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# α-Tocopherol derived lipid dimers as efficient gene transfection agents. Mechanistic insights into lipoplex internalization and therapeutic induction of apoptotic activity

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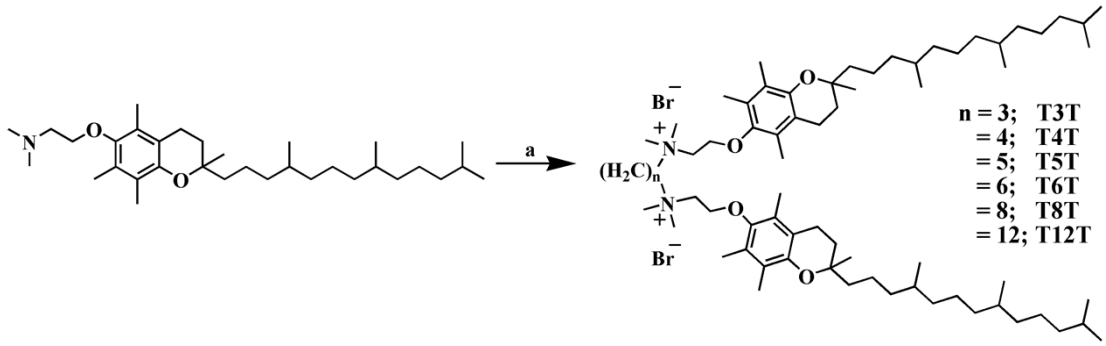
Scheme 1[a]




[a]Reagents and Conditions: a) α, ω-alkanediyl dibromide, MeOH-EtOAc, Pressure tube, 80 ˚C, ~14 days.

**Characterization Details[1].**

**T3T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H,-CH-C<u>H</u>$_3$, phytyl chain), 1.08-1.53 (*m*, 48H), 1.74-1.80 (*m*, 4H), 2.05 (*s*, 6H, -$CH_3$), 2.09 (*s*, 6H, -$CH_3$), 2.11 (*s*, 6H, -$CH_3$), 2.54 (*s*, 4H), 2.98 (*s*, 2H), 3.60 (*s*, 12H), 4.09-4.14 *(m*, 12H). ESI-MS (HRMS)**:** Calcd for $[C_{69}H_{124}N_2O_4Br_2]^{2+}/2$: 522.4775, Found: 522.4780. Anal. Calcd for $C_{69}H_{124}N_2O_4Br_2.3H_2O$: C 65.79, H 10.40, N 2.22. Found: C 65.76, H 10.39, N, 2.22.

**T4T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H,-CH-C<u>H</u>$_3$, phytyl chain), 1.07-1.57 (*m*, 48H), 1.71-1.78 (*m*, 4H), 2.05 (*s*, 6H, -$CH_3$), 2.10 (*s*, 6H, -$CH_3$), 2.13 (*s*, 6H, -$CH_3$), 2.23 (*s*, 2H), 2.53 (s, 4H), 3.6 (*s*, 12H), 4.09-4.12 (*m*, 12H). ESI-MS (HRMS): Calcd for $[C_{70}H_{126}N_2O_4Br_2]^{2+}/2$: 529.4853, Found: 529.4861. Anal. Calcd for $C_{70}H_{126}N_2O_4Br_2.2H_2O$: C 66.69; H 10.44; N 2.23. Found: C 66.68, H 10.42, N 2.23.

**T5T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H,-CH-C<u>H</u>$_3$, phytyl chain), 1.07-1.65 (*m*, 50H), 1.71-1.80 (*m*, 4H), 2.05 (*s*, 6H, -$CH_3$), 2.10 (*s*, 6H, -$CH_3$), 2.13 (*s*, 6H, -$CH_3$), 2.16-2.18 (*m*, 4H), 2.52 (*s*, 4H), 3.57 (*s*, 12H), 4.00-4.06 (*m*, 12H). ESI-MS (HRMS): Calcd for

$[C_{71}H_{128}N_2O_4Br_2]^{2+}/2$: 536.4931, Found: 536.4935. Anal. Calcd for $C_{71}H_{128}N_2O_4Br_2.2H_2O$: C 67.17, H 10.48, N 2.21. Found: C 67.19, H 10.47, N 2.20.

**T6T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H, -CH-C<u>H</u>$_3$, phytyl chain), 1.07-1.61 (*m*, 52H), 1.72-1.81 (*m*, 4H), 2.06-2.17 (*m*, 4H), 2.06 (*s*, 6H, -$CH_3$), 2.10 (*s*, 6H, -$CH_3$), 2.14 (*s*, 6H, -$CH_3$), 2.55 (*t*, 4H, $J$ = 5.6), 3.6 (*s*, 12H), 3.93 (*m*, 4H), 4.07-4.08 (*m*, 8H). ESI-MS (HRMS): Calcd for $[C_{72}H_{130}N_2O_4Br_2]^{2+}/2$ 536.6595, Found: 536.6587. Anal. Calcd for $C_{72}H_{130}N_2O_4Br_2.H_2O$: C 68.33, H 10.51, N 2.21. Found: C 68.32, H 10.52, N 2.22.

