Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 245–254

BIOORGANIC & MEDICINAL CHEMISTRY

Symmetrical Cationic Triglycerides: An Efficient Synthesis and Application to Gene Transfer

Satoshi Obika, Wei Yu, Atsuko Shimoyama, Takeshi Uneda, Kazuyuki Miyashita, Takefumi Doi and Takeshi Imanishi*

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Received 13 June 2000; accepted 25 August 2000

Abstract—Some cationic triglycerides **1Aa**–**1Cb** which have a symmetrical structure were effectively synthesized and formulated into cationic liposomes with the co-lipid dioleoylphosphatidylethanolamine (DOPE) and/or dilauroylphosphatidylcholine (DLPC). The plasmid encoding a luciferase was delivered into CHO cells by using these cationic liposomes. Our symmetrical cationic triglycerides showed high transfection activity when DOPE was used as a co-lipid. Among the symmetrical cationic triglycerides synthesized here, **1Ab** and **1Ac**, which have an oleoyl group at the 1- and 3-position in the glycerol backbone and also have a relatively long linker connecting the 2-hydroxy group in glycerol with the quaternary ammonium head group, were found to be the most suitable for gene delivery into cells. The transfection activity of the symmetrical cationic triglyceride **1Ab** was comparable with that of its asymmetrical congener **6** and several times higher than that of Lipofectin®.

Introduction

Genetic engineering, and molecular biology, gene therapy has recently received a considerable amount of attention owing to the rapid development of gene analytical techniques. One fundamental gene therapy technology is a gene delivery system. Among a large number of gene delivery systems, such as microinjection, electroporation, calcium phosphate precipitation, lipofection, and viral vector systems, the viral vector systems were thought to be one of the most practical transfection methods for in vivo applications.[1] Although successful viral vector-mediated gene transfers have been reported, there are serious safety concerns about the clinical use of these systems.[2]

On the other hand, one non-viral vector system, lipofection, has been the focus of much recent research for its safety, simple usage, and non-immunogenicity.[3] Since Felgner and co-workers reported the first example of efficient gene delivery into living cells with cationic liposomes (Lipofectin®) composed of a synthetic cationic lipid, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA),[4] numerous examples of such cationic liposome-mediated gene delivery have been reported.[5–11] As shown in Fig. 1, the great majority of the known cationic lipids for cationic liposome-mediated gene transfer have an asymmetric structure,[4,6–11] the same as natural phospholipids. The asymmetric synthesis of the triglyceride was very troublesome; therefore, many asymmetric cationic lipids were employed as a racemic mixture,[4,6,7,11] or some were synthesized via the enzymatic hydrolysis of a relatively expensive natural phospholipid.[7] A simple strategy to overcome these problems is to use a symmetrical cationic triglyceride instead of an asymmetric one; however, to our best knowledge, no application of cationic lipids with a symmetric structure has been reported to date, except for our previous results.[12–14] Here, we designed and synthesized several symmetrical cationic triglycerides **1** which were composed of biogenic materials, such as fatty acids, glycerin, and amino acid derivatives, to reduce the cytotoxicity of these cationic triglycerides. The transfection efficiency of the cationic liposomes composed of the symmetrical triglycerides **1** and natural phospholipids was also investigated.

Results and Discussion

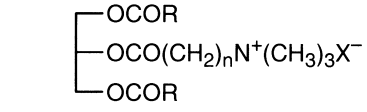
Synthesis of the symmetrical cationic triglycerides

The synthetic route for symmetrical cationic triglycerides **1Aa**–**1Cb** is illustrated in Scheme 1. According to

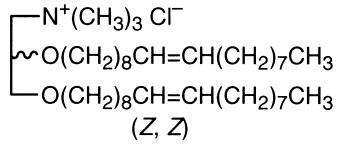
*Corresponding author. Tel.: +81-6-6879-8200; fax: +81-6-6879-8204; e-mail: imanishi@phs.osaka-u.ac.jp

0968-0896/01/$ - see front matter
PII: S0968-0896(00)00242-X

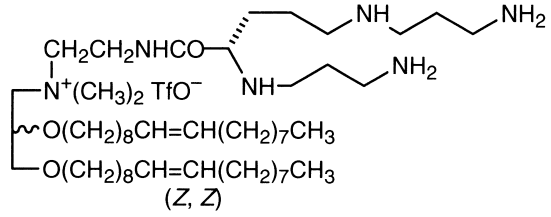
the literature,[15,16] dihydroxyacetone was coupled with fatty acids, such as oleic acid, elaidic acid and stearic acid, to afford the corresponding diesters **2A**–**2C**, respectively. The centered carbonyl group of **2A**–**2C** was reduced with $NaBH_4$[17] to give symmetrical alcohols **3A**–**3C** in 76–85% yields. In spite of the possibility for an acyl migration of the obtained alcohols **3A**–**3C**,[18] no asymmetrical product was observed in the ^1H and ^{13}C NMR spectra. The alcohols **3A**–**3C** were treated with chloroacetic anhydride or 4-chlorobutyryl chloride to give the corresponding triglycerides **4Aa**–**4Cb** in 76–95% yields. Moreover, another triglyceride **4Ac** was obtained in 87% yield by the reaction of **3A** with 5-chlorovaleryl chloride. Next, the chloroacetates **4Aa**,



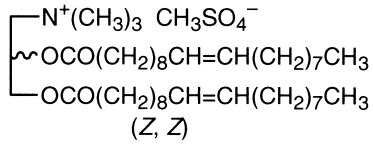
Symmetrical cationic triglycerides **1**



DOTMA

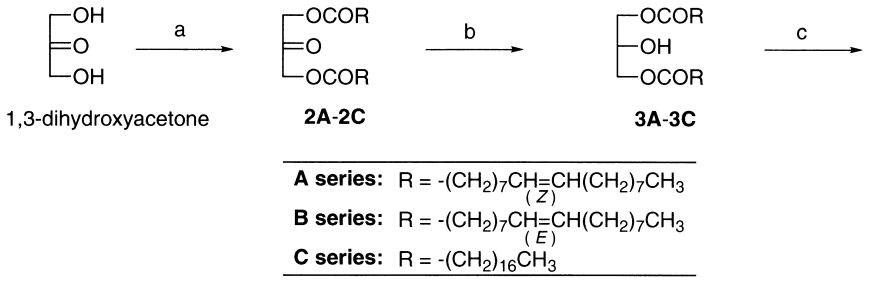


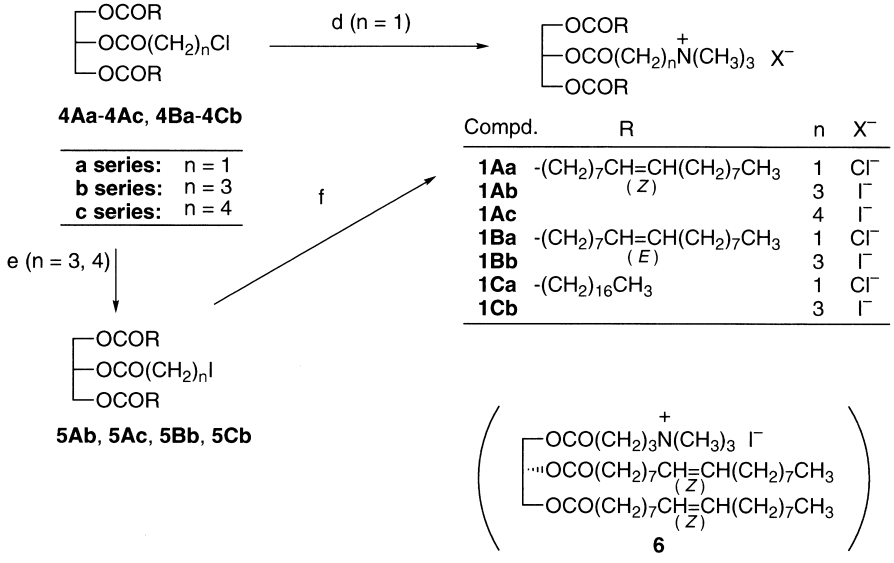
DOSPA



DOTAP

Figure 1. Structures of symmetrical cationic triglycerides **1** and other cationic lipids used in gene delivery vectors.





