6.25, 12.5, 25, 50 and 100 μM) in DMEM were added to the wells. After another 48 h, MTT (10 μL, 5 mg/mL) was added to each well and incubated at 37 °C for another 4 h. The MTT solutions were removed and purple precipitates (formazan) observed in plates, which were further

dissolved in dimethyl sulfoxide (DMSO, 100 μL). Microplate reader was used to measure the absorbance at 570 nm for each well.

5. Cell imaging

The confocal imaging experiments were performed using a 20-mm confocal dish and SH-SY5Y cells (1.5×10^4 cells/well). The cells were firstly passed and adhered for two days. The cells were firstly cultured with lipopolysaccharide (LPS) (20 μM) for 40 min. And then PBS was used to wash the cells for three times. Then, the freshly prepared stock solutions of **2** or **3** (5 μM in DMEM) were added to the cells, which were pretreated with LPS or none-pretreated ones. The probes were further cultured for 30 min at 37 °C. A Leica TCS SP8 confocal microscope (Germany) was used to perform Confocal imaging with a 63 × oil-immersion objective lens. The samples were excited at 633 nm with an argon ion laser and emissions were collected at 650-750 nm.

For the subcellular cell imaging, SH-SY5Y cells were chosen and cultured for for 48 h. And then, probe (5 μM) with Mito-tracker Green or Lyso-tracker Green (1 μM) in DMEM were used to culture the cells for 30 min. After washing, the cells with PBS for three times, Confocal imaging was performed with excitation at 488 nm and emissions at the range of 500-600 nm for the Mito-tracker and Lyso-tracker. The two near-infrared fluorescent probes were excited at 633 nm with an argon ion laser and emissions were collected at 650-750 nm.

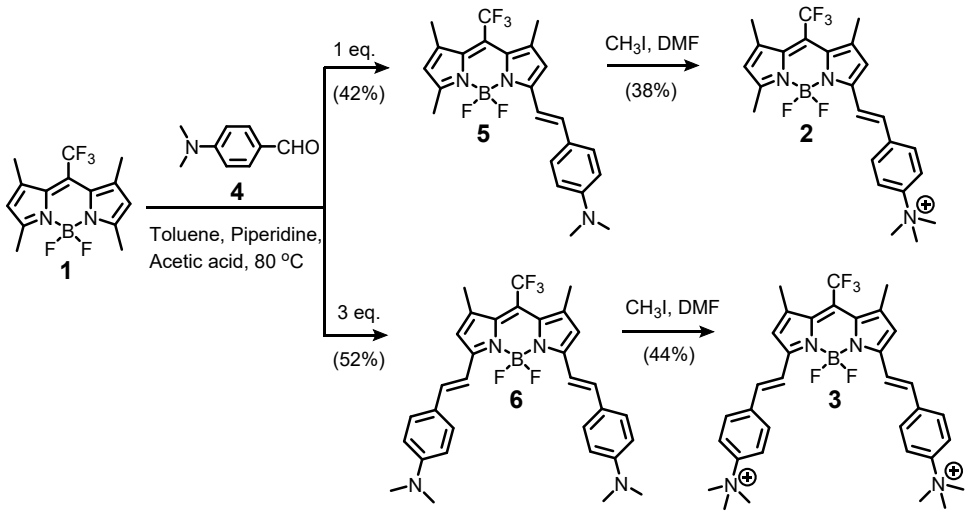
References

[S1] R. A. Velapoldi and H. H. Tønnesen, *J. Fluoresc*. 2004, **14**, 465.

[S2] I. Scalise and E. N. Durantini, *Bioorg. Med. Chem*. 2005, **13**, 3037.

[S3] X.-D. Jiang, T. Fang, X. Liu and D. Xi, *Eur. J. Org. Chem*. 2017, **34**, 5074.

[S4] A. Dragan, A. E. Graham and C. D. Geddes, *J. Fluoresc.* 2014, **24**, 397.



Scheme S1. Synthetic route of near-infrared fluorescent probes **2** and **3**.

