Supplementary Information

Delivering anti-cancer drugs with endosomal pH-sensitive anti-cancer liposomes

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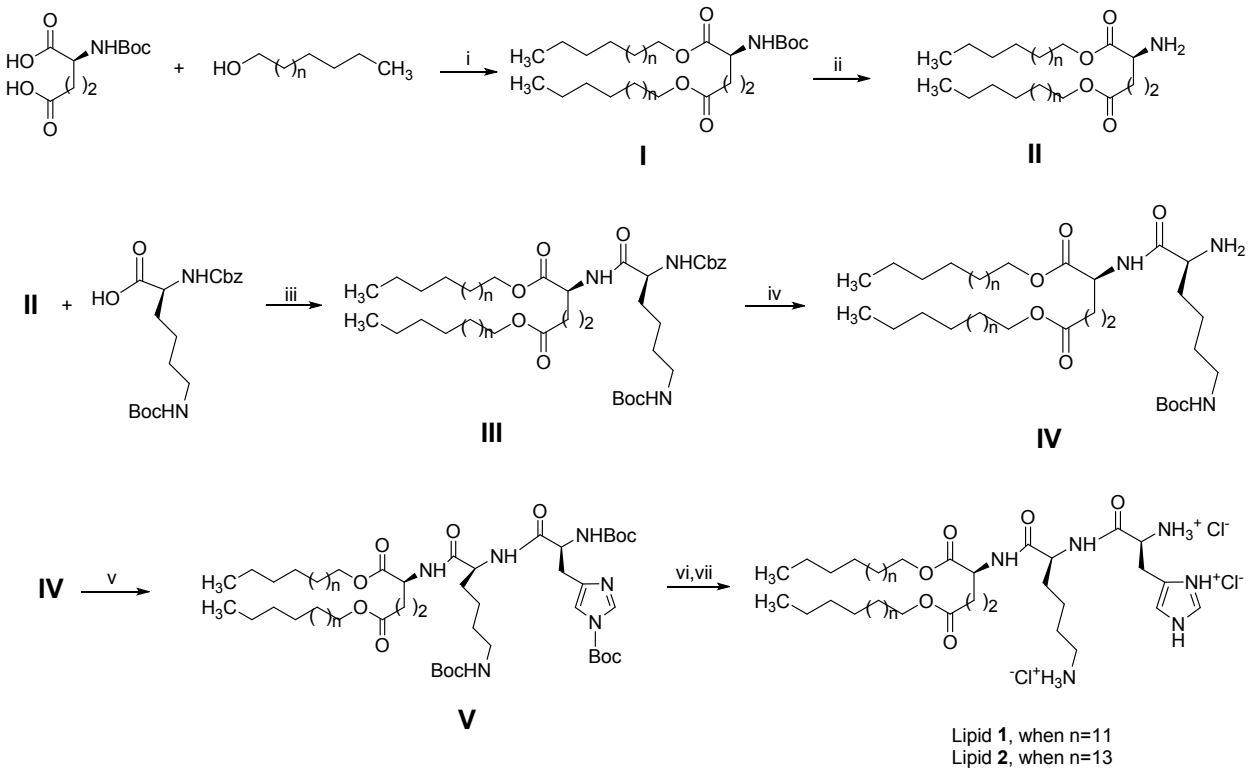
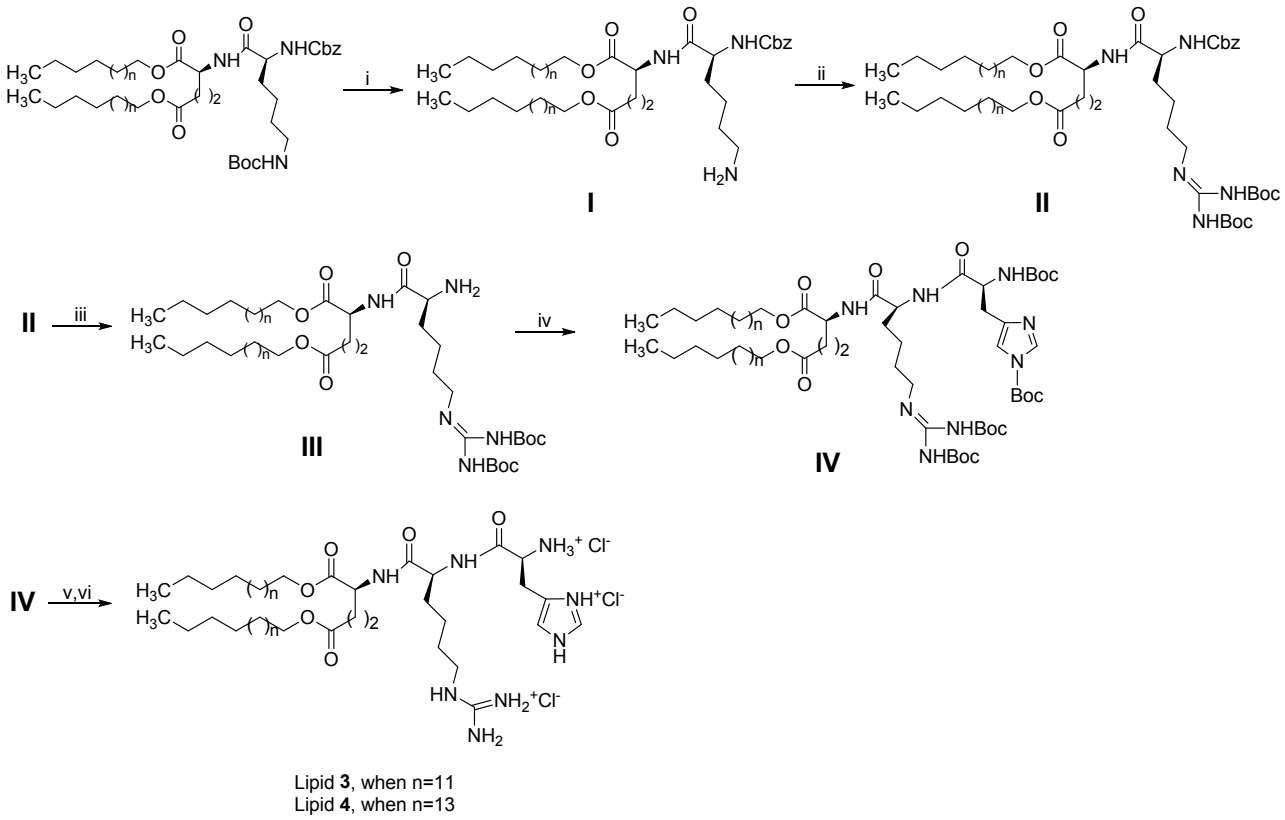
Scheme SI: Synthesis of Lipids 1-2



Reagents: i) EDCI, DIMAP, dry DCM; ii) TFA:DCM (1:2; v/v), 0 °C; iii) EDCI, HOBt, dry DCM; iv) $Pd(OH)_2/C$, MeOH, HCl, H_2; v) Nα, Nω-di-t-butyloxycarbonyl-L-Histidine, EDCI, HOBt, dry DCM; vi) TFA:DCM (1:2; v/v), 0 °C; vii) Amberlyst A-26 Cl^- ion-exchange resin.

Scheme SII: Synthesis of Lipids 3-4



Reagents: i) TFA:DCM (1:2; v/v), 0 °C; ii) Di Boc thiourea, Et_3N, $HgCl_2$, 0 °C; iii) $Pd(OH)_2/C$, MeOH, HCl, H_2; iv) Nα, Nω-di-t-butyloxycarbonyl-L-Histidine, EDCI, HOBt, dry DCM; v) TFA:DCM (1:2; v/v), 0 °C; vi) Amberlyst A-26 Cl^- loaded resin.

Synthesis of lipids 1-4:

Synthesis of 4-((S)-2-ammonio-3-(((S)-6-ammonio-1-(((S)-1,5-bis(hexadecyloxy)-1,5-dioxopentan-2-yl)amino)-1-oxohexan-2-yl)amino)-3-oxopropyl)-1H-imidazol-3-ium chloride (lipid 1, Scheme I)

Step (i): EDCI (2.6 g, 13.9 mmol) and DIMAP (0.29 g, 2.42 mmol) were added to an ice cold and stirred solution of N^{α}-Boc-L-Glutamic acid (1.5 g, 6.06 mmol) in 20 mL dry DCM. After ½ h, n-hexadecylalcohol (3.22 g, 13.34 mmol) was added to the reaction mixture, stirred at room temperature for 12 h. Reaction mixture was diluted in chloroform (70 mL) and washed with ice-cooled 1N HCl (2 x 70 mL), saturated sodium bicarbonate (2 x 80 mL) and brine (1 x 70 mL) sequentially. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent from the filtrate removed by rotary evaporation. The residue upon column chromatographic purification with 60-120 mesh silica gel using 0.1% methanol in chloroform (v/v) as eluent afforded 2.7 g (64% yield) of the pure intermediate **I**. (R_f = 0.9 using 5% methanol in chloroform, v/v).

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{15}$-]; 1.2-1.7 [m, 52H, -$(C\underline{H}_2)_{13}$-: m, 4H, -$(CH_2)_{13}$-C$\underline{H}_2$-CH_2-O-: s, 9H, CO-O-C(C$\underline{H}_3)_3$]; 2.0-2.5 [m, 4H, Glu $C^{\beta,\gamma}\underline{H}_2$]; 4.0-4.1 [m, 4H, -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.3[m, 1H, Glu$C^{\alpha}\underline{H}$]; 6.3[m, 1H, N$\underline{H}$-CO-O-$(CH_3)_3$]

ESIMS : m/z= 697 $[M+1]^+$ for $C_{42}H_{81}NO_6$

Step (ii): The intermediate **I** (0.6 g, 0.86 mmol) prepared above in step (i) was dissolved in 6 mL dry DCM and 3 mL TFA was added at 0 ^{o}C, left stirred for 3 h. Excess TFA was removed with nitrogen flow. The resulting compound was dissolved in chloroform (70 mL) and washed with

aqueous saturated $NaHCO_3$ (3 x 80 mL), brine (1 x 60 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent from the filtrate upon rotary evaporation and drying under vaccum pump for ½ h afforded 0.49 g (95% yield) of free amine as intermediate **II**. (R_f = 0.7 using 5% methanol in chloroform, v/v).

Step (iii): Solid HOBt (0.06 g, 0.43 mmol) and EDCI (0.08 g, 0.43 mmol) were added sequentially to an ice cold and stirred solution of N^{α}-Z-N^{ε}-Boc-L-Lysine (0.14 g, 0.36 mmol) in 10 mL dry DCM. After half an hour, the intermediate **II** (0.2 g, 0.336 mmol) obtained above in step (ii) dissolved in 15 mL dry DCM was added to the reaction mixture. DIPEA was added dropwise to the stirred reaction mixture until it became alkaline to litmus, left stirred at room temperature. After 12 h, the reaction mixture was diluted with chloroform (80 mL), washed sequentially with ice-cooled 1N HCl (2 x 60 mL), saturated sodium bicarbonate (2 x 60 mL) and brine (1 x 60 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent from the filtrate removed by rotary evaporation. The residue upon column chromatographic purification with 60-120 mesh silica gel using 2 % methanol in chloroform (v/v) as eluent afforded 0.29 g (90.4% yield) of the pure intermediate **III**. (R_f = 0.5 using 5% methanol in chloroform, v/v).

