Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information to

Cell Membrane Permeable Fluorescence Perylene Bisimide Derivatives for Cell Lysosome Imaging

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Table of Contents

1. General Information

Materials

4-(Bromomethyl)benzeneboronic acid pinacol ester were purchased from Jiangsu Sukailu Chemical Co., Ltd; Morpholino, N-Methyl pyrrolidone, Iodine, Sodium iodide, Bromine and AceticAcid were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd; $Pd(PPh_3)_4$ were purchased from BeiJing Greenchem Technology Co., Ltd; 3,4,9,10-Perylenetetracarboxylic dianhydride were purchased from Liaoyang Liangang Dyes Chemical Co., Ltd; Phosphate buffer saline (PBS), LysoTracker Green DND-26 and Hoechst 33342 were purchased from Life Technologies Co., Ltd. Solvents were either employed as purchased from Tianjin Kemiou Chemical Reagent Co., Ltd or dried according to procedures described in the literature. Deionized water was obtained from a Milli-Q water purification system (Millipore).

All reactions were monitored by thin-layer chromatography (TLC) on gel F254 plates. Flash chromatography was carried out on silica gel (200-300 mesh; Yantai City Chemical Industry Research Institute, Yantai, China). All pH measurements were performed with a PHS-3C digital pH-meter (Shanghai Yoke Instrument Co.,Ltd, Shanghai, China) with a combined glass-calomel electrode. The ^{1}H-NMR spectra were recorded at 20 ℃ on 600 MHz NMR spectrometer (Bruker). The^{13}C-NMR spectra were recorded at 20 ℃ on 150 MHz NMR spectrometer (Bruker). Chemical shifts are reported in ppm at room temperature using $CDCl_3$ as solvent, tetramethylsilane as internal standard unless indicated otherwise. Abbreviations used for splitting patterns are s = singlet, d = dublett, t = triplet, qui = quintet, m = multiplet. Mass spectra were carried out with Agilent LC/MSD XCT Trap. UV/Vis spectra were recorded with a Shimadzu WV-2550 spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer. The fluorescence quantum yields were determined by using N,N′-bis(ethylpropyl)perylene-3,4,9,10-tetracarboxylic diimide (Φ = 1 in DCM) for Lyso-APBI as the reference.

Preparation of Cell Cultures

HeLa cells were cultured in Dulbecco's modified Eagle's medium (high glucose, Gibco), supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37 ℃ in a 5% CO_2/95% air incubator in a humidified atmosphere, and culture media were replaced with fresh media two to three days. For fluorescence imaging, HeLa cells were grown in DMEM on a 35 mm glass bottom culture dishes at a density of 5 - 6 × 10^4 cells for at least 24 h to enable adherence to the bottom.

Cytotoxicity Test.

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazoilum bromide (MTT) reduction assay. HeLa cells with a density of 3 - 4×10^4 cells per mL were grown in 96-well plates to a total volume of 100 μL per well at 37 ℃ under an atmosphere of 5% CO_2 for 24 h. Subsequently, different concentrations Lyso-APBI probes of 1 μM, 5 μM, 2.5 μM 10 μM and 20 μM in fresh medium were incubated with HeLa cells for 24 h and 48 h, respectively, while cells in medium without probes were used as control. After incubation, MTT (10 μL, 5 mg/mL) was added to each well for 2 h. Then the medium was removed and DMSO (100 μL) was added to each well to dissolve the purple products. After shaking for 5 min, the absorbance was measured at 490 nm on a plate reader. Cell viability was expressed as a percentage of the control culture value.

Live cell imaging of probes Lyso-APBI and commercial dyes

HeLa cells were grown in Dulbecco's modified Eagle's medium (high glucose, Gibco) in a 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom. Before loading dyes, DMEM was removed, the dish was washed with PBS three times, and HeLa cells were incubated with probes Lyso-APBI (5 μmol/L) and LysoTracker Green DND-26 (1 μmol/L) in culture media at 37 ℃ for 30 min. Hoechst 33342 (5 μg/mL) was used to stain the cells for another 20 min. Afterward, the medium was removed and the dish was washed with PBS three times. The cells were observed in PBS. Images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000).

Accumulation rate in cell lysosome

HeLa cells were grown in Dulbecco's modified Eagle's medium (high glucose, Gibco) in a 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom. Before loading dyes, DMEM was removed, the dish was washed with PBS three times, and HeLa cells were incubated independently with probes Lyso-APBI-2 (1 μmol/L) and commercially available lysosome probes such as LysoTracker green (1 μmol/L) and LysoTracker red (1 μmol/L) in culture media at 37 ℃. The accumulation rate of probe Lyso-APBI-2 and commercial dyes in cell lysosome was investigated by *in situ* CLSM imaging at different time (10 min, 20 min, 30 min, 1 h and 2 h) .

Photostability of Lyso-APBI and commercial dyes in cell

HeLa cells were grown in Dulbecco's modified Eagle's medium (high glucose, Gibco) in a 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom. Before loading dyes, DMEM was removed, the dish was washed with PBS three times, and HeLa cells were incubated independently with probes Lyso-APBI (1 μmol/L) and commercially available lysosome probes LysoTracker (1 μmol/L) in culture media at 37 ℃ for 30min. Afterward, the medium was removed and the dish was washed with PBS three times. The cells were observed in PBS. Real-time changes in the fluorescence images of HeLa cells under continuous laser scanning were monitored to evaluate the photostability of Lyso-APBI in cells .

2. Synthesis and characterization of Lyso-APBI

Compound 2

4-(Bromomethyl)benzeneboronic acid pinacol ester (500 mg, 1.68 mmol) was dissolved in 5 mL CH_3CN. Then K_2CO_3 (698 mg, 5.05 mmol), morpholine (176 mg, 2.02 mmol) and NaI (5 mg, 0.3 mmol) were added and deaerated under nitrogen. The mixture was stirred at 23 ℃ overnight. With 50 mL H_2O to dilute the solution, the whole was extracted with $CHCl_3$. Then the organic layer was dried over Na_2SO_4 for 2 h and evaporated to dryness. The residue was washed three times (3×100 mL) by the mixture of CH_2Cl_2 and hexane (v/v = 1/12), and the solvent was evaporated. Compound **2** was obtained (light yellow solid, 272 mg, 53.3% yield). The ^{1}H-NMR spctrum of **2** is shown in Fig.S4. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.77 (d, J = 7.8 Hz, 2H), 7.34 (d, J = 7.7 Hz, 2H), 3.70 (t, J = 4.5 Hz, 4H), 3.51 (s, 2H), 2.43 (s, 4H), 1.34 (s, 12H). Electrospray ionization mass spectrum is shown in Fig.S6. MS m/z Calcd for $C_{17}H_{27}BNO_3$: 304.2082, found: 304.2082 [M+H]+.

