Supporting Information

Activable Rotor for Quantifying Lysosomal Viscosity in Living Cells

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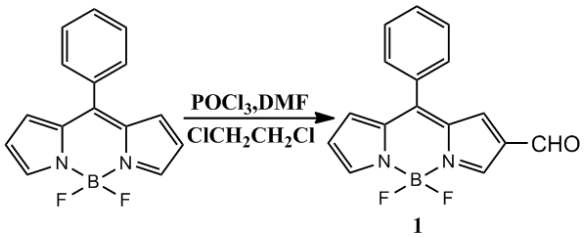
General information:

All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined using melting point apparatus (X-6) and uncorrected. ^{1}H NMR and ^{13}C NMR were measured on Varian MERCURY 400 spectrometer in $CDCl_3$ with TMS as internal reference. Chromatographic purification was conducted with silica gel (200 - 300 mesh). Fluorescence quantum yields were determined using fluorescein (Φ= 0.85). [1] Absorption spectra were recorded on TU-1901 UV-Vis absorption spectrometer. Fluorescence spectra were recorded on F-4500 spectrometer. The fluorescence lifetime imaging (FLIM) was measured with B&H DCS120. Fluorescence imaging was measured with Olympus FV1000. The fluorescence decays from solution samples were measured with Eninburgh instruments FL900. The viscosity of methanol/glycerol mixtures were measured using an advanced Brook field Rheo3000 R/S plus Rheometer. The temperature was kept at (25±0.1) °C with a bath circulator.

Experimental section:

Synthesis of compound 1

Compound **1** was synthesized according to previous literature. [2]



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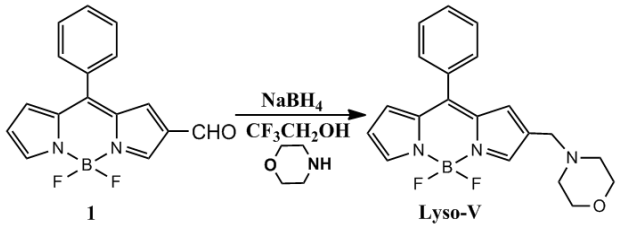
Synthesis of compound Lyso-V



Compound **1** (140 mg, 0.47mmol) in dry trifluoroethanol (20ml) was placed into a round-bottomed flask and stirred for 15 min under argon. The sodium borohydride (22 mg, 0.57mmol) and morpholine (45μL, 0.47mmol) were added and heated to 45□ for 2h. The TLC showed that the reaction went to completion. The mixture was evaporated under reduced pressure and purified by silica gel flash column (petroleum ether/ethyl acetate=3:1) to yield brown powder (117 mg, 68 %). Mp. = 117-119□. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 5.9 Hz, 2H), 7.75 – 7.42 (m, 5H), 6.91 –6.83 (d, *J* = 32.2 Hz, 2H), 6.54 (s, 1H), 3.70 (s, 4H), 3.39 (s, 2H), 2.44 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.02, 144.75, 143.98, 135.01, 133.86, 131.56, 131.14, 130.99, 130.87, 130.58, 128.59, 118.51, 66.95, 55.06, 53.59, 29.82. MALDI-TOF-MS: calcd. $[M-H]^+$for $C_{20}H_{19}BN_3OF_2$ 366.1595, found: 366.1589.

Effects on cell growth and cytotoxicity experiment

MCF-7 (human breast carcinoma) cells were obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS). All cell lines were maintained under standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37 °C) in RPMI 1640 medium, supplemented with 10% FBS (fetal calf serum). The cytotoxic effects of probe **Lyso-V** were assessed using the MTT assay (**Fig. S1**). Briefly, the cells in the exponential phase of growth were used in the experimentation. 1.5×10^3 cells/well were seeded onto 96-well plates and allowed to grow for 24 h prior to treatment with **Lyso-V** (5.0μM). The incubation time of probe **Lyso-V** were kept from 0 to 24 hours. At the end of this time, the **Lyso-V** -containing medium was replaced with PBS, and MTT was then added to each well (final concentration 0.5 mg/mL) for 4h at 37℃ and formazan crystals formed through MTT metabolism by viable cells were dissolved in DMSO. The optical density (O.D.) of the plate wells was recorded by an AC100-120 Automated Microplate Reader (TECAN, Switzerland) at a test wavelength of 570 nm and reference wavelength of 630 nm. The inhibition rate of each compound was calculated based on the following formula: Cell survival rate = average A_{570} nm of treated group/average A_{570} nm of control group × 100%. Each treatment was replicated six wells.

