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Design, Synthesis and Evaluation of Amphiphilic Silyl Lipids to Tune Lipid Nanoparticle
Properties for Nucleic Acid Delivery

By

LEAH AUTUMN PATTERSON

DISSERTATION

Submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Chemistry

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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Abstract

Silicon is the second most abundant element in Earth's crust and has no known element-specific toxicity. Despite being carbon's elemental "cousin", the silicon atom contains unique properties that can be harnessed in medicinal chemistry and chemical biology to improve chemical and biological properties. Some of these unique properties that are applicable to chemical biology are silicon's enhanced lipophilic character and larger size compared to carbon. Silicon's larger atomic radius results in 20% longer bond lengths compared to regular carbon-carbon bonds. These longer bond lengths in silicon-carbon bonds increase the surface area for Van der Waals intermolecular forces, allowing for greater hydrophobic interactions. Modification of hydrophobic interactions are particularly relevant in (i) medicinal chemistry for enhancing small molecule binding in hydrophobic binding pockets of proteins and increasing permeability of lipophilic and amphiphilic molecules across cell membranes, and (ii) chemical biology for modulating membrane fluidity and stability related to lipid nanoparticles (LNPs).

Chapter 1 summarizes the relevant properties of silicon in medicinal chemistry and chemical biology. A few specific examples are given that showcase how incorporation of silicon into small molecules increased their therapeutic properties such as potency and stability. Hydrosilylation is also described as a key synthetic strategy to access modular silicon-containing molecules. Chapter 1 also summarizes the use of LNPs and the history of amphiphilic lipids that have been synthesized with varying polar head groups and nonpolar tails to optimize LNP properties and nucleic acid delivery. LNPs have widely been used for nucleic acid delivery and are composed of amphiphilic lipids that contain a polar head group and nonpolar lipid tails. There are five classes of lipids used in LNPs. The cationic lipid component has a positively charged polar head group that facilitates in encapsulation of negatively charged RNA and DNA and cellular uptake. The

polar head group of ionizable lipids is pH dependent, with the head group becoming protonated in the endosome under acidic conditions, facilitating in endosomal release of nucleic acid inside the cell. Zwitterionic phospholipids and cholesterol are essential for controlling membrane fluidity and structural stability of the LNP. Lastly, PEGylated lipids increase in vivo stability, biodistribution and circulation time of LNPs. Several synthetic modifications have been made to the polar head group of amphiphilic lipids to control and improve LNP properties and nucleic acid delivery. However fewer examples of nonpolar tail modifications exist, with the most common examples including saturated tails with varying lengths, unsaturation, and branching.

Chapters 2 and 3 present my work for the synthesis and biophysical and biological evaluation of cationic and ionizable silyl lipids for their use in lipid nanoparticles and mRNA delivery. Lipid nanoparticles were formulated with ionizable and cationic silyl lipids and the following properties were evaluated: (i) size using dynamic light scattering (DLS) and cryo-TEM, (ii) encapsulation efficiency using the RiboGreen fluorescence assay, (iii) membrane fluidity using the C-Laurdan fluorescence assay and (iv) mRNA transfection efficiency in HEK293T cells. Chapter 2 demonstrates the synthesis and evaluation of cationic silyl lipids with modular tail structures to control particle properties, with key findings including (i) shorter silyl lipid tails (many containing phenyl rings) generally form particles with enhanced RNA encapsulation efficiency and mRNA transfection efficiency in HEK293T cells, (ii) the silyldimethyl group in the lipid tail enhances particle stability compared to a methylene and (iii) varying the silyl lipid tail structure enables control of membrane fluidity, with the silyldimethyl group imparting enhanced membrane fluidity compared to *cis* alkene and methylene-containing control lipids. Chapter 3 builds off this work by demonstrating the synthesis and evaluation of ionizable silyl lipids for mRNA delivery. Cryo-TEM, RiboGreen and C-laurdan assay, and cell transfection data

supports that modifying the silyl lipid tail structure and the number of silyl lipid tails (i.e. single vs double chain lipids) can be leveraged to control particle properties, with particles composed of single chain lipids demonstrating distinct membrane fluidity and mRNA delivery profiles compared to double chain lipids.

Appendix A discusses the evaluation of DNA encapsulation efficiency, encapsulation rate, and transfection efficiency for the cationic and ionizable silyl lipids.

Appendix B contains all relevant NMR data.

List of Symbols and Abbreviations

ACN	Acetonitrile
CDI	1,1'–Carbonyldiimidazole
δ	Chemical shift
chol	Cholesterol
Cy	Cyclohexyl
cod	1,5–Cyclooctadiene
DA	dialkylated or double chain
DCM	Dichloromethane
d	Doublet
DLS	Dynamic light scattering
EE	Encapsulation efficiency
ER	Encapsulation rate
equiv	Equivalents
EtOAc	Ethyl acetate
GP	Generalized polarization
g	Grams
GFP	Green fluorescent protein

HEK	Human embryotic kidney
Hex	Hexanes
HRMS	High resolution mass spectrometry
h	Hours
LNP	Lipid Nanoparticle
LAH	Lithium aluminum hydride
m/z	Mass-to-charge ratio
MHz	Megahertz
MeOH	Methanol
μL	Microliters
mg	Milligrams
mL	Milliliter
mM	Millimolar
mmol	Millimoles
min	Minutes
M	Molar
mol	Mole
MW	Molecular weight

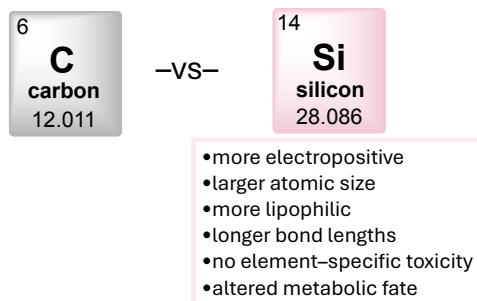
MA	monoalkylated or single chain
m	Multiplet
NMR	Nuclear magnetic resonance
ppm	Parts per million
Ph	Phenyl
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
q	Quartet
rt	Room temperature
s	Singlet
SM	Starting material
THF	Tetrahydrofuran
dvtms	1,1,3,3-Tetramethyl-1,3-divinyldisiloxane
TMS	Tetramethylsilane
TLC	Thin layer chromatography
TEM	Transmission electron microscopy
t	Triplet
TE	Tris-EDTA

Chapter 1: Introduction to Properties of Silicon, Hydrosilylation and Lipid Nanoparticles

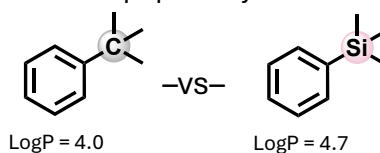
1.1. Harnessing the Unique Properties of Silicon in Medicinal Chemistry

Silicon is the second most abundant element in Earth's crust. Being one row below carbon on the periodic table, silicon is more electropositive and larger in size than carbon (Figure 1A). Silicon's larger atomic size results in 20% longer bonds for carbon–silicon bonds compared to carbon–carbon bonds. These longer bond lengths with silicon allow for increased surface area for Van der Waals intermolecular forces, resulting in higher lipophilicity for silicon–containing compounds compared to those containing carbon (Figure 1B). Additionally, silicon has no known element–specific toxicity. The lack of element–specific toxicity and enhanced lipophilicity of silicon combined with the synthetic accessibility of molecules containing silicon make it an interesting element to incorporate into medicinally–relevant molecules, with a selection of examples shown below (Figure 1C–F).^{1–4}

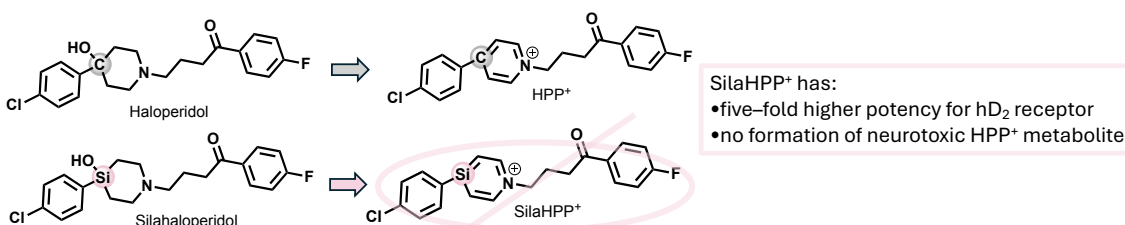
A) Unique properties of silicon



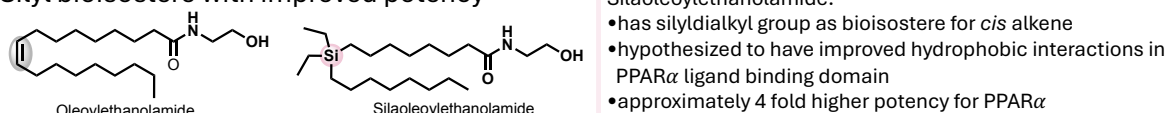
B) Enhanced lipophilicity of silicon



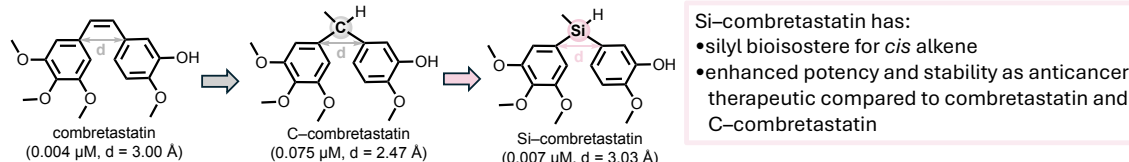
C) Altered metabolic fate



D) Silyl bioisostere with improved potency



E) Silyl bioisostere with enhanced potency and stability



F) Silyl AHLs with modular agonistic and antagonistic potencies

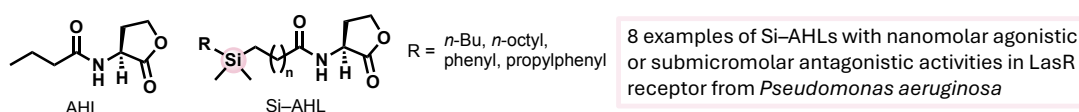


Figure 1. Unique properties of silicon (A–B) harnessed in medicinal chemistry to improve potency and selectivity, alter metabolic fate and improve ligand binding in hydrophobic pockets (C–E) and modulate bacterial cell–cell communication in *Pseudomonas aeruginosa* (F).

The unique properties of silicon have been utilized in the field of medicinal chemistry to enhance properties of small molecules (Figure 1C). Haloperidol is a dopamine receptor antagonist that is used as an antipsychotic agent. Previous work from Tacke and coworkers showed that the direct replacement of the carbon atom in Haloperidol with a silicon atom in

Silahloloperidol resulted in a five-fold increase in potency and selectivity for the hD₂ receptor and prevented the formation of the neurotoxic metabolite HPP⁺ (Figure 1C).¹

Oleoylethanolamide is a peroxisome proliferator-activated receptor (PPAR) agonist that is commonly used as an anti-inflammatory.^{5,6} In their work, Kajita and coworkers showed that replacing the *cis* alkene in oleoylethanolamide with a silyldiethyl group, a bioisostere for a *cis* alkene, to form silaoleoylethanolamide led to approximately a 4 fold improvement in potency for the PPAR α receptor (Figure 1D).² This was attributed to enhanced hydrophobic interactions in the PPAR α binding domain with the silyldialkyl group present. Combretastatin is an anticancer therapeutic that works by inhibiting tubulin polymerization.⁷ Nakamura and coworkers demonstrated that replacing the *cis* alkene in combretastatin with a silyl bioisostere enhanced potency and increased stability compared to native combretastatin and the carbon analog of combretastatin (Figure 1E).³ This example, along with the example described previously from Kajita and coworkers, emphasizes the benefit of using silyl bioisosteres in medicinal chemistry to enhance potency of small molecule therapeutics.

In a recent publication from the Franz lab, silyl groups were incorporated into the hydrophobic lipid tails of *N*-acyl homoserine lactones (AHLs) to enhance potency of AHLs as agonists and antagonists of the LasR receptor in *Pseudomonas aeruginosa*.⁴ This work demonstrated that incorporating various silyl lipid tail structures into AHLs resulted in modulation of potency and activity, where 8 silyl-AHL structures showed nanomolar agonist potencies or sub micromolar antagonist potencies (Figure 1F). These findings highlight the use of modular silyl groups in AHL lipid structures to modulate bacterial cell-cell communication.

1.2. Hydrosilylation as a Synthetic Strategy for Incorporating Silicon

Hydrosilylation is used to access functional organosilanes⁸ which are more broadly used in industry for the design of materials such as nanocoatings⁹ and electrochemical biosensors¹⁰. Hydrosilylation involves the addition of a silicon–hydrogen bond across a double bond in the presence of a catalyst (Figure 2).^{8,11,12} There are several products that can potentially be formed from hydrosilylation, depending on substrate and reaction conditions, all of which form from different mechanistic pathways in the catalytic cycle (Figure 3). The linear product (**F2a**) forms from the addition of the silicon atom of the hydrosilane to the terminal carbon of the alkene, and the branched product (**F2b**) forms when the silicon atom forms a bond with the internal carbon of the alkene (Figure 2). The dehydrogenative product (**F2c**) can form from the addition of the silicon atom to the terminal carbon of the alkene and the formation of an alkene beta to the silicon atom. Lastly, the reduced product (**F2d**) can also form from hydrogenation or reduction of the alkene.

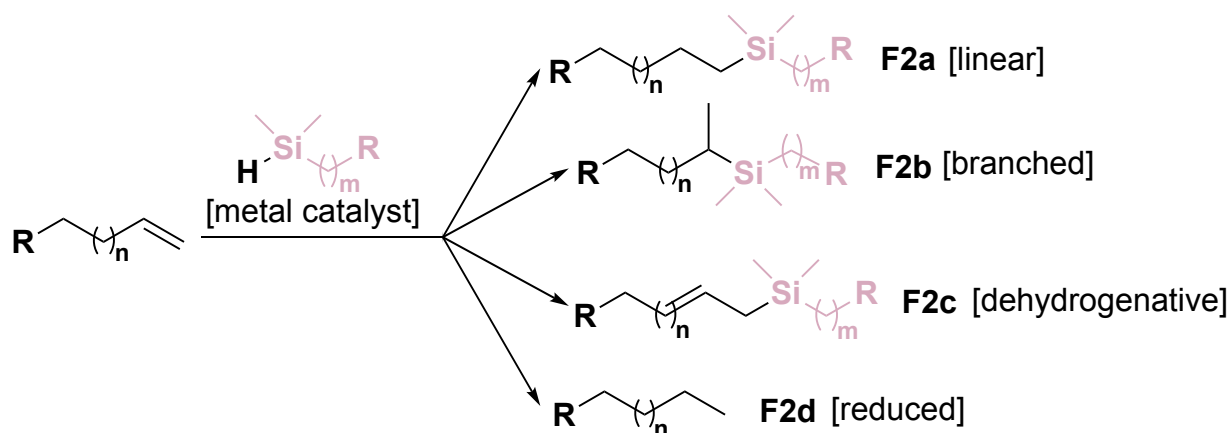


Figure 2. Linear, branched, dehydrogenative and reduced products that can be formed in the hydrosilylation reaction.

The Chalk–Harrod mechanism for hydrosilylation involves the oxidative addition of the hydrosilane into the metal center followed by migratory insertion of the alkene (Figure 3). This metal complex then undergoes reductive elimination to either give the linear or the branched

hydrosilylation products.^{11,12} However, during the reductive elimination step, the formation of a new double bond positioned beta to the silicon atom can also form, resulting in formation of the dehydrogenative silylation product^{11,13,14} and the release of hydrogen gas. The release of hydrogen gas can also lead to hydrogenation of the double bond,¹¹ forming the reduced product.

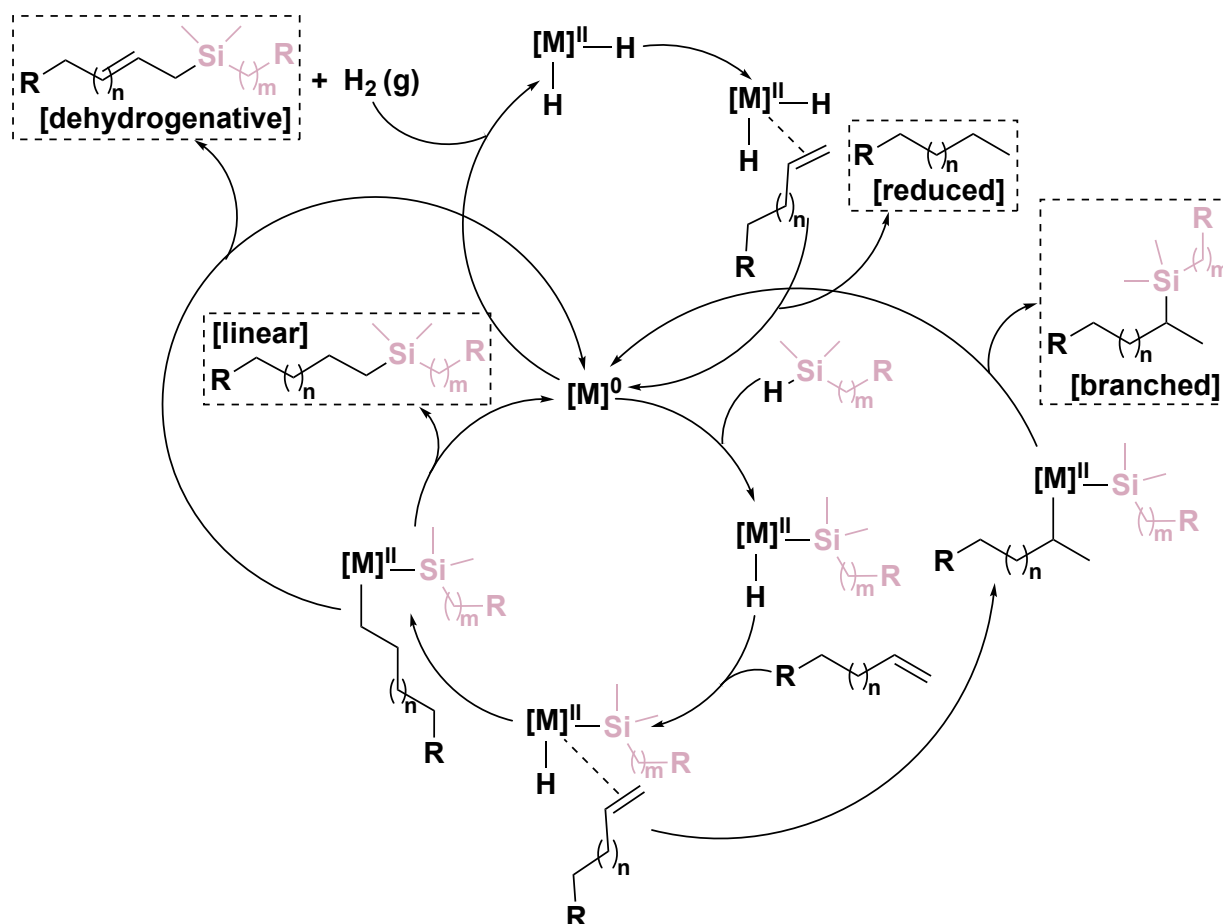


Figure 3. Plausible catalytic cycle to form linear, branched, dehydrogenative and reduced products from the hydrosilylation reaction

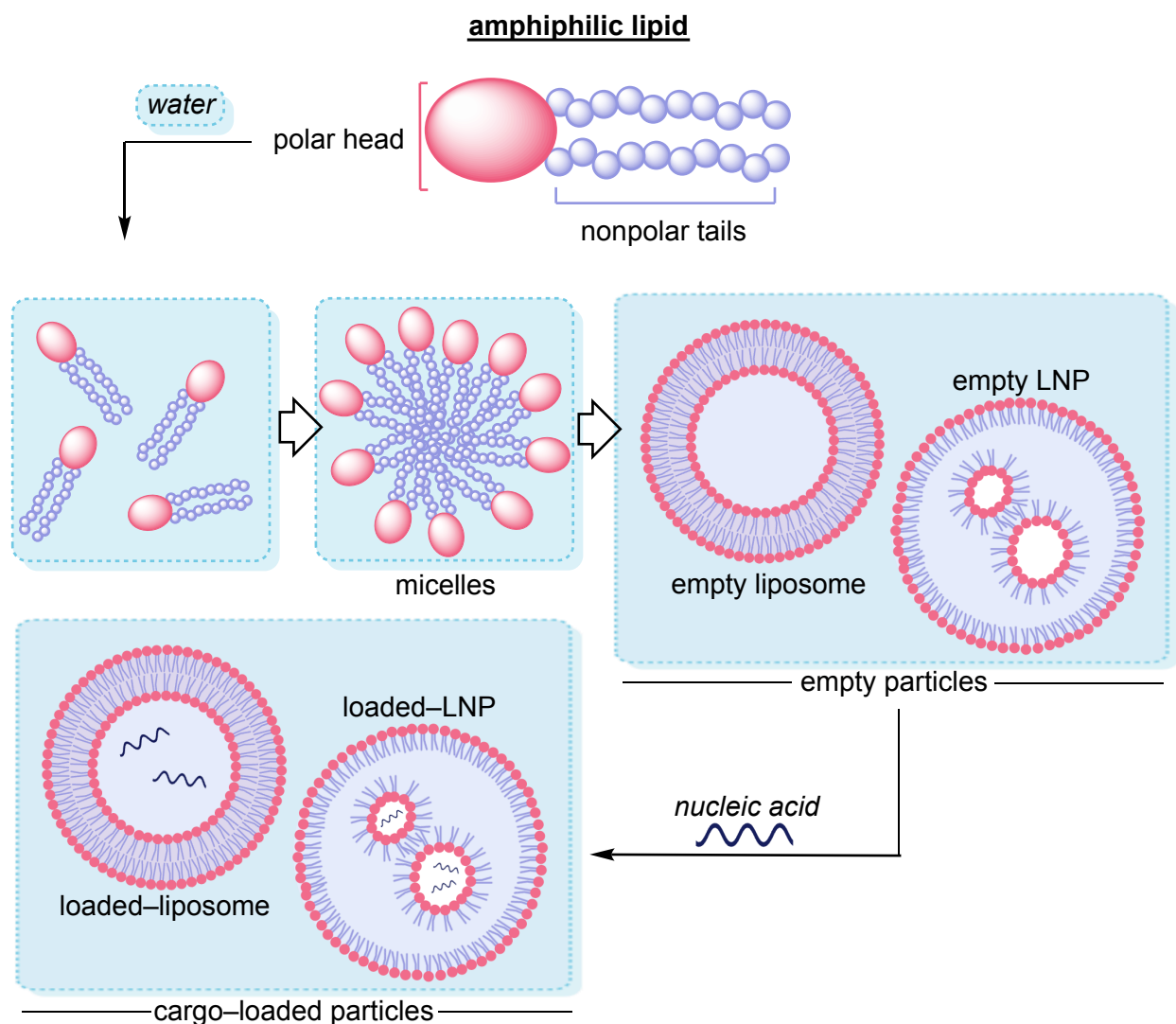
1.3. Use of Lipid Nanoparticles for Nucleic Acid Delivery

The development of disease treatments continues to be an important field of study in medicinal chemistry and chemical biology. To achieve effective disease treatment, therapeutics (i.e. small molecule drug or nucleic acid) must be safely and efficiently up taken into cells which heavily depends on several properties such as stability, biodistribution, metabolism and toxicity. One way

to overcome challenges related to therapeutic stability and biodistribution to achieve more efficient disease treatment is the development and utilization of liposomal-based and lipid nanoparticle (LNP)-based therapeutics that encapsulate the therapeutic cargo and facilitate in for more efficient delivery to cells.

LNPs have been used for drug delivery^{15,16} such as chemotherapy DOXIL^{17,18}, a liposomal-based therapy that encapsulates and delivers doxorubicin for breast cancer treatment. Along with small molecule drug delivery, LNPs have also widely been used for delivery of nucleic acids such as RNA^{19–22} and DNA.^{23–27} Targeted LNPs (tLNPs) have been utilized for delivery of RNA therapeutics, with a recent example demonstrating the use of anti-CD5-conjugated tLNPs containing FAP-CAR mRNA for the treatment of pancreatic cancer.²⁸ Along with cancer therapies, LNPs have also been used for the development of both animal and human vaccines, with more recent examples including DNA-encapsulated LNPs for the treatment of Influenza A in swine^{25,29} and BNT162b2 and mRNA-1273 for the treatment of Sars-CoV-2.^{30,31}

Amphiphilic lipids contain a polar head group and nonpolar lipid tails and can form different particle structures in aqueous solution (such as liposomes and LNPs) through the hydrophobic effect in which the polar head groups of the lipids preferentially interact with polar water molecules in solution and the nonpolar tails preferentially interact with other nonpolar lipid tails, driving particle formation (Figure 4).³² The RNA encapsulation process is driven by the electrostatic interactions between the negatively charged phosphodiester backbone of the nucleic acid with the polar head groups on the surface of the particle. The nucleic-acid loaded particles are then up-taken into cells through endocytosis, forming an endosome that undergoes a key conformational change to the Hexagonal II phase which allows for the release the cargo inside the cell.



Note: LNP = lipid nanoparticle | nucleic acid = RNA or DNA

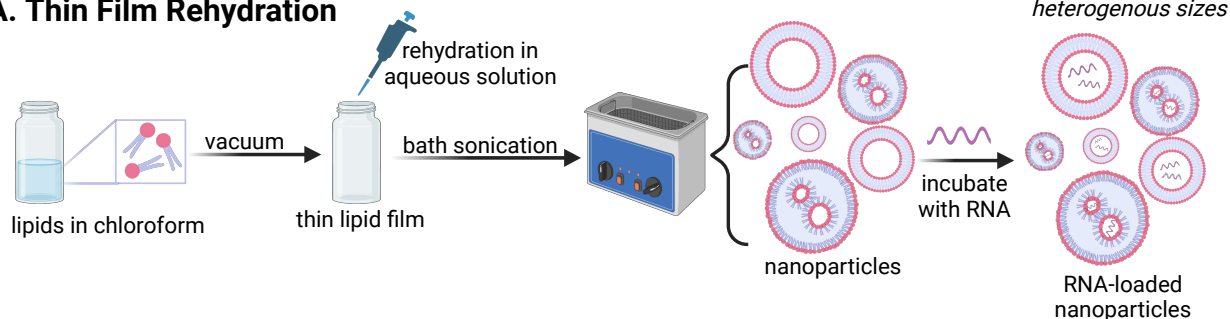
Figure 4. Formation of particles such as liposomes and LNPs for nucleic acid delivery. Images created using Chemdraw and BioRender software.

There are several methods for forming particles such as LNPs. A more classical method for forming LNPs includes thin-film rehydration and bath sonication to form the LNPs followed by subsequent incubation with nucleic acid (Figure 5A).^{33,34} This method offers a quick, cost-effective method to form LNPs in which negatively charged cargo is encapsulated after forming the LNPs but tends to result in less uniform particle sizes.³³ At the start of our project, this method was common in the literature. More recently, ethanol dilution and dialysis has become a method utilized in the literature for forming LNPs that involves incubating a lipid/ethanol solution with a

nucleic acid/citric acid buffer solution followed by dialysis; where mixing can be done either manually using a pipette or using a microfluidic mixing device (used for more careful control of particle size) (Figure 5B).^{33,34} This ethanol dilution/dialysis method for forming LNPs is higher in cost and takes more time, but allows the nucleic acid cargo to be encapsulated at the same time as LNPs are formed in solution, tending to produce smaller, more uniform-sized particles.³³

Different types of particles can form upon complexation with mRNA, some of which include liposomes and LNPs. Liposomes are characterized by their distinct membrane and a large aqueous core that encapsulates the negatively charged nucleic acid.³⁵ There are different morphologies of liposomes that can form that are distinguished by the number of membranes present. These morphologies include small unilamellar vesicles (SUVs; one membrane), large unilamellar vesicles (LUVs; one membrane) and multilamellar vesicles (MUVs; more than one membrane). LNPs are characterized by a lack of distinct membrane and a darker, denser core (sometimes referred to as “core-shell”) containing smaller protective compartments that encapsulate nucleic acid.³⁶ These different particle morphologies (liposomes vs LNPs) can be distinguished using cryo-TEM imaging, where liposomes tend to display particles with distinct membranes and large aqueous cores whereas LNPs tend to exhibit particles with darker, denser cores lacking a distinct membrane.^{35–38}

A. Thin Film Rehydration



B. Ethanol Dilution

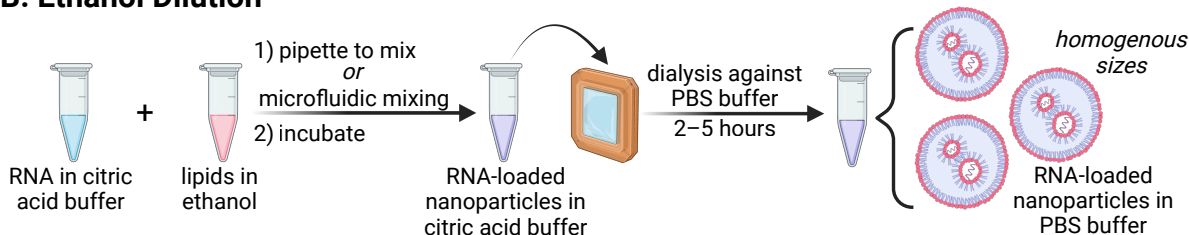


Figure 5. Methods for forming LNPs for nucleic acid delivery using (A) thin-film rehydration or (B) ethanol dilution.

Several classes of amphiphilic lipids are incorporated into LNPs, and each plays a unique yet critical role on efficient delivery of nucleic acid to the cell (Figure 6).^{19,21,39} Cationic lipids have a permanent positively-charged head group that facilitates in the efficient electrostatic coordination and encapsulation of negatively charged nucleic acid by the LNP⁴⁰ and the coordination of the LNP to the negatively-charged surface of the cell membrane.^{41,42,43,16} Ionizable lipids (also referred to as ionizable cationic lipids) contain a polar head whose charge depends on the pH of the surrounding solution. At physiological pH, the polar head group of ionizable lipids in the LNP preferentially remains neutral in charge.^{16,41,44} Once inside the endosome, the ionizable lipid head groups of the LNP become protonated due to the decrease in pH, promoting the release of nucleic acid inside the cell.^{16,45} PEGylated lipids (PEG = polyethylene glycol) are a third class of amphiphilic lipid that increase stability of LNPs by prolonging their circulation time and biodistribution in vivo.^{16,46,47-50} These lipids are characterized by their long PEG chains covalently linked to the surface of the polar head group of LNPs. Zwitterionic phospholipids are a fourth class of amphiphilic lipid that effect membrane fluidity and structural stability of LNPs.^{51,52} Cholesterol

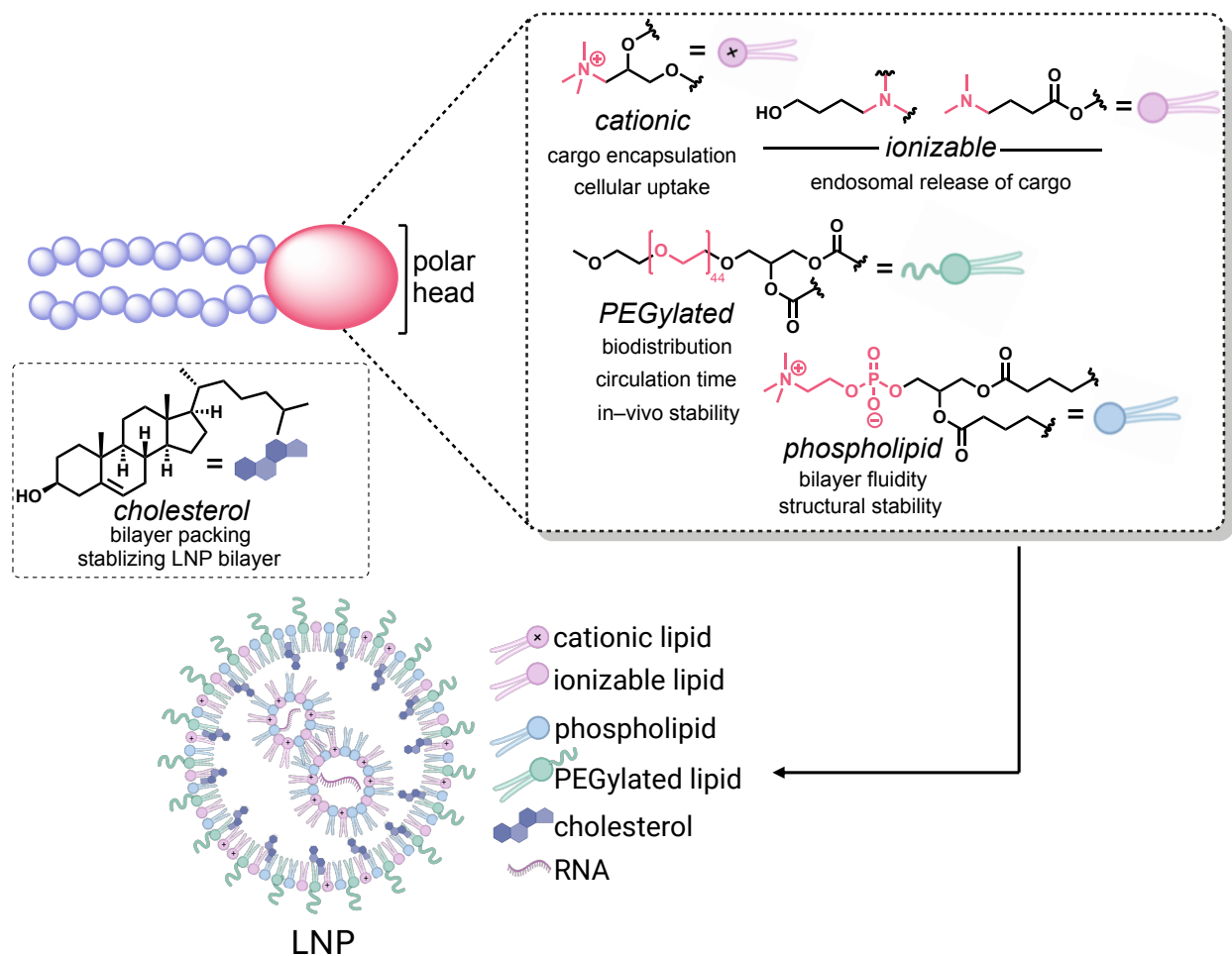
cellular uptake.^{16,53,54}

Figure 6. Classes of amphiphilic lipids and cholesterol used in LNPs for nucleic acid delivery.

At the time that I started this work, there were multiple examples in the field of LNPs related to polar headgroup modifications of amphiphilic lipids, with limited examples related to lipophilic tail modifications. This field has grown tremendously over the past few years since I began this work, with many more examples in the literature showing modifications to the polar head group, linker and lipophilic tails of amphiphilic lipids for use in LNPs. Recently, many synthetic modifications to the hydrophilic head group of amphiphilic lipids have been explored to optimize and improve LNP properties and RNA delivery (Figure 7). Examples of ionizable

amine-containing head groups previously evaluated for RNA delivery include guanidines for targeted cancer delivery,⁵⁵ polyamines for in utero delivery^{56,57} and delivery in mice,⁵⁸ and cyclic and branched amines for (i) enhanced vaccine immunogenicity⁵⁹ and in vivo mRNA delivery⁶⁰, (ii) targeted muscle delivery⁶¹ and (iii) co-delivery of siRNA and mRNA.⁶² Other head group designs include ionizable peptides for tissue-specific mRNA delivery,⁶³ epidermal growth factor receptor (EGFR)-conjugated head groups for targeted delivery to the placenta,⁶⁴ squaramides for enhanced interactions with nucleobases and sugar moieties of mRNA,⁶⁵ and piperidines for improved thermostability.⁶⁶ Also of interest are head groups containing tetrahydropyrimidines for enhanced muscle delivery and systemic delivery to the liver,⁶⁷ silicon-ethers for enhanced mRNA delivery and tissue clearance,⁶⁸ siloxane-containing amines for tissue-specific mRNA delivery,⁶⁹ mannose for enhanced potency of mRNA delivery^{70–72}, neurotransmitter amines for improved systemic blood-brain barrier delivery,⁷³ amino acids for improved cellular uptake and endosomal release of siRNA for cancer delivery⁷⁴ and dendritic arginine for enhanced membrane fusion for mRNA and pDNA delivery.⁷⁵ It is notable that although there have been a few more recent examples in the literature (as shown in Figure 7) of lipids containing silicon in the form of siloxanes or silyl ethers, these silyl modifications are in the polar head group and not in the lipid tail.

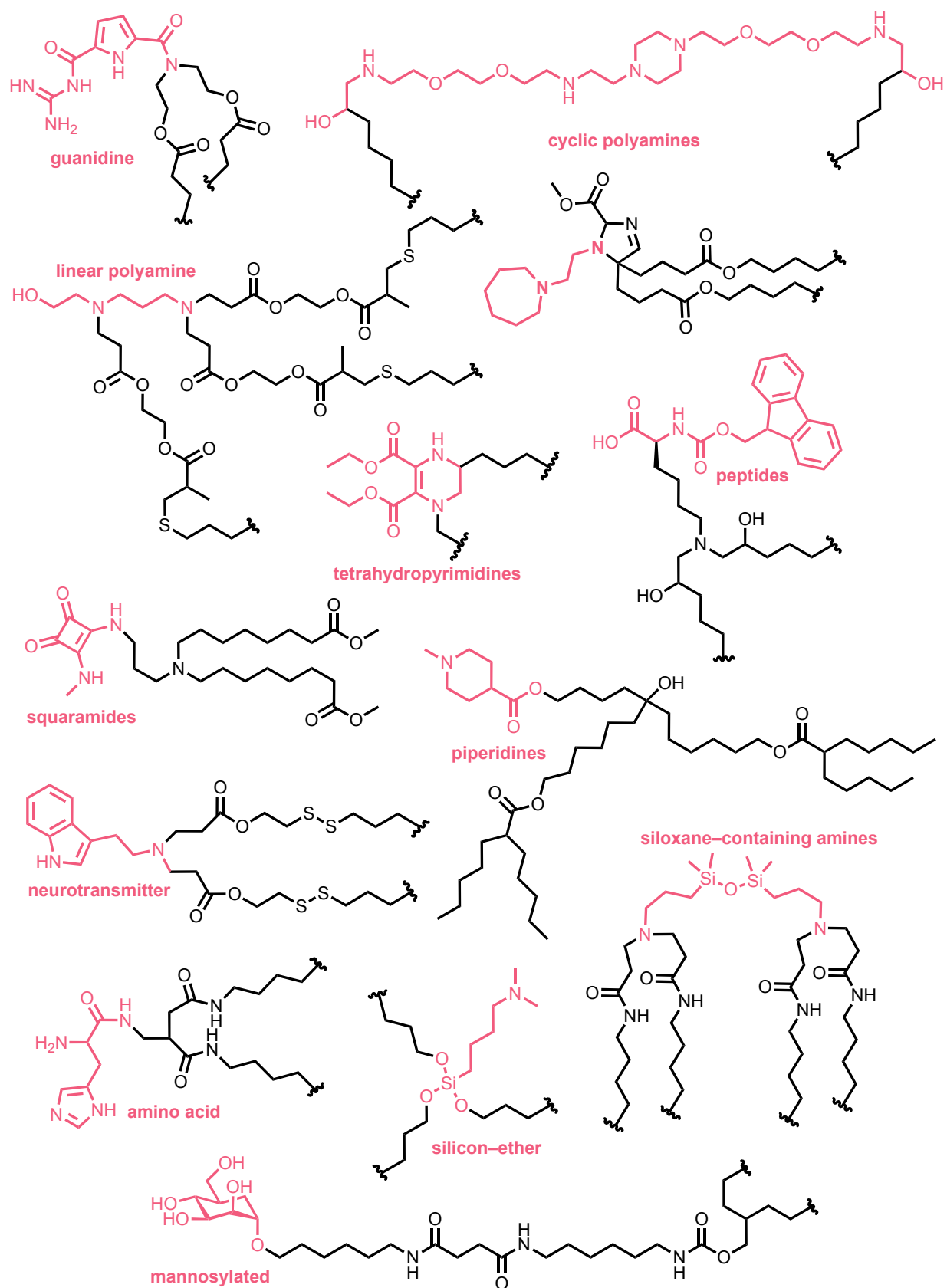


Figure 7. Examples of head group-modified lipids for improved mRNA delivery.

Along with varying the polar head group, the nonpolar lipid tails of these amphiphilic lipids have also been modified (Figure 8A). Some of these modifications include fully saturated tails with varying length^{76,77,78,79,80}, unsaturated tails^{55,76,81,82,83}, and branched tails^{84–89,90,91,92,93,94,95,96}. Other modifications of the hydrophobic tail include archaea–membrane lipids,⁸⁸ dendritic lipids with varying tail lengths,^{97,98} and highly–branched phenyl–containing poly(B–amino ester) lipid tails.⁹⁹ A selection of these tail–modified lipids is shown below (Figure 8B). Synthetic modification of the lipid tails has been shown to modulate LNP properties and nucleic acid delivery. Moreover, incorporating unsaturation and branching into the lipid tails of LNPs has been shown to enhance nucleic acid delivery^{44,83,93,100,101} by transiently disrupting the endosomal membrane and promoting endosomal release of cargo inside the cell. Varying tail length of saturated lipids and incorporating unsaturation and branching into tails has been shown to improve LNP stability^{93,101} and enhance tissue/organ^{77,88,91} and cell–type^{88,102} specificity of LNPs.

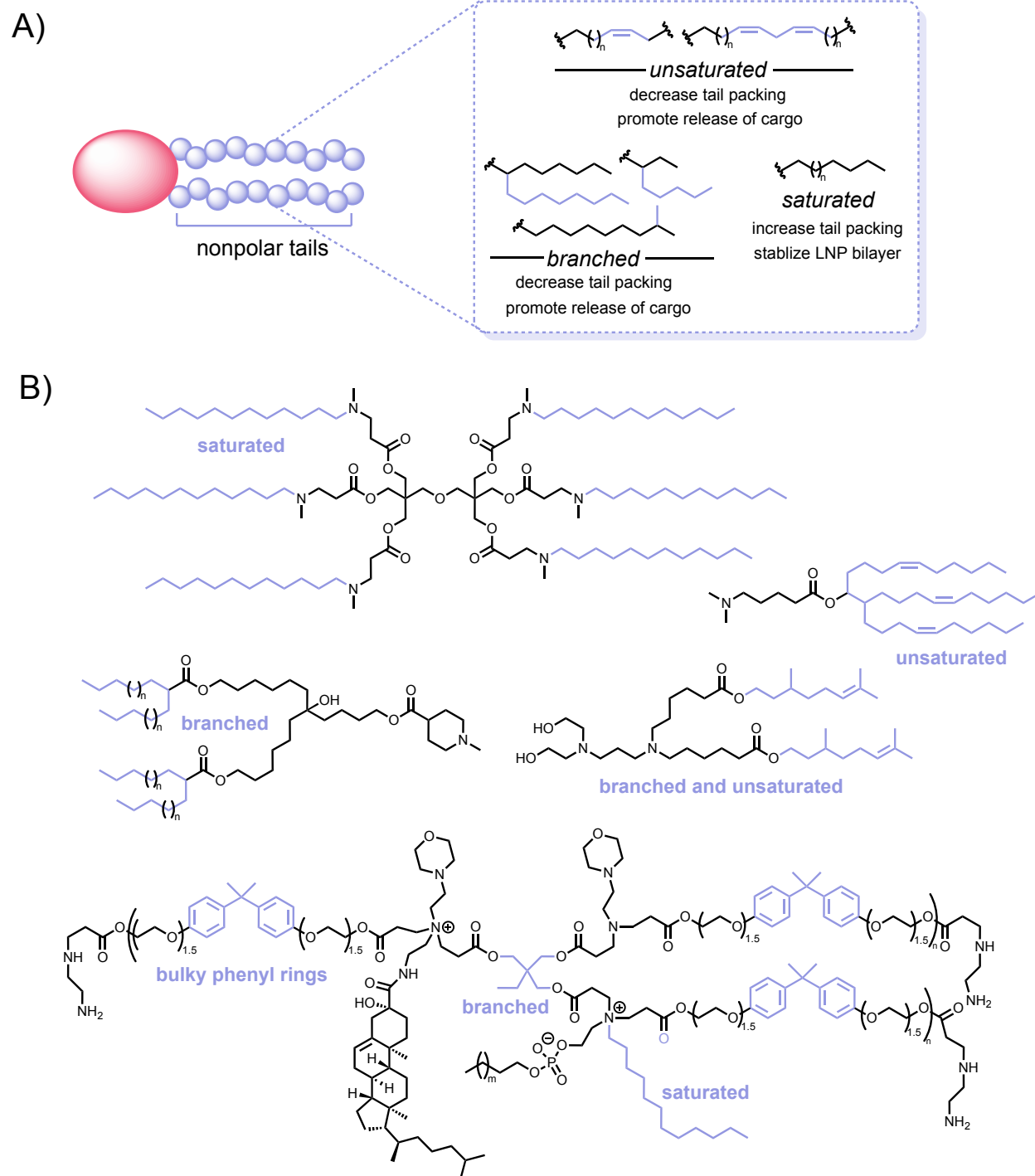
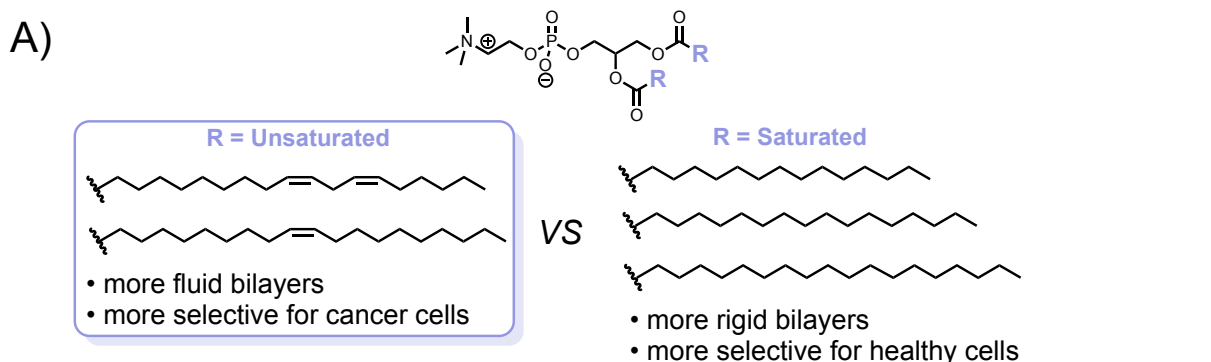
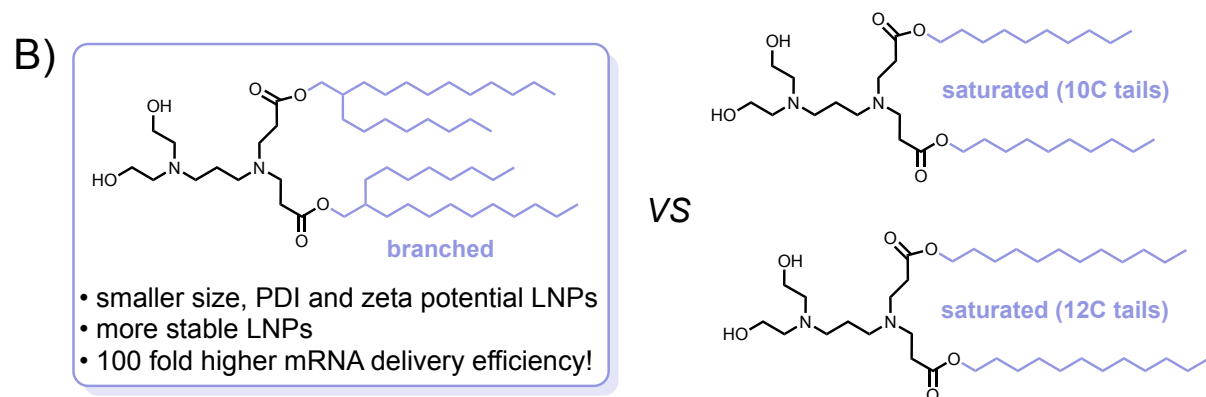


Figure 8. A) Lipid tails used in LNPs for nucleic acid delivery. B) Examples of tail-modified lipids for improved mRNA delivery.

An especially notable example of the influence of unsaturated lipid tails on LNP delivery is work from Bompard, et. al., in 2020.¹⁰² Their work demonstrated that LNPs with unsaturated lipid tails were more selective for targeting cancer cells due to their more fluid LNP membranes, while long-chain saturated tails were more selective for targeting healthy cells due to their more rigid membranes (determined by Laurdan assay using GP values) (Figure 9A). Another excellent example showcasing the influence of branched lipid tails on LNP delivery is work from Xu, et. al.⁹³ Their work showed that branched ionizable lipids formed LNPs with a smaller size, lower polydispersity index, lower zeta potential, greater stability and 100 fold higher mRNA delivery efficiency compared to LNPs with saturated ionizable lipids (Figure 9B).



Bompard, J.; et. al. Membrane Fluidity as a New Means to Selectively Target Cancer Cells with Fusogenic Lipid Carriers. *Langmuir* **2020**, *36* (19), 5134–5144.



Xu, H.; et. al. Tetra-Branched Ionizable Lipids Enhance the Stability and Transfection Efficiency of Lipid Nanoparticles for mRNA Delivery. *Chinese Chemical Letters* **2025**, *36* (11), 110833.

Figure 9. Unsaturated and branched lipid tails form LNPs with enhanced cell-type selectivity, stability, and/or mRNA delivery efficiency compared to saturated lipid tails.

1.4. Properties of LNPs that Affect Nucleic Acid Delivery

There are several properties of LNPs that influence the efficiency and effectiveness of nucleic acid delivery (Figure 10). Some of these properties include size and zeta potential, which are analyzed using dynamic light scattering (DLS).^{103,104} LNP size affects encapsulation efficiency, cellular uptake and biodistribution.^{105,106,107} Zeta potential (more generally thought of as surface charge) of LNPs is correlated with colloidal stability,¹⁰⁸ which is the ability of LNPs to resist aggregation in solution. Low zeta potential is associated with poor colloidal stability of LNPs and consequently the release of cargo during storage.¹⁰⁹ Zeta potential also influences cellular uptake efficiency.¹⁰⁶ Therefore, it is essential to control size and zeta potential in order to optimize subsequent LNP properties such as encapsulation efficiency, biodistribution, colloidal stability and storage stability.

Another property that is often evaluated for LNPs is encapsulation efficiency of cargo (Figure 10). Encapsulation efficiency is measured using a fluorescence assay in which a dye (RiboGreen for RNA^{85,87} or PicoGreen for DNA¹¹⁰) binds to any free/un-encapsulated nucleic acid and becomes fluorescent upon binding. LNP stability can also be assessed using a modified fluorescence assay in which LNPs are formed and their encapsulation efficiency is measured at increasing time points after LNP formation. A drop in encapsulation efficiency after longer time periods suggests lower LNP stability. Encapsulation rate can also be evaluated using a modified fluorescence assay in which the fluorescence is monitored over time. Fluorescence of the RNA- or DNA-bound dye decreases over time as the cargo is encapsulated by the LNP. Together, the size, zeta potential, RNA encapsulation efficiency and rate, stability and membrane fluidity of LNPs influences their overall mRNA transfection efficiency, which can be measured in vitro by monitoring %GFP expression in cells (Figure 10).

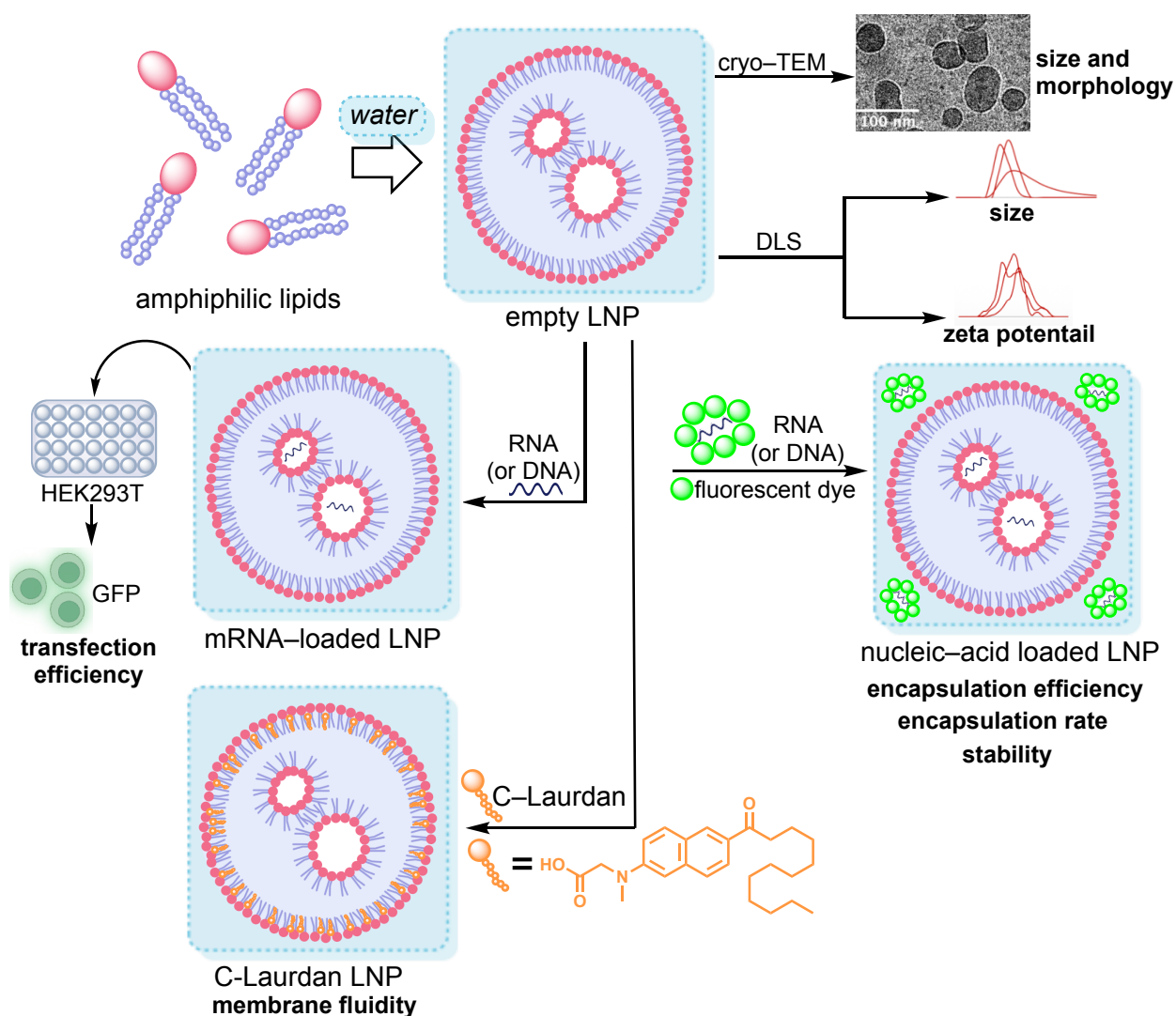


Figure 10. Properties of LNPs influential in nucleic acid delivery, and methods for measurements. Images created using Chemdraw and BioRender software.

Of particular note when assessing properties of engineered lipids, membrane fluidity of LNPs has previously been shown to influence their cellular binding¹⁰² and uptake¹¹¹ efficiency, making membrane fluidity a crucial property to evaluate (Figure 11). One method used to assess membrane fluidity of LNPs is the C-Laurdan fluorescence assay.¹¹² C-Laurdan is an amphiphilic fluorescent probe that embeds itself within the membrane of membranes. The emission wavelength of C-Laurdan is dependent on how tightly or loosely packed the membrane is. Tightly packed membranes result in a blue shifted (i.e. shorter wavelength) emission of C-Laurdan due to reduced

permeation of water into the membrane and consequently reduced stabilization of the fluorescent probe (Figure 11). Loosely packed membranes correspond to a red shift (i.e. longer wavelength) in emission for C-Laurdan caused by more water permeating into the membrane and thus enhanced stabilization of the probe. Generalized polarization (GP) is a quantitative value representing membrane packing and ranges from -1.0 to 1.0. Rigid membranes have higher (i.e. more positive) GP values and fluid membranes have lower (i.e. more negative) GP values.

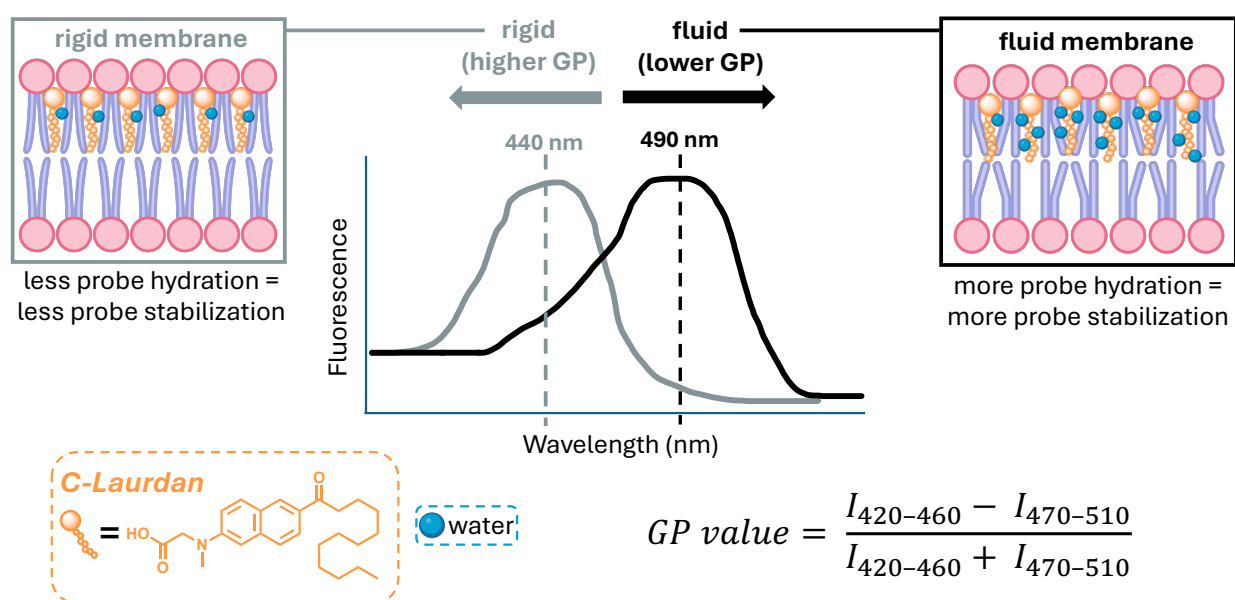


Figure 11. Using C-laurdan fluorescent probe for measuring membrane fluidity of LNP membranes upon partitioning of probe into membrane of LNPs. Images created using Chemdraw and BioRender software.

The work presented in my publication and dissertation offers unique, readily-accessible branched silyl lipid structures that can be utilized in LNP delivery. Due to the enhanced lipophilicity of silicon atom and the various degrees of branching provided by the tetraalkyl silyl group, we hypothesized that we could successfully modulate lipid tail packing and disruption within the LNP membrane (i.e. membrane fluidity). By tuning membrane fluidity of LNPs, we hypothesized that we could control subsequent LNP properties and mRNA delivery.

Chapter 2: Modular Cationic Silyl lipids Control Lipid Nanoparticle Properties and mRNA Delivery

The following work is adapted with permission from *ACS Appl. Bio Mater.* 2025, 8, 8, 7216–7227. Copyright © 2025 American Chemical Society. All HEK293T cell transfection data presented in this chapter was acquired in collaboration with Dr. Henriette O’Geen and Dr. David Segal in the Genome Center at UC Davis.

2.1. Abstract

Chapter 2 presents the design and synthesis of cationic silyl lipids containing structurally diverse tail structures for use in LNPs for mRNA delivery. Eighteen cationic silyl lipids were synthesized with the varying silyldimethyl positions, tail lengths, and silyldimethyl substituents (alkyl, cyclohexyl, or phenyl) in the lipid tail. The effect of these tail modifications on particle size, zeta potential, encapsulation efficiency and rate, stability, membrane fluidity and mRNA transfection efficiency was evaluated. The results support that incorporation of various silyl lipid tail structures into amphiphilic lipids controls particle properties, where shortening the silyl lipid tail and incorporation of phenyl rings generally resulted in higher rRNA encapsulation efficiency and mRNA transfection efficiency compared to other silyl lipids evaluated.

2.2. Cationic Silyl lipid Analogs of DOTAP

A classic cationic lipid used for mRNA delivery is *N,N,N*-trimethyl-2,3-bis(oleoyloxy)propan-1-aminium (DOTAP).¹¹³ DOTAP is characterized by its quaternary cationic amine head group, ester linkers, and *cis* alkene-containing hydrocarbon tails (Figure 12A). The *cis* alkene in DOTAP has been shown to increase mRNA delivery by disrupting lipid tail packing, destabilizing the endosomal membrane and promoting endosomal release of mRNA. However,

this unsaturation leaves DOTAP prone to oxidative degradation over time, potentially resulting in poor storage stability. Taking inspiration from DOTAP, we synthesized a direct silyl lipid analog in which the *cis* alkene is directly replaced by a silyldimethyl group (Figure 12B). The silyldimethyl group serves as a bioisostere for a *cis* alkene, with both DOTAP (Figure 12A) and silyl-DOTAP (Figure 12B) maintaining similar bond distances. We hypothesized that replacement of the *cis* alkene in DOTAP with the silyldimethyl moiety would maintain this disruption in tail packing *via* the branching methyl groups on silicon while improving storage stability by preventing oxidative degradation. Due to the enhanced lipophilicity of silicon atom and the various degrees of branching provided by the methyl groups and substituent on silicon (i.e. alkyl, cyclohexyl or phenyl), we hypothesized that we could modulate lipid tail packing and disruption within the LNP membrane (i.e. membrane fluidity). By tuning membrane fluidity of LNPs, we hypothesized that we could control subsequent LNP properties and mRNA delivery.

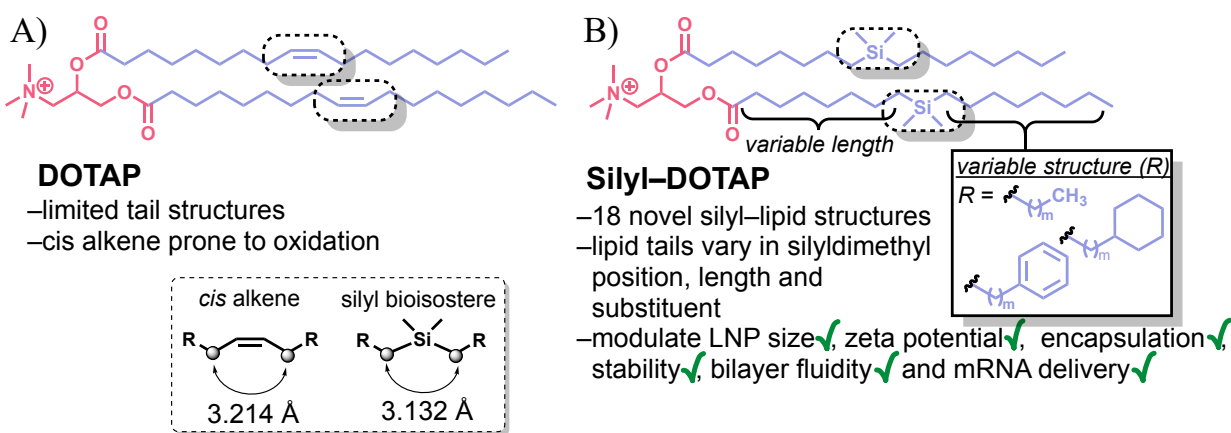


Figure 12. Cationic lipid DOTAP (A) and silyl lipid analogs (B) used for LNP formulations

At the time of this project, less work in the field existed that explored synthetic modifications of the polar head group and lipid tails of amphiphilic lipids used in LNP delivery. The field has grown immensely over the past few years. A significant amount of work has shown that synthetic

modifications of the polar head group of amphiphilic lipids influences LNP properties and nucleic acid delivery, however fewer examples of synthetic lipid tail modifications exist. Due to the more limited hydrophobic tail structures that are readily accessible using carbon-based synthetic organic chemistry, I sought to assess how varying silyldimethyl position in the lipid tail, the length of the silyl lipid tail, and the substituent in the silyl lipid tail would affect LNP properties and mRNA delivery (Figure 12). We hypothesized that these variations in silyl lipid tail structure, along with silicon's enhanced lipophilicity and the incorporation of branching methyl groups from the silyldimethyl, would effectively modulate lipid tail packing in the LNP membrane; this modulation of lipid tail packing would enable control and optimization of LNP properties and nucleic acid delivery. Hydrosilylation allows access to a diverse collection of lipid tail structures with varying degrees of branching that cannot be readily made with carbon. By incorporating the silyldimethyl moiety into lipid tails, we hypothesized that we could readily access a wide variety of lipid tail structures for incorporation into LNPs.

2.3. Synthesis of Cationic Silyl-DOTAP Lipid Analogs

To access cationic silyl lipids, diacylated alkene substrates **3a–3d** ($n = 1, 2, 3$ or 4) were first synthesized by coupling commercially available pentenoic (**1a**), hexenoic (**1b**), heptenoic (**1c**) or octanoic (**1d**) acid with deprotonated 3-(dimethylamino)propane-1,2-diol **2** using CDI and NaH to access **3a–3d** with isolated yields of 41–89% after column chromatography (Figure 13). A catalyst screen was then performed for hydrosilylation of alkene **3d** with silane **4** to access un-methylated silyl lipid **5d** (Table 1).

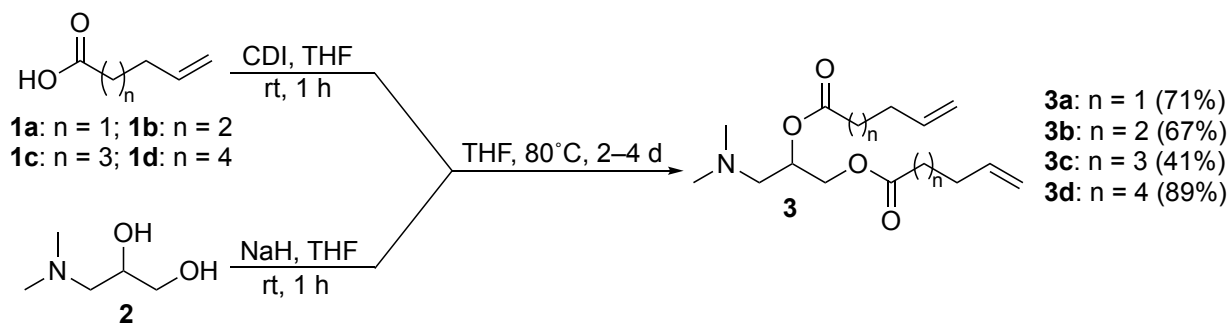


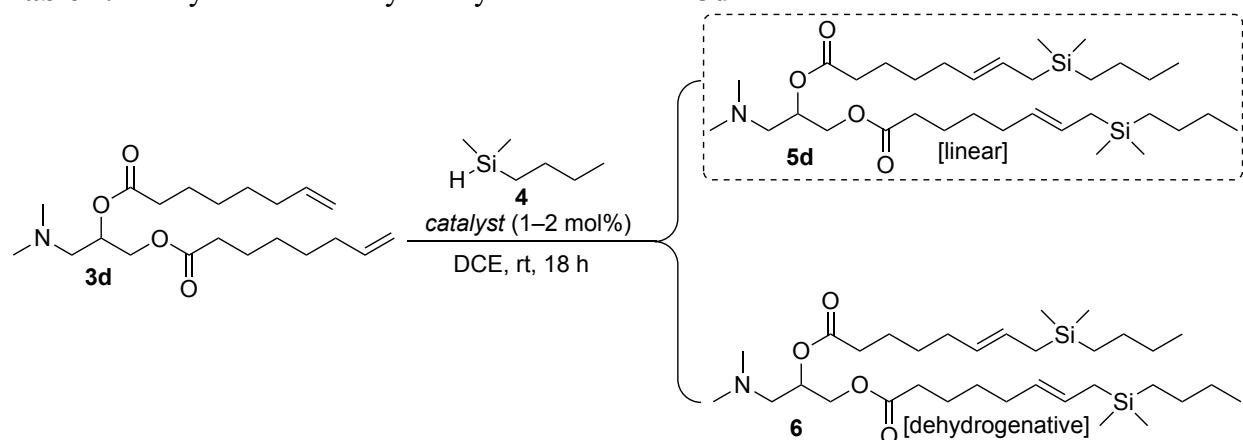
Figure 13. Synthesis of diacylated alkene precursors for hydrosilylation **3a–3d**

Upon screening with nine catalysts, $\text{Pt}_2(\text{dvtms})_3$, $\text{Pt}(\text{cod})\text{Cl}_2$, Pt octanol/octanal and $\text{Ir}_2(\text{cod})_2\text{Cl}_2$ gave a mixture of desired linear product **5d** and dehydrogenative silylation side product **6** determined by ^1H NMR using 3,4,5-trimethoxybenzoic acid as an internal standard (see Figure 14 for the crude ^1H NMR spectrum of **5d** mixed with **6** and unreacted starting material). All other catalysts (entries 5–9) yielded no product, showing only recovered starting material by NMR. Of the four catalysts in entries 1–4, $\text{Pt}_2(\text{dvtms})_3$ and $\text{Pt}(\text{cod})\text{Cl}_2$ gave the highest NMR yields of desired linear hydrosilylation product **5d** with yields of 36% and 21%. Hydrosilylation of **3a–3c** was thus performed using $\text{Pt}_2(\text{dvtms})_3$ due to the higher NMR yield observed and comparable product selectivity (3:1 **5d** to **6**) to $\text{Pt}(\text{cod})\text{Cl}_2$ (3.5:1 **5d** to **6**) by NMR (Table 2).

