# A viscosity-sensitive probe based on the structure of indole salt

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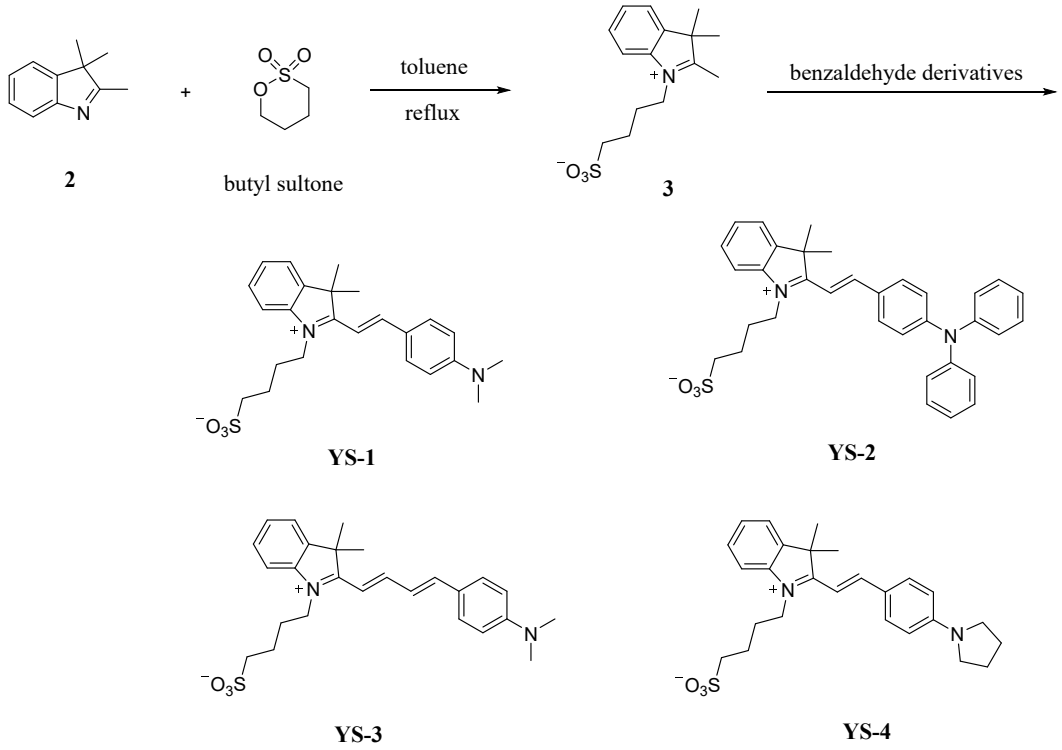
## General Information.

**Instruments**: $^{1}$H NMR and $^{13}$C NMR spectra were obtained using a BRAUNER ADVANCE III HD 600 MHz spectrometer. UV-vis absorption spectra measurements were performed on a FLUKE Ti95 spectrophotometer. Fluorescence emission spectroscopy measurements were performed on the Horiba Fluoromax-4 spectrofluorometer. High-resolution mass spectra (HRMS) of the compounds were collected on an Agilent liquid-phase high-resolution time-of-flight mass spectrometer (1260-6224). The fluorescence images were performed on the confocal microscope (Leica, TCS-SP8). Fluorescence emission/excitation spectra were measure done Fluorescence spectrometer (Fluoromax-4). Fluorescence quantum yield were measure done Steady State and Transient State Fluorescence Spectrometer (FluoroLog 3-TCSPC). The accumulation in vivo was real-time determined by In Vivo Imaging System (IVIS Lumina III, PerkinElmer).

**Materials:** All reagents used in the paper were purchased from commercial vendors, such as Adamas-beta Corporation, and were used without further purification. Column chro-matography was carried out using silica (200-300 mesh).

## Synthesis of probes.




**Scheme S1.** Synthesis of probes YSs

**Synthesis of compound 3** (*4-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)butane-1-sulfonate*). A 150 mL flask was added compound **2** (3.2 g, 20 mmol), butyl sultone (8.16 g, 60 mmol), 20 mL toluene stirring 5 min then heated to reflux for 14 h. After the reaction, mixtures cooled to room temperature, then the solvent was removed by evaporation under reduced pressure. The residue was dissolved in a small amount of methanol. Add the solution of methanol in ethyl acetate at vigorously stirred for 0.5 h. Following the mixture ultrasonic oscillation about 1.5 h, then filtration and vacumm durying to provide purple solid 3.54 g [1]. Yield 60%.

**Supplemental figures.**

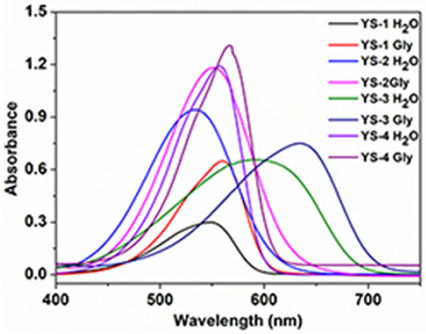



**Figure S1**. The UV-Vis spectra of **YS**s (10 μM) in $H_2O$ and glycerol.

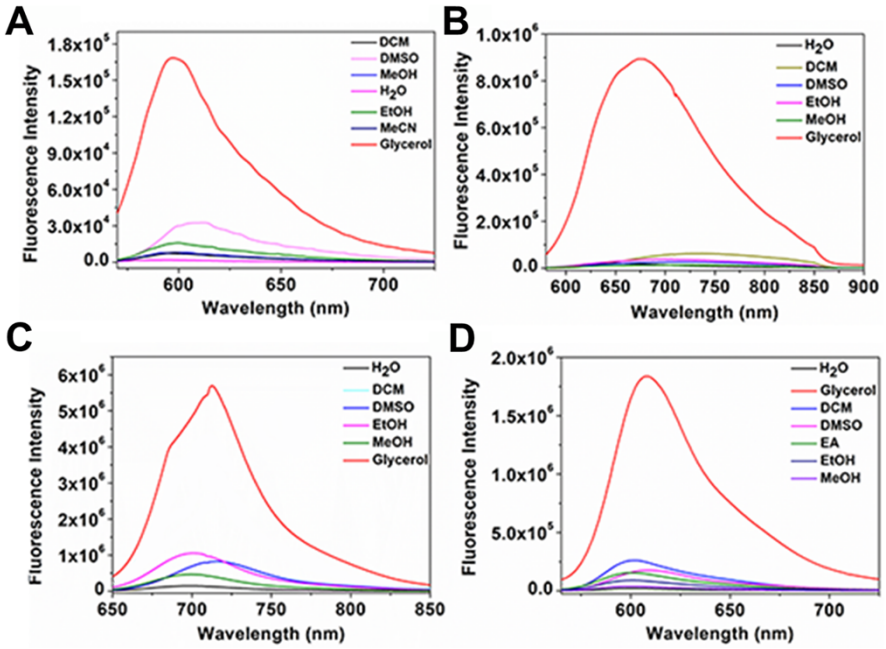



**Figure S2**. The fluorsecence spectra of A (**YS-1**, 10 μM, $\lambda_{ex}$ =560 nm), B (**YS-2**, 10 μM, $\lambda_{ex}$ =551 nm), C (**YS-3**, 10 μM, $\lambda_{ex}$ =634 nm) and D (**YS-4**, 10 μM, $\lambda_{ex}$ =567 nm) in different solvents (10 μM).