Scheme 1. (a) Ref. 14 for **2A**; elaidic acid, DCC, DMAP, CH_2Cl_2, 72% for **2B**; ref. 15 for **2C**; (b) ref. 16 for **3A**; $NaBH_4$, THF–H_2O (15:1), 76% for **3B**; $NaBH_4$, THF–benzene–H_2O (17:3:1), 84% for **3C**; (c) chloroacetic anhydride, 2,6-lutidine, CH_2Cl_2, 76–95% for **a** series; 4-chlorobutyryl chloride, 2,6-lutidine, CH_2Cl_2 or benzene, 81–92% for **b** series; 5-chlorovaleryl chloride, 2,6-lutidine, CH_2Cl_2, 87% for **4Ac**; (d) trimethylamine, Et_2O, 66–94%; (e) sodium iodide, methyl ethyl keone, 88–95%; (f) trimethylamine, Et_2O, 57–78%.

4Ba and **4Ca** were reacted with an excess amount of anhydrous trimethylamine at 0 °C to afford the desired symmetrical cationic triglycerides **1Aa**, **1Ba** and **1Ca**, respectively. On the other hand, the chlorobutyryl derivatives **4Ab**, **4Bb**, **4Cb** and chlorovaleryl derivative **4Ac** were converted to iodo derivatives **5Ab**, **5Bb**, **5Cb** and **5Ac**, respectively, in 88–95% yields by stirring in methyl ethyl ketone with sodium iodide. Formation of ammonium salts **1Ab**, **1Bb**, **1Cb** and **1Ac** was accomplished by reaction of the corresponding iodo compounds **5Ab**, **5Bb**, **5Cb** and **5Ac** with anhydrous trimethylamine. The symmetrical structure of the obtained ammonium salts **1** is clearly substantiated by the ^{13}C NMR spectrum (Table 1).

Transfection activity of the cationic triglycerides

Most of the reported asymmetric cationic lipids were formulated into cationic liposomes with the co-lipid dioleoylphosphatidylethanolamine (DOPE),[4,6–8,11] which is known to induce formation of an inverted hexagonal phase and promote destabilization of the membrane.[19] It has been reported that the replacement of DOPE with other natural lipids, which has no activity to form an inverted hexagonal phase, will not attain enhanced transfection ability similarly to that of DOPE.[20–23] To evaluate the effect of a co-lipid when it is employed with symmetrical cationic lipids, one of our symmetric triglycerides, **1Ab**, was formulated into cationic liposomes with a different amount of DOPE and/or dilauroylphosphatidylcholine[24] (DLPC). Their ability to deliver a plasmid encoding a luciferase into CHO cells was studied (Fig. 2). The cationic liposomes composed of the triglyceride **1Ab** alone demonstrated only moderate transfection efficiency; however, the addition of DOPE greatly enhanced the transfection activity of symmetric cationic triglyceride **1Ab**. On the other hand, the liposome formulations with DLPC lost their transfection activity. All of these findings are consistent with the known properties of asymmetric cationic lipids previously reported. We thus suggested that DOPE is quite effective as a co-lipid when it is used not only with an asymmetric cationic lipid but also with a symmetric triglyceride. It is noteworthy that the transfection activity of our cationic

Table 1. Selected ^{13}C NMR data (δ ppm in $CDCl_3$) for symmetrical cationic triglycerides **1Aa**–**1Cb**

Compound	—C(=O)—		-CH=CH-		CH_2CHCH_2	$-CH_2-N^+-(CH_3)_3$		$-CH_2CH_3$
1Aa	173.51	164.33	130.01	129.67	61.26	63.09	54.34	14.09
1Ab	173.39	171.23	130.01	129.67	61.60	65.59	53.84	14.11
1Ac	173.39	171.88	130.01	129.65	61.64	66.51	53.80	14.09
1Ba	173.46	164.31	130.44	130.10	61.19	63.06	54.23	14.07
1Bb	173.30	171.12	130.39	130.06	61.55	65.52	53.75	14.02
1Ca	173.49	164.33	—	—	61.21	63.07	54.29	14.09
1Cb	173.40	171.19	—	—	61.57	65.54	53.77	14.09

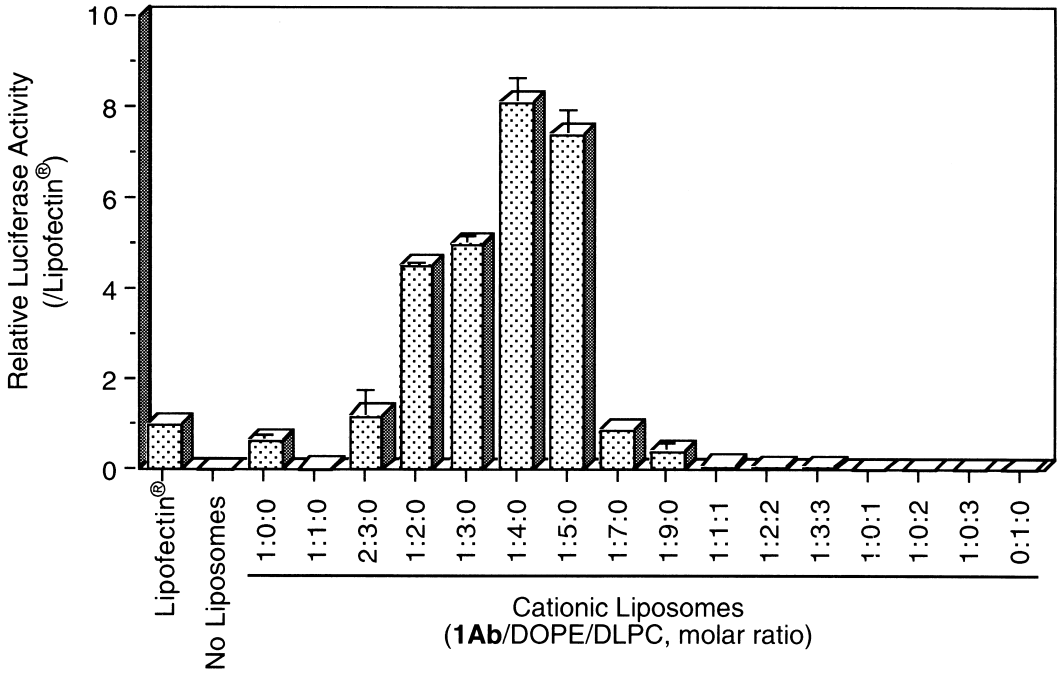


Figure 2. Effect of ratio of **1Ab**, DOPE and DLPC on transfection efficiency of cationic liposomes. CHO cells were inoculated at a density of 1×10^5 cells per 35-mm plate. After incubation for 18 h at 37 °C, the cells were washed twice with phosphate-buffered saline (PBS). Then, cationic liposomes–plasmid DNA (pGVC) complexes (liposome 10 μM, DNA 0.5 μg/mL) in 2 mL OPTI-MEM® medium were added to the cell culture plates. After incubation for 24 h, the cells were harvested and luciferase activity was measured using luciferase assay system (PicaGene™).

liposome, which is composed of symmetrical cationic triglyceride **1Ab** and DOPE (**1Ab**–DOPE = 1:4), was several times higher than that of the widely used Lipofectin® (DOTMA–DOPE = 1:1) in CHO cells.

To compare the transfection ability of a symmetrical cationic triglyceride with that of an asymmetric triglyceride, the symmetrical triglyceride **1Ab** and its asymmetric congener **6**[7] were formulated into cationic liposomes with DOPE (**1Ab** or **6**–DOPE = 1:4), respectively. The transfection efficiency of these liposomes was evaluated, and it was found that the symmetrical triglyceride **1Ab** worked as well as the asymmetrical triglyceride **6** (Fig. 3). This result clearly indicates that the asymmetrical cationic triglycerides, the synthesis of which is a troublesome procedure, could be replaced with easily prepared symmetrical cationic triglycerides.

