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Supplemental Information

**Fatty acid chain length drives
lysophosphatidylserine-dependent
immunological outputs**

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES (S1 – S7)

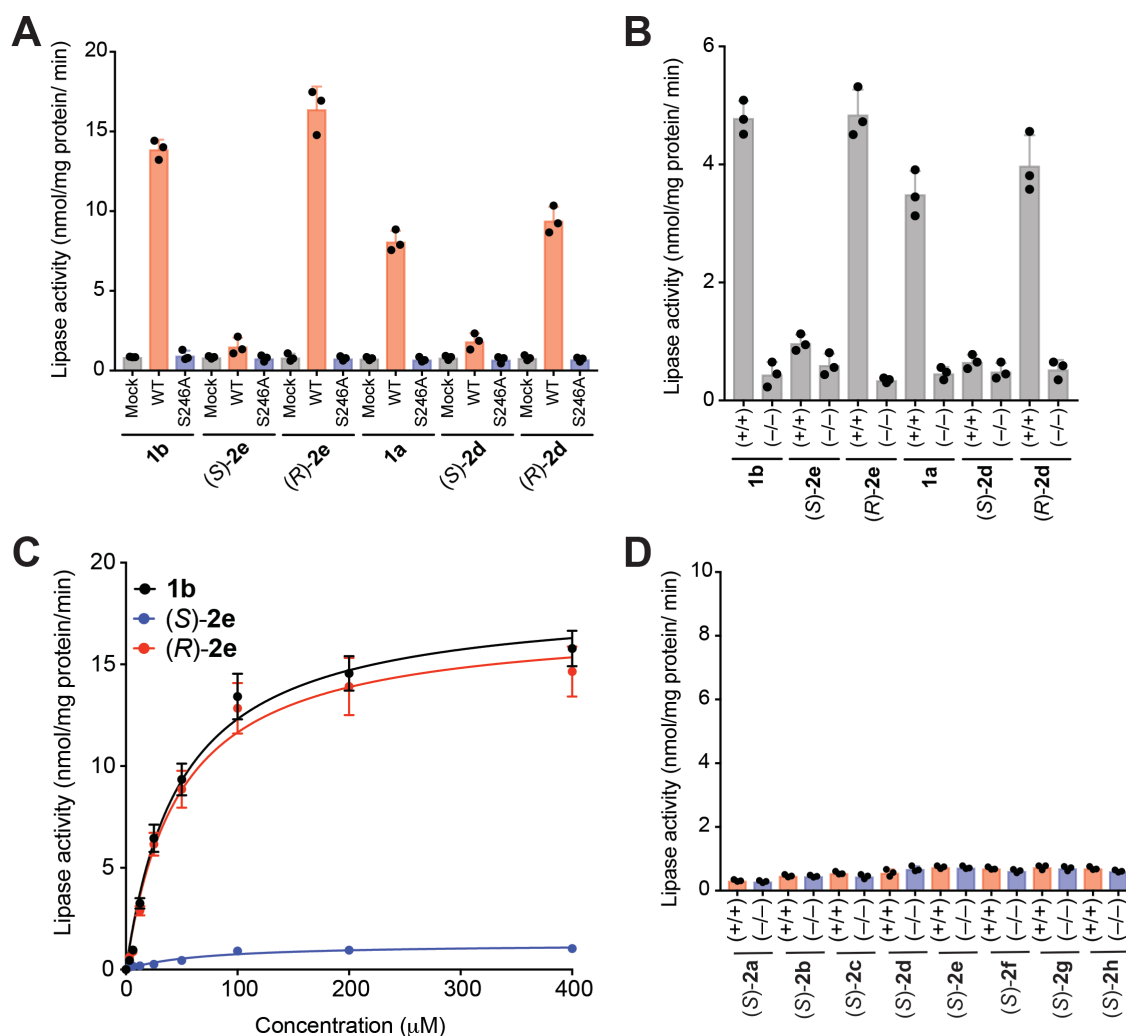


Figure S1. Lyso-PS lipase substrate assays performed with recombinant hABHD12 and endogenous mABHD12, Related to Figure 1. (A) HEK293T membrane lysates (10 μ g) transfected with wild type (WT) hABHD12 or the catalytically inactive S246A hABHD12 mutant or a mock-transfection control; and (B) Brain membrane lysates (20 μ g) from wild type (+/+) and ABHD12 knockout (-/-) mice were assayed against 100 μ M of commercial lyso-PSs (1a (C16:0) and 1b (C18:0)) or (R)-Me-lyso-PS (2d (C16:0) and 2e (C18:0)) or (S)-Me-lyso-PS (2d (C16:0) and 2e (C18:0)) using established protocols (Joshi et al., 2018, Singh et al., 2020). The data shows that hABHD12 and mABHD12 have similar lipase activity for a commercial canonical lyso-PS and the corresponding (R)-Me-lyso-PS of the same lipid tail, while the corresponding (S)-Me-lyso-PS is a poor substrate for this enzyme. (C) Enzyme kinetic assays for WT hABHD12 against varying concentrations of 1b (C18:0), (R)-2e (C18:0) and (S)-2e (C18:0) (0 – 400 μ M) showing comparative enzyme kinetic profiles for 1b and (R)-2e, but not (S)-2e (n = 3/data point). The line connecting the data points for a particular group represents a fit to a classical Michaelis-Menten enzyme kinetics

equation (Joshi et al., 2018). All enzyme kinetic parameters are reported in **Table 1. (D)** Brain membrane lysates from wild type (+/+) and ABHD12 knockout (–/–) mice were assayed against 100 μ M of (S)-Me-lyso-PS with varying chain lengths using established protocols (Joshi et al., 2018, Singh et al., 2020). The data shows that (S)-Me-lyso-PSs are not substrates for endogenous mABHD12, suggesting that the lyso-PS lipase activity of endogenous mABHD12 is highly stereoselective. The plot is at the same scale as the data presented in **Figure 1D** for relative comparison. All data is represented as mean \pm standard deviation from three independent experiments for all experimental groups. Complete nomenclature of the lipids can be found in **Figure 1B**.

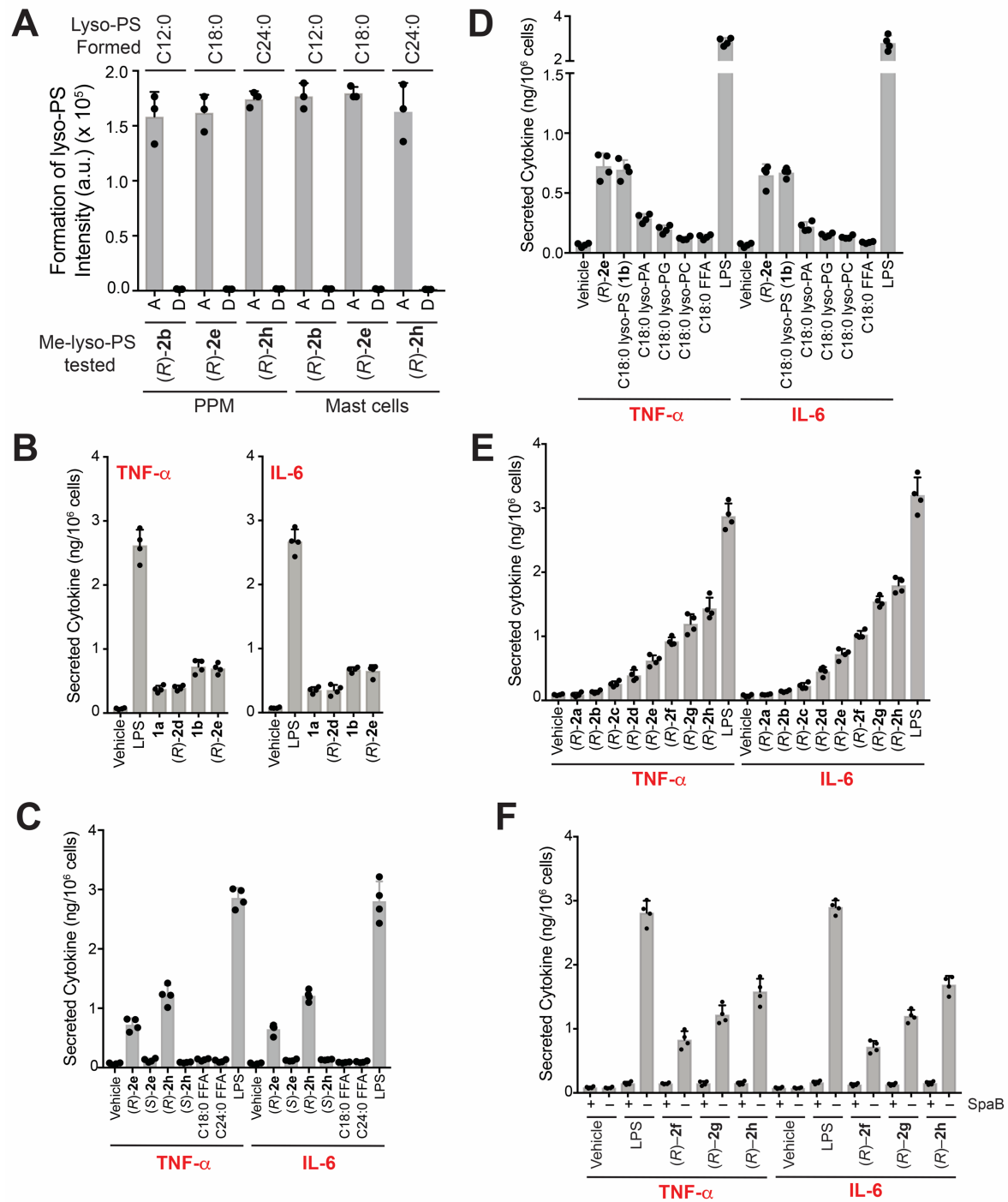


Figure S2. Metabolism of Me-lyso-PS, and pro-inflammatory cytokine secretions from mammalian macrophages following different Me-lyso-PS treatments, Related to Figure 2, 3. (A) LC-MS analysis of the lyso-PS products (loss of 14 Da from parent m/z): C12:0 (m/z [M-H]⁻ = 440.206) from (R)-2b, C18:0 (m/z [M-H]⁻ = 524.299) from (R)-2e and C24:0 (m/z [M-H]⁻ = 608.393) from (R)-2h, showing the conversion of the parent compound (Me-lyso-PS) to the corresponding lyso-PS following treatment with active (A), but not denatured (D), cellular lysates from ABHD12-null primary peritoneal macrophages (PPM) or ABHD12-null primary mast cells (20 μg lysate, 15 mins, 37 °C). In these assays, 100 μg of the (R)-Me-lyso-PS was used, and this LC-MS experiment was done thrice for each (R)-Me-lyso-PS, with reproducible result each time. (B) The treatment of WT PPM with commercially

available lyso-PSs **1a** (C16:0) and **1b** (C18:0) and their synthetic (*R*)-Me-lyso-PS counterparts (*R*)-**2d** (C16:0), and (*R*)-**2e** (C18:0) respectively (all at 1 μ M, 4 h, 37 $^{\circ}$ C), produce comparable secretion of TNF- α and IL-6, suggesting that canonical lyso-PS and (*R*)-Me-lyso-PS of the same chain length function similarly in producing pro-inflammatory cytokines from PPM. (C) Relative to the (*R*)-Me-lyso-PS, treatment of WT PPM with either the corresponding (*S*)-Me-lyso-PSs or free fatty acid (FFA) (all at 1 μ M, 4 h, 37 $^{\circ}$ C) fail to elicit secretion of TNF- α or IL-6. In this assay, LPS was used as a positive control. (D) Relative to the (*R*)-Me-lyso-PS ((*R*)-**2e** (C18:0)), only the corresponding canonical lyso-PS (**1b**, C18:0), but not the other lysophospholipids (lyso-PA, lyso-PG or lyso-PC) of the same lipid tail (C18:0), elicit equivalent secretion of TNF- α or IL-6 in WT PPM. In this assay, LPS was used as a positive control. (E) The secretion of pro-inflammatory cytokines TNF- α and IL-6 from human THP-1 macrophages following treatment with different (*R*)-Me-lyso-PSs (all at 1 μ M, 4 h, 37 $^{\circ}$ C). The data shows that VLC (*R*)-Me-lyso-PSs ((*R*)-**2g** (C22:0) and (*R*)-**2h** (C24:0)) produced the highest secretion of TNF- α and IL-6 from human THP-1 macrophages, consistent with results seen from mouse WT PPM presented in **Figure 2B**. (F) Pharmacological antagonism of TLR2 by SpaB (10 μ M, 4 h, 37 $^{\circ}$ C) (Liang et al., 2011, Liang et al., 2013) in human THP-1 macrophages results in significantly decreased secretion of TNF- α and IL-6 from these cells following treatment with different VLC (*R*)-Me-lyso-PSs ((*R*)-**2f** (C20:0), or (*R*)-**2g** (C22:0), or (*R*)-**2h** (C24:0)) (all at 1 μ M, 4 h, 37 $^{\circ}$ C), suggesting that these VLC (*R*)-Me-lyso-PSs signal through TLR2 in THP1 macrophages. These results are similar to those observed reported for PPM in **Figures 3A**. All the data presented in (B-F) in this figure, represents mean \pm standard deviation from four independent experiments for all experimental groups.

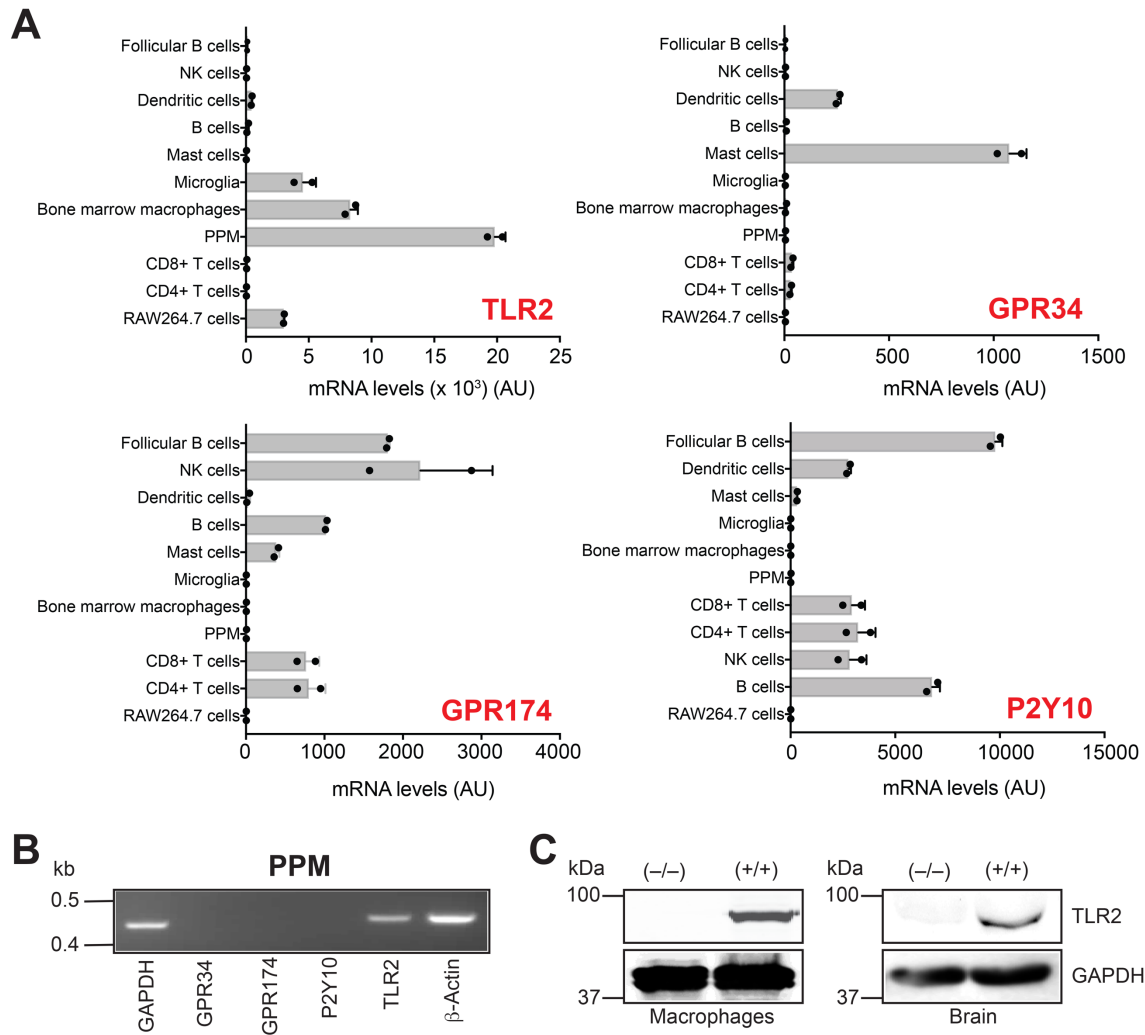


Figure S3. TLR2 is the only putative lyso-PS receptor expressed in macrophages, Related to Figure 3. (A) The mRNA expression profiles obtained from the large-scale gene expression database BioGPS (Wu et al., 2016), in different mouse immune cells, for the different putative lyso-PS receptors (TLR2, GPR34, GPR174 and P2Y10) postulated in literature (van der Kleij et al., 2002, Inoue et al., 2012), showing that TLR2 is the only receptor amongst these to have robust expression in all types of macrophages (PPM, bone marrow derived macrophages, microglia, and RAW264.7 cells). (B) RT-PCR analysis confirms the gene expression profile reported in (A), that TLR2, but not GPR34, GPR174 and P2Y10, is present in WT PPM. In this experiment, GAPDH and β -actin were used as controls. This RT-PCR experiment was done twice with reproducible results each time. (C) Representative western blot images showing the loss of TLR2 in PPM (*left*) and brain (*right*) lysates obtained from TLR2 knockout mice. In this experiment, GAPDH was used as a loading control. The western blot experiment was done twice with reproducible results each time.

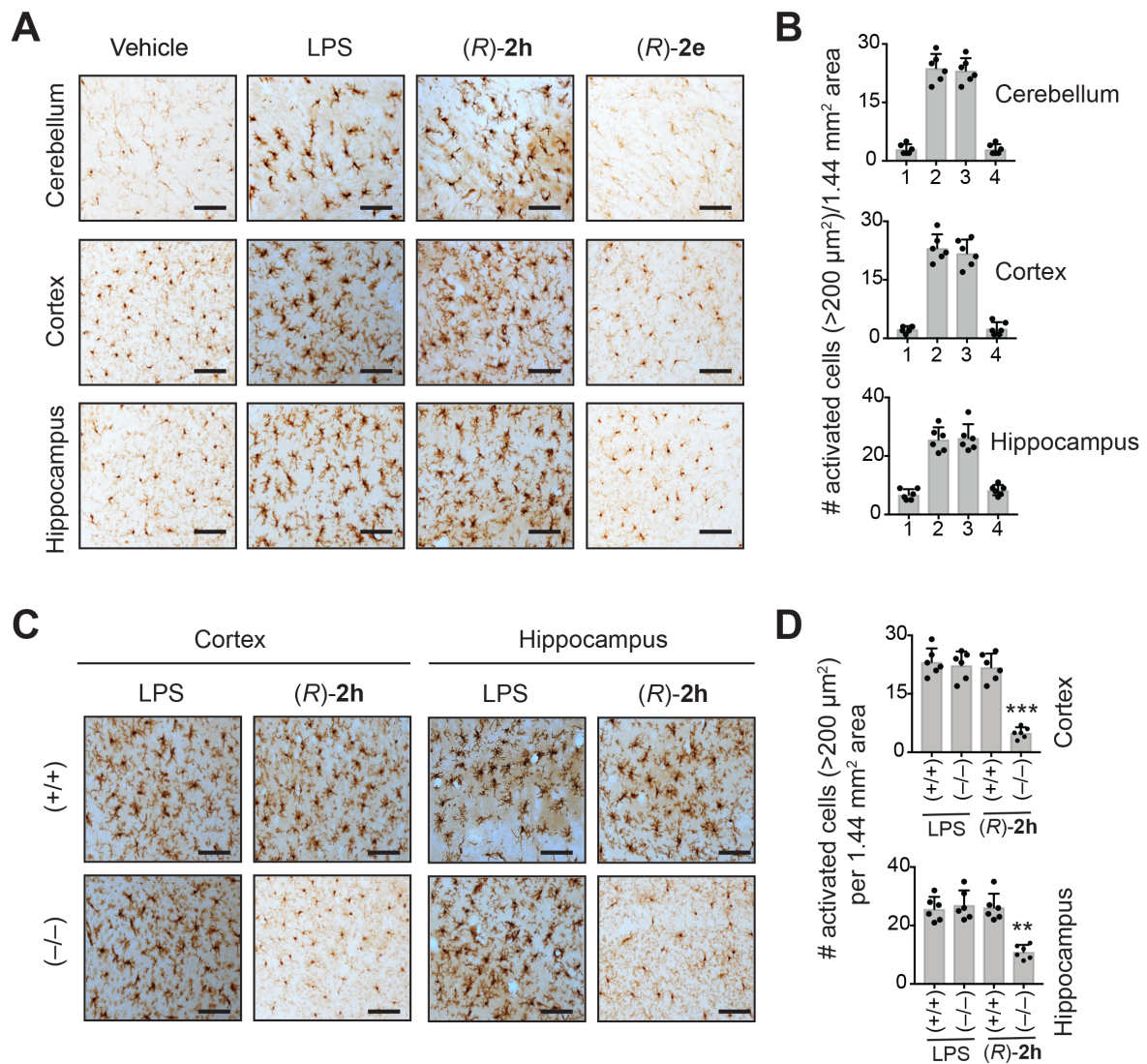


Figure S4. Analysis of activated microglia in the mouse brain following injection of a VLC (R)-Me-lyso-PS, Related to Figure 3. (A) Representative images from Iba-1 immunostaining for microglial activation (black bar = 250 μm) and (B) Quantification of enlarged cells (>200 μm^2) in the different brain regions (cerebellum, cortex and hippocampus) (per 1.44 mm^2) of wild type mice following intravenous injection of vehicle (PBS), LPS or (R)-2h (C24:0) or (R)-2e (C18:0) (all 1 mg/kg body weight, 10 h). The treatment groups are as follows: 1 = vehicle, 2 = LPS, 3 = (R)-2h (C24:0), and 4 = (R)-2e (C18:0) (C) Representative images from Iba-1 immunostaining for microglial activation (black bar = 250 μm) and quantification of enlarged cells (>200 μm^2) in the cortex and hippocampus (per 1.44 mm^2) of wild type (+/+) or TLR2 knockout (-/-) mice following intravenous injection of LPS or (R)-2h (C24:0) (both at 1 mg/kg body weight, 10 h), showing diminished activated microglia in TLR2 knockout mice. All data in (B) and (D) is represented as mean \pm standard deviation (n = 5/group). **p < 0.01, and ***p < 0.001 versus (+/+) group by Student's t-test.

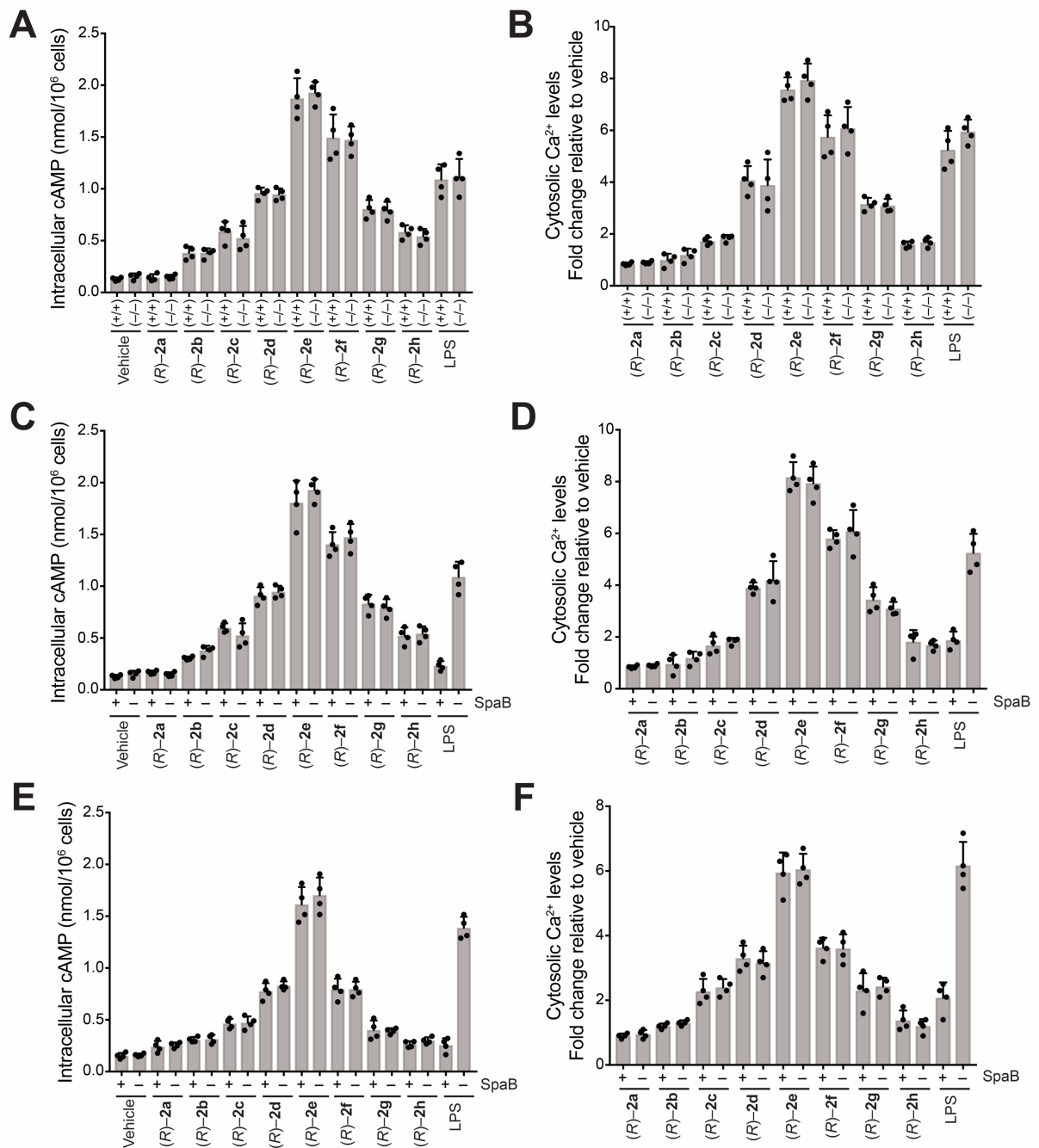


Figure S5. The intracellular cyclic AMP (cAMP) and relative cytosolic Ca²⁺ concentrations in mammalian macrophages following different (R)-Me-lyso-PS treatments, Related to Figure 4. (A, B) The concentrations of (A) intracellular cAMP and (B) cytosolic Ca²⁺ from PPM obtained from wild type (+/+) or TLR2 knockout (-/-) mice, following treatment with different (R)-Me-lyso-PSs (all at 1 μ M, 10 mins, 37 $^{\circ}$ C). The data shows in agreement with results in **Figures 4A** and **4B**, that LC (R)-Me-lyso-PSs (particularly (R)-2e (C18:0)) produced the highest increase for both phenotypes in (+/+) and (-/-) PPM. (C, D) The concentrations of (C) intracellular cAMP and (D) cytosolic Ca²⁺ from SpaB treated (10 μ M, 4 h, 37 $^{\circ}$ C) wild type PPM, following treatment with different (R)-Me-lyso-PSs (all at 1 μ M, 10 mins, 37 $^{\circ}$ C). The data shows in agreement with results in **Figures**

4A and **4B**, that LC (*R*)-Me-lyso-PSs (particularly (*R*)-**2e** (C18:0)) produced the highest increase for both phenotypes, and that SpaB treatment had no effect on either phenotype. (**E, F**) The concentrations of (**E**) intracellular cAMP and (**F**) cytosolic Ca²⁺ from SpaB treated (10 μM, 4 h, 37 °C) human THP-1 macrophages, following treatment with different (*R*)-Me-lyso-PSs (all at 1 μM, 10 mins, 37 °C). The data shows in agreement with results in **Figures 4A** and **4B**, that LC (*R*)-Me-lyso-PSs (particularly (*R*)-**2e** (C18:0)) produced the highest increase for both phenotypes, and that SpaB treatment had no effect on either phenotype. (**C-F**) Note: since SpaB is a dual TLR2-TLR4 antagonist (Liang et al., 2013, Liang et al., 2011), we see a decrease in the LPS (positive control in these assays) treated groups for both phenotypes. The data represented in this figure, together suggests that there exists a TLR2 independent signalling pathway that prefers long chain lyso-PS lipids (particularly C18:0 lyso-PS) as ligands in PPM. All the data presented in this figure represents mean ± standard deviation from four independent experiments for all experimental groups.

