SYNTHETIC APPROACHES TO CARDIOLIPIN ANALOGS

by

Michael A. Frasso

B.S. in Chemistry, Youngstown State University, 2012

M.S. in Chemistry University of Pittsburgh, 2014

Submitted to the Graduate Faculty of

The Kenneth P. Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

THE KENNETH P. DIETRICH SCHOOL OF ARTS AND SCIENCES

This dissertation was presented

by

Michael A. Frasso

It was defended on

June 19, 2020

and approved by

Kazunori Koide, Associate Professor, Department of Chemistry

W. Seth Horne, Associate Professor, Department of Chemistry

Valerian E. Kagan, Professor, Department of Occupational and Environmental Health

Peter Wipf: Distinguished University Professor, Department of Chemistry

SYNTHETIC APPROACHES TO CARDIOLIPIN ANALOGS

Michael A. Frasso, PhD

University of Pittsburgh, 2020

The first portion of this document describes the synthesis of three examples of the mitochondrial phospholipid cardiolipin with different fatty acid chain lengths and degrees of unsaturation. These compounds enabled the assessment of the phospholipase Cld1's hydrolysis specificity for different fatty acid esters in cardiolipin.

During the final deprotection reaction of the synthesis, trace hydrolysis of the cardiolipin acyl groups occurred. To ameliorate this issue, a new protecting group scheme was devised, along with conditions for the selective cleavage of a primary triethylsilyl ether in the presence of a vicinal, secondary triethylsilyl ether, of which there are a surprisingly limited number of examples.

Finally, a new method for cardiolipin synthesis was developed. The method was inspired by the biosynthetic remodeling of cardiolipin and allows for one of the fatty acids to be incorporated later in the synthetic, making it ideal for precious or sensitive fatty acids.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

4-AT 4-amino-TEMPO

4-HNE 4-hydroxynonenal

9-LOX 9-lipoxygenase

Ac ..... acetyl

ATR ..... attenuated total reflectance

Bn ..... benzyl

Bu ..... butyl

CDP-DAG ..... cytosine diphosphate diacylglycerol

CDP-DGS ..... cytosine diphosphate-diacylglycerol synthase

CK ..... choline kinase

CL ..... cardiolipin

CPT ..... cytosine diphosphate-choline:diacylglycerol cholinephosphotransferase

CT ..... cytosine triphosphate:phosphocholine cytidylyltransferase

CTP ..... cytosine triphosphate

DAG ..... diacylglycerol

DCC ..... *N*,*N*'-dicyclohexylcarbodiimide

DDQ 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DHAP ..... dihydroxyacetone phosphate

$DHQD_2$(AQN)……dihydroquinidine (anthraquinone 1,4-diyl) diether

$DHQD_2$(PHAL)……..…dihydroquinidine 1,4-phthalazinediyl diether

DIPEA..... diisopropylethylamine

DMAP..... *N*,*N*'-dimethylaminopyridine

DMB 3,4-dimethoxybenzyl

DMF ..... dimethylformamide

DMT..... 4,4'-dimethoxytrityl

EDCI ..... 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

Et..... ethyl

FT-IR..... Fourier transform infrared spectroscopy

G3P glycerol-3-phosphate

GPL ..... glycerophospholipid

HEPES ..... 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid

HODE ..... hydroxyoctadienoic acid

HOMO ..... highest occupied molecular orbital

HpODE ..... hydroperoxyoctadienoic acid

HRMS ..... high-resolution mass spectrometry

*i*Pr..... isopropyl

IR..... infrared spectroscopy

KODE ..... ketooctadienoic acid

LC-MS ..... liquid chromatography-mass spectrometry

m-CPBA..... *meta*-chloroperoxybenzoic acid

Me ..... methyl

Mp ..... melting point

NADPH..... nicotinamide adenine dinucleotide phosphate

NHS ..... *N*-hydroxysuccinimide

NMR ..... nuclear magnetic resonance

PA ..... phosphatidic acid

PAP-1 ..... phosphatidic acid phosphatase-1

PC..... phosphatidylchloline

PE..... phosphatidylethanolamine

PG ..... phosphatidylglycerol

PGP ..... phosphatidylglycerol phosphate

PI..... phosphatidylinositol

PIS..... phosphatidylinositol synthase

PMB ..... *p*-methoxybenzyl

PPTS ..... pyridinium *p*-toluenesulfonate

PS ..... phosphatidylserine

PSD ..... phosphatidylserine decarboxylase

PSS1 ..... phosphatidylserine synthase-1

PSS2..... phosphatidylserine synthase-2

SAM..... *S*-adenosylmethionine

SOMO..... singly occupied molecular orbital

TBAF ..... tetrabutylammonium fluoride

TBS ..... *tert*-butyldimethylsilyl

*t*Bu..... *tert*-butyl

TDBPS ..... *tert*-butyldiphenylsilyl

TES ..... triethylsilyl

Tf..... trifluoromethylsulfonyl

THF ..... tertrahydrofuran

THP ..... tetrahydropyran

TMS ..... trimethylsilyl

Tr..... trityl

Ts..... *p*-toluenesulfonyl

AKNOWLEDGEMENTS

I would like to thank Professor Peter Wipf for granting me the opportunity to work in his lab. The training that I have received under his supervision has allowed me progress farther than I would have ever dreamed possible when I began my journey into higher education. Additionally, I thank Professors Seth Horne, Kazunori Koide, Valerian Kagan, and Kay Brummond for serving on various committees throughout my graduate career.

Additional thanks to Professor Wipf is due for attracting a wonderful group of colleagues for me to work with over the years. They have provided endless intellectually stimulation both in chemistry and many other topics that have forced me to examine life in ways that I doubt I would otherwise have considered. In particular, I would like to express my deepest gratitude to John Milligan, Joseph Salamoun, and Michael Kerner. Most people are lucky to make one friend in a lifetime on whom they can rely on no matter the circumstance, and I have made three in a single place over only a few years.

Finally, I would like to thank my family for the unconditionally support. My parents Nancy and Anthony laid the foundation for my academic success very early in life by nurturing my natural curiosity and tolerating the ever-present question “why?”. My wife Molly has now taken over tolerating that question and her unwavering love and companionship is the single most important reason I have come this far.

1.0 INTRODUCTION

1.1 OVERVIEW OF LIPIDS

Lipids are essential for life. Their amphiphilic nature and tendency to form micelles allows for cell membrane and organelle formation. In addition to their vital structural role, many lipids and their metabolites serve as signaling agents in critical cellular processes such as apoptosis[1] and mitophagy.[2] In particular, the mitochondrial glycerophospholipid cardiolipin (CL) plays an integral role in mitochondrial structure and in efficient operation of the electron transport chain. Additionally, it is intimately involved in the apoptotic program.

There are two broad classes of fatty acid containing lipids, namely sphingolipids (Figure 1) and glycerolipids (Figure 2), which contain the aminoalcohol sphingosine and triol glycerol as the backbone units, respectively. Sphingolipids are classified by the substitution at the amine and primary alcohol. Acylation of the amine with a fatty acid gives the ceramides. The fatty acids found in this class of lipids are generally longer (the most abundant lipids containing 24 carbons) and contain fewer polyunsaturated residues than glycerolipids. Additionally, α-hydroxyl fatty acids are often found in the ceramides, as well as other sphingolipids.[3]

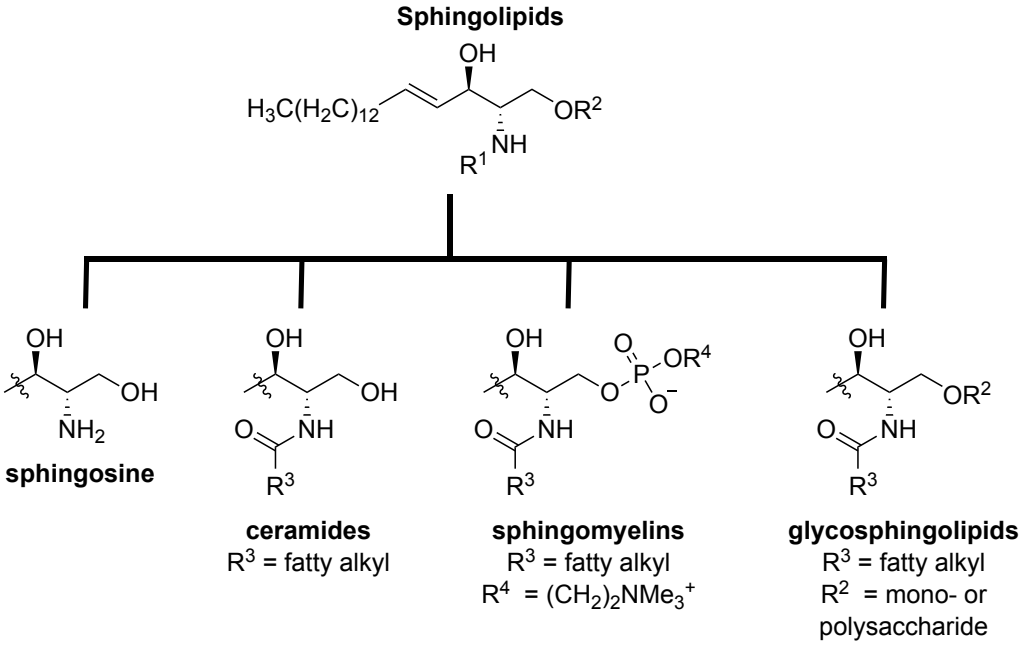


Figure 1: Major classes of sphingolipids.

Two different functionalizations of ceramide's primary alcohol lead to two additional classes of lipids. Glycosylation with a mono- or polysaccharide yields the glycosphingolipids. [4] Inclusion of a phosphate headgroup on the ceramides yields the sphingomyelins, and thus they can also be considered as phospholipids. These lipids share the same headgroup with the glycerophospholipid phosphatidylcholine (*vide infra*), and for some time, it was suspected that the former served primarily as a substitute for latter in membranes. However, the packing properties of the two classes are quite different, owing to the differing fatty acid composition of each (generally more unsaturated residues for phosphatidylcholine vs. more saturated for sphingomyelins) and the hydrogen bond donating capabilities that the amide provides.

Glycerolipids are the most abundant class of lipids and contain a three-carbon glycerol backbone (Figure 2). These can be further divided into two subclasses, namely the neutral mon/di/triacylglycerols and the charged (zwitterionic or anionic) glycerophospholipids. Glycerophospholipids (GPLs) can be categorized by two primary criteria: the substituents on the

glycerol backbone (–acyl, –alkyl, or –H, Figure 2) and the headgroup on the phosphate moiety (Table 1). Substitution on the backbone is derived from either fatty acids or fatty alcohols and gives rise to diacyl-GPLs or plasmalogens respectively. If no acyl or alkyl chain is present at one of the glycerol positions, the prefix *lyso*- is added to the parent name.

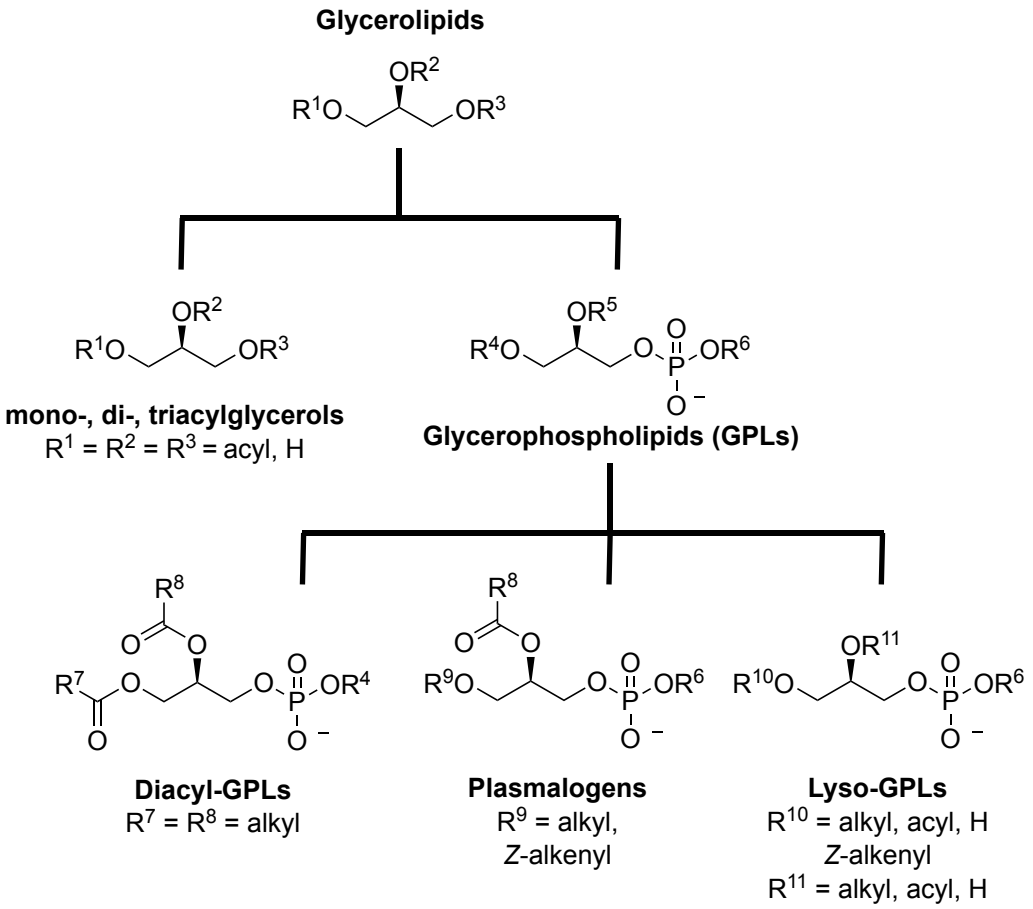
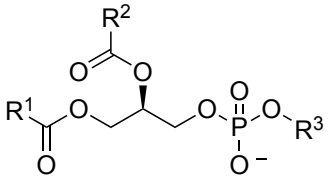


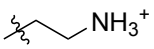
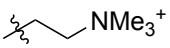
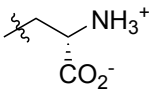
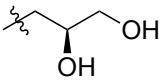
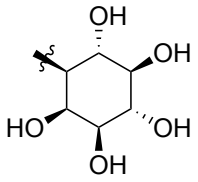
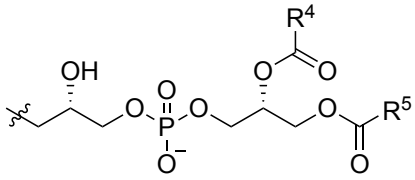
Figure 2: Major classes of glycerolipids. Only one of –R^4, –R^5 may be H in lyso-GPLs. See Table 1 below for phosphate headgroups (–R^6).

Diacyl-GPLs are the most abundant class of GPLs. The phosphate headgroup is commonly used when referring to a subclass of diacyl-GPLs, as this often determines a significant portion of the biological function (Table 1). Phosphatidic acid (PA) is structurally the simplest, with no additionally substitution on the phosphate (dianionic at physiological pH). There are three nitrogen-containing GPL headgroups, namely phosphatidylcholine (PC),

phosphatidylethanolamine (PE), and phosphatidylserine (PS), with PC and PE being the major membrane components in most cell types (Section 1.2).[5, 6] Phosphatidylglycerol (PG) and phosphatidylinositol (PI) both have alcohol-containing headgroups.

Table 1: Major phosphate headgroup structure of GPLs. $-R^1$, $-R^2$, $-R^4$, and $-R^5$ are alkyl, monounsaturated or polyunsaturated chains.



Name	R^1, R^2, R^4, R^5	R^3
Phosphatidic acid (PA)	alkyl or alkenyl	
Phosphatidylethanolamine (PE)	alkyl or alkenyl	
Phosphatidylcholine (PC)	alkyl or alkenyl	
Phosphatidylserine (PS)	alkyl or alkenyl	
Phosphatidylglycerol (PG)	alkyl or alkenyl	
Phosphatidylinositol (PI)	alkyl or alkenyl	
Cardiolipin (CL)	alkyl or alkenyl	

CL is unique among GPLs in that is contains three glycerol units, each containing a stereocenter. Two of these are 1,2-diacylglycerols (1,2-DAGs), giving CL the possibility of carrying up to four unique fatty acids. The two 1,2-DAG units are linked to the central glycerol by two phosphate groups that each carry a negative charge at physiological pH (pK_{a1} ~ 2.15,

pK_{a2} ~3.15).[7] These characteristic structural features are responsible for the distinctive effects of CL on membrane structure and protein binding.

1.2 STRUCURE OF LIPID MEMBRANES IN MITOCHONDRIA

The structure of biological membranes is governed by the complex interplay between lipid subclass abundance, fatty chain composition, and the effect of different lipid headgroups. The relative abundance of various classes of lipids can vary widely depending on cell type, organelle, and even between inner and outer layers (leaflets) of a particular membrane in some cases. Table 2 shows the percentage of total lipid content for various common classes of lipids.

Table 2: Percentage of total lipid composition by lipid subclass in mammalian cells.[8]

Name	% Total Lipids
Phosphatidylcholine	45-55
Phosphatidylethanolamine	15-25
Cholesterol	10-20
Phosphatidylinositol	10-15
Sphingomyelin	5-10
Phosphatidylserine	2-10
Cardiolipin	2-5
Glycosphingolipids	2-5
Phosphatidic acid	1-2

The fatty acids in lipids have a significant effect on membrane packing. Saturated chains tend to allow the formation of more densely packed gel phases that rigidify membranes and form a thicker bilayer. The *Z*-alkenes in mono- and polyunsaturated chains lead to varying degrees of increased membrane fluidity.[9] Polyunsaturated chains in particular lead to significantly decreased membrane thickness due to the conformational alterations that the multiple *Z*-alkenes

produce. These effectively introduce "packing defects" in the hydrophobic portion of the membrane relative to more ordered membranes composed of saturated chains. Monounsaturated chains introduce the defects closer to the center of membranes, whereas polyunsaturated chains provide shallower perturbations (Figure 3).[10] These defects allow for some proteins to recognize specific patterns in the lipid membrane.[11] Additionally, polyunsaturated lipids have been shown to aggregate preferentially in regions of high membrane curvature where the increased space between headgroups can be filled by the unsaturated chain's unique architecture.[10]

Figure 3: Illustration of the difference between deep defects (left, blue) and shallow defects (right, orange) induced in lipid membranes by mono- and polyunsaturated chains, respectively. Reproduced in part from *Phys. Chem. Chem. Phys.* 2015, 17, 15589-15597 with permission from the Royal Society of Chemistry; permission conveyed through Copyright Clearance Center.

GPL headgroups also impart their own unique effects on membrane structure. Different headgroups prefer different aggregation states and thus help determine the overall shape of biological membranes. GPLs like PC and PS have headgroups and hydrophobic tail regions that trace out a circle of similar radius and are thus referred to as "cylindrical" GPLs. These cylinders can pack together in an orderly fashion and tend to form flat bilayer structures (Figure 4). GPLs like PE have a headgroup that can be outlined by a circle with a smaller radius than the hydrophobic tails, which leads to a conically shaped lipid. These impart a negative curvature upon membranes, with the tendency to form inverted hexagonal phase (see Figure 5).[12] Lyso-GPLs and PIs show the opposite effect and lead to a positive curvature in membranes.

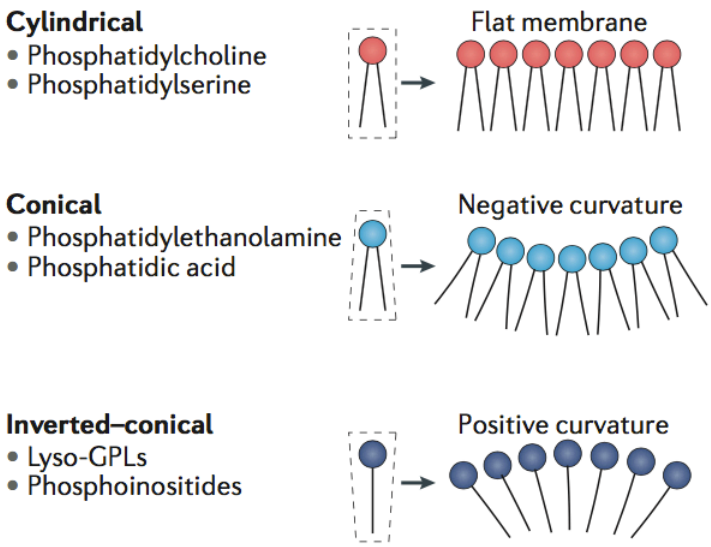


Figure 4: Effect of various phospholipids on membrane curvature. Reprinted with permission from *Nat. Rev. Mol. Cell Bio.* 2018, *19*, 281-296. Copyright 2018 Springer Nature; permission conveyed through Copyright Clearance Center.

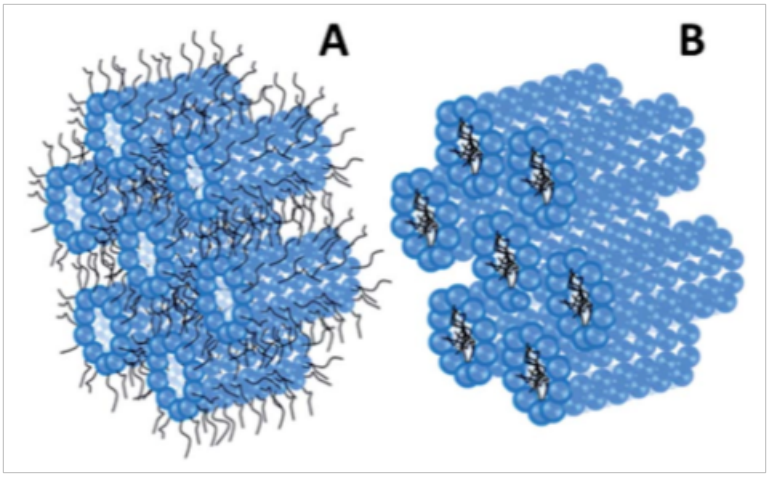


Figure 5: A: Inverted hexagonal phase in which the phosphate headgroups (blue) are oriented inwards and the hydrophobic tails (black) are outwards. B: Hexagonal phase with headgroups outwards and hydrophobic tails inwards. Reproduced in part from *Soft Matter* 2014, 10, 3978-3983 with permission from the Royal Society of Chemistry; permission conveyed through Copyright Clearance Center.

CL's unique dianionic structure and its tetra-acylated glycerol backbone impart a small headgroup and a large hydrophobic region. This correspondingly favors aggregation in an inverted hexagonal phase, though lamellar structures are possible.[13, 14] Additionally, CL's acyl

chains are commonly polyunsaturated and thus make it even more prone to forming a curved membrane environment (~70% of CL is tetralinoleoyl CL in humans).[15] CL's inherent bias for curved membranes is likely to contribute to its localization in the poles of bacterial membranes, as determined both computationally and experimentally.[16-18]

CL plays an important part in the structure of mitochondria. Mitochondria contain two membranes, of which CL is primarily confined to the inner mitochondrial membrane (See Figure 6 for mitochondrial membrane structure). The inner membrane contains an extensive network of inward folded tubules known as cristae. CL is found in the matrix-facing layer of the cristae with high concentrations at the most curved regions. Studies have shown that CL is essential for the proper formation of the mitochondrial cristae.

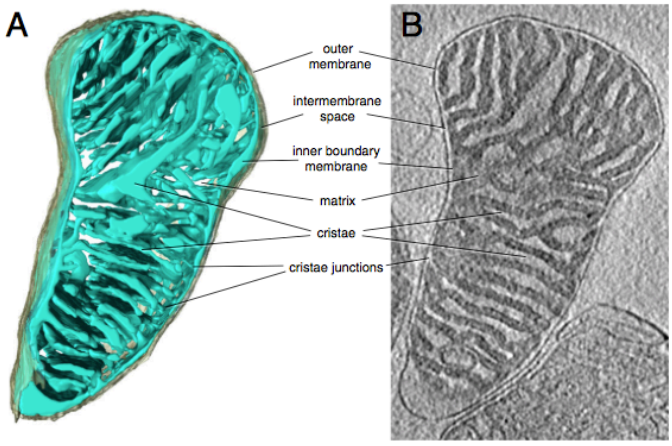


Figure 6: A: Three-dimensional cryo-electron tomography image of a mouse heart mitochondrion. Outer membrane = grey, inner membrane = cyan. B: A two-dimensional tomographic slice of the three-dimensional picture. Reproduced with permission from the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/); Originally by Werner Kühlbrandt from BMC Biology 2015, 13, 89, doi 10.1186/s12915-015-201-x.

Imbedded in the cristae is the electron transport chain, the assembly that gives mitochondria the often-used nickname "the powerhouse of the cell" (Figure 7). This series of protein complexes transfers electrons from NADPH and succinate to oxygen as the terminal

acceptor. During these transformations, a proton gradient is generated across the inner mitochondrial membrane, which ultimately drives oxidative phosphorylation and provides the cell with the majority of its ATP. CL plays an important role both in the function of individual complexes and in the higher order organization of multi-complex units known as supercomplexes. For example, it has been shown that there are 10 CLs that support normal activity of complex I, and that it promotes the association of complex III and complex IV by inserting into a hydrophobic cleft between the two complexes.[19, 20]

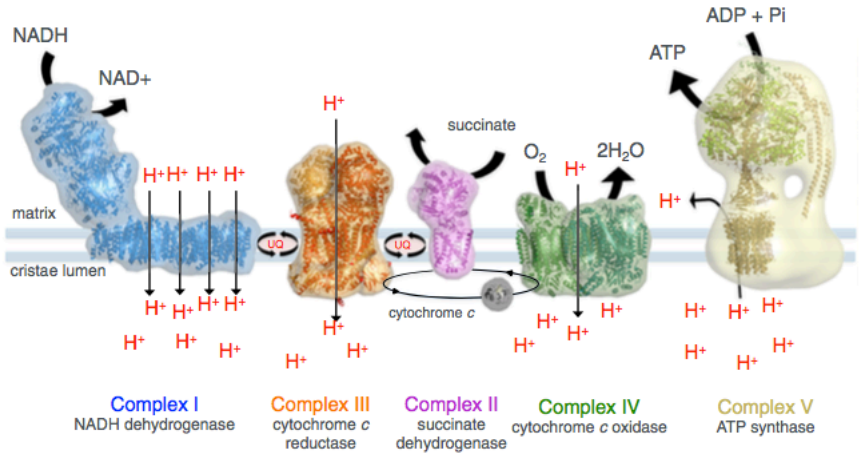


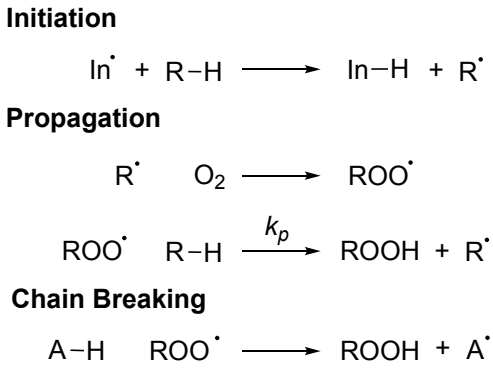
Figure 7: The electron transport chain. Reproduced with permission from the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/); Originally by Werner Kühlbrandt from BMC Biology 2015, 13, 89, doi 10.1186/s12915-015-201-x.

CL also plays a role in the degree of oligomerization of complex V dimers, which are primarily located at the cristae apex.[21] This is important not only for electron transport chain function, but also for the highly curved structure of the inner membrane, as complex V oligomers are thought to support normal cristae shape. Though CL has a preference for membrane regions of high curvature, individual cardiolipin molecules are not capable of supporting the membrane curvature alone, and larger aggregates of lipids and/or proteins are likely required.[22] In this respect, CL and complex V cooperatively enforce the native cristae structure.

1.3 OXIDATION CHEMISTRY OF LIPIDS

Cells must maintain a delicate balance to survive: they exist in an oxygen-rich environment and require oxidation chemistry for energy production, yet most major classes of biomolecules are susceptible to oxidative damage. Cells posses defenses against oxidative damage, such as the abundant antioxidant glutathione, but these can be overwhelmed for a variety of reasons. Notably, lipid oxidation is a key component of two types of cell death, namely apoptosis and ferroptosis. There is also significant evidence for oxidative damage to lipids in many diseases including cancer,[23] Alzheimer's,[24] Parkinson's,[25] diabetes,[26] and atherosclerosis.[27]

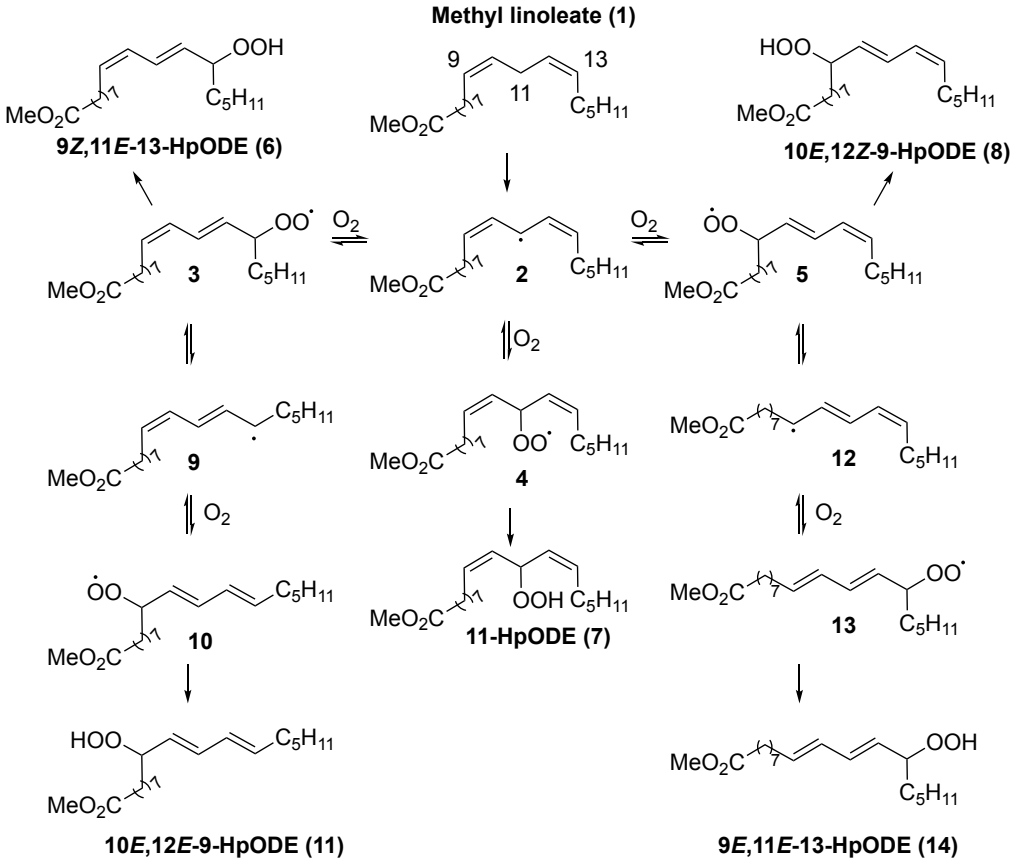
Free radical mediated oxidation of lipids, or lipid peroxidation, takes place via a typical radical chain mechanism (Scheme 1).[28] Initiators of the process in mitochondria often trace their source to electron leakage from the electron transport chain.[29] These leaked electrons form the superoxide radical anion ($O_2^{\bullet-}$), which is rapidly reduced to H_2O_2 by superoxide dismutases. H_2O_2 can subsequently act as a source of hydroxyl radicals that can initiate lipid peroxidation. The *bis*-allylic positions of polyunsaturated fatty acid-containing lipids are the most susceptible to oxidation due to the highly stabilized radicals that are generated and their comparatively weak C–H bond strength (~80 kcal/mol for *bis*-allylic C–H vs ~90 kcal/mol for allylic C–H).[30] The resulting allylic radicals react with oxygen nearly at the diffusion limit,[31] leading to peroxyl radicals in the first propagation step. These radicals are the chain carriers of the process and undergo rate limiting hydrogen atom abstraction to form hydroperoxides and an additional stabilized carbon-centered radical. The lipid peroxidation radical chain can be broken through the action of cellular antioxidants, such as glutathione. The precise fate of the resulting radical depends on the nature of the antioxidant.[29, 32]



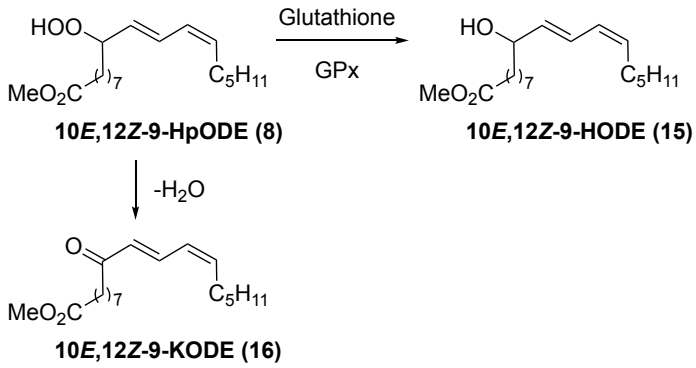
Scheme 1: Radical chain mechanism for lipid oxidation. In = initiator, k_p= rate constant for the rate-limiting step of propagation, A = cellular antioxidant.

There is a wide array of compounds that can be isolated from the nonspecific peroxidation of lipids, with the number of possible products increasing as the number of unsaturations increases in a given chain. There are two categories of products: primary peroxidation products, which consist of hydroperoxides, and secondary products that have undergone further transformations. The primary oxidation products of methyl linoleate **1** are shown in Scheme 2.[33] Hydrogen atom abstraction leads to a pentadienyl radical **2**, which can undergo a reversible reaction with oxygen at C13, C11, or C9 resulting in peroxyl radicals **3**, **4**, and **5** respectively. Hydrogen atom abstraction by the peroxyl radicals leads to the kinetic products 9*Z*,11*E*-13-hydroperoxyoctadienoic (HpODE) methyl ester **6**, 11-HpODE methyl ester **7**, and 10*E*,12*Z*-13-HpODE **8**. **4** is highly prone to reforming the pentadienyl radical **2** and can only be converted to **7** in the presence of high concentrations of α-tocopherol.[34] The corresponding thermodynamic products **11** and **14** (containing all *E*-alkenes) are also obtained and result from a similar pathway in which the reversible addition of oxygen to **2** allows for double bond isomerization. Two possible secondary products are shown in Scheme 3, resulting from either the reduction of hydroperoxide **11** by glutathione/glutathione peroxidase (10*E*, 12*Z*-9-hydroxyoctadecadienoic acid; HODE) or dehydration (10*E*, 12*Z*-9-ketooctadecadienoic

acid; KODE). While these reactions are shown with methyl linoleate, related reaction pathways can occur in more biologically relevant esters, such as those found in GPLs.

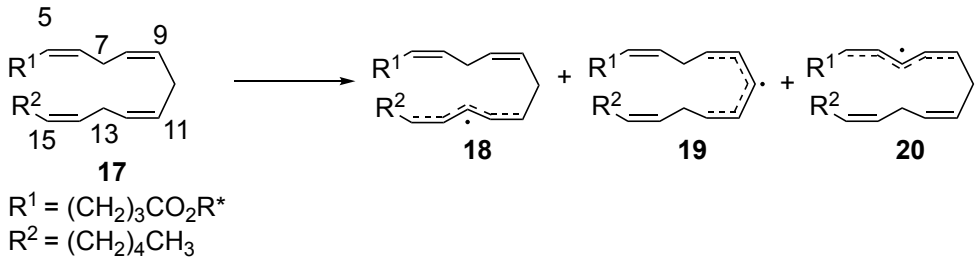


Scheme 2: Primary oxidation products of methyl linoleate.



Scheme 3: Two secondary oxidation products of linoleic acid. GPx = glutathione peroxidase.

In lipids with greater degrees of unsaturation than linoleic acid esters, such as those containing arachidonic acid esters, an even larger array of peroxidation products are possible.[28] Hydrogen atom abstraction can occur at C13, C10, or C7, resulting in pentadienyl radicals **18-20** (Scheme 4). Each of these can undergo a reaction with oxygen analogous to that shown in Figure 10 with methyl linoleate, as well as more complicated pathways.



Scheme 4: The three pentadienyl radicals derived from arachidonic acid and its esters.[35] R* is most commonly a glycerol unit.

1.4 OXIDATIVE DAMAGE OF CARDIOLIPIN AND APOPTOSIS

CL's location in the mitochondrial inner membrane predisposes it to oxidative damage even during normal cellular function, where an estimated 1-4% of the electrons in the electron transport chain result in the superoxide radical.[36] Additionally, it is also directly associated with

several of the complexes, which places it in close proximity to significant sources of oxidizing equivalents. CL's high polyunsaturated fatty acid content further exacerbates its susceptibility to oxidative damage.

Despite its close proximity to the electron transport chain, ~80% of the inner mitochondrial membrane is other GPLs and yet, under pro-apoptotic conditions, CL is preferentially oxidized over these more abundant lipids. For example, in model traumatic brain injury conditions (controlled cortical impact), CL was found to be the major phospholipid class that had undergone oxidation 3h following injury (Figure 8).[37] This is suggestive of an enzymatic pathway, and indeed, a CL-cytochrome *c* peroxidase complex has been found to preferentially oxidize CL under these and other pro-apoptotic conditions.[38, 39] The complex utilizes H_2O_2 as the oxidant, but can also use lipid hydroperoxides as well.[40, 41]

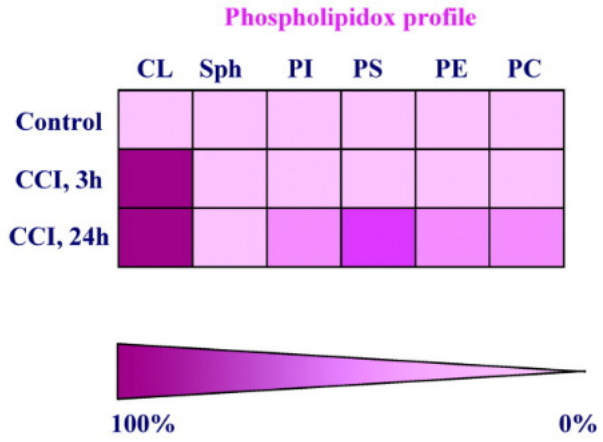


Figure 8: Phospholipid oxidation in control, 3h, and 24h following controlled cortical impact (determined by HPLC). "100%" is 110 ± 20 pmol phospholipid hydroperoxide per nmol of phospholipid. Approximate percentage of total phospholipids for each class are as follows: CL ~ 2%, Sph (sphingomyelin) ~ 1.5%, PI ~ 3%, PS ~ 12.5%, PE ~ 37%, PC ~ 43%. Reproduced in part from *Ann Neurol.* 2007, 62, 154-169 with permission from the Wiley Online Library; permission conveyed through Copyright Clearance Center.

Following oxidation of CL, oxygenated fatty acids are released by the action of calcium-independent phospholipase A_2 (iPLA$_2\gamma$).[39] The released oxygenated fatty acids are well

known lipid mediators in some cases, such as 9-HODE, 13-HODE, and their corresponding ketone derivatives. This has led to the suggestion that CL may function as a mitochondrial reservoir for lipid signaling agents and would explain the greater diversity of CL species found in tissues with important roles in signaling, such as the brain.

Given the integral role that oxidative damage to CL plays in the apoptotic program and its implication in a wide variety of diseases,[42-45] there has been significant interest in developing methods to prevent CL oxidation. Inhibitors of this process must be able to penetrate into the mitochondria and thus must possess unique properties. Many strategies for targeting mitochondria have been developed, only two of which will be presented here.[46, 47] The first involves the use of lipophilic cations.[48] Due to the pumping of protons into the intermembrane space by the electron transport chain, there is an electrical gradient across the inner membrane of about 140-180 mV. Lipophilic cations can pass through membranes, and as such, tend to localize in the matrix. The most prominent examples are based on triphenylphosphonium tagged antioxidants. High doses lead to mitochondrial membrane depolarization, which can alter ATP production and interfere with the transport of other cations across the membrane. Thus, all triphenylphosphonium-based anitoxidants suffer from dose-limiting toxicity.[49] The antioxidants themselves can act on a variety of reactive oxygen species, including superoxide, hydrogen peroxide, hydroxyl radicals, and lipid hydroperoxyl radicals (Figure 9).

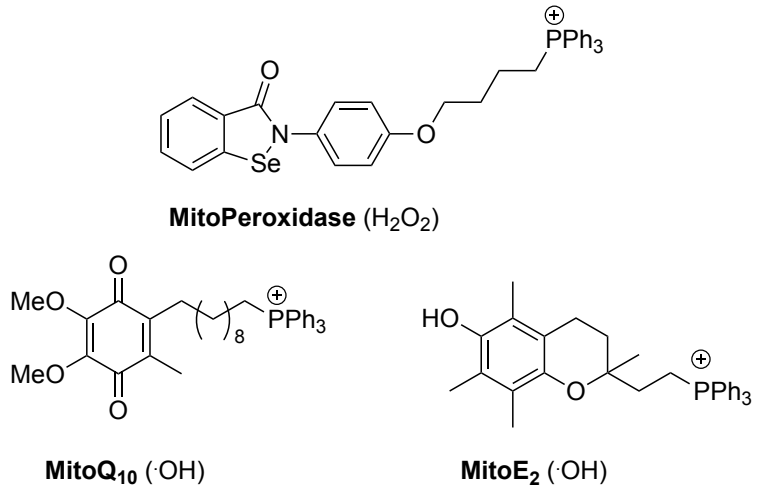
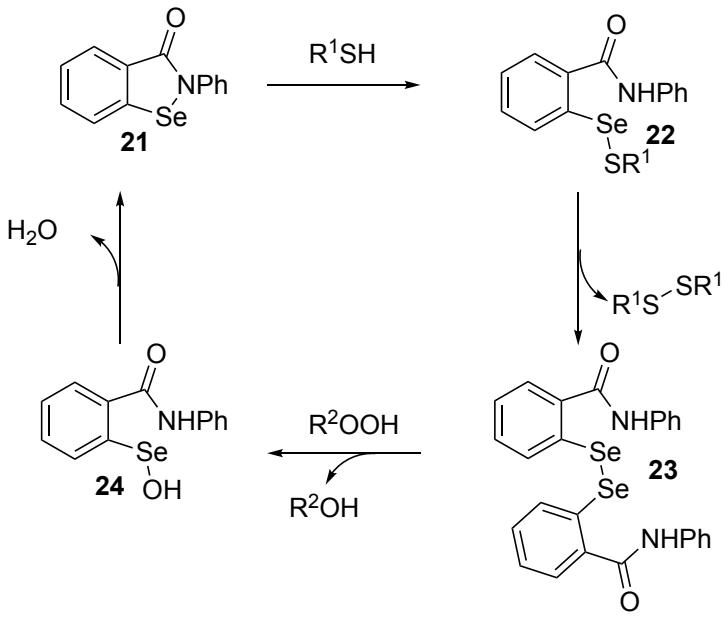


Figure 9: Mitochondria-targeted antioxidants based on triphenylphosphonium salts. The primary reactive oxygen species targeted by each is in parentheses.

$MitoE_2$ and $MitoQ_{10}$ are radical chain-breaking hydrogen atom donors and are particularly effective for lipid hydroperoxyl radicals ($MitoQ_{10}$ requires reduction to the active ubiquinol form by complex II of the electron transport chain).[48] Their lipophilic nature allows for them to remain embedded in the mitochondrial inner membrane in close proximity to where lipid hydroperoxides are formed. MitoPeroxidase is thought to operate through a more complicated mechanism based on information from the parent compound ebselen (**21**; originally designed as a glutathione peroxidase mimetic; see Scheme 5).[50] **21** readily reacts with cellular thiols to give adduct **22**. This species disproportionates providing diselenide **23**, which acts as the active reducing agent for cellular peroxides. The resulting selenic acid **24** can reform **21** with the loss of water.



Scheme 5: Proposed mechanism of action for ebselen. R^1SH = cellular thiols such as glutathione or cysteine residues of proteins. R^2 = H, alkyl.

