ms ID: bc-2009-00062z.R3

SUPPORTING INFORMATION

Synthesis and transfection properties of a series of lipidic neamine derivatives

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This material first provides more details about the synthesis and characteristics of the neamine derivatives **11**, **12a**, **14b**, **15a**, **18**, **22**, **24**, **27**, **30** and **32**. Next, the chemical structures of the 12 neamine derivatives evaluated in this study are indicated in Chart S1 and a comparison between some of their chemical features is shown in Table S1. In addition, the evaluation of the *in vitro* transfection efficiencies of the neamine derivatives is also more detailed: Figure S1 indicates the observed percentages of transfected HeLa cells (GFP-positive cells as estimated by flow cytometry) whereas Figure S2 shows the luciferase activities (luminometry assays) obtained when using the HeLa and 16HBE cell lines. For compound **13b**, supplementary data related to DNA condensation and relaxation as well as transfection activity when combined with small polyethylenimine (PEI 2 kDa, Sigma, France) are given in Figures S3 and S4, respectively. The literature cited here is detailed at the end of the document.

Synthesis and characteristics of neamine derivatives 11, 12a, 14b, 15a, 18, 22, 24, 27, 30 and 32.

General experimental and analytical conditions: ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a BRUKER ADVANCE 400 spectrometer using the residual solvent signal as internal standard. LRMS were achieved with NERMAG spectrometer for the FAB, DCI and EI techniques, with an AUTOFLEX BRUKER spectrometer for the MALDI and with a ZQ WATERS for the ESI. HRMS were obtained from the Mass Spectrometry Service, CRMPO, at the University of Rennes I, France, using a MICROMASS ZABSPEC-TOF spectrometer and a VARIAN MAT311 spectrometer. Melting points were determined with a BUCHI 510 apparatus. Thin layer chromatographies were performed on silica gel (Alugram Sil G/UV_{254}) or alumina gel (Alugram Alox N/UV_{254}) from Macherey-Nagel and spots were detected either by UV-absorption, by charring with ninhydrin or with cerium molybdate. Preparative chromatographies were performed on alumina gel (MP Ecochrom Biomedicals, Act II-III acc. to Brockman), silica gel (Acros Organic, 0.060-0.200 mm, 60 Å) or on C_{18} reversed phase (Macherey-Nagel polygoprep 60-50 C_{18}). All starting materials were obtained from suppliers and used without further purification unless otherwise noted. DMF was distilled in the presence of CaH_2, THF over sodium with benzophenone, CH_2Cl_2 and CH_3CN over P_2O_5 then stored under argon atmosphere prior to use.

The neamine derivatives **5**, **6**, **8**, **17** and **31** have been previously described (*1, 2*) and the amines **10a, 10b** (*3, 4*) and **28** (*5*) were synthesized according to the literature.

Synthesis of neamine derivatives 11, 12a, 14b, 15a, 18, 22, 24, 27, 30 and 32.

4'-*O*-[4-(triethylammonium)butyl] neamine derivative 11 (Scheme 2). Step a: A solution of compound **7** (450 mg, 0.27 mmol) and triethylamine (4 mL) in DMF (10 mL) under argon

atmosphere was stirred for 24 h at 70°C. After cooling, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on alumina gel with 6% MeOH in CH_2Cl_2 to lead to the protected derivative of compound **11** in 53% yield. LRMS (MALDI, DHB) *m/z*: 1687.8 $[M+H]^+$, 1445.7 $[M-Tr+H]^+$, 1202.5 $[M-2Tr+H]^+$, 960.4 $[M-3Tr+H]^+$, 718.3 $[M-4Tr+H]^+$. HRMS (ESI^+) *m/z*: $[M+H]^+$ calculated 1686.9137, found 1686.9140.

Step c: The protected compound was dissolved in CH_2Cl_2/TFA (1/1, v/v, 4 mL) with anisole (0.1 mL). After 2 h stirring at rt, solvents were removed under reduced pressure. H_2O and Et_2O were added and the aqueous layer was washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. **11** was eluted with a gradient of H_2O/MeOH. The pure compound was obtained as the TFA salt with 90% yield. ^1H NMR (CD_3OD) δ 5.88 (d, $J_{1'-2'}$= 4.0 Hz, 1H, H1'), 4.06 (m, 2H, H3', H5'), 3.97 (dd, 1H, J_{4-5}= J_{4-3}= 9.6 Hz, H4), 3.90 (m, 1H, OCH_2(CH_2)$_3$N), 3.62 (m, 1H, OCH_2(CH_2)$_3$N), 3.57 (dd, 1H, J_{4-5}= J_{5-6}= 9.2 Hz, H5), 3.05-3.48 (m, 15H, O(CH_2)$_3$$CH_2$N, N($CH_2CH_3$)$_3$, H1, H3, H6, H2', H4', 2H6'), 2.40 (ddd, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$= 12.8 Hz, 1H, $H2_{eq}$), 1.93 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.8 Hz, 1H, $H2_{ax}$), 1.55-1.78 (m, 4H, OCH_2(CH_2)$_2$$CH_2$N), 1.23 (t, *J*= 6.8 Hz, 9H, 3CH_3). ^{13}C NMR (CD_3OD) δ 97.1 (C1'), 81.1 (C4'), 79.3 (C4), 77.2 (C5), 74.4 (C6), 73.3 (OCH_2(CH_2)$_3$N), 70.4, 70.1 (C3', C5'), 58.0 (3NCH_2), 55.8 (C2'), 51.6 (C1), 50.2 (C3), 42.0 (C6'), 29.9 (C2), 28.1, 19.9 (OCH_2(CH_2)$_2$$CH_2$N), 7.8 (3$CH_3$). LRMS ($ESI^+$) *m/z*: 478.18 $[M+H]^+$. HRMS (ESI^+) m/z: $[M+H]^+$ calculated 478.3605, found 478.3602.

4'-*O*-[4-(*N,N*-di-*n*-dodecylamino)butyl] neamine derivative 12a (Scheme 2). Step b: To a solution of compound **7** (300 mg, 0.18 mmol) in DMF (10 mL) under argon atmosphere was added successively K_2CO_3 (180 mg, 1.3 mmol) and di-*n*-dodecylamine **9a** (64 mg, 0.18 mmol). The reaction mixture was stirred for 24 h at 70°C. After cooling, the reaction mixture was filtered then concentrated under reduced pressure. The residue was chromatographed on

alumina gel with cyclohexane/CH_2Cl_2 (65/35, v/v) to lead to the protected derivative of **12a** with 48% yield. **Step c:** The protected compound was dissolved in CH_2Cl_2/TFA (1/1, v/v, 4 mL) with anisole (0.1 mL). After 2 h stirring at rt, solvents were removed under reduced pressure. H_2O and Et_2O were added and the aqueous layer washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. After elution with a gradient of H_2O/MeOH, **12a** was obtained as the TFA salt with 89% yield. ^{1}H NMR (CD_3OD) δ 5.92 (d, $J_{1'-2'}$= 3.8 Hz, 1H, H1'), 4.04-4.15 (m, 2H, H3', H5'), 4.00 (dd, J_{4-5}= J_{4-3}= 9.7 Hz, 1H, H4), 3.94 (m, 1H, OCH_2(CH_2)$_3$N), 3.65 (m, 1H, OCH_2(CH_2)$_3$N), 3.60 (dd, J_{4-5}= J_{5-6}= 9.2 Hz, 1H, H5), 3.27-3.49 (m, 4H, H3, H6, H2', H6'b), 3.09-3.23 (m, 9H, H1, H4', H6'a, 3NCH_2), 2.45 (ddd, J_{2eq-1}= J_{2eq-3}= 4.1 Hz, $J_{2eq-2ax}$= 12.4 Hz, 1H, $H2_{eq}$), 2.00 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.5 Hz, 1H, $H2_{ax}$), 1.60-1.85 (m, 8H, OCH$_2$(CH_2)$_2$CH$_2$N, N[CH$_2$$CH_2$($CH_2$)$_9CH_3$]$_2$), 1.24-1.42 (m, 36H, N[CH$_2$CH$_2$(CH_2)$_9$CH$_3$]$_2$), 0.90 (t, J= 6.7 Hz, 6H, 2CH_3). ^{13}C NMR (CD_3OD) δ 97.3 (C1'), 81.3 (C4'), 79.6 (C4), 77.3 (C5), 74.5 (C6), 73.4 (OCH_2(CH_2)$_3$N), 70.4, 70.1 (C3', C5'), 55.8 (C2'), 54.3 (3NCH_2), 51.6 (C1), 50.2 (C3), 42.0 (C6'), 30.0 (C2), 33.2, 30.9, 30.8, 30.7, 30.6, 30.4, 28.5, 27.7, 25.0, 23.9, 21.9 (OCH$_2$(CH_2)$_2$CH$_2$N, N[CH$_2$(CH_2)$_{10}$CH$_3$]$_2$), 14.6 (2CH_3). LRMS (ESI$^+$) *m/z*: 730.4 $[M+H]^+$, 426.4, 408.4, 240.3. HRMS (ESI$^+$) *m/z*: $[M+H]^+$ calculated 730.6422, found 730.6425.