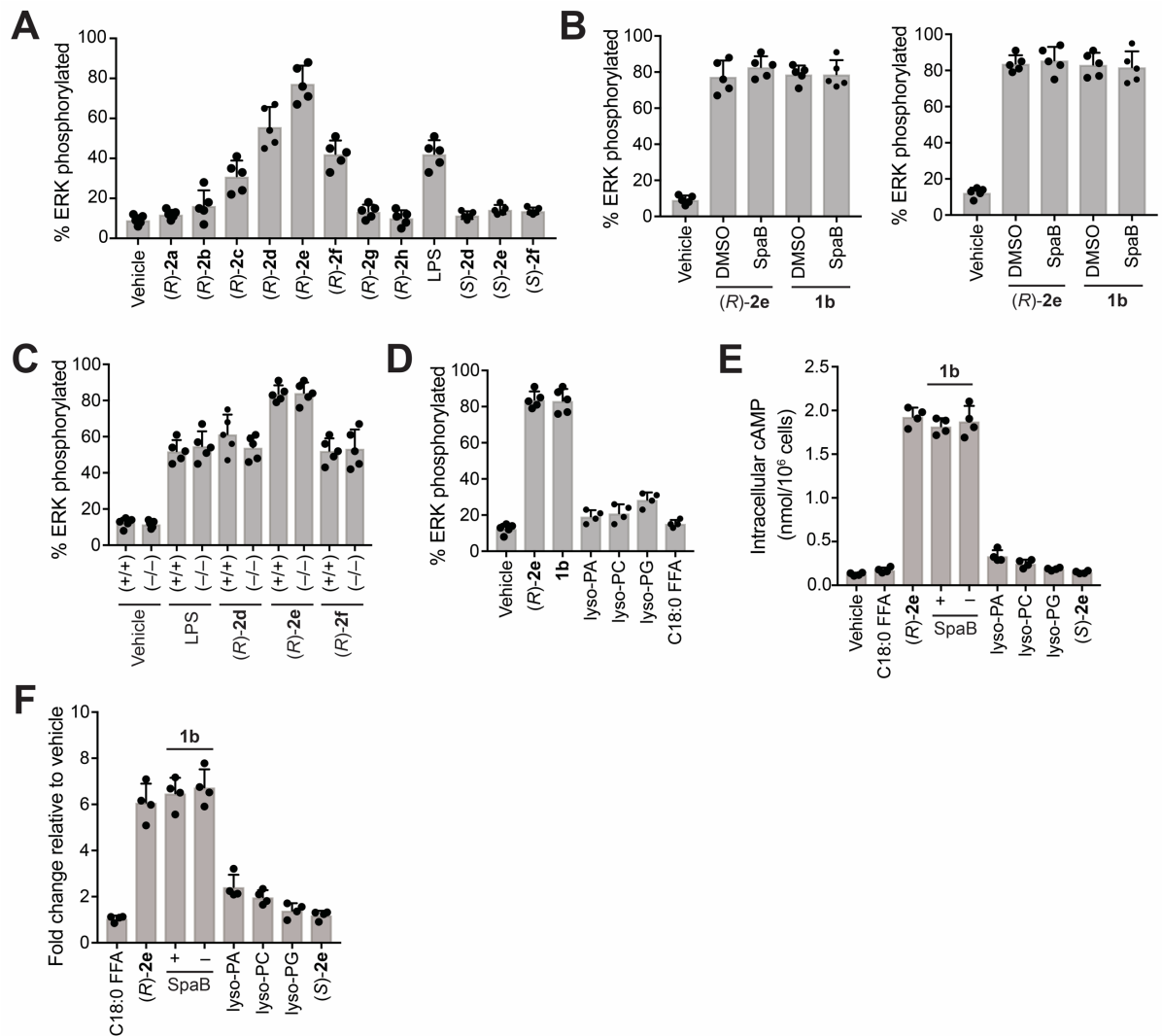


Figure S6. The phosphorylation of ERK following different (R)-Me-lyso-PS treatments, and effect of other lysophospholipid treatments on various phenotypes in mammalian macrophages, Related to Figure 4. (A) The percentage of ERK phosphorylated in human THP-1 macrophages following treatment with vehicle (DMSO) or LPS or different (R)-Me-lyso-PSs or different LC (S)-Me-lyso-PSs (all at 1 μ M, 10 mins, 37 $^{\circ}$ C). Consistent with results from **Figure 4C**, the LC (R)-Me-lyso-PSs, especially (R)-2e (C18:0), induces highest ERK phosphorylation in human THP-1 macrophages. (B) The antagonism of TLR2 by SpaB pre-treatment (10 μ M, 4 h, 37 $^{\circ}$ C) in WT PPM (*left*) or human THP-1 macrophages (*right*), followed by (R)-2e (C18:0) or 1b (C18:0 lyso-PS) treatment (1 μ M, 10 mins, 37 $^{\circ}$ C) does not change the ERK phosphorylation profile in either type of macrophages following (R)-2e or 1b treatment. Of note, (R)-2e and 1b behave similarly in this assay in both types of macrophages, suggesting that canonical lyso-PS and the corresponding (R)-Me-lyso-PS of the same chain length function similarly in this assay. (C) The percentage of ERK phosphorylated in PPM from wild type (+/+) or TLR2 (-/-) knockout mice, following treatment with vehicle (DMSO) or LPS or LC (R)-Me-lyso-PSs (R)-2d-f (all at 1 μ M, 10 mins, 37 $^{\circ}$ C), showing no significant changes in ERK phosphorylation following long chain (R)-Me-lyso-PS treatment between the two genotypes. The data represented (B) and (C), together

suggests that there exists a TLR2 independent signalling pathway that prefers LC lyso-PS lipids (particularly C18:0 lyso-PS) as ligands in mammalian macrophages that promotes phosphorylation of ERK. (**D, E, F**) Treatment of WT PPM with different lysophospholipids (all at 1 μ M, 10 mins, 37 °C) having the same lipid tail (C18:0), shows that the (*R*)-Me-lyso-PS, (*R*)-**2e**, and the canonical lyso-PS, **1b**, produce the highest (**D**) phosphorylation of ERK, (**E**) increased intracellular cAMP or (**F**) increased cytosolic Ca²⁺ flux, compared to the other lysophospholipids (lyso-PA, lyso-PG and lyso-PC) tested in these assays. In all these assays (**D, E, F**), (*R*)-**2e** and **1b** behave identically, and in (**E, F**), SpaB treatment has no effects on **1b** profiles, similarly to that seen in (**B**). All the data presented in this figure represents mean \pm standard deviation from four or more independent experiments for all experimental groups.

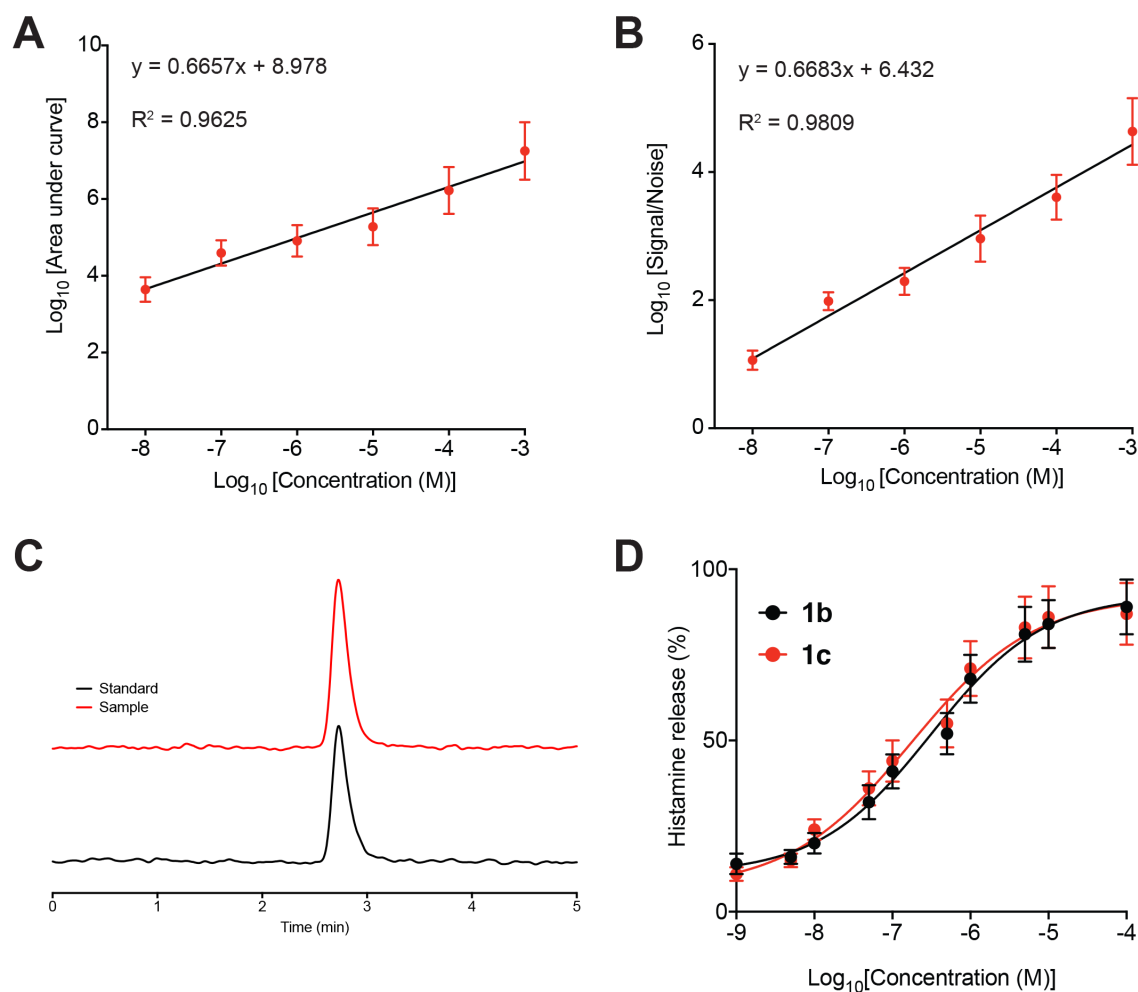
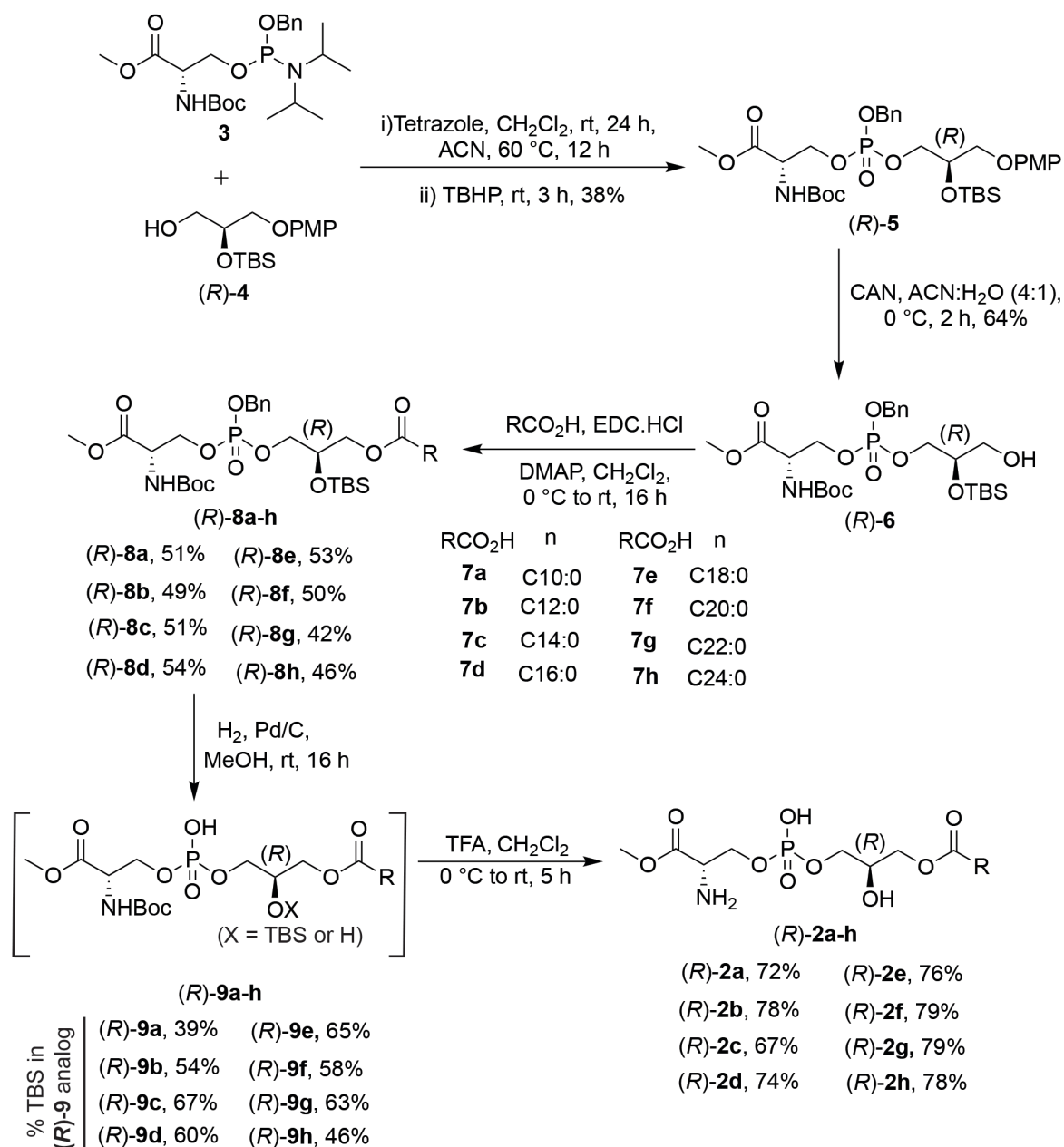
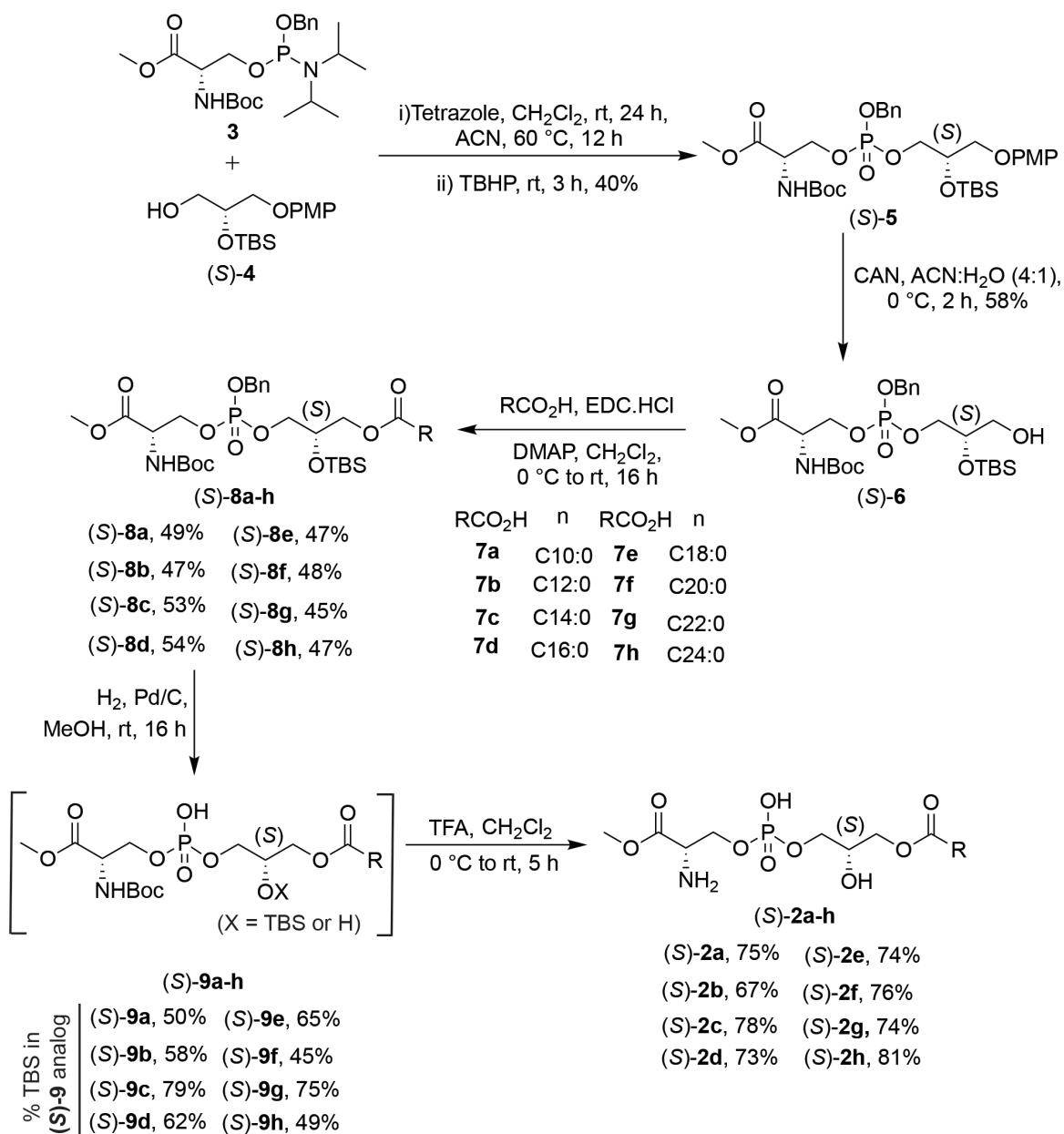


Figure S7. A LC-MS/MS method for measuring histamine release from mast cells (Chimalakonda et al., 2015), Related to Figure 5. Calibration curves using histamine standard showing the linearity of the histamine measurement > 5-orders of magnitude, based on **(A)** measuring the log of area under the curve to the standard concentration; and **(B)** measuring the log of the ratio of signal to noise as a function of the standard concentration. The data in **(A)** and **(B)** represent mean \pm standard deviation from four independent experiments. Based on these data, the limit of detection (LOD) and limit of quantification (LOQ) for histamine in this LC-MS assay are 1.2 nM and 7.5 nM respectively. **(C)** A representative LC-MS trace showing the co-elution of histamine standard (100 nM) (black trace) and experimental histamine extract (~ 100 nM) (red trace) in the LC-MS assay (Chimalakonda et al., 2015). **(D)** Comparing the histamine released from mast cells following treatment with authentic lyso-PSs **1b** (C18:0), and **1c** (C18:1), showing that increasing unsaturation does not seem to affect the mast cell degranulation process. Each data point represents mean \pm standard deviation from six independent experiments.

Data S1. General Synthetic Procedure: Compound Synthesis and Characterization, Related to Figure 1, and “Synthesis and compound characterization” section in the STAR Methods.



Scheme S1. Synthetic strategy for making (R)-Me-lyso-PSs. See **Supplementary Information** section “Chemical compound synthesis and characterization” for complete details.

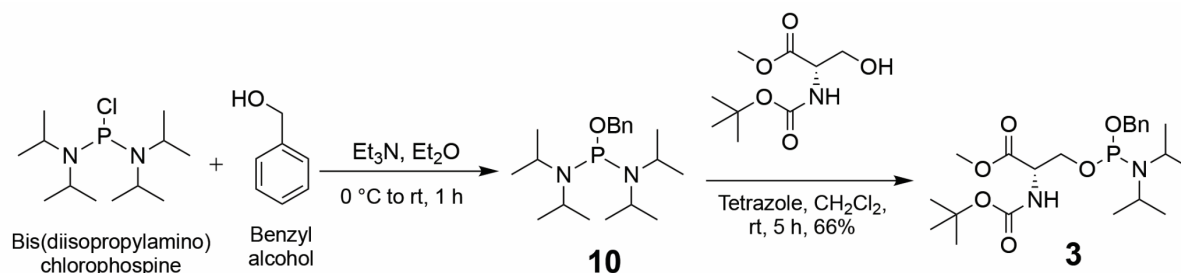


Scheme S2. Synthetic strategy for making (S)-Me-lyso-PSs. See **Supplementary Information** section “Chemical compound synthesis and characterization” for complete details.

General synthetic procedure:

All the chemicals were purchased from Sigma-Aldrich unless otherwise mentioned and used as received. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 pre-coated plates (0.25 mm thickness, Merck), and visualized by irradiation with a UV lamp and/or staining with phosphomolybdic acid, ninhydrin or potassium permanganate (KMnO₄) solutions. Column chromatography was performed on Rankem silica gel (60–120 mesh) and (100-200 mesh). ¹H, ¹³C, ³¹P and DEPT-135 spectra were recorded on JEOL 400 MHz or Bruker 400 MHz (or 100 MHz for ¹³C) NMR spectrometers. The internal standards for the recorded NMR spectra were either residual solvent signals (CHCl₃, δ_H = 7.26 ppm, δ_C = 77.2 ppm and CD₃OD, δ_H = 3.31, δ_C = 49.0) or an internal standard tetramethylsilane (δ_H = 0.00 ppm, δ_C = 0.00 ppm). Chemical shifts (δ) are reported in p.p.m. and coupling constants (*J*) in Hz, multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sext (sextet), m (multiplet), dd (doublet of doublet); broad multiplet are reported without abbreviation. High-resolution mass spectra (HRMS) were obtained from HRMS-ESI-QuadrupoleTime of Flight (QTOF) LC/MS (Sciex). Preparative High-Performance Liquid Chromatography (Prep-HPLC) was performed using a Combi flash EZ Prep UV using a Kromasil[®]-C-18 preparative column (250 mm × 21.2 mm, 10 μ m). Analytical HPLC was performed on an Agilent Technologies 1260 infinity equipped with a UV detector (λ = 214 and 254 nm) with an Eclipse plus C-18 reversed phase column (250 mm × 4.6 mm, 5 μ m).

Synthesis of methyl O-((benzyloxy)(diisopropylamino)phosphaneyl)-N-(tert-butoxycarbonyl)-L-serinate (Compound 3): The compound **10** described here was synthesized using a previously reported procedure, and the analytical data collected by us is consistent with literature reported values(Iwashita et al., 2009). The compound **3** described here was synthesized using a previously published methodology and the analytical data for compound **3** collected by us is comparable to a similar reported compound(Iwashita et al., 2009).



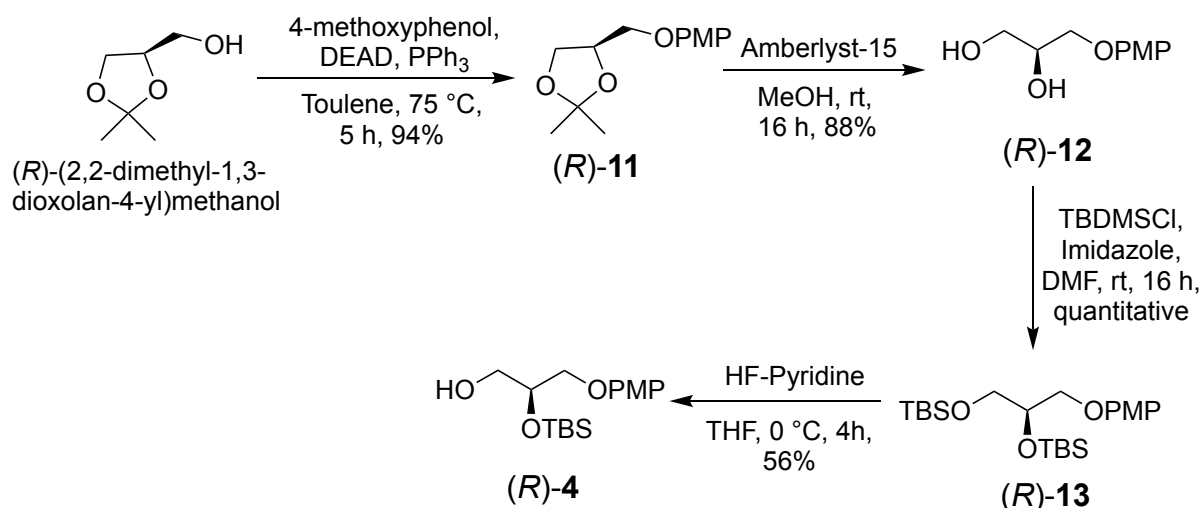
1-(benzyloxy)-N,N,N',N'-tetraisopropylphosphanediamine (Compound 10): To a solution of bis(diisopropylamino)chlorophosphine (3.0 g, 11.2 mmol) in dry diethyl ether (Et₂O) (30

mL) in Schlenk flask, a mixture of benzyl alcohol (1.0 mL, 10.0 mmol) and triethylamine (Et₃N) (1.4 mL, 10.0 mmol) in Et₂O (5 mL) was added at 0 °C under nitrogen (N₂) atmosphere. The reaction mixture was stirred for 30 min at 0 °C, then warmed to room temperature during 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 nitrogen atmosphere to yield compound **10**. The crude product was used as such for the next step without purification: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ 124.21.

Compound 3: The crude compound **10** (3.2 g, 94.5 mmol) was dissolved in anhydrous dichloromethane (CH₂Cl₂) (25 mL) in Schlenk flask and a solution of 1*H*-tetrazole (0.8 mL, 85.9 mmol) in 0.45 M acetonitrile (ACN) was added at room temperature. To this solution, *N*-Boc-*L*-Serine-methyl ester (1.8 g, 85.9 mmol) was added under N₂ atmosphere, and in a few minutes, a white solid precipitated. The mixture was stirred for 5 h at room temperature and the residual solvent was evaporated under reduced pressure. The residue was purified by neutral alumina column chromatography using 10% EtOAc in *n*-hexane to yield compound **3** (2.8 g, 61.3 mmol, 66% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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), 3.72 (2s, 3H), 3.67-3.54 (m, 2H), 1.45-1.43 (m, 9H), 1.22-1.13 (m, 12H); ³¹P NMR (400 MHz, CDCl₃) δ 149.68, 149.15; ¹³C NMR (100 MHz, CDCl₃) δ 171.0, 155.5 (2s), 139.3 (2d, *J*_{c-p} = 8 Hz), 128.4 (d, *J*_{c-p} = 5 Hz), 127.5 (d, *J*_{c-p} = 7 Hz), 127.1 (d, *J*_{c-p} = 13 Hz), 79.9 (2s), 65.5 (dd, *J*_{c-p} = 18, 9 Hz), 64.3 (2d, *J*_{c-p} = 16 Hz), 54.9 (t, *J*_{c-p} = 8 Hz), 52.5 (2s), 43.2 (d, *J*_{c-p} = 12 Hz), 28.4, 24.7, 24.6, 24.5; DEPT-135 NMR (100 MHz, CDCl₃) δ 128.4 (d, *J*_{c-p} = 4 Hz, CH), 127.4 (d, *J*_{c-p} = 8 Hz, CH), 127.1 (d, *J*_{c-p} = 12 Hz, CH), 65.5 (dd, *J*_{c-p} = 18, 8 Hz, CH₂), 64.2 (2d, *J*_{c-p} = 16 Hz, CH₂), 54.9 (t, *J*_{c-p} = 8 Hz, CH), 52.4 (2s, CH₃), 43.2 (d, *J*_{c-p} = 12 Hz, CH), 28.4 (CH₃), 24.7 (CH₃), 24.6 (CH₃), 24.5 (CH₃); HRMS-ESI: [(M + H)⁺-Boc] calcd for C₁₇H₃₀N₂O₄P, 357.1938; found, 357.1939.

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

(Compound (R)-4): The compounds (*R*)-**11**, (*R*)-**12**, (*R*)-**13** and (*R*)-**4** described in this section were synthesized using a previously reported procedure, and the analytical data collected for this compound by us is consistent with literature reported values (Iwashita et al., 2009).



(R)-4-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolane (Compound (R)-11): The commercially available (R)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (5.0 g, 37.83 mmol) was dissolved in anhydrous toluene, following which triphenylphosphine (PPh₃) (11.91 g, 45.40 mmol), and *p*-methoxyphenol (14.09 g, 113.49 mmol) were added under a N₂ atmosphere. To this solution, diethyl azodicarboxylate (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. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel (60-120 mesh) column chromatography using 10% EtOAc in *n*-hexane as an eluent to yield the desired product (R)-11 (8.5 g, 35.6 mmol, 94% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 6.86-6.80 (m, 4H), 4.45 (quint, *J* = 6.0 Hz, 1H), 4.14 (tt, *J* = 7.4, 1.2 Hz, 1H), 4.02 (ddt, *J* = 9.5, 5.4, 1 Hz, 1H), 3.89-3.85 (m, 2H), 3.75 (m, 3H), 1.46 (s, 3H), 1.39 (s, 3H); HRMS-ESI: [M + H]⁺ calcd for C₁₃H₁₉O₄, 239.1278; found, 239.1280.

(S)-3-(4-methoxyphenoxy)propane-1,2-diol (Compound (R)-12): The compound (R)-11 (8.5 g, 35.6 mmol) was dissolved in anhydrous methanol (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, the Amberlyst-15 was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel (60-120 mesh) column chromatography using 80% EtOAc in *n*-hexane as an eluent to give the desired product (R)-12 (6.2 g, 31.3 mmol, 88% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 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 C₁₀H₁₅O₄, 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 (Compound (R)-13): The compound (R)-12 (6.2 g, 31.3 mmol) was dissolved in anhydrous dimethylformamide (DMF) (50 mL) following which imidazole (6.4 g, 94 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl) (11.8 g, 78.2 mmol) were sequentially added at room temperature. The mixture was stirred for 16 h at room temperature after which it was diluted with water (30 mL) and Et₂O (30 mL), and the aqueous layer was separated and extracted three times with Et₂O (15 mL x 3). The combined organic layer was dried over Na₂SO₄ and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel (60-120 mesh) column chromatography using 5% Et₂O in *n*-hexane as an eluent to obtain the desired product (R)-13 (13.3 g, 31.1 mmol, quantitative) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 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), 0.13 (2s, 6 H), 0.09 (s, 6H); HRMS-ESI: [M + H]⁺ calcd for C₂₂H₄₃O₄Si₂, 427.2694; found 427.2693.

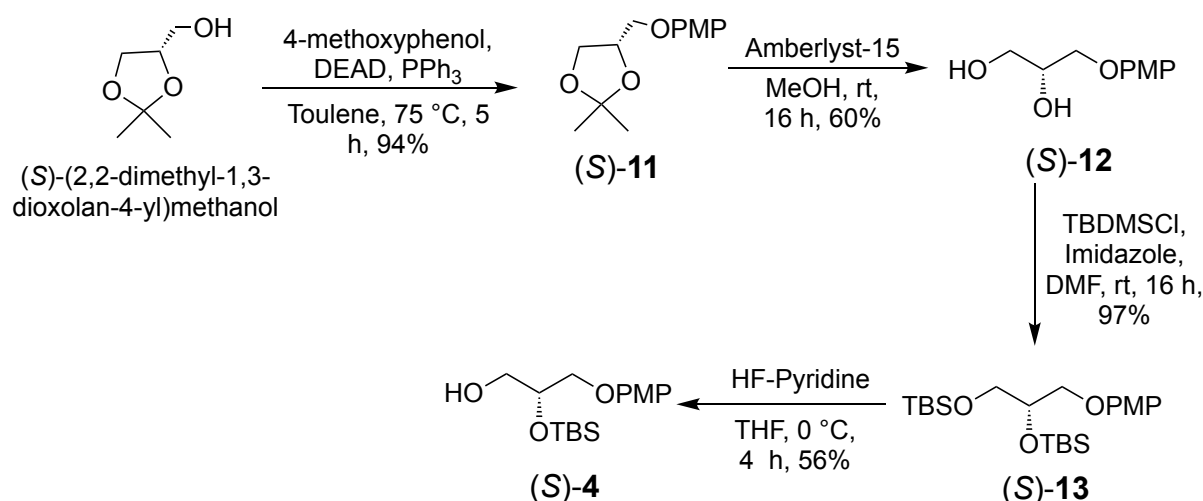
Compound (R)-4: The compound (R)-13 (13.3 g, 31.1 mmol) was dissolved in dry tetrahydrofuran (THF) (70 mL), following which the HF-pyridine complex (70%w/w) (5.3 mL) and pyridine (25 mL) were sequentially added at room temperature under N₂ atmosphere. The reaction mixture was stirred for 4 h at 0°C and 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 Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resultant residue was purified by silica gel (60-120 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to yield the desired product (R)-4 (5.2 g, 16.6 mmol, 56% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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 C₁₆H₂₉O₄Si, 313.1830; found, 313.1837.

