Synthesis of New Cationic Lipids from an Unsaturated Glycoside Scaffold.

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Supporting Information

Experimental procedures and NMR data for compounds **5** to **23**.
DNA complexation experiments.

General. All solvents were purchased from SDS (Peypin France). Dichloromethane was distilled from P_2O_5. DMF was dried over 3 Å molecular sieves and pyridine over KOH. All chemicals were purchased from Sigma-Aldrich-Fluka or Lancaster. Tosyl chloride was recrystallized in petroleum ether and triphenylphosphine in hexane. Other solvents and products were used without further purification. Reactions were monitored by thin layer chromatography using Merck precoated 60 F_{254} silica gel plates. Column chromatography was performed over SDS (Peypin France) 35-70μm silicagel according to the method of Still, Khan and Mitra[1]. HPLC were performed on a Waters Prep LC 4000 system at 235 nm on a Vydac-214 TP 1022 C4 column (25.2 mm). Mobile phases were H_2O and acetonitrile. Separation conditions were as follows: gradient H2O/MeCN; 0-5 min [80/20], 5-35 min [0/100], 35-45 min [0/100]; flow, 12 mL/min). Solid phase extractions were carried out using Supelco C-8 reverse-phase cartridges. Mobile phases were H_2O, acetonitrile and MeOH (HPLC grade). Separation conditions were as follows. H_2O/MeCN [1/0], H2O/MeCN [2/1], H2O/MeCN [1/2], H2O/MeCN [0/1], MeCN/MeOH [2/1]. For each concentration 30 mL of the mixture was eluted and the solutions were check by TLC. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER DRX-300 spectrometer. NMR spectra were processed using xwinnmr (Bruker) or SwaN-MR[2].

Octadec-9-enoic acid {3-hydroxy-6-[3-(2,2,2-trifluoro-acetylamino)-propoxy]-3,6-dihydro-2H-pyran-2-ylmethyl}-amide (**5**).

Triphenylphosphine (2.71 g, 10.34 mmol) was added to a solution of N-[3-(6-azidomethyl-5-hydroxy-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-2,2,2-trifluoro-acetamide (3.05 g, 9.4 mmol) in THF (47 mL) and the mixture was stirred at 30-40°C for 1 hour. Water (14 mL) was added and stirring was continued at 40 °C for 2 h. The solvents were removed under reduced pressure and the crude was redissolved in CH_2CI_2 (60 mL). Oleic acid (3 mL, 9.4 mmol) and dicyclohexylcarbodiimide (2.35 g, 11.28 mmol) was successively added. After two hours the reaction mixture was diluted with diethyl ether. *N,N'*-Dicyclohexylurea was remove by filtration. Concentration of the filtrate and final purification by flash chromatography (30 % heptane in ethyl acetate) afforded 4.7 g (50 %) of octadec-9-enoic acid (3-hydroxy-6-[3-(2,2,2-trifluoro-acetylamino)-propoxy]-3,6-dihydro-2H-pyran-2-ylmethyl)-amide (**5**).
^{1}H NMR ($CDCl_3$) 0.87 (t, 3 H, *J* = 6.73 Hz, H-18), 1.28 (m, 20 H, H-4,7 and H-12,17 octadecenoate), 1.63 (m, 2 H, H-3 octadecenoate), 1.89 (m, 2 H, H-2 propyl), 1.98 (m, 4 H, H-8 and H-11), 2.2 (dd, 2 H, *J* = 7.32, 7.95 Hz, H-2 octadecenoate), 3.10 (ddd, 1 H, *J* = 3.38,

4.73, 14.85 Hz, H-2'), 3.46 (m, 2 H, H-3 propyl), 3.56 (m, 2 H, H-2 and H-1 propyl), 3.89 (2 H, H-3 and H-1 propyl), 4.00 (ddd, 1 H, *J* = 3.08, 8.07, 14.85 Hz, H-2'), 4.28 (m, 1 H, OH), 4.92 (br s, 1 H, H-6), 5.28 (m, 2 H, H-9 and H-10), 5.61 (ddd, 1 H, *J* = 2.53, 2.57, 10.21 Hz, H-5), 6.00 (br d, *J* = 10.21 Hz, H-4), 6.11 (dd, 1 H, *J* = 4.98, 7.70 Hz, NHCO), 7.35 (m, 1 H, CF$_3$CONH).

^{13}C NMR (CDCl$_3$) δ 14.09 (C-18), 22.7 (C-17), 25.73 (C-3 octadecenoate), 27.24 (C-8 and C-11), 28.59 (C-2 propyl), 29.29 (C-4,7 and C-12,15), 31.93 (C-16), 36.55 (C-2 octadecenoate), 38.72 (C-3 propyl), 40.53 (C-2'), 64.2 (C-3), 67.16 (C-1 propyl), 71.57 (C-2), 95.33 (C-6), 124.91 (C-5), 129.74 and 130.09 ((C-9 and C-10), 134.4 (C-4), 175.42 (C-1 octadecenoate).

Octadec-9-enoic acid-[6-(3-amino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-ylmethyl]-amide (**6**).

A solution of potassium carbonate (10% in water, 20 mL) was added to a solution of octadec-9-enoic acid (3-hydroxy-6-[3-(2,2,2-trifluoro-acetylamino)-propoxy]-3,6-dihydro-2H-pyran-2-ylmethyl)-amide (**5**) (2.25 g, 4 mmol) in methanol/THF (90 mL 2:1). The reaction mixture was stirred overnight at 40° C. The solvent was evaporated under reduced pressure. The resulting oil was dissolved in ethanol, and the precipitate was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The resulting oil was purified by solid phase extraction (C-8 cartridge, 10 g). Extraction with acetonitrile gave octadec-9-enoic acid-[6-(3-amino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-ylmethyl]-amide (**6**) as an oil (1.9 g, 96 %).

