A novel cationic lipophosphoramide with di-unsaturated lipid chains: synthesis, physico-chemical properties, and transfection activities

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Figures S1–S3 provide additional informations about DNA condensation into, and its release from, lipoplexes formed by lipid 4–based liposomes or by the commercially available Lipofectamine (LFM)–based liposomes, under various experimental conditions.
Supporting Information Figure legends

**Figure S1.** DNA condensation and relaxation assays using LFM or lipid 4–based liposomes. The ability of lipid 4 and LFM–based liposomes to condense DNA was studied by mixing these cationic liposomes with plasmid DNA previously incubated with ethidium bromide. The required amounts of liposomes were used in order to form complexes characterized by different charge ratios, from 0.5 up to 8.0. The fluorescence decrease allowed the evaluation of the nucleic acid entrapment within complexes (see Experimental Section). Next, the effect of dextran sulfate as a counteranion was also studied, as this molecule can induce a dismantling of the preformed complexes, which results in DNA relaxation, and thus, recovery of fluorescence. Complexes formed at a charge ratio of 4 were mixed with increasing amounts of dextran sulfate, the recovery of fluorescence enabling to monitor the subsequent DNA relaxation. Assays were performed either in OptiMEM or in isotonically saline solution (0.9% NaCl). Data are expressed as a percentage of the maximum fluorescence, i.e., the fluorescence observed when ethidium bromide is intercalated into DNA in absence of any cationic lipid (mean ± SD with n ≥ 3). Of note, the data obtained with KLN47–based liposomes (not shown) were basically similar to those reported here for lipid 4–based liposomes.

**Figure S2.** DNA condensation and relaxation assays using LFM–based liposomes: correlation between results from fluorescence measurements (left) and agarose gel retardation assays (right). LFM–based lipoplexes were obtained by mixing, in 0.9% NaCl, liposomes with plasmid DNA previously incubated with ethidium bromide. The required amounts of liposomes were used in order to form complexes characterized by different charge ratios, from 0.5 up to 8.0. The fluorescence decrease allowed the evaluation of the nucleic acid entrapment within the complexes. These complexes were next mixed with 3.2 µg of dextran sulfate, as this amount was previously found to efficiently release the DNA from LFM–based lipoplex formed at CR4.0 (Supporting Information Figure S1). The recovery of fluorescence enabled monitoring of the DNA relaxation. Measurements were conducted before (1) and after (2) the addition of dextran sulfate (left part). Data are expressed as a percentage of the maximum fluorescence, i.e., the fluorescence observed when ethidium bromide is intercalated into DNA in absence of any cationic lipid (mean ± SD with n ≥ 3). Finally, each experimental condition was analysed by agarose gel electrophoresis (right part).

**Figure S3.** Time course of DNA condensation using LFM or lipid 4–based liposomes. Complexes were obtained by mixing, in OptiMEM, cationic liposomes at the required concentrations with plasmid DNA, previously incubated with ethidium bromide, in order to reach increasing charge ratios, from 1 up to 8. The fluorescence decrease allowed the evaluation of the nucleic acid entrapment within the lipoplexes. DNA condensation was evaluated for 14 h, with measurements repeated every 20 min. Data are expressed as a percentage of the maximum fluorescence, i.e., the fluorescence observed when ethidium bromide is intercalated into DNA in absence of any cationic lipid (mean ± SD with n ≥ 3). Of note, the data obtained with KLN47–based liposomes (not shown) were basically similar to those reported here for lipid 4–based liposomes.
Supporting Information Figures

A/ DNA condensation
1/ LFM

![Graph showing DNA condensation with LFM liposomes.](image)

2/ Lipid 4

![Graph showing DNA condensation with Lipid 4 liposomes.](image)

B/ DNA relaxation (at Charge Ratio 4)
1/ LFM

![Graph showing DNA relaxation with LFM liposomes.](image)

2/ Lipid 4

![Graph showing DNA relaxation with Lipid 4 liposomes.](image)

Figure S1. DNA condensation and relaxation assays using LFM or lipid 4–based liposomes.
**Figure S2.** DNA condensation and relaxation assays using LFM–based liposomes: correlation between results from fluorescence measurements (left) and agarose gel retardation assays (right).

1/ LFM–based lipoplexes

![1/ LFM–based lipoplexes](image)

2/ Lipid 4–based lipoplexes

![2/ Lipid 4–based lipoplexes](image)

**Figure S3.** Time course of DNA condensation using LFM or lipid 4–based liposomes.