# Synthesis of MAG and Lyso-PS Lipids with Varying Lipid Tails

A THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS
OF THE DEGREE OF

**DOCTOR OF PHILOSOPHY**

By
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**20173501**


**INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH (IISER) Pune -411008**
**2021**

Dedicated to…

***My Parents***

# CERTIFICATE

Certified that, the work incorporated in the thesis entitled "Synthesis of MAG and Lyso-PS Lipids with Varying Lipid Tails" submitted by Shaikh Minhaj Shamshoddin was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or Institution.

Dr. Siddhesh S. Kamat
(Supervisor)
Date: 23rd July-2021

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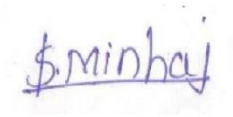

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Date: 23rd July 2021

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## GENERAL REMARKS

- $^{1}H$ spectra were recorded on a JEOL ECX 400 MHz or a Bruker 400 MHz spectrometer unless otherwise specified using an internal tetramethylsilane ($^{\delta}H$ = 0.00). Chemical shifts are expressed in ppm units downfield to TMS.
- $^{13}C$ spectra were recorded on a JEOL 100 MHz or a Bruker 100 MHz spectrometer unless otherwise specified using an internal tetramethylsilane ($^{\delta}C$ = 0.0).
- Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz.
- Mass spectra were obtained using HRMS-ESI-Q-Time of Flight LC-MS (Synapt G2, Waters) or MALDI TOF/TOF Analyser (Applied Biosystems 4800 Plus).
- FT-IR spectra were obtained using Bruker Alpha-FT-IR spectrometer and reported in $cm^{-1}$.
- All reactions were monitored by Thin-Layer Chromatography carried out on precoated Merck silica plates (F254, 0.25 mm thickness); compounds were visualized by UV light and different stains of a TLC plate.
- All reactions were carried out under nitrogen or argon atmosphere with dried solvents under anhydrous conditions and yields refer to chromatographically homogenous materials unless otherwise stated.
- All evaporations were carried out under reduced pressure on Büchi and Heildoph rotary evaporator below 40 °C unless otherwise specified.
- Silica gel (60-120) and (100-200) mesh were used for column chromatography.
- Materials were obtained from commercial suppliers and were used without further purification.
- Preparative HPLC purification was performed using high performance liquid
- Chromatography (HPLC) with C-18 preparative column (21.2 mm × 250 mm, 10 μm; Kromasil®C-18).

# ABBREVIATIONS

ABHD6- α/β hydrolase domain-containing protein 6

ABHD12- α/β hydrolase domain-containing protein 12

ABHD16A- α/β hydrolase domain-containing protein 16A

Ac – Acetyl

ACN – Acetonitrile

Calcd − Calculated

$CDCl_3$ – Chloroform-D

$CHCl_3$ – Chloroform

CNS – Central nervous system

Ctrl – Control

dd – Doublet of doublet

DCM – Dichloromethane

DMAP – *N*, *N*-Dimethylaminopyridine

DMF – *N*, *N*-Dimethylformamide

DPBS – Dulbecco's Phosphate-Buffered Saline

dt – Doublet of triplet

$\delta$ − Delta (in ppm)

eq. − Equivalent

ES − Esterase

$Et_3N$ – Triethylamine

EtOH – Ethanol

EtOAc – Ethyl acetate

$Et_2O$ – Diethyl ether

FFA- Free fatty acid

g – Gram

GPCR- g protein-coupled receptors

GPS- Glycerophosphoserine

h – Hour

HCl – Hydrochloric acid

HPLC – High performance liquid chromatography

HRMS – High-resolution mass spectrometry

Hz – Hertz

$H_2O$ – Water

IR – Infrared

IL6- Interleukin 6

*J* – Coupling constant

LC- Long-chain

LPS- Lipopolysaccharide

lyso-PA- lysophosphatidic Acid

lyso-PC- lysophosphatidylcholine

lyso-PE- lysophosphatidylethanolimine

lyso-PG- lysophosphatidylglycerol

lyso-PL- lysophospolipid

lyso-PS- lysophosphatidylserine

MAG- Monoacylglycerol

MAGL- Monoacylglycerol lipase

MALDI – Matrix-Assisted Laser Desorption Ionization

Me – Methyl

Me-lyso-PS- Methyl lysophsphatidylserine

MeOH – Methanol

mg – Milligram

min – Minute

MHz – Megahertz

mL – Millilitre

mM – Millimolar

mmol – Millimole

MS – Mass spectrum

MW – Molecular weight

*m/z* – Mass to Charge ratio

m – Multiplet

$NaHCO_3$ – Sodium bicarbonate

$NH_4Cl$ – Ammonium chloride

NMR – Nuclear magnetic resonance

nM – Nanomolar

PA – Phosphatidic acid

PBS – Phosphate buffered saline

PBMC- Peripheral blood mononuclear cell

PC – Phosphatidylcholine

PE – Phosphatidylethanolamine

PHARC- Polyneuropathy, Hearing loss, Ataxia, Retinitis Pigmentosa, Cataract

PLA1- Phospholipase A1

PLA2- Phospholipase A2

PLC- Phospholipase C

PLD- Phospholipase D

PI- Phosphatidylinositol

PI- Phosphatidylinositol

PS – Phosphatidylserine

$PPh_3$ – Triphenylphosphine

PTX- Pertussis Toxin

PS – Phosphatidylserine

Py – Pyridine

ppm – Parts per million

% – Percent

R*f* – Retention factor

RT – Room temperature

s – Singlet

S1P- Sipngosine-1-Phosphate

$SiO_2$ – Silica

*sn*- Stereospecific numbering

t – Triplet

TBHP – *tert*-butyl hydroperoxide

TEA – Triethylamine

TFA – Trifluoroacetic acid

THF – Tetrahydrofuran

TLC – Thin layer chromatography

TMS – Tetramethylsilane

TNFα – Tumour Necrosis Factor (α)

TLR2- Toll-like receptor 2

UV – Ultraviolet

VLC- Very-long-chain

µg – Microgram

µM- micromolar

µmol– Micromole

µL – Microlitre

µm – Micrometre

2-AG- 2-arachidonoyl glycerol

1-MAG- Mono-1-(fatty) acyl-glycerol

2-MAG- Mono-2-(fatty) acyl-glycerol

# ABSTRACT

## Synthesis of MAG and lyso-PS Lipids with Varying Lipid Tails (July 2021)

Minhaj S. Shaikh, M. Sc., University of Pune

Chair of Research Advisory Committee Dr. Siddhesh S. Kamat

My doctoral research described in this thesis involves the synthesis, and characterization of mono-acyl glycerol (MAG) and lysophosphatidylserine (lyso-PS) lipids with varying lipid tails. PHARC syndrome is a neurodegenerative disease abbreviated based on its symptoms i.e. polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract. It has been attributed to a mutation in the *abhd12* gene which encodes for lysophosphatidylserine (lyso-PS) lipase ABHD12. ABHD12 is an integral membrane enzyme which a member of the serine hydrolase family. Mice models for PHARC i.e. ABHD12 knock-out (KO) mice exhibits locomotor defects, microglial activation, and accumulation of lyso-PS in the cerebellum. Biochemical characterization and pathways downstream of lyso-PS is unclear in PHARC syndrome. The diastereomeric complexity in the lyso-PS structure has caused its commercial paucity to study its biological role in detail. Hence, in this study, I have chemically synthesized a library of lyso-PSs chain lengths such as medium, long, and very-long-chain fatty acyl chains to investigate their role in (neuro) immunological processes. We used these lipids to understand the enzyme kinetics of ABHD12. We found that ABHD12 is highly stereospecific and strongly prefers the (*R*) configuration of Me-lyso-PSs over (S) analogs. Next, we used our synthetic Me-lyso-PS library to investigate the pathways that can be triggered through lyso-PS signaling. We measured release of calcium, cytokines, and histamine that were involved in the immune cell activation and phosphorylation pathways in immune cells as a function of lyso-PS treatment. VLC lyso-PS have been found to elicit immune responses in the form of heightened cytokine release via the toll-like receptor 2 (TLR2) signalling pathway. We have also observed that upon LC lyso-PS stimulation, there is an increase in the cytosolic $Ca^{2+}$, cAMP, and phospho-ERK levels which we hypothesize might be due to the activation of a yet unknown GPCR. This study suggests the intricate balance between LC and VLC lysoPS which influence significant biological processes via specific receptors. Currently, I have synthesized a library of (*R*) (Me-lyso-PS) with unsaturated fatty acid chains. However, using this synthetic strategy we

are synthesizing bifunctional Me-lyso-PS lipid probes in an attempt to map the interacting partners of lyso-PS using chemical proteomics.

# CHAPTER 1

# INTRODUCTION

In the living organism, lipids are energy-rich organic substances and hydrophobic molecules which are soluble in organic solvents (Alcohol, Ether, Chloroform, Acetone, and Benzene, etc.)[1]. Like any organic molecule, it is made up of hydrogen, carbon, nitrogen, oxygen, and phosphorous. Phospholipids are one such class of lipid molecules. Predominantly, phospholipids have three main components; a hydrophilic head group, glycerol backbone and hydrophobic fatty acyl chains. Glycerol is trihydric alcohol i.e., with hydroxyl groups substituted at 1, 2, and 3 positions of the propane molecule. The three carbon atoms of glycerol are numbered stereospecifically which is denoted as '*sn*'. Based on the degree of unsaturation in the fatty acyl chain, they are classified into either saturated or unsaturated fatty acids. The fatty acids esterified at *sn*-1 and 2 positions of a glycerol backbone, and *sn*-3 positions are esterified with different head groups which results in various phospholipid species (X) such as phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidicacid (PA), phosphatidylcholine (PC), phosphatidylglycerol (PG) (Figure 1.1) [2,3].

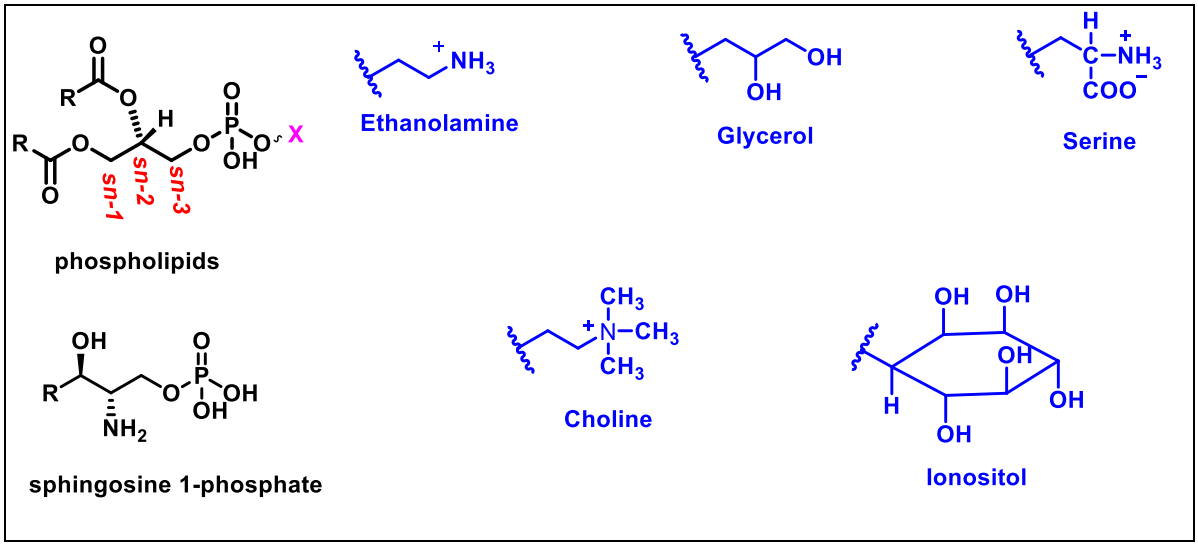


**Figure 1.1.** Structure and classification of phospholipids and sphingosine 1-phosphate. X represents the head group of phospholipids

Lysophospholipid (lyso-PL) have recently become the focus of special attention since it was discovered that lysophosphatidylserine (lyso-PS) are potent hormone-like signalling lipids. Lyso-PS have a common structure consisting of hydrophilic head portion of phosphate group and hydrophobic tail portion of fatty acid chain. Naturally occurring lyso-PS has two chiral centers such as 1) (*R*) configuration of *sn*-2 hydroxyl group of a glycerol backbone 2) Phospho-

*L*-serine head group on Cα-carbon atom. It shows amphiphilic properties due to the hydrophobic (fatty acid) and hydrophilic (glycerophosphoserine) head group. Lyso-PS induce several cellular responses through the interaction with specific receptors. To date, three kinds of specific receptors (GPR34, GPR174, and P2Y10) have been identified for lyso-PS respectively. In biological systems, lyso-PS are produced as a result of the action of phospholipase enzymes namely Phospholipase A1 (PLA1), Phospholipase A2 (PLA2) on phosphatidylserine. PLA1 and PLA2 hydrolytically cleave acyl moiety of PS at either the *sn*-1 or *sn*-2 position producing *sn*-1 lysophospholipids and *sn*-2 lysophospholipids as products respectively. (Figure 1.2)[4–6].

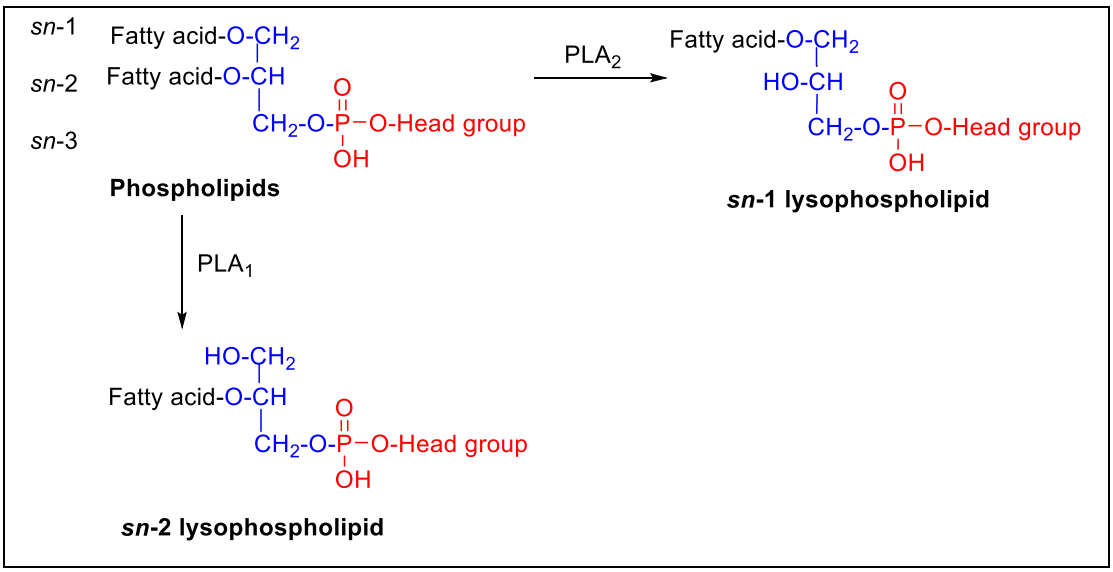


**Figure 1.2.** Enzymatic action of phospholipids and site of action of phospholipase

Lyso-PLs are the family of simple phospholipids in which one acyl chain is lacking and only one hydroxyl group of glycerol backbone is acylated (Figure 1.3). Lyso-PL has only one fatty acid group which is esterified to either *sn*-1 or *sn*-2 hydroxy group of a glycerol backbone. The most familiar lysophospholipids are lysophosphatidyl-ethanolamine (lyso-PE), lysophosphatidyl-inositol (lyso-PI), lysophosphatidyl-serine (lyso-PS), lysophosphatidic-acid (lyso-PA), lysophosphatidyl-choline (lyso-PC), lysophosphatidyl-glycerol (lyso-PG), and sphingosine 1-phosphate (S1P) (Figure 1.3). Defect in lyso-PL metabolism is often associated with various human diseases like cancer, neurological and inflammatory diseases. Among them, lyso-PA, and S1P are the best-studied examples for this lipid class and are also well-characterized over the past two decades. However, lyso-PA and S1P are well-established signalling biological lipid mediators in pathophysiology and their protein interactors have been

exploited as drug targets. Except for lyso-PA and S1P, other lyso-PL are not very well characterized as lyso-PS molecules. Recently, lyso-PS have emerged as an extra class of signalling lysophospholipids that showcased biological importance in the mammalian central nervous system (CNS) and immune system. As my thesis work describes in the coming chapter, I have developed methodologies, synthesized, and characterized numerous lyso-PS lipids with varying lipid tails to investigate lyso-PS metabolism and functions[7,8].

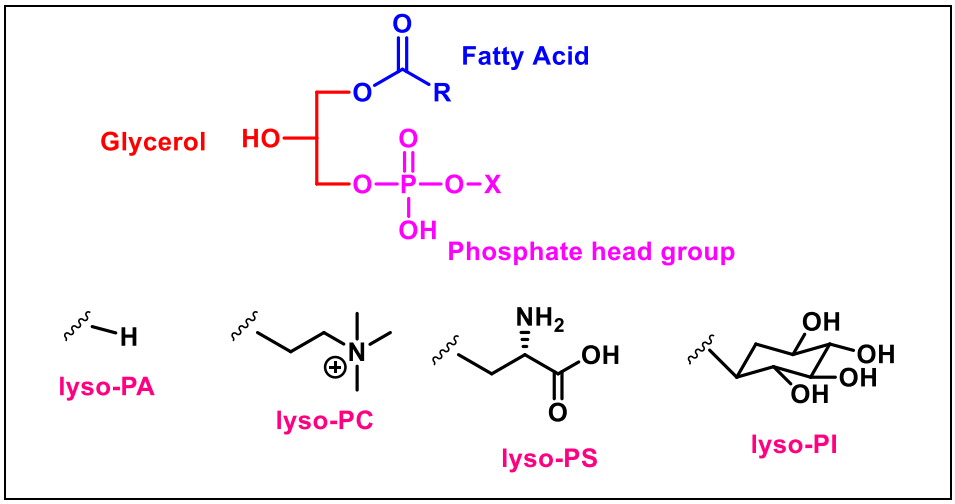


**Figure 1.3.** General chemical structure of lysophospholipids and the classification of lysophospholipids *(R)*-2-hydroxy-3-(phosphooxy) propyl fatty acid with the different alternative head group represented by X: lysophosphatidicacid (lyso-PA) with acid, lysophosphatidylcholine (lyso-PC) with choline, lysophosphatidylserine (lyso-PS) with serine, lysophosphatidylinositol (lyso-PI) with inositol. X represents the head group of lysophospholipids

## 1.1 Biological Functions of Lysophosphatidylserine (Lyso-PS)

Lyso-PS has been a potent bioactive lipid that induces several cellular responses *in vitro* and *in vivo* (Figure 1.4)[9,10]. Importantly, lyso-PS has a crucial role in biological processes like mast cell degranulation[11–16], inducing the chemotactic migration of human glioma cells and murine fibroblast[17,18], inhibition of lymphocyte proliferation[19–23], and macrophages clearance of apoptotic cells[24].

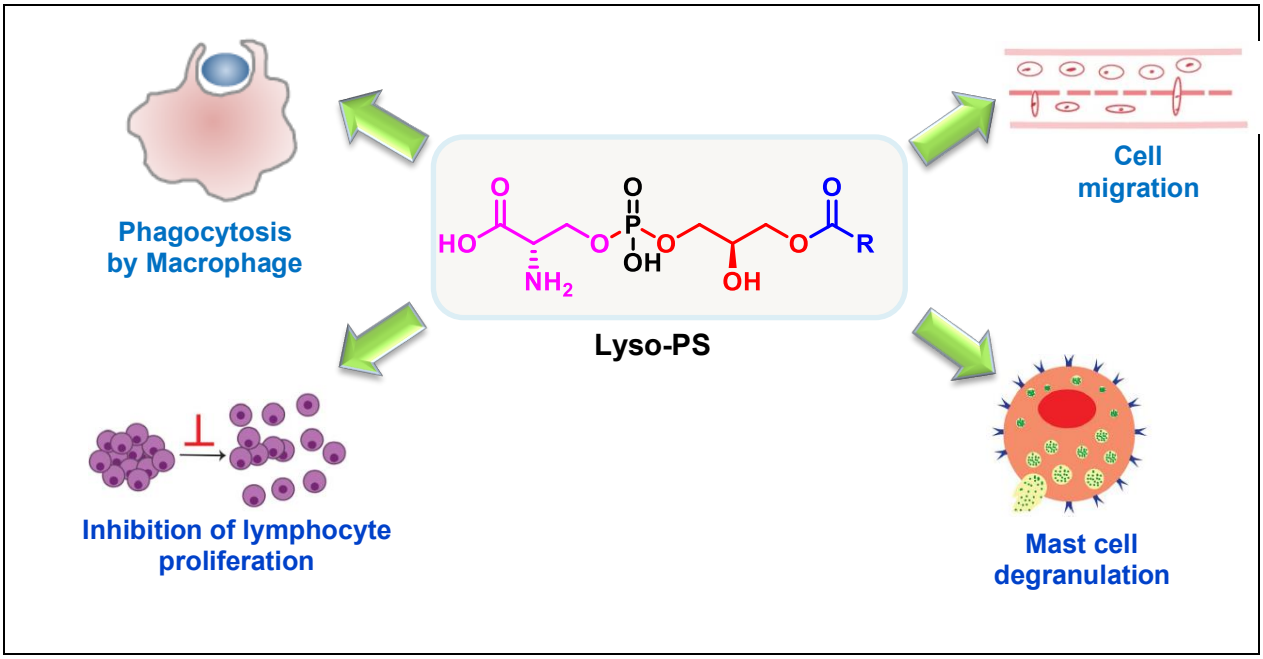


**Figure 1.4.** Biological role of lyso-PS. Lyso-PS mediates degranulation of mast cells, stimulation of chemotactic migration in glioma cells, suppresses T-cell proliferation, and acts as a mediator of macrophage-mediated phagocytosis.

## 1.2 Metabolism of Lyso-PS

α/β hydrolase domain (ABHD) protein family was first identified in 1992 and was found to be a part of the most diverse and universal protein family which include esterase, protease, lipase, and epoxide hydrolase, also it is a part of lyso-PS metabolizing enzymes. It has been showing lyso-PS majorly biosynthesized in CNS from PS precursors through a PS lipase activity. α/β hydrolase domain-containing protein 16 (ABHD16A) was first discovered in 2014 and has been shown to be highly expressed in the mammalian CNS and immune cells. Biochemical studies have shown that ABHD16A is the integral membrane enzyme that acts as a major PS lipase in CNS and primary macrophages. Another integral membrane lipase is α/β hydrolase domain-containing protein 12 (ABHD12) that hydrolysis of lyso-PS lipids. In short, the ABHD16A enzyme hydrolyses phosphatidylserine and gets *sn*-1 lyso-PS which is further hydrolyzed again by the ABHD12 enzyme to give glycerophosphoserine (GPS) head group and free fatty acid (FFA) (Figure 1.5)[25–27].

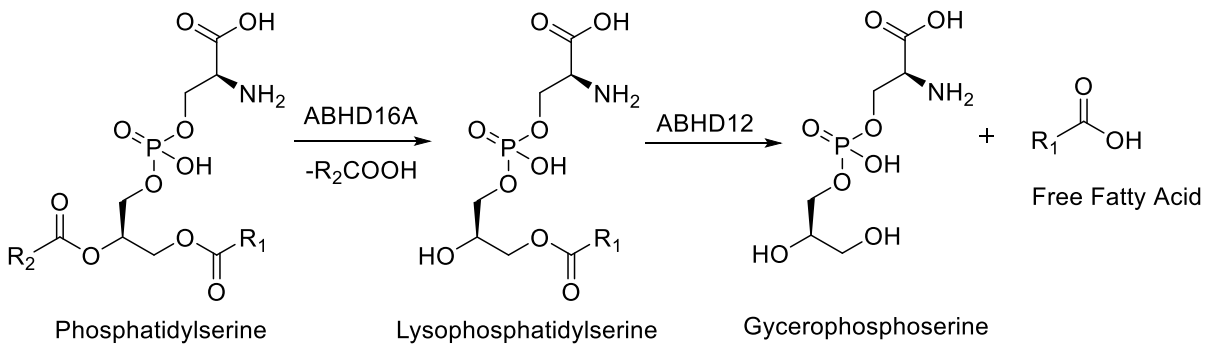


**Figure 1.5.** General scheme for the metabolism of lysophosphatidylserine

ABHD12 is a ~45 kDa transmembrane glycoprotein and is highly expressed in murine tissue and cells, including the brain, microglia, macrophages, and white adipose tissue. Of note, ABHD12 showed MAG lipase activity in which ABHD12 robustly hydrolyze the endocannabinoid 2-arachidonoyl glycerol (2-AG). The three serine hydrolases, namely monoacylglycerol lipase (MAGL) and the *α/β*-hydrolase domain-containing protein 6 (ABHD6) and ABHD12, approx. 99% of brain 2-AG hydrolase activity shows in CNS[28,29]. The previous report showed MAGL is a soluble membrane protein that is associated with cytoplasmic orientation. ABHD12 and ABHD6 are integral membrane proteins associated with luminal/extracellular orientation and cytoplasmic orientation (figure 1.6)[30].

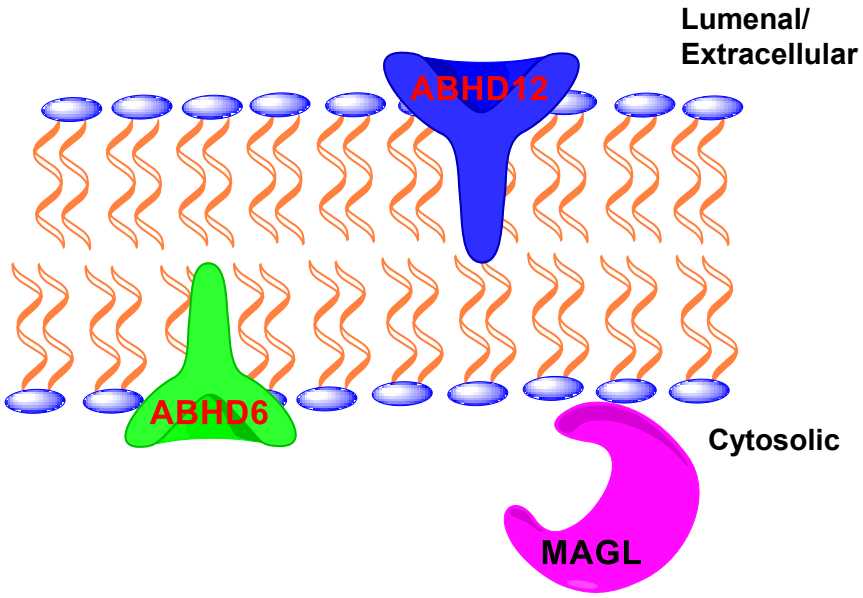


**Figure 1.6.** Cartoon model representation of 2-AG hydrolases in mouse brain

We recently demonstrated that ABHD12 is an integral membrane enzyme localized at the cellular compartment (ER membrane) where phosphatidylserine lipids and very-long-chain

(VLC) fatty acids are biosynthesized in major forms. Also, our data supported that ABHD12 prefers VLC lipids, it functions as a lyso-PS lipase, and lyso-PS is also constantly biosynthesized in ER membrane as well[31]. Our group recently quantified oxidized PS by LC-MS analysis. Importantly, we showed ABHD12 hydrolyzed oxidized PS to get *sn*-1 lyso-PS and then further hydrolyzed by ABHD12 to yield free fatty acid and glycerophosphoserine[32]. More recently, our group demonstrated ABHD12 prefers VLC lyso-PSs as substrates and also showed VLC lyso-PS easily produces a pro-inflammatory response from macrophages through Toll-like receptor 2 (TLR2)-dependant pathway resulting in neuroinflammation[33]. Our finding emphasizes the new aspect of the recent report PHARC subject that showcased a strong correlation between VLC lyso-PS activity and neuroinflammation. Disruption in ABHD12 activity increased level of lyso-PS result into PHARC subject. Blankman *et al.* have shown that very-long-chain (VLC ≥ C22) lyso-PS accumulation in the ABHD12 knockout mice brain leads to neuroinflammation and neurobehavioral disturbances seen in human PHARC subjects (Figure 1.7).

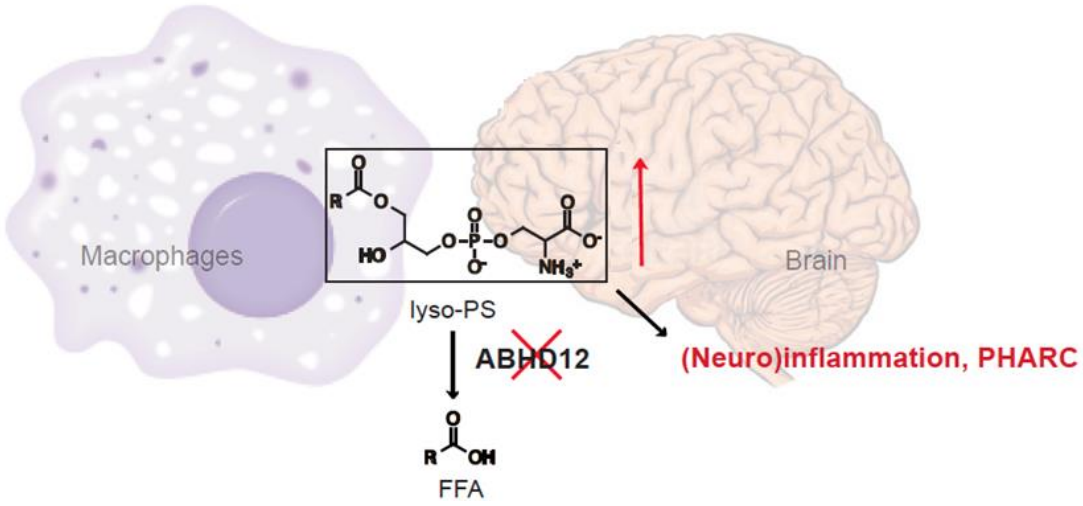


**Figure 1.7.** Mutation in ABHD12

Recently, the ABHD12 knockout mouse showed massive accumulation of lyso-PS lipid inside the brain. In this study, they observed the accumulation of lyso-PS significantly increase in particular very-long-chain (VLC ≥ C22) carbon chain length C22 and C24 chain length elevated (Figure 1.8) in the brain of ABHD12 knockout mice level[26].

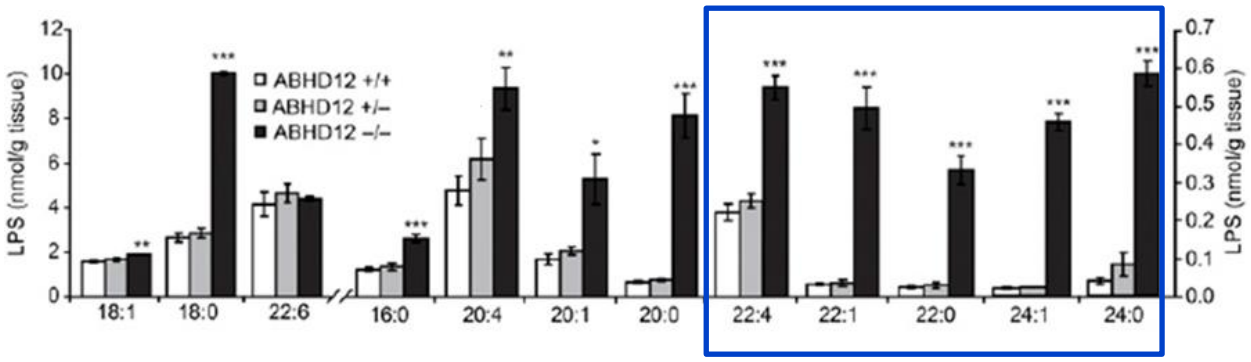


**Figure 1.8.** VLC lyso-PS accumulate in ABHD12 knockout mice brain[26]

PHARC disease was first time reported in a Norwegian family and found in both children and adult mutation in ABHD12 cause neurodegenerative disease. Also, homozygosity mapping was done and found particular gene is responsible for the disease, this gene was an ABHD12 gene. The symptoms of PHARC have seemed in late childhood or early teenage years and worsen progressively with age. The PHARC patient has characteristic symptoms of hearing loss, demyelination, and cerebellar ataxia. Towards this, to date, five well-defined ABHD12 mutations were identified in a patient with PHARC disease, and all those anticipated to lead to the complete loss of ABHD12 expression. Untargeted lipidomics has shown high levels of VLC lyso-PS in ABHD12 knockout mice brain, suggesting the role of the lyso-PS pathway in PHARC like symptoms displayed in ABHD12 knockout mice brain. Accumulation of lyso-PS in the brain of ABHD12 knockout mice contributes to neuroinflammation and PHARC behaviour (Figure 1.9)[34–37].

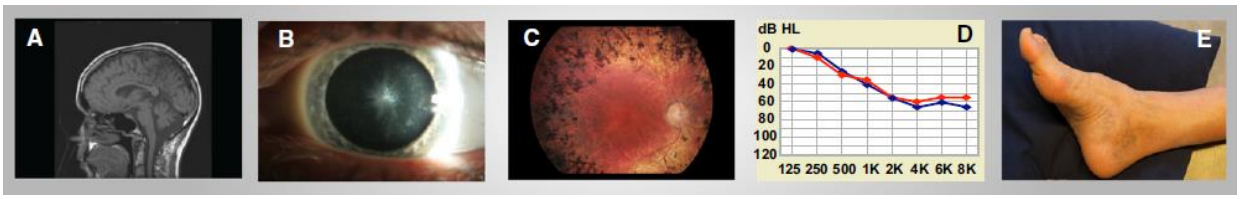


**Figure 1.9.** Symptoms of PHARC syndrome in humans[26]

ABHD12 hydrolyzed various lysophospholipid species and the highest hydrolysis activity was observed in lyso-PS and 1-monoacylglycerol (MAG) lipids (Figure 1.10) but did not display significant activity for doubly acylated glycerol backbone containing lipids (e.g. DAG, PA, PC, PE, PI, PG, PS)[26].

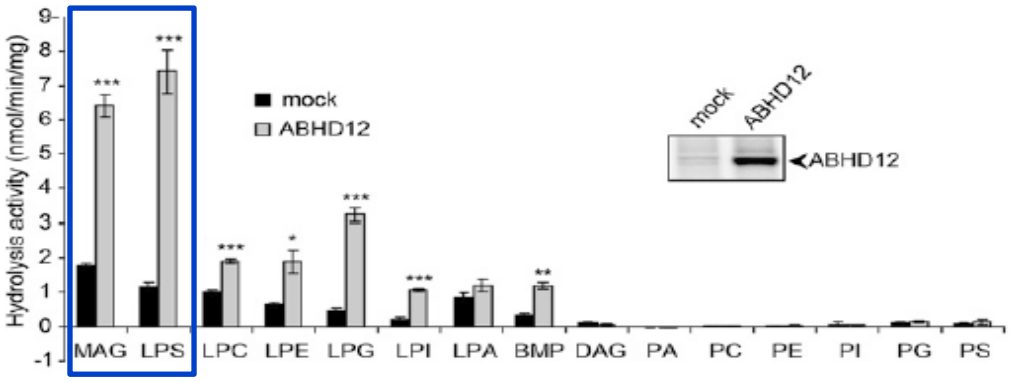


**Figure 1.10.** ABHD12 is a major brain LPS lipase[26]

Mammalian ABHD12 can use long-chain lipid-containing mono-1-(fatty) acyl-glycerol (1-MAG) and mono-2-(fatty) acyl-glycerol (2-MAG) lipid substrate at the comparable enzymatic role but mammalian ABHD12 does not use phospholipid, diacylglycerols, and triacylglycerol lipid as a substrate. The substrate scope of the enzyme is limited to only four lipids as lyso-PS, oxidised-PS, 2-MAG, and 1-MAG.

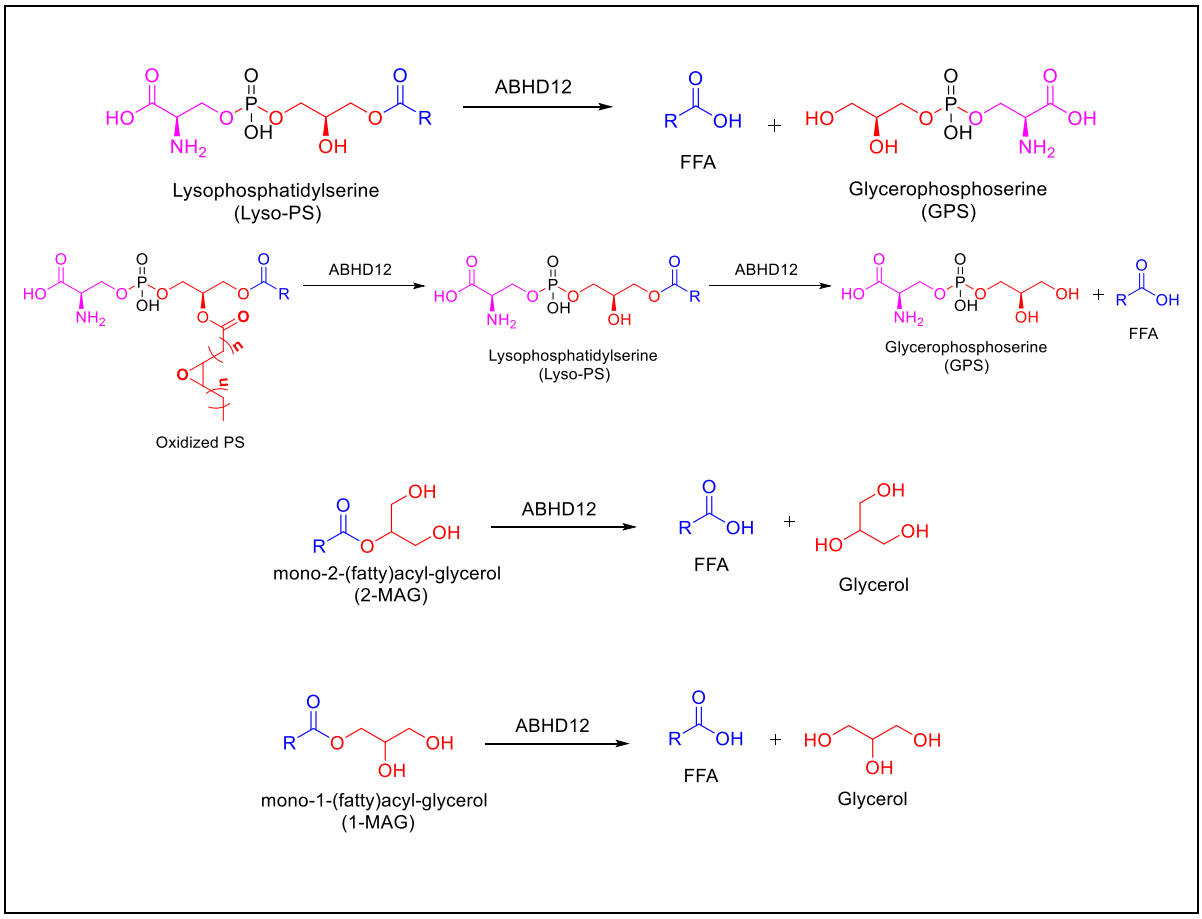


**Figure 1.11.** Mammalian ABHD12 catalyzed lipase reaction

## 1.3 Direction of research

Given the structural diversity and limited commercial availability, we took it upon ourselves to chemically synthesize the library of lyso-PS lipids with varying lengths of the fatty acid chain of medium to very-long-chain of lyso-PS. Only a few lyso-PS, MAG lipids are commercially available and to date, nobody has synthesized a library of lyso-PS and MAG lipids. To the best of our knowledge very-long-chain lyso-PS has not been tested, to date against ABHD12 except accumulation study in ABHD12 knockout mice brains. However, because of the lack of detailed structural activity relationship (SAR) of these lyso-PSs lipids, it remains unclear about the diverse role of lyso-PS in signalling functions and how these functions affect, balance, and/or modulate an immunological process. But, lyso-PS synthesis is extremely challenging compared to MAG lipids. We, therefore, initially started the synthesis of a relatively simple MAG library in order to optimize the synthetic routes that can help us to build the library of lyso-PS lipids, and then, moved to synthesize the more complex lyso-PS library.

As a part of this thesis, I developed methodologies, to synthesize physiologically relevant biomolecules, such as mono-acyl glycerol (MAG) and lysophosphatidylserine (lyso-PS) lipids with varying lipid tails, and study their roles in mammalian neuro and immune physiology. I generated a library of monoacylglycerols and lyso-PS with varying chain length species and used them in substrate assays to show that the ABHD12 enzyme (PHARC syndrome-associated enzyme) prefers very-long-chain free fatty acid esterified lipids (Chapter Ⅱ). I screened and utilized several different strategies to synthesize and purify lyso-PS followed by its structural elucidation using NMR and mass spectrometry (MS). I also utilized various spectroscopic methods for the structural determination of unexpected products along with the desired ones (Chapter Ⅲ). I used my lyso-PS library and we demonstrated that very long-chain lyso-PS is immune potent and it activates microglia and causes neuroinflammation in the brain. On the other hand, we found that long-chain lyso-PS species acted through some unidentified receptors and triggered calcium influx, ERK1/2 activation, and cAMP synthesis in the immune cells. (Chapter Ⅲ). I also generated a library of lyso-PS with unsaturated fatty acid chains and those libraries will be testing for biological assay (Chapter Ⅳ). On similar lines, currently, we are working on to generate lyso-PS probes with biorthogonal handles and this bifunctional lyso-PS probe in tandem with a recently established MS-based chemoproteomics platform can help in the identification of unknown lyso-PS protein and/ or receptors. To identify such proteins, we have prepared a set of probes that contains diazirine photoreactive group, an

alkyne handle, and the binding group as fatty acid with varying chain lengths and degree of unsaturation including palmitic Acid (C16:0), stearic Acid (C18:0), and oleic Acid (C18:1).

# CHAPTER 2

# SYNTHESIS OF 1-MAG LIPIDS FOR BIOCHEMICAL CHARACTERIZATION OF ABHD12

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## 2.1 Introduction and Classification of MAG Lipids

MAG lipids in which one acyl chain is lacking and only one hydroxyl group of glycerol backbone is acylated (Figure 2.1). MAG lipid has only one fatty acid group which is esterified to either *sn*-1 or *sn*-2 hydroxyl group of a glycerol backbone depends on which fatty acid is esterified. Fatty acid esterified with *sn*-1 hydroxyl group of glycerol backbone which is called 1-MAG lipid and fatty acid esterified with the *sn*-2 hydroxyl group of glycerol backbone which is 2-MAG lipid.

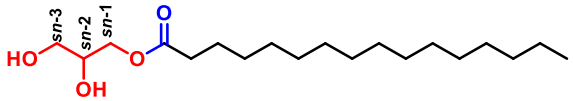
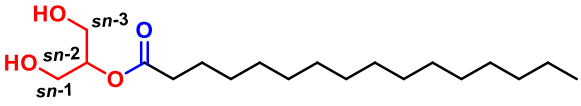
| Monoacylglycerol | Structure |
|---|---|
| 1-MAG 16:0 |  |
| 2-MAG 16:0 |  |

**Figure 2.1.** Structure of 1-MAG and 2-MAG lipids

In vitro, MAG lipids hydrolysis study has been shown in previous literature by ABHD12 enzyme. A mammalian ABHD12 substrate profiling study has been done with 1-MAG and 2-MAG lipid substrate at comparable enzymatic rates. This substrate profiling study was done at a single substrate concentration (25 to 100 μM) for the only medium (C8 to C12) and long-chain (C14 to C20) fatty acid MAG substrate but VLC (≥ C22) fatty acid MAG substrate profiling studies are lacking. Because only a few 1-MAG lipids are commercially available these are listed below. Also, VLC containing 1-MAG lipids is not commercially available because these are very expensive for such types of studies.

In 2012, Navia P. et. al. has performed an hABHD12 substrate profiling study. In this study, they showed that mammalian ABHD12 hydrolysis activity both 1-MAG vs 2-MAG lipid at comparable rates with slightly preferred 1-MAG lipid over 2-MAG lipid (Figure 2.2). For ex. hABHD12 preferred 1-arachidonoyl-glycerol (1-AG) compare to 2-arachidonoyl-glycerol (2-AG). Also, ABHD12 did not hydrolyze di-or triglyceride lipids[29].

## Table 2.1

## Commercial available MAG lipids

| Saturated 1-MAG lipids | Unsaturated 1-MAG lipids |
|---|---|
| C10:0 | C18:1 |
| C12:0 | C18:2 |
| C14:0 | |
| C16:0 | |

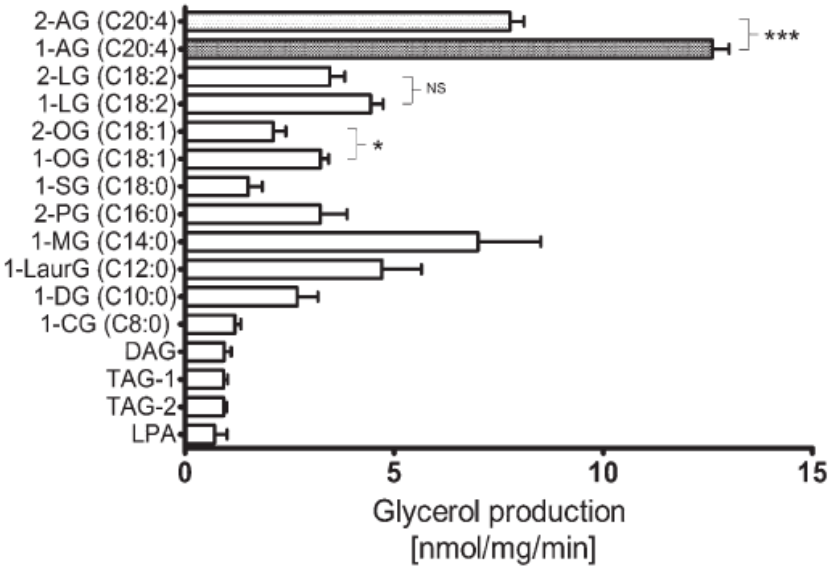


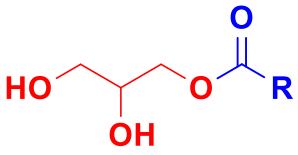

**Figure 2.2.** Substrate profile assay of 1-MAG and 2-MAG lipids against hABHD12

## 2.2. Synthesis of 1-MAG Lipids for Biochemical Characterization of ABHD12

Given the lack of commercial sources, we decided to perform a meticulous substrate profiling study of mammalian ABHD12. Concerning, we decided to develop a synthetic route to generate 1-MAG lipids of varying fatty acid chain lengths, and differing extent of unsaturation, to assess broadly the 1-MAG substrate preference of mammalian ABHD12. We wanted the synthesis route to be relatively easy, possible at a small scale (low milligram) using

commercially available free fatty acids, and able to generate good reaction yields. Towards this, we developed a two-step synthetic scheme (Scheme 2.1).

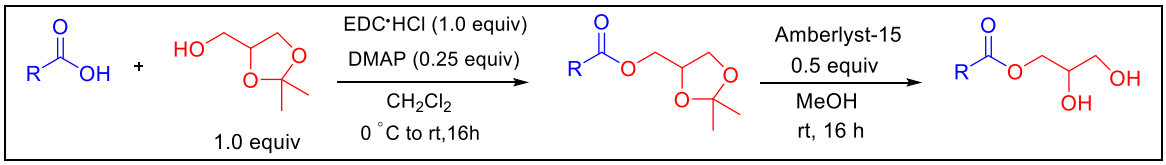
## EXPERIMENTAL SECTION:




R = Fatty acid chain

**Mono-1-(fatty) acyl-glycerol (1-MAG)**

## 2.3 Result and Discussion:




**Scheme 2.1.** Synthetic route for generating 1-MAG lipids from 1a' to 1p'.

We have done synthesis in two steps and both steps are well-reported synthetic reactions. In the first step, I prepared 1, 2-isopropylidene glycerol and esterified of a free fatty acid form (C10 to C24), and then we used 1-(3-dimethylamino propyl)-3-ethyl carbodiimide hydrochloride (EDC·HCl) as a coupling reagent[11]. The second step involved the deprotection of the isopropylidene group using the Amberlyst-15 catalyst to yield the corresponding 1-MAG lipid of interest[38]. All reactions were performed on a 10 – 20 mg scale depending on the cost and availability of the starting free fatty acid, and these reactions afforded yields from 50 – 94% (Table 2.2).

## 2.4. Substrate Profiling Study against Recombinant hABHD12

All substrate assays was performed by Alaumy, a project student in our lab. Having synthesized 16 1-MAG lipids in hand we decided to perform enzyme kinetics studies on recombinant hABHD12 against this lipid substrate library. Regarding this, firstly we have accessed the relationship between enzyme concentration and enzymatic rate.

## Table 2.2

## Reaction Yeild of 16 1-MAG Lipids

| Sr.No | 1-MAG lipids | Chain length | Types of FFA | Scale of reaction (mg) | % (Yield) |
|---|---|---|---|---|---|
| 1a’ | **Decanoic Acid** | 10:0 | Medium | 30 | 65 |
| 2b’ | **Dodecanoic Acid** | 12:0 | Medium | 30 | 65 |
| 3c’ | **Myristic Acid** | 14:0 | Long | 30 | 72 |
| 4d’ | **Palmitic acid** | 16:0 | Long | 20 | 94 |
| 5e’ | **Steric acid** | 18:0 | Long | 20 | 71 |
| 6f’ | **Oleic Acid** | 18:1 | Long | 20 | 71 |
| 7g’ | **α-Linolenic Acid** | 18:3 | Long | 10 | 83 |
| 8h’ | **Arachidic Acid** | 20:0 | Long | 20 | 89 |
| 9i’ | **Eicosenoic Acid** | 20:1 | Long | 20 | 50 |
| 10j’ | **Arachidonic Acid** | 20:4 | Long | 10 | 50 |
| 11k’ | **Behenic Acid** | 22:0 | Very long | 20 | 77 |
| 12l’ | **Erucic Acid** | 22:1 | Very long | 20 | 75 |
| 13m’ | **Docosatetraenoic Acid** | 22:4 | Very long | 10 | 50 |
| 14n’ | **Docosahexaenoic Acid** | 22:6 | Very long | 10 | 80 |
| 15o’ | **Lignoceric Acid** | 24:0 | Very long | 20 | 62 |
| 16p’ | **Nervonic Acid** | 24:1 | Very long | 20 | 50 |

For this study, we purchased commercially available C18:1 lyso-PS and C18:1 2-MAG used as a substrate profiling study. We used C18:1 lyso-PS, C18:1 1-MAG, and C18:1 2-MAG as substrate (all 100 µM) and assayed them against varying concentrations of WT hABHD12 transfected HEK293T membrane lysates. We found a good linear correlation between the enzyme concentration and substrates (Figure 2.3). We observed a good linear correlation between the enzyme concentration and all three substrates. Based on this study we chose 20 µg of membrane lysate. Towards this, we evaluated the relation between enzymatic rate and time of the assay. For this study again we used the same C18:1 lyso-PS, C18:1 1-MAG, and C18:1 2-MAG as substrate (all 100 µM) and assayed them against 20 µg of WT hABHD12 transfected HEK293T membrane lysates with different time (0-1 h) (Figure 2.3). Again, we

observed a good linear correlation between the enzyme rate and time of the assay up to 1 h of this lipase assay. After established the suitable condition we chose 20 µg of membrane lysate and time 30 min. As result, we quantified the release of free fatty acids in the LC-MS method[31].

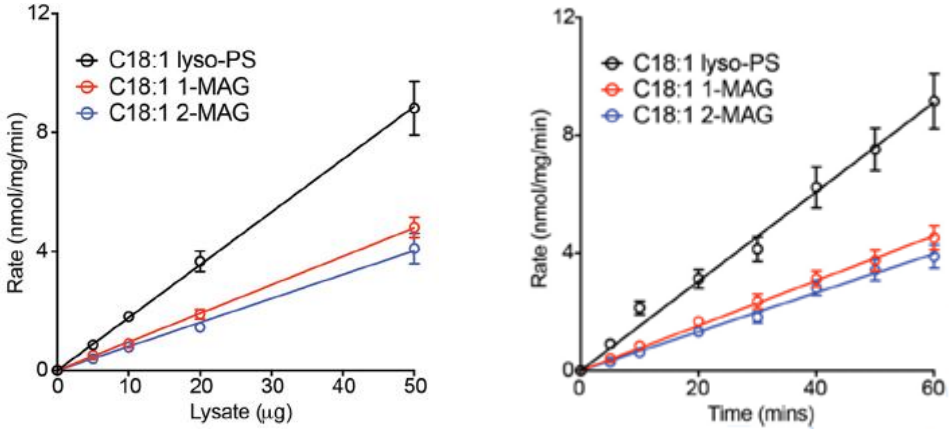



**Figure 2.3.** Optimization of wild-type hABHD12 lipase activity assay

Having demonstrated protocol assay conditions for enzyme kinetic studies then we have assayed both mock and wild-type hABHD12 against the 1-MAG substrate library at different substrate concentrations (0-400µM). We calculated the enzymatic rate for wild-type hABHD12 for a specific concentration of lipid got by subtracting the corresponding mock rate at that concentration for that lipid and the corrected wild-type hABHD12 enzymatic rate at different substrate concentrations for a specific lipid was plotted and fit to a classical Michaelis-Menten Kinetics equation. In a nutshell, we found hABHD12 prefers very-long-chain containing 1-MAG lipids from all kinetic constants, in addition not only $V_{max}$ but also $K_m$ value (Table 2.3).

Based on kinetic constants for the saturated fatty acid 1-MAG lipids we have found

$$V_{max} = C24:0 > C22:0 > C18:0 > C16:0 > C14:0 > C12:0 > C10:0$$

$$K_m = C24:0 < C22:0 < C18:0 < C16:0 < C14:0 < C12:0 < C10:0$$

## Table 2.3

## Kinetics for the 1-MAG lipid substrates tested *in vitro* against recombinant human ABHD12

| Lipid species | $V_{max}$ (nmol/mg protein/min) | $K_m$ (μM) |
|---|---|---|
| 1-MAG species | | |
| C10:0 | 0.14 ± 0.02 | 144 ± 14 |
| C12:0 | 0.16 ± 0.03 | 129 ± 21 |
| C14:0 | 0.38 ± 0.04 | 119 ± 22 |
| C16:0 | 3.2 ± 0.2 | 117 ± 21 |
| C18:0 | 5.1 ± 0.3 | 103 ± 19 |
| C18:1 | 5.6 ± 0.4 | 106 ± 15 |
| C18:3 | 5.4 ± 0.3 | 109 ± 15 |
| C20:0 | 12.0 ± 0.8 | 91 ± 11 |
| C20:1 | 12.3 ± 0.9 | 86 ± 14 |
| C20:4 | 12.6 ± 0.9 | 91 ± 12 |
| C22:0 | 15.7 ± 1.3 | 72 ± 12 |
| C22:1 | 15.6 ± 1.2 | 75 ± 13 |
| C22:4 | 15.1 ± 0.8 | 78 ± 8 |
| C22:6 | 14.9 ± 1.4 | 79 ± 9 |
| C24:0 | 17.7 ± 1.5 | 66 ± 9 |
| C24:1 | 17.9 ± 1.8 | 61 ± 8 |

When we compared 1-MAG lipids for particular chain lengths we found unsaturation of 1-MAG lipids (eg. for the C20:0, C20:1, and C20:4 group or the C22:0, C22:1, C22:4, and C22:6 group) doesn't affect the kinetic constants of ABHD12. From this study, we concluded that hABHD12 prefers VLC 1-MAG lipids (figure 2.4).

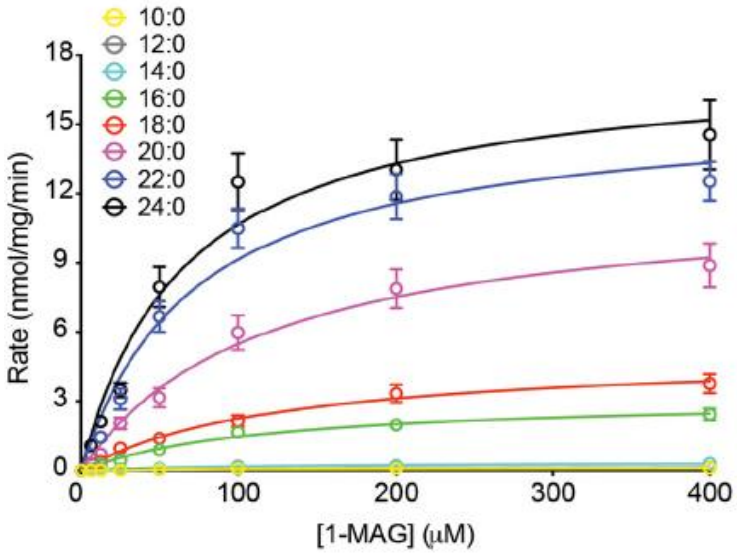



**Figure 2.4.** Enzyme kinetic studies with recombinant hABHD12

After successful 1-MAG substrate profiling studies, we bought three commercially available 2-MAG lipid substrates with increasing fatty acid chain length and have been done them against recombinant hABHD12. Nevertheless, for 2-MAG lipids, we found that hABHD12 prefers C20:4 > C18:1 > C16:0 from all the kinetic constants for these lipids. As we have observed consistent with the enzyme kinetics data from 1-MAG lipid profiling substrates (Table 2.4). We also bought three commercially available lyso-PS lipid substrates and we assayed them against recombinant hABHD12. As only C16 and C18 fatty acid chain length lyso-PS lipids are commercially available. However, in this case, we found that hABHD12 prefers C18:0 and C18:1 lyso-PS lipids in comparison to C16:0 lyso-PS as a substrate. Despite this, we didn't see any difference in the kinetic constants C18:0 and C18:1 lyso-PS (Table 2.5). In short, we concluded that the hABHD12 preference order is lyso-PS > 1-MAG > 2-MAG (Figure 2.5).

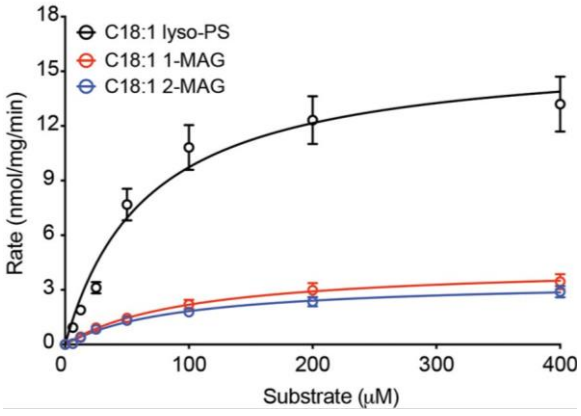



**Figure 2.5.** Enzyme kinetic study with lipid substrates against recombinant hABHD12

## Table 2.4

## Kinetics for the 2-MAG lipid substrates tested *in vitro* against recombinant human ABHD12

| 2-MAG species | | |
|---|---|---|
| C16:0 | 2.3 ± 0.4 | 148 ± 31 |
| C18:1 | 3.6 ± 0.5 | 129 ± 24 |
| C20:4 | 6.8 ± 0.5 | 95 ± 17 |

## Table 2.5

## Kinetic constants for the lyso-PS lipid substrates tested *in vitro* against recombinant human ABHD12

| Lyso-PS species | | |
|---|---|---|
| C16:0 | 7.5 ± 0.7 | 87 ± 14 |
| C18:0 | 14.8 ± 1.4 | 73 ± 12 |
| C18:1 | 14.3 ± 0.8 | 74 ± 11 |

In addition to this, we tested the membrane lysates from S246A ABHD12 transfected HEK293 cells, mock or wild-type ABHD12 tested against 1 MAG lipids at 100 μM and found negligible activity S246A hABHD12 mutant against any 1-MAG lipid substrate compared with wild-type ABHD12 (Figure 2.6).

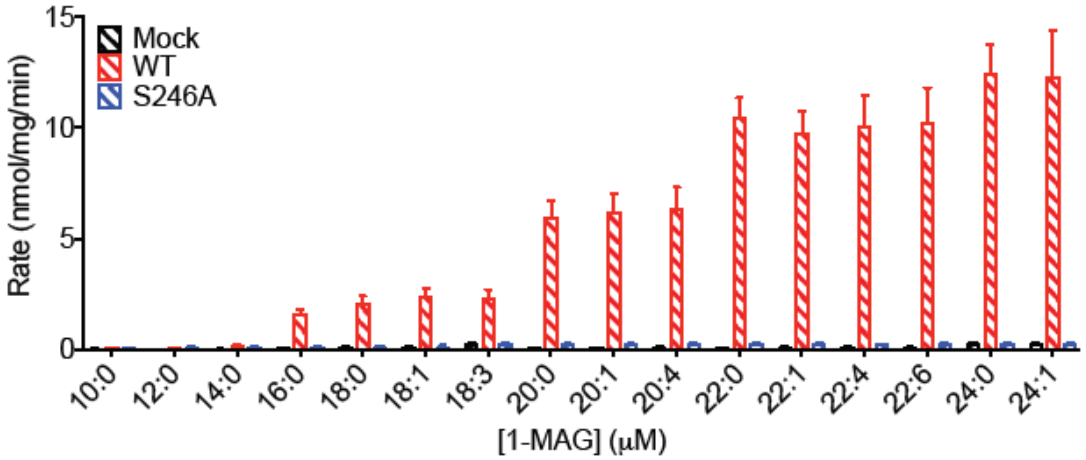



**Figure 2.6.** Enzymatic lipase assay for membrane lysate from Mock, wild-type, and S246A ABHD12

## 2.5 Substrate Profiling Study against Endogenous ABHD12 from Mouse Brain

After successfully enzyme kinetic study against recombinant hABHD12 then we decided to do an assay with 1-MAG lipid substrate against endogenous mammalian ABHD12. The previous report has shown mouse brain membrane lysate belongs to three enzymes are monoacylglycerol lipase (MAGL), ABHD12, and ABHD6 which does hydrolysis of MAG substrates. Fortunately, an inhibitor for both MAGL (JZL 184: MAGL inhibitor)[39], and ABHD6 (KT195: ABHD6 inhibitor)[40] are available. We treated wild-type and ABHD12 knockout brain lysates with the inhibitors (37 °C, 1 h, 1 μM each), and these inhibitor-treated brain membrane lysates were tested against the 1-MAG lipid substrates library. We found endogenous ABHD12 of mouse brain exhibited the best catalytic activity for VLC- 1-MAG lipids in the group of 1-MAG lipid substrates (Figure 2.7).

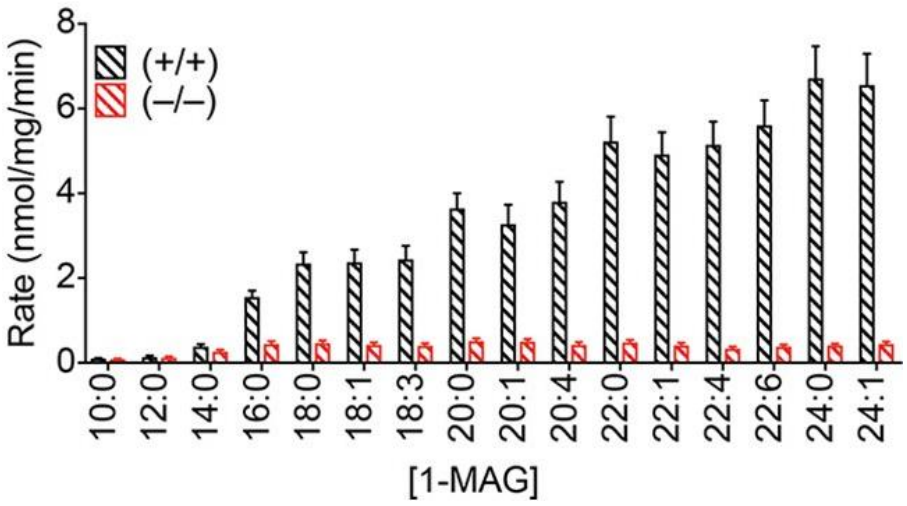



**Figure 2.7.** Enzymatic assay of 1-MAG lipids with endogenous mouse brain ABHD12

## 2.6 Conclusion

In a nutshell to describe, we have successfully synthesized the 1-MAG lipid library and we found recombinant hABHD12 prefer vary long-chain 1-MAG lipid. Also endogenous ABHD12 of the mouse brain displayed the best catalytic activity for VLC-1-MAG lipids.

## 2.7 Experimental Section:

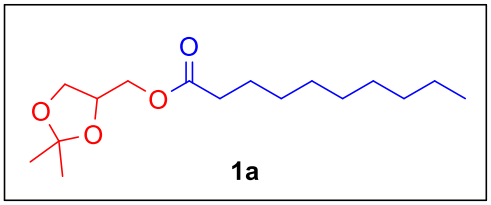
### 2.7.1 Synthesis and Characterization Data

General procedure (for reaction **1)** - To a round-bottomed flask with the fatty acid (1.0 equiv) were added 1, 2- isopropylidene glycerol (1.0 equiv), $CH_2Cl_2$ and *N,N*-dimethyl-4-amino pyridine (DMAP 0.25 equiv ) and 1-(3-dimethylamino propyl)-3-ethyl carbodiimide hydrochloride (EDC·HCl, 1.0 equiv) were added at 0 °C. After stirring the mixture for several hours (16 h) by monitoring reaction progress with TLC, the reaction was quenched with saturated $NaHCO_3$ extracted three times with $CH_2Cl_2$. The combined organic layer was dried over $Na_2SO_4$, filtrated, and concerted in vacuo. The residue was purified by column chromatography using 5% ethyl acetate/ hexane as an eluent to afford corresponding fatty acid ester's.

General procedure (for reaction **2**)- To a solution of fatty acid ester (1.0 equiv) in MeOH, Amberlyst-15 ($H^+$ form, 0.5 equiv) was added, and the whole mixture was stirred for 16 h at room temperature. After completion of the reaction (TLC analysis), amberlyst-15 was filtered off and the solvent of the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography using 40% ethyl acetate/ hexane as an eluent to afford corresponding monoacylglyceride (Table 2.2).

Compound **1a'** – **16p'** were synthesized using from above procedures and analytical data has shown below.

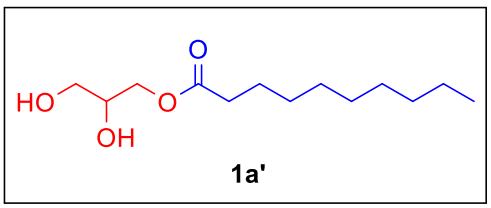
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl decanoate (1a)**



**1a**

According to general procedure **1a** (49 mg, 100% yield ) as a yellowish white solid was prepared from the corresponding Decanoic acid: $^1$H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.72, 11.5 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.1, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H ), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 12H), 0.88 (t, *J* = 6.6 Hz, 3H).

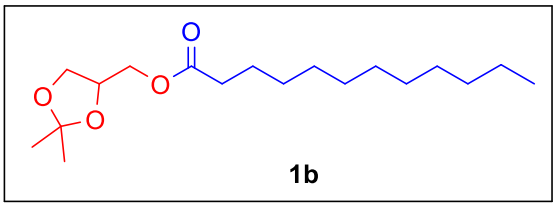
**Synthesis of 2,3-Dihydroxypropyl decanoate (1a')**



**1a'**

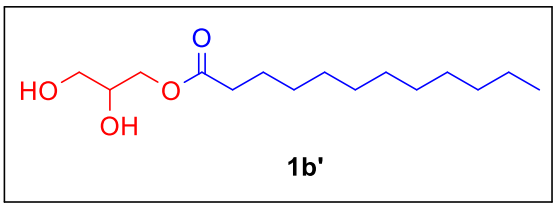
According to general procedure **1a'** (27 mg, 65% yield) as a yellowish white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl decanoate (**1a**): $^1$H NMR (400 MHz, $CDCl_3$) δ 4.20 (dd, *J* = 4.7, 11.6 Hz, 1H), 4.14 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.96−3.91 (m, 1H), 3.70 (dd, *J* = 3.6, 11.4 Hz, 1H), 3.60 (dd, *J* = 5.8, 11.4 Hz, 1H), 2.80 (s, 1H), 2.41 (s, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.26 (br, 12H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.0, 29.8, 29.5, 29.4, 29.2, 25.0, 22.8, 14.2; HRMS-ESI: $[M + H]^+$ calcd for $C_{13}H_{26}O_4$, 247.1904; found, 247.1897.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl dodecanoate (1b)**



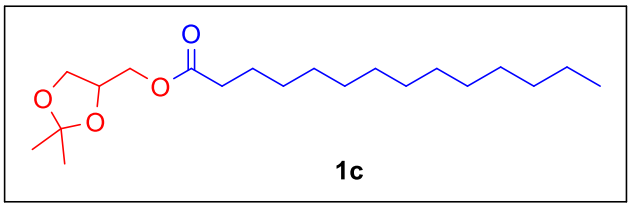

According to general procedure **1b** (47 mg**,** 100% ) as a yellowish white solid was prepared from the corresponding dodecanoic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.67,11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.3, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 1.29−1.26 (m, 16H), 0.88 (t, *J* = 6.6 Hz, 3H).

**Synthesis of 2,3-Dihydroxypropyl dodecanoate (1b')**




According to general procedure **1b'** (27 mg, 65% yield) as a yellowish white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl dodecanoate (**1b**): H NMR (400 MHz, $CDCl_3$) δ 4.19 (dd, *J* = 4.7, 11.6 Hz, 1H), 4.14 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.96-3.91 (m, 1H ), 3.70 (dd, *J* = 3.8, 11.5 Hz, 1H), 3.59 (dd, *J* = 5.8, 11.5 Hz, 1H), 2.88 (s, 1H), 2.51 (s, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.66-1.59 (m, 2H), 1.26 (br, 16H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.2, 63.5, 34.3, 32.0, 29.7, 29.6 (2C), 29.5, 29.4, 29.3, 25.0, 22.8, 14.2; HRMS-ESI: $[M + H]^+$ calcd for $C_{15}H_{30}O_4$, 275.2217; found, 275.2209.

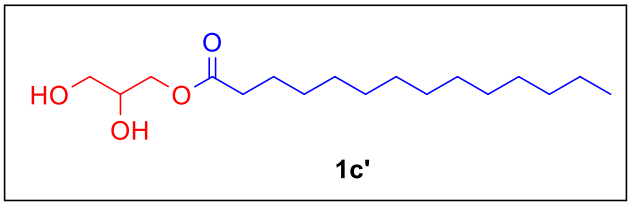
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl tetradecanoate (1c)**




According to general procedure **1c** (44 mg, 91% yield) as a yellowish white solid was prepared from the corresponding myristic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.16

(dd, $J$ = 4.6, 11.4 Hz, 1H), 4.11−4.06 (m, 2H ), 3.74 (dd, $J$ = 6.2, 8.4 Hz, 1H), 2.34 (t, $J$ = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 1.26−1.29 (m, 20H), 0.88 (t, $J$ = 6.6 Hz, 3H).

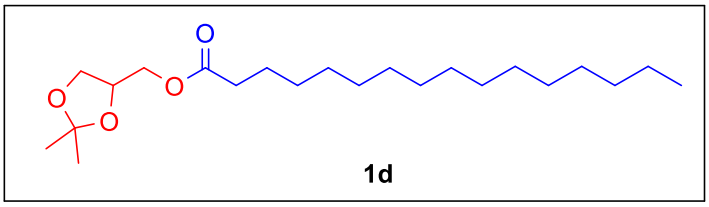
**Synthesis of 2,3-Dihydroxypropyl tetradecanoate (1c')**




**1c'**

According to general procedure **1c'** (25 mg, 72% yield) as a yellowish white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl tetradecanoate (**1c**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.21 (dd, $J$ = 4.6, 11.6 Hz, 1H), 4.15 (dd, $J$ = 6.0, 11.6 Hz, 1H), 3.94 (s, 1H), 3.70 (d, $J$ = 11.3 Hz, 1H), 3.60 (d, $J$ = 3.9 Hz, 1H), 2.56 (s, 1H), 2.35 (t, $J$ = 7.4 Hz, 2H), 2.12 (s, 1H, 1.66−1.59 (m, 2H), 1.26 (br, 20H), 0.88 (t, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.2, 63.5, 34.3, 32.1, 29.8, 29.8, 29.7, 29.6 (2C), 29.5, 29.4, 29.3, 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{17}H_{34}O_4$, 303.2530; found, 303.2520.

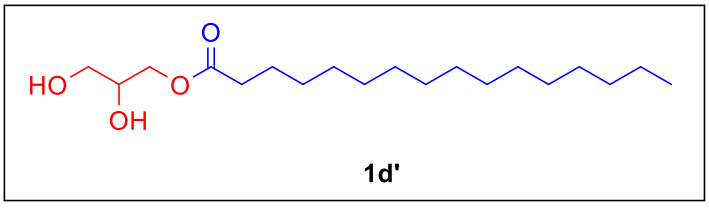
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl palmitate (1d)**




**1d**

According to general procedure **1d** (20 mg, 100% yield) as a white solid was prepared from the corresponding palmitic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.17 (dd, $J$ = 4.7, 11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, $J$ = 6.2, 8.4 Hz, 1H), 2.34 (t, $J$ = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 24H), 0.88 (t, $J$ = 6.6 Hz, 3H).

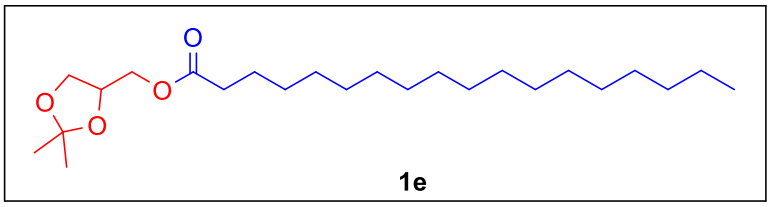
**Synthesis of 2,3-Dihydroxypropyl palmitate (1d')**




**1d'**

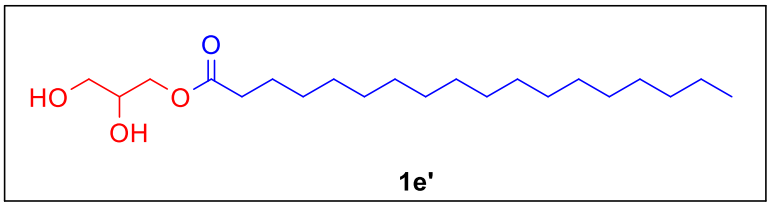
According to general procedure **1d'** (16 mg, 94% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl palmitate (**1d**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.21 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.15 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.96−3.91 (m, 1H), 3.70 (dd, *J* = 3.7, 11.3 Hz, 1H), 3.60 (dd, *J* = 5.7, 11.4 Hz, 1H), 2.60 (s, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.17 (s, 1H), 1.65−1.59 (m, 2H), 1.26 (br, 24H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6 (2C), 29.5, 29.4, 29.3, 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{19}H_{38}O_4$, 331.2843; found, 331.2847.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl stearate (1e)**



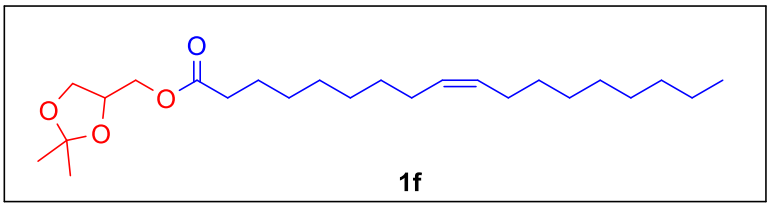

According to general procedure **1e** (20 mg, 100% yield) as a white solid was prepared from the corresponding stearic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.34−4.29 (m, 1H), 4.17 (dd, *J* = 4.7,11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.1, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 28H), 0.88 (t, *J* = 6.6 Hz, 3H).

**Synthesis of 2,3-Dihydroxypropyl stearate (1e')**




According to general procedure **1e'** (20 mg, 71%) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl stearate (**1e**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.20 (dd, *J* = 4.4, 11.5 Hz, 1H), 4.15 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.94−3.92 (m, 1H), 3.70 (dd, *J* = 3.7, 11.3 Hz, 1H), 3.60 (dd, *J* = 5.7, 11.4 Hz, 1H), 2.61(s, 1H), 2.35(t, *J* = 7.4 Hz, 2H), 2.17 (s, 1H), 1.64−1.59 (m, 2H), 1.25 (br, 28H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.8 (5C), 29.7, 29.6 (2C), 29.5 (2C), 29.4, 29.3, 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{21}H_{42}O_4$, 359.3156; found, 359.3155.

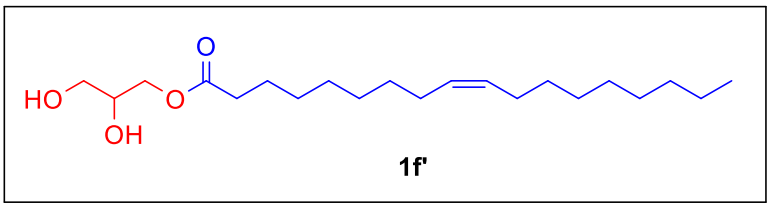
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl oleate (1f)**




**1f**

According to general procedure **1f** (20 mg, 71.4% yield) as a yellowish semisolid was prepared from the corresponding oleic acid: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.36−5.33 (m, 2H), 4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.6,11.5 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.2, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.05−1.98 (m, 4H), 1.64−1.58 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 20H), 0.88 (t, *J* = 6.4 Hz, 3H).

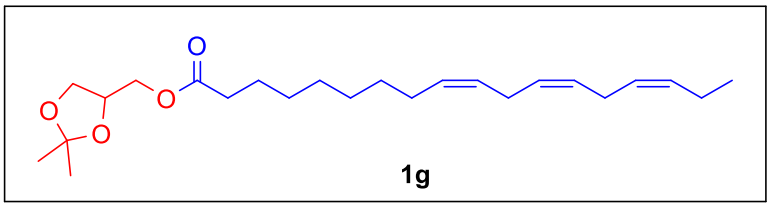
**Synthesis of 2,3-Dihydroxypropyl oleate (1f')**




**1f'**

According to general procedure **1f'** (12 mg, 71% yield) as a yellowish semisolid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl oleate (**1f**): $^1$H NMR (400 MHz, $CDCl_3$) δ 5.41−5.30 (m, 2H), 4.20 (dd, *J* = 6.0, 11.6 Hz, 1H), 4.14 (dd, *J* = 6.0,11.6 Hz, 1H), 3.96−3.91 (m, 1H), 3.69 (dd, *J* = 3.8, 11.4 Hz, 1H), 3.59 (dd, *J* = 5.8, 11.4 Hz,1H), 2.69 (s, 1H), 2.35(t, *J* = 7.4 Hz, 2H), 2.07−1.99 (m, 4H), 1.74 (s, 1H), 1.65−1.59 (m,2H), 1.26-1.30 (m, 20H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 130.2,129.8, 70.4, 65.3, 63.5, 34.3, 32.0, 29.9, 29.8, 29.7 (2C), 29.5 (2C), 29.3, 29.2, 27.3, 27.4,25.0, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{21}H_{40}O_4$, 357.2999; found, 357.2995.

**(2,2-Dimethyl-1,3-dioxoalan-4-yl)methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate (1g)**

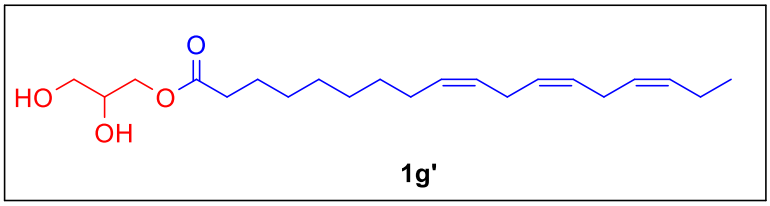



**1g**

According to general procedure **1g** (14mg, 100% yield) as a yellowish semisolid was prepared from the corresponding α-linolenic acid: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.43-5.27 (m, 6H),

4.34-4.28 (m, 1H), 4.17 (dd, $J$ = 4.6,11.5 Hz, 1H), 4.10-4.06 (m, 2H), 3.73 (dd, $J$ = 6.3, 8.4 Hz, 1H), 2.82-2.76 (m, 4H) 2.34 (t, $J$ = 7.4 Hz, 2H), 2.11-2.02 (m, 4H), 1.64-1.59 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 1.35−1.25 (m, 8H), 0.97 (t, $J$ = 7.4 Hz, 3H).

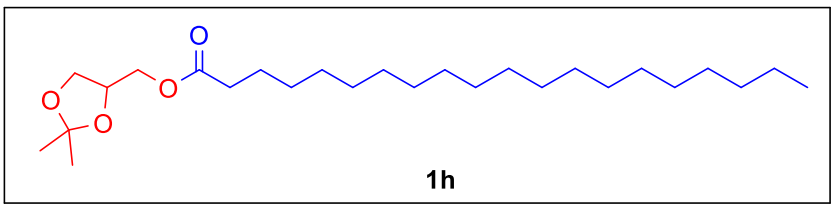
**Synthesis of 2,3-Dihydroxypropyl (9Z, 12Z, 15Z)-octadeca-9,12,15-trienoate (1g')**




**1g'**

According to general procedure **1g'** (8 mg, 83% yield) as a yellowish semisolid was prepared from the corresponding (2,2-dimethyl-1,3-dioxoalan-4-yl)methyl oleate (**1g**): $^1$H NMR (400 MHz, $CDCl_3$) δ 5.43−5.28 (m, 6H), 4.21 (dd, $J$ = 6.0, 11.6 Hz, 1H), 4.15 (dd, $J$ = 6.0, 11.6 Hz, 1H), 3.95−3.90 (m, 1H), 3.70 (dd, $J$ = 3.8, 11.4 Hz, 1H), 3.60 (dd, $J$ = 5.8, 11.4 Hz, 1H), 2.82−2.79 (m, 4H), 2.57 (s, 1H), 2.35 (t, $J$ = 7.4 Hz, 2H), 2.09−2.02 (m, 4H), 1.65−1.59 (m, 2H), 1.31 (br, 8H), 0.97 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.4, 132.1, 130.4, 128.4, 128.4, 127.9, 127.3, 70.4, 65.3, 63.5, 34.3, 29.7, 29.3 (2C), 29.2, ,27.3, 25.8, 25.7, 25.0, 20.7, 14.4; HRMS-ESI: $[M + H]^+$ calcd for $C_{21}H_{36}O_4$, 353.2686; found, 353.2687.

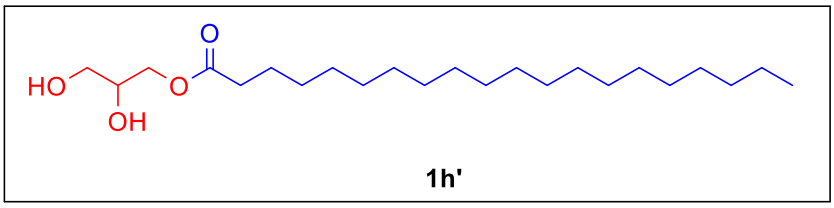
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl icosanoate (1h)**




**1h**

According to general procedure **1h** (20 mg, 100% yield) as a white solid was prepared from the corresponding arachidic acid: $^1$H NMR (400 MHz, $CDCl_3$) **δ** 4.35−4.29 (m, 1H), 4.17 (dd, $J$ = .7,11.5 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, $J$ = 6.1, 8.4 Hz, 1H), 2.34 (t, $J$ = 7.4 Hz, 2H), 1.64−1.59 (m, 2H), 1.43 (s, 3H),1.37 (s, 3H), 1.25 (br, 32H), 0.88 (t, $J$ = 6.6 Hz, 3H).

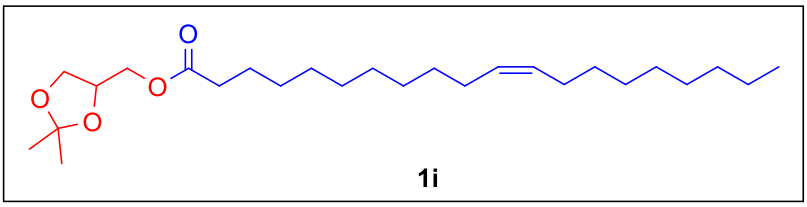
**Synthesis of 2,3-Dihydroxypropyl icosanoate (1h')**




**1h'**

According to general procedure **1h'** (16 mg, 89% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl icosanoate (**1h**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.21 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.15 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.93 (s, 1H), 3.68 (d, *J* = 11.0 Hz, 1H), 3.60 (dd, *J* = 5.3, 11.2 Hz, 1H), 2.57 (s, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.17 (s, 1H), 1.63−1.59 (m, 2H), 1.25 (br, 32H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.8 (9C), 29.7, 29.6, 29.5, 29.4, 29.3, 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{23}H_{46}O_4$, 387.3469; found, 387.3464.

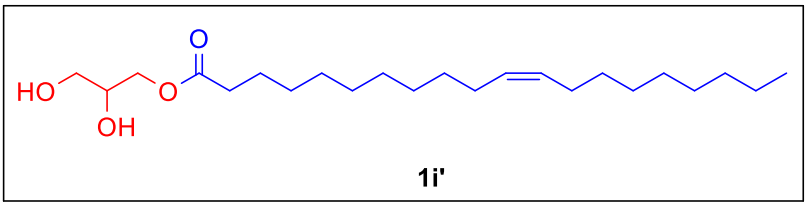
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl (*Z*)-icos-11-enoate (1i)**




**1i**

According to general procedure **1i** (18 mg, 67% yield) as a white solid was prepared from the corresponding eicosenoic acid: $^{1}$H NMR (400 MHz,$CDCl_3$) δ 5.36−5.33 (m, 2H),4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.7,11.5 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.1, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.03−1.99 (m, 4H), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 24H), 0.88 (t, *J* = 6.4 Hz, 3H).

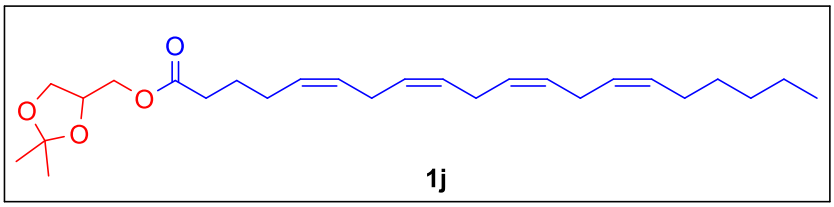
**Synthesis of 2,3-Dihydroxypropyl (*Z*)-icos-enoate (1i')**




**1i'**

According to general procedure **1i'** (8 mg, 50%) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl (*Z*)-icos-11-enoate (**1i**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.39−5.31 (m, 2H), 4.21 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.15 (dd, *J* = 6.1, 11.6 Hz, 1H), 3.93 (s, 1H), 3.71−3.69 (m, 1H), 3.59 (dd, *J* = 5.6, 11.3 Hz, 1H), 2.54 (s, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.10 (s, 1H) 2.03−1.99 (m, 4H), 1.68−1.61 (m, 2H), 1.27 (d, 24H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 130.1, 130.0, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9, 29.7 (2C), 29.6, 29.6, 29.5 (2C), 29.4, 29.4, 29.3, 27.4 (2C), 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{23}H_{44}O_4$, 385.3312; found, 385.3315.

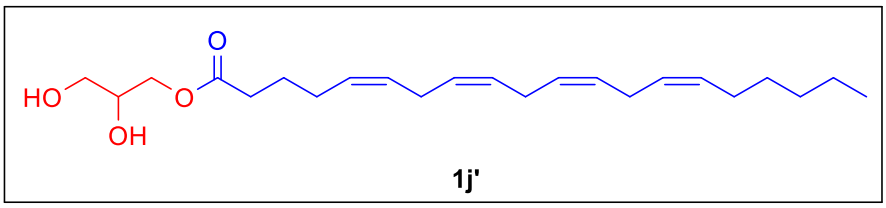
**(2,2-Dimethyl-1,3-dioxalan-4-yl)methyl (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate (1j)**




**1j**

According to general procedure **1j** (9 mg, 69% yield) as a yellowish semisolid was prepared from the corresponding arachidonic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.43−5.30 (m, 8H), 4.34−4.29 (m, 1H), 4.17 (dd, *J* = 4.6,11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.2, 8.4 Hz, 1H), 2.85−2.79 (m, 6H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.17−2.03 (m, 4H), 1.75−1.68 (m, 2H), 1.41 (s, 3H), 1.37 (s, 3H), 1.23 (br, 6H), 0.88 (t, *J* = 6.7 Hz, 3H).

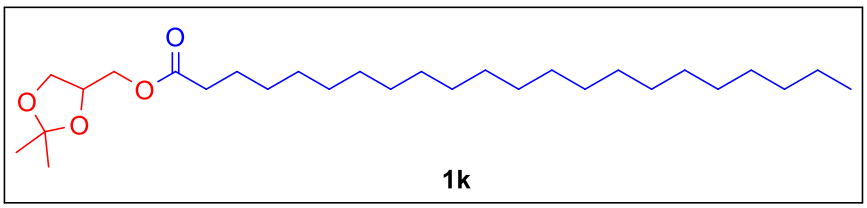
**Synthesis of 2,3-Dihydroxypropyl (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate (1j')**




**1j'**

According to general procedure **1j'** (3 mg, 50% yield) as a yellowish semisolid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate (**1j**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.44−5.30 (m, 8H), 4.21 (dd, *J* = 6.1, 11.6 Hz, 1H), 4.15 (dd, *J* = 4.6, 11.6 Hz, 1H), 3.93 (m, 1H), 3.70 (dd, *J* = 3.6, 11.6 Hz, 1H), 3.60 (dd, *J* = 5.7, 11.4 Hz, 1H), 2.84 (dd, *J* = 5.4, 11.4 Hz, 6H), 2.47 (s, 1H), 2.37(t, *J* = 7.4 Hz, 2H), 2.17−2.01 (m, 4H), 1.76−1.69 (m, 2H), 1.62 (s, 1H), 1.25 (br, 6H), 0.89 (t, *J* = 6.7 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.2, 130.7, 129.2, 128.9, 128.8, 128.4, 128.2, 28.0, 127.7, 70.4, 65.4, 63.5, 33.6, 31.7, 29.9, 29.5, 27.4, 26.7, 25.8 (2C), 24.9, 22.7, 14.2. HRMS-ESI: $[M + H]^+$ calcd for $C_{23}H_{38}O_4$, 379.2843; found, 379.2834.

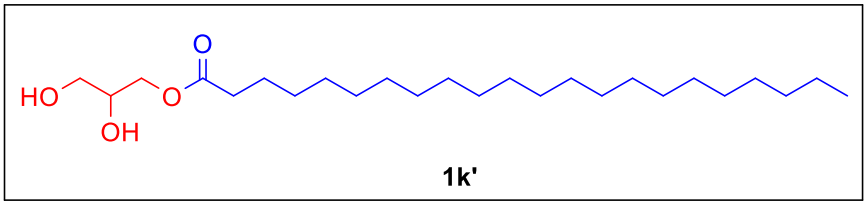
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl docosanoate (1k)**




**1k**

According to general procedure **1k** (18 mg, 75% yield) as a white solid was prepared from the corresponding behenic acid: $^1$H NMR (400 MHz, $CDCl_3$) δ 4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.7,11.6 Hz, 1H), 4.11−4.06 (m, 2H ), 3.74 (dd, *J* = 6.6, 8.4 Hz, 1H ), 2.34 (t, *J* = 7.4 Hz, 2H ), 1.69−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.25 (br, 36H), 0.88 (t, *J* = 6.6 Hz, 3H).

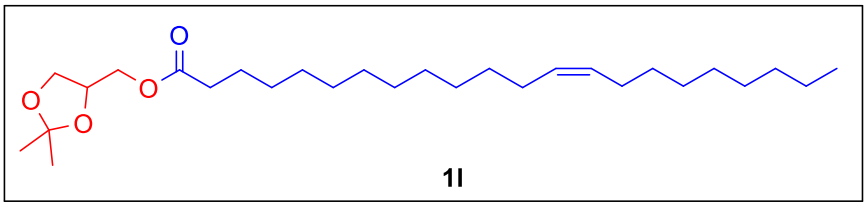
**Synthesis of 2,3-Dihydroxypropyl docasanoate (1k')**




**1k'**

According to general procedure **1k'** (10 mg, 77% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl docasanoate (**1k**): $^1$H NMR (400 MHz, $CDCl_3$,) δ 4.20 (dd, *J* = 4.7, 11.6 Hz, 1H), 4.14 (dd, *J* = 6.1, 11.6 Hz, 1H), 3.93 (d, *J* = 4.8 Hz, 1H), 3.70 (d, *J* = 11.4 Hz, 1H), 3.59 (dd, *J* = 5.76, 11.4 Hz, 1H), 2.47 (d, *J* = 5.0 Hz, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.04 (s, 1H), 1.68 −1.57 (m, 2H), 1.25 (br, 36H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9 (2C), 29.8 (3C), 29.6, 29.6, 29.5 (3C), 29.4 (3C), 29.3 (3C), 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{25}H_{50}O_4$, 415.3782; found, 415.3781.

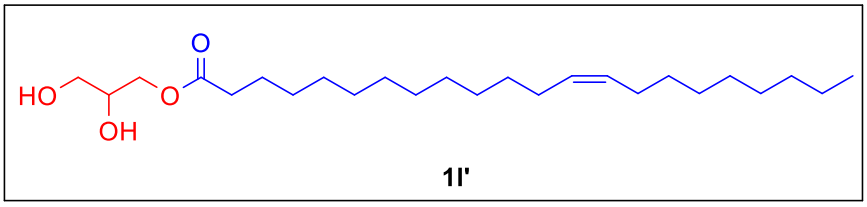
**(2,2-Dimethyl-1,3-dicoxolan-4-yl)methyl (*Z*)-docos-13-enoate (1l)**




**1l**

According to general procedure **1l** (18 mg, 69% yield) as a white solid was prepared from the corresponding erucic acid: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.39−5.31 (m, 2H), 4.34−4.29 (m, 1H), 4.17 (dd, *J* = 4.7, 11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.2, 8.4 Hz,1H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.04−1.96 (m, 4H), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.26 (br, 28H), 0.88 (t, *J* = 6.4 Hz, 3H).

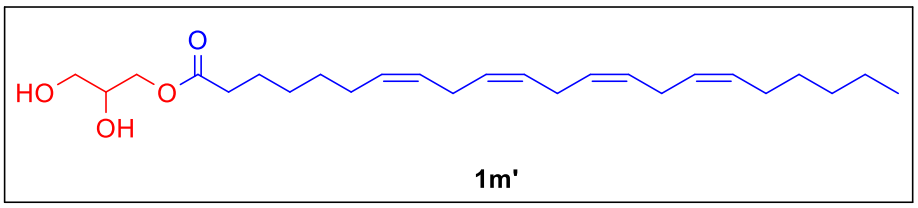
**Synthesis of 2,3-Dihydroxypropyl (*Z*)-docos-13-enoate (1l')**




**1l'**

According to general procedure **1l'** (12 mg, 75% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxalan-4-yl)methyl (*Z*)-docos-13-enoate (**1l**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.39−5.31 (m, 2H), 4.21 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.15 (dd, *J* = 6.0,11.6 Hz, 1H), 3.96−3.91 (m, 1H), 3.70 (dd, *J* = 3.8, 11.4Hz, 1H), 3.60 (dd, *J* = 5.7, 11.4 Hz,1H), 2.58 (s, 1H), 2.35(t, *J* = 7.4 Hz, 2H), 2.17 (s, 1H), 2.04−1.99 (m, 4H), 1.65−1.59 (m,2H), 1.26 (br, 28H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 130.1, 130.1, 130.0, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5 (2C), 29.4 (2C), 29.3, 27.4 (2C), 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{25}H_{48}O_4$, 413.3625; found, 413.3622.

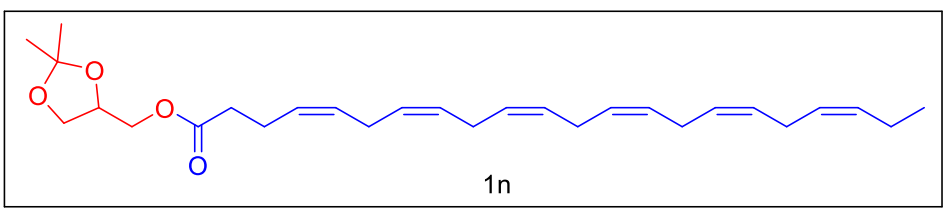
**Synthesis of 2,3-Dihydroxypropyl (7*Z*,10*Z*,13*Z*,16*Z*)-docosa-7,10,13,16-tetraenoate(1m')**




**1m'**

According to general procedure **1m'** (3 mg, 50% yield ) as a yellowish semisolid was prepared from the corresponding (2,2-dimethyl-1,3-dioxolan-4-yl)methyl (7Z,10Z,13Z,16Z)-icosa-7,10,13,17-tetraenoate: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.38−5.33 (m, 8H), 4.21 (dd, *J* = 6.6, 10.7 Hz, 1H), 4.14 (dd, *J* = 6.1, 11.6 Hz, 1H), 3.93 (s, 1H), 3.67 (d, *J* = 9.4 Hz, 1H), 3.60 (d, *J* = 6.0 Hz, 1H), 2.87−2.79 (m, 6H), 2.45 (s, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.19−2.16 (m, 1H) 2.07−2.01(m, 4H), 1.68−1.55 (m, 2H), 1.25 (br, 10H), 0.88 (t, *J* = 6.7 Hz, 3H); HRMS-ESI: $[M + H]^+$ calcd for $C_{25}H_{42}O_4$, 407.3156; found, 407.3156.

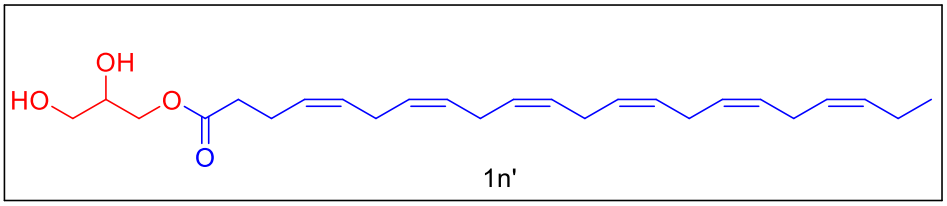
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa- 4,7,10,13,17,19-tetraenoate (1n)**




1n

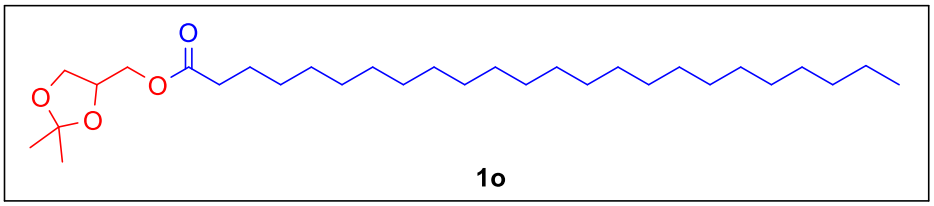
According to general procedure **1n** (6 mg, 47% yield) as a yellowish semisolid was prepared from the corresponding docosahexaenoic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.44−5.28 (m, 12H), 4.34−4.28 (m, 1H), 4.17 (dd, *J* = 4.6, 11.4 Hz, 1H), 4.12−4.06 (m, 2H), 3.74 (dd, *J* = 6.1, 8.4 Hz, 1H), 2.88−2.80 (m, 10H), 2.43−2.37 (m, 4H), 2.11−2.04 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 0.97 (t, *J* = 7.1 Hz, 3H).

**Synthesis of 2,3-Dihydroxypropyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-tetraenoate (1n')**



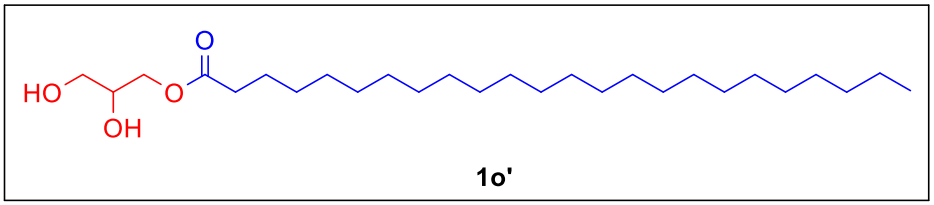

According to general procedure **1n'** (4 mg, 80% yield) as a yellowish semisolid was prepared from the corresponding (2,2-dimethyl-1,3-dioxolan-4-yl)methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,17,19-tetraenoate (**1n**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.46−5.28 (m, 12H), 4.24−4.13 (m, 2H), 3.95 (dd, *J* = 5.5, 9.9Hz, 1H), 3.70 (dd *J* = 3.9, 11.4Hz, 1H), 3.59 (dd, *J* = 5.7, 11.4Hz, 1H), 2.84 (dd, *J* = 4.2,15.2 Hz, 10H), 2.46−2.33 (m, 4H), 2.11−2.06 (m, 2H), 2.04 (s, 1H), 1.63 (s, 1H), 0.99 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.7, 132.2, 129.8, 128.7, 128.5 (2C), 128.4, 128.4, 128.2, 128.1, 128.0, 127.8, 127.2, 70.4, 65.5, 63.4, 34.2, 29.9 (2C), 25.8, 25.8, 25.7, 22.9, 20.7, 14.4; HRMS-ESI: $[M + H]^+$ calcd for $C_{25}H_{38}O_4$, 403.2843; found, 403.2837.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl tetracosanoate (1o)**




According to general procedure **1o** (18 mg, 69% yield) as a white solid was prepared from the corresponding lignoceric acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.34−4.29 (m, 1H), 4.17 (dd, *J* = 4.7,11.5 Hz, 1H), 4.11−4.06 (m, 2H), 3.73 (dd, *J* = 6.2, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.66−1.59 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 1.25 (br, 40H), 0.88 (t, *J* = 6.6 Hz, 3H).