Culture of MCF-7 cells and fluorescent imaging

MCF-7 was obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS) and cultured in RPMI 1640 supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Grow MCF-7 Cells in the exponential phase of growth on 35-mm glass-bottom culture dishes (Φ 20mm) for 1-2 days to reach 70-90%

confluency. These cells were used in co-localization experimentation (**Fig. S2**). The cells was washed three times with RPMI 1640, and then incubated with 2mL RPMI 1640 containing **Lyso-V** (5.0μM) and **Neutral Red** (NR) (5.0μM) in an atmosphere of 5% CO_2 and 95% air for 3 min at 37°C. Wash cells twice with 1mL PBS at room temperature, and then add 1mL RPMI 1640 culture medium and observe under a confocal microscopy (Olympus FV1000).

Viscosity measurements

The viscosity of mixtures was measured using an advanced Brook field Rheo3000 R/S plus Rheometer. The temperature was kept at (25±0.1) °C with a bath circulator. Each measurement used ca. 10 ml of sample. The viscosity was measured as a function of shear rate in the range from 0 to 100s^{-1}. In the **Fig. 2c**, we adjust the ratio of methanol and glycerol solution and achieve different viscosity mixtures (0.6cp~360cp). In the **Fig. 2d**, we adjust the ratio of water, methanol and glycerol solution and achieve different polarity and different viscosity mixtures (0.7cp, 10cp, 30cp and 69cp), the ratios as follows (mass ratio): 0.7cp (methanol: water =1:1), 10cp (methanol: water: glycerol =1:1:2.8), 30cp (methanol: water: glycerol =0.9:1.1:5) and 69cp (methanol: water: glycerol =0.8:1.2:8).

FLIM study of Lyso-V in MCF-7 cells

The fluorescence lifetime imaging (FLIM) was measured with B&H DCS120. The emission was collected through a 535±15nm band pass filter. The FLIM image obtained following single photon excitation of **Lyso-V** in MCF-7 cells is shown in **Fig. S3**. We find that these data are well fitted with monoexponential decay function. We use two drugs (dexamethasone and chloroquine) stimulated MCF-7cells. Firstly, MCF-7 cells were incubated with 2mL RPMI 1640 containing **Lyso-V** (5.0μM) in an atmosphere of 5% CO_2 and 95% air for 5 min at 37°C. Wash cells twice with 1mL PBS at room temperature, and then add 0.5mL RPMI 1640 culture medium and observe under a confocal microscopy. Secondly, MCF-7 cells were added dropwise 0.5mL RPMI 1640 containing dexamethasone (1mmol/L, 5μL) and collect images continuously at different times (**Fig. S4**). The final concentration of the dexamethasone is 5.0μM. The above method is applicable to chloroquine stimulation experiments. The final concentration of the chloroquine is 20.0Mm (**Fig. S5**). For the possible influence from protein, we performed the experiments. Experimental results show that the Bodipy dye don't be bound to protein inside the lysosome. (Fig. **S6** A-H)

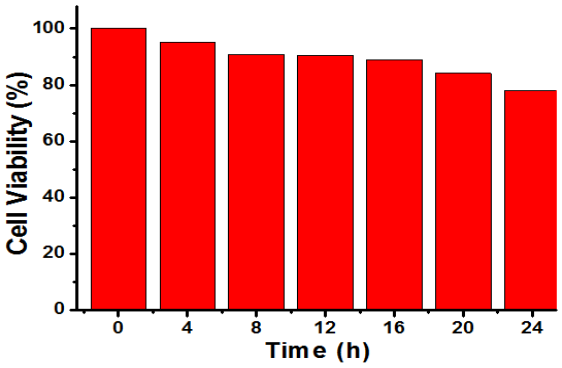


Figure S1 Cell viability of **Lyso-V** (5μM) at different time.

