Supporting Information

An Activatable Endoplasmic Reticulum-Targeted Probe For NIR Imaging-Guided Photothermal Therapy

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1. General

The chemical reagents and solvents required for the experiment were mainly purchased from commercial suppliers (including Sigma Aldrich, Adamas, TCI, Aladdin, J&K, etc.). Unless otherwise specified, the reagents can be used directly without purification. All solvents and reagents used were of analytical grade (A.R.). The characterization and testing instruments used in the experiment mainly include High Resolution Mass Spectrometer (Waters LCT Premier), Nuclear Magnetic Resonance Spectrometer (Bruker AM 400; TMS as internal standard), Ultraviolet Visible Spectrophotometer (Varian Cary 100), Fluorescence Spectrophotometer (Varian Cary Eclipse), pH meter (Mettler Toledo FE20), Thermostatic Shaker (Chem Star XHZ-032), Quartz Colorimeter (3 Ml), 808 nm Laser (MDL-III-690 nm-1 W-18071414), Laser confocal microscope (Carl Zeiss LSM710), carbon dioxide constant temperature incubator (Thermo), enzyme marker (Thermo), etc.

2. Synthesis

BOD-COOH, compound 1, compound 2, and compound 3 were prepared according to previously reported procedures. [1-4]

Synthesis and Characterization of Compound Ts-BOD: BOD-COOH (500 mg, 1.25 mmol) and HATU (955 mg, 7.52 mmol) were dissolved in anhydrous dichloromethane (20 mL). Then add compound 1 (538 mg, 2.51 mmol) after 10 min and add DIPEA (0.89 mL, 5.11 mmol). The system is then stirred overnight at room temperature The solvent was distilled off under reduced pressure. The residue was purified by column chromatography to get Ts-BOD that was a yellow solid (565 mg, 76%).^{1}H NMR (400 MHz, DMSO-d_6, δ) 8.18 (t, 1H), 7.68 (d, 3H), 7.38 (d, 2H), 7.27 (d, 2H), 7.12 (d, 2H), 6.17 (s, 2H), 4.49 (s, 2H), 3.19 (dd, 2H), 2.79 (dd, 2H), 2.45 (s, 6H), 2.36 (s, 3H),1.36 (s, 6H).13C NMR (101 MHz, DMSO-d_6, δ) 167.96, 158.58, 155.17, 143.13, 142.41, 137.89, 131.52, 130.11, 129.60, 126.99, 121.76, 115.94, 67.34, 42.25, 38.68, 21.39, 14.62. HRMS (ESI, m/z): calculated for $C_{30}H_{33}BF_2N_4O_4S$ $[M+H]^+$:595.2356, found: 595.2358.

Synthesis and Characterization of Compound Ts-BOD-N: Ts-BOD (200 mg, 0.34 mmol) was dissolved in toluene (15 mL), followed by the addition of piperidine (100 μL), glacial acetic acid (100 μL) and Compound 2 (280 mg, 1.70 mmol), and the mixture was refluxed at 100 ℃ for 12 h. The solvent was distilled off under reduced pressure. The residue was purified by column chromatography to afford Ts-BOD-N that was a green solid (160 mg, 54%).^{1}H NMR (400 MHz,

DMSO-d_6, δ) 8.19 (t, 1H), 7.69 (d, 3H), 7.41 (t, 4H), 7.13 (t, 4H), 7.02 (s, 2H), 6.85 (s, 2H), 6.54 (d, 2H), 5.77 (d, 2H), 4.50 (s, 2H), 3.88 (s, 6H), 3.21 (dd, 2H), 2.78 (d, 8H), 2.36 (s, 3H), 1.42 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6, δ) 170.82, 168.02, 158.39, 152.50, 146.90, 143.15, 141.69, 140.80, 138.16, 137.89, 135.99, 135.99, 132.99, 130.40, 130.13, 127.82, 127.00, 124.14, 123.31, 117.97, 115.69,113.39, 108.77, 107.79, 67.36, 60.23, 55.67, 42.26, 38.68, 29.90, 21.41, 21.23, 14.85, 14.55. HRMS (ESI, m/z): calculated for $C_{49}H_{52}BF_2N_5O_6S$ $[M-H]^-$:887.3579, found: 887.3580.

Synthesis and Characterization of Compound Ts-BOD-B: Compound 3 (30 mg, 1.80 mmol) was dissolved in anhydrous dichloromethane (5 mL) and Ts-BOD-N (40 mg, 0.30 mmol) was added at room temperature. The reaction mixture was stirred for 4 h at room temperature and partitioned between H_2O and dichloromethane. The separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by a silica gel column chromatography to afford Ts-BOD-B that was a blue solid (27.3 mg, 43%).^{1}H NMR (400 MHz, DMSO-d_6, δ) 8.20 (t, 1H), 7.66 (m, 7H), 7.55 (m, 4H), 7.36 (m, 7H), 7.27 (d, 7H), 7.15 (d, 2H), 6.97 (s, 2H), 5.08 (s, 4H), 4.52 (s, 2H), 3.86 (s, 6H), 3.21 (dd, 2H), 3.12 (s, 6H), 2.79 (dd, 2H), 2.36 (s, 3H), 1.46 (s, 6H), 1.26 (s, 24H). ^{13}C NMR (151 MHz, DMSO-d_6, δ) 167.98, 158.66, 155.39, 152.30, 143.15, 142.56, 140.79, 139.90, 137.89, 137.06, 136.93, 134.90, 133.66, 130.13, 130.05, 127.14, 127.00, 126.90, 119.66, 119.17, 118.98, 115.92, 84.10, 67.36, 66.53, 56.00, 42.26, 38.68, 37.54, 25.09, 21.41, 14.93. HRMS (ESI, m/z): calculated for $C_{76}H_{85}B_3F_2N_6O_{14}S$ $[M+Na]^+$: 1431.5984, Found 1431.5997.

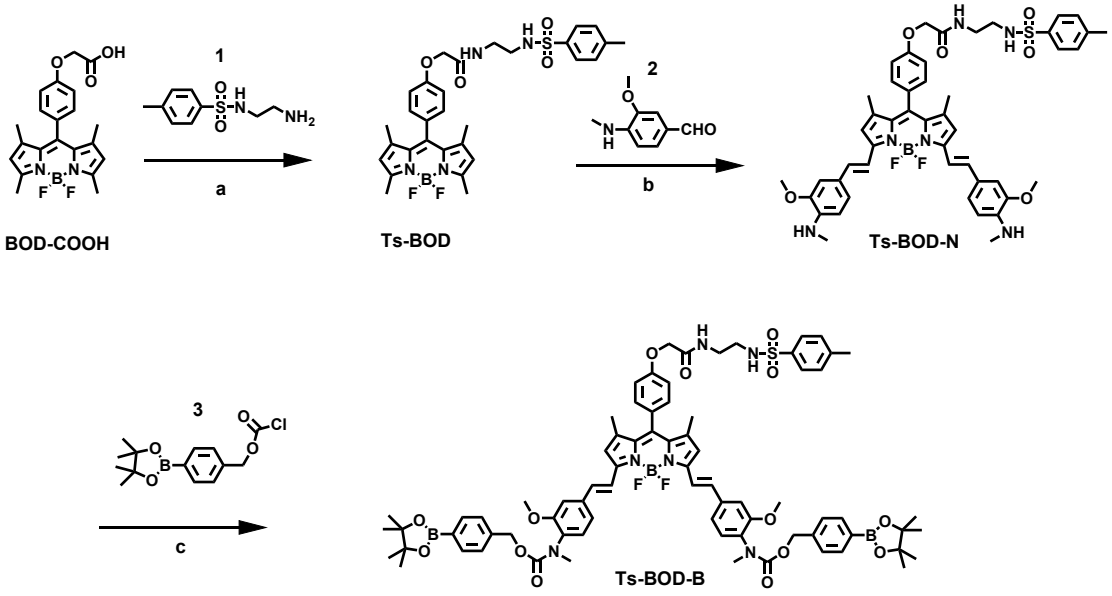

Fig. S1. The synthetic route for Ts-BOD-B. Reagents: (a) HATU, DIPEA, dichloromethane; (b)

piperidine, glacial acetic acid, toluene; (c) TEA, dichloromethane.