^{1}H NMR (300 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{15}$-]; 1.2-1.7 [m, 52H, -(C$\underline{H}_2$)$_{13}$-: m, 4H, -$(CH_2)_{13}$-C$\underline{H}_2$-CH_2-O-: s, 9H, CO-O-C(C$\underline{H}_3$)$_3$]; 1.8-2.0 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$]; 2.3-2.5 [m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.4-3.5 [m, 2H, LysC$^{\omega}\underline{H}_2$]; 4.0-4.3[m, 4H -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$]; 5.0 [d, 2H, -O-C$\underline{H}_2$-C_6H_5]; 5.5 [m, 1H, -N$\underline{H}$-Z]; 7.2-7.5 [m, 5H, O-CH_2-$C_6\underline{H}_5$]; 8.5 [m, 1H, N$\underline{H}$-CO-O-$(CH_3)_3$]

ESIMS : m/z= 981 $[M+Na]^+$ for $C_{56}H_{99}N_3O_9$

Step (iv): The intermediate **III** (0.13 g, 0.13 mmol) prepared in previous step (iii) was dissolved in 10 mL methanol and 5 ethyl acetate. $Pd(OH)_2/C$ (0.25 g) was added to the reaction mixture and air was removed, stirred at room temperature under hydrogen atmosphere (2 atm). After 12 h, the reaction mixture was filtered using celite, the filtrate was dried over anhydrous sodium sulphate and removal of the solvent from the filtrate by rotary evaporation afforded 0.09 g (88% yield) of pure intermediate **IV**. (R_f = 0.4, 5% methanol in chloroform, v/v).

Step (v): Solid HOBt (0.02 g, 0.14 mmol) and EDCI (0.02 g, 0.14 mmol) were added sequentially to an ice cold and stirred solution of N^{α}, N^{ω}-di-t-butyloxycarbonyl-L-Histidine (0.04 g, 0.11 mmol) in 10 mL dry DCM. After half an hour, the intermediate **IV** (0.09 g, 0.10 mmol) obtained above in step (iv) dissolved in 5 mL dry DCM was added to the reaction mixture. DIPEA was added dropwise to the stirred reaction mixture until it became alkaline to litmus, left stirred at room temperature. After 12 h, reaction mixture was diluted with chloroform (80 mL) and washed sequentially with ice-cooled 1N HCl (2 x 70 mL), saturated sodium bicarbonate (2 x 60 mL) and brine (1 x 70 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent from the filtrate removed by rotary evaporation. The residue upon column chromatographic purification with 60-120 mesh silica gel using 2.5 % methanol in chloroform (v/v) as eluent afforded 0.10 g (82.7% yield) of the pure intermediate **V**. (R_f = 0.5 using 5% methanol in chloroform, v/v).

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, $C\underline{H}_3$-$(CH_2)_{15}$-]; 1.2-1.7 [m, 52H, -$(C\underline{H}_2)_{13}$-: m, 4H, -$(CH_2)_{13}$-$C\underline{H}_2$-CH_2-O-: s, 27H, CO-O-$C(C\underline{H}_3)_3$]; 1.8-2.2 [m, 6H, Lys$C^{\gamma}\underline{H}_2$, Lys$C^{\delta}\underline{H}_2$, Lys$C^{\beta}\underline{H}_2$]; 2.3-2.5 [m, 4H, Glu $C^{\beta,\gamma}\underline{H}_2$]; 3.0-3.4 [m, 2H, Lys$C^{\omega}\underline{H}_2$: m, 2H, His$C^{\beta}\underline{H}$]; 4.0-4.2[m,

4H, -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$: m, 1H, HisC$^{\alpha}\underline{H}$]; 7.7-8.0 [m, 2H, His-ring]

ESIMS : m/z=1162 $[M+1]^+$ for $C_{64}H_{116}N_6O_{12}$

Step (vi, vii): To the ice cold solution of the intermediate **V** (0.09 g, 0.08 mmol) prepared above in step (v) was dissolved in 2 mL dry DCM, 1 mL of TFA was added and the mixture was allowed to stir for 3 h at 0 ^{o}C. TFA was removed with nitrogen flow and the residue was subjected to chloride ion exchange chromatography over amberlyst A-26 chloride ion exchange resin. The compound obtained after chloride ion exchange upon recrystalization from 1:5 (v/v) MeOH:Acetone afforded 0.05 g (65.7% yield) of the pure target compound lipid **1** as a white solid. (R_f = 0.2, 10% methanol in chloroform, v/v).

Lipid 1:

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C$\underline{H}_3$-$(CH_2)_{15}$-]; 1.1-1.7 [m, 52H, -(C$\underline{H}_2$)$_{13}$-: m, 4H, -$(CH_2)_{13}$-C$\underline{H}_2$-CH_2-O]; 2.0-2.5 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$]; 2.6-2.7 [m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.0-3.6 [m, 2H, LysC$^{\omega}\underline{H}_2$: m, 2H, HisC$^{\beta}\underline{H}$]; 4.0-4.2[m, 4H, -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$: m, 1H, HisC$^{\alpha}\underline{H}$]; 7.8-8.4[m, 2H, His-ring]

ESIMS: m/z= 861.7 for $C_{49}H_{95}Cl_3N_6O_6$

Synthesis of 4-((S)-2-ammonio-3-(((S)-6-ammonio-1-(((S)-1,5-bis(octadecyloxy)-1,5-dioxopentan-2-yl)amino)-1-oxohexan-2-yl)amino)-3-oxopropyl)-1H-imidazol-3-ium chloride (lipid 2, Scheme I)

Lipid **2** was synthesized following the same procedure described above for lipid **1**. The ^{1}H NMR and ESIMS spectral data are given below.

Intermediate I for lipid 2 (Scheme I)

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.2-1.7 [m, 60H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{15}$-C$\underline{H}_2$-CH_2-O-s: 9H, CO-O-C(C$\underline{H}_3)_3$]; 2.0-2.4 [m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 4.0-4.2 [m, 4H, -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.3[m, 1H, GluC$^{\alpha}\underline{H}$]

ESIMS : m/z= 789.7 $[M+K]^+$ for $C_{46}H_{89}NO_6$

Intermediate III for lipid 2 (Scheme I)

^{1}H NMR (300 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.2-1.7 [m, 60H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{15}$-C$\underline{H}_2$-CH_2-O-: s, 9H, CO-O-C(C$\underline{H}_3)_3$]; 1.8-2.4 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.0-3.2 [m, 2H, LysC$^{\omega}\underline{H}_2$]; 4.0-4.3[m, 4H -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$]; 5.1 [d, 2H, -O-C$\underline{H}_2$-C_6H_5]; 5.5 [m, 1H, -N$\underline{H}$-Z: m, 1H, N$\underline{H}$-CO-O-$(CH_3)_3$]; 7.2-7.5 [m, 5H, O-CH_2-$C_6\underline{H}_5$]

ESIMS : m/z= 1037 $[M+Na]^+$ for $C_{60}H_{107}N_3O_9$

Intermediate V for lipid 2 (Scheme I)

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.2-1.7 [m, 60H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{15}$-C$\underline{H}_2$-CH_2-O-: s, 27H, CO-O-C(C$\underline{H}_3)_3$]; 1.8-2.2 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.0-3.3 [m, 2H, LysC$^{\omega}\underline{H}_2$: m, 2H, HisC$^{\beta}\underline{H}$]; 4.0-4.2[m, 4H, -

$(CH_2)_{14}$-C<u>H</u>$_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}$<u>H</u>: m, 1H, LysC$^{\alpha}$<u>H</u>: m, 1H, HisC$^{\alpha}$<u>H</u>]; 7.7-7.9[m, 2H, His-ring]

ESIMS : m/z=1218 $[M+1]^+$ for $C_{68}H_{124}N_6O_{12}$

Lipid 2:

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C<u>H</u>$_3$-$(CH_2)_{17}$-]; 1.1-1.7 [m, 60H, -(C<u>H</u>$_2$)$_{15}$-: m, 4H, -$(CH_2)_{15}$-C<u>H</u>$_2$-CH_2-O]; 1.8-2.5 [m, 6H, LysC$^{\gamma}$<u>H</u>$_2$, LysC$^{\delta}$<u>H</u>$_2$, LysC$^{\beta}$<u>H</u>$_2$]; 2.6-2.7 [m, 4H, Glu C$^{\beta,\gamma}$<u>H</u>$_2$]; 3.0-3.2 [m, 2H, LysC$^{\omega}$<u>H</u>$_2$]; 3.6 [m, 2H, HisC$^{\beta}$<u>H</u>]; 4.0-4.2[m, 4H, -$(CH_2)_{14}$-C<u>H</u>$_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}$<u>H</u>: m, 1H, LysC$^{\alpha}$<u>H</u>: m, 1H, HisC$^{\alpha}$<u>H</u>]; 7.8-8.5 [m, 2H, His-ring]

ESIMS: m/z= 917.7 for $C_{53}H_{103}Cl_3N_6O_6$

Synthesis of 4-((S)-3-(((S)-6-((amino(iminio)methyl)amino)-1-(((S)-1,5-bis(hexadecyloxy)-1,5-dioxopentan-2-yl)amino)-1-oxohexan-2-yl)amino)-2-ammonio-3-oxopropyl)-1H-imidazol-3-ium chloride (lipid 3, Scheme II)

Step (i): Di hexadecyl 2-(2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanamido) pentanedioate (0.15 g, 0.15 mmol) was dissolved in 6 mL dry DCM and 3 mL TFA was added at 0 °C, left stirred for 3 h, excess TFA was removed with nitrogen flow. The resulting compound was dissolved in chloroform (70 mL) and washed with aqueous saturated $NaHCO_3$ (3 x 80 mL), brine (1 x 60 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent from the filtrate upon rotary evaporation and drying under vaccum pump for one hour afforded 0.12 g (89% yield) of free amine as

intermediate **I** (R_f = 0.4 using 5% methanol in chloroform, v/v) which was not purified and was used directly in the next step described below.

Step (ii): Mercury chloride (0.04 g, 0.18 mmol) was added to a mixture of intermediate **I** (prepared in step (i), 0.10 g, 0.12 mmol), bis-*N*-Boc-thiourea(0.05 g, 0.18 mmol) dissolved in dry *N*,*N*-dimethylformamide (DMF, 2 mL), Triethylamine (1 mL) and dry dichloromethane (DCM, 5 mL) at 0 °C with continuous stirring. The resulting mixture was stirred at 0 °C under nitrogen for 1 h, diluted with DCM (15 mL), and filtered through a pad of Celite. The filtrate was sequentially washed with water (2 × 40 mL) and brine solution (2 × 40 mL), dried over anhydrous sodium sulfate, and filtered, and the solvent from the filtrate was removed by rotary evaporation. The residue upon column chromatographic purification with 60-120 mesh silica gel using 1.5% methanol in chloroform (v/v) as eluent afforded 0.12 g (yield 90%) of the pure intermediate **II**. (R_f = 0.6, 5% methanol in chloroform, v/v).

