Supporting Information for

A Novel Fluorescence Probe for Simultaneous Detection of Mitochondrial Viscosity in Hepatic Ischemia Reperfusion Injury Models

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Experimental section

Materials and apparatus

All chemicals were commercially available and used without further purification. ^{1}HNMR and ^{13}C NMR spectra were recorded using Brucker Avance 500 MHz spectrometers. The spectra were reported in ppm (d) and referenced to a tetramethylsilane (TMS) standard in $CDCl_3$, DMSO-d_6. Thin layer chromatography (TLC) for reaction monitoring was performed on pre-coated silica gel plates (Merck 60 F254 nm) with a UV254 fluorescent indicator and column chromatography was conducted over silica gel (mesh 300–400). The fluorescence and UV-vis spectra were acquired on a SpectraMax M5 (Molecular Devices).

Measurement of Viscosity

PN solutions (5 μM) of different viscosity were obtained by water-glycerol mixture in different volume ratios. The solution was continuously shaken for 1 hour and then allowed to stand for 30 minutes to eliminate bubbles. The mixed solution was measured fluorescence spectra with $\lambda_{ex}/_{em}$ = 490/615 nm and both excitation and emission slit widths of 8 nm.

Quantum yield calculation

The fluorescence quantum yield Φ_s was estimated from the absorption and fluorescence spectra of probe according to equation, where the subscript s and r stand for the sample and reference (fluorescein as standard $\Phi_F = 0.85$), respectively. Φ is the quantum yields, a represents the absorbance at the excitation wavelength, S refers to the integrated emission band areas and n_D is the solvent refractive index. The fluorescence quantum yields (Φ_F) were estimated with equation as follows:

$$\varphi_s = \varphi_r \frac{S_s\, A_r\, n_{DS}^2}{S_R\, A_S\, n_{Dr}^2}$$

Cytotoxicity of probe PN

The cytotoxicity of probe **PN** was tested by MTT method. Miha cells were seeded at a density at 1×10^4 cells per well into 96-well plate, and incubated in 37 °C cell incubator

(containing 5% CO_2) for 12 hours. The culture medium was high glucose DMEM with fetal bovine serum and appropriate antibodies (penicillin and streptomycin). Then the probe **PN** (0, 1, 2, 5, 10, 20 μM) was added and incubated for 24 h. 50 μL MTT was added to each pore and the cells were incubated at 37 °C and 5% CO_2 for 4 h. Then, removed the medium and replaced it with DMSO (150μL), and detected the absorption values at 490 nm.

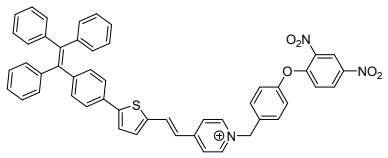
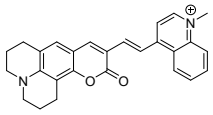
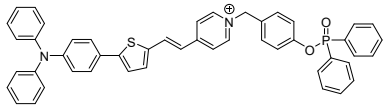
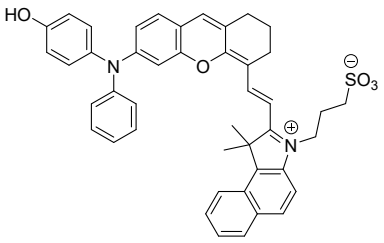
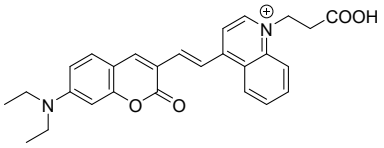
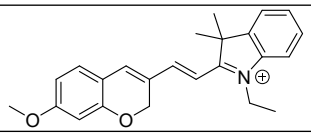
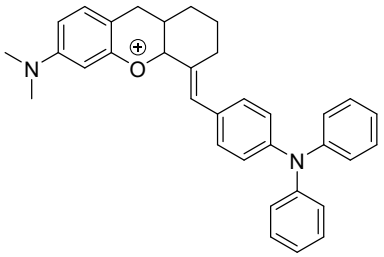
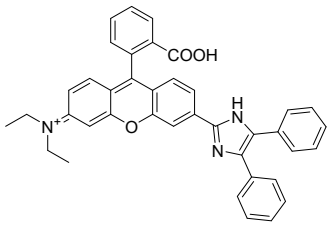
Cell culture and fluorescence imaging

