Supporting Information

Sterol-Modified Phospholipids: Cholesterol and Phospholipid Chimeras with Improved Biomembrane Properties

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Figure 10. Comparison of ^{31}P-NMR spectra of liposomes used in cholesterol exchange experiment. Upper: spectrum of the original liposome preparation; lower: spectrum of liposomes after the addition of shifting reagent 1 mM $Pr(NO_3)_3$. (A): MChcPC-DMPC-DMPG (5/4/1), (B): DMPC-Cholesterol-DMPG (5/4/1). The peak area ratio of the out /inside layer is about 1/1 indicating the unilamellarity of the liposome samples.

Synthesis of SMLs

*1-O-Trityl-2-palmitylcarbamoyl-sn-glycero-3-phosphocholine (**8b**):* This compound was synthesized according to the same procedure of **8a** and used directly for next step reaction.

*1-O-Trityl-2-myristylcarbamoyl-sn-glycero-3-phosphocholine (**8c**):* This compound was synthesized according to the same procedure of **8a** and used directly for next step reaction.

*1-Hydroxy-2-palmitylcarbamoyl-sn-glycero-3-phosphocholine (**9b**):* This compound was synthesized according to the same procedure of **9a**. TLC: R_f = 0.05 (eluent B). ^{1}H NMR ($CDCl_3$), δ 0.89 (t, J = 6.4, 3H); 1.21-1.31 (br, 26H); 1.48 (m, 2H); 3.03 (m, 1H); 3.11 (m, 1H); 3.30 (s, 9H); 3.66-3.76 (m, 4H); 4.0 (m, 2H); 4.30 (m, 2H); 4.79 (m, 1H); 6.52 (br, 1H). MALDI-MS calcd for $C_{25}H_{54}N_2O_7P^+$ $[M + H]^+$ 525.37, found 525.28.

*1-Hydroxy-2-myristylcarbamoyl-sn-glycero-3-phosphocholine (**9c**):* This compound was synthesized according to the same procedure of **9a**. TLC: R_f = 0.05 (eluent B). ^{1}H NMR ($CDCl_3$), δ 0.89 (t, J = 6.4, 3H); 1.21-1.31 (m, 22H); 1.47 (m, 2H); 3.03 (m, 1H); 3.11 (m, 1H); 3.29 (s, 9H); 3.66-3.76 (m, 4H); 3.99 (m, 2H); 4.30 (m, 2H); 4.78 (m, 1H); 6.52 (br, 1H). MALDI-MS calcd for $C_{23}H_{50}N_2O_7P^+$ $[M + H]^+$ 497.34, found 497.36.

*1-Cholesterycarbonoyl-2-palmitylcarbamoyl-sn-glycero-3-phosphocholine (**1b, Ch_cP_aPC**):* This compound was synthesized according to the same procedure of **1a**. TLC: R_f = 0.23 (eluent A). ^{1}H NMR ($CDCl_3$), δ0.69 (s, 3H); 0.85-1.65 (m, 64H); 1.78-2.01 (m, 5H); 2.37 (m, 2H); 3.0 (m, 1H); 3.16 (m, 1H); 3.37 (s, 9H); 3.88 (m, 2H); 3.98 (m, 2H); 4.24 (m, 1H); 4.36 (m, 4H); 5.07 (m, 1H); 5.39 (1H, d, J = 4.4); 6.17 (br, 1H). MALDI-MS calcd for $C_{53}H_{98}N_2O_9P^+$ $[M + H]^+$ 937.70, found 937.69.

*1-Cholesterylcarbonoyl-2-myristylcarbamoyl-sn-glycero-3-phosphocholine (**1c, Ch_cM_aPC**):* This compound was synthesized according to the same procedure of **1a**. TLC:

R_f = 0.25 (eluent A). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.78-2.06 (m, 5H); 2.38 (m, 2H); 3.03 (m, 1H); 3.17 (m, 1H); 3.38 (s, 9H); 3.88 (m, 2H); 4.0 (m, 2H); 4.25 (m, 1H); 4.36 (m, 4H); 5.08 (m, 1H); 5.40 (1H, d, J = 4.4); 6.02 (br, 1H). MALDI-MS calcd for $C_{51}H_{94}N_2O_9P^+$ $[M + H]^+$ 909.67, found 909.70.

*1,3-Benzylidene-2-palmityl-glycerol (**10b**):* This compound was synthesized according to the same procedure of **10a**.

*1,3-Benzylidene-2-myristyl-glycerol (**10c**):* This compound was synthesized according to the same procedure of **10a**.

*1,3-Benzylidene-2-oleyl-glycerol (**10d**):* This compound was synthesized according to the same procedure of **10a**.

*2-Palmityl-glycerol (**11b**):* This compound was synthesized according to the same procedure of **11a**. TLC: R_f = 0.16 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.86 (t, J = 6.4, 3H); 1.29 (br, 26H); 1.57 (m, 2H); 3.44-3.78 (m, 7H). MALDI-MS calcd for $C_{19}H_{41}O_3^+$ $[M + H]^+$ 317.31, found 317.28.

*2-Myristyl-glycerol (**11c**):* This compound was synthesized according to the same procedure of **11a**. TLC: R_f = 0.18 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 6.4, 3H); 1.29 (br, 22H); 1.58 (m, 2H); 3.44-3.78 (m, 7H). MALDI-MS calcd for $C_{17}H_{37}O_3^+$ $[M + H]^+$ 289.28, found 289.26

*2-Oleyl-glycerol (**11d**):* This compound was synthesized according to the same procedure of **11a**. TLC: R_f = 0.17 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 6.4, 3H); 1.29 (br, 22H); 1.58 (m, 2H); 2.0 (m, 4H); 3.44-3.78 (m, 7H); 5.34 (m, 2H). MALDI-MS calcd for $C_{21}H_{43}O_3^+$ $[M + H]^+$. 343.32, found 343.32.