**T8T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H, -CH-C<u>H</u>$_3$, phytyl chain), 1.08-1.57 (*m*, 56H), 1.72-1.81 (*m*, 4H), 1.98 (*s*, 4H), 2.06 (*s*, 6H, -$CH_3$), 2.10 (*s*, 6H, -$CH_3$), 2.14 (*s*, 6H, -$CH_3$), 2.55 (*m*, 4H), 3.58 (*s*, 12H), 3.9 (*s*, 4H), 4.07-4.10 (*m*, 8H). ESI-MS (HRMS): calcd for $[C_{74}H_{134}N_2O_4Br_2]^{2+}/2$ 557.5165, Found: 557.5172; Anal. Calcd for $C_{74}H_{134}N_2O_4Br_2.3H_2O$: C 66.84, H 10.61, N 2.11. Found: C 66.85, H 10.60, N 2.11.

**T12T:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.83-0.87 (*m*, 24H,-CH-C<u>H</u>$_3$, phytyl chain), 1.07-1.57 (*m*, 64H), 1.74-1.80 (*m*, 4H), 1.88 (*s*, 4H), 2.07 (*s*, 6H, -$CH_3$), 2.10 (*s*, 6H, -$CH_3$), 2.14 (*s*, 6H, -$CH_3$), 2.17 (*m*, 4H), 2.54 (*m*, 4H), 3.56 (*s*, 12H), 3.81-3.85 (*m*, 4H), 4.08-4.11 (*m*, 8H). ESI-MS (HRMS): Calcd for $[C_{78}H_{142}N_2O_4Br_2]^{2+}/2$ 585.5479, Found: 585.5470. Anal. Calcd for $C_{78}H_{142}N_2O_4Br_2.2H_2O$: C 68.49, H 10.76, N 2.05. Found: C 68.50, H 10.75, N 2.05.

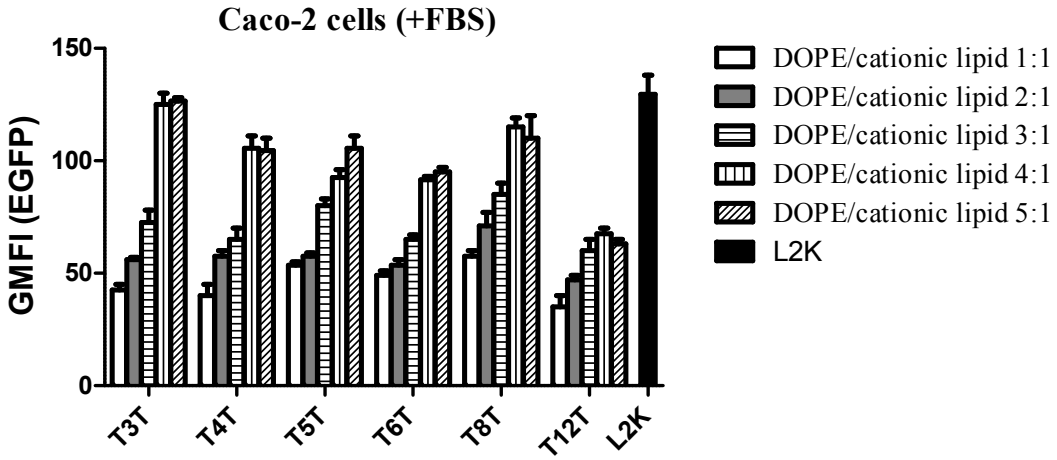



**Fig. S1** Optimization of DOPE content in different gemini lipid formulations. DOPE was mixed with each of the Gemini lipid at molar ratios 1:1 to 5:1 (DOPE/Gemini lipid) and transfections were performed at an N/P ratio of 0.5:1 (Gemini lipid/pDNA) in Caco-2 cells in the presence of serum. The EGFP expression levels were assessed 48 h post transfection under flow cytometry.