Intermediate II for lipid 3 (Scheme II)

^{1}H NMR (300 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C$\underline{H}_3$-$(CH_2)_{15}$-]; 1.2-1.7 [m, 52H, -(C$\underline{H}_2$)$_{13}$-: m, 4H, -$(CH_2)_{13}$-C$\underline{H}_2$-CH_2-O-: s, 18H, CO-O-C(C$\underline{H}_3$)$_3$]; 1.8-2.4 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.0-3.2 [m, 2H, LysC$^{\omega}\underline{H}_2$]; 4.0-4.3[m, 4H -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5-4.7 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$]; 5.1 [d, 2H, -O-C$\underline{H}_2$-C_6H_5]; 5.5 [m, 1H, -N$\underline{H}$-Z: m, 1H, N$\underline{H}$-CO-O-$(CH_3)_3$]; 7.3-7.5 [m, 5H, O-CH_2-$C_6\underline{H}_5$]

ESIMS : m/z= 1101 $[M]^+$ for $C_{62}H_{109}N_5O_{11}$

Step (iii): The intermediate **II** (0.10 g, 0.09 mmol) prepared in previous step (ii) was dissolved in 10 mL methanol and 5 mL ethyl acetate, 2 drops of 2N hydrochloric acid. $Pd(OH)_2$/C (0.25 g)

was added to the reaction mixture and air was removed. The resultant reaction mixture was stirred at room temperature for 12 h under hydrogen atmosphere (2 atm). The reaction mixture was filtered using celite, the filtrate was dried over anhydrous sodium sulfate and removal of the solvent from the filtrate by rotary evaporation afforded 0.08 g (90% yield) of amine intermediate **III** (R_f = 0.5, 5% methanol in chloroform, v/v) which was directly used in the next step described below without further purification.

Step (iv): Solid HOBt (0.01 g, 0.10 mmol) and EDCI (0.02 g, 0.10 mmol) were added sequentially to an ice cold and stirred solution of N^{α}, N^{ω}-di-t-butyloxycarbonyl-L-Histidine (0.03 g, 0.09 mmol) in 10 mL dry DCM. After half an hour, the intermediate **III** (0.08 g, 0.08 mmol) obtained above in step (iii) dissolved in 5 mL dry DCM was added to the reaction mixture. DIPEA was added dropwise to the stirred reaction mixture until it became alkaline to litmus, left stirred at room temperature for 12 h, diluted with chloroform (80 mL) and washed sequentially with ice-cooled 1N HCl (2 x 70 mL), saturated sodium bicarbonate (2 x 60 mL) and brine (1 x 70 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent from the filtrate removed by rotary evaporation. The residue upon column chromatographic purification with 60-120 mesh silica gel using 2.5 % methanol in chloroform (v/v) as eluent afforded 0.09 g (86% yield) of the pure intermediate **IV**. (R_f = 0.4 using 5% methanol in chloroform, v/v).

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, $C\underline{H}_3$-$(CH_2)_{15}$-]; 1.2-1.7 [m, 52H, -$(C\underline{H}_2)_{13}$-: m, 4H, -$(CH_2)_{13}$-$C\underline{H}_2$-CH_2-O-: s, 36H, CO-O-C($C\underline{H}_3)_3$]; 1.8-2.5 [m, 6H, Lys$C^{\gamma}\underline{H}_2$, Lys$C^{\delta}\underline{H}_2$, Lys$C^{\beta}\underline{H}_2$: m, 4H, Glu $C^{\beta,\gamma}\underline{H}_2$]; 3.0-3.5 [m, 2H, Lys$C^{\omega}\underline{H}_2$: m, 2H, His$C^{\beta}\underline{H}$]; 4.0-4.2[m, 4H, -

$(CH_2)_{14}$-C<u>H</u>$_2$-O-]; 4.4-4.6 [m, 1H, GluC$^{\alpha}$<u>H</u>: m, 1H, LysC$^{\alpha}$<u>H</u>: m, 1H, HisC$^{\alpha}$<u>H</u>]; 7.7-8.0 [m, 2H, His-ring]

ESIMS : m/z=1304 $[M+1]^+$ for $C_{70}H_{126}N_8O_{14}$

Step (v, vi): To the ice cold solution of the intermediate **IV** (0.08 g, 0.06 mmol) prepared above in step (iv) was dissolved in 2 mL dry DCM, 1 mL of TFA was added and the mixture was allowed to stir for 3 h at 0 ^{o}C. TFA was removed with nitrogen flow and the residue was subjected to chloride ion exchange chromatography over amberlyst A-26 chloride ion exchange resin. The compound obtained after chloride ion exchange upon recrystalization from 1:5 (v/v) MeOH:Acetone afforded 0.04 g (71% yield) of the pure target compound lipid **3** as a white solid. (R_f = 0.2, 10% methanol in chloroform, v/v).

Lipid 3:

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C<u>H</u>$_3$-$(CH_2)_{15}$-]; 1.1-1.7 [m, 52H, -(C<u>H</u>$_2$)$_{13}$-: m, 4H, -$(CH_2)_{13}$-C<u>H</u>$_2$-CH_2-O]; 2.0-2.5 [m, 6H, LysC$^{\gamma}$<u>H</u>$_2$, LysC$^{\delta}$<u>H</u>$_2$, LysC$^{\beta}$<u>H</u>$_2$: m, 4H, Glu C$^{\beta,\gamma}$<u>H</u>$_2$]; 3.0 [m, 2H, LysC$^{\omega}$<u>H</u>$_2$]; 3.4 [m, 2H, HisC$^{\beta}$<u>H</u>]; 4.0-4.2[m, 4H, -$(CH_2)_{14}$-C<u>H</u>$_2$-O-]; 4.3-4.5 [m, 1H, GluC$^{\alpha}$<u>H</u>: m, 1H, LysC$^{\alpha}$<u>H</u>]; 5.0 [m, 1H, HisC$^{\alpha}$<u>H</u>]; 7.7-8.1[m, 2H, His-ring]

ESIMS: m/z= 903 $[M]^+$ for $C_{50}H_{97}Cl_3N_8O_6$

Synthesis of 4-((S)-3-(((S)-6-((amino(iminio)methyl)amino)-1-(((S)-1,5-bis(octadecyloxy)-1,5-dioxopentan-2-yl)amino)-1-oxohexan-2-yl)amino)-2-ammonio-3-oxopropyl)-1H-imidazol-3-ium chloride (lipid 4, Scheme II)

Lipid **4** was synthesized following the same procedure as was used in preparing lipid **3**. The ^{1}H NMR and ESIMS spectral data are given below.

Intermediate II for lipid 4 (Scheme II):

^{1}H NMR (300 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.2-1.7 [m, 60H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{15}$-C$\underline{H}_2$-CH_2-O-: s, 18H, CO-O-C(C$\underline{H}_3)_3$]; 1.8-2.5 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.5 [m, 2H, LysC$^{\omega}\underline{H}_2$]; 4.0-4.3[m, 4H -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.5 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$]; 5.1 [d, 2H, -O-C$\underline{H}_2$-C_6H_5]; 7.3 [m, 5H, O-CH_2-$C_6\underline{H}_5$]; 8.3 [m, 1H, -N$\underline{H}$-Z: m, 1H, N$\underline{H}$-CO-O-$(CH_3)_3$]

ESIMS : m/z= 1180 $[M+Na]^+$ for $C_{66}H_{117}N_5O_{11}$

Intermediate IV for lipid 4 (Scheme II):

^{1}H NMR (300 MHz, $CDCl_3$):δ/ppm = 0.9 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.2-1.7 [m, 60H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{15}$-C$\underline{H}_2$-CH_2-O-: s, 36H, CO-O-C(C$\underline{H}_3)_3$]; 1.8-2.6 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$]; 3.0-3.3 [m, 2H, LysC$^{\omega}\underline{H}_2$: m, 2H, HisC$^{\beta}\underline{H}$]; 4.0-4.2[m, 4H, -$(CH_2)_{14}$-C$\underline{H}_2$-O-]; 4.4-4.6 [m, 1H, GluC$^{\alpha}\underline{H}$: m, 1H, LysC$^{\alpha}\underline{H}$]; 5.0 [m, 1H, HisC$^{\alpha}\underline{H}$]; 7.7-8.1 [m, 2H, His-ring]

ESIMS : m/z=1382 $[M+Na]^+$ for $C_{74}H_{134}N_8O_{14}$

Lipid 4:

^{1}H NMR (500 MHz, $CDCl_3$):δ/ppm = 0.8 [m, 6H, C$\underline{H}_3$-$(CH_2)_{17}$-]; 1.1-1.7 [m, 52H, -$(C\underline{H}_2)_{15}$-: m, 4H, -$(CH_2)_{13}$-C$\underline{H}_2$-CH_2-O]; 2.0-2.5 [m, 6H, LysC$^{\gamma}\underline{H}_2$, LysC$^{\delta}\underline{H}_2$, LysC$^{\beta}\underline{H}_2$: m, 4H, Glu C$^{\beta,\gamma}\underline{H}_2$];

3.0 [m, 2H, LysC$^{\omega}$H$_2$]; 3.4 [m, 2H, HisC$^{\beta}$H]; 4.0-4.2[m, 4H, -(CH$_2$)$_{14}$-CH$_2$-O-]; 4.3-4.5 [m, 1H, GluC$^{\alpha}$H: m, 1H, LysC$^{\alpha}$H]; 5.0 [m, 1H, HisC$^{\alpha}$H]; 7.8-8.4[m, 2H, His-ring]