Compound 3 and Compound 4

3,4,9,10-Perylenetetracarboxylic dianhydride (12.0 g, 30.59 mmol) was dissolved in 98.1 mL 18.4 mol/L H_2SO_4 and the solution was stirred at r.t. for 12 h. I_2 (0.2927 g, 1.147 mmol) was added, and the mixture was heated to 85 ℃. Subsequently, Br_2 was added dropwise within 3 h, and heating was continued at 85 ℃ for 16 h. Then the whole was cooled to r.t. and filtrated by a sand core. The filter cake washed with water until the filtrate was pH-neutral. The solid mixture was dried under vacuum and yield a red product (**3** and **4**, 14.0 g, 97% yield).

Compound 5 and Compound 6

Compound **3** and **4**, (1.0 g, 2.12 mmol) and compound **1** (1.31 g, 4.45 mmol) were dissolved in a mixture of AcOH (0.61 g, 10.18 mmol) and 25 mL N-methyl pyrrolidone and the solution was heated to 85 ℃ for 1hr. Subsequently, the solvent was evaporated under reduced pressure. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/C_2H_5OH = 40/1) provided **5** and **6** (red solid, **5**, 0.19g, 9% yield; **6**, 1.05 g, 45% yield). The ^{1}H-NMR spctrum of **5** is shown in Fig.S7. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.74 (d, J = 8.4 Hz, 1H), 8.87 (s, 1H), 8.66 (d, J = 6.6 Hz, 3H), 8.60 (m, 2H), 5.72 (m,

2H), 4.20 (m, 4H), 3.98 (m, 4H), 3.73 (m, 4H), 3.65~3.55 (m, 20H), 3.43 (m, 8H), 3.28 (d, J = 1.8 Hz, 12H); The ^{13}C-NMR spctrum of **5** is shown in Fig.S8. ^{13}C-NMR (150MHz, $CDCl_3$): δ 163.88, 163.58, 163.49, 162.74, 133.76, 133.46, 133.37, 133.29, 128.86, 128.50, 128.01, 127.88, 126.81, 123.79, 123.04, 120.79, 71.86, 70.50, 70.40, 69.26, 69.10, 58.95, 52.41, 52.25. Electrospray ionization mass spectrum is shown in Fig.S9. MS m/z Calcd for $C_{50}H_{61}BrN_2O_{16}$: 1024.3204, found: 1024.3089 [M+H]+. The ^{1}H-NMR spctrum of **6** is shown in Fig.S10. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.49 (d, J = 7.8 Hz, 2H), 8.90 (s, 2H), 8.68 (d, J = 7.2 Hz, 2H), 5.71 (m, 2H), 4.19 (m, 4H), 3.97 (m, 4H), 3.71 (m, 4H), 3.65~3.54 (m, 20H), 3.42 (t, J = 4.2 Hz, 8H), 3.29 (s, 12H); The ^{13}C-NMR spctrum of **6** is shown in Fig.S11. ^{13}C-NMR (150MHz, $CDCl_3$): δ 163.36, 162.87, 138.11, 132.93, 132.77, 130.16, 129.26, 128.46, 127.21, 123.37, 121.55, 120.72, 71.87, 70.49, 70.41, 69.04, 58.97, 52.42. Electrospray ionization mass spectrum is shown in Fig.S12. MS m/z Calcd for $C_{50}H_{60}Br_2N_2O_{16}$: 1102.2310, found: 1125.2204 [M+Na]+.

Compound Lyso-APBI-1

Compound **5** (150 mg, 0.146 mmol) and compound **2** (70 mg, 0.230 mmol) were dissolved in anhydrous toluene (60 mL). Then, 4.5 mL ethanol, 2M K_2CO_3 (4.5 mL, aq.) and $Pd(PPh_3)_4$ (9 mg, 0.008 mmol) were added. Then, the mixture was deaerated under nitrogen and heated to 80 ℃ for 18h. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/C_2H_5OH = 45/1) provided **Lyso-APBI-1** (red viscous liquid, 126.2 mg, 77% yield). The ^{1}H-NMR spctrum of **Lyso-APBI-1** is shown in Fig.S13. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.65 (d, J = 9.6 Hz, 2H), 8.60 (m, 2H), 8.53 (s, 1H), 8.07 (d, J = 5.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 7.8 Hz, 2H), 5.72 (m, 2H), 4.19 (m, 4H), 3.80 (t, J = 4.2 Hz, 4H), 3.73 (m, 4H), 3.65~3.54 (m, 20H), 3.42 (t, J = 4.8 Hz, 8H), 3.28 (d, J = 3.0 Hz, 12H), 3.55 (s, 4H); The ^{13}C-NMR spctrum of **Lyso-APBI-1** is shown in Fig.S14. ^{13}C-NMR (150MHz, $CDCl_3$): δ 163.99, 163.85, 163.79, 134.86, 132.55, 131.01, 129.89, 129.20, 128.55, 128.47, 128.04, 127.53, 123.56, 122.71, 71.87, 70.49,

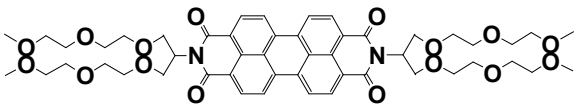
70.47, 70.39, 70.34, 69.29, 69.24, 67.04, 58.95, 52.22, 52.12; Electrospray ionization mass spectrum is shown in Fig.S15. MS m/z Calcd for $C_{61}H_{75}N_3O_{17}$: 1122.2, found: 1123.0 $[M+H]^+$.

Compound Lyso-APBI-2

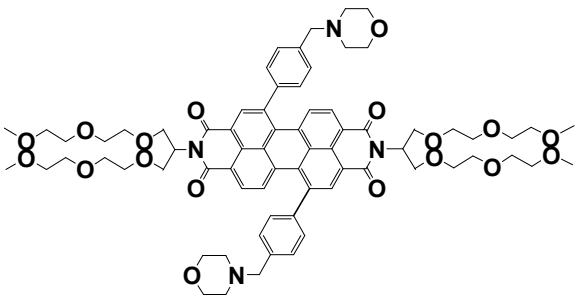
Compound **6** (100 mg, 0.091 mmol) and compound **2** (82 mg, 0.271 mmol) were dissolved in anhydrous toluene (60 mL). Then, 3.0 mL ethanol, 2M K_2CO_3 (3.0 mL, aq.) and $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) were added. Then, the mixture was deaerated under nitrogen and heated to 80 ℃ for 18h. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/C_2H_5OH = 45/1) provided **Lyso-APBI-2** (red viscous liquid, 91 mg, 77% yield). The ^{1}H-NMR spctrum of **Lyso-APBI-2** is shown in Fig.S16. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.58 (s, 2H), 8.07 (s, 2H), 7.84 (m, 2H), 7.50 (s, 8H), 5.69 (m, 2H), 4.15 (m, 4H), 3.95 (m, 4H), 3.79 (s, 8H), 3.68 (m, 4H), 3.62~3.54 (m, 22H), 3.42 (t, J = 4.8 Hz, 8H), 3.28 (s, 12H), 3.55 (s, 8H); The ^{13}C-NMR spctrum of **Lyso-APBI-2** is shown in Fig.S17. ^{13}C-NMR (150MHz, $CDCl_3$): δ 163.93, 163.87, 140.90, 134.91, 134.31, 132.80, 132.52, 130.87, 130.10, 129.63, 129.22, 129.02, 128.80, 127.91, 122.44, 122.12, 71.86, 70.47, 70.34, 69.25, 66.95, 62.94, 58.95, 53.69, 52.12; Electrospray ionization mass spectrum in Fig.S18. MS m/z Calcd for $C_{72}H_{88}N_4O_{18}$: 1297.5, found: 1298.8 $[M+H]^+$.