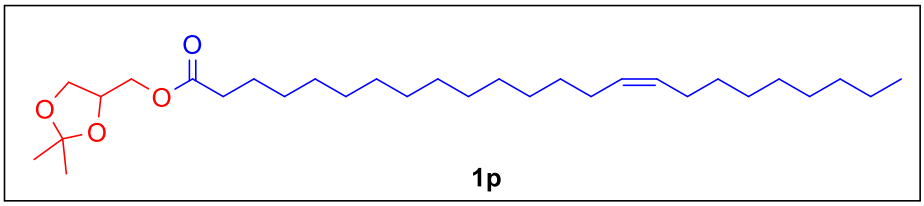
**Synthesis of 2,3-Dihydroxypropyl tetracosanoate (1o')**




1o'

According to general procedure **1o'** (5 mg, 32% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxolan-4-yl)methyl tetracosanoate (**1o**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.22 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.14 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.94 (s, 1H), 3.70 (d, J = 8.2 Hz, 1H), 3.60 (dd, *J* = 5.4, 11.3 Hz, 1H), 2.50 (s, 1H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.05 (s, 1H), 1.65−1.59 (m, 4H), 1.25 (br, 38H), 0.88 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9, 29.8, 29.8, 29.6 (6H), 29.5 (6H), 29.4 (2C), 29.3, 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{27}H_{54}O_4$, 443.4095; found, 443.4097.

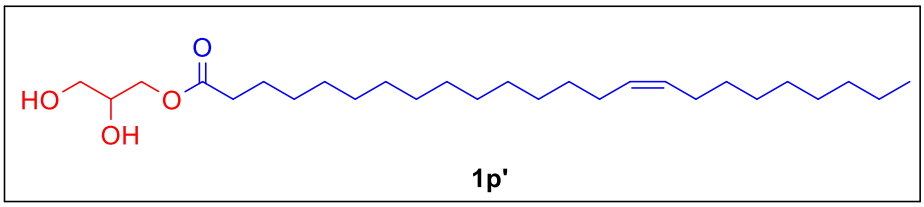
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl (*Z*)-tetracos-15-enoate (1p)**




1p

According to general procedure **1p** (18 mg, 69% yield) as a white solid was prepared from the corresponding nervonic acid: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.39−5.31 (m, 2H), 4.35−4.29 (m, 1H), 4.17 (dd, *J* = 4.6,11.4 Hz, 1H), 4.11−4.06 (m, 2H), 3.74 (dd, *J* = 6.2, 8.4 Hz, 1H), 2.34 (t, *J* = 7.4 Hz, 2H ), 2.04−1.99 (m, 4H), 1.66−1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.2 (br, 32H), 0.88 (t, *J* = 6.4 Hz, 3H).

**Synthesis of 2,3-Dihydroxypropyl (*Z*)-tetracos-15-enoate (1p')**



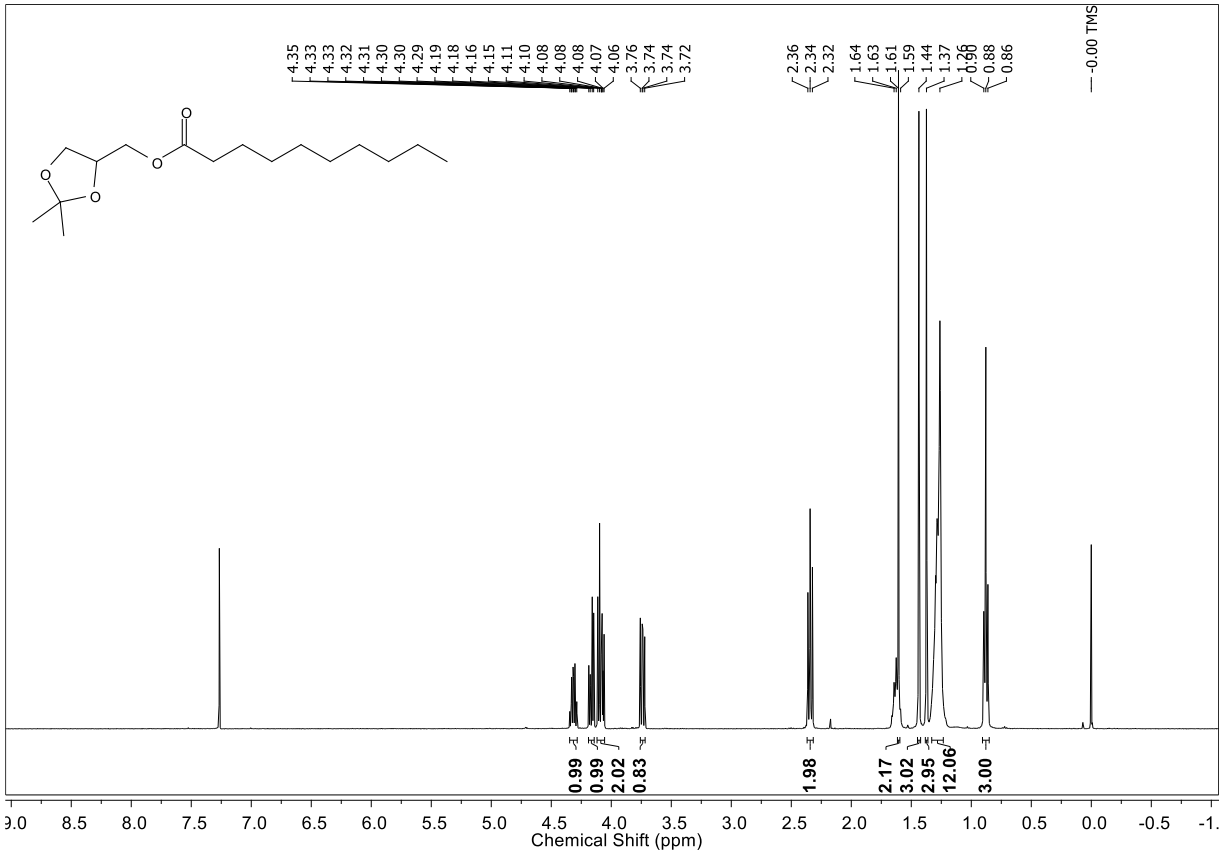

1p'

According to general procedure **1p'** (8 mg, 50% yield) as a white solid was prepared from the corresponding (2,2-dimethyl-1,3-dioxolan-4-yl)methyl (Z)-tetracos-15-enoate (**1p**): $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.39−5.31 (m, 2H), 4.21 (dd, *J* = 4.6, 11.6 Hz, 1H), 4.15 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.95−3.92 (m, 1H), 3.72−3.69 (m, 1H), 3.60 (dd, *J* = 5.8, 11.3 Hz, 1H), 2.58 (s, 1H),

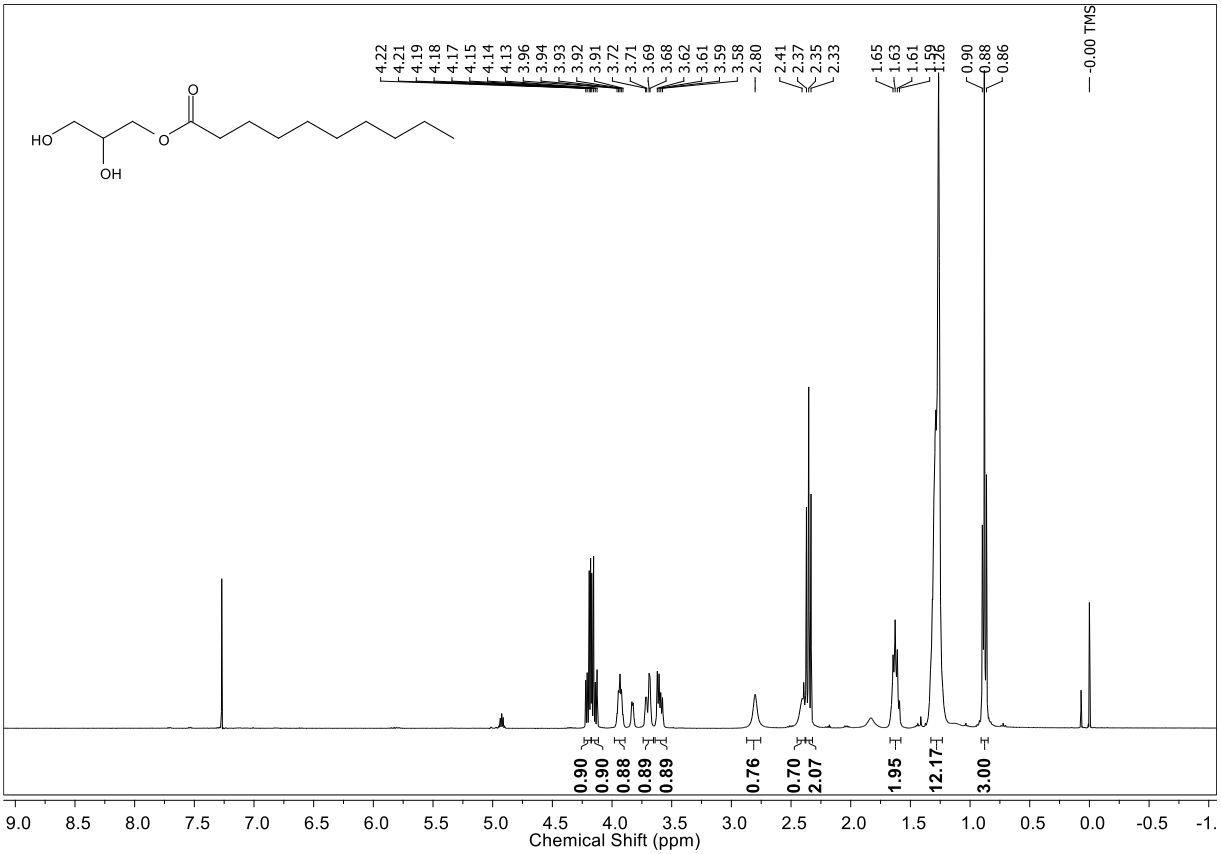
2.35 (t, $J$ = 7.4 Hz, 2H), 2.16 (s, 1H) 2.04−1.99 (m, 4H), 1.65−1.59 (m, 2H), 1.26 (br, 32H), 0.88 (t, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 130.0, 130.0, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5 (3C), 29.4 (2C), 29.3 (2C), 27.4 (2C), 25.1, 22.8, 14.3; HRMS-ESI: $[M + H]^+$ calcd for $C_{27}H_{52}O_4$, 441.3938; found, 441.3942.

## 2.8. Spectral Chart

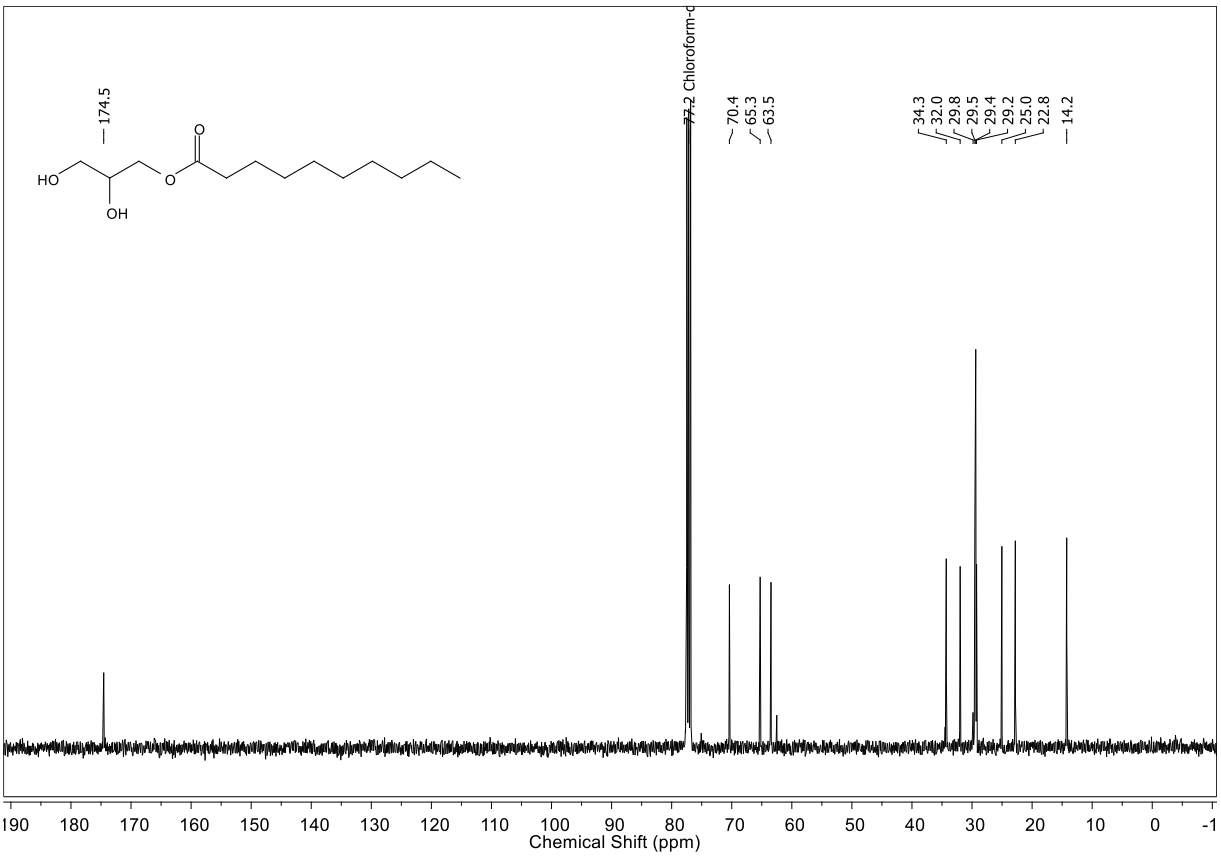
**$^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 a:**



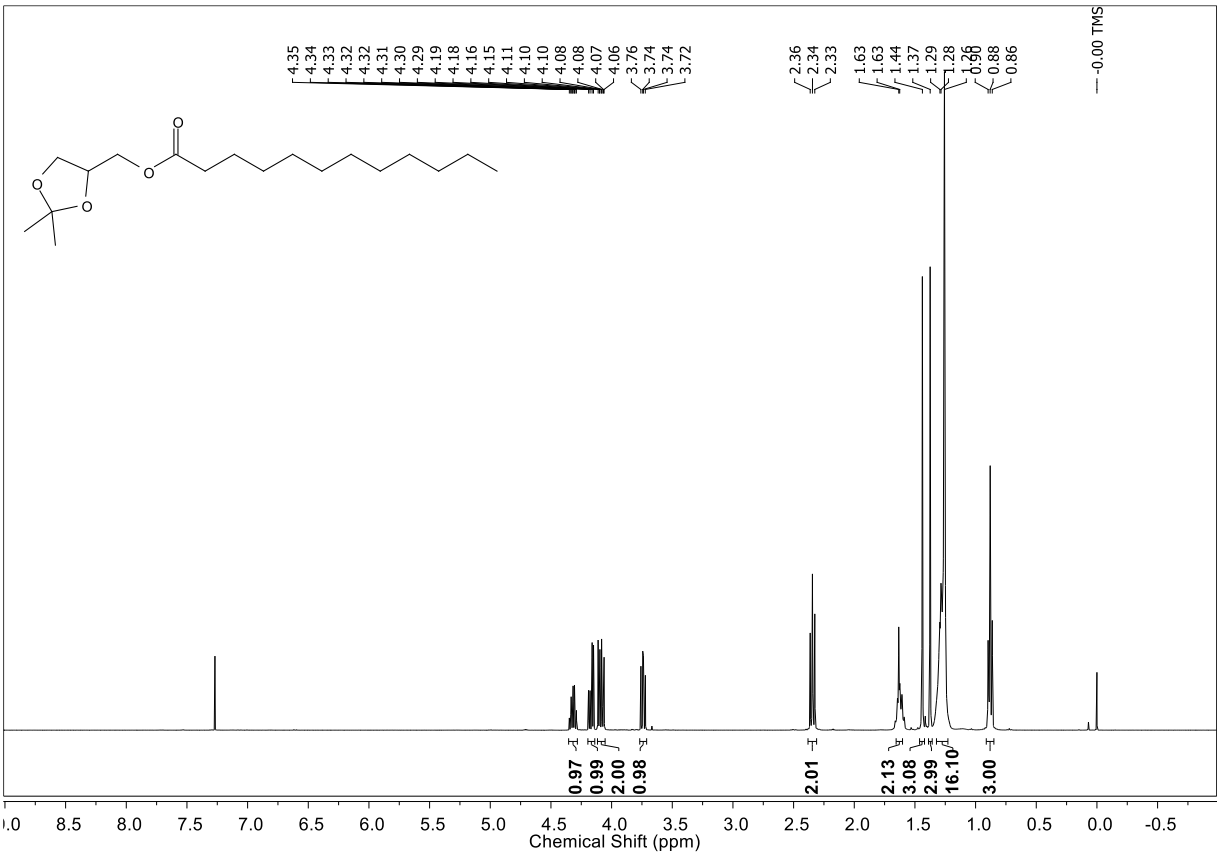

**$^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 a':**



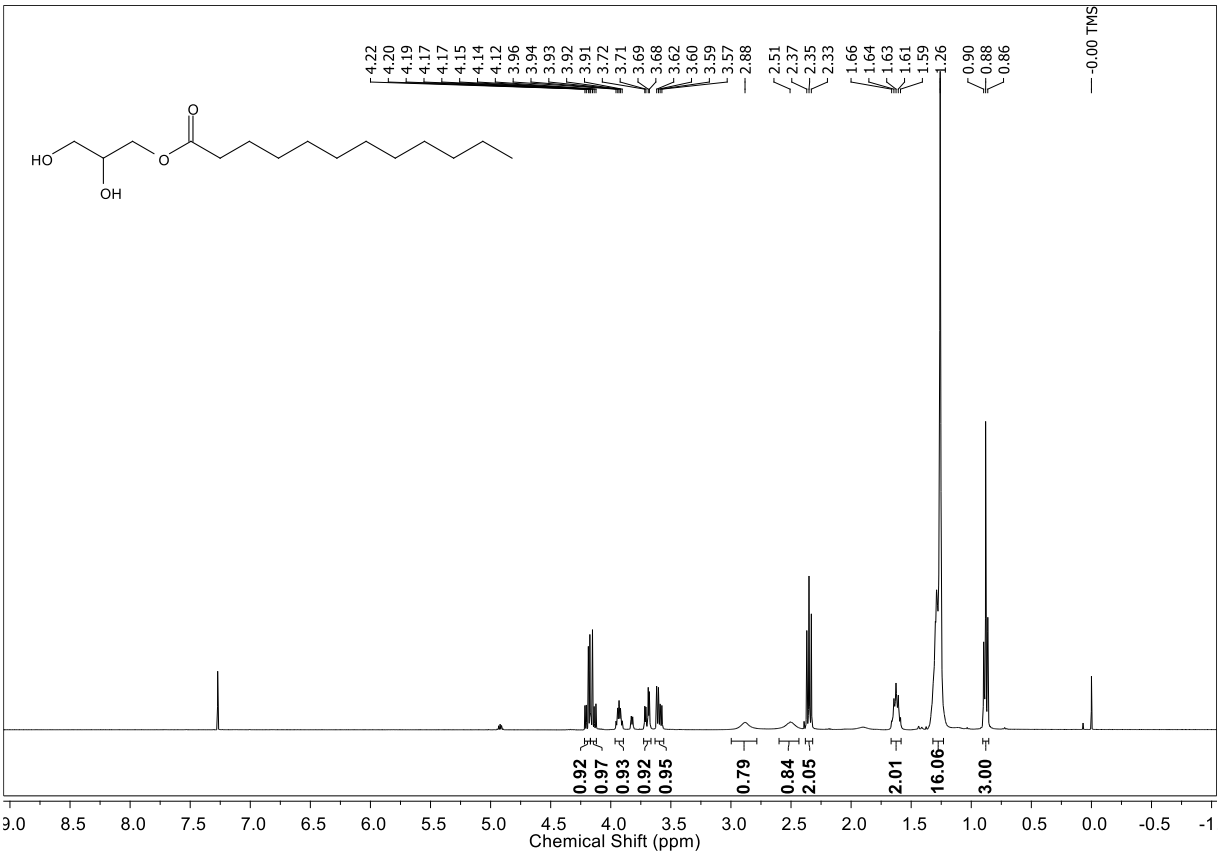

**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 1 a':**



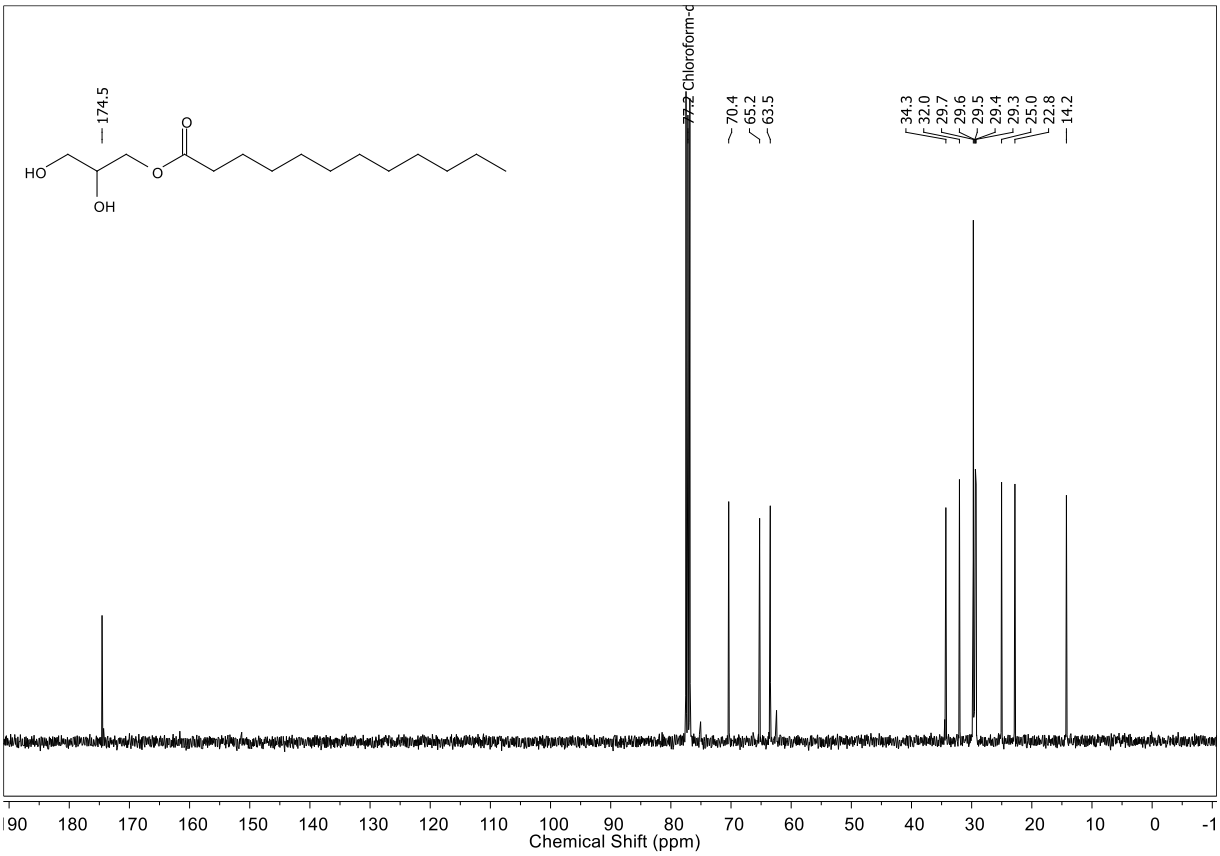
## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 b:



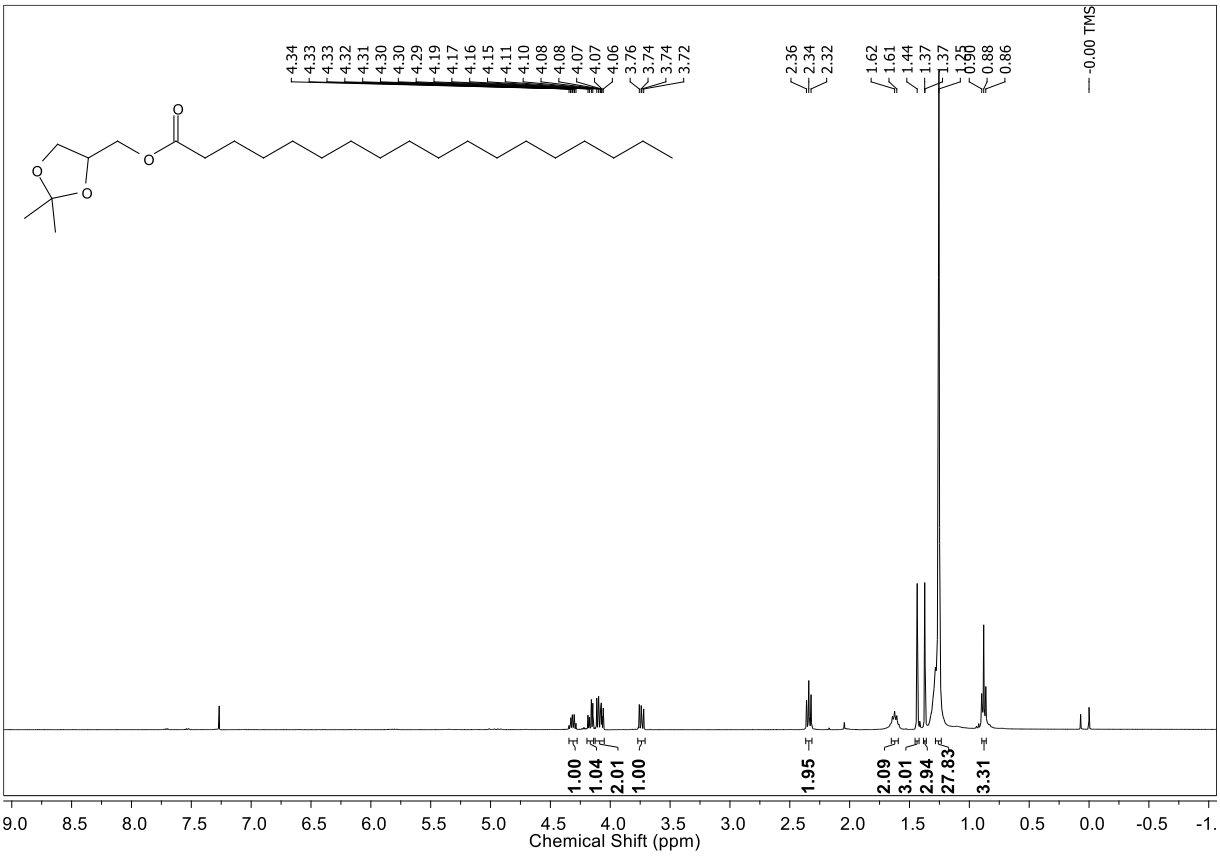

## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 b':



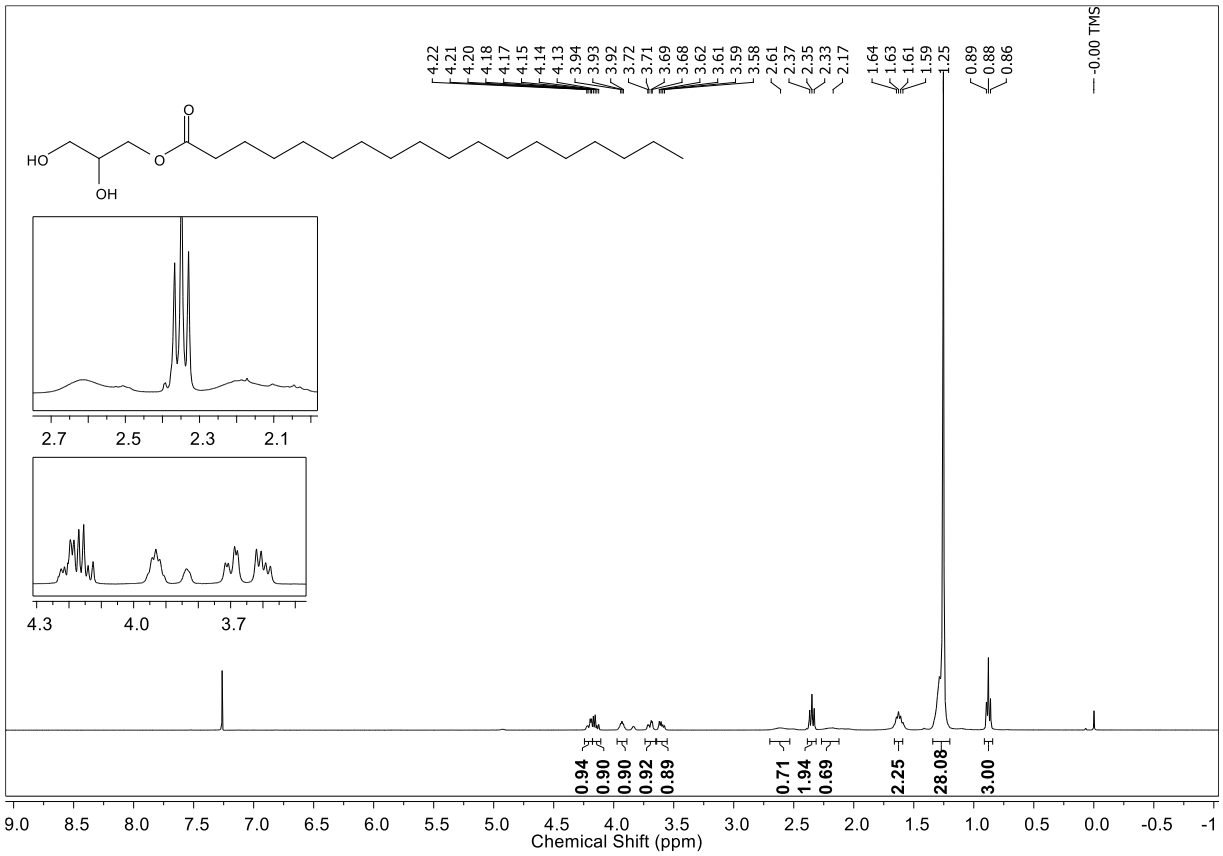
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 1 b':



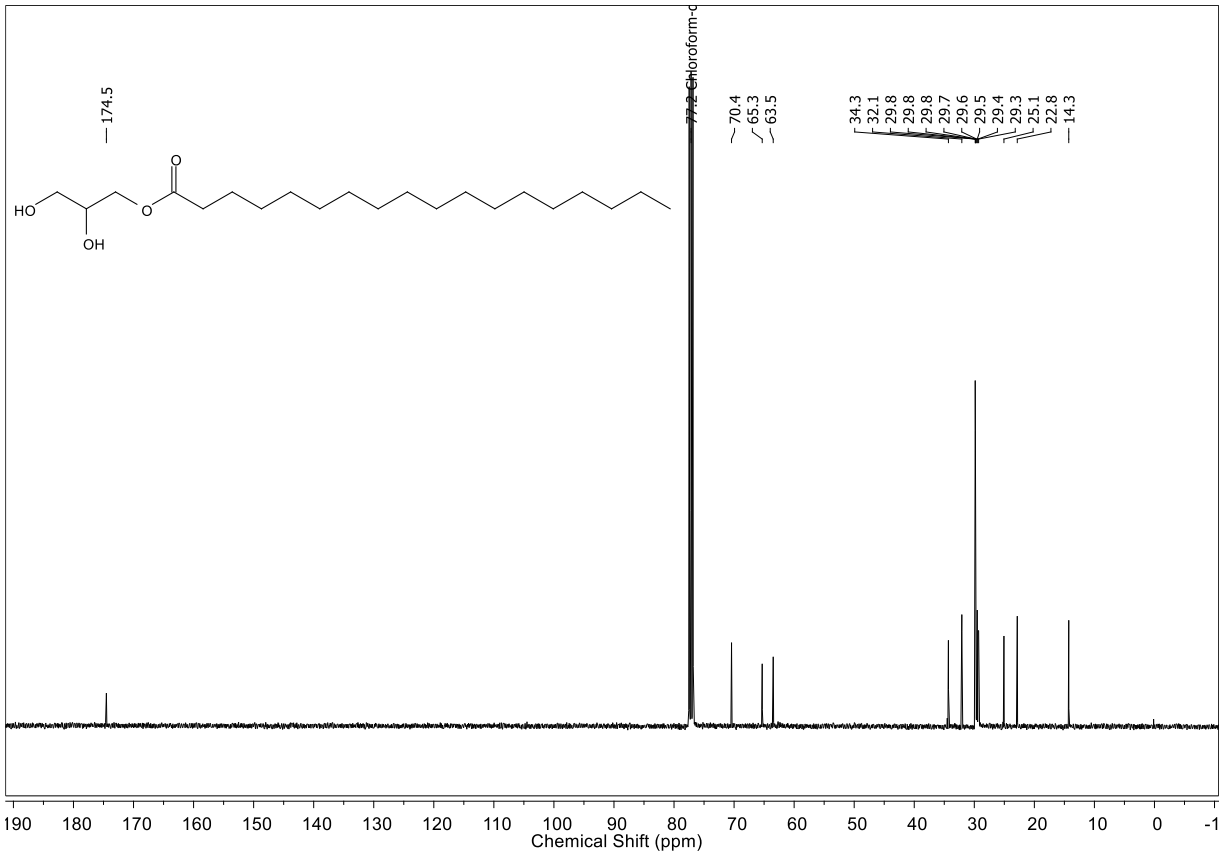

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 e:



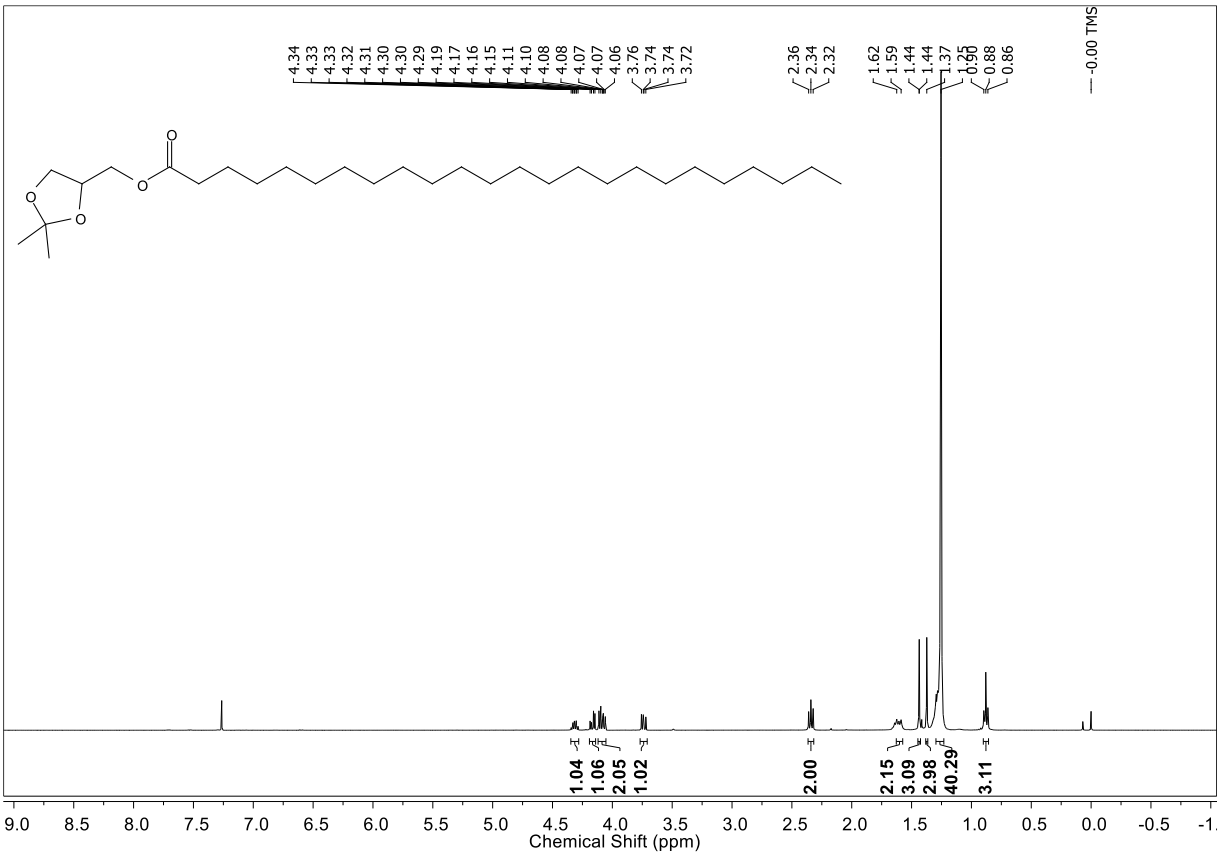
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 e';



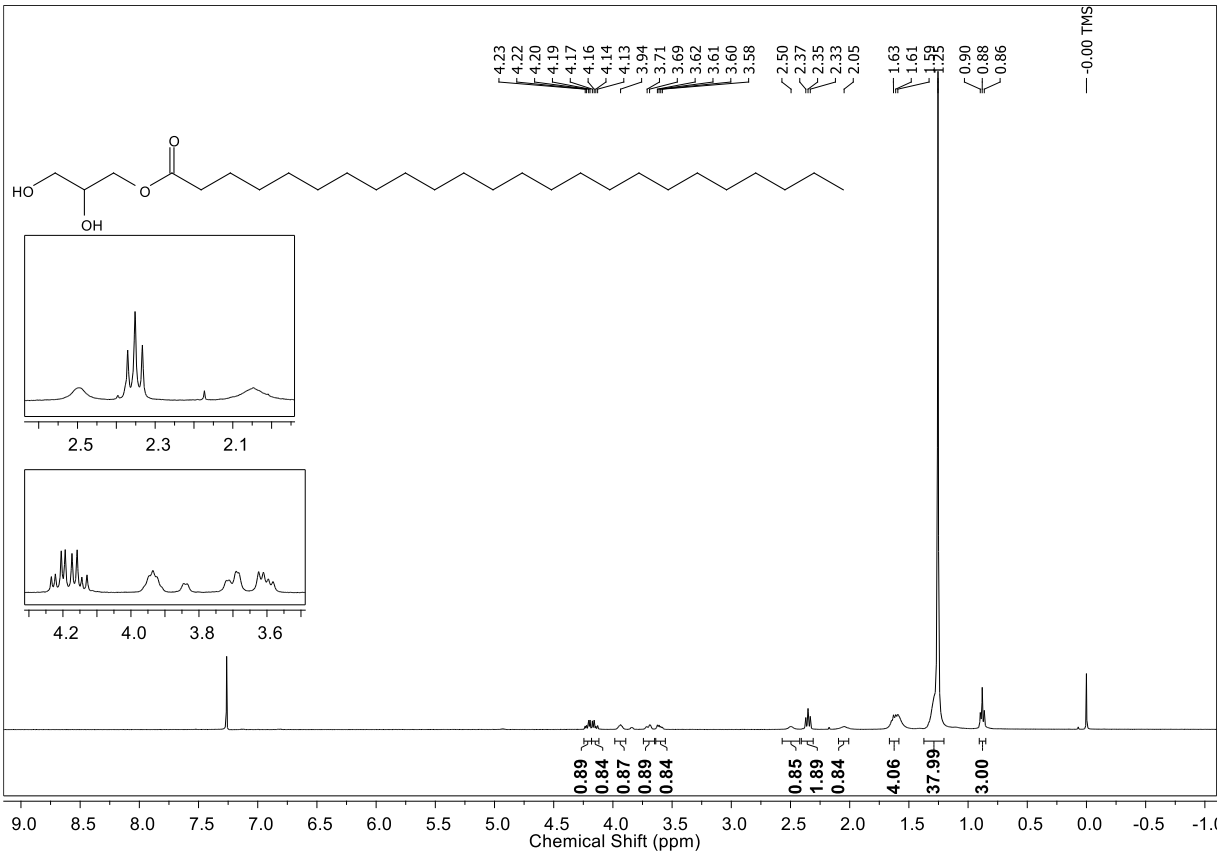

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 1 e':



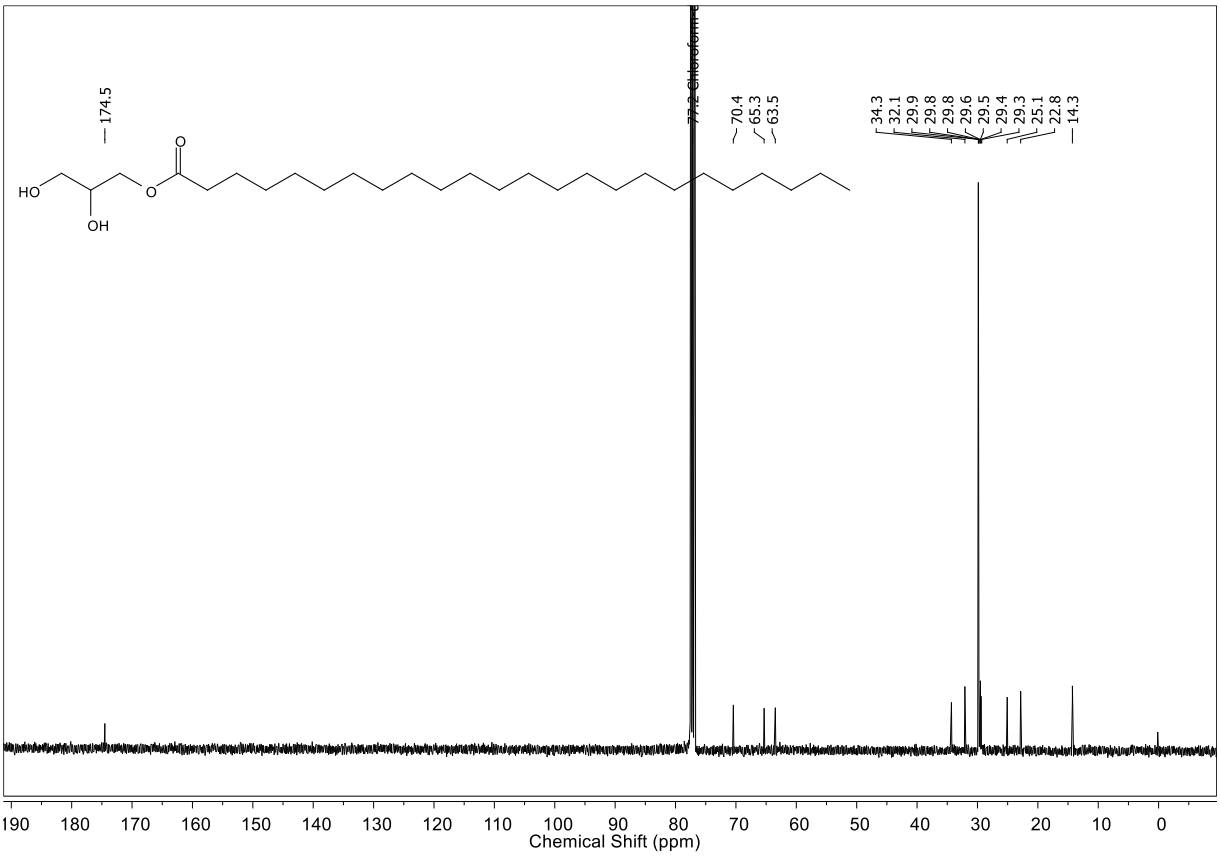
## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 o:



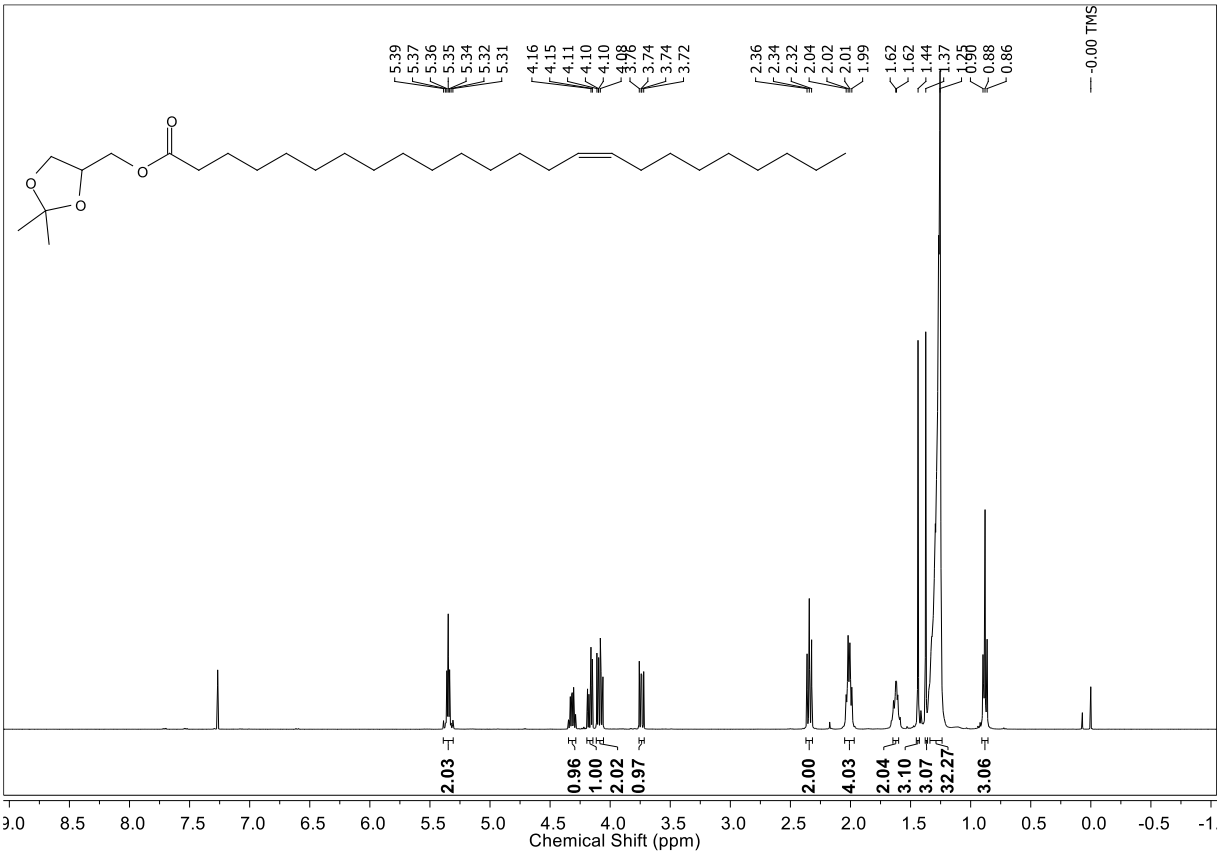

## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 o':



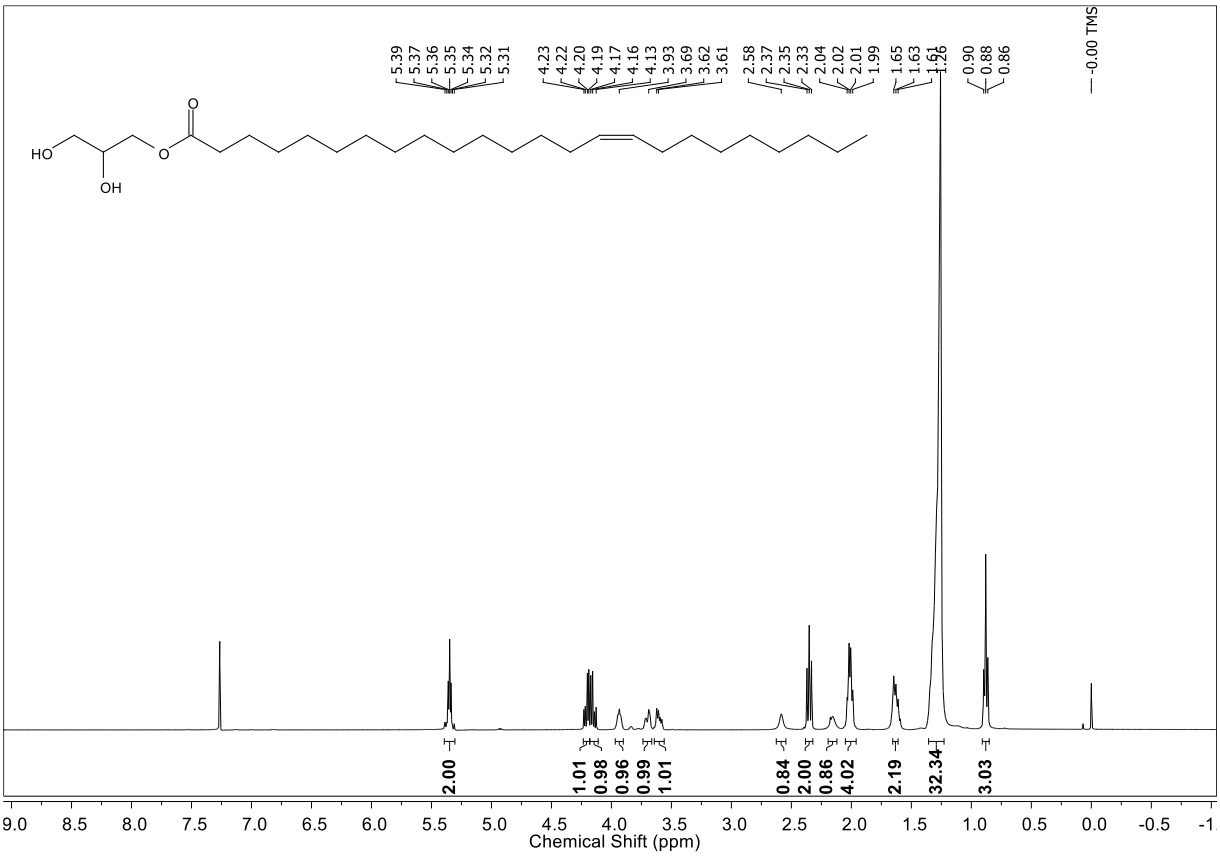
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 1 o':**



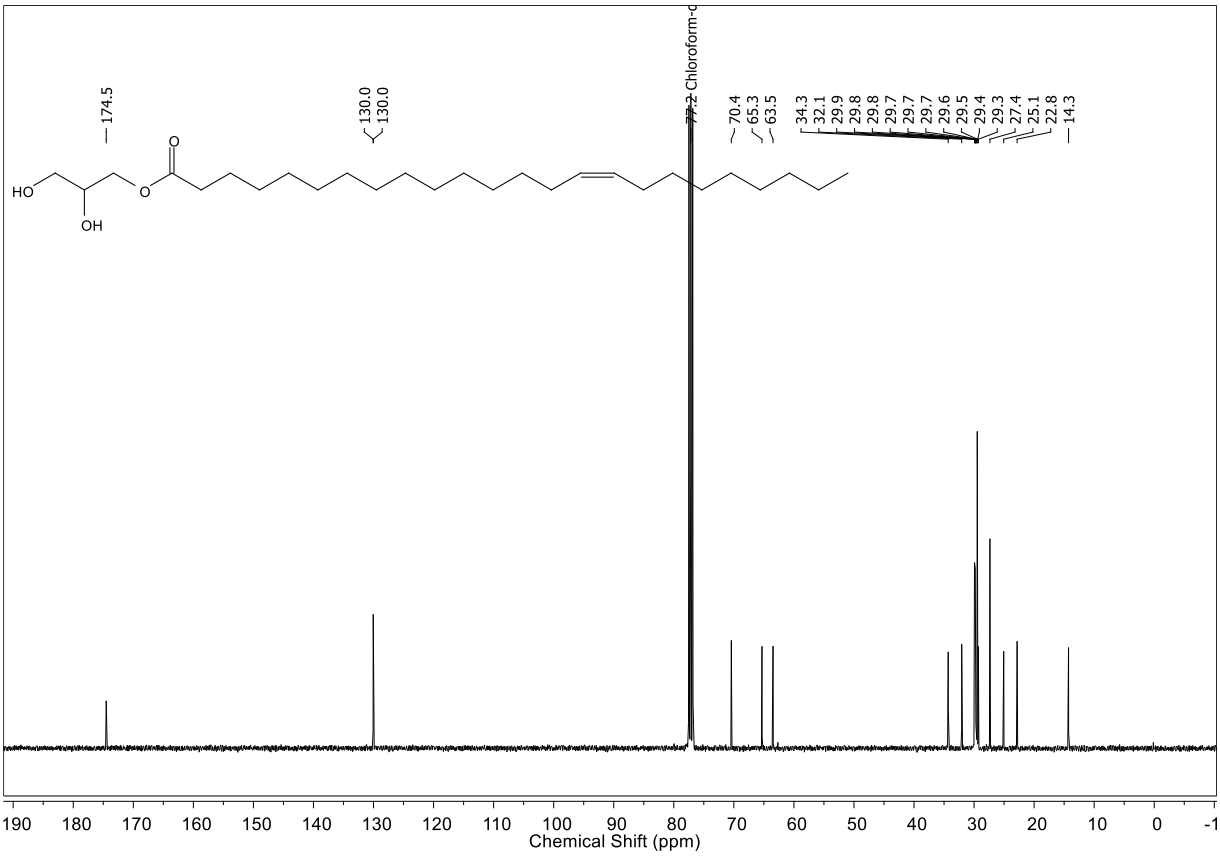

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 p:**



## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound 1 p':




## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 1 p':



# CHAPTER 3

# SYNTHESIS OF ME-LYSO-PS LIPIDS AND THEIR BIOLOGICAL APPLICATIONS

Adapted with permission from: Cell Chemical Biology, 2021; 28, pp 1-11

https://doi.org/10.1016/j.chembiol.2021.01.008

## 3.1 Introduction

A recent study has associated three lyso-PLs namely S1P, lyso-PA, and lyso-PS with different pathophysiological conditions like cancer and immunological condition[41–43]. Lyso-PS is a signaling lyso-PLs molecule that functions through TLR2 and GPCR receptors. As discussed in chapter 1, lyso-PS regulates macrophage activation, mast cell degranulation, chemotactic migration in U87 glioma cells, leukemia cell stimulation, and inhibition of lymphocyte proliferation.

Lyso-PS are further sub-classified into *sn*-1 and *sn*-2 lyso-PS (Figure 3.1). A recent study have suggested that *sn*-1 lyso-PS significantly to be more stable and abundant than *sn*-2 lyso-PS. However, *sn*-2 lyso-PS have been found to be substantially more stable at lower PH (< 4.0)[44–47]. Indeed, *sn*-1 and *sn*-2 lyso-PS showed similar levels present in various murine tissues.

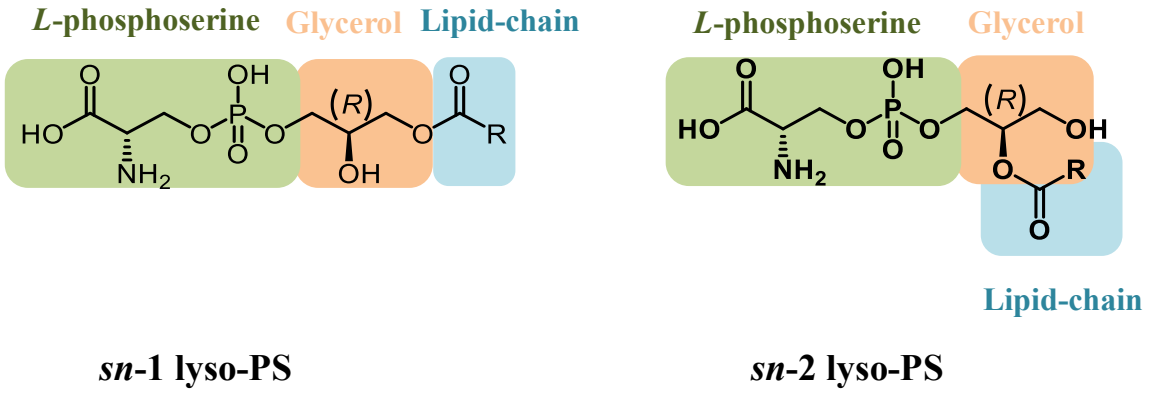



**Figure 3.1.** Classification of lysophosphatidylserine lipids

During my research program, I have tried to synthesize different derivatives of lyso-PS. However, glycerophosphoserine synthesis is extremely challenging as the initial precursor phosphorous and some phosphorous intermediate is moisture sensitive. To the best of our knowledge till date, there are no synthetic routes reported for the synthesis of lyso-PS derivatives. Here, I have mentioned certain challenges which I faced during the synthesis of the lyso-PS library.

## 3.2 Synthesis Challenges in Lyso-PS Lipids

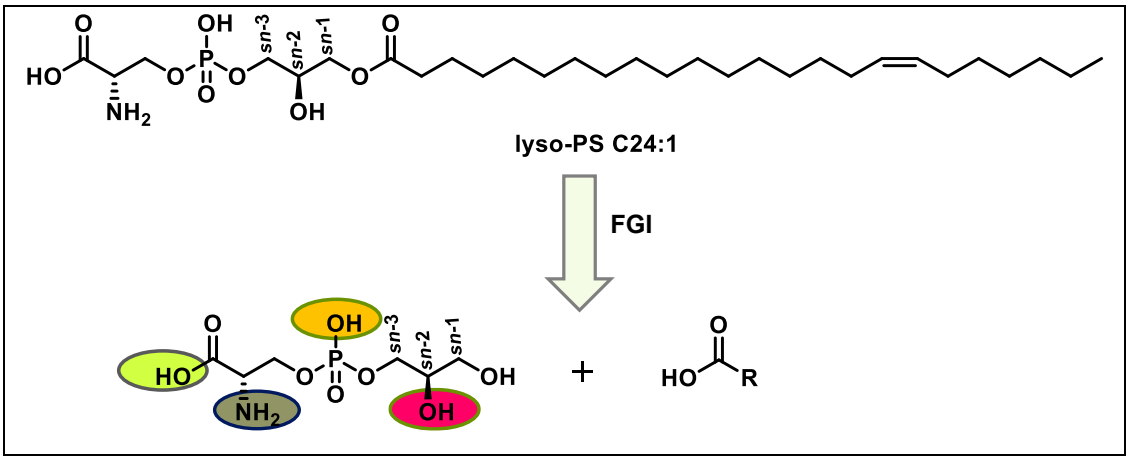



**Figure 3.2.** Retrosynthesis analysis for the synthesis of lyso-PS C24:1 lipid

In the above lyso-PS C24:1 lipid after the functional group disconnection, we would get two parts as lipids. One is glycerophosphoserine (GPS) and another is a free fatty acid (FFA). Although fatty acids are commercially available, the synthesis of the GPS head group can be quite challenging, and only a few chemical synthetic methods have been developed for the large-scale preparation of lyso-PS lipids[48–51]. Various difficulties that are faced during the synthesis of enantiomerically pure lyso-PSs are described below:

1) Lipophilicity of final compound

2) Combination of the leading hydrophilic and hydrophobic molecules

3) Choice of protecting group is crucial since these groups may cross-react and complicate the synthesis.

4) The major challenge in the lyso-PS synthesis construction of chiral synthesis for this need retention in the configuration of two chiral centers towards making the correct diastereomer.

5) To get the retention in configuration for this to require chiral synthon as a starting material.

6) Acyl group migration is another problem in selective synthesis of lyso-PS regioisomers which can intermolecular transfer of one fatty acid moiety from one hydroxyl group to the adjacent one.

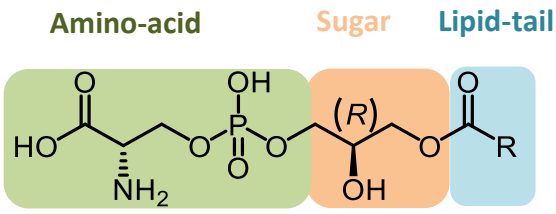
7) Since lipids are non-UV active and hence are difficult to visualize. Therefore, they must to either be monitored by different stains or by NMR spectroscopy

To tackle all these challenges we prefer the synthesis of MAG lipid library first followed by lyso-PS library synthesis.

## 3.3 Result and Discussions

### 3.3.1. Synthesis of Lyso-PSs Lipids

As a proof of concept, we decided to synthesize a library of the naturally occurring (*R*)-Me-lyso-PSs and unnatural (*S*)-Me-lyso-PSs with various saturated fatty acids. There are very few lyso-PSs are commercially available, and those too, are esterified only with long-chain (LC) fatty acids (figure 3.3).

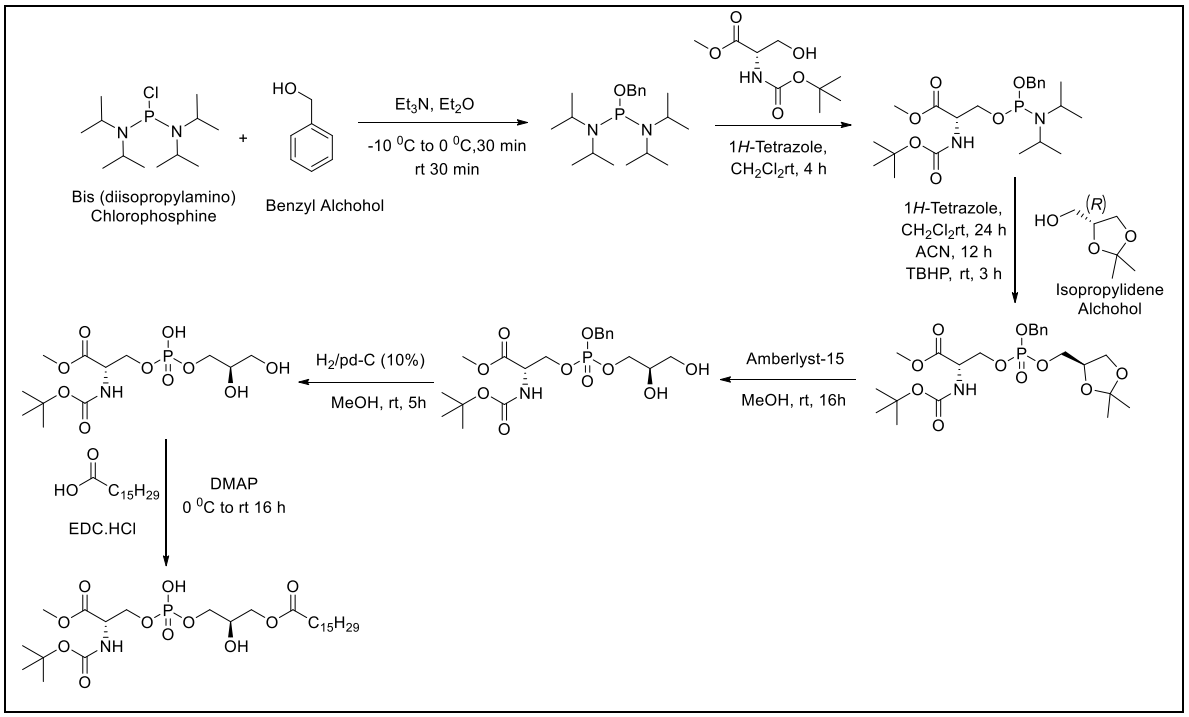



| -(COOR) | Fatty Acid |
|---|---|
| C16:0 | Palmetic Acid |
| C18:0 | Stearic Acid |
| C18:1 | Oleic Acid |

**Figure 3.3.** Commercial available long-chain lyso-PS lipid species

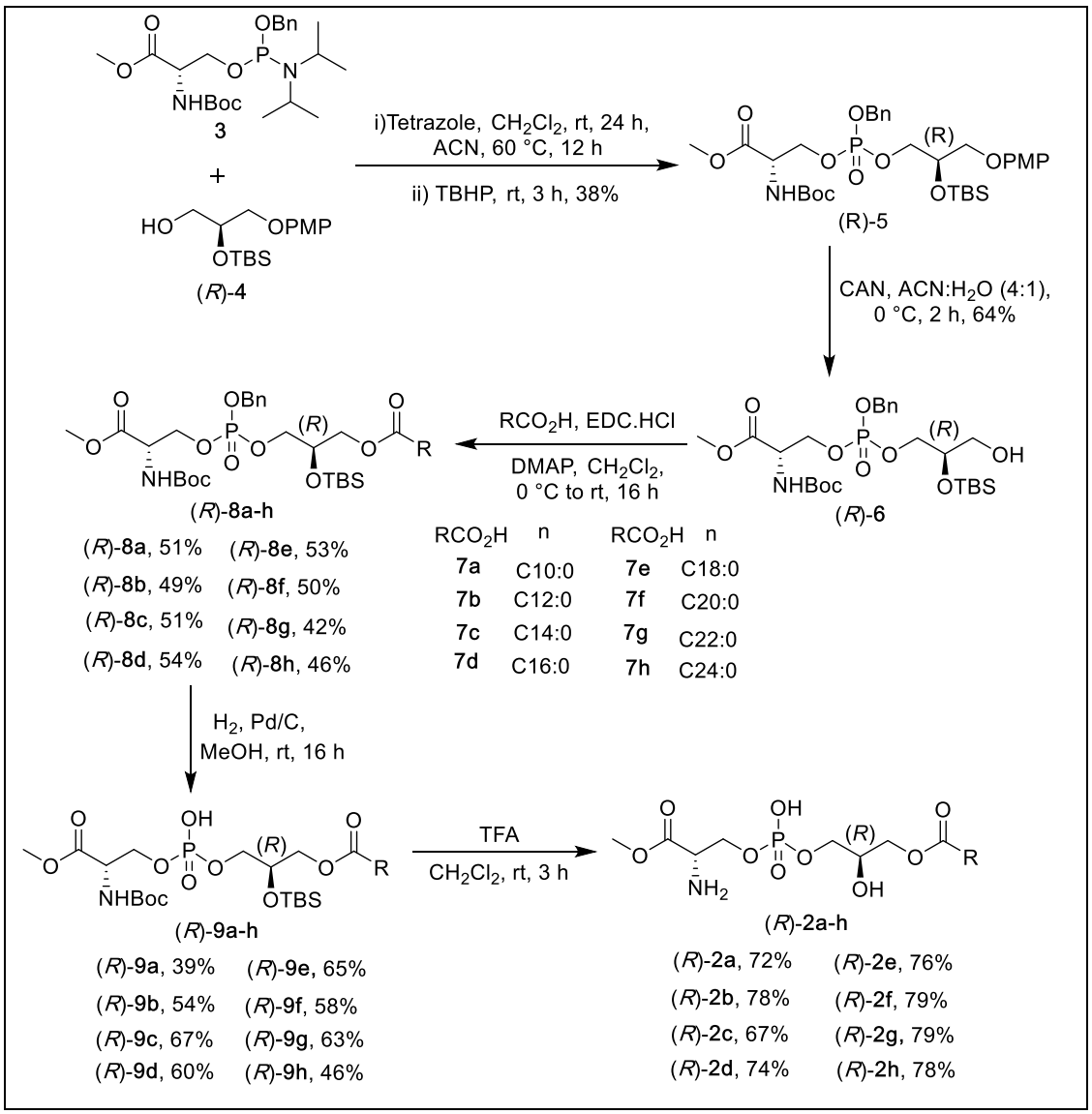
### 3.3.2 Limitation of Lyso-PS Synthesis Library

Initially, we had designed the following scheme 3.1. Unfortunately, after the coupling step, we were not able to purify the esterified compound since this compound was unstable on silica and the compounds were extremely polar and thus could not be purified.

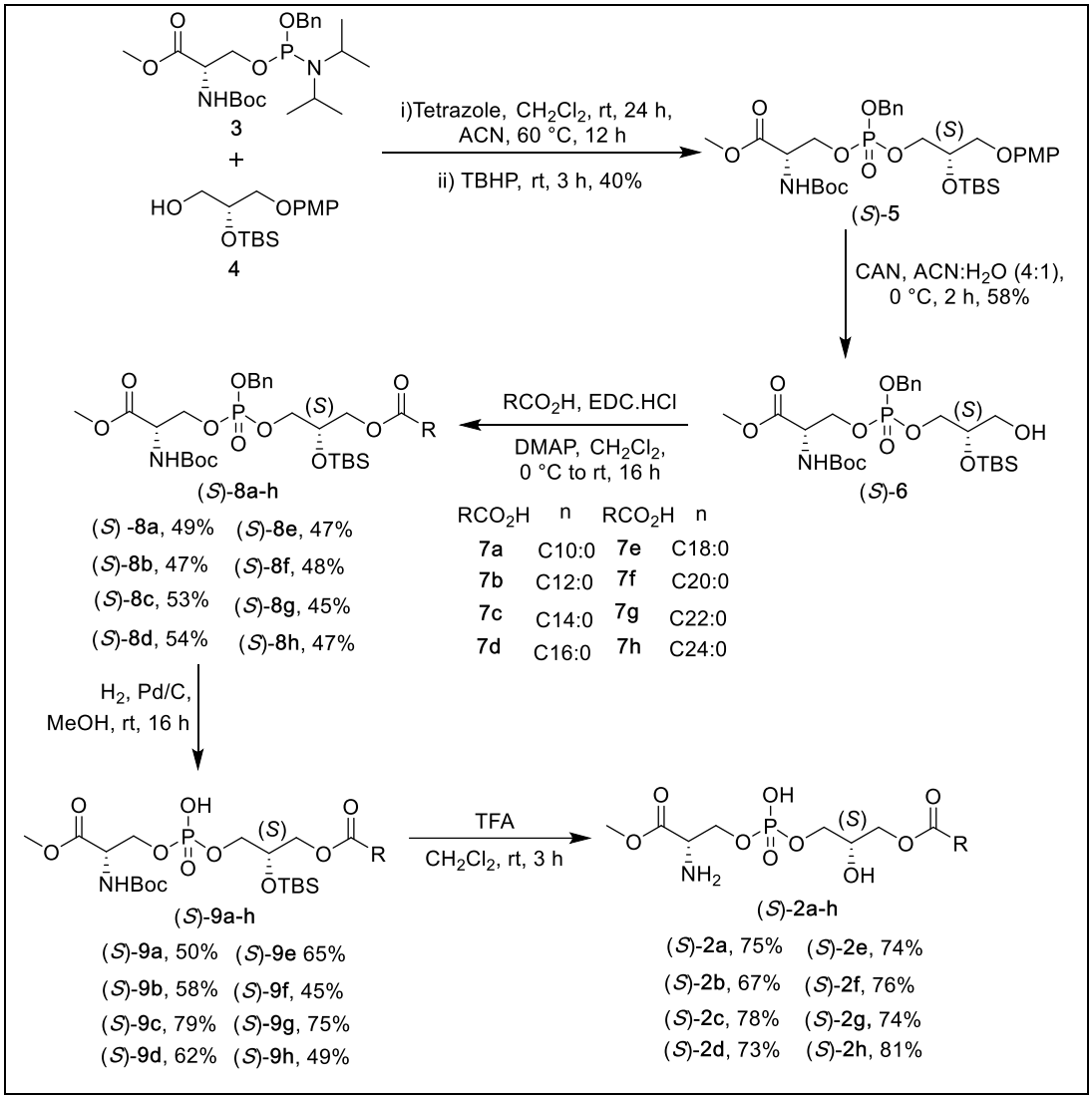



**Scheme 3.1.** Synthetic route of saturated (*R*) Me-lyso-PSs lipid library

After going through literature and using numerous approaches, we came up with the idea of protecting the secondary alcohol and phosphate group and can purify the compound at this stage. Therefore, we moved to the facile synthetic route and synthesized protected alcohol intermediate first and followed by coupling with phosphoramide moiety. In the following route, we could purify the esterified compound by column chromatography. Eventually, we were able to synthesize a library of the naturally occurring (*R*)-Me-lyso-PSs and unnatural (*S*)-Me-lyso-PSs with various saturated fatty acids.



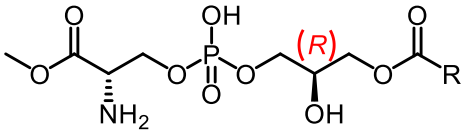

**Scheme 3.2.** Synthetic route of saturated (*R*) Me-lyso-PSs lipid library



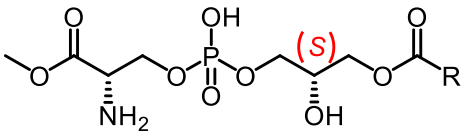

**Scheme 3.3.** Synthetic route of saturated (*S*) Me-lyso-PSs lipid library

## Table 3.1

## (*R*) and (*S*) Methyl ester-lysophosphatidylserine (Me-lyso-PS)



*(R)* - Natural



*(S)* - Unnatural

| ID | -(COOR) | ID | -(COOR) |
|---|---|---|---|
| (*R*)-2a | C10:0 | (*S*)-2a | C10:0 |
| (*R*)-2b | C12:0 | (*S*)-2b | C12:0 |
| (*R*)-2c | C14:0 | (*S*)-2c | C14:0 |
| (*R*)-2d | C16:0 | (*S*)-2d | C16:0 |
| (*R*)-2e | C18:0 | (*S*)-2e | C18:0 |
| (*R*)-2f | C20:0 | (*S*)-2f | C20:0 |
| (*R*)-2g | C22:0 | (*S*)-2g | C22:0 |
| (*R*)-2h | C24:0 | (*S*)-2h | C24:0 |

## 3.4. Substrate Profiling Study against Recombinant (hABHD12) and Endogenous Mouse Brain (mABHD12) Lysate

All substrate profile study was performed by Theja, a project student in our lab. Having synthesized a library of the (*R*)- and (*S*)-Me-lyso-PSs (Table 3.1), we first tested whether these lipids were substrates for ABHD12, the mammalian lyso-PS lipase. The kinetic assay was carried out at 8 different concentrations (0-400 µm) of Me-lyso-PS. Here, we used different (*R*)-Me-lyso-PS as a substrates i.e. C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0 and C24:0. The enzymatic rate for each reaction mixture with a particular substrate concentration was corrected by subtracting the rate of mock from wild-type rate and plotting the data to fit the Michaelis–Menten kinetics equation (figure 3.4). To further understand the endogenous preference of ABHD12 for (*R*)-Me-lyso-PS lipid substrates. We tested with ABHD12 knockout mice which displayed an accumulation of lyso-PS in the brain due to the absence of ABHD12. This assay was used as a control experiment to understand the ABHD12 specific lyso-PS lipase activity. Here wild-type and ABHD12 knockout mice brain membrane lysates were incubated with the library of (*R*)-Me-lyso-PS of varying chain lengths (figure 3.4)[33].

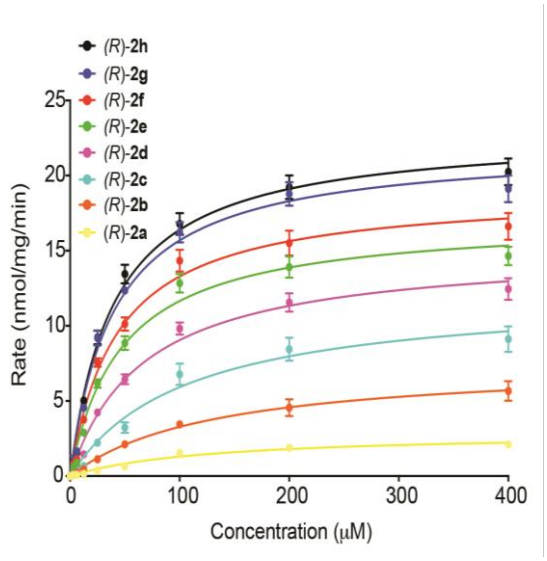
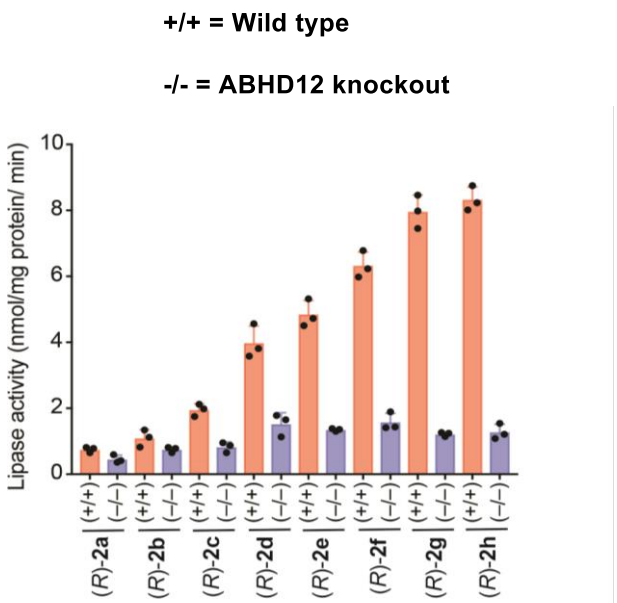
| Substrate assay with recombinant human ABHD12 (hABHD12) | Substrate assay with endogenous mouse brain ABHD12 (mABHD12) lysate |
|---|---|
|  |  |



**Figure 3.4.** Enzyme kinetic studies with recombinant hABHD12 and endogenous mouse brain (mABHD12) lysate with substrate Me-lyso-PS synthesized with different chain length R stands for natural substrate a,b,c,d,e,f,g,h for fatty acid chain lengths i.e. 2a - C10:0, 2b - C12:0, 2c - C14:0, 2d -C16:0, 2e - C18:0, 2f - C20:0, 2g - C22:0, 2h - C24:0.

## Table 3.2

## Kinetics for the *(R)*-Me-Lyso-PS Lipid Substrates tested in *vitro* against Recombinant Human hABHD12

| | $K_m$ (μM) | $V_{max}$ (nmoles/min) |
|---|---|---|
| ***(R)* Me-lyso-PS C10:0** | **121 ± 21** | **2.39 ± 0.02** |
| ***(R)* Me-lyso-PS C12:0** | **130 ± 16** | **7.65 ± 0.40** |
| ***(R)* Me-lyso-PS C14:0** | **105 ± 7** | **12.31 ± 0.77** |
| ***(R)* Me-lyso-PS C16:0** | **67 ± 9** | **15.21 ± 0.69** |
| ***(R)* Me-lyso-PS C18:0** | **46 ± 6** | **17.24 ± 0.69** |
| ***(R)* Me-lyso-PS C20:0** | **42 ± 5** | **18.94 ± 0.73** |
| ***(R)* Me-lyso-PS C22:0** | **39 ± 4** | **21.89 ± 0.70** |
| ***(R)* Me-lyso-PS C24:0** | **39 ± 4** | **22.85 ± 0.73** |

Based on kinetic constants for the saturated fatty acid (*R*)-Me-lyso PS lipids, we have found

$$V_{max} = C24:0 \approx C22:0 > C18:0 > C16:0 > C14:0 > C12:0 > C10:0$$

$$K_m = C24:0 < C22:0 < C18:0 < C16:0 < C14:0 < C12:0 < C10:0$$

### 3.5. VLC lyso-PSs elicit pro-inflammatory response through TLR2-dependant pathway

Lyso-PS lipids consist of a library of chemically divergent molecules whose structure consists of a fatty acid chain whose length ranges from medium to very long along with glycerophosphoserine head group and glycerol backbone. This complexity in lyso-PS structure has caused its commercial paucity to study its biological role in detail. Hence, in this study, we have chemically synthesized lyso-PS with medium, long and very-long-chain saturated lyso-PSs lipids to investigate their role in (neuro) immunological processes. In this chapter, our research unveils the distinct role of structurally variant lyso-PSs in immune system function

and its underlying molecular mechanism involving immune-specific cellular receptors. All biological study were performed by Neha, a postdoctoral fellow in our lab. The study reveals that lyso-PS exerts its signalling properties towards the activation of immune cells, such as macrophages and mast cells, by releasing inflammatory cytokines (TNF-α and IL-6) measurements and histamine respectively. Very-long-chain lyso-PS which was previously found to be associated with the pathology of a neurodegenerative disorder PHARC (Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa, and Cataract) signals through TLR2 (Toll-like Receptor 2) receptors and causes neuroinflammation and microgliosis. We proved this by using genetically engineered mice with TLR2 gene deletion where the inflammatory effect were absent upon Lyso-PS treatment. We also measured cytokine secretion at TNF-α and IL-6 from WT Primary peritoneal macrophages and interestingly found that VLC lyso-PS (C22:0) and (C24:0) produced highest cytokine secretion. LPS was used as a positive control here and it was already shown that LPS cytokine secrete through TLR4 dependant pathway (figure 3.5).

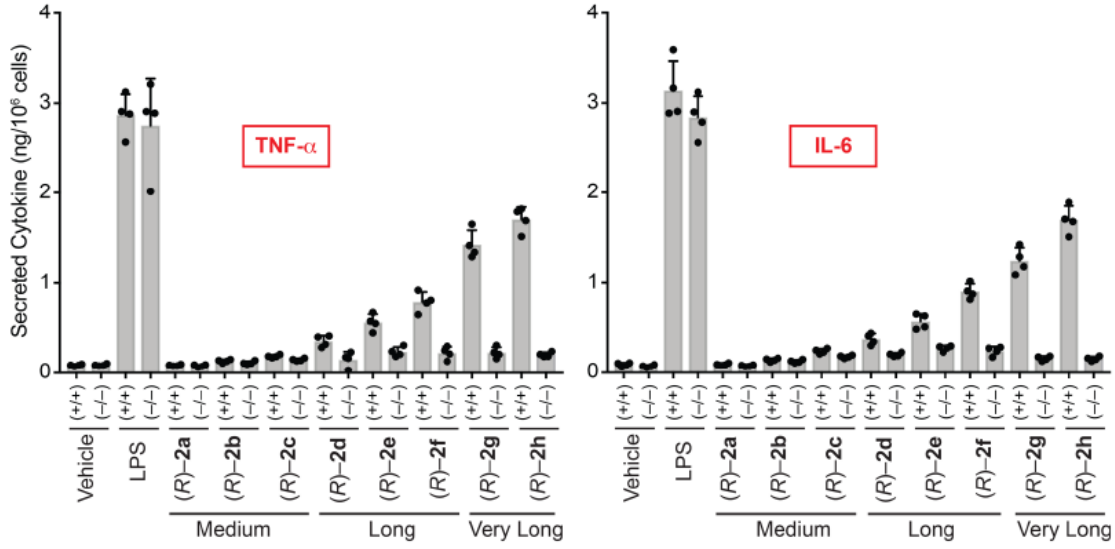
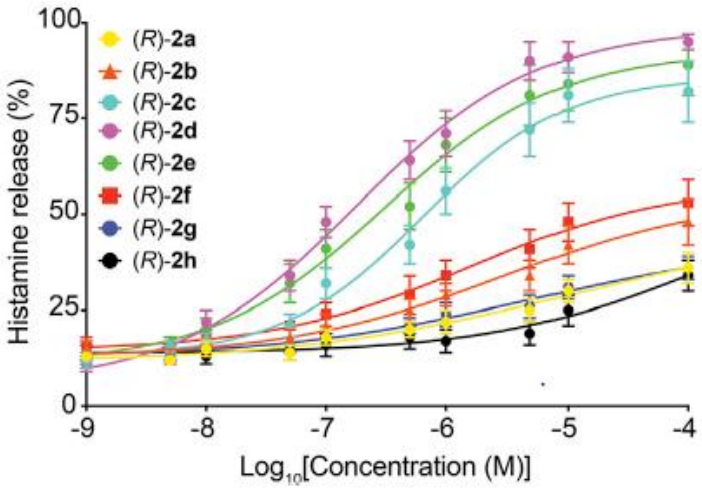



**Figure 3.5.** Very long chain Lyso-PS act show increased cytokine secretion as compared to other lyso-PS via TLR2 dependant pathway

Our further analysis also revealed the contribution of long-chain lyso-PS causing mast cell degranulation and histamine release, which is a signal for secondary immune response (figure 3.6). Interestingly, we observed that this function was mediated by an as-of-yet unknown, GPCR (G-protein Coupled Receptors, a class of cell surface receptors), and not TLR2. This indicates a distinct and immune-specific role of long and very-long-chain lyso-PS. In the mice

brain, lyso-PS is majorly regulated by a lyso-PS lipase enzyme called ABHD12, whose mutation causes PHARC syndrome




| Species | $EC_{50}$ (nM) |
|---|---|
| (*R*)-**2a** (10:0) | 11210 ± 4068 |
| (*R*)-**2b** (12:0) | 2942 ± 471 |
| (*R*)-**2c** (14:0) | 645 ± 75 |
| (*R*)-**2d** (16:0) | 179 ± 20 |
| (*R*)-**2e** (18:0) | 311 ± 37 |
| (*R*)-**2f** (20:0) | 1560 ± 221 |
| (*R*)-**2g** (22:0) | 4924 ± 1075 |
| (*R*)-**2h** (24:0) | 130500 ± 57282 |

**Figure 3.6.** LC lyso-PSs robustly cause histamine release from primary mast cells

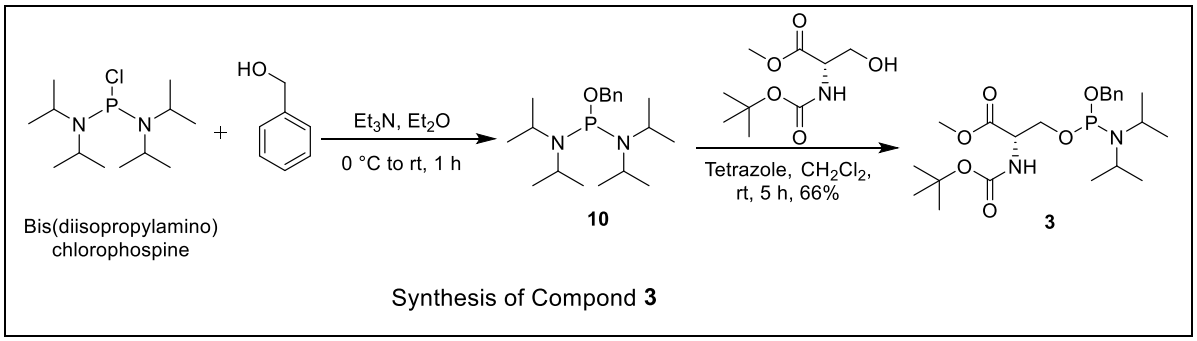
## 3.6. Conclusion

In this chapter, I have shown the synthesis of (*R*)- and (*S*)-Me-lyso-PSs lipid library and we found that hABHD12 strongly prefers VLC (*R*)-Me-lyso-PSs as substrates. Furthermore, endogenous ABHD12 of mouse brain membrane lysates indeed displayed the best catalytic activity for the (*R*)-Me-lyso-PS and we found that mABHD12 also prefers VLC (*R*)-Me-lyso-PSs. We assayed against (*S*)-Me-lyso-PS and discovered that these were very poor substrates against recombinant and endogenous mouse brain membrane lysates ABHD12 enzyme as well. In our cellular carboxyl esterase metabolize study we observed that synthesized Me-lyso-PSs got metabolized by cellular carboxylesterase to gives the corresponds to lyso-PSs. In nutshell, the synthetic Me-lyso-PSs serve as a stable prodrug-like biological surrogate to lyso-PSs. In addition, another study reveals that lyso-PS use as signaling molecules towards the activation of immune cells such as macrophages and mast cell degranulation by releasing inflammatory cytokine and histamine respectively. Interestingly, we find that VLC (*R*)-Me-lyso-PSs produce the highest secretion of a pro-inflammatory cytokine through the TLR2 receptor. In simple words, VLC lyso-PS induce neuroinflammation raises to fascinate possibility of human degenerative PHARC disease. However, in the further biological study, we found that long-chain lyso-PS cause mast cell degranulation and histamine release suggesting that another cryptic receptor likely GPCR prefers LC lyso-PSs[33].

## 3.7. EXPERIMENTAL SECTION

### 3.7.1 Synthesis and Characterization Data

### A) Synthesis of Compound 3



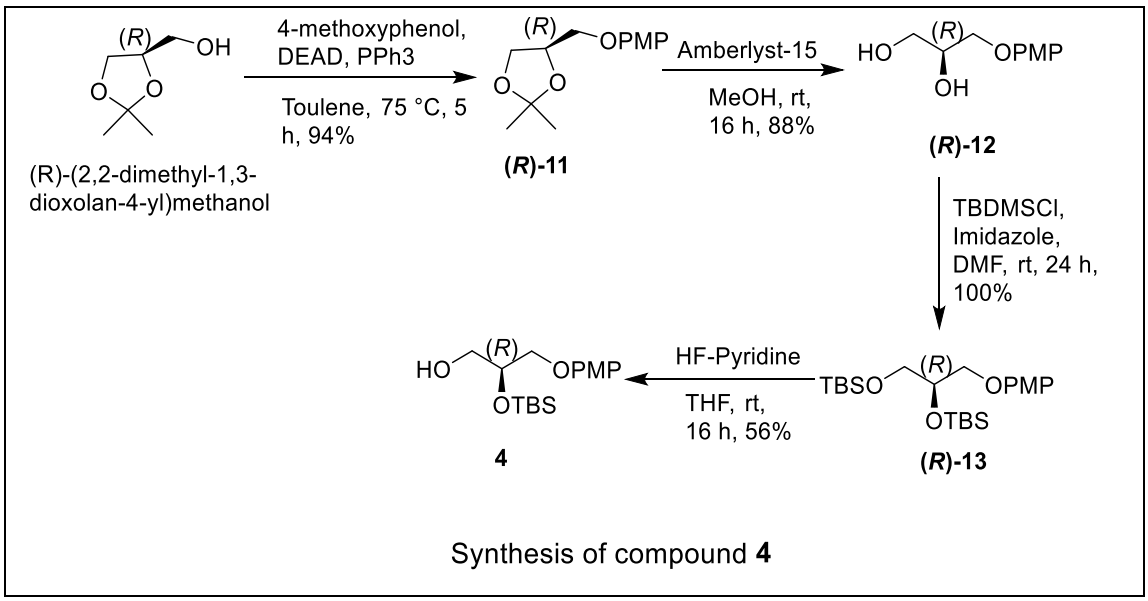

Synthesis of Compond **3**

**1-(benzyloxy)-*N,N,N′,N′*-tetraisopropylphosphanediamine (10)**: To a solution of bis(diisopropylamino) chlorophosphine (3.0 g, 11.2 mmol) in dry diethyl ether ($Et_2O$) (30 mL) in schlenk flask, a mixture of benzyl alcohol (1.0 mL, 10.0 mmol) and triethylamine ($Et_3N$) (1.4 mL, 10.0 mmol) in $Et_2O$ (5 mL) was added at 0 °C under nitrogen ($N_2$) atmosphere. The reaction mixture was stirred for 30 min at 0 °C, then warmed to room temperature for 30 min. The reaction mixture was diluted with cold hexane (15 mL), stirred for 10 min. The hexane solution was then transferred into another schlenk flask by cannula and concentrated under a nitrogen atmosphere to yield compound **10**. The crude product was used as such for next step without purification: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.31-7.22 (m, 5H), 4.58 (d, $J$ = 7.3 Hz, 2H), 3.53-3.46 (m, 4H), 1.13-1.10 (m, 24H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ 124.2082.

**Methyl *O*-((benzyloxy)(diisopropylamino)phosphaneyl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (3)**: The 1-(benzyloxy)-*N,N,N′,N′*-tetraisopropylphosphanediamine i.e. compound **10** (3.2 g, 94.5 mmol) was dissolved in anhydrous $CH_2Cl_2$ (25 mL) in schlenk flask and a solution of 1*H*-Tetrazole (0.8 mL, 85.9 mmol) in ~0.45 M ACN was added at room temperature. To this solution, *N*-Boc-L-Serine-methyl ester (1.8 g, 85.9 mmol) was added under a nitrogen atmosphere, in a few minutes, white solid was precipitated. The mixture was stirred for 5 h at room temperature and then the reaction was quenched with saturated $NaHCO_3$. The product was extracted in $CH_2Cl_2$ (3 x 50 mL) and then the combined organic layer was dried over the anhydrous $Na_2SO_4$, filtrated and concerted in vacuo. The residue was purified by neutral alumina column chromatography (EtOAc/Hexane 10:90) to yield the compound **3** (2.8 g, 61.3 mmol, 66%, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.38-7.25 (m, 5H), 5.55-5.37 (m,

1H), 4.77-4.60 (m, 2H), 4.45-4.43 (m, 1H), 4.13-4.02 (m, 1H), 3.91-3.79 (m, 1H), 3.73-3.71 (m, 3H), 3.67-3.54 (m, 2H), 1.45-1.43 (m, 9H), 1.22-1.13 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 171, 155.6, 155.5, 139.4, 139.3, 128.4, 128.4, 127.5, 127.4, 127.1, 127.0, 80.0, 79.9, 65.6, 65.6, 65.5, 66.4, 54.9, 54.8, 52.5, 52.4, 43.3, 43.1, 28.4, 24.7, 24.6, 24.5; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.4 (d, $J_{c\text{-}p}$ = 4 Hz, CH), 127.4 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 127.1 (d, $J_{c\text{-}p}$ =12 Hz, CH), 65.5 (dd, $J_{c\text{-}p}$ = 18, 8 Hz, $CH_2$), 64.2 (2d, $J_{c\text{-}p}$ = 16 Hz, $CH_2$), 54.9 (t, $J_{c\text{-}p}$ = 8 Hz, CH), 52.4 (2s, $CH_3$), 43.2 (d, $J_{c\text{-}p}$ = 12 Hz, CH), 28.4 ($CH_3$), 24.7 ($CH_3$), 24.6 ($CH_3$), 24.5 ($CH_3$); HRMS-ESI: [(M + H)$^+$-BOC] calcd for $C_{17}H_{30}N_2O_4P$, 357.1938; found, 357.1939.

**B) Synthesis of Compound 4**




Synthesis of compound **4**

**(*R*)-4-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolane ((*R*)-11)**: To synthesized compound **4** we followed previously been reported procedure[11]. The commercially available (*R*)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (5.0 g, 37.83 mmol) was dissolved in anhydrous toluene, followed by triphenylphosphine (11.91 g, 45.40 mmol), and *p*-methoxyphenol (14.09 g, 113.49 mmol) were added under a $N_2$ atmosphere. To the solution, DEAD (8.57 g, 49.18 mmol) in toluene (24.6 mL) was added dropwise, and the reaction mixture was stirred at 75 °C for 5 h. After completion the reaction, the mixture was evaporated, and the residue was purified by silica column chromatography using 10% EtOAc in *n*-hexane as an eluent to provide the desired product **(*R*)-11** (8.5 g, 35.6 mmol, 94% yield, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.87-6.81 (m, 4H), 4.45 (quintet, *J* = 5.9 Hz, 1H), 4.15 (dd, *J* = 8.4, 6.4 Hz, 1H), 4.02 (dd, *J* = 9.5, 5.4 Hz, 1H), 3.91-3.87 (m, 2H), 3.76 (s,

3H), 1.46 (s, 3H), 1.40 (s, 3H); HRMS-ESI: $[M + H]^+$ calcd for $C_{13}H_{18}O_4$, 239.1283; found, 239.1281.

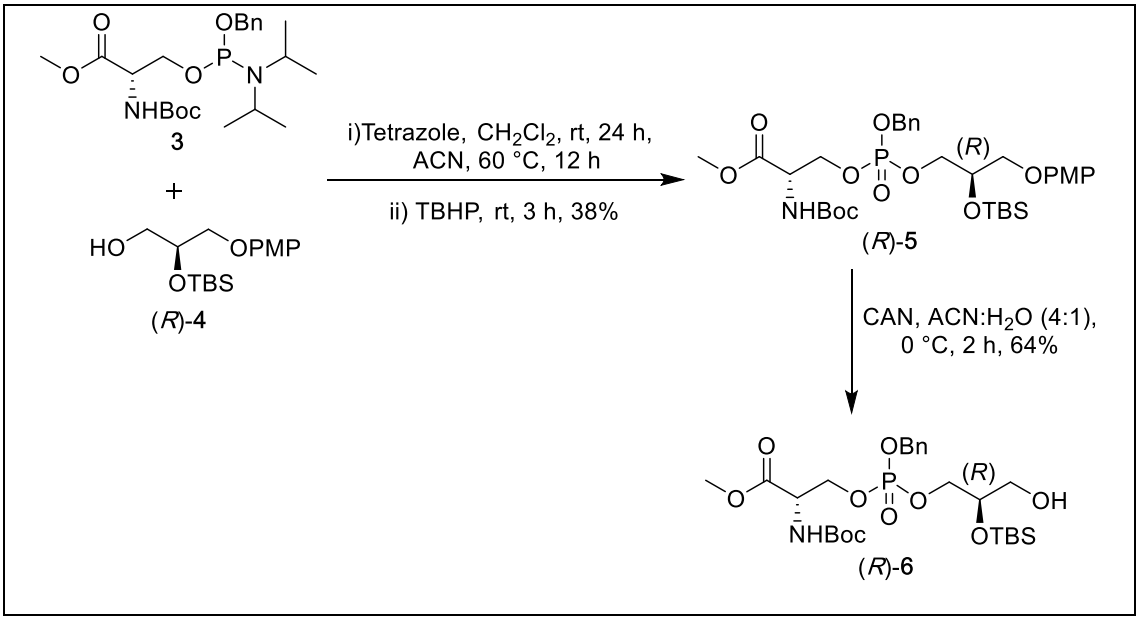
**3-(4-methoxyphenoxy)propane-1,2-diol ((*R*)-12)**: The compound **(*R*)-11** (8.5 g, 35.6 mmol) was dissolved in anhydrous MeOH (37 mL), and Amberlyst-15 (11.2 g, 35.6 mmol) was added at room temperature. The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, amberlyst-15 was filtered off and the solvent of the filtrate was evaporated under reduced pressure. The residue was purified by silica column chromatography using 50% EtOAc in *n*-hexane as an eluent to give the desired product **(*R*)-12** (6.2 g, 31.3 mmol, 88% yield, white solid). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.85-6.80 (m, 4H), 4.07 (sext, $J$ = 4.8 Hz, 1H), 4.00-3.94 (m, 2H), 3.84-3.79 (m, 1H), 3.76 (s, 3H), 3.74-3.69 (m, 1H), 3.08 (m, 1H), 2.62 (m, 1H) ; HRMS-ESI: $[M + H]^+$ calcd for $C_{10}H_{15}O_4$, 199.0965; found, 199.0967.

**(*R*)-5-((4-methoxyphenoxy)methyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane ((*R*)-13)**: The compound **(*R*)-12** (6.2 g, 31.3 mmol) was dissolved in anhydrous DMF (50 mL) followed by imidazole (6.4 g, 94 mmol) and TBSCl (11.8 g, 78.2 mmol) was sequentially added at room temperature. The mixture was stirred for 16 h at room temperature and then the mixture was diluted with water (30 mL) and $Et_2O$ (30 mL), and the aqueous layer was separated and extracted three times with $Et_2O$ (15 mL x 3). The combined organic layer was dried over $Na_2SO_4$ and the solvent was evaporated. The residue was purified by silica column chromatography using 5% $Et_2O$ in *n*-hexane as an eluent to obtain the desired product **(*R*)-13** (13.3 g, 31.1 mmol, 100% yield, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.87-6.82 (m, 4H), 4.07-4.02 (m, 2H), 3.86-3.81 (m, 1H), 3.78 (s, 3H), 3.66 (d, $J$ = 5.5 Hz, 2H), 0.92 (s, 18H), 0.12 (d, $J$ = 4.4 Hz, 6 H), 0.09 (broad, 6H); HRMS-ESI: $[M + H]^+$ calcd for $C_{22}H_{43}O_4Si_2$, 427.2694; found 427.2693.

**(*S*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propan-1-ol ((*R*)-4)**: The (*R*)-5-((4-methoxyphenoxy)methyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (13.3 g, 31.1 mmol) was dissolved in dry THF (70 mL). The HF-pyridine complex (70%w/w) (5.3 mL) and pyridine (25 mL) was sequentially added at room temperature under a $N_2$ atmosphere. The reaction mixture was stirred for 4 h at room temperature and then diluted with water (50 mL). The desired product was extracted three times with EtOAc (50 mL x 3), and washed with brine, dried over $Na_2SO_4$ and evaporated. The residue was purified by silica column chromatography using 60% Ethyl acetate in *n*-hexane as an eluent to provide the desired product **(*R*)-4** (5.2 g, 16.6 mmol, 56% yield, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.83 (s, 4H), 4.11-4.07 (m, 1H), 3.95-3.86 (m, 2H), 3.77 (s, 3H), 3.75-3.65 (m, 2H), 2.00 (broad, 1H), 0.92 (s, 9H),

0.14 (s, 3H), 0.13 (s, 3H); HRMS-ESI: $[M + H]^+$ calcd for $C_{16}H_{29}O_4Si$, 313.1830; found, 313.1837.