^{1}H NMR (CDCl$_3$) 0.86 (t, 3 H, *J* = 6.77 Hz, H-18), 1.25 (m, 16 H, H-4,6 and H-13,17), 1.33 (m, 4 H, H-7 and H-12), 1.60 (m, 2 H, H-3 octadecenoate), 1.72 (m, 2 H, H-2 propyl), 1.99 (m, 4 H, H-8 and H-11), 2.16 (dd, 2 H, *J* = 7.35, 7.97 Hz, H-2 octadecenoate), 2.74 (t, 2 H, *J* = 6.6 Hz, H-3 propyl), 3.20 (ddd, 1 H, *J* = 2.88, 4.84, 14.63 Hz, H-2'), 3.46 (m, 1 H, H-1 propyl), 3.64 (ddd, 1 H, *J* = 3.14, 3.5, 9.15 Hz, H-2), 3.73 (m, 1 H, H-1propyl), 3.88 (m, 2 H, H-5 and H-2'), 4.90 (ddd, 1 H, , *J* = 1.14, 1.5, 2.5 Hz, H-6), 5.31 (m, 2 H, H-9 and H-10), 5.63 (ddd, 1 H, *J* = 2.5, 2.5, 10.2 Hz, H-5), 6.01 (ddd, 1 H, *J* = 1.14, 1.4, 10.2 Hz, H-4), 6.36 (dd, 1 H, *J* = 5.08, 7.96 Hz, NHCO).

^{13}C NMR (CDCl$_3$) δ 14.9 (C-18), 22.71 (C-17), 25.79 (C-3 octadecenoate), 27.24 (C-8 and C-11), 29.76 (C-4,7, C-12,15 and C-2 propyl), 33.20 (C-16), 36.57 (C-2 octadecenoate), 39.34 (C-3 propyl), 40.50 (C-2'), 64.09 (C-3), 66.52 (C-1 propyl), 71.35 (C-2), 95.08 (C-6), 125.5 (C-5), 129.75 (C-10), 130.07 (C-9), 134.06 (C-4), 175.55 (C-1 octadecenoate).

Octadec-9-enoic acid-[6-(3-guanidino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-yl - methyl]-amide hydrogen sulfate (**7a**)

A solution of *O*-methylisourea hydrogen sulfate (0.68 g, 3.94 mmol) in water (4 mL) was slowly added to a solution of octadec-9-enoic acid-[6-(3-amino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-ylmethyl]-amide (**6**) (0.92 g, 1.97 mmol) and triethylamine (1.98 mL, 14.18 mmol) in methanol (4 mL). The reaction mixture was stirred overnight at 40°C. The solution was evaporated to dryness under reduced pressure. The resulting oil was purified by flash chromatography (CH$_2$Cl$_2$/MeOH 8:2) to yield octadec-9-enoic acid-[6-(3-guanidino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-yl-methyl]-amide hydrogen sulfate (**7a**) (0.77 g, 70 %).

7a ^{1}H NMR (CDCl$_3$) 0.86 (t, 3 H, *J* = 6.91Hz, H-18), 1.28 and 1.30 (m, 20 H, H-4,7 and H-12,17), 1.55 (m, 2 H, H-3 octadecenoate), 1.85 (m, 2 H, H-2 propyl), 1.97 (m, 4 H, H-8 and H-11), 2.23 (t, *J* = 7.24 Hz, H-2 octadecenoate), 3.31 (m, 2 H, H-3 propyl and H-2'), 3.51 (m, 1 H, H-1 propyl), 3.63 (m, 1 H, H-2), 3.70 (m, 1 H, H-2'), 3.75 (m, 1 H, H-1 propyl), 3.93 (br d, 1 H, *J* = 9.09 Hz, H-3), 4.93 (m, 1 H, H-6), 5.31 (m, 2 H, H-9 and H-10), 5.66 (br d, 1 H, *J*

= 10.18 Hz, H-5), 5.94 (br d, 1 H, *J* = 10.18 Hz, H-4), 7.02 (dd, 1 H, *J* = 6.34, 7.57 Hz, CONH), 7.64 (m, C=NH).

^{13}C NMR ($CDCl_3$) δ 14.04 (C-18), 22.62 (C-17), 25.86 (C-3 octadecenoate), 27.20 (C-8 and C-11), 29.44 (C-4,7 and C-12,15), 29.77 (C-2 propyl), 31.85 (C-16), 36.27 (C-2 octadecenoate), 38.36 (C-3 propyl), 40.30 (C-2'), 64.25 (C-3), 65.11 (C-1 propyl), 71.04 (C-2), 94.74 (C-6), 125.39 (C-5), 129.58 and 129.95 (C-9 and C-10), 134.08 (C-4), 157.60 (C=NH), 175.5 (C-1 octadecenoate).

Octadec-9-enoic acid-[6-(3-guanidino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-yl-methyl]-amide hydrogen hydrochloride (**7b**).

Using the same procedure octadec-9-enoic acid-[6-(3-amino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-ylmethyl]-amide (**6**) (0.86 g, 1.85 mmol) was dissolved in methanol (3.7 mL) then triethylamine (0.52 mL, 3.7 mmol) and a water solution (3.7 mL) of *O*-methylisourea hydrochloride (0.307 g, 2.77 mmol) were added successively. Purification by solid phase extraction (C-8 10 g cartridge water/acetonitrile 1:2 to 2:1) yielded 0.75 g (74.4 %) of octadec-9-enoic acid-[6-(3-guanidino-propoxy)-3-hydroxy-3,6-dihydro-2H-pyran-2-yl methyl]-amide hydrochloride (**7b**).

7b ^{1}H NMR ($CDCl_3$) 0.86 (t, 3 H, *J* = 6.92 Hz, H-18), 1.26 (m, 16 H, H-4,6 and H-13,17), 1.28 (m, 4 H, H-7 and H-12), 1.44 (m, 2 H, H-3 octadecenoate), 1.70 (m, 2 H, H-2 propyl), 1.96 (m, 4 H, H-8 and H-11), 2.04 (dd, 2 H, *J* = 7.19, 7.71 Hz, H-2 octadecenoate), 3.12 (m, 2 H, H-3 propyl), 3.17 (m, 1 H, H-2'), 3.41 (m, 1 H, H-1 propyl), 3.46 (m, 2 H, H-2 and H-2'), 3.65 (m, 1 H, H-1 propyl), 3.73 (m, 1 H, H-3), 4.90 (br dd, 1 H, *J* = 1.2, 2.25, Hz, H-6), 5.17 (d, 1 H, *J* = 6.19 Hz, OH), 5.31 (m, 2 H, H-9 and H-10), 5.63 (ddd, 1 H, *J* = 2.24, 2.48, 10.27 Hz, H-5), 5.81 (br d, 1 H, *J* = 10.27 Hz, H-4), 7.75 (t, 1 H, *J* = 5.6 Hz, C=NH guanidine), 7.92 (t, 1 H, *J* = 5.7 Hz, NHCO).

