# Development of ionizable lipids for gene delivery to the lung using an Ugi four-component reaction

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## INTRODUCTION

The development of efficient delivery systems for therapeutic agents with organ-specific targeting is critical for advancing targeted therapies for effective treatment in extra-hepatic tissues. In this study, we present a novel approach utilizing a four-component reaction for the synthesis of a diverse library of lipids that exhibit a distinctive and remarkable tropism toward the lung. These findings hold significant promise for the development of therapeutic interventions for pulmonary disorders.

## METHODS

- Firefly Luciferase (FLuc) mRNA was encapsulated in LNPs using the NanoAssemblr (Precision Nanosystems, Inc.). The LNPs were characterized for their size, PDI, mRNA encapsulation efficiency, and zeta potential. Cryo-electron microscopy (CryoEM) was utilized to assess the morphology of the LNPs.
- Systematic evaluation of various amine core structures, isonitrile components, and tail configurations was performed *in vivo* by intravenous injection of 0.1mg/kg mRNA in Balb/c mice, followed by luminescence imaging using IVIS system (Perkin Elmer).
- Biodistribution studies were performed using Immunohistochemistry in ai9 mice after Cre recombinase mRNA delivery. CRISPR Cas9-based gene editing was quantified using Next-Generation Sequencing (NGS).

## RESULTS: Characterization of LNPs

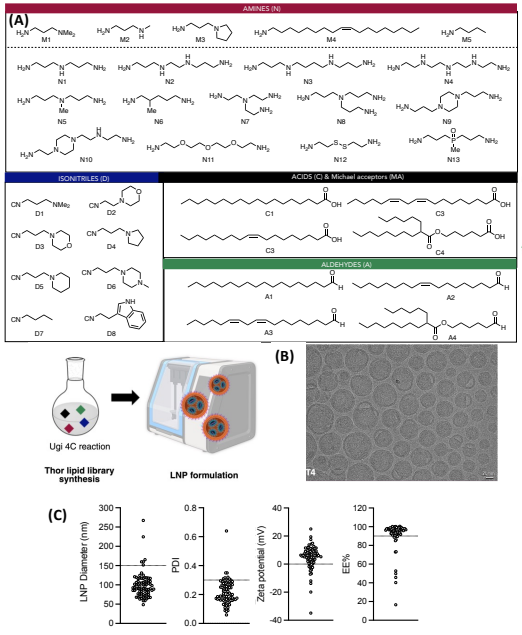



**Fig 1** (A) Condensed substrate table utilized for the synthesis of Thor lipids (B) CryoEM image of representative Thor LNP. (C) Physicochemical characterization of Thor LNPs including size, PDI, mRNA encapsulation efficiency, and Zeta potential