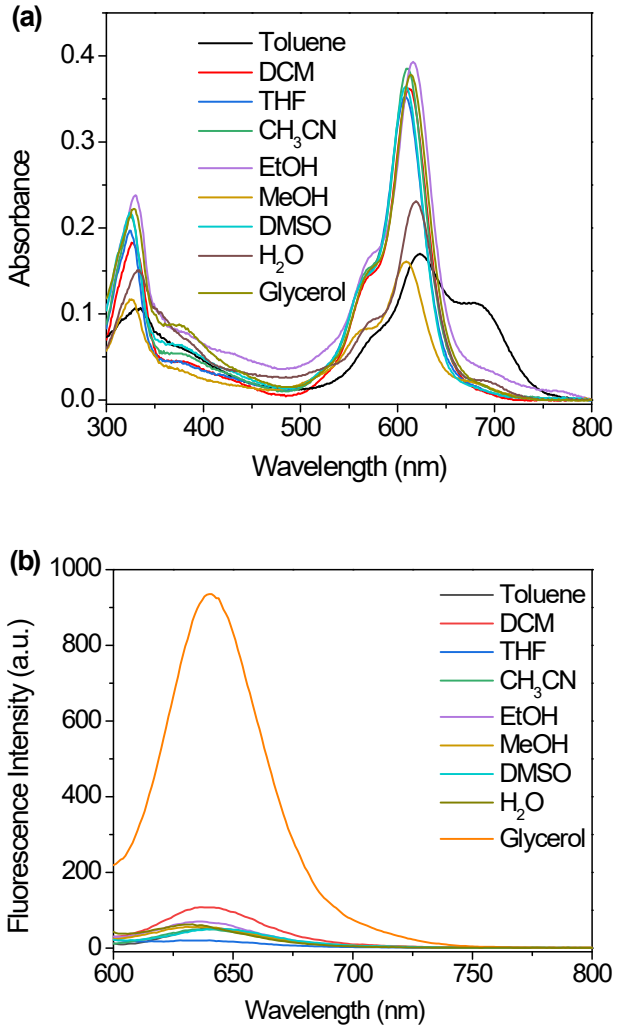


Fig. S1. (a) UV-Vis and (b) fluorescence spectra of **2** (10 μM) in different solvents. (λ_{ex} = 580 nm)

Table S1. The spectroscopic properties of **2** in different solvents.

Solvents	λ_{max} (nm) (log ε)	λ_{em} (nm)	Φ_F[a]
Toluene	335 (4.03), 623 (4.23), 684 (4.05)	643	0.03
DCM	325 (4.26), 611 (4.56)	640	0.03
THF	325 (4.29), 608 (4.55)	641	0.006
CH_3CN	325 (4.33), 611 (4.59)	638	0.009
EtOH	330 (4.38), 616 (4.59)	638	0.015
MeOH	326 (4.07), 609 (4.21)	638	0.025
DMSO	325 (4.33), 608 (4.56)	645	0.015
H_2O	332 (4.18), 618 (4.36), 700 (3.28)	625	0.033
Glycerol	329 (4.35), 615 (4.58)	636	0.26

[a] All was excited at 580 nm and using zinc phthalocyanine in DMF as the reference (Φ_F = 0.28).

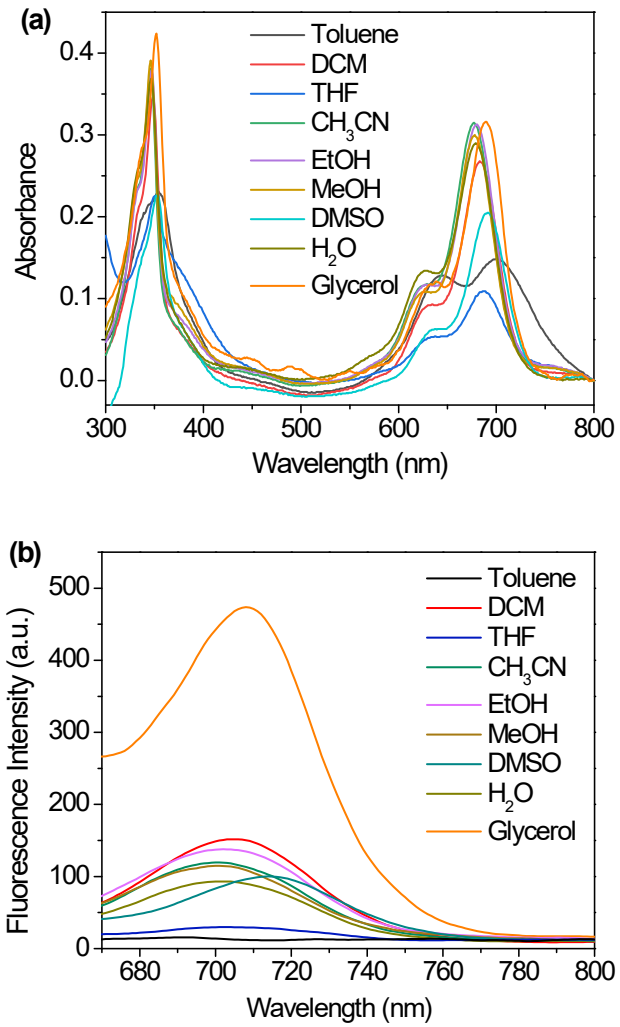


Fig. S2. (a) UV-Vis and (b) fluorescence spectra of **3** (10 μM) in different solvents.

(λ_{ex} = 630 nm)

Table S2. The spectroscopic properties of **3** in different solvents.

Solvents	λ_{max} (nm) (log ε)	λ_{em} (nm)	Φ_F[a]
Toluene	346 (4.58), 677 (4.50)	---	---
DCM	353 (4.35), 691 (4.31)	705	0.01
THF	346 (4.57), 679 (4.50)	705	0.002
CH_3CN	352 (4.62), 690 (4.50)	701	0.002
EtOH	347 (4.58), 680 (4.50)	703	0.007
MeOH	346 (4.59), 679 (4.47)	702	0.005
DMSO	353 (4.35), 691 (4.31)	715	0.006
H_2O	347 (4.57), 679 (4.46)	703	0.006
Glycerol	352 (4.63), 689 (4.50)	714	0.039

[a] All was excited at 630 nm and using zinc phthalocyanine in DMF as the reference (Φ_F = 0.28).

