Supplementary Information

Engineered Triphenylphosphonium-Based, Mitochondrial-Targeted Liposomal Drug Delivery System Facilitates Cancer Cell Killing Actions of Chemotherapeutics

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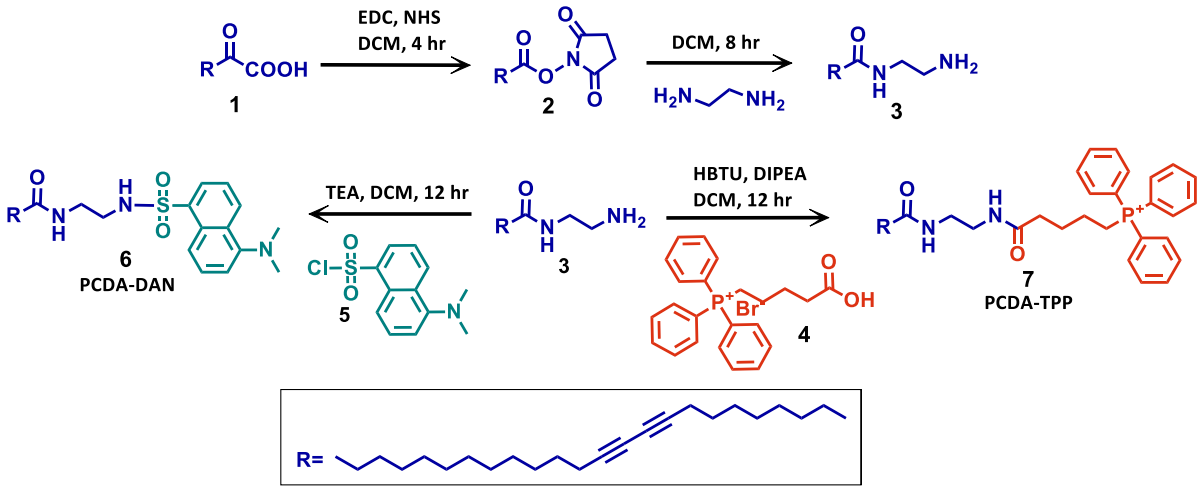
Experimental section



Scheme S1: Synthetic methodologies adopted for the synthesis of **PCDA-DAN(6) and PCDA-TPP (7)**.

Synthesis of N-Hydroxysuccinimide ester of 10,12-pentacosadiynoic acid (NHS-PCDA) (2):

500 mg of PCDA (1.33 mmol) was dissolved in 5 ml of dichloromethane. N-hydroxysuccinimide (NHS) (248 mg, 1.60 mmol)), and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (248, 1.60 mmol) were added to the mixture solution. The resulting organic solution was stirred for 4 h at ambient temperature. After evaporation of the solvent in vacuo, the product was extracted using ethyl acetate and water. To remove the remaining water, magnesium sulfate was added to the organic layer. The organic solvent part was separated by centrifugation and filtering, and then the solvent was removed in vacuo. The product was confirmed using thin-layer chromatography (TLC, hexane/ethyl acetate 3:1). The product was powdered and obtained in 513 mg (81%) (Scheme S1).[1]

Synthesis of N-(2-aminoethyl)-10,12-pentacosadiynamide using NHS-PCDA and ethylenediamine (NH_2-PCDA) (3):

NHS-PCDA (**2**) (513 mg, 1.08 mmol) was dissolved in 6 ml of dichloromethane. To the organic solvent, 1.35 ml of ethylenediamine was added. The mixture solution was stirred at ambient temperature for 8 h. After stirring, the product solution was extracted with water and dichloromethane in ratio 2:1. Then the organic layer was dried with magnesium sulfate and filtered, and the solvent was removed by evaporation. The product was then crystallized and the white product was obtained in 377 mg (83%). The product was confirmed with ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS).[1]

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.12 (s, 1H), 3.32 (s, 2H), 2.85 (s, 2H), 2.23 (t, J=6.8 Hz, 4H), 2.18 (t, J=7.5 Hz, 2H), 1.63-1.61 (m, 2H), 1.52-1.49 (m, 4H), 1.37-1.35 (m, 4H), 1.29-1.25 (m, 22H), 0.88 (t, J=6.9 Hz, 3H).

ESI MS (m/z): $[M+H]^+$= 417.6908 (calculated); 417.4000 (observed).

Synthesis of N-(2-(5-(dimethylamino)naphthalene-1-sulfonamido)ethyl)pentacosa-10,12-diynamide (PCDA-DAN) (6):

80 mg of N-(2-aminoethyl)-10,12-pentacosadiynamide (**3**) (0.19 mmol) was dissolved in 5 ml. 59 mg of Dansyl chloride (**5**) (0.22 mmol) and trimethylamine (1.2 eq.) were added to this solution and the reaction mixture was allowed to stir overnight under a nitrogen atmosphere. Progress of the reaction was monitored through TLC. On completion, the reaction mixture was

diluted with DCM and washed with brine. Organic layer was collected, dried over sodium sulphate and evaporated. The residue was then purified by column chromatography (35% ethyl acetate in hexane) and the pure product was obtained in 75.7 mg (61% yield).[2]

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.55 (d, J= 8.5 Hz, 1H), 8.27 (d, J=8.6, 1H), 8.23-8.21 (m, 1H), 7.59-7.51 (m, 2H), 7.19 (d, J=7.5 Hz, 1H), 5.95 (s, 1H), 5.69 (s,1H), 3.29 (q, J=5.6 Hz, 5.3 Hz, 2H), 3.02 (q, J= 5.7 Hz, 5.1 Hz, 2H), 2.89 (s, 6H), 2.23 (t, J=6.9 Hz, 4H), 1.96 (t, J=7.7 Hz, 2H), 1.53-1.45 (m, 6H), 1.30-1.22 (m, 26 H), 0.87 (t, J= 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 174.21, 152.12, 134.25, 130.71, 129.91, 129.76, 129.47, 128.61, 123.23, 118.55, 115.28, 65.29, 65.321, 45.44, 43.40, 39.12, 36.42, 31.94, 29.66, 29.64, 29.51, 29.38, 29.18, 29.15, 29.13, 28.93, 28.89, 28.79, 28.37, 28.32, 25.47, 22.72, 19.23.

ESI MS (m/z): $[M+Na]^+$= 672.9588 (calculated); 672.3500 (observed).