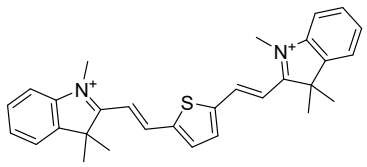
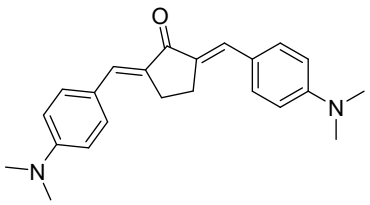
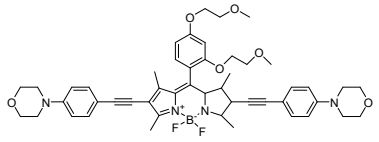
DMEM containing 10% fetal bovine serum and 1% penicillin was used for HepG2 cell culture in an incubator supplemented with 95% air and 5% CO_2 at 37 °C. Then cells were seeded into cell culture dishes at a density of 2.0×10^4 in growth medium. For stimuli experiments, the cells were pretreated with nystatin for 30 min and then treated with 10 μM **PN** for 15 min. Then, nutrient solution was removed and cells were washed three times with PBS buffer (pH=7.0, 10 mM) before imaging. FL imaging was recorded by confocal luminescence microscope.

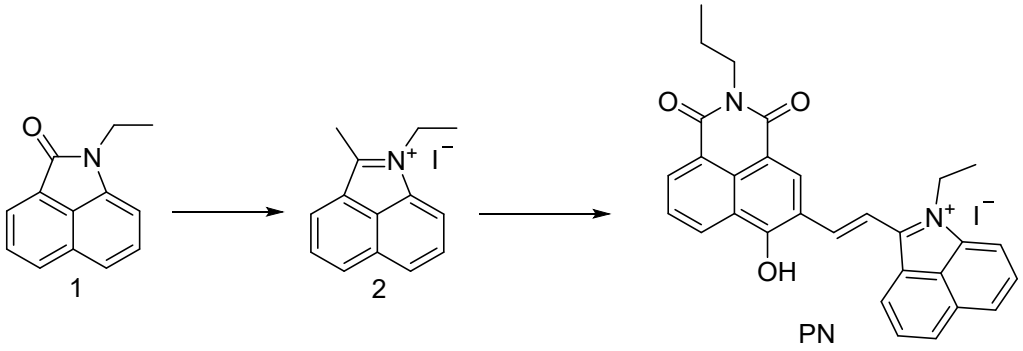
Viscosity monitoring in HIRI mouse model

Before initiating experiments, mice underwent a 7-day acclimation period upon their arrival. Each study group comprised n = 3 mice. To construct HIRI mice model, an already established methodology was employed. All mice were intravenously injected with probe **PN** (100 μL, 0.1 mM in ultrapure water, with 2% DMSO, v/v), and then were fully anesthetized. After laparotomy, vascular clamps were used to block the blood flow to the left and median hepatic lobes, resulting in ~70% of the liver ischemia. After 90 min of ischemia, we removed the clamps, the blood supply to the mice's liver was restored for 1 hours. For normal group, the liver was exposed as a control. The abdominal cavity was then opened and the liver was removed for fluorescence imaging.

Table S1 previous viscosity probes and this work

Probes	λ_{ex}/nm	λ_{em}/nm	Stokes shift/nm	sensitivity	
	440	620	180	31 times	[1]
	600	710	110	437 times	[2]
	488	671	183	Not mention	[3]
	734	911	177	10 times	[4]
	550	675	125	125 times	[5]
	500	628	128	20 times	[6]
	640	725	85	140 times	[7]
	570	655	85	48.5-fold	[8]

	525	595	70	Not mention	[9]
	450	508	58	less than 10-fold	[10]
	550	586	36	Nearly 20-fold	[11]
This work	600	790	190	32 times	



Scheme S1. Synthesis route of **PN**.

