Supporting Information

Water-soluble fluorescent probes for differentiating cancer cells and normal cells by tracking lysosomal viscosity

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Contents

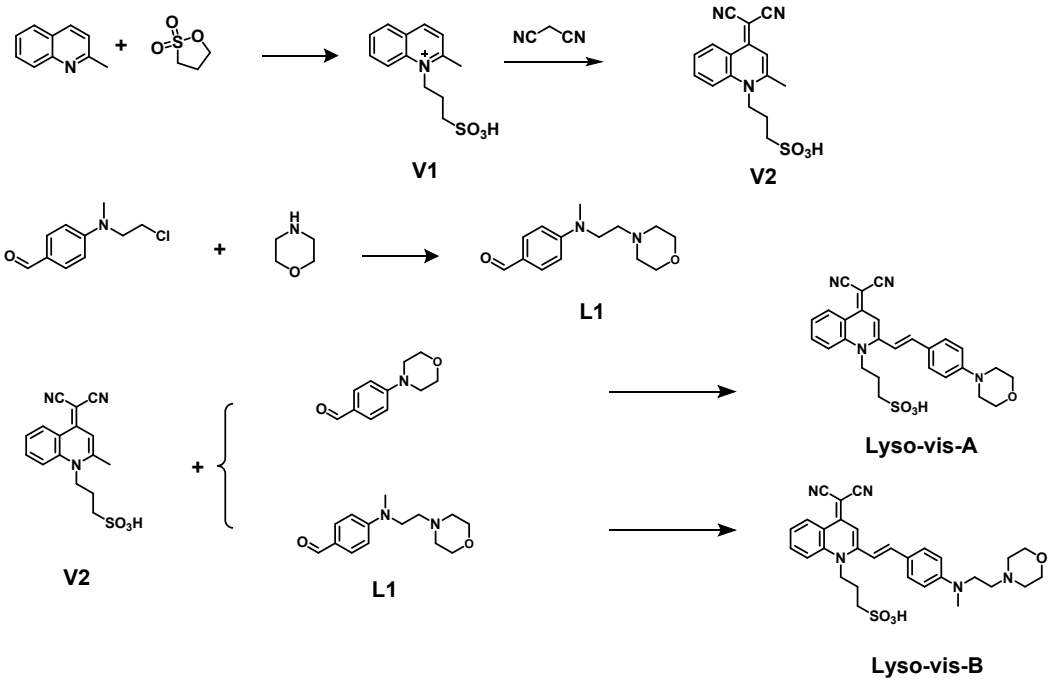
1. Experiment

1.1 Materials and methods

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker 400 MHz spectrometer. Mass spectrometry was performed with PC Sciex API 150EX ESI-MS system and Thermo TSQ Endura Triple Quadrupole Mass Spectrometer. UV-vis absorption spectra were recorded on Shimadzu UV-3600 Plus UV-VIS-NIR Spectrophotometer. Fluorescence spectra were acquired with a FluoroMax-4 fluorescence photometer. Cell imaging was performed by a ZEISS LSM 800 With Airscan Confocal Laser Scanning Microscope. Ultra-performance liquid chromatography (UPLC) was carried out on a Waters ACQUITY UPLC H-CLASS. Milli-Q water was applied in all experiments.

Metal ion (Cu^{2+}, Cu^{+}, Fe^{2+}, Zn^{2+}, K^{+}, Ca^{2+}) stock solutions (500 μM) were obtained by diluting the standard solutions of the corresponding nitrate salt, respectively. The anions (NO_2^-, NO_3^-, SO_3^{2-}, HSO_3^-, HCO_3^-, HClO) stock solutions (500 μM) were prepared by diluting the standard solutions of the corresponding sodium salt, respectively.

1.2 Synthesis and Characterization of Lyso-vis-A and Lyso-vis-B



Scheme S1 The synthetic route of **Lyso-vis-A** and **Lyso-vis-B**.

1.2.1 Synthesis of intermediates

The intermediates **V1, V2** and **L1** were prepared following the procedure shown in Scheme S1 using the method described in previous report.

1.2.2 Synthesis of Lyso-vis-A and Lyso-vis-B

Synthesis of Lyso-vis-A. V2 (0.56 mmol, 200 mg) and 4-Morpholinobenzaldehyde (0.68 mmol, 129.8 mg), piperidine (50 μL) was added into 5 mL of acetonitrile, and was refluxed for 20 h under argon. After completion, acid was added into mixture and the solid was isolated by filtration. The product was purified by column chromatograph with dichloromethane and methanol (v:v = 10:1) to give a red solid of **Lyso-vis-A** (0.08 g, 26%). ^{1}HNMR (400 MHz, DMSO-d6) δ 8.92 (dd, J = 8.5, 1.3 Hz, 1H), 8.22 (d, J = 8.9 Hz, 1H), 7.89 (t, J = 8.0 Hz, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.62 – 7.56 (m, 1H), 7.53 (d, J = 15.6 Hz, 1H), 7.38 (d, J = 15.6 Hz, 1H), 7.09 (s, 1H), 6.98 (d, J = 8.9 Hz, 2H), 4.74 (t, J = 8.4 Hz, 2H), 3.80 – 3.70 (m, 4H), 3.27 – 3.22 (m, 4H), 2.70 (t, J = 6.3 Hz, 2H), 2.14 (t, J = 8.8 Hz, 2H). ^{13}CNMR (101 MHz, DMSO-d_6) δ 152.05, 150.73, 150.63, 141.57, 138.75, 133.92, 131.02, 125.50, 125.22, 123.32, 121.16, 118.86, 114.54, 112.06, 105.98, 66.44, 53.86, 48.18, 47.76, 45.62, 44.02, 38.81, 25.00, 22.58, 22.21.