A second method for targeting antioxidants to the mitochondria is based on naturally occurring peptides. The SS peptides developed by Szeto and Schiller are one example of this.[51, 52] These compounds were developed from dermorphin, a μ opioid receptor agonist (Figure 10).[53] Subsequently, these compounds were found to preferentially localize in the mitochondrial inner membrane, though they do not cause membrane depolarization.[52, 54] Development ultimately led to SS-31, which uses a dimethyltyrosine as the active antioxidant for prevention of CL oxidation. Like $MitoE_2$, this compound functions as a chain-breaking hydrogen atom donor.

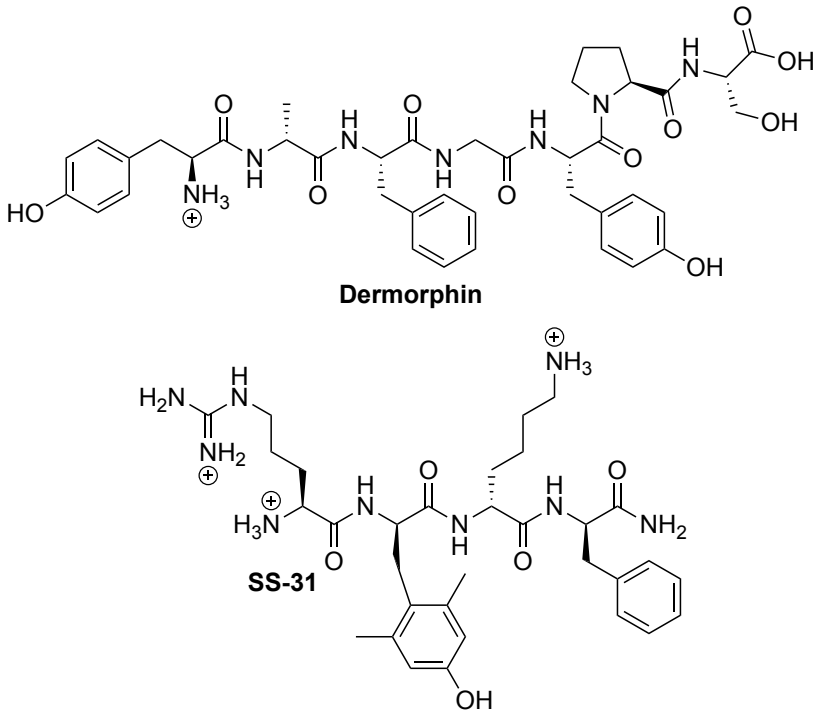


Figure 10: Structures of Dermorphin and SS-31.

A second class of peptide-based targeting agents is based on the antibiotic Gramicidin S and the stable radical 4-amino-TEMPO (4-AT) developed by Wipf and coworkers (Figure 11). Gramicidin S is a cyclic peptide whose rigid secondary structure is stabilized by four intramolecular hydrogen bonds. Its antibiotic effect stems from the disruption of bacterial cell membranes and it also possesses the ability to perturb mitochondrial membrane potential.[55, 56] These properties suggested that Gramicidin S or a fragment that retained the type-II' β turn (D-Phe-Pro) could be an effective mitochondria targeting agent for an antioxidant payload. 4-AT was selected as the antioxidant due to its ability to undergo recycling and superoxide dismutase mimetic activity.[57, 58] Analogs that did not contain the type-II' β turn (D-Phe-Pro) showed markedly lower mitochondrial localization.[59] Additionally, an (*E*)-alkene was used as an effective isostere for one of the peptide bonds and can presumably lead to better bioavailability through reduced sensitivity to proteases.[46, 60, 61] This compound has found applications in several

conditions in which preventing CL oxidation reduces apoptotic cell death, including hemorrhagic shock, traumatic brain injury, and Huntington's disease.[62-64] A truncated analog (JP4-039) that maintains significant mitochondrial targeting ability was also developed and has been used as a radioprotective and radiomitigative agent (Figure 12).[65-67]

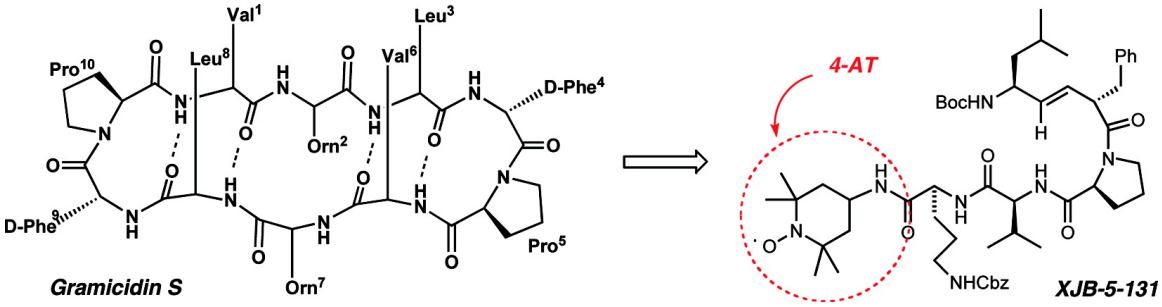
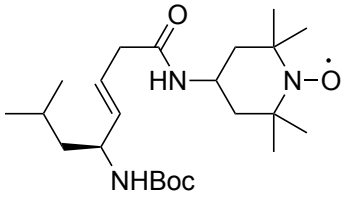


Figure 11: Structures of Gramicidin S and XJB-5-131. Reproduced with permission from *Acc. Chem. Res.* 2008, *41*, 87-97. Copyright 2008 American Chemical Society.



JP4-039

Figure 12: Structure of JP4-039.

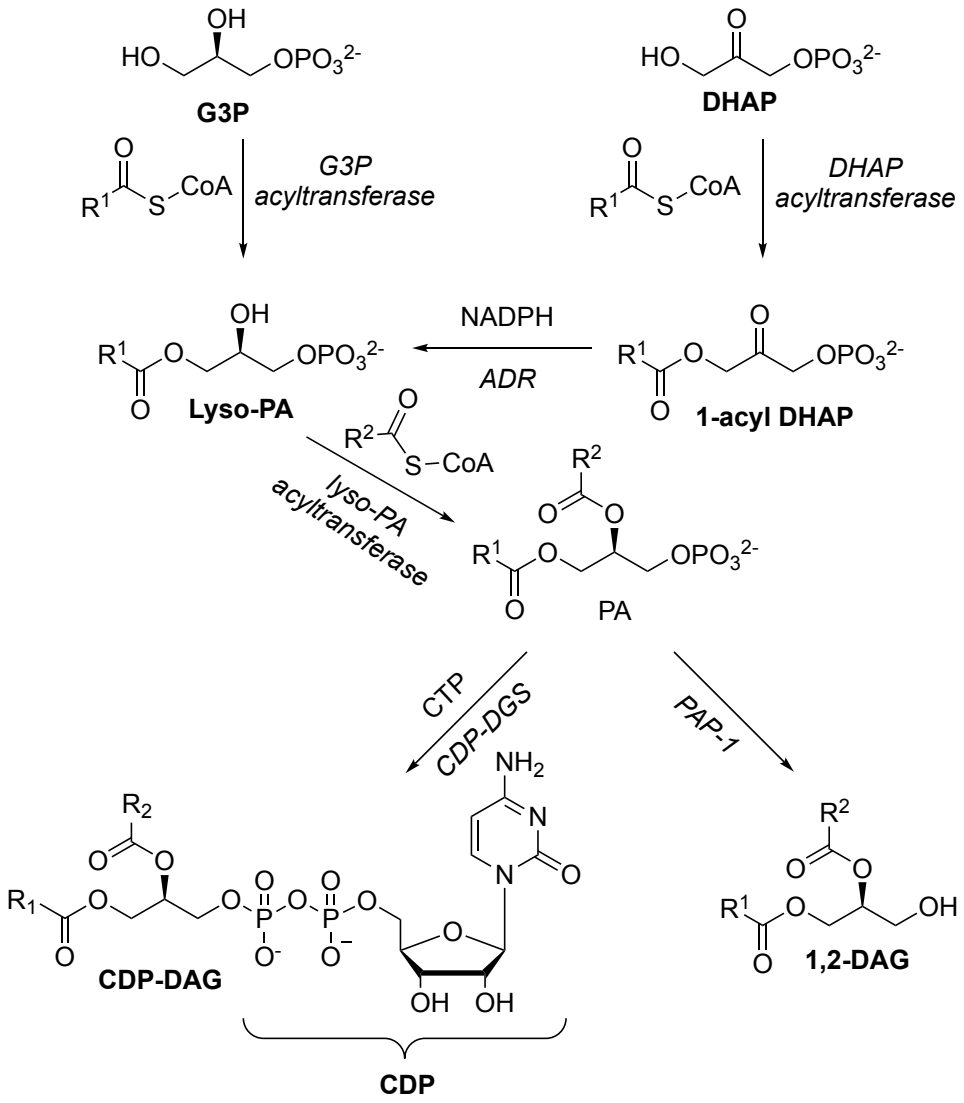
1.5 SYNTHESIS OF GLYCEROPHOPHOLIPIDS

1.5.1 Cardiolipin Biosynthesis

GPL biosynthesis in mammals is centered on two key intermediates, namely cytidine diphosphate diacylglycerol (CDP-DAG) and 1,2-DAG, both of which are utilized in CL

biosynthesis (Scheme 6).[68] The first step in CDP-DAG synthesis is the acyl-CoA dependent acylation of glycerol-3-phosphate (G3P) at the *sn*-1 (*s*tereospecific *n*umbering nomenclature is based on Fischer projection of glycerol with central hydroxyl group to the left; see Figure 13) position by G3P acyltransferase and yields lyso-phosphatidic acid (lyso-PA). Lyso-PA can subsequently be acylated by lyso-PA acyltransferase and generates PA. Phosphorylation or dephosphorylation of PA by CDP-DAG synthase (CDP-DGS) or PA phosphatase-1 (PAP-1) gives CDP-DAG and 1,2-DAG, respectively. The synthetic logic of the dephosphorylative route to 1,2-DAG has been reflected in a number of chemical syntheses (*vide infra*).

An alternative reductive pathway to lyso-PA utilizes dihydroxyacetone phosphate (DHAP) as the starting point, and to which no analogous synthetic route has been developed.[69] DHAP is acylated in an acyl-CoA dependent reaction catalyzed by DHAP acyltransferase. NADPH-dependent reduction by 1-acyl-DHAP reductase (ADR) completes the second pathway to lyso-PA.



Scheme 6: Biosynthetic route to key intermediates of GPL synthesis in mammals.

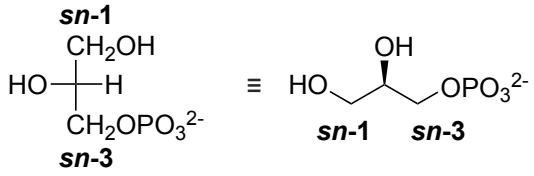
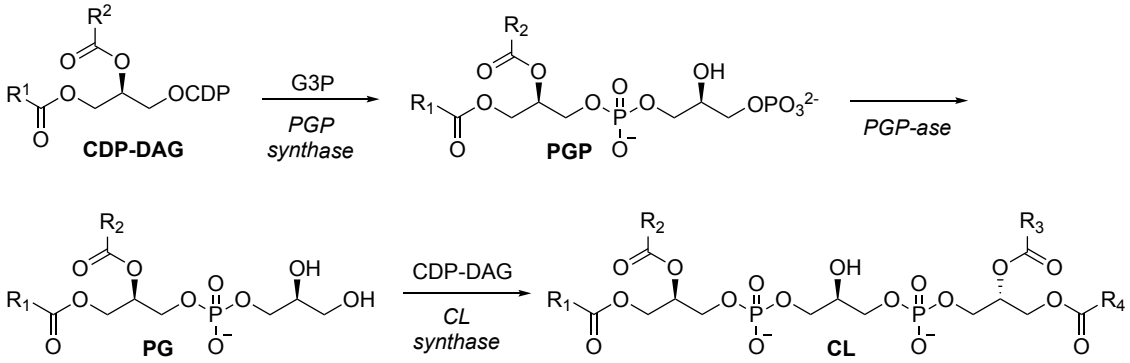


Figure 13: Stereospecific numbering nomenclature using G3P as an example.

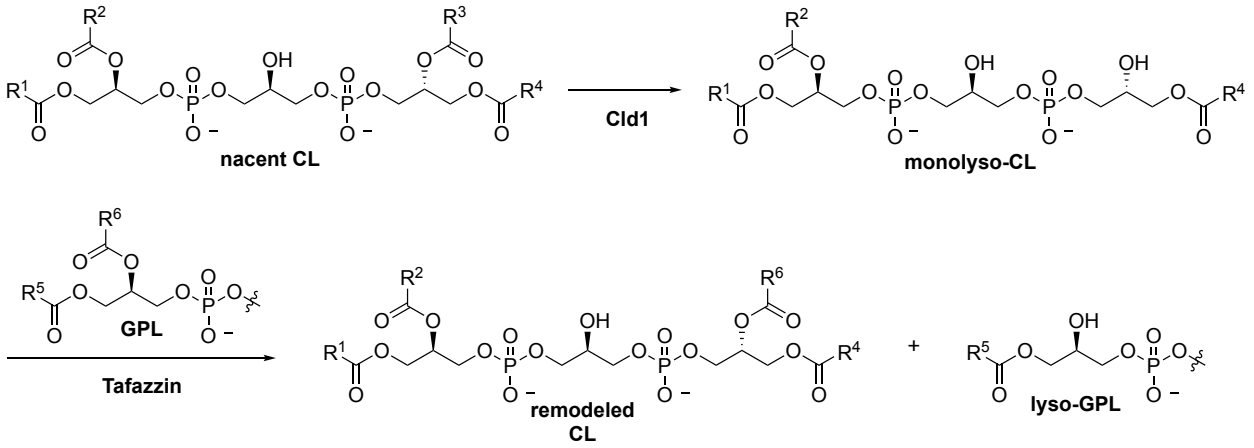
CL biosynthesis takes place exclusively in the mitochondrial inner membrane where CL primarily resides (Scheme 7).[70] The first step towards CL involves the reaction of CDP-DAG with G3P catalyzed by PG glycerol phosphate synthase (PGP synthase). Subsequent

dephosphorylation by phosphatidylglycerolphosphatase (PGP-ase) results in PG. The final step in the assembly of the parent CL architecture is the incorporation of a second PA residue via the reaction of PG with a second equivalent of CDP-DAG.



Scheme 7: Biosynthesis of CL in mammals.

The CL that results from CL synthase undergoes further modifications, termed "remodeling" of the fatty acid composition (Scheme 8). The first step is hydrolysis of one of the acyl chains, resulting in a monolyso-CL. Subsequently, a fatty acid is transferred from a donor GPL (PC, PE, etc.) and yields a tetra-acylated-CL and lyso-GPL. This reaction is catalyzed by the acyltransferase tafazzin in both yeast and higher eukaryotes. The selectivity of this process is of interest because nascent CL and remodeled CL have markedly different fatty acid composition.[71] Additionally, there is a vast difference in the CL species composition in different tissue types and it is unknown how this diversity is regulated. For example, tetra-linoleoyl-CL predominates in heart muscle,[72] whereas there is a much greater occurrence of higher order polyunsaturated fatty acids in the brain.[73]



Scheme 8: CL remodeling in yeast. The donor GPL can is most commonly PC or PE.

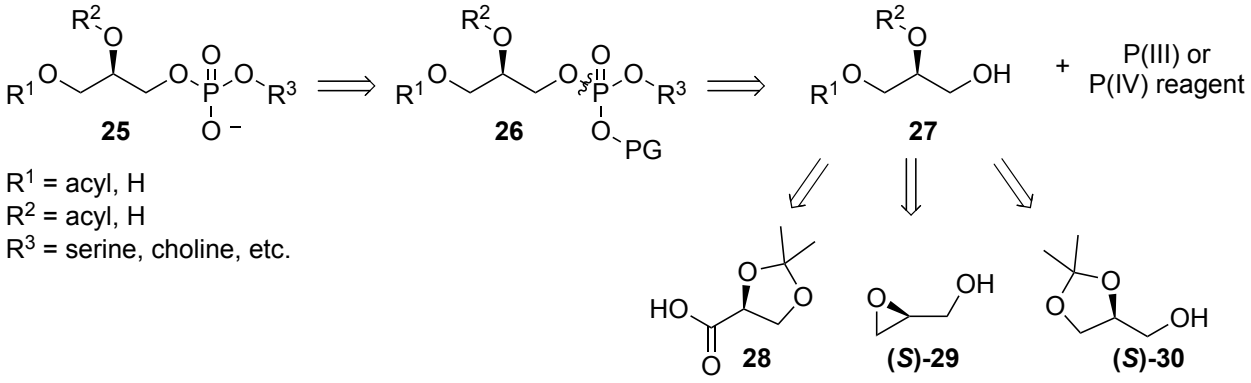
S. cerevisiae (yeast) is a useful organism for studying CL remodeling, as it only utilizes a single enzyme for each of the remodeling steps, whereas there are multiple enzymes capable of acylating CL in mammalian cells.[44] There is strong evidence that Cld1, a cardiolipin-specific phospholipase, is responsible for the first step in CL remodeling.[71, 74] Originally, this was described by Leber and coworkers and they found that Cld1 shows a preference for the cleavage of palmitic acid residues, though no direct evidence was provided.[71] This may provided a partial explanation for the transition from more saturated CLs prior to remodeling to a higher content of unsaturated fatty acids afterwards.

Further interest in CL remodeling is generated by Barth syndrome, in which patients show significantly altered CL composition. Defects in the tafazzin gene lead to impaired reacylation, and thus accumulation of monolyso-CL. This results in a collapse of the heavily folded nature of the inner mitochondrial membrane due to CL's pivotal role in supporting this architecture.[75, 76] Impaired electron transport chain function has also been observed, likely due to CL's role in proper electron transport chain organization.[43, 77] An improperly functioning electron transport chain does not effectively consume NADH and $FADH_2$, which further exacerbates poor energy production by feedback inhibition of other mitochondrial pathways.[78]

The collective result of these abnormalities is that patients experience an enlarged heart that inefficiently circulates blood (dilated cardiomyopathy), exercise intolerance, low white blood cell counts (neutropenia), and in rare severe cases, death.[43, 44]

1.5.2 General Retrosynthesis for Glycerophospholipids

Given the great significance and large structural diversity of GPLs, they have attracted the attention of the synthetic community for many years. Though variations exist, most GPL syntheses follow a similar retrosynthetic outline (Scheme 9). GPLs are most commonly manipulated with a protected phosphate (**26**) to facilitate isolation and purification. If a lyso-GPL (R^1 or R^2 = H) is desired, there will be an additional protecting group involved at the requisite position. Intermediate **26** can be prepared from the appropriate glycerol precursor and a phosphorus (III) or phosphorus (V) coupling reagent that contains the desired phosphate protecting group (*vide infra*). The most laborious facet of phospholipid synthesis is the assembly of the requisite glycerol fragment(s), which is usually derived from L-glyceric acid (**28**), (*S*)-glycidol (**(*S*)-29**), or (*S*)- solketal (**(*S*)-30**).



Scheme 9: Overview of GPL retrosynthesis.

1.5.3 Phosphate Assembly in GPLs

The protecting group scheme governs much of the chemistry at phosphorus during GPL synthesis. Figure 14 shows the most commonly used protecting groups. The methyl, benzyl, and cyanoethyl groups (**31, 33, 34**) have found wide application in a variety of GPLs,[79-82] whereas the others are more limited.

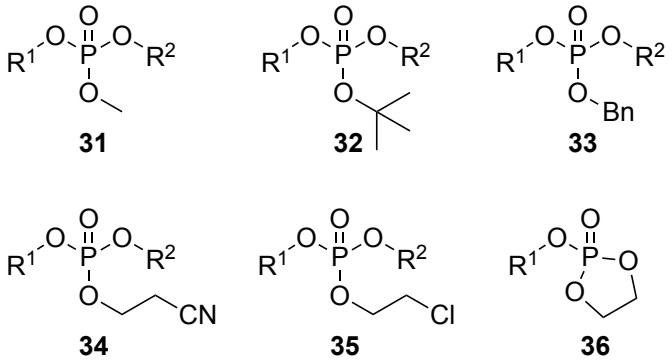
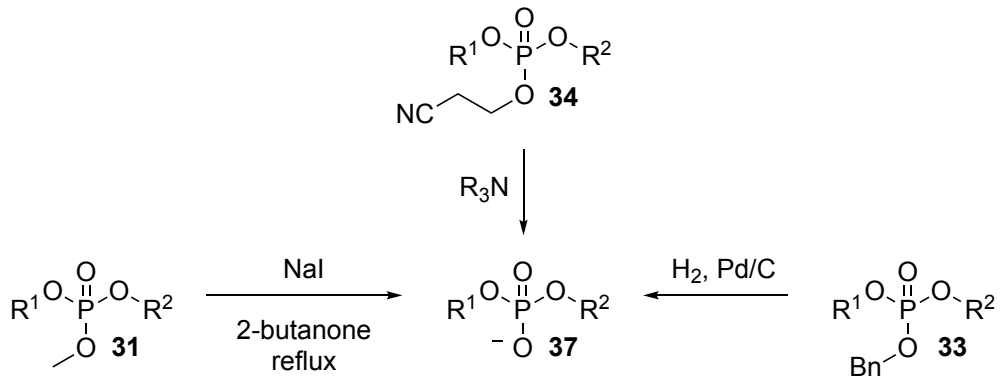


Figure 14: The most common phosphate protecting groups used in phospholipid synthesis.

Scheme 10 shows common conditions used for the removal of the most broadly utilized phosphate protecting groups. Methyl phosphates such as **31** are most often cleaved with sodium iodide in refluxing 2-butanone.[82] This method is convenient in that it directly prepares the GPL sodium salt without the need for cation exchange. Trimethylsilyl bromide has also been used

occasionally. The conditions were originally developed for the cleavage of dialkylphosphonates[83] and subsequently first applied to phospholipid synthesis for the demethylation of fluorinated 2-acyl-lysophosphatidic acid derivatives.[84]



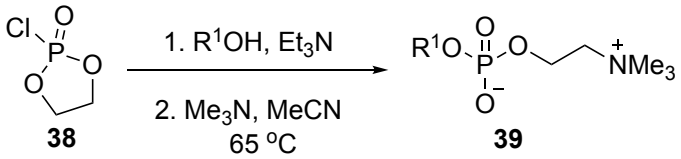
Scheme 10: Common cleavage conditions for the most widely applied phosphate protecting groups.

Benzyl phosphates **33** can be cleaved using standard hydrogenolysis conditions.[85] This is a facile method, but its use precludes the preparation of unsaturated GPLs due to competing olefin reduction. While they are stable to a wide variety of conditions, benzyl phosphates can be cleaved under prolonged nucleophilic reaction conditions.[86]

Arguably, the most universal phosphate protecting group is the cyanoethyl, which has found extensive use in oligonucleotide synthesis.[87, 88] However, its application to phospholipids has been less widespread. Cleavage occurs under mildly basic conditions, most often using trialkylamines or ammonia (Scheme 10, **34** to **37**).[89] These conditions tolerate a wide variety of functionality, most notably the skipped dienes in polyunsaturated fatty acid chains. Cyanoethyl protecting groups have been successfully applied to the synthesis of some of the more complex GPLs including lysobisphosphatidic acid,[79, 90] cardiolipin,[91, 92] and phosphoinositides.[93].

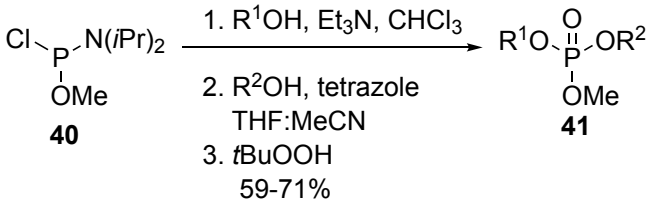
The first protocols that were developed for GPL synthesis were based on P(V) reagents. There has been a large body of work amassed on the topic,[94] but these methods have largely been supplanted by the use of P(III) reagents. A versatile method that continues to find applications is

the dioxaphospholane **38** developed by Hadju (Scheme 11).[95] First, substitution with the desired glycerol fragment leads to a protected phosphate. This intermediate dioxaphospholane can then undergo a nucleophilic ring opening with anhydrous trimethylamine leading to PCs (**39**) or with various nitrogen, oxygen, and sulfur nucleophiles to access other analogs.



Scheme 11: Use of dioxaphospholane 38 in PC synthesis.

The original impetus for the development of P(III) reagents for phospholipid synthesis was their great success in nucleotide synthesis.[96] In 1986, Stec and co-workers developed the first method broadly applicable to prepare phospholipids using P(III) reagents (Scheme 12).[97] Alcohols undergo monosubstitution under basic conditions with chlorophosphoramidite reagent **40**. The intermediate phosphoramidite can then be coupled to the desired alcohol using tetrazole as the promoter. Oxidation using *tert*-butyl hydroperoxide (*t*-BuOOH) led to the synthesis of protected PCs, PEs, and PGs. This method could also be used to prepare phosphorothioyl analogs, which had previously been used in mechanistic studies of phospholipases,[98, 99] by replacing *t*-BuOOH with elemental sulfur.

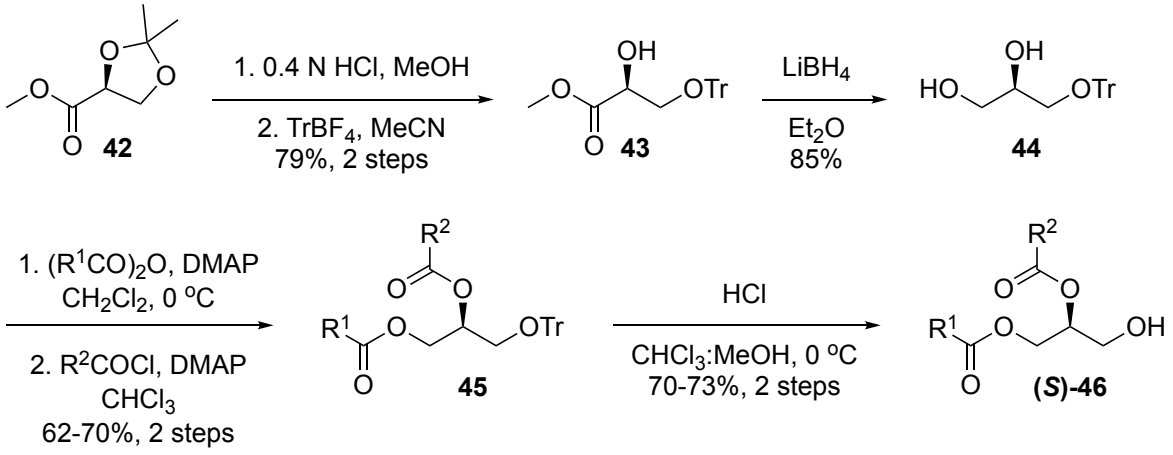


Scheme 12: The original chlorophosphoramidite coupling developed by Stec.

The closely related phosphordiamidite (replace -Cl with -N(*i*Pr)$_2$ in **40**) reagents were applied to a triphosphoinositide two years later.[100] Both phosphoramidite methods have become the preferred P-O bond formation strategies in the more complex phospholipids.

1.5.4 Functionalized Glycerol Precursors

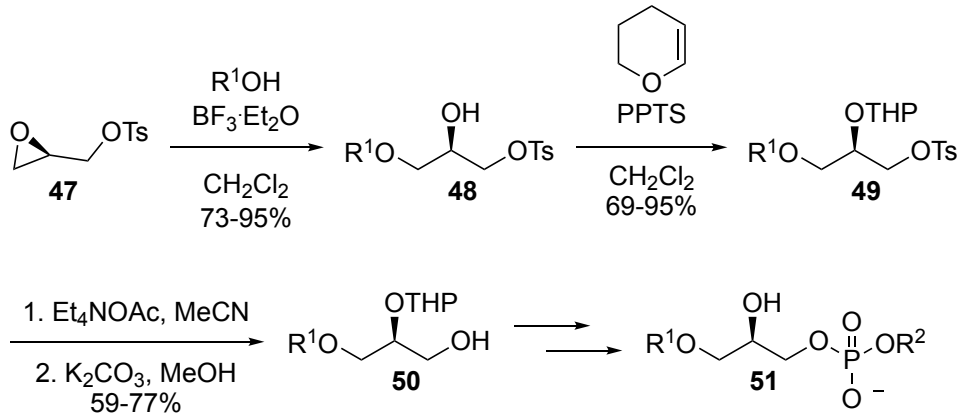
The most common functionalized glycerol precursors are derived from the chiral pool. Hadju and coworkers developed a synthesis of 1,2-diacylglycerols beginning with commercially available protected L-glyceric acid derivative **42** (Scheme 13).[101] Cleavage of the isopropylidene using dilute HCl in methanol followed by selective trityl (Tr) protection of the primary alcohol gave **43** in 79% yield over two steps. Subsequent reduction using $LiBH_4$ gave diol **44** in 85% yield. Sequential, selective acylations could be performed to install the desired fatty acid chains in **45**. While this method provides good yields, it requires two equivalents of the fatty acid for the selective acylation of the primary alcohol and thus is not ideal for precious fatty acids. Finally, the trityl group could be cleaved without acyl migration using HCl in a chloroform:methanol mixture at 0 °C.



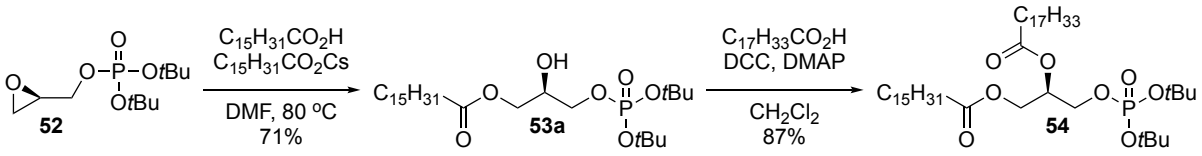
Scheme 13: 1,2-DAG synthesis derived from L-glyceric acid developed by Hadju.

Glycidol derivatives are the second category that has commonly served as functionalized glycerol precursors. Originally, the desired enantiomer could be accessed from the asymmetric epoxidation of allyl alcohol,[102] but both are now commercially available. (*R*)-Glycidyl tosylate **47** has been used in the preparation of ether phospholipids (Scheme 14).[103] Selective opening of

the epoxide with alcohols promoted by $BF_3{\cdot}Et_2O$ proceeded in good to excellent yields. $BF_3{\cdot}Et_2O$ was found to be advantageous over $TiCl_4$ or $Ti(OiPr)_4$, as competitive substitution of the tosylate can occur with chloride or isopropoxide ions.[104] THP protection of **48** gave protected glycerol **49**. The tosylate could be displaced using tetraethylammonium acetate (Et_4NOAc) in acetonitrile, though rigorous exclusion of moisture was essential for good yields. Subsequent acetate hydrolysis using potassium carbonate in methanol resulted in alcohol **50**. The THP was cleaved following the incorporation of the phosphate leading to ether GPLs **51**.



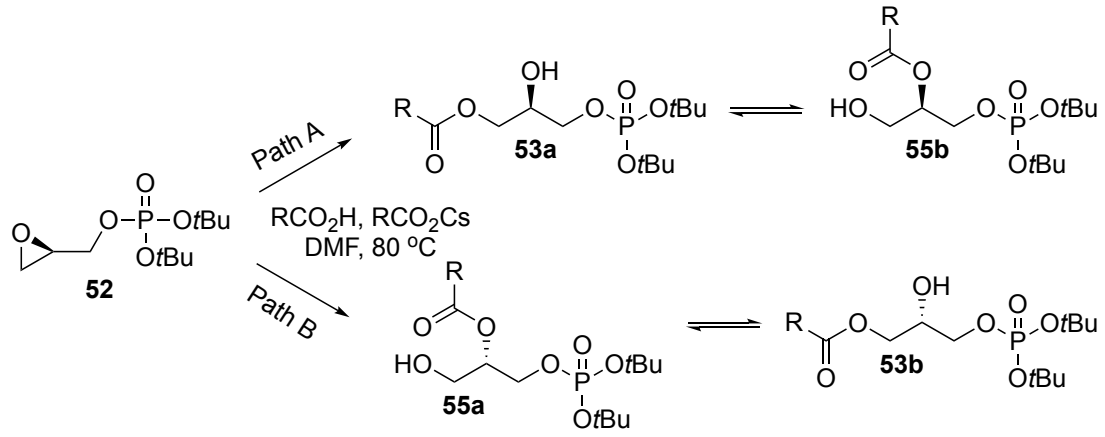
Scheme 14: Preparation of functionalized glycerol 31 used in ether phospholipid synthesis.



Scheme 15: Use of phosphorylated glycerol 52 in GLP synthesis.

Konradsson demonstrated that epoxide **52** could be opened regioselectively using a mixture of palmitic acid and its cesium salt in DMF at 80 °C (Scheme 15).[86] A minor (undisclosed) amount of the undesired isomer was also obtained. This could be obtained from either a 1,2-acyl shift (path A, Scheme 16) or epoxide opening at the secondary carbon of the epoxide (path B, Scheme 16). The undesired isomer **55b** was presumed to be from path A due to the facility with which epoxide opening occurs at the primary carbon. This hypothesis was

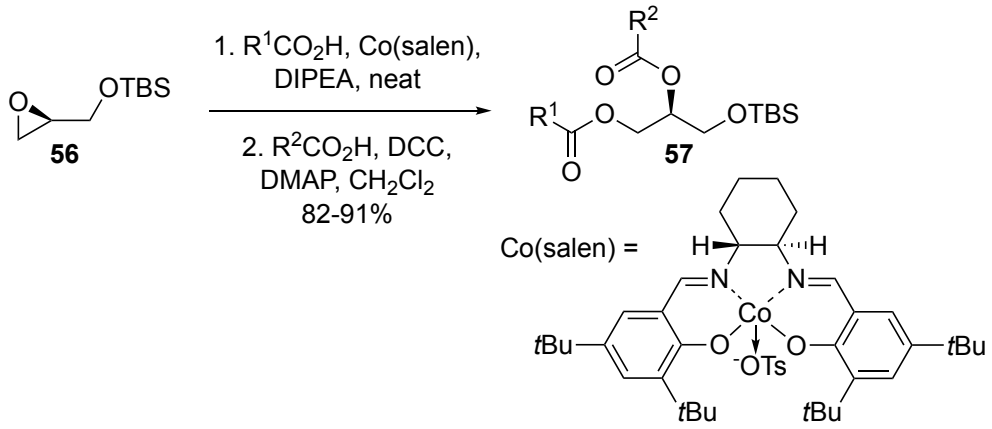
confirmed using a Mosher's ester analysis to show retention of stereochemistry at the secondary carbon.



Scheme 16: Possible isomerization pathway investigated by Konradsson.

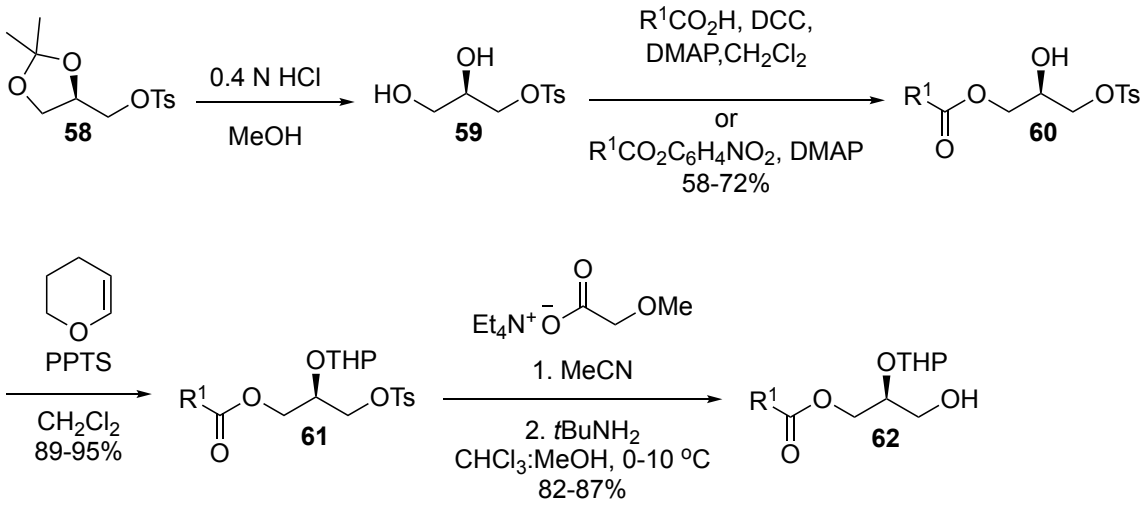
The second fatty acid was then installed using a DCC coupling under standard conditions. While this method allows for expedient access to differentially acylated phosphatidic acid and phosphatidylcholines, it requires a four-fold excess of the fatty acid, which is again undesirable for precious acids.

Minnaard has developed a related method for opening TBS-protected glycidol **56** using a cobalt-salen catalyst (Scheme 17).[105] The conditions utilize equimolar amounts of **57** and the desired fatty acid and avoid the acyl migration issue observed in Konradsson's work.



Scheme 17: Co(salen) catalyzed glycidol opening using an equimolar amount of the fatty acid.

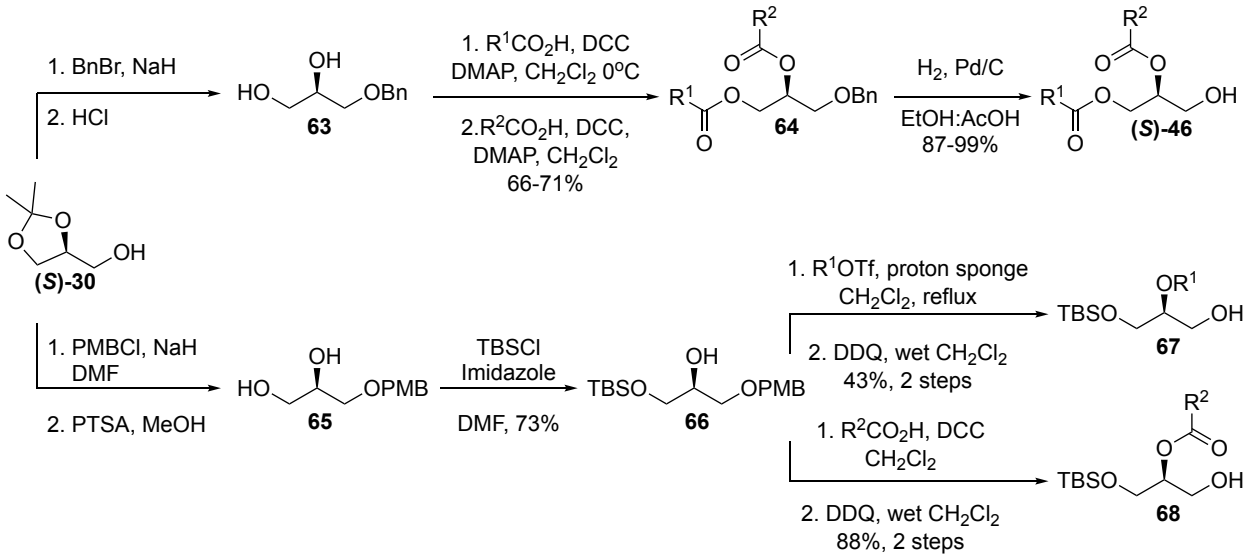
The most widely used glycerol precursor is solketal, which is commercially available as both enantiomers. Hadju and coworkers developed a method to access various lysophospholipids from (*S*)-solketal (Scheme 18)[106] that is related to the method presented in Scheme 14 above. The isopropylidene of **58** can be selectively cleaved under acidic conditions. Subsequent selective acylation of the primary alcohol of **59** can be accomplished using either a DCC coupling or the appropriate *p*-nitrophenyl ester and DMAP in 58-72% yield followed by THP protection. Notably, good selectivity could only be achieved in the acylation if a two-fold excess of the diol was used. Previously, displacement of the tosylate with Et_4NOAc followed by methanolysis was used for the two-step conversion to the desired alcohol (**49** to **50** in Scheme 14). However, when the same sequence was attempted using **61**, poor selectivity was observed (there was no second ester in **49**). The use of the methoxyacetate, as well as the modified methanolysis conditions allowed for significantly improved selectivity over the related sequence in Scheme 14.



Scheme 18: Preparation of functionalized glycerol 62 used in lysophospholipid synthesis.

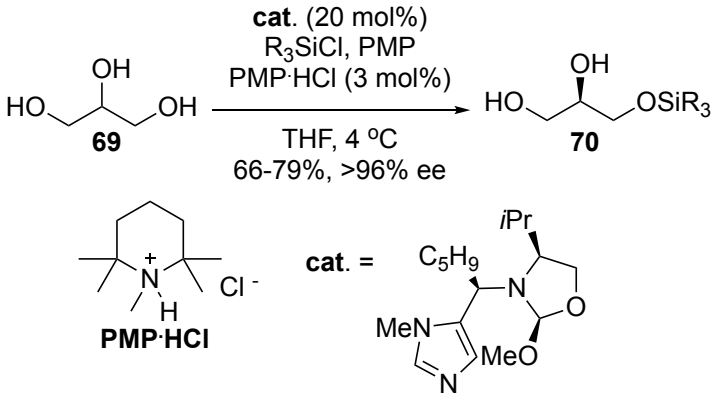
(*S*)-Solketal **(*S*)-30** has often been protected as a benzyl ether and subjected to acidic isopropylidene cleavage (Scheme 19).[107] Benzyl protected diol **63** can be subjected to sequential, selective acylation reactions and subsequently has the advantage of only requiring a single step to deprotect, unlike the Ts group in Scheme 18. A significant disadvantage is that unsaturated fatty acids cannot be incorporated with this method, due to alkene reduction under the reaction conditions. However, an analogous sequence can be performed with a *p*-methoxybenzyl (PMB) ether that circumvents this issue.[85]

PMB-protected diol **65** has also been used to prepare analogs of both natural lysobisphosphatidic acid and its ether analogs.[79, 90] Selective TBS protection of the primary alcohol followed by alkylation or acylation leads to lysobisphosphatidic acid precursors **67** and **68**. Notably, the PMB cleavage leading to alcohol **68** proceeds without any 1,2-acyl shift.



Scheme 19: Routes to functionalized glycerols from solketal.

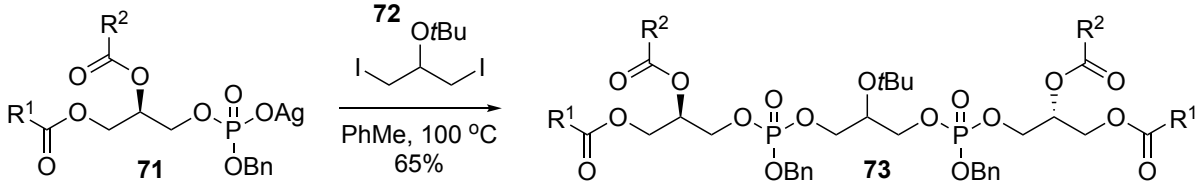
The desymmetrization of glycerol is an attractive synthetic alternative to more traditional chiral pool derived solketal methods (Scheme 20).[108] Tan and coworkers found that a chiral imidazole efficiently acts as a scaffolding catalyst for the enantioselective silylation of glycerol **69** in both good yield and enantioselectivity.[109] Notably, decreasing the catalyst loading to 5 mol% still allows for the product to be isolated in greater than 90% *ee*, though yields do suffer slightly (65% vs 78%). While other methods to desymmetrize glycerol have been developed,[110-112] this is the most direct and highly selective method to date.



Scheme 20: Tan's enantioselective silylation of glycerol.