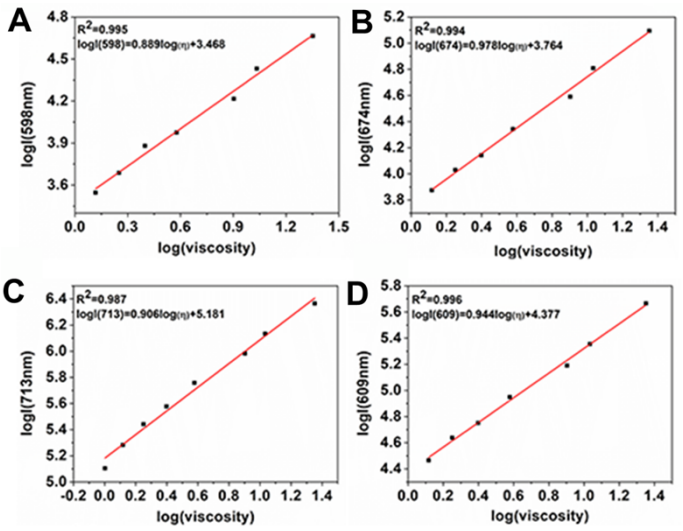



**Figure S3**. A (**YS-1**, 10 μM, $\lambda_{ex}$ =560 nm), B (**YS-2**, 10 μM, $\lambda_{ex}$ =551 nm), C (**YS-3**, 10 μM, $\lambda_{ex}$ =634 nm) and D (**YS-4**, 10 μM, $\lambda_{ex}$ =567 nm) Linearity between log *I* and log $\eta$ in different proportions of glycerol aqueous solution.

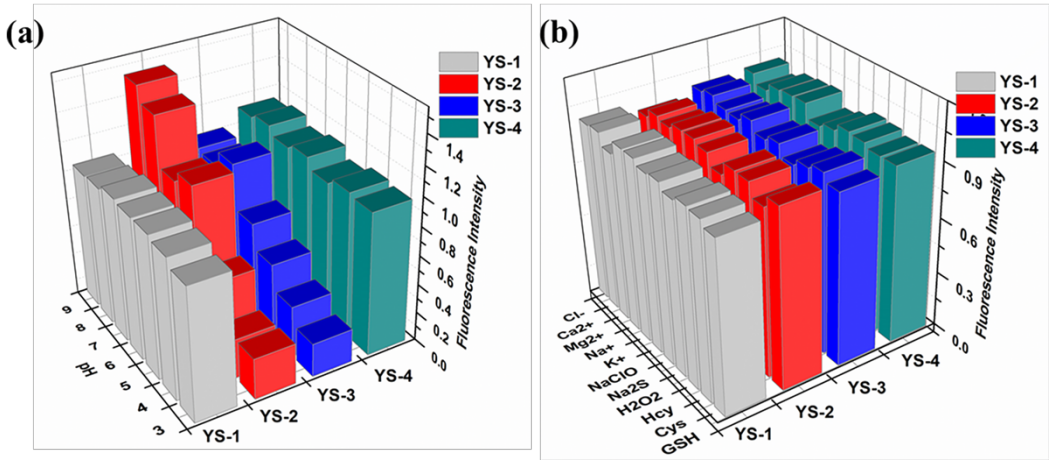



**Figure S4.** All probes (10 μM) tasted in aqueous solution. (a) The influences of pH on fluorescence; (b) The influences of bioactive molecules on fluorescence. GSH, Cys, Hcy, 200 μM; others 100 μM.

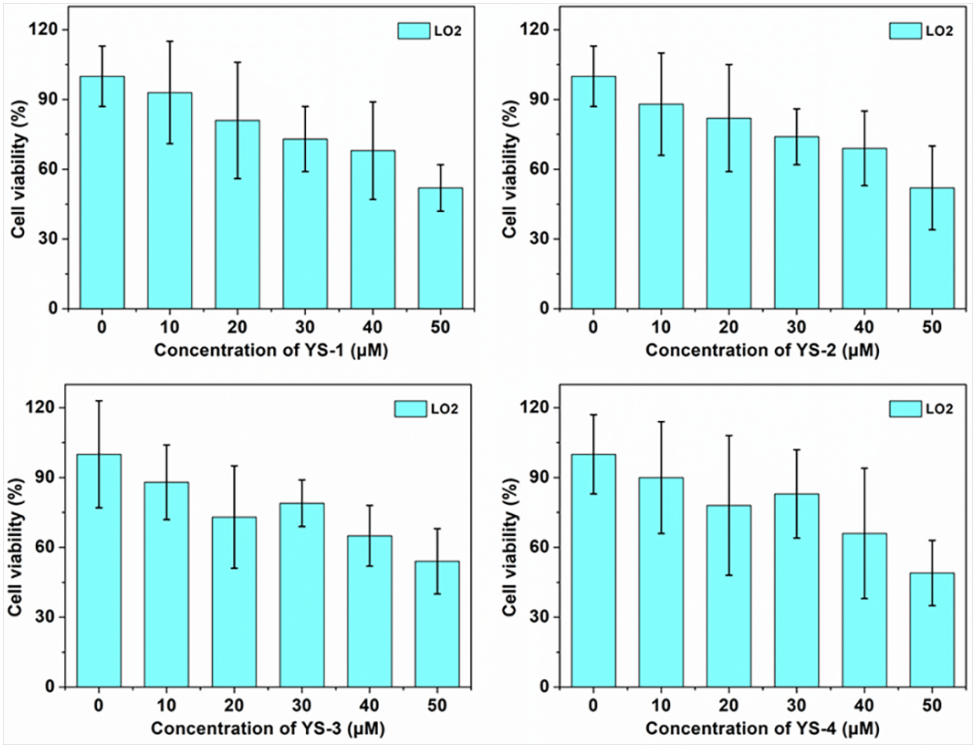



**Figure S5.** Cell cytotoxicity of **YS**s against LO2 for 24 h evaluated by MTT assay.

**Table S1.** Average fluorescence intensity of **YS-2** in cells.

| | Area | Mean | StdDev |
|---|---|---|---|
| MCF-7 | 0.02 | 35.128 | 33.039 |
| 4T1 | 0.278 | 12.747 | 7.083 |
| HepG2 | 0.044 | 38.015 | 28.639 |
| LO2 | 0.047 | 8.174 | 5.911 |

Area: range; Mean: average fluorescence intensity; StdDev: standard deviation.

