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A two-step continuous flow synthesis of multi-tail ionizable lipids

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A robust and safe continuous flow synthesis of multi-tail ionizable lipids, key components in LNP formulations for RNA therapeutic delivery, is reported. A nearly quantitative conversion in the epoxidation of long-chain terminal alkenes is safely achieved in continuous flow using the Oxone/acetone system, which prevents the buildup of hazardous dimethyldioxirane. A catalyst-free polyalkylation of amine nucleophiles *via* high-temperature epoxide ring-opening reduces reaction time dramatically—from several days in batch to just twenty minutes of residence time in continuous flow. Alkylation of the crude epoxidation product enabled the production of representative ionizable lipids with a throughput exceeding 10 g h^{-1} in a laboratory setting, demonstrating strong potential for rapid and safe lipid manufacturing at scale.

In recent years, messenger RNA (mRNA) has emerged as a transformative technology for multiple therapeutic applications, including vaccines, cancer immunotherapies and gene editing.[1] Its immense clinical potential was demonstrated by the use of mRNA vaccines to combat the 2020 pandemic caused by the novel coronavirus (SARS-CoV-2).[2] However, the direct use of RNA therapeutics is limited: they are prone to nuclease-mediated degradation, and their substantial molecular weight and polyanionic nature result in poor cellular permeability. As a result, RNA molecules must be encapsulated to reach target cells effectively. Lipid nanoparticles (LNPs) based on ionizable lipids have become the predominant delivery platform for mRNA therapeutics.[3] These specialized lipids feature an ionizable headgroup and multiple hydrophobic tails. They are positively charged at acidic pH, facilitating RNA condensation, but remain neutral at physiological pH to minimize toxicity. Multi-tail ionizable lipids—defined as lipids containing three or more hydrophobic tails—were initially developed for siRNA delivery,[4] but their use was later extended to efficient mRNA delivery through optimization of LNP composition.[5]

One of the main disadvantages in the batch production of ionizable lipids is the lengthy synthetic process.[4a] Methods that rely on the ring-opening of epoxides by amines as a key step often require reaction times of several days.[4b,6] Although microwave irradiation can shorten reaction times to about five hours by increasing the reaction temperature,[7] this approach is difficult to scale up to industrially relevant levels. To address these limitations, we developed an efficient, reproducible, and scalable continuous flow protocol for the S_N2 reaction between long-chain epoxides and polyamine nucleophiles (Scheme 1). Continuous flow chemistry emerges as the preferred approach for this synthesis, as it enables the safe use of high temperatures and pressures while enhancing heat and mass transfer.[8] Notably, flow-based processes have a proven track record of being scaled to produce metric tons of material annually,[9] underscoring their potential to meet increasing clinical demand with greater efficiency and safety. There are a few examples in the literature addressing the aminolysis of epoxides facilitated by continuous flow chemistry. Heterogeneous catalysis in continuous flow aminolysis has been reported using solid-supported Lewis acids (such as Sc-based catalysts[10] or molybdenum oxide[11]) and biocatalysts (Lipozyme TL IM[12]). Homogeneous, catalyst-free epoxide aminolysis in a continuous flow microreactor has also been described, enabled by the use of high temperatures typically not achievable in batch and microwave-assisted reactions.[13] However, the existing literature primarily focuses on monoalkylation of select amines. Herein, we present a homogeneous, catalyst-free, and broadly applicable continuous flow procedure for scalable multi-alkylation of various polyamine nucleophiles *via* epoxide ring-opening.[14]

Another challenge in the synthesis of large libraries of β-amino alcohol ionizable lipids is the limited availability of long-chain terminal epoxides. Monosubstituted terminal alkenes are attractive starting materials for epoxide synthesis due to their accessibility from large-scale industrial processes