1.5.5 Chemical Synthesis of Cardiolipins

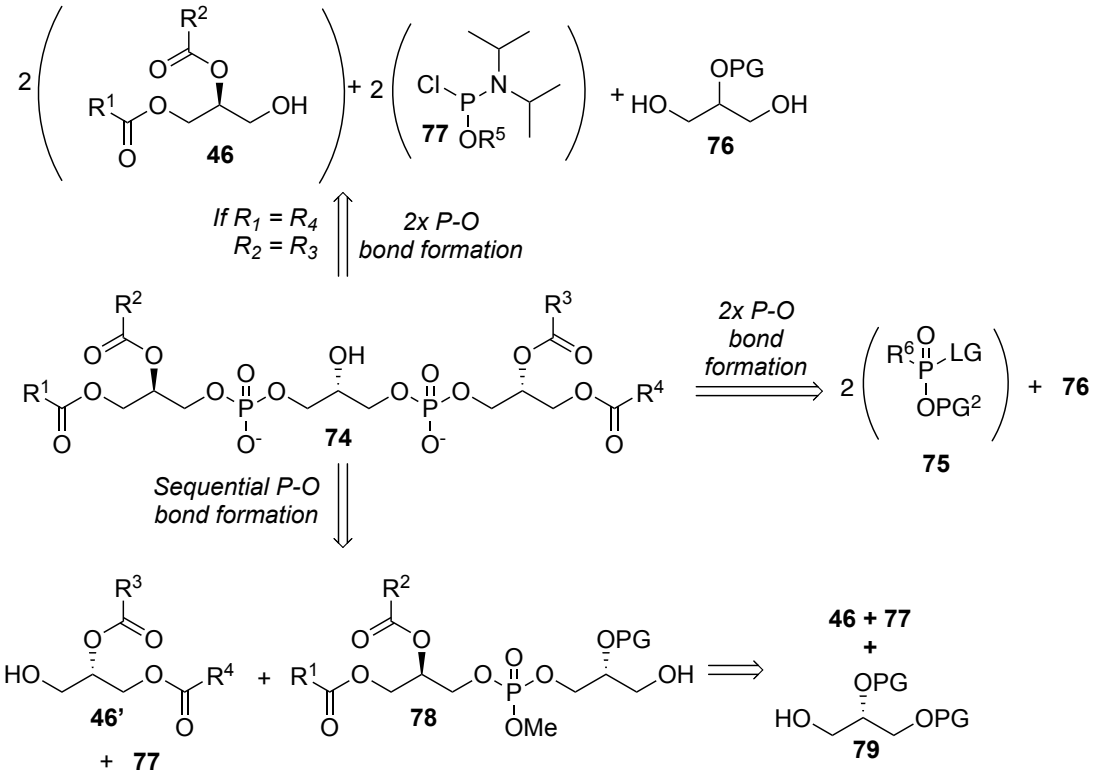
The essential role of CL in mitochondrial structure,[13] bioenergetics,[113] and signaling[114] has attracted the attention of the synthetic chemistry community so that a better understanding of its multifaceted biological roles can be obtained. Additionally, CL's unique structure among GPLs adds to its synthetic appeal. Indeed, it was curiosity about CL's structure that motivated its original synthesis by van Deenen in 1965 (Scheme 12).[115] The key step in the synthesis was the alkylation of silver phosphate salt **71** with diiodide **72** to assemble the CL scaffold. While this method was successful, the silver salts used were photosensitive.



Scheme 21: First synthesis of the cardiolipin scaffold by van Deenen.

Since the original synthesis, Umpolung approaches for phosphate assembly in which glycerol alcohols act as the nucleophiles on activated phosphorus electrophiles have become the dominate method by which CLs are synthesized (Figure 24). Previous methods were based on

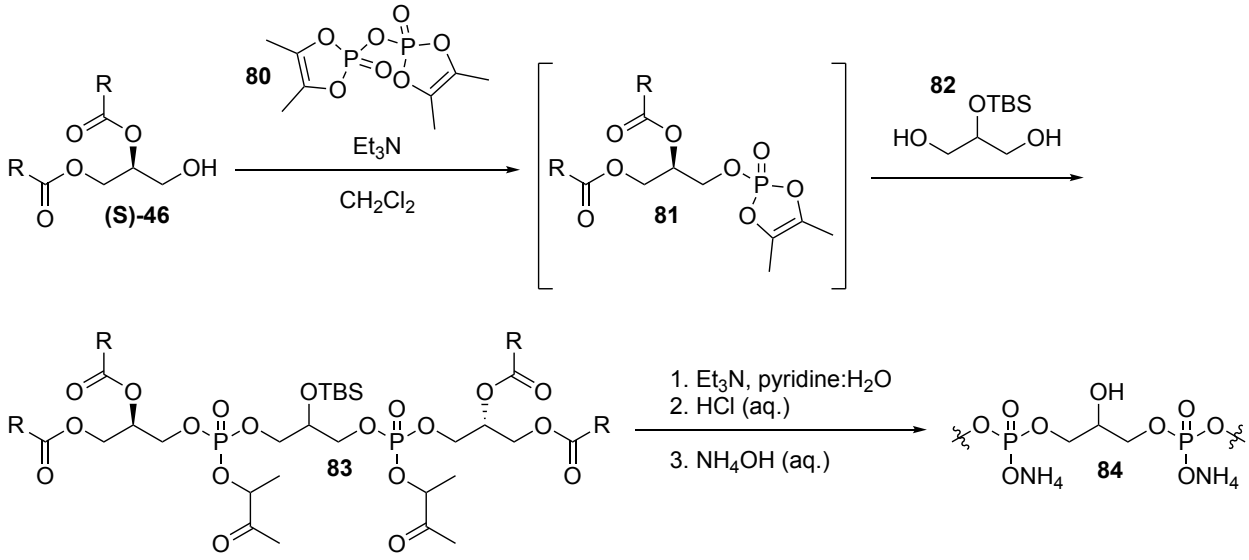
using an activated P(V) reagent (**76**) and generally formed both PA-glycerol linkages in a single operation (Schem 22, right). More recent developments have adapted phosphoramidite chemistry to constructing the CL scaffold. If identical DAG segments are desired, a one-pot reaction, in which two equivalents of DAG **46** can be coupled to diol **76** using the appropriate phosphoramidite coupling reagent **77** (Scheme 22 top). If non-identical acyl chain composition on the two DAG fragments is required, sequential phosphate formations can be conducted through the intermediacy of an appropriately protected monophosphate like **78** (Scheme 22, bottom).



Scheme 22: The three general synthetic strategies to access CLs.

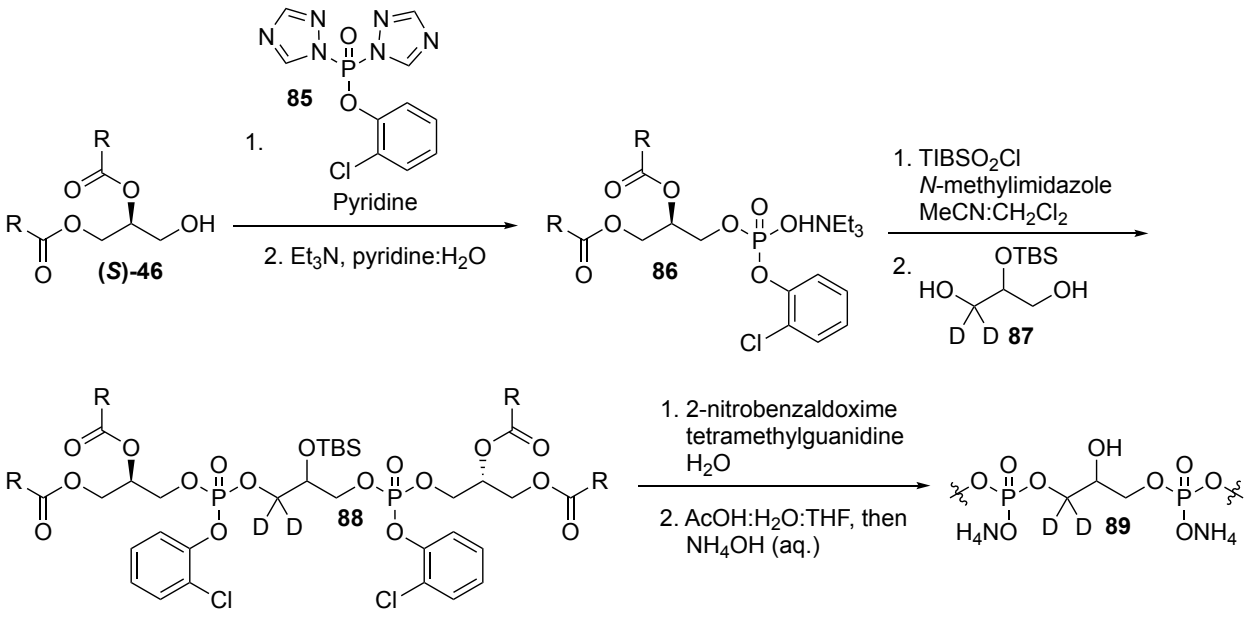
Ramirez and coworkers utilized protected phosphoanhydride **80** for this purpose along with 1,2-DAGs using triethylamine as the base (Scheme 23).[116] Cyclic phosphate **81** is activated enough to undergo further substitution with protected glycerol **82** in a one-pot fashion, yielding protected CL **83** in 30-37% yield. The phosphate protecting group undergoes E2 elimination with

trientylamine as the base, releasing the free phosphates and methyl vinyl ketone. Subsequent acidic cleavage of the TBS ether and formation of the diammonium salt gave the desired CLs **84** in 68-78% yield.



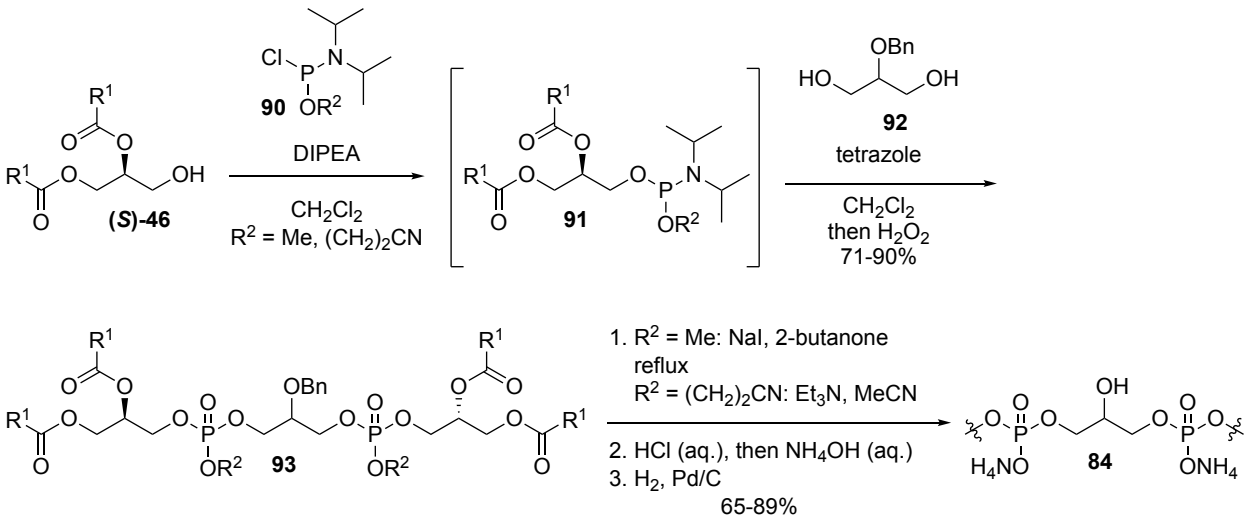
Scheme 23: Ramirez and coworkers synthesis of CL using phosphoanhydride 80.

Watts and coworkers performed a related synthesis of deuterated CLs that were of interest for studying CL biophysics by deuterium NMR spectroscopy. They utilized phosphorylating reagent **85** prepared *in-situ* from the triazole and the phosphoryldichloride (Scheme 24).[117] Hydrolysis of the intermediate gave the triethylammonium salt **86**. This compound can be activated for further substitution using 2,4,6-triisopropylbenzenesulfonyl chloride ($TIBSO_2Cl$) and *N*-methylimidazole, leading to protected CLs **88**. Selective cleavage of the 2-chlorophenol protecting group with the aldoximate anion followed by hydrolysis and diammonium salt formation gave the desired CLs **89**. A closely related synthesis by Ahmad and coworkers later found that the parent dichlorophosphate derivative of **85** can also be used directly.[118]



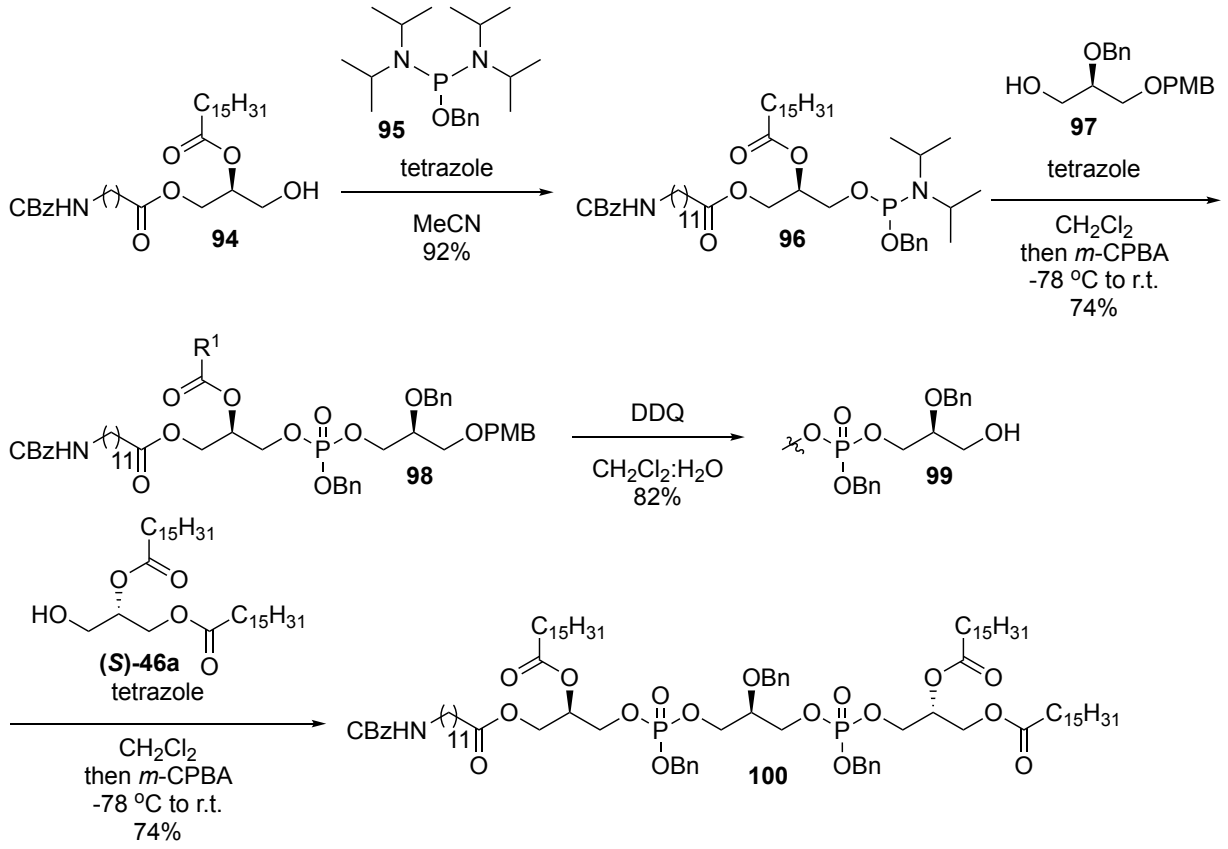
Scheme 24: Watts and coworkers' synthesis of CL using phosphorylating reagent 85.

While P(V)-based methodologies are successful, they are accompanied by an increased risk of acyl migration due to the lower reactivity of P(V) reagents vs P(III) reagents, as well as other side reactions.[119] This prompted Ahmad and coworkers to apply phosphoramidite chemistry to CL synthesis (Scheme 25).[91, 120] They demonstrated that chlorophosphoramidite reagents with either a methyl or cyanoethyl protecting group undergo successful coupling of DAGs and benzyl-protected glycerol **92** in a CL synthesis. Subsequent phosphate deprotection, salt exchange, and hydrogenolysis delivered the desired CL diammonium salts in good yields. This method has been widely adopted for the assembly of symmetrically substituted CLs, including for cyclopropane-containing derivatives.[92]



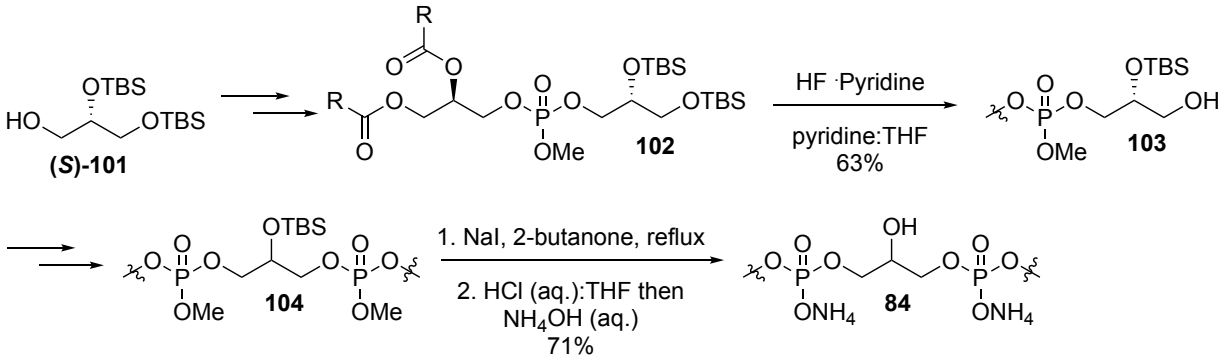
Scheme 25: Ahmad and coworkers' chlorophosphoramidite methods using either a methyl or cyanoethyl protecting group.

While these approaches are effective, they do not permit the incorporation of differentially substituted phosphatidic acid units. The approach was refined by Holmes in order to prepare CLs that could be linked to a resin (Scheme 26).[121] The key difference in this approach compared to previous methods is the use of the orthogonally protected glycerol **97**, which allows the two phosphates to be incorporated sequentially. DAG **94** was selectively monocoupled with phosphoramidite **95** in 92% yield. The isolated intermediate P(III) species **97** was then submitted to a second coupling with glycerol **96** followed by oxidation with *m*-chloroperoxybenzoic acid (*m*-CPBA), resulting in phosphate **98** in 74% yield. Selective cleavage of the PMB ether under oxidative conditions gave alcohol **99** in good yield. The final step in assembling the CL scaffold was a second phosphoramidite coupling and oxidation that proceeded in similar yields to the first.

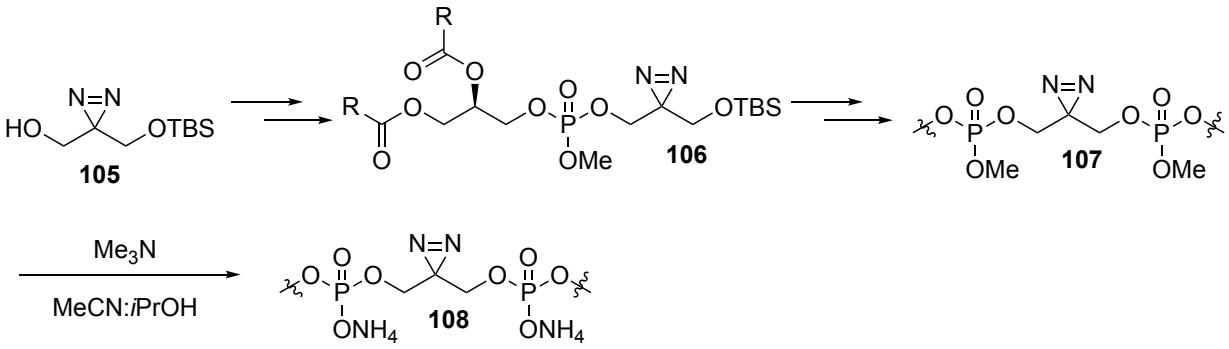


Scheme 26: Holmes phosphoramidite method for installing distinct phosphatidic acid units in CL. The Bn and CBz protecting group were successfully cleaved as well but have not been shown for the sake of brevity.

Miyoshi and coworkers expanded the method to allow for the incorporation of oxidation-prone acid chains by altering the protecting group scheme used on the central glycerol (Scheme 27).[122] Bis-TBS-protected glycerol **(*S*)-101** was used to prepare compound **102**, which contains two linoleic acid residues. Selective deprotection of the primary TBS ether with Olah's reagent provided alcohol **103** in 63% yield. The TBS ether on the central glycerol was ultimately cleaved using dilute aqueous HCl in THF and provided CL **84** in 71% yield over the final two deprotection steps. They also extended this work to diazirine-containing CL derivatives for use in a photoaffinity labeling study (Scheme 28).[123]



Scheme 27: Miyoshi's modification for incorporating polyunsaturated acyl chains.



Scheme 28: Miyoshi and coworkers' application of their methodology to the synthesis of a diazirine containing CL derivative.

1.6 RESULTS AND DISCUSSION

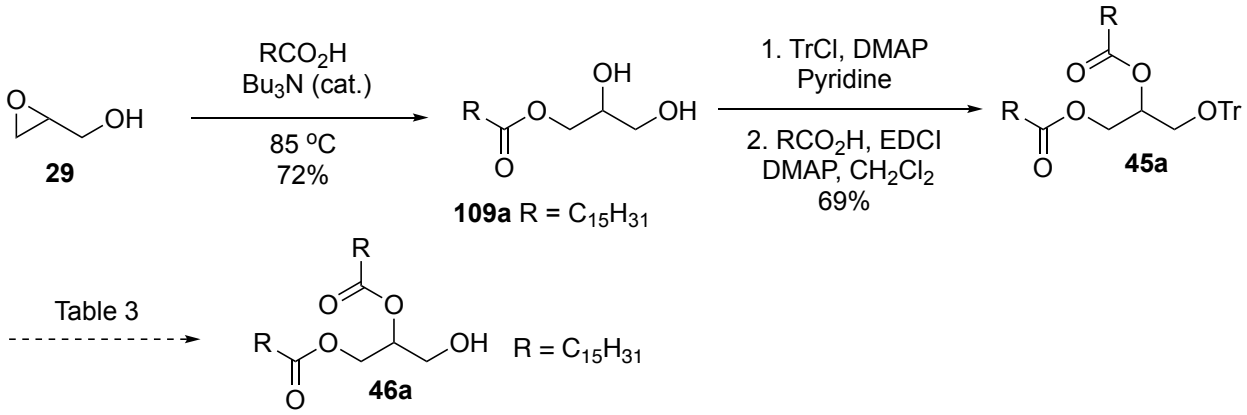
CL undergoes remodeling in which the fatty acid composition changes from a higher content of saturated fatty acids to primarily unsaturated fatty acids in mature CL. What is the underlying significance of this process and how is selectivity achieved? These changes are likely not driven by improved bioenergetic function, as mitochondria containing either remodeled or unremodeled CL are functionally equivalent.[124] However, CL's role as a source of oxidized polyunsaturated fatty acids[39] is dependent upon the presence of polyunsaturated residues, which may provide a biological role for remodeling.

S. cerevisiae provides a convenient system for studying CL biosynthesis as all the enzymes are well characterized and the remodeling process only utilizes Cld1 and tafazzin for the deacylation and reacylation steps respectively. Since Cld1 begins the remodeling process, it is possible that it plays a large role in the observed fatty acid compositional changes of CL. Indeed, results by Leber and coworkers suggest that Cld1 selectively cleaves palmitic acid residues from CL, though their experiments were conducted using whole cells and did not provide direct evidence of this claim.[71] A clearer understanding of Cld1 selectivity would provide a partial explanation for the observed fatty acid observed alterations. Commercial CL sources are sold mostly as uniformly tetra-acylated derivatives or mixtures isolated from various sources (bovine heart, *E. coli*, etc.), which prompted the synthesis of a targeted group of CLs to help improve the understanding of Cld1 selectivity. The method of sequential phosphate synthesis developed by Holmes and Miyoshi was selected as a starting point for the synthetic efforts due to the ability to incorporate different 1,2-DAGs.

1.6.1 Synthesis of Cardiolipins

As mentioned above, the synthetic strategy was closely based on prior work by Holmes and Miyoshi.[121, 122] Glycidol **29** was chosen as the starting point as opposed to solketal to avoid the need for the selective acylation reactions, which normally require the use of a large excess of either the glycerol or fatty acid component. Nearly equimolar quantities of glycidol **29** and the desired fatty acid can be reacted with catalytic tributylamine, resulting in monoacylglycerol **109a** in good yield (Scheme 29).[125] Protection of the primary alcohol using TrCl followed by EDCI coupling with palmitic acid gave protected 1,2-diacylglycerol **45a** in 69% yield. Subsequent attempts to cleave the Tr group without acyl migration were not successful (Table 3). Weakly

acidic conditions using either boric acid[126] or PPTS failed to provide useful conversions (entries 1 and 2). Stronger acids such as *p*-toluenesulfonic acid (*p*-TsOH) and hydrochloric acid[101] both lead to partial acyl migration during the course of the reaction or purification, respectively (entries 3 and 4). Lewis acidic conditions using bismuth(III) chloride[127] or standard hydrogenation conditions with Pd/C also gave similarly unsatisfactory results (entries 5 and 6).



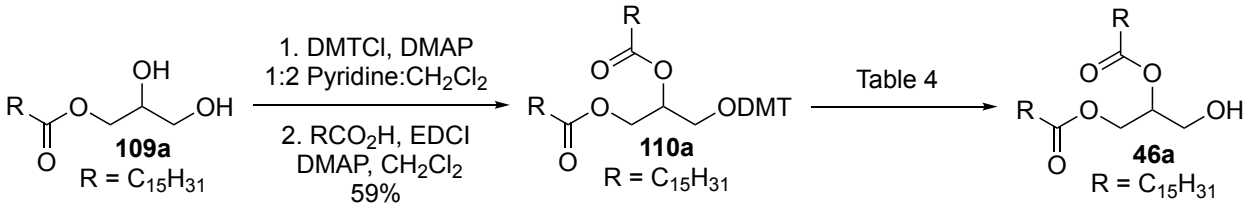
Scheme 29: Attempted synthesis of 1,2-DAG 46a from Tr protected intermediate 45a. *Migration occurs during purification.

Table 3: Conditions used in attempted conversion of 45a to 46a. *Migration occurs during purification.

Entry	Reagent	Equiv.	Temperature (°C)	Solvent	Result
1	$B(OH)_3$	5	r.t.	$MeNO_2$	R.S.M.
2	PPTS	2	r.t.	1:1 $CHCl_3$:MeOH	incomplete conversion
3	*p*-TsOH	0.08	r.t.	1:1 CH_2Cl_2:MeOH	acyl migration
4	HCl	1.0	r.t.	1:1 $CHCl_3$:MeOH	acyl migration*
5	$BiCl_3$	0.07	r.t. to 60	1:1 $CHCl_3$:MeCN	incomplete conversion
6	H_2, Pd/C	—	r.t.	EtOAc	R.S.M.

Changing the protecting group to the more acid labile 4,4'-dimethoxytrityl (DMT) group was more successful (Scheme 30). Boric acid also failed to cleave the DMT group and the mixed solvent $CHCl_3$:AcOH:H_2O gave incomplete conversion (Table 4, entries 1 and 2). However, a

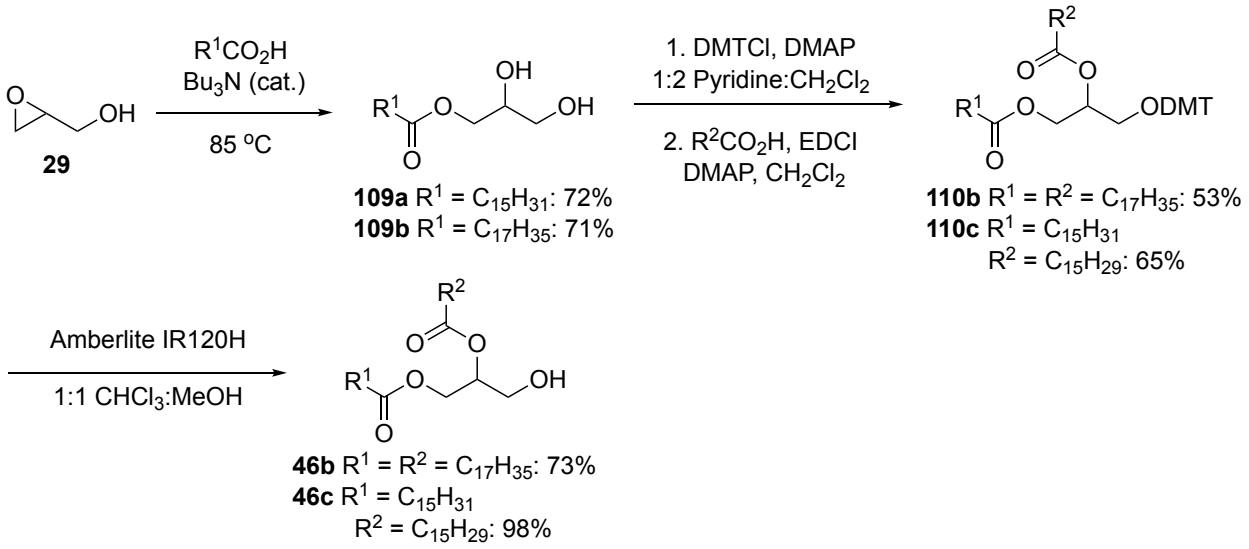
resin-bound sulfonic acid (Amberlite-IR120H) gave complete conversion to the desired 1,2-DAG **46a** in good yield without acyl migration (entry 3). This method was then further applied to the synthesis of two additional DAGs (Scheme 31).



Scheme 30: Deprotection of DMT protected DAG 110a. All reactions were conducted at room temperature.

Table 4: Screen of reaction conditions for the cleavage of DMT protecting group in 110a.

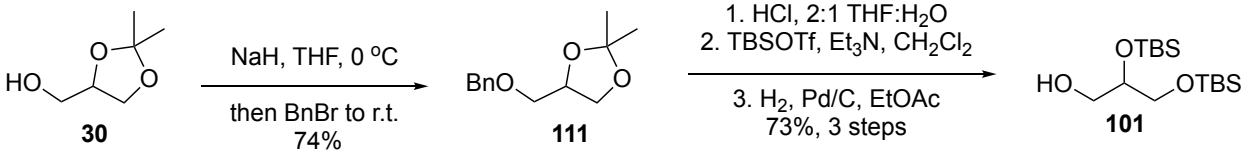
Entry	Reagent	Amount	Solvent	Result
1	$B(OH)_3$	5 equiv.	$MeNO_2$	R.S.M.
2	—	—	$CHCl_3$:AcOH:H_2O	incomplete conversion
3	Amberlite IR120H	439 mg/mmol	1:1 $CHCl_3$:MeOH	87%



Scheme 31: Synthesis of DAGs 46b-c.

With the three requisite 1,2-diacylglycerol segments in hand, attention turned to the synthesis of the protected central glycerol of CL. This was accomplished through *bis*-TBS

protected alcohol **101** in 4 steps (Scheme 32). Beginning from solketal **30**, protection of the alcohol with benzyl bromide gave intermediate **112** in 74% yield. Initially, hydrolysis of the isopropylidene was conducted using 2:1 AcOH:H_2O and though acceptable yields could be obtained (~70% of the intermediate diol), the removal of excess AcOH was cumbersome. This method was ultimately supplanted by using aqueous HCl in 2:1 THF:H_2O, which simplified both reaction setup and workup. Thus, the desired alcohol **111** could be obtained from **101** following hydrolysis of the isopropylidene, TBS protection of the intermediate diol, and hydrogenolysis of the benzyl ether in 73% yield over three steps.

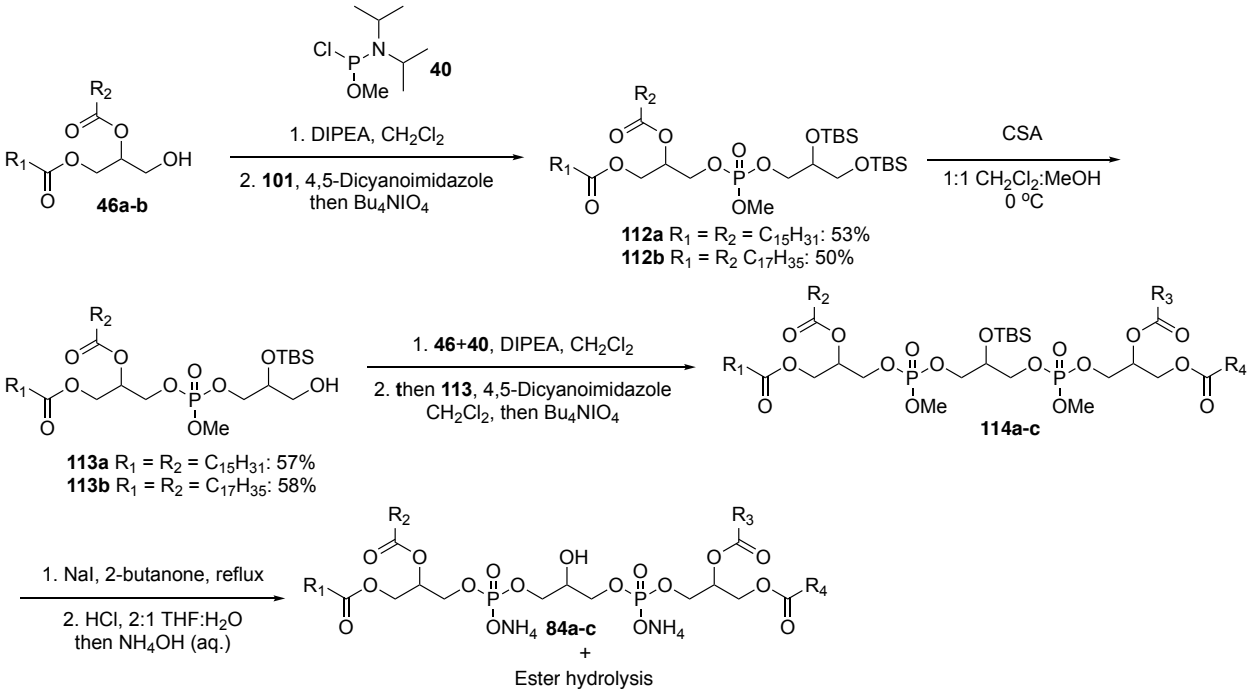


Scheme 32: Synthesis of *bis*-TBS protected alcohol 101.

Synthesis of CLs **84a-c** was accomplished using phosphoramidite chemistry (Scheme 32). Compounds **46a-b** were converted to a phosphoramidite using chlorophosphoramidite **40** and coupled in the same pot to alcohol **101** using 4,5-dicyanoimidazole as the promoter.[128] Though 1*H*-tetrazole is a more commonly used promoter, 4,5-dicyanoimidazole has been found to be more active, is substantially less expensive, and less hazardous.[129] Oxidation to the phosphate using tetrabutylammonium periodate (Bu_4NIO_4) gave **112a-b** in good yields. Selective TBS deprotection has been reported in closely related systems using Olah's reagent,[122] but the use of camphorsulfonic acid (CSA) proved a more convenient and reproducible method that gave similar yields. With alcohols **113a-b** in hand, the second phosphate was installed using phosphoramidite coupling, delivering protected CLs **114a-c**.

Deprotection of CLs was performed as a two-step sequence. Demethylation of **114a-c** with NaI in 2-butanone at reflux occurs smoothly and the crude disodium salt can be used

directly in the next step. The TBS group was surprisingly resistant to cleavage under both acidic and fluoride-mediated conditions, with all conditions tested showing either partial ester cleavage or difficulties in exchanging undesired counterions (such as pyridinium from Olah's reagent or tetrabutylammonium from TBAF). Treatment with HCl in 2:1 THF:H_2O followed by quenching with aqueous ammonium hydroxide provided the cleanest product, and ester hydrolysis byproducts could be removed by preparative TLC prior to use in biological assays.



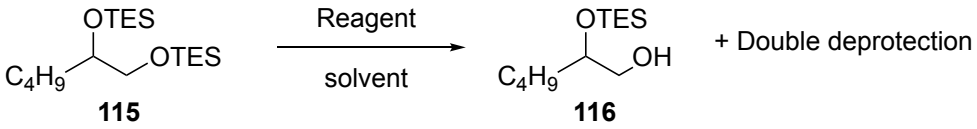
Scheme 33: Synthesis of Cardiolipins 84a-c. See Table 5 for yields and R definitions of compounds 114a-c and 84a-c.

Table 5: R group definitions and yields of compounds 114a-c and 84a-c.

Compound	R_1	R_2	R_3	R_4	Yield (%)
114a	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{31}$	67%
114b	$C_{18}H_{35}$	$C_{18}H_{35}$	$C_{18}H_{35}$	$C_{18}H_{35}$	40%
114c	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{29}$	$C_{15}H_{31}$	39%
84a	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{31}$	73%
84b	$C_{18}H_{35}$	$C_{18}H_{35}$	$C_{18}H_{35}$	$C_{18}H_{35}$	38%
84c	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{15}H_{29}$	$C_{15}H_{31}$	70%

1.6.2 Circumventing Ester Hydrolysis

In order to circumvent the undesired ester hydrolysis during the final desilylation, the more labile triethylsilyl (TES) group was used in place of the TBS. The TES group is cleaved ~300 times more rapidly under acid conditions than TBS,[130] which should preclude any ester hydrolysis. Selective cleavage of a primary TES ether over a secondary TES ether is a known process, though with significantly fewer examples than the same transformation of TBS ethers.[131] However, there is very little known about the selective cleavage of vicinal $1^o/2^o$ TES ethers. In order to explore possible conditions for this transformation, 1,2-hexanediol was protected with TESCl and several different deprotection conditions were investigated (Scheme 34 and Table 6). 2,4,6-Trimethylbenzoic acid and triphenylacetic acid were used as bulky proton sources and failed to provide any conversion even after extended reaction times (entries 1 and 2). A similar result was observed for the more acidic organophosphinic acid (entry 3). PPTS gave primarily recovered starting material, but also a significant amount of the desired monodeprotection (29%, entry 4). Lewis acids such as $FeCl_3$,[132] $NaAuCl_4$,[133] and $Sc(OTf)_3$, gave either complex reaction mixtures or complete double deprotection (entries 5-7). Conditions reported by Phillips using catalytic fluoride ion under neutral conditions gave poor selectivity and resulted in primarily double deprotection (entry 8).[134] Two different oxidative cleavages were attempted as well. Cerium(IV) nitrate adsorbed on SiO_2[135] again gave a complex mixture (entry 9). Finally, Swern oxidation conditions have been shown to selectively oxidize primary TES and TMS ethers to the corresponding aldehydes in closely related systems.[136] However, these conditions resulted in an approximately 2:1 mixture of the desired aldehyde (cleavage of primary TES ether) to undesired ketone (cleavage of secondary TES ether, entry 10).



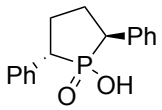
115 **116**

Scheme 34: Model system used for investigating selective TES group cleavage conditions.

Table 6: Conditions screened for the selective deprotection of model *bis*-silyl ether 115.

Entry	Reagent	mol%	Temperature (°C)	Solvent	Yield of **116**
1	2,4,6-trimethylbenzoic acid	5 to 18	-20 to r.t	1:1 CH_2Cl_2:MeOH	R.S.M.
2	triphenylacetic acid	5 to 100	-20 to r.t	1:1 CH_2Cl_2:MeOH	R.S.M.
3	**117**	5 to 18	-20 to r.t	1:1 CH_2Cl_2:MeOH	R.S.M.
4	PPTS	10	-50	1:1 CH_2Cl_2:MeOH	29%
5	$FeCl_3$	2	r.t	MeOH	complex mixture
6	$NaAuCl_4{\cdot}2H_2O$	1 to 5	-20 to 0	THF:MeOH	complex mixture
7	$Sc(OTf)_3$	30	0	CH_2Cl_2	double deprotection
8	TBAF	24	r.t	THF:buffer	double deprotection
9	CAN/SiO_2	24	r.t	1:1 CH_2Cl_2:*i*PrOH	complex mixture
10	Swern	400	-78	CH_2Cl_2	Low Selectivity*

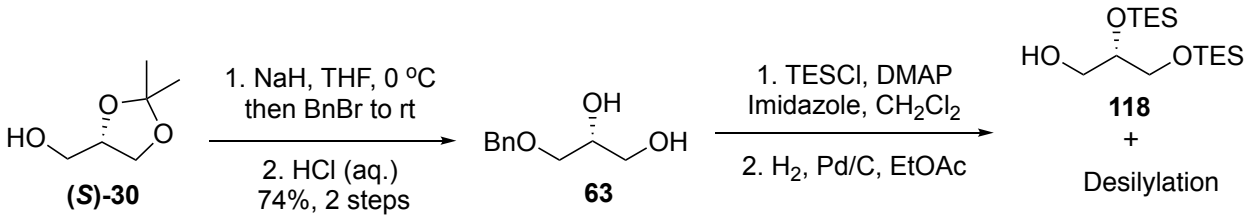
* ~2:1 mixture of aldehyde and ketone



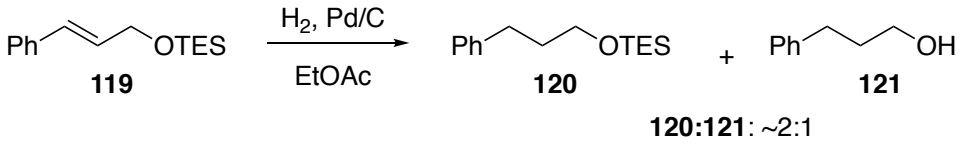
117

Encouraged by the results obtained with PPTS, the synthesis of *bis*-TES-protected glycerol **118** was attempted in a similar manner to *bis*-TBS protected **101**, this time beginning from commercially available (*S*)-solketal (Scheme 35). Benzyl protection, isopropylidene hydrolysis, and TES protection all proceeded in good yields. Surprisingly however, the hydrogenation to remove the benzyl group led to partial cleavage of a TES ether (presumably the primary ether). This is a known side reaction in some instances for relatively unhindered silyl

groups such as trimethylsilyl (TMS) and TES, even when controlling for traces of acid in the catalyst that may be left over from commercial production (Scheme 36; residual acid can be quite different with different suppliers).[137, 138] Prior work suggested that this undesired side reaction could be suppressed by using acetonitrile as the solvent.[138] However, this also led to a complete shutdown of the desired hydrogenolysis of the benzyl group, perhaps due to the poor solubility of the starting material.

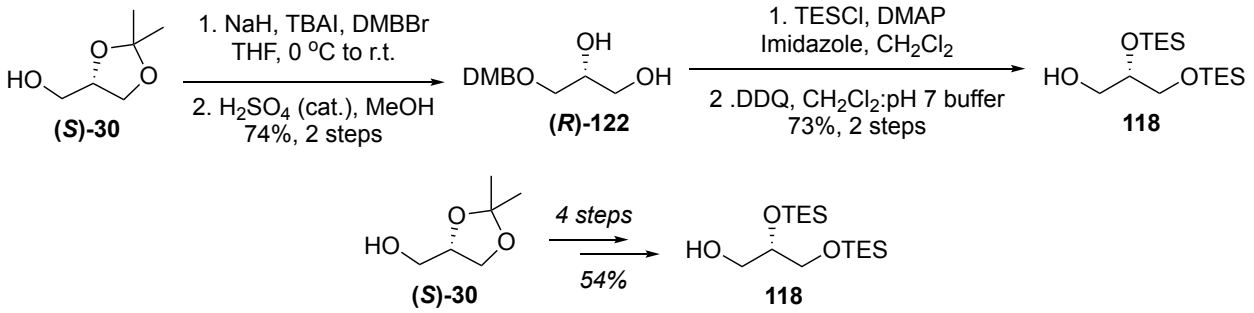


Scheme 35: TES cleavage under hydrogenation conditions.