**Table S2.** Average fluorescence intensity of **YS-3** in cells。

| | Area | Mean | StdDev |
|---|---|---|---|
| HeLa | 0.543 | 123.63 | 82.215 |
| MCF-7 | 0.015 | 65.271 | 21.455 |
| 4T1 | 0.188 | 27.963 | 8.411 |
| HepG2 | 0.057 | 74.861 | 19.486 |
| LO2 | 0.031 | 9.343 | 6.036 |

Area: range; Mean: average fluorescence intensity; StdDev: standard deviation.

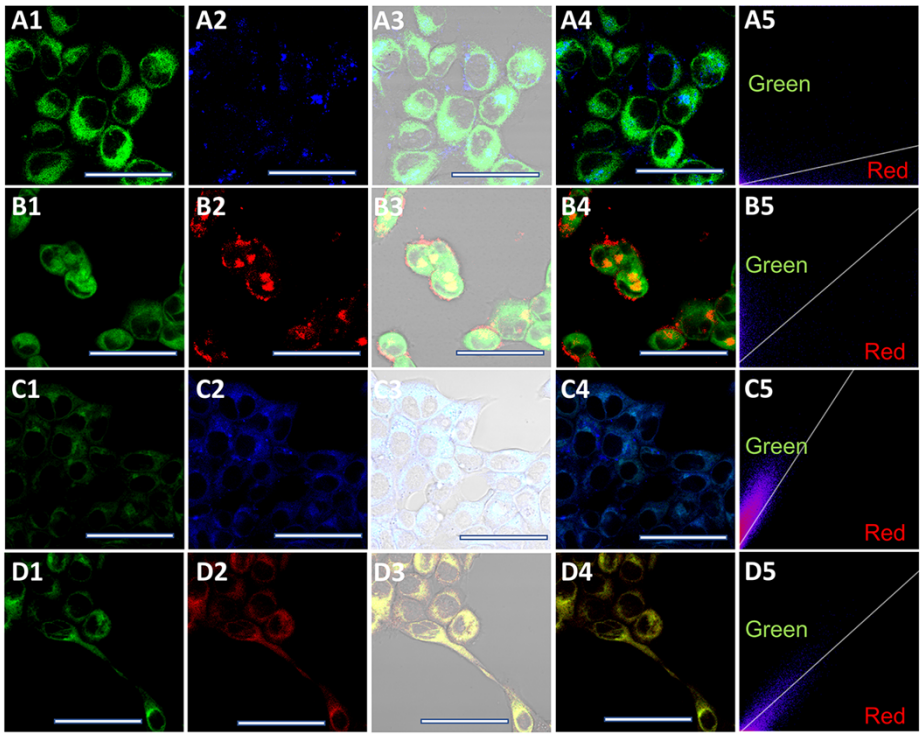



**Figure S6.** Samples were MCF-7 cells (A and B) and 4T1 cells (C and D). A1 - D1 ER-Tracker Green (100 nM, $\lambda_{ex}$ = 480 nm, $\lambda_{ex}$ = 500 nm); A2 and C2 **YS-2** (1 μM, $\lambda_{ex}$ = 560 nm, $\lambda_{ex}$ = 650 nm); B2 and D2 **YS-3** (1 μM, $\lambda_{ex}$ = 650 nm, $\lambda_{ex}$ = 750 nm); A3-D3 were the co-localization image in bright field; A4-D4 were the co-localization image in dark field; A5 Pearson's colocalization coefficient of **YS-2** and ER-Tracker Green, the Pearson's correlation factor is 0.28; B5 Pearson's colocalization coefficient of **YS-3** and Lyso-Tracker Green, the Pearson's correlation factor is 0.42; C5 Pearson's colocalization coefficient of **YS-2** and Mito-Tracker Green, the Pearson's correlation factor is 0.83; D5 Pearson's colocalization coefficient of **YS-3** and Lyso-Tracker Green, the Pearson's correlation factor is 0.92. The scale bar is 30 μm.

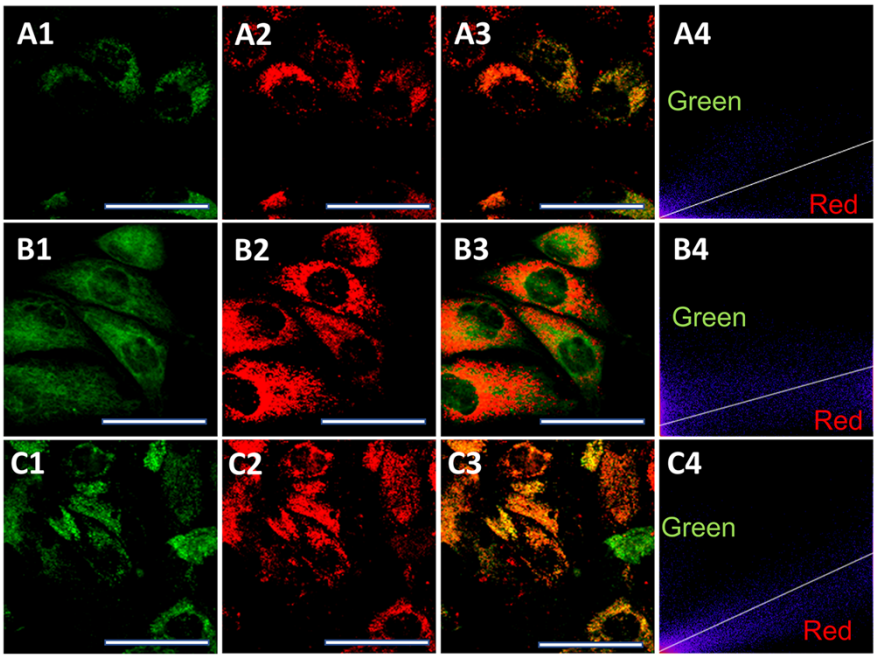
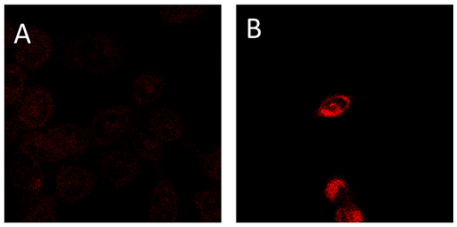



**Figure S7.** Samples were HepG2 cells. A1 Mito-Tracker Green (100 nM, $\lambda_{ex}$ = 480 nm, $\lambda_{ex}$ = 500 nm); B1 ER-Tracker Green (100 nM, $\lambda_{ex}$ = 480 nm, $\lambda_{ex}$ = 500 nm); C1 Lyso-Tracker Green (50 nM, $\lambda_{ex}$ = 480 nm, $\lambda_{ex}$ = 500 nm); A2 - C2 **YS-3** (0.5 μM, $\lambda_{ex}$ = 650 nm, $\lambda_{ex}$ = 750 nm); A3-C3 were the co-localization image; A4 Pearson's colocalization coefficient of **YS-3** and Mito-Tracker Green, the Pearson's correlation factor is 0.71; B4 Pearson's colocalization coefficient of **YS-3** and ER-Tracker Green, the Pearson's correlation factor is 0.58; C4 Pearson's colocalization coefficient of **YS-3** and Lyso-Tracker Green, the Pearson's correlation factor is 0.76. The scale bar is 30 μm.

