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Supplementary Information

Redox-Responsive Tetraphenylethylene-Buried Cross-Linked Vesicle for Enhanced Drug Loading and Efficient Drug Delivery Monitoring

Yunlong Yu,[†] Yun Chen,[†] Jingsheng Huang,[†] Liang Wang,[†] Zhongwei Gu,[‡] and Shiyong Zhang[*,†]*

[†] National Engineering Research Center for Biomaterials, College of Chemistry, Sichuan University, 29 Wangjiang Road, Chengdu 610064, China.

[‡] College of Materials Science and Engineering, Nanjing Tech University, Nanjing 210009, Jiangsu, China.

EXPERIMENTAL SECTION

Materials

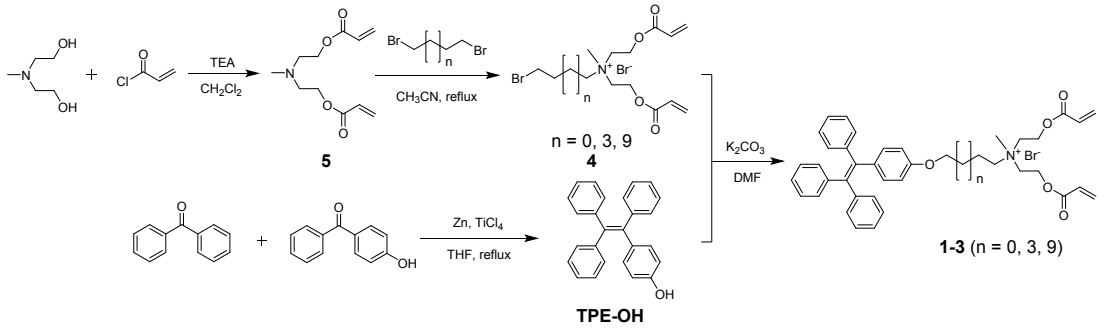
All reagents were obtained from commercial suppliers (such as acrylyl chloride purchased from Adamas-beta Ltd.) which were used without further purification. Cell counter kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Nude mice (14 ± 2 g, 4-5 weeks old) were purchased from Dashuo experimental animal company (Sichuan, China).

General Method

^{1}H NMR spectra were obtained by a Bruker AV II-400, and the chemical shifts were labeled relative to $CDCl_3$ and D_2O ($CDCl_3$: δ 7.26 ppm; D_2O: δ 4.79 ppm) using tetramethylsilane (TMS) as internal reference. The fluorescence emission was carried out on a Hitachi F-7000 fluorescence spectrometer. The particle size and zeta potential were characterized by the Dynamic Light Scattering (DLS) Analyzer (Malvern ZetasizerNano ZS90). TEM images were obtained on a Tecnai G2F20S-TWIN instrument. The samples were stained with an aqueous solution of 2% phosphotungstic acid, dried for 0.5 h under infrared lamps. Human liver cancer (HepG2) was obtained from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China) which was used for all cell experiments and animal xenograft model. The cell line was grown in Dulbecco's modification of Eagle's medium Dulbecco supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin in an incubator under 5% CO_2 at 37 ^oC. Cell toxicity was evaluated by measuring the percentage of cell viability via the Cell Counting Kit-8 assay (CCK-8). The absorbance

was then measured using a microplate reader Varioscan Flash (ThermoFisher SCIENTIFIC). The cell viability (%) was obtained according to the manufacturer's instructions. The cellular uptake of HepG2 cells incubated with free DOX, TPE@CLV **III**, DOX@TPE@CLV **III** and T-DOX@TPE@CLV **III** were observed under confocal laser scanning microscopy (CLSM, LSM780) and flow cytometry (BD FACSCalibur/Calibur) provided by College of Chemistry Experiment Platform Center. *In vivo* fluorescence images were acquired by Live Cell Imaging System (LCIS, Maestro CRi, Inc., USA).

Synthesis



Scheme 1S. Synthesis of compounds **1-3**.

Synthesis of compound 5. Acryloyl chloride (6.84 g, 75.5 mmol) was added dropwise to a solution of *N*-methyldiethanolamine (3.0 g, 25.2 mmol) and triethylamine (TEA, 10.2 g, 100.7 mmol) in 70 mL of dry dichloromethane (CH_2Cl_2) at 0 ^oC. The reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was concentrated and the residue was purified by column chromatography over silica gel (petroleum ether: ethyl acetate = 2:1) to give **3** (3.0 g, 53%) as a clear oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3 H), 2.77 (t, J = 5.8 Hz, 4 H), 4.26 (t, J = 5.8 Hz, 4 H), 5.82. (dd, J = 1.2, 1.6 Hz, 2 H).

Synthesis of compound 4 (n = 0). Under N_2 atmosphere, compound **3** (361.7 mg,

1.5 mmol) and 1,3-Dibromopropane (200 mg, 1.0 mmol) were refluxed in dry acetonitrile (CH_3CN) for 36h. After cooling to room temperature, the reaction mixture was concentrated, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The combine organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The combine organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography over silical gel (dichloromethane: CH_3OH = 10:1) to give the product as yellow liquid (234.3 mg, 51 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 2 H), 3.06 (s, 2 H), 3.57 (s, 2 H), 3.54 (s, 3 H), 3.86-3.88 (m, 2 H), 4.19 (s, 4 H), 4.74 (m, 4 H), 5.92-5.94 (m, 2 H), 6.10-6.17 (m, 2 H), 6.43-6.47 (m, 2 H).

Synthesis of compound 4 (n = 3). Under N_2 atmosphere, compound **3** (361.7 mg, 1.5 mmol) and 1,6-Dibromohexane (244 mg, 1.0 mmol) were refluxed in dry acetonitrile (CH_3CN) for 36h. After cooling to room temperature, the reaction mixture was concentrated, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The combine organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The combine organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography over silical gel (dichloromethane: CH_3OH = 10:1) to give the product as yellow liquid (220 mg, 56 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76-1.91 (m, 8 H), 3.41 (t, J = 6.2 Hz, 2 H), 3.54 (s, 2 H), 3.54 (s, 3 H), 3.67-3.71 (m, 2 H), 4.14-4.25 (m, 4 H), 4.72 (t, J = 4.6 Hz, 4 H), 5.95-5.98 (m, 2 H), 6.09-6.16 (m, 2 H), 6.46-6.51 (m, 2 H).

Synthesis of compound 4 (n = 9). Compound **3** (1.023 g, 4.5 mmol) and 1,12-

dibromododecane (984 mg, 3.0 mmol) were refluxed in dry acetonitrile (CH_3CN) under N_2 atmosphere for 36 h. After cooling to room temperature, the reaction mixture was concentrated, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The combine organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography over silical gel (dichloromethane: CH_3OH = 10:1) to give the product as yellow liquid (752 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24-1.42 (m, 16 H), 1.80-1.97 (m, 4 H), 3.40 (t, J = 6.8 Hz, 2 H), 3.54 (s, 3 H), 3.58-3.62 (m, 2 H), 4.14-4.24 (m, 4 H), 4.18-4.20 (m, 4 H), 4.72 (s, 4 H), 5.93-5.96 (m, 2 H), 6.08-6.14 (m, 2 H), 6.45-6.94 (m, 2 H).

Synthesis of TPE-OH.[1] Under N_2 atmosphere, a three-necked flask equipped with a magnetic stirrer was charged with zinc powder (1.6 g, 24 mmol) and dry tetrahydrofuran (THF, 40 mL). The mixture was cooled to -5 to 0 ^oC, and dry $TiCl_4$ (1.3 mL, 12 mmol) was slowly added by a syringe with the temperature kept under 10 ^oC. The suspending mixture was warmed to room temperature and stirred for 0.5 h, then heated over refluxing for 2.5 h. The mixture was again cooled to -5 to 0 ^oC, charged with pyridine (0.5 mL, 6 mmol) and stirred for 10 min. The solution of diphenyl ketone and 4-hydroxy diphenyl ketone (2.4 mmol, in 1:1.2 mole ratio) in 15 mL THF was added slowly. After addition, the reaction mixture was heated over refluxing until the carbonyl compounds were consumed (monitored by TLC). The reaction was quenched with 10% K_2CO_3 aqueous solution and taken up with CH_2Cl_2. The organic layer was collected. After solvent evaporation, the crude product was purified on a silica gel column (petroleum ether: ethyl acetate from 20:1 to 7:1) to give the desired product as

a white powder (600 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 1 H), 6.56 (d, *J* = 8 Hz, 2 H), 6.89 (d, *J* = 8 Hz, 2 H), 7.00-7.12 (m, 15 H).

Synthesis of compound 1 (n = 0**)**. Potassium carbonate (103.7 mg, 0.75 mmol) was added to a solution of TPE-OH (174 mg, 0.5 mmol) in DMF (4 mL) at room temperature. After the mixture was stirred at 60 ^{o}C for 2 h, compound **2** (n = 0, 213.5 mg, 0.5 mmol) was added slowly. The resulting reaction mixture was further stirred overnight at 60 ^{o}C under N_2, cooled to room temperature, and poured over 50 mL of icy water. The mixture was then extracted with CH_2Cl_2 (100 mL × 3). The combined organic phase was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo.* The crude product was purified on a silica gel column with CH_2Cl_2/CH_3OH (10/1) as the eluent to give the product as yellow liquid (269.4 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 2 H), 3.58 (s, 3 H), 3.87 (s, 2 H), 4.02 (s, 2 H), 4.25 (s, 4 H), 4.66-4.73 (m, 4 H), 5.87-5.90 (m, 2 H), 6.07-6.14 (m, 2 H), 6.43-6.47 (m, 2 H), 6.56-6.58 (m, 2 H),6.91-6.93 (m, 2 H), 7.00-7.03 (m, 15 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.9, 50.6, 50.6, 58.0, 61.6, 61.7, 64.2, 113.8, 126.6, 126.7, 127.2, 127.8, 127.9, 128.0, 131.5, 132.8, 133.5, 140.7, 144.0, 144.1, 156.7, 165.3. High resolution ESI-MS: $[M\text{-}Br]^+$ calcd for $C_{40}H_{42}NO_5^+$ 616.3057, found 616.3056.