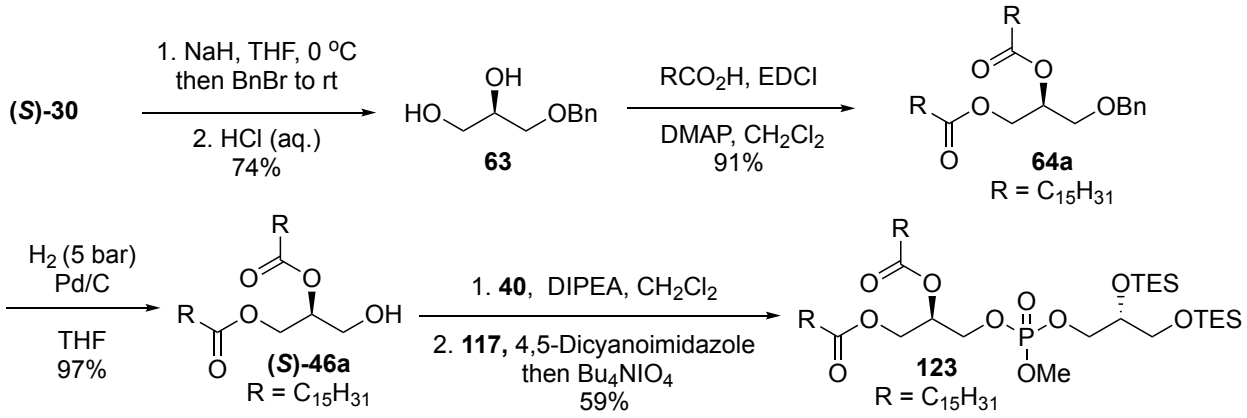
Scheme 36: Known TES cleavage under hydrogenation conditions[138]

In order to improve the selectivity of the final deprotection in the synthesis of *bis*-TES glycerol **118**, the benzyl ether was replaced by a 3,4-dimethoxylbenzyl (DMB) ether, which was envisioned to allow for selective cleavage under oxidative conditions (Scheme 37). Protection of the alcohol in **(*S*)-30** with freshly prepared 3,4-dimethoxybenzyl bromide followed by transacetalization gave the desired diol **(*R*)-122** in 74% yield over two steps. TES protection followed by DMB ether cleavage with DDQ gave a substantially improved yield of 73% over two steps compared to similar efforts with the benzyl ether (note: a PMB ether can also be carried through this sequence but is more challenging to purify in the final step). Subsequent optimizations allowed this sequence to be performed with only a single purification in nearly identical yield (54% over 4 steps).



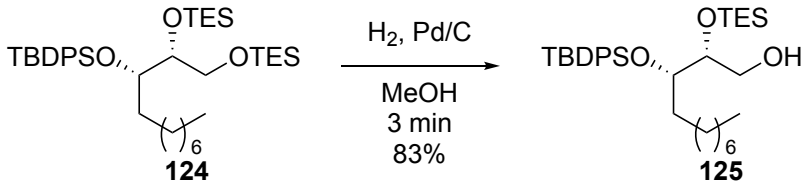
Scheme 37: Successful preparation of alcohol 118 utilizing the DMB ether protecting group.

The alcohol **118** was then used in preparing phosphate **123** (Scheme 38**)**. (*S*)-Solketal **30** was benzyl protected with sodium hydride and benzyl bromide, followed by acidic isopropylidene cleavage yielding diol **63**. The diol was subjected to EDCI coupling leading to protected dipalmitoylglycerol **64a** in 91% yield. Subsequent hydrogenolysis of the benzyl group in a Parr hydrogenator (5 bar) with Pd/C catalyst provided diacylglycerol **(*S*)-46a** in near quantitative yields, with no purification required. 1,2-Diacylglycerols are known to be prone to isomerization,[79, 139] and thus elevated pressures were used to minimize reaction times and reduce the risk of unwanted byproduct formation. One pot phosphate formation was accomplished using chlorophosphoramidite **40** followed by activation of the intermediate phosphoramidite with 4,5-dicyanoimidazole and coupling to alcohol **118**. Finally, oxidation with Bu_4NIO_4 gave phosphate **123** in 59% yield.



Scheme 38: Synthesis of phosphate 123.

Further optimization of the selective TES deprotection was then conducted on compound **123**. Incidentally, the only reported example uses reducing conditions with H_2 and Pd/C (Scheme 39).[140] The reaction times reported were very short (<5 min) and in most cases, no selectivity between the TES ethers was observed. Thus it is likely that steric effect of the *tert*-butyldiphenylsilyl (TBDPS) ether in **124** plays a significant role in shielding the secondary TES ether. Based on the prior discussion about silyl group cleavage under these conditions and the varying quality of Pd/C, it was postulated that this method would be very difficult to reproduce.

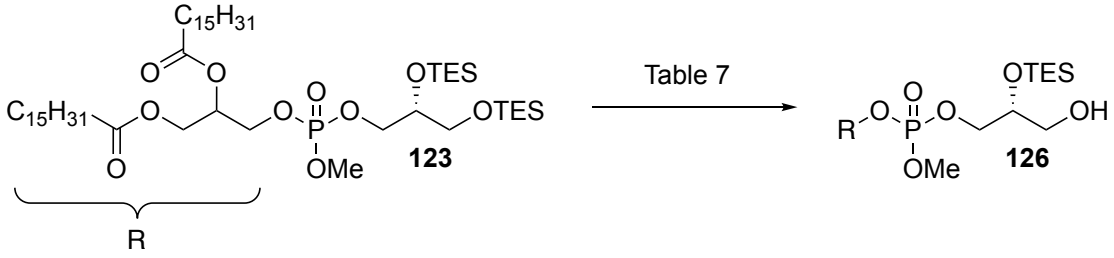


Scheme 39: The only selective deprotection of 1°/2° 1,2-*bis*-TES ethers found in the literature.

Compound **123** was found to be less reactive than model substrate **115 (**Scheme 40 and Table 7**)**. This is likely due at least in part to the very low solubility of compound **123** in 1:1 CH_2Cl_2:MeOH below –25°C. Performing the reaction at –10 °C provided a satisfactory yield (entry 1). Other acidic deprotection conditions were explored as well. Olah's reagent, while providing slightly lower yields than PPTS, also proved difficult to reproduce and so was not

investigated further (entry 2). Weaker acids such as acetic acid (AcOH) or hexafluoroisopropanol (HFIP) used in large excess failed to improve results (entries 3 and 4). The significantly stronger acid triphenylphosphine hydrobromide provided complete double deprotection within 30 min (entry 5). TBAF buffered with acetic acid resulted in nonselective deprotection (entry 5).

Given the lack of encouraging results with deprotection conditions other than PPTS, efforts were made to fine-tune this reaction. Reducing the temperature to –20 °C did not affect the overall yield, but improved the reliability with which the unreacted starting material could be recovered (entry 7). Changing the counterion from tosylate to trifluoroacetate greatly decreased the rate of the reaction but did not affect the overall yield (entry 8). Dimethylaniline hydrochloride is a slightly stronger acid (pKa 2.5 vs 3.4 in DMSO) and gave similar results to PPTS, but since it is not commercially available and is substantially more hygroscopic, was not used further (entry 9). Similarly, changing the alcoholic component of the solvent from methanol to isopropanol greatly decreased the reaction rate without improving the yield. Thus, the most reliable conditions were found to be 10 mol% PPTS in 1:1 CH_2Cl_2:MeOH at –20 °C and provided the desired deprotection product **126** in 43% yield (78% BRSM).

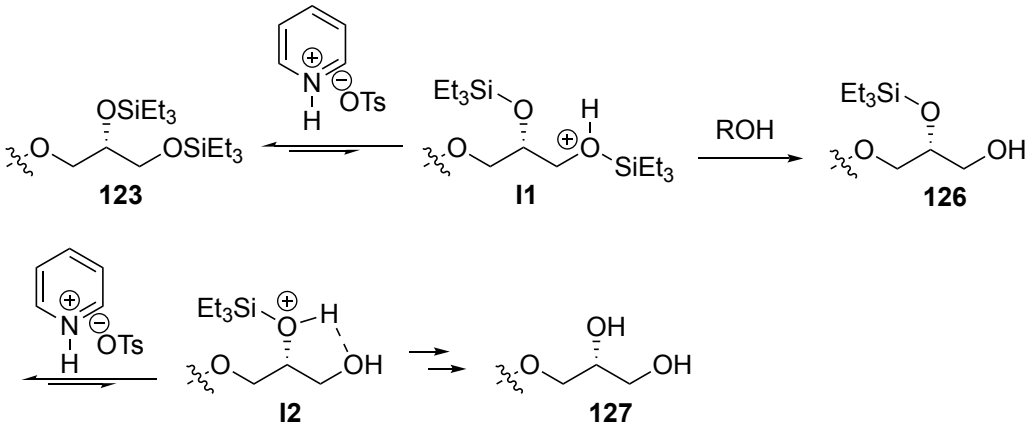


Scheme 40: Conversion of 123 to 126.

Table 7: Optimization of selective TES ether cleavage conditions.

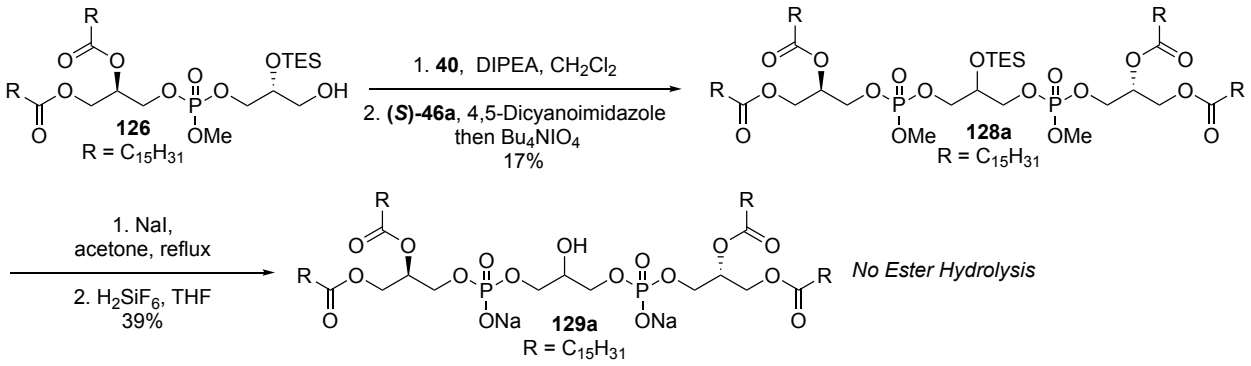
Entry	Reagent	mol%	Temperature (°C)	Solvent	Yield of **126** (%)
1	PPTS	5	-10	1:1 CH_2Cl_2:MeOH	43
2	HF·Py	100	0	THF	31
3	AcOH	—	r.t	2:1:1 THF:AcOH:H_2O	33
4	HFIP	—	r.t	CH_2Cl_2:HFIP:MeOH	R.S.M.
5	Ph_3PHBr	5	-10	1:1 CH_2Cl_2:MeOH	0
6	TBAF	100	-10	THF:AcOH	low selectivity
7	PPTS	5	-20	1:1 CH_2Cl_2:MeOH	43
8	PyTFA	5	-20	1:1 CH_2Cl_2:MeOH	40
9	$PhNMe_2$·HCl	5	-20	1:1 CH_2Cl_2:MeOH	43
10	PPTS	5	r.t	1:1 CH_2Cl_2:*i*PrOH	26

A potential explanation for the inability to increase the yield of the desired product is shown in Scheme 41. It should be noted that in all cases with tractable product mixtures, some combination of unreacted starting material, monodeprotection, and double deprotection occurs cleanly. Reversible protonation of the silyl ethers likely occurs selectively at the less hindered primary position giving intermediate **I1**. Following cleavage of the Si–O bond, a second protonation can occur at the remaining silyl ether oxygen. It is possible that the neighboring alcohol oxygen can participate in stabilizing the protonated silyl ether in intermediate **I2** and thus increase the rate at which the second TES is cleaved.



Scheme 41: Proposed rational for the difficulties in increase the yield of the selective cleavage of TES ethers of 1,2-diols.

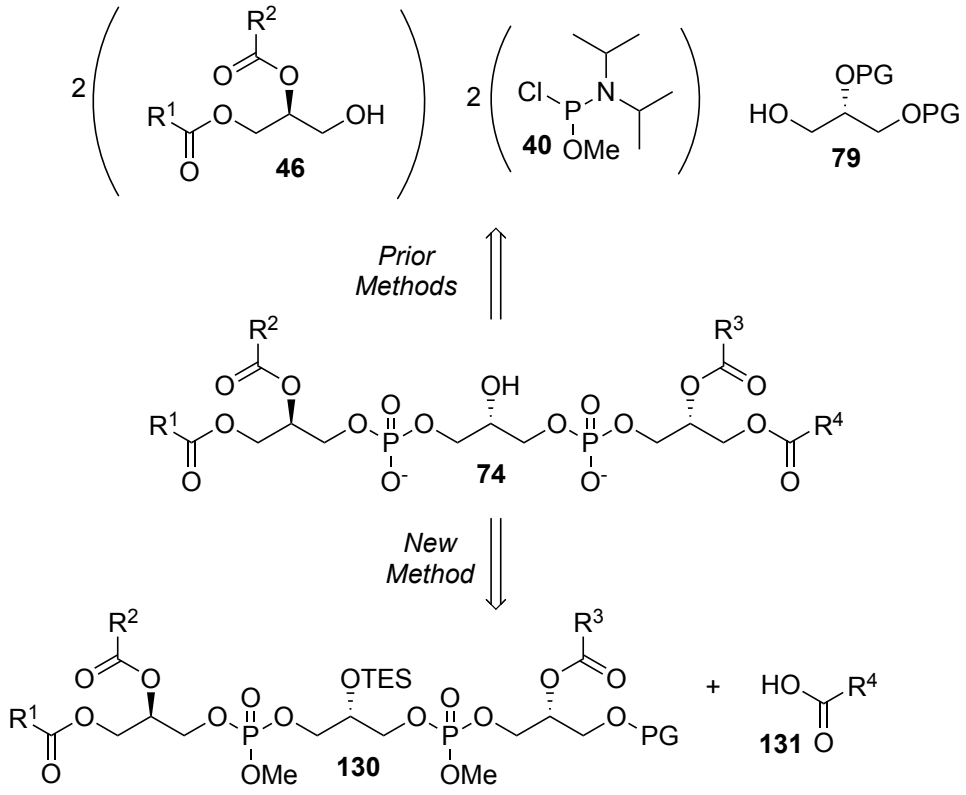
Alcohol **126** was then carried through a second phosphoramidite coupling/oxidation sequence, yielding protected CL **128a** in low yield (Scheme 42). Subsequent demethylation with sodium iodide was carried out in acetone at reflux, as the higher temperature of 2-butanone was found to be unnecessary and reaction mixtures were generally cleaner. TES ether cleavage using H_2SiF_6[141, 142] in THF gave the desired CL **129a** in a modest yield of 39%. In prior work, THF gave significantly reduced reaction rates compared to MeCN, but this reaction was complete in 30 min with no ester hydrolysis.



Scheme 42: Successful preparation of CL 129a without ester hydrolysis.

1.6.3 Development of a Bioinspired Late Stage Acylation

With the knowledge that the final deprotection could be achieved without ester hydrolysis, focus was shifted to designing a more streamlined synthesis of CL analogs. The two general approaches to CL synthesis (Scheme 43) both utilize 1,2-diacylglycerols that are prepared by early incorporation of the acyl residues, which makes them inconvenient for analog synthesis. A more streamlined approach was inspired by the biosynthesis of CL, in which the full CL is first synthesized, then exchange of fatty acid residues occurs as needed (Section 1.3). Methods for enzymatic deacylation of CL have been developed,[143, 144] but they use unprotected CLs and generally do not provide high yields.

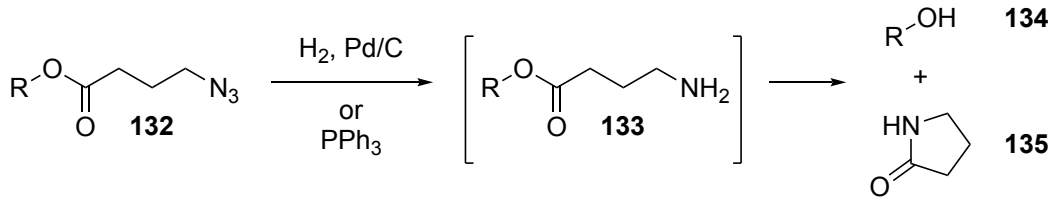


Scheme 43: Proposed method for incorporating fatty acids after CL backbone assembly.

In practice, this strategy is equivalent to using a protecting group on one of the glycerol positions that can be cleaved under mild conditions orthogonal both to the TES ether and methyl phosphates (**130**, Scheme 43). Additionally, migration of the acyl group must be avoided and thus the reaction should be conducted under neutral conditions. Finally, the subsequent acylation reaction should be conducted using the crude material from the deprotection step to avoid any issues of acyl migration during purification.

Initial inspiration was drawn from the 4-azidobutyryl esters developed by Kusumoto (Scheme 44).[145] These can be cleaved under mild hydrogenation or Staudinger reduction conditions, were the azide is first reduced to the amine, followed by cyclization to release the desired alcohol. They have been applied in sensitive substrates previously[146] and the lactam byproduct **135** was not expected to interfere with subsequent acylation. Additionally, two

variants were developed that have increasing degrees of conformational restriction and should allow for even more facile cleavage if needed.[147, 148]

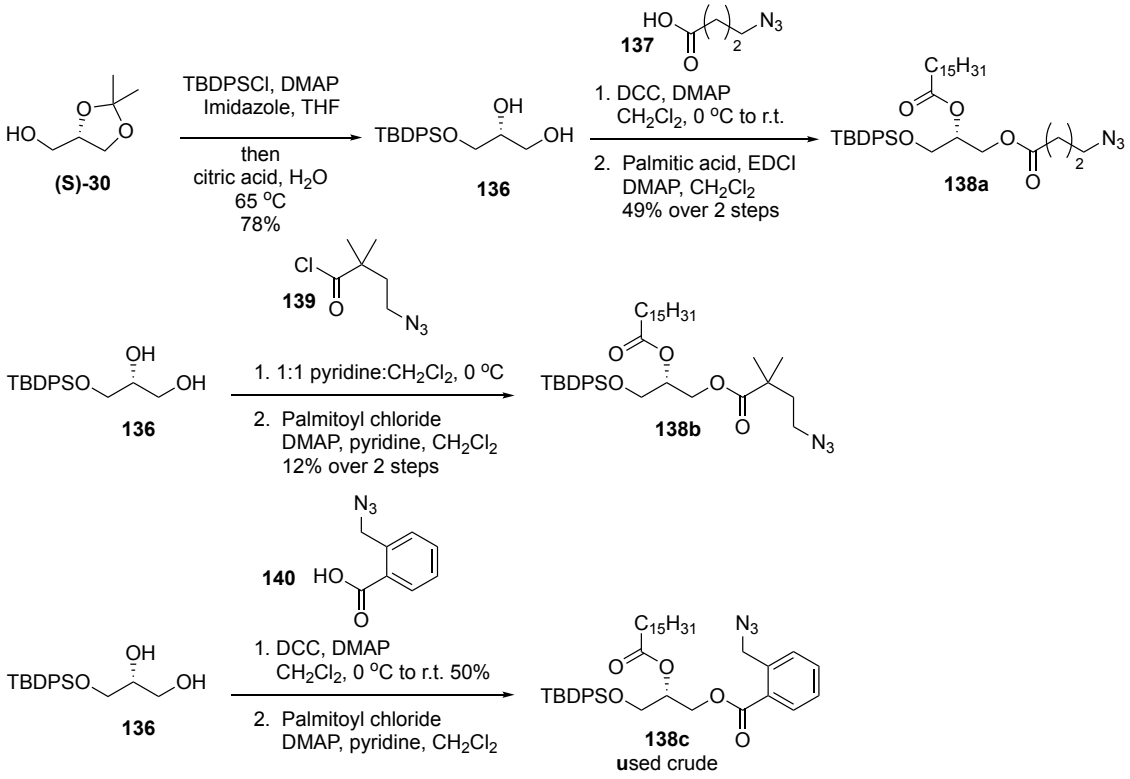


Scheme 44: Kusumoto's 4-azidobutryl ester protecting group.

Protected glycerol synthesis began from (*S*)-solketal **30** (Scheme 45). TBPDS protection of the alcohol was selected due to its moderate acid stability (to survive isopropylidene cleavage) and orthogonal cleavage conditions to the azido-esters. Isopropylidine cleavage on related systems has been performed using $FeCl_3$ adsorbed on SiO_2[149] or 90% acetic acid.[150] $FeCl_3/SiO_2$ gave poor conversion and it was undesirable to remove large quantities of acetic acid. An adaptation using citric acid was developed that not only uses significantly less acid (5 equiv. vs >1500 equiv.) but allows for a telescoped TBDPS protection/isopropylidene deprotection with a simplified purification (the prior synthesis uses a two flask approach that requires a solvent switch from DMF to 90% acetic acid with both chromatography and crystallization required for purification after the second step).

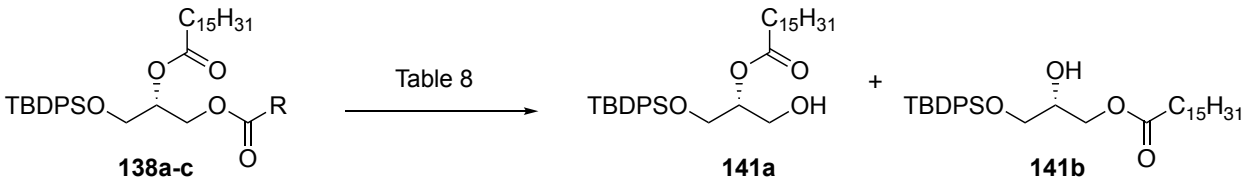
The resulting diol **136** could then be converted to diesters **138a-c** by sequential acylation reactions. Compound **138a** was prepared by a selective acylation with *N*,*N*-dicyclohexylcarbodiimide (DCC) and acid **137** followed by acylation of the secondary alcohol with palmitic acid under EDCI coupling conditions in 49% over 2 steps. Compound **138b** was prepared in an unoptimized 12% yield by sequential acylation with acid chloride **139** and palmitoyl chloride. Compound **138c** was prepared using the same DCC coupling used in preparing **138a** and the intermediate alcohol was isolated in an unoptimized 49% yield.

Subsequent acylation with palmitoyl chloride gave the desired diester **138c** in sufficient purity to use in test deprotection reaction (product contaminated with palmitic acid).



Scheme 45: Synthesis of azide-esters 138a-c with varying degrees of conformational restriction.

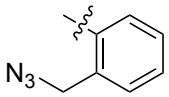
Cleavage of the azido-esters proved to be challenging (Scheme 46, Table 8). Attempted cleavage under Staudinger reduction conditions or hydrogenation with Pd/C successfully reduced the azide to the amine (**138***), but subsequent cyclization and ester cleavage was incomplete in all cases. This is consistent with prior results that required elevated temperatures for ester cleavage.[145, 146] Additionally, isomerization of the desired compound **141a** to **141b** occurred to varying degrees. Similar results for all azido-esters **138a-c** were obtained and further efforts to improve this step were abandoned.



138a-c **141a** **141b**

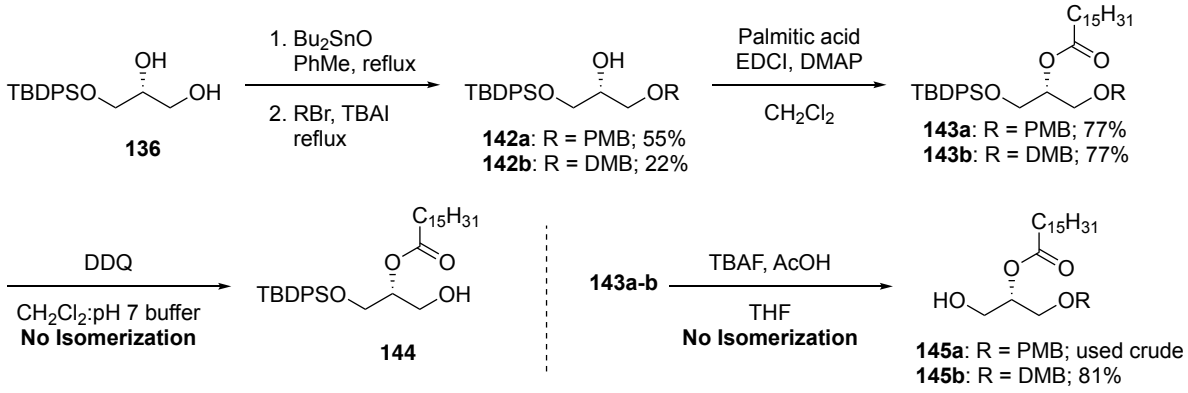
Scheme 46: Attempted cleavage of azido-esters 138a-c.

Table 8: Attempted cleavage of azido-ester protecting groups. 138* is 138 containing an amine (i.e. azide reduction was always successful but ester cleavage was incomplete).

Entry	R	Solvent	Temp (ºC)	Reagent	**138*:141a:141b**
1	$(CH_2)_3N_3$	THF	r.t.-65	PPh_3, H_2O	2:1:1
2	$(CH_2)_3N_3$	*i*-PrOH	r.t	H_2, Pd/C	1:1:1
3	$(CH_2)_3N_3$	THF	r.t	DPPE, H_2O	1.3:1:1.9
4	$C(CH_3)_2(CH_2)_2N_3$	*i*-PrOH	r.t	H_2, Pd/C	1.7:1.7:1
5	$C(CH_3)_2(CH_2)_2N_3$	THF	r.t	PPh_3, H_2O	1:1.3:1
6	$C(CH_3)_2(CH_2)_2N_3$	*i*-PrOH	r.t	H_2, AcOH, Pd/C	2.8:1.1:1
7		THF	r.t	PBu_3, H_2O	3.8:1:1.1

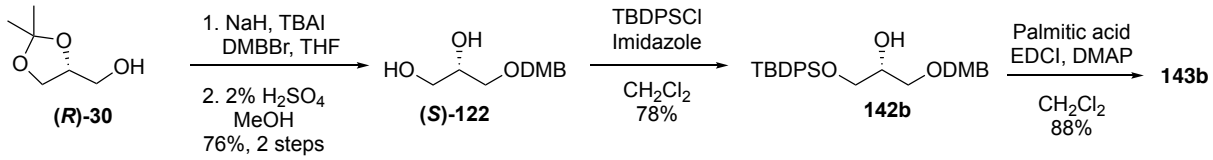
Oxidation-labile benzyl ethers (PMB, DMB) also possess the requisite orthogonality for the synthesis of the protected fragment. One challenge associated with the use of these protecting groups is their selective installation on diol **136**. Dibutyltin oxide has been used with great success in related selective alkylations and acylations in carbohydrate chemistry[151, 152] and related 1,2-diols.[153, 154] PMB protection of diol **136** proceeded with acceptable efficiency, but the corresponding DMB protection gave 22% yield, likely due to the lower stability of this ether (Scheme 34). In both cases, purification proved to be quite challenging and ultimately led to the redesign of the synthesis for these compounds (*vide infra*). EDCI coupling proceeded in 77% yield for both **142a** and **142b** giving protected glycerols **143a-b**. Both the PMB and DMB ethers

can be cleaved using DDQ under buffered conditions without acyl migration. Likewise, TBAF buffered with acetic acid[139] allows for orthogonal cleavage of the TBDPS groups.



Scheme 47: Synthesis of protected glycerols 145a-b.

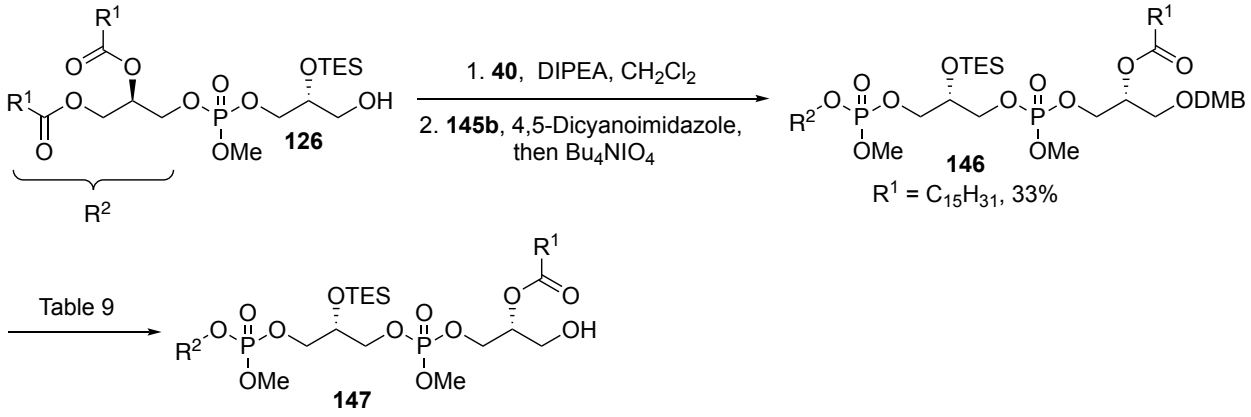
Given that the DMB protected glycerol **145b** ultimately proved to be the most useful (*vide infra*), the synthesis was optimized to allow for more efficient material throughput. The simplest solution was to use the opposite enantiomer used to prepare alcohol **142b** and utilize reaction conditions already known to be effective for installing the DMB ether on solketal and selective isopropylidene cleavage (Scheme 48). Using **(*R*)-30**, this sequence proceeded in similar yield to the opposite enantiomer. Selective protection of the primary alcohol with TBDPSCl gave **142b** in 78% yield with complete selectivity. Finally, EDCI coupling gave the desired protected glycerol **143b** in 88% yield, an improvement of 36% over the sequence compared to using the Bu_2SnO method (49% from **(*R*)-30** to **143b** vs 13% for **(*S*)-30** to **143b**).



Scheme 48: Alternative synthesis of 143b.

Key CL precursor **146** was prepared through phosphoramidite coupling of phosphate **126** and protected glycerol **145b** as performed previously (Scheme 49, Table 9). Three different

oxidative cleavages of the DMB ether were attempted. CAN on SiO_2[135] gave approximately 50% conversion but also lead to the cleavage of the TES ether. Catalytic DDQ using manganese(III) acetate as the terminal oxidant[155] gave only trace conversion. Using stoichiometric DDQ in a biphasic CH_2Cl_2:phosphate buffer system provided full conversion to the desired alcohol and was used directly in subsequent transformations.



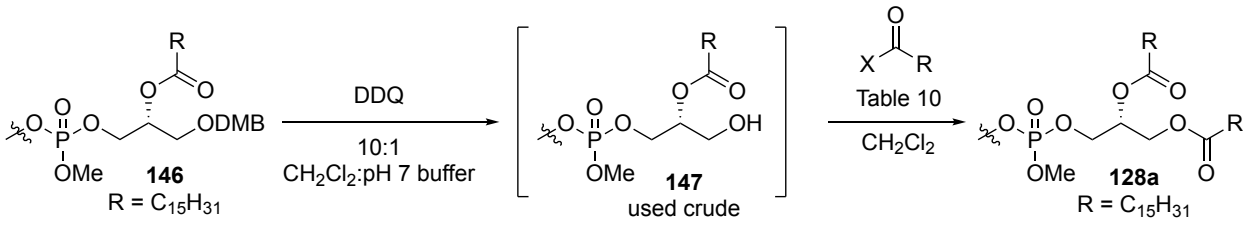
Scheme 49: Synthesis of DMB protected CL precursor 146 and optimization of DMB cleavage.

Table 9: DMB cleavage conditions for the conversion of 146 to 147.

Entry	Reagents	Equiv.	Solvent	Result
1	CAN/SiO_2	0.45	CH_2Cl_2	Incomplete conversion TES loss
2	DDQ, $Mn(OAc)_3$	0.1, 3.0	CH_2Cl_2	Trace conversion
3	DDQ	1.2	10:1 CH_2Cl_2:buffer	Complete conversion

The crude alcohol **147** was directly used in the installation of the final fatty acid residue (Scheme 50, Table 10). DCC performed poorly and had the additional complication that the urea byproduct could not be separated from the desired compound (entry 1). T3P® gave a cleaner product but a similarly poor yield (entry 2). The commercially available palmitoyl chloride proceeded well in 66% yield (entry 3). An activated *N*-hydroxysuccinimide (NHS) ester with

catalytic DMAP was relatively unreactive proceeding with low conversion (entry 4). The mixed pivaloyl anhydride with catalytic DMAP was found to be equally effective to the acid chloride (entry 5), but contained a small amount of TES cleavage in the product (likely due to traces of HCl). Increasing the amount of triethylamine to 3 equiv. suppressed this side reaction. Ultimately, the mixed pivaloyl anhydrides were selected for further use, as they are generally less moisture sensitive than the corresponding acid chlorides.



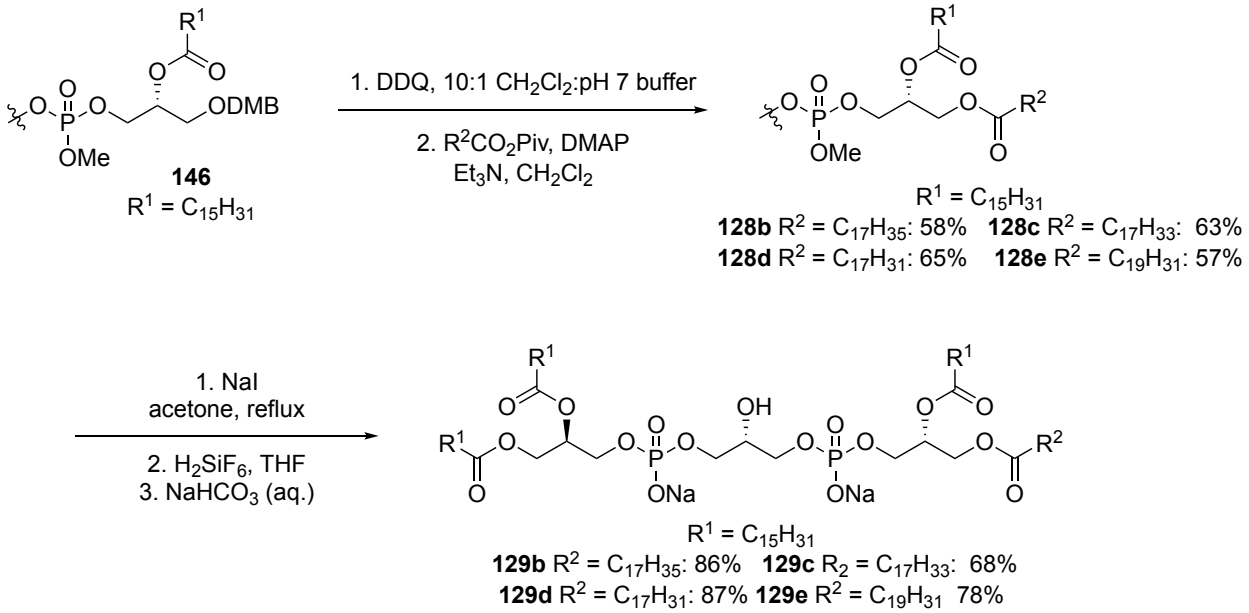
Scheme 50:Conversion of DMB-protected 146 to protected CL 128a.

Table 10: Screening of reaction conditions for deprotection/acylation sequence.

Entry	X	Reagents	Yield **128a** (%)	Entry	X	Reagents	Yield **128a** (%)
1	OH	DCC, DMAP	<30	4	NHS	DMAP, Et_3N	<20
2	OH	T3P, DMAP, Et_3N	33	5	PivO	DMAP, Et_3N	66*
3	Cl	Et_3N	66	6	PivO	DMAP, Et_3N**	66

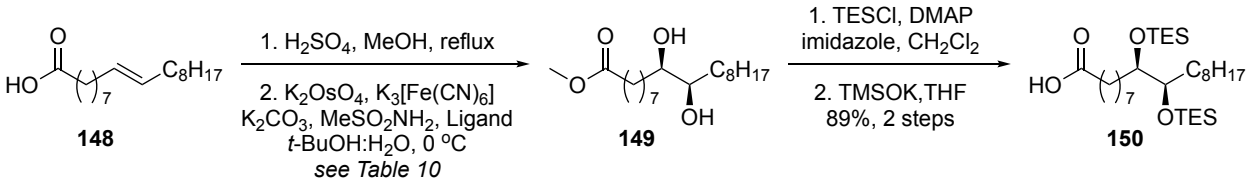
* contains small amount of TES cleavage
**3 equiv. Et_3N used

The bioinspired acylation sequence was then used to incorporate fatty acids of various degrees of unsaturation (Scheme 51). Stearic (C18, saturated) and oleic (C18, monounsaturated) acids can be installed in 58% and 63% yields respectively. The method is also successful for polyunsaturated fatty acids. Linoleic (C18, diunsaturated) and arachidonic (C20, tetraunsaturated) acid residues, which contain one and three easily oxidized *bis*-allylic positions respectively, can be installed in similar yields of 65% and 57%.



Scheme 51: Synthesis of CLs 129b-e.

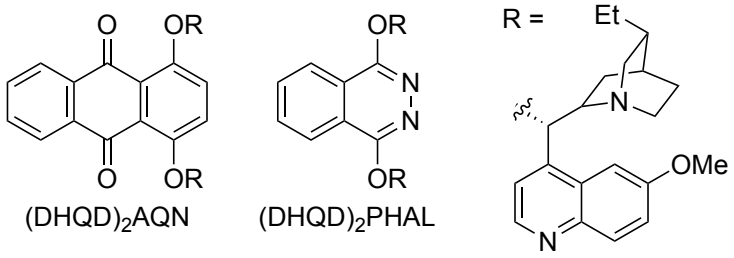
Given the importance of oxidized CLs in apoptosis,[156, 157] efforts were made to demonstrate that oxygenated lipids could be incorporated as well. One of the simplest way to prepare an oxidized fatty acid is from a monounsaturated fatty acid through a Sharpless asymmetric dihydroxylation.[158] The *cis*-fatty acids are known to give poor enantioselectivities, and thus the *trans*-18:1 elaidic acid was chosen as the starting point (Scheme 52, Table 11). The anthraquinone based ligand $(DHQD)_2AQN$ has been shown to provide superior results for isolated *trans*-alkenes compared to the more commonly used $(DHQD)_2PHAL$.[159] This was indeed found to be the case, but conversion was significantly lower (entries 1, 2). Increasing the ligand loading from 1 to 1.6 mol% of $(DHQD)_2PHAL$ allowed for a more satisfactory 93% yield and >95% ee (based on comparison of specific rotation to literature value) of **149** (entry 3). This also permitted the use of the commercially available AD-mix β with a small amount of additional ligand, which simplified reaction setup. Subsequent protection of the diol **149** with TESCl and mild ester hydrolysis conditions using potassium trimethylsilanoate (TMSOK) gave the desired compound **150** in 89% yield over two steps.



Scheme 52: Preparation of protected fatty acid diol 150.

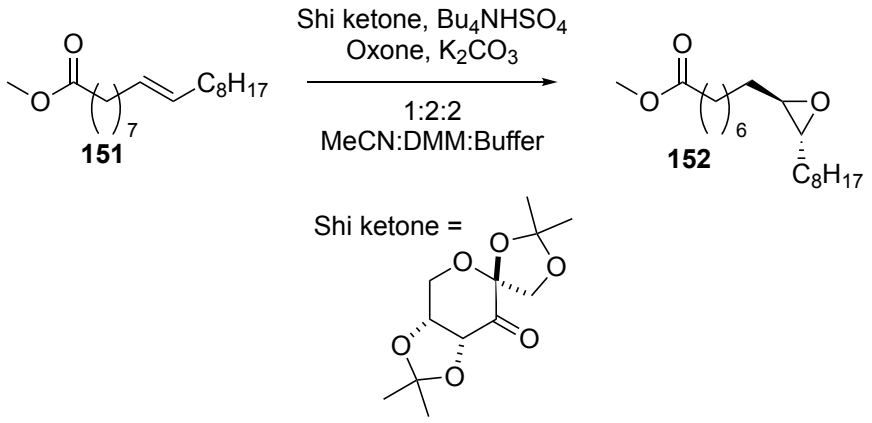
Table 11: Conditions screened for the dihydroxylation of 148-methyl ester.

Entry	Ligand	Loading (mol%)	yield (%)	ee (%)
1	$(DHQD)_2AQN$	1	68	>95
2	$(DHQD)_2PHAL$	1	96	85
3	$(DHQD)_2PHAL$	1.6	93	>95



A similar direct conversion of a simple fatty acid to an epoxidized derivative proved more challenging (Scheme 53, Table 12). Direct Shi epoxidation of elaidic acid methyl ester **151** was first attempted. Under standard reaction conditions using *D*-fructose derived catalyst Shi ketone with 1.4 equiv. of oxone, no reaction was observed. Performing the reaction at a slightly higher temperature had no effect (entry 2). It is well known that the Shi ketone decomposes via a Baeyer-Villiger oxidation if the pH is too low, which significantly hampers the epoxidation (Scheme 54, **153** to **154** and **155**).[160, 161] In the current experiments, the pH was measured between 7 and 8 following the addition of all reagents (entry 3), the same range that had led to rapid catalyst decomposition in prior studies. Adjustment of the pH to ~9 with K_2CO_3 followed by additional portions of both the catalyst and oxone also did not lead to any further conversion. A final attempt to promote epoxidation using superstoichiometric Shi ketone and a large excess

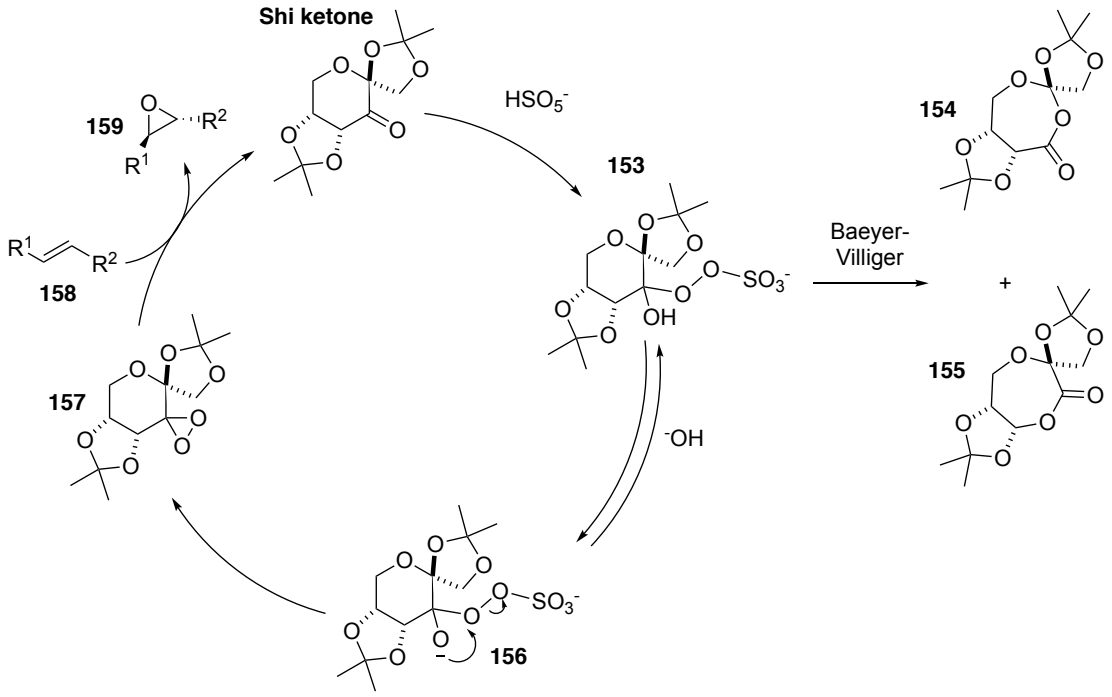
of oxone[162] led to only 10% conversion to the desired epoxide (entry 4). One possible reason for the difficulties in promoting this reaction may be due to **151** forming micelles in the highly polar reaction medium. This would aggregate the alkenes away from the bulk solution in the center of the micelle, which could slow epoxidation enough to lead to either decomposition of the Shi ketone via the Baeyer-Villiger pathway or oxone decomposition preferentially.



Scheme 53: Shi epoxidation of 151.