Synthesis of (R)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propan-1-ol

(Compound (S)-4): The compounds (S)-11, (S)-12, (S)-13 and (S)-4 described in this section were synthesized using a previously reported procedure, and the analytical data collected for this compound by us is consistent with literature reported values(Ikubo et al., 2015).

(S)-4-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolane (Compound (S)-11): The commercially available (S)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (5.0 g, 37.83 mmol) was dissolved in anhydrous toluene, following which PPh₃ (11.91 g, 45.40 mmol), and *p*-methoxyphenol (14.09 g, 113.49 mmol) were added under N₂ 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 completing the reaction, the mixture was

concentrated under reduced pressure, and the residue was purified by silica gel (60-120 mesh) 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) as a colourless oil: ^1H NMR (400 MHz, CDCl_3) δ 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: $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{19}\text{O}_4$, 239.1278; found, 239.1279.



(R)-3-(4-methoxyphenoxy)propane-1,2-diol (Compound (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 filtrate was concentrated under reduced pressure. The residue was purified by silica gel (60-120 mesh) column chromatography using 80% EtOAc in *n*-hexane as an eluent to give the desired product (S)-**12** (4.19 g, 21.2 mmol, 60% yield) as a white solid: ^1H NMR (400 MHz, CDCl_3) δ 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: $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{15}\text{O}_4$, 199.0965; found, 199.0966.

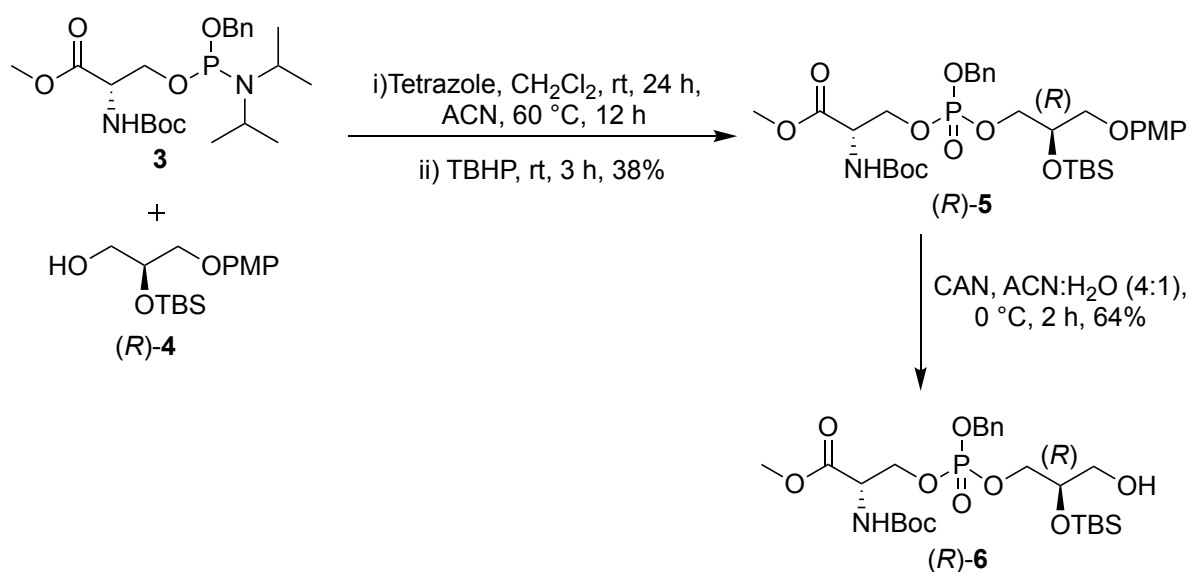
(S)-5-((4-methoxyphenoxy)methyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-

disiladecane (Compound (S)-13): The compound (S)-**12** (4.1 g, 20.7 mmol) was dissolved in anhydrous DMF (33 mL), following which imidazole (4.23 g, 62.1 mmol) and TBDMSCl (7.8 g, 51.8 mmol) was sequentially added at room temperature. The mixture was stirred for 16 h at room temperature after which it 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 \times 3). The combined organic layer was dried over Na_2SO_4 , filtered and the filtrate was concentrated

under reduced pressure. The resultant residue was purified by silica gel (60-120 mesh) column chromatography using 5% Et₂O in *n*-hexane as an eluent to obtain the desired product (*S*)-**13** (8.55 g, 20.1 mmol, 97% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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 (2s, 6H), 0.08 (s, 6H); HRMS-ESI: [M + H]⁺ calcd for C₂₂H₄₃O₄Si₂, 427.2694; found 427.2692.

Compound (*S*)-4: The compound (*S*)-**13** (8.5 g, 19.9 mmol) was dissolved in dry THF (45 mL), following which the HF-pyridine complex (70%w/w) (3.5 mL) and pyridine (16 mL) were sequentially added at room temperature under N₂ atmosphere. The reaction mixture was stirred for 4 h at 0 °C and then diluted with water (30 mL). The desired product was extracted three times with EtOAc (30 mL x 3). The combined organic layer was washed with brine, dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resultant residue was purified by silica gel (60-120 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (*S*)-**4** (3.32 g, 10.7 mmol, 56% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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 C₁₆H₂₉O₄Si, 313.1830; found, 313.1832.

Synthesis of Methyl *O*-((benzyloxy)((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (Compound (*R*)-5): The compound (*R*)-**5** described in this section were synthesized using a previously published methodology with minor modifications, and the analytical data for (*R*)-**5** collected by us is comparable to similar compounds reported in literature(Ikubo et al., 2015).



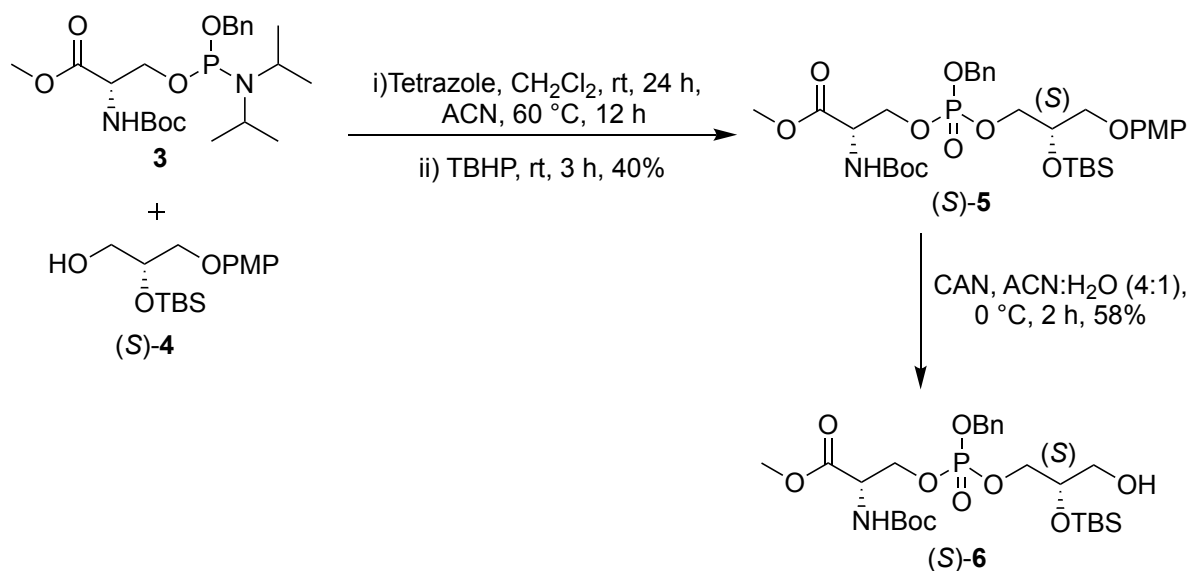
The compound **3** (2.2 g, 4.82 mmol) was dissolved in anhydrous CH₂Cl₂ (2.5 mL), and this solution was co-evaporated with ACN three times (3 x 2.5 mL). The residue was dissolved in anhydrous CH₂Cl₂ (25 mL), to which a solution of 1*H*-tetrazole in ACN (~0.45 M) (1.1 mL, 1.2 mmol) was added at room temperature. To this, compound (*R*)-**4** (3.7 g, 11.8 mmol) in CH₂Cl₂ (5 mL) was added dropwise under N₂ atmosphere, and the resulting mixture was stirred at room temperature for 24 h. To this, anhydrous ACN (30 mL) was added and the resulting mixture was heated to 60 °C for 12 h. The reaction mixture was cooled to room temperature and the intermediate formation was confirmed by TLC, following which a solution of *t*-butyl hydroperoxide (TBHP) 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 another 3 h. The reaction mixture was diluted with 15 mL water and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layer was washed with brine solution, dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography using 85% MeOH in H₂O as an eluent to afford compound (*R*)-**5** (1.25 g, 1.83 mmol, 38% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -0.41; ¹³C NMR (100 MHz, CDCl₃) δ 169.8, 155.4, 154.2, 152.8, 135.7 (d, *J*_{C-P} = 7 Hz), 128.9, 128.8, 128.1 (d, *J*_{C-P} = 4 Hz), 115.6 (2s), 114.8, 80.5, 70.0 (d, *J*_{C-P} = 7 Hz), 69.8 (t, *J*_{C-P} = 6 Hz), 69.6 (d, *J*_{C-P} = 6 Hz), 69.0 (t, *J*_{C-P} = 7 Hz), 67.7 (broad), 55.9, 54.1 (d, *J*_{C-P} = 8 Hz), 52.9, 28.4, 25.9, 18.3, -4.5, -4.6; DEPT-135 NMR (100 MHz, CDCl₃) δ 128.7 (CH), 128.6 (CH), 127.9 (d, *J*_{C-P} = 4 Hz, CH), 115.3 (2s, CH), 114.6 (CH), 69.8 (d, *J*_{C-P} = 9 Hz, CH), 69.6 (t, *J*_{C-P} = 5 Hz, CH₂), 69.4 (d, *J*_{C-P} = 5 Hz, CH₂), 68.8 (t, *J*_{C-P} = 7 Hz, CH₂), 67.5 (broad, CH₂), 55.7 (CH₃), 53.9 (d, *J*_{C-P} = 7 Hz, CH), 52.7 (CH₃), 28.2 (CH₃), 25.7 (CH₃), -4.8 (CH₃), -4.9 (CH₃); HRMS-ESI: [(M + H)⁺-Boc] calcd. for C₂₇H₄₃NO₉PSi, 584.2439; found, 584.2441.

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

The compound (*R*)-**6** described in this section were synthesized using a previously published methodology, and the analytical data for (*R*)-**6** collected by us is comparable to similar compounds reported in literature (Ikubo et al., 2015). To a solution of (*R*)-**5** (1.2 g, 1.76 mmol) in ACN: H₂O (4:1) (10 mL), ceric ammonium nitrate (CAN) (2.41 g, 4.4 mmol) was added dropwise at 0 °C under N₂ atmosphere. The reaction mixture was stirred for 2 h at 0 °C and then diluted with water (5 mL). The whole mixture was extracted three times with EtOAc (3 x 20 mL), and the combined organic layer was washed with brine solution, dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The crude

product was purified by silica gel (100-200 mesh) column chromatography using 60% EtOAc in *n*-hexane as an eluent to afford the desired product (*R*)-**6** (0.648 g, 1.12 mmol, 64% yield) as a brown oil: ^1H NMR (400 MHz, CDCl_3) δ 7.36-7.29 (m, 5H), 5.64-5.45 (m, 1H), 5.06-5.00 (m, 2H), 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) δ -0.70, -0.85; ^{13}C NMR (100 MHz, CDCl_3) δ 169.8, 155.3 (2s), 135.5 (d, $J_{\text{C-P}} = 6\text{ Hz}$), 128.8, 128.7, 128.1, 80.4, 71.2 (d, $J_{\text{C-P}} = 8\text{ Hz}$), 69.8 (2d, $J_{\text{C-P}} = 5\text{ Hz}$), 67.9 (t, $J_{\text{C-P}} = 5\text{ Hz}$), 67.7 (2d, $J_{\text{C-P}} = 5\text{ Hz}$), 62.9, 54.0 (d, $J_{\text{C-P}} = 7\text{ Hz}$), 52.8 (2s), 28.3, 25.8, 18.1, -4.7, -4.8; DEPT-135 NMR (100 MHz, CDCl_3) δ 128.8 (CH), 128.7 (CH), 128.0 (CH), 71.1 (d, $J_{\text{C-P}} = 7\text{ Hz}$, CH), 69.7 (2d, $J_{\text{C-P}} = 6\text{ Hz}$, CH_2), 67.9 (t, $J_{\text{C-P}} = 5\text{ Hz}$, CH_2), 67.6 (2d, $J_{\text{C-P}} = 6\text{ Hz}$, CH_2), 62.9 (CH_2), 53.9 (d, $J_{\text{C-P}} = 8\text{ Hz}$, CH), 52.7 (2s, CH_3), 28.3 (CH_3), 25.7 (CH_3), -4.8 (CH_3), -4.9 (CH_3); HRMS-ESI: $[(\text{M} + \text{H})^+ - \text{Boc}]$ calcd. for $\text{C}_{20}\text{H}_{37}\text{NO}_8\text{PSi}$, 478.2021; found, 478.2023.

Synthesis of Methyl *O*-((benzyloxy)((*S*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(4-methoxyphenoxy)propoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (Compound (*S*)-5**):** The compound (*S*)-**5** described in this section were synthesized using a previously published methodology with minor modifications, and the analytical data for (*S*)-**5** collected by us is comparable to similar compounds reported in literature(Ikubo et al., 2015).



The compound **3** (1.0 g, 2.19 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mL), and this solution was co-evaporated with ACN three times (3 x 1.5 mL). The residue was dissolved in anhydrous CH_2Cl_2 (12 mL), to which a solution of 1*H*-tetrazole in ACN (~0.45 M) (0.5 mL, 0.55 mmol) was added at room temperature. To this, compound (*S*)-**4** (1.68 g, 5.36 mmol) in CH_2Cl_2 (2.3 mL) was added dropwise under N_2 atmosphere, and the resulting mixture stirred

at room temperature for 24 h. To this, anhydrous ACN (14 mL) was added and the resulting reaction mixture was heated to 60 °C. The reaction mixture was stirred for 12 h at this temperature. The reaction mixture was cooled to room temperature and the intermediate formation was confirmed by TLC, following which a solution of TBHP in decane (5.0-6.0 M) (0.64 mL, 6.57 mmol) was added dropwise and the reaction mixture was stirred for another 3 h at room temperature. The reaction mixture was diluted with 7.5 mL water and extracted with CH₂Cl₂ (3 X 15 mL). The combined organic layer was washed with brine solution, dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography using 95% MeOH in H₂O as an eluent to afford the compound (S)-**5** (0.6 g, 1.46 mmol, 40%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -0.40; ¹³C NMR (100 MHz, CDCl₃) δ 169.7, 155.3, 154.1, 152.7, 135.6 (d, *J*_{C-P} = 6 Hz), 128.8, 128.7, 128.1 (d, *J*_{C-P} = 3 Hz), 115.4, 114.7, 80.4, 69.9 (d, *J*_{C-P} = 8 Hz), 69.7 (t, *J*_{C-P} = 6 Hz), 69.5 (broad), 68.9 (d, *J*_{C-P} = 6 Hz), 67.6 (broad), 55.8, 54.0 (d, *J*_{C-P} = 7 Hz), 52.8, 28.4, 25.8, 18.2, -4.6, -4.7; DEPT-135 NMR (100 MHz, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, *J*_{C-P} = 2 Hz, CH), 115.4 (CH), 114.7 (CH), 69.9 (d, *J*_{C-P} = 8 Hz, CH), 69.7 (t, *J*_{C-P} = 5 Hz, CH₂), 69.5 (broad, CH₂), 68.9 (d, *J*_{C-P} = 6 Hz, CH₂), 67.6 (broad, CH₂), 55.8 (CH₃), 54.0 (d, *J*_{C-P} = 7 Hz, CH), 52.8 (CH₃), 28.4 (CH₃), 25.8 (CH₃), -4.7 (CH₃), -4.8 (CH₃); HRMS-ESI: [(M + H) – Boc]⁺ calcd for C₂₇H₄₃NO₉PSi, 584.2445; found, 584.2443.

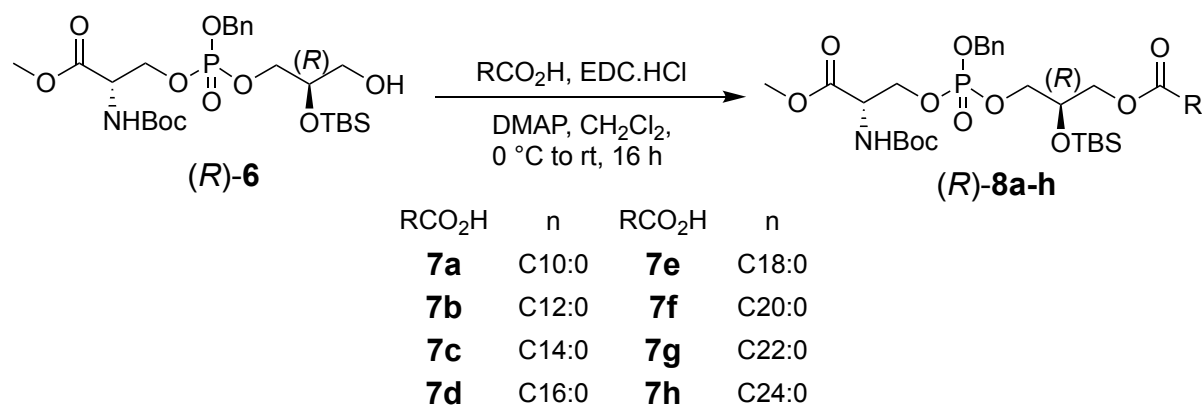
Synthesis of Methyl O-((benzyloxy)((S)-2-((tert-butyldimethylsilyl)oxy)-3-hydroxypropoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (Compound (S)-6):

The compound (S)-**6** described in this section were synthesized using a previously published methodology, and the analytical data for (S)-**6** collected by us is comparable to similar compounds reported in literature (Ikubo et al., 2015). To a solution of (S)-**5** (0.45 g, 0.658 mmol) in ACN: H₂O (4:1) (3.8 mL), CAN (0.9 g, 1.65 mmol) was added dropwise at 0 °C under N₂ atmosphere. The reaction mixture was stirred for 2 h at 0 °C and then diluted with H₂O (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 Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel (100-200 mesh) column chromatography using 60% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**6** (0.220 g, 0.381 mmol, 58% yield) as a brown oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -0.54, -

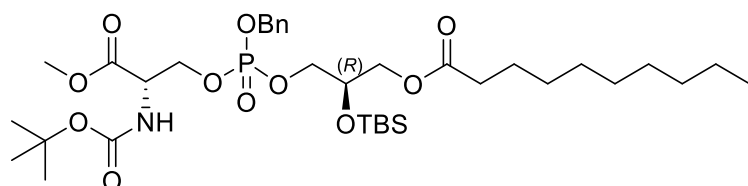
0.81; ^{13}C NMR (100 MHz, CDCl_3) δ 169.9, 155.4, 135.6 (2d, $J_{\text{C-P}} = 6$ Hz), 128.9 (d, $J_{\text{C-P}} = 2$ Hz), 128.8 (d, $J_{\text{C-P}} = 1$ Hz), 128.2 (d, $J_{\text{C-P}} = 5$ Hz), 80.5, 71.1 (2d, $J_{\text{C-P}} = 5$ Hz), 69.9 (2d, $J_{\text{C-P}} = 4$ Hz), 67.8 (2d, $J_{\text{C-P}} = 6$ Hz), 67.5 (2d, $J_{\text{C-P}} = 6$ Hz), 63.0, 54.0 (d, $J_{\text{C-P}} = 7$ Hz), 52.9 (2s), 28.4, 25.8, 18.2, -4.6, -4.8; DEPT-135 NMR (100 MHz, CDCl_3) δ 128.8 (d, $J_{\text{C-P}} = 2$ Hz, CH), 128.7 (broad, CH), 128.1 (d, $J_{\text{C-P}} = 5$ Hz, CH), 71.0 (2d, $J_{\text{C-P}} = 5$ Hz, CH), 69.8 (2d, $J_{\text{C-P}} = 5$ Hz, CH_2), 67.7 (2d, $J_{\text{C-P}} = 6$ Hz, CH_2), 67.4 (2d, $J_{\text{C-P}} = 5$ Hz, CH_2), 62.9 (CH_2), 53.9 (d, $J_{\text{C-P}} = 7$ 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: : $[(M + H) - \text{Boc}]^+$ calcd. for $\text{C}_{20}\text{H}_{37}\text{NO}_8\text{PSi}$, 478.2021; found, 478.2023.

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

The compounds (R)-8a-h described in this section were synthesized using a previously reported methodology in literature (Ikubo et al., 2015).



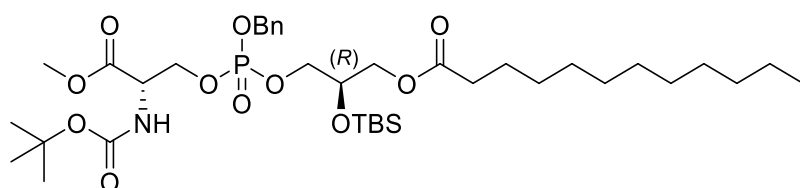
To a solution of the alcohol (R)-6 (1.0 equiv) and fatty acid **7** (0.9 equiv) in anhydrous CH_2Cl_2 , 4-dimethylaminopyridine (DMAP 0.25 equiv) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide hydrochloride ($\text{EDC} \cdot \text{HCl}$, 0.9 equiv) were sequentially added at 0 °C. After stirring 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 , filtered and the filtrate was concentrated under reduced pressure below 25 °C. The residue was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the corresponding desired product (R)-8.



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

(Compound (R)-8a): Following the general procedure (A1), (R)-6 (80 mg, 0.138 mmol), **7a**

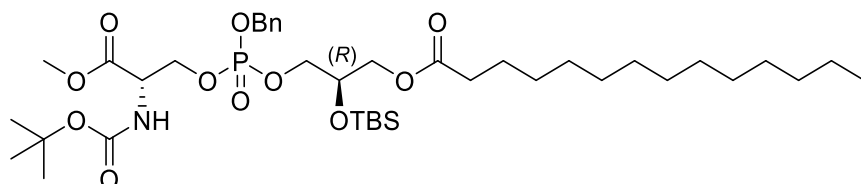
(Decanoic acid, C10:0) (21 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0346 mmol) and CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (*R*)-**8a** (52 mg, 0.0710 mmol, 51%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.08; ¹³C NMR (100 MHz, CDCl₃) δ 173.5, 169.8, 155.3, 135.8 (d, *J*_{c-p} = 6.0 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 2 Hz), 80.5, 69.9 (2d, *J*_{c-p} = 5 Hz), 69.3 (d, *J*_{c-p} = 8 Hz), 68.6 (t, *J*_{c-p} = 6 Hz), 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, CDCl₃) δ 128.7 (CH), 128.6 (CH), 127.9 (d, *J*_{c-p} = 2 Hz, CH), 69.7 (2d, *J*_{c-p} = 5 Hz, CH₂), 69.1 (d, *J*_{c-p} = 8 Hz, CH), 68.5 (t, *J*_{c-p} = 7 Hz, CH₂), 67.5 (m, CH₂), 64.7 (CH₂), 54.1 (m, CH), 52.6 (CH₃), 34.1 (CH₂), 31.8 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.8 (CH₂), 22.6 (CH₂), 14.0 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₃₅H₆₂NO₁₁PSi [M+K]⁺: calcd., 770.34; found, 770.35; [M+Na]⁺: calcd., 754.37; found, 754.38.



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

(Compound (*R*)-**8b**): 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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (*R*)-**8b** (45 mg, 0.0592 mmol, 49%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 136.0 (d, *J*_{c-p} = 7 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 2 Hz), 80.5, 69.8 (t, *J*_{c-p} = 5 Hz), 69.1 (d, *J*_{c-p} = 8 Hz), 68.5 (t, *J*_{c-p} = 6 Hz), 67.7 (m), 64.8, 54.0 (d, *J*_{c-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, -

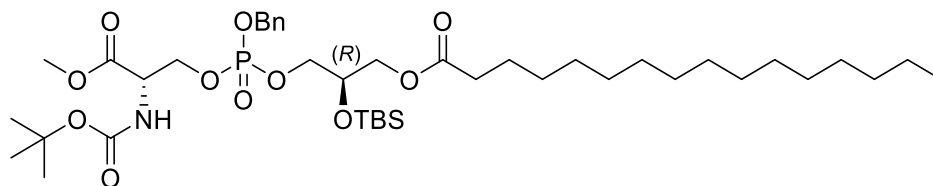
4.7, -4.8; DEPT-135 NMR (100 MHz, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{\text{C-P}}$ = 2 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH₂), 69.0 (d, $J_{\text{C-P}}$ = 8 Hz, CH), 68.4 (t, $J_{\text{C-P}}$ = 6 Hz, CH₂), 67.6 (m, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}}$ = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₃₇H₆₆NO₁₁PSi [M+K]⁺: calcd., 798.38, found., 798.35, [M+Na]⁺: calcd., 782.40; found, 782.38.



(2R)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

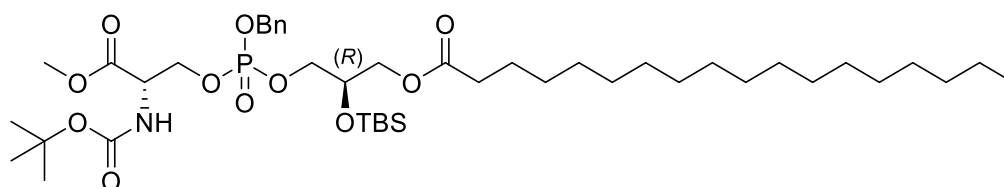
oxopropoxy)phosphoryl)oxy)-2-((tert-butylidimethylsilyl)oxy)propyl tetradecanoate

(Compound (R)-**8c**): 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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (R)-**8c** (56 mg, 0.0711 mmol, 51%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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), 4.28-4.19 (m, 1H), 4.13-4.04 (m, 1H), 4.02-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, J = 7.5, 2.2 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.08; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, $J_{\text{C-P}}$ = 7 Hz), 128.9, 128.8, 128.3 (d, $J_{\text{C-P}}$ = 2 Hz), 80.4, 69.8 (t, $J_{\text{C-P}}$ = 5 Hz), 69.1 (d, $J_{\text{C-P}}$ = 9 Hz), 68.5 (t, $J_{\text{C-P}}$ = 7 Hz), 67.7 (t, $J_{\text{C-P}}$ = 4 Hz), 64.8, 54.0 (d, $J_{\text{C-P}}$ = 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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{\text{C-P}}$ = 2 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH₂), 69.0 (d, $J_{\text{C-P}}$ = 9 Hz, CH), 68.4 (t, $J_{\text{C-P}}$ = 7 Hz, CH₂), 67.6 (m, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}}$ = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.5 (CH₂), 22.3 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₃₉H₇₀NO₁₁PSi [M+K]⁺: calcd., 826.41; found, 826.44; [M+Na]⁺: calcd., 810.43; found, 810.44.



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

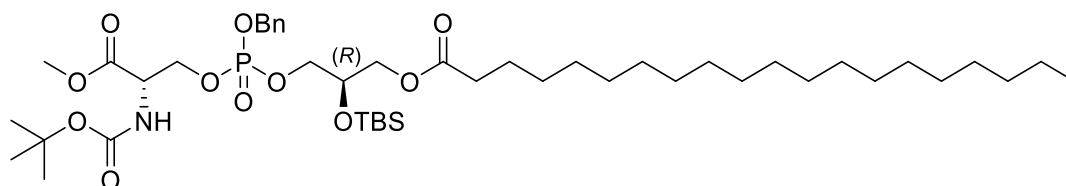
(Compound (R)-8d): Following the general procedure (**A1**), (**R**)-**6** (80 mg, 0.138 mmol), **7d** (Palmitic acid, C16:0) (32 mg, 0.124 mmol), EDC·HCl (24 mg, 0.124 mmol), DMAP (4 mg, 0.0345 mmol) and CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (**R**)-**8d** (61 mg, 0.0747 mmol, 54%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.4, 169.8, 155.3, 135.6 (d, *J*_{c-p} = 5 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 3 Hz), 80.4, 69.8 (t, *J*_{c-p} = 5 Hz), 69.1 (d, *J*_{c-p} = 9 Hz), 68.5 (t, *J*_{c-p} = 7 Hz), 67.7 (t, *J*_{c-p} = 4 Hz), 64.8, 54.0 (d, *J*_{c-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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, *J*_{c-p} = 2 Hz, CH), 69.7 (t, *J*_{c-p} = 5 Hz, CH₂), 69.0 (d, *J*_{c-p} = 9 Hz, CH), 68.4 (t, *J*_{c-p} = 7 Hz, CH₂), 67.6 (t, *J*_{c-p} = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, *J*_{c-p} = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₁H₇₄NO₁₁PSi [M+K]⁺: calcd., 854.44; found, 854.44; [M+Na]⁺: calcd., 838.46; found, 838.47.



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

(Compound (R)-8e): 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 CH₂Cl₂ (4 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to

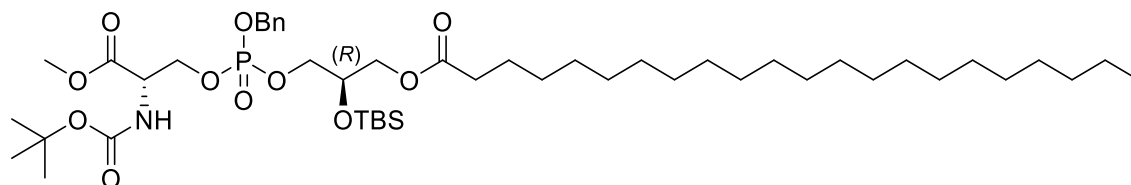
provide the desired product (*R*)-**8e** (54 mg, 0.0640 mmol, 53%) as a colourless oil: ^1H NMR (400 MHz, CDCl_3) δ 7.41-7.31 (m, 5H), 5.47 (dd, J = 14.2, 8.5 Hz, 1H), 5.06 (dd, J = 8.7, 3.2 Hz, 2H), 4.53-4.36 (m, 2H), 4.27-4.19 (m, 1H), 4.13-4.04 (m, 1H), 4.03-3.85 (m, 4H), 3.73 (2s, 3H), 2.29 (td, 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) δ -1.08; ^{13}C NMR (100 MHz, CDCl_3) δ 173.6, 169.8, 155.3, 135.6 (d, J = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{\text{C-P}}$ = 2 Hz), 80.5, 69.8 (t, $J_{\text{C-P}}$ = 5 Hz), 69.1 (d, $J_{\text{C-P}}$ = 8 Hz), 68.5 (t, $J_{\text{C-P}}$ = 7 Hz), 67.7 (t, $J_{\text{C-P}}$ = 4 Hz), 64.8, 54.0 (d, $J_{\text{C-P}}$ = 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) δ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{\text{C-P}}$ = 2 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH_2), 69.0 (d, $J_{\text{C-P}}$ = 8 Hz, CH), 68.4 (t, $J_{\text{C-P}}$ = 7 Hz, CH_2), 67.6 (t, $J_{\text{C-P}}$ = 4 Hz, CH_2), 64.7 (CH_2), 53.9 (d, $J_{\text{C-P}}$ = 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 $\text{C}_{43}\text{H}_{78}\text{NO}_{11}\text{PSi}$ $[\text{M}+\text{K}]^+$: calcd., 882.47; found, 882.47; $[\text{M}+\text{Na}]^+$: calcd., 866.49; found, 866.50.



