Supporting Information for

Simultaneous Imaging of Lysosomal and Mitochondrial Viscosity during Mitophagy Using Molecular Rotors with Dual-color Emission

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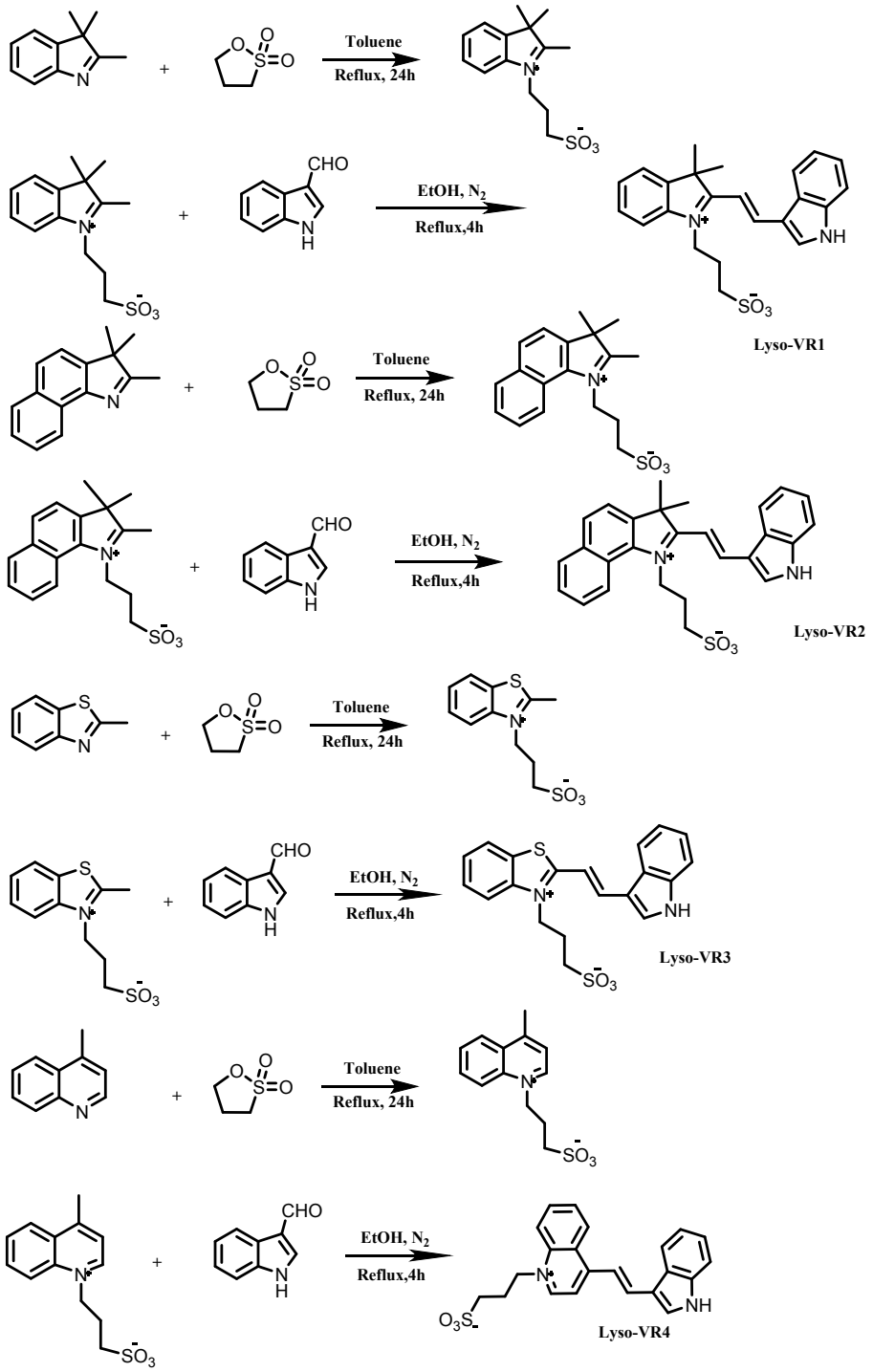
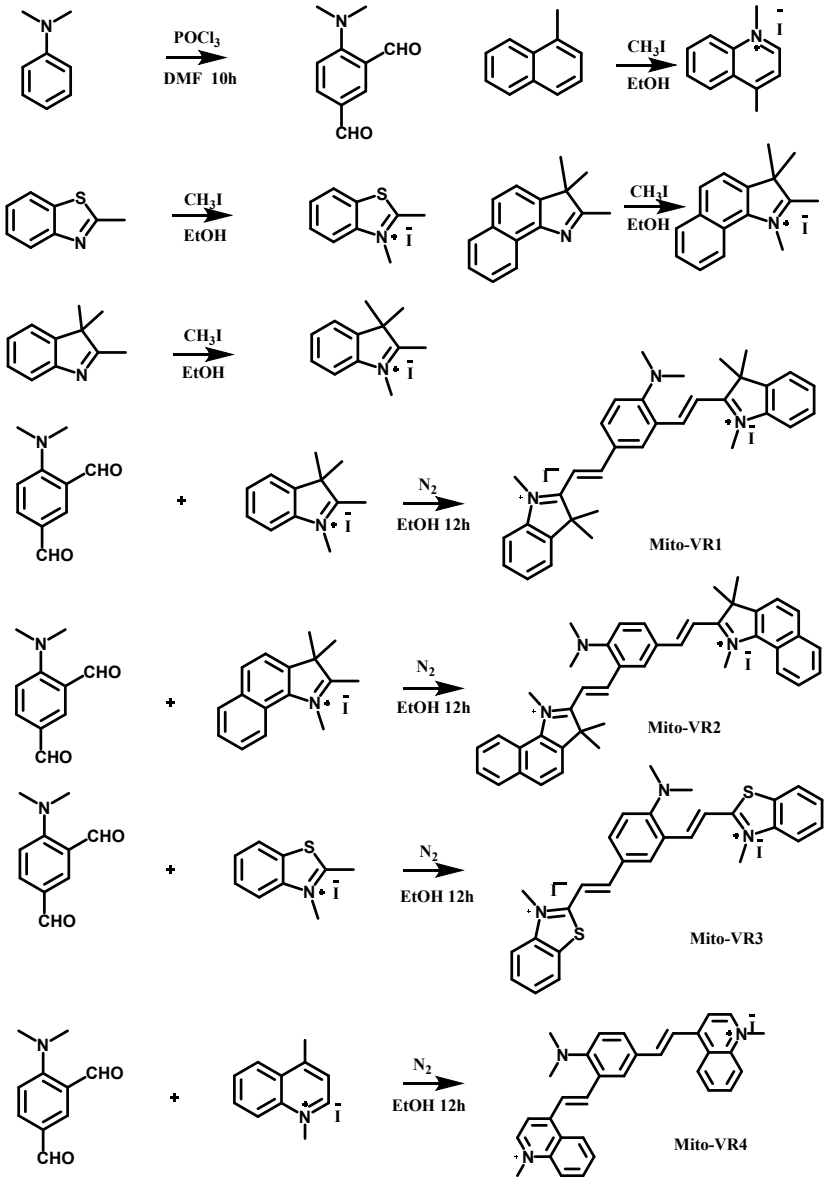
1. Synthetic schemes



Scheme S1. Synthetic routes for Lyso-VR1, Lyso-VR2, Lyso-VR3 and Lyso-VR4.



Scheme S2. Synthetic routes for Mito-VR1, Mito-VR2, Mito-VR3 and Mito-VR4.

2. MATERIALS AND METHODS

2.1. Materials and Apparatus

All reactions were performed under an argon atmosphere using Schlenk techniques, monitored by thin layer chromatography (TLC). Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Reagent grade solvents were dried by the standard procedures and were freshly distilled prior to use. Ultra-pure water with an electric resistance>18.25 MΩ was used throughout the experiments and it was obtained from a Millipore Milli-Q water purification system (Billerica, MA, USA). All solvents used for optical measurements were of chromatographic grade. Product separations were performed by column chromatography using 200−300 mesh silica gel. UV-vis absorption spectra were recorded with a Shimadzu UV-2450 spectrophotometer with an interval of 2 nm. Fluorescence spectra were recorded on an F-7000 (Hitachi). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-III 400 instrument (Bruker) using tetramethylsilane (TMS) as an internal standard. Mass spectroscopy (MS) analysis was performed on an LCQ advantage ion trap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Flow cytometer measurements were acquired from a BD FACSCalibur flow cytometer (Becton & Dickinson Company, Franklin Lakes, NJ).

2.2. Synthesis, purification and characterization

Synthesis of 3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)-propane-1-sulfonate. 2,3,3-Trimethylindolenine (3.00g, 18.8 mmol) was heated under reflux with an equimolar of 1,3-propanesultone (2.30 g,18.8 mmol) in toluene (50 mL) for 24 h, with constant stirring. Upon cooling, the product was separated by filtration, dried and washed with toluene. The product was collected as a dry red powder and used without further purification. Yield: 5.18 g (98%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.05 (d, *J*=4.0 Hz, 1H), 7.83 (d, *J*=4.0 Hz, 1H), 7.65-7.59(m, 2H), 4.66(t, *J*=8.0 Hz, 2H), 2.64 (t, *J*=8.0 Hz, 2H), 2.20-2.13(m, 2H), 1.53(s, 6H).

Synthesis of 3-(2,3,3-trimethyl-3H-benzo[g]indol-1-ium-1-yl)-propane-1-sulfonate. 2,3,3-Trimethyl-3H-benzo[g]indole (2.09g, 10.0 mmol) was heated under reflux with an equimolar of 1,3-propanesultone (1.22 g,10.0 mmol) in toluene (40 mL) for 24 h, with constant stirring. Upon cooling, the product was separated by filtration, dried and washed with toluene. The product was collected as a colorless powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.36 (d, *J*=4.0 *Hz*, 1H), 8.30-8.20 (m, *3*H), 7.80-7.70(m, 2H), 4.78(t, *J*=8.0 *Hz*, 2H), 2.94(s, 3H), 2.68 (t, *J*=8.0 *Hz,* 2H), 2.26-2.19(m, 2H), 1.76(s, 6H).

Synthesis of 3-(2-methylbenzo[d]thiazol-3-ium-3-yl)propane-1-sulfonate.. 2-methylbenzo[d]thiazole (1.64 g 10 mM) was heated under reflux with an equimolar of 1,3-propanesultone (1.22 g, 10 mmol) in toluene (40 mL) for 24 h, with constant stirring. Upon cooling, the product was separated by filtration, dried and washed with toluene. The product was collected as a colorless powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.45-8.41 (m, 2H), 7.91-7.87 (m, *1*H), 7.81-7.78(m, 1H), 4.92(t, *J*=8.0 *Hz*, 2H), 3.01(s, 3H), 2.65 (s, 2H), 2.17(s, 2H), 1.76(s, 6H).

Synthesis of 3-(4-methylquinolin-1-ium-1-yl)-propane-1-sulfonate. 4-methylquinoline was heated under reflux with an equimolar of 1, 3-propanesultone (1.22 g, 10.0 mmol) in toluene (40 mL) for 24 h, with constant stirring. Upon cooling, the product was separated by filtration, dried and washed with washed with toluene. The product was collected as a dry red powder and used without further purification. ^{1}H-NMR (400 MHz, Methanol-d4) δ (ppm): 9.35 (d, *J*=4.0 *Hz*, 1H), 8.69 (d, *J*=4.0 *Hz*, 1H), 8.60(d, *J*=4.0 *Hz*, 1H), 8.31-8.28(m, 1H), 8.10-8.06(m, 1H), 8.02 (d, *J*=4.0 *Hz*, 1H), 5.32-5.28(m, 2H), 3.10(s, 3H),

3.02(m, 2H), 2.58-2.53(m, 2H).

Synthesis of Lyso-VR1. 3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl) propane-1-sulfonate was dissolved in anhydrous ethanol (20 mL) followed with the addition of substituted indole-3-carboxaldehyde (2.0 mmol). The mixture was allowed to reflux for 4 h in an atmosphere of pure nitrogen. The reaction was monitored by TLC until the disappearance of starting materials. Then the reaction mixture was cooled to room temperature and extracted with dichloromethane (3×50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Removal of excess solvent was carried on by rotary evaporation under reduced pressure to get the crude product. The residue was further purified by column chromatography using ethyl acetate/petroleum ether (3:2, v/v) as the eluent to give pure product. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 12.89 (s, 1H), 8.85 (s, 1H), 8.70 (d, *J*=8.0 Hz, 1H), 8.42-8.40(m, 1H), 7.84-7.78 (m, 2H), 7.58-7.52 (m, 2H), 7.49-7.45 (m, 2H), 7.35-7.33 (m, 2H), 4.72 (t, *J* = 8.0 Hz, 2H), 2.72 (t, *J* = 4.0 Hz, 2H), 2.21-2.14 (m, 2H), 1.81 (s, 6H).

Synthesis of Lyso-VR2. 3-(2,3,3-trimethyl-3H-benzo[g]indol-1-ium-1-yl) propane-1-sulfonate was dissolved in anhydrous ethanol (20 mL) followed the addition of substituted indole-3-carboxaldehyde (2.0 mmol). The mixture was allowed to reflux for 4 h in an atmosphere of pure nitrogen. The reaction was monitored by TLC until the disappearance of starting materials. Then the reaction mixture was cooled to room temperature and extracted with dichloromethane (3×50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Removal of excess solvent was carried on by rotary evaporation under reduced pressure to get the crude product. The residue was further purified by column chromatography using ethyl acetate/petroleum ether (3:2, v/ v) as the eluent to give pure products. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 12.87(s, 1H), 8.90-8.18 (m, 6H), 7.98(s, 2H), 7.64(d, *J*=12 Hz, 2H), 7.39(s, 2H), 4.87(s, 2H), 4.38 (s, 2H), 2.26(s, 2H), 1.08(s, 6H).

Synthesis of Lyso-VR3. 2,3-dimethylbenzo[d]thiazol-3-ium was dissolved in anhydrous ethanol (20 mL) followed the addition of substituted indole-3-carboxaldehyde (2.0 mmol). The mixture was allowed to reflux for 4 h in an atmosphere of pure nitrogen. The reaction was monitored by TLC until the disappearance of starting materials. Then the reaction mixture was cooled to room temperature and removal of excess solvent was carried on by rotary evaporation under reduced pressure to get the crude product. The residue was further purified by column chromatography to give pure products. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.57(d, *J*=16 Hz 1H), 8.46(d, *J*=4.0 Hz 2H), 8.06(t, *J*=4.0 Hz, *J*=4.0 Hz, 1H), 7.97(d, *J*=8.0 Hz 1H), 7.67-7.64(m, *3*H), 7.44-7.31(m, 3H), 4.83(t, *J*=8.0 Hz, *J*=8.0 Hz, 2H), 3.02-2.97 (m, 2H), 2. 76(t, *J*=8.0 Hz, *J*=4.0 Hz, 2H).

Synthesis of Lyso-VR4. 3-(4-methylquinolin-1-ium-1-yl) propane-1-sulfonate and indole-3-carboxaldehyde were dissolved in anhydrous ethanol. The mixture was allowed to reflux for 6 h in an atmosphere of pure nitrogen. The reaction was monitored by TLC until the disappearance of starting materials. Then the mixture was cooled to room temperature and removal of excess solvent by rotary evaporation under reduced pressure to get the crude product. The residue was further purified by column chromatography to give pure products. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.13(d, *J*=2.0 Hz, 1H), 8.96 (d, *J*=4.0 Hz 1H), 8.57-8.53(m, 2H), 8.43(d, *J*=2.0 Hz 1H), 8.28(d, *J*=4.0 Hz 1H), 8.03-7.96(m, *2*H), 7.62(d, *J*=4.0 Hz 1H), 7.39-7.32(m, 2H), 5.05-5.01(m, 2H), 2.59-2.56(m, 2H), 2.29-2.22(m, 2H).

