Electronic Supporting information

A deep-red fluorescent molecular rotor based on donor-two-acceptor modular system for imaging mitochondrial viscosity

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1. Materials and instruments

Chemical reagents used in the experiment were purchased from TCI or Sigma-Aldrich companies without secondary treatment. The bio-reagents were purchased from Beyotime Institute of Biotechnology. The deionized water for analysis was obtained from a Milli-Q ultrapure system. NMR spectra were recorded on a Bruker spectrometer at 400 (^{1}H NMR) MHz and 100 (^{13}C NMR) MHz. High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument. All UV absorption spectra in this paper were done by a Shimadzu UV-2550 UV/Vis spectrophotometer and all fluorescence emission spectra were done by a Hitachi F-4600 fluorophotometer. Fluorescence lifetime test were measured by Edinburgh FLS1000 steady state transient fluorescence spectrometer. Cells were imaged on an Olympus FV100-IX81confocal microscope. All cell images were analyzed with Olympus FV1000-ASW.

2. Viscosity spectroscopy measurement

The stock solution of **DpCy7** was 5×10^{-3} M in DMSO. Solvents with different viscosities are obtained by mixing deionized water and glycerol in different ratios. A working solution (e. g. 5.0 μM) of **DpCy7** of different viscosity was prepared by adding 3 μL of stock solution to 3 mL of the required solvent mixture. The prepared solutions were mixed uniformly by using an oscillator for 1 min and standing for 5 min before UV-vis and fluorescence spectral analysis. Fluorescence spectra of solution were excited at 570 nm with excitation slit width and emission slit width set at 5 nm and 2.5 nm, respectively.

3. Measurement of fluorescence quantum yield

Fluorescence quantum yield (Φ_F) was measured by using rhodamine B in ethanol (Φ_F = 0.73, in ethanol) as the reference. The quantum yield was calculated by the following equation. [1]

$$\emptyset_{F(x)} = \emptyset_{F(x)}\left(A_s F_x / A_x F_s\right)\left(\eta_x/\eta_s\right)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the certain excitation wavelength, F is the area under the corrected emission curve, and η is the refractive index of the solvent. Subscripts X and S refer to the unknown and to the standard, respectively.