5-*O*-[6-(*N*,*N*-di-*n*-octadecylamine)hexyl] neamine derivative 14b (Scheme 2). The same procedure as described for the synthesis of **12a** (steps b and c) was applied to compound **8** with di-*n*-octadecylamine **9b**. **14b** was obtained with 32% yield for two steps. ^{1}H NMR (CD_3OD) δ 5.85 (d, $J_{1'-2'}$= 4.0 Hz, 1H, H1'), 4.00-4.18 (m, 4H, H4, H3', H5', OCH_2(CH_2)$_5$N), 3.77 (m, 1H, OCH_2(CH_2)$_5$N), 3.49-3.66 (m, 3H, H3, H5, H6), 3.37-3.48 (m, 3H, H2', H4', H6'b), 3.20-3.32 (m, 2H, H1, H6'a), 3.08-3.20 (m, 6H, O(CH_2)$_5$$CH_2$N, N[CH$_2$$CH_2$($CH_2$)$_9CH_3$]$_2$), 2.43 (ddd, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$= 12.8 Hz 1H, $H2_{eq}$), 1.98

(ddd, $J_{2ax\text{-}1}$= $J_{2ax\text{-}3}$= $J_{2eq\text{-}2ax}$= 12.8 Hz, 1H, $H2_{ax}$), 1.62-1.78 (m, 8H, $OCH_2CH_2(CH_2)_2$, CH_2CH_2N, $N[CH_2CH_2(CH_2)_{15}CH_3]_2$), 1.22-1.50 (m, 64H, $OCH_2CH_2(CH_2)_2CH_2CH_2N$, $N[CH_2CH_2(CH_2)_{15}CH_3]_2$), 0.90 (t, *J*= 6.8 Hz, 9H, $3CH_3$). ^{13}C NMR (CD_3OD) δ 93.0 (C1'), 83.2 (C5), 74.3 (C4), 73.4 (C6), 72.1 ($OCH_2(CH_2)_5N$), 71.4 (C5'), 70.8 (C4'), 68.2 (C3'), 53.4 (C2'), 52.7 ($2NCH_2$), 50.2, 49.2 (C1, C3), 40.1 (C6'), 33.9, 31.7, 29.7, 29.4, 29.2, 29.1, 28.8, 28.1, 26.2, 25.1, 23.6, 23.4, 22.3 (C2, $OCH_2(CH_2)_4CH_2N$, $[NCH_2(CH_2)_{16}CH_3]_2$), 13.0 ($2CH_3$). HRMS (ESI^+) *m/z*: $[M+H]^+$ calculated 926.8613, found 926.8634.

5-*O*-[6-(*N*-2-aminoethyl-*N,N*-di-*n*-dodecylamine)hexyl] neamine derivative 15a (Scheme 2). The same procedure as described for the synthesis of **12a** (steps b and c) was applied to compound **8** with *N*-2-aminoethyl-*N,N*-di-*n*-dodecylamine **10a**. **15a** was obtained with 39% yield for two steps. ^{1}H NMR (D_2O) δ 5.70 (d, $J_{1'\text{-}2'}$= 4.0 Hz, 1H, H1'), 3.75-3.95 (m, 4H, H4, H3', H5', $OCH_2(CH_2)_5N$), 3.51-3.65 (m, 4H, H3, H5, H6, $OCH_2(CH_2)_5N$), 3.36-3.48 (H2', H4', $3NCH_2$), 3.15-3.21 (m, 5H, H6'b, $2NCH_2$), 2.90-3.03 (m, 2H, H1, H6'a), 2.32 (ddd, $J_{2eq\text{-}1}$= $J_{2eq\text{-}3}$= 4.0 Hz, $J_{2eq\text{-}2ax}$= 12.8 Hz, 1H, $H2_{eq}$), 1.70 (ddd, $J_{2ax\text{-}1}$= $J_{2ax\text{-}3}$= $J_{2eq\text{-}2ax}$= 12.8 Hz, 1H, $H2_{ax}$), 1.35-1.57 (m, 8H, $OCH_2CH_2(CH_2)_2$ CH_2CH_2N, $N[CH_2CH_2(CH_2)_{15}CH_3]_2$), 1.03-1.27 (m, 64H, $OCH_2CH_2(CH_2)_2CH_2CH_2N$, $N[CH_2CH_2(CH_2)_{15}CH_3]_2$), 0.74 (t, *J*= 6.8 Hz, 9H, $3CH_3$). ^{13}C NMR (D_2O) δ 93.0 (C1'), 82.6 (C5), 73.6 (C4), 72.7 (C6), 72.2 ($OCH_2(CH_2)_5N$), 70.1 (C5'), 69.9 (C4'), 68.4 (C3'), 61.6 ($3NCH_2$), 53.2 (C2'), 49.7, 48.8 (C1, C3), 39.9 (C6'), 32.0, 31.1, 29.7, 29.2, 28.0, 24.9, 24.8, 22.6 (C2, $OCH_2(CH_2)_4CH_2N$, $[NCH_2(CH_2)_{16}CH_3]_2$), 13.8 ($2CH_3$). LRMS (ESI^+) *m/z*: 845.6 $[M+K]^+$, 801.7 $[M+H]^+$, 685.5, 633.4, 423.3, 380.5.

2-(*N*-(*N',N'*-di-*n*-dodecyl)ethylamino)aminodiethanoic acid 16 (Scheme 3). Step a: To a solution of amine **10a** (209 mg, 0.52 mmol) in dry CH_3CN (5 mL) under argon atmosphere were added K_2CO_3 (218 mg, 1.6 mmol) and TBAI (194 mg, 0.5 mmol). The mixture was stirred under reflux 15 min before the addition of bromoethylbenzoate (362 mg, 1.58 mmol)

then 3 days. After cooling, the mixture was filtrated and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed with water, an aqueous saturated $NaHCO_3$ solution then brine. The organic solution was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a mixture of CH_2Cl_2/MeOH (98/2, v/v) to lead to the benzylic ester of **16** with 98% yield. ^{1}H NMR ($CDCl_3$) δ 7.32-7.40 (m, 10H, H arom.), 5.13 (s, 4H 2CO*CH*$_2$Ph), 4.14 (m, 2H, N*CH*$_2$CH$_2$N), 3.80 (td, *J*= 4.8 and 12.8 Hz, 2H, N[*CH*$_2$(CH$_2$)$_{10}$CH$_3$]$_2$), 3.57 (td, *J*= 4.4 and 12.8 Hz, 2H, N[*CH*$_2$(CH$_2$)$_{10}$CH$_3$]$_2$), 3.46 (s, 4H, 2CO*CH*$_2$*N*), 3.24 (m, 2H, N*CH*$_2$CH$_2$N), 1.72 (m, 4H, 2NCH$_2$*CH*$_2$(CH$_2$)$_9$CH$_3$), 1.23-1.36 (m, 36H, 2NCH$_2$CH$_2$(*CH*$_2$)$_9$CH$_3$), 0.89 (t, *J*= 6.4 Hz, 6H, 2*CH*$_3$). ^{13}C NMR ($CDCl_3$) δ 170.6 (*C*OOBn), 135.13 (*C* arom.), 128.8-128.4 (*CH* arom.), 68.1 (N*CH*$_2$CO), 66.9 (*CH*$_2$Ph), 60.9, 55.2, 48.2 (4N*CH*$_2$), 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 26.3, 22.7 (20*CH*$_2$), 15.3 (2*CH*$_3$). LRMS (DCI$^+$) *m/z*: 693 [M+H]$^+$, 366, 294, 250. **Step b:** The benzylic ester of **16** (130 mg, 0.19 mmol) was stirred in EtOH (2 mL) at rt with Pd/C (10%, 130 mg) and cyclohexadiene (177 µL, 1.87 mmol) for 1 h. The mixture was filtrated on kieselguhr and concentrated under reduce pressure. The diacid **16** was obtained as a white solid with 86% yield and enough pure for doing the next step without purification. ^{1}H NMR ($CDCl_3$) δ 4.28 (m, 2H, N*CH*$_2$CH$_2$N), 3.77 (m, 2H, N[*CH*$_2$(CH$_2$)$_{10}$CH$_3$]$_2$), 3.48-3.67 (m, 6H, 2CO*CH*$_2$*N*, N[*CH*$_2$(CH$_2$)$_{10}$CH$_3$]$_2$), 3.26 (m, 2H, N*CH*$_2$CH$_2$N), 1.63 (m, 4H, 2NCH$_2$*CH*$_2$(CH$_2$)$_9$CH$_3$), 1.22-1.35 (m, 36H, 2NCH$_2$CH$_2$(*CH*$_2$)$_9$CH$_3$), 0.89 (t, *J*= 6.4 Hz, 6H, 2*CH*$_3$). ^{13}C NMR ($CDCl_3$) δ 173.5 (*C*OOH), 59.5 (N*CH*$_2$CO), 58.4, 55.5, 47.8 (4N*CH*$_2$), 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 26.4, 22.7 (20*CH*$_2$), 14.1 (2*CH*$_3$). LRMS (FAB$^+$, NBA) *m/z*: 513 [M+H]$^+$, 496, 468, 412, 366.