Synthesis of 4-(dimethylamino)-isophthalaldehyde. Phosphorus oxychloride (15 ml) was added to DMF (40 ml) in drops. Then Me_2NPh (5 ml) was added in drops into the solution, and the reaction lasted for 10 h at 80℃. The reactant was poured into the mixture of ice and 80 mL of 38% NaOH solution, and kept at 0℃ to give a large amount of precipitate. The precipitate was filtered and purified by column chromatography on silica gel (eluting with petroleum ether: ethyl acetate 5 : 1) to give 4.5 g (25% of

yield). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.959(s, 1H), 9.773(s, 1H), 8.111(s, 1H), 7.832(d, 1H), 6.939(d, 1H), 3.039(s, 6H).

Synthesis of 1,2,3,3-tetramethyl-3H-indol-1-ium. To a solution of 2,3,3-trimethylindolenine in anhydrous ethanol were added methyl iodide (1.5 eq.) and the reaction mixture was heated at reflux for 12 h. The resulting solid was filtered under vacuum, washed with Et_2O and dried to afford indolium. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 7.92(d, *J*=4.0 Hz 1H), 7.65-7.60(m, 2H), 3.99(s, 3H), 2.79(s, 3H), 1.54(s, 6H).

Synthesis of 1,2,3,3-tetramethyl-3H-benzo[g]indol-1-ium iodide. To a solution of 1,1,2-trimethylbenz[e]indole in anhydrous ethanol were added methyl iodide (1.5 eq.) and the reaction mixture was heated at reflux for 12 h. The resulting solid was filtered under vacuum, washed with Et_2O and dried to afford indolium. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.38(d, *J*=4.0 Hz, 1H), 8.30(d, *J*=4.0 Hz, 1H), 8.23(d, *J*=4.0 Hz, 1H), 8.13(d, *J*=4.0 Hz, 1H), 7.80(t, *J*=4.0 Hz, 1H), 7.73(t, *J*=4.0 Hz, 1H), 4.124(s, 3H), 2.905(s, 3H), 1.769(s, 6H).

Synthesis of 2,3-dimethylbenzo[d]thiazol-3-ium. 2-methylbenzo[d]thiazole and methyl iodide were refluxed in anhydrous ethanol for 12 h, with constant stirring. Upon cooling, the product was separated by filtration, dried and washed with anhydrous ethanol. The product was collected as a colorless powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.46 (d, *J*=4.0 Hz, 1H), 8.30(d, *J*=4.0 Hz, 1H), 7.92-7.88(m, 1H), 7.83-7.79 (m, 1H), 4.22(s, 3H), 3.19(s, 3H).

Synthesis of 1,4-dimethylquinolin-1-ium. 4-methylquinoline and methyl iodide were refluxed in toluene for 12 h. The resulting solid was filtered under vacuum, washed with toluene and dried to afford a yellow product. 9.39 (d, *J*=2.0 Hz, 1H), 8.55-8.48(m, 2H), 8.29-8.25(m, 1H), 4.59(s, 3H), 3.01(s, 3H).

Synthesis of Mito-VR1. To a solution of 4-(dimethylamino)-isophthalaldehyde in anhydrous ethanol were added 1,2,3,3-tetramethyl-3H-indol-1-ium and the reaction mixture was heated at reflux for 12 h. Then the reaction mixture was cooled to room temperature and extracted with dichloromethane (3×50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Removal of excess solvent was carried on by rotary evaporation under reduced pressure to get the crude product. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.77(s, 1H), 8.47(d, *J*=8.0 Hz,1H), 8.38(d, *J*=4.0 Hz, 1H), 8.30(d, *J*=8.0 Hz,1H), 7.96-7.85(m, 4H),7.65-7.57 (m, 6H),7.30(d, *J* = 9.2 Hz,1H), 4.24(s, 3H), 4.15(s, 3H), 3.18(s, 6H),1.82(d, *J*=6.0 Hz, 12H).

Synthesis of Mito-VR2. To a solution of 4-(dimethylamino)-isophthalaldehyde in anhydrous ethanol were added 1,2,3,3-tetramethyl-3H-benzo[g]indol-1-ium iodide and the reaction mixture was heated at reflux for 12 h. Then the reaction mixture was cooled to room temperature and extracted with dichloromethane (3×50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Removal of excess solvent was carried on by rotary evaporation under reduced pressure to get the crude product. ^{1}H-NMR (400 MHz, DMSO-d_6) *δ* (ppm): 8.83 (s, 1H), 8.58 (d, *J*=8.0 Hz, 1H), 8.46-8.41 (m,3H), 8.36 (d, *J*=4.0 Hz, 1H), 8.32 (d, *J*=4.0 Hz, 1H), 8.28 (d, *J*=6.0 Hz, 1H), 8.10 (d, *J*=6.0 Hz, 1H), 7.86-7.80 (m, 2H), 7.76-7.64 (m, 1H), 7.34 (d, *J*=4.0 Hz, 1H), 4.39 (s, 3H), 4.30 (s, 3H), 3.21 (s, 6H), 2.08 (s, 3H), 2.06 (s, 6H).

Synthesis of Mito-VR3. To a solution of 4-(dimethylamino)-isophthalaldehyde in anhydrous ethanol were added 2,3-dimethylbenzo[d]thiazol-3-ium and the reaction mixture was heated at reflux for 12 h. Then the reaction mixture was cooled to room temperature and removal of excess solvent was by rotary evaporation under reduced pressure to get the crude product. ^{1}H-NMR (400 MHz, DMSO-d_6) *δ* (ppm): 8.55 (s, 1H), 8.43 (d, *J*=8.0 Hz, 2H), 8.30 (d, *J*=8.0 Hz ,1H), 8.25-8.13 (m,4H), 8.01-7.77 (m,6H), 7.29 (d, *J*=8.0 Hz, 1H), 4.42 (s, 3H), 4.37 (s, 3H), 3.10 (s, 6H).

Synthesis of Mito-VR4. To a solution of 4-(dimethylamino)-isophthalaldehyde in anhydrous ethanol were added 1,4-dimethylquinolin-1-ium and the reaction mixture was heated at reflux for 12 h. Then the reaction mixture was cooled to room temperature and removal of excess solvent was by rotary evaporation under reduced pressure to get the crude product.

1.3 Characterizations of the probes. Solutions of different viscosity values were obtained by mixing phosphate buffer saline (PBS)-glycerol systems in different proportions. Probes in different viscous solutions were prepared by adding the corresponding stock solution (1.0 mM of probes in DMSO) to 1.0 mL of PBS-glycerol systems. The mixtures were shook for 3 min to eliminate air bubbles and stood at 25 ^{o}C for 10 min. All the *in vitro* measurements were performed at 25 ^{o}C unless otherwise indicated. Absorbance spectra were recorded with a UV spectrophotometer and fluorescence spectra for Lyso-VR1, Lyso-VR2 and Lyso-VR4, and Mito-VR1, Mito-VR2 and Mito-VR3 were recorded with a fluorescence spectrophotometer using excitation wavelengths of 488 nm, while Lyso-VR3 and Mito-VR4 were recorded with a fluorescence spectrophotometer using excitation wavelengths of 405 nm and 640nm, respectively. For studying the effect of solvents on the emission spectra of the probes, stock solutions of probes were diluted with 1 mL of 1.Acetone, 2.DCM, 3.DMSO, 4.EA, 5.glycerol, 6.MeOH, 7.DMF, 8.PBS, and 9.CH_3CN, respectively. Fluorescence spectra were recorded after thorough mixing. To test the pH effect on the response of Lyso-VR1 and Mito-VR2, probe solutions in 10%, 50% and 95% glycerol with different pH values in the range of 4.0 and 7.4 were prepared and fluorescence spectra were obtained and analyzed. The viscosity sensitivity studies of Lyso-VR1 and Mito-VR2 were investigated by measuring their fluorescence spectra in PBS-glycerol binary system with different mass fractions of glycerol. The logarithm of maximum emissions at wavelengths of 550 nm and 660 nm were plotted against that of the viscosity, respectively, which was then fitted with the Föster-Hoffmann equation. For selectivity assay, Lyso-VR1 and Mit-VR2 were treated with possible interfering substances in physiological conditions including cysteine (1.0 mM), glutathione (10 mM), cationic compounds and anionic compounds (10 mM), except sodium chloride (150 mM), respectively. Then, the mixture was incubated at 37 °C for 60 min. Fluorescence spectra were then obtained at 25 ^{o}C.

1.4 Cell culture and Fluorescent Imaging

HeLa cells were grown in RPMI-1640 supplemented with 10% FBS (fetal bovine serum), penicillin (100 μ/mL) and streptomycin (100 μ/mL) in an atmosphere of 5% CO_2 at 37 °C. The cells were plated on a 35-mm Petri dish with 10-mm bottom well in the folate-free RPMI-1640 medium for 24 h, then the dishes were washed three times with PBS. The cells were then incubated with 1 mL cell growth medium supplemented with 1.0 μM probe for 0.5 h at 37 °C. After washing with PBS three times to remove the remaining probe, the HeLa cells were incubated in the absence or presence of serum in the culture medium, the cells were then visualized by fluorescence imaging. All Fluorescence imaging were recorded at 25 ^{o}C using a water objective 60×, on a confocal laser scanning fluorescence microscope setup consisting of a Nikon inverted microscope. Ar+ laser (488 nm, 559 nm) was used as excitation sources. Excitation wavelength for Lyso-VR1: 488 nm; Emission collection: 500 nm-580 nm. Excitation wavelength for Mito-VR2: 488 nm; Emission collection: 680 nm-750 nm. Excitation wavelength for Mito-Tracker Green: 488 nm; Emission collection: 500 nm-550 nm. Excitation wavelength for Lyso-Tracker Red: 559 nm; Emission collection: 595 nm-640 nm.

1.5 Cytotoxicity assay

The cytotoxicity of probe against HeLa cells were studied using a WST-1 cell proliferation and cytotoxicity assay following the kit protocol. Briefly, cells were incubated with various concentrations of probe (1.0-40 μM) for 24 h, then WST-1 was added and incubated for another 4 h. Cell viability was determined by measuring the absorbance at 450 nm with a microplate reader.

1.6 Flow Cytometry (FCM) Analysis

HeLa cells were cultured at 1.0×10^7 cells/well in cell plates. Probes were added and the cells were further incubated for another 30 min, and washed with PBS to remove unbound probes. Then Earle's

Balanced Salts (EBS) solution was applied for autophagy induced by starvation. After 2 h, the cells were digested by trypsin, the supernatant is then removed by centrifugation, then added 0.5 mL PBS and analysis by a flow cytometer.

3. Reference

[S1] Jean-Alexandre Richard, *Organic & Biomolecular Chemistry*, **2015**, 13, 8169 –8172.

3. Additional figures

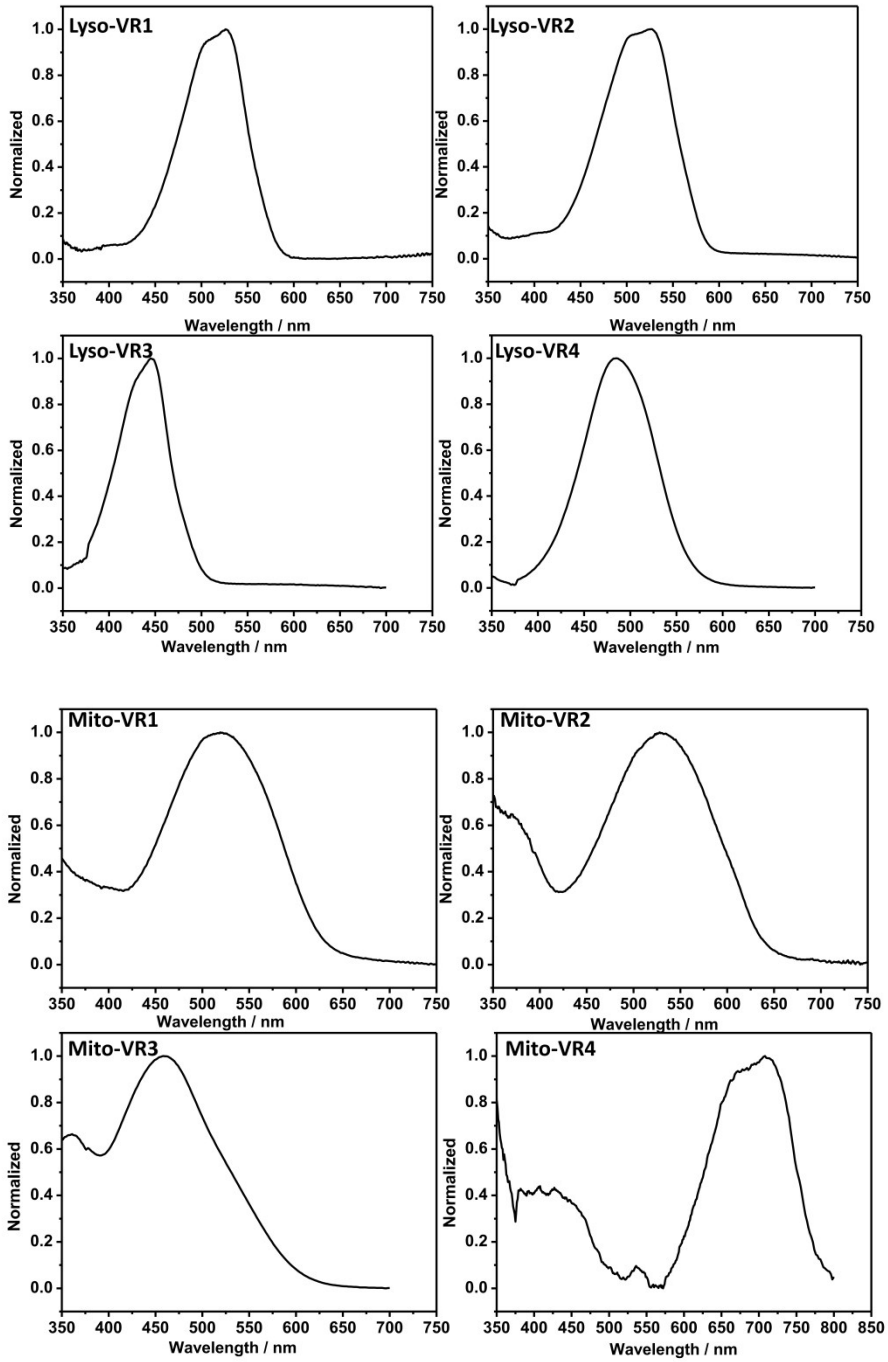


Fig. S1. Normalized absorption spectra of Lyso-VR1, Lyso-VR2, Lyso-VR3, Lyso-VR4, Mito-VR1, Mito-VR2, Mito-VR3 and Mito-VR4 in glycerin.

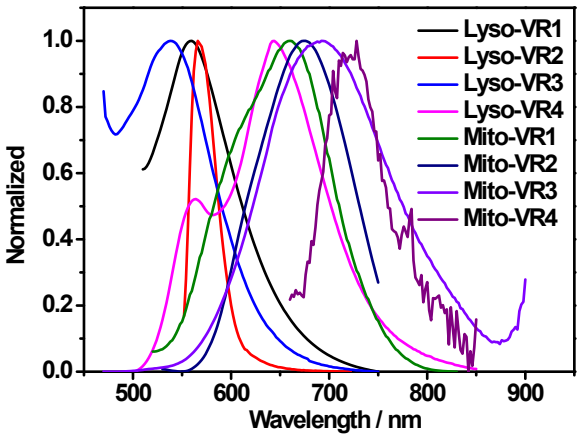


Fig. S2. Normalized fluorescence spectra of Lyso-VR1, Lyso-VR2, Lyso-VR3, Lyso-VR4, Mito-VR1, Mito-VR2, Mito-VR3 and Mito-VR4 in glycerin.