3. Photothermal conversion efficiency (PCE)

Ts-BOD-B (40 μM) activated by H_2O_2 (300 μM) exposed to 2.0 W/cm^2 at 690 nm for 10 min, then removed the laser and the solution was cooled down to room temperature.[5] (Note: Recording the the temperature of the heating and cooling circle every 30 seconds). The calculation method of photothermal conversion efficiency is as follows:

$$\theta = \frac{T - T_{\text{surr}}}{T_{max} - T_{surr}} \tag{1}$$

T corresponds to the temperature with different irradiation times, T_{max} is the highest temperature of the test system after 10 minutes of laser irradiation, and T_{surr} is the initial temperature of the test system. The ϑ corresponding to the driving temperature was obtained from the equation (1).

$$t = -\tau_s \ln \theta \tag{2}$$

$$hs = \frac{mC}{\tau_s} \tag{3}$$

The *t* represents time period corresponding to the cooling of the system after laser irradiation. The *m* is the mass of the sample solution, and C is the heat capacity of the solution. The τ_s is the relative time constant of cooling period determined by equation (2).

$$\eta = \frac{hs(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A})} \tag{4}$$

The *h* represents the heat transfer coefficient and *s* represents the surface area of the container. The value of *hs* is calculated according to equation (3), Q_{dis} is the heat dissipated from the laser by the solvent and container. The *I* represents the laser power and *A* is the absorbance at 690 nm.

4. Cell lines and culture conditions

The human non small cell lung (A549) cancer cells line, human embryonic kidney (HEK-293T) cells line and mouse breast (4T1) cancer cells was purchased from the Institute of Cell Biology (Shanghai, China). A549 cells and normal HEK-293T cells were cultured in Dulbecco's Modified Eagle Medium (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10 % fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1 % penicillin-streptomycin (10,000 U mL^{-1} penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing,

China) in a sterile incubator with 5% CO_2 and 37 ℃. Mouse breast cancer cells (4T1) were cultured in Roswell Park Memorial Institute (RPMI-1640) medium (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10 % fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1 % penicillin-streptomycin (10,000 U mL^{-1} penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing, China) in a sterile incubator with 5% CO_2 and 37 ℃.

5. Cytotoxicity detection

For the dark cytotoxicity and phototoxicity assay, A549 cells, 4T1 cells and HEK-293T cells were seeded in six 96-well plates (2.0×10^4 cells per well) and incubated for 24 h. After observing the stability of cell adhesion under the microscope, one group of A549 cells, 4T1 cells and HEK-293T cells were incubated with various concentrations of Ts-BOD-B for 24 h without laser light, while the other group of A549 cells, 4T1 cells and HEK-293T cells were incubated with various concentrations of Ts-BOD-B for 8 h with laser light (690 nm, 2.0 W cm^{-2}) for 10 min. Then the cells were incubated for another 12 h and carefully washed with PBS three times, and the medium (100 μL) containing 10% CCK-8 was added to each well and cultured for 2 h under the same conditions. The absorption spectrum at 450 nm was measured by a microplate reader, and the cell survival rate was calculated by Equation (5)

$$Cells\ viability\ (\%) = \frac{A - A_{blank}}{A_0 - A_{blank}} \times 100\% \quad (5)$$

In the above equation, A represents the experimental groups, A_0 represents the control groups, A_{blank} represents the blank groups.

6. Calcein-AM/PI staining experiment.

For examination of cell death induced by Ts-BOD-B mediated PTT, four groups of experiments were conducted: (1) untreated cells; (2) cells were incubated with Ts-BOD-B (40 μM) for 8 h; (3) cells were irradiated with laser (690 nm, 2.0 W cm^{-2}) for 10 mins; (4) cells were incubated with Ts-BOD-B (40 μM) for 8 h and then exposed to laser (690 nm, 2.0 W cm^{-2}) for 10 mins; After treatment, the four groups of cells were placed in the sterile incubator again for 12 hours. Then the incubated cells were stained (5 μM) calcein acetoxymethyl ester (AM) and (10 μM) propidium iodide (PI) staining reagents for 30 min at 37 °C, the four groups of cells were washed three times in PBS and imaging experiments were performed using a Carl Zeiss LSM710 instrument with a 20x microscope.

The excitation wavelengths of Calcein-AM and PI were 488 nm and 561 nm, respectively. And the emission wavelength range was collected between 490-520 nm and 580-640 nm for Calcein-AM and PI, respectively.

7. Subcellular localization analysis

A549 cells and 4T1 cells were planted and incubated in confocal culture plates for 24 hours for adherence. Following cultivation, the cells were incubated with probe Ts-BOD-B (40 μM) for 8 hours, and then washed with HBSS three times. Then the cells were cocultured with commercial endoplasmic reticulum targeted probe (ER Tracker Green, 1 μM) for 30 minutes, and then washed with HBSS at least three times. Subsequently, imaging was performed using Carl Zeiss LSM710 instrument with a 60x oil mirror. The excitation wavelengths of Ts-BOD-B and ER Tracker Green were 633 nm and 488 nm, respectively. And the emission wavelength range was collected between 740-800 nm and 500-540 nm, respectively.

8. the vitro NIR fluorescence imaging

A549 cells were planted and incubated in confocal culture plates for 24 hours for adherence. Subsequently, three groups of experiments were conducted: (1) A549 cells were incubated with Ts-BOD-B (40 μM) for 8 hours; (2) A549 cells were incubated with 2 μg/mL LPS for 12 hours in advance, and then incubated with probe Ts-BOD-B (40 μM) for 8 hours; (3) A549 cells were incubated with 10 mM NAC for 1 hour in advance, and then incubated with probe Ts-BOD-B (40 μM) for 8 hours. All confocal imaging experiments were performed using a Carl Zeiss LSM710 instrument with an excitation light wavelength of 633 nm. And the emission wavelength range was collected between 740-800 nm.

9. Supplementary figures

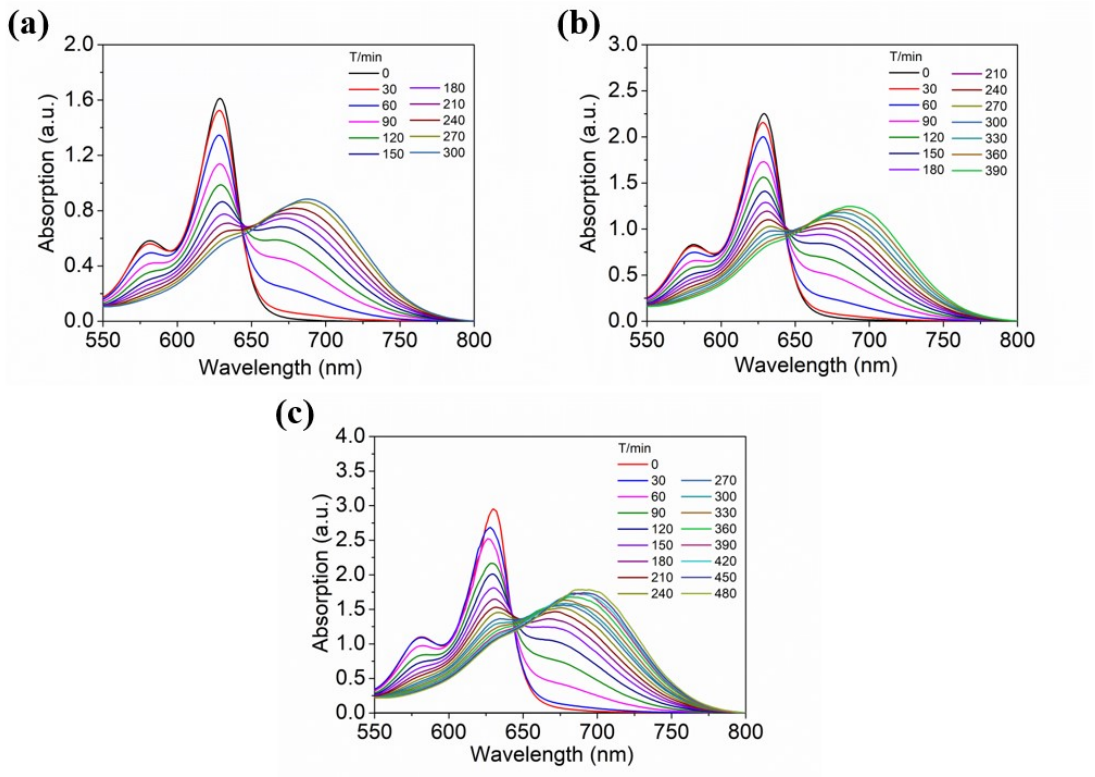

Fig. S2. Time-dependent absorption changes of Ts-BOD-B in the presence of 300 μM H_2O_2. (a) 20 μM, (b) 30 μM, (c) 40 μM.