5,5-Dineamine derivative 18 (Scheme 3). Step c: Compound **16** (19.5 mg, 0.04 mmol) was dissolved in dry DMF (3 mL) under argon atmosphere. To the solution were added HOBT

(15.4 mg, 0.11 mmol) and EDC (21.7 mg, 0.11 mmol), then the mixture was stirred at rt for 30 min before the addition of a solution of **17** (200 mg, 0.11 mmol) in DMF (2 mL). The mixture was stirred at 60°C for 12 h. After cooling and concentration of the solution under reduced pressure, the crude product was dissolved in CH_2Cl_2 and washed successively with water, an aqueous saturated $NaHCO_3$ solution then brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a mixture of CH_2Cl_2/MeOH (99/1, v/v) to lead to the protected dineamine compound with 55% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 12 h stirring at rt, the solvents were removed under reduced pressure. H_2O and Et_2O were added and the aqueous layer washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. **18** was eluted with a gradient of H_2O/MeOH and obtained as the TFA salt with 91% yield. ^{1}H NMR (CD_3OD) δ 5.85 (d, $J_{1'-2'}$= 3.8 Hz, 2H, H1'), 4.20 (m, 2H), 4.00-4.16 (m, 6H), 3.71-3.84 (m, 4H), 3.3-3.65 (m, 20H), 3.18-3.29 (m, 8H), 3.02-3.11 (m, 2H), 2.41-2.51 (m, 2H, $H2_{eq}$), 1.88-2.05 (m, 2H, $H2_{ax}$), 1.49-1.82 (m, 8H), 1.24-1.48 (m, 36H), 0.92 (t, *J*= 6.8 Hz, 6H, 2CH_3). ^{13}C NMR (CD_3OD) δ 171.2 (2*C*ONH), 162.0, 161.7 (*C*OCF_3), 121.1, 118.2, 115.3, 112.4 (CO*C*F_3), 92.7 (2C1'), 83.2 (2C5), 74.2 (2C4), 73.5 (2C6), 72.3 (2OCH_2(CH_2)$_5$N), 71.4 (2C4'), 70.8 (2C5'), 68.3 (2C3'), 60.3, 57.9, 57.5, 56.5 (6 NCH_2), 53.5 (2C2'), 50.2 (2C1), 49.2 (2C3), 40.2 (2C6'), 38.9 (2 N*CH2*CONH), 31.7, 29.8, 29.3, 29.2, 29.1, 29.0, 28.7, 28.0, 26.6, 25.8, 25.1, 22.3, 21.6 (2C2, 2OCH_2(CH_2)$_4$$CH_2$N, N[$CH_2$($CH_2$)$_{10}$$CH_3$]$_2$), 14.6 (2$CH_3$). LRMS (MALDI, DHB) *m/z*: 1782.6 [M+4TFA]$^+$, 1320.2 [M+H]$^+$, 966.8.

Dibenzyl 2-(octadecyl)malonate 20 (Scheme 4). Step a: To a THF solution of dibenzyl malonate **19** (0.5 mL, 2 mmol, 20 mL) under argon atmosphere at 0°C, were added NaH (60%

suspension, 96 mg, 2.4 mmol) and after 30 min stirring at rt, bromooctadecane (667 mg, 2 mmol). The mixture was stirred for 24 h, then, an aqueous saturated NH_4Cl solution was added. After separation of the organic and aqueous layers, the latter was washed 3 times with Et_2O. The organic layers were dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel with a mixture of cyclohexane/CH_2Cl_2 (40/60, v/v) to lead to the alkylated product **20** with 64% yield. ^{1}H NMR ($CDCl_3$) δ 7.28-7.35 (m, 10H, *CH* arom.), 5.19 (s, 4H $2CH_2Ph$), 3.46 (t, *J*= 7.6 Hz, 1H, $CH_2CH(CO_2Bn)_2$), 1.95 (m, 2H, $CH_3(CH_2)_{16}CH_2CH(CO_2Bn)_2$), 1.23-1.36 (m, 32H, $CH_3(CH_2)_{16}CH_2CH(CO_2Bn)_2$), 0.89 (t, *J*= 6.8 Hz, 3H, CH_3). LRMS (FAB^+,NBA) *m/z*: 559 $[M+Na]^+$, 538 $[M+H]^+$, 427.

2-(Octadecyl)malonic acid 21 (Scheme 4). Step b: The dibenzylic ester **20** (320 mg, 0.59 mmol) was stirred in EtOH/THF (4 mL, 1/1, v/v) at rt with Pd/C (10%, 320 mg) and cyclohexadiene (570 µL, 5.96 mmol) for 3 h. The mixture was filtrated on kieselguhr and concentrated under reduce pressure. The diacid **21** was obtained as a white solid with 95% yield and enough pure for doing the next step without purification. ^{1}H NMR (DMSO-d_6) δ 3.14 (t, *J*= 7.6 Hz, 1H, $CH_2CH(CO_2H)_2$), 1.69 (m, 2H, $CH_3(CH_2)_{16}CH_2CH(CO_2H)_2$), 1.17-1.30 (m, 32H, $CH_3(CH_2)_{16}CH_2CH(CO_2H)_2$), 0.86 (t, *J*= 6.8 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.6 (CO_2H), 51.9 ($CH_2CH(CO_2H)_2$), 31.2 ($CH_3(CH_2)_{16}CH_2CH(CO_2H)_2$), 29.5, 29.2, 28.9, 27.3, 22.6 ($CH_3(CH_2)_{16}CH_2CH(CO_2H)_2$), 14.4 ($CH_3$). LRMS ($FAB^+$, NBA) *m/z*: 396 $[M+K]^+$, 357 $[M+H]^+$.

5,5-Dineamine derivative 22 (Scheme 4). Step c: Compound **21** (14.3 mg, 0.04 mmol) was dissolved in dry DMF (3 mL) under argon atmosphere. To the solution were added HOBT (13.6 mg, 0.1 mmol) and EDC (19.2 mg, 0.1 mmol). The mixture was stirred at rt for 40 min before the addition of a solution of neamine derivative **17** (180 mg, 0.1 mmol) in DMF (3

mL). After 12 h stirring, the mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed successively with water, an aqueous saturated $NaHCO_3$ solution then brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a mixture of CH_2Cl_2/cyclohexane (50/50, v/v) to lead to the dineamine protected compound with 37% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 12 h stirring at rt, solvents were removed under reduced pressure. H_2O and Et_2O were added and the aqueous phase washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. **22** was eluted with a gradient of H_2O/MeOH and obtained as the TFA salt with 35% yield. ^{1}H NMR (CD_3OD) δ 5.83 (d, $J_{1'-2'}$= 3.6 Hz, 2H, H1'), 4.01-4.16 (m, 8H, H4, H3', H5', OCH_2(CH_2)$_5$N), 3.74 (m, 2H, OCH_2(CH_2)$_5$N), 3.45-3.62 (m, 4H, H5, H6), 3.46-3.35 (m, 6H, H3, H4', H6'b), 3.32-3.15 (m, 6H, H1, H2', H6'a), 2.43 (ddd, J_{2eq-1}= J_{2eq-3}= 4.4 Hz, $J_{2eq-2ax}$ = 12.4 Hz, 2H, $H2_{eq}$), 2.19 (m, 1H, *CH*CONH), 1.98 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.4 Hz, 2H, $H2_{ax}$), 1.57-1.69 (m, 4H, O(CH_2)$_5$$CH_2$N), 1.48-1.55 (m, 2H), 1.25-1.45 (m, 48H), 0.92 (t, *J*= 6.8 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD) δ 174.9 (2*C*ONH), 98.2 (2C1'), 83.3 (2C5), 74.4 (2C4), 73.4 (2C6), 72.3 (2OCH_2(CH_2)$_5$N), 71.6 (2C4'), 70.6 (2C5'), 68.2 (2C3'), 53.4 (2C2'), 50.1 (2C1), 49.2 (2C3), 47.9 (2*CH*CONH), 40.9 (2C6'), 38.7 (2O(CH_2)$_5$$CH_2$N), 35.8, 31.7, 29.8, 29.4, 29.1, 28.9, 26.3, 25.8, 25.0, 22.3 (2C2, 25CH_2), 13.0 (CH_3). LRMS (MALDI, DHB) *m/z*: 1185.7 $[M+Na]^+$, 1163.8 $[M+H]^+$, 716.5.