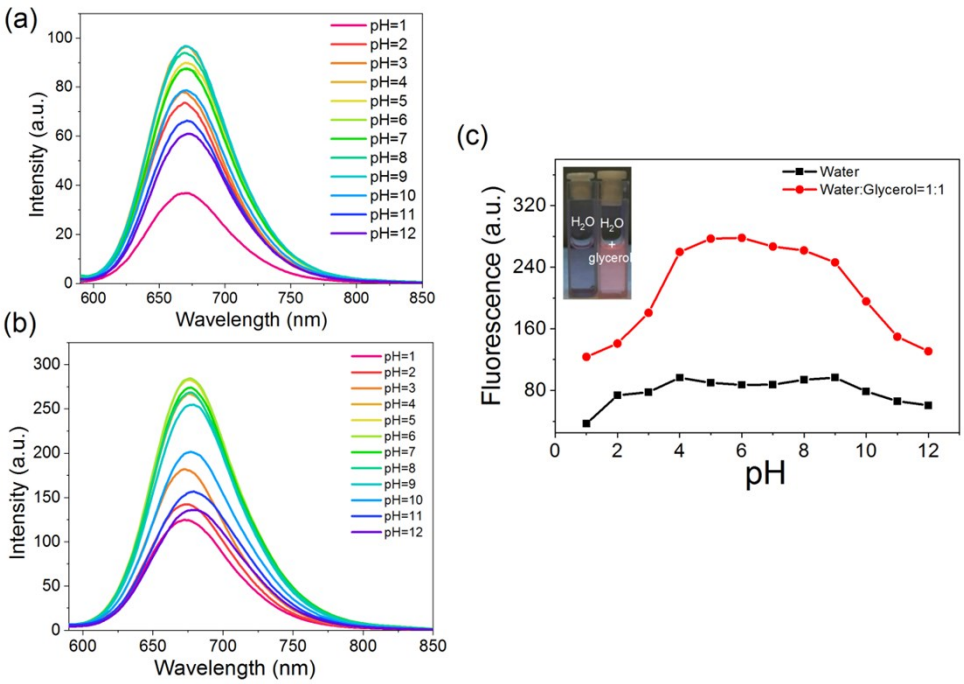

Fig. S1 (a) Fluorescence spectra of **DpCy7** (5 μM) in pure water at different pH. (b) Fluorescence spectra of **DpCy7** (5 μM) in 50% glycerol aqueous solution at different pH. (c) Fluorescence dot plots spectra of **DpCy7** (5 μM) at 670 nm among different pH in water (black) and 50% glycerol aqueous solution (red). Inset: Color changes of **DpCy7** in the water and glycerol under UV irradiation.

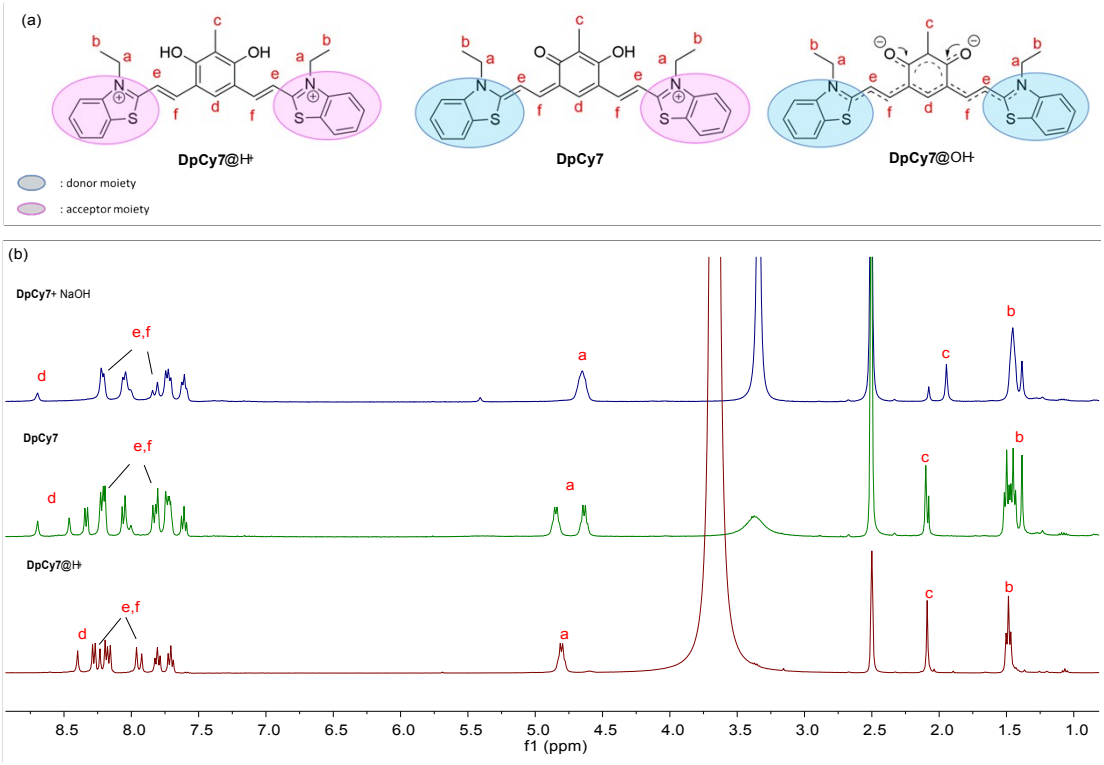

Fig S2 (a) Three ionization states of the probe **DpCy7** under different pH conditions. (b) ^{1}H NMR titration of **DpCy7** with NaOH in DMSO-d_6.