To study the relationship between the structure of cationic triglyceride and transfection activity, the cationic liposomes composed of triglycerides **1Aa**–**1Cb** and DOPE (**1**–DOPE = 1:4) were prepared, and their transfection efficiency was evaluated (Table 2). These results clearly show that the transfection efficiency depends upon both the structure of the acyl chain at the 1- and the 3-position of glycerol backbone and the length of the linker connecting the hydrophilic head group with the glycerol backbone. The transfection activity was increased when the unsaturated structure was introduced in the middle of a long acyl chain (**1Ab** > **1Bb** > **1Cb**). The transfection activity of the cationic triglyceride containing an oleoyl group (**1Ab**) was higher than that of **1Bb** which has an elaidoyl group. It was known that the bilayer stiffness decreased when an unsaturated structure was introduced into the hydrophobic chain of the lipid.[21] The decrease in bilayer stiffness probably enhances the fusion ability of cationic liposomes.[21] That seems to be the reason why cationic triglycerides **1Bb** and **1Ab** show higher transfection activity than **1Cb**. It is interesting that low transfection activity was observed when cationic triglycerides with a short linker (**1Aa**, **1Ba** and **1Ca**) were used; on the contrary, high transfection activity was observed when cationic triglycerides with a relatively long linker (**1Ab**, **1Bb**, **1Cb** and **1Ac**) were used. In the case of cationic triglycerides with a short linker, the hydrophilic quaternary ammonium group of the cationic triglycerides would be embedded in the lipid bilayer of liposomes. This would have prevented the positive charge of the head group in cationic lipid from interacting with the negative charge of DNA or cell membrane. Therefore, cationic lipid with a relatively long linker is suggested to be essential for high transfection activity.

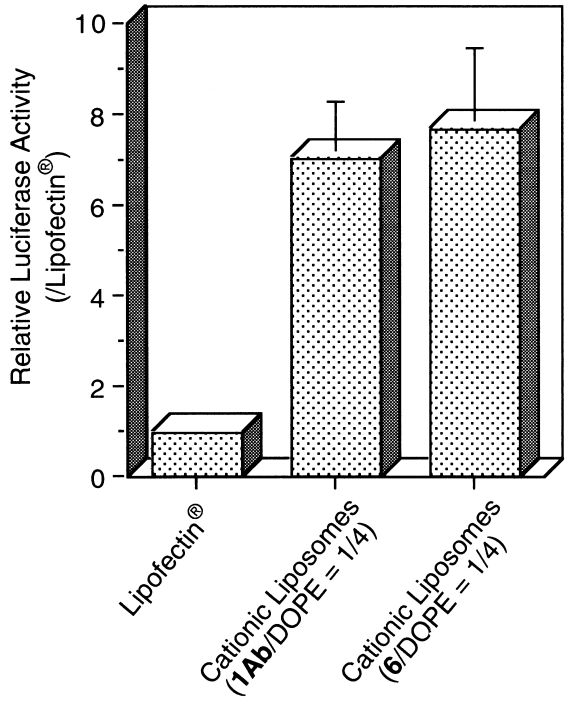


Figure 3. Transfection efficiency of cationic liposomes (**1Ab**–DOPE = 1:4) compared with that of cationic liposomes (**6**–DOPE = 1:4). See caption of Figure 2.

Conclusion

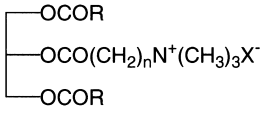
DOPE is essential for effective transfection when a symmetrical cationic triglyceride is used, and an asymmetrical structure of cationic lipids was not essential for efficient transfection activity. The optimal structure of our cationic triglyceride is the one with an unsaturated (*Z*-olefine) acyl chain at the 1- and 3-positions and a relatively long linker at the 2-position of the glycerol backbone.

Experimental

General considerations

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H

Table 2. Relationship between the structure of **1** and the transfection efficiency of the cationic liposomes[a]



Triglycerides	R	*n*	Relative luciferase activity (/Lipofectin®)
1Aa	-$(CH_2)_7CH=CH(CH_2)_7CH_3$ (*Z*)	1	0.0±0.0
1Ba	-$(CH_2)_7CH=CH(CH_2)_7CH_3$ (*E*)	1	0.0±0.0
1Ca	-$(CH_2)_{16}CH_3$	1	0.0±0.0
1Ab	-$(CH_2)_7CH=CH(CH_2)_7CH_3$ (*Z*)	3	10.4±0.7
1Bb	-$(CH_2)_7CH=CH(CH_2)_7CH_3$ (*E*)	3	3.2±0.4
1Cb	-$(CH_2)_{16}CH_3$	3	1.7±0.5
1Ac	-$(CH_2)_7CH=CH(CH_2)_7CH_3$ (*Z*)	4	8.0±0.9
Lipofectin®			1

[a]See captions in Figure 2.

and ^{13}C NMR spectra were recorded on a Varian VXR-200 (^{1}H, 200 MHz), JEOL EX-270 (^{1}H, 270 MHz; ^{13}C, 67.5 MHz) or JEOL GX-500 (^{1}H, 500 MHz) spectrometer. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. Mass spectra were measured on JEOL JMS-D300 or JMS-600 mass spectrometers. Luciferase activity was measured on an EG&G Berthold Lumat LB9501. For column chromatography, Merck Kieselgel 60 (70–200 mesh) or Fuji Silysia BW-127ZH (100–200 mesh) was used. DOPE, DLPC and the luciferase expression plasmid pGVC were purchased from WAKO Pure Chemical Industries (Osaka, Japan). Lipofectin® and OPTI-MEM® were purchased from Gibco BRL (NY, USA). Luciferase assay system PicaGene™ was obtained from Toyo Inki (Tokyo, Japan). Asymmetrical cationic triglyceride **6** was prepared according to the literature.[7]

Chemistry

1,3-Dioleoyloxypropan-2-one (2A). Compound **2A** was prepared according to the literature.[15]

Mp 40–41.5 °C (MeOH) (lit.[15] 43 °C). ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.14–1.44 (40H, m, CH_2), 1.67 (4H, m, $OCOCH_2C\underline{H}_2$), 2.02, 2.17 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.42 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.75 (4H, s, CH_2COCH_2), 5.34 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$).

1,3-Dielaidoyloxypropan-2-one (2B). To a stirred solution of 1,3-dihydroxyacetone (0.82 g, 8.87 mmol) in CH_2Cl_2 (18 mL) were added elaidic acid (5.04 g, 17.7 mmol) and 4-dimethylaminopyridine (2.16 g, 17.7 mmol). To this reaction mixture a solution of 1,1-dicyclohexylcarbodiimide (3.70 g, 18.0 mmol) in CH_2Cl_2 (7 mL) was added dropwise with stirring at room temperature. After stirring for 20 h at the same temperature, the precipitated cyclohexylurea was removed by filtration. The filtrate was washed with 5% HCl, H_2O, and sat. NaCl. The organic layer was dried (Na_2SO_4) and concentrated, and the residue was recrystallized from AcOEt–EtOH to give colorless crystals **2B** (3.96 g, 72%).

Mp 57.5–59 °C (AcOEt–EtOH). IR ν (KBr): 2918, 2850, 1731, 1469, 1420, 1301, 1251, 1210 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.06–1.44 (40H, m, CH_2), 1.62 (4H, m, $OCOCH_2C\underline{H}_2$), 1.95, 1.97 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.42 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.75 (4H, s, CH_2COCH_2), 5.38 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($CDCl_3$) δ: 198.10, 172.90, 130.48, 130.17, 130.20, 96.12, 66.13, 33.72, 32.61, 32.55, 31.90, 29.65, 29.55, 29.49, 29.40, 29.32, 29.18, 29.09, 29.03, 28.92, 24.78, 22.69, 14.12. MS (EI) m/z: 618 (M^+, 2.5), 265 (35.7), 55 (100). Anal. calcd for $C_{39}H_{70}O_5$: C, 75.67; H, 11.40. Found: C, 75.56; H, 11.28.

1,3-Distearoyloxypropan-2-one (2C). Compound **2C** was prepared according to the literature.[16]

Mp 83–85 °C (hexane–$CHCl_3$) (lit.[16] 84–86 °C). ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.18–1.36 (56H, m, CH_2), 1.62 (4H, m, $OCOCH_2C\underline{H}_2$), 2.42 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.74 (4H, s, CH_2CO-CH_2).