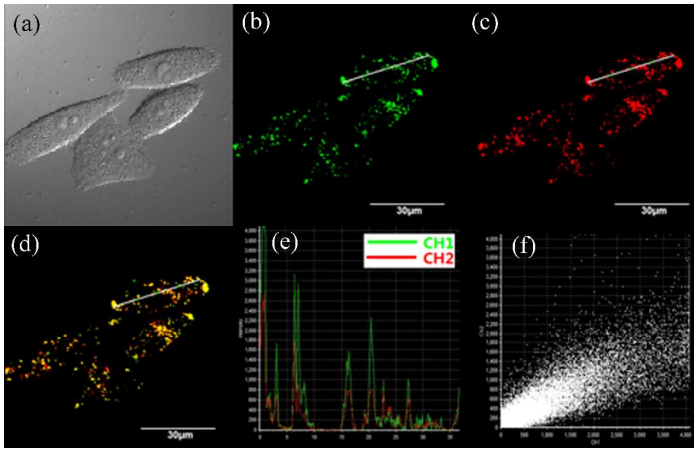


Figure S2 Colocalization imaging experiment with **Lyso-V** (5.0μM) and **NR** (5.0μM) to co-stain MCF-7 cells for 3 min at 37°C. (a) Bright field image. (b) Confocal image from **Lyso-V** on Channel 1: λ_{ex} = 488 nm, λ_{em} = 515–545 nm and (c) Confocal image from **NR** Channel 2: λ_{ex} = 559 nm, λ_{em} = 585–610 nm. (d) Merged image of channel 1 and 2. (e) Intensity profile of regions of interest (ROI) across MCF-7 cells. (f) Intensity correlation plot of stain **Lyso-V** and **NR**.

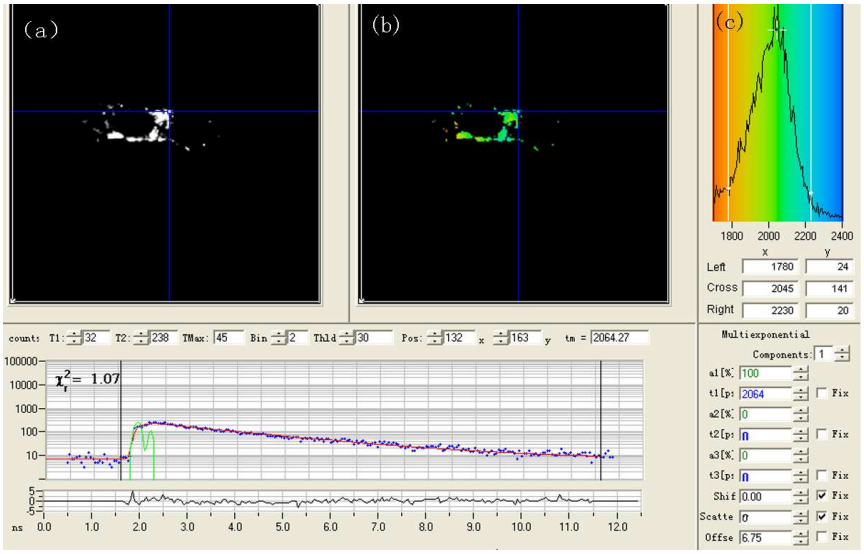


Figure S3 (a) Fluorescence image obtained following 405 nm excitation and 535±15 nm detection from MCF-7 cells incubated with 5μM solution of **Lyso-V** (b) FLIM image obtained following 405 nm pulsed excitation of the same layer of cells (c) histogram of lifetimes The histogram of the fluorescence lifetime distribution inside MCF-7 cells gives the value of (2.05±0.3) ns. According to the lifetime calibration graph (**Fig 2c**), the lifetime range corresponds to the viscosity value of 50cP ~90cP.

