Development of a solid-phase synthetic approach for triazine-based lipids

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ABSTRACT

Introduction: Ionizable cationic lipids have significantly advanced lipid nanoparticle (LNP)-mediated drug, gene, and vaccine delivery, enabling improved transfection efficiency and biocompatibility (1). However, efficient synthetic approaches to achieve compositionally diverse libraries remain limited by synthetic challenges (2). Building on prior solution-phase synthesis of triazine-based lipids with unique properties and transfection efficiency, this study employs a solid-phase approach to rapidly generate diverse lipids for structure-activity studies to optimize in vitro and in vivo performance.

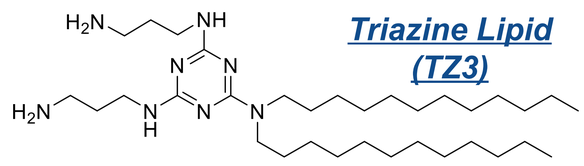
Methods: To expand this library, we developed a solid-phase synthesis using a 4-nitrophenyl carbonate-functionalized resin as an acid-labile platform. The diamine headgroup was first reacted to form an amine-functionalized resin, followed by nucleophilic aromatic substitution with cyanuric chloride, serving as a thermally controlled, chemoselective linker, to yield a dichlorotriazine-functionalized resin. At room temperature, dialkylamine substitution introduced lipid tails, and diverse amine nucleophiles completed the final substitution at 80°C. Trifluoroacetic acid cleavage yielded highly pure lipids, characterized by NMR, ESI-MS, and HPLC.

Results: These data demonstrate the versatility of solid-phase lipid synthesis using cyanuric chloride as a linker to generate diverse libraries. The first iteration used a C12 dialkyl amine lipid tail with nine distinct headgroups. Six of eight lipids reached 100% yield without additional purification as confirmed by mass spectrometry and NMR. The remaining two lipids showed 80–90% conversion, likely due to steric hindrance from t-butyl and Boc-protected reagents. These data suggest that protecting groups significantly impact reaction efficiency. Based on these findings, a second iteration is evaluating lipid tail diversity (C12, C14, C16, C18) to establish structure-activity relationships, aiming to enhance lipid-mediated mRNA delivery. In vitro and in vivo studies are ongoing.

Conclusion: Solid-phase synthesis using cyanuric chloride enables rapid generation of diverse lipid libraries with high yield and purity. This method allows precise control over headgroup and tail incorporation, facilitating evaluation of their stability on lipid nanoparticle formulation and mRNA transfection efficiency. This approach streamlines synthesis and optimization for improved delivery applications.

OBJECTIVE

Develop a solid-phase synthetic strategy using cyanuric chloride for efficient combinatorial lipid libraries to investigate mRNA delivery using triazine lipids.



CONCLUSION

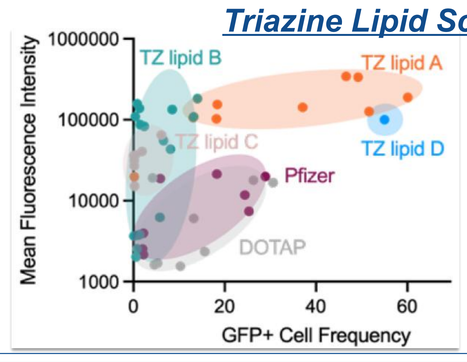
- Solid phase synthesis of triazine lipids is efficient and feasible
- Lipids are synthesized in 100% yield without the need for additional purification
- The efficiency and yield facilitate improved combinatorial synthesis

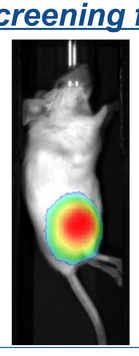
Future Directions

- In vitro screening of lipids for transfection efficiency
- Structure activity relationship on headgroup chemistry and alkyl tail length
- Ongoing project for use of cationic lipids in the Venditto lab:
  - mRNA therapeutic delivery
  - Protein subunit vaccination
  - mRNA vaccine development