Synthesis of compound 2 (n = 3**)**. Potassium carbonate (103.7 mg, 0.75 mmol) was added to a solution of TPE-OH (174 mg, 0.5 mmol) in DMF (4 mL) at room temperature. After the mixture was stirred at 60 ^{o}C for 2 h, compound **2** (n = 3, 234.5 mg, 0.5 mmol) was added slowly. The resulting reaction mixture was further stirred overnight at 60 ^{o}C under N_2, cooled to room temperature, and poured over 50 mL of

icy water. The mixture was then extracted with CH_2Cl_2 (100 mL × 3). The combined organic phase was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo.* The crude product was purified on a silica gel column with CH_2Cl_2/CH_3OH (10/1) as the eluent to give the product as yellow liquid (250.7 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41-1.51 (m, 4 H), 1.71 -1.83 (m, 4 H), 3.53 (s, 3 H), 3.63-3.66 (m, 2 H), 3.83-3.86 (m, 2 H), 4.13-4.18 (m, 4 H), 4.73 (s, 4 H), 5.86-5.89 (m, 2 H), 6.06-6.13 (m, 2 H), 6.43-6.47 (m, 2 H), 6.58-6.60 (m, 2 H), 6.89-6.91 (m, 2 H), δ 7.00-7.08 (m, 15 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.8, 22.8, 25.9, 26.2, 29.1, 50.0, 57.9, 61.2, 63.7, 67.3, 113.6, 126.3, 127.1, 127.8, 131.4, 132.6, 133.2, 136.2, 144.0, 144.1, 157.5, 165.2. High resolution ESI-MS: $[M+K]^+$ calcd for $C_{43}H_{48}NO_5^+$ 658.3527, found 658.3506.

Synthesis of compound 3 (n = 9**)**. Potassium carbonate (103.7 mg, 0.75 mmol) was added to a solution of TPE-OH (174 mg, 0.5 mmol) in DMF (4 mL) at room temperature. After the mixture was stirred at 60 ^{o}C for 2 h, compound **2** (n = 9, 276.6 mg, 0.5 mmol) was added slowly. The resulting reaction mixture was further stirred overnight at 60 ^{o}C under N_2, cooled to room temperature, and poured over 50 mL of icy water. The mixture was then extracted with CH_2Cl_2 (100 mL × 3). The combined organic phase was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo.* The crude product was purified on a silica gel column with CH_2Cl_2/CH_3OH (10/1) as the eluent to give the product as yellow liquid (300 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24-1.40 (m, 18 H), 1.69 -1.73 (m, 2 H), 3.53 (s, 3 H), 3.58-3.62 (m, 2 H), 3.83-3.86 (m, 2 H), 4.13-4.20 (m, 4 H), 4.72 (s, 4 H), 5.93-6.08 (m,

2 H), 6.11-6.14 (m, 2 H), 6.45-6.50 (m, 2 H), 6.59-6.61 (m, 2 H), 6.89-6.91 (m, 2 H), δ 7.00-7.08 (m, 2 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.9, 23.1, 26.2, 26.4, 29.3, 29.4, 29.5, 29.6, 50.0, 57.9, 61.1, 63.9, 66.6, 67.9, 113.7, 114.6, 126.3, 126.4, 127.1, 127.2, 127.7, 127.8, 128.9, 131.0, 131.4, 131.5, 132.6, 133.0, 133.2, 136.0, 140.1, 140.7, 144.1, 157.8, 165.2. High resolution ESI-MS: $[M+Na]^+$ calcd for $C_{49}H_{60}NO_5^+$ 742.4466, found 742.4488.

Synthesis and characterization of tetraphenylethylene-buried vesicles (TPE@LV **III).** Compound **3** (50 μL, 0.12 M in n-propanol) was added dropwise into 5.0 mL of deionized water with the speed of 1.2 mL/min under ultrasonic vibration at room temperature. The TPE@LV **III** stayed for several minutes and formed spontaneously as a pale blue emulsion with strong Tyndall effect.

Carboxyfluorescein leakage assay. Briefly, compound **3** (50 μL, 0.12 M in co-solvent) was added dropwise into 5.0 mL of 5(6)-Carboxyfluorescein (CF, 1.5 mM) solution with a speed of 1.2 mL/min ultrasonic vibration. A portion (1.0 mL) of the resulting nanoparticle solution was passed through a column of Shepadex G-50 using millipore water as the eluent to remove the extravesicular CF. The nanoparticle fractions were combined and diluted to 5.0 mL with deionized water. The concentration of compound **3** in the solution was 0.24 mM. At this moment, the fluorescence emission at 520 nm (λex = 490 nm) was recorded. After that, 20 μL of Triton X-100 (1%) was added to lyse the vesicles, and the fluorescence emission (λex = 490 nm) was measured again. The leakage assay results of **TPE@LVs I-III** were shown in Figure **2S**.

Typical preparation of tetraphenylethylene-buried vesicles loading

hydrophilic and hydrophobic doxorubicin (DOX@TPE@CLV III). Compound **3** (100 μL, 0.12 M in n-propanol) and hydrophobic DOX (10 μL, 0.2 M in DMSO) was added dropwise into 8.0 mL of hydrophilic DOX solution ([DOX] = 4 mM) under ultrasonic vibration at room temperature. The linker **4** (2.1 mg, 0.006 mmoL) and $K_2S_2O_8$ (0.24 mg, 2 mol%) was stirred for 24 h at 50 ^oC and dialyzed against deionized water (Spectra/Pore, MWCO 2000) for 48 h to give red DOX@TPE@CLV **III** solution.

To determine the loading content of hydrophobic DOX, vesicles carrying with only hydrophobic DOX was also prepared. Compound **3** (100 μL, 0.12 M in n-propanol) and hydrophobic DOX (10 μL, 0.2 M in DMSO) was added dropwise into 8.0 mL MiliQ water under ultrasonic vibration at room temperature. The linker **4** (2.1 mg, 0.006 mmoL) and $K_2S_2O_8$ (0.24 mg, 2 mol%) was stirred for 24 h at 50 ^oC and dialyzed against deionized water (Spectra/Pore, MWCO 2000) for 48 h.

Typical preparation of doxorubicin entrapped tetraphenylethylene-induced cross-linked vesicles III (DOX@TPE@CLVs III). Briefly, mixture of DOX@TPE@CLV III and cross-linker 3,3'-disulfanediylbis(2-acrylamidopropanoic acid) ([III] = 1.2 mM, [linker] = 1.2 mM, 5 mL) was treated with $K_2S_2O_8$ (0.032 mg, 2%). The reaction mixture was stirred for 24 h at 60 ^oC. The DOX@TPE@CLVs III was afforded after dialyzing against deionized water for 48h (Spectra/Pore, MWCO 1000).

Typical preparation of Targeted doxorubicin entrapped tetraphenylethylene-induced cross-linked vesicles III (T-DOX@TPE@CLVs III). The DOX@TPE@CLVs III ([III] = 12 mM, 5 mL), triethylamine (8 μL, 1.0 equvi.), EDCI

(2.3 mg, 20 mol%) and NHS (2.1 mg, 30 mol%) was stirred for 6 h at 35 ^{o}C. Then, the D-Glucosamine hydrochloride (2.6 mg, 20 mol%) was added dropwise and stirred overnight. The final T-DOX@TPE@CLVs III was given by dialyzing against deionized water for 48 h.

For calculation of drug loading content (DLC) and encapsulation efficiency (EE).[2] DOX@TPE@CLV **III** was divided to two parts, one was destroyed in GSH (0.1 M HCl), followed by freeze drying, and redissolved in DMSO. The other was followed by freeze drying directly to calculate the total weight. The fluorescence intensity of the DOX was detected (Ex = 480 nm) in DMSO solution using a pre-stablished calibration curve with various DOX concentrations. The DLC and EE was calculated as following formula:

DLC (%) = (weight of loaded DOX/total weight of DOX@TPE@CLV **III**) × 100%

EE (%) = (weight of loaded DOX/total weight of DOX in feeding) × 100%

***In vitro* stability assay.**[3] To be brief, the TPE@CLV **III** ([**3**] = 1.0 mg/mL) was diluted to different concentrations (1000 μg/mL, 200 μg/mL, 50 μg/mL and 20 μg/mL). Afterwards, the sizes of above solutions were measured by DLS to evaluate its dilute stability of low concentration.

The FBS stability of TPE@LV **III and** TPE@CLV **III** was investigated by incubation with 10% (V/V) Fetal bovine serum (FBS). Briefly, TPE@LV **III and** TPE@CLV **III** ([**3**] = 1.2 mM, in 1.8 mL water) were mixed with 0.4 mL FBS, respectively. The particle sizes were recorded to prove its stability after 0 h, 4 h and 24

h incubation at 37 °C.

***In vitro* release assay of DOX@**TPE@CLV **III.** Each 1.0 mL of the aliquot sample, DOX@TPE@CLV **III** was added into a dialysis bag (MWCO 6000−8000) and dialyzed against 25.0 mL of pH 7.4 PBS and pH 7.4 PBS mixed with glutathione (GSH, 10 mM) with gentle shaking (100 rpm) at 37 °C. At predetermined periods, 1.0 mL of the solution was collected from the corresponding different reservoirs and the samples were detected by fluorescence spectra (λex = 480 nm, λem = 580 nm). To keep a constant volume after each sampling, 1.0 mL of corresponding buffer solution was added to the reservoir. The release experiments were conducted in triplicate, and the results were demonstrated as mean ± SD.