1,3-Dioleoylglycerol (3A). According to the literature,[17] compound **3A** was prepared in the following manner. To a stirred solution of compound **2A** (1.00 g, 1.62 mmol) in THF–H_2O (15:1, 16 mL) at 0 °C was added $NaBH_4$ (100 mg, 2.59 mmol). The reaction mixture was stirred for 20 min at the same temperature. The solvent was concentrated, and the residue was dissolved in Et_2O and washed with water. The organic layer was dried ($MgSO_4$) and concentrated. The residue was washed with *n*-pentane at −78 °C to give a colorless oil **3A** (0.86 g, 85%).

^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=7.0$ Hz, CH_3), 1.18–1.46 (40H, m, CH_2), 1.64 (4H, m, $OCOCH_2C\underline{H}_2$), 2.01 (8H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.34 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.14–4.16 (5H, m, CH_2CHCH_2), 5.34 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($CDCl_3$) δ: 174.33, 130.44, 130.14, 68.79, 65.46, 34.52, 33.03, 32.34, 30.11, 29.95, 29.89, 29.76, 29.68, 29.53, 29.41, 27.63, 27.59, 25.30, 23.11, 14.55.

1,3-Dielaidoylglycerol (3B). To a stirred solution of compound **2B** (1.00 g, 1.62 mmol) in THF–H_2O (15:1, 16 mL) at 0 °C was added $NaBH_4$ (100 mg, 2.59 mmol). The reaction mixture was stirred for 20 min at the same temperature. The solvent was concentrated, and the residue was dissolved in Et_2O and washed with water. The organic layer was dried ($MgSO_4$) and concentrated. The residue was washed with *n*-pentane at −78 °C to give a colorless oil **3B** (0.76 g, 76%).

^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.0$ Hz, CH_3), 1.15–1.40 (40H, m, CH_2), 1.63 (4H, m, $OCOCH_2C\underline{H}_2$), 2.00 (8H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.35 (4H, t, $J=7.0$ Hz, $OCOCH_2$), 4.10–4.20 (5H, m, CH_2CHCH_2), 5.38 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.87, 130.48, 130.17, 68.39, 65.03, 34.07, 32.58, 32.52, 31.88, 29.63, 29.54, 29.47, 29.29, 29.17, 29.08, 28.92, 24.87, 22.66, 14.09.

1,3-Distearoylglycerol (3C). To a stirred solution of compound **2C** (0.12 g, 0.1 mmol) in THF–benzene–H_2O (17:3:1, 21 mL) at 0 °C was added $NaBH_4$ (12.5 mg, 0.33 mmol). The reaction mixture was stirred for 1 h at the same temperature. The solvent was concentrated, and the residue was dissolved in chloroform and washed with water. The organic layer was dried ($MgSO_4$) and concentrated. The residue was recrystallized from $CHCl_3$ to give colorless crystals **3C** (0.10 g, 84%).

Mp 75–77 °C ($CHCl_3$) (lit.[16] 76–78 °C). ^{1}H NMR ($CDCl_3$) δ: 0.87 (6H, t, $J=6.0$ Hz, CH_3), 1.28–1.38 (56H, m, CH_2), 1.62 (4H, m, $OCOCH_2C\underline{H}_2$), 2.32 (4H, t, $OCOCH_2$), 4.06–4.22 (5H, m, CH_2CHCH_2). ^{13}C NMR ($CDCl_3$) δ: 173.90, 68.34, 65.00, 34.08, 31.93, 29.86, 29.70, 29.61, 29.53, 29.46, 29.36, 29.26, 29.12, 24.87, 24.80, 22.69, 14.14.

1,3-Dioleoyl-2-chloroacetylglycerol (4Aa). To a stirred solution of compound **3A** (1.00 g, 1.61 mmol) in CH_2Cl_2 (5 mL) at room temperature were added 2,6-lutidine (5 mL) and chloroacetic anhydride (0.80 g, 4.68 mmol). The reaction mixture was stirred for 3 h, and the solvent was concentrated. The residue was dissolved in AcOEt, washed with H_2O, sat. $NaHCO_3$, H_2O, 2% HCl, H_2O, and sat. NaCl. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified by flash column chromatography (hexane–AcOEt, 6:1) to afford a colorless oil **4Aa** (0.86 g, 77%).

IR ν (KBr): 2924, 2855, 2032, 1748, 1653, 1457, 1158 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.0$ Hz, CH_3), 1.00–1.40 (40H, m, CH_2), 1.60 (4H, m, $OCOCH_2C\underline{H}_2$), 2.00, 2.02 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.07 (2H, s, CH_2Cl), 4.17, 4.36 (4H, ABX, $J=6.0$, 3.5 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.31 (1H, m, $CH_2C\underline{H}CH_2$), 5.34 (4H, m, $CH_2CH{=}CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.63, 167.04, 130.44, 130.26, 130.14, 71.70, 62.16, 41.08, 34.41, 32.35, 30.53, 30.45, 30.21, 30.13, 29.97, 29.77, 29.53, 27.90, 27.66, 27.62, 25.25, 23.13, 14.56. MS (EI) m/z: 696 (M^+, 8.8), 417 (17.2), 415 (38.6), 339 (5.4), 265 (80.3), 55 (100). Anal. calcd for $C_{41}H_{73}O_6Cl$: C, 70.60; H, 10.55; Cl, 5.08. Found: C, 70.79; H, 10.48; Cl, 4.87.

1,3-Dioleoyl-2-(4-chlorobutanoyl)glycerol (4Ab). Compound **4Ab** was prepared as described for **4Aa** using **3A** (2.33 g, 3.75 mmol) and 4-chlorobutyryl chloride (1.59 g, 11.3 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 30:1) to afford a colorless oil **4Ab** (2.20 g, 81%).

IR ν (KBr): 2930, 2854, 2676, 2031, 1746, 1652, 1456, 1377, 1166, 1049 cm^{-1}. ^1H NMR($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.18–1.42 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 2.02, 2.00 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.10 (2H, m, $COCH_2C\underline{H}_2CH_2Cl$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.53 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2Cl$), 3.60 (2H, t, $J=6.5$ Hz, $COCH_2CH_2C\underline{H}_2Cl$), 4.14, 4.33 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.27 (1H, m, $CH_2C\underline{H}CH_2$), 5.34 (4H, m, $CH_2CH{=}CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.23, 171.73, 129.99, 129.69, 69.36, 61.96, 43.79, 33.98, 31.88, 31.09, 29.74, 29.67, 29.51, 29.29, 29.13, 29.06, 27.50, 27.19, 27.14, 24.82, 22.66, 14.09. MS (EI) m/z: 724 (M^+, 6.1), 602 (14.2), 445 (40.1), 443 (100), 339 (12.8), 265 (58.5), 181 (14.3), 179 (43.1), 107 (31.1), 105 (84.3). Anal. calcd for $C_{43}H_{77}O_6Cl$: C, 71.18; H, 10.70; Cl, 4.89. Found: C, 71.19; H, 10.65; Cl, 4.83.

1,3-Dioleoyl-2-(5-chlorovaleryl)glycerol (4Ac). Compound **4Ac** was prepared as described for **4Aa** using **3A** (0.38 g, 0.61 mmol) and 5-chlorovaleryl chloride (0.29 g, 1.87 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 30:1) to afford a colorless oil **4Ac** (0.40 g, 87%).

IR ν (KBr): 2922, 2855, 2677, 2030, 1745, 1655, 1459, 1377, 1162 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=7.0$ Hz, CH_3), 1.20–1.42 (42H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 1.82 (2H, m, $CH_2C\underline{H}_2(CH_2)_2Cl$), 2.00, 2.02 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.35 (2H, t, $J=6.5$ Hz, $OCOC\underline{H}_2(CH_2)_3Cl$), 3.18 (2H, t, $J=6.0$ Hz, $(CH_2)_3C\underline{H}_2Cl$), 4.14, 4.32 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.27 (1H, m, $CH_2C\underline{H}CH_2$), 5.34 (4H, m, $CH_2CH{=}CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.24, 172.18, 130.01, 129.70, 69.22, 62.03, 44.30, 34.02, 33.28, 31.90, 31.72, 29.76, 29.71, 29.53, 29.31, 29.17, 29.11, 29.08, 27.23, 27.17, 24.84, 22.68, 22.16, 14.11. MS (EI) m/z: 738 (M^+, 3.3), 602 (14.9), 457 (80.2), 339 (10.6), 265 (39.9), 247 (10.9), 195 (9.5), 193 (27.6), 121 (22.0), 119 (49.7), 55 (100). Anal. calcd for $C_{44}H_{79}O_6Cl$: C, 71.46; H, 10.77; Cl, 4.79. Found: C, 71.39; H, 10.64; Cl, 4.90.

