**Summary**

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1. Capacity of compounds 5, 6, 9a/b and 11a/b to compact pDNA without DOPE as colipid.

Fig. S1. Ability for pDNA complexation of compounds 5, 6, 9a/b and 11a/b, all formulated without DOPE, at various CR. For each compound, the profile of retardation assay (panel A) and the corresponding relative fluorescence intensity (panel B) of the lower DNA band (i.e. the supercoiled pDNA form are shown.
2. Transfection efficacies of compounds 5 and 6 formulated without DOPE.

Fig. S3. Transfection efficacies (TE) of compounds 5 and 6, formulated without DOPE on cell lines A549, 16HBE and SKMel28 using luciferase encoding pDNA. TE are expressed in RLU/mg of proteins (n=3). Naked pDNA was used as negative control.

3. Transfection efficacies of compounds 9a/b and 11a/b formulated without DOPE.

Fig. S4. Transfection efficacies (TE) of compounds 9a/b and 11a/b, all formulated without DOPE, on cell lines A549, 16HBE and SKMel28 using luciferase encoding pDNA. TE are expressed in RLU/mg of proteins (n=3). Lipofectamine (LFM) and naked pDNA was used as positive and negative control, respectively.
4. Transfection efficacies of compounds 5, 6, 9a/b and 11a/b formulated with DOPE.

**Figure S2-1.** Transfection efficiency and viability following incubation of four cell lines with lipoplexes prepared with quaternary ammonium sophorolipids 5 and 6, hydroxylated quaternary ammonium salts 9a and 9b, or non-hydroxylated quaternary ammonium salts 11a and 11b. Lipids (formulated alone or with the colipid DOPE at 1:1 molar ratio) were complexed with the luciferase-encoding pDNA pEGFP-Luc at various CR. Lipofectamine (“LFM”) and naked (uncomplexed) pDNA (“DNA”) were used as positive and negative control, respectively. The transfection efficacy (evaluated in the unit of RLU/mg of protein; panel A) and the cell viability (evaluated using untransfected cells as reference; panel B) are color coded. LX CR, lipoplex charge ratio (n=3); nd, not determined.
Figure S2-2. Transfection efficiency of C2C12 cell line with lipoplexes prepared with quaternary ammonium sophorolipids 5 and 6, hydroxylated quaternary ammonium salts 9a and 9b, or non-hydroxylated quaternary ammonium salts 11a and 11b. Lipids (formulated with the colipid DOPE at 1:1 molar ratio) were complexed with the luciferase-encoding pDNA pEGFP-Luc at various CR. Lipofectamine ("LFM") and naked (uncomplexed) pDNA ("DNA") were used as positive and negative control, respectively. The transfection efficacies were evaluated in the unit of RLU/mg of protein.
5. Cell viability (C2C12) after incubation with 5, 6, 9a/b and 11a/b-based lipoplexes formulated with DOPE.

**Figure S7:** Cell viability following incubation of C2C12 cell line with lipoplexes prepared with quaternary ammonium sophorolipids 5 and 6, hydroxylated quaternary ammonium salts 9a and 9b, or non-hydroxylated quaternary ammonium salts 11a and 11b. Lipids (formulated with the colipid DOPE at 1:1 molar ratio) were complexed with the luciferase-encoding pDNA pEGFP-Luc at various CR. Lipofectamine ("LFM") and naked (uncomplexed) pDNA ("DNA") were used as positive and negative control, respectively.
6. Cell viability (A549, 16HBE, SKMEL28) after incubation with 5 and 6-based lipoplexes formulated without DOPE.

Fig. S5. Cell viability determined 48 h after incubation of the cells with lipoplexes prepared with compounds 5 and 6, formulated without DOPE. Naked pDNA was used as negative control. Values are expressed as a percentage of the viability determined with untransfected cells.
7. Cell viability (A549, 16HBE, SKMEL28) after incubation with 9a/b and 11a/b-based lipoplexes formulated without DOPE.

Fig. S6. Cell viability determined 48 h after incubation of the cells with lipoplexes prepared with compounds 9a/b and 11a/b, all formulated without DOPE. Naked pDNA was used as negative control. Values are expressed as a percentage of the viability determined with untransfected cells.
8. NMR spectra.

Fig. S6. $^1$H-NMR spectrum for (S)-9-(methyl(octadecyl)amino)nonan-2-ol (8)
Fig. S7. $^{13}$C-NMR spectrum for (S)-9-(methyl(octadecyl)amino)nonan-2-ol (8).
Fig. S8. 1H-NMR spectrum for N-(S)-8-hydroxynonyl)-N,N-dimethyloctadecan-1-ammonium iodide (9a)
Fig. S9. $^{13}$C-NMR spectrum for N-((S)-8-hydroxynonyl)-N,N-dimethyloctadecan-1-ammonium iodide (9a).
Fig. S10. $^1$H-NMR spectrum for $N$-butyl,$N$-((S)-8-hydroxynonyl)$N$-methyleneoctadecan-1-aminium iodide (9b)
Fig. S11. $^{13}$C-NMR spectrum for N-butyl, N-((S)-8-hydroxynonyl)-N-methyloctadecan-1-ammonium iodide (9b).
Fig. S12. H-NMR spectrum for N-methyl-N-nonyl octadecan-1-amine (10)
Fig. S13. 13C-NMR spectrum for N-methyl-N-nonyloctadecan-1-amine (10)
Fig. S15. \( ^13 \)C-NMR spectrum for \( \text{N}, \text{N}-\text{dimethyl, N}-\text{nonyl octadecan-1-ammonium iodide} \ (11\text{a}) \).
Fig. S16. $^1$H-NMR spectrum for N-buty1,N-methyl,N-nonyl-octadec-1-aminonium iodide (11b)
Fig. S17. 1H-NMR spectrum for N-butyl, N-methyl, N-nonyloctadecan-1-ammonium iodide (11b)