3 Supplementary Figures, Tables

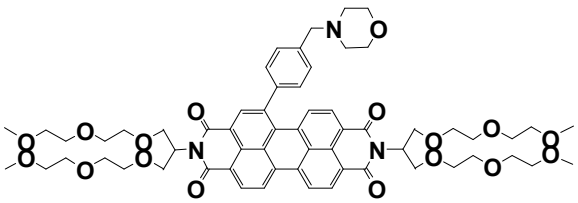
Chart.S1 The structural formula of various compounds mentioned in the paper



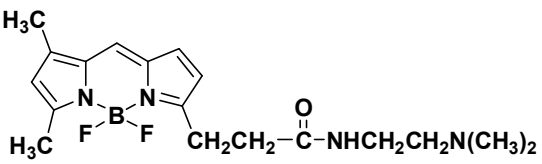
APBI



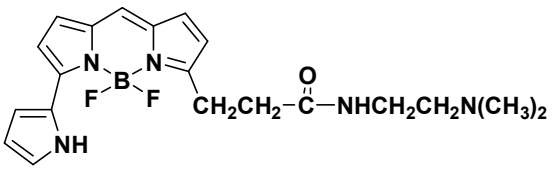
Lyso-APBI-2



Lyso-APBI-1



LysoTracker- Green DND-26



LysoTracker- Red DND-99

Table S1 The properties of Lyso-APBI and LysoTracker Probes

	$\lambda_{ex}/\lambda_{em}$ (nm)	Stokes shift (nm)	Water solubility (g/L)	pK_a	acid activation ratio [a]
Lyso-APBI-1	512/607	97	164.2±2.3	5.7 ±0.2	70
Lyso-APBI-2	551/624	73	235.7±1.6	5.4 ±0.2	190
LysoTracker Green DND-26	504/511	7	——[b]	——[c]	low
LysoTracker Red DND-99	577/590	13	——[b]	——[c]	low

[a] the intensity of fluorescence at pH 4/ pH 8; [b] Insoluble; [c] not obtain.

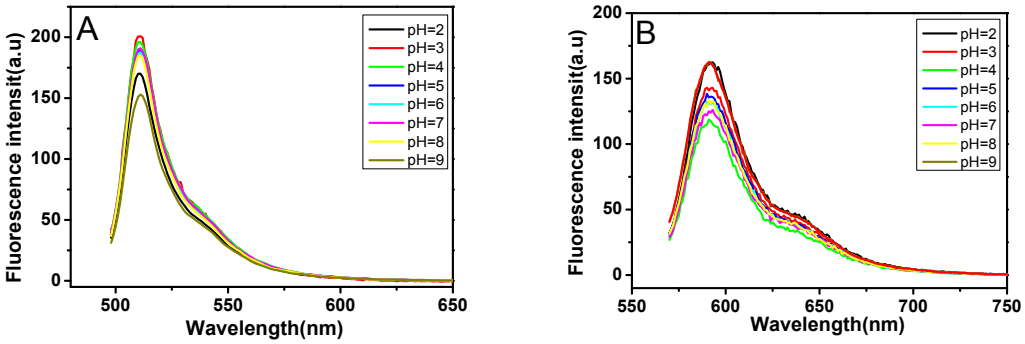


Fig.S1 The fluorescence spectra of LysoTracker Green (A) and LysoTracker Red (B) recorded at different pH values.

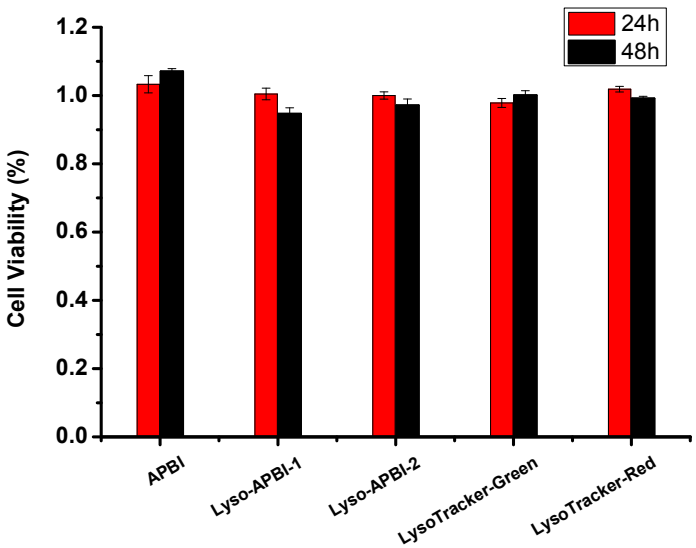


Fig.S2 In vitro viability of Hela cells treated with **APBI, Lyso-APBI** and commercial dye LysoTracker for 24 h and 48 h. The concentration of all probes is 10uM.

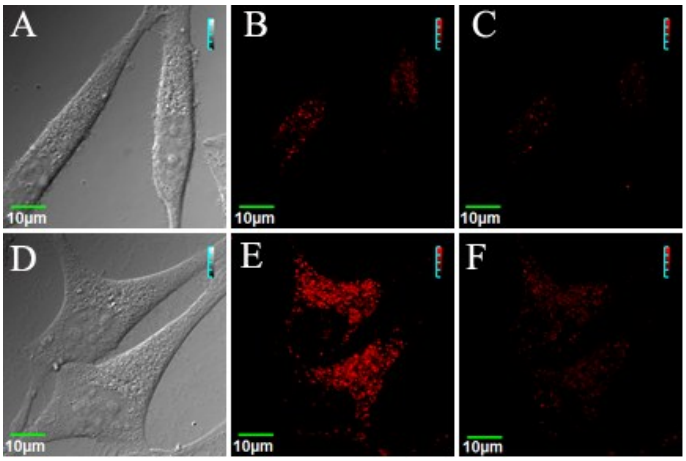


Fig.S3 Confocal images of HeLa cells stained with 5.0 μM Lyso-APBI and stimulated using 100 μM chloroquine for 2 min. Lyso-APBI-1: (A) bright-field image; (B) images of the stained cells before chloroquine stimulation; (C) images of cells stimulated with 100 μM chloroquine for 2 min. Lyso-APBI-2: (D) bright-field image; (E) images of the stained cells before chloroquine stimulation; (F) images of cells stimulated with 100 μM chloroquine for 2 min.