**C) Synthesis of Compound (*R*)-5 & (*R*)-6**




**Methyl *O*-((benzyloxy)((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate ((*R*)-5)**:

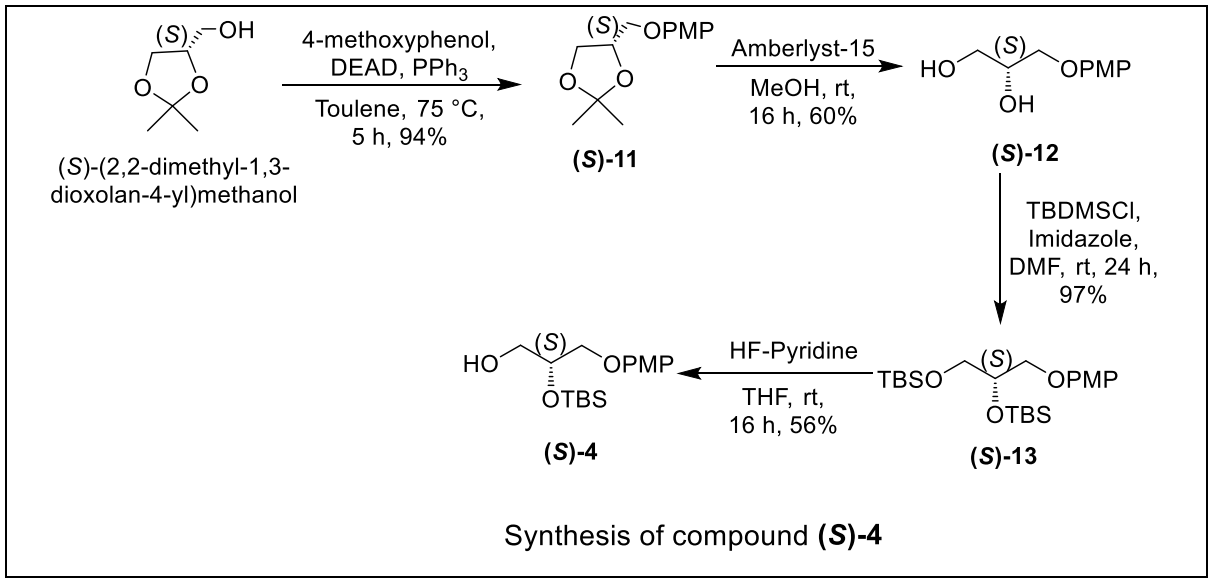
To synthesized compound (R)-**5** and (R)-**6** we followed previously been reported procedure[52]. The Phosphonamidite **3** (2.2 g, 4.82 mmol) was dissolved in anhydrous $CH_2Cl_2$ (2.5 ml), and then the solution was co-evaporated with ACN three times (3 x 2.5 mL). The residue was dissolved in anhydrous $CH_2Cl_2$ (25 mL), and the solution of 1*H*-tetrazole in ACN (~0.45 M) (1.1 mL, 1.2 mmol) was added at room temperature. The solution of alcohol **(*R*)-4** (3.7 g, 11.8 mmol) in $CH_2Cl_2$ (5 mL) was added dropwise under $N_2$ atmosphere, and then the mixture stirred at room temperature for 24 h. The anhydrous ACN (30 mL) was added and then the reaction mixture was heated to 60 °C for 12 h. The intermediate formation was confirmed by TLC then *t*-butyl hydroperoxide (TBHP) solution in decane (5.0-6.0 M) (1.4 mL, 14.46 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with 15 mL water and extracted with $CH_2Cl_2$ (3 X 25 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by flash column chromatography using MeOH/$H_2O$ (85:15) as an eluent to afford the compound **(*R*)-5** (1.25 g,

1.83 mmol, 38%, colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.40-7.29 (m, 5H), 6.85-6.77 (m, 4H), 5.58-5.51(m, 1H), 5.09-5.02 (m, 2H), 4.54-4.37 (m, 2H), 4.29-4.22 (m, 1H), 4.18-4.09 (m, 2H), 4.06-3.99 (m, 1H), 3.92-3.80 (m, 2H), 3.74 (s, 3H), 3.73-3.70 (m, 3H), 1.44 (s, 9H), 0.89 (s, 9H), 0.13-0.08 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -0.41; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.8, 155.4, 154.2, 152.8, 135.7 (d, $J_{c\text{-}p}$ = 7Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 4Hz), 115.6 (2s), 114.8, 80.5, 70.0 (d, $J_{c\text{-}p}$ = 7 Hz), 69.8 (t, $J_{c\text{-}p}$ = 6Hz), 69.6 (d, $J_{c\text{-}p}$ = 6 Hz), 69.0 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (broad), 55.9, 54.1 (d, $J_{c\text{-}p}$ = 8Hz), 52.9, 28.4, 25.9, 18.3, -4.5, -4.6; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.7 (CH), 128.6 (CH), 127.9 (d, $J_{c\text{-}p}$ = 4 Hz, CH), 115.3 (2s, CH), 114.6 (CH), 69.8 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 69.6 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.4 (d, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 68.8 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.5 (broad, $CH_2$), 55.7 ($CH_3$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 28.2 ($CH_3$), 25.7 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); HRMS-ESI: [(M + H)$^+$-BOC] calcd. for $C_{27}H_{43}NO_9PSi$, 584.2439; found, 584.2441.

**Methyl O-((benzyloxy)((R)-2-((tert-butyldimethylsilyl)oxy)-3-hydroxypropoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate ((*R*)-6)**:

To the solution of PMP-protected alcohol **(*R*)-5** (1.2 g, 1.76 mmol) in ACN: $H_2O$ (4:1) (10 mL), the Ceric Ammonium Nitrate (CAN) (2.41 g, 4.4 mmol) was added dropwise at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred for 2 h at 0 °C and then diluted with $H_2O$ (5 mL). The whole was extracted three times with EtOAc (3 x 20 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by column chromatography using EtOAc/Hexane (60:20) as an eluent to afford the desired product **(*R*)-6** (0.648 g, 1.12 mmol, 64%, brown oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.36-7.29 (m, 5H), 5.64-5.45 (m, 1H), 5.06-5.00 (m, *2*H), 4.50-4.32 (m, 2H), 4.26-4.16 (m, 1H), 4.03-3.89 (m, 2H), 3.86-3.79 (m, 1H), 3.70 (2s, 3H), 3.59-3.47 (m, 2H), 2.60 (broad, 1H), 1.41 (s, 9H), 0.85 (2s, 9H), 0.05 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -0.70, -0.85; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.8, 155.3 (2s), 135.5 (d, $J_{c\text{-}p}$ = 6Hz), 128.8, 128.7, 128.1, 80.4, 71.2 (d, $J_{c\text{-}p}$ = 8Hz), 69.8 (2d, $J_{c\text{-}p}$ = 5Hz), 67.9 (t, $J_{c\text{-}p}$ = 5Hz), 67.7 (2d, $J_{c\text{-}p}$ = 5Hz), 62.9, 54.0 (d, $J_{c\text{-}p}$ = 7Hz), 52.8 (2s), 28.3, 25.8, 18.1, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (CH), 71.1 (d, $J_{c\text{-}p}$ = 7Hz, CH), 69.7 (2d, $J_{c\text{-}p}$ = 6Hz, $CH_2$), 67.9 (t, $J_{c\text{-}p}$ = 5Hz, $CH_2$), 67.6 (2d, $J_{c\text{-}p}$ = 6Hz, $CH_2$), 62.9 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 8Hz, CH), 52.7 (2s, $CH_3$), 28.3 ($CH_3$), 25.7 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); HRMS-ESI: [(M + H)$^+$-BOC] calcd. for $C_{20}H_{37}NO_8PSi$, 478.2021; found, 478.2023.

**D) Synthesis of Compound (*S*)-4**




**(*S*)-4-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolane ((*S*)-11)**:

To synthesized compound **(*S*)-4** we followed the previously been reported procedure[52]. The commercially available (*S*)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (5.0 g, 37.83 mmol) was dissolved in anhydrous toluene followed by triphenylphosphine (11.91 g, 45.40 mmol), and *p*-methoxyphenol (14.09 g, 113.49 mmol) were added under an $N_2$ atmosphere. To the solution, DEAD (8.57 g, 49.18 mmol) in toluene (24.6 mL) was added dropwise, and the reaction mixture was stirred at 75 °C for 5 h. After completion of the reaction, the mixture was evaporated, and the residue was purified by silica column chromatography using 10% EtOAc in *n*-hexane as an eluent to provide the desired product **(*S*)-11** (8.5 g, 35.6 mmol, 94% yield, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.86-6.80 (m, 4H), 4.45 (quint, $J$ = 5.9 Hz, 1H), 4.15 (dd, $J$ = 8.4, 6.4 Hz, 1H), 4.01 (dd, $J$ = 9.4, 5.5 Hz, 1H), 3.90-3.86 (m, 2H), 3.75 (s, 3H), 1.46 (s, 3H), 1.40 (s, 3H); HRMS-ESI: $[M]^+$ calcd for $C_{13}H_{18}O_4$, 238.1205; found 238.1204.

**(*S*)-3-(4-methoxyphenoxy)propane-1,2-diol ((*S*)-12)**:

The compound **(*S*)-11** (8.4 g, 35.3 mmol) was dissolved in anhydrous MeOH (36 mL), and Amberlyst-15 (11.1 g, 35.3 mmol) was added at room temperature. The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, amberlyst-15 was filtered off and the solvent of the filtrate was evaporated under reduced pressure. The residue was purified by silica column chromatography using 90% EtOAc in *n*-hexane as an eluent to give the desired product **(*S*)-12** (4.19 g, 21.2 mmol, 60% yield, white solid). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.87-6.82 (m, 4H), 4.09 (sext, $J$ = 4.9 Hz, 1H), 4.03-3.97 (m, 2H), 3.86-3.81

(m, 1H), 3.77 (s, 3H), 3.76-3.72 (m, 1H), 2.65 (m, 1H), 2.08 (m, 1H); HRMS-ESI: $[M]^+$ calcd for $C_{10}H_{14}O_4$, 198.0892; found, 198.0892.

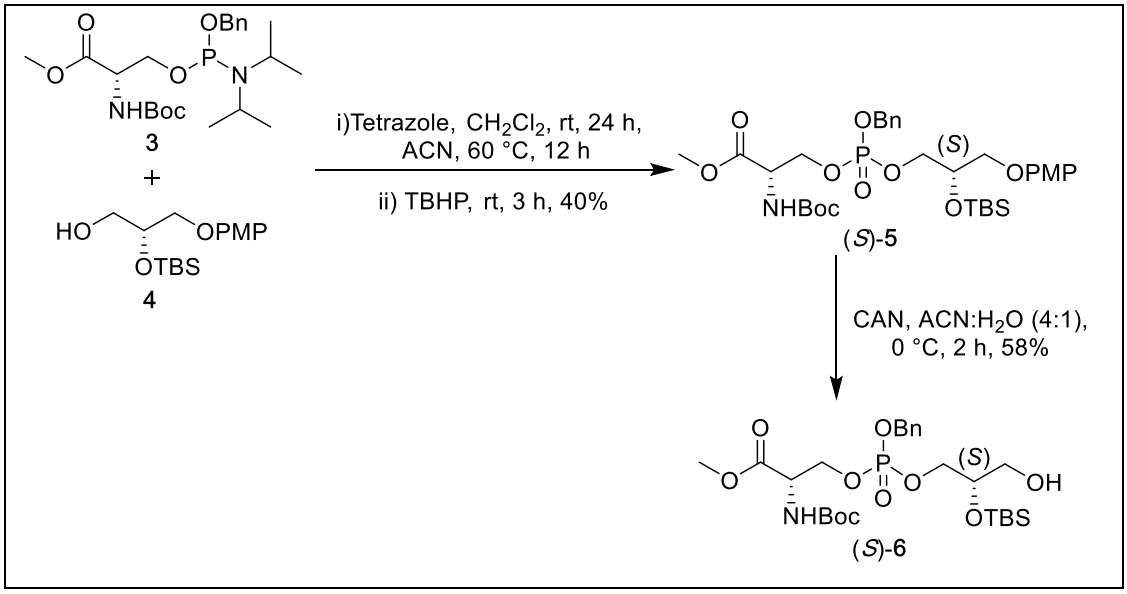
**(*S*)-5-((4-methoxyphenoxy)methyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane ((*S*)-13)**:

The compound **(*S*)-12** (4.1 g, 20.7 mmol) was dissolved in anhydrous DMF (33 mL) followed by imidazole (4.23 g, 62.1 mmol) and TBSCl (7.8 g, 51.8 mmol) was sequentially added at room temperature. The mixture was stirred for 16 h at room temperature and then the mixture was diluted with water (25 mL) and $Et_2O$ (25 mL), and the aqueous layer was separated and extracted three times with $Et_2O$ (15 mL x 3). The combined organic layer was dried over $Na_2SO_4$ and the solvent was evaporated. The residue was purified by silica column chromatography using 5% $Et_2O$ in *n*-hexane as an eluent to obtain the desired product **(*S*)-13** (8.55 g, 20.1 mmol, 97% yield, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.87-6.81 (m, 4H), 4.06-4.02 (m, 2H), 3.84-3.80 (m, 1H), 3.77 (s, 3H), 3.65 (d, $J$ = 5.5 Hz, 2H), 0.91 (s, 18H), 0.12-0.11 (m, 6H), 0.08 (s, 6H); HRMS-ESI: [M + H]+ calcd for $C_{22}H_{43}O_4Si_2$, 427.2694; found 427.2692.

**(*S*)-2-((tert-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propan-1-ol ((*S*)-4)**:

The (*S*)-5-((4-methoxyphenoxy)methyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (8.5 g, 19.9 mmol) was dissolved in dry THF (45 mL). The HF-pyridine complex (70%w/w) (3.5 mL) and pyridine (16 mL) was sequentially added at room temperature under a $N_2$ atmosphere. The reaction mixture was stirred for 4 h at room temperature and then diluted with water (30 mL). The desired product was extracted three times with EtOAc (30 mL x 3), and washed with brine, dried over $Na_2SO_4$ and evaporated. The residue was purified by silica column chromatography using 60% EtOAc in *n*-hexane as an eluent to provide the desired product **(*S*)-4** (3.32 g, 10.7 mmol, 56% yield, colourless oil, recovered starting compound (*S*)-13, (1.1 g, 2.6 mmol, 13%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.83 (s, 4H), 4.12-4.07 (m, 1H), 3.95-3.86 (m, 2H), 3.76 (s, 3H), 3.75-3.65 (m, 2H), 1.98 (broad, 1H), 0.91 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H); HRMS-ESI: $[M + H]^+$ calcd for $C_{16}H_{29}O_4Si$, 313.1830; found, 313.1832.

**E) Synthesis of Compound (*S*)-5 & (*S*)-6**




**Methyl *O*-((benzyloxy)((*S*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate ((*S*)-5)**:

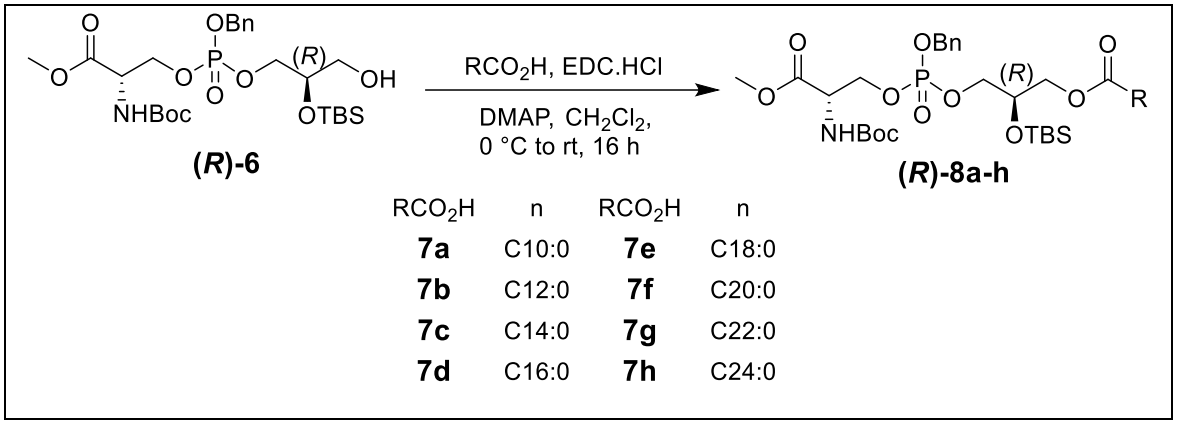
To synthesized compound **(*S*)-5** and **(*S*)-6** we followed previously been reported procedure[52] .The phosphoramidite **3** (1.0 g, 2.19 mmol) was dissolved in anhydrous $CH_2Cl_2$ (1.5 ml), and then the solution was co-evaporated with ACN three times (3 x 1.5 mL). The residue was dissolved in anhydrous $CH_2Cl_2$ (12 mL), and the solution of 1*H*-tetrazole in ACN (~0.45 M) (0.5 mL, 0.55 mmol) was added at room temperature. The solution of alcohol **4** (1.68 g, 5.36 mmol) in $CH_2Cl_2$ (2.3 mL) was added dropwise under $N_2$ atmosphere, and then the mixture stirred at room temperature for 24 h. The anhydrous ACN (14 mL) was added and then the reaction mixture was heated to 60 °C for 12 h. The intermediate formation was confirmed by TLC then *tert*-butyl hydroperoxide (TBHP) solution in decane (5.0-6.0 M) (0.64 mL, 6.57 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with 7.5 mL water and extracted with $CH_2Cl_2$ (3 X 15 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography using MeOH/$H_2O$ (95:5) as an eluent to afford the compound **(*S*)-5** (0.6 g, 1.46 mmol, 40%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.39-7.29 (m, 5H), 6.84-6.76 (m, 4H), 5.52-5.42 (m, 1H), 5.09-5.01 (m, 2H), 4.52-4.56 (m, 2H), 4.27-4.20 (m, 1H), 4.17-4.07 (m, 2H), 4.04-3.97 (m, 1H), 3.90-3.78 (m, 2H), 3.75 (s, 3H), 3.72-3.70 (m, 3H), 1.43 (s, 9H), 0.88 (2s, 9H), 0.11-0.07 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -0.40. $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.7, 155.3, 154.1, 152.7, 135.6 (d, $J_{c\text{-}p}$ = 6Hz), 128.8, 128.7, 128.1 (d, $J_{c\text{-}p}$ =

3Hz), 115.4, 114.7, 80.4, 69.9 (d, $J_{c\text{-}p}$ = 8 Hz), 69.7 (t, $J_{c\text{-}p}$ = 6Hz), 69.5 (broad), 68.9 (d, $J_{c\text{-}p}$ = 6Hz), 67.6 (broad), 55.8, 54.0 (d, $J_{c\text{-}p}$ = 7Hz), 52.8, 28.4, 25.8, 18.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 115.4 (CH), 114.7 (CH), 69.9 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.5 (broad, $CH_2$), 68.9 (d, $J_{c\text{-}p}$ = 6 Hz, $CH_2$), 67.6 (broad, $CH_2$), 55.8 ($CH_3$), 54.0 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.8 ($CH_3$), 28.4 ($CH_3$), 25.8 ($CH_3$), -4.7 ($CH_3$), -4.8 ($CH_3$); HRMS-ESI: [(M + H)$^+$-BOC] calcd for $C_{27}H_{43}NO_9PSi$, 584.2445; found, 584.2443.

**Methyl *O*-((benzyloxy)((*S*)-2-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate ((*S*)-6)**:

To the solution of PMP protected alcohol **(*S*)-5** (0.45 g, 0.658 mmol) in ACN: $H_2O$ (4:1) (3.8 mL), the Ceric Ammonium Nitrate (CAN) (0.9 g, 1.65 mmol) was added dropwise at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred for 1 h at 0 °C and then diluted with $H_2O$ (1.9 mL). The whole was extracted three times with EtOAc (3 x 20 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (100-200 mesh silica gel) using EtOAc/Hexane (60:20) as an eluent to afford the desired product **(*S*)-6** (0.220 g, 0.381 mmol, 58%, brown oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.32 (m, 5H), 5.70-5.46 (m, 1H), 5.12-5.02 (m, 2H), 4.53-4.34 (m, 2H), 4.30-4.19 (m, 1H), 4.07-3.90 (m, 2H), 3.88-3.81 (m, 1H), 3.74 (2s, 3H), 3.63-3.52 (m, 2H), 2.31 (broad, 1H), 1.44 (s, 9H), 0.88 (2s, 9H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -0.54, -0.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.9, 155.4, 135.6 (2d, $J_{c\text{-}p}$ = 6 Hz), 128.9 (d, $J_{c\text{-}p}$ = 2 Hz), 128.8 (d, $J_{c\text{-}p}$ = 1Hz), 128.2 (d, $J_{c\text{-}p}$ = 5 Hz), 80.5, 71.1 (2d, $J_{c\text{-}p}$ = 5 Hz), 69.9 (2d, $J_{c\text{-}p}$ = 4 Hz), 67.8 (2d, $J_{c\text{-}p}$ = 6 Hz), 67.5 (2d, $J_{c\text{-}p}$ = 6Hz), 63.0, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9 (2s), 28.4, 25.8, 18.2, -4.6, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (d, $J_{c\text{-}p}$ = 2Hz, CH), 128.7 (broad, CH), 128.1 (d, $J_{c\text{-}p}$ = 5 Hz, CH), 71.0 (2d, $J_{c\text{-}p}$ = 5 Hz, CH), 69.8 (2d, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 67.7 (2d, $J_{c\text{-}p}$ = 6Hz, $CH_2$), 67.4 (2d, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 62.9 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.8 (2s, $CH_3$), 28.3 ($CH_3$), 25.7 ($CH_3$), -4.7 ($CH_3$), -4.9 ($CH_3$); HRMS-ESI: [(M + H)$^+$-BOC] calcd. for $C_{20}H_{37}NO_8PSi$, 478.2021; found, 478.2023.

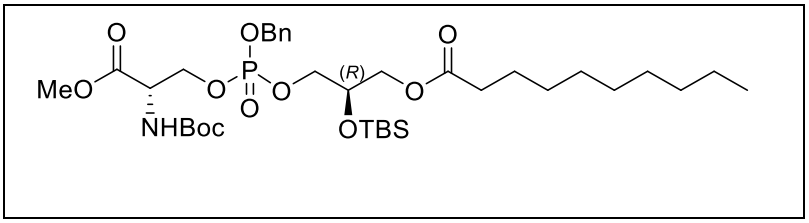
**General procedure (A1) for the synthesis of compounds (*R*)-8a-h**:




| $RCO_2H$ | n | $RCO_2H$ | n |
|---|---|---|---|
| **7a** | C10:0 | **7e** | C18:0 |
| **7b** | C12:0 | **7f** | C20:0 |
| **7c** | C14:0 | **7g** | C22:0 |
| **7d** | C16:0 | **7h** | C24:0 |

To synthesized compound **(*R*)-8** we followed previously been reported procedure[52–54]. To a solution of alcohol **(*R*)-6** (1.0 equiv) and fatty acid **7** (0.9 equiv) in anhydrous $CH_2Cl_2$, the 4-Dimethylaminopyridine (DMAP 0.25 equiv) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 0.9 equiv) were sequentially added at 0 °C. After stirring the mixture 16 h at room temperature, the reaction was quenched with saturated solution of $NaHCO_3$ and extracted three times with $CH_2Cl_2$. The combined organic layer was dried over $Na_2SO_4$, filtrated, and concentrated under reduced pressure at 25 °C. The residue was purified by column chromatography (100-200 silica gel mesh) using 25-30% Ethyl Acetate in hexane as an eluent to afford the corresponding desired product **(*R*)-8.**

**(*R*)-8a**

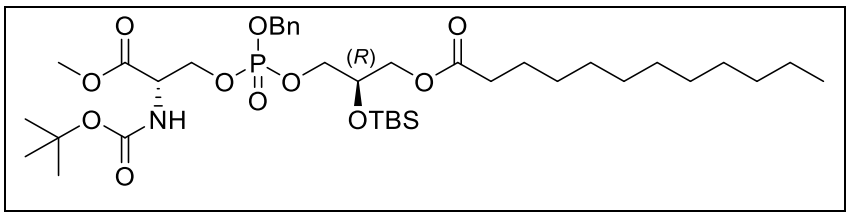



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl decanoate**

Following the general procedure (**A1**), **(*R*)-6** (80 mg, 0.138 mmol), **7a** (Decanoic acid, C10:0) (21 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0346 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane,30:70) to yield **(*R*)-8a** (52 mg, 0.0710 mmol, 51%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.31 (m, 5H), 5.43 (m, 1H), 5.06 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.54-4.34 (m, 2H), 4.29-4.20 (m, 1H), 4.15-4.06 (m, 1H), 4.04-3.86 (m, 4H),

3.74 (2s, 3H), 2.29 (td, $J$ = 7.6, 2.2 Hz, 2H), 1.61 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.36-1.21 (m, 12H), 0.92-0.82 (m, 12H), 0.08 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.08; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.5, 169.8, 155.3, 135.8 (d, $J_{c\text{-}p}$ = 6.0 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.5, 69.9 (2d, $J_{c\text{-}p}$ = 5 Hz), 69.3 (d, $J_{c\text{-}p}$ = 8 Hz), 68.6 (t, $J_{c\text{-}p}$ = 6Hz), 67.7 (m), 64.9, 54.2 (m), 52.8, 34.3, 32.0, 29.5, 29.4, 29.3, 28.5, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.7 (CH), 128.6 (CH), 127.9 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (2d, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.5 (m, $CH_2$), 64.7 ($CH_2$), 54.1 (m, CH), 52.6 ($CH_3$), 34.1 ($CH_2$), 31.8 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 29.1 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.8 ($CH_2$), 22.6 ($CH_2$), 14.0 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{35}H_{62}NO_{11}PSi$ $[M+K]^+$: calcd., 770.34; found, 770.35; $[M+Na]^+$: calcd., 754.37; found, 754.38.

**(*R*)-8b**

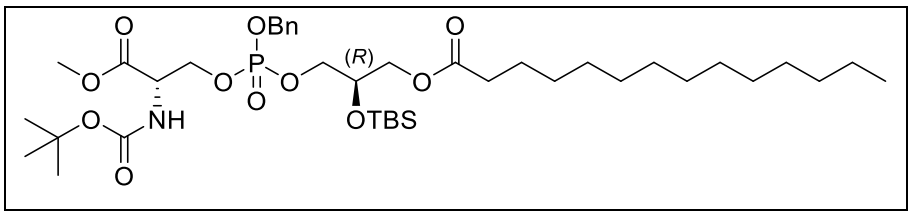



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl dodecanoate**

Following the general procedure (**A1**), **(*R*)-6** (70 mg, 0.121 mmol), **7b** (Dodecanoic acid, C12:0) (22 mg, 0.108 mmol), EDC·HCl (20 mg, 0.109 mmol), DMAP (3 mg, 0.0302 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-8b** (45 mg, 0.0592 mmol, 49%, colourless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.31 (m, 5H), 5.47 (dd, $J$ = 14.4, 8.4 Hz, 1H), 5.05 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.35 (m, 2H), 4.27-4.18 (m, 1H), 4.13-4.04 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.2 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 16H), 0.90-0.82 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 136.0 (d, $J_{c\text{-}p}$ = 7 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5Hz), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (t, $J_{c\text{-}p}$ = 6 Hz), 67.7 (m), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 8 Hz), 52.9, 34.2, 32.0, 29.7, 29.6, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (t, $J_{c\text{-}}$

$_p$ = 6 Hz, $CH_2$), 67.6 (m, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{37}H_{66}NO_{11}PSi$ $[M+K]^+$ : calcd., 798.38, found., 798.35, $[M+Na]^+$ : calcd., 782.40; found, 782.38.

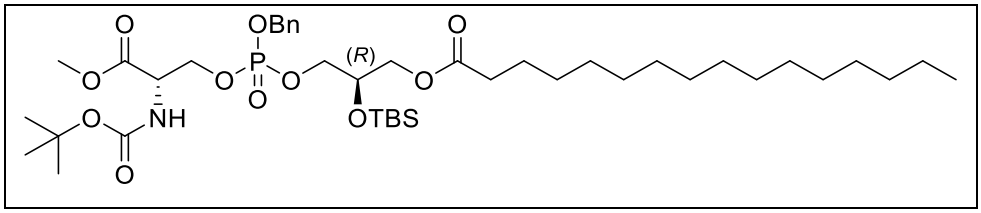
**(*R*)-8c**




**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate**

Following the general procedure (**A1**), **(*R*)-6** (80 mg, 0.138 mmol), **7c** (Myristic acid, C14:0) (28 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0345 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-8c** (56 mg, 0.0711 mmol, 51%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (dd, $J$ = 14.2, 8.4 Hz, 1H), 5.05 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.36 (m, 2H), 4.28-419 (m, 1H), 4.13-4.04 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.2 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 20H), 0.90-0.83 (m, 12H), 0.07 (s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.08; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 7 Hz), 128.9, 128.8, 128.3 (d, $J_{c\text{-}p}$ = 2 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (m, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.5 ($CH_2$), 22.3 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{39}H_{70}NO_{11}PSi$ $[M+K]^+$: calcd., 826.41; found, 826.44; $[M+Na]^+$: calcd., 810.43; found, 810.44.

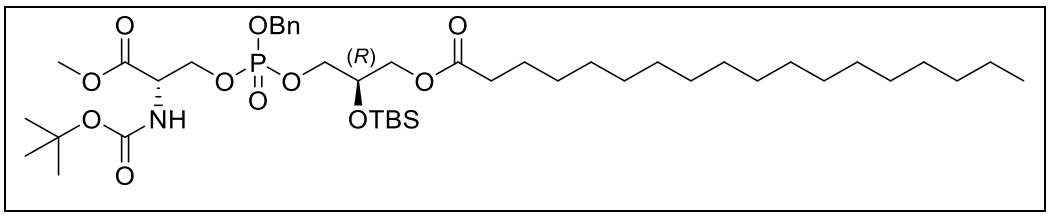
**(*R*)-8d**



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate**

Following the general procedure (**A1**), **(*R*)-6** (80 mg, 0.138 mmol), **7d** (Palmitic acid, C16:0) (32 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0345 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to afford the desired product **(*R*)-8d** (61 mg, 0.0747 mmol, 54%, colourless oil). $^1$H NMR (400 MHz, CDCl3) $\delta$ 7.41-7.31 (m, 5H), 5.47 (dd, $J$ = 14.5, 8.4 Hz, 1H), 5.05 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.36 (m, 2H), 4.27-4.19 (m, 1H), 4.13-4.04 (m, 1H), 4.02-3.84 (m ,4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.6 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.34-1.20 (m, 24H), 0.89-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.4, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 3 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{41}H_{74}NO_{11}PSi$ $[M+K]^+$: calcd., 854.44; found, 854.44; $[M+Na]^+$: calcd., 838.46; found, 838.47.

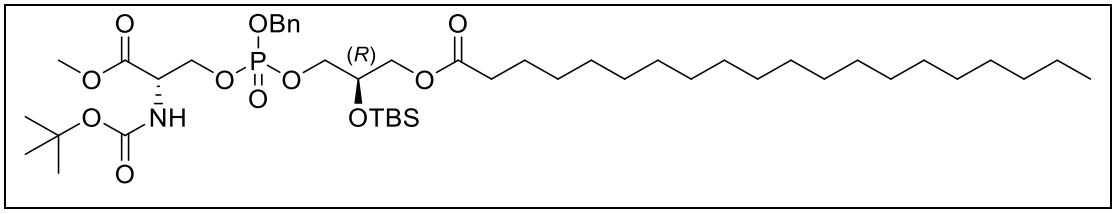
**(*R*)-8e**



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate**

Following the general procedure (**A1**), **(*R*)-6** (70 mg, 0.121 mmol), **7e** (Stearic acid, C18:0) (31 mg, 0.109 mmol), EDC·HCl (20 mg, 0.109 mmol), DMAP (3 mg, 0.0209 mmol) and $CH_2Cl_2$ (4 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane 30:70) to provide **(*R*)-8e** (54 mg, 0.0640 mmol, 50%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.31 (m, 5H), 5.47 (dd, $J$ = 14.2, 8.5 Hz, 1H), 5.06 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.36 (m, 2H), 4.27-4.19 (m, 1H), 4.13-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.4 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 28H), 0.89-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.08; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.3, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{43}H_{78}NO_{11}PSi$ $[M+K]^+$: calcd., 882.47; found, 882.47; $[M+Na]^+$: calcd., 866.49; found, 866.50.

**(*R*)-8f**

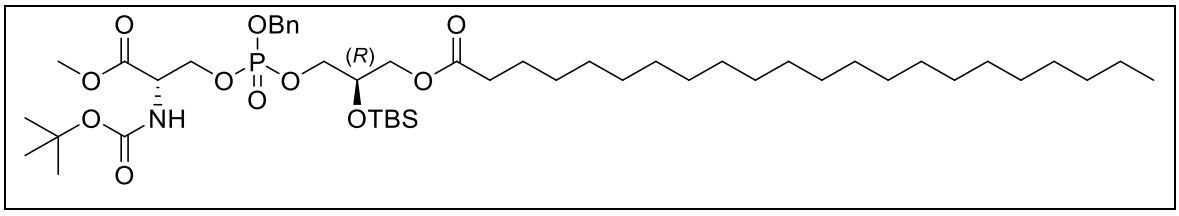



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate**

Following the general procedure (**A1**), **(*R*)-6** (90 mg, 0.155 mmol), **7f** (Arachidic acid, C20:0) (43 mg, 0.140 mmol), EDC·HCl (27 mg, 0.140 mmol), DMAP (5 mg, 0.0388 mmol) and $CH_2Cl_2$ (5.5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 25:75) to afford **(*R*)-8f** (67 mg, 0.0768 mmol, 50%,

colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (dd, $J$ = 14.4, 8.4 Hz, 1H), 5.05 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.54-4.35 (m, 2H), 4.27-4.19 (m, 1H), 4.13-4.04 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.4 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 32H), 0.90-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 7 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7Hz), 67.7 (t, $J_{c\text{-}p}$ = 5 Hz), 64.8, 54.1 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.3, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{45}H_{82}NO_{11}PSi$ $[M+K]^+$: calcd., 910.50; found, 910.49; $[M+Na]^+$: calcd., 894.52; found, 894.52.

**(*R*)-8g**

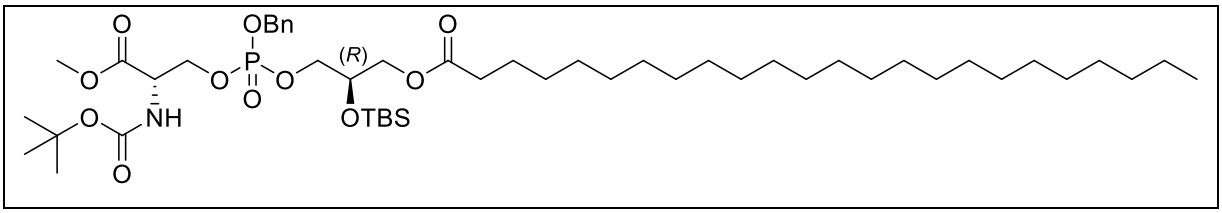



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docosanoate**

Following the general procedure (**A1**), **(*R*)-6** (80 mg, 0.139 mmol), **7g** (Behenic acid, C22:0) (38 mg, 0.125 mmol), EDC·HCl (24 mg, 0.125 mmol), DMAP (4 mg, 0.0348 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 25:75) to yield **(*R*)-8g** (53 mg, 0.0589 mmol, 42%, colourless oil. $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.42-7.31 (m, 5H), 5.47 (dd, $J$ = 14.0, 8.5 Hz, 1H), 5.06 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.36 (m, 2H), 4.28-4.19 (m, 1H), 4.14-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.5 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.21 (m, 36H), 0.91-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.08; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.4, 135.6 (d, $J_{c\text{-}p}$ = 6 Hz), 128.9, 128.8, 128.1 (d, $J$ = 2 Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.2 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (t, $J_{c\text{-}p}$ =

7 Hz), 67.8 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.3, 32.1, 29.8, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.3, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.8 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{47}H_{86}NO_{11}PSi$ $[M+K]^+$: calcd., 938.53; found, 938.54; $[M+Na]^+$: calcd., 922.56; found, 922.57.

**(*R*)-8h**

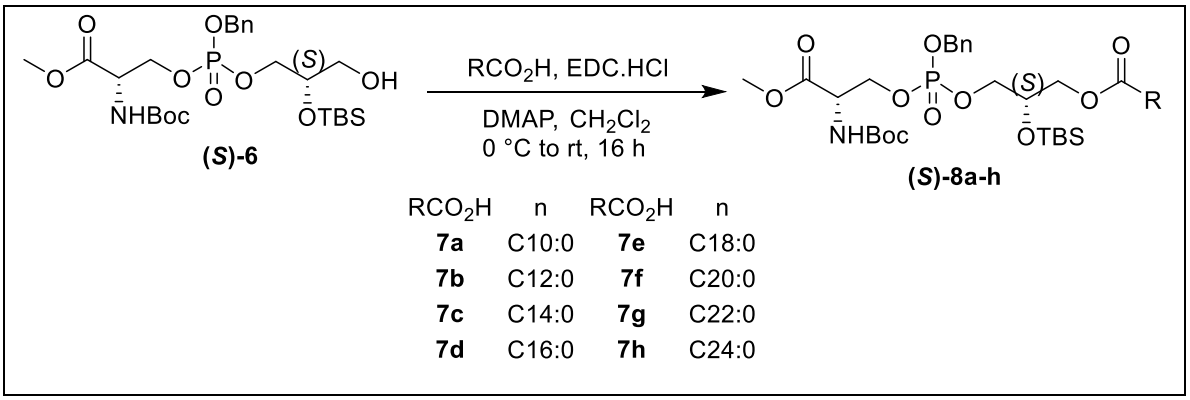



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetracosanoate**

Following the general procedure (**A1**), **(*R*)-6** (80 mg, 0.139 mmol), **7h** (Lignoceric acid, C24:0) (46 mg, 0.125 mmol), EDC·HCl (24 mg, 0.125 mmol), DMAP (4 mg, 0.0348 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 25:75) to yield **(*R*)-8h** (59 mg, 0.0589 mmol, 46%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (dd, $J$ = 14.6, 8.4 Hz, 1H), 5.06 (dd, $J$ = 8.7, 3.2 Hz, 2H), 4.53-4.34 (m, 2H), 4.27-4.19 (m, 1H), 4.14-4.04 (m, 1H), 4.03-3.84 (m, 4H), 3.73 (2s, 3H), 2.29 (td, $J$ = 7.5, 2.4 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 40H), 0.89-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 6 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.1 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.01 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (t, $J_{c\text{-}p}$ = 7 Hz, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.5 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -

4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{49}H_{90}NO_{11}PSi$ $[M+K]^+$: calcd., 966.57; found, 966.58; $[M+Na]^+$: calcd., 950.59; found, 950.61.

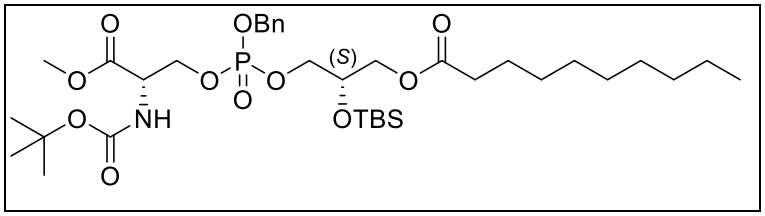
## General procedure (A2) for the synthesis of compounds (*S*)-8a-h:




| $RCO_2H$ | n | $RCO_2H$ | n |
|---|---|---|---|
| **7a** | C10:0 | **7e** | C18:0 |
| **7b** | C12:0 | **7f** | C20:0 |
| **7c** | C14:0 | **7g** | C22:0 |
| **7d** | C16:0 | **7h** | C24:0 |

To synthesized compound **(*S*)-8** we followed previously been reported procedure[52–54]. To a solution of alcohol **(*S*)-6** (1.0 equiv) and fatty acid **7** (0.9 equiv) in anhydrous $CH_2Cl_2$, the 4-Dimethylaminopyridine (DMAP 0.25 equiv) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 0.9 equiv) were sequentially added at 0 °C. After stirring the mixture 16 h at room temperature, the reaction was quenched with saturated solution of $NaHCO_3$ and extracted three times with $CH_2Cl_2$. The combined organic layer was dried over $Na_2SO_4$, filtrated and concentrated under reduced pressure at 25 °C. The residue was purified by column chromatography (100-200 silica gel mesh) using 25-30% Ethyl Acetate in hexane as an eluent to afford the corresponding desired product **(*S*)-8.**

**(*S*)-8a**

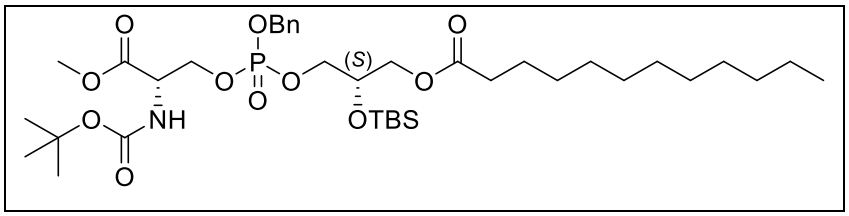



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl decanoate**

Following the general procedure (**A2**), **(*S*)-6** (85 mg, 0.147 mmol), **7a** (Decanoic acid, C10:0) (21 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0346 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-

200 silica gel mesh) (EtOAc/Hexane,30:70) to yield **(*S*)-8a** (56 mg, 0.0683 mmol, 49%, colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.46 (t, $J$ = 9.7 Hz, 1H), 5.05 (t, $J$ = 8.1 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-4.19 (m, 1H), 4.14-4.05 (m, 1H), 4.03-3.85 (m, 4H), 3.74 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (t, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.32-1.20 (m, 12H), 0.89-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.05, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 6.0 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4Hz), 64.8, 54.1 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.6 (CH), 128.1 (d, $J_{c\text{-}p}$ = 1 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.1 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{35}H_{62}NO_{11}PSi$ $[M+K]^+$: calcd., 770.34; found, 770.35; $[M+Na]^+$: calcd., 754.37; found, 754.37.

**(*S*)-8b**

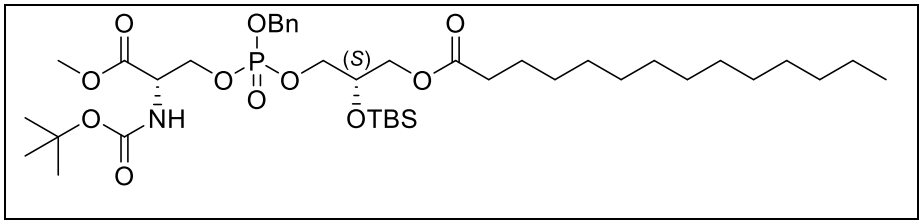



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl dodecanoate**

Following the general procedure (**A2**), **(*S*)-6** (90 mg, 0.155 mmol), **7b** (Dodecanoic acid, C12:0 (28 mg, 0.140 mmol), EDC·HCl (26 mg, 0.140 mmol), DMAP (4 mg, 0.0389 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*S*)-8b** (43 mg, 0.0566 mmol, 47%, colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.31 (m, 5H), 5.47 (t, $J$ = 9.7 Hz, 1H), 5.06 (t, $J$ = 8.0 Hz, 2H), 4.54-4.36 (m, 2H), 4.28-4.19 (m, 1H), 4.14-4.05 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.6 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 16H), 0.90-0.83 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 7 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5Hz), 69.2 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4Hz),

64.8, 54.1 (d, $J_{c\text{-}p}$ = 6 Hz), 52.9, 34.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.8 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{37}H_{66}NO_{11}PSi$ $[M+K]^+$ : calcd., 798.38, found., 798.35, $[M+Na]^+$ : calcd., 782.40; found, 782.38.

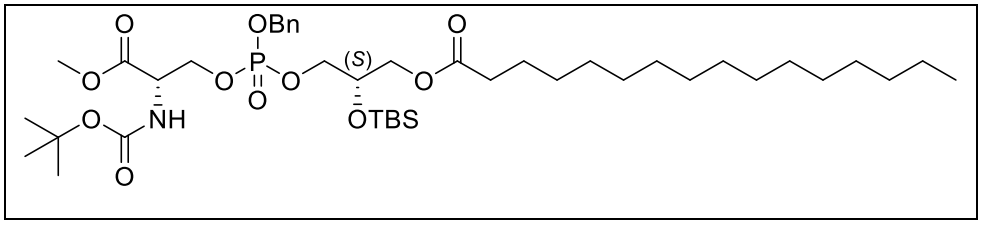
**(*S*)-8c**




**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate**

Following the general procedure (**A2**), **(*S*)-6** (80 mg, 0.138 mmol), **7c** (Myristic acid, C14:0) (28 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0345 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*S*)-8c** (54 mg, 0.0686 mmol, 53%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (t, $J$ = 9.7 Hz, 1H), 5.06 (t, $J$ = 8.0 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-419 (m, 1H), 4.14-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.19 (m, 20H), 0.91-0.81 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 7 Hz), 128.9, 128.8, 128.1(broad), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (t, $J_{c\text{-}p}$ = 7 Hz), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.1 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (broad, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{39}H_{70}NO_{11}PSi$ $[M+K]^+$ : calcd., 826.41; found, 826.43; $[M+Na]^+$: calcd., 810.43; found, 810.44.

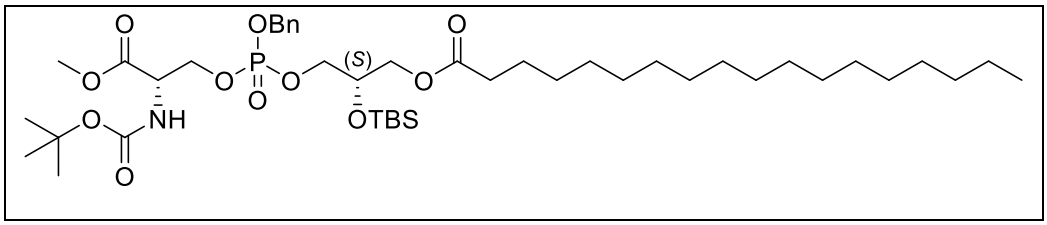
**(*S*)-8d**



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate**

Following the general procedure (**A2**), **(*S*)-6** (80 mg, 0.138 mmol), **7d** (Palmitic acid, C16:0) (32 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0346 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to afford the desired product **(*S*)-8d** (53 mg, 0.0649 mmol, 54%, colourless oil; $^1$H NMR (400 MHz, CDCl3) $\delta$ 7.41-7.30 (m, 5H), 5.47 (t, $J$ = 9.7 Hz, 1H), 5.05 (t, $J$ = 8.1 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-4.19 (m, 1H), 4.15-4.05 (m, 1H), 4.03-3.84 (m ,4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.35-1.19 (m, 24H), 0.91-0.82 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.05, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 1 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.8, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{41}H_{74}NO_{11}PSi$ $[M+K]^+$: calcd., 854.44; found, 854.47; $[M+Na]^+$: calcd., 838.46; found, 838.48.

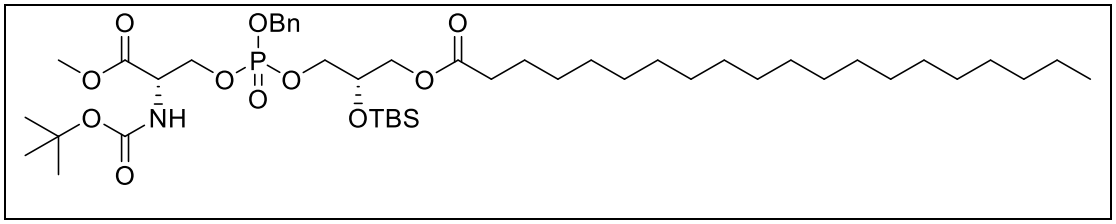
**(*S*)-8e**



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate**

Following the general procedure (**A2**), **(*S*)-6** (80 mg, 0.0138 mmol), **7e** (Stearic acid, C18:0) (35 mg, 0.124 mmol), EDC·HCl (23 mg, 0.124 mmol), DMAP (3 mg, 0.0311 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane 30:70) to provide **(*S*)-8e** (55 mg, 0.0652 mmol, 47%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.31 (m, 5H), 5.47 (t, $J$ = 9.7 Hz, 1H), 5.06 (t, $J$ = 8.0 Hz, 2H), 4.54-4.34 (m, 2H), 4.28-4.19 (m, 1H), 4.14-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (quint, $J$ = 6.9 Hz, 2H), 1.44 (s, 9H), 1.33-1.18 (m, 28H), 0.90-0.83 (m, 12 H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 2 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.2 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.1 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{c\text{-}p}$ = 1 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{43}H_{78}NO_{11}PSi$ $[M+K]^+$: calcd., 882.47; found, 882.48; $[M+Na]^+$: calcd., 866.49; found, 866.50.

**(*S*)-8f**

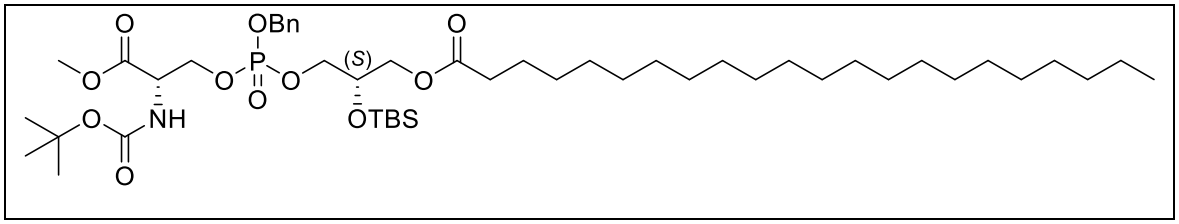



**(2*R*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate**

Following the general procedure (**A2**), **(*S*)-6** (70 mg, 0.121 mmol), **7f** (Arachidic acid C20:0), (34 mg, 0.109 mmol), EDC·HCl (20 mg, 0.109 mmol), DMAP (3 mg, 0.0303 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography

(EtOAc/Hexane 25:75) to provide **(*S*)-8f** (62 mg, 0.0711 mmol, 48%, colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (t, $J$ = 9.8 Hz, 1H), 5.05 (t, $J$ = 7.9 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-4.19 (m, 1H), 4.13-4.05 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5, Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.31-1.21 (m, 32H), 0.90-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.10; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 1Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.9, 34.3, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 1Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{45}H_{82}NO_{11}PSi$ $[M+K]^+$: calcd., 910.50; found, 910.52; $[M+Na]^+$: calcd., 894.52; found, 894.53.

**(*S*)-8g**

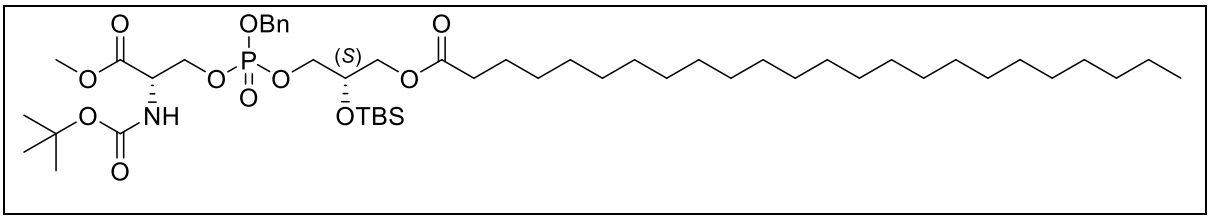



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docasanoate**

Following the general procedure (**A2**), **(*S*)-6** (80 mg, 0.139 mmol), **7g** (Behenic acid, C22:0) (38 mg, 0.125 mmol), EDC·HCl (24 mg, 0.125 mmol), DMAP (4 mg, 0.0348 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 25:75) to yield **(*S*)-8g** (57 mg, 0.0633 mmol, 45%, colourless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (t, $J$ = 9.7 Hz, 1H), 5.06 (t, $J$ = 8.0 Hz, 2H), 4.54-4.34 (m, 2H), 4.29-4.19 (m, 1H), 4.14-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.21 (m, 36H), 0.90-0.84 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.09; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 6 Hz), 128.9, 128.8, 128.1 (d, $J$ = 2 Hz), 80.5, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.2 (d, $J_{c\text{-}p}$ = 8 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz),

64.8, 54.1 (d, $J_{c\text{-}p}$ = 8 Hz), 52.9, 34.3, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{c\text{-}p}$ = 2 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 9 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 6 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{47}H_{86}NO_{11}PSi$ $[M+K]^+$: calcd., 938.53; found, 938.55; $[M+Na]^+$: calcd., 922.56; found, 922.58.

**(*S*)-8h**

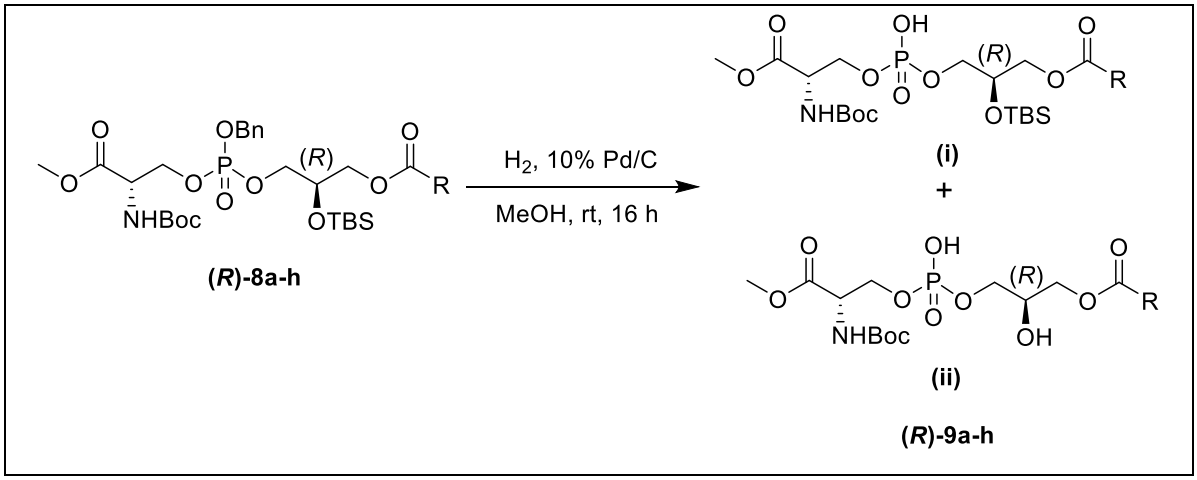



**(2*S*)-3-(((benzyloxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetracosanoate**

Following the general procedure (**A2**), **(*S*)-6** (90 mg, 0.156 mmol), **7h** (Lignoceric acid, C24:0) (42 mg, 0.140 mmol), EDC·HCl (27 mg, 0.140 mmol), DMAP (5 mg, 0.039 mmol) and $CH_2Cl_2$ (5 mL) were used. The crude mixture was purified by column chromatography (100-200 silica gel mesh) (EtOAc/Hexane, 25:75) to yield **(*S*)-8h** (68 mg, 0.0589 mmol, 47%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41-7.30 (m, 5H), 5.47 (t, $J$ = 9.8 Hz, 1H), 5.05 (t, $J$ = 8.0 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-4.18 (m, 1H), 4.14-4.05 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (t, $J$ = 7.5 Hz, 2H), 1.60 (quint, $J$ = 7.0 Hz, 2H), 1.44 (s, 9H), 1.33-1.20 (m, 40H), 0.90-0.82 (m, 12H), 0.07 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -1.06, -1.10; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.8, 155.3, 135.6 (d, $J_{c\text{-}p}$ = 6 Hz), 128.9, 128.8, 128.1 (d, $J_{c\text{-}p}$ = 1 Hz), 80.4, 69.8 (t, $J_{c\text{-}p}$ = 5 Hz), 69.1 (d, $J_{c\text{-}p}$ = 9 Hz), 68.5 (m), 67.7 (t, $J_{c\text{-}p}$ = 4 Hz), 64.8, 54.0 (d, $J_{c\text{-}p}$ = 7 Hz), 52.8, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.7, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{c\text{-}p}$ = 1 Hz, CH), 69.7 (t, $J_{c\text{-}p}$ = 5 Hz, $CH_2$), 69.0 (d, $J_{c\text{-}p}$ = 8 Hz, CH), 68.4 (m, $CH_2$), 67.6 (t, $J_{c\text{-}p}$ = 4 Hz, $CH_2$), 64.7 ($CH_2$), 53.9 (d, $J_{c\text{-}p}$ = 7 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.6 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF)

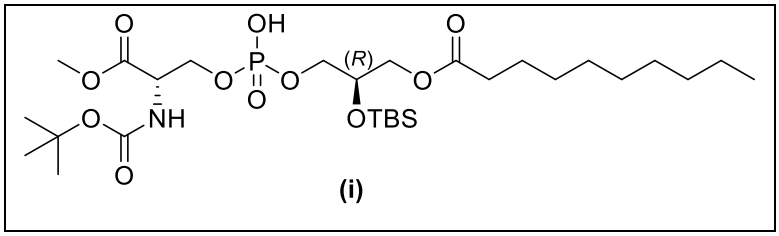
for $C_{49}H_{90}NO_{11}PSi$ $[M+K]^+$: calcd., 966.57; found, 966.57; $[M+Na]^+$: calcd., 950.59; found, 950.60.

## General procedure (B1) for the synthesis of compounds (*R*)-9a-h (debenzylation):

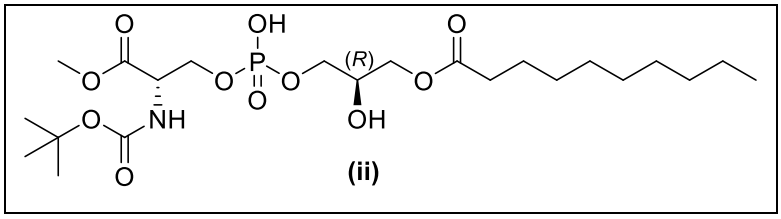



To synthesized compound **(*R*)-9** we followed previously been reported procedure[52–54]. A benzyl-protected substrate **(*R*)-8** was dissolved in dry MeOH in two necks round bottom flask. The Pd/C (10%) was added into the solution under $N_2$ atmosphere, then the round bottom flask was equipped with hydrogen-filled rubber bladder. The reaction mixture was stirred for the next 12 to 16 h at room temperature. The reaction solution was filtered through a celite pad followed by washing with methanol (3 × 10 mL). The filtrate was concentrated under reduced pressure at 25 °C to afford the product **(*R*)-9** which were used without further purification to the next step. We were also observed here 30-60% TBDMS deprotection (characterized by $^1$H NMR) under this condition due to solvent effect[55–58].

**(*R*)-9a**



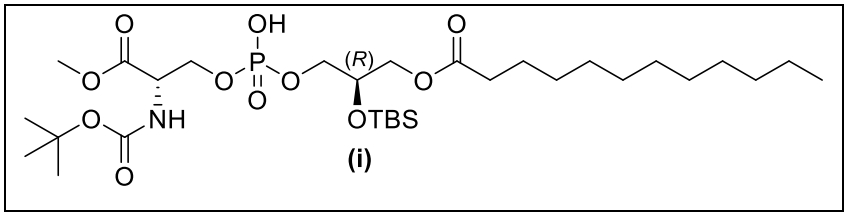

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl decanoate**



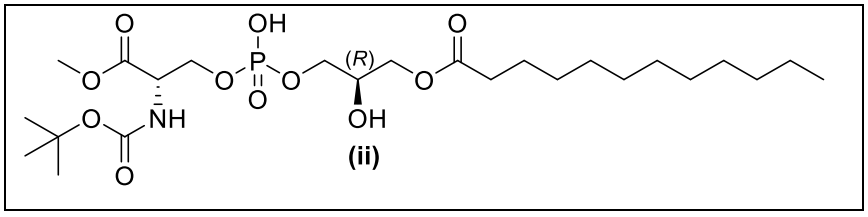

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl decanoate**

Following the general procedure (**B1**), **(*R*)-8a** (49 mg, 0.0669 mmol), Pd-C (25 mg), and MeOH (5 mL) were used. The product is obtained as a mixture of **(*R*)-9a (i)** (39%, calcd. by $^{1}$H nmr) and **(ii)** (61%, calcd. by $^{1}$H nmr) (30 mg, colourless oil) and used without purify to the next step. $^{1}$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.37 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.77 (m, 2H), 3.75 (s, 3H), 2.4o-2.31 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.23 (m, 12H), 0.94-0.86 (m, 7H), 0.15-0.09 (m, 2H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ -0.19, -0.46.

**(*R*)-9b**



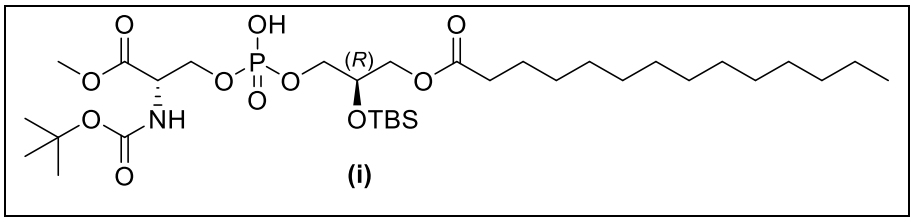

**(2*R*)-3-((((*S*)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl dodecanoate**



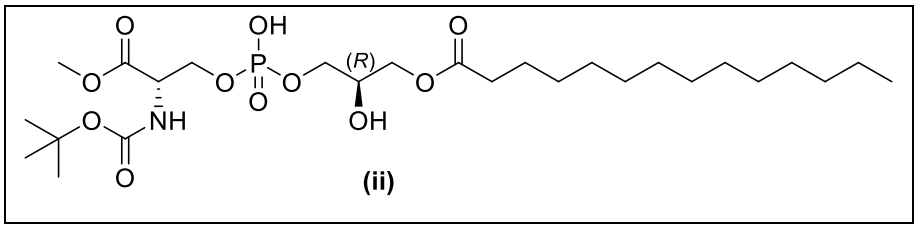

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate**

Following the general procedure (**B1**), **(*R*)-8b** (45 mg, 0.0592 mmol), Pd-C (23 mg), and MeOH (4.5 mL) were used. The product is obtained as a mixture of **(*R*)-9b (i)** (54%, calcd. by $^1$H nmr) and **(ii)** (43%, calcd. by $^1$H nmr) (32 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.39-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.74 (s, 3H), 2.38-2.33 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.36-1.23 (m, 16H); 0.94-0.86 (m, 8H), 0.16-0.10 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ 0.14, -0.21.

**(*R*)-9c**



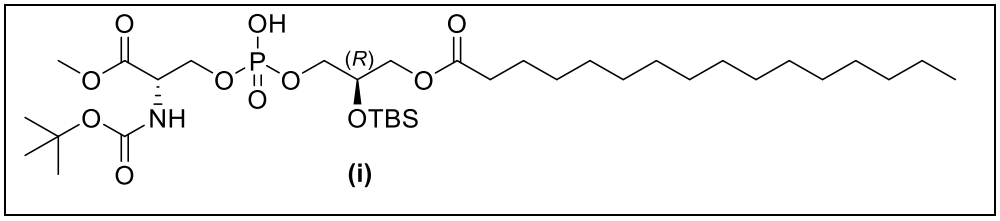

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate**



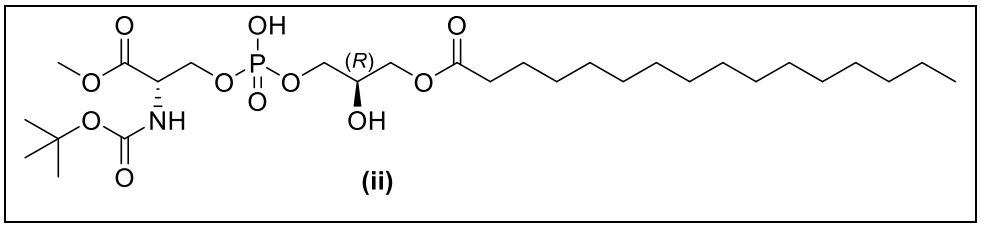

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate**

Following the general procedure (**B1**), **(*R*)-8c** (44 mg, 0.0558 mmol), Pd-C (22 mg), and MeOH (4.4 mL) were used. The product is obtained as a mixture of **(*R*)-9c (i)** (67%, calcd. by $^1$H nmr) and **(ii)** (33%, calcd. by $^1$H nmr) (28 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.39-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.74 (s, 3H), 2.41-2.29 (m, 2H), 1.62 (q, $J$ = 6.9 Hz, 2H), 1.45 (s, 9H), 1.36-1.22 (m, 20H), 0.93-0.86 (m, 9H), 0.17-0.08 (m , 4H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ 0.21, -0.15.

**(*R*)-9d**



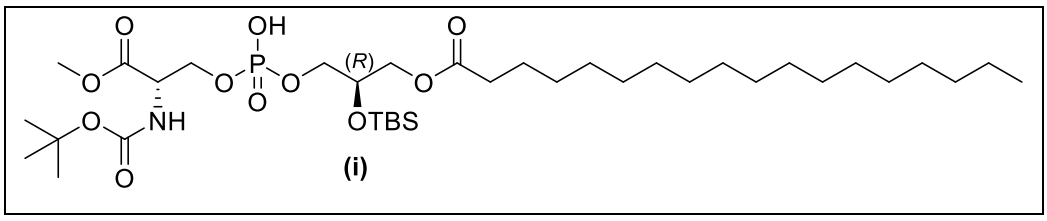

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate**



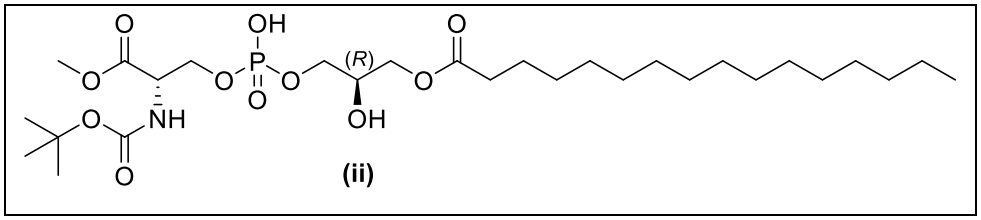

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate**

Following the general procedure (**B1**), **(*R*)-8d** (55 mg, 0.0674 mmol), Pd-C (28 mg), and MeOH (5.5 mL) were used. The product is obtained as a mixture of **(*R*)-9d (i)** (60%, calcd. by $^{1}$H nmr) and **(ii)** (40%, calcd. by $^{1}$H nmr) (36 mg, colourless oil) and used without purify to the next step. $^{1}$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.40-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.90-3.76 (m, 2H), 3.75 (m, 3H), 2.39-2.30 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.24 (m, 24H), 0.93-0.86 (m, 8H), 0.17-0.09 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.27, -0.12.

**(*R*)-9e**



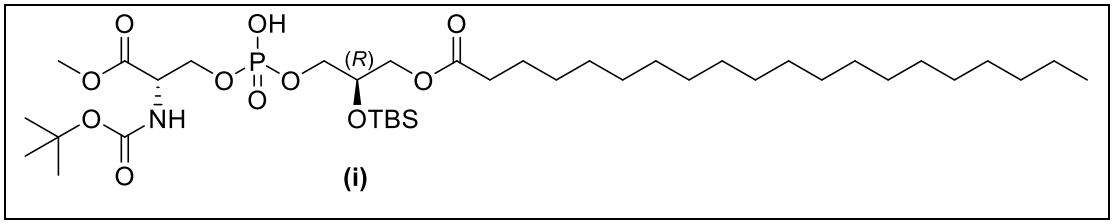

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate**



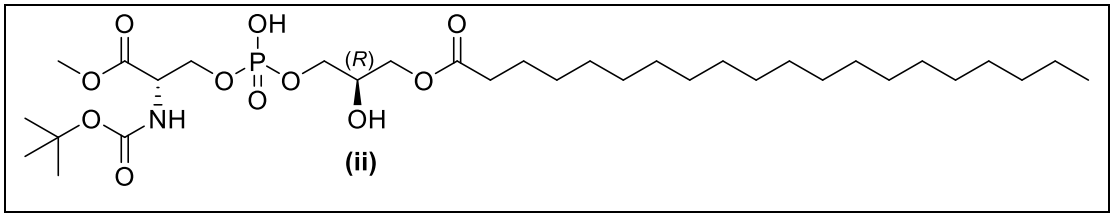

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate**

Following the general procedure (**B1**), **(*R*)-8e** (45 mg, 0.0533 mmol), Pd-C (22 mg), and MeOH (4.5 mL) were used. The product is obtained as a mixture **(*R*)-9e (i)** (65%, calcd. by $^{1}$H nmr) and **(ii)** (35%, calcd. by $^{1}$H nmr) (30 mg, colourless oil) and used without purify to the next step. $^{1}$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.40-4.32 (m 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.92-3.76 (m, 2H), 3.74 (s, 3H), 2.40-2.30 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.36-1.24 (m, 28H), 0.93-0.86 (m, 9H), 0.17-0.09 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.24, -0.14.

**(*R*)-9f**




**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate**

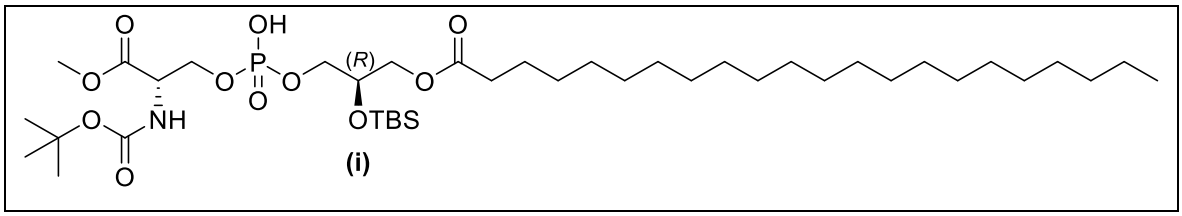



**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate**

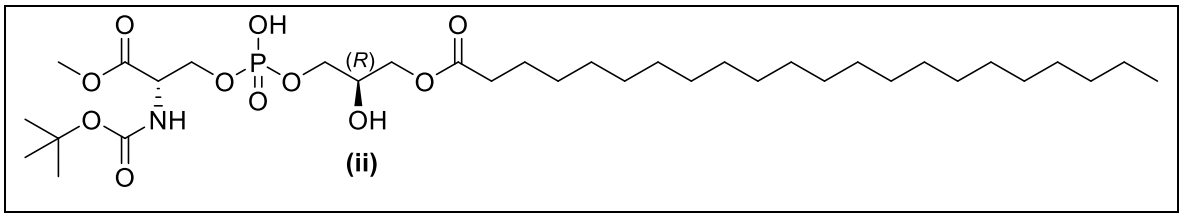
Following the general procedure (**B1**), **(*R*)-8f** (65 mg, 0.0745 mmol), Pd-C (33 mg), and MeOH (6.5 mL) were used. The product is obtained as a mixture **(*R*)-9f (i)** (58%, calcd. by $^{1}$H

nmr) and **(ii)** (42%, calcd. by $^1$H nmr) (49 mg, white solid) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.40-4.34 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.75 (s, 3H), 2.38-2.27 (m, 2H), (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.24 (m, 32H), 0.95-0.85 (m, 9H), 0.17-0.09 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ -0.79, -0.93.

**(*R*)-9g**



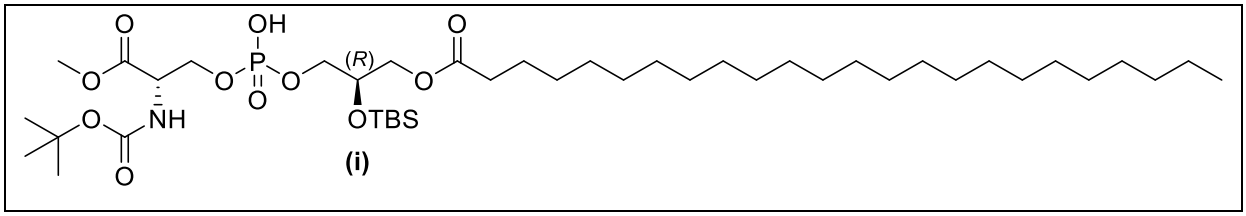

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docasanoate**



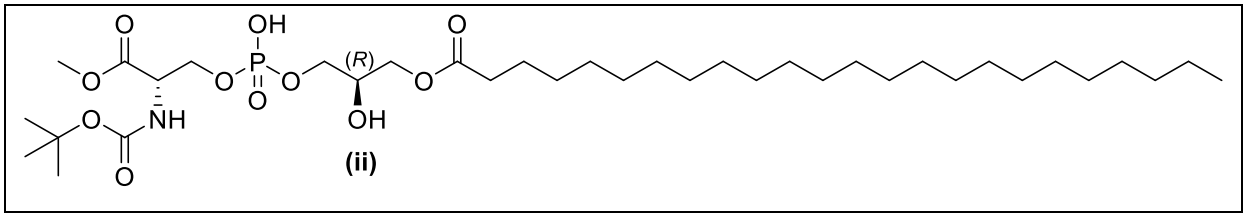

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docosanoate**

Following the general procedure (**B1**), **(*R*)-8g** (52 mg, 0.0578 mmol), Pd-C (26 mg), and MeOH (5.2 mL) were used. The product is obtained as a mixture **(*R*)-9g (i)** (63%, calcd. by $^1$H nmr) and **(ii)** (37%, calcd. by $^1$H nmr) (35 mg, white solid) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.40-4.32 (m, 1H), 4.31-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.90-3.77 (m, 2H), 3.74 (s, 3H), 2.42-2.28 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.21 (m, 36H), 0.96-0.84 (m, 10H), 0.18-0.07 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ -0.56.

**(*R*)-9h**



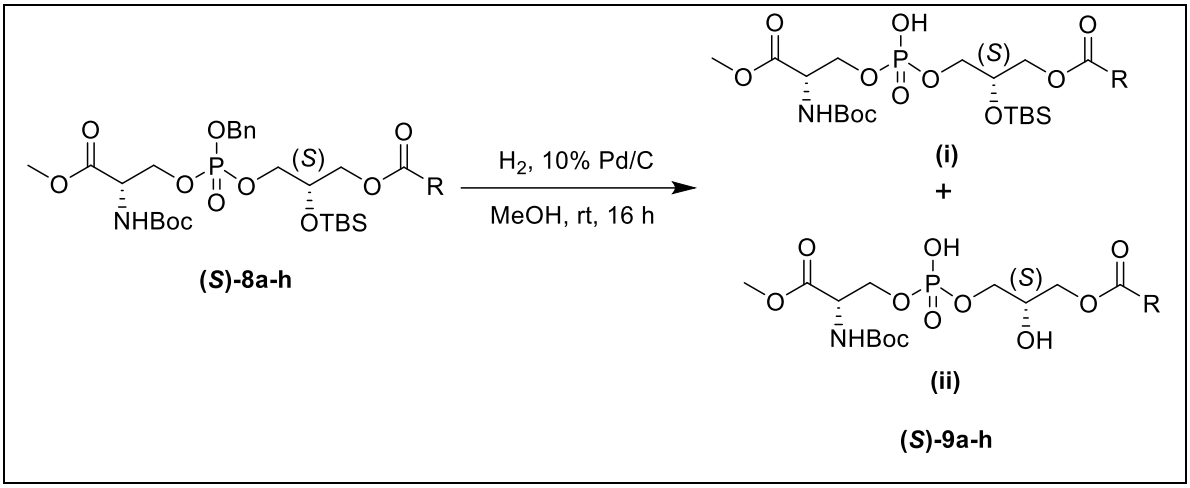

**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyltetracosanoate**




**(2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate**

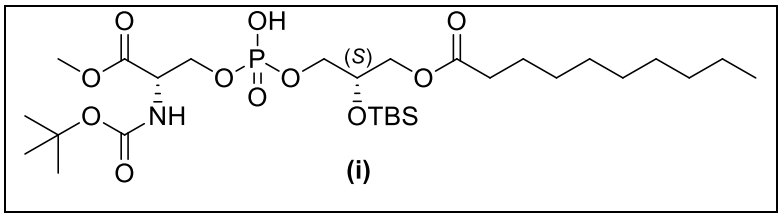
Following the general procedure (**B1**), **(*R*)-8h** (59 mg, 0.0589 mmol), Pd-C (30 mg), and MeOH (5.9 mL) were used. The product is obtained as a mixture **(*R*)-9h (i)** (46%, calcd. by $^{1}$H nmr) and **(ii)** (54%, calcd. by $^{1}$H nmr) (40 mg, white solid) and used without purify to the next step. $^{1}$H NMR (400 MHz, MeOH-$d_4$ + CDCl3) $\delta$ 4.45-4.36 (m, 1H), 4.33-4.03 (m, 4H), 4.02-3.83 (m, 3H), 3.75 (s, 3H), 2.41-2.23 (m, 2H), 1.62 (m, 2H), 1.45 (s, 9H), 1.36-1.18 (m, 40H), 0.96-0.80 (m, 9H), 0.17-0.03 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ -0.50, -0.90.

## General procedure (B2) for the synthesis of compounds (*S*)-9a-h (debenzylation):

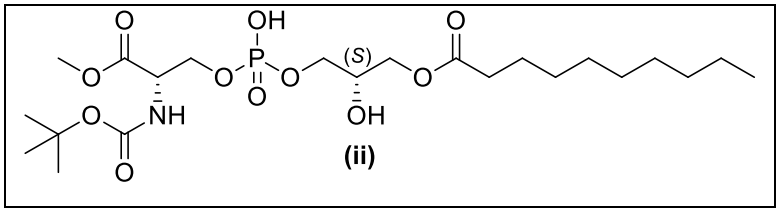



To synthesized compound **(*S*)-9** we followed previously been reported procedure[52–54]. A benzyl-protected substrate **(*S*)-8** was dissolved in dry MeOH in two neck round bottom flask. The Pd/C (10%) was added into the solution under the $N_2$ atmosphere, then the round bottom flask was equipped with hydrogen-filled rubber bladder. The reaction mixture was stirred for the next 12 to 16 h at room temperature. The reaction solution was filtered through a celite pad followed by washing with methanol (3 × 10 mL). The filtrate was concentrated under reduced pressure at 25 °C to afford the product **(*S*)-9** which were used without further purification to the next step. We were also observed 30-60% TBDMS deprotection (characterized by $^1$H NMR) under this condition due to solvent effect.

**(*S*)-9a**



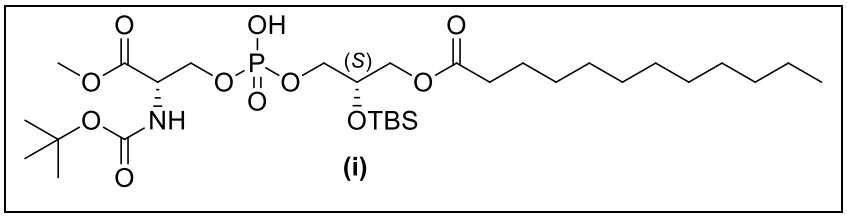

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl decanoate**



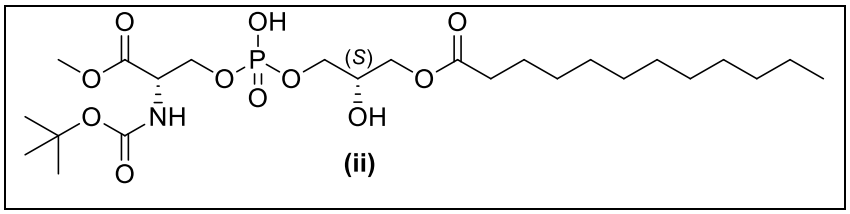
**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl decanoate**

Following the general procedure (**B2**), **(*S*)-8a** (50 mg, 0.0683 mmol), Pd-C (25 mg), and MeOH (5.0 mL) were used. The product is obtained as a mixture of **(*S*)-9a (i)** (50%, calcd. by $^1$H nmr) and **(ii)** (50%, calcd. by $^1$H nmr) (36 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.39-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.89-3.77 (m, 2H), 3.75 (s, 3H), 2.40-2.31 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.23 (m, 12H), 0.94-0.86 (m, 8H), 0.16-0.07 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-d$_4$) $\delta$ 0.22, -0.16.

**(*S*)-9b**




**(2*S*)-3-((((*S*)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl dodecanoate**

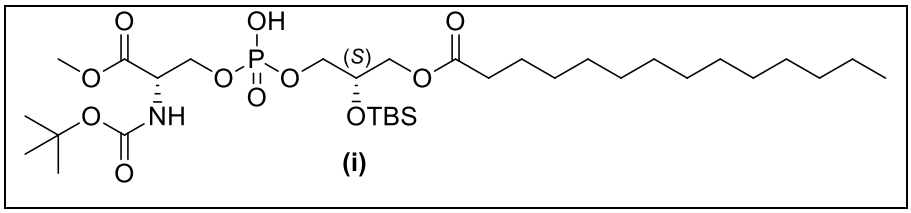



**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate**

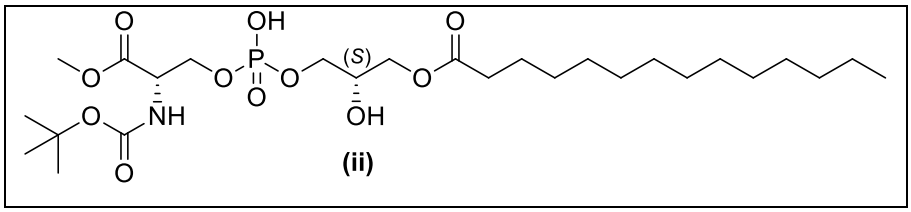
Following the general procedure (**B2**), **(*S*)-8b** (42 mg, 0.0553 mmol), Pd-C (21 mg), and MeOH (4.2 mL) were used. The product is obtained as a mixture of **(*S*)-9b (i)** (58%, calcd. by $^1$H nmr) and **(ii)** (42%, calcd. by $^1$H nmr) (30 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-d$_4$) $\delta$ 4.39-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.89-3.76 (m, 2H), 3.74 (s, 3H), 2.39-2.30 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s,

9H), 1.36-1.23 (m, 16H); 0.94-0.86 (m, 8H), 0.16-0.09 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.16, -0.21.

**(*S*)-9c**



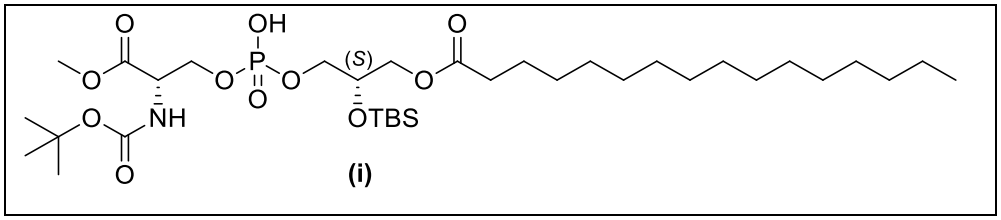

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate**



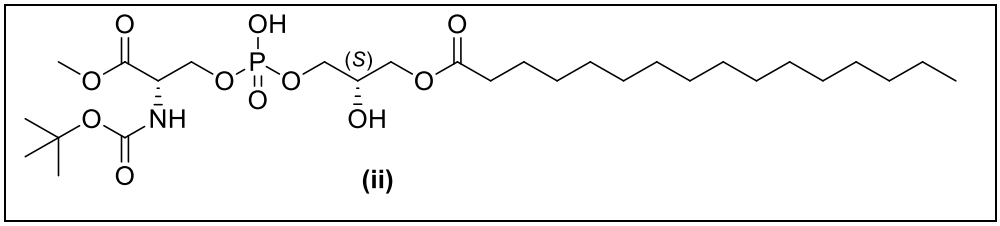

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate**

Following the general procedure (**B2**), **(*S*)-8c** (54 mg, 0.0686 mmol), Pd-C (27 mg), and MeOH (5.5 mL) were used. The product is obtained as a mixture of **(*S*)-9c (i)** (79%, calcd. by $^1$H nmr) and **(ii)** (21%, calcd. by $^1$H nmr) (40 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.39-4.32 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.74 (s, 3H), 2.41-2.29 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.36-1.22 (m, 20H), 0.93-0.85 (m, 10H), 0.17-0.08 (m , 5H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.16, -0.22.

**(*S*)-9d**



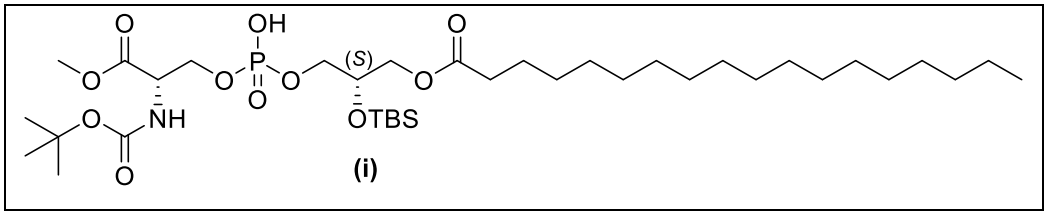
**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate**



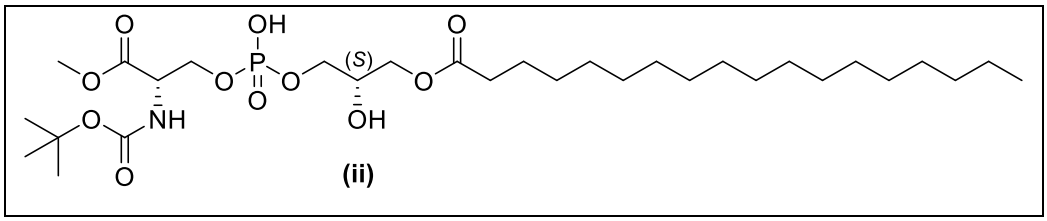

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate**

Following the general procedure (**B2**), **(*S*)-8d** (53 mg, 0.0649 mmol), Pd-C (27 mg), and MeOH (5.3 mL) were used. The product is obtained as a mixture of **(*S*)-9d (i)** (62%, calcd. by $^1$H nmr) and **(ii)** (38%, calcd. by $^1$H nmr) (38 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.40-4.32 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.90-3.76 (m, 2H), 3.74 (s, 3H), 2.39-2.30 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37-1.24 (m, 24H), 0.93-0.86 (m, 9H), 0.17-0.09 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.23, -0.16.

**(*S*)-9e**



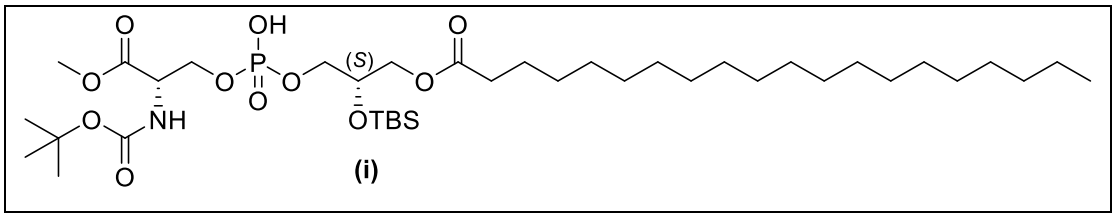

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate**



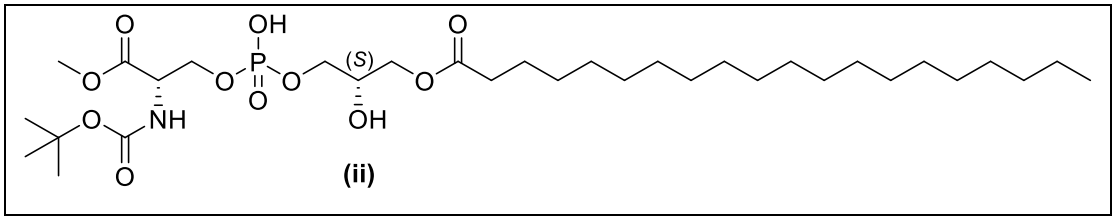

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate**

Following the general procedure (**B2**), **(*S*)-8e** (55 mg, 0.0652 mmol), Pd-C (28 mg), and MeOH (5.5 mL) were used. The product is obtained as a mixture **(*S*)-9e (i)** (65%, calcd. by $^1$H nmr) and **(ii)** (35%, calcd. by $^1$H nmr) (43 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.40-4.32 (m 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.74 (s, 3H), 2.40-2.31 (m, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.45 (s, 9H), 1.36-1.24 (m, 28H), 0.93-0.86 (m, 9H), 0.17-0.09 (m, 4H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.22, -0.17.

**(*S*)-9f**



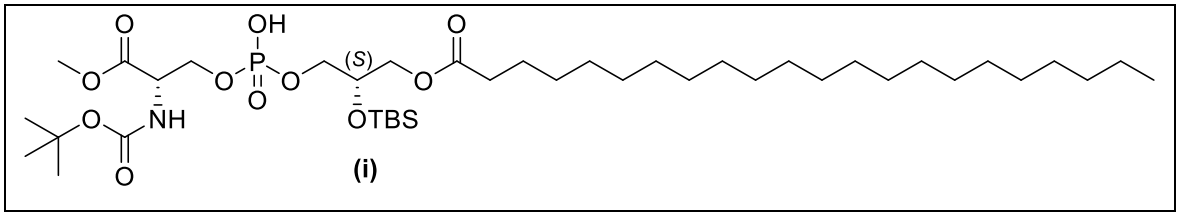

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate**



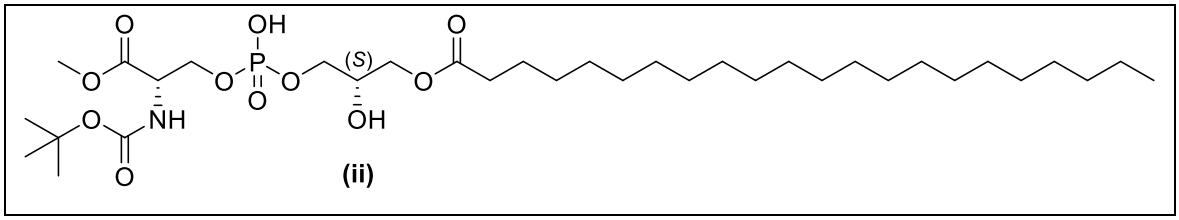

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate**

Following the general procedure (**B2**), **(*S*)-8f** (62 mg, 0.0711 mmol), Pd-C (31 mg), and MeOH (6.2 mL) were used. The product is otained as a mixture **(*S*)-9f (i)** (60%, calcd. by $^1$H nmr) and **(ii)** (40%, calcd. by $^1$H nmr) (48 mg, colourless oil) and used without purify to the next step. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.47 (m, 1H), 4.31-3.87 (m, 7H), 3.76 (s, 3H), 2.32-2.28 (m, 2H), 1.60-1.59 (m, 2H), 1.43 (s, 9H), 1.25 (m, 32H), 0.90-0.84 (m, 12H), 0.08 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ 0.67, 0.37.

**(*S*)-9g**



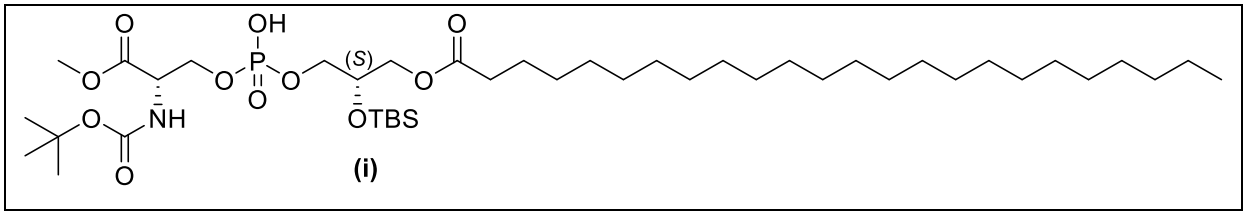

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docasanoate**



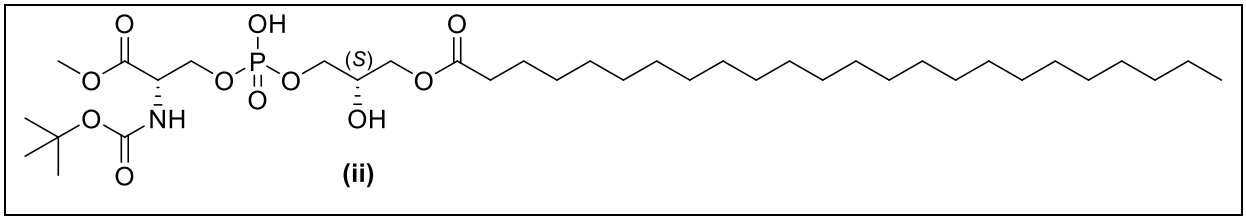

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docasanoate**

Following the general procedure (**B2**), **(*S*)-8g** (57 mg, 0.0633 mmol), Pd-C (28 mg), and MeOH (5.5 mL) were used. The product is obtained as a mixture **(*S*)-9g (i)** (68%, calcd. by $^{1}$H nmr) and **(ii)** (32%, calcd. by $^{1}$H nmr) (52 mg, colourless oil) and used without purify to the next step. $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.47 (m, 1H), 4.30-3.97 (m, 7H), 3.76 (s, 3H), 2.32-2.28 (m, 2H), 1.60-1.58 (m, 2H), 1.43 (s, 9H), 1.25 (m, 36H), 0.90-0.87 (m, 12H), 0.08 (m, 5H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -0.88, -1.36.

**(*S*)-9h**



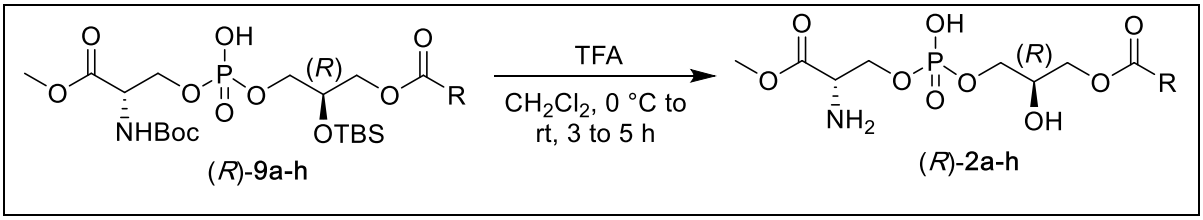

**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetracosanoate**




**(2*S*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate**

Following the general procedure (**B2**), **(*S*)-8h** (68 mg, 0.0589 mmol), Pd-C (34 mg), and MeOH (6.5 mL) were used. The product is obtained as a mixture **(*S*)-9h (i)** (65%, calcd. by $^{1}$H nmr) and **(ii)** (35%, calcd. by $^{1}$H nmr) (30 mg, colourless oil) and used without purify to the next step. $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.45 (m, 1H), 4.29-3.95 (m, 7H), 3.76 (s, 3H), 2.32-2.28 (m, 2H), 1.60-1.58 (m, 2H), 1.43 (s, 9H), 1.25 (m, 40H), 0.94-0.87 (m, 12H), 0.08 (m, 4H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ 1.02, 0.58.

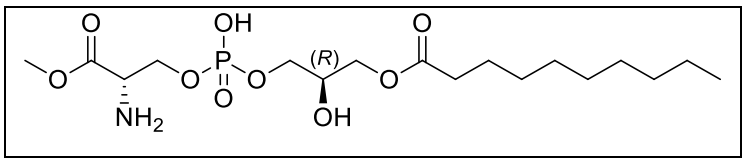
## General procedure (C1) for the synthesis of compounds (*R*)-2a-h (TBS and *t*-BOC deprotection)




To synthesized compound **(*R*)-2** we followed previously been reported procedure[52]. The compound **(*R*)-9** in dry $CH_2Cl_2$ was charged in two necks round bottom flask which was equipped with $N_2$ balloon. The solution was cooled to -10 °C and then the TFA was added dropwise. After TFA addition, the reaction temperature and stirring time was variable for different analogues. For the starting moieties **(*R*)-9a-d**, the reaction solution were stirred at 0 °C for 1 h and then at room temperature for 4 h and got (above 90% pure compound). For the moiety **(*R*)-9e** the reaction solution was stirred at 0 °C for 4 h and then at room temperature for 1 h and got (above 90% pure compound) . For the longer fatty acid chain length moieties **(*R*)-9f-h** were required to stir the reaction solution at 0 °C for 5 h got (above 70% pure compound). Once the reaction was complete, the reaction solution was concentrated under reduced pressure

at < 25 °C and then the dried residue was washed with *n*-Pentane: $Et_2O$ (3:1) three times, dried under high vacuum to afford the TFA salt of the desired product i.e. **(*R*)-2** The purity of the final compounds **(*R*)-2a-h** was determined based on the NMR spectra, and LC-MS analysis.

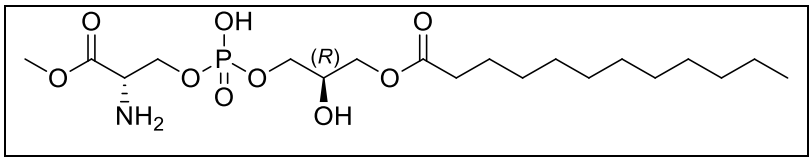
**(*R*)-2a**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl decanoate**

Following the general procedure (**C1**), **(*R*)-9a** (30 mg), TFA (0.300 mL) and $CH_2Cl_2$ (0.170 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2a** (21 mg, 0.0491 mmol, 72% yield relative to **(*R*)-8a**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.60 (m, 1H), 4.40-4.07 (m, 4H), 4.05-3.89 (m, 3H), 3.86 (2s, 3H), 3.79-3.64 (m, 1H), 2.36 (t, $J$ = 7.4 Hz, 2H), 1.61 (q, $J$ = 7.0 Hz, 2H), 1.40-1.22 (m, 12H), 0.90 (t, $J$ = 6.8 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{17}H_{35}NO_9P$) $[M + H]^+$ 428.2049; found, 428.2048. Purity: > 97%.

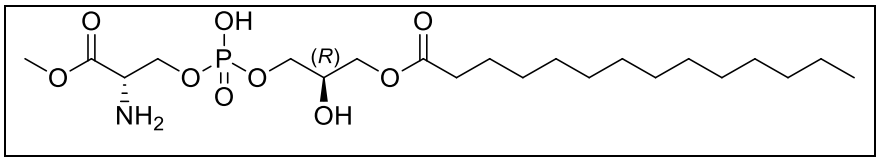
**(*R*)-2b**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate**

Following the general procedure (**C1**), **(*R*)-9b** (32 mg), TFA (0.320 mL) and $CH_2Cl_2$ (0.180 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2b** (21 mg, 0.0461 mmol, 78% yield relative to **(*R*)-8b**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.60 (m, 1H), 4.40-4.07 (m, 4H), 4.06-3.88 (m, 3H), 3.86 (2s, 3H), 3.77-3.64 (m, 1H), 2.36 (td, $J$ = 7.4, 3.4 Hz, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.38-1.23 (m, 16H), 0.90 (t, $J$ = 6.8 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{19}H_{39}NO_9P$) $[M + H]^+$ 456.2362; found, 456.2363. Purity > 95%.

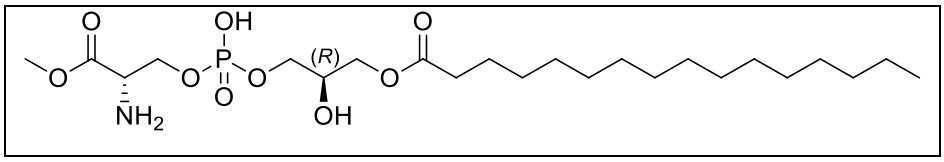
**(*R*)-2c**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate**

Following the general procedure (**C1**), **(*R*)-9c** (28 mg), TFA (0.280 mL) and $CH_2Cl_2$ (0.160 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2c** (20 mg, 0.0414 mmol, 67% yield relative to **(*R*)-8c**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.49-4.30 (m, 1H), 4.28-4.00 (m, 4H), 3.98-3.82 (m, 3H), 3.79 (s, 3H), 3.73-3.55 (m, 1H), 2.30 (m, 2H), 1.56 (m, 2H), 1.33-1.15 (m, 20H), 0.83 (t, *J* = 6.3 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{21}H_{43}NO_9P$) $[M + H]^+$ 484.2675; found, 484.2677. Purity > 95%.

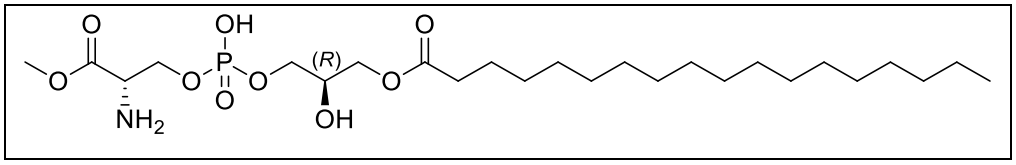
**(*R*)-2d**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate**

Following the general procedure (**C1**), **(*R*)-9d** (36 mg), TFA (0.360 mL) and $CH_2Cl_2$ (0.210 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2d** (26 mg, 0.0508 mmol, 74% yield relative to **(*R*)-8d**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.61(m, 1H), 4.37-4.07 (m, 4H), 4.06-3.89 (m, 3H), 3.86 (2s, 3H), 3.77-3.64 (m, 1H), 2.36 (td, *J* = 7.4, 2.5 Hz, 2H), 1.62 (q, *J* = 7.0 Hz, 2H), 1.38-1.21 (m, 24H), 0.90 (t, *J* = 6.8 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{23}H_{47}NO_9P$) $[M + H]^+$ 512.2988; found, 512.2988. Purity > 98%.