^{13}C NMR ($CDCl_3$) δ 14.44 (C-18), 22.6 (C-17), 25.86 (C-3 octadecenoate), 27.12 (C-8 and C-11), 29.22 (C-4,7 and C-12,17), 29.63 (C-2 propyl), 31.78 (C-16), 35.81 (C-2 octadecenoate), 38.61 (C-3 propyl), 39.30 (C-2'), 64.67 (C-3), 64.99 (C-1 propyl), 71.28 (C-2), 93.78 (C-6), 125.88 (C-5), 129.67 (C-9 C-10), 134.79 (C-4), 157.65 (C=NH), 173.2 (C-1 octadecenoate).

Tetradecanoic acid-[3-hydroxy-6-[3-(2,2,2-trifluoro-acetylamino)-propoxy]-3,6-dihydro-2H-pyran-2-ylmethylamide (**8**)**.**

Triphenylphosphine (0.91 g, 3.45 mmol) was added to a solution of N-(3-(6-azidomethyl-4-hydroxy-5,6-dihydro-2H-pyran-2-yloxy)-propyl)-2,2,2-trifluoroacetamide (**4**) (0.97 g, 3 mmol) in THF (15 mL) then the solution was stirred for 1 hour at 30-40°C. Water (4 mL) was added and the reaction mixture was stirred at 40 °C for 2 h. After removal of the solvents the crude was dissolved in CH_2CI_2 (20 mL). Tetradecanoic acid (0.68 g, 3 mmol) and dicyclohexylcarbodiimide (0.825 g, 4 mmol) were successively added. After two hours the reaction mixture was diluted with diethyl ether and filtered through a celite pad. The filtrate was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography (30 % heptane in ethyl acetate) to yield 1.07 g (70 %) of tetradecanoic acid-[3-hydroxy-6-[3-(2,2,2-trifluoro-acetylamino)-propoxy]-3,6-dihydro-2H-pyran-2-ylmethyl-amide (**8**).

^{1}H NMR ($CDCl_3$) 0.87 (t, 3 H, *J* = 6.76Hz, H-14), 1.28 (m, 20 H, H-4 ,13 tetradecanoate), 1.63 (m, 2 H, H-3 tetradecanoate), 1.89 (m, 2 H, H-2 propyl), 2.2 (dd, 2 H, *J* = 7.23, 8.08 Hz, H-2 tetradecanoate), 3.15 (ddd, 1 H, *J* = 2.87, 4.91, 14.84 Hz, H-2'), 3.51 (m, 2 H, H-3 propyl), 3.61 (m, 2 H, H-2 and H-1 propyl), 3.85-3.93 (2 H, H-3 and H-1 propyl), 4.07 (ddd, 1 H, *J* = 3.02, 8.30, 14.84 Hz, H-2'), 4.2 (d, 1 H *J* = 5.18 Hz, OH), 4.94 (ddd, 1 H, *J* = 1.35, 1.54, 2.48 Hz, H-6), 5.65 (ddd, 1 H, *J* = 2.48, 2.52, 10.21 Hz, H-5), 6.07 (ddd, *J* = 1.54, 1.57, 10.21 Hz, H-4), 6.07 (overlap, 1 H, <u>NH</u>CO), 7.28 (m, 1 H, $CF_3CO\underline{NH}$).

1-chloromethoxy-tetradecane (**9**).

1-Tetradecanol (0.54 g, 2.5 mmol) was added portionwise to a suspension of paraformaldehyde (0.075 g, 2.5 mmol) in trimethylchlorosilane (1.25 mL). The reaction mixture was stirred for 2 h at room temperature. The clear solution was evaporated under reduced pressure to yield 1-chloromethoxy-tetradecane as colorless oil that was used without further purification.

Tetradecanoic acid 3-tetradecyloxymethoxy-6-(3-(2,2,2-trifluoro-acetylamino)-propoxy)-3,6-dihydro-2H-pyran-2-ylmethyl)-amide (**10**)

A solution of freshly prepared 1-chloromethoxy-tetradecane (0.636 g, 2.43 mmol) in THF (0.5 mL) was added to a solution of tetradecanoic acid (3-hydroxy-6-(3-(2,2,2-trifluoro-acetylamino)-propoxy)-3,6-dihydro-2H-pyran-2-ylmethyl)-amide (0.41 g, 0.81 mmol), diisopropylethylamine (0.562 mL, 3.24 mmol) and tetrabutylammonium iodide (0.3 g, 0.81 mmol) in THF (2.5 mL), The solution was stirred at 60° C for 4 h. The reaction mixture was cooled to room temperature, treated with a saturated $NaHCO_3$ solution, and extracted with diethyl ether. The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was subjected to flash chromatography (heptane ethyl acetate 9:1 to remove the excess of tetradecanol then heptane ethyl acetate 1:1) to yield 0.42 g of **10** (70 %).

^{1}H NMR ($CDCl_3$) 0.86 1 (t, 6 H, *J* = 7.48 Hz, H-14), 1.22 (m, 42 H, H-4,13 tetradecanoate and H-3,13 tetradecyl), 1.59 (m, 4 H, H-3 tetradecanoate and H-2 tetradecyl), 1.89 (m, 2 H, H-2 propyl), 2.12 (t, 2 H, *J* = 7.73Hz, H-2 tetradecanoate), 3.39 (ddd, 1 H, *J* = 4.45, 4.47, 13.64 Hz, H-6'), 3.47 (m, 2 H, H-3 propyl), 3.59 (m, 2 H, H-1 propyl), 5.61 (ddd, 2 H, *J* = 5.41, 5.91, 10.25 Hz, H-1 tetradecyl), 3.68 (ddd, 1 H, *J* = 4.84, 7.06, 13.65 Hz, H-6'), 3.76 (m, 1 H, H-6), 3.88 (m, 1 H, H-1 propyl), 3.94 (ddd, 1 H, *J* = 5.47, 5.87, 10.29 Hz, H-5), 4.70 (d, 1 H, *J* = 6.94 Hz, OCH_2O), 4.90 (d, 1 H, *J* = 6.94 Hz, OCH_2O), 4.94 (m, 1 H, H-2), 5.72 (ddd, 1 H, *J* = 1.72, 2.86, 10.27 Hz, H-3), 5.9 (dd, 1 H, *J* = 4.63, 6.9 Hz, NHCO), 5.99 (ddd, 1 H, *J* = 1.31, 1.45, 10.27 Hz, H-4), 7.48 (m, 1 H, NHCO),

^{13}C NMR ($CDCl_3$) δ 14.17 (C-14), 22.71 (C-13), 25.77 (C-3 tetradecanoate), 26.22 (C-3 tetradecyl), 28.64 (C-2 propyl), 29.68 (C4,11 tetradecanoate and tetradecyl), 31.95 (C-12), 36.85 (C-2 tetradecanoate), 38.68 (C-3 propyl), 40.55 (C-6'), 67.33 (C-1 tetradecyl), 68.67 (C-5 et C-1 propyl), 71.52 (C-6), 94.73 (C-2), 95.52 (OCH_2O), 125.73 (C-3), 132.0 (C-4).

