Supporting Information

A lysosome-targeting viscosity-sensitive fluorescent probe based on a novel functionalised near-infrared xanthene-indolium dye and its application in living cells

Chang Liu,[a,b] Tong Zhao,[a,b] Song He,[a,b] Liancheng Zhao[a,b,c] and Xianshun Zeng*[a,b]

[a] *Tianjin Key Laboratory for Photoelectric Materials and Devices, School of Materials Science & Engineering, Tianjin University of Technology, Tianjin 300384, China. Fax: (+86)22-60215226; Tel: (+86)22-60216748; E-mail: xshzeng@tjut.edu.cn.*

[b] *Key Laboratory of Display Materials and Photoelectric Devices, Ministry of Education, School of Materials Science & Engineering, Tianjin University of Technology, Tianjin, 300384, China*

[c] *School of Materials Science and Engineering, Institute of Information Functional Materials& Devices, Harbin Institute of Technology, Harbin, 150001, China.*

Contents

Materials and equipment

All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Lyso-Tracker Green DND 26 and Mito-Tracker Green were purchased from Beyotime Institute of Biotechnology. ^{1}H NMR and ^{13}C NMR were measured on a Bruker AVANCE III HD 400MHz spectrometer. Chemical shifts (d values) were reported in ppm down field from internal Me_4Si. High resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Melting points were recorded on a melting point apparatus (RY-2, Tianjin, China). UV-vis absorption spectra were obtained with UV-2550 (Shimadzu, Japan) spectrophotometer. A Hitachi F-4600 spectrophotometer (Tokyo, Japan) was used for fluorescence measurements with a 700 V PMT voltage. The pH values were reported by a Mettler Toledo Seven Excellence PH meter (Shanghai, China). The absorbance for MTT analysis was recorded on a microplate reader (PL-9602). The confocal microscopy imaging was used Olympus FV1000-IX81 inverted fluorescence microscope. Image processing was analyzed with Olympus software (FV1000-ASW) and Image J software.

Detection of fluorescence quantum yield

Rhodamine B was used as a standard[S1] to calculate the relative fluorescence quantum yields according to the following equation:

$$\Phi_B = \Phi_1(F_B/F_1)(A_1/A_B)(\lambda_{ex1}/\lambda_{exB})(\eta_B/\eta_1)^2$$

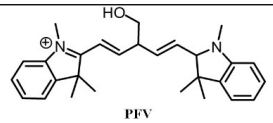
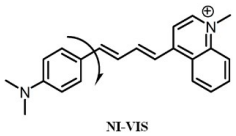
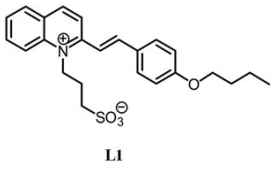
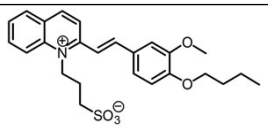
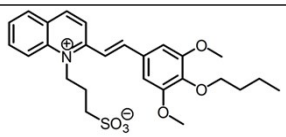
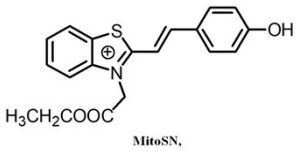
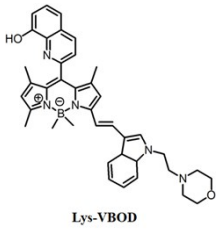
Here, Φ represents quantum yield; F stands for integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution ; and the subscripts 1 and B refer to the unknown and the standard, respectively.

Colocalization experiment in HeLa cells

Group 1: HeLa cells were pre-treated with the probe (0.2 μM) for 30 min and exposed to Rapamycin (2 μM) for another 30 min, then cells were treated with Lyso-Tracker Green (200 nM) for another 30 min.

Group 2: HeLa cells were pre-treated with the probe (0.2 μM) for 30 min and exposed to Nystatin (2 μM) for another 30 min, then cells were treated with Mito-Tracker Green (200 nM) for another 30 min.

Table S1 Fluorescent probes for viscosity reported in the literatures.