2-(Dioctadecyl)malonic acid 23 (Scheme 4). Step a: To a solution of the dibenzyl monoalkylmalonate **20** (300 mg, 0.56 mmol) in THF (20 mL) under argon atmosphere at 0°C, were added NaH (60% suspension, 50 mg, 1.25 mmol), and after 30 min stirring at rt, bromooctadecane (667 mg, 2 mmol). The mixture was stirred for 24 h at reflux. After cooling

at rt, an aqueous saturated NH_4Cl solution was added. After separation of the organic and aqueous layers, the latter was washed 3 times with Et_2O. The organic layers were dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel with a mixture of cyclohexane/CH_2Cl_2 (70/30, v/v) to lead to the dibenzyl dialkylmalonate with 30% yield. ^{1}H NMR ($CDCl_3$) δ 7.27-7.35 (m, 10H, *CH* arom.), 5.13 (s, 4H $2CH_2Ph$), 1.91 (m, 4H, $(CH_3(CH_2)_{16}CH_2)_2C(CO_2Bn)_2$), 1.20-1.36 (m, 30H, $(CH_3CH_2(CH_2)_{15}CH_2)_2C(CO_2Bn)_2$), 1.08 (m, 4H, $(CH_3CH_2(CH_2)_{15}CH_2)_2C(CO_2Bn)_2$), 0.92 (t, *J*= 6.8 Hz, 6H, $2CH_3$). ^{13}C NMR ($CDCl_3$) δ 171.7 (2*C*ONH), 135.7 (2*C* arom.), 128.2, 128.5 (10*CH* arom.), 66.7 (2*CH*Ph), 57.8 ($(CH_3(CH_2)_{17})_2C(CO_2Bn)_2$), 32.2, 32.0, 29.8, 29.7, 29.6, 29.4, 29.3, 23.9, 22.7 ($(CH_3(CH_2)_{17})_2C(CO_2Bn)_2$), 14.2 ($2CH_3$). LRMS ($FAB^+$, NBA) *m/z*: 812 $[M+Na]^+$, 790 $[M+H]^+$, 613. **Step b:** The dialkylated product was debenzylated (124 mg, 0.16 mmol) following the same procedure as for the dibenzyl monoalkylmalonate **20**. The diacid **23** was obtained as a white solid with 91% yield and enough pure for doing the next step without purification. ^{1}H NMR ($CDCl_3$) δ 1.94 (m, 4H, $(CH_3(CH_2)_{16}CH_2)_2C(CO_2H)_2$), 1.18-1.45 (m, 64H, $(CH_3(CH_2)_{16}CH_2)_2C(CO_2H)_2$), 0.90 (t, *J*= 6.8 Hz, 6H, $2CH_3$). ^{13}C NMR ($CDCl_3$) δ 57.9 ($(CH_3(CH_2)_{17})_2C(CO_2H)_2$), 32.0, 29.7, 29.4, 29.3, 24.9, 22.7 ($(CH_3(CH_2)_{17})_2C(CO_2H)_2$), 14.1 ($2CH_3$). LRMS ($FAB^+$, NBA) *m/z*: 632 $[M+Na]^+$, 610 $[M+H]^+$, 564, 546.

5-Neamine derivative 24 (Scheme 4). Step c: Compound **23** (15.8 mg, 0.026 mmol) was dissolved in dry DMF (3 mL) under argon atmosphere. To the solution were added HOBT (10.8 mg, 0.08 mmol) and EDC (15.2 mg, 0.08 mmol). The mixture was stirred at rt for 40 min before the addition of a solution of neamine derivative **17** (120 mg, 0.068 mmol) in DMF (3 mL). After 12 h stirring, the mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed successively with water, an aqueous saturated

$NaHCO_3$ solution then brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a mixture of CH_2Cl_2/cyclohexane (40/60, v/v) to lead to the mononeamine protected compound with 35% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 12 h stirring at rt, the solvents were removed under reduced pressure. H_2O and Et_2O were added and the aqueous layer was washed twice with Et_2O before being concentrated and poured on a C18 reversed phase column. After elution with a gradient of H_2O/MeOH, **24** was obtained as the TFA salt with a quantitative yield. ^{1}H NMR (CD_3OD) δ 5.82 (d, $J_{1'-2'}$= 3.6 Hz, 1H, H1'), 3.99-4.08 (m, 4H, H4, H3', H5', OCH_2(CH_2)$_5$N), 3.76 (m, 1H, OCH_2(CH_2)$_5$N), 3.49-3.61 (m, 2H, H5, H6), 3.32-3.45 (m, 3H, H3, H4', H6'b), 3.15-3.27 (m, 3H, H1, H2', H6'a), 2.40 (ddd, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$ = 12.8 Hz, 1H, $H2_{eq}$), 2.18 (m, 1H, *CH*CONH), 1.89 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.8 Hz, 1H, $H2_{ax}$), 1.64 (m, 2H, O(CH_2)$_5$$CH_2$N), 1.49-1.60 (m, 4H), 1.20-1.50 (m, 72H), 0.92 (t, *J*= 6.8 Hz, 6H, 2CH_3). ^{13}C NMR (CD3OD) δ 177.6 (*C*ONH), 162.1, 161.7 (*C*OCF_3), 121.1, 118.2, 115.2, 112.4 (CO*C*F$_3$), 93.2 (C1'), 83.3 (C5), 75.0 (C4), 73.5 (C6), 72.2 (OCH_2(CH_2)$_5$N), 71.2 (C4'), 71.0 (C5'), 68.5 (C3'), 53.6 (C2'), 50.3 (C1), 49.2 (C3), 46.8 (*CH*CONH), 40.2 (C6'), 38.6 O(CH_2)$_5$$CH_2$N, 32.7, 31.7, 29.8, 29.4, 29.3, 29.2, 29.1, 28.7, 27.2, 26.4, 25.0, 22.3 (C2, 38CH_2), 13.1 (2CH_3). LRMS (MALDI, DHB) *m/z*: 990.9 $[M+Na]^+$, 968.9 $[M+H]^+$. HRMS (ESI^+) *m/z*: $[M+H]^+$ calculated 968.8718, found 968.8718.

2-(Dodecylamido)malonic acid 26 (Scheme 5). Step a: To a solution of dodecanoic acid (254 mg, 1 mmol) in CH_2Cl_2 (14 mL) at rt were added EDC (288 mg, 1.5 mmol) and HOBT (203 mg, 1.5 mmol) then, after 45 min stirring, the chlorhydrate **25** (254 mg, 1.2 mmol) and Et_3N (1 mL). 12 h later, the mixture was washed successively with water, HCl 1M, an aqueous saturated $NaHCO_3$ solution then brine. The organic layer was dried over $MgSO_4$,

filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on silica gel with a mixture of CH_2Cl_2/MeOH (99:1, v/v) to lead to diethyl 2-(dodecylamido)malonate with 95% yield. ^{1}H NMR ($CDCl_3$) δ 6.45 (d, *J*= 6.8 Hz, 1H, *NH*), 5.18 (d, *J*= 6.8 Hz, 1H, NH*CH*(CO_2)$_2$), 4.29 (m, 4H, 2CH_3*CH_2*OCO), 2.30 (t, *J*= 7.6 Hz, 2H, *CH_2*CONH), 1.66 (m, 2H, *CH_2*), 1.32 (t, *J*= 7.2 Hz, 6H, 2*CH_3*), 1.27 (m, 16H, 8*CH_2*), 0.86 (t, *J*= 6.8 Hz, 3H, *CH_3*). ^{13}C NMR ($CDCl_3$) δ 172.9 (*C*ONH), 166.5 (2*C*OOEt), 62.6 (2CH_3*CH_2*OCO), 56.4 (NH*CH*(CO_2)$_2$), 36.1 (*CH_2*CONH), 31.9, 29.6, 29.5, 29.3, 29.2, 25.4, 22.7 (9*CH_2*), 14.1, 14.0 (3*CH_3*). LRMS (FAB$^+$, NBA) *m/z*: 358 [M+H]$^+$, 342, 328, 316, 297, 284, 277, 256, 228. **Step b:** This compound in EtOH (9 mL) was treated with NaOH aq. 2M at 70°C for 2 h. After cooling, the solution was neutralized with HCl aq. 1M. The solution was filtrated and concentrated under reduced pressure to give the diacid **26** with 90% yield, enough pure for doing the next step without purification. ^{1}H NMR (CD_3OD) δ 4.87 (m, 1H, NH*CH*(CO_2)$_2$), 2.25 (t, *J*= 7.2 Hz, 2H, *CH_2*CONH), 1.55 (m, 2H, *CH_2*), 1.24 (m, 16H, 8*CH_2*), 0.84 (t, *J*= 6.8 Hz, 3H, *CH_3*). ^{13}C NMR (CD3OD) δ 174.8 (*C*ONH), 168.1 (2*C*OOH), 48.2 (NH*CH*(CO_2H)$_2$), 35.0 (*CH_2*CONH), 31.7, 29.4, 29.2, 29.1, 28.2, 25.4, 22.4 (9*CH_2*), 13.0 (*CH_3*). LRMS (FAB$^+$, NBA) *m/z*: 302 [M+H]$^+$, 284, 256, 228, 200.

