Research & Reviews: Journal of Pharmacy and Pharmaceutical Sciences

e-ISSN: 2320-1215
p-ISSN: 2322-0112

Combinatorial Design and Synthesis of Novel Ionizable Lipids for mRNA Delivery with Efficient Chemical Reactions

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Mini Review

Received: 23-Dec-2024, Manuscript No. JPPS-24-156126; **Editor assigned:** 26-Dec-2024, Pre QC No. JPPS-24-156126 (PQ); **Reviewed:** 09-Jan-2025, QC No. JPPS-24-156126; **Revised:** 11-Apr-2025, Manuscript No. JPPS-24-156126 (R); **Published:** 18-Apr-2025, DOI: 10.4172/2320-1215.14.1.007

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Citation: Zhang G, et al. Combinatorial Design and Synthesis of Novel Ionizable Lipids for mRNA Delivery with Efficient Chemical Reactions. RRJ Pharm Pharm Sci. 2025;14:007.

ABSTRACT

Lipid nanoparticles are the most clinically advanced mRNA delivery system. Ionizable lipid is the most important component of the four components comprising lipid nanoparticles. However, the structure-activity relationship of ionizable lipid is not clear. Traditional complicated design and synthesis procedures of ionizable lipids are time-consuming and laborious. Combinatorial design and synthesis of ionizable lipids with efficient chemical reaction can largely decrease the time of developing novel functional ionizable lipids. Michael addition and epoxy ring-opening reaction are the most used two-component reactions for developing novel lipids. Ugi reaction, A_3-coupling, van Leusen imidazole synthesis reaction is efficient multi-component reaction used for ionizable lipid development. However, efficient chemical reactions used for combinatorial design and synthesis of ionizable lipids are still in lack. Therefore, utilizing more novel efficient chemical reactions for ionizable lipid development will certainly provide more novel functional ionizable lipids for efficient mRNA delivery.

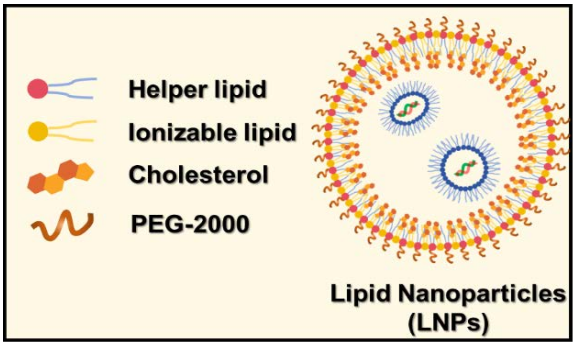
Keywords: Lipid nanoparticles; Ionizable lipids; mRNA delivery systems; RNA therapeutics

e-ISSN: 2320-1215
p-ISSN: 2322-0112

INTRODUCTION

Since the outbreak of COVID-19, mRNA vaccines have brought about significant economic and social benefits. Lipid nanoparticles are the most clinically advanced nucleic acid delivery vectors. Ionizable lipids, phospholipids, cholesterol, and PEGylated lipids comprise the FDA approved lipid nanoparticle formulations (Figure 1) [1-3].

Figure 1. Four components of lipid nanoparticles.