Elemental analysis calcd.(%) C 72.07, H 9.15, N 6.46; found: C 70.85, H 9.63, N 6.89

(5-((2-(pentacosa-10,12-diynamido)ethyl)amino)-5-oxo-pentyl)triphenylphosphonium bromide (PCDA-TPP) (7):

To a solution of N-(2-aminoethyl)-10,12-pentacosadiynamide (**3**) (50 mg, 0.11 mmol) at 0°C in DCM under nitrogen atmosphere was added HBTU (50 mg, 0.13 mmol) and DIPEA (1.2 eq). The reaction mixture was allowed to stir for 15 minutes and then (4-carboxybutyl)triphenylphosphonium bromide (**4**) (1.2 eq. 58.5mg, 0.13 mmol) was added. Reaction was then allowed to stir overnight. Progress of the reaction was monitored through TLC. On completion, the reaction mixture was diluted with DCM and washed with brine. Organic layer was collected, dried over sodium sulphate and evaporated. A sticky residue was obtained which was further washed with pentane to get the pure compound in 67.2 mg (80% yield).[2]

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.83-7.80 (m, 3H), 7.72-7.63 (m, 12H), 6.79 (s, 1H), 6.59 (s, 1H), 3.29 (s, 4H), 2.32 (t, J= 7.2 Hz, 2H), 2.22 (q, J= 7.4 Hz, 7.6 Hz, 4H), 2.15 (t, J= 7.6 Hz, 2H), 1.91-1.85 (m, 2H), 1.73-1.67 (m, 2H), 1.51-1.41(m, 10 H), 1.37-1.29 (m, 24 H), 0.87 (t, J=6.7 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 174.40, 173.29, 135.36, 133.39, 133.33, 133.29, 130.72, 130.66, 130.60, 125.84, 124.75, 118.15, 117.29, 110.73, 65.25, 65.19, 64.50, 42.68, 39.72,

39.53, 38.63, 36.39, 34.49, 31.93, 29.66, 29.63, 29.50, 29.37, 29.21, 29.12, 28.94, 28.88, 28.84, 28.36, 26.10, 25.93, 25.71, 22.71, 21.51.

^{31}P NMR (162 MHz, CDCl3): δ 24.4

ESI MS (m/z): $[M]^+$= 761.5175 (calculated); 761.6000 (observed).

Elemental analysis calcd.(%) C 78.80, H 9.26, N 3.68; found: C 77.93, H 9.71, N 3.91

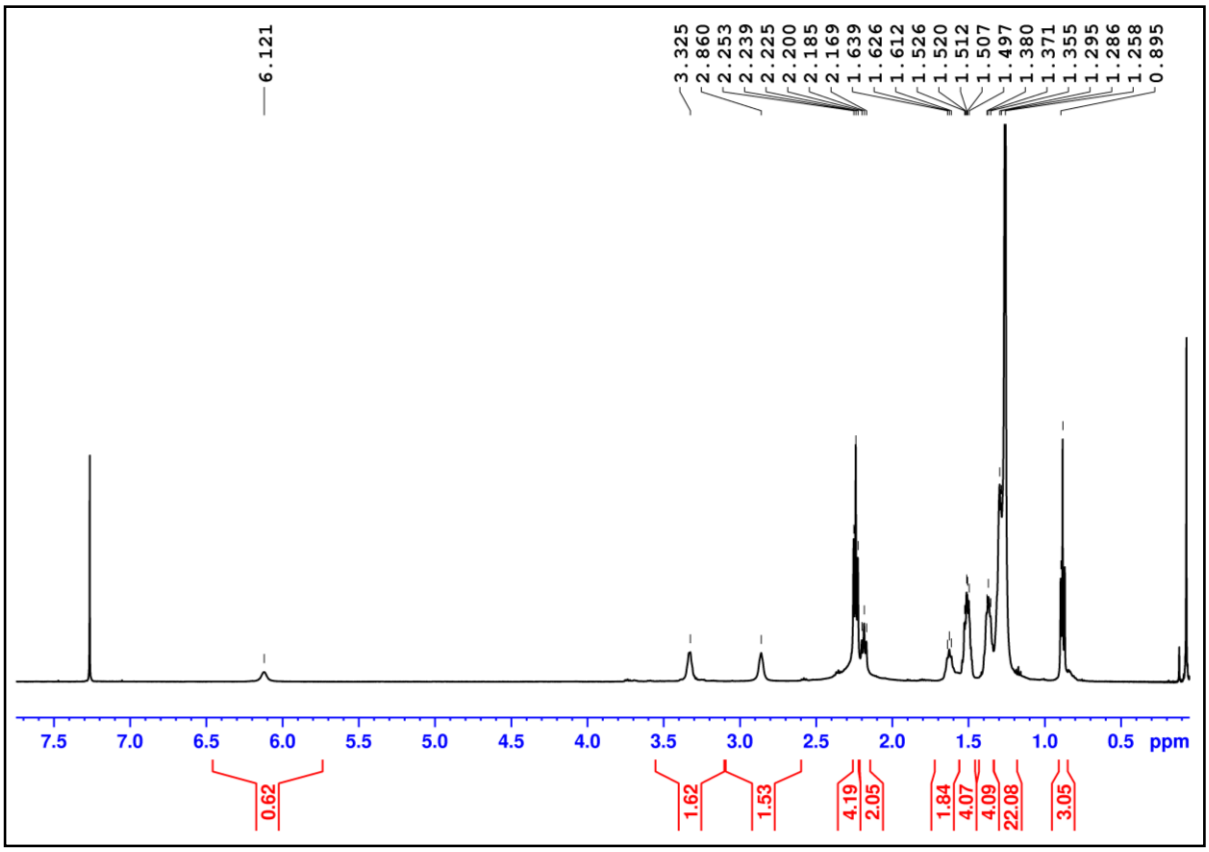


Figure S1. ^{1}H NMR ($CDCl_3$, 500 MHz, δppm) of **PCDA-NH_2 (3)**.