*1-Cholesterycarbonoyl-2-palmityl-glycerol (**12b**):* This compound was synthesized according to the same procedure of **12a**. TLC: R_f = 0.39 (eluent E). ^{1}H NMR ($CDCl_3$), δ

0.69 (s, 3H); 0.85-1.65 (m, 64H); 1.79-2.01 (m, 5H); 2.39 (m, 2H); 2.80 (m, 1H); 3.51 (m, 2H); 3.61 (m, 2H); 3.72 (m, 1H); 4.21 (m, 2H); 5.39 (1H, d, J = 4.4); MALDI-MS calcd for $C_{47}H_{85}O_5^+$ $[M + H]^+$ 729.64, found 729.61.

*1-Cholesterycarbonoyl-2-myristyl-glycerol (**12c**):* This compound was synthesized according to the same procedure of **12a**. TLC: R_f = 0.40 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.78-2.02 (m, 5H); 2.38 (m, 2H); 2.82 (m, 1H); 3.51 (m, 2H); 3.60 (m, 2H); 3.72 (m, 1H); 4.22 (m, 2H); 5.41 (1H, d, J = 4.4); MALDI-MS calcd for $C_{45}H_{81}O_5^+$ $[M + H]^+$ 711.69, found 711.68.

*1-Cholesterylcarbonoyl-2-oleyl-glycerol (**12d**):* This compound was synthesized according to the same procedure of **12a**. TLC: R_f = 0.40 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.78-2.01 (m, 9H); 2.38 (m, 2H); 3.51 (m, 2H); 3.60 (m, 2H); 3.71 (m, 1H); 4.22 (m, 2H); 4.46 (m, 1H); 5.35 (m, 2H); 5.40 (d, J = 4.4, 1H); MALDI-MS calcd for $C_{49}H_{87}O_5^+$ $[M + H]^+$ 755.66, found 755.62.

*1-Cholesterylcarbonoyl-2-palmityl-rac-glycero-3-phosphate (**13b**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A). MALDI-MS calcd for $C_{47}H_{85}NaO_8P^+$ $[M + Na]^+$ 831.59, found 831.61.

*1-Cholesterylcarbonoyl-2-myristyl-rac-glycero-3-phosphate (**13c**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A). MALDI-MS calcd for $C_{45}H_{81}NaO_8P^+$ $[M + Na]^+$ 803.56, found 803.55.

*1-Cholesterylcarbonoyl-2-oleyl-rac-glycero-3-phosphate (**13d**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A). MALDI-MS calcd for $C_{49}H_{87}NaO_8P^+$ $[M + Na]^+$ 857.60, found 857.62.

*1-Cholesterylcarbonoyl-2-palmityl-rac-glycero-3-phosphocholine (**2b, Ch_cP_ePC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.3 (eluent A). δ 0.69 (s, 3H); 0.85-1.65 (m, 64H); 1.78-2.04 (m,

5H); 2.38 (m, 2H); 3.41 (s, 9H); 3.52 (m, 2H); 3.68 (m, 1H); 3.88 (m, 4H); 4.19 (m, 1H); 4.35 (m, 4H); 5.39 (1H, d, J = 4.4); MALDI-MS calcd for $C_{52}H_{97}NO_8P^+$ $[M + H]^+$ 894.69, found 894.70.

*1-Cholesterylcarbonoyl-2-myristyl-rac-glycero-3-phosphocholine (**2c, Ch_cM_ePC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.3 (eluent A). δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.78-2.04 (m, 5H); 2.38 (m, 2H); 3.41 (s, 9H); 3.52 (m, 2H); 3.68 (m, 1H); 3.88 (m, 4H); 4.19 (m, 1H); 4.35 (m, 4H); 5.39 (1H, d, J = 4.4); MALDI-MS calcd for $C_{52}H_{97}NO_8P^+$ $[M + H]^+$ 866.66, found 866.67.

*1-Cholesterylcarbonoyl-2-oleyl-rac-glycero-3-phosphocholine (**2d, Ch_cO_ePC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.3 (eluent A). δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.78-2.04 (m, 9H); 2.39 (m, 2H); 3.40 (s, 9H); 3.54 (m, 2H); 3.70 (m, 1H); 3.84-3.97 (m, 4H); 4.20 (m, 1H); 4.35-4.44 (m, 4H); 5.35 (m, 2H); 5.39 (1H, d, J = 4.4); MALDI-MS calcd for $C_{54}H_{99}NO_8P^+$ $[M + H]^+$ 920.71, found 920.73.

*1-Cholesteryl-2-palmitoyl-3-trityl glycerol (**17b**):* This compound was synthesized according to the same procedure of **17a**. TLC: TLC: R_f = 0.5 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.68 (s, 3H); 0.85-1.65 (m, 62H); 1.80 (m, 3H); 1.99 (m, 2H); 2.10 (m, 1H); 2.27 (m, 1H); 2.35 (t, J = 7.2, 2H); 3.12 (m, 1H); 3.25 (m, 2H); 3.66 (m, 2H); 5.16 (m, 1H); 5.32 (d, J = 4.4, 1H); 7.26 (m, 9H); 7.42 (m, 6H). MALDI-MS calcd for $C_{65}H_{97}O_4^+$ $[M + H]^+$ 941.74, found 941.72.

*1-Cholesteryl-2-myristoyl-3-trityl glycerol (**17c**):* This compound was synthesized according to the same procedure of **17a**. TLC: R_f = 0.5 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 58H); 1.81 (m, 3H); 2.0 (m, 2H); 2.19 (m, 1H); 2.26 (m, 1H); 2.35 (t, J = 7.2, 2H); 3.12 (m, 1H); 3.25 (m, 2H); 3.66 (m, 2H); 5.17 (m, 1H); 5.32 (d, J = 4.4, 1H); 7.27 (m, 9H); 7.44 (m, 6H). MALDI-MS calcd for $C_{63}H_{93}O_4^+$ $[M + H]^+$ 913.71, found 913.71.