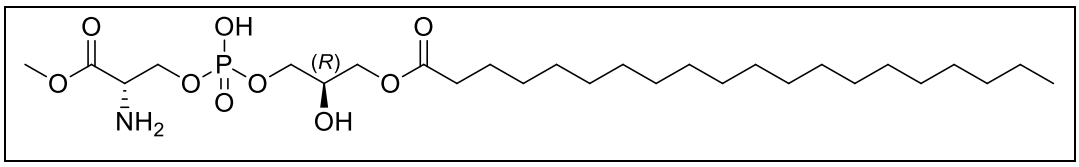
**(*R*)-2e**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate**

Following the general procedure (**C1**), **(*R*)-9e** (18 mg), TFA (0.180 mL) and $CH_2Cl_2$ (0.110 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 1 mL) to yield desired final compound **(*R*)-2e** (13 mg, 0.0241 mmol, 76% yield relative to **(*R*)-8e**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.30-4.23 (m, 3H), 4.22-4.04 (m, 2H), 4.02-3.89 (m, 2H), 3.86 (s, 3H), 3.75-3.65 (m, 1H), 2.34 (t, $J$ = 6.6 Hz, 2H), 1.61 (m, 2H), 1.36-1.19 (m, 28H), 0.88 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{25}H_{51}NO_9P$) $[M + H]^+$ 540.3296; found, 540.3297. Purity > 98%.

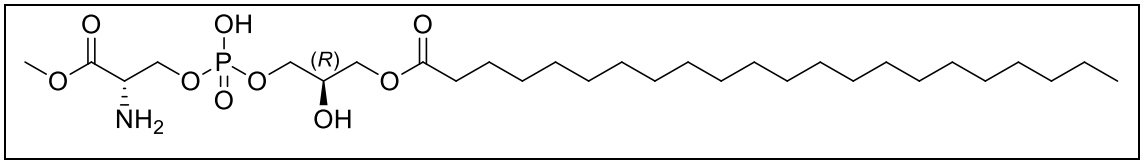
**(*R*)-2f**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate**

Following the general procedure (**C1**), **(*R*)-9f** (25 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.350 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2f** (17 mg, 0.0299 mmol, 79% yield relative to **(*R*)-8f**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.37-4.14 (m, 3H), 4.13-4.04 (m, 2H), 4.03-3.88 (m, 2H), 3.84 (s, 3H), 3.75-3.55 (m, 1H), 2.32 (t, $J$ = 7.3 Hz, 2H), 1.58 (m, 2H), 1.32-1.20 (m, 32H), 0.85 (t, $J$ = 6.6 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.45; HRMS (ESI): m/z calcd. for ($C_{27}H_{55}NO_9P$) $[M + H]^+$ 568.3609; found, 568.3610. Purity > 95%. .

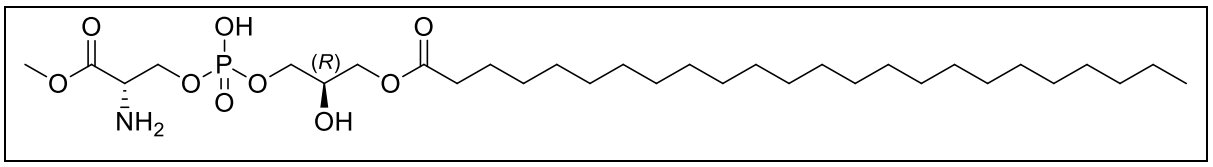
**(*R*)-2g**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docasanoate**

Following the general procedure (**C1**), **(*R*)-9g** (28 mg), TFA (0.225 mL) and $CH_2Cl_2$ (0.390 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2g** (22 mg, 0.0369 mmol, 79% yield relative to **(*R*)-8g**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.33-4.05 (m, 5H), 4.04-3.90 (m, 2H), 3.83 (s, 3H), 3.77-3.55 (m, 1H), 2.31 (m, 2H), 1.58 (m, 2H), 1.32-1.19 (m, 36H), 0.84 (t, $J$ = 6.3 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{29}H_{59}NO_9P$) $[M + H]^+$ 596.3922; found, 596.3923. Purity > 95%.

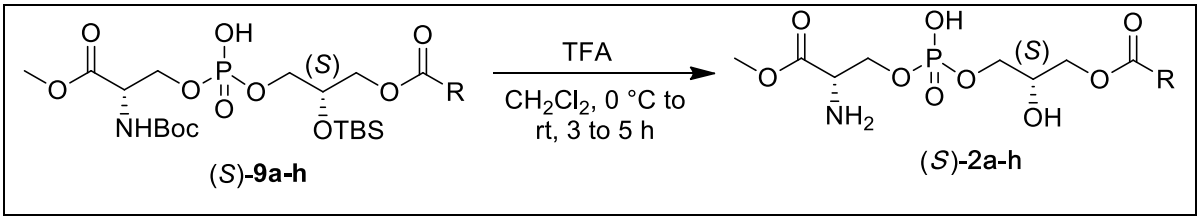
**(*R*)-2h**



**(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate**

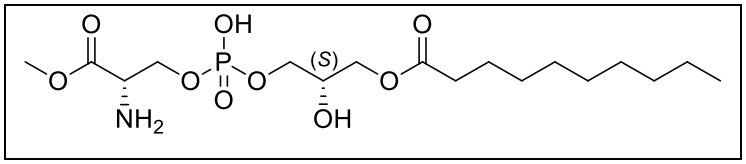
Following the general procedure (**C1**), **(*R*)-9h** (25 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.350 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-2h** (18 mg, 0.0289 mmol, 78% yield relative to **(*R*)-8h**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.33-4.05 (m, 5H), 4.04-3.88 (m, 3H), 3.83 (s, 3H), 3.72-3.52 (m, 1H), 2.31 (m, 2H), 1.58 (m, 2H), 1.34-1.14 (m, 40H), 0.84 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; ; HRMS (ESI): m/z calcd. for ($C_{31}H_{63}NO_9P$) $[M + H]^+$ 624.4235; found, 624.4236. Purity > 96%.

## General procedure (C2) for the synthesis of compounds (*S*)-2a-h (TBS and *t*-BOC deprotection)




To synthesized compound **(*S*)-2** we followed previously been reported procedure[52]. The compound **(*S*)-9** in dry $CH_2Cl_2$ was charged in two necks round bottom flask which was equipped with $N_2$ balloon. The solution was cooled to -10 °C and then the TFA was added dropwise. After TFA addition, the reaction temperature and stirring time was variable for different analogues. For the starting moieties **(*S*)-9a-d**, the reaction solution were stirred at 0 °C for 1 h and then at room temperature for 4 h and got (above 90% pure compound). For the moiety **(*S*)-9e** the reaction solution was stirred at 0 °C for 4 h and then at room temperature for 1 h and got (above 90% pure compound). For the longer fatty acid chain length moieties **(*S*)-9f-h** were required to stir the reaction solution at 0 °C for 5 h and got (above 75% pure compound). Once the reaction was complete, the reaction solution was concentrated under reduced pressure at < 25 °C and then the dried residue was washed with *n*-Pentane: $Et_2O$ (3:1) three times, dried under high vacuum to afford the TFA salt of the desired product i.e. **(*S*)-2** The purity of the final compounds **(*S*)-2a-h** was determined based on the NMR spectra, and LC-MS analysis.

**(*S*)-2a**

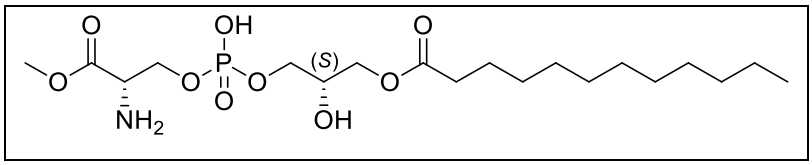


**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl decanoate**

Following the general procedure (**C2**), **(*S*)-9a** (36 mg), TFA (0.360 mL) and $CH_2Cl_2$ (0.210 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2a** (22 mg, 0.0515 mmol, 75% yield relative to **(*S*)-8a**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.41-4.05 (m, 4H), 4.04-3.85 (m, 4H), 3.82 (s, 3H), 3.75-3.58 (m, 1H), 2.40-2.29 (m, 2H), 1.66-1.51 (m, 2H), 1.36-1.19 (m, 12H), 0.87 (t, $J$ = 6.4

Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{17}H_{35}NO_9P$) $[M + H]^+$ 428.2049; found, 428.2048. Purity > 95%.

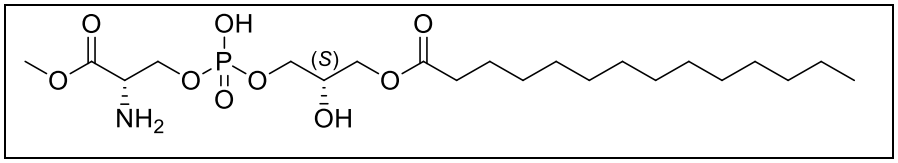
**(*S*)-2b**




**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate**

Following the general procedure (**C2**), **(*S*)-9b** (30 mg), TFA (0.300 mL) and $CH_2Cl_2$ (0.170 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2b** (17 mg, 0.0373 mmol, 67% yield relative to **(*S*)-8b**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.59 (m, 1H), 4.40-4.07 (m, 4H), 4.06-3.88 (m, 3H), 3.86 (2s, 3H), 3.78-3.61 (m, 1H), 2.36 (t, $J$ = 7.4 Hz, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.38-1.23 (m, 16H), 0.90 (t, $J$ = 6.7 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{19}H_{39}NO_9P$) $[M + H]^+$ 456.2362; found, 456.2361. Purity > 98%.

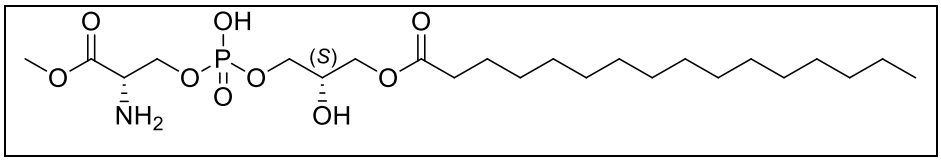
**(*S*)-2c**




**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate**

Following the general procedure (**C2**), **(*S*)-9c** (40 mg), TFA (0.400 mL) and $CH_2Cl_2$ (0.230 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2c** (26 mg, 0.0538 mmol, 78% yield relative to **(*S*)-8c**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.60 (m, 1H), 4.40-4.07 (m, 4H), 4.06-3.89 (m, 3H), 3.85 (2s, 3H), 3.76-3.65 (m, 1H), 2.36 (t, $J$ = 7.4 Hz, 2H), 1.62 (m, 2H), 1.38-1.23 (m, 20H), 0.90 (t, $J$ = 6.7 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{21}H_{43}NO_9P$) $[M + H]^+$ 484.2675; found, 484.2676. Purity > 96%.

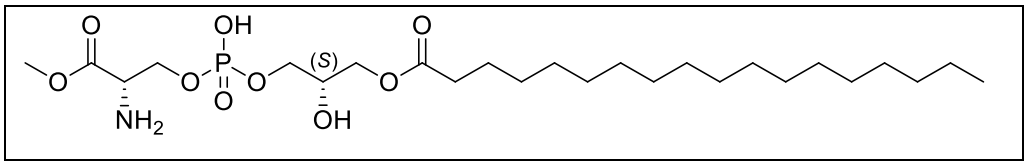
**(*S*)-2d**



**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate**

Following the general procedure (**C2**), **(*S*)-9d** (38 mg), TFA (0.380 mL) and $CH_2Cl_2$ (0.220 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2d** (24 mg, 0.0469 mmol, 73% yield relative to **(*S*)-8d**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.62 (m, 1H), 4.40-4.08 (m, 4H), 4.04-3.89 (m, 3H), 3.86 (2s, 3H), 3.76-3.65 (m, 1H), 2.36 (td, $J$ = 7.4, 2.8 Hz, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.37-1.24 (m, 24H), 0.90 (t, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.43; HRMS (ESI): m/z calcd. for ($C_{23}H_{47}NO_9P$) $[M + H]^+$ 512.2988; found, 512.2989. Purity > 96%.

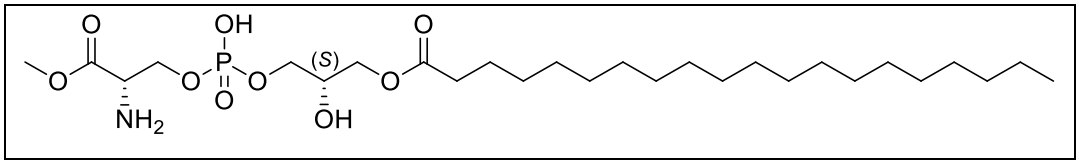
**(*S*)-2e**



**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate**

Following the general procedure (**C2**), **(*S*)-9e** (43 mg), TFA (0.430 mL) and $CH_2Cl_2$ (0.250 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2e** (26 mg, 0.0482 mmol, 74% yield relative to **(*S*)-8e**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$) $\delta$ 4.61 (m, 1H), 4.37-4.07 (m, 5H), 4.06-3.88 (m, 2H), 3,86 (2s, 3H), 3.77-3.65 (m, 1H), 2.36 (td, $J$ = 7.4, 2.5 Hz, 2H), 1.62 (q, $J$ = 7.0 Hz, 2H), 1.37-1.24 (m, 28H), 0.90 (t, $J$ = 6.9 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.43; HRMS (ESI): m/z calcd. for ($C_{25}H_{51}NO_9P$) $[M + H]^+$ 540.3296; found, 540.3298. Purity > 98%.

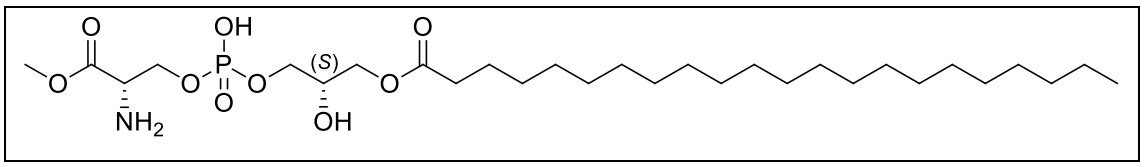
**(*S*)-2f**



**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate**

Following the general procedure (**C2**), **(*S*)-9f** (25 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.350 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2f** (16 mg, 0.0282 mmol, 76% yield relative to **(*S*)-8f**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.37-4.14 (m, 3H), 4.13-4.04 (m, 2H), 4.03-3.89 (m, 2H), 3.84 (s, 3H), 3.75-3.54 (m, 1H), 2.32 (m, 2H), 1.58 (m, 2H), 1.32-1.17 (m, 32H), 0.84 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.43; HRMS (ESI): m/z calcd. for ($C_{27}H_{55}NO_9P$) $[M + H]^+$ 568.3609; found, 568.3610. Purity > 97%.

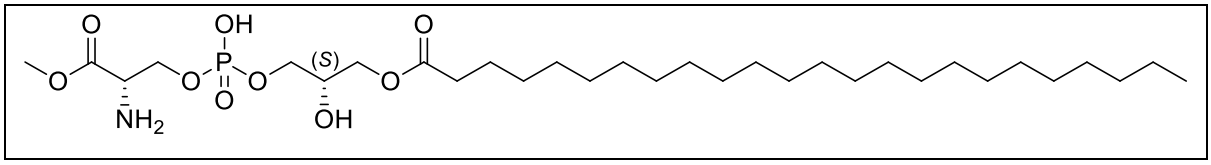
**(*S*)-2g**



**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docasanoate**

Following the general procedure (**C2**), **(*S*)-9g** (30 mg), TFA (0.240 mL) and $CH_2Cl_2$ (0.420 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2g** (21 mg, 0.0352 mmol, 74% yield relative to **(*S*)-8g**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.36-4.15 (m, 3H), 4.14-4.04 (m, 2H), 4.03-3.90 (m, 2H), 3.84 (s, 3H), 3.77-3.55 (m, 1H), 2.32 (t, $J$ = 7.4Hz, 2H), 1.59 (m, 2H), 1.33-1.19 (m, 36H), 0.85 (t, $J$ = 6.3 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.45; HRMS (ESI): m/z calcd. for ($C_{29}H_{59}NO_9P$) $[M + H]^+$ 596.3922; found, 596.3924. Purity > 95%.

**(*S*)-2h**

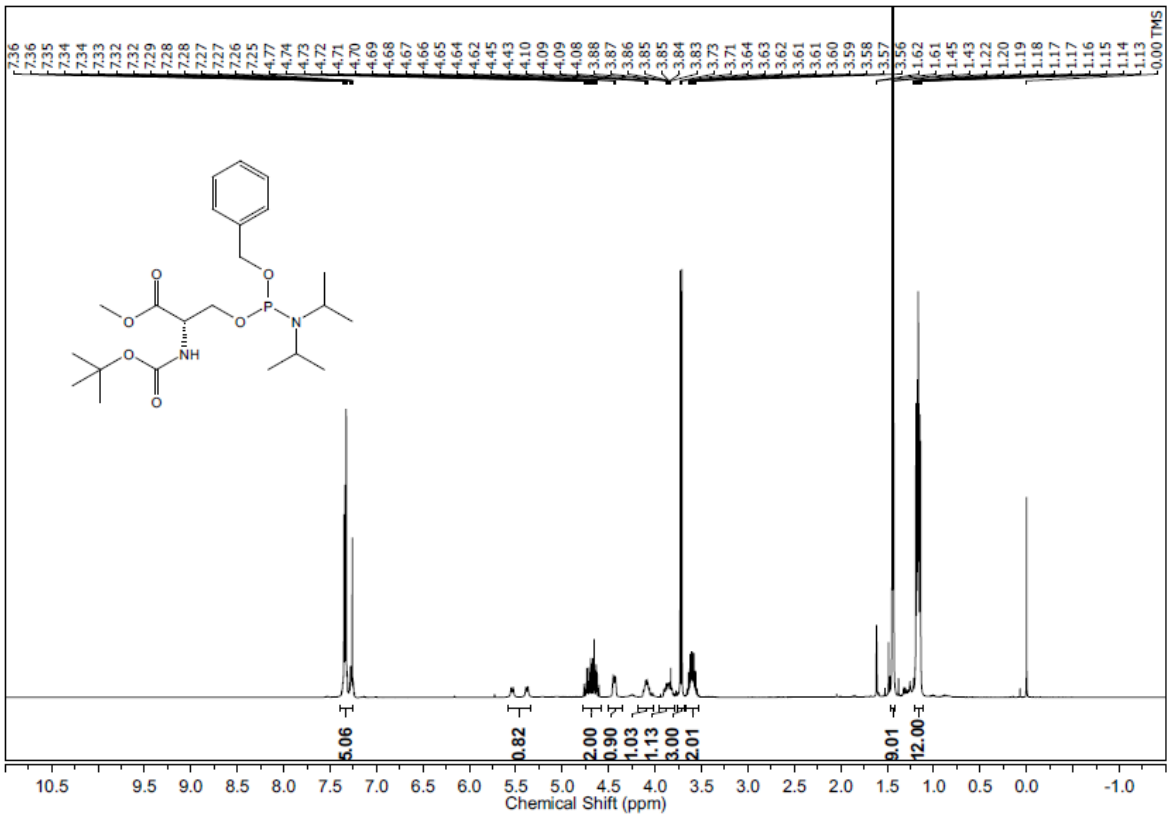


**(2*S*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate**

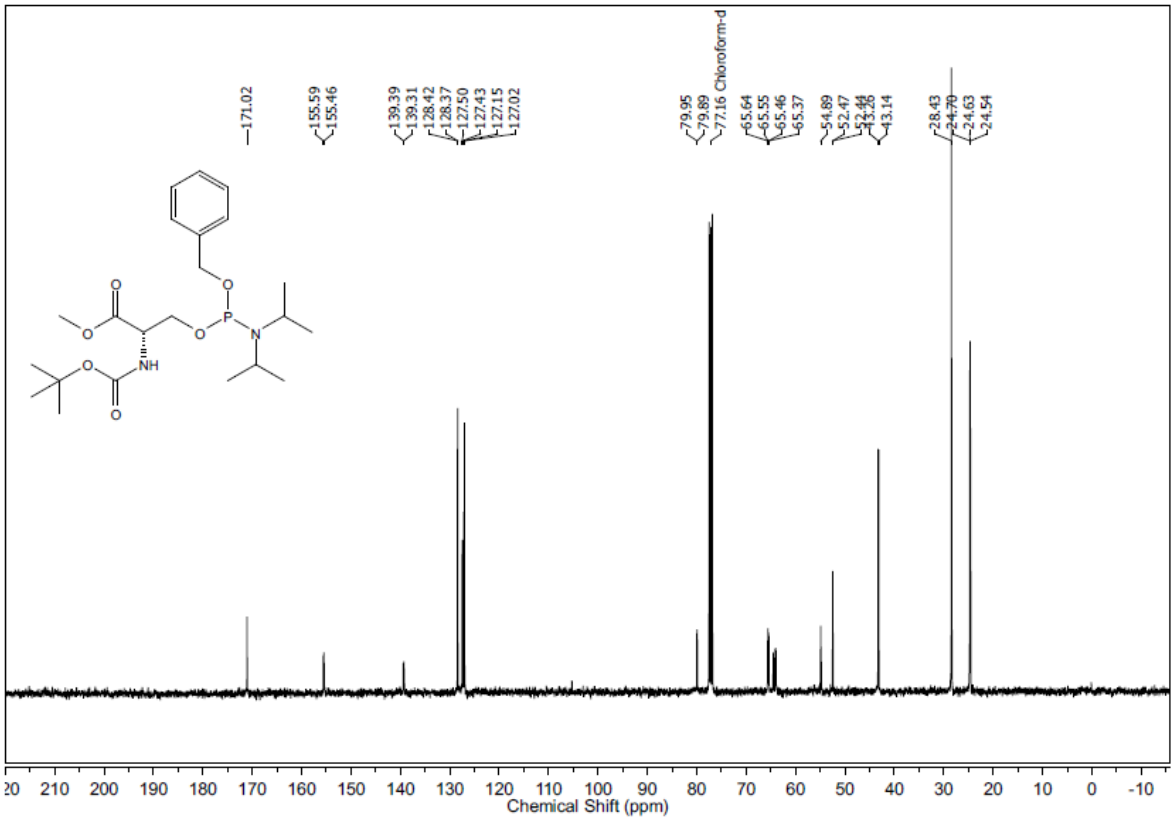
Following the general procedure (**C2**), **(*S*)-9h** (26 mg), TFA (0.210 mL) and $CH_2Cl_2$ (0.360 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield the desired final compound **(*S*)-2h** (15 mg, 0.0240 mmol, 81% yield relative to **(*S*)-8h**) as a white solid: $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) $\delta$ 4.34-4.05 (m, 5H), 4.03-3.88 (m, 3H), 3.84 (s, 3H), 3.72-3.53 (m, 1H), 2.31 (m, 2H), 1.58 (m, 2H), 1.33-1.14 (m, 40H), 0.85 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$) $\delta$ 0.44; HRMS (ESI): m/z calcd. for ($C_{31}H_{63}NO_9P$) $[M + H]^+$ 624.4235; found, 634.4233. Purity > 97%.

## 3.8 Spectral Data

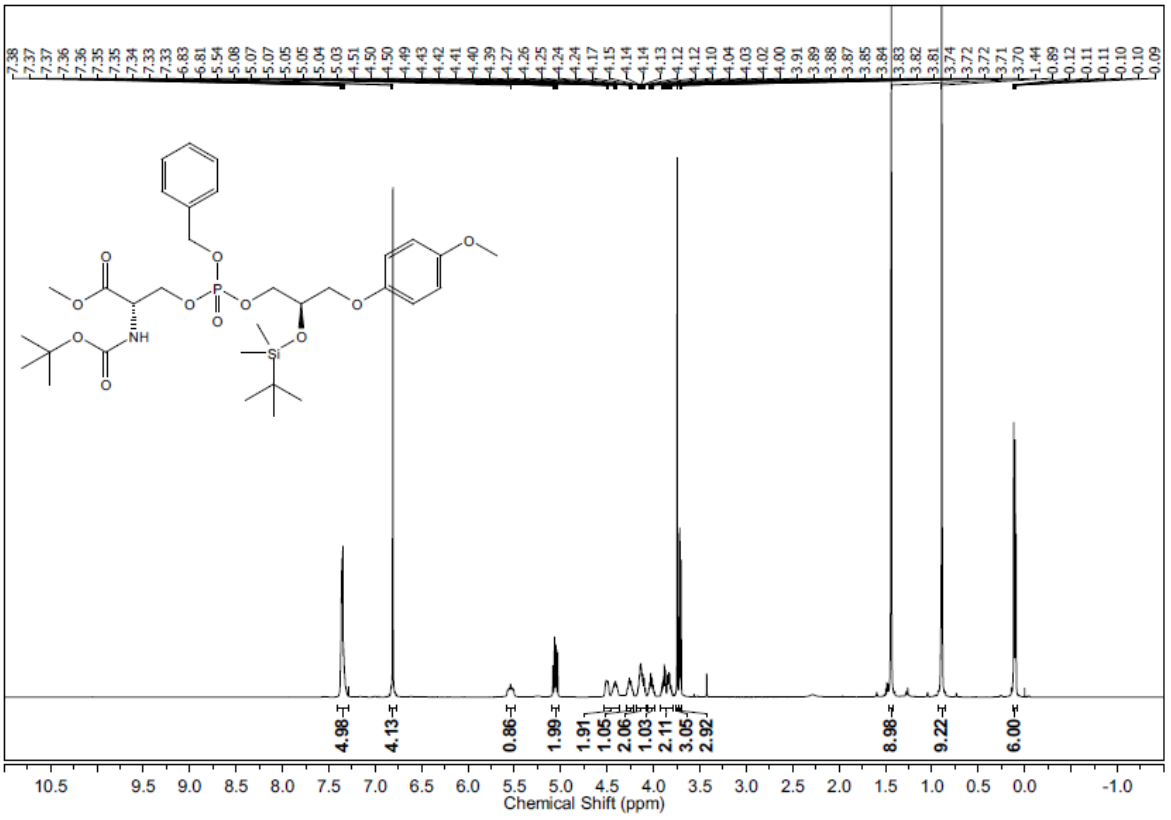
**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound 3**



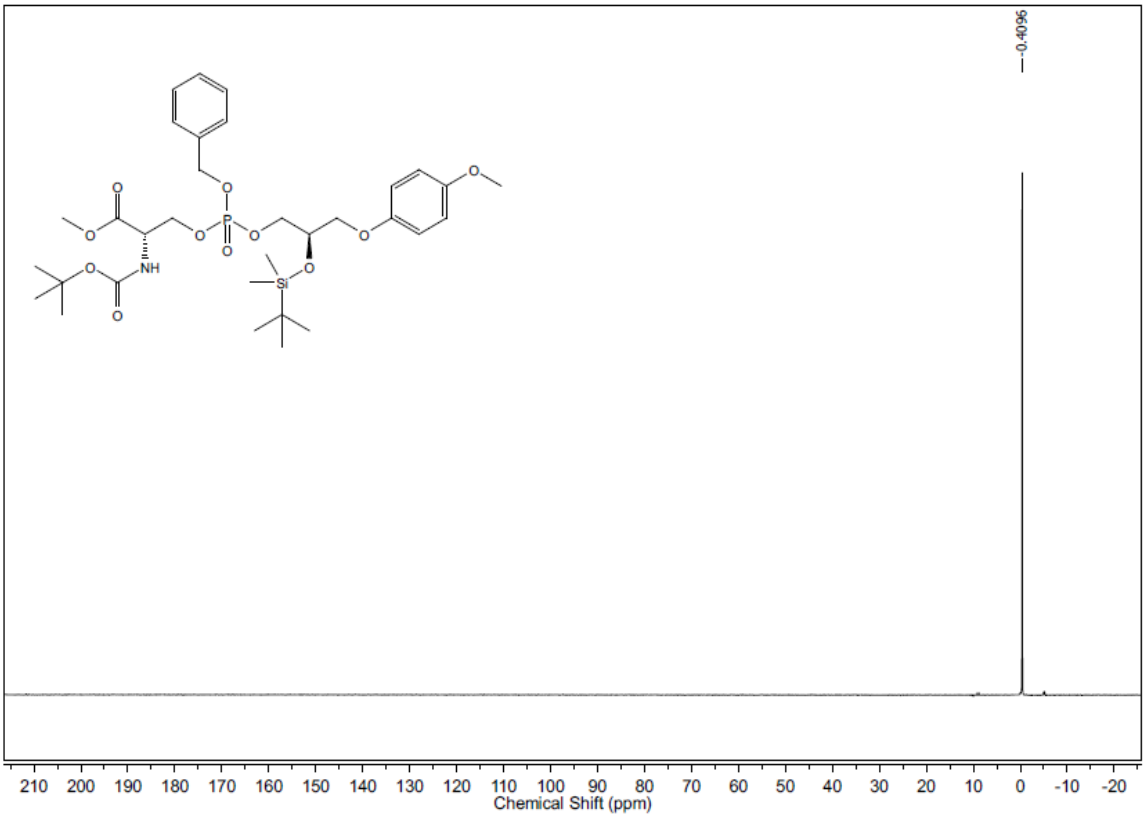
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound 3



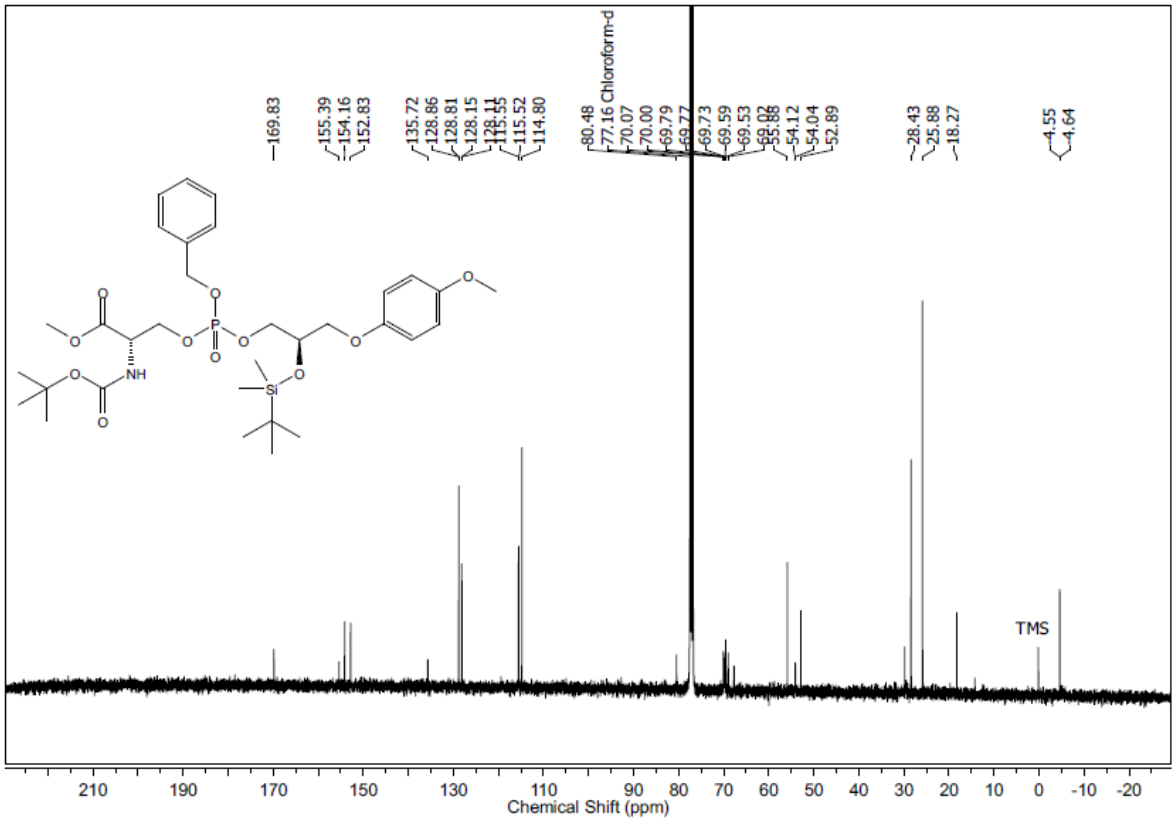

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-5



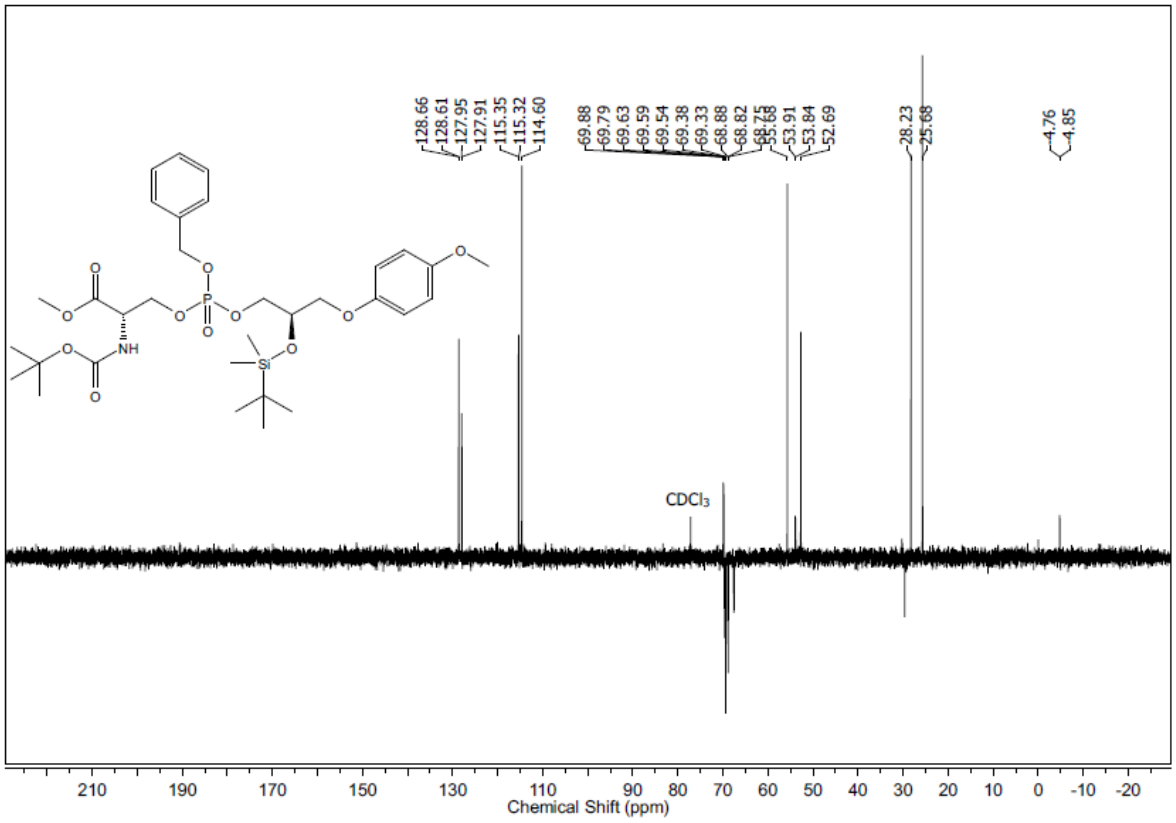
**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Compound (*R*)-5**



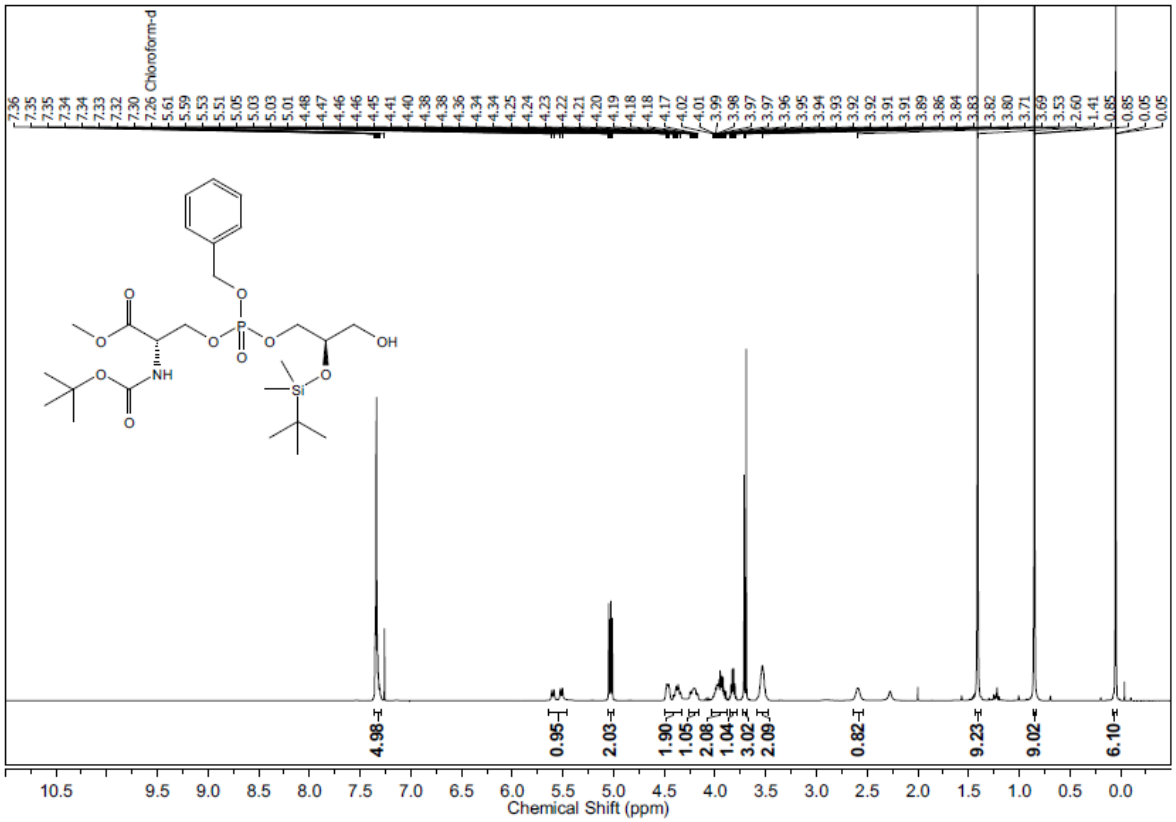

**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-5**



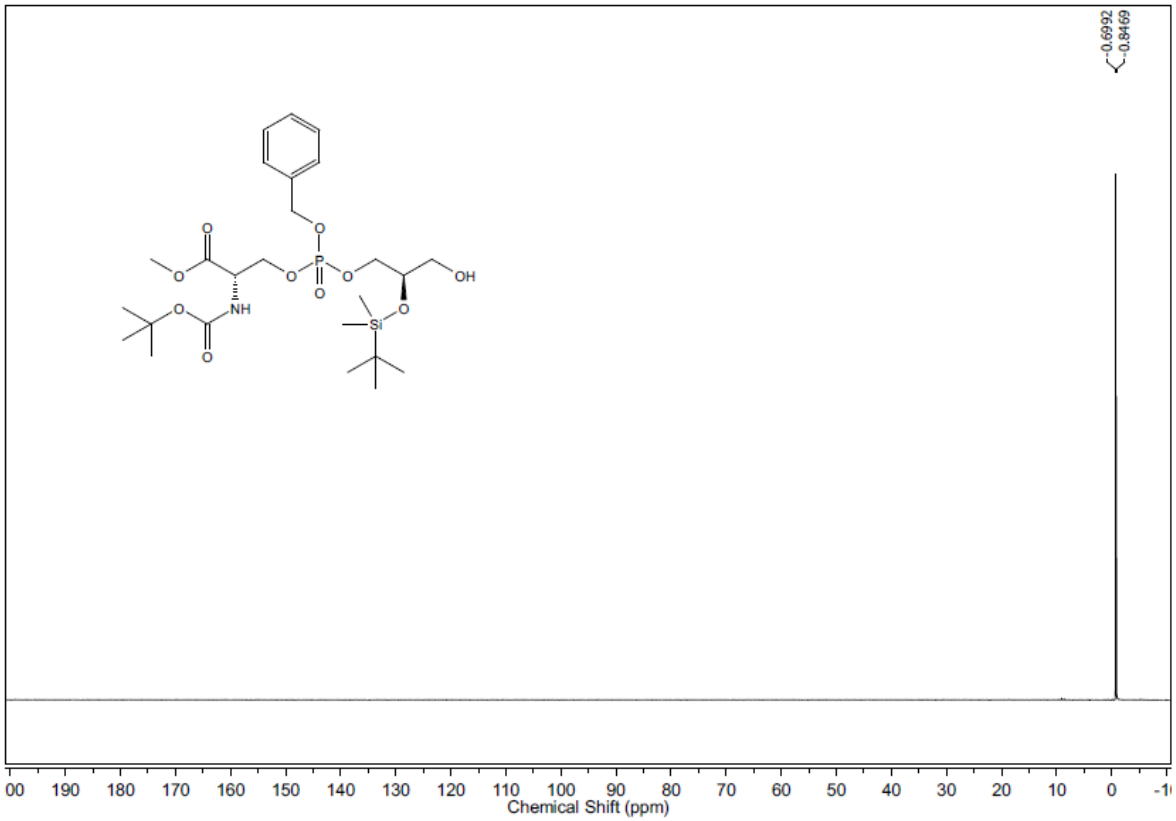
**DEPT-135 NMR ($CDCl_3$, 100 MHz) for compound (*R*)-5**



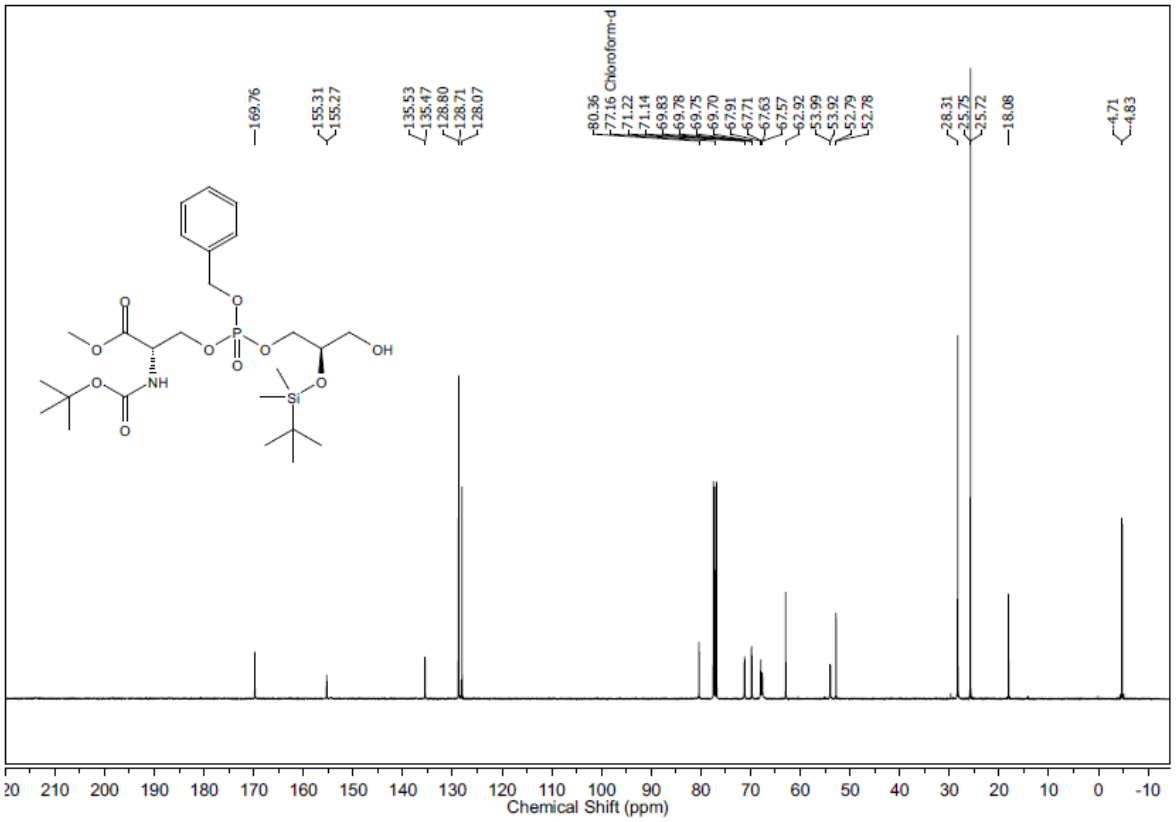

**$^1$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-6**



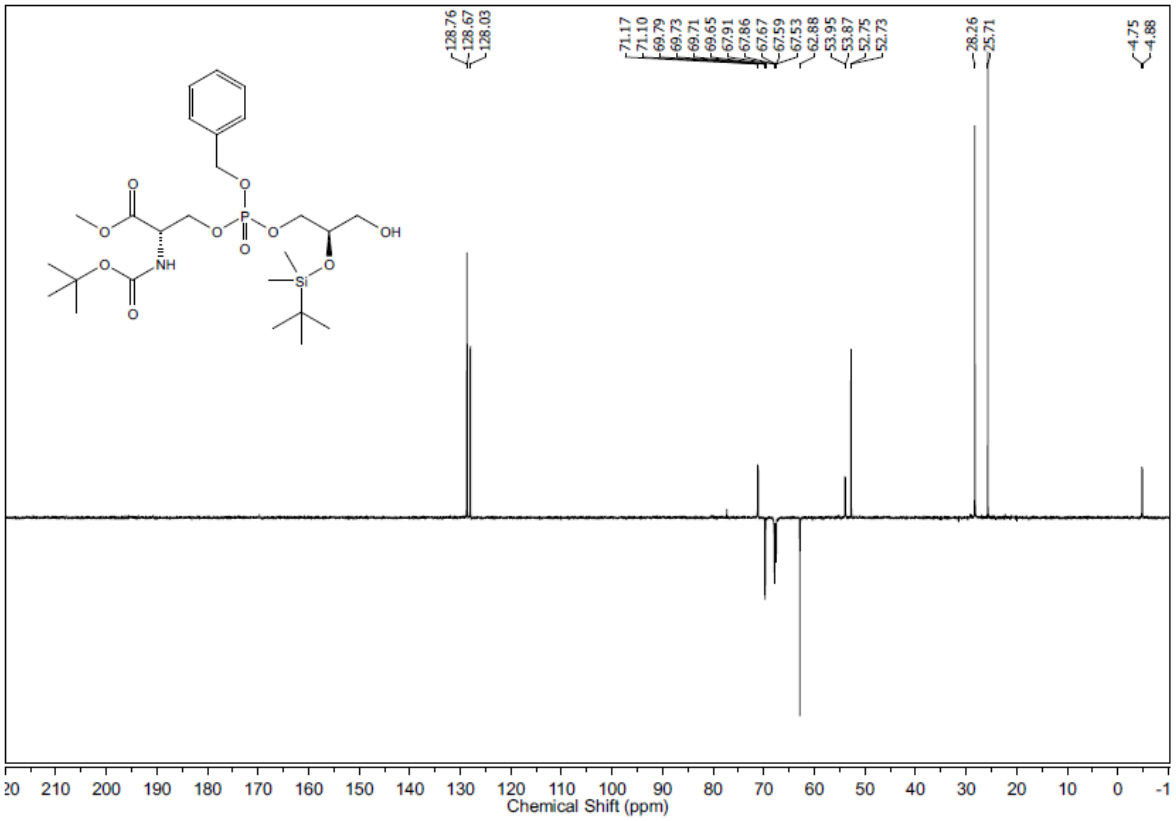
**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Compound (*R*)-6**



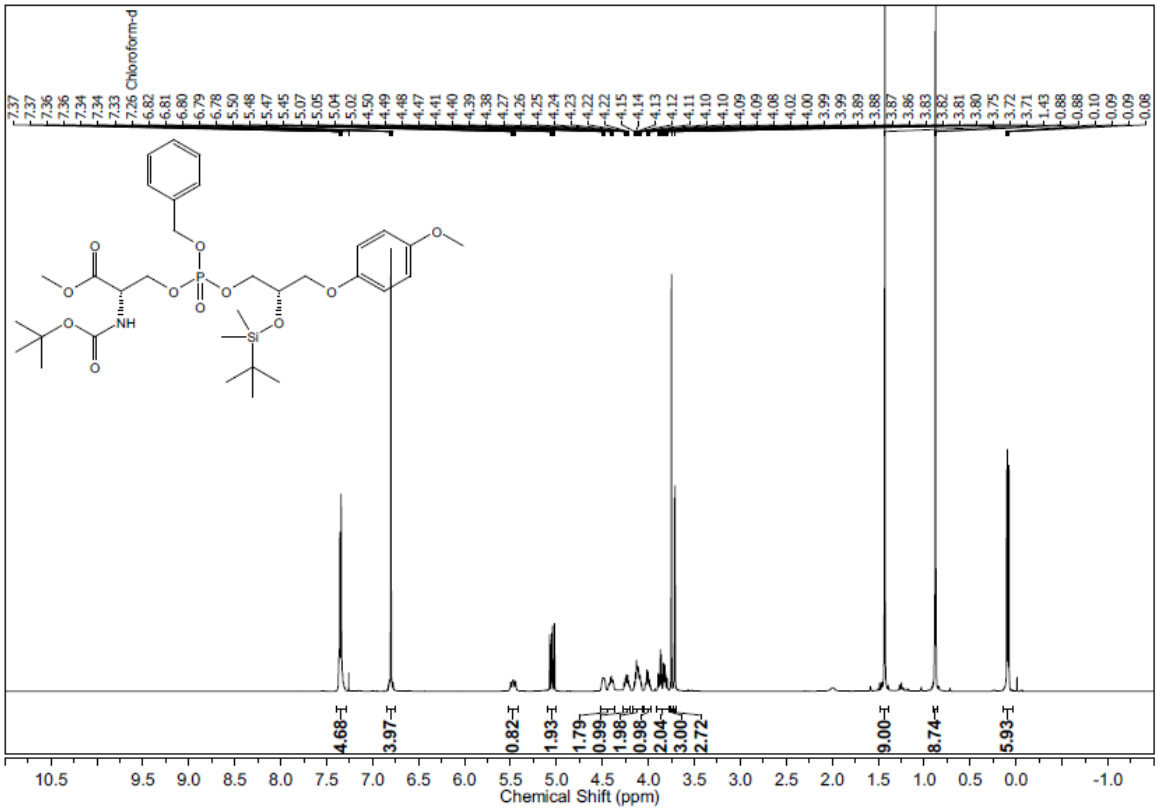

**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-6**



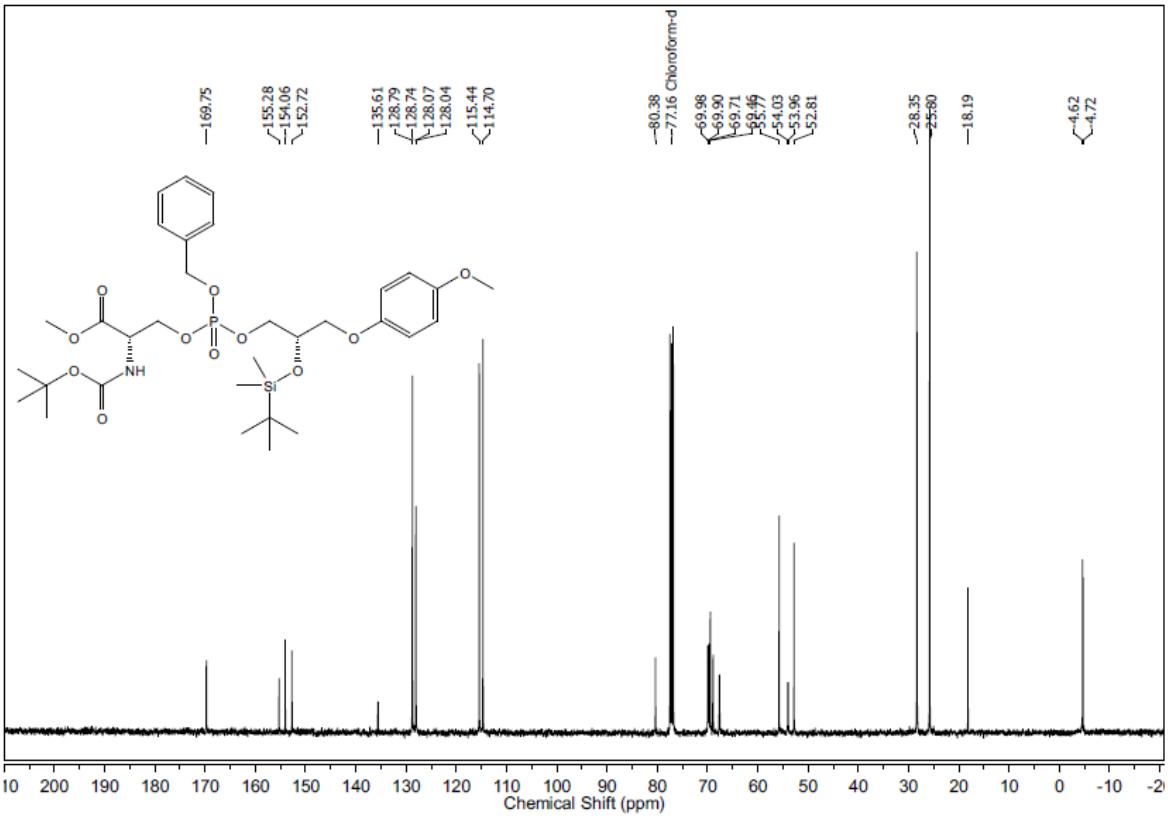
**DEPT-135 NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-6**



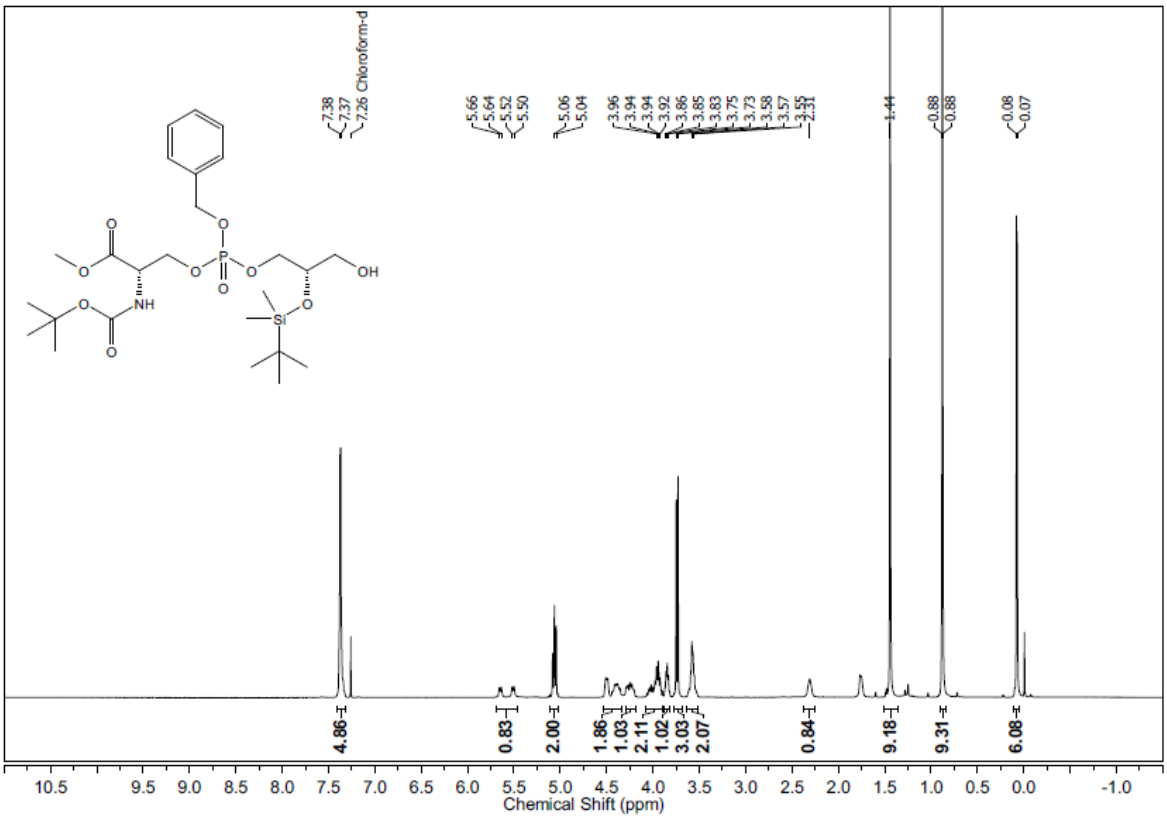

**$^1$H-NMR ($CDCl_3$, 400 MHz) for Compound (*S*)-5**



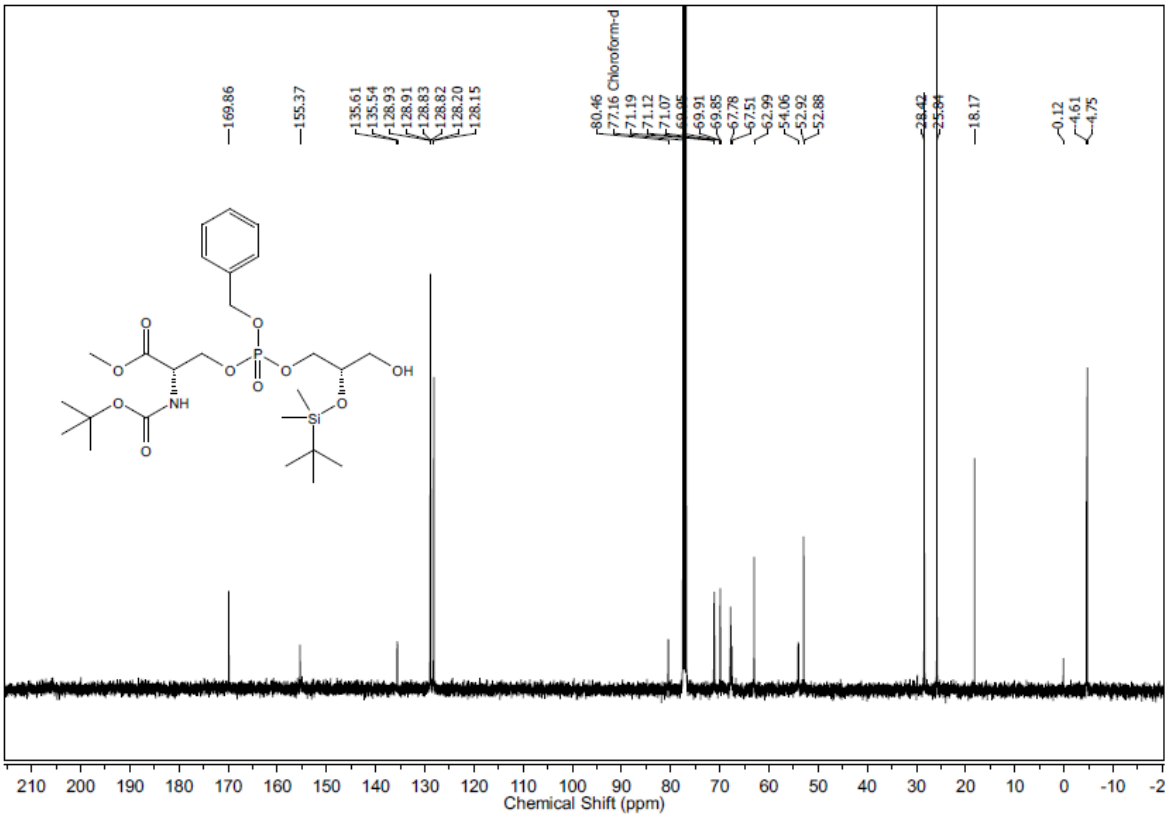
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*S*)-5**



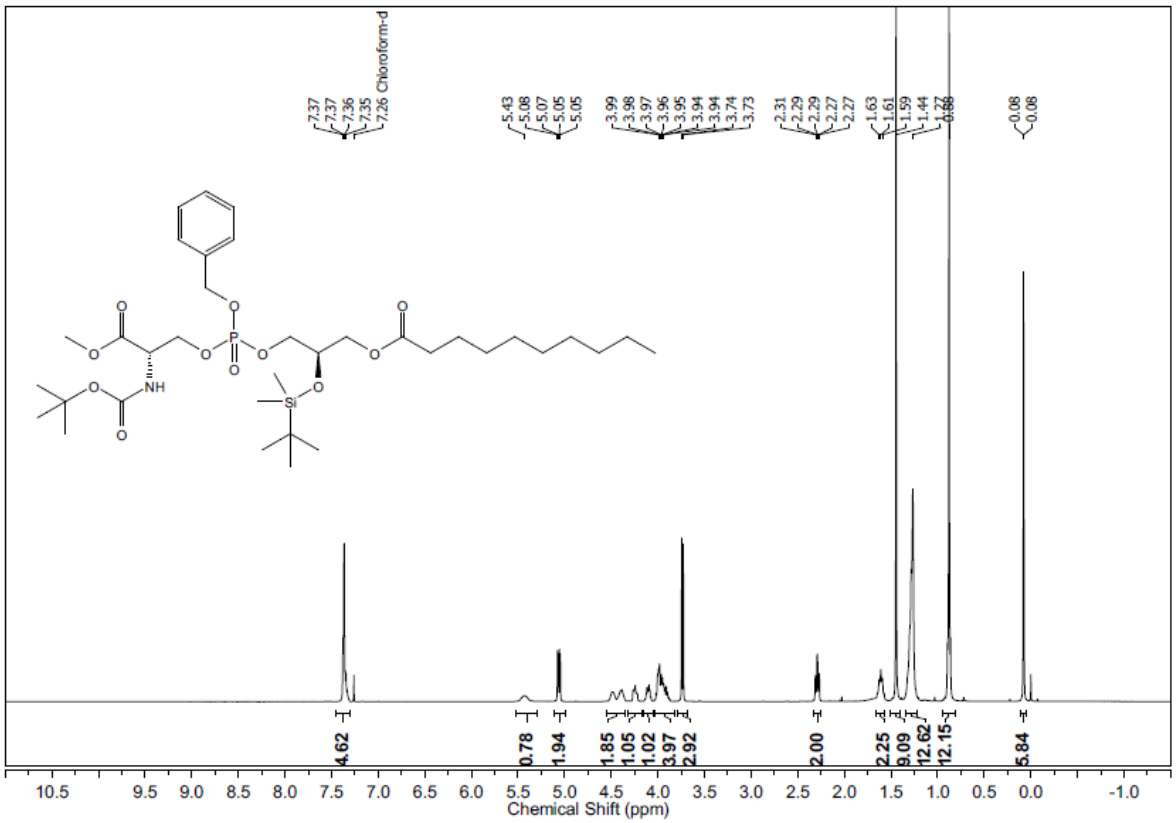

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*S*)-6**



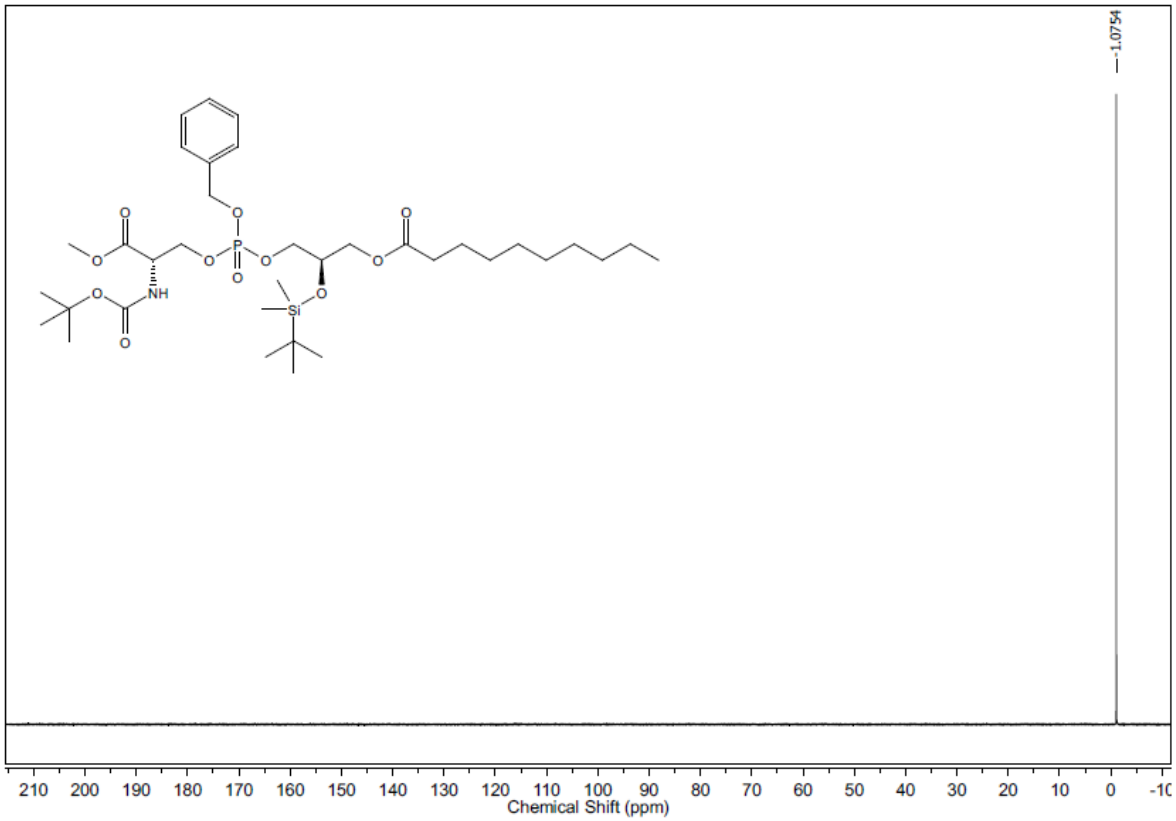
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*S*)-6**



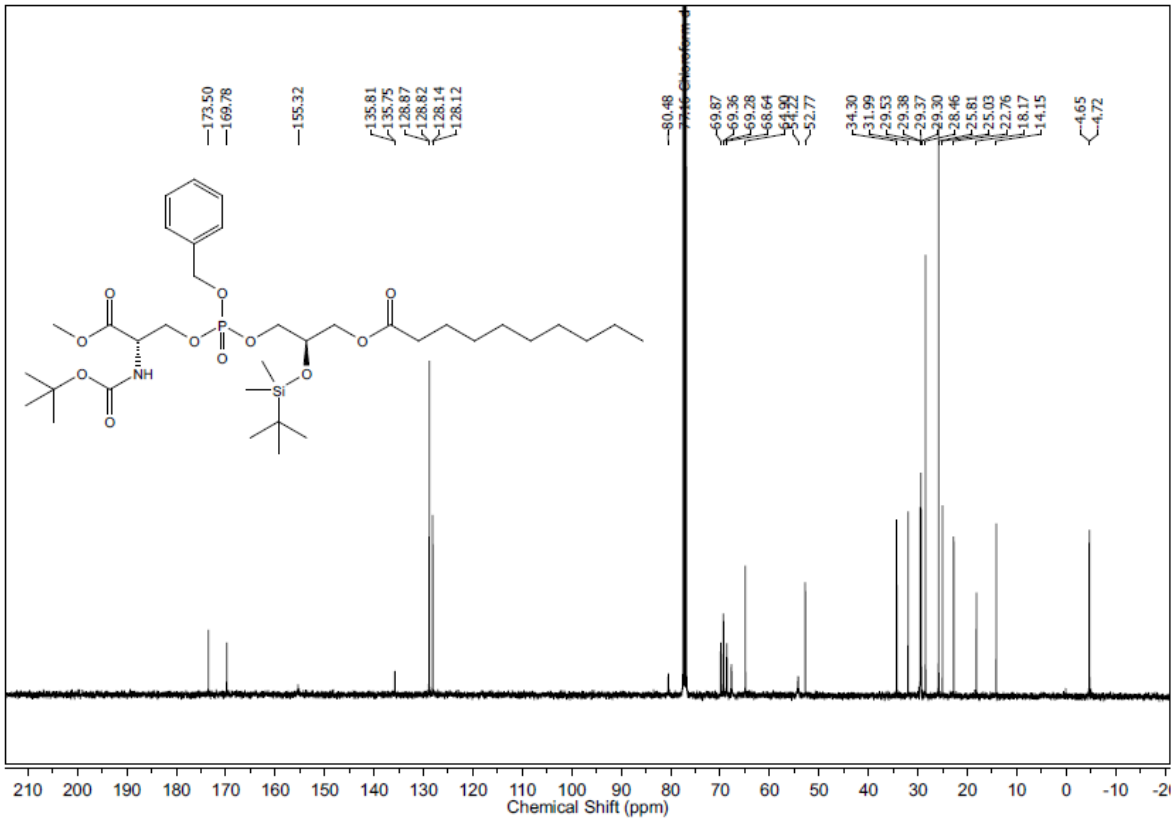

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-8a**



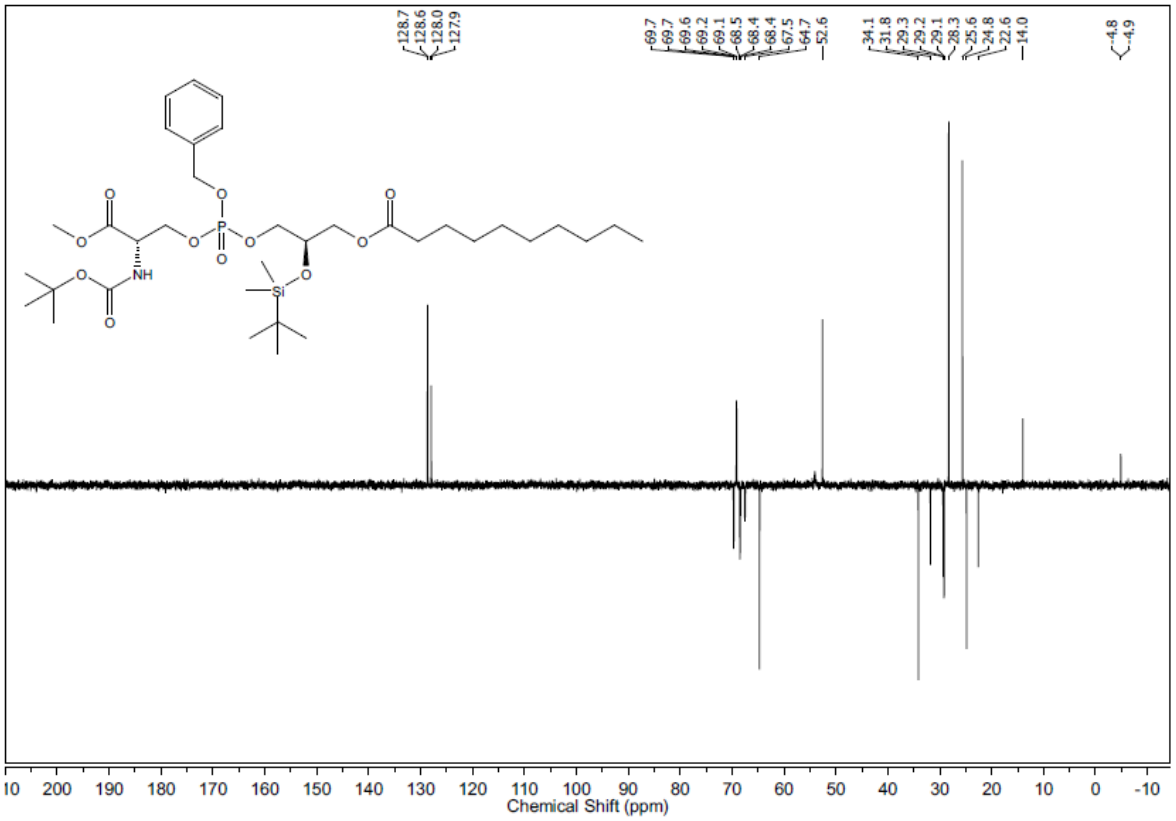
## $^{31}$P-NMR ($CDCl_3$, 400MHz) for Compound (*R*)-8a



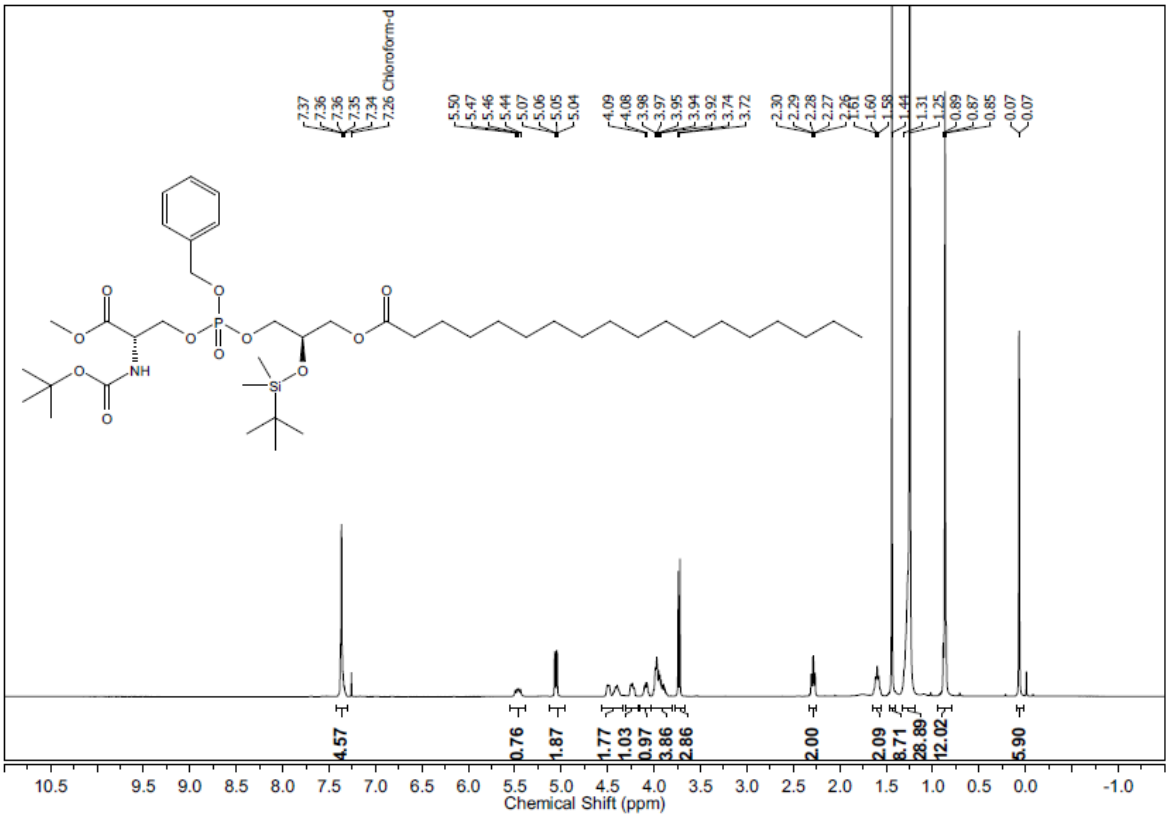

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-8a



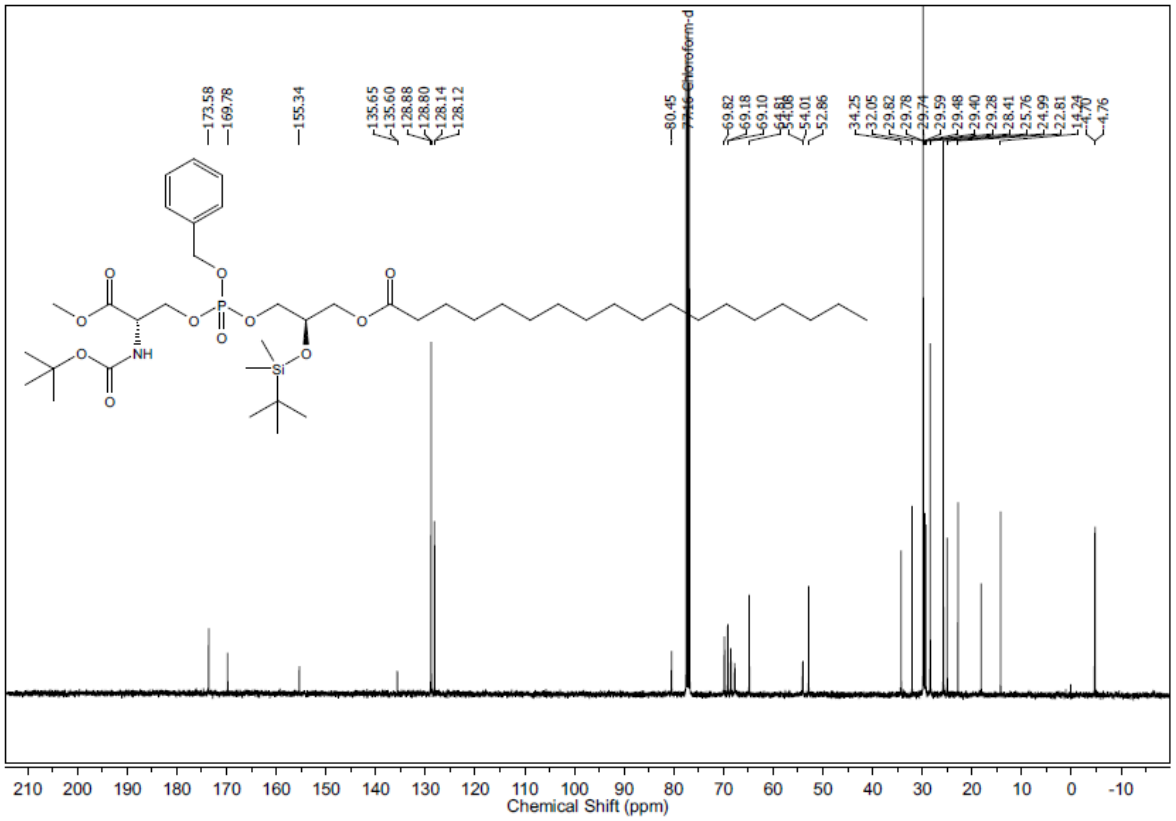
**DEPT-135 NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-8a**



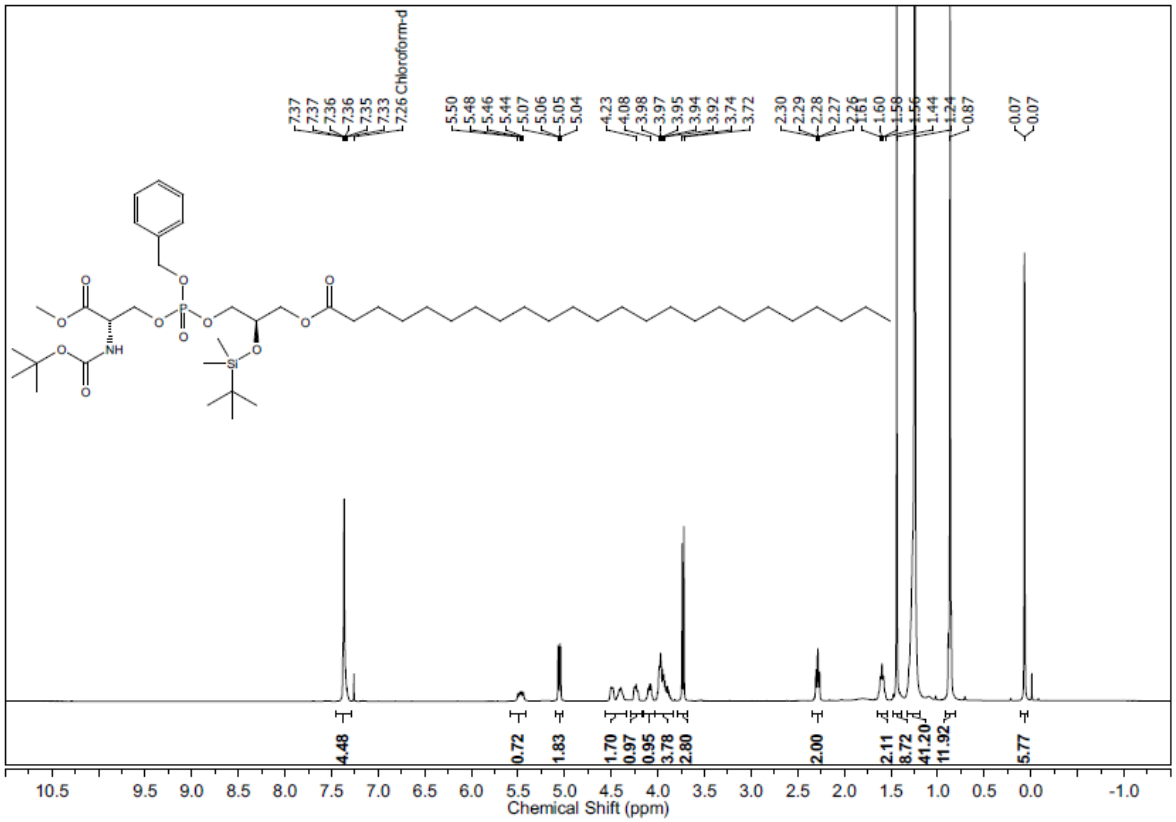

**$^1$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-8e**



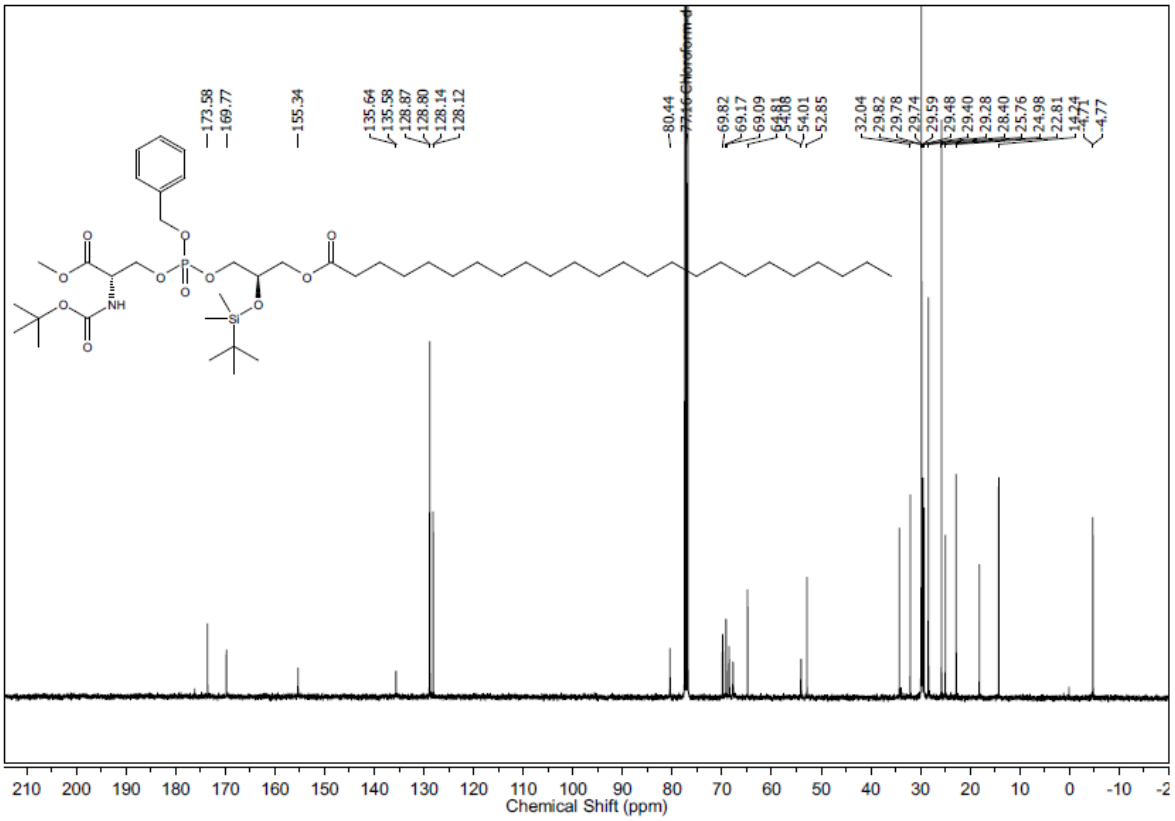
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-8e**



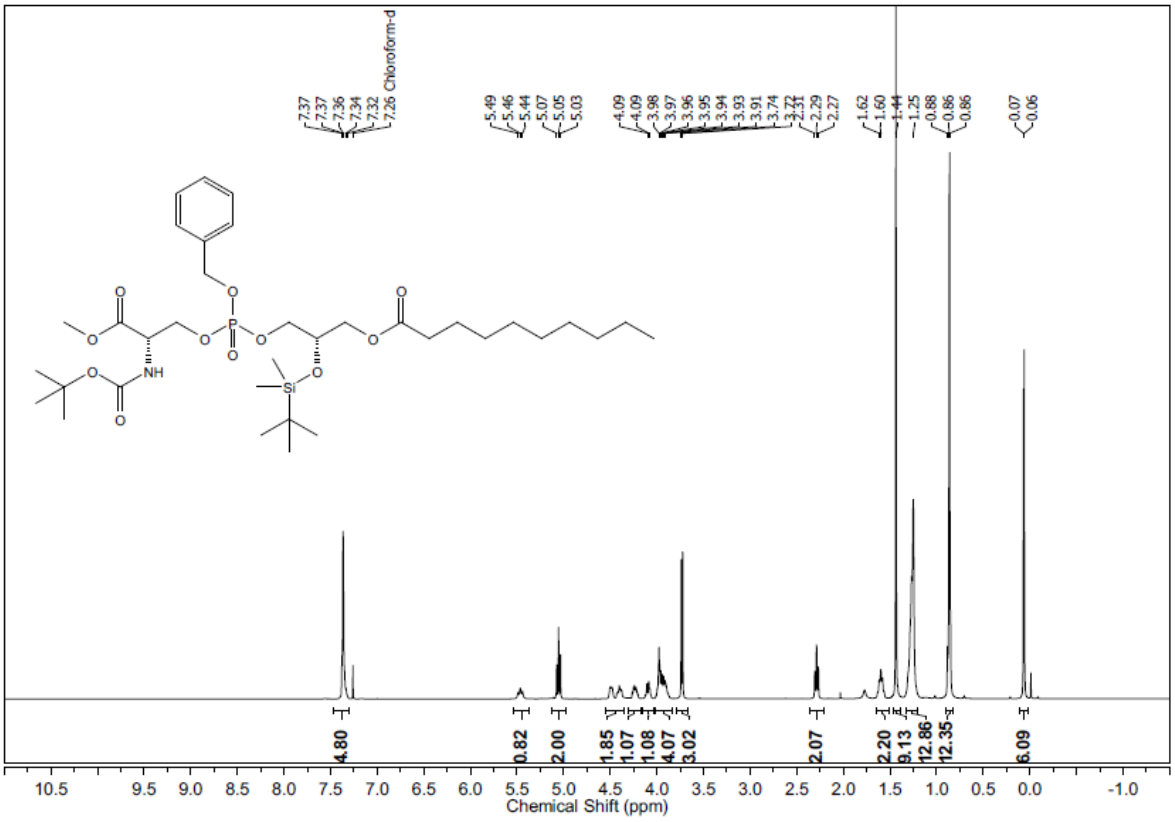

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-8h**



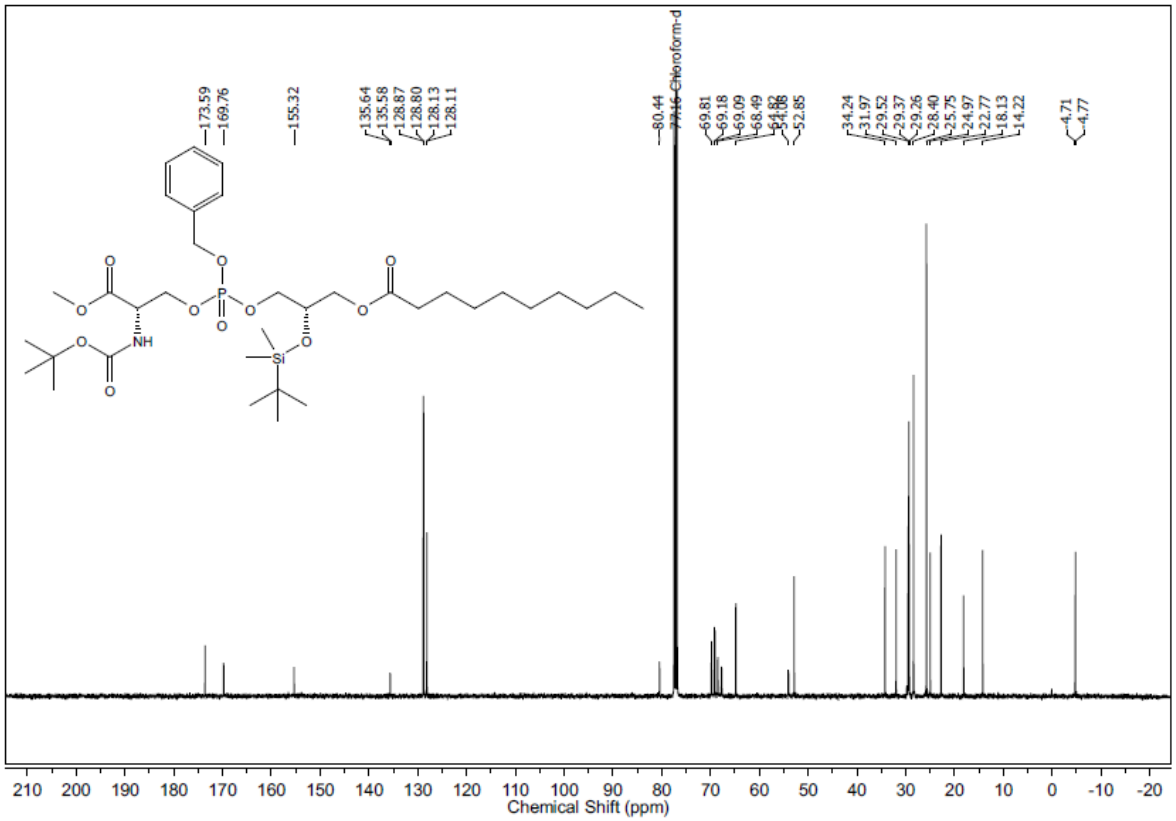
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-8h**



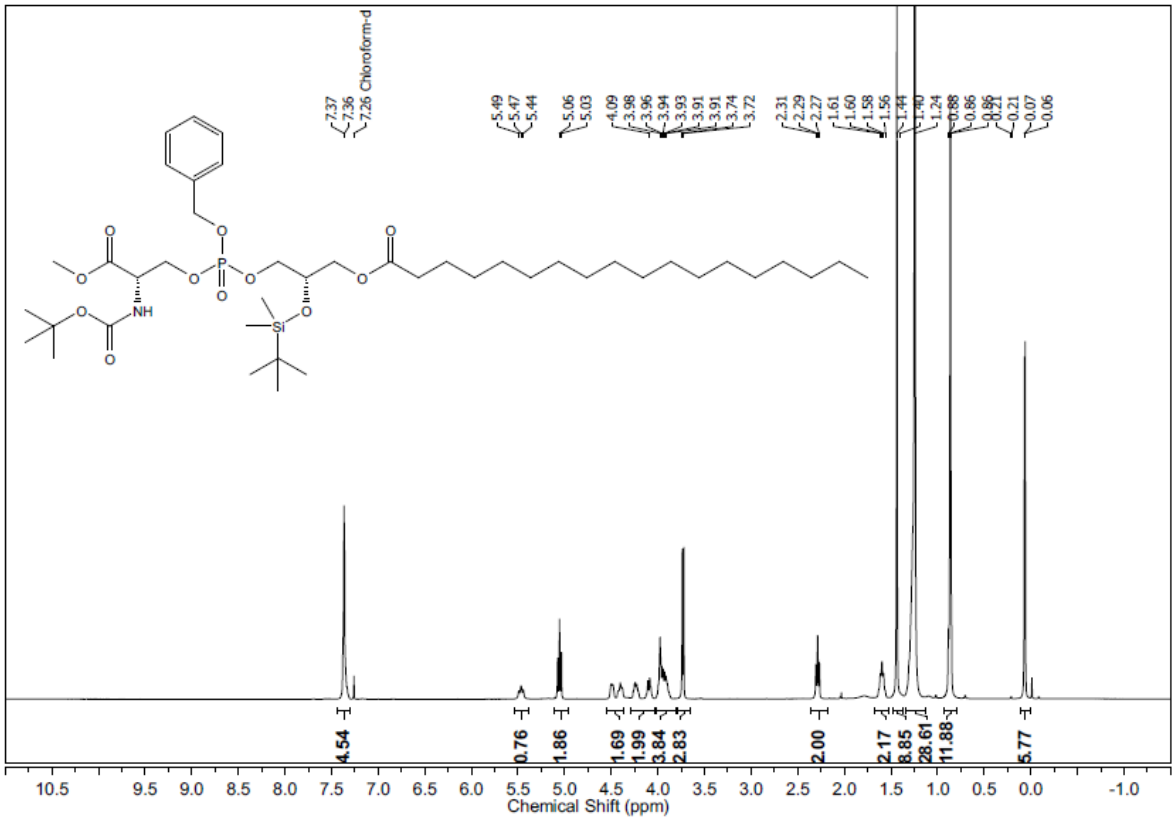

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*S*)-8a**



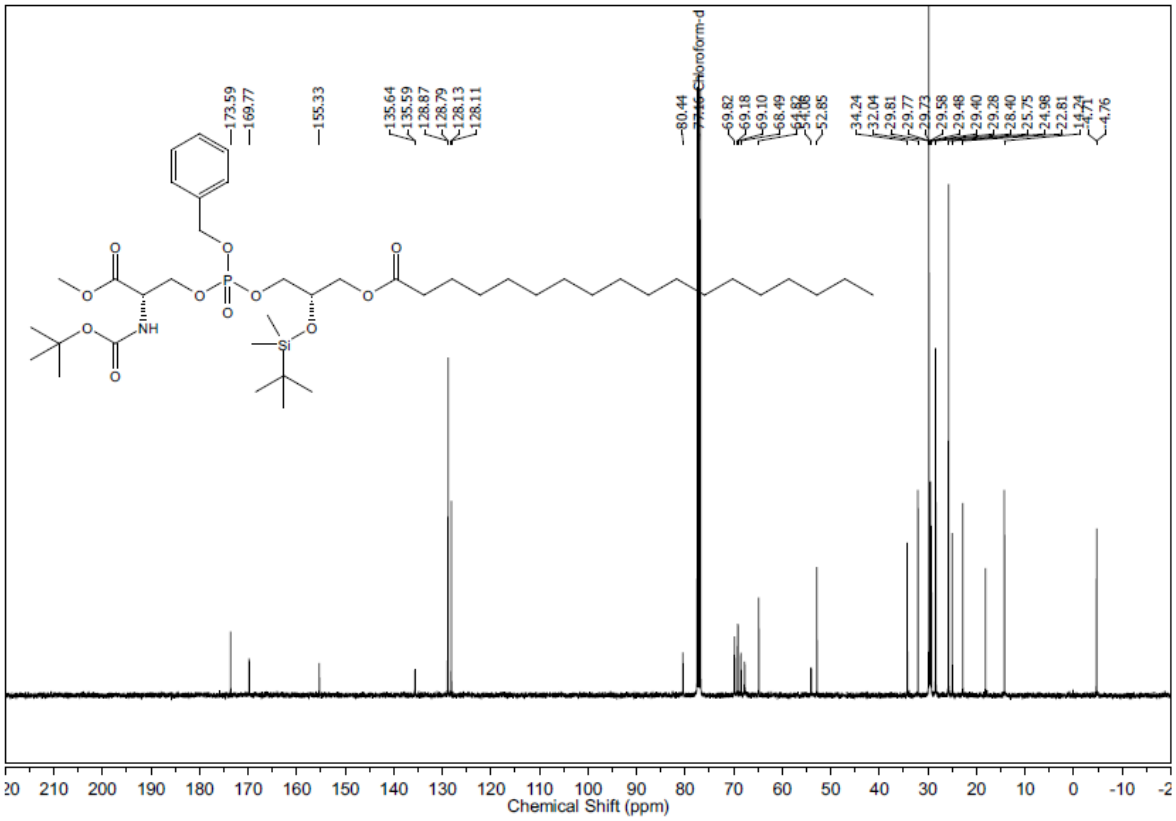
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*S*)-8a**



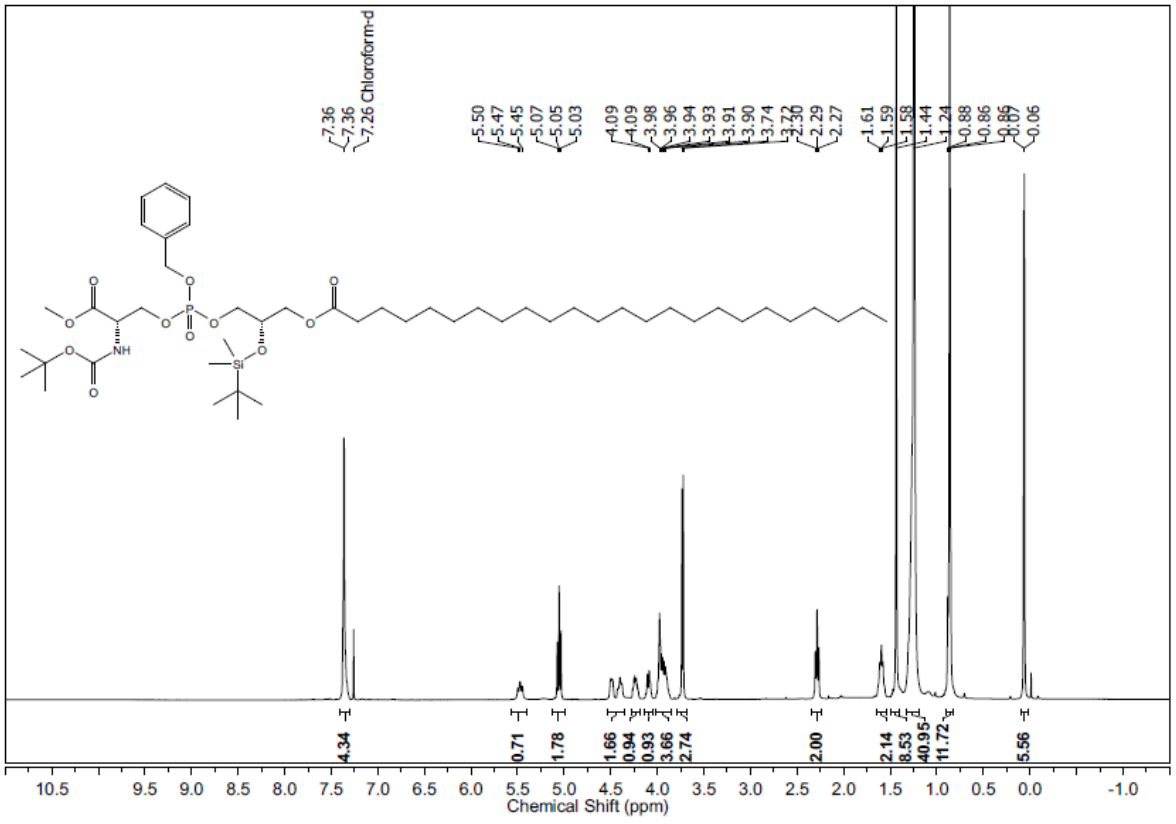

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*S*)-8e**



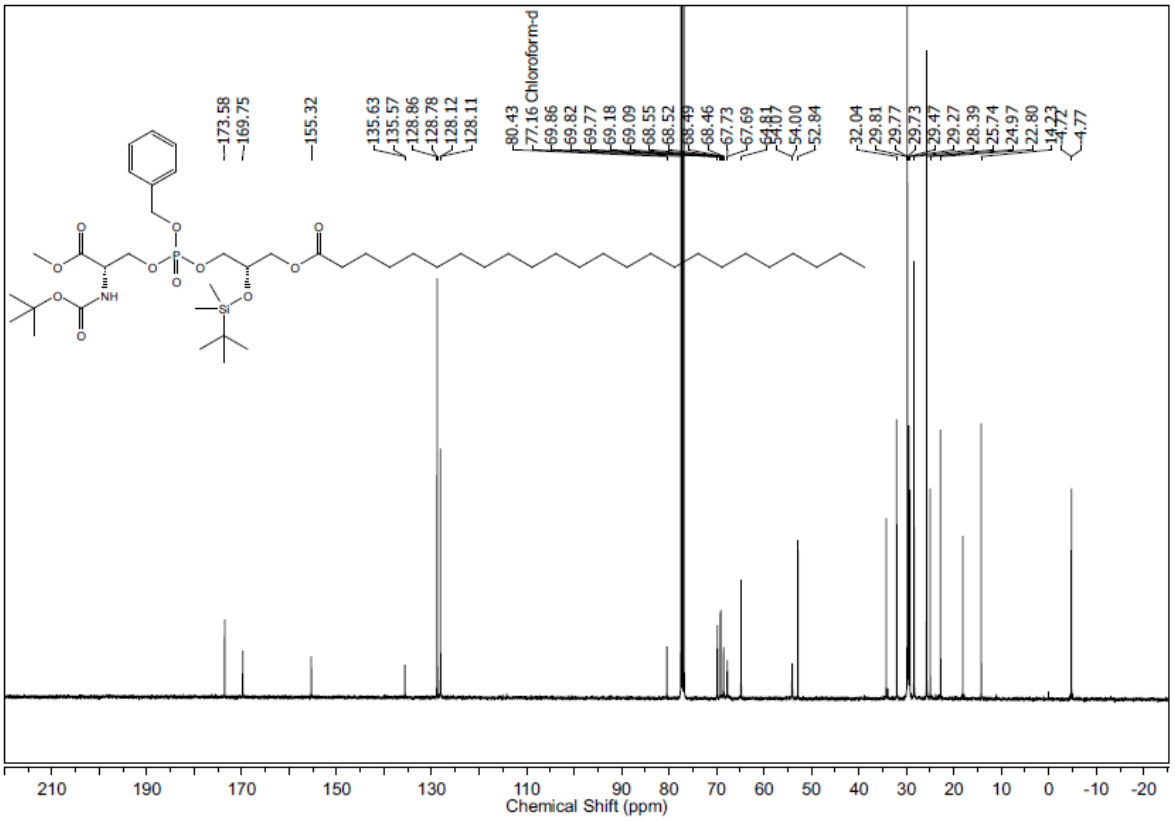
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*S*)-8e**