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

(Compound (*R*)-8f**):** 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 silica gel (100-200 mesh) column chromatography using 25% EtOAc in n-hexane as an eluent to provide the desired product (*R*)-**8f** (67 mg, 0.0768 mmol, 50% yield) as a colourless oil: ^1H NMR (400 MHz, CDCl_3) δ 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) δ -1.09; ^{13}C NMR (100 MHz, CDCl_3) δ 173.6, 169.8, 155.3, 135.6 (d, $J_{\text{C-P}}$ = 7 Hz), 128.9, 128.8, 128.1 (d, $J_{\text{C-P}}$ = 2 Hz), 80.4, 69.8 (t, $J_{\text{C-P}}$ = 5 Hz), 69.1 (d, $J_{\text{C-P}}$ = 9 Hz), 68.5 (t, $J_{\text{C-P}}$ = 7 Hz), 67.7 (t, $J_{\text{C-P}}$ = 5 Hz), 64.8, 54.1 (d, $J_{\text{C-P}}$ = 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) δ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{\text{C-P}}$ = 2 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH_2), 69.0 (d, $J_{\text{C-P}}$ = 8 Hz, CH), 68.4 (t, $J_{\text{C-P}}$ = 7 Hz, CH_2), 67.6 (t, $J_{\text{C-P}}$ = 4 Hz, CH_2), 64.7 (CH_2), 53.9 (d, $J_{\text{C-P}}$ = 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).

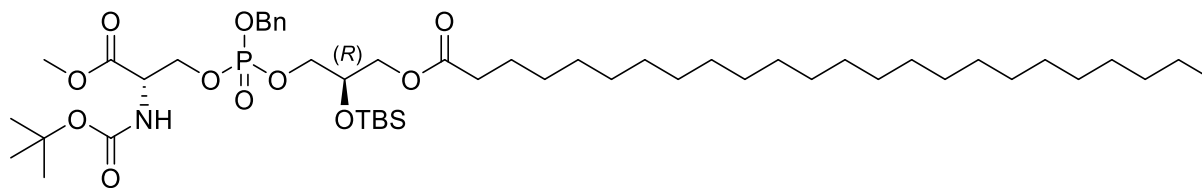
$\rho = 7$ Hz, CH₂), 67.6 (t, $J_{\text{C-P}} = 4$ Hz, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}} = 7$ Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₅H₈₂NO₁₁PSi [M+K]⁺: calcd., 910.50; found, 910.49; [M+Na]⁺: calcd., 894.52; found, 894.52.



(2R)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl docosanoate

(Compound (R)-**8g**): 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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (R)-**8g** (53 mg, 0.0589 mmol, 42% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.08; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.4, 135.6 (d, $J_{\text{C-P}} = 6$ Hz), 128.9, 128.8, 128.1 (d, $J = 2$ Hz), 80.5, 69.8 (t, $J_{\text{C-P}} = 5$ Hz), 69.2 (d, $J_{\text{C-P}} = 8$ Hz), 68.5 (t, $J_{\text{C-P}} = 7$ Hz), 67.8 (t, $J_{\text{C-P}} = 4$ Hz), 64.8, 54.0 (d, $J_{\text{C-P}} = 7$ 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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, $J_{\text{C-P}} = 2$ Hz, CH), 69.7 (t, $J_{\text{C-P}} = 5$ Hz, CH₂), 69.0 (d, $J_{\text{C-P}} = 8$ Hz, CH), 68.4 (t, $J_{\text{C-P}} = 7$ Hz, CH₂), 67.6 (t, $J_{\text{C-P}} = 4$ Hz, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}} = 7$ Hz, CH), 52.8 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₇H₈₆NO₁₁PSi [M+K]⁺: calcd., 938.53; found, 938.54; [M+Na]⁺: calcd., 922.56; found, 922.57.



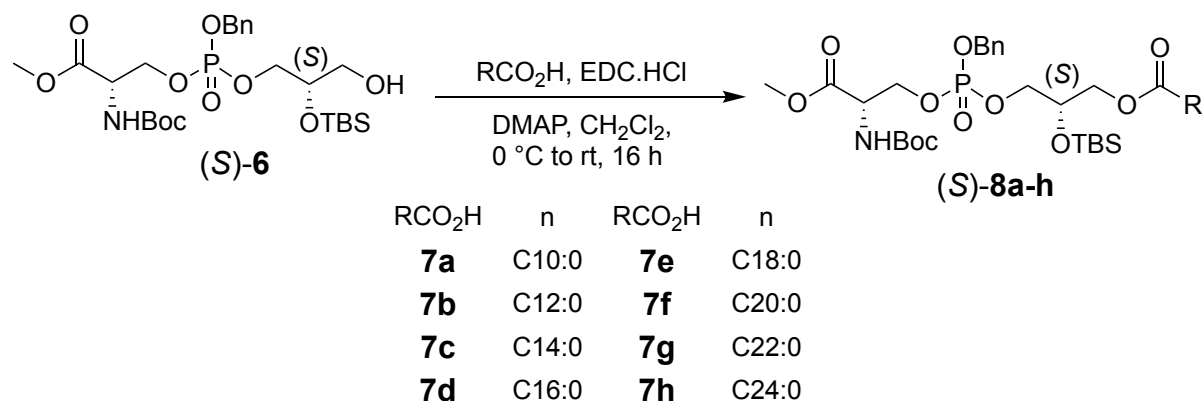
(2R)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl tetracosanoate

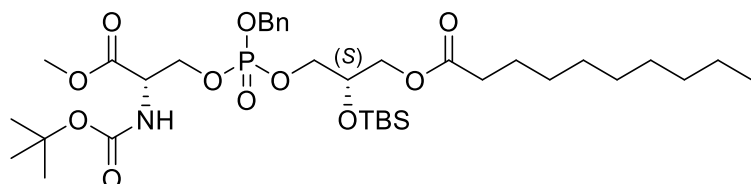
(Compound (R)-**8h**): Following the general procedure (**A1**), (R)-**6** (80 mg, 0.139 mmol), **7h** (Lignoceric acid, C₂₄:0) (46 mg, 0.125 mmol), EDC·HCl (24 mg, 0.125 mmol), DMAP (4 mg, 0.0348 mmol) and CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to provide the desired product (R)-**8h** (59 mg, 0.0589 mmol, 46% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{C-P} = 6 Hz), 128.9, 128.8, 128.1 (d, *J*_{C-P} = 2 Hz), 80.4, 69.8 (t, *J*_{C-P} = 5 Hz), 69.1 (d, *J*_{C-P} = 8 Hz), 68.5 (t, *J*_{C-P} = 7 Hz), 67.7 (t, *J*_{C-P} = 4 Hz), 64.8, 54.1 (d, *J*_{C-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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.01 (d, *J*_{C-P} = 2 Hz, CH), 69.7 (t, *J*_{C-P} = 5 Hz, CH₂), 69.0 (d, *J*_{C-P} = 9 Hz, CH), 68.4 (t, *J*_{C-P} = 7 Hz, CH₂), 67.6 (t, *J*_{C-P} = 4 Hz), 64.7 (CH₂), 53.9 (d, *J*_{C-P} = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.5 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₉H₉₀NO₁₁PSi [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:

The compounds (S)-8a-h described in this section were synthesized using a previously reported methodology in literature (Ikubo et al., 2015).



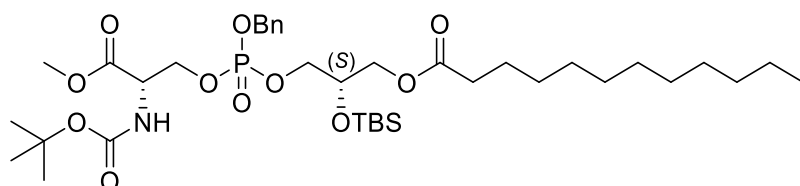
To a solution of the alcohol (S)-6 (1.0 equiv) and fatty acid 7 (0.9 equiv) in anhydrous CH₂Cl₂, DMAP (0.25 equiv) and EDC·HCl (0.9 equiv) were sequentially added at 0 °C. After stirring the mixture 16 h at room temperature, the reaction was quenched with saturated solution of NaHCO₃ and extracted three times with CH₂Cl₂. The combined organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure below 25 °C. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the corresponding product (S)-8.



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

(Compound (S)-8a): Following the general procedure (A2), (S)-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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-8a (50 mg, 0.0683 mmol, 49%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.05, -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{c-p} = 6.0 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 2Hz),

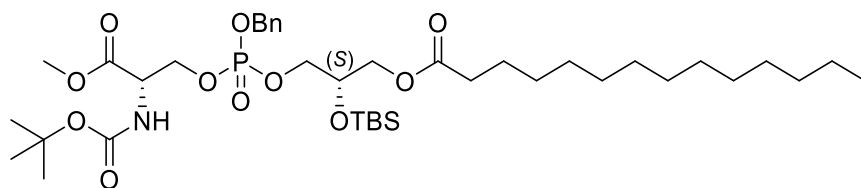
80.4, 69.8 (t, $J_{\text{C-P}} = 5$ Hz), 69.1 (d, $J_{\text{C-P}} = 9$ Hz), 68.5 (m), 67.7 (t, $J_{\text{C-P}} = 4$ Hz), 64.8, 54.1 (d, $J_{\text{C-P}} = 7$ Hz), 52.9, 34.2, 32.0, 29.5, 29.4, 29.3, 28.4, 25.8, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, CDCl_3) δ 128.8 (CH), 128.6 (CH), 128.1 (d, $J_{\text{C-P}} = 1$ Hz, CH), 69.7 (t, $J_{\text{C-P}} = 5$ Hz, CH_2), 69.0 (d, $J_{\text{C-P}} = 8$ Hz, CH), 68.4 (m, CH_2), 67.6 (t, $J_{\text{C-P}} = 4$ Hz, CH_2), 64.7 (CH_2), 53.9 (d, $J_{\text{C-P}} = 8$ 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 $\text{C}_{35}\text{H}_{62}\text{NO}_{11}\text{PSi}$ $[\text{M}+\text{K}]^+$: calcd., 770.34; found, 770.35; $[\text{M}+\text{Na}]^+$: calcd., 754.37; found, 754.37.



(2S)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl dodecanoate

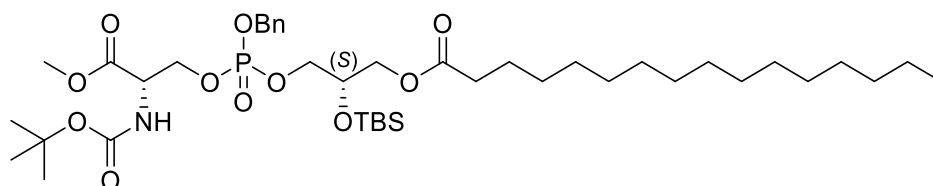
(Compound (S)-**8b**): Following the general procedure (**A2**), (S)-**6** (65 mg, 0.112 mmol), **7b** (Dodecanoic acid, C12:0) (20 mg, 0.101 mmol), EDC·HCl (19 mg, 0.101 mmol), DMAP (2.8 mg, 0.0280 mmol) and CH_2Cl_2 (4.6 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8b** (43 mg, 0.0566 mmol, 47%) as a colourless oil: ^1H NMR (400 MHz, CDCl_3) δ 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) δ -1.06, -1.09; ^{13}C NMR (100 MHz, CDCl_3) δ 173.6, 169.8, 155.3, 135.6 (d, $J_{\text{C-P}} = 7$ Hz), 128.9, 128.8, 128.1 (d, $J_{\text{C-P}} = 2$ Hz), 80.5, 69.8 (t, $J_{\text{C-P}} = 5$ Hz), 69.2 (d, $J_{\text{C-P}} = 9$ Hz), 68.5 (m), 67.7 (t, $J_{\text{C-P}} = 4$ Hz), 64.8, 54.1 (d, $J_{\text{C-P}} = 6$ 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) δ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{\text{C-P}} = 2$ Hz, CH), 69.7 (t, $J_{\text{C-P}} = 5$ Hz, CH_2), 69.0 (d, $J_{\text{C-P}} = 9$ Hz, CH), 68.4 (m, CH_2), 67.6 (t, $J_{\text{C-P}} = 4$ Hz, CH_2), 64.7 (CH_2), 53.9 (d, $J_{\text{C-P}} = 7$ 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 $\text{C}_{37}\text{H}_{66}\text{NO}_{11}\text{PSi}$ $[\text{M}+\text{K}]^+$: calcd., 798.38, found., 798.35, $[\text{M}+\text{Na}]^+$: calcd., 782.40; found, 782.38.



(2S)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl tetradecanoate

(Compound (S)-**8c**): Following the general procedure (**A2**), (S)-**6** (75 mg, 0.129 mmol), **7c** (Myristic acid, C14:0) (26 mg, 0.116 mmol), EDC·HCl (23 mg, 0.116 mmol), DMAP (3.8 mg, 0.0323 mmol) and CH₂Cl₂ (4.8 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8c** (54 mg, 0.0686 mmol, 53%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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), 4.28-4.19 (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), 1.33-1.19 (m, 20H), 0.91-0.81 (m, 12H), 0.07 (2s, 6H); ³¹P NMR (400 MHz, CDCl₃) δ -1.06, -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{C-P} = 7 Hz), 128.9, 128.8, 128.1 (broad), 80.5, 69.8 (t, *J*_{C-P} = 5 Hz), 69.1 (d, *J*_{C-P} = 8 Hz), 68.5 (t, *J*_{C-P} = 7 Hz), 67.7 (t, *J*_{C-P} = 4 Hz), 64.8, 54.1 (d, *J*_{C-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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.0 (broad, CH), 69.7 (t, *J*_{C-P} = 5 Hz, CH₂), 69.0 (d, *J*_{C-P} = 8 Hz, CH), 68.4 (m, CH₂), 67.6 (t, *J*_{C-P} = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, *J*_{C-P} = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₃₉H₇₀NO₁₁PSi [M+K]⁺: calcd., 826.41; found, 826.43; [M+Na]⁺: calcd., 810.43; found, 810.44.

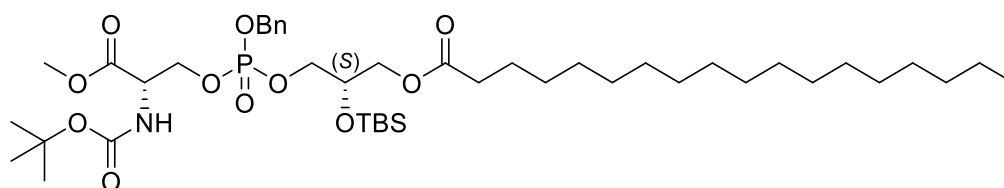


(2S)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-

oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl palmitate

(Compound (S)-**8d**): Following the general procedure (**A2**), (S)-**6** (70 mg, 0.121 mmol), **7d** (Palmitic acid, C16:0) (28 mg, 0.109 mmol), EDC·HCl (21 mg, 0.109 mmol), DMAP (3.5 mg, 0.0302 mmol) and CH₂Cl₂ (4.5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8d** (53 mg, 0.0649 mmol, 54%) as a colourless oil: ¹H NMR

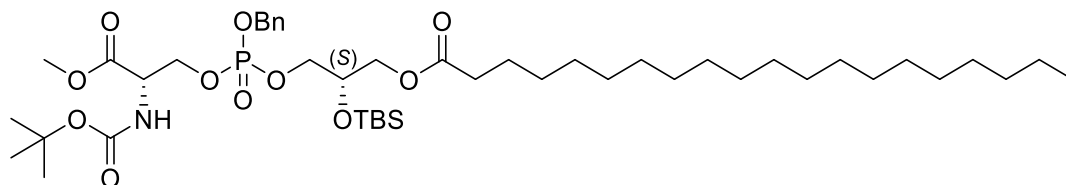
(400 MHz, CDCl₃) δ 7.41-7.30 (m, 5H), 5.47 (t, J = 9.7 Hz, 1H), 5.05 (t, J = 8.1 Hz, 2H), 4.54-4.35 (m, 2H), 4.28-4.19 (m, 1H), 4.15-4.05 (m, 1H), 4.03-3.84 (m, 4H), 3.73 (2s, 3H), 2.29 (t, J = 7.5 Hz, 2H), 1.60 (quint, J = 7.0 Hz, 2H), 1.44 (s, 9H), 1.35-1.19 (m, 24H), 0.91-0.82 (m, 12H), 0.07 (2s, 6H); ³¹P NMR (400 MHz, CDCl₃) δ -1.05, -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, $J_{\text{C-P}}$ = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{\text{C-P}}$ = 1 Hz), 80.4, 69.8 (t, $J_{\text{C-P}}$ = 5 Hz), 69.1 (d, $J_{\text{C-P}}$ = 9 Hz), 68.5 (m), 67.7 (t, $J_{\text{C-P}}$ = 4 Hz), 64.8, 54.0 (d, $J_{\text{C-P}}$ = 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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{\text{C-P}}$ = 2 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH₂), 69.0 (d, $J_{\text{C-P}}$ = 8 Hz, CH), 68.4 (m, CH₂), 67.6 (t, $J_{\text{C-P}}$ = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}}$ = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₁H₇₄NO₁₁PSi [M+K]⁺: calcd., 854.44; found, 854.47; [M+Na]⁺: calcd., 838.46; found, 838.48.



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

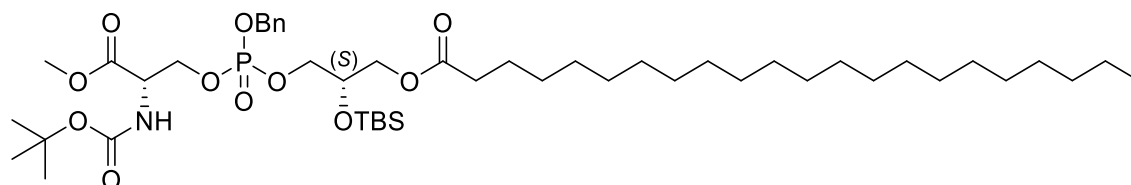
(Compound (S)-**8e**): Following the general procedure (**A2**), (S)-**6** (80 mg, 0.138 mmol), **7e** (Stearic acid, C18:0) (35 mg, 0.125 mmol), EDC·HCl (23 mg, 0.125 mmol), DMAP (3.5 mg, 0.0239 mmol) and CH₂Cl₂ (4.5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8e** (55 mg, 0.0652 mmol, 47%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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, 12H), 0.07 (2s, 6H); ³¹P NMR (400 MHz, CDCl₃) δ -1.06, -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, J = 5 Hz), 128.9, 128.8, 128.1 (d, $J_{\text{C-P}}$ = 2 Hz), 80.4, 69.8 (t, $J_{\text{C-P}}$ = 5 Hz), 69.2 (d, $J_{\text{C-P}}$ = 8 Hz), 68.5 (m), 67.7 (t, $J_{\text{C-P}}$ = 4 Hz), 64.8, 54.1 (d, $J_{\text{C-P}}$ = 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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.0 (d, $J_{\text{C-P}}$ = 1 Hz, CH), 69.7 (t, $J_{\text{C-P}}$ = 5 Hz, CH₂), 69.0 (d, $J_{\text{C-P}}$ = 9 Hz, CH), 68.4 (m, CH₂), 67.6 (t, $J_{\text{C-P}}$ = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, $J_{\text{C-P}}$ = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂),

29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₃H₇₈NO₁₁PSi [M+K]⁺: calcd., 882.47; found, 882.48; [M+Na]⁺: calcd., 866.49; found, 866.50.



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

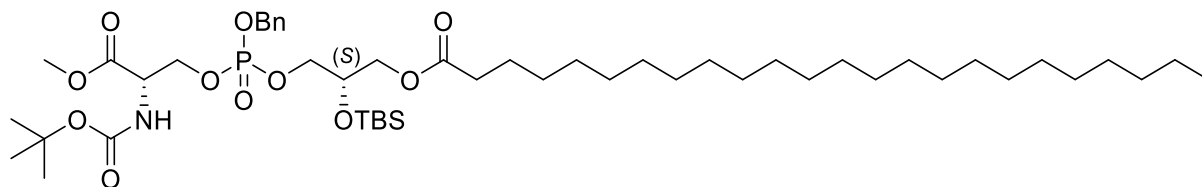
(Compound (S)-**8f**): Following the general procedure (**A2**), (S)-**6** (85 mg, 0.147 mmol), **7f** (Arachidic acid, C_{20:0}) (41 mg, 0.132 mmol), EDC·HCl (26 mg, 0.132 mmol), DMAP (4.7 mg, 0.0366 mmol) and CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in n-hexane as an eluent to afford the desired product (S)-**8f** (62 mg, 0.0711 mmol, 48%) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.06, -1.10; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{c-p} = 5 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 1Hz), 80.5, 69.8 (t, *J*_{c-p} = 5 Hz), 69.1 (d, *J*_{c-p} = 8 Hz), 68.5 (m), 67.7 (t, *J*_{c-p} = 4 Hz), 64.8, 54.0 (d, *J*_{c-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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, *J*_{c-p} = 1Hz, CH), 69.7 (t, *J*_{c-p} = 5 Hz, CH₂), 69.0 (d, *J*_{c-p} = 9 Hz, CH), 68.4 (m, CH₂), 67.6 (t, *J*_{c-p} = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, *J*_{c-p} = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₅H₈₂NO₁₁PSi [M+K]⁺: calcd., 910.50; found, 910.52; [M+Na]⁺: calcd., 894.52; found, 894.53.



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

(Compound (S)-**8g**): 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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8g** (57 mg, 0.0633 mmol, 45% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.06, -1.09; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{c-p} = 6 Hz), 128.9, 128.8, 128.1 (d, *J* = 2 Hz), 80.5, 69.8 (t, *J*_{c-p} = 5 Hz), 69.2 (d, *J*_{c-p} = 8 Hz), 68.5 (m), 67.7 (t, *J*_{c-p} = 4 Hz), 64.8, 54.1 (d, *J*_{c-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, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.1 (d, *J*_{c-p} = 2 Hz, CH), 69.7 (t, *J*_{c-p} = 5 Hz, CH₂), 69.0 (d, *J*_{c-p} = 9 Hz, CH), 68.4 (m, CH₂), 67.6 (t, *J*_{c-p} = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, *J*_{c-p} = 6 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₇H₈₆NO₁₁PSi [M+K]⁺: calcd., 938.53; found, 938.55; [M+Na]⁺: calcd., 922.56; found, 922.58.



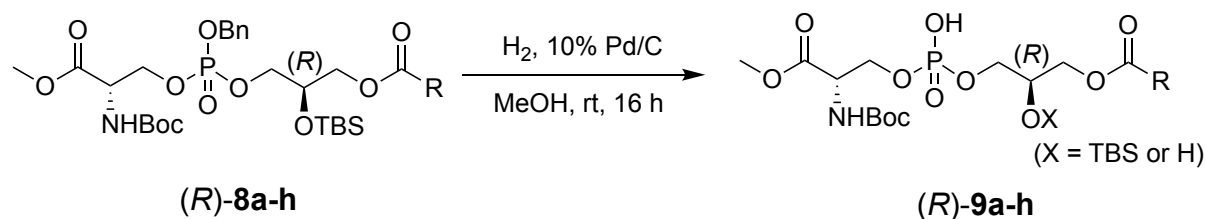
(2S)-3-(((benzyloxy)((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl tetracosanoate

(Compound (S)-**8h**): 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 CH₂Cl₂ (5 mL) were used. The crude mixture was purified by silica gel (100-200 mesh) column chromatography using 25% EtOAc in *n*-hexane as an eluent to afford the desired product (S)-**8h** (68 mg, 0.0589 mmol, 47% yield) as a colourless oil: ¹H NMR (400 MHz, CDCl₃) δ 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); ³¹P NMR (400 MHz, CDCl₃) δ -1.06, -1.10; ¹³C NMR (100 MHz, CDCl₃) δ 173.6, 169.8, 155.3, 135.6 (d, *J*_{c-p} = 6 Hz), 128.9, 128.8, 128.1 (d, *J*_{c-p} = 1 Hz), 80.4, 69.8 (t, *J*_{c-p} = 5 Hz), 69.1 (d, *J*_{c-p} = 9 Hz), 68.5 (m), 67.7 (t, *J*_{c-p} = 4 Hz), 64.8, 54.0 (d, *J*_{c-p}

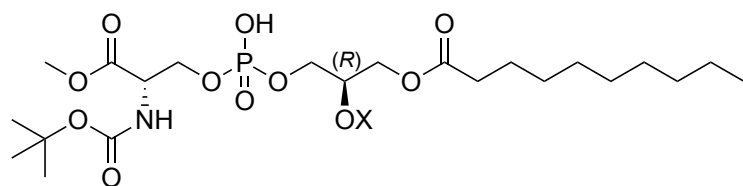
= 7 Hz), 52.8, 34.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.7, 25.0, 22.8, 18.1, 14.2, -4.7, -4.8; DEPT-135 NMR (100 MHz, CDCl₃) δ 128.8 (CH), 128.7 (CH), 128.0 (d, J_{C-P} = 1 Hz, CH), 69.7 (t, J_{C-P} = 5 Hz, CH₂), 69.0 (d, J_{C-P} = 8 Hz, CH), 68.4 (m, CH₂), 67.6 (t, J_{C-P} = 4 Hz, CH₂), 64.7 (CH₂), 53.9 (d, J_{C-P} = 7 Hz, CH), 52.7 (CH₃), 34.1 (CH₂), 31.9 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.3 (CH₃), 25.6 (CH₃), 24.9 (CH₂), 22.7 (CH₂), 14.1 (CH₃), -4.8 (CH₃), -4.9 (CH₃); MALDI (ESI-TOF) for C₄₉H₉₀NO₁₁PSi [M+K]⁺: calcd., 966.57; found, 966.57; [M+Na]⁺: calcd., 950.59; found, 950.60.

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

The intermediates (*R*)-9a-h described in this section were synthesized using a previously reported methodology in literature (Ikubo et al., 2015).

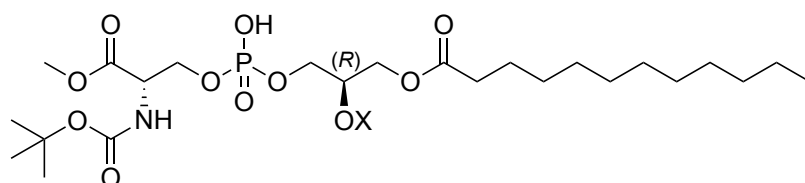


The benzylated compound (*R*)-8 was dissolved in dry MeOH in a two-neck round bottom flask, following which, Pd/C (10%) was added to this solution under N₂ atmosphere, and the round bottom flask was subsequently equipped with hydrogen filled rubber bladder. The reaction mixture was stirred for next 12 to 16 h at room temperature. The reaction solution was filtered through a celite pad, and washed with MeOH (3 × 10 mL). The filtrate was then concentrated under reduced pressure at 25 °C to yield (*R*)-9 intermediates (> 95% pure, > 80% yield), which were used as such, without any further purification for the next step. The debenzylated (*R*)-9 intermediates consisted of a mixture of two compounds where the *sn*-2 hydroxyl was either TBS protected or deprotected because of solvent methanolysis. As per our reaction scheme (**Supplementary Fig. 1**), both compounds would yield the final desired products, hence we did not try to purify or isolate them separately, and proceeded with the intermediate to the next step.

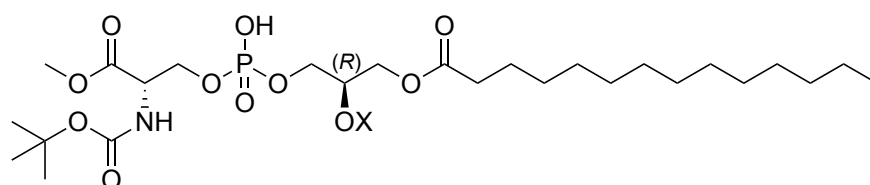


Intermediate (*R*)-9a: TBS protected: (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl decanoate + **TBS deprotected:** (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-

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 intermediate (*R*)-**9a** (30 mg) was obtained in a ratio of 39:61 (TBS protected: TBS deprotected; based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.15-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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), 2.40-2.31 (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, 7H), 0.15-0.09 (m, 2H); ^{31}P NMR (400 MHz, MeOH-d₄) δ -0.19, -0.46.

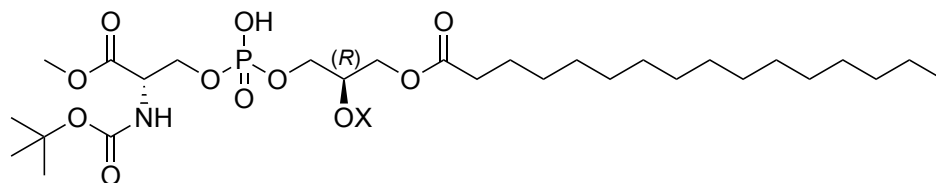


Intermediate (*R*)-9b: TBS protected: (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl dodecanoate + **TBS deprotected:** (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (*R*)-**9b** (32 mg) was obtained in a ratio of 54:46 (TBS protected: TBS deprotected; based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.16-0.10) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.14, -0.22.