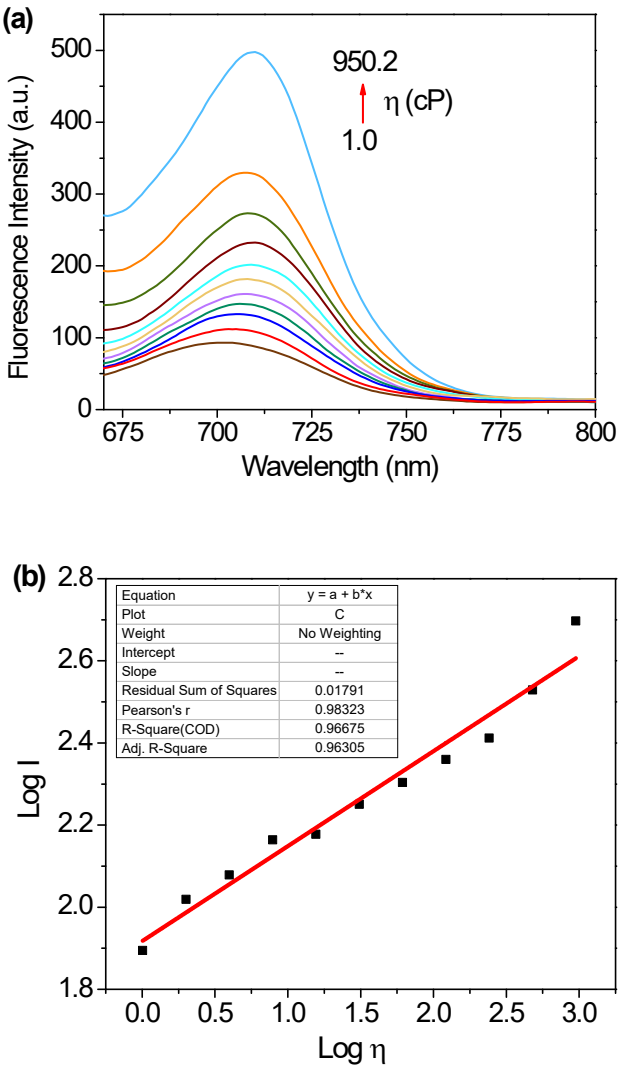


Fig. S3. (a) Fluorescence spectra of **3** (10 μM) in glycerol-H_2O mixtures with increasing viscosity from 1.0 to 950.2 cp. (b) The linear relationship of **3** (10 μM) between Log I (I: fluorescence intensity at 714 nm) and Log η (η: viscosity). (λ_{ex} = 630 nm)

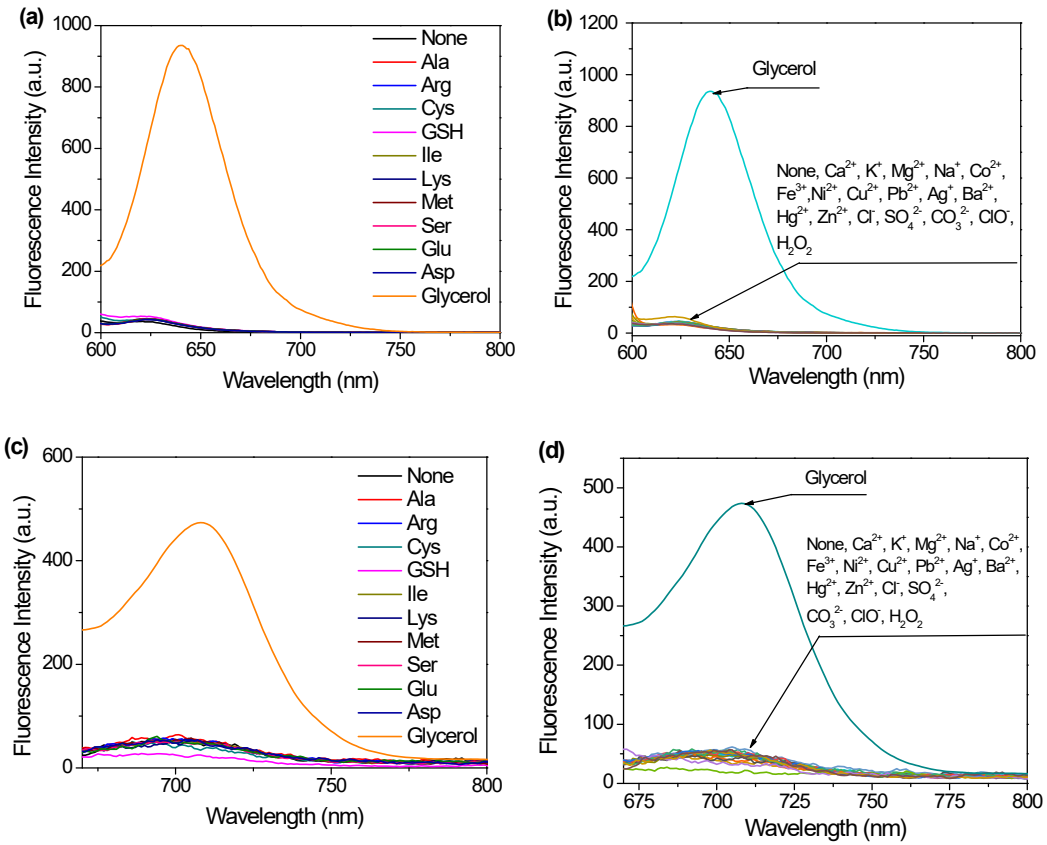


Fig. S4. Fluorescence spectra of (a and b) **2** and (c and d) **3** (10 μM) in PBS solutions (0.1 M PBS, pH 7.4) before and after the addition of (a and c) biologically relevant amino acid and (b and d) metal ions, anions and oxidizing reagents (100 μM). All the spectra were compared with that in glycerol. (λ_{ex} = 580 nm for **2** and 630 nm for **3**)