ESIMS: m/z= 960 $[M]^+$ for $C_{54}H_{105}Cl_3N_8O_6$

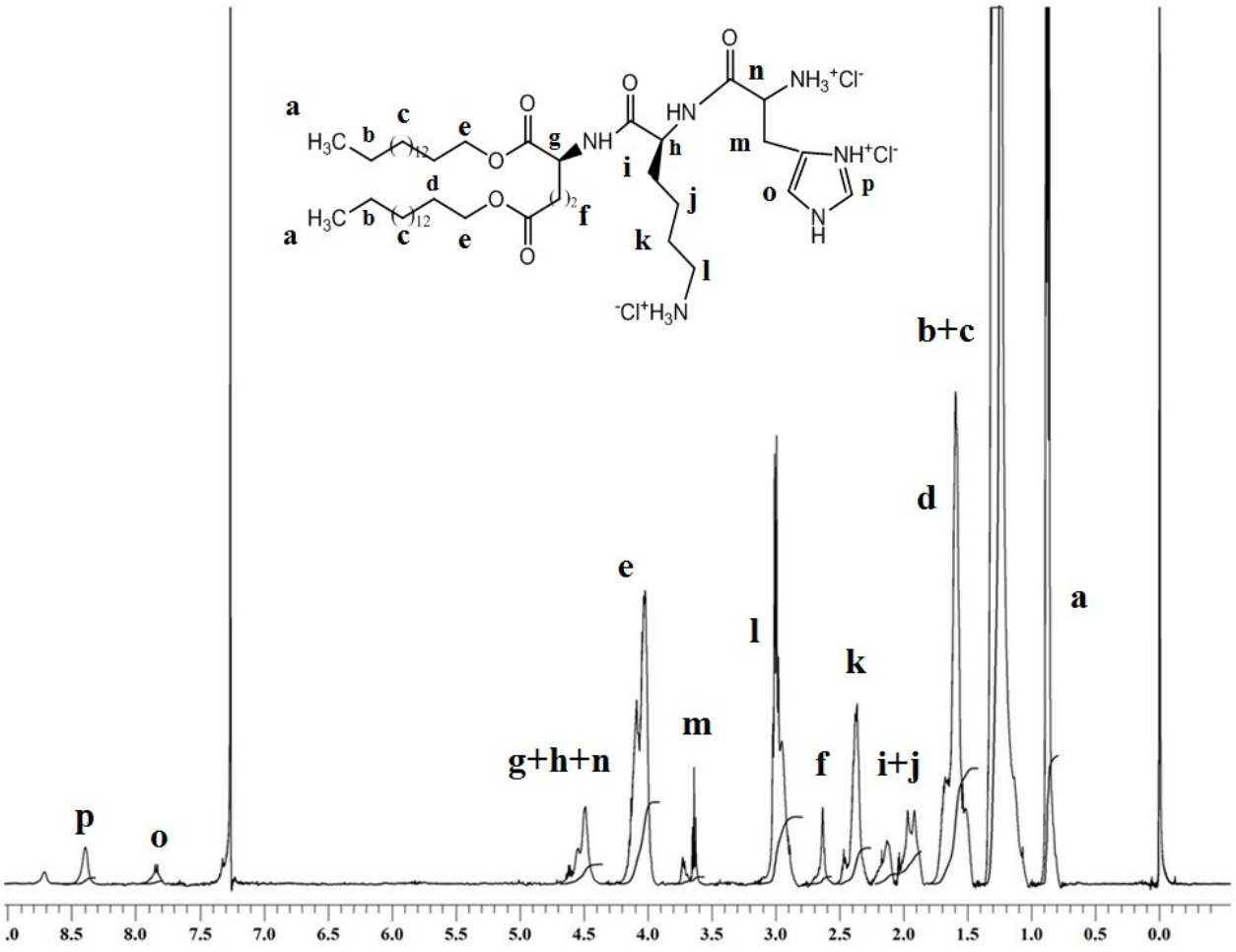


Fig. S1. ^{1}H NMR (500 MHz, $CDCl_3$) Spectrum for lipid **1**

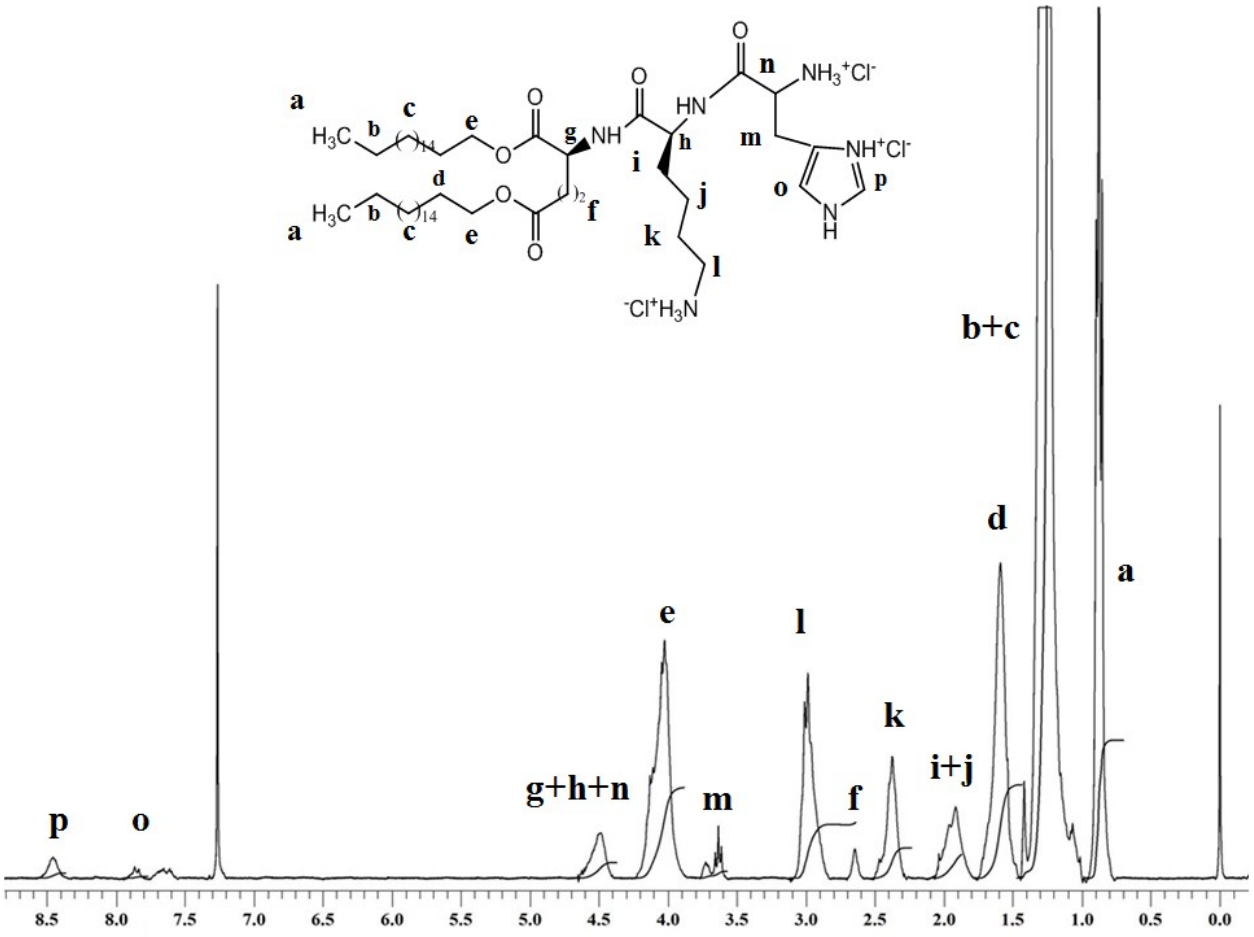


Fig. S2. ^{1}H NMR (500 MHz, $CDCl_3$) Spectrum for lipid **2**

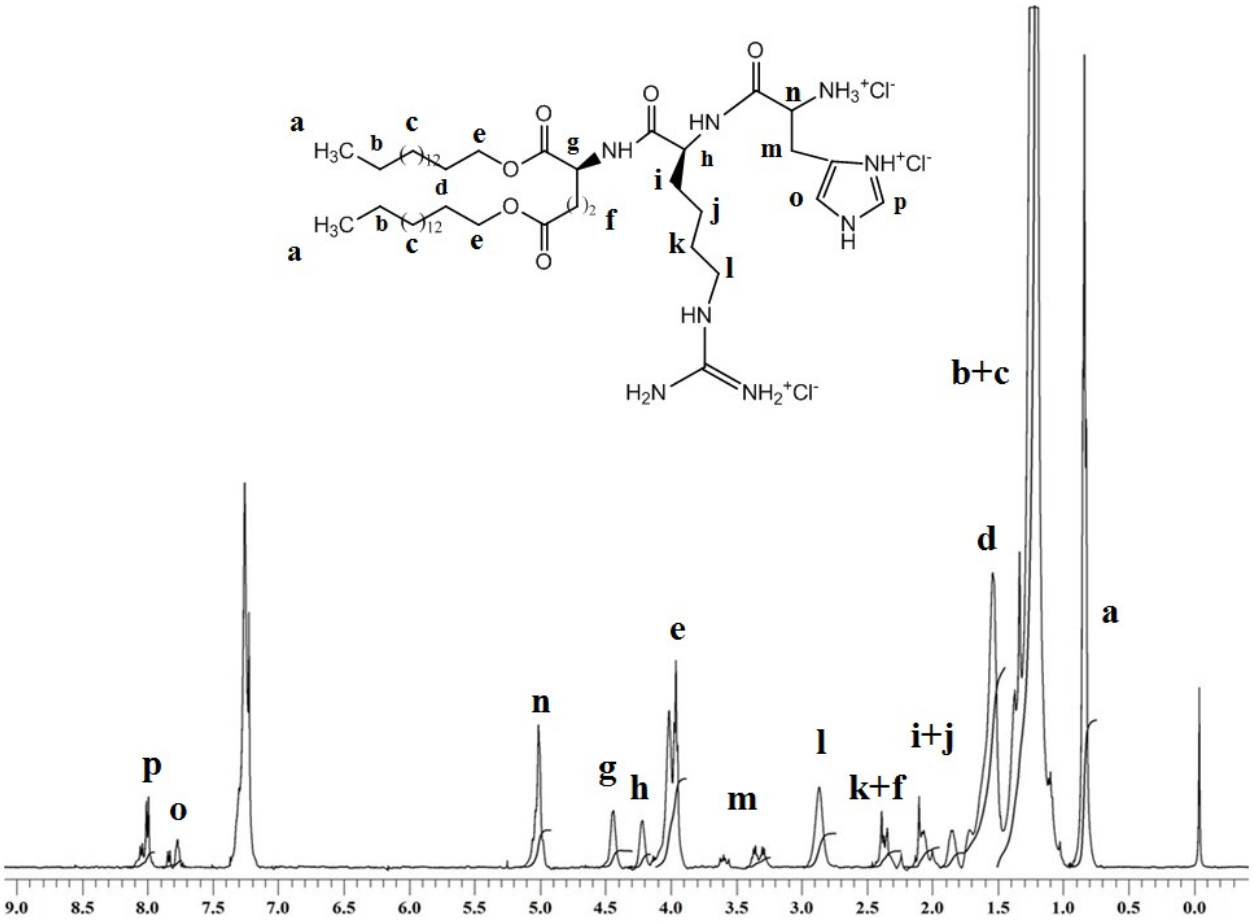


Fig. S3. ^{1}H NMR (500 MHz, $CDCl_3$) Spectrum for lipid **3**

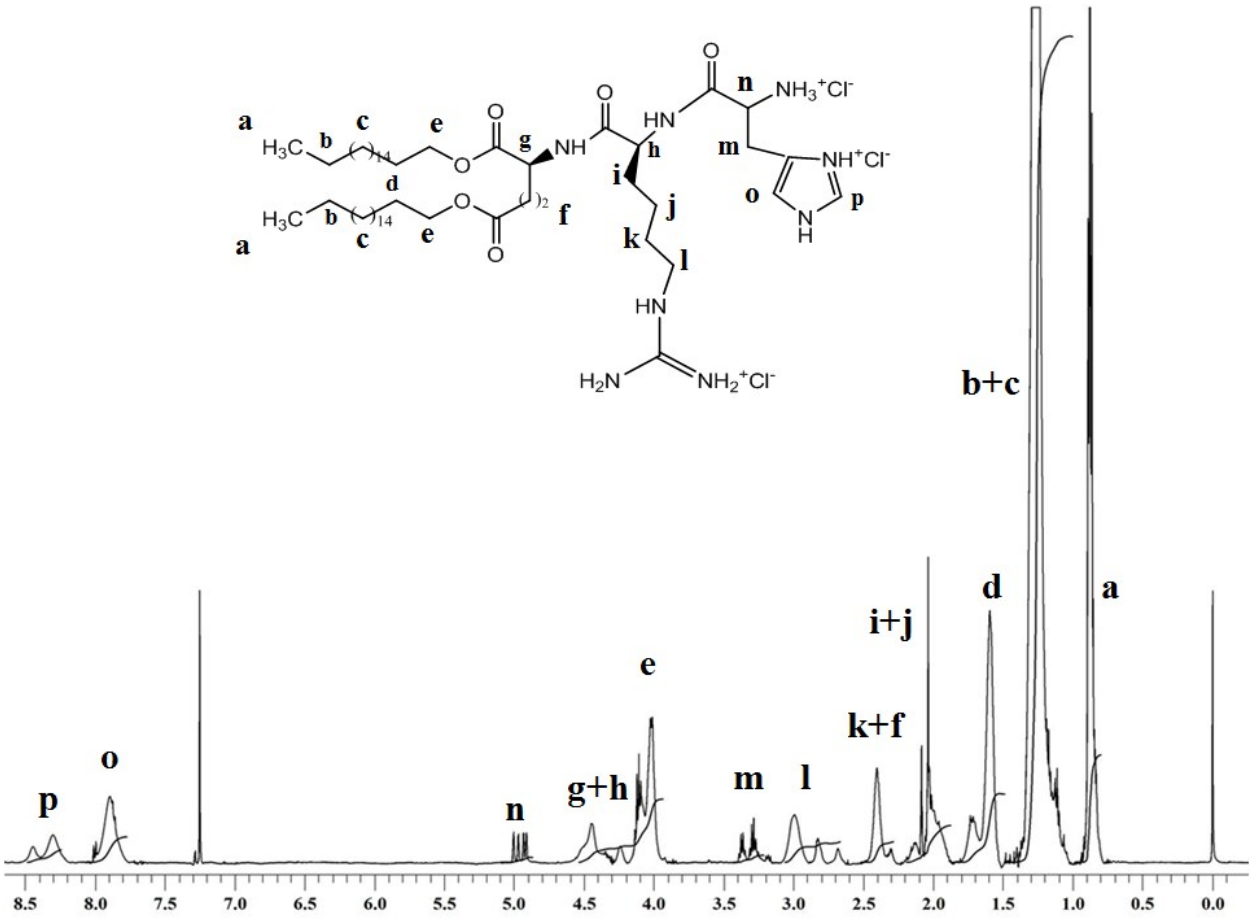


Fig. S4. ^{1}H NMR (500 MHz, $CDCl_3$) Spectrum for lipid **4**

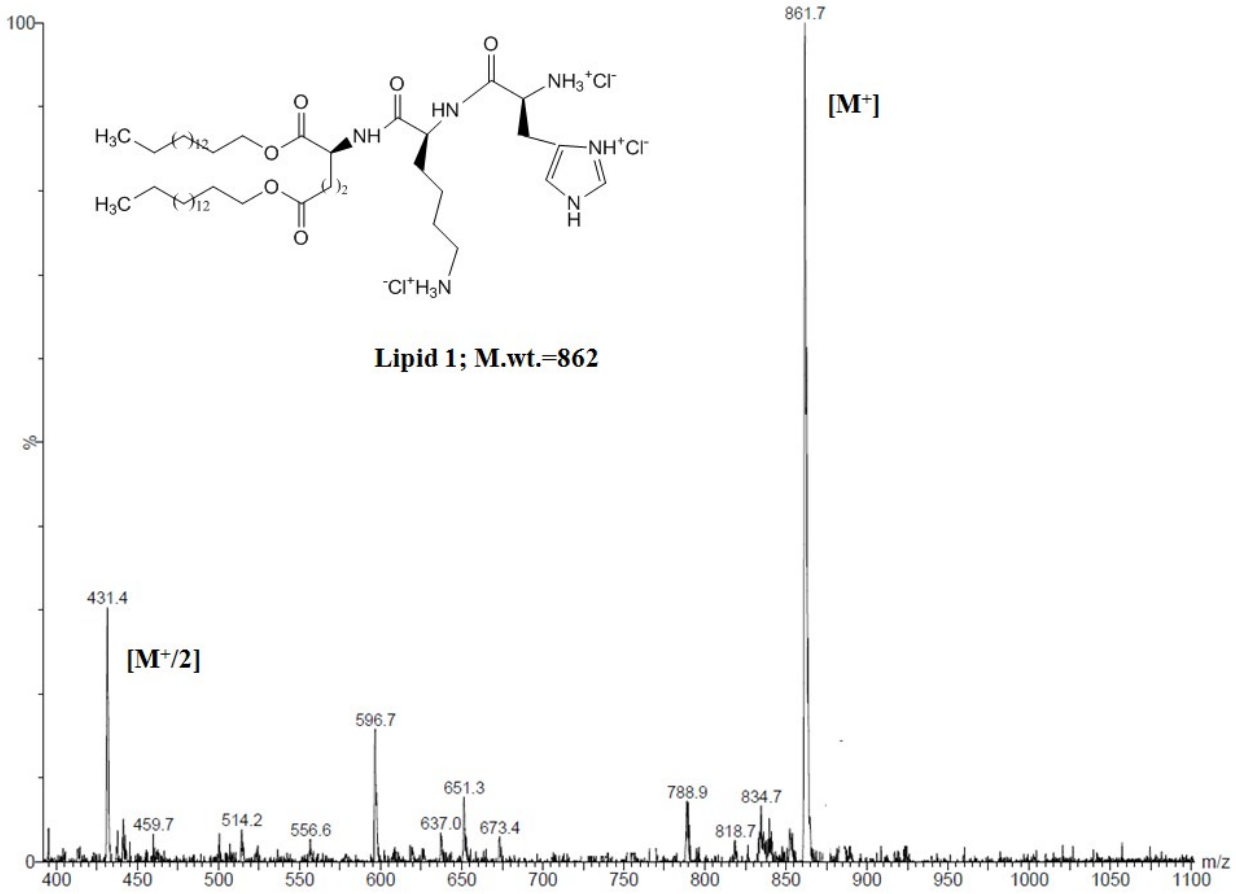


