Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Water-soluble AIE-active fluorescent organic nanoparticles for ratiometric detection of SO_2 in mitochondria of living cells

Experimental Section

Materials and instruments

Unless otherwise stated, all chemicals and solvents are purchased as reagent grade and can be used without further purification. All reactions are carried out under magnetic stirring. Reactions are monitored by the analytical thin layer chromatography (TLC) on the silica F254 glass plate and displayed by UV light (254nm or 365nm) or immersed in EtOH-H_2SO_4 (4%) before heating. Column chromatography was conducted over silica gel (mesh 200-300) or BioGel P - 2 fine resins (Bio-Rad, Hercules, CA). Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were measured at room temperature with JEOL 's NMR (400 or 600 MHz) spectrometer. Mass spectra were recorded on a waters LCT Premier XEmass spectrometer or a Bruker MALDI-TOF mass spectrometer. Transmission electron microscope (TEM) images were recorded on FEI Talos F200S. Dynamic Light Scattering (DLS) measurements were performed on a Zeta potential analyzer (Brookhaven Instruments Corporation, America). All optical testing experiments were performed in PBS solution (10 mM, pH 7.4).

UV–vis/fluorescence measurements

The probe TYDL was dissolved in water to prepare a 0.2 mM stock solution. The solutions of different analytes, including NaF(F^-) NaCl(Cl^-), KBr(Br^-), KI(I^-), NaAc(Ac^-), K_3PO_4, (PO_4^{3-}), Na_2HPO_4(HPO_4^{2-}), K_2CO_3(CO_3^{2-}), $KHCO_3$(HCO_3^-), Na_2SO_4(SO_4^{2-}), NaClO4(ClO_4^-), $NaNO_3$(NO_3^-), KSCN(SCN^-), $Na_2S_2O_3$($S_2O_3^{2-}$), NaHS(HS^-), GSH, Cys, BSA, Vc, $NaHSO_3$(HSO_3^-), TBACN(CN^-) were prepared in phosphate buffer solution (PBS, 10 mM, pH 7.4).

Confocal imaging of living cells

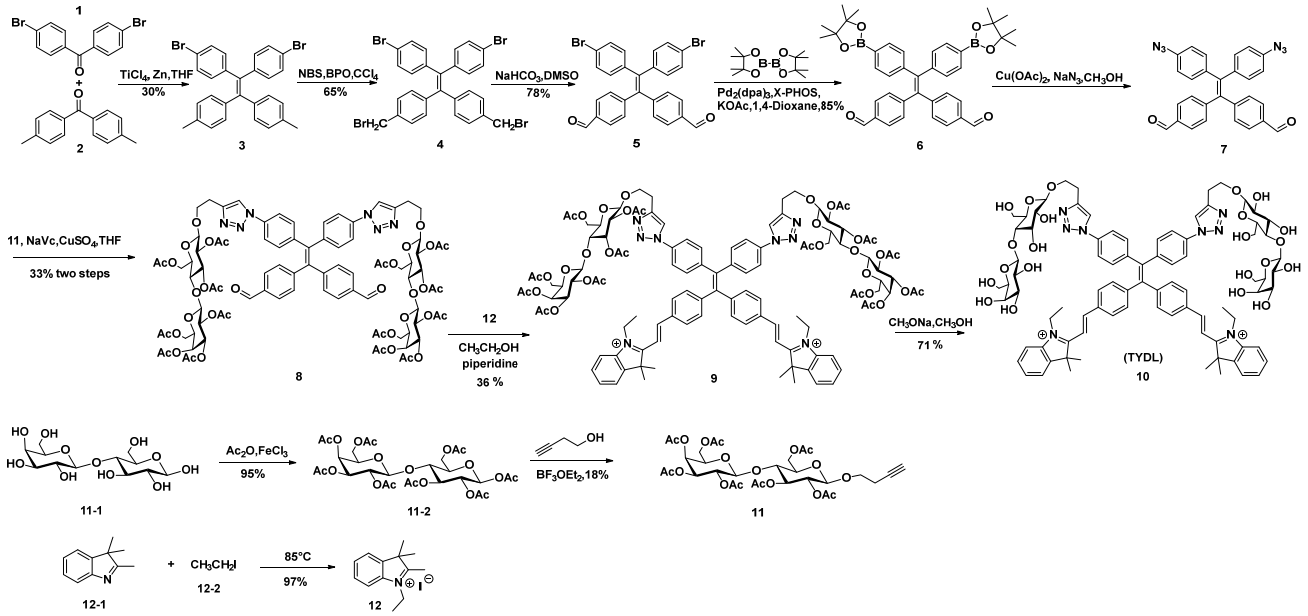
HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator, and culture media were replaced with fresh media every day. The cells were further incubated with diverse concentration of probes in culture media at 37 °C and then washed 3 times with PBS buffer before cell fluorescence imaging experiments with confocal laser scanning microscopy. LO2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% FBS. Other processes were consistent with those of HepG2 cells.

MTT assay

HepG2 cells were seeded in a 96-well plate with culture media. Each concentration of **TYDL** was incubated with cell for 24 h. After incubation, cells were incubated with cell culture media containing 0.5 mg/mL MTT (thiazolyl blue tetrazolium bromide) for 3 h and removed the media. Then the cells were dissolved in DMSO of 0.5 mL, the absorbance at 570 nm was measured. The cell viability(%) was calculated according to the following equation:

$$\text{Cell viability} = \frac{\text{OD}_{sample} - \text{OD}_{blank}}{\text{OD}_{control} - \text{OD}_{blank}} \times 100\%$$

Synthesis of TYDL



Scheme S1. Synthetic route for probe **TYDL**

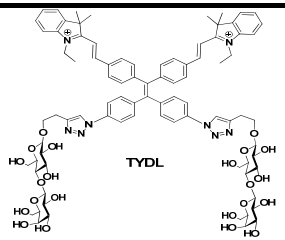
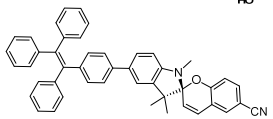
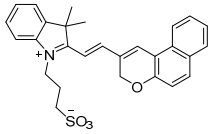
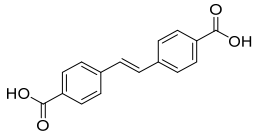
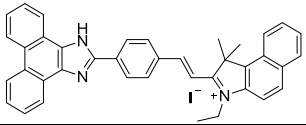
Compounds **3-8** [17]and **11**[18] were synthesized according to the procedures in the literatures.

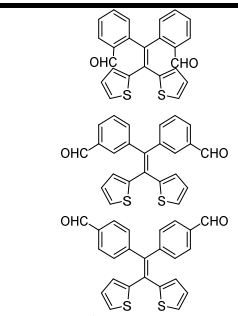
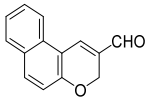
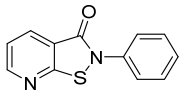
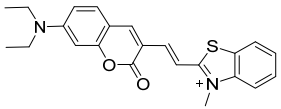
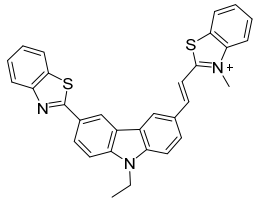
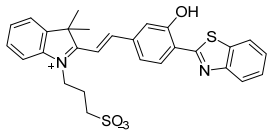
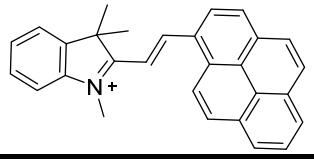
Synthesis of Compound 12: Compound**12-1** (1.6 ml, 9.9mmol) and Compound **12-2** (8 ml, 9.9 mmol) were added to a 50 mL two-necked flask. The reaction mixture was heated to 85℃ overnight under reflux. After cooling to room temperature, precipitate were filtered and washed by hexane to afford the products.(3.0 g, yield 97%) ^{1}H NMR (600 MHz, Chloroform-d): δ 7.75 (dd, J = 7.34, 2.24 Hz ,1H), 7.59 (m, 3H), 4.77(q, J =7.48Hz), 3.16(s, 3H), 1.66(s, 6H), 1.63(t, J= 7.48Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.57, 181.55, 170.44, 170.39, 170.09, 169.72, 169.11, 148.54, 145.48, 143.63, 142.48, 141.14, 140.43, 136.36, 134.35, 132.73, 131.95, 130.98, 130.02, 129.69, 128.90, 124.80, 122.76, 119.96, 114.91, 101.29, 96.23, 72.86, 71.12, 70.73, 69.20, 66.70, 61.93, 60.85, 60.83, 53.52, 52.39, 52.39, 45.92, 45.92, 44.02, 29.76, 27.12, 22.76, 20.72, 19.80, 14.19 ; MS (ESI): m/z calcd for [$C_{13}H_{18}N^+$]: 188.14; Found: 188.13.