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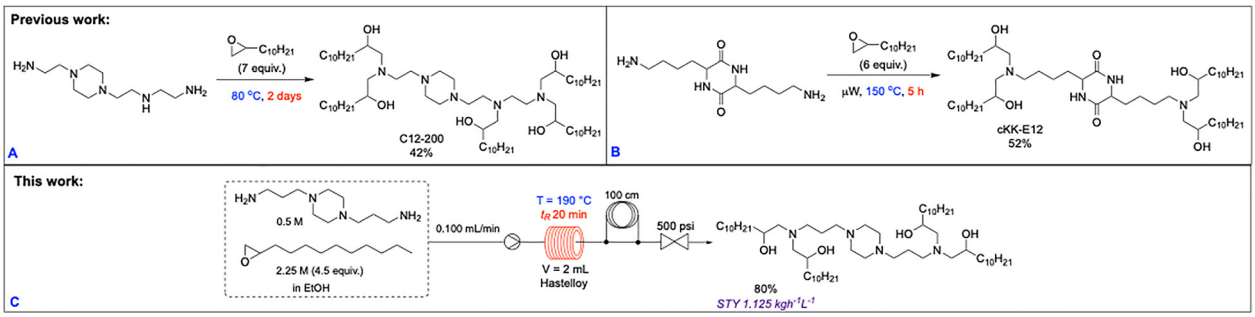


Scheme 1 Synthesis of ionizable lipids under batch and flow conditions.

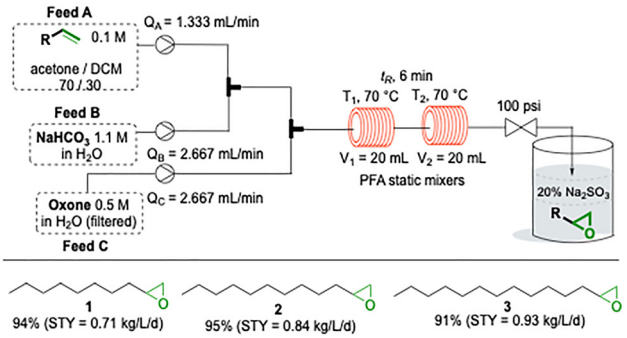
and biomass.[15] However, they exhibit reduced nucleophilicity compared to disubstituted internal alkenes, requiring an excess of highly electrophilic oxidants for effective epoxidation, reagents that are inherently unstable. Once again, flow technologies provide solutions by avoiding the accumulation of hazardous materials through the generation and immediate consumption of reactive species within a single, continuous process.[16] This approach is particularly relevant at the industrial scale, where the accumulation of oxidants can result in dangerous thermal runaways, which are difficult to control in large-scale batch epoxidation reactions.[17] Several examples of continuous flow synthesis of long-chain epoxides have been reported in the literature, including epoxidation using *in situ* generated HOF·MeCN from fluorine and wet acetonitrile,[18] continuous semi-batch Payne epoxidation with a cyanamide–potassium carbonate catalytic system,[19] and biocatalytic processes.[20] We focused our efforts on developing a continuous flow epoxidation process using dimethyldioxirane (DMDO), generated *in situ* from the reaction of acetone and Oxone. Oxone, a triple salt ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), is a solid, easy-to-handle, non-toxic, water-soluble, stable, and inexpensive oxidant.[21] The synthesis of epoxides using dioxiranes, typically generated from Oxone and ketones, has been reported in the literature under both batch[22] and continuous flow conditions as a mean to improve safety.[23] However, none of these reports describe an efficient oxidation (in terms of high conversion and yield) of monosubstituted long-chain alkenes. Due to their relatively low reactivity, these substrates require stronger electrophilic oxidants—such as methyl(trifluoromethyl)dioxirane (TFDO)[24] or harsher reaction conditions to undergo efficient epoxidation. Herein, we demonstrate a high-yielding, highly selective, safe, and operationally simple continuous flow epoxidation of long-chain terminal alkenes using *in situ* generated TFDO or DMDO, followed by efficient ring-opening reactions to produce ionizable lipids of interest in a streamlined two-step continuous flow process.