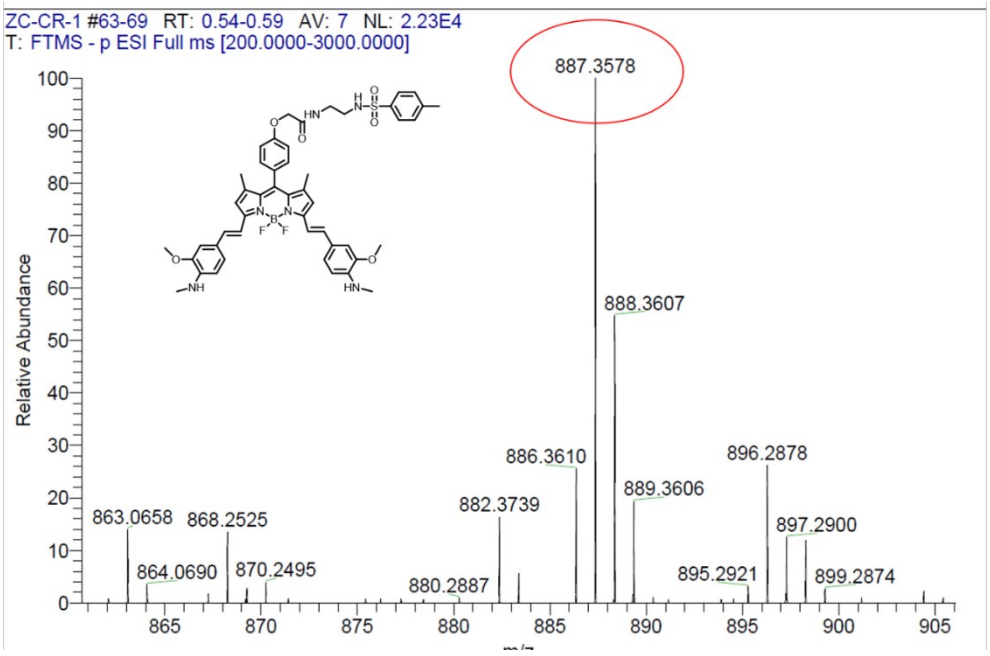

Fig. S3. HRMS spectrum of the products from the reaction of Ts-BOD-B with H_2O_2

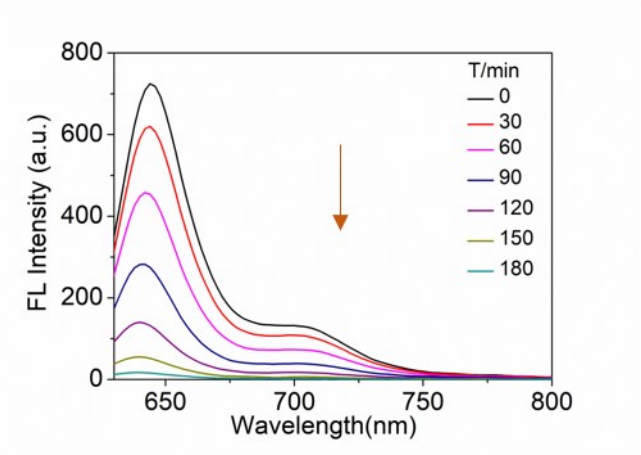

Fig. S4. Emission spectra changes of Ts-BOD-B (10 μM) in the presence of 300 μM H_2O_2. λ_{ex} = 620 nm.

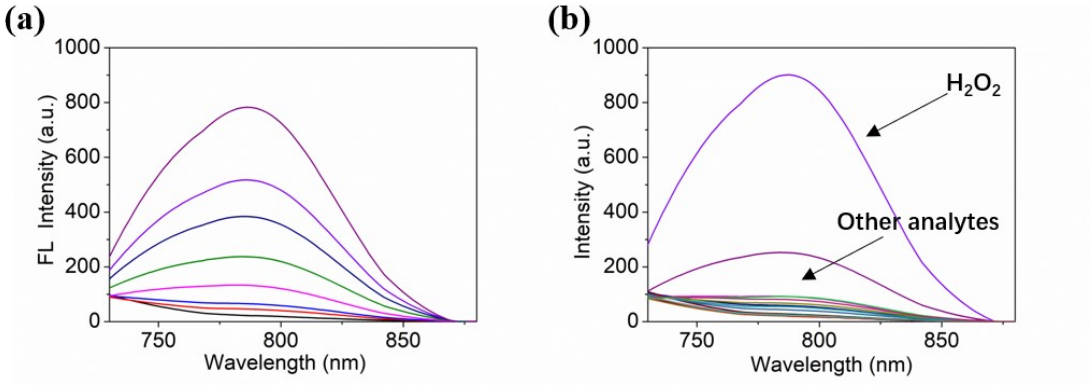

Fig. S5. (a) Fluorescence spectra of Ts-BOD-B (10 μM) upon addition of 0-60 μM H_2O_2 in 24 hours. (b) Fluorescence intensity changes of Ts-BOD-B in the presence of 100 μM H_2O_2 in 24 hours. Data shown are for 1 mM GSH, 1 mM Cys, and 100 μM for other: 1) Blank; 2) NO^{2-}; 3) NO^{3-}; 4) $S_2O_3^{2-}$; 5) SO_4^{2-}; 6) ClO^-; 7) Gly; 8) Hcy; 9) Cys; 10) GSH^-; 11) ClO^-; 12) NaHS; 13) DHA; 14) L-AA; 15) Na+; 16) K+; 17) Fe^{2+}; 18) Fe^{3+}; 19) Cu^{2+}; 20) $O_2^{\cdot -}$; 21)·OH; 22) $ONOO^-$; 23) H_2O_2.

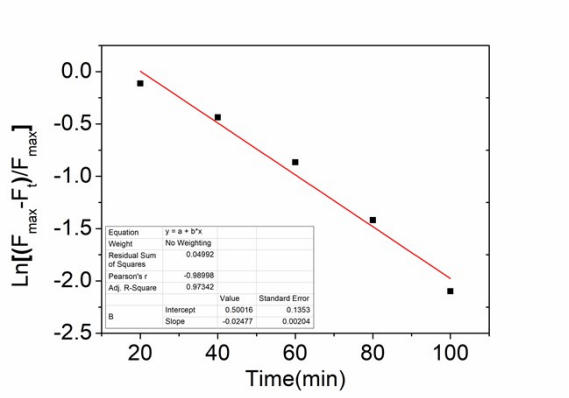

Fig. S6 Pseudo first-order kinetic plots of the reaction of Ts-BOD-B (10 μM) with H_2O_2 (1 mM). From the plot k_{obs} (0.0248 min^{-1}) can be obtained according to equation: $Ln((F_{max}-F_t)/F_{max})) = -k_{obs}t$. Where F_t and F_{max} are the fluorescence intensities at 790 nm at time t and the maximum value obtained after the reaction is complete, respectively, and k' is the pseudo-first-order rate constant.