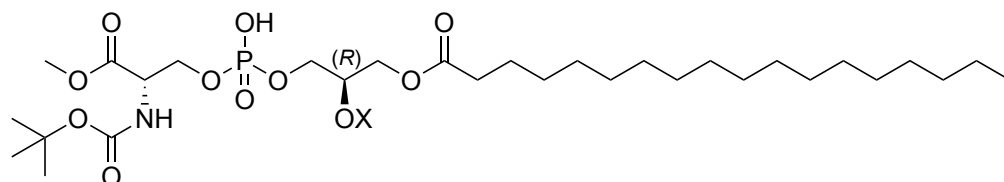


Intermediate (*R*)-9c: TBS protected: (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate + **TBS deprotected:** (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (*R*)-**9c** (28 mg) was obtained in a ratio of 67:33 (TBS protected: TBS deprotected; based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.08) as a colourless oil: ^1H NMR (400 MHz,

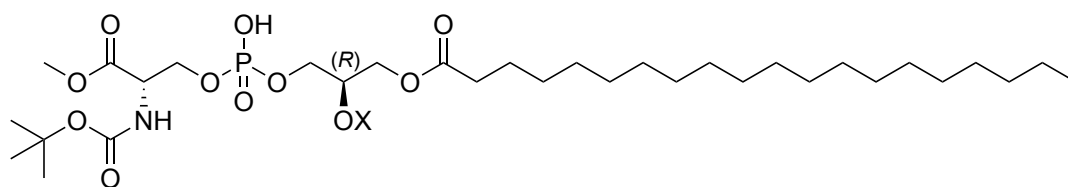
MeOH- d_4) δ 4.39-4.33 (m, 1H), 4.30-4.14 (m, 2H), 4.13-3.92 (m, 3H), 3.91-3.76 (m, 2H), 3.74 (s, 3H), 2.41-2.29 (m, 2H), 1.62 (q, J = 6.9 Hz, 2H), 1.45 (s, 9H), 1.36-1.22 (m, 20H), 0.93-0.86 (m, 9H), 0.17-0.08 (m, 4H); ^{31}P NMR (400 MHz, MeOH- d_4) δ 0.22, -0.16.



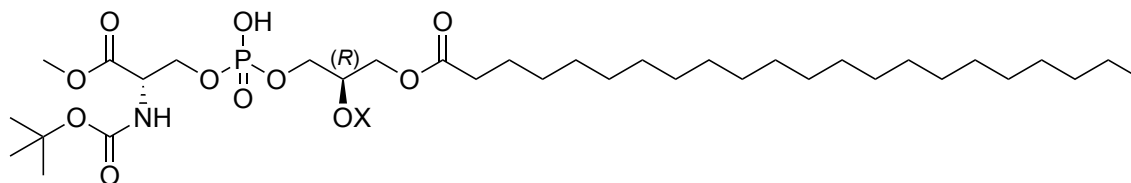
Intermediate (R)-9d: TBS protected: (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate + **TBS deprotected:** (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (**R**)-**9d** (36 mg) was obtained in a ratio of 60:40 (TBS protected: TBS deprotected; based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe $_2$) peak from δ 0.17-0.08) as a colourless oil: ^1H NMR (400 MHz, MeOH- d_4) δ 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) δ 0.28, -0.12.



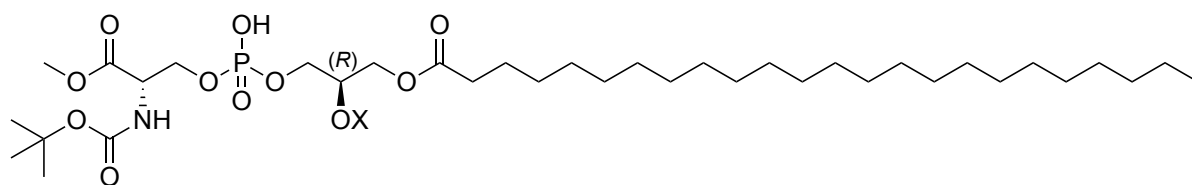
Intermediate (R)-9e: TBS protected: (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate + **TBS deprotected:** (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (**R**)-**9e** (30 mg) was obtained in a ratio of 65:35 (TBS protected: TBS deprotected; based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe $_2$) peak from δ 0.17-0.08) as a colourless oil: ^1H NMR (400 MHz, MeOH- d_4) δ 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) δ 0.24, -0.14.



Intermediate (R)-9f: TBS protected: (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate + **TBS deprotected:** (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 intermediate (*R*)-**9f** (49 mg) was obtained in a ratio of 58:42 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-*d*₄) δ 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) δ -0.79, -0.93.



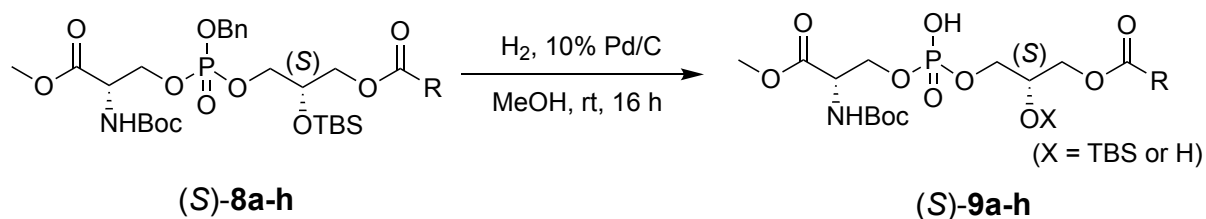
Intermediate (R)-9g: TBS protected: (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docosanoate + **TBS deprotected:** (2*R*)-3-((((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (*R*)-**9g** (35 mg) was obtained in a ratio of 63:37 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-*d*₄) δ 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), 0.17-0.09 (m, 4H); ^{31}P NMR (400 MHz, MeOH-*d*₄) δ -0.57.



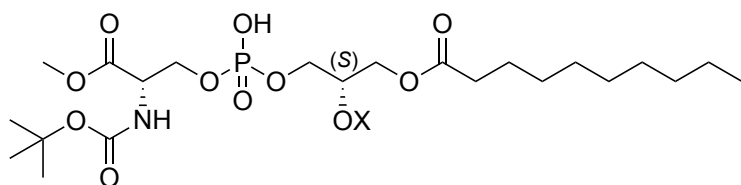
Intermediate (R)-9h: TBS protected: (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetracosanoate + **TBS deprotected:** (2R)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (**R**)-**9h** (40 mg) was obtained in a ratio of 46:54 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.06) as a colourless oil: ^1H NMR (400 MHz, MeOH-*d*₄ + CDCl₃) δ 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), 0.17-0.06 (m, 3H); ^{31}P NMR (400 MHz, MeOH-*d*₄ + CDCl₃) δ -0.50, -0.91.

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

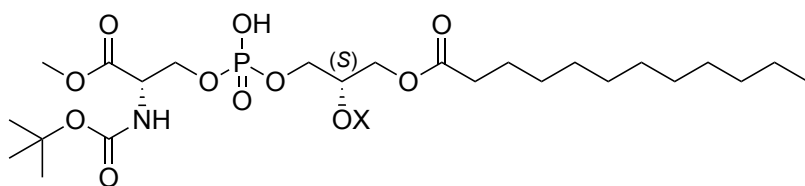
The intermediates (**S**)-**9a-h** described in this section were synthesized using a previously reported methodology in literature(Ikubo et al., 2015).



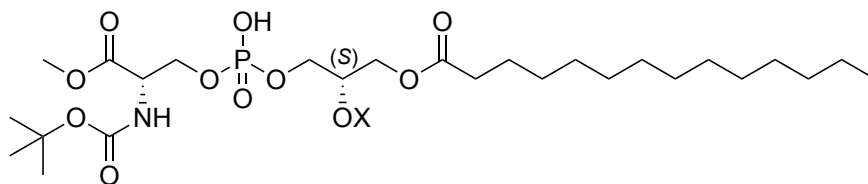
The benzylated compound (**S**)-**8** was dissolved in dry MeOH in a two-neck round bottom flask, following which, Pd/C (10%) was added to this solution under N₂ atmosphere, and the round bottom flask was subsequently equipped with hydrogen filled rubber bladder. The reaction mixture was stirred for next 12 to 16 h at room temperature. The reaction solution was filtered through a celite pad, and washed with MeOH (3 × 10 mL). The filtrate was then concentrated under reduced pressure at 25 °C to yield (**S**)-**9** intermediates (> 95% pure, > 80% yield), which were used as such, without any further purification for the next step. The debenzylated (**S**)-**9** intermediate consisted of a mixture of two compounds where the *sn*-2 hydroxyl was either TBS protected or deprotected because of solvent methanolysis. As per our reaction scheme (**Supplementary Fig. 2**), both compounds would yield the final desired products, hence we did not try to purify or isolate them separately, and proceeded with the intermediate to the next step.



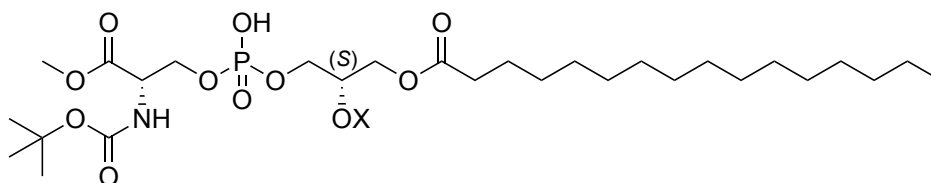
Intermediate (S)-9a: TBS protected: (2S)-3-((((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl decanoate + **TBS deprotected:** (2S)-3-((((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (S)-**9a** (36 mg) was obtained in a ratio of 50:50 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.16-0.07) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.22, -0.17.



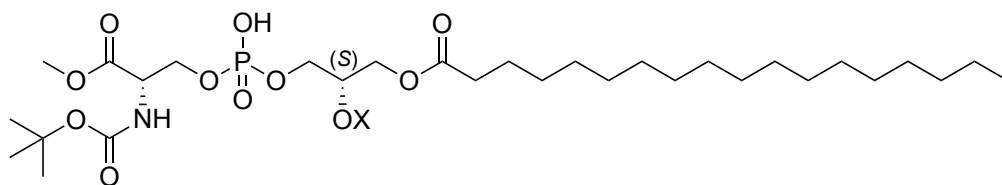
Intermediate (S)-9b: TBS protected: (2S)-3-((((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((tert-butyldimethylsilyl)oxy)propyl dodecanoate + **TBS deprotected:** (2S)-3-((((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (S)-**9b** (30 mg) was obtained in a ratio of 58:42 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.16-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.16, -0.21.



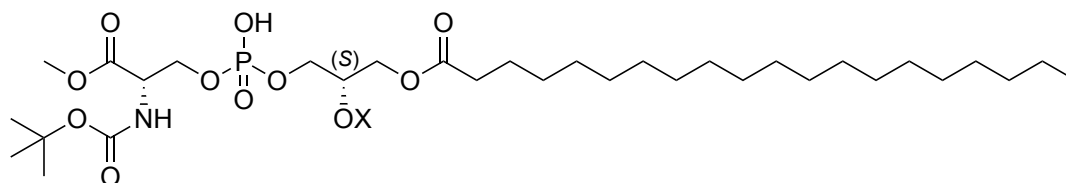
Intermediate (S)-9c: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetradecanoate + **TBS deprotected:** (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate: Following the general procedure (**B2**), (S)-**8c** (54 mg, 0.0686 mmol), Pd/C (27 mg), and MeOH (5.4 mL) were used. The intermediate (S)-**9c** (40 mg) was obtained in a ratio of 79:21 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.08) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.16, -0.22.



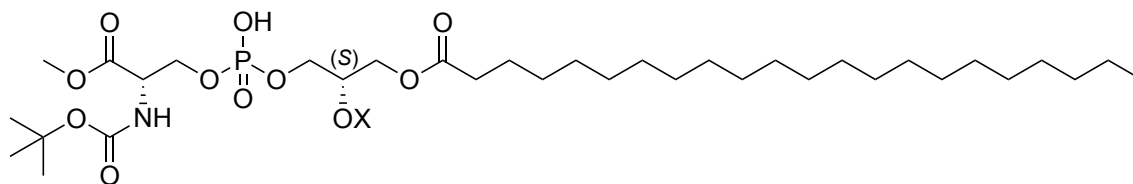
Intermediate (S)-9d: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl palmitate + **TBS deprotected:** (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (S)-**9d** (38 mg) was obtained in a ratio of 62:38 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.23, -0.16.



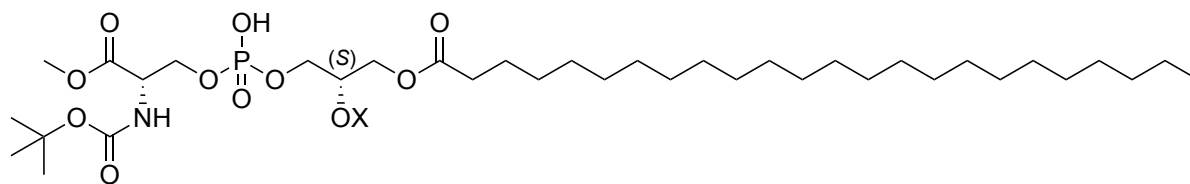
Intermediate (S)-9e: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl stearate + **TBS deprotected:** (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl 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 intermediate (S)-**9e** (43 mg) was obtained in a ratio of 65:35 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 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-d₄) δ 0.22, -0.17.



Intermediate (S)-9f: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl icosanoate + **TBS deprotected:** (2S)-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 intermediate (S)-**9f** (48 mg) was obtained in a ratio of 45:55 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under (-SiMe₂) peak from δ 0.17-0.09) as a colourless oil: ^1H NMR (400 MHz, MeOH-d₄) δ 4.41-4.33 (m, 1H), 4.31-4.25 (m, 1H), 4.24-4.00 (m, 4H), 3.98-3.80 (m, 2H), 3.75 (s, 3H), 2.42-2.29 (m, 2H), 1.62 (q, J = 7.0 Hz, 2H), 1.45 (s, 9H), 1.35-1.26 (m, 32H), 0.94-0.85 (m, 8H), 0.17-0.09 (m, 3H); ^{31}P NMR (400 MHz, MeOH-d₄) δ 0.05, -0.39.

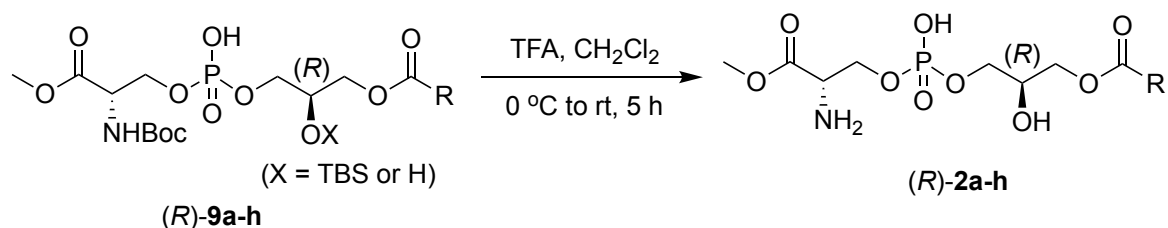


Intermediate (S)-9g: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl docosanoate + **TBS deprotected:** (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docosanoate: Following the general procedure (**B2**), (S)-**8g** (57 mg, 0.0633 mmol), Pd/C (28 mg), and MeOH (5.7 mL) were used. The intermediate (S)-**9g** (40 mg) was obtained in a ratio of 75:25 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under $(-\text{SiMe}_2)$ peak from δ 0.17-0.08) as a colourless oil: ^1H NMR (400 MHz, MeOH- d_4) δ 4.40-4.32 (m, 1H), 4.30-4.25 (m, 1H), 4.24-4.00 (m, 4H), 3.98-3.80 (m, 2H), 3.74 (s, 3H), 2.42-2.29 (m, 2H), 1.62 (m, 2H), 1.45 (s, 9H), 1.38-1.24 (m, 36H), 0.94-0.85 (m, 10H), 0.17-0.08 (m, 5H); ^{31}P NMR (400 MHz, MeOH- d_4) δ -0.44.

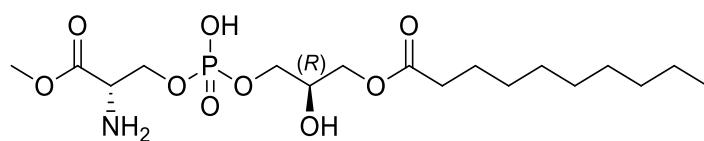


Intermediate (S)-9h: TBS protected: (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-((*tert*-butyldimethylsilyl)oxy)propyl tetracosanoate + **TBS deprotected:** (2S)-3-((((S)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate: Following the general procedure (**B2**), (S)-**8h** (68 mg, 0.0589 mmol), Pd/C (34 mg), and MeOH (6.8 mL) were used. The intermediate (S)-**9h** (30 mg) was obtained in a ratio of 49:51 (TBS protected: TBS deprotected based the percentage abundant is calculated based on the ^1H NMR integration under $(-\text{SiMe}_2)$ peak from δ 0.18-0.06) as a colourless oil: ^1H NMR (400 MHz, MeOH- d_4 + CDCl_3) δ 4.62-4.44 (m, 1H), 4.40-3.91 (m, 6H), 3.89-3.80 (m, 1H), 3.75 (s, 3H), 2.42-2.27 (m, 2H), 1.62 (m, 2H), 1.44 (s, 9H), 1.37-1.21 (m, 40H), 0.95-0.81 (m, 9H), 0.18-0.06 (m, 3H); ^{31}P NMR (400 MHz, MeOH- d_4) δ 0.32, -0.14.

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

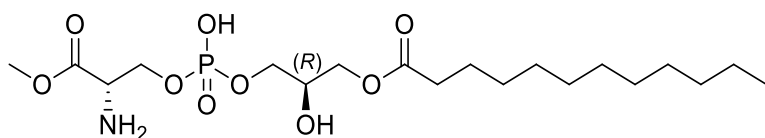


The intermediate (*R*)-**9** in dry CH₂Cl₂ was charged into a two-neck round bottom flask, which was equipped with a N₂ balloon. The solution was cooled to -10 °C, following which the solution of trifluoroacetic acid (TFA) was added dropwise. After TFA addition, the reaction temperature and stirring time was different, depending on the compound that was synthesized. For the intermediates (*R*)-**9a-d**, the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for another 4 h. For intermediate (*R*)-**9e** the reaction solution was stirred at 0 °C for 4 h and then at room temperature for 1 h. For the intermediates (*R*)-**9f-h**, the reaction mixture was stirred only at 0 °C for 5 h. The rationale for using different temperatures, was to minimize hydrolysis of the fatty acid moiety. Our observation was that as the fatty acid chain length of this moiety increases, temperatures of the reaction mixture had to be maintained at 0 °C. After completion the deprotection reaction, the remaining solution was concentrated under reduced pressure at 25 °C. The resultant residue was washed three times with *n*-Pentane: Et₂O (3:1), and then dried under high vacuum to afford the desired final product (*R*)-**2**. The purity of the final compounds (*R*)-**2a-h** was determined based on the NMR analysis, and LC-MS analysis, and found to be ≥ 95% for all compounds.

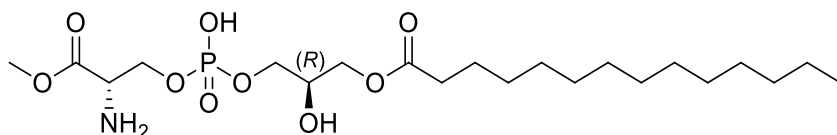


(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-

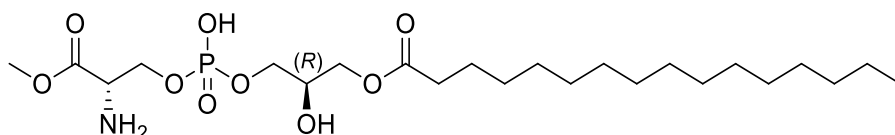
hydroxypropyl decanoate (Compound (*R*)-2a**):** Following the general procedure (**C1**), (*R*)-**9a** (30 mg), TFA (0.300 mL) and CH₂Cl₂ (0.170 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield desired final compound (*R*)-**2a** (21 mg, 0.0491 mmol, 72% yield relative to (*R*)-**8a**) as a white solid: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.44; HRMS (ESI): *m/z* calcd. for (C₁₇H₃₅NO₉P) [*M* + *H*]⁺ 428.2049; found, 428.2048.



(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate (Compound (R)-2b): Following the general procedure (**C1**), (**R**)-**9b** (32 mg), TFA (0.320 mL) and CH₂Cl₂ (0.180 mL) were used. The product was washed with *n*-pentane: Et₂O (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: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.44; HRMS (ESI): *m/z* calcd. for (C₁₉H₃₉NO₉P) [M + H]⁺ 456.2362; found, 456.2363.

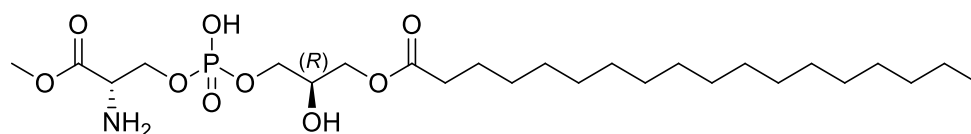


(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate (Compound (R)-2c): Following the general procedure (**C1**), (**R**)-**9c** (28 mg), TFA (0.280 mL) and CH₂Cl₂ (0.160 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield desired final compound (**R**)-**2c** (20 mg, 0.0414 mmol, 67% yield relative to (**R**)-**8c**) as a white solid: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.44; HRMS (ESI): *m/z* calcd. for (C₂₁H₄₃NO₉P) [M + H]⁺ 484.2675; found, 484.2677.

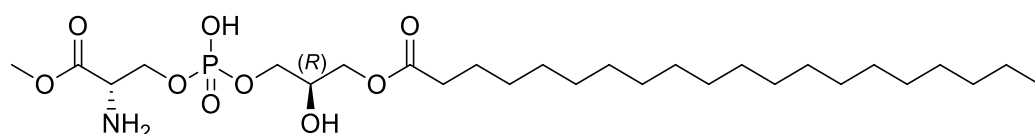


(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate (Compound (R)-2d): Following the general procedure (**C1**), (**R**)-**9d** (36 mg), TFA (0.360 mL) and CH₂Cl₂ (0.210 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield desired final compound (**R**)-**2d** (26 mg, 0.0508 mmol, 74% yield relative to (**R**)-**8d**) as a white solid: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR

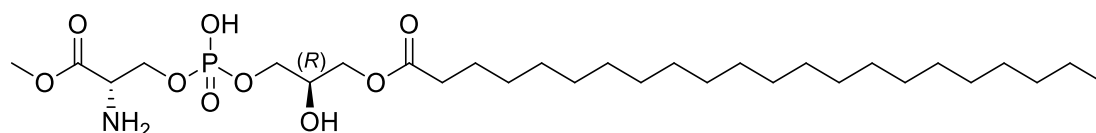
(400 MHz, MeOH- d_4) δ 0.44; HRMS (ESI): m/z calcd. for ($C_{23}H_{47}NO_9P$) [$M + H$] $^+$ 512.2988; found, 512.2988.



(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate (Compound (R)-2e): 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: Et₂O (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: 1H NMR (400 MHz, MeOH- d_4 + $CDCl_3$ (1:2 ratio)) δ 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) δ 0.44; HRMS (ESI): m/z calcd. for ($C_{25}H_{51}NO_9P$) [$M + H$] $^+$ 540.3296; found, 540.3297.

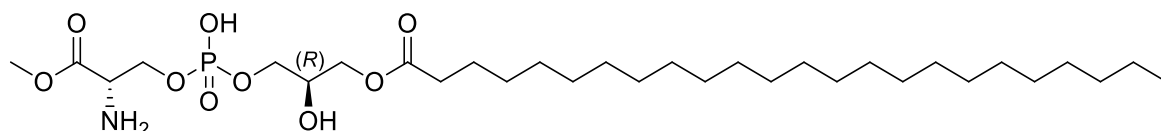


(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate (Compound (R)-2f): Following the general procedure (C1), (R)-9f (25 mg), TFA (0.200 mL) and CH_2Cl_2 (0.350 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield desired final compound (R)-2f (17 mg, 0.0299 mmol, 79% yield relative to (R)-8f) as a white solid: 1H NMR (400 MHz, MeOH- d_4 + $CDCl_3$ (1:2 ratio)) δ 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) δ 0.45; HRMS (ESI): m/z calcd. for ($C_{27}H_{55}NO_9P$) [$M + H$] $^+$ 568.3609; found, 568.3610.



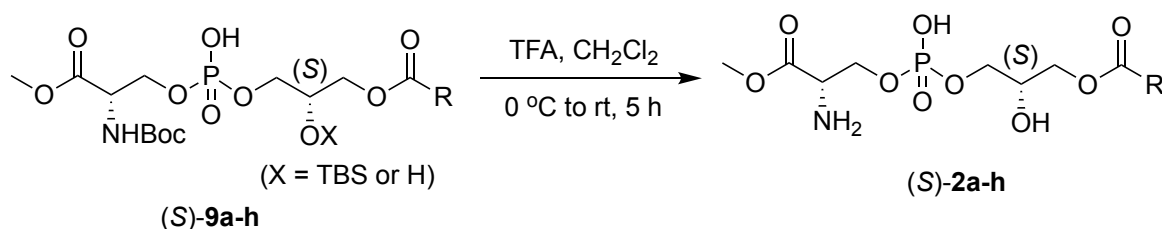
(2R)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docosanoate (Compound (R)-2g): Following the general procedure (C1), (R)-9g (28 mg), TFA (0.225 mL) and CH_2Cl_2 (0.390 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield desired final compound (R)-2g (22 mg,

0.0369 mmol, 79% yield relative to (*R*)-**8g**) as a white solid: ^1H NMR (400 MHz, MeOH- d_4 + CDCl_3 (1:2 ratio)) δ 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) δ 0.44; HRMS (ESI): m/z calcd. for ($\text{C}_{29}\text{H}_{59}\text{NO}_9\text{P}$) [$\text{M} + \text{H}$] $^+$ 596.3922; found, 596.3923.



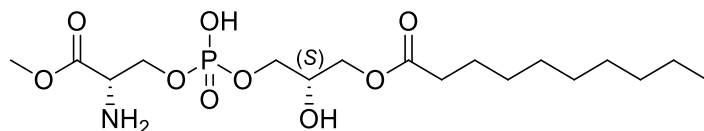
(2*R*)-3-((((*S*)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate (Compound (*R*)-2h**):** Following the general procedure (**C1**), (*R*)-**9h** (25 mg), TFA (0.200 mL) and CH_2Cl_2 (0.350 mL) were used. The product was washed with *n*-pentane: Et_2O (4:1) (3 x 2 mL) to yield desired final compound (*R*)-**2h** (18 mg, 0.0289 mmol, 78% yield relative to (*R*)-**8h**) as a white solid: ^1H NMR (400 MHz, MeOH- d_4 + CDCl_3 (1:2 ratio)) δ 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) δ 0.44; ; HRMS (ESI): m/z calcd. for ($\text{C}_{31}\text{H}_{63}\text{NO}_9\text{P}$) [$\text{M} + \text{H}$] $^+$ 624.4235; found, 624.4236.

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

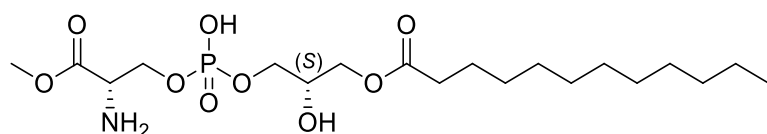


The intermediate (*S*)-**9** in dry CH_2Cl_2 was charged into a two-neck round bottom flask, which was equipped with a N_2 balloon. The solution was cooled to $-10\text{ }^\circ\text{C}$, following which the solution of trifluoroacetic acid (TFA) was added dropwise. After TFA addition, the reaction temperature and stirring time was different, depending on the compound that was synthesized. For the intermediates (*S*)-**9a-d**, the reaction mixture was stirred at $0\text{ }^\circ\text{C}$ for 1 h and then at room temperature for another 4 h. For intermediate (*S*)-**9e** the reaction solution was stirred at $0\text{ }^\circ\text{C}$ for 4 h and then at room temperature for 1 h. For the intermediates (*S*)-**9f-h**, the reaction mixture was stirred only at $0\text{ }^\circ\text{C}$ for 5 h. The rationale for using different temperatures, was to minimize hydrolysis of the fatty acid moiety. Our observation was that as the fatty acid chain length of this moiety increases, temperatures of the reaction mixture had to be maintained at $0\text{ }^\circ\text{C}$. After completion the deprotection reaction, the remaining solution was concentrated under reduced pressure at $25\text{ }^\circ\text{C}$. The resultant residue was

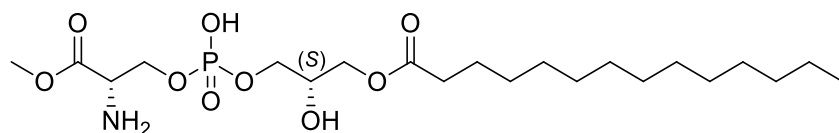
washed three times with *n*-Pentane: Et₂O (3:1), and then dried under high vacuum to afford the desired final product (S)-**2**. The purity of the final compounds (S)-**2a-h** was determined based on the NMR analysis, and LC-MS analysis, and found to be $\geq 95\%$ for all compounds.



(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl decanoate (Compound (S)-2a**):** Following the general procedure (**C2**), (S)-**9a** (36 mg), TFA (0.360 mL) and CH₂Cl₂ (0.210 mL) were used. The product was washed with *n*-pentane: Et₂O (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: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.44; HRMS (ESI): *m/z* calcd. for (C₁₇H₃₅NO₉P) [M + H]⁺ 428.2049; found, 428.2048.

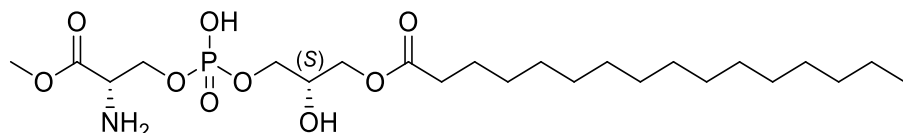


(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl dodecanoate (Compound (S)-2b**):** Following the general procedure (**C2**), (S)-**9b** (30 mg), TFA (0.300 mL) and CH₂Cl₂ (0.170 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2b** (17 mg, 0.0373 mmol, 67% yield relative to (S)-**8b**) as a white solid: ¹H NMR (400 MHz, MeOH-d₄) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.44; HRMS (ESI): *m/z* calcd. for (C₁₉H₃₉NO₉P) [M + H]⁺ 456.2362; found, 456.2361.

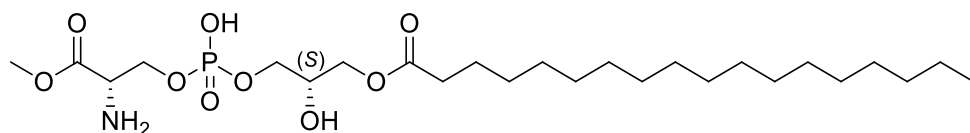


(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetradecanoate (Compound (S)-2c**):** Following the general procedure (**C2**), (S)-**9c** (40 mg), TFA (0.400 mL) and CH₂Cl₂ (0.230 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2c** (26 mg,

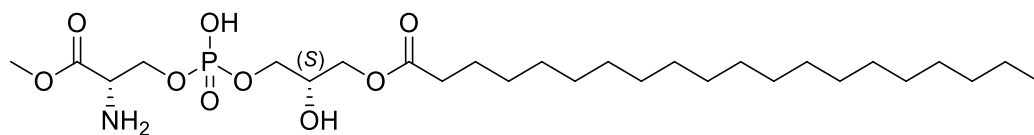
0.0538 mmol, 78% yield relative to (S)-**8c** as a white solid: ^1H NMR (400 MHz, MeOH- d_4) δ 4.60 (m, 1H), 4.40-4.07 (m, 4H), 4.06-3.89 (m, 3H), 3.85 (2s, 3H), 3.76-3.65 (m, 1H), 2.36 (t, J = 7.4 Hz, 2H), 1.62 (m, 2H), 1.38-1.23 (m, 20H), 0.90 (t, J = 6.7 Hz, 3H); ^{31}P NMR (400 MHz, MeOH- d_4) δ 0.44; HRMS (ESI): m/z calcd. for ($\text{C}_{21}\text{H}_{43}\text{NO}_9\text{P}$) [$\text{M} + \text{H}$] $^+$ 484.2675; found, 484.2676.