The desired linear hydrosilylation product **5a** (79%) and **5d** (93%) was isolated by column chromatography and observed by ^1H NMR upon hydrosilylation of **3a** and **3d** with silane **4a** using $\text{Pt}_2(\text{dvtms})_3$ (Table 2, entries 1 and 4). However, when attempting hydrosilylation of **3b** with silane **4a** a complex mixture of hydrosilylation products and unreacted alkene starting material was observed by ^1H NMR that were inseparable by column chromatography (entry 2). Unlike **3a–b** and **3d**, hydrosilylation of alkene **3c** with silane **4b** gave only monosilylation product **7** by ^1H NMR with an isolated yield of 41% after column chromatography (see Figure 15 for the ^1H NMR spectrum of isolated product **7**).

Due to the inability to access the desired linear hydrosilylation product using $\text{Pt}_2(\text{dvtms})_3$ with diacylated alkenes **3b–3c**, a different synthetic route was used in which hydrosilylation of hexenoic or heptenoic acid was first performed with the corresponding silane **4** to access silyl carboxylic acid precursor **8** (Table 3). Hydrosilylation of hexenoic acid **1b** and heptenoic acid **1c** with corresponding silane **4a** or **4b** was first attempted using $\text{Pt}_2(\text{dvtms})_3$, however this gave no product formation and showed only unreacted starting material by ^1H NMR. When switching to $\text{Pt}(\text{cod})\text{Cl}_2$ as the hydrosilylation catalyst, the desired linear silyl–carboxylic acid products **8a** (35%) and **8b** (80%) were observed by ^1H NMR after column chromatography. However, a loss in yield with this synthetic route was observed (particularly for **8a**) due to the formation of branched side product **9** and the inability to separate the remaining linear product from the branched using column chromatography (see Figure 16 for ^1H NMR spectrum of **8a** mixed with **9**). Another potential cause of the reduced yield of linear product **8a** observed with silane **4a** may be due to the lower catalyst loading (0.2 mol%) compared to silane **4b** (0.4 mol% catalyst loading).

Table 1. Catalyst screen for hydrosilylation of alkene **3d** with silane **4**



Entry	Catalyst	Mole %	5d (%) ^a	6 (%) ^a	5d : 6 ^b
1	Pt ₂ (dvtms) ₃	1	36	12	3 : 1
2	Pt(cod)Cl ₂	2	21	6	3.5 : 1
3	Pt octanol/octanal	1	9	6	1.5 : 1
4	Ir ₂ (cod) ₂ Cl ₂	2	14	32	1 : 2.3
5	Pt(dvtms)NHC	2	No product observed, recovered starting material		
6	Pt(acac) ₂	2			
7	PtO ₂	2			
8	Rh(PPh ₃) ₃ Cl	2			
9	BCF	2			

^aYields determined using ¹H NMR spectroscopy with 3,4,5-trimethoxybenzoic acid as an internal standard. ^bRatio of linear to dehydrogenative products determined using ¹H NMR spectroscopy with 3,4,5-trimethoxybenzoic acid as an internal standard. See Figure 14 for NMR of entry 1.

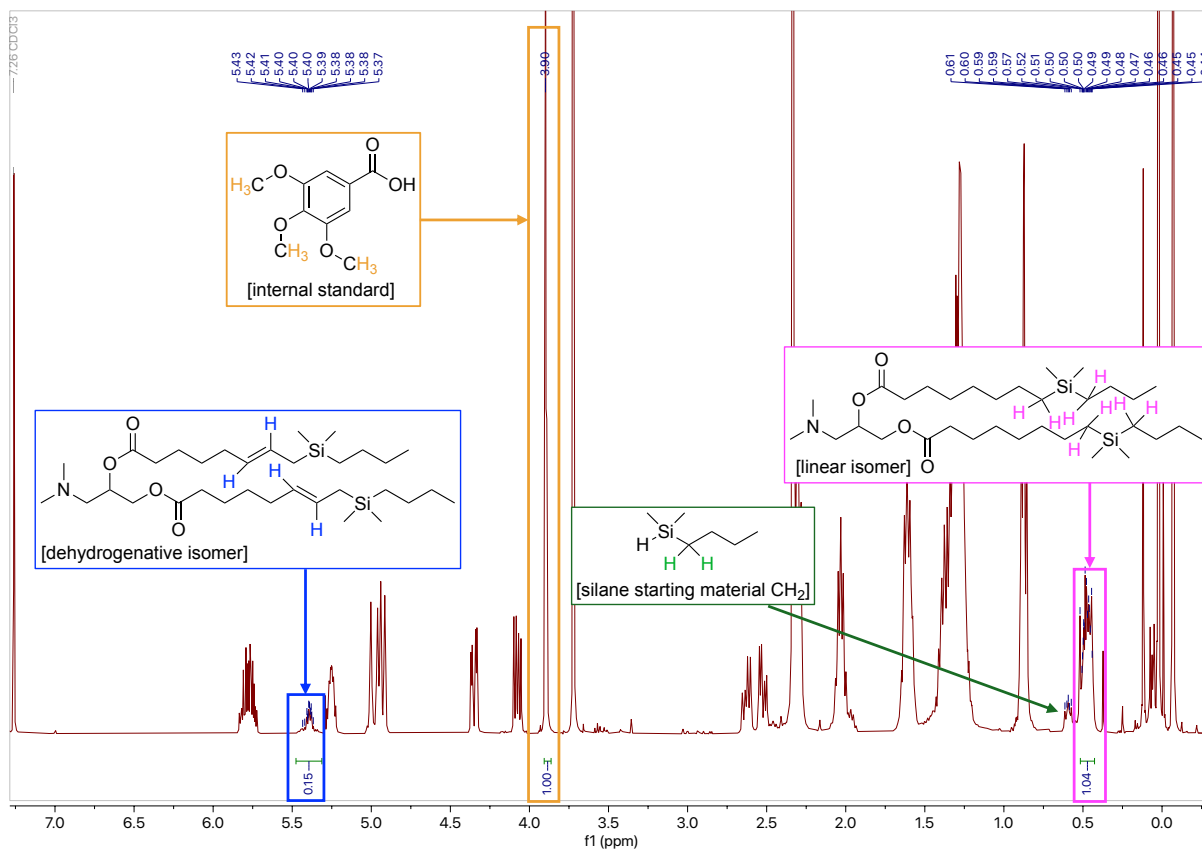
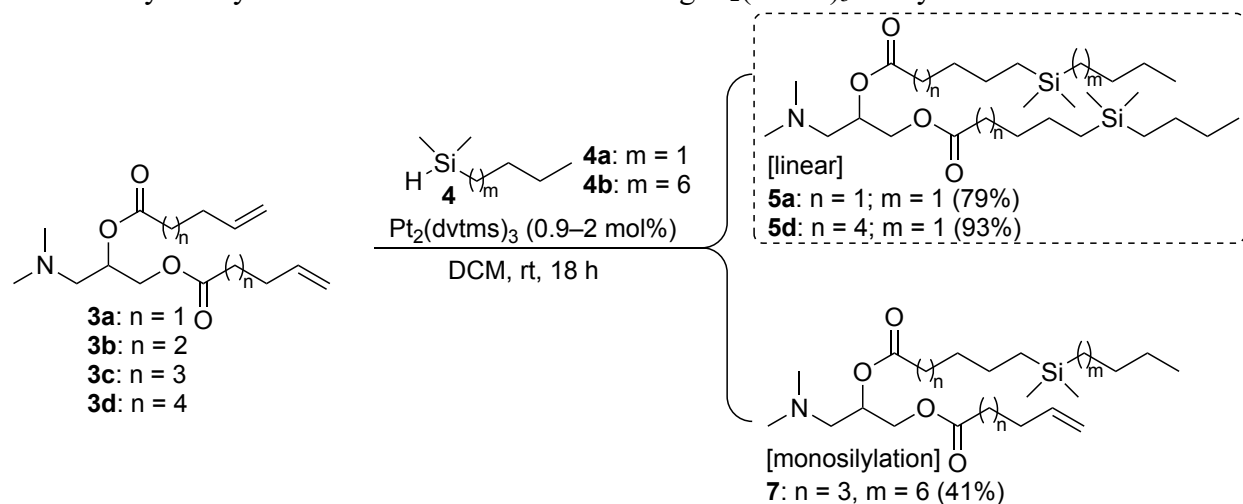
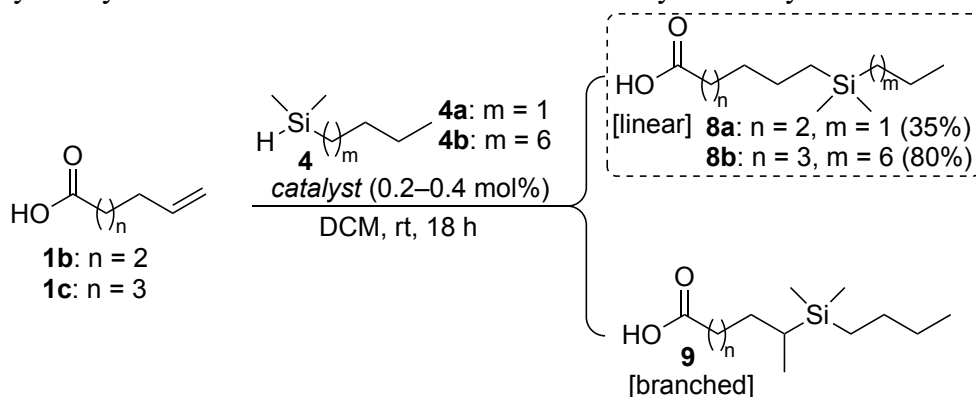


Figure 14. The crude ^1H NMR spectrum of **5d** containing dehydrogenative side product **6** and unreacted starting material (see entry 1, Table 1).

Table 2. Hydrosilylation of **3a–3d** with silane **4** using $\text{Pt}_2(\text{dvtms})_3$ catalyst

Entry	Alkene	Silane	Catalyst (mol %)	Product (by ^1H NMR)	Isolated yield (column chromatography)
1	3a	4a	0.9	5a	79%
2	3b	4a	1.5	complex mixture	inseparable mixture
3	3c	4b	1	7	41%
4	3d	4a	0.9	5d	93%

Table 3. Hydrosilylation of alkenoic acids to access linear silyl-carboxylic acids **8a** and **8b**

Entry	Alkene	Silane	Catalyst	Mole %	Product (by ^1H NMR)	Isolated yield (column chromatography)
1	1b	4a	$\text{Pt}(\text{cod})\text{Cl}_2$	0.2	8a ^a	35%
2	1c	4b	$\text{Pt}(\text{cod})\text{Cl}_2$	0.4	8b	80%
3	1b	4a	$\text{Pt}_2(\text{dvtms})_3$		no reaction, recovered starting material	
4	1c	4b	$\text{Pt}_2(\text{dvtms})_3$			

^aSee Figure 16 for ^1H NMR spectrum of **8a** containing **9**.

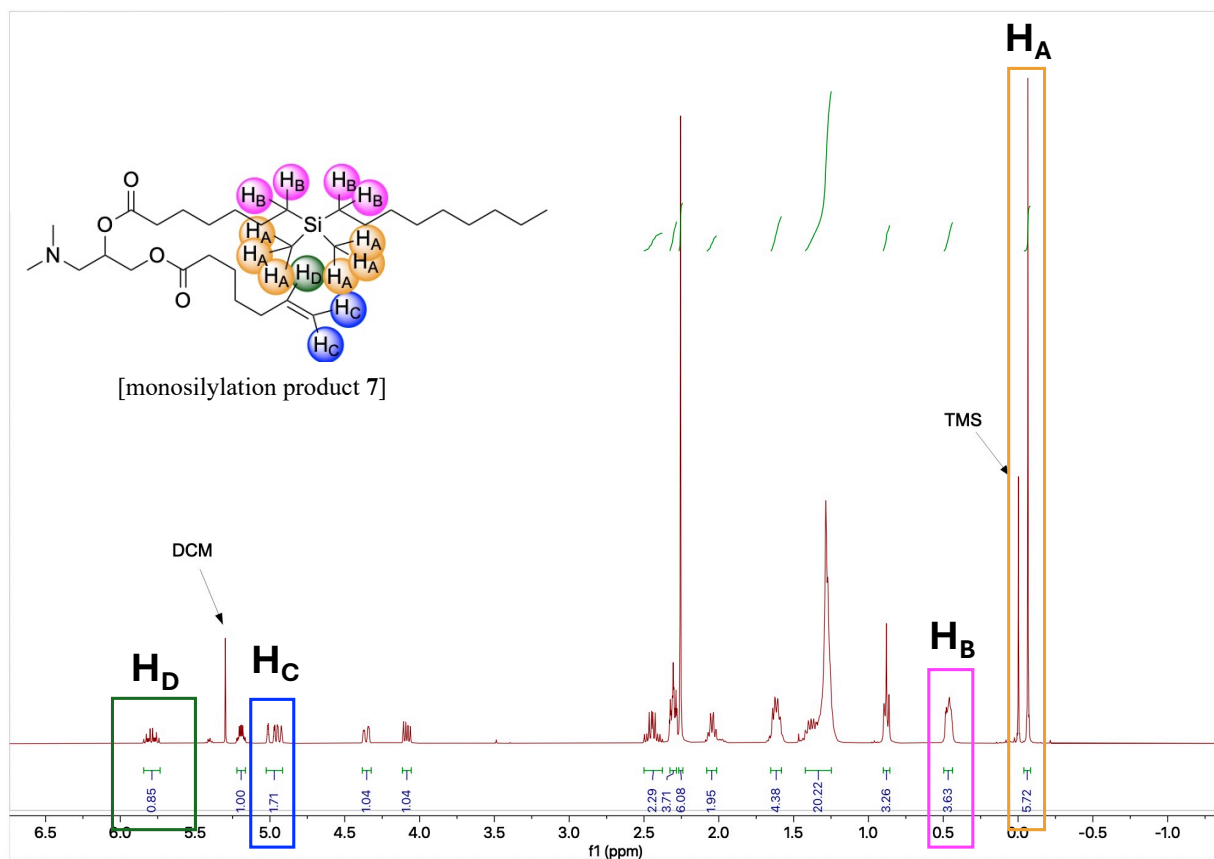


Figure 15. The 1H NMR spectrum of monosilylation product **7** isolated by column chromatography.

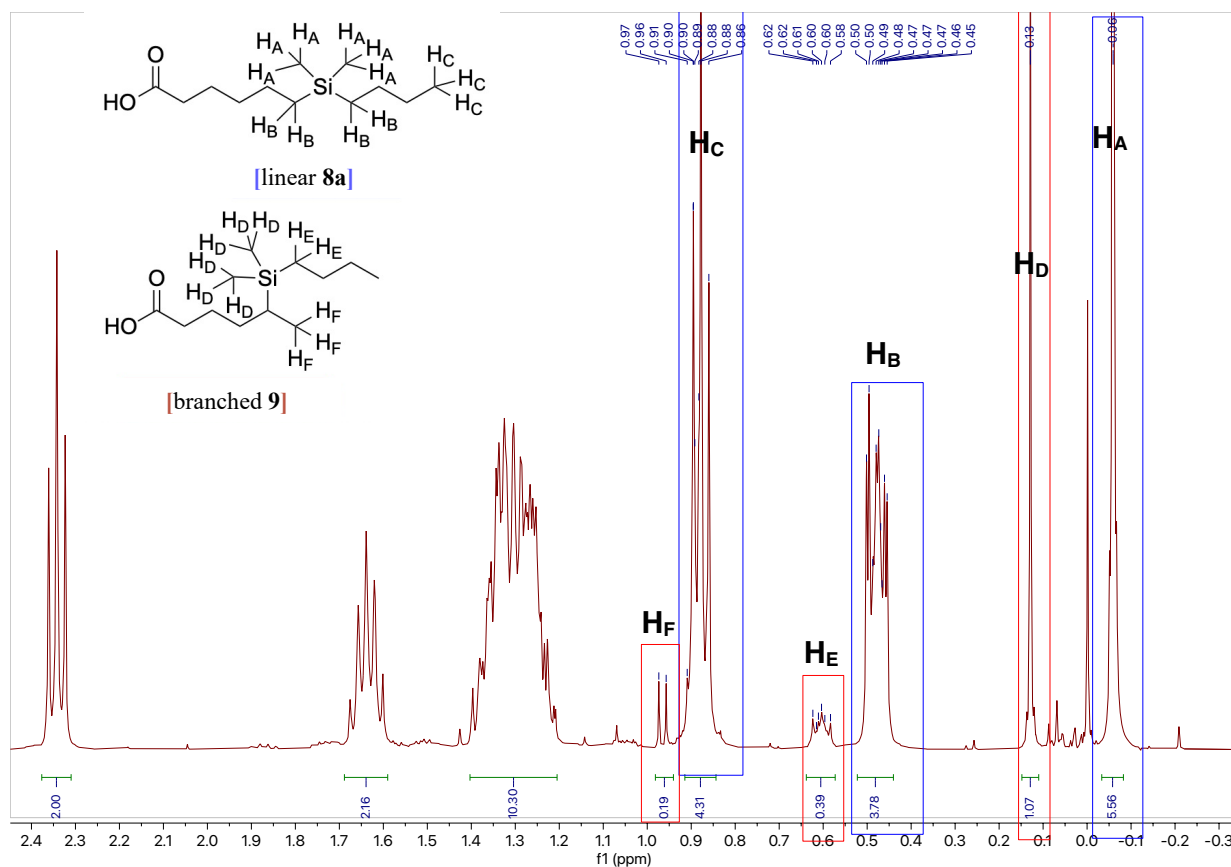


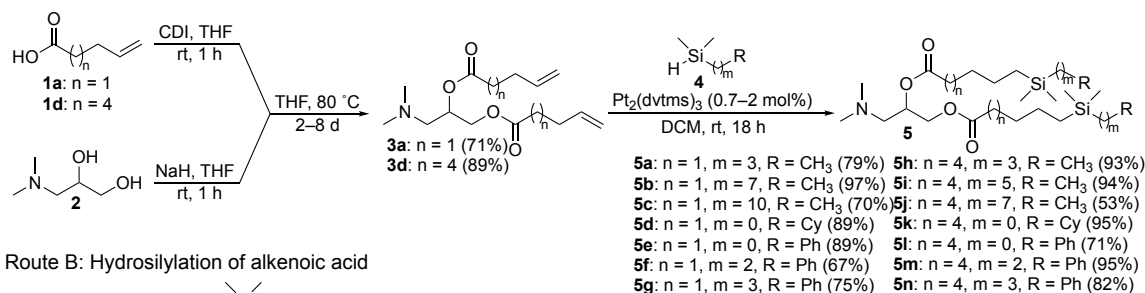
Figure 16. The ¹H NMR spectrum of **8a** containing **9** after column chromatography

The results described above emphasize the substrate and catalyst specificity of the hydrosilylation reaction, with the optimal catalyst and synthetic route for each cationic silyl lipid depending on the length of the alkene precursor. Based on these findings, two synthetic routes were used to access the desired cationic silyl lipids (Figure 17). Route A was used to access cationic silyl lipids **10a–10g** and **10l–10r** and Route B was used to access silyl lipids **10h–10k**. In Route A, diacylated alkene **3a** and **3d** underwent hydrosilylation with corresponding silane **4** using Pt₂(dvtms)₃ to access unmethylated precursors **5a–5n** with isolated yields of 67–97% after column chromatography. In Route B, hexenoic acid **1b** and heptenoic acid **1c** underwent hydrosilylation with corresponding silane **4** using Pt(cod)Cl₂ to access silyl carboxylic acid **8a–8d** with isolated yields of 18–80% after column chromatography. Loss in yield of **8a–8d** was due

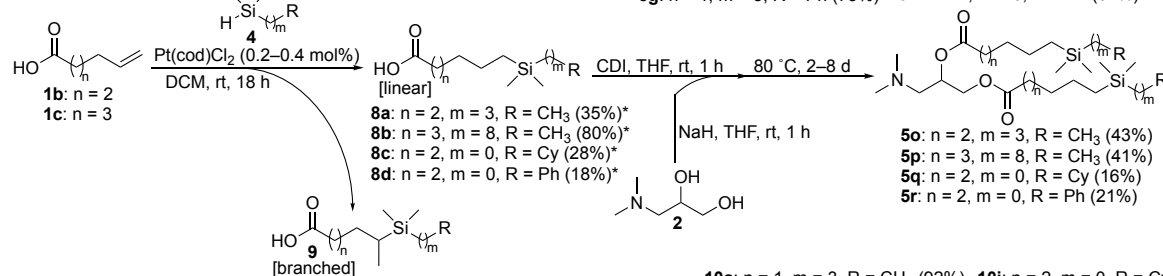
to the inability to separate all of the linear product **8** from the branched side product **9** by column chromatography. Silyl carboxylic acid **8a–8d** was then coupled to 3-(dimethylamino)propane-1,2-diol **2** using CDI and NaH to access unmethylated precursors **5a–5r** with isolated yields of 16–43% after column chromatography. The methylation of **5a–5r** with methyl iodide was performed to access **10a–10r** with isolated yields of 20–95% (see Figure 18 for synthesis of silanes **4b–4f** to access **5c**, **5i**, **5f–5g**, **5m–5n** and **5p**).

Two synthetic routes to access un-methylated silyl-lipid precursors

Route A: Hydrosilylation of diacylated alkene



Route B: Hydrosilylation of alkenoic acid



Methylation to access cationic silyl-lipid DOTAP analogs

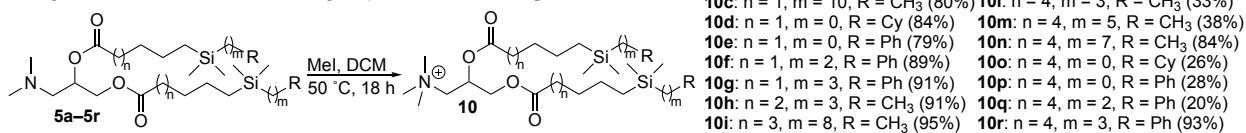


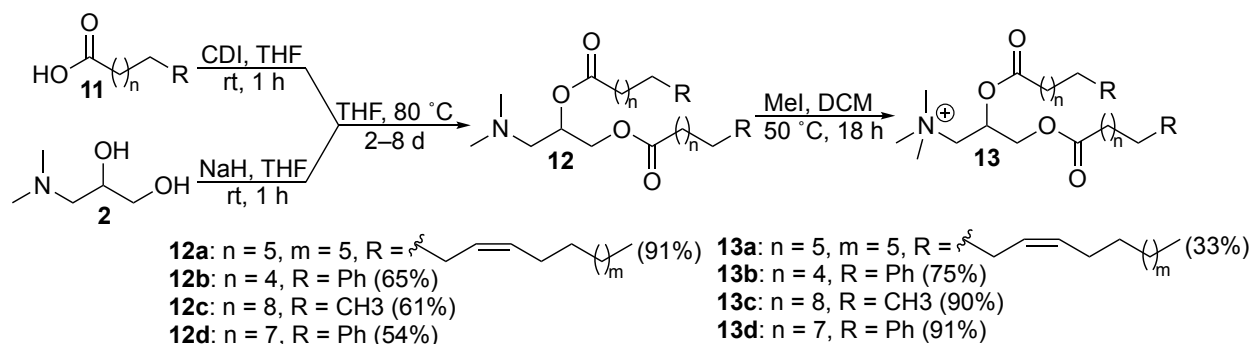
Figure 17. Two synthetic routes to access cationic silyl lipid DOTAP analogs

B: Synthesis of silanes **4d–4e to access **5i**, **5f–5g** and **5m–5n****
4b: *m* = 5 (76%)
4c: *m* = 7 (90%)

$$\text{Cl}-\text{Si}(\text{R})_m \xrightarrow[-78^\circ\text{C to rt, 18 h}]{\text{LiAlH}_4 (4 \text{ M}), \text{DCM}} \text{H}-\text{Si}(\text{R})_m$$

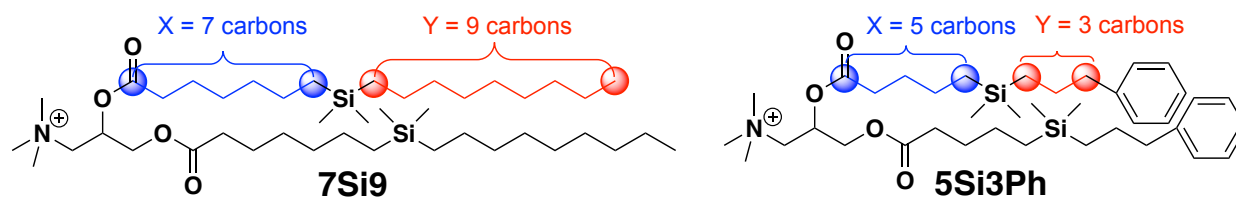
4d: $m = 5$, $\text{R} = \text{CH}_3$ (59%)
4e: $m = 2$, $\text{R} = \text{Ph}$ (94%)
4f: $m = 3$, $\text{R} = \text{Ph}$ (93%)

Cationic lipid controls (including DOTAP) were also synthesized with $-\text{CH}_2$ in place of $-\text{SiMe}_2$ for comparison to the silyl lipids **10a–10r** (Figure 19 and Figure 20). Note that the CMe_2 (carbondimethyl) containing starting materials were not commercially available, further emphasizing the benefit of the hydrosilylation strategy to access a larger toolbox of branched silyl lipid structures. These control lipids were synthesized by coupling commercially available carboxylic acid **11** with 3-(dimethylamino)propane-1,2-diol **2** using CDI and NaH to access **12a–12d** with isolated yields of 54–91% after column chromatography. The methylation of **12a–12d** was performed with methyl iodide to access cationic control lipids **13a–13d** with isolated yields of 33–91% after column chromatography.



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All silyl lipid analogs of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) are named according to the following naming system: XSiY where Si = SiMe₂, X = number of carbons (including the carbonyl carbon) before the silicon and Y = number of carbons in the alkyl chain after the silicon (before any phenyl or cyclohexyl ring). For example, X = 7 and Y = 9 for 7Si9 so there are 7 carbons before the silyl group (including the carbonyl carbon) and 9 carbons after the silyl group. Another example is for 5Si3Ph, where X = 5 and Y = 3 so there are 5 carbons before the silyl group and 3 carbons after the silyl group followed by a phenyl ring. See the examples below.



Using the synthetic routes described above, eighteen cationic silyl lipids were synthesized with varying silyldimethyl positions, silyl substituents (i.e. alkyl, cyclohexyl, phenyl), and tail lengths to evaluate the influence of modular silyl lipid tail structure on LNP formation and properties (Figure 20). An XSiY naming system is used for these silyl lipids where X = the number of carbon atoms in the alkyl chain before the silicon atom (including the carbonyl carbon), Si = SiMe₂ and Y = the number of carbon atoms in the chain after the silicon atom (before any phenyl or cyclohexyl ring). For example, 5Si3Ph-DOTAP (**10g**) has 5 carbon atoms before the silicon atom (X = 5) and 3 carbon atoms after the silicon atom (Y = 3) but before the phenyl ring.

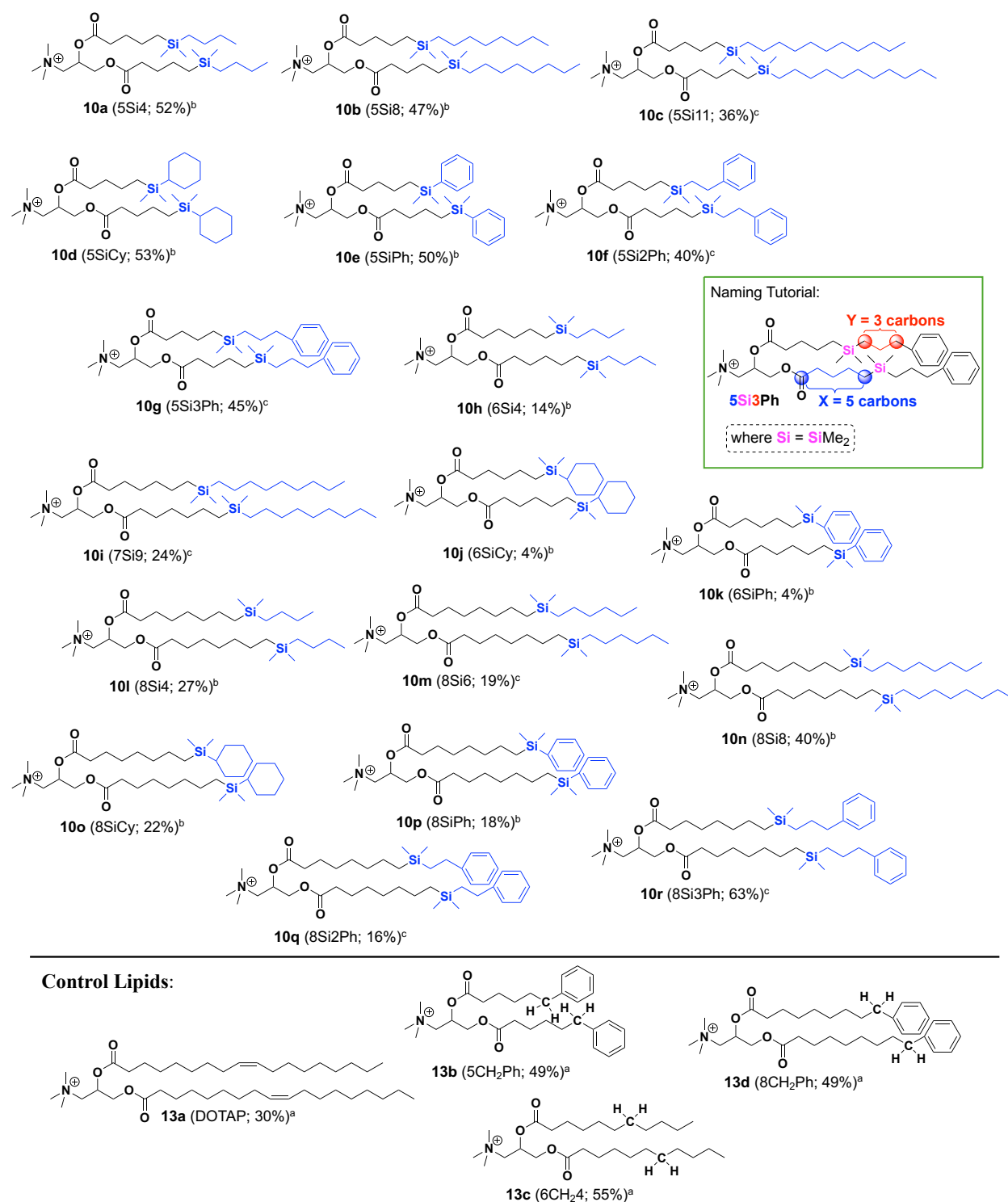


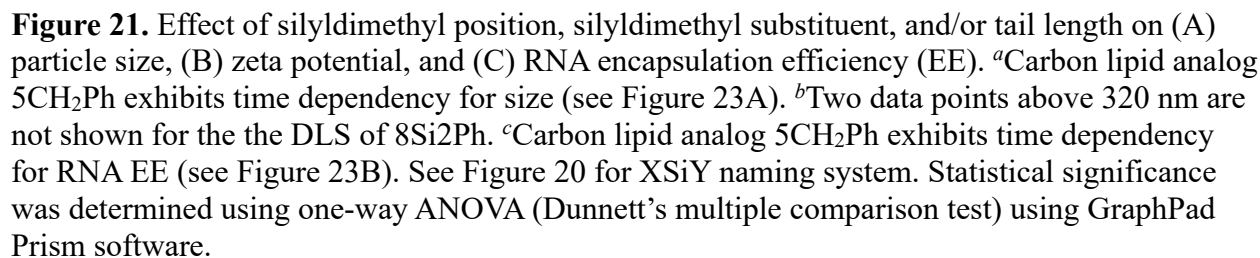
Figure 20. Cationic silyl lipid DOTAP analogs and cationic control lipids. Isolated yields are reported over ^atwo, ^bthree or ^cfour steps. An XSiY naming system is used where X = number of carbon atoms before the silicon atom (including the carbonyl carbon), Si = SiMe₂ and Y = number of carbon atoms after the silicon atom (before any cyclohexyl or phenyl ring).

2.4. Size and Zeta Potential of Liposomes Containing Cationic Silyl lipids

Particle size and zeta potential was evaluated for cationic silyl lipids using dynamic light scattering (DLS). A stock solution of the cationic silyl lipid was first prepared in chloroform. An aliquot of this stock solution was transferred to a glass vial, concentrated to dryness under vacuum to form a thin lipid film, hydrated in Milli Q water, and bath sonicated at room temperature for thirty minutes to form a 1 mg/mL solution for analysis. The results support that the size does not significantly change compared to DOTAP liposomes upon variation of the silyl lipid tail structure (Figure 21A, Table 4). However, some silyl lipids resulted in bimodal particle populations as observed in the size distribution plots (Figure 22), potentially contributing to the larger error bars observed for some of the samples such as 8Si2Ph. Note that for particles with bimodal size distributions (i.e. 6Si4, 6SiPh, 8SiPh, 8Si2Ph, and 8Si3Ph), only the first peak (i.e. smaller-sized population) in the distribution was used to calculate the average size. A time dependent relationship was observed for liposomes containing cationic control lipid 5CH₂Ph (synthesized based on high encapsulation efficiency observed for direct silyl lipid comparison 5SiPh, *vide infra*). Upon immediate formation of 5CH₂Ph particles, their size was observed to be around 165 nm (Figure 23A). However, as the time increased between liposome formation and size measurement, 5CH₂Ph particles decreased in size to around 5 nm and “flaking” was observed. During this flaking, the cationic lipid appeared to be precipitating out of solution, potentially indicating the degradation of the particles over time. This observed “flaking” and time-dependence of size may be contributing to the large error bars observed for 5CH₂Ph (Figure 21A). This “flaking” or degradation was not observed for 5SiPh, suggesting that the silyldimethyl group itself is imparting some increased stability potentially due to the enhanced lipophilic character of the silicon atom. Note that cryo-TEM was also used to evaluate the size distribution and morphology of 5Si3Ph liposomes (this silyl lipid was chosen for evaluation of

LNP size and morphology due to having the best performance for in vitro mRNA transfection, *vide infra*) at 0.2 mg/mL in nuclease-free water (Figure 24).

A larger change in zeta potential was observed, with the incorporation of the silyl lipid tails into liposomes generally increasing zeta potential compared to liposomes containing DOTAP (Figure 21B, Figure 25, Table 4). It is notable that 14 of the 18 silyl lipids formed liposomes with higher zeta potentials compared to DOTAP. As previously stated, a higher zeta potential has been associated with greater colloidal stability and thus improved storage stability of liposomes over time, showing promise in using these cationic silyl lipids to increase colloidal stability and potentially storage stability of liposomes (although further experiments would need to be conducted to directly assess this such as measuring the size and zeta potential of the same liposome sample over an extended period of time from weeks to months).



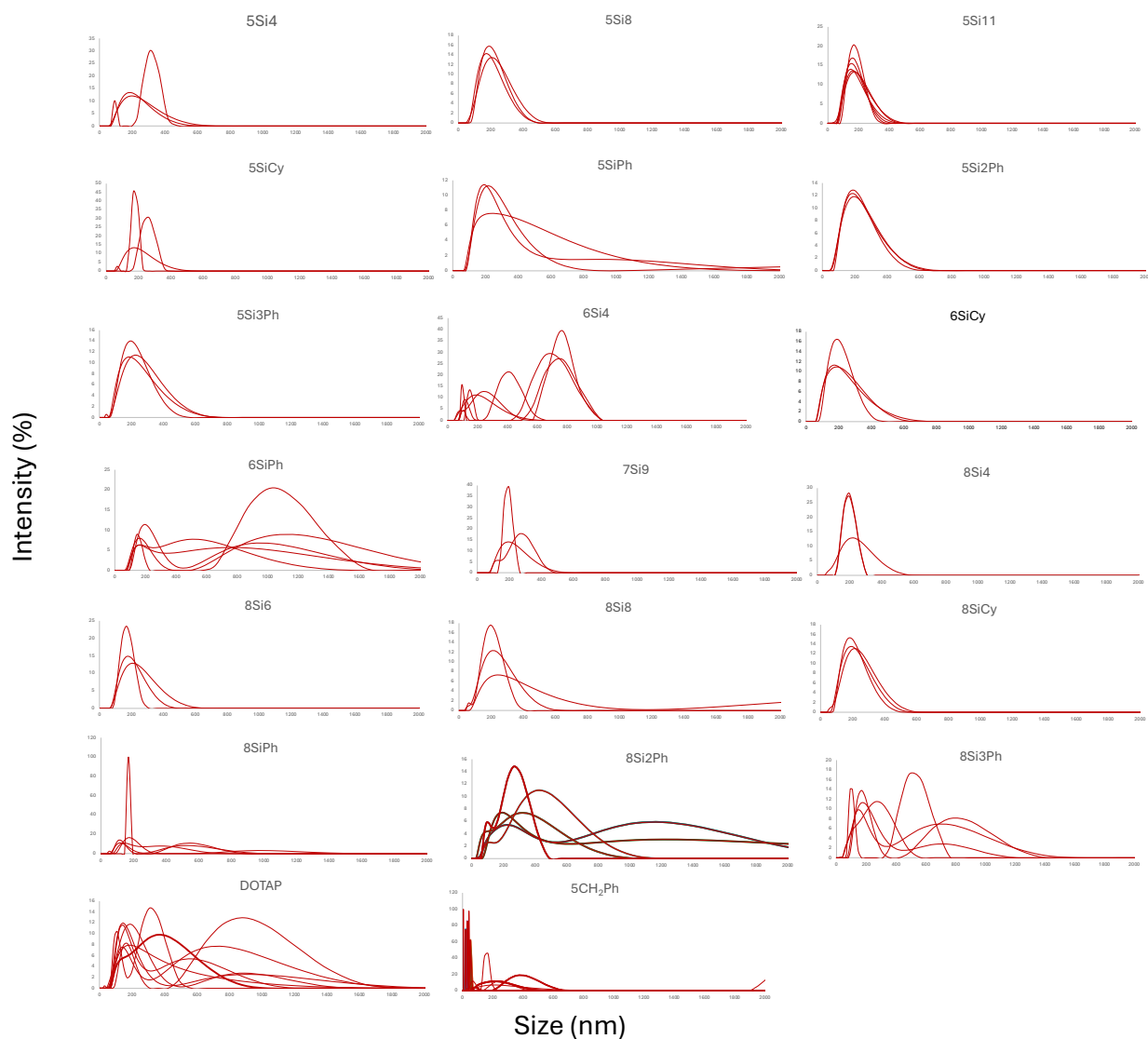


Figure 22. Size by intensity (%) distributions of cationic silyl lipid containing liposomes measured using DLS. Note size by intensity (%) refers to the percentage of total light that is scattered by particles within a given size or population. Each red curve corresponds to one 1 mg/mL liposome sample (i.e. one trial). The size for each trial was defined as the peak of the size distribution curve. For liposomes with bimodal distributions (i.e. 6Si4, 6SiPh, 8SiPh, 8Si2Ph, and 8Si3Ph), only the first peak (i.e. smaller-sized population) in the distribution was used in the average size calculation. See Table 4 for average size of liposomes. See Figure 20 for XSiY naming system.

Table 4. Average size, zeta potential, rRNA EE and ER of particles

Liposome	Size (nm)	Zeta (mV)	rRNA EE (%) ^a	rRNA ER ^a
DOTAP	168 ± 37 ^{d,h}	50 ± 14 ^d	65 ± 2	3.4 ± 0.5
5CH₂Ph	69 ± 63 ^{f,i,j}	70 ± 11 ^f	64 ± 15 ^{g,k}	2.0 ± 0.5
5Si4	163 ± 60 ^a	80 ± 3 ^a	83 ± 5	5.1 ± 0.2
5Si8	171 ± 26 ^a	63 ± 14 ^a	74 ± 6	7.8 ± 0.5
5Si11	166 ± 10 ^c	78 ± 11 ^c	27 ± 13 ^c	1.9 ± 1.4 ^c
5SiCy	169 ± 1 ^a	88 ± 5 ^a	85 ± 4	5.5 ± 0.4
5SiPh	180 ± 30 ^a	77 ± 6 ^a	88 ± 9	4.3 ± 1.3
5Si2Ph	198 ± 0 ^a	76 ± 2 ^a	55 ± 4	—————
5Si3Ph	208 ± 19 ^a	78 ± 3 ^a	65 ± 8 ^e	—————
6Si4	354 ± 53 ^{c,i}	90 ± 6 ^c	65 ± 5 ^c	—————
6SiCy	188 ± 16 ^a	77 ± 5 ^a	38 ± 16 ^d	—————
6SiPh	171 ± 18 ^{b,i}	87 ± 8 ^b	76 ± 1	—————
8Si4	198 ± 0 ^a	70 ± 2 ^a	44 ± 7	—————
8Si6	164 ± 36 ^a	84 ± 5 ^a	48 ± 1	—————
8Si8	221 ± 40 ^a	73 ± 15 ^a	50 ± 7	2.3 ± 0.7
8SiCy	199 ± 30 ^a	70 ± 3 ^a	49 ± 4	—————
8SiPh	138 ± 28 ^{c,i}	79 ± 6 ^c	62 ± 14 ^h	—————
8Si2Ph	311 ± 85 ^{b,i}	65 ± 11 ^b	56 ± 10	—————
8Si3Ph	174 ± 62 ^{b,i}	89 ± 4 ^b	53 ± 8	—————
7Si9	180 ± 30 ^{a,i}	73 ± 4 ^a	59 ± 1	4.8 ± 0.1

The values reported represent the average ± SD of ^athree trials, ^bfive trials, ^csix trials, ^deight trials, ^enine trials, ^ften trials, ^gtwelve trials, or ^hseventeen trials. One trial = one 1 mg/mL liposomal solution for size and zeta potential. One trial = one aliquot of rRNA-loaded liposomes (10:1 w/w cationic lipid/rRNA) for EE. ⁱThe data showed a bimodal size distribution (see Figure 22) with two populations. ^jThe DLS data shows that 5CH₂Ph size is time dependent (see Figure 23A), contributing to the large standard deviation observed for size. ^kData points represent the EE of 5CH₂Ph at three different time points based on the time dependence of size and EE (see Figure 23A–B). Measuring size and zeta potential for each sample using DLS varied within 15–120 minutes after sample preparation. For liposome samples with bimodal size distributions, only the peak from the first population was used in the average size calculation. EE measurements were collected immediately after bath sonication/liposome formation unless otherwise noted. See Figure 20 for XSiY naming system.

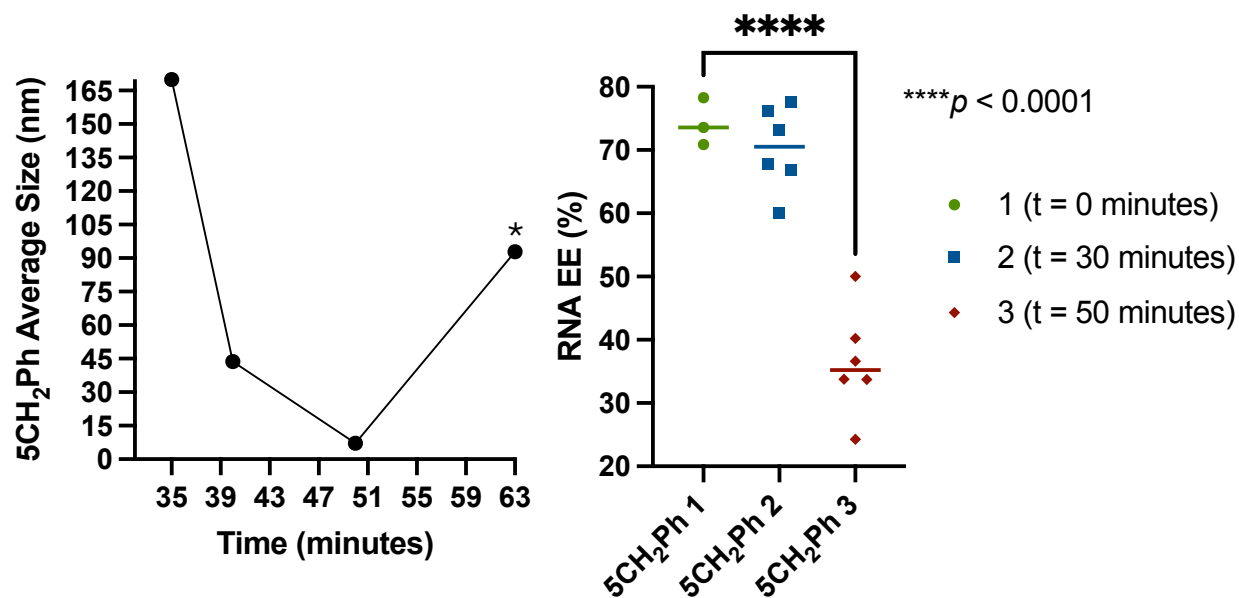


Figure 23. Time-dependent size and RNA encapsulation efficiency (RNA EE). A) Size by intensity decreased over time and white solid/precipitate (i.e. lipid) was observed to be crashing out of solution as time progressed for a single batch of 5CH₂Ph liposomes. *After the size was measured at $t = 50$ minutes, the same sample was re-mixed manually with inversion, resulting in an increase in particle size up to ~90 nm. B) Average RNA EE decreased over time. The time points of 0, 30, and 50 minutes represent the time between bath sonication to form the liposomes and incubation of the liposomes with rRNA. When letting the liposomes sit at room temperature for 30 minutes after bath sonication, the EE is maintained around 70% compared to when $t = 0$ (i.e. the liposomes were incubated with rRNA immediately after bath sonication). However, when letting the liposomes sit for 50 minutes after bath sonication before incubating with rRNA, a large decrease in EE is observed around 35%.

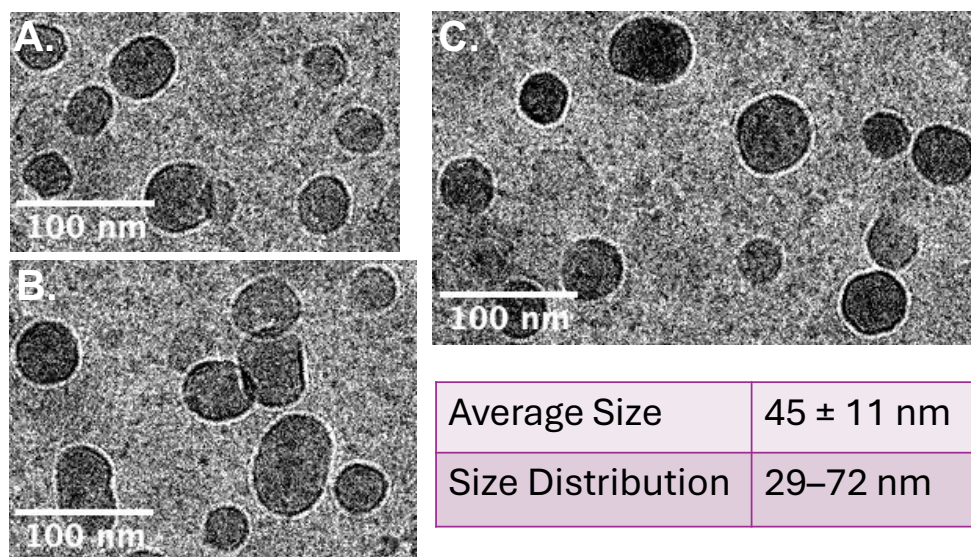


Figure 24. Cryo-TEM images (A–C) of 5Si₃Ph liposomes used in HEK293T cell transfection. Liposomes are 0.2 mg/mL in nuclease-free water and 1:0 w/w cationic silyl lipid/DOPE.

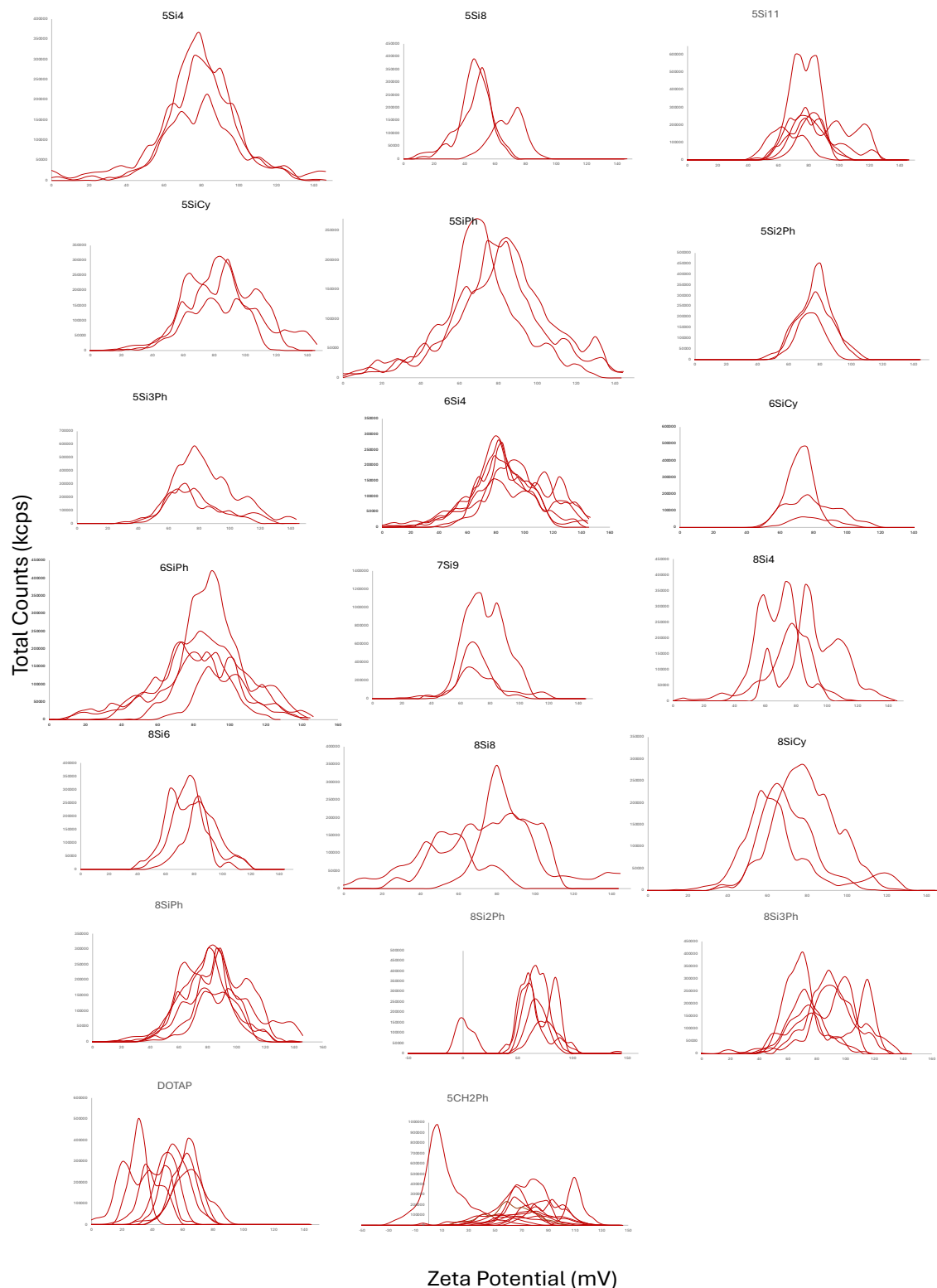


Figure 25. Zeta potential distributions of cationic silyl lipid containing liposomes measured using DLS. Each red curve corresponds to one 1 mg/mL liposome sample (i.e. one trial). The zeta potential for each trial was given/reported by the instrument. See Table 4 for average zeta potential of liposomes. See Figure 20 for XSiY naming system.

2.5. rRNA Encapsulation Efficiency (EE), Rate (ER) and Stability

RNA encapsulation efficiency was evaluated using the Quant-It Ribogreen assay kit.

Liposomes were prepared using the same procedure described for DLS but instead hydrating and sonicating in 1X Tris–EDTA buffer to prepare a 1 mg/mL liposomal solution. An aliquot of this liposome solution (15 µL) was mixed with an aliquot of rRNA standard from the kit (15 µL, 100 µg/mL) and left to incubate at room temperature for 15 minutes to form rRNA-loaded liposomes (10:1 w/w cationic lipid/rRNA). An aliquot of this sample was then plated in triplicate in a 96 well–plate. Row A contained 1 µL of sample, 99 µL of 1X Tris–EDTA and 100 µL of 1:200 Ribogreen solution (in 1X Tris–EDTA), representing the “in–tact” liposomes. Row B contained 1 µL of sample, 99 µL of 2% v/v Triton–X and 100 µL of 1:200 Ribogreen solution (in 1X Tris–EDTA), representing the “lysed” liposomes. The RNA EE was then determined using Equation 1 by subtracting the fluorescence of Row B from Row A (“un–encapsulated” or “free” RNA) and dividing this by the fluorescence of Row B (“total RNA”). See Figure 26 for a diagram of the assay set–up.

$$RNA\ EE = \frac{fluorescence_{Row\ B-A}}{fluorescence_{Row\ B}} \times 100 \quad (1)$$

Varying silyl lipid tail structure modulated the RNA EE of liposomes (Figure 21C, Table 4). Direct replacement of the *cis* alkene in DOTAP with the silyldimethyl moiety in 8Si8 resulted in a subtle decrease in average EE from $65 \pm 2\%$ (DOTAP) to $50 \pm 7\%$ (8Si8). However, moving the silyldimethyl moiety one position closer to the cationic head group resulted in an increase in EE to $59 \pm 1\%$ (7Si9). Subsequently moving the silyldimethyl moiety closest to the cationic head group resulted in a noticeable drop in EE to $27 \pm 13\%$ (5Si11). These results support that RNA EE can be controlled by the strategic positioning of the silyldimethyl moiety in the lipid tail.

Modulation of lipid tail length also had an observable effect on EE. When the silyldimethyl moiety is closest to the cationic head group, EE decreases as tail length increases from $83 \pm 5\%$ (5Si4) to $74 \pm 6\%$ (5Si8) to $27 \pm 13\%$ (5Si11). When moving the silyldimethyl farther down in the alkyl chain, the same trend is not observed with EE being mostly maintained as tail length increases from $44 \pm 7\%$ (8Si4) to $48 \pm 1\%$ (8Si6) to $50 \pm 7\%$ (8Si8). These findings support that (1) varying tail length can modulate EE of liposomes and (2) the effect of varying tail length on EE depends on the strategic positioning of the silyldimethyl moiety in the lipid tail.

	LNP-1 Rep. 1	LNP-1 Rep. 2	LNP-1 Rep. 3	LNP-2 Rep. 1	LNP-2 Rep. 2	LNP-2 Rep. 3	LNP-3 Rep. 1	LNP-3 Rep. 2	LNP-3 Rep. 3	LNP-4 Rep. 1	LNP-4 Rep. 2	LNP-4 Rep. 3
A	1 μ l X 99 μ l 1xTE 100 μ l W											
B	1 μ l X 99 μ l Z 100 μ l W											
	LNP-5 Rep. 1	LNP-5 Rep. 2	LNP-5 Rep. 3	LNP-6 Rep. 1	LNP-6 Rep. 2	LNP-6 Rep. 3	LNP-7 Rep. 1	LNP-7 Rep. 2	LNP-7 Rep. 3	LNP-8 Rep. 1	LNP-8 Rep. 2	LNP-8 Rep. 3
C	1 μ l X 99 μ l 1xTE 100 μ l W											
D	1 μ l X 99 μ l Z 100 μ l W											

Figure 26. Quant-It RiboGreen EE assay set-up. Solution W = 1:200 RiboGreen solution. Solution X = rRNA-loaded liposomes (10:1 w/w cationic lipid/RNA). Solution Z = 2% v/v Triton-X buffer prepared in 1x Tris-EDTA (1xTE) buffer. Fluorescence was measured at 485 nm (ex) and 528 nm (em).

Lipids with cyclic substituents directly attached to the silicon atom were also evaluated for their effect on EE. When the silyldimethyl moiety is farthest from the cationic head group, the incorporation of a phenyl ring appeared to increase average EE to $62 \pm 14\%$ (8SiPh). Switching the phenyl ring for a cyclohexyl had a detrimental effect on EE, dropping to $49 \pm 4\%$ (8SiCy). A

similar effect of the phenyl ring is observed when the silyldimethyl moiety is moved closer to the head group, with 6SiPh ($76 \pm 1\%$) having a notably higher EE than the 6SiCy ($38 \pm 16\%$).

Switching from the phenyl ring to the cyclohexyl ring does not appear to have the same effect on EE when the silyldimethyl moiety is closest to the head group, with 5SiPh ($88 \pm 9\%$) and 5SiCy ($85 \pm 4\%$) having approximately the same high EE. These results support that (1) incorporation of a phenyl and cyclohexyl substituent in the tail can be used to control EE and (2) the effect of the phenyl ring and cyclohexyl ring on EE depends on the position of the silyldimethyl moiety and the length of the lipid tail.

In addition to direct incorporation of the phenyl ring, a series of phenyl-containing lipids were synthesized to evaluate how increasing the number of carbons between the silicon atom and the phenyl ring influences EE. When the silyldimethyl moiety is positioned closest to the head group, increasing the distance between the silicon and the phenyl ring had a detrimental effect on EE with a decrease from $88 \pm 9\%$ for 5SiPh to $55 \pm 4\%$ and $65 \pm 8\%$ for 5Si2Ph and 5Si3Ph. A similar effect is observed when the silyldimethyl is farthest from the head group, where a drop in average EE from $62 \pm 14\%$ (8SiPh) to $56 \pm 10\%$ (8Si2Ph) and $53 \pm 8\%$ (8Si3Ph) is observed. These results support that (1) the distance between silicon atom and the phenyl ring in the lipid tail can be carefully controlled to modulate EE and (2) increasing the distance between the silicon and the phenyl ring generally decreases EE, with a more noticeable decrease observed when the silyldimethyl moiety is closest to the head group.

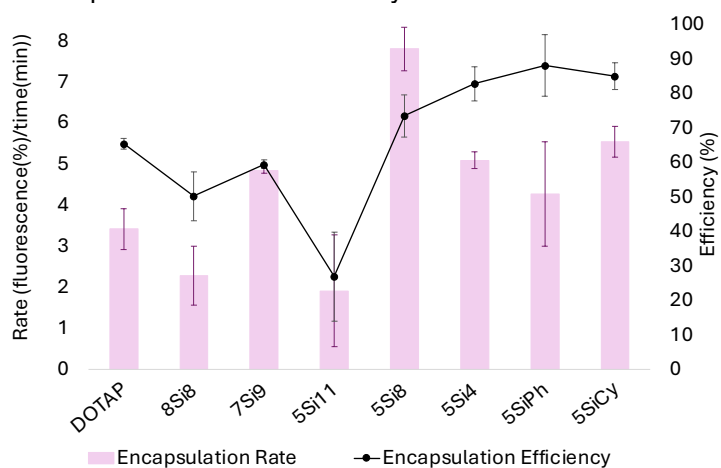
The rRNA ER of the liposomes was evaluated using a modified RiboGreen assay in which the following was added sequentially to the same well in a 96-well plate: i) 1X Tris-EDTA buffer (98 μ L), (ii) 1 mg/mL liposome solution prepared as previously described for DLS but with hydration in 1X Tris-EDTA (1 μ L), iii) 1:200 RiboGreen solution (100 μ L), and iv) rRNA

standard (1 μ L). The ER was evaluated by measuring the decrease in fluorescence (%) over time (Figure 27). The fluorescence (%) was calculated according to Equation (2). The time initial is defined as the first fluorescence reading taken immediately after plating the samples, and time x is defined as the subsequent fluorescence readings taken at 1, 2, 3, 5, 10, 15, 25, 45, 65, 105, 160, and 180 minutes after the initial reading. The ER was compared quantitatively for each rRNA-loaded liposome by plotting the fluorescence (%) at x = 0, 1, 2, 3 and 5 minutes (where x = 0 is time initial) in Excel for all three trials. A linear trendline was fitted to the data for all three trials, and the ER for each trial was defined as the magnitude of the slope of the equation for the trendline (Figure 28, Table 4).

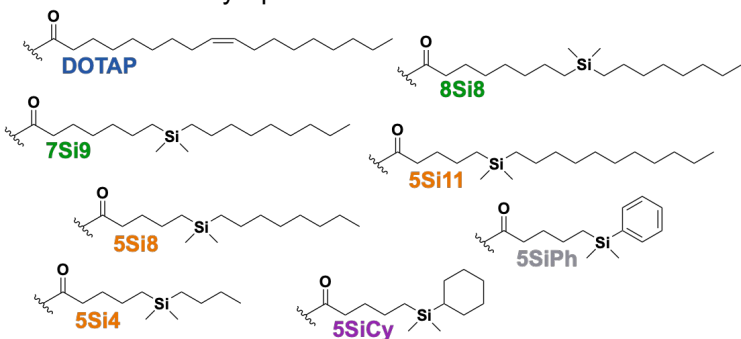
$$fluorescence\ (\%) = \frac{fluorescence_{time\ x}}{fluorescence_{time\ initial}} \times 100 \quad (2)$$

A synergistic relationship was observed between EE and ER (Figure 27A). As encapsulation rate increased so did encapsulation efficiency, and vice versa. Direct replacement of the *cis* alkene in DOTAP with a silyl dimethyl moiety in 8Si8 showed a drop in EE and ER, followed by an increase in EE and ER when moving the silyldimethyl moiety one carbon closer to the head group 7Si9. This was followed by a noticeable drop in EE and ER when moving the silyldimethyl moiety closest to the head group (5Si11). In general, these results support that EE and ER are highest when the tail length was shorter and the silyldimethyl moiety is closest to the cationic head group. Liposomes formed with 5Si8 and 5SiCy lipids demonstrated the highest EE and ER. The 5Si4, 5Si8, 5SiPh, 5SiCy, and 6SiPh liposomes had the highest EE, and 5SiPh, 5Si4, 5Si8, 5SiPh, and 5SiCy liposomes had the highest ER. It is also notable that many of these top-performing liposomes contained a cyclohexyl or phenyl ring in the tail, suggesting that these ring structures are beneficial for controlling and improving encapsulation efficiency of RNA cargo.

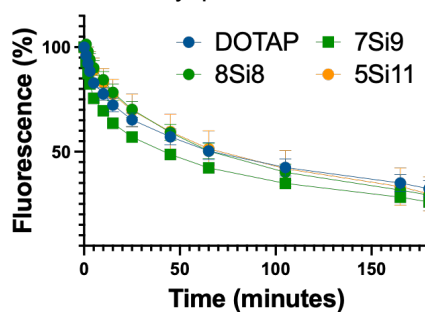
A. Encapsulation rate vs efficiency



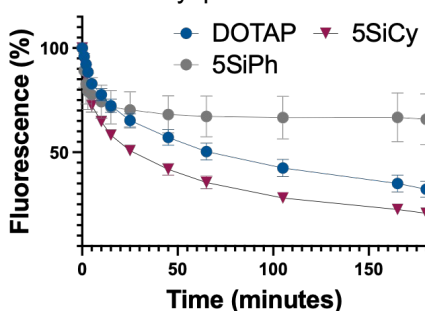
B. Structures of silyl lipid tails



C. Variable silyl position



D. Variable silyl position/substituent



E. Variable silyl position/length

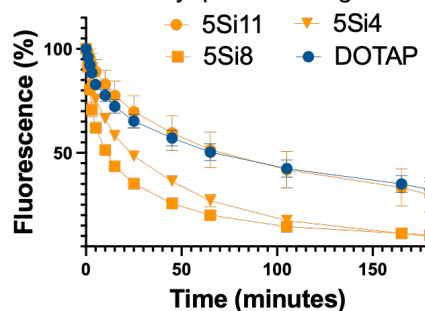


Figure 27. Synergistic relationship between rRNA EE and ER of liposomes containing cationic silyl lipids. A) Encapsulation rate vs efficiency. B) Structures of silyl lipid tails. Encapsulation rate changes with variable C) silyl position, D) silyl position and substituent, and E) silyl position and length. All data represents the average $\pm$ SD of three trials. Fluorescence (%) was calculated using Equation (2) above. The slope of fluorescence (%) over time (minutes) was defined as the encapsulation rate for the liposome samples (Figure 28). See Figure 20 for XSiY naming system.

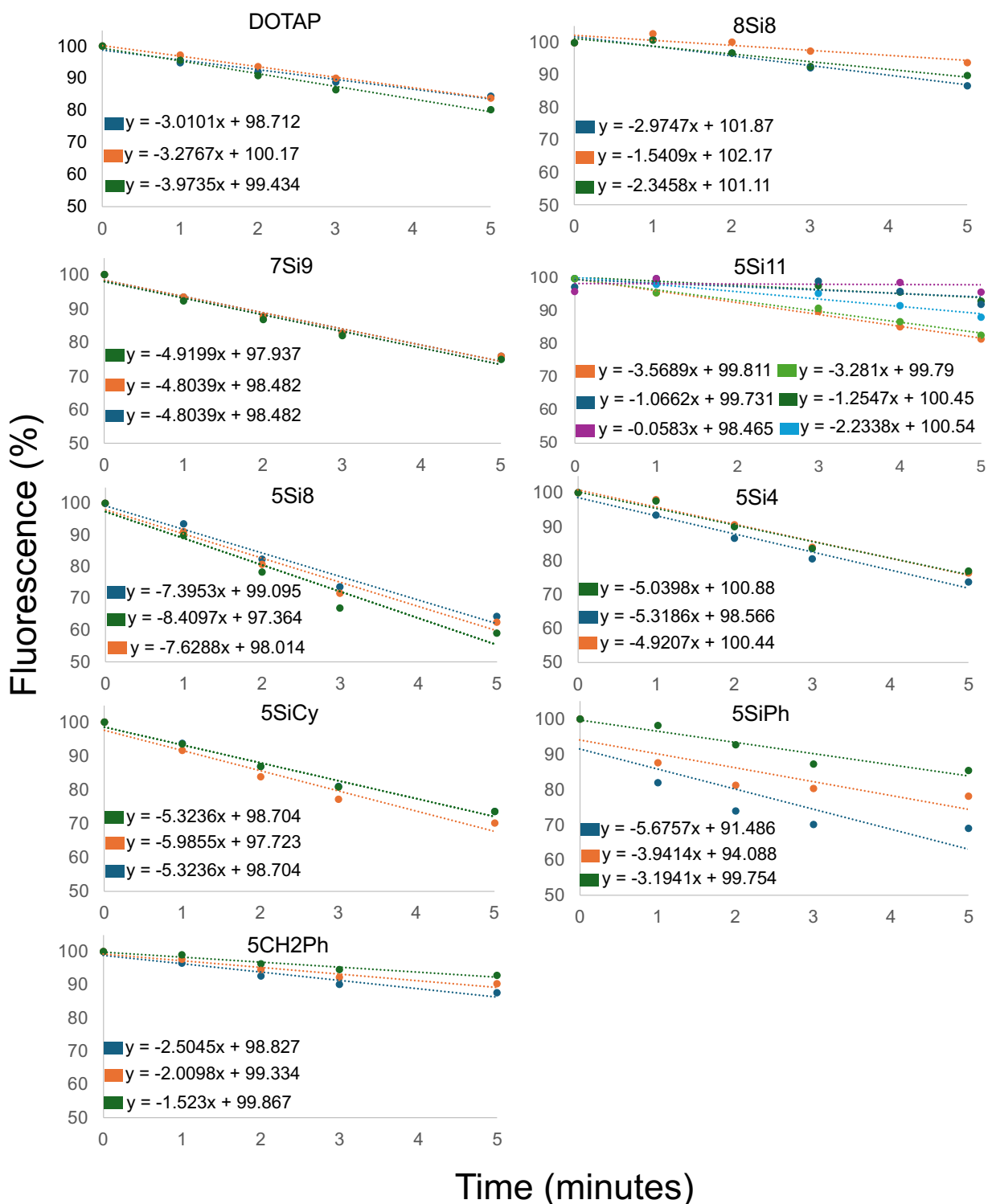


Figure 28. Linear regressions and trend lines used to determine the rRNA encapsulation rate of the liposomes. Each line corresponds to one trial. The magnitude of the slope of each line was denoted as the rate for that trial. The average $\pm$ SD ER of three trials is reported in Table 4. See Figure 20 for XSiY naming tutorial.