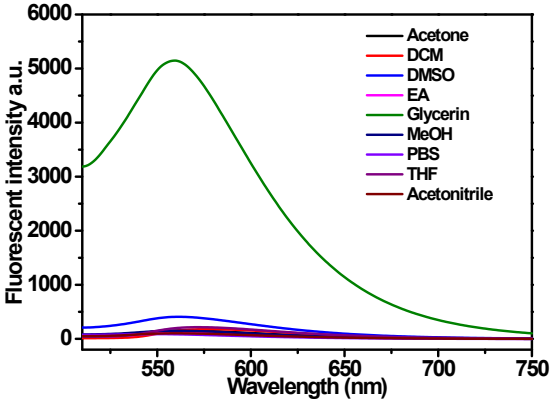


Fig. S3 Fluorescence spectra of **Lyso-VR1** in different solvents.

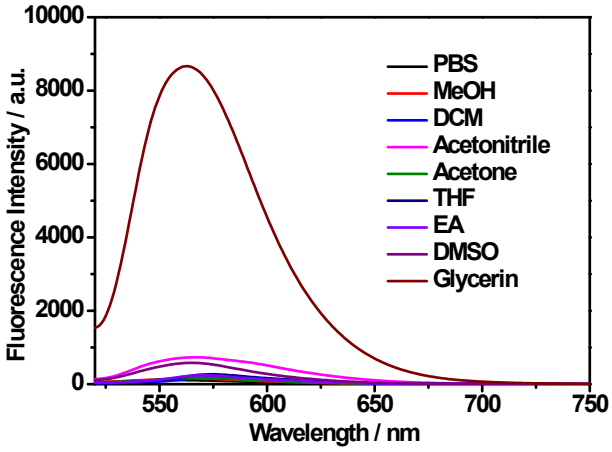


Fig. S4 Fluorescence spectra of Lyso-VR2 in different solvents.

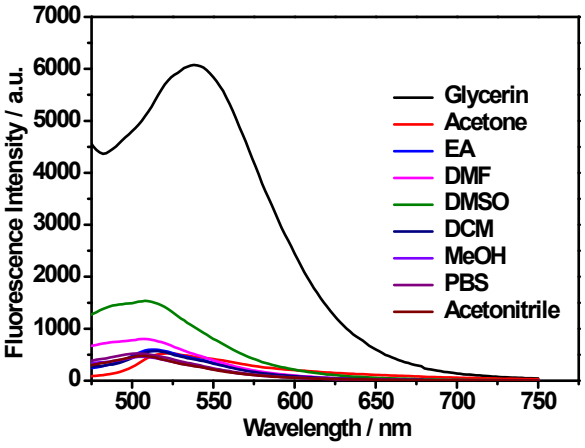


Fig. S5 Fluorescence spectra of **Lyso-VR3** in different solvents.

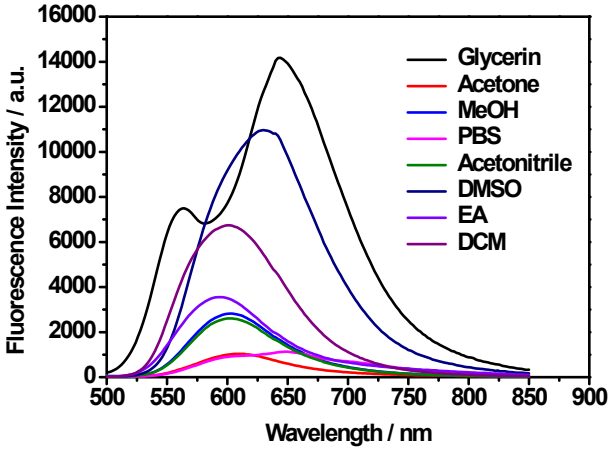


Fig. S6 Fluorescence spectra of **Lyso-VR4** in different solvents.

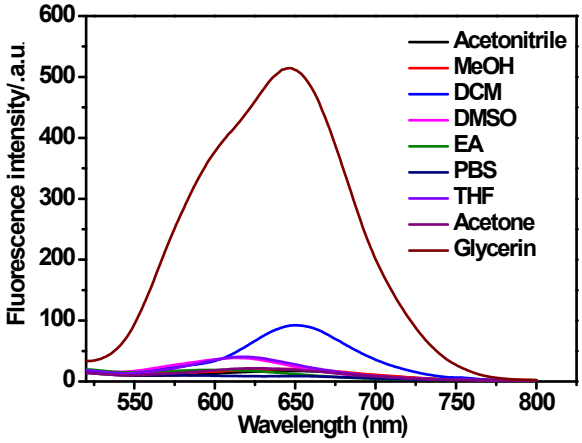


Fig. S7 Fluorescence spectra of **Mito-VR1** in different solvents.

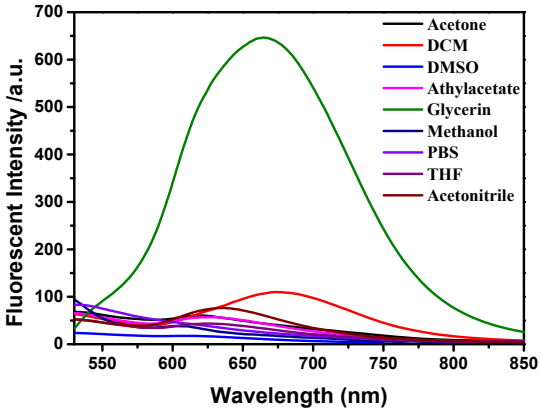


Fig. S8 Fluorescence spectra of **Mito-VR2** in different solvents.

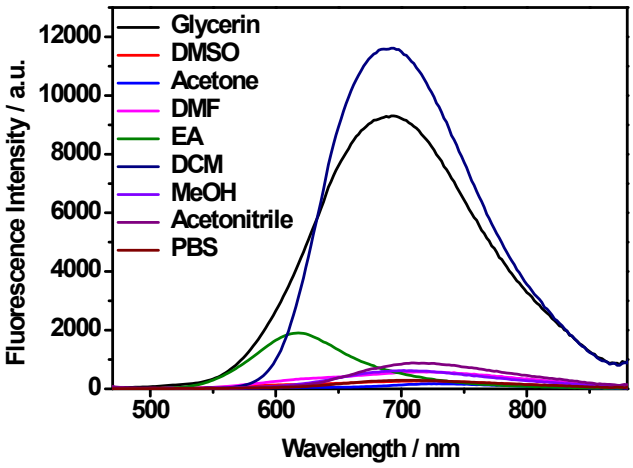


Fig. S9 Fluorescence spectra of **Mito-VR3** in different solvents.

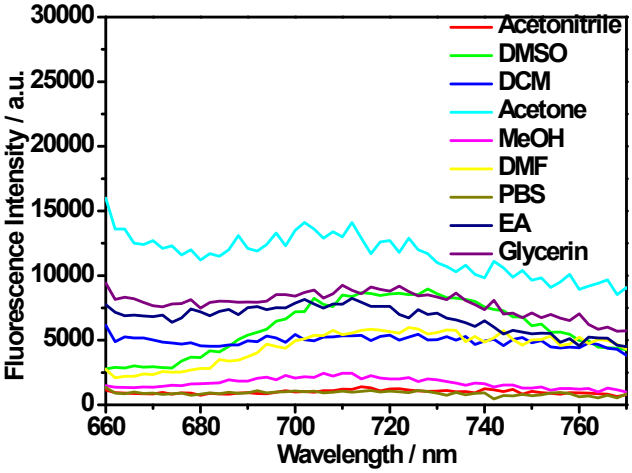


Fig. S10 Fluorescence spectra of **Mito-VR4** in different solvents.

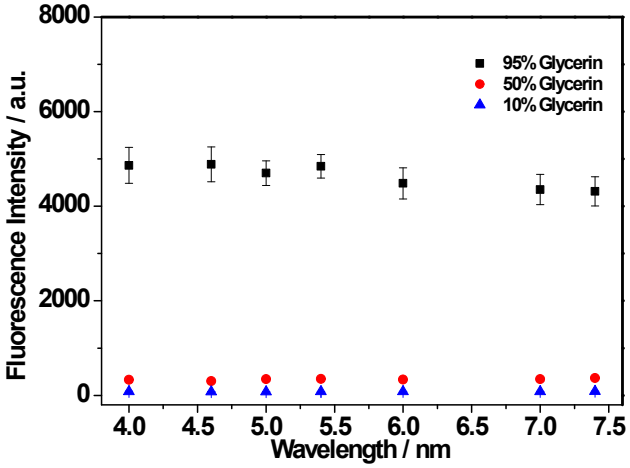


Fig. S11. Fluorescence intensity of **Lyso-VR1** at 550 nm in different pH values in solutions of different viscosity**.**

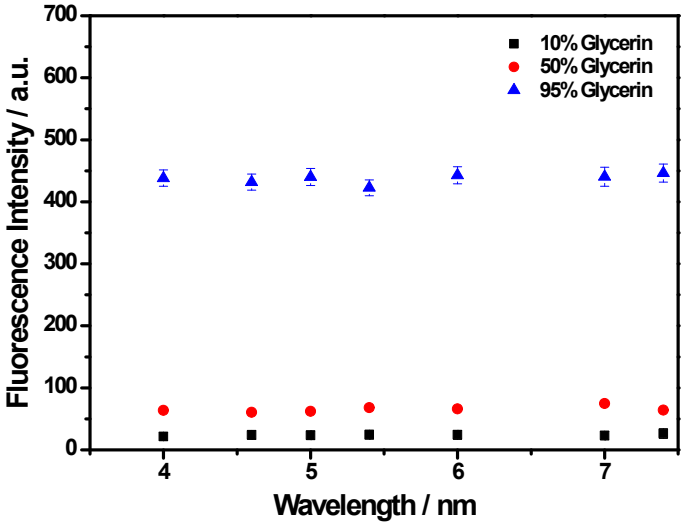


Fig. S12 Fluorescence intensity of **Mito-VR2** at 660 nm in different pH values in solutions of different viscosity**.**

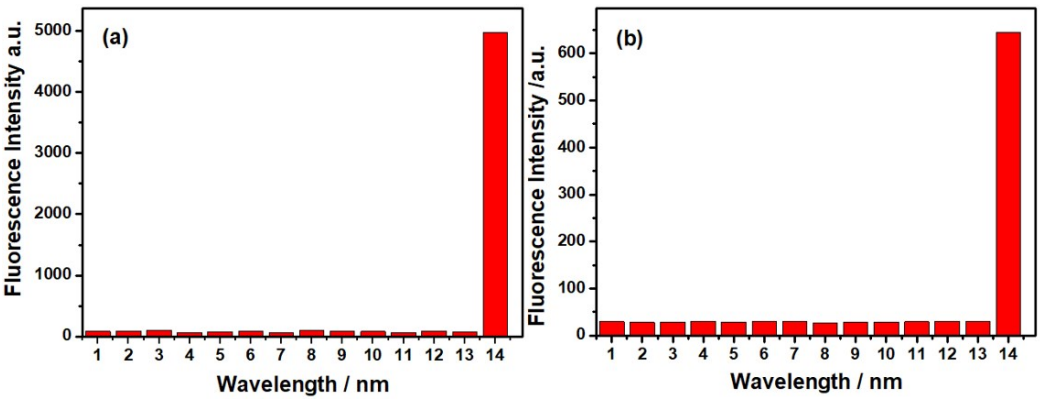


Fig. S13 Fluorescence intensity of **Lyso-VR1** at 550 nm (a), and **Mito-VR2** at 660 nm (b) in the presence of (1) $CaCl_2$ (10 mM), (2) K_2CO_3 (10 mM), (3) KF (10 mM), (4) $MgCl_2$ (10 mM), (5) Na_2HPO_4 (10 mM), (6) Na_2SO_4 (10 mM), (7) NaOAc (10 mM), (8) NaCl (150 mM), (9) $NaHCO_3$ (10 mM), (10) $NaNO_3$ (10 mM), (11) $ZnCl_2$ (10 mM), (12) cysteine (1 mM), (13) glutathione (10 mM), (14) glycerin (99%).

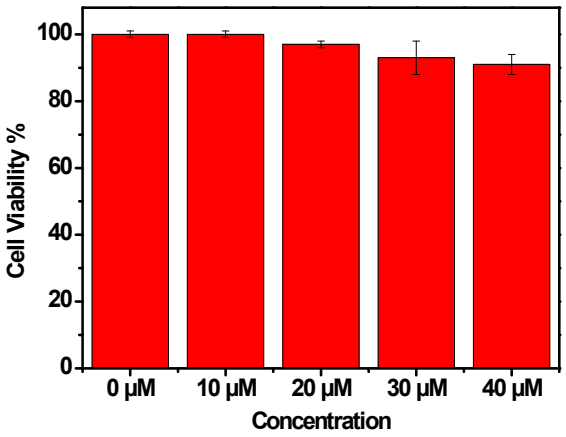


Fig. S14 Cytotoxicity assessment of **Lyso-VR1** by a WST assay in 1640 for 24 h.

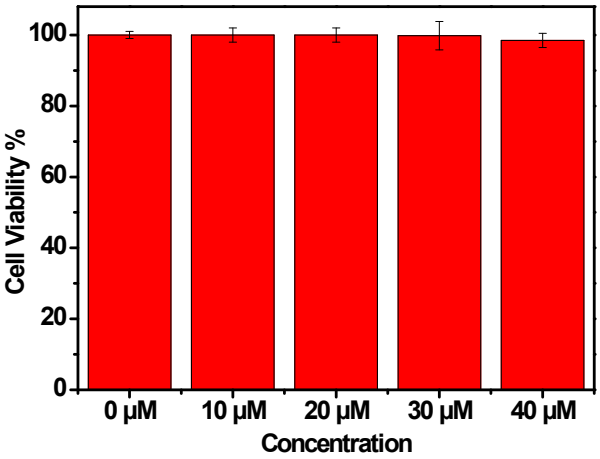


Fig. S15 Cytotoxicity assessment of **Mito-VR2** by a WST assay in 1640 for 24 h.

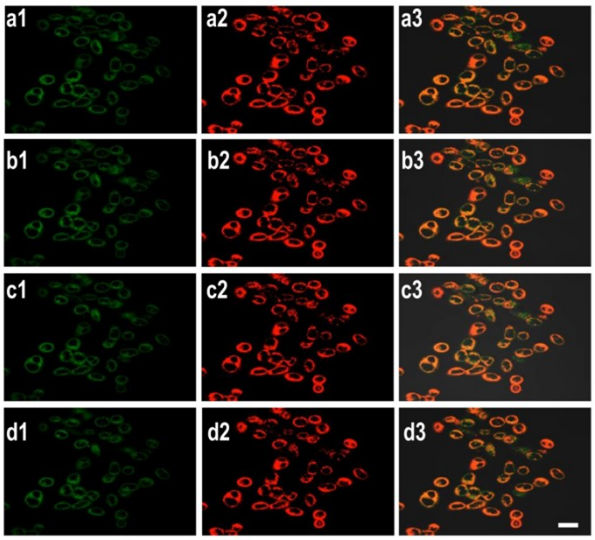


Fig. S16 Confocal fluorescence images of cells incubated with Lyso-VR1 (a1-d1) and Mito-VR2 (a2-d2) in normal medium at 30 min (a1-**a**3), 60 min (b1-b3), 90 min (c1-c3) and 120 min (d1-d3). Scale bar=20 μm.

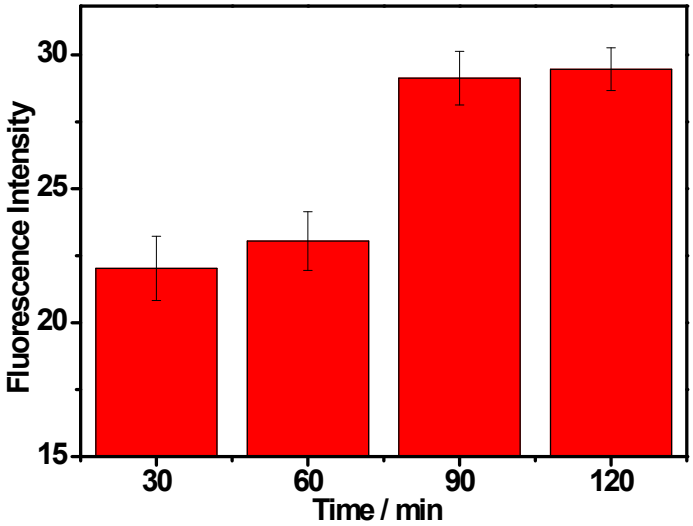


Fig. S17 Averaged fluorescence intensity of five typical regions in fluorescence images of HeLa cells for Figure 4 (a1-a4) in the manuscript. Analysis was conducted by ImageJ software. Error bars represent SD.