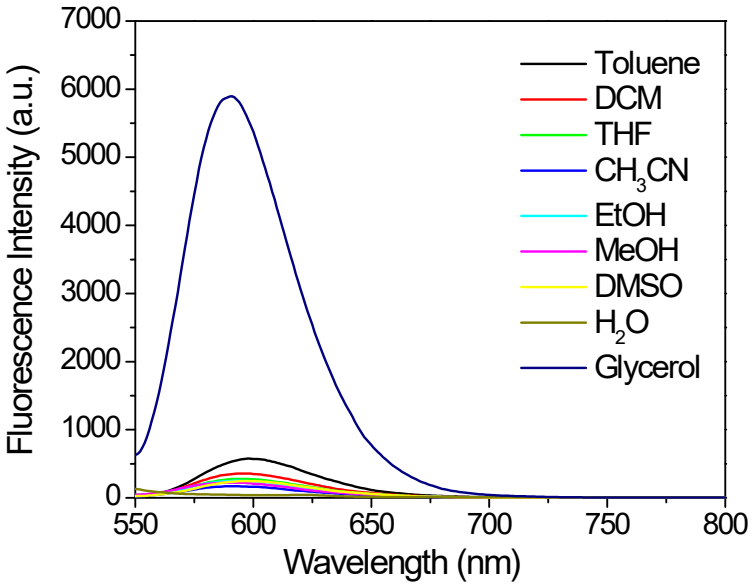


Fig. S5. Fluorescence spectra of **1** (10 μM) in different solvents. (λ_{ex} = 530 nm)

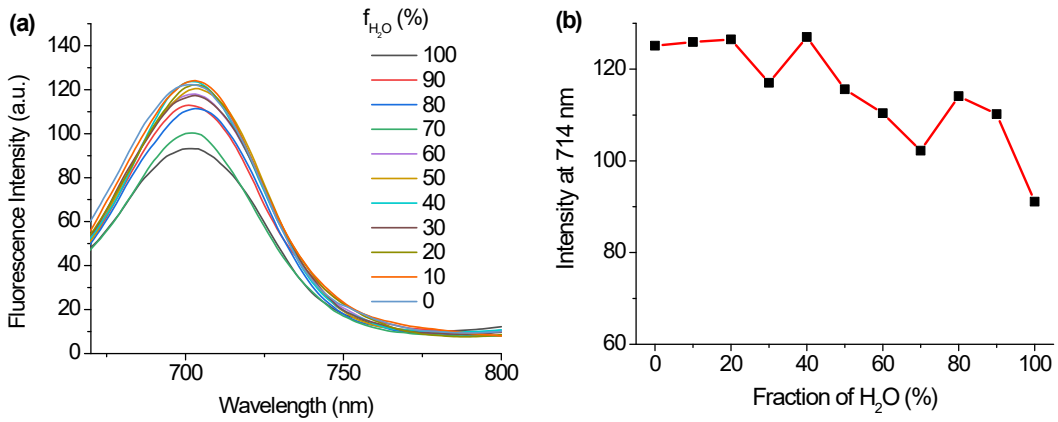


Fig. S6. (a) Fluorescence spectra of **3** (10 μM) in acetonitrile-H_2O mixtures with increasing fraction of H_2O. (b) The intensity changes at 714 nm for **3** versus the increasing fraction of H_2O. (λ_{ex} = 630 nm)

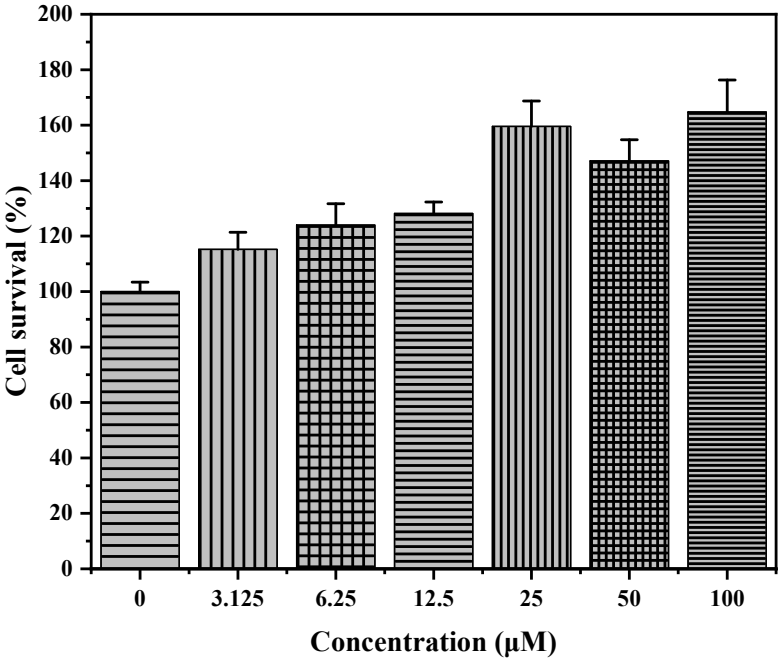


Fig. S7. MTT assay of SH-SY5Y cells with different concentration of **2** (0-100 μM) for 24 h.