Probes	λ_{em}(nm)	Targeted Localization	Response multiple	Journal[S2-S6]
 PFV	570	Mitochondria	>3-fold	Dyes. Pigments. 2019, **168**, 134
 NI-VIS	670	Mitochondria	167-fold	Anal. Chem. 2019, **91**, 10302
 L1	535	-	14-fold	Polyhedron. 2019, **170**, 440
 L2	578	-	180-fold	Polyhedron. 2019, **170**, 440
 L3	603	-	120-fold	Polyhedron. 2019, **170**, 440
 MitoSN,	520	Mitochondria	35-fold	Spectrochim. Acta. A. 2018, **203**, 127.
 Lys-VBOD	637	Lysosome	3.7-fold	Sens. Actuat. B Chem. 2020, **304**,127271.

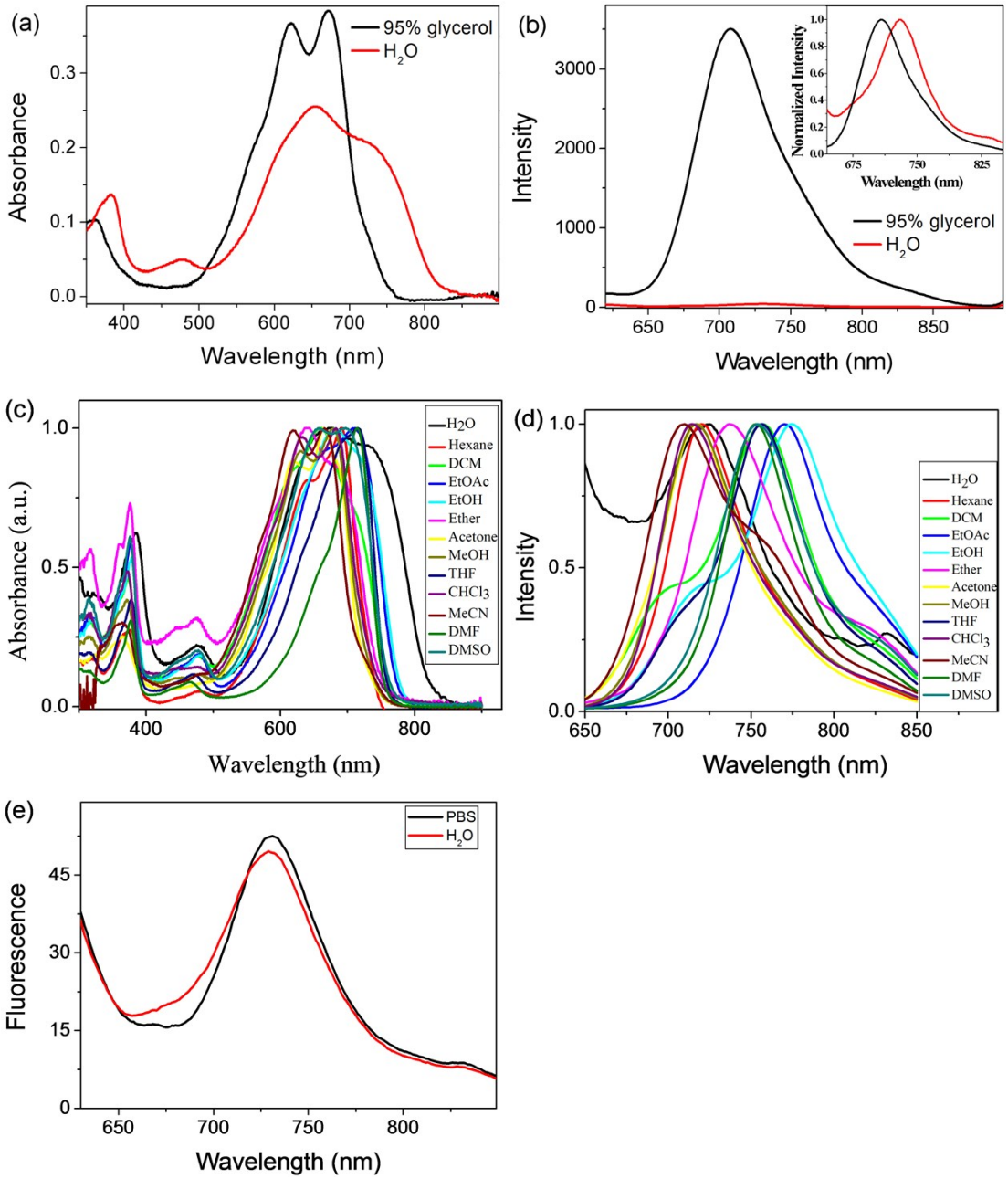


Fig. S1 Absorption and fluorescence spectra of **Lyso-cy**. a) Absorption spectra of **Lyso-cy** (10 μM) and b) fluorescence spectra of **Lyso-cy** (5 μM) in 95% glycerol and water, respectively. c) Absorption