Synthesis of Lyso-vis-B. V2 (0.56 mmol, 200 mg) and L1 (0.68 mmol, 168.6 mg), piperidine (50 μL) was added into 5 mL of acetonitrile, and was refluxed for 20 h under argon. After completion, acid was added into mixture and the solid was isolated by filtration. The product was purified by column chromatograph with dichloromethane and methanol (v:v = 10:1) to give a red solid of Lyso-vis-B (0.075 g, 23%). ^{1}HNMR (400 MHz, DMSO-d6) δ 8.91 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.88 (t, J = 8.4 Hz, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.61 – 7.54 (t, 1H), 7.41 (q, J = 15.5 Hz, 2H), 7.11 (s, 1H), 6.73 (d, J = 8.8 Hz, 2H), 4.73 (t, J = 8.6 Hz, 2H), 3.58 (t, 4H), 3.50 (t, J = 5.1 Hz, 2H), 3.01 (s, 3H), 2.69 (t, J = 6.2 Hz, 2H), 2.44 (t, 2H), 2.12 (t, 2H), 1.59 (dt, J = 26.4, 5.4 Hz, 4H). ^{13}CNMR (101 MHz, DMSO-d_6) δ 152.58, 152.30, 150.36, 140.95, 138.68, 134.02, 130.53, 126.16, 125.51, 125.29, 121.14, 118.87, 116.61, 114.71, 66.45,

48.18, 47.87, 44.15, 22.63, 22.16.

1.3 General procedure for absorption and fluorescent measurement

5 mM stock solution of probe **Lyso-vis-A** and **Lyso-vis-B** were prepared in appropriate DMSO firstly. Cys, Hcy and GSH were dissolved in PBS buffer (pH=7.4, 10 mM) to form 500 mM stock solution. $CuCl_2$, CuCl, $ZnCl_2$, $FeCl_2$, K_2CO_3, $CaCl_2$, $NaNO_2$, KNO_3, Na_2SO_3, $NaHSO_3$ and $NaHCO_3$ were prepared as 500 mM stock solution in water. Concentrations of H_2O_2 and HOCl were detected by UV absorption experiment by Beer-Lambert Law. Specifically, the extinction coefficient (ε) of ClO^- and H_2O_2 were 350 M^{-1} cm-1 and 43.6 M^{-1} cm^{-1} as well as the determined absorption was at 292 nm and 240 nm, respectively.

1.4 Determination of viscosity

In this study, we have used mixtures of water (low viscosity solvent) and anhydrous glycerol (high viscosity solvent) in different proportions to change the viscosity of the solution of **Lyso-vis-A** and **Lyso-vis-B** in a broad range without considerable change in the solvent polarity (the dielectric constant). At 20 °C water (polar protic solvent) has a dielectric constant (ε) of 80.18 and viscosity (η) of 1.00 cP, and glycerol (polar protic solvent) has ε=42.5 and η=945.0 cP. Viscosity for the mixtures was calculated using the following equation:

$$\ln(\eta_{mix}) = \sum_i w_i \times \ln(\eta_i)$$

where η_{mix} is the viscosity of the mixture, η_i is the viscosity of each component, and w_i is the weighting factor ($0<w<1$) of each component.

1.5 The Förster-Hoffmann equation

The Förster-Hoffmann equation was utilized to correlate the relationship between the fluorescence emission intensity of the Lyso-MC and the solvent viscosity.

$$\log I = C + x\log\eta$$

in which η is the viscosity, I is the emission intensity, C is a constant and x represents

the sensitivity of the fluorescent probe to the viscosity.

1.6 General procedure of cell culture and MTT assay

MCF-7 cells were cultured in DMEM containing 10% FBS (fetal bovine serum) and 1% antibiotics (penicillin-streptomycin, 10000 U/mL) in the constant-temperature incubation with an atmosphere of 5% CO_2 and 95% air at 37 ℃. The MTT assay was used to measure the cytotoxicity of **Lyso-vis-A** to MCF-7 cells. About 1.0×10^4 cells/well was seeded into a 96-well cell-culture plate. After 24 h, various concentrations of **Lyso-vis-A** (1, 2, 5, 10, 15, 20 and 30 μM) were added to the wells. After another 12 h, 20 μL of MTT (5 mg/mL) was added to each well and incubated at 37 ℃ under 5% CO_2 for 4 h. Remove the MTT solution and purple precipitates (formazan) observed in plates were dissolved in 150 μL DMSO. Microplate reader was used to measure the absorbance at 490 nm for each well.

1.7 Living cell imaging experiments

As to co-localization experiments, MCF-7 cells were seeded at a density of 2×10^5 cells per well in 35 mm confocal dish (Φ = 20 mm), 24 h before the addition of **Lyso-vis-A**. The cells were first treated with **Lyso-vis-A** (5 μM, 30 min), and then incubated with Lyso Tracker Green (500 nM) or Mito Tracker Green (200 nM) for another 10 min. All the cells were washed for three times with PBS before being taken pictures using CLSM (63× objective lens, Zeiss LSM 800).

As to pH effect experiments, HeLa cells were seeded at a density of 2×10^5 cells per well in 35 mm confocal dish (Φ = 20 mm), 24 h before the addition of **Lyso-vis-A**. The cells were treated with **Lyso-vis-A** (10 μM) and Lyso Tracker Green (500 nM) at 37 ℃. The chloroquine group was treated first with **Lyso-vis-A** (10 μM) and Lyso Tracker Green (500 nM) at 37 ℃ for 20 mins, and then treated with chloroquine (50 μM) for another 30 mins. All the cells were washed for three times with PBS before being taken pictures using CLSM (40× oil lens, Zeiss LSM 800).

As to viscosity titration experiments, MCF-7 cells were seeded at a density of 2 × 10^5 cells per well in 35 mm confocal dish (Φ = 20 mm), 24 h before the addition of **Lyso-vis-A**. The cells were treated with **Lyso-vis-A** (10 μM) at 37 ℃. The dexamethasone group was treated first with **Lyso-vis-A** (10 μM) at 37 ℃ for 30 mins, and then treated with 20 μM dexamethasone for another 10 mins. The Cu^{2+} group was treated first with **Lyso-vis-A** (10 μM) at 37 ℃, and then treated with 20 μM $CuCl_2$ for another 10 min. All the cells were washed for three times with PBS before being taken pictures using CLSM (40 x oil lens, Zeiss LSM 800).

