

Supplementary information for:

Asymmetric cationic lipid based non-viral vectors for an efficient nucleic acid delivery

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Fig S1. $^1\text{H-NMR}$ spectra of Di-Octadecylamine.

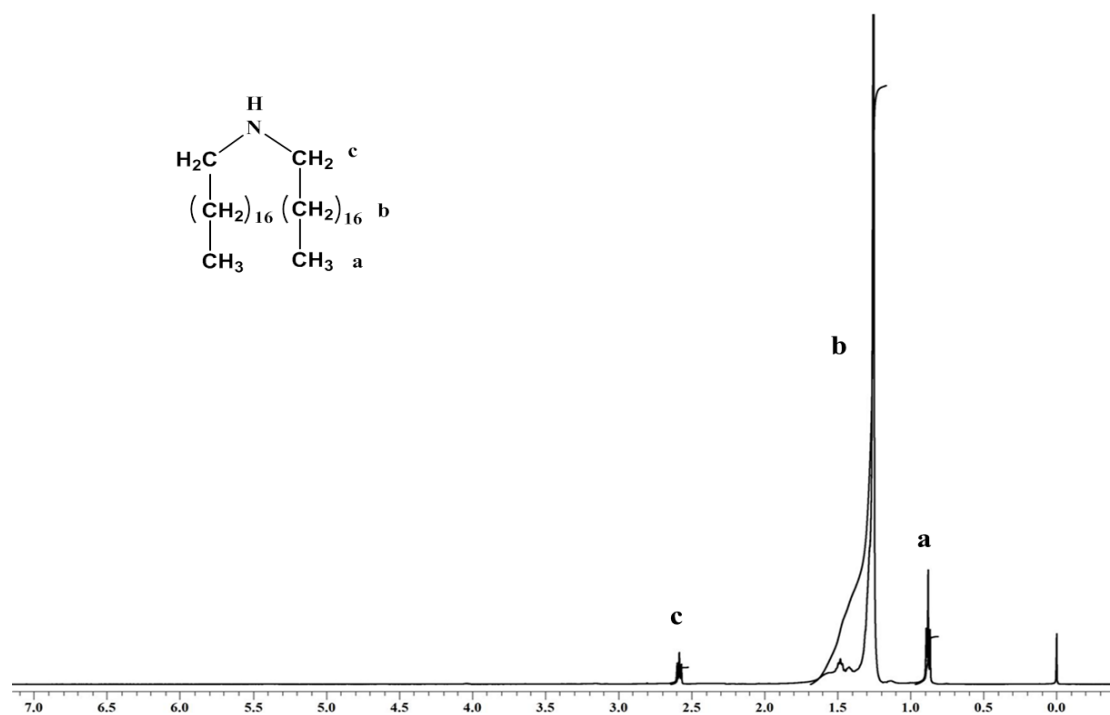


Fig S2. ESI-MASS spectra of Di-Octadecylamine

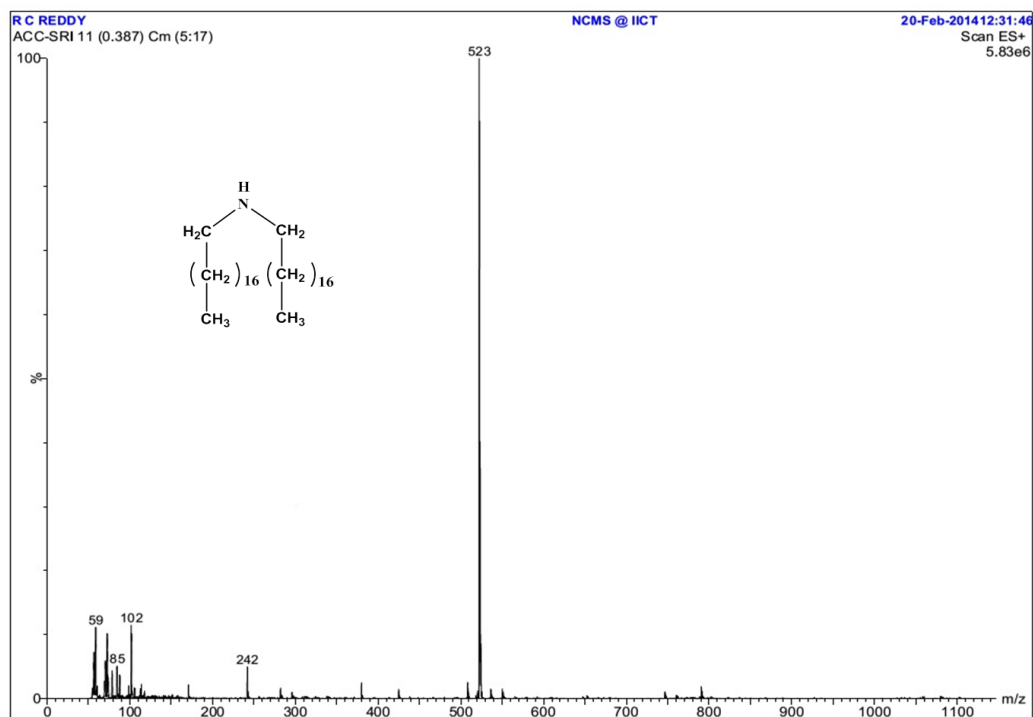


Fig S3. $^1\text{H-NMR}$ spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadecan-1-Aminium

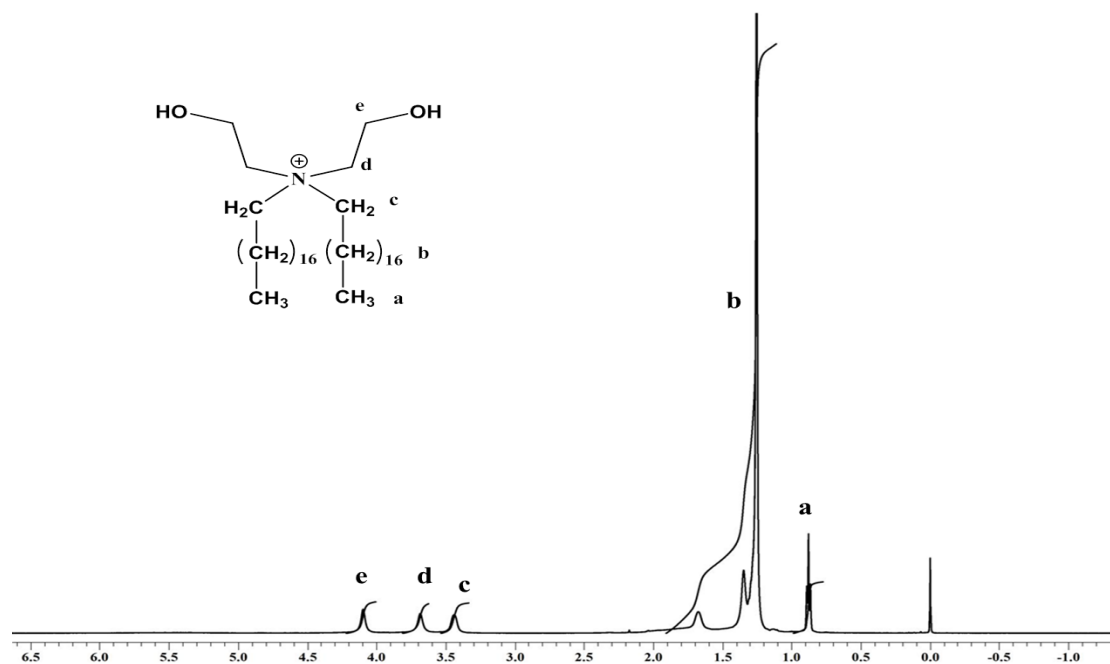


Fig S4. ESI-MASS spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadecan-1-Aminium