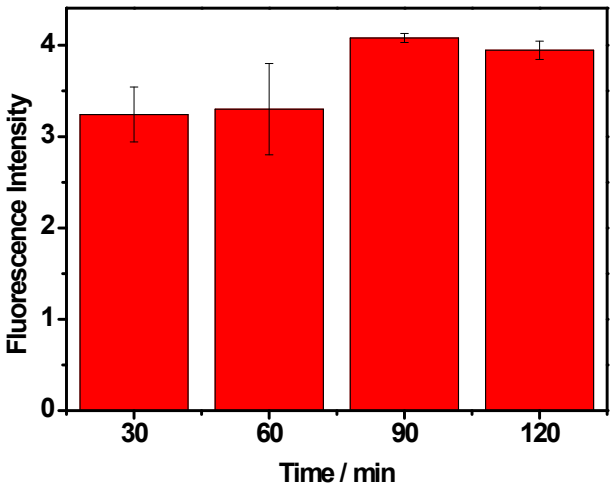


Fig. S18 Averaged fluorescence intensity of five typical regions in fluorescence images of HeLa cells for Figure 4 (b2-b4) in the manuscript. Analysis was conducted by ImageJ software. Error bars represent SD.

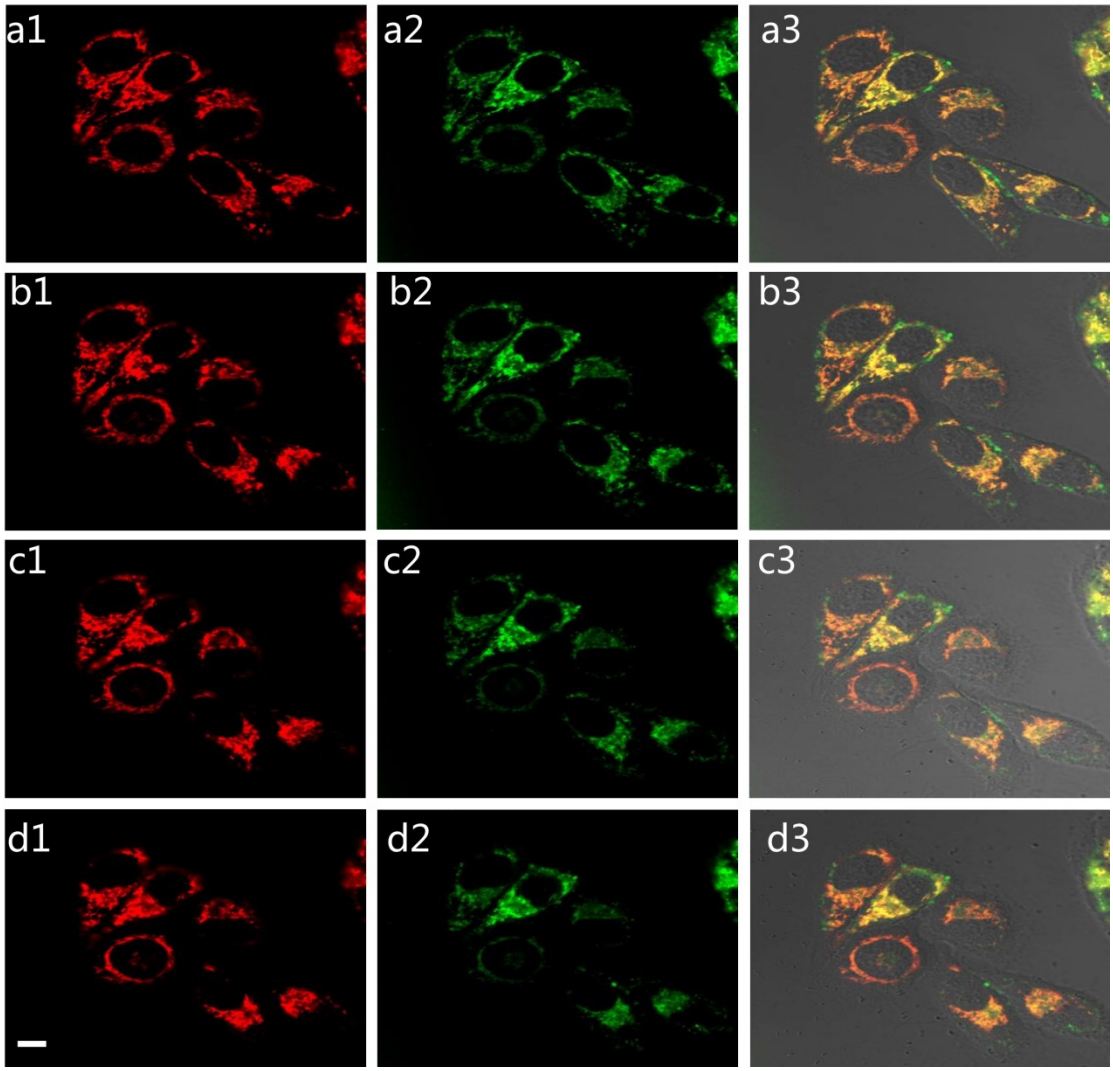


Fig. S19. Confocal fluorescence images of cells incubated with **Mito-VR2** (a1-d1) and Mito-Tracker Green (a2-d2) during starvation-induced autophagy at 30 min, 60 min, 90 min and 120 min. Scale bar=20 μm.

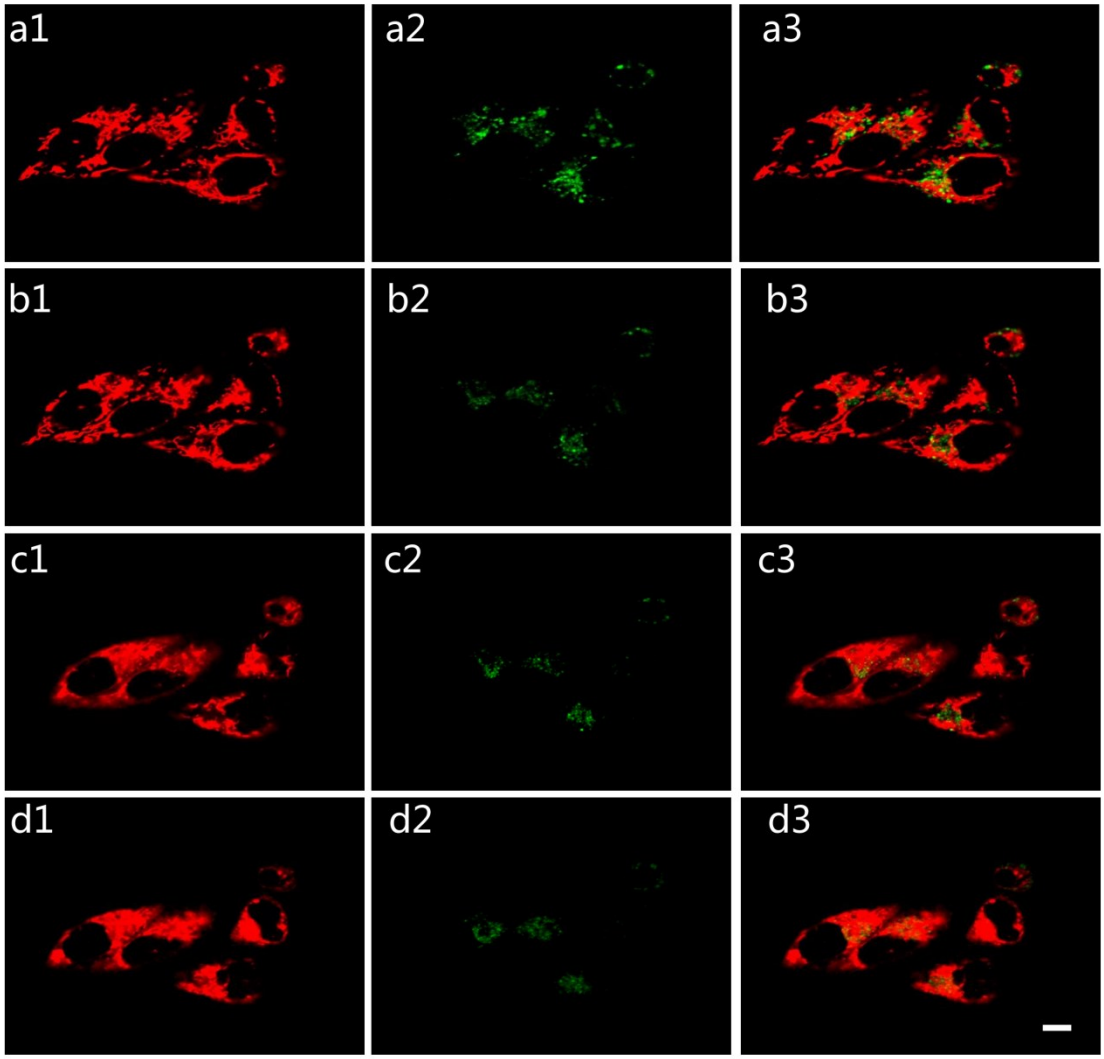


Fig. S20. Confocal fluorescence images of cells incubated with **Mito-VR2** (a1-d1) and Lyso-Tracker Green (a2-d2) during starvation-induced autophagy at 30 min (a1-a3), 60 min (b1-b3), 90 min (c1-c3) and 120 min (d1-d3). scale bar=20 μm.

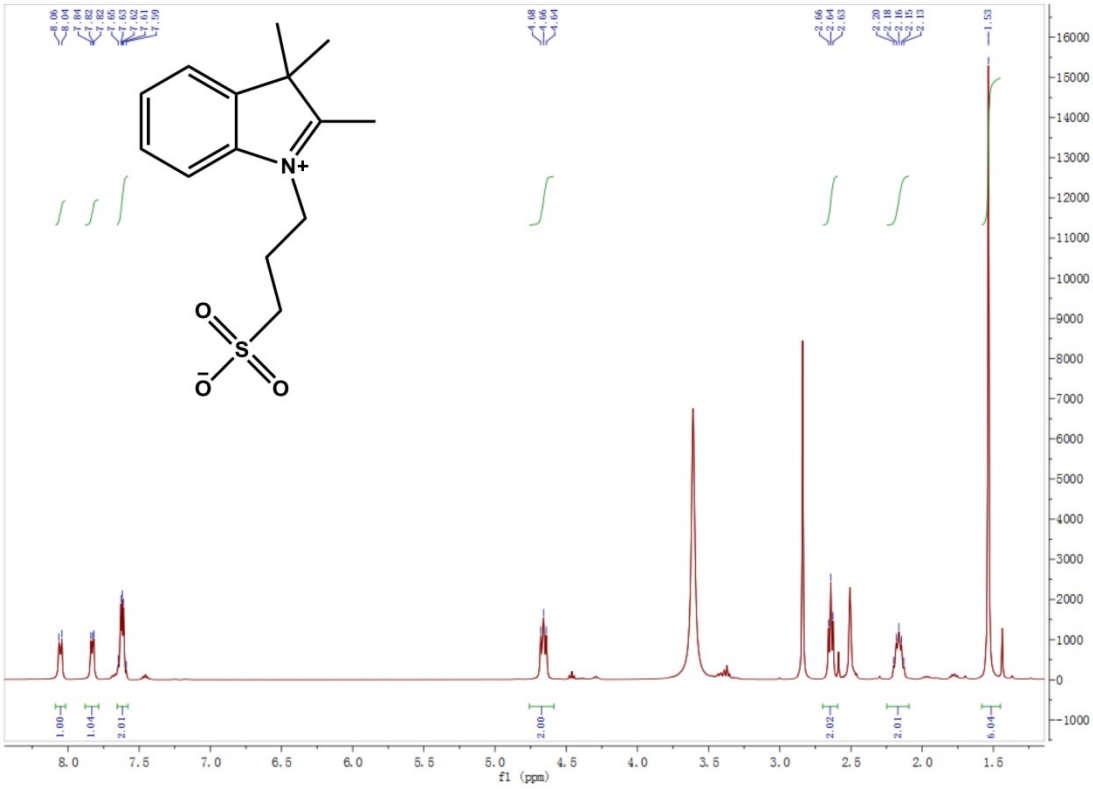


Fig. S21 ^{1}H NMR spectrum of 3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)-propane-1-sulfonate.

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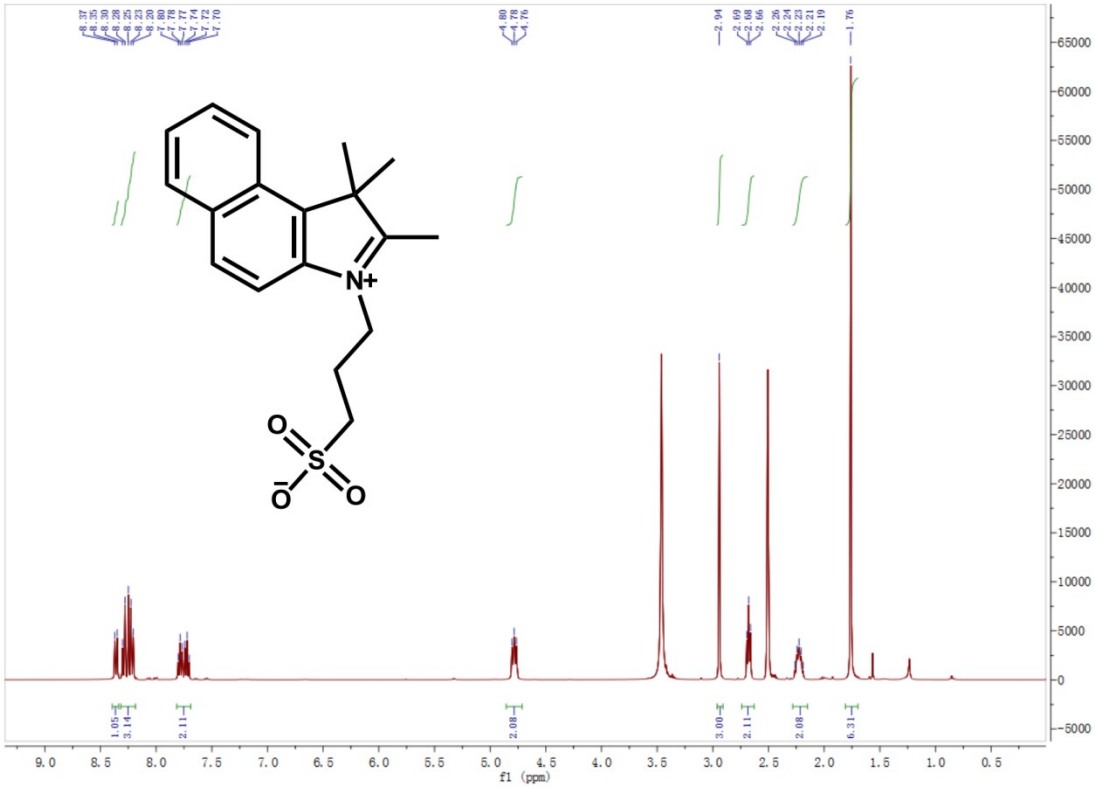

Fig. S22 ^{1}H NMR spectrum of 3-(2,3,3-trimethyl-3H-benzo[g]indol-1-ium-1-yl)-propane-1-sulfonate.