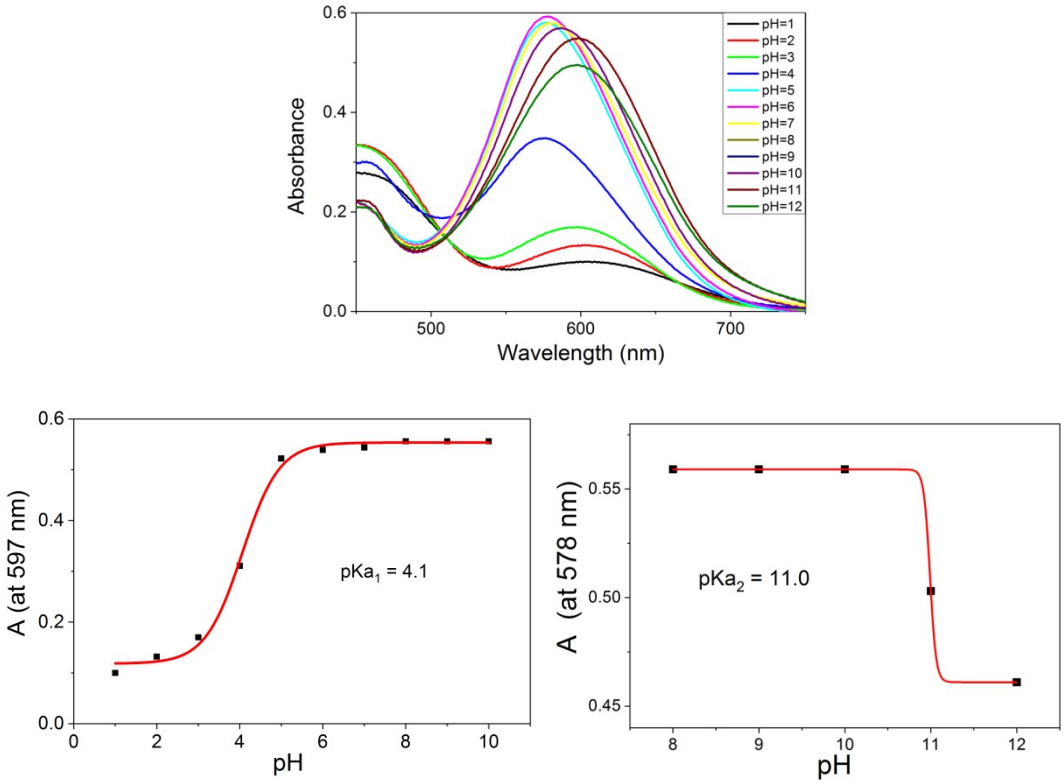

Fig.S3 a) UV-vis spectra of probe **DpCy7** (10 μM) in different pH aqueous solution; b) and c) pH-absorbance fit sigmoidal of **DpCy7**

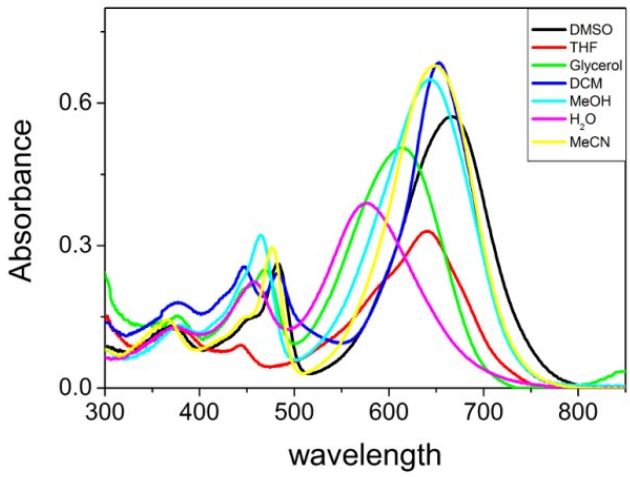

Fig. S4 UV-vis responses of **DpCy7** (10 μM) upon the different solvents: DMSO, tetrahydrofuran, dichloromethane, methanol, H_2O, acetonitrile and glycerol.