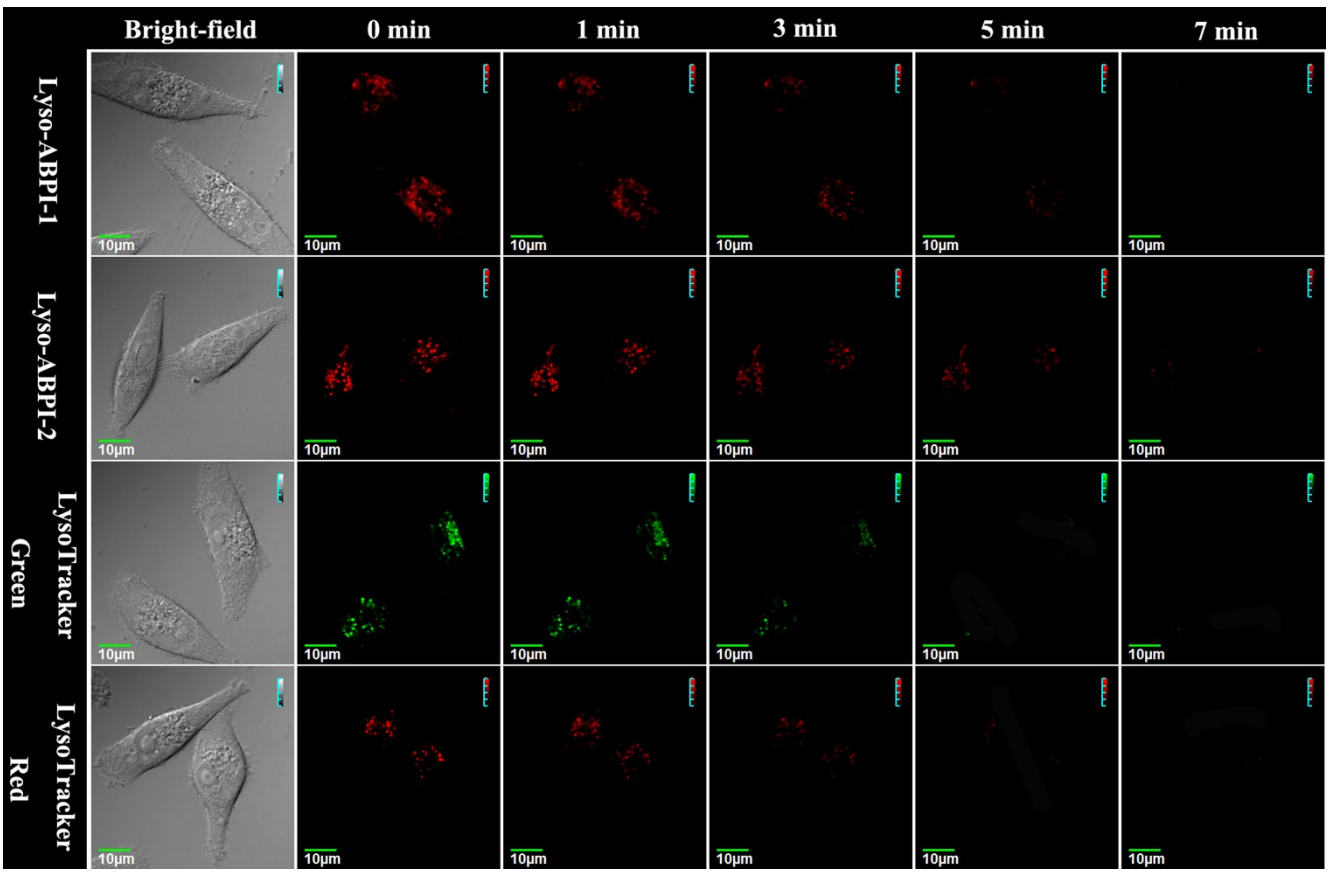


Fig.S4 Real-time changes in the fluorescence images of HeLa cells stained by **Lyso-APBI** and Lyso Tracker under continuous laser scanning were monitored to evaluate the photostability of probes in cells.