As to experiments about difference in viscosity between normal cells and cancer, HEK293 cells, HeLa cells and MCF-7 cells were seeded at a density of 2 × 10^5 cells per well in 35 mm confocal dish (Φ = 20 mm), 24 h before the addition of **Lyso-vis-A**. The cells were treated with **Lyso-vis-A** (10 μM) at 37 ℃. All the cells were washed for three times with PBS before being taken pictures using CLSM (40× oil lens, Zeiss LSM 800).

2. Supplementary Figures

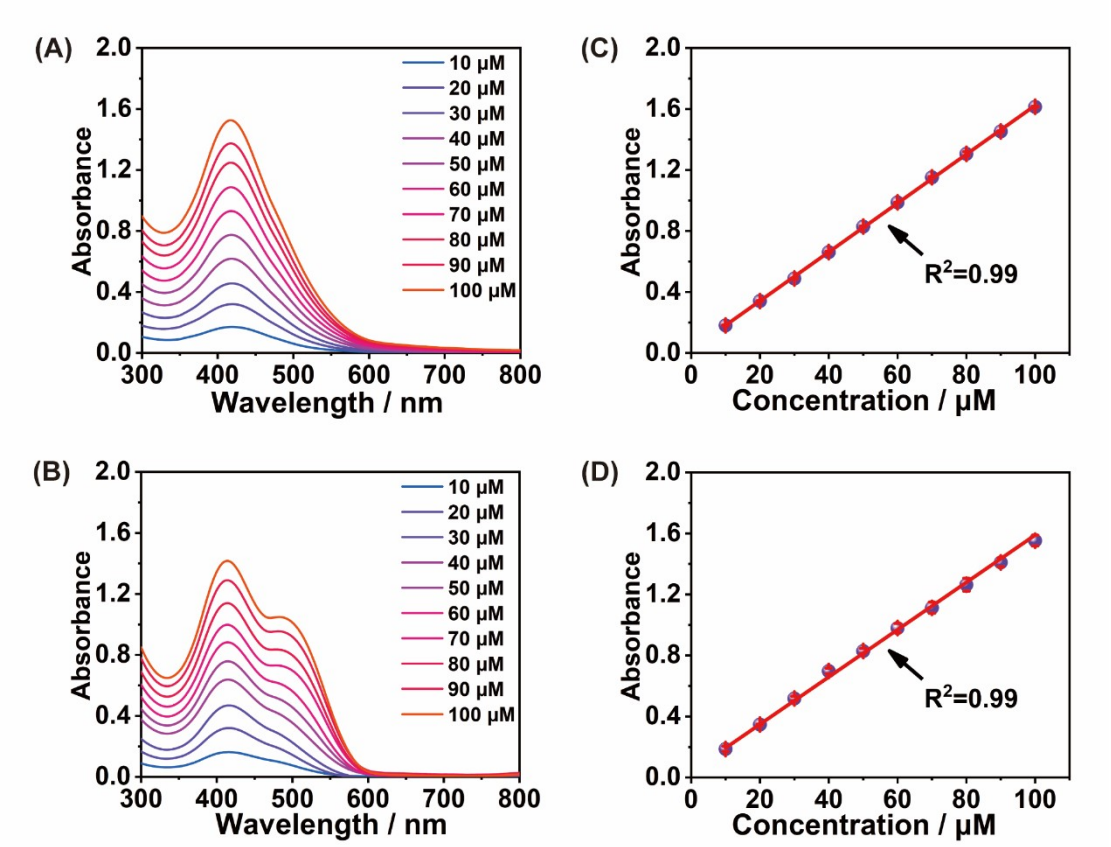


Fig. S1 Water solubility of **Lyso-vis-A** and **Lyso-vis-B**. UV-vis spectra of **Lyso-vis-A** (A) and **Lyso-vis-B** (B) in water with different concentration (10-100 μM). Linear correlation of the absorbance intensities at 420 nm and concentration of **Lyso-vis-A** (C) and **Lyso-vis-B** (D) within the range of 10-100 μM.