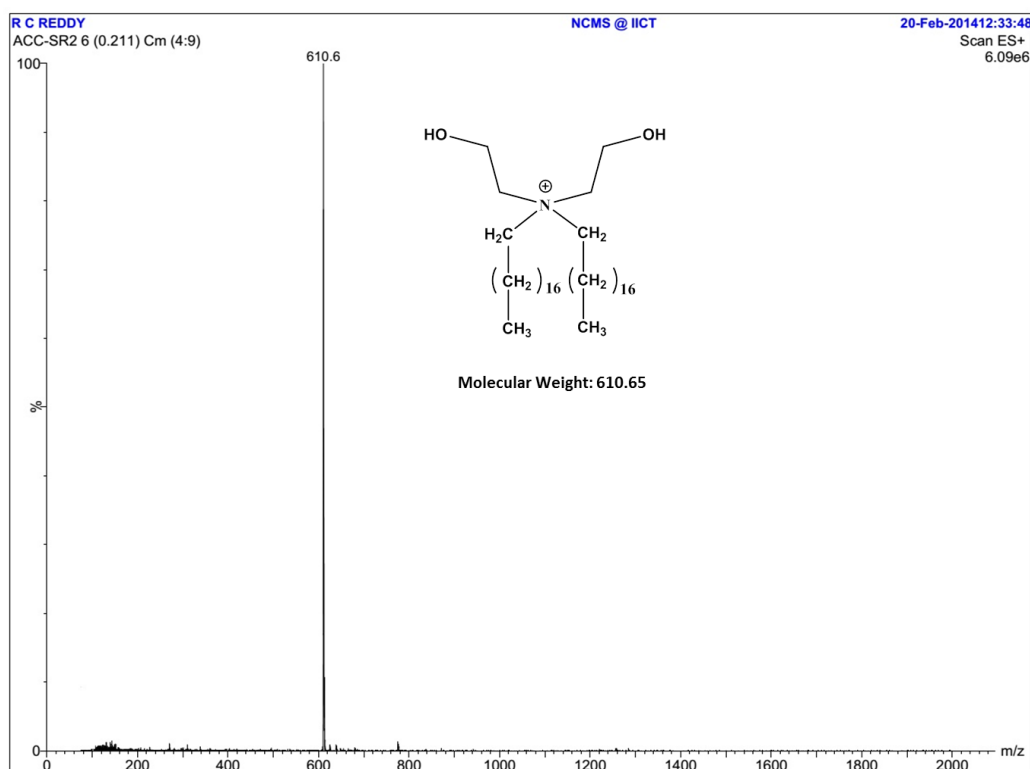


Fig S5. $^1\text{H-NMR}$ spectra of N-Octadecyloctadec-9-en-1 amine

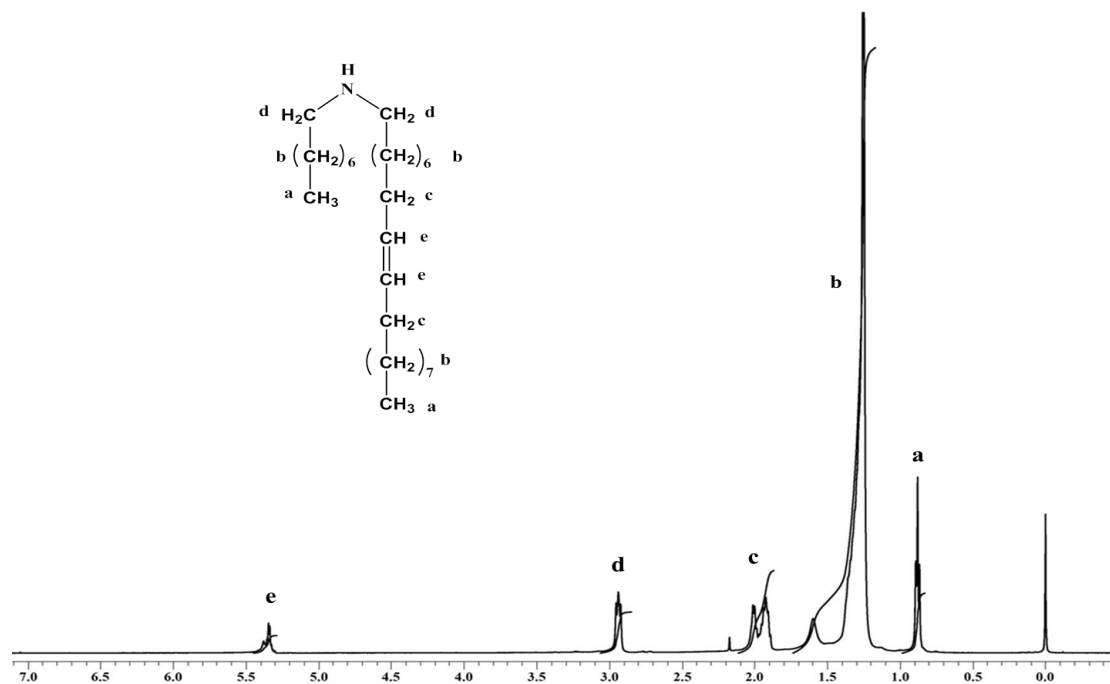


Fig S6. ESI-MASS spectra of N-Octadecyloctadec-9-en-1 amine

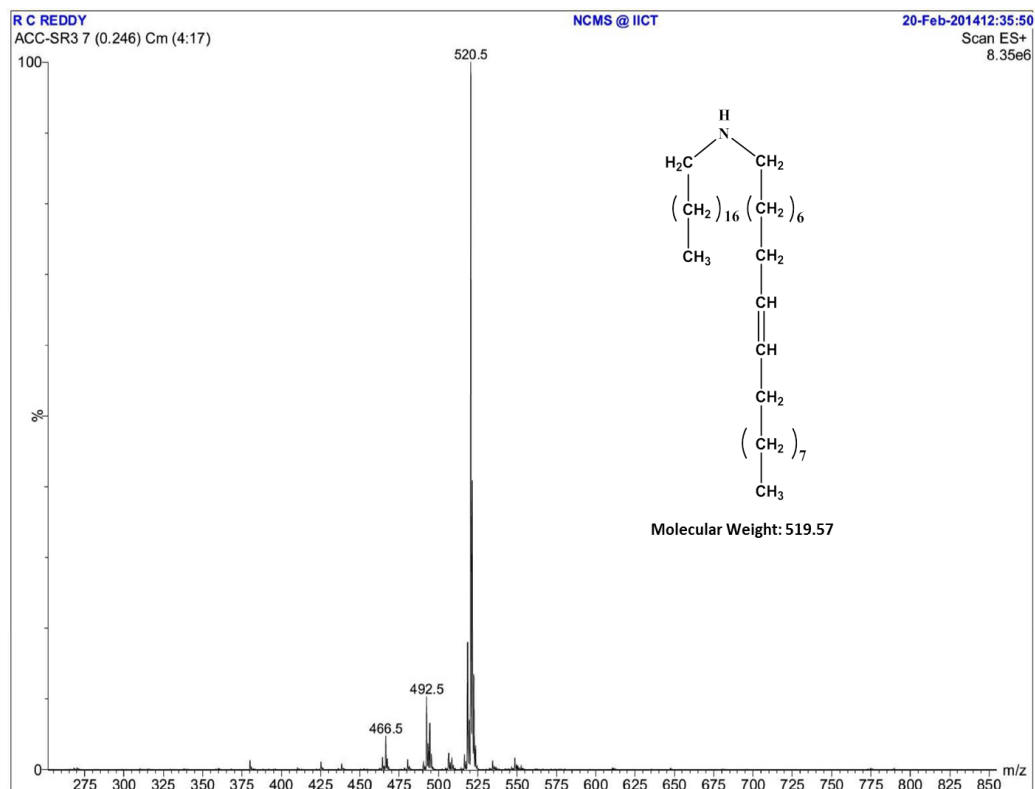