Part B: ^{1}H-NMR spectrum, ^{13}C NMR spectrum and MALDI-TOF spectrum

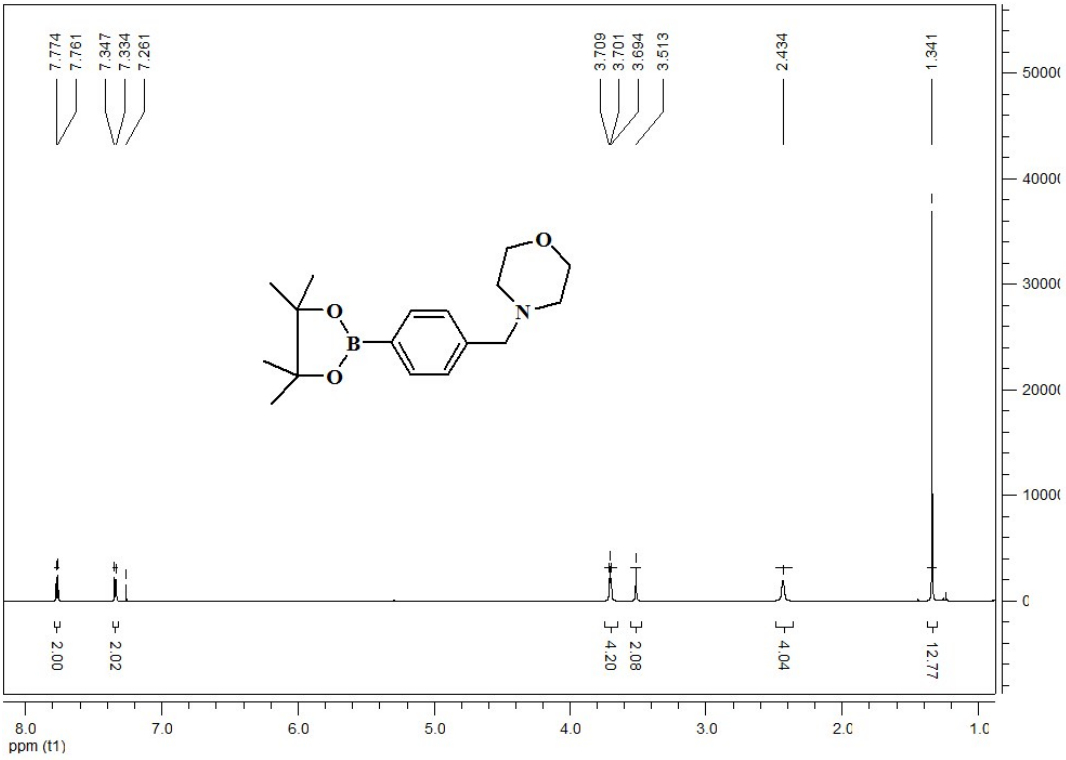


Fig.S5 ^{1}H-NMR spectrum(600MHz, $CDCl_3$, 20 ℃) of **2**

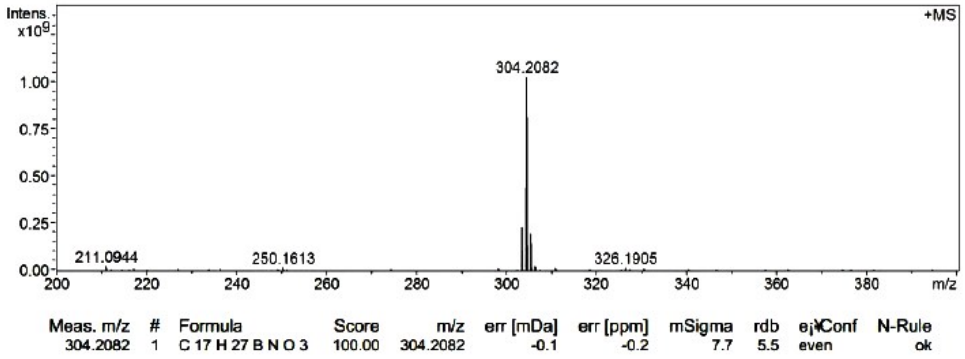


Fig.S6 Electrospray ionization mass spectrum of **2**

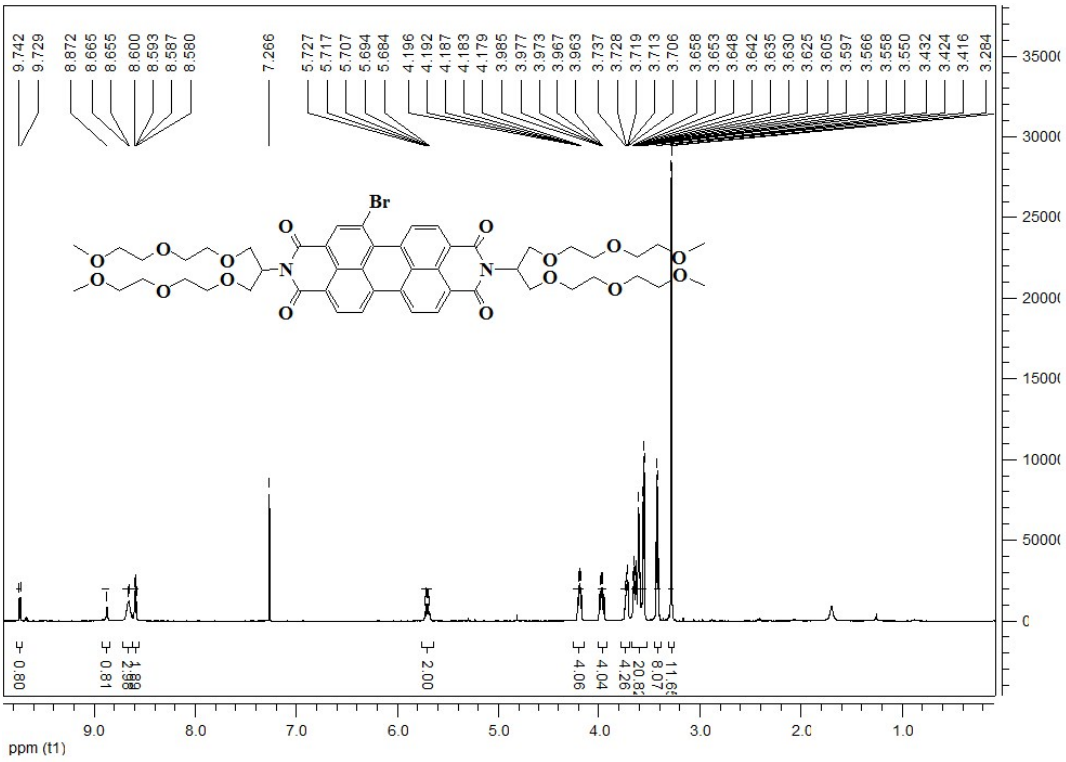


Fig.S7 ^{1}H-NMR spectrum(600MHz, $CDCl_3$, 20 ℃) of **5**

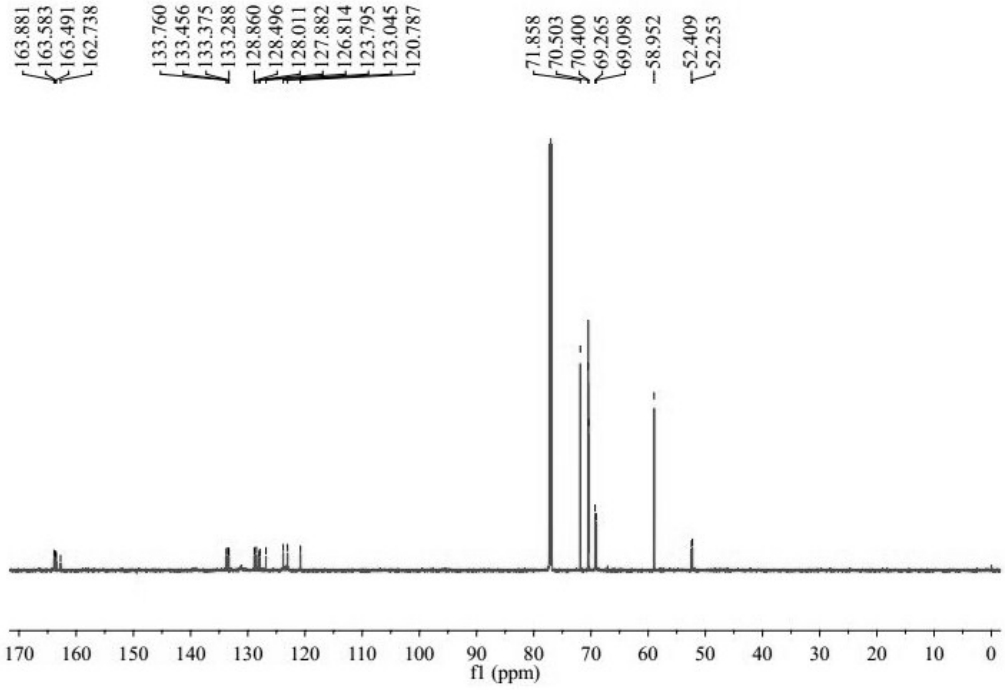
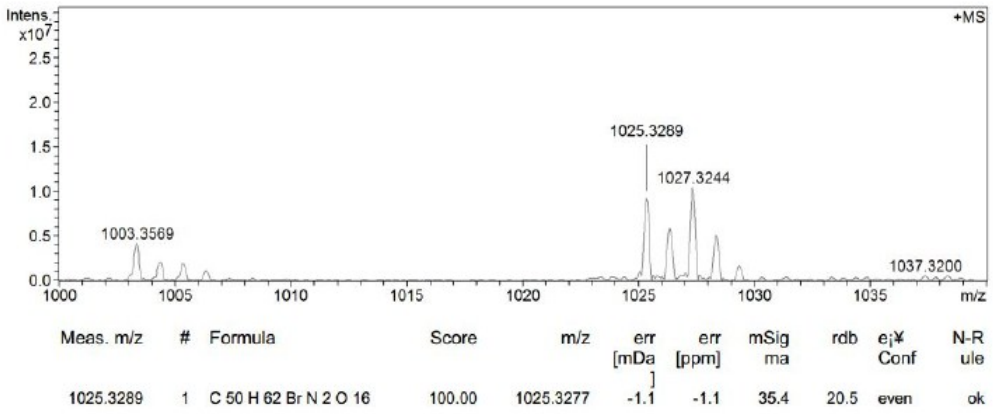