Tetradecanoic acid (6-(3-amino-propoxy)-3-tetradecyloxymethoxy-3,6-dihydro-2H-pyran-2-ylmethyl)-amide (**11**).

Tetradecanoic acid 3-tetradecyloxymethoxy-6-(3-(2,2,2-trifluoro-acetylamino)-propoxy)-3,6-dihydro-2H-pyran- 2-ylmethyl)-amide (**10**) (1.8 g, 2.45 mmol) in methanol/THF (57 mL 2:1) was treated by potassium carbonate (10% solution in water, 12.25 mL). The reaction mixture was stirred overnight at 40° C then the solvent was evaporated under reduced pressure. The resulting oil was dissolved in ethanol. The precipitated was removed by filtration and the filtrate was evaporated to dryness. The resulting oil was purified by solid phase extraction on a C-8 cartridge (10 g). Extraction with acetonitrile afforded 1.55 g of the amine (**11**) (99 %).

^{1}H NMR ($CDCl_3$) 0.86 5 (t, 6 H, *J* = 6.7, Hz, H-14), 1.22 (m, 40 H, H-4,13 tetradecanoate and tetradecyl), 1.30 (m, 2 H, H-3 tetradecyl), 1.53 (m, 2 H, H-2 tetradecyl), 1.59 (m, 2 H, H-3 tetradecanoate), 1.75 (m, 2 H, H-2 propyl), 2.16 (t, 2 H, *J* = 7.6 Hz, H-2 tetradecanoate), 2.83 (dd, 2 H, *J* = 6.29, 6.29 Hz, H-3 propyl), 3.43 (m, 1 H, H-6'), 3.53 (m, 2 H,H-1 propyl), 3.59 (m, 1 H, H-6'), 3.78 (m, 1 H, H-6), 3.84 (m, 1 H, H-1 propyl), 3.90 (ddd, 1 H, *J* = 1.58, 2.69, 9.12 Hz, H-5), 4.68 (d, 1 H, *J* = 7.07 Hz, OCH_2O), 4.78 (d, 1 H, *J* = 7.1 Hz, OCH_2O), 4.92 (m, *J* = 1.11, 1.29 Hz, H-2), 5.70 (ddd, 1 H, *J* = 1.77, 2.8, 10.29 Hz, H-3), 5.95 (ddd, 1 H, *J* = 1.11, 3.28, 10.26 Hz, H-4), 6.29 (dd, *J* = 4.88, 4.94 Hz, CONH).

^{13}C NMR ($CDCl_3$) δ 14.4 (C-14), 22.71 (C-13), 25.86 (C-3 alkyl), 26.23 (C-3 alkyl), 29.69 (C-4 C-11 alkyl), 31.96 (C-3 tetradecyl), 32.92 (C-12), 33.1 (C-2 propyl), 36.80 (C-2 tetradecanoate), 39.16 (C-3 propyl), 40.35 (C-6'), 66.36 (C-1 propyl), 68.64 (C-6 and C-1 tetradecyl), 71.12 (C-5), 94.41 (C-2), 95.49 (OCH_2O), 126.29 (C-3), 131.61 (C-4), 173.5 (C-1).

Tetradecanoic acid (6-(3-guanidino-propoxy)-3-tetradecyloxymethoxy-3,6-dihydro-2H-pyran-2-ylmethyl)-amide hydrochloride (**12**).

A solution of *O*-methylisourea (0.17 g, 1.53 mmol) in water (2.04 mL) was added slowly to a solution of tetradecanoic acid (6-(3-amino-propoxy)-3-tetradecyloxymethoxy-3, 6-dihydro-2H-pyran-2-ylmethyl)-amide (**11**) (0.65 g, 1.02 mmol) and triethylamine (0.28 mL, 2.04 mmol). in methanol (2.04 mL). The mixture was stirred at 40 °C overnight. The solution was evaporated to dryness. The residue was purified by chromatography (CH_2Cl_2/MeOH 9:1 then 8:2) to give 0.36 g (50 %) of the guanidine **12**.

^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, *J* = 6.58 Hz, H-14), 1.20 (m, 42 H, H-3,13 tetradecyl and H-4,13 tetradecanoate), 1.44 (m, 2 H, H-3 tetradecanoate), 1.69 (m, 2 H, H-2 propyl), 2.06 (m, 2 H, H-2 tetradecanoate), 3.16 (m, 2 H, H-3 propyl), 3.22 (m, 1 H, H-6'), 3.31 (m, 1 H, H-6'), 3.41 (m, 1 H, H-1 propyl), 3.47 (m, 2 H, H-1 tetradecyl), 3.59 (m, 1 H, H-6), 3.65 (m, 1 H, H-1 propyl), 3.80 (m, 1 H, H-5), 4.62 (d, 1 H, *J* = 7.07 Hz, OCH_2O), 4.70 (d, 1 H, *J* = 7.07 Hz, OCH_2O), 4.88 (m, 1 H, H-2), 5.70 (ddd, 1 H, *J* = 2.12, 2.15, 10.21 Hz, H-3), 5.91 (br d, 1 H, *J* = 10.21 Hz, H-4), 7.67 (dd, 1 H, *J* = 4.17, 4.67 Hz, NH guanine), 7.81 (dd, 1 H, *J* = 4.79, 5.63 Hz, NHCO).

^{13}C NMR ($CDCl_3$) δ 14.67 (C-14), 22.91 (C-13), 26.2 and 26.6 (C-3 tetradecyl and tetradecanoate), 29.75 (C-2 propyl, C-3,11 tetradecanoate and C-4,11 tetradecyl), 32.02 (C-12), 36.06 (C-2 tetradecanoate), 38.69 (C-3 propyl), 40.09 (C-6'), 68.32 (C-1 propyl and C-1 tetradecyl), 69.37 (C-6), 70.77 (C-5), 94.26 (C-2), 95.14 (OCH_2O), 127.40 (C-3), 131.78 (C-4),

1-chloromethoxy-*cis*-octadec-9-ene (**13**).

