Supporting Information (Ms. No. ja0704683)

Dramatic Influence of the Orientation of Linker between Hydrophilic and Hydrophobic Lipid Moiety in Liposomal Gene Delivery.

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Complete author lists for references 3 & 7i.

3. Hacein-Bey-Abina, S.; Von Kalle, C.; Schmidt, M.; McCormack, M.P.; Wulffraat, N.; Leboulch, P.; Lim, A.; Osborne, C.S.; Pawliuk, R.; Morillon, E.; Sorensen, R.; Forster, A.; Fraser, P.; Cohen, J.I.; de Saint Basile, G.; Alexander; I.; Wintergerst, U.; Frebourg, T.; Aurias, A.; Stoppa- Lyonnet, D.; Romanana, S.; Radford-Weiss, I.; Gross, F.; Valensi, F.; Delabesse, E.; Macintyre, E.; Sigaux, F.; Soulier, J.; Leiva, L.E.; Wissler, M.; Prinz, C.; Rabbitts, T.H.; Le Deist, F.; Fischer, A.; Cavazzana-Calvo, M. *Science* **2003**, *401*, 415-419.

7i. Kirby, A. J.; Carmilleri, P.; Engberts, J. B. F. N.; Feiters, M. C.; Nolte, R. J. M.; Soderman, O.; Bergsma, M.; Bell, P. C.; Fielden, M. L.; Garcia Rodriguez. C. L.; Guedat, P.; Kremer, A.; McGregor, C.; Perrin, C.; Ronsin, G.; Van Eijik.; M. C. P. *Angew. Chem.. Int. Ed.. Eng*. **2003**, *42*, 1448-1457.

Figure S1. ^{1}H NMR (200 MHz, $CDCl_3$) of the Intermidiate **I**, Scheme I, Part **A**.