Cytotoxicity assay. *In vitro* cytotoxicity was processed by the Cell Counting Kit-8 assay (CCK-8). Briefly, HepG2 cells (5000/well) were seeded in 96-well culture plates and incubated at 37 ^{o}C/5% CO_2. After 24 h, culture media was removed and fresh media (200 μL) containing the DOX concentration of DOX@TPE@CLV **III** which ranging from 0.05 μg/mL to 20 μg/mL was added to each well, separately. Cells without any treatment were set as control. After 24 h, culture media was removed and fresh media (100 μL) containing CCK-8 (10 μL) was added to each well and the plates were incubated at 37 ^{o}C for another 2 h. Then, the absorbance at 450 nm of each sample was measured using amicroplate reader Varioscan Flash. The Cell viability of TPE@CLV **III** with different concentrations of compound **3** from 1 μg/mL to 200 μg/mL were similar to the above procedures.

Cellular uptake evaluation by confocal laser scanning microscopy (CLSM).

HepG2 cells (5×10^4 cells/mL) were incubated at 37 °C/5% CO_2 for 24 h in glass Petri dish. Subsequently, the cells were cultured with concentration of TPE@CLV **III**, DOX•HCl, DOX@TPE@CLV **III** and T-DOX@TPE@CLV **III** ([DOX] = 10 μg/mL, [**3**] = 32 μg/mL), at 37 °C for 3 h, 6 h and 12 h, respectively. The culture medium was removed and the cells were washed by 10 mM PBS and another 1 mL PBS was added. The cellular uptake was then observed under confocal laser-scanning microscopy.

Cellular uptake evaluation by flow cytometry study. HepG2 cells were seeded on 6-well plates at a density of 2×10^5 cells/well for 24 h incubation. Then, DOX•HCl and DOX@TPE@CLV **III** and T-DOX@TPE@CLV **III** ([DOX] = 10 μg/mL, [**3**] = 32 μg/mL) with DMEM media were added to culture HepG2 cell. After incubated for 3 h, the cells were digested with trypsinization and washed with PBS. The cells were collected and centrifugated (1000 rpm/min, 5 min) and measured by BD flow cytometry.

***In vivo* anti-tumor study.** All animal experiments were performed in compliance with the relevant laws and guidelines, which were approved by the Animal Care Committee of Sichuan University (Chengdu, China). The tumor accumulation was investigated by the HepG2 xenograft model. 1×10^6 HepG2 cells suspended in 100 μL of PBS were inoculated subcutaneously in 5 weeks old female nude mice. After 2−3 weeks, the solid tumors reached about 100 mm^3. Then, the mice were randomly separated into four groups which had five mice every group, and injected intravenously via tail vein with 0.9% NaCl, DOX•HCl, DOX@TPE@CLV **III** and T-DOX@TPE@CLV **III** ([DOX] = 5 mg kg^{-1},

respectively). The DOX dose of 5 mg/kg was injected twice one week. The body weights of mice and the volume or weight of tumors were recorded, owing to the behaviors of mice were determined by the therapeutic effect. At 12 h postinjection, major organs (heart, liver, spleen, lung, and kidney) and tumor were excised and washed with saline (3 × 10 mL) for semi-quantitative analyses by detecting their fluorescence signals. Fluorescence signals were collected by a fluorescence imaging system.

***In vivo* pharmacokinetic study.** BALB/c mice (~20 g, six weeks old) were randomly divided into three groups (n=3) In order to measure the DOX concentration in plasma, DOX•HCl, DOX@TPE@CLV **III** and T-DOX@TPE@CLV **III** with DOX dose of 5 mg/kg were injected intravenously via the tail vein. The blood samples were taken from the eye socket at the 3 min, 0.5 h, 1 h, 2 h, 6 h, 12 h and 24 h time points after injection. The blood (about 0.6 mL) samples were centrifuged (3000 g, 10 min) at 4 ^{o}C. A 100 μL aliquot of plasma was treated with 50 μL of HCl (5 M) and incubated for 3 h at 50 ^{o}C. Then 50 μL of NaOH (1.0 M) and GSH (10 mM) were added and incubated for 30 min at room temperature. The mixed 200 μL solvents (chloroform/isopropanol, 4:1, v/v) was extracted twice and separated by centrifugation (10 000 g, 5 min). The organic phase was collected and pooled. Then, the organic phase was dried by Termovap Sample Concentrator. At last, the samples was dissolved in 1 mL DMSO and directly examined by using fluorescence spectroscopy. A standard curve was made by adding various concentrations of DOX to the plasma. All samples

can be calculated according to the standard curve. Pharmacokinetic parameters were analyzed using the pharmacokinetic software DAS 3.0 by fitting to the two-compartment model.

References

1. Huang, J.; Yu, Y.; Wang, L.; Wang, X.; Gu, Z.; Zhang, S. Tetraphenylethylene-induced cross-linked vesicles with tunable luminescence and controllable stability. *ACS Appl. Mater. Interfaces* **2017**, *9*, 29030–29037.
2. He, Q.; Shi, J. Mesoporous silica nanoparticle based nano drug delivery systems: synthesis, controlled drug release and delivery, pharmacokinetics and biocompatibility. *Journal of Materials Chemistry*, **2011**, *21*, 5845-5855.
3. Chen, Y.; Chen, H.; Ma, M.; Chen, F.; Guo, L.; Zhang, L.; Shi, J. Double mesoporous silica shelled spherical/ellipsoidal nanostructures: synthesis and hydrophilic/hydrophobic anticancer drug delivery. *Journal of Materials Chemistry*, **2011**, *21*, 5290-5298.

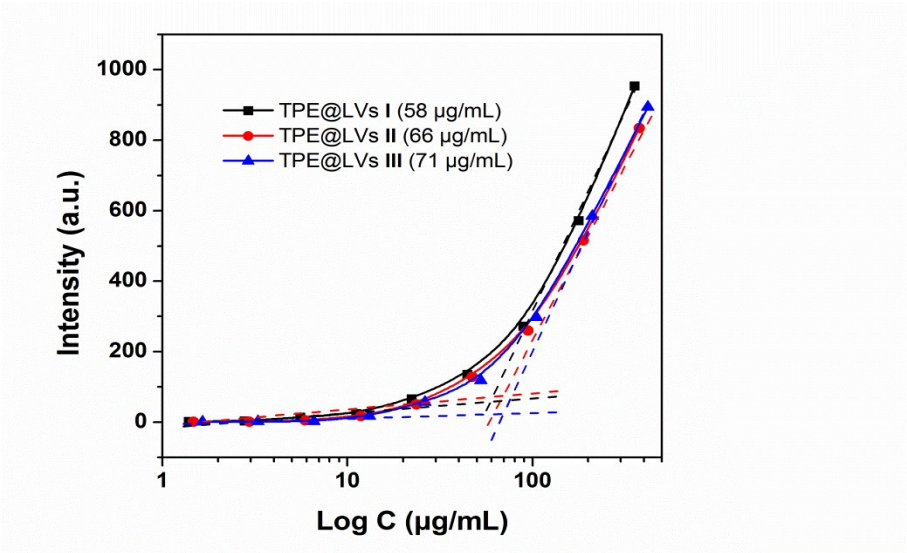


Fig. S1 Emission intensity at 475 nm (F_{475}) of compound **1** as a function of **1-3** in water. λ_{ex} = 373 nm.

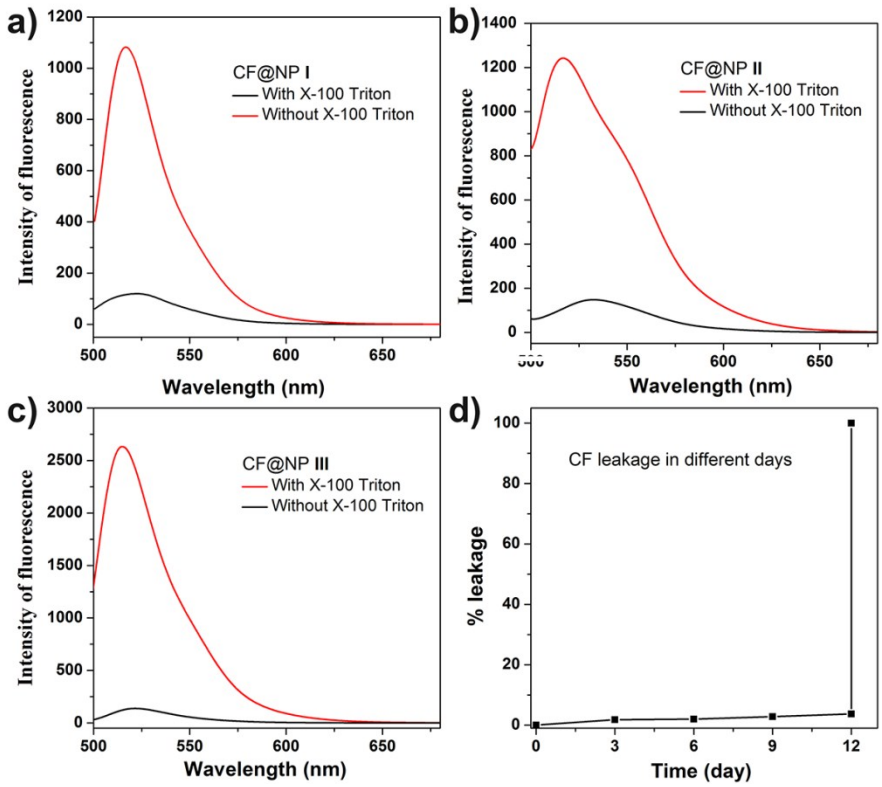


Fig. S2 Emission intensity at 475 nm (F_{475}) of compound **1** as a function of [3] in water. λ_{ex} = 373 nm.

