Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Syntheses, Transfection Efficacy and Cell Toxicity Properties of Novel Cholesterol-based Gemini Lipids having Hydroxyethyl Head group

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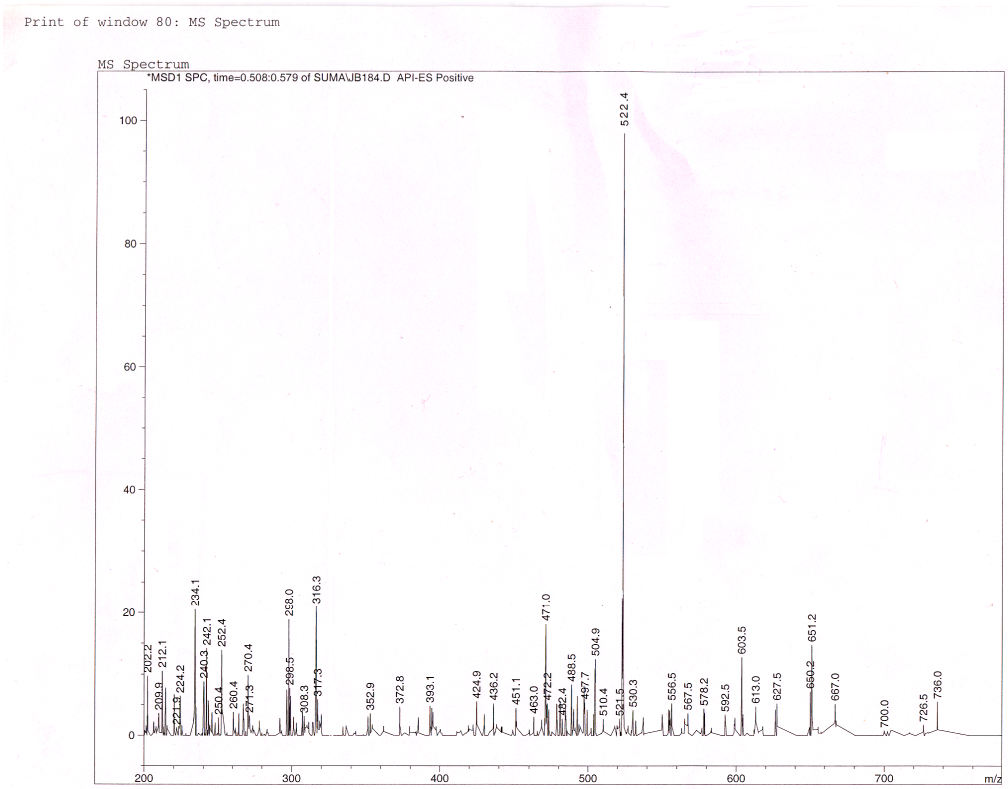
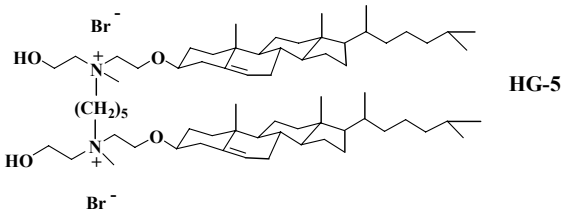
Table S1. Elemental analysis values of new cholesterol based monomeric and gemini lipids.

Lipid	Formula	Calculated			Found		
		C	H	N	C	H	N
H-M	$C_{33}H_{60}O_2NBr \cdot 0.5H_2O$	66.98	10.39	2.37	67.28	10.45	2.06
HG-3	$C_{67}H_{120}Br_2N_2O_4$	68.30	10.27	2.38	68.34	9.95	2.49
HG-4	$C_{68}H_{122}Br_2N_2O_4 \cdot 1.5H_2O$	67.03	10.34	2.30	66.87	10.41	2.21
HG-5	$C_{69}H_{124}Br_2N_2O_4 \cdot 2H_2O$	66.75	10.39	2.26	66.49	10.12	2.37
HG-6	$C_{70}H_{126}Br_2N_2O_4$	68.94	10.41	2.30	68.69	10.23	2.44
HG-12	$C_{76}H_{138}Br_2N_2O_4$	70.02	10.67	2.15	69.82	10.43	2.36





HG-5

Figure S1. Mass spectra of HG-5.