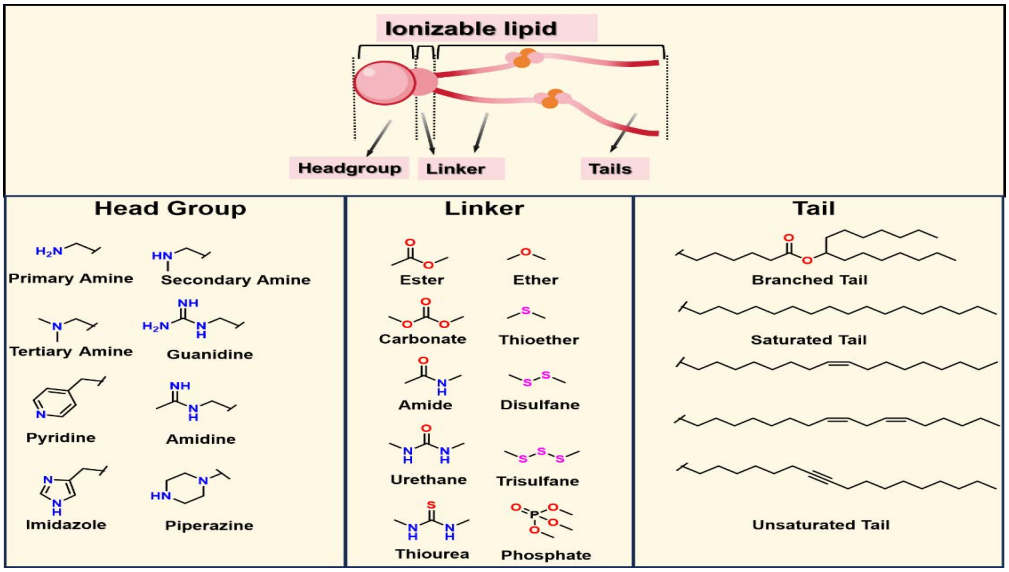


LITERATURE REVIEW

Of the four components, ionizable lipids are the most important but have strict patent barriers. Besides, ionizable lipids determine the *in vivo* mRNA delivery efficiency of lipid nanoparticles. Developing novel functional ionizable lipids is a hot spot in both scientific research and industry. However, as the structure-activity relationship of ionizable lipids is not very clear, traditional ionizable lipid development is laborious and time-consuming, requiring complicate synthesis procedures. For instance, ALC0315, the FDA approved ionizable lipid developed by Pfizer, took four years for its development. Recently, combinatorial chemistry has been introduced into the development of novel ionizable lipids. Compared to traditional complicate design and synthesis procedure, development of novel ionizable lipids with efficient chemical reactions in a way of combinatorial chemistry can decrease the time from years to months, even weeks. Therefore, combinatorial design and synthesis of novel ionizable lipids have become a hot research topic recently [4].

The structure of ionizable lipids can be divided into three parts, ionizable head groups, linkers and hydrophobic tails (Figure 2). Ionizable head groups include amines, guanidines, pyridines, amidines, imidazoles and piperazines. Linkers are even more variable, including ester, ether, carbonate, thioether, disulfane, trisulfane, amide, urethane, thiourea and phosphate. Hydrophobic tails include branched tails, saturated or unsaturated tails. The three parts are designed in a combinatorial way and linked together by efficient organic chemical reactions to produce novel ionizable lipids [5].

Figure 2. Structures of ionizable lipids.



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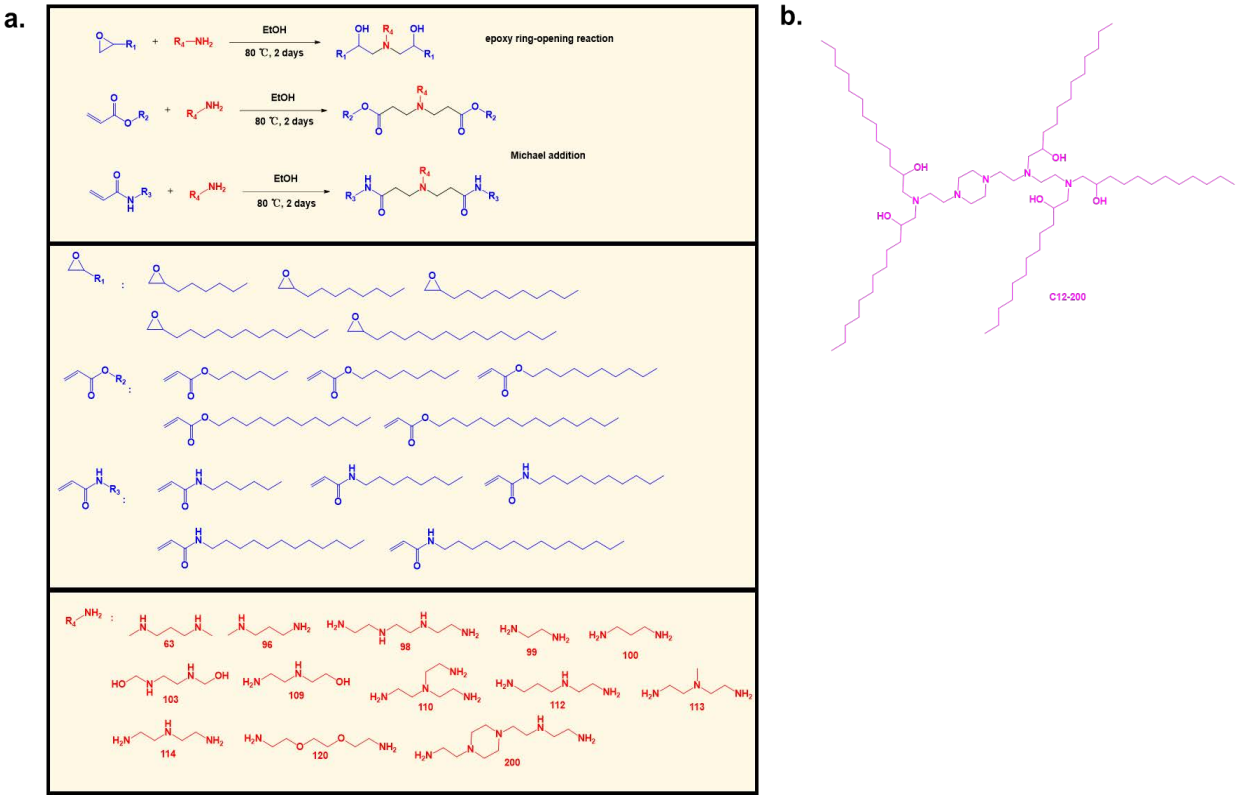
DISCUSSION