To a stirred suspension of paraformaldehyde (0.225 g, 7.5 mmol) in trimethylchlorosilane (3.75 mL) was added portionwise *cis*-octadec-9-ene-1-ol (2.04 g, 7.5 mmol). After two hours the solution was evaporated under vacuum to yield 1-chloromethoxy- *cis*-octadec-9-ene (**13**) as a colorless oil that was used without further purification.

2,2,2-Trifluoro-N-[3-(5-hydroxy-6-octadec-9-enyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**14**).

2,2,2-Trifluoro-N-[3-(5-hydroxy-6-hydroxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**3**) (0.9 g, 3 mmol) was dissolved in THF (10.5 mL), and this solution was treated sequentially with diisopropylethylamine (1.55 mL, 9 mmol), tetrabutylammonium iodide (1.108 g, 3 mmol) and 1-chloromethoxy-*cis*-octadec-9-ene (**13**) (2.38 g, 7.5 mmol). The mixture was stirred at room temperature for 2 hours. The excess of chloromethoxy ether was decomposed with a saturated $NaHCO_3$ solution. The two layers were separated and the aqueous phase was extracted with three portions of diethyl ether. The combined organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was subjected to flash chromatography (heptane-AcOEt 9:1 then 5:5) to yield 2.1 g (70 %).

^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, *J* = 7.3 Hz, H-18), 1.32 (m, 22 H, H-7,3 and H-12, 17), 1.57 (m, 2 H, H-2 octadecenyl), 1.87 (m, 2 H, H-2 propyl), 2.02 (m, 4 H, H-8 and H-11), 2.3 (d, 1 H, *J* = 6.53 Hz, OH), 3.45 (m, 4 H, H-3 propyl and H-1 octadecenyl), 3.67 (m, 1 H, H-1 propyl), 3.72 (m, 1 H, H-6), 3.80 (m, 1 H, H-6'), 3.86 (m, 1 H, H-6'), 3.92 (m, 1 H, H-1 propyl), 4.17 (m, H-5), 4.72 (m, 2H, OCH$_2$O), 4.97 (m, 1 H, H-2), 5.31 (m, 2 H, H-9,10), 5.74 (ddd, 1 H, *J* = 1.95, 3.05, 10.4 Hz, H-3), 5.95 (ddd, 1 H, *J* = 1.34, 2.62, 10.4 Hz, H-4), 7.28 (m, 1 H, NHCO).

^{13}C NMR (CDCl$_3$) 14.50 (C-18), 23.09 (C-17), 26.42 (C-3 octadecenyl), 27.47 (C-8 and C-11), 28.52 (C-2 propyl), 29.75 (C-4,8 and C-12,15), 29.93 (C-2 octadecenyl), 32.20 (C-16), 38.87 (C-3 propyl), 65.16 (C-5), 67.44 (C-6'), 68.14 (C-1 propyl), 68.67 (C-1 octadecenyl), 71.12 (C-6), 94.79 (C-2), 96.02 (OCH$_2$O), 125.99 (C-3), 130.03 and 130.38 (C-9 and C-10), 133.71 (C-4), 168.59 (NHCO).

2,2,2-Trifluoro-N-[3-(5-hydroxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**15**).

2,2,2-Trifluoro-N-[3-(5-hydroxy-6-hydroxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**3**) (1.15 g, 3.85 mmol) was dissolved in THF (11 mL) and this solution was treated sequentially with diisopropylethylamine (1.1 mL, 5.78 mmol, tetrabutylammonium iodide (1.41 g, 3.85 mmol) and with a freshly prepared solution of 1-chloromethoxy-tetradecane (1.52 g, 5.78 mmol) in THF (2.5 mL). The mixture was stirred at room temperature for 2 hours. The excess of chloromethoxy ether was decomposed with a saturated NaHCO$_3$ solution. The two layers were separated and the aqueous phase was extracted with three portions of diethyl ether. The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated. The residue was subjected to flash chromatography (heptane-AcOEt 5:5 then 3:7) to give 1.44 g (71 %) of the tetradecyloxy acetal **15**.

^{1}H NMR (CDCl$_3$) δ 0.89 (t, 3 H, *J* = 7.09 Hz, H-14), 1.20 (m, 20 H, H-4,13), 1.3 (m, 2 H, H-3 tetradecyl), 1.53 (m, 2 H, H-2 tetradecyl), 1.87 (m, 2 H, H-2 propyl), 2.37 (d, 1 H, *J* = 6.4 Hz, OH), 3.52 (m, 2 H, H-3 propyl), 3.56 (m, 2 H, H-1 tetradecyl), 3.69 (m, 1 H, H-1 propyl), 3.73 (m, 2 H, H-6 and H-6'), 3.86 (m, 1 H, H-6'), 3.95 (m, 1 H, H-1 propyl), 4.15 (br t, 1 H, *J* = 7.72 Hz, H-5), 4.72 (s, 2 H, OCH$_2$O), 4.96 (br s, 1 H, Hz, H-2), 5.72 (ddd, 1 H, *J* = 2.02, 2.7, 10.18 Hz, H-3), 5.93 (ddd, 1 H, *J* = 1.18, 1.9, 10.18 Hz, H-4), 7.28 (m, NH).

^{13}C NMR (CDCl$_3$) δ 14.50 (C-14), 23.09 (C-13), 26.59 (C-3), 28.35 (C-2 propyl), 29.93 (C-4,11), 32.20 (C-12), 38.87 (C-3 propyl), 65.16 (C-5), 67.44 (C-6'), 68.14 (C-1 propyl), 68.67 (C-1 tetradecyl), 71.12 (C-6), 94.79 (C-2), 96.02 (OCH$_2$O), 125.82 (C-3), 133.88 (C-4),

2,2,2-Trifluoro-N-[3-(5-tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**16**).

2,2,2-Trifluoro-N-[3-(5-hydroxy-6-hydroxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**3**) (3 g, 10 mmol) was dissolved in THF (30 mL), and this solution was treated sequentially with diisopropylethylamine (10.41 mL, 60 mmol), tetrabutylammonium iodide (7.35 g, 20 mmol) and a freshly prepared solution of 1-chloromethoxy-tetradecane (13.14 g, 50 mmol). This mixture was stirred at 60° C for 4 h then cooled to room temperature. The excess of chloromethoxy ether was decomposed with a saturated NaHCO$_3$ solution. The two layers were separated and the aqueous phase was extracted with three portions of diethyl ether. The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated. The residue was subjected to flash chromatography (heptane-AcOEt 9:1 then 5:5) to afford **16** (5.26 g, 70 %).