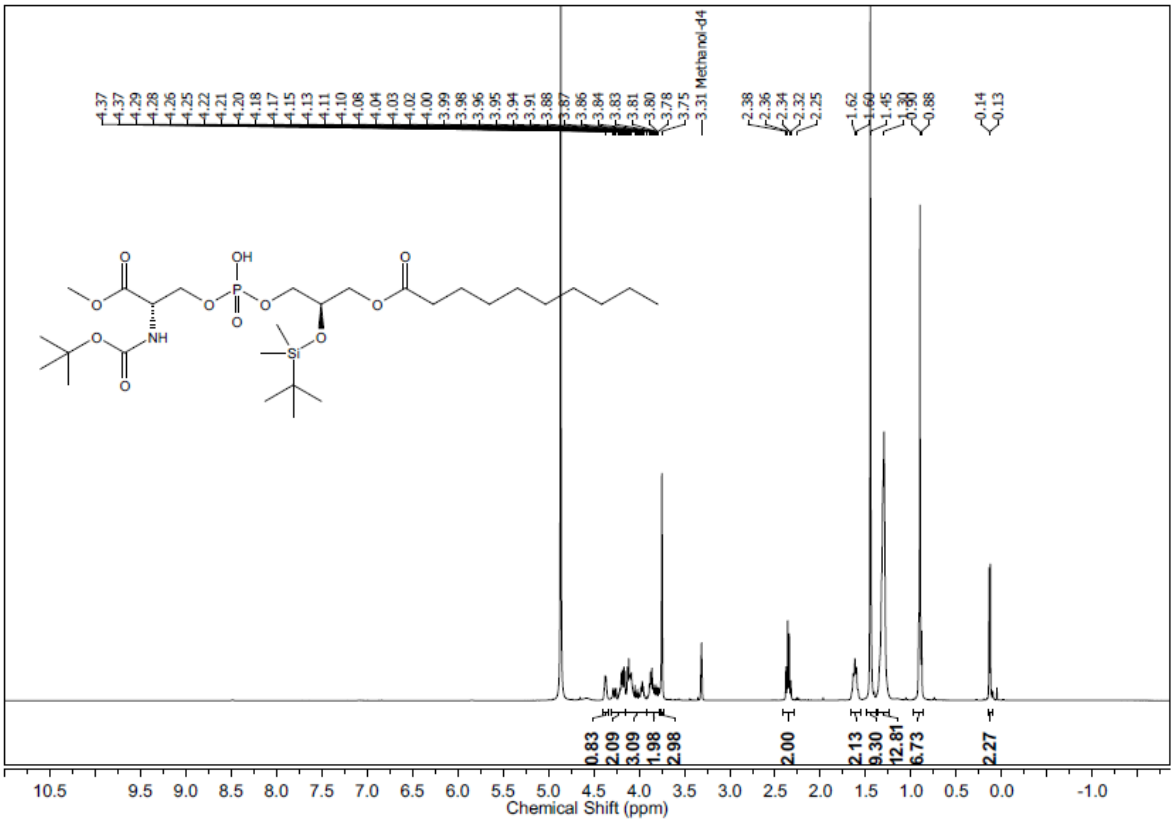

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*S*)-8h**



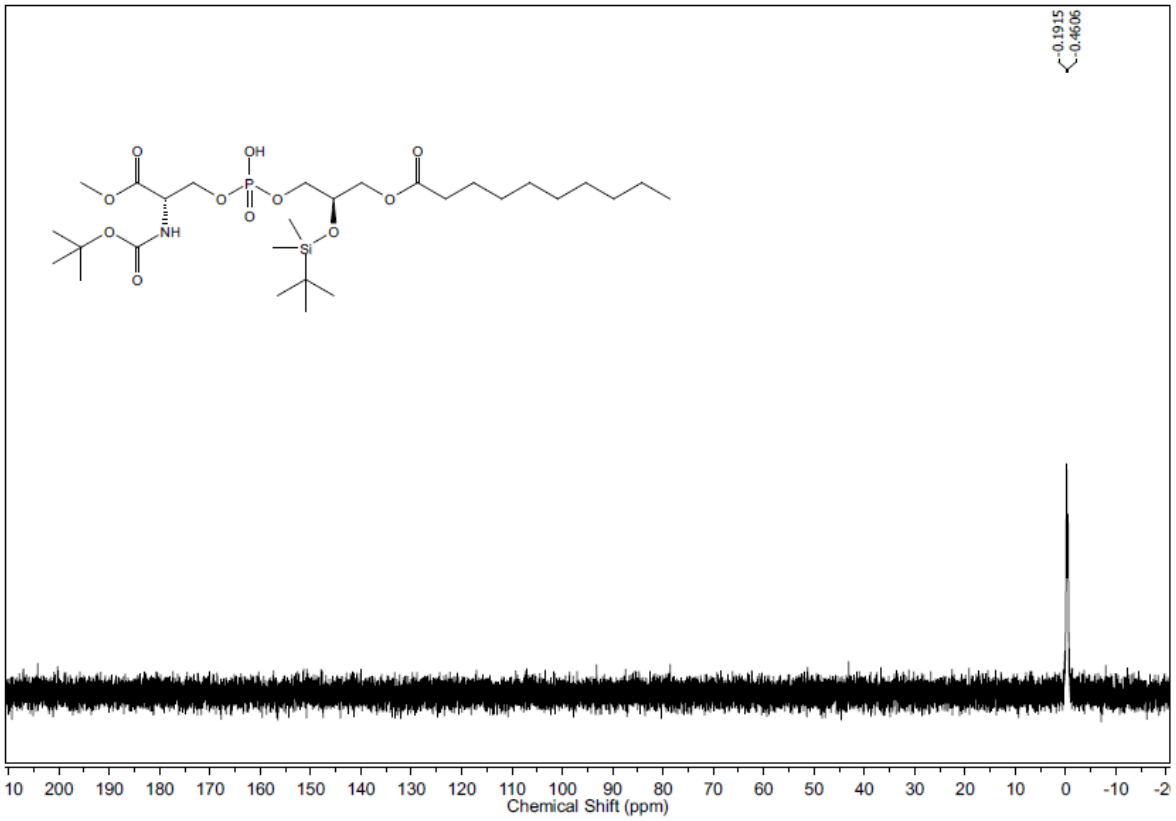
**$^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*S*)-8h**



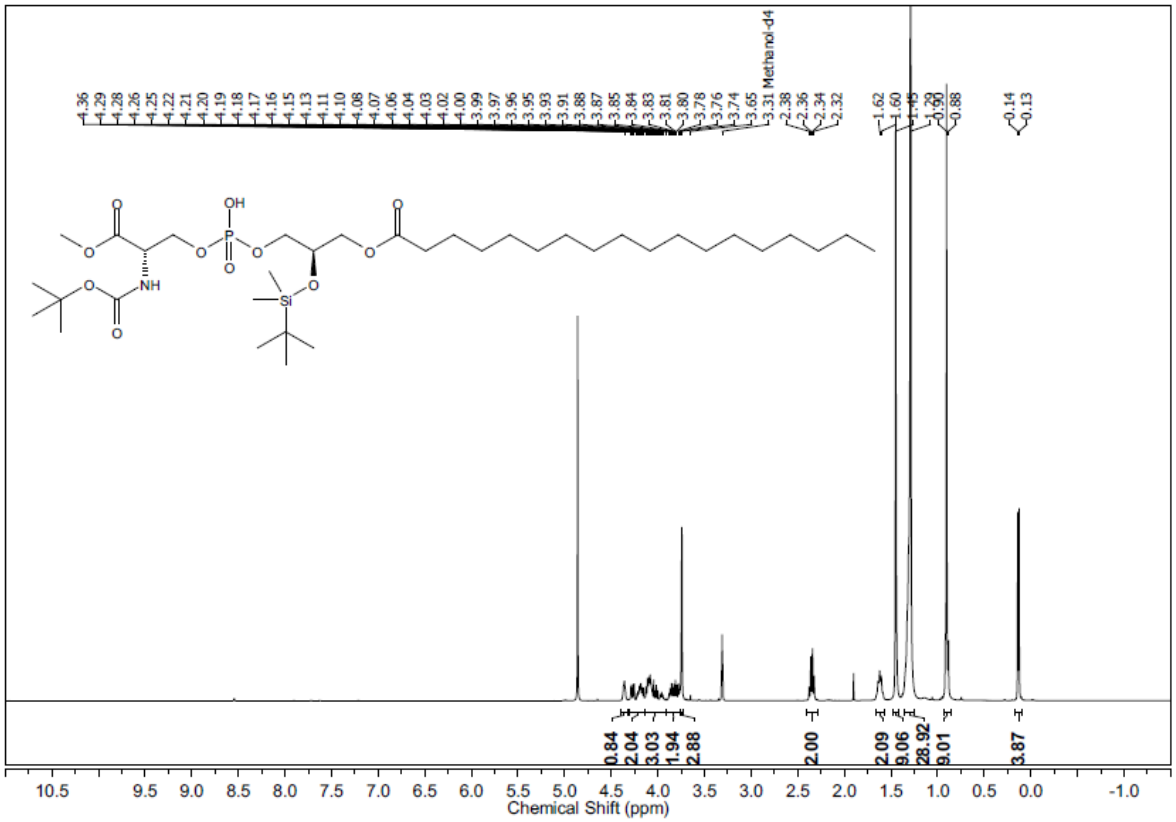

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*R*)-9a**



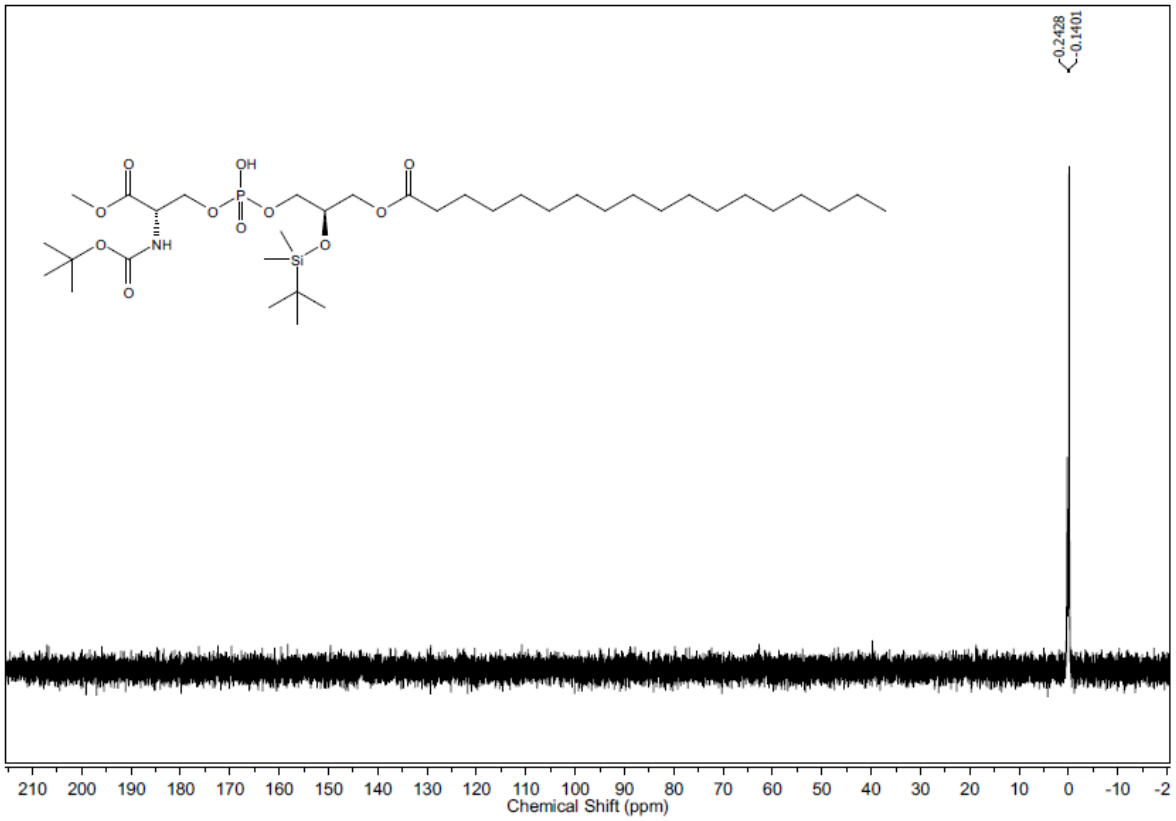
**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Intermediate (*R*)-9a**



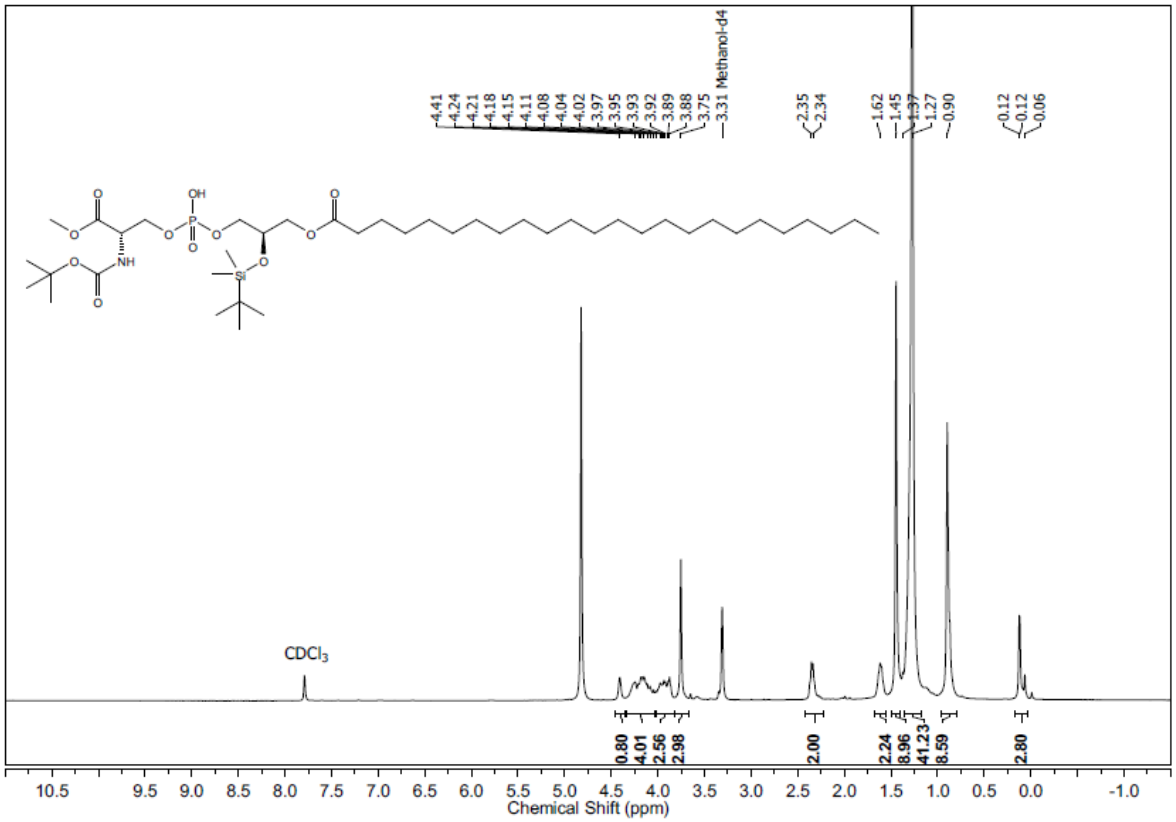

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*R*)-9e**



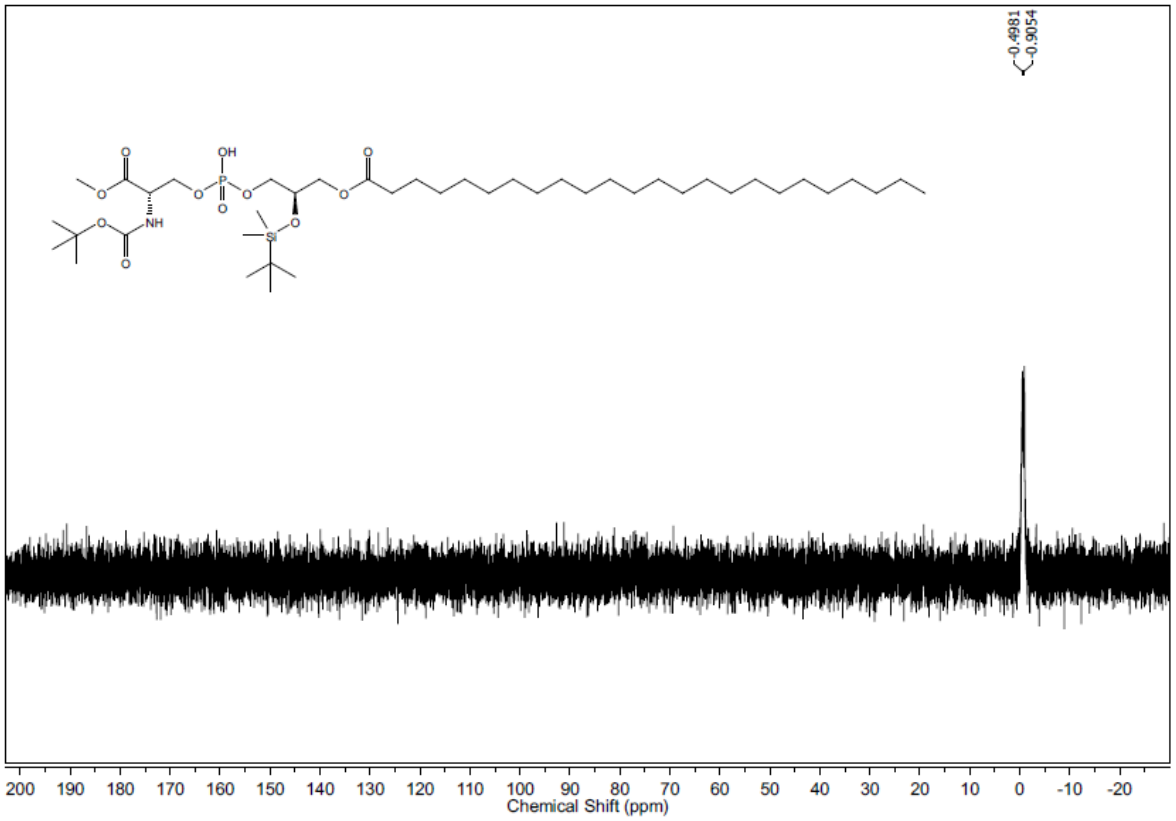
**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Intermediate (*R*)-9e**



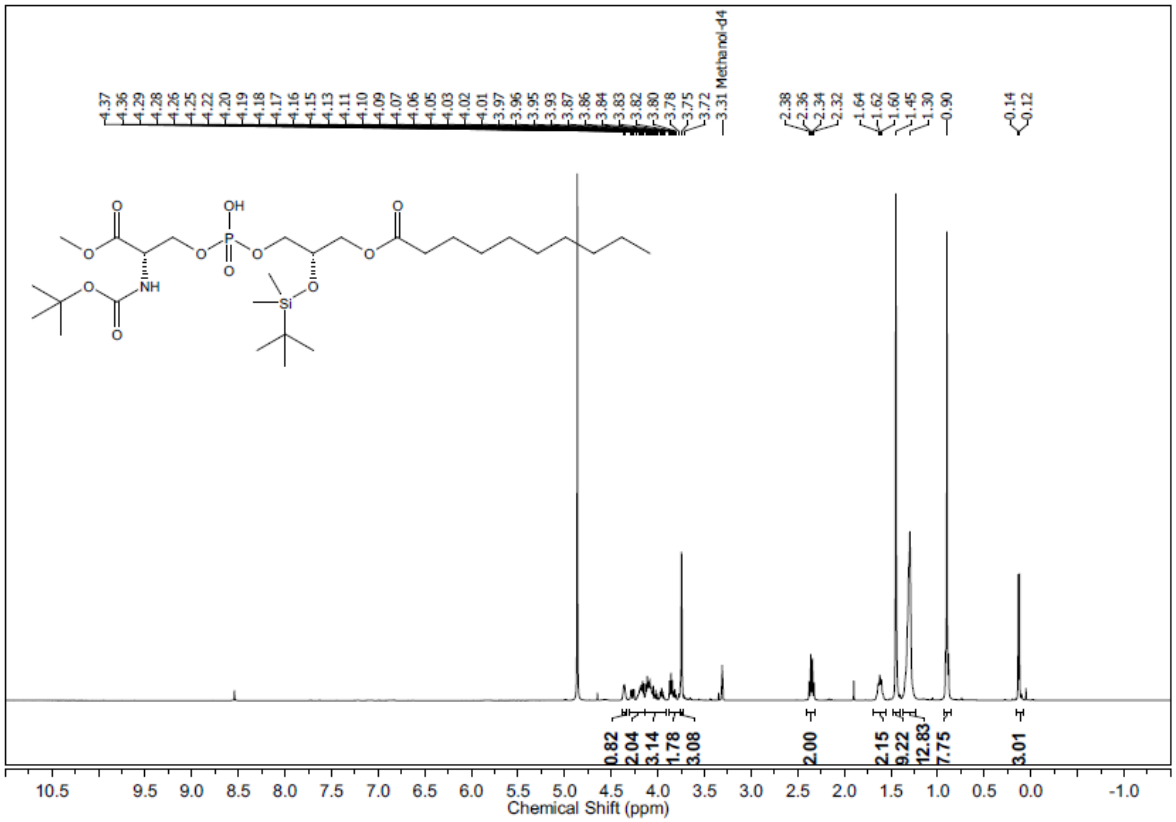

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*R*)-9h**



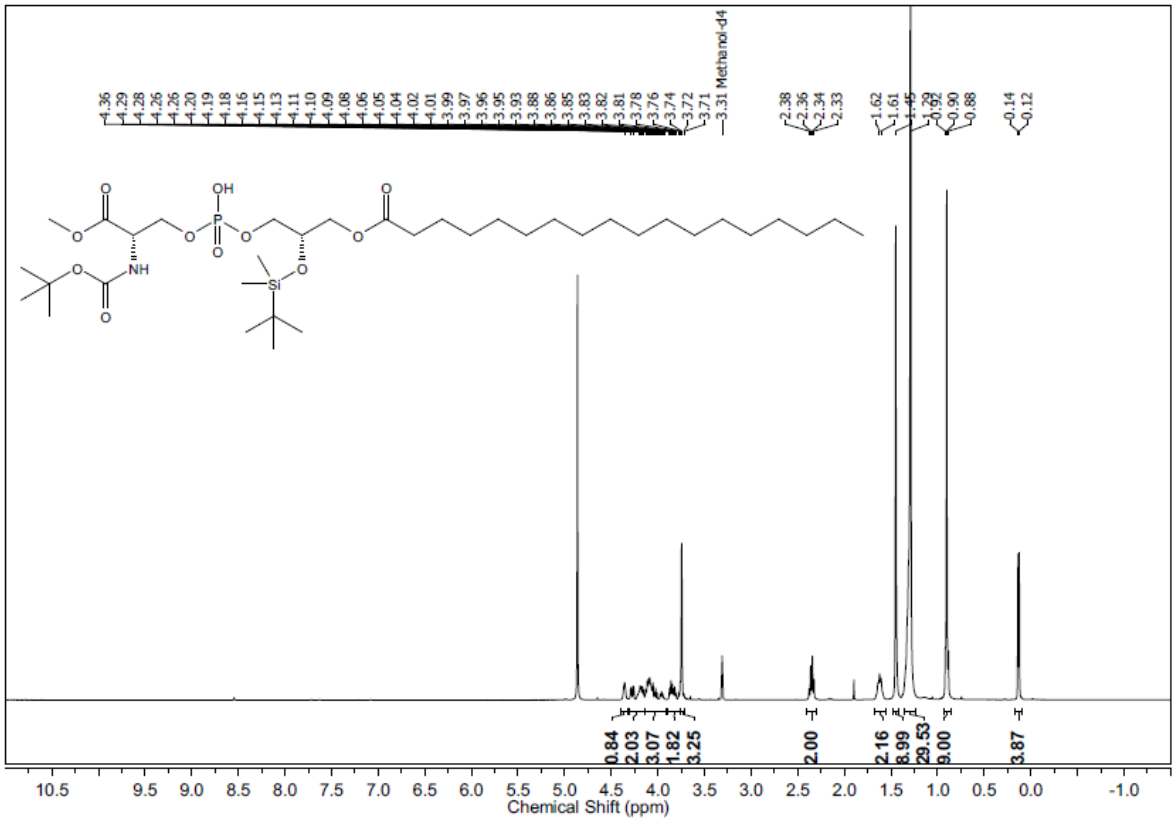
**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Intermediate (*R*)-9h**



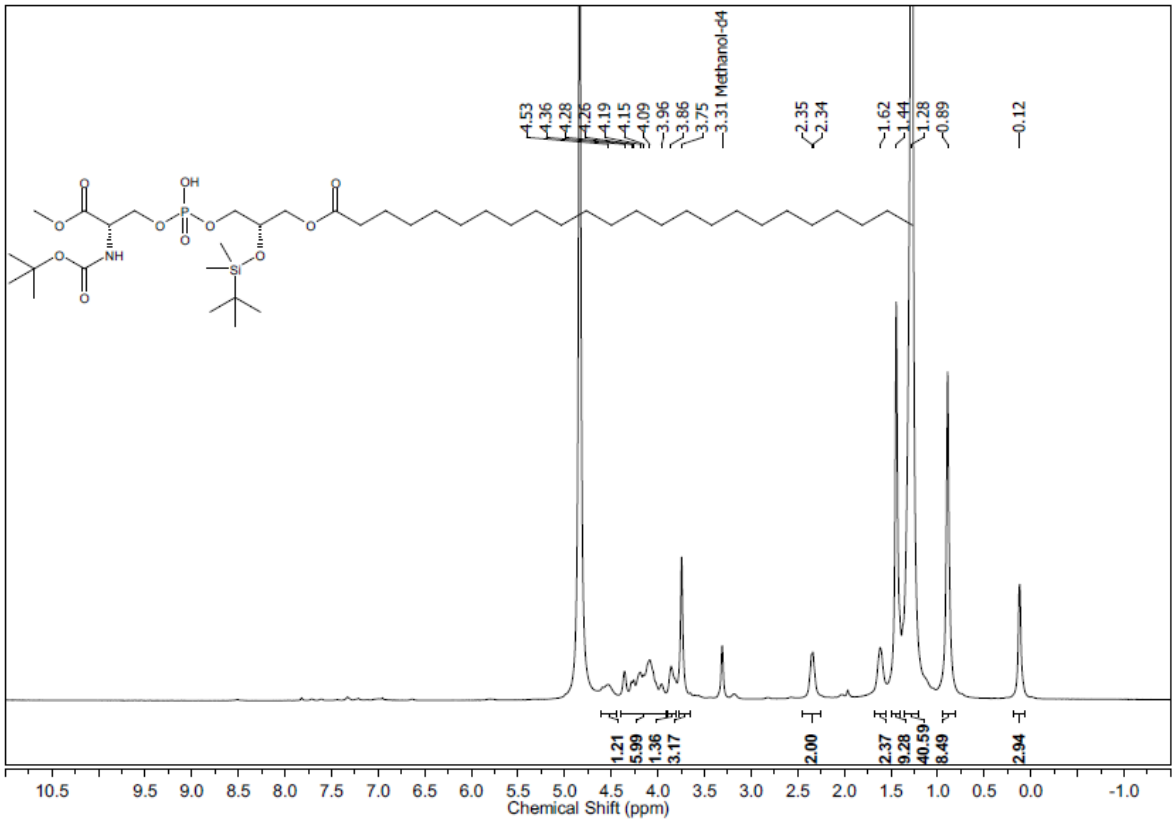

**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*S*)-9a**



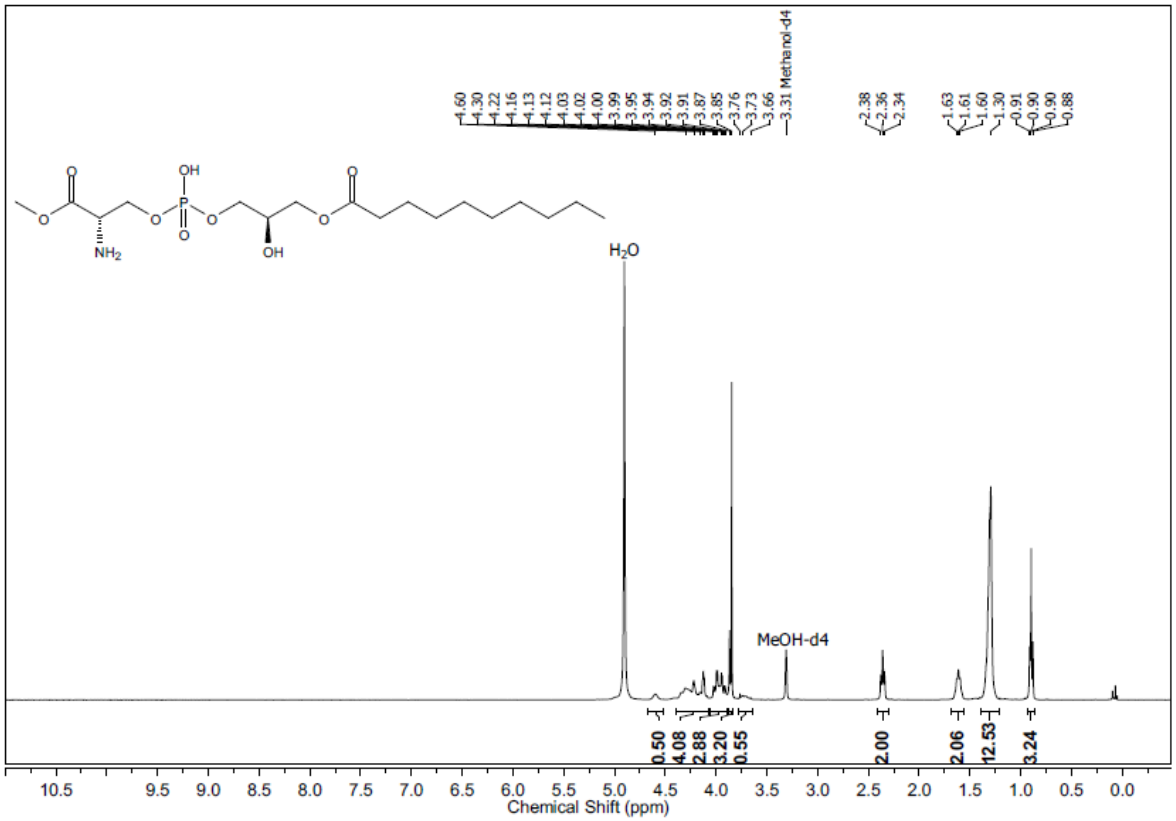
**$^1$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*S*)-9e**



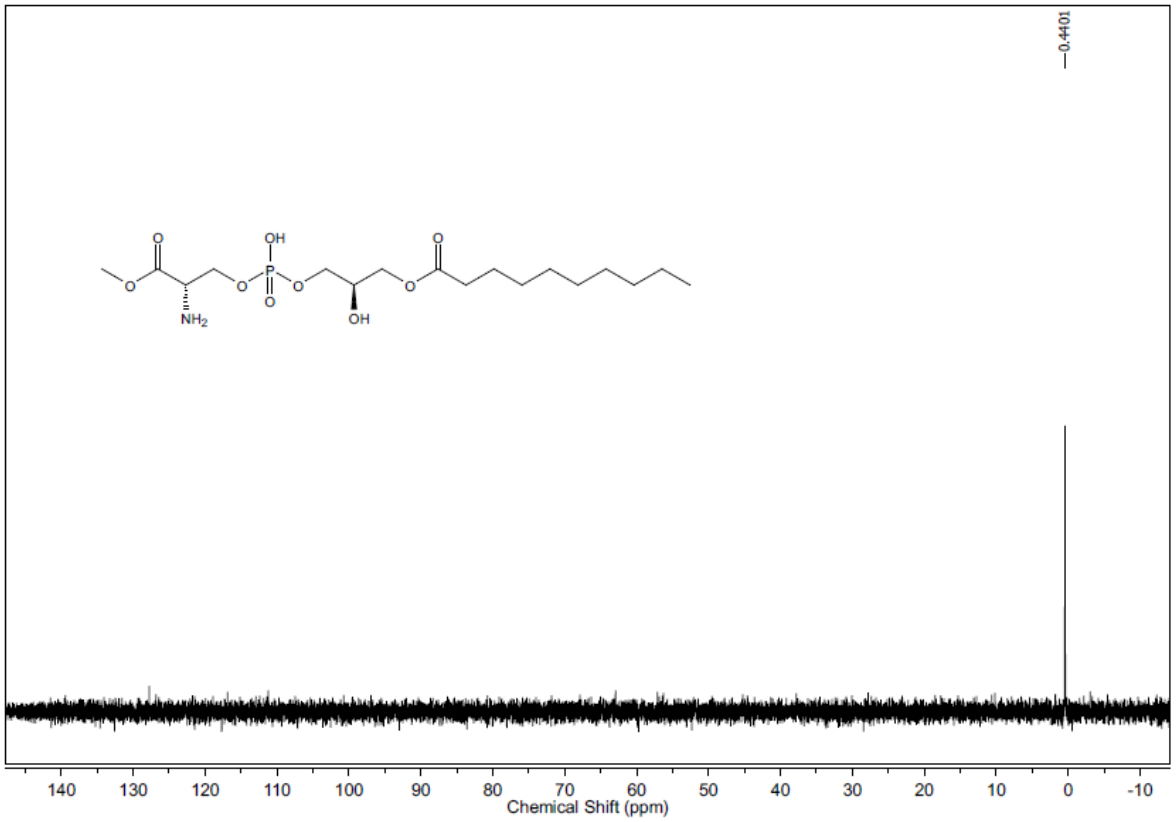

**$^1$H-NMR ($CDCl_3$, 400 MHz) for Intermediate (*S*)-9h**



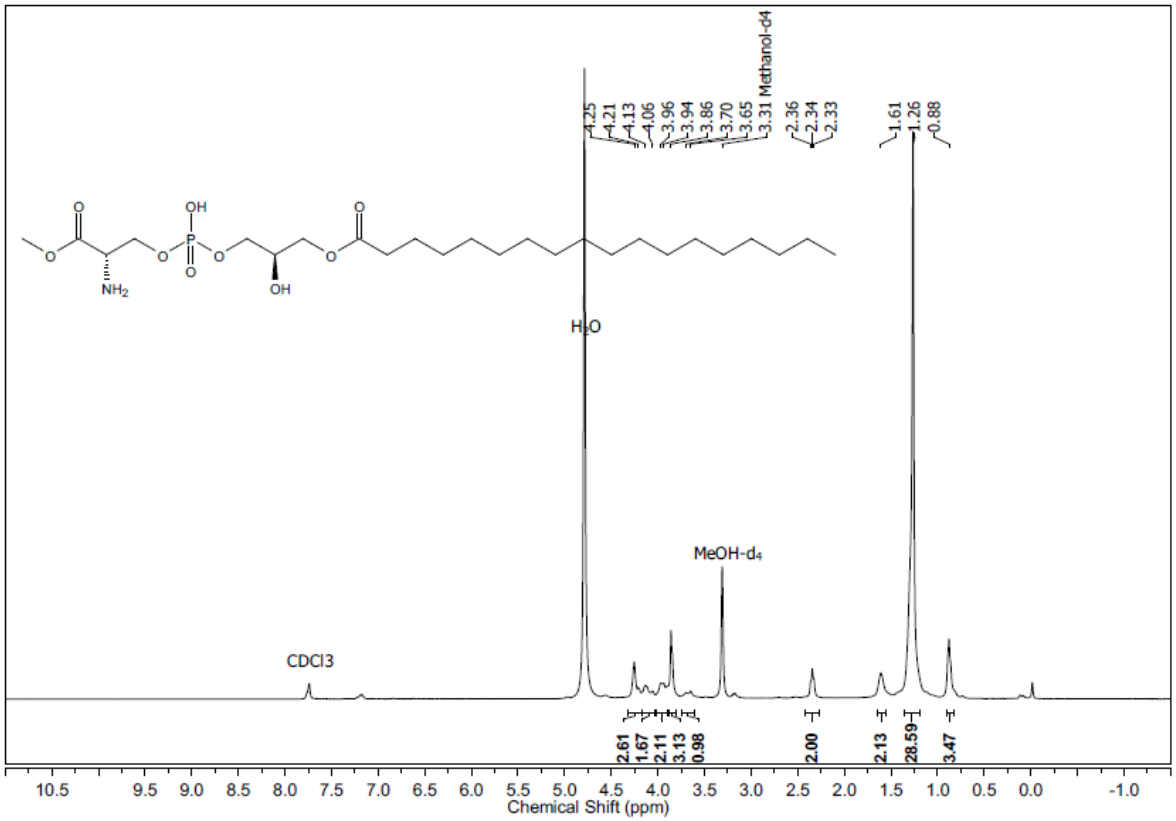
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*R*)-2a



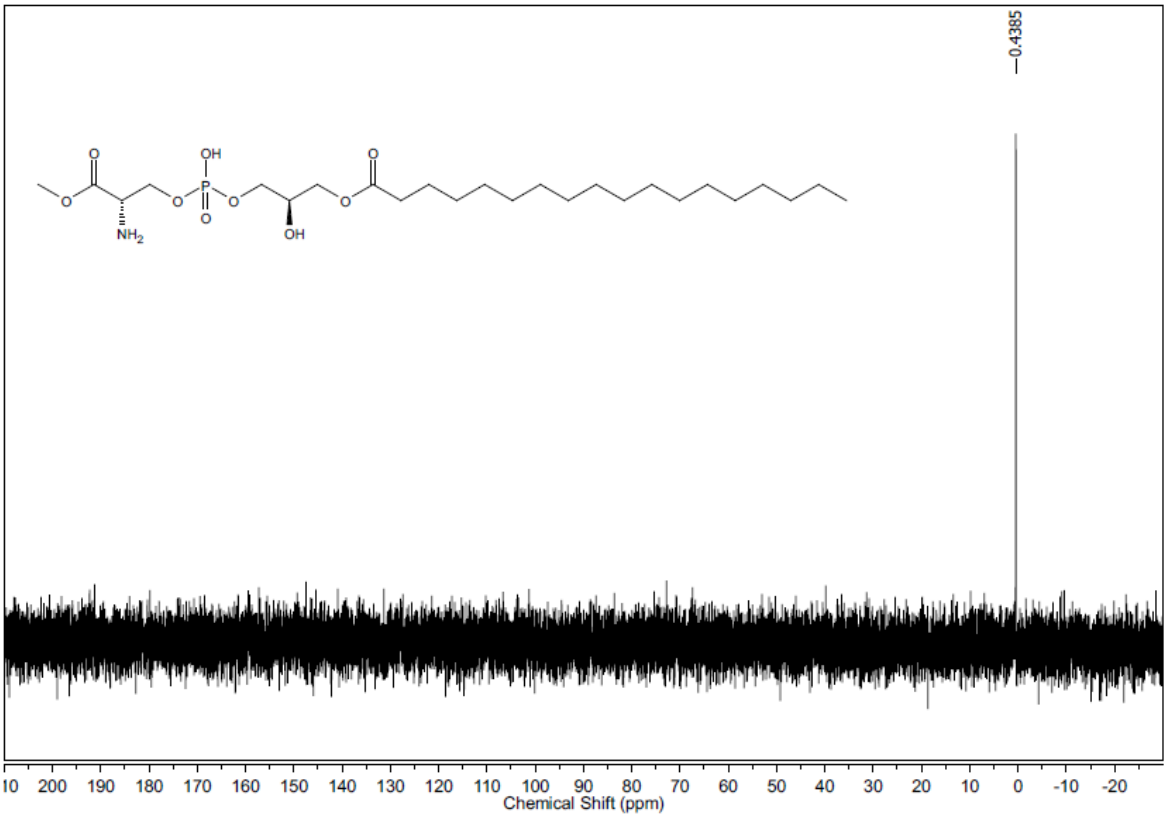

## $^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*R*)-2a



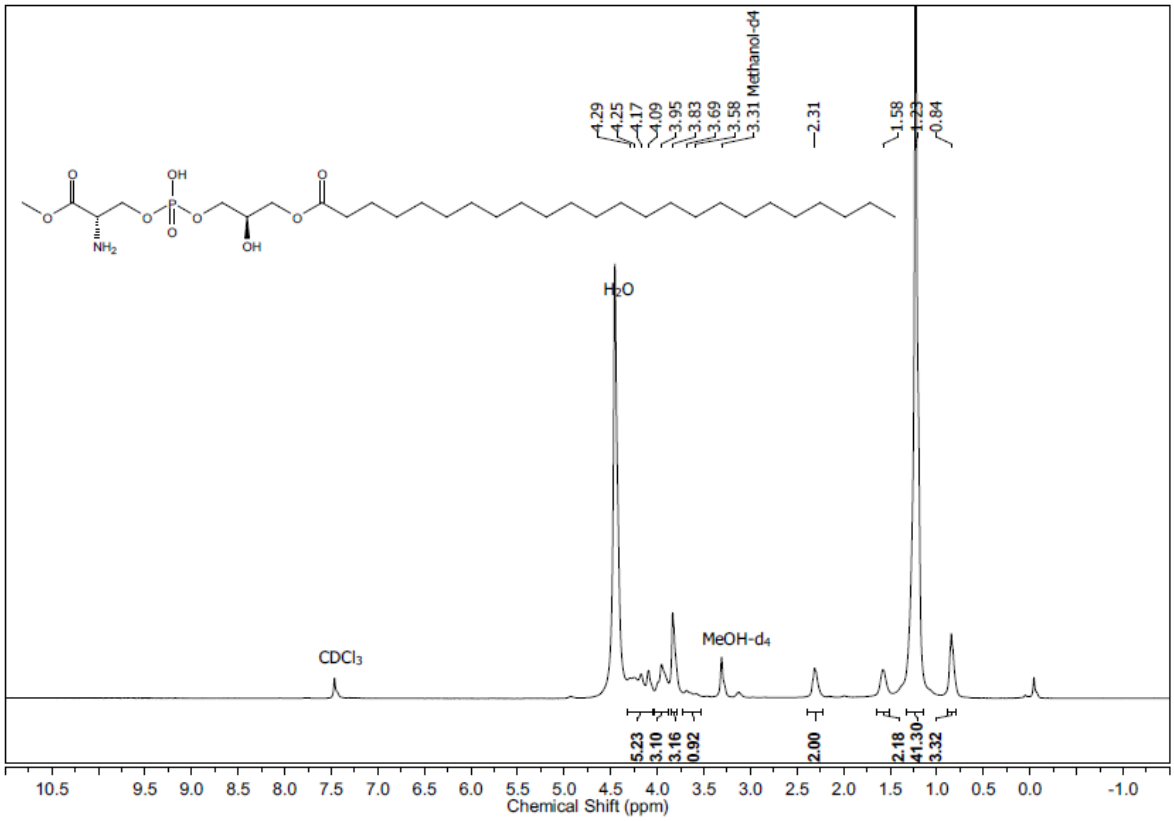
**$^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*R*)-2e**



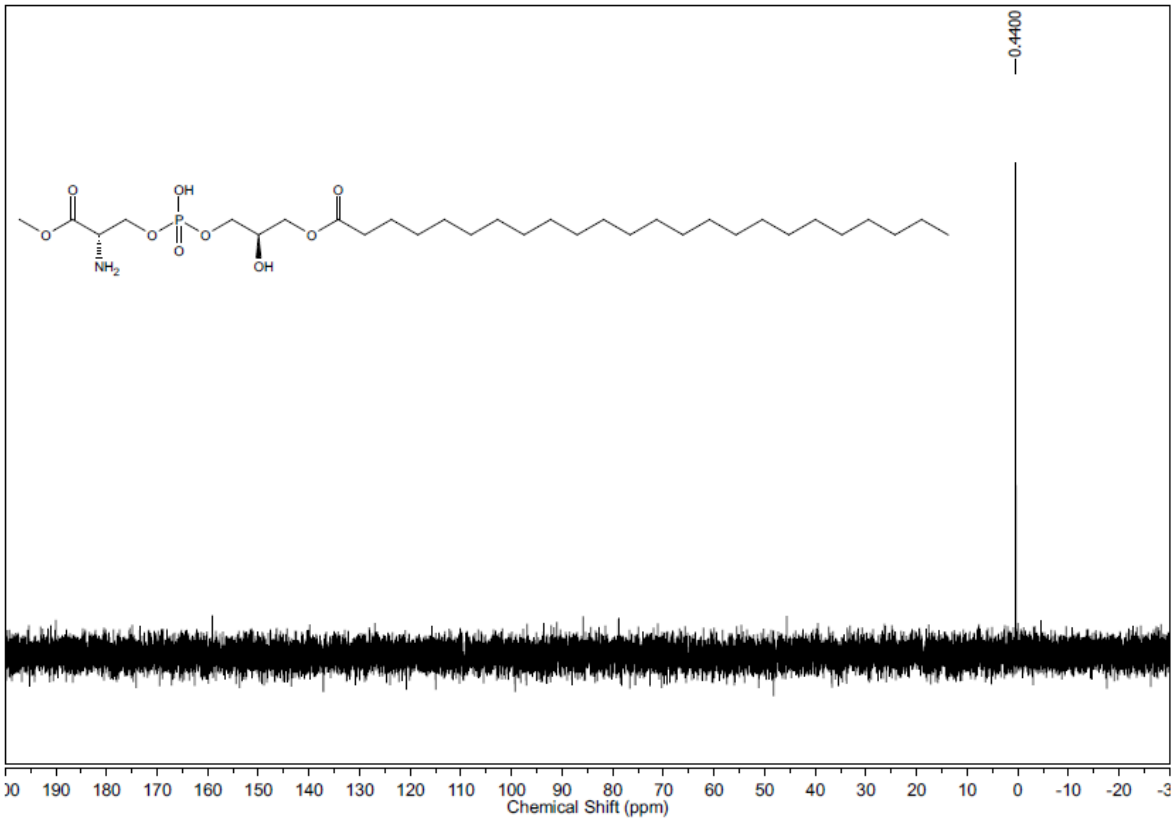

**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*R*)-2e**



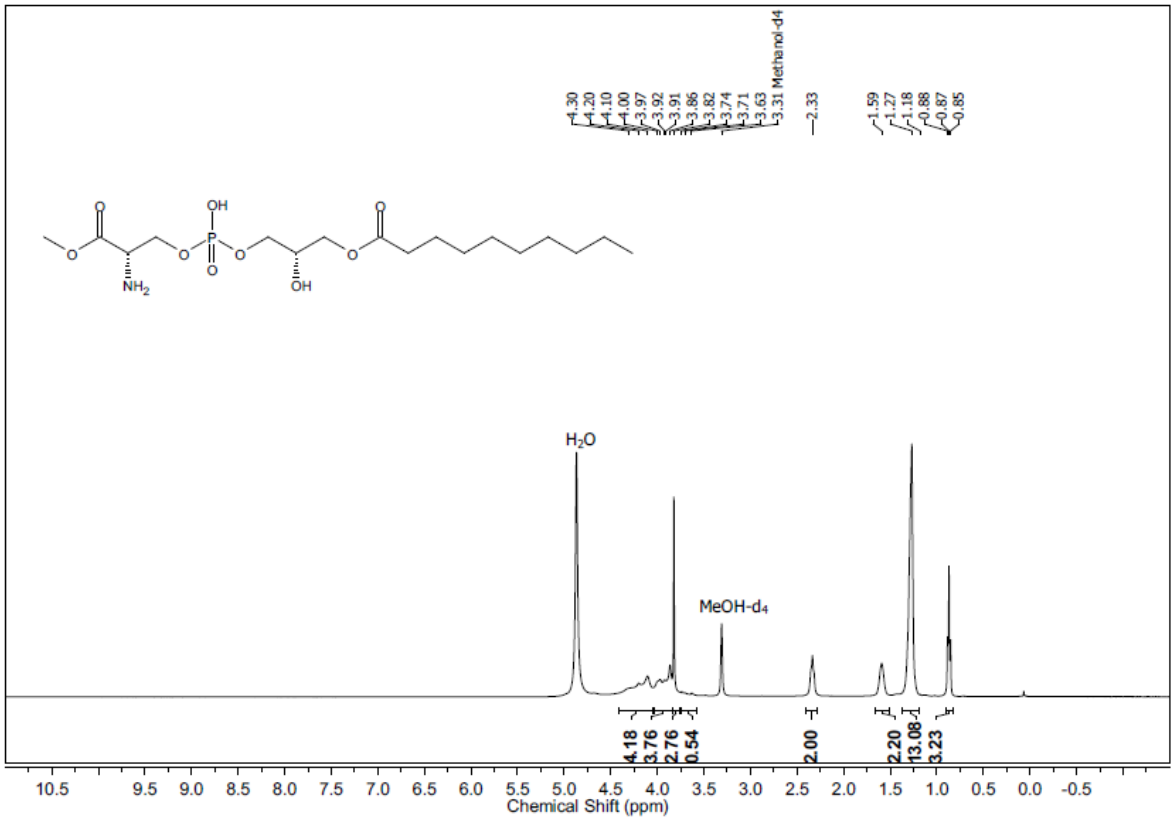
**$^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*R*)-2h**



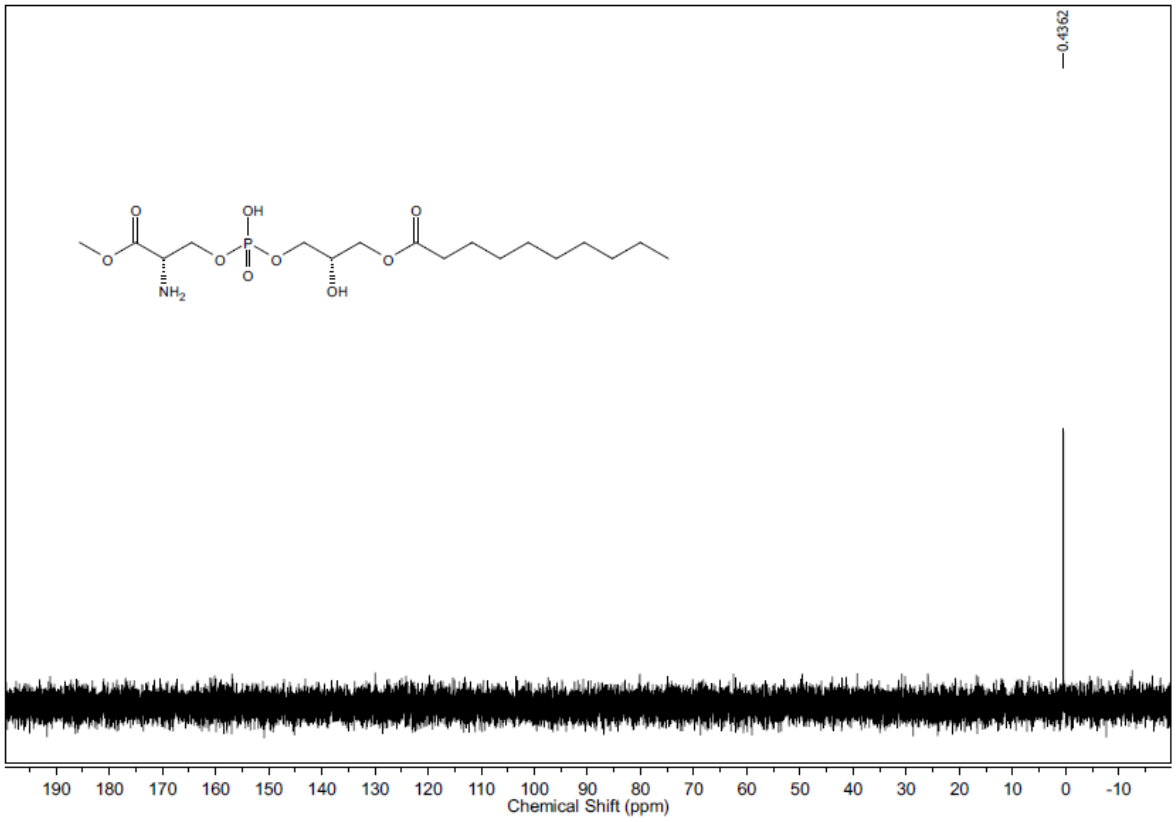

**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*R*)-2h**



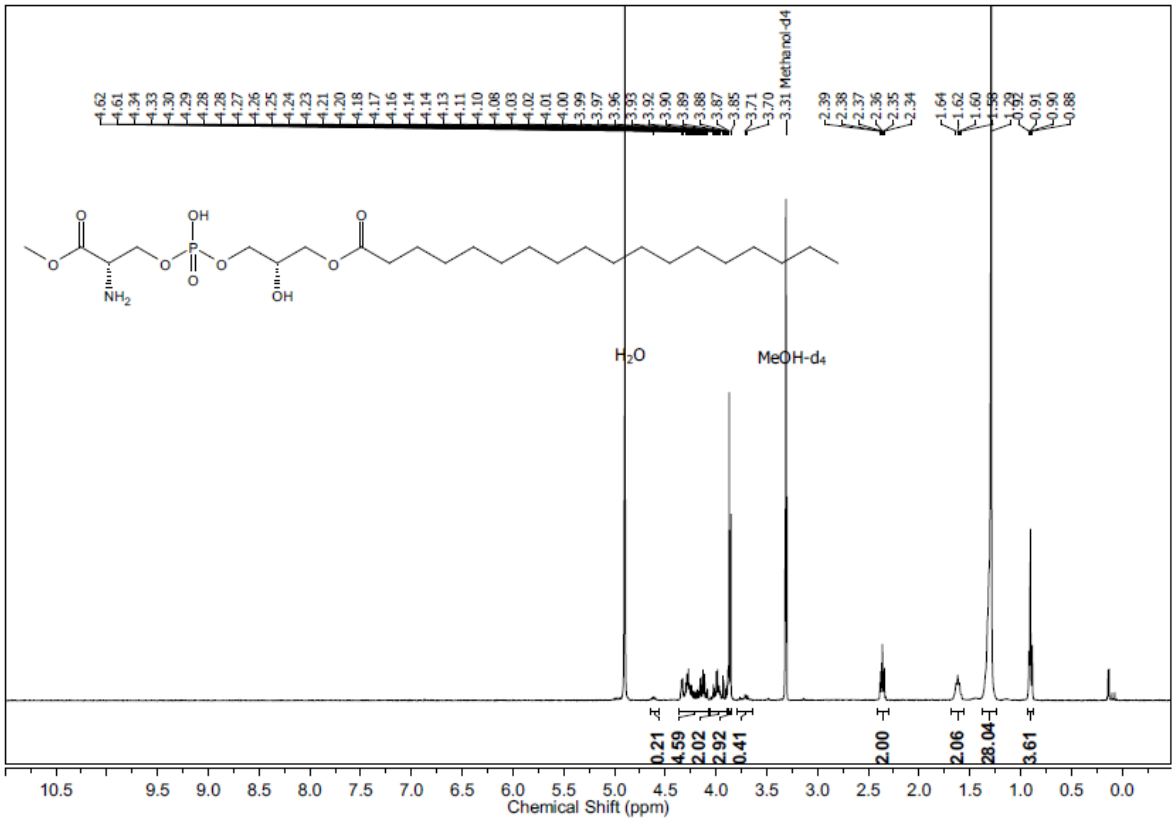
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*S*)-2a



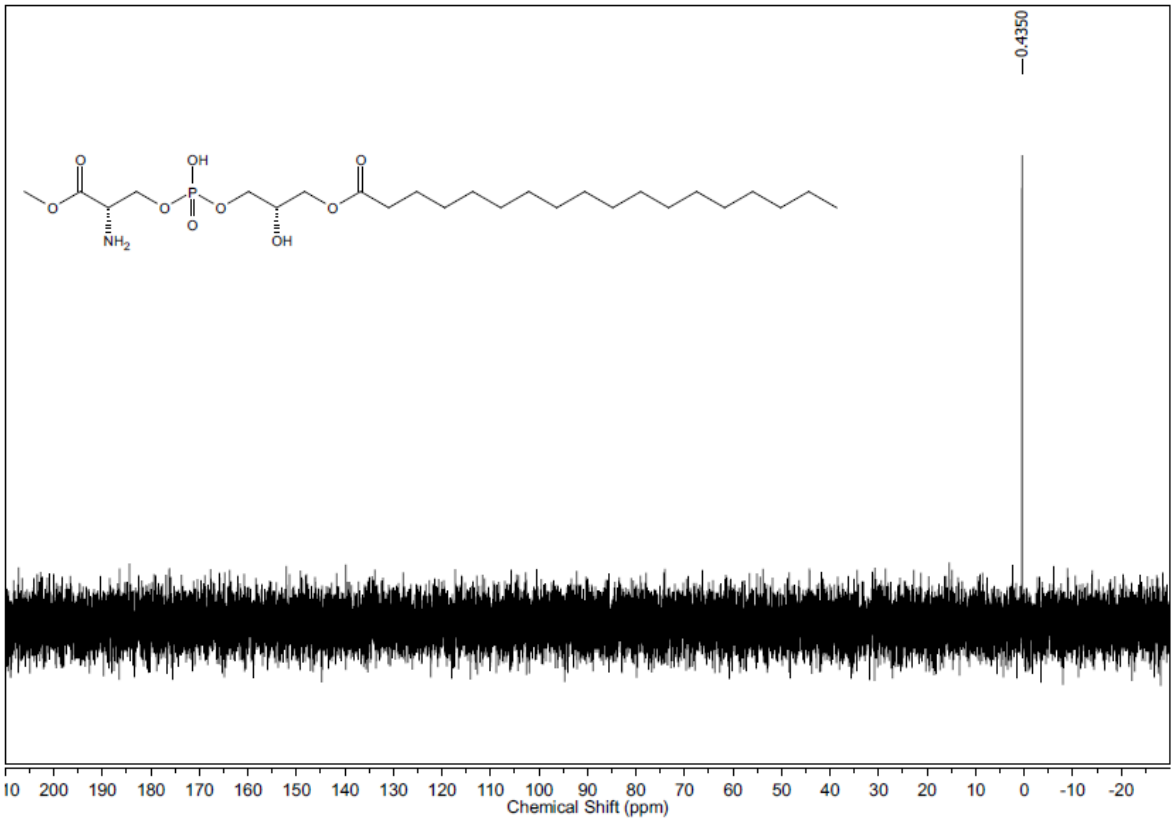

## $^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*S*)-2a



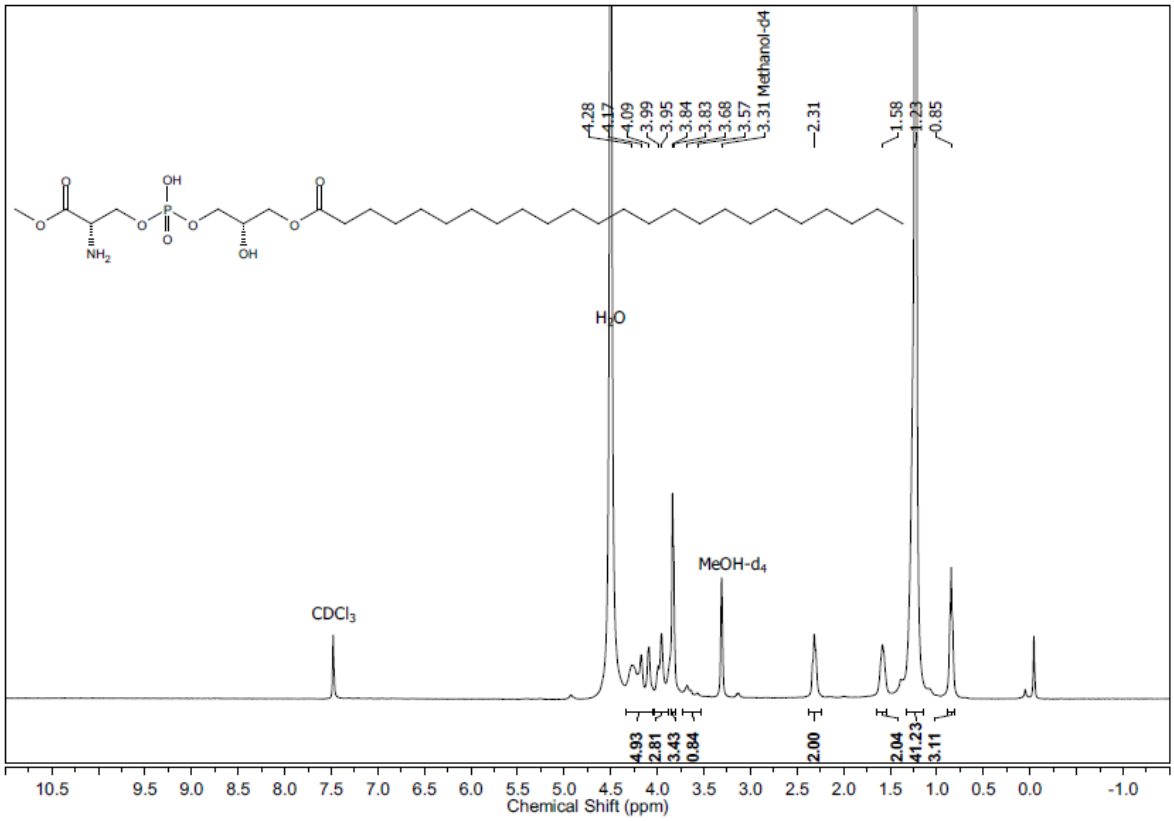
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*S*)-2e



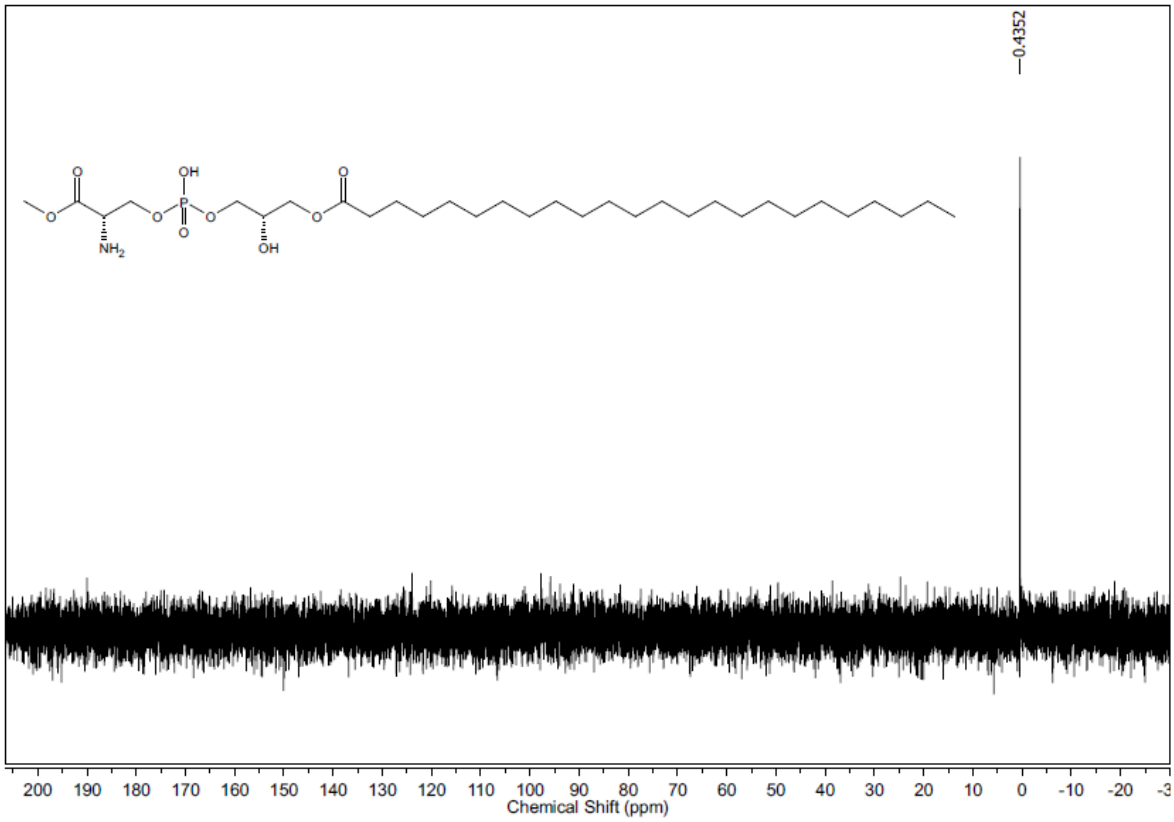

## $^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*S*)-2e



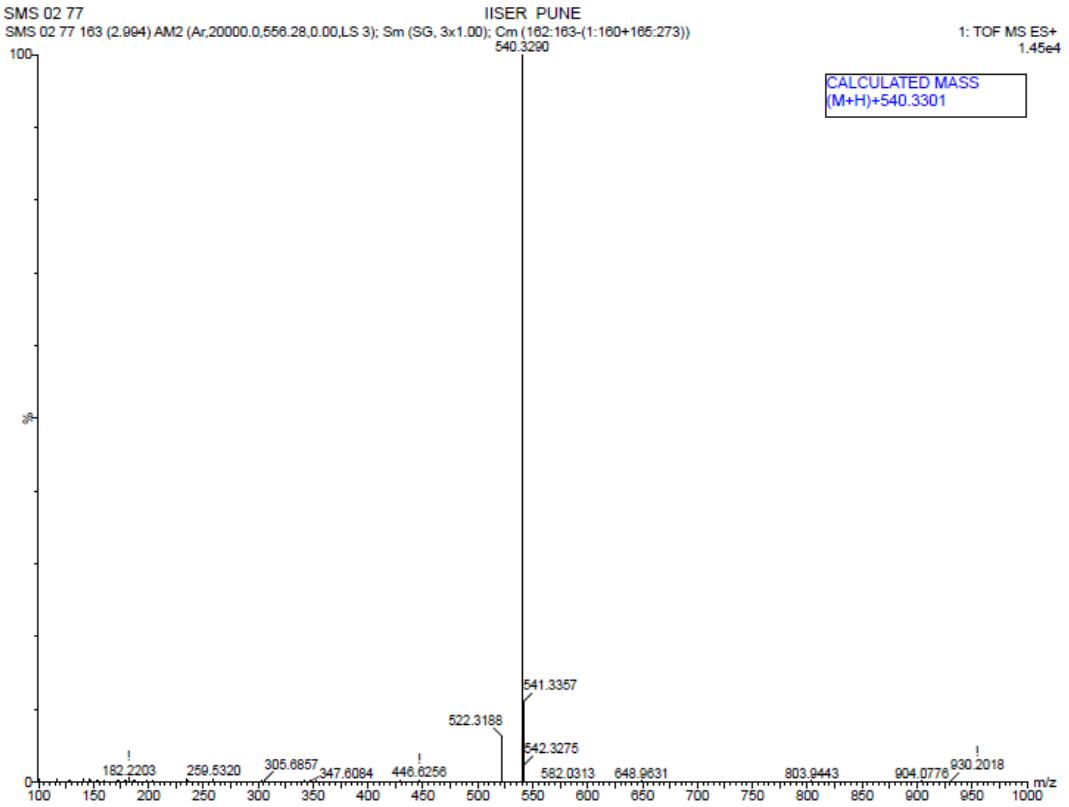
**$^{1}$H-NMR ($CDCl_3$, 400 MHz) of Final Compound (*S*)-2h**



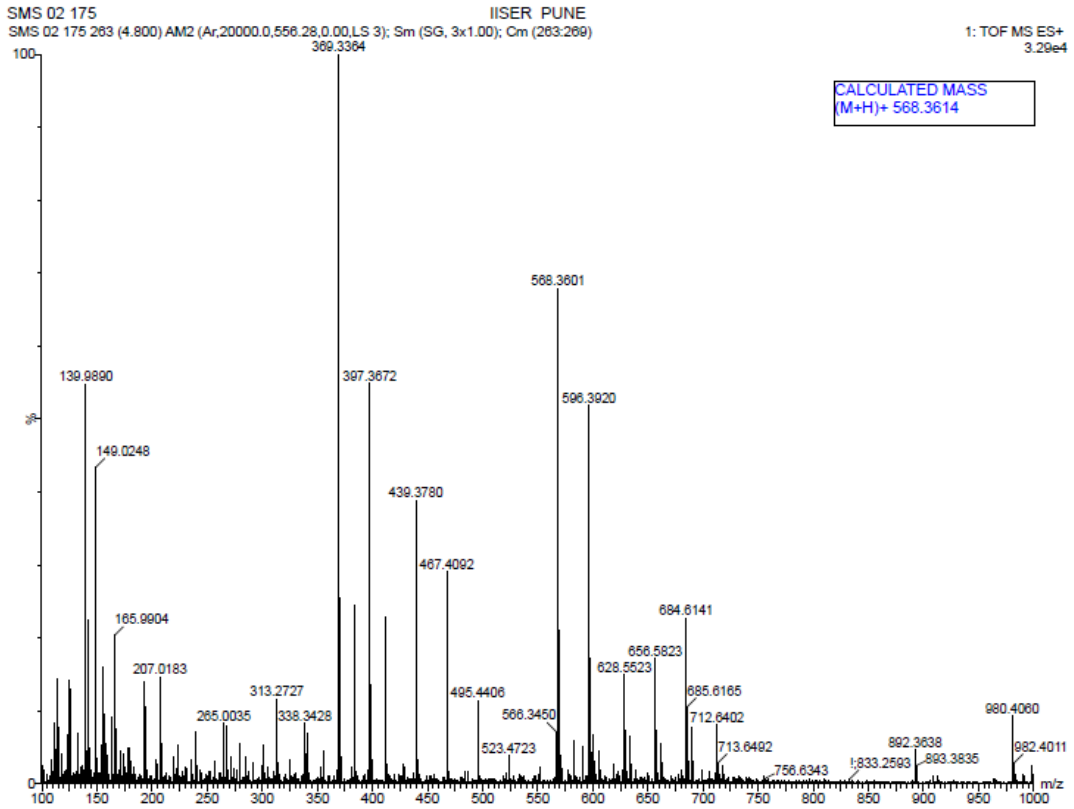

**$^{31}$P-NMR ($CDCl_3$, 400MHz) for Final Compound (*S*)-2h**



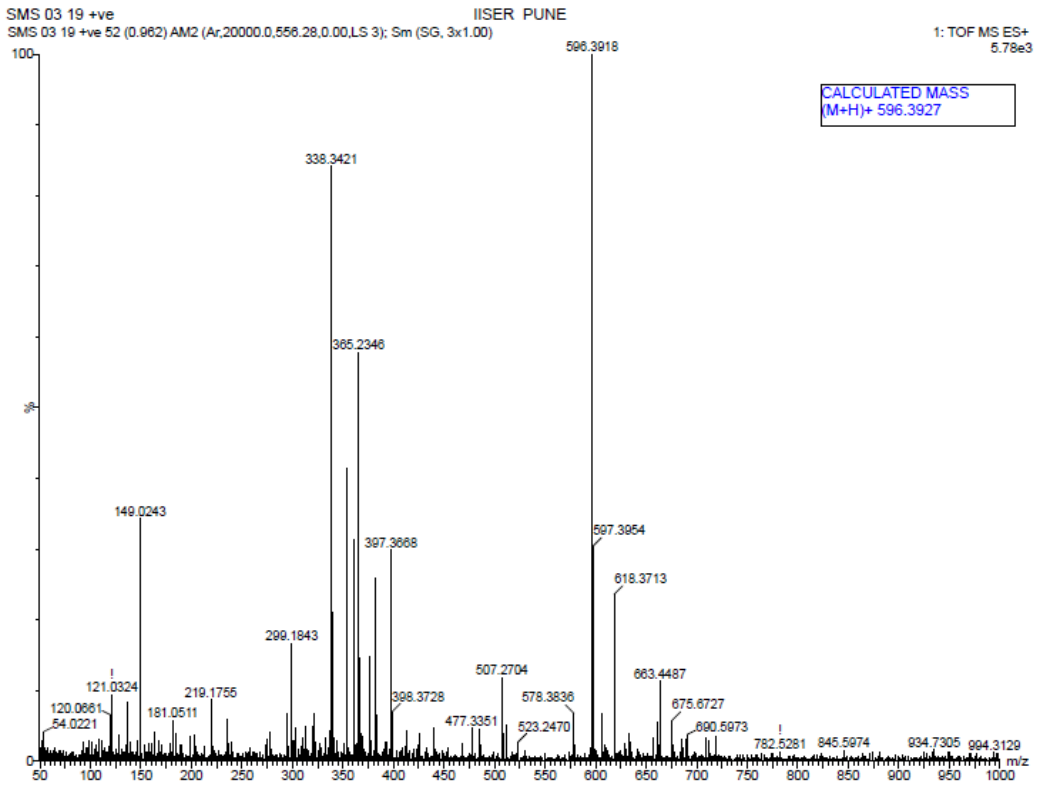
## HRMS of Compound (*R*)-2e



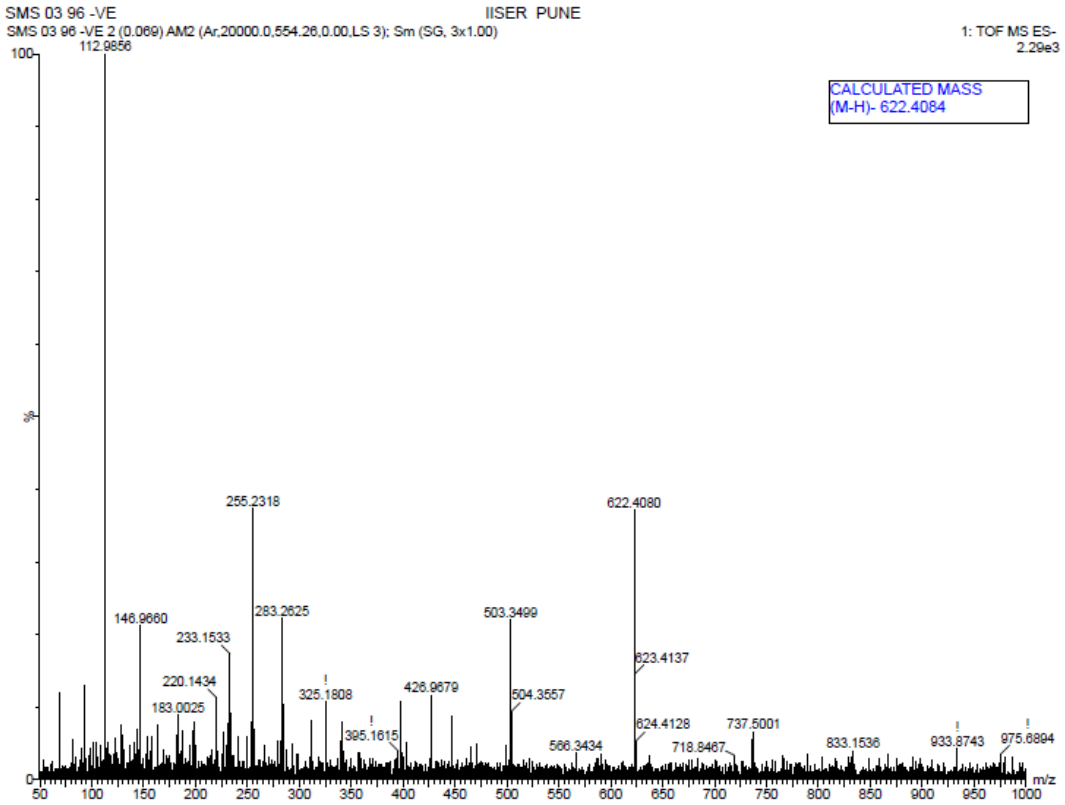

## HRMS of Compound (*R*)-2f



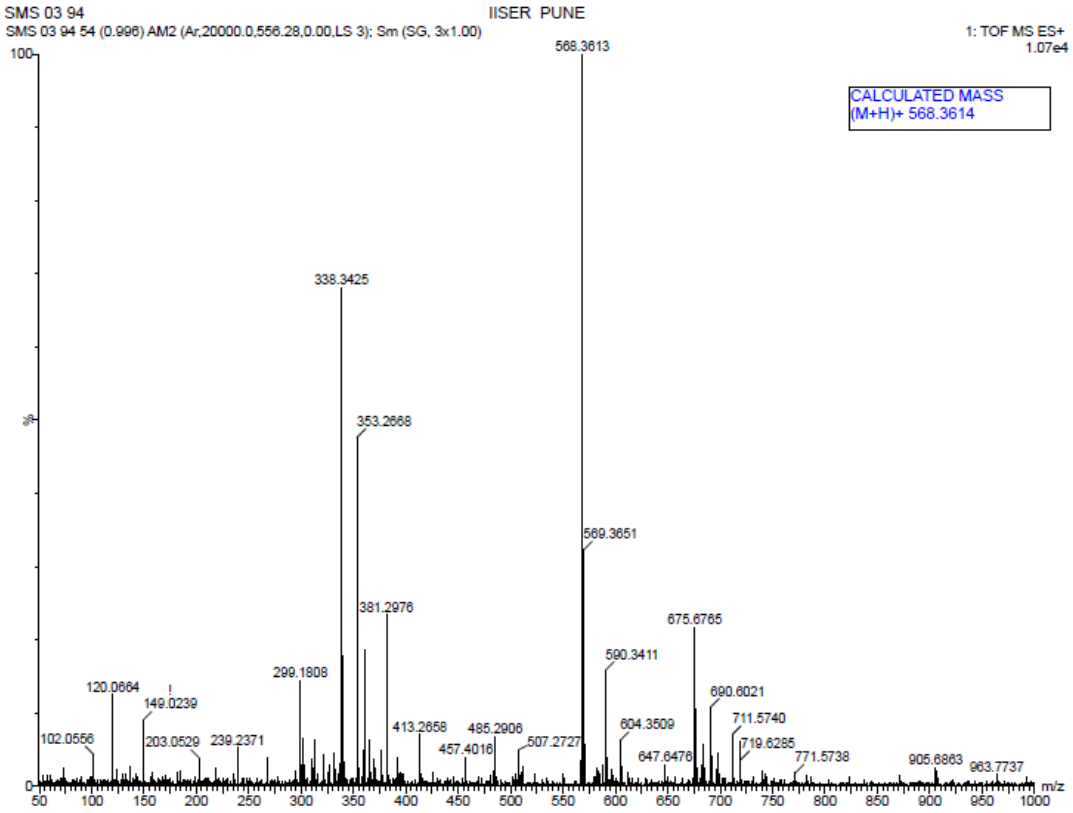
## HRMS of Compound (*R*)-2g



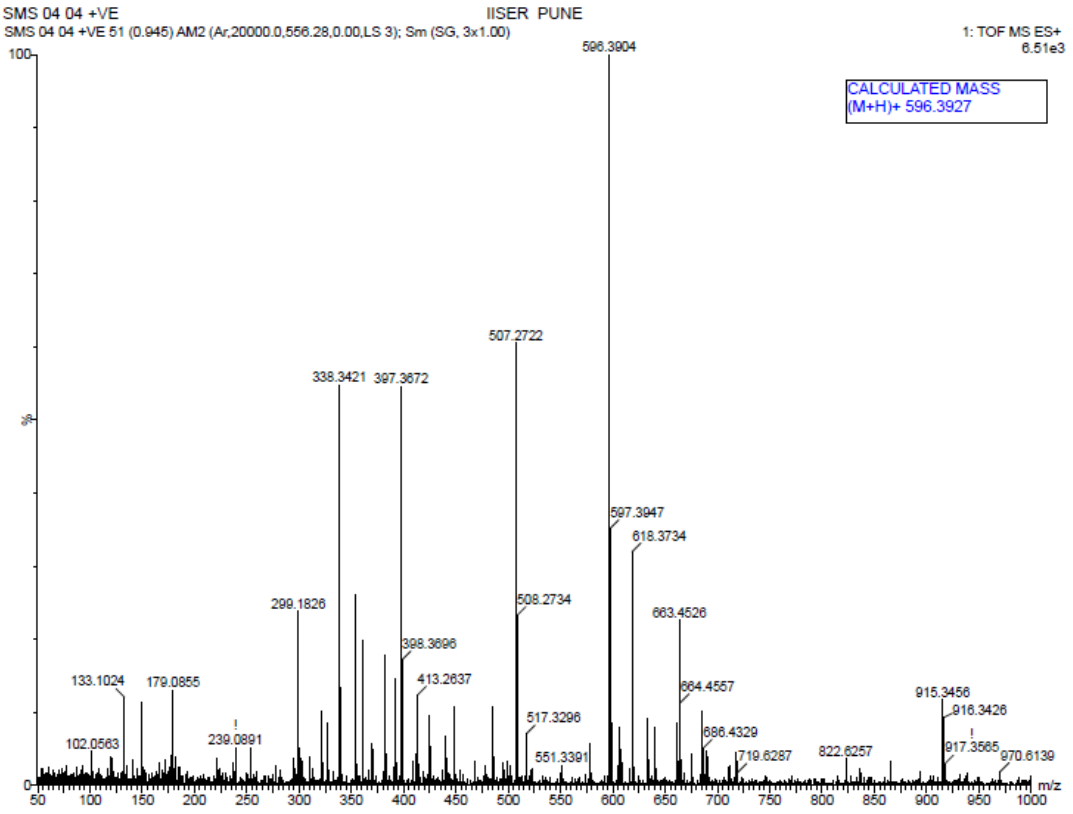

## HRMS of Compound (*R*)-2h



## HRMS of Compound (*S*)-2f




## HRMS of Compound (*S*)-2g



# CHAPTER 4

# SYNTHESIS OF UNSATURATED ME-LYSO-PS LIPIDS

## 4.1 Introduction of Unsaturated Lyso-PS

Recently lyso-PS have emerged as an important class of lipid mediators that play a significant role in various physiological processes such as macrophage activation, mast cell degranulation, and the development and maturation of T regulatory cells. Lyso-PS has been found to be most abundant in the central nervous system (NCS) and immune cells (macrophages and microglia). Given lyso-PSs ability to influence a multitude of immunological pathways, its physiological concentrations are tightly regulated by dedicated biosynthetic and degradative enzymes. To date, ABHD12 has been identified as the only degradative enzyme that metabolizes both saturated and unsaturated lyso-PS lipids into glycerophosphoserine and free fatty acid. PHARC subject due to loss of function mutation in ABHD12 gene have been found to be associated to PHARC, a progressive neurodegenerative disorder. Seminal studies have shown that ABHD12 K/O mice display age dependant behavioural defects noticed in PHARC like phenotype in which hearing loss, auditory brain steam response, and motor behaviour. The study suggested that very-long-chain (VLC) saturated as well as unsaturated lyso-PS lipids were highly accumulated in the ABHD12 K/O mice brain.

## 4.2 Classification of Fatty Acids and their Structure

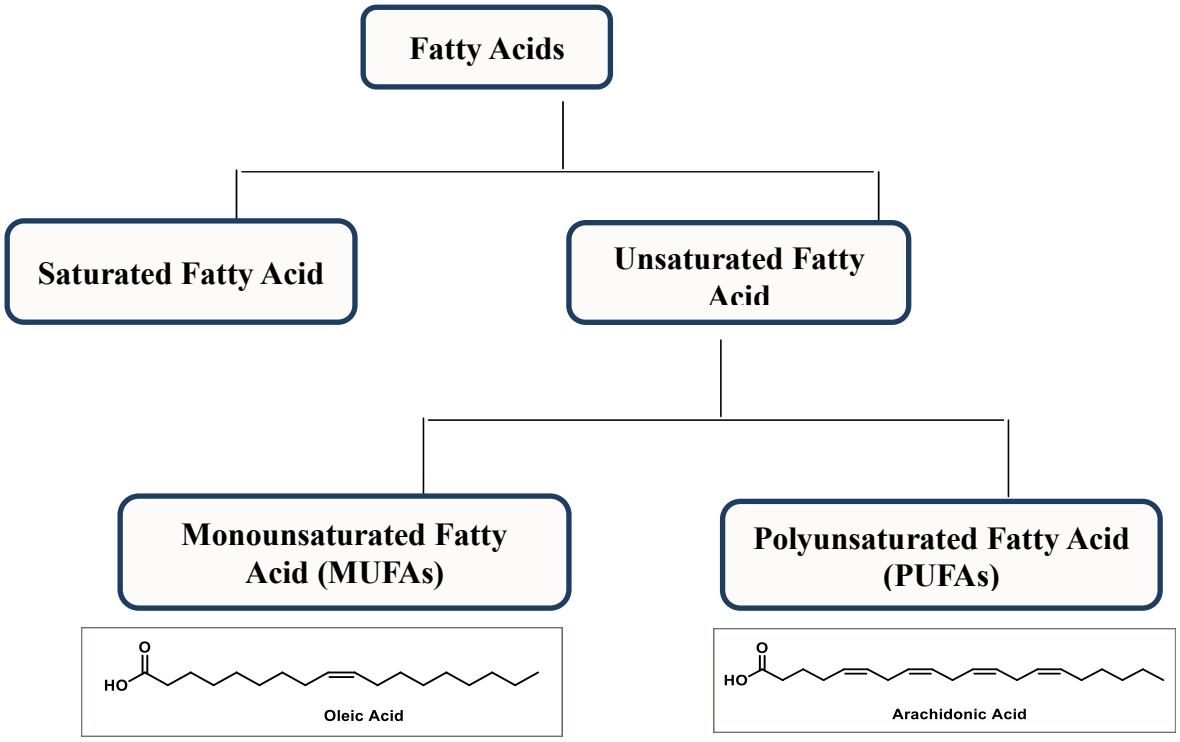



**Figure 4.1:** Classification and chemical structure of fatty acid (FAs)

Fatty acids are divided into two general categories: 1) Saturated 2) Unsaturated. Saturated fatty acids have no double bond in the structure. However, unsaturated fatty acids have one or more double bonds. Only one double bond presented fatty acid is called monounsaturated fatty acid (MUFAs) ex. Oleic acid. More than one double bond presented fatty acid is called polyunsaturated fatty acid (PUFAs) ex. α-linoleic acid, Arachidonic acid (AA).

## 4.3 Biological role of Polyunsaturated Fatty Acids (PUFA)

Fatty acid is an important class of lipids and is an important constituent of the cell membrane. Fatty acids are involved in various physiological processes and any perturbation in their metabolism poses biomedical relevance. Polyunsaturated fatty acids have crucial role in several biological functions such as control in inflammatory cascades, reducing oxidative stress, presenting neuroprotection, and cardiovascular protection. and is also an important source of energy. Of note, polyunsaturated fatty acids stimulate the activation of immune cells. Omega-3 and omega-6 polyunsaturated fatty acids are essential in humans and their role in anti-inflammatory and immunomodulatory functions are well documented. For example linoleic acid, omega-3 polyunsaturated fatty acid is associated with several chronic diseases such as rheumatoid arthritis, diabetes, and neurodegenerative disease. Omega-3 PUFA is plays a crucial role in the lower the risk in cardiovascular disease. Heart and blood vessel-related diseases can cause a heart attack or stroke this process is called the atherosclerosis process. Importantly, omega-3 also plays an important protective role in inflammation in the atherosclerosis process[59,60].

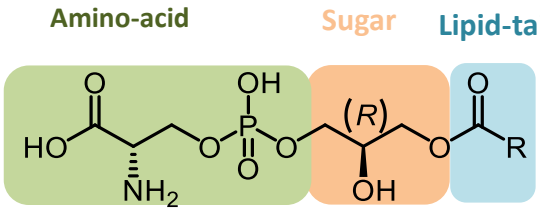
It has been already studied the effect of omega-3 PUFA in inflammatory cytokine production. Moreover, previous research demonstrated that increase levels of omega-3 PUFA (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), particularly) decrease the circulating concentration of inflammatory cytokines such as interleukin (IL6), tumor necrosis factor α (TNF α)[61,62]. Nowadays, strong evidence from in vitro and in vivo studies supports the biomedical importance of omega-3 PUFA that is involved in various pathologies such as, cardiovascular, neurodegenerative, rheumatoid arthritis, diabetes disease[63].

PUFAs, as well as MUFAs, exert immunomodulatory effects on both T cell and B cell functions. To date, as per our best knowledge, nobody has worked on unsaturated lyso-PS library towards immune cells. Projecting forwards, the aforementioned problem we will test our synthetic unsaturated Me-lyso-PS library in a variety of biological assays. We are interesting to know these synthesized unsaturated Me-lyso-PS how they influence signalling

properties towards the activation of immune cells, such as macrophages and mast cells by secretion of pro-inflammatory cytokines and histamine release. In addition, we want to know how the unsaturated fatty acid chain length of lyso-PSs produce intracellular cAMP, cytosolic $Ca^{2+}$ flux, and ERK phosphorylation level.

## 4.4 Result and Discussions:

As a proof of concept, we decided to synthesize a library of the naturally occurring (*R*)-Me-lyso-PSs with unsaturated fatty acids only. Not only we designed simplest synthetic route but also we synthesized in low milligram as per cost of fatty acid and their availability. Only one (18:1 lyso-PS) unsaturated lyso-PS lipids are commercially available these is esterified only with long-chain (LC) fatty acids (figure 4.1). Also, VLC containing unsaturated lyso-PS lipids is not commercially available because these are very expensive for such types of studies.

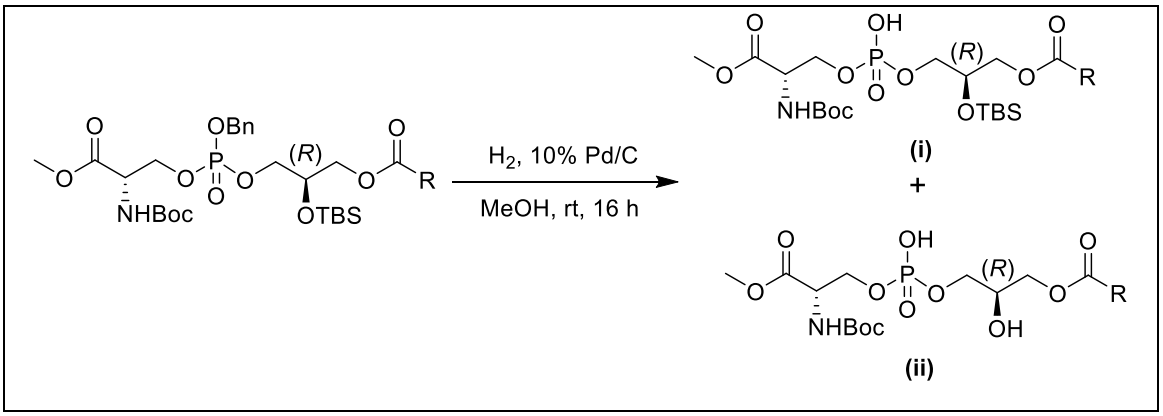



| -(COOR) | Fatty Acid |
|---|---|
| C18:1 | Oleic Acid |

**Figure 4.2:** Commercial available long-chain lyso-PS lipid species

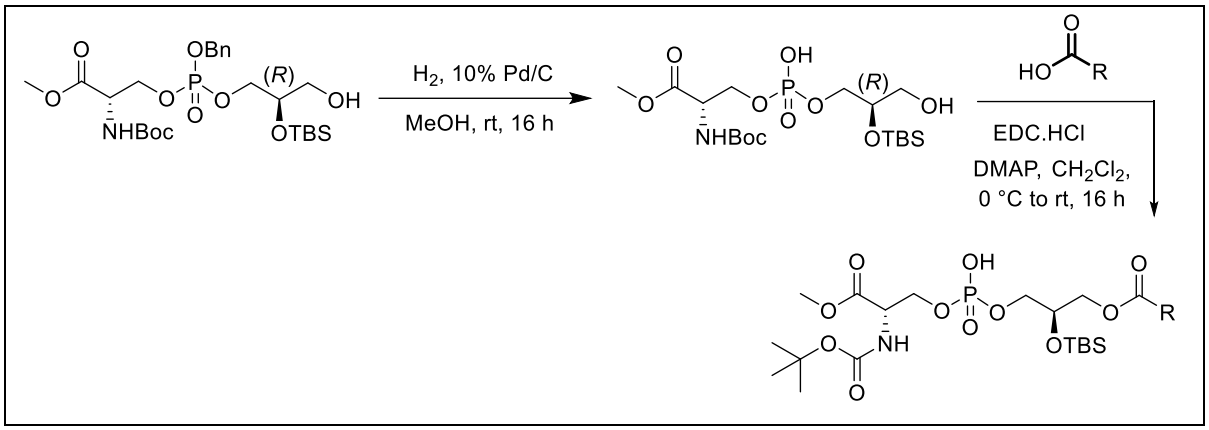
### 4.4.1 Limitation of Unsaturated Me-lyso-PS Library

### Route A



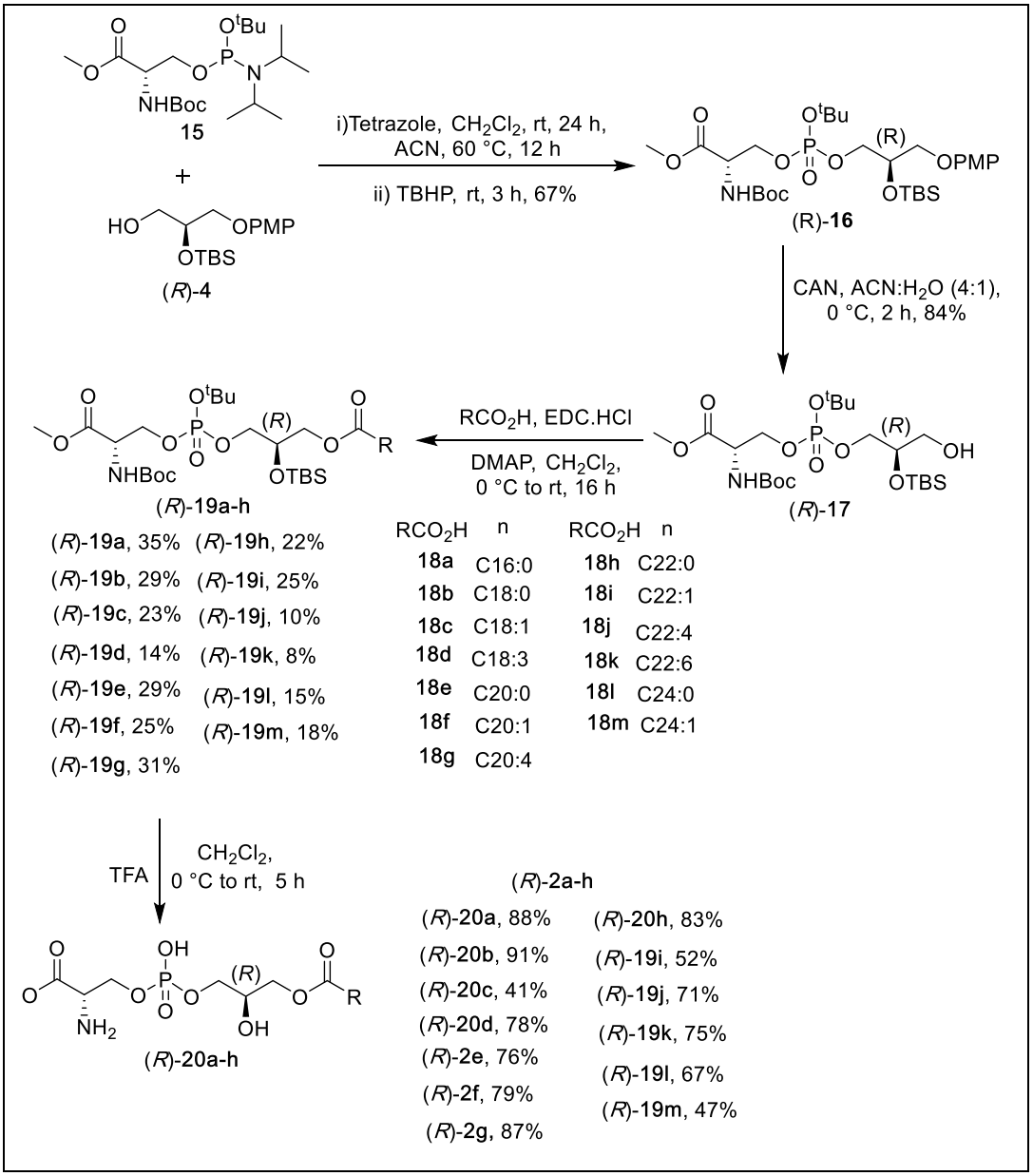
In the above **Route A**, the unsaturated double bond along with the benzyl group will be reduced during the hydrogenolysis reaction. We thus, choosed another route for the synthesis of unsaturated Me-lyso-PS library.

## Route B




In the above **Route B**, debenzylation of the initial substrate in the presence of hydrogen gas successfully lead to the synthesis of the desired intermediate, which upon coupling with the acid in the presence of EDC·HCl furnished the desired final compound. However, due to the presence of a free hydroxyl group of phosphate, the compounds were extremely polar and thus could not be purified.

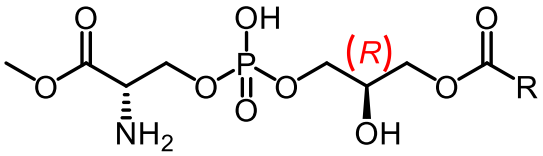
We then envisage that the incorporation of the *tert*-butyl group at the hydroxyl group of phosphate will make the compounds non-polar, necessary for better purification, and on the other hand, the debenzylation while using the benzyl protection in the previous routes can be avoided. For the synthesis of the unsaturated Me-lyso-PS library, we followed the following scheme (4.1).




**Scheme 4.1:** Synthetic route of unsaturated (*R*)-Me-lyso-PSs

## Table 4.1

## Saturated and unsaturated methyl ester-lysophosphatidylserine (Me-lyso-PS)




***(R)* - Natural**

| ID | -(COOR) | ID | -(COOR) |
|---|---|---|---|
| (*R*)-20a | C16:0 | (*R*)-20h | C22:0 |
| (*R*)-20b | C18:0 | (R)-20i | C22:1 |
| (*R*)-20c | C18:1 | (*R*)-20j | C22:4 |
| (*R*)-20d | C18:3 | (*R*)-20k | C22:6 |
| (*R*)-20e | C20:0 | (*R*)-20l | C24:0 |
| (*R*)-20f | C20:1 | (*R*)-20m | C24:1 |
| (*R*)-20g | C20:4 | | |

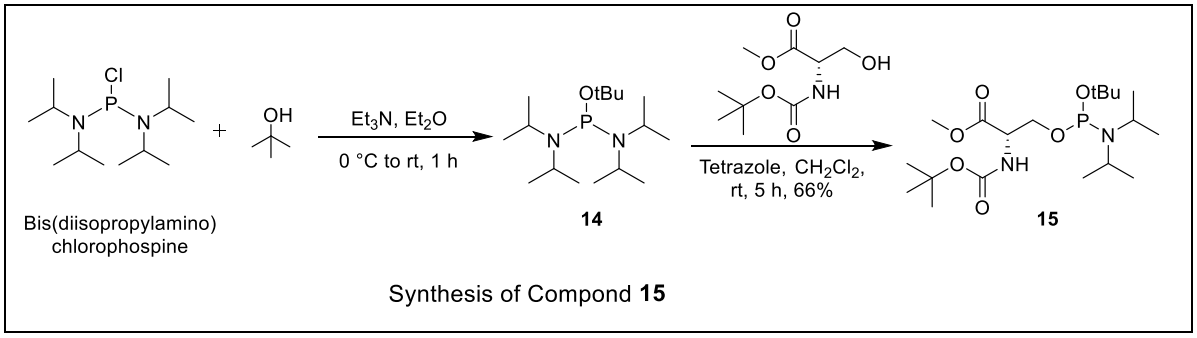
## 4.5 Conclusion

For the first time, we have shown that the ABHD12 enzyme is also the principal lyso-PS lipase that regulates mast cell biology. In this chapter, we have successfully synthesized saturated and unsaturated (*R*)-Me-lyso-PSs lipid library with different route. The study presents a potential scope to further dissect the structure-activity relationship of the lyso-PS bearing polyunsaturated fatty acid chain. We have shown the specific contribution of lyso-PSs in the brain and immune cell functions. However, currently, not much information is known about it's role in other mammalian organs. Hence, understanding the role of unsaturated Me-lyso-PS and its underlying mechanism under various physiological or pathological conditions in specific tissues/organs will be intriguing.

