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## Effect of Heterocyclic-based Head group Modifications on the Structure-Activity Relationship of Tocopherol-based Lipids for Non-viral Gene Delivery

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## **Supporting information**

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- 1) <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and ESI-MS (HRMS) spectra of all lipid molecules including the intermediate (Fig. S1-S21)
- 2) Agarose gel binding assay using L2K (Fig. S22)
- 3) Ethidium bromide displacement assay using L2K (Fig. S23)
- Comparative analysis of cell viability (%) of L2K: DNA complex using MTT assay (Fig. S24).

(1) Toc-Br





(2) Toc-Tme







Fig. S3: <sup>13</sup>C-NMR Spectrum of Toc-Tme



Fig. S4: ESI-MS (HRMS) Spectrum of Toc-Tme

(3) Toc-Pyr



Fig. S5: <sup>1</sup>H-NMR Spectrum of Toc-Pyr



Fig. S6: <sup>13</sup>C-NMR Spectrum of Toc-Pyr





(4) Toc-Dm



Fig. S8: <sup>1</sup>H-NMR Spectrum of Toc-Dm



Fig. S9: <sup>13</sup>C-NMR Spectrum of Toc-Dm



Fig. S10: ESI-MS (HRMS) Spectrum of Toc-Dm

(5) Toc-Db



Fig. S12: <sup>13</sup>C-NMR Spectrum of Toc-Db



Fig. S12: ESI-MS (HRMS) Spectrum of Toc-Db

(6) Toc-Pip



Fig. S13: <sup>1</sup>H-NMR Spectrum of Toc-Pip



Fig. S14: <sup>13</sup>C-NMR Spectrum of Toc-Pip



Fig. S15: ESI-MS (HRMS) Spectrum of Toc-Pip

(7) Toc-Mor



Fig. S17: <sup>13</sup>C-NMR Spectrum of Toc-Mor











Fig. S20: <sup>13</sup>C-NMR Spectrum of Toc-Im





**Fig. S22:** Agarose gel binding assay using Toc-Pip with four different charge ratios (0.3:1, 1:1, 3:1 and 9:1) and Lipofectamine-2000 (L2K) as the control for comparison. L2K with two different concentrations (L2K-1: 0.8 µl) and (L2K-2: 1.4 µl optimal concentration) according to the manufacturer's protocol. 400 ng of plasmid pCMVβ- gal DNA/well was used. Gel depicts retention of lipoplexes prepared with Toc-Pip at 3:1 in the well.



**Fig. S23:** Ethidium bromide displacement assay using Lipofectamine-2000 (L2K) at three different conditions 4.6  $\mu$ L, 8.05  $\mu$ L (optimal concentration), 11.5  $\mu$ L. The amount of plasmid DNA per titration is 2.3  $\mu$ g and L2K was used according to the manufacturer's protocol.



**Fig. S24:** Graph depicts comparative analysis of cell viability (%) of Lipid: DNA complex using MTT assay in Neuro-2a cell line: Lipoplexes were prepared with Toc-Pip formulated with DOPE and L2K was used as the positive control and assayed according to the protocol mentioned in methods. Diagonal lines (1.8  $\mu$ L), vertical lines (3.15  $\mu$ L), checks (4.5  $\mu$ L).