^{1}H NMR (CDCl$_3$) δ 0.89 (t, 3 H, *J* = 6.8 Hz, H-14), 1.30 (m, 20 H, H-4,13 tetradecyl), 1.34 (m, 2 H, H-3 tetradecyl), 1.57 (m, 2 H, H-2 tetradecyl), 1.89 (m, 2 H, H-2 propyl), 3.49 (m, 2 H,H-3 propyl), 3.53 (m, 1 H, *J* = 6.63 Hz, H-1 tetradecyl), 3.63 (m, 2 H, H-1 propyl H-1), 3.68 (m, 1 H, H-6'), 3.86 (m, 1 H, H-6), 3.92 (m, 1 H, H-1 propyl), 4.06 (m, 1 H, H-6), 4.1 (br d, 1 H, *J* = 9.27 Hz, H-5), 4.70 (d, 1 H, *J* = 6.86 Hz, OCH$_2$O), 4.80 (d, 1 H, *J* = 7.08 Hz, OCH$_2$O), 4.99 (m, 1 H, H-2), 5.72 (ddd, 1 H, *J* = 1.83, 2.79, 10.3 Hz, H-3), 6.03 (ddd, 1 H, *J* = 0.8, 2.11, 10.3 Hz, H-4), 7.36 (m, NH).

^{13}C NMR ($CDCl_3$) δ 14.10 (C-14), 22.72 (C-13), 26.27 (C-3 tetradecyl), 28.31 (C-2 propyl), 28.31 (C-2 propyl), 29.70 (C-4,11 tetradecyl), 31.97 (C-12), 38.57 (C-3 propyl), 66.94 (C-1 propyl C-6'), 68.24 (C-1 tetradecyl), 68.66 (C-1 tetradecyl), 69.73 (C-6), 69.98 (C-5), 94.54 (C-2), 95.12 (OCH20), 95.63 (OCH_2O), 125.91 (C-3), 131.9 (C-4).

6-(3-Amino-propoxy)-2-octadec-9-enyloxymethoxymethyl-3,6-dihydro-2H-pyran-3-ol (**17).**

A solution of **14** (2.25 g, 4 mmol) in methanol/THF (90 mL 2:1) was treated with potassium carbonate (10% in water, 20 mL). The reaction mixture was stirred overnight at 40 °C then the solvent was evaporated under reduced pressure. The resulting oil was dissolved in ethanol. The precipitated was removed by filtration and the filtrate was evaporated to dryness to give an oil that was used without further purification.

^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.14, Hz, H-18), 1.30 (m, 20 H, H-4,7 and H-12,17), 1.36 (m, 2 H, H-3 octadecenyl), 1.55 (m, 2 H, H-2 octadecenyl), 1.75 (m, 2 H, H-2 propyl), 1.98 (m, 4 H, H-8 and H-11), 2.79 (t, 2 H, J = 6.70, Hz, H-3 propyl), 3.53 (m, 2 H, H-1 octadecenyl), 3.70 (m, 1 H, H-2'), 3.76 (m, 1 H, H-2), 3.84 (m, 2 H, H-1 propyl and H-2'), 4.19 (br d, 1 H, J = 8.98 Hz, H-3), 4.72 (m, 2 H, OCH_2O), 4.96 (br s, 1 H, H-2), 5.33 (m, 2 H, H-9 and H-10), 5.72 (ddd, 1 H, J = 1.89, 3.1, 10.3 Hz, H-5), 5.95 (ddd, 1 H, J = 0.84, 2.24, 10.3 Hz, H-4).

^{13}C NMR ($CDCl_3$) δ 14.32 (C-18), 23.09 (C-17), 26.59 (C-3 octadecenyl), 27.47 (C-8 and C-11), 29.75 (C-2, C-4,7 and C-12,15 octadecenyl), 32.03 (C-16), 33.78 (C-2 propyl), 39.74 (C-3 propyl), 64.99 (C-5), 66.74 (C-6'), 67.97 (C-1 propyl), 68.49 (C-1 octadecenyl), 70.77 (C-6), 94.61 (C-1), 96.02 (OCH_2O), 126.3 (C-3), 130.20 (C-9 and C-10), 133.71 (C-4).

6-(3-Amino-propoxy)-2-tetradecyloxymethoxymethyl-3,6-dihydro-2H-pyran-3-ol (**18**).

A solution of 2,2,2-trifluoro-N-[3-(5-hydroxy-6-tetradecyloxymethoxymethyl-tetrahydro-pyran-2-yloxy)-propyl]-acetamide (**15**) (2.8 g, 5.31 mmol) in methanol/THF (125 mL 2:1) was treated with potassium carbonate (10% in water, 26 mL). The reaction mixture was stirred overnight at 40° C then the solvent was evaporated under reduced pressure. The resulting oil was dissolved in ethanol. The precipitated was removed by filtration then the solution was evaporated to dryness to give an oil that was used without further purification.

^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3 H, J = 6.71Hz, H-14), 1.22 (m, 20 H, H-4,13 tetradecyl), 1.30 (m, 2 H, H-3 tetradecyl), 1.55 (m, 2 H, H-2 tetradecyl), 1.71 (m, 2 H, H-2 propyl), 2.75 (t, 2 H, J = 6.71Hz, H-3 propyl), 3.66 (m, 2 H, H-2 and H-2'), 3.76 (m, 1 H, H-2'), 3.82 (m, 1 H, H-1 propyl), 4.12 (ddd, 1 H, H-3), 4.68 (m, 2 H,OCH_2O), 4.92 (ddd, 1 H, J = 1.12, 1.37, 2.7, Hz, H-6), 5.68 (ddd, 1 H, J = 2.2, 2.7, 10.19 Hz, H-5), 5.91 (m, 1 H, J = 1.12, 1.73, 10.19 Hz, H-4),

^{13}C NMR ($CDCl_3$) δ 14.67 (C-14), 23.09 (C-13), 26.59 (C-3 tetradecyl), 30.10 (C-4,C11 tetradecyl), 32.2 (C-12), 33.61 (C-2 propyl), 39.57 (C-3 propyl), 64.29 (C-3), 66.56 (C-2'), 67.62 (C1-propyl), 68.32 (C-1 tetradecyl), 70.60 (C-2), 94.61 (C-6), 95.84 (OCH_2O), 126.17 (C-5), 134.06 (C-4).

3-(5-Tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propylamine (**19**).

