A Library of Novel Polyvalent Ionizable Lipids for Targeted Co-Delivery of mRNA and DNA

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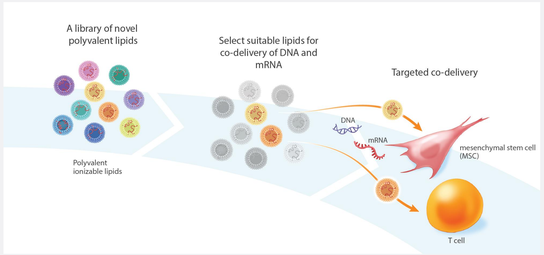
Summary

Lipid delivery systems containing ionizable lipids have entered broad clinical use with the introduction of COVID-19 mRNA vaccines and are now generally considered the gold standard for in vivo mRNA delivery. However, intravenously administered lipid delivery systems are still limited by undesirable toxicity profiles, poor accumulation at in vivo target sites, minimal uptake by cells of interest, and low levels of endosomal escape. To address these deficiencies, we have investigated strategic incremental modifications to the chemical structure of DLinDHS, a key component of the ToRNAdo™ Lipid Delivery System. ToRNAdo™ has shown effective delivery of mRNA to a variety of cell types both ex vivo and in vivo, including mRNA encoding chimeric antigen receptors (CARs) targeting CD19 and the receptor tyrosine kinase like orphan receptor 1 (ROR1), a receptor found on many solid tumor cancers. iPSC-derived macrophages transfected with such mRNA efficiently bound recombinant CD19 or ROR1 and demonstrated improved cytotoxicity towards tumor cells. We hypothesized that combining various structural elements with the polyvalent DLinDHS backbone could lead to improved transfection efficiency and enhanced tissue targeting. In designing the novel lipids, structural characteristics such as biodegradability, lipid tail length, level of branching, branched tail symmetry, and increased hydrogen bonding ability were considered.



Conclusions

Ester functional groups were incorporated to increase potential for in vivo biodegradability via hydrolysis by esterases, generating byproducts consisting of functional groups which may be metabolized more readily. Lipid tail design was influenced by length, level of branching, and branched tail symmetry in order to optimize the physicochemical characteristics of the resulting lipid-nucleic acid complexes. Increased hydrogen bonding capacity was achieved through the introduction of hydroxyl substituents, promoting stronger intermolecular association with anionic nucleic acid phosphate groups. Using these principles, we designed 4,200 novel ionizable lipids and synthesized a library consisting of 84 polyvalent ionizable lipids sorted into 11 families. We tested this library for delivery of mRNA to cells and identified candidate vehicles for further development, which will ideally yield an effective and versatile lipid delivery system with potential to enable effective therapies that can positively impact patient outcomes for countless diseases.

1 Scope of Lipid Tails

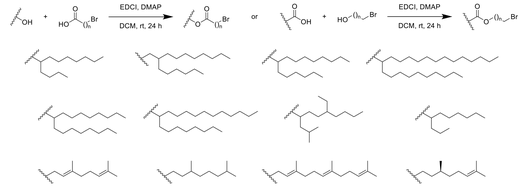


Figure 1. The scope of lipid tails (R) encompass branched or terpene derived alcohols or carboxylic acids. Tethered ester chains range from n=2 to n=6.

2 Synthesis of Branched Ester Substituted Spermine Analogs

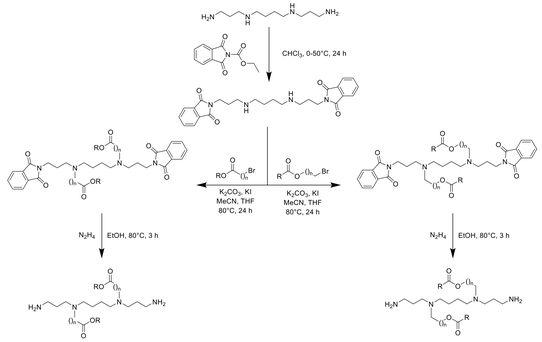


Figure 2. Synthesis of branched ester substituted spermine analogs derived from phthalimide protected spermine.

3 Synthesis of Branched Ester Substituted Bis Hydroxyethyl Ethylenediamine Analogs

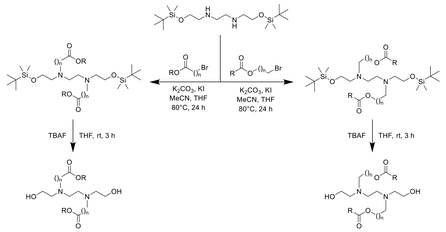


Figure 3. Synthesis of branched ester substituted bis hydroxyethyl ethylenediamine analogs derived from TBS protected bis hydroxyethyl ethylenediamine.