Since shorter silyl lipid tails resulted in optimal RNA EE and ER, the role of the silyldimethyl moiety in enhancing these properties was further explored. A methylene control of top-performing silyl lipid 5SiPh was synthesized where the silyldimethyl was replaced with a methylene (5CH₂Ph). Removing the silyl group resulted in a drop in EE from $88 \pm 9\%$ (5SiPh) to $64 \pm 15\%$ (5CH₂Ph) (Table 4). An even larger change in average ER was observed from 4.3 ± 1.3 (5SiPh) to 2.0 ± 0.5 (5CH₂Ph) fluorescence(%)•min⁻¹ when removing the silyldimethyl group. It is also notable that the EE of 5CH₂Ph decreases over time, suggesting that the absence of the silyl group may be decreasing the stability of the liposomes potentially contributing to the drop in EE observed (Figure 23B, Figure 29). These results suggest that the silyldimethyl group in addition to the short tail length and phenyl ring are all playing a role in enhancing EE and ER.

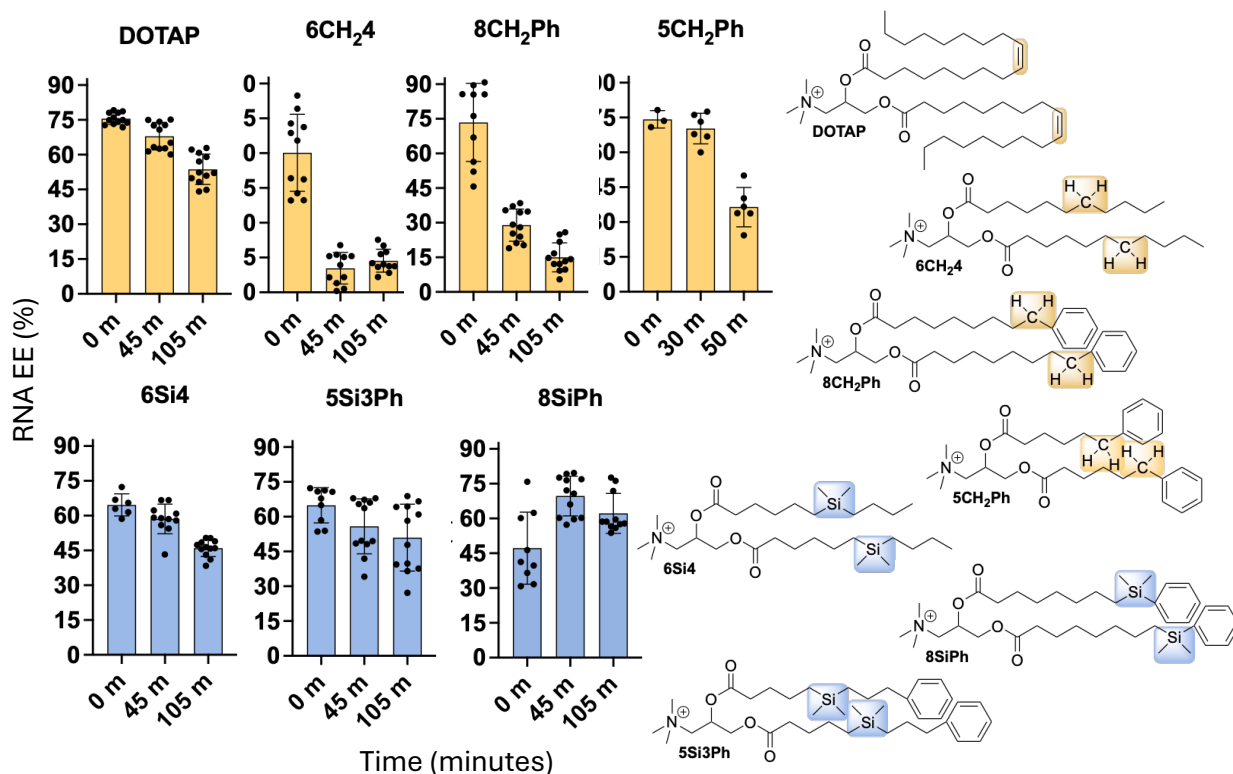


Figure 29. Liposome stability evaluated by measuring RNA EE as a function of time (i.e. minutes between bath sonication to form liposomes and incubation of liposomes with rRNA standard). Initial start times (indicated t = 0 min) have variation between 0–5 min. Each time point represents data collected from two assays (n = 3–6 trials per assay at each time point for a total of 6–12 trials per experiment). See Figure 20 for XSiY naming system

Methylene control lipids 8CH₂Ph and 6CH₂4 were also synthesized for comparison to 8SiPh, 5Si3Ph and 6Si4 due to their high mRNA (EGFP) transfection efficiency in HEK293T cells (vide infra). The stability of these methylene and silyl-containing liposomes was probed using a modified RiboGreen assay in which 1 mg/mL liposomes were first formed in 1X Tris–EDTA buffer using the procedure described for DLS and incubated with rRNA either (i) immediately after bath sonication (time $t = 0$ minutes), (ii) 45 minutes after bath sonication or (iii) 105 minutes after bath sonication to evaluate the change in EE over time depending on the structure of the lipid tail (Figure 29). The silyl-liposomes (results shown in blue) showed similar EE over time profiles as DOTAP, with the average EE only slightly dropping over time for DOTAP, 6Si4 and 5Si3Ph and average EE increasing from 0 to 45 minutes for 8SiPh. This data suggests that the silyldimethyl group is imparting a similar effect as the *cis* alkene on liposome stability. However, replacing the silyldimethyl with a methylene appeared to have a detrimental effect on stability, with a significant drop in EE observed from 0 to 45 minutes for 6CH₂4 compared to 6Si4 (in which only a slight decrease in EE is observed for 6Si4 from 0 to 105 minutes). A similar trend is observed for 8CH₂Ph, where a large drop in EE is observed from approximately 74% to 29% from 0 to 45 minutes. An opposite trend is observed for 8SiPh, with EE increasing from approximately 45% to 65% from 0 to 45 minutes. These results support that the silyldimethyl group is imparting an increase in liposome stability that is not present when the silyldimethyl group is replaced with a methylene.

2.6. Membrane Fluidity of Liposomes Containing Cationic Silyl lipids

The experimental procedure for membrane fluidity analysis was designed by me and performed with assistance from my undergraduate summer student Ebube Agwaramgbo from Xavier University. The decrease in RNA EE and ER as a function of increasing tail length was

notable. I hypothesized that longer tails in the liposomal membrane may be increasing lipid tail packing due to greater surface area for Van der Waals intermolecular forces, which may be decreasing RNA permeability and consequently decreasing EE and ER. To test this hypothesis, the membrane fluidity of liposomes was evaluated for a selection of cationic silyl lipids and DOTAP and 6CH₂4 as controls using the C-laurdan fluorescence assay (Figure 11, Figure 30A–D).

The membrane fluidity of liposomes was qualitatively compared by measuring the fluorescence from 400–600 nm of liposomes that were incubated with C-laurdan (Figure 30B–D). A 0.3 mg/mL liposomal solution was prepared in PBS buffer according to the procedure for DLS. A 0.4 mg/mL stock solution of C-Laurdan was prepared by dissolving 2 mg of C-Laurdan in 5 mL of DMSO. An aliquot of 0.4 mg/mL C-Laurdan stock solution was transferred to the 0.3 mg/mL liposome solution to prepare a lipid/C-Laurdan solution (592:1 mol/mol), and this solution was incubated at room temperature in the dark for 2 h. This lipid/C-laurdan solution (592:1 mol/mol) was diluted in 9 mL of PBS buffer and the fluorescence was measured using a Cary Eclipse Fluorescence Spectrophotometer (ex: 350 nm, em: 400–600 nm) and normalized to 1. The normalized fluorescence intensity versus wavelength was graphed in Excel. Fluid membranes are more hydrated due to being more loosely packed, resulting in greater dipolar relaxation of C-laurdan and a red shift in emission wavelength towards 490 nm. Rigid membranes are less hydrated due to being more tightly packed, resulting in less dipolar relaxation of C-laurdan and a blue shift in emission wavelength towards 440 nm. The generalized polarization (GP) value was calculated using Equation 3 to quantitatively compare the membrane fluidity of liposomes, where $I_{420-460}$ is the normalized fluorescence intensity from 420–460 nm (i.e. the “rigid” membrane state) and $I_{470-510}$ is the normalized fluorescence intensity from 470–

510 nm (i.e. the “fluid” membrane state) (Figure 3.8A). The GP value ranges from -1.0 to 1.0, with a more negative GP value corresponding to more fluid membrane structures and a more positive GP value corresponding to more rigid membrane structures.

$$GP = \frac{I_{420-460} - I_{470-510}}{I_{420-460} + I_{470-510}} \quad (3)$$

The influence of the silyldimethyl group on membrane fluidity was first evaluated. The results show a more red-shifted emission spectrum for 8Si8 (pink) compared to DOTAP (brown), indicating a more hydrated membrane with the silyldimethyl compared to the *cis* alkene (Figure 3.8B). This is consistent with the more negative GP value observed for 8Si8 (-0.41 ± 0.02) compared to DOTAP (-0.21 ± 0.005) (Figure 30A, Table 5), which indicates a more red-shifted emission spectrum and thus a more loosely packed membrane for 8Si8. These results support the direct replacement of a *cis* alkene with a branching silyldimethyl group to increase membrane fluidity of liposomes.

The influence of the silyldimethyl group on membrane fluidity was also evaluated by comparison to a methylene-containing control lipid. The results show a more red-shifted emission spectrum for 6Si4 (blue) compared to the methylene control lipid 6CH₂4 (purple), indicating a more hydrated membrane with the silyldimethyl than with the methylene (Figure 30C). This is consistent with the more negative GP value observed for 6Si4 (-0.29 ± 0.004) compared to 6CH₂4 (-0.17 ± 0.004) (Figure 3.8A), which demonstrates a more red shifted emission spectrum for 6Si4. The same effect is observed when comparing 8SiPh to 8CH₂Ph, where GP increases from -0.19 ± 0.02 to -0.11 ± 0.002 when replacing the silyldimethyl with a methylene (Table 5). These results (along with the results for 8Si8 compared to DOTAP) emphasize the ability to increase membrane fluidity by incorporating a branching silyldimethyl

group in place of a *cis* alkene and methylene. This emphasizes the benefit and promise of using these modular silyl lipids in liposome and LNP applications for controlling membrane packing and structural stability.

The influence of tail length and silyldimethyl position on membrane fluidity was also evaluated. The results show a more red-shifted emission spectrum as tail length decreases from 5Si11 (green) to 5Si8 (orange) to 5Si4 (black), indicating a more hydrated membrane with shorter silyl lipid tails (Figure 30D). This is consistent with the decrease in GP observed when decreasing tail length from -0.27 ± 0.006 (5Si11) to -0.32 ± 0.001 (5Si8) to -0.36 ± 0.01 (5Si4) (Figure 3.8A), which demonstrates an increase in red-shifted emission with decreasing tail length. This relationship between tail length of and membrane fluidity aligns with the relationship between tail length and EE for these 5SiX lipids. Shortening of the tails results in an increase in membrane fluidity that corresponds to the increase in EE observed for 5SiX lipids, suggesting that shortening the silyl lipid tails increases the permeability of RNA through the lipid membrane, resulting in greater encapsulation of RNA cargo inside the liposome. When the silyldimethyl group is positioned farther down the tail, the same trend in GP is not observed with decreasing tailing length. There is a large increase in GP from -0.39 ± 0.02 to -0.29 ± 0.004 when increasing tail length from 8Si4 (12 carbons) to 8Si6 (14 carbons), demonstrating a blue-shift towards more rigid membranes with increasing tail length (Table 5). A further increase in tail length from 8Si6 (14 carbons) to 8Si8 (16 carbons) results in a notable drop in GP from -0.29 ± 0.004 to -0.41 ± 0.02 , indicating a red-shift to a more fluid membrane. These results support that (i) synthetic modification of silyl lipid tail length and silyldimethyl position are useful strategies to control membrane fluidity of liposomes, and (ii) the effect of tail length on membrane fluidity depends on the position of the silyldimethyl group in the lipid tail.

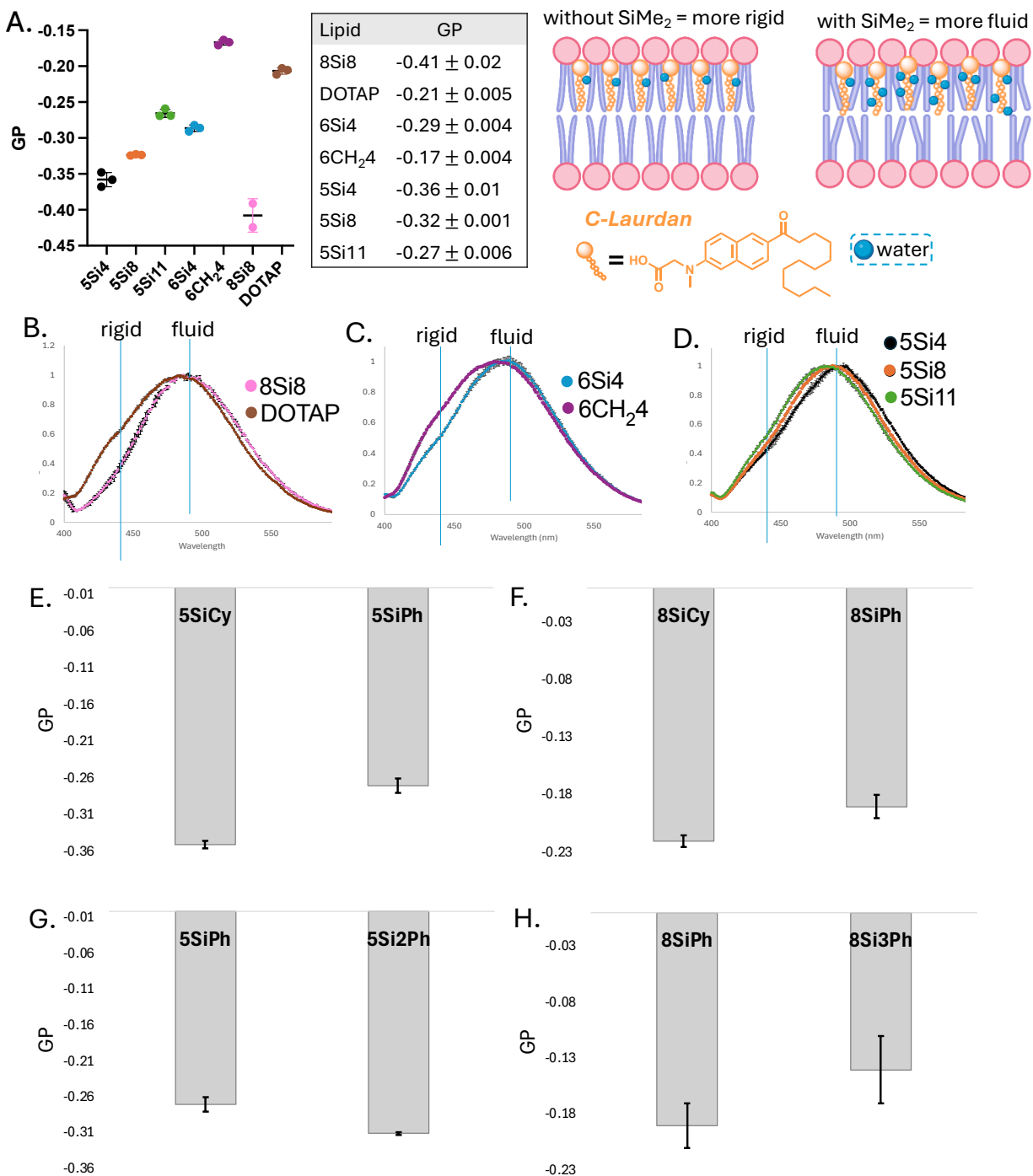


Figure 30. Evaluation of liposome membrane fluidity using C-laurdan fluorescence assay. (A) Generalized polarization (GP) values represent the average $\pm$ SD of $n = 2-3$ trials (see Equation 3 and Figure 1.9 for GP calculation). (B–D) The average normalized fluorescence intensity ($n = 2$ or 3) vs wavelength from 400–600 nm. (E–F) Effect of cyclohexyl vs phenyl ring on GP. (G–H) Effect of phenyl ring distance from silicon on GP. See Figure 20 for XSiY naming system.

Table 5. GP values for DOTAP and cationic silyl lipids.

Cationic Lipid	GP
DOTAP	-0.21 ± 0.005^b
5Si4	-0.36 ± 0.01^b
5Si8	-0.32 ± 0.001^b
5Si11	-0.27 ± 0.006^b
5SiCy	-0.35 ± 0.005^b
5SiPh	-0.27 ± 0.01^b
5Si2Ph	-0.31 ± 0.002^b
6Si4	-0.29 ± 0.004^b
6CH ₂ 4	-0.17 ± 0.004^b
6SiCy	-0.32 ± 0.004^b
8Si4	-0.39 ± 0.02^b
8Si6	-0.29 ± 0.004^b
8Si8	-0.41 ± 0.02^a
8SiCy	-0.22 ± 0.009^b
8SiPh	-0.19 ± 0.02^b
8CH ₂ Ph	-0.11 ± 0.002^b
8Si3Ph	-0.14 ± 0.03^b

Values represent the average $\pm$ standard deviation of ^atwo or ^bthree trials.

The influence of a cyclohexyl versus a phenyl ring on membrane fluidity was also compared (Figure 30E–F). When the silyldimethyl group is positioned closest to the cationic head group, a notable increase in GP from -0.35 ± 0.005 (5SiCy) to -0.27 ± 0.01 (5SiPh) is observed upon replacement of a cyclohexyl with a phenyl ring; this indicates a more rigid membrane with a phenyl ring in the tail (Figure 30E, Table 5). When moving the silyldimethyl group farthest from the head group, an increase in GP is also observed, albeit more subtle, from -0.22 ± 0.009 (8SiCy) to -0.19 ± 0.02 (8SiPh) upon replacing the cyclohexyl with a phenyl ring (Figure 30F, Table 5). It is possible that phenyl-containing tails have more rigid membranes than cyclohexyl-containing tails due to pi–pi stacking between the phenyl rings causing greater lipid tail packing. These results support that (i) phenyl rings can be incorporated into lipid tails to increase rigidity of membranes, and (ii) the extent of increased membrane rigidity depends on the position of the

silyldimethyl group, where data suggests that shorter silyl lipid tails will experience a more significant increase in tail packing upon incorporation of a phenyl ring.

Membrane fluidity was also evaluated in relation to the distance between the phenyl ring and the silyldimethyl group (Figure 3.8G–H). There is a decrease in GP from -0.27 ± 0.01 (5SiPh) to -0.31 ± 0.002 (5Si2Ph) when the silyldimethyl group is closest to the cationic head group, indicating an increase in membrane fluidity upon moving the phenyl ring farther away from the silyldimethyl group (Figure 30G, Table 5). The opposite effect is observed when the silyldimethyl group is moved farthest from the head group. The GP increases from -0.19 ± 0.02 (8SiPh) to -0.14 ± 0.03 (8Si3Ph) upon increasing the distance between the phenyl ring and the silyldimethyl group, indicating a shift to more rigid membranes when increasing the distance between the silyldimethyl group and the phenyl ring (Figure 30H, Table 5). This data supports that (i) membrane fluidity can be controlled by varying the phenyl ring distance from the silyldimethyl group, and (ii) the effect of phenyl ring distance on membrane fluidity depends on the silyldimethyl position in the lipid tail.

2.7. mRNA Transfection Efficiency in HEK293T Cells

The transfection efficiency of the liposomes was evaluated in HEK293T cells. One day prior to transfection, two separate 5 mg/mL stock solutions, one with cationic lipid and one with ionizable helper lipid DOPE were prepared in chloroform. Separate 3:1, 1:0 and 1:1 w/w cationic lipid/DOPE solutions were prepared by combining the appropriate volume of corresponding cationic lipid and DOPE solutions into the same vial. The following volumes of each lipid solution were used: i) 40 μ L of cationic lipid and 13.4 μ L of DOPE (3:1 w/w), ii) 40 μ L of cationic lipid and 0 μ L of DOPE (1:0 w/w), iii) 40 μ L of cationic lipid and 40 μ L of DOPE (1:1 w/w). The chloroform was removed under vacuum overnight to form a thin lipid film. The

morning of transfection, this lipid film was hydrated in the appropriate volume of nuclease-free water and bath sonicated for 20 minutes at 40°C to form the working liposomal solution for transfection (0.5 mg/mL). The following volumes of nuclease-free were used: i) 0.53 mL (3:1 w/w), ii) 0.40 mL (1:0 w/w), and iii) 0.8 mL (1:1 w/w). This process was repeated for all 20 cationic lipids to form two batches of 3:1, 1:0, and 1:1 w/w cationic lipid/DOPE liposomal solutions for transfection.

HEK293T cells (ATCC #CRL-3216) were maintained in DMEM media supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C under 5% CO₂. Twenty-four hours prior to transfection, cells were plated in 48-well culture plates at a density of 50,000 cells per well in antibiotic free maintenance media. Transfections were performed with a base-modified Cap-1 GFP mRNA (TriLink). GFP mRNA (0.5 µg) was premixed with Optimem (25 µL), while the 3:1, 1:1, or 1:0 w/w cationic lipid/DOPE liposomes (3.5 µg) were diluted in Optimem (25 µL) in a separate tube. After 10 minutes, these liposomal and mRNA solutions were mixed and incubated for 25 minutes at room temperature to form the mRNA-loaded liposomal solution. This entire mRNA-liposomal solution was transferred to a single well of a 48-well plate. GFP expression was monitored using the EVOS FL Auto 2 cell imaging system (Thermo Fisher Scientific). Two or 5 days after transfection, cells were detached using TrypLE and washed twice with 1% BSA in DPBS buffer. The number of GFP positive cells was assessed using Flow Cytometry on the CytoFLEX (Beckman Coulter). The fold change in %GFP positive cells of the mRNA-liposomal solution was compared to DOTAP for all 20 cationic lipids (3:1 w/w cationic lipid/DOPE) (Figure 3.9A). The fold change in %GFP was determined by dividing the %GFP positive cells for each silyl lipid by %GFP positive cells for DOTAP (Equation 4). The %GFP positive cells for 1:0, 3:1, and 1:1 w/w DOTAP/DOPE, 5Si3Ph/DOPE, 8SiPh/DOPE, and 6Si4/DOPE was

graphed (Figure 3.9B). Transfection efficiencies were determined from four biological replicates (n=4) and significance was calculated using a one-way ANOVA (Dunnett's multiple comparison test) using GraphPad Prism software.

$$\%GFP = \frac{\%GFP_{silyl-lipid}}{\%GFP_{silyl-lipid}} \quad (4)$$

Of the 20 cationic lipids (including DOTAP and 5CH₂Ph control lipids), 8SiPh, 6Si4 and 5Si3Ph demonstrated the highest mRNA transfection efficiency (Figure 31A). These lipids all contain shorter tails and variable silyldimethyl positions, with two of them containing a phenyl ring. It is notable that two of these top-performing lipids 5Si3Ph and 8SiPh have the same tail structure but vary only in the position of the silyldimethyl group. This supports the strategic positioning of the silyldimethyl group to control and optimize mRNA transfection efficiency. These results support that shorter tails containing phenyl rings and variable silyldimethyl position are beneficial to enhancing transfection efficiency for cationic silyl lipid containing liposomes.

Further optimization studies were performed to evaluate the influence of cationic lipid/DOPE weight ratio on mRNA transfection efficiency (Figure 31B–C). The results demonstrate that the optimal weight ratio varied depending on the cationic silyl lipid tail structure, with 1:0 w/w cationic lipid/DOPE being preferred for 6Si4 and 1:0 and 3:1 w/w being preferred for 8SiPh. Interestingly, 5Si3Ph performed the best at all three weight ratios (1:0, 3:1 and 1:1 w/w), emphasizing the benefit of incorporating this cationic silyl lipid into LNP formulations. Overall, these results support the incorporation of cationic silyl lipids into LNP formulations for controlling and optimizing mRNA delivery.

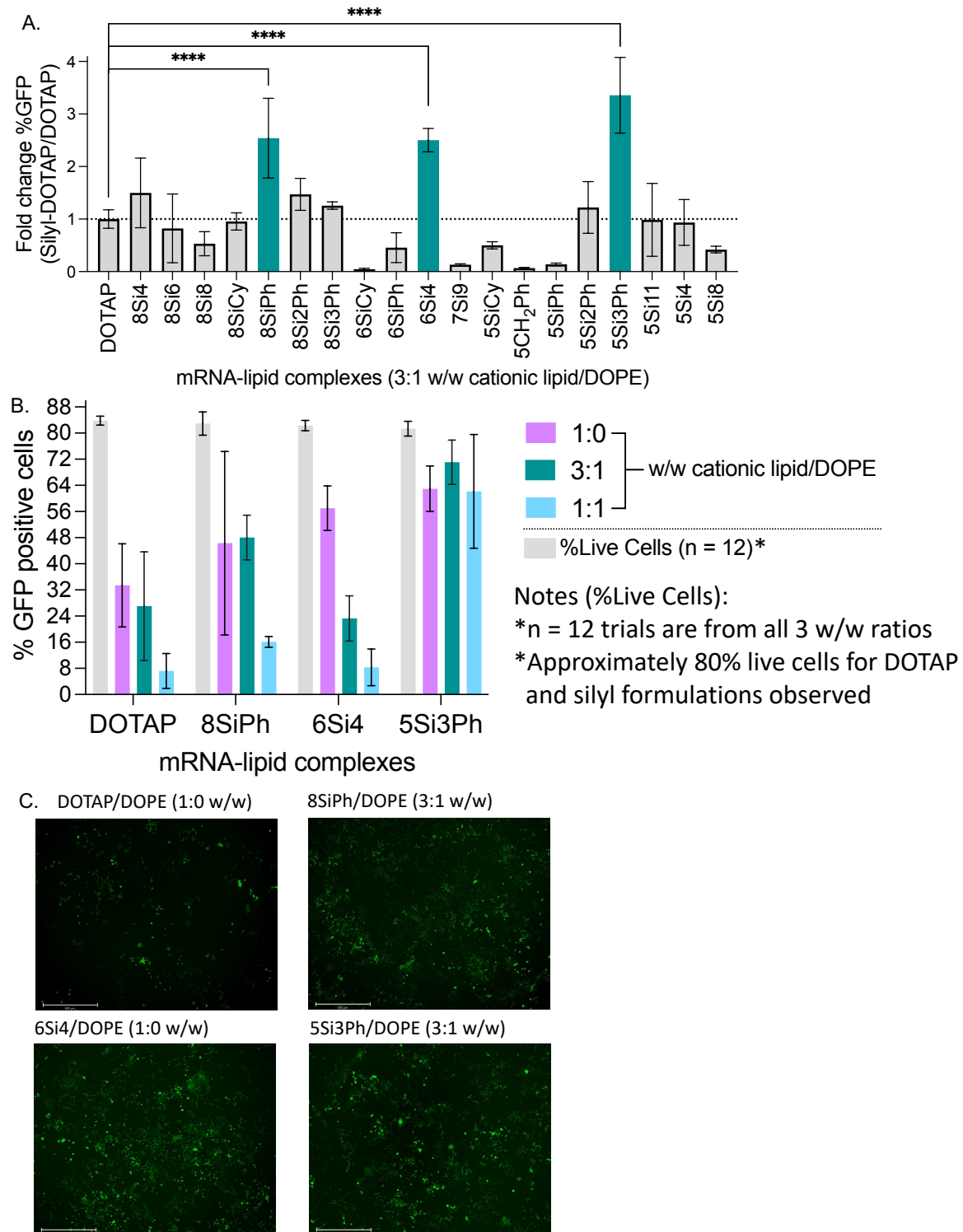


Figure 31. mRNA transfection efficiency of liposomes containing cationic silyl lipids and/or DOPE at A) 3:1 w/w cationic silyl lipid to DOPE helper lipid or B) 1:0, 3:1 or 1:1 w/w cationic silyl lipid/DOPE. Results are reported as the average $\pm$ SEM (n = 4) and significant increase is indicated (****p<0.0001). C) Representative fluorescent microscope images of transfected HEK293T cells at optimized w/w ratios taken 5 days after transfection using the EVOS FL Auto 2 cell imaging system (ThermoFisher Scientific). Each microscope image was captured from one

biological replicate. Cell images were processed using ImageJ software to subtract background fluorescence and sharpen. Scale bars are 650 μm . See Figure 20 for XSiY naming system.

2.8. Conclusion – Cationic Silyl lipids

Engineered lipids with silyl-lipid tails expand the current chemical space of branched-tail lipids used for liposomes, LNPs and nanomaterials. Hydrosilylation is an efficient key step to access novel lipids containing varied silyl lipid tail groups with structurally diverse features that would otherwise be difficult to access using carbon-based synthetic chemistry. A modular synthesis provides access to a toolbox of silyl lipids with different silyldimethyl positions, tail lengths, and substituents in the lipid tails that allow control nanomaterial properties to improve mRNA delivery.

Compared to DOTAP and carbon-based lipid controls, five silyl lipids demonstrated enhanced EE and ER and three silyl lipids demonstrated enhanced stability and mRNA delivery efficiency in HEK293T cells. Incorporating a silyl group (in place of a *cis* alkene or methylene) increased membrane fluidity in liposomes. The position and structure of the silyl group impacts mRNA delivery, where shortening the tail length and the strategic positioning of the silyldimethyl group modulate mRNA delivery efficiency. Notably, these engineered silyl lipids can afford high transfection efficiency without the addition of any helper lipids such as DOPE. These novel silyl lipid components are expected to have broad implications for the design of amphiphilic and lipophilic lipids for both materials and medicinal applications, and were applied for the development of ionizable silyl lipids which is discussed in Chapter 3.

2.9. General Synthetic Procedures and Characterization for Cationic Silyl Lipids

Synthesis, Characterization and Instrumentation:

All nuclear magnetic resonance (NMR) spectra were obtained on the Bruker Nanobay AVIIIHD 400 (400 MHz for ^1H ; 100 MHz for ^{13}C ; 80 MHz for ^{29}Si) or 300 MHz Bruker Advance-NEO at room temperature. Chemical shifts are reported in parts per million (δ scale) and referenced according to the following: the solvent residual signal of deuterated chloroform at δ 7.26, deuterated chloroform for $^{13}\text{C}\{^1\text{H}\}$ signals (middle peak is δ 77.1 respectively), and tetramethylsilane external standard in CDCl_3 for $^{29}\text{Si}\{^1\text{H}\}$ DEPT20 signals (δ 0.00). Coupling constants are reported in Hertz (Hz) and multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p) and multiplet (m). Compounds were analyzed for HRMS on a Thermo Fisher Orbitrap XL (Davis, CA) using electrospray in the positive ion mode or negative ion mode at >60,000 resolution and using 5 kV spray voltage, with a curtain plate temperature of 275 °C and sheath gas setting of 15. These settings result in mass accuracies <5 ppm. Samples were analyzed via flow injection analysis by injecting 5 μL samples into a stream of 50% acetonitrile and 50% aqueous solution of 0.1% formic acid, flowing at 200 $\mu\text{L}/\text{minute}$.

Commercially available reagents were purchased and used without further purification. All hydro, ethoxy, and chlorosilanes were purchased from Gelest or Sigma–Aldrich. 3-(Dimethylamino)propane-1,2-diol was purchased from TCI America and the alkenoic acids were purchased from Ambeed. Karstedt's catalyst (abbreviated $\text{Pt}_2(\text{dvtms})_3$ where dvtms is divinyltetramethylsiloxane), dichloro(1,5-cyclooctadiene)platinum(II) catalyst (abbreviated $\text{Pt}(\text{cod})\text{Cl}_2$ where cod is cyclooctadiene), and methyl iodide (molecular formula MeI) were purchased from Sigma-Aldrich. 1,1'-Carbonyldiimidazole (CDI) and sodium hydride (NaH , 57-

63% oil dispersion) were purchased from Fisher. 4-Amino-1-butanol, 4-(methylamino)butan-1-ol, 6-bromohex-1-ene, potassium carbonate, and anhydrous acetonitrile were purchased from Sigma–Aldrich.

Reactions were analyzed by thin-layer chromatography (TLC) on EMD glass plates that were pre-coated with silica gel 60 F254, and the reactions were purified by column chromatography using Acros silica gel 60 Å (0.035-0.070 mm). The following abbreviations are used throughout: ethyl acetate (molecular formula EtOAc), hexanes (Hex), tetrahydrofuran (THF), dichloromethane (DCM), lithium aluminum hydride (LAH), and 1,1'-carbonyldiimidazole (CDI). Anhydrous DCM and THF were used for synthesis and were collected from a solvent still that removes water from solvents by pushing them through two dry, alumina columns. All reagents and solvents used for synthesis and purification were purchased from commercial sources unless specified otherwise. All reactions were carried out under inert, argon conditions. The reaction vessels used for all reactions were flame dried under vacuum to remove moisture and purged with argon.

Dynamic Light Scattering experiments were performed with the Malvern Zetasizer Pro instrument in Ghaussi Hall in the lab of Dr. Veronica Morales at UC Davis. Copper grids were prepped for cryo-TEM analysis using the TFS Vitrobot Mark IV at the Biological Electron Microscopy Core Facility in Briggs Hall at UC Davis. Cryo-TEM Images were captured using the TFS Glacios Cryo-TEM (Gatan K3 Direct Electron Detector, TFS Ceta 4k × 4k) at the Biological Electron Microscopy Facility in Briggs Hall at UC Davis. Fluorescence spectrophotometry experiments for evaluating membrane fluidity were performed using the Cary Eclipse instrument located in 3480 Chemistry Annex at UC Davis. Flow cytometry was performed using the EVOS FL Auto 2 cell imaging system (Thermo Fisher Scientific) in the Flow Cytometry Shared Laboratory at UC Davis.

Reagents for Biophysical Evaluation and Cell Work:

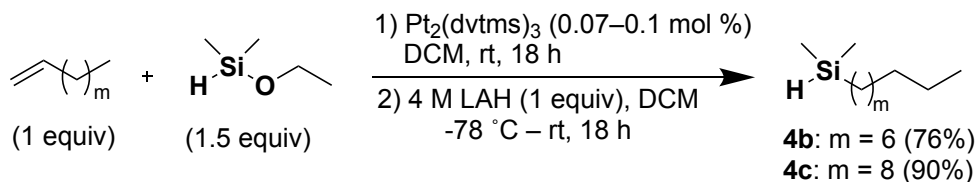
Milli Q water from Sigma–Aldrich or nuclease–free water from Thermo Fisher Scientific was used to prep particles for DLS analysis. 1x Tris-EDTA buffer from Thermo Fisher Scientific was used to prep samples for the RiboGreen RNA fluorescence assay. The Quant–It RiboGreen RNA Assay Kit used to perform the RNA encapsulation efficiency assay was purchased from Thermo Fisher Scientific (catalog number R11490). The kit contained 20x Tris-EDTA buffer, RiboGreen dye, and RNA standard that were used for the assay. 100x Triton-X from Sigma Aldrich was used to prepare the 2% v/v Triton-X solution used for the assay. The Quant–It PicoGreen dsDNA Assay Kit used to perform the DNA encapsulation efficiency assay was purchased from Thermo Fisher Scientific (catalog number P11496). The kit contained 20x Tris-EDTA buffer, PicoGreen dye, and dsDNA standard that were used for the assay. Nuclease-free water from Thermo Fisher scientific was used to prepare particles for HEK293T cell transfection. CleanCap EGFP mRNA (1 mg/mL in 1 mM Sodium Citrate pH 6.4) used for HEK293T cell transfection was purchased from TriLink Biotechnologies (catalog number L-7601-100). The 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipid used for HEK293T cell transfection was purchased from Ambeed (catalog number 4004-05-1). The 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC, catalog number P1138), 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol–2000 (DMG–PEG–2000, catalog number TA9H9ABDD3D8) and cholesterol (catalog number C8667) used for HEK293T transfection was purchased from Sigma–Aldrich. The following carbon grids were purchased from Quantifoil TEM Substrates and used for cryo–TEM imaging: Ultrathin Carbon on Quantifoil, Orthogonal Array of 1.2 μ m Diameter Holes - 1.3 μ m Separation, mounted on a 200M Cu grid, package of 10 grids (catalog number 668-200-CU). The 10x PBS buffer used for membrane fluidity (GP)

analysis was purchased from Thermo Fisher Scientific. The C-laurdan probe used for membrane fluidity (GP) analysis was purchased from TOCRIS (catalog number 7273).

Additional mRNA encapsulation efficiency, size and polydispersity index, and zeta potential experiments were performed with Cre-mRNA before in vivo evaluation of silyl-LNPs in Ai14 mice (will be performed in May of 2026). The CleanCap Cre-mRNA (5moU) (1 mg/mL in 1 mM sodium citrate pH 6.4) used for these experiments was purchased from TriLink BioTechnologies (catalog number L-7211-1000). The dialysis cassettes (Thermo Fisher Scientific™ Slide-A-Lyzer™ Dialysis Cassettes, 20K MWCO) used for LNP formulation for these experiments were purchased from Fisher Scientific (catalog number PI66003). The 1 M citrate buffer solution used for LNP preparation via ethanol dilution and dialysis was prepared from commercially available sodium citrate and citric acid in nuclease-free water at a concentration of 0.1 M and pH 4.

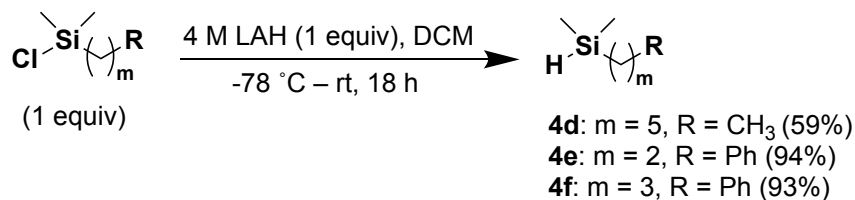
2.9A. General Synthetic Procedures

A.1. Synthesis of silanes to access 7Si9 and 5Si11 analogs of DOTAP



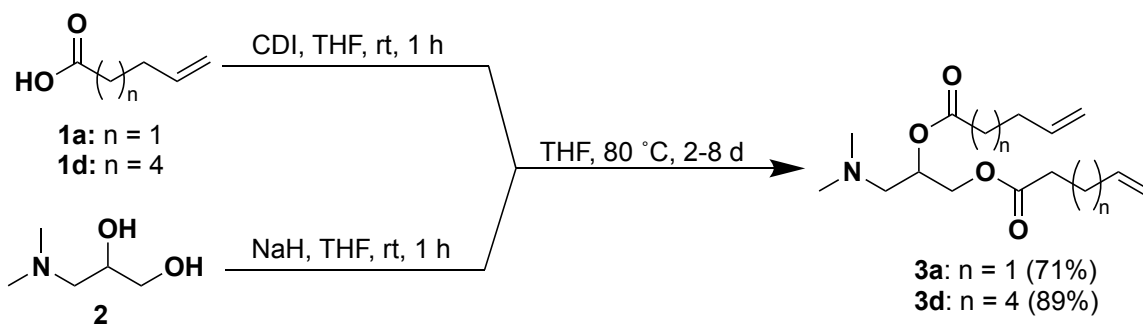
Alkene (1 equiv), dimethylethoxysilane (1.5 equiv), and $\text{Pt}_2(\text{dvtms})_3$ (0.07–0.1 mol %) were stirred in DCM at room temperature for 18 h. The next day, 4M LAH (1 equiv) was added dropwise to the crude reaction mixture at -78°C and stirred to room temperature for 18 h. The reaction was quenched via dropwise addition of aqueous sodium potassium tartrate in an ice bath. The crude reaction mixture was washed with DCM (20 mL) and sodium potassium tartrate (20 mL), dried in anhydrous MgSO_4 , filtered, and concentrated to dryness. The product **4** was purified using column chromatography with silica gel as the stationary phase and the product eluted in 100% hexanes.

A.2. Synthesis of silanes to access 5Si2Ph, 5Si3Ph, 8Si2Ph, 8Si3Ph, and 8Si6 analogs of DOTAP



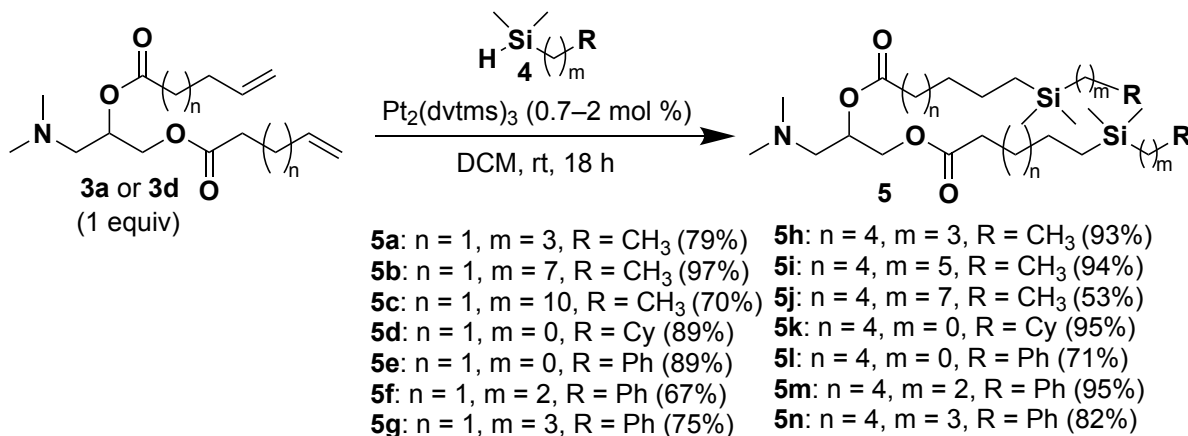
A 4.0 M solution of LAH (1 equiv) was added dropwise to a solution of chloroalkyldimethylsilane (1 equiv) in DCM at -78°C and stirred to room temperature for 18 h. The reaction was quenched via dropwise addition of aqueous sodium potassium tartrate in an ice bath. The crude reaction mixture was washed with DCM (20 mL) and sodium potassium tartrate (20 mL), dried in anhydrous MgSO_4 , filtered, and concentrated to dryness. The product **4** was purified using column chromatography with silica gel as the stationary phase and the product eluted in 100% hexanes.

B.1. Synthesis of diacylated alkene precursor (Procedure adapted from reference ¹¹⁴)



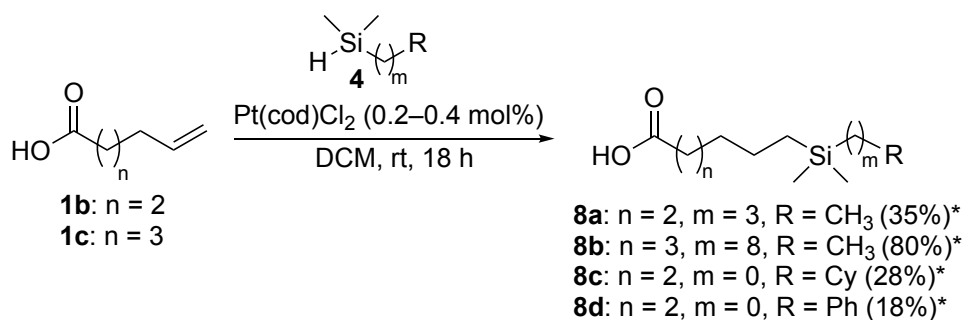
In one round bottom flask, alkenoic acid **1** (1 equiv) was added dropwise to a solution of CDI (1.5 equiv) in THF. In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.4 equiv) was added dropwise to a solution of rinsed/wash NaH (1.35 equiv) in THF. Both reactions were stirred at room temperature for 1 h. The two reaction mixtures were combined and stirred at 80 °C for 2–8 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **3** eluted in 1–2% MeOH/DCM.

B.2. Hydrosilylation of diacylated alkene precursor



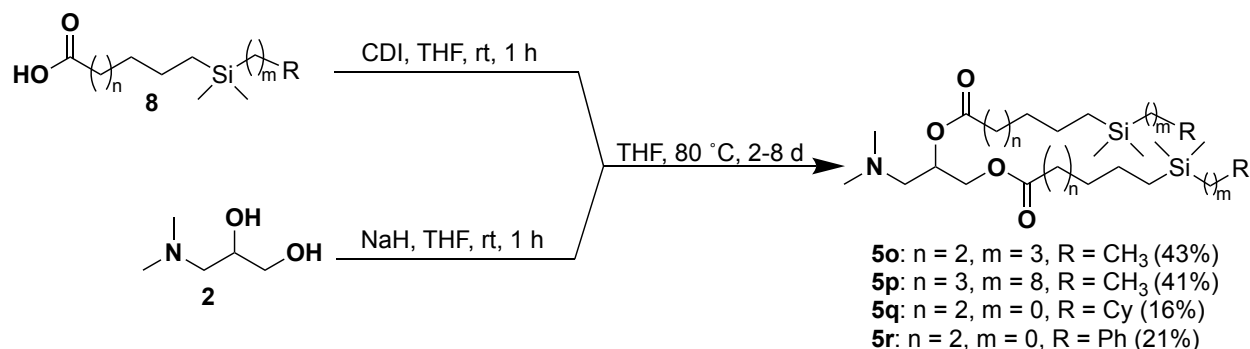
Diacylated alkene **3** (1 equiv), silane **4** (2.5 equiv), and $\text{Pt}_2(\text{dvtms})_3$ (0.7–2 mol %) were stirred in DCM at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and the product **5** eluted in 1–2% MeOH/DCM.

C.1. Hydrosilylation of alkenoic acid



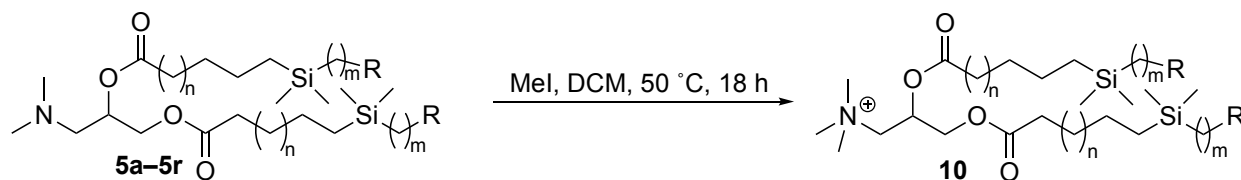
Alkenoic acid **1** (1 equiv), silane **4** (1.5 equiv), and an aliquot of $\text{Pt}(\text{cod})\text{Cl}_2$ stock solution (20 mg/mL in DCE, 0.2–0.4 mol %) were stirred in DCM at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and Hex/EtOAc as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 6:4 Hex/EtOAc solvent mixture. The product **8** eluted in 1–2% EtOAc/Hex.

C.2. Synthesis of diacylated silyl lipid precursor



In one round bottom flask, carboxylic acid **8** (1 equiv) was added dropwise to a solution of CDI (1.5 equiv) in THF. In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.4 equiv) was added dropwise to a solution of rinsed/wash NaH (1.35 equiv) in THF. Both reactions were stirred at room temperature for 1 h. The two reaction mixtures were combined and stirred at 80 °C for 2–8 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **5** eluted in 1–2% MeOH/DCM.

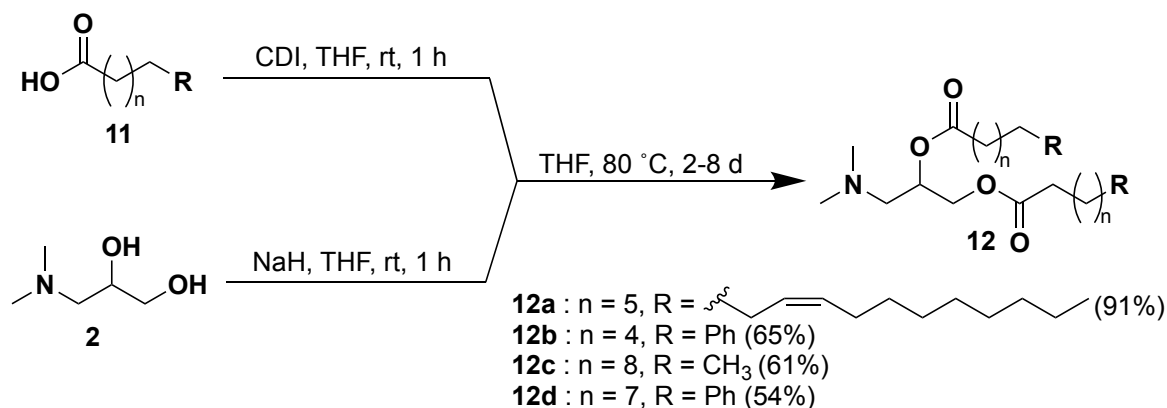
*D. Methylation to Access Silyl Lipids **10a–10r** (Procedure adapted from reference ¹¹⁵)*



10a (5Si4): n = 1, m = 3, R = CH ₃ (92%)	10j (6SiPh): n = 2, m = 0, R = Ph (94%)
10b (5Si8): n = 1, m = 7, R = CH ₃ (68%)	10k (7Si9): n = 3, m = 8, R = CH ₃ (95%)
10c (5Si11): n = 1, m = 10, R = CH ₃ (80%)	10l (8Si4): n = 4, m = 3, R = CH ₃ (33%)
10d (5SiCy): n = 1, m = 0, R = Cy (84%)	10m (8Si6): n = 4, m = 5, R = CH ₃ (38%)
10e (5SiPh): n = 1, m = 0, R = Ph (79%)	10n (8Si8): n = 4, m = 7, R = CH ₃ (84%)
10f (5Si2Ph): n = 1, m = 2, R = Ph (89%)	10o (8SiCy): n = 4, m = 0, R = Cy (26%)
10g (5Si3Ph): n = 1, m = 3, R = Ph (91%)	10p (8SiPh): n = 4, m = 0, R = Ph (28%)
10h (6Si4): n = 2, m = 3, R = CH ₃ (91%)	10q (8Si2Ph): n = 4, m = 2, R = Ph (20%)
10i (6SiCy): n = 2, m = 0, R = Cy (92%)	10r (8Si3Ph): n = 4, m = 3, R = Ph (93%)

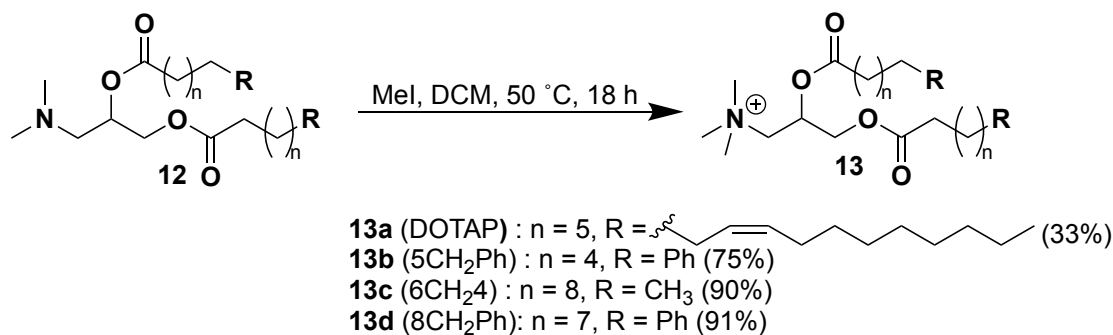
Silyl lipid **5** (1 equiv) and MeI (20 equiv) were stirred at 50 °C in DCM for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted lipid **5** eluted at 1–2% MeOH/DCM, and quaternized silyl lipid **10** eluted at 4–10% MeOH/DCM.

E.1. Synthesis of diacylated precursor (Procedure adapted from reference ¹¹⁴)



In one round bottom flask, carboxylic acid **11** (1 equiv) was added dropwise to a solution of CDI (1.5 equiv) in THF. In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.4 equiv) was added dropwise to a solution of rinsed/wash NaH (1.35 equiv) in THF. Both reactions were stirred at room temperature for 1 h. The two reaction mixtures were combined and stirred at 80 °C for 2–8 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **12** eluted in 1–2% MeOH/DCM.

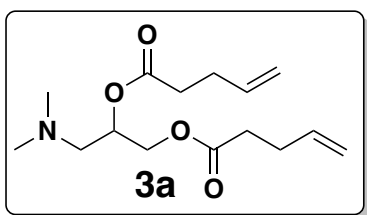
E.2. Methylation to access control lipids **13a–13d** (Procedure adapted from reference ¹¹⁵)



Lipid **12** (1 equiv) and MeI (20 equiv) were stirred at 50 °C in DCM for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **12** eluted at 1–2% MeOH/DCM and quaternized product **13** eluted at 4–10% MeOH/DCM.

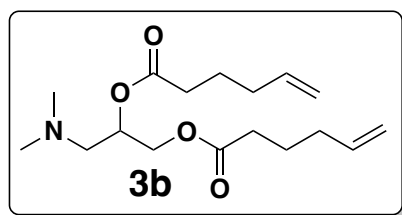
2.9B. Characterization of Cationic Silyl lipids

A. Alkene Precursors used in Route A



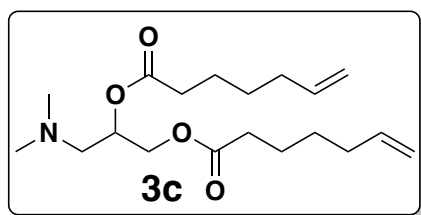
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** was synthesized according to Figure 17, Route A and General Synthetic Procedure B.1. In one round bottom flask, 4-pentenoic acid **1a** (0.72 mL, 7.05 mmol) was added dropwise to a solution of CDI (1.72 g, 10.6 mmol) in THF (12 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.33 mL, 2.82 mmol) was added dropwise to a solution of dried/washed NaH (228 mg, 9.5 mmol) in THF (12 mL). Both reactions were stirred

at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 6 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **3a** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 71% (570 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.81 (m, 2H), 5.19 (tt, J = 6.4, 3.1 Hz, 1H), 5.01 (m, 4H), 4.36 (dd, J = 11.9, 3.2 Hz, 1H), 4.09 (dd, J = 12.0, 6.4 Hz, 1H), 2.38 (m, 8H), 2.25 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 172.8, 172.6, 136.7, 115.7, 115.6, 69.5, 64.2, 59.4, 46.1, 33.7, 33.5, 28.9, 28.9. Exact mass: 283.18; acquired mass: 282.2796, 287.2123. HRMS (ESI) m/z: calc for C₁₅H₂₅NO₄H⁺ [M + H]⁺ 284.1862, found: 284.1609.



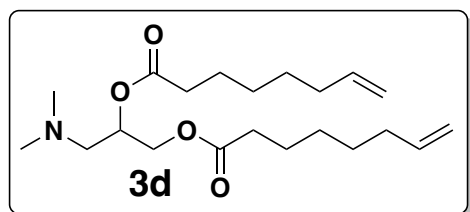
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(dimethylamino)propane-1,2-diyl bis(hex-5-enoate) **3b** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.1*. In one round bottom flask, 5-hexenoic acid **1b** (0.48 mL, 4 mmol) was added dropwise to a solution of CDI (973 mg, 6 mmol) in THF (10 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.19 mL, 1.6 mmol) was added dropwise to a solution of dried/washed NaH (130 mg, 5.4 mmol) in THF (10 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 2 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed

with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **3b** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 67% (343 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.77 (ddtd, $J = 16.9, 10.2, 6.7, 1.8$ Hz, 2H), 5.19 (qd, $J = 6.5, 3.1$ Hz, 1H), 5.01 (m, 4H), 4.37 (dd, $J = 12.0, 3.1$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.43 (m, 2H), 2.32 (td, $J = 7.5, 2.6$ Hz, 4H), 2.25 (s, 6H), 2.09 (dddd, $J = 12.8, 6.4, 2.6, 1.3$ Hz, 4H), 1.72 (pd, $J = 7.5, 1.5$ Hz, 4H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.4, 173.1, 137.8, 137.8, 115.5, 115.5, 69.5, 64.1, 59.5, 46.2, 33.8, 33.5, 33.1, 33.1, 24.2, 24.1.



This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(dimethylamino)propane-1,2-diyl bis(hept-6-enoate) **3c** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.1*. In one round bottom flask, 6-heptenoic acid **1c** (0.54 mL, 4 mmol) was added dropwise to a solution of CDI (973 mg, 6 mmol) in THF (6 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.19 mL, 1.6 mmol) was added dropwise to a solution of dried/washed NaH (134 mg, 5.6 mmol) in THF (5 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 76° C for 2 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 ,

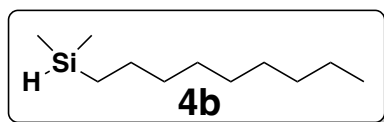
filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **3c** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 41% (229 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.78 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 2H), 5.21 (m, 1H), 4.96 (m, 4H), 4.35 (dd, *J* = 11.9, 3.3 Hz, 1H), 4.06 (dd, *J* = 11.9, 6.3 Hz, 1H), 2.53 (dd, *J* = 13.0, 7.0 Hz, 1H), 2.45 (m, 1H), 2.31 (td, *J* = 7.5, 4.1 Hz, 4H), 2.27 (s, 6H), 2.06 (m, 4H), 1.63 (m, 5H), 1.42 (ddq, *J* = 10.6, 5.8, 2.9 Hz, 4H).



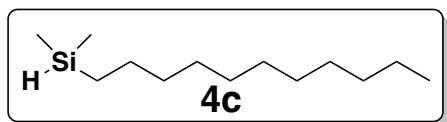
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.1*. In one round bottom flask, 7-octenoic acid **1d** (1.23 mL, 8 mmol) was added dropwise to a solution of CDI (1.95 g, 12 mmol) in THF (12 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.37 mL, 3.2 mmol) was added dropwise to a solution of dried/washed NaH (260 mg, 10.8 mmol) in THF (12 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 6 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber

contained a 9:1 DCM/MeOH solvent mixture. The product **3d** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 89% (1.05 g). ^1H NMR (400 MHz, CDCl_3) δ 5.79 (ddt, $J = 16.9, 10.2, 6.7$ Hz, 2H), 5.18 (tt, $J = 6.4, 3.1$ Hz, 1H), 4.95 (m, 4H), 4.36 (dd, $J = 11.9, 3.2$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.45 (m, 2H), 2.30 (td, $J = 7.5, 2.5$ Hz, 4H), 2.25 (s, 6H), 2.04 (q, $J = 7.0$ Hz, 4H), 1.62 (pd, $J = 7.5, 1.8$ Hz, 4H), 1.36 (m, 7H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 138.9, 138.9, 114.6, 69.4, 64.1, 59.5, 46.2, 34.5, 34.2, 33.7, 28.7, 28.7, 24.9, 24.9. HRMS (ESI) m/z : calc for $\text{C}_{21}\text{H}_{37}\text{NO}_4\text{Na}^+ [\text{M} + \text{Na}]^+$ 391.2654, found: 391.2681.

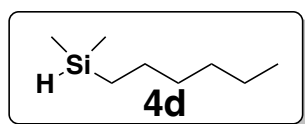
B. Silanes used in Hydrosilylation



Dimethyl(nonyl)silane **4b** was synthesized according to Figure 18A and General Synthetic Procedure *A.1*. Nonene (3 mmol, 0.52 mL), dimethylethoxysilane (7.5 mmol, 1.0 mL), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.07 mol%) were stirred in DCM at room temperature for 18 h. 4M LAH (3 mmol, 0.75 mL) was added dropwise to this reaction mixture at room temperature and stirred for 18 h. The reaction was quenched in an ice bath via the dropwise addition of aqueous sodium potassium tartrate and washed with DCM (20 mL) and sodium potassium tartrate (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The product **4b** was purified using column chromatography with silica gel as the stationary phase and **4b** eluted in 100% hexanes to obtain an isolated yield of 76% (348 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.83 (ts, $J = 7.1, 3.6$ Hz, 1H), 1.29 (m, 17H), 0.88 (t, $J = 6.8$ Hz, 3H), 0.58 (m, 2H), 0.06 (d, $J = 3.6$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 33.4, 32.1, 29.7, 29.6, 29.5, 24.5, 22.9, 14.3, 14.3, -4.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ -13.11 . HRMS (ESI) m/z : calc for $\text{C}_{11}\text{H}_{26}\text{SiK}^+ [\text{M} + \text{K}]^+$ 226.1437, found 226.1444.

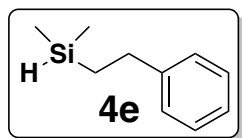


Dimethyl(undecyl)silane **4c** was synthesized according to Figure 18A and General Synthetic Procedure A.1. Undecene (2 mmol, 0.41 mL), dimethylethoxysilane (3 mmol, 0.41 mL), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.1 mol%) were stirred in DCM at room temperature for 18 h. 4M LAH (3 mmol, 0.75 mL) was added dropwise to this reaction mixture at room temperature and stirred for 18 h. The reaction was quenched in an ice bath via the dropwise addition of aqueous sodium potassium tartrate and washed with DCM (20 mL) and sodium potassium tartrate (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The product **4c** was purified using column chromatography with silica gel as the stationary phase and **4c** eluted in 100% hexanes to obtain an isolated yield of 90% (385 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.83 (ts, $J = 7.1, 3.6$ Hz, 1H), 1.30 (m, 26H), 0.89 (t, $J = 6.4$ Hz, 4H), 0.58 (m, 2H), 0.06 (d, $J = 3.7$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 33.4, 32.1, 29.9, 29.8, 29.8, 29.6, 24.5, 22.9, 14.3, 14.3, -4.3. ^{29}Si NMR (79 MHz, CDCl_3) δ -13.12. HRMS (ESI) m/z : calc for $\text{C}_{13}\text{H}_{30}\text{SiK}^+ [\text{M} + \text{K}]^+$ 254.1787, found: 254.2301.

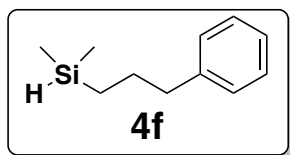


Hexyldimethylsilane **4d** was synthesized according to Figure 18B and General Synthetic Procedure A.2. 4 M LAH (4 mmol, 1.0 mL) was added dropwise to a solution of hexyldimethylchlorosilane (4 mmol, 0.82 mL) in DCM (2 mL) and stirred at room temperature for 18 h. The reaction was quenched in an ice bath via the dropwise addition of aqueous sodium potassium tartrate and washed with DCM (20 mL) and sodium potassium tartrate (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under

vacuum. The product **4d** was purified using column chromatography with silica gel as the stationary phase and **4d** eluted in 100% hexanes to obtain an isolated yield of 59% (342 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.84 (ts, $J = 7.1, 3.5$ Hz, 1H), 1.31 (m, 12H), 0.89 (t, 3H), 0.58 (m, 2H), 0.06 (d, $J = 3.6$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 33.0, 31.8, 24.5, 22.8, 14.3, 14.3, –4.3. ^{29}Si NMR (79 MHz, CDCl_3) –13.10. HRMS (ESI) m/z : calc for $\text{C}_8\text{H}_{20}\text{SiK}^+$ $[\text{M} + \text{K}]^+$ 183.0971, found 183.0806.



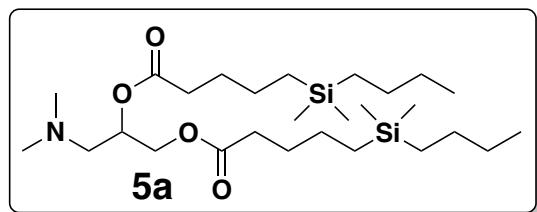
Dimethyl(phenylethyl)silane **4e** was synthesized according to Figure 18B and General Synthetic Procedure A.2. 4 M LAH (2 mmol, 0.5 mL) was added dropwise to a solution of chlorodimethyl(2-phenylethyl)silane (2 mmol, 0.4 mL) in DCM (2 mL) and stirred at room temperature for 18 h. The reaction was quenched in an ice bath via the dropwise addition of aqueous sodium potassium tartrate and washed with DCM (20 mL) and sodium potassium tartrate (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The product **4e** was purified using column chromatography with silica gel as the stationary phase and **4e** eluted in 100% hexanes to obtain an isolated yield of 94% (310 mg). ^1H NMR (300 MHz, CDCl_3) δ 7.21 (m, 6H), 3.90 (ts, $J = 7.1, 3.6$ Hz, 1H), 2.68 (m, 2H), 0.96 (dt, 2H), 0.08 (d, $J = 3.7$ Hz, 6H). ^{13}C NMR (76 MHz, CDCl_3) δ 144.9, 128.5, 127.9, 125.7, 30.7, 16.3, –4.4. ^{29}Si NMR (79 MHz, CDCl_3) δ –13.12. HRMS (ESI) m/z : calc for $\text{C}_{10}\text{H}_{16}\text{SiH}^+$ $[\text{M} + \text{H}]^+$ 165.1100, found: 165.1136.



Dimethyl(3-phenylpropyl)silane **4f** was synthesized according to Figure 18B and General Synthetic Procedure A.2. 4 M LAH (2 mmol, 0.5 mL) was added dropwise to a solution of

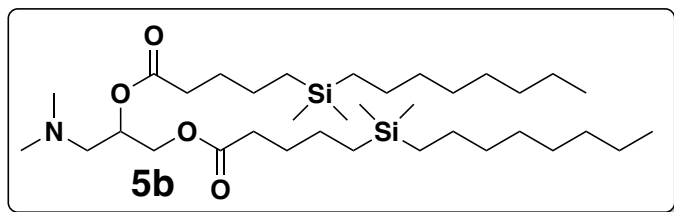
chlorodimethyl(3-phenylpropyl)silane (2 mmol, 0.44 mL) in DCM (2 mL) and stirred at room temperature for 18 h. The reaction was quenched in an ice bath via the dropwise addition of aqueous sodium potassium tartrate and washed with DCM (20 mL) and sodium potassium tartrate (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The product **4f** was purified using column chromatography with silica gel as the stationary phase and **4f** eluted in 100% hexanes to obtain an isolated yield of 93% (330 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.25 (m, 2H), 7.15 (m, 3H), 3.84 (ts, $J = 7.1, 3.6$ Hz, 1H), 2.61 (t, 2H), 1.63 (m, 2H), 0.60 (m, 2H), 0.52 (m, 0H), 0.04 (d, $J = 3.6$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 142.7, 128.6, 128.4, 125.8, 39.6, 26.6, 14.1, -4.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ -13.11 . HRMS (ESI) m/z : calc for $\text{C}_{11}\text{H}_{18}\text{SiH}^+$ $[\text{M} + \text{H}]^+$ 179.1256, found: 179.1262.

C. Un-Methylated Silyl Lipid Precursors



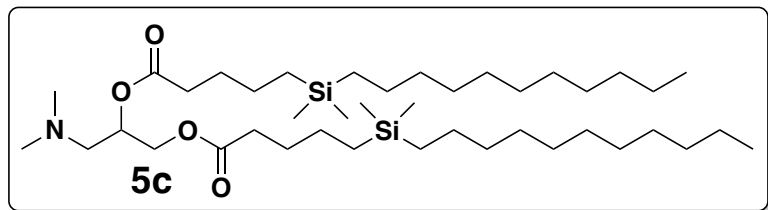
3-(Dimethylamino)propane-1,2-diyl bis(5-(butyldimethylsilyl)pentanoate) **5a** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (80 mg, 0.3 mmol), butyldimethylsilane **4a** (0.12 mL, 0.7 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4a** eluted in 100% DCM and product **5a** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 79% (116 mg). ^1H NMR

(400 MHz, CDCl₃) δ 5.19 (tt, J = 6.4, 3.2 Hz, 1H), 4.35 (dd, J = 12.0, 3.2 Hz, 1H), 4.08 (dd, J = 11.9, 6.4 Hz, 1H), 2.45 (m, 2H), 2.31 (td, J = 7.6, 2.7 Hz, 4H), 2.26 (s, 6H), 1.64 (qd, J = 7.5, 2.4 Hz, 4H), 1.28 (m, 11H), 0.87 (t, J = 7.0 Hz, 6H), 0.48 (dt, J = 12.7, 4.9 Hz, 8H), -0.06 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.7, 173.4, 69.4, 64.1, 59.5, 46.2, 34.3, 34.1, 28.9, 28.9, 26.8, 26.3, 23.7, 23.7, 15.2, 15.1, 13.9, -3.3. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.31. HRMS (ESI) m/z : calc for C₂₇H₅₇NO₄Si₂Li⁺ [M + Li]⁺ 524.4053, found: 524.4134.