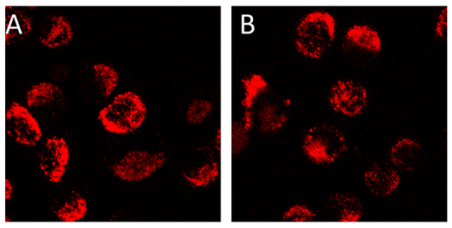



C

| | Area | Mean | StdDev |
|---|---|---|---|
| A | 0.041 | 7.944 | 6.425 |
| B | 0.04 | 36.842 | 23.538 |

**Figure S8.** A LO2 cells with **YS-3** (1 μM, 30 min, $\lambda_{ex}$ = 650 nm, $\lambda_{em}$ = 750 nm); B LO2 cells were cultured using antifungal drug nystatin (0.1 μM, 30 min) and subsequently stained with **YS-3** (1 μM, 30 min, $\lambda_{ex}$ = 650 nm, $\lambda_{em}$ = 750 nm); C The fluorescence intensity of A and B. Area: range; Mean: average fluorescence intensity; StdDev: standard deviation.

After incubating HeLa cells with the endocytosis inhibitor Ikarugamycin for 1 hour, wash them twice with PBS and stain with YS-3 (1 μM, λex = 650 nm, λem = 750 nm) for 30 minutes before capturing images.




C

| | Area | Mean | StdDev |
|---|---|---|---|
| A | 0.253 | 119.24 | 26.371 |
| B | 0.211 | 114.94 | 35.716 |

Figure S9. A Without Ikarugamycin; B Ikarugamycin (20 nM); C Average fluorescence intensity of A and B. Area: range; Mean: average fluorescence intensity; StdDev: standard deviation.

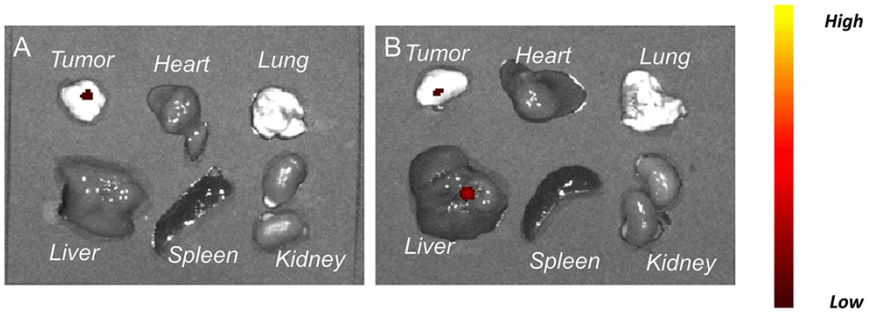



**Figure S10.** The fluorescence imaging of organs and tumors (A, **YS-2**, $\lambda_{ex}$ = 550 nm, $\lambda_{em}$ = 680 nm; B, **YS-3**, $\lambda_{ex}$ = 640 nm, $\lambda_{em}$ = 720 nm).