Efficient chemical reactions used for combinatorial design and synthesis of ionizable lipids

Michael addition and epoxy ring-opening reactions are the mostly used reactions for developing ionizable lipids in a combinatorial way, which were first used by Daniel G. Anderson. Michael addition reactions always involve acrylates or acrylamides as hydrophobic tails to react with amine heads to form novel ionizable lipids, while epoxy ring-opening reactions utilize alkyl epoxides as hydrophobic tails to form new lipids (Figure 3a). For example, the famous lipid C12-200, is a multi-tailed ionizable lipid which has higher *in vivo* transfection efficiency than the benchmark lipid D-Lin-MC3-DMA (Figure 3b) [6,7].

Figure 3. a) Michael addition or epoxy ring-opening reaction used in combinatorial design of ionizable lipids. B) Molecular structure of C12-200.



Ugi reaction is the first multi-component reaction used for the combinatorial design and synthesis of ionizable lipids. Compared to two-component reactions, multi-component reactions can provide a higher dimension for the combinatorial design of novel ionizable lipid library. For Michael addition, only amine heads and hydrophobic tails can be changed to maximize the number of ionizable lipid libraries. For Ugi reactions, besides amine heads and alkyl ketones as hydrophobic tails, isocyanides can act as linkers to provide a higher dimension for the number of lipid library (Figure 4). Daniel G. Anderson et al. utilized this reaction to generate a lipid library over 1000 lipids. After high-throughput screening, they found that ionizable lipids generated by Ugi reaction using heterocyclic amines had the effect of activating STING pathway thus promoting mRNA vaccine efficacy [8,9].

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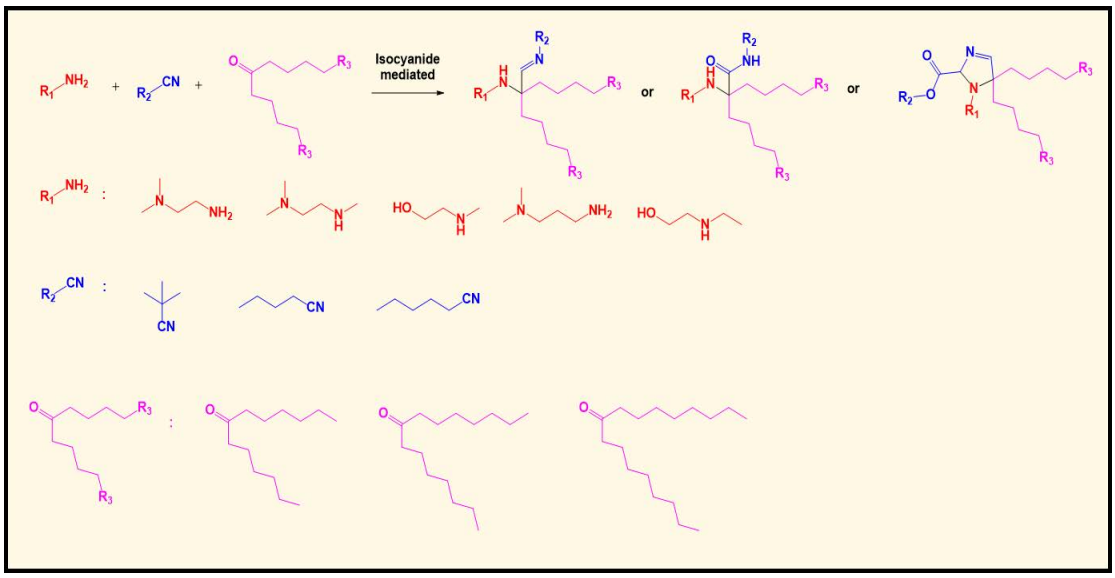
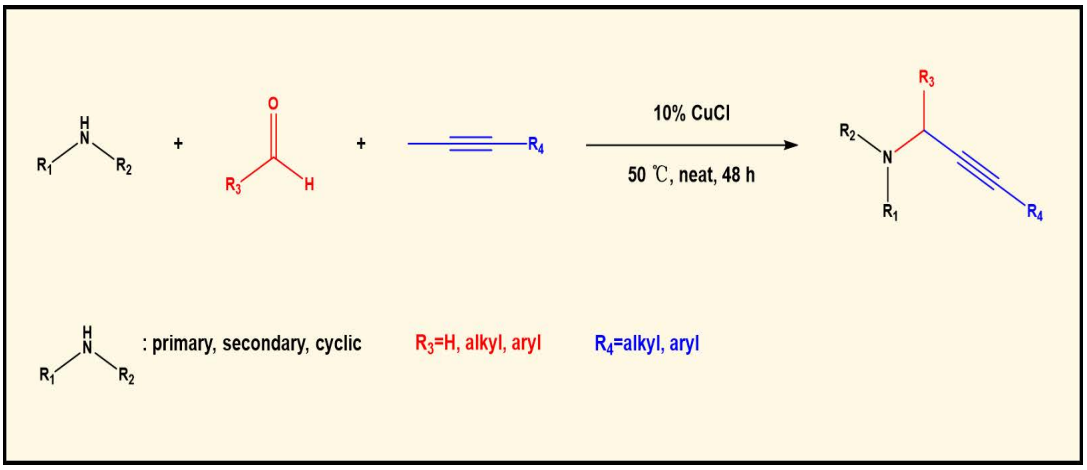
Figure 4. Ionizable lipid library generated by three-component Ugi reaction.