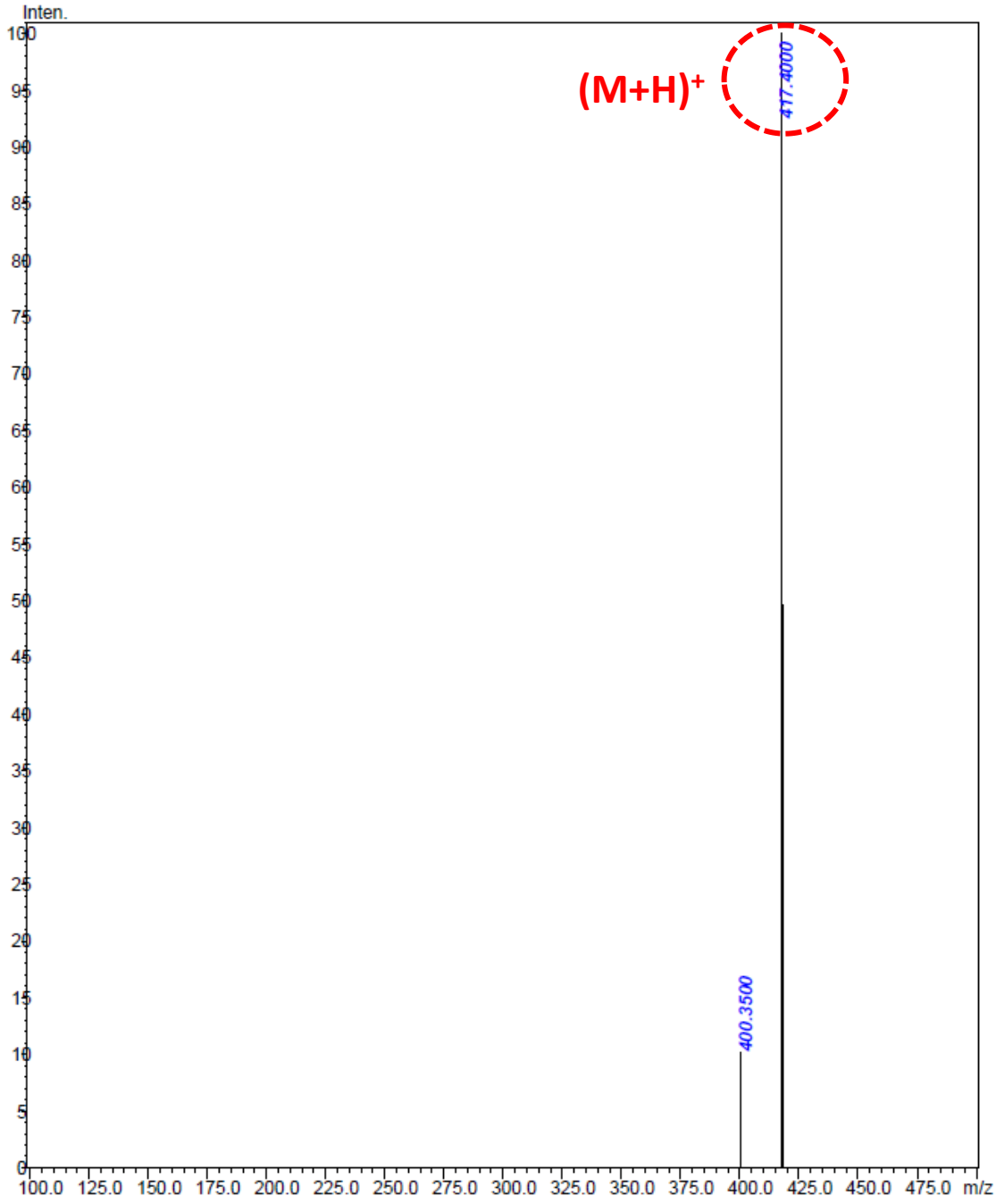


Figure S2. ESI Mass spectra of **PCDA-NH_2 (3).**

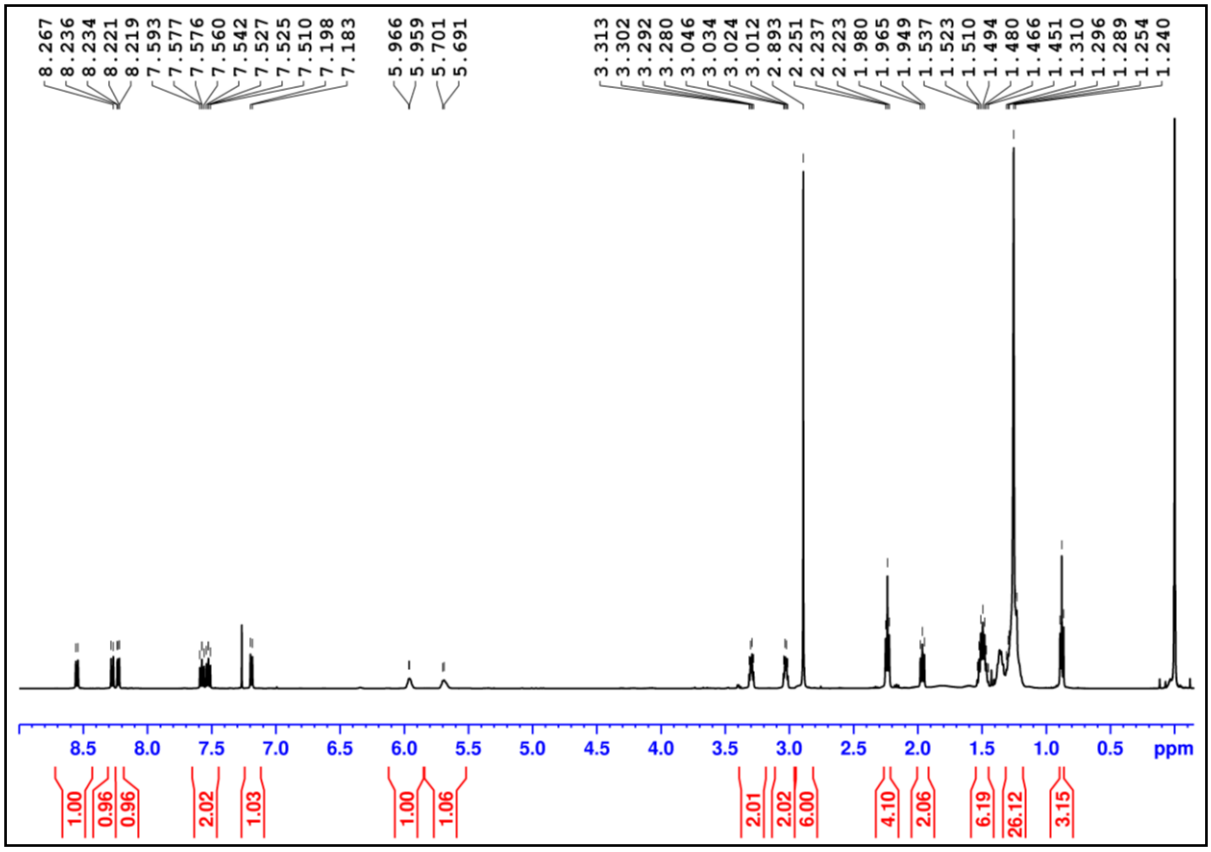


Figure S3. ^{1}H NMR ($CDCl_3$, 500 MHz, δppm) of **PCDA-DAN (6)**.