The epoxidation of 1-dodecene was first examined under batch conditions using a biphasic organic solvent–water system with Oxone (2 equiv.), acetone (10 equiv.), and sodium bicarbonate (5 equiv.). In dichloromethane, the reaction gave a 48% yield after two hours. Conversion was slower in ethyl acetate, while in acetonitrile the substrate showed poor solubility, separating as oily droplets. No reaction occurred without acetone. The moderate yield obtained in DCM indicated it was a suitable starting point for optimization under continuous flow conditions. In the initial flow setup, a solution of 1-dodecene (0.05 M) and acetone (2 M) was sequentially mixed with a large excess of aqueous sodium bicarbonate (1.1 M) and Oxone (0.5 M) to generate DMDO *in situ*. The reaction stream was collected into a stirred biphasic mixture of DCM and 20% aqueous sodium sulfite to quench any residual DMDO. Under initial conditions (25 °C for 5.5 min in a static mixer, then 60 °C for 2.75 min in a tubular reactor), 1,2-epoxydodecane was obtained in 17% yield. Replacing acetone with trifluoroacetone (TFDO precursor) gave full conversion, but due to its high cost, a 1 : 9 trifluoroacetone-acetone mix was tested, yielding only 43% conversion. The strategy then shifted to using acetone alone with intensified conditions. Running the reaction entirely at 60 °C for 8.2 min increased conversion to 35%. By extending residence time to 10 min and raising acetone concentration to 6.45 M (50% with DCM), conversion reached 84%. Higher temperatures had little effect, but at 70 °C with a 12 min residence time and reduced flow rate, conversion rose to 93%. Increasing 1-dodecene to 0.1 M and acetone to 9.45 M (70% of solvent) maintained productivity, though higher acetone levels caused precipitation. At 60 °C and 5 min, conversion was 76%, and reducing the organic flow rate further (6 min residence time) yielded 97% conversion.

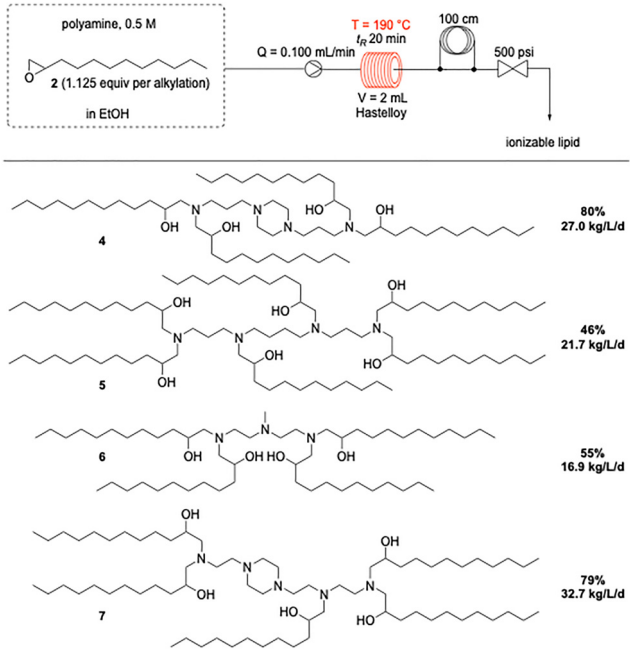
A loss of approximately 5% in recovered mass was observed with the increased acetone volume, resulting in a lower NMR yield compared to the measured conversion. This discrepancy is attributed to the reduced solubility of both the starting material and product in the reaction medium, along with hydrophobic interactions with the PFA reactor material, leading to partial adhesion to the reactor walls. This effect diminishes as the reaction scale increases.

The optimized reaction conditions were applied to the multigram synthesis of three pharmaceutically relevant long-chain terminal epoxides.† Starting from the corresponding alkenes, 1,2-epoxydecane (**1**), 1,2-epoxydodecane (**2**), and 1,2-epoxytetradecane (**3**) were isolated by column chromatography in 94%, 95%, and 91% yield, respectively (Scheme 2).

The amine polyalkylation reaction was optimized using 1,2-epoxydodecane and 1,4-bis(3-aminopropyl)piperazine as partners. The reaction was initially investigated under microwave irradiation, and its progress was qualitatively monitored by



Scheme 2 Substrate scope of the epoxidation reaction.