Fig. S5. ESIMS for lipid **1**

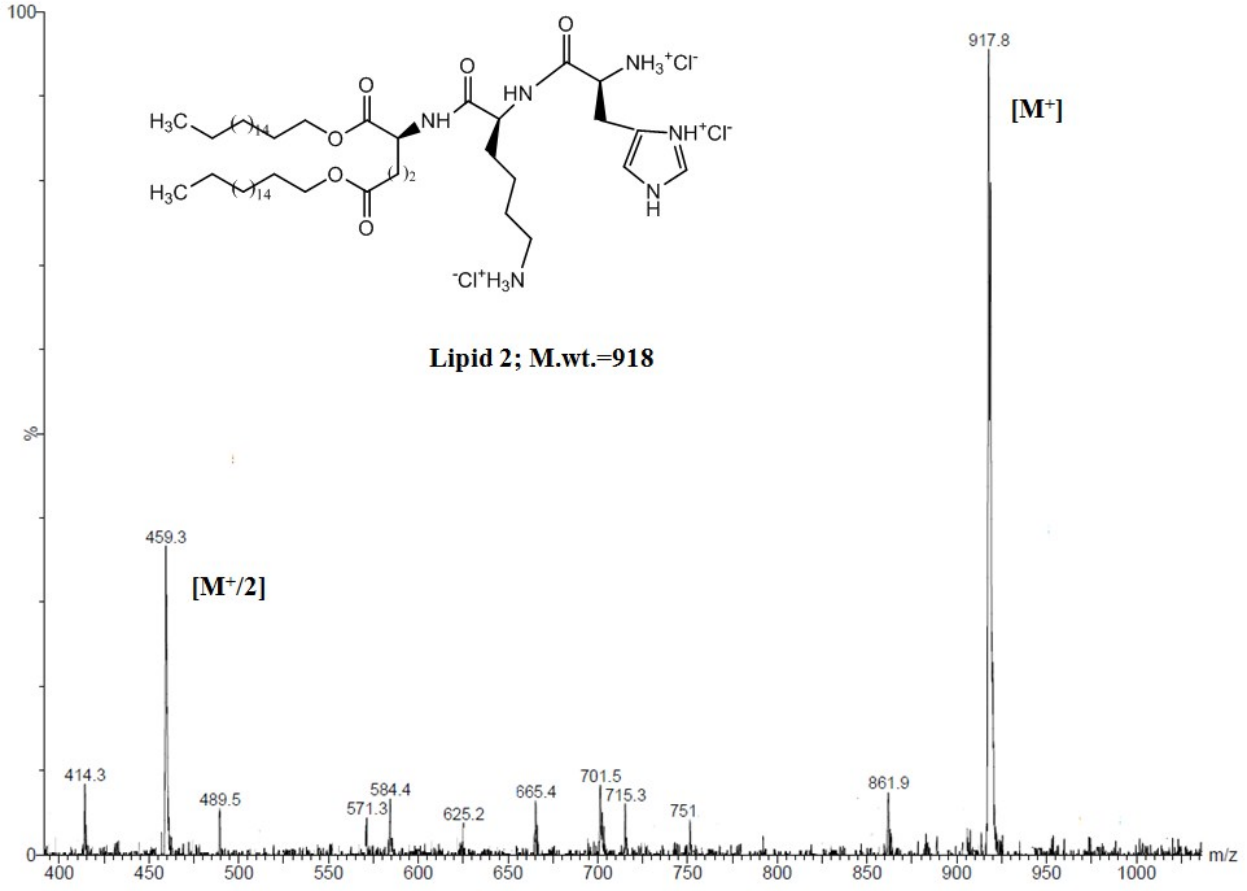


Fig. S6. ESIMS for lipid **2**

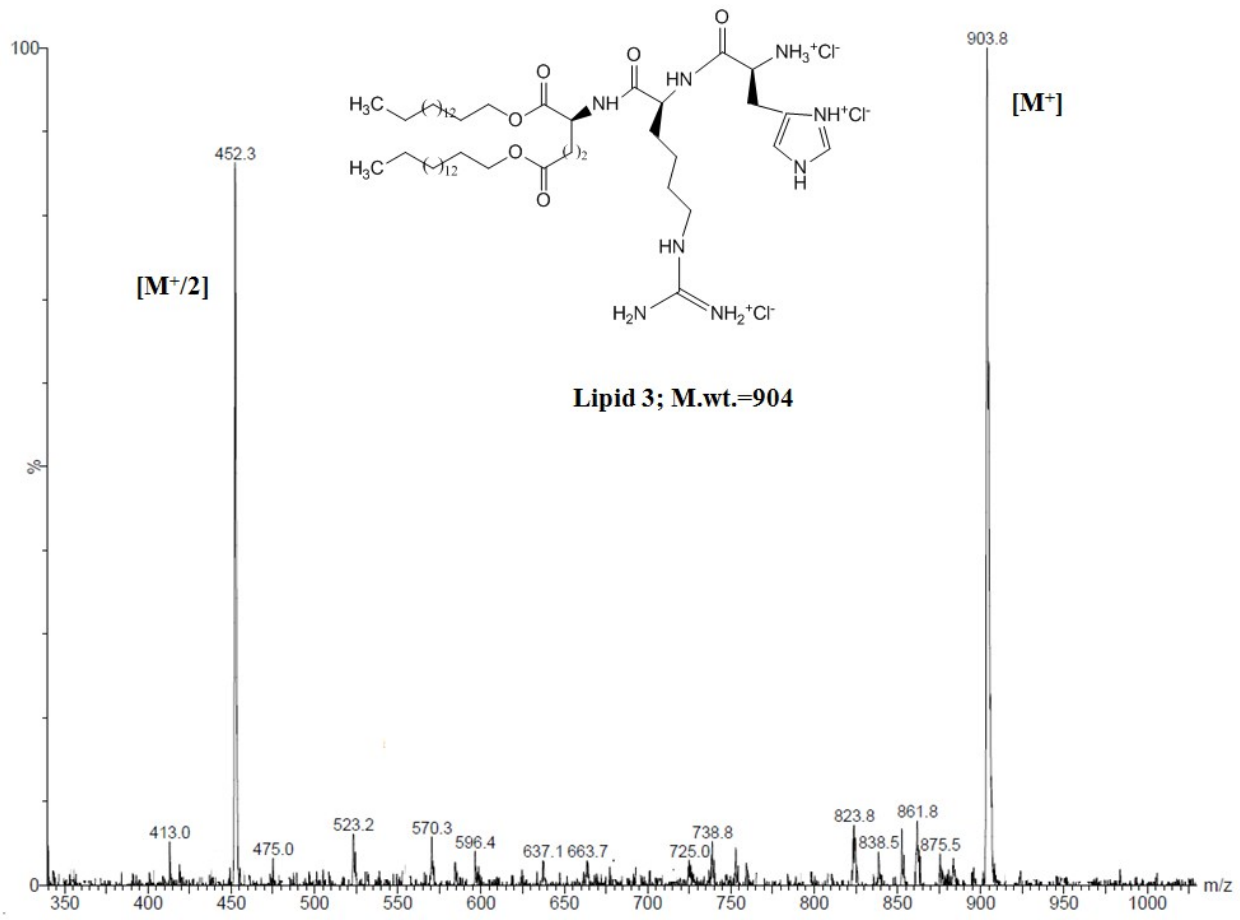


Fig. S7. ESIMS for lipid **3**

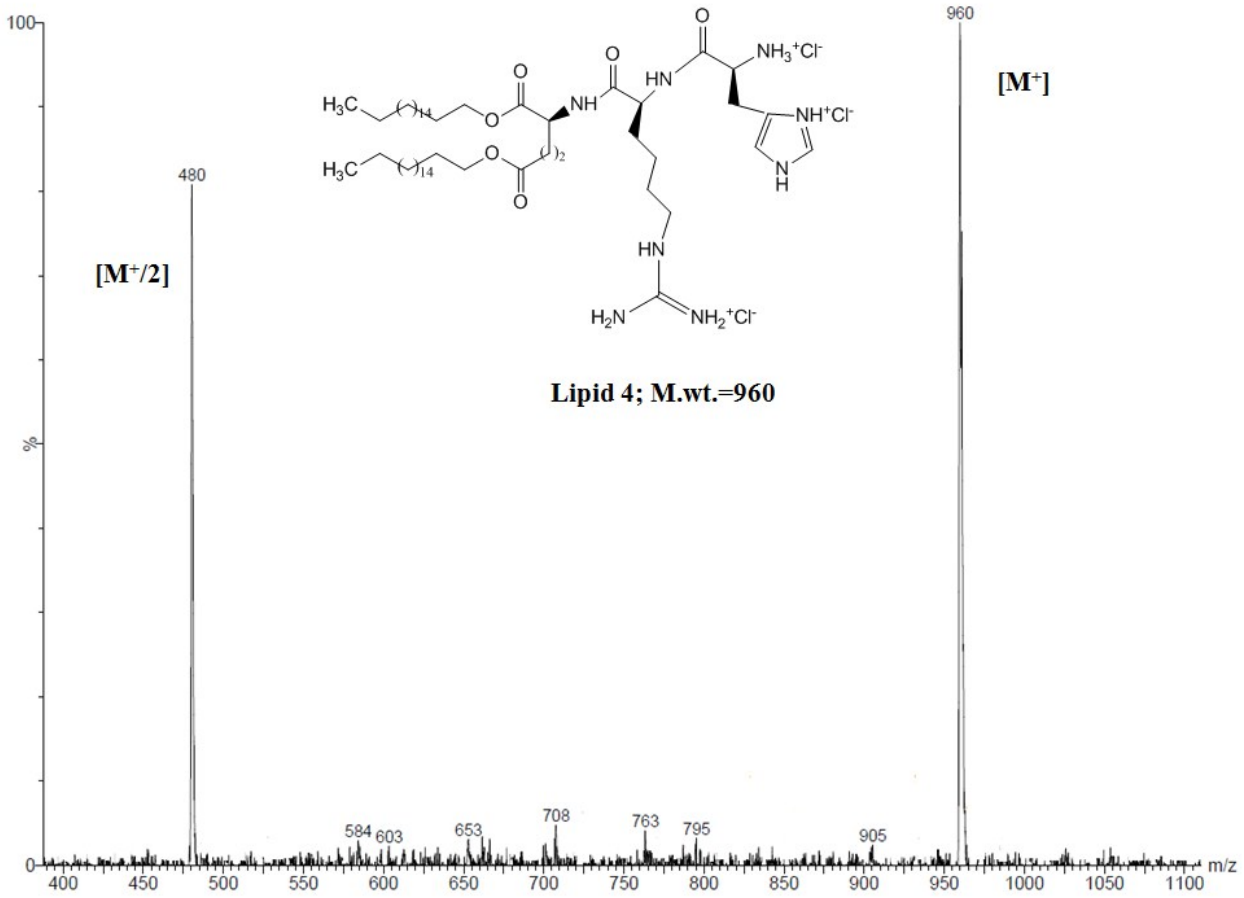
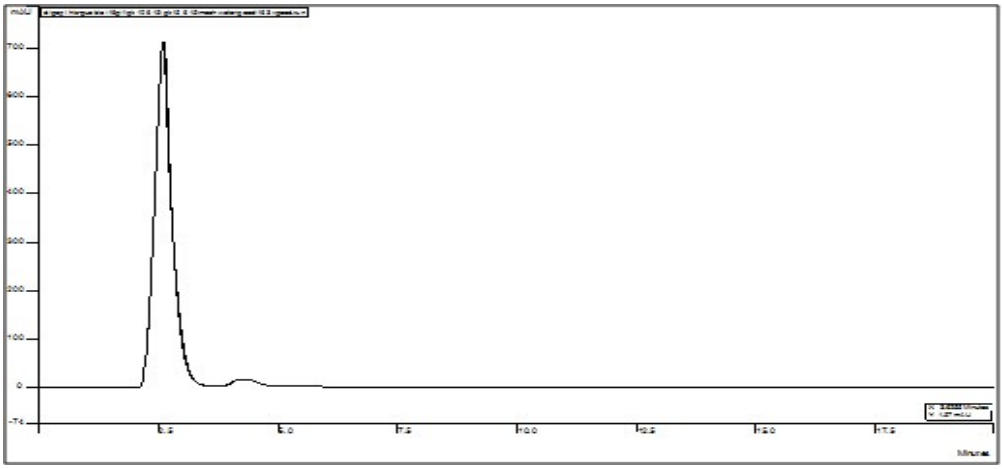


Fig. S8. ESIMS for lipid **4**

A.



B.

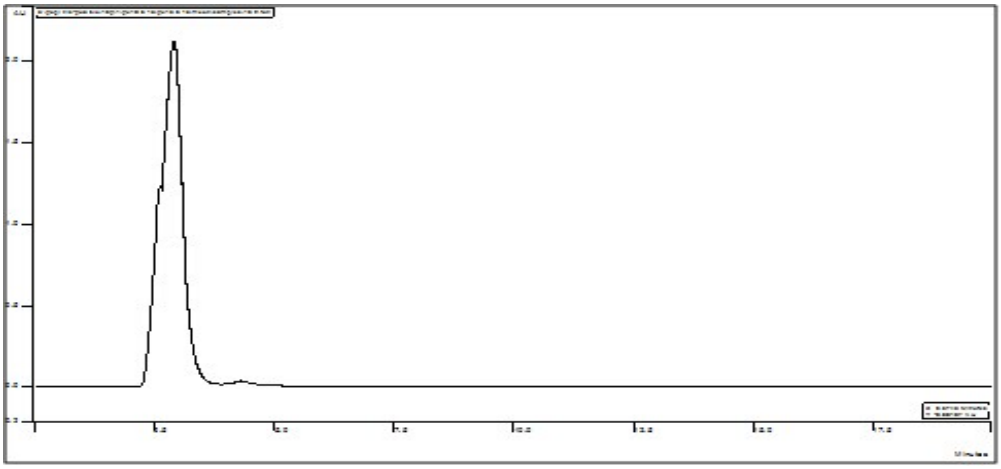
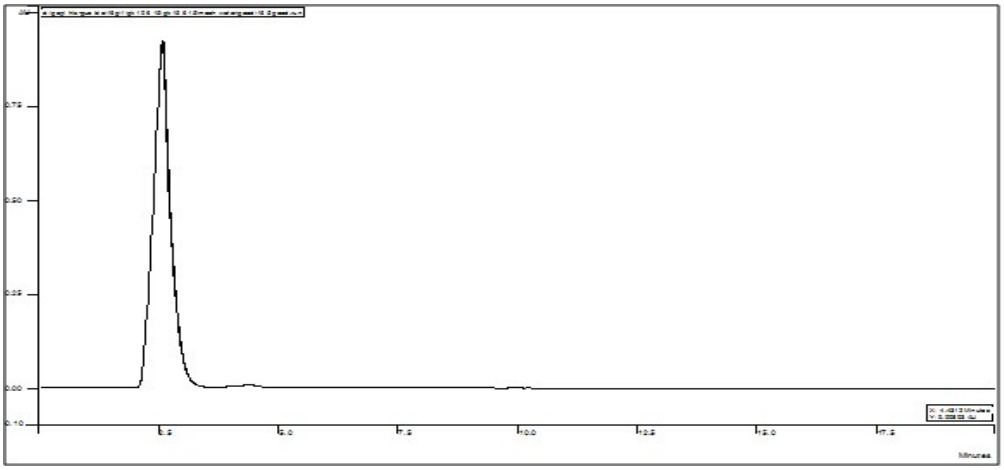


Fig. S9. Representative HPLC Chromatograms for lipid **1** using pure methanol as mobile phase (**A**) and using 95:5 methanol:water, v/v, as the mobile phase (**B**).