spectra of **Lyso-cy** (10 μM) and d) fluorescence spectra of **Lyso-cy** (5 μM) in solvents with different polarity. e) fluorescence spectra of **Lyso-cy** (5 μM) in PBS buffer and water. λ_{ex} = 600 nm, slit: 10 nm.

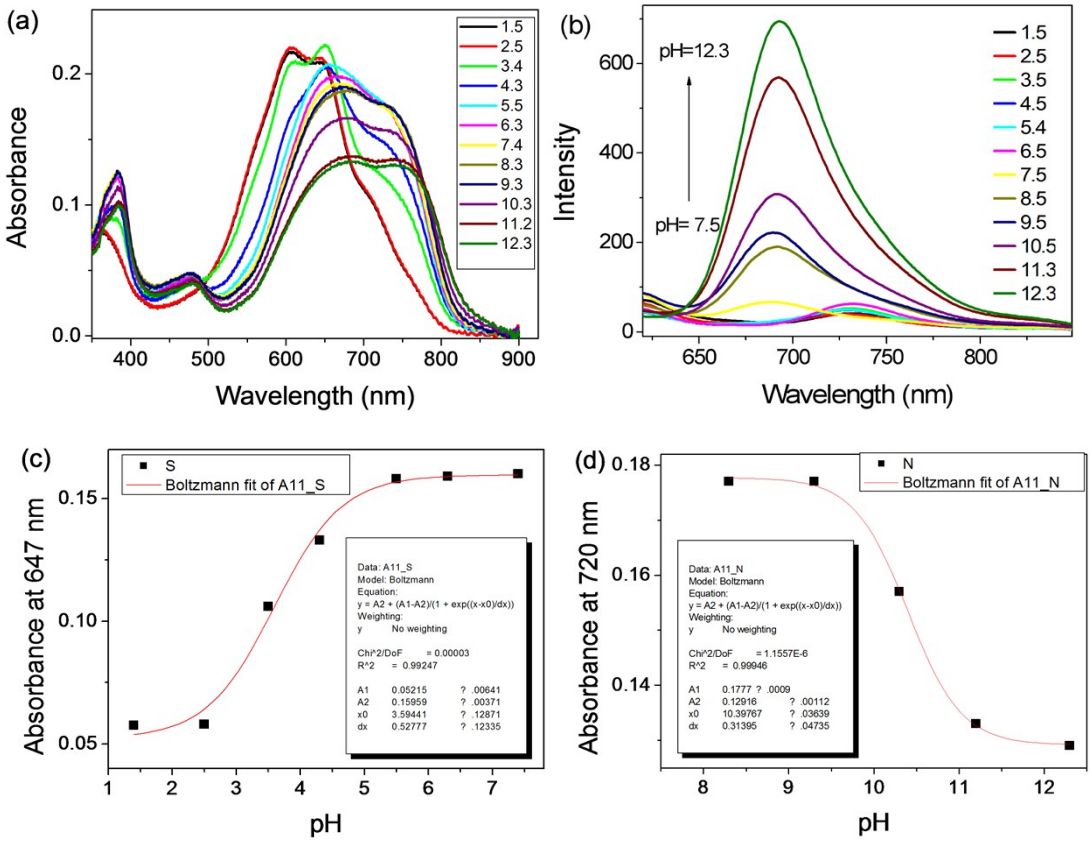


Fig. S2 a) The pH-dependence of absorption spectra of **Lyso-cy** (10 μM). b) pH-dependence of fluorescence spectra of **Lyso-cy** (5 μM). c) Curve of absorbance at 647 nm of the probe versus increasing pH from 1.3 to 7.4. The pKa was deduced to be 3.59 (with correlation coefficient $R^2 = 0.992$). d) Curve of absorbance at 720 nm of the probe versus increasing pH from 8.3 to 12.3. The pKa was deduced to be 10.39 (with correlation coefficient $R^2 = 0.999$). λ_{ex} = 600 nm, slit: 10 nm.