## 4.6 EXPERIMENTAL SECTION

## 4.6.1 Synthesis and characterization Data

### A) Synthesis of Compound 14




Synthesis of Compond **15**

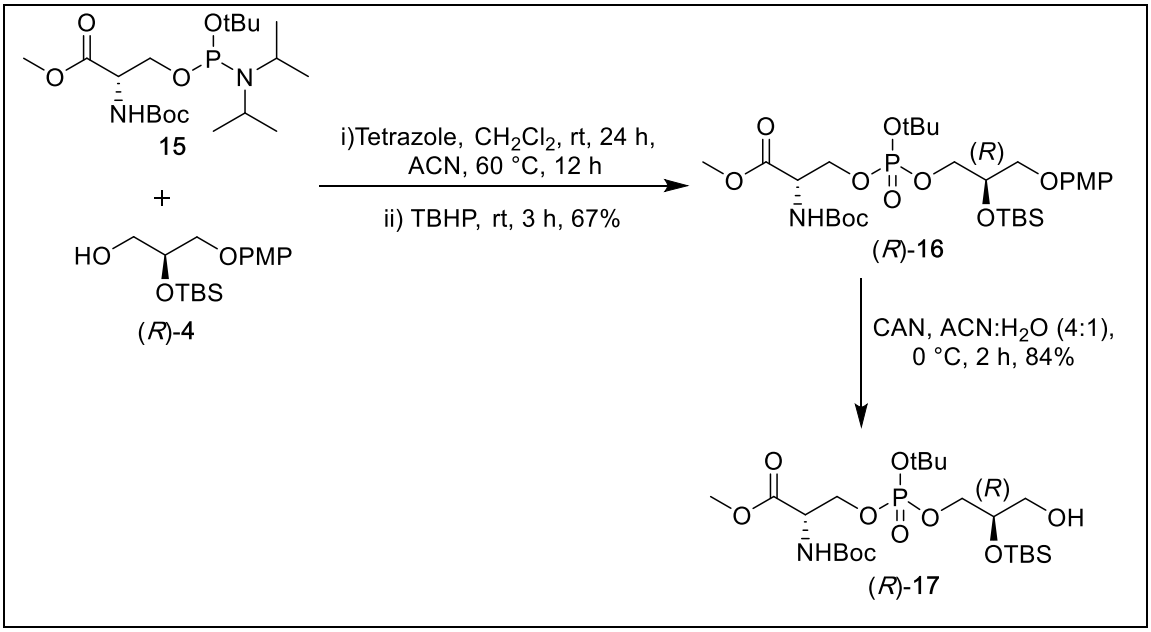
**1-*tert*-butoxy-*N,N,N',N'*-tetraisopropylphosphanediamine (14) :** To a solution of bis(diisopropylamino) chlorophosphine (5.0 g, 18.7 mmol) in dry diethyl ether ($Et_2O$) (30 mL) in Schlenk flask, a mixture of benzyl alcohol (1.3 mL, 16.8 mmol) and triethylamine ($Et_3N$) (2.3 mL, 16.8 mmol) in $Et_2O$ (5 mL) was added at 0 °C under nitrogen ($N_2$) atmosphere. The reaction mixture was stirred for 30 min at 0 °C, and then warmed to room temperature for 30 min. The reaction mixture was diluted with cold hexane (15 mL), stirred for 10 min. The hexane solution was then transferred into another Schlenk flask by cannula and concentrated under a nitrogen atmosphere to yield compound **15**. The crude product was used as such for next step without purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.61-3.50 (m, 4H), 1.32 (s, 9H), 1.19-1.14 (m, 24H), $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ 98.88.

### B) Synthesis of Compound 15

**Methyl *O*-((benzyloxy)(diisopropylamino) phosphaneyl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (15)**: 1-*tert*-butoxy-*N,N,N',N'*-tetraisopropylphosphanediamine **(14)** (5.2 g, 17.0 mmol) was dissolved in anhydrous $CH_2Cl_2$ (40 mL) in Schlenk flask and to this solution of 1*H*-Tetrazole (1.3 mL, 30.7 mmol) in ~0.45 M ACN was added at room temperature. To this solution, *N*-Boc-L-Serine-methyl ester (3.3 g, 15.3 mmol) was added under a nitrogen atmosphere, in a few minutes, a white solid was precipitated. The whole was stirred for 5 h at room temperature and then the reaction was quenched with saturated $NaHCO_3$. The whole was extracted in $CH_2Cl_2$ (3 x 100 mL) and then the combined organic layer was dried over the anhydrous $Na_2SO_4$, evaporated in vacuo. The residue was neutral alumina column chromatographed (EtOAc/Hexane 10:90) to yield the compound **15** (4.3 gm, 10.1 mmol, 60 %, colorless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.67-5.61 (m, 1/2H), 5.41-5.39 (m, 1/2H),

4.38-4.31 (m, 1H), 4.00-3.92 (m, 1H), 3.78-3.70 (m, 1H), 3.68 (2s, 3H), 3.59-3.46 (m, 2H), 1.40 (m, 9H), 1.31 (m, 9/2H), 1.26 (m, 9/2H), 1.12-1.07 (m, 12H), $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ 138.85, 138.72.

**C) Synthesis of Compound (*R*)-16 & (*R*)-17**




**Methyl O-(*tert*-butoxy((R)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate ((*R*)-16):**

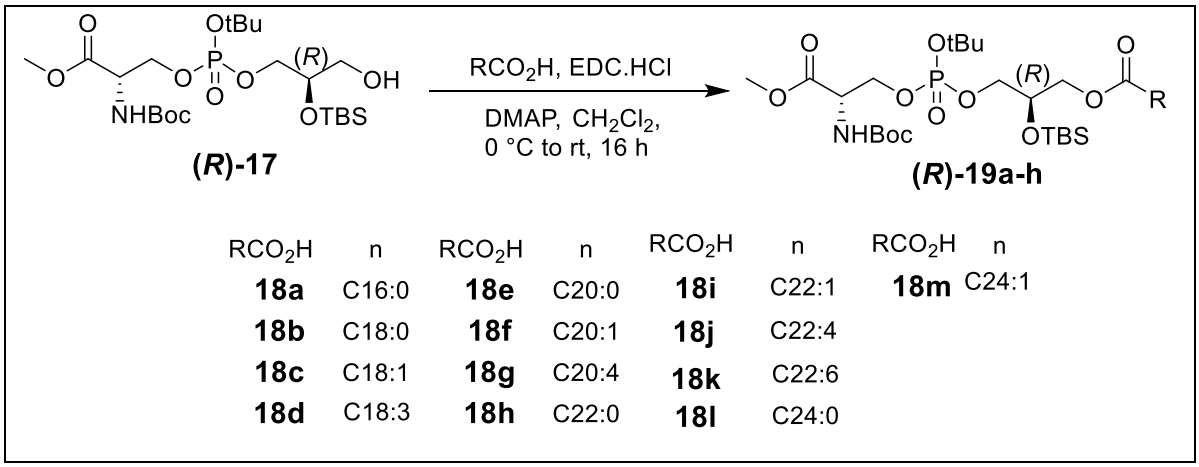
To synthesis compounds **(*R*)-16** and **(*R*)-17,** we followed previously reported procedure[64]. The Phosphonamidite **15** (2.3 g, 5.44 mmol) was dissolved in anhydrous $CH_2Cl_2$ (5 ml), and then the solution was co-evaporated with ACN three times (3 x 5 mL). Under nitrogen atmosphere, the residue was dissolved in anhydrous $CH_2Cl_2$ (25 mL), and subsequently, the solution of 1*H*-tetrazole in ACN (~0.45 M) (1.4 mL, 1.3 mmol) was added at room temperature. The solution of alcohol **(*R*)-4** (3.0 g, 10.8 mmol) in $CH_2Cl_2$ (5 mL) was added dropwise under $N_2$ atmosphere, after a few minutes, a white solid precipitated. The mixture was stirred at room temperature for 24 h. The anhydrous ACN (15 mL) was added and then the reaction mixture was heated to 60°C for 12 h. The intermediate formation was confirmed by TLC then *t*-butyl hydroperoxide (TBHP) solution in decane (5.0-6.0 M) (1.5 mL, 16.2 mmol) was added dropwise and the reaction mixture was stirred for 3 h at room temperature under nitrogen atmosphere. The reaction mixture was diluted with 15 mL water and extracted with $CH_2Cl_2$ (3 X 30 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtrated, and concentrated under reduced pressure. The residue was column chromatographed

(EtOAc/Hexane 40:60) to yield the compound **(*R*)-16** (1.2 g, 1.85 mmol, 67 %, colorless oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.80 (s, 4H), 5.59-5.54 (m, 1H), 4.48-4.35 (m, 2H), 4.26-4.13 (m, 2H), 4.10-4.03 (m, 1H), 4.00-3.90 (m, 2H), 3.86-3.81 (m, 1H), 3.74 (s, 3H), 3.73 (2s, 3H), 1.46 (s, 9H), 1.42 (2s, 9H), 0.88 (s, 9H), 0.11 (m, 3H), 0.09 (m, 3H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.55, -5.81, HRMS-ESI: [(M + H)$^+$-TBS, BOC] calcd. for $C_{20}H_{36}NO_9PSi$, 494.1975; found, 494.1980.

**Methyl O-((*tert*-butoxy)((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropoxy)phosphoryl)-N-(*tert*-butoxycarbonyl)-L-serinate ((*R*)-17)**:

To the solution of PMP-protected alcohol **(*R*)-16** (1.2 g, 1.85 mmol) in ACN: $H_2O$ (4:1) (15 mL), the Ceric Ammonium Nitrate (CAN) (2.5 g, 4.6 mmol) was added dropwise at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred for 45 min at 0 °C and then diluted with $H_2O$ (5 mL). The whole was extracted three times with EtOAc (3 x 25 mL). The combined organic layer was washed with brine solution, dried over $Na_2SO_4$, filtrated and concentrated under reduced pressure. The residue was column chromatographed, EtOAc/Hexane (60:40) used as an eluent to afford the desired product **(*R*)-17** (0.400 g, 0.736 mmol, 84 %, brown oil). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.63-5.56 (m, 1H), 4.51-4.49 (m, 1H), 4.41-4.36 (m, 1H), 4.26-4.20 (m, 1H), 3.98-3.94 (m, 2H), 3.90-3.86 (m, 1H), 3.77 (2d, 3H), 3.65-3.56 (m, 3H), 2.16 (s, 1H), 1.48 (s, 9/2H), 1.47 (s, 9/2H), 1.44 (s, 9H), 0.89 (s, 9/2H), 0.88 (s, 9/2H), 0.09 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -4.98, -5.36. HRMS-ESI: [(M + H)$^+$-TBS, BOC] calcd. for $C_{13}H_{30}NO_8PSi$, 388.1557; found, 388.1557.

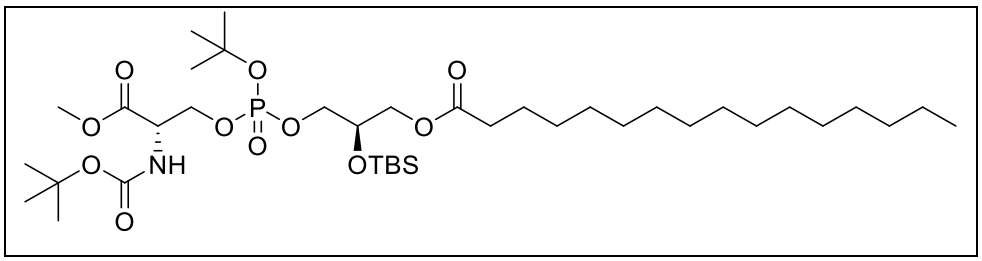
**General procedure (D1) for the synthesis of compounds (*R*)-19a-h**:




| $RCO_2H$ | n | $RCO_2H$ | n | $RCO_2H$ | n | $RCO_2H$ | n |
|---|---|---|---|---|---|---|---|
| **18a** | C16:0 | **18e** | C20:0 | **18i** | C22:1 | **18m** | C24:1 |
| **18b** | C18:0 | **18f** | C20:1 | **18j** | C22:4 | | |
| **18c** | C18:1 | **18g** | C20:4 | **18k** | C22:6 | | |
| **18d** | C18:3 | **18h** | C22:0 | **18l** | C24:0 | | |

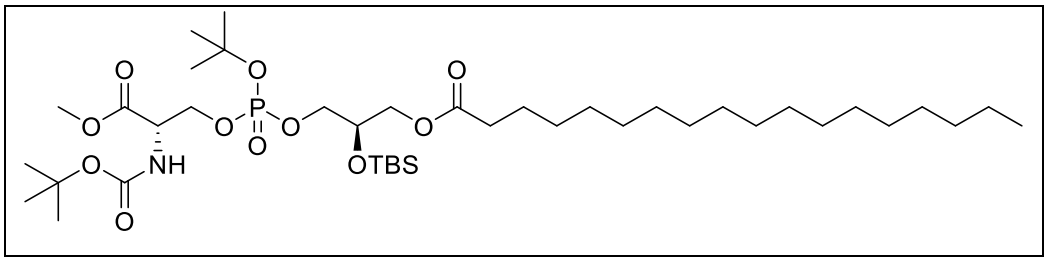
To synthesize a compound **(*R*)-19,** we followed previously reported procedure. To a solution of alcohol **(*R*)-17** (1.0 equiv) and fatty acid **18** (0.9 equiv) in anhydrous $CH_2Cl_2$, the 4-Dimethylaminopyridine (DMAP 0.25 equiv) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 0.9 equiv) were sequentially added at 0 °C. After stirring the mixture 16 h at room temperature, the reaction was quenched with a saturated solution of $NaHCO_3$ and extracted three times with $CH_2Cl_2$. The combined organic layer was dried over $Na_2SO_4$, filtrated, and concentrated under reduced pressure at 25 °C. The residue was column chromatographed (100-200 silica gel mesh) using 25-30% Ethyl Acetate in hexane as an eluent to afford the corresponding desired product **(*R*)-19.**

**(*R*)-19a**



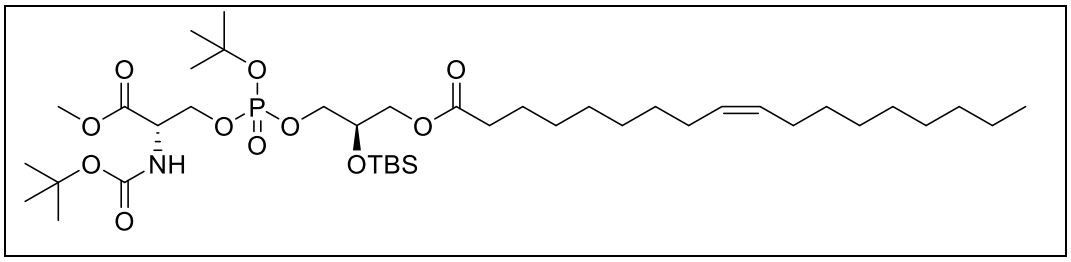

**(2*R*)-3-(((*tert*-butoxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propylpalmitate** (Compound **(*R*)-19a**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18a** (Palmitic acid, C16:0) (34 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19a** (40 mg, 0.0511 mmol, 35%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 4.51-4.36 (m, 2H), 4.27-4.18 (m, 1H), 4.15-4.08 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.32-2.28 (m, 2H), 1.62-1.57 (m, 2H), 1.47 (2d, 9H), 1.44 (s, 9H), 1.27-1.24 (m, 24H), 0.88-0.85 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.60, -5.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 169.9 (d, $J_{c\text{-}p}$ = 4.7 Hz), 155.4, 84.2 (d, $J_{c\text{-}p}$ = 7.0 Hz), 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.8 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.7 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.5 Hz), 65.0, 54.1 (d, $J_{c\text{-}p}$ = 7.7 Hz), 52.8, 34.2, 32.0, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 69.1 (d, $J_{c\text{-}p}$ = 8.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.6 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.4 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.6 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_3$), 29.7 ($CH_3$), 29.7 ($2CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$).

**(*R*)-19b**



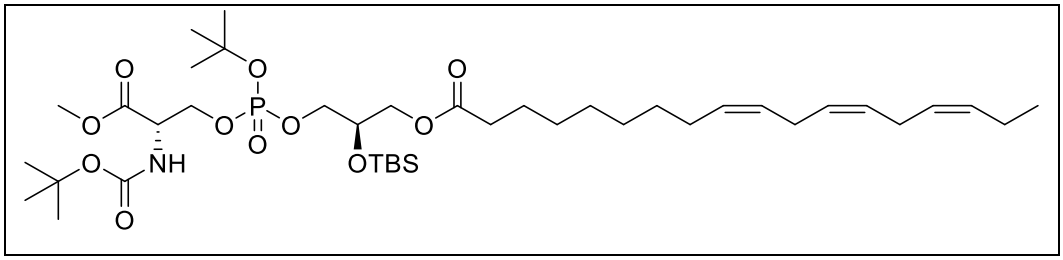
**(2*R*)-3-(((*tert*-butoxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate** (Compound **(*R*)-19b**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18b** (Stearic acid, C18:0) (38 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19b** (34 mg, 0.0420 mmol, 29%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 4.52-4.36 (m, 2H), 4.27-4.18 (m, 1H), 4.16-4.10 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.32-2.28 (m, 2H), 1.62-1.59 (m, 2H), 1.47 (2d, 9H), 1.44 (s, 9H), 1.27-1.24 (m, 28H), 0.88-0.85 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.60, -5.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.4, 84.2 (d, $J_{c\text{-}p}$ = 6.8 Hz), 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.8 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.7 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.6 Hz), 52.8, 34.3, 32.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 69.1 (d, $J_{c\text{-}p}$ = 8.5 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.6 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 8.0 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_3$), 29.7 ($CH_3$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{40}H_{80}NO_{11}PSi$ $[M+K]^+$: calcd., 848.4; found, 848.6; $[M+Na]^+$: calcd., 832.5; found, 832.7.

**(*R*)-19c**



**(2*R*)-3-(((*tert*-butoxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl oleate** (Compound **(*R*)-19c**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18c** (Oleic acid, C18:1) (37 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19c** (27 mg, 0.0334 mmol, 23%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 5.39-3.19 (m, 2H), 4.51-4.37 (m, 2H), 4.28-418 (m, 1H), 4.15-4.13 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.33-2.29 (m, 2H), 2.06-1.98 (m, 4H), 1.63-1.58 (m, 2H), 1.48 (2d, 9H), 1.45 (s, 9H), 1.32-1.25 (m, 20H), 0.89-0.84 (m, 12H), 0.09 (d, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.59, -5.80; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.4, 130.1, 129.9, 84.3, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.8 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.6 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.6 Hz), 52.8, 34.3, 32.0, 29.9, 29.9, 29.9, 29.7, 29.5, 29.3, 29.3, 29.2, 28.4, 27.4, 27.3, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 130.0 (CH), 129.7 (CH), 69.1 (d, $J_{c\text{-}p}$ = 8.6 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.9 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.6 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_3$), 29.7 ($CH_3$), 29.7 ($CH_2$), 29.5 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 29.1 ($CH_2$), 28.3 ($CH_3$), 27.2 (d, $J_{c\text{-}p}$ = 3.7 Hz, $CH_2$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{40}H_{78}NO_{11}PSi$ $[M+K]^+$: calcd., 846.4; found, 846.6; $[M+Na]^+$: calcd., 830.4; found, 830.6.

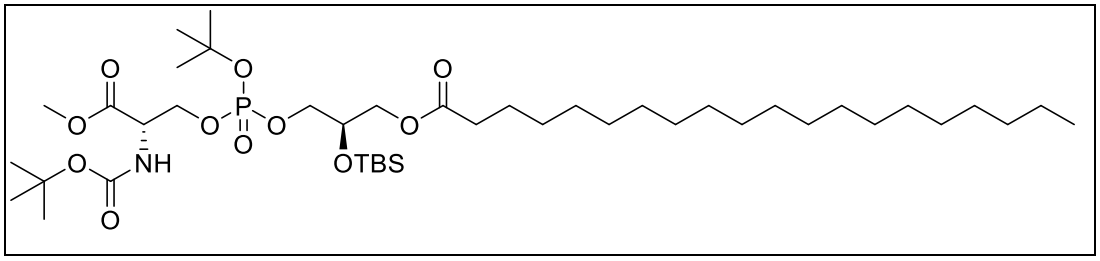
**(*R*)-19d**




**(2*R*)-3-(((*tert*-butoxy)((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl (9Z, 12Z, 15Z)-octadeca-9,12,15-trienoate** (Compound **(*R*)-19d**): Following the general procedure (**D1**), **(*R*)-17** (70 mg, 0.128 mmol), **18d** (α-Linolenic acid, C18:3) (32 mg, 0.115 mmol), EDC·HCl (22 mg, 0.115 mmol), DMAP (4 mg, 0.0320 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19d** (14 mg, 0.0174 mmol, 14%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.59-5.55 (m, 1H), 5.43-5.27 (m, 6H), 4.52-4.37 (m, 2H), 4.28-418 (m, 1H), 4.16-4.13 (m, 1H), 4.06-3.86 (m,

4H), 3.77 (2s, 3H), 2.80 (*t*, *J* = 6.4 Hz, 4H), 2.33-2.29 (m, 2H), 2.11-2.02 (m, 4H), 1.63-1.60 (m, 2H), 1.48 (2d, 9H), 1.45 (s, 9H), 1.33-1.25 (m, 8H), 0.97 (*t*, *J* = 7.5 Hz, 3H), 0.88 (s, 9H), 0.09 (d, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.60, -5.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.9 Hz), 155.4, 132.1, 130.4, 128.4, 128.4, 127.9, 127.2, 84.0, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 5.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.4 Hz), 67.4 (d, $J_{c\text{-}p}$ = 3.7 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 5.5 Hz), 52.9, 34.3, 32.1, 29.9, 29.9, 29.9, 29.8, 29.7, 29.3, 29.3, 29.3, 28.4, 27.4, 25.8, 25.7, 25.7, 25.0, 20.7, 18.2, 14.4, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 132.0 (CH), 130.3 (CH), 128.3 (CH), 128.3 (CH), 127.7 (CH), 127.1 (CH), 69.1 (d, $J_{c\text{-}p}$ = 5.8 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 3.6 Hz, $CH_2$), 64.9 ($2CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 5.0 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.4 (CH), 30.2 (@), 29.8 ($2CH_3$), 29.7 ($CH_3$), 29.6 ($CH_2$), 29.2 ($CH_2$), 29.1 ($2CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 25.7 ($CH_3$), 25.6 ($CH_2$), 25.5 ($CH_2$), 24.9 ($CH_2$), 20.6 ($CH_2$), 14.3 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{40}H_{74}NO_{11}PSi$ $[M+K]^+$: calcd., 842.4; found, 842.6; $[M+Na]^+$: calcd., 826.4; found, 826.6.

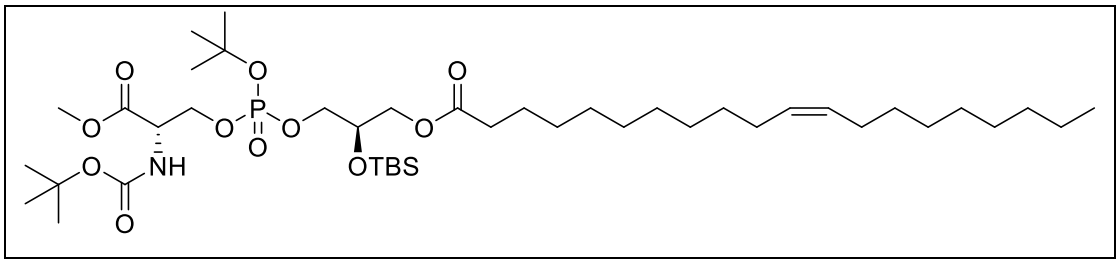
**(*R*)-19e**




**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl icosanoate** (Compound **(*R*)-19e**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18e** (Arachidic acid, C20:0) (41 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19e** (35 mg, 0.0394 mmol, 29%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 4.51-4.36 (m, 2H), 4.27-4.18 (m, 1H), 4.17-4.11 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.32-2.28 (m, 2H), 1.62-1.57 (m, 2H), 1.47 (2d, 9H), 1.44 (s, 9H), 1.27-1.24 (m, 32H), 0.88-0.85 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.61, -5.82; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.7 Hz), 155.4, 84.2 (d, $J_{c\text{-}p}$ = 7.0 Hz), 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.6 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.5 Hz), 65.0, 54.1 (d, $J_{c\text{-}p}$ = 7.8 Hz), 52.8, 34.3, 32.0, 29.9, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 69.1 (d, $J_{c\text{-}p}$ = 8.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.6 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.4 Hz,

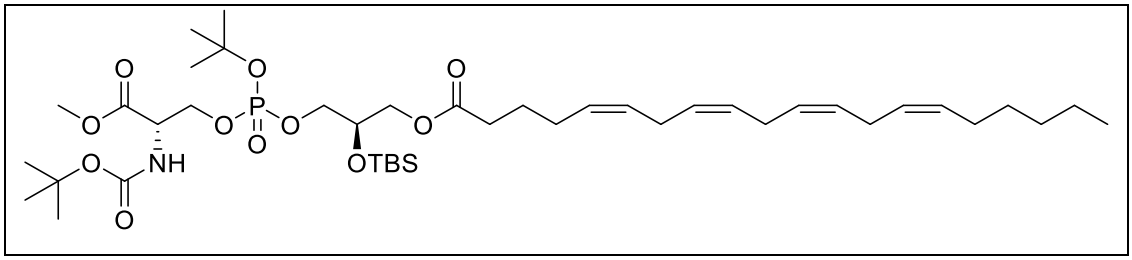
$CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.3 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($2CH_3$), 29.7 ($CH_3$), 29.7 ($2CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{42}H_{84}NO_{11}PSi$ $[M+K]^+$: calcd., 876.5; found, 876.6; $[M+Na]^+$: calcd., 860.5; found, 860.7.

**(*R*)-19f**



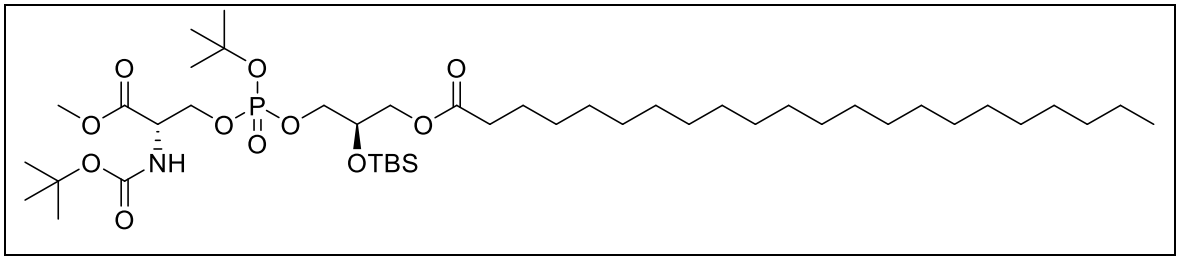

**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (Z)-icos-11-enoate** (Compound **(*R*)-19f**): Following the general procedure (**D1**), **(*R*)-17** (60 mg, 0.110 mmol), **18f** (Eicosenoic acid, C20:1) (31 mg, 0.0993 mmol), EDC·HCl (19 mg, 0.0993 mmol), DMAP (3 mg, 0.0275 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19f** (23 mg, 0.0266 mmol, 25%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 5.38-5.30 (m, 2H), 4.51-4.37 (m, 2H), 4.28-4.18 (m, 1H), 4.15-4.13 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.33-2.28 (m, 2H), 2.02-1.98 (m, 4H), 1.63-1.57 (m, 2H), 1.48 (2d, 9H), 1.44 (s, 9H), 1.32-1.26 (m, 24H), 0.89-0.83 (m, 12H), 0.09 (d, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.60, -5.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.6 Hz), 155.4, 130.1, 130.0, 84.2, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.6 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.9 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.8 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.4 Hz), 52.8, 34.3, 32.0, 29.9, 29.9, 29.9, 29.6, 29.6, 29.5, 29.4, 29.3, 28.4, 27.3, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 129.9 (CH), 129.8 (CH), 69.1 (d, $J_{c\text{-}p}$ = 8.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.4 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_3$), 29.8 ($CH_2$), 29.7 ($CH_3$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{42}H_{82}NO_{11}PSi$ $[M+K]^+$: calcd., 874.5; found, 874.6; $[M+Na]^+$: calcd., 858.5; found, 858.5.

**(*R*)-19g**



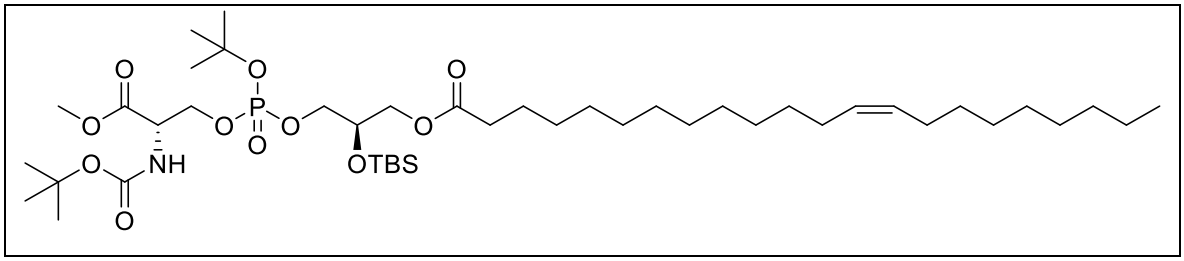
**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate** (Compound **(*R*)-19g**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18g** (Arachidonic acid, C20:4) (40 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19g** (35 mg, 0.0421 mmol, 31%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 5.42-5.29 (m, 8H), 4.52-4.32 (m, 2H), 4.28-418 (m, 1H), 4.16-4.11 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.87-2.78 (m, 6H), 2.36-2.31 (m, 2H), 2.13-2.02 (m, 4H), 1.74-1.66 (m, 2H), 1.48 (2d, 9H), 1.44 (s, 9H), 1.39-1.24 (m, 6H), 0.93-0.83 (m, 12H), 0.09 (d, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.58, -5.79; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.3, 170.0 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.4, 130.6, 129.0, 129.0, 128.7, 128.4, 128.3, 128.0, 127.7, 84.3, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.7 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.4 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.9 Hz), 52.8, 33.7, 31.6, 29.9, 29.9, 29.4, 28.4, 27.3, 26.7, 25.8, 25.8, 25.7, 24.9, 22.7, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 130.5 (CH), 128.9 (CH), 128.9 (CH), 128.6 (CH), 128.2 (CH), 128.1 (CH), 127.8 (CH), 127.5 (CH), 69.1 (d, $J_{c\text{-}p}$ = 8.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.4 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.3 Hz, $CH_2$), 65.0 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.7 Hz, CH), 52.7 ($CH_3$), 33.5 ($CH_2$), 31.5 ($CH_2$), 29.7 ($CH_3$), 29.3 ($CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 26.5 ($CH_2$), 25.7 ($CH_3$), 25.6 ($CH_2$), 24.7 ($CH_2$), 22.6 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{42}H_{76}NO_{11}PSi$ $[M+K]^+$: calcd., 868.4; found, 868.5; $[M+Na]^+$: calcd., 852.4; found, 852.5.

**(*R*)-19h**



**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl docosanoate** (Compound **(*R*)-19h**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18h** (Behenic acid, C22:0) (44 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19h** (27 mg, 0.0311 mmol, 22%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 4.52-4.37 (m, 2H), 4.28-4.18 (m, 1H), 4.16-4.11 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.33-2.28 (m, 2H), 1.63-1.59 (m, 2H), 1.48 (2d, 9H), 1.44 (s, 9H), 1.29-1.24 (m, 36H), 0.89-0.85 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.61, -5.82; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.5, 169.9 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.3, 84.2 (d, $J_{c\text{-}p}$ = 7.3 Hz), 80.3, 69.1 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 67.9 (t, $J_{c\text{-}p}$ = 5.6 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.0 Hz), 64.9, 54.0 (d, $J_{c\text{-}p}$ = 7.1 Hz), 52.7, 34.2, 31.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.3, 25.7, 24.9, 22.7, 18.0, 14.1, -4.8, -4.9; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 69.1 (d, $J_{c\text{-}p}$ = 8.6 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.6 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.2 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 8.2 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($2CH_3$), 29.7 ($CH_3$), 29.7 ($2CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{44}H_{88}NO_{11}PSi$ $[M+K]^+$: calcd., 904.5; found, 904.7; $[M+Na]^+$: calcd., 888.5; found, 888.7.

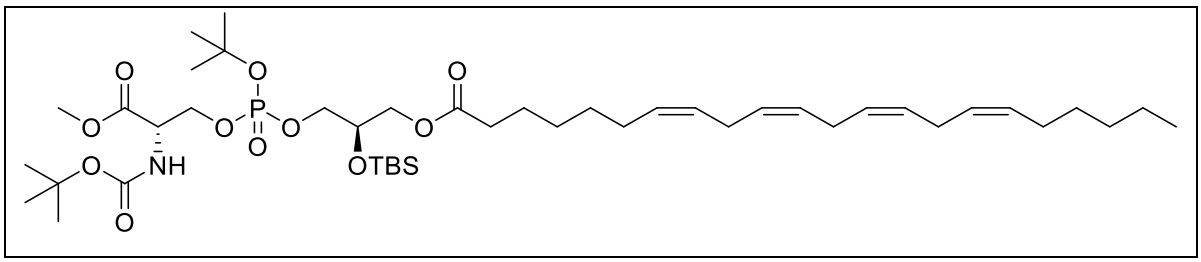
**(*R*)-19i**




**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (Z)-docos-13-enoate** (Compound **(*R*)-19i**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18i** (Erucic acid, C22:1) (44 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19i** (32 mg, 0.0370 mmol, 25%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 5.37-5.30 (m, 2H), 4.51-4.36 (m, 2H), 4.27-418 (m, 1H), 4.16-4.13 (m, 1H), 4.06-3.86 (m, 4H), 3.76 (2s, 3H), 2.32-2.28 (m,

2H), 2.02-1.98 (m, 4H), 1.62-1.57 (m, 2H), 1.47 (2d, 9H), 1.44 (s, 9H), 1.31-1.25 (m, 28H), 0.88-0.85 (m, 12H), 0.09 (d, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.61, -5.82; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.4, 130.0, 130.0, 84.3, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.8 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.8 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.1 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.7 Hz), 52.8, 34.3, 32.0, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 28.4, 27.3, 25.8, 25.0, 22.8, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 129.9 (CH), 129.9 (CH), 69.1 (d, $J_{c\text{-}p}$ = 8.8 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.7 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 4.9 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.6 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_2$), 29.7 ($2CH_3$), 29.6 ($2CH_2$), 29.5 ($2CH_2$), 29.3 ($2CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{44}H_{86}NO_{11}PSi$ $[M+K]^+$: calcd., 902.5; found, 902.7; $[M+Na]^+$: calcd., 886.5; found, 886.8.

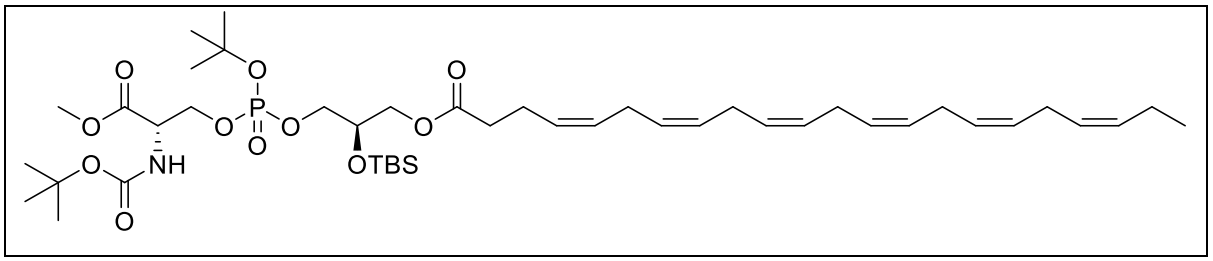
**(*R*)-19j**




**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (7Z,10Z,13Z,16Z)-docosa-7,10,13,16-tetraenoate** (Compound **(*R*)-19j**): Following the general procedure (**D1**), **(*R*)-17** (60 mg, 0.110 mmol), **18j** (Docosatetraenoic acid, C22:4) (30 mg, 0.0993 mmol), EDC·HCl (19 mg, 0.0993 mmol), DMAP (3 mg, 0.0248 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19j** (11 mg, 0.0128 mmol, 10%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.53 (m, 1H), 5.43-5.30 (m, 8H), 4.52-4.37 (m, 2H), 4.28-419 (m, 1H), 4.17-4.14 (m, 1H), 4.07-3.85 (m, 4H), 3.77 (2s, 3H), 2.85-2.79 (m, 6H), 2.35-2.30 (m, 2H), 2.07-2.03 (m, 4H), 1.67-1.63 (m, 2H), 1.48 (2d, 9H), 1.45 (s, 9H), 1.39-1.25 (m, 10H), 0.90-0.87 (m, 12H), 0.10-0.05 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.37, -5.58; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.6, 170.0 (d, $J_{c\text{-}p}$ = 4.6 Hz), 155.4, 130.6, 130.2, 130.1, 128.7, 128.5, 128.2, 128.1, 128.0, 127.7, 84.3, 80.4, 72.1, 69.2 (2d, $J_{c\text{-}p}$ = 5.8 Hz), 68.0, 67.4 (d, $J_{c\text{-}p}$ = 3.5 Hz), 65.1 (d, $J_{c\text{-}p}$ = 2.3 Hz), 64.4, 54.1 (d, $J_{c\text{-}p}$ = 4.8 Hz), 52.9, 34.2, 31.7, 29.9, 29.9, 29.9, 29.5, 29.4, 29.0, 28.4, 27.4, 27.2, 26.0, 25.9, 25.8, 25.8, 24.9, 22.7, 18.2, 14.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 130.5 (CH), 130.0 (CH), 130.0 (CH), 128.6 (CH), 128.4 (CH), 128.1 (CH), 127.9

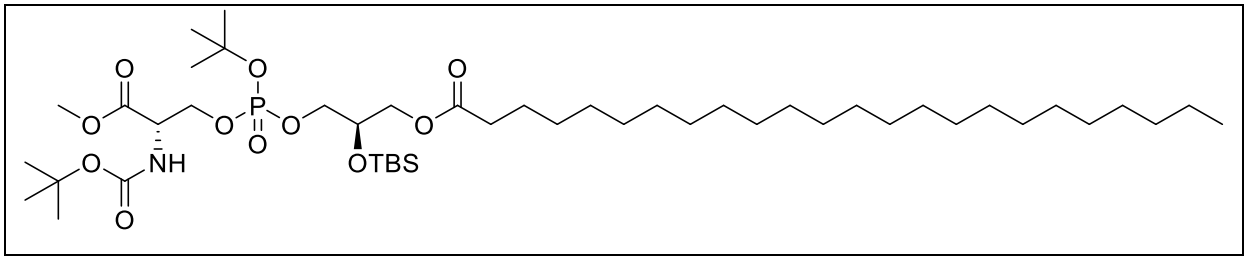
(CH), 127.6 (CH), 69.1 (d, $J_{c\text{-}p}$ = 5.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.7 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 3.6 Hz, $CH_2$), 65.0 ($CH_2$), 64.2 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 5.2 Hz, CH), 52.7 ($CH_3$), 34.1 ($CH_2$), 31.5 ($CH_2$), 29.8 ($2CH_3$), 29.3 ($2CH_2$), 28.8 ($CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 27.1 ($CH_2$), 25.7 ($CH_3$), 25.6 ($CH_2$), 24.8 ($CH_2$), 22.6 ($CH_2$), 14.1 ($CH_3$), -4.7 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{44}H_{80}NO_{11}PSi$ $[M+K]^+$: calcd., 896.4; found, 896.5; $[M+Na]^+$: calcd., 880.5; found, 880.5.

**(*R*)-19k**



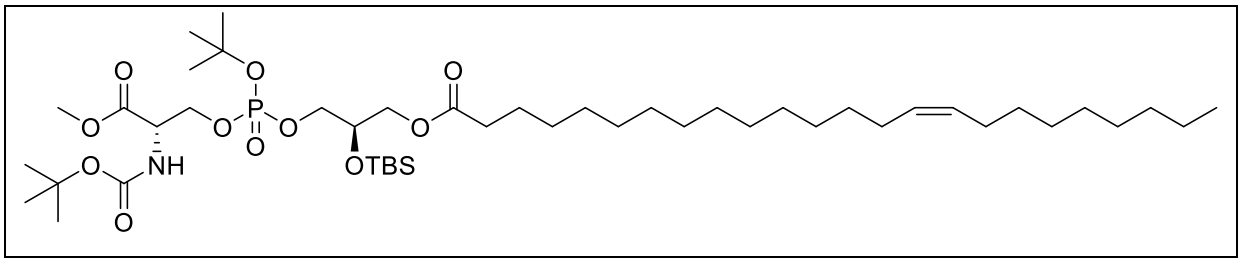

**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate** (Compound **(*R*)-19k**): Following the general procedure (**D1**), **(*R*)-17** (60 mg, 0.110 mmol), **18k** (Docosahexaenoic acid, C22:6) (30 mg, 0.0993 mmol), EDC·HCl (19 mg, 0.0993 mmol), DMAP (4 mg, 0.0275 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19k** (7 mg, 0.00820 mmol, 8%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.55 (m, 1H), 5.43-5.28 (m, 12H), 4.50-4.32 (m, 2H), 4.28-4.19 (m, 1H), 4.17-4.12 (m, 1H), 4.06-3.88 (m, 4H), 3.77 (2s, 3H), 2.85-2.80 (m, 10H), 2.42-2.39 (m, 4H), 2.11-2.04 (m, 2H), 1.61 (m, 2H), 1.49 (2d, 9H), 1.45 (s, 9H), 0.97 (t, $J$ = 7.5 Hz, 3H), 0.89 (m, 9H), 0.10-0.09 (m, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.58, -5.80; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.9, 170.0 (d, $J_{c\text{-}p}$ = 5.0 Hz), 155.4, 132.2, 129.6, 129.5, 128.7, 128.4, 128.2, 128.2, 128.1, 128.0, 128.0, 127.8, 127.2, 84.3, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 5.7 Hz), 68.0, 67.4 (d, $J_{c\text{-}p}$ = 3.9 Hz), 65.2, 62.0, 54.1 (d, $J_{c\text{-}p}$ = 4.6 Hz), 52.9, 34.1, 29.9, 28.5, 27.6, 26.0, 26.0, 25.8, 25.8, 25.7, 25.7, 22.8, 20.7, 18.2, 14.4, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 132.1 (CH), 129.5 (CH), 129.4 (CH), 128.6 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.1 (CH), 128.1 (CH), 127.9 (CH), 127.8 (CH), 127.0 (CH), 69.1 (d, $J_{c\text{-}p}$ = 5.9 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.2 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 2.9 Hz, $CH_2$), 65.1 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 4.7 Hz, CH), 52.7 ($CH_3$), 34.0 ($CH_2$), 29.8 ($CH_3$), 28.3 ($CH_3$), 25.7 ($CH_3$), 25.6 ($2CH_2$), 25.5 ($CH_2$), 22.6 ($CH_2$), 14.3 ($CH_3$), -4.8 ($2CH_3$.

**(*R*)-19l**



**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl tetracosanoate** (Compound **(*R*)-19l**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147 mmol), **18l** (Lignoceric acid, C24:0) (48 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19l** (19 mg, 0.0201 mmol, 15%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.58-5.54 (m, 1H), 4.52-4.37 (m, 2H), 4.28-4.18 (m, 1H), 4.16-4.13 (m, 1H), 4.06-3.86 (m, 4H), 3.77 (2s, 3H), 2.33-2.29 (m, 2H), 1.63-1.60 (m, 2H), 1.48 (2d, 9H), 1.45 (s, 9H), 1.30-1.25 (m, 40H), 0.89-0.86 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.58, -5.79; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.7, 170.0 (d, $J_{c\text{-}p}$ = 4.8 Hz), 155.4, 84.2, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.5 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.6 Hz), 67.3 (d, $J_{c\text{-}p}$ = 5.4 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.4 Hz), 52.8, 34.3, 32.1, 29.9, 29.8, 29.8, 29.6, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.2, 14.3, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 69.1 (d, $J_{c\text{-}p}$ = 8.7 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.8 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.5 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_3$), 29.7 ($CH_3$), 29.7 ($2CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{46}H_{92}NO_{11}PSi$ $[M+K]^+$: calcd., 932.5; found, 932.5; $[M+Na]^+$: calcd., 916.6; found, 916.6.

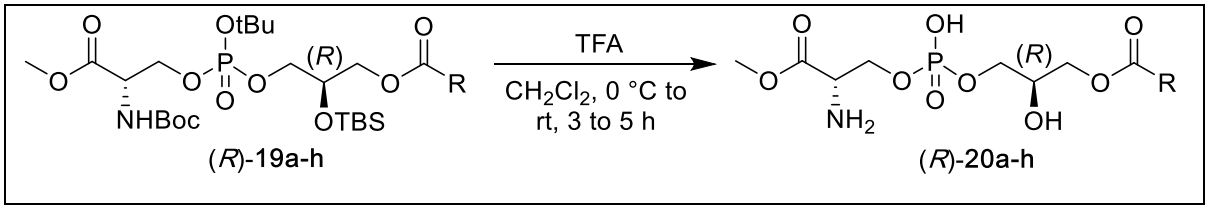
**(*R*)-19m**



**(2R)-3-((tert-butoxy((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl (Z)-tetracos-15-enoate** (Compound **(*R*)-19k**): Following the general procedure (**D1**), **(*R*)-17** (80 mg, 0.147

mmol), **18m** (Nervonic acid, C24:1) (40 mg, 0.132 mmol), EDC·HCl (25 mg, 0.132 mmol), DMAP (4 mg, 0.0367 mmol) and $CH_2Cl_2$ (5 mL) were used. The residue was column chromatographed (100-200 silica gel mesh) (EtOAc/Hexane, 30:70) to yield **(*R*)-19m** (23 mg, 0.0246 mmol, 18%, colourless oil). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.59-5.55 (m, 1H), 5.35-5.33 (m, 2H), 4.51-4.37 (m, 2H), 4.28-418 (m, 1H), 4.16-4.13 (m, 1H), 4.06-3.86 (m, 4H), 3.77 (2s, 3H), 2.33-2.29 (m, 2H), 2.03-1.98 (m, 4H), 1.63-1.59 (m, 2H), 1.48 (2d, 9H), 1.45 (s, 9H), 1.33-1.25 (m, 32H), 0.89-0.86 (m, 12H), 0.09 (2s, 6H); $^{31}$P NMR (400 MHz, $CDCl_3$) $\delta$ -5.60, -5.81; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 173.7, 170.0 (d, $J_{c\text{-}p}$ = 5.2 Hz), 155.4, 130.0, 130.0, 84.3, 80.4, 69.2 (2d, $J_{c\text{-}p}$ = 8.7 Hz), 68.0 (t, $J_{c\text{-}p}$ = 5.7 Hz), 67.4 (d, $J_{c\text{-}p}$ = 5.2 Hz), 65.1, 54.1 (d, $J_{c\text{-}p}$ = 7.7 Hz), 52.8, 34.3, 32.0, 29.9, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 27.4, 25.8, 25.0, 22.8, 18.2, 14.3, -4.6, -4.7; DEPT-135 NMR (100 MHz, $CDCl_3$) $\delta$ 129.9 (CH), 129.9 (CH), 69.1 (d, $J_{c\text{-}p}$ = 8.8 Hz, CH), 67.9 (t, $J_{c\text{-}p}$ = 5.8 Hz, $CH_2$), 67.2 (d, $J_{c\text{-}p}$ = 5.5 Hz, $CH_2$), 64.9 ($CH_2$), 54.0 (d, $J_{c\text{-}p}$ = 7.5 Hz, CH), 52.7 ($CH_3$), 34.2 ($CH_2$), 31.9 ($CH_2$), 29.8 ($CH_2$), 29.7 ($CH_3$), 29.7 ($CH_2$), 29.6 ($2CH_2$), 29.5 ($2CH_2$), 29.3 ($CH_2$), 29.2 ($CH_2$), 28.3 ($CH_3$), 27.2 ($CH_2$), 25.7 ($CH_3$), 24.9 ($CH_2$), 22.7 ($CH_2$), 14.1 ($CH_3$), -4.8 ($CH_3$), -4.9 ($CH_3$); MALDI (ESI-TOF) for $C_{46}H_{90}NO_{11}PSi$ $[M+K]^+$: calcd., 930.5; found, 930.5; $[M+Na]^+$: calcd., 914.5; found, 914.6.

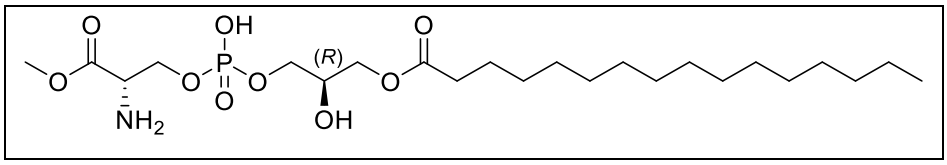
## General procedure (E1) for the synthesis of compounds (*R*)-20a-h (TBS, *t*-Bu, and *t*-BOC deprotection):




To synthesized compound **(*R*)-20** we followed previously been reported procedure[54,64]. The compound **(*R*)-19** in dry $CH_2Cl_2$ was charged in two necks round bottom flask which was equipped with $N_2$ balloon. The solution was cooled to -10 °C and then the TFA was added dropwise. After TFA addition, the reaction temperature and stirring time was variable for different analogues. For the starting moieties **(*R*)-19a-b**, the reaction solution were stirred at 0 °C for 1 h and then at room temperature for 4 h and got (above 85% pure compound). For the moiety **(*R*)-19c-g** the reaction solution was stirred at 0 °C for 3.5 h and then at room temperature for 1 h and got (above 90% pure compound). For the longer fatty acid chain length moieties **(*R*)-19h-m** were required to stir the reaction solution at 0 °C for 5 h got (above 70%

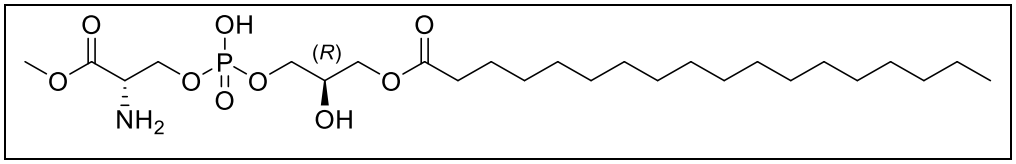
pure compound). Once the reaction was complete, the reaction solution was concentrated under reduced pressure at < 25 °C and then the dried residue was washed with *n*-Pentane: $Et_2O$ (3:1) three times, dried under high vacuum to afford the TFA salt of the desired product i.e. **(*R*)-20** The purity of the final compounds **(*R*)-20a-h** was determined based on the NMR spectra, and HRMS analysis.

**(*R*)-20a**



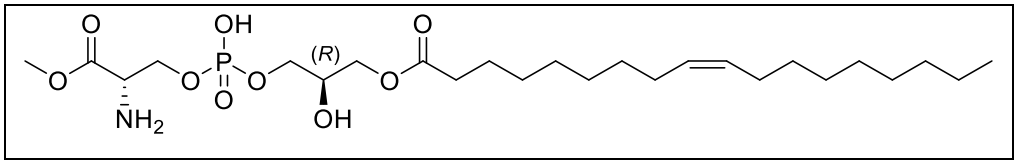

**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate** (Compound **20a**): Following the general procedure (**E1**), **(*R*)-19a** (40 mg), TFA (0.400 mL) and $CH_2Cl_2$ (0.200 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20a** (21 mg, 0.0449 mmol, 88%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.17-3.87 (m, 4H), 3.76-3.73 (m, 3H), 3.61-3.59 (2s, 3H), 3.46 (m, 1H), 2.09 (t, J = 7.1 Hz, 2H), 1.36-1.34 (m, 2H), 1.00 (brs, 24H), 0.61 (t, J = 6.6 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.18.

**(*R*)-20b**



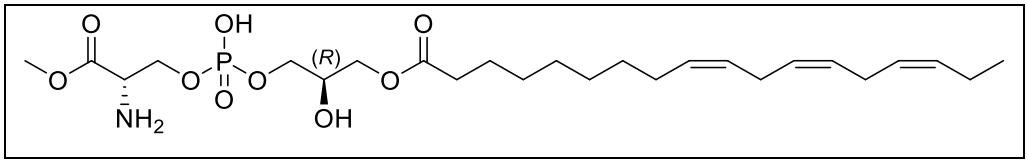

**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate** (Compound **20b**): Following the general procedure (**E1**), **(*R*)-19b** (34 mg), TFA (0.340 mL) and $CH_2Cl_2$ (0.170 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20b** (20 mg, 0.0370 mmol, 91 %, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 3.98-3.96 (m, 2H), 3.89-3.84 (m, 5H), 3.72-3.70 (2s, 3H), 3.56 (m, 1H), 2.19 (t, J = 7.4 Hz, 2H), 1.53-1.44 (m, 2H), 1.11 (brs, 28H), 0.73 (t, J = 6.6 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.05.

**(*R*)-20c**



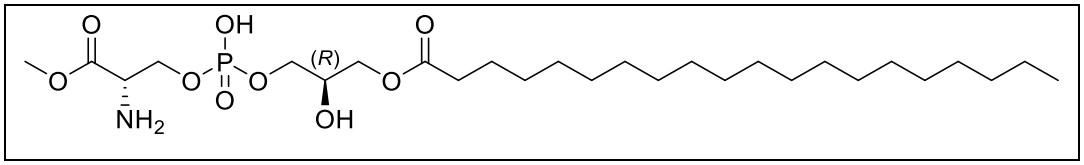
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl oleate** (Compound **20c**): Following the general procedure (**E1**), **(*R*)-19c** (27 mg), TFA (0.270 mL) and $CH_2Cl_2$ (0.160 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20c** (7 mg, 0.0130 mmol, 41%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.03-5.02 (m, 2H), 4.12-3.83 (m, 4H), 3.71-3.68 (m, 3H), 3.57-3.56 (2s, 3H), 3.42 (m, 1H), 2.05 (t, J = 7.2 Hz, 2H), 1.72-1.70 (m, 4H), 1.32 (m, 2H), 1.01-0.97 (m, 20H), 0.59-0.56 (m, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 22.11, 4.09; HRMS (ESI): m/z calcd. for ($C_{25}H_{48}NO_9P$) $[M + H]^+$ 538.3145; found, 538.3140.

**(*R*)-20d**



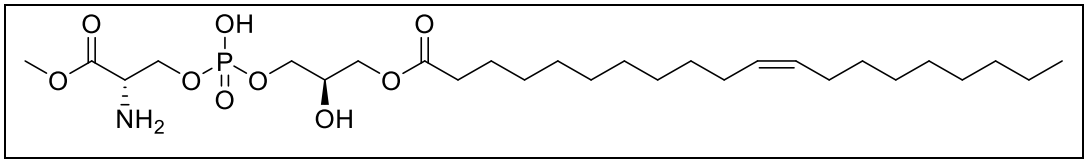
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate** (Compound **20d**): Following the general procedure (**E1**), **(*R*)-19d** (14 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.100 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20d** (7 mg, 0.0131 mmol, 78%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.22-5.08 (m, 6H), 4.03-3.93 (m, 4H), 3.80-3.78 (m, 3H), 3.67-3.66 (2s, 3H), 3.53-3.52 (m, 1H), 2.62-2.59 (m, 4H), 2.15 (t, J = 7.4 Hz, 2H), 1.92-1.85 (m, 4H), 1.42 (m, 2H), 1.14-1.06 (m, 8H), 0.78 (t, J = 7.5 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.97, 4.26; HRMS (ESI): m/z calcd. for ($C_{25}H_{44}NO_9P$) $[M + H]^+$ 534.2832; found, 534.2838.

**(*R*)-20e**



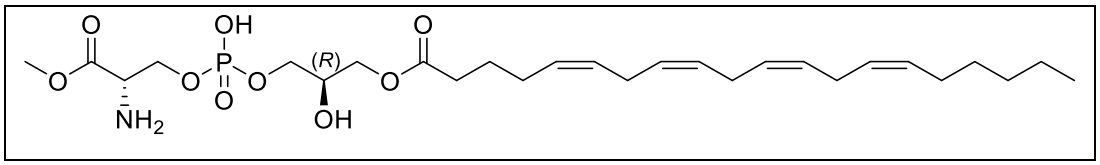
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate** (Compound **20e**): Following the general procedure (**E1**), **(*R*)-19e** (33 mg), TFA (0.330 mL) and $CH_2Cl_2$ (0.170 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20e** (19 mg, 0.0334 mmol, 86%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 3.95-3.74 (m, 4H), 3.71-3.62 (m, 3H), 3.55-3.54 (2s, 3H), 3.41 (m, 1H), 2.03 (t, J = 7.4 Hz, 2H), 1.32-1.28 (m, 2H), 0.94 (brs, 32H), 0.56 (t, J = 6.6 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.08.

**(*R*)-20f**




**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (*Z*)-icos-11-enoate** (Compound **20f**): Following the general procedure (**E1**), **(*R*)-19f** (23 mg), TFA (0.300 mL) and $CH_2Cl_2$ (0.180 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20f** (11 mg, 0.0194 mmol, 73%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.18-5.10 (m, 2H), 4.07-3.92 (m, 4H), 3.84-3.80 (m, 3H), 3.67-3.66 (2s, 3H), 3.53-3.52 (m, 1H), 2.17-2.12 (m, 2H), 1.82-1.79 (m, 4H), 1.42-1.40 (m, 2H), 1.07 (brs, 24H), 0.68 (t, J = 6.5 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.81, 4.37; HRMS (ESI): m/z calcd. for ($C_{27}H_{52}NO_9P$) $[M + H]^+$ 566.3458; found, 566.3448.

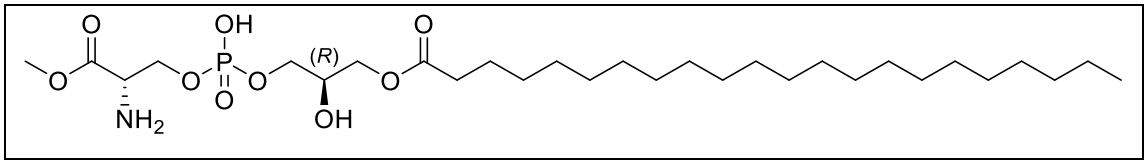
**(*R*)-20g**




**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate** (Compound **20g**): Following the general procedure (**E1**), **(*R*)-19g** (35 mg), TFA (0.350 mL) and $CH_2Cl_2$ (0.200 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20g** (20 mg, 0.0357 mmol, 87%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.31-5.08 (m, 8H), 4.18-3.94 (m, 4H), 3.89-3.79 (m, 3H), 3.74-3.73 (2s, 3H), 3.64

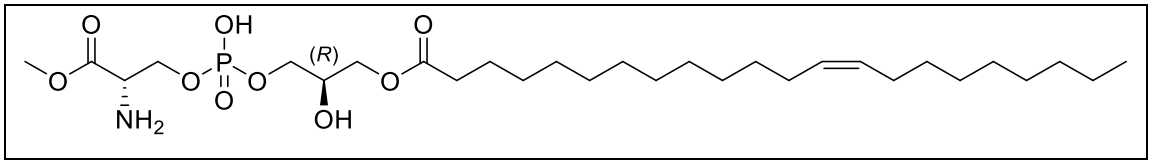
(m, 1H), 2.73-2.68 (m, 6H), 2.25 (t, J = 7.5 Hz, 2H), 1.99-1.90 (m, 4H), 1.58-1.55 (m, 2H), 1.25-1.10 (m, 6H), 0.75 (t, J = 6.4 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.93, 4.46; HRMS (ESI): m/z calcd. for ($C_{27}H_{46}NO_9P$) $[M + H]^+$ 560.2988; found, 560.2972.

**(*R*)-20h**



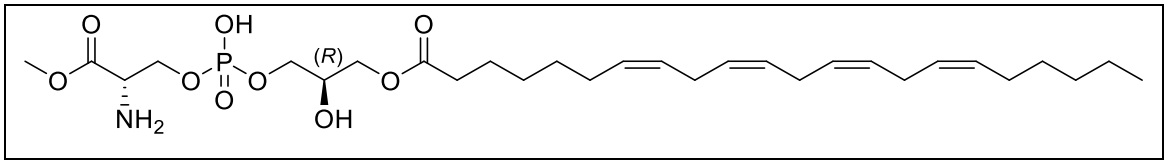
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docasanoate** (Compound **20h**): Following the general procedure (**E1**), **(*R*)-19h** (27 mg), TFA (0.350 mL) and $CH_2Cl_2$ (0.200 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20h** (15 mg, 0.0251 mmol, 83%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.18-3.87 (m, 4H), 3.75-3.72 (m, 3H), 3.61-3.59 (2s, 3H), 3.47 (m, 1H), 2.08 (t, J = 7.1 Hz, 2H), 1.35 (m, 2H), 0.99 (brs, 36H), 0.61 (t, J = 6.1 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 3.97.

**(*R*)-20i**



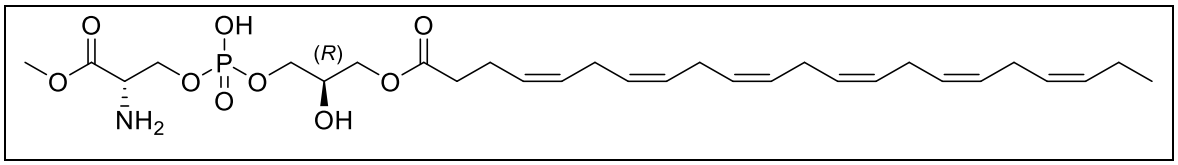
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (Z)-docos-13-enoate** (Compound **20i**): Following the general procedure (**E1**), **(*R*)-19i** (32 mg), TFA (0.350 mL) and $CH_2Cl_2$ (0.200 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20i** (11 mg, 0.0185 mmol, 52%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.51-5.03 (m, 2H), 4.00-3.87 (m, 4H), 3.78-3.66 (m, 3H), 3.61-3.59 (2s, 3H), 3.46 (m, 1H), 2.08 (t, J = 7.4Hz, 3H), 1.76-1.73 (m, 4H), 1.37-1.34 (m, 2H), 1.01 (brs, 28H), 0.61 (t, J = 6.4 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.82, 4.16; HRMS (ESI): m/z calcd. for ($C_{29}H_{56}NO_9P$) $[M + H]^+$ 594.3771; found, 594.3770.

**(*R*)-20j**



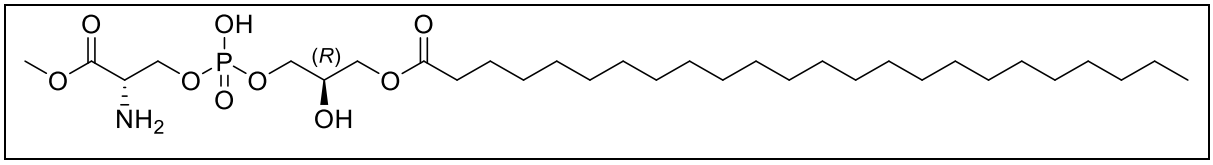
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (7Z,10Z,13Z,16Z)-docosa-7,10,13,16-tetraenoate** (Compound **20j**): Following the general procedure (**E1**), **(*R*)-19j** (11 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.150 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20j** (5 mg, 0.0851 mmol, 71%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.20-5.17 (m, 8H), 4.05-3.92 (m, 4H), 3.82-3.75 (m, 3H), 3.69-3.67 (2s, 3H), 3.43 (m, 1H), 2.66-2.62 (m, 6H), 2.17 (t, J = 7.4 Hz, 2H), 1.90-1.85 (m, 4H), 1.45-1.43 (m, 2H), 1.18-1.08 (m, 10H), 0.71 (t, J = 6.6 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.81, 4.40; HRMS (ESI): m/z calcd. for ($C_{29}H_{50}NO_9P$) $[M + H]^+$ 588.3301; found, 588.3300.

**(*R*)-20k**



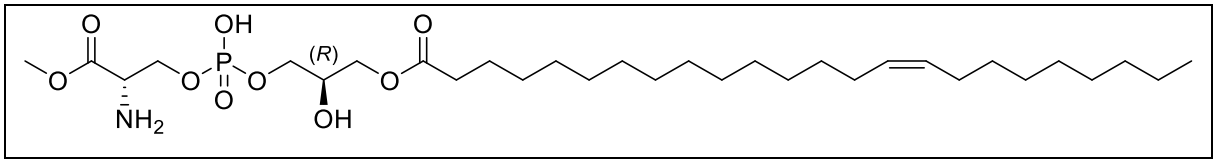
**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate** (Compound **20k**): Following the general procedure (**E1**), **(*R*)-19k** (7 mg), TFA (0.200 mL) and $CH_2Cl_2$ (0.150 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20k** (3 mg, 0.0514 mmol, 75%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.24-5.11 (m, 12H), 3.95-3.88 (m, 4H), 3.83-3.75 (m, 3H), 3.71-3.69 (2s, 3H), 3.43 (m, 1H), 2.69-2.63 (m, 10H), 2.25-2.21 (m, 4H), 1.91 (t, J = 7.5 Hz, 2H), 0.81 (t, J = 7.5 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.91, 4.50; HRMS (ESI): m/z calcd. for ($C_{29}H_{46}NO_9P$) $[M + H]^+$ 584.2988; found, 584.2982.

**(*R*)-20l**



**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate** (Compound **20l**): Following the general procedure (**E1**), **(*R*)-19l** (18 mg), TFA (0.250 mL) and $CH_2Cl_2$ (0.180 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20l** (8 mg, 0.0128 mmol, 67%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.06-3.94 (m, 4H), 3.85-3.75 (m, 3H), 3.68-3.67 (2s, 3H), 3.54 (m, 1H), 2.16 (t, J = 7.4 Hz, 2H), 1.43 (m, 2H), 1.07 (brs, 40H), 0.69 (t, J = 6.5 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 4.43.

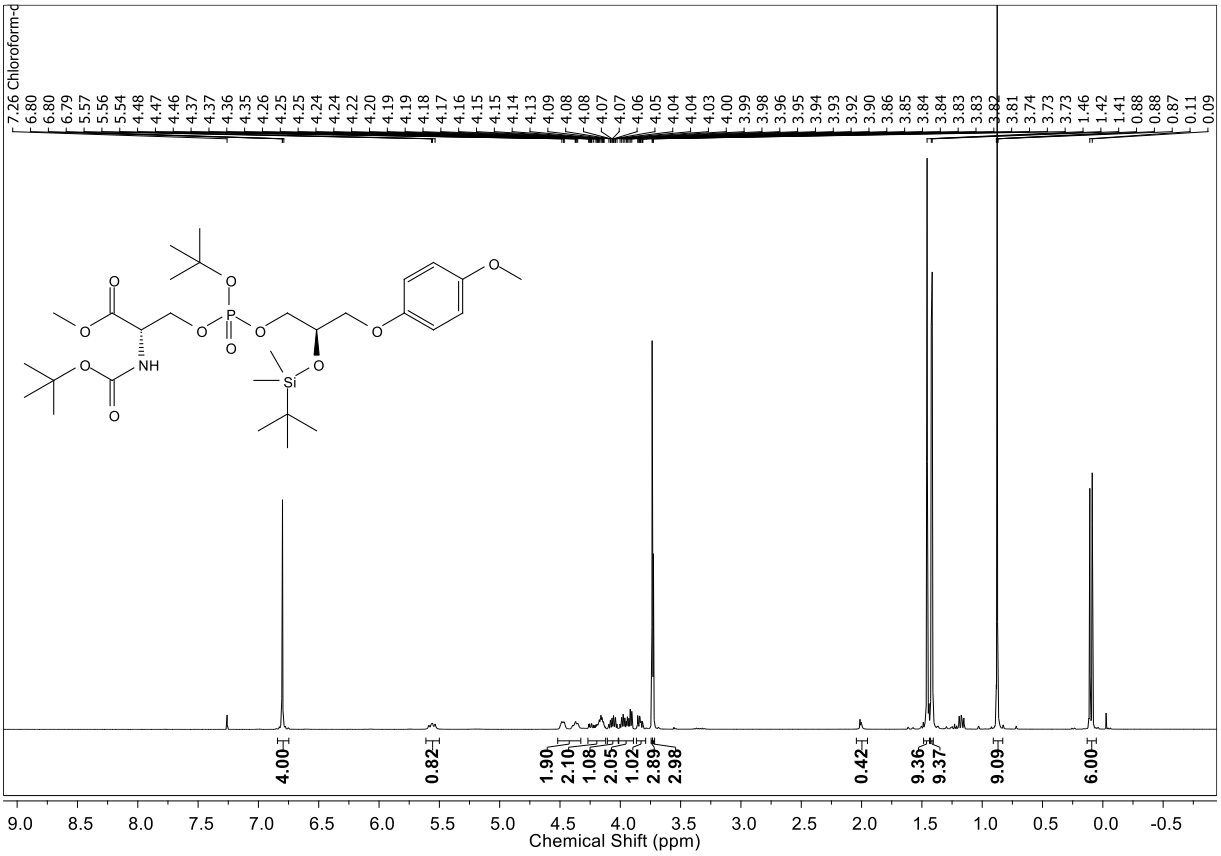
**(*R*)-20m**



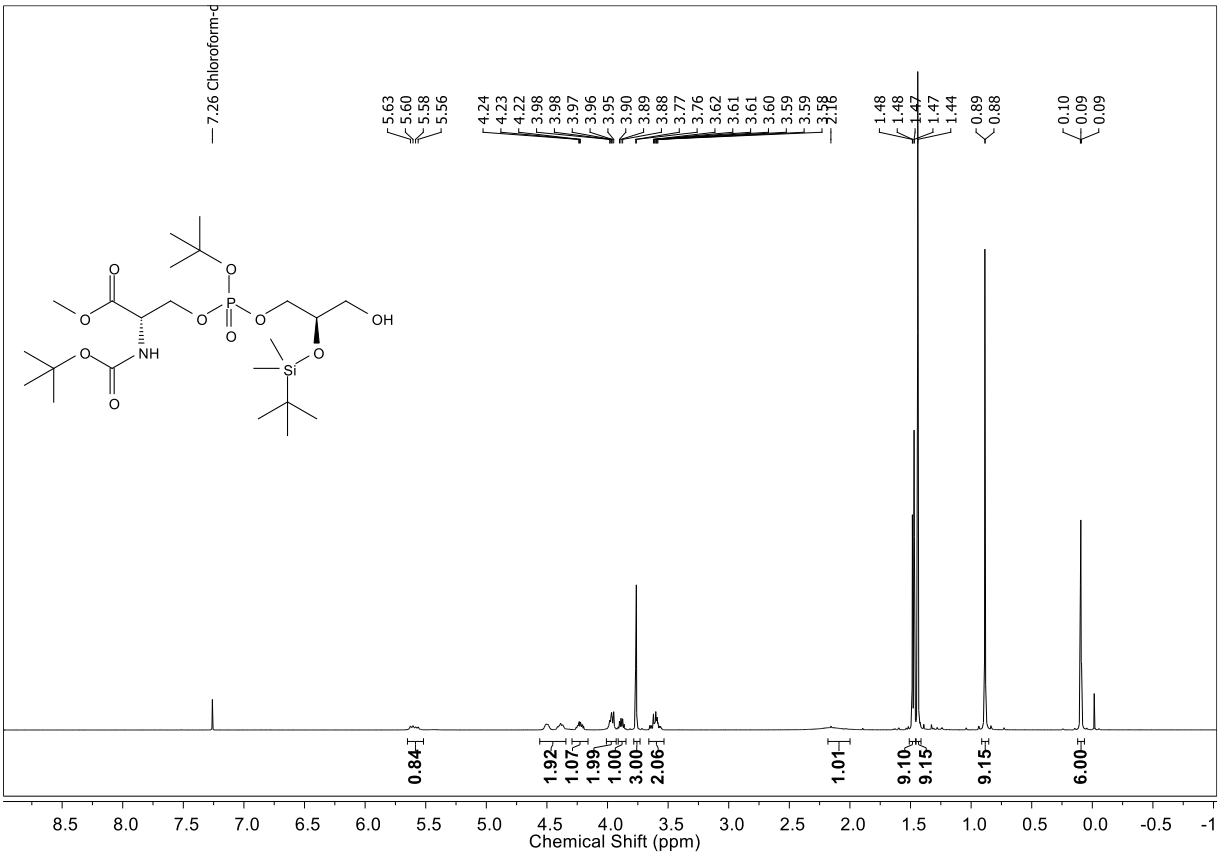

**(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl (Z)-tetracos-15-enoate** (Compound **20m**): Following the general procedure (**E1**), **(*R*)-19m** (22 mg), TFA (0.300 mL) and $CH_2Cl_2$ (0.150 mL) were used. The product was washed with *n*-pentane: $Et_2O$ (4:1) (3 x 2 mL) to yield desired final compound **(*R*)-20m** (7 mg, 0.0112 mmol, 47%, white solid): $^1$H NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 5.21-5.14 (m, 2H), 4.01-3.95 (m, 4H), 3.84-3.76 (m, 3H), 3.70-3.69 (2s, 3H), 3.56 (m, 1H), 2.17 (t, J = 7.3 Hz, 3H), 1.85-1.82 (m, 4H), 1.45 (m, 2H), 1.10 (brs, 32H), 0.71 (t, J = 6.5 Hz, 3H); $^{31}$P NMR (400 MHz, MeOH-$d_4$ + $CDCl_3$) δ 21.95, 4.33; HRMS (ESI): m/z calcd. for ($C_{31}H_{60}NO_9P$) $[M + H]^+$ 622.4084; found, 622.4081.

## 4.7 Spectral Data

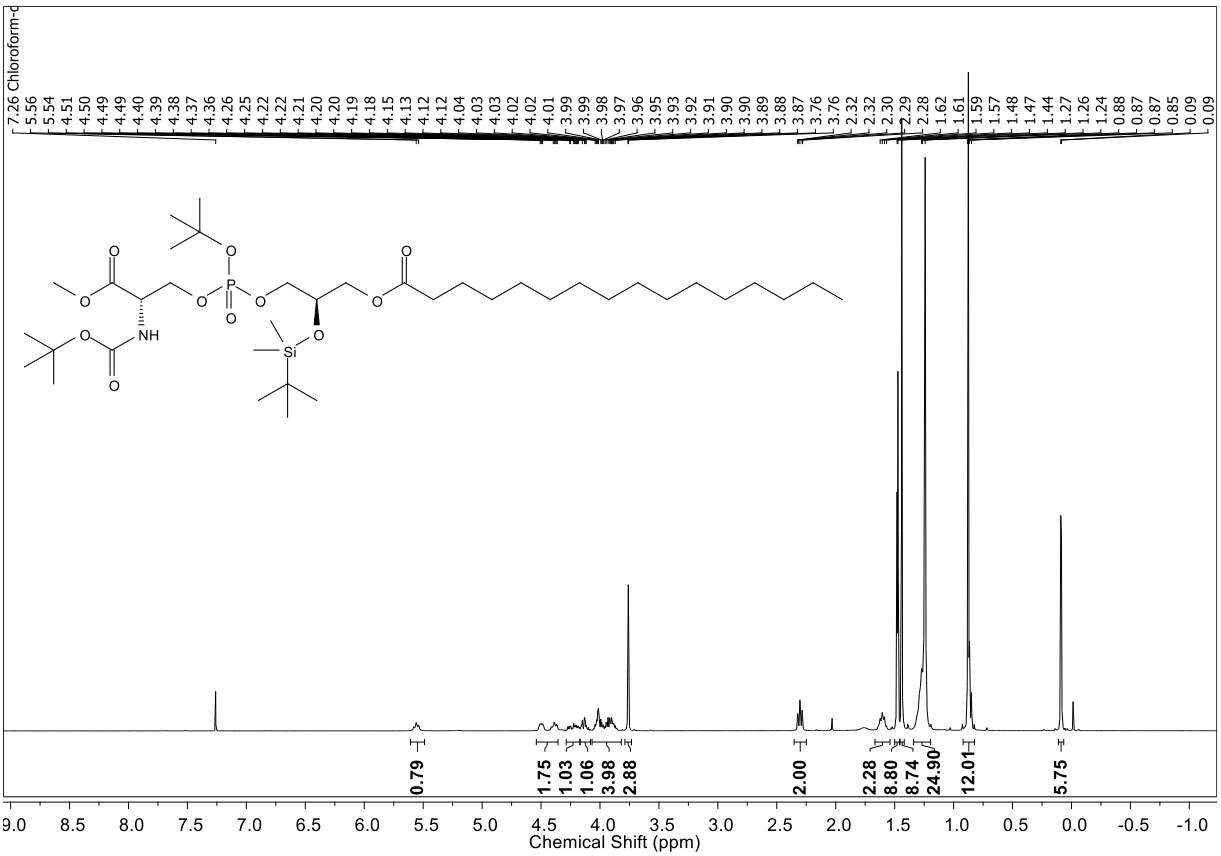
**$^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-16**



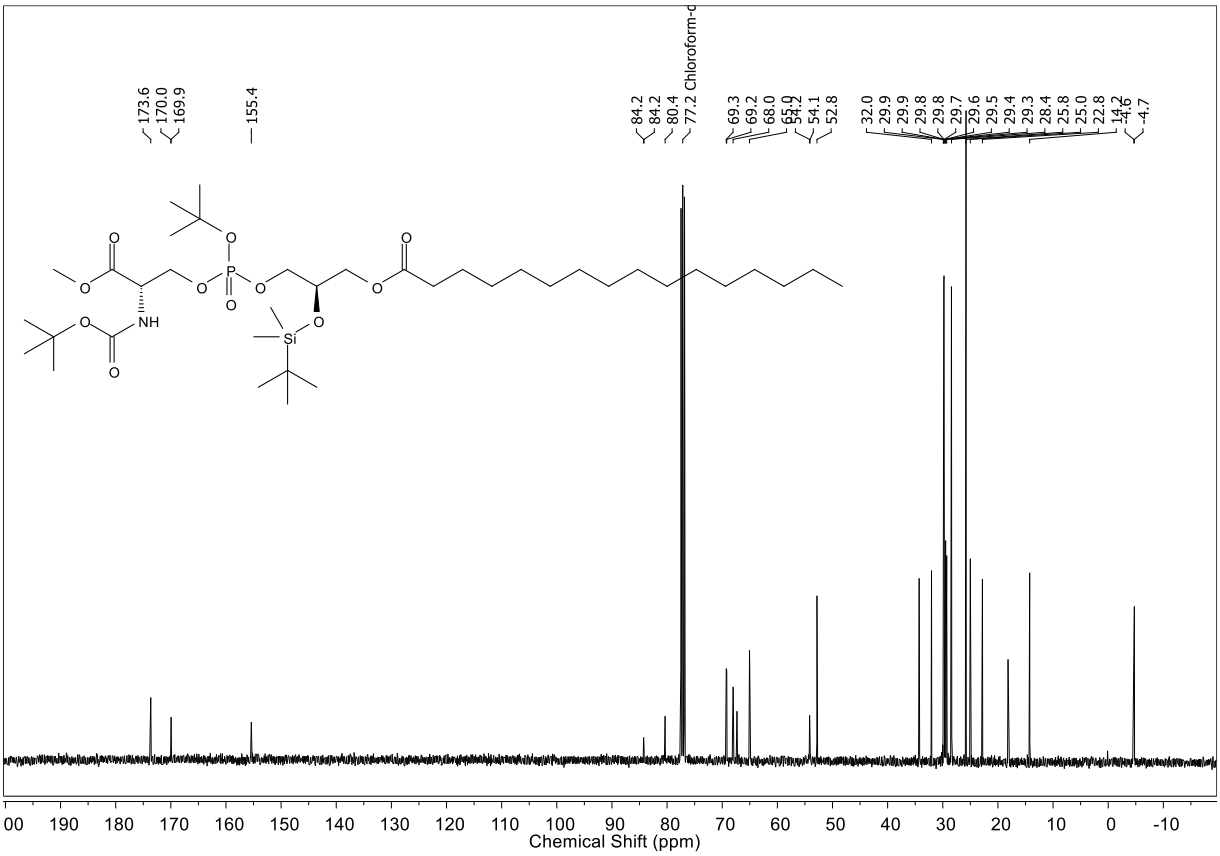
## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-17



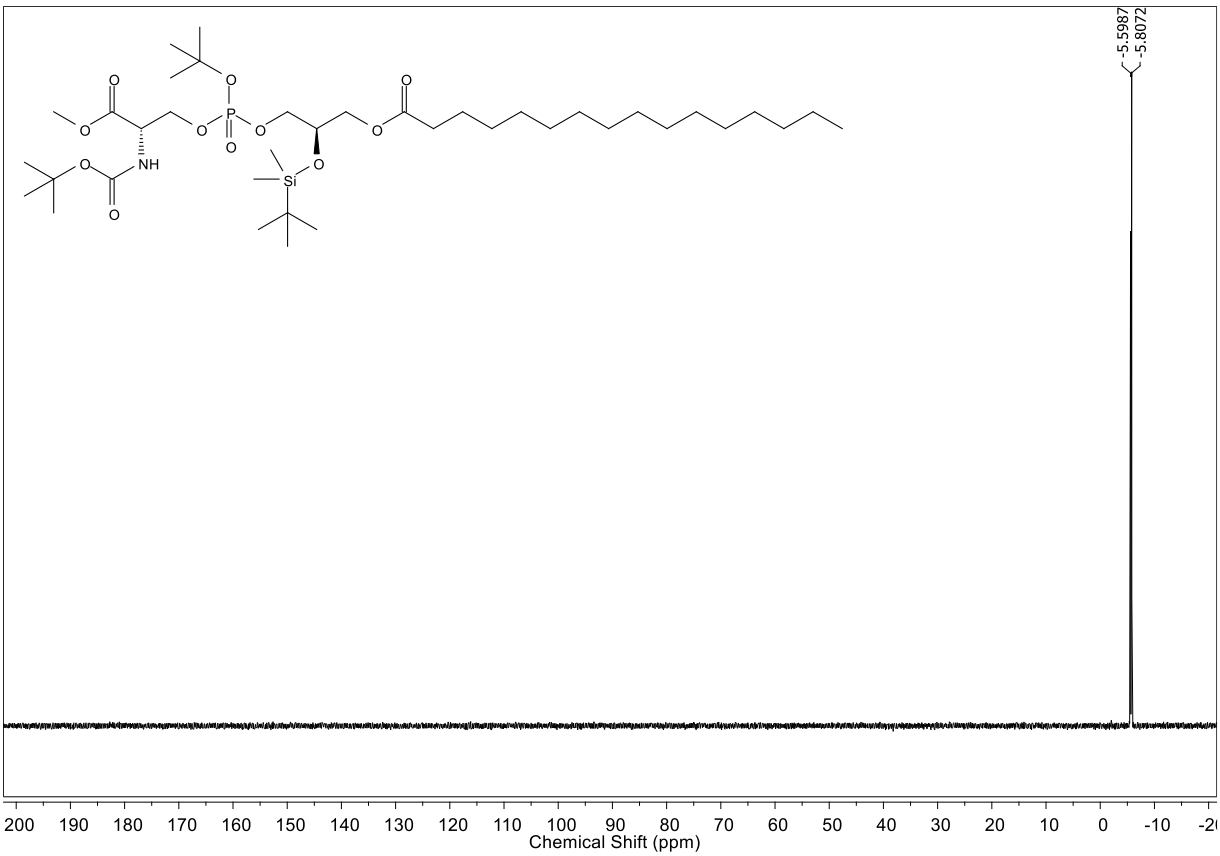

## $^1$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19a



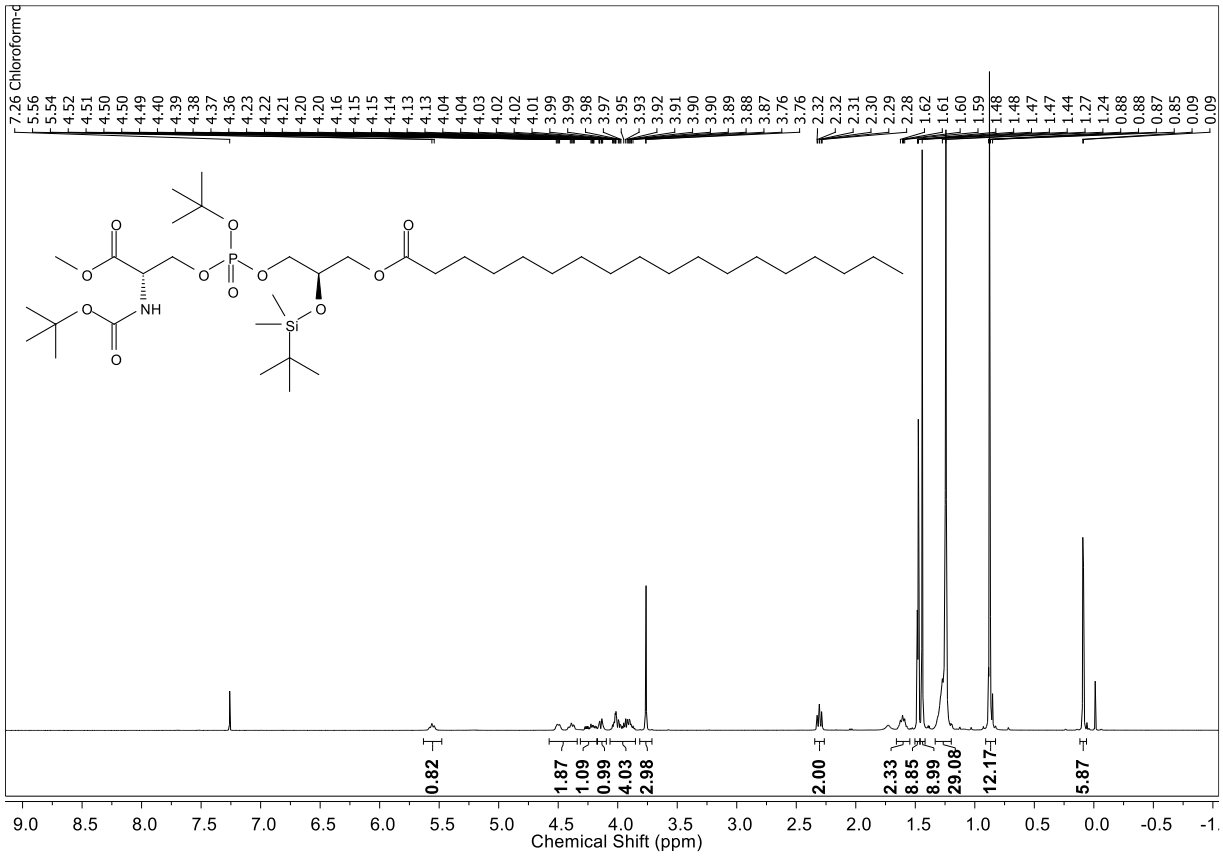
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19a



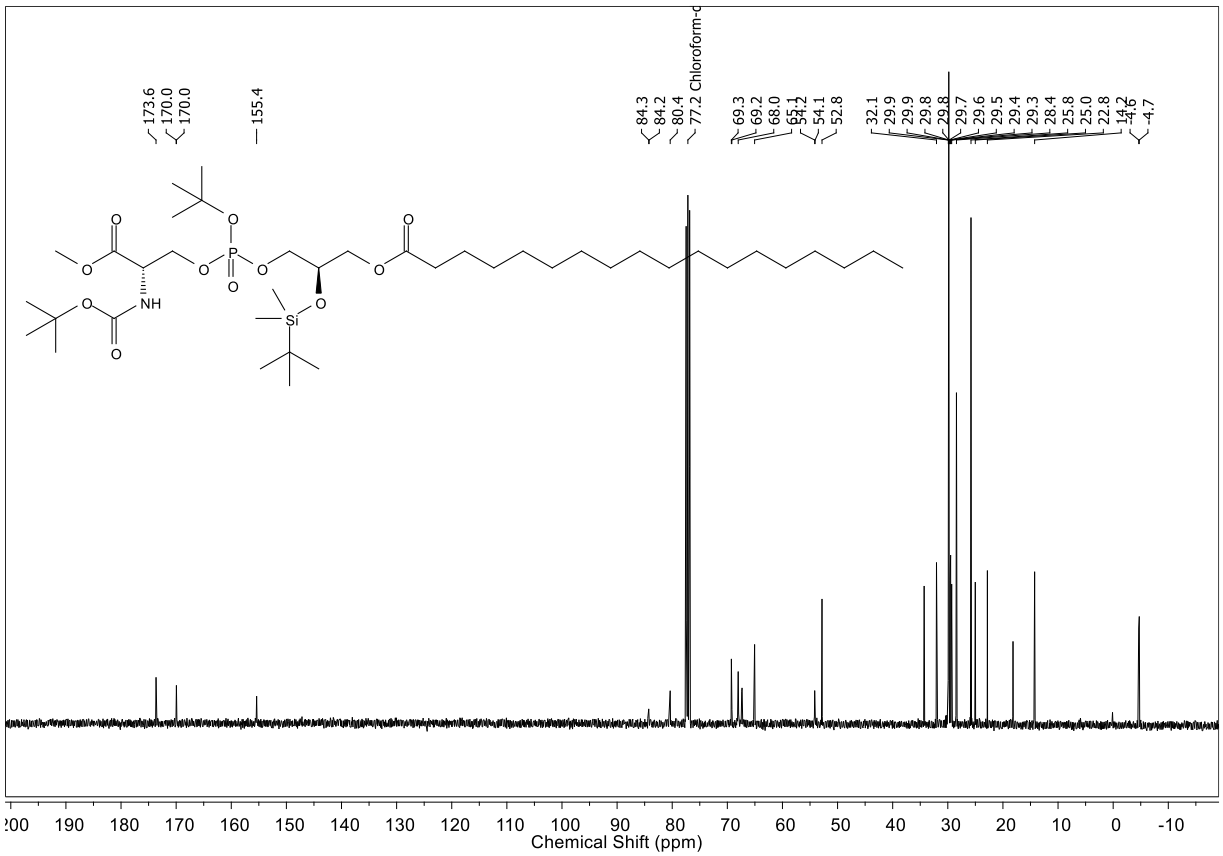

## $^{31}$P-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19a



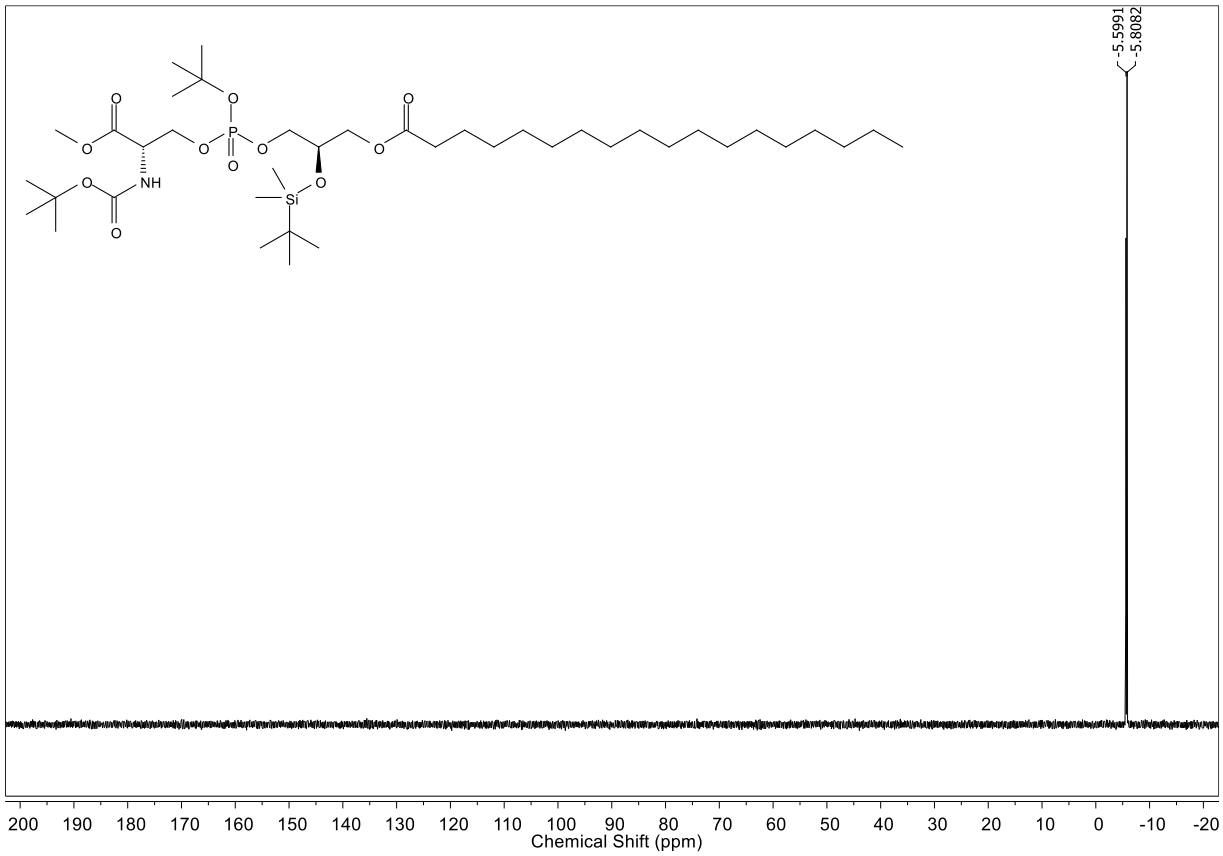
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19b



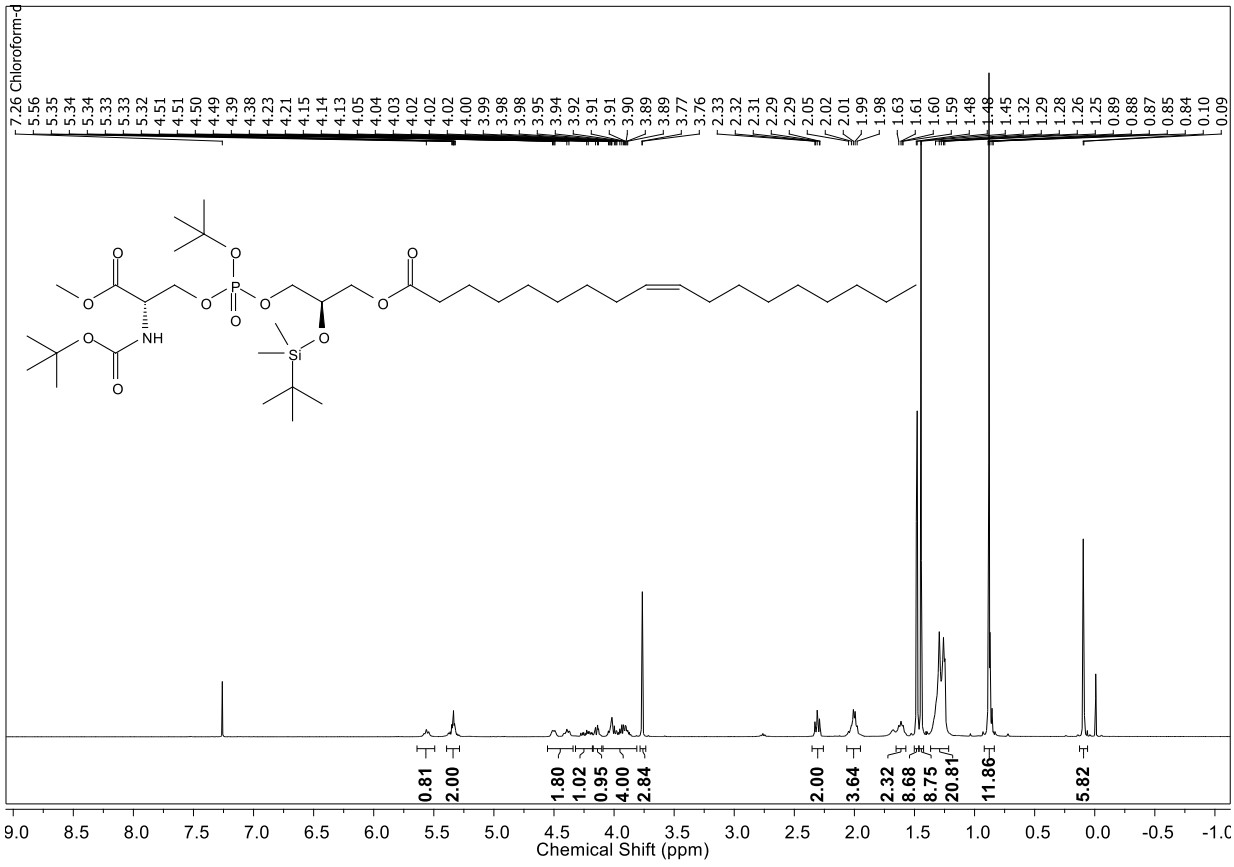

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19b



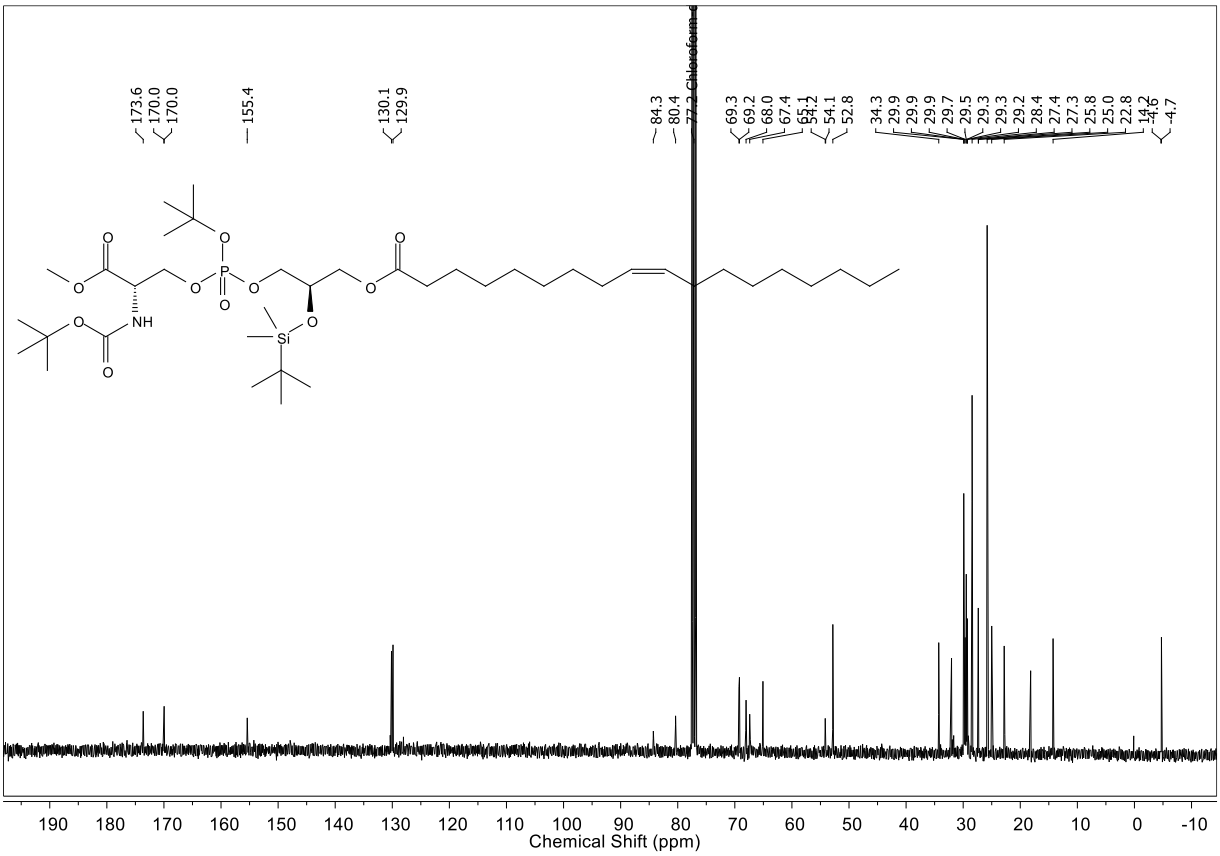
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19b