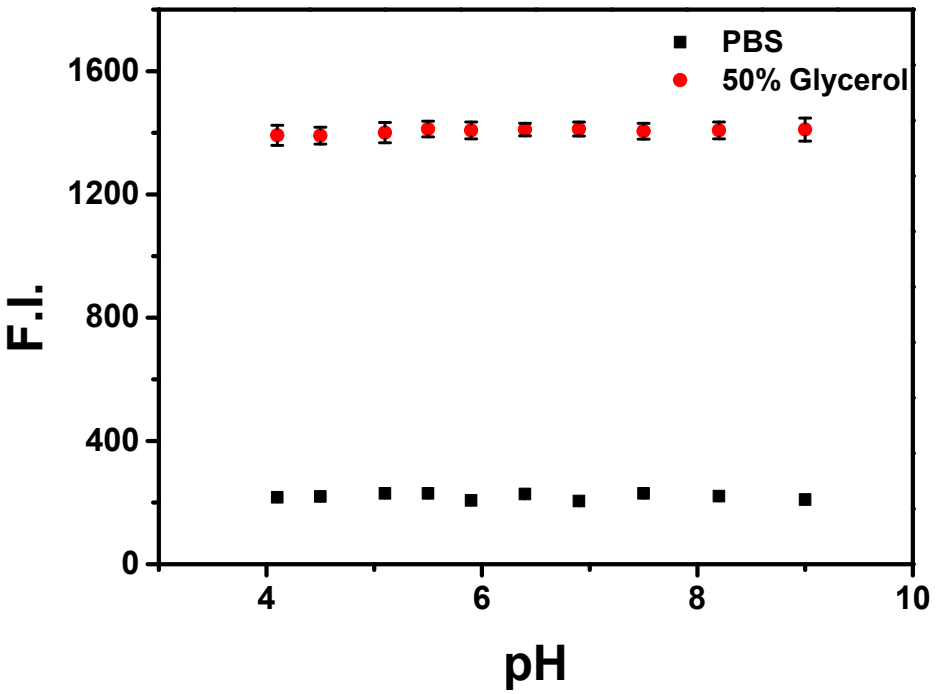


Fig. S1 The effects of pH on the fluorescence intensity of 10 μM **PN** in presence of varied glycerol volumetric ratios.

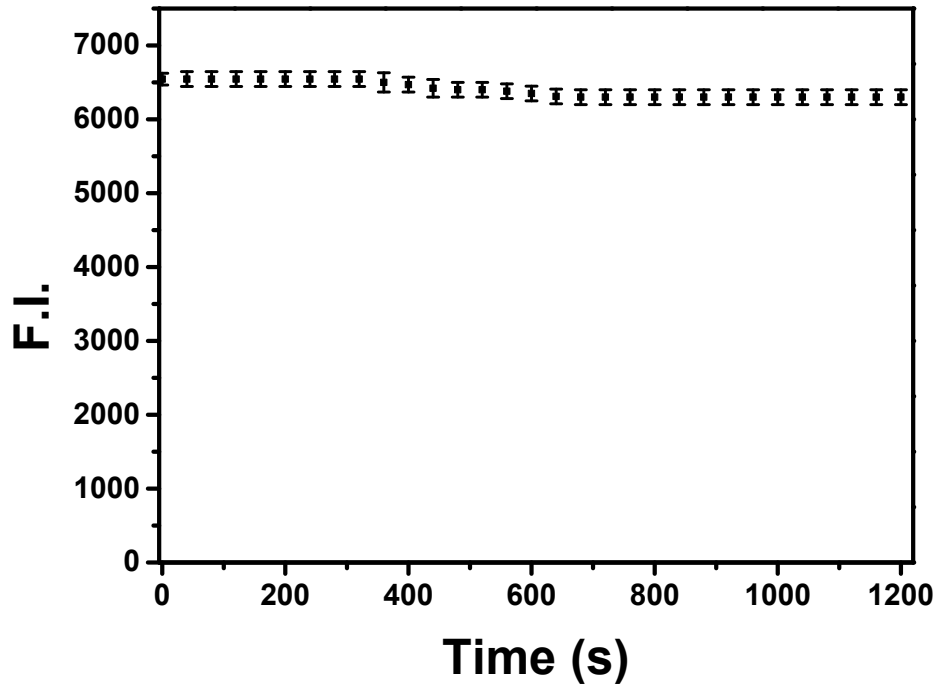


Fig. S2 Photobleaching of **PN** (10 μM) in 95% glycerol solution under irradiation with 660 nm laser (300 mW/cm^2).