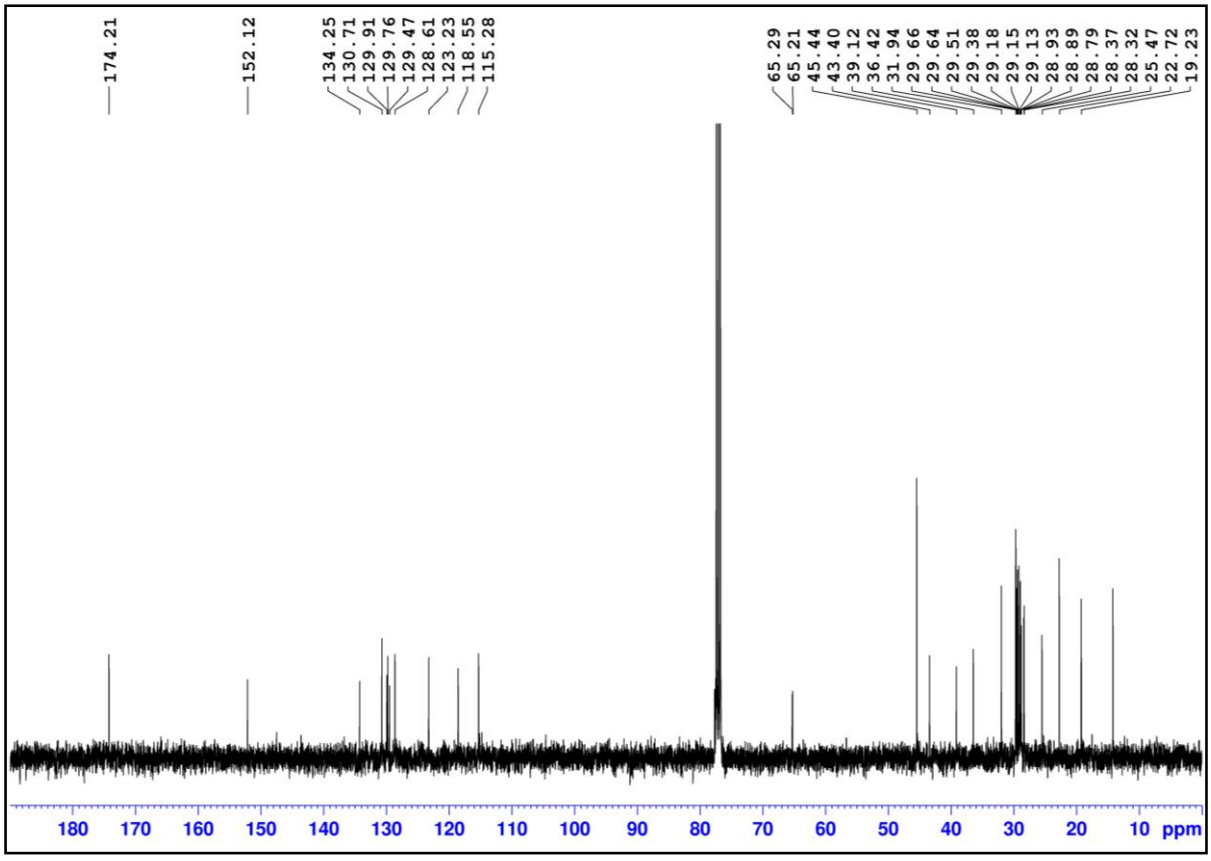


Figure S4. ^{13}C NMR ($CDCl_3$, 100 MHz, δppm) of **PCDA-DAN (6)**.