Table S1 Spectral properties of **DpCy7** in different solvents

Solvents	λ_{abs}(nm)	λ_{em}(nm)	Stokes shift (nm)	Φ
DMSO	667	700	33	0.08
THF	642	693	51	0.12
DCM	657	694	37	0.09
MeOH	643	683	40	0.13
H_2O	577	670	93	0.05
MeCN	648	689	41	0.10
Glycerol	615	688	73	0.39

Table S2 HOMO and LUMO energy of **DpCy7**@H^+ and **DpCy7** calculated at B3LYP/6-31G (d) level.

	LUMO (eV)	HOMO (eV)	ΔE (eV)
DpCy7@H^+	-7.91	-10.92	3.01
DpCy7	-5.20	-7.47	2.27

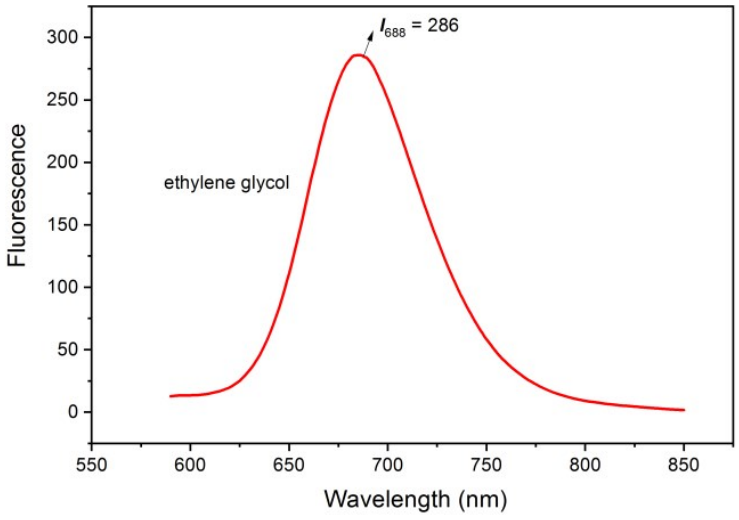

Fig. S5 Fluorescence spectrum of **DpCy7** (5 μM) in ethylene glycol.

Table S3 The comparison of the experimental and predicated viscosity for ethylene glycol

	I_{688}	Exp. log η[#]	Calc. log η[2]	Error%
Ethylene glycol	286	1.169	1.244	6.0%

[#]: calculated by the Förster-Hoffmann equation of log I_{688} = 1.79 + 0.57 log η

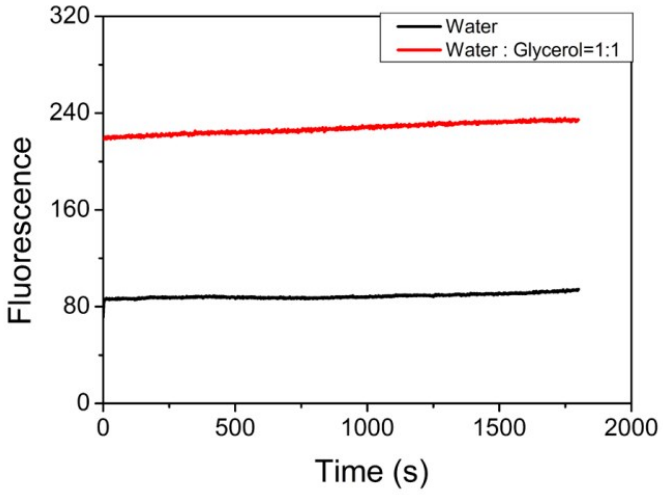

Fig. S6 Time-dependent fluorescence spectrum of probe **DpCy7** (5μM) in water and 50% water-glycerol.