Fig S7. $^1\text{H-NMR}$ spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadec-9- en-1-aminium

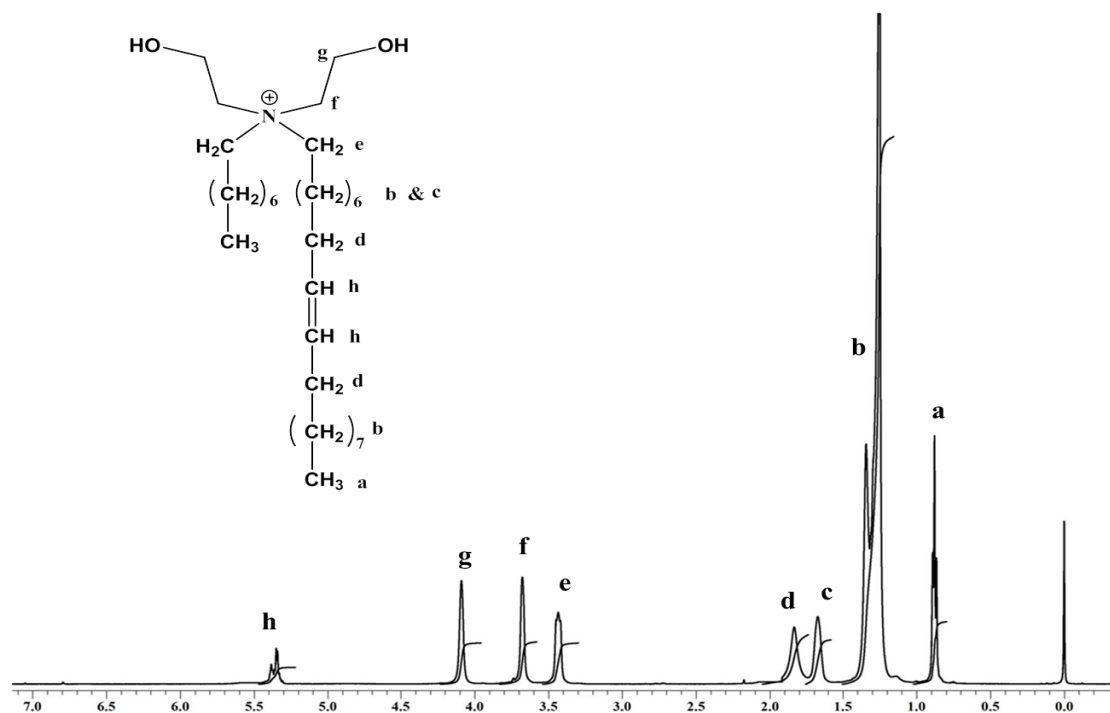


Fig S8. ESI-MASS spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadec-9- en-1-aminium