## RESULTS: Structure-Activity Relationship

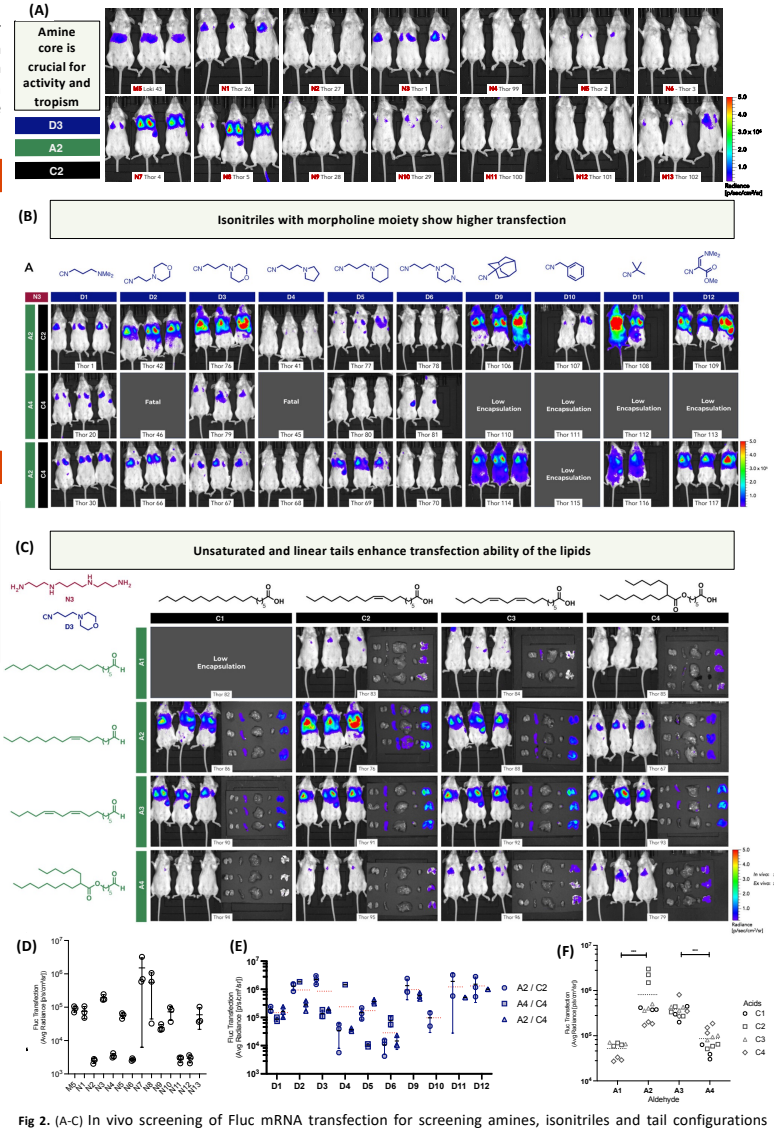



**Fig 2.** (A-C) In vivo screening of Fluc mRNA transfection for screening amines, isonitriles and tail configurations respectively (0.1 mg/kg mFluc per mouse). (D-F) Quantification of *in vivo* mRNA delivery efficacy of amines, isonitriles and tail configurations respectively.

## RESULTS: Biodistribution

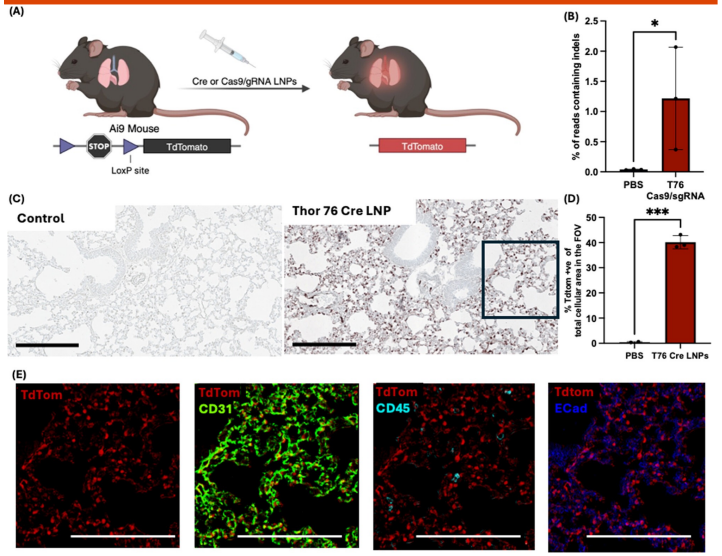
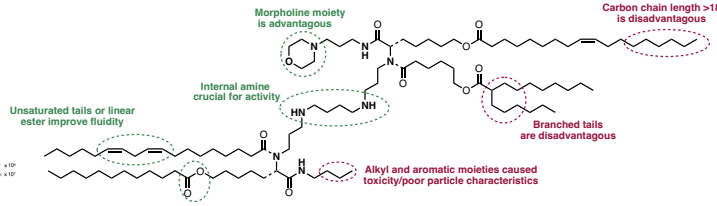



**Fig 3:** Gene editing in Ai9 mice. (A) Schematic representing ai9 reporter mice and its application. (B) Quantitative analysis of CRISPR/Cas9 + sgAi9-treated mice 3 days post-injection. (C) Multiplex IHC images of tdTomato expression in ai9 mice lungs treated with Thor 76 LNPs encapsulating Cre mRNA 5 days post injection (scale bar = 200 µm). (**D**). Quantification of tdtom expression in lungs of ai9 mice from IHC images. Data are presented as means ± SD ($n$ = 3), ($p$ < 0.05) (**E**) Zoomed in image of **Thor 76** cre LNP lung IHC to identify cell types transfected (CD31-endothelial cell marker; CD45-immune cell marker; Ecad-epithelial cell marker; scale bar = 200 µm).

## SUMMARY




## REFERENCES

Renner, J., Vittala Murthy, N. T., Gautam, M., Bodi, E., Jozic, A., & Sahay, G. (2025). Synthesis of Ionizable Lipids for Gene Delivery to the Lung Using an Ugi Four Component Reaction. *Journal of the American Chemical Society*, *147*(20), 17459-17467.

## CONFLICT OF INTEREST AND ACKNOWLEDGEMENTS

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