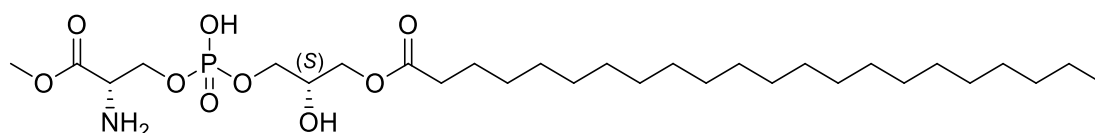
(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate (Compound (S)-2d): Following the general procedure (**C2**), (S)-**9d** (38 mg), TFA (0.380 mL) and CH_2Cl_2 (0.220 mL) were used. The product was washed with *n*-pentane: Et_2O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2d** (24 mg, 0.0469 mmol, 73% yield relative to (S)-**8d**) as a white solid: ^1H NMR (400 MHz, MeOH- d_4) δ 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) δ 0.43; HRMS (ESI): m/z calcd. for ($\text{C}_{23}\text{H}_{47}\text{NO}_9\text{P}$) [$\text{M} + \text{H}$] $^+$ 512.2988; found, 512.2989.



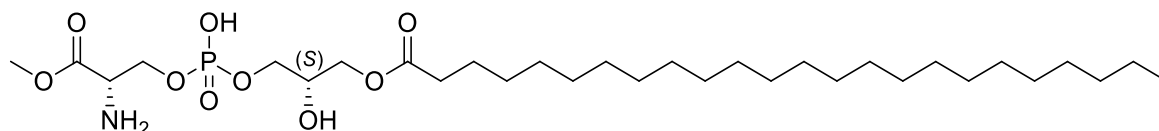
(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl stearate (Compound (S)-2e): Following the general procedure (**C2**), (S)-**9e** (43 mg), TFA (0.430 mL) and CH_2Cl_2 (0.250 mL) were used. The product was washed with *n*-pentane: Et_2O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2e** (26 mg, 0.0482 mmol, 74% yield relative to (S)-**8e**) as a white solid: ^1H NMR (400 MHz, MeOH- d_4) δ 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) δ 0.43; HRMS (ESI): m/z calcd. for ($\text{C}_{25}\text{H}_{51}\text{NO}_9\text{P}$) [$\text{M} + \text{H}$] $^+$ 540.3296; found, 540.3298.



(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl icosanoate (Compound (S)-2f): Following the general procedure (**C2**), (S)-**9f** (25 mg), TFA (0.200 mL) and CH₂Cl₂ (0.350 mL) were used. The product was washed with *n*-pentane: Et₂O (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: ¹H NMR (400 MHz, MeOH-d₄ + CDCl₃ (1:2 ratio)) δ 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); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.43; HRMS (ESI): m/z calcd. for (C₂₇H₅₅NO₉P) [M + H]⁺ 568.3609; found, 568.3610.



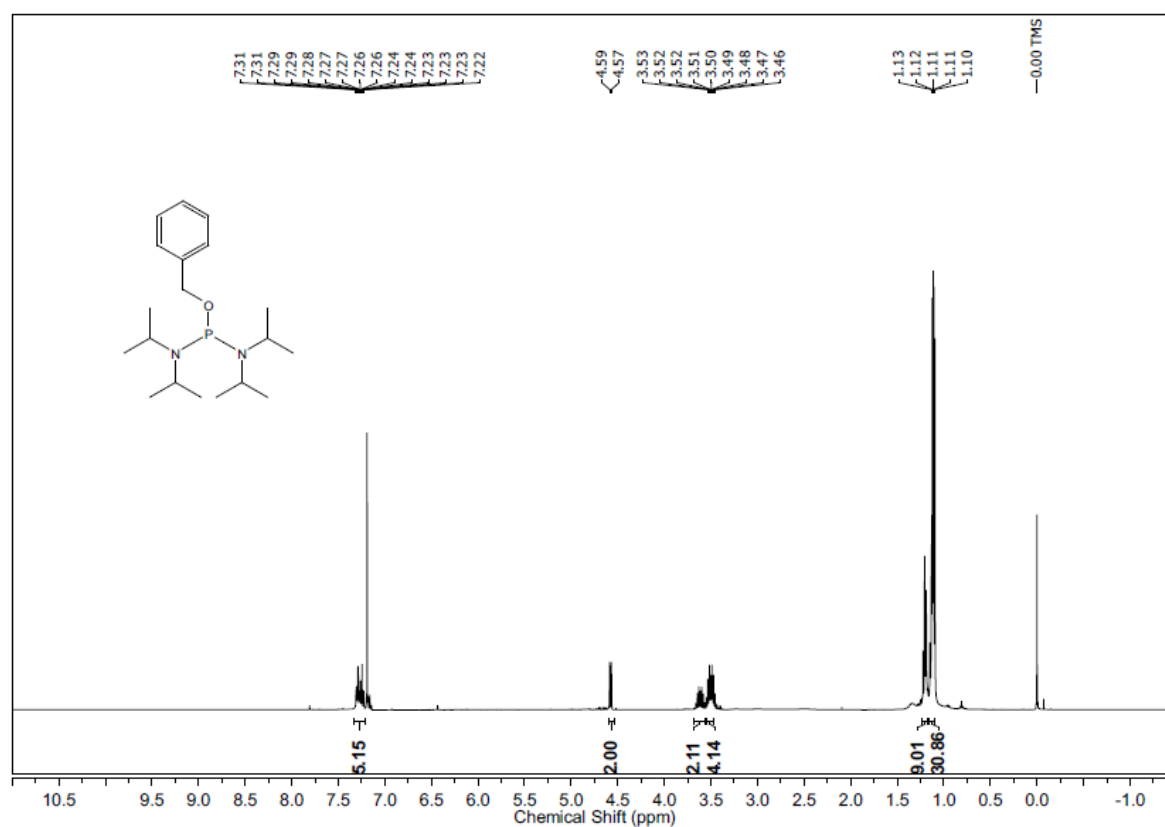
(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl docosanoate (Compound (S)-2g): Following the general procedure (**C2**), (S)-**9g** (30 mg), TFA (0.240 mL) and CH₂Cl₂ (0.420 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2g** (21 mg, 0.0352 mmol, 74% yield relative to (S)-**8g**) as a white solid: ¹H NMR (400 MHz, MeOH-d₄ + CDCl₃ (1:2 ratio)) δ 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* = 7.4 Hz, 2H), 1.59 (m, 2H), 1.33-1.19 (m, 36H), 0.85 (t, *J* = 6.3 Hz, 3H); ³¹P NMR (400 MHz, MeOH-d₄) δ 0.45; HRMS (ESI): m/z calcd. for (C₂₉H₅₉NO₉P) [M + H]⁺ 596.3922; found, 596.3924.



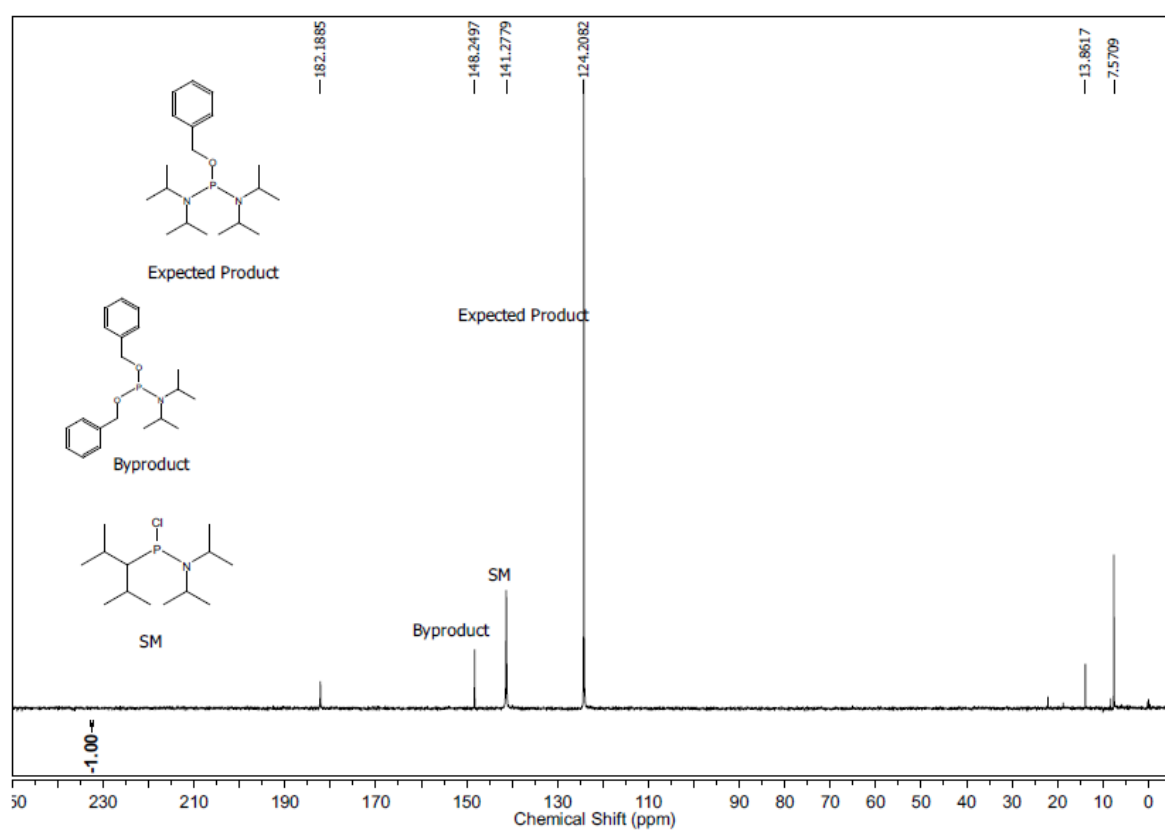
(2S)-3-((((S)-2-amino-3-methoxy-3-oxopropoxy)(hydroxy)phosphoryl)oxy)-2-hydroxypropyl tetracosanoate (Compound (S)-2h): Following the general procedure (**C2**), (S)-**9h** (26 mg), TFA (0.210 mL) and CH₂Cl₂ (0.360 mL) were used. The product was washed with *n*-pentane: Et₂O (4:1) (3 x 2 mL) to yield the desired final compound (S)-**2h** (15 mg, 0.0240 mmol, 81% yield relative to (S)-**8h**) as a white solid: ¹H NMR (400 MHz, MeOH-

$d_4 + CDCl_3$ (1:2 ratio)) δ 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, $MeOH-d_4$) δ 0.44; HRMS (ESI): m/z calcd. for $(C_{31}H_{63}NO_9P)$ $[M + H]^+$ 624.4235; found, 624.4233.