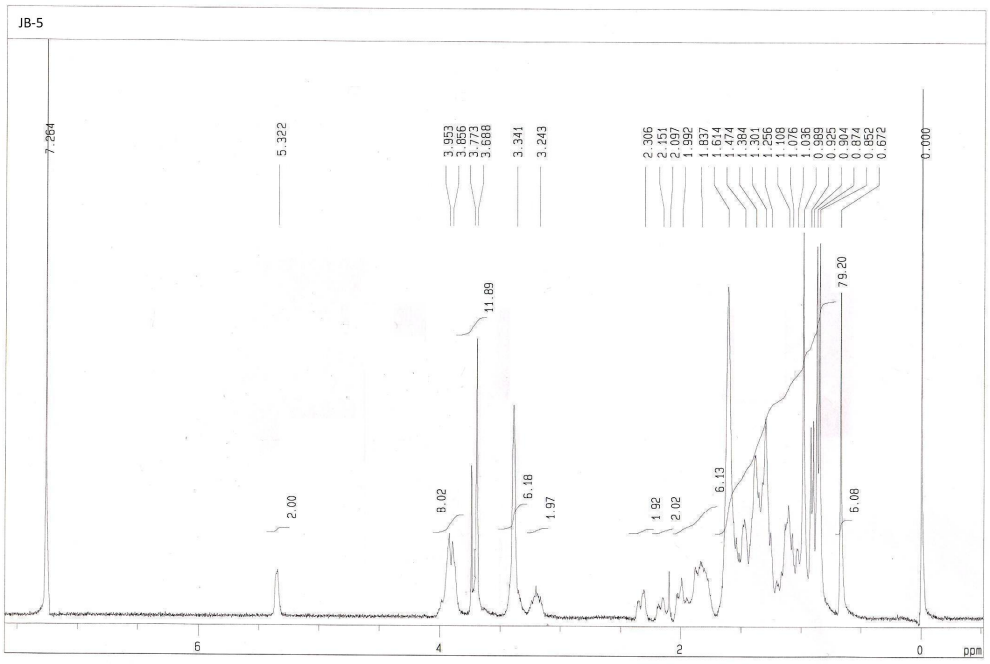


Figure S2. ^{1}H-NMR spectra of HG-5.