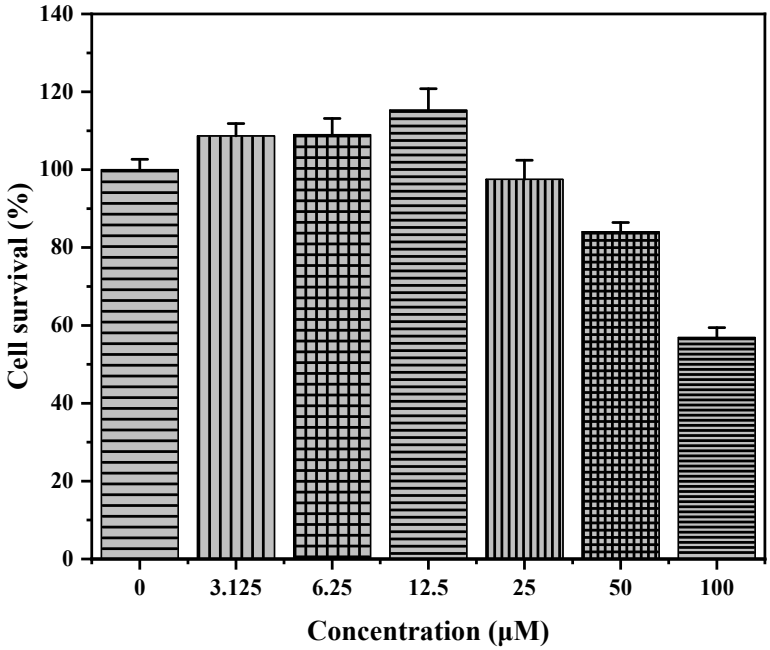


Fig. S8. MTT assay of SH-SY5Y cells with different concentration of **3** (0-100 μM) for 24 h.

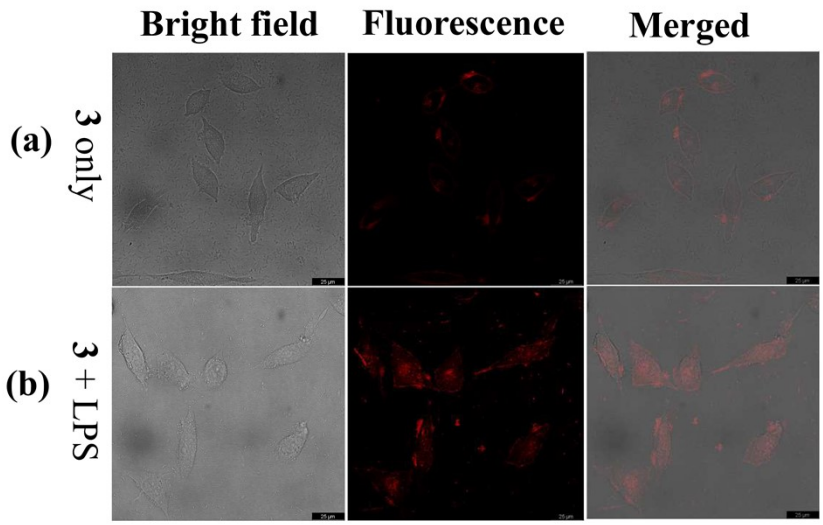


Fig. S9. Confocal fluorescence images of **3** in SH-SY5Y cells pretreated with (a) DMEM or (b) LPS (20 μM) for 40 min; and then further treated with **3** (5 μM) for another 30 min. (λ_{ex} = 633 nm, λ_{em} = 650-750 nm)