HPLC Conditions:
System: Varian Prostar series
Column: Lichrospher® 100, RP-18e (5 μm)
Mobile Phase: Methanol (**A**); Methanol:Water, 95:5, v/v, (**B**).
Flow Rate: 1.0 mL/min
Typical Column Pressure: 60-65 Bars
Detection: UV at 210 nm**A.**



B.

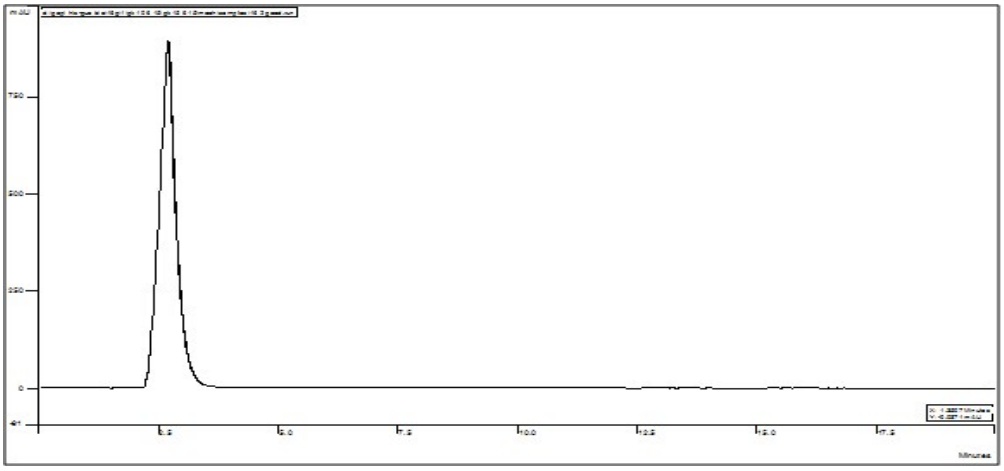
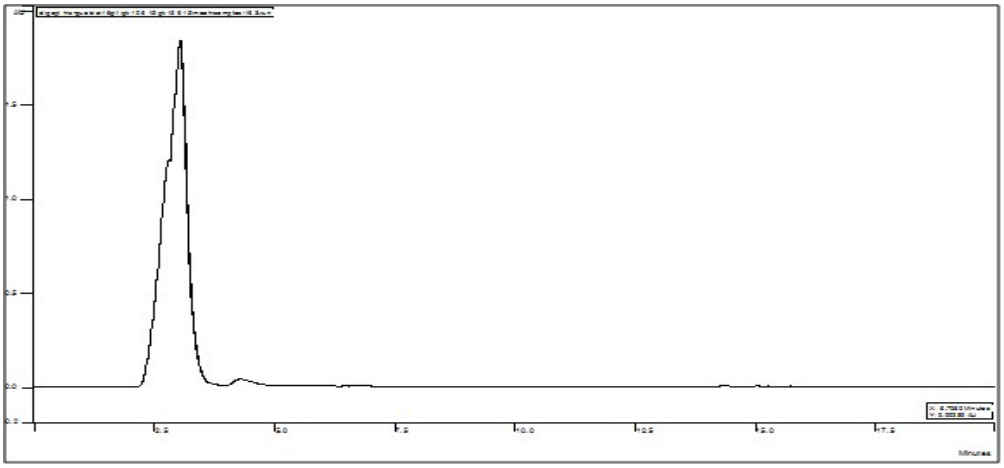


Fig. S10. Representative HPLC Chromatograms for lipid **2** using pure methanol as mobile phase (**A**) and using 95:5 methanol:water, v/v, as the mobile phase (**B**).