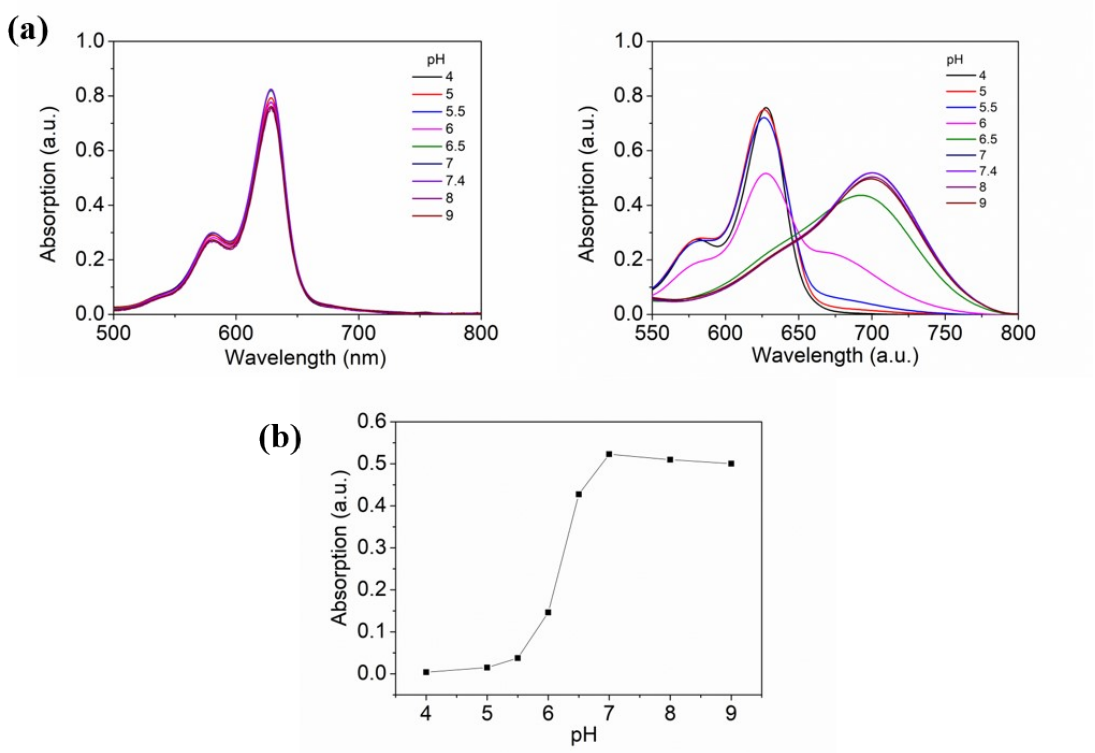

Fig. S7. (a) The absorption intensity of Ts-BOD-B (10 μM) reacts in the absence/presence of H_2O_2 (300 μM) with pH (4-9) in 24 hours. (b) The absorption changes of Ts-BOD-B (10 μM) in the

presence of H_2O_2 (300 μM) at 700 nm changes with pH (4-9) in 24 hours.

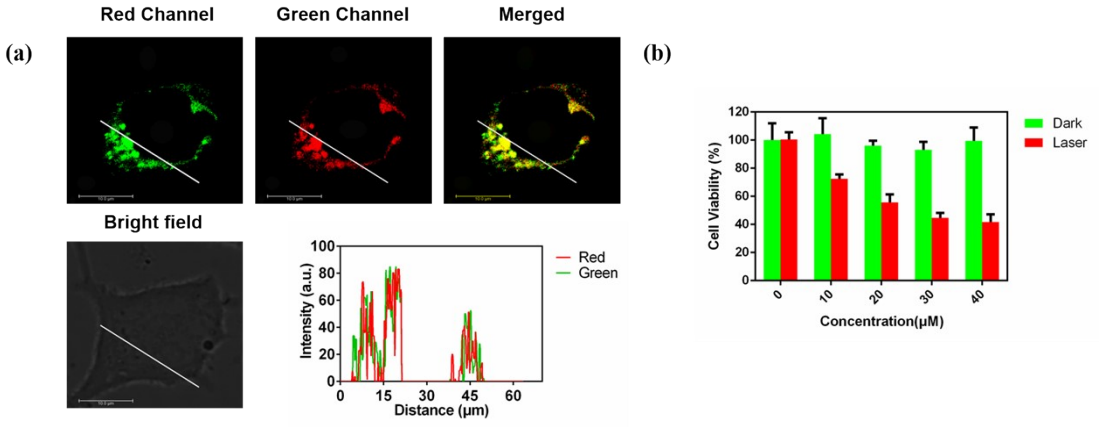

Fig. S8. (a) Colocalization images of 4T1 cells stained with Ts-BOD-B (40 μM, red channel, λ_{ex} = 633 nm, λ_{em} = 740-800 nm) and ER-Tracker™Green (1 μM, green channel, λ_{ex} = 488 nm, λ_{em} = 500-540 nm). Scale bar = 10 μm. (b) The cytotoxicity of Ts-BOD-B toward 4T1 cells under dark and 690 nm light irradiation (2 W/cm^2).

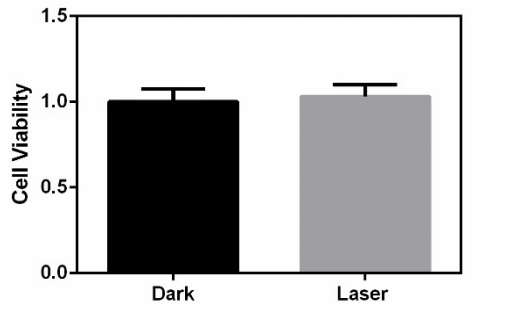

Fig. S9 MTT assay of cell viability of A549 cells under dark and laser irradiation (2.0 W/cm^2).