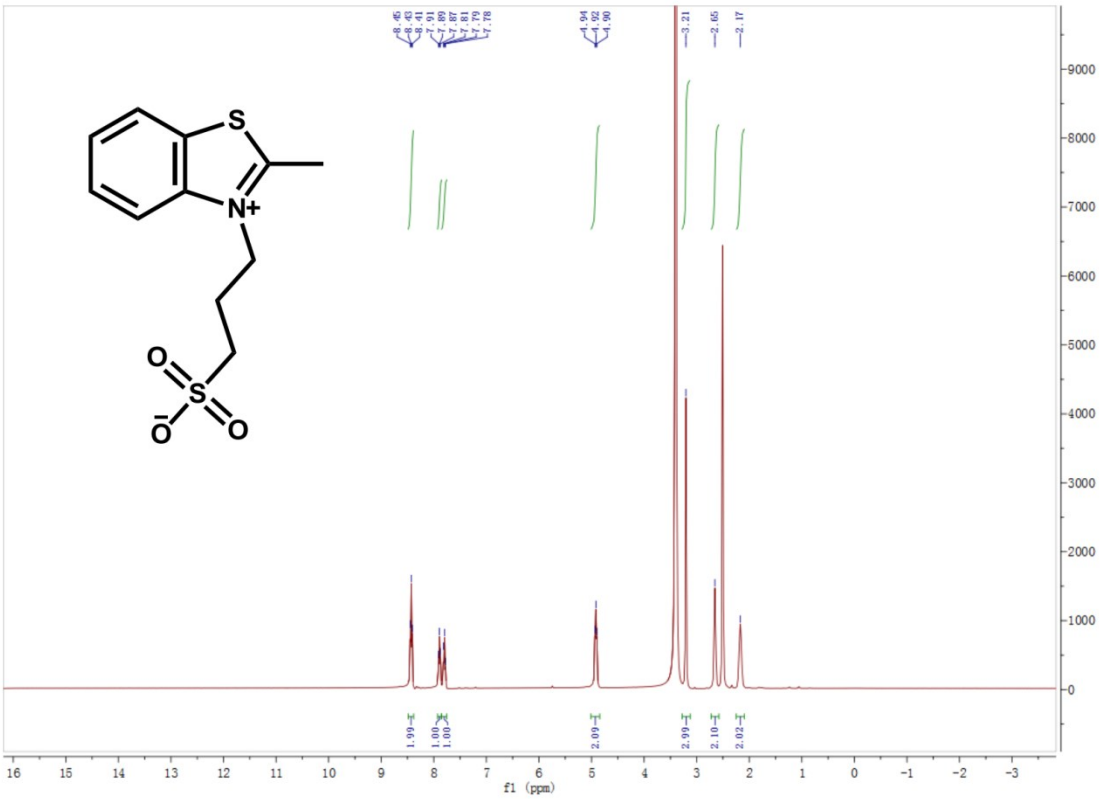

Fig. S23 ^{1}H NMR spectrum of 2,3-dimethylbenzo[d]thiazol-3-ium.

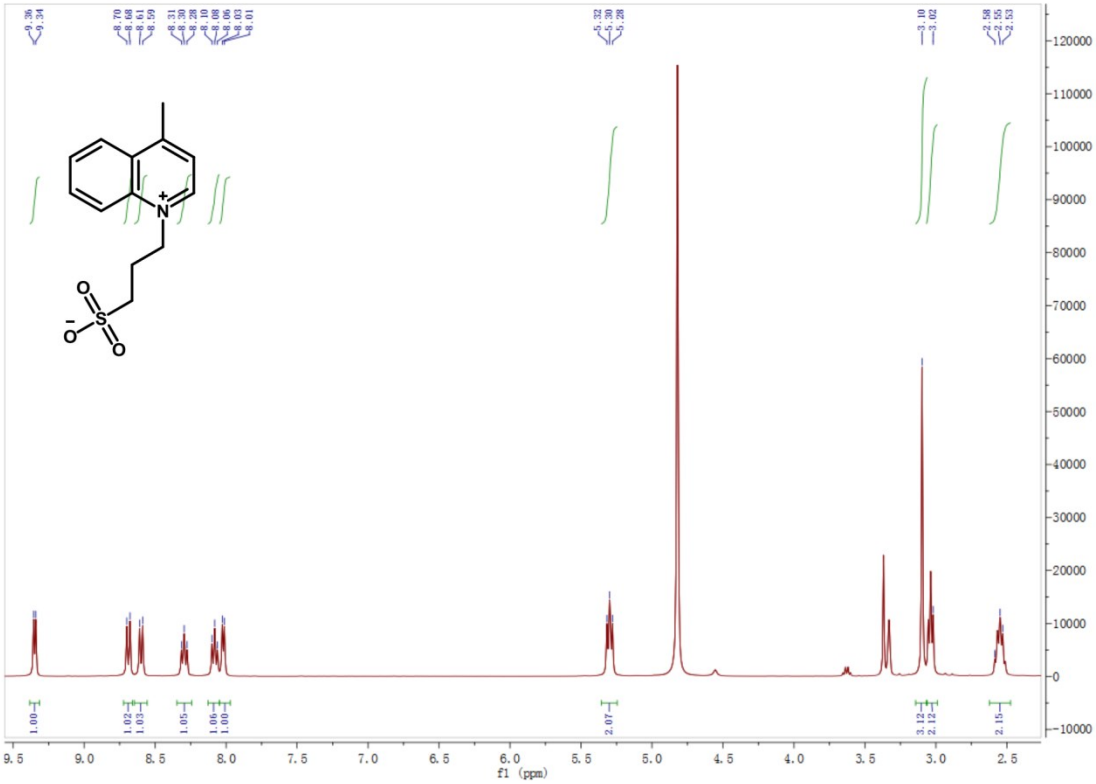

Fig. S24 ^{1}H NMR spectrum of 3-(4-methylquinolin-1-ium-1-yl)-propane-1-sulfonate.