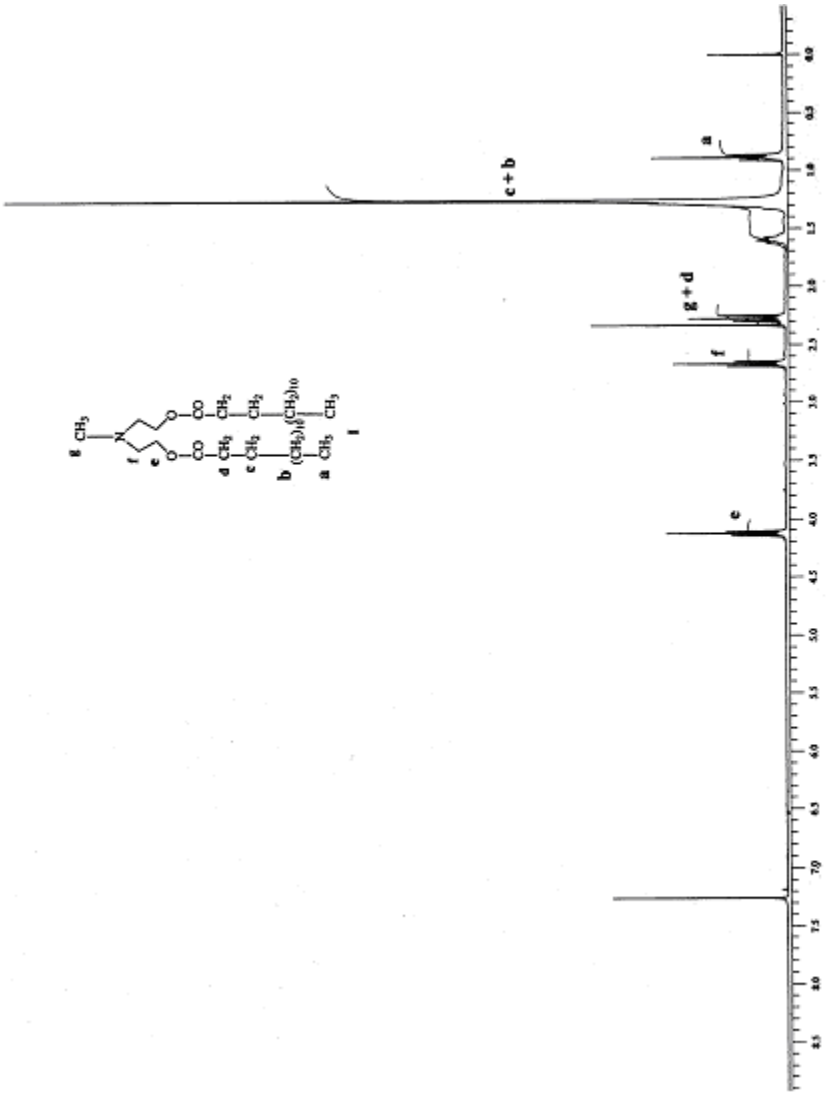


Figure S2. ^{1}H NMR (300 MHz, $CDCl_3$) of Lipid **1**

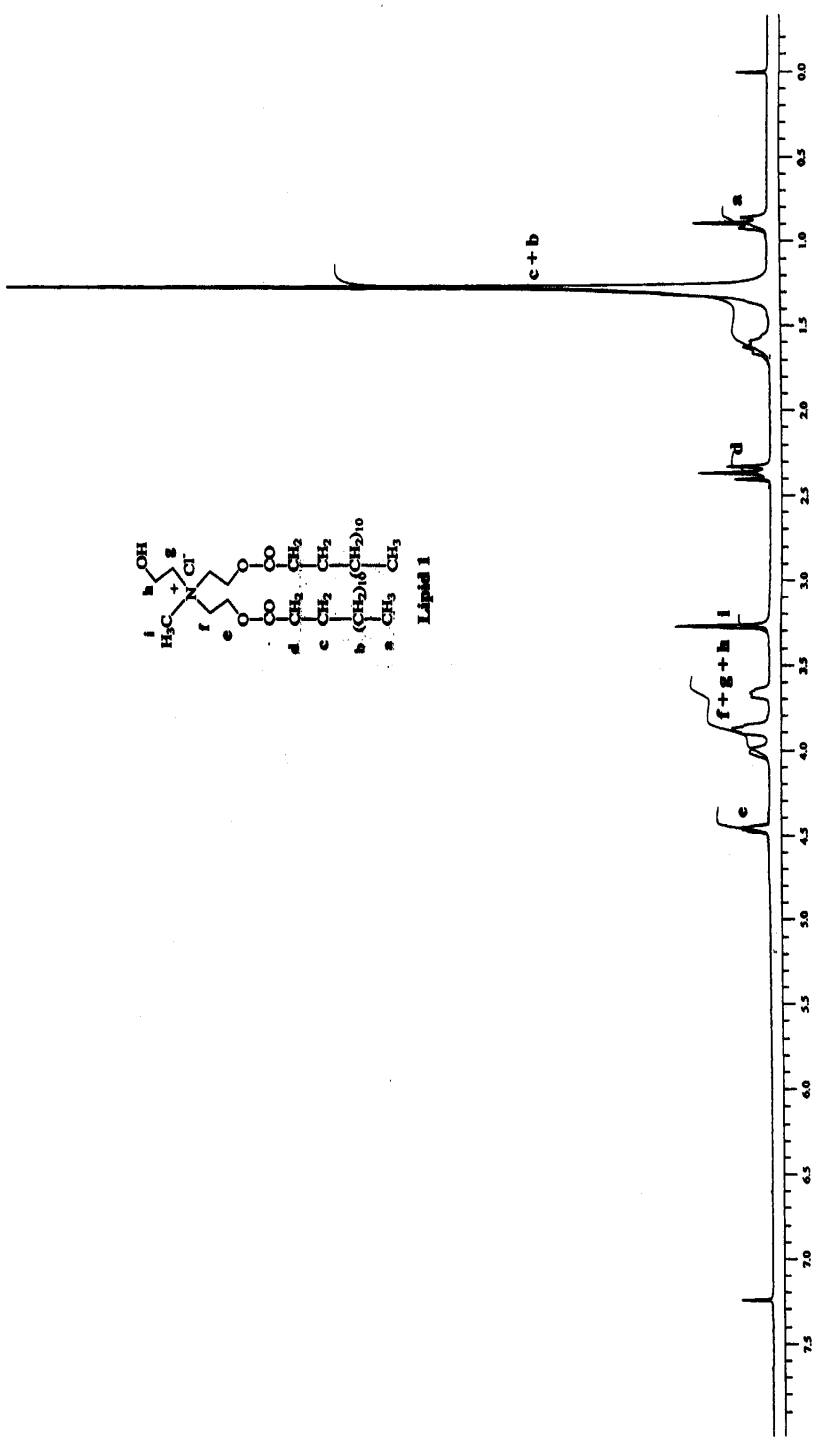


Figure S3. ^{1}H NMR (200 MHz, $CDCl_3$) of the Intermidiate **II**, Scheme I, Part **B**.

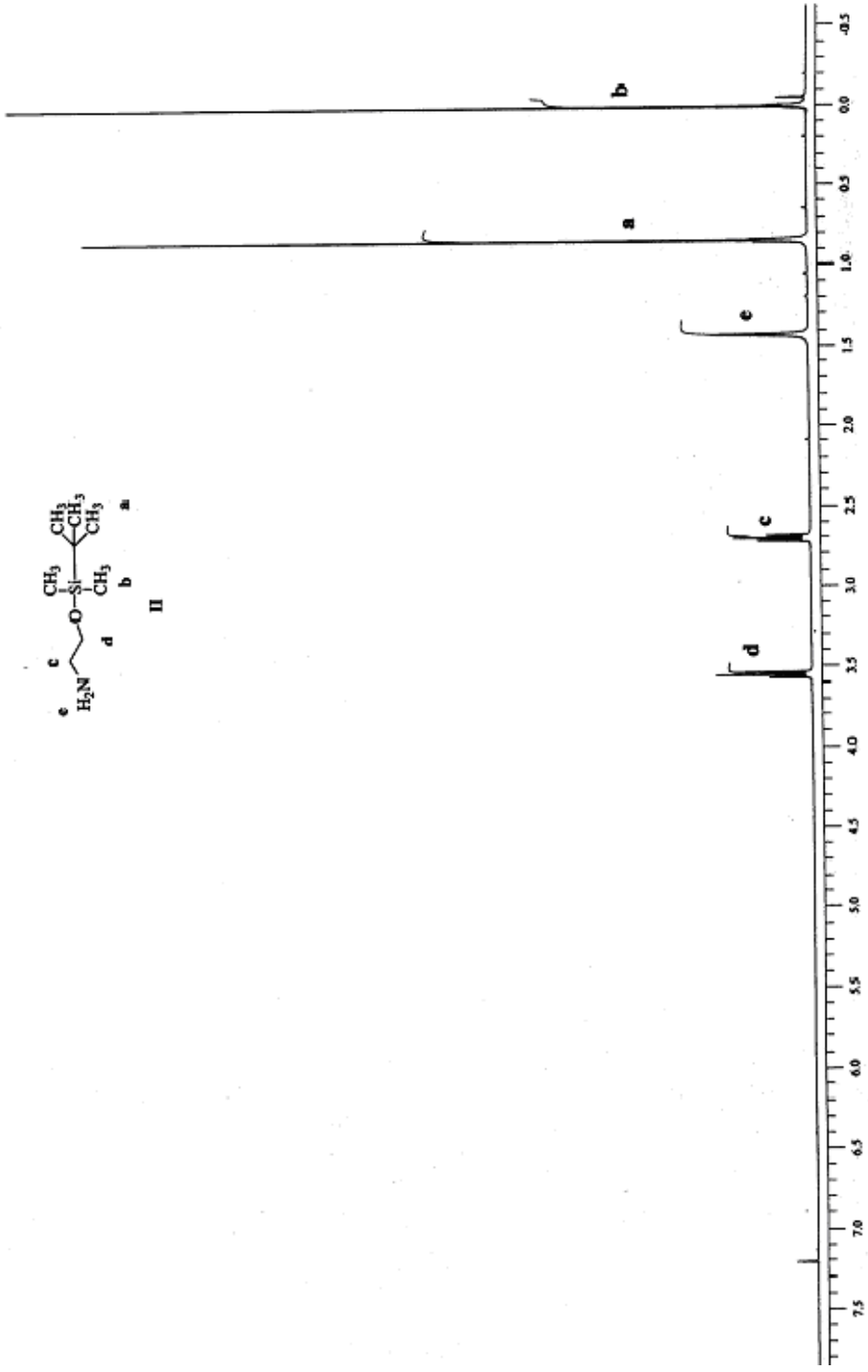


Figure S4. ^{1}H NMR (200 MHz, $CDCl_3$) of the Intermidiate **III**, Scheme I, Part **B**.