Fig.S8 ^{13}C-NMR spectrum(150MHz, $CDCl_3$, 20 ℃) of **5**



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¡¥ Conf	N-Rule
1025.3289	1	C 50 H 62 Br N 2 O 16	100.00	1025.3277	-1.1	-1.1	35.4	20.5	even	ok

Fig.S9 Electrospray ionization mass spectrum of **5**

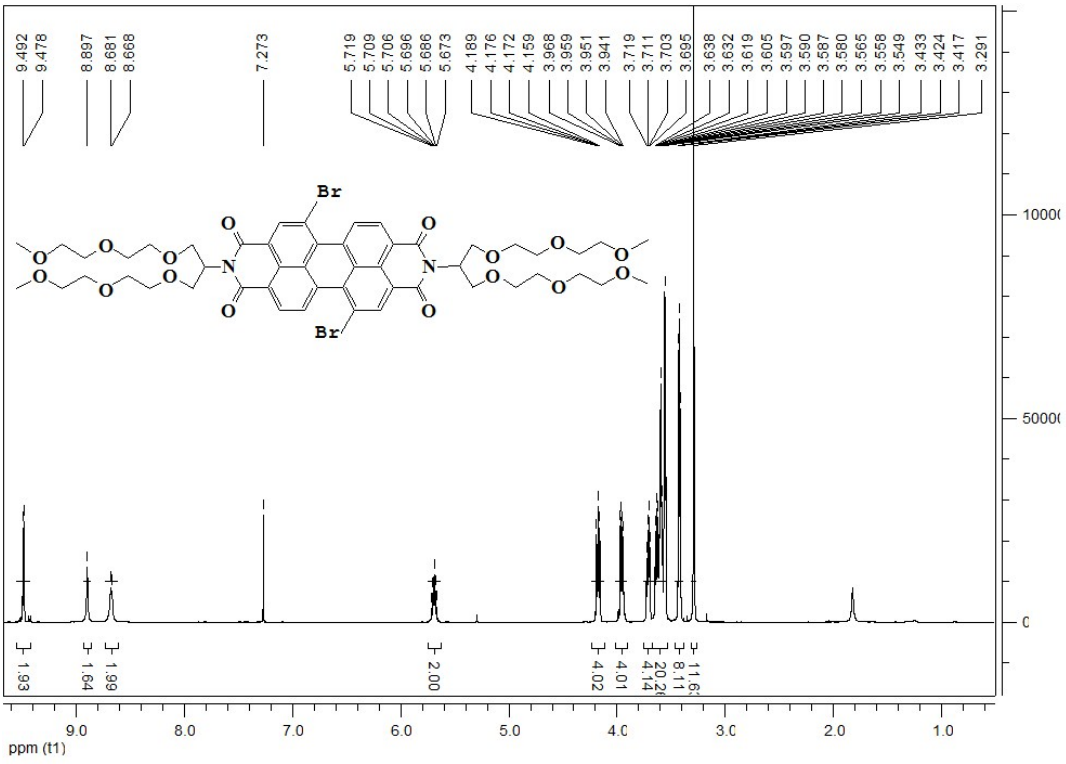


Fig.S10 ^{1}H-NMR spectrum(600MHz, $CDCl_3$, 20 ℃) of **6**

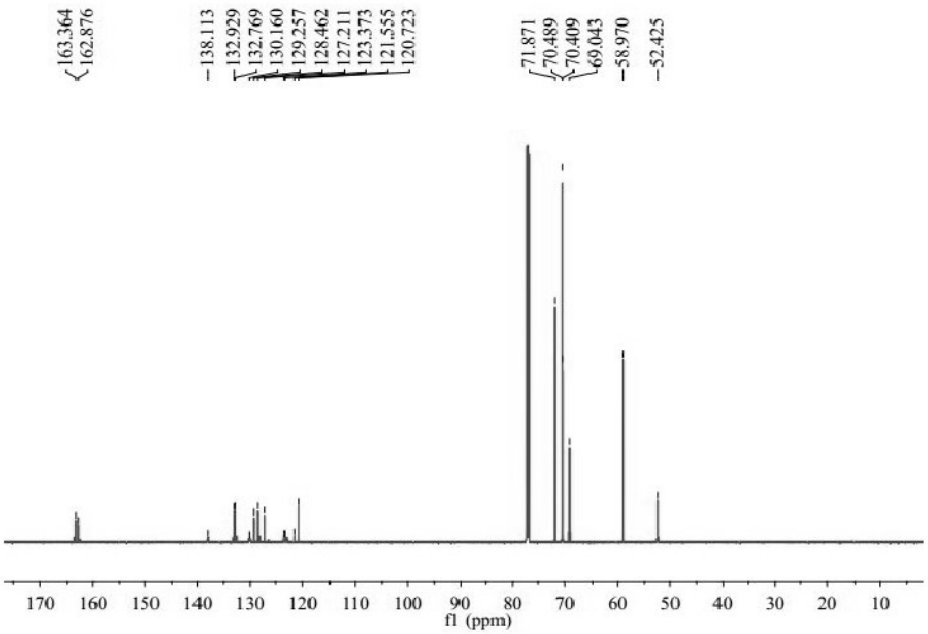
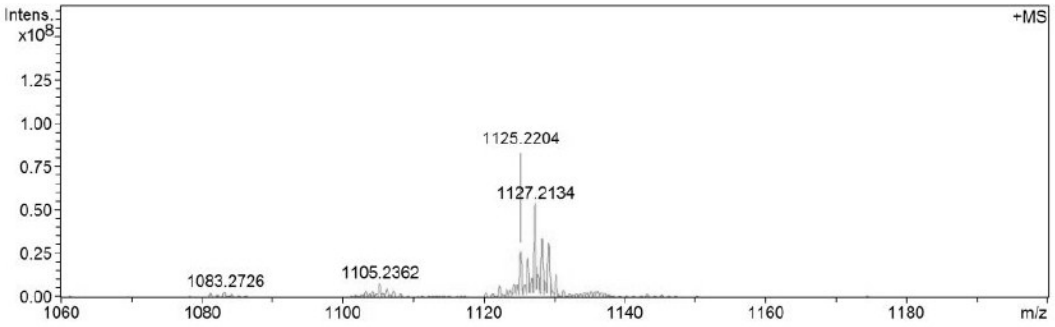


Fig.S11 ^{13}C-NMR spectrum(150MHz, $CDCl_3$, 20 ℃) of **6**



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¡¥ Conf	N-Rule
1125.2204	1	C 50 H 60 Br 2 N 2 Na O 16	100.00	1125.2202	-0.2	-0.2	71.5	20.5	even	ok