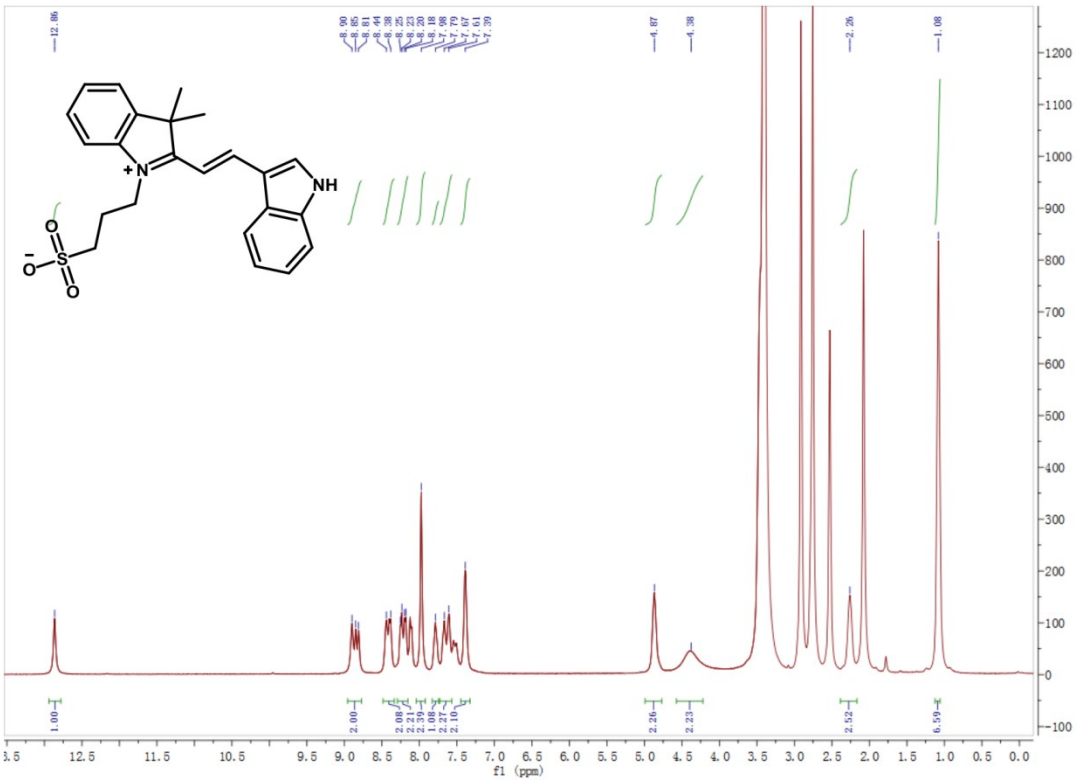

Fig. S25 ^{1}H NMR spectrum of **Lyso-VR1**

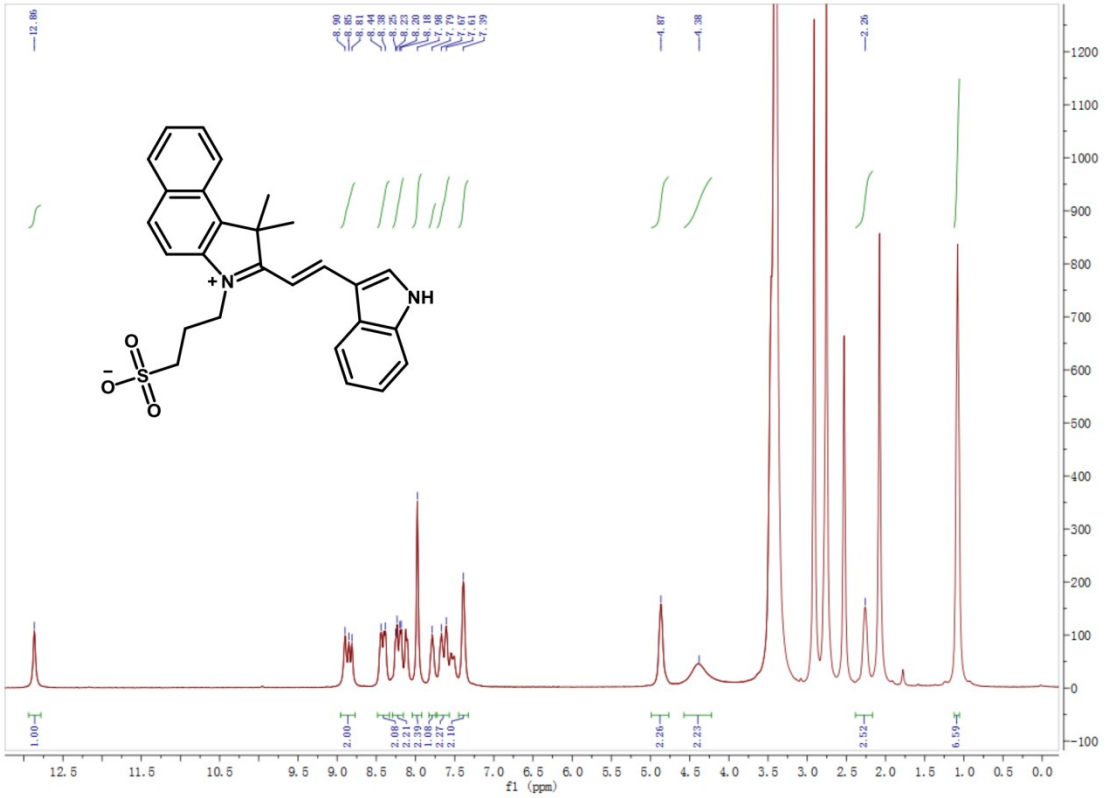

Fig. S26 ^{1}H NMR spectrum of **Lyso-VR2**

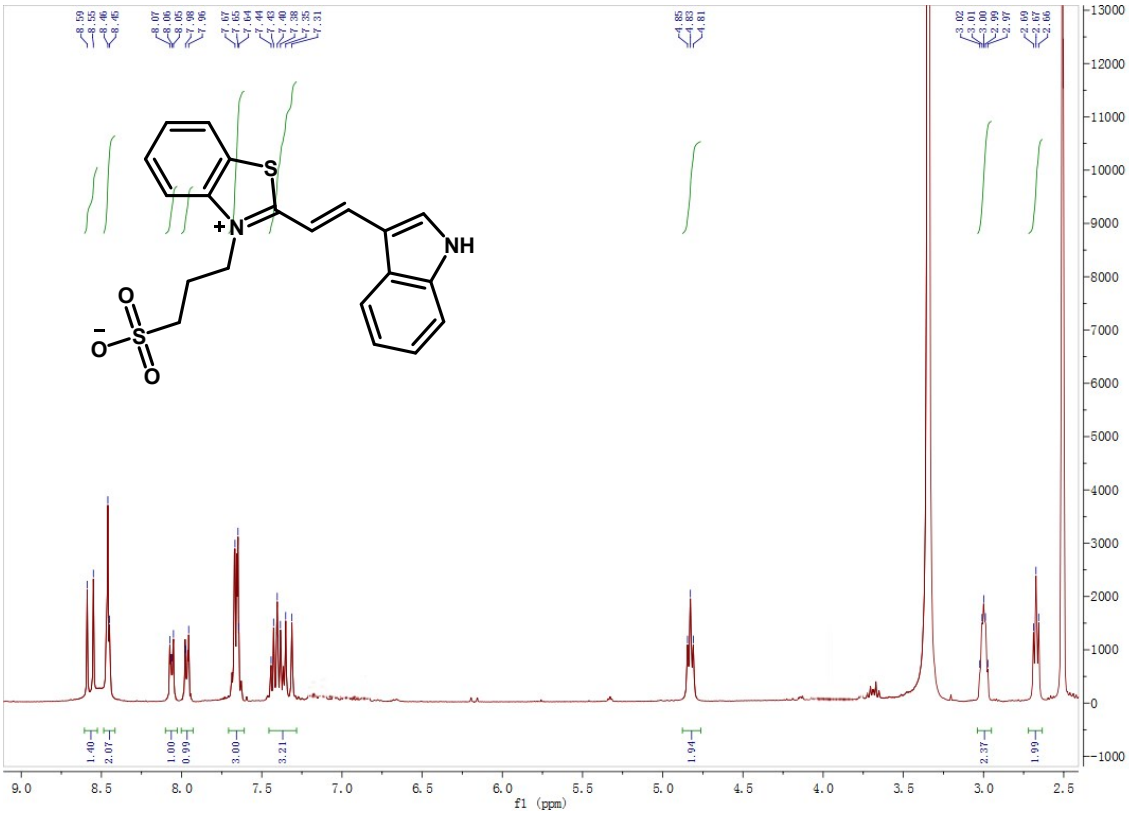

Fig. S27 ^{1}H NMR spectrum of **Lyso-VR3**

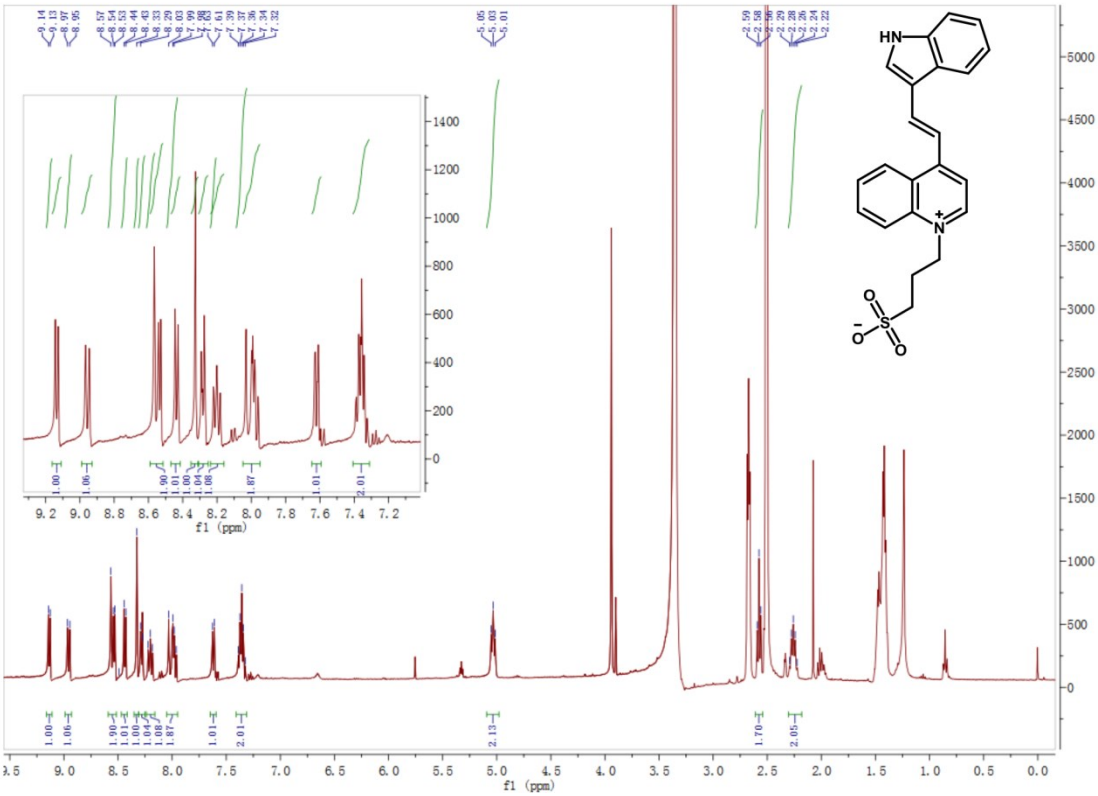

Fig. S28 ^{1}H NMR spectrum of **Lyso-VR4**

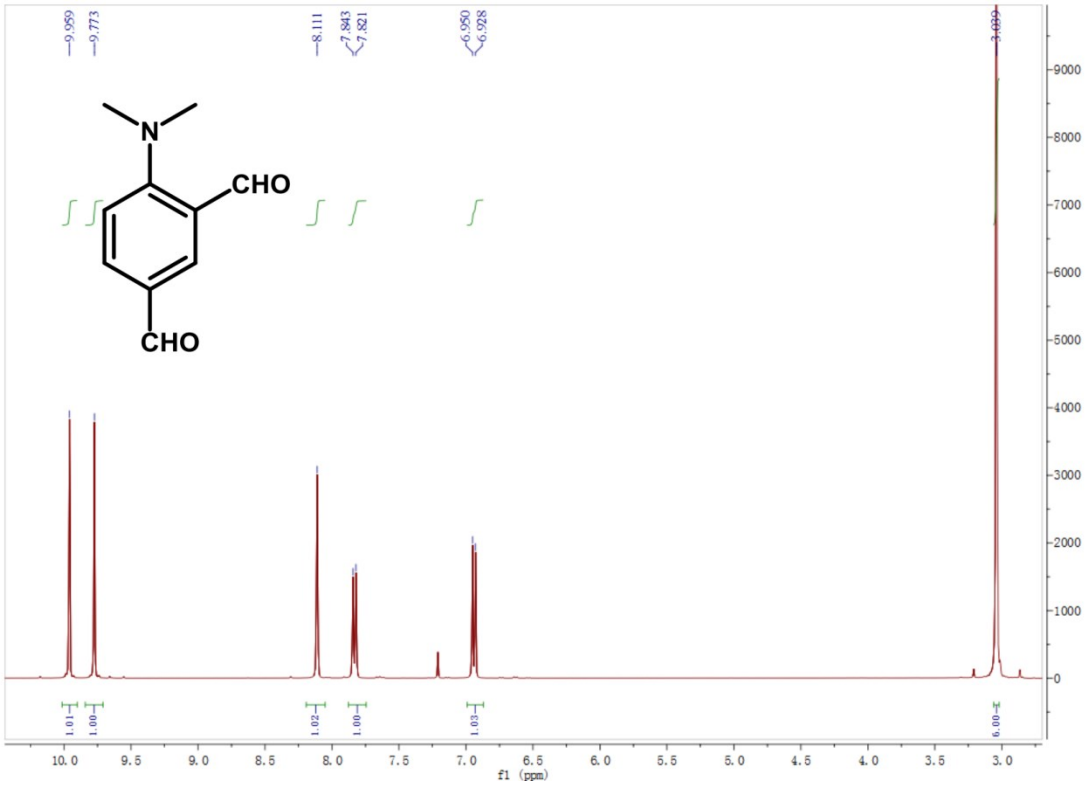

Fig. S29 ^{1}H NMR spectrum of 4-(dimethylamino)-isophthalaldehyde.

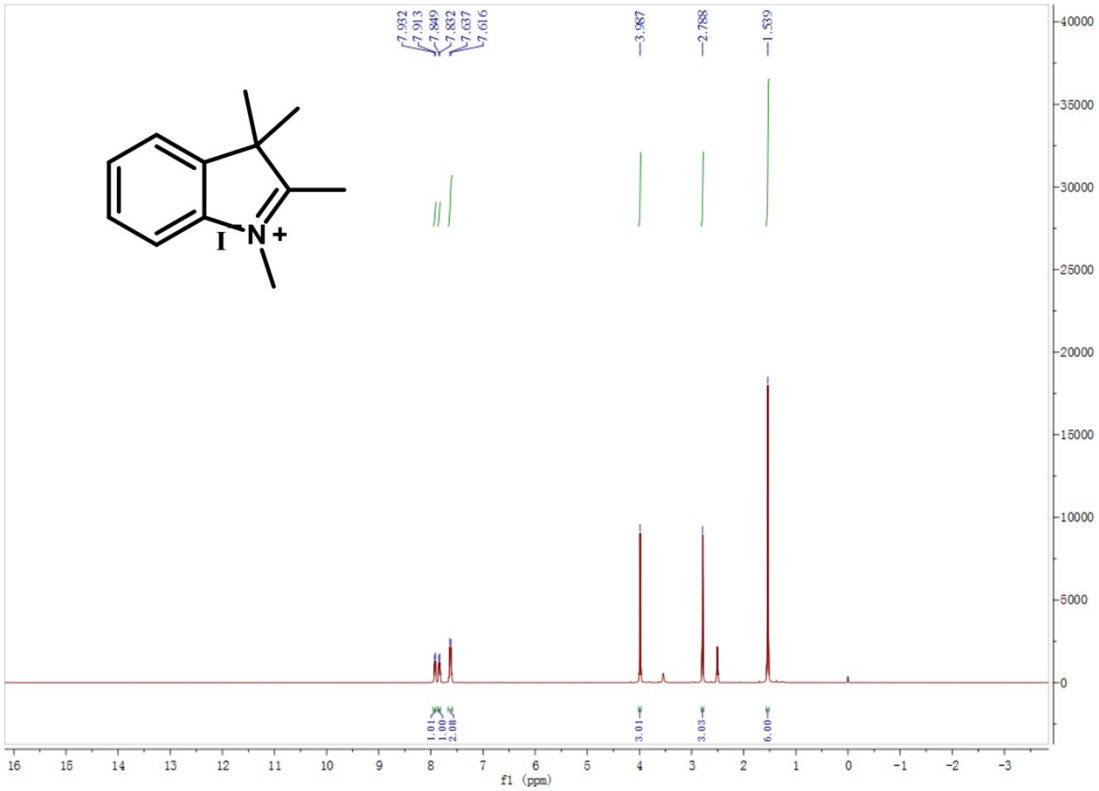

Fig. S30 ^{1}H NMR spectrum of 1,2,3,3-tetramethyl-3H-indol-1-ium.

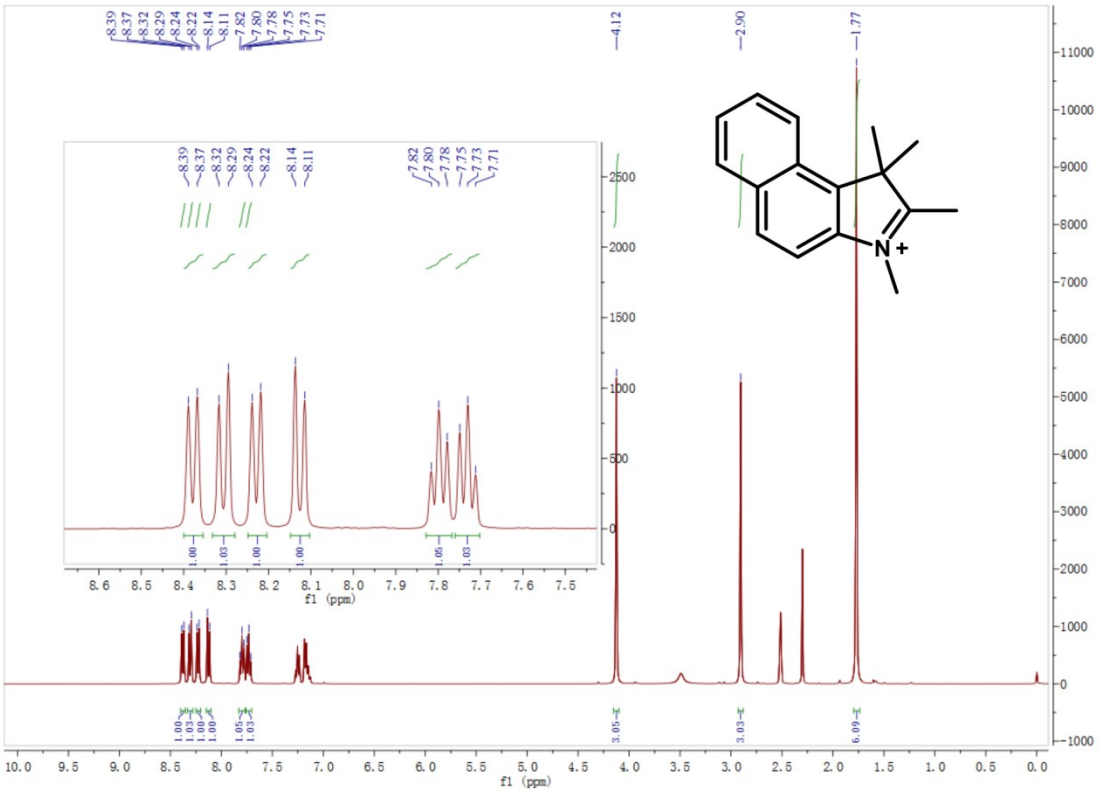

Fig. S31 ^{1}H NMR spectrum of 1,2,3,3-tetramethyl-3H-benzo[g]indol-1-ium iodide.

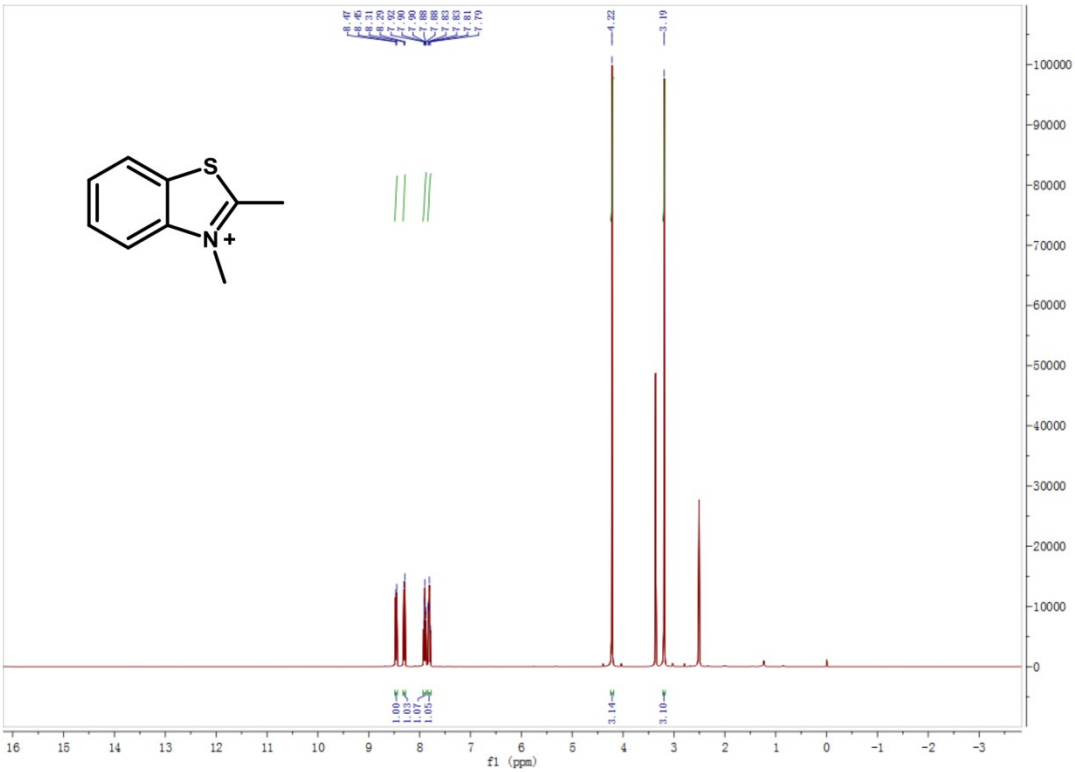

Fig. S32 ^{1}H NMR spectrum of 2,3-dimethylbenzo[d]thiazol-3-ium.

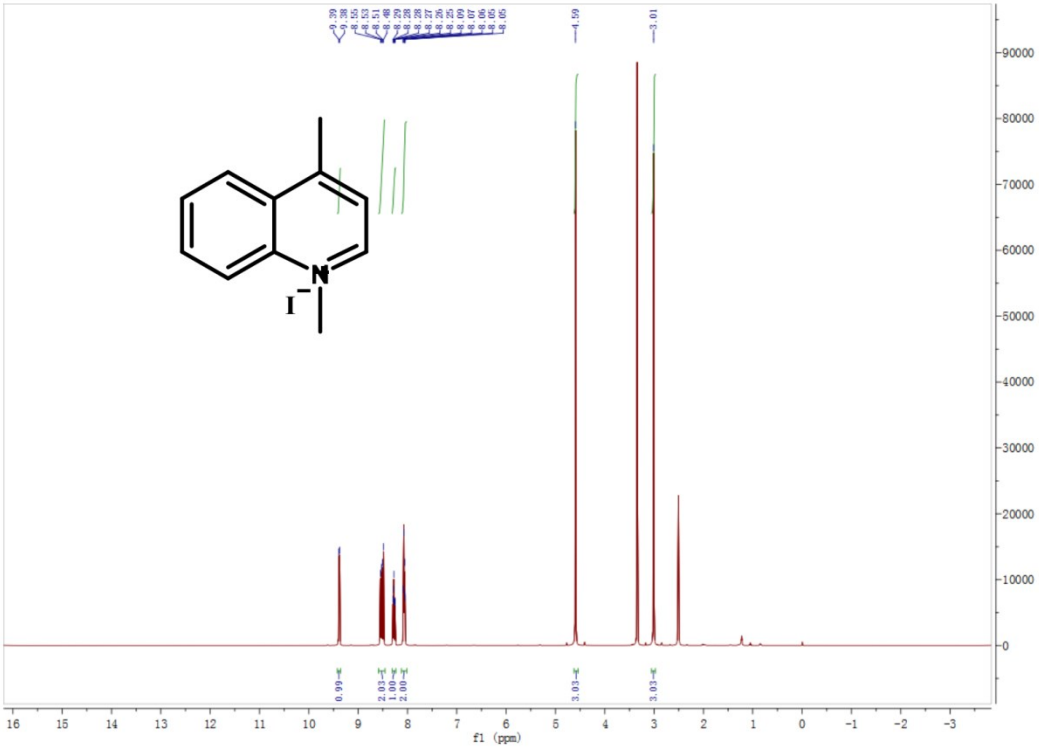

Fig. S33 ^{1}H NMR spectrum of 1,4-dimethylquinolin-1-ium.