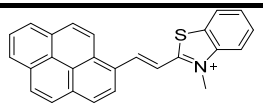
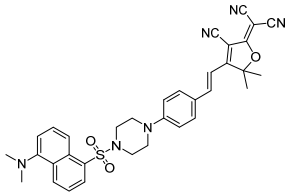
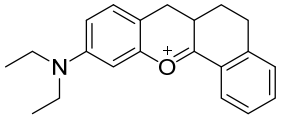
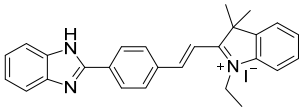
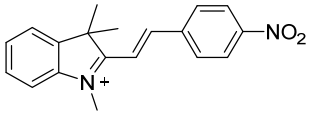
Synthesis of Compound 9: To a 25 mL two-necked flask, compound **8** (50 mg, 0.0271 mmol) and compound **12** (34.1 mg, 0.1082 mmol) were added and dissolved in dry EtOH (4 mL). Pyridine (20 mL) was added and the mixture was heated under reflux for 8h. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel column using DCM / MeOH (50: 2, v / v) as an eluent to obtain compound **9** as an orange solid (24 mg, yield 36%). ^{1}H NMR (600 MHz, Chloroform-d): δ 8.12 (s, 2H), 7.87 (d, J = 38.4 Hz, 2H), 7.63 – 7.48 (m, 12H), 7.16 (dq, J = 17.5, 10.7, 9.5 Hz, 10H) ,5.41 (t, J = 9.5 Hz, 1H), 5.28 (s, 2H), 5.23 (s, 1H), 5.15 – 5.09 (m, 2H), 5.03 (d, J = 7.5 Hz, 2H), 4.94 (s, 1H), 4.89 (dd, J = 9.2, 4.7 Hz, 2H), 4.86 – 4.81 (m, 1H), 4.72 (d, J = 10.0 Hz, 1H), 4.42 (d, J = 10.3 Hz, 5H), 4.13 – 3.94 (m, 10H), 3.87 (s, 2H), 3.84 – 3.79 (m, 2H), 3.75 – 3.63 (m, 5H), 3.57 – 3.52 (m, 1H), 3.03 (s, 6H),2.10 – 2.04 (m, 10H), 2.02 (d, J = 5.4 Hz, 3H), 1.98 (s, 22H), 1.90 (d, J = 5.2 Hz, 7H), 1.85 – 1.69 (m, 14H), 1.54 (s, 4H); ^{13}C NMR (151 MHz, Chloroform-d) δ 191.57, 181.55, 170.44, 170.39, 170.09, 169.72, 169.11, 148.54, 145.48, 143.63, 142.48, 141.14, 140.43, 136.36, 134.35, 132.73, 131.95, 130.98, 130.02, 129.69, 128.90, 124.80, 122.76, 119.96, 114.91, 101.29, 96.23, 72.86, 71.12, 70.73, 69.20, 66.70, 61.93, 60.85, 60.83, 53.52, 52.39, 52.39, 45.92, 45.92, 44.02, 29.76, 27.12, 22.76, 20.72, 19.80, 14.19; HRMS (ESI): m/z calcd for $[C_{114}H_{130}N_8O_{36}]^{2+}$: 1093.9305; Found: 1093.9267.

Synthesis of Compound 10 (TYDL): To a 25mL eggplant shaped bottle, compound **9** (50 mg, 0.0204 mmol) in dry MeOH (8 mL) was added and 1 M NaOMe in MeOH was slowly added dropwise at room temperature. The resulting mixture was stirred for 1 hour at room temperature. The reaction pH was then adjusted with Amberlite IR-120 plus (H^+) to pH 6 and the solvent was removed under reduced pressure. (27 mg, yield: 71%)**.**^{1}H NMR (600 MHz, DMSO-d6): δ 8.62 (d, J = 18.9 Hz, 2H), 8.05 (d, J = 7.3 Hz, 1H), 7.87 (d, J = 24.2 Hz, 1H), 7.73 (d, J = 8.0 Hz, 2H), 7.69 – 7.63 (m, 12H), 7.37 – 7.21 (m, 10H), 5.30 (s, 2H), 5.06 (s, 4H), 4.77 (s, 1H), 4.70 – 4.64 (m, 2H), 4.61 (s, 2H), 4.55 (s, 2H), 4.49 (s, 2H), 4.26 (s, 1H), 4.12 – 4.08 (m, 3H), 4.02 (s, 1H), 3.73 – 3.67 (m, 4H), 3.58 (s, 5H), 3.50 – 3.40 (m, 10H), 3.02 (s, 1H), 2.93 (s, 4H), 1.99 – 1.91 (m, 5H), 1.65 – 1.56 (m, 18H), 1.51 (d, J = 10.7 Hz, 3H), 1.45 – 1.42 (m, 2H). HRMS (ESI): m/z calcd for $[C_{86}H_{102}N_8O_{22}]^{2+}$: 799.3549; Found: 799.3545.

Table S1. Comparison of **TYDL** with some other reported HSO_3^- selective fluorescent probes.

Entry	Probe Structure	AIE active	Response mode	Water solubility	Can nanoparticles be formed?	Cell experiment	LOD	Ref.
1		Yes	Ratiometric	PBS buffer	Yes	Yes	75 nM	This work
2		Yes	Ratiometric	CH_3CN: H_2O=3:7 (v/v)	No	No	1.04 ppm (1×10^{-5}M)	[1]
3		Yes	Ratiometric	PBS buffer/ DMSO=9/1	No	Yes	0.17 μM	[2]
4		Yes	Turn- on	DMF/H_2O (v: v = 1:9)	No	No	38.4 nM	[3]
5		Yes	Turn- on	EtOH /PBS buffer = 4:6(v/v)	No	Yes	26 nM	[4]

6		Yes	Turn-off	CH_3OH/H_2O=1/9 (v/v)	No	No	97.1nM 100.2nM 77.1nM	[5]
7		Yes	Turn-off	DMSO/HEPES =1/9	No	Yes	203.5 nM	[6]
8		Yes	Turn-off	THF/water =1/999 (v/v)	No	No	0.39 mM	[7]
9		No	Ratiometric	PBS buffer ,containing 30% DMF	No	Yes	0.38 mM	[8]
10		No	Ratiometric	PBS buffer containing 20% glycero	No	Yes	0.34 mM	[9]
11		No	Ratiometric	PBS buffer	No	Yes	0.34 mM	[10]
12		No	Ratiometric	DMSO/PBS buffer =4/6, pH = 7.4	No	Yes	15.1 nM	[11]

13		No	Ratiometric	HEPES buffer /DMF= 7:3	No	Yes	1.45 ×10^{-7}M	[12]
14		No	Ratiometric	EtOH/PBS buffer = 5:5	No	Yes	66 nM	[13]
15		No	Turn off	PBS containing 20% CH_3CH_2OH	No	Yes	6.29×10^{-6}M	[14]
16		No	Turn on	EtOH/Tris-HCl=1:9	No	Yes	0.14 mM	[15]
17		No	Turn off	DMSO/PBS buffer =1:9	No	No	8.7 μM	[16]

Supplementary spectra

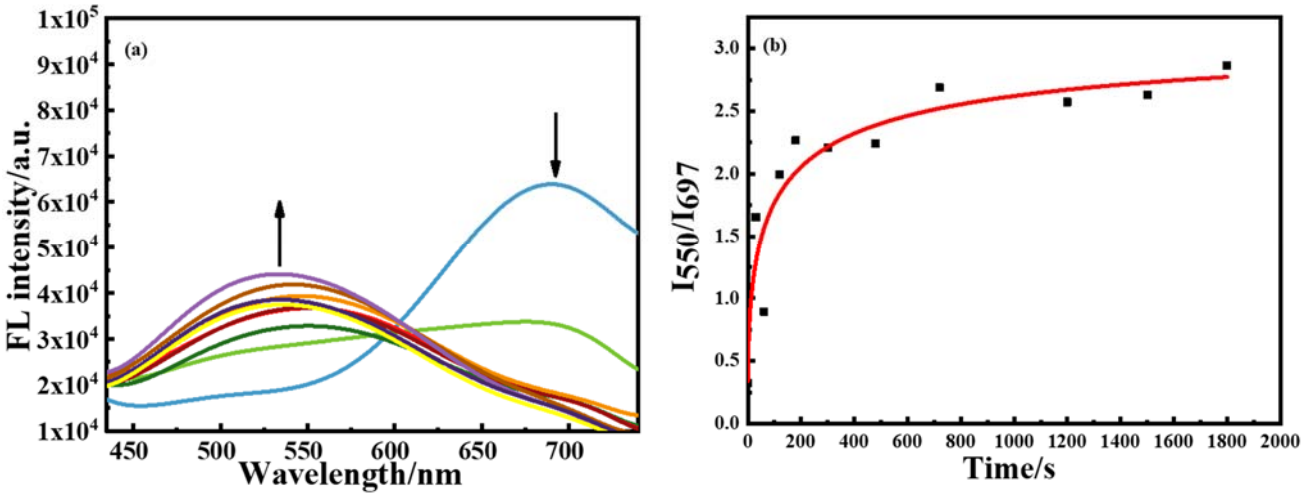


Fig. S1. Time-dependent fluorescence spectral change of probe **TYDL** (5 μM) upon addition of HSO_3^- (200 μM) in pure water. (b) Fluorescence intensity ratio (I_{550}/I_{697}) of probe **TYDL** (5 μM) upon addition of HSO_3^- (200 μM) in pure water. λ_{ex} = 300 nm.