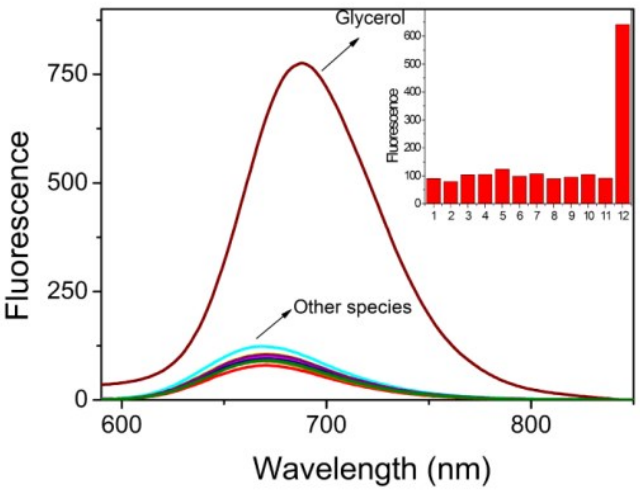

Fig. S7 Fluorecence spectra of **DpCy7** (5 μM) upon the addition of various anion (50 μM). (1) Cl^-, (2) ClO^-, (3) Cys, (4) GSH, (5) H_2O_2, (6) Hcy, (7) HSO_3^-, (8) HSO_4^-, (9) SO_3^{2-}, (10) SO_4^{2-}, (11) blank, (12) glycerol.

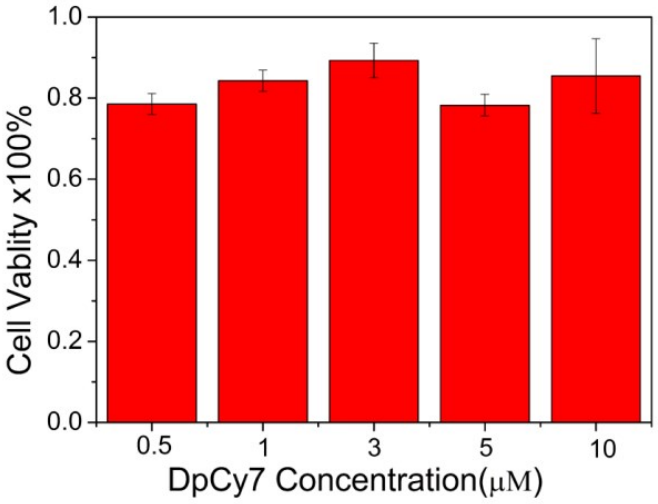

Fig. S8 The MTT assay of **DpCy7**.