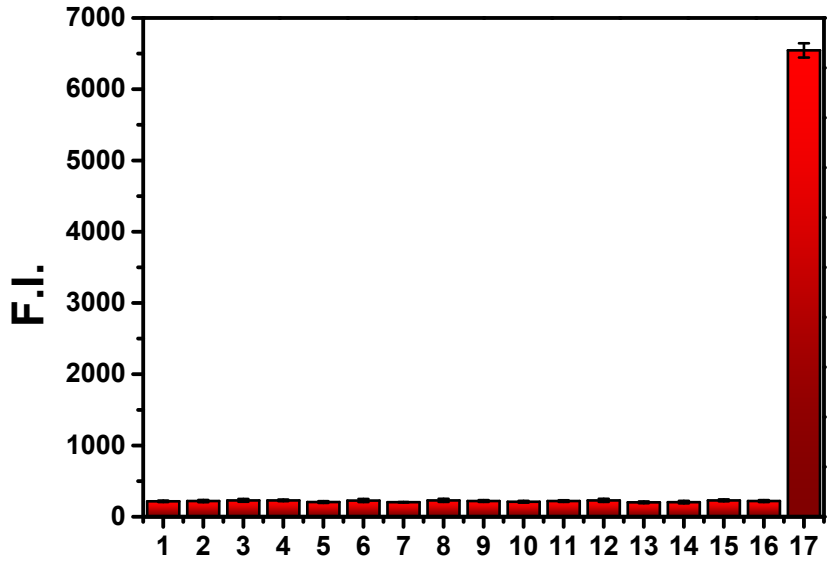


Fig. S3 Fluorescence intensity of 10 μM **PN** toward various species (100 μM) in PBS/glycerol (v:v = 1:1) mixture (down), respectively. (1) Blank; (2)Ser; (3)Cys; (4)GSH; (5)Hcy; (6)BSA; (7)SO_3^{2-}; (8)ClO^-; (9)ClO_4^-; (10)H_2O_2; (11)CO_3^{2-}; (12)OAc^-; (13)NO_3^-; (14)Cl^-; (15)Fe^{3+}; (16)Zn^{2+}; (17) Glycerol.

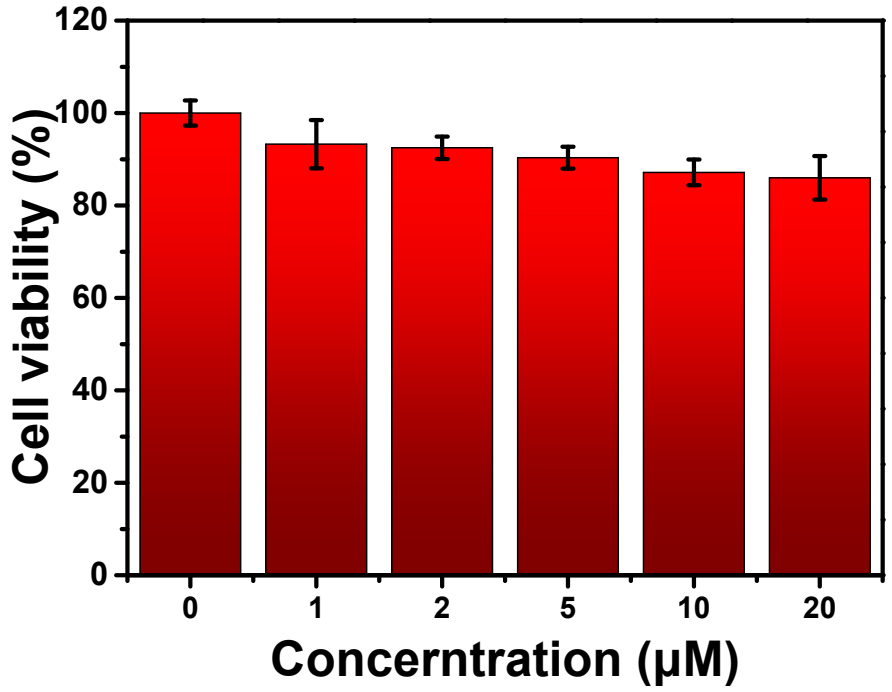


Fig. S4 MTT results of HepG2 cells viabilities after incubation with **PN** for 24 h. Data are expressed as mean ± SD (*$p < 0.05$, experiment times $n = 3$).