1,3-Dielaidoyl-2-chloroacetylglycerol (4Ba). Compound **4Ba** was prepared as described for **4Aa** using **3B** (0.47 g, 0.75 mmol) and chloroacetic anhydride (0.39 g, 2.28 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 20:1) to afford a colorless oil **4Ba** (0.40 g, 76%).

IR ν (KBr): 2924, 2854, 1744, 1465, 1285, 1242, 1163, 1092 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.10–1.45 (40H, m, CH_2), 1.60 (4H, m, $OCOCH_2C\underline{H}_2$), 1.95, 1.97 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 4.08 (2H, s, CH_2Cl), 4.17, 4.36 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.34 (1H, m, $CH_2C\underline{H}CH_2$), 5.38 (4H, m, $CH_2CH{=}CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.30, 173.26, 166.63, 71.27, 61.74, 40.66, 33.97, 32.62, 62.56, 32.38, 31.91, 29.67, 29.58, 29.50, 29.33, 29.20, 29.11, 29.06, 28.95, 28.53, 24.81, 24.68, 22.69, 14.14. MS (EI) m/z: 696 (M^+, 10.8), 417 (20.1), 415 (44.0), 265 (86.8), 264 (100). Anal. calcd for $C_{41}H_{73}O_6Cl$: C, 70.60; H, 10.55; Cl, 5.08. Found: C, 70.62; H, 10.30; Cl, 5.08.

1,3-Dielaidoyl-2-(4-chlorobutanoyl)glycerol (4Bb). Compound **4Bb** was prepared as described for **4Aa** using **3B** (0.76 g, 1.22 mmol) and 4-chlorobutyryl chloride (0.68 g, 3.66 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 20:1) to afford a colorless oil **4Bb** (0.76 g, 86%).

IR ν (KBr): 2924, 2853, 2675, 2025, 1746, 1465, 1377, 1167, 1096 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=7.0$ Hz, CH_3), 1.16–1.40 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 1.95, 1.97 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.10 (2H, m, $COCH_2C\underline{H}_2CH_2Cl$), 2.31 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.53 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2Cl$), 3.60 (2H, t, $J=6.5$ Hz, $COCH_2C\underline{H}_2CH_2Cl$), 4.14, 4.32 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.27 (1H, m, $CH_2C\underline{H}CH_2$), 5.38 (4H, m, $CH_2CH{=}CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.26, 171.73, 130.48, 130.15, 69.36, 61.96, 43.81, 34.06, 32.58, 32.54, 31.88, 31.11, 29.74, 29.63, 29.56, 29.47, 29.29, 29.17, 29.09, 29.06, 28.93, 27.50, 24.82, 22.66, 14.09. MS (EI) m/z: 724 (M^+, 4.8), 706 (1.6), 602 (13.6), 445 (38.2), 443 (94.7), 339 (12.6), 265 (29.8), 181 (14.4), 179 (43.5), 107 (32.9), 105 (91.4), 55 (100). Anal. calcd for $C_{43}H_{77}O_6Cl$: C, 71.19; H, 10.70; Cl, 4.89. Found: C, 71.10; H, 10.43; Cl, 4.88.

1,3-Distearoyl-2-chloroacetylglycerol (4Ca). To a stirred solution of compound **3C** (1.70 g, 2.72 mmol) in CH_2Cl_2 (16 mL) at room temperature were added 2,6-lutidine (16 mL) and chloroacetic anhydride (1.40 g, 8.19 mmol). The reaction mixture was stirred for 3 h, and the solvent was concentrated. The residue was dissolved in $CHCl_3$, washed with H_2O, sat. $NaHCO_3$, H_2O, 2% HCl, H_2O, and sat. NaCl. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified by SiO_2 column chromatography (CH_2Cl_2–AcOEt, 25:1) to afford a white powder **4Ca** (1.81 g, 95%).

Mp 58–60 °C. IR ν (KBr): 2915, 2849, 1739, 1467, 1416, 1254, 1168, 1105 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.10–1.40 (56H, m, CH_2), 1.60 (4H, m, $OCOCH_2C\underline{H}_2$), 2.32 (4H, t, $J=7.0$ Hz, $OCOCH_2$), 4.08 (2H, s, $C\underline{H}_2Cl$), 4.17, 4.36 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.33 (1H, m, $CH_2C\underline{H}CH_2$). ^{13}C NMR ($\underline{C}DCl_3$) δ: 173.24, 166.60, 96.10, 77.47, 77.20, 77.00, 76.51, 71.25, 61.71, 40.63, 33.96, 31.92, 30.21, 29.67, 29.60, 29.44, 29.35, 29.22, 29.08, 24.80, 22.68, 22.68, 14.09. MS (EI) m/z: 700 (M^+, 2.7), 419 (15.9), 417 (43.1), 341 (11.3), 267 (61.8), 57 (100). Anal. calcd for $C_{41}H_{77}O_6Cl$: C, 70.20; H, 11.06; Cl, 5.05. Found: C, 70.10; H, 10.79; Cl, 4.85.

1,3-Distearoyl-2-(4-chlorobutanoyl)glycerol (4Cb). To a stirred solution of compound **3C** (0.77 g, 1.23 mmol) in benzene (10 mL) at room temperature were added 2,6-lutidine (10 mL) and 4-chlorobutyryl chloride (0.53 g, 3.75 mmol). The reaction mixture was stirred for 3 h, and the solvent was concentrated. The residue was dissolved in $CHCl_3$, washed with H_2O, sat. $NaHCO_3$, H_2O, 2% HCl, H_2O, and sat. NaCl. The organic layer was dried ($MgSO_4$) and concentrated, and the residue was recrystallized from AcOEt to afford colorless crystals **4Cb** (0.83 g, 92%).

Mp 56–57 °C (AcOEt). IR ν (KBr): 2914, 2848, 1733, 1471, 1255, 1175, 1108, 1066, 1041 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.18–1.38 (56H, m, CH_2), 1.64 (4H, m, $OCOCH_2C\underline{H}_2$), 2.10 (2H, m, $COCH_2C\underline{H}_2CH_2Cl$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.53 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2Cl$), 3.60 (2H, t, $J=6.0$ Hz, $COCH_2CH_2C\underline{H}_2Cl$), 4.14, 4.32 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.27 (1H, m, $CH_2C\underline{H}CH_2$). ^{13}C NMR ($\underline{C}DCl_3$) δ: 173.28, 171.75, 96.12, 69.40, 61.96, 43.81, 34.04, 31.92, 31.13, 29.69, 9.62, 29.47, 29.36, 29.27, 29.11, 27.51, 24.85, 22.68, 14.11. MS (EI) m/z: 728 (M^+, 1.1), 606 (13.1), 445 (92.4), 341 (21.3), 267 (72.1), 181 (19.3), 179 (57.2), 107 (31.2), 105 (92.4), 57 (100). Anal. calcd for $C_{43}H_{81}O_6Cl$: C, 70.79; H, 11.19; Cl, 4.85. Found: C, 70.50; H, 10.97; Cl, 4.59.

1,3-Dioleoyl-2-(4-iodobutanoyl)glycerol (5Ab). To a stirred solution of sodium iodide (4.40 g, 29.7 mmol) in anhydrous methyl ethyl ketone (32 mL) was added **4Ab** (2.20 g, 3.03 mmol). The stirring was continued for 48 h at room temperature and the reaction mixture was concentrated. The residue was dissolved in AcOEt, washed with H_2O, 10% $NaHSO_3$, H_2O, 5% $NaHCO_3$, H_2O and sat. NaCl. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography (hexane–AcOEt, 30:1) to afford a colorless oil **5Ab** (2.18 g, 88%).