PREMISE

Triazine Lipid Screening for mRNA Delivery

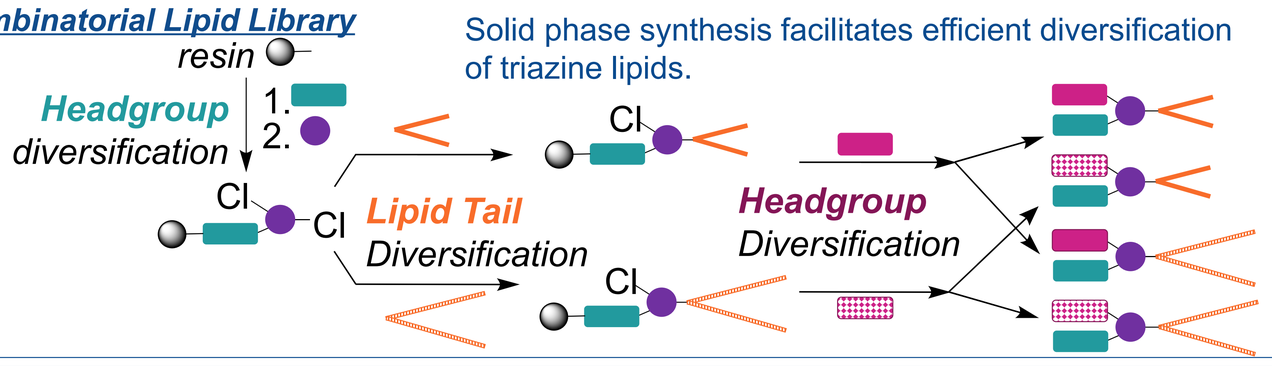




In vitro lipid screening with triazine lipids identified candidates with improved transfection.

Efficient synthetic strategies to access compositionally diverse libraries are critical.

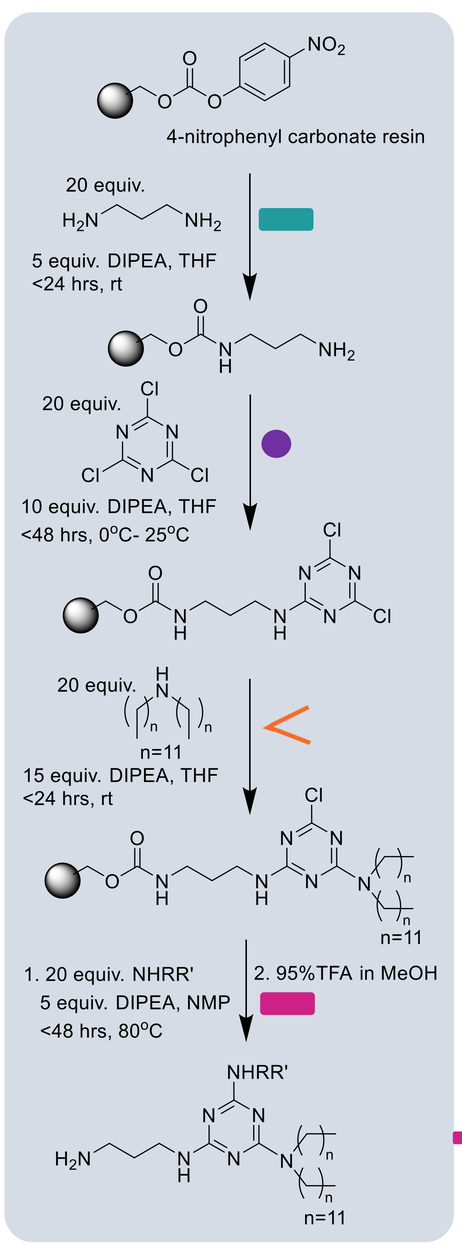
Combinatorial Lipid Library



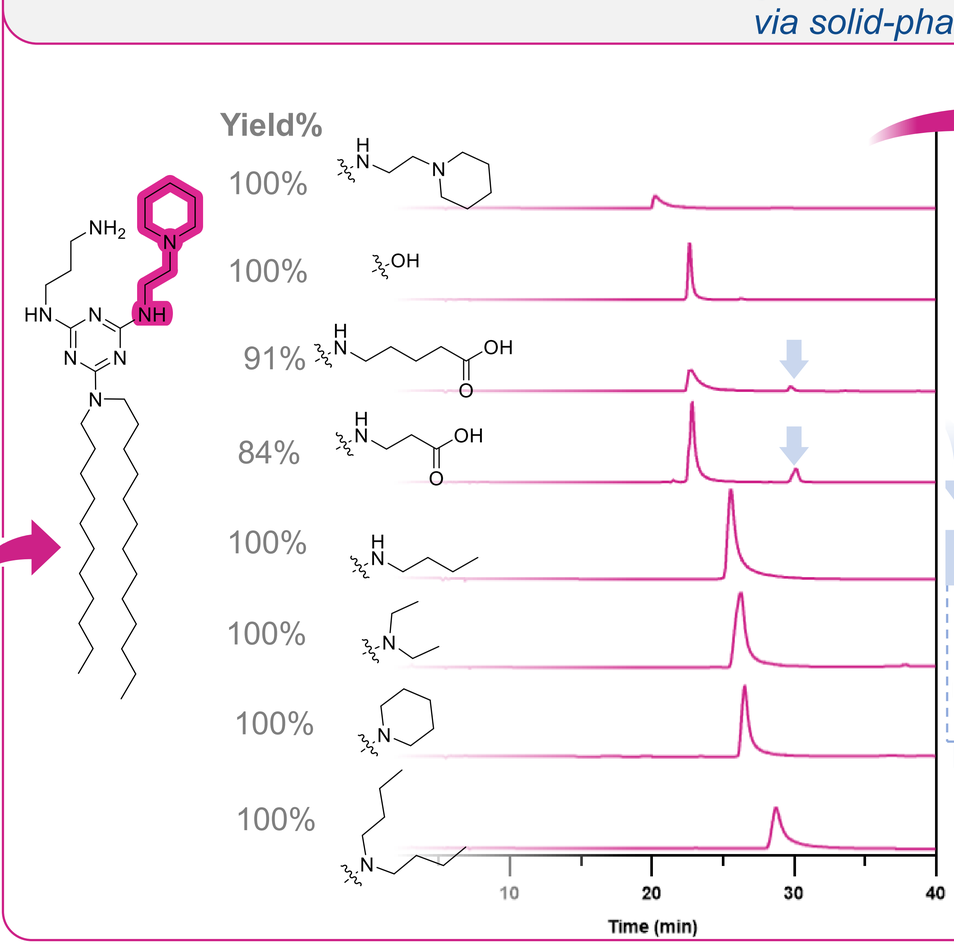
Solid phase synthesis facilitates efficient diversification of triazine lipids.