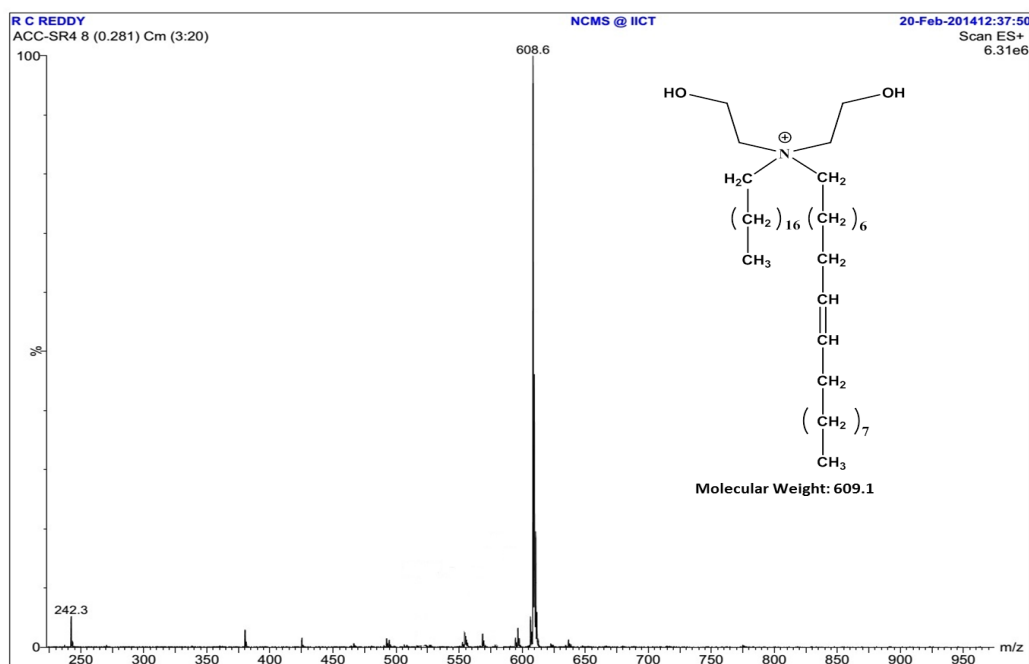


Fig S9. $^1\text{H-NMR}$ spectra of Di (Octadec-9-en-1yl) amine

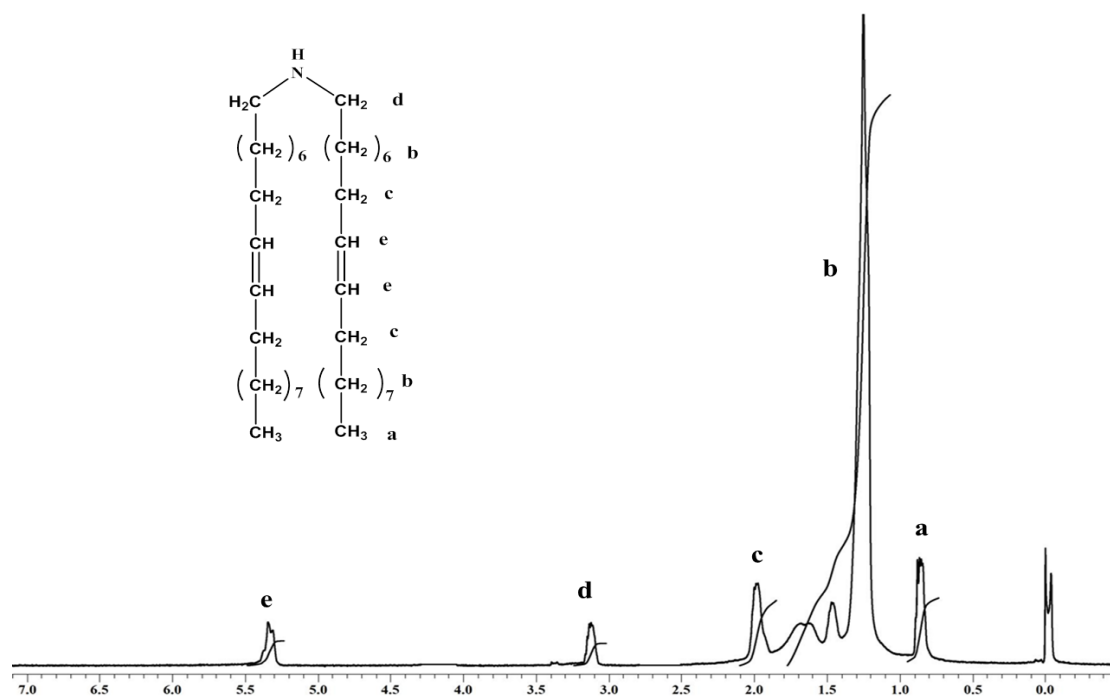


Fig S10. ESI-MASS spectra of Di (Octadec-9-en-1yl) amine

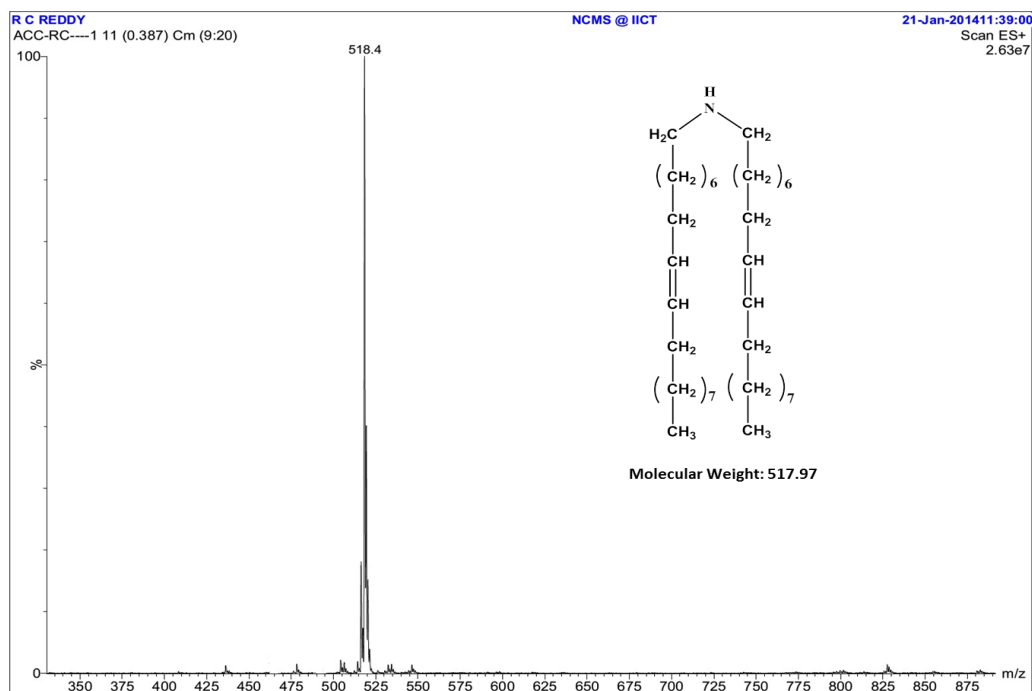


Fig S11. $^1\text{H-NMR}$ spectra of N, N-bis (2-hydroxyethyl) –N-(octadec-9-en-1-yl) Octadec-9-1-aminium

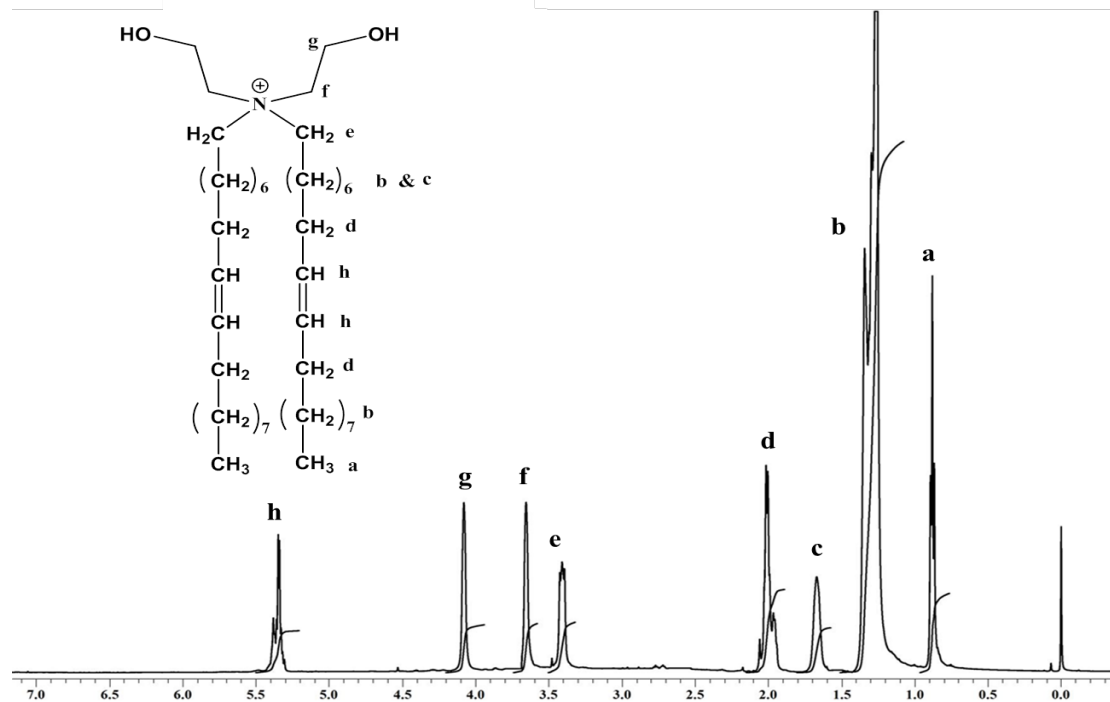


Fig S12. ESI-MASS spectra of N, N-bis (2-hydroxyethyl) –N-(octadec-9-en-1-yl) Octadec-9-1-aminium