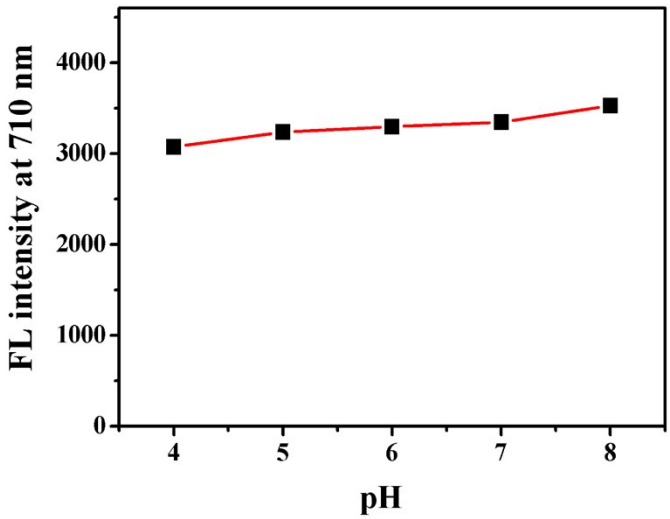


Fig. S3 The pH-dependence of the fluorescence intensity of **Lyso-cy** (5 μM) with 95% glycerol. λ_{ex} = 600 nm, slit: 10 nm.

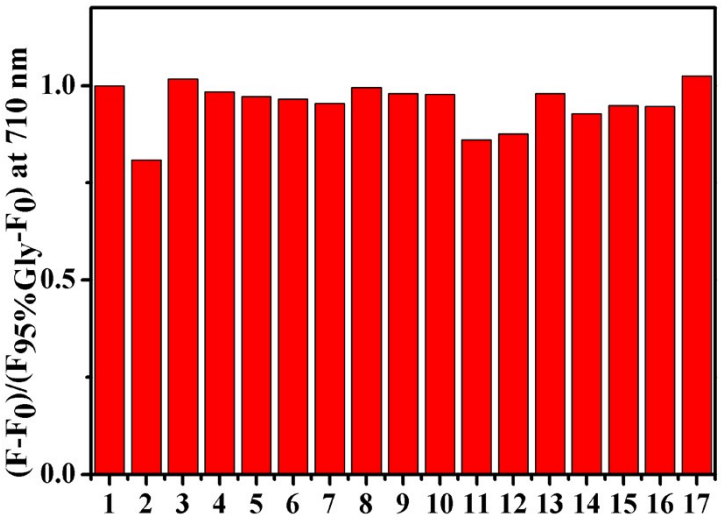


Fig. S4 Fluorescence intensity changes [$(F_i-F_{Probe})/(F_{95\%Gly}-F_{probe})$] of the probe **Lyso-cy** (5 μM) at 710 nm in the presence of other relevant species (10 equiv.) in water. 1: **Lyso-cy** in 95% glycerol, 2: **Lyso-cy** + $NaNO_2$, 3: **Lyso-cy** + $AgNO_3$, 4: **Lyso-cy** + $MgCl_2$, 5: **Lyso-cy** + $CaCl_2$, 6: **Lyso-cy** + $Fe(NO_3)_2$, 7: **Lyso-cy** +$Ni(NO_3)_2$, 8: **Lyso-cy** + $Hg(NO_3)_2$, 9: **Lyso-cy** + $Zn(NO_3)_2$, 10: **Lyso-cy** + $Cu(NO_3)_2$, 11: **Lyso-cy** + GSH, 12: **Lyso-cy** + Hcy, 13: **Lyso-cy** + Cys, 14: **Lyso-cy** + ClO^-, 15: **Lyso-cy** + $ONOO^-$, 16: **Lyso-cy** + H_2O_2 and 17: **Lyso-cy** + TBHP; λ_{ex} = 600 nm; slit: 10 nm.