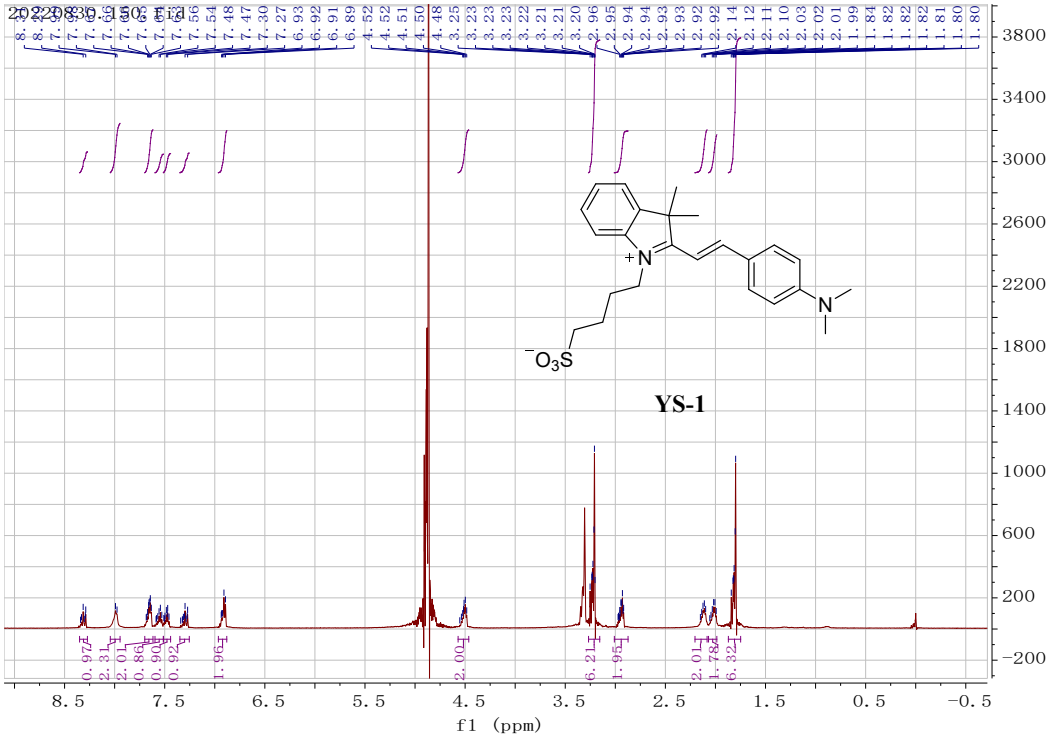



**Figure S11.** $^{1}$H NMR spectrum of **YS-1** (Methanol-$d_4$)

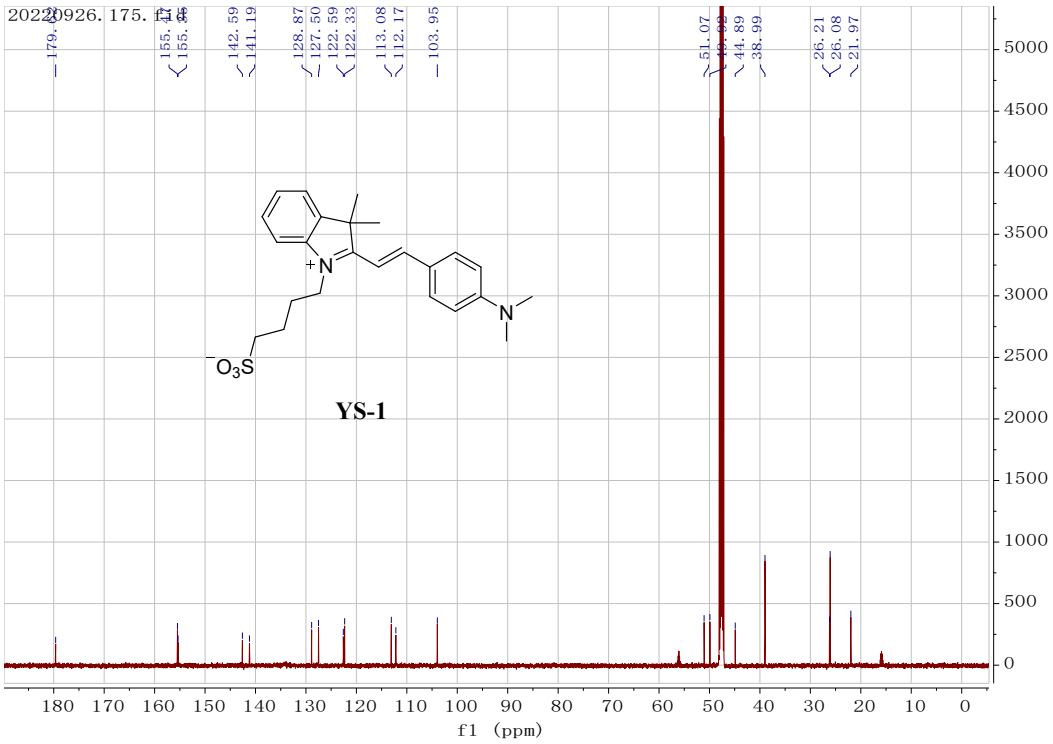



**Figure S12.** $^{13}$C NMR spectrum of **YS-1** (Mechanol-$d_4$)

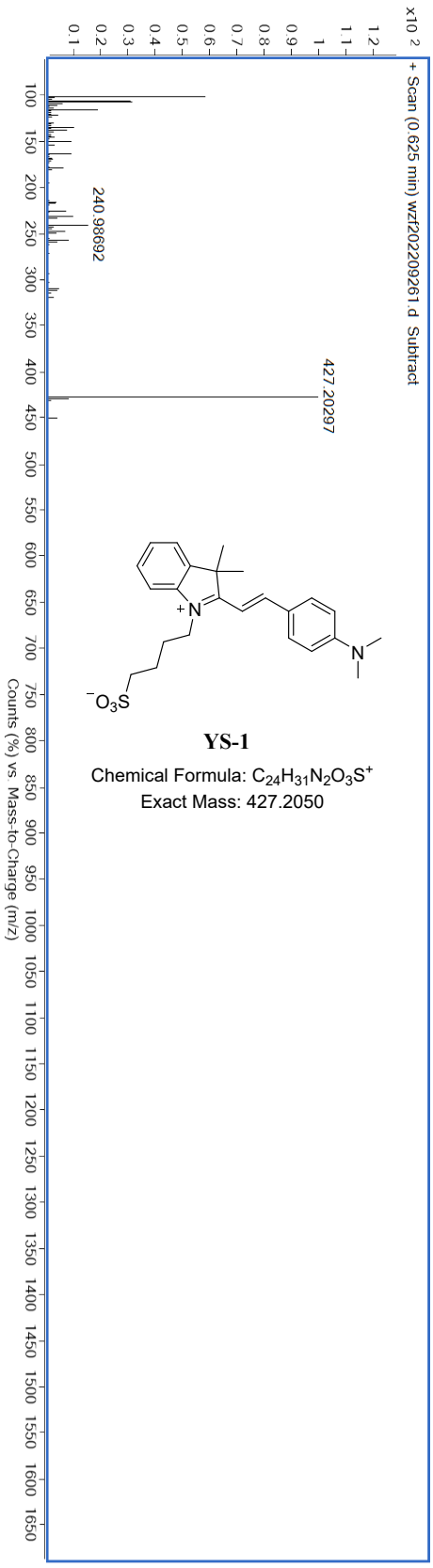



**Figure S13.** HR MS spectra of **YS-1**.

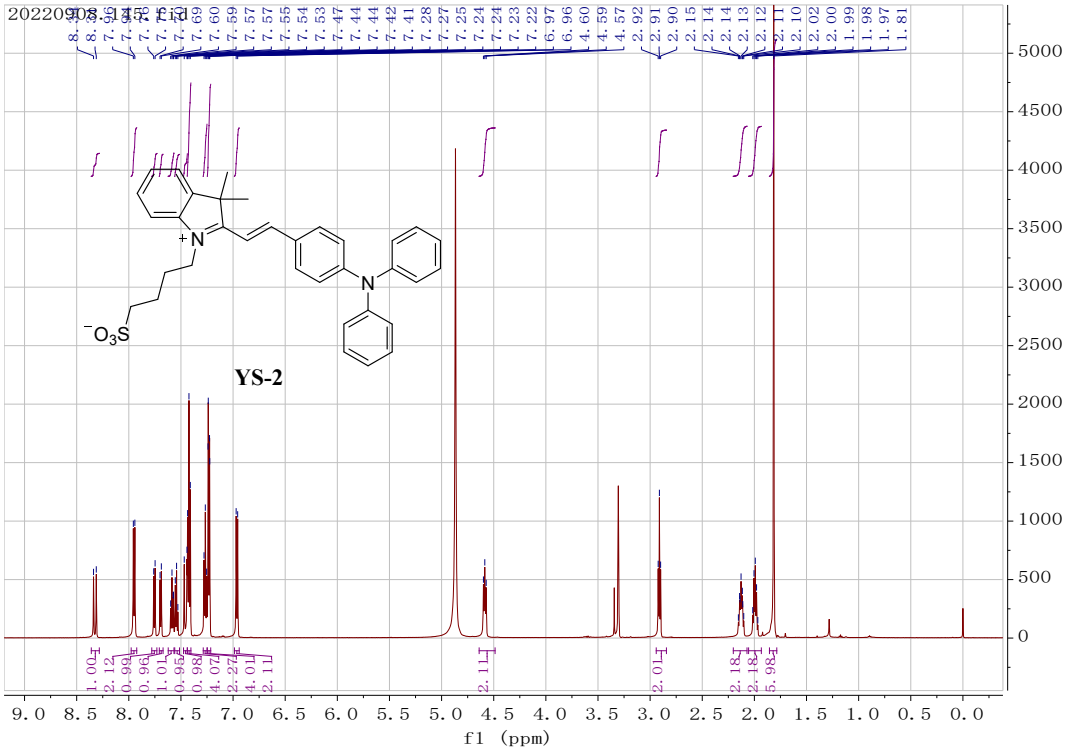