*1-Cholesteryl-2-oleoyl-3-trityl glycerol (**17d**):* This compound was synthesized according to the same procedure of **17a**. TLC: R_f = 0.5 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 58H); 1.81 (m, 3H); 2.01 (m, 6H); 2.11 (m, 1H); 2.27 (m, 1H); 2.37 (t, J = 7.2, 2H); 3.13 (m, 1H); 3.24 (m, 2H); 3.66 (m, 2H); 5.17 (m, 1H); 5.35 (m, 3H); 7.28 (m, 9H); 7.45 (m, 6H). MALDI-MS calcd for $C_{67}H_{99}O_4^+$ $[M + H]^+$ 967.75, found 967.74.

*1-Cholesteryl-2-palmitoyl glycerol (**18b**):* The removal of trityl group was carried out according to the general procedure. The crude product was used directly for next step reaction. TLC: R_f = 0.08 (eluent E).

*1-Cholesteryl-2-myristoyl glycerol (**18c**):* The removal of trityl group was carried out according to the general procedure. The crude product was used directly for next step reaction. TLC: R_f = 0.08 in hexane/EtOAc (10/1).

*1-Cholesteryl-2-oleoyl glycerol (**18d**):* The removal of trityl group was carried out according to the general procedure. The crude product was used directly for next step reaction. TLC: R_f = 0.08 (eluent E).

*1-Cholesteryl-2-palmitoyl-rac-glycero-3-phosphate (**19b**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A).

*1-Cholesteryl-2-myristoyl-rac-glycero-3-phosphate (**19c**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A).

*1-Cholesteryl-2-oleoyl-rac-glycero-3-phosphate (**19d**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.05 (eluent A).

*1-Cholesteryl-2-palmitoyl-rac-glycero-3-phosphocholine (**3b, Ch_ePPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.31 (eluent A). ^{1}H NMR ($CDCl_3$), δ0.68 (s, 3H); 0.85-1.65 (m, 62H); 1.83 (m, 3H); 1.99 (m, 2H); 2.11 (m, 1H); 2.29 (m, 3H); 3.14 (m, 1H); 3.38 (s, 9H); 3.64 (m, 2H); 3.82 (m, 2H); 4.24-4.44 (m, 5H); 5.32 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{51}H_{95}NO_7P^+$ $[M + H]^+$ 864.68, found 864.70.

*1-Cholesteryl-2-myristoyl-rac-glycero-3-phosphocholine (**3c, Ch_eMPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.31 (eluent A). ^{1}H NMR ($CDCl_3$), δ0.68 (s, 3H); 0.85-1.65 (m, 58H); 1.83 (m, 3H); 1.99 (m, 2H); 2.12 (m, 1H); 2.30 (m, 3H); 3.14 (m, 1H); 3.39 (s, 9H); 3.62 (m, 2H); 3.83 (m, 2H); 4.22-4.42 (m, 5H); 5.33 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{49}H_{91}NO_7P^+$ $[M + H]^+$ 836.65, found 836.65.

*1-Cholesteryl-2-oleoyl-rac-glycero-3-phosphocholine (**3d, Ch_eOPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.30 (eluent A). ^{1}H NMR ($CDCl_3$), δ0.68 (s, 3H); 0.85-1.65 (m, 58H); 1.83 (m, 3H); 2.0 (m, 6H); 2.12 (m, 1H); 2.29 (m, 3H); 3.13 (m, 1H); 3.36 (s, 9H); 3.60 (m, 2H); 3.82 (m, 2H); 4.25-4.45 (m, 5H); 5.32 (m, 3H). MALDI-MS calcd for $C_{53}H_{97}NO_7P^+$ $[M + H]^+$ 890.70, found 890.67.

*1-Palmityl-2,3-isopropylidene glycerol (**20b**):* To a suspension of NaH (9 g, 0.225 mol) in 50 mL anhydrous toluene, was added solketal (19.8 g, 0.15 mol) dropwise. After the addition, the reaction mixture was stirred at 120 ºC for 15 min. Then 1-bromotetradecane (40 mL, 0.134 mol) was added to the mixture and the reaction was kept at 120 ºC overnight. After the reaction mixture was cooled to r.t., water (400 mL) was carefully added to destroy the excessive NaH, followed by the addition of hexane (400 mL). The organic layer was then washed with water (200 mL × 2), dried over sodium sulfate, filtered, and evaporated. The residue was used directly for next step reaction.

*1-Myristyl-2,3-isopropylidene glycerol (**20c**):* This compound was synthesized according to the same procedure of **20b**.

*1-Oleyl-2,3-isopropylidene glycerol (**20d**):* This compound was synthesized according to the same procedure of **20b**.

*1-Palmityl glycerol (**21b**):* The crude product **20b** was dissolved in 200 mL methanol and 40 mL conc. HCl and refluxed for 1.5 h. Then the mixture was cooled to r.t. and placed at 4 ºC overnight. The crystal was collected and recrystallized from methanol. TLC: R_f = 0.1 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 7.2, 3H); 1.27 (br, 26H); 1.53 (m, 2H); 2.52 (br, 2H); 3.30-3.90 (m, 7H). MALDI-MS calcd for $C_{19}H_{41}O_3^+$ $[M + H]^+$ 317.31, found 317.30.

*1-Myristyl glycerol (**21c**):* This compound was synthesized according to the same procedure of **21b**. TLC: R_f = 0.1 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 7.2, 3H); 1.27 (br, 22H); 1.52 (m, 2H); 2.50 (br, 2H); 3.31-3.90 (m, 7H). MALDI-MS calcd for $C_{17}H_{37}O_3^+$ $[M + H]^+$ 289.28, found 289.25.

*1-Oleyl glycerol (**21d**):* This compound was synthesized according to the same procedure of **21b**, but purified by HPFC (30-70% ethyl acetate in hexane). TLC: R_f = 0.1 (eluent D). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 7.2, 3H); 1.27 (br, 22H); 1.57 (m, 2H); 2.0 (m, 4H); 2.62 (br, 2H); 3.36-3.54 (m, 4H); 3.68 (m, 2H); 3.85 (m, 1H); 5.34 (m, 2H). MALDI-MS calcd for $C_{21}H_{43}O_3^+$ $[M + H]^+$ 343.32, found 343.36.