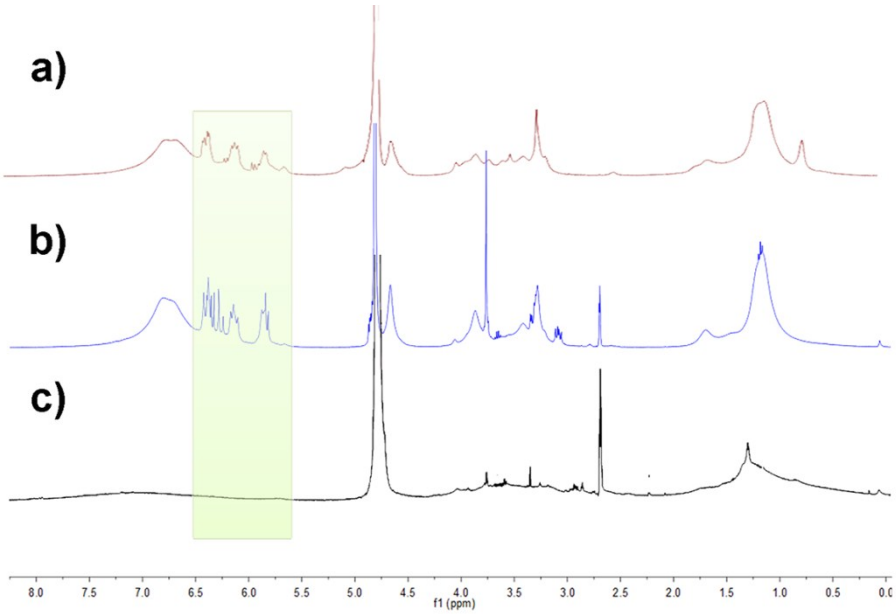


Fig. S3 ^{1}H NMR spectra of a 6 mM aqueous solution of TPE@LVs **III** in D_2O (a), TPE@LVs **III** and linker **4** in D_2O before (b) and after (c) cross-linking.

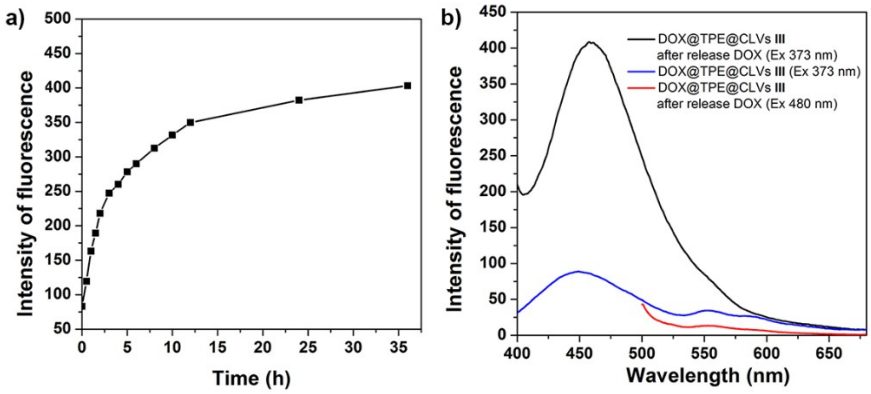


Fig. S4 a) Fluorescence intensity of TPE with DOX release from DOX@TPE@CLVs **III** at 37 °C over time, λ_{ex} = 373 nm and λ_{em} = 475 nm. b) Fluorescence spectra of DOX@TPE@CLVs **III** after 36 h release at 37 °C. (NP **III** λ_{ex} = 373 nm, DOX λ_{ex} = 480 nm, [TPE] = 50 μM, [DOX] = 10 μg/mL).

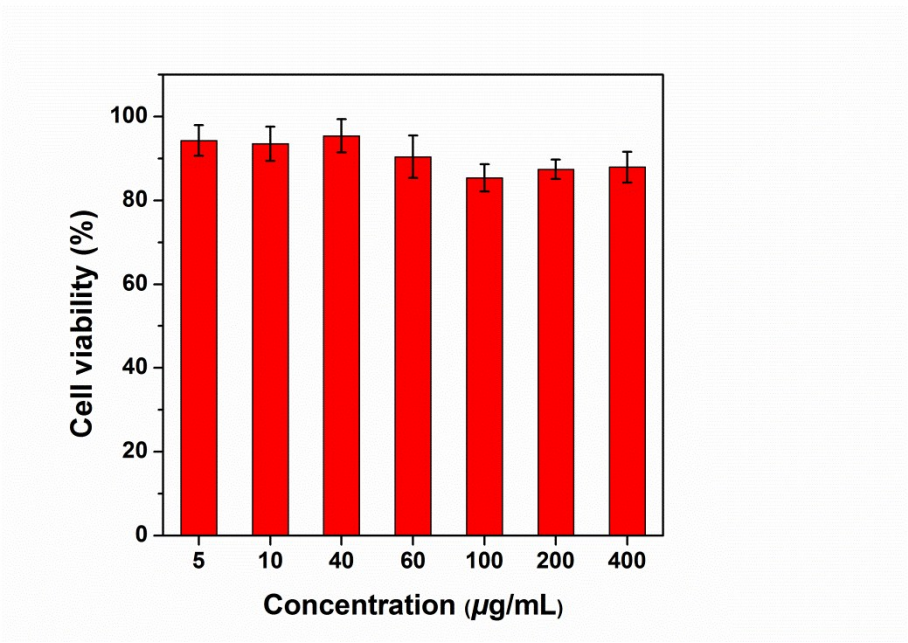
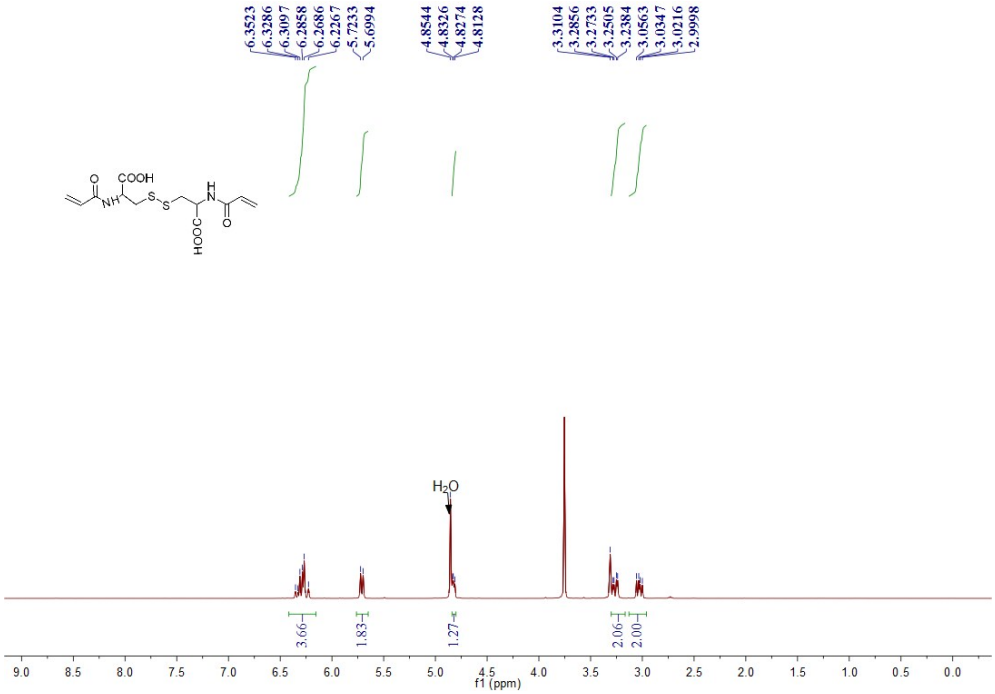
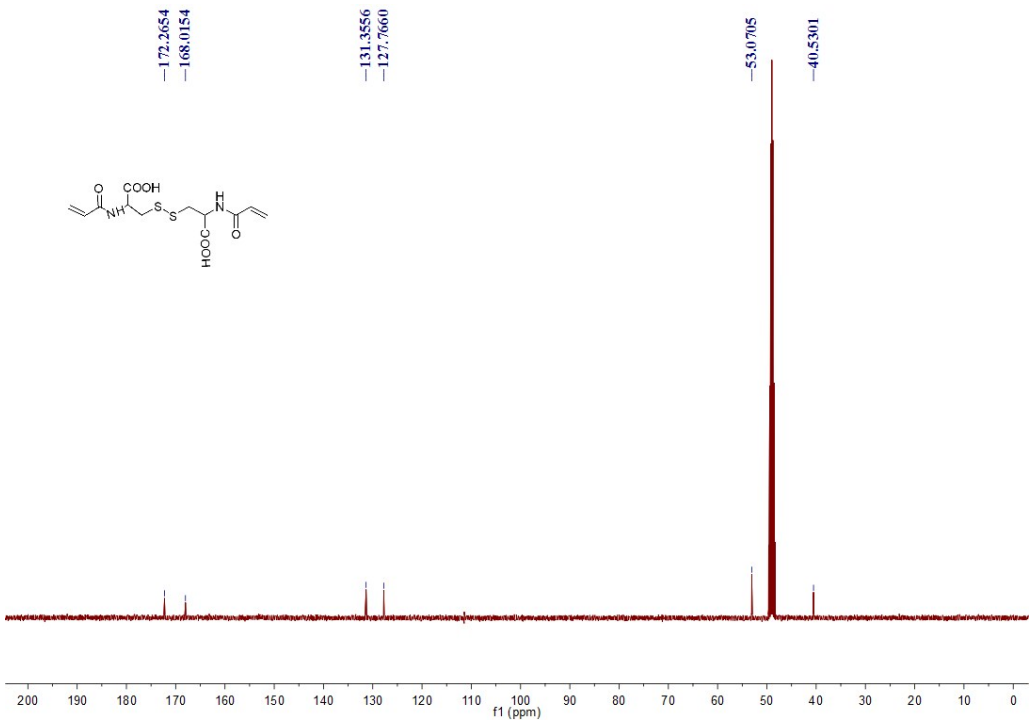


Fig. S5 Cell viability of TPE@CLVs against HepG2 cells after incubation for 24 h at 37 °C with a series of concentrations. HepG2 cells incubated without any materials were used as the control (mean ± SD, n = 5).