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[2] Liu, Y.; Feng, S.; Gong, S.; Feng, G., *Anal. Chem.*, 2022, ***94***, 17439-17447.
[3] Liu, W.; Wang, Y.; Wang, T.; Wang, L.; Hu, S.; Tian, D., *Anal. Chim. Acta.*, 2023, ***1253***, 341099.
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[11] Li, L.-L.; Li, K.; Li, M.-Y.; Shi, L.; Liu, Y.-H.; Zhang, H.; Pan, S.-L.; Wang, N.; Zhou, Q.; Yu, X.-Q., *Anal. Chem.*, 2018, ***90***, 5873-5878.

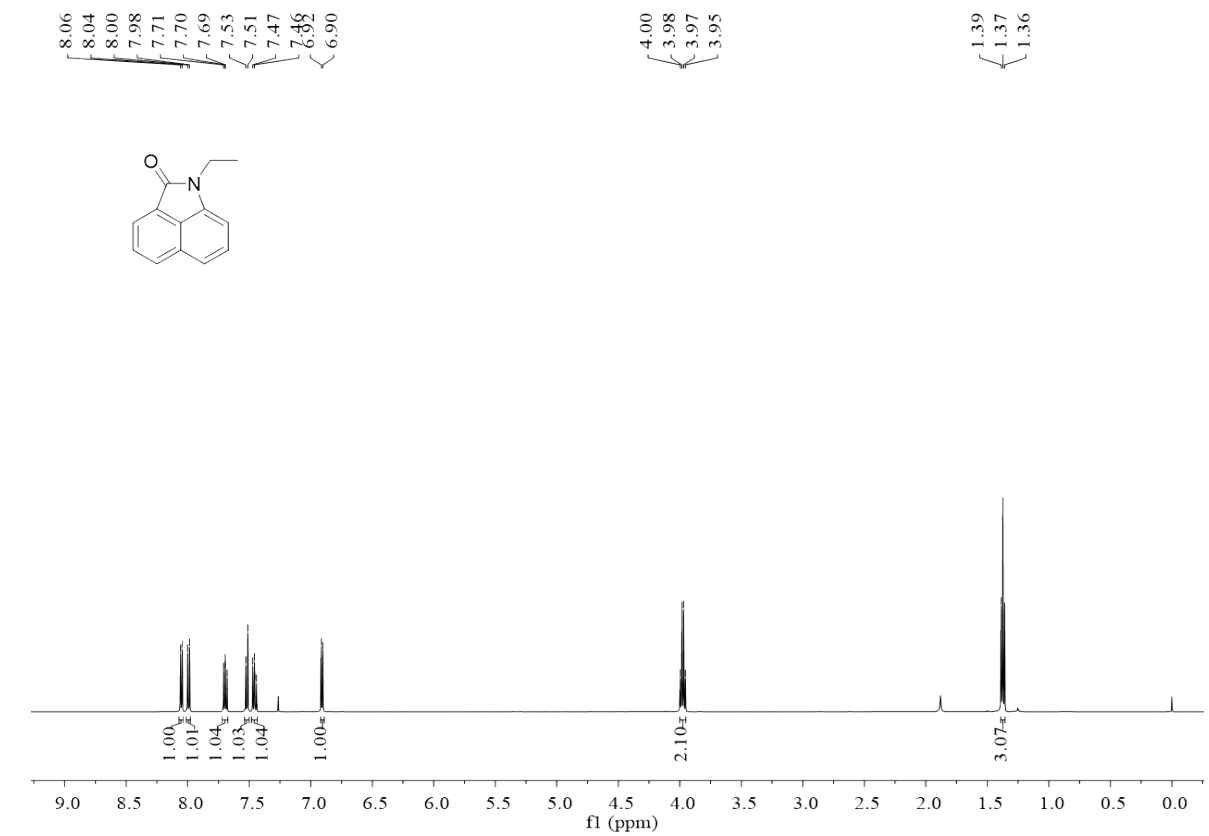

Fig. S5 ^{1}H NMR spectrum of **compound 1** in $CHCl_3$-d_6.