2,2,2-Trifluoro-N-[3-(5-tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-acetamide (**16**) (7.29 g, 10.05 mmol) in methanol/THF (220 mL 2:1) was treated with potassium carbonate (10% in water, 48 mL). The reaction mixture was stirred overnight at 40 °C then the solvent was evaporated under reduced pressure. The resulting oil was dissolved in ethanol. The precipitated was removed by filtration then the solution was evaporated to dryness to afford an oil that was used without further purification.

^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6 H, *J* = 7.30 Hz, H-14), 1.24 (m, 40 H, H-3,13 tetradecyl), 1.34 (m, 4 H, H-3 tetradecyl), 1.53 (m, 4 H, H-2 tetradecyl), 1.73 (m, 2 H, H-2 propyl), 2.77 (t, 2 H, *J* = 6.91 Hz, H-3 propyl), 3.51 (m, 5 H, H-1 propyl and H-1 tetradecyl), 3.72 (m, 1 H, H-6'), 3.82 (m, 3 H, H-6, H-6', H-1propyl), 4.15 (dddd, 1 H, *J* = 1.51, 1.69, 1.81, 7.93 Hz, H-5), 4.72 (d, 2 H, *J* = 7.68 Hz, OCH_2O), 4.78 (d, 2 H, *J* = 7.68 Hz, OCH_2O), 4.96 (m, H-2), 5.72 (ddd, 1 H, *J* = 1.75, 3.31, 10.4 Hz, H-3), 6.01 (ddd, 1 H, *J* = 1.56, 2.66, 10.4 Hz, H-4).
^{13}C NMR ($CDCl_3$) δ 14.39 (C-14), 23.15 (C-13), 26.53 (C-3), 30.08 (C-4,11 tetradecyl), 32.36 (C-12), 33.94 (C-2 propyl), 39.68 (C-3 propyl), 66.59 (C-6'), 66.89 (C-1 propyl), 68.47 (C-1 tetradecyl), 68.91 (C-1 tetradecyl), 69.65 (C-6), 70.09 (C-5), 94.86 (C-2), 95.43 (OCH_20), 95.99 (OCH_2O), 126.76 (C-3), 132.02 (C-4).

N-[3-(5-Hydroxy-6-octadec-9-enyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-guanidinium hydrochloride(**20**).

A solution of *O*-methylisourea hydrochloride (0.188 g, 1.7 mmol) in water (1.7 mL) was added slowly to a solution of 6-(3-amino-propoxy)-2-octadec-9-enyloxymethoxymethyl-3,6-dihydro-2H-pyran-3-ol (**17**) (0.41 g, 0.85 mmol) and triethylamine (0.356 mL, 2.55 mmol) in methanol (1.7 mL). The reaction mixture was stirred overnight at 40°C. The solvents were evaporated. The resulting oil was purified by HPLC (16-18 mn) to yield 0.33 g (70 %) of guanidinium chloride (**20**).
^{1}H NMR (CD_3OD) δ 0.91 (t, 3 H, *J* = 6.77 Hz, H-18), 1.35 (s, 20 H, H-4.6 and H-13.17 octadecenyl), 1.36 (m, 4 H, H-7 and H-14), 1.40 (m, 2 H, *J* = Hz, H-3 octadecenyl), 1.59 (m, 2 H, H-2 octadecenyl), 1.89 (m, 2 H, H-2 propyl), 2.05 (m, 4 H, H-8 and H-11 octadecenyl), 3.30 (m, 2 H, H-3 propyl), 3.56 (m, 2 H, H-1 octadecenyl and H-1 propyl), 3.72 (m, 1 H, H-6'), 3.73 (m, 1 H, H-6), 3.83 (m, 1 H, H-6'), 3.88 (m, 1 H, H-1 propyl), 4.03 (m, 1 H, H-5), 4.72 (m, 2 H, OCH_2O), 4.99 (m, 1 H, *J* = 1.05, 1.47, 2.71 Hz,H-2), 5.35 (m, 2 H, H-9 and H-10), 5.74 (ddd, 1 H, *J* = 2.12, 2.71, 10.19 Hz,H-3), 5.94 (ddd, 1 H, *J* = 1.37, 1.47, 10.19 Hz, H-4).
^{13}C NMR (CD_3OD) δ 14.4 (C-18), 23.7 (C-17), 27.0 (C-8 and C-11), 28.1 (C-2 propyl), 30.4, 30.6 and 30.8 (C-4,7 and C-12,15), 32.5 (C-16), 40.0 (C-3 propyl), 64.2 (C-5), 66.5 (C-6'), 68.5 (C-1 propyl), 69.2 (C-1 octadecenyl), 72.8 (C-6), 95.9 (C-2), 96.8 (OCH_2O), 126.9 (C-3), 130.9 (C-9 C-10), 135 (C-4).

N-[3-(5-Hydroxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-guanidinium chloride (**21**).

A solution of *O*-methylisourea hydrochloride (0.65 g, 5.9 mmol) in water (8 mL) was added slowly to a solution of 6-(3-amino-propoxy)-2-tetradecyloxymethoxymethyl-3, 6-dihydro-2H-pyran-3-ol (**18**), (1.74 g, 3.93 mmol) and triethylamine (1.1 mL, 7.86 mmol) in methanol (8 mL). The reaction mixture was heated overnight at 40°C then evaporated to dryness. The resulting oil was purified by HPLC (16-21 mn) to yield N-[3-(5-Hydroxy-6-tetradecyloxy methoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-guanidinium hydrochloride (**21**) (0.33 g, 70 %).
^{1}H NMR (dmso d_6) δ 0.86 5 (t, 3 H, *J* = 6.75 Hz, H-14), 1.23 (br s, 20 H, H-4,13 tetradecyl), 1.30 (m, 2 H, H-3 tetradecyl), 1.48 (m, 2 H, H-2 tetradecyl), 1.76 (m, 2 H, H-2 propyl), 3.15 (br t, 1 H, *J* = 6.8 Hz , H-3 propyl), 3.46 (m, 1 H, H-1 propyl), 3.47 (m, 2 H, H-6'), 3.63 m, 1 H, H-6), 3.67 (H-1 propyl), 3.83 (H-5), 4.62 (OCH_2O), 4.91 (ddd, 1 H, *J* = 1.27, 2.11, 2.66 Hz, H-2), 5.20 (d, 1 H, *J* = 5.81 Hz, OH), 5.66 (ddd, 1 H, *J* = 1.97, 2.66, 10.25 Hz, H-3), 5.84 (ddd, 1 H, *J* = 1.27, 2.39, 10.25 Hz, H-4), 7.68 (NH).
^{13}C NMR (dmso d_6) δ 14.4 (C-14), 22.6 (C-13), 26.2 (C-3 tetradecyl), 29.5 (C-2 propyl, C-4,11 tetradecyl), 31.8 (C-12 tetradecyl), 38.7 (C-3 propyl), 63.0 (C-5), 65.3 (C-6'), 67.3 (C-1

tetradecyl), 67.6 (C-1 propyl), 71.8 (C-6), 94.3 (C-2), 95.4 (OCH_2O), 125.9 (C-3), 134.9 (C-4), 158.0 (C=NH).