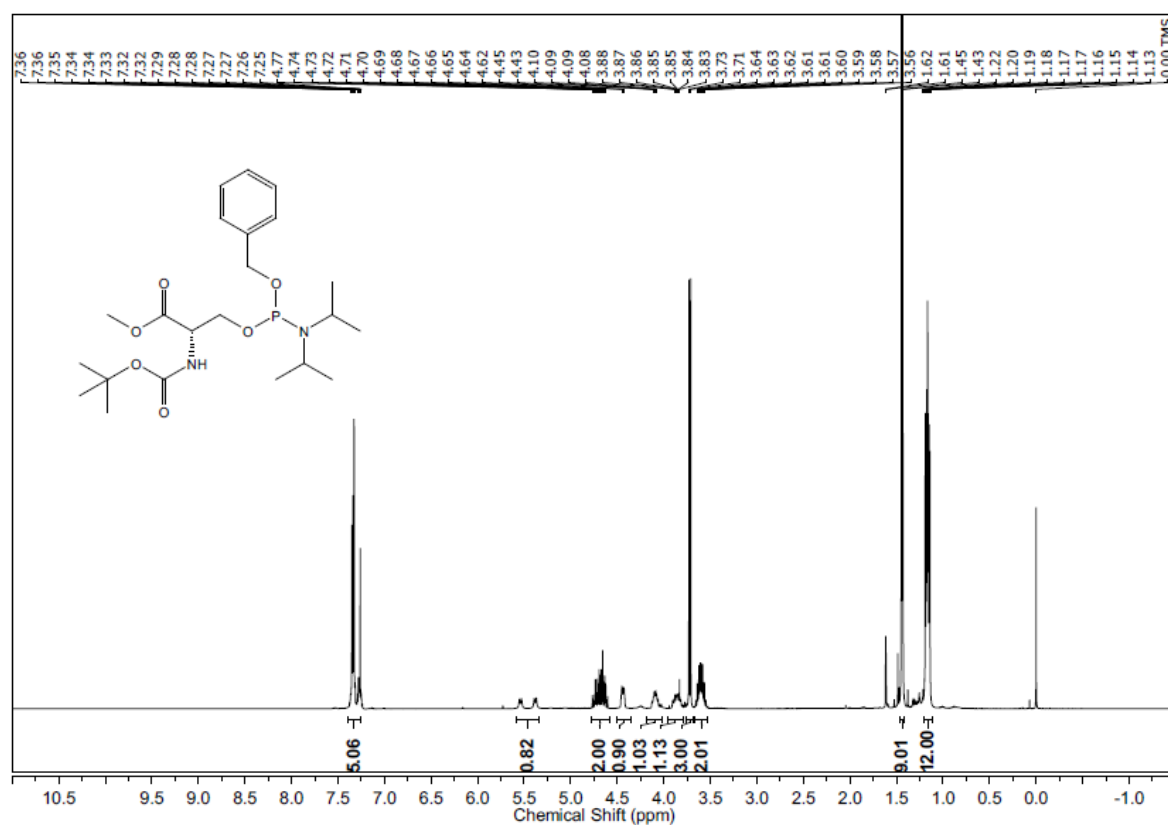
¹H NMR of compound **10**



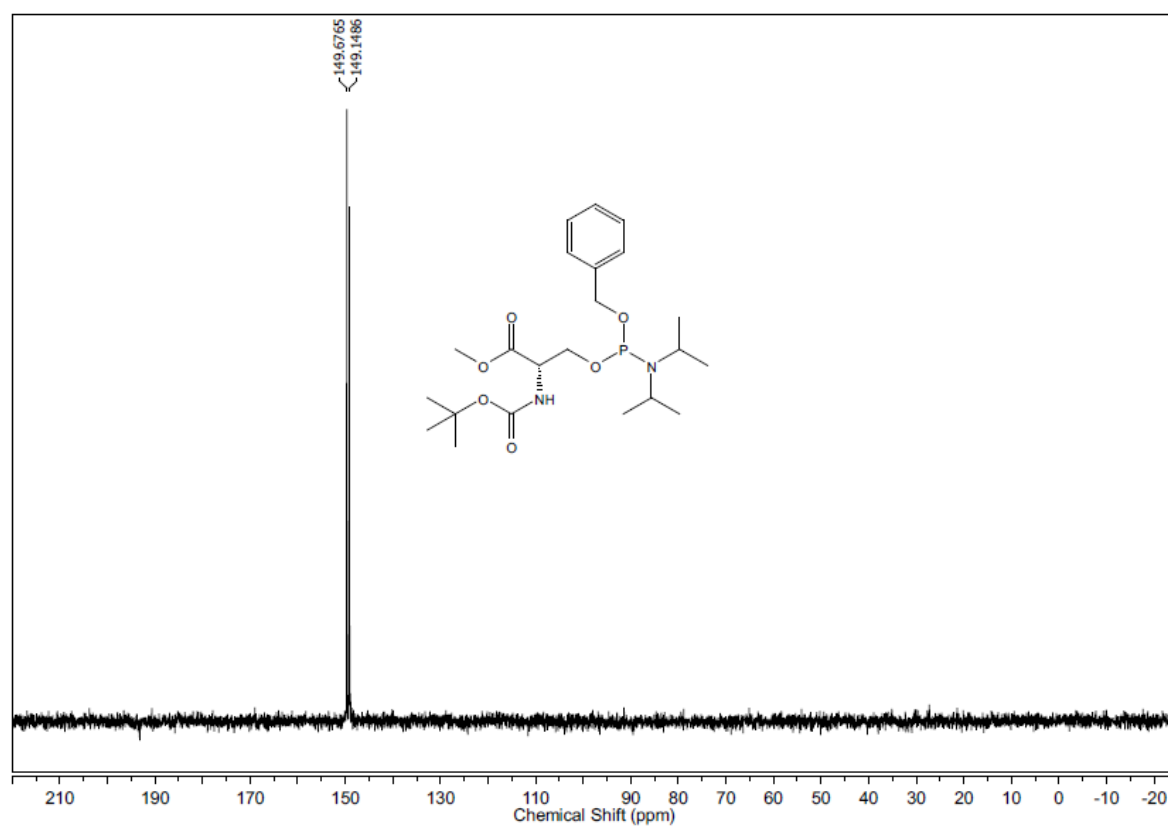
³¹P NMR of compound **10**



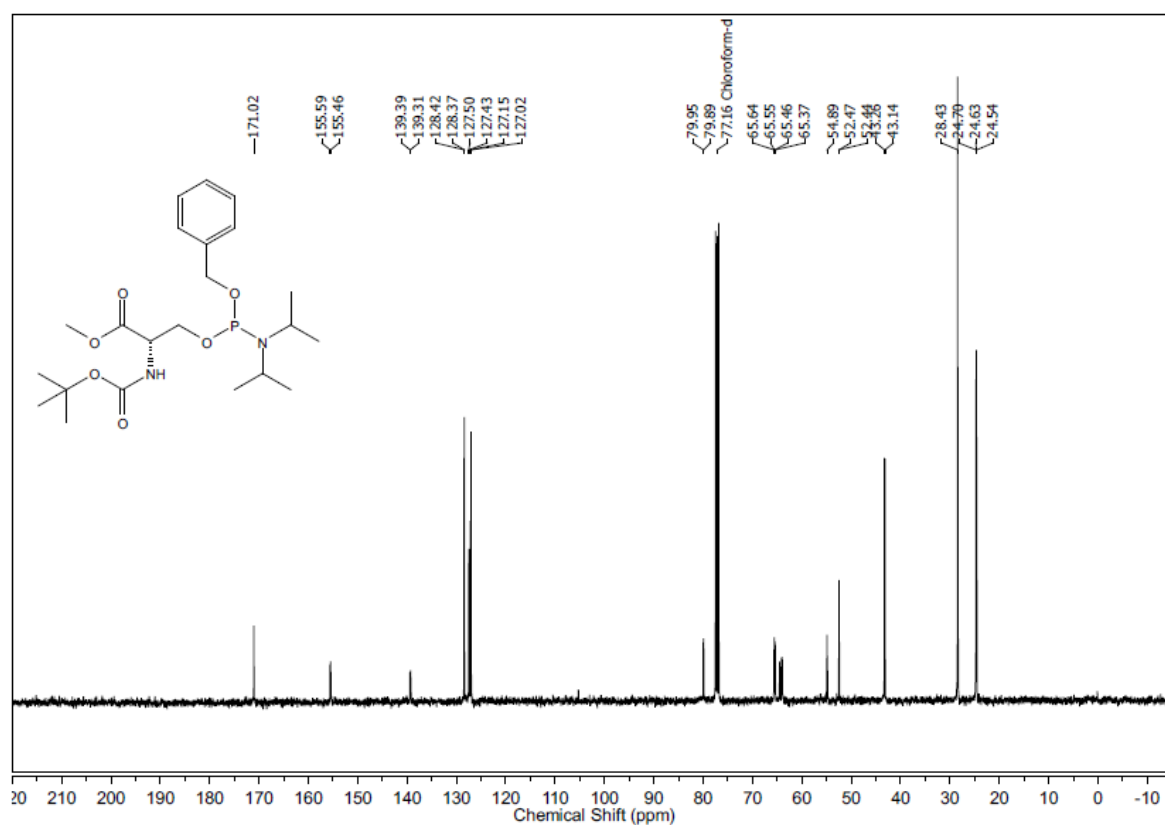
¹H NMR of compound **3**



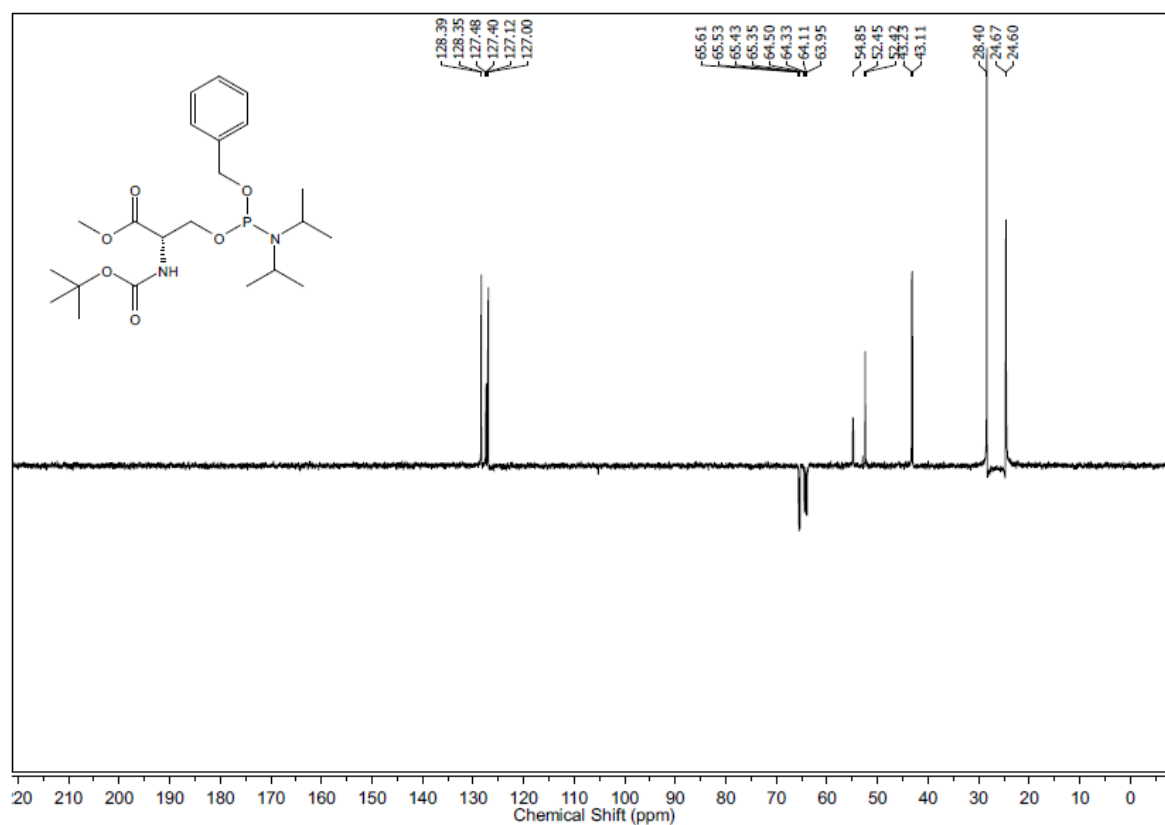
³¹P NMR of compound **3**



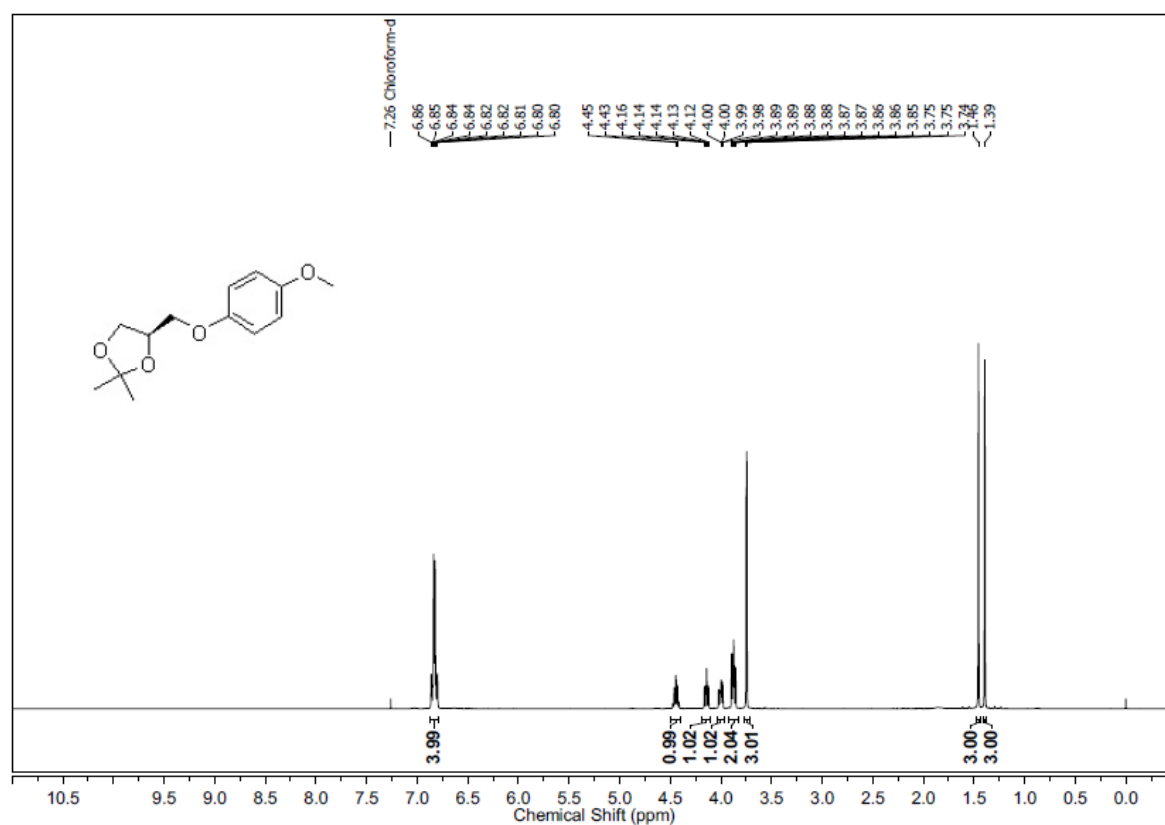
¹³C NMR of compound **3**



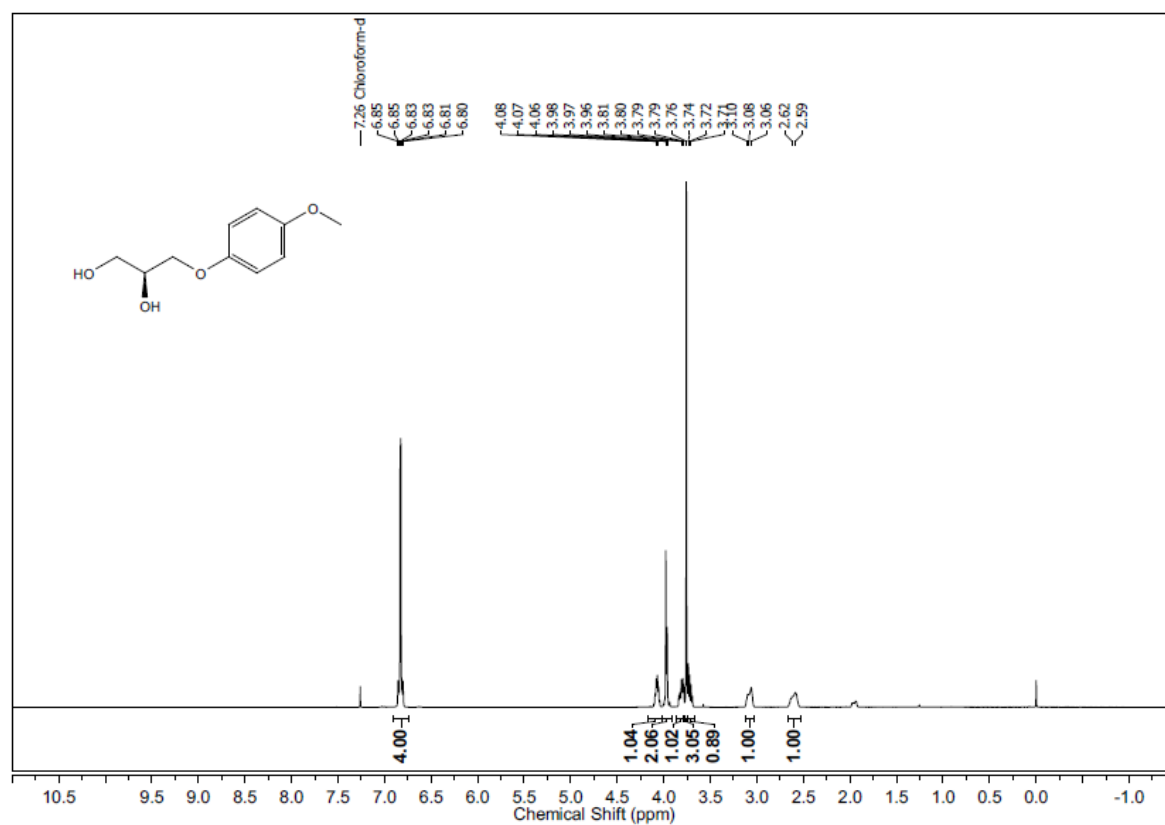
DEPT-135 NMR of compound **3**

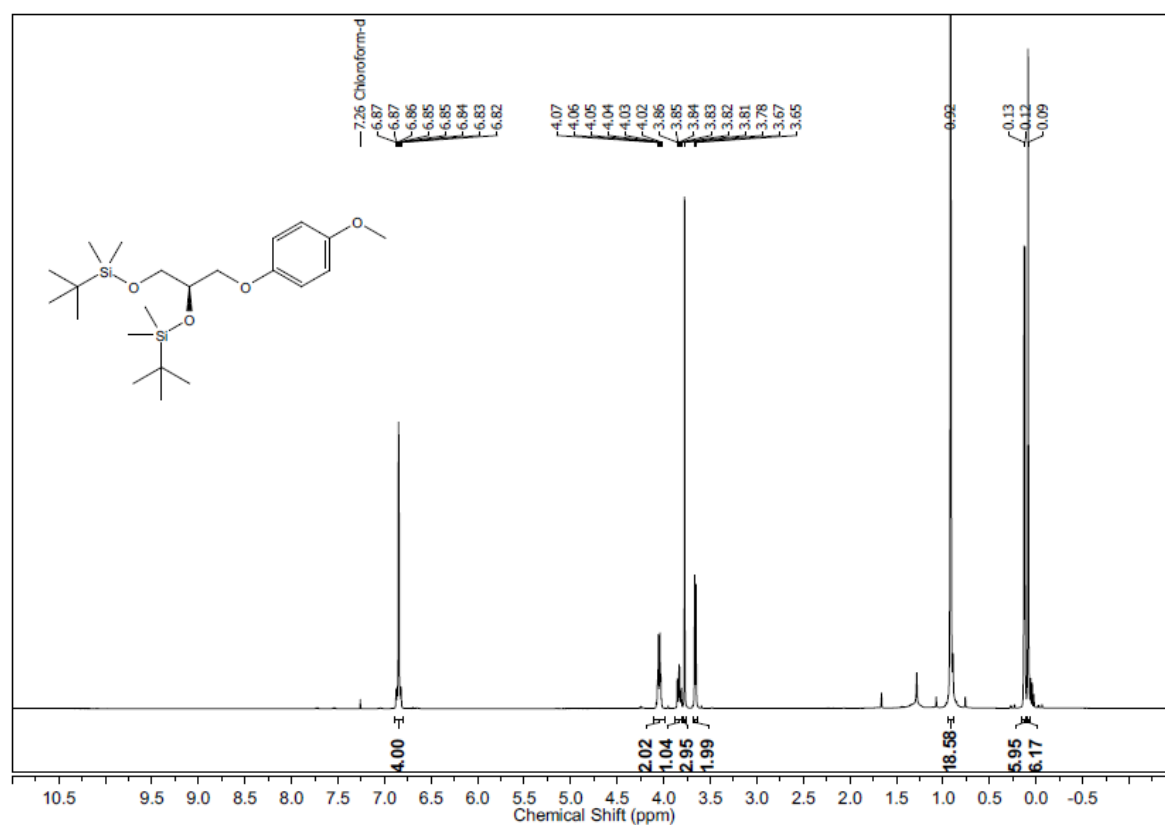


^1H NMR of compound (*R*)-11

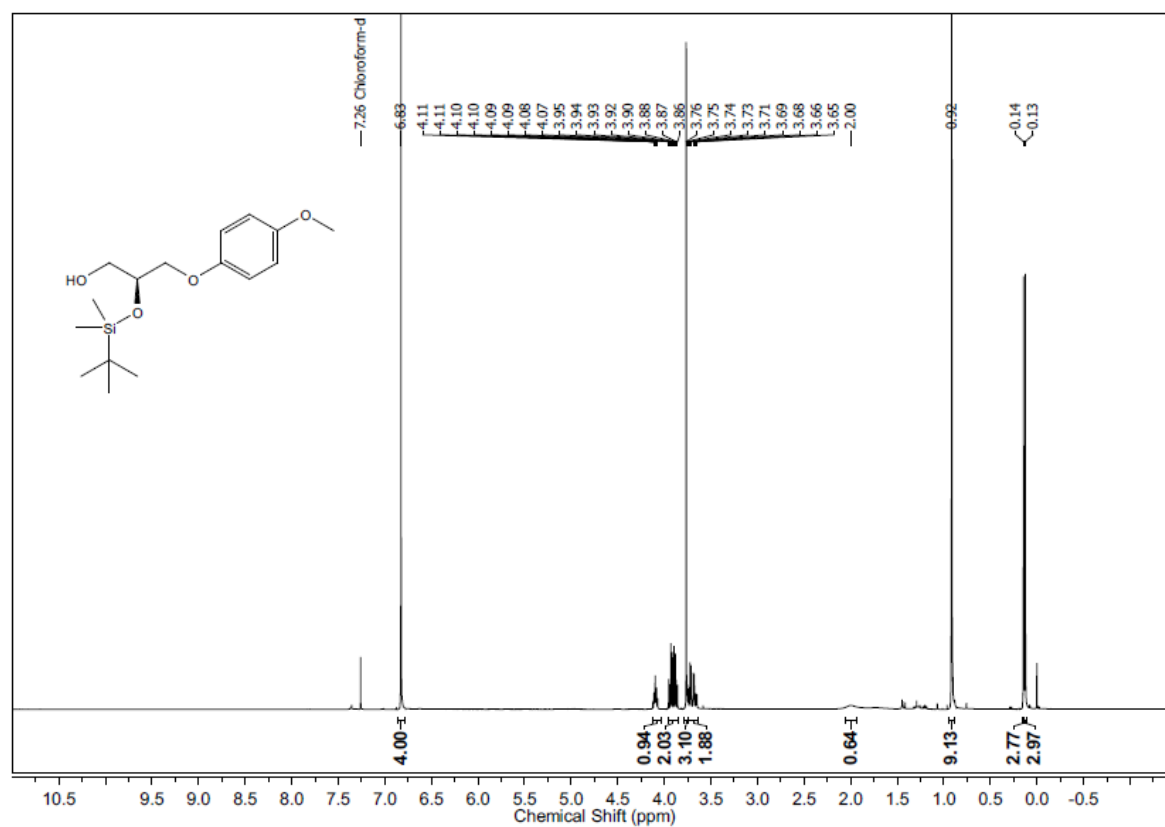


^1H NMR of compound (*R*)-12

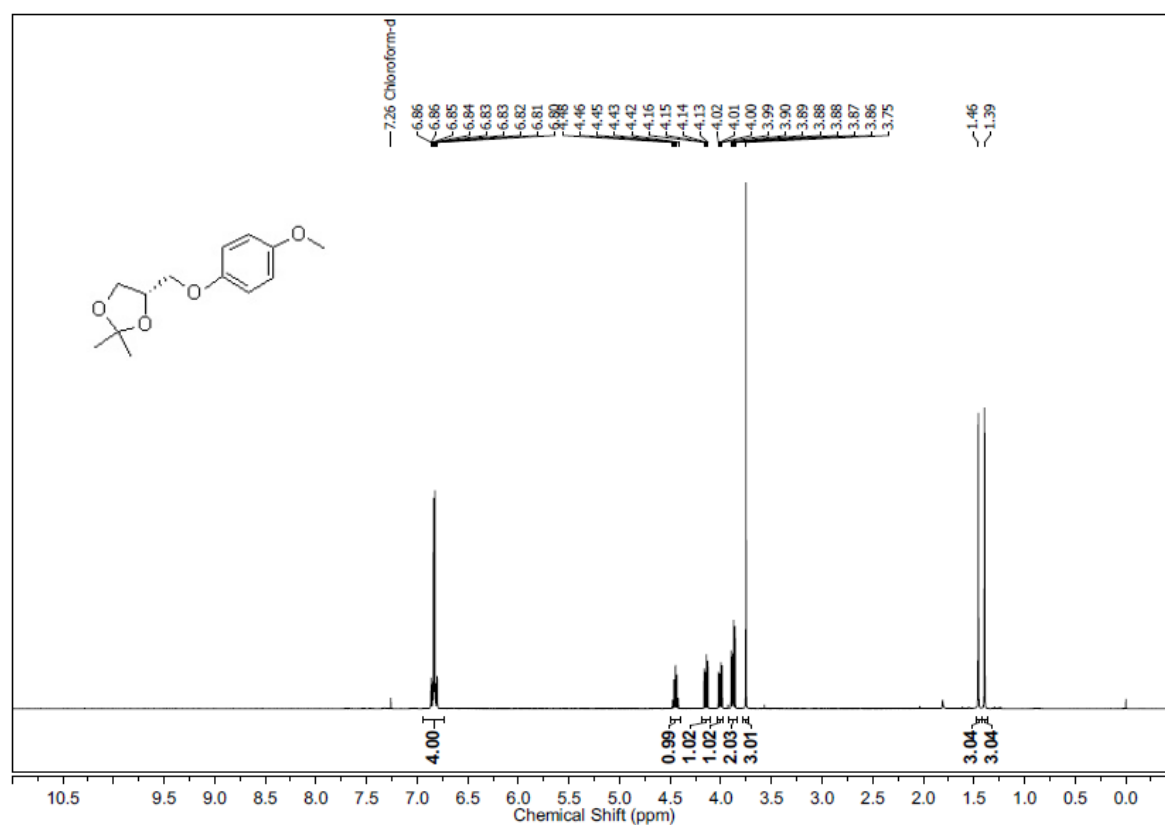


¹H NMR of compound (*R*)-**13**

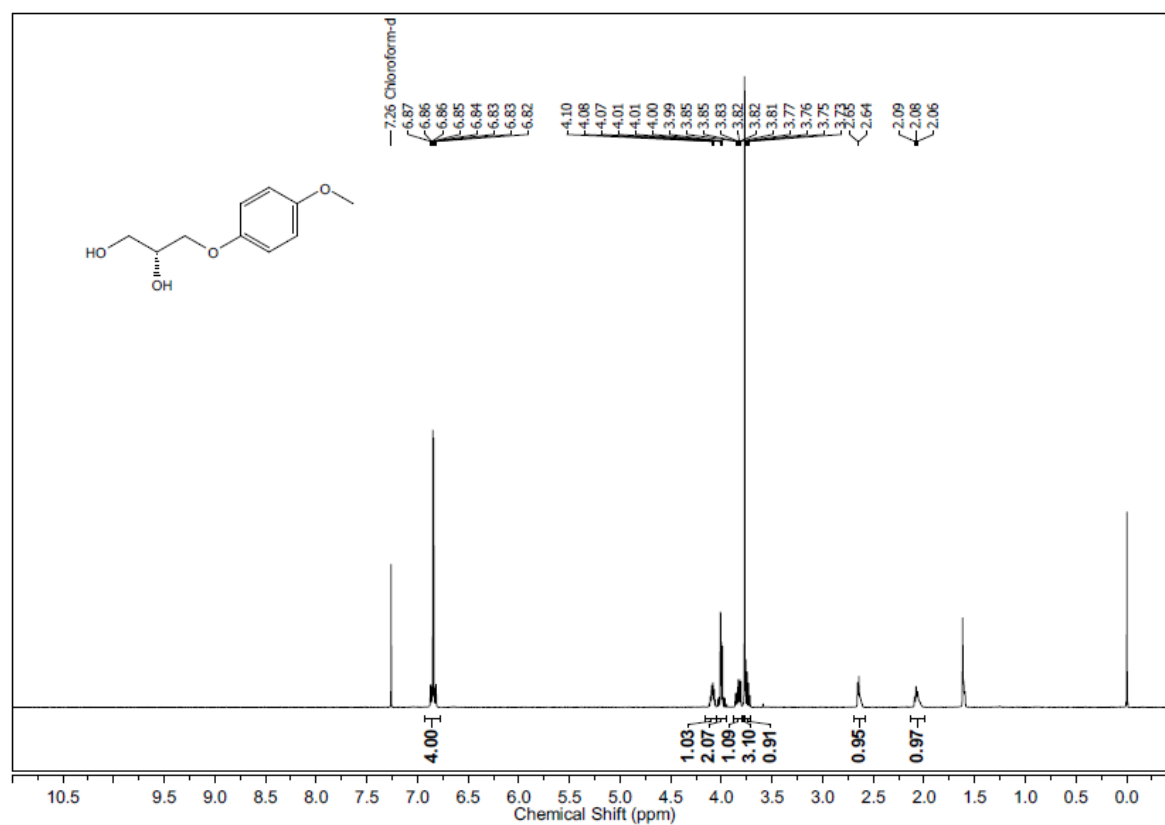
¹H NMR of compound (*R*)-**4**



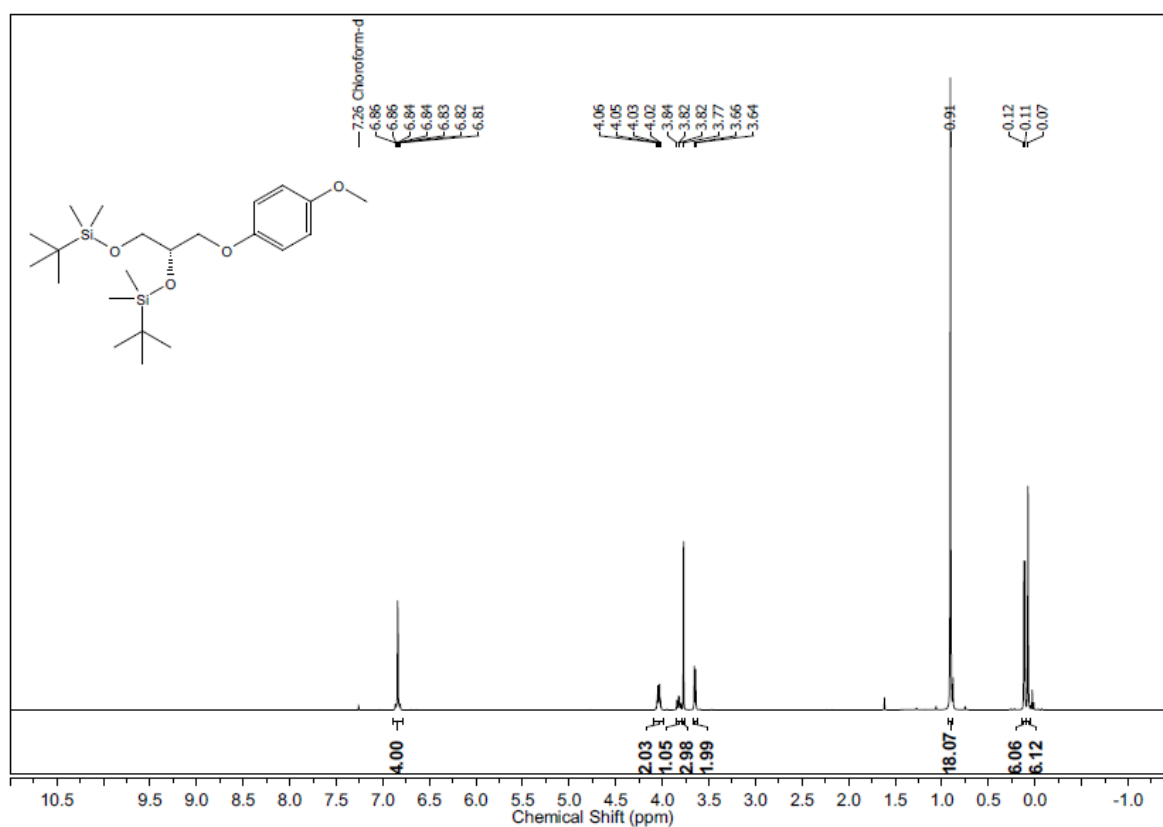
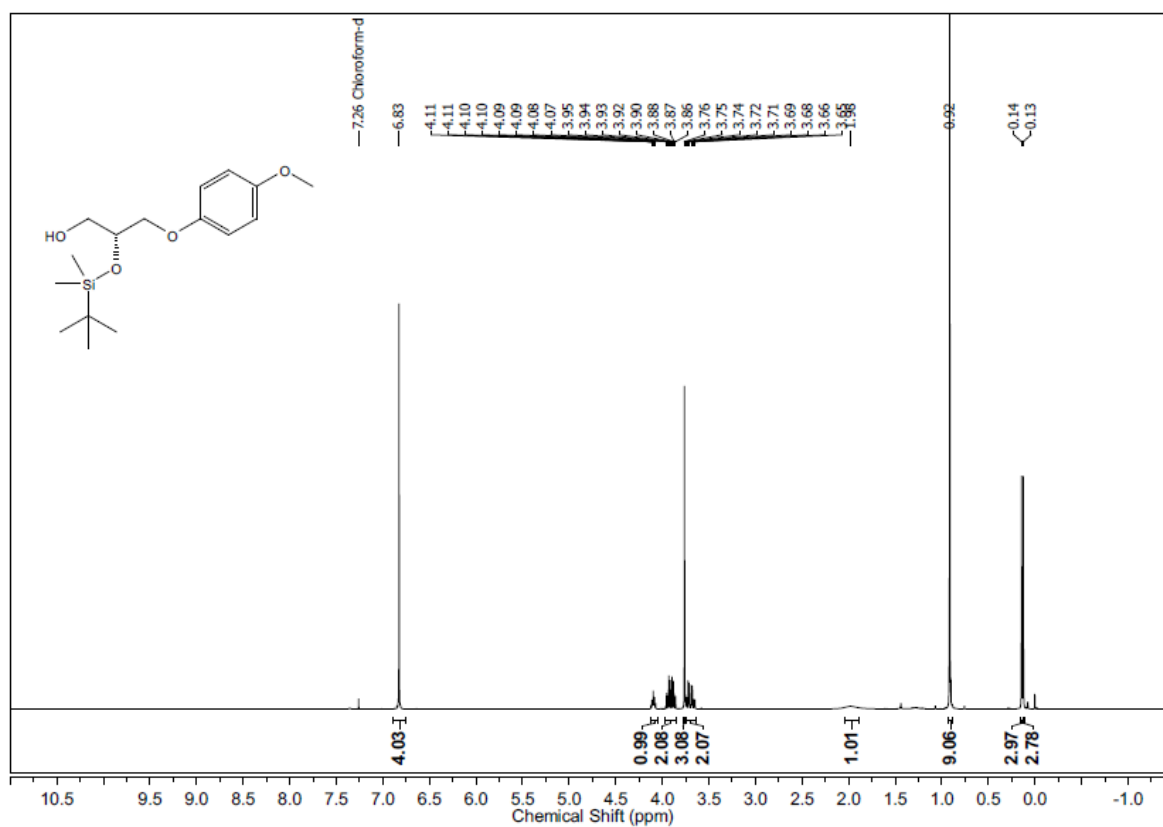
¹H NMR of compound (S)-11

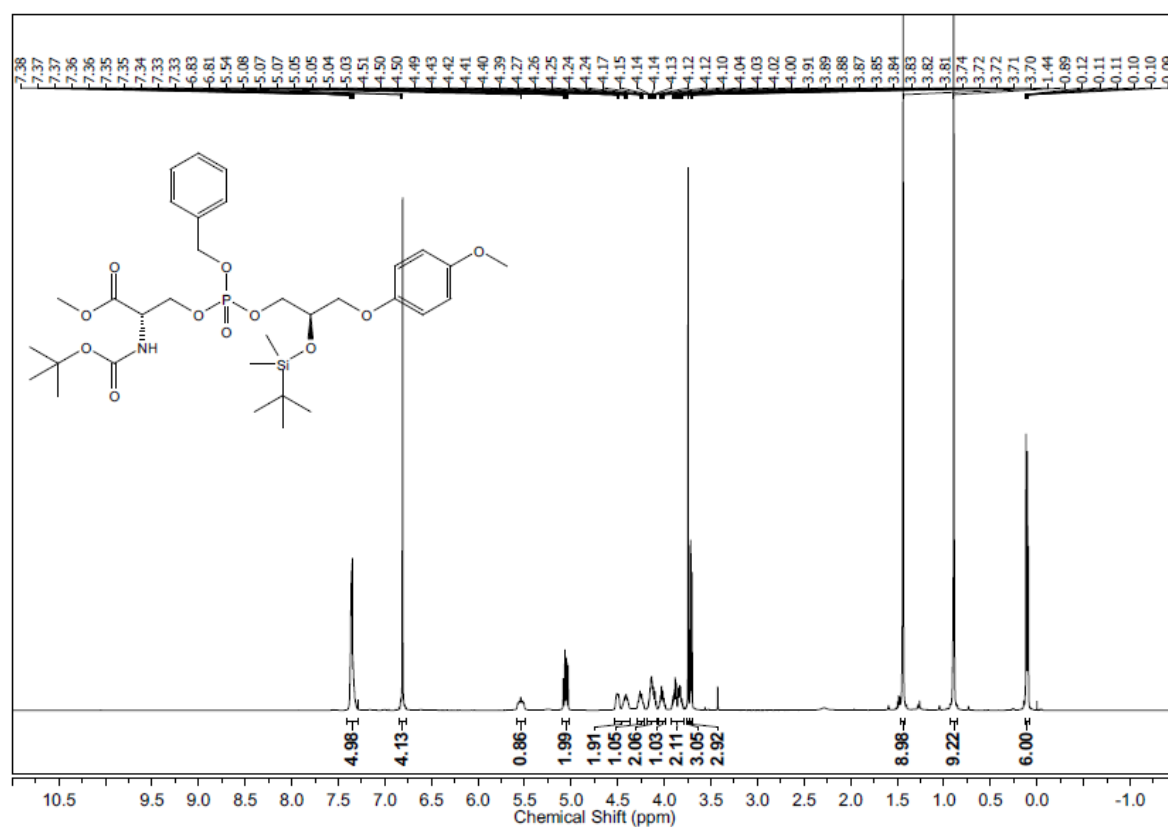
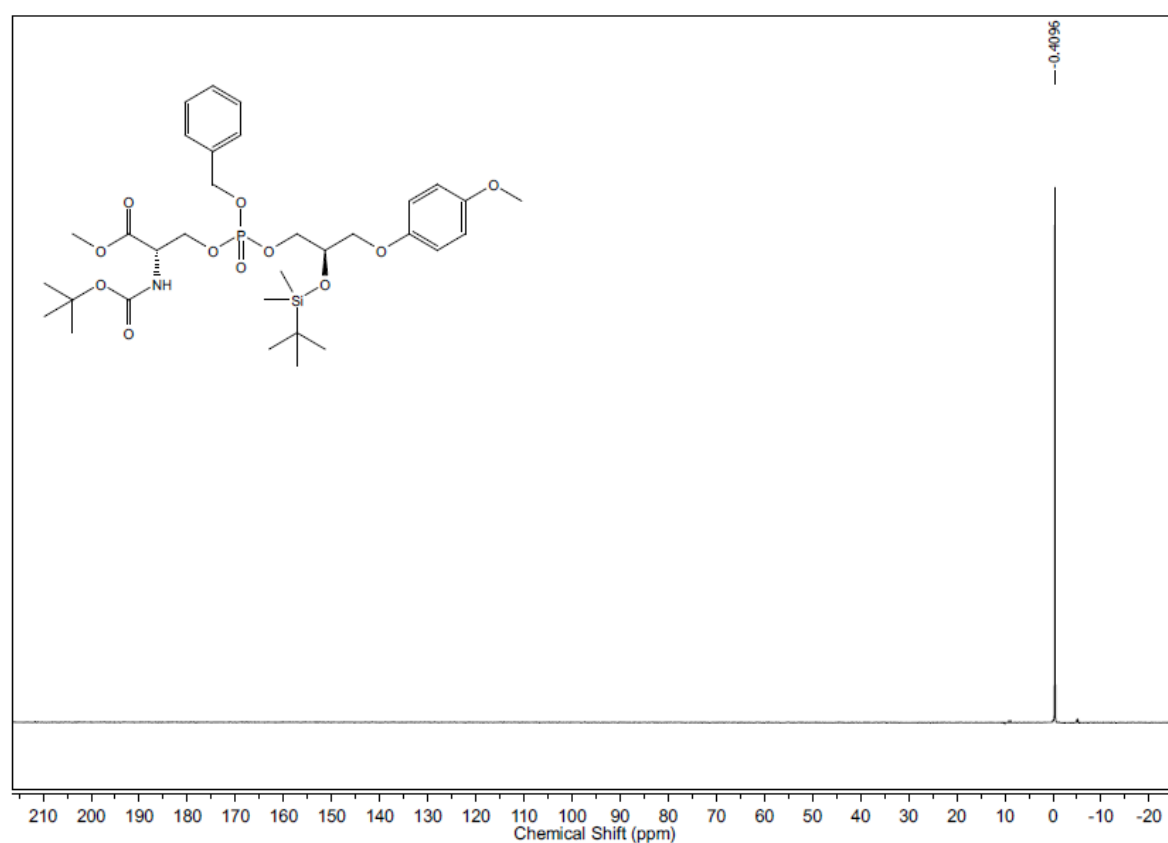