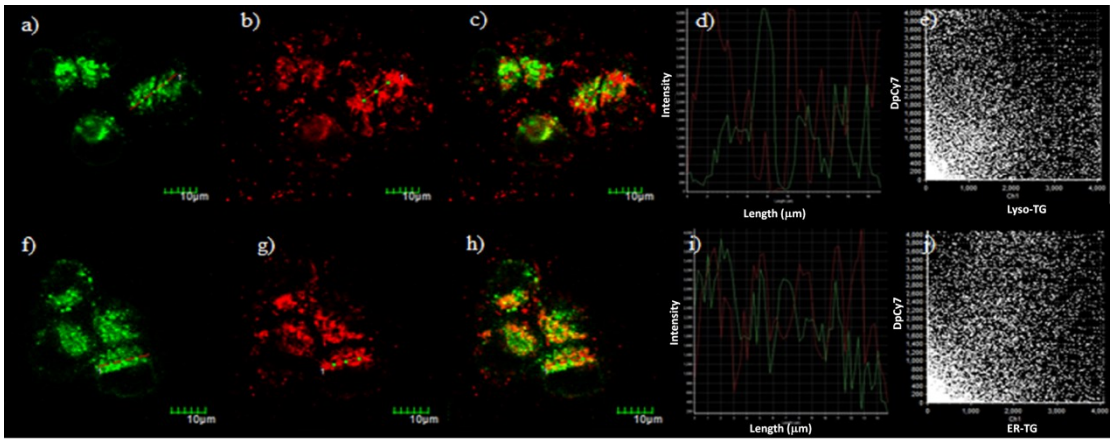

Fig. S9 Co-localization experiments involving with probe **DpCy7** (0.1 μM) and Lyso Tracker Green (200 nM, a-e) and ER Tracker Green (200 nM). f-j) in HeLa cells incubated with 1 μM monensin at 37°C for 30 min. a) and f): Confocal image with co-localization agents on green channel; b) and g): Confocal image from 0.1 μM probe **DpCy7** on red channel; c) and h): Overlay of green and red channels; d) and i): The intensity profile of ROI lines; e) and j): Fluorescence intensity plot of co-localization agent channel and **DpCy7** channel. Scale bar = 10 μm

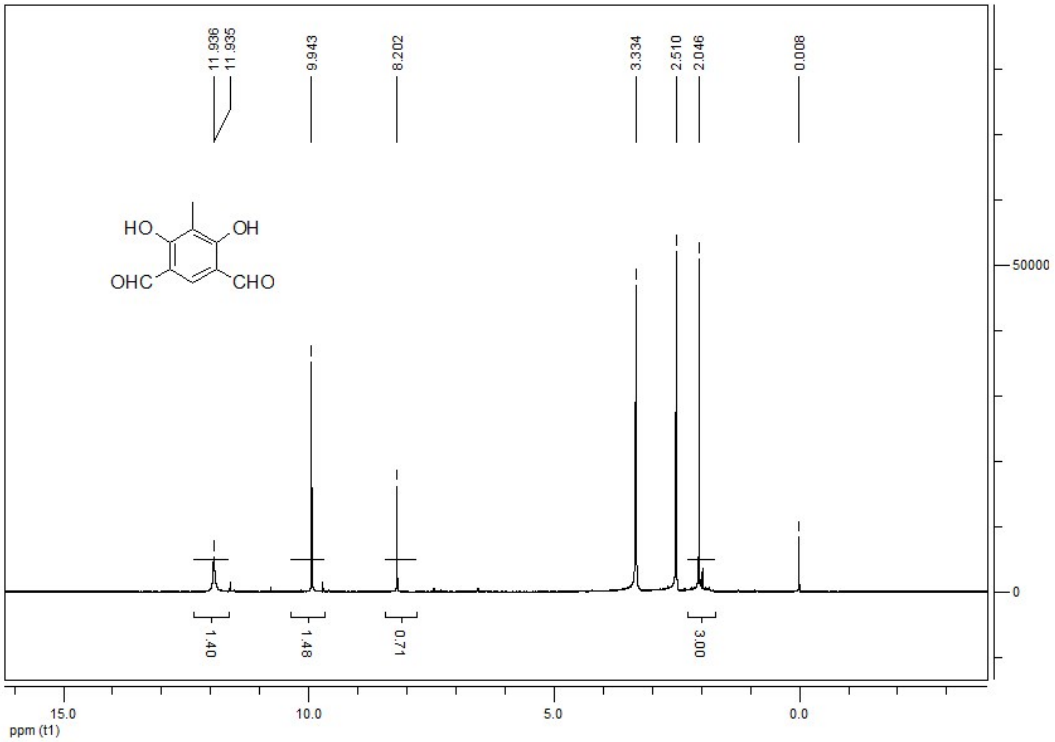

Fig. S10 ^{1}H NMR of 2, 4-Dihydroxy-3-methylisophtalaldehyde (**1**) (400 MHz, DMSO-d_6).