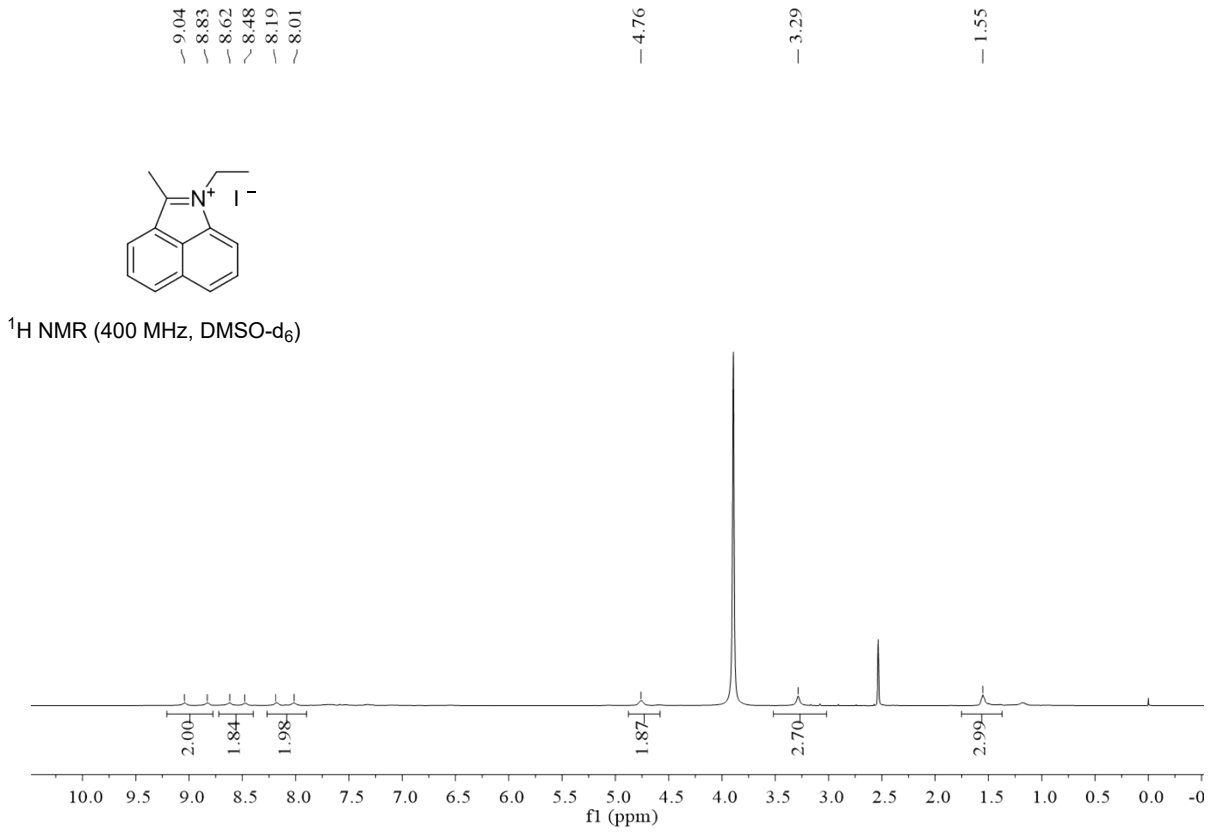

Fig. S6 ^{1}H NMR spectrum of **compound 2** in DMSO-d_6.