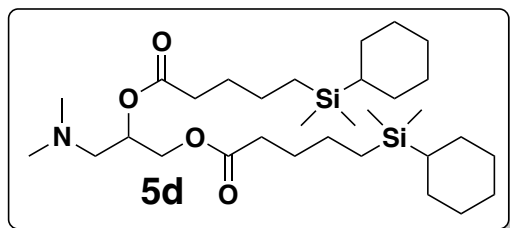


3-(Dimethylamino)propane-1,2-diyl bis(5-(dimethyl(octyl)silyl)pentanoate) **5b** was synthesized according to Figure 17, Route A and General Synthetic Procedure B.2. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (80 mg, 0.3 mmol), octyldimethylsilane **4** (0.16 mL, 0.7 mmol), and catalytic Pt₂(dvtms)₃ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5b** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 97% (172 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.19 (tt, J = 6.4, 3.2 Hz, 1H), 4.35 (dd, J = 11.9, 3.2 Hz, 1H), 4.08 (dd, J = 11.9, 6.4 Hz, 1H), 2.45 (m, 2H), 2.31 (td, J = 7.6, 2.8 Hz, 4H), 2.25 (s, 6H), 1.63 (pd, J = 7.5, 2.4 Hz, 4H), 1.33 (m, 31H), 0.88 (t, J = 6.8 Hz, 6H), 0.48 (m, 8H), -0.06 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.6, 173.3, 69.3, 64.0, 59.5, 46.2, 34.3, 34.0, 33.8, 32.1, 29.5, 29.4, 28.9, 28.9,

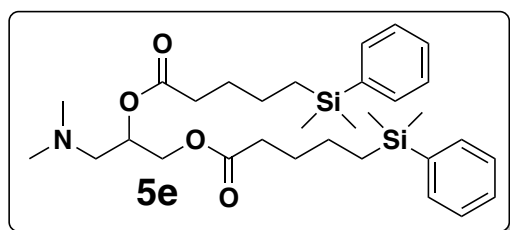
23.9, 23.7, 23.6, 22.8, 15.3, 15.1, 14.2, -3.3. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.27. HRMS (ESI) m/z : calc for $\text{C}_{35}\text{H}_{73}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ Calculated mass: 628.5156, found: 628.5151.



3-(Dimethylamino)propane-1,2-diyl bis(5-dimethyl(undecyl)silyl)pentanoate) **5c** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Dimethyl(undecyl)silane **4c** (147 mg, 0.7 mmol), diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (78 mg, 0.3 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4c** eluted in 100% DCM and product **5c** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 70% (172 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.19 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.35 (dd, $J = 11.9, 1.3$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.45 (m, 2H), 2.31 (td, $J = 7.6, 3.1$ Hz, 4H), 2.26 (s, 8H), 1.64 (m, 6H), 1.32 (m, 41H), 0.88 (t, $J = 6.7$ Hz, 6H), 0.48 (m, 8H), -0.06 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 173.4, 69.4, 65.7, 64.1, 62.1, 59.6, 46.7, 46.2, 34.3, 34.1, 33.9, 32.1, 31.1, 29.9, 29.8, 29.8, 29.6, 29.5, 28.9, 28.9, 24.0, 23.7, 23.7, 22.8, 15.4, 15.2, 14.3, -3.3. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.27. HRMS (ESI) m/z : calc for $\text{C}_{41}\text{H}_{85}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ 712.6095, found: 712.6095.

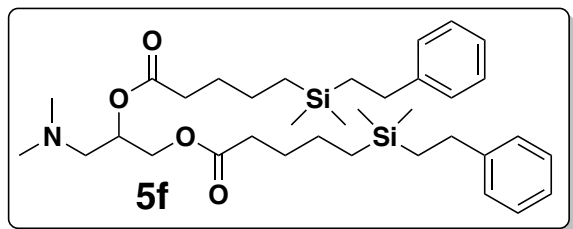


3-(Dimethylamino)propane-1,2-diyl bis(5-cyclohexyldimethylsilyl)pentanoate) **5d** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (80 mg, 0.3 mmol), cyclohexyldimethylsilane **4** (0.12 mL, 0.7 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5d** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 89% (143 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.19 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.35 (dd, $J = 11.9, 3.2$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.45 (m, 2H), 2.31 (td, $J = 7.6, 2.8$ Hz, 4H), 2.25 (s, 6H), 1.64 (m, 16H), 1.21 (m, 14H), 0.57 (tt, $J = 12.5, 3.1$ Hz, 2H), 0.47 (m, 4H), -0.10 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 69.3, 63.9, 59.4, 46.1, 34.2, 33.9, 28.9, 28.9, 28.2, 27.5, 27.1, 25.3, 23.7, 23.6, 13.3, 13.3, -5.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 3.01. HRMS (ESI) m/z : calc for $\text{C}_{31}\text{H}_{61}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ 568.4217, found: 568.4257.



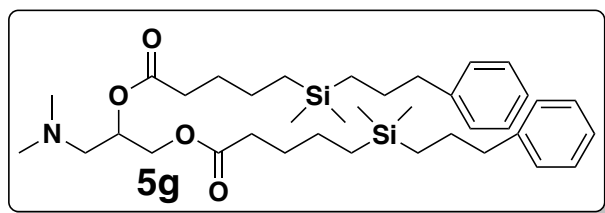
3-(Dimethylamino)propane-1,2-diyl bis(5-(dimethyl(phenyl)silyl)pentanoate) **5e** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-

(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (80 mg, 0.3 mmol), phenyldimethylsilane **4** (0.11 mL, 0.7 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5e** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 89% (139 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.49 (m, 4H), 7.35 (m, 6H), 5.16 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.33 (dd, $J = 11.9, 3.2$ Hz, 1H), 4.05 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.42 (m, 2H), 2.29 (m, 4H), 2.24 (s, 6H), 1.63 (pd, $J = 7.5, 2.7$ Hz, 5H), 1.33 (m, 5H), 0.75 (m, 4H), 0.25 (s, 13H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 139.5, 133.7, 129.1, 127.9, 69.4, 64.1, 59.6, 46.2, 34.3, 34.0, 28.9, 28.8, 23.7, 23.7, 15.7, –2.9. ^{29}Si NMR (79 MHz, CDCl_3) δ –3.10. HRMS (ESI) m/z : calc for $\text{C}_{31}\text{H}_{49}\text{NO}_4\text{Si}_2\text{H}^+ [\text{M} + \text{H}]^+$ 556.3278, found: 556.3280.



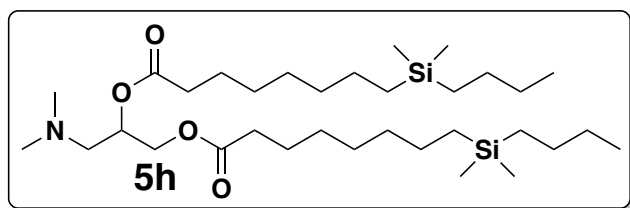
3-(Dimethylamino)propane-1,2-diyl bis(5-(dimethyl(phenethyl)silyl)pentanoate) **5f** was synthesized according to Figure 17, Route A and General Synthetic Procedure B.2. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (80 mg, 0.3 mmol), dimethyl(phenylethyl)silane **4e** (140 mg, 0.8 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified via a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with

KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4e** eluted in 100% DCM and product **5f** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 67% (116 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 5H), 7.17 (m, 6H), 5.19 (tt, *J* = 6.4, 3.2 Hz, 1H), 4.36 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.08 (dd, *J* = 11.9, 1.9 Hz, 1H), 2.61 (m, 4H), 2.43 (m, 2H), 2.30 (td, *J* = 7.5, 3.4 Hz, 4H), 2.25 (s, 6H), 1.63 (pd, *J* = 7.5, 3.1 Hz, 4H), 1.33 (m, 3H), 0.87 (m, 4H), 0.52 (m, 4H), –0.01 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.4, 173.2, 145.2, 128.3, 127.7, 125.5, 69.3, 63.9, 59.4, 46.1, 34.1, 33.9, 29.9, 28.7, 28.7, 23.5, 23.5, 17.2, 14.9, –3.5. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.5. HRMS (ESI) *m/z*: calc for C₃₅H₅₇NO₄Si₂Na⁺ [*M* + Na]⁺ 634.3724, found: 634.3507.

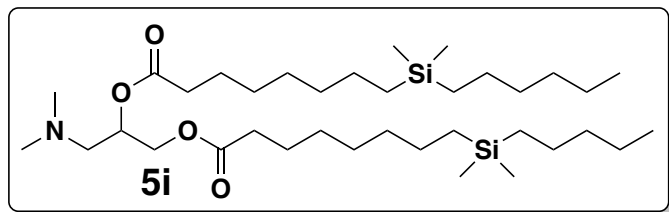


3-(Dimethylamino)propane-1,2-diyl bis(5-(dimethyl(3-phenylpropyl)silyl)pentanoate) **5g** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(pent-4-enoate) **3a** (100 mg, 0.35 mmol), dimethyl(3-phenylpropyl)silane **4f** (189 mg, 1 mmol), and catalytic Pt₂(dvtms)₃ (0.7 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4f** eluted in 100% DCM and product **5g** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 75% (170 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 4H), 7.17 (ddd, *J* = 7.8, 5.8, 1.6 Hz, 6H), 5.19 (tt, *J* = 6.4, 3.2 Hz, 1H), 4.35 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.08 (m, 1H),

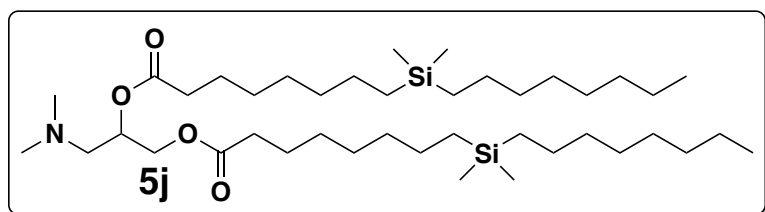
2.61 (t, $J = 7.7$ Hz, 4H), 2.43 (m, 3H), 2.30 (dt, $J = 7.7, 3.8$ Hz, 3H), 2.25 (s, 6H), 1.62 (m, 8H), 1.30 (m, 4H), 0.52 (m, 8H), -0.05 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 142.8, 128.6, 128.4, 125.8, 69.4, 64.1, 59.5, 53.6, 46.2, 40.1, 34.3, 34.0, 28.9, 28.9, 26.2, 23.7, 23.6, 15.3, 15.1, -3.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.37. HRMS (ESI) m/z : calc for $\text{C}_{37}\text{H}_{61}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ 640.4217, found: 640.4222.



3-(Dimethylamino)propane-1,2-diyl bis(8-(butyldimethylsilyl)octanoate) **5h** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (100 mg, 0.3 mmol), butyldimethylsilane **4a** (0.11 mL, 0.7 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted butyldimethylsilane **4a** eluted in 100% DCM and product **5h** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 93% (151 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.20 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.35 (dd, $J = 11.9, 3.2$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.3$ Hz, 1H), 2.46 (m, 2H), 2.30 (td, $J = 7.5, 3.0$ Hz, 5H), 2.26 (s, 6H), 1.61 (m, 6H), 1.27 (m, 29H), 0.88 (t, $J = 7.0$ Hz, 6H), 0.47 (m, 8H), -0.06 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 173.4, 69.2, 64.1, 59.5, 46.0, 34.6, 34.3, 33.7, 29.3, 29.2, 29.2, 29.2, 26.8, 26.3, 25.1, 25.1, 24.0, 15.4, 15.1, 13.9, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.22. HRMS (ESI) m/z : calc for $\text{C}_{33}\text{H}_{69}\text{NO}_4\text{Si}_2\text{K}^+$ $[\text{M} + \text{K}]^+$ 624.4631, found: 624.8127.

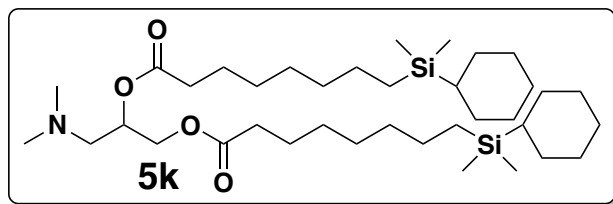


3-(Dimethylamino)propane-1,2-diyl bis(8-(hexyldimethylsilyl)octanoate) **5i** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (130 mg, 0.14 mmol), hexyldimethylsilane **4d** (86 mg, 0.6 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (2 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted hexyldimethylsilane **4d** eluted in 100% DCM and product **5i** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 94% (122 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.20 (tt, $J = 6.3, 3.2$ Hz, 1H), 4.35 (dd, $J = 12.0, 3.3$ Hz, 1H), 4.08 (dd, $J = 11.9, 6.3$ Hz, 1H), 2.46 (m, 2H), 2.30 (td, $J = 7.6, 2.8$ Hz, 5H), 2.26 (s, 6H), 1.62 (m, 6H), 1.28 (m, 34H), 0.88 (t, 7H), 0.46 (m, 8H), -0.07 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 173.4, 69.2, 64.1, 59.4, 46.0, 34.6, 34.3, 33.7, 33.5, 31.8, 29.3, 29.2, 29.2, 25.1, 25.1, 24.0, 24.0, 22.8, 15.4, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.18. HRMS (ESI) m/z : calc for $\text{C}_{37}\text{H}_{77}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ 656.5469, found: 656.5472.



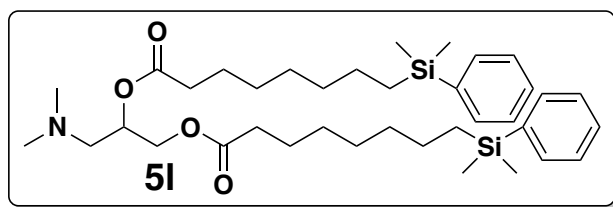
3-(Dimethylamino)propane-1,2-diyl bis(8-(dimethyl(octyl)silyl)octanoate) **5j** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-

(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (51 mg, 0.14 mmol), octyldimethylsilane **4** (80 μ L, 0.35 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (2 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5j** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 53% (51 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.22 (tt, J = 6.2, 3.3 Hz, 1H), 4.34 (dd, J = 11.9, 3.3 Hz, 1H), 4.07 (dd, J = 11.9, 6.3 Hz, 1H), 2.50 (m, 2H), 2.31 (m, 4H), 2.28 (s, 5H), 1.61 (m, 5H), 1.28 (m, 48H), 0.88 (t, J = 6.8 Hz, 6H), 0.46 (m, 8H), -0.07 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.4, 69.0, 64.0, 59.3, 45.8, 34.5, 34.3, 33.9, 33.7, 33.7, 33.7, 32.1, 29.5, 29.5, 29.3, 29.2, 29.2, 29.2, 24.1, 22.8, 15.5, 15.4, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.18. HRMS (ESI) m/z : calc for $\text{C}_{41}\text{H}_{85}\text{NO}_4\text{Si}_2\text{NH}_4^+$ $[\text{M} + \text{NH}_4]^+$ 729.6361, found: 729.6310.



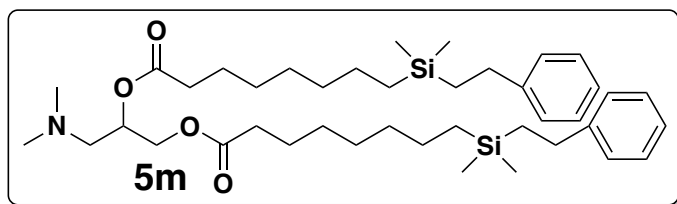
3-(Dimethylamino)propane-1,2-diyl bis(8-(cyclohexyldimethylsilyl)octanoate) **5k** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (100 mg, 0.3 mmol), cyclohexyldimethylsilane **4** (0.12 mL, 0.7 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (0.9 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with

KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5k** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 95% (200 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.20 (tt, *J* = 6.4, 3.2 Hz, 1H), 4.35 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.08 (dd, *J* = 11.9, 6.4 Hz, 1H), 2.46 (m, 2H), 2.31 (dt, *J* = 7.6, 3.8 Hz, 4H), 2.26 (s, 6H), 1.72 (m, 7H), 1.63 (tt, *J* = 13.5, 4.9 Hz, 12H), 1.15 (m, 34H), 0.57 (tt, *J* = 12.5, 3.1 Hz, 2H), 0.45 (m, 4H), –0.11 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.7, 173.4, 69.3, 64.1, 59.5, 46.1, 34.6, 34.3, 33.8, 29.3, 29.2, 29.2, 29.2, 29.1, 29.1, 28.3, 27.6, 27.6, 27.2, 25.5, 25.1, 25.1, 24.1, 13.7, –5.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 17.50, 2.93. HRMS (ESI) *m/z*: calc for C₃₇H₇₃NO₄Si₂Li⁺ [M + Li]⁺ 658.5238, found: 658.5233.

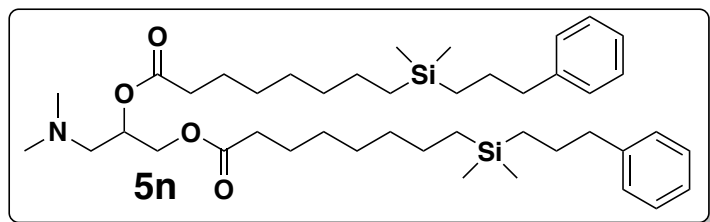


3-(Dimethylamino)propane-1,2-diyl bis(8-(dimethyl(phenyl)silyl)octanoate) **5I** was synthesized according to Figure 17, Route A and General Synthetic Procedure *B.2*. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (80 mg, 0.27 mmol), phenyldimethylsilane **4** (80 μL, 0.68 mmol), and catalytic Pt₂(dvtms)₃ (1 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4** eluted in 100% DCM and product **5I** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 71% (124 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.50 (m, 4H), 7.34 (m, 6H), 5.18 (tt, *J* = 6.5, 3.2 Hz, 1H), 4.35 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.08 (dd, *J* = 11.9, 6.4 Hz, 1H), 2.44 (m, 2H), 2.30 (m, 1H), 2.25 (s, 5H), 1.60 (m,

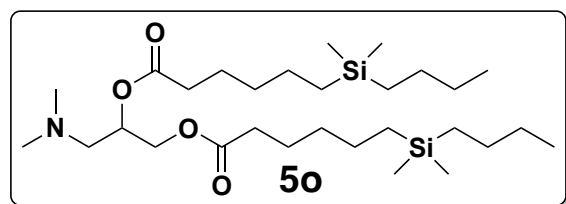
8H), 1.28 (m, 22H), 0.73 (m, 5H), 0.25 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.4, 139.8, 133.7, 128.9, 127.8, 69.4, 64.1, 59.5, 46.2, 34.5, 34.3, 33.5, 29.2, 29.2, 29.1, 25.1, 25.1, 23.9, 15.8, -2.9 . ^{29}Si NMR (79 MHz, CDCl_3) δ -3.14 . HRMS (ESI) m/z : calc for $\text{C}_{37}\text{H}_{61}\text{NO}_4\text{Si}_2\text{NH}_4^+ [\text{M} + \text{NH}_4]^+$ 657.4483, found: 657.5501.



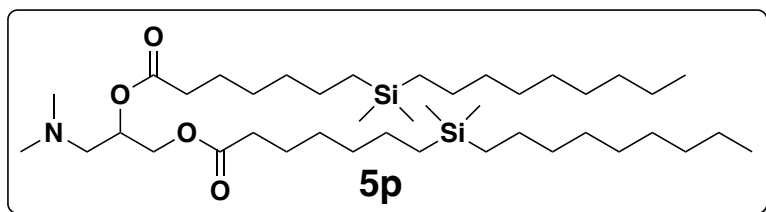
3-(Dimethylamino)propane-1,2-diyl bis(8-((dimethyl(phenethyl)silyl)octanoate) **5m** was synthesized according to Figure 17, Route A and General Synthetic Procedure B.2. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (57 mg, 0.14 mmol), dimethyl(phenylethyl)silane **4e** (56 mg, 0.34 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (1.8 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4e** eluted in 100% DCM and product **5m** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 95% (90 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.06 (m, 10H), 5.04 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.21 (dd, $J = 11.9, 1.4$ Hz, 1H), 3.94 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.46 (m, 3H), 2.29 (m, 2H), 2.15 (td, $J = 7.6, 2.6$ Hz, 4H), 2.10 (s, 6H), 1.45 (m, 6H), 1.14 (m, 18H), 0.72 (m, 5H), 0.35 (m, 3H), -0.16 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 145.4, 128.3, 127.8, 125.5, 69.3, 63.9, 59.5, 46.1, 34.5, 34.2, 33.6, 30.1, 29.2, 29.1, 29.1, 29.0, 25.1, 25.0, 25.0, 24.9, 23.9, 17.4, 15.2, -3.4 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.47. HRMS (ESI) m/z : calc for $\text{C}_{41}\text{H}_{69}\text{NO}_4\text{Si}_2\text{K}^+ [\text{M} + \text{K}]^+$ 734.4402, found: 734.4474.



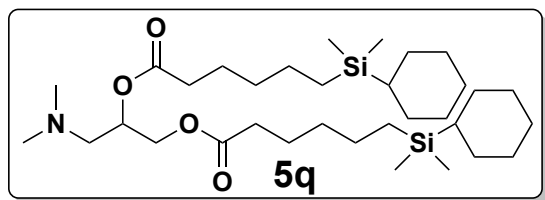
3-(Dimethylamino)propane-1,2-diyl bis(8-(dimethyl(3-phenylpropyl)silyl)octanoate) **5h** was synthesized according to Figure 17, Route A and General Synthetic Procedure B.2. Diacylated alkene 3-(dimethylamino)propane-1,2-diyl bis(oct-7-enoate) **3d** (50 mg, 0.14 mmol), dimethyl(3-phenylpropyl)silane **4f** (75 mg, 0.42 mmol), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (1.8 mol%) were stirred in DCM (1 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. Unreacted silane **4f** eluted in 100% DCM and product **5n** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 82% (82 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.27 (m, 6H), 7.18 (ddd, $J = 7.6, 5.5, 2.0$ Hz, 6H), 5.19 (tt, $J = 6.4, 3.2$ Hz, 1H), 4.36 (dd, $J = 11.9, 1.1$ Hz, 1H), 4.09 (dd, $J = 11.9, 6.4$ Hz, 1H), 2.63 (m, 4H), 2.43 (m, 3H), 2.30 (td, $J = 7.6, 2.5$ Hz, 5H), 2.25 (s, 7H), 1.60 (m, 12H), 1.27 (m, 21H), 0.53 (m, 4H), 0.46 (t, $J = 7.9$ Hz, 4H), -0.06 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 173.4, 142.9, 128.7, 128.4, 125.8, 69.5, 64.1, 59.6, 46.3, 40.2, 34.6, 34.4, 33.7, 29.3, 29.3, 29.2, 26.3, 25.2, 25.1, 24.0, 15.4, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.27. HRMS (ESI) m/z : calc for $\text{C}_{43}\text{H}_{73}\text{NO}_4\text{Si}_2\text{H}^+$ $[\text{M} + \text{H}]^+$ 724.5156, found: 724.5138.



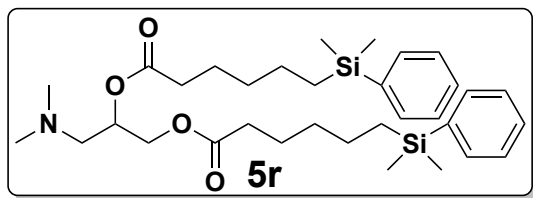
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(6-(butyldimethylsilyl)hexanoate) **5o** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.2. In one round bottom flask, 6-(butyldimethylsilyl)hexanoic acid **8a** (114 mg, 0.5 mmol) was added dropwise to a solution of CDI (120 mg, 0.7 mmol) in THF (2 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (23 μ L, 0.2 mmol) was added dropwise to a solution of dried/washed NaH (16 mg, 0.7 mmol) in THF (2 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 6 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with anhydrous DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **5o** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 43% (45 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.19 (tt, J = 6.4, 3.1 Hz, 1H), 4.36 (dd, J = 11.9, 3.2 Hz, 1H), 4.09 (dd, J = 11.9, 6.4 Hz, 1H), 2.45 (m, 2H), 2.30 (td, J = 7.5, 2.3 Hz, 4H), 2.25 (s, 7H), 1.62 (m, 5H), 1.27 (m, 17H), 0.88 (t, J = 7.0 Hz, 6H), 0.47 (m, 7H), –0.06 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.7, 173.4, 69.4, 64.1, 59.6, 46.2, 34.5, 34.3, 33.2, 33.2, 26.8, 26.3, 24.8, 24.8, 23.7, 15.3, 15.3, 15.1, 13.9, –3.3. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.25. HRMS (ESI) m/z : calc for C₂₉H₆₁NO₄Si₂H⁺ [M + H]⁺ 544.4217, found: 544.4212.



This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(7-(dimethyl(nonyl)silyl)heptanoate) **5p** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.2. In one round bottom flask, 7-(dimethyl(nonyl)silyl)heptanoic acid **8b** (180 mg, 0.57 mmol) was added dropwise to a solution of CDI (247 mg, 1.5 mmol) in THF (7 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (30 μ L mg, 0.25 mmol) was added dropwise to a solution of dried/washed NaH (112 mg, 4.7 mmol) in THF (7 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 ° C for 4 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with anhydrous DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **5p** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 41% (75 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.19 (tt, J = 6.4, 3.2 Hz, 1H), 4.36 (dd, J = 11.9, 3.2 Hz, 1H), 4.09 (dd, J = 11.9, 6.4 Hz, 1H), 2.45 (m, 2H), 2.30 (td, J = 7.5, 2.4 Hz, 4H), 2.25 (s, 7H), 1.63 (m, 5H), 1.29 (m, 45H), 0.88 (t, J = 6.8 Hz, 6H), 0.46 (m, 8H), –0.07 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.9, 66.4, 65.8, 62.9, 54.9, 34.4, 34.1, 33.9, 33.5, 33.4, 32.1, 29.7, 29.5, 29.5, 28.9, 28.9, 24.9, 24.7, 24.0, 23.9, 23.9, 22.8, 15.4, 15.4, 14.3, –3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.20. HRMS (ESI) m/z : calc for C₄₁H₈₅NO₄Si₂NH₄⁺ [M + NH₄]⁺ 729.6361, found: 729.6284.

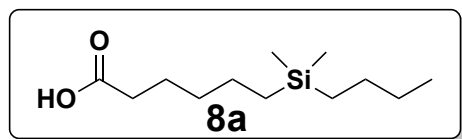


This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(6-(cyclohexyldimethylsilyl)hexanoate) **5q** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.2. In one round bottom flask, 6-(cyclohexyldimethylsilyl)hexanoic acid **8c** (64 mg, 0.35 mmol) was added dropwise to a solution of CDI (59 mg, 0.5 mmol) in THF (2 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (12 μ L, 0.14 mmol) was added dropwise to a solution of dried/washed NaH (8 mg, 0.5 mmol) in THF (2 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 7 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with anhydrous DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **5q** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 16% (13 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.19 (tt, *J* = 6.4, 3.2 Hz, 1H), 4.36 (dd, *J* = 11.9, 3.2 Hz, 1H), 4.09 (dd, *J* = 11.9, 6.4 Hz, 1H), 2.43 (m, 1H), 2.31 (m, 4H), 2.25 (s, 5H), 1.71 (m, 7H), 1.63 (m, 11H), 1.30 (m, 15H), 1.18 (d, *J* = 10.1 Hz, 7H), 1.06 (m, 5H), 0.56 (tt, *J* = 12.5, 3.1 Hz, 2H), 0.46 (m, 4H), –0.11 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.7, 173.4, 69.4, 64.1, 59.6, 46.2, 34.5, 34.3, 33.4, 33.3, 28.3, 27.6, 27.2, 25.5, 24.8, 24.7, 23.8, 13.5, 13.5, –5.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.96. HRMS (ESI) *m/z*: calc for C₃₃H₆₅NO₄Si₂Na⁺ [*M* + Na]⁺ 618.4350, found: 618.4351.

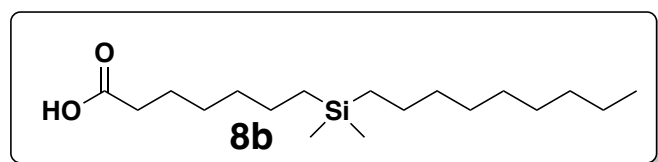


This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(6-(dimethyl(phenyl)silyl)hexanoate) **5r** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.2. In one round bottom flask, 6-(dimethyl(phenyl)silyl)hexanoic acid **8d** (86 mg, 0.34 mmol) was added dropwise to a solution of CDI (84 mg, 0.5 mmol) in THF (2 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (16 μ L, 0.14 mmol) was added dropwise to a solution of dried/washed NaH (11 mg, 0.5 mmol) in THF (2 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 7 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with anhydrous DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **5r** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 21% (17 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.49 (ddd, J = 6.7, 3.7, 1.6 Hz, 4H), 7.34 (dd, J = 5.7, 2.0 Hz, 6H), 5.17 (tt, J = 6.3, 3.3 Hz, 1H), 4.35 (m, 1H), 4.07 (m, 1H), 2.43 (m, 2H), 2.29 (m, 4H), 2.24 (s, 6H), 1.61 (m, 7H), 1.31 (m, 12H), 0.73 (m, 4H), 0.25 (s, 13H). ¹³C NMR (101 MHz, CDCl₃) δ 173.6, 173.3, 139.6, 133.7, 128.9, 127.9, 69.4, 64.1, 59.5, 46.2, 34.5, 34.2, 33.1, 33.1, 24.8, 24.7, 24.7, 24.7, 23.7, 15.7, 15.7, –2.9. ²⁹Si NMR (79 MHz, CDCl₃) δ –3.13. HRMS (ESI) m/z : calc for C₃₃H₅₃NO₄Si₂Na⁺ [M + Na]⁺ 606.3411, found: 606.3415.

D. Silyl Carboxylic Acid Precursors

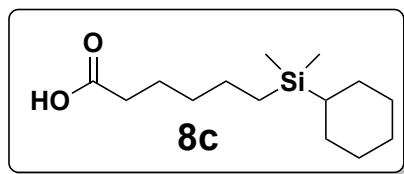


6-(n-Butyldimethylsilyl)hexanoic acid **8a** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.1. 5-Hexenoic acid **1b** (0.23 mL, 2 mmol), n-butyldimethylsilane **4a** (0.49 mL, 3 mmol), and a stock solution of Pt(cod)Cl₂ in DCE (20 mg/mL, 0.2 mol%) was stirred in DCM (2 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and Hex/EtOAc as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 6:4 Hex/EtOAc solvent mixture. The product **8a** eluted in 1–2% EtOAc/Hex to obtain an isolated yield of 35% (158 mg). ¹H NMR (400 MHz, CDCl₃) δ 2.34 (t, *J* = 7.5 Hz, 2H), 1.64 (p, *J* = 7.4 Hz, 2H), 1.30 (m, 7H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.48 (m, 4H), –0.06 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 180.3, 34.1, 33.2, 26.8, 26.3, 24.5, 23.7, 15.3, 15.1, 13.9, –3.3. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.27. HRMS (ESI) *m/z*: calc for C₁₂H₂₅O₂Si[–] [M – H][–] 229.1624, found: 229.1626.

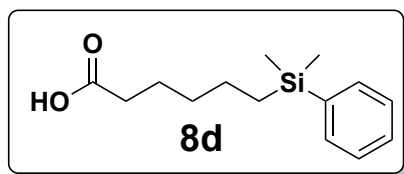


7-(dimethyl(nonyl)silyl)heptanoic acid **8b** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.1. 6-Heptenoic acid **1c** (0.10 mL, 0.74 mmol), dimethyl(nonyl)silane **4b** (207 mg, 1.11 mmol), and a stock solution of Pt(cod)Cl₂ in DCE (20 mg/mL, 0.4 mol%) was stirred in DCM (2 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and Hex/EtOAc as the mobile phase. The fractions were spotted on TLC

plates and stained with KMnO_4 . The solvent chamber contained a 6:4 Hex/EtOAc solvent mixture. The product **8b** eluted in 1–2% EtOAc/Hex to obtain an isolated yield of 80% (185 mg). ^1H NMR (400 MHz, CDCl_3) δ 2.35 (t, $J = 7.5$ Hz, 2H), 1.64 (m, 2H), 1.30 (m, 24H), 0.88 (t, 4H), 0.47 (dt, $J = 10.0, 5.5$ Hz, 4H), -0.06 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.7, 35.1, 34.3, 34.1, 33.7, 32.3, 32.0, 30.0, 29.8, 29.2, 25.1, 24.3, 24.1, 23.1, 15.7, 14.5, -2.9 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.19. HRMS (ESI) m/z : calc for $\text{C}_{18}\text{H}_{38}\text{O}_2\text{SiNa}^+ [\text{M} + \text{K}]^+$ 353.2278, found: 353.2178.

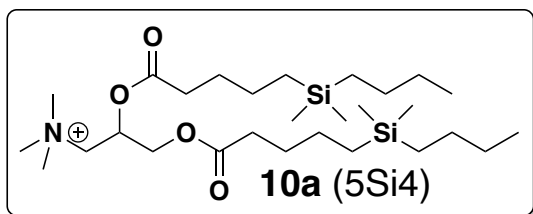


6-(cyclohexyldimethylsilyl)hexanoic acid **8c** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.I. 5-Hexenoic acid **1b** (0.23 mL, 2 mmol), cyclohexyldimethylsilane **4** (0.53 mL, 3 mmol), and a stock solution of $\text{Pt}(\text{cod})\text{Cl}_2$ in DCE (20 mg/mL, 0.2 mol%) was stirred in DCM (2 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and Hex/EtOAc as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 6:4 Hex/EtOAc solvent mixture. The product **8c** eluted in 1–2% EtOAc/Hex to obtain an isolated yield of 28% (137 mg). ^1H NMR (400 MHz, CDCl_3) δ 2.35 (td, $J = 7.5, 2.5$ Hz, 2H), 1.68 (m, 8H), 1.24 (m, 8H), 0.57 (tt, $J = 12.5, 3.1$ Hz, 1H), 0.47 (m, 2H), -0.10 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.9, 34.1, 33.3, 28.3, 27.6, 27.2, 25.5, 24.5, 23.8, 13.5, -5.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.97. HRMS (ESI) m/z : calc for $\text{C}_{14}\text{H}_{27}\text{O}_2\text{Si}^- [\text{M} - \text{H}]^-$ 255.1780, found: 255.1785.



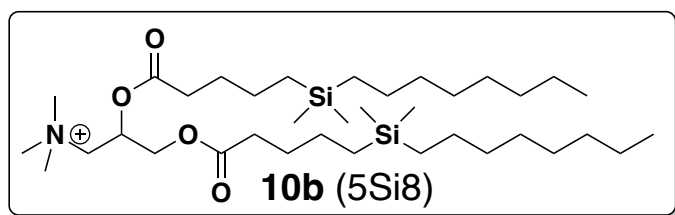
6-(dimethyl(phenyl)silyl)hexanoic acid **8d** was synthesized according to Figure 17, Route B and General Synthetic Procedure C.1. 5-Hexenoic acid **1b** (0.23 mL, 2 mmol), phenyldimethylsilane **4** (0.46 mL, 3 mmol), and a stock solution Pt(cod)Cl₂ in DCE (20 mg/mL, 0.2 mol%) was stirred in DCM (2 mL) at room temperature for 18 h. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary phase and Hex/EtOAc as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 6:4 Hex/EtOAc solvent mixture. The product **8d** eluted in 1–2% EtOAc/Hex to obtain an isolated yield of 18% (86 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.50 (m, 2H), 7.35 (m, 3H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.63 (m, 3H), 1.34 (tt, *J* = 6.3, 2.5 Hz, 5H), 0.75 (m, 2H), 0.26 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 180.1, 139.6, 133.7, 128.9, 127.9, 34.1, 34.1, 33.0, 24.5, 24.4, 23.7, 15.7, –2.9. ²⁹Si NMR (79 MHz, CDCl₃) δ –3.11. HRMS (ESI) *m/z*: calc for C₁₄H₂₁O₂Si[–] [M – H][–] 249.1311, found: 249.1315.

E. Target Cationic Silyl Lipids



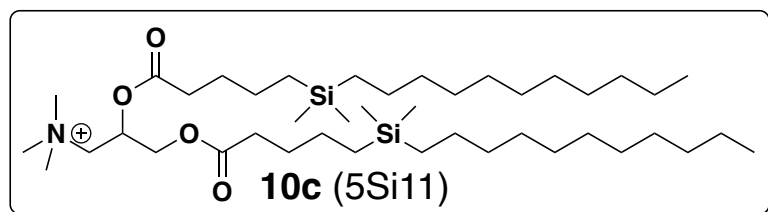
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(butyldimethylsilyl)pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10a** (5Si4) was synthesized according to Figure 17 and General Synthetic Procedure D. Methyl iodide (0.34 mL, 5.5 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-(butyldimethylsilyl)-pentanoate) **5a** (172 mg, 0.27 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude

product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5a** eluted at 1–2% MeOH/DCM, and **10a** (5Si4) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 92% (109 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.61 (m, 1H), 4.51 (m, 2H), 4.13 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.87 (dd, *J* = 14.3, 8.7 Hz, 1H), 3.54 (s, 9H), 2.35 (m, 4H), 1.62 (m, 4H), 1.28 (m, 13H), 0.87 (t, *J* = 7.0 Hz, 6H), 0.47 (m, 8H), –0.06 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.1, 172.7, 66.1, 65.6, 62.8, 54.7, 33.9, 33.7, 28.6, 28.4, 26.6, 26.0, 23.5, 15.0, 14.9, 14.8, 14.8, 13.8, –3.5, –3.5. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.36. HRMS (ESI) *m/z*: calc for C₂₈H₆₀NO₄Si₂⁺ [M]⁺ 530.4056, found: 530.4076.

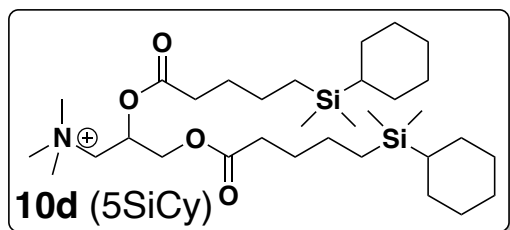


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(dimethyl(octyl)silyl)pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10b** (5Si8) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.34 mL, 5.5 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-(dimethyl(octyl)silyl)-pentanoate) **5b** (172 mg, 0.27 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5b** eluted at 1–2% MeOH/DCM, and **10b** (5Si8) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 68% (120 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.62 (m, 1H), 4.52 (m, 2H), 4.14

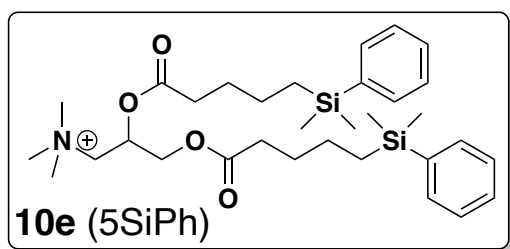
(dd, $J = 12.2, 5.5$ Hz, 1H), 3.88 (dd, $J = 14.3, 8.7$ Hz, 1H), 3.55 (s, 9H), 2.37 (m, 4H), 1.64 (m, 5H), 1.32 (m, 31H), 0.88 (t, $J = 6.7$ Hz, 6H), 0.48 (m, 9H), -0.05 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 172.8, 66.3, 65.7, 62.9, 54.9, 34.0, 33.8, 33.8, 32.0, 29.4, 29.4, 28.7, 28.5, 23.9, 23.6, 22.7, 14.2, $-3.3, -3.4$. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.32, 2.29. HRMS (ESI) m/z : calc for $\text{C}_{36}\text{H}_{76}\text{NO}_4\text{Si}_2^+$ $[\text{M}]^+$ 642.5308, found: 642.5333.



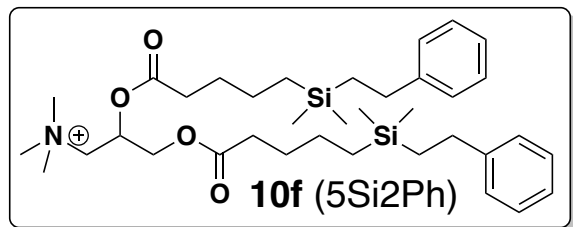
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(dimethyl(undecyl)silyl)pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10c** (5Si11) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (42 μL , 0.67 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-dimethyl(undecyl)silyl)pentanoate **5c** (24 mg, 0.03 mmol) were stirred in DCM (1 mL) at 50 $^{\circ}\text{C}$ for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5c** eluted at 1–2% MeOH/DCM, and **10c** (5Si11) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 80% (20 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.61 (m, 1H), 4.52 (dd, $J = 12.2, 4.1$ Hz, 1H), 4.17 (m, 2H), 3.87 (dd, $J = 14.2, 8.8$ Hz, 1H), 3.54 (s, 8H), 2.37 (m, 4H), 1.63 (m, 4H), 1.26 (m, 48H), 0.88 (t, $J = 6.8$ Hz, 6H), 0.48 (m, 9H), -0.05 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.2, 172.8, 66.3, 65.7, 62.9, 54.8, 33.9, 33.8, 31.9, 29.8, 29.7, 29.4, 29.4, 28.6, 28.5, 23.9, 23.6, 22.7, 15.2, 15.2, 15.1, 15.1, 14.1, -3.4 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.32. HRMS (ESI) m/z : calc for $\text{C}_{42}\text{H}_{88}\text{NO}_4\text{Si}_2^+$ $[\text{M}]^+$ 726.6247, found: 726.6255.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(cyclohexyldimethylsilyl)-pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10d** (5SiCy) was synthesized according to Figure 17 and General Synthetic Procedure D. Methyl iodide (0.31 mL, 5 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-cyclohexyldimethylsilyl)pentanoate **5d** (143 mg, 0.25 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5d** eluted at 1–2% MeOH/DCM, and **10d** (5SiCy) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 84% (124 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.60 (m, 1H), 4.51 (m, 2H), 4.14 (dd, *J* = 12.1, 5.4 Hz, 1H), 3.87 (dd, *J* = 14.3, 8.7 Hz, 1H), 3.54 (s, 9H), 2.36 (m, 4H), 1.65 (m, 15H), 1.21 (m, 14H), 0.57 (tt, *J* = 12.7, 3.1 Hz, 2H), 0.47 (m, 4H), –0.11 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.9, 66.3, 65.8, 63.0, 54.9, 34.1, 33.9, 28.8, 28.7, 28.2, 28.2, 27.6, 27.1, 27.1, 25.4, 25.4, 23.7, 13.4, 13.4, –5.2, –5.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 3.03. HRMS (ESI) *m/z*: calc for C₃₂H₆₄NO₄Si₂⁺ [M]⁺ 582.4369, found: 582.4380.

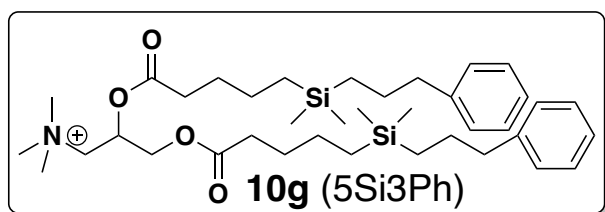


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(dimethyl(phenyl)silyl)pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10e** (5SiPh) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.31 mL, 5 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-(dimethyl(phenyl)silyl)-pentanoate) **5e** (139 mg, 0.25 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5e** eluted at 1–2% MeOH/DCM, and **10e** (5SiPh) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 79% (113 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.48 (m, 4H), 7.34 (m, 6H), 5.54 (m, 1H), 4.46 (m, 2H), 4.10 (dd, *J* = 12.2, 5.4 Hz, 1H), 3.81 (dd, *J* = 14.3, 8.7 Hz, 1H), 3.46 (s, 9H), 2.34 (m, 4H), 1.63 (m, 6H), 1.33 (m, 5H), 0.74 (m, 4H), 0.25 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.2, 172.8, 139.4, 139.2, 133.7, 129.1, 129.0, 127.9, 127.9, 66.3, 65.8, 62.9, 54.9, 33.9, 33.8, 28.6, 28.4, 23.7, 23.6, 15.6, –2.9. ²⁹Si NMR (79 MHz, CDCl₃) δ –2.93, –2.97. HRMS (ESI) *m/z*: calc for C₃₂H₅₂NO₄Si₂⁺ [M]⁺ 570.3430, found: 570.3446.



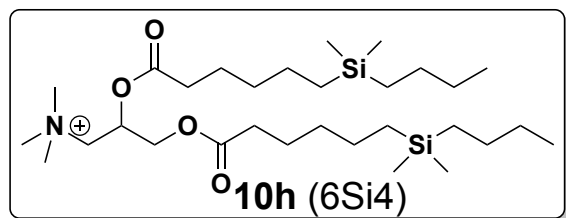
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(dimethyl(phenethyl)silyl)pentanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10f** (5Si2Ph) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.24 mL, 3.8 mmol) and 3-(dimethylamino)-propane-1,2-diyl bis(5-(dimethyl(phenethyl)silyl)pentanoate) **5f** (116 mg, 0.19 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The

crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMNO₄. Unreacted **5f** eluted at 1–2% MeOH/DCM, and **10f** (5Si2Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 89% (106 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.26 (m, 5H), 7.16 (m, 6H), 5.58 (m, 1H), 4.49 (m, 2H), 4.13 (dd, *J* = 12.2, 5.4 Hz, 1H), 3.86 (dd, *J* = 14.3, 8.7 Hz, 1H), 3.49 (s, 8H), 2.60 (m, 4H), 2.37 (m, 4H), 1.63 (m, 6H), 1.32 (m, 5H), 0.86 (m, 4H), 0.52 (m, 4H), –0.01 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.8, 145.3, 145.2, 128.5, 128.4, 127.9, 125.7, 125.7, 66.3, 65.8, 63.0, 54.9, 34.0, 33.8, 30.1, 30.1, 28.7, 28.5, 23.6, 23.6, 17.4, 17.3, 15.1, 15.0, –3.3, –3.4. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.60, 2.57. HRMS (ESI) *m/z*: calc for C₃₆H₆₀NO₄Si₂⁺ [M]⁺ 654.4369, found: 654.4362.



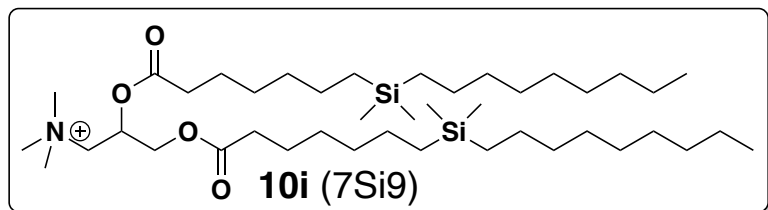
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((5-(dimethyl(3-phenylpropyl)silyl)-pentanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10g** (5Si3Ph) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.28 mL, 4.6 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(5-(dimethyl(3-phenylpropyl)silyl)pentanoate) **5g** (146 mg, 0.23 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMNO₄. Unreacted **5g** eluted at 1–2% MeOH/DCM, and **10g**

(5Si3Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 91% (135 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.28 (m, 5H), 7.18 (m, 6H), 5.59 (m, 1H), 4.51 (m, 2H), 4.13 (dd, $J = 12.1$, 5.5 Hz, 1H), 3.86 (dd, $J = 14.3$, 8.6 Hz, 1H), 3.53 (s, 9H), 2.61 (t, $J = 7.7$ Hz, 4H), 2.36 (m, 4H), 1.62 (m, 8H), 1.30 (m, 5H), 0.50 (m, 8H), -0.05 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 172.8, 142.8, 142.8, 128.6, 128.4, 125.8, 125.8, 66.3, 65.8, 62.9, 54.9, 40.1, 40.1, 34.0, 33.8, 28.7, 28.5, 26.2, 26.2, 23.6, 15.2, 15.2, 15.1, 15.1, -3.3 , -3.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.41, 2.38. HRMS (ESI) m/z : calc for $\text{C}_{38}\text{H}_{64}\text{NO}_4\text{Si}_2^+ [\text{M}]^+$ 654.4369, found: 654.4362.

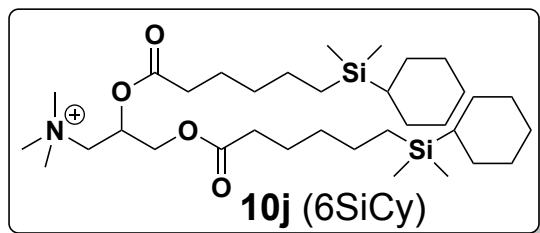


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((6-(butyldimethylsilyl)hexanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10h** (6Si4) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.1 mL, 1.7 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(6-(butyldimethylsilyl)-hexanoate) **5o** (45 mg, 0.08 mmol) were stirred in DCM (1 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5o** eluted at 1–2% MeOH/DCM, and **10h** (6Si4) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 91% (42 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.61 (m, 1H), 4.52 (m, 2H), 4.14 (dd, $J = 12.2$, 5.5 Hz, 1H), 3.87 (dd, $J = 14.3$, 8.6 Hz, 1H), 3.54 (s, 9H), 2.35 (tt, $J = 7.2$, 1.7 Hz, 4H), 1.60 (m, 5H), 1.27 (m, 17H), 0.87 (t, $J = 7.0$ Hz, 6H), 0.47 (m, 8H), -0.06 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 172.9, 66.4, 65.8, 62.9, 54.9, 34.3, 34.1, 33.2, 26.8, 26.3, 24.6, 24.5,

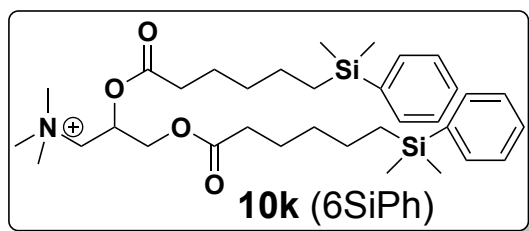
23.8, 23.7, 15.3, 15.1, 15.1, 13.9, -3.3, -3.3. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.28, 2.26. HRMS (ESI) m/z : calc for $\text{C}_{30}\text{H}_{64}\text{NO}_4\text{Si}_2^+$ $[\text{M}]^+$ 558.4369, found: 558.4383.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((7-(dimethyl(nonyl)silyl)heptanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10i** (7Si9) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (50 μL , 0.8 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(7-(dimethyl(nonyl)silyl)-heptanoate) **5p** (20 mg, 0.03 mmol) were stirred in DCM (3 mL) at 50 $^\circ\text{C}$ for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5p** eluted at 1–2% MeOH/DCM, and **10i** (7Si9) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 95% (19 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.60 (m, 1H), 4.53 (m, 2H), 4.15 (dd, $J = 12.2, 5.5$ Hz, 1H), 3.87 (dd, $J = 14.3, 8.6$ Hz, 1H), 3.54 (s, 9H), 2.36 (m, 4H), 1.60 (m, 8H), 1.29 (m, 42H), 0.88 (t, 6H), 0.46 (t, $J = 7.9$ Hz, 7H), -0.06 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 172.9, 66.4, 65.8, 62.9, 54.9, 34.4, 34.1, 33.9, 33.5, 33.4, 32.1, 29.7, 29.5, 29.5, 28.9, 28.9, 24.9, 24.7, 24.0, 23.9, 23.9, 22.8, 15.4, 15.4, 14.3, -3.2. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.20. HRMS (ESI) m/z : calc for $\text{C}_{42}\text{H}_{88}\text{NO}_4\text{Si}_2^+$ $[\text{M}]^+$ 726.6247, found: 726.6254.

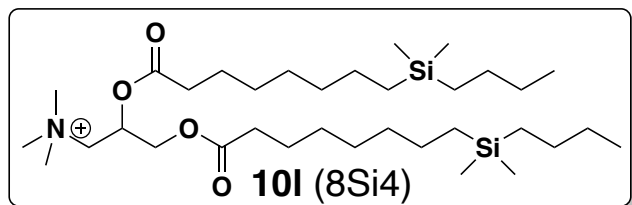


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((6-(cyclohexyldimethylsilyl)hexanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10j** (6SiCy) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (30 μ L, 0.44 mmol) and 3-(dimethylamino)-propane-1,2-diyl bis(6-(cyclohexyldimethylsilyl)hexanoate) **5q** (13 mg, 0.02 mmol) were stirred in DCM (1 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5q** eluted at 1–2% MeOH/DCM, and **10j** (6SiCy) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 92% (12 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.60 (m, 1H), 4.54 (m, 2H), 4.14 (m, 1H), 3.85 (dd, *J* = 14.4, 8.3 Hz, 1H), 3.53 (s, 9H), 2.36 (m, 5H), 1.64 (m, 20H), 1.21 (m, 25H), 0.57 (m, 2H), 0.46 (m, 4H), –0.10 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.4, 172.9, 66.4, 65.8, 63.0, 54.9, 34.3, 34.1, 33.3, 29.8, 28.3, 27.6, 27.2, 25.4, 25.4, 24.6, 24.5, 23.8, 23.8, 13.5, –5.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.97. HRMS (ESI) *m/z*: calc for C₃₄H₆₈NO₄Si₂⁺ [M]⁺ 610.4682, found: 610.4695.



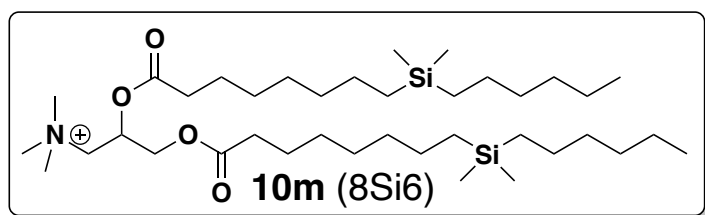
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((6-(dimethyl(phenyl)silyl)hexanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10k** (6SiPh) was

synthesized according to Figure 17 and General Synthetic Procedure D. Methyl iodide (40 μ L, 0.58 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(6-(dimethyl(phenyl)silyl)-hexanoate) **5r** (17 mg, 0.03 mmol) were stirred in DCM (1 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5o** eluted at 1–2% MeOH/DCM, and **10k** (6SiPh) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 94% (16 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.49 (ddd, J = 6.9, 3.7, 1.6 Hz, 4H), 7.35 (dd, J = 4.0, 1.6 Hz, 6H), 5.58 (m, 1H), 4.51 (m, 2H), 4.13 (dd, J = 12.0, 5.6 Hz, 1H), 3.83 (dd, J = 14.1, 8.8 Hz, 1H), 3.51 (s, 10H), 2.33 (m, 5H), 1.59 (m, 8H), 1.31 (m, 13H), 0.73 (t, J = 7.7 Hz, 4H), 0.25 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.8, 139.6, 139.5, 133.7, 128.9, 128.9, 127.9, 127.9, 66.3, 65.8, 62.9, 54.9, 34.2, 34.0, 33.0, 33.0, 24.5, 24.4, 23.7, 23.7, 15.7, –2.9. ²⁹Si NMR (79 MHz, CDCl₃) δ –3.11. HRMS (ESI) m/z : calc for C₃₄H₅₆NO₄Si₂⁺ [M]⁺ 598.3743, found: 598.3768.



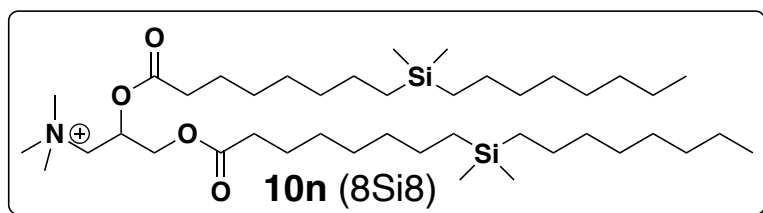
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(butyldimethylsilyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10l** (8Si4) was synthesized according to Figure 17 and General Synthetic Procedure D. Methyl iodide (0.25 mL, 4 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(butyldimethylsilyl)octanoate) **5h** (120 mg, 0.2 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the

stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMNO₄. Unreacted **5h** eluted at 1–2% MeOH/DCM, and **10l** (8Si4) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 33% (41 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.60 (m, 1H), 4.53 (m, 2H), 4.14 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.88 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.54 (s, 9H), 2.36 (m, 4H), 1.60 (m, 6H), 1.31 (m, 26H), 0.87 (t, *J* = 7.0 Hz, 6H), 0.47 (m, 8H), –0.06 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.5, 173.0, 66.5, 65.9, 63.1, 55.1, 34.5, 34.3, 33.8, 33.8, 29.4, 29.3, 29.3, 29.2, 26.9, 26.4, 25.1, 24.9, 24.2, 24.1, 15.6, 15.6, 15.2, 14.1, –3.1. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.23. HRMS (ESI) *m/z*: calc for C₃₄H₇₂NO₄Si₂⁺ [M]⁺ 614.4995, found: 614.5000.

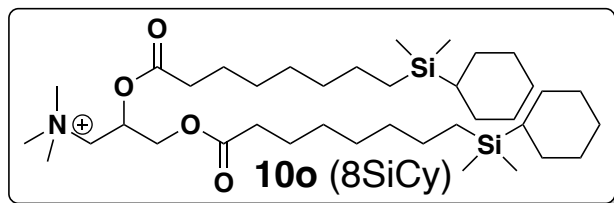


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(hexyldimethylsilyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10m** (8Si6) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.23 mL, 3.7 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(hexyldimethylsilyl)-octanoate) **5i** (122 mg, 0.19 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMNO₄. Unreacted **5i** eluted at 1–2% MeOH/DCM, and **10m** (8Si6) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 38% (47 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.60 (m, 1H), 4.54 (m, 2H), 4.15 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.87 (dd, *J* = 14.3, 8.5 Hz, 1H), 3.54 (s, 9H), 2.37 (m, 4H), 1.61 (m,

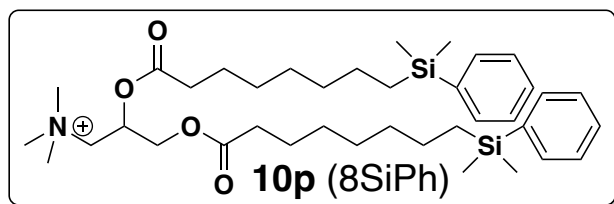
2H), 1.26 (m, 37H), 0.88 (t, 6H), 0.47 (m, 8H), -0.06 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.3, 172.9, 66.3, 65.8, 63.0, 54.9, 34.4, 34.1, 33.7, 33.6, 33.5, 31.8, 29.8, 29.2, 29.2, 29.2, 29.1, 24.9, 24.8, 24.0, 24.0, 23.9, 22.8, 15.5, 15.4, 15.4, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.19. HRMS (ESI) m/z : calc for $\text{C}_{38}\text{H}_{80}\text{NO}_4\text{Si}_2^+ [\text{M}]^+$ 670.5621, Found: 670.5630.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(dimethyl(octyl)silyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10n** (8Si8) was synthesized according to Figure 17 and General Synthetic Procedure D. Methyl iodide (0.52 mL, 8.4 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(dimethyl(octyl)silyl)-octanoate) **5j** (300 mg, 0.42 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5j** eluted at 1–2% MeOH/DCM, and **10n** (8Si8) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 84% (255 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.61 (m, 1H), 4.53 (m, 2H), 4.14 (dd, $J = 12.2, 5.5$ Hz, 1H), 3.88 (dd, $J = 14.3, 8.6$ Hz, 1H), 3.54 (s, 9H), 2.36 (m, 4H), 1.60 (m, 6H), 1.27 (m, 45H), 0.88 (t, $J = 6.8$ Hz, 6H), 0.46 (m, 9H), -0.07 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.1, 172.6, 66.1, 65.5, 62.7, 54.6, 34.1, 33.8, 33.6, 33.4, 33.4, 31.8, 29.2, 29.1, 28.9, 28.9, 28.8, 24.7, 24.5, 23.7, 22.5, 15.2, 15.2, 15.1, 13.9, -3.5 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.19. HRMS (ESI) m/z : calc for $\text{C}_{42}\text{H}_{88}\text{NO}_4\text{Si}_2^+ [\text{M}]^+$ 726.6247, found: 726.6245.

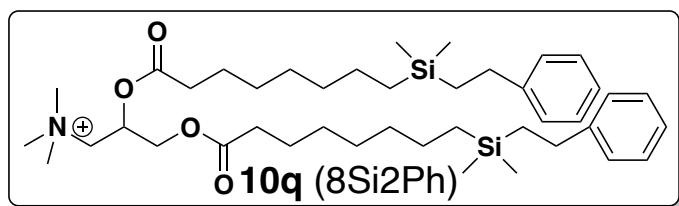


This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(cyclohexyldimethylsilyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10o** (8SiCy) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.21 mL, 3.4 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(cyclohexyldimethylsilyl)octanoate) **5k** (110 mg, 0.17 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5k** eluted at 1–2% MeOH/DCM, and **10o** (8SiCy) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 26% (29 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.61 (m, 1H), 4.52 (m, 2H), 4.15 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.87 (dd, *J* = 14.2, 8.6 Hz, 1H), 3.54 (s, 9H), 2.36 (m, 5H), 1.71 (m, 6H), 1.63 (m, 5H), 1.16 (m, 30H), 0.57 (tt, *J* = 12.5, 3.1 Hz, 2H), 0.45 (m, 4H), –0.11 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.4, 172.9, 66.5, 65.9, 63.1, 55.0, 34.4, 34.2, 33.9, 33.8, 29.3, 29.3, 29.2, 29.1, 28.4, 27.7, 27.2, 25.5, 24.9, 24.9, 24.1, 24.1, 13.7, –5.1. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.94. HRMS (ESI) *m/z*: calc for C₃₈H₇₆NO₄Si₂⁺ [M]⁺ 666.5308, found: 666.5309.



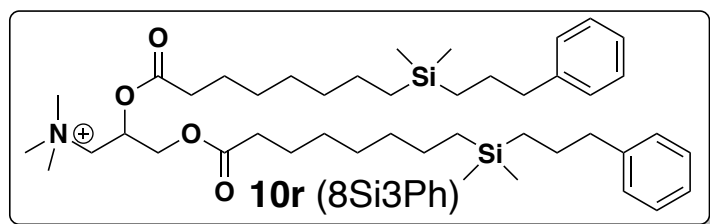
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(dimethyl(phenyl)silyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10p** (8SiPh) was

synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.23 mL, 3.8 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(dimethyl(phenyl)silyl)-octanoate) **5l** (120 mg, 0.19 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **5l** eluted at 1–2% MeOH/DCM, and **10p** (8SiPh) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 28% (34 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.49 (ddd, *J* = 6.7, 3.3, 1.8 Hz, 4H), 7.34 (ddd, *J* = 5.0, 2.7, 1.3 Hz, 6H), 5.59 (m, 1H), 4.51 (m, 2H), 4.13 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.85 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.51 (s, 10H), 2.34 (m, 4H), 1.58 (m, 7H), 1.27 (m, 19H), 0.73 (m, 4H), 0.24 (s, 11H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.9, 139.8, 133.7, 128.9, 127.9, 66.4, 65.8, 62.9, 54.9, 34.3, 34.1, 33.6, 33.5, 29.2, 29.1, 29.1, 28.9, 24.9, 24.8, 23.9, 23.9, 15.8, –2.9. ²⁹Si NMR (79 MHz, CDCl₃) δ –3.13. HRMS (ESI) *m/z*: calc for C₃₈H₆₄NO₄Si₂⁺ [*M*]⁺ 654.4369, found: 654.4365.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(dimethyl(phenethyl)silyl)octanoyl)oxy)-N,N,N-trimethylpropan-1-aminium **10q** (8Si₂Ph) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.15 mL, 2.4 mmol) and 3-(dimethylamino)-propane-1,2-diyl bis(8-(dimethyl(phenethyl)silyl)-octanoate) **5m** (80 mg, 0.11 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column

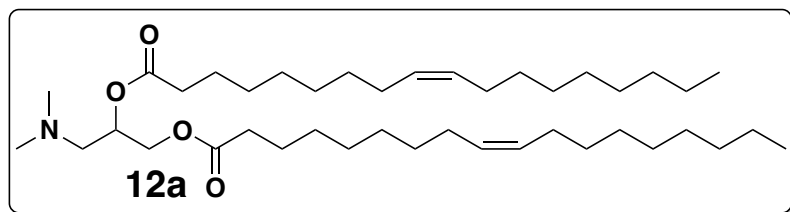
with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5m** eluted at 1–2% MeOH/DCM, and **10q** (8Si2Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 20% (16 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.26 (m, 6H), 7.18 (m, 6H), 5.60 (m, 1H), 4.51 (m, 2H), 4.14 (dd, $J = 12.2, 5.5$ Hz, 1H), 3.86 (dd, $J = 14.3, 8.5$ Hz, 1H), 3.51 (s, 9H), 2.61 (m, 4H), 2.36 (m, 4H), 1.60 (m, 10H), 1.29 (m, 19H), 0.86 (m, 4H), 0.50 (t, $J = 7.7$ Hz, 4H), -0.02 (s, 11H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.4, 172.9, 145.5, 128.4, 127.9, 125.6, 66.4, 65.8, 63.0, 54.9, 34.3, 34.1, 33.7, 33.6, 30.2, 29.2, 29.2, 29.1, 24.9, 24.8, 23.9, 23.9, 17.5, 15.3, -3.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.48. HRMS (ESI) m/z : calc for $\text{C}_{42}\text{H}_{72}\text{NO}_4\text{Si}_2^+ [\text{M}]^+$ 710.4995, found: 710.4995.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 2,3-Bis((8-(dimethyl(3-phenylpropyl)silyl)-octanoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **10r** (8Si3Ph) was synthesized according to Figure 17 and General Synthetic Procedure *D*. Methyl iodide (0.21 mL, 3.4 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(8-(dimethyl(3-phenylpropyl)silyl)octanoate) **5n** (124 mg, 0.17 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO_4 . Unreacted **5n** eluted at 1–2% MeOH/DCM, and **10r** (8Si3Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 93% (118 mg). ^1H NMR (400

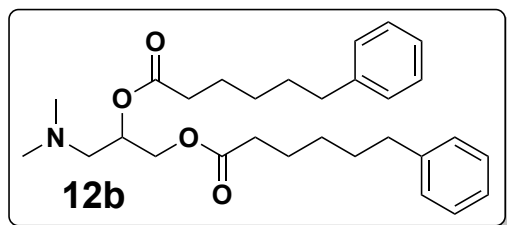
MHz, CDCl₃) δ 7.27 (m, 6H), 7.17 (m, 5H), 5.60 (m, 1H), 4.52 (m, 2H), 4.14 (dd, J = 12.2, 5.5 Hz, 1H), 3.88 (m, 1H), 3.53 (s, 10H), 2.61 (t, J = 7.7 Hz, 4H), 2.36 (m, 5H), 1.60 (m, 11H), 1.28 (m, 18H), 0.53 (m, 4H), 0.46 (t, J = 7.8 Hz, 4H), -0.06 (s, 10H). ¹³C NMR (101 MHz, CDCl₃) δ 173.2, 172.7, 142.8, 128.5, 128.2, 125.6, 66.2, 65.7, 62.9, 54.8, 40.0, 34.2, 34.0, 33.5, 33.5, 29.1, 29.1, 29.0, 28.9, 26.1, 24.8, 24.7, 23.9, 23.8, 17.9, 15.2, -3.38 . ²⁹Si NMR (79 MHz, CDCl₃) δ 2.28. HRMS (ESI) m/z : calc for C₄₄H₇₆NO₄Si₂⁺ [M]⁺ 738.5308, found: 738.5321.