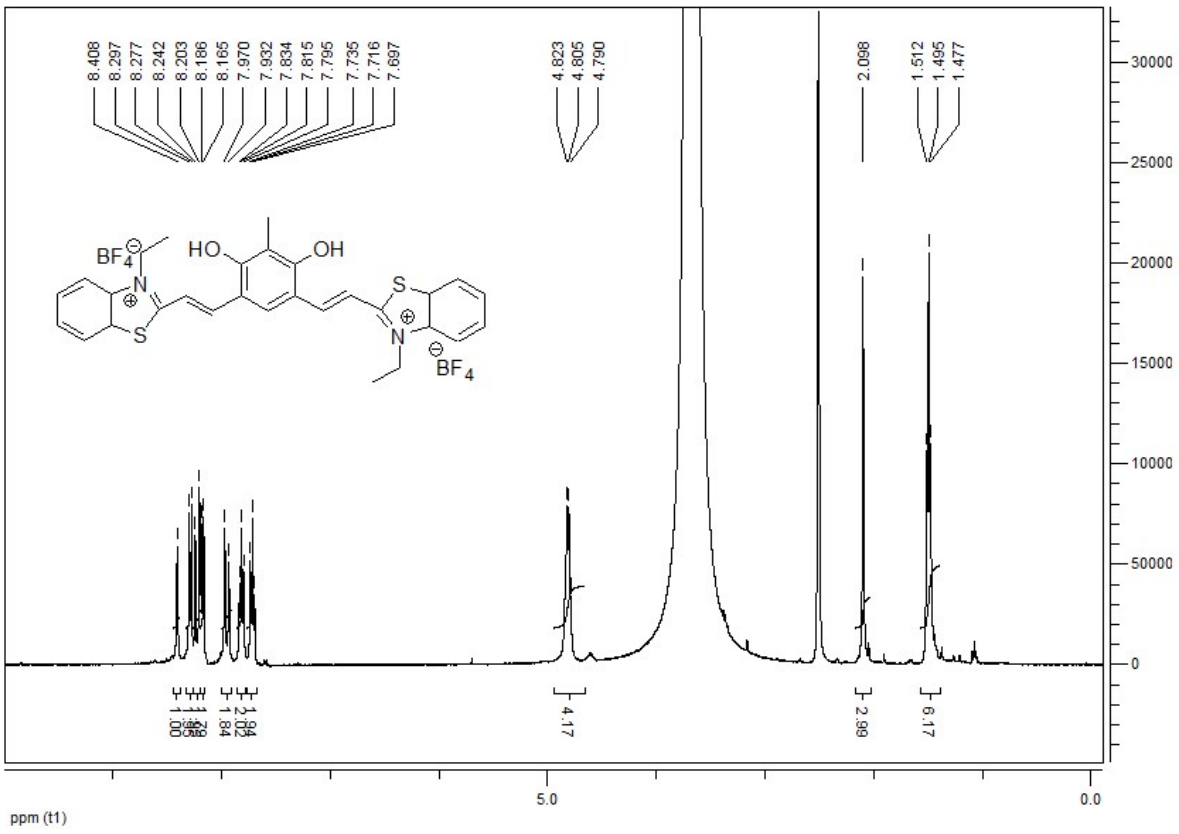

Fig. S11 ^{1}H NMR of the protonated **DpCy7** (400 MHz, DMSO-d_6).