Fig.S12 Electrospray ionization mass spectrum of **6**

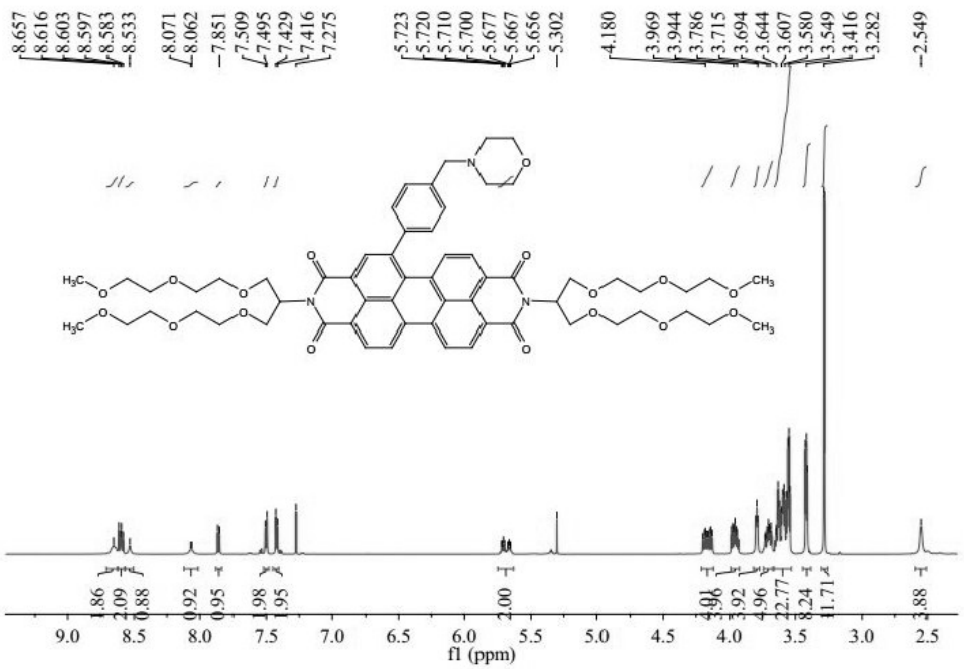


Fig.S13 ^{1}H-NMR spectrum(600MHz, $CDCl_3$, 20 ℃) of **Lyso-APBI-1**

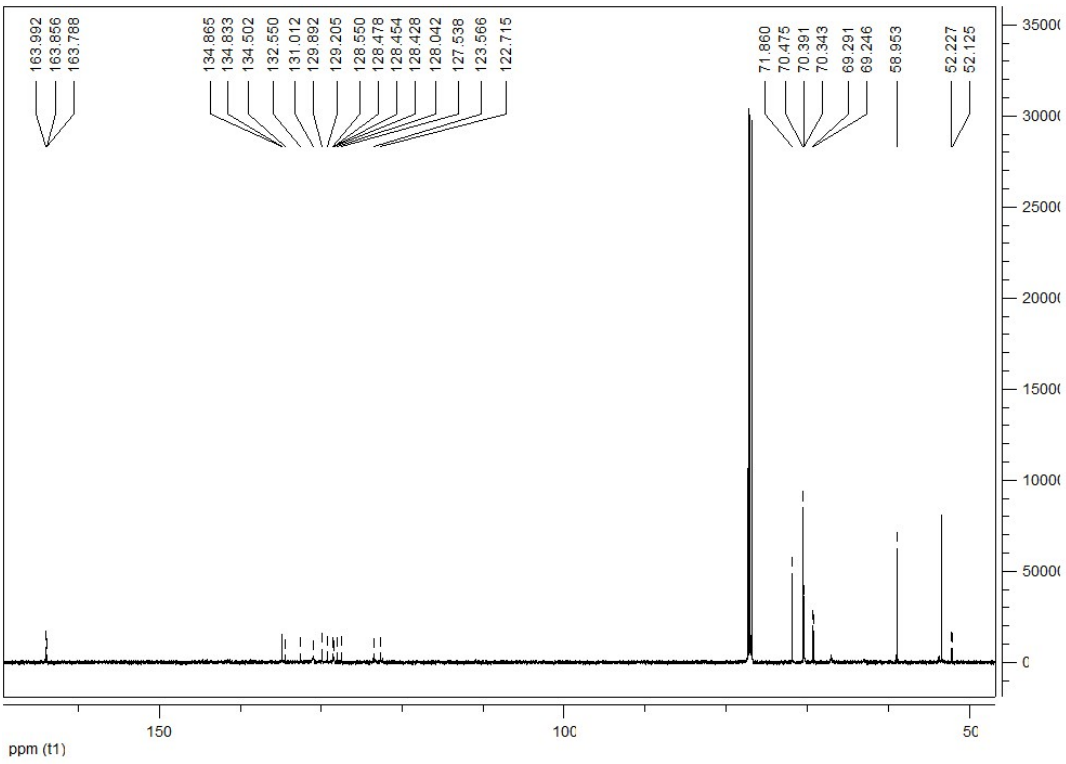


Fig.S14 ^{13}C-NMR spectrum(150MHz, $CDCl_3$, 20 ℃) of **Lyso-APBI-1**

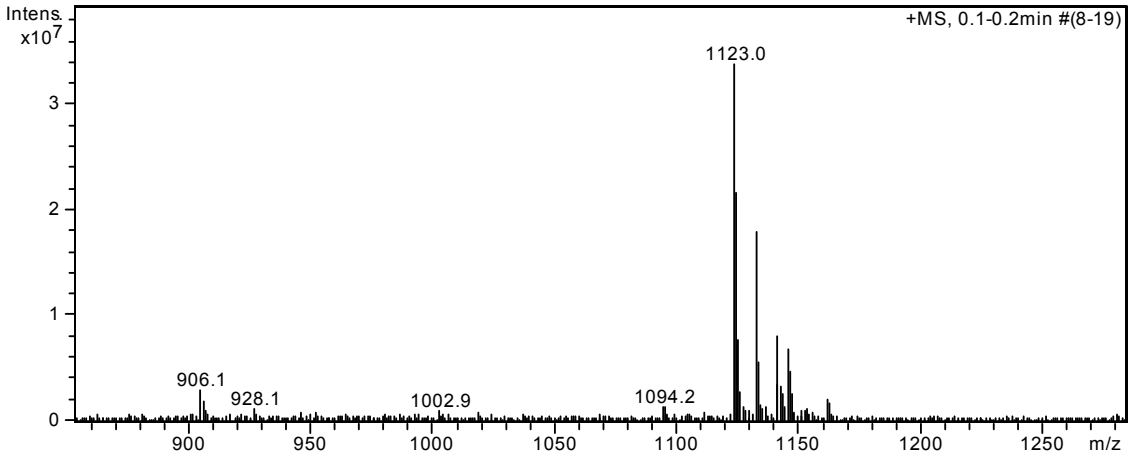


Fig.S15 Electrospray ionization mass spectrum of **Lyso-APBI-1**