F. Control Lipids DOTAP, 5CH₂Ph, 6CH₂4 and 8CH₂Ph (Figure 2.7)



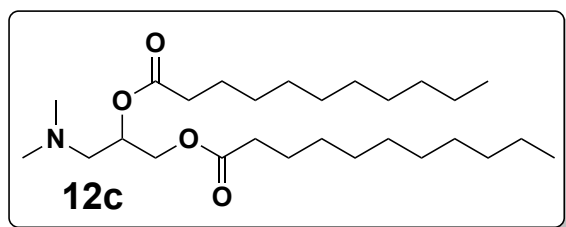
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl dioleate **12a** was synthesized according to Figure 19 and General Synthetic Procedure *E.1* with an isolated yield of 91% (510 mg). In one round bottom flask, oleic acid **11** (0.63 mL, 2 mmol) was added dropwise to a solution of CDI (357 mg, 2.2 mmol) in THF (12 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.1 mL, 0.8 mmol) was added dropwise to a solution of dried/washed NaH (67 mg, 2.8 mmol) in THF (12 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 5 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **12a** eluted in 1–2% MeOH/DCM. ¹H NMR (400 MHz,

CDCl₃) δ 5.36 (m, 4H), 5.24 (m, 1H), 4.33 (dd, J = 11.9, 3.4 Hz, 1H), 4.06 (dd, J = 11.9, 6.1 Hz, 1H), 2.60 (m, 1H), 2.49 (dd, J = 13.1, 5.4 Hz, 1H), 2.33 (m, 4H), 2.30 (s, 3H), 2.02 (m, 9H), 1.62 (m, 5H), 1.31 (m, 49H), 0.88 (t, 7H). ¹³C NMR (101 MHz, CDCl₃) δ 173.6, 130.1, 129.9, 129.8, 68.7, 63.9, 59.1, 45.4, 34.5, 34.2, 32.0, 31.7, 29.9, 29.9, 29.8, 29.7, 29.7, 29.5, 29.4, 29.3, 29.3, 29.2, 27.4, 27.3, 25.8, 24.9, 24.9, 22.8, 22.7, 14.2. HRMS (ESI) m/z : calc for C₄₁H₇₇NO₄Na⁺ [M + Na]⁺ 670.5750, found: 669.5939.



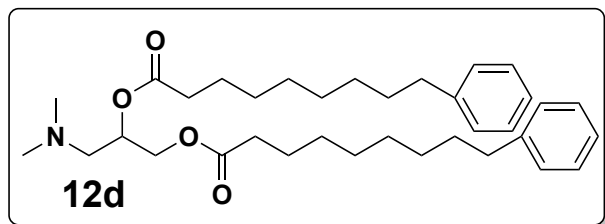
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(Dimethylamino)propane-1,2-diyl bis(6-phenylhexanoate) **12b** was synthesized according to Figure 19 and General Synthetic Procedure *E.1*. In one round bottom flask, 6-phenylhexanoic acid **11** (0.75 mL, 4 mmol) was added dropwise to a solution of CDI (973 mg, 6 mmol) in THF (12 mL). In another round bottom flask, 3-(dimethylamino)propane-1,2-diol **2** (0.19 mL, 1.6 mmol) was added dropwise to a solution of dried/washed NaH (130 mg, 5.4 mmol) in THF (12 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 2 days. The reaction was quenched via the dropwise addition of aqueous NH₄Cl and washed with DCM (20 mL) and NH₄Cl (20 mL). The organic layer was dried with anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO₄. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **12b** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 65% (465 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.26 (m, 5H), 7.17 (m, 6H), 5.20 (m, 1H),

4.34 (dd, $J = 11.9, 3.3$ Hz, 1H), 4.06 (dd, $J = 11.9, 6.3$ Hz, 1H), 2.60 (t, 4H), 2.46 (m, 2H), 2.30 (td, $J = 7.4, 4.0$ Hz, 4H), 2.26 (s, 5H), 1.65 (m, 8H), 1.36 (m, 4H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.5, 173.2, 142.6, 128.5, 128.5, 128.4, 128.4, 125.8, 69.1, 64.1, 59.3, 45.9, 35.9, 34.4, 34.2, 31.3, 31.2, 28.9, 28.9, 28.8, 24.9, 24.9. HRMS (ESI) m/z : calc for $\text{C}_{29}\text{H}_{41}\text{NO}_4\text{H}^+$ $[\text{M} + \text{H}]^+$ 468.3114, found: 468.3122.

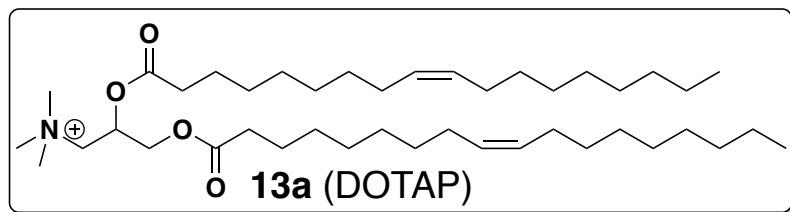


This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(dimethylamino)propane-1,2-diyl diundecanoate **12c** was synthesized according to Figure 19 and General Synthetic Procedure *E.1*. In one microwave vial, undecanoic acid **11** (200 mg, 1.1 mmol) and CDI (261 mg, 1.6 mmol) were dissolved in THF (3 mL). In another microwave vial, 3-(dimethylamino)propane-1,2-diol **2** (50 μL , 0.43 mmol) was added dropwise to a solution of dried/washed NaH (35 mg, 1.5 mmol) in THF (3 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 °C for 7 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **12c** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 61% (120 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.19 (qd, $J = 6.4, 3.2$ Hz, 1H), 4.36 (dd, $J = 11.9, 3.2$ Hz, 1H), 4.09 (dd, $J = 11.9, 6.5$ Hz, 1H), 2.43 (m, 2H), 2.30 (td, $J = 7.6, 2.3$ Hz, 4H), 2.25 (s, 6H), 1.60 (m, 4H), 1.27 (m, 29H), 0.87

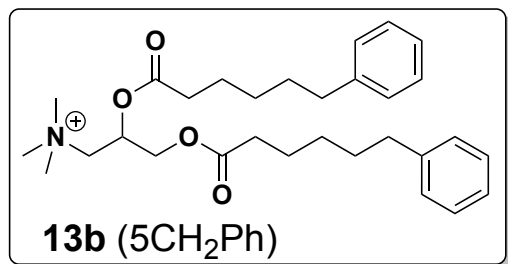
(t, 5H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 173.4, 69.4, 64.1, 59.6, 46.2, 34.6, 34.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 25.1, 25.1, 22.8, 14.3.



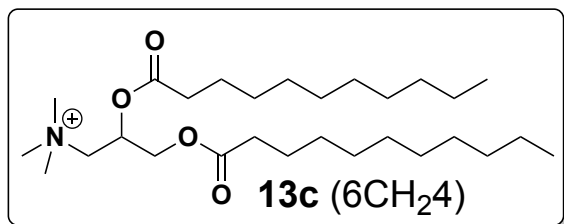
This procedure was adapted from Massing, U. et al.¹¹⁴ 3-(dimethylamino)propane-1,2-diyl bis(9-phenylnonanoate) **12d** was synthesized according to Figure 19 and General Synthetic Procedure *E.1*. In one microwave vial, 9-phenylnonanoic acid **11** (200 mg, 0.85 mmol) and CDI (208 mg, 1.3 mmol) were dissolved in THF (3 mL). In another microwave vial, 3-(dimethylamino)propane-1,2-diol **2** (40 μL , 0.35 mmol) was added dropwise to a solution of dried/washed NaH (28 mg, 1.2 mmol) in THF (3 mL). Both reactions were stirred at room temperature for 1 h. The two reactions were combined and refluxed in anhydrous THF at 80 $^{\circ}\text{C}$ for 7 days. The reaction was quenched via the dropwise addition of aqueous NH_4Cl and washed with DCM (20 mL) and NH_4Cl (20 mL). The organic layer was dried with anhydrous MgSO_4 , filtered and concentrated to dryness under vacuum. The crude product was purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. The fractions were spotted on TLC plates and stained with KMnO_4 . The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The product **12d** eluted in 1–2% MeOH/DCM to obtain an isolated yield of 54% (104 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.28 (m, 5H), 7.17 (m, 6H), 5.19 (m, 1H), 4.36 (dd, J = 11.9, 3.2 Hz, 1H), 4.09 (dd, J = 11.9, 6.4 Hz, 1H), 2.58 (t, 4H), 2.43 (m, 2H), 2.30 (td, J = 7.5, 2.5 Hz, 4H), 2.25 (s, 6H), 1.60 (m, 10H), 1.31 (m, 19H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 173.3, 142.9, 128.5, 128.4, 125.7, 69.4, 64.1, 59.6, 46.2, 36.1, 34.5, 34.3, 31.6, 29.5, 29.4, 29.4, 29.3, 29.2, 25.1, 25.0.



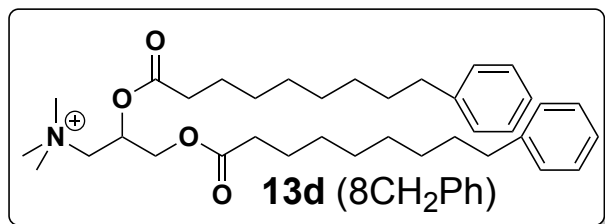
This procedure was adapted from Hurley, C. A. et al.¹¹⁵ 3-(((Z)-Hexadec-9-enoyl)oxy)-*N,N,N*-trimethyl-2-(oleoyloxy)propan-1-aminium **13a** (DOTAP) was synthesized according to Figure 19 and General Synthetic Procedure *E.2*. Methyl iodide (0.96 mL, 15.4 mmol) and 3-(dimethylamino)propane-1,2-diyl dioleate **12a** (500 mg, 0.77 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified using a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **12a** eluted at 1–2% MeOH/DCM, and **13a** (DOTAP) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 33% (169 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.61 (m, 1H), 5.35 (m, 4H), 4.53 (m, 2H), 4.15 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.89 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.55 (s, 9H), 2.37 (t, *J* = 1.5 Hz, 4H), 2.01 (m, 9H), 1.61 (m, 5H), 1.30 (m, 46H), 0.88 (t, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 173.2, 172.7, 130.1, 130.0, 129.7, 129.6, 66.2, 65.7, 62.9, 54.8, 34.2, 33.9, 31.9, 29.8, 29.8, 29.7, 29.5, 29.3, 29.3, 29.3, 29.2, 29.2, 29.1, 29.1, 29.1, 27.2, 27.2, 27.2, 24.8, 24.6, 22.7, 14.1. HRMS (ESI) *m/z*: calc for C₄₂H₈₀NO₄⁺ [M]⁺ 662.6082, found: 662.6086.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ *N,N,N*-Trimethyl-2,3-bis((6-phenylhexanoyl)oxy)propan-1-aminium **13b** (5CH₂Ph) was synthesized according to Figure 19 and General Synthetic Procedure E.2. Methyl iodide (0.53 mL, 8.6 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(6-phenylhexanoate) **12b** (200 mg, 0.43 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified via a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **12b** eluted at 1–2% MeOH/DCM, and **13b** (5CH₂Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 75% (155 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.29 (m, 5H), 7.19 (m, 6H), 5.60 (m, 1H), 4.53 (m, 2H), 4.15 (dd, *J* = 12.2, 5.4 Hz, 1H), 3.88 (dd, *J* = 14.3, 8.7 Hz, 1H), 3.51 (s, 9H), 2.62 (td, *J* = 7.8, 2.1 Hz, 4H), 2.38 (td, *J* = 7.5, 1.5 Hz, 4H), 1.64 (m, 9H), 1.37 (m, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 173.2, 172.7, 142.5, 142.4, 128.5, 128.4, 128.4, 125.9, 125.8, 66.2, 65.8, 62.9, 54.8, 35.8, 35.7, 34.2, 34.0, 31.2, 31.1, 28.8, 28.7, 24.7, 24.6. HRMS (ESI) *m/z*: calc for C₃₀H₄₄NO₄⁺ [M]⁺ 482.3265, found: 482.3260.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ *N,N,N*-trimethyl-2,3-bis(undecanoyloxy)propan-1-aminium **13c** (6CH₂4) was synthesized according to Figure 19 and General Synthetic Procedure *E.2*. Methyl iodide (0.33 mL, 5.3 mmol) and 3-(dimethylamino)propane-1,2-diyl diundecanoate **12c** (120 mg, 0.26 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified via a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMnO₄. Unreacted **12c** eluted at 1–2% MeOH/DCM, and **13c** (6CH₂4) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 90% (111 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.60 (m, 1H), 4.55 (ddd, *J* = 18.2, 13.3, 3.0 Hz, 2H), 4.15 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.87 (dd, *J* = 14.3, 8.5 Hz, 1H), 3.54 (s, 9H), 2.36 (t, 4H), 1.60 (m, 5H), 1.27 (m, 31H), 0.88 (t, *J* = 6.7 Hz, 5H). ¹³C NMR (101 MHz, CDCl₃) δ 173.4, 172.9, 66.4, 65.8, 62.9, 54.9, 34.4, 34.1, 32.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 24.9, 24.8, 22.8, 14.3. Note: For NMR analysis, I had to dissolve sample in more CDCl₃ with TMS than for the corresponding silyl lipid 6Si4 due to poorer solubility, resulting in large solvent peaks in the spectra. HRMS (ESI) *m/z*: calc for C₂₈H₅₆NO₄⁺ [*M*]⁺ 470.4204, found: 470.4203.



This procedure was adapted from Hurley, C. A. et al.¹¹⁵ *N,N,N*-trimethyl-2,3-bis((9-phenylnonanoyl)oxy)propan-1-aminium **13d** (8CH₂Ph) was synthesized according to Figure 19 and General Synthetic Procedure E.2. Methyl iodide (0.23 mL, 3.7 mmol) and 3-(dimethylamino)propane-1,2-diyl bis(9-phenylnonanoate) **12d** (104 mg, 0.19 mmol) were stirred in DCM (3 mL) at 50 °C for 18 h in a microwave vial. The crude product was concentrated to dryness under vacuum and purified via a gradient column with silica gel as the stationary DCM/MeOH as the mobile phase. The solvent chamber contained a 9:1 DCM/MeOH solvent mixture. The fractions were spotted on TLC plates and stained with KMNO₄. Unreacted **12d** eluted at 1–2% MeOH/DCM, and **13d** (8CH₂Ph) eluted at 4–10% MeOH/DCM to obtain an isolated yield of 91% (97 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 9H), 7.17 (m, 6H), 5.59 (m, 1H), 4.53 (m, 2H), 4.15 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.85 (dd, *J* = 14.3, 8.5 Hz, 1H), 3.51 (s, 9H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.36 (m, 4H), 1.60 (m, 9H), 1.31 (m, 18H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 172.8, 142.9, 142.9, 128.5, 128.4, 125.7, 125.7, 66.3, 65.8, 62.9, 54.9, 36.1, 34.3, 34.1, 31.6, 31.6, 29.5, 29.4, 29.4, 29.4, 29.3, 29.2, 29.2, 24.9, 24.7. For NMR analysis, I had to dissolve sample in more CDCl₃ with TMS than for the corresponding silyl lipids 8SiPh and 5Si3Ph due to poorer solubility, resulting in large solvent peaks in the spectra. HRMS (ESI) *m/z*: calc for C₃₆H₅₆NO₄⁺ [M]⁺ 566.4204, found: 566.4202.

Chapter 3: Single and Double Chain Ionizable Silyl lipids Demonstrate Distinct Membrane Fluidity and mRNA Delivery Profiles

The following work is adapted from my manuscript that is accepted for publication in *ACS Chemical Biology*: Patterson, L.A.; Tran, W.N.; Humeniuk, Y.; Coppage, D.A.; Agwarambo, E.; Downey, O.; O'Geen, H.; Segal, D.J.; Franz, A.K. Ionizable Silyl Lipids with Distinct Membrane Fluidity and mRNA Delivery Profiles. *ACS Chemical Biology* (accepted). All HEK293T cell transfection data presented in this chapter was acquired in collaboration with Dr. Henriette O'Geen and Dr. David Segal in the Genome Center at UC Davis.

3.1. Abstract

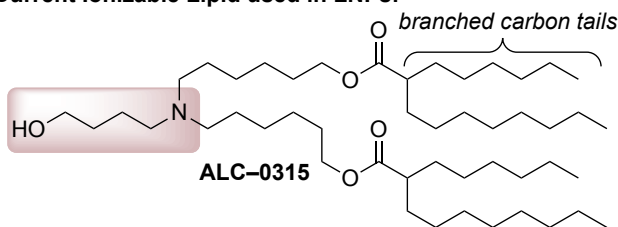
Chapter 3 presents the design and synthesis of ionizable silyl lipids containing structurally diverse tail structures for use in nanomaterials and mRNA delivery. Sixteen ionizable silyl lipids were synthesized with varying tail lengths and silyldimethyl substituents (alkyl, cyclohexyl, or phenyl) in the lipid tail, with tail length selection based on my previous work which demonstrated that cationic silyl lipid tails generally showed enhanced rRNA encapsulation efficiency and mRNA transfection efficiency (Chapter 2). There continues to be a need for insight into the structure-function relationships of lipids that influence self-assembly into various nanostructures. Due to the uniqueness of these silyl lipid tail structures, I also explored single chain (monoalkylated, MA) and double chain (dialkylated, DA) ionizable silyl lipids for their use in nanomaterials for mRNA delivery. The results demonstrate that (i) single chain silyl lipids can form particles with higher rRNA encapsulation efficiency compared to double chain lipids, and (ii) single chain lipids formed particles with more fluid membranes compared to double chain lipids which formed particles with more rigid membranes. These findings support the

incorporation of various silyl lipid tail structures into ionizable lipids to control particle properties, where single vs double chain silyl lipids can be strategically leveraged to control membrane fluidity and subsequent particle properties.

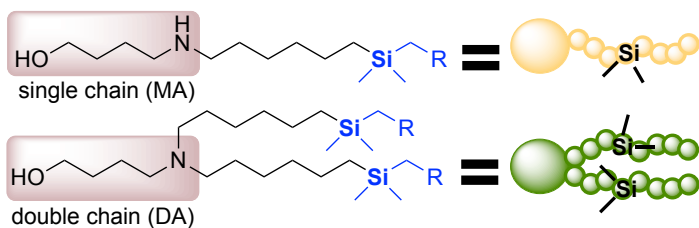
3.2. Ionizable Silyl lipid Analogs of ALC-0315

Ionizable lipids play a critical role in the endosomal release of cargo, becoming protonated in the endosome and promoting the Hexagonal II phase conformation and release of cargo inside the cell.^{19,41,44,54,81,86,116} There is growing interest to develop new ionizable lipid structures that can be incorporated into LNPs to tune their properties and improve mRNA transfection. A new class of branched ionizable silyl lipids inspired by ALC-0315 were synthesized for biophysical and biological evaluation. ALC-0315 is a current ionizable lipid^{100,117,118} used in LNP formulations for mRNA delivery (Figure 32A). The branched ionizable silyl lipids (Figure 32B) synthesized for evaluation are composed of the same head group as ALC-0315 but differ in the silyldimethyl tail structure, with alkyl, cyclohexyl, or phenyl-containing silyl substituents (Figure 32C). I hypothesized that, similar to the cationic silyl lipids previously discussed, varying the silyl lipid tail structure of ionizable lipids would also modulate LNP properties and mRNA delivery, allowing for the formulation of novel LNP formulations containing both cationic and ionizable silyl lipids for optimized transfection efficiency. There continues to be a need for insight into the structure-function relationships of lipids that influence self-assembly into various nanostructures. Due to the uniqueness of the silyl lipid tail structures, I also explored single chain (mono-alkylated, MA) and double chain (di-alkylated, DA) ionizable silyl lipids for their use in nanomaterials for mRNA delivery.

A) Current Ionizable Lipid used in LNPs:



B) This Work: Branched Silyl Ionizable Lipids in LNPs



C) Toolbox of Silyl Lipid Building Blocks:

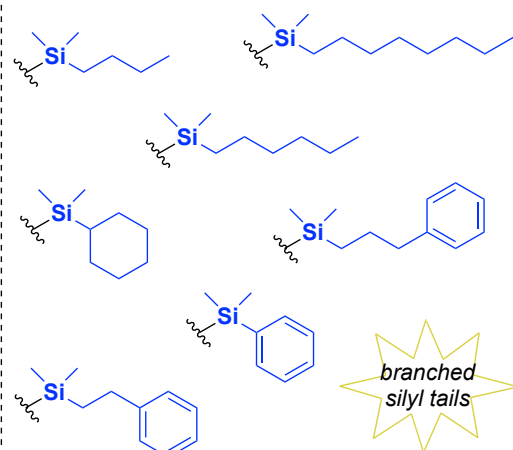
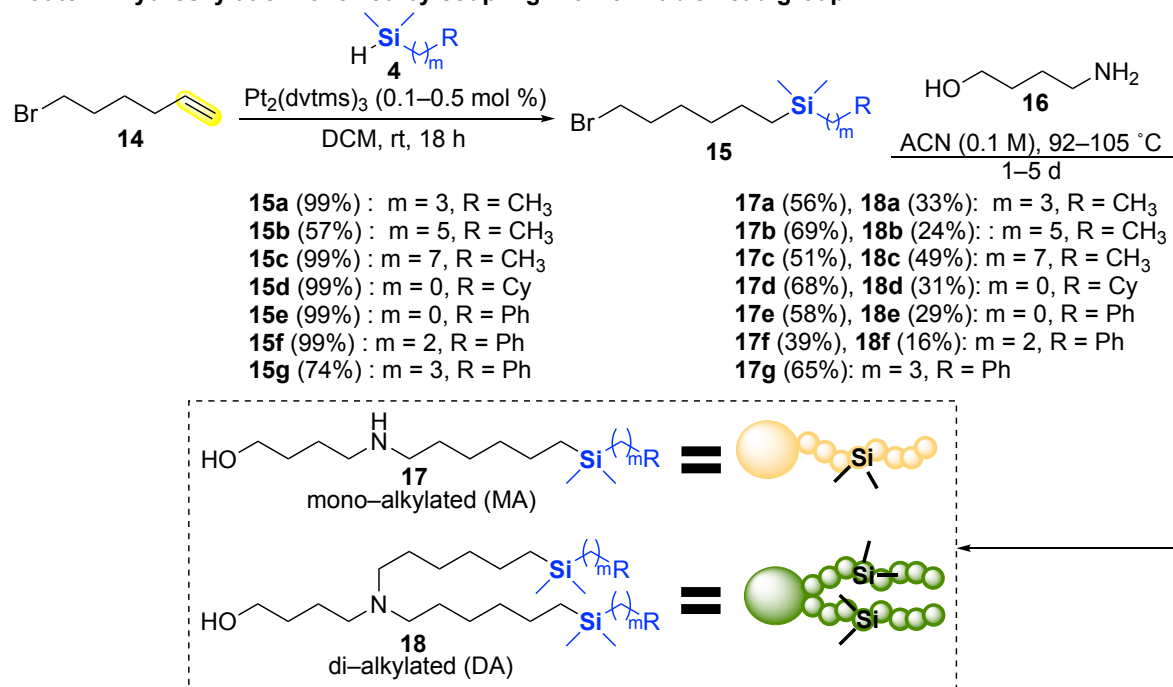


Figure 32. Ionizable lipid ALC-0315 (A) and single and double chain ionizable silyl lipids (B–C)

3.3. Synthesis of Ionizable Silyl lipids

The ionizable silyl lipids were synthesized by subjecting 6-bromohexene **14** to hydrosilylation with silane **4** using Karstedt's catalyst ($\text{Pt}_2(\text{dvtms})_3$) to afford bromoalkylsilane precursor **15a–15g** with isolated yields of 57–99% after column chromatography (Figure 33, Route A). Bromoalkylsilane **15** was then coupled with 4-aminobutan-1-ol **16** under reflux in acetonitrile to afford either MA product **17a–17g** (39–69% isolated yield) or DA product **18a–18f** (16–49% isolated yield) after column chromatography. It is notable that DA silyl lipid was not observed when coupling (6-bromohexyl)dimethyl(3-phenylpropyl)silane **15g** with 4-aminobutan-1-ol **16**, with only MA product **17g** and un-reacted starting material observed by TLC and NMR after column chromatography.

Route A: Hydrosilylation followed by coupling with ionizable head group



Route B: Coupling with ionizable head group followed by hydrosilylation

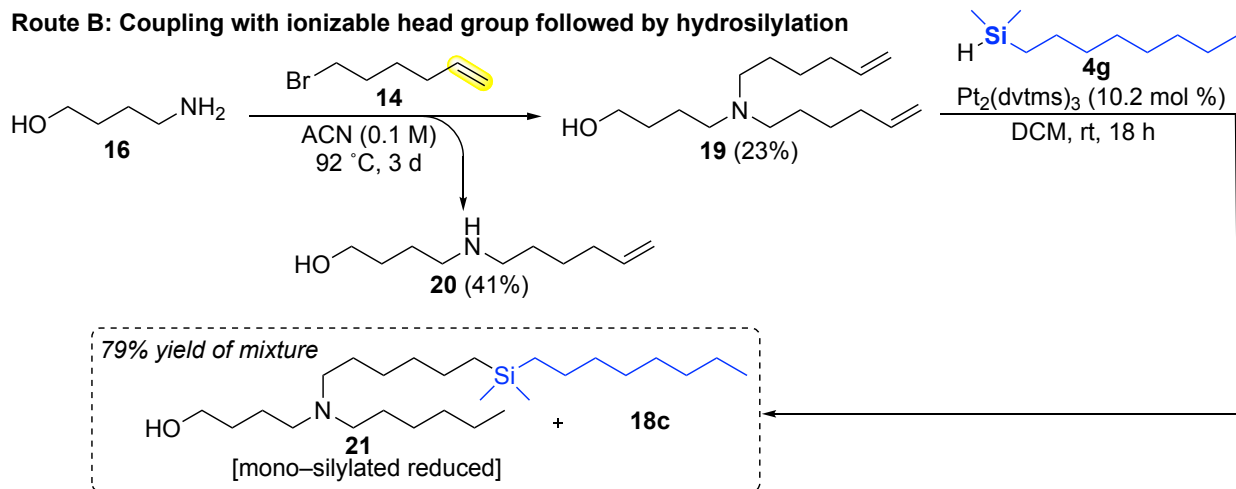


Figure 33. A) Synthetic route used to access all single and double chain ionizable silyl lipids. B) Alternative route explored to access silyl lipids. All yields are isolated after column chromatography.

An alternative route was explored initially in which 4-aminobutan-1-ol **16** was first coupled with 6-bromohexene **14** under reflux in acetonitrile to afford dialkylated alkene **19** (23%, Figure 34) and monoalkylated alkene **20** (41%, Figure 35) observed as a byproduct (Figure 33, Route B). Hydrosilylation of dialkylated alkene precursor **19** with octyldimethylsilane **4g** (see General

Procedures Section 3.9, Reduction of Chlorosilane) was performed with catalytic $\text{Pt}_2(\text{dvtms})_3$ to afford mostly monosilylated reduced byproduct **21** mixed with trace **18c** by ^1H NMR (Figure 36) and HRMS (Figure 37) after column chromatography. Based on these findings, synthetic Route A was used to access the ionizable silyl lipids with isolated yields of 16–69% over 2–3 steps (Figure 38).

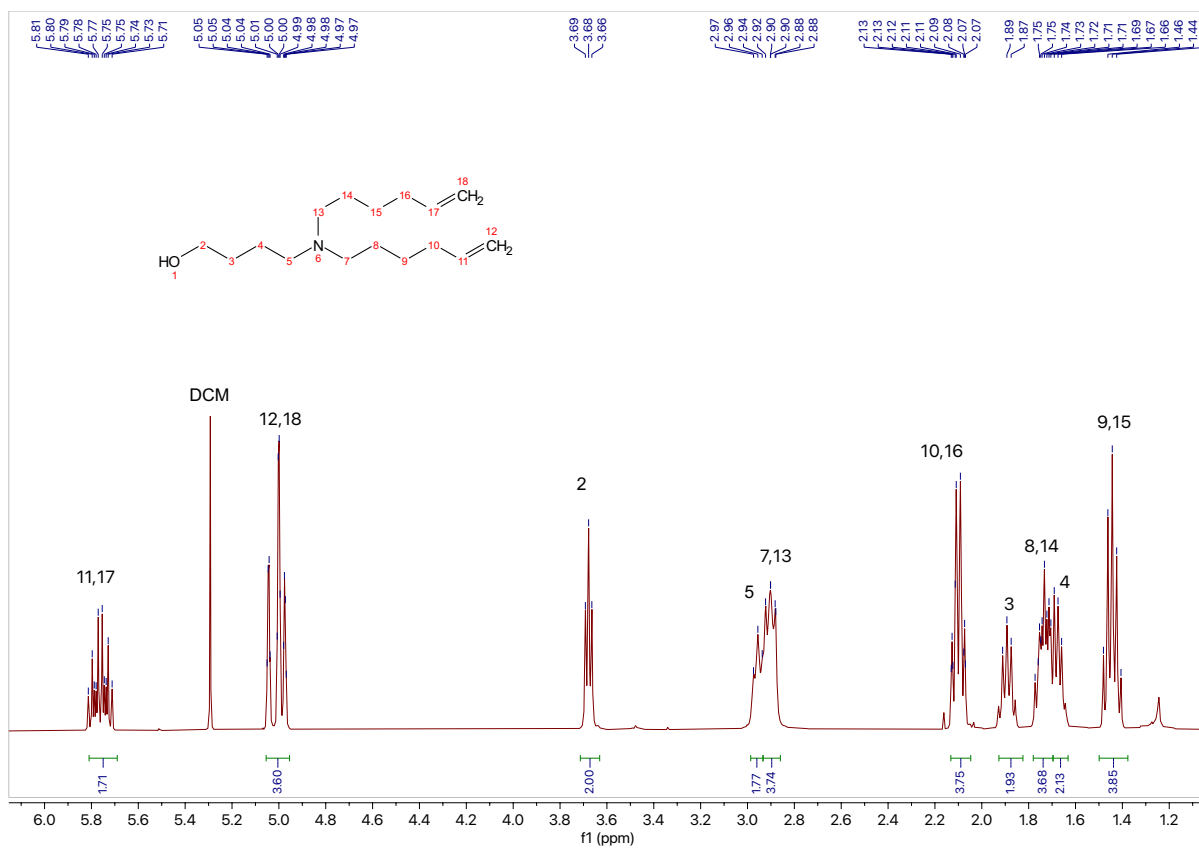


Figure 34. The ^1H NMR spectrum of di-alkylated alkene **19**.

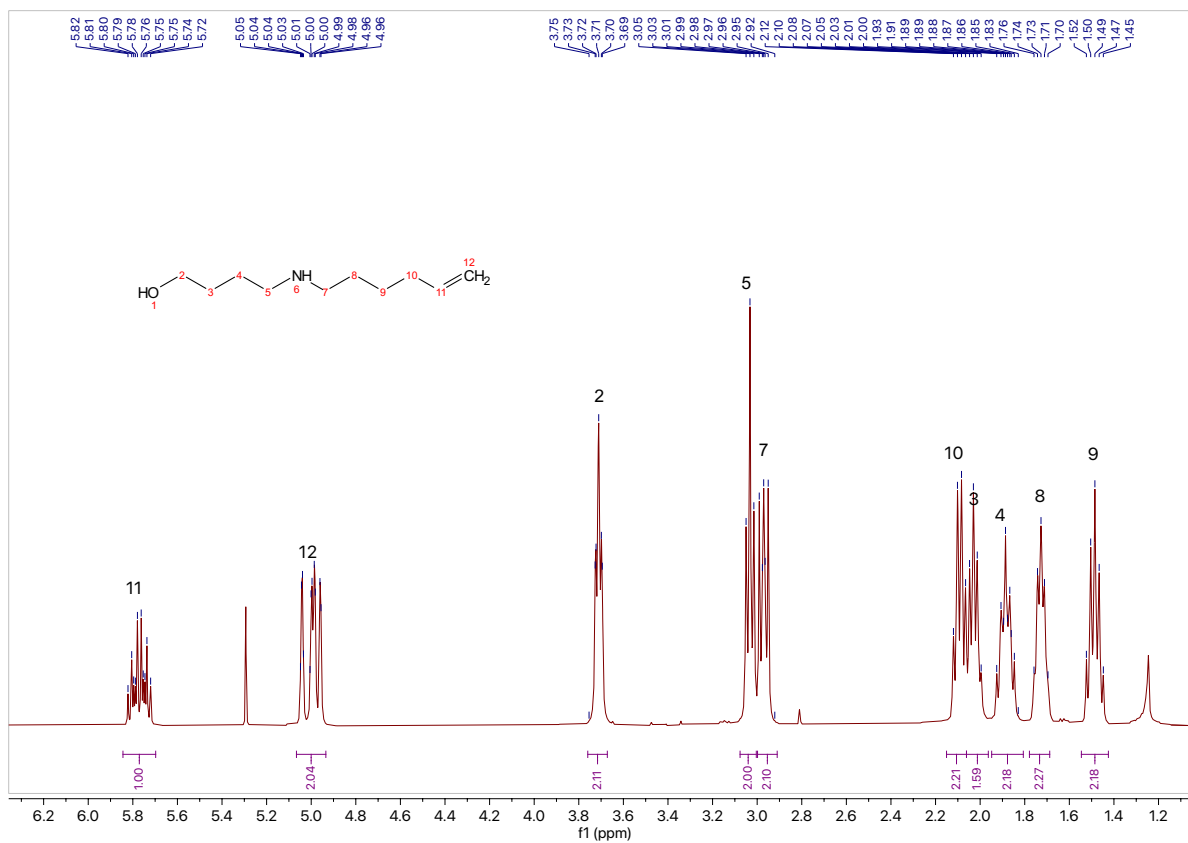


Figure 35. The ¹H NMR spectrum of mono-alkylated alkene **20**.

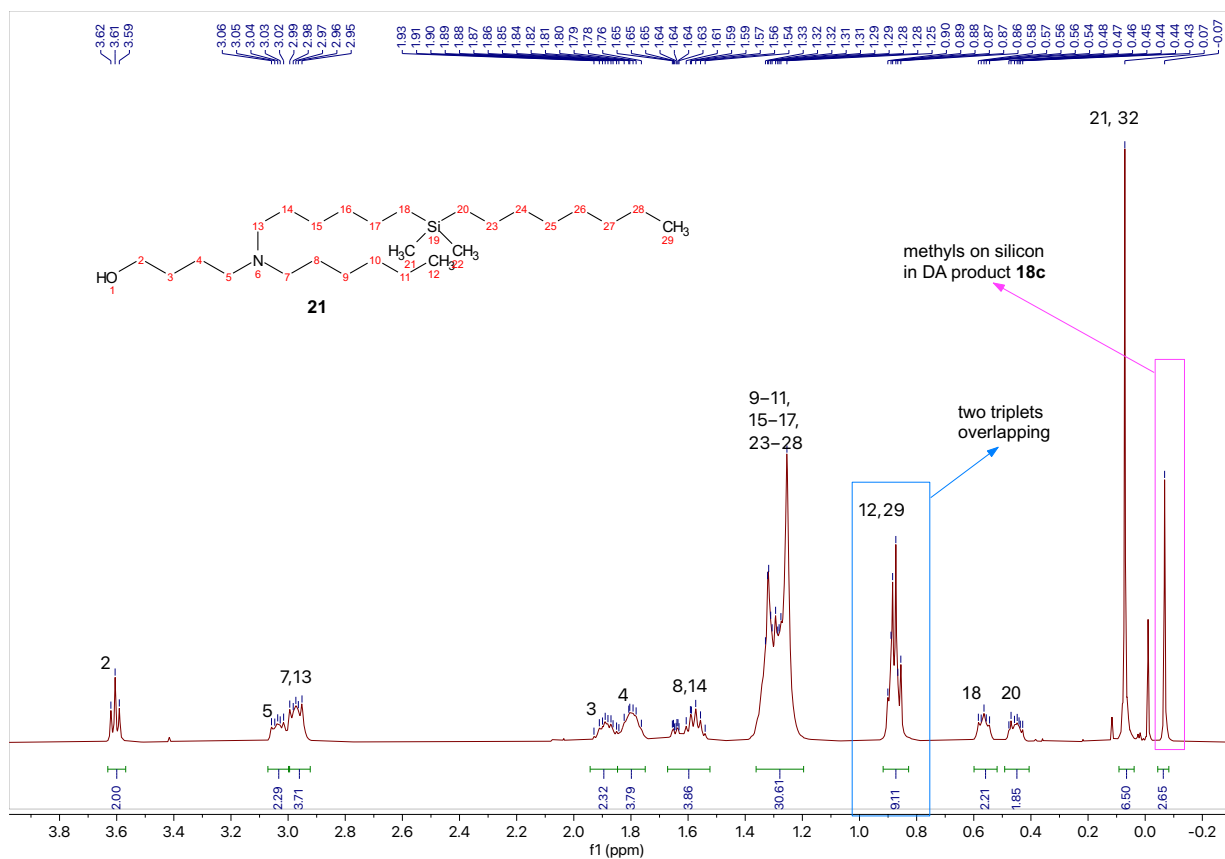


Figure 36. The ^1H NMR spectrum with mono-silylated reduced hydrosilylation byproduct **21** (with trace **18c**).

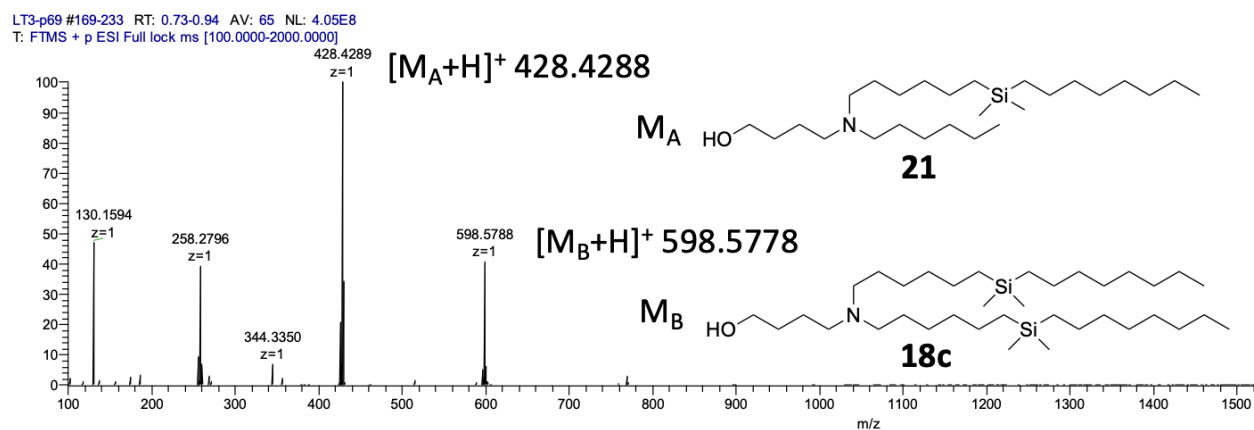


Figure 37. HRMS spectrum of **21** mixed with **18c** from Route B (Figure 3.6).

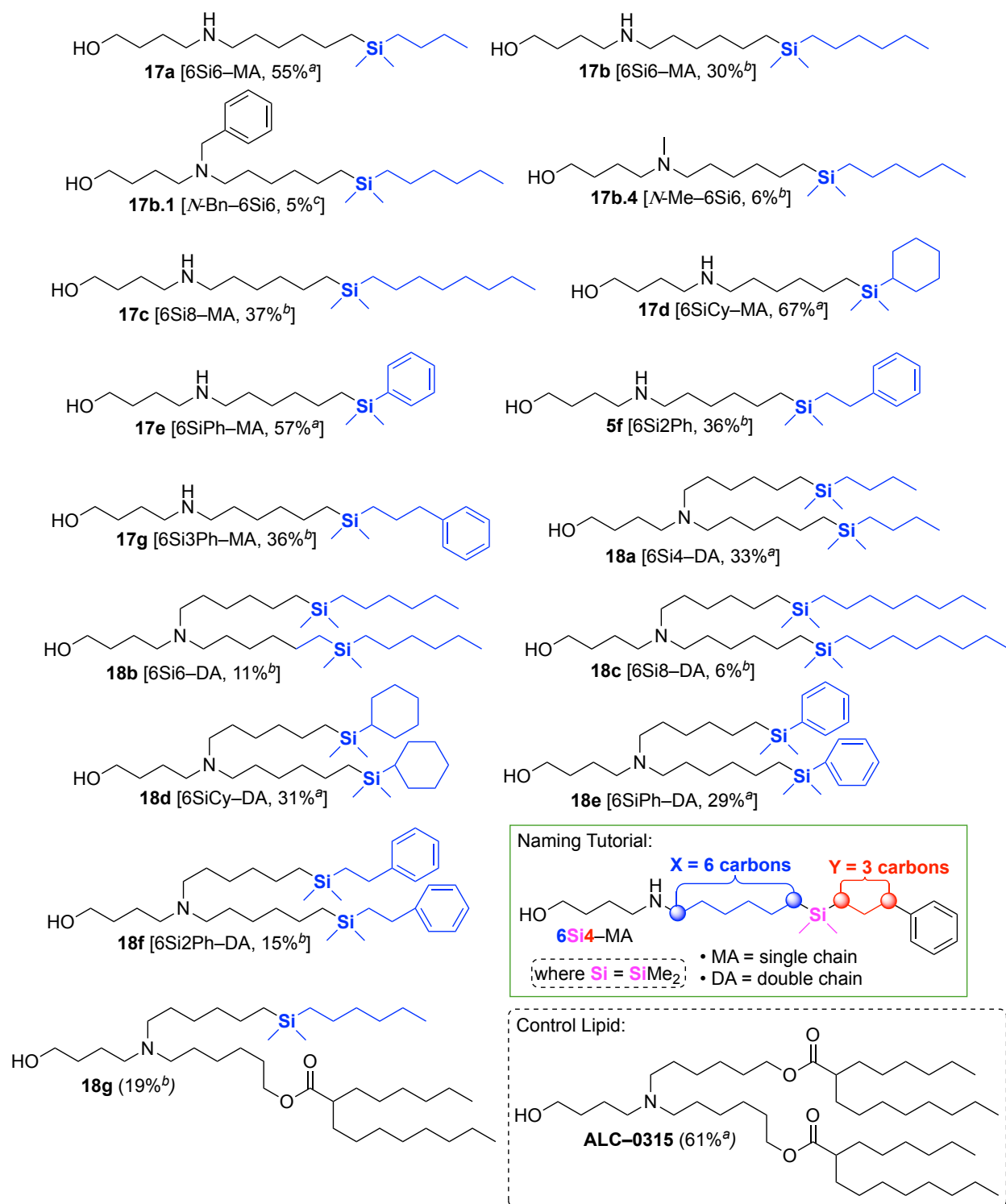


Figure 38. Ionizable silyl lipids and ALC-0315 control. Isolated yields are reported over ^atwo, ^bthree or ^cfour steps. An XSiY naming system is used where X = number of carbon atoms before the silicon atom, Si = SiMe₂, Y = number of carbon atoms after the silicon atom (before any cyclohexyl or phenyl ring), MA = single chain (monoalkylated) and DA = double chain (dialkylated).

3.4. rRNA EE and Stability of Particles Containing Ionizable Silyl lipids

The RNA EE of the ionizable silyl lipids was evaluated using the RiboGreen fluorescence assay (see Section 2.4 and Equation 1 for procedure and calculation of EE). An aliquot of 10 mg/mL ionizable lipid stock solution (20 μ L) was transferred to a vial, the chloroform was removed under vacuum to form a thin lipid film, and the film was hydrated in 400 μ L of 1X Tris–EDTA buffer and batch sonicated at 40 °C for 20 minutes to form a 0.5 mg/mL solution for EE analysis. The results support that varying the silyl lipid tail structure and the number of alkyl chains (i.e. single vs double chain) of ionizable lipids modulates EE (Figure 39). First, the ability of the ionizable silyl lipids was evaluated to encapsulate RNA. The RNA encapsulation efficiency (EE) was evaluated for particles composed of only ionizable silyl lipids with comparison to ALC–0315 (Figure 39A, Table 6). It was observed that two of the single chain (mono–alkylated, MA) silyl lipids 6Si6–MA and 6Si8–MA demonstrated significantly higher EEs (63–64%, **** p <0.0001) compared to ALC–0315 control (20 $\pm$ 8%, Figure 39A–B). The 6Si6–MA and 6Si6–MA formulations demonstrated overall higher EE than 6Si4–MA, 6SiCy–MA, 6SiPh–MA and 6Si2Ph–MA and all double chain (DA) lipids (6Si4–DA, 6Si6–DA, 6Si8–DA, 6SiCy–DA and 6SiPh–DA) (19–36%) (Figure 39A). It is notable that the hybrid structure 6Si6–ALC with a silyl lipid and branching ester tail also performed with low EE (Figure 39A). These findings support that silyl lipid tail structure in conjunction with single vs double chain ionizable lipids can be used as a strategy to control EE. Increasing tail length had a positive impact on EE for MA lipids, with average EE increasing from 24 $\pm$ 5% (6Si4–MA) to 63 $\pm$ 6% (6Si6–MA) and 64 $\pm$ 8% (6Si8–MA). These results support that (i) varying tail length for alkyl containing tails can be used to control EE, and (ii) the effect of increasing tail length on EE depends on the structure having one vs two lipid tails (i.e. single vs double chain).

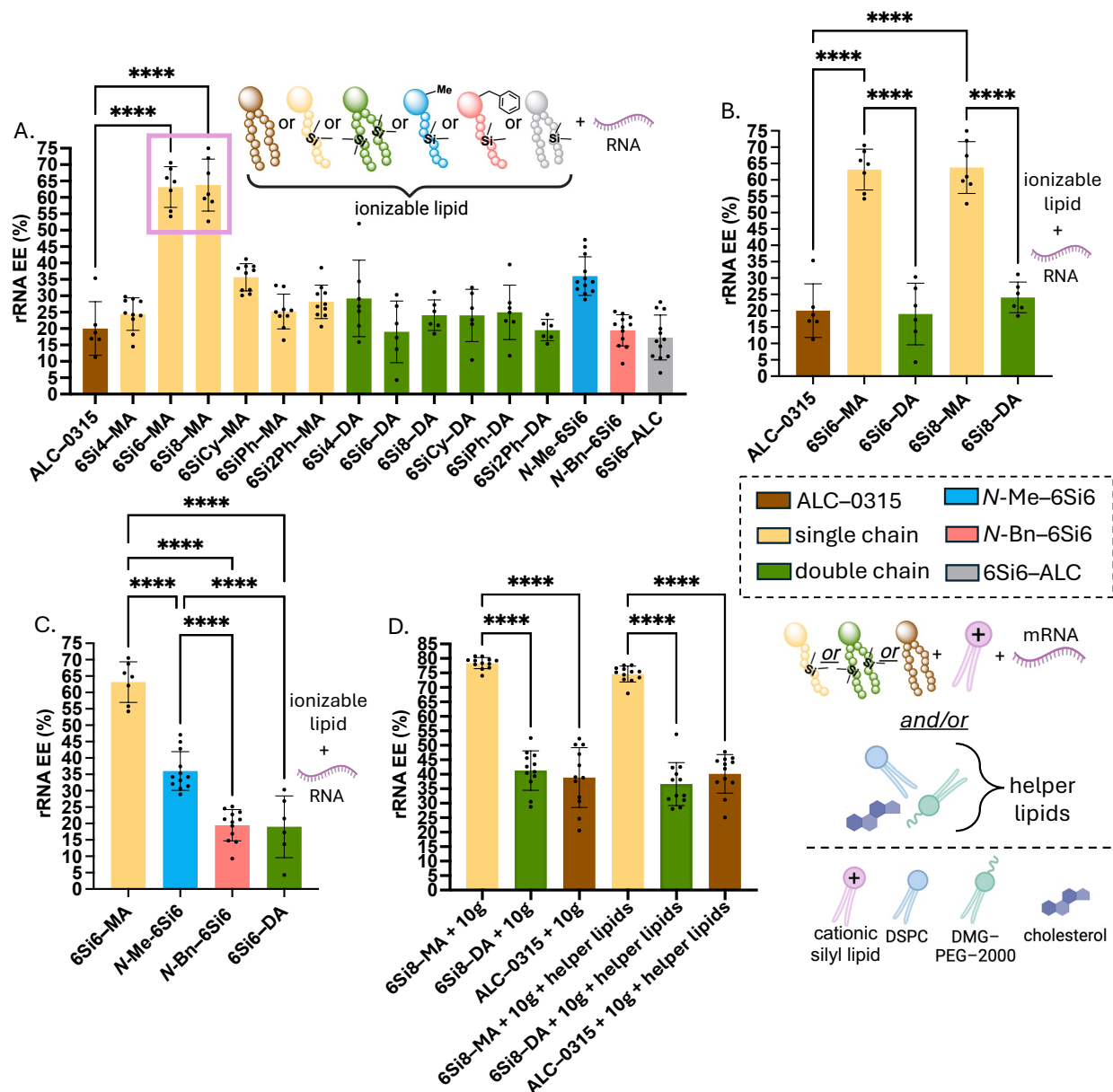


Figure 39. rRNA encapsulation efficiency of (A–C) particles composed of only ionizable lipid and (D) particles composed of either ionizable and cationic lipid (i.e. 6Si8–MA + **10g** (5Si3Ph–DOTAP)) or ionizable, cationic and helper lipids (i.e. 6Si8–MA + **10g** + helper lipids). Helper lipids are DSPC, cholesterol and DMG–PEG–2000. D) Ionizable + **10g** formulations are 1:1 w/w and ionizable + **10g** + helper lipid formulations are 10:10:2:4:1 w/w. See Figure 38 for XSiY–MA and XSiY–DA naming system. Data represents the average $\pm$ standard deviation ($n = 6–12$, **** $p < 0.0001$; p values determined using one-way ANOVA Turkey test in GraphPad Prism). Images created using Chemdraw and BioRender software.

A statistically significant increase (**** $p<0.0001$) in EE is observed when switching from double chain lipids 6Si6-DA ($19 \pm 9\%$) and 6Si8-DA ($24 \pm 5\%$) to single chain lipids 6Si6-MA ($63 \pm 6\%$) and 6Si8-MA ($64 \pm 8\%$) (Figure 39B). We were interested to understand if this notable increase in EE is attributed solely to changing the number of lipid tails (i.e. single vs double chain) or if it is in part due the free N-H group that is present for MA lipids. To test this hypothesis, the *N*-benzyl analog of 6Si6-MA was synthesized in which the N-H was alkylated with a benzyl moiety (*N*-Bn-6Si6). Replacement of the N-H group with an *N*-benzyl moiety resulted in a statistically significant drop in EE from $63 \pm 6\%$ (6Si6-MA) to $19 \pm 5\%$ (*N*-Bn-6Si6) (**** $p<0.0001$, Figure 39C). These results suggest that it is both the single lipid chain and the presence of the N-H group that are contributing to the enhanced EE observed for 6Si6-MA and 6Si8-MA compared to their DA lipid counterparts. We sought to understand if any steric effects from the bulky benzyl ring were also contributing to the drop in EE for *N*-Bn-6Si6 compared to 6Si6-MA. To evaluate this, a methylated analog *N*-Me-6Si6 was synthesized and evaluated in which the N-H group was replaced with an *N*-methyl. A statistically significant drop in EE is also observed upon methylation of the amine, with EE decreasing from $63 \pm 6\%$ (6Si6-MA) to $36 \pm 6\%$ (*N*-Me-6Si6) (**** $p<0.0001$) (Figure 39C). This further supports the importance of the free N-H group in enhancing EE for 6Si6-MA and 6Si8-MA, potentially due to hydrogen-bonding effects. Interestingly, the EE for *N*-Me-6Si6 ($36 \pm 6\%$) is significantly higher than *N*-Bn-6Si6 ($19 \pm 5\%$) but significantly lower than 6Si6-MA ($63 \pm 6\%$, **** $p<0.0001$). However, since the EE for *N*-Me-6Si6 falls within the range of the other DA lipids (10–41 % EE), this suggests that hydrogen bonding (from the free N-H group of the secondary amine) is the largest contributor to the higher EE observed for 6Si6-MA and 6Si8-MA compared to the other lipids evaluated. Along with hydrogen bonding, differences in pKa

between the secondary amine and the tertiary alkyl and tertiary benzyl amine may also be contributing to enhanced EE for 6Si6–MA compared to N-Me–6Si6 and N-Bn–6Si6.

EE was also evaluated for 1:1 w/w and 10:10:2:4:1 w/w formulations containing 6Si6–MA/DA and 6Si8–MA/DA based on their distinct mRNA transfection efficiency profiles in HEK293T cells (*vida infra*). EE is significantly higher ($****p < 0.0001$) for 1:1 w/w 6Si8–MA + **10g** formulations (~78%) compared to 6Si8–DA + **10g** (~40%) (Figure 39D). These results align with the enhanced %GFP positive cells observed for 1:1 w/w ionizable/cationic formulations containing 6Si8–MA (~55–65%) compared to 6Si8–DA (~12–40%) (*vida infra*). These results suggest that the enhanced EE of MA ionizable/cationic (1:1 w/w) formulations compared to DA may attribute to their enhanced %GFP positive cells. A lack of correlation between EE and %GFP positive cells is observed when combining ionizable and cationic lipids with helper lipids (10:10:2:4:1 w/w ionizable/cationic/DSPC/cholesterol/PEG). The %GFP positive cells appear to generally be higher for DA formulations (green) than MA formulations (*vida infra*), where 6Si8–DA (~75–100%) shows significantly higher %GFP positive cells than 6Si6–MA (15–20%) ($*p = 0.0327$, *vida infra*). However, EE appears to be higher for particles with MA lipid than DA lipid, with a significantly higher EE observed for 6Si8–MA (~65–80%) compared to 6Si8–DA (~25–55%) ($****p < 0.0001$, Figure 39D). This lack of correlation between EE and %GFP positive cells for MA and DA formulations containing helper lipids may be attributed to their more complex membrane composition (as determined by a C-laurdan assay, *vida infra*). A wider fluorescence emission spectra is observed for MA formulations compared to DA formulations (*vida infra*), suggesting the presence of both rigid and fluid membrane structures for MA particles. Other plausible reasons for the low %GFP positive cells despite high EE for MA

formulations containing helper lipids may be (i) poor cellular uptake (as supported by the low %GFP positive cells, *vide infra*) and (ii) poor endosomal release of mRNA.

A modified RiboGreen assay was used to evaluate particle stability by measuring EE from 0–105 minutes after bath sonication for (i) particles composed of only ionizable lipid for direct comparison and (ii) particles composed of ionizable, cationic and helper lipids (DPSC, cholesterol and DMG–PEG–2000) (see Section 2.4 for procedure to measure EE vs time). Lipids 6Si6–MA and 6Si8–MA were selected for analysis based on their high EE and compared to 6SiCy, 6Si3Ph–MA, 6Si6–DA, 6Si8–DA and ALC–0315. Stability was evaluated by measuring EE at increasing time points from bath sonication/particle formation ($t = 0, 45,$ and 105 minutes). To prepare samples containing only ionizable lipid, an aliquot of 10 mg/mL ionizable lipid stock solution ($20\text{ }\mu\text{L}$) was transferred to a vial, the chloroform was removed under vacuum to form a thin lipid film, and the film was hydrated in $400\text{ }\mu\text{L}$ of 1X Tris–EDTA buffer and batch sonicated at $40\text{ }^{\circ}\text{C}$ for 20 minutes to form a 0.5 mg/mL solution for analysis. Samples containing ionizable, cationic and helper lipids ($10:10:2:4:1$ w/w ionizable lipid, cationic lipid, DPSC, cholesterol and DMG–PEG–2000) were prepared by transferring the following volume of each 10 mg/mL stock solution to the same vial: ionizable lipid ($20\text{ }\mu\text{L}$), cationic lipid ($20\text{ }\mu\text{L}$), DPSC ($4\text{ }\mu\text{L}$), cholesterol ($8\text{ }\mu\text{L}$) and DMG–PEG–2000 ($2\text{ }\mu\text{L}$). The chloroform was removed under vacuum to form a thin lipid film, and this film was hydrated in 1X Tris–EDTA buffer ($1,080\text{ }\mu\text{L}$) and bath sonicated at $40\text{ }^{\circ}\text{C}$ for 20 minutes to form the 0.5 mg/mL solution for analysis. The EE is generally maintained around 45–60% for particles containing MA silyl lipids or ALC–0315 and helper lipids (Figure 41A–E, Table 6). When comparing to particles composed of only ionizable lipid, 6Si8–MA maintained EE between 50–70% by 105 minutes without the presence of helper lipids (Figure 41F), while a notable drop in EE from to 5–25% by 105 minutes for 6Si8–DA

(Figure 41G) and ALC-0315 (Figure 41H) was observed without the presence of helper lipids (Table 6). Single chain lipids are known to form particles with different/unique morphology compared to double chain lipids,^{119,120} and the incorporation of additional hydrogen bonding groups into lipid structures has been shown to enhance mRNA binding/uptake in particle structures.^{121,122} Therefore, this drop in EE observed for double chain lipid 6Si8-DA (Figure 41G) that is not observed for 6Si8-MA (Figure 41F) suggests that this single chain lipid may be forming particles with different morphology compared to DA lipids and possibly undergoing hydrogen-bonding between the N-H group of 6Si8-MA and RNA that may be enhancing EE and having a stabilizing effect on the particle. This also suggests that incorporation of some MA silyl lipids into particles containing helper lipids may support particle stability.

Table 6. rRNA EE and stability of particles containing ionizable silyl lipids

Liposome	rRNA EE (%) ^f	Stability (EE vs time)		
		t = 0 m ^a	t = 45 m ^a	t = 105 m ^a
6Si4-MA	27 ± 5 ^{d,h} ; 24 ± 5 ^{d,i}	—	—	—
6Si6-MA	69 ± 7 ^{b,h} ; 63 ± 6 ^{b,i}	63 ± 4 ^g	65 ± 3 ^g	65 ± 3 ^g
6Si8-MA	72 ± 11 ^{b,h} ; 64 ± 8 ^{b,i}	70 ± 5 ^f ; 48 ± 10 ^g	57 ± 4 ^f ; 61 ± 2 ^g	52 ± 9 ^f ; 56 ± 2 ^g
6SiCy-MA	39 ± 4 ^{d,h} ; 36 ± 4 ^{d,i}	54 ± 2 ^g	60 ± 3 ^g	58 ± 3 ^g
6SiPh-MA	28 ± 6 ^{c,h} ; 25 ± 5 ^{c,i}	—	—	—
6Si2Ph-MA	31 ± 6 ^{d,h} ; 28 ± 5 ^{d,i}	—	—	—
6Si3Ph-MA	—	58 ± 3 ^g	61 ± 1 ^g	58 ± 4 ^g
6Si4-DA	33 ± 13 ^{b,h} ; 29 ± 12 ^{b,i}	—	—	—
6Si6-DA	21 ± 10 ^{a,h} ; 19 ± 9 ^{a,i}	—	—	—
6Si8-DA	27 ± 5 ^{a,h} ; 24 ± 5 ^{a,i}	—	—	—
N-Bn-6Si6	22 ± 5 ^{a,h} ; 19 ± 5 ^{a,i}	—	—	—
N-Me-6Si6	38 ± 7 ^{e,h} ; 36 ± 6 ^{e,i}	—	—	—
6SiCy-DA	27 ± 8 ^{e,h} ; 24 ± 8 ^{e,i}	—	—	—
6SiPh-DA	28 ± 10 ^{a,h} ; 25 ± 8 ^{a,i}	—	—	—
6Si2Ph-DA	22 ± 4 ^{b,h} ; 20 ± 3 ^{b,i}	—	—	—
6Si6-ALC	19 ± 7 ^{a,h} ; 17 ± 7 ^{a,i}	—	—	—
ALC-0315	23 ± 9 ^{e,h} ; 20 ± 8 ^{e,i}	17 ± 8 ^f ; 61 ± 9 ^g	10 ± 4 ^f ; 55 ± 2 ^g	18 ± 6 ^f ; 58 ± 2 ^g

Values are reported as the average ± standard deviation of ^asix, ^bseven or ^cnine, ^dten or ^etwelve trials. ^fParticles are composed of only ionizable silyl lipid (or ALC-0315) without any helper lipids. ^gParticles are composed of ionizable lipid (silyl or ALC-0315), cationic silyl lipid (5Si3Ph-DOTAP), DSPC, cholesterol, and DMG-PEG-2000 at a 10:10:2:4:1 w/w ratio. ^hrRNA EE (%) was calculated using Equation (1) above. ⁱrRNA EE (%) was calculated using Equation (5), where [rRNA] is the concentration of rRNA determined from the rRNA calibration curve in Figure 40. All particles analyzed were prepared at 0.5 mg/mL concentration in 1x TE buffer. See Figure 38 for XSiY-MA and XSiY-DA naming system.

$$EE (\%) = \frac{[rRNA_{RowB}] - [rRNA_{RowA}]}{[rRNA_{RowB}]} \times 100 \quad (5)$$

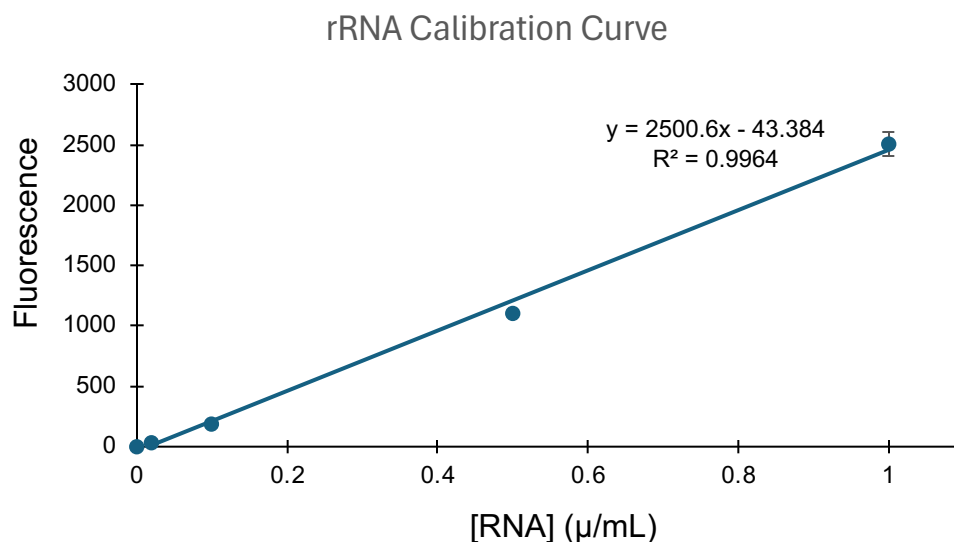


Figure 40. rRNA calibration curve used to calculate EE (%) using Equation (5) based on [rRNA] in Row B (“lysed” LNPs) and Row A (“in-tact” LNPs). Data represents the average $\pm$ standard deviation of $n = 3$ trials. The [rRNA] is the rRNA concentration “x” calculated by plugging in the fluorescence reading for that well into the equation for “y”. This rRNA calibration curve represents the average of three trials.

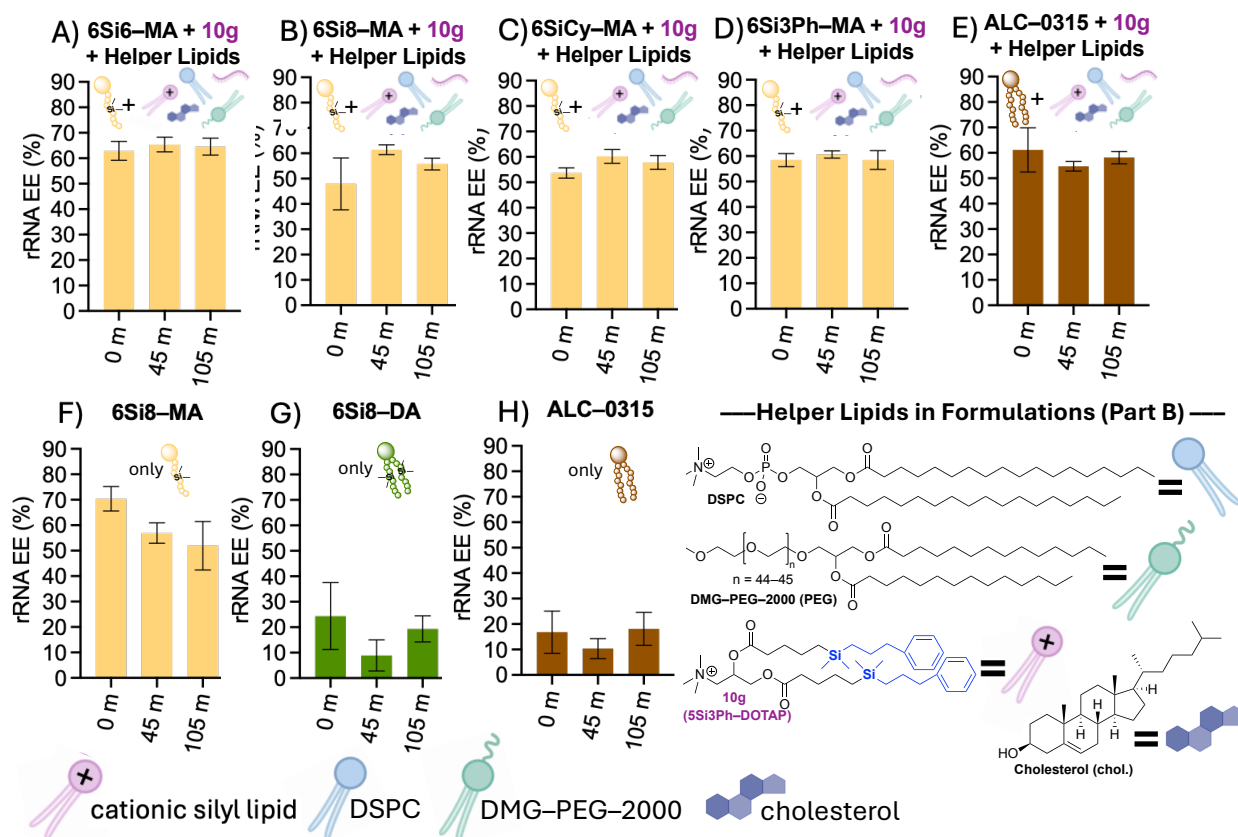


Figure 41. Evaluation of rRNA EE over time for particles composed of (A–E) ionizable lipid, cationic lipid **10g** (5Si3Ph–DOTAP) and helper lipids DSPC, cholesterol and DMG–PEG–2000 or (F–H) only ionizable lipid. Formulations in A–E are 10:10:2:4:1 w/w ionizable lipid, cationic lipid, DSPC, cholesterol, DMG–PEG–2000. All samples were prepared via thin–film hydration with nuclease–free water (0.5 mg/mL) and bath sonication at 40 °C for 20 minutes. Images created using Chemdraw and BioRender software. See Figure 38 for XSiY naming system.