IR ν (KBr): 2926, 2854, 1745, 1656, 1462, 1377, 1167 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.17–1.41 (40H, m, CH_2), 1.62 (4H, m, $OCOCH_2C\underline{H}_2$), 2.00, 2.02 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.13 (2H, m, $COCH_2C\underline{H}_2CH_2I$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.48 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2\ CH_2CH_2I$), 3.24 (2H, t, $J=7.0$ Hz, $COCH_2CH_2C\underline{H}_2I$), 4.16, 4.32 (4H, ABX, $J=6.0$, 4.5 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.27 (1H, m, $CH_2C\underline{H}CH_2$), 5.34 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($\underline{C}DCl_3$) δ: 173.23, 171.41, 130.01, 129.69, 69.40, 69.18, 34.70, 34.02, 31.88, 30.30, 29.74, 29.69, 29.51, 29.31, 29.15, 29.09, 28.32, 27.21, 27.15, 24.82, 22.66, 14.09, 4.94. MS (EI) m/z: 816 (M^+, 7.5), 798 (1.7), 602 (17.5), 535 (100), 339 (14.7), 271 (30.1), 265 (51.9), 197 (47.4). Anal. calcd for $C_{43}H_{77}O_6I$: C, 63.21; H, 9.50; I, 15.53. Found: C, 63.27; H, 9.41; I, 15.31.

1,3-Dioleoyl-2-(5-iodovaleryl)glycerol (5Ac). Compound **5Ac** was prepared as described for **5Ab** using **4Ac** (0.31 g, 0.42 mmol) and sodium iodide (0.70 g, 4.67 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 100:1) to afford a colorless oil **5Ac** (0.33 g, 95%).

IR ν (KBr): 2926, 2854, 2026, 1748, 1653, 1456, 1373, 1161 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=7.0$ Hz, CH_3), 1.19–1.41 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 1.75 (2H, m, $CH_2C\underline{H}_2(CH_2)_2I$), 1.85 (2H, m, $CH_2C\underline{H}_2(CH_2)_2I$), 2.00, 2.02 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.36 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2I$), 3.18 (2H, t, $J=7.0$ Hz, $COCH_2CH_2C\underline{H}_2I$), 4.14, 4.32 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.26 (1H, m, $CH_2C\underline{H}CH_2$), 5.34 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($\underline{C}DCl_3$) δ: 173.23, 172.11, 130.01, 129.69, 62.22, 62.04, 34.02, 32.92, 32.56, 31.88, 29.74, 29.69, 29.51, 29.29, 29.15, 29.04, 27.21, 27.15, 25.66, 24.82, 22.66, 14.09, 5.48. MS (EI) m/z: 830 (M^+, 4.8), 602 (13.3), 549 (69.2), 339 (13.8), 285 (19.6), 265 (29.8), 211 (20.7), 55 (100). Anal. calcd for $C_{44}H_{79}O_6I\cdot 1/10$ hexane: C, 63.80; H, 9.65; I, 15.11. Found: C, 63.99; H, 9.53; I, 14.96.

1,3-Dielaidoyl-2-(4-iodobutanoyl)glycerol (5Bb). Compound **5Bb** was prepared as described for **5Ab** using **4Bb** (0.76 g, 1.05 mmol) and sodium iodide (1.59 g, 10.6 mmol). The residue was purified by flash column chromatography (hexane–AcOEt, 40:1) to afford a colorless oil **5Bb** (0.79 g, 92%).

IR ν (KBr): 2922, 2853, 1744, 1457, 1375, 1165 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.17–1.42 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 1.95, 1.97 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.13 (2H, m, $COCH_2C\underline{H}_2CH_2I$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.47 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2I$), 3.24 (2H, t, $J=6.5$ Hz, $COCH_2CH_2C\underline{H}_2I$), 4.14, 4.32 (4H, ABX, $J=6.0$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.26 (1H, m, $CH_2C\underline{H}CH_2$), 5.37 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($\underline{C}DCl_3$) δ: 173.24, 171.41, 130.48, 130.15, 69.38,

61.96, 34.68, 34.00, 32.58, 32.54, 32.28, 31.88, 29.63, 29.56, 29.47, 29.29, 29.17, 29.09, 29.06, 28.95, 28.30, 24.82, 22.66, 14.11, 4.98. MS (EI) *m/z*: 816 (M^+, 8.1), 798 (1.8), 602 (13.6), 535 (80.5), 339 (14.9), 265 (44.7), 271 (30.5), 197(49.4), 55 (100). Anal. calcd for $C_{43}H_{77}O_6I$•1/10 hexane: C, 63.74, H, 9.68; I, 15.13. Found: C, 63.59; H, 9.38; I, 14.84.

1,3-Distearoyl-2-(4-iodobutanoyl)glycerol (5Cb). To a stirred solution of sodium iodide (1.56 g, 10.5 mmol) in anhydrous methyl ethyl ketone (11 mL) was added **4Cb** (0.83 g, 1.14 mmol). The stirring was continued for 48 h at room temperature and the reaction mixture was concentrated. The residue was dissolved in $CHCl_3$, washed with H_2O, 10% $NaHSO_3$, H_2O, 5% $NaHCO_3$, H_2O and sat. NaCl. The organic layer was dried ($MgSO_4$) and concentrated. The residue was recrystallized from hexane AcOEt (20:1) to afford colorless crystals **5Cb** (0.82 g, 88%).

Mp 61–62 °C (hexane–AcOEt). IR ν (KBr): 2914, 2849, 1733, 1652, 1378, 1471, 1378, 1174, 1108, 1066 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.18–1.36 (56H, m, CH_2), 1.61 (4H, m, $OCOCH_2CH_2$), 2.13 (2H, m, $COCH_2CH_2CH_2I$), 2.32 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.48 (2H, t, $J=7.0$ Hz), 3.24 (2H, t, $J=7.0$ Hz, $COCH_2CH_2CH_2I$), 4.13, 4.31 (4H, ABX, $J=6.0$, 4.5 Hz, CH_2CHCH_2), 5.27 (1H, m, CH_2CHCH_2). ^{13}C NMR ($CDCl_3$) δ: 173.30, 171.43, 96.10, 69.38, 61.96, 34.68, 34.04, 31.93, 29.71, 29.49, 29.36, 29.27, 29.11, 28.29, 24.85, 22.70, 14.14, 5.03. MS (EI) *m/z*: 820 (M^+, 2.9), 607 (100), 537 (33.1), 341 (17.4), 267 (30.0), 271 (16.2), 197 (37.4). Anal. calcd for $C_{43}H_{81}O_6I$: C, 62.91; H, 9.94; I, 15.46. Found: C, 63.05; H, 9.71; I, 15.13.

***N*-[2-(1,3-Dioleoyloxy)propoxycarbonylmethyl]-*N*,*N*,*N*-trimethylammonium chloride (1Aa).** Excess amount of anhydrous trimethylamine was added to a solution of **4Aa** (1.40 g, 2.01 mmol) in dry Et_2O (4 mL) at 0 °C. The reaction mixture was stirred for 12 h at the same temperature and concentrated. The residue was purified by SiO_2 column chromatography (hexane) to give a white wax **1Aa** (1.01 g, 66%).

Mp 35–39 °C. IR ν (KBr): 2924, 2853, 2062, 1746, 1634, 1464, 1399, 1255, 1196, 1091, 1008 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.00–1.46 (40H, m, CH_2), 1.58 (4H, m, $OCOCH_2CH_2$), 2.00, 2.02 (4H each, m, $CH_2CH=CHCH_2$), 2.35 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 3.67 (9H, s, $N(CH_3)_3$), 4.11, 4.48 (4H, ABX, $J=5.5$, 4.5 Hz, CH_2CHCH_2), 4.92 (2H, s, $COCH_2N$), 5.22 (1H, m, CH_2CHCH_2), 5.34 (4H, m, $CH_2CH=CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.51, 164.33, 130.01, 129.67, 72.30, 63.09, 61.21, 54.34, 33.95, 31.88, 29.74, 29.69, 29.60, 29.49, 29.29, 29.17, 29.09, 27.21, 27.15, 24.78, 22.66, 14.09. MS (EI) *m/z*: 705(M^+-CH_3Cl, 81.6), 602 (10.0), 265 (26.6), 55 (100). Anal. calcd for $C_{44}H_{82}NO_6Cl{\cdot}2H_2O$: C, 66.68; H, 10.43; N, 1.77; Cl, 4.47. Found: C, 66.96; H, 10.72; N, 1.99; Cl, 4.27.