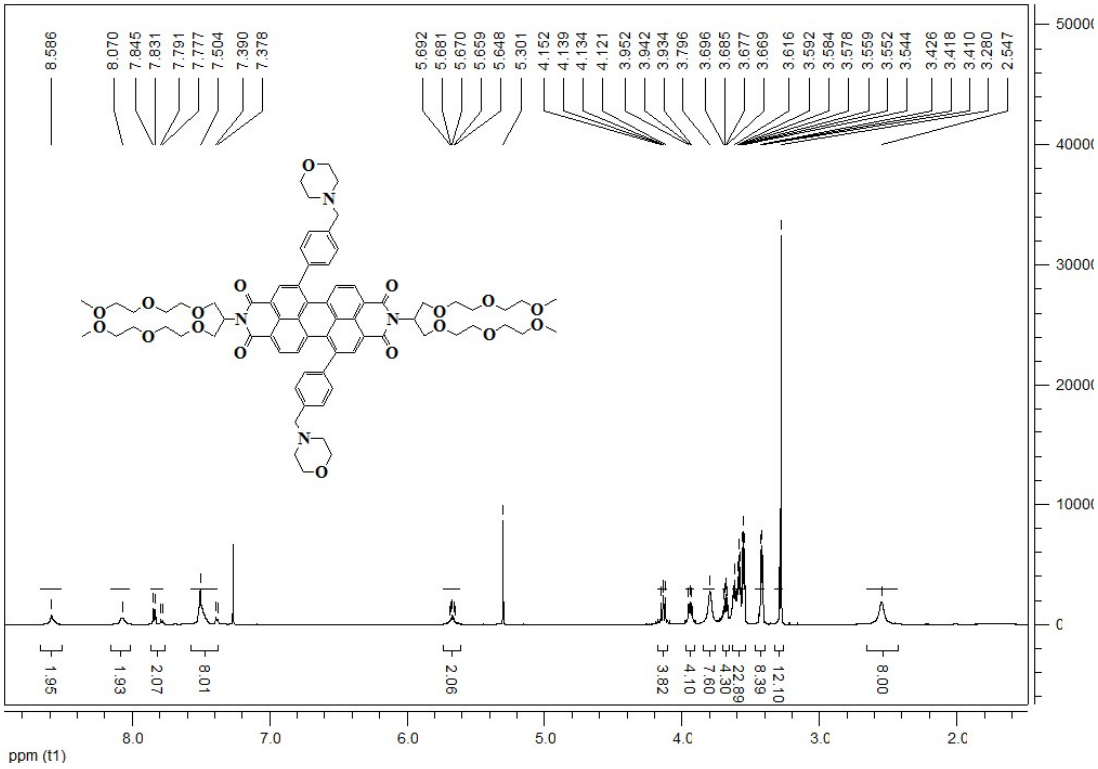


Fig.S16 ^{1}H-NMR spectrum(600MHz, $CDCl_3$, 20 ℃) of **Lyso-APBI-2**

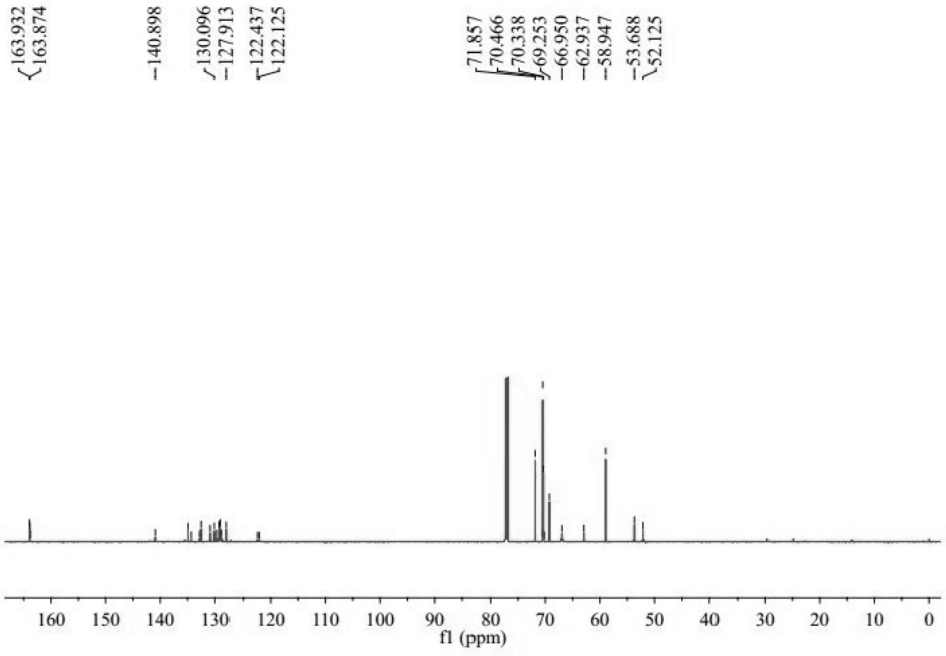


Fig.S17 ^{13}C-NMR spectrum(150MHz, $CDCl_3$, 20 ℃) of **Lyso-APBI-2**

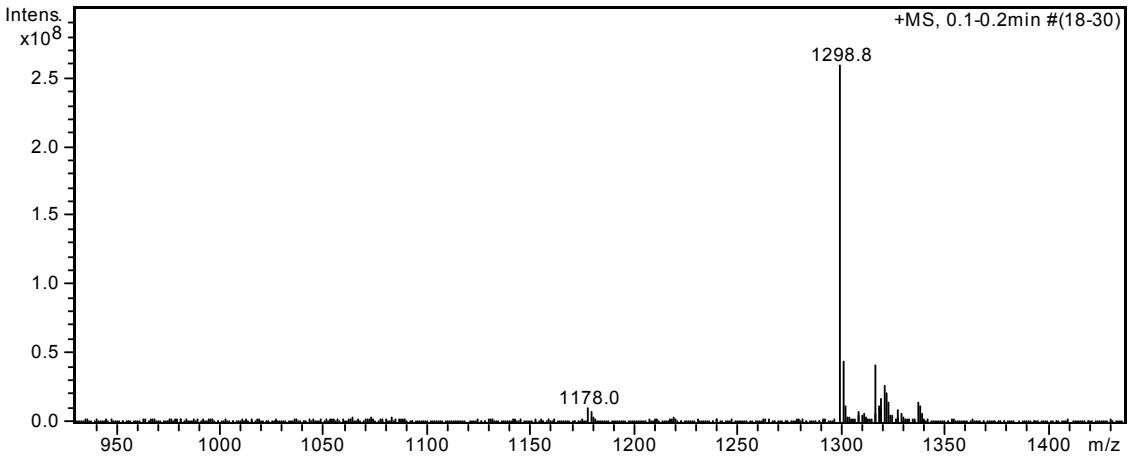


Fig.S18 Electrospray ionization mass spectrum of **Lyso-APBI-2**