5,5-Dineamine derivative 27 (Scheme 5). Step c: The diacid **26** (13 mg, 0.043 mmol) was dissolved in dry DMF (3 mL) under argon atmosphere. To the solution were added HOBT (17.4 mg, 0.13 mmol) and EDC (24.7 mg, 0.13 mmol). The mixture was stirred at rt for 40 min before the addition of a solution of neamine derivative **17** (230 mg, 0.12 mmol) in DMF (2 mL). After 12 h stirring, the mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed successively with water, an aqueous saturated $NaHCO_3$ solution then brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on alumina

gel with a mixture of CH_2Cl_2/MeOH (99/1, v/v) to lead to the protected compound with 30% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 12 h stirring at rt, the solvents were removed under reduced pressure. H_2O and Et_2O were added then the aqueous layer was washed twice with Et_2O before being concentrated. The crude product was eluted on a C18 reversed phase column with a gradient of H_2O/MeOH. Pure compound **27** was obtained as the TFA salt with a quantitative yield. ^{1}H NMR (D_2O) δ 5.69 (d, $J_{1'-2'}$= 3.6 Hz, 2H, H1'), 3.71-3.94 (m, 8H, H4, H3', H5', OCH_2(CH_2)$_5$N), 3.68 (s, 1H, NCH(CONH)$_2$), 3.51-3.65 (m, 6H, OCH_2(CH_2)$_5$N, H5, H6), 3.30-3.49 (m, 6H, H4', O(CH_2)$_5$$CH_2$N), 3.10-3.27 (m, 6H, H3, H2', H6'b), 2.97-3.08 (m, 2H, H1, H6'a), 2.32 (ddd, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$= 12.8 Hz, 1H, H2$_{eq}$), 2.13 (t, J= 7.6 Hz, 4H, CH_3(CH_2)$_9$$CH_2$CO), 1.72 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.4 Hz, 1H, H2$_{ax}$), 1.28-1.50 (m, 10H), 1.01-1.22 (m, 20H), 0.71 (t, J= 7.2 Hz, 6H, 2CH_3). ^{13}C NMR (D_2O) δ 176.9 (CONH), 171.1 (2NHCO(CH_2)$_2$$C$ONH), 163.3, 163.0, 162.6, 162.3 (COCF_3), 120.7, 117.7, 114.9, 112.0 (COCF$_3$), 92.81 (2C1'), 82.6 (2C5), 73.3, 72.8 (2C4, 2C6, NCH(CONH)$_2$), 72.2 (2OCH_2(CH_2)$_5$N), 70.1 (2C4', 2C5'), 68.3 (2C3'), 53.2 (2C2'), 49.7 (2C1), 48.8 (2C3), 42.6, 40.0, 39.2, 35.6, 31.6, 29.3, 29.2, 29.0, 28.7, 28.3, 27.8, 25.8, 25.4, 24.6, 22.3 (2C2, 2C6', 20CH_2), 13.6 (CH_3). LRMS (MALDI, DHB) *m/z*: 1316.9 [M+K]$^+$, 1300.9 [M+Na]$^+$, 1278.9 [M+H]$^+$.

***N*-2-(1,3-diaminopropane)dodecylamide 29 (Scheme 5). Step a:** To a solution of dodecanoic acid (101.1 mg, 0.5 mmol) in CH_2Cl_2 (7 mL) were added at rt EDC (144 mg, 0.75 mmol) and HOBT (101.5 mg, 0.75 mmol) then, after 45 min stirring, the amine **28** (175.3 mg, 0.6 mmol). 2 h later, the mixture was washed successively with water, an aqueous saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue obtained was chromatographed on silica gel

with a mixture of CH_2Cl_2/MeOH (96/4, v/v) to lead to *N*-2-(N^1,N^3-di-t-boc-1,3-diaminopropane)dodecylamide with 90% yield. ^{1}H NMR ($CDCl_3$) δ 7.0 (s, 1H, CH_2CONH), 5.40 (m, 2H, 2N*H*COOtBu), 3.76 (m, 1H, NH*CH*($CH_2NHBoc)_2$), 3.36 (m, 2H, NHCH($CH_2NHBoc)_2$), 3.19 (m, 2H, NHCH($CH_2NHBoc)_2$), 2.17 (t, *J*= 8.0 Hz, 2H, CH_2CONH), 1.60 (m, 2H, CH_2), 1.46 (s, 18H, 6CH_3 tBu), 1.27 (m, 16H, 8CH_2), 0.89 (t, *J*= 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 174.0 (CH_2CONH), 157.5 (2*C*OOtBu), 79.8 (2*C* tBu), 52.7 (NH*CH*($CH_2NHBoc)_2$), 40.8 (NHCH($CH_2NHBoc)_2$), 36.9 (CH_2CONH), 31.9, 29.6, 29.5, 29.3, 29.2 (7CH_2), 28.3 (6CH_3 tBu), 25.6, 22.7 (2CH_2), 14.1 (CH_3). LRMS (FAB^+, NBA) *m/z*: 472 $[M+H]^+$, 416, 372, 360. **Step e:** This compound was treated in a solution of CH_2Cl_2/TFA (4 mL, 1/1, v/v) at rt for 1 h. After concentration of the mixture under reduce pressure, the diamine **29** was obtained with a quantitative yield enough pure for doing the next step without purification. ^{1}H NMR (CD_3OD) δ 7.0 (s, 1H, CH_2CONH), 5.40 (m, 2H, 2N*H*COOtBu), 3.76 (m, 1H, NH*CH*($CH_2NHBoc)_2$), 3.36 (m, 2H, NHCH($CH_2NHBoc)_2$), 3.19 (m, 2H, NHCH($CH_2NHBoc)_2$), 2.17 (t, J = 8.0 Hz, 2H, CH_2CONH), 1.60 (m, 2H, CH_2), 1.46 (s, 18H, 6CH_3 tBu), 1.27 (m, 16H, 8CH_2), 0.89 (t, *J*= 6.8 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD) δ 178.0 (CH_2CONH), 47.6 (NH*CH*($CH_2NHBoc)_2$), 42.1 (NHCH(CH_2NH_2), 37.1 (CH_2CONH), 33.2, 30.9, 30.8, 30.7, 30.6, 26.3, 23.9 (9CH_2), 14.6 (CH_3). LRMS (FAB^+, NBA) *m/z*: 272 $[M+H]^+$, 254, 225.

5,5-Dineamine derivative 30 (Scheme 5). Step f: The diamine **29** (24 mg, 0.047 mmol) was dissolved in dry DMF (4 mL) under argon atmosphere. To the solution were added successively K_2CO_3 (32 mg, 0.23 mmol) and neamine derivative **8** (200 mg, 0.12 mmol). After 24 h stirring under reflux, the mixture was cooled to rt and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed successively with water and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced

pressure. The residue obtained was chromatographed on alumina gel with a mixture of cyclohexane/CH_2Cl_2 (50/50, v/v) then CH_2Cl_2 to lead to the protected compound with 50% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 24 h stirring at rt, the solvents were removed under reduced pressure. H_2O and Et_2O were added. The aqueous layer was washed twice with Et_2O before being concentrated. The crude product was eluted on a C18 reversed phase column with a gradient of H_2O/MeOH. The pure compound **30** was obtained as the TFA salt with 80% yield. ^{1}H NMR (D_2O) δ 5.69 (d, $J_{1'-2'}$= 3.6 Hz, 2H, H1'), 3.73-3.92 (m, 9H, H4, H3', H5', OCH_2(CH_2)$_5$N, NCH(CH_2NHCO)$_2$), 3.49-3.68 (m, 6H, OCH_2(CH_2)$_5$N, H5, H6), 3.31-3.43 (m, 12H, H3, H2', H4', H6'b, NCH(CH_2NHCO)$_2$), 3.12-3.25, (m, 8H, H1, H6'a, O(CH_2)$_5$$CH_2$N), 2.32 (ddd, 2H, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$= 12.4 Hz, 1H, $H2_{eq}$), 1.71 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.4 Hz, 1H, $H2_{ax}$), 1.32-1.51 (m, 12H), 1.05-1.29 (m, 24H), 0.76 (t, J= 7.2 Hz, 6H, 2CH_3). ^{13}C NMR (D_2O) δ 173.6 (CONH), 163.1, 162.7 (COCF_3), 120.6, 117.7, 114.8, 111.9 (COCF$_3$), 92.9 (2C1'), 82.6 (2C5), 73.3 (2C4), 72.7 (2C6), 72.2 (2OCH_2(CH_2)$_5$N), 70.0 (2C4', 2C5'), 68.3 (2C3'), 61.6 (NCH(CH_2NH)$_2$), 53.1 (2C2'), 49.7 (2C1), 48.8 (2C3), 39.9 (2C6'), 31.0, 29.2, 27.9, 24.8, 24.7 (2C2, CH_2), 13.5 (CH_3). LRMS (MALDI, DHB) *m/z*: 1102.9 $[M+Na]^+$, 1080.9 $[M+H]^+$, 800.6.