^{1}H, ^{13}C NMR, and MS Spectra of Key Compounds

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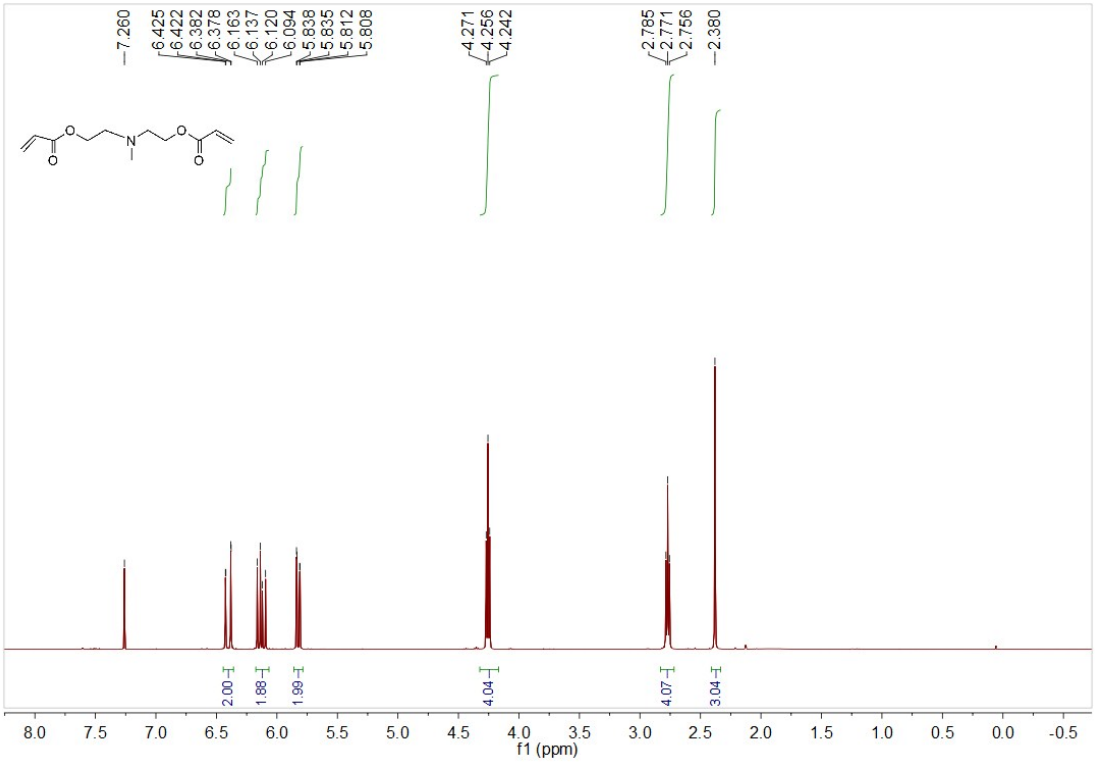
131.3556
127.7660
53.0705
40.5301
COOH
HOOC
f1 (ppm)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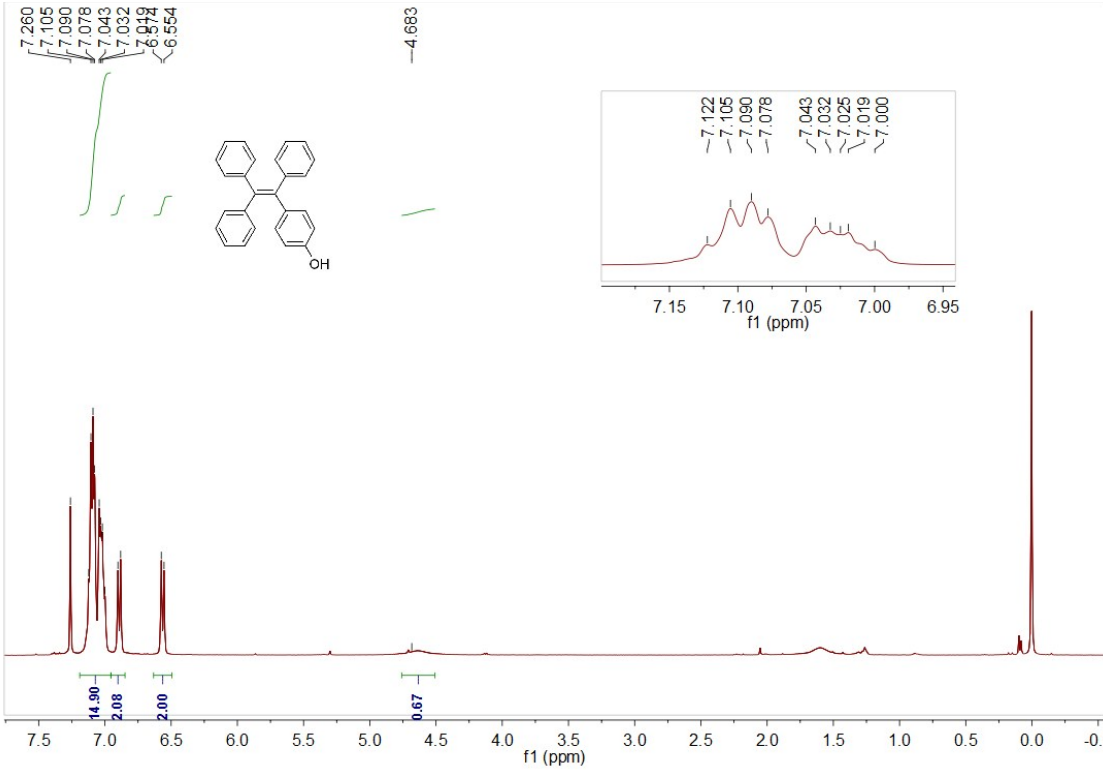
6.425
6.422
6.382
6.378
6.163
6.137
6.120
6.094
5.838
5.835
5.812
5.808
4.271
4.256
4.242
2.785
2.771
2.756
2.380
2.00
1.88
1.99
4.04
4.07
3.04
f1 (ppm)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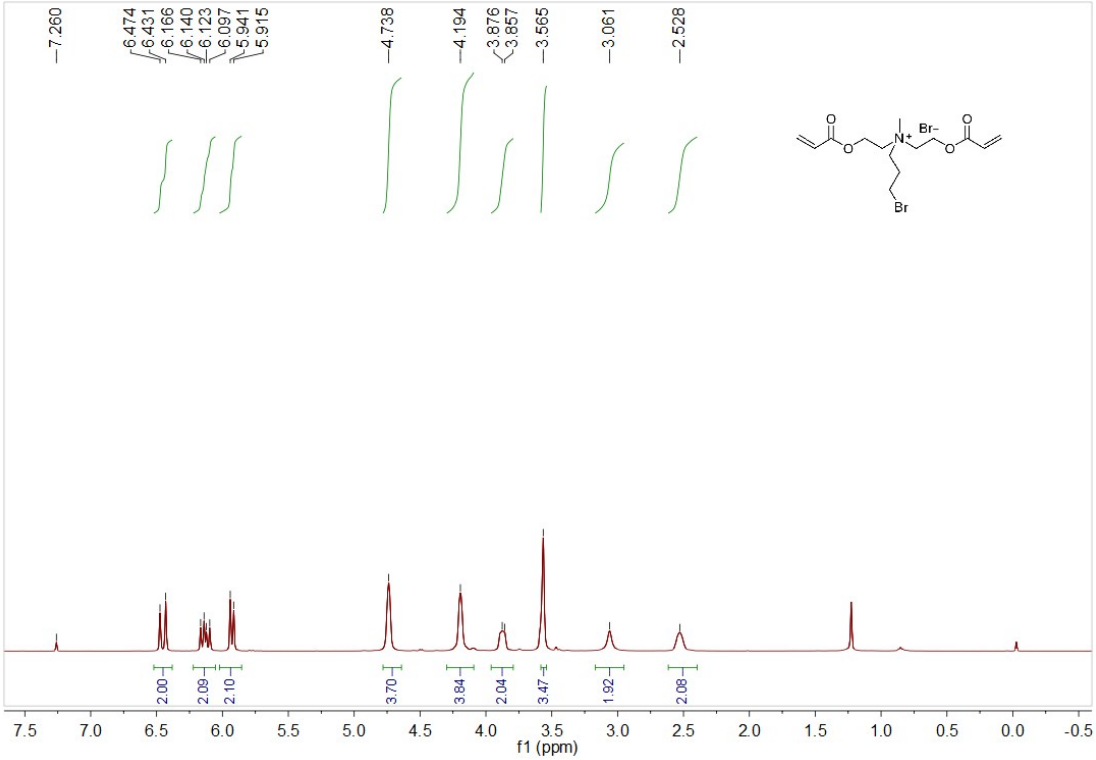
7.105
7.090
7.078
7.043
7.032
6.554
4.683
OH
7.122
7.105
7.090
7.078
7.043
7.032
7.025
7.019
7.000
f1 (ppm)
14.90
2.08
2.00
0.67
f1 (ppm)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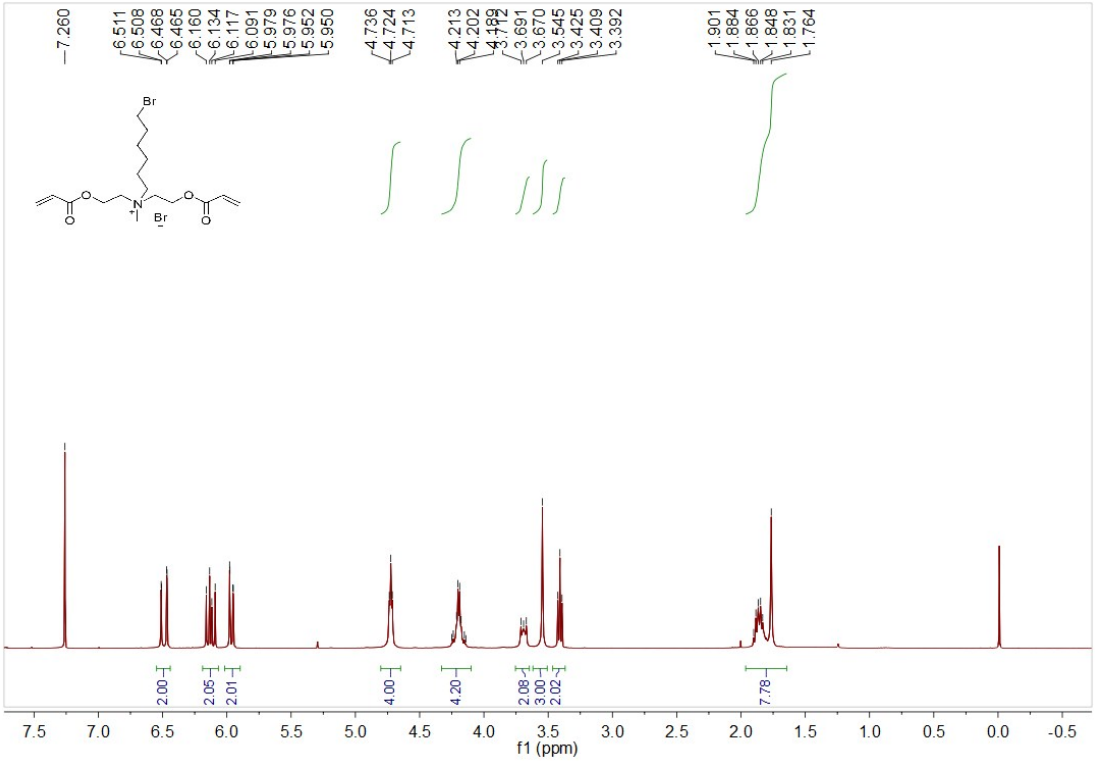
6.431
6.166
6.140
6.123
6.097
5.941
5.915
4.738
4.194
3.876
3.857
3.565
3.061
2.528
Br-
Br
2.00
2.09
2.10
3.70
3.84
2.04
3.47
1.92
2.08
f1 (ppm)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6.508
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6.465
6.160
6.134
6.117
6.091
5.979
5.976
5.952
5.950
4.736
4.724
4.713