*1-Palmityl-3-trityl glycerol (**22b**):* The 3-trityl group was introduced according to the general procedure. TLC: R_f = 0.11 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 7.2, 3H); 1.27 (br, 26H); 1.55 (m, 2H); 2.42 (br, 1H); 3.18 (m, 2H); 3.32-3.53 (m, 4H). 3.94 (m, 1H); 7.22 (m, 9H); 7.42 (m, 6H). MALDI-MS calcd for $C_{38}H_{55}O_3^+$ $[M + H]^+$ 559.42, found 559.40.

*1-Myristyl-3-trityl glycerol (**22c**):* The 3-trityl group was introduced according to the general procedure. TLC: R_f = 0.11 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.87 (t, J = 7.2, 3H); 1.27 (br, 22H); 1.55 (m, 2H); 2.41 (br, 1H); 3.14 (m, 2H); 3.34-3.53 (m, 4H). 3.94 (m, 1H); 7.27 (m, 9H); 7.44 (m, 6H). MALDI-MS calcd for $C_{36}H_{51}O_3^+$ $[M + H]^+$ 531.39, found 531.38.

*1-Oleyl-3-trityl glycerol (**22d**):* The 3-trityl group was introduced according to the general procedure. TLC: R_f = 0.11 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.88 (t, J = 7.2, 3H); 1.28 (br, 22H); 1.56 (m, 2H); 2.0 (m, 4H); 2.42 (br, 1H); 3.20 (m, 2H); 3.36-3.54 (m, 4H); 3.96 (m, 1H); 5.35 (m, 2H); 7.25 (m, 9H); 7.45 (m, 6H). MALDI-MS calcd for $C_{40}H_{57}O_3^+$ $[M + H]^+$ 585.43, found 585.44.

*1-Palmityl-2-cholesterylcarbonoyl-3-trityl glycerol (**23b**):* This compound was synthesized according to the same procedure of **23a**. TLC: R_f = 0.43 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 64H); 1.79-2.02 (m, 5H); 2.42 (m, 2H); 3.24 (m, 2H); 3.37 (m, 2H); 3.61 (m, 2H); 4.48 (m, 1H); 5.04 (m, 1H); 5.39 (d, J = 4.4, 1H); 7.27 (m, 9H); 7.43 (m, 6H). MALDI-MS calcd for $C_{66}H_{99}O_5^+$ $[M + H]^+$ 971.75, found 971.78.

*1-Myristyl-2-cholesterylcarbonoyl-3-trityl glycerol (**23c**):* This compound was synthesized according to the same procedure of **23a**. TLC: R_f = 0.43 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.79-2.02 (m, 5H); 2.42 (m, 2H); 3.23 (m, 2H); 3.37 (m, 2H); 3.61 (m, 2H); 4.48 (m, 1H); 5.04 (m, 1H); 5.39 (d, J = 4.4, 1H); 7.27 (m, 9H); 7.43 (m, 6H). MALDI-MS calcd for $C_{64}H_{95}O_5^+$ $[M + H]^+$ 943.72, found 943.71.

*1-Oleyl-2-cholesterylcarbonoyl-3-trityl glycerol (**23d**):* This compound was synthesized according to the same procedure of **23a**. TLC: R_f = 0.43 (eluent E). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 60H); 1.79-2.02 (m, 9H); 2.40 (m, 2H); 3.23 (m, 2H); 3.37 (m, 2H); 3.61 (m, 2H); 4.48 (m, 1H); 5.04 (m, 1H); 5.33-5.39 (m, 3H); 7.27 (m, 9H); 7.44 (m, 6H). MALDI-MS calcd for $C_{68}H_{101}O_5^+$ $[M + H]^+$ 997.77, found 997.74.

*1-Palmityl-2-cholesterylcarbonoyl glycerol (**24b**):* This compound was synthesized according to the general procedure of removal of trityl group. TLC: R_f = 0.63 (eluent D).

*1-Myristyl-2-cholesterylcarbonoyl glycerol (**24c**):* This compound was synthesized according to the general procedure of removal of trityl group. TLC: R_f = 0.63 (eluent D).

*1-Oleyl-2-cholesterylcarbonoyl glycerol (**24d**):* This compound was synthesized according to the general procedure of removal of trityl group. TLC: R_f = 0.63 (eluent D).

*1-Palmityl-2-cholesterylcarbonoyl-rac-glycero-3-phosphate (**25b**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.57 (eluent C).

*1-Myristyl-2-cholesterylcarbonoyl-rac-glycero-3-phosphate (**25c**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.57 (eluent C).

*1-Oleyl-2-cholesterylcarbonoyl-rac-glycero-3-phosphate (**25d**):* This compound was synthesized according to the general procedure of phosphorylation. TLC: R_f = 0.57 (eluent C).

*1-Palmityl-2-cholesterylcarbonoyl-rac-glycero-3-phosphocholine (**4b, P_eCh_cPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.53 (eluent A). ^{1}H NMR ($CDCl_3$), δ 0.68 (s, 3H); 0.85-1.65 (m, 62H); 1.84-2.05 (m, 5H); 2.36 (m, 2H); 3.38 (s, 9H); 3.45 (m, 2H); 3.61 (m, 2H); 3.82 (m, 2H); 4.02 (m, 2H); 4.35 (m, 2H); 4.44 (m, 1H); 4.98 (m, 1H); 5.39 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{52}H_{97}NO_8P^+$ $[M + H]^+$ 894.69, found 894.68.

*1-Myristyl-2-cholesterylcarbonoyl-rac-glycero-3-phosphocholine (**4c, M_eCh_cPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.53 (eluent A). ^{1}H NMR ($CDCl_3$), δ 0.68 (s, 3H); 0.85-1.65 (m,

58H); 1.84-2.05 (m, 5H); 2.37 (m, 2H); 3.39 (s, 9H); 3.44 (m, 2H); 3.61 (m, 2H); 3.83 (m, 2H); 4.02 (m, 2H); 4.35 (m, 2H); 4.44 (m, 1H); 4.98 (m, 1H); 5.39 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{50}H_{93}NO_8P^+$ $[M + H]^+$ 866.66, found 866.64.