N-[3-(5-Tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-guanidinium sulfate (**22**).

A solution of *O*-Methylisourea hydrogensulfate (0.22 g, 1.3 mmol) in water (1.3 mL) was added slowly to a solution 3-(5-tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propylamine (**19**) (0.41 g, 0.65 mmol) and triethylamine (0.65 mL, 4.68 mmol) in methanol (1.3 mL). The reaction mixture was stirred overnight at 40°C then evaporated to dryness. Ethanol was added to the resulting oil leaving an insoluble solid, which was washed twice with ethanol. The combined ethanol solution was half evaporated in order to allow **22** to crystallized (0.73 g, 75 %).

^{1}H NMR (dmso d_6) δ 0.85 (t, 3 H, *J* = 6.8 Hz, H-14), 1.20 (m, 20 H, H-4,13 tetradecyl), 1.32 (m, 2 H, H-3 tetradecyl), 1.48 (m, 2 H, H-2 tetradecyl), 1.79 (m, 2 H, H-2 propyl), 3.12 (m, 2 H, H-3 propyl), 3.39 (m, 2 H, H-1 tetradecyl), 3.47 (m, 2 H, H-1 propyl), 3.66 (m, 1 H, H-6'), 3.78 (m, 2 H,H-6 and H-6'), 4.15 (m, 1 H, H-5), 4.60 (m, 2 H, OCH_2O), 4.72 (m, 2 H,OCH_2O), 4.94 (br s 1 H, H-2), 5.72 (br d, *J* = 10.3 Hz, H-3), 5.97 (br d, *J* = 10.3 Hz, H-4), 7.95 (m, C=NH).

^{13}C NMR (dmso d_6) δ 14.51 (H-14), 23.09 (H-13), 26.66 (H-3 tetradecyl), 29.42 (H-2 propyl), 30.11 (C-4,11 tetradecyl), 32.33 (C-12), 38.67 (C-3 propyl), 64.73 (H-6'), 68.60 (H-1 tetradecyl), 68.94 (H-1 propyl), 69.78 (C-5 and C-6), 94.9 (C-2), 95.52 (OCH_2O), 96.03 (OCH_2O), 126.79 (C-3), 131.90 (C-4), 157.85 (C=NH).

N-[3-(5-Tetradecyloxymethoxy-6-tetradecyloxymethoxymethyl-5,6-dihydro-2H-pyran-2-yloxy)-propyl]-guanidinium chloride (**23**).

A solution of **22** (0.071 g, 0.0485 mmol) in methanol (0.5 mL) was added to the top of an ion-exchange column (Dowex 21K). The product was eluted (20 % water in THF) then most of the THF was evaporated under reduced pressure. The aqueous solution was lyophilized overnight to yield 0.031 g of guanidine **23** (43 %)

^{1}H NMR (dmso d_6) δ 0.87 (t, 6H, *J* = 6.5 Hz, H-14), 1.15 to 1.40 (m, 44H, H-3,13 tetradecyl) , 1.51 (m , 4H, H-2 tetradecyl) , 1.76 (m , 2H, H-2 propyl) , 3.17 (m , 2H, H-3 propyl) , 3.40 to 3.80 (m , 9 H, H-1 propyl, H-1 tetradecyl, H-6, H-6’), 4.03 (broad d, , 1H, *J* = 9 Hz, H-5) , 4.60 to 4.70 (m , 3H, OCH_2O) , 4.79 (d, 1 H, *J* = 8 Hz, OCH_2O) , 4.98 (broad s, 1H, H-2), 5.28 (ddd, *J* = 2, 2 and 10.5 Hz , 1H) , 6.02 (broad d, 1 H, *J* = 10.5 Hz , H-4) , from 6.70 to 7.60 (very broad band , 4H) , 7.54 (t, 1H, *J* = 5 Hz, C=NH).

DNA complexation experiments[3].

Lipoplex preparation

Lipoplexes of different charge ratios (expressed as moles of positive charge per moles of negative charge) were obtained by mixing equal volumes of various concentrations of cationic lipid with plasmid DNA at the desired concentration. The resultant solutions contained either 10 μg/mL plasmid DNA during complexation. These solutions contained solutes at final concentrations of either 150 mM NaCl.

Physical properties

Size determinations of the lipopolyamine/plasmid DNA complexes were performed using a Coulter N4 Plus particle analyzer (Coulter). The mean particle diameter was obtained from the unimodal fit analysis following dynamic light scattering done at a 90° angle at 20 °C. All samples were measured at a DNA concentration of 10 μg/mL.

Fluorescence Studies

A Jobin-Yvon Spex Fluoromax-2 spectrofluorometer (Longjumeau, France) was used to evaluate the amount of plasmid DNA involved in complexation. Ethidium bromide (5 μM) was added to solutions of lipoplexes (10 μg DNA/mL) and fluorescence was monitored at excitation and emission wavelengths of 260 nm and 590 nm respectively. Values were expressed as arbitrary fluorescence units.

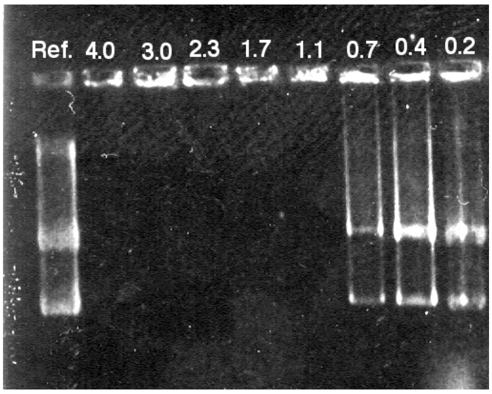

Figure 1 .Analysis of complex formation by agarose gel electrophoresis for UGG **23.**. Above N/P > 1 (N/P was the molar ratio of UGG over DNA phosphates), the entire DNA was complexed and remains in the well. Free pXL 3031 plasmid was the reference.

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