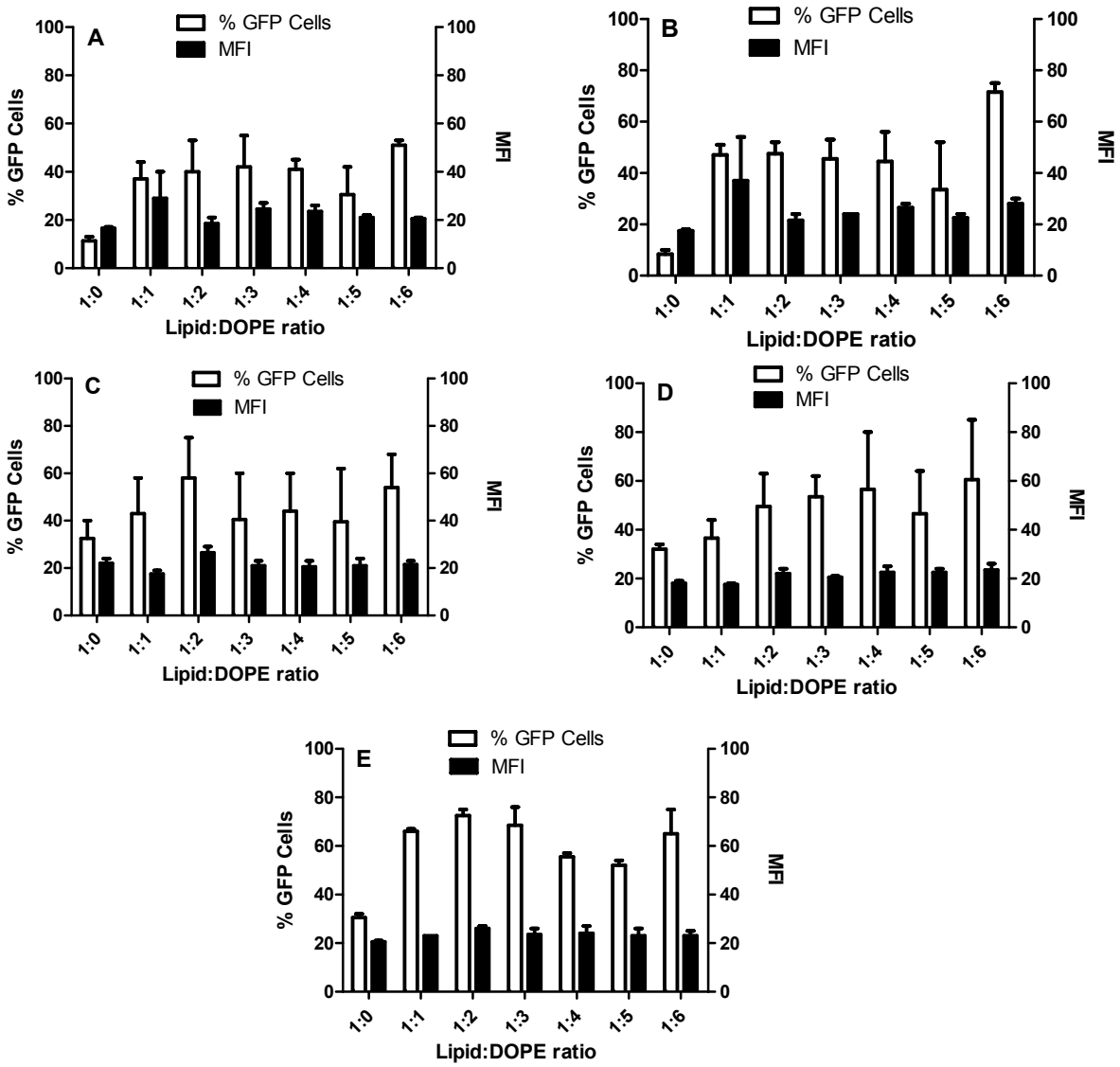


Figure S3. EGFP-c3 DNA transfection efficacies of cholesterol based gemini lipids mixed with various amount of DOPE in absence of serum. (A) HG-3; (B) HG-4; (C) HG-5; (D) HG-6 and (E) HG-12. Concentrations of DNA = 0.8 μg/well and lipid were used at N/P ratio of 1. Data are expressed as the number of transfected cells and MFI is obtained from flow cytometry analysis after 48 h of transfection.