*1-Oleyl-2-cholesterylcarbonyl-rac-glycero-3-phosphocholine (**4d, O_eCh_cPC**):* This compound was synthesized according to the general procedure of phosphocholine synthesis. TLC: R_f = 0.53 (eluent A). ^{1}H NMR ($CDCl_3$), δ 0.69 (s, 3H); 0.85-1.65 (m, 58H); 1.84-2.05 (m, 9H); 2.37 (m, 2H); 3.38 (s, 9H); 3.45 (m, 2H); 3.62 (m, 2H); 3.84 (m, 2H); 4.02 (m, 2H); 4.35 (m, 2H); 4.45 (m, 1H); 4.98 (m, 1H); 5.35-5.39 (m, 3H). MALDI-MS calcd for $C_{54}H_{99}NO_8P^+$ $[M + H]^+$ 920.71, found 920.71.

*1-Palmitoyl-2-cholesterylcarbonoyl-sn-glycero-3-phosphocholine (**5b, PCh_cPC**):* This compound was synthesized according to the same procedure of **5a**. TLC: R_f = 0.54 (eluent C). ^{1}H NMR ($CDCl_3$/MeOH-d_4/pyridine-d_5, 10:2:1), δ 0.68 (s, 3H); 0.85-1.65 (m, 62H); 1.84-2.05 (m, 5H); 2.31 (t, J = 7.6, 2H); 2.36 (m, 2H); 3.27 (s, 9H); 3.66 (m, 2H); 4.08 (m, 2H); 4.23 (m, 1H); 4.30 (m, 2H); 4.44 (m, 2H); 5.08 (m, 1H); 5.40 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{52}H_{95}NO_9P^+$ $[M + H]^+$ 908.67, found 908.67.

*1-Myristoyl-2-cholesterylcarbonoyl-sn-glycero-3-phosphocholine (**5c, MCh_cPC**):* This compound was synthesized according to the same procedure of **5a**. TLC: R_f = 0.54 (eluent C). ^{1}H NMR ($CDCl_3$/MeOH-d_4/pyridine-d_5, 10:2:1), δ 0.69 (s, 3H); 0.85-1.65 (m, 58H); 1.84-2.05 (m, 5H); 2.33 (t, J = 7.6, 2H); 2.39 (m, 2H); 3.27 (s, 9H); 3.67 (m, 2H); 4.09 (m, 2H); 4.24 (m, 1H); 4.31 (m, 2H); 4.45 (m, 2H); 5.09 (m, 1H); 5.40 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{52}H_{95}NO_9P^+$ $[M + H]^+$ 908.67, found 908.67.

*1-Oleoyl-2-cholesterylcarbonoyl-sn-glycero-3-phosphocholine (**5d, OCh_cPC**):* This compound was synthesized according to the same procedure of **5a**. TLC: R_f = 0.54 (eluent C). ^{1}H NMR ($CDCl_3$/MeOH-d_4/pyridine-d_5, 10:2:1), δ 0.69 (s, 3H); 0.85-1.65 (m, 58H); 1.84-2.06 (m, 9H); 2.33 (t, J = 7.6, 2H); 2.39 (m, 2H); 3.28 (s, 9H); 3.68 (m, 2H); 4.10 (m, 2H); 4.24 (m, 1H); 4.32 (m, 2H); 4.47 (m, 2H); 5.10 (m, 1H); 5.36 (m, 2H); 5.40 (d, J = 4.4, 1H). MALDI-MS calcd for $C_{54}H_{97}NO_9P^+$ $[M + H]^+$ 934.69, found 934.68.

Freeze-Fracture Electron Microscopy (FF-EM)

The FF-EM images were taken on a JEOL 100 CX electron microscope (Nanoanalytical Laboratory, San Francisco, CA). Sonicated PChcPC liposome sample (20 mM) was quenched using sandwich technique and liquid nitrogen-cooled propane. Using this technique a cooling rate of 10,000 Kelvin per second is reached avoiding ice crystal formation and artifacts possibly caused by the cryo-fixation process. The fracturing process was carried out in JEOL JED-9000 freeze etching equipment and the freshly exposed fracture planes were shadowed with Pt for 30 seconds in an angle of 25-35 degree and with carbon for 35 seconds (2 kV/60-70 mA, 1×10^{-5} Torr). The replicas produced this way were cleaned with concentrated, fuming HNO_3 for 24 hours followed by repeating agitation with fresh chloroform/methanol (1/1 by volume) at least 5 times. The cleaned replicas were then examined by the electron microscope. As shown in Figure 9A, the PChcPC vesicles displayed clearly convex and concave fracture planes (shadow behind and shadow in front of the structures respectively) which are characteristic for bilayer vesicles such as liposomes.

Negative Stain Transmission Electron Microscope (TEM)

For transmission electron microscopy(TEM), a liposome dispersion (ca. 5 mM) was deposited onto the sample grid, stained with 2% uranyl acetate for 10 minutes, dried with Whatman® filter paper, and observed with a Tecnai 12 transmission electron microscope (Electron Microscope Lab, Berkeley, CA). The doxorubicin loaded liposome (SeChcPC-PEGDSPE-αT, 94.8/5/0.2) has a average diameter around 100 nm (Fig. 9B).

^{31}P-NMR Spectra

NMR measurements were performed on a Bruker Avance III 300 spectrometer operating at 121 MHz for ^{31}P using a 5 mm PABBO probe. Spectra were acquired using a phase cycled Hahn echo with proton broad band decoupling (WALTZ16). Typical acquisition parameters were: 90° pulse length 10 μs; recycle delay 2 s; spectral width 400 ppm (49 kHz). Before Fourier transformation, the signal was exponentially multiplied with 10 Hz line broadening. Each spectrum was the average of 2-15 k scans. Sample concentration ranges from 35 mM to 50 mM with 1 mM $Pr(NO_3)_3$ as the shifting reagent.

Biodistribution Study of the Encapsulated Doxorubicin in Tumored Mice Using Fluorescence Detection.