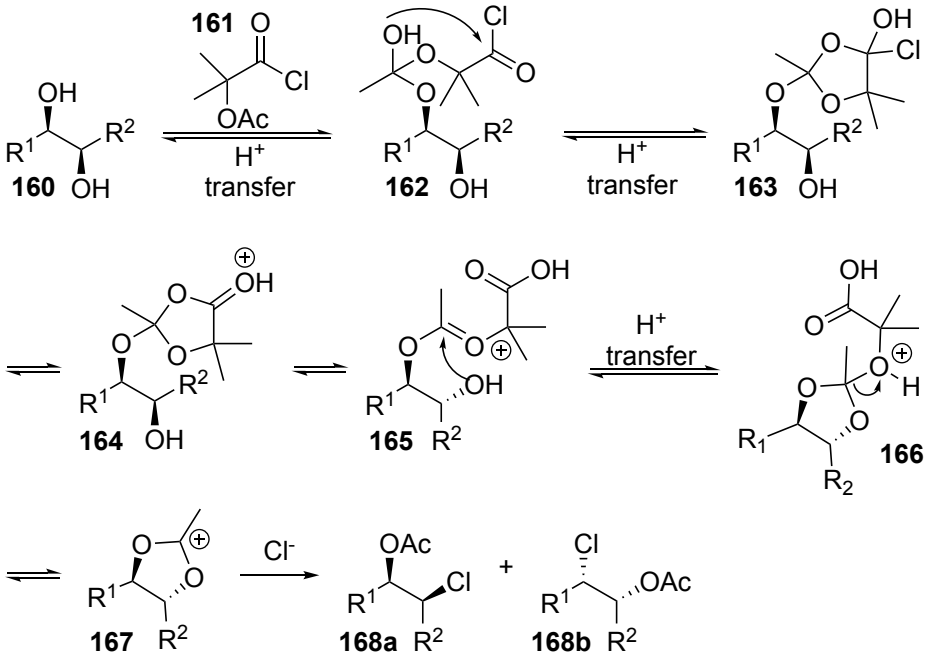
Table 12: Attempted Shi epoxidation. DMM = dimethoxymethane buffer = 0.05 M $Na_2B_4O_7$ in disodium ethylenediaminetetraacetic acid (4 x 10^{-4} M).

Entry	Shi ketone (equiv.)	Oxone (equiv.)	Temp (^oC)	Yield **152**
1	0.3	1.4	-10	R.S.M.
2	0.3	1.4	-5-r.t.	R.S.M.
3	0.3 (x3)	2.1	-5	R.S.M.
4	2.0	3.8	-5	10%



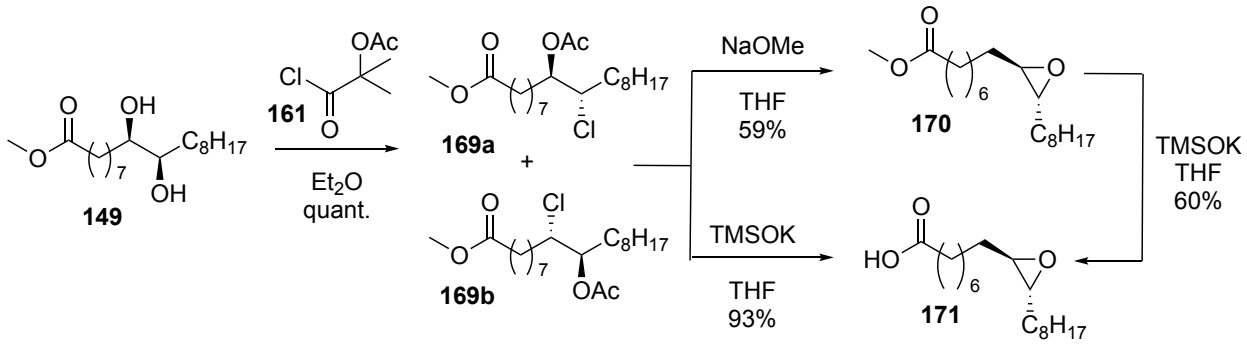
Scheme 54: Mechanism of Shi epoxidation.

Since diol **149** is easily obtained, it was used to access the desired epoxide. The Moffatt reagent **161**[163] has been used to stereospecifically convert vicinal diols to epoxides via the intermediacy of vicinal chloro-acetates.[163, 164] The chloro-acetates are generated through a unique mechanistic pathway (Scheme 55). Because the acid chloride of **161** is sterically hindered, it has been proposed that nucleophilic attack by one alcohol in the diol **160** occurs preferentially at the acetate carbonyl, followed by cyclization onto the acid chloride. Intermediate **163** can then eject a chloride ion. The resulting protonated dioxolanone **164** is poised to undergo ring opening to acetoxonium ion **165**, which is subsequently attacked by the pendant alcohol. Following another proton transfer, loss of the α-hydroxy acid generates a second acetoxonium ion **167**, which can undergo substitution at either carbon resulting in vicinal chloro-acetates **168a-b**. These compounds can be converted to a single epoxide product upon cleavage of the acetates under basic conditions.



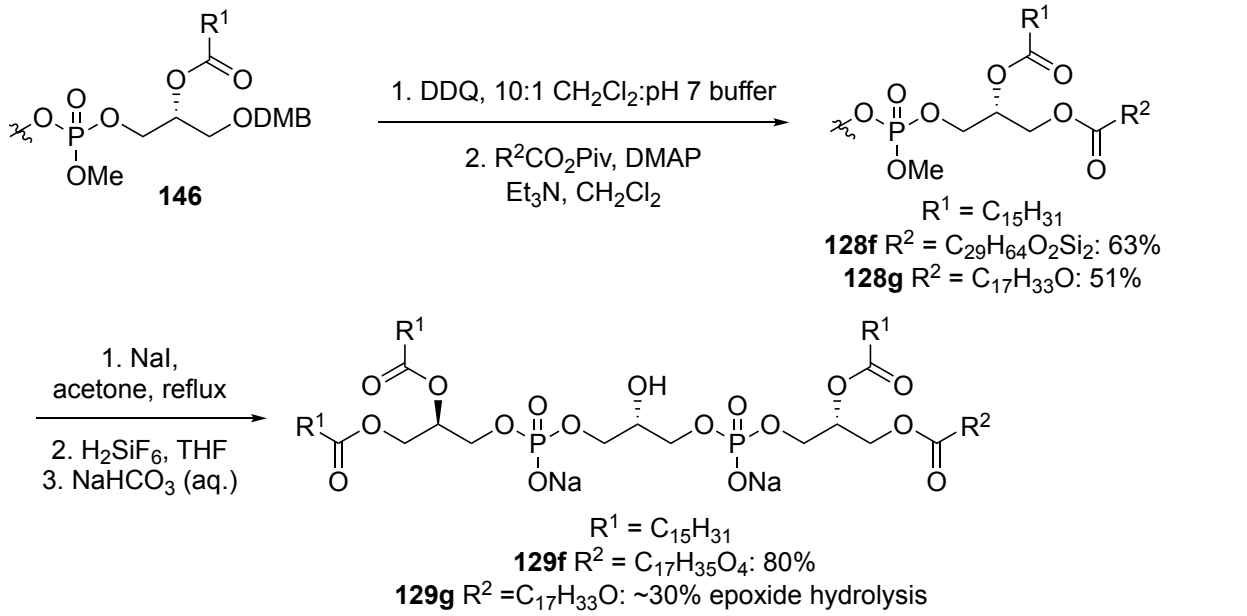
Scheme 55: Generation of vicinal chloro-acetates using the Moffatt reagent.

This method proved to be highly effective, converting diol **149** to 1:1 mixture of vicinal chloro-acetates **169a-b** in quantitative yield (Scheme 56). Initially, stepwise acetate cleavage/epoxide formation with NaOMe and subsequent ester hydrolysis with TMSOK gave the desired epoxy acid **171** in 35% yield over two steps. Ultimately, a one-pot epoxide formation and ester hydrolysis using TMSOK was found to be more effective and delivered the desired product in 93% yield in a single step.



Scheme 56: Preparation of epoxy acid 171.

The oxidized fatty acids could then be incorporated into protected CLs using the bioinspired acylation method (Scheme 57) in comparable yields to the naturally occurring fatty acids. Protected diol derivative **146f** could then be deprotected smoothly using the same demethylation/desilylation sequence used previously, giving 80% of the desired CL **147f**. However, epoxide **146g** undergoes partial epoxide hydrolysis during the final desilylation reaction, likely promoted by the acidic aqueous conditions (H_2SiF_6 is sold as a solution in water).



Scheme 57: Incorporation of oxidized fatty acids using the developed method.

1.6.4 Biological Results

One major conundrum in CL biosynthesis is how the acyl chain composition is controlled during the remodeling process. Two possible explanations are 1) fatty acid availability in donor GPLs and 2) enzymatic selectivity in either the hydrolysis and/or reacylation step(s). *S. cerevisiae* is well suited for studying this process because it only utilizes a single CL remodeling pathway.[165] In studies performed by the Bayir, Greenberg, Hüttemann, and Kagan labs, supplementation of yeast cells with oleic or linoleic acid led to the majority of CL species being tetraacylated with the supplemented acid. This effect was not observed with longer chain polyunsaturated fatty acids arachidonic acid (C20, tetraunsaturated) and docosahexaenoic acid (C22, hexaunsaturated), suggesting that a mechanism exists to select for certain fatty acids and is not controlled by fatty acid availability alone. An increase in the oleic acid and linoleic acid content of donor GPLs PE and PC with corresponding decrease in C16 fatty acid content was also observed. LC/MS analysis of the lyso-PE /lyso-PC content of these experiments also suggested that PEs and PCs containing oleic or linoleic acid were preferentially utilized in reacylating CL.

In order to assess the inherent selectivity of Cld1 for the hydrolysis of particular fatty acid residues, compounds **84a-c**, as well as commercially obtained tetramyristoyl CL (TMCL, saturated C14), tetraoleoyl CL (TOCL, monounsaturated C18), and tetralinoleoyl-CLs (TLCL, diunsaturated C18) were each incubated with purified Cld1 from *S. cerevisiae*. Cld1 showed selectivity for hydrolyzing CLs containing C16 and C18 saturated fatty acids (**84a**, **84c**, and **84b** respectively, shown in pink and red, Figure 15) over shorter chain and C18 unsaturated fatty acid containing CLs (shown in blue) as judged by the appearance of monolyso-CL by HPLC-MS.[74] Additional in vitro experiments using isolated Cld1 and liposomes containing CLs isolated from *cld1Δ* cells (1:1 dioleoyl-PC:CL) also showed primarily the release of palmitic acid. These

studies demonstrate that enzymes involved in CL remodeling have a fundamental selectivity that is at least partially responsible for the observed trend towards unsaturation in CL biosynthesis.

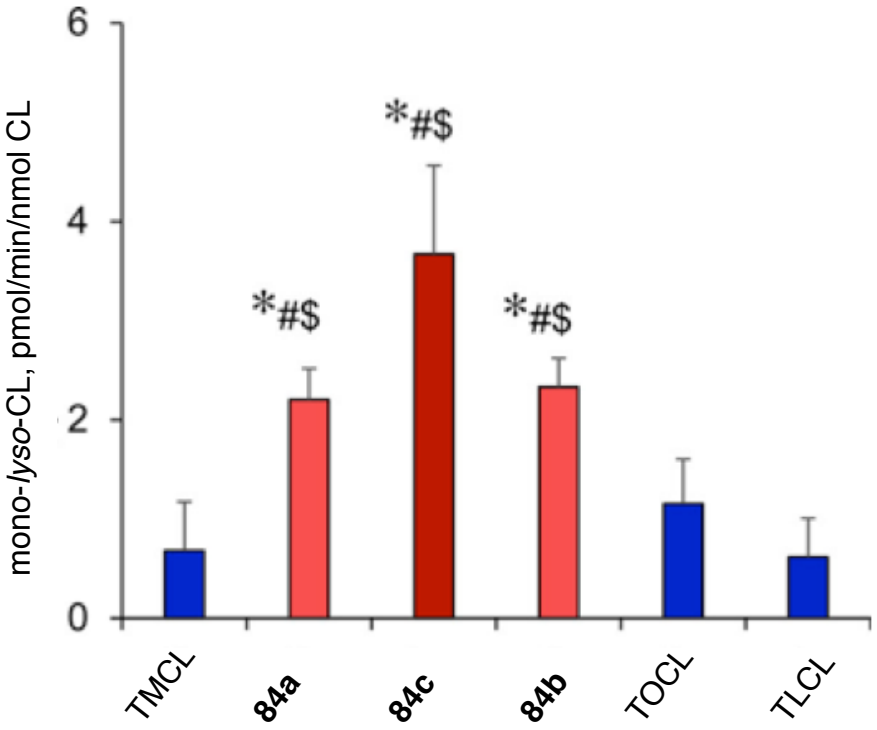


Figure 15: Cld1-catalyzed hydrolysis of various CLs. CL liposomes (1:1 CL:dioleoyl PC) were incubated with Cld1 in 50 mM HEPES buffer for 20 min at 37 °C. Analysis of mono-lyso-CL content was performed after lipid extraction by the Folchs method using LC-MS. *$p < 0.05$ vs mono-*lyso* TMCL, #$p < 0.002$ vs mono-*lyso* TOCL, \$$p < 0.005$ vs mono-*lyso* TLCL ($n = 4–10$). Reprinted with permission from *ACS Chem. Bio.* 2017, *12*, 265-281. Copyright 2017 American Chemical Society.

1.6.5 Conclusion

CLs **84a-c** were prepared as mixtures of diastereomers and enabled the testing of Cld1's hydrolysis selectivity. This has begun to clarify the reason why CLs posses a different fatty acid composition following the remodeling process. During this work, it became clear that there were

no facile ways in which to incorporate precious or unstable fatty acids into the CL scaffold late in the synthesis. The biosynthetic remodeling of CL inspired a strategy that allows for the late stage incorporation of saturated, monounsaturated, oxidation-prone polyunsaturated, and unnatural fatty acids. This method eliminates 3 steps per analog led to the synthesis of enantiopure CLs **129a-f**. While the method was only applied to CLs, it should be equally applicable to other major phospholipids classes as well.

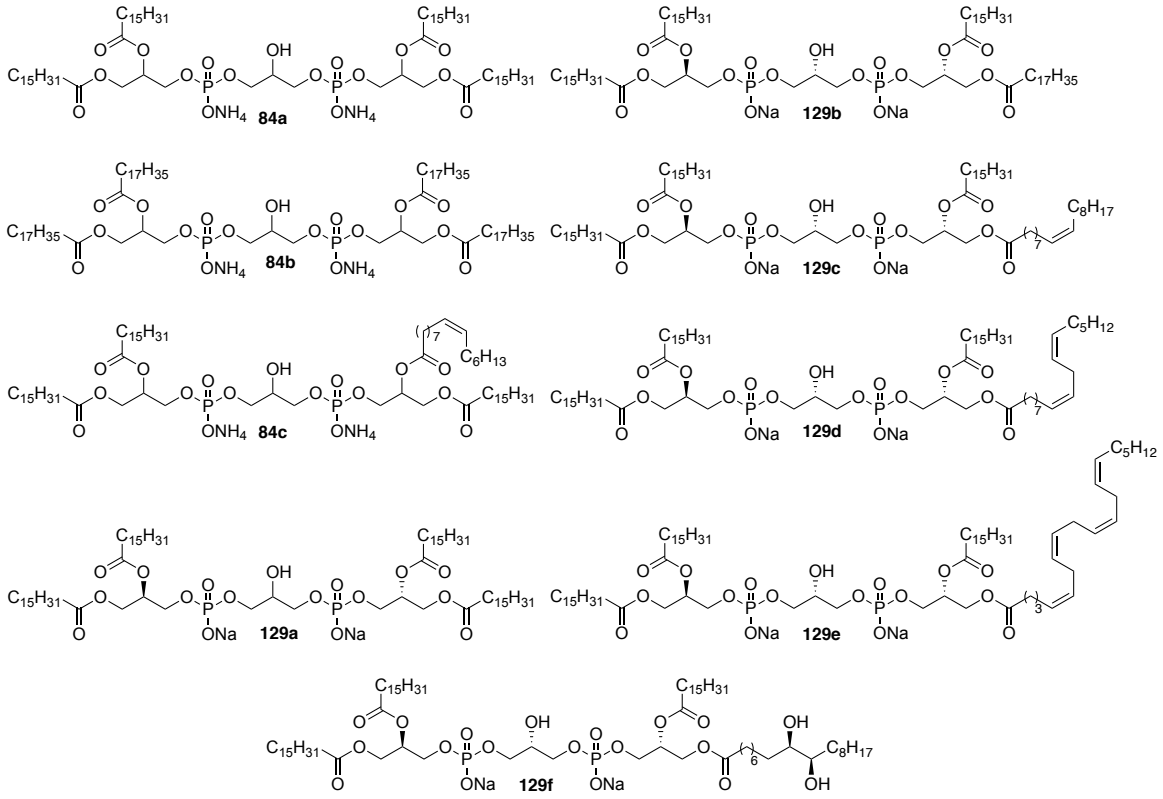


Figure 16: Structure of all CL analogs prepared.

2.0 EXPERIMENTAL SECTION

2.1 GENERAL

All air and moisture sensitive reactions were carried out under a nitrogen or argon atmosphere. All reactions carried out above room temperature were performed using an oil bath or aluminum bead bath set to the specified temperature and monitored with an external thermometer. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone ketyl. Dichloromethane was distilled from CaH_2. Diisopropylethylamine (DIPEA) and diisopropylamine were distilled from and stored over KOH. Deoxygenation was performed by bubbling nitrogen through the solvent for 10 minutes. All other reagents were used as received. Concentration following workups and chromatography was performed using a rotary evaporator connected to a PIAB Lab Vac H40. Traces of solvents were removed from products by high vacuum.

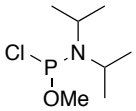
Reactions were monitored by thin layer chromatography analysis (pre-coated silica gel 60 F254 plates, 250 μm layer thickness) and visualized with a 254 nm UV light or by staining with a $KMnO_4$ solution. Flash chromatography on SiO_2 (Silicycle, 40-63 μm) was used for purification where indicated.

Melting points were determined using a Laboratory Devices Mel-Temp II and are uncorrected. Infrared spectra were obtained from neat solids or oils on a Perkin Elmer ATR IR or

Smiths Detection IdentifyIR FT-IR spectrometer. High-resolution mass spectra were obtained on a Thermo Scientific Exactive Orbitrap mass spectrometer using electrospray ionization.

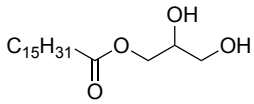
^{1}H NMR spectra were obtained on a Bruker Advance at 300 MHz, 400 MHz, or 500 MHz in $CDCl_3$ or CD_3OD. Chemical shifts (δ) were reported in parts per million with the residual solvent peak used as an internal standard δ ^{1}H / ^{13}C (solvent): 7.26 / 77.00 ($CDCl_3$); 3.31 (CD_3OD). ^{1}H NMR spectra were obtained and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, bs = broad singlet), number of protons, and coupling constant(s). ^{13}C NMR spectra were recorded using a proton-decoupled pulse sequence run at 100 MHz or 125 MHz and are tabulated by observed peaks. ^{31}P NMR spectra were recorded using a complete decoupled pulse sequence run at 202 MHz.

2.2 EXPERIMENTAL PROCEDURES

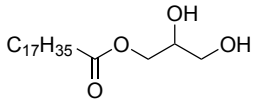


***N,N*-Diisopropylamidomethylchlorophosphite (40)**.[166] To a solution of methyl phosphorodichloridite (10.06 g, 71.91 mmol, 1.0 equiv.) in Et_2O (205 mL) at 0°C under nitrogen was added diisopropylamine (20.2 mL, 144 mmol, 2.0 equiv.) over 45 min. The reaction mixture was then stirred for 16 h at room temperature. The salt was filtered off under nitrogen, rinsed with Et_2O (2x80 mL), and the solvent was distilled off under nitrogen. Purification by distillation under high vacuum gave *N,N*-diisopropylamidomethylchloro-phosphite (**40**, 12.78 g, 64.66 mmol, 90%) as a clear colorless oil. This compound was stored diluted in CH_2Cl_2 (0.5-1.5 M)

at -20 °C under inert gas. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.80-3.72 (m, 2 H), 3.60 (d, 3 H, J = 14.0 Hz), 1.28-1.19 (m, 12 H); ^{31}P NMR ($CDCl_3$, 202 MHz) δ 184.0.

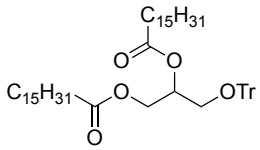


General Procedure A: 1-*O*-Palmitoylglycerol (**109a**).[125, 167] A solution of palmitic acid (4.99 g, 19.5 mmol, 1.0 equiv.), 50% glycidol in CH_2Cl_2 (2.70 mL, 21.4 mmol, 1.1 equiv.) and tributylamine (0.090 mL, 0.37 mmol, 0.02 equiv.) was heated to 85 °C and stirred for 5 h. The crude product was recrystallized from *i*-Pr_2O:Et_2O (1:1) to yield 1-*O*-palmitoylglycerol (**109a**, 4.57 g, 13.8 mmol, 71%) as a colorless solid: Mp 71.9-72.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.22, 4.16 (ABX, 2 H, J_{AB} = 11.7 Hz, J_{AX} = 4.7 Hz, J_{BX} = 6.1 Hz), 3.97-3.90 (m, 1 H), 3.70, 3.60 (ABX, 2 H, J_{AB} = 11.6 Hz, J_{AX} = 3.8 Hz, J_{BX} = 5.8 Hz), 2.35 (t, 2 H, J = 7.7 Hz), 1.66-1.58 (m, 2 H), 1.29-1.25 (m, 24 H), 0.85 (t, 3 H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 70.3, 65.2, 63.3, 34.1, 31.9, 29.7-29.6 (m), 29.4, 29.3, 29.1, 24.9, 22.7, 14.1. IR ν 3293, 2913, 2848, 1728 cm^{-1}.

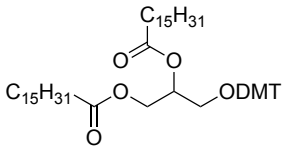


1-*O*-Stearoylglycerol (**109b**).[168] Prepared according to General Procedure A using stearic acid (5.14 g, 17.2 mmol, 1.0 equiv.), 50% glycidol in dichloromethane (2.4 mL, 18.1 mmol, 1.05 equiv.) and tributylamine (0.08 mL, 0.3 mmol, 0.02 equiv.). The crude product was recrystallized from *i*-Pr_2O:Et_2O (1:1) to yield 1-*O*-stearoylglycerol (**109b**, 4.36 g, 12.2 mmol, 71%) as a colorless solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.2, 4.15 (ABX, 2 H, J_{AB} = 11.7 Hz, J_{AX} = 4.6 Hz, J_{BX} = 5.9 Hz), 3.96-3.90 (m, 1 H), 3.70, 3.61 (ABX, 2 H, J_{AB} = 11.4 Hz, J_{AX} = 3.8 Hz,

J_{BX} = 6.1 Hz), 2.71 (bs, 2 H), 2.35 (t, 2 H, J = 7.7 Hz), 1.65-1.60 (m, 2 H), 1.28-1.25 (m, 28 H), 0.88 (t, 3 H, J = 6.6 Hz).

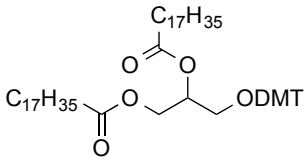


1,2-*O*-Dipalmitoyl-3-*O*-triylglycerol (**45a**).[101] To a solution of **109a** (2.09 g, 6.32 mmol, 1.0 eq.) in pyridine (12 mL) was added TrCl (1.91 g, 6.85 mmol, 1.08 eq.) and DMAP (78 mg, 0.63 mmol, 0.1 eq.). The reaction mixture was heated to 50 °C and stirred for 22 h. The reaction mixture was poured into water (50 mL) and extracted with CH_2Cl_2 (2x25 mL). The organic layers were rinsed with brine (25 mL), dried ($MgSO_4$), and concentrated. A solution of this alcohol in CH_2Cl_2 (22 mL) was then added dropwise to a solution of EDCI (1.24 g, 6.40 mmol, 1.1 eq.), palmitic acid (1.64 g, 6.40 mmol, 1.1 eq.) and DMAP (0.362 g, 2.94 mmol, 0.5 eq.) in CH_2Cl_2 (15 mL) that had been stirring for 15 min prior to addition of the alcohol. Stirring was continued for 4 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL), rinsed with water (15 mL), brine (15 mL), dried ($MgSO_4$), and concentrated. Purification by filtration through SiO_2 (95:5 hexanes:ethyl acetate containing 1% Et_3N) gave 1,2-*O*-dipalmitoyl-3-*O*-triylglycerol (**45a**, 3.81 g 4.00 mmol, 69%, 85% pure) as a colorless solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42 (d, 2.0 H, J = 2 Hz), 7.43-7.41 (m, 6 H), 7.32-7.23 (m, 10 H), 5.27-5.22 (m, 1 H), 4.34, 4.24 (ABX, 2 H, J_{AB} = 11.9 Hz, J_{AX} = 3.6 Hz, J_{BX} = 6.3 Hz), 3.23 (dd, 2 H, J_1 = 2.4 Hz, J_2 = 4.8 Hz), 2.33 (t, 2 H, J = 7.5 Hz), 2.22 (t, 2 H, J = 7.5 Hz), 1.65-1.54 (m, 4 H), 1.28-1.25 (m, 48 H), 0.91 (t, 3 H, J = 6.6 Hz).

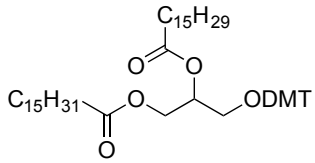


General Procedure B: 1,2-*O*-Dipalmitoyl-3-*O*-dimethoxyltriylglycerol (**110a**). To a solution of **109a** (4.57 g, 13.8 mmol, 1.0 equiv.) in 1:2 pyridine:CH_2Cl_2 (35 mL) at 0°C was added dimethoxyltrityl chloride (DMTCl) (4.82 g, 14.1 mmol, 1.0 equiv.) and DMAP (0.177 g, 1.43 mmol, 0.1 equiv.). The orange solution was stirred at this temperature for 15 min., then warmed to room temperature and stirred for 13 h. The orange reaction mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2x50 mL). The organic portion was rinsed with water (40 mL), brine (40 mL), dried ($MgSO_4$), and concentrated. The residue was then concentrated from PhMe (2x20 mL) and the resulting orange oil used without further purification. A solution of EDCI (2.93 g, 15.3 mmol, 1.1 equiv.), palmitic acid (3.91 g, 15.3 mmol, 1.1 equiv.) and DMAP (0.851 g, 6.90 mmol, 0.5 equiv.) in CH_2Cl_2 (60 mL) was allowed to stir at room temperature under nitrogen for 15 min. Then, the previously obtained oil from DMT protection (8.77 g, 13.9 mmol 1.0 eq) in CH_2Cl_2 (30 mL) was added and stirring continued for 4 h. The reaction mixture was diluted with water (50 mL) and the layers separated. The organic layer was rinsed with water (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated. Purification by filtration through SiO_2 (9:1 CH_2Cl_2:hexanes) gave 1,2-*O*-dipalmitoyl-3-*O*-dimethoxyltriylglycerol (**110a**,7.94 g, 9.11 mmol, 66%) as a colorless solid. Acceptable purity can also be obtained by trituration with MeOH several times. Mp 64.5-66.0 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42 (d, 2.0 H, J = 2 Hz), 7.40-7.20 (m, 7 H), 6.82 (d, 4 H, J = 8.7 Hz), 5.28-5.22 (m, 1 H), 4.34, 4.24 (ABX, 2 H, J_{AB} = 11.7 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.7 Hz), 3.79 (s, 6 H), 3.22 (d, 2 H, J = 5.1 Hz), 2.33 (t, 2 H, J = 7.5 Hz), 2.23 (t, 2 H, J = 7.5 Hz), 1.65-1.53 (m, 4 H), 1.28-1.25 (m, 48 H), 0.91 (t, 3 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 173.0, 158.5, 144.5, 135.7, 130.0,

128.1, 127.8, 126.8, 113.1, 86.0, 70.5, 62.9, 62.0, 55.1 34.4, 34.1, 31.9, 29.7-29.1 (m), 25.0, 24.8, 22.7, 14.1; IR ν 2915, 2846, 1729 cm^{-1}; HRMS (ESI^+) *m/z* calculated $C_{56}H_{87}O_7$ $[M+H]^+$ 871.6452; found 303.14 (DMT^+).

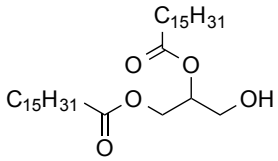


1,2-*O*-Distearoyl-3-*O*-dimethoxyltriylglycerol (**110b**). Prepared according to General Procedure B using **109b** (4.36 g, 12.2 mmol, 1.0 equiv.), DMTCl (4.29 g, 12.5 mmol, 1.03 equiv.) and DMAP (0.145 g, 1.18 mmol, 0.1 equiv.). For the second step, EDCI (2.57 g, 13.4 mmol, 1.1 equiv.), stearic acid (4.01 g, 13.4 mmol, 1.1 equiv.) and DMAP (0.754 g, 6.11 mmol, 0.5 equiv.) were used. Purification by trituration with MeOH gave 1,2-*O*-distearoyl-3-*O*-dimethoxyltriylglycerol (**110b**, 6.68 g, 6.48 mmol, 53%) as a colorless solid. Mp 67-68.4 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, 2 H, *J* = 7.2 Hz), 7.35-7.24 (m, 7 H), 6.82 (d, 4 H, *J* = 8.7 Hz), 5.29-5.28 (m, 1 H), 4.34, 4.24 (ABX, 2 H, J_{AB} = 11.7 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.7 Hz), 3.82 (s, 6 H), 3.25 (d, 2 H, *J* = 3.3 Hz), 2.36 (t, 2 H, *J* = 7.5 Hz), 2.27 (t, 2 H, *J* = 7.5 Hz), 1.69-1.56 (m, 4 H), 1.29 (m, 54 H), 0.91 (t, 3 H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 173.0, 158.5, 144.6, 135.8 (2C), 130.0 (2C), 128.1, 127.8, 126.8, 113.1, 113.0, 86.1, 70.5, 62.9, 62.1, 55.2, 34.4, 34.1, 31.9, 31.8, 29.7-29.1 (m), 25.0, 24.9, 22.7, 14.1; IR ν 2915, 2848, 1730 cm^{-1}.

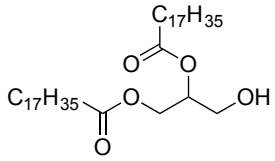


1-*O*-Palmitoyl-2-*O*-palmitoleoyl-3-*O*-dimethoxyltriylglycerol (110c). Prepared according to General Procedure B using **109a** (2.04 g, 6.17 mmol, 1.0 equiv.), DMTCl (2.15 g, 6.28 mmol,

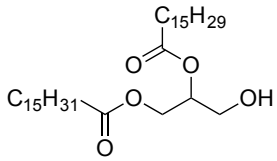
1.0 equiv.), and DMAP (0.079 g, 0.64 mmol, 0.1 equiv.). For the second step, EDCI (228 mg, 1.19 mmol, 1.1 equiv.), palmitoleic acid (309 mg, 1.19 mmol, 1.1 equiv.) and DMAP (64 mg, 0.52 mmol, 0.47 equiv.) were used. Purification by chromatography on SiO_2 (95:5 hexanes:ethyl acetate) gave 1-*O*-palmitoyl-2-*O*-palmitoleoyl-3-*O*-dimethoxyltriylglycerol (**110c**, 0.619 g, 0.712 mmol, 65%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (d, 2 H, J = 8.4 Hz), 7.31-7.25 (m, 7 H), 6.82 (d, 4 H, J = 8.7 Hz), 5.40-5.30 (m, 2 H), 5.29-5.22 (m, 1 H), 4.35, 4.24 (ABX, 2 H, J_{AB} = 11.7 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.7 Hz), 3.79 (s, 6 H), 3.23-3.21 (m, 2 H), 2.33 (t, 2 H, J = 7.5 Hz), 2.23 (t, 2 H, J = 7.7 Hz), 2.02-2.00 (m, 4 H), 1.67-1.53 (m, 4 H), 1.30-1.26 (m, 40 H), 0.88 (t, 6 H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 173.0, 158.5, 144.6, 135.8 (2C), 130.0 (2C), 128.1, 127.8, 126.8, 113.1, 113.0, 86.1, 70.5, 62.9, 62.0, 55.2, 34.4, 34.1, 31.9, 31.8, 29.7-29.0 (m), 25.0, 27.2, 25.0, 24.9, 22.7, 22.6, 14.1 (2C); IR ν 2923, 2853, 2093, 1741, 1250 cm^{-1}.



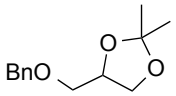
General Procedure C: 1,2-*O*-Dipalmitoylglycerol (46a).[169] To a solution **110a** (7.94 g, 9.11 mmol, 1.0 equiv.) in 1:1 $CHCl_3$:MeOH (90 mL) was added Amberlite IR-120H (4.0 g). The reaction mixture was stirred 2.5 h, then filtered through basic alumina (ethyl acetate). The resulting yellow oil was precipitated from 3:1 Et_2O:hexanes and filtered giving 1,2-*O*-dipalmitoylglycerol (**46a**, 3.75 g, 5.50 mmol, 60%, 90% pure) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.08 (pent, 1 H, J = 5.0 Hz), 4.32, 4.24 (ABX, 2 H, J_{AB} = 11.8 Hz, J_{AX} = 4.5 Hz, J_{BX} = 5.9 Hz), 3.74-3.73 (m, 2 H), 2.33 (apparent q, 4 H, J = 8.0 Hz), 1.65-1.58 (m, 4 H), 1.28-1.25 (m, 48 H), 0.88 (t, 3 H, J = 6.6 Hz).



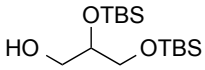
1,2-*O*-Distearoylglycerol (46b).[169] Prepared according to General Procedure C using **110b** (4.34 g, 4.68 mmol, 1.0 equiv.) and Amberlite IR-120H (2.07 g). Purification by trituration with 3:1 Et_2O:hexanes gave 1,2-*O*-distearoylglycerol (**46b**, 2.08 g, 3.39 mmol, 72%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.08 (pent, 1 H, J = 5.0 Hz), 4.32, 4.25 (ABX, 2 H, J_{AB} = 12.0 Hz, J_{AX} = 4.5 Hz, J_{BX} = 5.5 Hz), 3.79-3.72 (m, 2 H), 2.34 (t, 2 H, J = 7.7 Hz), 2.32 (t, 2 H, J = 7.5 Hz), 2.00 (t, 1 H, J = 6.25 Hz), 1.64-1.54 (m, 4 H), 1.29-1.25 (m, 56 H), 0.88 (t, 3 H, J = 7.0 Hz).



1-*O*-Palmitoyl-2-*O*-palmitoleoylglycerol (46c). Prepared according to General Procedure C using **110c** (100 mg, 0.115 mmol, 1.0 equiv.) and Amberlite IR-120H (52 mg). Purification by chromatography on SiO_2 (1:0 to 5:1 CH_2Cl_2:ethyl acetate) gave 1-*O*-palmitoyl-2-*O*-palmitoleoylglycerol (**46c**, 64 mg, 0.113 mmol, 98%) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40-5.30 (m, 2 H), 5.10-5.05 (m, 1 H), 4.32, 4.25 (ABX, 2 H, J_{AB} = 12.2 Hz, J_{AX} = 4.4 Hz, J_{BX} = 5.5 Hz), 3.78-3.70 (m, 2 H), 2.33 (apparent q, 4 H, J = 7.3 Hz), 2.02-2.00, (m, 4 H), 1.64-1.54 (m, 4 H), 1.30-1.27 (m, 40 H), 0.88 (t, 6 H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 173.4, 130.0, 129.7 128.1, 72.1, 62.0, 61.6, 34.3, 34.1, 31.9, 31.8, 29.7-29.0 (m), 27.2, 25.0, 24.9, 22.7, 22.6, 14.1; IR ν 3502, 2919, 2850, 2093, 1737, 1163 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{35}H_{67}O_5$ $[M+H]^+$ 567.4983, found 567.4960.



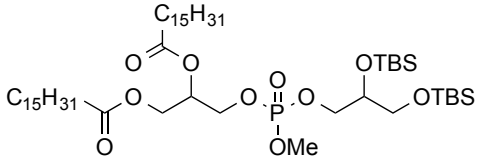
4-((Benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (111).[169] A slurry of 60% NaH in mineral oil (360 mg, 9.00 mmol, 1.2 equiv.) in THF (10 mL) at 0 °C under nitrogen was added a solution of 2,2-dimethyl-1,3-dioxolane-4-methanol (1.0 mL, 7.8 mmol, 1.0 equiv.) in THF (5 mL). The reaction mixture was stirred for 30 min., then BnBr (0.98 mL, 1.4 mmol, 1.1 equiv.) was added in a drop wise fashion. The reaction mixture was warmed to room temperature and stirred for 2.5 h. The reaction was quenched with water (8 mL), diluted with Et_2O (20 mL), and the layers separated. The aqueous portion extracted with Et_2O (25 mL) and the combined organic fractions were rinsed with, brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (95:5 to 9:1 to 85:15 hexanes: ethyl acetate) gave 4-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (**111**, 1.15 g, 5.17 mmol, 67%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35-7.29 (m, 5 H), 4.60, 4.56 (ABq, 2 H, J_{AB} = 12.0 Hz), 4.31 (pent, 1 H, J = 6.0 Hz), 4.06 (dd, 1 H, J_1 = 6.4 Hz, J_2 = 8.0 Hz), 3.75 (dd, 1 H, J_1 = 6.4 Hz, J_2 = 8.4 Hz), 3.56, 3.46 (ABX, 2 H, J_{AB} = 9.8 Hz, J_{AX} = 5.9 Hz, J_{BX} = 5.7 Hz), 1.42 (s, 3 H), 1.37 (s, 3 H).



2,3-*O*-Bis(*tert*-butyldimethylsilyl)glycerol (101).[170] **111** (1.15 g, 5.17 mmol, 1.0 equiv.) was dissolved in 2:1 THF:H_2O (10 mL), 12 M HCl (2.1, 25 mmol, 4.9 equiv.) added, and the reaction mixture was stirred for 1 h. The reaction was poured into sat. $NaHCO_3$ (30 mL) and extracted with Et_2O (3x 25 mL). The organic portion was rinsed brine (25 mL), dried ($MgSO_4$), and concentrated. The resulting yellow oil was dissolved in CH_2Cl_2 (8.7 mL) at 0 °C under nitrogen Et_3N (1.4 mL, 9.9 mmol, 2.3 equiv.) and TBSOTf (2.2 mL, 9.5 mmol, 2.2 equiv.) added, and the

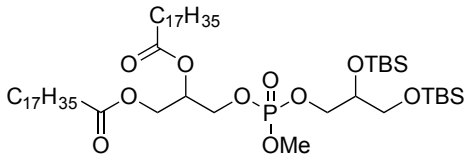
cloudy white reaction mixture was stirred for 3 h. The reaction mixture was quenched with H_2O (8 mL) and diluted with CH_2Cl_2 (10 mL). The layers were separated and the organic portion rinsed with brine (8 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (99:1 to 95:5 hexanes:ethyl acetate) gave 1-*O*-benzyl-2,3-*O*-bis(*tert*-butyldimethylsilyl)glycerol (1.72, 4.31 mmol, 83% over 2 steps) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.33 (m, 4 H), 7.28-7.26 (m, 1 H), 4.54 (s, 2 H), 3.86 (pent, 1 H, J = 5.3 Hz), 3.61 (dd, 1 H, J_1 = 6 Hz, J_2 = 10.0 Hz), 3.57-3.52 (m, 2 H), 3.42 (dd, 1 H, J_1 = 5.5 Hz, J_2 = 10.0 Hz), 0.87 (s, 18 H), 0.070 (d, 6 H, J = 1.5 Hz), 0.042 (s, 6 H).

To a solution of the intermediate (1.62 g, 4.06 mmol, 1.0 equiv.) in ethyl acetate (20 mL) was added 10% Pd/C (0.161 g, 0.152 mmol, 0.037 equiv.). The mixture was submitted to hydrogenation on a Parr hydrogenator under H_2 (5 atm) for 1 h. The mixtures were filtered through Celite (ethyl acetate) and concentrated giving 2,3-*O*-bis(*tert*-butyldimethylsilyl)glycerol (**101**, 1.15 g, 3.59 mmol, 88%) as a clear colorless oil that can be used without further purification. An analytically pure sample can be obtain by chromatography on SiO_2 (95:5 to 9:1 hexanes:ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.79-3.74 (m, 1 H), 3.69-3.53 (m, 4 H), 2.10 (bs, 1 H), 0.89 (s, 18 H), 0.10-0.06 (m, 12 H).



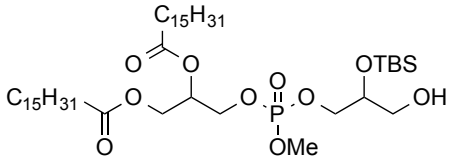
General Procedure D: 3-(((2,3-Bis((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy) phosphoryl)oxy)propane-1,2-diyl dipalmitate (112a). To a solution of **46a** (1.73 g, 2.89 mmol, 1.0 equiv.) and DIPEA (0.53 mL, 3.2 mmol, 1.1 equiv.) in CH_2Cl_2 (30 mL) under nitrogen was added 0.5 M **40** (6.0 mL, 3.0 mmol, 1.1 equiv.) and the reaction mixture was stirred for 1 h. To

this solution was added **101** (0.921 g, 2.87 mmol, 0.99 equiv.) and 4,5-dicyanoimidazole (0.700 g, 5.80 mmol, 2 equiv.) in THF (4.9 mL) in a dropwise fashion. The reaction mixture was stirred for 1 h, cooled to 0 °C, Bu_4NIO_4 (1.46 g, 3.36 mmol, 1.2 equiv.) added, and stirring continued for 30 min. The reaction mixture was diluted with water (15 mL) and the layers separated. The combined organic layers were rinsed with sat. $Na_2S_2O_3$ (15 mL), sat. $NaHCO_3$ (15 mL), brine (15 mL), dried ($MgSO_4$), and concentrated. The crude material was then filtered through a plug of SiO_2 (1:1 hexanes:ethyl acetate) and concentrated. Purification by chromatography on SiO_2 (5:1 to 3:1 hexanes:ethyl acetate) gave 3-(((2,3-bis((*tert*-butyldimethylsilyl)oxy)propoxy) (methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**112a**, 1.42 g, 1.47 mmol, 51%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.22 (pent, 1 H, J = 4.8 Hz), 4.36-4.30 (m, 1 H), 4.19-4.09 (m, 4 H), 3.97-3.83 (m, 2 H), 3.76 (d, 3 H, J = 11.1 Hz; diasteromers visible), 3.56-3.54 (m, 2 H), 2.31 (q, 4 H, J = 7.10 Hz), 1.61-1.56 (m, 4 H), 1.28-1.25 (m, 48 H), 0.88-0.85 (m, 24 H), 0.08 (s, 3 H), 0.07 (s, 3 H), 0.05 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.7, 72.0 (d, $^3J_{CP}$ = 7.5 Hz), 69.4 (d, $^3J_{CP}$ = 7.5 Hz), 69.2-69.1 (m), 65.4-65.3 (m), 64.0, 61.6, 55.4 (d, $^2J_{CP}$ = 6.3 Hz), 34.1, 34.0, 31.9, 31.6, 29.7-29.1 (m), 25.8, 25.7, 24.8, 22.7, 22.6, 18.3, 18.1,14.1, -4.7, -4.8, -5.5 (2C); IR ν 2922, 2854, 1741, 1252, 835 cm^{-1}, HRMS (ESI$^+$) *m/z* calculated for $C_{51}H_{106}O_{10}PSi_2$ $[M+H]^+$ 965.7057; found 965.7086.