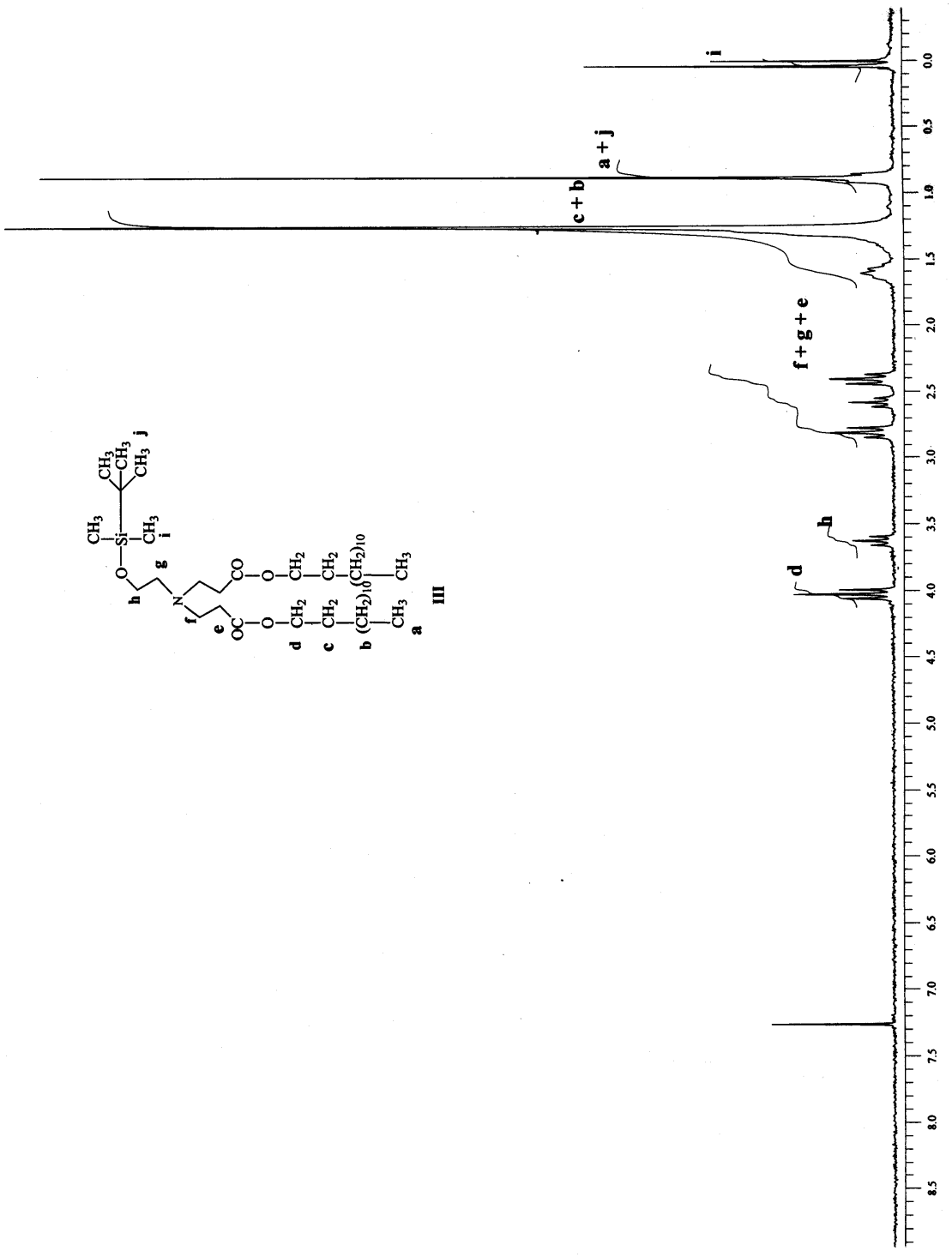


Figure S5. ^{1}H NMR(200 MHz, $CDCl_3$) of the Intermidiate **IV**, Scheme I, Part **B**.

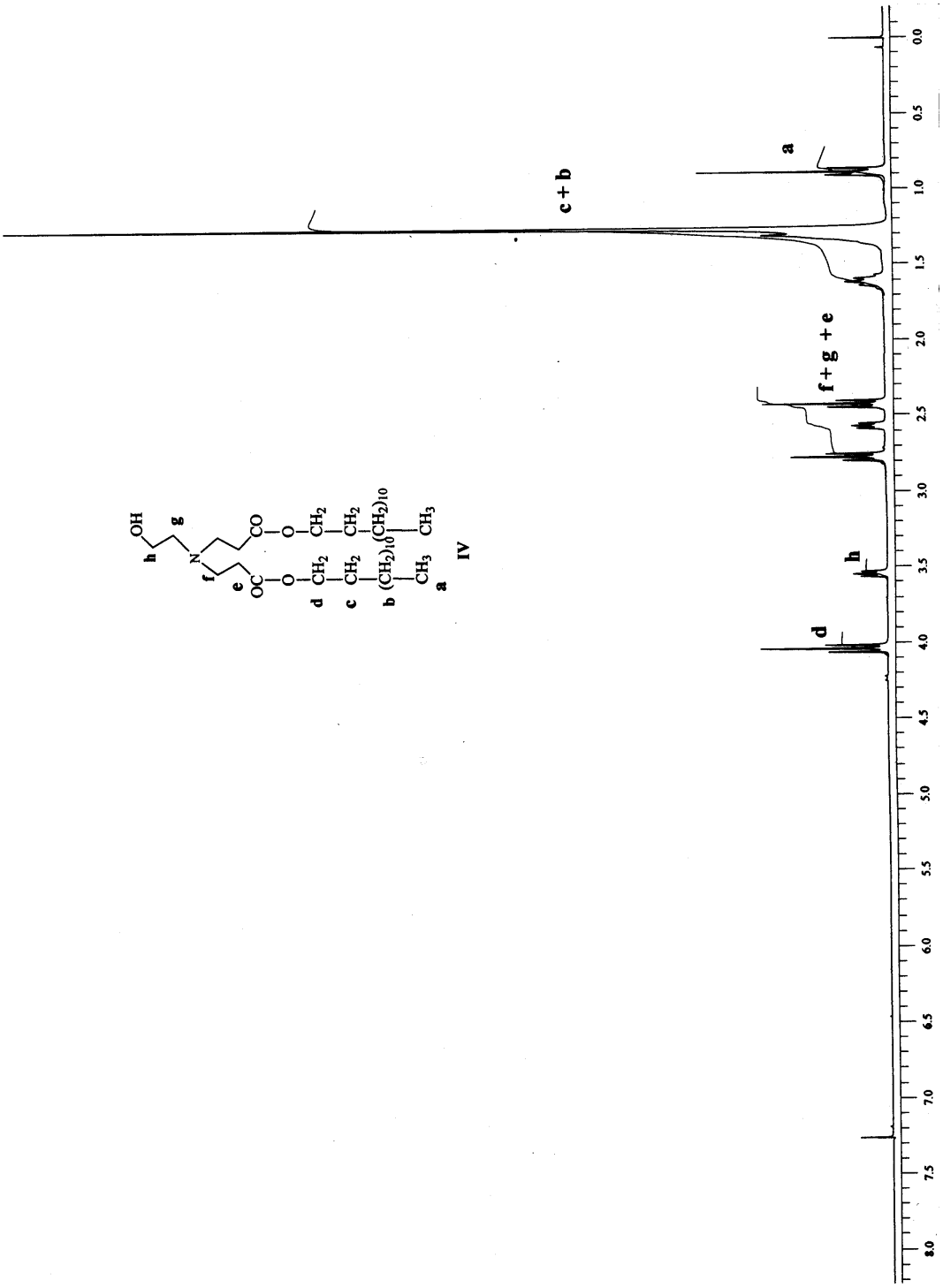


Figure S6. ^{1}H NMR (200 MHz, $CDCl_3$)of the Intermidiate **V**, Scheme I, Part **C**.