Eleven days after tumor inoculation, when the tumors were approximately 5 mm wide, mice (3 per group) were injected via the tail vain with either 15 mg/kg of doxorubicin as Doxil™ or encapsulated in PChcPC/PEGDSPE/alpha-tocopherol: 94.8/5.0/0.2 mole ratio in ~200 μL of PBS. Two control mice received 200 μL of PBS. Blood was collected from the mice via the orbital sinus 6, and 24, after dosing; at 48 h, the group was sacrificed for tissue collection. Prior to euthanasia, mice were anesthetized with a ketamine-xylazine-acepromazine cocktail by intraperitoneal injection. For all mice, blood was collected by heart puncture, and the whole tumors were dissected and their weights recorded. Up to 300 mg of each tumor was placed in a 2 mL tube containing 1 mL of acidified alcohol (90% isopropanol / 0.075 M HCl) and zirconia beads. The tumor was homogenized by bead beating (Bead Beater, Biospec, Bartlesville, OK) at 5,000 rpm. The blood was allowed to coagulate at 4 ºC and then centrifuged for 10 min at 14,000 rpm. The serum (upper phase) was collected and its volume recorded. Up to 400 □L of

serum was transferred to a 2 mL tube containing 1 mL of acidified alcohol. The homogenized tumor samples and the sera were stored at 4 °C overnight to extract the drug. The samples were then centrifuged at 14,000 rpm for 5 min at 4 ºC. An aliquot (400 □L) of each supernatant was transferred to tubes containing 1.6 mL of acidified alcohol. Fluorescence emission from the drug was measured (Ex: 490 nm, Em: 590 nm) using a Spex Fluorolog photon counting instrument (Model FL1/2, Jobin Yvon, Edison, NJ) equipped with a 150 W xenon light source. A calibration curve was prepared for DOX using concentrations from 0.04 to 5 μg/mL in acidified alcohol. To subtract the background fluorescence from each tissue type, a calibration curve was prepared using serial dilutions of the tumor and sera extracts of the control mice.

Complete Ref. 27.

(1)Fahy, E.; Subramaniam, S.; Brown, H. A.; Glass, C. K.; Merrill, A. H.; Murphy, R. C.; Raetz, C. R. H.; Russell, D. W.; Seyama, Y.; Shaw, W.; Shimizu, T.; Spener, F.; van Meer, G.; VanNieuwenhze, M. S.; White, S. H.; Witztum, J. L.; Dennis, E. A. *J. Lipid Res.* **2005**, *46*, 839-861.

Table 1. The main transition[a] and enthalpy[b] of SML/diacylphosphocholine[c] liposomes
[a]Gel-to-liquid crystal transition, unit: ºC. [b]The total enthalpy of main transition and pretransition (not observed in most formulations containing cholesterol) , unit: Kcal/mol.
[c]Lipids containing the chain of the same length were mixed.

	Ch_cP_aPC		Ch_cP_ePC		Ch_ePPC		P_eCh_cPC		SCh_cPC		PCh_cPC		MCh_cPC		OCh_cPC	
%Chol	Tm	ΔH	Tm	ΔH	Tm	ΔH	Tm	ΔH	Tm	ΔH	Tm	ΔH	Tm	ΔH	Tm	ΔH
0	42	8.8	42	8.8	42	8.8	42	8.8	55.2	11.5	42	8.8	24.4	5.6	55.2	11.5
5	40.8	5.9	40.6	6.6	40.8	7.2	40.9	7.1	54.7	11.6	41.4	6.9	23.5	3.6	54.1	11.3
10	40	3.7	38.9	3.0	39.9	3.6	40.1	6.2	54.4	9.8	40.3	5.1	22.7	2.4	53.5	8.9
20	37.7	1.2	35.8	0.3	38.3	3.3	38.4	1.4	52.5	6.6	38.6	2.8	20.7	1.3	56.9	5.6
30	35.4	0.9			35	1.7	38.2	1.1	50	3.4	35.2	0.1			48.5	2.1
35					32	0.4			48.9	2.2					47.3	2.0
40															43.7	0.4

Table 2. Diameter of Liposomes Used in Cholesterol Exchange Experiments

Liposome	Diameter (nm)	Polydispersity
PChcPC/DPPC/DPPG	100	0.19
Chol/DPPC/DPPG	110	0.18
POPC	115	0.43
MChcPC/DMPC/DMPG	129	0.26
ChcMaPC/DMPC/DMPG	98	0.19
DMPC/Chol/DMPG	97	0.14

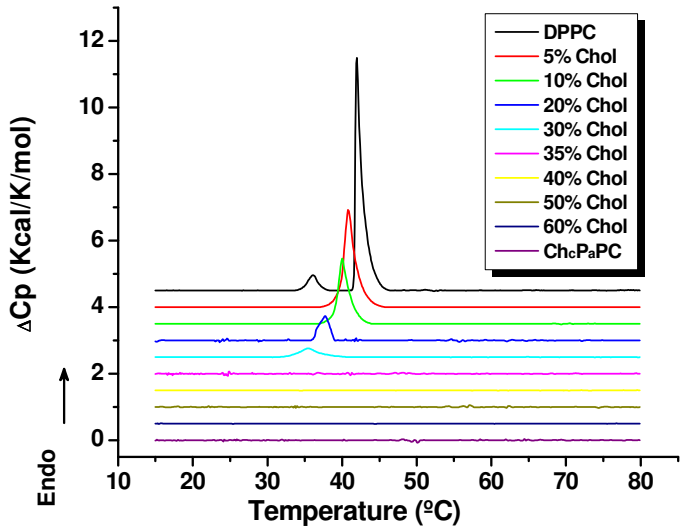
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
DPPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
ChcPaPC

Figure 1.