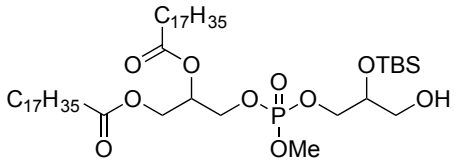
3-(((2,3-Bis((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl distearate (112b). Prepared according to General Procedure D using **46b** (542 mg, 0.884 mmol, 1.0 equiv.), DIPEA (0.16 mL, 0.98 mmol, 1.1 equiv.), and 0.5 M **40** (1.9 mL, 0.98 mmol, 1.1 equiv.). For the second step, **101** (283 mg, 0.884 mmol, 1.1 equiv.) and 4,5-dicyanoimidazole

(213 mg, 1.77 mmol, 2.0 equiv.), and Bu_4NIO_4 (455 mg, 1.05 mmol, 1.2 equiv.) were used. Purification by chromatography on SiO_2 (5:1 hexanes:ethyl acetate) gave 3-(((2,3-bis((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl distearate (**112b**, 0.605 g, 0.592 mmol, 67%) as a waxy colorless solid. Mp = 31.0-31.9 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.24-5.20 (m, 1 H), 4.37-4.31 (m, 1 H), 4.22-4.09 (m, 4 H), 3.97-3.84 (m, 2 H), 3.77 (d, 3 H, J =11.1 Hz; diastereomers visible), 3.59-3.49 (m, 2 H), 2.31 (apparent q, 4 H, J = 7.0 Hz), 1.61-1.57 (m, 4 H), 1.28-1.25 (m, 56 H), 0.88-0.86 (m, 24 H), 0.09 (s, 3 H), 0.08 (s, 3 H), 0.05 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.7, 72.0 (d, $^3J_{CP}$ = 8.8 Hz), 69.4 (d, $^3J_{CP}$ = 7.5 Hz), 69.2-69.1 (m), 65.4-65.3 (m), 64.0, 61.7, 54.4 (d, $^2J_{CP}$ = 5.0 Hz), 34.2, 34.0, 31.9, 29.7-29.1 (m), 25.9, 25.7, 24.8, 22.7, 22.6, 18.3, 18.1, 14.1, -4.7, -4.8, -5.4, -5.5; IR ν 2923, 2852, 1743, 1252, 835 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{55}H_{114}O_{10}PSi_2$ $[M+H]^+$ 1021.7688, found 1021.7766.

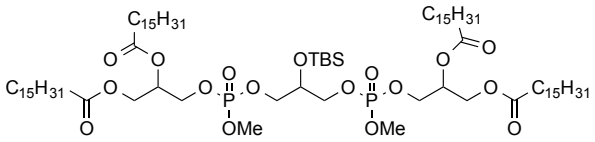


General Procedure E 3-(((2-((*Tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)(methoxy) phosphoryl)oxy) propane-1,2-diyl dipalmitate (113a). To a solution of **112a** (800 mg, 0.829 mmol, 1.0 equiv.) in 1:1 CH_2Cl_2:MeOH (8.3 mL) at 0 °C was added camphorsulfonic acid (195 mg, 0.823 mmol, 1.0 equiv.). The reaction mixture was maintained between -5 and 5 °C and stirred for 5.5 h. The reaction mixture was filtered through a small plug of basic alumina (ethyl acetate) and concentrated. Purification by chromatography on SiO_2 (3:1 to 1:1 hexanes:ethyl acetate) gave 3-(((2-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)(methoxy)phosphoryl) oxy)propane-1,2-diyl dipalmitate (**113a**, 435 mg, 0.511 mmol, 62%) as a white solid. Mp 27.8-28.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.29-5.20 (m, 1 H), 4.33 (dd, 1 H, J_1 = 4.4 Hz, J_2

=12.0 Hz), 4.21-4.14 (m, 3 H), 4.06-4.01 (m, 2 H), 3.93-3.89 (m, 1 H), 3.80-3.76 (m, 3 H), 3.67-3.50 (m, 2 H), 2.35-2.31 (m, 5 H), 1.62-1.60 (m, 4 H), 1.28-1.25 (m, 48 H), 0.90-0.86 (m, 15 H), 0.11 (s, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 172.8 (diastereomers visible), 71.1 (d, $^3J_{CP}$ = 7.0 Hz), 69.4-69.3 (m), 67.7 (m), 65.6-65.5 (m), 63.0, 61.6, 54.6 (m), 34.1, 34.0, 31.9, 29.7-29.1 (m), 25.7, 24.8, 22.7, 18.0, 14.1, -4.8, -4.9; IR ν 2922, 2854, 1741, 1252, 835 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{45}H_{92}O_{10}PSi$ $[M+H]^+$ 851.6192, found 851.6180.

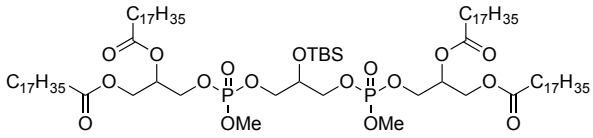


3-(((2-((*Tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl distearate (113b). Prepared according to General Procedure E using **112b** (961 mg, 0.941 mmol, 1.0 equiv.) and CSA (221 mg, 0.932 mmol, 0.99 equiv.). Purification by chromatography on SiO_2 (4:1 to 1:1 hexanes:ethyl acetate) gave 3-(((2-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl distearate (**113b**, 470 mg, 0.518 mmol, 55%) as a waxy colorless solid. Mp = 41.2-41.7 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.25-5.22 (m, 1 H), 4.34 (dd, 1 H, J_1 = 4.2 Hz, J_2 = 12.0 Hz), 4.19-4.11 (m, 3 H), 4.05-4.02 (m, 2 H), 3.95-3.90 (m, 1 H), 3.78 (d, 3 H, J = 11.4 Hz; diastereomers visible), 3.67-3.60 (m, 2 H), 2.36-2.29 (m, 5 H), 1.62-1.58 (m, 4 H), 1.28-1.25 (m, 56 H), 0.90-0.86 (m, 15 H), 0.11 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 172.8, 71.1 (d, $^3J_{CP}$ = 7.1 Hz), 69.4 (m), 67.7 (m), 65.6 (m), 63.0, 61.6, 54.6 (m), 34.2, 34.0, 31.9, 29.7-29.1 (m), 25.7, 24.8, 22.7, 18.0, 14.1, -4.7, -4.9; IR ν 3535, 2921, 2850, 1739, 733 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{49}H_{100}O_{10}PSi$ $[M+H]^+$ 907.6818, found 907.6785.



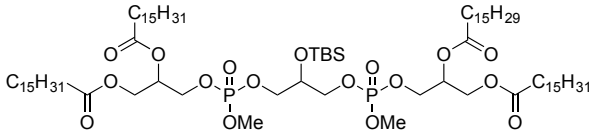
General Procedure F: 3-(((3-(((2,3-(Palmitoyloxy)propoxy)(methoxy) phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propan e-1,2-diyldipalmitate (114a). To a solution of **113a** (322 mg, 0.378 mmol, 1.0 equiv.) in CH_2Cl_2 (3.7 mL) at 0 °C under nitrogen was added DIPEA (0.069 mL, 0.42 mmol, 1.1 equiv.) and 0.5 M **40** in CH_2Cl_2 (0.83 mL, 0.42 mmol, 1.1 equiv.). The reaction mixture was warmed to room temperature and stirred for 1.5 h. To this solution was added **46a** (364 mg, 0.416 mmol, 1.1 equiv.) and 4,5-dicyanoimidazole (90 mg, 0.76 mmol, 2.0 equiv.) in THF (1.5 mL) in a dropwise fashion. The reaction mixture was stirred for 2 h, cooled to 0 °C, Bu_4NIO_4 (186 mg, 0.429 mmol, 1.1 equiv.) added, and stirring continued 40 min. The reaction mixture was diluted with water (3 mL) and CH_2Cl_2 (5 mL) and the layers separated. The combined organic layers were rinsed with sat. $Na_2S_2O_3$ (3 mL), brine (3 mL), dried ($MgSO_4$), and concentrated. The crude product was filtered through a plug of SiO_2 (1:1 hexanes:ethyl acetate) and concentrated. Purification by chromatography on SiO_2 (3:1 to 2:1 to 1:1 hexanes:ethyl acetate) gave 3-(((3-(((2,3-(palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy) propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**114a**, 381 mg, 0.255 mmol, 67%) as a colorless solid. Mp 36.5-37.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.25-5.22 (m, 2 H), 4.36-4.31 (m, 2 H), 4.22-4.12 (m, 6 H), 4.05-3.97 (m, 5 H), 3.78 (d, 6 H, J = 14.0 Hz; diastereomers visible), 2.32 (apparent q, 8 H, J = 8.0 Hz), 1.61-1.57 (m, 8 H), 1.28-1.25 (m, 96 H), 0.89-0.86 (m, 21 H), 0.11 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 172.7, 69.8 (m), 69.4 (d, $^3J_{CP}$ = 6.3 Hz), 67.8 (d, $^3J_{CP}$ = 6.3 Hz), 65.7 (m), 61.6, 54.6 (d, $^2J_{CP}$ = 6.3 Hz), 34.2,

34.0, 31.9, 29.7-29.1 (m), 25.6, 24.9, 22.7, 18.0, 14.1, -4.9; IR ν 2913, 2848, 1737, 1465, 913, 744 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{81}H_{161}O_{17}P_2Si$ $[M+H]^+$ 1496.0973, found 1496.0903.



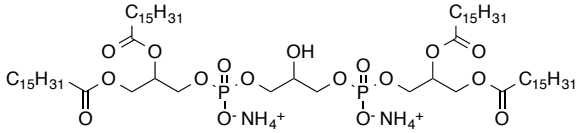
General Procedure G: 3-(((3-(((2,3-(Stearoyloxy)propoxy)(methoxy) phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propan e-1,2-diyl distearate (114b). To a solution of **46b** (356 mg, 0.570 mmol, 1.0 equiv.) and DIPEA (0.10 mL, 0.63 mmol, 1.1 equiv.) under nitrogen was added 0.5 M **44** (1.2 mL, 0.63 mmol, 1.1 equiv.) in CH_2Cl_2 and the reaction mixture stirred for 1 h. To this solution was added a solution of **113b** (470 mg, 0.518 mmol, 1.0 equiv.) and 4,5-dicyanoimidazole (125 mg, 1.04 mmol, 2.0 equiv.) in THF (1.5 mL) in a dropwise fashion The reaction mixture was stirred for 3 h, cooled to 0 °C, Bu_4NIO_4 (173 mg, 0.399 mmol, 1.2 equiv.) added, and stir 45 min. The reaction mixture was diluted with water (2 mL) and CH_2Cl_2 (5 mL) and the layers separated. The combined organic layers were rinsed with sat. $Na_2S_2O_3$ (2x3 mL), sat. $NaHCO_3$ (3 mL), brine (3 mL), dried ($MgSO_4$), and concentrated. The crude product was filtered through a plug of SiO_2 (1:1 hexanes:ethyl acetate) and concentrated. To aide in separation of starting alcohols from the desired product, this material was dissolved in CH_2Cl_2 (4 mL) and TBSCl (43 mg, 0.29 mmol 0.5 equiv.), imidazole (21 mg, 0.31 0.6 equiv.) and DMAP (3 mg, 0.024 mmol 0.04 equiv.) added. The mixture was stirred for 4.5 h. The reaction was quenched with water (4 mL) and diluted with CH_2Cl_2 (4 mL). The layers were separated, and the organic layer rinsed with brine (4 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (5:1 to 3:1 to 1:1 hexanes:ethyl acetate) gave 3-(((3-(((2,3-(stearoyloxy)propoxy)(methoxy) phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,

2-diyl distearate (**114b**, 335 mg, 0.208 mmol, 40%) as a waxy colorless solid. Mp = 48.1-48.2 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.25-5.22 (m, 2 H), 4.36-4.30 (m, 2 H), 4.29-4.04 (m, 11 H), 3.78 (d, 6 H, J = 11.4 Hz; diastereomers visible), 2.32 (apparent q, 8 H, J = 7.2 Hz), 1.61-1.57 (m, 8 H), 1.25 (m, 112 H), 0.89-0.86 (m, 21 H), 0.11 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.8, 69.7 (m), 69.4 (m), 67.9 (m), 65.6 (m), 61.6, 54.6 (d, $^{2}J_{CP}$ = 5.9 Hz), 34.2, 34.0, 31.9, 29.7-29.1 (m), 25.6, 24.8, 22.7, 18.0, 14.1, -4.9; IR ν 2918, 2851, 1741, 1043 cm^{-1}; HRMS (ESI^{+}) *m/z* calculated for $C_{89}H_{177}O_{17}P_2Si$ $[M+H]^{+}$ 1608. 2225, found 1608.2190.

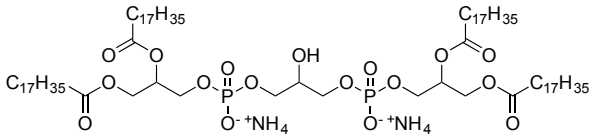


3-(((3-(((2-Palmitoleoyloxy-3-(palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (127c). Prepared according to General Procedure G using **46c** (156 mg, 0.275 mmol, 1.0 equiv.) and DIPEA (0.050 mL, 0.30 mmol, 1.1 equiv.) under nitrogen was added 0.5 M **40** (0.60 mL, 0.30 mmol, 1.1 equiv.). For the second step, **113a** (212 mg, 0.249 mmol, 1.0 equiv.), 4,5-dicyanoimidazole (60 mg, 0.50 mmol, 2.0 equiv.), and Bu_4NIO_4 (128 mg, 0.30 mmol, 1.2 equiv.) were used. For the third step, TBSCl (19 mg, 0.5 equiv.), imidazole (17 mg, 1 equiv.) and DMAP (3 mg, 0.1 equiv.) were used. Purification by chromatography on SiO_2 (5:1 to 3:1 to 1:1 hexanes:ethyl acetate) gave 3-(((3-(((2-palmitoleoyloxy-3-(palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**114c**, 145 mg, 0.0970 mmol, 39%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.39-5.33 (m, 2 H), 5.25-5.22 (m, 2 H), 4.36-4.32 (m, 2 H), 4.23-4.12 (m, 6 H), 4.05-4.00 (m, 5 H), 3.78 (d, 6 H, J = 11.4 Hz; diastereomers visible), 2.32 (apparent q, 8 H, J = 7.2 Hz), 2.02-2.00 (m, 4 H), 1.61-1.54 (m, 8 H), 1.30-1.26 (m, 88 H), 0.89-0.86 (m, 21 H), 0.11

(s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.8, 130.0, 129.7, 69.7 (m), 69.4 (d, $^3J_{CP}$ = 6.5 Hz), 67.8 (d, $^3J_{CP}$ = 5.5 Hz), 65.6 (m), 61.6, 54.6 (d, $^2J_{CP}$ = 6.0 Hz), 34.1, 34.0, 31.8, 29.7-29.0 (m), 25.6, 24.8, 22.7, 22.6 18.0, 14.1, -4.9; IR ν 2919, 2850, 1741, 1465, 1034 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{81}H_{159}O_{17}P_2Si$ $[M+H]^+$ 1494.0816, found 1494.0825.

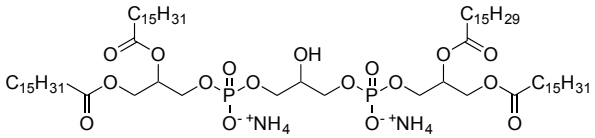


General Procedure H: Tertrapalmitoylcardiolipin diammonium salt (84a). To a solution of **114a** in 2-butanone (0.8 mL) was added NaI (24 mg, 0.16 mmol, 4.0 equiv.). The solution was heated at reflux for 13.5 h and concentrated. The resulting crude waxy yellow solid was dissolved in 0.1:2:1 1 M HCl:THF:H_2O (1 mL) and the resulting yellow solution stirred for 12 h. The orange-brown reaction mixture was quenched with 30% NH_4OH (2 mL), the resulting white slurry extracted with $CHCl_3$ (3x5 mL), and concentrated. The resulting faint yellow solid was triturated with Et_2O giving the tertrapalmitoylcardiolipin diammonium salt (**84a**, 40 mg, 0.029 mmol, 73%) as a colorless solid containing 5-7% (ESI-MS$^-$) monolysocardiolipin impurity that can be removed by thin layer chromatography. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (bs, 8 H), 5.21(bs, 2 H), 4.37 (m, 2 H), 4.17-4.13 (m, 2 H), 3.91 (bs, 9 H), 2.29 (apparent q, 8 H, *J* = 8.5 Hz), 1.58 (m, 8 H), 1.28-1.25 (m, 98 H), 0.87 (t, 12 H, *J* = 6.8 Hz); HRMS (ESI$^-$) *m/z* calculated for $C_{73}H_{141}O_{17}P_2$ $[M-H]^-$ 1351.9639, found 1351.9648.

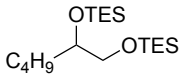


Tertastearoylcardiolipin diammonium salt (84b). Prepared according to General Procedure H using **114b**. Purification by precipitation from hot THF gave the tertastearoylcardiolipin diammonium salt (**84b**, 53 mg, 0.035 mmol, 38%) as a colorless powder containing 5-7%

(ESI-MS$^-$) monolysocardiolipin impurity that can be removed by thin layer chromatography. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 (bs, 8 H), 5.21 (bs, 2 H), 4.37-4.35 (m, 2 H), 4.17-4.13 (m, 2 H), 3.92 (bs, 9 H), 2.30 (apparent q, 8 H, J = 8.3 Hz), 1.59-1.58 (m, 8 H), 1.28-1.25 (m, 112 H), 0.88 (t, 12 H, J = 6.8 Hz); MS (ESI$^-$) *m/z* calculated for $C_{81}H_{156}O_{17}P_2$ $[M-2H]^{2-}$ 731.5, found 731.8.

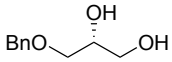


Tripalmitoyl-monopalmitoleoylcardiolipin diammonium salt (84c). Prepared according to General Procedure H using **114c**. Purification by trituration with Et_2O gave tripalmitoyl-monopalmitoleoylcardiolipin diammonium salt (**84c**, 83 mg, 0.060 mmol, 70%) as a colorless powder containing 5-7% (ESI-MS$^-$) monolysocardiolipin impurity that can be removed by thin layer chromatography. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 (bs, 8 H), 5.35-5.32 (m, 2 H), 5.21 (bs, 2 H), 4.39-4.35 (m, 2 H), 4.17-4.13 (m, 2 H), 3.84 (bs, 9 H), 2.30 (apparent q, 8 H, J = 8.1 Hz), 2.01-1.99 (m, 4 H), 1.66-1.58 (m, 8 H), 1.28-1.25 (m, 106 H), 0.88 (t, 12 H, J = 6.4 Hz); MS (ESI$^-$) *m/z* calculated for $C_{73}H_{138}O_{17}P_2$ $[M-2H]^{2-}$ 674.5, found 674.7.

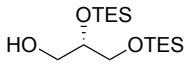


5-Butyl-3,3,8,8-tetraethyl-4,7-dioxa-3,8-disiladecane (115). To a solution of 1,2-hexanediol (0.200 mL, 1.56 mmol, 1.0 equiv.) in CH_2Cl_2 (4.4 mL) at 0 °C under nitrogen was added imidazole (244 mg, mmol, 2.2 equiv.), and TESCl (0.543 mL, 3.20 mmol, 2.1 equiv.). The cloudy white slurry was warmed to room temperature and stirred for 21.5 h. The reaction mixture was diluted with CH_2Cl_2 (8 mL), rinsed with water (4 mL), brine (4 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on SiO_2 (98:2 hexanes:ethyl acetate) gave 5-butyl-3,3,8,8-tetraethyl-4,7-dioxa-3,8-disiladecane (**115**, 384 mg, 1.11 mmol,

67%, 95% pure by ^{1}H NMR) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.66 (pent, 1 H, J = 6.0 Hz), 3.52, 3.42 (ABX, 2 H, J_{AB} = 9.8 Hz, J_{AX} = 5.6 Hz, J_{BX} = 6.4 Hz), 1.60-1.53 (m, 1 H), 1.40-1.28 (m, 5 H), 0.97-0.91 (m, 21 H), 0.63-0.57 (m, 12 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 73.3, 67.2, 31.4, 27.4, 22.9, 14.0, 6.9, 6.8, 6.7, 6.5, 5.1, 4.3; IR ν 2954, 2876, 1109, 1003, 723 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{18}H_{43}O_2Si_2$ $[M+H]^+$ 347.2796, found 347.2811.



(*R*)-3-*O*-Benzylglycerol (63). [169] To a slurry of NaH (1.24 g, 31.0 mmol, 1.8 equiv.) and BnBr (2.1 mL, 17.5 mmol, 1.0 equiv.) in THF (57 mL) at 0 °C under nitrogen was added (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (2.2 mL, 17.3 mmol, 1.0 equiv.) in a dropwise fashion. The reaction mixture stirred for 15 min then warmed to room temperature for 18 h. 5 M HCl (17 mL, 85 mmol, 5 equiv.) was added and stirring continued for 0.5 h. The reaction was quenched with sat. $NaHCO_3$ (90 mL), and the mixture extracted with MTBE (3x65 mL). The combined organic fractions were rinsed with sat. $NaHCO_3$ (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (1:1 to 1:2 to 1:3 hexanes:ethyl acetate) gave (*R*)-3-*O*-benzylglycerol (**63,** 2.30 g, 12.6 mmol, 73%) as a clear yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38-7.29 (m, 5 H), 4.56 (s, 2 H), 3.90 (bs, 1 H), 3.73-3.71 (m, 1 H), 3.66-3.63 (m, 1 H), 3.59, 3.56 (ABX, 2 H, J_{AB} = 9.5 Hz, J_{AX} = 3.8 Hz, J_{BX} = 6.1 Hz), 2.60 (bs, 1 H), 2.08 (bs, 1 H).



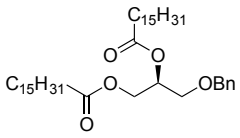
(*S*)-2,3-*O*-Bis(triethylsilyl)glycerol (118). To a suspension of NaH (2.92 g, 122 mmol, 1.5 equiv.) in THF (225 mL) at 0°C under nitrogen was added 3,4-dimethoxybenzyl bromide (crude, quantitative yield assumed, 1.1 equiv; transferred using 50 mL THF), TBAI (1.50 g, 4.06 mmol, 0.05 eq.), and (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (7.00 mL, 55.5 mmol, 1.00 equiv.;

dropwise). The white suspension was warmed to room temperature and stirred for 18 h. The mixture was diluted with water (120 mL), extracted with Et_2O (3x200 mL). The combined organic portions were rinsed with brine (120 mL), dried (Na_2SO_4), and concentrated.

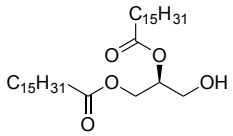
The resulting dark orange-brown oil was dissolved in MeOH (500 mL), 2% H_2SO_4 (aq., 4.5 mL, mmol, 0.02 equiv.) was added, and the resulting solution stirred for 18 h. The reaction mixture was quenched by adding solid $KHCO_3$ (1 g) and stirring for 15 min. The MeOH was removed under reduced pressure, the residue taken up in PhMe (200 mL) and rinsed with water (100 mL). The aqueous portion was extracted with ethyl acetate (2x200 mL) and the combined organic layers rinsed with brine (150 mL), dried (Na_2SO_4) and concentrated.

The dark orange oil was placed under nitrogen, dissolved in CH_2Cl_2 (160 mL), cooled to 0 °C, and DMAP (0.698 g, 5.66 mmol, 0.1 equiv.), imidazole (8.42 g, 124 mmol, 2.2 equiv.), and TESCl (19.5 mL, 115 mmol, 2.1 equiv.) added. The cloudy pale yellow slurry was warmed to room temperature and stirred for 14.5 h. The reaction mixture was rinsed with water (200 mL) and the aqueous layer extracted with CH_2Cl_2 (200 mL). The combined organic layers were rinsed with brine (200 mL) and dried (Na_2SO_4). The solution of the crude product was diluted to a 10:1 CH_2Cl_2:pH 7 0.1 M sodium phosphate buffer solution (560 mL total) and cooled to 0 °C. DDQ (14.13 g, 61.64 mmol, 1.1 equiv.) was added and the mixture immediately turns dark green. The reaction mixture was warmed to room temperature and stirred for 35 min. The precipitate was filtered off and the filtrate rinsed with water (100 mL), sat. $NaHCO_3$ (3x100 mL), and brine (100 mL). The organic layer was decolorized with carbon, dried (Na_2SO_4), and concentrated. The resulting purple-brown oil was diluted with hexanes, the dark purple-brown precipitate filtered off, and the filtrated concentrated. Further purification was performed by filtration through SiO_2 (97:3 to 93:7 hexanes:ethyl acetate), resulting in (*S*)-2,3-*O*-bis(triethylsilyl)glycerol (**118**, 14.87

g, 44.06 mmol, 54% over 4 steps) as a clear pale yellow oil. $[\alpha]_D^{21}$ = -19.3 (*c* = 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.81-3.76 (m, 1 H), 3.68-3.57 (m, 4 H), 2.21 (dd, 1 H, J_1 = 4.8 Hz, J_2 = 7.6 Hz), 0.96 (t, 9 H, *J* = 8.0 Hz), 0.96 (t, 9 H, *J* = 7.8 Hz), 0.61 (pent, 12 H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 72.3, 65.0, 64.7, 6.7 (2C), 4.8, 4.2; IR ν 3463, 2954, 2877, 723 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{15}H_{37}O_3Si_2$ $[M+H]^+$ 321.2281, found 343.2094 [M+Na].

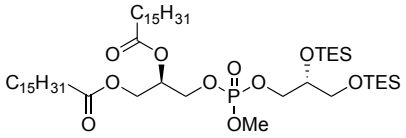


(*S*)-1,2-*O*-Dipamitoyl-3-*O*-benzylglycerol (64a).[107] To a solution of **63** (1.44 g, 7.90 mmol 1.0 equiv.), DMAP (0.098 g, 0.79 mmol, 0.1 equiv.), EDCI (3.54 g, 17.5 mmol, 2.2 equiv.) in CH_2Cl_2 (52 mL) under nitrogen was added palmitic acid (4.71 g, 17.5 mmol, 2.2 equiv.). The reaction mixture was stirred for 14 h and the reaction mixture rinsed with water (30 mL), brine (30 mL), dried ($MgSO_4$) and concentrated. Purification by chromatography on SiO_2 (97:3 to 95:5 to 93:7 hexanes:ethyl acetate) gave (*S*)-1,2-*O*-dipamitoyl-3-*O*-benzylglycerol (**64**, 4.72 g, 7.16 mmol, 91%) as a colorless solid. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.29 (m, 5 H), 5.26-5.22 (m, 1 H), 4.56, 4.52 (ABq, 2 H, J_{AB} = 12.3 Hz), 4.34, (dd, 2 H, J_1 = 3.5 Hz, J_2 = 12.0 Hz), 4.18, (dd, 2 H, J_1 = 6.5 Hz, J_2 = 12.0 Hz), 3.60, 3.58 (ABX, 2 H, J_{AB} = 10.5 Hz, J_{AX} = 1.6 Hz, J_{BX} = 1.4 Hz), 2.32 (t, 2 H, *J* = 7.5 Hz), 2.27 (t, 2 H, *J* = 7.5 Hz), 1.63-1.57 (m, 4 H), 1.30-1.25 (m, 48 H), 0.88 (t, 6 H, *J* = 7.0 Hz).



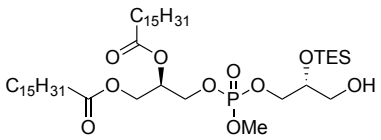
(*S*)-1,2-*O*-Dipalmitoylglycerol ((*S*)-46a).[107] To three batches of **64a** (2.52 g, 3.82 mmol, 1.00 equiv.) in THF (19 mL) was added 10% Pd/C (0.082 g, 0.23 mmol, 0.02 equiv.) to each batch. The mixtures were submitted to hydrogenation on a Parr hydrogenator under H_2 (5 bar) for 3.5 h.

The combined reaction mixtures were filtered through Celite ($CHCl_3$) and concentrated giving (*S*)-1,2-*O*-dipalmitoylglycerol (**(*S*)-46a**, 6.52 g, 11.48 mmol, quant.) as a colorless solid that was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.08 (apparent pent, 1 H, J = 5.2 Hz), 4.32, 4.24 (ABX, 2 H, J_{AB} = 11.6 Hz, J_{AX} = 7.4 Hz, J_{BX} = 2.6 Hz), 3.73-3.55 (m, 2 H), 2.33 (q, 4 H, J = 8.0 Hz), 1.99 (bs, 1 H), 1.64-1.60 (m, 4 H), 1.28-1.25 (m, 48 H), 0.88 (t, 6 H, J = 6.6 Hz).



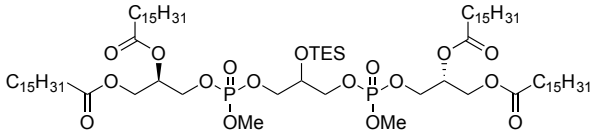
(2*R*)-3-((((*R*)-2,3-Bis((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl) oxy)propane-1,2-diyl dipalmitate (123). Performed according to general procedure D using **(*S*)-46a** (732 mg, 1.29 mmol, 1.0 equiv.), and DIPEA (0.25 mL, 1.4 mmol, 1.1 equiv.), and 0.5 M **40** (2.8 mL, 1.4 mmol, 1.1 equiv.). For the second step, **118** (458 mg, 1.43 mmol, 1.1 equiv.), 4,5-dicyanoimidazole (307 mg, 2.57 mmol, 2.0 equiv.), and Bu_4NIO_4 (618 mg, 1.43 mmol, 1.1 equiv.) were used. The crude material was then filtered through a plug of SiO_2 (1:1 hexanes:ethyl acetate) and concentrated. Purification by chromatography on SiO_2 (7:1 to 6:1 to 5:1 hexanes:ethyl acetate) gave (2*R*)-3-((((*R*)-2,3-bis((triethylsilyl)oxy)propoxy)(methoxy) phosphoryl)oxy) propane-1,2-diyl dipalmitate (**123**, 737 mg, 0.763 mmol, 59%; mixture of diastereomers at phosphorus) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.23 (pent, 1 H, J = 5.0 Hz), 4.36-4.32 (m, 1 H), 4.24-4.11 (m, 4 H), 3.98-3.93 (m, 1 H), 3.86 (apparent pent., 1 H, J = 5.3 Hz), 3.77 (d, $^3J_{HP}$ = 11.0 Hz, diastereomers), 3.58-3.53 (m, 2 H), 2.33 (t, 2 H, J = 7.8 Hz), 2.30 (t, 2 H, J = 7.8 Hz), 1.63-1.59 (m, 4 H), 1.28-1.25 (m, 48 H), 0.96 (t, 9 H, J = 8.0 Hz), 0.95 (t, 9 H, J = 7.8 Hz), 0.88 (t, 6 H, J = 7.0 Hz), 0.62 (q, 6 H, J = 8.0 Hz), 0.59 (q, 6 H, J = 8.0 Hz); ^{13}C NMR (CDCl3, 125 MHz) δ 173.2, 172.8 (2C), 71.8 (d, $^3J_{CP}$ = 8.1 Hz), 69.4 (d, $^3J_{CP}$ =

7.9 Hz), 69.1 (t, $^{2}J_{CP}$ = 4.9 Hz), 65.3 (t, $^{2}J_{CP}$ = 6.4 Hz), 63.7, 61.6 (d, $^{3}J_{CP}$ = 4.3 Hz), 54.4 (d, $^{2}J_{CP}$ = 5.8 Hz), 34.1, 34.0, 31.9, 29.7-29.1 (m), 24.8, 22.7, 14.1, 6.7 (2C), 4.8, 4.3; IR ν 2919, 2850.1741,1034 cm^{-1}; HRMS (ESI^{+}) *m/z* calculated for $C_{51}H_{106}O_{10}PSi_2$ $[M+H]^{+}$ 965.7057, found 965.7031.

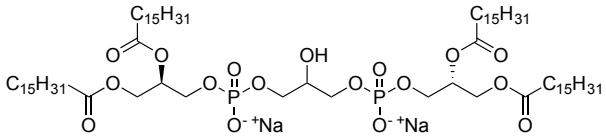


(2*R*)-3-((((*R*)-3-Hydroxy-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (126). To a solution of **123** (2.25 g, 2.33 mmol, 1.0 equiv.) in 1:1 CH_2Cl_2:MeOH (23 mL) at -20 °C was added PPTS (0.029 g, 0.11 mmol, 0.05 equiv.) in CH_2Cl_2 (0.8 mL). The reaction mixture was maintained between -25 and -20 °C and stirred for 2.5 h. The reaction mixture was quenched by adding Et_3N (0.065 mL), warmed to room temperature, and concentrated. Purification by chromatography on SiO_2 (3:1 to 1:1 hexanes:ethyl acetate) gave (2*R*)-3-((((*R*)-3-Hydroxy-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**126**, 0.853 g, 1.00 mmol, 43%; mixture of diastereomers at phosphorus) as a waxy colorless solid. 0.780 g of **123** was also recovered (35% recovery). Mp = 27.1-28.4 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26-5.21 (m, 1 H), 4.33 (dd, 1 H, J_1 = 4.4 Hz, J_2 = 12.0 Hz), 4.23-4.13 (m, 3 H), 4.08-4.03 (m, 2 H), 3.93-3.90 (m, 1 H), 3.79 (d, $^{3}J_{HP}$ = 11.2 Hz, diastereomers visible), 3.65, 3.61 (ABX, 2 H, J_{AB} = 11.4 Hz, J_{AX} = 4.9 Hz, J_{BX} = 3.9 Hz), 2.32 (q, 2 H, *J* = 8.0 Hz), 2.35-2.29 (bs, 1 H), 1.62-1.57 (m, 4 H), 1.28-1.25 (m, 48 H), 0.97 (t, 9 H, *J* = 8.0 Hz), 0.88 (t, 6 H, *J* = 6.8 Hz), 0.63 (q, 6 H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 172.9 (2C), 70.9 (d, $^{3}J_{CP}$ = 7.5 Hz), 69.3 (d, $^{3}J_{CP}$ = 10.0 Hz), 67.6 (t, $^{2}J_{CP}$ = 5.0 Hz), 65.6 (t, $^{2}J_{CP}$ = 5.6 Hz), 63.0 (d, $^{4}J_{CP}$ = 2.5 Hz), 61.6, 54.6-6 (t, $^{2}J_{CP}$ = 5.6 Hz), 34.1, 34.0, 31.9,

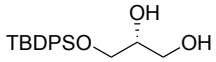
29.7-29.1 (m), 24.8, 22.7, 14.1, 6.7, 4.7. IR ν 2919, 2850, 1741, 1034 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{49}H_{100}O_{10}PSi$ $[M+H]^+$ 851.6197, found 851.6193.



3-(((3-(((2,3-(Palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128a). Prepared according to general procedure G using **126** (130 mg, 0.153 mmol, 1.0 equiv.), DIPEA (0.03 mL, 0.2 mmol, 1.1 equiv.), and 0.5 M **40** in CH_2Cl_2 (0.34 mL, 0.17 mmol, 1.1 equiv.). For the second step, **(*S*)-46a** (102 mg, 0.167 mmol, 1.1 equiv.), 4,5-dicyanoimidazole (37 mg, 0.31 mmol, 2.0 equiv.) in THF (0.60 mL), and Bu_4NIO_4 (74 mg, 0.17 mmol, 1.1 equiv.) were used. Purification by chromatography on SiO_2 (3:1 to 2:1 to 1:1 hexanes:ethyl acetate) gave 3-(((3-(((2,3-(palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)(methoxy) phosphoryl)oxy)propane-1,2-diyl dipalmitate (**128a**, 40 mg, 0.027 mmol, 17%) as a colorless solid. Mp = 37.3-38.1 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.26-5.20 (m, 2 H), 4.35-4.31 (m, 2 H), 4.24-4.11 (m, 6 H), 4.09-4.00 (m, 5 H), 3.78 (d, $^3J_{HP}$ = 11.2 Hz, diasteromers visible), 2.32 (q, 8 H, *J* = 8.0 Hz), 1.61-1.58 (m, 8 H), 1.30-1.25 (m, 96 H), 0.96 (t, 9 H, *J* = 8.0 Hz), 0.88 (t, 12 H, *J* = 6.8 Hz), 0.63 (q, 6 H, *J* = 8.0 Hz); ^{13}C NMR (CDCl3, 125 MHz) δ 173.2, 172.8, 69.4 (d, $^3J_{CP}$ = 8.8 Hz), 67.8 (m), 65.6 (m), 61.6, 54.7 (d, $^3J_{CP}$ = 5.0 Hz), 34.1, 34.0, 31.9, 29.7-29.1 (m), 24.8, 22.7, 14.1, 6.6, 4.7; IR ν 2916, 2850, 1739, 1036 cm^{-1}. HRMS (ESI^+) *m/z* calculated for $C_{81}H_{161}O_{17}P_2Si$ $[M+H]^+$ 1496.0978, found 1518.0823 $[M+Na]^+$.

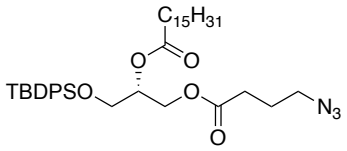


General Procedure I: Tetrapalmitoylcardiolipin disodium salt (129a). To a solution of **128a** (39 mg, 0.026 mmol, 1.0 equiv.) in acetone (0.52 mL) was added NaI (12 mg, 0.080 mmol, 3.0 equiv.) and the solution heated to reflux for 10 h. Following concentration, the resulting white solid was dissolved in CH_2Cl_2 (10 mL) and rinsed with sat. brine:H_2O (1:1, 3 mL). The organic layer was concentrated. The resulting light yellow solid dissolved in THF (0.52 mL) was added H_2SiF_6 (20% wt. in H_2O, 1 drop) and the yellow reaction mixture stirred for 0.5 h. The reaction mixture was quenched with sat. $NaHCO_3$ (3 mL) and extracted with $CHCl_3$ (2x5 mL). The combined organic layers were rinsed with brine (3 mL), dried (Na_2SO_4) and concentrated. The crude orange-yellow solid was triturated with Et_2O, dissolved in hot THF (0.1 mL) and allowed to cool to room temperature. The resulting precipitate was filtered, rinsed with Et_2O, and dried giving tetrapalmitoylcardiolipin disodium salt (**129a**, 14 mg, 0.010 mmol, 39%) as a faintly brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.2 (bs, 2 H), 4.41-4.48 (m, 2 H), 4.18 (m, 2 H), 3.90 (bs, 9 H), 2.30-2.28 (m, 8 H), 1.63-1.58 (m, 8H), 1.25 (m, 96 H), 0.88 (t, 12 H, J = 7.0 Hz); MS (ESI$^-$) *m/z* calculated for $C_{73}H_{140}O_{17}P_2$ $[M-2H]^{2-}$ 675.5, found 676.2.



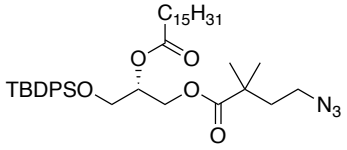
(*R*)-3-*O*-(*tert*-Butyldiphenylsilyl)glycerol (136).[150] To a solution of (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (2.0 mL, 15.6 mmol, 1.0 equiv.) in THF (35 mL) 0 °C under nitrogen was added was added imidazole (1.18 g, 17.3 mmol, 1.1 equiv.), DMAP (0.189 g, 1.53 mmol, 0.1 equiv.) and TBDPSCl (4.50 mL, 17.3 mmol, 1.1 equiv.). The resulting white slurry was stirred for 17 h. The reaction mixture was diluted with water (10 mL), citric acid monohydrate (15.18 g, 78.22 mmol, 5.0 equiv.) added, and the cloudy white emulsion

heated to 65 °C for 1.25 h (clears when temperature reaches 60 °C). The reaction mixture was poured into sat. $NaHCO_3$ (100 mL) and extracted with methyl *t*-butyl ether (3x50 mL). The organic portion was rinsed sat. $NaHCO_3$ (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated. Recrystallization from 95:5 hexanes:ethyl acetate (20 mL) at -20 °C gave (*R*)-3-*O*-(*tert*-butyldiphenylsilyl) glycerol (**136**, 3.77 g, 11.4 mmol, 73%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67-7.65 (m, 4 H), 7.45-7.38 (m, 6 H), 3.83-3.79 (m, 1 H), 3.76-3.62 (m, 4 H), 2.56 (d, 1 H, J = 4.8 Hz), 1.95 (bs, 1 H), 1.07 (s, 9 H).



(*R*)-1-*O*-(4-Azidobutanoyl)-3-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-palmitoylglycerol (138a). To a solution of **136** (68 mg, 0.0.21 mmol, 1.0 equiv.), 4-azidobutyric acid (29 mg, 0.22 mmol, 1.1 equiv.), and DMAP (1 mg, 0.01 mmo, 0.05 eq.) in CH_2Cl_2 (1.3 mL) at 0 °C under nitrogen was added DCC (88 mg, 0.36 mmol, 1.9 eq. in CH_2Cl_2 (0.17 mL) in a dropwise fashion over 2 min. The reaction mixture was then stirred for 2 h at 0 °C, then allowed to warm to room temperature for an additional 21 h. The reaction mixture filtered through Celite (CH_2Cl_2) and concentrated. Purification by chromatography on SiO_2 (7:1 to 5:1 hexanes:ethyl acetate) gave (*R*)-3-*O*-(*tert*-butyldiphenylsilyl)-1-*O*-(4-azidobutanoyl)glycerol (56 mg, 0.13 mmol, 62% yield) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4 H), 7.45-7.34 (m, 6 H), 4.20, 4.18 (ABX, 2 H, J_{AB} = 9.6 Hz, J_{AX} = 2.6 Hz, J_{BX} = 3.8 Hz), 3.94 (sextet, 1 H, J = 5.6 Hz), 3.72, 3.67 (ABX, 2 H, J_{AB} = 10.4 Hz, J_{AX} = 4.7 Hz, J_{BX} = 5.7 Hz), 3.34 (t, 2 H, J = 6.6 Hz), 2.51 (d, 1 H, J = 5.6 Hz), 2.40 (t, 2 H, J = 7.2 Hz), 1.88 (pent, 2 H, J = 7.2 Hz), 1.07 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.7, 135.5, 132.8, 129.9, 127.8, 70.0, 65.3, 64.4, 50.5, 31.0, 26.8, 24.2, 19.2; IR ν 3463, 2932, 2098, 1737cm^{-1}.

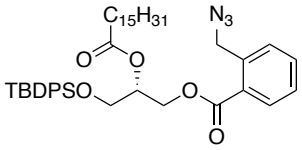
To a solution of the intermediate alcohol (56 mg, 0.13 mmol, 1.0 eq.), EDCI (37 mg, 0.19 mmol, 1.5 eq.), and DMAP (2 mg, 0.02 mmo, 0.1 eq.) in CH_2Cl_2 (0.84 mL) under nitrogen was added palmitic acid (37 mg, 0.14 mmol, 1.1 eq.). The reaction mixture was then stirred for 2 d. The reaction mixture was diluted with MTBE (8 mL), and rinsed with 1 N $NaHSO_4$ (3 mL), water (3 mL), and brine (3 mL). The organic portion was dried ($MgSO_4$) and concentrated. Purification by filtration through a plug of SiO_2 (9:1 hexanes:ethyl acetate) gave (*R*)-3-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-palmitoyl-1-*O*-(4-azidobutanoyl)glycerol (**138a**, 68 mg, 0.10 mmol, 79%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4 H), 7.45-7.34 (m, 6 H), 5.20-5.15 (m, 1 H), 4.43, 4.23 (ABX, 2 H, J_{AB} = 11.8 Hz, J_{AX} = 3.7 Hz, J_{BX} = 6.3 Hz), 3.78, 3.75 (ABX, 2 H, J_{AB} = 10.6 Hz, J_{AX} = 4.8 Hz, J_{BX} = 4.8 Hz), 3.33 (t, 2 H, *J* = 6.8 Hz), 2.38 (t, 2 H, *J* = 7.2 Hz), 2.34-2.20 (m, 2 H), 1.87 (pent, 2 H, *J* = 6.8 Hz), 1.61-1.54 (m, 2 H), 1.30-1.25 (m, 24 H), 1.05 (s, 9 H), 0.88 (t, 3 H, *J* = 6.8 Hz).