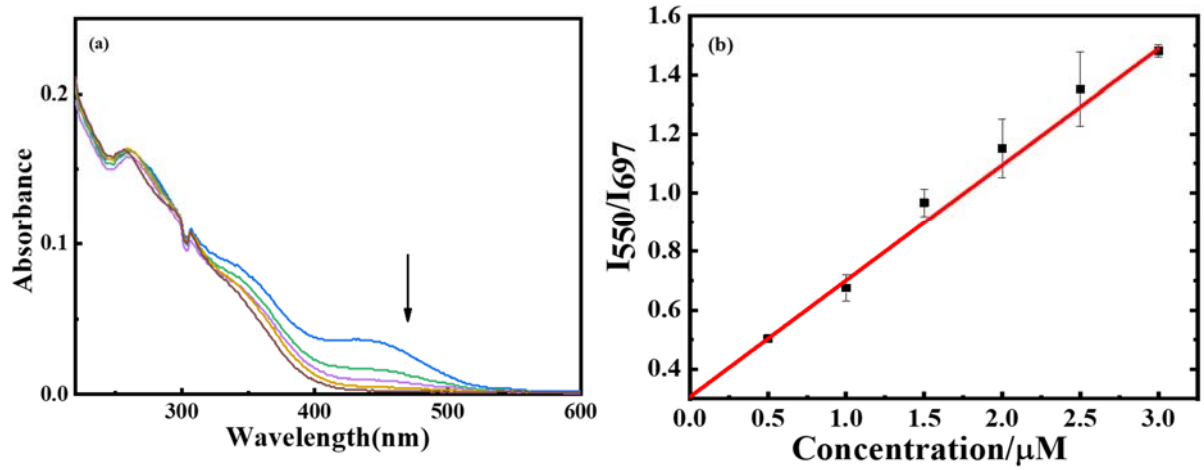


Fig. S2. a) Absorption spectral changes of **TYDL** (10 μM) upon titration with HSO_3^-(0-500 μM) in pure water. b) the detection limit of **TYDL**.

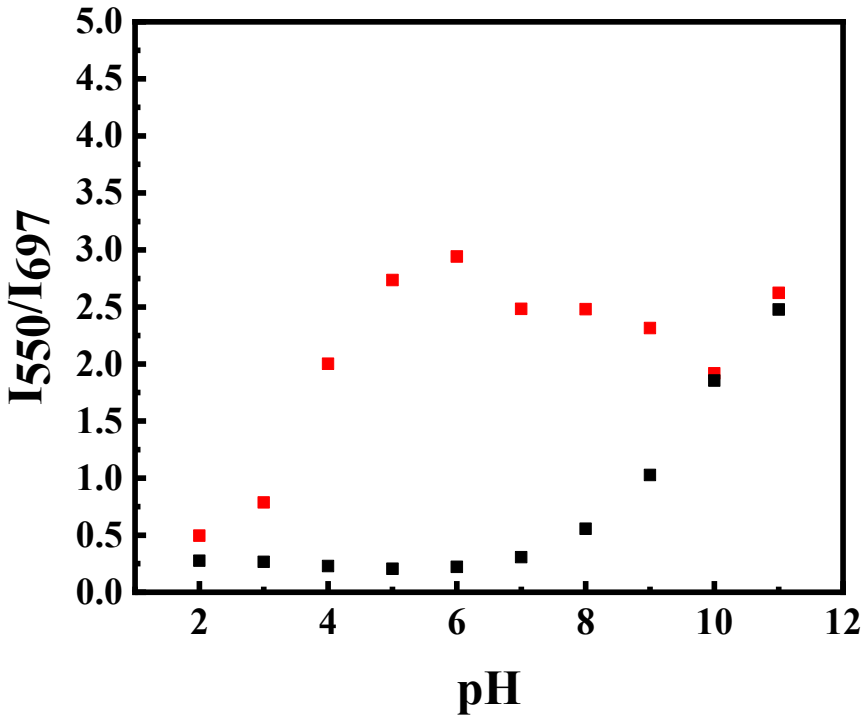


Fig. S3. The effect of various pH values on the fluorescence ratio (I_{550}/I_{697}) of **TYDL** (5 μM) in the presence or absence of HSO_3^- (200 μM). Spectra were recorded after 10 min incubation of **TYDL** with HSO_3^-.

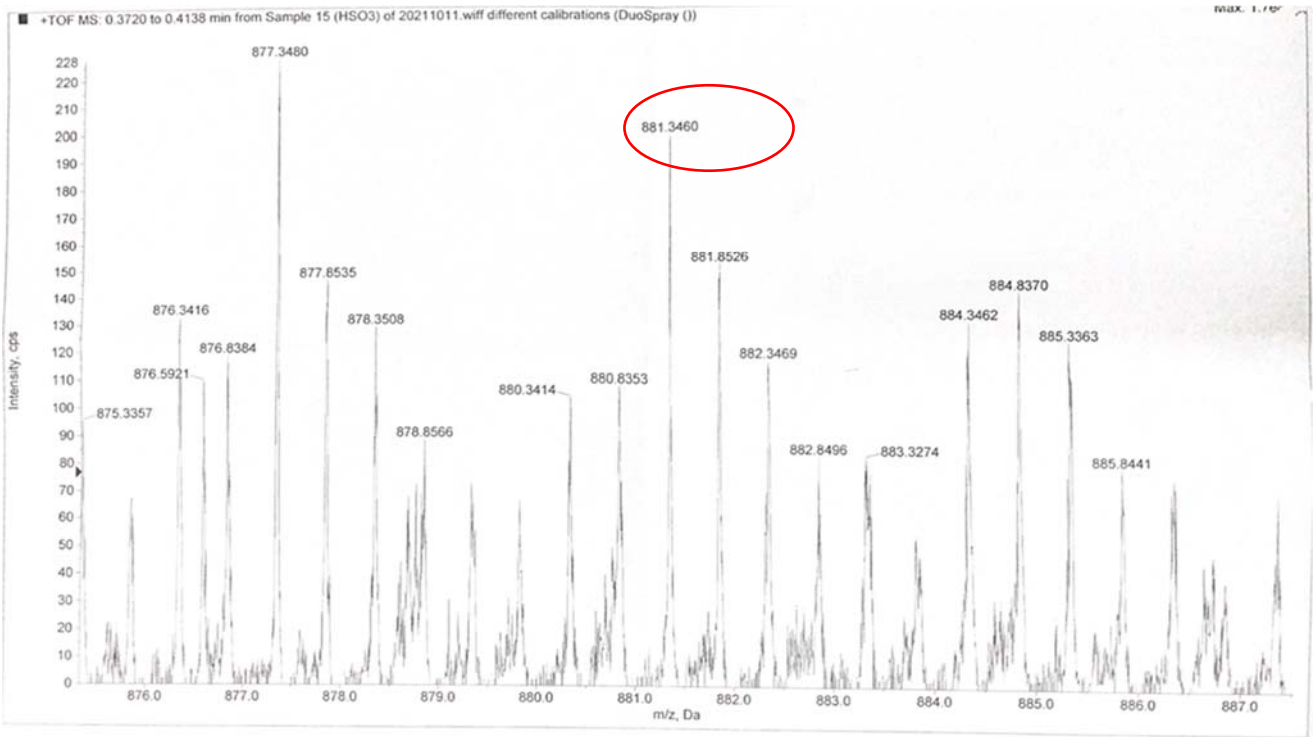


Fig. S4. MS of Compound **TYDL'** $[(TYDL\text{-}HSO_3)^{2+}]$.

