Branched Lipid Architecture Enhances LNP-mediated mRNA Delivery to the Liver via Enhanced Endosomal Escape

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Statement of Purpose: Lipid nanoparticles (LNPs) have emerged as the preeminent platform for efficacious nucleic acid delivery. This is largely due to the ionizable lipid (IL), which is a component that both entraps nucleic acids during formulation and disrupts the endosome, enhancing both encapsulation efficiency and endosomal escape. While an enormous effort has been placed on optimizing the ionizable core of ILs, fewer studies have investigated the role of the lipid tails. Recently, it was shown that small terminally branched tails can enhance mRNA transfection; however, such studies are limited by the commercial sparsity of structurally diverse lipids, which are not found in nature.[1,2] Therefore, to investigate the phenomenon of lipid architecture in a more rigorous fashion we developed a method for the facile synthesis of ILs with unique branched lipid tails. These novel ILs form LNPs that enhance mRNA delivery in the liver by facilitating greater endosomal disruption **(Fig. 1A)**.

Methods: A small library of branched epoxides were synthesized via copper-catalyzed Grignard C-C coupling, followed by epoxidation **(Fig. 1B)**. The epoxides were then reacted with different lipid cores via S_N2 chemistry to form the corresponding ILs. These ILs were formulated into LNPs encapsulating luciferase mRNA using a microfluidic device. To probe endosomal escape, artificial endosomes composed of DOPS, DOPC, and DOPE, and containing a lipid FRET pair, were mixed with LNPs containing branched or non-branched ILs. The degree of membrane disruption was monitored over time by examining an increase in FRET donor fluorescence. To evaluate efficacy, the LNPs were injected by *i.v.* administration with 0.1 mg/kg mRNA into C57BL/6 mice, and organs were imaged after 12 h.

Results: Twenty-four ILs were synthesized consisting of four unique terminal branching groups using different lipid lengths and polyamine cores. The branching groups included sec-butyl, tert-butyl, isopropyl, and normal (non-branch). LNPs containing branched ILs showcased superior endosomal disruption in an artificial endosome model **(Fig. 1C)**. The LNPs were tested for liver delivery against C12-200 and MC3, two industry-standard ILs. In all cases, branching performed as well as or better than the respective linear versions for liver delivery. While none of the non-branched 494-core LNPs performed better than MC3, several branched 494-core LNPs showed statistically significant increase in liver delivery **(Fig. 1D,E)**. Additionally, several branched 200-core LNPs, especially those with shorter chains, outperformed C12-200 for liver delivery **(Fig. 1F,G)**.

Conclusions: This works explores the importance of lipid architecture as it relates to overall LNP efficacy, where small changes in lipid structure can significantly increase mRNA potency. Additionally, we have developed a robust synthetic method that can generate a multitude of branched ILs. Future work will analyze the role of more pronounced branching groups as well as investigate the relationship between protein corona formation and lipid structure, especially for liver delivery.

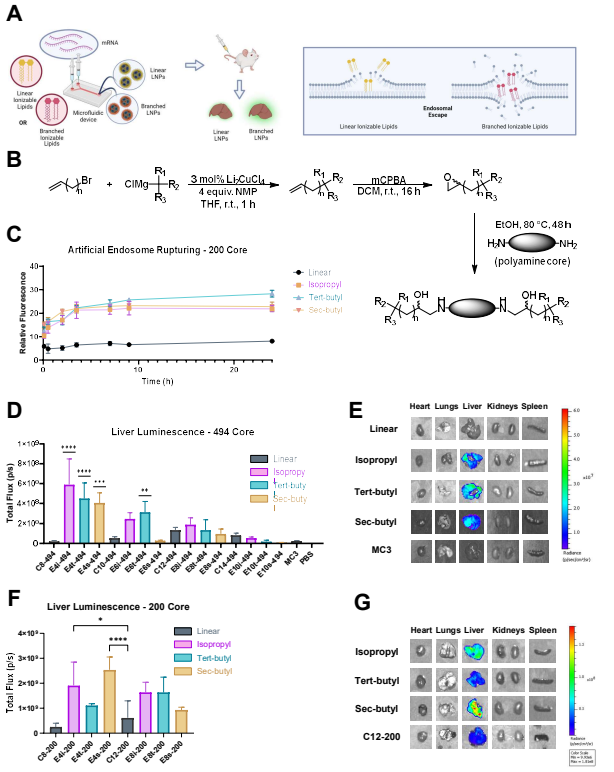

Figure 1. (A) Enhanced liver delivery of LNPs via branched ILs that induce greater endosomal escape. **(B)** Synthetic scheme to generate branched ILs. **(C)** Fluorescence time course after mixing artificial endosomes containing a FRET pair with LNPs formulated with branched ILs. **(D)** *In vivo* delivery of branched and non-branched 494-core LNPs encapsulating Luc mRNA, using an MC3 LNP as a control. **(E)** Luminescence images of organs from representative 494-core groups. **(F)** *In vivo* delivery of branched and non-branched 200-core LNPs encapsulating Luc mRNA, using a C12-200 LNP as a control. **(G)** Luminescence images of organs from representative 200-core groups. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$

Ref: (1) Hajj, K. A., *et al. Small*. (2019). (2) Hajj, K. A., *et al. Nano Lett*. (2020).