**Figure S14.** $^{1}$H NMR spectrum of **YS-2** (Mechanol-$d_4$).

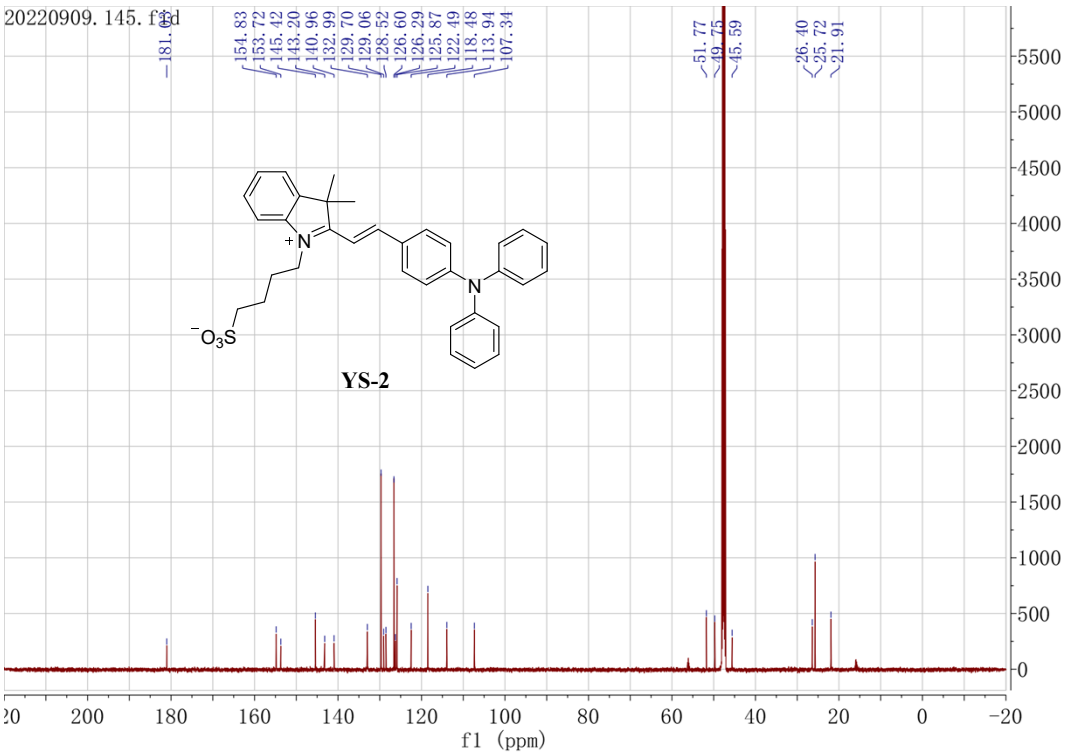



**Figure S15.** $^{13}$C NMR spectrum of **YS-2** (Mechanol-$d_6$)

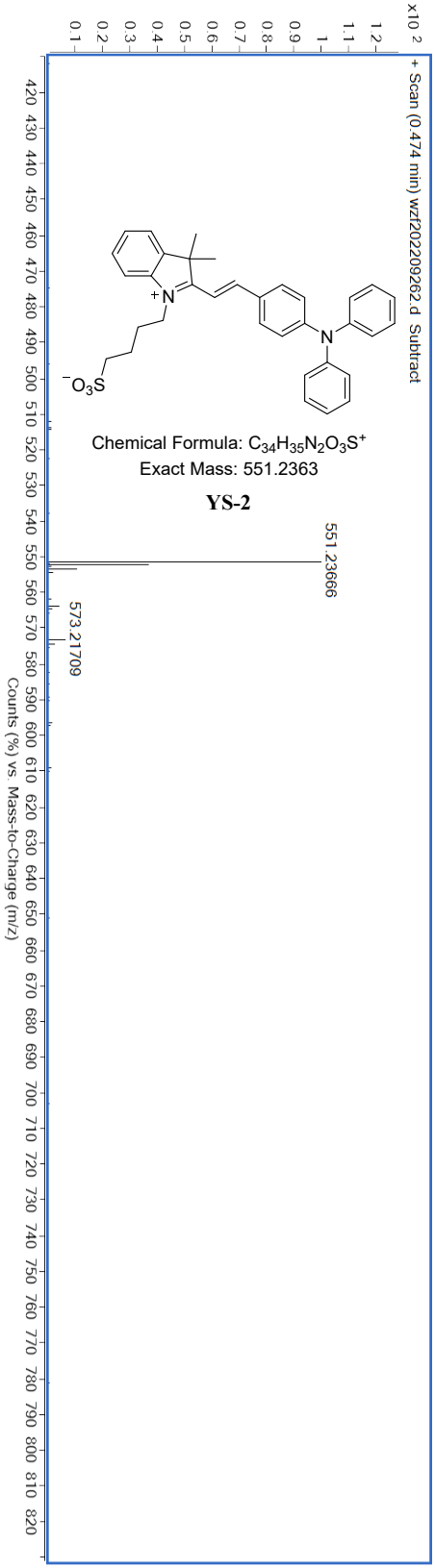



**Figure S16.** HR MS spectra of **YS-2**.

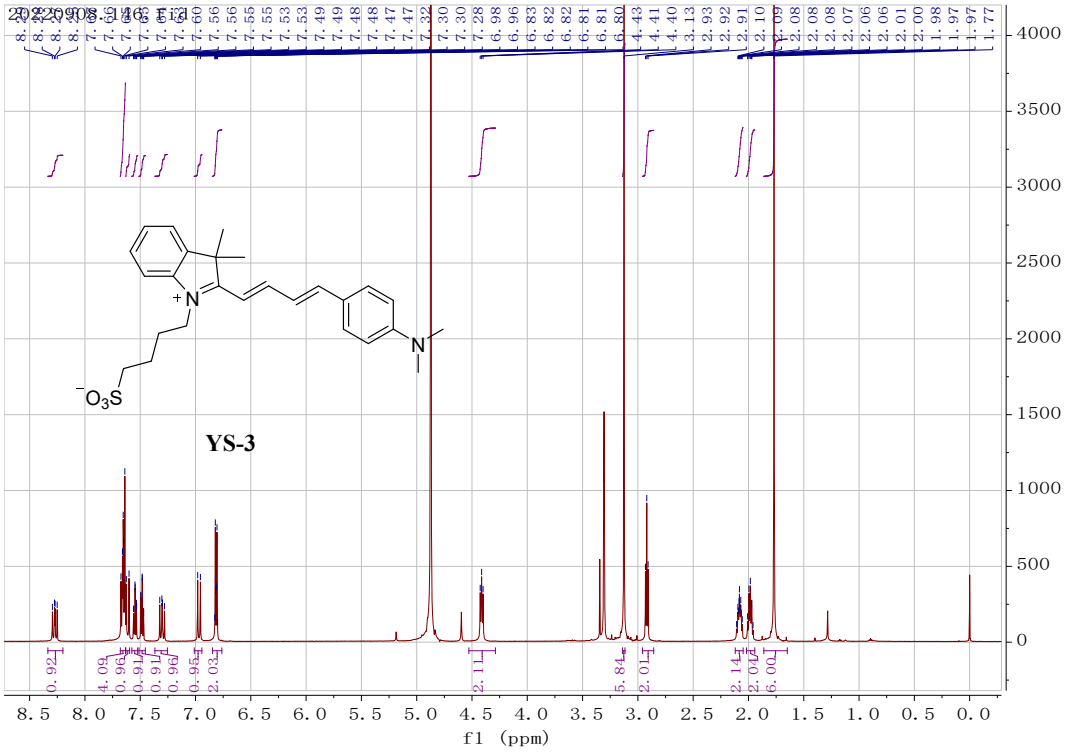



**Figure S17.** $^{1}$H NMR spectrum of **YS-3** (Methanol-$d_4$).