4.213
4.202
3.691
3.670
3.545
3.425
3.409
3.392
1.901
1.884
1.866
1.848
1.831
1.764
Br
Br
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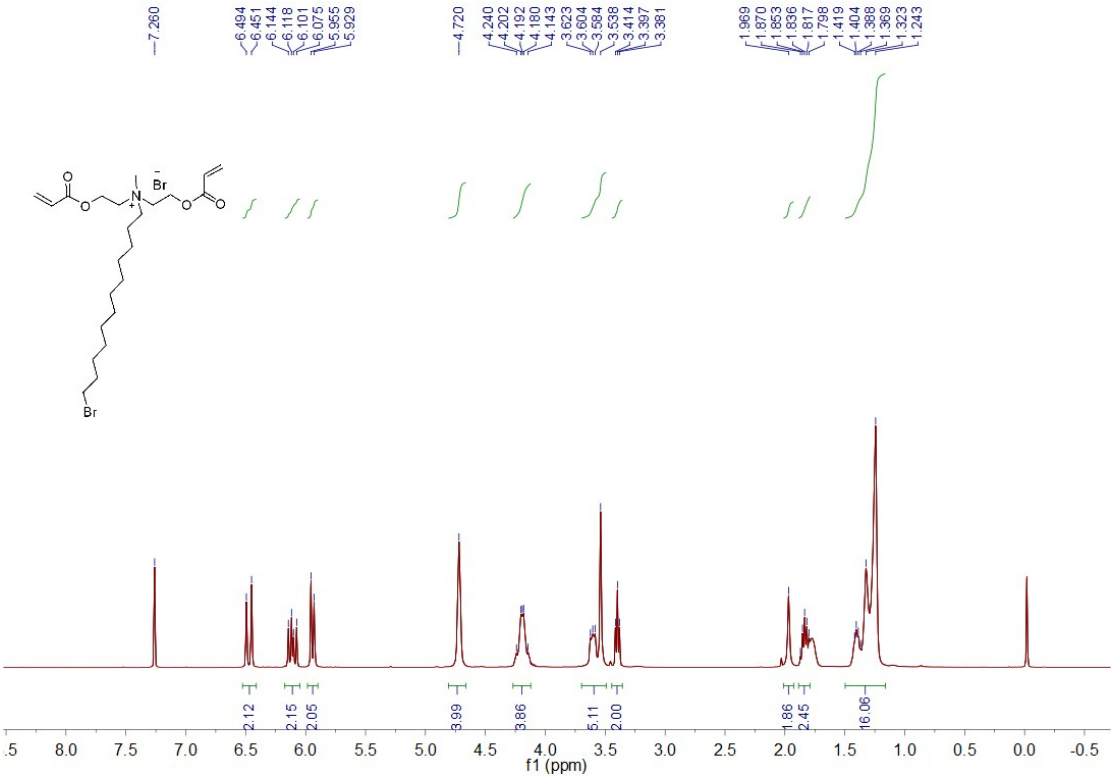
2.05
2.01
4.00
4.20
2.08
3.00
2.02
7.78
f1 (ppm)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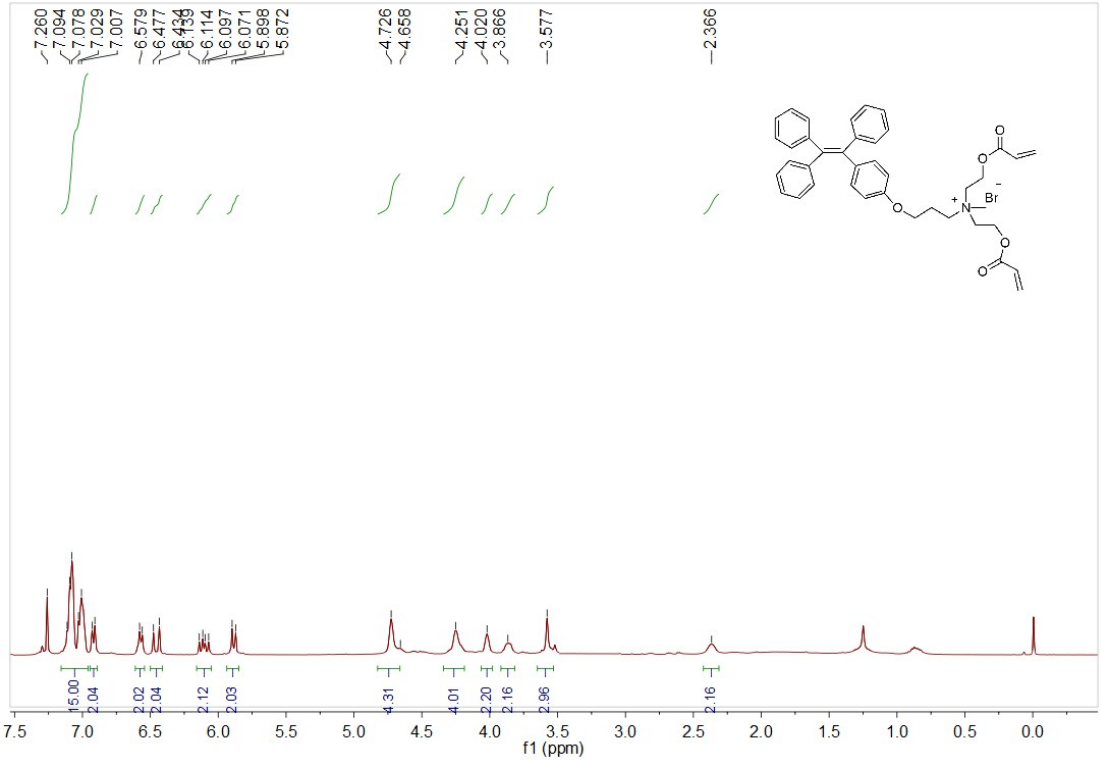
6.451
6.144
6.118
6.101
6.075
5.955
5.929
4.720
4.240
4.202
4.192
4.180
4.143
3.623
3.604
3.584
3.538
3.414
3.397
3.381
1.969
1.870
1.853
1.836
1.817
1.798
1.419
1.404
1.388
1.369
1.323
1.243
Br
Br
2.12
2.15
2.05
3.99
3.86
5.11
2.00
1.86
2.45
16.06
f1 (ppm)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6.579
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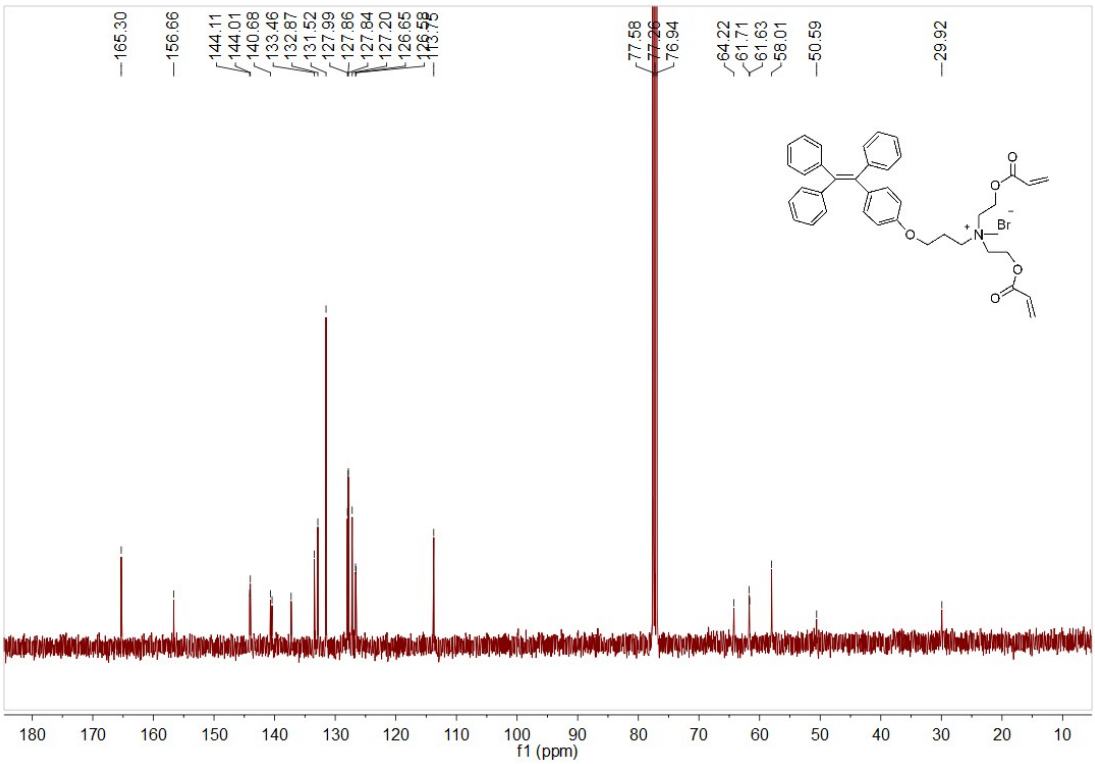
6.434
6.114
6.097
6.071
5.898
5.872
4.726
4.658
4.251
4.020
3.866
3.577
2.366
Br
15.00
2.04
2.02
2.04
2.12
2.03
4.31
4.01
2.20
2.16
2.96
2.16
f1 (ppm)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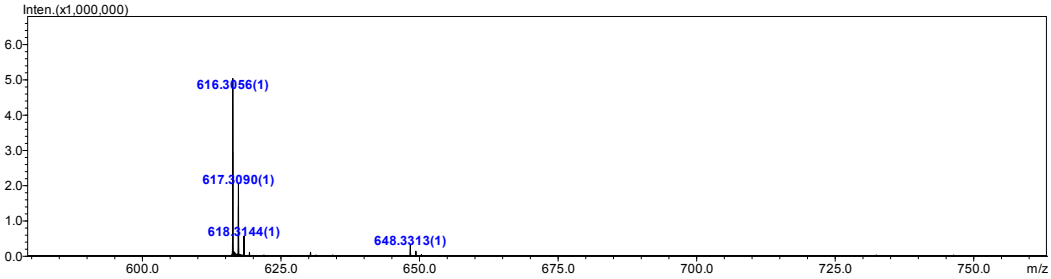
156.66
144.11
144.01
140.68
133.46
132.87
131.52
127.99
127.86
127.84
127.20
126.65
126.58
113.75
77.58
77.26