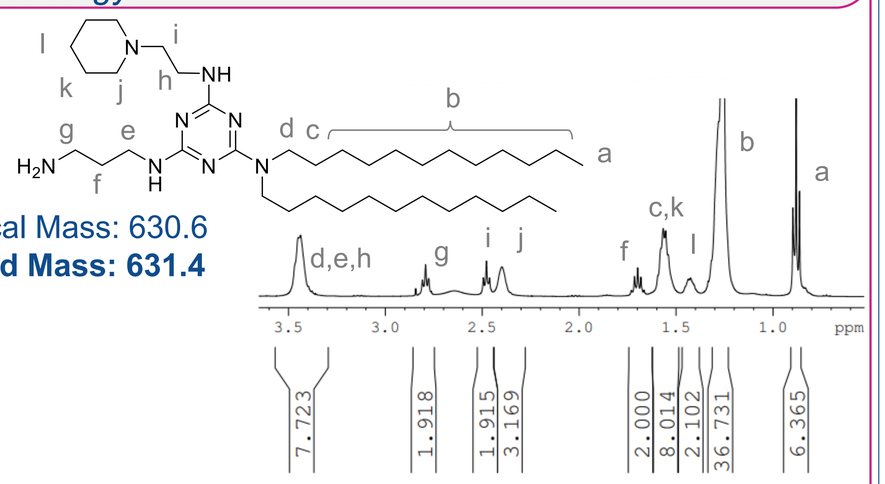
RESULTS

Solid Phase Synthetic Scheme for Combinatorial Lipid Synthesis

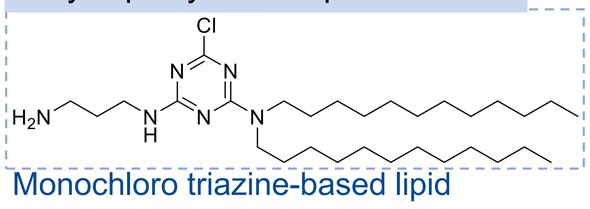


Headgroup diversification of ionizable lipids via solid-phase synthetic strategy





Only impurity = incomplete reaction



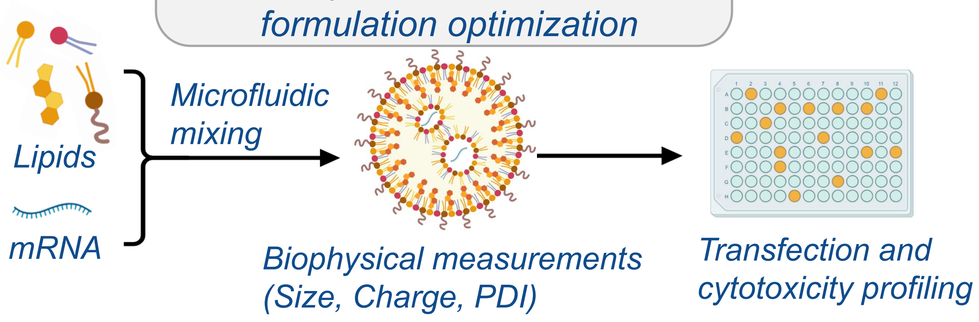
Monochloro triazine-based lipid

Theoretical Mass: 538.5
Observed Mass: 539.3

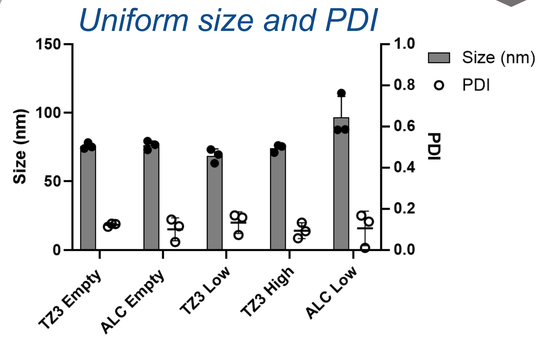
Steric hinderance prevents complete substitution when protecting groups are used