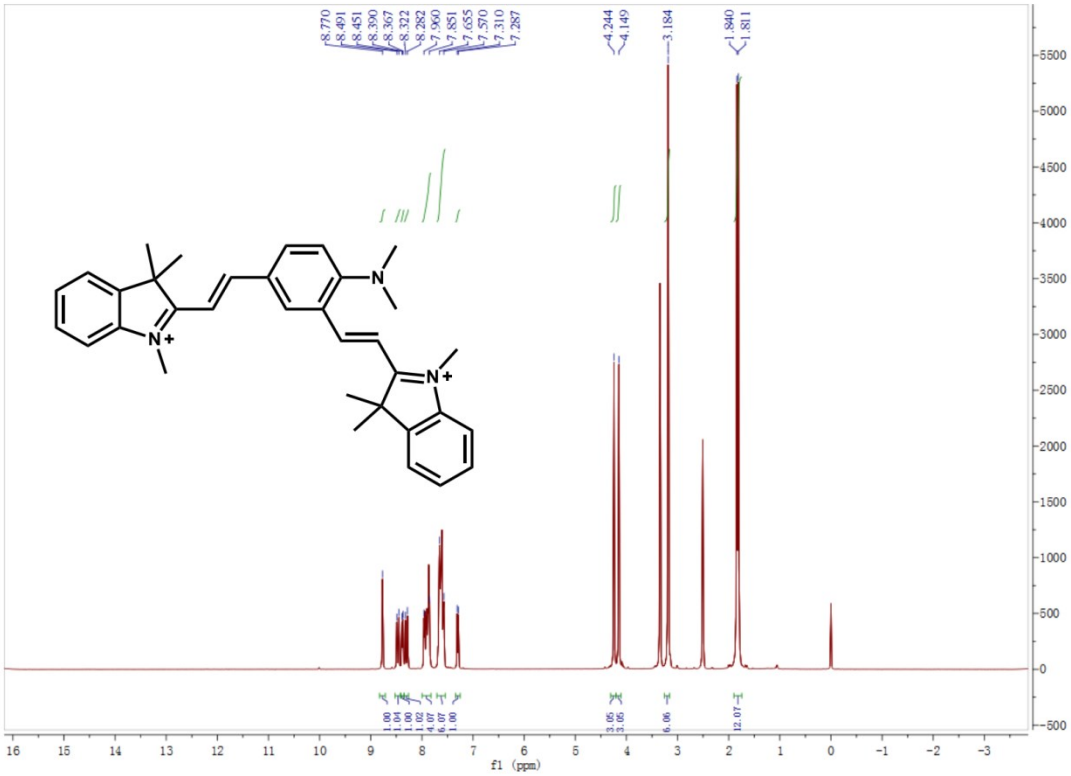

Fig. S34 ^{1}H NMR spectrum of **Mito-VR1**.

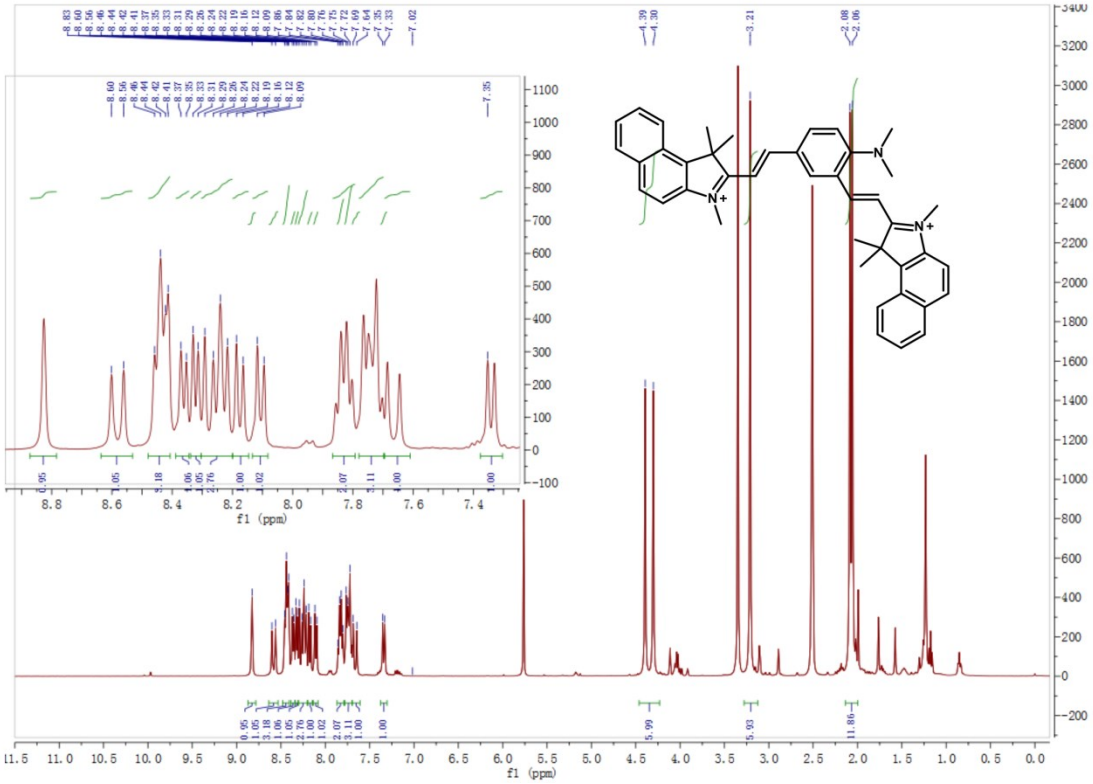

Fig. S35 ^{1}H NMR spectrum of **Mito-VR2**.

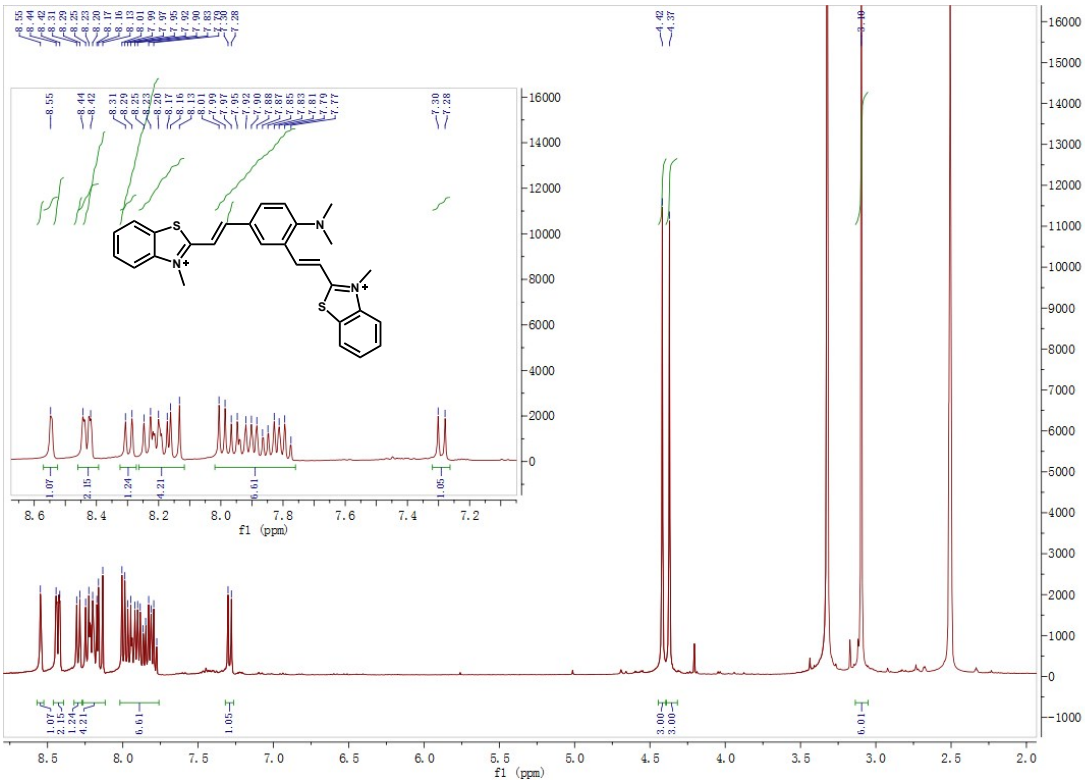

Fig. S36 ^{1}H NMR spectrum of **Mito-VR3**.

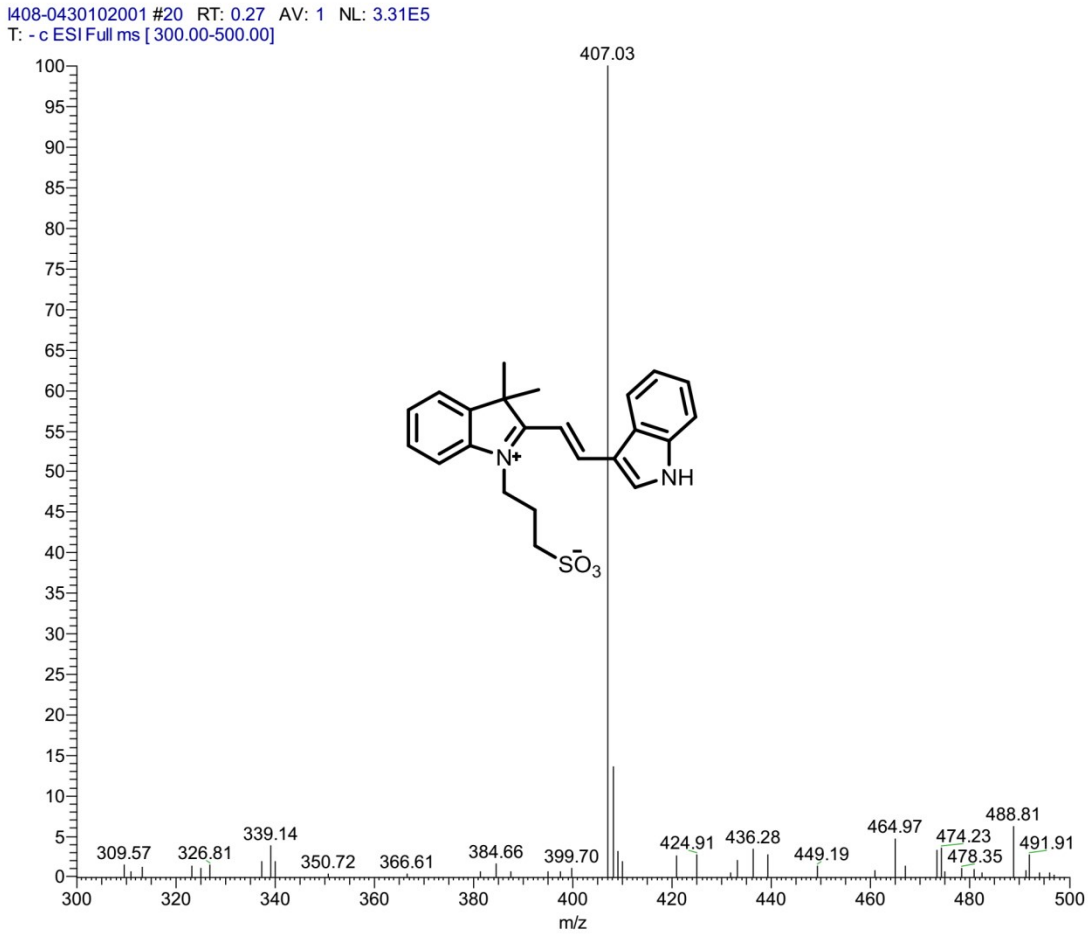

Fig. S37 Mass spectrum of **Lyso-VR1**.

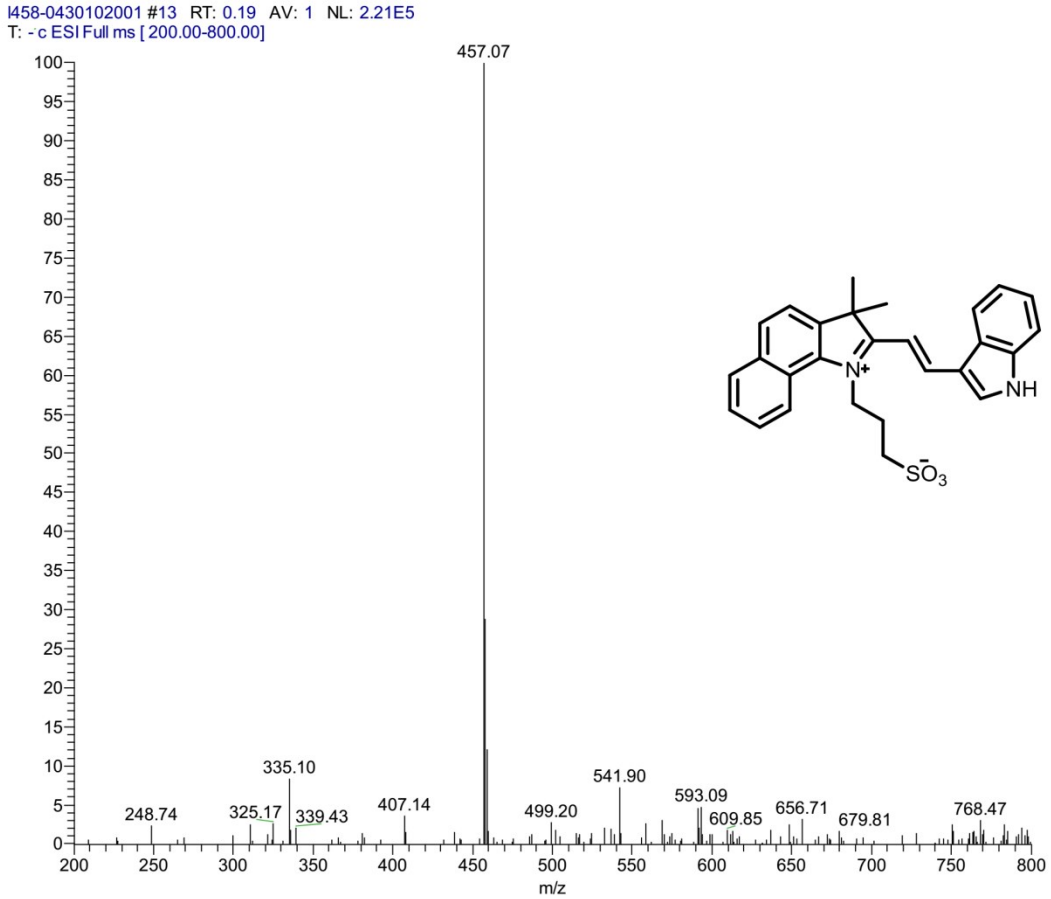

Fig. S38 Mass spectrum of **Lyso-VR2**.