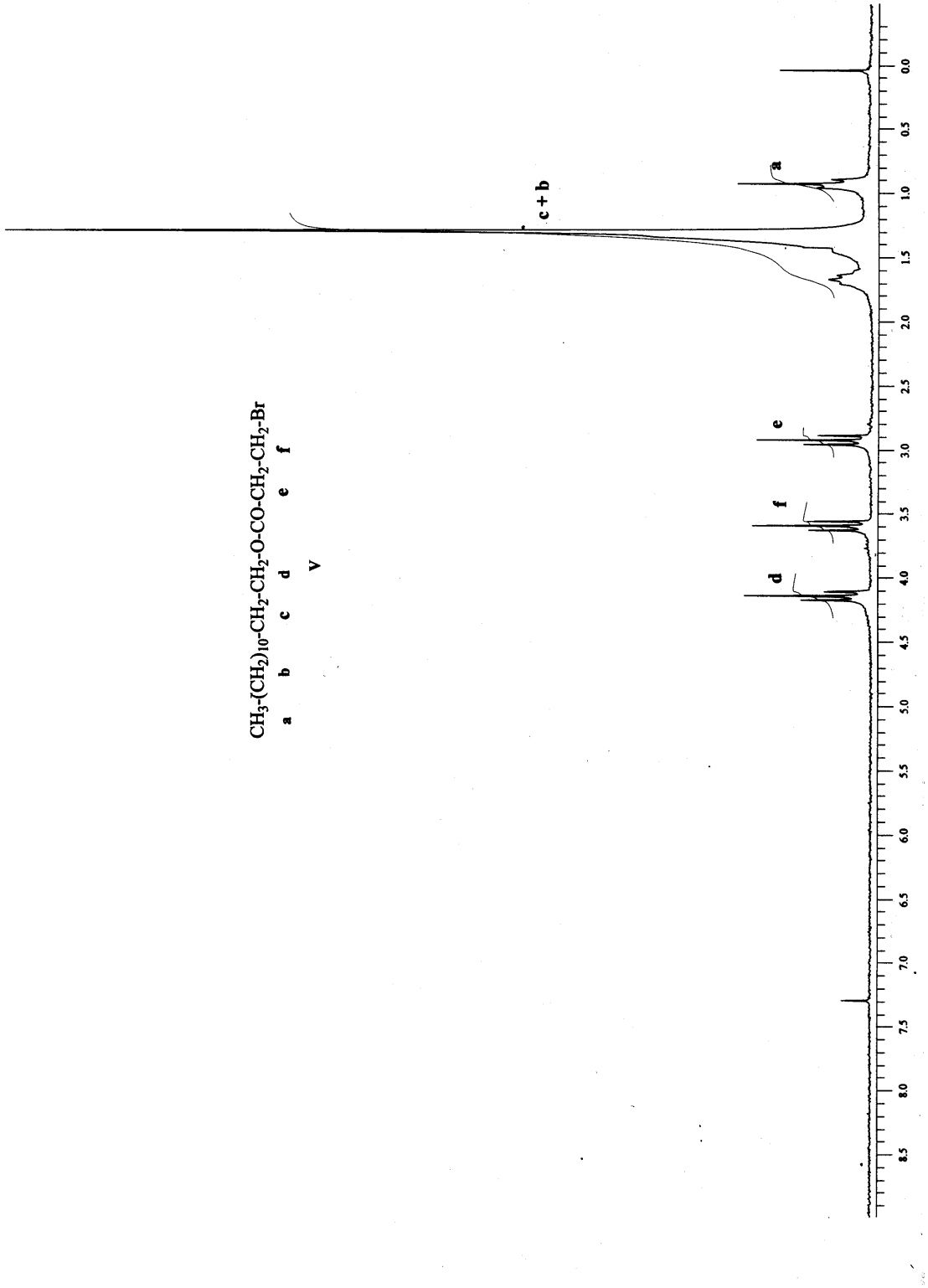


Figure S7. ^{1}H NMR (200 MHz, $CDCl_3$) of Lipid **2**.

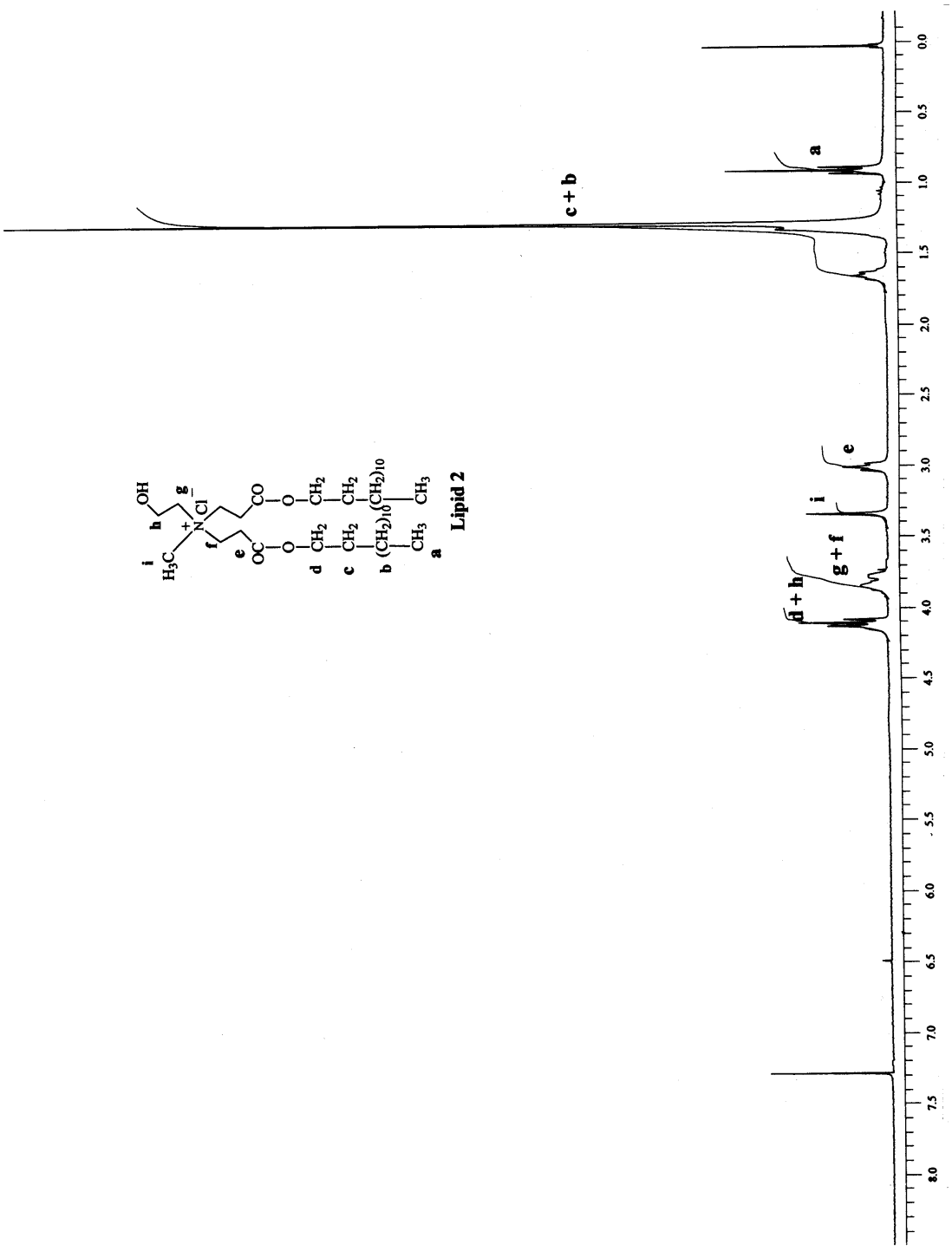


Figure S8. ESI Mass Spectrum of Lipid **1**.