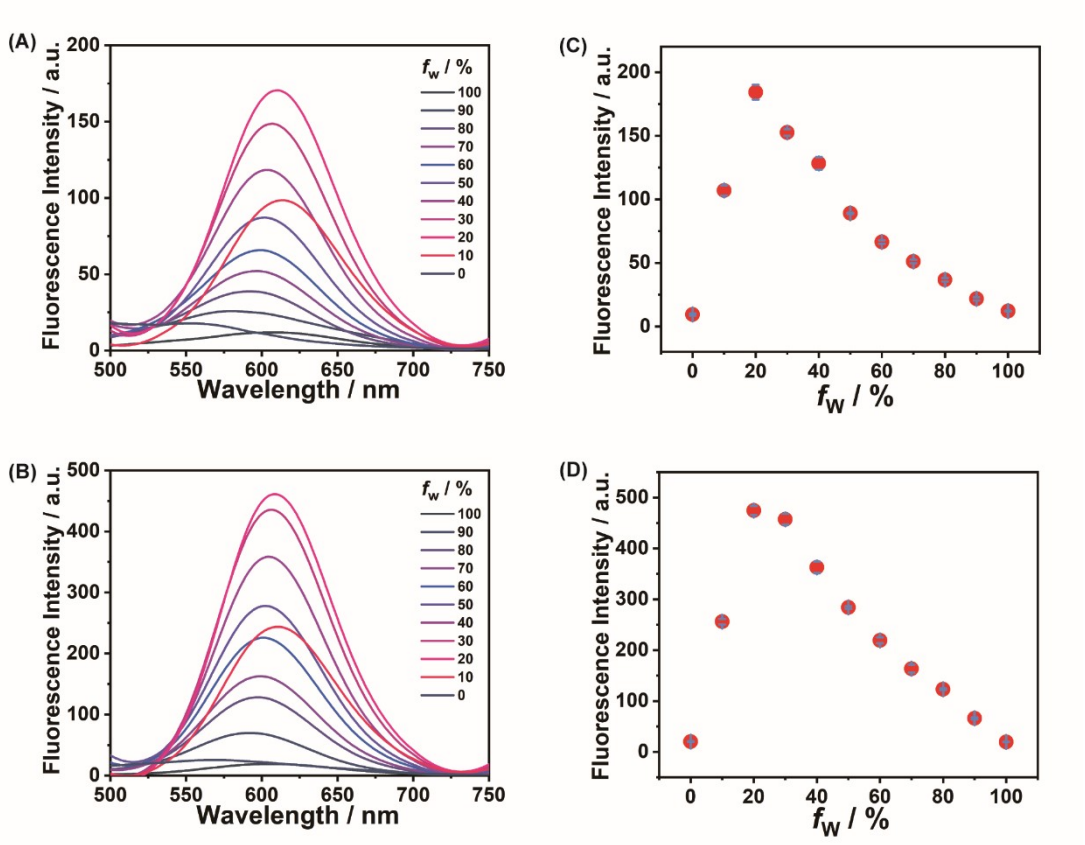


Fig. S2 Fluorescence spectra of **Lyso-vis-A** (A) and **Lyso-vis-B** (B) in the THF-water mixture with different water fractions (f_W). Variations of fluorescence intensities at 610 nm for **Lyso-vis-A** (C) and 590 nm for **Lyso-vis-B** (D) with different f_W. **Lyso-vis-A**: 5 μM, **Lyso-vis-B**: 5 μM, Ex=470 nm.

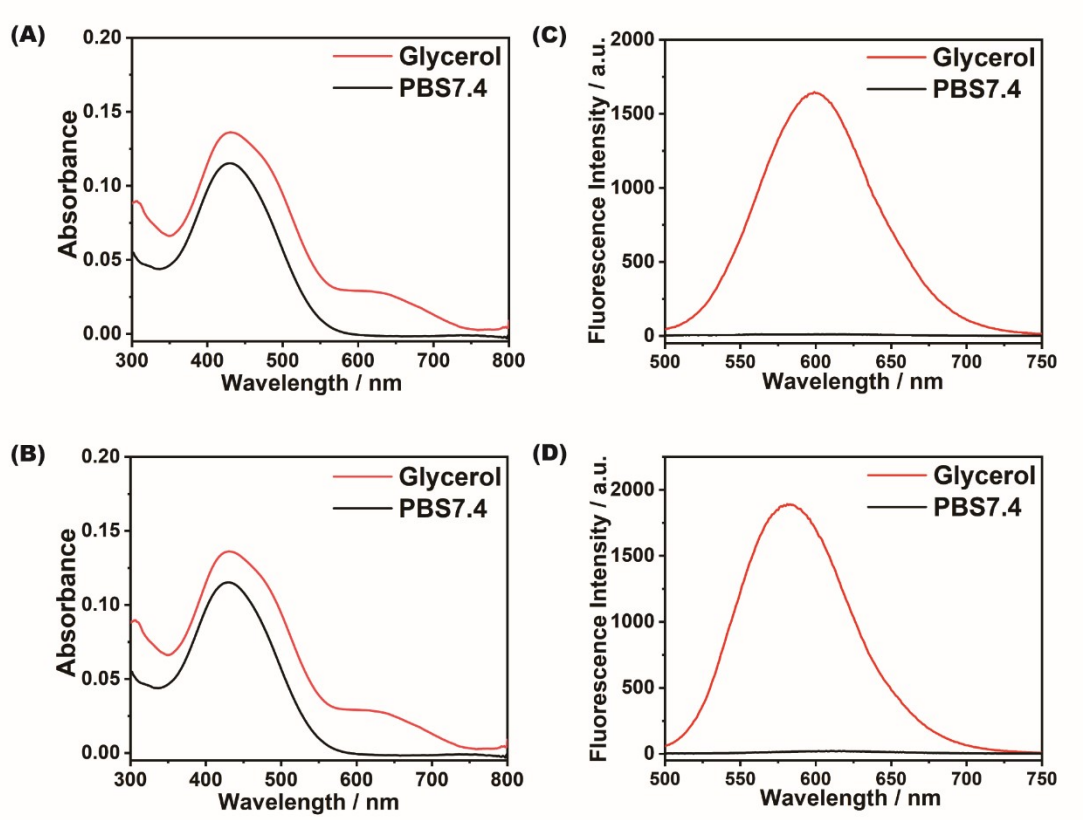


Fig. S3 UV-vis spectra of **Lyso-vis-A** (A) and **Lyso-vis-B** (B) in PBS (pH =7.4, 10 mM) and 90% glycerol; Fluorescence spectra of **Lyso-vis-A** (C) and **Lyso-vis-B** (D) in PBS (pH =7.4, 10 mM) and glycerol. **Lyso-vis-A**: 5 μM, **Lyso-vis-B**: 5 μM, Ex=470 nm.