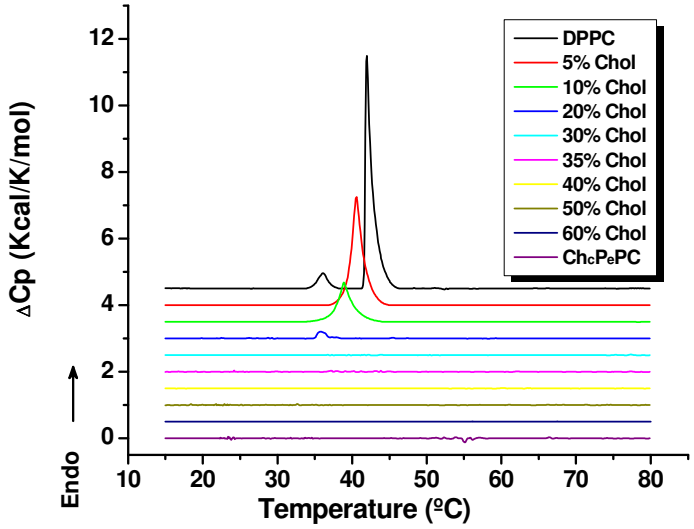
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
DPPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
ChcPePC

Figure 2.

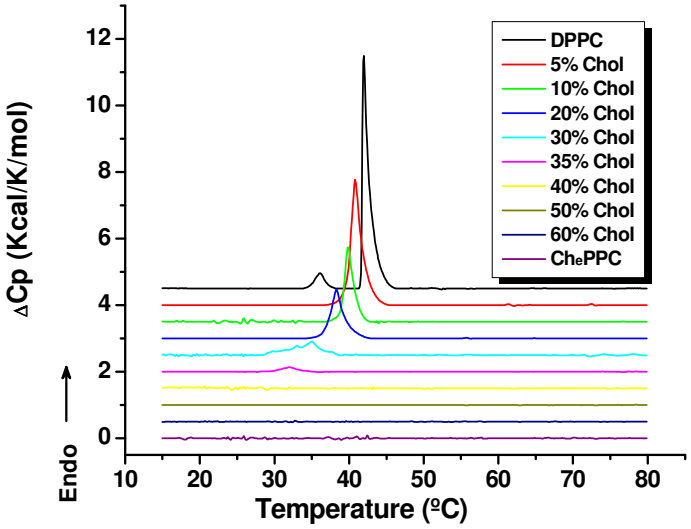
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
DPPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
ChePPC

Figure 3.

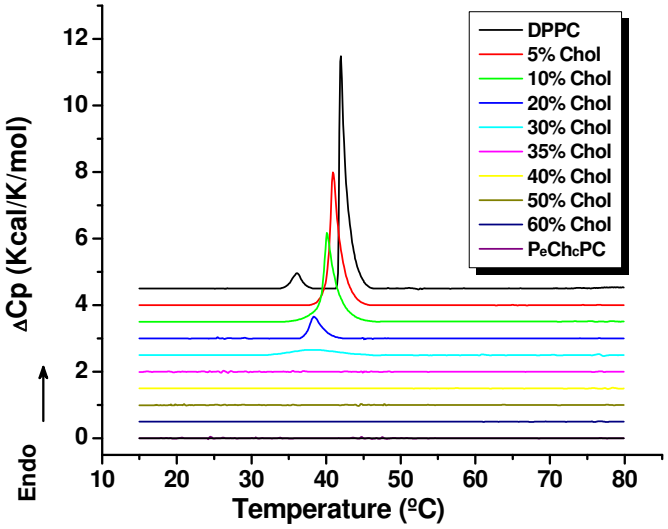
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
DPPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
PeChcPC

Figure 4.

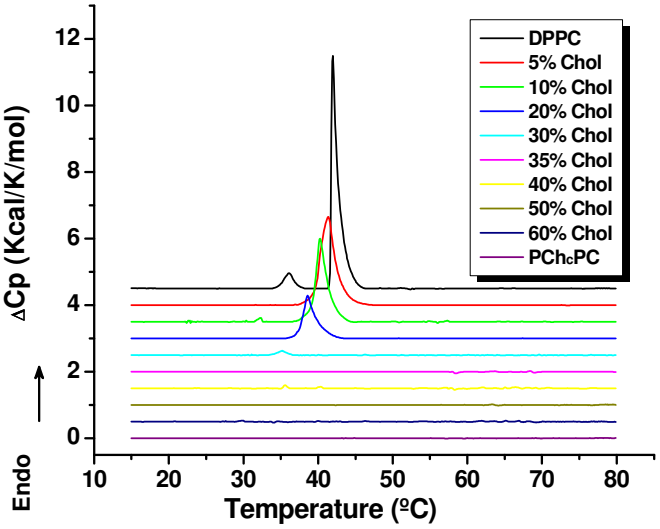
DPPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
PChcPC
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
0
2
4
6
8
10
12
10
20
30
40
50
60
70
80

Figure 5.

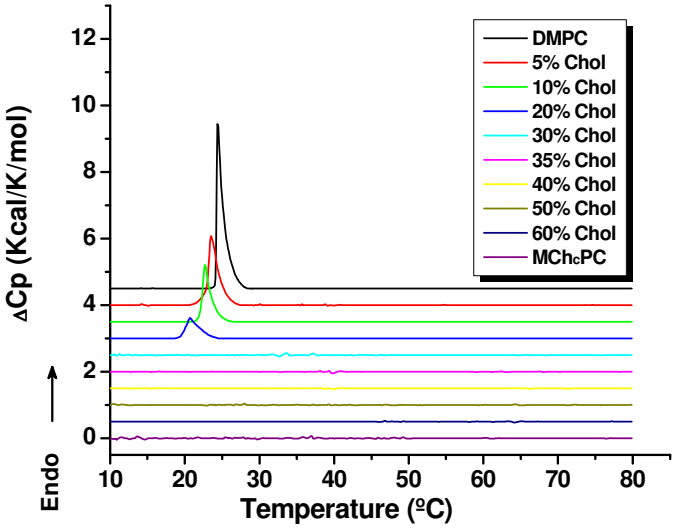
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
DMPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
MChcPC

Figure 6.