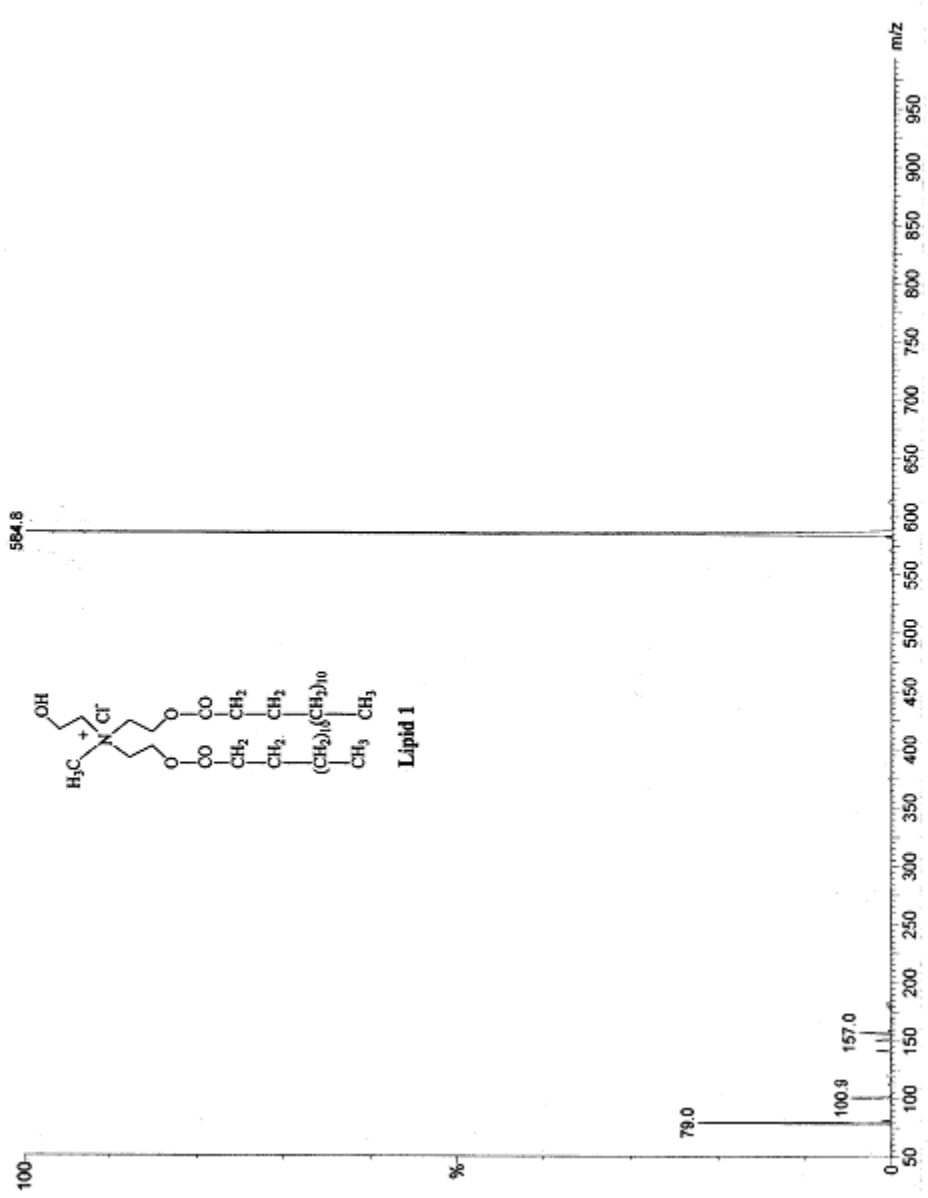


Figure S9. ESI Mass Spectrum of Lipid **2**.