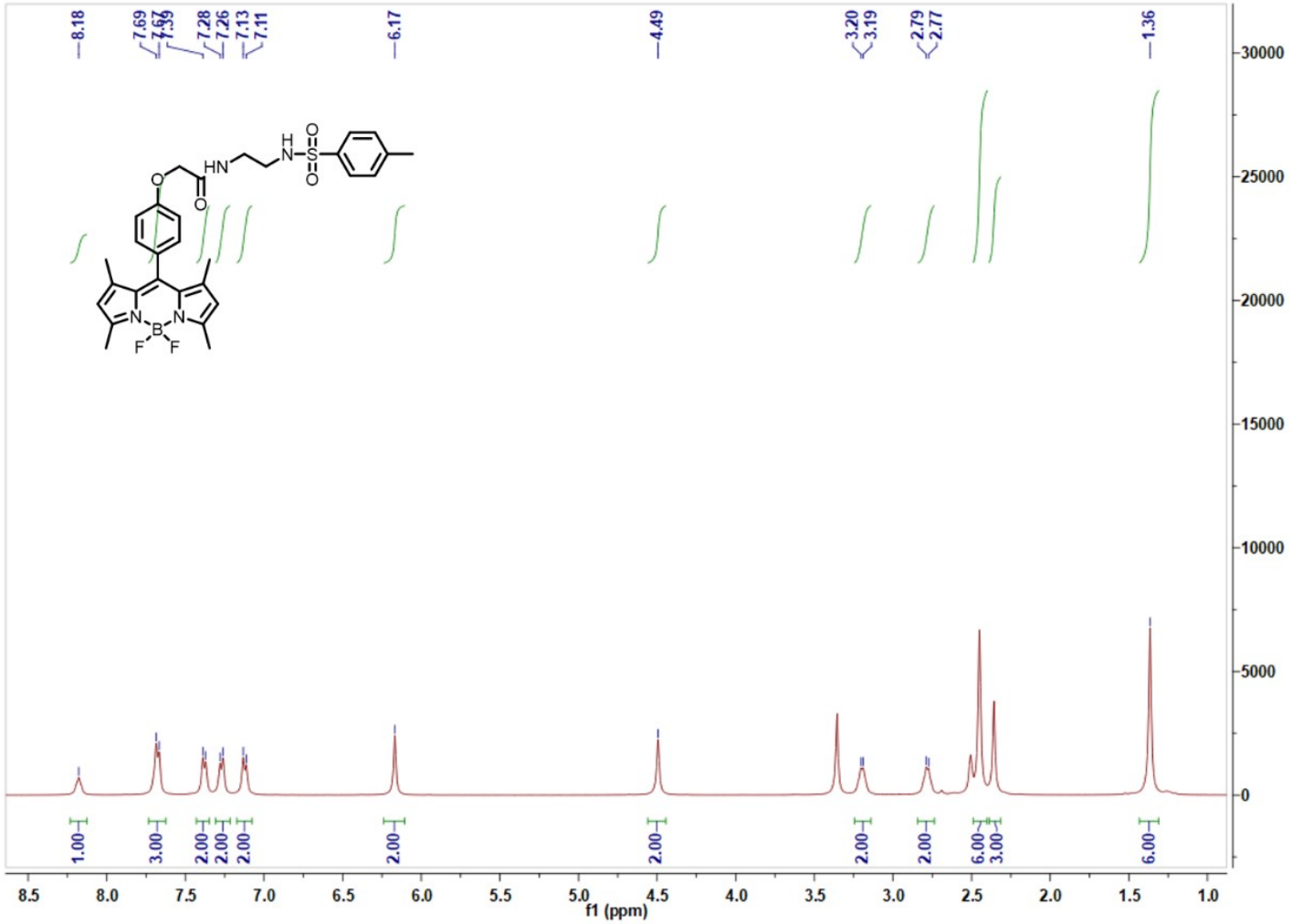

Fig. S10. ^{1}H NMR spectrum of Ts-BOD in DMSO-d_6

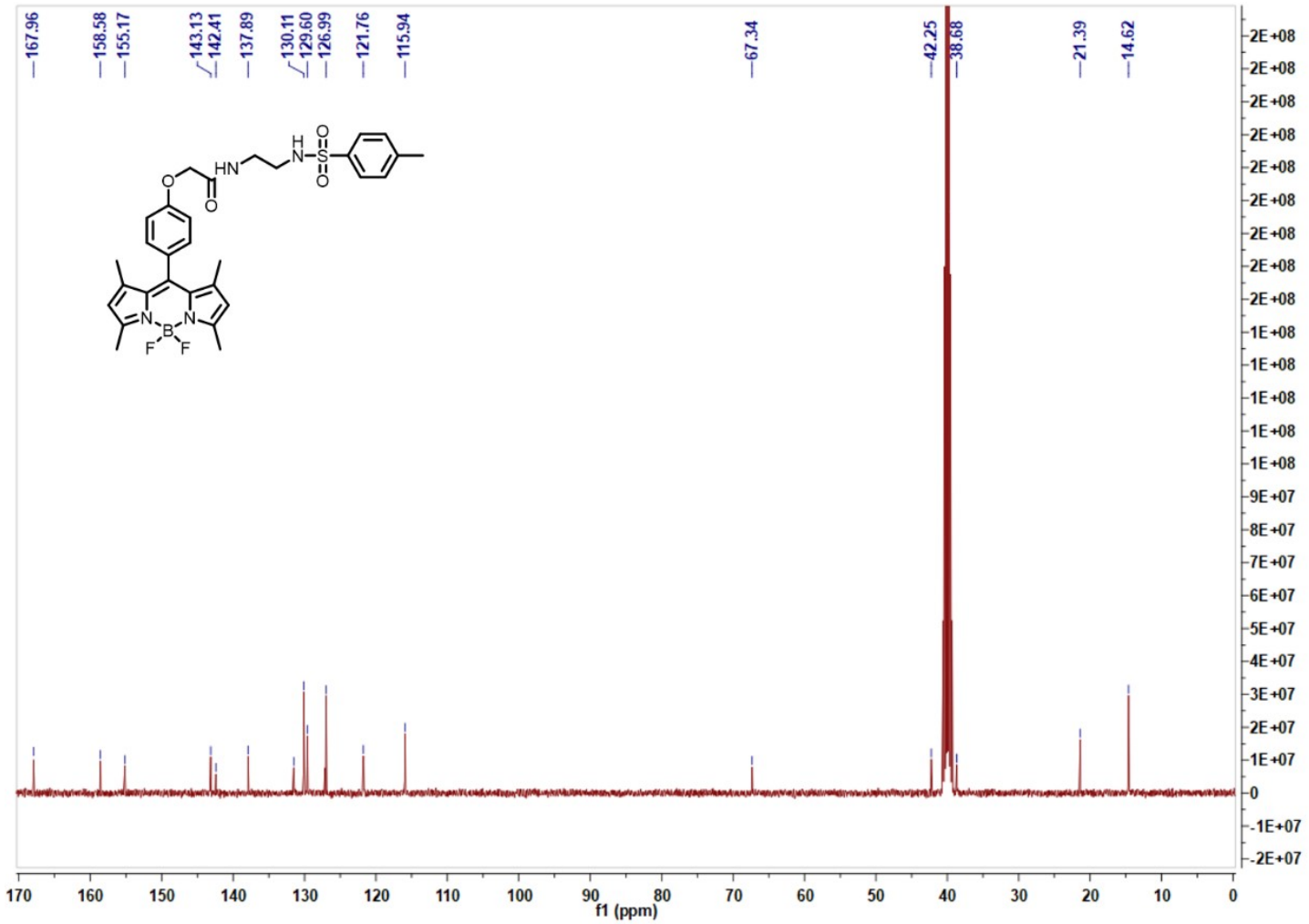

Fig. S11. ^{13}C NMR spectrum of Ts-BOD in DMSO-d_6