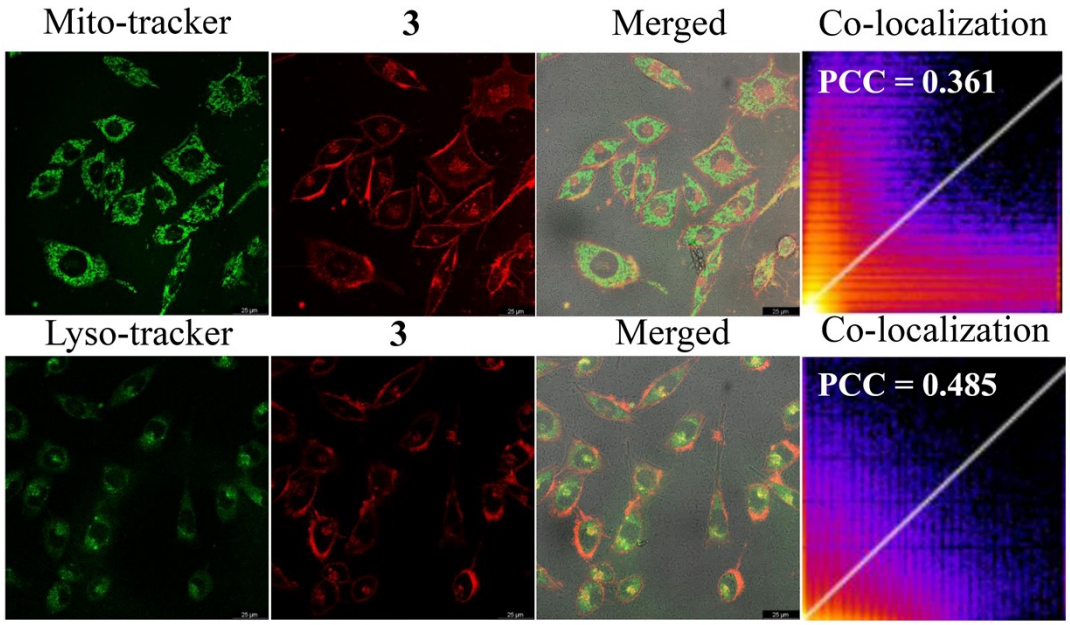


Fig. S10. Fluorescent images of SH-SY5Y cells co-incubated with **3** (5 μM) and Lyso- or Mito-tracker for 30 min at 37℃, respectively. For **3**, λ_{ex} = 633 nm, λ_{em} = 650-750 nm; for Mito-tracker or Lyso-tracker, λ_{ex} = 488 nm, λ_{em} = 500-600 nm.

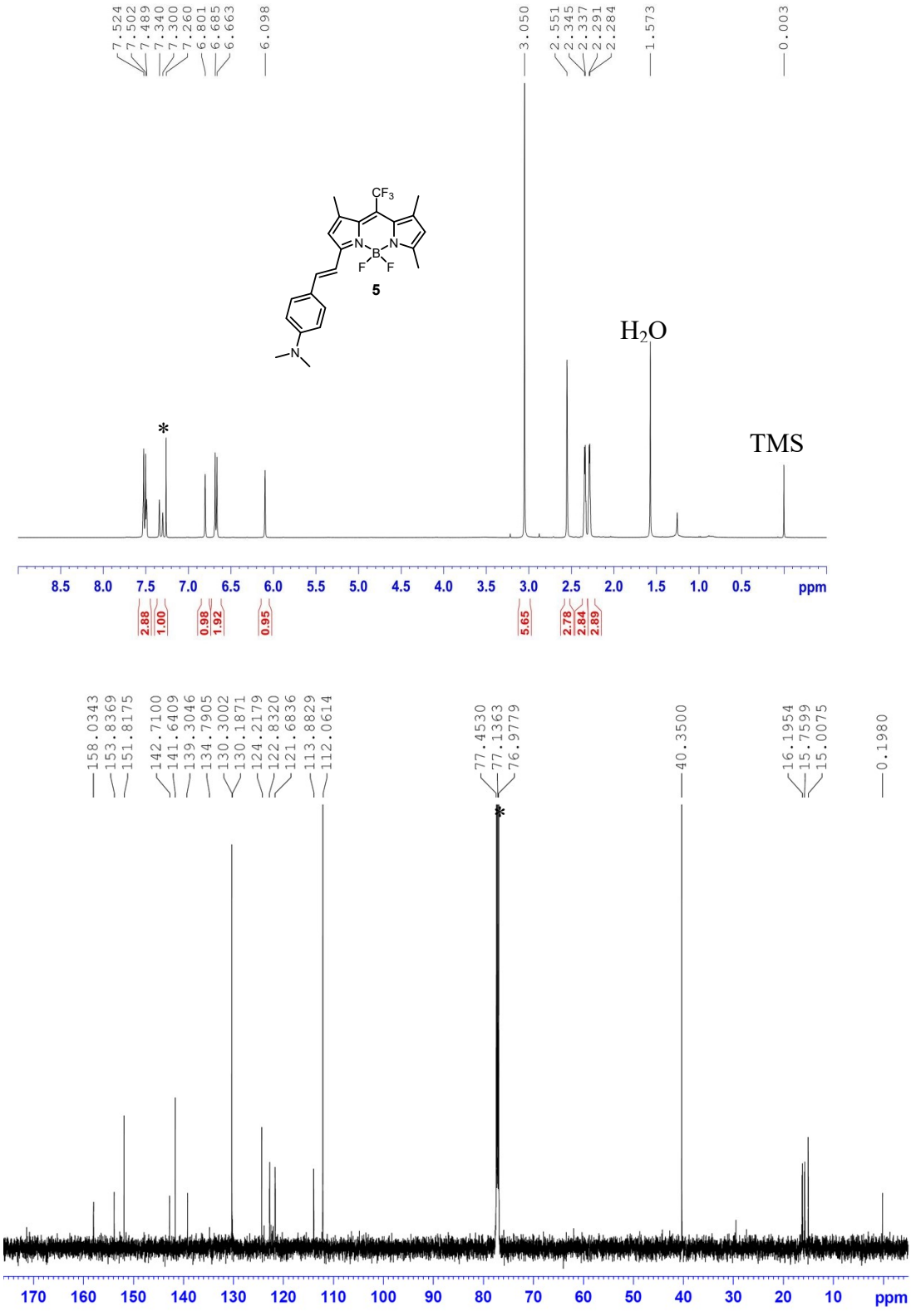


Fig. S11. ^{1}H and ^{13}C NMR spectrum of **5** in $CDCl_3$.