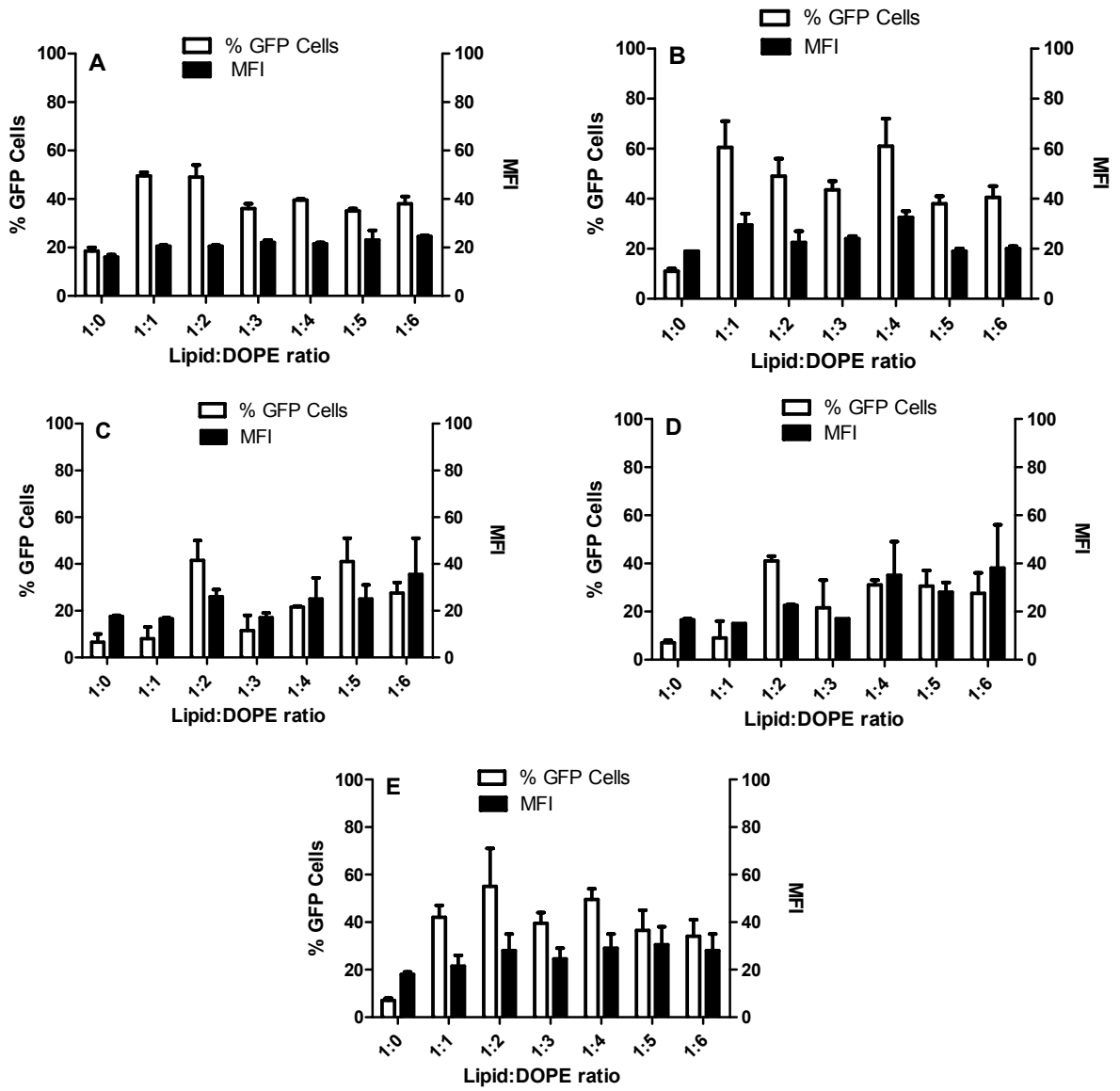


Figure S4. Transfection efficiencies of EGFP-c3 DNA of gemini lipids with various compositions of DOPE in presence of serum. (A) HG-3; (B) HG-4; (C) HG-5; (D) HG-6 and (E) HG-12. Concentrations of DNA = 0.8 μg/well and lipid were used at N/P ratio of 1. Data are expressed as the number of transfected cells and MFI as obtained from the flow cytometry analysis.