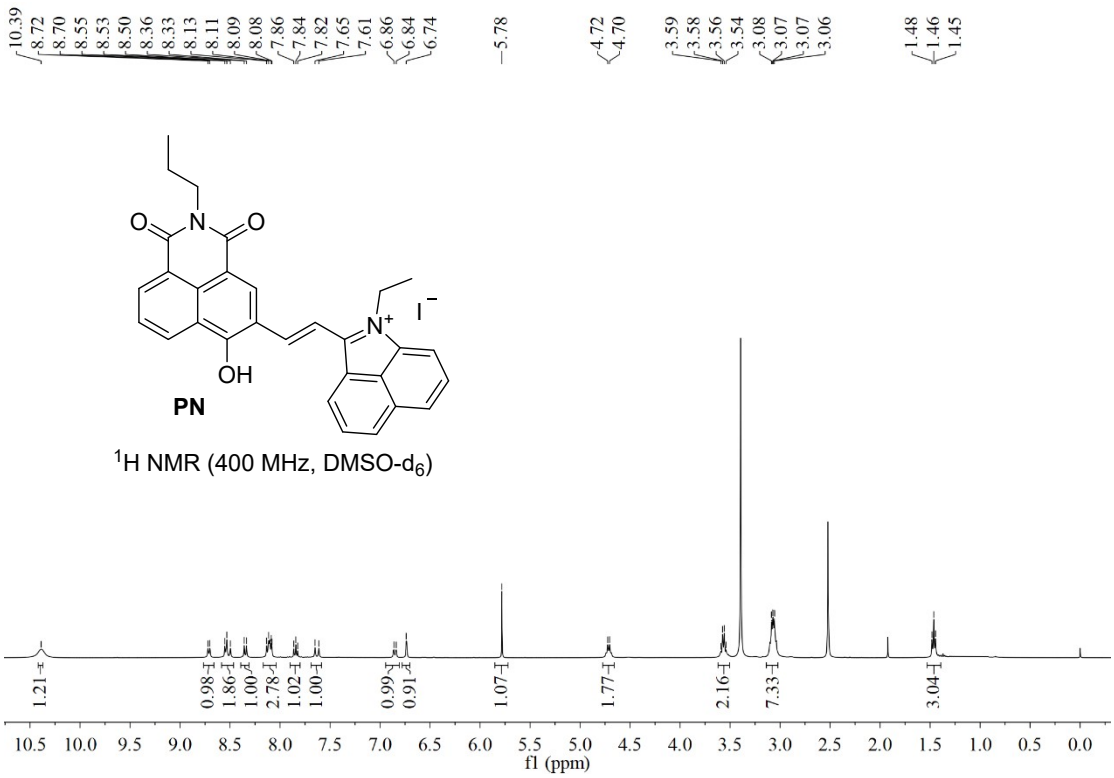

Fig. S7 ^{1}H NMR spectrum of **PN** in DMSO-d_6.