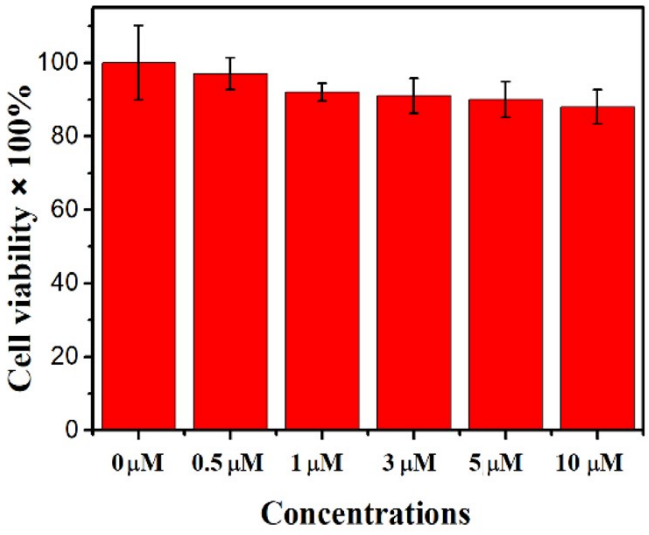


Fig. S5 Cytotoxicity of **Lyso-cy** in HeLa cells. The cells were incubated with **Lyso-cy** at corresponding concentrations (0 μM, 0.5 μM, 1 μM, 3 μM, 5μM, 10 μM) for 24 h.

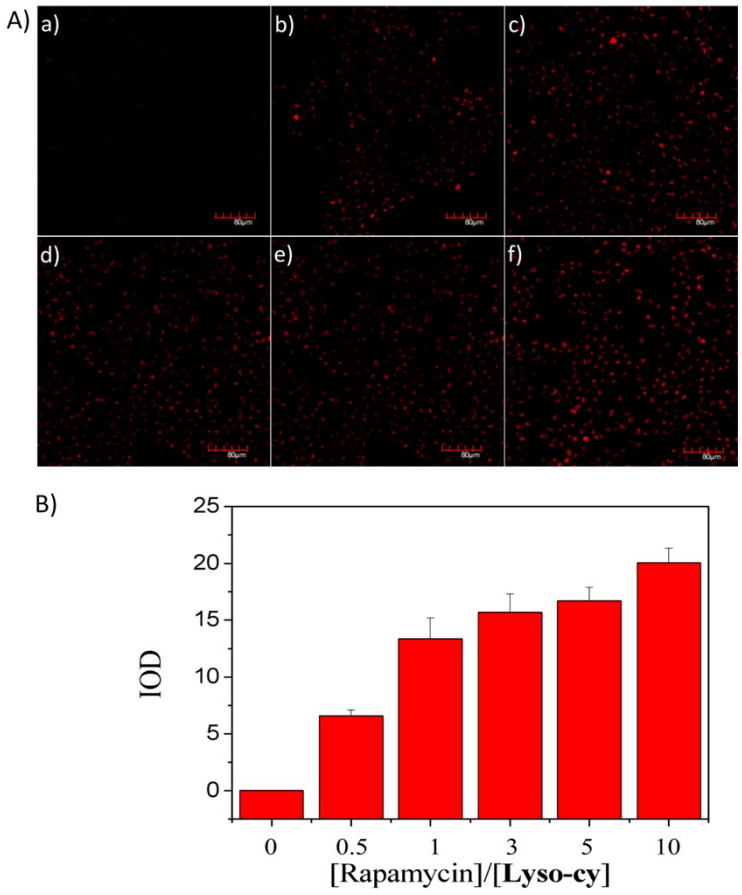


Fig. S6 Rapamycin stimulated fluorescence increase of the probe **Lyso-cy**. A) Fluorescence imaging of **Lyso-cy** in HeLa cells. The cells were treated with **Lyso-cy** (1 μM) for 30 min, washed with PBS, and then incubated with different concentrations of Rapamycin (0-10 μM) for 20 min, respectively. Concentrations of Rapamycin, a): 0 μM, b): 0.5 μM, c): 1.0 μM,. d): 3.0 μM, d): 5.0 μM, f): 10.0 μM. Red channel: λ_{ex} = 635 nm, λ_{em} = 670-770 nm. Scale bar: 80 μm. B) Relative fluorescence intensities of HeLa in panels (a)-(f).