76.94
64.22
61.71
61.63
58.01
50.59
29.92
Br
f1 (ppm)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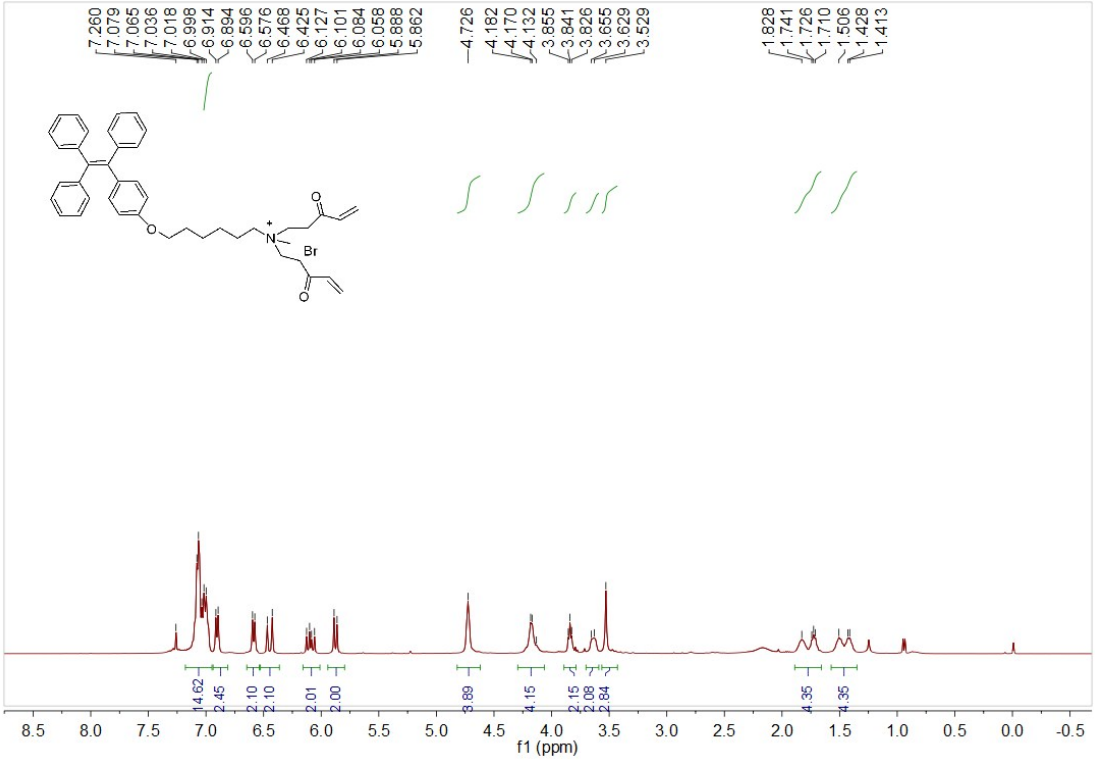
m/z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7.018
6.998
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6.596
6.576
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6.425
6.127
6.101
6.084
6.058
5.888
5.862
4.726
4.182
4.170
4.132
3.855
3.841
3.826
3.655
3.629
3.529
1.828
1.741
1.726
1.710
1.506
1.428
1.413
Br
14.62
2.45
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2.10
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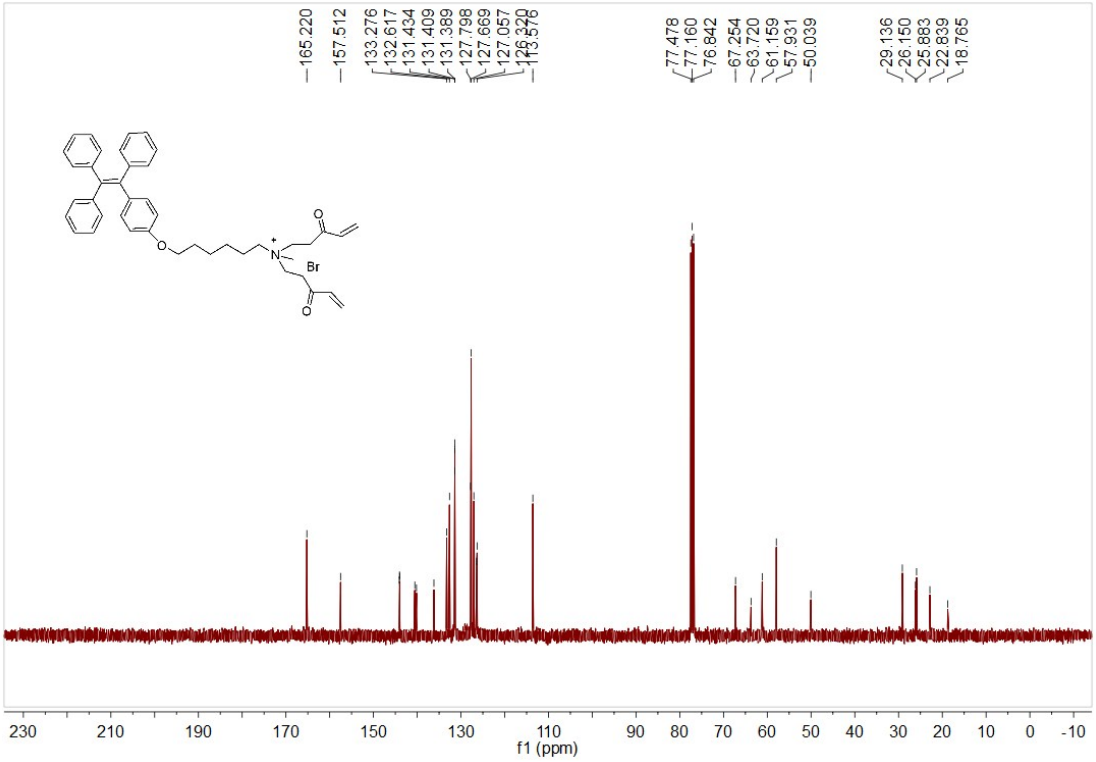
2.00
3.89
4.15
2.15
2.08
2.84
4.35
4.35
f1 (ppm)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131.409
131.389
127.798
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127.057
126.320
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77.160
76.842
67.254
63.720
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57.931
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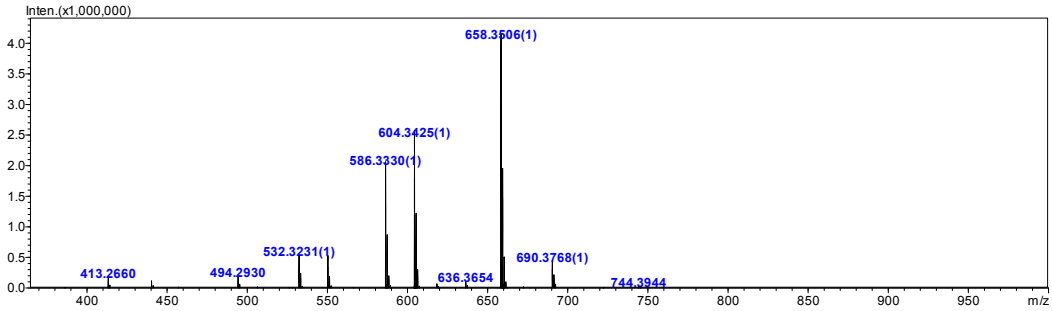
29.136
26.150
25.883