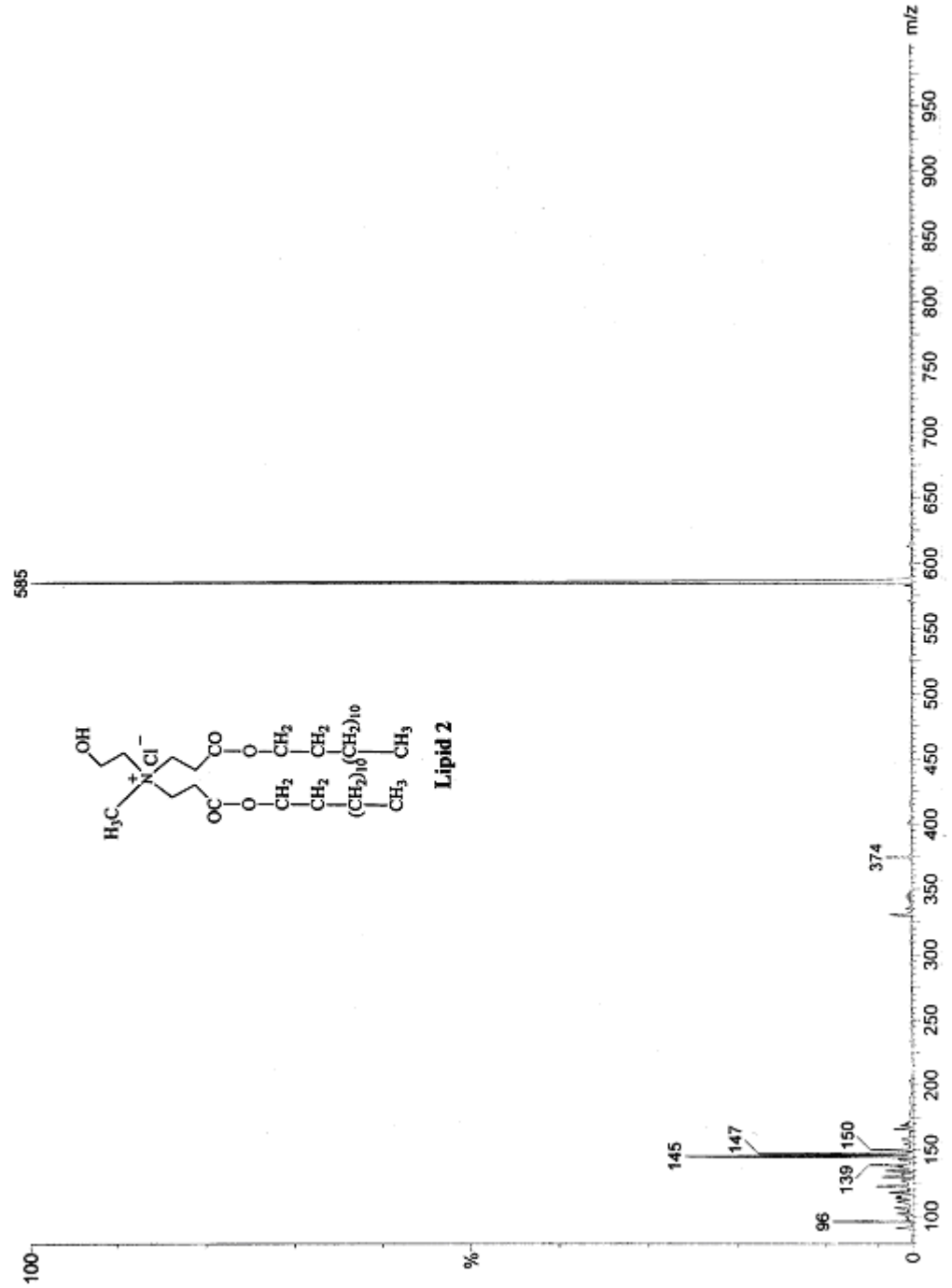
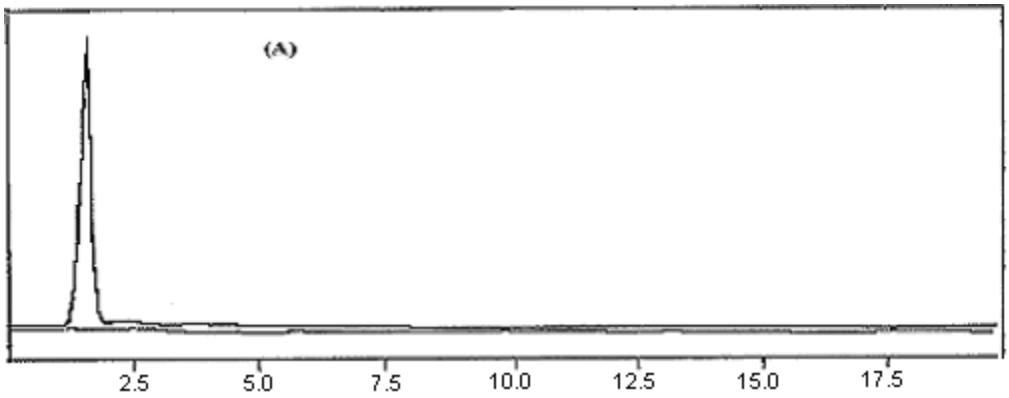
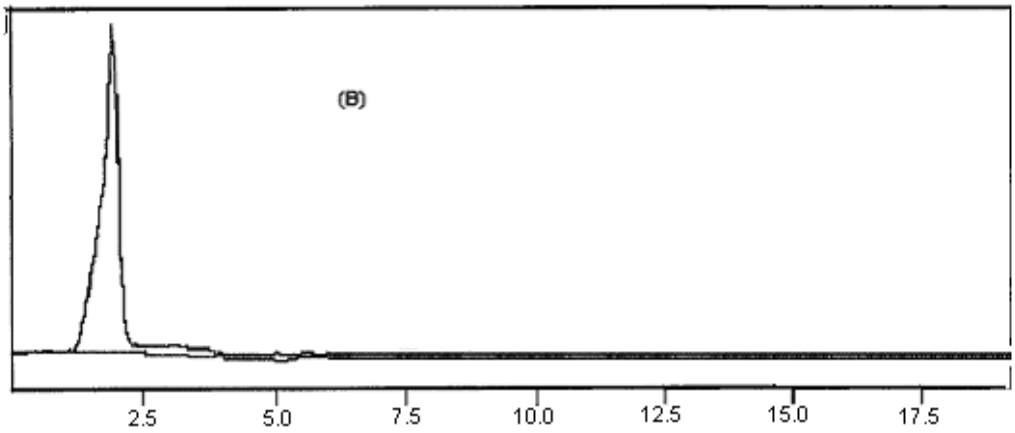


Figure S10. HPLC Chromatograms for purified Lipid **1**.





HPLC CONDITIONS:

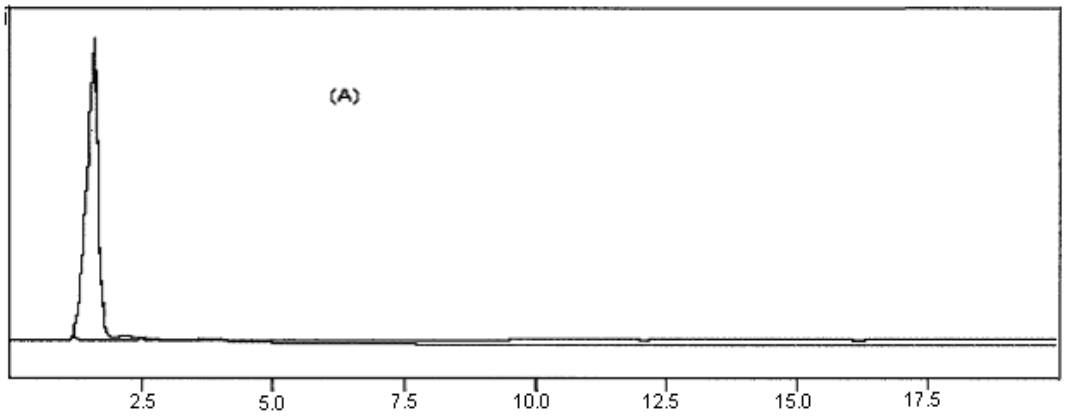
System: Varian Prostar 210; Column: Varian microsorb 4.6x 250 mm Reverse Phase C18.

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

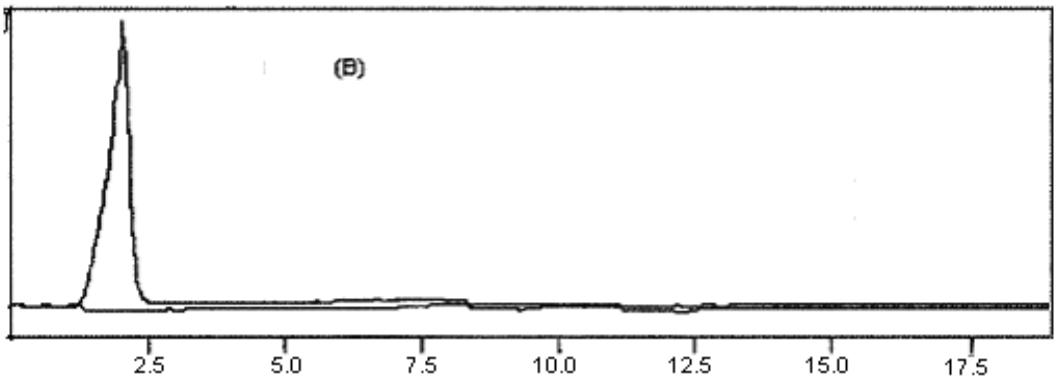
Flow Rate: 1.5 mL/min; Detection : UV at 210 nm; Temperature: 20 ˚C

Typical Column Pressure: 300-305 psi.