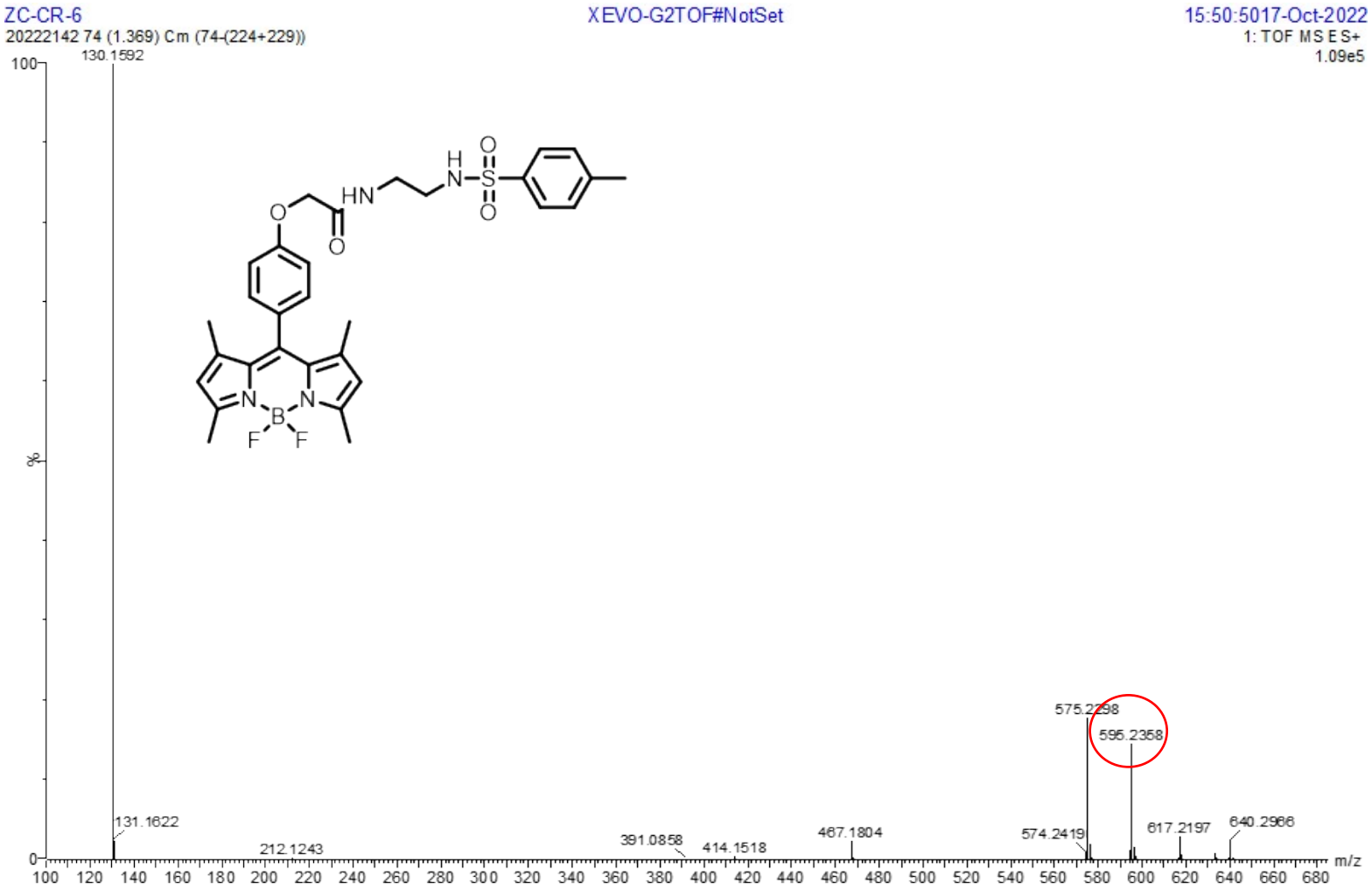

Fig. S12. HRMS of compound Ts-BOD

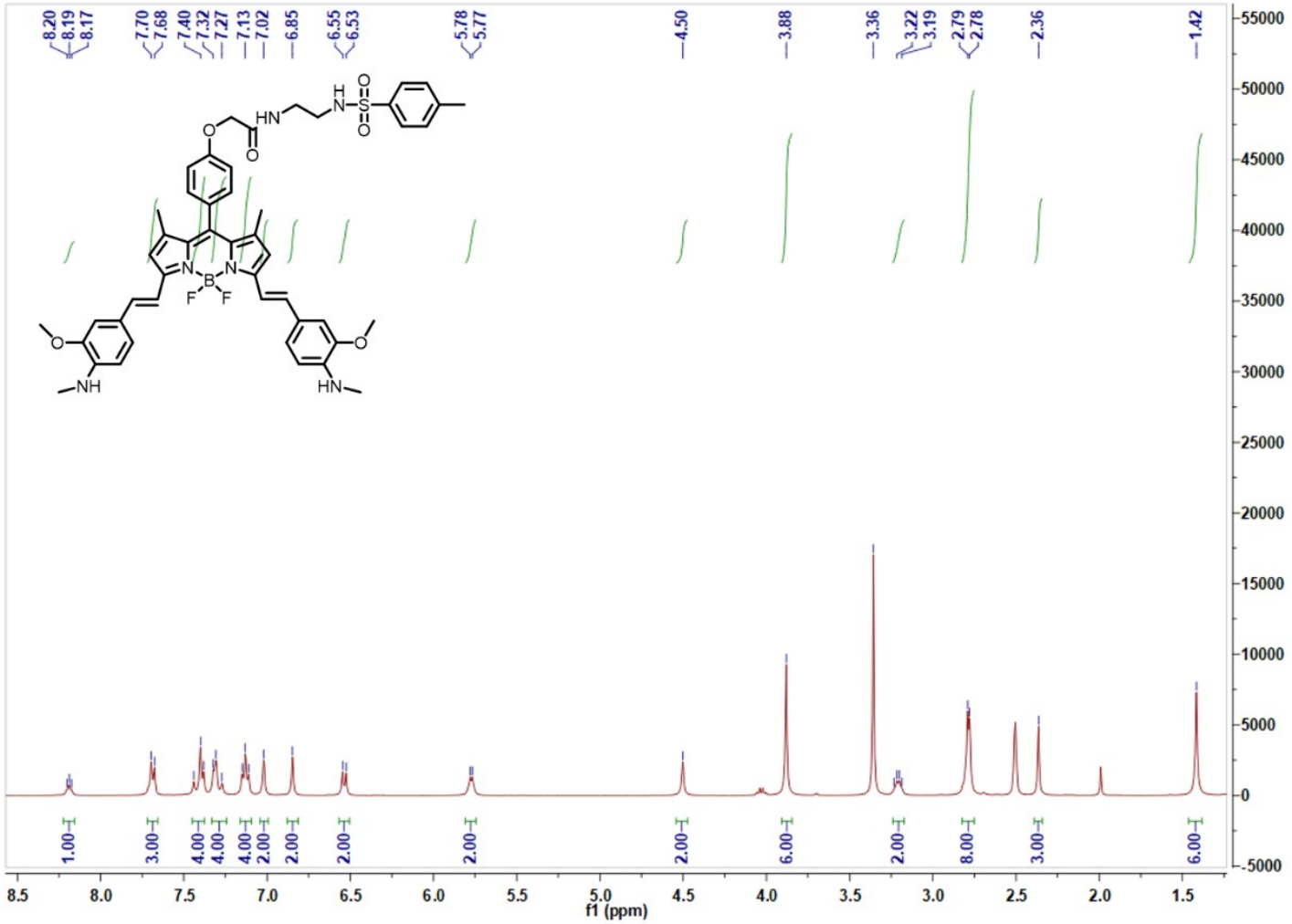

Fig. S13. ^{1}H NMR spectrum of Ts-BOD-N in DMSO-d_6