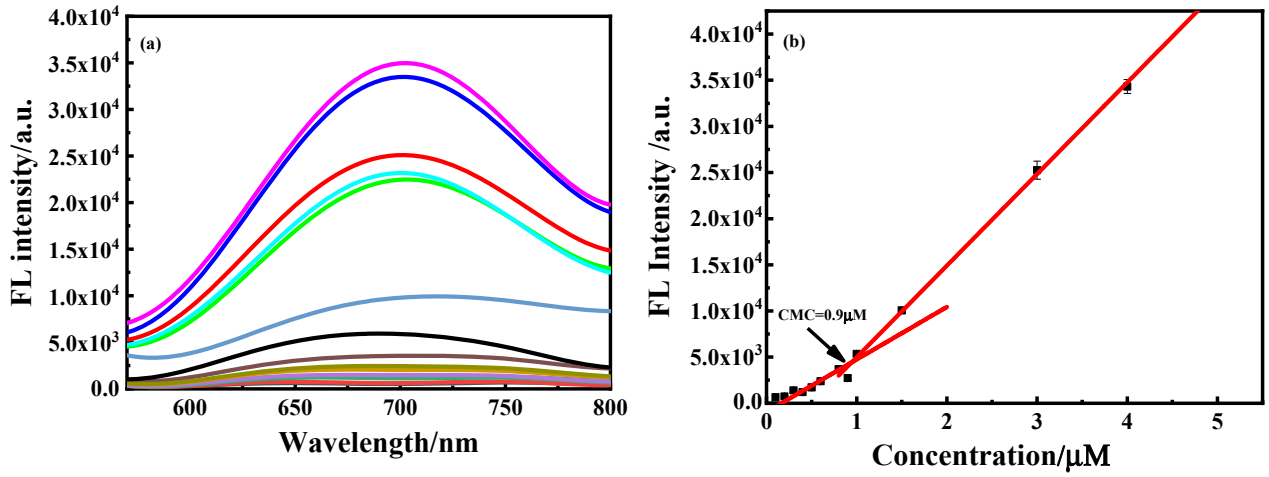


Fig. S5. (a) Fluorescence spectra of different concentrations of TYDL in pure water. (b) The curve of fluorescence intensity at 697 nm versus the concentration of TYDL in pure water. The crosspoint corresponds to the CMC of TYDL. λex = 300 nm

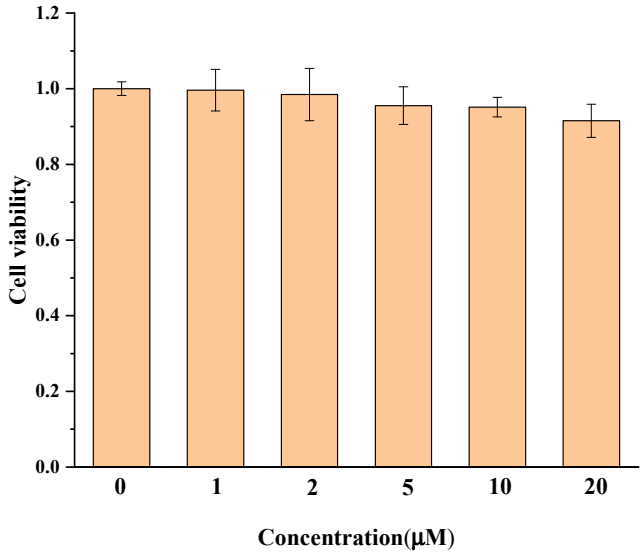


Fig. S6. Effects of different concentrations of **TYDL** on the viability of HepG2 cells. The results are the mean standard deviation of three separate measurements.

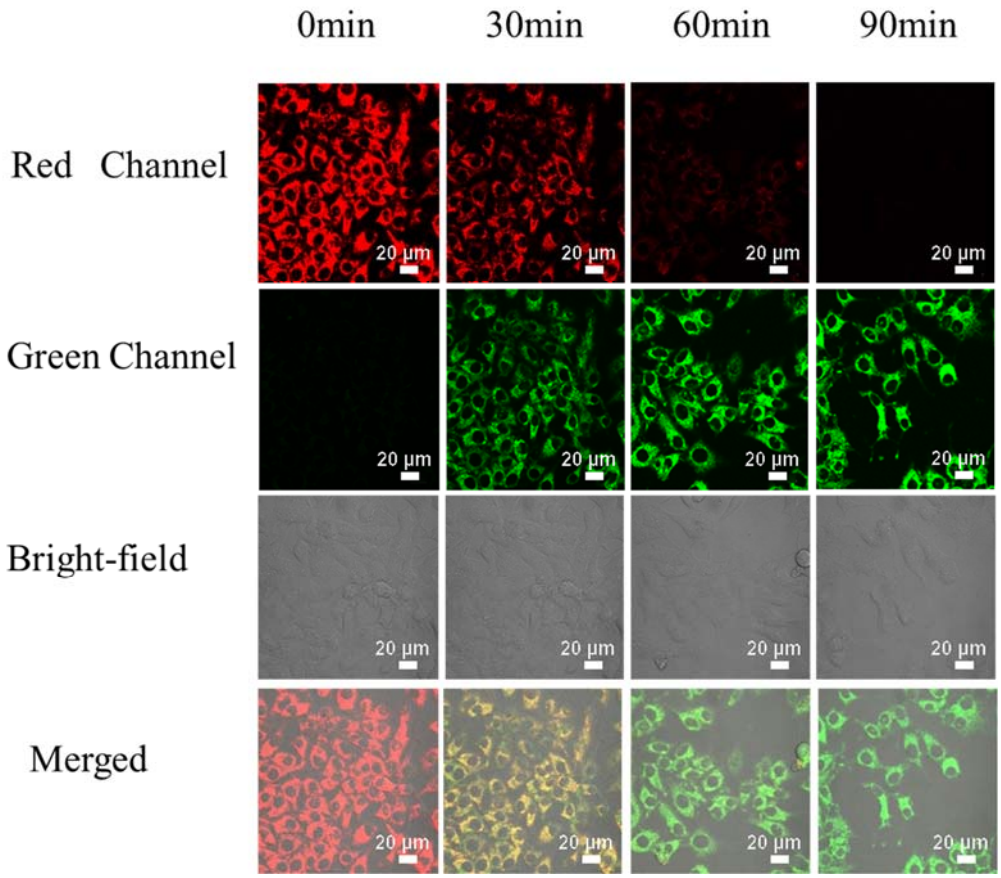


Fig. S7. Fluorescence images of **TYDL** responding to HSO_3^- in HepG2 cells. From left to right: HepG2 cells pretreated with TYDL (5 μM) for 30 min, incubated with 200 μM $NaHSO_3$ for 0, 30, 60, 90 min, and then imaged. From up to down: Red channel (λ_{em} = 600-750 nm); Green channel (λ_{em} = 500-550 nm); Bright-field; Merge.

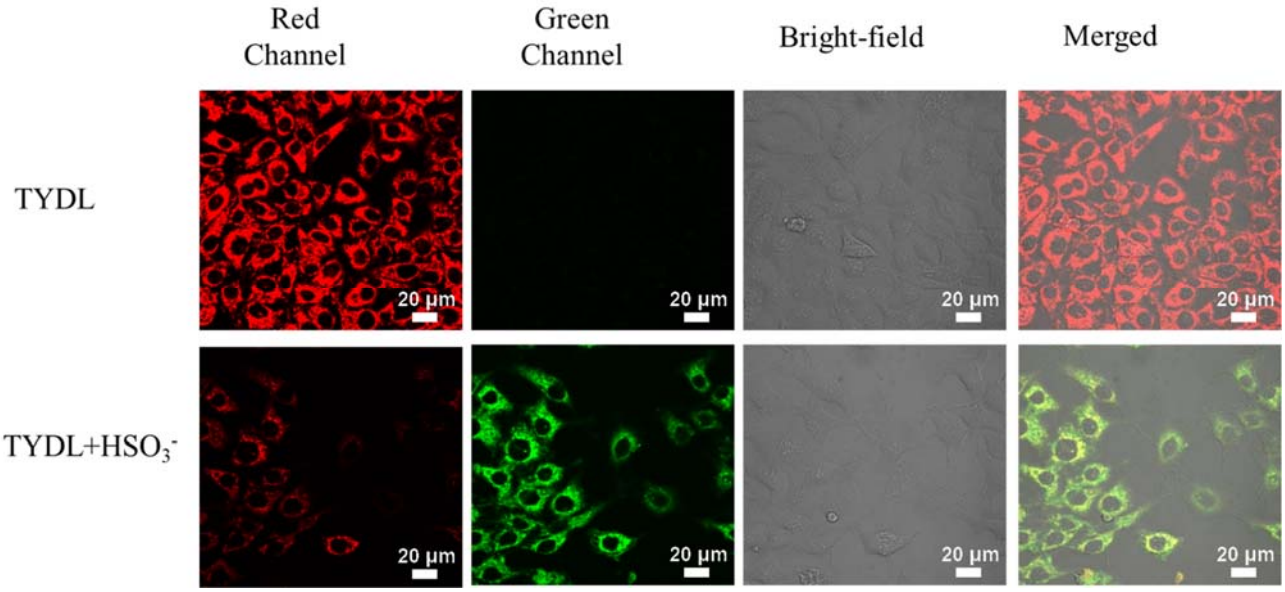


Fig. S8. From up to down: HepG2 cells pretreated with **TYDL** (5 μM) alone for 60 min, sequentially incubated with 200 μM $NaHSO_3$ (60 min) and 5 μM **TYDL** (60 min), and then imaged. From left to right: Red channel (λ_{em} = 600-750 nm); Green channel (λ_{em} = 500-550 nm); Bright-field; Merge.