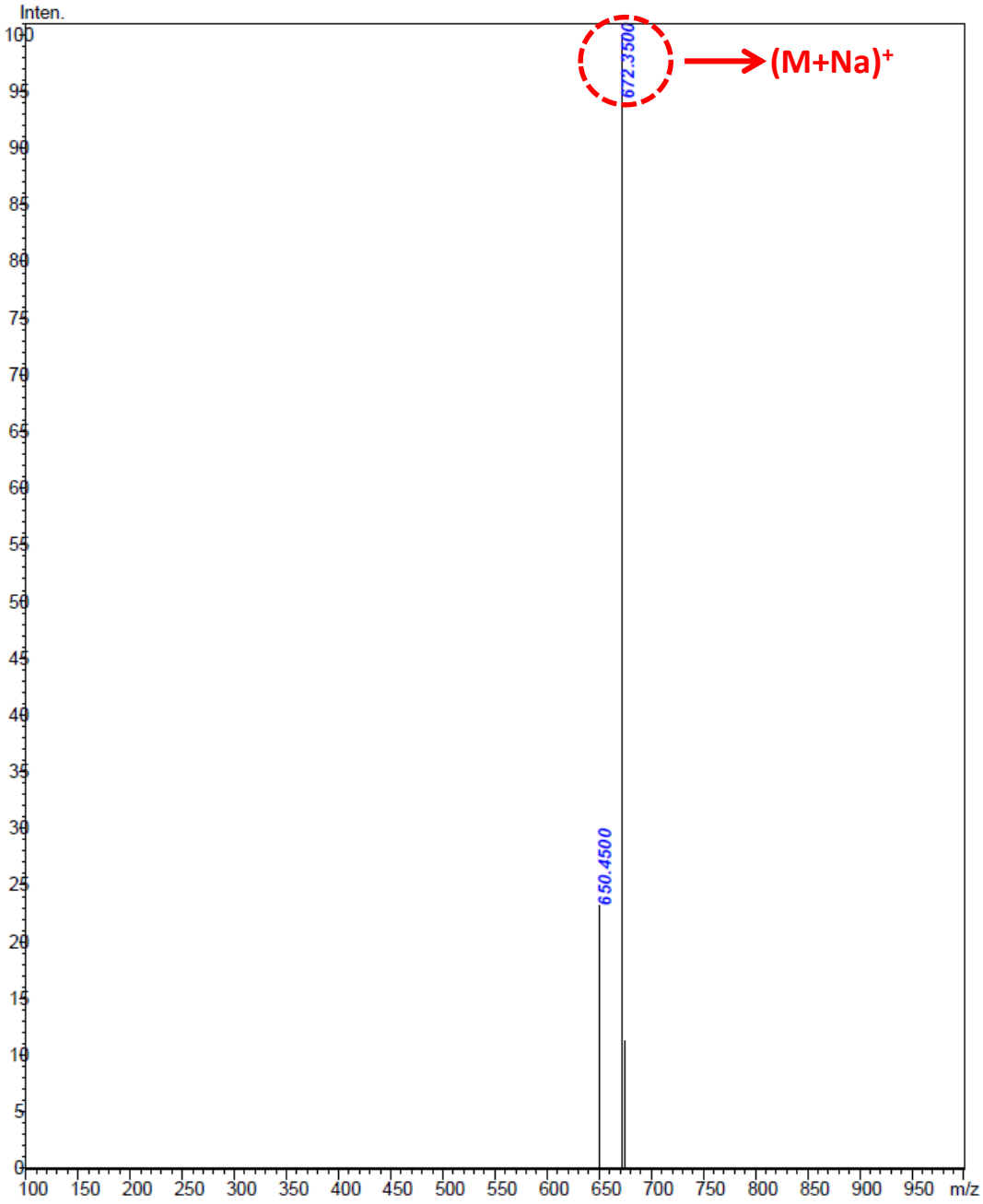


Figure S5. ESI Mass spectra of **PCDA-DAN (6).**

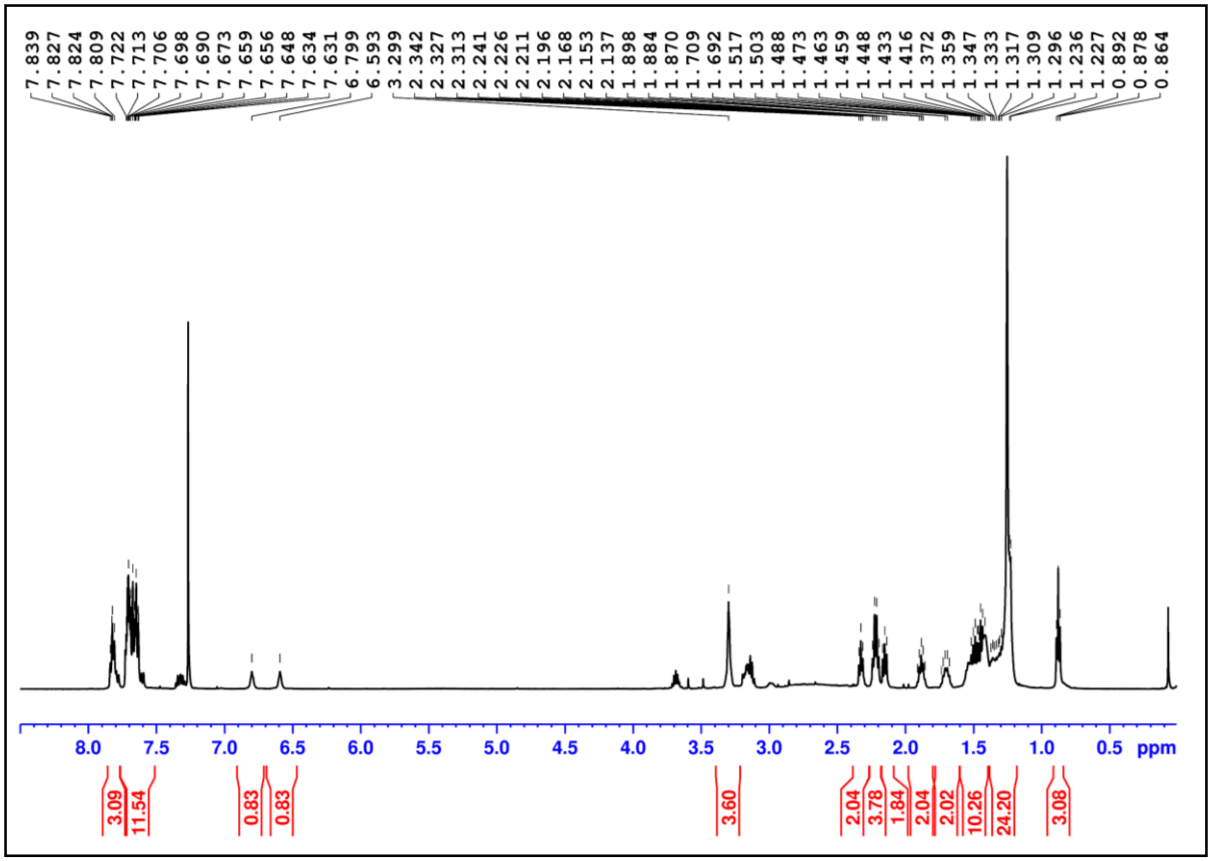


Figure S6. ^{1}H NMR ($CDCl_3$, 500 MHz, δppm) of **PCDA-TPP (7)**.