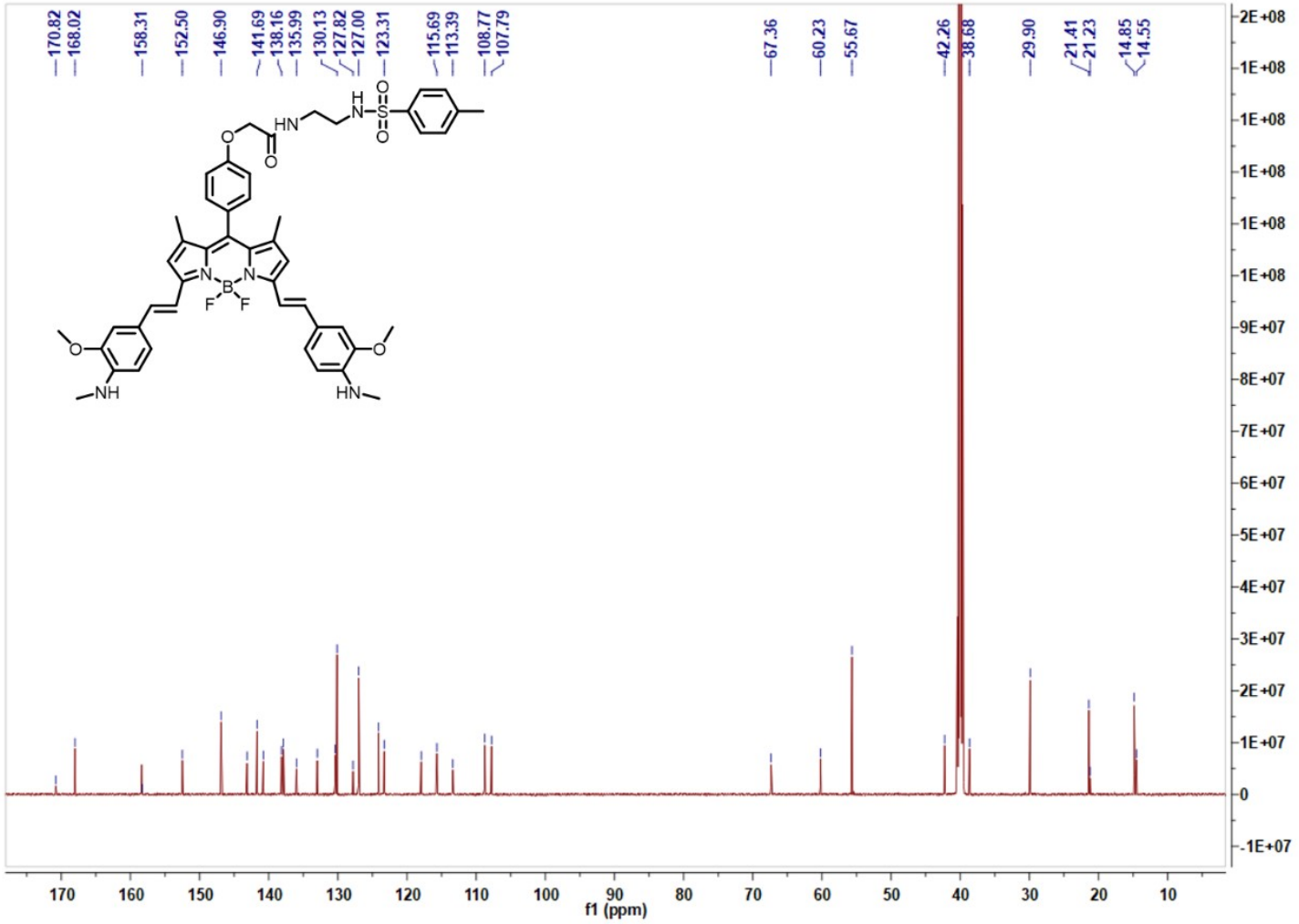

Fig. S14. ^{13}C NMR spectrum of Ts-BOD-N in DMSO-d_6

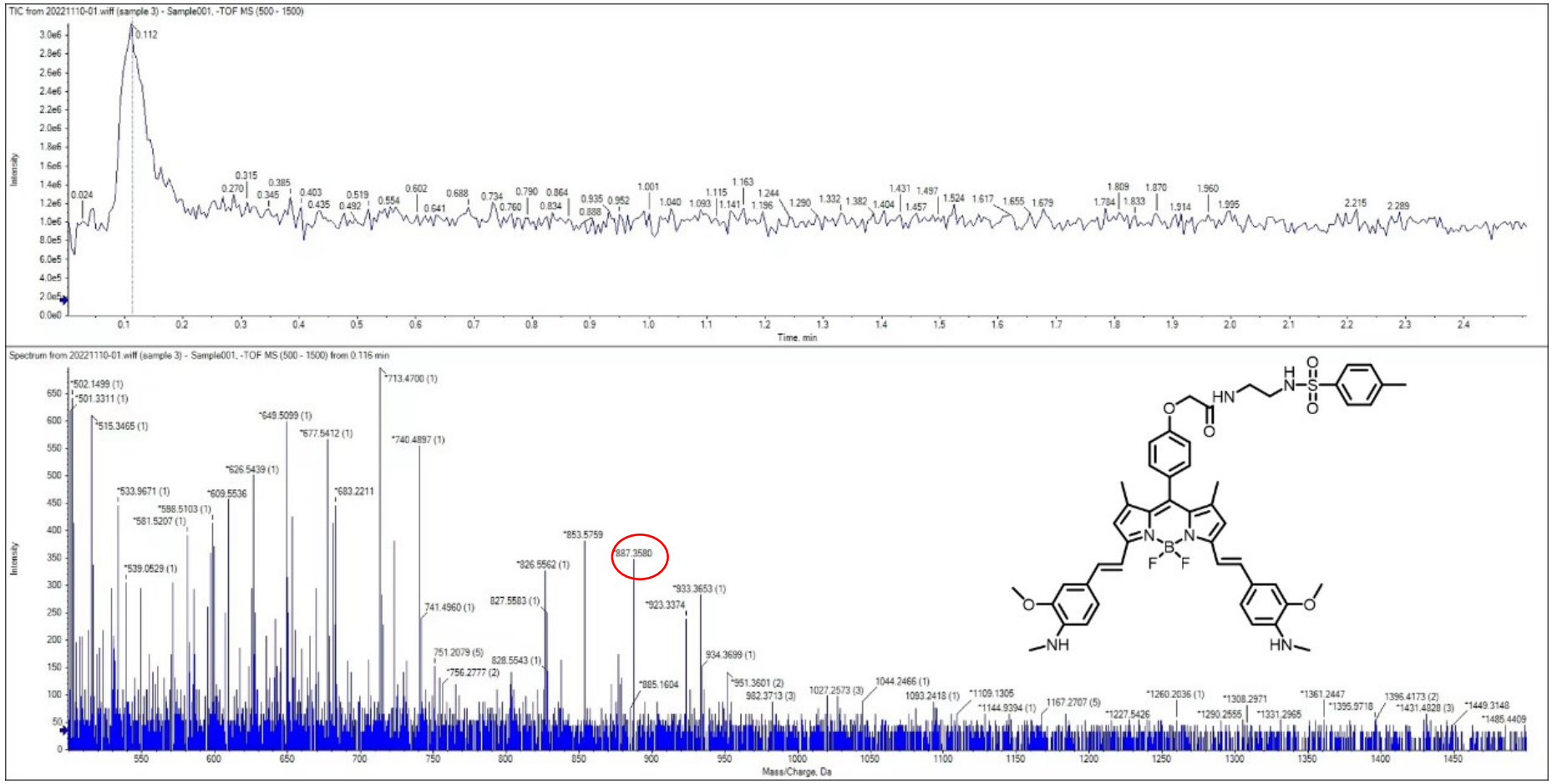

Fig. S15. HRMS of compound Ts-BOD-N.