***N*-[3-[2-(1,3-Dioleoyloxy)propoxycarbonyl]propyl]-*N*,*N*,*N*-trimethylammonium iodide (1Ab).** Compound **1Ab** was prepared as described for **1Aa** using **5Ab** (1.58 g, 1.94 mmol). The residue was purified by SiO_2 column chromatography ($CHCl_3$–MeOH, 20:1) to afford a white powder **1Ab** (1.32 g, 78%).

Mp 47–50 °C. IR ν (KBr): 2924, 2852, 1738, 1466, 1412, 1364, 1252, 1176, 1117, 1067, 1025, 968, 938, 879, 851, 798, 725 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.17–1.40 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2CH_2$), 2.02 (8H, m, $CH_2CH=CHCH_2$), 2.12 (2H, m, $COCH_2CH_2CH_2N$), 2.33 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.54 (2H, t, $J=6.5$ Hz, $COCH_2CH_2CH_2N$), 3.48 (9H, s, $N(CH_3)_3$), 3.75 (2H, m, $COCH_2CH_2CH_2N$), 4.13, 4.40 (4H, ABX, $J=5.5$, 4.5 Hz, CH_2CHCH_2), 5.16 (1H, m, CH_2CHCH_2), 5.34 (4H, m, $CH_2CH=CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.40, 171.23, 130.01, 129.67, 70.39, 61.58, 53.78, 34.00, 31.88, 29.72, 29.67, 29.49, 29.29, 29.17, 29.09, 29.06, 27.19, 27.14, 24.82, 22.66, 18.31, 14.11. MS (EI) *m/z*: 733 (M^+-CH_3I, 100), 265 (5.7), 142 (55.8). Anal. calcd for $C_{46}H_{86}NO_6I$: C, 63.06; H, 9.89; N, 1.60; I, 14.19. Found: C, 62.92; H, 9.60; N, 1.51; I, 14.14.

***N*-[4-[2-(1,3-Dioleoyloxy)propoxycarbonyl]butyl]-*N*,*N*,*N*-trimethylammonium iodide (1Ac).** Compound **1Ac** was prepared as described for **1Aa** using **5Ac** (0.27 g, 0.33 mmol). The residue was purified by SiO_2 column chromatography ($CHCl_3$–MeOH, 20:1) to afford a white wax **1Ac** (0.217 g, 75%).

Mp 46.5–50 °C. IR ν (KBr): 2924, 2853, 1739, 1653, 1457, 1418, 1373, 1173, 1096 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=7.0$ Hz, CH_3), 1.18–1.40 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2CH_2$), 1.78 (2H, m, $COCH_2CH_2(CH_2)_2N$), 1.90 (2H, m, $CO(CH_2)_2CH_2CH_2N$), 2.00, 2.33 (4H each, m, $CH_2CH=CHCH_2$), 2.33 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.45 (2H, t, $J=6.0$ Hz, $OCOCH_2CH_2$), 3.46 (9H, s, $N(CH_3)_3$), 3.71 (2H, m, $CO(CH_2)_2CH_2CH_2N$), 4.11, 4.41 (4H, ABX, $J=5.5$, 4.5 Hz, CH_2CHCH_2), 5.17 (1H, m, CH_2CHCH_2), 5.34 (4H, m, $CH_2CH=CHCH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.39, 171.88, 130.01, 129.65, 69.72, 66.51, 61.64, 53.80, 34.04, 32.80, 31.86, 29.72, 29.67, 29.49, 29.29, 29.15, 29.09, 29.06, 27.19, 27.14, 24.85, 22.64, 22.18, 20.95, 14.09. MS (EI) *m/z*: 747 (M^+-CH_3I, 100), 265 (5.1), 142 (53.8), 128 (64.3). Anal. calcd for $C_{47}H_{88}NO_6I$•1/2H_2O: C, 62.78; H, 9.89; N, 1.56; I, 14.11. Found: C, 62.82; H, 9.63; N, 1.59; I, 13.89.

***N*-[2-(1,3-Dielaidoyloxy)propoxycarbonylmethyl]-*N*,*N*,*N*-trimethylammonium chloride (1Ba).** Compound **1Ba** was prepared as described for **1Aa** using **4Ba** (0.60 g, 0.86 mmol). The residue was washed with Et_2O and dried under reduced pressure to afford a white powder **1Ba** (0.57 g, 88%).

Mp 71–75 °C. IR ν (KBr): 2917, 2851, 1742, 1629, 1470, 1277, 1163, 1026 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.87 (6H, t, $J=6.5$, CH_3), 1.15–1.40 (40H, m, CH_2), 1.60 (4H, m, $OCOCH_2CH_2$), 1.94, 1.96 (4H each, m, $CH_2CH=CHCH_2$), 2.34 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 3.66 (9H, s, $N(CH_3)_3$), 4.10, 4.47 (4H, ABX, $J=5.5$, 4.0 Hz, CH_2CHCH_2), 4.89 (2H, s, $COCH_2N$), 5.21 (1H, m, CH_2CHCH_2), 5.37 (4H, m, $CH_2CH=CHCH_2$). ^{13}C

NMR ($CDCl_3$) δ: 173.46, 164.31, 130.44, 130.10, 72.20, 63.06, 61.19, 54.23, 33.93, 32.56, 31.88, 29.65, 29.45, 29.31, 29.24, 29.15, 29.09, 28.93, 24.78, 22.64, 14.07. MS (EI) *m/z*: 706 (M^+-CH_3Cl, 6.9), 602 (1.1), 265 (1.9), 58 (100). Anal. calcd for $C_{44}H_{82}NO_6Cl\cdot 1/2H_2O$: C, 69.03; H, 10.93; N, 1.83; Cl, 4.63. Found: C, 68.78; H, 10.63; N, 2.02; Cl, 4.59.

***N*-[3-[2-(1,3-Dielaidoyloxy)propoxycarbonyl]propyl]-*N*,*N*,*N*-trimethylammonium iodide (1Bb).** Compound **1Bb** was prepared as described for **1Aa** using **5Bb** (0.79 g, 0.97 mmol). The residue was washed with Et_2O and dried under reduced pressure to afford a white powder **1Bb** (0.67 g, 78%).

Mp 74–77 °C. IR ν (KBr): 2920, 2850, 1754, 1465, 1416, 1363, 1252, 1176, 1116, 1065, 1022 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.5$ Hz, CH_3), 1.19–1.50 (40H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 1.95, 1.97 (4H each, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.14 (2H, m, $COCH_2C\underline{H}_2CH_2N$), 2.33 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.54 (2H, t, $J=7.0$ Hz, $COC\underline{H}_2CH_2CH_2N$), 3.49 (9H, s, $N(CH_3)_3$), 3.76 (2H, m, $CO\underline{C}H_2CH_2C\underline{H}_2N$), 4.13, 4.39 (4H, ABX, $J=5.0$, 4.5 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.17 (1H, m, $CH_2C\underline{H}CH_2$), 5.38 (4H, m, $CH_2C\underline{H}{=}C\underline{H}CH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.30, 171.12, 130.39, 130.06, 70.23, 65.52, 61.55, 53.75, 33.96, 32.51, 32.47, 31.81, 29.71, 29.56, 29.40, 29.38, 29.22, 29.09, 29.04, 28.99, 28.90, 24.76, 22.57, 18.30, 14.02. MS (EI) *m/z*: 733 (M^+-CH_3I, 21.6), 339 (1.1), 265 (1.0), 142 (75.9), 58 (100). Anal. calcd for $C_{46}H_{86}NO_6I$: C, 63.06; H, 9.89; N, 1.60; I, 14.49. Found: C, 62.77; H, 9.65; N, 1.63; I, 14.52.

***N*-[2-(1,3-Distearoyloxy)propoxycarbonylmethyl]-*N*,*N*,*N*-trimethylammonium chloride (1Ca).** Compound **1Ca** was prepared as described for **1Aa** using **4Ca** (0.92 g, 1.31 mmol). The residue was washed with Et_2O and dried under reduced pressure to afford a white powder **1Ca** (0.94 g, 94%).