Recently, some other efficient chemical reactions have also been utilized for developing novel ionizable lipids, thus providing more chances for ionizable lipid discovery.

A_3-coupling reaction is an efficient three-component reaction which can occur in aqueous reactions, developed by Chao-Jun Li. Aldehydes, alkynes, and amines in the presence of various transition metal catalysts can directly undergo dehydration and condensation to form propargylamines (Figure 5). Michael J. Mitchell et al utilized this reaction to develop novel biodegradable lipids. After five rounds of directed chemical evolution, several biodegradable ionizable lipids with asymmetry structures were identified to be superior to the benchmark ionizable lipid, D-Lin-MC3-DMA.

Figure 5. A_3-coupling reactions.



van Leusen imidazole synthesis reaction is another efficient three-component reaction which has been used for ionizable lipid synthesis. Amines, aldehydes and tosylmethylisocyanides undergo coupling reactions to form imidazoles under room temperature and basic environments. Yucai Wang et al. utilized this reaction to generate a large library for high-throughput *in vivo* screening of lipids with high spleen-selective transfection efficiency. After three batches of screening, amine heads, hydrophobic tails and linkers were sequentially optimized for the highest spleen specific transfection. The optimal spleen-targeting LNP formulation containing A3B7C2 ionizable lipid was reported to be much 18.3 fold more efficient than the SORT spleen targeting formulation (Figure 6).

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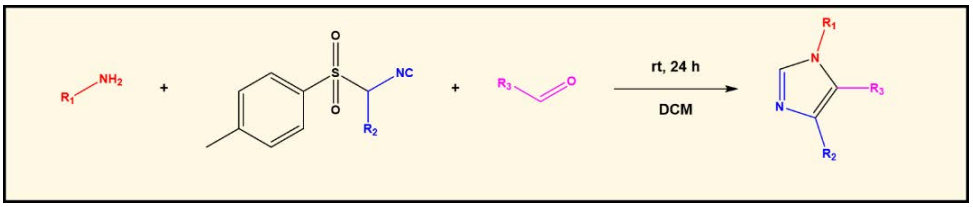
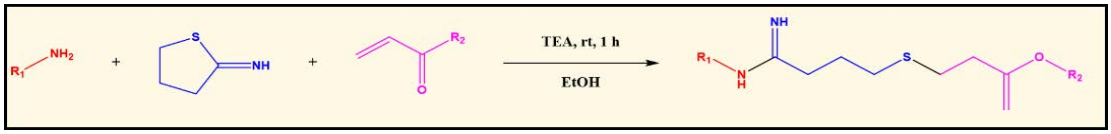
Figure 6. van Leusen imidazole synthesis reaction.