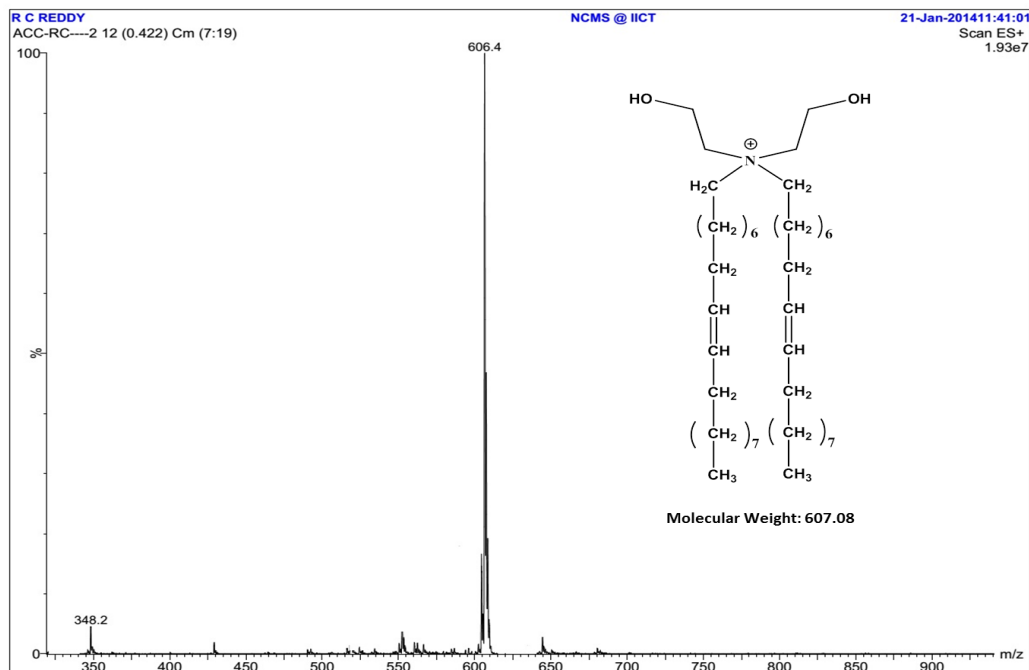


Fig S13. HRMS spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadecan-1-
Aminium

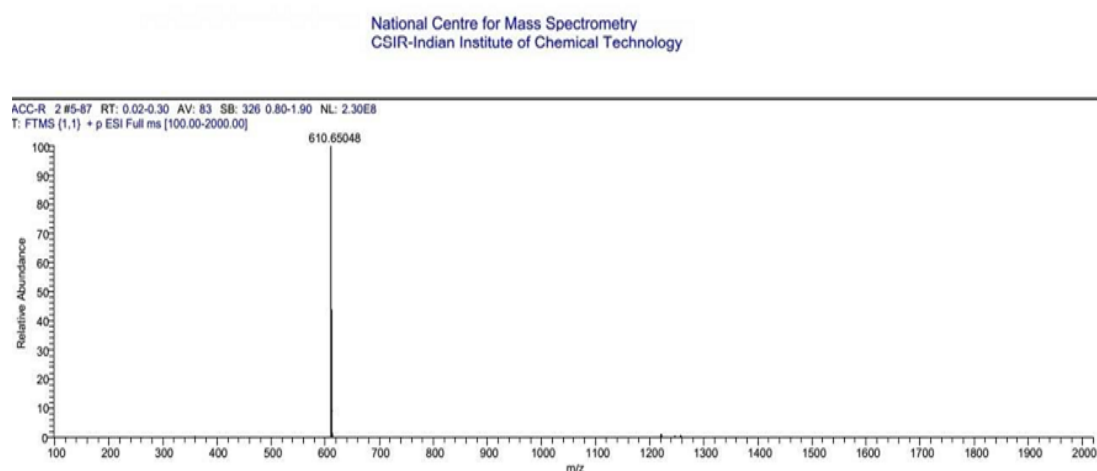


Fig S14. HRMS spectra of N, N-bis(2-hydroxyethyl) –N-octadecyloctadec-9- en-1-
aminium

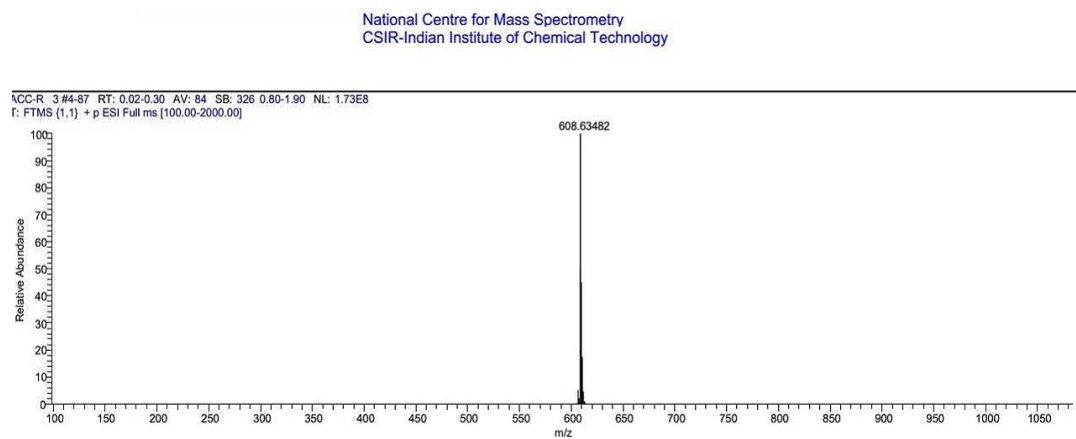


Fig S15.HRMS spectra of N, N-bis (2-hydroxyethyl) –N-(octadec-9-en-1-yl) Octadec-9-1-aminium