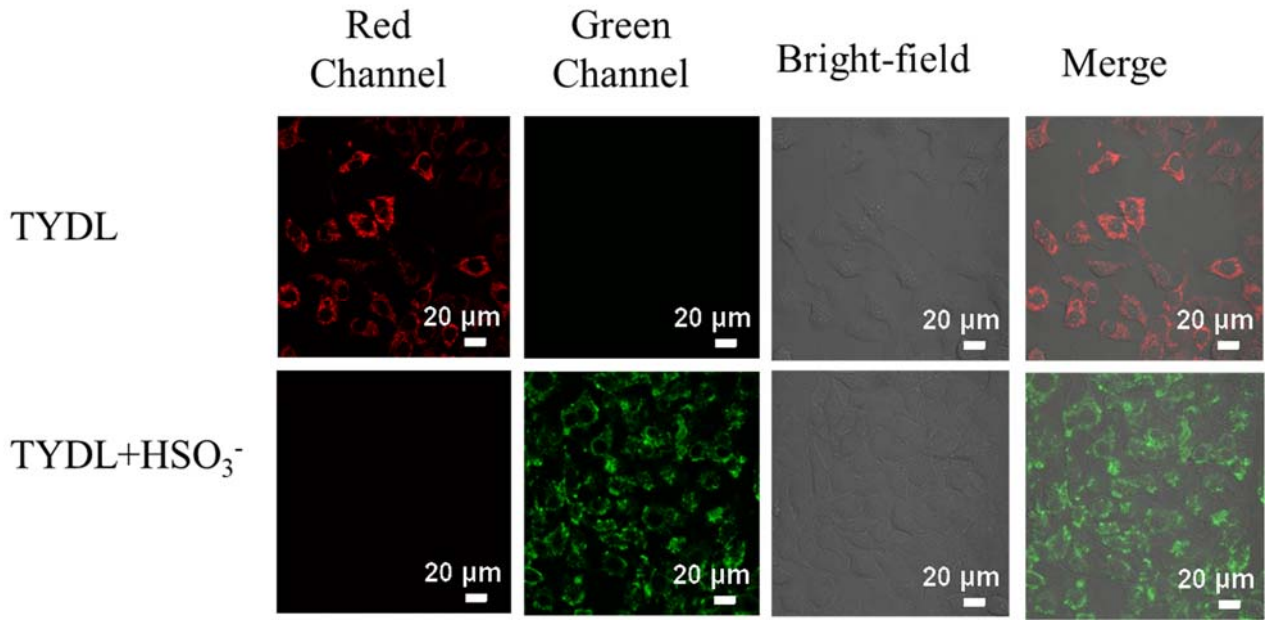


Fig. S9. From up to down:LO2 cells pretreated with **TYDL** (5 μM) alone for 60 min, sequentially incubated with 200 μM NaHSO3 (60 min) and 5 μM **TYDL** (60 min), and then imaged. From left to right: Red channel (λ_{em} = 600-750 nm); Green channel (λ_{em} = 500-550 nm); Bright-field; Merge.