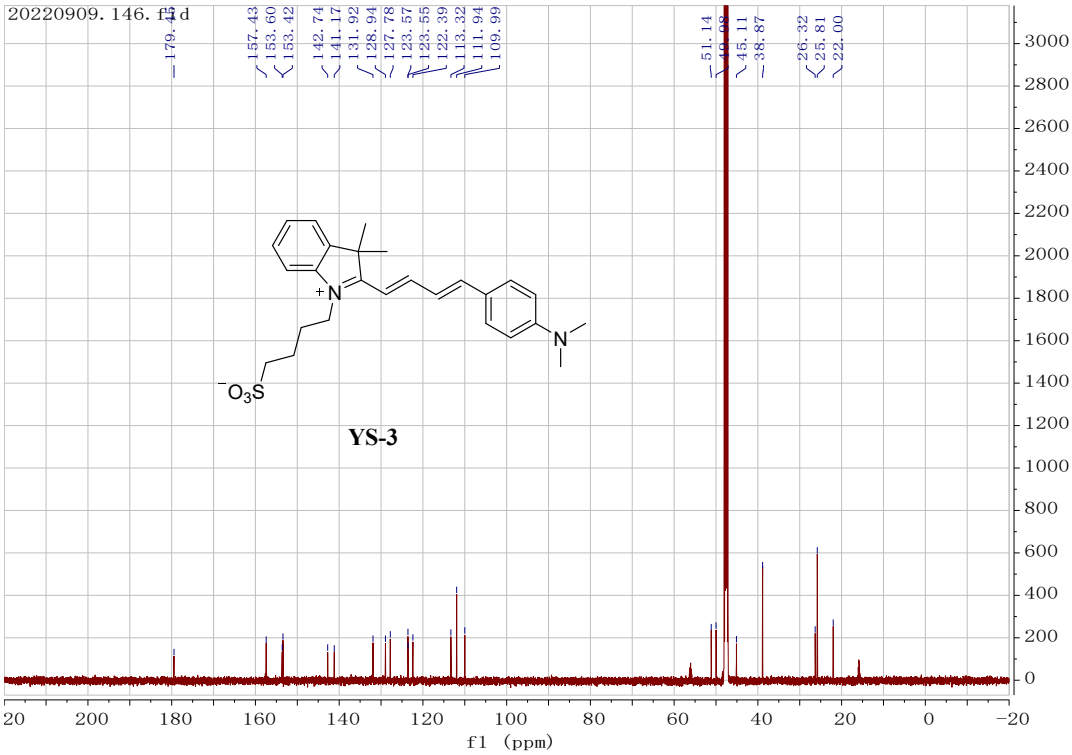



**Figure S18.** $^{13}$C NMR spectrum of **YS-3** (Methanol-$d_4$).

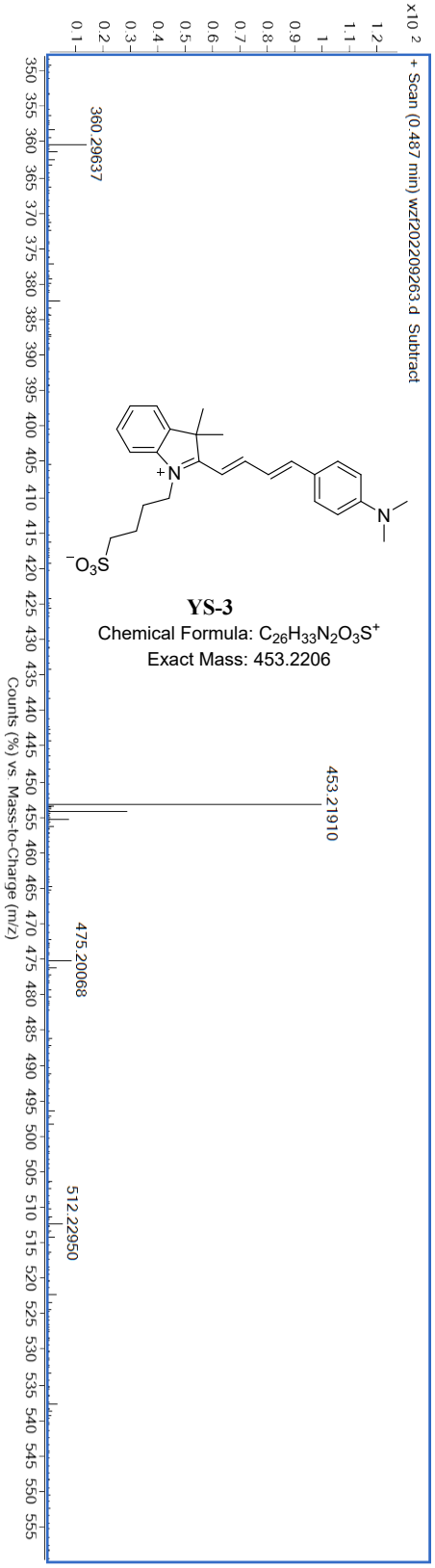



**Figure S19.** HR MS spectra of **YS-3**.

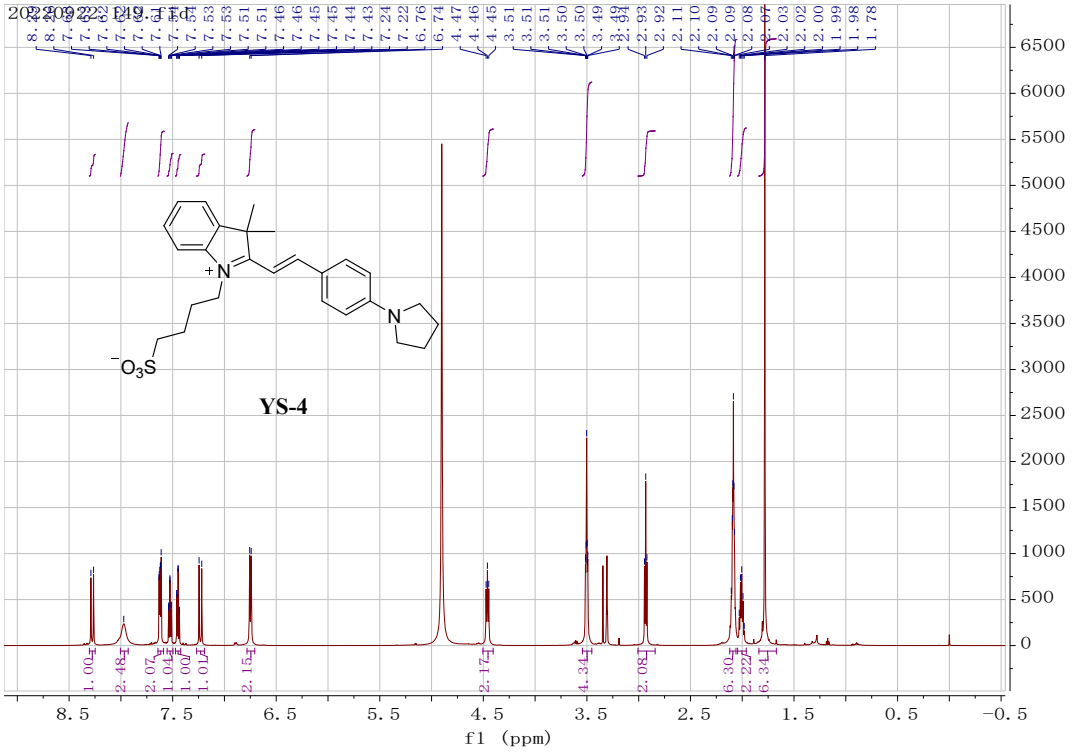



**Figure S20.** $^{1}$H NMR spectrum of **YS-4** (Methanol-$d_4$).

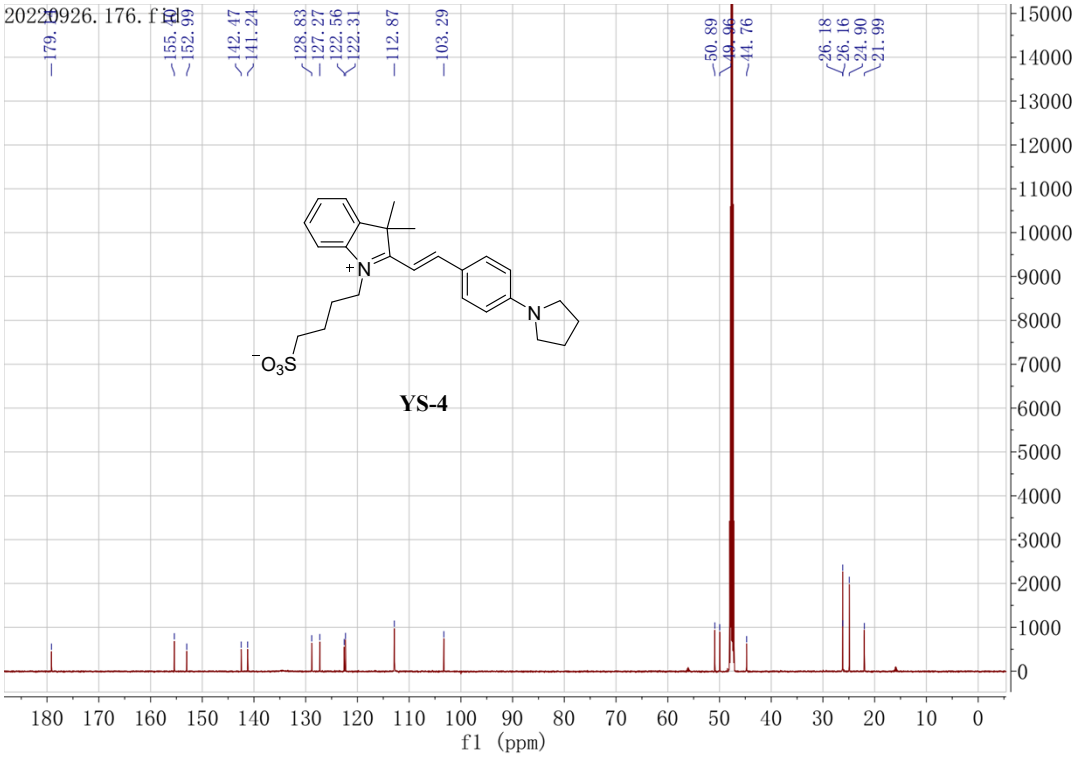