Figure S11. HPLC Chromatograms for purified Lipid **2**



Time (min)



Time (min)

.

HPLC CONDITIONS:

System: Varian Prostar 210; Column: Varian microsorb 4.6x 250 mm Reverse Phase C18.

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

Flow Rate: 1.5 mL/min; Detection : UV at 210 nm; Temperature: 20 ˚C

Typical Column Pressure: 300-305 psi.

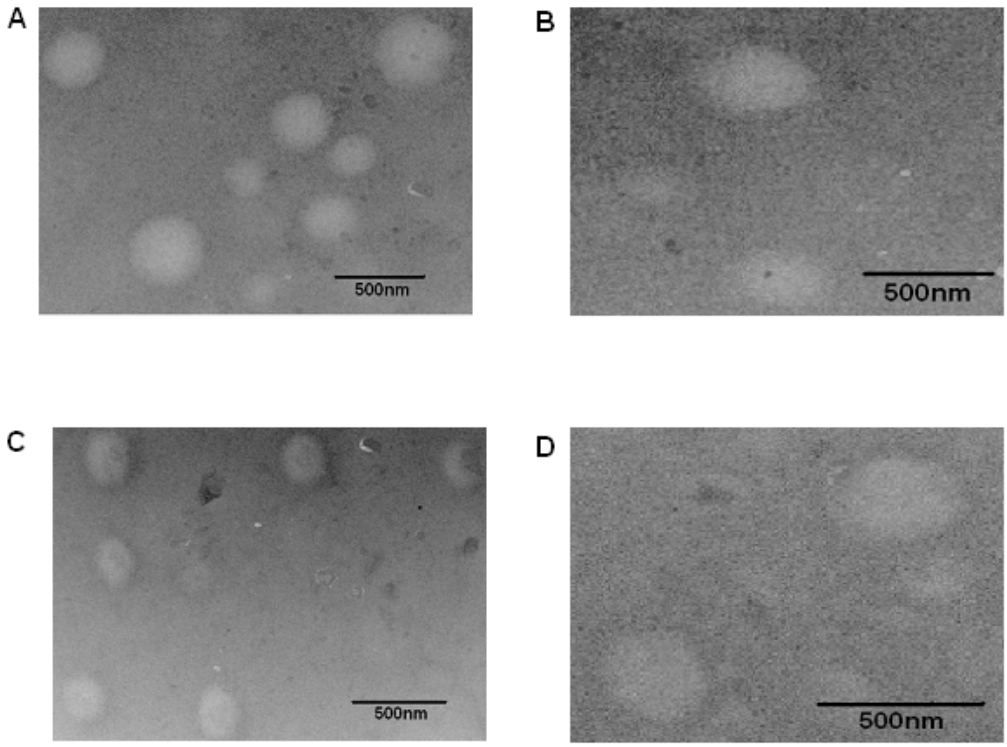


Figure S12. Transmission electron micrographs of representative liposomes prepared from lipid **1** (**A**) and lipid **2** (**C**) and representative lipoplexes prepared using lipid **1** (**B**) and lipid **2** (**D**) at a lipid/DNA charge ratio of 1:1. Bars in Parts **A**-**D** correspond to 500 nm. Experimental details are described in the text.

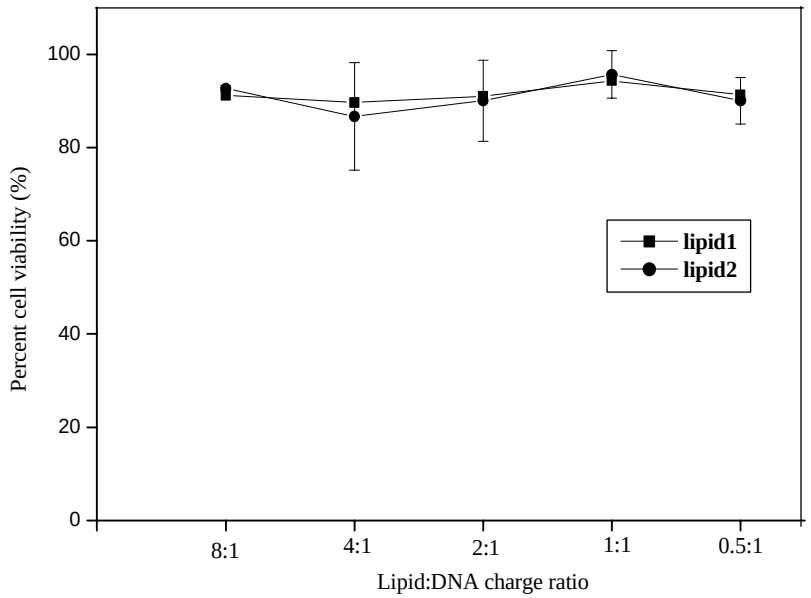


Figure S13. MTT-assay based percent cell viabilities when representive CHO cells are treated with lipoplexes of lipids **1** and **2** with increasing lipid:DNA charge ratios. The cell viability values shown are the average of triplicate experiments performed on same day. Details of cell viability assay are as described in the text.