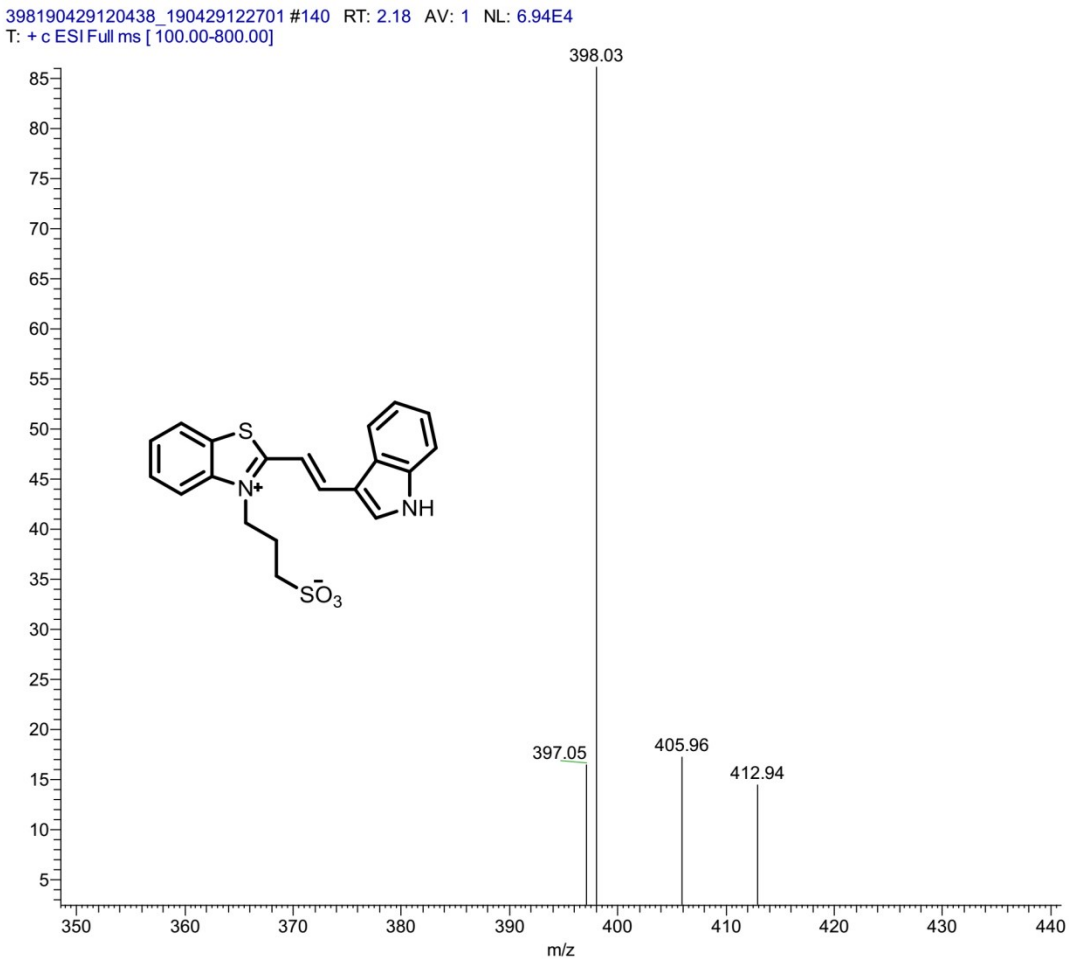

Fig. S39 Mass spectrum of **Lyso-VR3**.

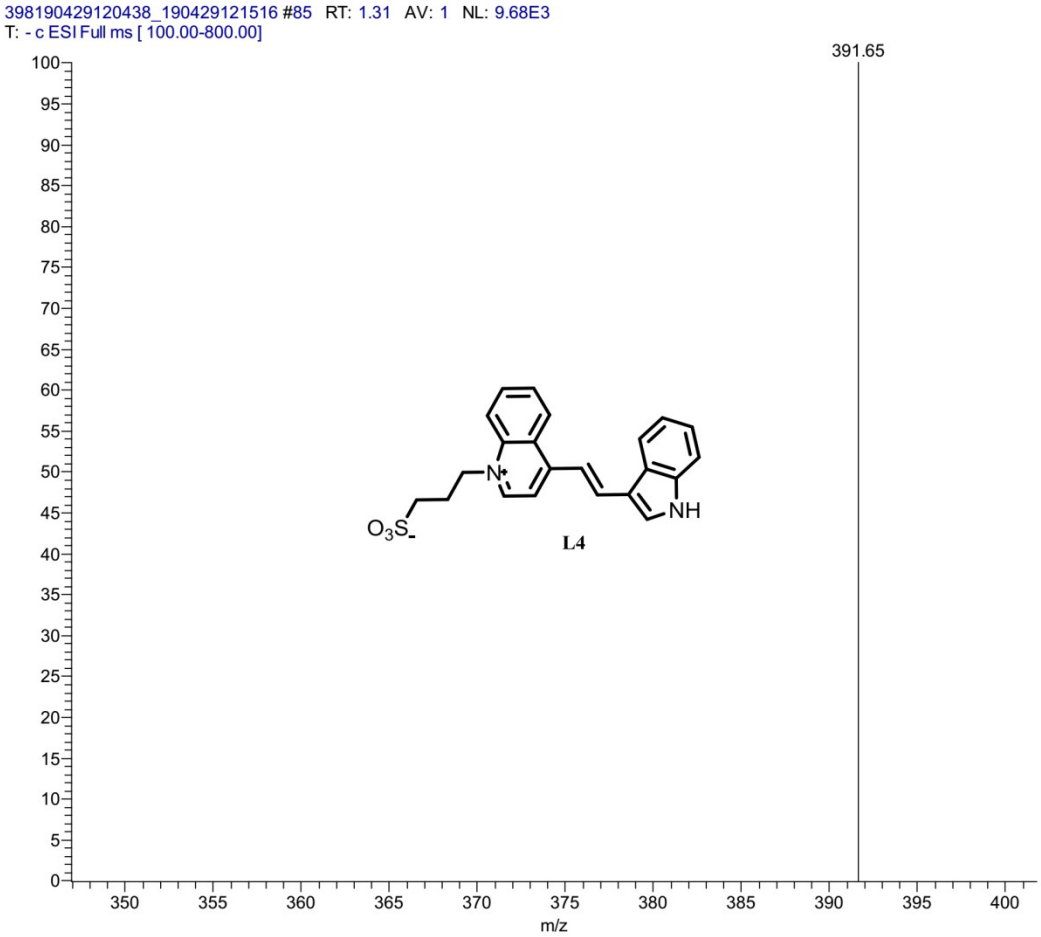

Fig. S40 Mass spectrum of **Lyso-VR4**.

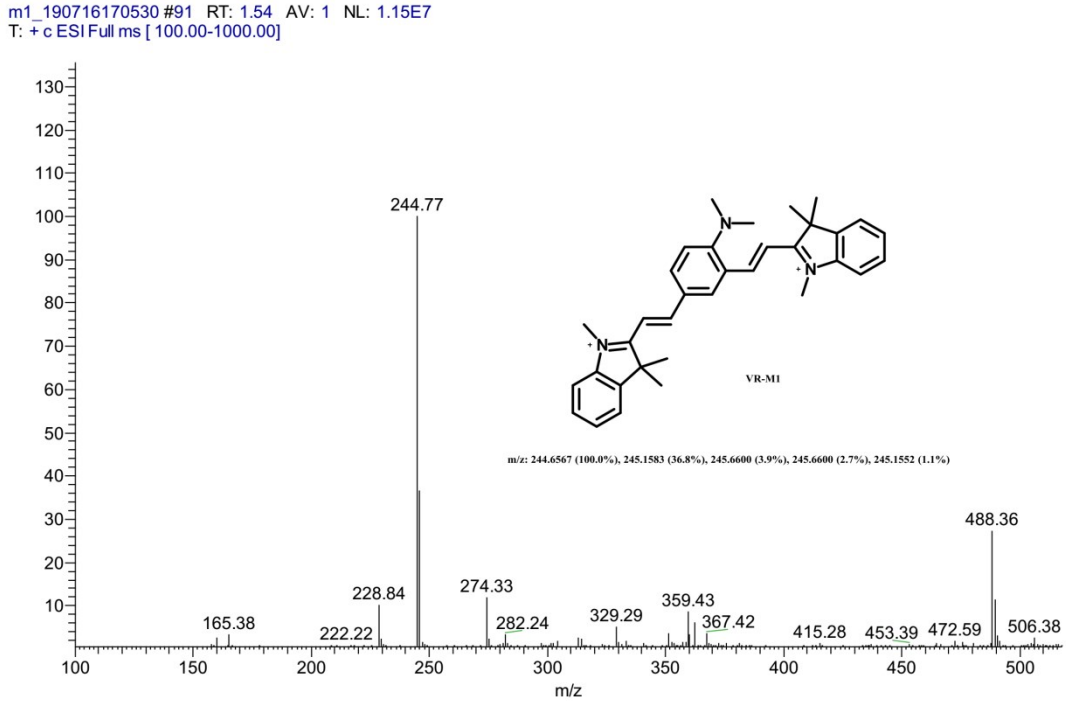

Fig. S41 Mass spectrum of **Mito-VR1**.

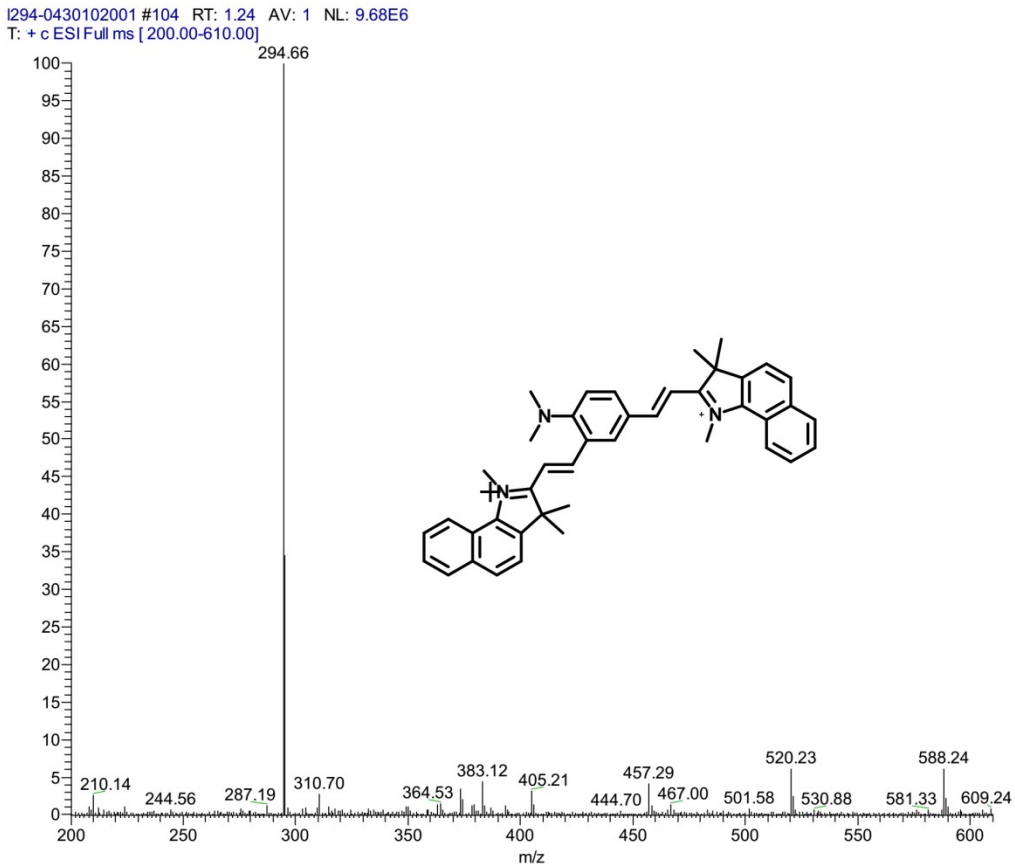

Fig. S42 Mass spectrum of **Mito-VR2**.

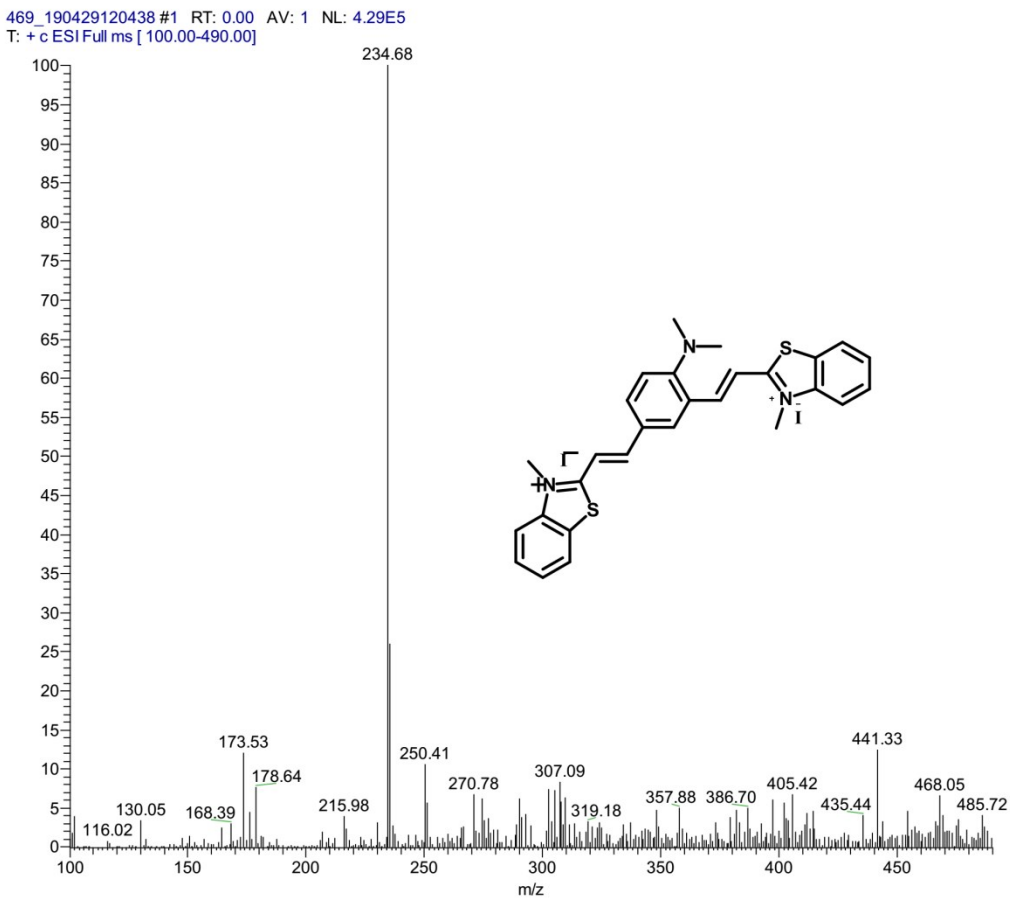

Fig. S43 Mass spectrum of **Mito-VR3**.

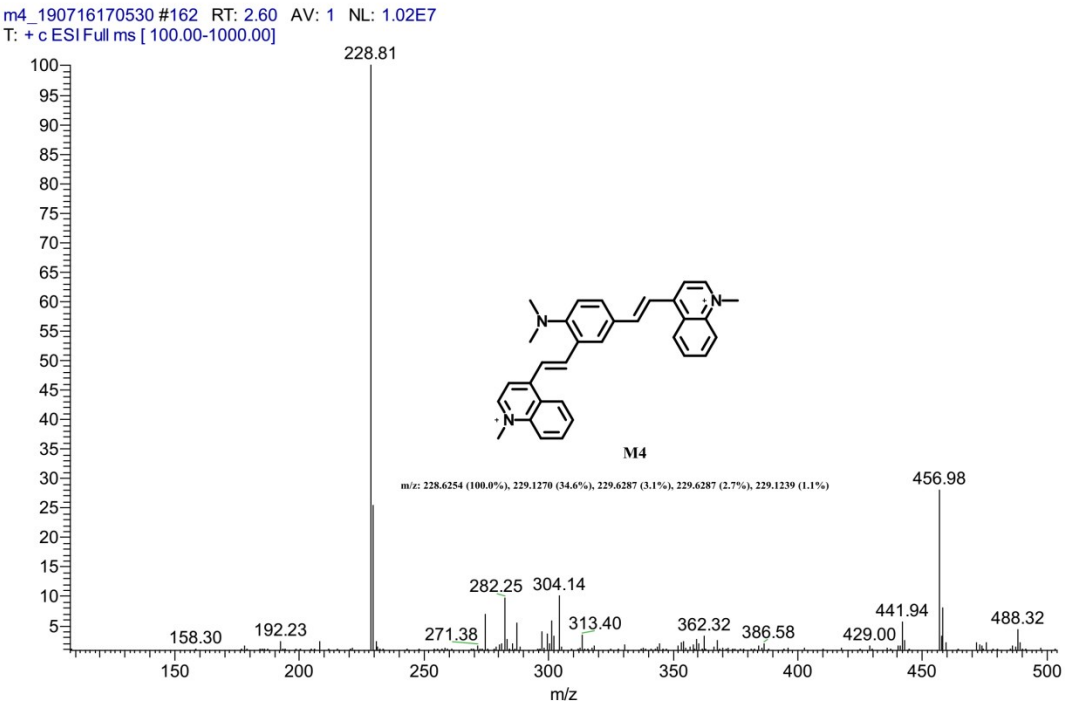

Fig. S44 Mass spectrum of **Mito-VR4**.