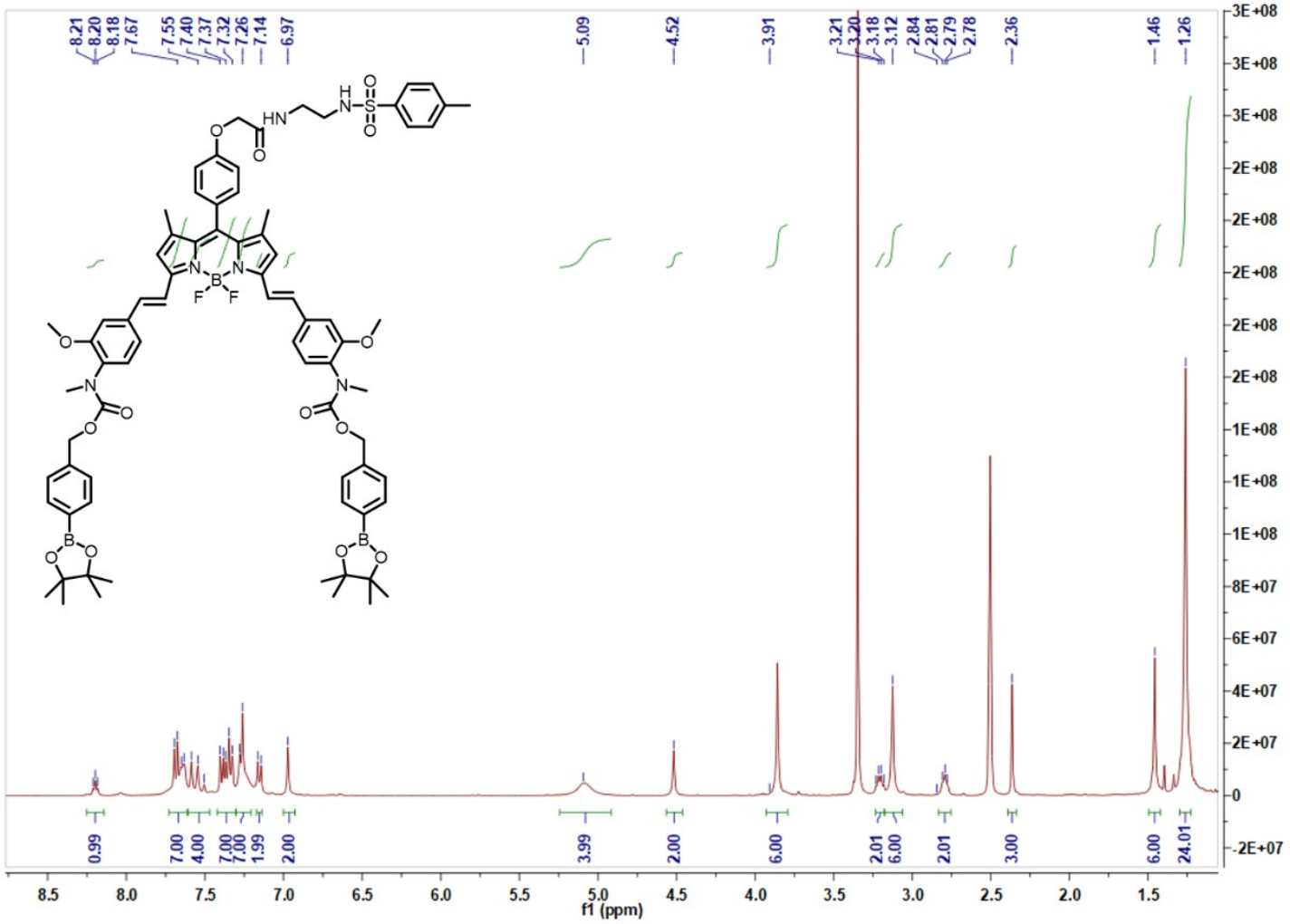

Fig. S16. ^{1}H NMR spectrum of Ts-BOD-B in DMSO-d_6

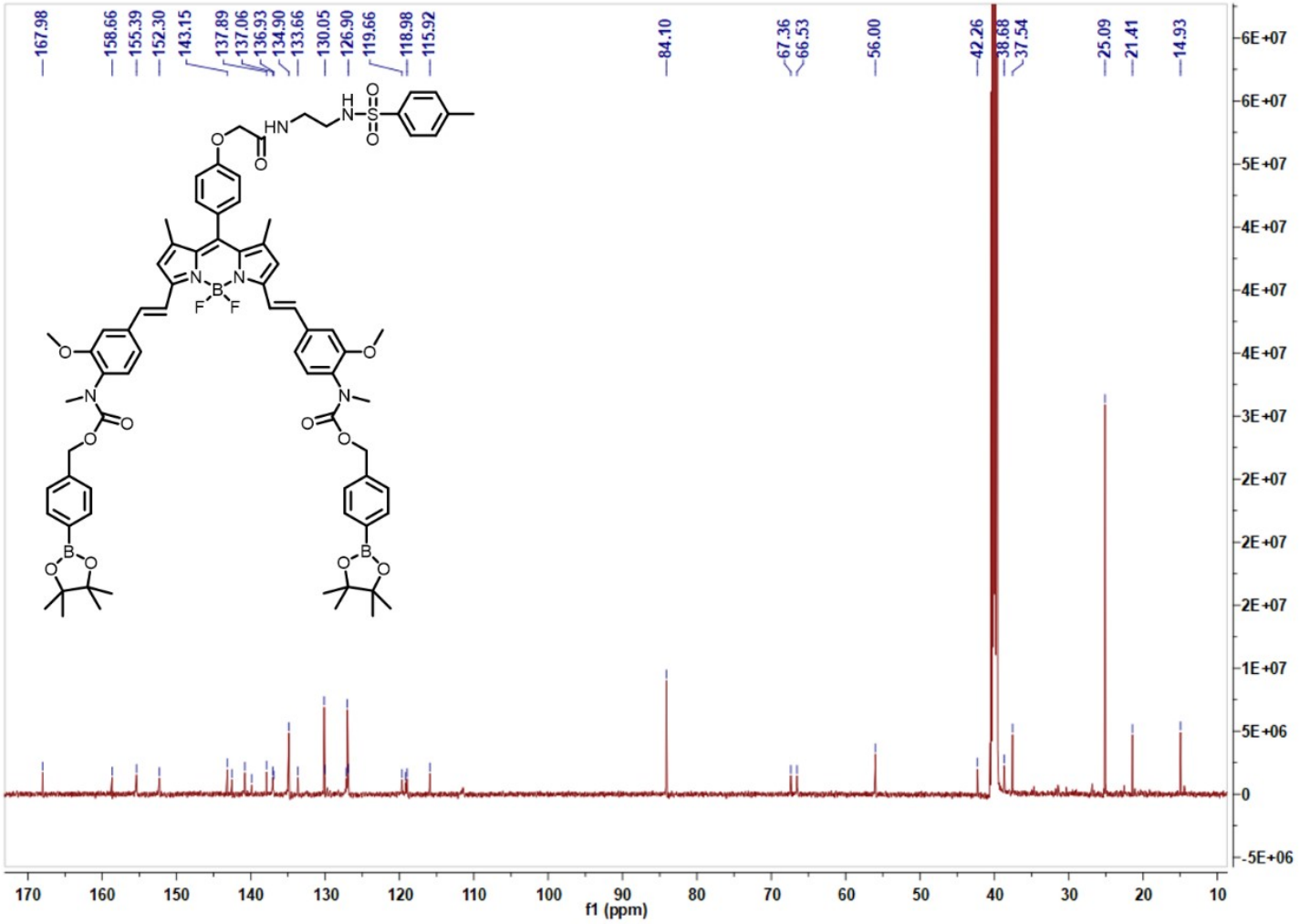

Fig. S17. ^{13}C NMR spectrum of Ts-BOD-B in DMSO-d_6

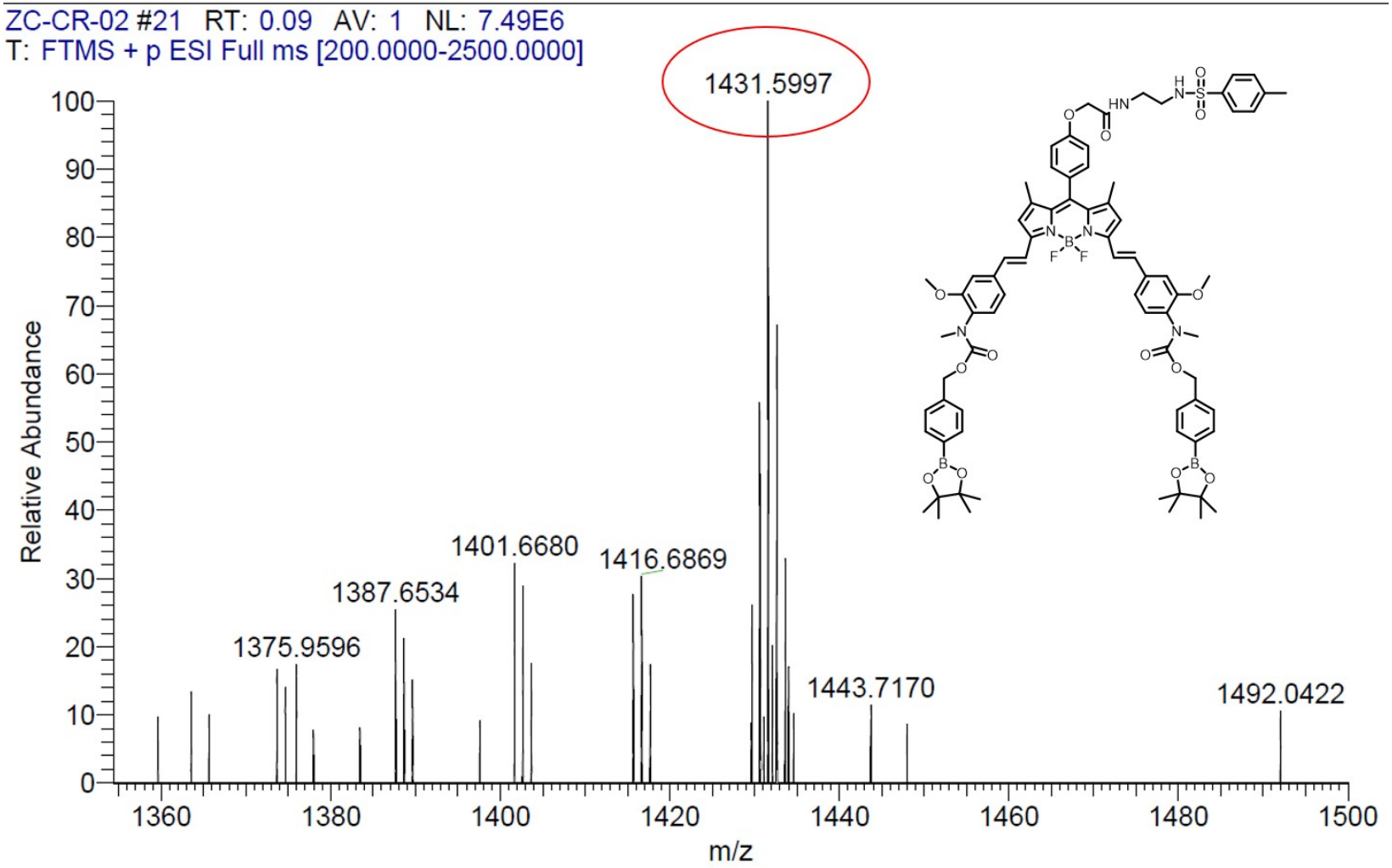

Fig. S18. HRMS of compound Ts-BOD-B

Notes and references

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