22.839
18.765
Br
f1 (ppm)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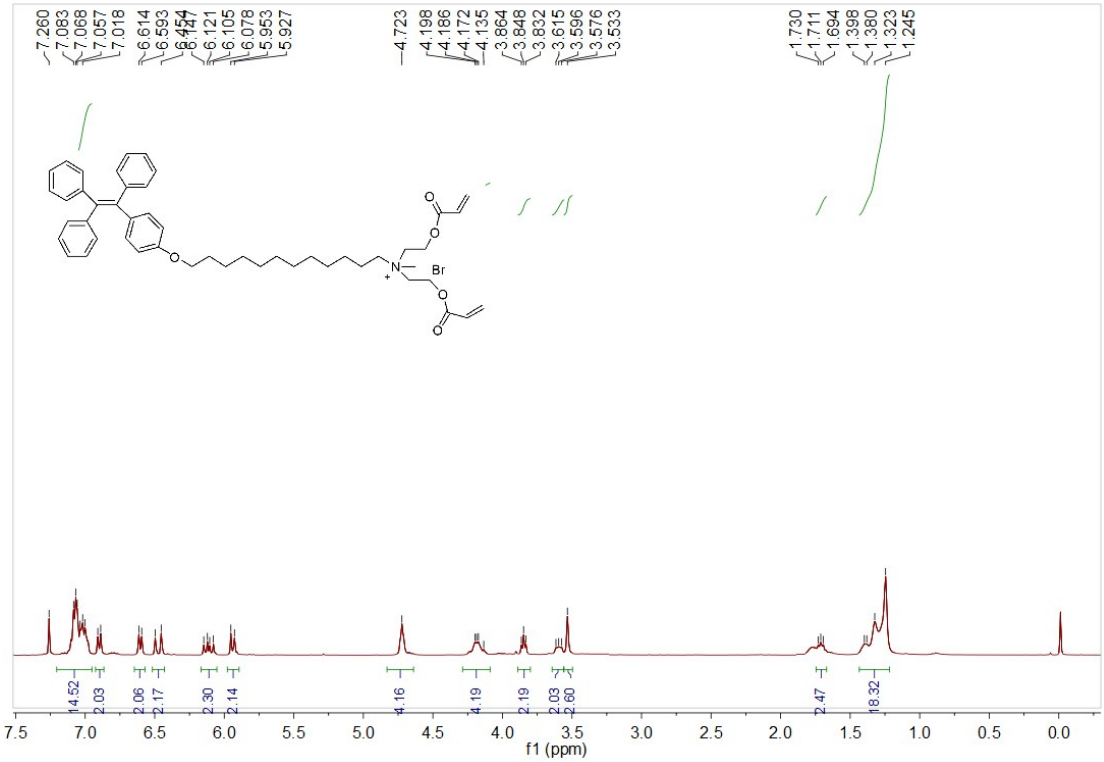
m/z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7.018
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6.078
5.953
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4.723
4.198
4.186
4.172
4.135
3.864
3.848
3.832
3.615
3.596
3.576
3.533
1.730
1.711
1.694
1.398
1.380
1.323
1.245
Br
14.52
2.03
2.06
2.17
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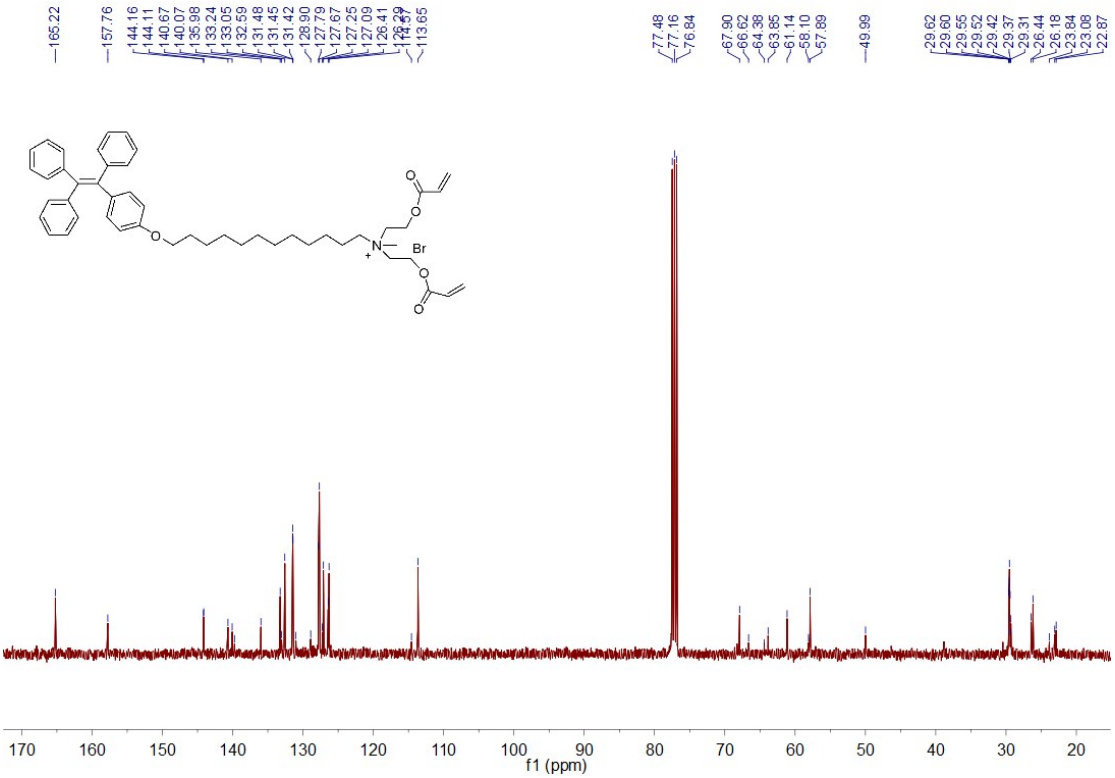
2.14
4.16
4.19
2.19
2.03
2.60
2.47
18.32
f1 (ppm)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144.11
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132.59
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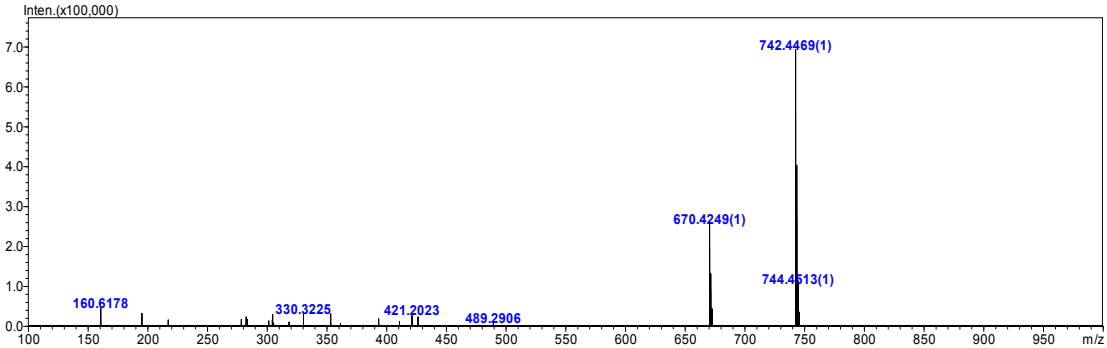
131.42
128.90
127.79
127.67
127.25
127.09
126.41
113.65
77.48
77.16
76.84
67.90
66.62
64.38
63.85
61.14
58.10
57.89
49.99
29.62
29.60
29.55
29.52
29.42
29.37
29.31
26.44
26.18
23.84
23.08
22.87
Br
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)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