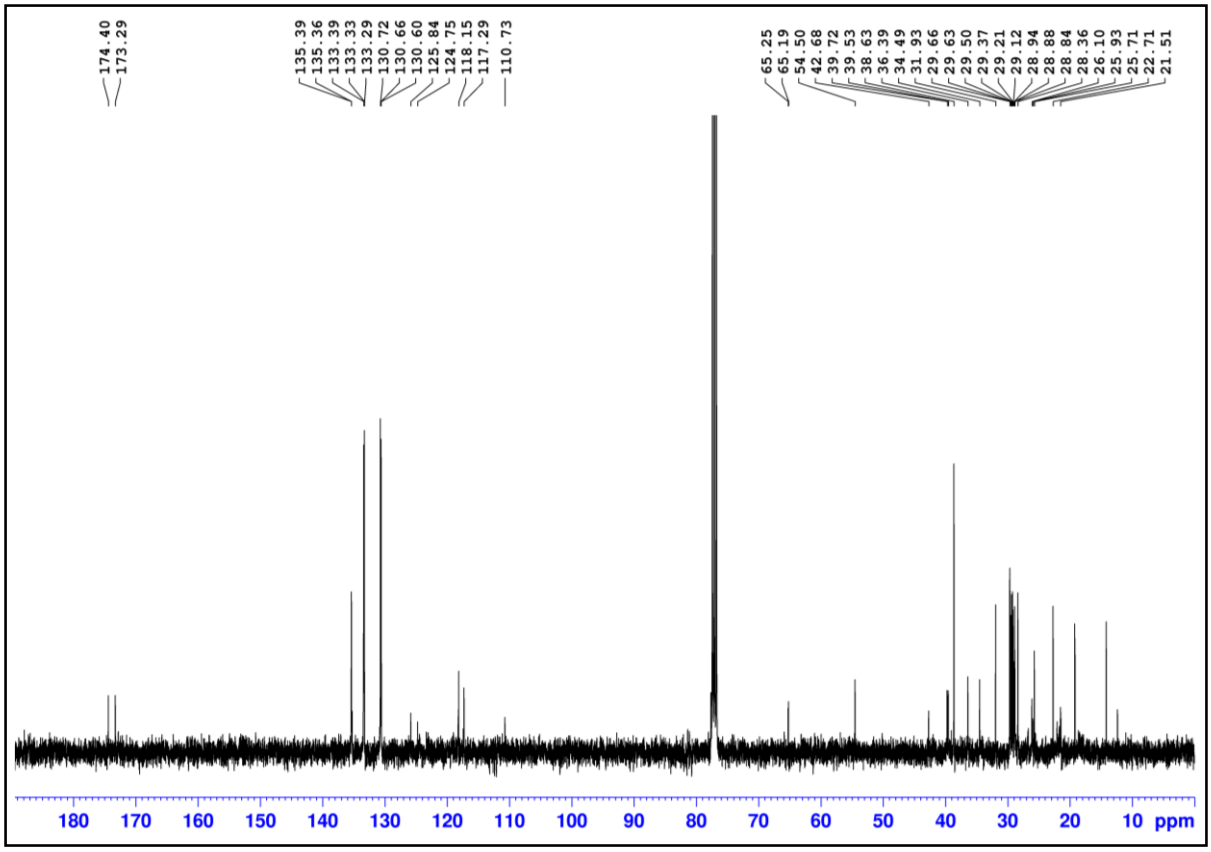


Figure S7. ^{13}C NMR ($CDCl_3$, 100 MHz, δppm) of **PCDA-TPP (7)**.

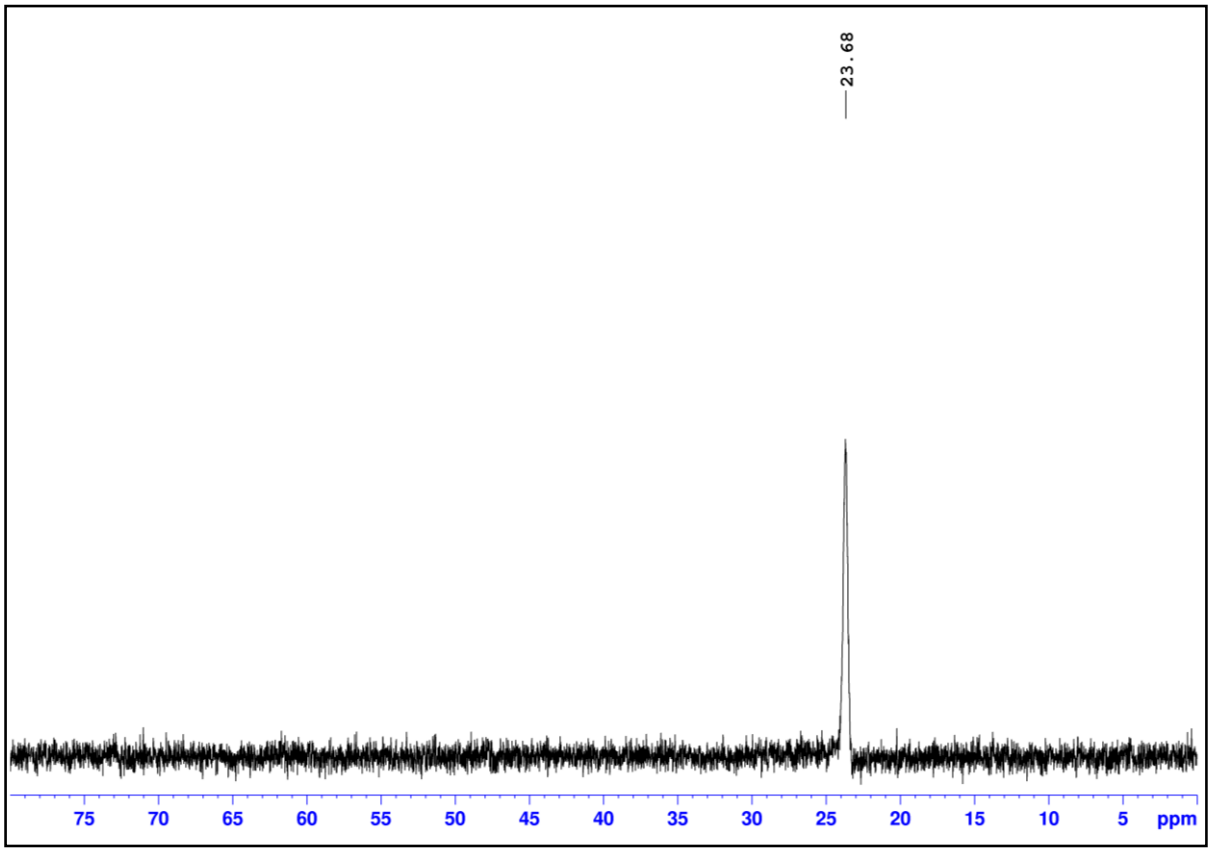


Figure S8. ^{31}P NMR ($CDCl_3$, 162 MHz, δppm) of **PCDA-TPP (7)**.