^{1}H NMR, ^{13}C NMR and HRMS Spectra of Compounds

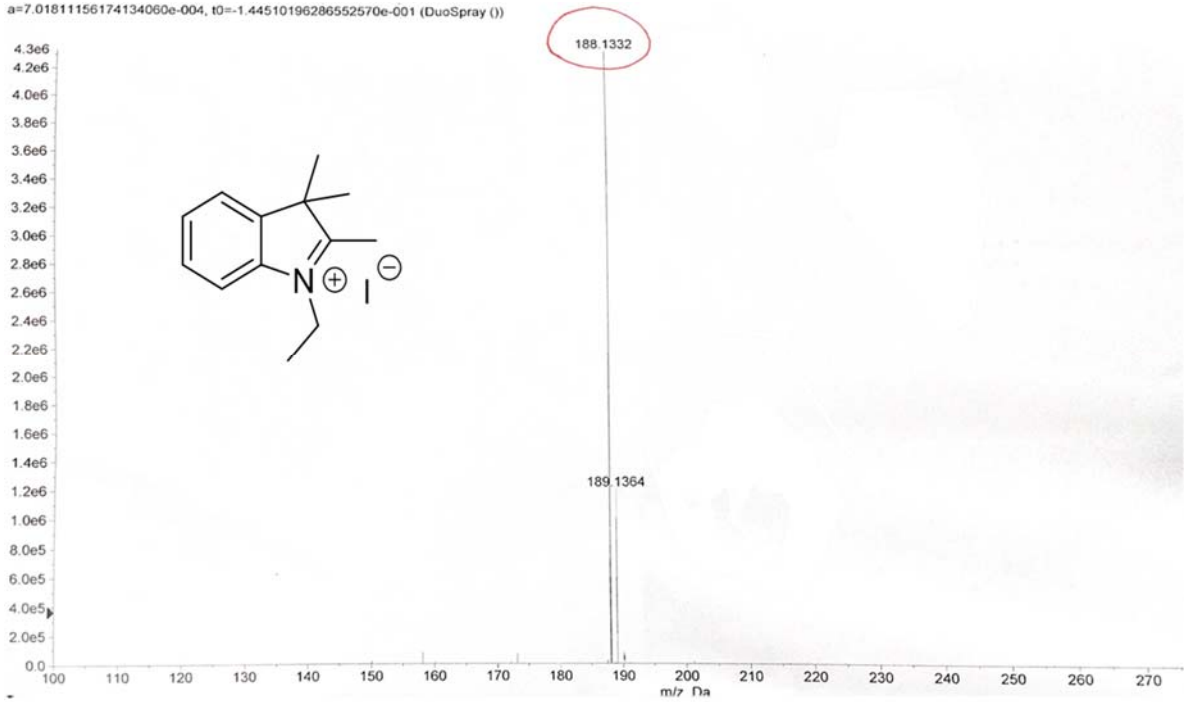


Fig. S10.MS Spectrum of Compound **12**

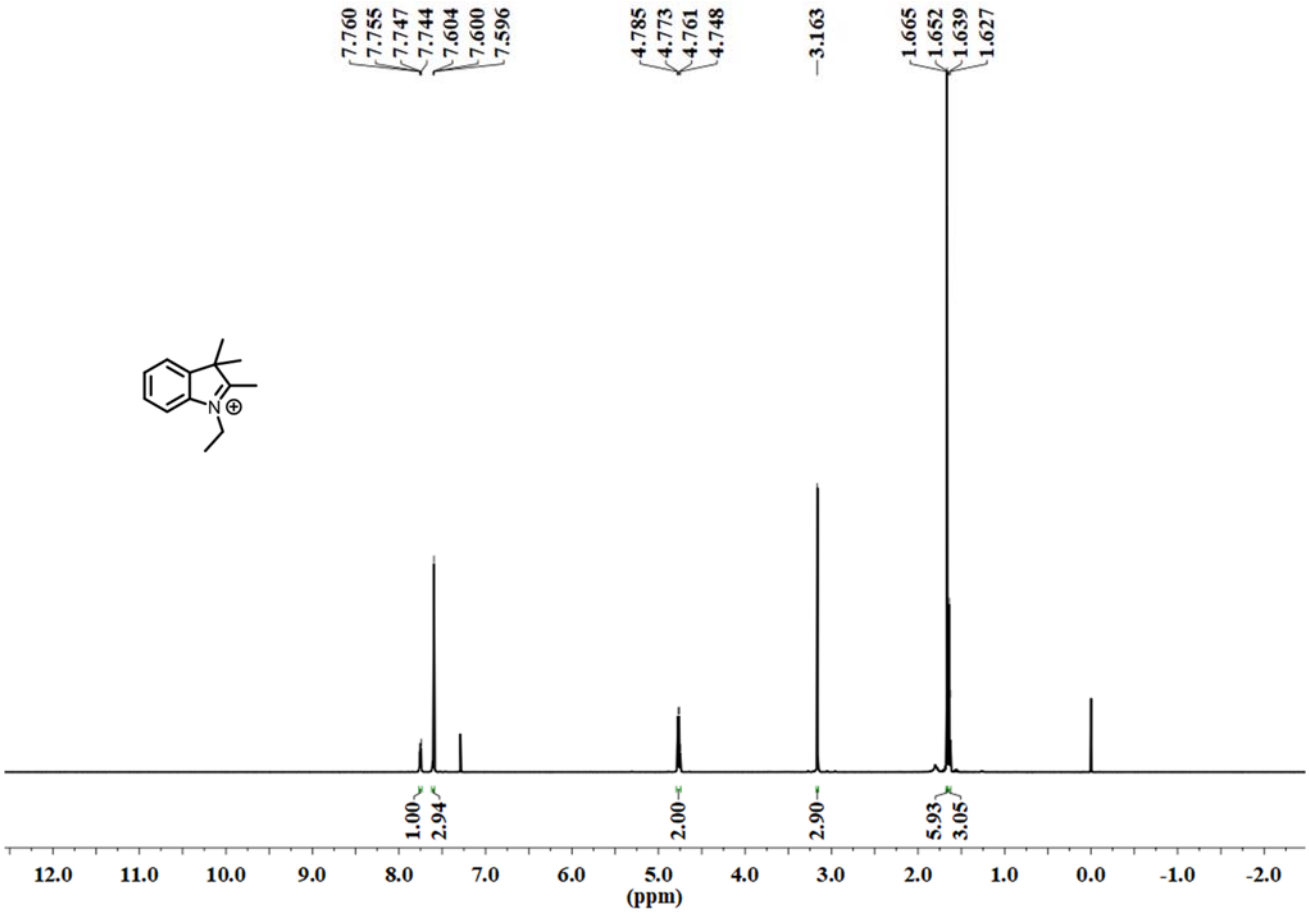


Fig. S11. ^{1}H NMR Spectrum of Compound **12**

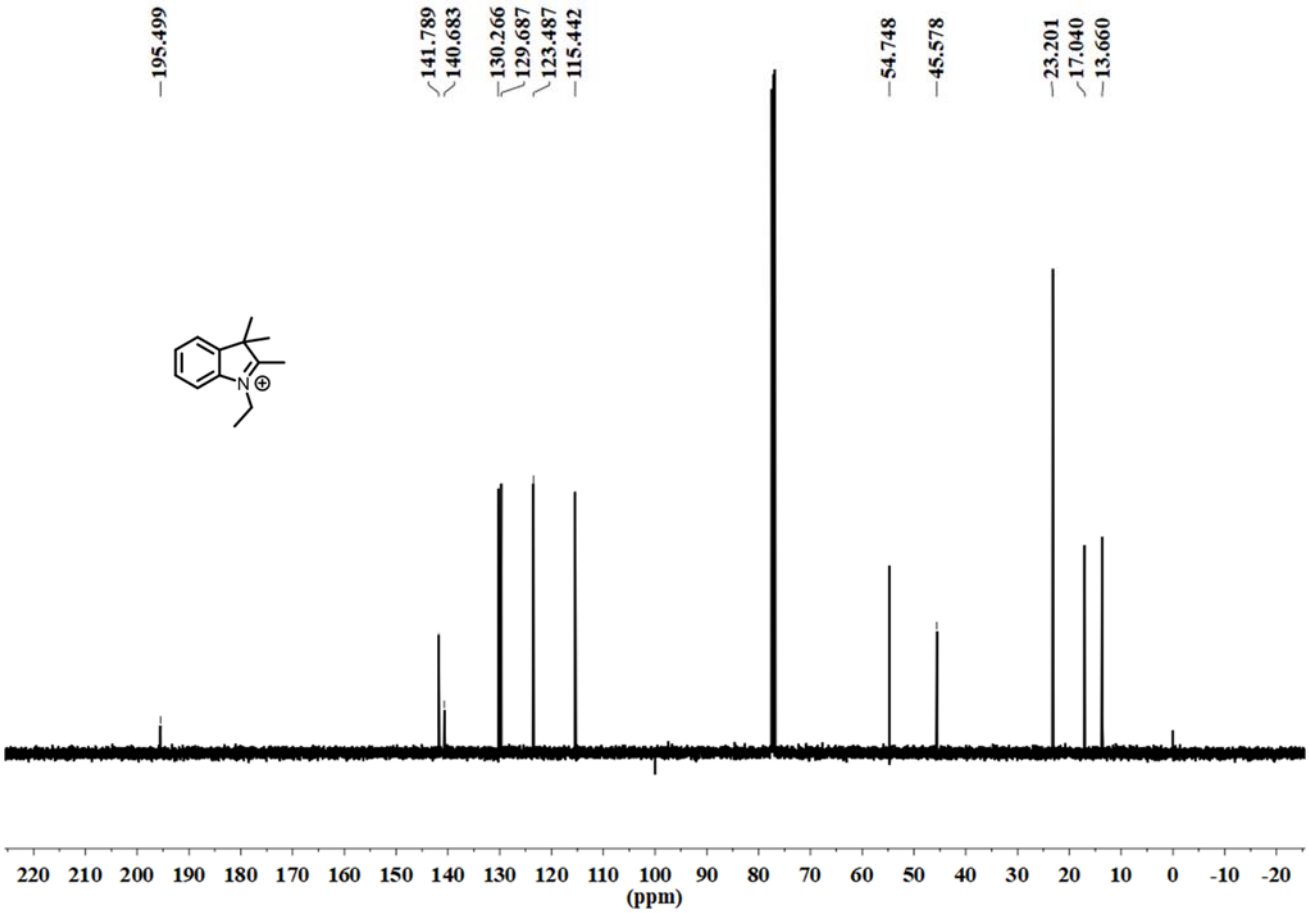
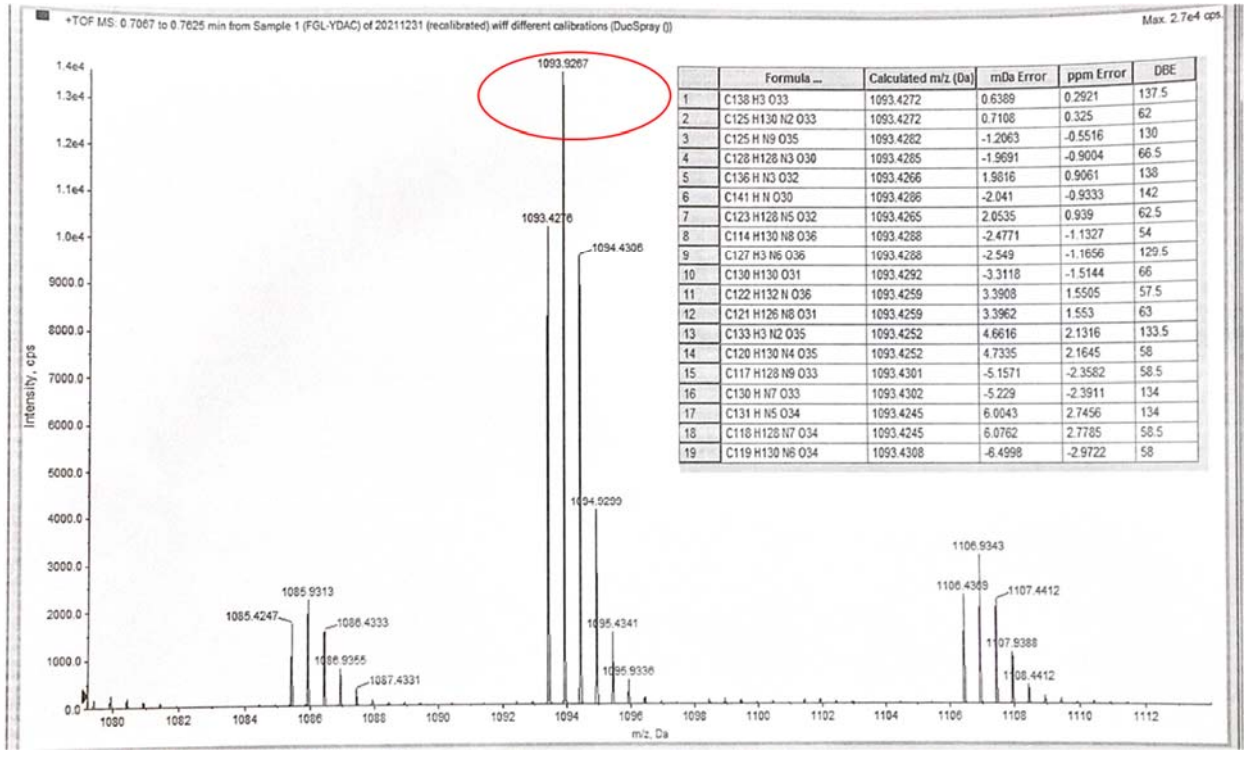