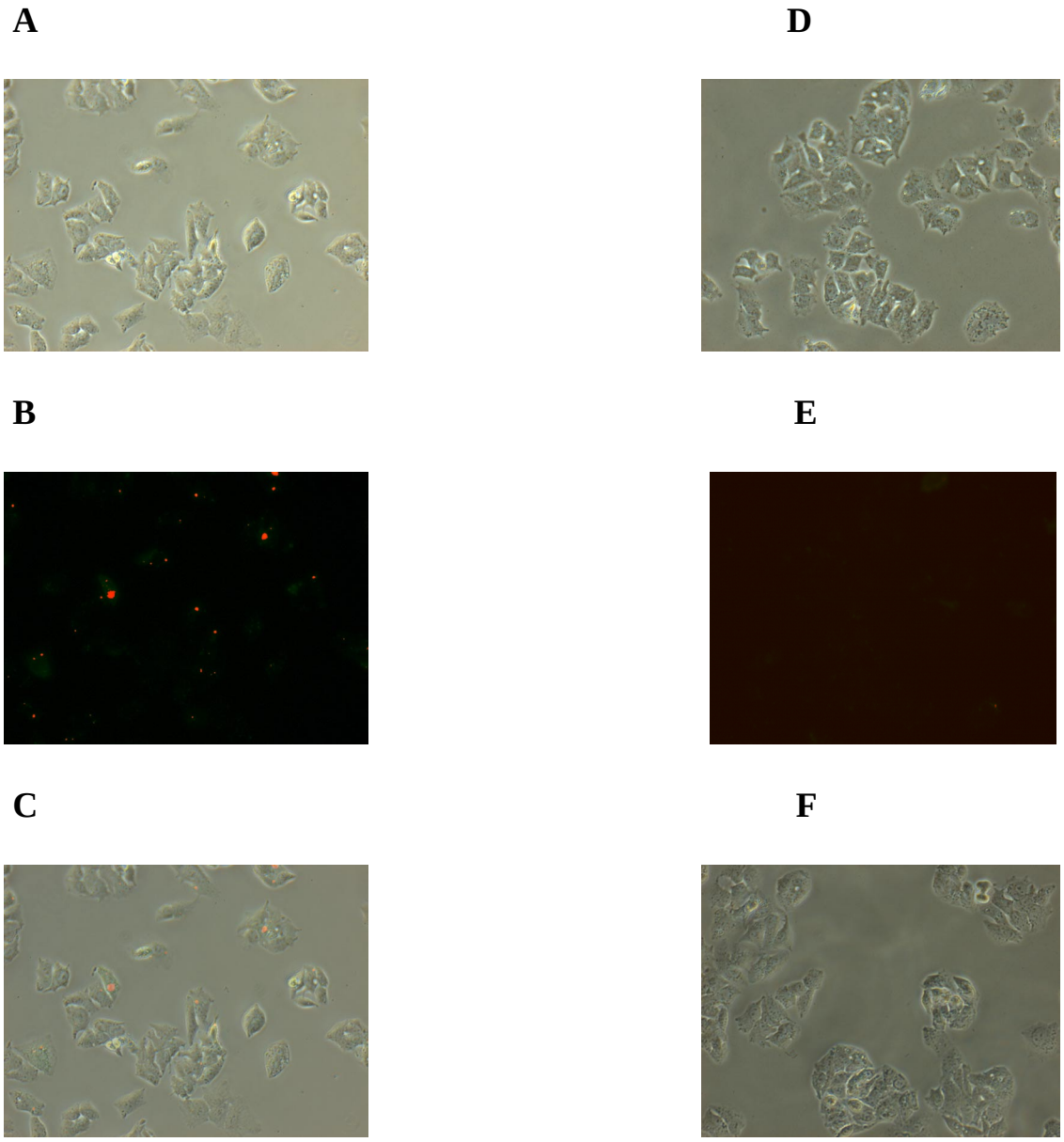


Figure S14. Epifluorescence microscopic images of HepG2 cells trasfected with Rh-PE labeled lipid **1** lipoplexes (**A**-**C**) and Rh-PE labeled lipid **2** lipoplexes (**D**-**F**). Lipid:DNA charge ratios in both the lipoplexes were maintained at 1:1**. A** & **D**: phase contrast bright field images**; B** & **E**: fluorescent images; and **C** & **F**: overlay images. The details of the cellular uptake experiments are as described in the text. Images were obtained using Nikon inverted fluorescence microscope (TE 2000E), Japan.

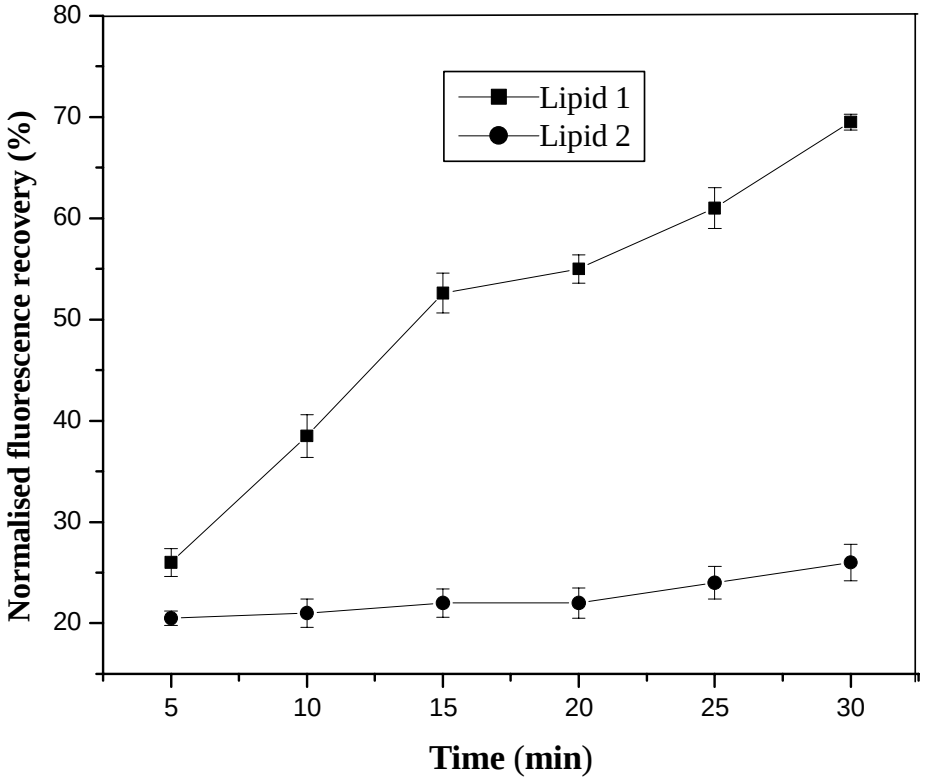


Figure S15. FRET studies on the biomembrane fusogenicities of lipid **1**/Cholesterol (1:1 mole ratio) & lipid **2**/Chol liposomes (1:1 mole ratio) based on the increase of the NBD fluorescence. Fusion was induced by adding the cationic lipid **1**/Chol & lipid **2**/Chol liposomes to the double fluorophore labeled biomembrane mimicking DOPC/DOPE/DOPS/Chol liposomal formulations. The values shown are representative of two independent measurements. The details of FRET experiments are as discussed in text.

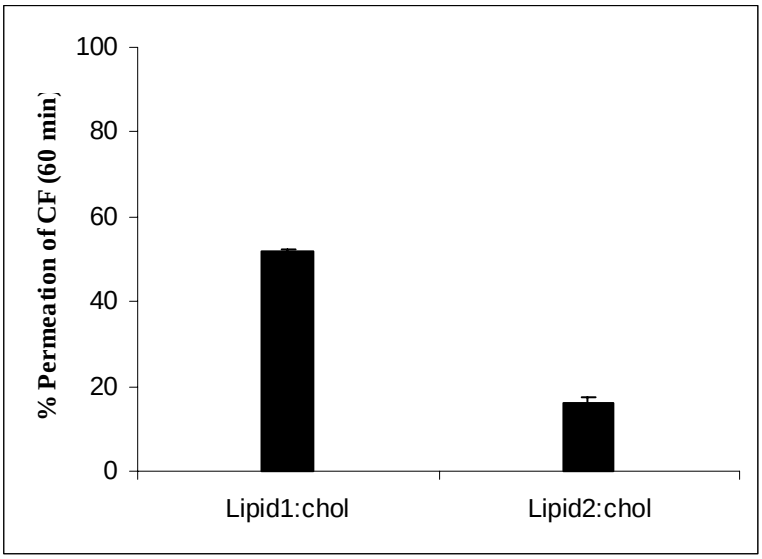


Figure S16. A bar plot of the percentage CF leakage in 60 min of incubation at 25 ˚C from lipid **1**:cholesterol (1:1 mole ratio) and lipid **2**:cholesterol (1:1 mole ratio) liposomes. The details of the experiments are as described in the text.