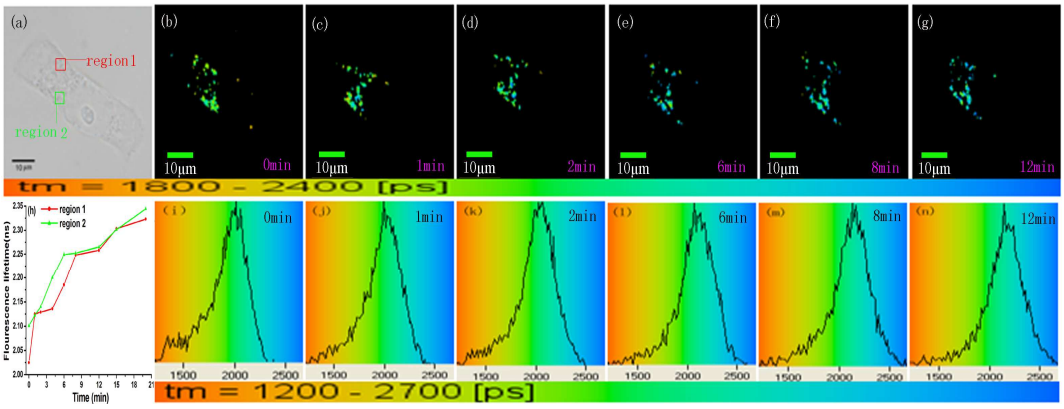


Figure S4. Fluorescence lifetime imaging of MCF-7 cell stained with 5μM **Lyso-V** and stimulated different time (0~20minutes) by 5μM dexamethasone at 535±15 nm fluorescence detection. (a) Bright field image of MCF-7 cell. (b) ~ (g) Fluorescence lifetime imaging of MCF-7 cell stimulated different time by 5μM dexamethasone. (h) Changes of fluorescence lifetime plot of **Lyso-V** (region **1** and region **2**) stimulated different time by 5μM dexamethasone. (i) ~ (n) Changes of fluorescence lifetime histogram of **Lyso-V** (total cell) stimulated different times by 5μM dexamethasone.

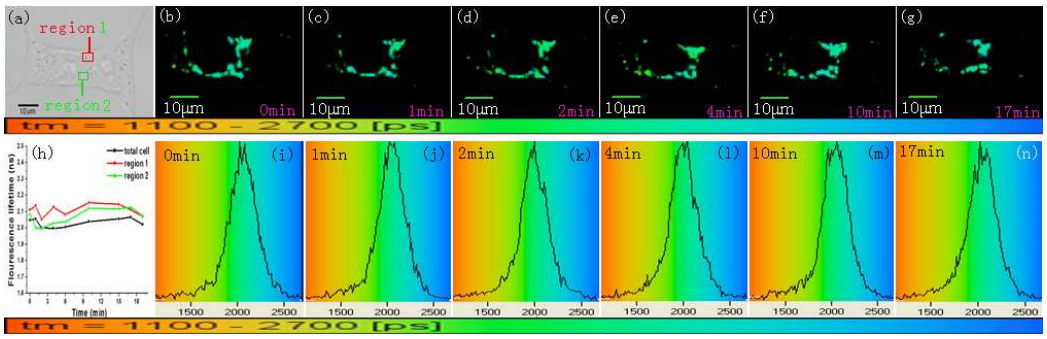


Figure S5. Fluorescence lifetime imaging of MCF-7 cell stained with 5μM **Lyso-V** and stimulated different time (0~17minutes) by 20μM chloroquine at 535±15 nm fluorescence detection. (a) Bright field image of MCF-7 cell. (b) ~ (g) Fluorescence lifetime imaging of MCF-7 cell stimulated different time by 20μM chloroquine. (h) Changes of fluorescence lifetime plot of **Lyso-V** (region **1**, region **2** and total cell) stimulated different time by 20μM chloroquine. (i) ~ (n) Changes of fluorescence lifetime histogram of **Lyso-V** (total cell) stimulated different times by 20μM chloroquine.