*Synthesized lipids **>90% purity** characterized by NMR and LCMS*

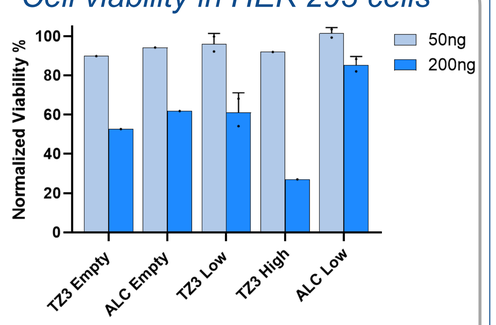
Multiplexed LNP-mRNA formulation optimization



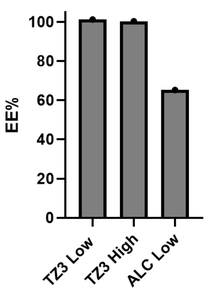
Uniform size and PDI



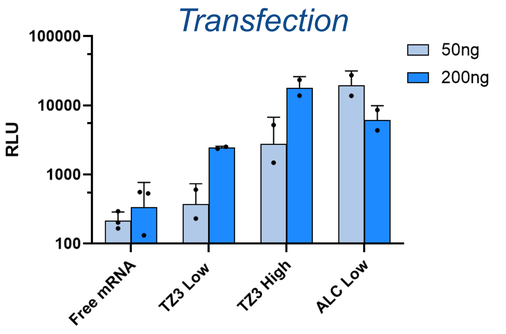
Cell viability in HEK 293 cells



Encapsulation efficiency



Transfection

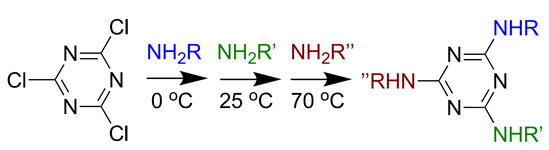


Low and high concentrations of lipid and aqueous solutions

LNP Formulation Development	Physical Characterization	In Vitro Testing	Data Analysis and Optimization
Design lipid Composition	***Particle size and PDI***	*Cell line selection*	*Statistical analysis*
Microfluidic mixing	***Encapsulation efficiency***	***Transfection efficiency***	*DOE validation*
		Cytotoxicity assessment	*Iterate and refine*

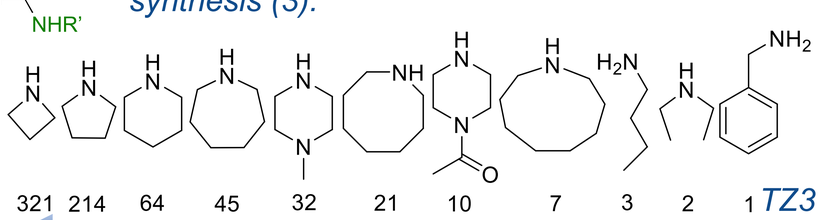
Synthetic feasibility ✓ *Formulation throughput* ✓ *In vitro assessment* ✓

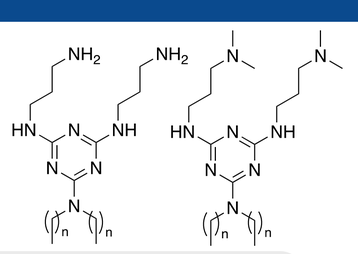
BACKGROUND



Cyanuric chloride enables thermally controlled, chemo-selective reactivity for modular lipid synthesis (3).

Amine Nucleophilicity Trend: Cyclic secondary > primary > linear secondary amines (3).





LD_{50} (μM)	130	340

DOTMA: 80 μM
DMPC: 970 μM

TZ3 showed higher in vitro toxicity but enhanced in vivo delivery and antibody response compared to DOTAP and DOTMA (4).

(1) Tang X, Zhang Y, Han X. Adv Nano Biomed Res. 2023;3. (2) Zhang Y, Sun C, Wang C, Jankovic KE, Dong Y. Chem Rev. 2021;121:12181–277. (3) Simanek, E et al. 2009 *Proc RSC A* 466:1445-1468. (4) Nardo , D; et al. (Venditto, VJ). RSC Adv, 2021, 24752-24761.

ACKNOWLEDGEMENTS

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CPRI Bridging Science With Possibility

NIH

KSCHIRT Kentucky Spinal Cord & Head Injury Research Trust

The Venditto lab