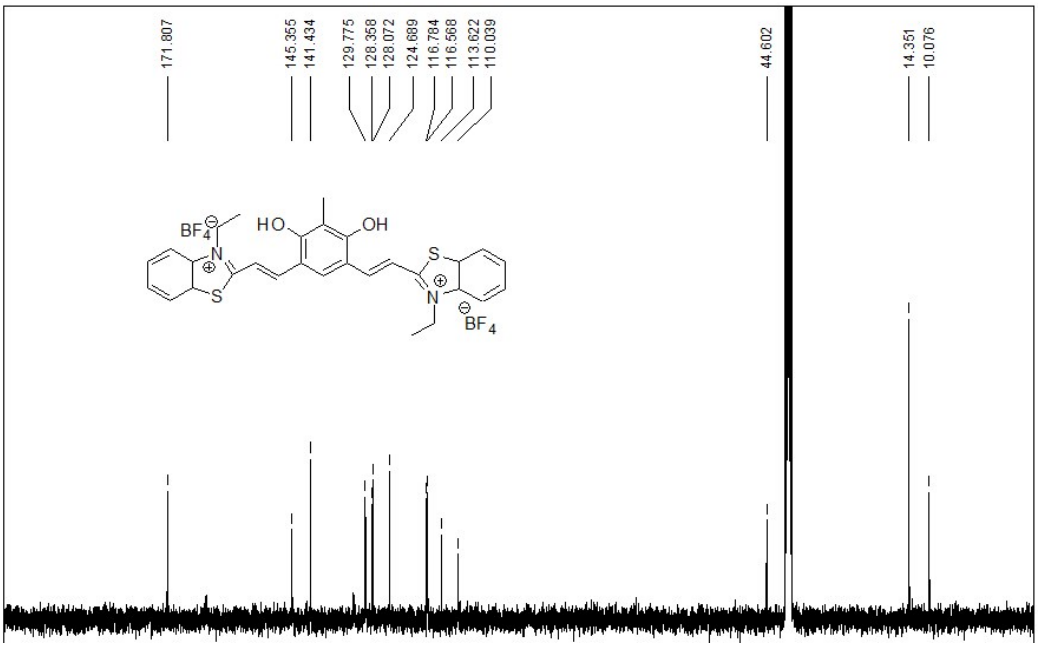

Fig. S12 ^{13}C NMR of the protonated **DpCy7** (100 MHz, DMSO-d_6).

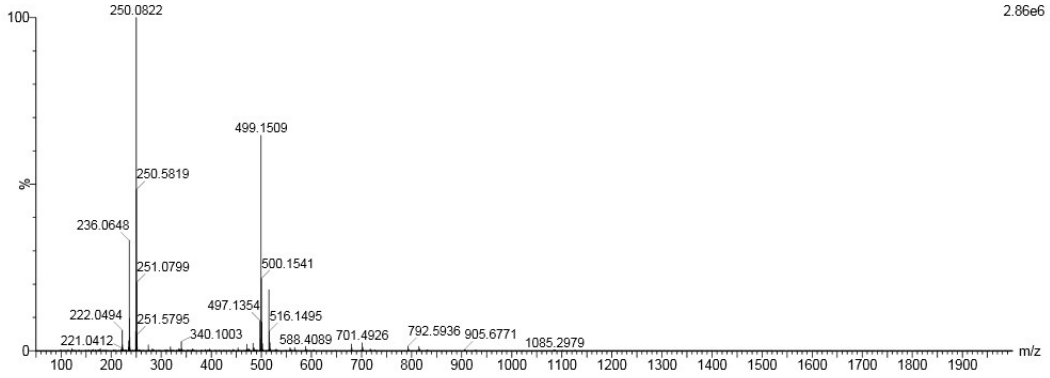

Fig. S13 HRMS (LC/MS) spectra of the protonated **DpCy7**. The peak at m/z = 250.0822 was assigned to the mass of [**DpCy7**-2BF_4^-] $^{2+}$.

References

[1]. D. Oushiki , H. Kojima , T. Terai, M. Arita, K. Hanaoka, Y. Urano and T Nagano, *J. Am. Chem. Soc*., 2010,**132**,2795.

[2]. A. R. Katritzky, K. Chen, Y. Wang, M. Karelson, B. Lucic, N. Trinajstic, T. Suzuki, G. Schüürmann *J. Phys. Org. Chem.* 2000, **13**, 80.