(*R*)-1-*O*-(4-Azido-2,2-dimethylbutanoyl)-3-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-palmitoylglycerol (138b). To a solution of **136** (481 mg, 1.46 mmol, 1.0 eq.) in 1:1 pyridine:CH_2Cl_2 (4.8 mL) at 0 °C under nitrogen was added 4-azido-2,2-dimethylbutanoyl chloride[148] (crude product, quantitative yield assumed, 1.0 equiv.) in CH_2Cl_2 (0.5 mL) in a dropwise fashion. The reaction mixture was then stirred for 3 h. The reaction mixture was quenched with water, diluted with CH_2Cl_2 (5 mL), and the layers separated. The organic layer was rinsed with 1 M $NaHSO_4$ (2x3 mL), brine (3 mL), dried ($MgSO_4$), and concentrated. Significant pyridine remains, so the oil was taken up in CH_2Cl_2 (10 mL), rinsed with 1 M, HCl (2x3 mL), brine (3 mL), dried ($MgSO_4$), and

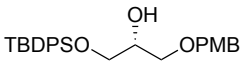
concentrated. Purification by chromatography on SiO_2 (9:1 to 85:15 to 1:1 hexanes:ethyl acetate) gave (*R*)-3-*O*-(*tert*-butyldiphenylsilyl)-1-*O*-(4-azido-2,2-dimethylbutanoyl)glycerol (182 mg, 0.329 mmol, 23%, 85% pure, hydrolyzed acid chloride as impurity) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4 H), 7.46-7.40 (m, 2 H), 7.38-7.35 (m, 4 H), 4.20 (d, 2 H, J = 4.4 Hz), 3.95 (m, 1 H), 3.71, 3.66 (ABX, 2 H, J_{AB} = 8.4 Hz, J_{AX} = 3.6 Hz, J_{BX} = 4.8 Hz), 3.26 (t, 2 H, J = 6.0 Hz), 2.54 (d, 1 H, J = 3.2 Hz), 1.82 (t, 2 H, J = 6.0 Hz), 1.18 (s, 6 H), 1.07 (s, 9 H).

To a solution of the intermediate alcohol (182 mg, 0.323 mmol, 1.0 equiv.), DMAP (4 mg, 0.03 mmol, 0.1 equiv.), and pyridine (0.05 mL, 0.7 mmol, 2 equiv.) in CH_2Cl_2 (2.1 mL) under nitrogen at 0 °C was added palmitoyl chloride (0.14 mL, 4.6 mmol, 1.4 equiv.) in a dropwise fashion. The reaction mixture was then stirred for 16.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and quenched with 1 N HCl (4 mL). The layers were separated and the organic portion rinsed with sat. $NaHCO_3$ (4 mL), brine (4 mL), dried ($MgSO_4$) and concentrated. Removal of impurities attempted by rinsing with MeOH, but was unsuccessful (286 mg isolated). Major byproduct appears to be methyl palmitate. A portion of the unpurified material was used in a separate reaction without further purification (105 mg). The remaining portion (181 mg) was purified by chromatography on SiO_2 (98:2 to (95:5 hexanes:ethyl acetate) giving 129 mg of **138b**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.63 (m, 4 H), 7.45-7.37 (m, 6 H), 5.20-5.18 (m, 1 H), 4.42, 4.22 (ABX, 2 H, J_{AB} = 11.8 Hz, J_{AX} = 3.8 Hz, J_{BX} = 6.2 Hz), 3.78, 3.75 (ABX, 2 H, J_{AB} = 10.6 Hz, J_{AX} = 4.8 Hz, J_{BX} = 4.8 Hz), 3.24 (t, 2 H, J = 7.6 Hz), 2.27-2.24 (m, 2 H), 1.81 (t, 2 H, J = 7.6 Hz), 1.61-1.54 (m, 2 H), 1.30-1.25 (m, 24 H),1.17 (s, 6 H), 1.05 (s, 9 H), 0.88 (t, 3 H, J = 6.8 Hz).

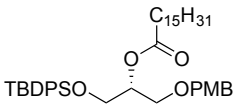


(*R*)-1-*O*-(2-(Azidomethyl)benzoyl)-3-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-palmitoylglycerol (138c). To a solution of **136** (72 mg, 0.22 mmol, 1.0 equiv.), 2-(azidomethyl)benzoic acid[171] (43 mg, 0.0.24 mmol, 1.1 equiv.), and DMAP (2 mg, 0.02 mmo, 0.07 equiv.) in CH_2Cl_2 (1.3 mL) at 0 °C under nitrogen was added DCC (86 mg, 0.38 mmol, 1.7 equiv. in CH_2Cl_2 (0.3 mL) in a dropwise fashion over 6 min. The reaction mixture was then stirred for 2 h at 0 °C, then allowed to warm to room temperature for an additional 21 h. The reaction mixture filtered through Celite (CH_2Cl_2) and concentrated. Purification by chromatography on SiO_2 (7:1 to 5:1 hexanes:ethyl acetate) gave (*R*)-1-*O*-(2-(azidomethyl)benzoyl)-3-*O*-(*tert*-butyldiphenylsilyl)glycerol (53 mg, 0.11 mmol, 50% yield) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96-7.94 (m, 2 H), 7.66-7.65 (m, 4 H), 7.55-7.53 (m, 1 H), 7.48-7.33 (m, 7 H), 4.43 (d, 2 H, J = 5.2 Hz), 4.39 (s, 2 H), 4.13-4.06 (m, 1 H), 3.82, 3.78 (ABX, 2 H, J_{AB} = 10.4 Hz, J_{AX} = 4.7 Hz, J_{BX} = 5.3 Hz), 2.58 (d, 1 H, J = 5.6 Hz), 1.82 (t, 2 H, J = 6.0 Hz), 1.08 (s, 9 H).

To a solution of the intermediate alcohol (336 mg, impure), DMAP (8 mg, 0.07 mmol, 0.1 equiv.) and pyridine (0.11 mL, 0.1.4 mmol, 2 equiv.) in CH_2Cl_2 (4.6 mL) under nitrogen at 0 °C was added palmitoyl chloride (0.30 mL, 0.96 mmol, 1.4 equiv.) in a dropwise fashion. The reaction mixture was then stirred for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and quenched with 1 N HCl (4 mL). The layers were separated and the organic portion rinsed with sat. $NaHCO_3$ (2x4 mL), brine (4 mL), dried ($MgSO_4$) and concentrated. Purification by chromatography on SiO_2 (95:5 hexanes:ethyl acetate) gave the desire product **138c** containing significant quantities of palmitic acid impurity (66% pure). This compound was used without further purification.

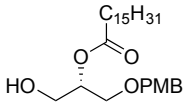


(*R*)-1-*O*-(*t*ert-butyldiphenylsilyl)-3-*O*-(4-methoxylbenzyl)glycerol (142a). **136** (1.06 g, 3.21 mmol, 1.0 equiv.) and Bu_2SnO (1.06 g, 4.17 mmol, 1.3 equiv.) were suspended in PhMe (14 mL) under nitrogen and heated to reflux with azeotropic removal of water for 21 h. The solution was cooled to room temperature, TBAI (0.079 g, 0.32 mmol, 0.1 equiv.) and a solution of freshly prepared PMBBr[172] in PhMe (1 mL). The reaction mixture was returned to reflux for 5.5 h, diluted with methyl *t*-butyl ether (20 mL), rinsed with water (2x10 mL), brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (6:1 to 4:1 hexanes:ethyl acetate gave (*R*)-1-*O*-(*t*ert-butyldiphenylsilyl)-3-*O*-(4-methoxylbenzyl)glycerol (**142a,** 556 mg, 1.23 mmol) as a clear, colorless oil. The a second column was performed on a substantial mixed fraction (5:1 to 4:1 hexanes:ethyl acetate) giving an additional 231 mg. Combined yield 49%, 90% pure by ^{1}H NMR. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4 H), 7.45-7.35 (m, 6 H), 7.24-7.21 (m, 2 H), 6.88-6.85 (m, 2 H), 3.94-3.87 (m, 1 H), 3.80 (s, 3 H), 3.71 (d, 2 H, J = 5.6 Hz), 3.56, 3.53 (ABX, 2 H, J_{AB} = 9.6 Hz, J_{AX} = 4.7 Hz, J_{BX} = 6.1 Hz), 2.49 (d, 1 H, J = 5.2 Hz), 1.05 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 135.4, 133.2, 130.1, 129.8, 129.3, 127.7, 113.8, 73.1, 70.8, 70.7, 64.8, 55.3, 26.8, 19.3; IR ν 3457, 2931, 2857, 1112, 702 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{27}H_{35}O_4Si$ $[M+H]^+$ 451.2305, found 473.2124 $[M+Na]^+$.

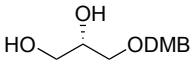


(*R*)-1-*O*-(*t*ert-butyldiphenylsilyl)-2-*O*-palmitoyl-3-*O*-(4-methoxylbenzyl)glycerol (143a). To a solution of **142a** (785 mg, 1.74 mmol, 1.0 equiv.), EDCI (415 mg, 2.14 mmol, 1.2 equiv.), and DMAP (65 mg, 0.53 mmol , 0.3 equiv.) in CH_2Cl_2 (11 mL) under nitrogen was added palmitic acid (584 mg, 2.16 mmol, 1.2 equiv.). The reaction mixture was then stirred for 21 h. The

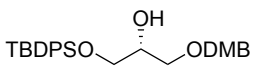
reaction mixture was diluted with CH_2Cl_2 (5 mL), and rinsed with water (5 mL), brine (5mL), dried ($MgSO_4$) and concentrated. Purification by chromatography on SiO_2 (1:0 to 98:2 to 96:4 hexanes:ethyl acetate) gave (*R*)-1-*O*-(*t*ert-butyldiphenylsilyl)-2-*O*-palmitoyl-3-*O*-(4-methoxylbenzyl) glycerol (**143a**, 926 mg, 1.34 mmol, 77%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.63 (m, 4 H), 7.44-7.34 (m, 6 H), 7.22-7.20 (m, 2 H), 6.86-6.84 (m, 2 H), 5.15 (pent, 1 H, J = 5.2 Hz), 4.46, 4.44 (ABq, 2 H, J_{AB} = 11.6 Hz), 3.80-3.79 (m, 5 H), 3.65, 3.62 (ABX, 2 H, J_{AB} = 10.4 Hz, J_{AX} = 3.5 Hz, J_{BX} = 4.5 Hz), 2.34-2.22 (m, 2 H), 1.60-1.55 (m, 2 H), 1.30-1.25 (m, 24 H), 1.02 (s, 9 H), 0.88 (t, 3 H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 159.2, 135.6, 135.5 133.4, 133.3, 130.1, 129.7, 129.2, 127.7 (2C), 113.8, 72.8, 72.7, 68.1, 62.6, 55.3, 34.5, 31.9, 29.7-29.2 (m), 26.7, 25.0, 22.7, 19.2, 14.1; IR ν 2923, 2854, 1737,1112, 701 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{43}H_{65}O_5Si$ $[M+H]^+$ 689.4601, found 711.4421 $[M+Na]^+$.



(*S*)- 2-*O*-palmitoyl-3-*O*-(4-methoxylbenzyl)glycerol (145a). To a solution of **143a** (72 mg, 0.10 mmol, 1.0 equiv.) in THF (0.9 mL) was added 1 M AcOH in THF (0.14 mL, 0.14 mmol, 1.4 equiv.) followed by 1 M TBAF in THF (0.11 mL, 0.11 mmol, 1.1 equiv.). The solution was stirred for 4.5 h. The reaction mixture was quenched with pH 7 phosphate buffer (3 mL) and extracted with methyl *t*-butyl ether (10 mL). The organic layer was rinsed with pH 7 phosphate buffer (3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated. 72 mg isolated and used without further purification.

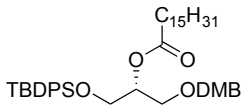


(*S*)-3-*O*-(3,4-dimethoxybenzyl)glycerol ((*S*)-12).[173] To as suspension of NaH (0.770 g, 32.1 mmol, 2.0 equiv.), TBAI (0.196 g, 0.792 mmol, 0.05 eq.), and freshly prepare 3,4-dimethoxybenzyl bromide[174] (crude, quantitative yield assumed, 1.1 equiv; transferred using 5 mL THF) in THF (50 mL) at 0°C under nitrogen was added (*R*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (2.00 mL, 15.8 mmol, 1.00 equiv.) in a dropwise fashion over 5 min. The white suspension was warmed to room temperature and stirred for 15 h. The reaction mixture was quenched with water (50 mL), extracted with Et_2O (3x70 mL). The combined organic portions were rinsed with brine (50 mL), dried (Na_2SO_4), and concentrated. The resulting orange oil was dissolved in MeOH (105 mL), 2% H_2SO_4 was added (aq., 0.88 mL, 0.32 mmol, 0.02 equiv.), and the resulting solution stirred for 22 h. The reaction mixture was quenched by adding solid $KHCO_3$ (0.300 g) and stirring for 5 min. $MgSO_4$ was added, and about 2/3 of the MeOH removed under reduced pressure. The resulting slurry was filtered through Celite (ethyl acetate) and concentrated. Purification by chromatography on SiO_2 (1:3 hexanes:ethyl acetate to 97:3 ethyl acetate:MeOH) gave (*S*)-3-*O*-(3,4-dimethoxybenzyl)glycerol (**(*S*)-122**, 2.89 g, 11.93 mmol, 76%) as a clear pale yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.87-6.84 (m, 3 H), 4.50 (s, 2 H), 3.89-3.88 (m, 7 H), 3.73-3.71 (m, 1 H), 3.66-3.64 (m, 1 H), 3.59-3.52 (m, 2 H), 2.52 (d, 1 H, J = 2.0 Hz), 2.01 (bs, 1 H).



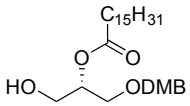
(*R*)-1-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-(3,4-dimethoxylbenzyl)glycerol (142b). To a solution of **(*S*)-122** (9.64 g, 39.8 mmol, 1.0 equiv.) and imidazole (3.52 g, 51.7 mmol, 1.3 equiv.) at 0°C under nitrogen in CH_2Cl_2 (133 mL) was added TBDPSCl (10.3 mL, 40.0 mmol, 1.0 equiv.) in a dropwise fashion over 20 min. The reaction mixture was allowed to warm to room temperature

and stirred for 21 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), rinsed with water (100 mL), 0.5 M HCl (100 mL), brine (100 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on SiO_2 (3:1 to 2:1 hexanes:ethyl acetate) gave (*R*)-1-*O*-(*t*ert-butyldiphenylsilyl)-3-*O*-(3,4-dimethoxylbenzyl) glycerol (**142b**, 14.8 g, 30.7 mmol, 68%; 90% pure by ^{1}H NMR, contains ethyl acetate) as a clear, faintly yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4 H), 7.45-7.35 (m, 6 H), 6.85-6.80 (m, 3 H), 4.47 (s, 1 H), 3.93-3.91 (m, 1 H), 3.87 (s, 3 H), 3.84 (s, 3 H), 3.73-3.71 (m, 2 H), 3.56, 3.53 (ABX, 2 H, J_{AB} = 9.8 Hz, J_{AX} = 4.6 Hz, J_{BX} = 5.8 Hz), 2.48 (d, 1 H, J = 5.2 Hz), 1.05 (s, 9 H).

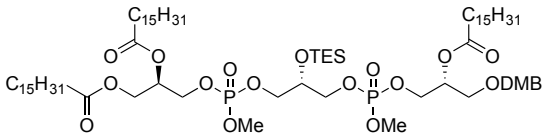


(*R*)-1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-palmitoyl-3-*O*-(3,4-dimethoxylbenzyl)glycerol (143b). To a solution of **142b** (2.66 g, 5.53 mmol, 1.0 equiv.), EDCI (1.34 g, 6.64 mmol, 1.2 equiv.), DMAP (0.210 g, 1.70 mmol, 0.31 equiv.) in CH_2Cl_2 under nitrogen was added palmitic acid (1.78 g, 6.59 mmol, 1.2 equiv.). The reaction mixture was stirred for 6 h, diluted with CH_2Cl_2 (10 mL), rinsed with water (20 mL), brine (20 mL), dried ($MgSO_4$) and concentrated. Purification by chromatography on SiO_2 (9:1 to 5:1 hexanes:ethyl acetate) (*R*)-1-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-palmitoyl-3-*O*-(3,4-dimethoxylbenzyl) glycerol (**143b**, 3.49 g, 4.85 mmol, 88%) as a clear colorless oil. $[\alpha]_D^{21}$ = +4.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 125 MHz) δ 7.66-7.63 (m, 4 H), 7.43-7.40 (m, 2 H), 7.38-7.34 (m, 4 H), 6.84-6.79 (m, 3 H), 5.17 (pent, 1 H, J = 5.0 Hz), 4.47, 4.44 (ABq, 2 H, J_{AB} = 11.8 Hz), 3.87 (s, 3 H), 3.84-3.78 (m, 5 H), 3.66, 3.63 (ABX, 2 H, J_{AB} = 10.8 Hz, J_{AX} = 4.8 Hz, J_{BX} = 6.2 Hz), 2.34-2.24 (m, 2 H), 1.60-1.55 (m, 2 H), 1.30-1.25 (m, 24 H), 1.03 (s, 9 H), 0.88 (t, 3 H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 173.3, 149.0, 148.6, 135.6, 135.5, 133.3 (2 C), 130.6, 129.7, 127.7 (2 C),

120.2, 111.0, 110.9 73.1, 72.7, 68.2, 62.7, 55.9, 55.8, 34.5, 31.9, 29.7-29.2 (m), 26.7, 25.0, 22.7, 19.2, 14.1; IR ν 2924, 2854, 1737,1112, 701 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{44}H_{66}O_6Si$ $[M+H]^+$ 719.4707; found 741.4514 $[M+Na]^+$.



(*S*)-2-*O*-palmitoyl-3-*O*-(3,4-dimethoxylbenzyl)glycerol (145b). To a solution of **143b** (5.29 g, 7.36 mmol, 1.0 equiv.) in THF (49 mL) was added AcOH (0.51 mL, 8.8 mmol, 1.2 equiv.) followed by 1.0 M TBAF in THF (8.1 mL, 8.1 mmol, 1.1 equiv.). The solution was stirred for 3.75 h. The reaction mixture was quenched with 0.1 M pH 7 sodium phosphate buffer (40 mL), and extracted with Et_2O (2x80 mL). The organic layer was rinsed with pH 7 phosphate buffer (40 mL), brine (40 mL), dried (Na_2SO_4), and concentrated. Purification by recrystallization from 9:1 hexanes:Et_2O (30 mL) gave (*S*)-2-*O*-palmitoyl-3-*O*-(3,4-dimethoxylbenzyl)glycerol (**145b**, 2.97 g, 5.93 mmol, 81%, 96% pure by ^1H NMR) as colorless crystals. $[\alpha]_D^{21}$ = -3.7 (*c* = 1.0, $CHCl_3$). Mp = 56.9-59.0 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.87-6.82 (m, 3 H), 5.05 (app. pent, 1 H, *J* = 5.0 Hz), 4.51, 4.47 (ABq, 2 H, J_{AB} = 11.8 Hz), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.83-3.70 (m, 2 H), 3.66, 3.63 (ABX, 2 H, J_{AB} = 11.0 Hz, J_{AX} = 5.9 Hz, J_{BX} = 6.1 Hz), 2.35 (t, 2 H, *J* = 7.5 Hz), 2.02 (t, 1 H, *J* = 6.3 Hz), 1.64-1.60 (m, 2 H), 1.29-1.25 (m, 24 H), 0.88 (t, 3 H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 149.1, 148.8, 130.2, 120.3, 111.0, 110.9, 73.4, 72.9, 68.8, 62.8, 55.9 (2C), 34.4, 31.9, 29.7-29.1 (m), 22.7, 14.1; IR ν 3548, 2917, 2849, 1729 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{28}H_{49}O_6$ $[M+H]^+$ 481.3529, found 503.3347 $[M+Na]^+$.'

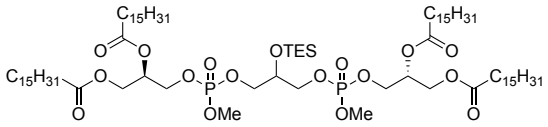


(2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((3,4-dimethoxybenzyl)oxy)-2-(palmitoyloxy)propoxy) phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (146). Prepared according to General Procedure G using **126** (1.22 g, 1.43 mmol, 1.0 equiv.), DIPEA (0.36 mL, 1.6 mmol, 1.1 equiv.), and 1.0 M **40** in CH_2Cl_2 (1.57 mL, 1.57 mmol, 1.1 equiv.). For the second step, **145b** (0.756 g, 1.57 mmol, 1.1 equiv.), 4,5-dicyanoimidazole (0.258 g, 2.16 mmol, 1.5 equiv.), Bu_4NIO_4 (0.708 g, 1.60 mmol, 1.1 equiv.) were used. Purification by chromatography on SiO_2 (2:1 to 1:1 2:3 hexanes:ethyl acetate) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((3,4-dimethoxybenzyl)oxy)-2-(palmitoyloxy)propoxy)pho sphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**146**, 0.662 g, 0.470 mmol, 33%; mixture of diastereomers at phosphorus) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86-6.83 (m, 3 H), 5.23-5.20 (m, 2 H), 4.50-4.43 (m, 2 H), 4.35-4.31 (m, 1 H), 4.27-4.10 (m, 5 H), 4.05-3.96 (m, 5 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.79-3.74 (m, 6 H), 3.60-3.58 (m, 2 H), 2.31 (apparent q, 6 H, J = 8.0 Hz), 1.65-1.55 (m, 6 H), 1.28-1.25 (m, 72 H), 0.96 (t, 9 H, J = 8.0 Hz), 0.88 (t, 9 H, J = 6.6 Hz), 0.62 (q, 6 H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.0, 172.8, 149.1, 148.8, 130.2, 120.3, 111.0, 110.9, 73.3 (d, $^3J_{CP}$ = 2.5 Hz), 70.5 (d, $^3J_{CP}$ = 7.5 Hz), 69.5-69.3 (m), 67.8-67.5 (m), 66.2-66.0 (m), 65.6-65.5 (m), 61.6, 55.9, 55.8, 54.5-54.4 (m), 34.2, 34.1, 34.0, 31.9, 31.5, 29.7-29.1 (m), 24.9 (2C), 22.7, 14.1, 6.6, 4.7; IR ν 2917, 2850, 2122, 1743, 1028 cm^{-1}. HRMS (ESI$^+$) *m/z* calculated for $C_{74}H_{141}O_{18}P_2Si$ $[M+H]^+$ 1407.9362; found 1429.9199 $[M+Na]^+$.

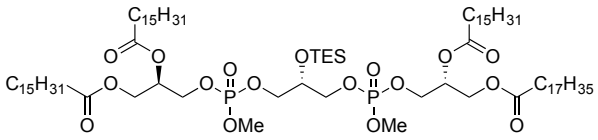
General Procedure J: *Pivaloyl anhydride formation:* To a solution of fatty acid (1.0 equiv.) and Et_3N (1.2 equiv.) in CH_2Cl_2 (0.2 M) under nitrogen was added pivaloyl chloride (1.0 equiv.) and the reaction mixture stirred for 1.5-3.0 h. The resulting solution of fatty acid anhydride was used directly.

*DMB cleavage of **146**:* To a biphasic mixture of **146** (1.00 equiv.) in 10:1 CH_2Cl_2:pH 7 phosphate buffer (0.1 M) was added DDQ (1.2 equiv.). The reaction mixture immediately turns dark green and was stirred for 2.5 h. The reaction mixture was diluted with CH_2Cl_2 (7 mL) and rinsed with water (3 mL), sat. $NaHCO_3$ (3x3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated. The resulting crude alcohol (a brown oil) was used without further purification.

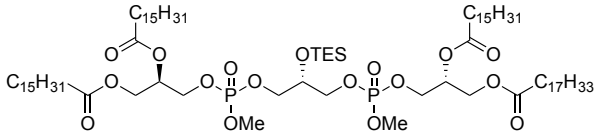
Acylation: To the solution of the mixed pivaloyl-fatty acid anhydride (1.2 equiv.) under nitrogen was added Et_3N (3.0 equiv.), crude alcohol from DMB cleavage reaction of **146** (transferred as a 0.9 M solution in CH_2Cl_2,) and DMAP (0.2 equiv.) and the resulting pale brown reaction mixture stirred for 18-20 h. The reaction mixture was diluted with CH_2Cl_2 (7 mL), rinsed with water (3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated. Purification was performed as specified.



3-(((3-(((2,3-(Palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128a). Prepared according to general procedure J. Purification by chromatography on SiO_2 (2:1 to 3:2 to 1:1 hexanes:ethyl acetate) gave 3-(((3-(((2,3-(palmitoyloxy)propoxy)(methoxy)phosphoryl)oxy)-2((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**128a**, 74 mg, 0.049 mmol, 63%) as a waxy colorless solid. Characterization data were identical to those reported above.

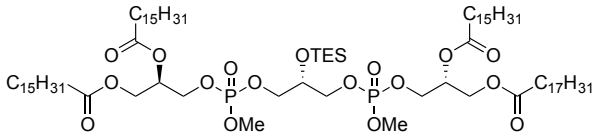


(2*R*)-3-((Methoxy(3-((methoxy((*R*)-3-((stearoyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128b). Prepared according to general procedure J. Purification by chromatography on SiO_2 (3:1 to 2:1 to 3:2 to 1:1 hexanes:ethyl acetate) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((stearoyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane -1,2-diyl dipalmitate (**128b**, 71 mg, 0.047 mmol, 63%) as a waxy colorless solid. Mp = 38.6-39.2 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26-5.20 (m, 2 H), 4.35-4.31 (m, 2 H), 4.24-4.10 (m, 6 H), 4.08-3.99 (m, 5 H), 3.78 (d, $^3J_{HP}$ = 11.6 Hz, diastereomers), 2.32 (q, 8 H, *J* = 8.0 Hz), 1.61-1.58 (m, 8 H), 1.30-1.25 (m, 100 H), 0.96 (t, 9 H, *J* = 8.0 Hz), 0.88 (t, 12 H, *J* = 6.8 Hz), 0.63 (q, 6 H, *J* = 7.9 Hz); ^{13}C NMR (CDCl3, 125 MHz) δ 173.2, 172.8, 69.4-69.3 (m), 67.8 (m), 65.6 (m), 61.6, 54.6 (d, $^3J_{CP}$ = 6.3 Hz), 34.1, 34.0, 31.9, 29.7-29.1 (m), 24.8, 22.7, 14.1, 6.6, 4.7; IR ν 2916, 2850, 1739, 1036 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{83}H_{164}O_{17}P_2Si$ $[M+H]^+$ 1524.1291; found 1546.1132 $[M+Na]^+$.



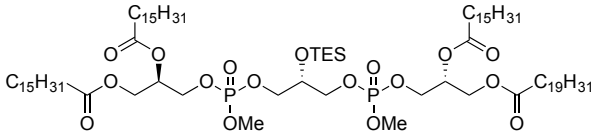
(2*R*)-3-((Methoxy(3-((methoxy((*R*)-3-((oleyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128c). Prepared according to general procedure J. Purification by chromatography on SiO_2 (3:1 to 2:1 to 3:2 to 1:1 hexanes: ethyl acetate) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((oleyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy) propane-1,2-diyl dipalmitate (**128c**, 72 mg, 0.047

mmol, 64%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.36-5.31 (m, 2 H), 5.25-5.20 (m, 2 H), 4.36-4.32 (m, 2 H), 4.25- 4.13 (m, 6 H), 4.06-3.95 (m, 5 H), 3.78 (d, $^{3}J_{HP}$ = 11.0 Hz, diastereomers visible), 2.33 (t, 4 H, *J* = 7.8 Hz), 2.31 (t, 4 H, *J* = 7.5 Hz) 2.03-1.99 (m, 4 H), 1.63-1.59 (m, 8 H), 1.30-1.25 (m, 94 H), 0.96 (t, 9 H, *J* = 8.0 Hz), 0.88 (t, 12 H, *J* = 6.8 Hz), 0.63 (q, 6 H, *J* = 8.0 Hz); ^{13}C NMR (CDCl3, 125 MHz) δ 173.2, 172.8, 130.2, 130.0, 129.7, 69.4-69.3 (m), 67.8 (m), 65.6 (m), 61.6, 54.6 (d, $^{3}J_{CP}$ = 6.3 Hz), 34.1, 34.0 (2C), 31.9 (2C), 29.8-29.1 (m), 27.2 (2C), 24.8, 22.7, 14.1, 6.6, 4.7; IR ν 2922, 2853, 1743, 1035 cm^{-1}; HRMS (ESI^{+}) *m/z* calculated for $C_{83}H_{163}O_{17}P_2Si$ $[M+H]^+$ 1522.1135, found 1522.1135, 1544.0970 $[M+Na]^+$.



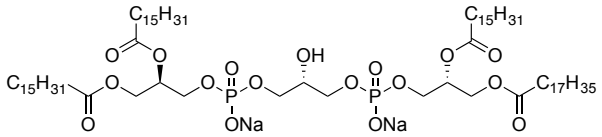
(2*R*)-3-((Methoxy(3-((methoxy((*R*)-3-((linoleyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128d). Prepared according to general procedure J using degassed CH_2Cl_2 and shielding from light during the reaction. Purification by chromatography on SiO_2 (3:1 to 2:1 to 3:2 hexanes: ethyl acetate) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((linoleyloxy)-2-(palmitoyloxy) propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**128d**, 76 mg, 0.050 mmol, 65%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.39-5.25 (m, 4 H), 5.24-5.22 (m, 2 H), 4.35-4.31 (m, 2 H), 4.23-4.12 (m, 6 H), 4.06-3.99 (m, 5 H), 3.78 (d, $^{3}J_{HP}$ = 11.6 Hz, diastereomers visible), 2.77 (t, 2 H, *J* = 6.4 Hz), 2.32 (q, 8 H, *J* = 8.0 Hz), 2.05 (q, 2 H, *J* = 6.8 Hz), 1.62-1.59 (m, 18 H), 1.39-1.25 (m, 86 H), 0.96 (t, 9 H, *J* = 7.8 Hz), 0.90-0.86 (m, 12 H), 0.63 (q, 6 H, *J* = 8.0 Hz); ^{13}C NMR (CDCl3, 125 MHz) δ 173.2 (2C), 172.8, 130.2, 130.0, 128.1, 127.9, 69.4-69.3 (m), 67.8 (m), 65.6 (m), 61.6, 54.6 (d, $^{3}J_{CP}$ = 6.3 Hz), 34.1, 34.0 (2C), 31.9, 31.5, 29.7-29.1 (m), 27.2, 25.6, 24.8, 22.7, 22.6 14.1 (2C),

6.6, 4.7; IR ν 2922, 2853, 1743, 1036 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{83}H_{161}O_{17}P_2Si$ $[M+H]^+$ 1520.09783, found 1542.0802 $[M+Na]^+$.

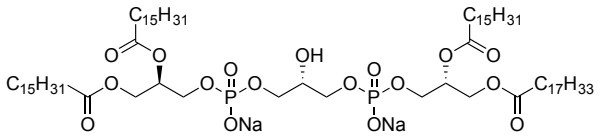


(2*R*)-3-((Methoxy(3-((methoxy((*R*)-3-((arachidonoyloxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128e). Prepared according to general procedure J using degassed CH_2Cl_2 and shielding from light during the reaction. The following modifications were used for the workup and purification: butylated hydroxytoluene (BHT, 2 mg; to prevent oxidation during workup) was added, the reaction mixture was diluted with CH_2Cl_2 (7 mL), rinsed with water (3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on SiO_2 (4:1 to 3:1 to 2:1 to 1:1 hexanes:ethyl acetate; 1 mg BHT added before concentrating fractions) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((arachidonoyloxy)-2-(palmitoyloxy)propoxy)phosphoryloxy)-2-((triethylsilyl)oxy) propoxy)phosphoryl) oxy)propane-1,2-diyl dipalmitate (**128e**, 52 mg, 0.029 mmol, 57%; mixture of diastereomers at phosphorus) as a clear colorless oil. Product contains ~2% BHT as a stabilizer. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.38-5.32 (m, 8 H), 5.25-5.22 (m, 2 H), 4.35-4.32 (m, 2 H), 4.23-4.13 (m, 6 H), 4.06-3.98 (m, 5 H), 3.78 (d, $^3J_{HP}$ = 11.0 Hz, diastereomers visible), 2.84-2.80 (m, 6 H), 2.34-2.30 (m, 8 H), 2.11 (q, 2 H, *J* = 7.0 Hz), 2.05 (q, 2 H, *J* = 7.2 Hz), 1.70 (pent, 2 H, *J* = 7.5 Hz), 1.63-1.60 (m, 8 H), 1.39-1.25 (m, 78 H), 0.96 (t, 9 H, *J* = 8.0 Hz), 0.90-0.87 (m, 12 H), 0.63 (q, 6 H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 172.9, 172.7, 130.5, 129.0, 128.8, 128.6, 128.3, 128.1, 127.9, 127.6, 69.6-69.5 (m), 67.9 (m), 65.6 (m), 61.8-61.7 (m), 54.5 (d, $^3J_{CP}$ = 6.3 Hz), 34.2, 34.0, 33.4, 31.9, 31.5, 30.4, 29.7-29.1 (m), 27.2, 26.5, 25.7, 24.9, 24.7, 22.7, 22.6 (2C), 14.1, 6.6, 4.8; IR ν 2922, 2853, 1742, 1036

cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{85}H_{161}O_{17}P_2Si$ $[M+H]^+$ 1544.0978, found 1544.1090 $[M+Na]^+$.

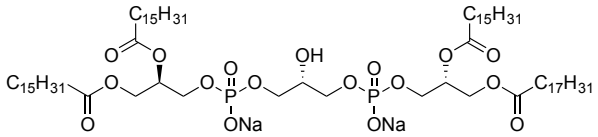


Tripalmitoyl-monostearoyl cardiolipin disodium salt (**129b**). Prepared according to General Procedure I using **129b** (53 mg, 0.035 mmol, 1.0 equiv.) and NaI (15 mg, 0.053 mmol, 2.9 equiv.). For the second step, H_2SiF_6 (0.05 mL, 0.09 mmol, 2 equiv.) was used. Trituration with Et_2O gave tripalmitoyl-monostearoyl cardiolipin disodium salt (**129b**, 42 mg, 0.029 mmol, 86%) as a colorless solid. $[\alpha]_D^{20}$ = +5.1 (*c* = 0.54, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.26-5.22 (m, 2 H), 4.47 (dd, 2 H, J_1 = 3.0, J_2 = 12.0 Hz), 4.20 (dd, 2 H, J_1 = 6.5, J_2 = 12.0 Hz), 4.02-3.89 (m, 9 H), 2.36-2.30 (m, 8 H), 1.62-1.59 (m, 8 H), 1.33-1.29 (m, 100 H), 0.90 (t, 12 H, *J* = 6.8 Hz); HRMS (ESI$^-$) *m/z* calculated for $C_{75}H_{144}O_{17}P_2$ $[M]^{2-}$ 688.4940, found 689.4955.

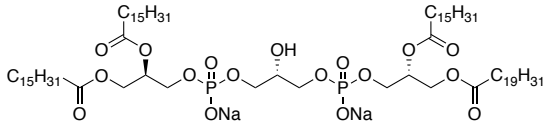


Tripalmitoyl-monooleoyl cardiolipin disodium salt (**129c**). Prepared according to General Procedure I using NaI (19 mg, 0.13 mmol, 3.0 equiv.). For the second step H_2SiF_6 (0.05 mL, 0.08 mmol, 2 equiv.) was used. The residue was purified by dissolving in hot 1:3 hexanes:Et_2O and allowed to precipitate in the -20°C freezer overnight. The precipitate was collected by vacuum filtration and gave tripalmitoyl-monooleoyl cardiolipin disodium salt (**129c**, 40 mg, 0.028 mmol, 68%) as a colorless solid. $[\alpha]_D^{20}$ = +2.7 (*c* = 0.43, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.38-5.31 (m, 2 H), 5.26-5.22 (m, 2 H), 4.47 (dd, 2 H, J_1 = 2.5, J_2 = 12.0 Hz), 4.20 (dd, 2 H, J_1 = 7.0, J_2 = 12.0 Hz), 4.01 (t, 4 h, *J* = 5.5 Hz), 3.95-3.89 (m, 5 H), 2.36-2.30 (m, 8 H),

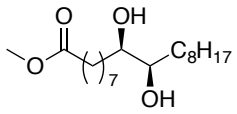
2.04-2.02 (m, 4 H), 1.62-1.59 (m, 8 H), 1.33-1.29 (m, 94 H), 0.90 (t, 12 H, J = 9.0 Hz); HRMS (ESI$^-$) *m/z* calculated for $C_{75}H_{142}O_{17}P_2$ $[M]^{2-}$ 688.4861, found 688.4885.



Tripalmitoyl-monolinoleoyl cardiolipin disodium salt (**129d**). Prepared using a modified version of General Procedure I: To a solution of NaI (21 mg, 0.14 mmol, 3.3 equiv.) in a nitrogen-flushed reflux apparatus was added a solution of **128d** (65 mg, 0.043 mmol, 1.0 equiv.) in degassed acetone (0.43 mL), followed by an additional portion of degassed acetone (0.43 mL, used to rinse the vial that starting material came from). The pale yellow solution was shielded from light and heated to reflux for 14.5 h. The acetone was removed under reduced pressure and the resulting pale yellow solid dissolved in CH_2Cl_2 (10 mL). The organic portion was rinsed with sat. brine:H_2O (1:1, 3 mL), dried (Na_2SO_4), concentrated. The resulting pale yellow solid was dissolved in degassed THF (0.78 mL) under nitrogen, H_2SiF_6 (0.06 mL, 0.1 mmol, 2 equiv.) added, and the reaction mixture stirred for 0.5 h shielded from light. The reaction mixture was quenched with sat. $NaHCO_3$ (3 mL), and extracted with $CHCl_3$ (2x7 mL). The combined organic layers were rinsed with brine (3 mL), dried (Na_2SO_4) and concentrated. Purification by precipitation from hot 3:1 Et_2O:hexanes and further cooling to -20°C gave tripalmitoyl-monolinoleoyl cardiolipin disodium salt (**129d**, 53 mg, 0.037 mmol, 87%) as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 5.36-5.30 (m, 4 H), 5.25-5.28 (m, 2 H), 4.47 (dd, 2 H, J_1 = 2.8, J_2 = 12.0 Hz), 4.20 (dd, 2 H, J_1 = 6.8, J_2 = 12.0 Hz), 4.01 (t, 4 H, J = 5.4 Hz), 3.95-3.88 (m, 5 H), 2.78 (t, 2 H, J = 6.2 Hz), 2.35 (t, 4 H, J = 7.4 Hz), 2.32 (t, 4 H, J = 7.6 Hz), 2.07 (q, 4 H, J = 6.5 Hz), 1.61-1.59 (m, 8 H), 1.37-1.29 (m, 86 H), 0.93-0.89 (m, 12 H); HRMS (ESI$^-$) *m/z* calculated for $C_{75}H_{140}O_{17}P_2$ $[M]^{2-}$ 687.4783; found 687.4800.

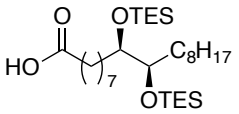


Tripalmitoyl-monoarachidoyl cardiolipin disodium salt (**129e**). Performed using a modified version of General Procedure I: To NaI (18 mg, 0.12 mmol, 3.1 equiv.) in a nitrogen flushed reflux apparatus was added a solution of **128e** (60 mg, 0.39 mmol, 1.0 equiv.) in degassed acetone (0.4 mL), followed by an additional portion of degassed acetone (0.38 mL, used to rinse the vial that starting material came from). The pale yellow solution was shielded from light and heated to relax for 13.5 h. The acetone was removed under reduced pressure and the resulting pale yellow solid dissolved in CH_2Cl_2 (7 mL). The organic portion was rinsed with 20% $Na_2S_2O_3$ (3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated. The residue was dissolved in degassed THF (0.78 mL) under nitrogen, H_2SiF_6 (0.05 mL, 0.08 mmol, 2 equiv.) added, and the reaction mixture stirred for 0.5 h shielded from light. The reaction mixture was quenched with sat. $NaHCO_3$ (3 mL) and extracted with $CHCl_3$ (2x7 mL). The combined organic layers were rinsed with brine (3 mL), dried (Na_2SO_4) and concentrated giving tripalmitoyl-monoarachidoyl cardiolipin disodium salt (**129e**, 44 mg, 0.030 mmol, 78%) as a pale yellow solid. $[\alpha]_D^{20} = +2.5$ (*c* = 0.52, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.39-5.30 (m, 8 H), 5.24 (bs, 2 H), 4.46 (d, 2 H, *J* = 11.5 Hz), 4.20 (dd, 2 H, J_1 = 6.5 Hz, J_2 = 11.5 Hz), 4.01-4.00 (m, 4 H), 3.94-3.91 (m, 5 H), 2.86-2.83 (m, 6 H), 2.36-2.30 (m, 8 H), 2.13-2.06 (m, 4 H), 1.69-1.60 (m, 8 H), 1.37-1.29 (m, 78 H), 0.91-0.89 (m, 12 H); HRMS (ESI^-) *m/z* calculated for $C_{77}H_{140}O_{17}P_2$ $[M]^{2-}$ 699.4783; found 699.4788.

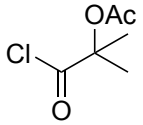


Methyl (9*R*,10*R*)-9,10-dihydroxyoctadecanoate (149).[158] Elaidic acid (0.510 g, 1.72 mmol, 1.0 equiv.) was dissolved MeOH (5.7 mL) and one drop of conc. H_2SO_4 added. The solution was then heated to reflux for 9.25 h. The reaction mixture was neutralized with sat. $NaHCO_3$ and the MeOH removed under reduced pressure. Water (3 mL) was added and the mixture extracted with Et_2O (15 mL). The organic layer was then rinsed with sat. $NaHCO_3$ (3 mL), brine (3 mL), dried (Na_2SO_4), and concentrated giving methyl elaidate (0.507 g, 1.71 mmol, quant.) as a clear oil.

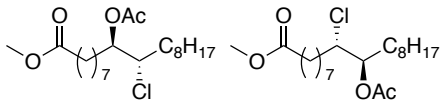
AD-mix β (1.40 g), additional $(DHQD)_2PHAL$ (0.005 g, 0.006 mmol, 0.006 equiv.) and methane sulfonamide (0.098 g, 1.0 mmol, 1.0 equiv.) were dissolved in (4 mL) and H_2O (5 mL) at room temperature (takes about 10 min). The orange biphasic mixture was cooled to 0 °C, a portion of the methyl elaidate (0.296 g, 0.998 mmol, 1.0 equiv. in 0.5 mL; flask rinsed with 0.5 mL *t*-BuOH) added, and the mixture stirred for 21 h at this temperature. The now yellow reaction mixture was quenched with Na_2SO_3 (1.40 g) and allowed to warm to room temperature. The mixture was extracted with Et_2O (3x20 mL), and the combined organic layers rinsed with 2 M NaOH (15 mL), brine (15 mL), dried (Na_2SO_4), and concentrated. The crude mixture was filtered through a plug of SiO_2 (4:1 to 1:1 hexanes:ethyl acetate) giving methyl (9*R*,10*R*)-9,10-dihydroxyoctadecanoate (**149**, 0.307 g, 0.929 mmol, 93%, >95% ee) as a colorless solid. $[\alpha]_D^{21}$ = +22.1 (*c* = 1.0, MeOH; lit. +22.5); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.67 (s, 3 H), 3.40 (bs, 2 H), 2.30 (t, 2 H, *J* = 7.5 Hz), 1.98 (d, 1 H, *J* = 4.2 Hz), 1.94 (d, 1 H, *J* = 4.5 Hz), 1.64-1.59 (m, 2 H), 1.47-1.28 (m, 24 H), 0.88 (t, 3 H, *J* = 6.8 Hz).