Fig. S12. ^{13}C NMR Spectrum of Compound **12**



	Formula ...	Calculated m/z (Da)	mDa Error	ppm Error	DBE
1	C138 H3 O33	1093.4272	0.6389	0.2921	137.5
2	C125 H130 N2 O33	1093.4272	0.7108	0.325	62
3	C125 H N9 O35	1093.4282	-1.2063	-0.5516	130
4	C128 H128 N3 O30	1093.4285	-1.9691	-0.9004	66.5
5	C136 H N3 O32	1093.4266	1.9816	0.9061	138
6	C141 H N O30	1093.4286	-2.041	-0.9333	142
7	C123 H128 N5 O32	1093.4265	2.0535	0.939	62.5
8	C114 H130 N8 O36	1093.4288	-2.4771	-1.1327	54
9	C127 H3 N6 O36	1093.4288	-2.549	-1.1656	129.5
10	C130 H130 O31	1093.4292	-3.3118	-1.5144	66
11	C122 H132 N O36	1093.4259	3.3908	1.5505	57.5
12	C121 H126 N8 O31	1093.4259	3.3962	1.553	63
13	C133 H3 N2 O35	1093.4252	4.6616	2.1316	133.5
14	C120 H130 N4 O35	1093.4252	4.7335	2.1645	58
15	C117 H128 N9 O33	1093.4301	-5.1571	-2.3582	58.5
16	C130 H N7 O33	1093.4302	-5.229	-2.3911	134
17	C131 H N5 O34	1093.4245	6.0043	2.7456	134
18	C118 H128 N7 O34	1093.4245	6.0762	2.7785	58.5
19	C119 H130 N6 O34	1093.4308	-6.4998	-2.9722	58