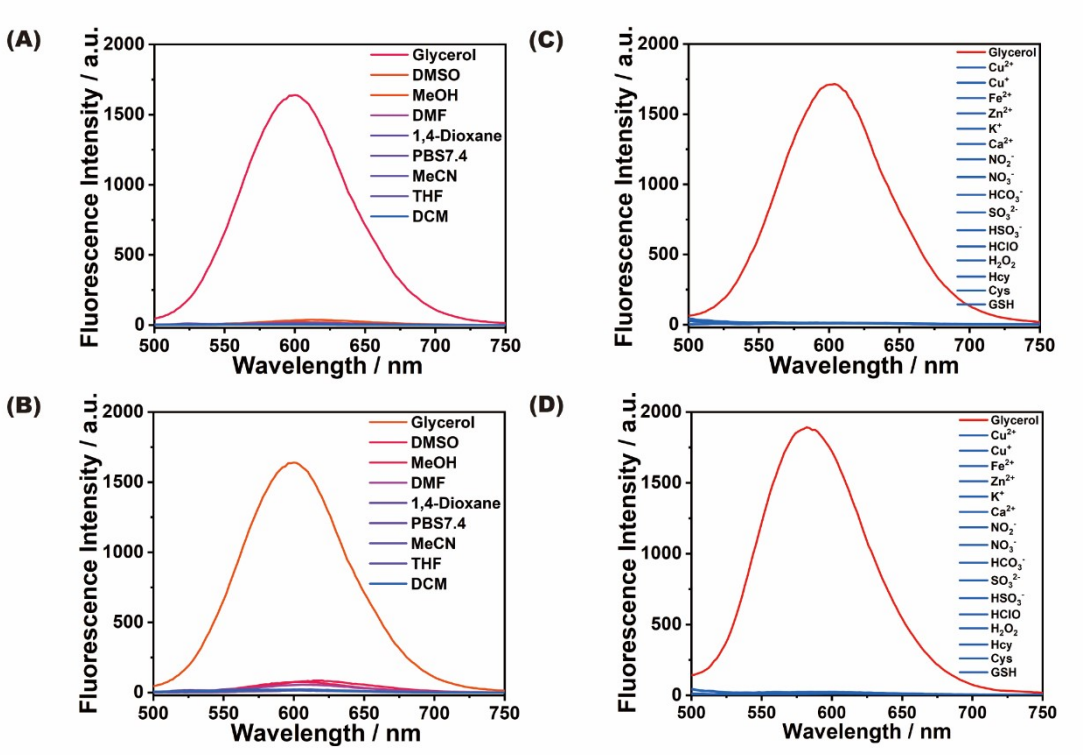


Fig. S4 Selectivity test of **Lyso-vis-A** and **Lyso-vis-B**. Fluorescence spectra of **Lyso-vis-A** (A) and **Lyso-vis-B** (B) in 90% glycerol or different polarity solvents; Fluorescence spectra of **Lyso-vis-A** (C) and **Lyso-vis-B** (D) in 90% glycerol or in PBS buffer (pH=7.4, 10 mM) with various relevant molecules and ions (500 μM). **Lyso-vis-A**: 5 μM, **Lyso-vis-B**: 5 μM, Ex=470 nm.

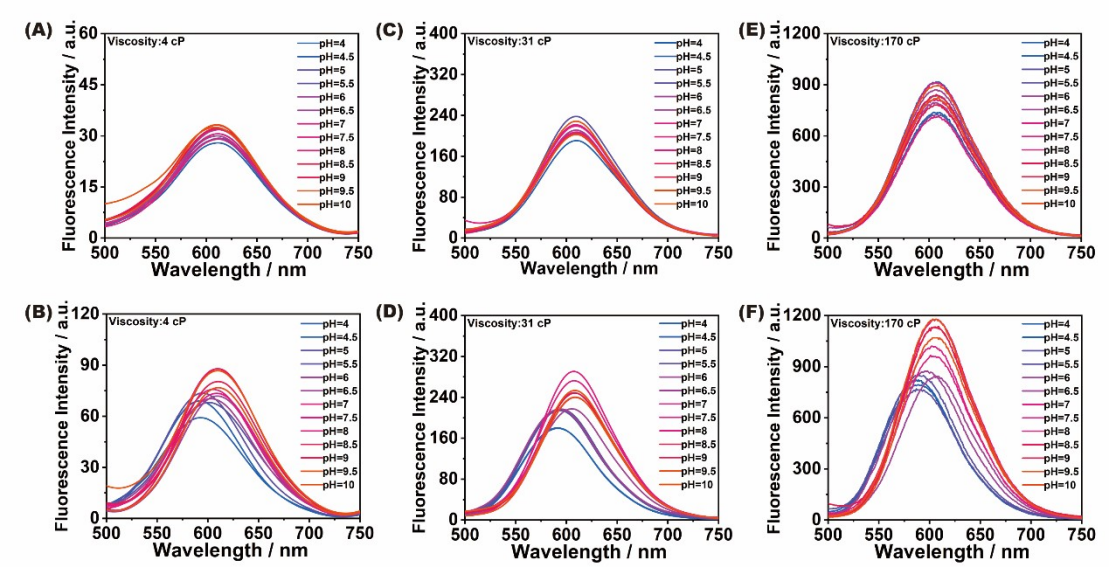


Fig. S5 Fluorescence spectra of **Lyso-vis-A** (A, C and E) and **Lyso-vis-B** (B, D and F) in different pH buffer (4-10) with different viscous solution (η=4 cP, 31 cP, 170 cP). **Lyso-vis-A**: 5 μM, **Lyso-vis-B**: 5 μM, Ex=470 nm.

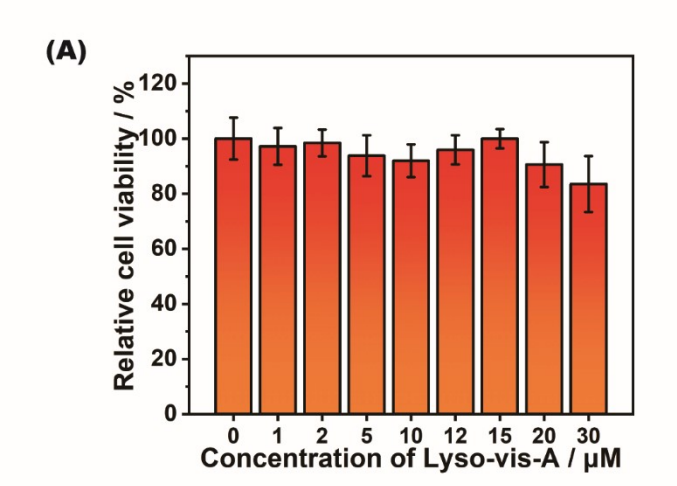
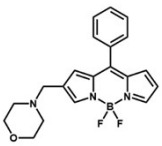
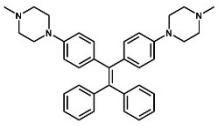
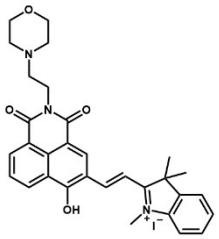
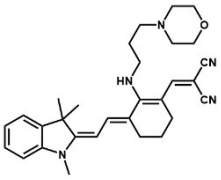
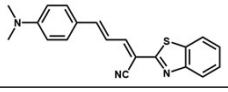
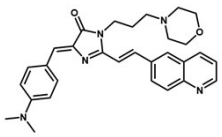
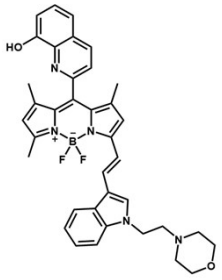
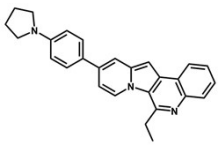
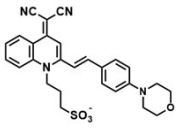


Fig. S6 Cytotoxicity data of **Lyso-vis-A** in MCF-7 cells.