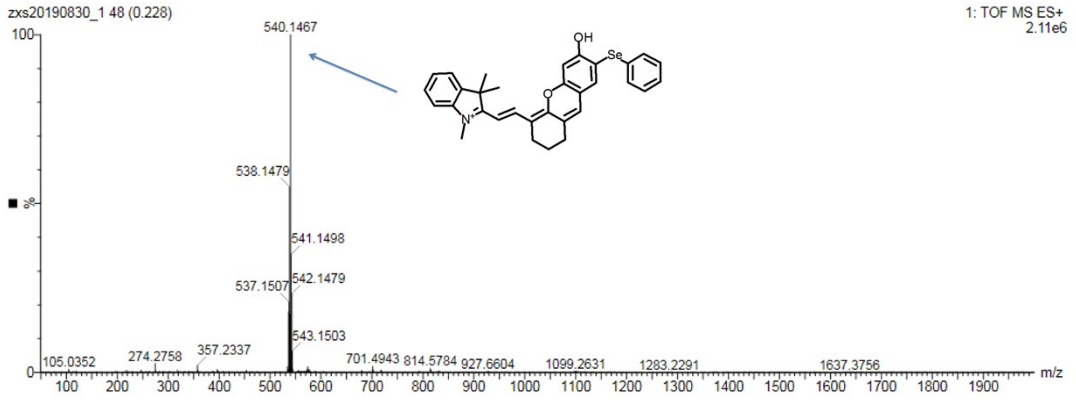


Fig. S7 HRMS (LC/MS) spectra of **Lyso-cy**. The peak at m/z = 540.1467 was assigned to the mass of [**Lyso-cy - BF_4^-**]$^+$.