Scheme 3 Substrate scope of the aminolysis reaction.

comparing total ion current chromatograms obtained by HPLC-MS.

The solvent-free reaction conducted at 180 °C for 30 minutes afforded the desired tetraalkylated amine as the main product. However, these conditions could not be translated to continuous flow due to the formation of precipitate during the reaction. Among the solvents tested, ethanol provided better conversion than isopropanol and *n*-butanol. However, the pressure limitations of the microwave setup restricted operation to temperatures below 160 °C. In all cases, a significant amount of partially alkylated amines was detected, and side reactions occurred, as evidenced by HPLC-MS signals and the darkening of the reaction mixtures. Both limitations can be addressed in continuous flow, where the use of backpressure enables superheating of solvents and enhanced heat transfer allows for more selective transformations. Ethanol emerges as an excellent solvent choice for continuous flow optimization due to its low toxicity, low cost, and good solubility for all substrates and products.

In the initial flow experiment, a solution of 1,4-bis(3-aminopropyl)piperazine (0.2 M) and 1,2-epoxydodecane (0.8 M, 4 equivalents) in ethanol was heated to 150 °C with a residence time of 20 minutes. Under these conditions, the product mixture predominantly contained amines bearing three and four alkyl chains. Increasing the temperature to 190 °C (500 psi backpressure regulator) further enhanced the proportion of tetraalkylated products, although significant amounts of lower alkylated species remained. Increasing the amine concentration to 0.5 M and using 4.5 equivalents of epoxide at 190 °C (20-minute residence time) resulted in a product mixture highly enriched in tetraalkylated compounds. The crude mixture was purified by flash chromatography, affording the desired product **4** in 80% yield (space-time yield: 27.0 kg L^{-1} d^{-1}) (Scheme 3).

Shorter residence times of 5 and 10 minutes under these conditions did not afford satisfactory conversion to the target products. Likewise, reducing the epoxide equivalents to 4.1 increased the proportion of trialkylated species in the mixture. Raising the temperature to 200 °C enabled an isolated yield of 73% in just 10 minutes, thereby increasing the space-time yield to 49.2 kg L^{-1} d^{-1}. However, the reaction mixture was more complex, making isolation of the desired product by column chromatography more challenging. The addition of 4% v/v of 37% aqueous HCl at 190 °C similarly increased the space-time yield (STY) to 51.0 kg L^{-1} d^{-1}, affording a 76% isolated yield with a 10-minute residence time. Nevertheless, acid-catalyzed conditions were not considered optimal for broader substrate scope due to the variable solubility of amine substrates in the reaction medium. Optimized conditions (0.5 M amine, 4.5 epoxide equivalents, 190 °C, 20 min residence time) were successfully applied to produce 10 g (80% yield) of desired ionizable lipid in a total process time of 70 minutes, with a throughput of 11.2 g h^{-1}.‡ When the crude product from the epoxidation reaction was used as the starting material in the amine polyalkylation reaction, the isolated yield of desired ionizable lipid dropped slightly to 75%. Eliminating the need for purification of the epoxide allows to significantly reduce operational time and complexity and opens a possibility for scalable, telescoped procedure of valuable ionizable lipids production starting from readily available terminal alkenes and amines. Three other ionizable lipids (**5**–**7**), which have been previously described and successfully used in LNP formulations,[4b,6,25] were produced and isolated using the optimized conditions (Scheme 3).

In summary, a two-step continuous flow process was developed for synthesizing multi-tail ionizable lipids, important in pharmaceuticals. First, long-chain terminal alkenes were efficiently converted to epoxides using *in situ* generated DMDO. In the second step, amines were polyalkylated *via* epoxide ring opening at high temperature without a catalyst, using a backpressure-controlled setup. This reduced reaction time from days to 20 minutes and avoided pressure buildup.

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Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: **https://doi.org/10.1039/d5cc04692k**.

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† For detailed optimisation, see Table S1.
‡ For detailed optimisation, see Table S3.

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