5,5-Dineamine derivative 32 (Scheme 5). Step g: The neamine derivative **31** (102 mg, 0.055 mmol) was dissolved in dry DMF (3 mL) under argon atmosphere. To the solution were added HOBT (15 mg, 0.11 mmol) and EDC (21.3 mg, 0.11 mmol). The mixture was stirred at rt for 40 min before the addition of a solution of diamine **29** (11.1 mg, 0.022 mmol) in DMF (2 mL). After 24 h stirring, the mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed successively with water, an aqueous saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtrated and

concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a mixture of CH_2Cl_2/MeOH (98/2, v/v) to lead to the protected compound with 20% yield. **Step d:** This compound dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and anisole (0.1 mL). After 12 h stirring at rt, the solvents were removed under reduced pressure. H_2O and Et_2O were added. The aqueous layer was washed twice with Et_2O before being concentrated. The compound **32** was eluted on a C18 reversed phase column with a gradient of H_2O/MeOH. The pure compound **32** was obtained as the TFA salt with 80% yield. ^{1}H NMR (D_2O) δ 5.67 (d, $J_{1'-2'}$= 3.6 Hz, 2H, H1'), 3.96 (m, 1H, N*CH*($CH_2NHCO)_2$), 3.76-3.90 (m, 8H, H4, H3', H5', O*CH*$_2$($CH_2)_5$N), 3.48-3.63 (m, 6H, O*CH*$_2$($CH_2)_5$N, H5, H6), 3.18-3.39 (m, 20H, H1, H3, H2', H4', H6'a, H6'b, O($CH_2)_5$*CH*$_2$N, NCH(*CH*$_2$NHCO)$_2$), 2.34 (s, 8H, NHCO(*CH*$_2)_2$CONH), 2.28 (ddd, J_{2eq-1}= J_{2eq-3}= 4.0 Hz, $J_{2eq-2ax}$ = 12.4 Hz, 1H, $H2_{eq}$), 2.07 (t, *J*= 7.2 Hz, 2H, $CH_3(CH_2)_9$*CH*$_2$CO), 1.71 (ddd, J_{2ax-1}= J_{2ax-3}= $J_{2eq-2ax}$= 12.4 Hz, 1H, $H2_{ax}$), 1.28-1.52 (m, 10H), 1.04-1.25 (m, 24H), 0.92 (t, *J*= 7.2 Hz, 6H, 2*CH*$_3$). ^{13}C NMR (D_2O) δ 177.4 (*C*ONH), 174.9, 174.2 (2NH*C*O($CH_2)_2$*C*ONH), 163.1, 162.8 (*C*OCF_3), 120.6, 117.7, 114.8, 111.9 (CO*C*F_3), 93.1 (2C1'), 82.8 (2C5), 73.9 (2C4), 72.9 (2C6), 72.2 (2O*CH*$_2$($CH_2)_5$N), 70.4 (2C4'), 69.7 (2C5'), 68.6 (2C3'), 53.3 (2C2'), 49.8 (2C1), 48.8 (2C3), 40.4, 40.2, 39.2 (2C6', 2O($CH_2)_5$*CH*$_2$N, NCH(*CH*$_2$NHCO)$_2$), 35.9 (N*CH*(CH_2NHCO)$_2$), 31.2, 29.3, 28.7, 28.6, 28.4, 28.2, 25.9, 25.3, 24.7, 22.0 (2C2, 22*CH*$_2$), 13.4 (*CH*$_3$). LRMS (MALDI, DHB) *m/z*: 1300.9 $[M+Na]^+$, 1278.9 $[M+H]^+$, 871.

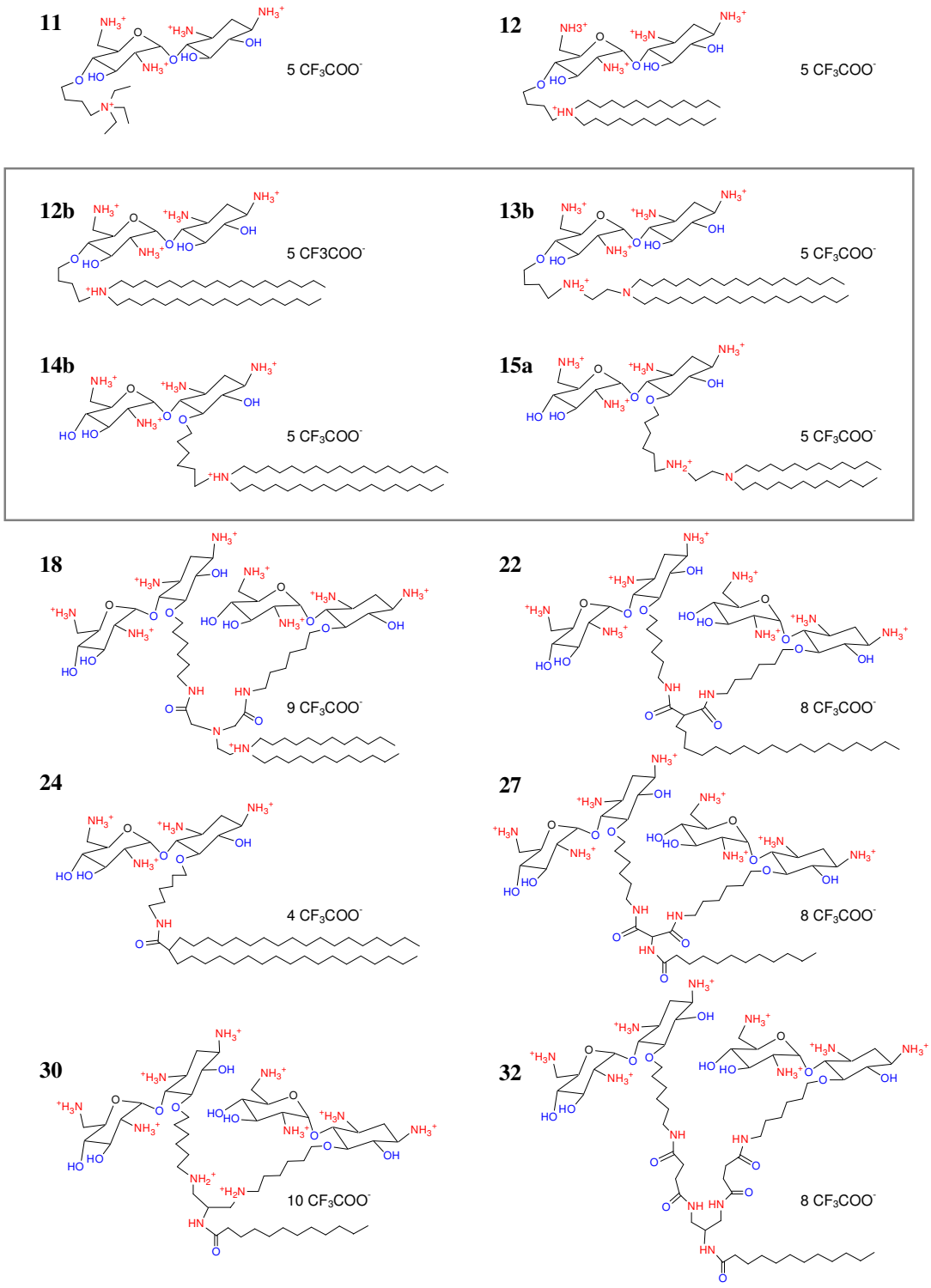

Chart S1. Chemical structure of the twelve neamine derivatives studied. For each derivative, the most likely charged species at physiological pH 7.4 is indicated, as determined with the MarvinSketch software [Marvin 5.0.2.1, 2008, ChemAxon (http://www.chemaxon.com)]. The four derivatives, which displayed the highest transfection activity, are here boxed.

Table S1. Comparison of some chemical features of lipidic derivatives of neamine and some others published cationic lipids.