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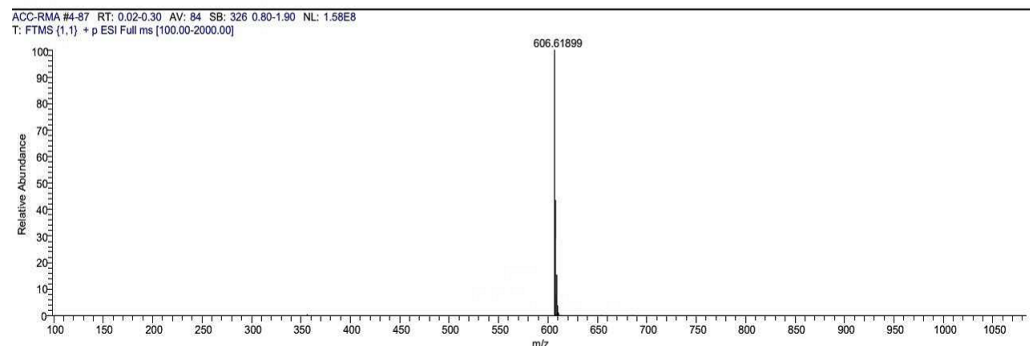
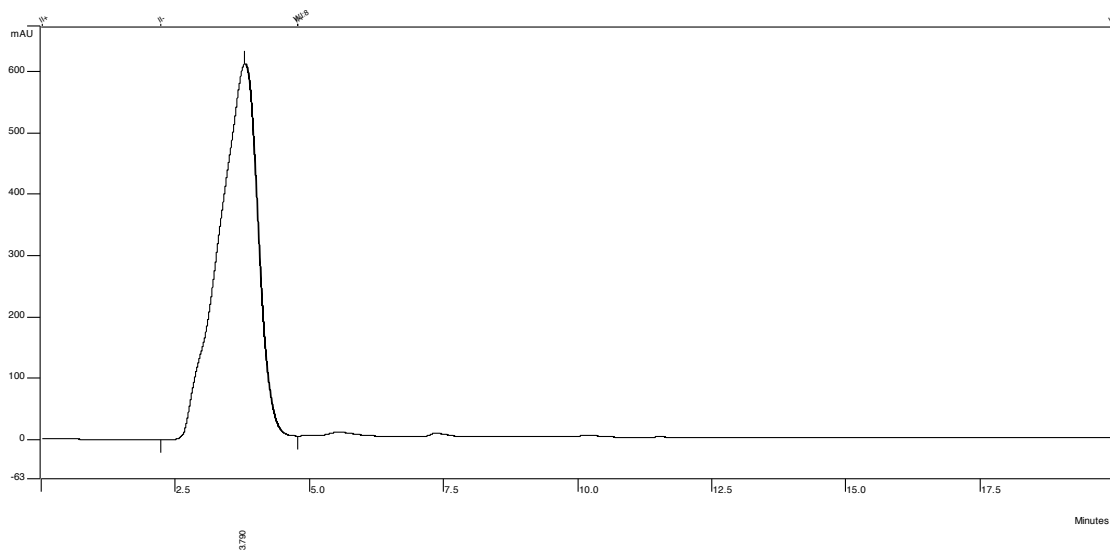


Figure S16. HPLC spectra of **Lipid S-S**

A. Solvent system: 100% Methanol



B. Solvent system: 5% Water in Methanol

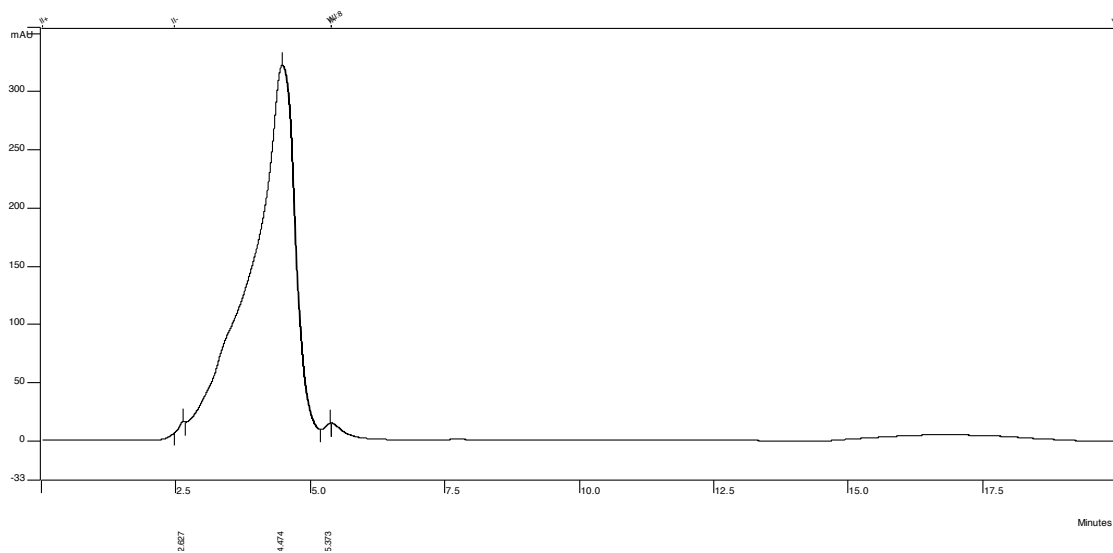
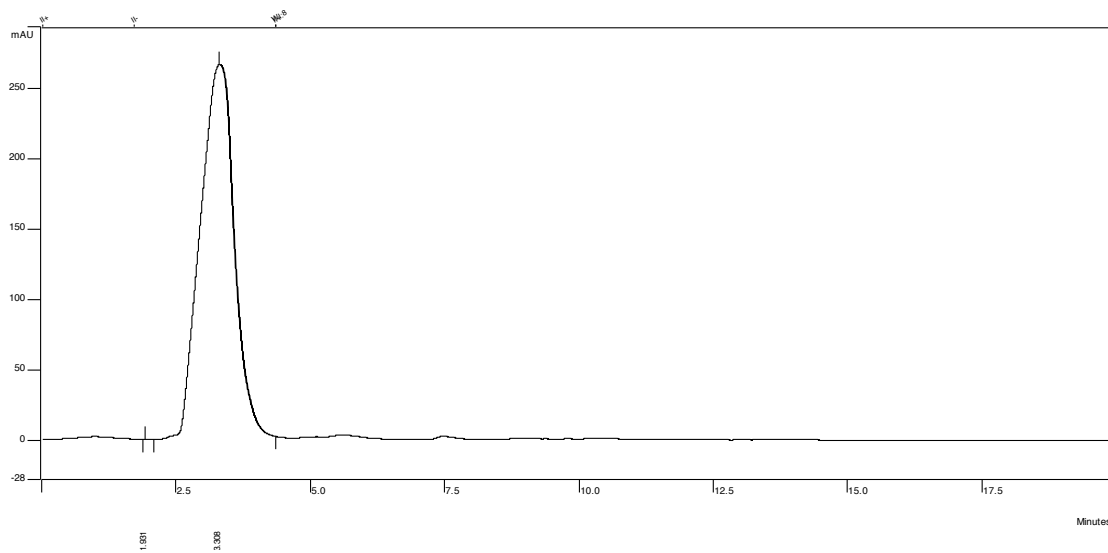