(9*R*,10*R*)-9,10-bis((Triethylsilyl)oxy)octadecanoic acid (150). To a yellow solution of **149** (0.400 g, 1.21 mmol, 1.0 equiv.) in CH_2Cl_2 (3.5 mL) at 0 °C under nitrogen was added DMAP (0.015 g, 0.12 mmol, 0.1 equiv.), imidazole (0.181 g, 2.66 mmol, 2.2 equiv.), and TESCl (0.43 mL, 2.53 mmol, 2.1 equiv.). The cloudy, pale yellow slurry was warmed to room temperature and stirred for 18.5 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL), rinsed with water (4 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. Purification by filtration through SiO_2 (97:3 hexanes:ethyl acetate) gave a pale yellow oil. This oil was dissolved in THF (6.3 mL), TMSOK (297 mg, 2.08 mmol, 2.1 equiv.) added, and the reaction was stirred 4 h. The reaction was quenched with 0.5 M citric acid (1 mL) and stirred for 5 min. The mixture was diluted with water (3 mL) and the resulting mixture was extracted with Et_2O (2x10 mL). The combined organic fractions were rinsed with brine (3 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on SiO_2 (9:1 to 6:1 to 4:1 to 2:1 hexanes:ethyl acetate) gave (9*R*,10*R*)-9,10-bis((triethylsilyl) oxy)octadecanoic acid (**150**, 464 mg, 0.854 mmol, 89%) as a clear pale yellow oil. $[\alpha]_D^{20}$ = +33.1 (*c* =1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.53 (d, 2 H, *J* = 7.5 Hz), 2.35 (t, 2 H, *J* = 7.5 Hz), 1.65-1.58 (m, 4 H), 1.50-1.40 (m, 2 H), 1.31-1.18 (m, 20 H), 0.96, (t, 18 H, *J* = 8.0 Hz), 0.88 (t, 3 H, *J* = 6.8 Hz), 0.58, (q, 12 H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.0, 75.7, 33.9, 31.9, 30.2-29.2 (m), 26.7 (2C), 24.7, 22.7, 14.1, 6.9, 5.2. IR ν 2952, 2923, 2876, 2855, 2847, 1710, 722 cm^{-1}; HRMS (ESI^-) *m/z* calculated for $C_{30}H_{64}O_4Si_2$ $[M-H]^-$ 543.4259; found 543.4262.

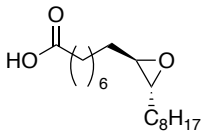


1-Chloro-2-methyl-1-oxopropan-2-yl acetate (161).[163] 2-Hydroxyisobutyric acid (4.05 g, 38.1 mmol, 1.0 equiv.) was cooled in an ice bath and acetyl chloride (7.1 mL, 98 mmol, 2.6 equiv.) was added dropwise. When gas evolution has ceased (~10 min), the mixture was heated to reflux for 2 h. Excess acetyl chloride was removed by distillation. To the resulting oil was added $SOCl_2$ (2.60mL, 34.6 mmol, 0.91 equiv) and the mixture heated to reflux for 2 h. The crude oil was then distilled under high vacuum (~4 mm Hg) giving 1-chloro-2-methyl-1-oxopropan-2-yl acetate (**161**, 3.85 g, 23.4 mmol, 61%, b.p. = 50 °C) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.09 (s, 3 H). 1.60 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.8, 169.8, 83.0, 23.9, 20.7.

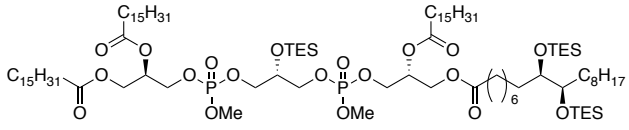


(9*R*,10*S*)-9-Acetoxy-10-chlorooctadecanoic acid methyl ester (169a); (9*R*,10*S*)-10-acetoxy-9-chlorooctadecanoic acid methyl ester (169b). To a suspension of **149** (116 mg, 0.351 mmol, 1.0 equiv.) in Et_2O (0.45 mL) was added **161** (124 mg, 0.753 mmol, 2.1 equiv.) in Et_2O (0.45 mL) a dropwise fashion. The resulting clear solution was then stirred for 21 h. The reaction mixture was diluted with Et_2O (10 mL), rinsed with sat. $NaHCO_3$ (3x3 mL), brine (3 mL), dried (Na_2SO4), and concentrated. Purification by filtration through SiO_2 (4:1 hexanes:ethyl acetate) gave (9*R*,10*S*)-9-acetoxy-10-chlorooctadecanoic methyl ester and (9*R*,10*S*)-10-acetoxy-9-chlorooctadecanoic acid methyl ester (**169a**+**169b**, 137 mg, 0.350 mmol, quant.) as a clear colorless oil. Product is a ~1:1 mixture and was used without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.99-4.96 (m, 1 H), 3.99-3.96 (s, 1 H), 3.67 (s, 3 H),

2.30 (t, 2 H, J = 7.5 Hz; both isomers visible), 2.09 (s, 3 H), 1.74-1.60 (m, 6 H), 1.31-1.26 (m, 20 H), 0.89-0.87 (m, 3 H).

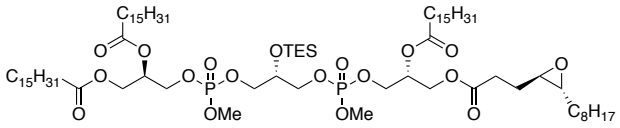


8-((2*R*,3*R*)-3-Octyloxiran-2-yl)octanoic acid (171). To solution of **169a** and **169b** (135 mg, 0.345 mmol, 1.0 equiv., 1:1 mixture) in THF (3.5 mL) under nitrogen was added TMSOK (197 mg, 1.38 mmol, 4.0 equiv.) and the reaction mixture was stirred for 9 h. The reaction was quenched with 0.5 M citric acid (3 mL) and stirred for 5 min. The mixture was extracted with Et_2O (3x8 mL). The combined organic fractions were rinsed with brine (6 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on SiO_2 (3:1 to 2:1 hexanes ethyl acetate) gave 8-((2*R*,3*R*)-3-octyloxiran-2-yl)octanoic acid (**171**, 92 mg, 0.31 mmol, 89%) as a colorless solid. $[\alpha]_{546}^{20}$ = +24 (*c* = 0.245, MeOH); Mp = 54.0-54.4 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.65 (t, 2 H, J = 5.0 Hz), 2.35 (t, 2 H, J = 7.5 Hz), 1.65-1.27 (m, 24 H), 0.89-0.86 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.7, 58.9 (2 C), 33.6, 32.1 (2 C), 31.8, 29.5-28.9 (m), 26.0, 24.7, 22.6, 14.1; IR ν 3058, 2918, 2847, 1709, 1689, 878 cm^{-1}; HRMS (ESI$^-$) *m/z* calculated for $C_{18}H_{33}O_3$ $[M-H]^-$ 297.2430; found 297.24233.



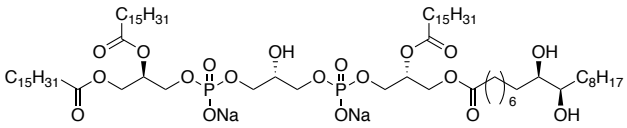
(2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((14-((9*R*,10*R*)-9,10-bis((triethylsilyl)oxy)octadecanoyl)oxy)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128f). Prepared according to general procedure J. Purification by chromatography on SiO_2 (2:1 to 3:2 hexanes: ethyl acetate) gave (2*R*)-3-((methoxy(3-((methoxy((*R*)-3-((14-((9*R*,10*R*)-9,10-bis((triethylsilyl)oxy)octadecanoyl)ox

y)-2-(palmitoyloxy)propoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**128f**, 77 mg, 0.043 mmol, 63%; mixture of diastereomers at phosphorus) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.25-5.23 (m, 2 H), 4.36-4.31 (m, 2 H), 4.25-4.10 (m, 6 H), 4.05-3.97 (m, 5 H), 3.93 (t, 2 H, J = 6.5 Hz), 3.78 (d, $^3J_{HP}$ = 11.2 Hz, diastereomers), 3.59 (apparent d, 2 H, J = 7.20 Hz), 2.32 (q, 8 H, J = 8.1 Hz), 1.1.61-1.58 (m, 10 H), 1.50-1.40 (m, 2 H), 1.46-1.25 (m, 96 H), 0.96 (t, 9 H, J = 7.8 Hz), 0.95 (t, 18 H, J = 7.8 Hz), 0.88 (t, 9 H, J = 6.8 Hz), 0.69-0.61 (m, 18 H); 13C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.8, 75.7 (2C), 69.4 (m), 68.2, 67.8 (m), 65.6 (m), 61.7, 54.6 (d, $^3J_{CP}$ = 6.3 Hz), 34.1, 34.0, 31.9 (2C), 30.2, 29.9-29.1 (m), 26.8, 26.7, 24.9, 22.7, 14.1, 6.9, 6.6, 5.2, 4.7; IR ν 2922, 2853, 1743, 1016, 741 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{95}H_{196}O_{19}P_2Si_3$ $[M+H]^+$ 1787.3153; found 1807.2811 $[M+Na]^+$.



(2*R*)-3-((((2*S*)-3-((((*R*)-2-palmitoyloxy-3-((9-((2*R*,3*R*)-3-heptyloxiran-2-yl)nonanoyl)oxy)propoxy)(methoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (128g). Prepared according to general procedure J. Purification by chromatography on SiO_2 (prerinsed with 1% Et_3N in 2:1 hexanes ethyl acetate; eluted with 3:1 to 2:1 to 1:1 hexanes:ethyl acetate) gave (2*R*)-3-((((2*S*)-3-((((*R*)-2-palmitoyloxy-3-((9-((2*R*,3*R*)-3-heptyloxiran-2-yl)nonanoyl)oxy)propoxy)(methoxy)phosphoryl)oxy)-2-((triethylsilyl)oxy)propoxy)(methoxy)phosphoryl)oxy)propane-1,2-diyl dipalmitate (**128g**, 64 mg, 0.041 mmol, 51%; mixture of diastereomers at phosphorus) as a waxy, colorless solid. Mp = 31.4-32.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.25-5.23 (m, 2 H), 4.36-4.31 (m, 2 H), 4.25-4.12 (m, 6 H), 4.05-3.94 (m, 5 H), 3.78 (d, $^3J_{HP}$ = 11.2 Hz,

diastereomers), 2.64 (t, 2 H, J = 4.8 Hz), 2.32 (apparent q, 8 H, J = 8.0 Hz), 2.01-1.98 (m, 4 H), 1.61-1.54 (m, 6 H), 1.50-1.43 (m, 6 H), 1.28-1.25 (m, 92), 0.96 (t, 9 H, J = 7.8 Hz), 0.88 (t, 9 H, J = 6.6 Hz), 0.63 (m, 6 H, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.7, 75.7, 69.5-69.4 (m), 67.8 (m), 65.6 (m), 61.7, 58.8 (2 C), 54.5 (d, $^{3}J_{CP}$ = 6.3 Hz), 34.1, 34.0 (2 C), 31.2 (2 C), 32.0, 31.0, 29.7-29.0 (m), 26.1, 26.0, 24.8 (2 C), 22.7, 22.6, 14.1 (2 C), 6.6, 4.7; IR ν 2918, 2850, 1739, 1035, 722 cm^{-1}; HRMS (ESI$^+$) m/z calculated for $C_{85}H_{161}O_{18}P_2Si$ $[M+H]^+$ 1560.0927, found 1560.0913.



Tripalmitoyl-mono(9*R*,10*R*)-9,10-dihydroxyoctadecanoyl cardiolipin disodium salt (**129f**). Prepared according to General Procedure I using **128f** (75 mg, 0.042 mmol, 1.0 equiv.) and NaI (19 mg, 0.13 mmol, 3.0 equiv.). For the second step, was used. The resulting crude solid was triturated with Et_2O giving tripalmitoyl-monostearoyl diol cardiolipin disodium salt (**129g**, 49 mg, 0.034 mmol, 80%) as a colorless solid. $[\alpha]_D^{20}$ = +6.0 (c = 0.46, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.26-5.22 (m, 2 H), 4.47-4.41 (m, 2 H), 4.20 (dd, 2 H, J_1 = 6.5, J_2 = 12.0 Hz), 4.01 (t, 4 H, J = 5.3 Hz), 3.94-3.89 (m, 5 H), 3.37 (d, 2 H, J = 7.5 Hz), 2.36-2.31 (m, 8 H), 1.62-1.59 (m, 8 H), 1.50 (m, 4 H), 1.49-1.29 (m, 94 H), 0.90 (t, 12 H, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.9, 174.6, 75.3, 72.1 (m), 71.6 (m), 67.8 (m), 64.8 (m), 63.8 (m), 35.2, 35.0, 34.1, 33.1, 30.9-30.3 (m), 27.1, 27.0, 26.0 (2C), 23.7, 14.4; HRMS (ESI$^-$) *m/z* calculated for $C_{75}H_{144}O_19P_2$ $[M]^{2-}$ 705.4889, found 705.4883.

REFERENCES

1. Chaurio, R. A.; Janko, C.; Munoz, L. E.; Frey, B.; Herrmann, M.; Gaipl, U. S. *Molecules* **2009,** *14*, 4892-4914.

2. Chu, C. T.; Ji, J.; Dagda, R. K.; Jiang, J. F.; Tyurina, Y. Y.; Kapralov, A. A.; Tyurin, V. A.; Yanamala, N.; Shrivastava, I. H.; Mohammadyani, D.; Wang, K. Z. Q.; Zhu, J. H.; Klein-Seetharaman, J.; Balasubramanian, K.; Amoscato, A. A.; Borisenko, G.; Huang, Z. T.; Gusdon, A. M.; Cheikhi, A.; Steer, E. K.; Wang, R.; Baty, C.; Watkins, S.; Bahar, I.; Bayir, H.; Kagan, V. E. *Nat. Cell Biol.* **2013,** *15*, 1197-1205.

3. DeVries, G. H.; Norton, W. T. *J. Neurochem.* **1973,** *22*, 251-257.

4. D'Angelo, G.; Capasso, S.; Sticco, L.; Russo, D. *FEBS J.* **2013,** *280*, 6338-6353.

5. McMaster, C. R. *FEBS Lett.* **2017**, 1-17.

6. van der Veen, J. N.; Kennelly, J. P.; Wan, S.; Vance, J. E.; Vance, D. E.; Jacobs, R. L. *Biochim. Biophys. Acta Biomembr.* **2017,** *1859*, 1558-1572.

7. Olofsson, G.; Sparr, E. *PLoS One* **2013,** *8*, e73040.

8. Vance, J. E.; Steenbergen, R. *Prog. Lipid Res.* **2005,** *44*, 207-234.

9. Rawicz, W.; Olbrich, K. C.; McIntosh, T.; Needham, D.; Evans, E. *Biophys. J.* **2000,** *79*, 328-339.

10. Pinot, M.; Vanni, S.; Pagnotta, S.; Lacas-Gervais, S.; Payet, L. A.; Ferreira, T.; Gautier, R.; Goud, B.; Antonny, B.; Barelli, H. *Science* **2014,** *345*, 693-697.

11. Antonny, B. *Annu. Rev. Biochem.* **2011,** *80*, 101-123.

12. Cullis, P. R.; Dekruijff, B. *Biochim. Biophys. Acta* **1979,** *559*, 399-420.

13. Lewis, R. N. A. H.; McElhaney, R. N. *Biochim. Biophys. Acta Biomembr.* **2009,** *1788*, 2069-2079.

14. Writoban, B. B.; Neff, J. K.; Gohil, V. M. *FEBS Lett.* **2017**.

15. Feillet-Coudray, C.; Fouret, G.; Casas, F.; Coudray, C. *J. Bioenerg. Biomembr.* **2014,** *46*, 447-457.

16. Huang, K. C.; Mukhopadhyay, R.; Wingreen, N. S. *PLoS Comput. Biol.* **2006,** *2*, 1357-1364.

17. Mukhopadhyay, R.; Huang, K. C.; Wingreen, N. S. *Biophys. J.* **2008,** *95*, 1034-1049.

18. Renner, L. D.; Weibel, D. B. *Proc. Nat. Acad. Sci. U.S.A.* **2011,** *108*, 6264-6269.

19. Pfeiffer, K.; Gohil, V.; Stuart, R. A.; Hunte, C.; Brandt, U.; Greenberg, M. L.; Schagger, H. *J. Biol. Chem.* **2003,** *278*, 52873-52880.

20. Sharpley, M. S.; Shannon, R. J.; Draghi, F.; Hirst, J. *Biochemistry* **2006,** *45*, 241-8.

21. Acehan, D.; Malhotra, A.; Xu, Y.; Ren, M.; Stokes, D. L.; Schlame, M. *Biophys. J.* **2011,** *100*, 2184-2192.

22. Huang, K. C.; Ramamurthi, K. S. *Mol. Microbiol.* **2010,** *76*, 822-832.

23. Reuter, S.; Gupta, S. C.; Chaturvedi, M. M.; Aggarwal, B. B. *Free Radic. Biol. Med.* **2010,** *49*, 1603-1616.

24. Huang, W. J.; Zhang, X.; Chen, W. W. *Biomed. Rep.* **2016,** *4*, 519-522.

25. Hwang, O. *Exp. Neurobiol.* **2013,** *22*, 11-17.

26. Giacco, F.; Brownlee, M. *Circ. Res.* **2010,** *107*, 1058-1070.

27. Kattoor, A. J.; Pothineni, N. V. K.; Palagiri, D.; Mehta, J. L. *Curr. Atheroscler. Rep.* **2017,** *19*, doi:10.1007/s11883-017-0678-6.

28. Yin, H. Y.; Xu, L. B.; Porter, N. A. *Chem. Rev.* **2011,** *111*, 5944-5972.

29. Bochkov, V. N.; Oskolkova, O. V.; Birukov, K. G.; Levonen, A. L.; Binder, C. J.; Stockl, J. *Antioxid. Redox Signal.* **2010,** *12*, 1009-1059.

30. Pratt, D. A.; Mills, J. H.; Porter, N. A. *J. Am. Chem. Soc.* **2003,** *125*, 5801-5810.

31. Maillard, B.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1983,** *105*, 5095-5099.

32. Gaschler, M. M.; Stockwell, B. R. *Biochem. Biophys. Res. Commun.* **2017,** *482*, 419-425.

33. Yin, H. Y.; Zhu, M. J. *Free Radic. Res.* **2012,** *46*, 959-974.

34. Brash, A. R. *Lipids* **2000,** *35*, 947-952.

35. Zielinski, Z. A. M.; Pratt, D. A. *J. Org. Chem.* **2017,** *82*, 2817-2825.

36. Milne, G. L.; Yin, H.; Hardy, K. D.; Davies, S. S.; Roberts, L. J. *Chem. Rev.* **2011,** *111*, 5973-5996.

37. Bayir, H.; Tyurin, V. A.; Tyurina, Y. Y.; Viner, R.; Ritov, V.; Amoscato, A. A.; Zhao, Q.; Zhang, X. J.; Janesko-Feldman, K. L.; Alexander, H.; Basova, L. V.; Clark, R. S.; Kochanek, P. M.; Kagan, V. E. *Ann. Neurol.* **2007,** *62*, 154-169.

38. Kagan, V. E.; Tyurin, V. A.; Jiang, J.; Tyurina, Y. Y.; Ritov, V. B.; Amoscato, A. A.; Osipov, A. N.; Belikova, N. A.; Kapralov, A. A.; Kini, V.; Vlasova, II; Zhao, Q.; Zou, M.; Di, P.; Svistunenko, D. A.; Kurnikov, I. V.; Borisenko, G. G. *Nat. Chem. Biol.* **2005,** *1*, 223-232.

39. Tyurina, Y. Y.; Poloyac, S. M.; Tyurin, V. A.; Kapralov, A. A.; Jiang, J. F.; Anthonymuthu, T. S.; Kapralova, V. I.; Vikulina, A. S.; Jung, M. Y.; Epperly, M. W.; Mohammadyani, D.; Klein-Seetharaman, J.; Jackson, T. C.; Kochanek, P. M.; Pitt, B. R.; Greenberger, J. S.; Vladimirov, Y. A.; Bayir, H.; Kagan, V. E. *Nat. Chem.* **2014,** *6*, 542-552.

40. Kagan, V. E.; Tyurin, V. A.; Jiang, J. F.; Tyurina, Y. Y.; Ritov, V. B.; Amoscato, A. A.; Osipov, A. N.; Belikova, N. A.; Kapralov, A. A.; Kini, V.; Vlasova, I. I.; Zhao, Q.; Zou, M. M.; Di, P.; Svistunenko, D. A.; Kurnikov, I. V.; Borisenko, G. G. *Nat. Chem. Biol.* **2005,** *1*, 223-232.

41. Genaro-Mattos, T. C.; Queiroz, R. F.; Cunha, D.; Appolinario, P. P.; Di Mascio, P.; Nantes, I. L.; Augusto, O.; Miyamoto, S. *Biochemistry* **2015,** *54*, 2841-2850.

42. Pointer, C. B.; Klegeris, A. *Cell Mol. Neurobiol.* **2017,** *37*, 1161-1172.

43. Ikon, N.; Ryan, R. O. *Lipids* **2017,** *52*, 99-108.

44. Claypool, S. M.; Koehler, C. M. *Trends Biochem. Sci.* **2012,** *37*, 32-41.

45. Monteiro-Cardoso, V. F.; Oliveira, M. M.; Melo, T.; Domingues, M. R.; Moreira, P. I.; Ferreiro, E.; Peixoto, F.; Videira, R. A. *J. Alzheimer's Dis.* **2015,** *43*, 1375-1392.

46. Hoye, A. T.; Davoren, J. E.; Wipf, P.; Fink, M. P.; Kagan, V. E. *Acc. Chem. Res.* **2008,** *41*, 87-97.

47. Rin Jean, S.; Tulumello, D. V.; Wisnovsky, S. P.; Lei, E. K.; Pereira, M. P.; Kelley, S. O. *ACS Chem. Biol.* **2014,** *9*, 323-333.

48. James, A. M.; Sharpley, M. S.; Manas, A. R.; Frerman, F. E.; Hirst, J.; Smith, R. A.; Murphy, M. P. *J. Biol. Chem.* **2007,** *282*, 14708-14718.

49. Murphy, M. P.; Smith, R. A. *Annu. Rev. Pharmacol. Toxicol.* **2007,** *47*, 629-656.

50. Bhowmick, D.; Mugesh, G. *Org. Biomol. Chem.* **2015,** *13*, 10262-10272.

51. Szeto, H. H. *Br. J. Pharmacol.* **2013,** *171*, 2029-2050.

52. Szeto, H. H.; Schiller, P. W. *Pharm. Res.* **2011,** *28*, 2669-2679.

53. Schiller, P. W.; Nguyen, T. M.; Chung, N. N.; Lemieux, C. *J. Med. Chem.* **1989,** *32*, 698-703.

54. Birk, A. V.; Liu, S.; Soong, Y.; Mills, W.; Singh, P.; Warren, J. D.; Seshan, S. V.; Pardee, J. D.; Szeto, H. H. *J. Am. Soc. Nephrol.* **2013,** *24*, 1250-1261.

55. Ashrafuzzaman, M.; Andersen, O. S.; McElhaney, R. N. *Biochim. Biophys. Acta* **2008,** *1778*, 2814-2822.

56. Sholtz, K. F.; Solovjeva, N. A.; Kotelnikova, A. V.; Snezhkova, L. G.; Miroshnikov, A. I. *FEBS Lett.* **1975,** *58*, 140-144.

57. Zhang, R.; Goldstein, S.; Samuni, A. *Free Radic. Biol. Med.* **1999,** *26*, 1245-1252.

58. Krishna, M. C.; Grahame, D. A.; Samuni, A.; Mitchell, J. B.; Russo, A. *Pro. Nat. Acad. Sci. U.S.A.* **1992,** *89*, 5537-5541.

59. Jiang, J.; Kurnikov, I.; Belikova, N. A.; Xiao, J.; Zhao, Q.; Amoscato, A. A.; Braslau, R.; Studer, A.; Fink, M. P.; Greenberger, J. S.; Wipf, P.; Kagan, V. E. *J. Pharmacol. Exp. Ther.* **2007,** *320*, 1050-1060.

60. Xiao, J.; Weisblum, B.; Wipf, P. *J. Am. Chem. Soc.* **2005,** *127*, 5742-5743.

61. Wipf, P.; Xiao, J.; Jiang, J.; Belikova, N. A.; Tyurin, V. A.; Fink, M. P.; Kagan, V. E. *J. Am. Chem. Soc.* **2005,** *127*, 12460-12461.

62. Macias, C. A.; Chiao, J. W.; Xiao, J.; Arora, D. S.; Tyurina, Y. Y.; Delude, R. L.; Wipf, P.; Kagan, V. E.; Fink, M. P. *Ann. Surg.* **2007,** *245*, 305-314.

63. Xun, Z.; Rivera-Sanchez, S.; Ayala-Pena, S.; Lim, J.; Budworth, H.; Skoda, E. M.; Robbins, P. D.; Niedernhofer, L. J.; Wipf, P.; McMurray, C. T. *Cell Rep.* **2012,** *2*, 1137-1142.

64. Ji, J.; Kline, A. E.; Amoscato, A.; Samhan-Arias, A. K.; Sparvero, L. J.; Tyurin, V. A.; Tyurina, Y. Y.; Fink, B.; Manole, M. D.; Puccio, A. M.; Okonkwo, D. O.; Cheng, J. P.; Alexander, H.; Clark, R. S.; Kochanek, P. M.; Wipf, P.; Kagan, V. E.; Bayir, H. *Nat. Neurosci.* **2012,** *15*, 1407-1413.

65. Rajagopalan, M. S.; Gupta, K.; Epperly, M. W.; Franicola, D.; Zhang, X.; Wang, H.; Zhao, H.; Tyurin, V. A.; Pierce, J. G.; Kagan, V. E.; Wipf, P.; Kanai, A. J.; Greenberger, J. S. *In Vivo* **2009,** *23*, 717-726.

66. Shinde, A.; Berhane, H.; Rhieu, B. H.; Kalash, R.; Xu, K.; Goff, J.; Epperly, M. W.; Franicola, D.; Zhang, X.; Dixon, T.; Shields, D.; Wang, H.; Wipf, P.; Parmar, K.; Guinan, E.; Kagan, V.; Tyurin, V.; Ferris, R. L.; Zhang, X.; Li, S.; Greenberger, J. S. *Radiat. Res.* **2016,** *185*, 134-150.

67. Brand, R. M.; Epperly, M. W.; Stottlemyer, J. M.; Skoda, E. M.; Gao, X.; Li, S.; Huq, S.; Wipf, P.; Kagan, V. E.; Greenberger, J. S.; Falo, L. D., Jr. *J. Invest. Dermatol.* **2017,** *137*, 576-586.

68. Vance, J. E. *Traffic* **2015,** *16*, 1-18.

69. Athenstaedt, K.; Daum, G. *J. Biol. Chem.* **2000,** *275*, 235-240.

70. Schlame, M.; Greenberg, M. L. *Biochem. Biophys. Acta Mol. Cell Biol.* **2017,** *1862*, 3-7.

71. Beranek, A.; Rechberger, G.; Knauer, H.; Wolinski, H.; Kohlwein, S. D.; Leber, R. *J. Biol. Chem.* **2009,** *284*, 11572-11578.

72. Maguire, J. J.; Tyurina, Y. Y.; Mohammadyani, D.; Kapralov, A. A.; Anthonymuthu, T. S.; Qu, F.; Amoscato, A. A.; Sparvero, L. J.; Tyurin, V. A.; Planas-Iglesias, J.; He, R. R.; Klein-Seetharaman, J.; Bayir, H.; Kagan, V. E. *Biochim. Biophys. Acta Mol. Cell Biol.* **2017,** *1862*, 8-24.

73. Kiebish, M. A.; Han, X.; Cheng, H.; Lunceford, A.; Clarke, C. F.; Moon, H.; Chuang, J. H.; Seyfried, T. N. *J. Neurochem.* **2008,** *106*, 299-312.

74. Tyurina, Y. Y.; Lou, W. J.; Qu, F.; Tyurin, V. A.; Mohammadyani, D.; Liu, J.; Huttemann, M.; Frasso, M. A.; Wipf, P.; Bayir, H.; Greenberg, M. L.; Kagan, V. E. *ACS Chem. Biol.* **2017,** *12*, 265-281.

75. Acehan, D.; Khuchua, Z.; Houtkooper, R. H.; Malhotra, A.; Kaufman, J.; Vaz, F. M.; Ren, M.; Rockman, H. A.; Stokes, D. L.; Schlame, M. *Mitochondrion* **2009,** *9*, 86-95.

76. Bissler, J. J.; Tsoras, M.; Goring, H. H.; Hug, P.; Chuck, G.; Tombragel, E.; McGraw, C.; Schlotman, J.; Ralston, M. A.; Hug, G. *Lab. Invest.* **2002,** *82*, 335-344.

77. Spencer C. T.; Bryant, B. B.; Margossian, R.; Maisenbacher, M.; Breitenger, P.; Benni P. B.; Redfearn, S.; Marcus, E.; Cade, W.T. *Am. J. Physiol. Heart Circ. Physiol.* **2011,** *301,* H2122-H2129.

78. Gabriel, J. L.; Plaut, G. W. *Biochemistry* **1984,** *23*, 2773-2778.

79. Jiang, G. W.; Xu, Y.; Prestwich, G. D. *J. Org. Chem.* **2006,** *71*, 934-939.

80. Kubiak, R. J.; Bruzik, K. S. *J. Org. Chem.* **2003,** *68*, 960-968.

81. Xu, Y. J.; Sculimbrene, B. R.; Miller, S. J. *J. Org. Chem.* **2006,** *71*, 4919-4928.

82. Martin, S. F.; Josey, J. A. *Tetrahedron Lett.* **1988,** *29*, 3631-3634.

83. Mckenna, C. E.; Higa, M. T.; Cheung, N. H.; Mckenna, M. C. *Tetrahedron Lett.* **1977**, 155-158.

84. Xu, Y.; Qian, L.; Prestwich, G. D. *J. Org. Chem.* **2003,** *68*, 5320-5330.

85. Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996,** *61*, 6305-6312.

86. Lindberg, J.; Ekeroth, J.; Konradsson, P. *J. Org. Chem.* **2002,** *67*, 194-199.

87. Roy, S.; Caruthers, M. *Molecules* **2013,** *18*, 14268-14284.

88. Tener, G. M. *J. Am. Chem. Soc.* **1961,** *83*, 159-168.

89. Hebert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992,** *57*, 1777-1783.

90. Jiang, G. W.; Xu, Y.; Falguieres, T.; Gruenberg, J.; Prestwich, G. D. *Org. Lett.* **2005,** *7*, 3837-3840.

91. Ali, S. M.; Ahmad, M. U.; Koslosky, P.; Kasireddy, K.; Krishna, U. M.; Ahmad, I. *Tetrahedron* **2006,** *62*, 6990-6997.

92. Inuki, S.; Ohta, I.; Ishibashi, S.; Takamatsu, M.; Fukase, K.; Fujimoto, Y. *J. Org. Chem.* **2017,** *82*, 7832-7838.

93. Gu, Q. M.; Prestwich, G. D. *J. Org. Chem.* **1996,** *61*, 8642-8647.

94. Stepanov, A. E.; Shvets, V. I. *Chem. Phys. Lipids* **1986,** *41*, 1-51.

95. Kim, U. T.; Hajdu, J. *J. Chem. Soc. Chem. Comm.* **1993**, 70-72.

96. Roy, B.; Depaix, A.; Perigaud, C.; Peyrottes, S. *Chem. Rev.* **2016,** *116*, 7854-7897.

97. Bruzik, K. S.; Salamonczyk, G.; Stec, W. J. *J. Org. Chem.* **1986,** *51*, 2368-2370.

98. Bruzik, K.; Jiang, R. T.; Tsai, M. D. *Biochemistry* **1983,** *22*, 2478-2486.

99. Tsai, T. C.; Hart, J.; Jiang, R. T.; Bruzik, K.; Tsai, M. D. *Biochemistry* **1985,** *24*, 3180-3188.

100. Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; Vandermarel, G. A.; Vanboom, J. H. *Tetrahedron Lett.* **1988,** *29*, 6513-6516.

101. Roodsari, F. S.; Wu, D. P.; Pum, G. S.; Hajdu, J. *J. Org. Chem.* **1999,** *64*, 7727-7737.

102. Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980,** *102*, 5974-5976.

103. Kazi, A. B.; Shidmand, S.; Hajdu, J. *J. Org. Chem.* **1999,** *64*, 9337-9347.

104. Guivisdalsky, P. N.; Bittman, R. *Tetrahedron Lett.* **1988,** *29*, 4393-4396.

105. Fodran, P.; Minnaard, A. J. *Org. Biomol. Chem.* **2013,** *11*, 6919-6928.

106. Rosseto, R.; Bibak, N.; DeOcampo, R.; Shah, T.; Gabrielian, A.; Hajdu, J. *J. Org. Chem.* **2007,** *72*, 1691-1698.

107. Martin, S. F.; Josey, J. A.; Wong, Y. L.; Dean, D. W. *J. Org. Chem.* **1994,** *59*, 4805-4820.

108. Behr, A.; Eilting, J.; Irawadi, K.; Leschinski, J.; Lindner, F. *Green Chem.* **2008,** *10*, 13-30.

109. Giustra, Z. X.; Tan, K. L. *Chem. Commun.* **2013,** *49*, 4370-4372.

110. Sakakura, A.; Umemura, S.; Ishihara, K. *Adv. Synth. Catal.* **2011,** *353*, 1938-1942.

111. Trost, B. M.; Malhotra, S.; Mino, T.; Rajapaksa, N. S. *Chem. -Eur. J.* **2008,** *14*, 7648-7657.

112. Lewis, C. A.; Sculimbrene, B. R.; Xu, Y. J.; Miller, S. J. *Org. Lett.* **2005,** *7*, 3021-3023.

113. Paradies, G.; Paradies, V.; De Benedictis, V.; Ruggiero, F. M.; Petrosillo, G. *Biochim. Biophys. Acta Bioenerg.* **2014,** *1837*, 408-417.

114. Kagan, V. E.; Bayir, H.; Tyurina, Y. Y.; Bolevich, S. B.; Maguire, J. J.; Fadeel, B.; Balasubramanian, K. *Biochem. Biophys. Res. Commun.* **2017,** *482*, 482-490.

115. Dehaas, G. H.; Vandeene. L. *Recl. Trav. Chim. Pays-Bas* **1965,** *84*, 436-438.

116. Ramiez, F.; Ioannou, P. V.; Marecek, J. F.; Dodd, G. H.; Golding, B. T. *Synthesis* **1977**, 769-770.

117. Duralski, A. A.; Spooner, P. J. R.; Rankin, S. E.; Watts, A. *Tetrahedron Lett.* **1998,** *39*, 1607-1610.

118. Lin, Z.; Ahmad, M. U.; All, S. M.; Ahmad, M. *Tetrahedron Lett.* **2004,** *45*, 6923-6925.

119. Ramirez, F. I., P. V.; Marecek, J. F.; Dodd, G. H.; Golding, B. T. *Tetrahedron* **1977,** *33*, 599-608.

120. Lin, Z.; Ahmad, M. U.; Ali, S. M.; Ahmad, I. *Lipids* **2004,** *39*, 285-290.

121. Johns, M. K.; Yin, M. X.; Conway, S. J.; Robinson, D. E. J. E.; Wong, L. S. M.; Bamert, R.; Wettenhall, R. E. H.; Holmes, A. B. *Org. Biomol. Chem.* **2009,** *7*, 3691-3697.

122. Abe, M.; Kitsuda, S.; Ohyama, S.; Koubori, S.; Murai, M.; Miyoshi, H. *Tetrahedron Lett.* **2010,** *51*, 2071-2073.

123. Abe, M.; Nakano, M.; Kosaka, A.; Miyoshi, H. *Tetrahedron Lett.* **2015,** *56*, 2258-2261.

124. Baile, M. G.; Sathappa, M.; Lu, Y. W.; Pryce, E.; Whited, K.; McCaffery, J. M.; Han, X.; Alder, N. N.; Claypool, S. M. *J. Biol. Chem.* **2014,** *289*, 1768-1778.

125. Lok, C. M.; Mank, A. P. J.; Ward, J. P. *Chem. Phys. Lipids* **1985,** *36*, 329-334.

126. Neef, A. B.; Schultz, C., Selective Fluorescence Labeling of Lipids in Living Cells. *Angew. Chem. Int. Ed.* **2009,** *48*, 1498-1500.

127. Sabitha, G.; Reddy, E. V.; Swapna, R.; Reddy, N. M.; Yadav, J. S. *Synlett* **2004**, 1276-1278.

128. Vargeese, C.; Carter, J.; Yegge, J.; Krivjansky, S.; Settle, A.; Kropp, E.; Peterson, K.; Pieken, W. *Nucleic Acids Res.* **1998,** *26*, 1046-1050.

129. Sanghvi, Y. S.; Guo, Z. Q.; Pfundheller, H. M.; Converso, A. *Org. Process Res. Dev.* **2000,** *4*, 175-181.

130. Greene, T. W; Wuts, P. G. M. *Protective Groups In Organic Synthesis, 3rd ed.*; John Wiley & Sons: New York, 1991.

131. Crouch, R. D. *Tetrahedron* **2004,** *60*, 5833-5871.

132. Yang, Y. Q.; Cui, J. R.; Lhu, L. G.; Sun, Y. P.; Wu, Y. K. *Synlett* **2006**, 1260-1262.

133. Zhang, Q.; Kang, X. Q.; Long, L.; Zhu, L. J.; Chai, Y. H. *Synthesis* **2015,** *47*, 55-64.

134. DiLauro, A. M.; Seo, W. J.; Phillips, S. T. *J. Org. Chem.* **2011,** *76*, 7352-7358.

135. Hwu, J. R.; Jain, M. L.; Tsai, F. Y.; Tsay, S. C.; Balakumar, A.; Hakimelahi, G. H. *J. Org. Chem.* **2000,** *65*, 5077-5088.

136. Rodriguez, A.; Nomen, M.; Spur, B. W.; Godfroid, J. J. *Tetrahedron Lett.* **1999,** *40*, 5161-5164.

137. Ikawa, T.; Sajiki, H.; Hirota, K. *Tetrahedron* **2004,** *60*, 6189-6195.

138. Sajiki, H.; Ikawa, T.; Hattori, K.; Hirota, K. *Chem. Commun.* **2003**, 654-655.

139. Chevallier, J.; Sakai, N.; Robert, F.; Kobayashi, T.; Gruenberg, J.; Matile, S. *Org. Lett.* **2000,** *2*, 1859-1861.

140. Kim, S.; Jacobo, S. M.; Chang, C. T.; Bellone, S.; Powell, W. S.; Rokach, J. *Tetrahedron Lett.* **2004,** *45*, 1973-1976.

141. Pilcher, A. S.; Deshong, P. *J. Org. Chem.* **1993,** *58*, 5130-5134.

142. Pilcher, A. S.; Hill, D. K.; Shimshock, S. J.; Waltermire, R. E.; Deshong, P. *Abstr. Pap. Am. Chem. Soc.* **1992,** *204*, 210-212.

143. Sedlak, E.; Panda, M.; Dale, M. P.; Weintraub, S. T.; Robinson, N. C. *Biochemistry* **2006,** *45*, 746-754.

144. Boldyrev, I. A.; Pavlova, Y. B.; Molotkovsky, J. G. *Russ. J. Bioorg. Chem.* **2009,** *35*, 219-224.

145. Kusumoto, S.; Keiichiro, S.; Tetsuo, S. *Bull. Chem. Soc. Jpn.* **1986,** *59*, 1296-1298.

146. Gardinier, K. M.; Leahy, J. W. *J. Org. Chem.* **1997,** *62*, 7098-7099.

147. Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001,** *66*, 8165-8176.

148. Castelli, R.; Overkleeft, H. S.; van der Marel, G. A.; Codee, J. D. C. *Org. Lett.* **2013,** *15*, 2270-2273.

149. Kim, K. S.; Song, Y. H.; Lee, B. H.; Hahn, C. S. *J. Org. Chem.* **1986,** *51*, 404-407.

150. Leftheris, K.; Goodman, M. *J. Med. Chem.* **1990,** *33*, 216-223.

151. Dong, H.; Zhou, Y. X.; Pan, X. L.; Cui, F. C.; Liu, W.; Liu, J. Y.; Ramstrom, O. *J. Org. Chem.* **2012,** *77*, 1457-1467.

152. Jager, M.; Minnaard, A. J. *Chem. Commun.* **2016,** *52*, 656-664.

153. Wei, A.; Kishi, Y. *J. Org. Chem.* **1994,** *59*, 88-96.

154. Kumar, C. R.; Tsai, C. H.; Chao, Y. S.; Lee, J. C. *Chem. -Eur. J.* **2011,** *17*, 8696-8703.

155. Sharma, G. V. M.; Lavanya, B.; Mahalingam, A. K.; Krishna, P. R. *Tetrahedron Lett.* **2000,** *41*, 10323-10326.

156. Kagan, V. E.; Bayir, H. A.; Belikova, N. A.; Kapralov, O.; Tyurina, Y. Y.; Tyurin, V. A.; Jiang, J. F.; Stoyanovsky, D. A.; Wipf, P.; Kochanek, P. M.; Greenberger, J. S.; Pitt, B.; Shvedova, A. A.; Borisenko, G. *Free Radic. Bio. Med.* **2009,** *46*, 1439-1453.

157. Mao, G. W.; Qu, F.; St Croix, C. M.; Tyurina, Y. Y.; Planas-Iglesias, J.; Jiang, J. F.; Huang, Z. T.; Amoscato, A. A.; Tyurin, V. A.; Kapralov, A. A.; Cheikhi, A.; Maguire, J.; Klein-Seetharaman, J.; Bayir, H.; Kagan, V. E. *ACS Chem. Biol.* **2016,** *11*, 530-540.

158. Plate, M.; Overs, M.; Schafer, H. J. *Synthesis* **1998**, 1255-1258.

159. Becker, H.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **1996,** *35*, 448-451.

160. Wang, Z. X.; Tu, Y.; Frohn, M.; Shi, Y. *J. Org. Chem.* **1997,** *62*, 2328-2329.

161. Wang, Z. X.; Tu, Y.; Frohn, M.; Zhang, J. R.; Shi *J. Am. Chem. Soc.* **1997,** *119*, 11224-11235.

162. Hoard, D. W.; Moher, E. D.; Martinelli, M. J.; Norman, B. H. *Org. Lett.* **2002,** *4*, 1813-1815.

163. Greenberg, S.; Moffatt, J. G. *J. Am. Chem. Soc.* **1973,** *95*, 4016-4025.

164. Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990,** *112*, 5583-5601.

165. Ren, M. D.; Phoon, C. K. L.; Schlame, M. *Prog. Lipid Res.* **2014,** *55*, 1-16.

166. Stadlbauer, S.; Frank, R.; Maulana, I.; Lonnecke, P.; Kirchner, B.; Hey-Hawkins, E. *Inorg. Chem.* **2009,** *48*, 6072-6082.

167. Mori, K. *Tetrahedron* **2012,** *68*, 8441-8449.

168. Degenhardt, A. G.; Hofmann, T. *J. Agric. Food Chem.* **2010,** *58*, 12906-12915.

169. Lee, J. D.; Ueno, M.; Miyajima, Y.; Nakamura, H. *Org. Lett.* **2007,** *9*, 323-326.

170. Harried, S. S.; Croghan, M. D.; Kaller, M. R.; Lopez, P.; Zhong, W. G.; Hungate, R.; Reider, P. J. *J. Org. Chem.* **2009,** *74*, 5975-5982.

171. Takeshi, W.; Ohkubo, A.; Mochizuki, A.; Sekine, M. *Tetrahedron Lett.* **2001,** *42*, 1069-1072.

172. Khartulyari, A. S.; Kapur, M.; Maier, M. E. *Org. Lett.* **2006,** *8*, 5833-5836.

173. Lebeau, L.; Oudet, P.; Mioskowski, C. *Helv. Chim. Acta* **1991,** *74*, 1697-1706.

174. Louafi, F.; Hurvois, J. P.; Chibani, A.; Roisnel, T. *J. Org. Chem.* **2010,** *75*, 5721-5724.