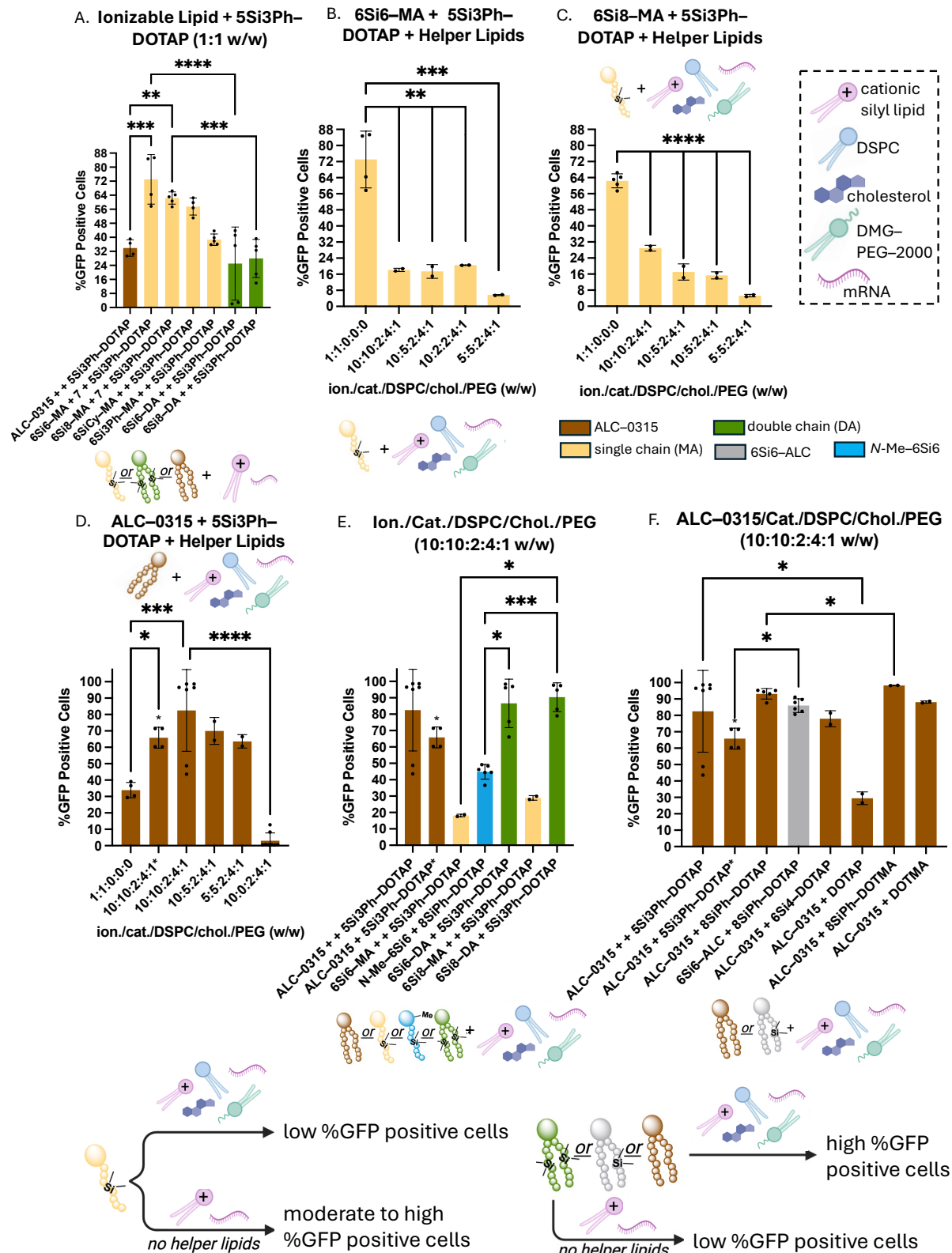
3.5. mRNA Transfection Efficiency in HEK293T Cells

Particle formulations with ionizable silyl lipids 6Si6–MA, 6Si8–MA, 6SiCy–MA or 6Si3Ph–MA were selected for in vitro transfection experiments based on the enhanced EE for 6Si6–MA and 6Si8–MA (Figure 39A–B) and their EE being maintained over time when combined with cationic and helper lipids (see Figure 41A–D). Particles formulated using only ionizable lipid and cationic silyl lipid **10g** 5Si3Ph–DOTAP (1:1 w/w) were first evaluated. These particles were prepared by (i) combining 20 µL of 10 mg/mL ionizable lipid stock solution with 20 µL of 10 mg/mL cationic lipid stock solution in the same vial, (ii) removing the chloroform under vacuum to form a thin lipid film, (iii) hydrating the lipid film in 800 µL of nuclease–free water and (iv) bath sonicating at 40 °C for 20 minutes to form the 0.5 mg/mL solution for analysis. Higher %GFP positive cells are observed for particles containing 6Si6–MA, 6Si8–MA, or 6SiCy–MA (~56–72%) compared to ALC–0315 (~33%), with 6Si6–MA (**** $p=0.0001$) and 6Si8–MA (** $p=0.0058$) having significantly higher %GFP positive cells compared to ALC–0315 and generally higher %GFP positive cells compared to all other ionizable/cationic lipid formulations evaluated (Figure 42A). When comparing these MA lipids with their DA lipid counterparts, a statistically significant decrease in %GFP positive cells to below 48% is observed for 6Si6–DA (compared to 6Si6–MA, **** $p<0.0001$) and 6Si8–DA (compared to 6Si8–MA, *** $p=0.0002$) (Figure 42A). These results suggest that the presence of a single vs double chain ionizable lipid in the formulation is playing a key role in enhancing mRNA transfection for particles

composed of only ionizable and cationic lipids. This enhanced transfection efficiency may be attributed to the enhanced EE previously observed for MA silyl lipids compared to DA silyl lipids and ALC-0315 (Figure 39A–B).

Further experiments were performed for particles containing 6Si6–MA or 6Si8–MA to optimize the weight ratio of each component for mRNA delivery (Figure 42B–C). The following weight ratios of ionizable lipid (ion.), cationic silyl lipid **10g** 5Si3Ph–DOTAP (cat.) and helper lipid components DSPC, cholesterol (chol.) and DMG–PEG–2000 (PEG) were chosen for analysis: 10:10:2:4:1, 10:5:2:4:1, 10:2:2:4:1, and 5:5:2:4:1 w/w ion./cat./DSPC/chol./PEG. These lipid solutions were prepared by (i) transferring the appropriate microliter volume of ionizable lipid, cationic lipid, DSPC, cholesterol and DMG–PEG–2000 (PEG) stock solutions (10 mg/mL) to the same glass vial, (ii) removing the chloroform under vacuum to form a thin lipid film, (iii) hydrating this lipid film in the appropriate volume of nuclease–free water and (iv) bath sonicating at 40 °C for 20 minutes to form a 0.5 mg/mL solution for analysis. A large drop in transfection efficiency is observed at all weight ratios when incorporating DSPC, cholesterol and PEG into the formulation for particles containing 6Si6–MA or 6Si8–MA ionizable lipids and cationic silyl lipid 5Si3Ph–DOTAP (Figures 42B–C). Significance is noted, with lower %GFP positive cells observed for 10:10:2:4:1 w/w (** $p=0.0016$), 10:5:2:4:1 w/w (** $p=0.0015$), 10:2:2:4:1 w/w (** $p=0.0021$) and 5:5:2:4:1 w/w (** $p=0.0005$) 6Si6–MA formulations compared to 1:1:0:0:0 w/w (Figure 42B). Significantly lower %GFP positive cells are also observed for all 6Si8–MA w/w ratios containing DSPC, cholesterol and PEG (**** $p<0.0001$) compared to 1:1:0:0:0 w/w (no DSPC, cholesterol or PEG) (Figure 42C). These findings suggest that MA silyl lipids perform best when combined only with cationic silyl lipid, with lower performance upon formulation with other helper lipid components. Previous work has shown

that incorporation of hydrogen bonding groups in ionizable lipids improves mRNA uptake efficiency and delivery by enhancing binding to mRNA.^{121,122} I hypothesize that the decrease in transfection efficiency when adding helper lipids may be attributed to their disruption of intermolecular hydrogen bonding between the N–H group of MA lipids and mRNA. Single chain lipid structures are known to give unique particle morphology (such as the formation of micelles) compared to double chain lipids which are known to form more liposomal-type structures.^{119,120} Therefore, the single chain and double chain lipids may be giving rise to different particle morphologies when combined with helper lipids, potentially contributing to the distinct transfection efficiency profiles observed for MA and DA lipids. This is supported by cryo-TEM images which demonstrate that MA lipids in combination with helper lipids form particles with less uniform spherical morphologies compared to MA lipids without helper lipids, while DA lipids with and without helper lipids form particles with more uniform spherical morphologies (see cryo-TEM section below, *vide infra*).



bath sonication at 40 °C for 20 minutes. LNPs were prepared with (A) ion./cat. 5Si3Ph–DOTAP (1:1 w/w), or (B–F) varying weight ratios of ion./cat./DSPC/chol./PEG, where cat.= 6Si4–DOTAP, 5Si3Ph–DOTAP, 8SiPh–DOTAP, 8SiPh–DOTMA, DOTAP or DOTMA. Note: cat. = cationic lipid, ion. = ionizable lipid, chol. = cholesterol and PEG = DMG–PEG–2000. Images created using Chemdraw and BioRender software. *LNPs were extruded using the Avanti Mini Extruder Kit (100 nm membrane filter; eleven passes through extruder). See Figure 38 for XSiY naming system.

Our results demonstrate that particles formulated with ALC–0315 generally perform best when combined with cationic silyl lipid, DSPC, cholesterol and PEG, performing worse when combined with only cationic lipid (Figure 42D). Significantly higher %GFP positive cells are observed for 10:10:2:4:1 w/w* (* $p=0.0419$) and 10:10:2:4:1 w/w (*** $p=0.0002$) ALC–0315 formulations compared to 1:1:0:0:0 w/w. An additional weight ratio experiment was performed with ALC–0315 at 10:0:2:4:1 w/w to understand if cationic silyl lipid **10g** was contributing to the enhanced transfection efficiency for particles with ALC–0315. Upon removal of **10g** from the formulation, a statistically significant drop in %GFP positive cells (**** $p<0.0001$) is observed from approximately 80% (10:10:2:4:1 w/w) to below 15% (10:0:2:4:1 w/w). These results (i) suggest the key role of the cationic silyl lipid in enhancing mRNA transfection efficiency for particles containing ALC–0315 and (ii) support the incorporation of cationic silyl lipid **10g** into currently existing particle formulations containing ALC–0315 to further optimize mRNA delivery.

The improved mRNA delivery observed for particles containing **10g** and ALC–0315 (Figure 42D) compared to 6Si6–MA (Figure 42B) and 6Si8–MA (Figure 42C) may be due to MA lipids leading to less stable membranes (compared to DA lipids such as ALC–0315) due to reduced surface area for Van der Waals intermolecular forces and consequently less lipid tail packing. To test this hypothesis, transfection efficiency was evaluated for particles containing 6Si6–DA and 6Si8–DA (10:10:2:4:1 w/w ion./cat. **10g**/DSPC/chol./PEG) (Figure 42E). A general increase in average %GFP positive cells is observed from below 30% for MA silyl lipids (yellow) to

between 80–90% for DA silyl lipids (green), with DA silyl lipids performing similarly to ALC-0315 (64–85%, brown). Significance is noted, with 6Si8–DA (green) demonstrating significantly higher %GFP positive cells ($*p=0.0327$) compared to 6Si6–MA (yellow). These results support our hypothesis and further support the incorporation of DA silyl lipids into current particle formulations (with helper lipids) to optimize mRNA delivery efficiency.

Cryo–TEM data was also acquired for 1:1 w/w ionizable/cationic and 10:10:2:4:1 w/w ionizable/cationic/helper lipid particles due to the distinct differences in %GFP positive cells observed for MA vs DA formulations (see cryo–TEM section below, *vide infra*). Less uniform spherical morphology is observed for MA/cationic/helper lipid particles compared to MA/cationic and DA/cationic/helper lipid particles. This lack of uniform spherical morphology for MA/cationic/helper lipid formulations may contribute to their lower average %GFP positive cells (Figure 42E, yellow) when compared to MA/cationic (Figure 42A, yellow) and DA/cationic/helper lipid formulations (Figure 42E, green). Altogether, these findings contribute to a deeper understanding of the complex relationships between lipid structure, particle formulation and morphology and mRNA transfection efficiency.

The role of the cationic lipid on mRNA transfection efficiency was also evaluated for particles containing ALC-0315 (Figure 42F). When replacing cationic silyl lipid 5Si3Ph–DOTAP (**10g**) with cationic silyl lipid 8SiPh–DOTAP (**10p**), a general increase in %GFP positive cells is observed from ~65% to ~95%. The structures of cationic lipids 5Si3Ph–DOTAP and 8SiPh–DOTAP both contain a phenyl ring in the lipid tail but only differ in the position of the silyldimethyl group. These results support that silyldimethyl position in the lipid tail is contributing to the enhanced transfection efficiency observed for particles containing cationic silyl lipid 8SiPh–DOTAP. Particles with cationic silyl lipid 6Si4–DOTAP (**10h**) generally exhibit

lower average %GFP positive cells (~76%) than 8SiPh-DOTAP (~95%) but higher %GFP positive cells than 5Si3Ph-DOTAP (~65%). When replacing cationic silyl lipids 5Si3Ph-DOTAP, 8SiPh-DOTAP and 6Si4-DOTAP with DOTAP, a non-silyl containing cationic lipid, a notable drop in average %GFP positive cells is observed to below ~32%. Especially notable is the statistically significant decrease in average %GFP positive cells from ~83% to below ~32% when replacing cationic silyl lipid 5Si3Ph-DOTAP with DOTAP (* $p=0.0211$). Cationic silyl lipid 8SiPh-DOTMA was also evaluated and compared to DOTMA (Figure 42F). A subtle increase in %GFP positive cells from ~88% to ~98% is observed when switching from DOTMA to 8SiPh-DOTMA (~98%). Interestingly, the two cationic silyl lipids 8SiPh-DOTAP and 8SiPh-DOTMA that gave the highest average %GFP positive cells (between ~90–100%) have the same tail structure, which includes the same silyldimethyl position and a phenyl ring at the end of the lipid tail, with 8SiPh-DOTMA having slightly higher %GFP positive cells (* $p=0.0211$) compared to 8SiPh-DOTAP. Overall, these results demonstrate the benefit of (i) incorporating cationic silyl lipids (in place of DOTAP and DOTMA) into particles containing ALC-0315 to enhance mRNA delivery, and (ii) incorporating phenyl rings into cationic silyl lipid tails to enhance transfection efficiency of particles containing ALC-0315.

Due to the distinct differences in EE, membrane fluidity (see membrane fluidity section below, *vide infra*) and mRNA transfection efficiency for 6Si6-MA and 6Si6-DA, a hybrid, unsymmetrical lipid 6Si6-ALC was synthesized and evaluated containing one ALC-0315 tail and one 6Si6 tail (gray, Figure 42F). The 6Si6-ALC (gray) formulation demonstrates high %GFP positive cells above 80%. A significantly higher %GFP (* $p=0.0415$) is observed for 6Si6-ALC + 8SiPh-DOTAP particles compared to ALC-0315 + 5Si3Ph-DOTAP* (brown). Overall,

these findings support the incorporation of unsymmetrical, hybrid silyl–ALC lipids into LNP formulations to further optimize mRNA delivery efficiency.

Distinct differences in GFP intensity are observed for cells treated with MA vs DA lipid formulations (Figure 43A–B). Histograms were plotted from flow cytometry data demonstrating GFP intensity vs cell count for some of the key particle formulations containing MA and DA lipids. Interestingly, 6Si6–MA/5Si3Ph–DOTAP and 6Si8–MA/5Si3Ph–DOTAP (1:1 w/w) formulations resulted in sufficient average %GFP positive cells (~60–72% GFP cells, Figure 42A–C) but demonstrated lower overall GFP intensity across all cells (blue, Figure 43A). This data suggests that although a high number of cells were transfected with these 1:1 w/w formulations (as indicated by the sufficient %GFP positive cells), less mRNA–encapsulated particles were overall uptaken per cell via endocytosis and/or less mRNA was released from the endosome, resulting in lower overall GFP intensity across all cells. This differs from 6Si6–DA and 6Si8–DA formulations (10:10:2:4:1 w/w ionizable/5Si3Ph–DOTAP/DSPC/cholesterol/PEG) which showed high %GFP positive cells (Figure 42E) and overall high GFP intensity across a larger number of cells (pink, Figure 43B), suggesting high cellular uptake of these mRNA–encapsulated particles, sufficient endosomal release of mRNA and subsequently high GFP intensity across all cells. Future experiments evaluating endosomal release rate will be performed to further elucidate the underlying mechanisms behind the distinct mRNA delivery profiles for these MA and DA formulations.

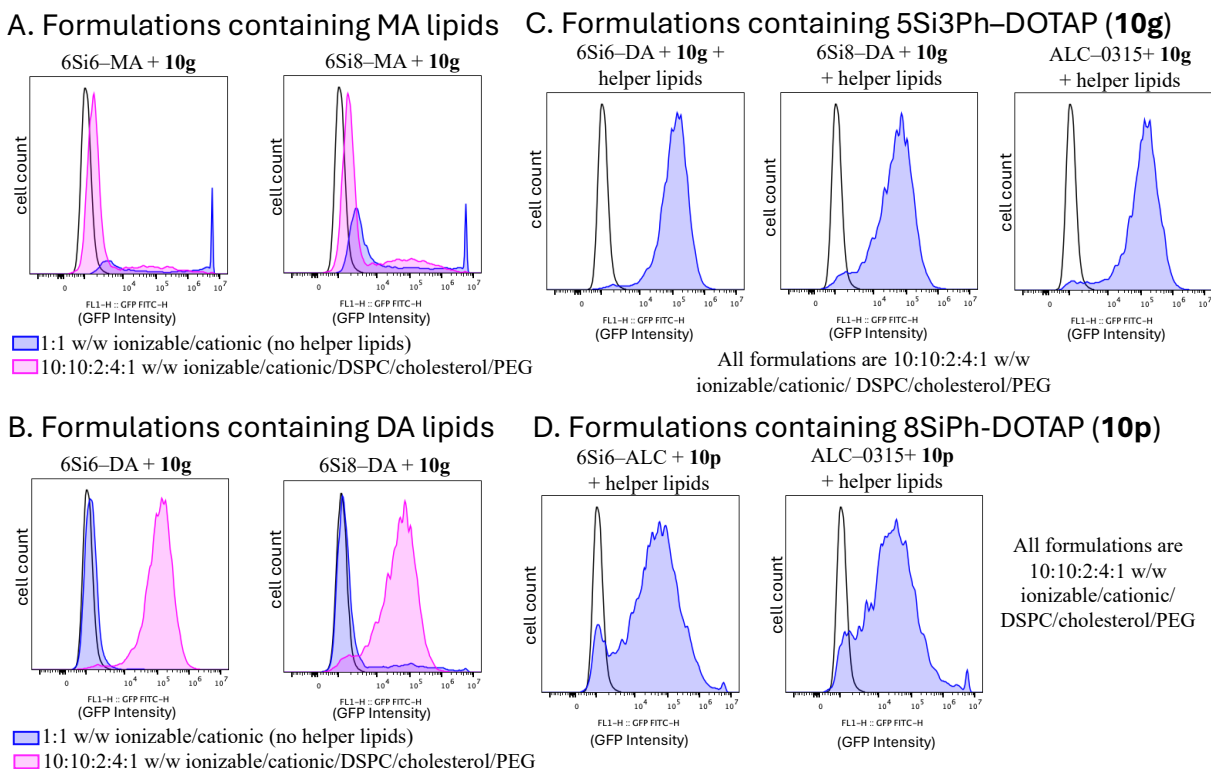


Figure 43. Flow cytometry analysis of fluorescence of transfected HEK293T cells. Histograms were acquired from flow cytometry for a selection of LNP formulations. Data represents the GFP Intensity (x-axis; FL1-H::GFP FITC-H) vs Cell Count (y-axis) collected from one biological replicate/sample. See Figure 38 for XSiY naming system.

A correlation between silyldimethyl position in the lipid tail and GFP intensity was observed for cationic lipids (Figure 43C–D). Histograms plots for formulations containing 5Si3Ph-DOTAP (10g) or 8SiPh-DOTAP (10p) demonstrate sufficiently high GFP intensity across cells (Figure 43C–D). However, the distribution of GFP intensity is slightly wider/more broad for cells treated with formulations containing 8SiPh-DOTAP (Figure 43D) compared to 5Si3Ph-DOTAP (Figure 43C). This emphasizes the influence of cationic silyl lipid structure on transfection efficiency and highlights the critical role of silyldimethyl position in the lipid tail on overall transfection efficiency (since 5Si3Ph-DOTAP and 8SiPh-DOTAP have identical lipid structures but only vary in the position of the silyl-dimethyl moiety).

Cell viability was assessed by flow cytometry 48 hours after HEK293T transfection (Figure 44). Forward Scatter (FSC) and Side Scatter (SSC) profiles were used to differentiate live cells from dead cells and cell debris. The percentage of live cells for a selection of representative ionizable lipid formulations is reported, with all formulations showing an average of 80% live cells post transfection. This data is comparable to standard transfection reagents and does not suggest toxicity of these ionizable lipid formulations. There is no statistically significant difference (determined using one-way ANOVA Turkey test in GraphPad Prism) in %live cells between treated cells and untreated cells (denoted as NT), further supporting no cytotoxicity for any of the ionizable lipid formulations evaluated.

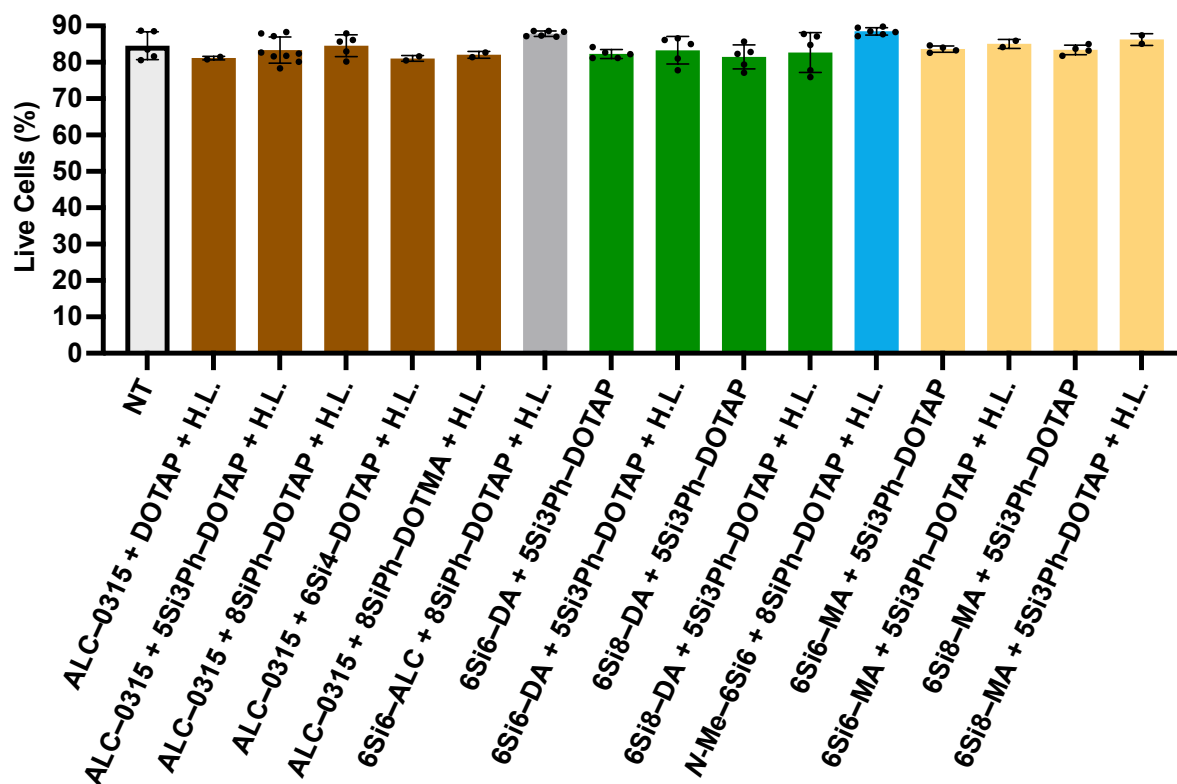


Figure 44. Cell viability 48 hours HEK293T transfection with Ionizable/Cationic and Ionizable/Cationic/Helper Lipid Formulations. Live Cells (%) was determined by flow cytometry. See Figure 38 for XSiY naming system.

3.6. Membrane Fluidity of Particles Containing Ionizable Silyl lipids

The data presented above demonstrates a correlation between the number of lipid tails (i.e. single vs double chain), EE and transfection efficiency. To better understand this correlation, the membrane fluidity of particles composed of only MA or DA lipids was evaluated and compared to particles containing only ALC-0315 using the C-Laurdan assay (Figure 45A).^{123–125}

Membrane fluidity was evaluated by calculating the generalized polarization (GP) value from the fluorescence emission spectrum of liposomal solutions containing C-laurdan. GP values range from -1.0 to 1.0, where lower GP values closer to -1.0 indicate more fluid membranes and a red-shifted emission spectrum. The GP data shows a clear relationship between single vs double chain lipids and membrane fluidity of particles. All MA silyl lipids formed particles with more fluid membrane structures as indicated by their negative GP values (Table 7, Figure 45A, yellow) and red-shifted emission spectra (Figure 45B, Figure 46, yellow). The opposite is observed for DA lipids and ALC-0315, which formed particles with more rigid membrane structures as indicated by GP values >0 (Table 7, Figure 45A, green) and blue-shifted emission spectra (Figure 45B, Figure 47, green). These findings may explain the lower transfection efficiency observed for particles with MA lipids. The MA lipids may be forming particles with more fluid membranes, decreasing the structural stability and consequently transfection efficiency of the particles.

The relationship between tail length and membrane fluidity was evaluated and compared for single and double chain lipids (Figure 45A, Table 7). An increase in GP is observed from -0.38 ± 0.02 (6Si4-MA) to -0.30 ± 0.02 (6Si6-MA) and -0.33 ± 0.04 (6Si8-MA) when increasing the number of carbon atoms in the alkyl chain after the silicon, indicating a blue-shift to more rigid membranes upon increasing tail length for single chain lipids. Increasing tail length for double

chain lipids also results in more rigid membranes, with GP increasing from 0.11 ± 0.05 (6Si4–DA) to 0.14 ± 0.01 (6Si6–DA). Upon further increasing tail length, the formation of 6Si8–DA particles with two membrane populations is observed, one with more fluid membranes and a GP value closer to 0 and another with more rigid membranes and a GP value closer to 0.25 (Figure 45A). These results support that (i) varying tail length can control membrane fluidity of particles and (ii) chain alkylation (i.e. single vs double chain lipids) influences the effect of tail length on membrane fluidity.

Silyl lipid tails with a cyclohexyl ring were also investigated. Incorporation of a cyclohexyl in place of the alkyl group in the silyl lipid tails showed a subtle affect in membrane fluidity for MA lipids, with 6SiCy–MA (-0.37 ± 0.05) having an average GP value similar to 6Si4–MA (-0.38 ± 0.02) but slightly lower than 6Si6–MA (0.30 ± 0.02) and 6Si8–MA (-0.33 ± 0.04) (Figure 45A, Table 7). Replacing an alkyl group with a cyclohexyl ring results in a subtle increase in membrane rigidity for DA lipids, with 6SiCy–DA (0.18 ± 0.01) having a higher average GP value compared to 6Si4–DA (0.11 ± 0.05) and 6Si6–DA (0.14 ± 0.01). These results demonstrate the ability to modulate membrane fluidity of particles by incorporating a cyclohexyl ring into the lipid tail, where the effect of the cyclohexyl group on membrane fluidity depends on the number of tails in the ionizable lipid structure (i.e. single vs double chain).

Engineering a phenyl ring into the silyl lipid tails also influences membrane fluidity. When replacing an alkyl group with a phenyl ring for MA lipids, an increase in membrane rigidity is observed as indicated by the increase in average GP from -0.38 –(-0.33) (6Si4–, 6Si6–, 6Si8– and 6SiCy–MA) to -0.24 ± 0.007 for 6SiPh–MA (Figure 45A, Table 7). The opposite is observed for some of the DA lipids, with GP decreasing from 0.14 ± 0.01 (6Si6–DA) and 0.18 ± 0.01 (6SiCy–DA) to 0.10 ± 0.007 (6SiPh–DA) indicating a red–shift to more fluid membranes upon

incorporation of a phenyl ring. These results demonstrate (i) the utility of incorporating a phenyl ring into ionizable lipid tails to control membrane fluidity and (ii) the influence of phenyl ring on membrane fluidity depends on the number of lipid tails (i.e. single vs double chain).

The relationship between membrane fluidity and phenyl distance from the silyldimethyl group was also evaluated. Increasing the number of carbon atoms between the phenyl ring and the silicon caused an increase in membrane fluidity for MA lipids as shown by the decrease in GP from -0.24 ± 0.007 (6SiPh-MA) and -0.24 ± 0.03 (6Si2Ph-MA) to -0.30 ± 0.008 (6Si3Ph-MA) (Figure 45A, Table 7). However, an increase in GP is observed for DA lipids from 0.10 ± 0.007 (6SiPh-DA) to 0.15 ± 0.01 (6Si2Ph-DA) upon increasing the number of carbon atoms between the phenyl ring and the silicon, indicating a blue-shift towards more rigid membranes. Overall, these results (i) support the use of variable-length phenyl-containing silyl lipid tails for controlling membrane fluidity and (2) demonstrate that the influence of phenyl distance from the silyldimethyl group on membrane fluidity depends on the number of lipid tails (i.e. single vs double chain).

Membrane fluidity of particles containing MA or DA ionizable lipids (6Si6-MA/DA, 6Si8-MA/DA or ALC-0315), cationic silyl lipids (5Si3Ph-DOTAP or 8SiPh-DOTAP) and helper lipids was also investigated based on the high transfection efficiency observed for particles with DA ionizable lipids and the distinct differences in transfection efficiency observed for MA vs DA lipids. All particles evaluated have average GP values between -23 – 0.09 (Table 7), indicating the formation of membranes that generally are more fluid than those formed from only DA lipids but more rigid than those formed from only MA lipids. Although the GP values of particles containing MA and DA lipids are similar, their fluorescence emission spectra are noticeably different (Figure 45C, Figure 48). Particles formed with 6Si6-DA, 6Si8-DA and ALC-0315 all

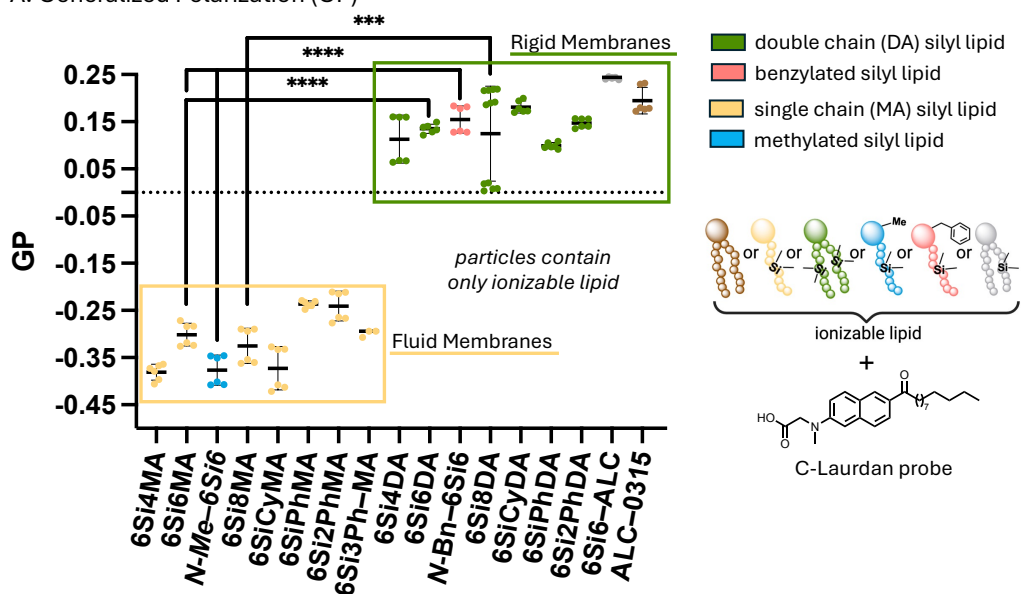
have a narrower fluorescence emission peak compared to those with MA lipids, suggesting the presence of more uniform rigid membranes (green, Figure 45C, Figure 48). However, particles formed with 6Si6–MA and 6Si8–MA have notably wider fluorescence emission peaks (yellow, Figure 45C, Figure 48), indicating a more complex membrane composition potentially due to the presence of both rigid and fluid membranes. This complex membrane profile observed for particles with MA lipids may contribute to their lower transfection efficiencies compared to particles containing DA lipids. These results support the incorporation of MA and DA silyl lipids into particles to control their membrane fluidity profiles.

I was interested to understand if the distinct membrane fluidity and EE profiles for 6Si6– and 6Si8–MA and DA silyl lipids is solely due to the single vs double chain lipid tail(s) or if the presence of the free N–H group in MA lipids is a determining factor. To understand this, the benzyl–containing lipid *N*-Bn–6Si6 (**5b.1**) was also evaluated. Benzylation of the N–H group caused a significant increase (**** $p < 0.0001$) in membrane rigidity as indicated by the large increase in GP from -0.30 ± 0.02 (6Si6–MA, yellow) to 0.15 ± 0.03 (*N*-Bn–6Si6, pink) (Table 7, Figure 45A). This increase in GP correlates with the blue–shifted emission spectrum observed for *N*-Bn–6Si6 (pink, Figure 45B and Figure 49) compared to 6Si6–MA (yellow, Figure 45B and Figure 46). This increase in membrane rigidity is also correlated with the decrease in EE from $69 \pm 7\%$ (6Si6–MA) to $22 \pm 5\%$ (*N*-Bn–6Si6) when benzylating the N–H position (pink, Figure 39A, Figure 39C, Table 6). The increase in membrane rigidity for the benzylated lipid *N*-Bn–6Si6 may be resulting in decreased permeability of RNA through the lipid membrane and consequently reduced EE of cargo. Overall, these findings suggest that (1) alkylation of the N–H position of MA silyl lipids can be used to further modulate particle properties and (2) it is both the single chain and the free N–H group that are contributing to the distinct membrane fluidity

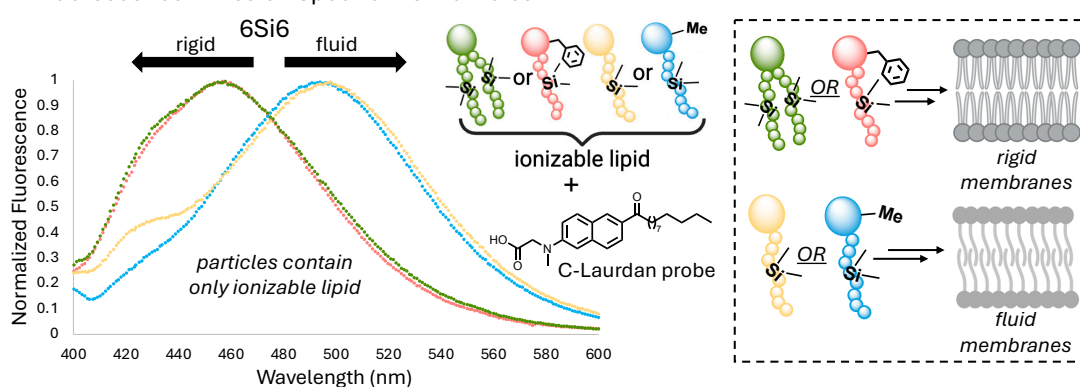
and EE profiles observed for 6Si6– and 6Si8–MA silyl lipids. I sought to understand if the bulkiness of the benzyl group in *N*-Bn–6Si6 was have any steric effects that may be causing the significant increase in membrane rigidity compared to 6Si6–MA. To evaluate this, the GP value of *N*-Me–6Si6 was also determined. Compared to the benzyl group, the methyl group replacement of the N–H resulted in no significant change membrane rigidity, with fluid membranes being observed for *N*-Me–6Si6 and 6Si6–MA as indicated by their negative GP values of -0.38 ± 0.03 (*N*-Me–6Si6, Figure 45A, blue) and -0.30 ± 0.02 (6Si6–MA, Figure 45A, yellow) and red-shifted emission spectra (Figure 45B, blue for *N*-Me–6Si6, yellow for 6Si6–MA). These results suggest that the size of the substituent replacing the N–H group is also having an effect in modulating/tuning the membrane fluidity of these particles, where the increased bulkiness of the benzyl group may be having a steric effect that is contributing to the higher GP value observed for *N*-Bn–6Si6 compared to *N*-Me–6Si6 and 6Si6–MA.

Due to the enhanced EE of 6Si6–MA particles (Figure 39A, Table 6) and the enhanced mRNA transfection efficiency of 6Si6–MA particles (containing only ionizable and cationic lipid, Figure 42A) and 6Si6–DA particles (containing ionizable, cationic and helper lipids, Figure 42E), a hybrid analog ALC–0315 was synthesized with one of the branching ester tails replaced with the 6Si6 lipid tail (6Si6–ALC). Incorporation of the silyl lipid tail increased membrane rigidity as indicated by the increase in average GP from 0.19 ± 0.03 (ALC–0315) to 0.24 ± 0.006 (6Si6–ALC, Figure 45A, grey, Table 7) and the blue-shifted emission spectrum (Figure 49, grey). This data highlights the strategy of incorporating silyl lipid tails to access hybrid unsymmetrical silyl–ALC-type lipids that can be used to further optimize and modulate membrane fluidity and particle properties.

A. Generalized Polarization (GP)



B. Fluorescence Emission Spectrum of Particles



C. Fluorescence Emission Spectrum of Particles

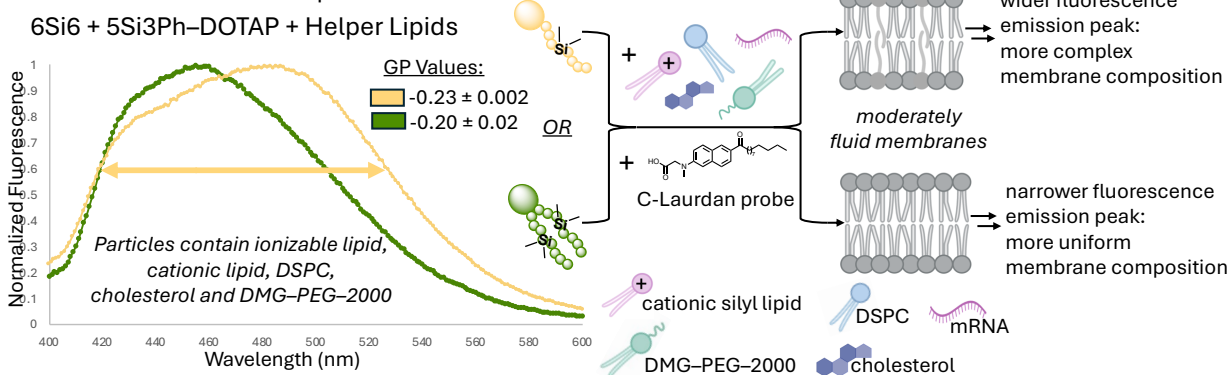


Figure 45. Membrane fluidity of particles. A–B) Particles containing only single chain (MA) or double chain (DA) ionizable lipid. C) LNPs are 10:10:2:4:1 w/w ionizable lipid/cationic lipid/DSPC/chol./PEG (ionizable lipid = 6Si6–MA, 6Si6–DA, 6Si8–MA, 6Si8–DA or ALC–0315; cationic lipid = 5Si3Ph–DOTAP). Data represents the average ± standard deviation (n = 3–12, *** $p=0.0009$, **** $p<0.0001$; p values determined using one-way ANOVA Turkey test in GraphPad Prism). Images created using Chemdraw and BioRender software. See Figure 38 for XSiY naming system.

Table 7. General polarization (GP) of particles containing MA and DA ionizable silyl lipids

Lipid Components	λ_{max} (nm)	GP
6Si4-MA	499 ^b	-0.38 ± 0.02 ^b
6Si6-MA	498 ^b	-0.30 ± 0.02 ^b
N-Me-6Si6	494 ^b	-0.38 ± 0.03 ^b
N-Bn-6Si6	457 ^b	0.15 ± 0.03 ^b
6Si8-MA	497 ^b	-0.33 ± 0.04 ^b
6SiCy-MA	496 ^b	-0.37 ± 0.05 ^b
6SiPh-MA	486 ^b	-0.24 ± 0.007 ^b
6Si2Ph-MA	494 ^b	-0.24 ± 0.03 ^b
6Si3Ph-MA	498 ^a	-0.30 ± 0.008 ^a
6Si4-DA	460 ^b	0.11 ± 0.05 ^b
6Si6-DA	458 ^b	0.14 ± 0.01 ^b
6Si8-DA	456 ^d	0.12 ± 0.10 ^d
6SiCy-DA	456 ^b	0.18 ± 0.01 ^b
6SiPh-DA	457 ^b	0.10 ± 0.007 ^b
6Si2Ph-DA	455 ^b	0.15 ± 0.01 ^b
6Si6-ALC	450 ^b	0.24 ± 0.006 ^b
ALC-0315	452 ^b	0.19 ± 0.03 ^b
6Si6-MA + 10g + helper Lipids	481 ^a	-0.23 ± 0.002 ^a
6Si8-MA + 10g + helper Lipids	459 ^a	-0.15 ± 0.009 ^a
6Si6-DA + 10g + helper Lipids	455 ^a	-0.20 ± 0.02 ^a
6Si8-DA + 10g + helper Lipids	455 ^c	0.09 ± 0.02 ^c
ALC-0315 + 10g + helper Lipids	453 ^a	-0.15 ± 0.007 ^a
ALC-0315 + 10p + helper Lipids	471 ^c	-0.05 ± 0.11 ^c

Helper Lipids = DSPC, cholesterol (chol.) and DMG-PEG-2000 (PEG) (10:10:2:4:1 w/w ionizable lipid, cationic lipid 5Si3Ph-DOTAP (**10g**) or 8SiPh-DOTAP (**10p**), DSPC, cholesterol and /DMG-PEG-2000). The λ_{max} values are associated with the corresponding fluorescence emission spectra in Figures 46–49. Data represents the average ± standard deviation of ^athree, ^bsix, ^cseven, or ^dtwelve trials (n = 3–12). See Figure 38 for XSiY naming system.

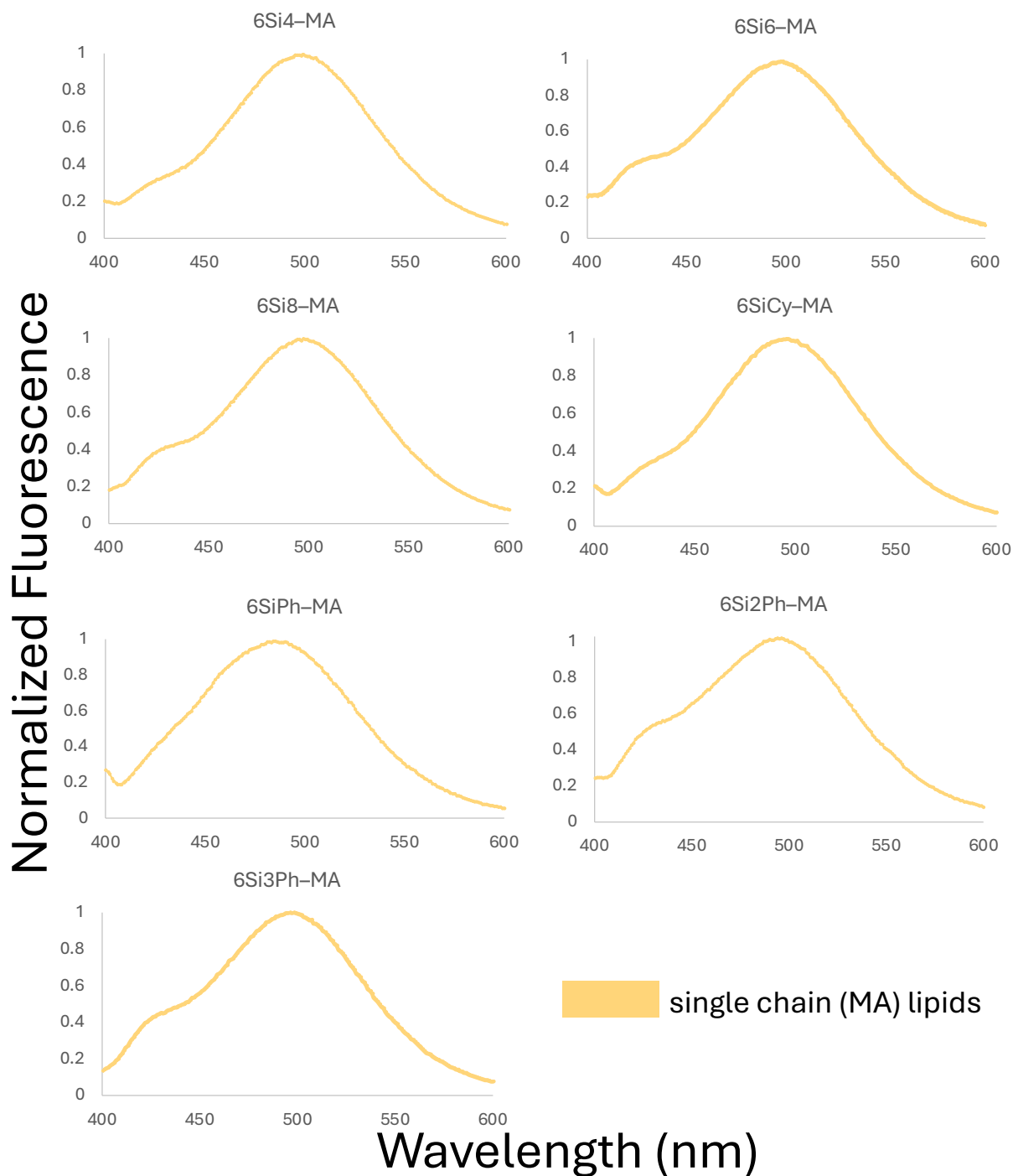


Figure 46. Fluorescence spectra of particles composed of only single chain (MA) lipids. Particles were prepared at a concentration of 0.3 mg/mL in PBS buffer. Data represents the average normalized fluorescence vs wavelength ($n = 3-6$). See Figure 38 for XSiY naming system and Table 7 for λ_{max} values.

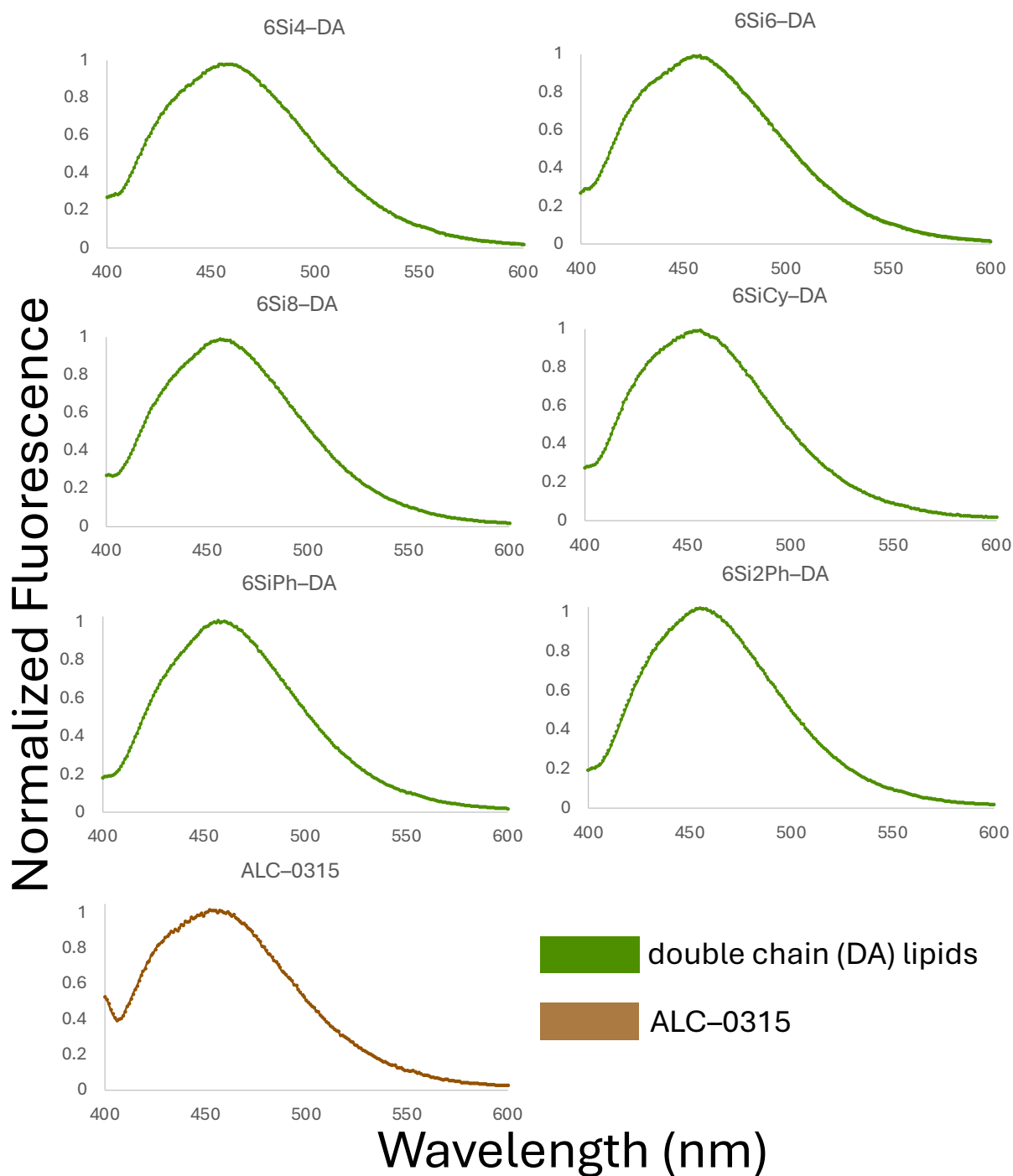


Figure 47. Fluorescence spectra of particles composed of only double chain (DA) lipids. Particles were prepared at a concentration of 0.3 mg/mL in PBS buffer. Data represents the average normalized fluorescence vs wavelength ($n = 6-12$). See Figure 38 for XSiY naming system and Table 7 for λ_{max} values.

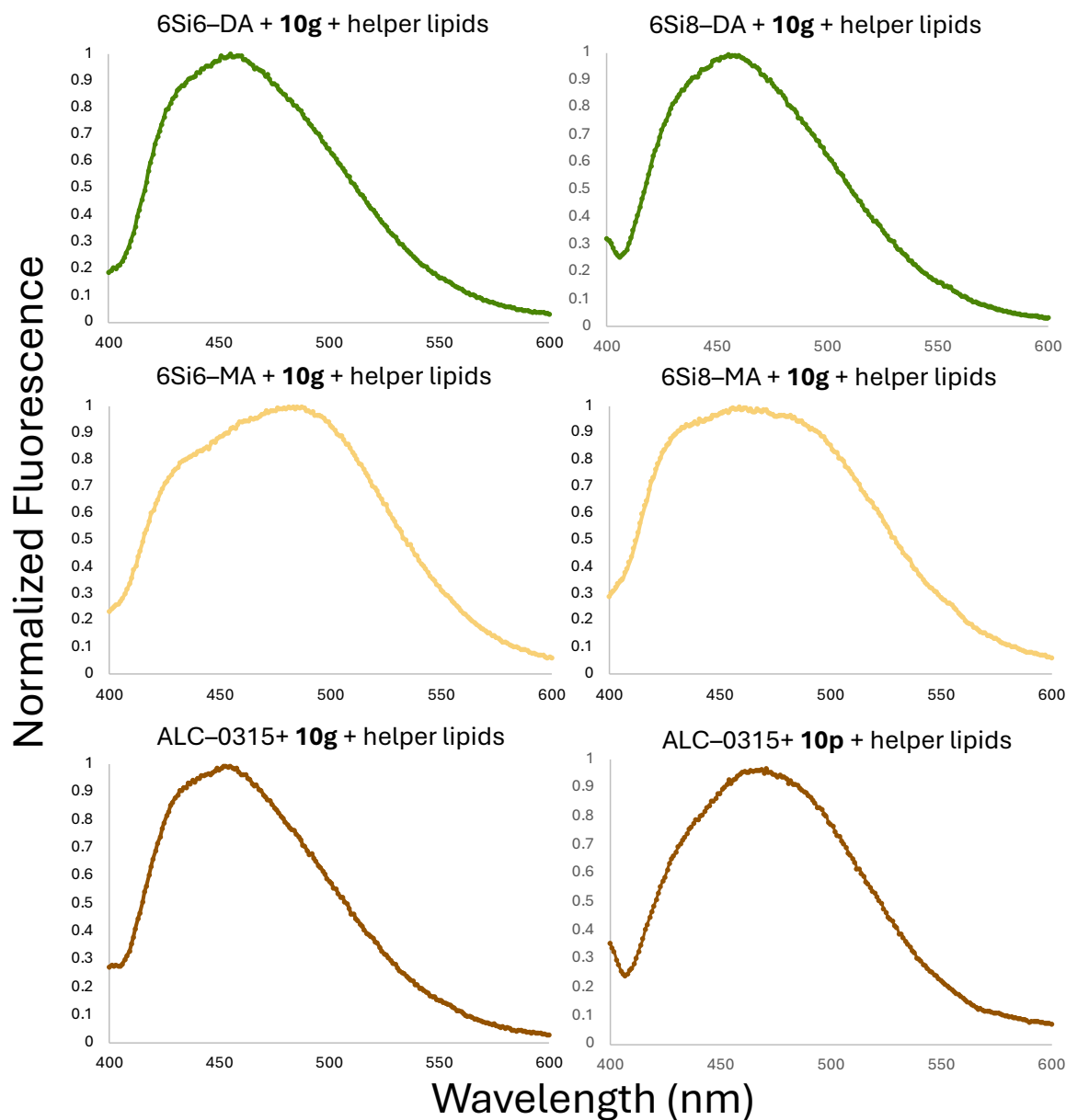


Figure 48. Fluorescence spectra of particles composed of ionizable lipid, cationic lipid 5Si3Ph-DOTAP (**10g**) or 8SiPh-DOTAP (**10p**), DSPC, cholesterol and DMG-PEG-2000 (10:10:2:4:1 w/w). Particles were prepared at a concentration of 0.3 mg/mL in PBS buffer. Data shown is the average normalized fluorescence vs wavelength ($n = 3-7$). See Figure 38 for XSiY naming system and Table 7 for λ_{max} values.

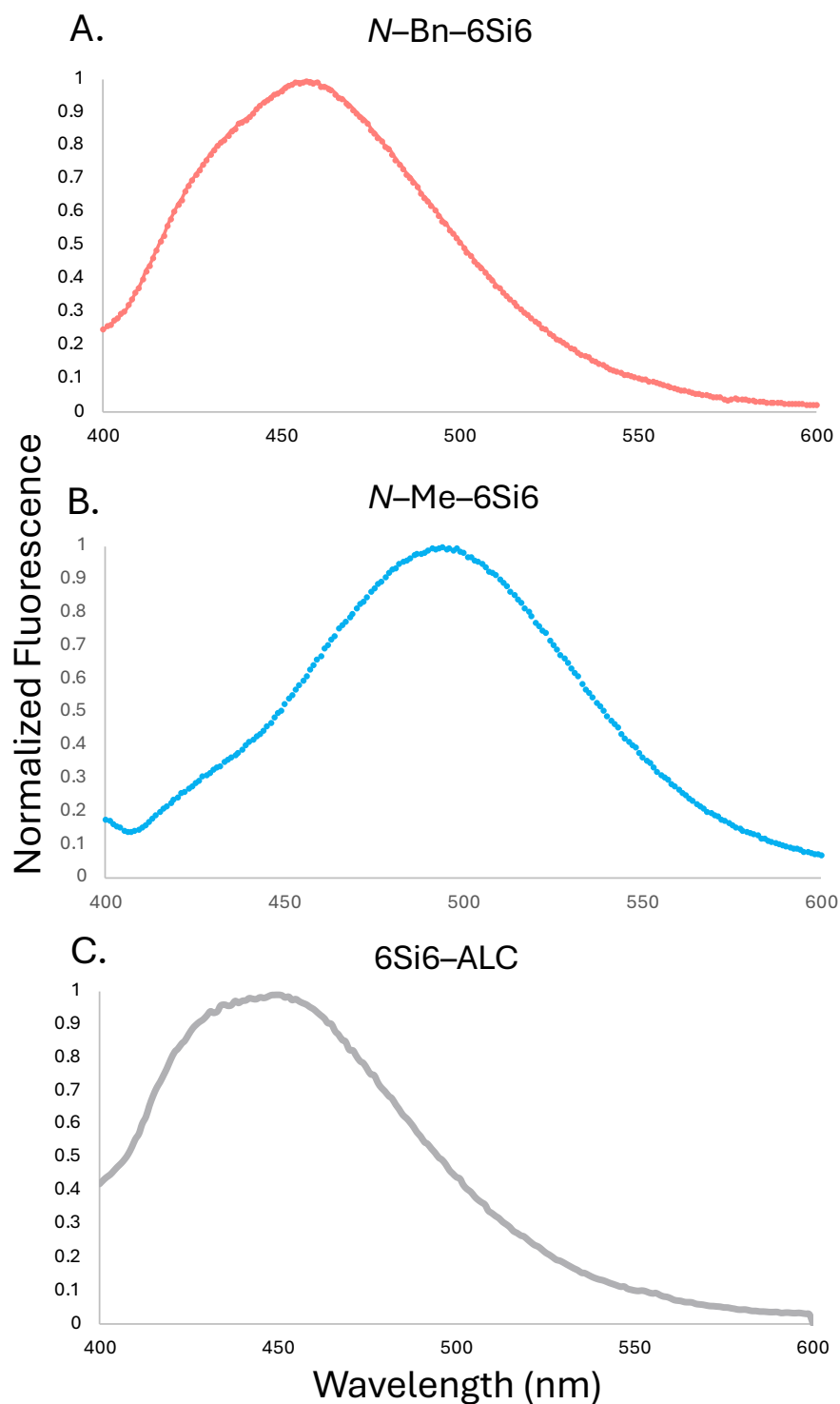


Figure 49. Fluorescence spectrum of particles composed of only (A) benzylated silyl lipid *N*-Bn-6Si6, (B) methyl silyl lipid *N*-Me-6Si6 and (C) 6Si6-ALC. Particles were prepared at a concentration of 0.3 mg/mL in PBS buffer. Data shown is the average normalized fluorescence vs wavelength ($n = 6$). See Figure 38 for XSiY naming system and Table 7 for λ_{max} values.

3.7. Size of Particles Containing Ionizable Silyl lipids by DLS and Cryo-TEM

Size and zeta potential were compared for particles containing 6Si6-MA, 6Si6-DA, 6Si8-MA or 6Si8-DA (with cationic and helper lipids) based on their distinct differences in EE and transfection efficiency. All ionizable lipids formed particles (containing ionizable, cationic and helper lipids) with bimodal size by intensity distributions with one smaller-sized population with average particle sizes between 40–240 nm (Figure 50A, pink), and one larger sized population with sizes up to 1000 nm (see Figure 51 and Table 8) likely corresponding to large aggregates or dust particulates in solution (since these larger particles are not observed in cryo-TEM images (Figure 50E–L). These bimodal size distributions correspond to the range of polydispersity index (PDI) values between 0.4–0.1 (Figure 50C, pink). The smaller-sized population in size by intensity correlates with the size by number, which shows average particle sizes between 35–120 nm (Figure 50B, pink). Cryo-TEM also shows similar particle sizes as observed in size by number, with sizes ranging from 44–92 nm (Figure 50I–L). Zeta potential was also evaluated, with MA and DA lipids forming particles with similar zeta potentials ranging from 60–80 mV (Figure 50D, pink, Table 8).

The size by intensity was also evaluated for particles composed of only 6Si6-MA or 6Si6-DA due to the distinct differences in EE observed for these single and double chain lipids . Interestingly, 6Si6-MA formed very small particles of less than 10 nm in size compared to 6Si6-DA which formed much larger particles between 146–568 nm (Figure 52A). It is known that smaller particles such as micelles are formed from single chain lipids with typical sizes between 2–30 nm^{119,126,127} while larger particles such as LNPs and liposomes are formed from double chain lipids with characteristic sizes of around 80 nm for LNPs, less than 200 nm for small

unilamellar vesicles (SUVs) and between 200–1000 nm for large unilamellar vesicles (LUVs).^{119,120,127} Cryo–TEM data (Figure 50E–L) shows that these MA and DA silyl lipids form particles (containing cationic and/or helper lipids) of less than 160 nm in size with darker, more dense cores that lack a visible bilayer and aqueous compartment (features which are typically associated with liposomal–type particle morphology), suggesting a lack of unilamellar morphology such as SUVs or LUVs for particles containing these single and double chain lipids.

Particles containing only ionizable and cationic lipids (no helper lipids) were also evaluated with DLS and cryo–TEM based on their distinct differences in EE and transfection efficiency. Average particle sizes between 100–130 nm were observed in size by intensity for 6Si8–MA (Figure 50A, blue), with slightly smaller average sizes by number (between 40–108 nm, Figure 50B, blue, Table 8). Similar particle sizes (between 45–143 nm) were observed in cryo–TEM for 6Si6– and 6Si8–MA (Figure 50E–F) and 6Si6– and 6Si8–DA (Figure 50G–H) particles containing cationic lipid. Ionizable/cationic formulations demonstrated similar average zeta potentials (58–88 mV, blue) as ionizable/cationic/helper lipid formulations (66–75 mV, pink) (Figure 50D, Table 8; see Figure 53 for size and zeta potential distributions).

It is notable that MA ionizable/cationic/helper lipid formulations have less overall spherical morphology (Figure 50I–J, see particles highlighted in purple box) compared to their DA counterparts (Figure 50K–L). This difference in particle morphology may be contributing to the distinct differences in %GFP positive cells (*vida infra*), membrane fluidity and EE observed for MA (yellow) and DA (green) formulations. These findings (i) support the hypothesis that the single vs double chain feature of MA and DA lipids may contribute to morphological differences in particle structure that may influence particle properties and (ii) contribute to a deeper

mechanistic understanding of the unique interplay between lipid structure, particle formulation and morphology, and subsequent particle properties such as EE and transfection efficiency.

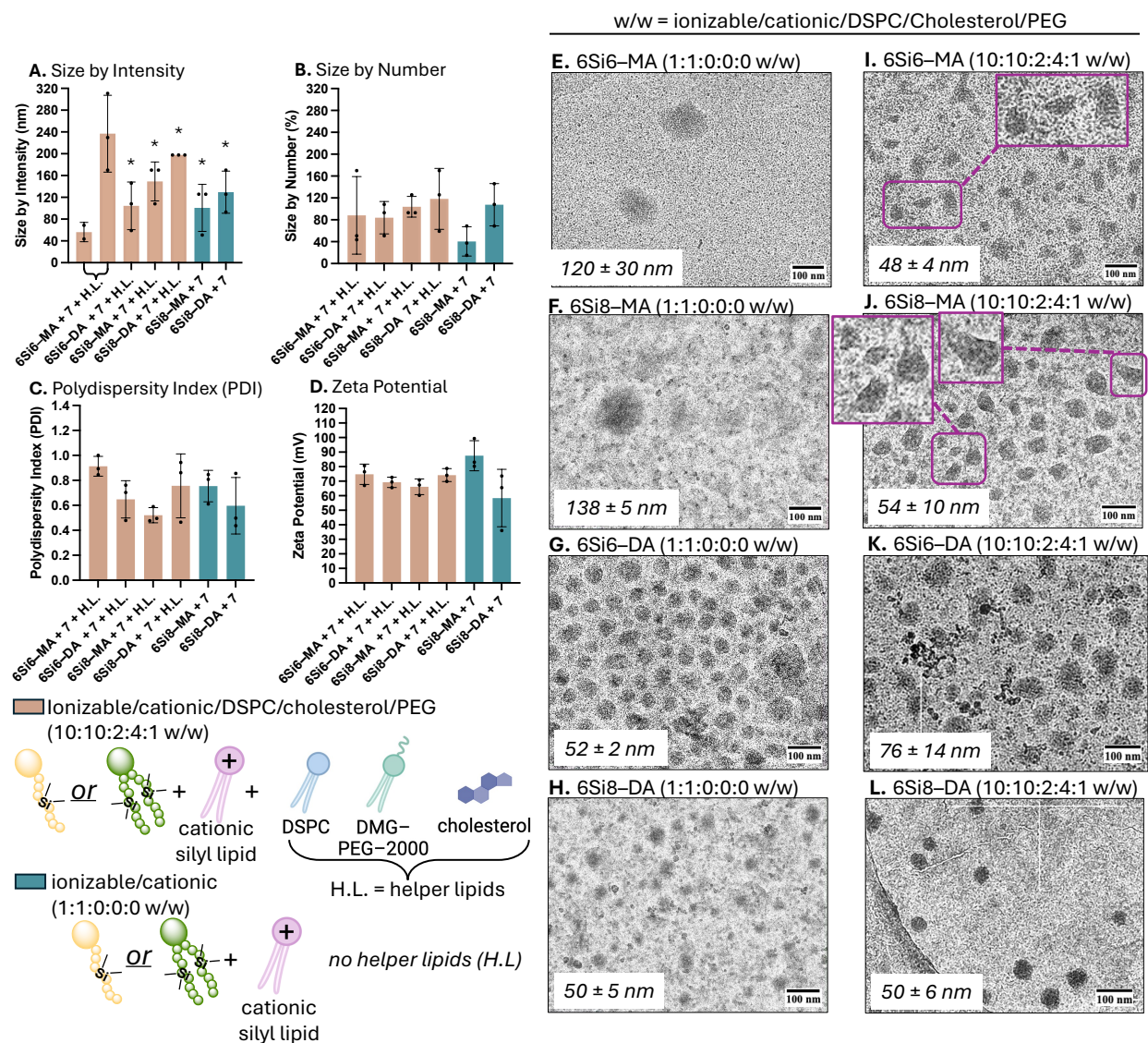


Figure 50. Size, zeta potential and polydispersity index (PDI) profiles for particles containing (i) ionizable lipid (6Si6-MA, 6Si6-DA, 6Si8-MA, 6Si8-DA or ALC-0315), cationic silyl lipid **10g**, and helper lipids (H.L. = DSPC, cholesterol and DMG-PEG-2000) (10:10:2:4:1 w/w), and (ii) ionizable and cationic silyl lipid **10g** (no H.L.) (1:1 w/w). Formulations are 0.5 mg/mL in nuclease-free water. *Two populations were observed in size by intensity, a smaller population below 350 nm (A) and one larger population above 400 nm (not shown) likely representing large aggregates or dust contamination in the sample (see Table 8 for average sizes of both populations). A–D) Data was collected using Dynamic Light Scattering and represents the average ± standard deviation of n = 3 trials where 1 trial = 1 sample/solution. Cryo-TEM images of ionizable/**10g** (1:1 w/w) (E–H) and ionizable/**10g**/DSPC/cholesterol/PEG (10:10:2:4:1 w/w) (I–L) formulations. Scale bars are 100 nm. Images were processed and average ± standard deviation was determined using ImageJ software [also see Table 8]. Images were created using

Chemdraw and BioRender software. See Table 8 for average size, zeta potential and PDI values for (A–D) and Figure 38 for XSiY naming system.

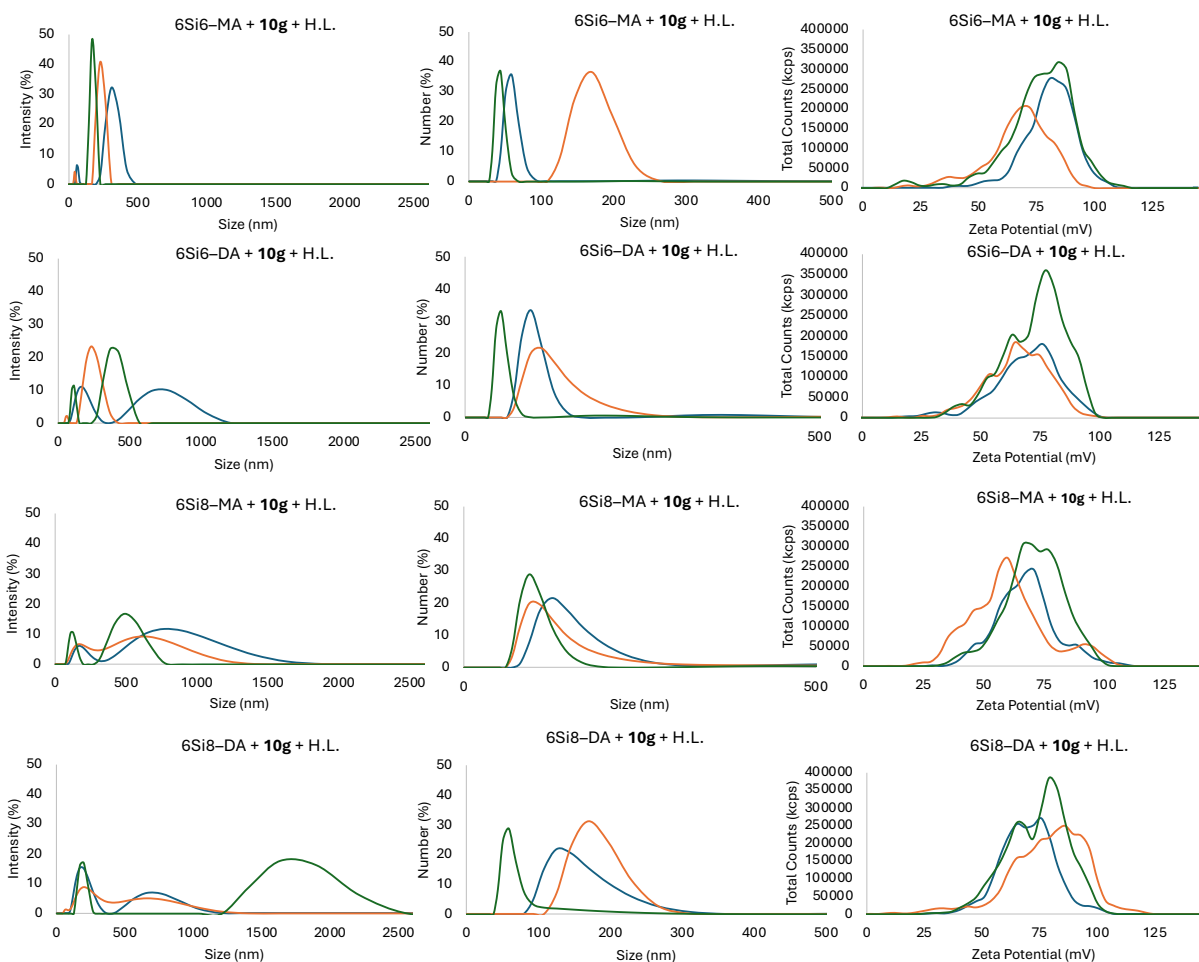


Figure 51. Size by intensity, size by number and zeta potential distributions for particles composed of ionizable lipid, cationic lipid **10g**, and helper lipids (H.L. = DSPC, cholesterol and DMG–PEG–2000) (10:10:2:4:1 w/w). Particles were prepared at a concentration of 0.5 mg/mL in nuclease-free water. Each color represents the data for one trial (i.e. one particle solution). A total of three particle solutions (i.e. $n = 3$ trials) were prepared and analyzed for each lipid evaluated. See Table 8 for the corresponding average size and zeta potential values and Figure 38 for XSiY naming system.