	Cationic lipid	Mw (g/mol)	Z+ pH 7.4	Z+ pH 5.0	HG type	SP size (g/mol)	LD type	LogP	LogP+	References
Lipidic Neamine Derivatives	**30**, 10 TFA^-	2220	10	10	5,5-dinea	257	12C (1)	-39.9	1.0	This study
	32, 8 TFA^-	1964	8	8	5,5-dinea	455	12C (1)	-35.5	-1.0	This study
	27, 8 TFA^-	2020	8	8	5,5-dinea	285	12C (1)	-33.7	1.0	This study
	18, 9 TFA^-	2345	9	9	5,5-dinea	374	12C (2)	-32.5	5.5	This study
	22, 8 TFA^-	2075	8	8	5,5-dinea	270	18C (1)	-29.7	5.0	This study
	11, 5 TFA^-	1047	5	5	4'-mononea	73	2C (3)	-21.9	3.5	This study
	12a, 5 TFA^-	1300	5	5	4'-mononea	73	12C (2)	-14.4	12.0	This study
	15a, 5 TFA^-	1371	5	6	5-mononea	145	12C (2)	-12.8	12.5	This study
	13b, 5 TFA^-	1511	5	6	4'-mononea	117	18C (2)	-8.8	16.5	This study
	12b, 5 TFA^-	1468	5	5	4'-mononea	74	18C (2)	-8.7	17.0	This study
	14b, 5 TFA^-	1496	5	5	5-mononea	102	18C (2)	-7.9	17.5	This study
	24, 4 TFA^-	1424	4	4	5-mononea	143	18C (2)	-3.8	18.3	This study
Reference cationic lipids	DOSN, 6 TFA^-	1957	6	6	Neomycin B	176	18C (2)	-17.3	-6.5	Desigaux et al., 2007
	DOSP, 4 TFA^-	1671	4	4	Paromomycin	101	18C (2)	-10.8	11.5	Desigaux et al., 2007
	DOST, 4 TFA^-	1523	4	4	Tobramycin	101	18C (2)	-8.6	14.0	Desigaux et al., 2007
	DOSK, 3 TFA^-	1426	3	3	Kanamycin	101	18C (2)	-5.8	5.0	Desigaux et al., 2007
	BGTC, 2 Cl^-	719	2	3	Guanidinium	89	Chol (1)	-1.9	14.0	Vigneron et al., 1996
	DOTAP, $CH_3SO_4^-$	774	1	1	Ammonium	76	18C (2)	7.7	18.5	Leventis et al., 1990
	KLN47, I^-	871	1	1	Arsonium	125	18C (2)	14.2	24.0	Picquet et al., 2005

Mw, molecular weight; Z+ pH 7.4 or pH 5.0, most likely positive charge number at physiological pH 7.4 or pH 5.0, respectively; HG type, headgroup structure distinguishing, for neamine derivatives, between compounds containing one or two neamines and the attachment position of the spacer onto the neamine core (4'-mononea, 5-mononea or 5,5-dinea); LD type, lipid domain structure indicating the length and the number of aliphatic chains (except for BGTC which is a cholesteryl derivative); SP size, sum of atomic masses in the spacer (SP) domain; LogP, partitioning value of ionic species (average value of octanol-water portioning coefficient); LogP+, partitioning value estimation for a formulation combining one molecule of cationic lipid + one molecule of neutral colipid DOPE (molar ratio 1/1). All those values were calculated using the MarvinSketch software [Marvin 5.0.2.1, 2008, ChemAxon (http://www.chemaxon.com)]. Cationic lipids are listed according to their logP values. Lipidic derivatives of neamine (see Scheme S1); DOST, DOSP, DOSK, DOSN [lipidic derivatives of tobramycin, paromomycin, kanamycin and neomycin, respectively (*6*)]; BGTC (*7*); KLN47 (*8*).

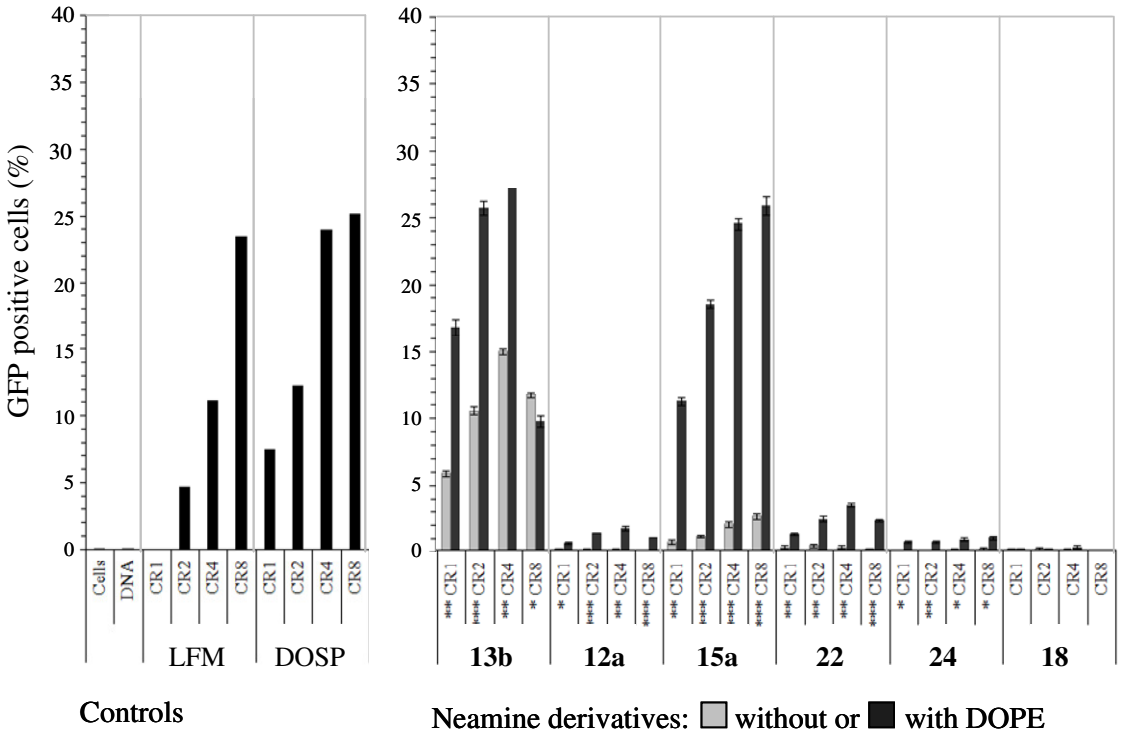

Figure S1. Flow cytometry measurements (GFP assays): transfection efficiencies of lipidic derivatives of neamine used alone or formulated with the colipid DOPE (molar ratio 1/1). The reagents indicated were complexed with DNA (pEGFP-Luc, see "Experimental Procedure" section) in order to obtain increasing charge ratios (CR), from CR1.0 up to CR8.0. The commercial Lipofectamine (LFM, DOSPA/DOPE, Invitrogen) and the paromomycin derivative [DOSP, DOSP/DOPE (*6*)] were used as cationic lipid references. Cell samples were analysed by flow cytometry in order to estimate the gene delivery efficiency as a percentage of positive cells (GFP positive cells). Other derivatives (**11**, **27**, **30**, and **32**) were found completely inefficient and, thus, they are not indicated here. These results were obtained with HeLa cells. Tests were performed in triplicates so efficiencies of formulations of neamine derivatives with or without DOPE can be compared (student t-test: *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$).