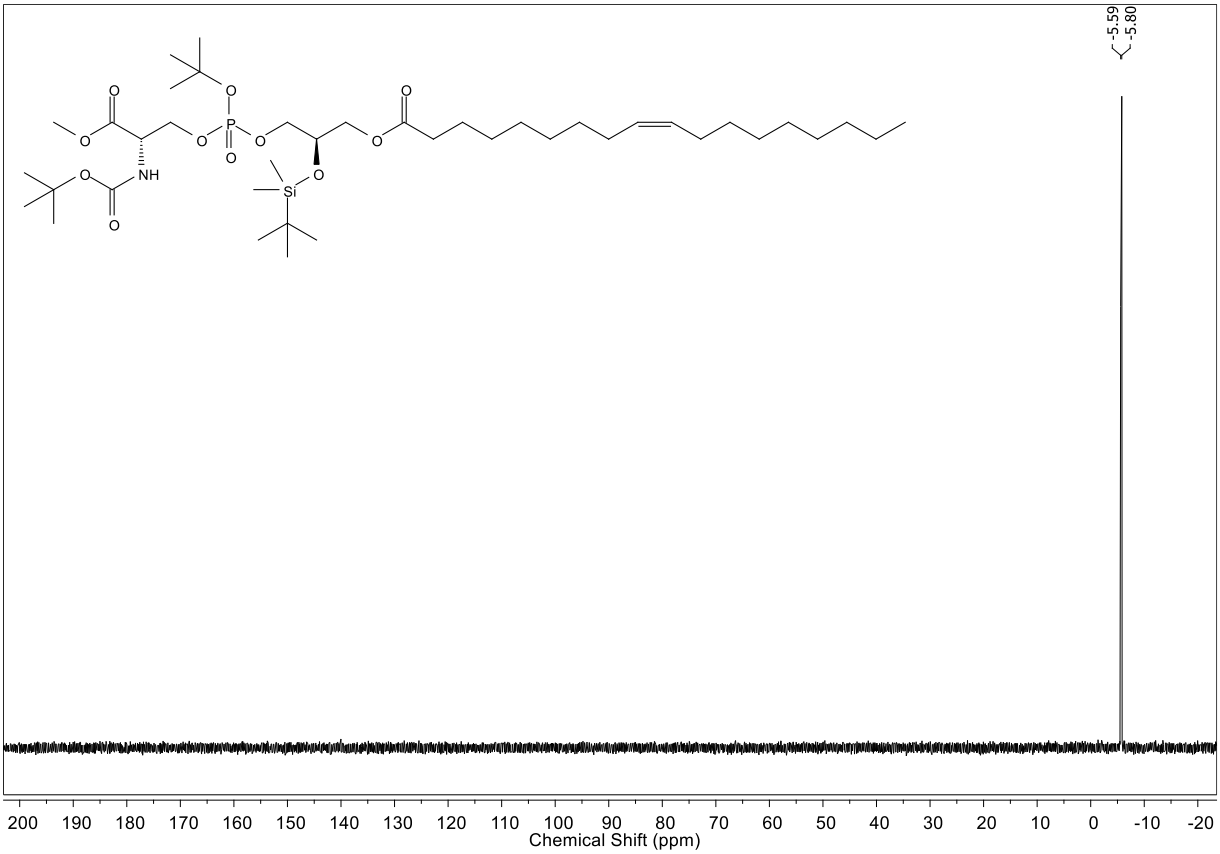

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19c



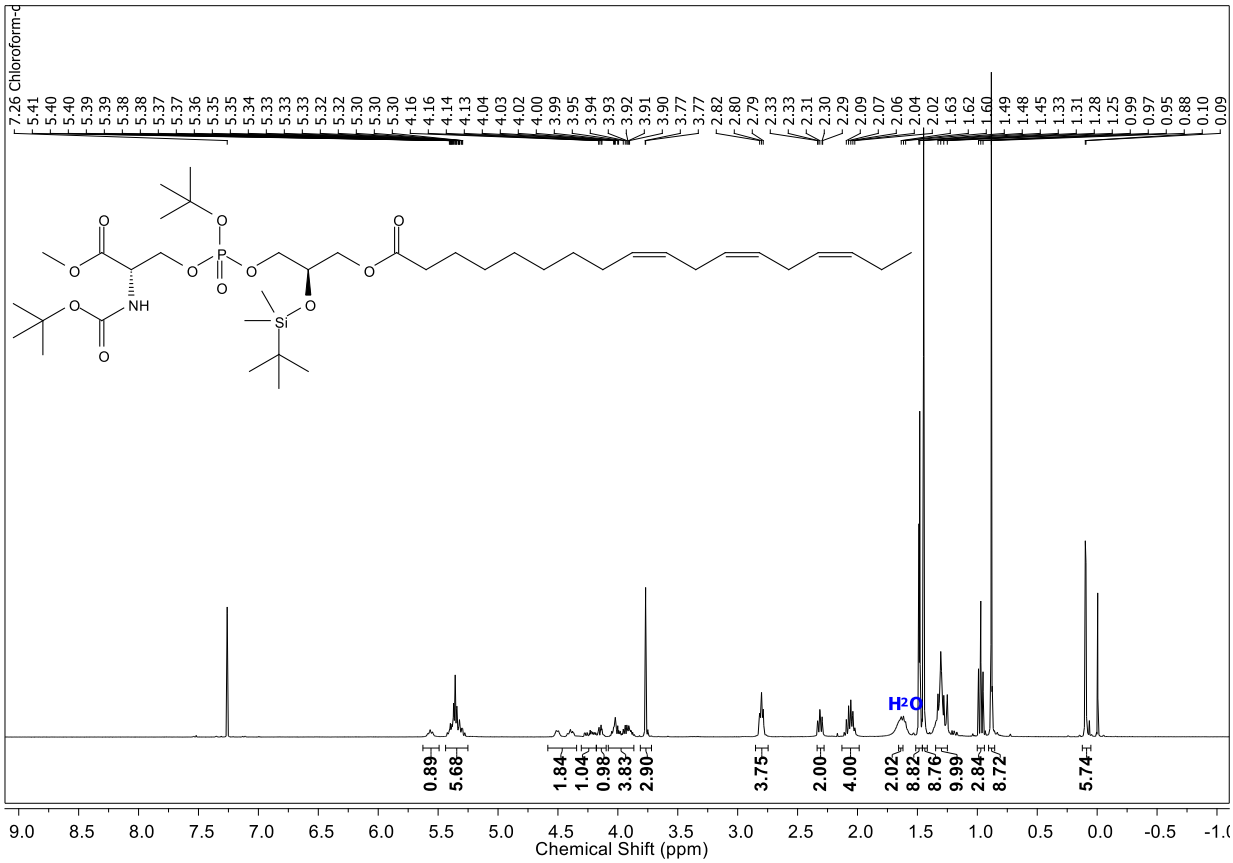
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19c



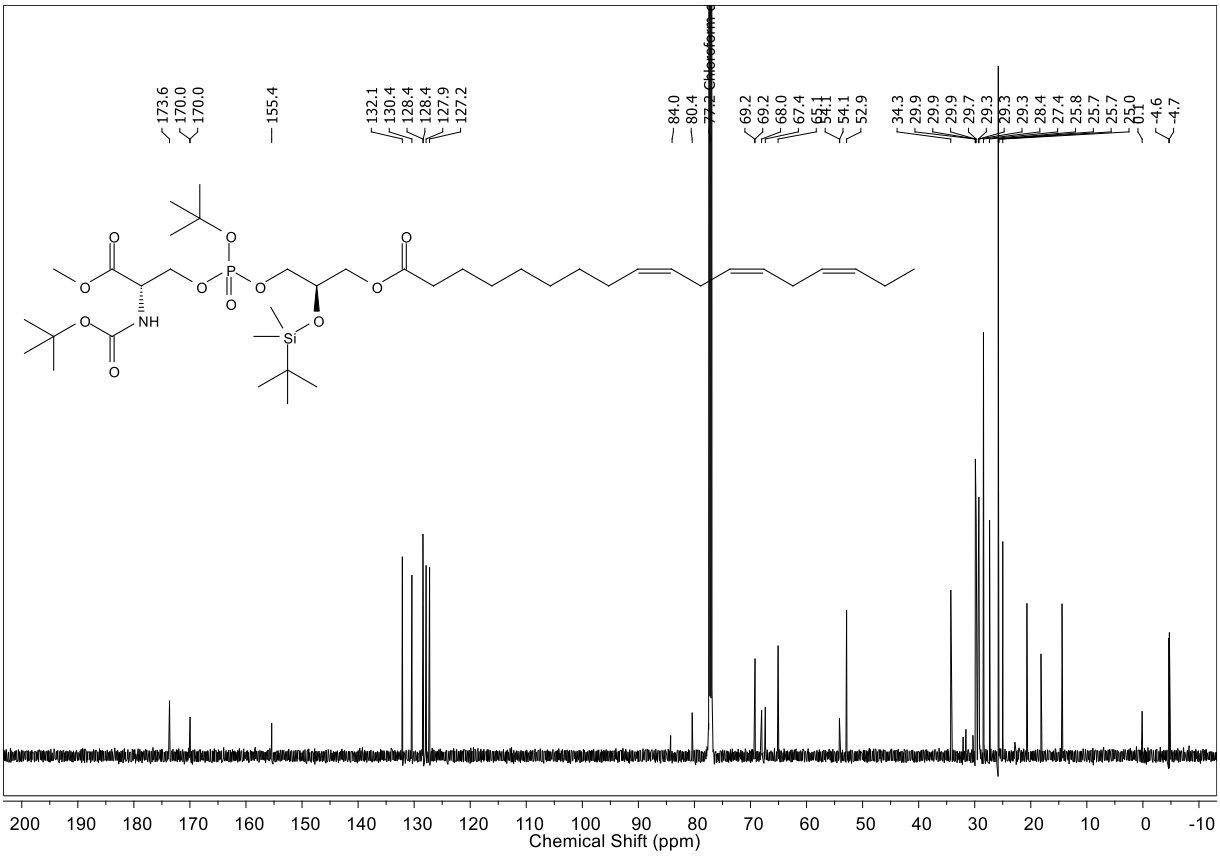

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19c



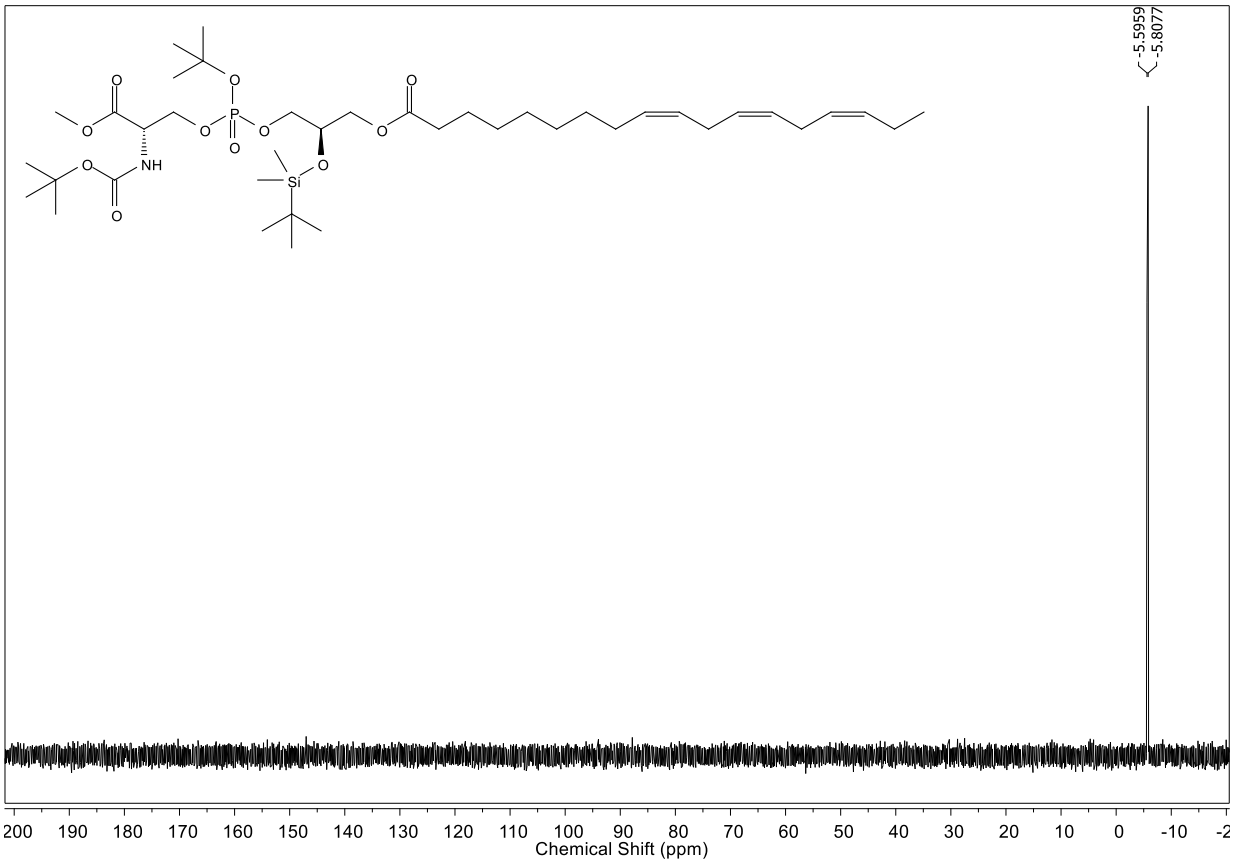
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19d



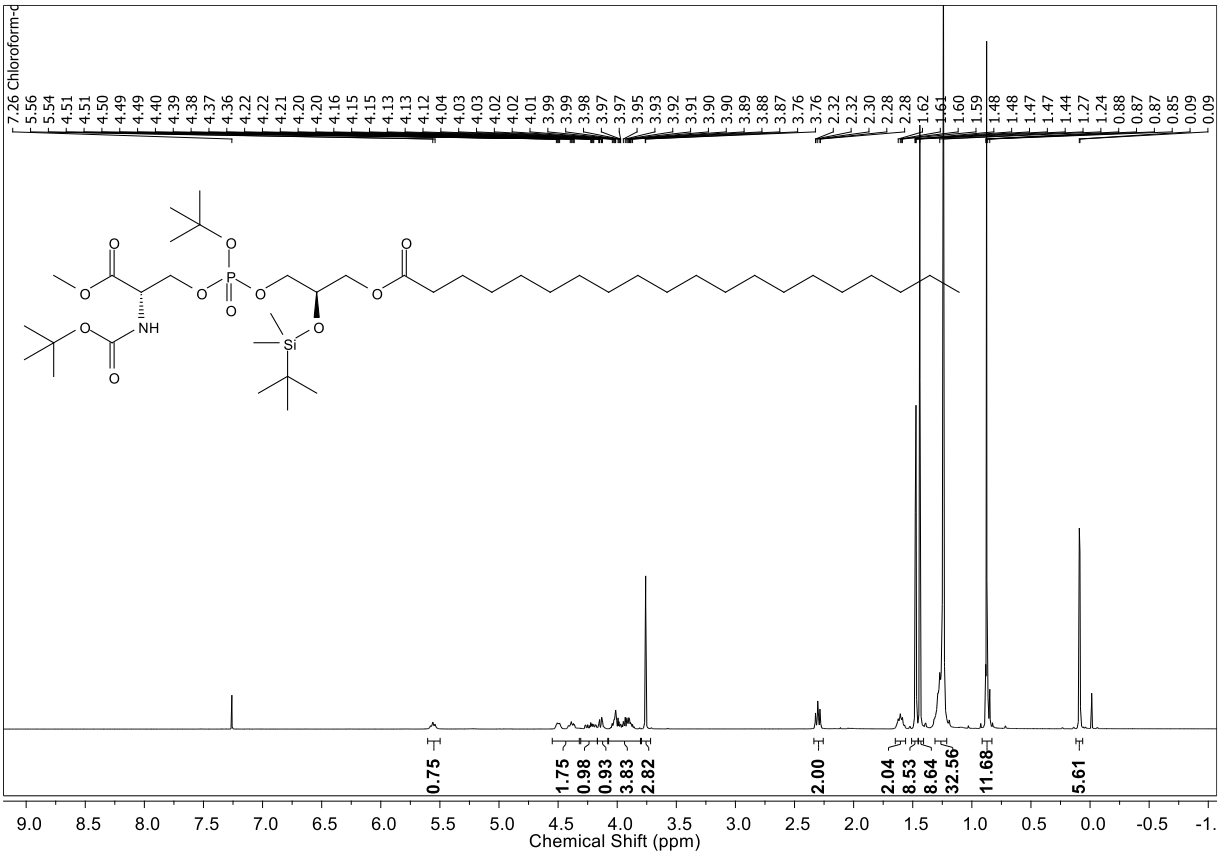

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19d



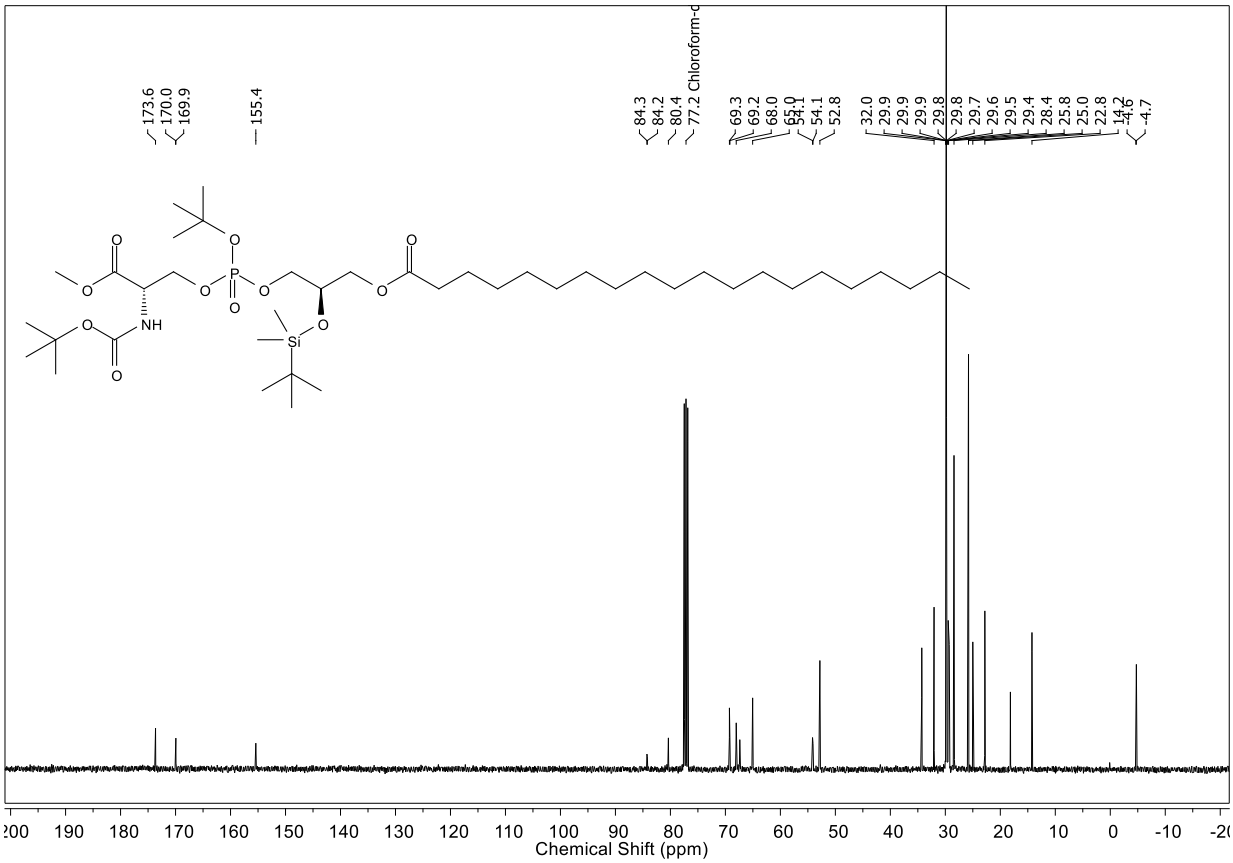
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19d



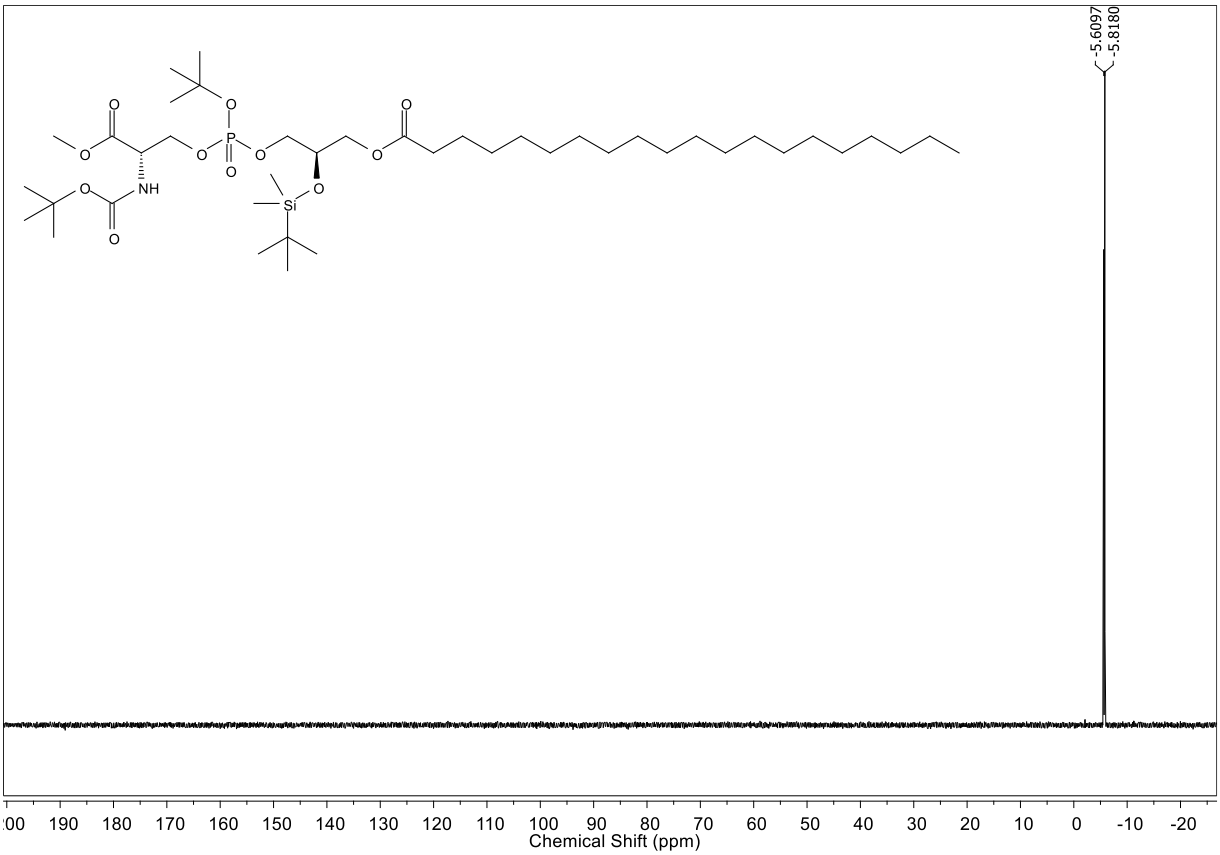

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19e



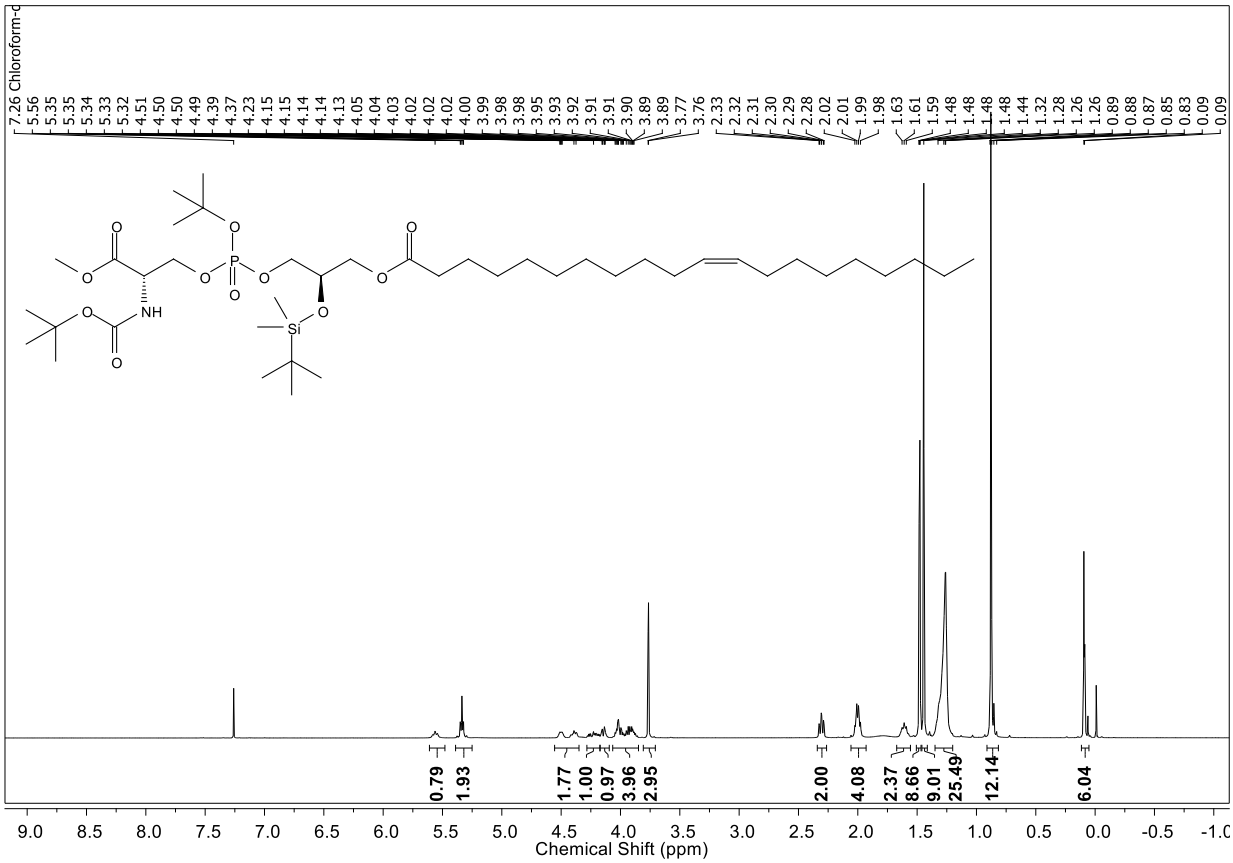
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19e



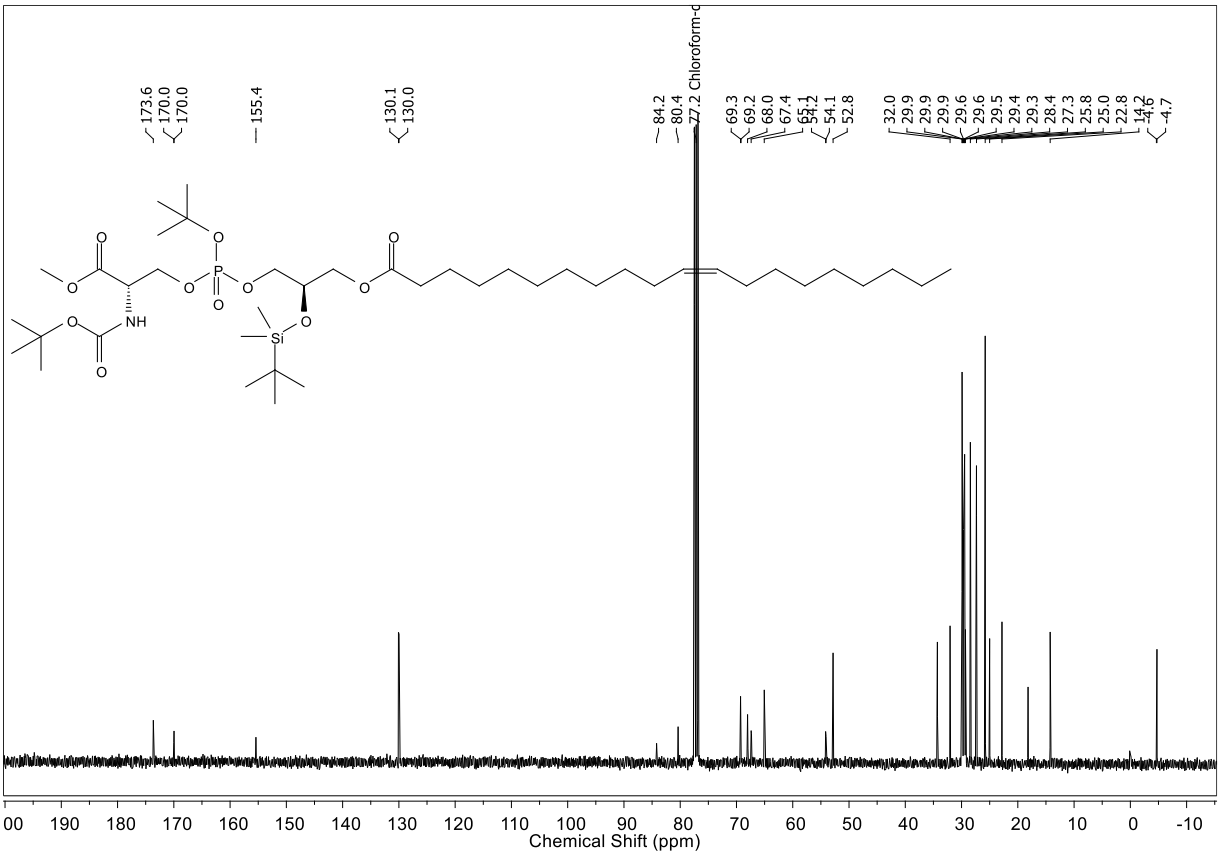

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19e



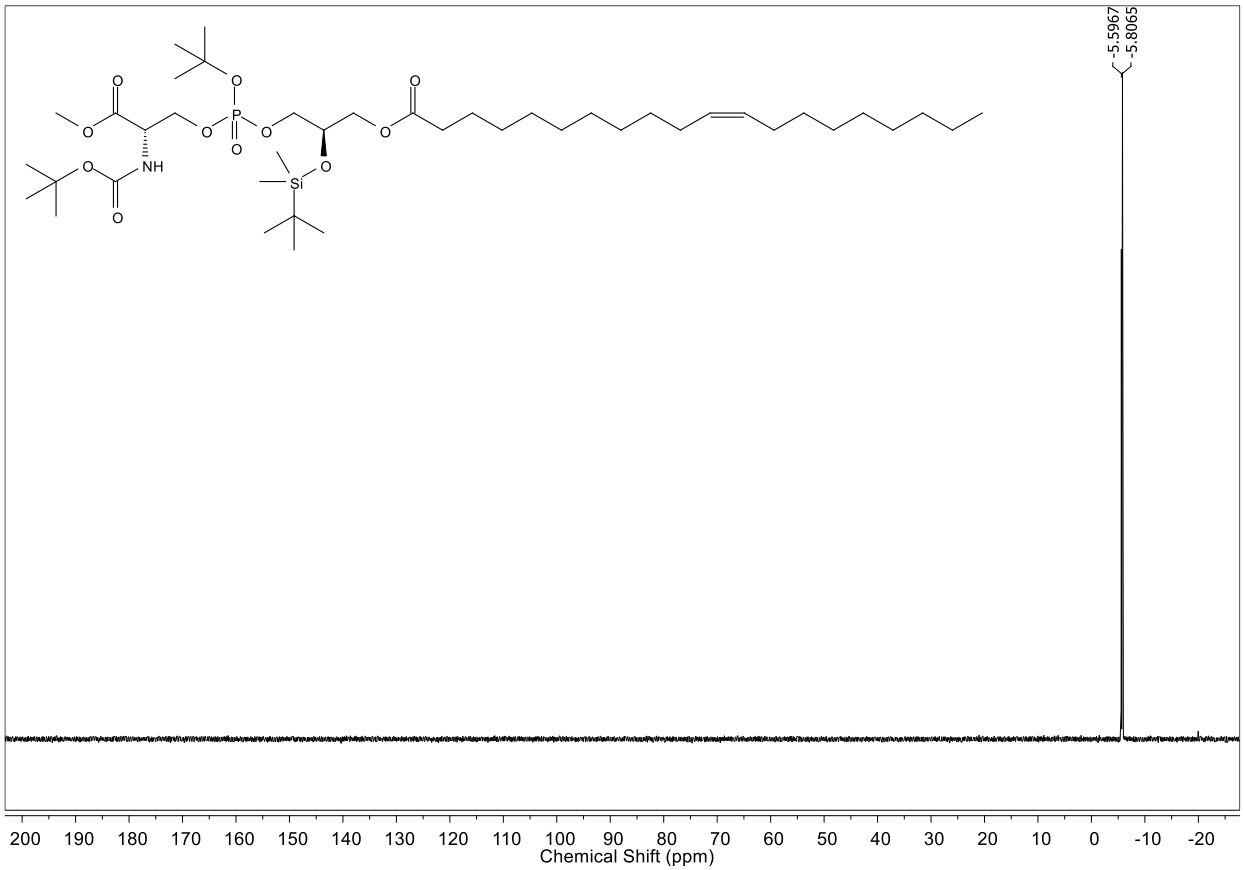
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19f



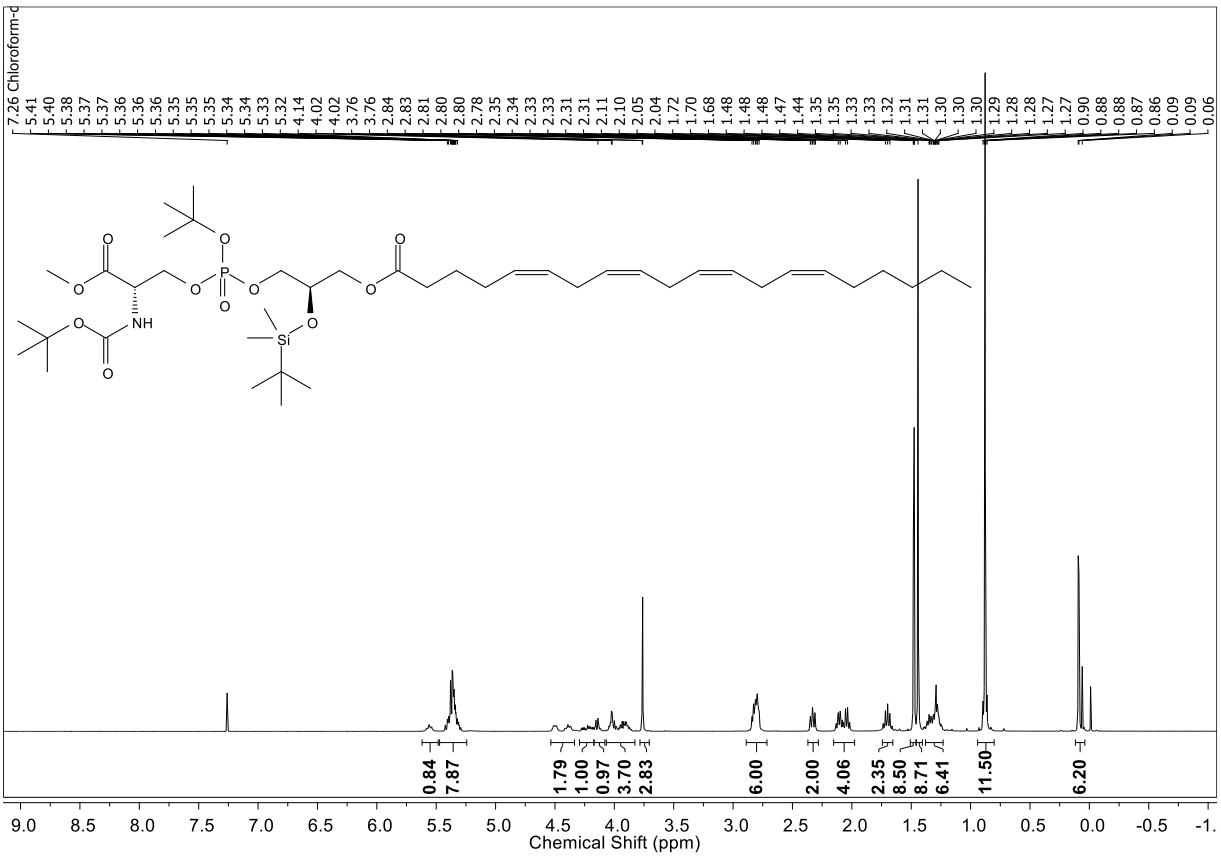

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19f



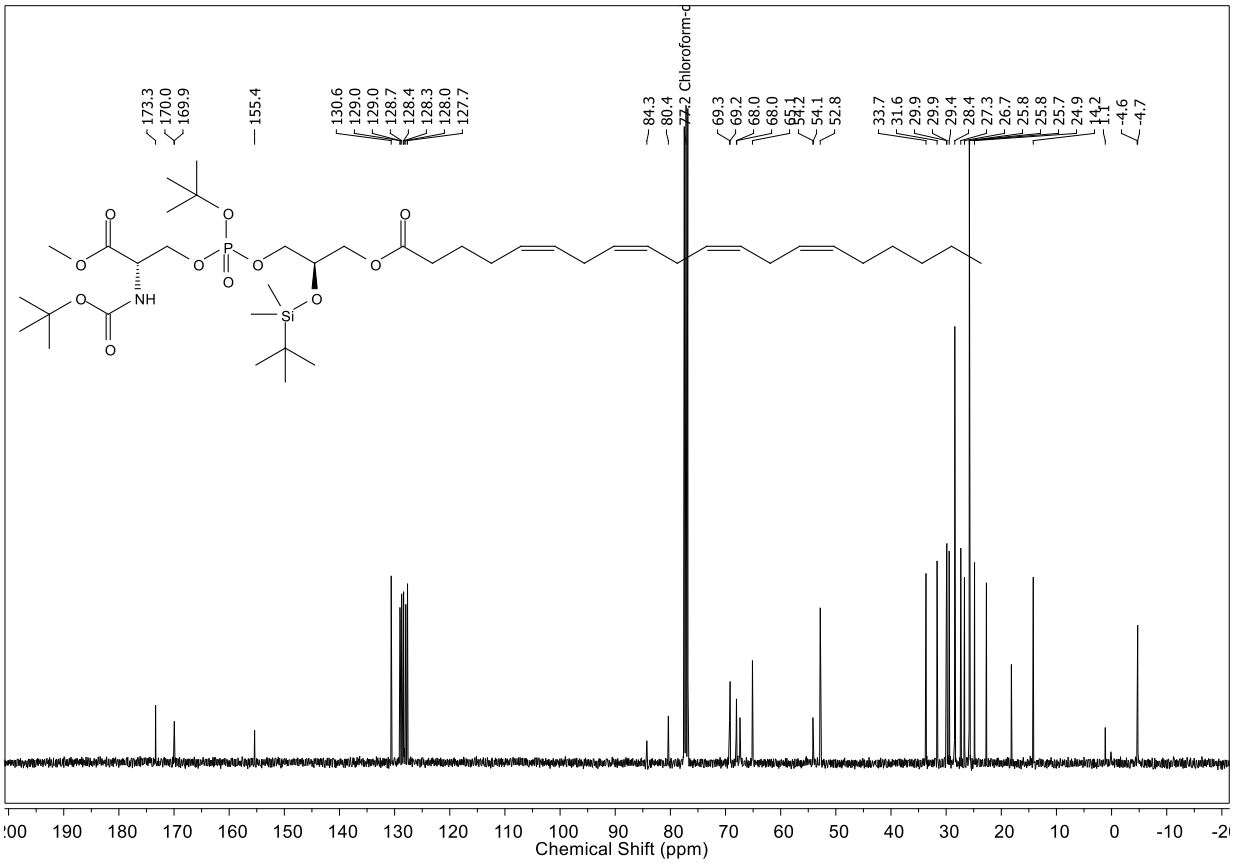
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19f



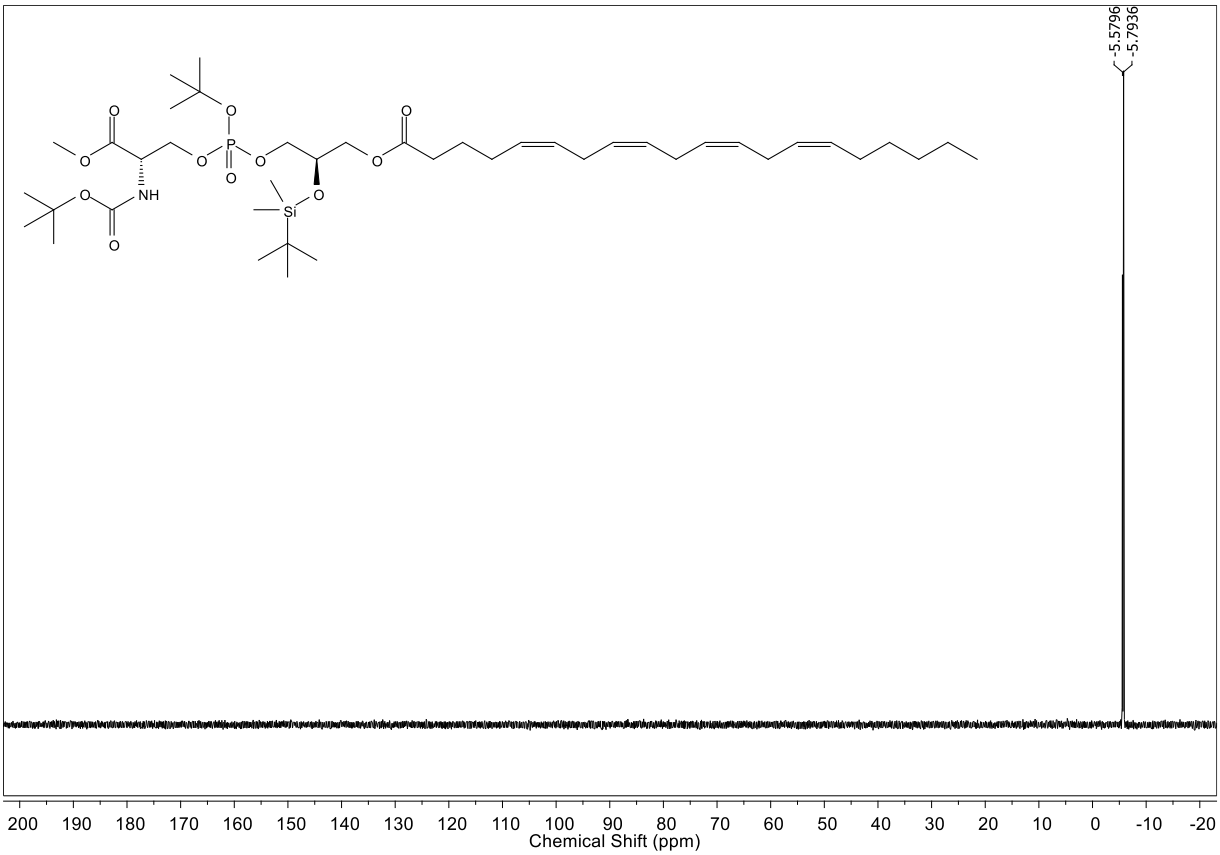

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19g



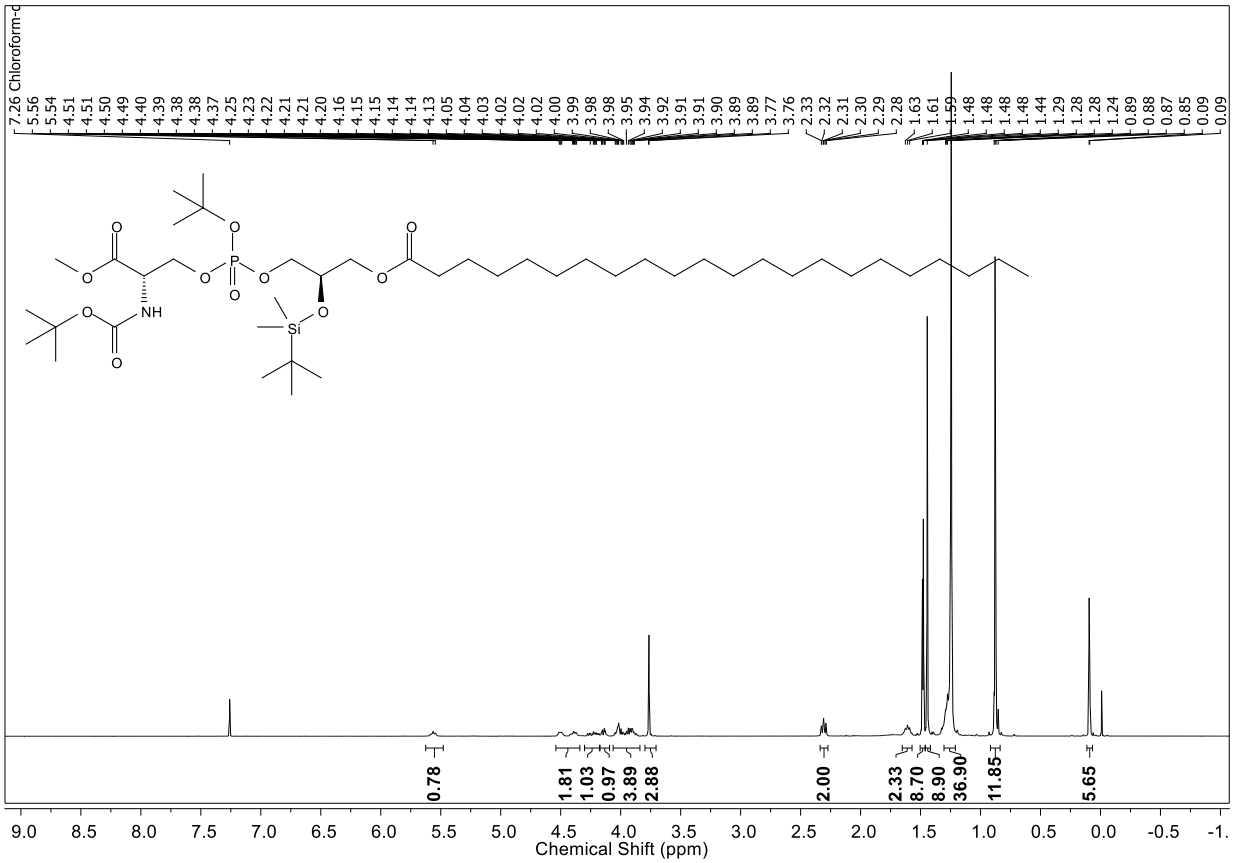
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19g



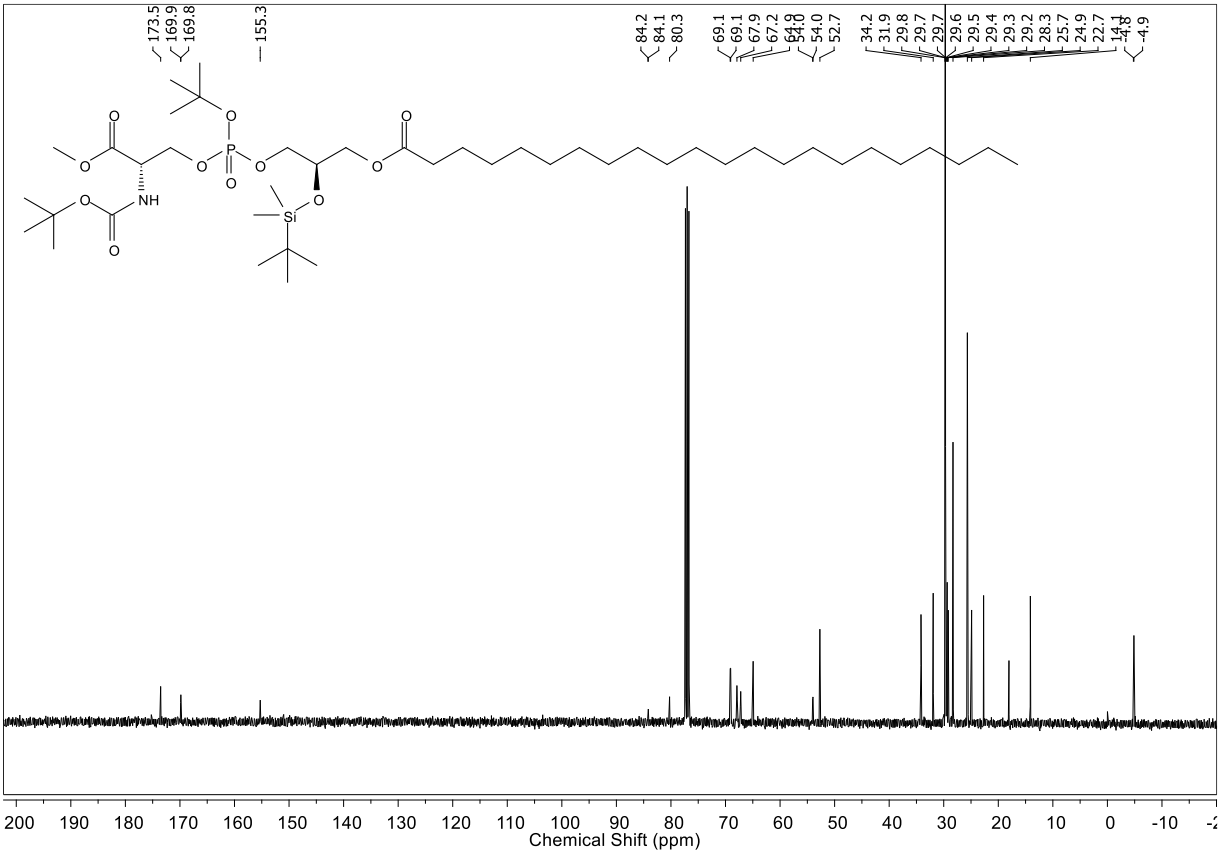

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19g



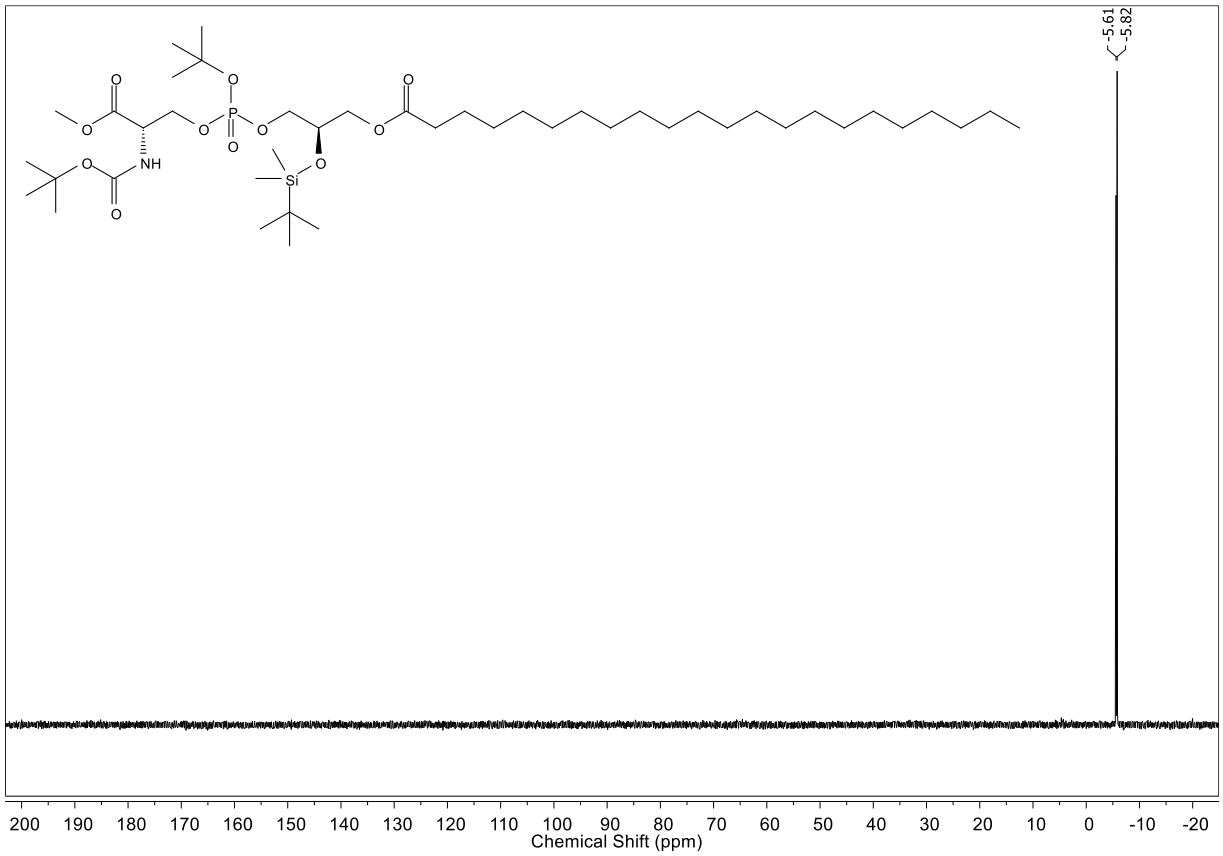
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19h



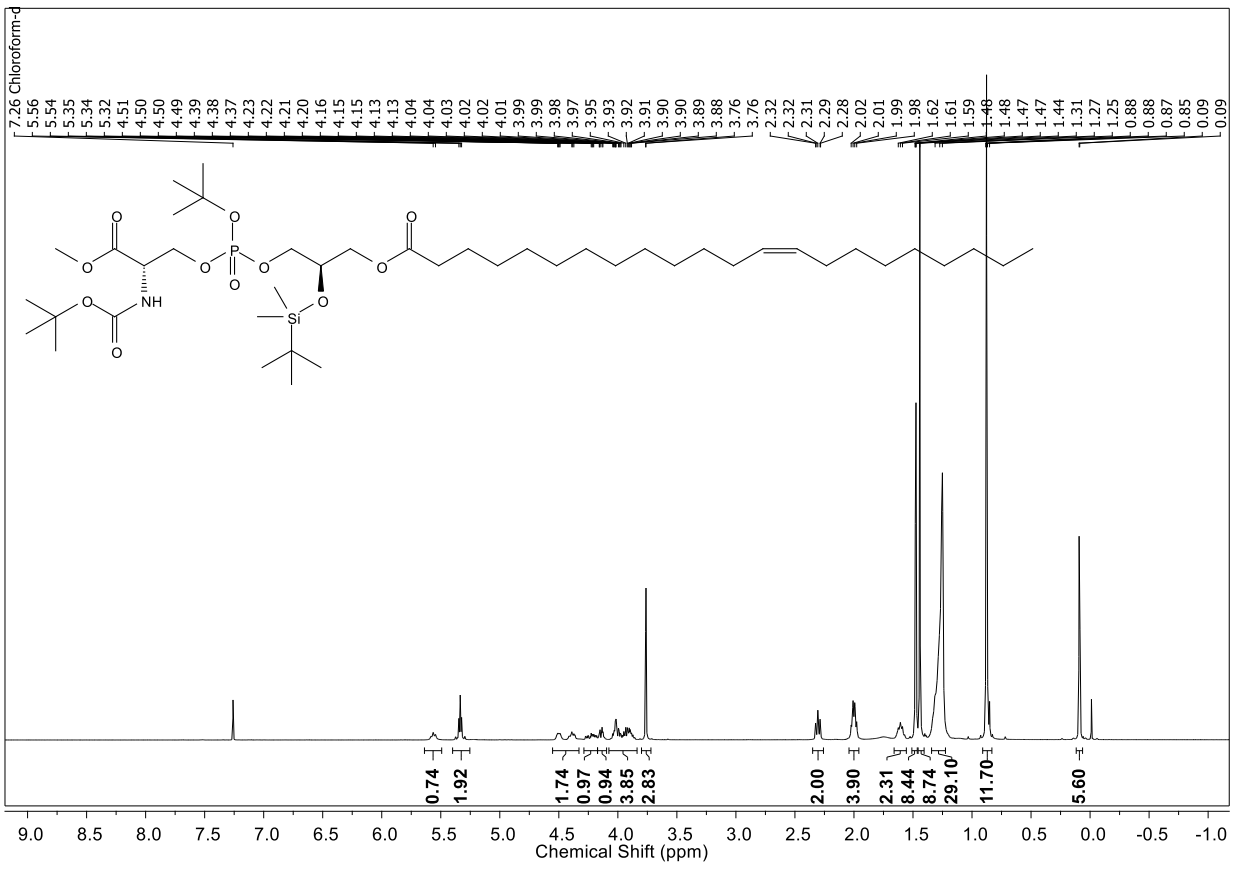

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19h



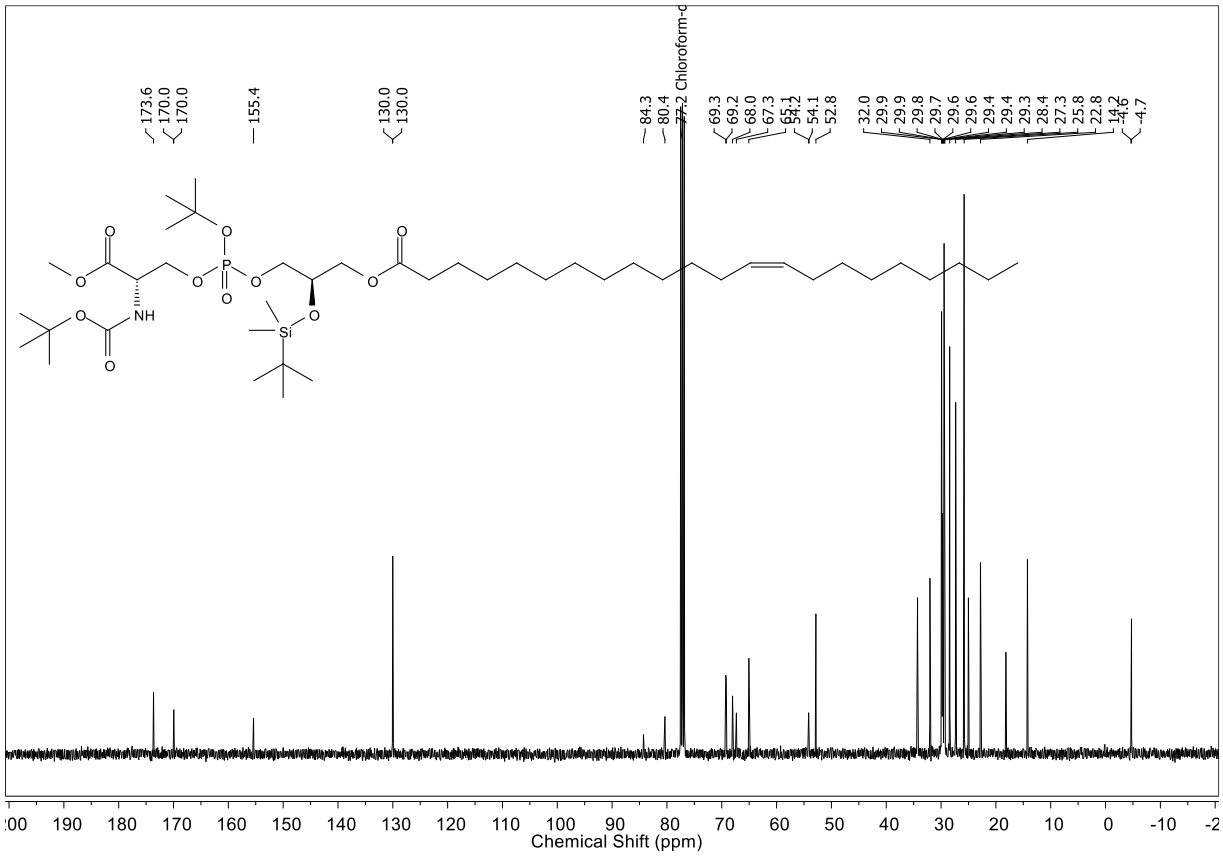
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19h



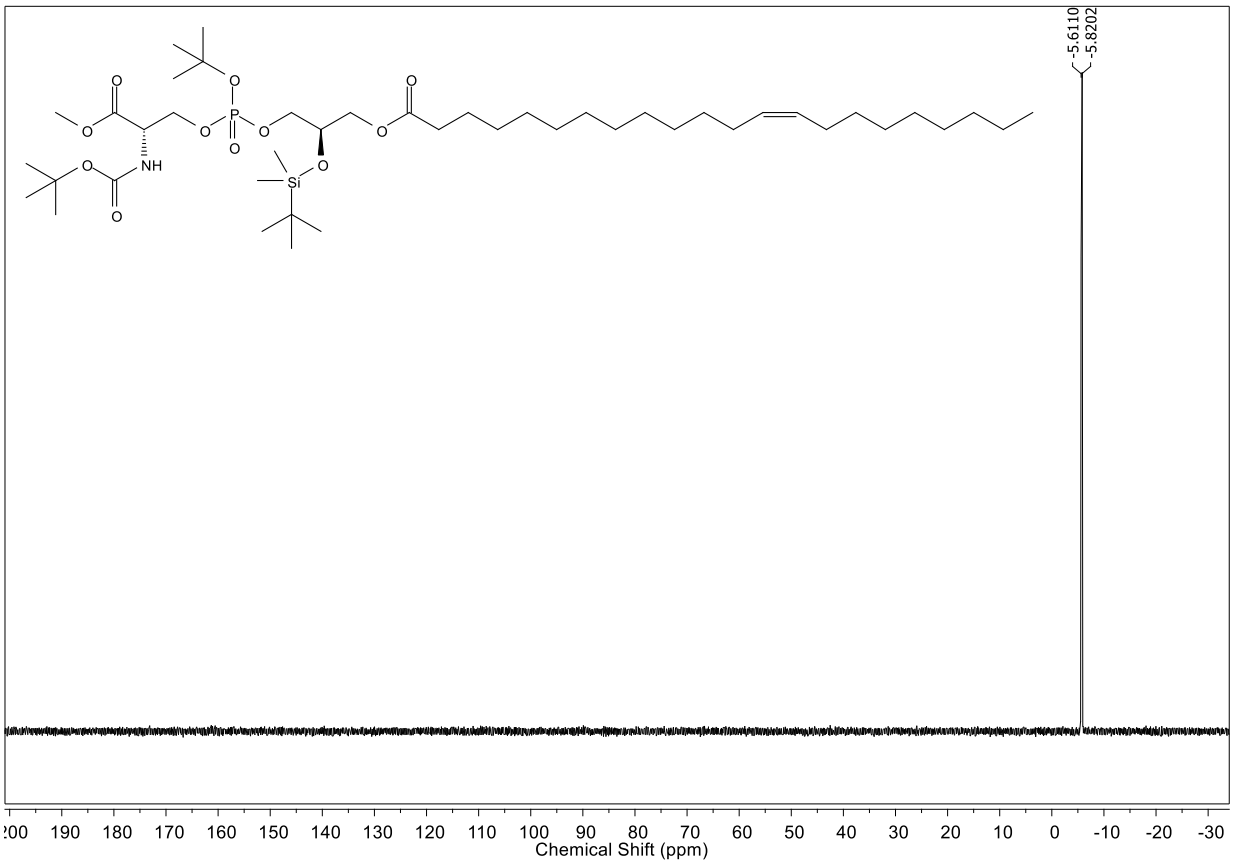

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19i



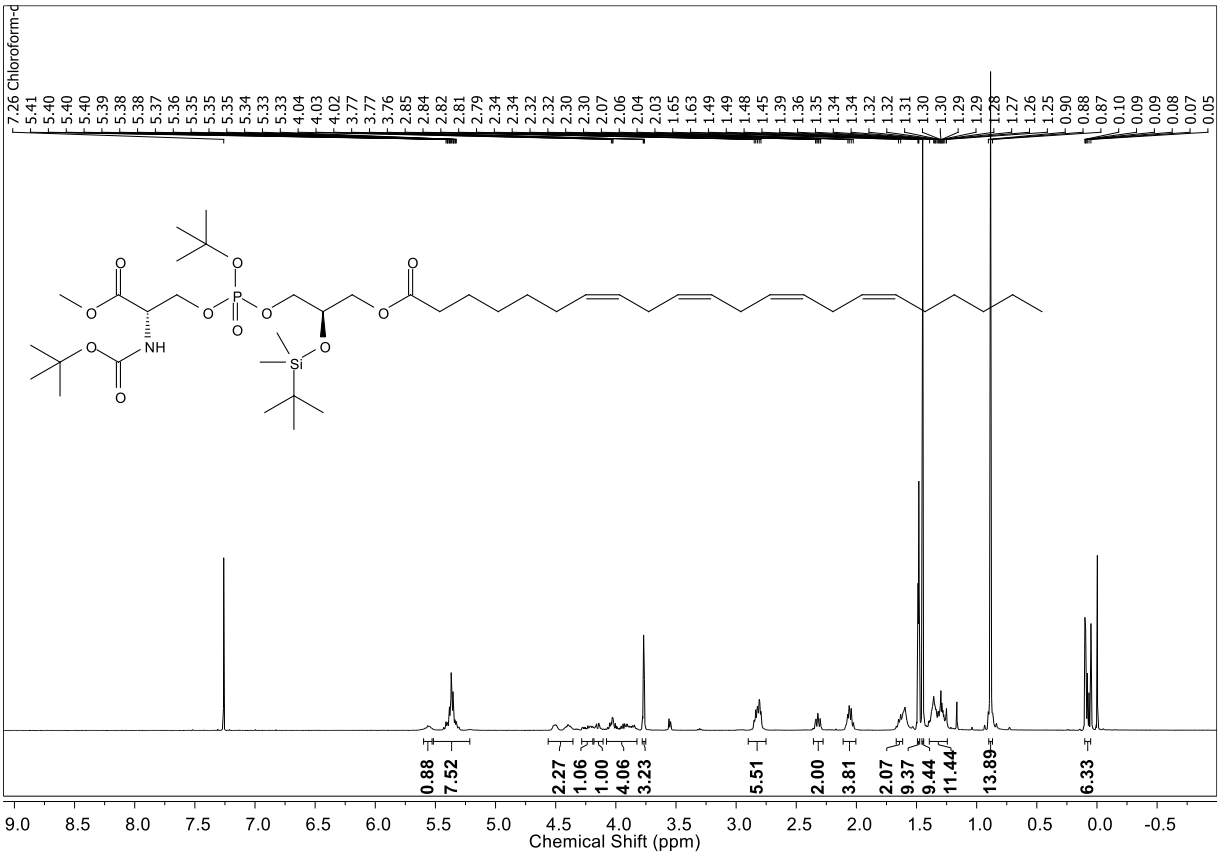
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19i



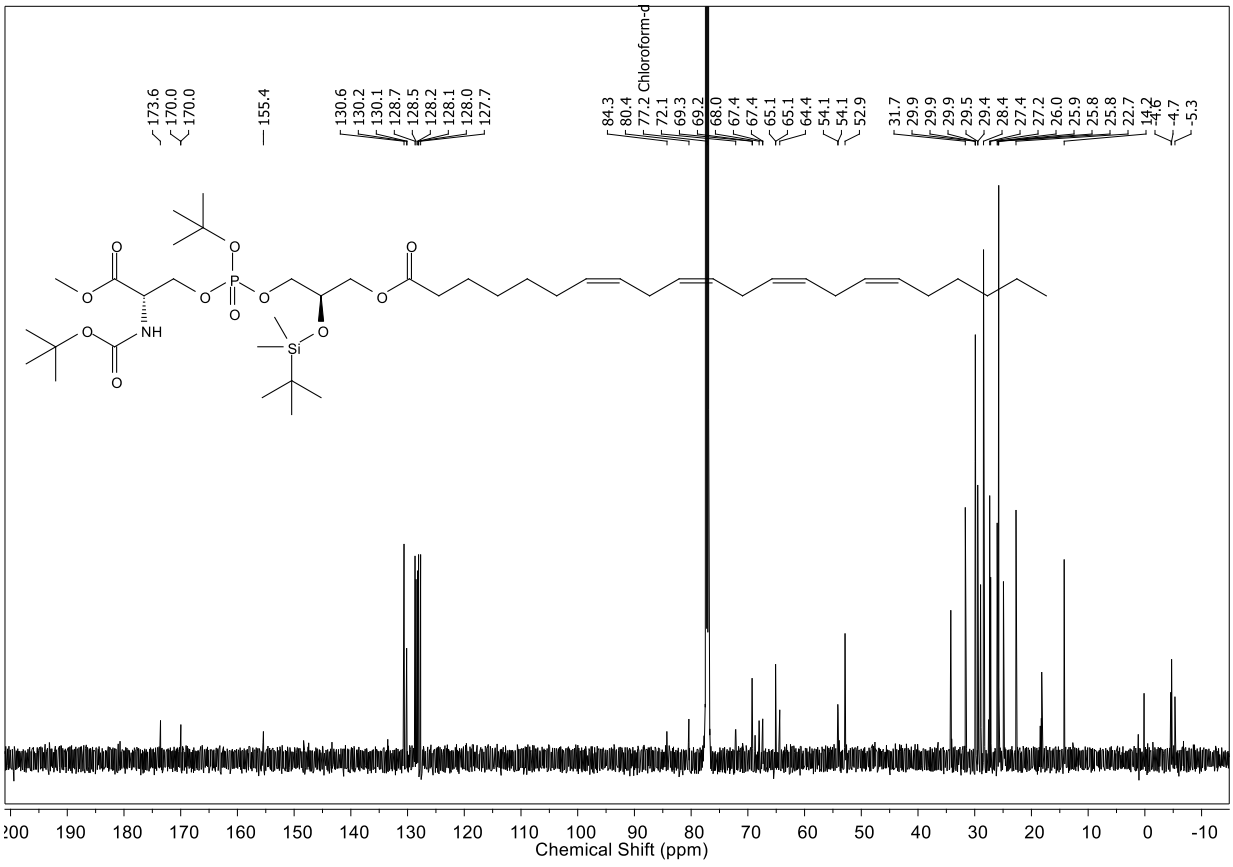

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19i



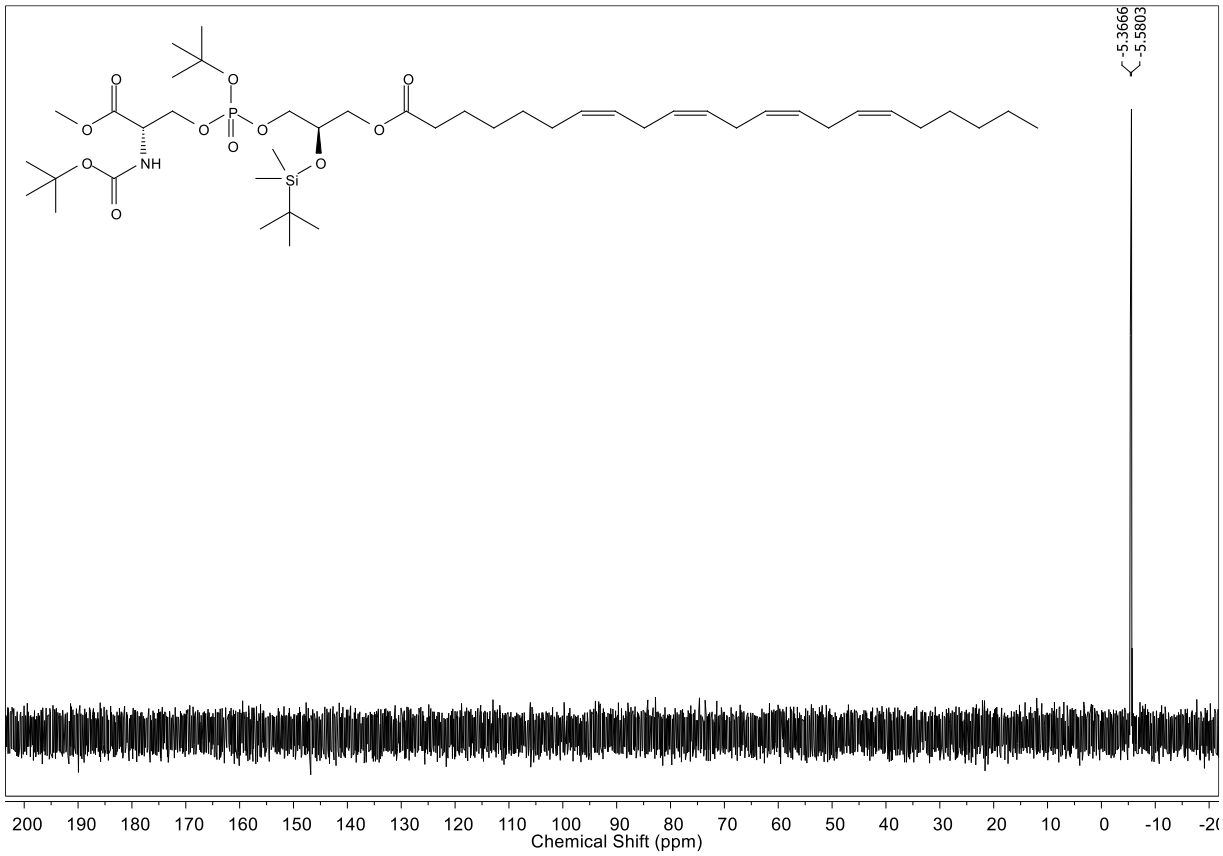
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19j



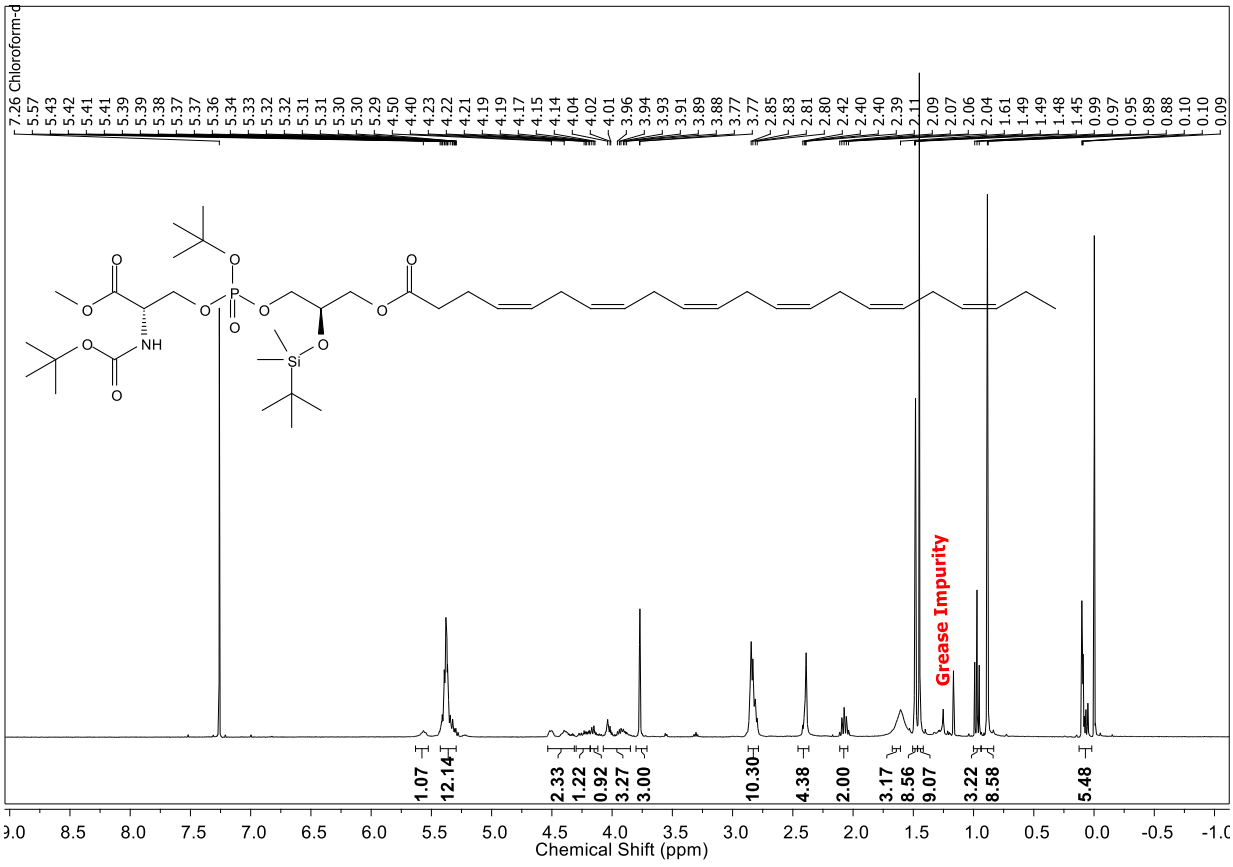

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19j



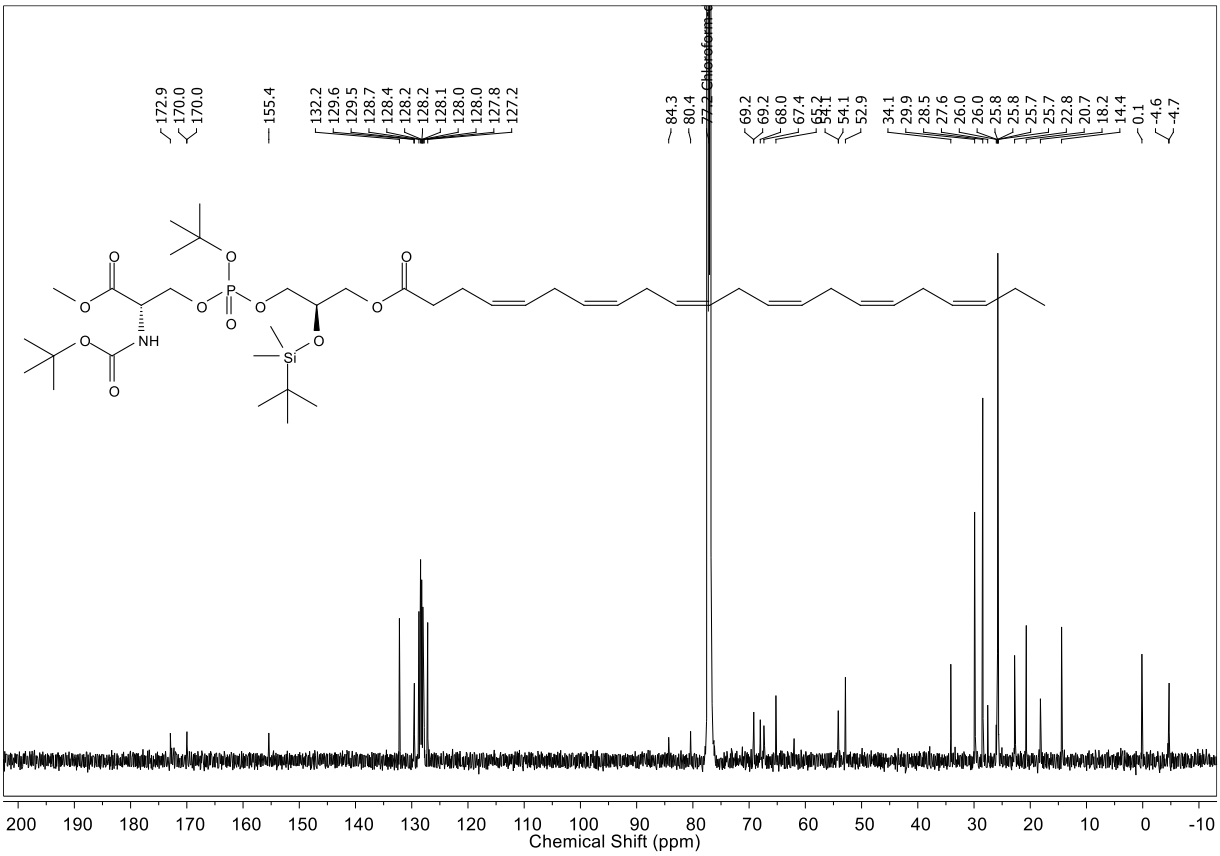
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19j



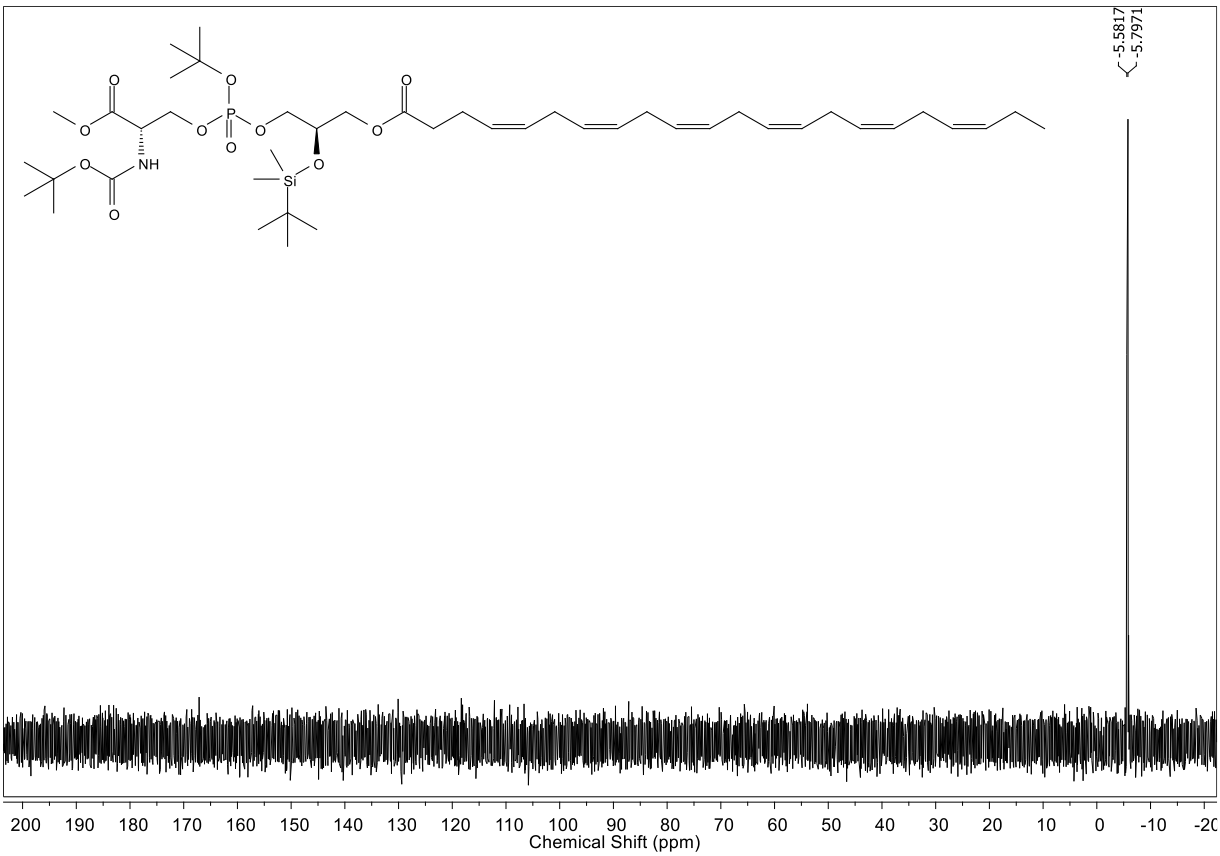

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19k



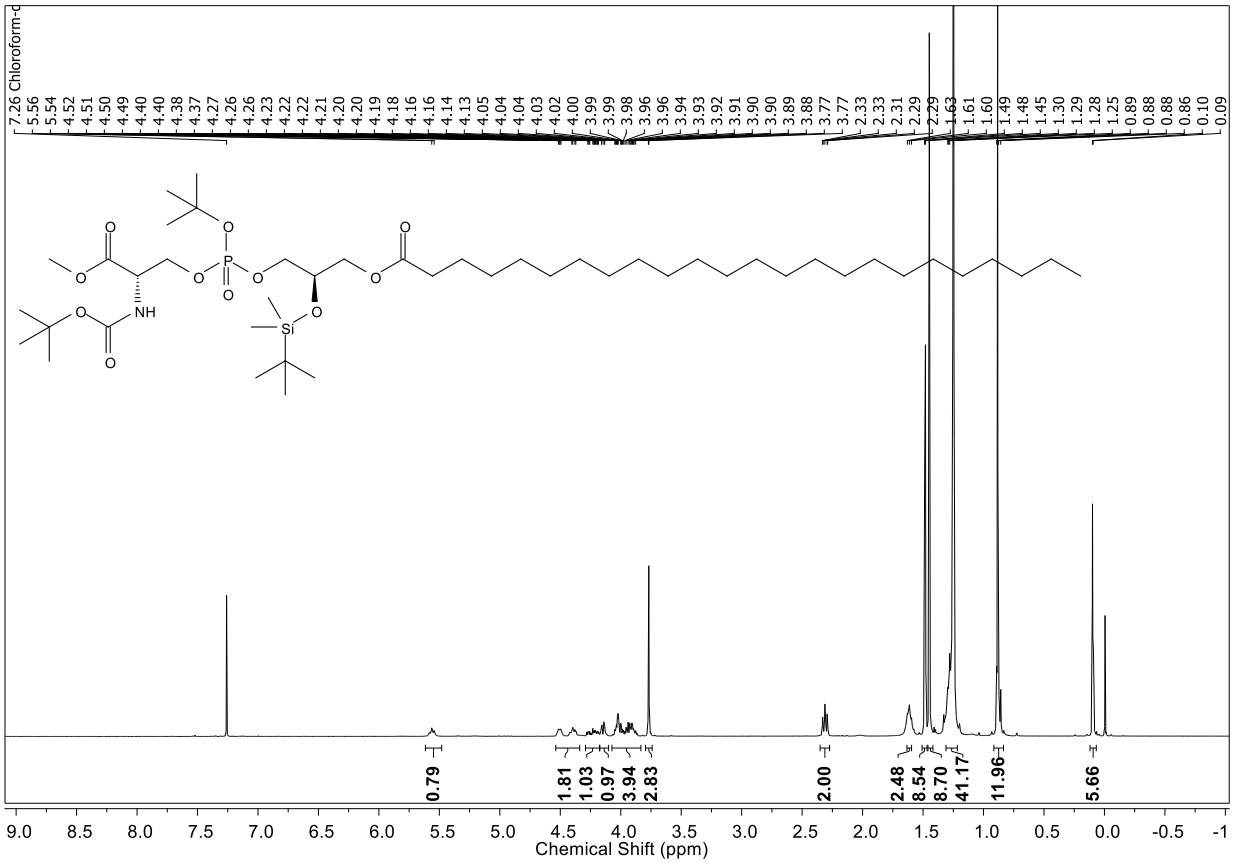
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19k



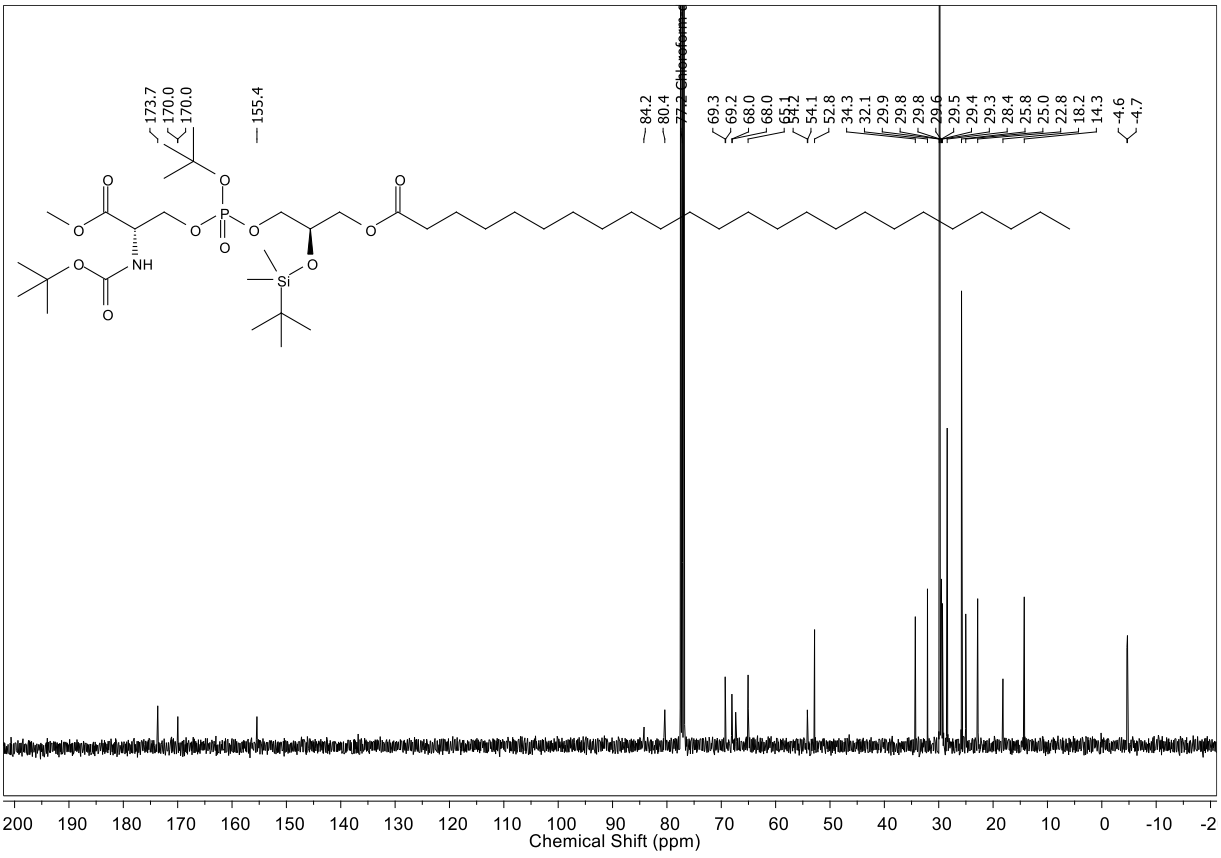

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19k



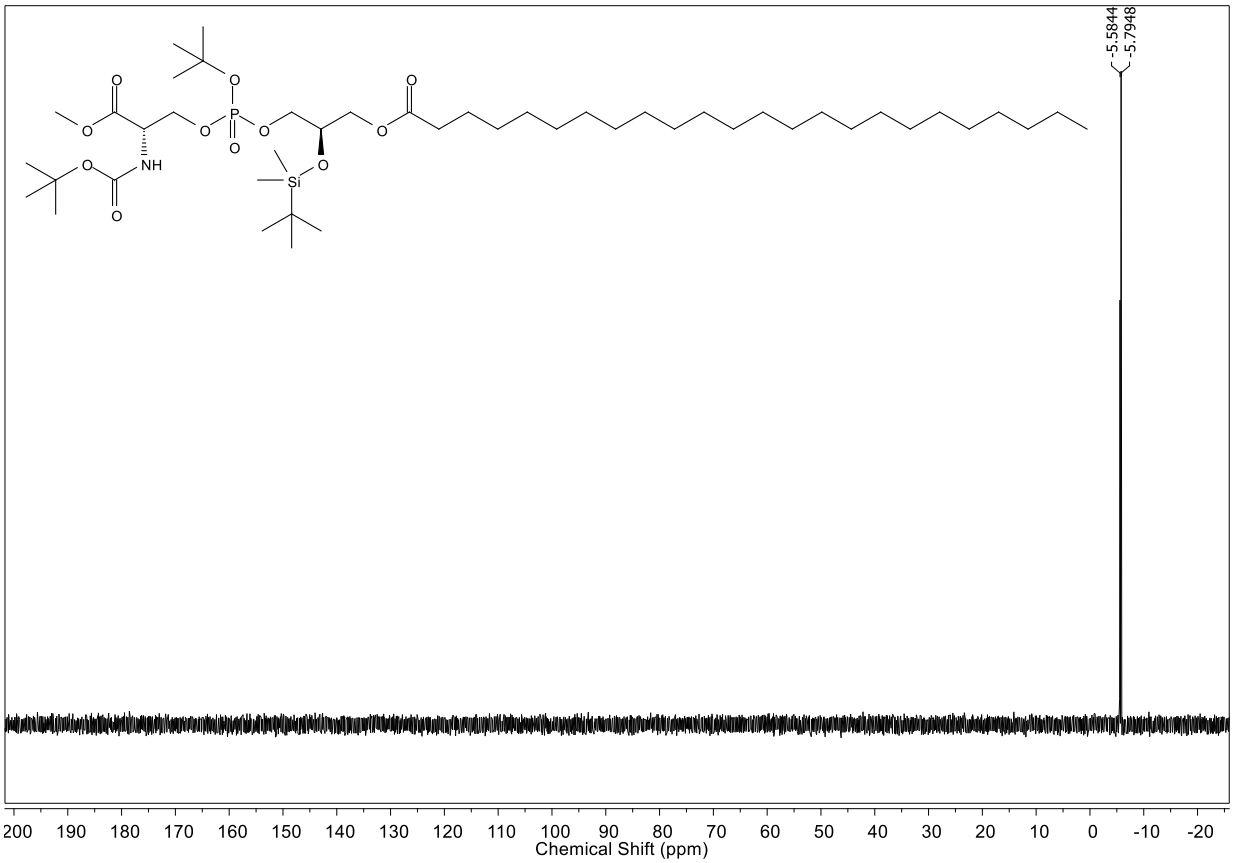
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19l



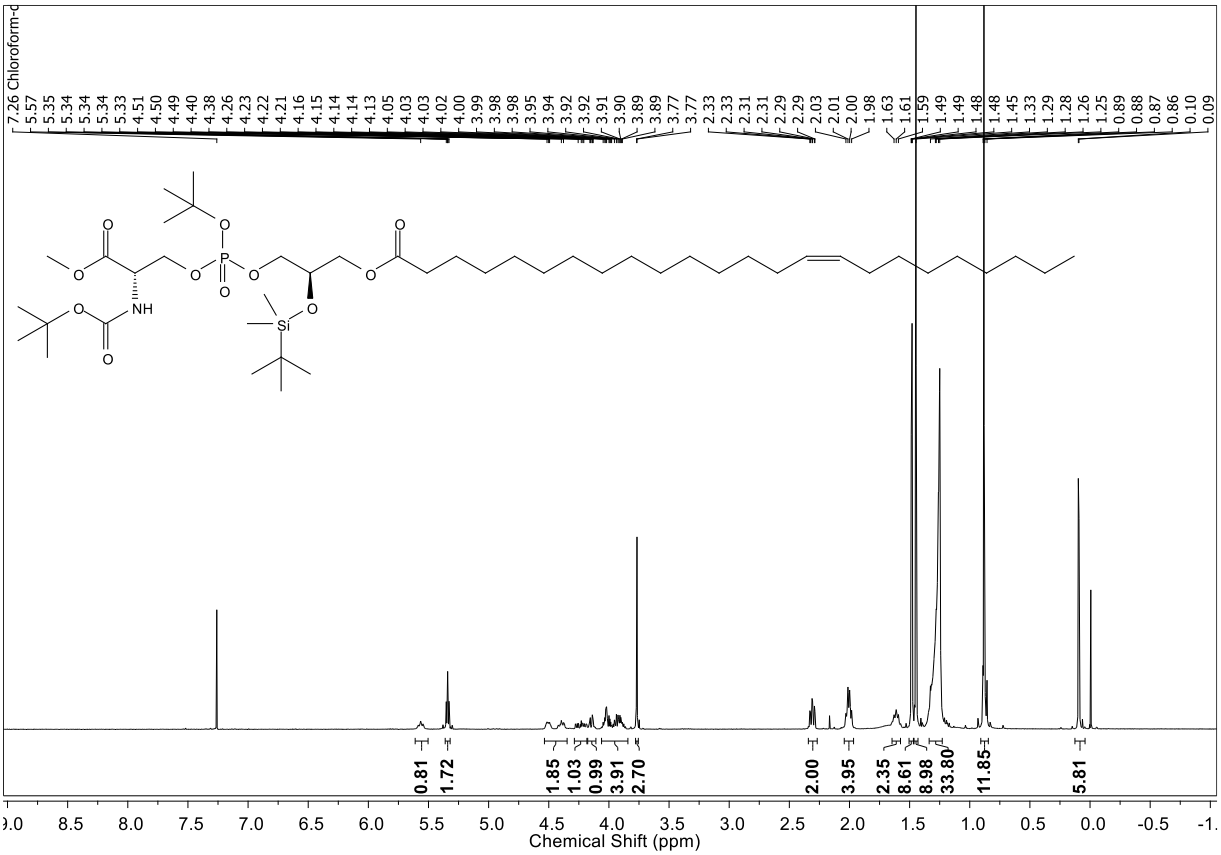

## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19l



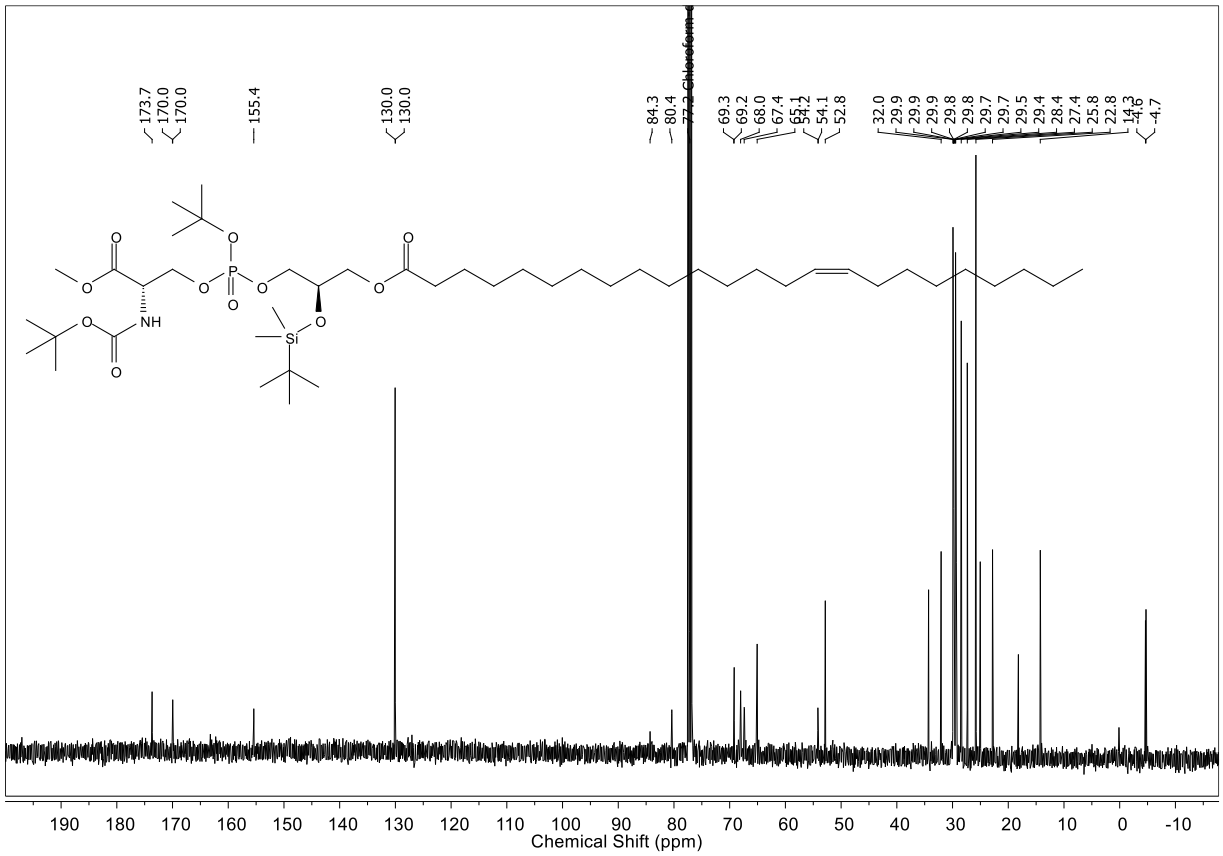
## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19l



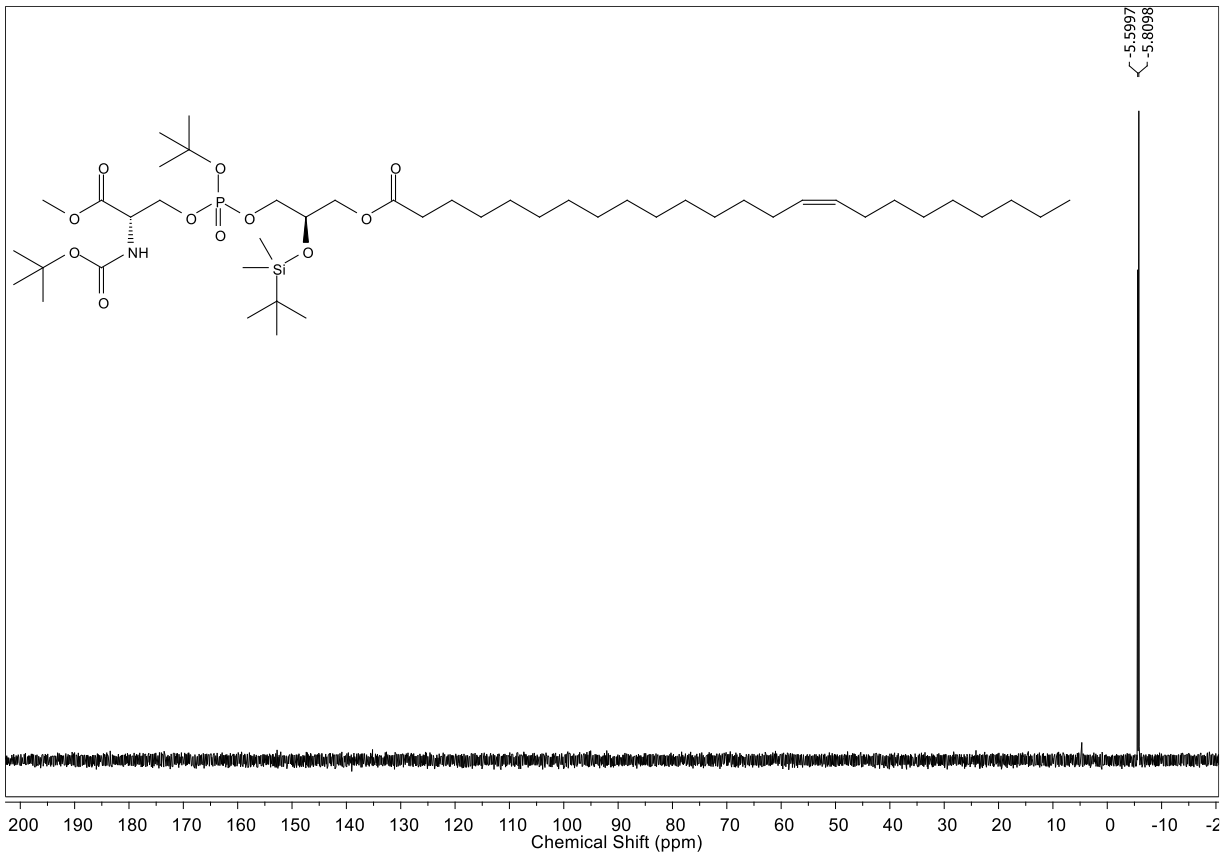

## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-19m



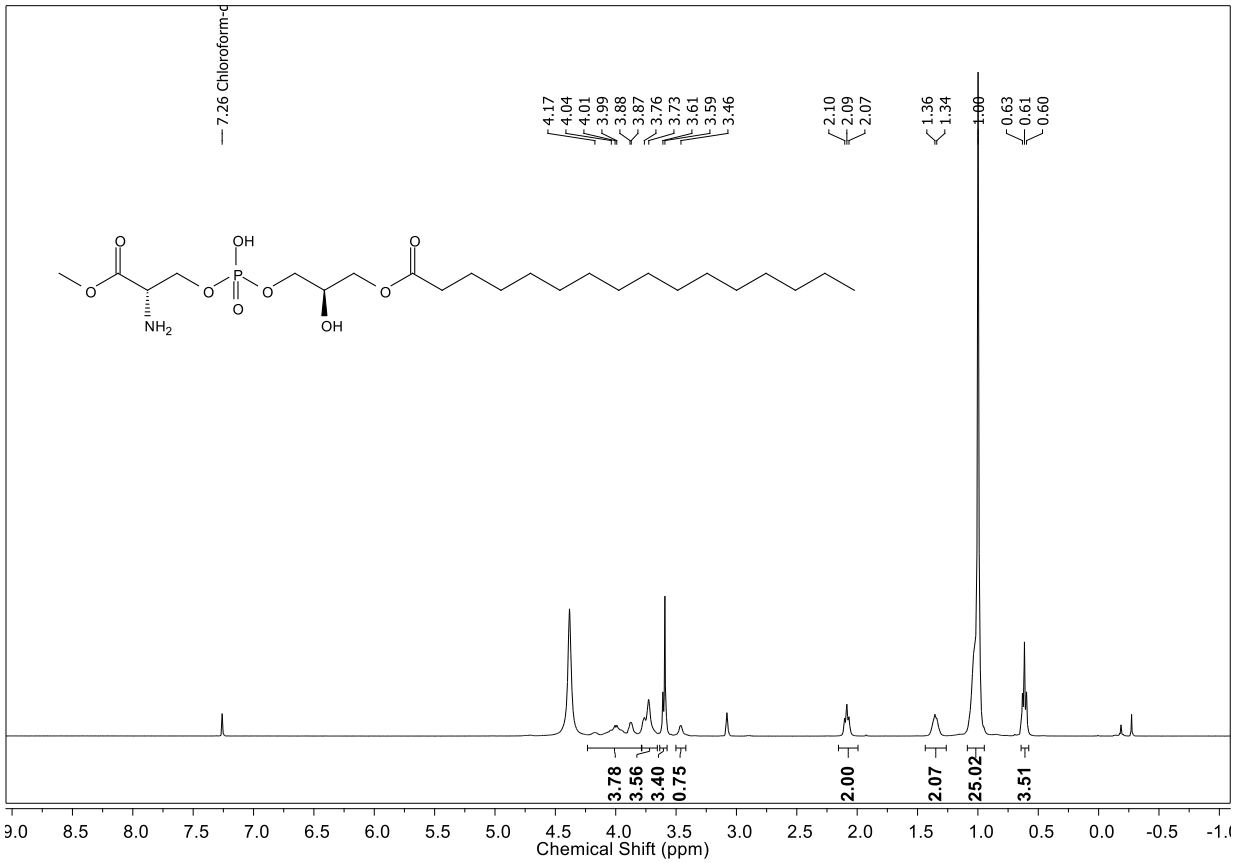
## $^{13}$C-NMR ($CDCl_3$, 100 MHz) for Compound (*R*)-19m