Mp 99.5–101.5 °C. IR ν (KBr): 2915, 2848, 1740, 1647, 1471, 1398, 1255, 1236, 1196, 1182 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.0$ Hz, CH_3), 1.15–1.36 (56H, m, CH_2), 1.60 (4H, m, $OCOCH_2C\underline{H}_2$), 2.35 (4H, t, $J=8.0$ Hz, $OCOCH_2$), 3.63 (9H, s, $N(CH_3)_3$), 4.07, 4.55 (4H, ABX, $J=7.0$, 5.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 4.92 (2H, s, CH_2), 5.20 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 173.49, 164.33, 72.26, 63.07, 61.21, 54.29, 33.96, 31.90, 29.67, 29.47, 29.33, 29.26, 29.11, 24.80, 22.66, 14.09. MS (EI) *m/z*: 709 (M^+-CH_3Cl, 6.9), 605 (5.4), 341 (7.2), 267 (20.1), 85 (100). Anal. calcd for $C_{44}H_{86}NO_6Cl\cdot 1/2\ H_2O$: C, 68.67; H, 11.39; N, 1.82; Cl, 4.61. Found: C, 68.72; H, 11.31; N, 1.92; Cl, 5.04.

***N*-[3-[2-(1,3-Distearoyloxy)propoxycarbonyl]propyl]-*N*,*N*,*N*-trimethylammonium iodide (1Cb).** Compound **1Cb** was prepared as described for **1Aa** using **5Cb** (0.82 g, 1.00 mmol). The residue was washed with Et_2O and dried under reduced pressure to afford a white powder **1Cb** (0.50 g, 57%).

Mp 100–102 °C. IR ν (KBr): 2915, 2849, 1733, 1471, 1417, 1254, 1175, 1115 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=5.5$ Hz, CH_3), 1.16–1.38 (56H, m, CH_2), 1.61 (4H, m, $OCOCH_2C\underline{H}_2$), 2.12 (2H, m, $COCH_2C\underline{H}_2CH_2N$), 2.33 (4H, t, $J=7.5$ Hz, $OCOCH_2$), 2.54 (2H, t, $J=6.5$ Hz, $COC\underline{H}_2CH_2CH_2N$), 3.47 (9H, s, $N(CH_3)_3$), 3.73 (2H, m, $COCH_2CH_2C\underline{H}_2N$), 4.12, 4.41 (4H, ABX, $J=5.5$, 4.0 Hz, $C\underline{H}_2CHC\underline{H}_2$), 5.16 (1H, m, $CH_2C\underline{H}CH_2$). ^{13}C NMR ($CDCl_3$) δ: 173.40, 171.19, 96.07, 70.33, 65.54, 61.57, 53.77, 34.00, 31.88, 29.67, 29.45, 29.33, 29.24, 29.08, 24.84, 22.64, 18.30, 14.09. MS (EI) *m/z*: 737 (M^+-CH_3I, 4.4), 341 (1.4), 267 (2.2), 142 (23.1), 114 (14.9), 58 (100). Anal. calcd for $C_{46}H_{90}NO_6I$: C, 62.78; H, 10.30; N, 1.59; I, 14.41. Found: C, 62.49; H, 10.03; N, 1.63; I, 14.48.

Biological assay

Prepartion of cationic liposomes and transfection experiments were carried out according to the procedure previously reported.[12]

Acknowledgement

Part of this work was supported by a Grant-in-Aid for Scientific Research (B), No. 11470469, from Japan Society for the Promotion of Science.

References and Notes

1. Kasid, A.; Morecki, S.; Aebersold, P.; Cornetta, K.; Culver, K.; Freeman, S.; Director, E.; Lotze, M. T.; Blaese, R. M.; Anderson, W. F.; Rosenberg, S. A. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 473; Anderson, W. F. *Science* **1992**, *256*, 808; Mulligan, R. C. *Science* **1993**, *260*, 926; Dunbar C. E. *Annu. Rev. Med.* **1996**, *47*, 11.
2. Vile, R. G.; Tuszynski, A.; Castleden, S. *Mol. Biotechnol.* **1996**, *5*, 139; Moolten, F. L.; Cupples, L. A. *Hum. Gene Ther.* **1992**, *3*, 479; Cornetta, K.; Morgan, R. A.; Anderson, W. F. *Hum. Gene Ther.* **1991**, *2*, 5.
3. Lee, R. J.; Huang, L. *Crit. Rev. Ther. Drug Carrier Syst.* **1997**, *14*, 173.
4. Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7413; Felgner, P. L.; Ringold, G. M. *Nature* **1989**, *337*, 387.
5. For reviews: Miller, A. D. *Angew. Chem. Int. Ed.* **1998**, *37*, 1768; Bennett, M. J.; Aberle, A. M.; Balasubramaniam, R. P.; Malone, J. G.; Nantz, M. H.; Malone, R. W. *J. Liposome Res.* **1996**, *6*, 545; Felgner, P. L. *J. Liposome Res.* **1993**, *3*, 3; Brigham, K. L.; Schreier H. *J. Liposome Res.* **1993**, *3*, 31.
6. Felgner, J. H.; Kumar, R.; Sridhar, C. N.; Wheeler, C. J.; Tsai, Y. J.; Border, R.; Ramsey, P.; Martin, M.; Felgner, P. L. *J. Biol. Chem.* **1994**, *269*, 2550.
7. Leventis, R.; Silvius, J. R. *Biochim. Biophys. Acta* **1990**, *1023*, 124.
8. Gao, X.; Huang, L. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 280.
9. Behr, J.-P.; Demeneix, B.; Loeffler, J.-P.; Perez-Mutul, J. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6982.
10. Demeneix, B. A.; Abdel-Taweb, H.; Benoist, C.; Seugnet, I.; Behr, J.-P. *BioTechniques* **1994**, *16*, 496.
11. Hawley-Nelson, P.; Ciccarone, V.; Gebeyehu, G.; Iessee, J.; Felgner, P. L. *Focus* **1993**, *1*, 573.

12. Obika, S.; Yu, W.; Shimoyama, A.; Uneda, T.; Miyashita, K.; Doi, T.; Imanishi, T. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1817.
13. Obika, S.; Yu, W.; Shimoyama, A.; Uneda, T.; Minami, T.; Miyashita, K.; Doi, T.; Imanishi, T. *Biol. Pharm. Bull*. **1999**, *22*, 187.
14. Yu, W.; Shimoyama, A.; Uneda, T.; Obika, S.; Miyashita, K.; Doi, T.; Imanishi, T. *J. Biochem*. **1999**, *125*, 1034.
15. Kodali, D. R.; Atkinson, D.; Redgrave, T. G.; Small, D. M. *J. Lipid Res*. **1987**, *28*, 403.
16. Morrisett, J. D. *Lipids* **1974**, *9*, 726.
17. Bentley, P. H.; McCrare, W. *J. Org. Chem*. **1970**, *35*, 2082.
18. Cockman, S. J.; Joll, C. A.; Mortimer, B.-C.; Redgrave, T. G.; Stick, R. V. *Aust. J. Chem*. **1990**, *43*, 2093.
19. Verkleij, A. *J. Biochim. Biophys. Acta* **1984**, *779*, 43.
20. Walker, S.; Sofia, M. J.; Kakarla, R.; Kogan, N. A.; Wierichs, L.; Longley, C. B.; Bruker, K.; Axelrod, H. R.; Midha, S.; Babu, S.; Kahne, D. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1585.
21. Felgner, J. H.; Kumar, R.; Sridhar, C. J.; Wheeler, C. J.; Tsai, Y. J.; Border, R.; Ramsey, P.; Martin, M.; Felgner, P. L. *J. Biol. Chem*. **1994**, *269*, 2550.
22. Farhood, H.; Bottega, R.; Epand, R. M.; Huang, L. *Biochim. Biophys. Acta* **1992**, *1111*, 239.
23. Farhood, H.; Serbina, N.; Huang, L. *Biochim. Biophys. Acta* **1995**, *1235*, 28.
24. DLPC was utilized as a co-lipid in the cationic liposomes containing *N*-(α-trimethylammonioacetyl)didodecyl-D-glutamate chloride (TMAG). See: Koshizaka, T.; Hayashi, Y.; Yagi, K. *J. Clin. Biochem. Nutr*. **1989**, *7*, 185.