Table S1. The comparison of lysosomal viscosity fluorescent probes.

Probes	λex/λem (nm)	Fluorescence Enhancement (fold)	Detection Range (cP)	Biological experiments
Lyso-V (*J. Am. Chem. Soc.*, 2013, **135**, 2903) 	~/517	~	0.6-359.6	MCF-7 cells colocalization; chloroquine and dexamethasone induced MCF-7 cells
PIP–TPE (*Chem. Sci.*, 2017, **8**, 7593) 	360/493	~	2.15-438.40	Hela cells colocalization; chloroquine induced Hela cells
Lyso-NA (*J. Mater. Chem. B*, 2018, **6**, 580) 	550/610	50	1-1410	RAW264.7 cells colocalization; control the cellular viscosity by changing the temperature
Lyso-MC (*Chem. Commun.*, 2019, **55**, 2688) 	565/620	33	1-945	SH-SY5Y cells colocalization; control the cellular viscosity by changing the temperature and induced with dexamethasone
Lys-BTC (*Org. Biomol. Chem.*, 2019, **17**, 6398) 	470/685	5.4	1.99-1138	PC3 cells colocalization; chloroquine incubated PC3 cells
C25 (*Sens. Actuators B*	500/600	13	1-945	SH-SY5Y cells colocalization;

Chem., 2020, **305**, 127509) 				control the cellular viscosity by changing the temperature and induced with dexamethasone
Lys-VBOD (*Sens. Actuators B Chem.*, 2020, **304**, 127271) 	565/640	65	1.01-1412	A549 cells colocalization; dexamethasone induced Bel-7402 cells
IQ-LV70 (*Sens. Actuators B Chem.*, 2020, **309**, 127764) 	433/590	116	1.0-302.7	Hela cells colocalization; dexamethasone induced MCF7 cells
Lyso-vis-A (This work) 	470/610	160	1.98-476.30	Hela cells colocalization; Cu^{2+} and dexamethasone induced Hela cells; distinguish cancer cells (HeLa and MCF-7) from normal cells (HEK293) due to the disparity of viscosity

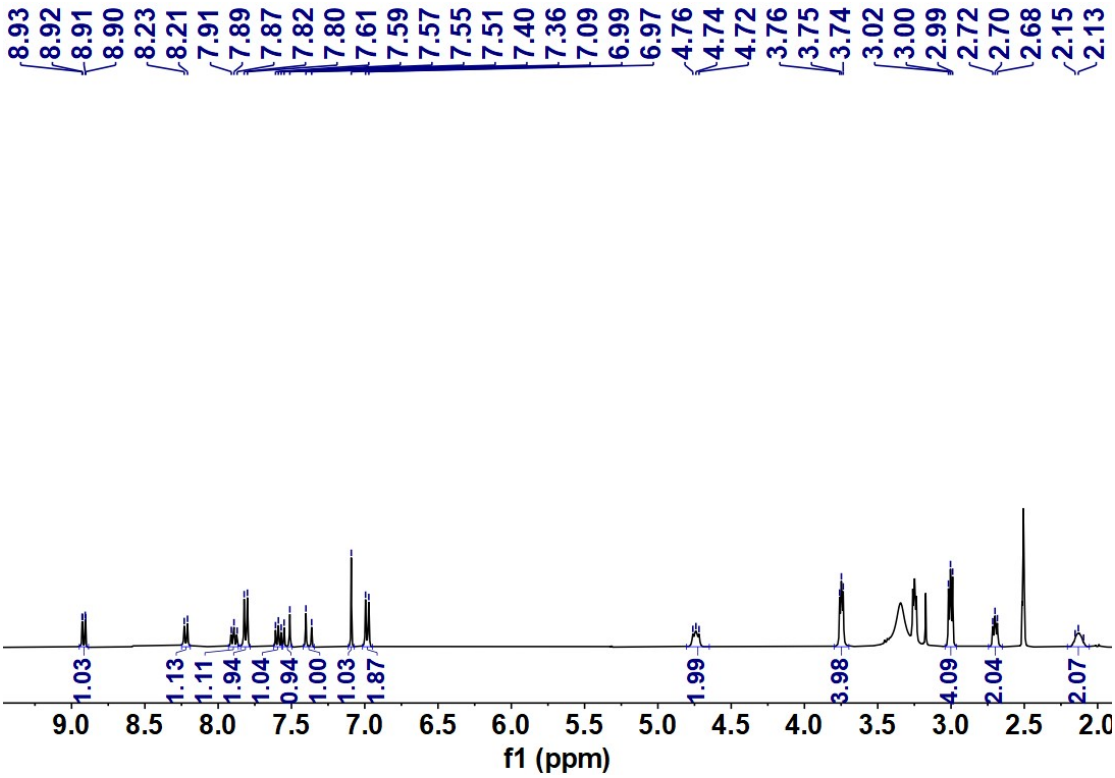


Fig. S7 ^{1}H NMR spectrum of **Lyso-vis-A** (DMSO-d_6).