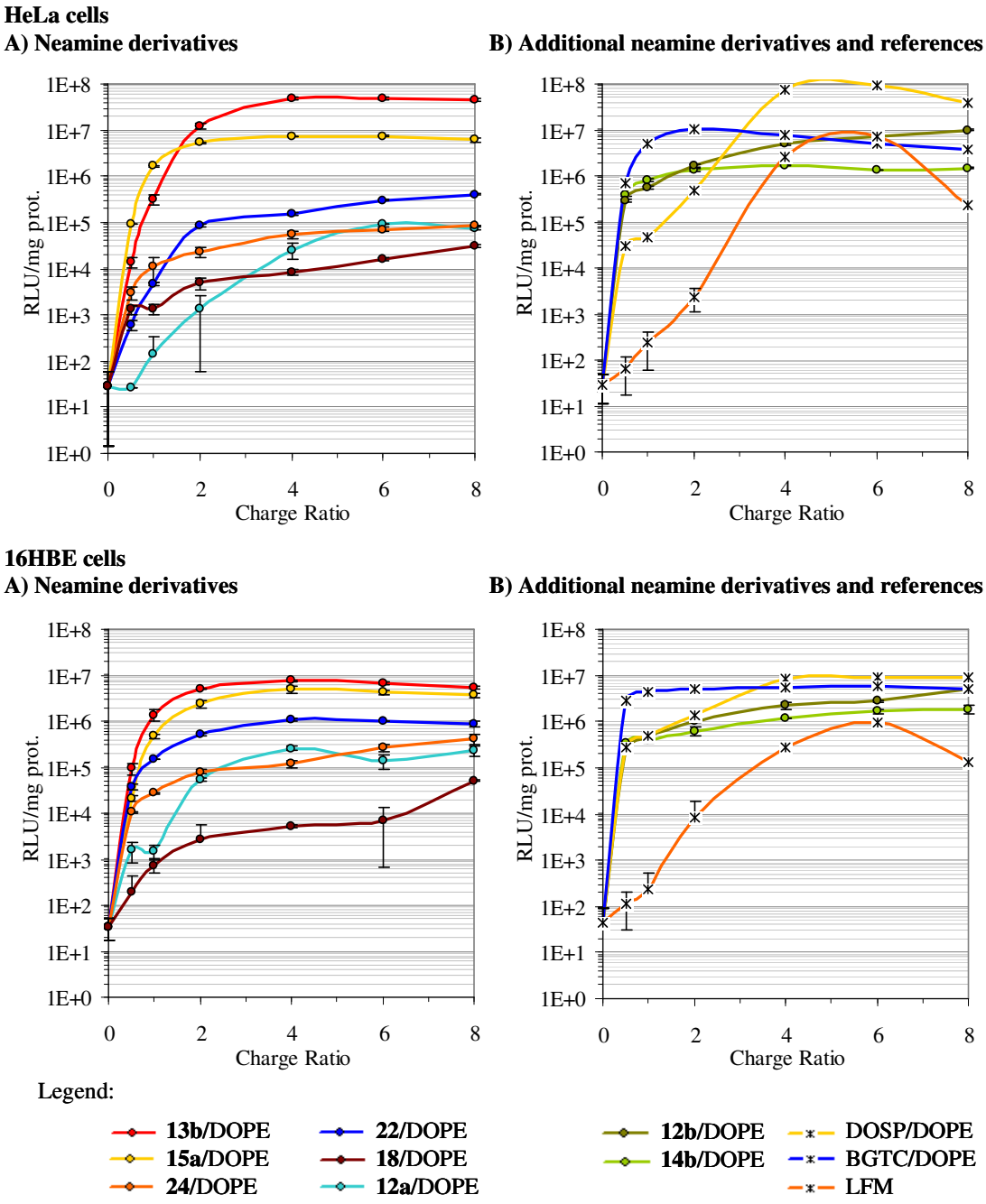

Figure S2. Luminometry measurements (luciferase assays): dose responses of the *in vitro* transfection activity of lipidic neamine derivatives formulated with the colipid DOPE (molar ratio 1/1). Luciferase reporter gene expression is indicated as a function of the charge ratio (CR) of lipoplexes, from CR0.5 up to CR8.0. The commercial Lipofectamine (LFM, Invitrogen), the paromomycin derivative [DOSP, (*6*)] and the bisguanidinium tren-cholesterol BGTC [BGTC/DOPE 3/2, (*7*)] derivative were used as cationic lipid references in those tests. Cells were transfected as described in "Experimental Procedure" section, using lipoplexes prepared by mixing DNA (pEGFP-Luc) with the required amounts of lipids. Data are expressed as relative light units (RLU) per mg of total proteins (mean +/- SD with n=3). These results were obtained using HeLa and 16HBE cells.

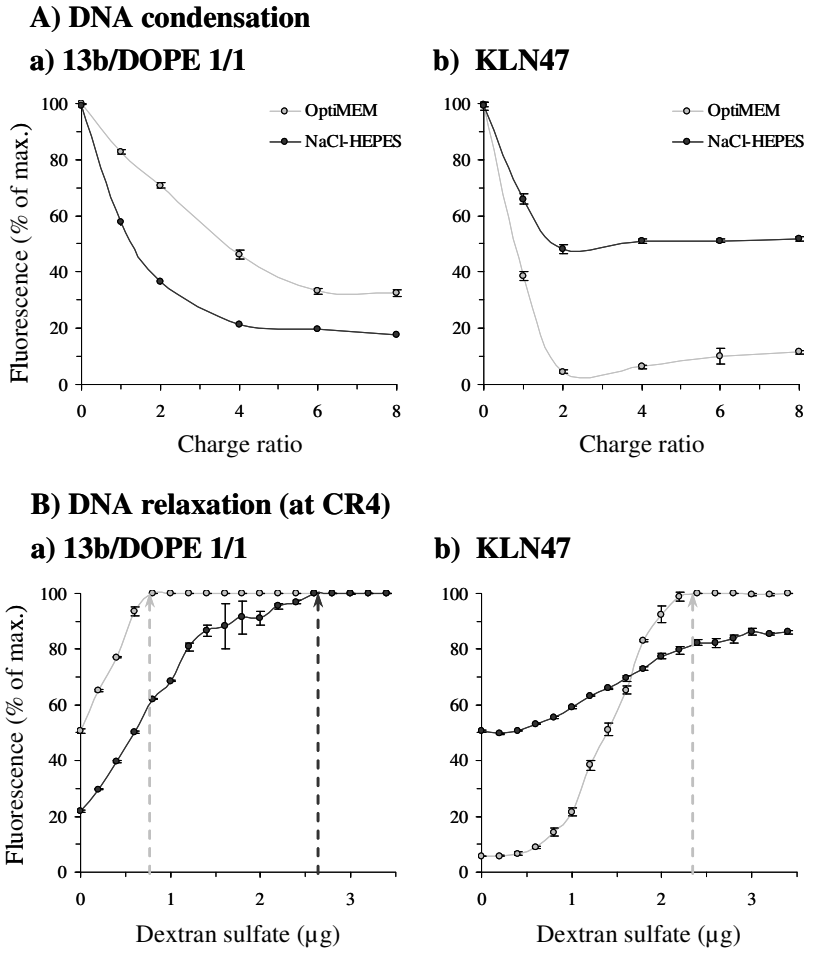

Figure S3. DNA condensation and relaxation assays with cationic liposomes 13b/DOPE 1/1 and KLN47. Complexes were obtained by mixing cationic liposomes at the required concentrations with plasmid DNA incubated first with ethidium bromide (EtB). The fluorescence decrease allowed the evaluation of nucleic acid entrapment within complexes (A; see "Experimental Procedure" section). Complexes at charge ratio 4 were then mixed with increasing quantities of dextran sulfate as a counter-anion. The recovery of fluorescence enabled to follow the DNA relaxation (B). The minimal quantity of counter-anion required to recover maximum fluorescence intensity can then be estimated (arrows). Assays were performed either in OptiMEM or in NaCl-HEPES (0.9% NaCl, 20 mM HEPES). Data are expressed as a percentage of the EtB fluorescence intercalated into DNA in absence of carrier (mean +/- SD with $n \geq 3$).

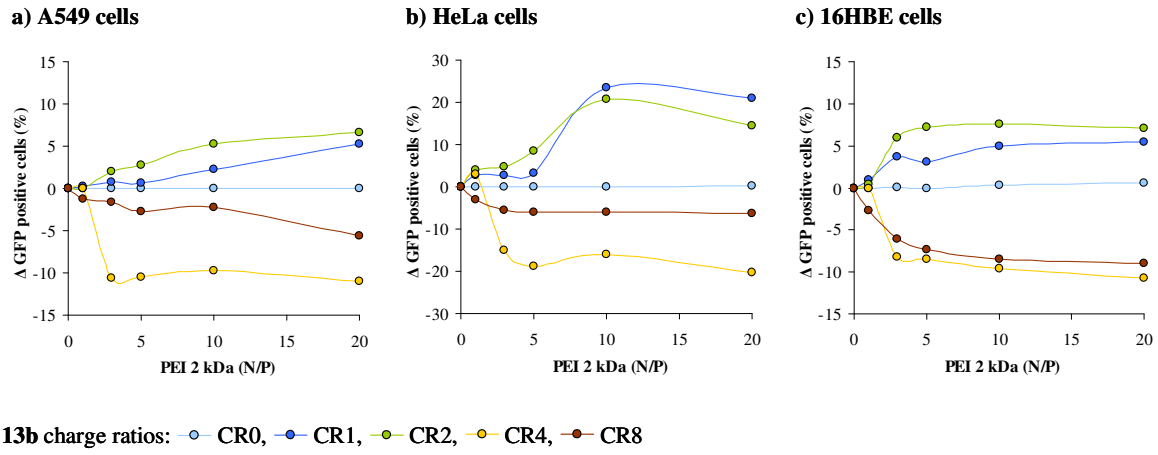

Figure S4. ***In vitro*** **transfection activity for combinations of PEI 2 kDa and 13b/DOPE 1/1 with three different cell lines.** Lipoplexes (**13b**/DOPE mixed with DNA, from CR1.0 up to CR8.0), polyplexes (PEI 2 kDa with DNA, from N/P1 up to N/P20) and lipopolyplexes (combinations of PEI 2 kDa with DNA, from N/P 1 up to 20, + **13b**/DOPE, from CR1.0 up to CR8.0) were used to transfect cell lines as described in "Experimental Procedure" section. For each CR, the variation of %GFP positive cells (Δ = % lipopolyplex - % lipoplex) is indicated [polyplexes alone (CR0) are ineffective, whatever the N/P tested]. Thus, compared with a lipoplex at a given CR, a lipopolyplex is more efficient if a Δ value higher than 0 is measured whereas it is less if a Δ value lower than 0 is obtained.

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FOOTNOTES

LFM, Lipofectamine; CR, charge ratio; EtB, ethidium bromide; RLU, relative light unit; TFA, trifluoroacetic acid; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; HOBT, 1-hydroxybenzotriazole; rt, room temperature; Δ, heating; Tr, trityl; PMB, *p*-methoxybenzyl.