4 Synthesis of Branched Ester Substituted 1,4-Diaminobutane Analogs

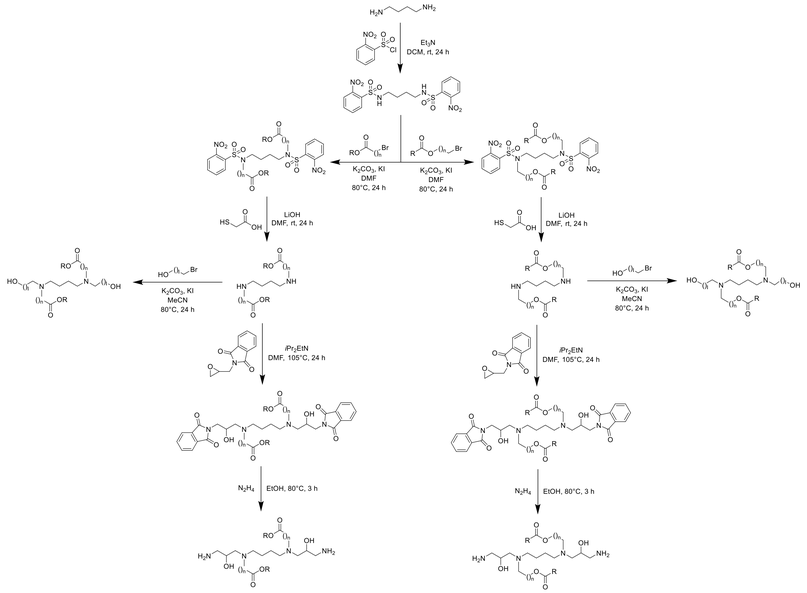


Figure 4. Synthesis of branched ester substituted 1,4-diaminobutane analogs derived from bis Ns protected 1,4-diaminobutane via divergent synthetic pathway. The deprotected secondary amines were alkylated with bromoalcohols ranging from l=1 to l=3 or glycidyl phthalimide followed by hydrazinolysis to the primary amine.

5 Synthesis of Branched Hydroxy Ester Substituted Spermine Analogs

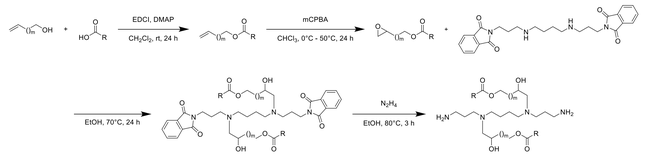


Figure 5. Synthesis of branched hydroxy ester substituted spermine analogs derived from phthalimide protected spermine via epoxide ring opening reaction. Tethered ester chains range from m=1 to m=4.

6 mRNA Lipoplex Delivery to THP-1 Monocytes and iMSCs in vitro

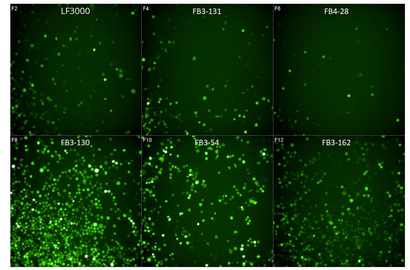


Figure 6. Lipoplex transfection data of THP-1 monocytes. Lipids were complexed with mRNA encoding GFP at varying lipid:mRNA weight ratios as determined by gel electrophoresis. Lipoplexes containing 500 ng mRNA were delivered to wells containing 30,000 THP-1 monocytes. Images were taken 24 hours post transfection. Images are organized by increasing ester tether length from n=2 to n=6. All lipids successfully transfected cells, with lipid FB3-130 (n=4) exhibiting the highest amount of GFP positive cells.

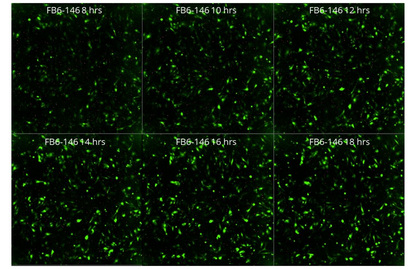


Figure 7. Lipoplex transfection data of iMSCs. Lipid FB6-146 was complexed with mRNA encoding GFP at a 2.5:1 lipid:mRNA weight ratio. Lipoplexes containing 200 ng mRNA were delivered to wells containing 10,000 iMSCs. Images were taken every 2 hours from 8 to 18 hours post transfection to determine the timepoint at which GFP expression peaks. Peak expression was noted at 18 hours post transfection for lipid FB6-146. Other lipids were tested under the same experimental conditions, yielding similar peak expression times.

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