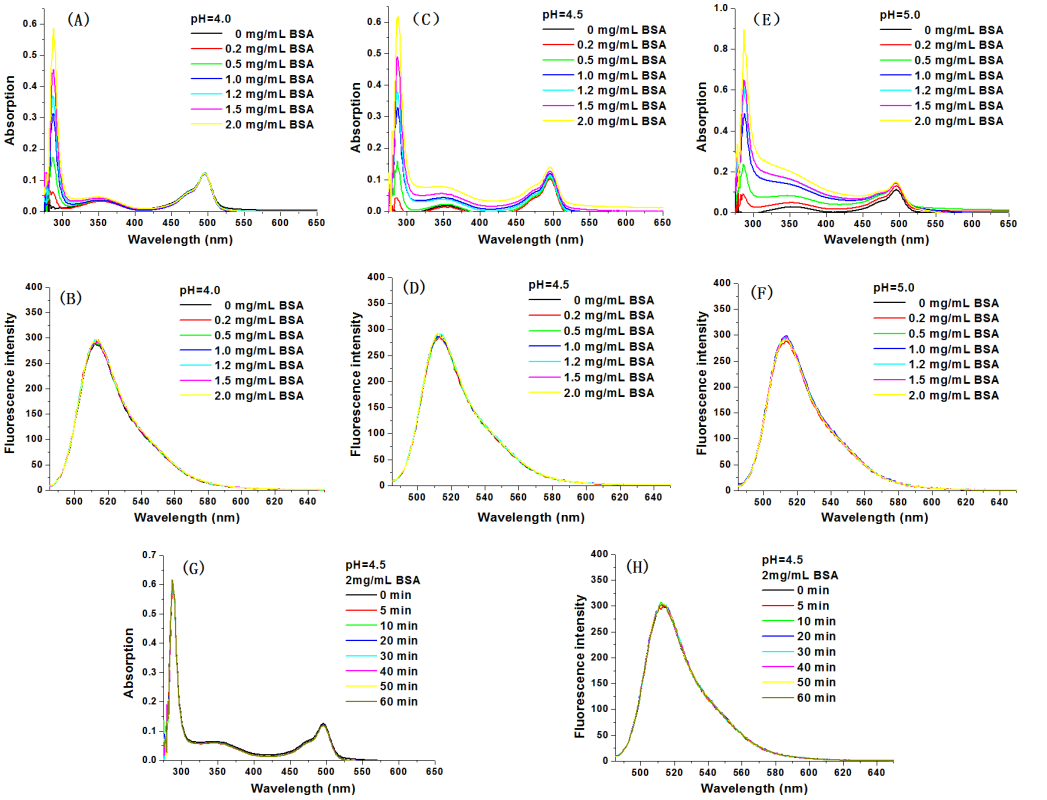
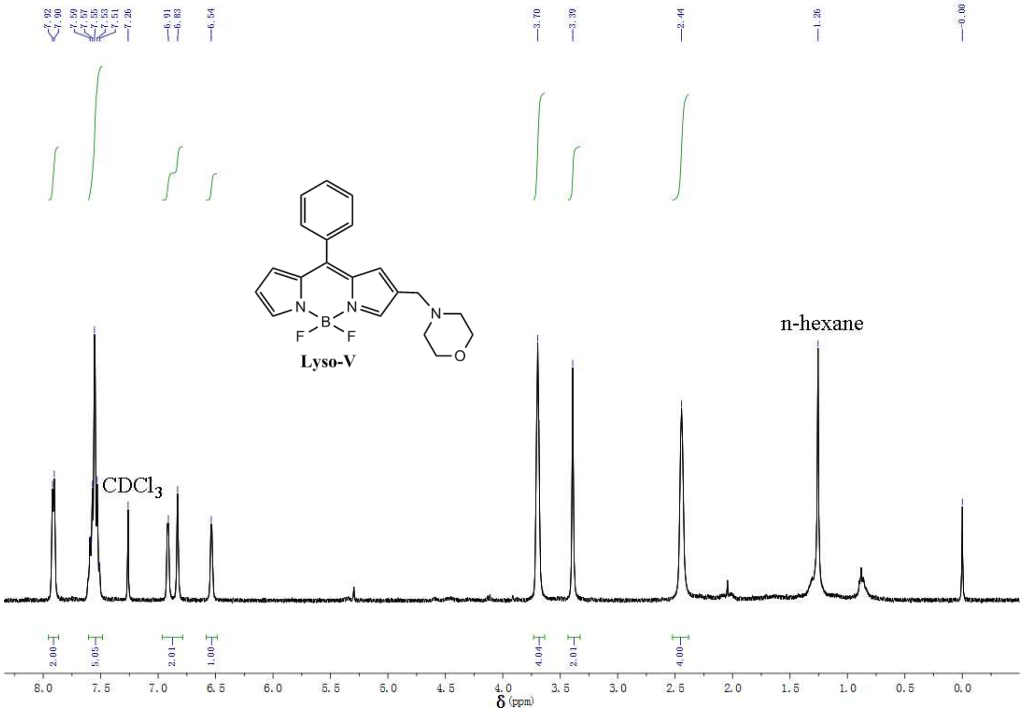
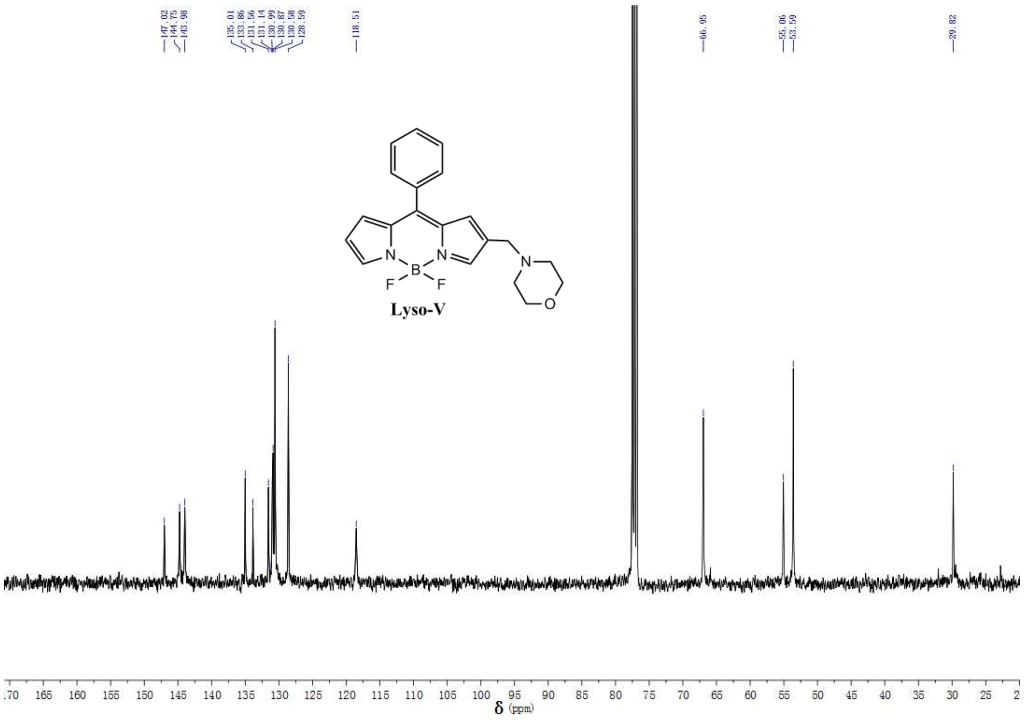


Figure S6. When Lyso-V (2.0μM) in water-methanol mixture solution (volume ratio, water:methanol=9:1, pH=4.0, 4.5, 5.0, Fig. **S6** A-F) is titrated with 0-2.0mg/ml BSA (Albumin from bovine serum), the change in absorbance (except for a new absorption peak of BSA at 280nm), fluorescence intensity are all negligible, even after the mixture is stirred for more than 60 min (Fig. **S6** G-H)

Reference:
[1] Matsumoto, T.; Urano, Y.; Shoda, T.; Kojima, H.; Nagano, T. *Org. Lett.* **2007**, 9, 3375-3377.
[2] Chang, J. Y.; Li, J. J; Hao, Y.; Jin, Y. Z; Wei, D. P.; Yang, C. W.; Zhao, Y. W.; Gao, S.Y.; Erhong, H. *Eur. J. Org. Chem.* **2011**, 28, 5460–5468.

NMR spectra of Compound Lyso-V




147.02
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143.98
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Lyso-V
δ (ppm)