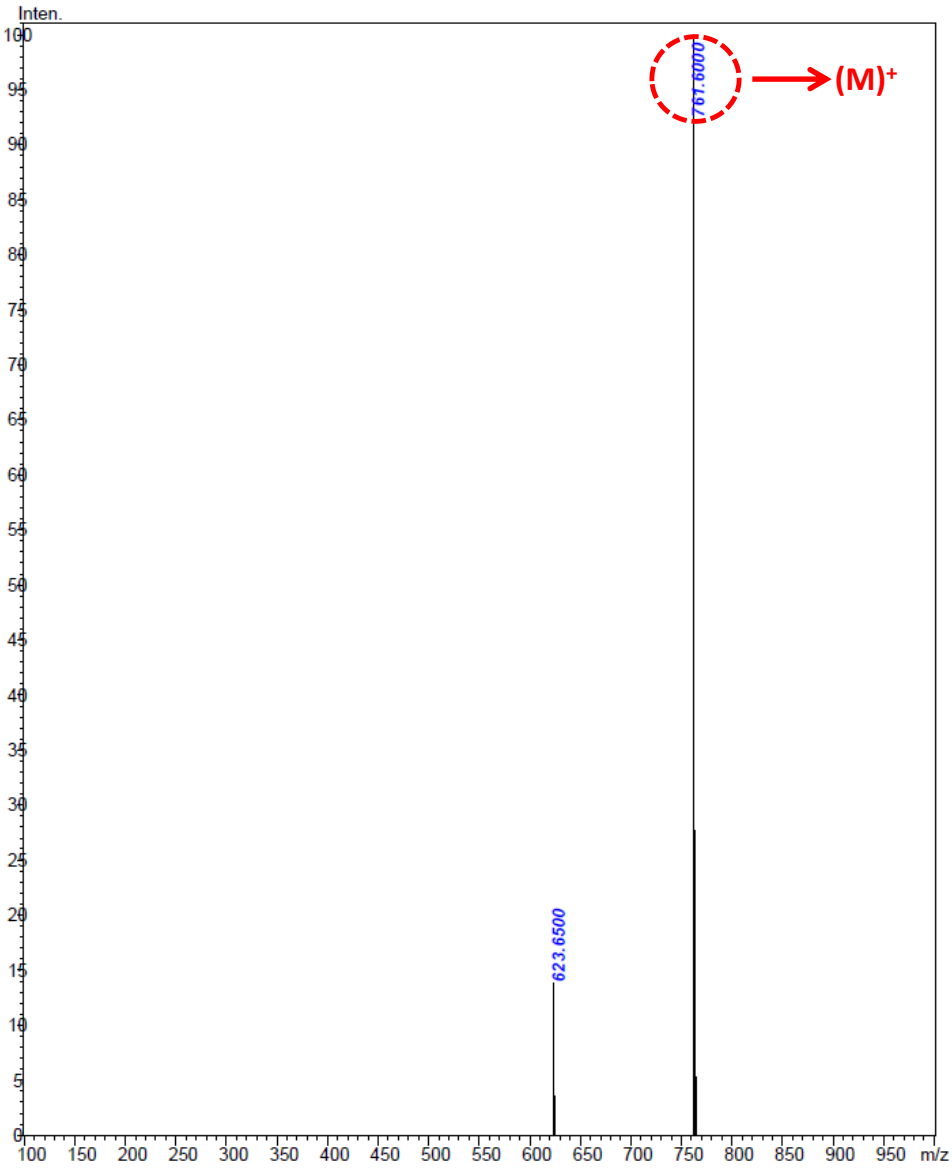


Figure S9. ESI Mass spectra of **PCDA-TPP (7).**

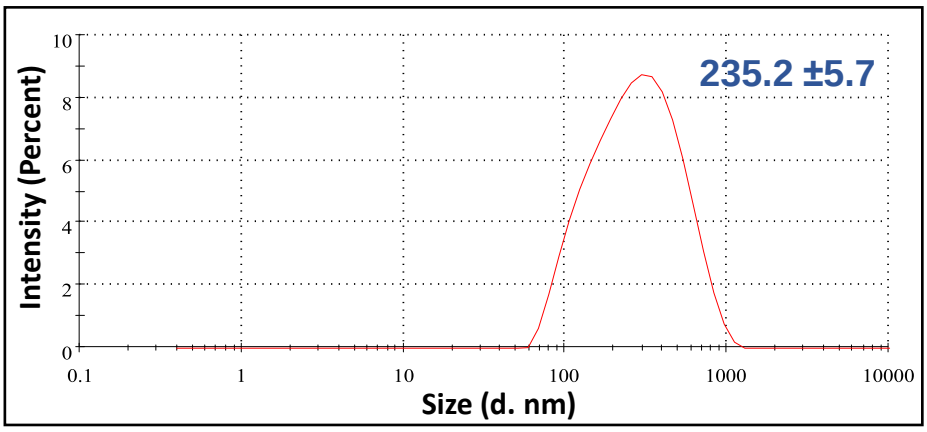


Figure S10. Size distribution of **Lip-DT** obtained from Dynamic light scattering (DLS) measurement.