Table 8. Average size, polydispersity index (PDI) and zeta potential of particles

Formulation	size by intensity (nm) ^a	size by number (nm)	PDI	zeta potential (mV)
6Si6-MA + 10g (with H.L.)	56 ± 18; 237 ± 71	88 ± 71	0.91 ± 0.08	75 ± 7
6Si6-DA + 10g (with H.L.)	104 ± 44; 453 ± 281	84 ± 30	0.65 ± 0.15	69 ± 4
6Si8-MA + 10g (no H.L.)	111 ± 43; 932 ± 685	40 ± 27	0.75 ± 0.13	88 ± 10
6Si8-MA + 10g (with H.L.)	149 ± 36; 639 ± 141	104 ± 19	0.52 ± 0.06	66 ± 5
6Si8-DA + 10g (no H.L.)	129 ± 39; 527 ± 327	108 ± 39	0.60 ± 0.23	58 ± 20
6Si8-DA + 10g (with H.L.)	198; 986 ± 562	118 ± 56	0.76 ± 0.26	74 ± 4

Data represents the average ± standard deviation of n = 3 trials (i.e. three separate LNP solutions). LNPs contain ionizable lipid (6Si6-MA, 6Si6-DA, 6Si8-MA or 6Si8-DA), cationic silyl lipid 5Si3Ph-DOTAP (**10g**), DSPC, cholesterol and DMG-PEG-2000 (10:10:2:4:1 w/w) and were prepared at a concentration of 0.5 mg/mL in nuclease-free water via thin film rehydration and bath sonication at 40 °C for 20 minutes. ^aBimodal size by intensity distributions were observed for all LNPs evaluated. The smaller-sized population between 38–198 nm was best represented in the size by number distributions and cryo-TEM images (Figure 50). See Figure 38 for XSiY naming system.

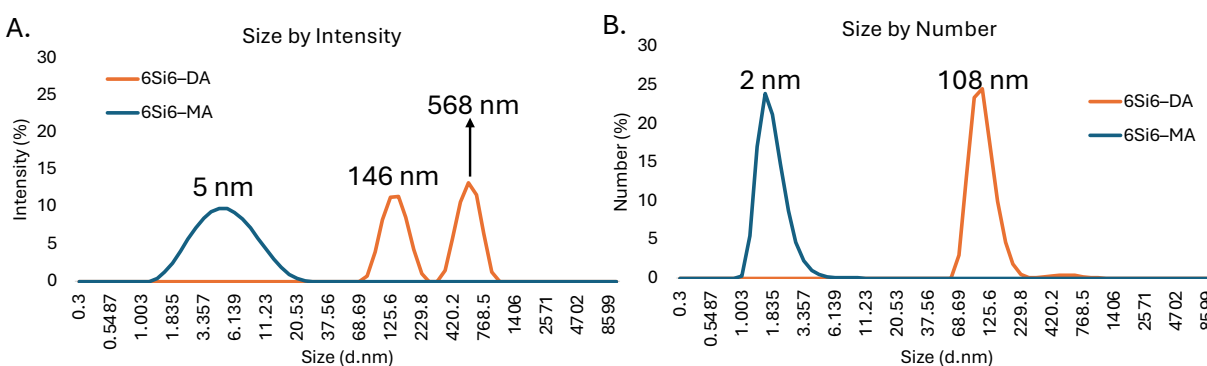


Figure 52. Size by intensity and size by number profiles for particles composed of only ionizable lipid 6Si6-MA or 6Si6-DA (no cationic or helper lipids). Particles were prepared at a 0.5 mg/mL concentration in nuclease-free water via thin film rehydration and bath sonication at 40 °C for 20 minutes. See Figure 38 for XSiY naming system.

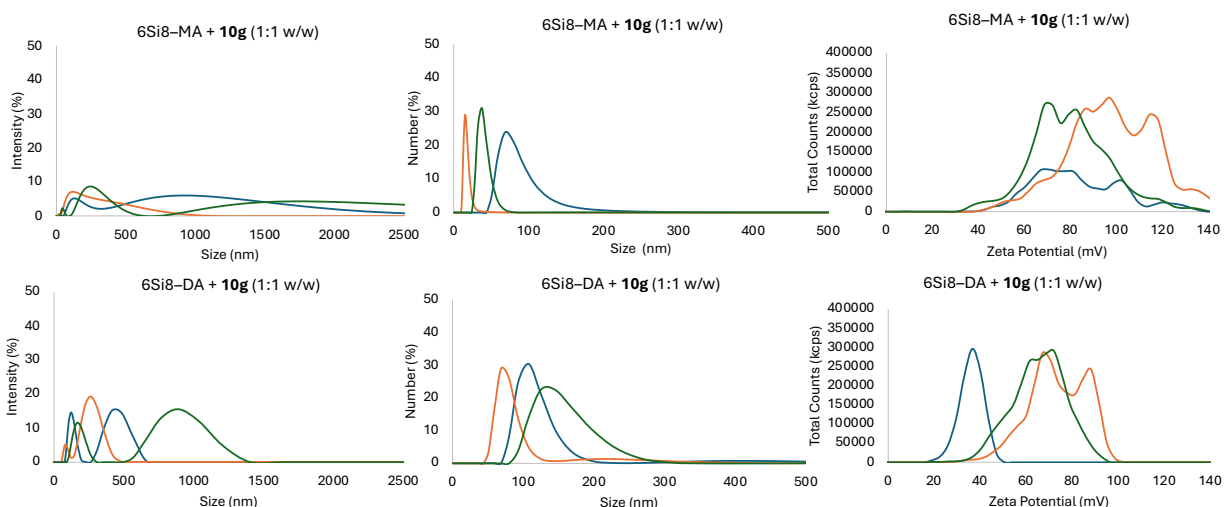


Figure 53. Size by intensity, size by number and zeta potential distributions of particles composed of ionizable lipid and cationic lipid **10g** (1:1 w/w; no helper lipids included in formulation). Particles were prepared at a concentration of 0.5 mg/mL in nuclease-free water. Each color represents the data for one trial (i.e. one solution/sample). A total of three solutions/samples (i.e. $n = 3$ trials) was analyzed for each lipid evaluated. See Table 8 for average size and zeta potential values and Figure 38 for XSiY naming system.

Additional Cryo-TEM images were obtained for LNPs containing ionizable lipid 6Si6-DA, 6Si8-DA or ALC-0315, cationic lipid 5Si3Ph-DOTAP (**10g**) and helper lipids (Figure 54) based on their top performance in vitro cell transfection. LNPs were prepared with a 10:10:2:4:1 w/w ratio of ionizable lipid/cationic lipid/DSPC/cholesterol/PEG and a concentration of 0.5 mg/mL in nuclease-free water via thin film rehydration and bath sonication at 40 °C for 20 minutes. These particles displayed average sizes of 78 ± 14 nm (Figure 54A, 6Si6-DA), 50 ± 6 nm (Figure 54B, 6Si8-DA), and 136 ± 56 nm (Figure 54C, ALC-0315).

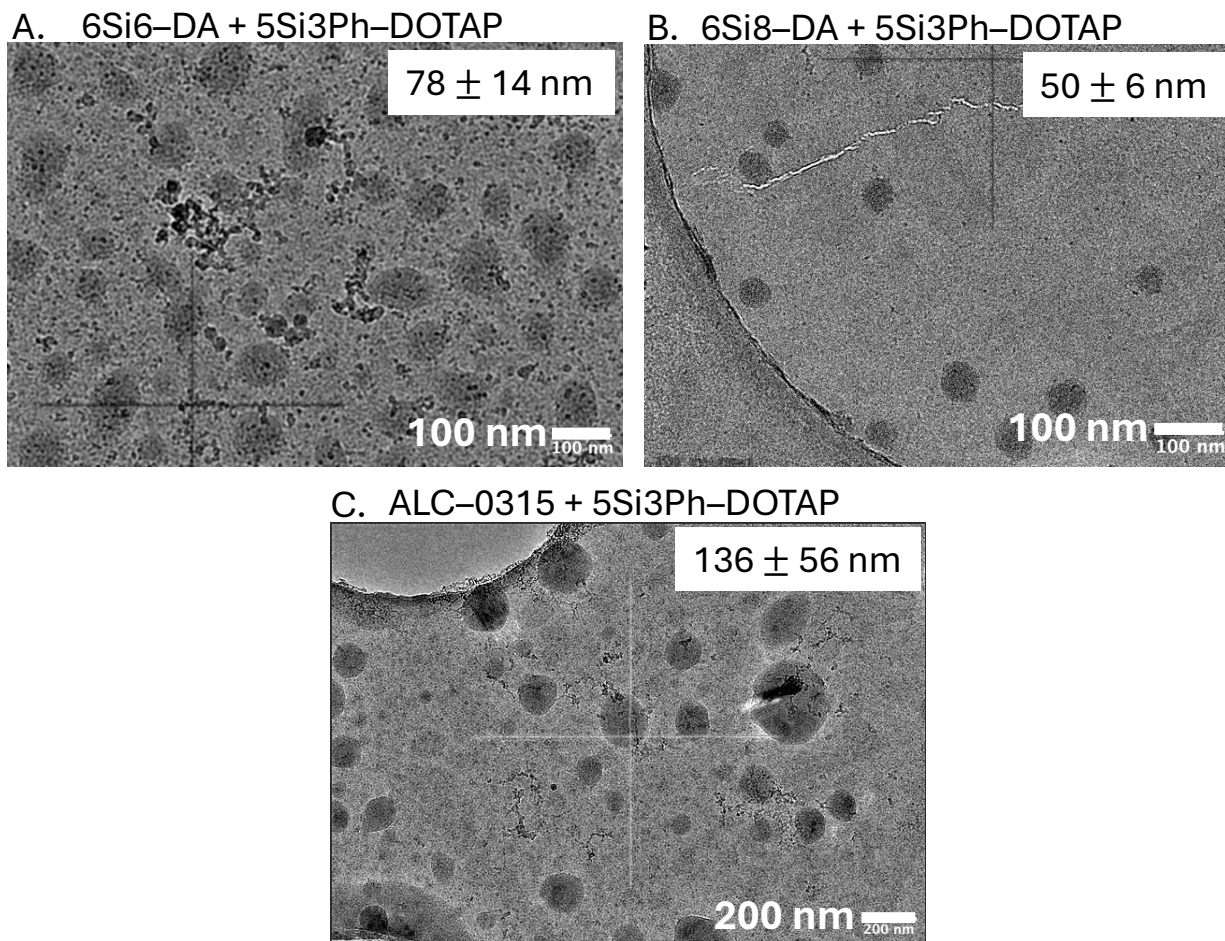


Figure 54. Cryo-TEM images of LNPs containing ionizable lipid (6Si6-DA, 6Si8-DA or ALC-0315), cationic silyl lipid 5Si3Ph-DOTAP (**10g**), DSPC, cholesterol and DMG-PEG-2000 (10:10:2:4:1 w/w). LNPs were prepared at 0.5 mg/mL concentration in nuclease-free water via thin film rehydration and bath sonication at 40 °C for 20 minutes. See Figure 38 for XSiY naming system.

Particle size was also evaluated for LNPs containing ALC-0315, cationic silyl lipid 8SiPh-DOTAP or 8SiPh-DOTMA, DSPC, cholesterol and DMG-PEG-2000 (10:10:2:4:1 w/w) (Figure 55) based on their high %GFP expression (between 95–98%). Two images were taken from the same sample/grid for ALC-0315 + 8SiPh-DOTAP (Figure 53A–B) and ALC-0315 + 8SiPh-DOTMA (Figure 55C–D). The ALC-0315 + 8SiPh-DOTAP LNPs showed sizes between 40–100 nm (Figure 55A–B). Similar sizes between 40–100 nm were observed ALC-0315 + 8SiPh-DOTMA LNPs (Figure 55C–D).

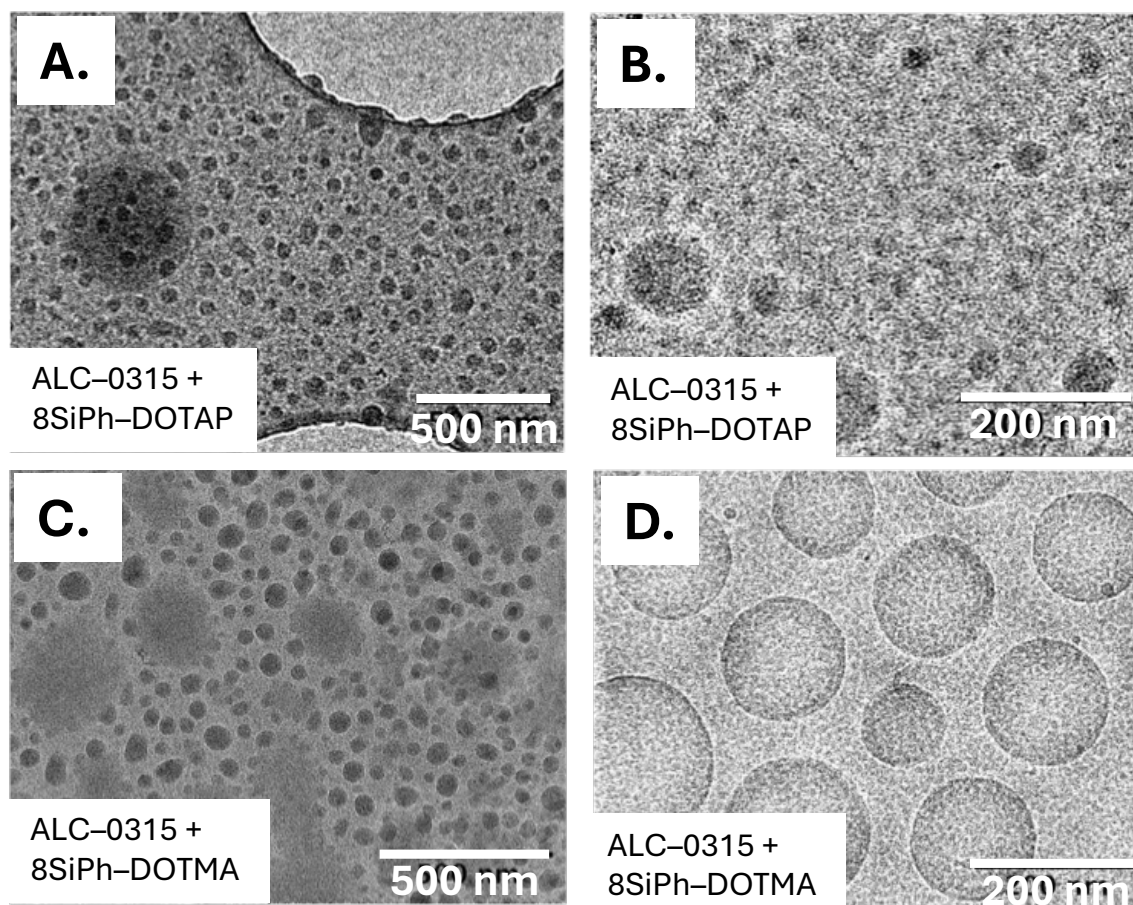


Figure 55. Cryo-TEM images of LNPs containing ALC-0315, cationic silyl lipid 8SiPh-DOTAP or 8SiPh-DOTMA, DSPC, cholesterol and DMG-PEG-2000 (10:10:2:4:1 w/w). LNPs were prepared at a concentration of 0.5 mg/mL in nuclease-free water via thin film rehydration and bath sonication at 40 °C for 20 minutes. Two images were taken from the same sample/grid for ALC-0315 + 8SiPh-DOTAP LNPs (A–B) and ALC-0315 + 8SiPh-DOTMA LNPs (C–D). See Figure 38 for XSiY naming system.

3.8. Conclusion – Ionizable Silyl lipids

Incorporation of branching silyl groups into ionizable lipid tails and the strategic use of single- vs double-chain lipids can be used to control particle properties. When forming particles with only the ionizable lipid, the single chain (MA) silyl lipids form particle with more fluid membranes compared to double chain (DA) lipids. Several MA silyl lipids gave higher transfection efficiencies than DA lipids when forming particles with only ionizable and cationic lipids. However, when forming particles with the combination of ionizable, cationic and helper lipids (DSPC, cholesterol

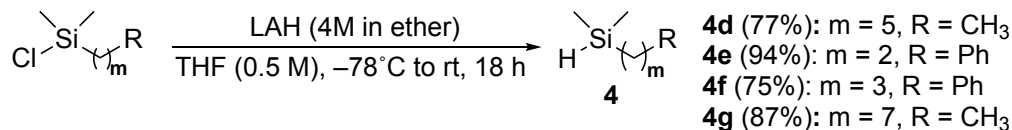
and DMG-PEG-2000), the DA silyl lipids demonstrated higher transfection efficiencies compared to MA lipids. Particles formed from only MA ionizable lipid showed much smaller sizes compared to those formed from only DA lipids, highlighting the ability to control particle size by the strategic use of single versus double chain lipids. This key difference in particle size along with the key role of the N-H group in MA lipids may contribute to the distinct EE, mRNA delivery and membrane fluidity profiles observed for single versus double chain lipids. Overall, this work supports the use of ionizable silyl lipids in LNP delivery, where incorporation of branching silyl groups into the lipid tail and use of single or double chain silyl lipids can be strategically leveraged to control particle properties. Incorporating cationic silyl lipids into LNPs in combination with ALC-0315 can also enhance mRNA delivery, with cationic silyl lipids containing a phenyl group (i.e. 5Si3Ph-DOTAP, 8SiPh-DOTAP and 8SiPh-DOTMA) having a profoundly positive effect on mRNA transfection efficiency compared to cationic lipid controls DOTAP and DOTMA. Overall, these findings emphasize the benefit of incorporating ionizable and cationic silyl lipids as a key LNP design feature to tune LNP properties and enhance mRNA delivery efficiency. This work contributes deeper insight into the structure-function relationships and the unique interplay between lipid structure, particle assembly and particle properties. Future work includes evaluation of LNP biodistribution and in vivo efficacy of our lead ionizable and cationic silyl lipid LNP formulations.

3.9. General Synthetic Procedures and Characterization and for Ionizable Silyl lipids

See section 2.9 for synthesis, characterization and instrumentation information and reagents for biophysical evaluation and cell work.

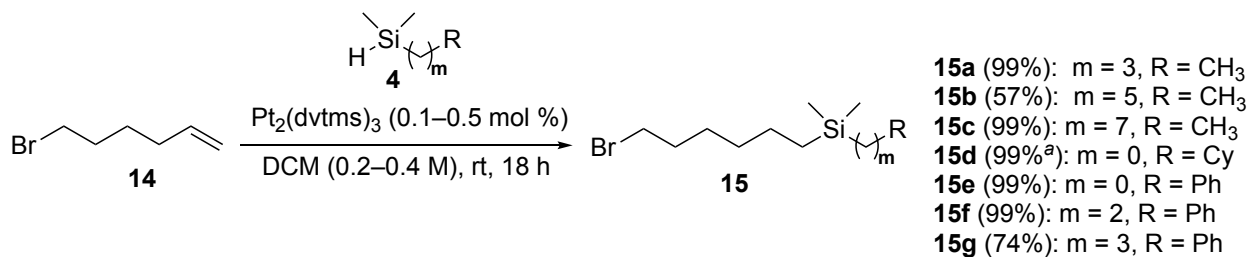
3.9A. General Procedures:

A. Reduction of Chlorosilane



Dimethylchlorosilane (1 equiv) and THF (0.5 M) were added to a round-bottom flask. The round-bottom flask was placed in a -78°C dry ice/acetone bath, and LAH (1 equiv) was added to the reaction mixture dropwise. The reaction was stirred overnight and allowed to warm to room temperature. The crude reaction was then dried with rotary evaporation, being careful to monitor vacuum and not to leave on to dry for too long as to avoid losing the silane under vacuum due to its volatility. The crude product was purified using a column with silica gel as the stationary phase and hexanes as the mobile phase. Column fractions were analyzed with TLC using a KMnO_4 stain. The solvent chamber in which the TLC plates were developed contained a mixture of 9:1 hexanes/EtOAc. The product **4** eluted in 100% hexanes.

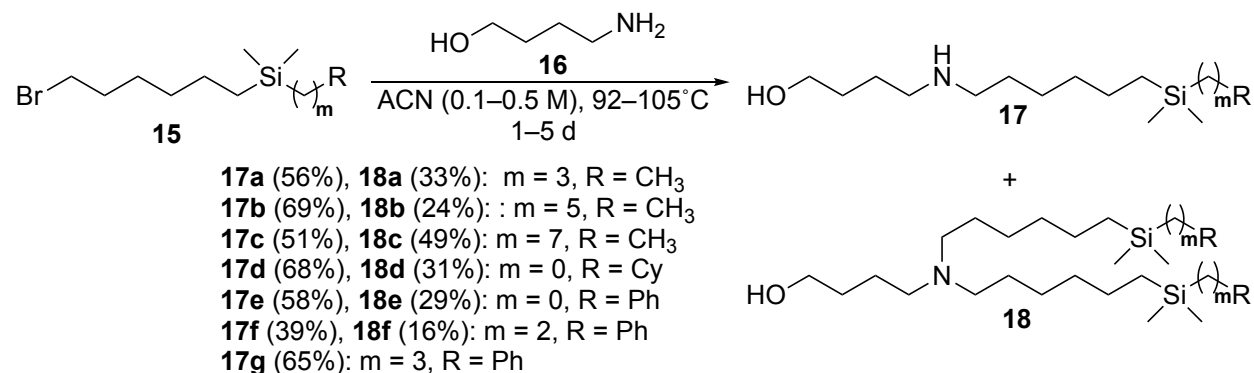
B. Hydrosilylation of Bromoalkene



In a round-bottom flask, bromohexene **14** (1 equiv), dimethylalkyl silane **4** (1.5 equiv), and catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.1–0.5 mol %) were stirred in DCM (0.2–0.4 M) overnight at room temperature. The crude reaction was analyzed with TLC to determine if unreacted starting material (**14** or **4**) was present. The crude reaction was dried under rotary evaporation, and the remaining oil was flushed through a silica gel column. Column fractions were analyzed with thin-layer chromatography (TLC) using a KMnO_4 stain. The solvent chamber in which the TLC plates were

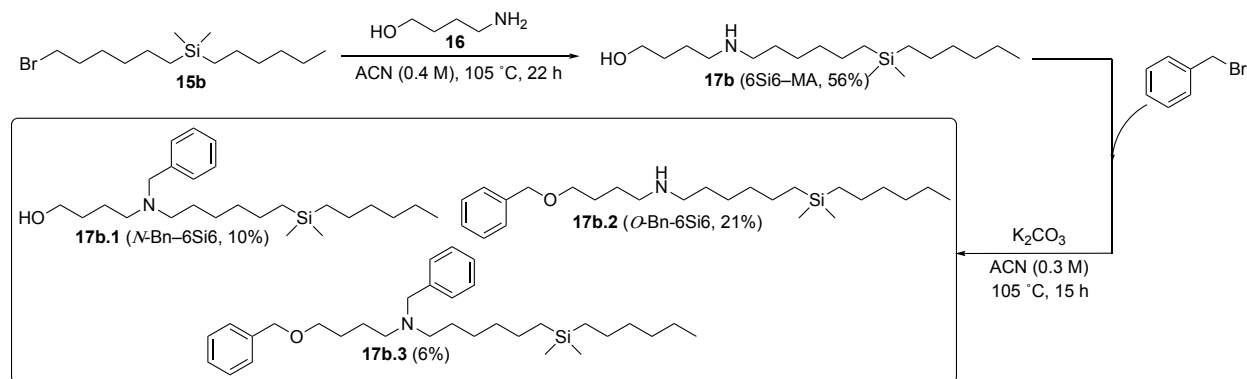
developed contained a mixture of 9:1 hexanes/EtOAc. The product **15** eluted in 100% hexanes. Any unreacted starting material (**14** or **4**) was removed during rotary evaporation and drying of **15** under vacuum. ^aNote that some unreacted cyclohexyldimethylsilane **4** was observed by ¹³C NMR for **15d** that was successfully removed after purification in the next synthetic step to afford pure products **17d** and **18d**.

C. Alkylation with Bromoalkylsilane



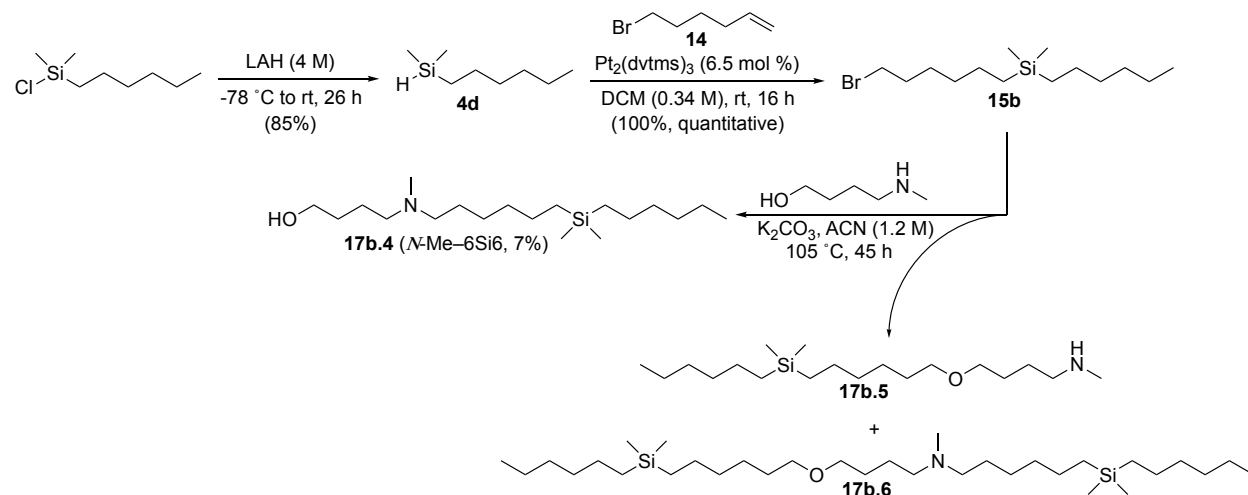
In one round-bottom flask, bromoalkylsilane **15** (3–5 equiv) and butanol amine **16** (1 equiv) were refluxed in ACN (0.1 M) at 92–105 °C for 1–5 d. The crude reaction was dried using rotary evaporation and purified using a gradient column with silica gel as the stationary phase and DCM/MeOH as the mobile phase. Column fractions were analyzed with TLC using a KMnO₄ stain. The solvent chamber in which the TLC plates were developed contained a mixture of 85:15 DCM/MeOH. The double chain (DA) product **18** elutes first in 1–2% MeOH and the single chain (MA) product **17** elutes second in 2–6% MeOH.

D. Synthesis of benzylated silyl lipids **17b.1**, **17b.2** and **17b.3**



Alkylation of 4-amino-1-butanol **16** (0.6 mmol, 0.055 mL) with **15b** (1.5 mmol, 460 mg) was carried out in ACN (1.5 mL, 0.4 M) and stirred at 105 °C for 22 h. The crude reaction was dried with under rotary evaporation and **17b** was isolated from silica gel column chromatography. Column fractions were analyzed using TLC where TLC plates were developed in 85:15 DCM/MeOH and stained with KMnO₄. Product **17b** eluted in 1–4% MeOH/DCM with an isolated yield of (182 mg, 56%). Compound **17b** (0.58 mmol, 182 mg) was heated with benzylbromide (1.7 mmol, 0.21 mL) and K₂CO₃ (2.3 mmol, 319 mg) in ACN (2.0 mL, 0.3 M) at 105 °C for 15 h. The crude reaction was dried with rotary evaporation and purified with silica gel column chromatography. Column fractions were analyzed using TLC where TLC plates were developed in 85:15 DCM/MeOH and stained with KMnO₄. Benzylated product **17b.1** (N-Bn-6Si6, 24.5 mg, 10%) eluted from the column in 1% MeOH/DCM, **17b.2** (O-Bn-6Si6, 52.5 mg, 21%) eluted in 2–3% MeOH/DCM, and **17b.3** (15.5 mg, 6%) eluted in 3–5% MeOH/DCM. Unreacted starting material **17b** (53 mg) was also recovered from the column in 5–10% MeOH/DCM.

E. Synthesis of methylated silyl lipid **17b.4**

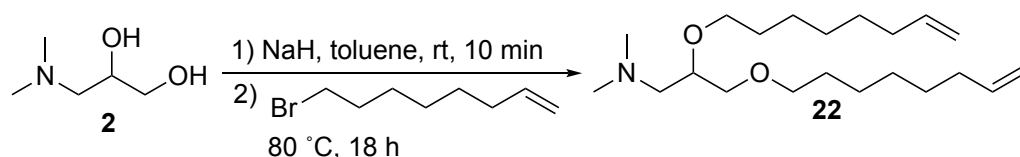


Chloro(hexyl)dimethylsilane (0.80 mL, 4.1 mmol) was added dropwise to a round-bottom flask containing LAH (1.0 mL, 4.0 mmol) with stirring at -78 °C and the reaction was allowed to warm to room temperature overnight. After 26 hours, the reaction was transferred to an ice bath with stirring and quenched with dropwise addition of NH_4Cl (aq). The crude reaction was washed with NH_4Cl (aq) (20 mL) and the organic layer was extracted with hexanes. The crude reaction was then flushed through a silica gel column in 100% hexanes, and the fractions were visualized on TLC plates that were developed in 9:1 hexanes/EtOAc and stained with KMnO_4 . Pure silane **4d** was isolated with a yield of 85% (499 mg). Hydrosilylation of 6-bromohex-1-ene **14** with silane **4d** and $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 6.5 mol %) was carried out in DCM (0.50 mL, 0.34 M) and stirred at room temperature for 16 hours. The crude reaction was flushed through a silica gel column in 100% hexanes, and the fractions were visualized on TLC plates that were developed in 9:1 hexanes/EtOAc and stained with KMnO_4 . 6-Bromoheptyl(hexyl)dimethylsilane **15b** was isolated quantitatively (529 mg) after column chromatography. Reflux of 6-Bromoheptyl(hexyl)dimethylsilane **15b** (529 mg, 1.7 mmol) was carried out with 4-(methylamino)butan-1-ol (0.13 mL, 1.1 mmol) and K_2CO_3 (317 mg, 2.3 mmol) in ACN (1.0 mL, 1.2 M) at 105 °C for 45 hours. The crude reaction was concentrated to dryness under vacuum and

purified with a gradient column using a silica gel stationary phase and a DCM/MeOH mobile phase. Column fractions were analyzed with TLC plates that were developed in 85:15 DCM/MeOH and stained with KMnO₄. 4-((6-(Hexyldimethylsilyl)hexyl)(methyl)amino)butan-1-ol **17b.4** (*N*-Me-6Si6) eluted from the column in approximately 4% MeOH/DCM with an isolated yield of 7% (26 mg). Note that the low isolated yield is due to the formation of byproducts (**17b.5** and **17b.6**) and coelution of **17b.4** with **17b.5** that were not completely separable after two columns.

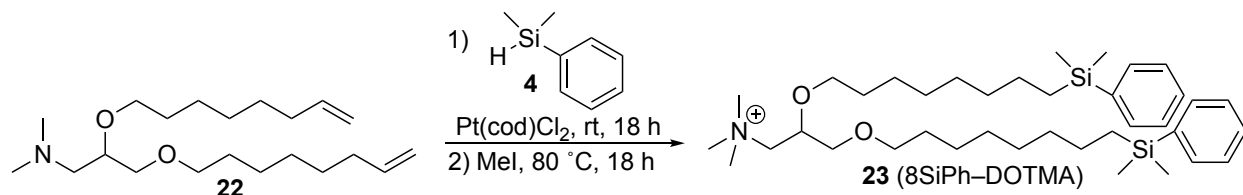
F. Synthesis of cationic silyl lipid **23** (8SiPh-DOTMA)

1. Alkylation to form alkene **22**



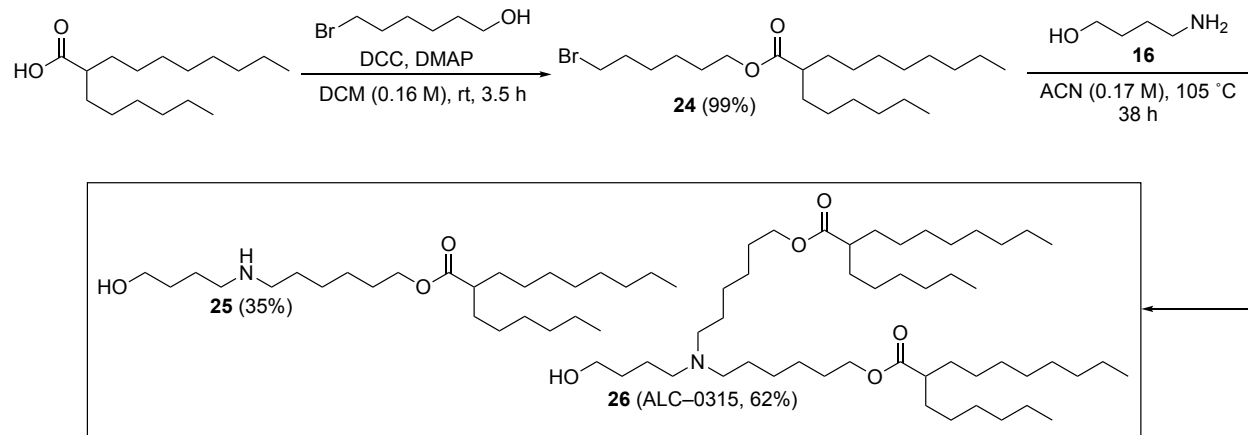
3-(Dimethylamino)propane-1,2-diol **2** (0.694 mL, 6.0 mmol) was stirred in toluene (30 mL) with NaH (1.92 g, 48 mmol) at room temperature for 10 min. 8-Bromooct-1-ene (2.51 mL, 15.0 mmol) was then added to the flask dropwise and heated for 18 h at 80 °C. The crude reaction was quenched with dropwise addition of aqueous NH₄Cl and washed with DCM (approx. 20 mL) and brine (approx. 20 mL). The organic layer was dried over anhydrous MgSO₄ and dried under rotary evaporation. The remaining oil was purified using a gradient column with silica gel as the stationary phase. Column fractions were analyzed with TLC, where TLC plates were developed in 9:1 DCM/MeOH and stained with KMnO₄. A DCM/MeOH mobile phase was used, starting at 0% MeOH, then increasing to 3% and 5% MeOH. Three drops of triethylamine was added for every 10 mL of solvent/mobile phase. The product *N,N*-dimethyl-2,3-bis(oct-7-en-1-yloxy)propan-1-amine **22** eluted from the column in 3–5% MeOH/DCM with an isolated yield of 66% (1.35 g).

2. Quaternization to form cationic silyl lipid **23** (8SiPh–DOTMA)

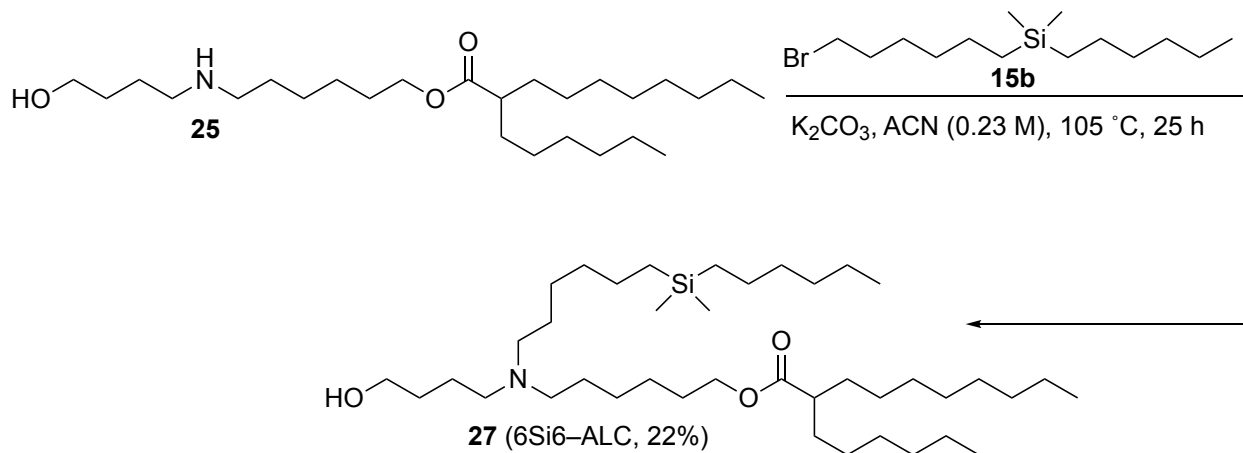


Alkene **22** (212 mg, 0.62 mmol), dimethylphenylsilane **4** (0.24 mL, 1.56 mmol) and a spatula tip of $\text{Pt}(\text{cod})\text{Cl}_2$ were combined in the same flask and stirred at room temperature for 18 hours to afford 2,3-bis((8-(dimethyl(phenyl)silyl)octyl)oxy)-*N,N*-dimethylpropan-1-amine (8SiPh–DOTMA) which was pushed through a silica plug to remove the catalyst, concentrated to dryness under vacuum, and combined with MeI (0.40 mL, 5.89 mmol) in a sealed microwave vial and heated at 80 °C for 18 h. The crude reaction was concentrated to dryness under rotary evaporation and purified with a gradient column using silica gel as the stationary phase. Column fractions were analyzed using TLC, where TLC plates were developed in 9:1 DCM/MeOH and stained using KMnO_4 . A DCM/MeOH mobile phase was used, starting at 0% MeOH, then increasing to 3% and 5% MeOH. Three drops of triethylamine were added for every 10 mL of solvent/mobile phase. Cationic silyl lipid **23** (8SiPh–DOTMA) eluted in 3–5% MeOH/DCM with an isolated yield of 21% (81 mg) over two steps.

G. Synthesis of ALC lipids **25**, **26** and **27**



2-Hexyldecanoic acid (5.8 mmol, 1.7 mL), 6-bromohexan-1-ol (2.4 mmol, 0.31 mL), *N,N'*-dicyclohexylmethanediimine (DCC, 5.8 mmol, 1.2 g) and *N,N*-dimethylpyridin-4-amine (DMAP, 5.7 mmol, 0.70 g) were stirred at room temperature in DCM (15 mL) for 3.5 hours. The crude reaction was concentrated to dryness under rotary evaporation and purified with silica gel chromatography. Column fractions were analyzed using TLC, where TLC plates were developed in 8:2 Hex/EtOAc and stained using KMnO₄. The product **24** eluted in 1% EtOAc with an isolated yield of 99% (0.99 g). 4-Aminobutan-1-ol **16** (0.54 mmol, 0.05 mL) and **24** (2.4 mmol, 0.99 g) were stirred at 105 °C in ACN (3.2 mL) for 38 hours and the crude reaction was concentrated to dryness under rotary evaporation and purified with silica gel chromatography. Column fractions were analyzed using TLC, where TLC plates were developed in 9:1 DCM/MeOH and stained using KMnO₄. Double chain product ALC-0315 (**26**) eluted in 1–2% MeOH with an isolated yield of 62% (256 mg) and single chain product 6-((4-hydroxybutyl)amino)hexyl 2-hexyldecanoate (**25**) eluted in 5–15% MeOH with an isolated yield of 35% (144 mg).

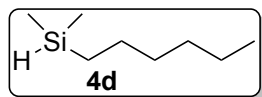


6-((4-Hydroxybutyl)amino)hexyl 2-hexyldecanoate **25** (0.23 mmol, 97 mg), (6-bromohexyl)(hexyl)dimethylsilane **15b** (0.68 mmol, 209 mg) and K₂CO₃ (0.90 mmol, 125 mg) were stirred at 105 °C in ACN (1 mL) for 25 hours. The crude reaction was filtered through celite to remove K₂CO₃, concentrated to dryness under rotary evaporation and purified with silica gel

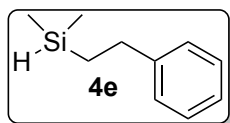
chromatography. Column fractions were analyzed using TLC, where TLC plates were developed in 85:15 DCM/MeOH and stained using KMnO₄. Unsymmetrical silyl–ALC hybrid **27** (6Si6–ALC) eluted in 1–2% MeOH with an isolated yield of 22% (32 mg). Unreacted starting material **25** eluted in 4–10% MeOH.

3.9B. Characterization:

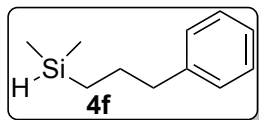
A. Silanes used in Hydrosilylation



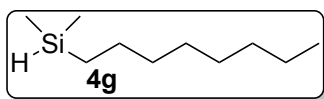
Hexyldimethylsilane **4a** was synthesized according to General Synthetic Procedure A. Chloro(hexyl)dimethylsilane (1 mmol, 0.200 mL) was stirred in THF (2.00 mL) in a –78 °C dry ice/acetone bath. LAH (4.0 M, 1 mmol, 0.250 mL) was added dropwise to this mixture, and the reaction was stirred for 18 hours and allowed to warm to room temperature from –78 °C over 18 h. The crude reaction was dried with rotary evaporation and flushed through a silica gel column with 100% hexanes. The fractions were analyzed using TLC with KMnO₄ as the stain. The product **4a** eluted in 100% hexanes and was obtained with an isolated yield of 77% (113 mg).



Dimethyl(phenethyl)silane **4e** was synthesized according to General Synthetic Procedure A. Chlorodimethyl(phenethyl)silane (1 mmol, 0.200 mL) was stirred in THF (2.00 mL) in a –78 °C dry ice/acetone bath. LAH (4.0 M, 1 mmol, 0.250 mL) was added dropwise to this mixture, and the reaction was stirred for 18 hours and allowed to warm to room temperature from –78 °C over 18 h. The crude reaction was dried with rotary evaporation and flushed through a silica gel column with 100% hexanes. The fractions were analyzed using TLC with KMnO₄ as the stain. The product **4e** was obtained with an isolated yield of 94% (155 mg).

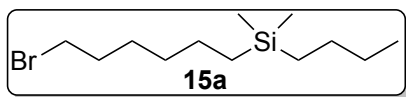


Dimethyl(3-phenylpropyl)silane **4f** was synthesized according to General Synthetic Procedure A. Chlorodimethyl(3-phenylpropyl)silane (1 mmol, 0.220 mL) was stirred in THF (2.00 mL) in a -78°C dry ice/acetone bath. LAH (4.0 M, 1 mmol, 0.250 mL) was added dropwise to this mixture, and the reaction was stirred for 18 hours and allowed to warm to room temperature from -78°C over 18 h. The crude reaction was dried with rotary evaporation and flushed through a silica gel column with 100% hexanes. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **4f** was obtained with an isolated yield of 75% (134 mg).



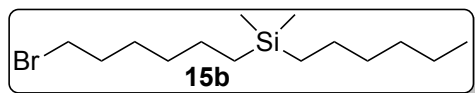
Octyldimethylsilane **4g** was synthesized according to General Synthetic Procedure A. Chloro(octyl)dimethylsilane (1 mmol, 0.240 mL) was stirred in THF (2.00 mL) in a -78°C dry ice/acetone bath. LAH (4.0 M, 1 mmol, 0.250 mL) was added dropwise to this mixture, and the reaction was stirred for 18 hours and allowed to warm to room temperature from -78°C over 18 h. The crude reaction was dried with rotary evaporation and flushed through a silica gel column with 100% hexanes. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **4g** was obtained with an isolated yield of 87% (153 mg).

B. Bromoalkylsilanes (Figure 4.1, Route A)

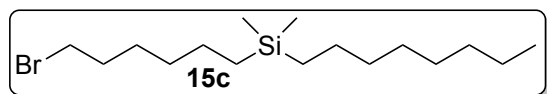


Compound (6-bromohexyl)(butyl)dimethylsilane **15a** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure B. Compounds 6-bromohex-1-ene **14** (2 mmol, 0.270 mL) and butyldimethylsilane **4** (3 mmol, 0.490 mL) were stirred in DCM (5.00 mL) with catalytic

Pt₂(dvtms)₃ (50 μ L, 0.13 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMNO₄. The product **15a** eluted in 100% hexanes with an isolated yield of 99% (553 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.41 (t, J = 6.9 Hz, 2H), 1.85 (m, 2H), 1.32 (m, 9H), 0.88 (t, J = 7.1 Hz, 3H), 0.48 (m, 4H), -0.05 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 34.2, 32.9, 32.9, 28.0, 26.7, 26.3, 23.8, 15.3, 15.1, 13.9, -3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.24.

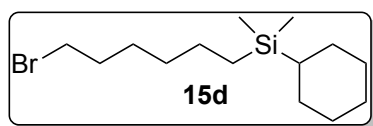


Compound (6-bromohexyl)(hexyl)dimethylsilane **15b** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure *B*. Compounds 6-bromohex-1-ene **14** (0.52 mmol, 70 μ L) and hexyldimethylsilane **4a** (0.78 mmol, 113 mg) were stirred in DCM (2.0 mL) with catalytic Pt₂(dvtms)₃ (50 μ L, 0.5 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMNO₄. The product **15b** eluted in 100% hexanes with an isolated yield of 57% (92 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.41 (t, J = 6.9 Hz, 2H), 1.85 (m, 2H), 1.42 (m, 2H), 1.29 (m, 13H), 0.89 (t, 3H), 0.48 (m, 4H), -0.06 (s, 5H). ¹³C NMR (101 MHz, CDCl₃) δ 34.2, 33.5, 32.9, 32.9, 31.8, 28.1, 24.0, 23.9, 22.8, 15.4, 15.4, 14.3, -3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.21. HRMS (ESI) m/z : calculated for C₁₄H₃₂BrSi⁺ [M + H]⁺ 307.1457; found: 307.1894.



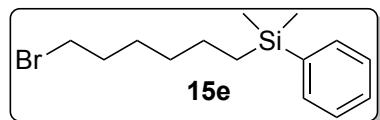
Compound (6-bromohexyl)dimethyl(octyl)silane **15c** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure *B*. Compounds 6-bromohex-1-ene **14** (0.49 mmol, 60

μL) and octyldimethylsilane **4g** (0.74 mmol, 153 mg) were stirred in DCM (2.0 mL) with catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.53 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMnO_4 . The product **15c** eluted in 100% hexanes with an isolated yield of 99% (149 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.41 (t, $J = 6.9$ Hz, 2H), 1.85 (p, $J = 6.9$ Hz, 2H), 1.42 (m, 2H), 1.32 (m, 18H), 0.88 (m, 3H), 0.48 (m, 4H), -0.06 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 34.2, 33.8, 32.9, 32.9, 32.1, 29.5, 29.4, 28.0, 24.0, 23.9, 22.8, 15.4, 15.3, 14.2, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.21. HRMS (ESI) m/z : calculated for $\text{C}_{16}\text{H}_{36}\text{BrSi}^+ [\text{M} + \text{H}]^+$ 335.1770; found: 335.1492.

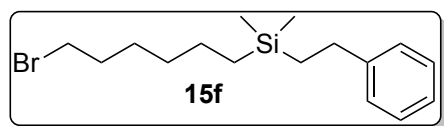


Compound (6-bromohexyl)(cyclohexyl)dimethylsilane **15d** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure B. Compounds 6-bromohex-1-ene **14** (1 mmol, 0.13 mL) and cyclohexyldimethylsilane **4** (1.5 mmol, 0.26 mL) were stirred in DCM (3.0 mL) with catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.26 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMnO_4 . The product **15d** eluted in 100% hexanes with an isolated yield of 99% (302 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.41 (t, $J = 6.9$ Hz, 2H), 1.85 (p, $J = 6.9$ Hz, 2H), 1.67 (m, 6H), 1.42 (qd, $J = 6.8, 3.9$ Hz, 2H), 1.30 (m, 4H), 1.20 (m, 3H), 1.08 (m, 2H), 0.57 (tt, $J = 12.5, 3.1$ Hz, 1H), 0.47 (m, 2H), -0.10 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 34.2, 33.0, 32.9, 28.3, 28.0, 27.6, 27.1, 26.9, 25.4, 23.9, 13.6, -1.4 , -5.1 . ^{29}Si NMR (79 MHz, CDCl_3) δ 3.71. The additional signal at -1.4 ppm in ^{13}C NMR is unreacted

cyclohexyldimethylsilane starting material **4** that was successfully removed after purification in the next synthetic step to afford pure products **17d** and **18d**.

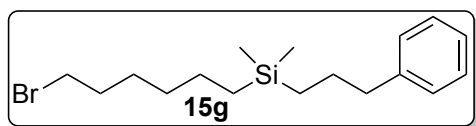


Compound (6-bromohexyl)dimethyl(phenyl)silane **15e** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure *B*. Compounds 6-bromohex-1-ene **14** (1 mmol, 0.13 mL) and phenyldimethylsilane **4** (1.5 mmol, 0.23 mL) were stirred in DCM (3.0 mL) with catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.26 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMnO_4 . The product **15e** eluted in 100% hexanes with an isolated yield of 99% (297 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.55 (m, 2H), 7.39 (dp, $J = 5.3, 1.9$ Hz, 4H), 3.41 (t, $J = 6.8$ Hz, 2H), 1.85 (p, $J = 6.9$ Hz, 2H), 1.44 (m, 2H), 1.36 (m, 4H), 0.77 (m, 2H), 0.29 (s, 7H). ^{13}C NMR (101 MHz, CDCl_3) δ 139.6, 133.6, 128.9, 127.8, 34.1, 32.8, 32.7, 27.9, 23.8, 15.7, -2.9 . ^{29}Si NMR (79 MHz, CDCl_3) δ -1.88 . HRMS (ESI) m/z : calculated for $\text{C}_{14}\text{H}_{24}\text{BrSi}^+ [\text{M} + \text{H}]^+$ 299.0831; found: 299.0793.



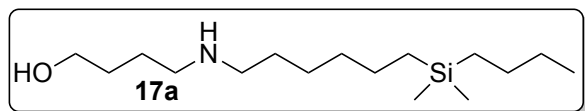
Compound (6-bromohexyl)dimethyl(phenethyl)silane **15f** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure *B*. Compounds 6-bromohex-1-ene **14** (0.63 mmol, 80 μL) and dimethyl(phenethyl)silane **4e** (0.94 mmol, 155 mg) were stirred in DCM (2.0 mL) with catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.42 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMnO_4 . The product **15f** eluted in

100% hexanes with an isolated yield of 99% (194 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.25 (m, 6H), 3.44 (t, J = 6.8 Hz, 2H), 2.65 (m, 2H), 1.88 (m, 2H), 1.39 (m, 6H), 0.92 (m, 2H), 0.54 (m, 2H), 0.02 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 145.4, 128.4, 127.9, 125.6, 34.1, 32.9, 32.8, 30.1, 28.0, 23.8, 17.4, 15.2, -3.3. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.51. HRMS (ESI) m/z : calculated for $\text{C}_{16}\text{H}_{28}\text{BrSi}^+ [\text{M} + \text{H}]^+$ 327.1144; found: 327.1592.



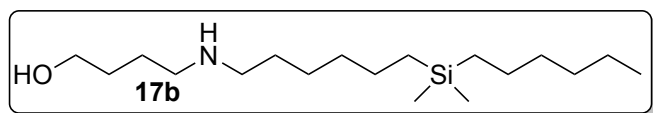
Compound (6-bromohexyl)dimethyl(3-phenylpropyl)silane **15g** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure B. Compounds 6-bromohex-1-ene **14** (0.5 mmol, 70 μL) and dimethyl(3-phenylpropyl)silane **4f** (0.75 mmol, 134 mg) were stirred in DCM (2.0 mL) with catalytic $\text{Pt}_2(\text{dvtms})_3$ (50 μL , 0.52 mol %) for 18 hours at room temperature. The crude reaction was dried with rotary evaporation and purified with column chromatography using silica gel as the stationary phase. The fractions were analyzed with TLC using KMnO_4 . The product **15g** eluted in 100% hexanes with an isolated yield of 74% (133 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.36 (m, 2H), 7.26 (m, 3H), 3.48 (t, J = 6.9 Hz, 2H), 2.69 (t, J = 7.7 Hz, 2H), 1.92 (p, J = 7.0 Hz, 2H), 1.68 (m, 2H), 1.49 (m, 2H), 1.36 (m, 4H), 0.62 (m, 2H), 0.56 (m, 2H), 0.03 (s, 6H).

C. Monoalkylated Silyl Lipids (Figure 4.1, Route A)

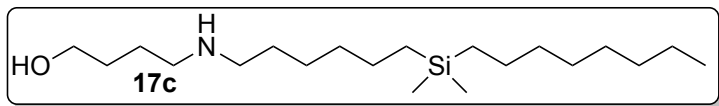


Compound 4-((6-(butyldimethylsilyl)hexyl)amino)butan-1-ol **17a** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(butyl)dimethylsilane **15a** (2.11 mmol, 590 mg) was refluxed with 4-aminobutan-1-ol **16** (0.42 mmol, 40 μL) at 102°C in ACN (4.2 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary

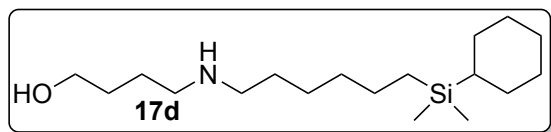
phase. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **17a** eluted in 3–10% MeOH/DCM with an isolated yield of 56% (118 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.71 (t, $J = 5.5$ Hz, 2H), 3.03 (t, $J = 7.0$ Hz, 2H), 2.94 (m, 2H), 2.03 (p, $J = 7.0$ Hz, 2H), 1.86 (p, $J = 7.2$ Hz, 2H), 1.72 (p, $J = 6.4$ Hz, 2H), 1.28 (m, 8H), 0.87 (t, $J = 7.0$ Hz, 3H), 0.46 (m, 4H), -0.07 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.4, 48.2, 48.0, 33.2, 29.6, 26.7, 26.6, 26.2, 26.0, 23.9, 23.7, 15.3, 15.0, 13.9, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.24. HRMS (ESI) m/z : calculated for $\text{C}_{16}\text{H}_{38}\text{NOSi}^+ [\text{M} + \text{H}]^+$ 288.2723; found: 288.2713.



Compound 4-((6-(hexyldimethylsilyl)hexyl)amino)butan-1-ol **17b** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(hexyl)dimethylsilane **15b** (0.98 mmol, 300 mg) was refluxed with 4-aminobutan-1-ol **16** (0.27 mmol, 25 μL) at 105°C in ACN (2.0 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **17b** eluted in 3–10% MeOH/DCM with an isolated yield of 69% (104 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.73 (t, $J = 5.4$ Hz, 2H), 3.03 (t, $J = 6.8$ Hz, 2H), 2.95 (m, 2H), 2.05 (p, $J = 6.8$ Hz, 2H), 1.86 (m, 2H), 1.75 (m, 2H), 1.29 (m, 16H), 0.88 (t, $J = 8.0$ Hz, 3H), 0.45 (m, 4H), -0.07 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.6, 48.1, 48.0, 33.5, 33.2, 31.7, 29.6, 26.7, 26.0, 23.9, 23.9, 23.8, 22.7, 15.3, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.20. HRMS (ESI) m/z : calculated for $\text{C}_{18}\text{H}_{42}\text{NOSi}^+ [\text{M} + \text{H}]^+$ 316.3036; found: 316.3030.

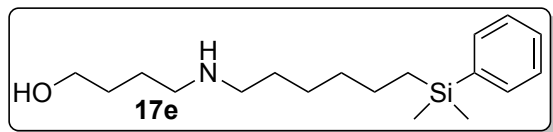


Compound 4-((6-(dimethyl(octyl)silyl)hexyl)amino)butan-1-ol **17c** was synthesized according to Figure 4.1 Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(octyl)silane **15c** (0.45 mmol, 150 mg) was refluxed with 4-aminobutan-1-ol **16** (0.27 mmol, 25 μ L) at 92 °C in ACN (1.6 mL) for 7 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **17c** eluted in 3–10% MeOH/DCM with an isolated yield of 43% (69 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.71 (m, 2H), 3.03 (t, J = 7.0 Hz, 2H), 2.94 (m, 2H), 2.04 (p, J = 7.0 Hz, 2H), 1.86 (p, J = 7.2 Hz, 2H), 1.73 (p, J = 6.4 Hz, 2H), 1.32 (m, 19H), 0.87 (t, J = 6.7 Hz, 3H), 0.45 (m, 4H), –0.07 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 61.4, 48.2, 48.0, 33.8, 33.2, 32.0, 29.6, 29.4, 29.4, 26.6, 26.0, 24.0, 23.9, 23.7, 22.8, 15.3, 15.3, 14.2, –3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.20. HRMS (ESI) m/z : calculated for C₂₀H₄₆NOSi⁺ [M + H]⁺ 344.3349; found: 344.3337.

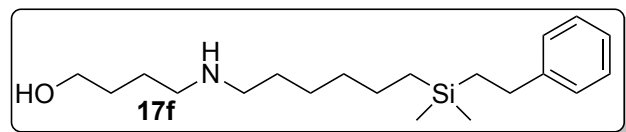


Compound 4-((6-(cyclohexyldimethylsilyl)hexyl)amino)butan-1-ol **17d** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(cyclohexyl)dimethylsilane **15d** (2.04 mmol, 623 mg) was refluxed with 4-aminobutan-1-ol **16** (0.41 mmol, 38 μ L) at 102 °C in ACN (4.1 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **17d** eluted in 3–10% MeOH/DCM with an isolated yield of 68% (151 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.71 (t, 2H), 3.02 (t, J = 7.0 Hz, 2H), 2.94 (m, 2H), 2.03 (p, J = 7.0 Hz, 2H), 1.85 (p, J = 7.8 Hz, 2H), 1.66 (m, 7H), 1.19 (m, 9H), 0.55 (tt, J = 12.5, 3.1 Hz, 1H), 0.44 (m, 2H), –

0.12 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.4, 53.5, 48.2, 48.0, 33.3, 29.6, 28.2, 27.5, 27.1, 26.6, 26.0, 25.4, 23.9, 23.7, 13.5, -5.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.94. HRMS (ESI) m/z : calculated for $\text{C}_{18}\text{H}_{40}\text{NOSi}^+ [\text{M} + \text{H}]^+$ 314.2879; found: 314.2873.

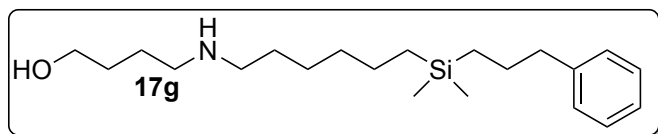


Compound 4-((6-(dimethyl(phenyl)silyl)hexyl)amino)butan-1-ol **17e** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(phenyl)silane **15e** (2.37 mmol, 708 mg) was refluxed with 4-aminobutan-1-ol **16** (0.47 mmol, 44 μL) at 102°C in ACN (4.7 mL) for 24 hours. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **17e** eluted in 3–10% MeOH/DCM with an isolated yield of 58% (146 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.49 (m, 2H), 7.34 (m, 3H), 3.68 (t, $J = 5.7$ Hz, 2H), 2.99 (t, $J = 7.0$ Hz, 2H), 2.90 (m, 2H), 2.00 (p, $J = 7.0$ Hz, 2H), 1.82 (p, $J = 8.0$ Hz, 2H), 1.70 (p, $J = 6.0$ Hz, 2H), 1.30 (m, 6H), 0.71 (m, 2H), 0.24 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 139.5, 133.6, 128.9, 127.8, 61.4, 48.1, 48.0, 32.9, 29.6, 26.5, 25.9, 23.8, 23.7, 15.7, -2.9 . ^{29}Si NMR (79 MHz, CDCl_3) δ -3.13 . HRMS (ESI) m/z : calculated for $\text{C}_{18}\text{H}_{34}\text{NOSi}^+ [\text{M} + \text{H}]^+$ 309.2443; found: 309.2411.



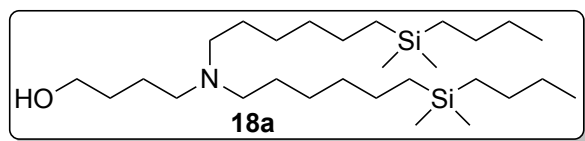
Compound 4-((6-(dimethyl(phenethyl)silyl)hexyl)amino)butan-1-ol **17f** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(phenethyl)silane **15f** (0.63 mmol, 206 mg) was refluxed with 4-aminobutan-1-ol **16** (0.33 mmol, 30 μL) at 92°C in ACN (2.2 mL) for 7 days. The crude reaction

was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **17f** eluted in 3–10% MeOH/DCM with an isolated yield of 39% (74 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 5H), 3.76 (m, 2H), 3.08 (t, *J* = 7.1 Hz, 2H), 3.00 (m, 2H), 2.67 (m, 2H), 2.09 (p, *J* = 6.8 Hz, 2H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.77 (p, *J* = 6.4 Hz, 2H), 1.36 (m, 6H), 0.92 (m, 2H), 0.55 (m, 2H), 0.04 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 145.3, 128.3, 128.3, 127.8, 125.5, 61.3, 48.2, 48.0, 33.1, 30.0, 29.6, 26.6, 26.0, 23.8, 23.6, 17.3, 15.2, –3.3. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.50. HRMS (ESI) *m/z*: calculated for C₂₀H₃₈NOSi⁺ [*M* + *H*]⁺ 336.2723; found: 336.2724.

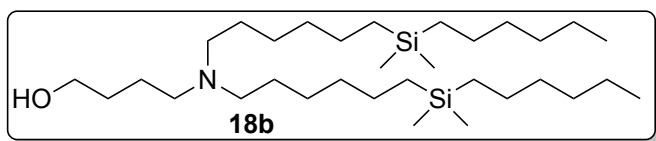


Compound 4-((6-(dimethyl(3-phenylpropyl)silyl)hexyl)amino)butan-1-ol **17g** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(3-phenylpropyl)silane **15g** (0.39 mmol, 133 mg) was refluxed with 4-aminobutan-1-ol **16** (0.15 mmol, 14 μL) at 92°C in ACN (1.6 mL) for 7 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **17g** eluted in 3–10% MeOH/DCM with an isolated yield of 65% (60 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.28 (m, 3H), 7.17 (m, 3H), 3.72 (t, *J* = 5.4 Hz, 2H), 3.02 (t, *J* = 6.8 Hz, 2H), 2.93 (m, 2H), 2.61 (t, *J* = 7.7 Hz, 2H), 2.03 (p, *J* = 6.8 Hz, 2H), 1.85 (m, 2H), 1.74 (p, *J* = 6.1 Hz, 2H), 1.59 (m, 2H), 1.29 (m, 8H), 0.53 (m, 2H), 0.45 (m, 2H), –0.06 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 142.8, 128.6, 128.3, 125.7, 61.6, 48.1, 48.0, 40.1, 33.1, 29.7, 26.6, 26.2, 26.0, 23.9, 15.3, –3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.29. HRMS (ESI) *m/z*: calculated for C₂₁H₄₀NOSi⁺ [*M* + *H*]⁺ 350.2879; found: 350.2873.

D. Dialkylated Silyl Lipids (Figure 4.1, Route A)

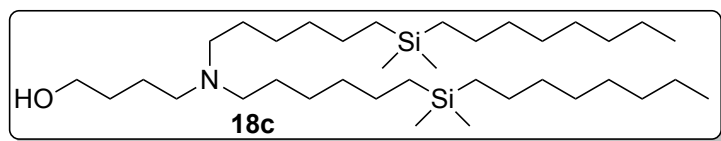


Compound 4-(bis(6-(butyldimethylsilyl)hexyl)amino)butan-1-ol **18a** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(butyl)dimethylsilane **15a** (2.11 mmol, 590 mg) was refluxed with 4-aminobutan-1-ol **16** (0.42 mmol, 40 μ L) at 102°C in ACN (4.2 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **18a** eluted in 1–2% MeOH/DCM with an isolated yield of 33% (70 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.70 (t, J = 5.7 Hz, 2H), 3.05 (t, J = 7.7 Hz, 2H), 2.97 (t, J = 8.0 Hz, 4H), 1.93 (m, 2H), 1.76 (m, 4H), 1.68 (p, J = 6.3 Hz, 2H), 1.27 (m, 15H), 0.87 (t, J = 7.1 Hz, 6H), 0.46 (m, 8H), –0.07 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 61.5, 53.0, 52.5, 33.2, 26.7, 26.7, 26.2, 23.9, 23.3, 15.3, 15.0, 13.9, –3.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.26. HRMS (ESI) m/z : calculated for C₂₈H₆₄NOSi₂⁺ [M + H]⁺ 486.4526; found: 486.4544.

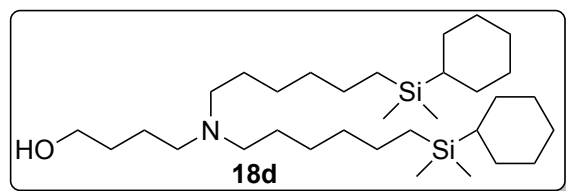


Compound 4-(bis(6-(hexyldimethylsilyl)hexyl)amino)butan-1-ol **18b** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(hexyl)dimethylsilane **15b** (0.98 mmol, 300 mg) was refluxed with 4-aminobutan-1-ol **16** (0.27 mmol, 25 μ L) at 105°C in ACN (2.0 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **18b** eluted

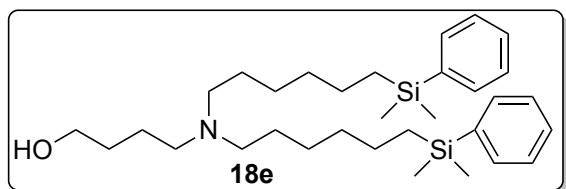
in 1–2% MeOH/DCM with an isolated yield of 24% (36 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.70 (t, J = 5.6 Hz, 2H), 2.95 (m, 6H), 1.91 (m, 2H), 1.70 (m, 6H), 1.28 (m, 31H), 0.88 (t, J = 8.0 Hz, 6H), 0.46 (m, 8H), -0.06 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.8, 52.6, 33.5, 33.2, 31.7, 26.8, 23.9, 23.9, 22.7, 15.3, 15.3, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.22. HRMS (ESI) m/z : calculated for $\text{C}_{32}\text{H}_{72}\text{NOSi}_2^+$ $[\text{M} + \text{H}]^+$ 542.5152; found: 542.5168.



Compound 4-(bis(6-(dimethyloctyl)silyl)hexyl)amino)butan-1-ol **18c** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(octyl)silane **15c** (0.45 mmol, 150 mg) was refluxed with 4-aminobutan-1-ol **16** (0.27 mmol, 25 μL) at 92°C in ACN (1.6 mL) for 7 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **18c** eluted in 1–2% MeOH/DCM with an isolated yield of 7% (12 mg). ^1H NMR (400 MHz, CDCl_3) δ 3.73 (t, J = 5.6 Hz, 2H), 3.08 (t, J = 7.8 Hz, 2H), 3.00 (t, J = 8.8 Hz, 4H), 1.97 (p, J = 6.9 Hz, 2H), 1.71 (m, 5H), 1.30 (m, 37H), 0.88 (m, 7H), 0.46 (m, 8H), -0.06 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.5, 52.8, 52.4, 33.8, 33.2, 32.1, 29.5, 29.4, 26.7, 24.0, 23.9, 23.2, 22.8, 15.3, 14.2, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.23. HRMS (ESI) m/z : calculated for $\text{C}_{36}\text{H}_{80}\text{NOSi}_2^+$ $[\text{M} + \text{H}]^+$ 598.5778; 598.5781.