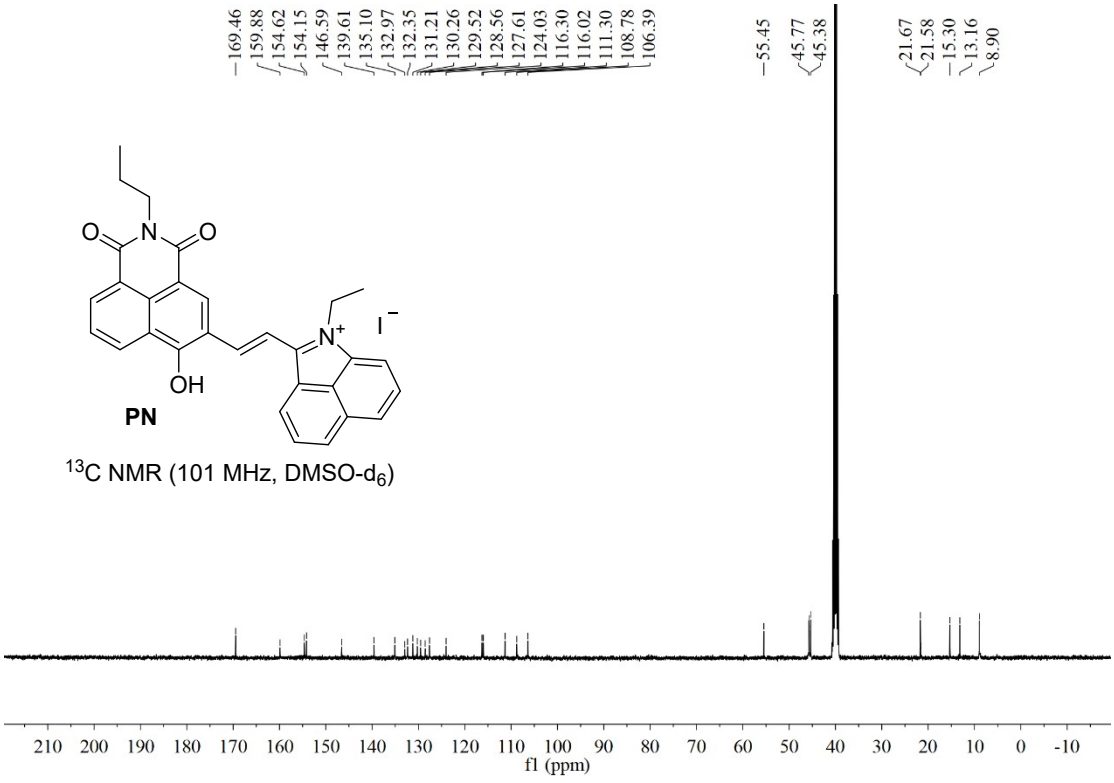

Fig. S8 ^{13}C NMR spectrum of **PN** in DMSO-d_6.

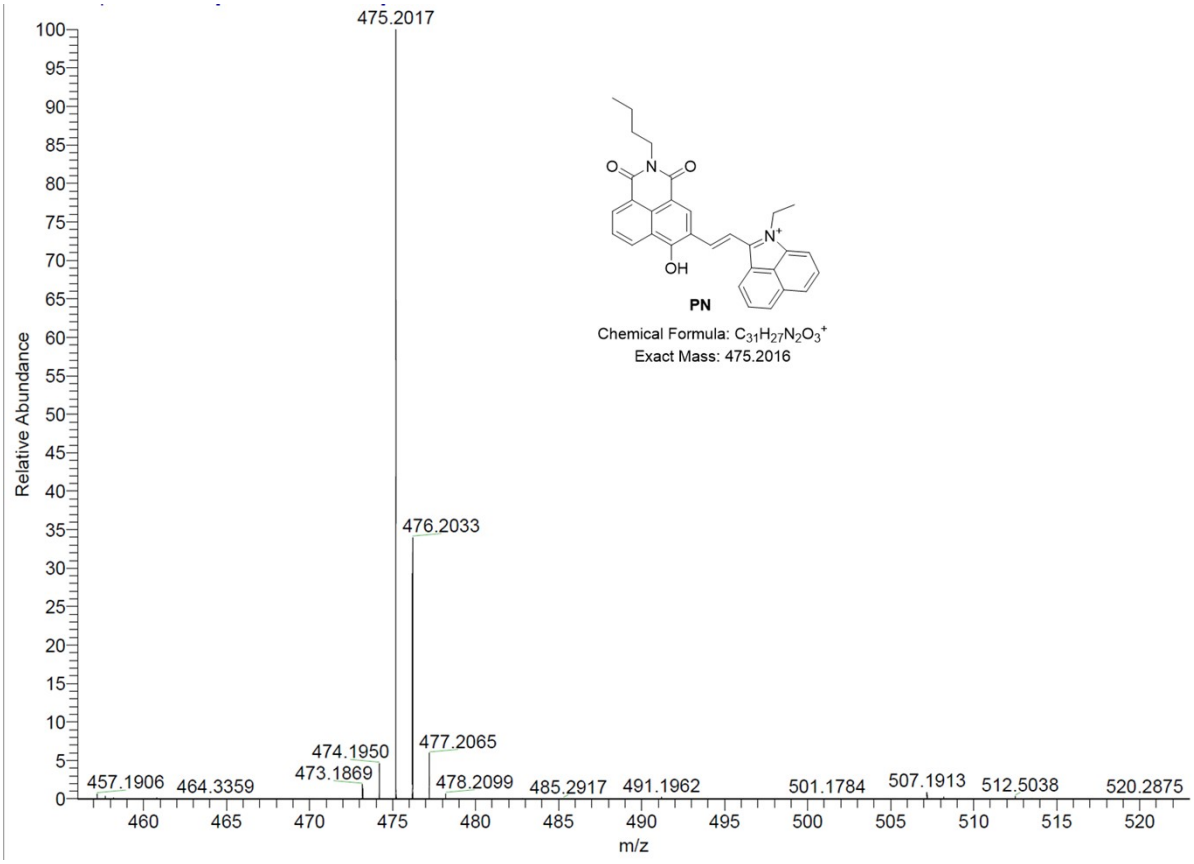

Fig. S9 HRMS result of compound **PN**' cation part.