HPLC Conditions:
System: Varian Prostar series
Column: Lichrospher® 100, RP-18e (5 μm)
Mobile Phase: Methanol (**A**); Methanol:Water, 95:5, v/v, (**B**).
Flow Rate: 1.0 mL/min
Typical Column Pressure: 60-65 Bars
Detection: UV at 210 nm

A.



B.

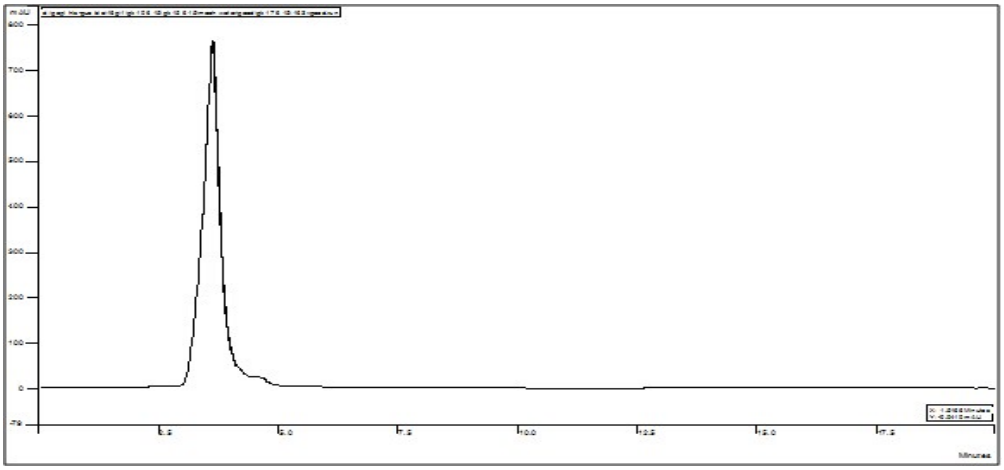
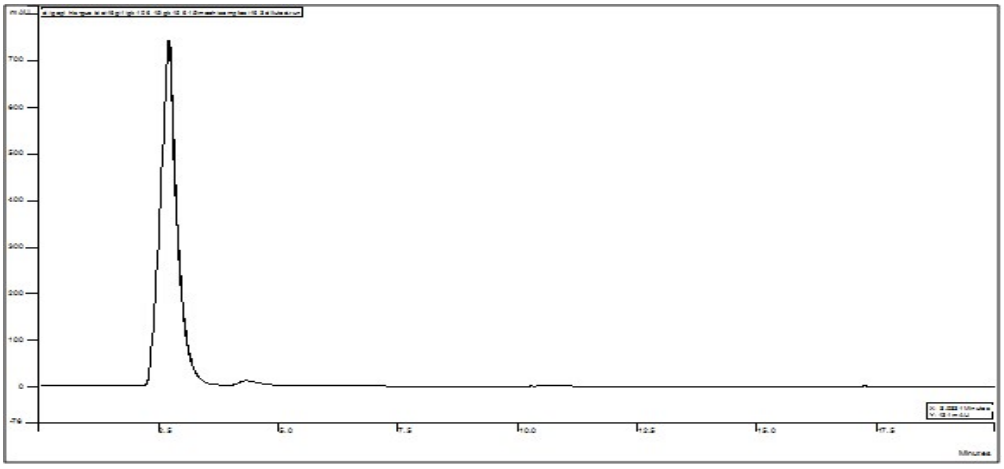


Fig. S11. Representative HPLC Chromatograms for lipid **3** using pure methanol as mobile phase (**A**) and using 95:5 methanol:water, v/v, as the mobile phase (**B**).

HPLC Conditions:
System: Varian Prostar series
Column: Lichrospher® 100, RP-18e (5 μm)
Mobile Phase: Methanol (**A**); Methanol:Water, 95:5, v/v, (**B**).
Flow Rate: 1.0 mL/min
Typical Column Pressure: 60-65 Bars
Detection: UV at 210 nm

A.



B.

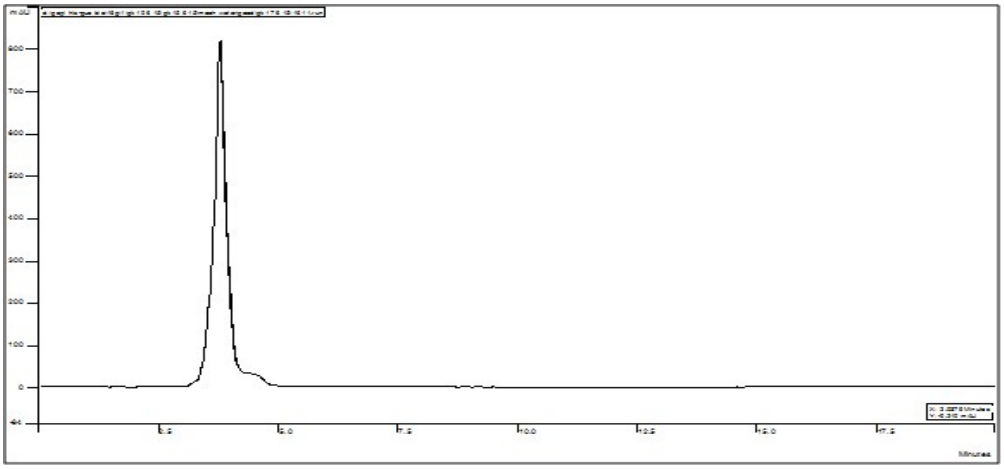


Fig. S12. Representative HPLC Chromatograms for lipid **4** using pure methanol as mobile phase (**A**) and using 95:5 methanol:water, v/v, as the mobile phase (**B**).

HPLC Conditions:
System: Varian Prostar series
Column: Lichrospher® 100, RP-18e (5 μm)
Mobile Phase: Methanol (**A**); Methanol:Water, 95:5, v/v, (**B**).
Flow Rate: 1.0 mL/min
Typical Column Pressure: 60-65 Bars
Detection: UV at 210 nm

Table S1. Size and zeta potentials of the liposomal formulations of lipids **1**-**4**.

Liposomal formulations of	Hydrodynamic Diameters (nm)	PDI	Zeta potentials (mv)
lipid **1**	167 ± 7	0.11 ± 0.02	3.1 ± 1.1
lipid **2**	145 ± 5	0.19 ± 0.03	4.5 ± 2.8
lipid **3**	157 ± 4	0.15 ± 0.01	4.6 ± 1.3
lipid **4**	171 ± 8	0.21 ± 0.03	5.6 ± 2.1

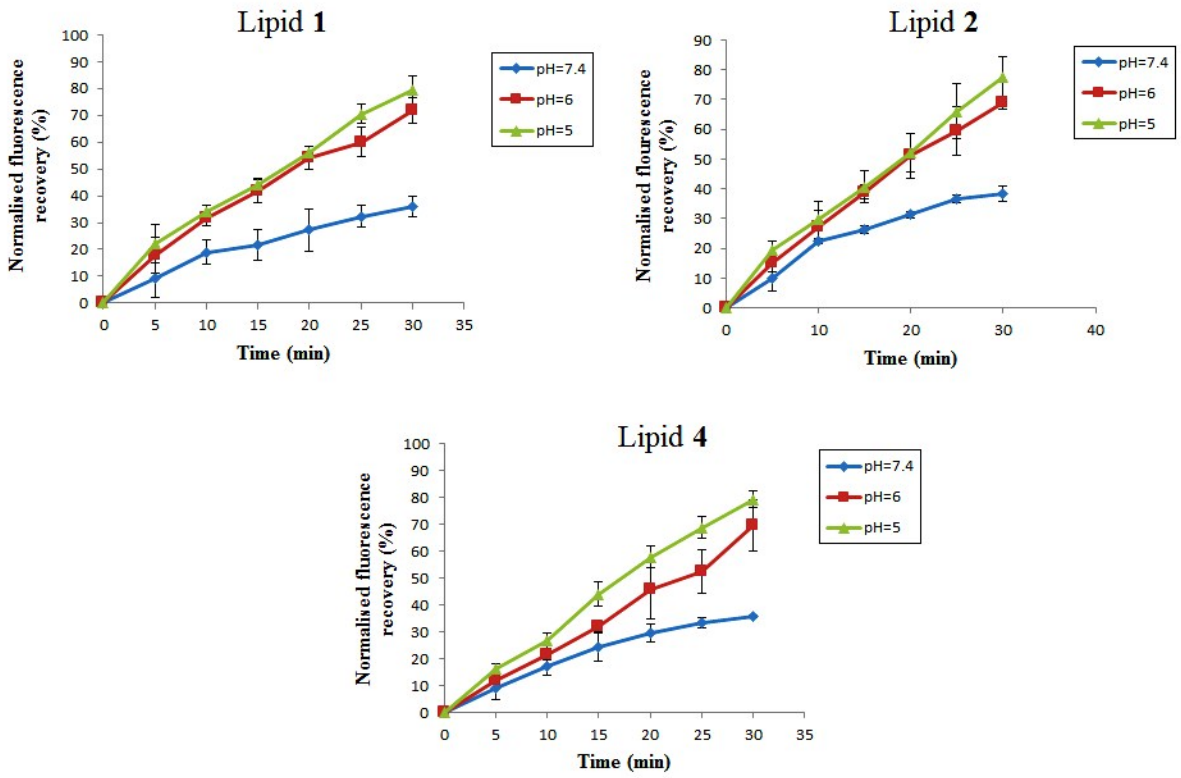


Fig. S13. The membrane fusion activity of liposomal formulations of lipids **1, 2** & **4**. Fusion versus pH. Fusion was induced by adding the biomembrane mimetic DOPC/DOPE/DOPS/Cholesterol/NBD-PE/Rho-PE liposomes (with 0.5 mM total lipid concentration) to liposomes of equimolar amount of liposomal formulations of lipids **1, 2** & **4** (with 0.5 mM total lipid concentration) in 0.5 mL 10 mM Hepes buffer at pH 7.4, pH 6.0, and pH 5.0).