Michael J. Mitchell et al. also developed a multi-component reaction for fast synthesis of ionizable lipids. Traut's reagent were introduced to react with amine to turn it from a weak nucleophile into a strong nucleophile to easily react with acrylates. The reaction can occur under room temperature and finish in just one hour, which greatly accelerates the development of novel ionizable lipids. Although the top-performing ionizable lipid 12T-014 is less efficient than the commercial ionizable lipid SM102 for *in vivo* delivery of mRNA, it can be as a fifth component for traditional lipid nanoparticle formulation for efficient organ selective targeting (Figure 7).

Figure 7. Fast and facile synthesis of amidine-incorporated ionizable lipid.



As artificial intelligence is thriving in these years, utilizing efficient chemical reactions for automated high-throughput synthesis and screening of ionizable lipids has become even more necessary. Bowen Li et al. has developed a high-throughput automated synthesis and screening platform to accelerate the discovery of ionizable lipids with excellent performances. However, the research is limited to lipid libraries designed by Ugi reaction, still needing other highly efficient chemical reactions.

CONCLUSION

As the most clinically advanced RNA delivery system, lipid nanoparticles are the hotpot of global scientific research. Of the four components, ionizable lipids are the most significant but lack enough understanding of the structure-activity relationship, thus needing combinatorial design of large lipid libraries for screening and discovering novel functional ionizable lipids for efficient mRNA *in vivo* delivery. However, only a few efficient chemical reactions, including Michael addition, epoxy ring-opening reaction, Ugi reaction, A_3-coupling reactions and Van Leusen imidazole synthesis reaction have been utilized for combinatorial design and synthesis of novel ionizable lipids. Traut's reagent is a good choice for accelerating traditional azo-Michael addition reaction, but the top performing ionizable lipid 12T-014 does not outperform SM102 for *in vivo* RNA delivery, thus needing further investigation for more efficient ionizable lipids. As artificial intelligence is thriving in these years, combining automated high-throughput synthesis and screening platform with highly efficient chemical reactions is quite essential. However, there is still lack of highly efficient chemical reactions for automated high-throughput synthesis and screening of novel ionizable lipids. The chemical reactions should be performed under mild temperatures and in non-toxic solvents, and can be finished in hours to achieve fast and facile synthesis of ionizable lipids, easy for scale-up industrial production. Therefore, combining click chemistry with combinatorial design and synthesis of ionizable lipids can be a good research topic. However, although click chemistry can provide chances for fast and facile synthesis of novel ionizable lipids. It still needs high-throughput screening and artificial intelligence to further summarize and predict top performing lipids to outperform the benchmark commercial ionizable lipids, D-Lin-MC3-DMA, SM102 and ALC0315. For more efficiently developing novel functional ionizable lipids, it needs not only biologists, but also organic chemists and scientists in the field of artificial intelligence. Only by working together can more and more novel functional ionizable lipids can be discovered and go to clinical practice for better RNA therapy for our human beings all over the world.

ACKONWLEDGMENTS

This work was supported by the National Key Research & Development Program of China (grant Nos. 2021YFA1201000, 2023YFC2605000), NSFC key project (grant No. 32030060), NSFC international collaboration key project (grant No. 51861135103). The authors also appreciated the support of "the Chinese Academy of Sciences (CAS-NSTDA) International

Partnership Program" (121D11KYSB20210003) and the Science Fund for Creative Research Groups of Nature Science Foundation of Hebei Province (B2021201038).

REFERENCES

1. Albertsen CH, et al. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022;188:114416.
2. El Moukhtari SH, et al. Lipid nanoparticles for siRNA delivery in cancer treatment. J Control Release. 2023;361:130-146.
3. Melamed JR, et al. Ionizable lipid nanoparticles deliver mRNA to pancreatic β cells *via* macrophage-mediated gene transfer. Sci Adv. 2023;9:eade1444.
4. Saadati F, et al. A route to lipid ALC-0315: A key component of a COVID-19 mRNA vaccine. Chemistry. 2022;28:e202200906.
5. Xu Y, et al. Rational design and combinatorial chemistry of ionizable lipids for RNA delivery. J Mater Chem B. 2023;11:6527-6539.
6. Love KT, et al. Lipid-like materials for low-dose, *in vivo* gene silencing. Proc Natl Acad Sci U S A. 2010;107:1864-1869.
7. Akinc A, et al. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nat Biotechnol. 2008;26:561-569.
8. Miao L, et al. Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. Nat Biotechnol. 2019;37:1174-1185.
9. Han X, et al. Optimization of the activity and biodegradability of ionizable lipids for mRNA delivery *via* directed chemical evolution. Nat Biomed Eng. 2024;8:1412-1424.