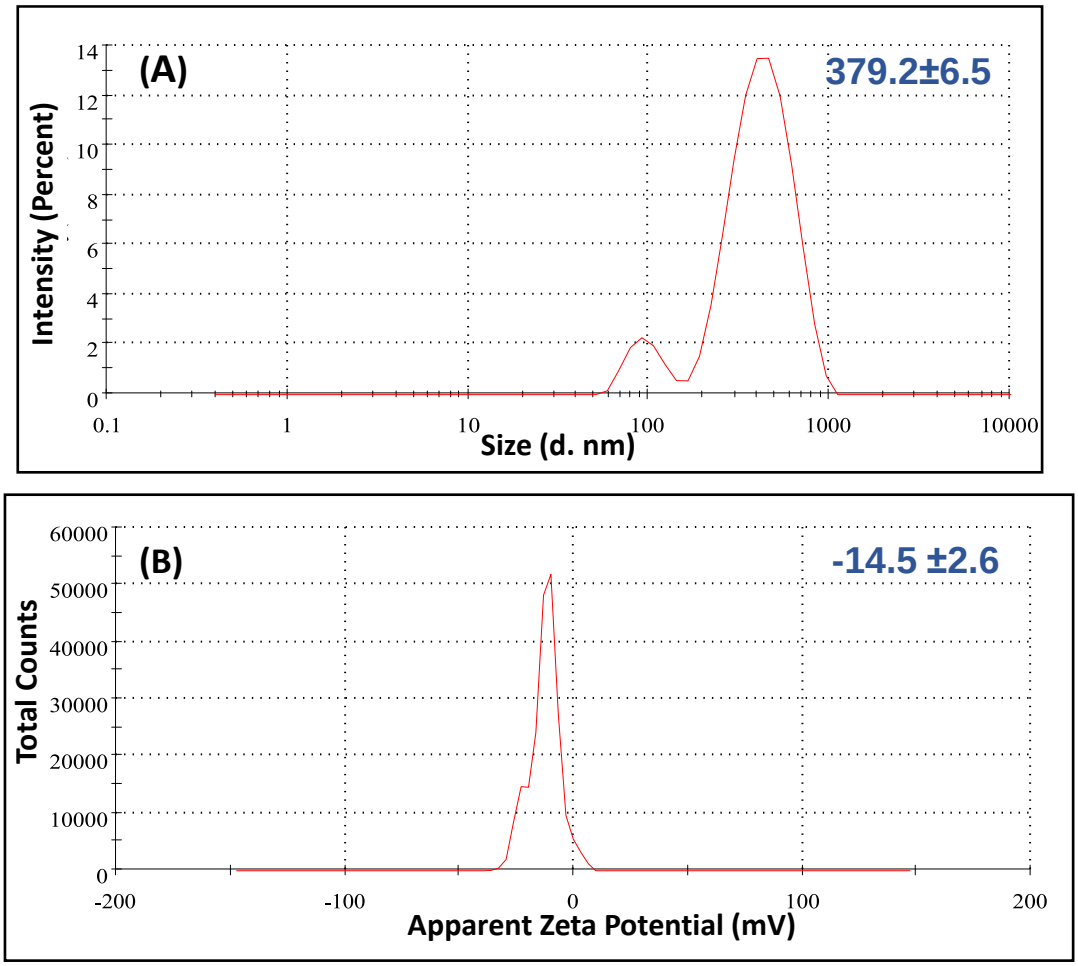


Figure S11. (A) Size and (B) Zeta potential distribution obtained from Dynamic lighting scattering measurements for the PCDA/DMPC-based liposome (without functionalization). The average size distribution of the vesicles is 379.2±6.5 nm.

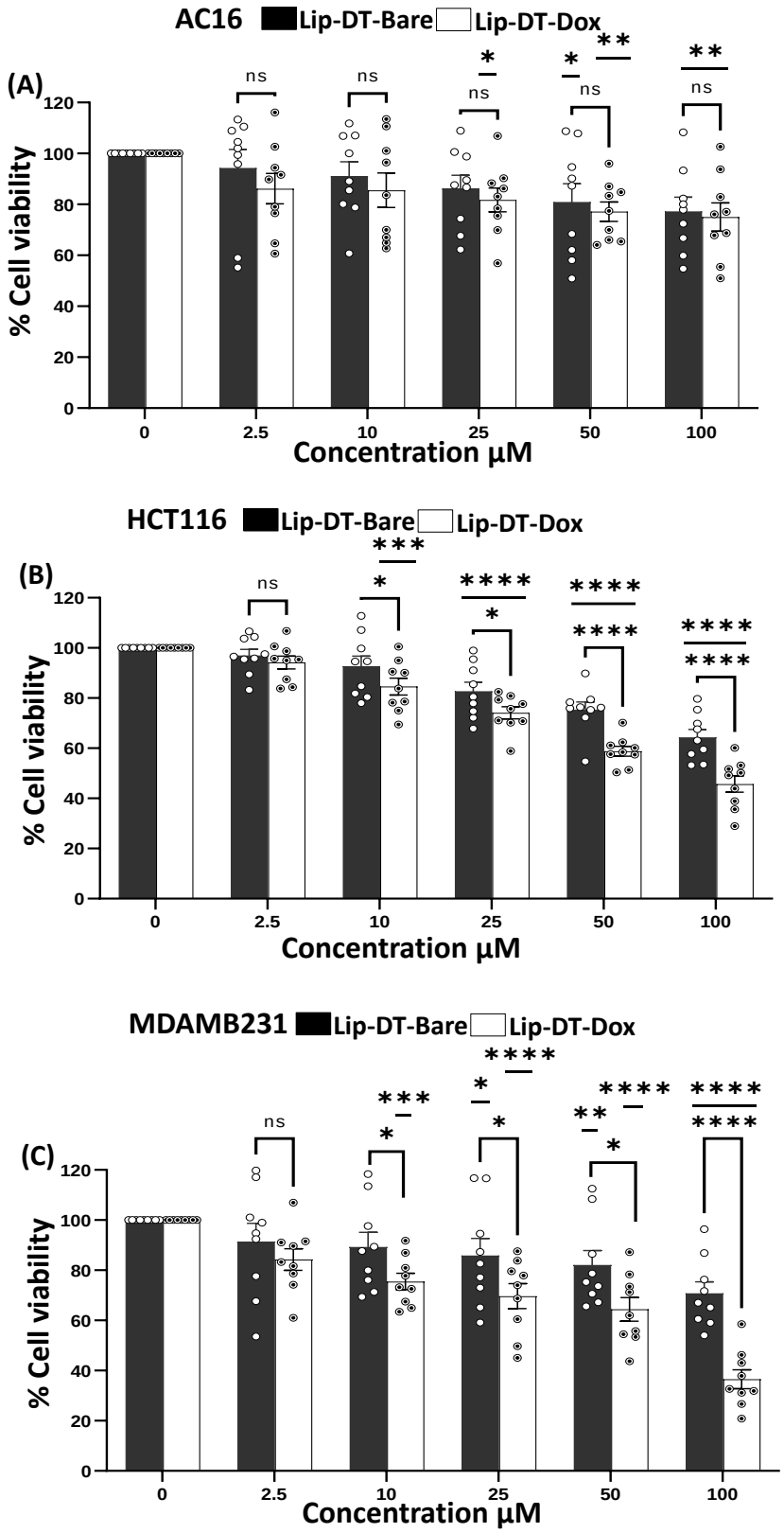


Figure S12. Cytotoxicity and incorporation of **Lip-DT** compound in cell. MTT assay was used to measure the cellular toxicity following incubation with **Lip-DT**, and **Lip-DT-Dox** (1 µM) at 36 h (A) The survival of human cardiomyocytes AC16 (B) Cytotoxicity of cancer cell HCT116 and (C) triple negative breast cancer cell MDAMB231. All results are presented as means $\pm$ S.E. of multiple experiments (n=9, *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001 compared to control group).

Reference

1 D. Yun, D. Jeong, E. Cho and S. Jung, Colorimetric Detection of Some Highly Hydrophobic Flavonoids Using Polydiacetylene Liposomes Containing Pentacosa-10,12-diynoyl Succinoglycan Monomers, *PLoS One*, 2015, **10**, e0143454.

2 S. Pandey, S. Patil, N. Ballav and S. Basu, Spatial targeting of Bcl-2 on endoplasmic reticulum and mitochondria in cancer cells by lipid nanoparticles, *J. Mater. Chem. B*, 2020, **8**, 4259–4266.