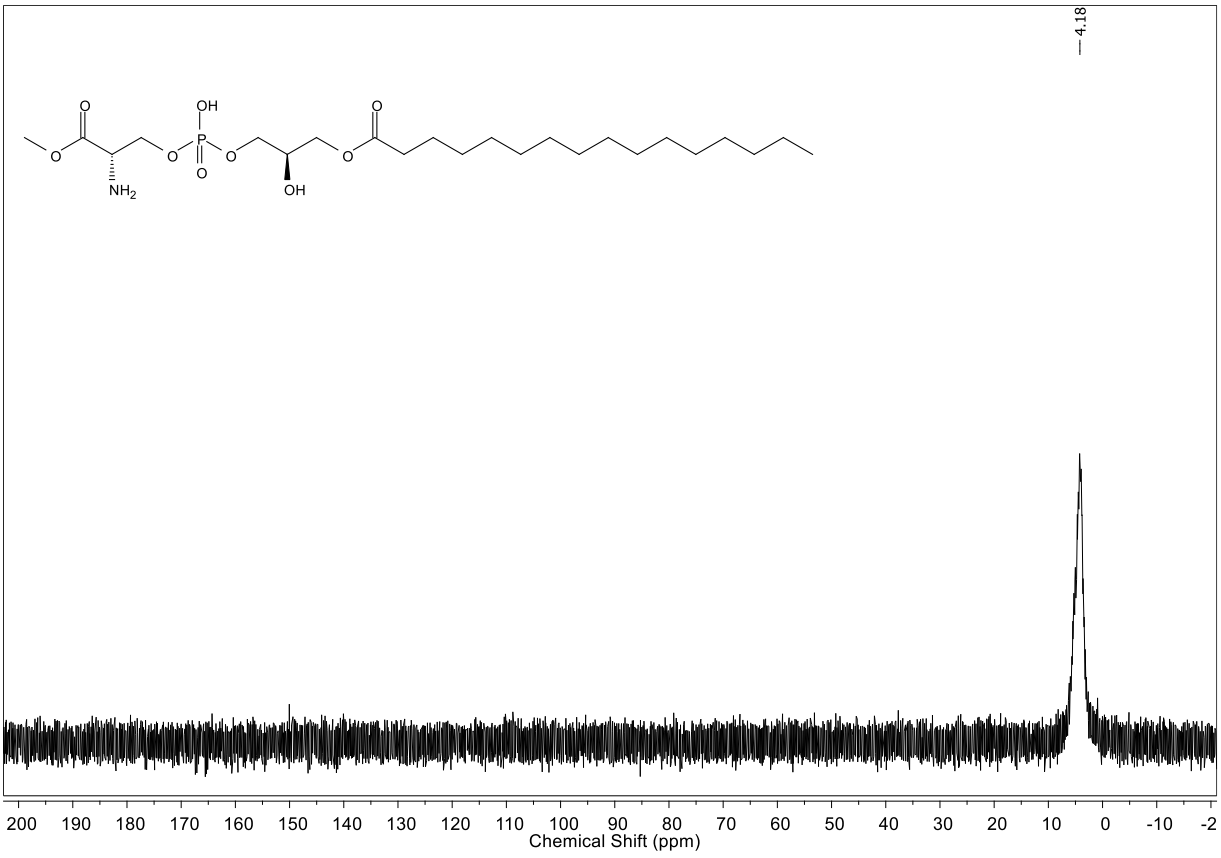

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20m



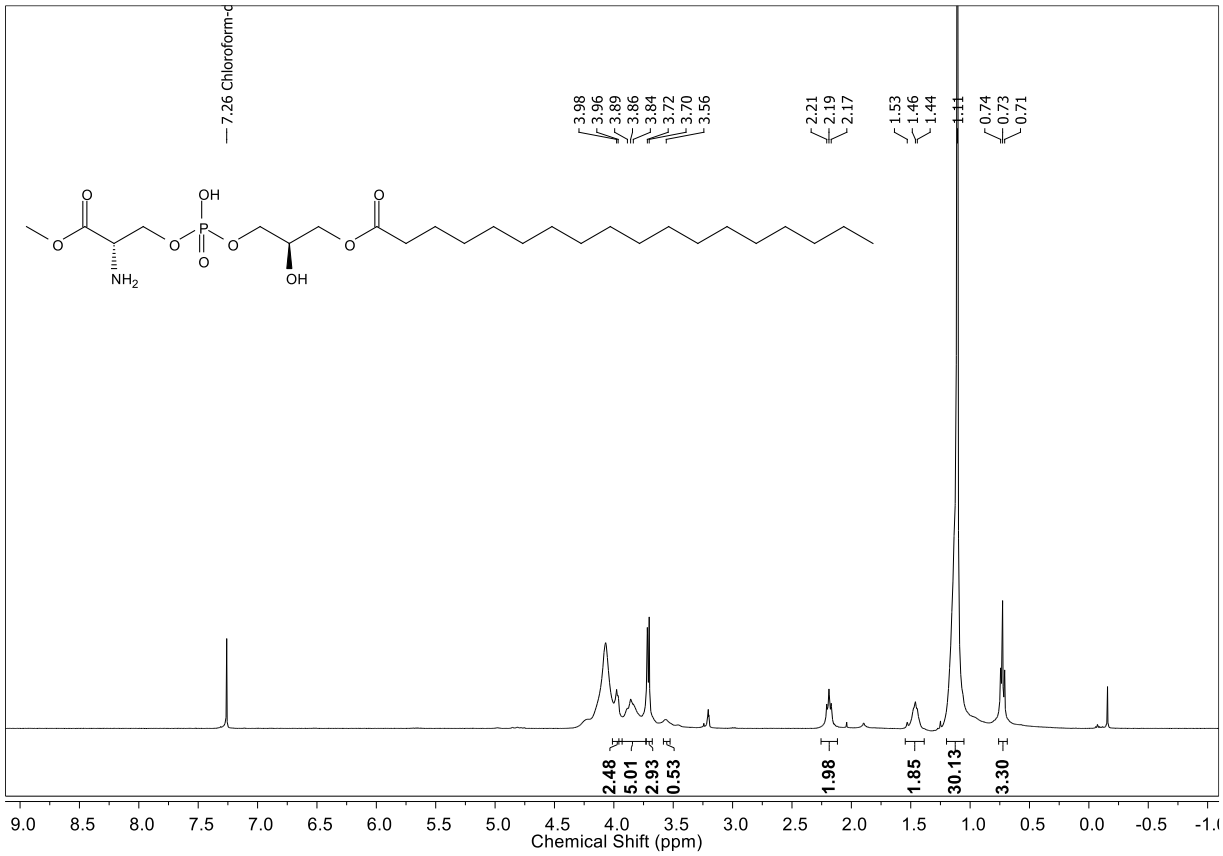
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20a



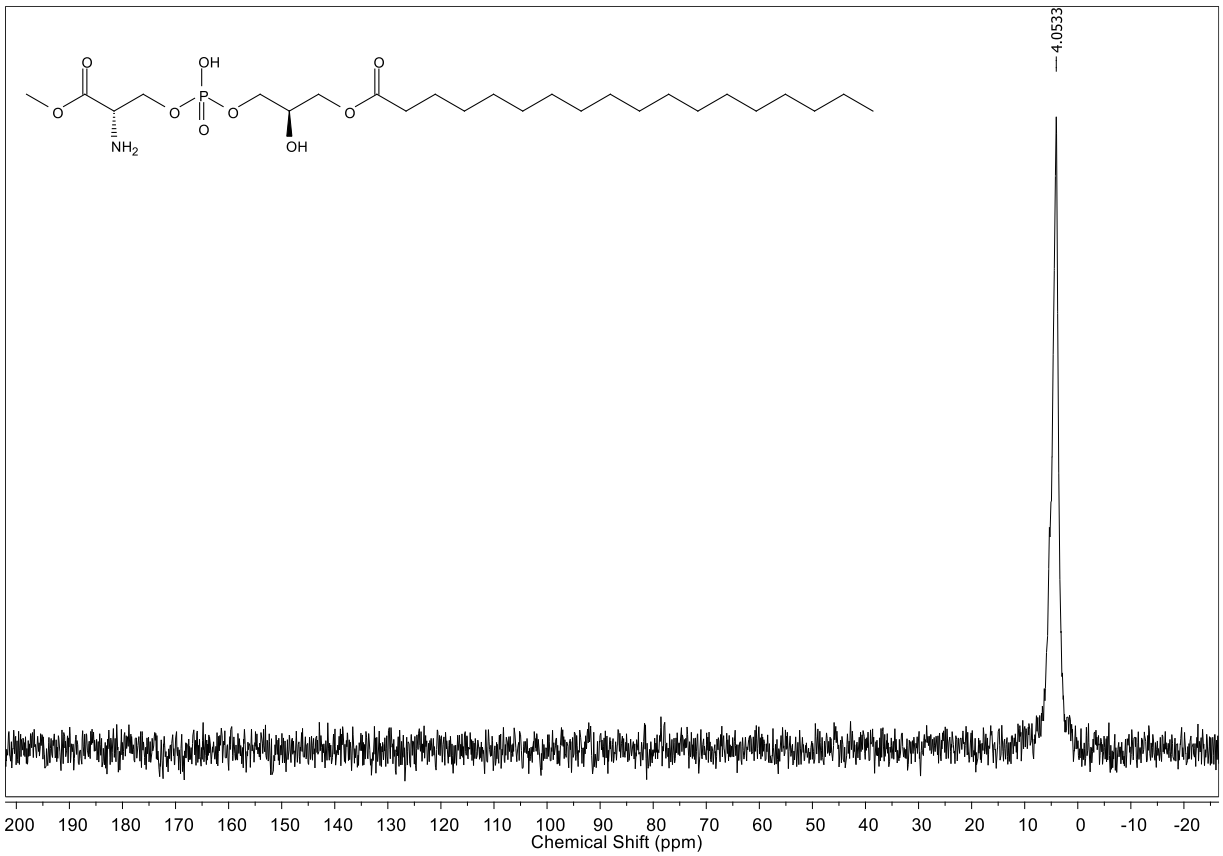

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20a



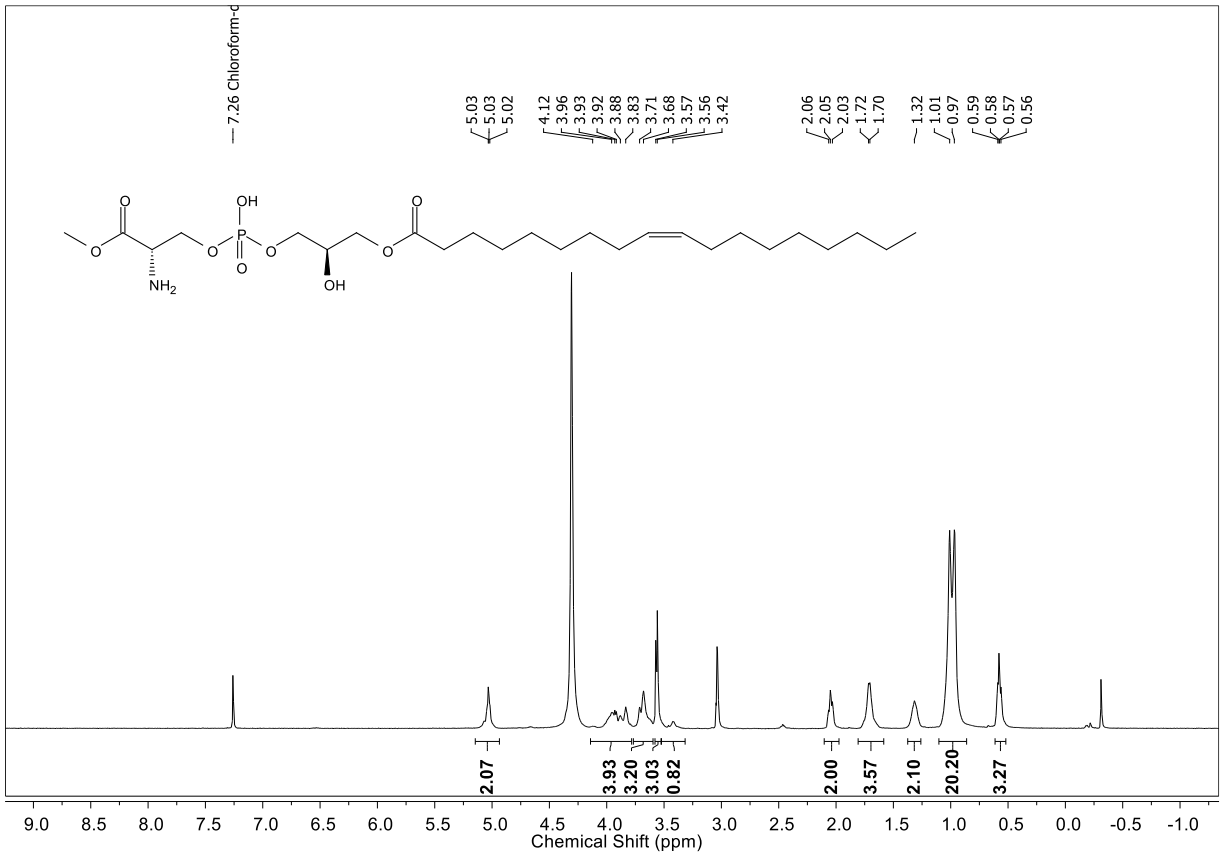
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20b



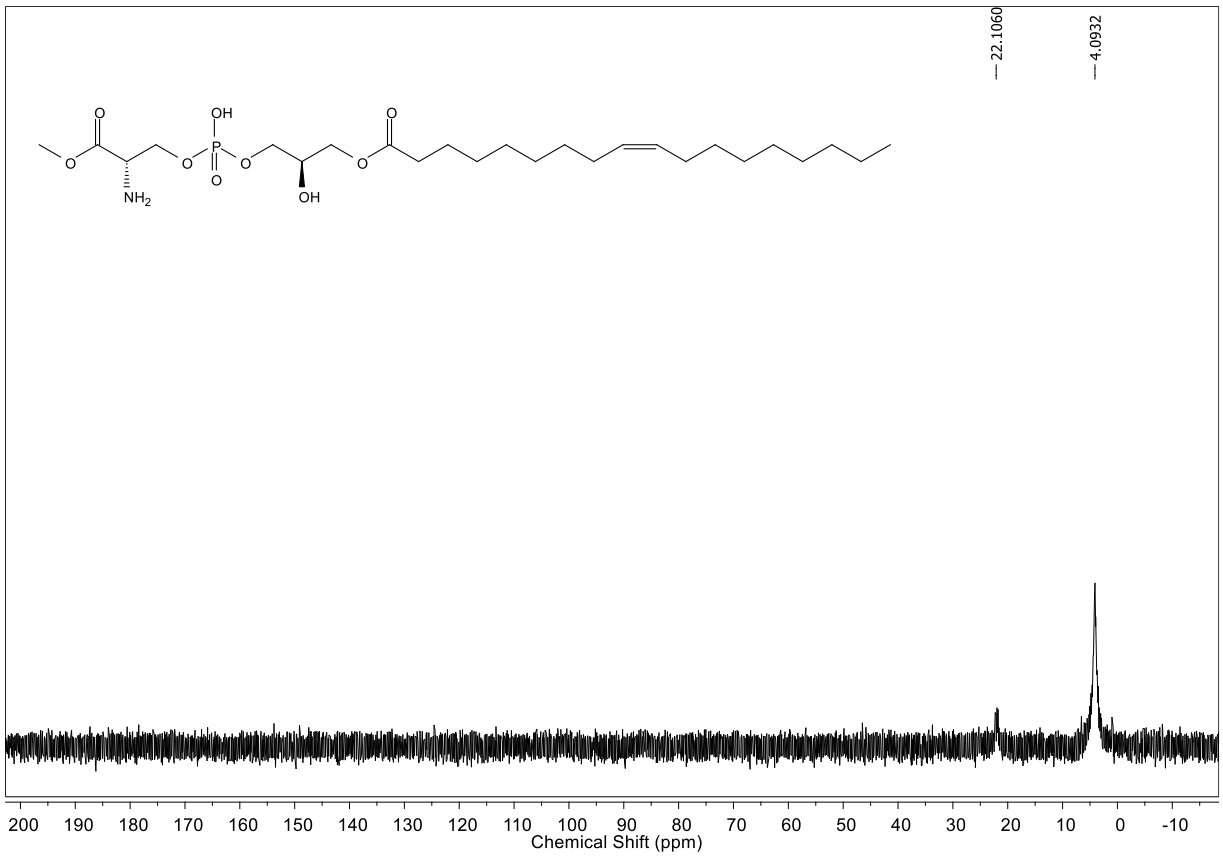

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20b



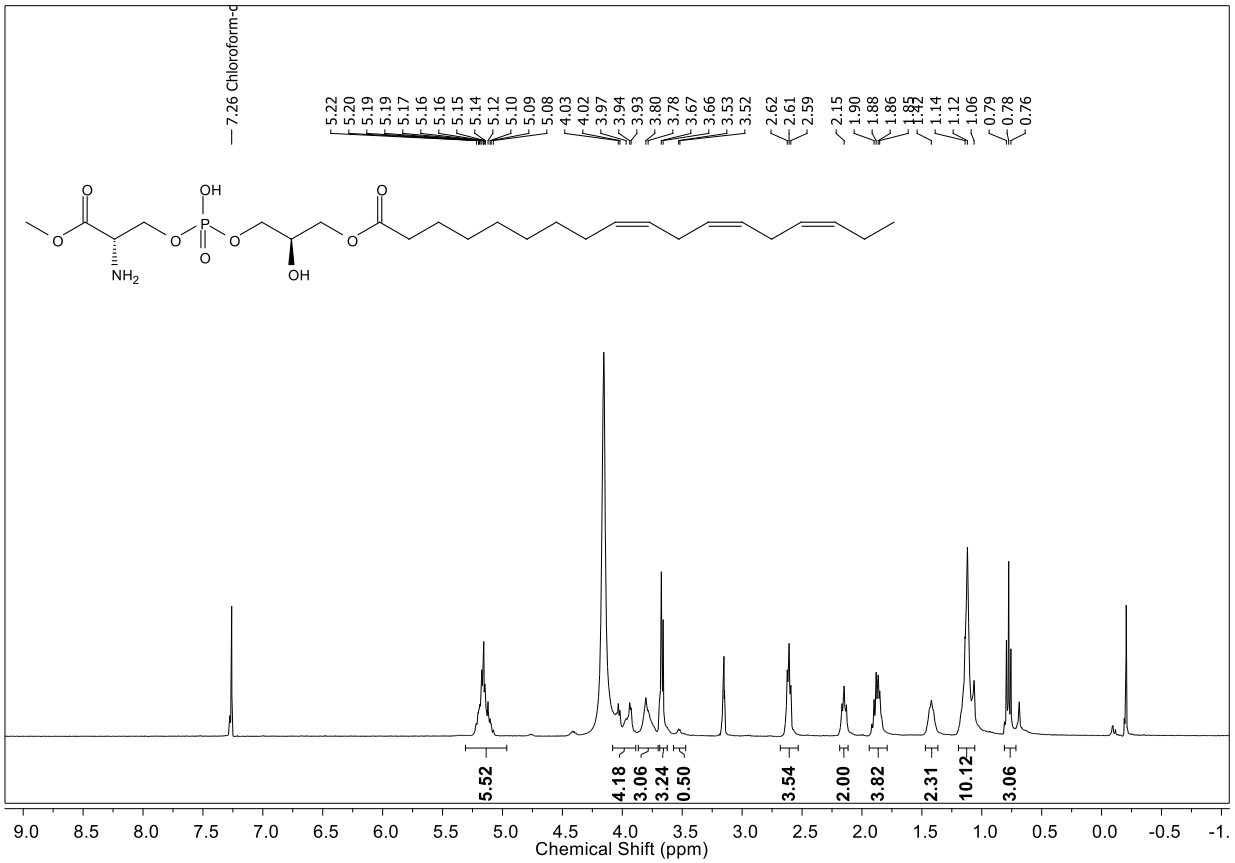
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20c



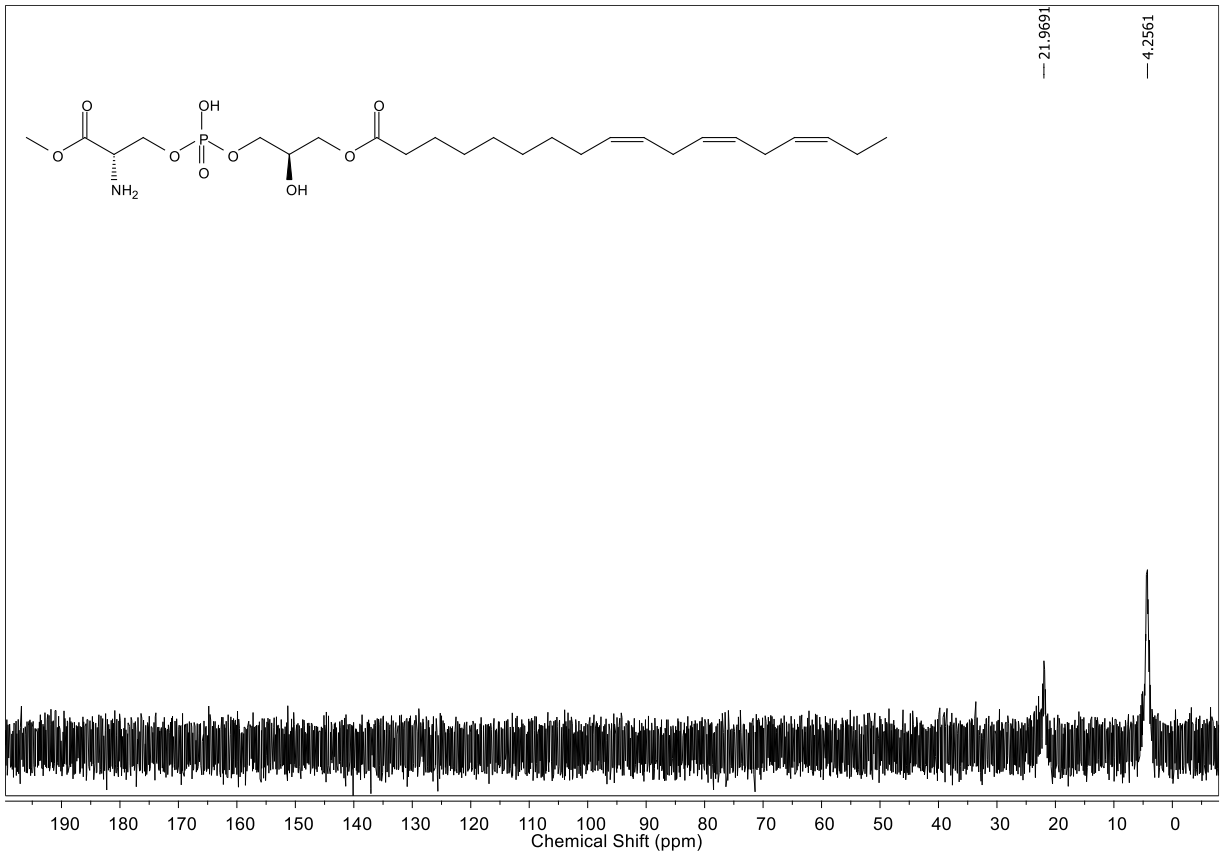

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20c



## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20d




## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20d



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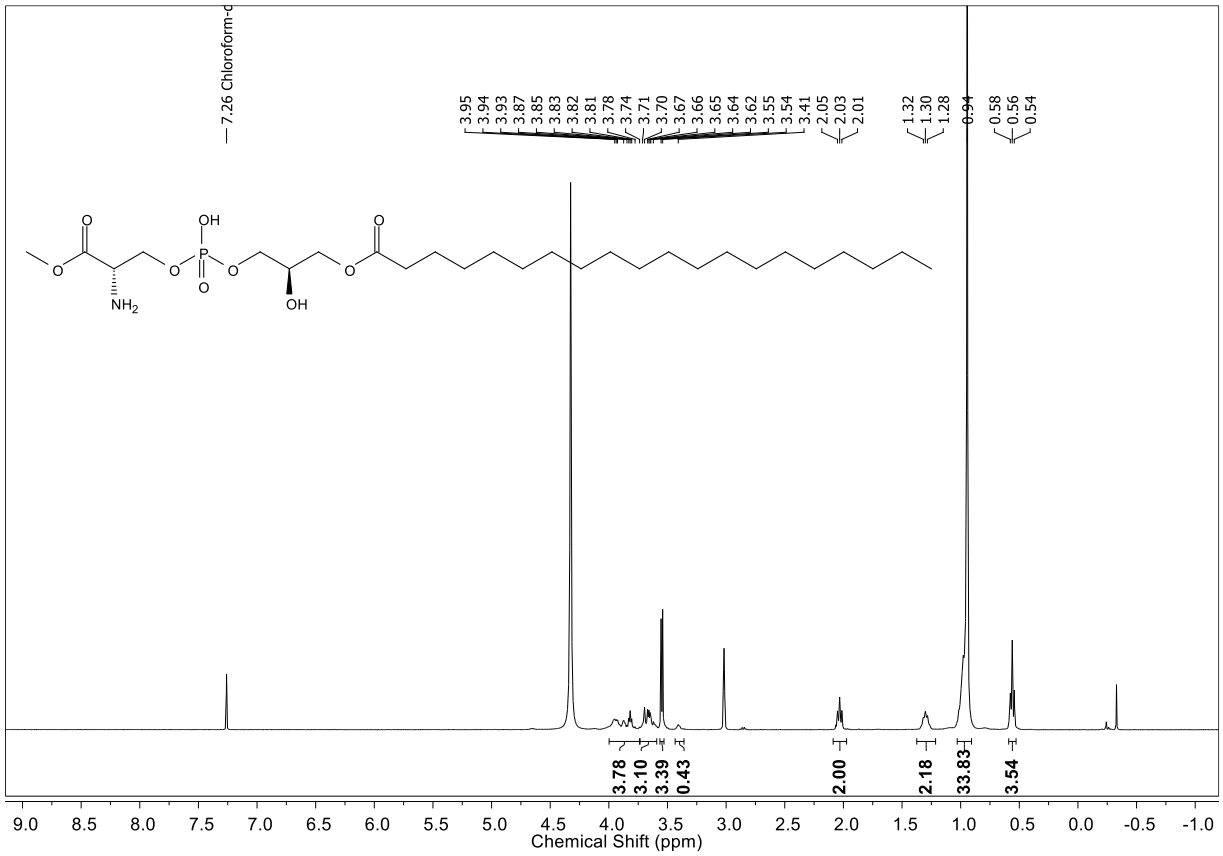


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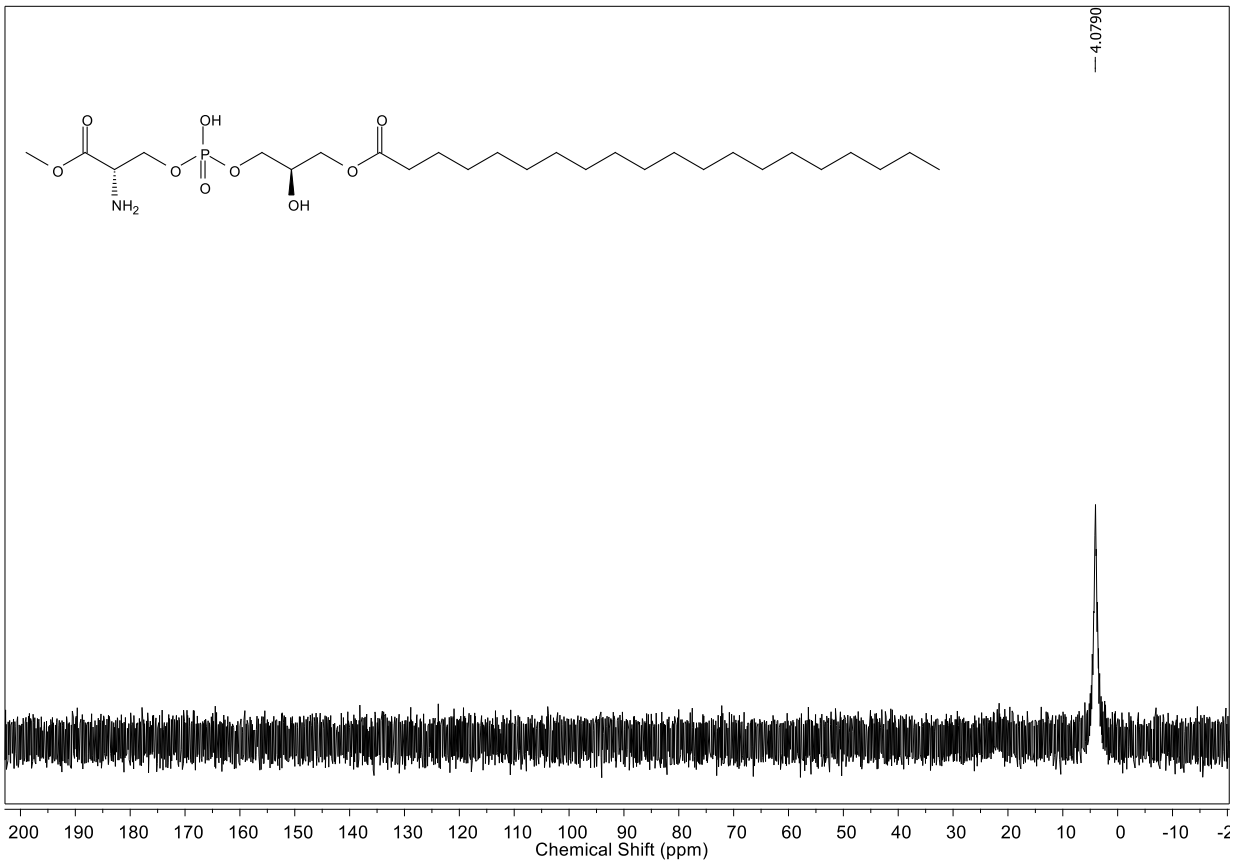

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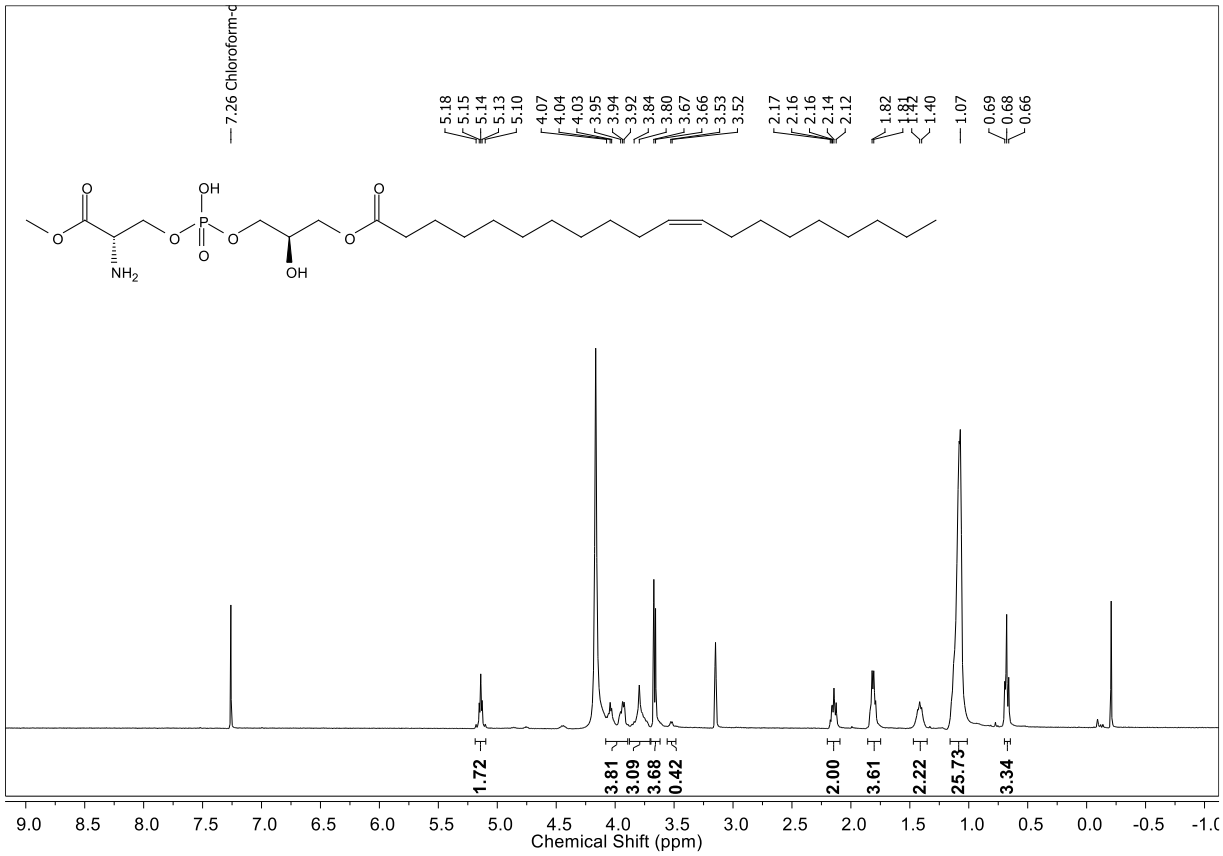


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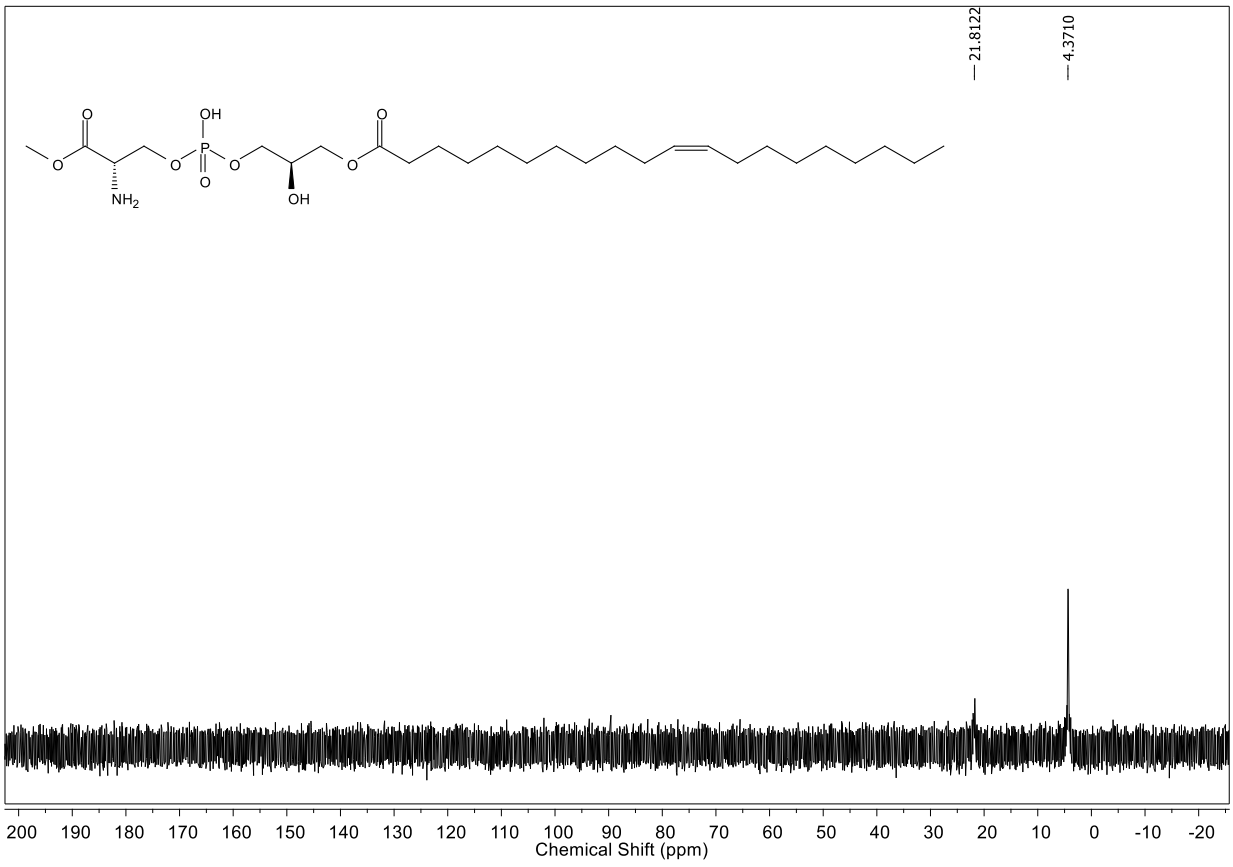

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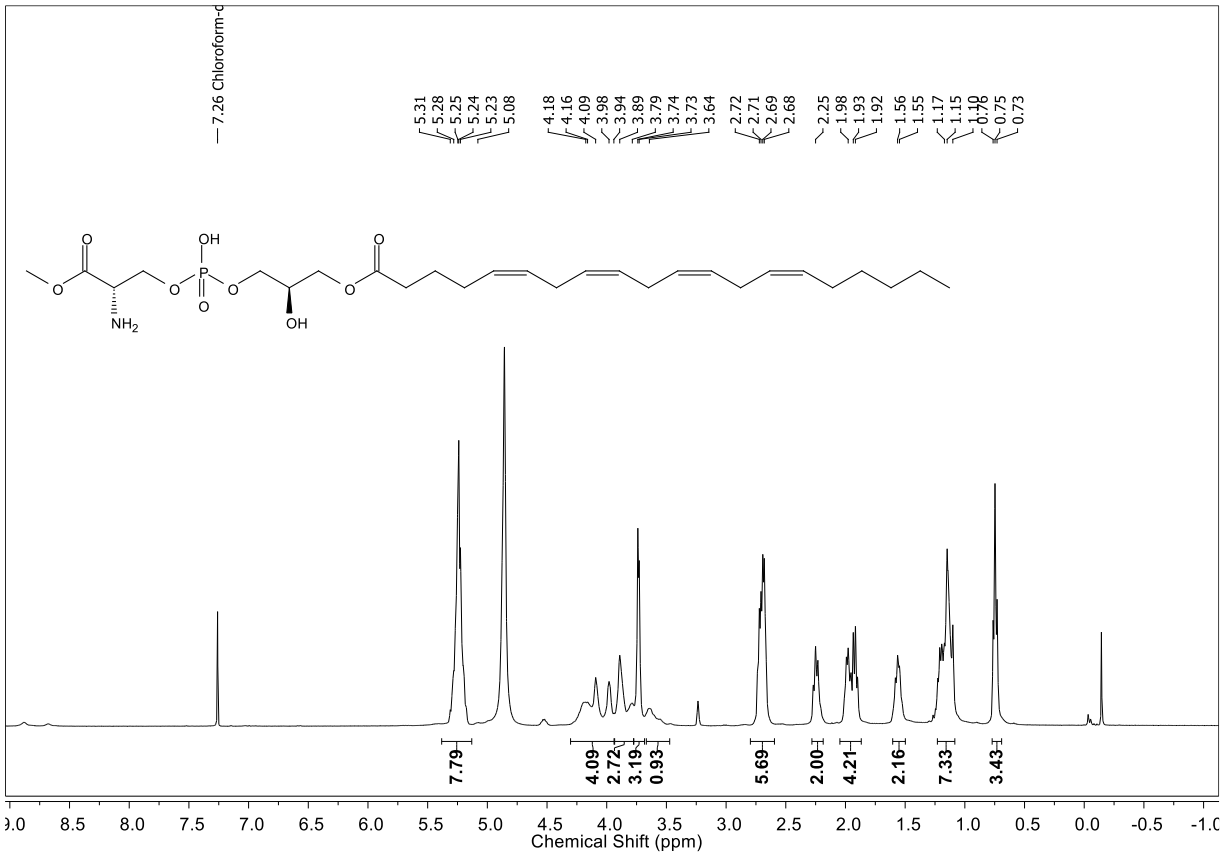
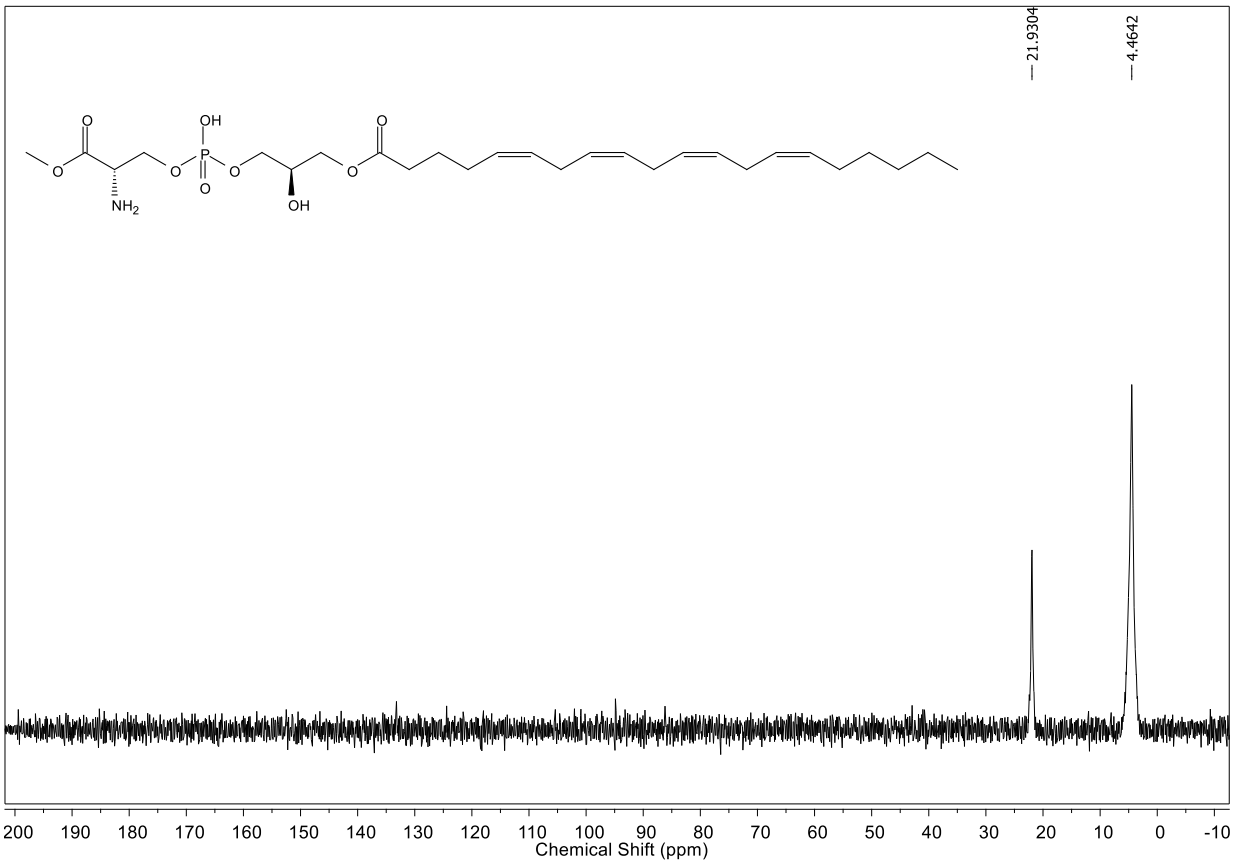
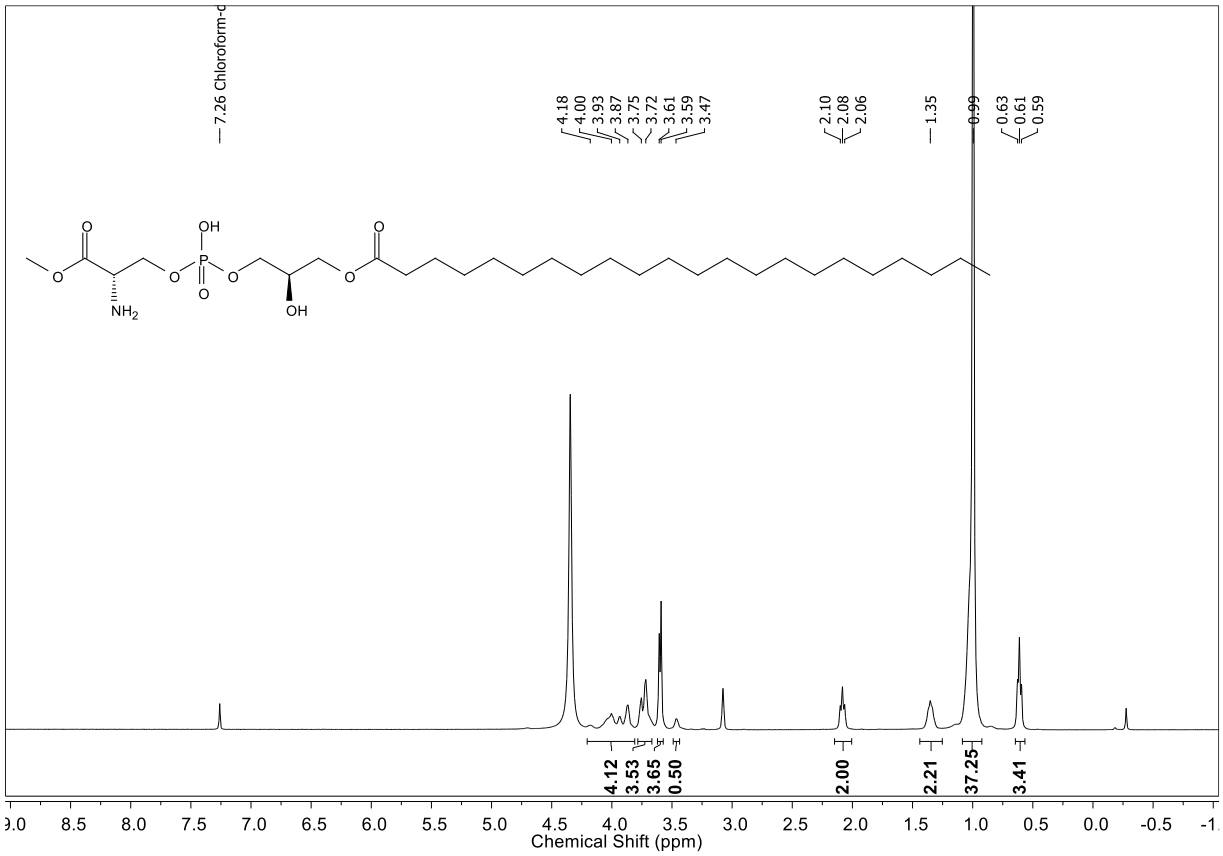


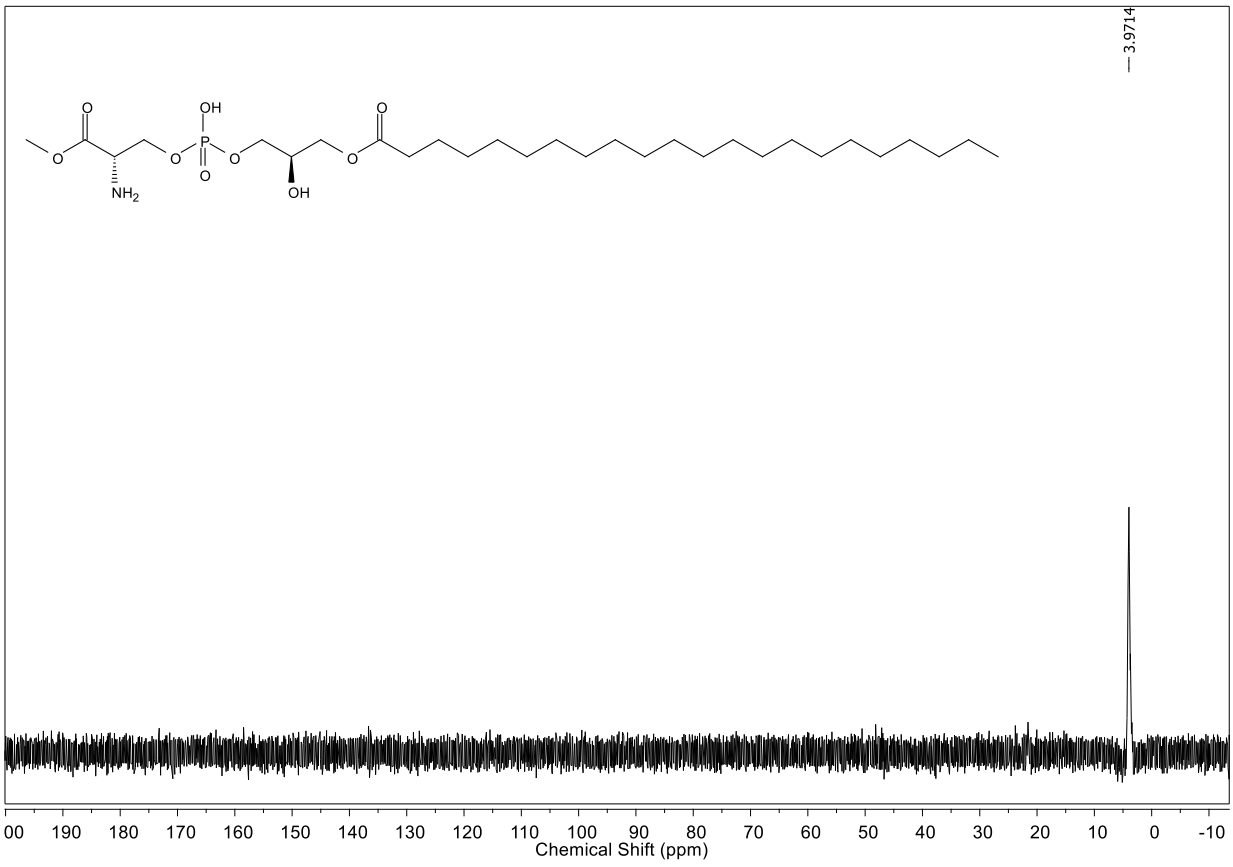

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20g



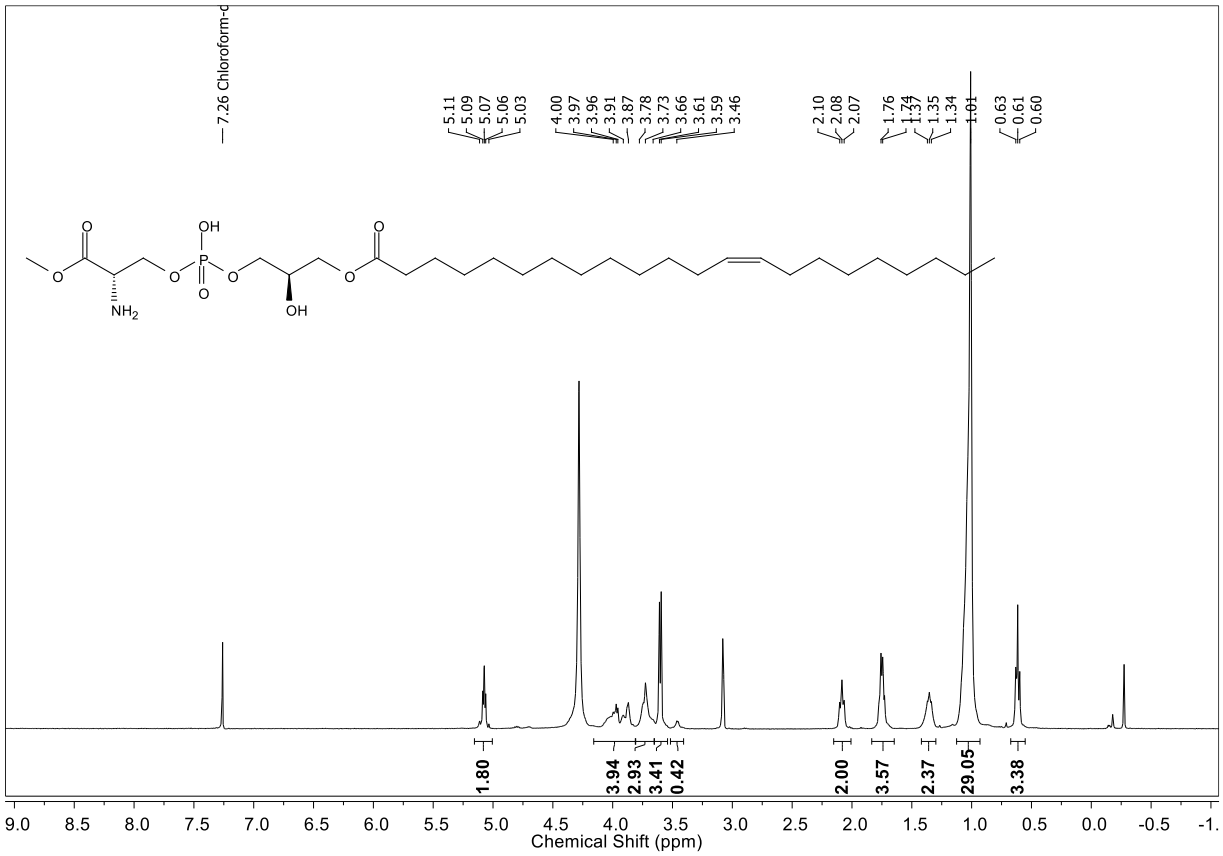
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20h



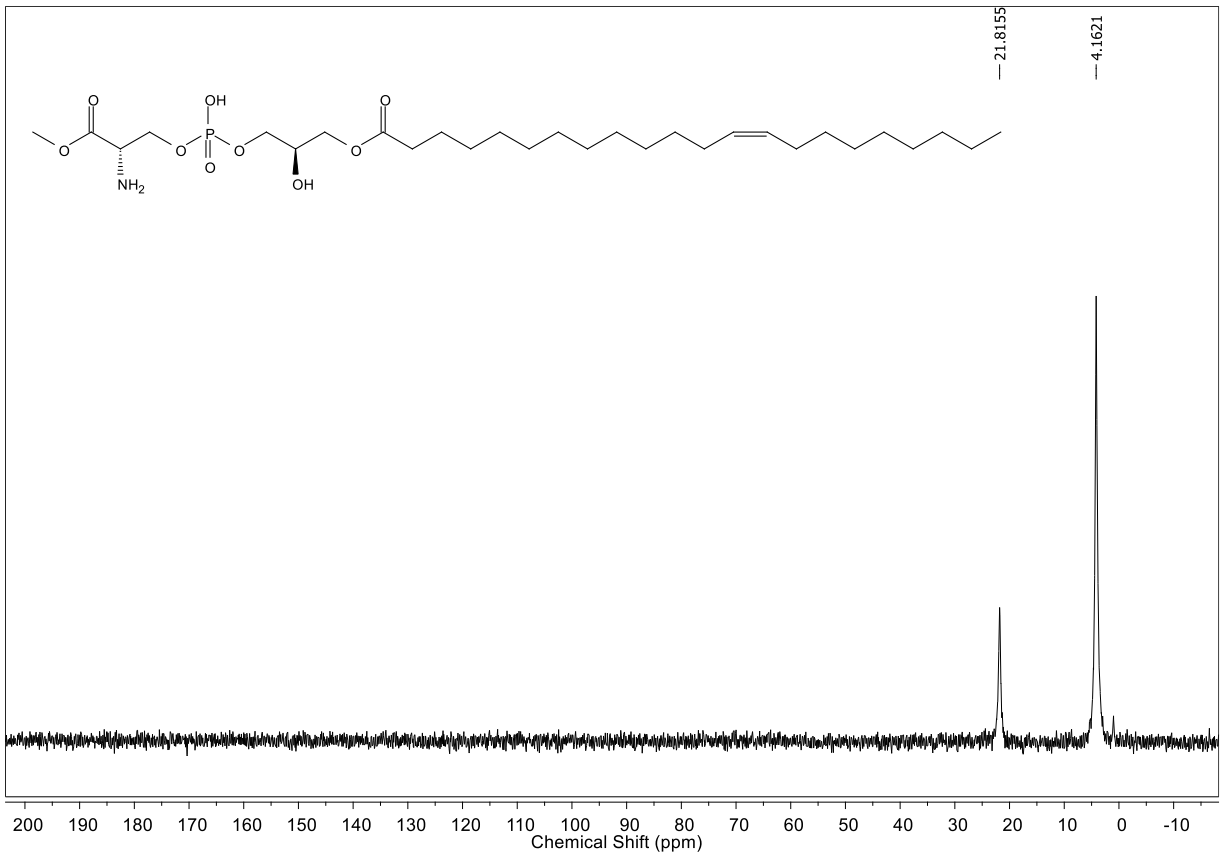

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20h



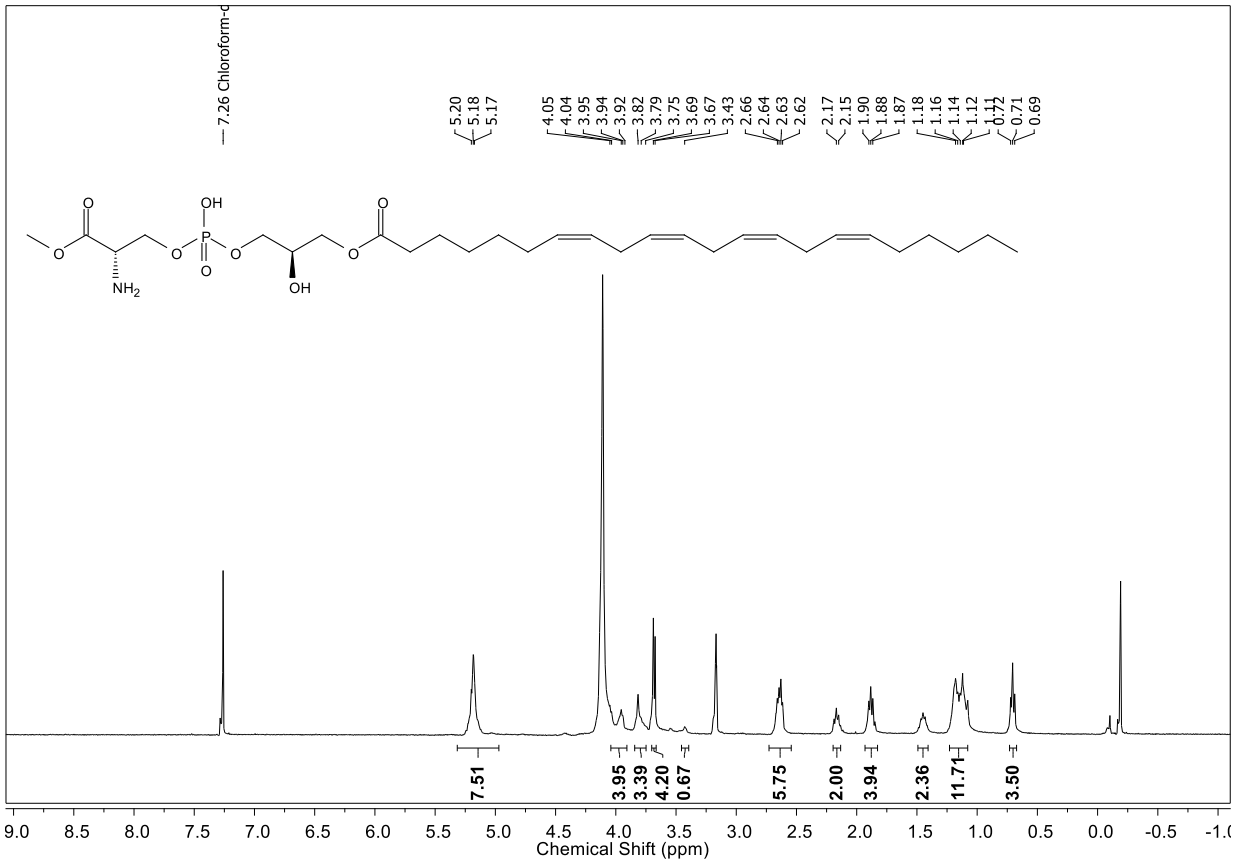
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20i



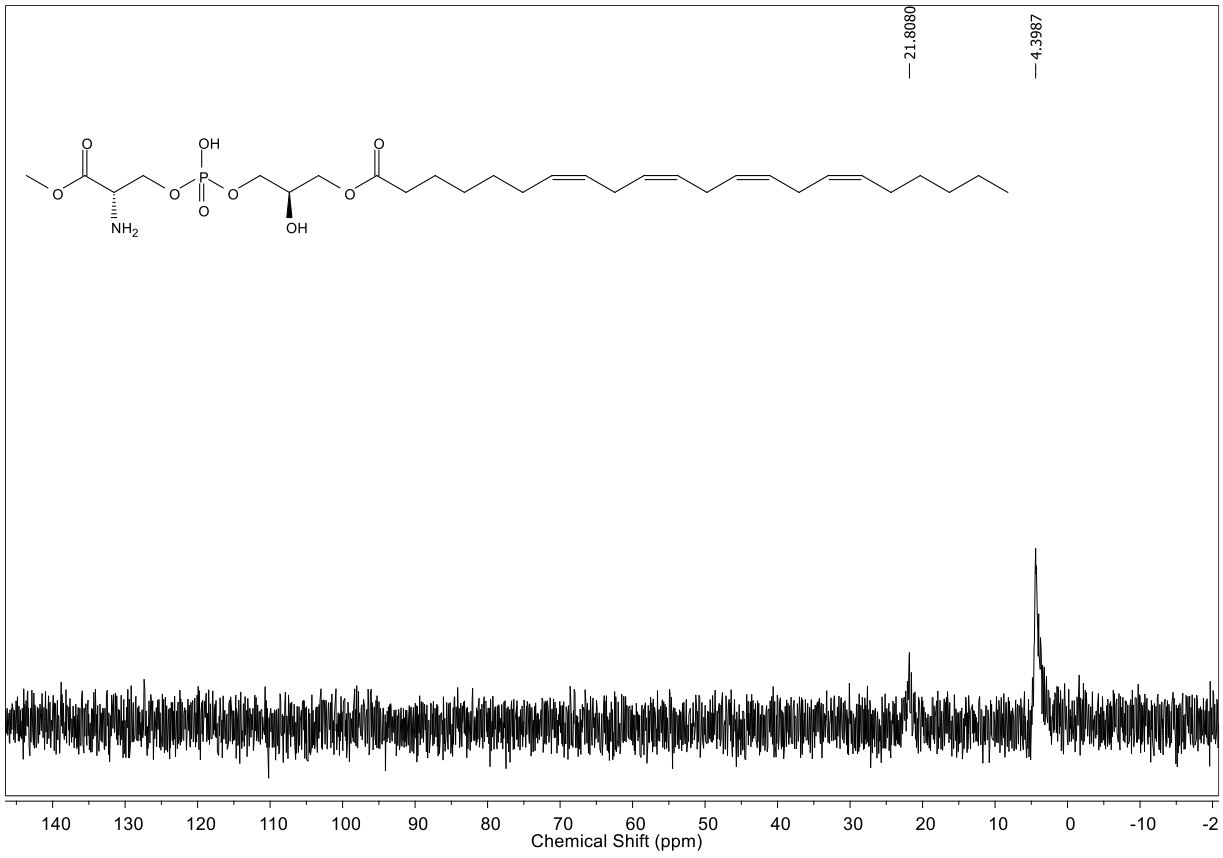

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20i



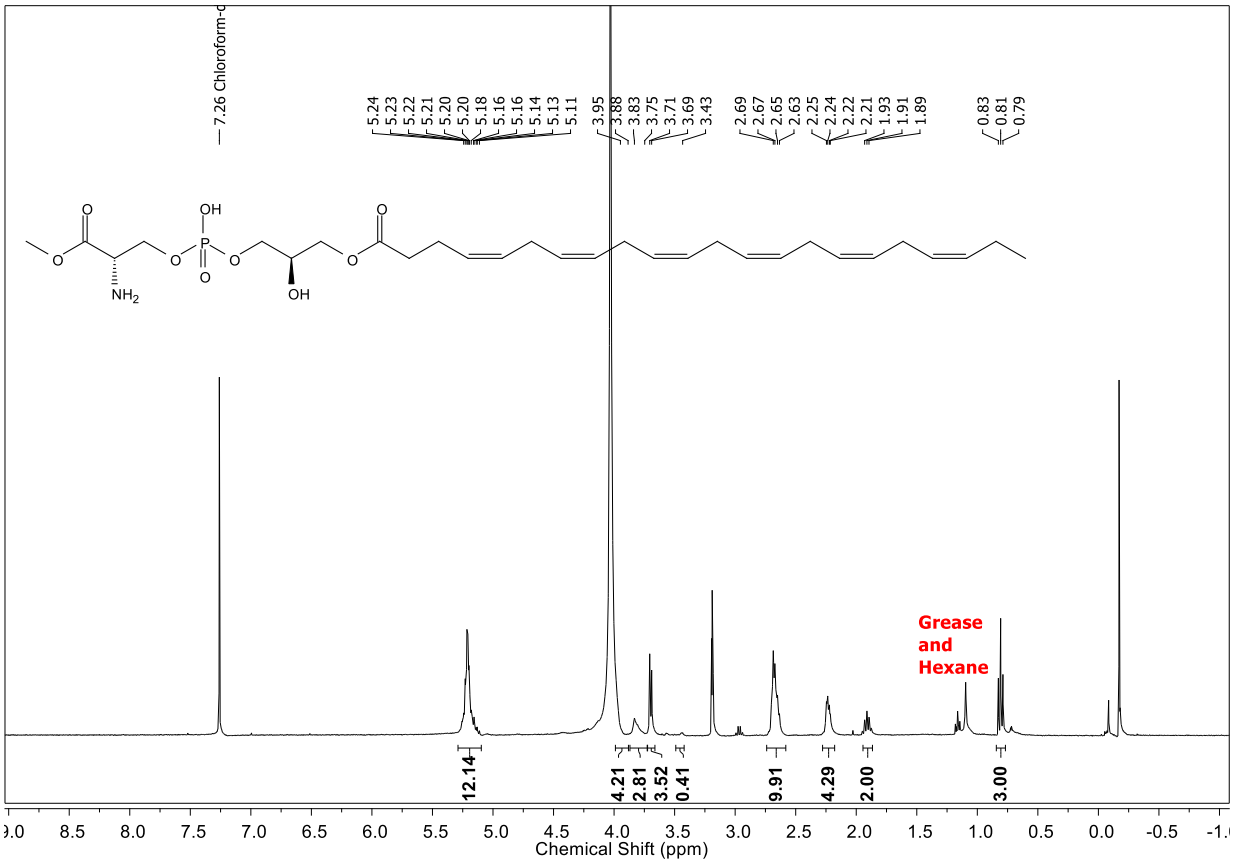
## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20j



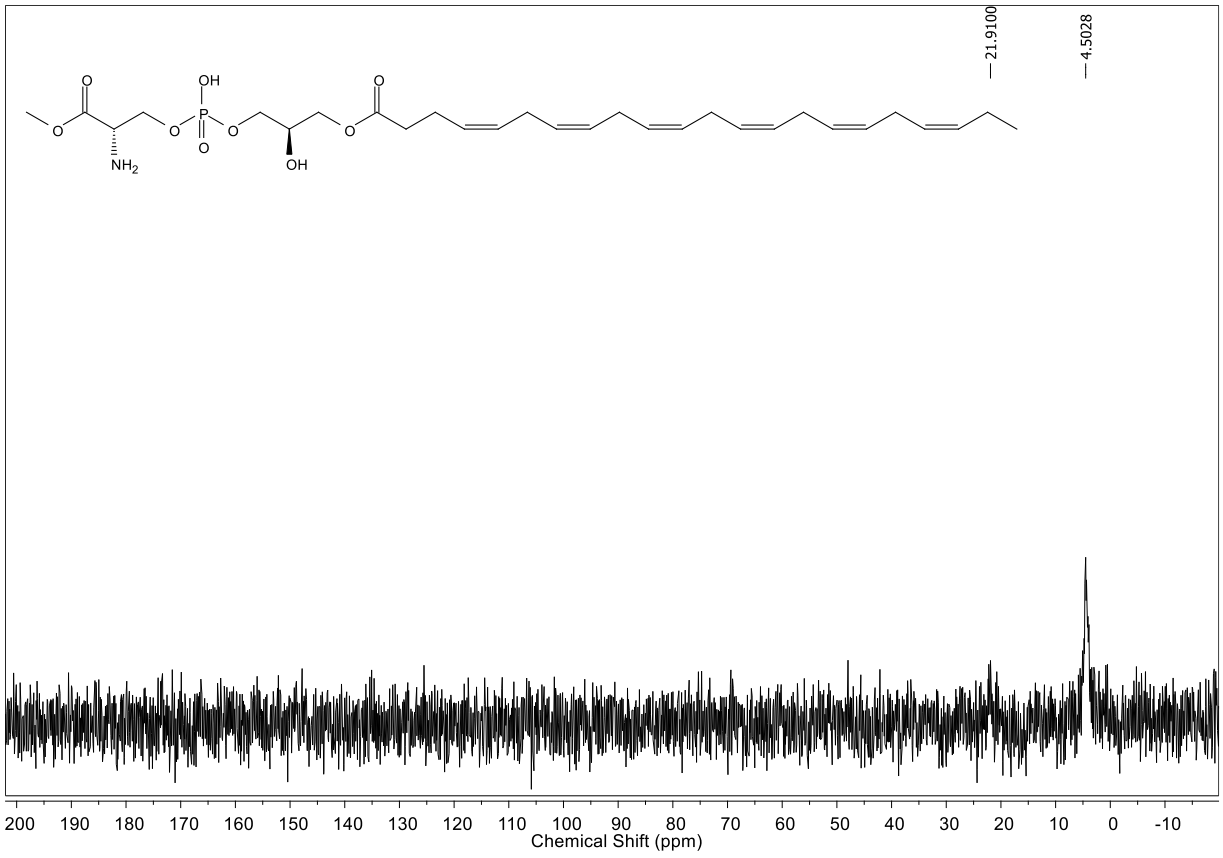

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20j



## $^{1}$H-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20k




## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20k



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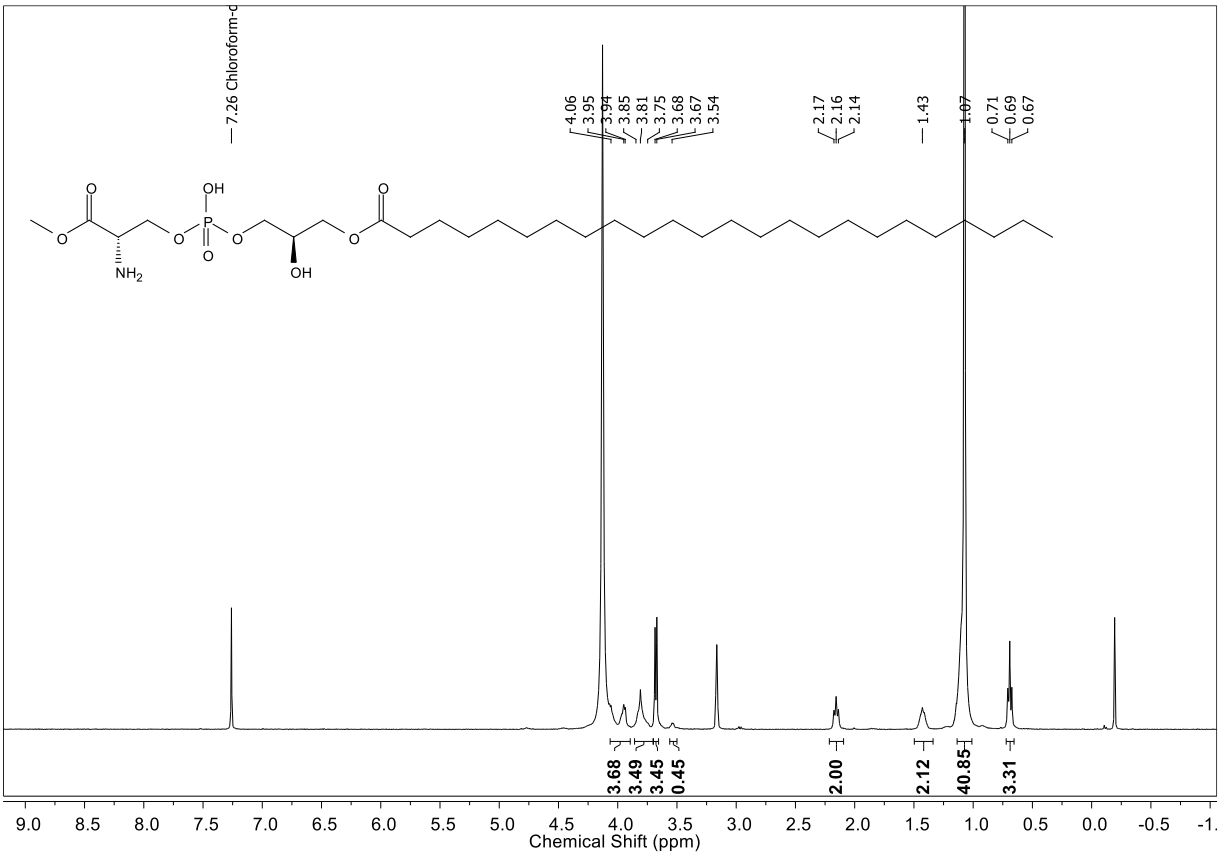
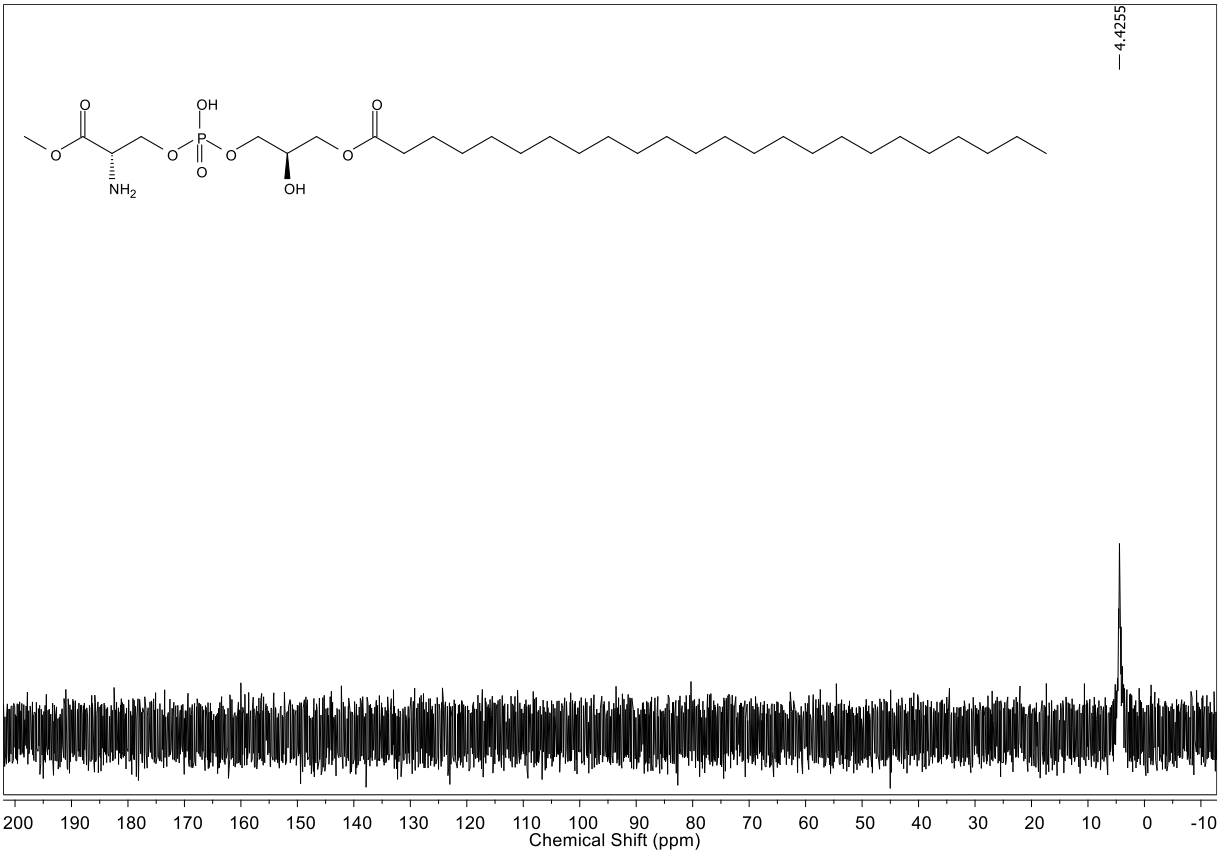



## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20l



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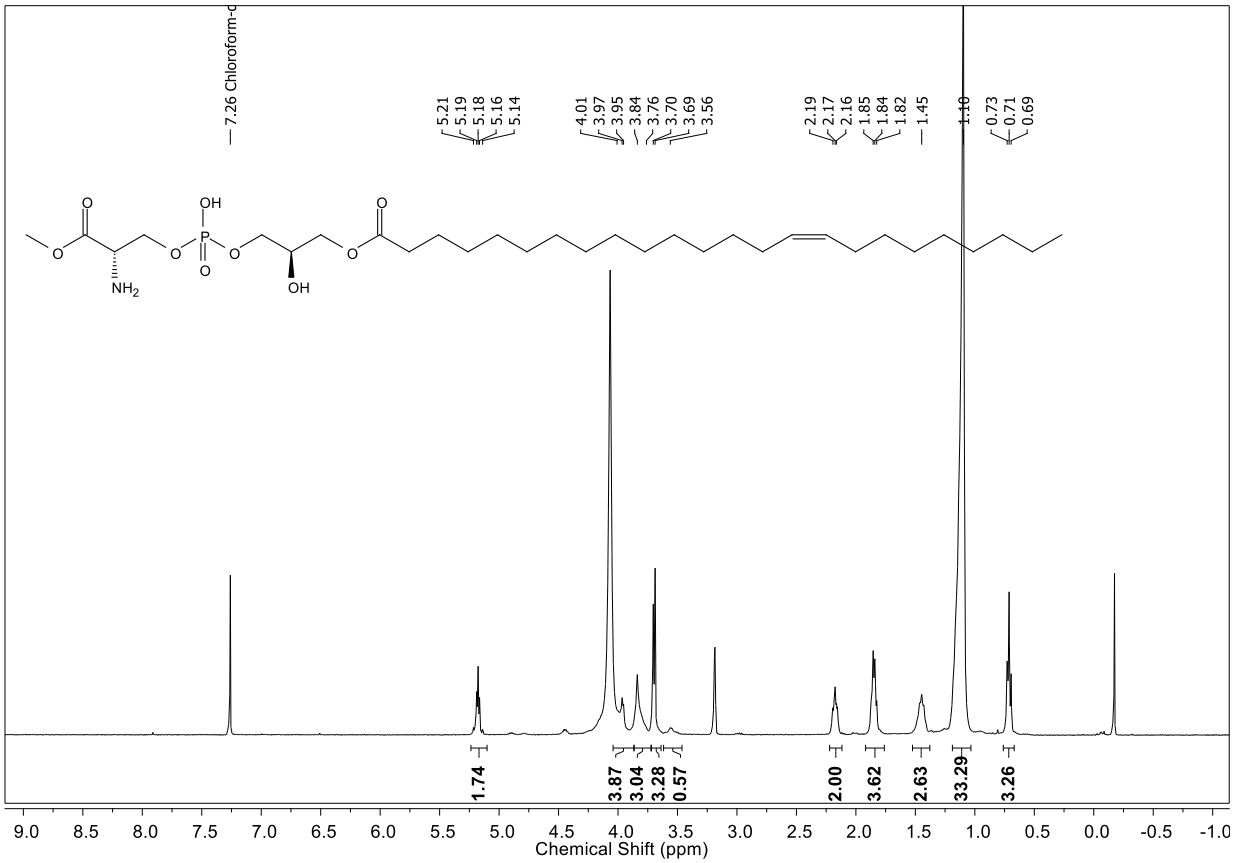
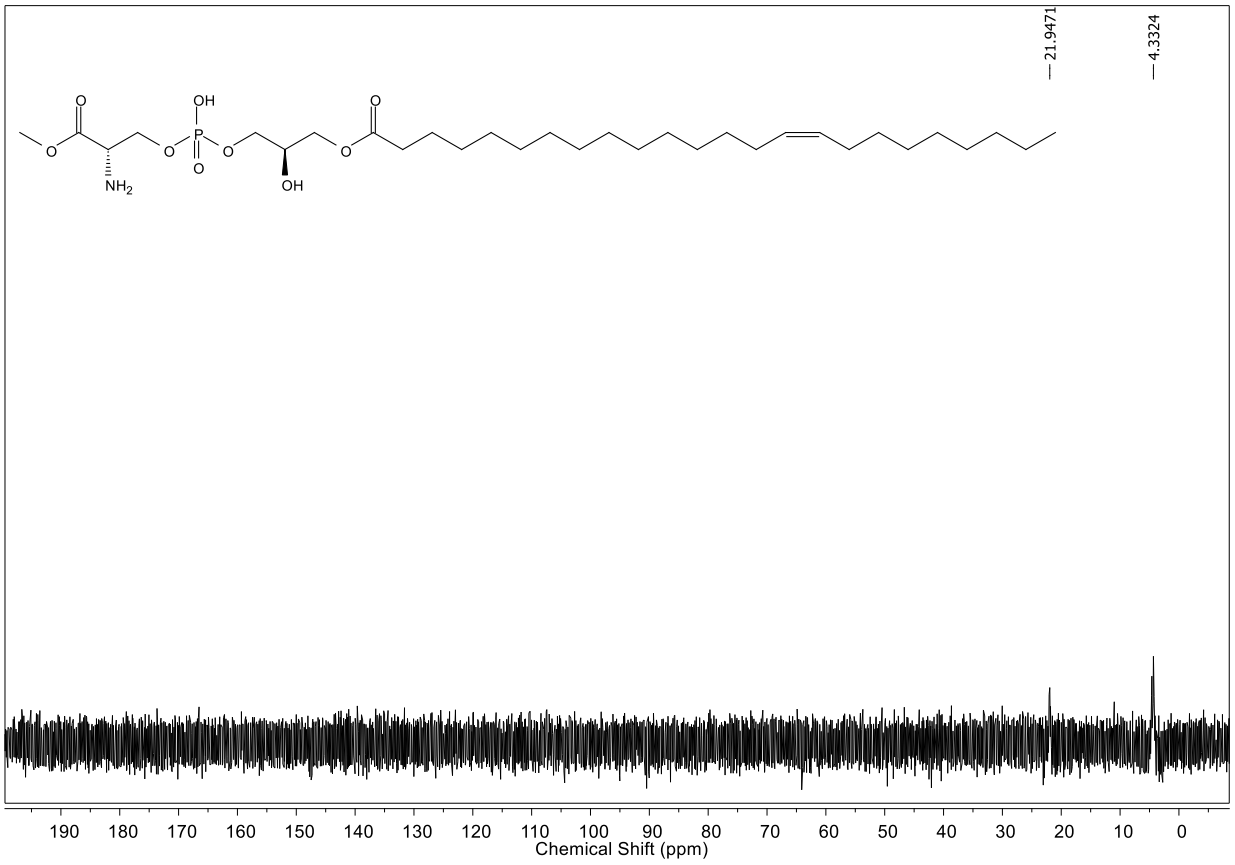
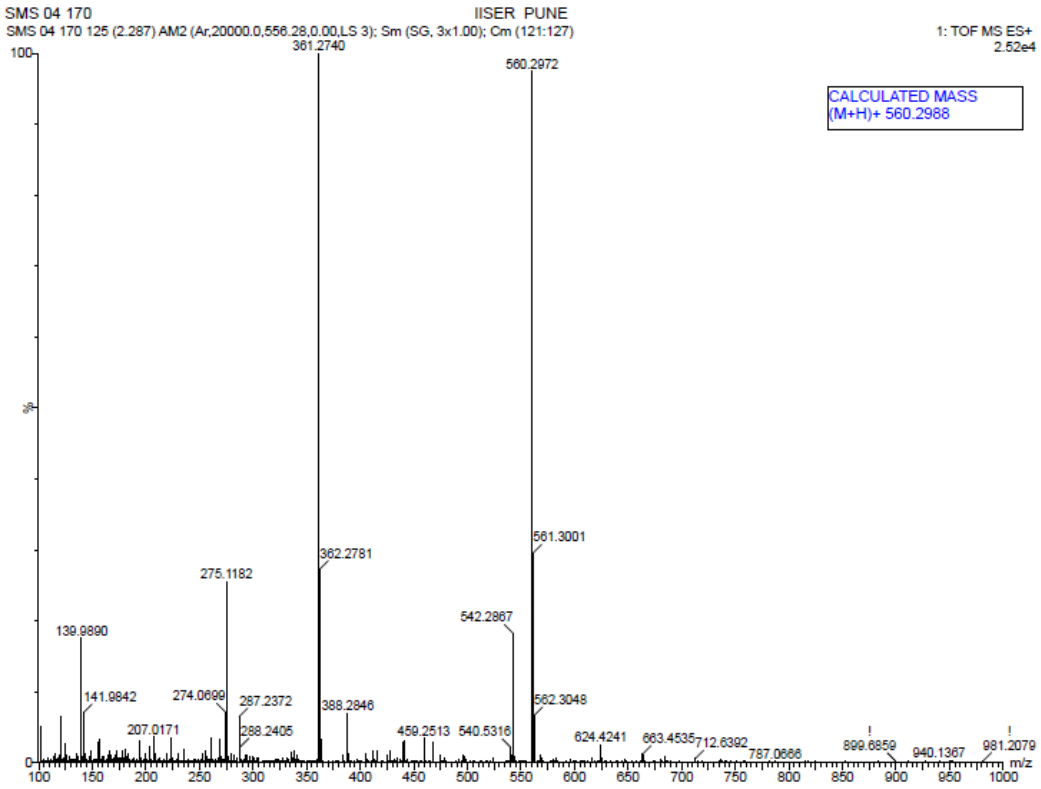


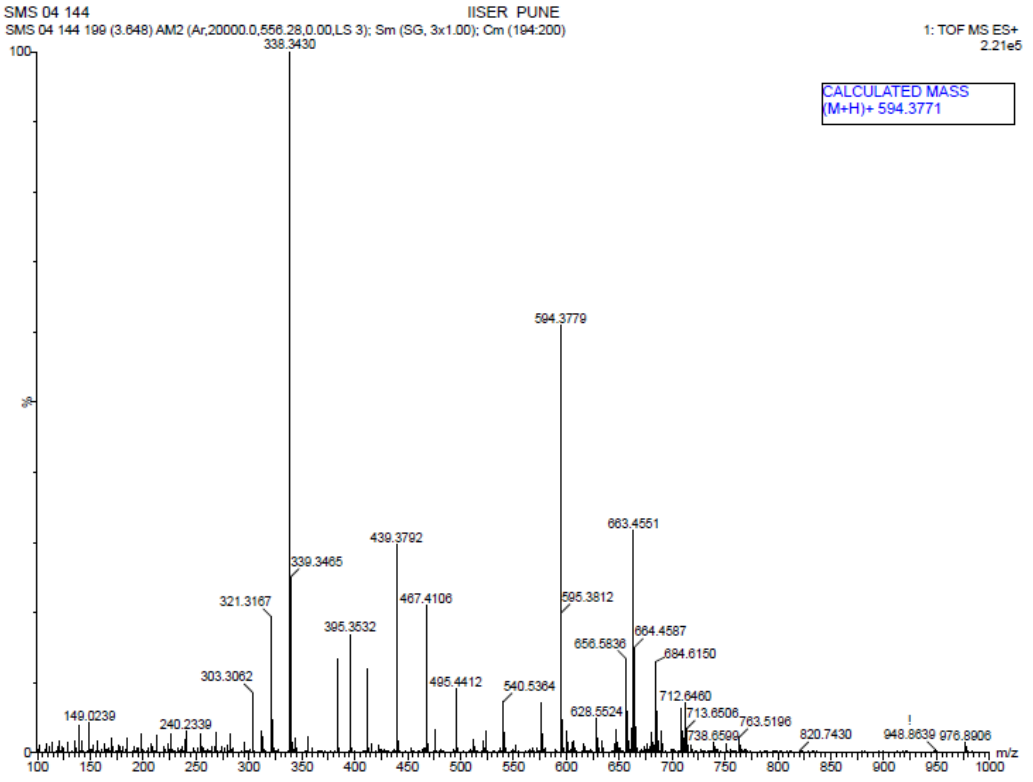

## $^{31}$P-NMR ($CDCl_3$, 400 MHz) for Compound (*R*)-20m



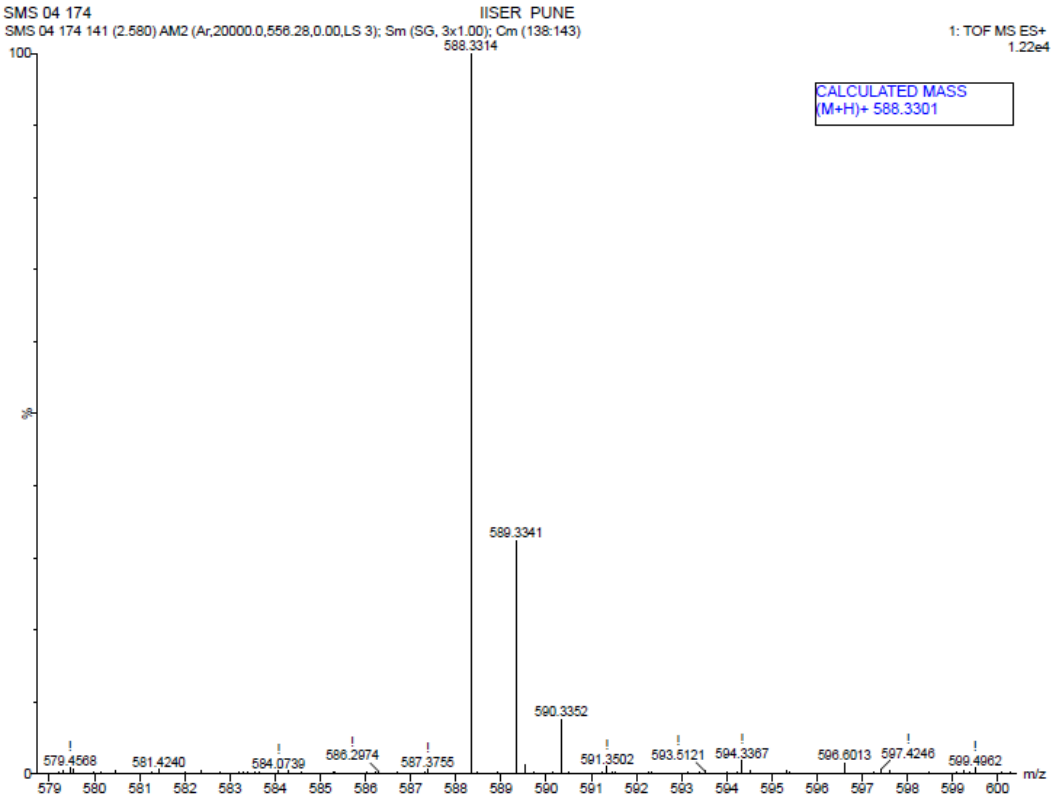
## HRMS of Compound (*R*)-20g



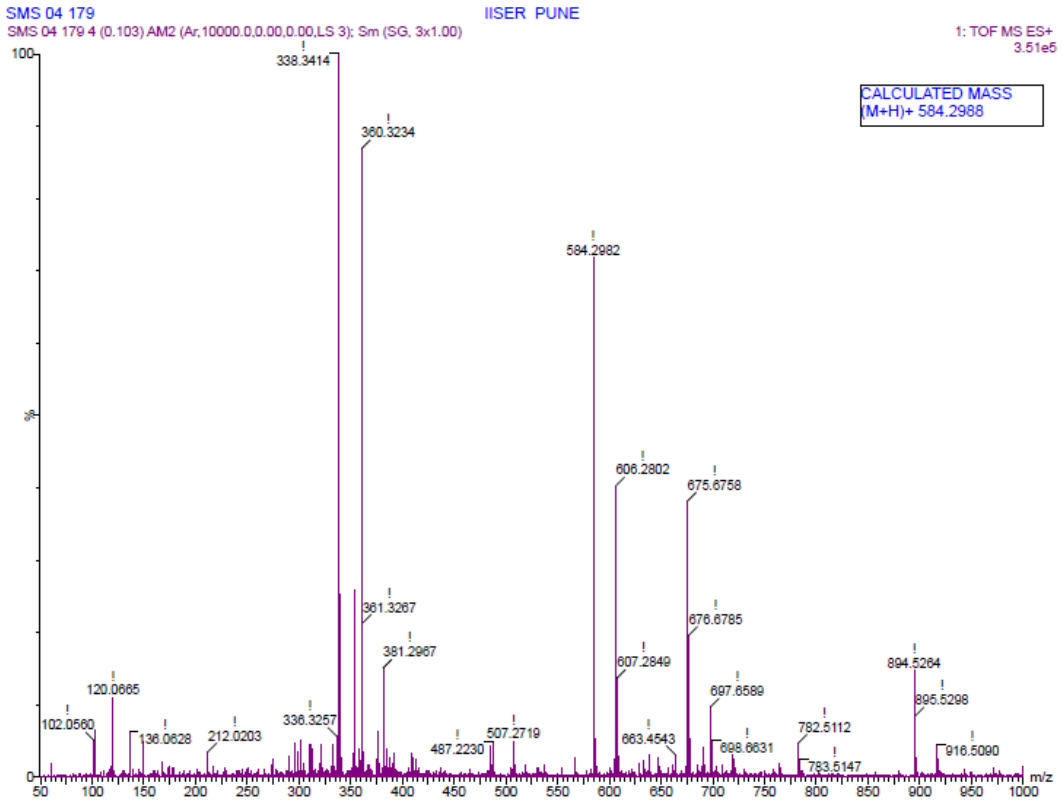

## HRMS of Compound (*R*)-20i



## HRMS of Compound (*R*)-20j




## HRMS of Compound (*R*)-20k



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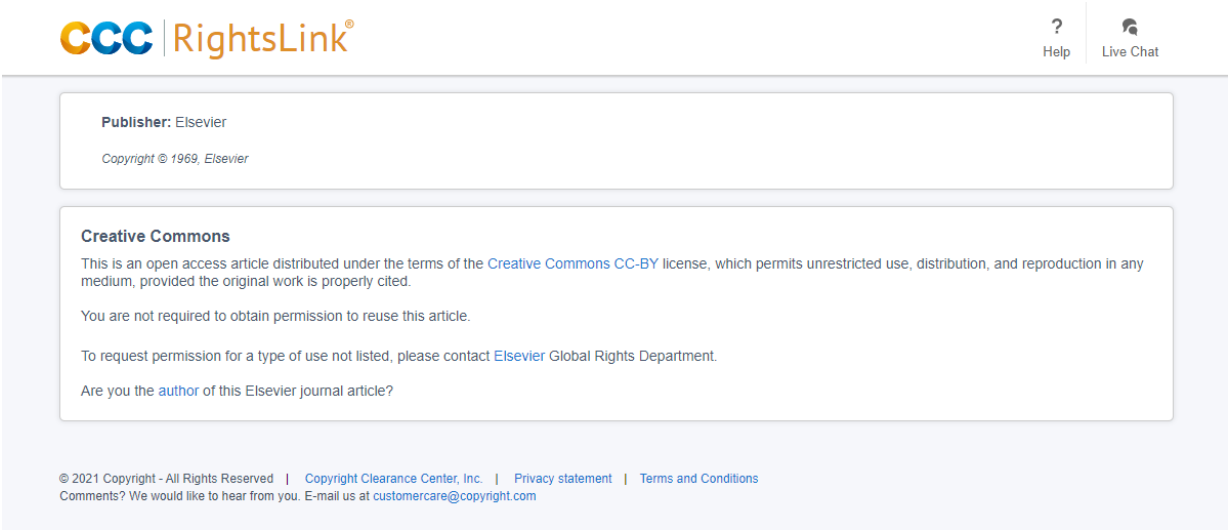


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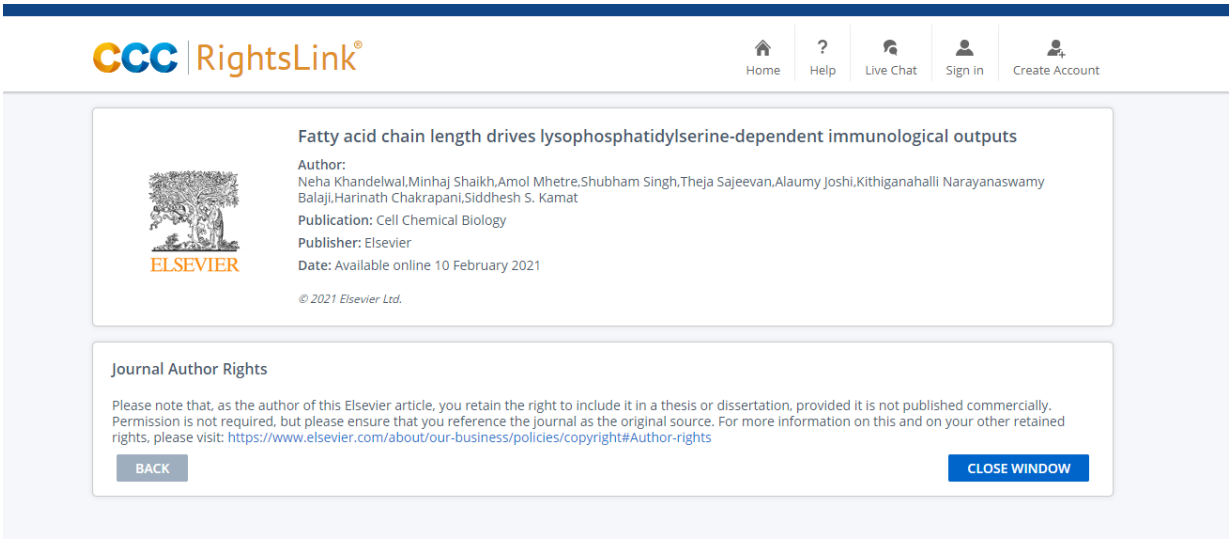

# PUBLICATIONS

1. Alaumy J[#], Minhaj S[#], Shubham S[#], Abinaya R[§], Amol M[§], Siddhesh S. Kamat[ϕ]. Biochemical characterization of the PHARC-associated serine hydrolase ABHD12 reveals its preference for very-long-chain lipids. *J. Biol. Chem.*, 2018. https.//doi.org/10.1074/jbc.RA118.005640
([#]Equal contribution)

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