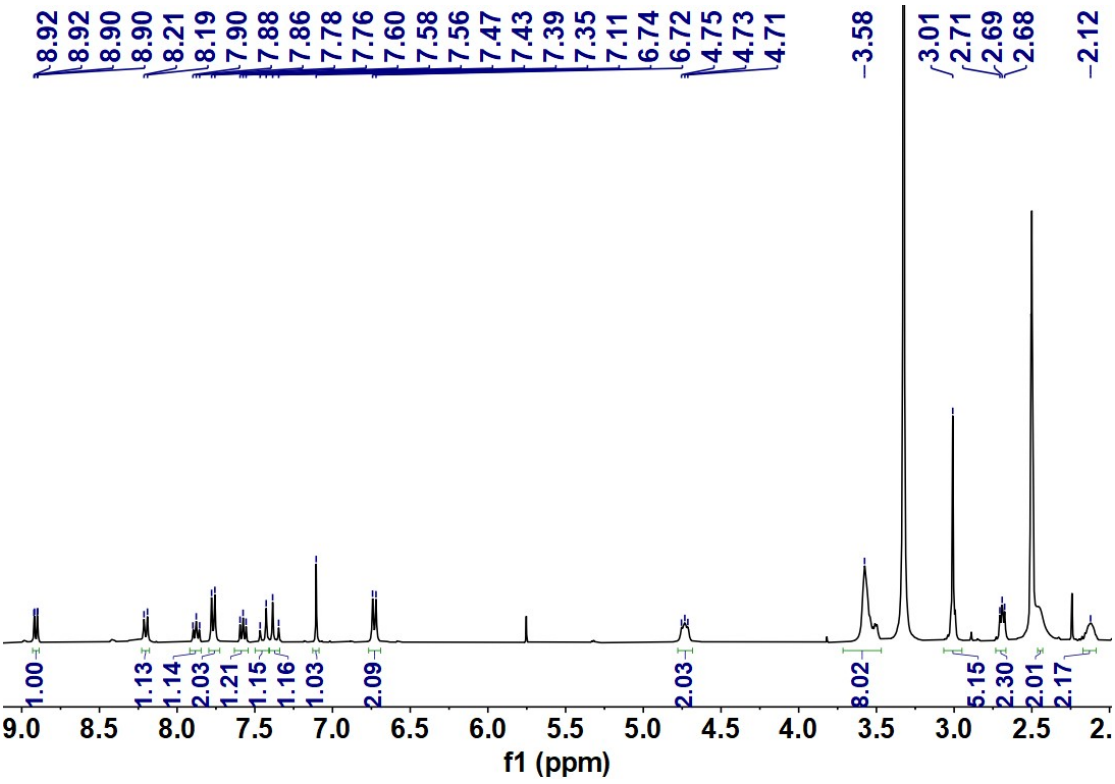


Fig. S8 ^{1}H NMR spectrum of **Lyso-vis-B** (DMSO-d_6).

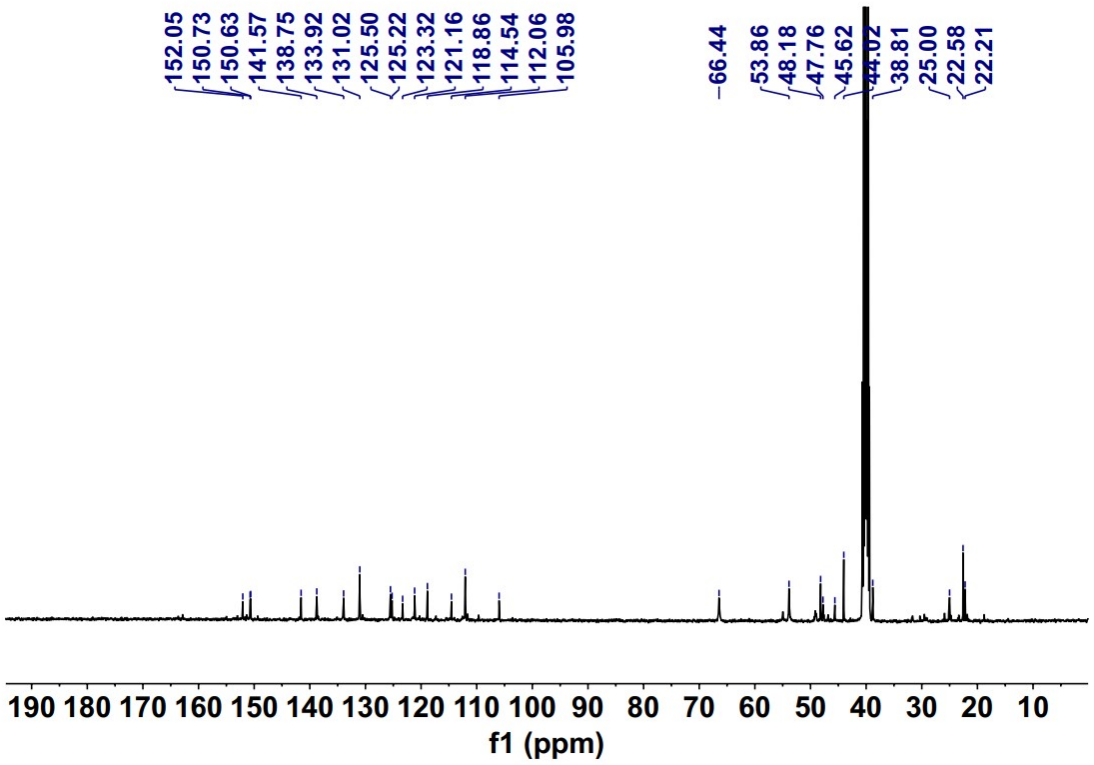


Fig. S9 ^{13}C NMR spectrum of **Lyso-vis-A** (DMSO-d_6).