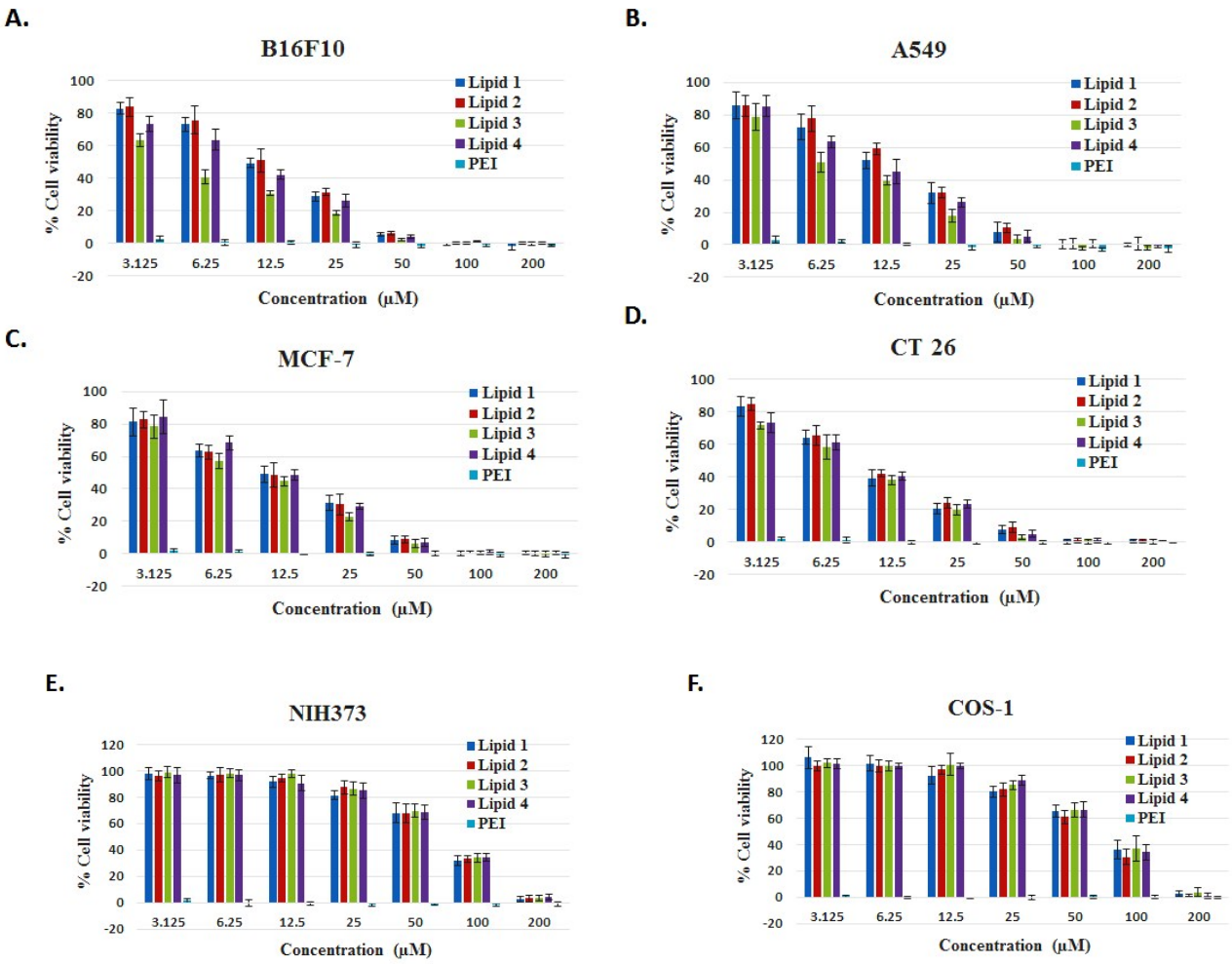


Fig. S14. Cytotoxicity profiles of the liposomal formulations of lipids **1-4**. Cells were treated with liposomal formulations of lipids **1-4** at various concentrations as indicated, in DMEM/RPMI medium containing 10% FBS. Polyetheleneimine (PEI) was used as positive control. The MTT assays were done 24 h after treating the cells with the liposomes of lipids shown in the inset for each figure. The details of the experimental protocols are provided in the main text.

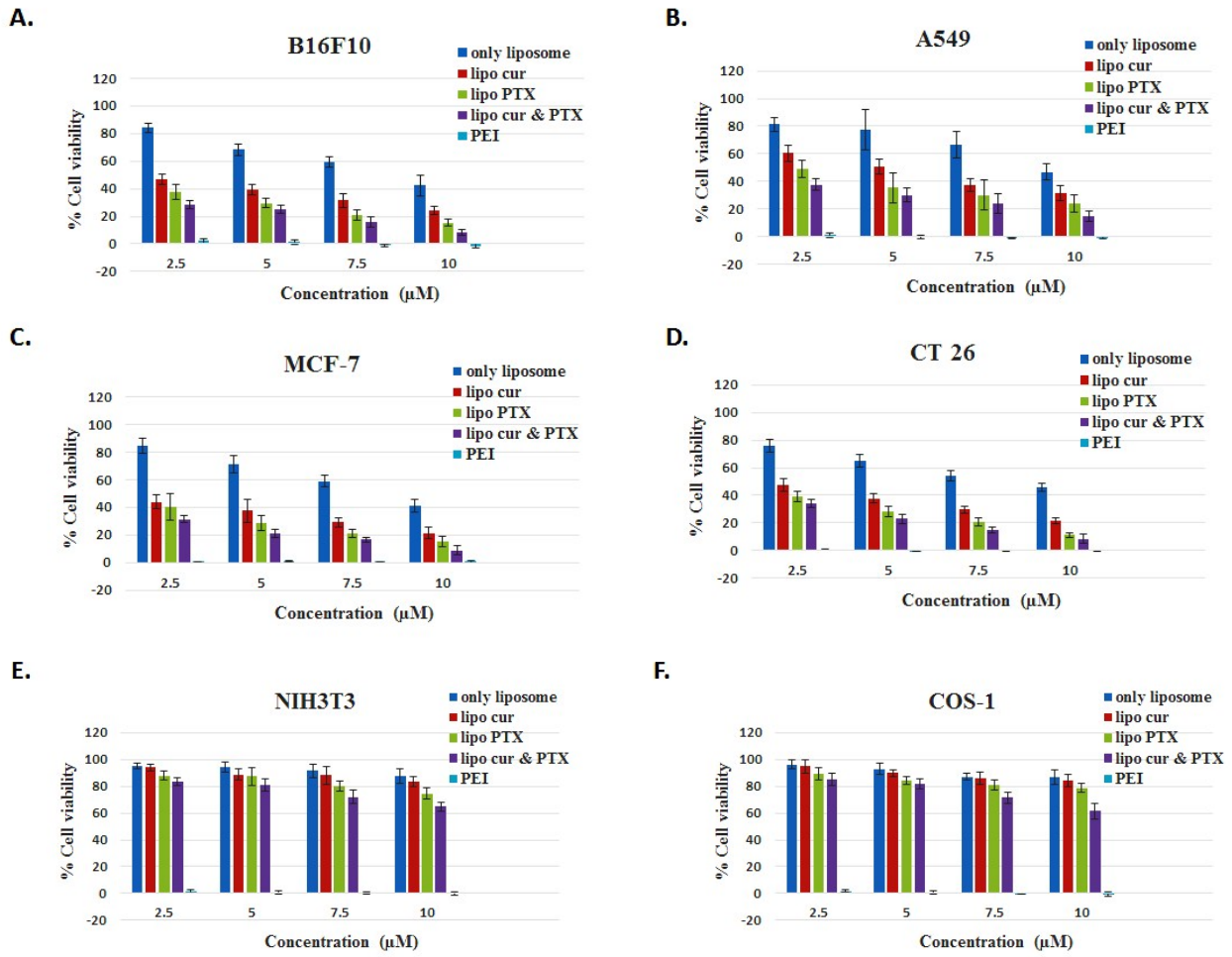


Fig. S15. Curcumin and PTX co-encapsulated liposomal formulations of lipid **3** show significant cytotoxicity in cancerous (A549, B16F10, MCF 7 & CT26) cells but not in non-cancerous cells (COS-1 & NIH3T3). Cells were treated with liposome containing: cationic lipid (2.5µM, 5 µM, 7.5 µM, 10 µM) cationic lipid (2.5µM, 5 µM, 7.5 µM, 10 µM) & curcumin (2.5µM, 5 µM, 7.5 µM, 10 µM), cationic lipid (2.5µM, 5 µM, 7.5 µM, 10 µM) & PTX (2.5nM, 5 nM, 7.5 nM, 10 nM) and cationic lipid (2.5µM, 5 µM, 7.5 µM, 10 µM), PTX (1.25 nM, 2.5 nM, 3.75 nM, 5 nM) & curcumin (1.25 µM, 2.5 µM, 3.75 µM, 5 µM) for 24 h. Cellular cytotoxicities of liposomally formulated drugs were measured by MTT assay. Polyetheleneimine (PEI) was used as positive control.

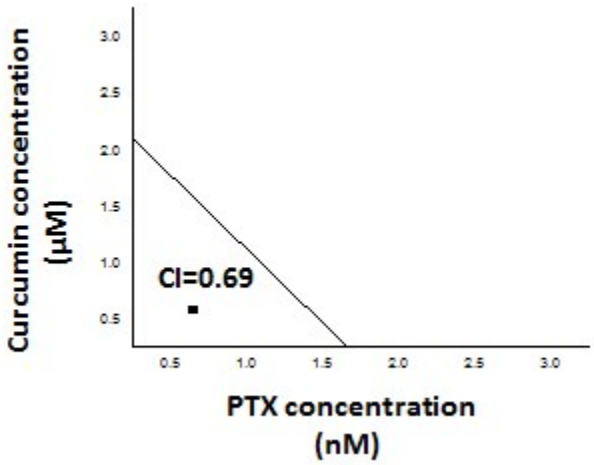


Fig S16. Isobologram for liposomal formulation of curcumin and paclitaxel (PTX) in B16F10 cells. First, the individual doses of liposomal PTX and liposomal curcumin required to achieve 50% (straight line) growth inhibition were plotted on the x- and y-axes, respectively. The concentrations of lipid **3** used in both these formulations were kept constant at 2.5 μM. Combination index (CI) value of 0.69 (calculated using origin software) is shown by the black square in the graph.

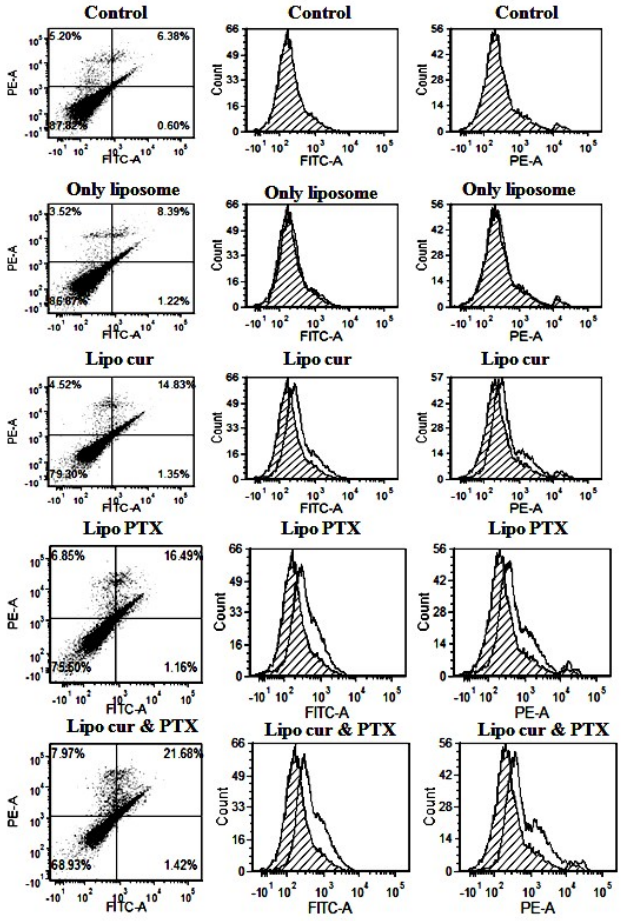


Fig. S17. Liposomal curcumin & PTX does not induce apoptosis in NIH3T3 cells. NIH3T3 cells were treated with liposome containing: cationic lipid (10 μM), cationic lipid (10 μM) & curcumin (7 μM), cationic lipid (10 μM) & PTX (10 nM) and cationic lipid (10 μM), curcumin (3.5 μM) & PTX (5 nM). The experimental details are as provided in the main text.