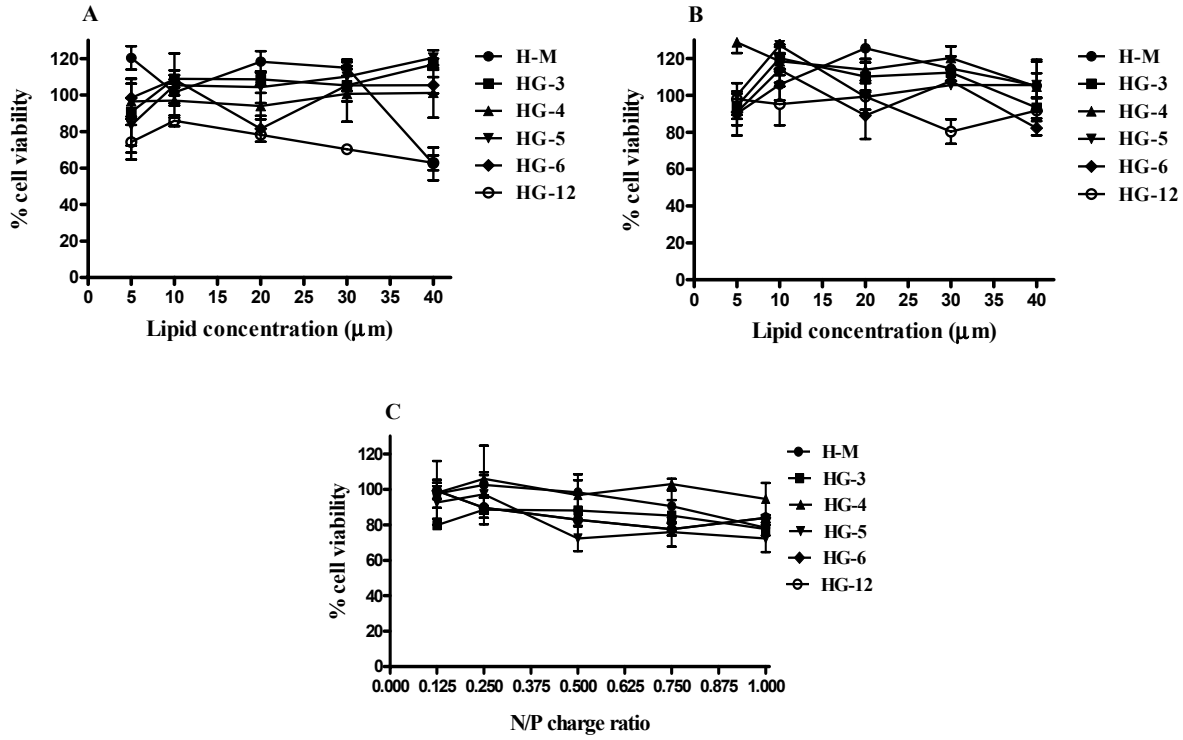


Figure S5. MTT based cellular cytotoxicity assay of gemini lipids against HeLa cells compared to that of monomeric lipid. Cytotoxicity of gemini lipids at high concentration **(A)** without serum; **(B)** in presence of serum and **(C)** cytotoxicity of gemini lipids at optimized lipid: DOPE molar ratio as a function of N/P charge ratio in presence of serum. The %-viability values shown are the averages of triplicate experiments performed on the same day.

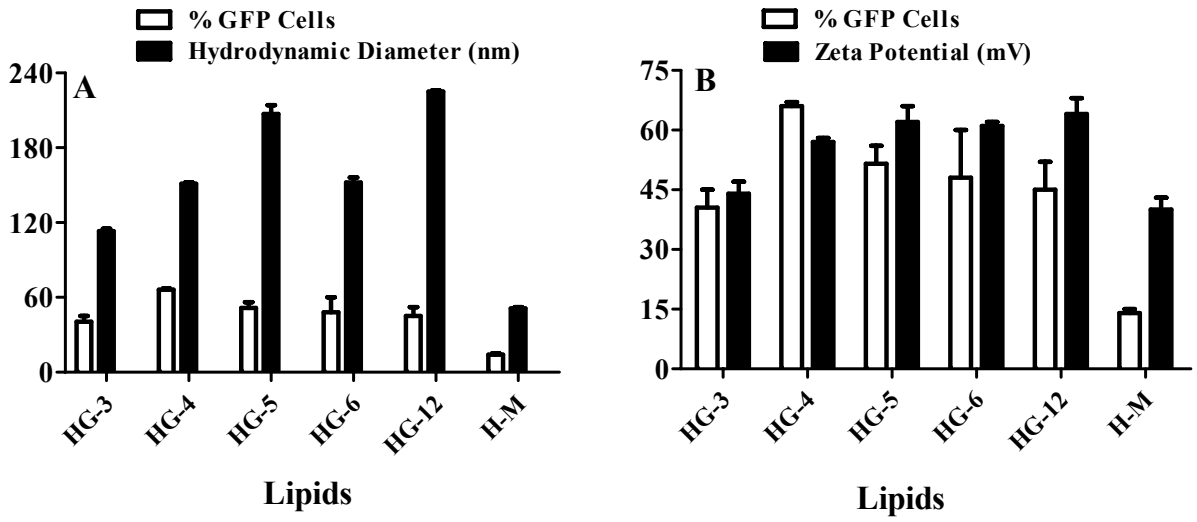


Figure S6. Relationship between physicochemical parameters *e.g.*, **(A)** hydrodynamic diameters and **(B)** zeta potential of various lipid suspensions.