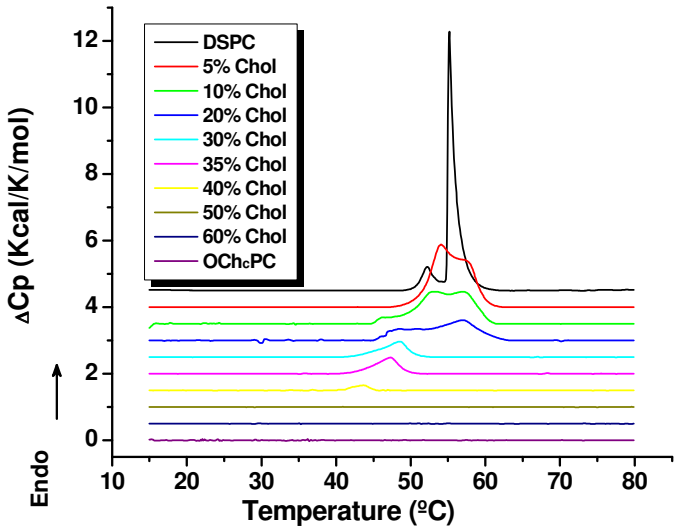
DSPC
5% Chol
10% Chol
20% Chol
30% Chol
35% Chol
40% Chol
50% Chol
60% Chol
OChcPC
ΔCp (Kcal/K/mol)
Endo
Temperature (ºC)
0
2
4
6
8
10
12
10
20
30
40
50
60
70
80

Figure 7.

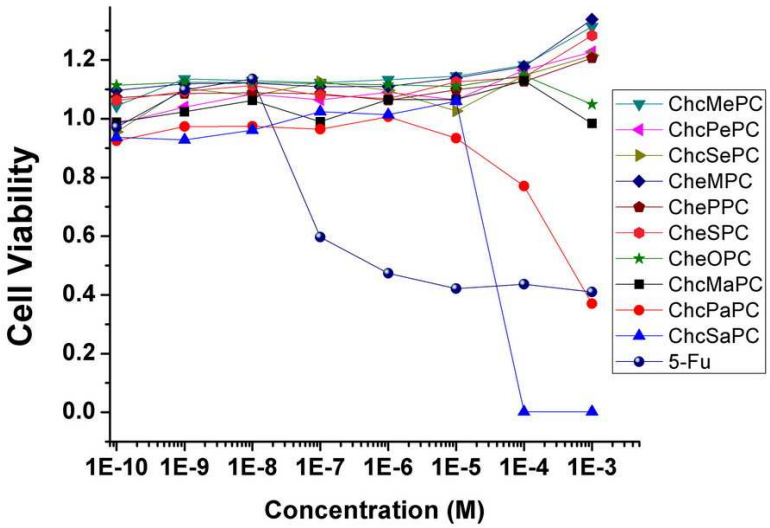
Cell Viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1E-10 1E-9 1E-8 1E-7 1E-6 1E-5 1E-4 1E-3
Concentration (M)
ChcMePC
ChcPePC
ChcSePC
CheMPC
ChePPC
CheSPC
CheOPC
ChcMaPC
ChcPaPC
ChcSaPC
5-Fu

Figure 8.

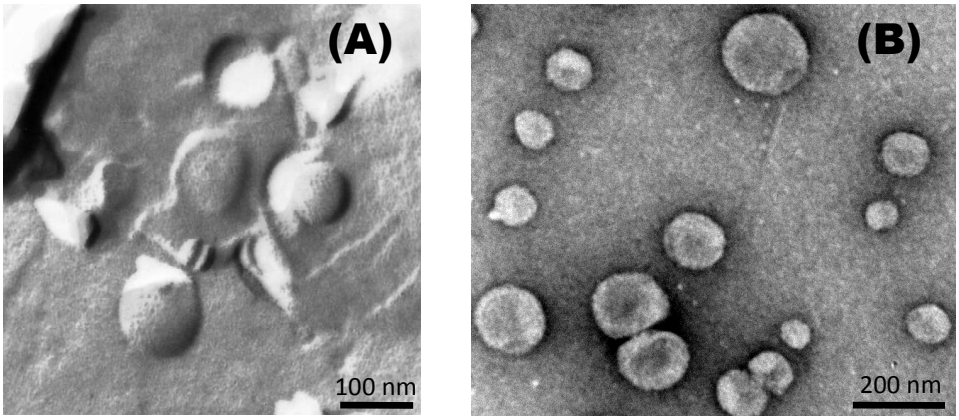

Figure 9.

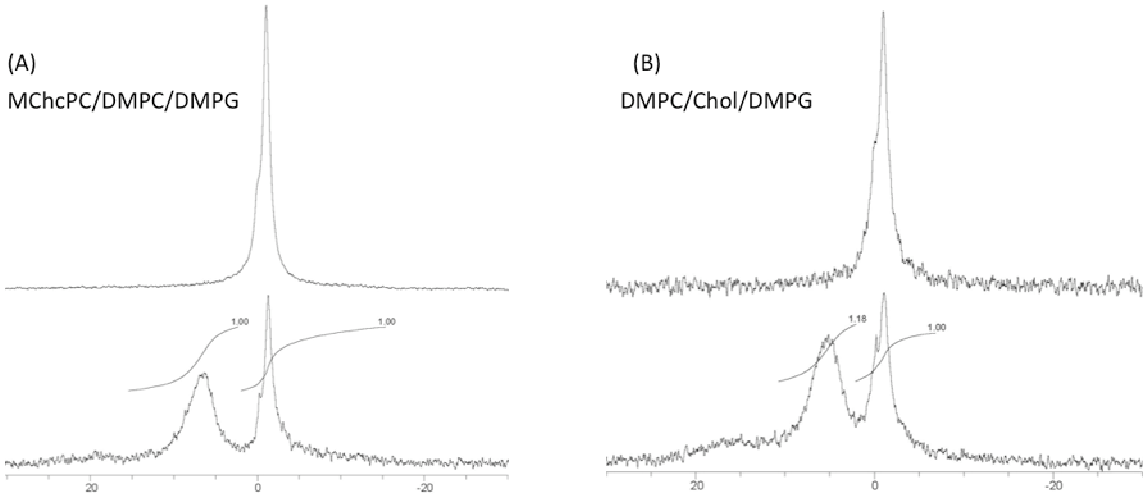
(A)
MChcPC/DMPC/DMPG
1.00
1.00
20
0
-20
(B)
DMPC/Chol/DMPG
1.18
1.00
20
0
-20

Figure 10.