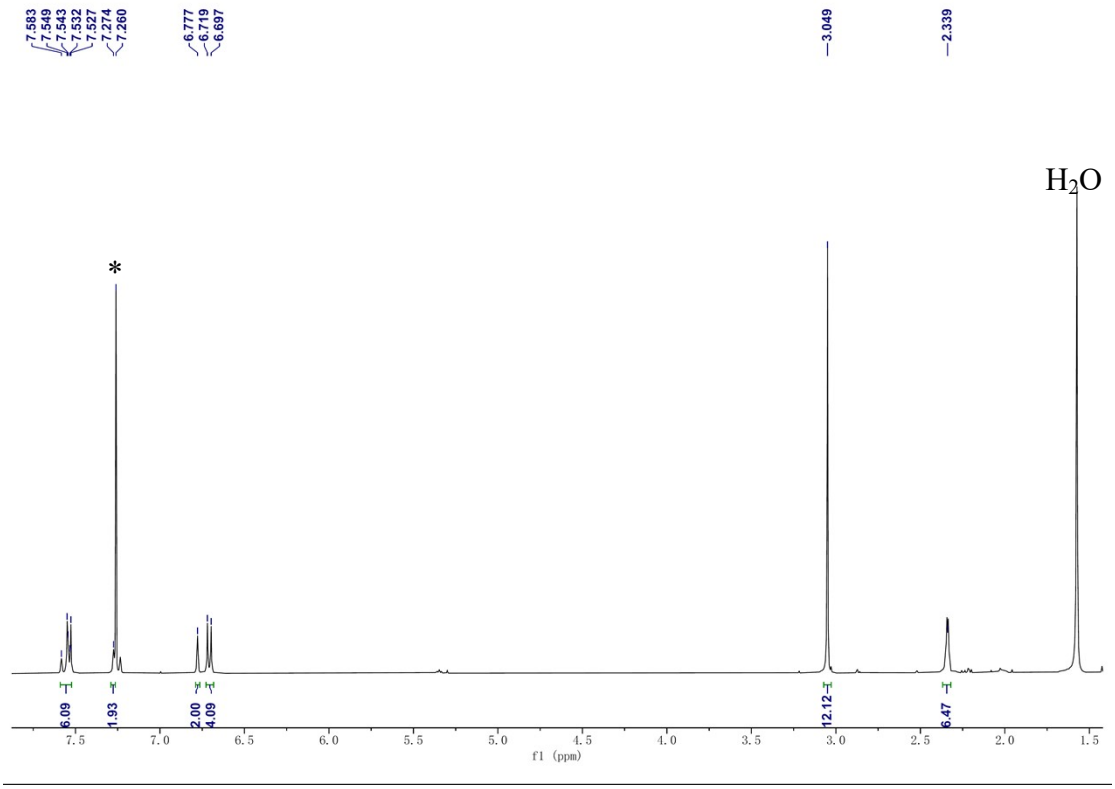


Fig. S12. ^{1}H NMR spectrum of **6** in $CDCl_3$.