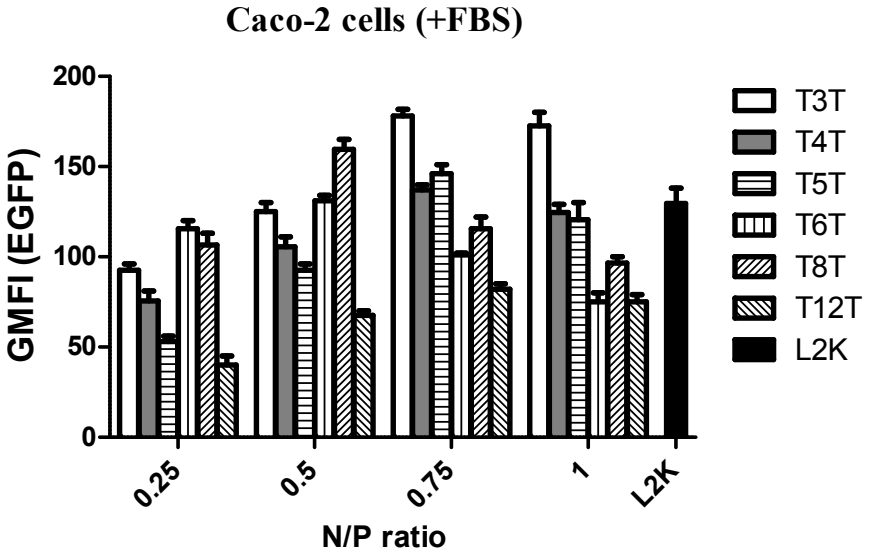



**Fig. S2** Optimization to achieve maximum gene expression levels for transfections mediated by different optimized gemini co-liposomal formulations at different N/P ratios (*p*DNA, 0.8 μg) in Caco-2 cells in the presence of serum. The EGFP expression levels were assessed 48 h post transfection under flow cytometry.

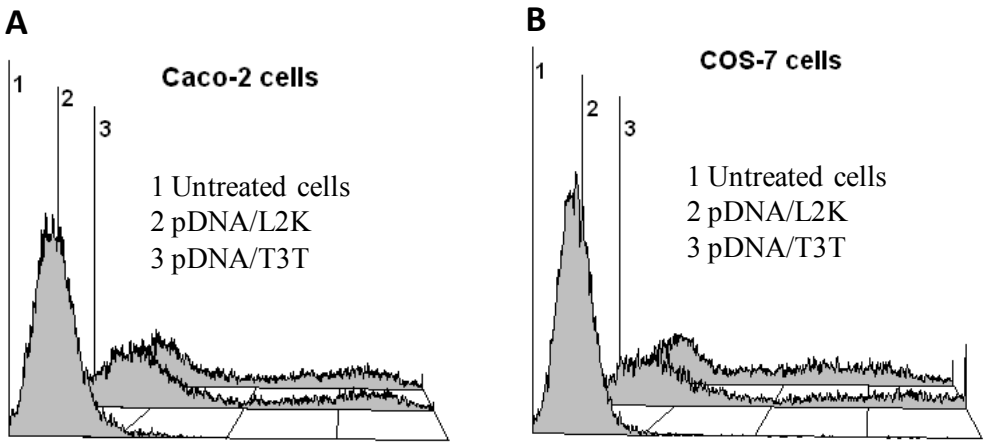



**Fig. S3** Representative flow cytometry histograms depicting EGFP expression levels for transfection mediated by most efficient co-liposomal formulation, T3T in comparison with L2K in the presence of serum in Caco-2 and COS-7 cells.

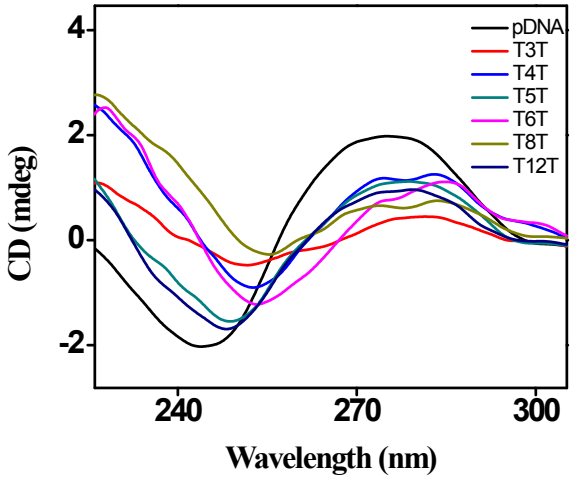



**Fig. S4** Circular dichroism (CD) spectra of *p*DNA alone (50 µg/mL) and its complexes with different dimeric lipid suspensions at their optimized ratios of maximum transfection (T3T, T4T, T5T and T12T at N/P ratios of 0.75; T6T and T8T at N/P ratios of 0.5). The spectra were recorded on a Jasco J-815 CD spectropolarimeter in 1 mm quartz cuvette at room temperature.

1. a) B. Kedika and S. V. Patri, J. Med. Chem., 2011, 54, 548.

b) K. Kumar, B. Maiti, P. Kondaiah and S. Bhattacharya, Mol. Pharmaceutics, 2014, DOI: 10.1021/mp500620e