**Figure S21.** $^{13}$C NMR spectrum of **YS-4** (Methanol-$d_4$).

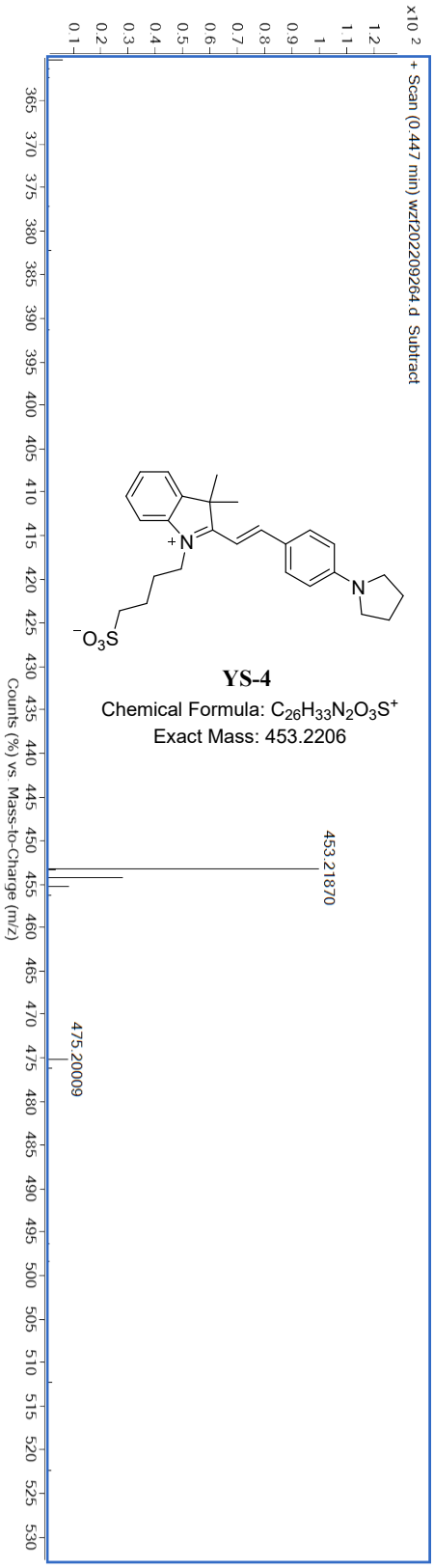



**Figure S22.** HR MS spectra of **YS-4**.

## References

[1] Narayanan, N.; Patonay, G. A New Method for the Synthesis of Heptamethine Cyanine Dyes: Synthesis of New Near-Infrared Fluorescent Labels. *J. Org. Chem.* **1995**, 60, 2391-2395.