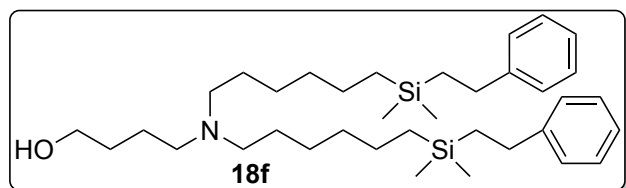


Compound 4-(bis(6-(cyclohexyldimethylsilyl)hexyl)amino)butan-1-ol **18d** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)(cyclohexyl)dimethylsilane **15d** (2.04 mmol, 623 mg) was refluxed with 4-aminobutan-1-ol **16** (0.41 mmol, 38 μ L) at 102 °C in ACN (4.1 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **18d** eluted in 1–2% MeOH/DCM with an isolated yield of 31% (69 mg). ¹H NMR (400 MHz, CDCl₃) δ 3.72 (t, *J* = 5.6 Hz, 2H), 3.05 (t, *J* = 7.8 Hz, 2H), 2.96 (t, 8Hz, 5H), 1.95 (p, *J* = 7.0 Hz, 2H), 1.66 (m, 12H), 1.19 (m, 26H), 0.56 (tt, *J* = 12.5, 3.1 Hz, 2H), 0.45 (m, 4H), –0.11 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 61.5, 53.5, 52.9, 52.5, 33.3, 28.2, 27.6, 27.1, 26.7, 25.4, 23.9, 23.3, 13.5, –5.2. ²⁹Si NMR (79 MHz, CDCl₃) δ 2.96. HRMS (ESI) *m/z*: calculated for C₃₂H₆₈NOSi₂⁺ [M + H]⁺ 538.4839; found: 538.4860.



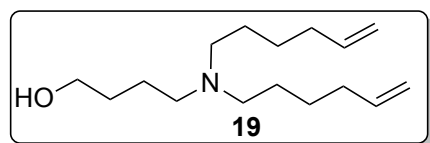
Compound 4-(bis(6-(dimethyl(phenyl)silyl)hexyl)amino)butan-1-ol **18e** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(phenyl)silane **15e** (2.37 mmol, 708 mg) was refluxed with 4-aminobutan-1-ol **16** (0.47 mmol, 44 μ L) at 102 °C in ACN (4.7 mL) for 24 hours. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **18e** eluted in 1–2% MeOH/DCM with an isolated yield of 29% (73 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.49 (m, 4H), 7.35 (m, 6H), 3.69 (t, *J* = 5.6 Hz, 2H), 2.99 (t, *J* = 7.7 Hz, 2H), 2.90

(t, $J = 8\text{ Hz}$, 4H), 1.90 (p, $J = 7.0\text{ Hz}$, 2H), 1.68 (m, 6H), 1.31 (m, 13H), 0.72 (m, 4H), 0.25 (s, 13H). ^{13}C NMR (101 MHz, CDCl_3) δ 139.5, 133.6, 128.9, 127.8, 61.5, 52.8, 52.5, 32.9, 26.6, 23.8, 15.7, -2.9 . ^{29}Si NMR (79 MHz, CDCl_3) δ -3.10 . HRMS (ESI) m/z : calculated for $\text{C}_{32}\text{H}_{56}\text{NOSi}_2^+$ $[\text{M} + \text{H}]^+$ 526.3900; found: 526.3909.

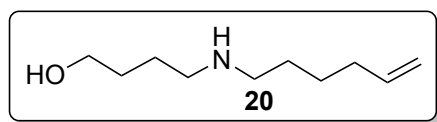


Compound 4-(bis(6-(dimethyl(phenethyl)silyl)hexyl)amino)butan-1-ol **18f** was synthesized according to Figure 4.1, Route A and General Synthetic Procedure C. In a microwave vial, compound (6-bromohexyl)dimethyl(phenethyl)silane **15f** (0.63 mmol, 206 mg) was refluxed with 4-aminobutan-1-ol **16** (0.33 mmol, 30 μL) at 92°C in ACN (2.2 mL) for 7 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMnO_4 as the stain. The product **18f** eluted in 1–2% MeOH/DCM with an isolated yield of 16% (31 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.26 (m, 12H), 3.76 (m, 2H), 3.12 (m, 2H), 3.04 (t, $J = 8\text{ Hz}$, 4H), 2.66 (m, 4H), 2.01 (p, $J = 7.1\text{ Hz}$, 2H), 1.83 (m, 4H), 1.73 (m, 2H), 1.37 (m, 12H), 0.92 (m, 4H), 0.56 (m, 3H), 0.04 (s, 12H). ^{13}C NMR (101 MHz, CDCl_3) δ 145.3, 128.4, 127.8, 125.6, 61.4, 52.8, 52.5, 33.1, 30.1, 26.6, 23.8, 23.2, 17.3, 15.2, -3.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.52. HRMS (ESI) m/z : calculated for $\text{C}_{36}\text{H}_{64}\text{NOSi}_2^+$ $[\text{M} + \text{H}]^+$ 582.4526; found 582.4530.

D. Compounds **19** and **20** (Figure 4.1, Route B)

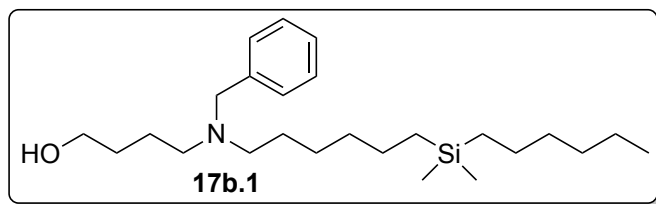


4-(di(hex-5-en-1-yl)amino)butan-1-ol **19** was synthesized according to Figure 4.1, Route B. In a microwave vial, compound 6-bromohexene **14** (5 mmol, 0.67 mL) was refluxed with 4-aminobutan-1-ol **16** (2 mmol, 0.18 mL) at 92 °C in ACN (20.0 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **19** eluted in 1–2% MeOH/DCM with an isolated yield of 23% (115 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.76 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 2H), 5.01 (m, 4H), 3.71 (t, *J* = 5.6 Hz, 2H), 3.05 (t, 2H), 2.98 (t, 4H), 2.11 (m, 4H), 1.94 (p, 2H), 1.77 (m, 4H), 1.69 (p, 2H), 1.46 (p, *J* = 7.4 Hz, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 137.6, 115.8, 61.5, 60.5, 52.9, 52.3, 33.1, 29.5, 26.1, 22.7.

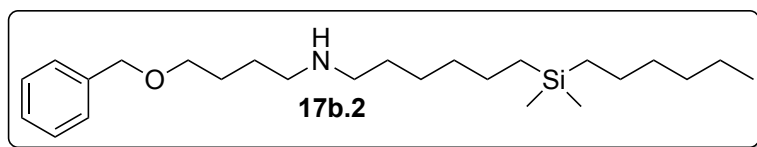


4-(hex-5-en-1-ylamino)butan-1-ol **20** was synthesized according to Figure 4.1, Route B. In a microwave vial, compound 6-bromohexene **14** (5 mmol, 0.67 mL) was refluxed with 4-aminobutan-1-ol **16** (2 mmol, 0.18 mL) at 92 °C in ACN (20.0 mL) for 3 days. The crude reaction was dried with rotary evaporation and purified using column chromatography with silica gel as the stationary phase. The fractions were analyzed using TLC with KMNO₄ as the stain. The product **20** eluted in 3–10% MeOH/DCM with an isolated yield of 41% (205 mg). ¹H NMR (400 MHz, CDCl₃) δ 5.77 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.00 (m, 2H), 3.71 (t, 2H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.97 (t, 2H), 2.09 (m, 2H), 2.02 (p, 2H), 1.88 (p, 2H), 1.73 (p, *J* = 6.3 Hz, 2H), 1.49 (p, *J* = 7.6 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 137.7, 115.6, 61.5, 48.1, 48.0, 33.1, 29.7, 26.1, 25.4, 23.8.

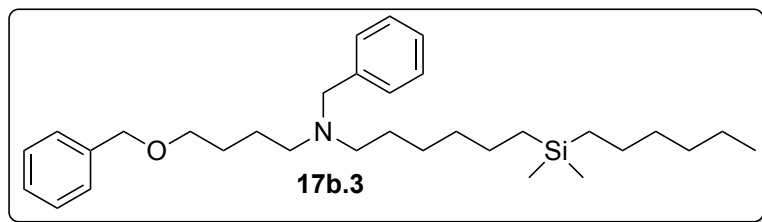
D. Benzylated and Methylated Silyl lipids



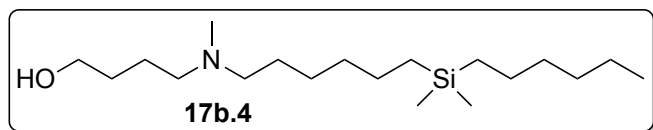
4-(Benzyl(6-(hexyldimethylsilyl)hexyl)amino)butan-1-ol **17b.1** was synthesized using General Synthetic Procedure *D* with an isolated yield of 10% (24.5 mg) after silica gel column chromatography. ^1H NMR (400 MHz, CDCl_3) δ 7.30 (m, 5H), 3.62 (s, 2H), 3.59 (t, $J = 5.0$ Hz, 2H), 2.49 (t, $J = 5.5$ Hz, 2H), 2.42 (m, 2H), 1.65 (m, 4H), 1.49 (p, $J = 7.2$ Hz, 2H), 1.26 (m, 16H), 0.88 (t, $J = 4.0$ Hz, 3H), 0.45 (m, 4H), -0.07 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 129.7, 128.5, 127.4, 62.8, 58.6, 54.5, 53.5, 33.7, 33.5, 32.4, 31.8, 27.4, 25.8, 25.6, 24.0, 22.8, 15.4, 15.4, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.17. HRMS (ESI) m/z : calculated for $\text{C}_{25}\text{H}_{48}\text{NOSi}^+$ $[\text{M} + \text{H}]^+$ 406.3505; found 406.3503.



N-(4-(benzyloxy)butyl)-6-(hexyldimethylsilyl)hexan-1-amine **17b.2** was synthesized using General Synthetic Procedure *D* with an isolated yield of 21% (52.5 mg) after silica gel column chromatography. ^1H NMR (400 MHz, CDCl_3) δ 7.53 (m, 2H), 7.42 (m, 3H), 4.06 (s, 2H), 3.68 (t, $J = 5.6$ Hz, 2H), 2.91 (m, 2H), 2.79 (m, 2H), 1.94 (m, 2H), 1.73 (m, 2H), 1.66 (m, 2H), 1.27 (m, 15H), 0.88 (t, $J = 4.0$ Hz, 3H), 0.45 (m, 4H), -0.07 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 130.9, 129.3, 61.8, 57.3, 52.9, 52.4, 33.5, 33.3, 31.8, 26.8, 23.9, 23.9, 22.8, 15.4, 15.3, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.20. HRMS (ESI) m/z : calculated for $\text{C}_{25}\text{H}_{48}\text{NOSi}^+$ $[\text{M} + \text{H}]^+$ 406.3505; found 406.3502.

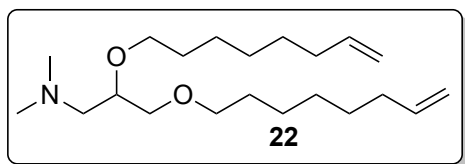


N-Benzyl-*N*-(4-(benzyloxy)butyl)-6-(hexyldimethylsilyl)hexan-1-amine **17b.3** was synthesized using General Synthetic Procedure *D* with an isolated yield of 6% (15.5 mg) after silica gel column chromatography. ^1H NMR (400 MHz, CDCl_3) δ 7.31 (m, 10H), 4.48 (s, 2H), 3.56 (s, 2H), 3.44 (t, $J = 6.2$ Hz, 2H), 2.42 (m, 4H), 1.61 (m, 4H), 1.46 (m, 2H), 1.26 (m, 16H), 0.89 (t, $J = 4.0$ Hz, 3H), 0.46 (m, 4H), -0.06 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 138.8, 133.7, 129.9, 129.8, 129.0, 128.9, 128.5, 128.3, 128.0, 127.8, 127.7, 127.6, 126.8, 73.0, 70.4, 58.7, 53.9, 53.6, 33.8, 33.5, 31.8, 29.9, 27.7, 27.3, 24.0, 24.0, 22.8, 15.5, 15.4, 14.3, -3.2 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.17. HRMS (ESI) m/z : calculated for $\text{C}_{32}\text{H}_{54}\text{NOSi}^+ [\text{M} + \text{H}]^+$ 496.3975; found 496.3976.

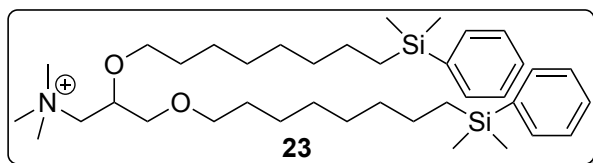


4-((6-(hexyldimethylsilyl)hexyl)(methyl)amino)butan-1-ol **17b.4** was synthesized using General Synthetic procedure *E* with an isolated of 7% (26 mg) after silica gel chromatography. ^1H NMR (400 MHz, CDCl_3) δ 3.74 (t, $J = 5.6$ Hz, 2H), 3.12 (t, $J = 8.2$ Hz, 2H), 3.02 (t, $J = 6.6$ Hz, 2H), 2.78 (s, 2H), 2.02 (m, 2H), 1.83 (m, 2H), 1.72 (m, 4H), 1.30 (m, 21H), 0.89 (t, $J = 6.8$ Hz, 3H), 0.47 (m, 5H), -0.06 (s, 5H). ^{13}C NMR (101 MHz, CDCl_3) δ 61.6, 56.3, 56.0, 40.1, 33.5, 33.2, 31.8, 29.2, 26.6, 23.9, 23.9, 23.7, 22.8, 21.7, 15.4, 14.3, 1.2, -3.3 . ^{29}Si NMR (79 MHz, CDCl_3) δ 2.23.

F. Cationic Silyl Lipid 8SiPh-DOTMA **23**



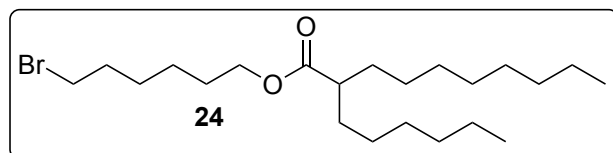
N,N-Dimethyl-2,3-bis(oct-7-en-1-yloxy)propan-1-amine **22** was synthesized using General Synthetic Procedure *F.1*. 3-(Dimethylamino)propane-1,2-diol **2** (0.694 mL, 6.0 mmol) was stirred in toluene (30.0 mL) with NaH (1.92 g, 48 mmol) at room temperature for 10 minutes. 8-Bromooct-1-ene (2.51 mL, 15.0 mmol) was then added to the flask dropwise and heated for 18 h at 80 °C to form *N,N*-dimethyl-2,3-bis(oct-7-en-1-yloxy)propan-1-amine **22** with an isolated yield of 66% (1.35 g) after silica gel column chromatography. ¹H NMR (400 MHz, CDCl₃) δ 5.80 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 2H), 4.95 (m, 4H), 3.49 (m, 8H), 2.38 (m, 2H), 2.26 (s, 6H), 2.03 (m, 4H), 1.56 (p, *J* = 6.7 Hz, 4H), 1.34 (m, 14H).



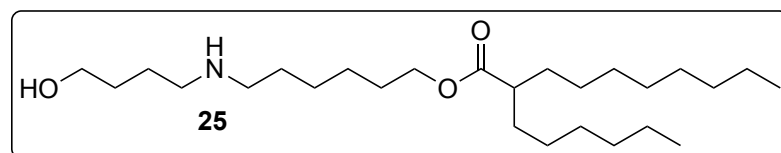
2,3-Bis((8-(dimethyl(phenyl)silyl)octyl)oxy)-*N,N,N*-trimethylpropan-1-aminium **23** was synthesized using General Synthetic Procedure *F.2*. Compound **22** (212 mg, 0.62 mmol), dimethylphenylsilane **4** (0.24 mL, 1.56 mmol) and a sprinkle of Pt(cod)Cl₂ were combined in the same flask and stirred at room temperature for 18 h to afford 2,3-bis((8-(dimethyl(phenyl)silyl)octyl)oxy)-*N,N*-dimethylpropan-1-amine which was pushed through a silica plug to remove the catalyst, concentrated to dryness under vacuum, and combined with MeI (0.40 mL, 5.89 mmol) in a sealed microwave vial and heated at 80 °C for 18 h to afford cationic silyl lipid **23** (8SiPh-DOTMA) with an isolated yield of 21% (81 mg) over two steps. ¹H NMR (400 MHz, CDCl₃) δ 7.50 (m, 4H), 7.34 (m, 6H), 4.05 (m, 2H), 3.68 (m, 1H), 3.59 (m, 3H), 3.48 (s, 9H), 3.43 (m, 3H), 1.55 (m, 8H), 1.27 (m, 29H), 0.73 (t, *J* = 7.7 Hz, 4H), 0.24 (s, 11H). ¹³C

NMR (101 MHz, CDCl₃) δ 139.7, 133.5, 132.9, 128.7, 127.7, 73.5, 72.0, 69.3, 68.04, 55.3, 33.5, 33.5, 31.8, 29.9, 29.3, 29.3, 29.2, 29.1, 26.2, 26.0, 23.8, 22.6, 15.7, 14.1, -3.00. ²⁹Si NMR (79 MHz, CDCl₃) δ -3.13. HRMS (ESI) m/z : calculated for C₂₅H₄₈NOSi⁺ [M]⁺ 626.4784; found 626.4789.

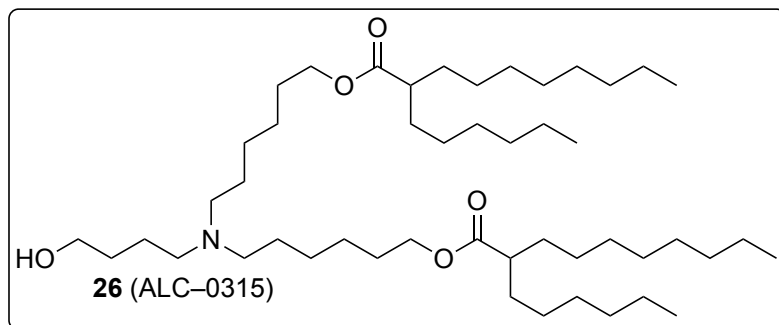
G. ALC Lipids 25, 26 and 27



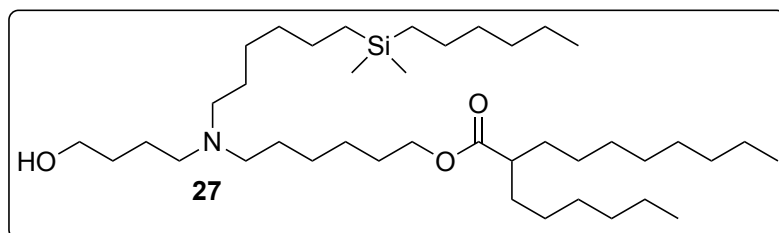
6-bromohexyl 2-hexyldecanoate **24** was synthesized using General Synthetic Procedure *G* with an isolated yield of 99% (0.99 g). See General Synthetic Procedure *G* for reaction set-up and purification details. ¹H NMR (300 MHz, CDCl₃) δ 4.07 (t, J = 6.6 Hz, 2H), 3.53 (t, J = 6.7 Hz, 1H), 3.40 (t, J = 6.8 Hz, 1H), 2.31 (tt, J = 8.9, 5.3 Hz, 1H), 1.82 (m, 2H), 1.51 (m, 6H), 1.25 (m, 22H), 0.86 (t, J = 7.5 Hz, 6H).



6-((4-hydroxybutyl)amino)hexyl 2-hexyldecanoate **25** was synthesized using General Synthetic Procedure *G* with an isolated yield of 35% (144 mg). See General Synthetic Procedure *G* for reaction set-up and purification details. ¹H NMR (400 MHz, CDCl₃) δ 4.04 (t, J = 6.7 Hz, 2H), 3.70 (t, J = 5.4 Hz, 2H), 3.01 (t, J = 6.9 Hz, 2H), 2.94 (m, 2H), 2.29 (tt, J = 8.8, 5.5 Hz, 1H), 2.02 (m, 2H), 1.87 (p, J = 7.4 Hz, 2H), 1.72 (p, J = 6.3 Hz, 2H), 1.60 (m, 2H), 1.41 (m, 6H), 1.25 (m, 22H), 0.87 (t, J = 6.8 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 176.9, 63.9, 61.6, 48.0, 47.9, 45.9, 32.6, 32.0, 31.8, 29.7, 29.6, 29.4, 29.4, 28.6, 27.6, 27.6, 26.5, 25.9, 25.5, 23.9, 22.8, 22.7, 14.3, 14.2. HRMS (ESI) m/z : Calculated for C₂₆H₅₄NO₃⁺ [M + H]⁺ 429.4137; 429.4103.



((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate) **26** (ALC-0315) was synthesized using General Synthetic Procedure *G* with an isolated yield of 62% (256 mg). See General Synthetic Procedure *F* for reaction set-up and purification details. ^1H NMR (300 MHz, CDCl_3) δ 4.04 (t, J = 6.6 Hz, 4H), 3.68 (t, J = 5.4 Hz, 2H), 2.95 (m, 2H), 2.87 (t, J = 8.7 Hz, 4H), 2.30 (tt, J = 8.8, 5.4 Hz, 2H), 1.88 (p, J = 7.1 Hz, 2H), 1.63 (t, J = 7.5 Hz, 12H), 1.40 (dp, J = 10.7, 3.5, 3.0 Hz, 9H), 1.24 (m, 40H), 0.85 (m, 12H). ^{13}C NMR (76 MHz, CDCl_3) δ 176.9, 63.8, 61.8, 53.1, 52.8, 45.9, 32.6, 31.9, 31.8, 29.7, 29.6, 29.4, 29.3, 28.7, 27.6, 27.5, 26.8, 25.7, 23.9, 22.8, 22.7, 14.2, 14.2. HRMS (ESI) m/z : calculated for $\text{C}_{48}\text{H}_{96}\text{NO}_5^+$ $[\text{M} + \text{H}]^+$ 766.7289; 766.7257.



6-((6-(hexyldimethylsilyl)hexyl)(4-hydroxybutyl)amino)hexyl 2-hexyldecanoate **27** was synthesized using General Synthetic Procedure *G* with an isolated yield of 22% (32 mg). See General Synthetic Procedure *G* for reaction set-up and purification details. ^1H NMR (400 MHz, CDCl_3) δ 4.04 (t, J = 6.7 Hz, 2H), 3.68 (t, J = 5.6 Hz, 2H), 2.98 (t, J = 7.5 Hz, 2H), 2.91 (t, J = 8.5 Hz, 4H), 2.29 (tt, J = 8.8, 5.4 Hz, 1H), 1.90 (p, J = 6.9 Hz, 2H), 1.63 (m, 5H), 1.26 (m, 40H), 0.87 (m, 9H), 0.46 (m, 4H), -0.07 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.9, 63.9, 61.7, 53.1, 52.7, 45.9, 33.5, 33.3, 32.6, 31.9, 31.82, 31.7, 29.8, 29.7, 29.6, 29.4, 29.3, 28.6, 27.6, 27.5, 26.8, 26.8,

25.7, 23.9, 23.9, 23.8, 23.6, 22.8, 22.7, 22.7, 15.3, 14.3, 14.2, 14.2, -3.3. ^{29}Si NMR (79 MHz, CDCl_3) δ 2.22. HRMS (ESI) m/z : calculated for $\text{C}_{40}\text{H}_{84}\text{NO}_3\text{Si}^+$ $[\text{M} + \text{H}]^+$ 654.6220; 654.6200.

Summary of Dissertation:

My dissertation work encompasses the breadth of lipid nanoparticle properties (i.e. size, encapsulation efficiency, stability, membrane fluidity and mRNA transfection efficiency) that can be controlled by strategic tuning of both the head group and silyl lipid tail structures (Figure 56). Hydrosilylation is a robust reaction that allows efficient access to a structurally diverse toolbox of novel silyl lipid tails with varying degrees of branching that would otherwise be more difficult to access with traditional carbon-based synthetic chemistry. I have synthesized 34 novel silyl lipid structures with cationic and ionizable head groups and varying silyl lipid tails, including both single and double chain silyl lipids. Varying the silyl lipid tail structure and head group of these lipid structures resulted in the formulation of particles that span the full spectrum of membrane fluidity. Single chain (MA) ionizable silyl lipids and cationic silyl lipids generally demonstrated the formation of particles with more fluid membranes, while double chain (DA) ionizable silyl lipids generally formed particles with more rigid membranes. This variation in membrane fluidity corresponded to variation in subsequent properties such as size, RNA encapsulation efficiency and mRNA transfection efficiency. Cationic silyl lipids generally performed better when the silyl lipid tail was shorter in length, where phenyl rings in the tails showed enhanced RNA encapsulation efficiency and rate and greater mRNA transfection efficiency compared to non-phenyl containing tails. Membrane fluidity of particles containing ionizable silyl lipids was successfully controlled by varying the structure of the silyl lipid tail, with distinct membrane fluidity and mRNA transfection efficiencies observed for single vs double chain silyl lipids. Double chain ionizable silyl lipids demonstrated enhanced mRNA transfection efficiency when combined with cationic silyl lipids (i.e. 5Si3Ph-DOTAP and 8SiPh-DOTAP) and helper lipids DSPC, cholesterol and DMG-PEG-2000, which corresponded to the

formation of more rigid membrane structures. Single chain ionizable silyl lipids showed enhanced mRNA transfection when combined with only cationic silyl lipids and no helper lipids, which corresponded to a wider membrane fluidity profile suggesting the presence of both rigid and fluid membrane structures.

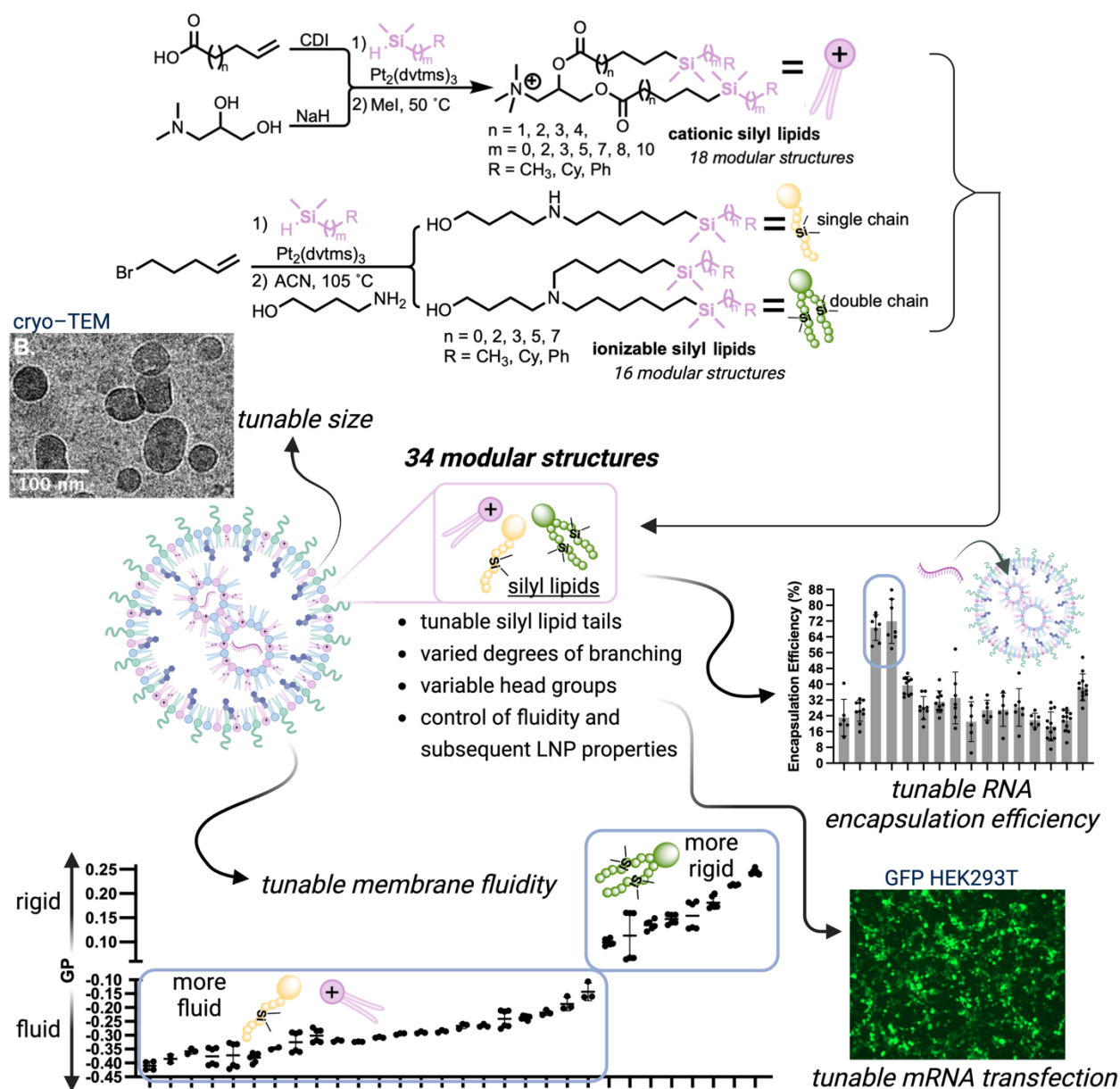


Figure 56. Summary of Dissertation: Evaluation of a new class of cationic and ionizable silyl lipids with modular tail structures that successfully modulate membrane fluidity, RNA encapsulation efficiency, size, mRNA transfection efficiency and other nanomaterial properties influential in efficient LNP delivery.

Along with membrane fluidity, other particle properties such as size, zeta potential, polydispersity index, RNA encapsulation efficiency and rate, stability (measured by RNA encapsulation efficiency over time), and mRNA transfection efficiency were also tuned by varying the structure of the cationic and ionizable silyl lipid tails. Overall, this work highlights the promise of these ionizable and cationic silyl lipid structures for use in nanomaterials for mRNA delivery.

Biophysical and In Vivo Evaluation of Cre–mRNA LNPs:

In vivo experiments will be performed in June 2026 evaluating biodistribution and Cre–recombinase mRNA delivery efficiency of silyl–LNPs in Ai14 mice, selected due to carrying the LoxP STOP cassette that is recognized and excised by Cre-recombinase which subsequently activates tdTomato expression. Lead silyl lipid LNP formulations containing ionizable lipid 6Si6–DA or 6Si8–DA, cationic lipid 5Si3Ph–DOTAP or 8SiPh–DOTAP, and helper lipids DSPC, cholesterol and DMG–PEG–2000 will be evaluated for in vivo efficacy, toxicity and biodistribution in Ai14 mice, and both IV and ICV injections will be performed to evaluate systemic mRNA delivery efficiency and biodistribution and delivery efficiency in the brain.

To prepare for in vivo experiments, LNPs were prepared using ethanol dilution as the more current preparation method instead of thin-film rehydration, due to forming particles with lower polydispersity index and more homogenous sizes. Cre mRNA encapsulation efficiency, size, polydispersity index, and zeta potential was evaluated for the top–performing silyl–LNP formulations formed using ethanol dilution (note that for all experiments in Chapters 2–3, the traditional thin film–rehydration method was used to form particles). The Cre mRNA EE (%) was first determined for LNPs with a 45:5:10:38.5:1.5 mol % ratio of

ionizable/cationic/DSPC/cholesterol/PEG based on a recently published paper that reported forming ALC-0315 DOTAP LNPs for in vitro mRNA delivery.¹²⁸ When forming LNPs at this mol % ratio, low average EEs were observed below 60% (Figure 57A, purple). I hypothesized that the low EEs may be attributed to not having high enough mol % ionizable lipid (45 mol %) in the formulation. To test this hypothesis, I increased the ionizable lipid in the formulation to 65 mol % however this still resulted in average EEs below 60% (Figure 57A, green).

Based on the lack of improvement in EE upon varying the ratio of lipid components, I hypothesized that the total lipid/mRNA w/w ratio may not be high enough to allow for sufficient RNA encapsulation. The calculated w/w ratios used in the two LNP formulations previously tested (Figure 57A) are around 6–8:1 w/w total lipid/mRNA. To enhance the w/w lipid/mRNA ratio, I formed LNPs using the 10:10:2:4:1 w/w ratio ionizable/cationic/DSPC/cholesterol/PEG that I used previously for in vitro transfection experiments in Chapter 3. This 10:10:2:4:1 w/w ratio corresponds to a 16.4:1 w/w total lipid/mRNA, which is about twice as high as the ratio used for the previous formulations (6–8:1 w/w). Increasing the total lipid/mRNA w/w ratio in the formulation resulted in significantly higher Cre mRNA EE for 6Si8-DA LNPs around 90% (Figure 57B, blue). Based on these findings, the 10:10:2:4:1 w/w ratio ionizable/cationic/DSPC/cholesterol/PEG will be used to form LNPs via ethanol dilution for in vivo experiments. It is notable that all LNPs formed using ethanol dilution resulted in the formation of more unimodal, less polydisperse particle sizes as indicated by their unimodal size by intensity distributions (Figure 57C) and low polydispersity index (PDI) values <0.2 (Table 9). This is distinct from the size distribution profiles and PDIs observed for these 10:10:2:4:1 w/w LNPs formed using thin film rehydration, which had bimodal size distributions and PDI values between 0.4–1 (see Chapter 3). These results further support the use of ethanol dilution sample

preparation of Cre mRNA LNPs to form particles with more uniform, homogenous sizes for in vivo evaluation in Ai14 mouse studies.

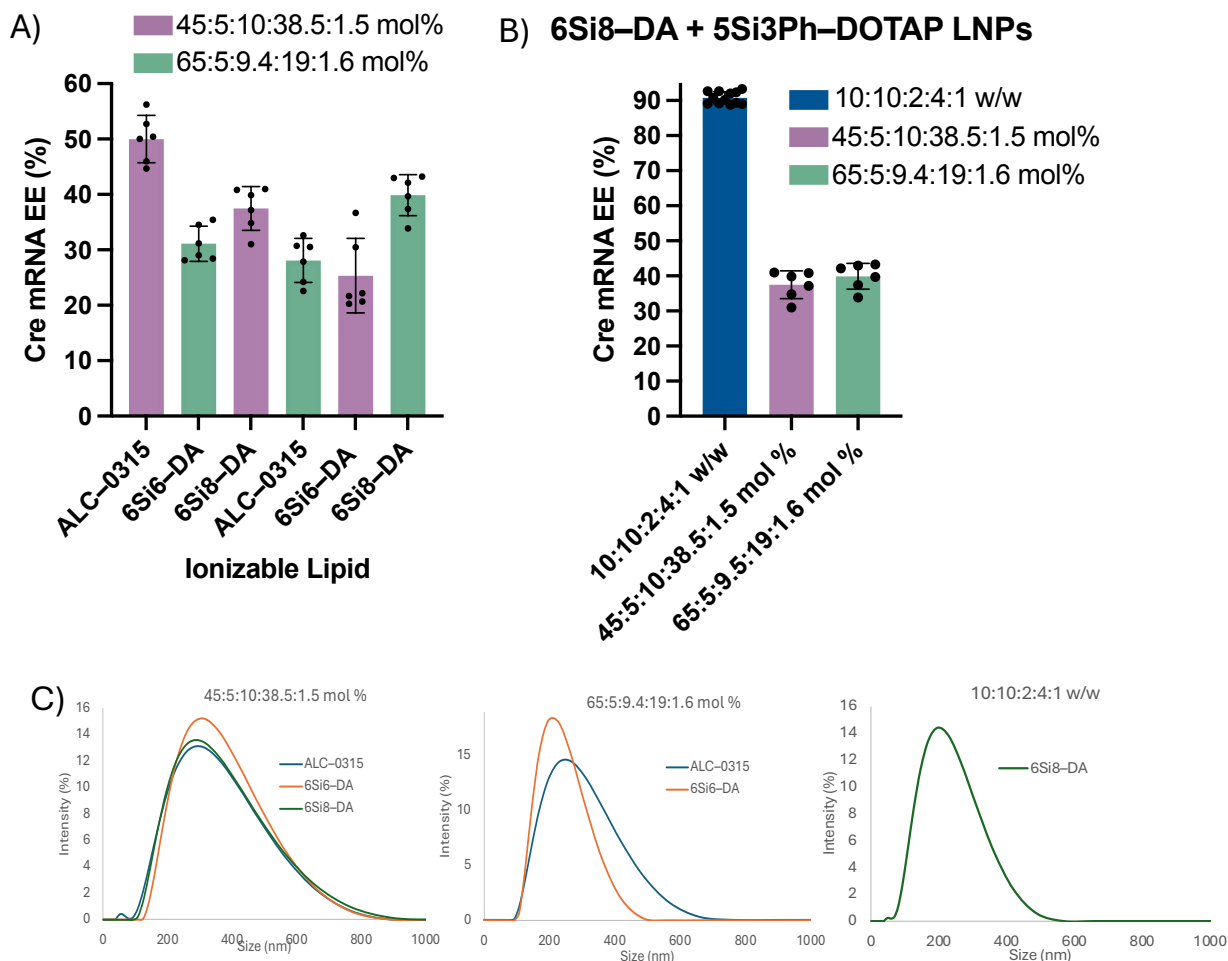


Figure 57. Cre mRNA-LNP (A–B) EE % at varying ratios of ionizable lipid/cationic 5Si3Ph-DOTAP (**10g**)/DSPC/ cholesterol/ DMG-PEG-2000 and (C) size distribution profiles of LNPs formed using ethanol dilution. LNPs were formed at a 1:3.5 v/v lipid solution in ethanol/mRNA solution in citric acid buffer (0.1 M, pH 4). Formulations in (A) are 6–8:1 w/w total lipid mRNA. Formulations in (B) are 16.4:1 w/w total lipid/mRNA. EE values in Figure (A) represent the average $\pm$ standard deviation of $n = 6$ trials, where $n = 3$ trials were taken from one batch of mRNA-LNPs and $n = 3$ trials were taken from another batch of mRNA-LNPs. EE values in (B) (blue, ~90%) represent the average $\pm$ standard deviation of $n = 12$ trials taken from one mRNA-LNP solution. A Cre-mRNA calibration curve was prepared with the RiboGreen fluorescence assay and used to calculate EE according to Equation 5. See Figure 58A for sample Cre mRNA calibration curve.

Table 9. Size, PDI and zeta potential of Cre mRNA–LNPs formed using ethanol dilution.

	45:5:10:38.5:1.5 mol %			10:10:2:4:1 w/w
	ALC–0315 ^a	6Si6–DA ^a	6Si8–DA ^b	6Si8–DA ^c
Size (nm)	269 ± 28	263 ± 69 ^a	289	198
PDI	0.16 ± 0.007	0.15 ± 0.04	0.17	0.13
Zeta Potential (mV)	-16 ± 1	-12 ± 8	-20	12

All LNPs were prepared at 0.1 mg/mL concentration in nuclease water from the Cre–mRNA LNP stock solutions that were prepared for EE analysis. Cre mRNA–LNP formulations are composed of ionizable lipid, 5Si3Ph–DOTAP (**10g**), DSPC, cholesterol and DMG–PEG–2000. ^aValues represent the average ± standard deviation of n = 2 trials, where 1 trial = 1 mRNA–LNP solution. ^bValues represent the reading from n = 1 trial (the other trial was too dilute for detection from the instrument). ^cValues represent the readings from n = 1 trial, where 1 trial = 1 mRNA–LNP solution.

For in vivo studies, 3 male and 3 female mice (n = 6) will be injected per Cre mRNA–LNP formulation evaluated. The desired dose of mRNA per mouse is 0.5 mg/kg of body weight and the approximate weight per mouse is approximately 25 g, equating to a total of 0.075 mg (75 µg) of Cre mRNA needed per LNP formulation. The experiments performed using the previous Cre mRNA–LNPs (Figure 57, Table 9) contained a total of 0.033 mg (33 µg) of Cre mRNA per LNP formulation. Therefore, to ensure that the total minimum amount of Cre mRNA was encapsulated per LNP formulation, the mass of Cre mRNA was tripled in the formulation (0.099 mg, 99 µg) and the volumes of total lipid/ethanol and Cre mRNA/citrate buffer solutions were tripled to maintain a 1:3.5 v/v ratio of total lipid/mRNA solution in the formulation. The EE (%) and size was re-evaluated with this larger batch of Cre mRNA–LNPs (Figure 58). The EE (%) of ALC–0315 formulation increased to ~60% when switching to the FDA–approved mol % formulation and tripling the batch size (yellow, Figure 58B) compared to the previous mol % formulation which resulted in ~50% EE (Figure 57A). While a drop in EE from ~90% (blue, Figure 57B) to ~80% (blue, Figure 58B) is observed for 6Si8–DA LNPs when tripling the batch size, the amount of mRNA actually encapsulated by these particles (0.80 x 0.099 mg = **0.079 mg mRNA**

encapsulated) is still sufficient based on the minimum amount needed for mice injections (0.075 mg Cre mRNA needed).

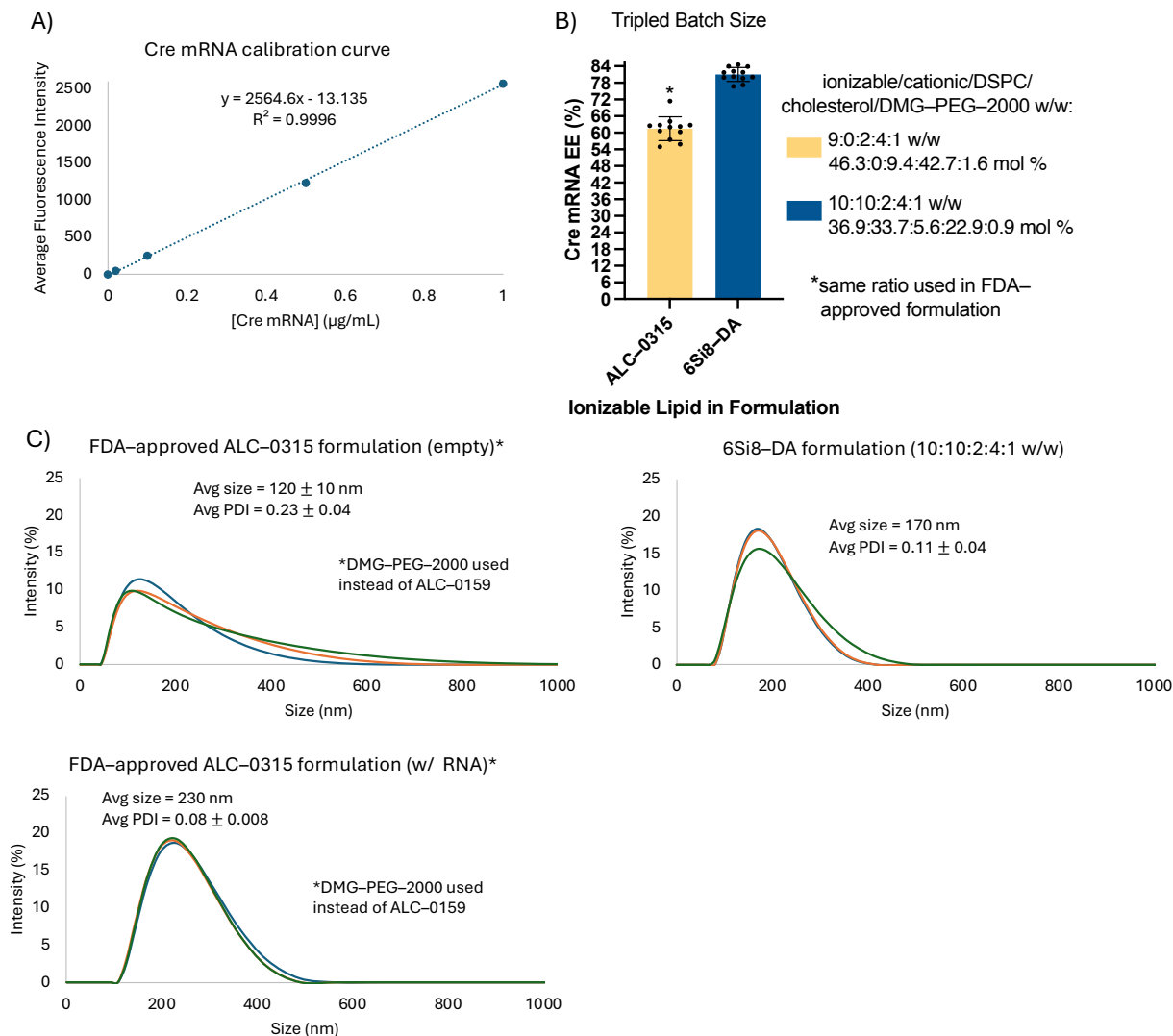


Figure 58. Cre mRNA EE (B) and size distribution profiles (C) of Cre mRNA-LNPs with tripled batch size for Ai14 mice studies. Cre mRNA EE (%) calculated according to Equation 5 using a Cre mRNA calibration curve (A). Values in the calibration curve represent the average $\pm$ standard deviation of $n = 2$ trials. All formulations in (B–C) are composed of ionizable lipid and/or 5Si3Ph-DOTAP (**10g**), DSPC, cholesterol and DMG-PEG-2000. ALC-0315 Cre mRNA-LNPs are 20:1 w/w total lipid/mRNA and 6Si8-DA Cre mRNA-LNPs are 16.4:1 w/w total lipid/mRNA. All LNPs were formed at a 1:3.5 v/v lipid solution in ethanol/mRNA solution in citric acid buffer (0.1 M, pH 4).

Size distribution data were acquired for ALC-0315 (mol % ratio from FDA-approved formulation) and 6Si8-DA Cre mRNA-LNPs with the tripled batch size (Figure 58C). Cre mRNA-LNPs formed with both ALC-0315 and 6Si8-DA demonstrated average sizes between 170–230 nm with low PDI values between 0.07–0.15, indicating the presence of unimodal, homogenous particle sizes sufficient for in vivo mouse studies. To confirm LNP formation without the presence of Cre mRNA, empty ALC-0315 formulations were prepared using the same method (ethanol dilution) and their sizes were compared to LNPs formed with Cre mRNA. Interestingly, empty ALC-0315 LNPs formed particles with smaller sizes (110–130 nm) and a larger PDI (0.23 ± 0.04) compared to Cre mRNA-LNPs (230 nm, 0.08 ± 0.008). This data confirms the use of ethanol dilution as a sufficient method for forming both empty and mRNA-encapsulated particles with particle sizes between 100–300 nm and PDIs < 0.25 . These findings confirm the use of these Cre mRNA-LNPs with tripled batch size (triple Cre mRNA mass and triple volume of lipid/ethanol and citric acid/Cre mRNA solutions) for in vivo evaluation in Ai14 mice. This tripled batch size was used to prepare all Cre mRNA-LNP formulations via ethanol dilution, and these formulations (see Table 10 for specific formulations) were used for IV and ICV injections in Ai14 mice. Biodistribution of these Cre mRNA-LNP formulations will be determined via evaluation of tdTomato expression in the next 1–2 weeks. See Table 11 and Table 12 for volumes of lipid stock solutions, ethanol, buffers and Cre mRNA used to prepare LNP samples for mouse studies.

Note that terminal filtration with a 0.22 μm filter cannot be performed with LNPs because the particles will adhere to the hydrophilic membrane of the filter and be completely removed from the sample prior to IV and ICV injection. Instead, LNPs are prepared aseptically using (i) autoclaved vials, pipette tips and Eppendorf tubes, (ii) 0.22 μm (low-protein-binding membranes

such as PVDF or equivalent) filtered PBS and citric acid buffer and (iii) prepared fresh the day of IV and ICV injections, achieving equivalent sterility to terminal filtration. This method is consistent with the sterility methods described in FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing (2004) which recognizes the use of aseptic sterilization techniques instead of terminal filtration when terminal filtration is incompatible with the sample. Aseptic sterilization is also used instead of terminal filtration for the FDA-approved LNP-based therapeutic Onpattro.

Table 10. Cre mRNA–LNP formulations used for IV and ICV injections in Ail4 mice.

Formulation	Prepared with Cre mRNA	Injections	Ratio of Components (mol %)	Ratio of components (w/w)
ALC–0315 + DSPC + cholesterol + DMG–PEG–2000 [reference] ^{*a}	Yes	IV	46.3 : 9.4 : 42.7 : 1.6	9: 2 : 4 : 1
6Si8–DA + 5Si3Ph–DOTAP + DSPC + cholesterol + DMG–PEG–2000 ^b	Yes	IV and ICV	36.9 : 33.7 : 5.6 : 22.9 : 0.9	10 : 10 : 2 : 4 : 1
6Si8–DA + 8SiPh–DOTAP + DSPC + cholesterol + DMG–PEG–2000 ^b	No (empty)	IV	36.9 : 33.7 : 5.6 : 22.9 : 0.9	10 : 10 : 2 : 4 : 1

All LNPs evaluated were prepared with a 1:3.5 v/v ratio of lipid/ethanol to citric acid/Cre mRNA solution and dialyzed against 1X PBS for ~2 hours in the dark with stirring. See Tables 11–12 for volumes of lipid stock solutions, ethanol, citric acid buffer and Cre mRNA used to prepare samples at a 1:3.5 v/v ratio. Samples were prepared fresh the day of injections. *Used the same ratio of components as the FDA–approved formulation to prepare Cre mRNA–ALC–0315 LNPs. ^aALC–0315 LNPs were prepared at a 20:1 w/w ratio of total lipid components to Cre mRNA to match FDA–approved formulation. ^bSilyl LNPs were prepared at a 16.4:1 w/w ratio of total lipid components to Cre mRNA.

Table 11. Volumes used to prepare lipid solution in ethanol for LNP formation

ionizable + cationic lipid in formulation	ionizable lipid ^a (μL)	cationic lipid ^a (μL)	DSPC ^a (μL)	cholesterol ^a (μL)	DMG–PEG–2000 ^a (μL)	ethanol added (μL)	total volume (μL)
ALC–0315 + no cationic lipid	91	0	19	42	10	246	408
6Si8–DA + 5Si3Ph–DOTAP	60	60	12	24	6	246	408
6Si8–DA + 8SiPh–DOTAP	60	60	12	24	6	246	408

^aVolume taken from a 10 mg/mL stock solution prepared in ethanol.

Table 12. Volumes used to prepare Cre mRNA solution in citric acid buffer for LNP formation

Formulation	Volume of Cre mRNA ^a (μL)	Volume of citric acid buffer ^b (μL)	Total volume of Cre mRNA/citric acid solution (μL)
ALC–0315 + DSPC + cholesterol + DMG–PEG–2000	81	1,341	1,422
6Si8–DA + 5Si3Ph–DOTAP + DSPC + cholesterol + DMG–PEG–2000	99	1,323	1,422
6Si8–DA + 8SiPh–DOTAP + DSPC + cholesterol + DMG–PEG–2000	99	1,323	1,422

^aVolume taken from a 1 mg/mL Cre mRNA stock solution. ^bVolume taken from a 0.1 M citric acid buffer solution (pH 4).

Appendix A: DNA EE, ER and Transfection Efficiency of Particles Containing Silyl lipids

All HEK293T cell transfection data presented in below was acquired in collaboration with Dr. Henriette O'Geen and Dr. David Segal in the Genome Center at UC Davis.

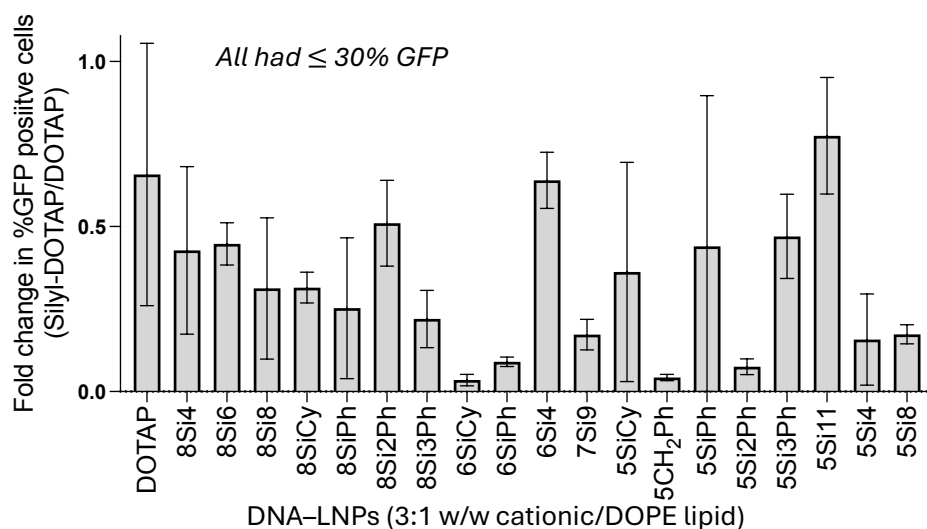
A.1. DNA Transfection Efficiency of Particles Containing Cationic and Ionizable Silyl lipids

The pDNA transfection efficiency of particles containing the cationic silyl lipid analogs of DOTAP were evaluated in HEK293T cells using pMax DNA (GFP) [pDNA] and compared to DOTAP (Figure 59A–B). Overall, particles containing the cationic silyl lipids appeared to be less effective for transfection with pDNA compared to mRNA, with all cationic silyl lipids and DOTAP showing less than 30% GFP positive cells after pDNA transfection when mixed with the DOPE ionizable lipid (3:1 w/w cationic/DOPE lipid). Of the cationic silyl lipids tested, 8Si2Ph, 6Si4, 5Si3Ph and 5Si11 cationic silyl lipids appeared to have the highest average %GFP with the smallest standard deviation between biological replicates. These results support continued efforts towards optimizing LNP formulations containing cationic silyl lipids for improved pDNA transfection.

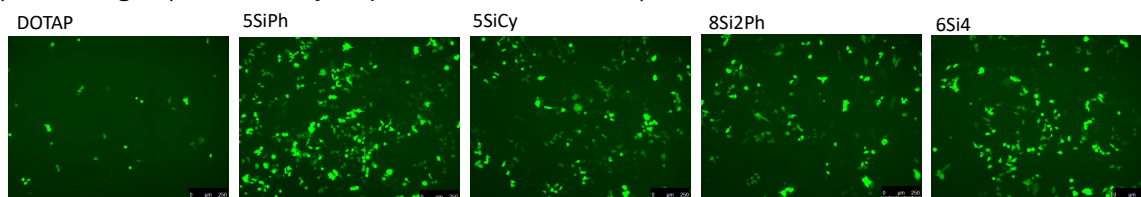
Particles containing ionizable silyl lipid analogs of ALC–0315 with and without cationic silyl lipid 5Si3Ph–DOTAP were evaluated for pDNA transfection efficiency in HEK293T cells (Figure 59C). Cationic silyl lipid analog **10g** was chosen for evaluation with ionizable silyl lipids based on the high mRNA transfection observed for 5Si3Ph–particles (see Chapter 2). Particle containing only single chain ionizable silyl lipids (1:0 w/w ionizable/cationic) showed 2–10% GFP positive cells compared to ALC–0315 which demonstrated less than 1% GFP positive cells. Three of the four ionizable silyl lipids evaluated showed toxicity indicated by dead cells

observed post-transfection. An increase in %GFP is observed for all ionizable silyl lipids and ALC-0315 when combined with cationic silyl lipid **10g**, with these 1:1 w/w ionizable/cationic lipid formulations showing no toxicity. These results suggest that, while suitable for mRNA transfection, more work needs to be done to improve and optimize LNPs containing cationic and ionizable silyl lipids for pDNA transfection.

A) pDNA Transfection with Cationic Silyl-Lipid DOTAP Analogs



B) Cell Images (Cationic Silyl-Lipid DOTAP DNA-LNPs)



C) pDNA Transfection with Ionizable Silyl-Lipid ALC-0315 Analogs + **10g** (5Si3Ph-DOTAP Analog)

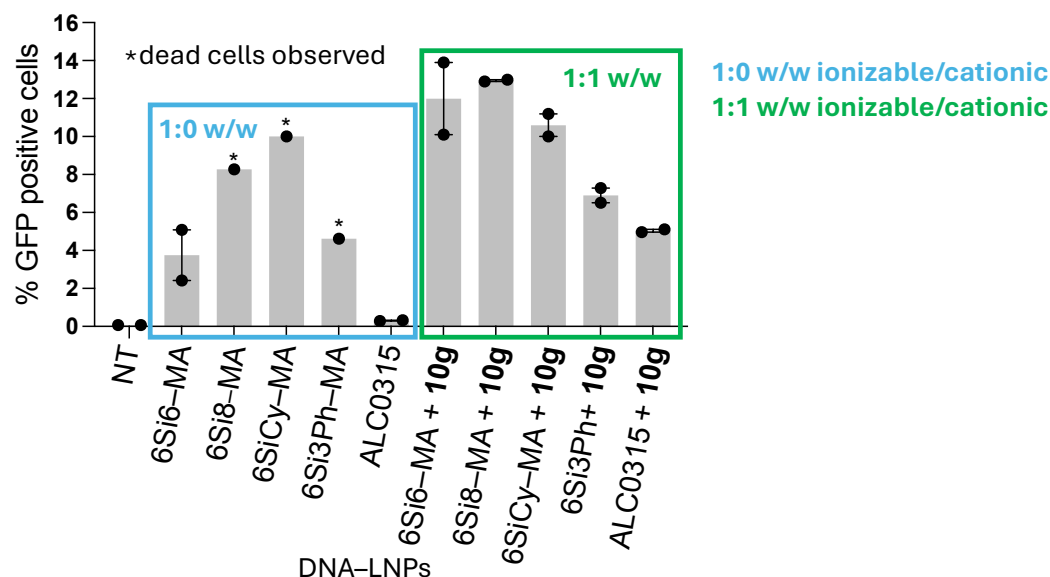


Figure 59. pMax-GFP DNA transfection efficiency in HEK293T cells. LNPs prepared for transfection were (A-B) 3:1 w/w cationic lipid/DOPE (DOPE is used as the ionizable lipid) and n = 4 replicates or (C) 1:0 or 1:1 w/w ionizable/cationic silyl lipid **10g** (where the ionizable lipid is ALC-0315 or a MA silyl lipid analog of ALC-0315) and n = 1-2 replicates.

A.2. DNA EE and ER of Particles Containing Cationic Silyl lipids

The DNA encapsulation efficiency (EE) was evaluated for particles composed of cationic silyl lipid analogs of DOTAP for comparison to DOTAP particles. The Quant-It PicoGreen assay was used to evaluate DNA EE using the same liposome preparation and assay procedure as the RiboGreen assay that was used to determine RNA EE and ER. The DNA EE was first evaluated for a selection of the cationic silyl lipids with variable position (8Si8 and 5Si11) and variable lengths and substituents in the tail (5Si3Ph, 6Si4 and 8Si2Ph) for comparison to DOTAP based on their in vitro pDNA transfection efficiency (Figure 60A). The results demonstrate that direct replacement of the *cis* alkene in DOTAP with the silyldimethyl group in 8Si8 results in a decrease in DNA EE from ~50% (DOTAP) to less than 20% (8Si8) (Figure 60A). However, moving the silyldimethyl group closer to the cationic head group results in an increase in DNA EE to ~50% (5Si11). These results emphasize the value of synthetically controlling silyldimethyl position to optimize DNA EE. Keeping the silyldimethyl position the same but shortening the tail and incorporating a bulky phenyl ring resulted in only a subtle increase in DNA EE to ~55% (5Si3Ph). Subsequent silyl lipid tail modifications did not appear to have a significant effect on DNA EE, with 6Si4 and 8Si2Ph maintaining similar DNA EEs as 5Si3Ph and DOTAP of ~55–60%. These findings demonstrate that (1) varying silyldimethyl position but keeping the tail length the same as DOTAP is a useful strategy for controlling DNA EE, and (2) subsequent modifications of tail length, substituent and/or silyldimethyl position do not appear to have a significant effect on DNA EE; this differs from encapsulation with rRNA, where variations in silyldimethyl position, tail length and/or substituent resulted in much greater changes in RNA EE (see Chapter 2).

DNA encapsulation rate (ER) was also evaluated with a modified Quant–It PicoGreen assay using the same procedure as for RNA ER. Direct replacement of the *cis* alkene in DOTAP with the silyldimethyl in 8Si8 did not show a significant change in DNA ER (Figure 60B), with DOTAP and 8Si8 both having a DNA ER of less than 0.2 fluorescence(%)•min⁻¹ (Figure 60C). Varying the silyldimethyl position but keeping tail length the same showed little effect on rate, with DNA ER being maintained around 0.2 fluorescence(%)•min⁻¹ for 5Si11 (Figure 60B–C). No significant change in DNA ER was observed when shortening the silyl lipid tail and varying silyldimethyl position and substituent, with 6Si4 and 8Si2Ph demonstrating a DNA ER of less than 0.2 fluorescence(%)•min⁻¹ (Figure 60B–C). Interestingly, 5Si3Ph showed the highest DNA ER of all cationic lipids including DOTAP, with an average DNA ER around 1 fluorescence(%)•min⁻¹ (Figure 60C) and a large drop in fluorescence (%) observed from 1–50 minutes (Figure 60B). It is notable that DOTAP, 8Si8, 5Si11, 6Si4 and 8Si2Ph particlees showed an increase in fluorescence (%) from 0–5 minutes, with a decrease in fluorescence (%) not being observed until around 10–15 minutes after incubation with DNA (Figure 60B). These observations suggest that the DNA was not being encapsulated by the particles until around 10–15 minutes after incubation, which may have caused an increase in fluorescence (%) from 0–5 minutes due to coordination of the PicoGreen dye to the un–encapsulated DNA sitting outside of the particles. The cationic particles did not show this behavior for RNA ER, with an immediate drop in fluorescence (%) being observed immediately after incubation with RNA (see Chapter 2). Also notable is the lack of correlation between DNA EE and ER with varying the cationic silyl lipid tail (Figure 60C). This differs from RNA EE and ER which did appear correlated with varying the cationic silyl lipid tail (see Chapter 2). Overall, these findings (i) demonstrate that varying the cationic silyl lipid tail structure has a larger effect on RNA ER compared to DNA

ER, (ii) suggest that DNA EE is not correlated with DNA ER, and (iii) more experiments are needed to fully elucidate the relationship between lipid tail structure and DNA EE for the cationic lipids.

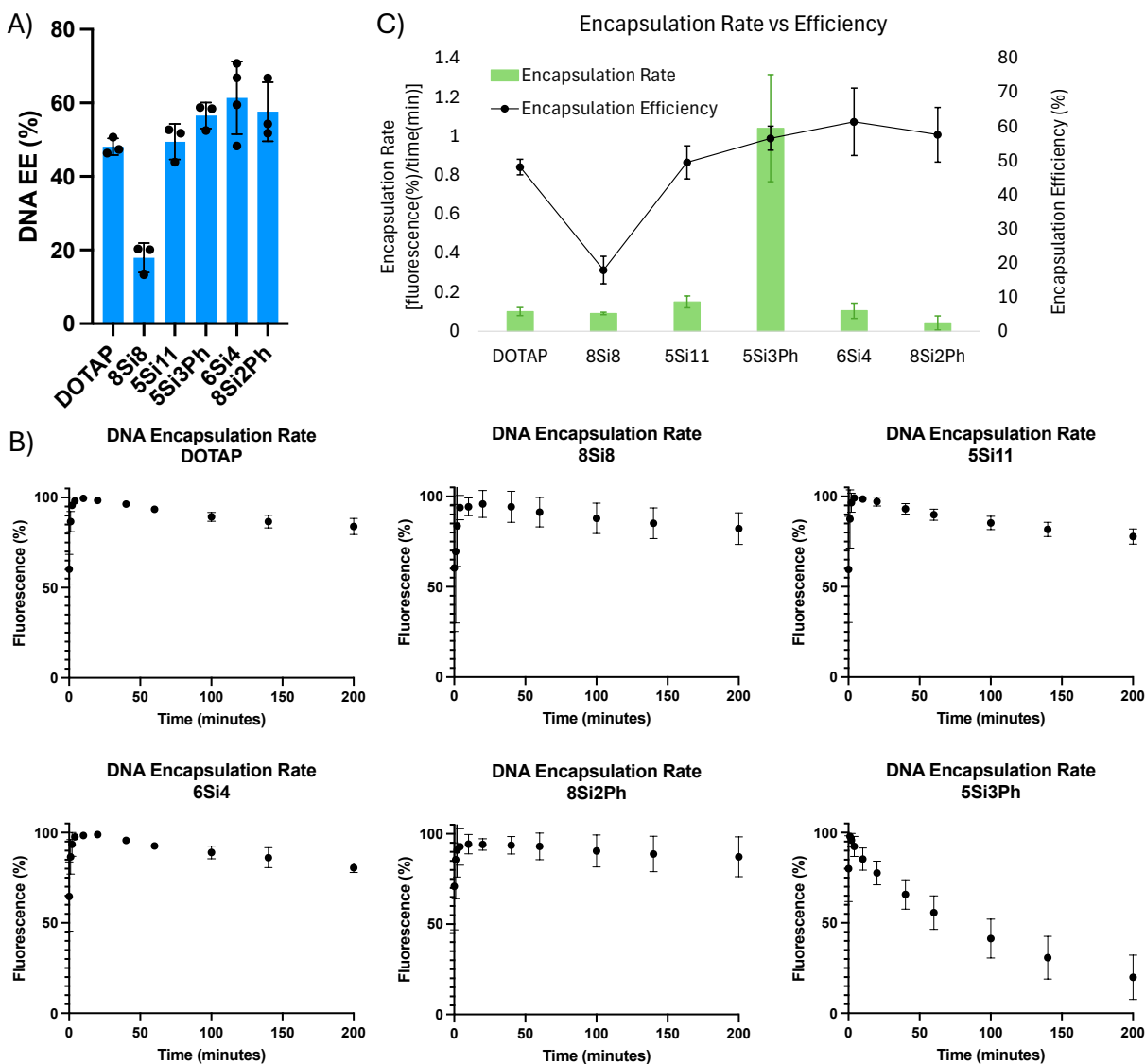


Figure 60. DNA encapsulation efficiency (EE) and rate (ER) of cationic silyl lipid DOTAP analogs using Quant-IT PicoGreen Assay. The procedure used for the RiboGreen assay to determine rRNA EE and ER was used for the PicoGreen assay to determine DNA EE and ER. EE (%) was calculated using Equation (1) but with [pDNA] instead of [rRNA] (see Chapter 2).

The significantly lower DNA EE observed for 8Si8 particles compared to the other cationic lipids evaluated may be attributed to differences in particle membrane fluidity (Figure 61A). There is a large decrease in GP from -0.21 ± 0.005 for DOTAP to -0.41 ± 0.02 for 8Si8; this indicates much more fluid membranes for 8Si8 particles compared to DOTAP particles. DOTAP, 5Si11 and 6Si4 particles all have more rigid membranes than 8Si8 particles, as indicated by their more positive GP values of between -0.29 to -0.21 (Figure 61A). These changes in GP correlate with the changes in DNA EE observed for these lipids. There is a significant drop in both GP (purple) and DNA EE (orange) when switching from DOTAP to 8Si8 (Figure 61B). This is followed by a notable increase in GP and DNA EE when switching to 5Si11 and 6Si4. This data demonstrates a correlation between DNA EE and membrane fluidity, where lipids that form more rigid membranes (i.e. DOTAP, 5Si11 and 6Si4) are better suited for encapsulating DNA compared to lipids that form more fluid membranes (such as 8Si8). A potential explanation may be that 8Si8 particles lack structural stability due to their highly fluid membranes, causing DNA to “leak” out of the particle after encapsulation and resulting in a low DNA EE.

A. Membrane Fluidity

Cationic Lipid	GP
DOTAP	-0.21 ± 0.005
8Si8	-0.41 ± 0.02
6Si4	-0.29 ± 0.004
5Si11	-0.27 ± 0.006

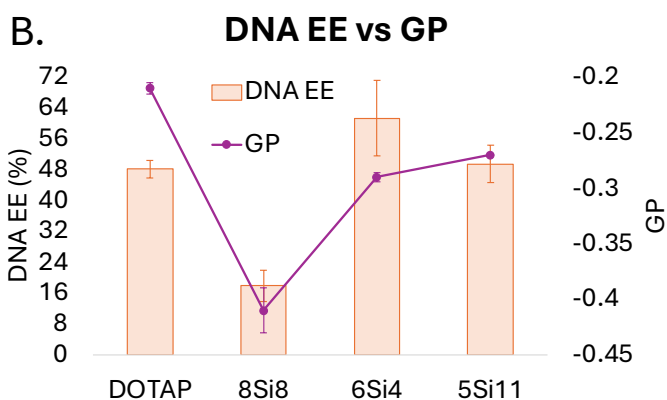


Figure 61. A) GP values for membrane fluidity and (B) the relationship between DNA EE for DOTAP and 8Si8, 5Si11 and 6Si4 cationic silyl lipid analogs of DOTAP. Membrane fluidity was measured using the C-laurdan fluorescence assay (see Chapter 2).

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Appendix B: NMR Spectra