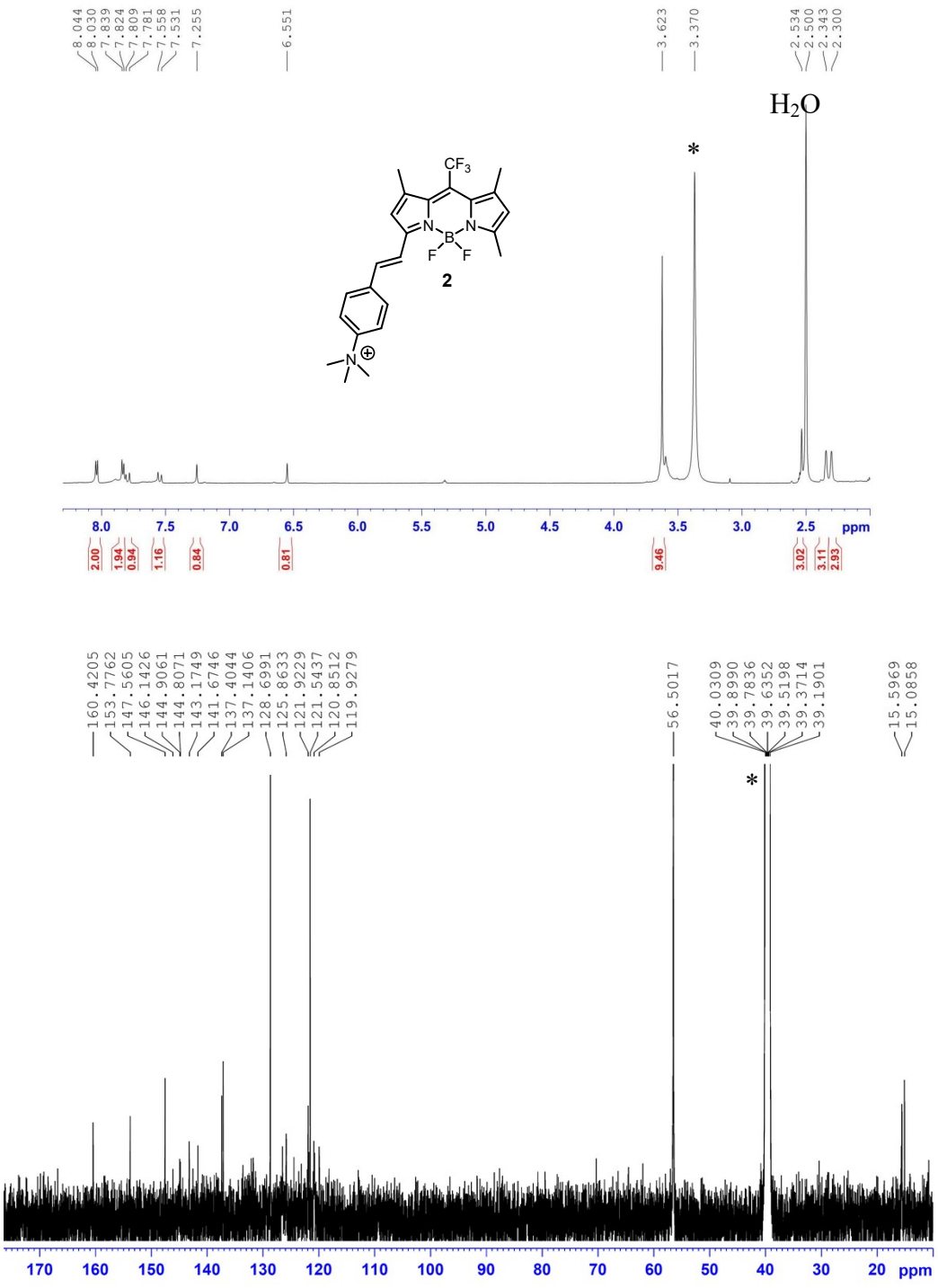


Fig. S13. ^{1}H and ^{13}C NMR spectrum of **2** in DMSO-d_6.

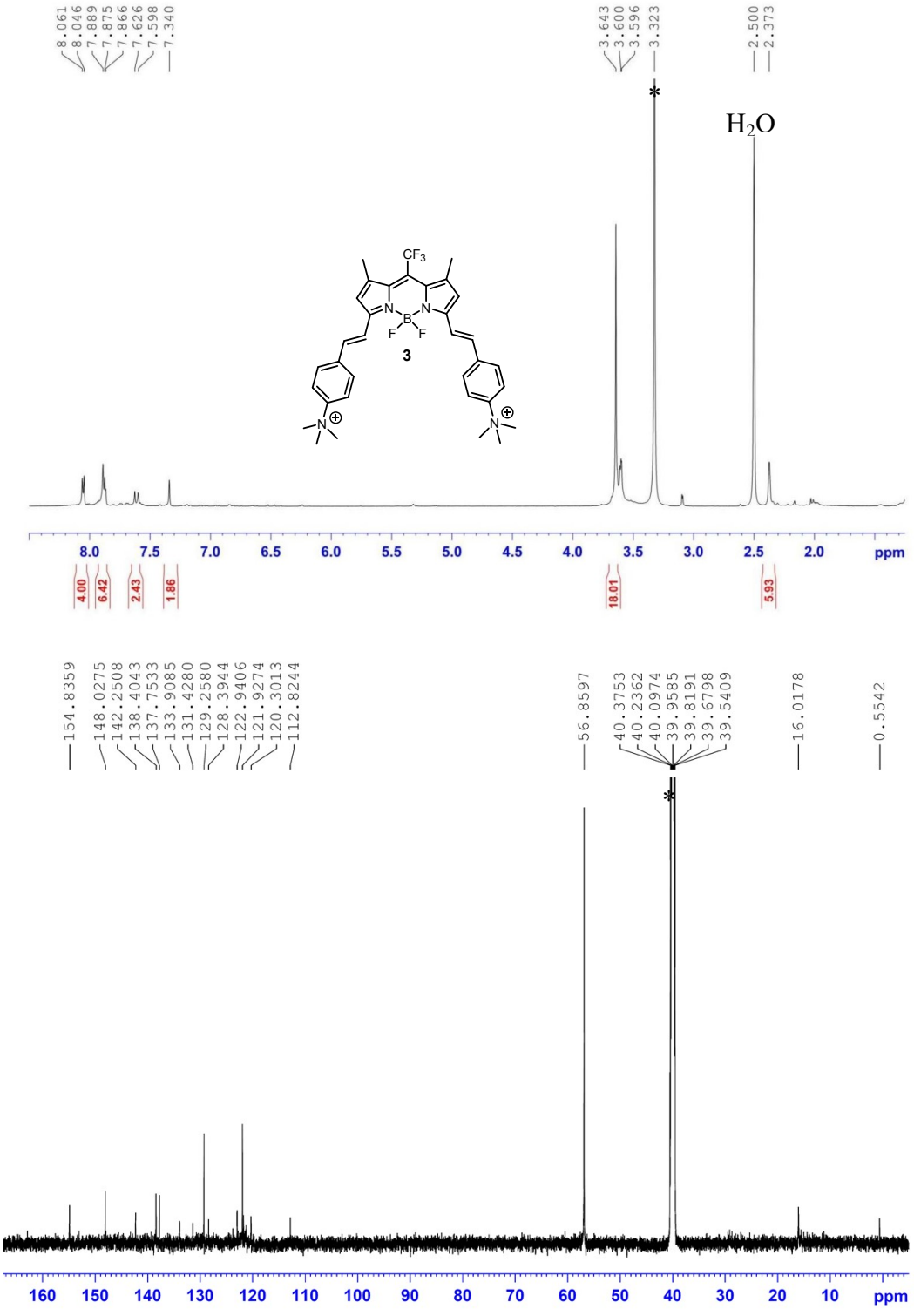


Fig. S14. ^{1}H and ^{13}C NMR spectrum of **3** in DMSO-d_6.