Figure S 17. HPLC spectra of **Lipid S-U**

A. Solvent system: 100% Methanol



B. Solvent system: 5% Water in Methanol

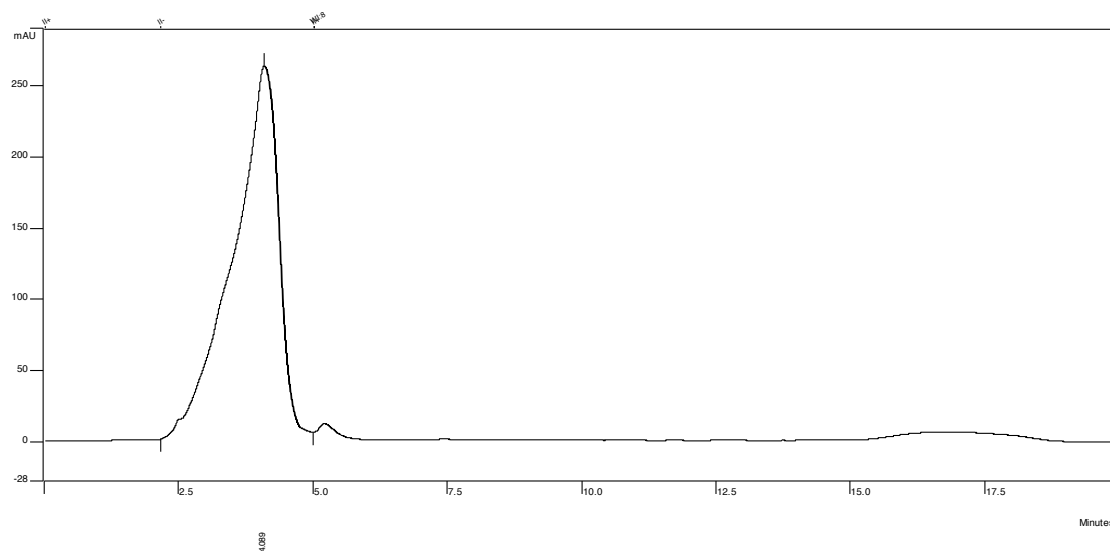
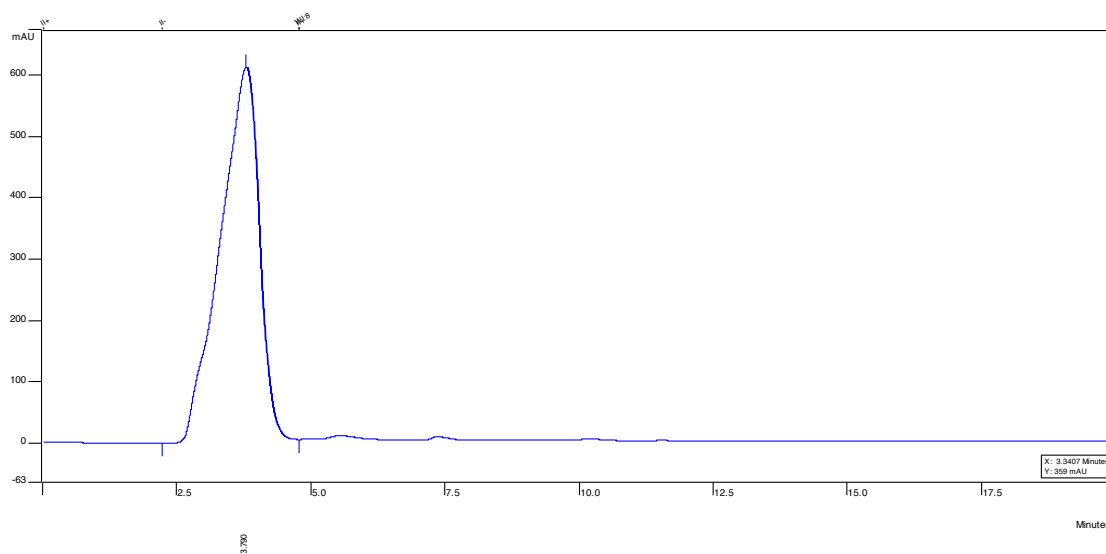
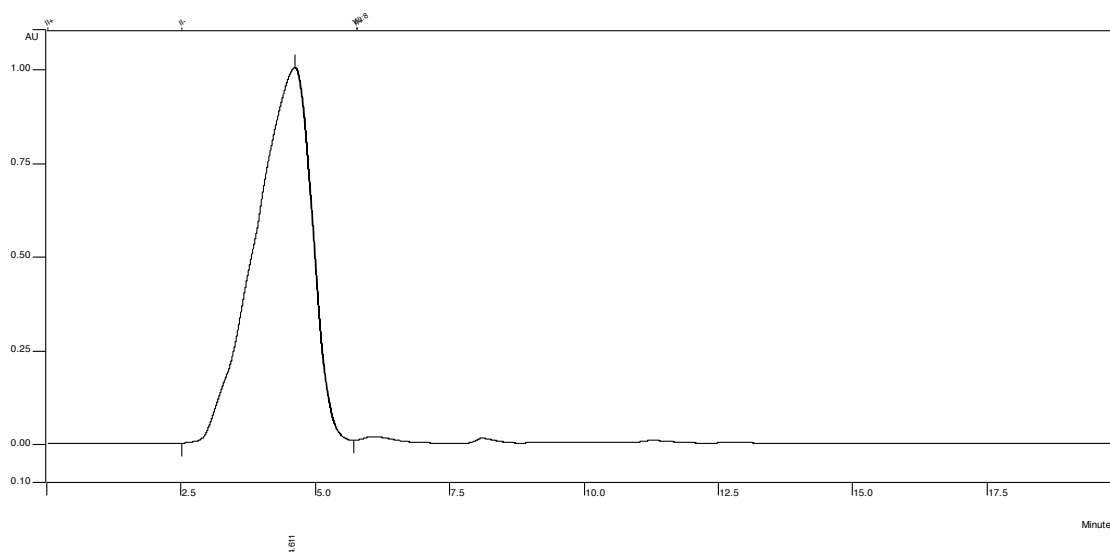


Figure S 18. HPLC spectra of Lipid U-U

A. Solvent system: 100% Methanol



B. Solvent system: 5% Water in Methanol



HPLC Conditions:

System: Varian series

Column: Lichrospher® 100, RP-18e (5 µm)

Mobile Phases: Methanol (A); Methanol:Water, 95:5, v/v, (B).

Flow Rate: 2.0 mL/min

Typical Column Pressure: 60-65 Bars

Detection: UV at 210 nm

Figure S19. A) DNA binding assay for three lipids with varying lipid/DNA charge ratio, B) DNA degradation profile post DNase I treatment for three lipid formulations with varying lipid/DNA charge ratio.

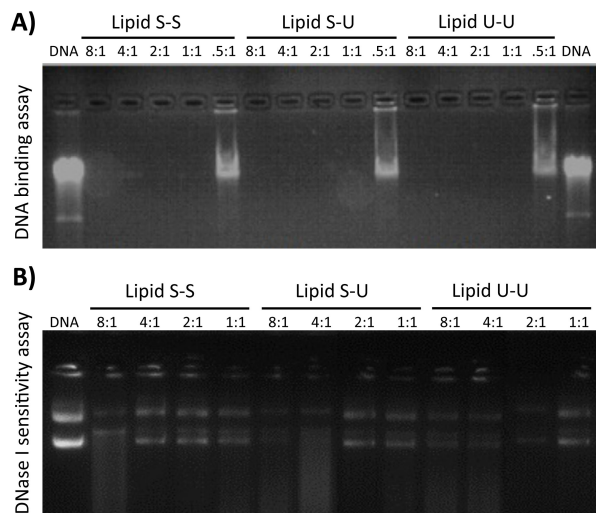


Figure S20: Cytotoxic effect of lipid-DNA complexes was studied with a) B16F10 (murine melanoma) and b) CHO (Chinese hamster ovary) cells at varying lipid/DNA charge ratio.