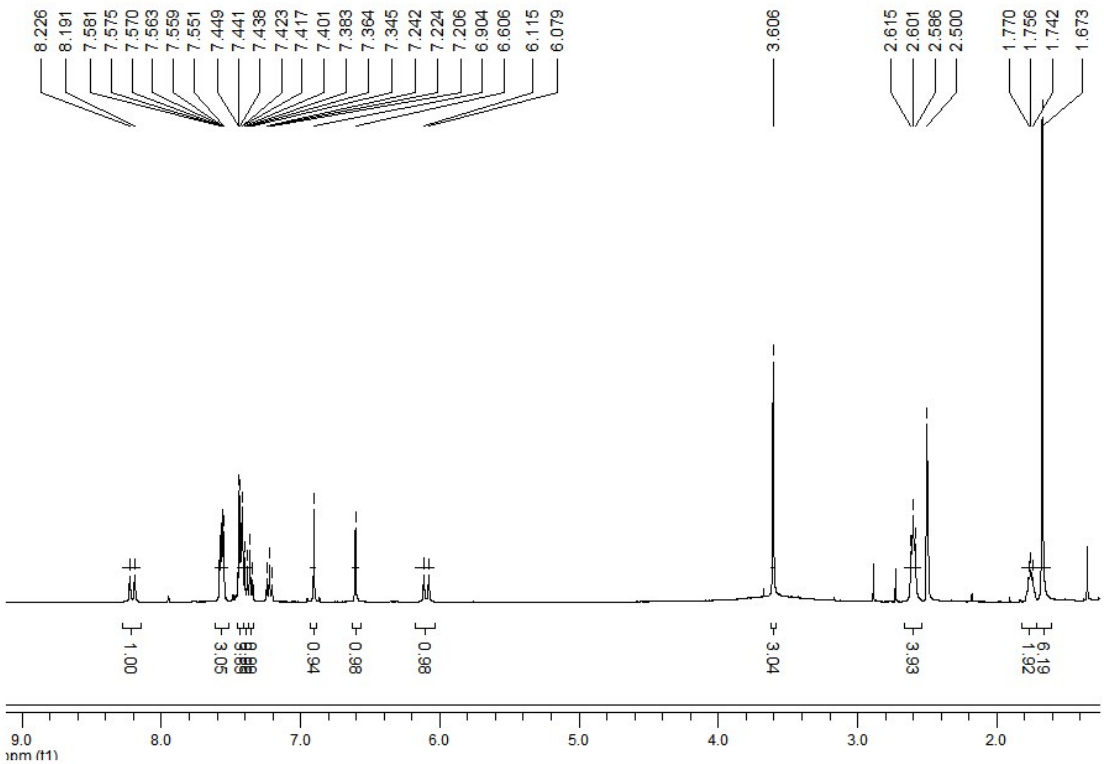


Fig. S8 ^{1}H NMR of **Lyso-cy** (400 MHz, DMSO-d_6).

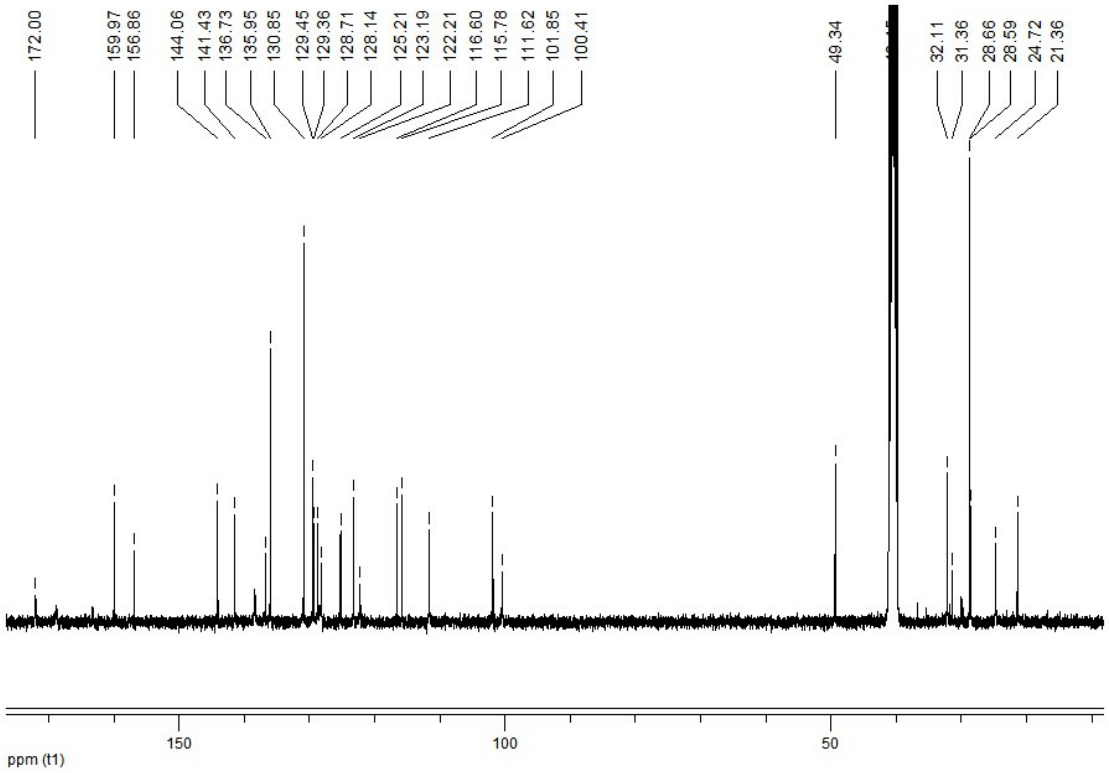


Fig. S9 ^{13}C NMR of **Lyso-cy** (100 MHz, DMSO-d_6).

Reference

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S5 H. Wang, B. Fang, L. F. Xiao, D. Li, L. Zhou, L. Kong, Y. Yu, X. Z. Li, Y. J. Wu, and Z. J. Hu, Spectrchim. Acta. A., 2018, **203**, 127-131.

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