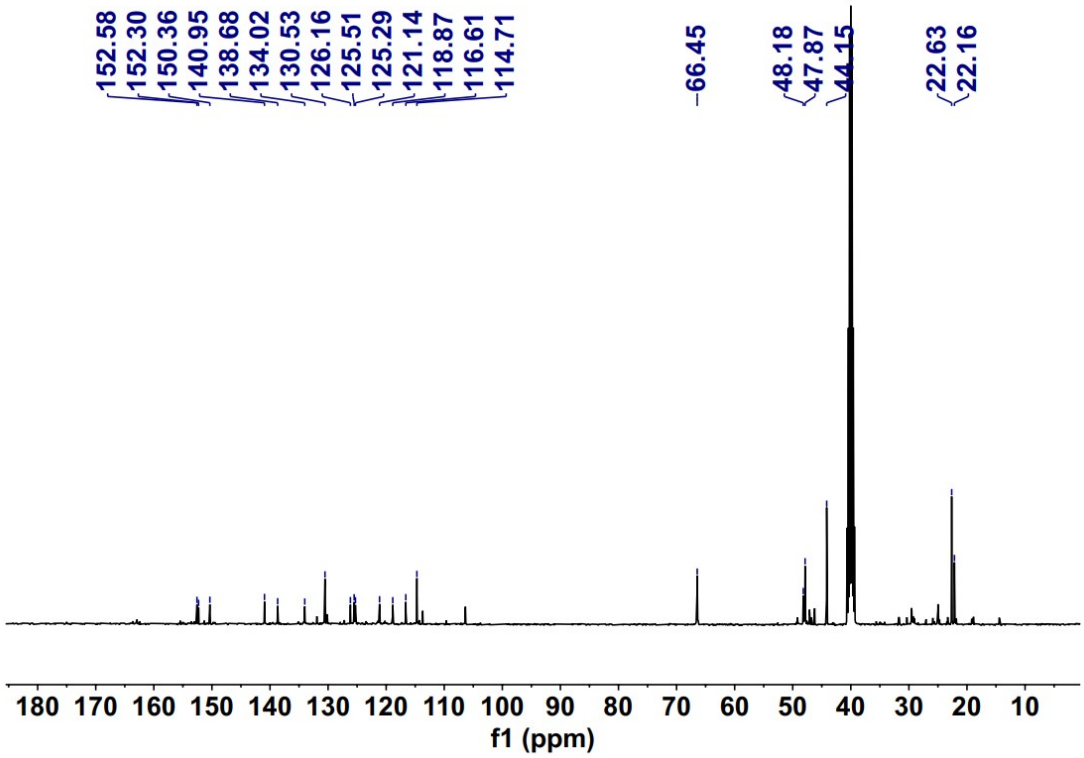


Fig. S10 ^{13}C MR spectrum of **Lyso-vis-B** (DMSO-d_6).

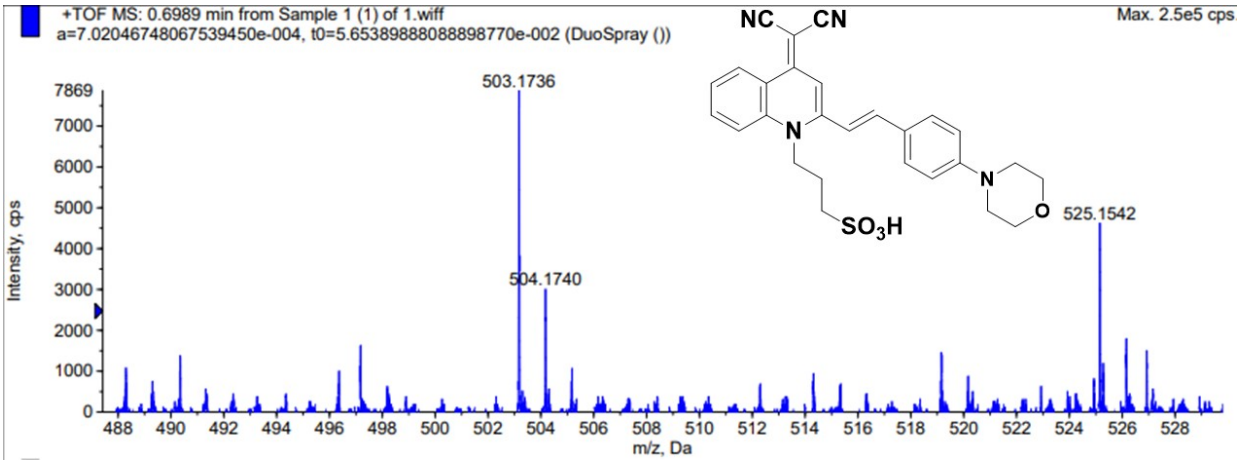


Fig. S11 ESI-MS spectrum of **Lyso-vis-A**.

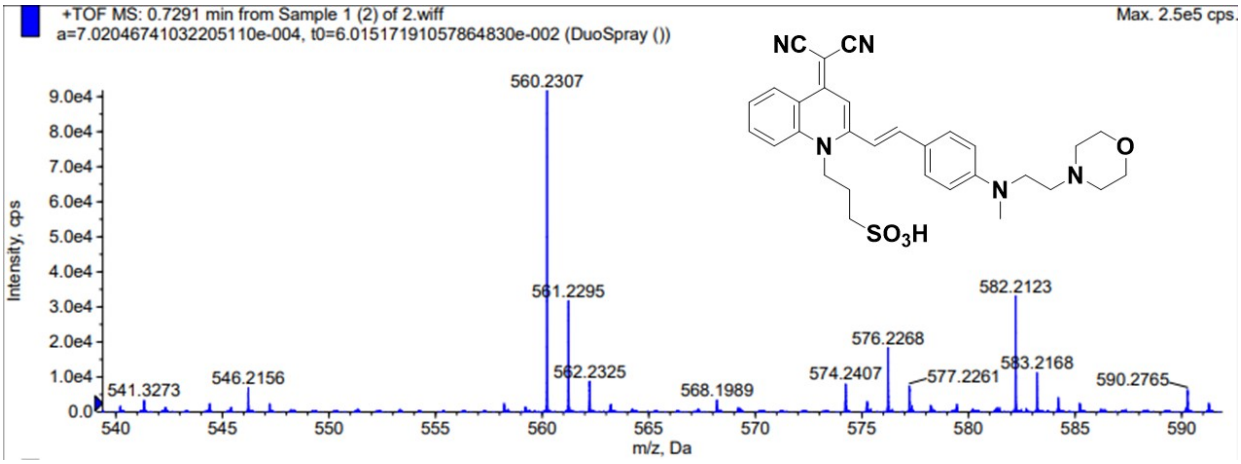


Fig. S12 ESI-MS spectrum of **Lyso-vis-B**.