Fig. S13.MS Spectrum of Compound **9**

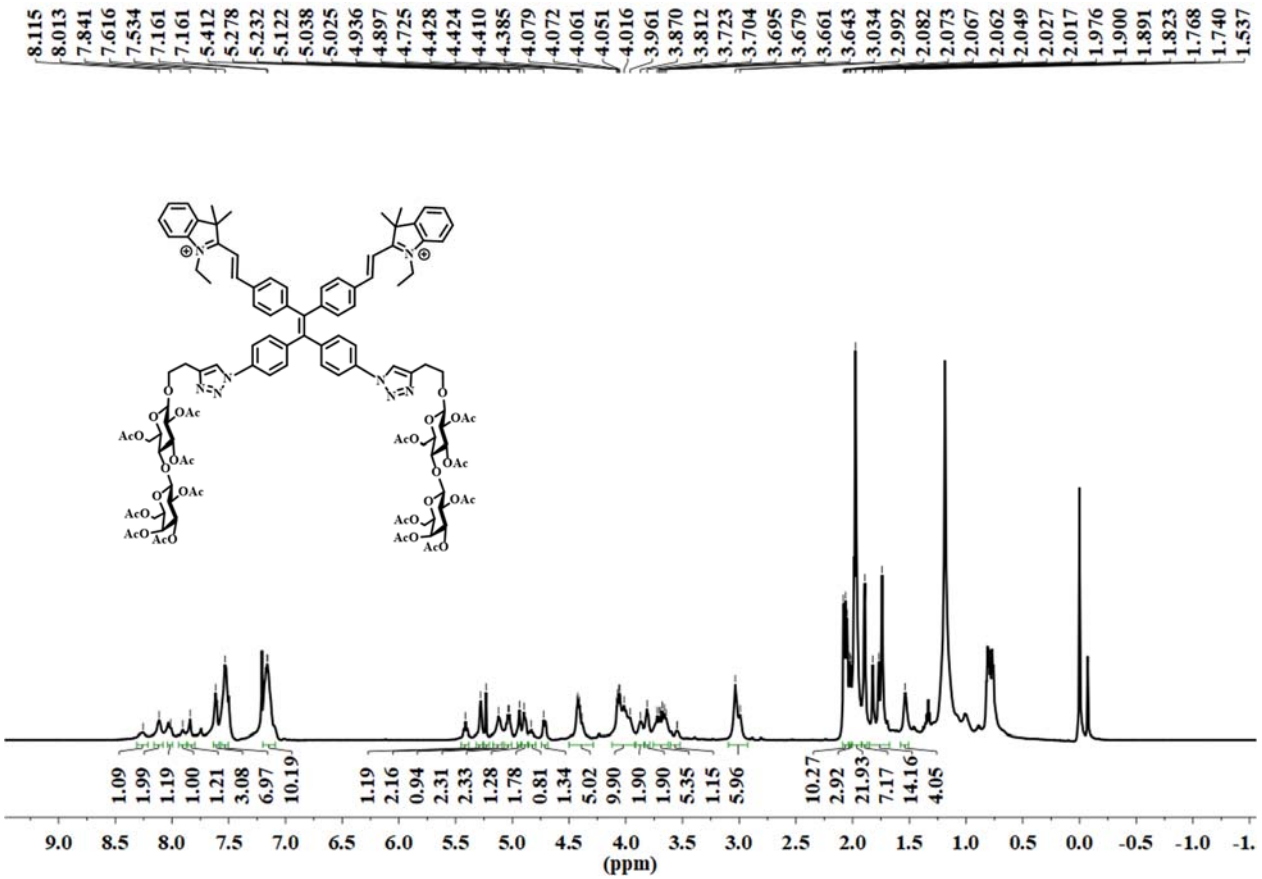


Fig. S14. ^{1}H NMR Spectrum of Compound **9**

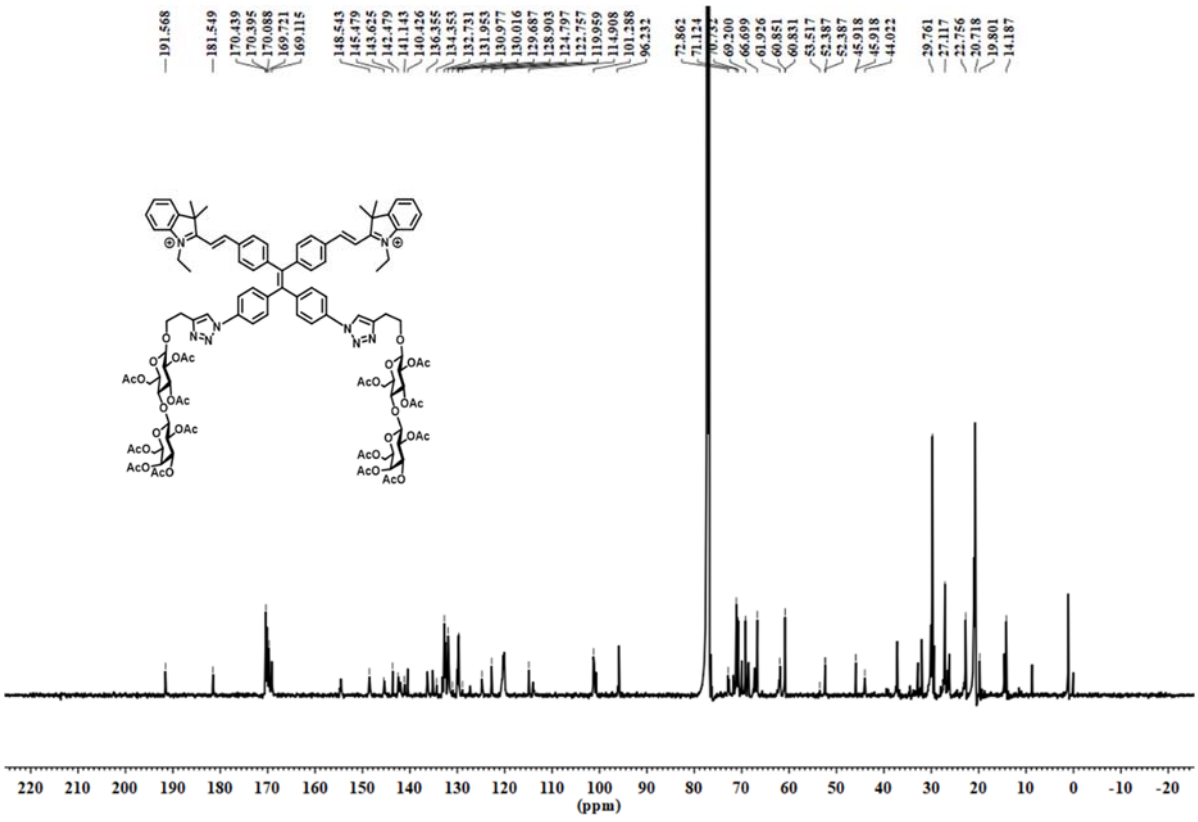
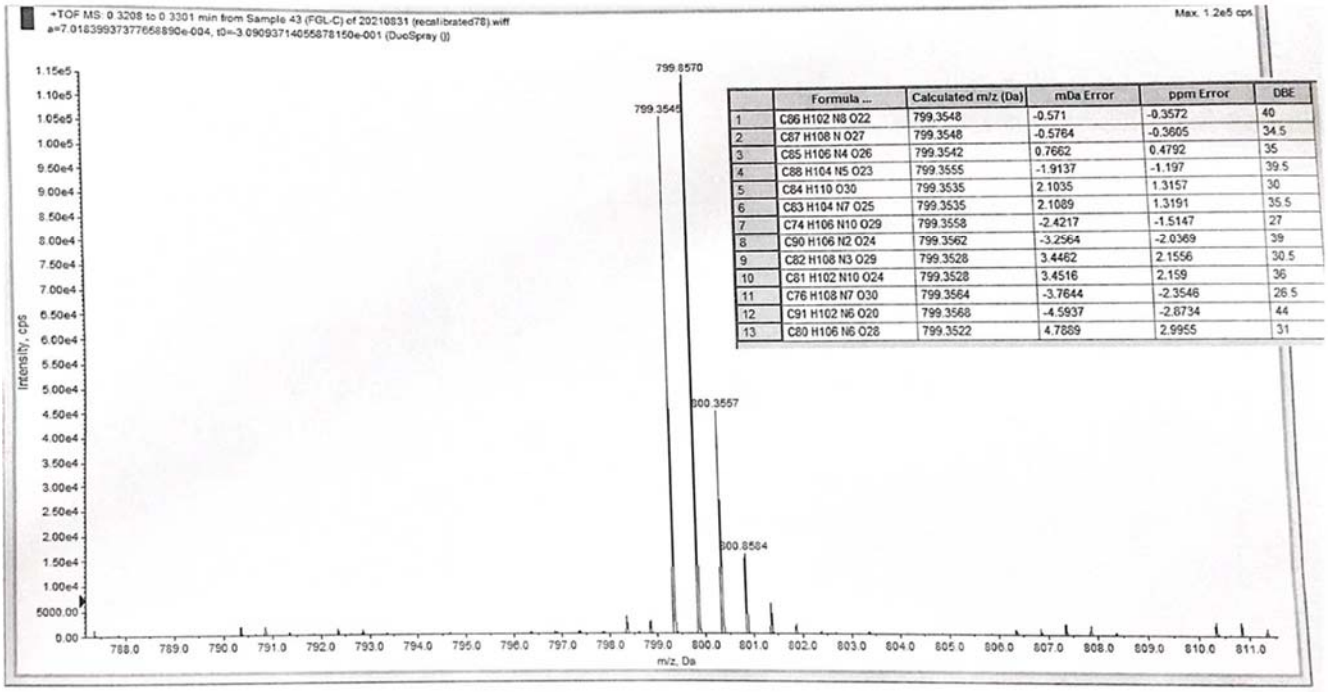


Fig. S15. ^{13}C NMR Spectrum of Compound **9**



	Formula ...	Calculated m/z (Da)	mDa Error	ppm Error	DBE
1	C86 H102 N8 O22	799.3548	-0.571	-0.3572	40
2	C87 H108 N O27	799.3548	-0.5764	-0.3605	34.5
3	C85 H106 N4 O26	799.3542	0.7662	0.4792	35
4	C88 H104 N5 O23	799.3555	-1.9137	-1.197	39.5
5	C84 H110 O30	799.3535	2.1035	1.3157	30
6	C83 H104 N7 O25	799.3535	2.1089	1.3191	35.5
7	C74 H106 N10 O29	799.3558	-2.4217	-1.5147	27
8	C90 H106 N2 O24	799.3562	-3.2564	-2.0369	39
9	C82 H108 N3 O29	799.3528	3.4462	2.1556	30.5
10	C81 H102 N10 O24	799.3528	3.4516	2.159	36
11	C76 H108 N7 O30	799.3564	-3.7644	-2.3546	26.5
12	C91 H102 N6 O20	799.3568	-4.5937	-2.8734	44
13	C80 H106 N6 O28	799.3522	4.7889	2.9955	31

Fig. S16.MS Spectrum of Compound **10**

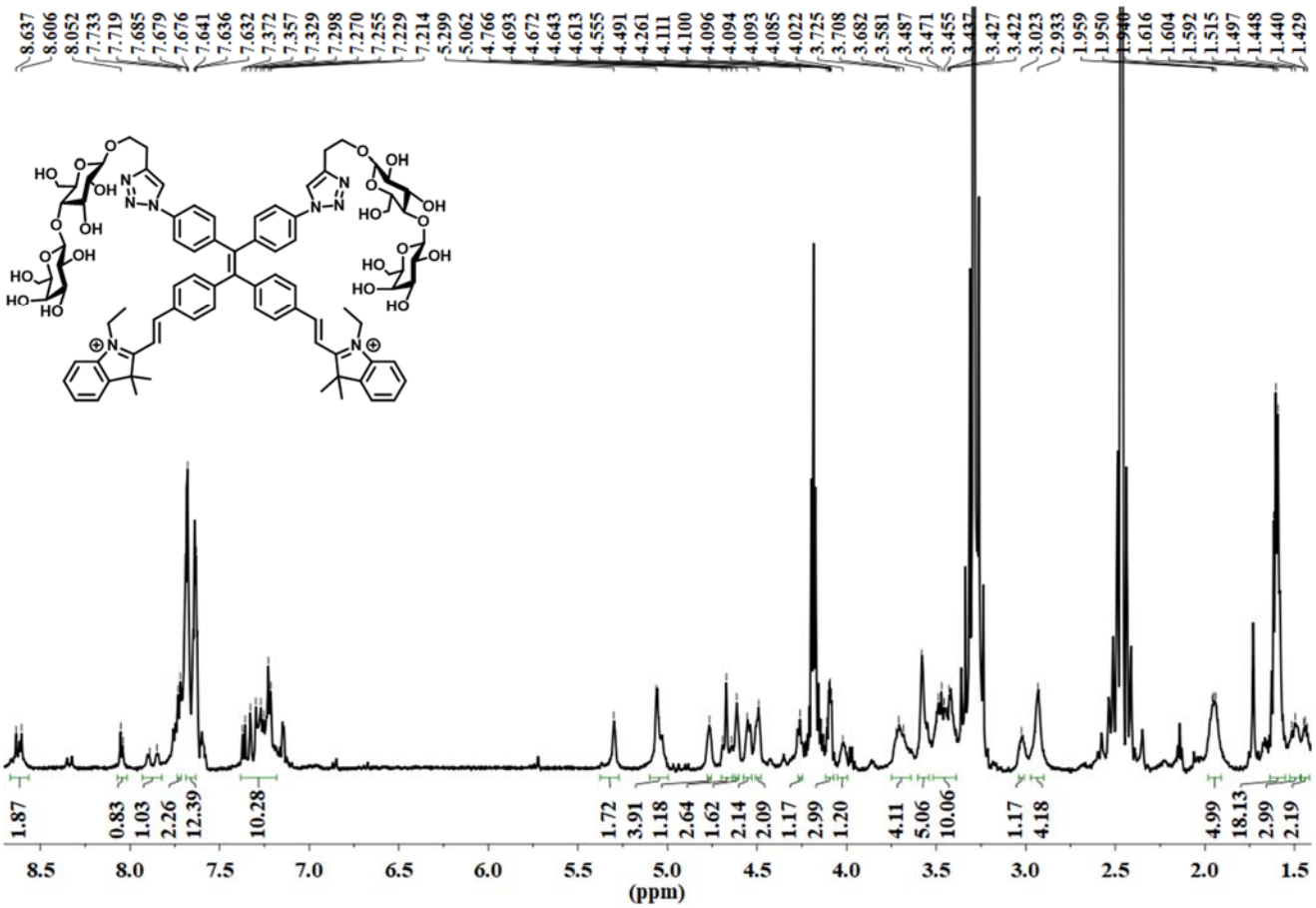


Fig. S17. [1]H NMR Spectrum of Compound **10**

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