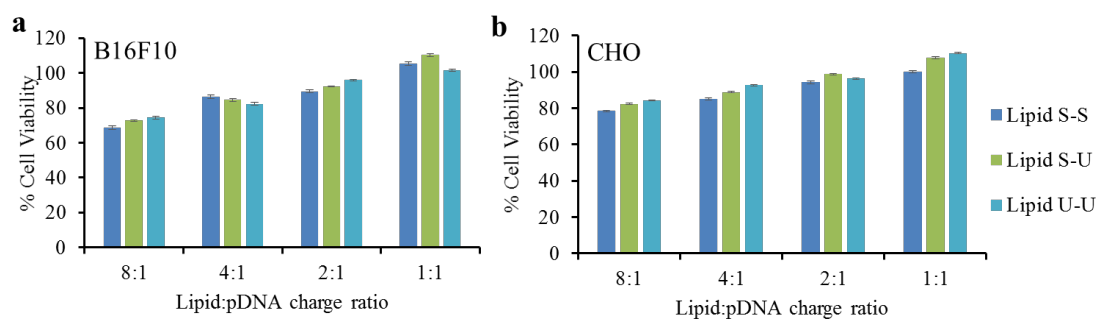


Figure S21: Comparative cytotoxic assays with commercial transfection agent lipofectamine 2000 and cationic lipids using CHO (Chinese hamster ovary) cells.

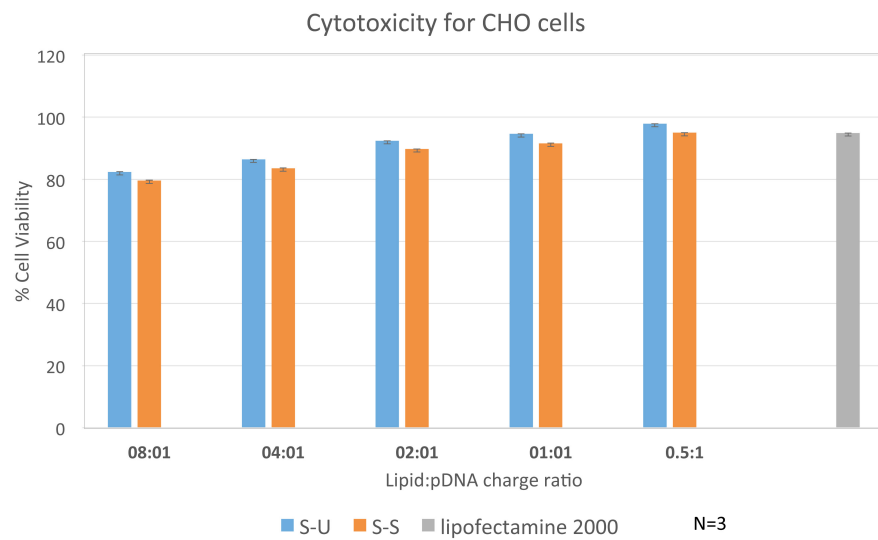


Figure S22. Confocal laser scanning microscope images for endosomal escape of lipoplexes with liposome tagged with NBD-PE (green) and lysosomes trailed with lysotracker red.

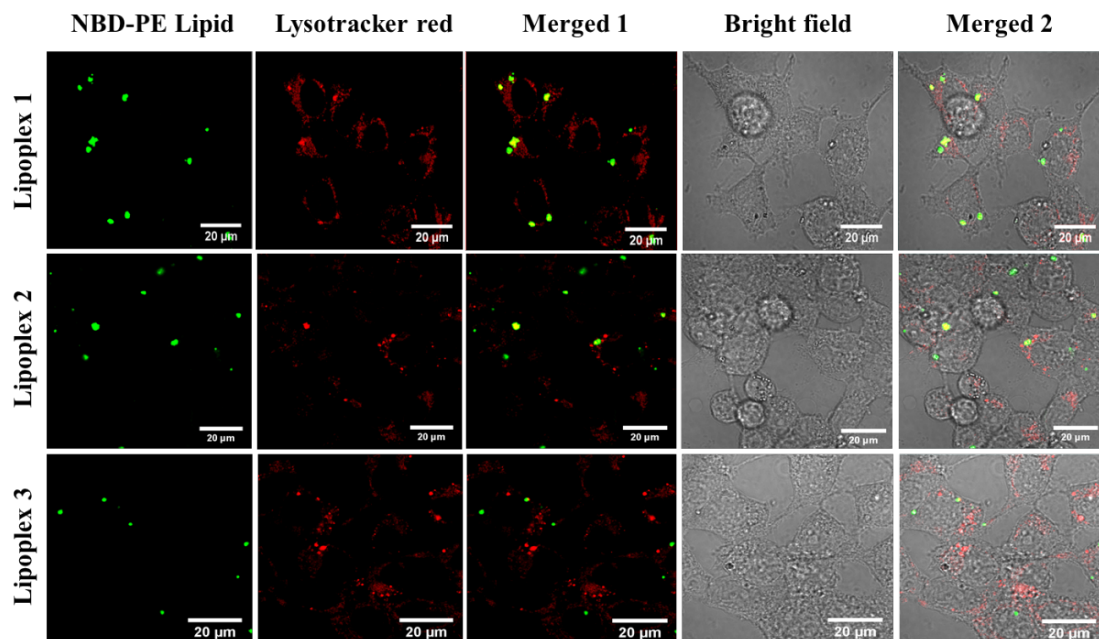


Table TS1: Size and Zeta potential measurements for **Lipid S-S, S-U** and **U-U** with varying lipid/DNA charge ratio.

Liposomes	Lipid:DNA Charge Ratio							
	8:1		4:1		2:1		1:1	
	HDD (nm)	Zeta Potential (mEV)	HDD (nm)	Zeta Potential (mEV)	HDD (nm)	Zeta Potential (mEV)	HDD (nm)	Zeta Potential (mEV)
Lipid S-S	197.3	+21.4	205.7	+11.7	367.6	+2.8	474.8	-21.5
Lipid S-U	172.5	+25.3	211.8	+15.7	294.7	+6.5	332.8	-08.1
Lipid U-U	187.5	+14.3	234.0	+12.8	369.2	+3.1	441.2	-17.3

Table TS2: Size and Zeta potential measurements for **Lipid S-S, S-U** and **U-U** in **DI water**

Liposome	Zeta Size (nm)	Potentials(mV)
1	113.2 ± 2.6	28.5 ± 5.82
2	117.5 ± 3.2	46.1 ± 7.82
3	136.7 ± 5.7	30.3 ± 8.24