¹H NMR of compound (S)-12

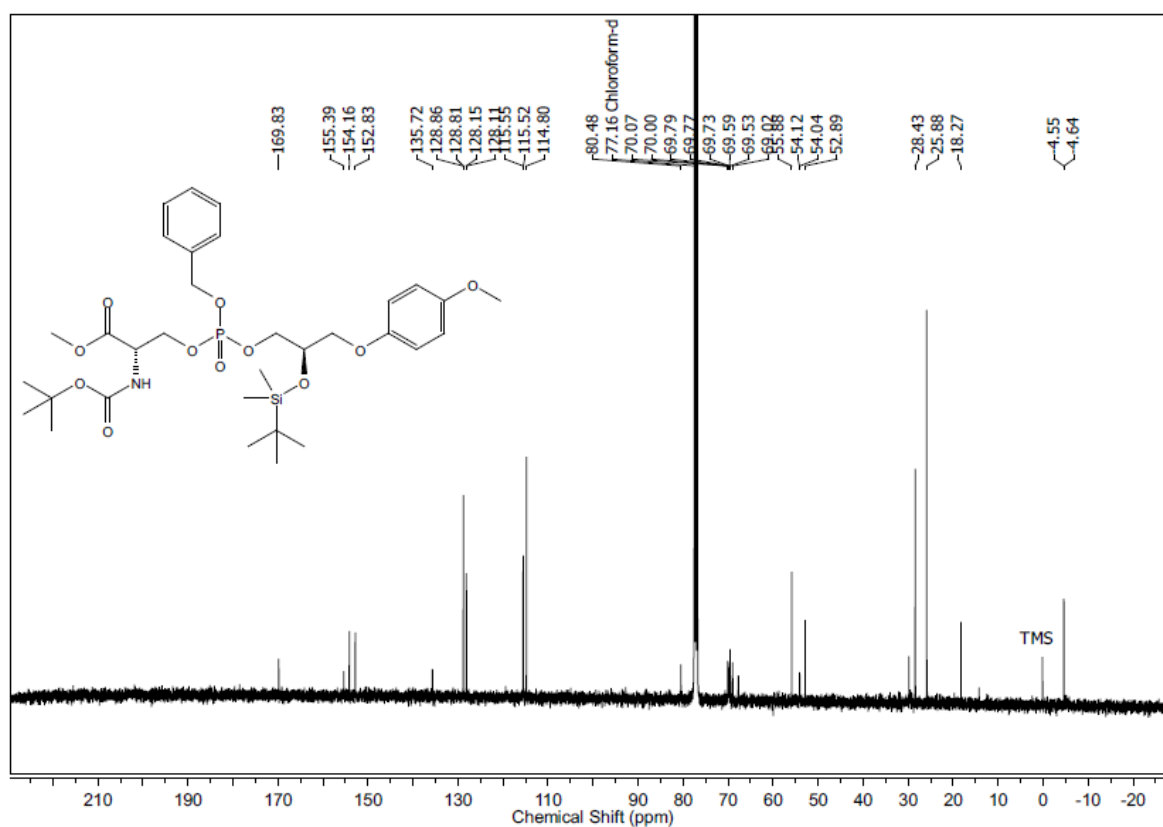


¹H NMR of compound (S)-13

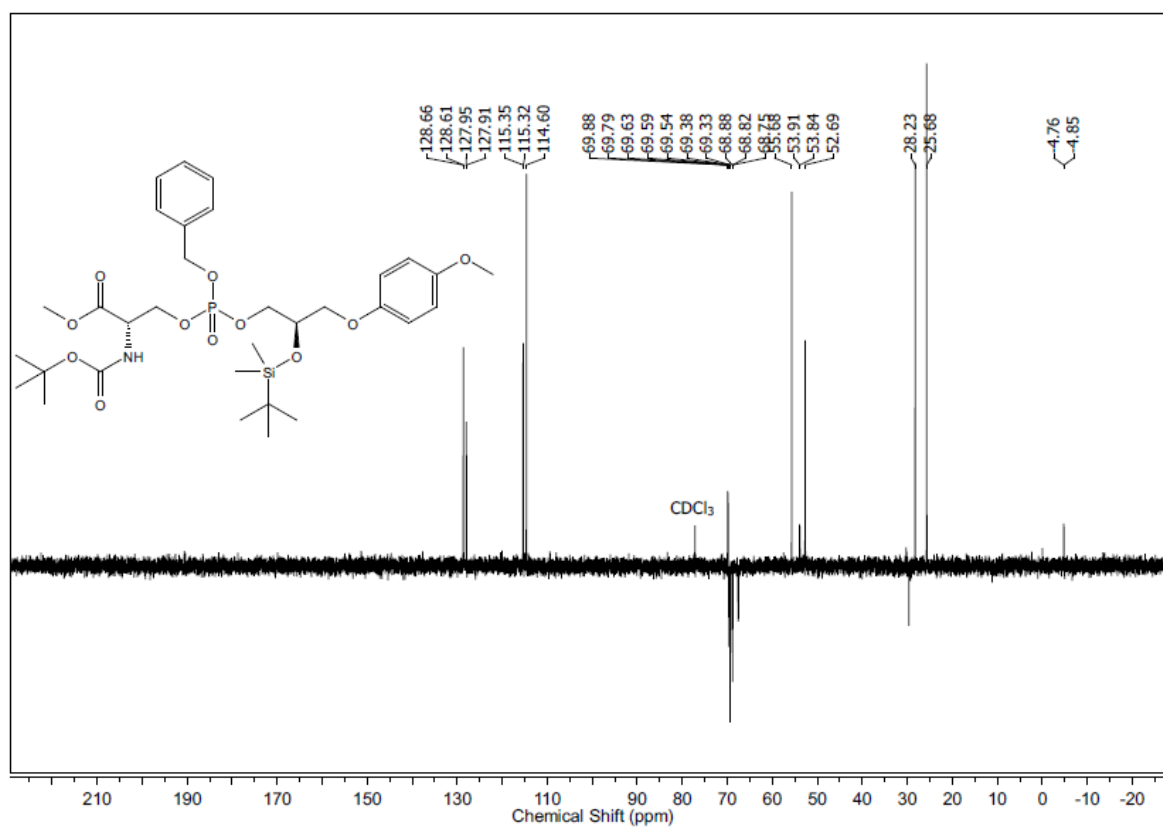
¹H NMR of compound (S)-4

¹H NMR of compound (*R*)-**5**³¹P NMR of compound (*R*)-**5**

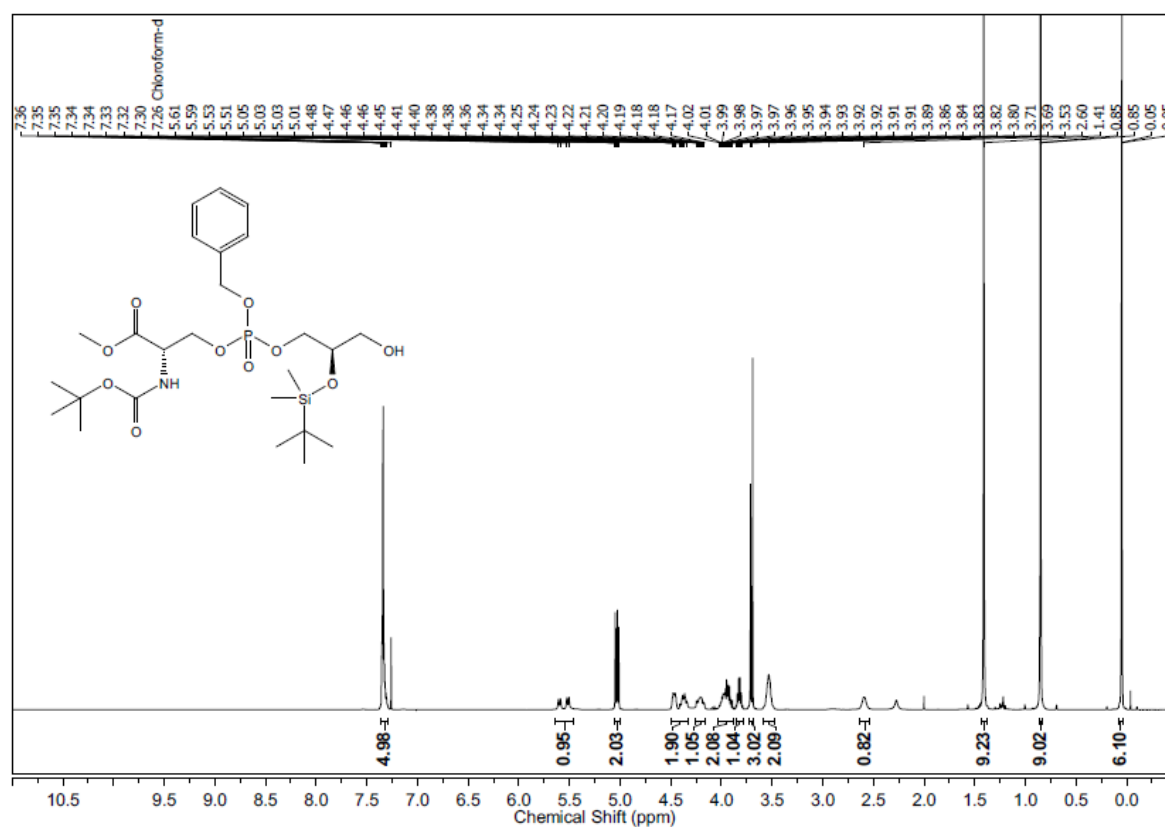
^{13}C NMR of compound (*R*)-5



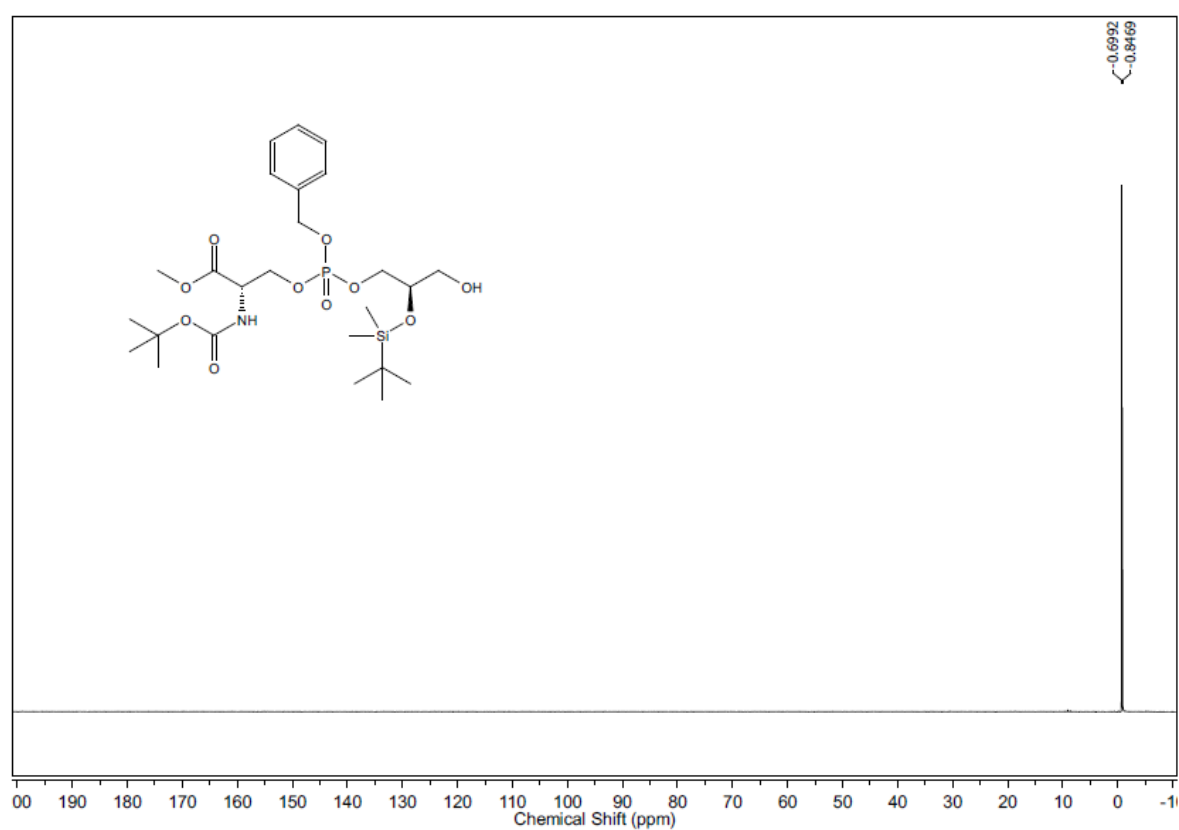
DEPT-135 NMR of compound (*R*)-5



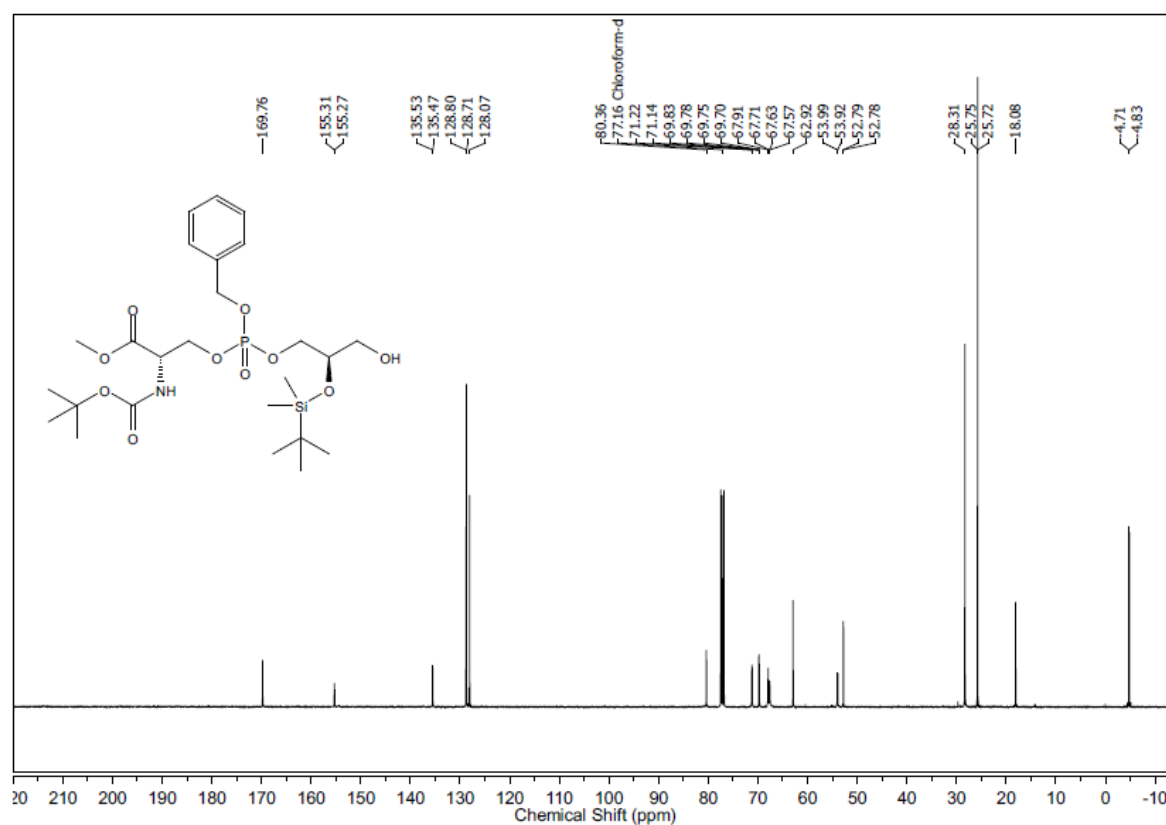
¹H NMR of compound (R)-6



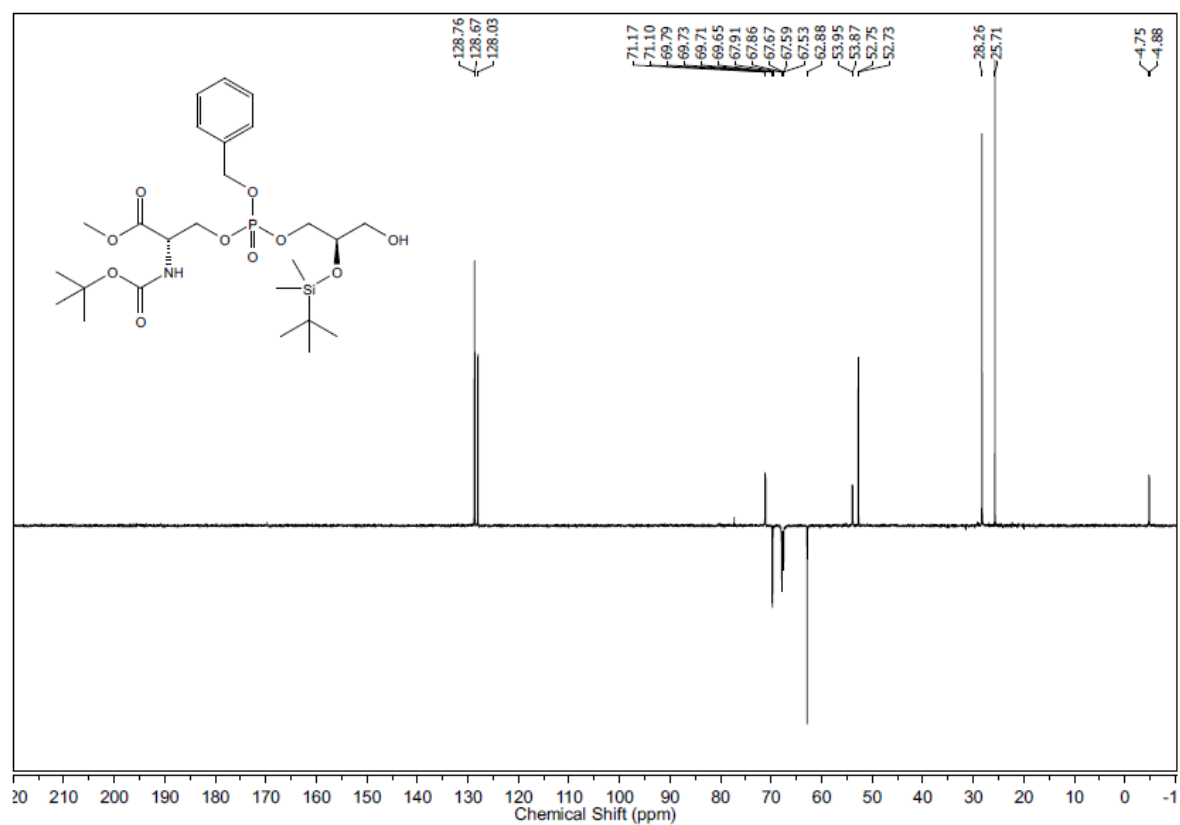
³¹P NMR of compound (R)-6



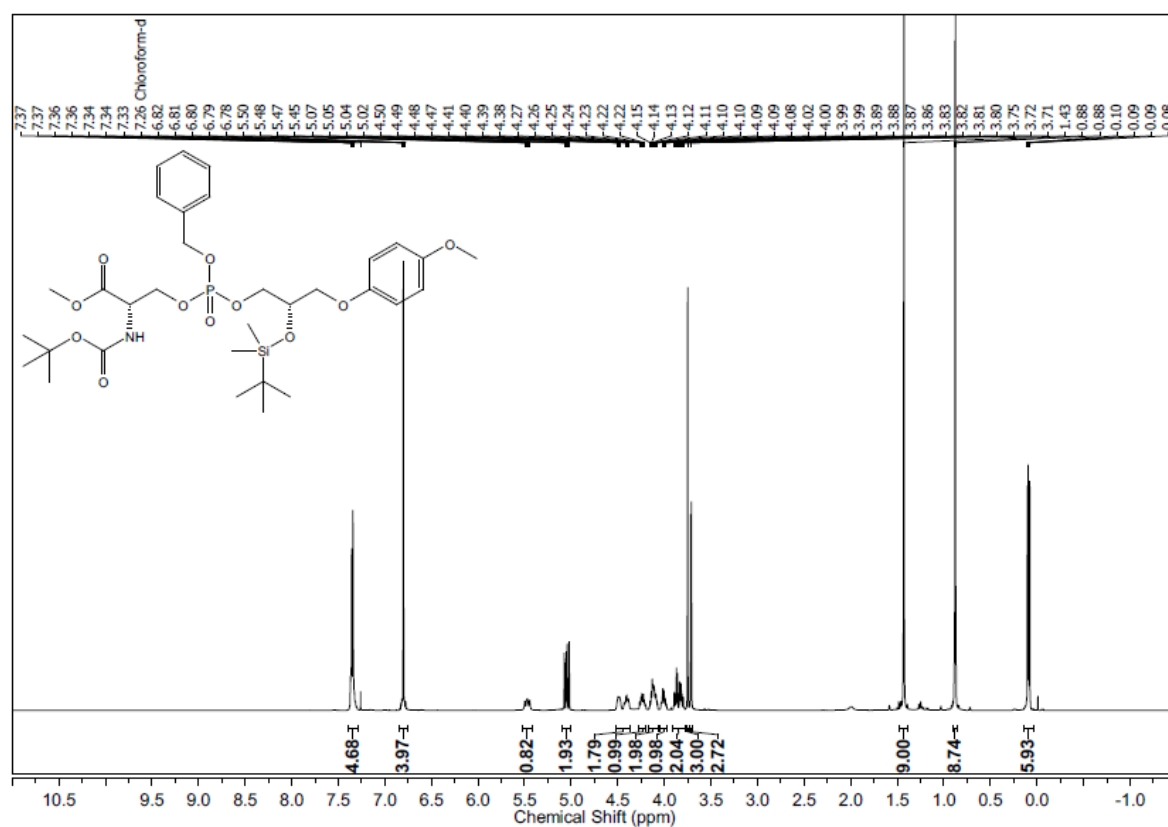
^{13}C NMR of compound (*R*)-6



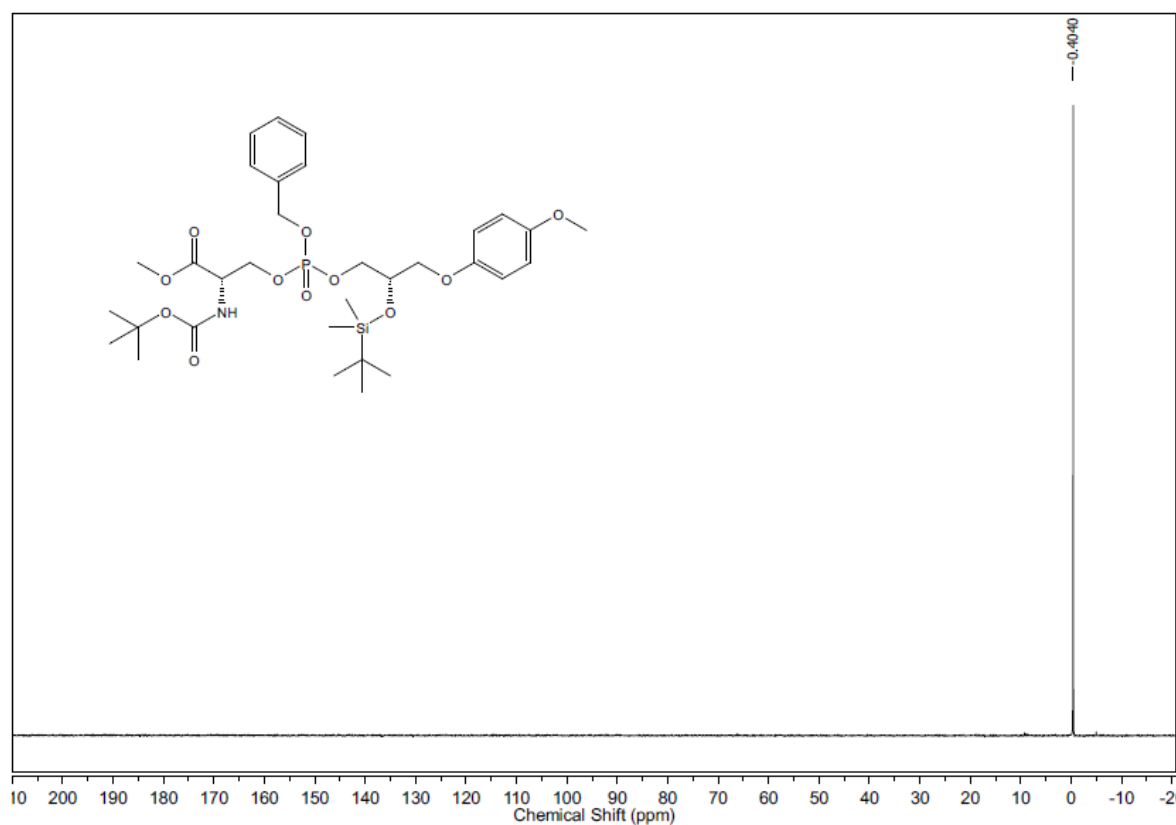
DEPT-135 NMR of compound (*R*)-6



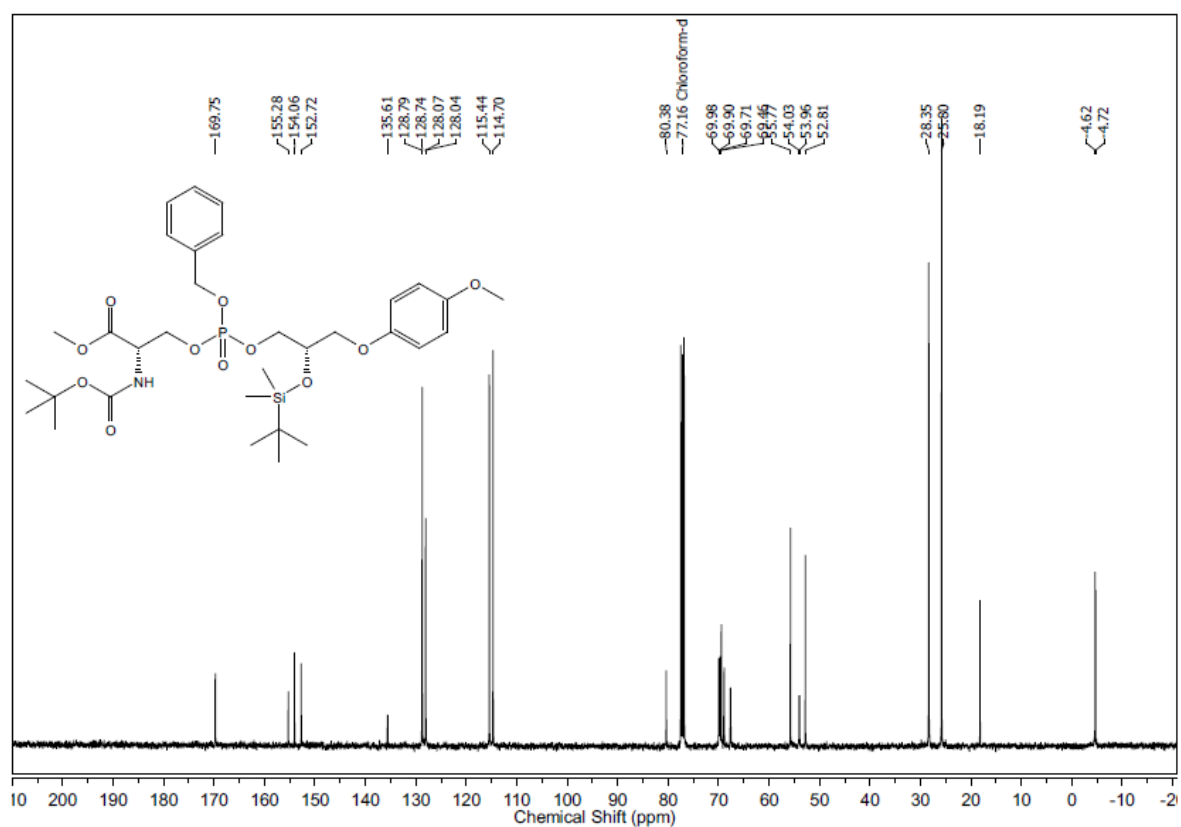
¹H NMR of compound (S)-5



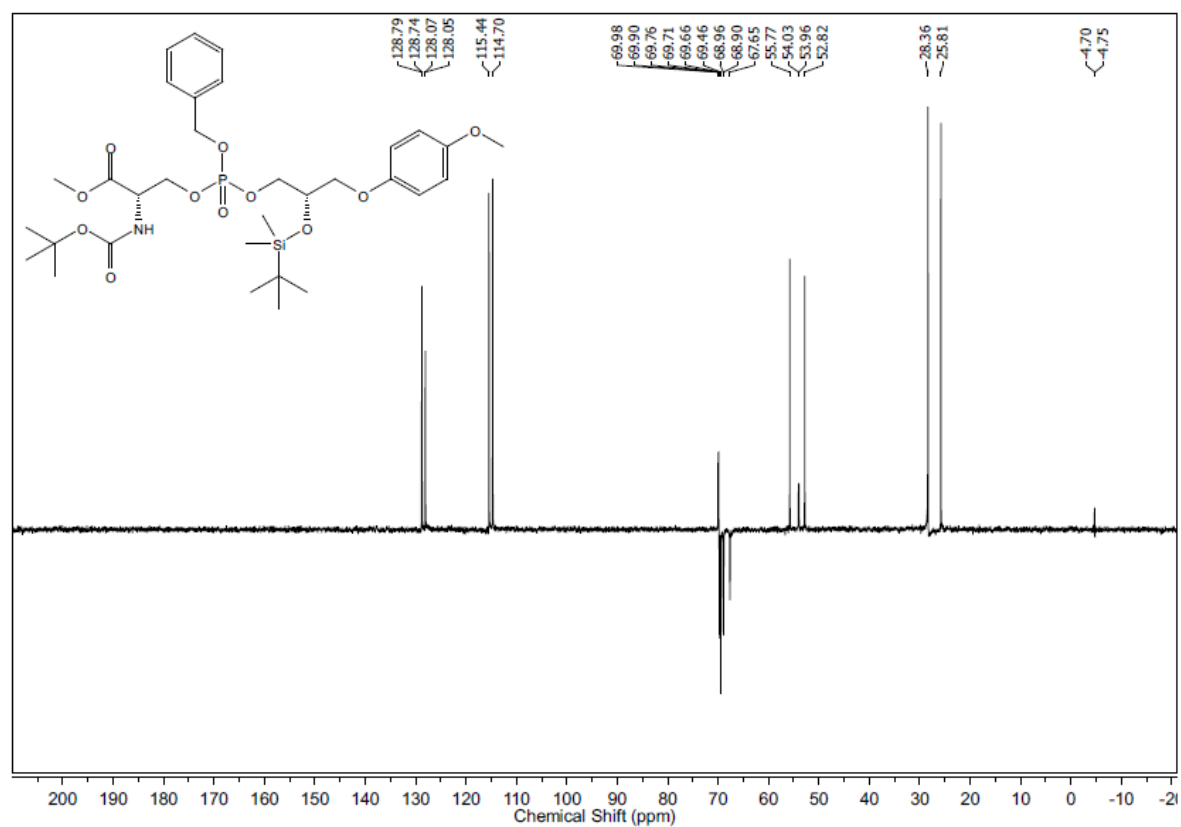
³¹P NMR of compound (S)-5



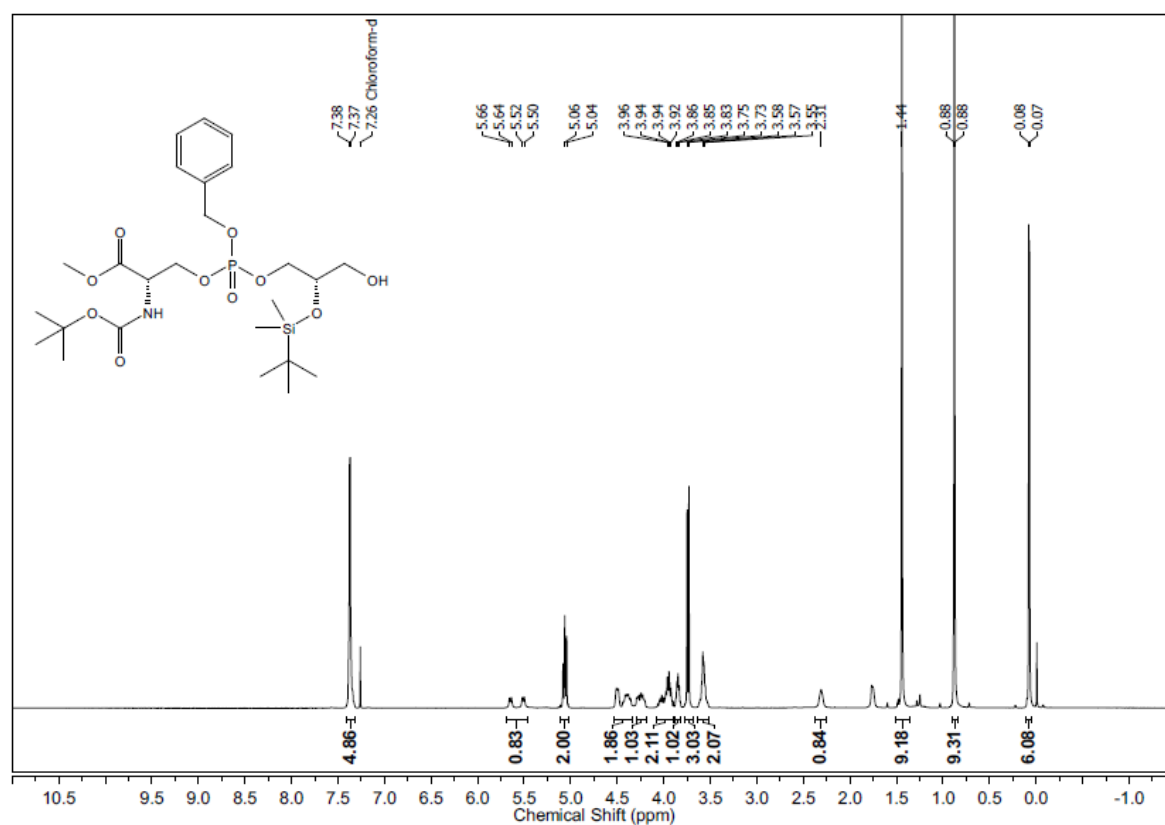
^{13}C NMR of compound (S)-5



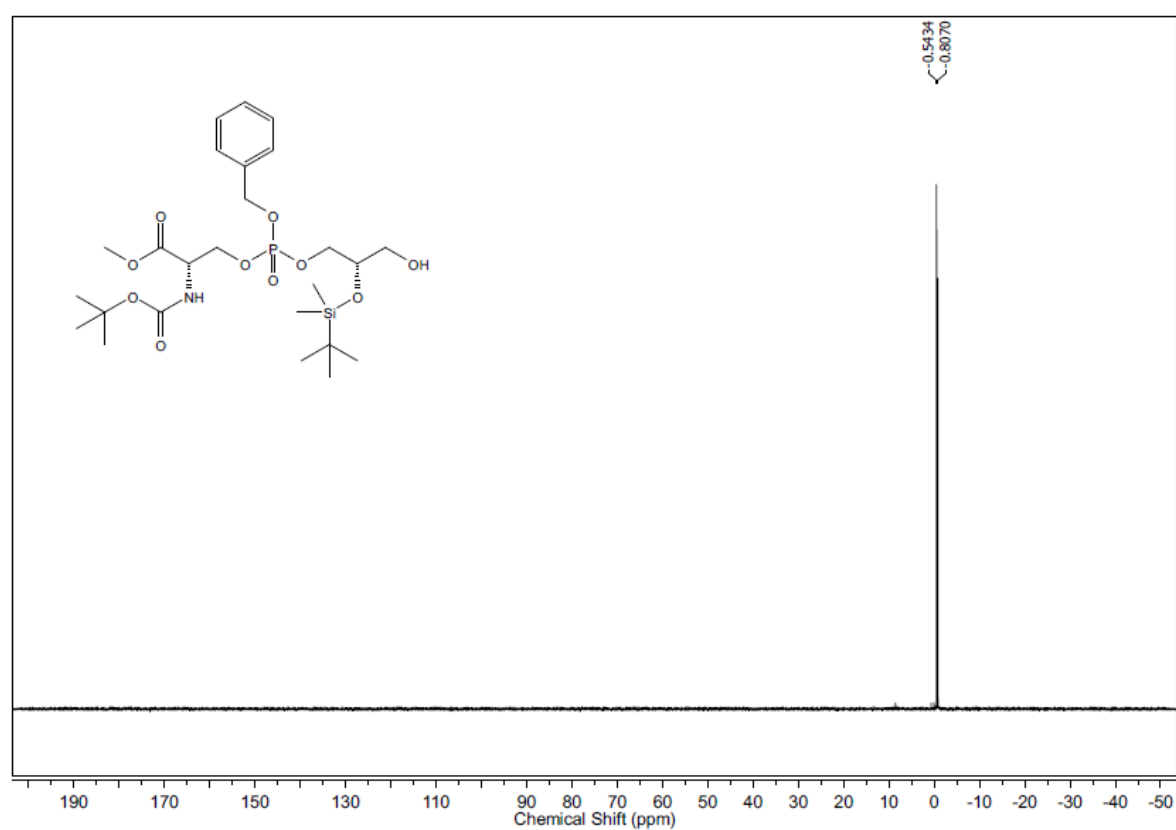
DEPT-135 NMR of compound (S)-5



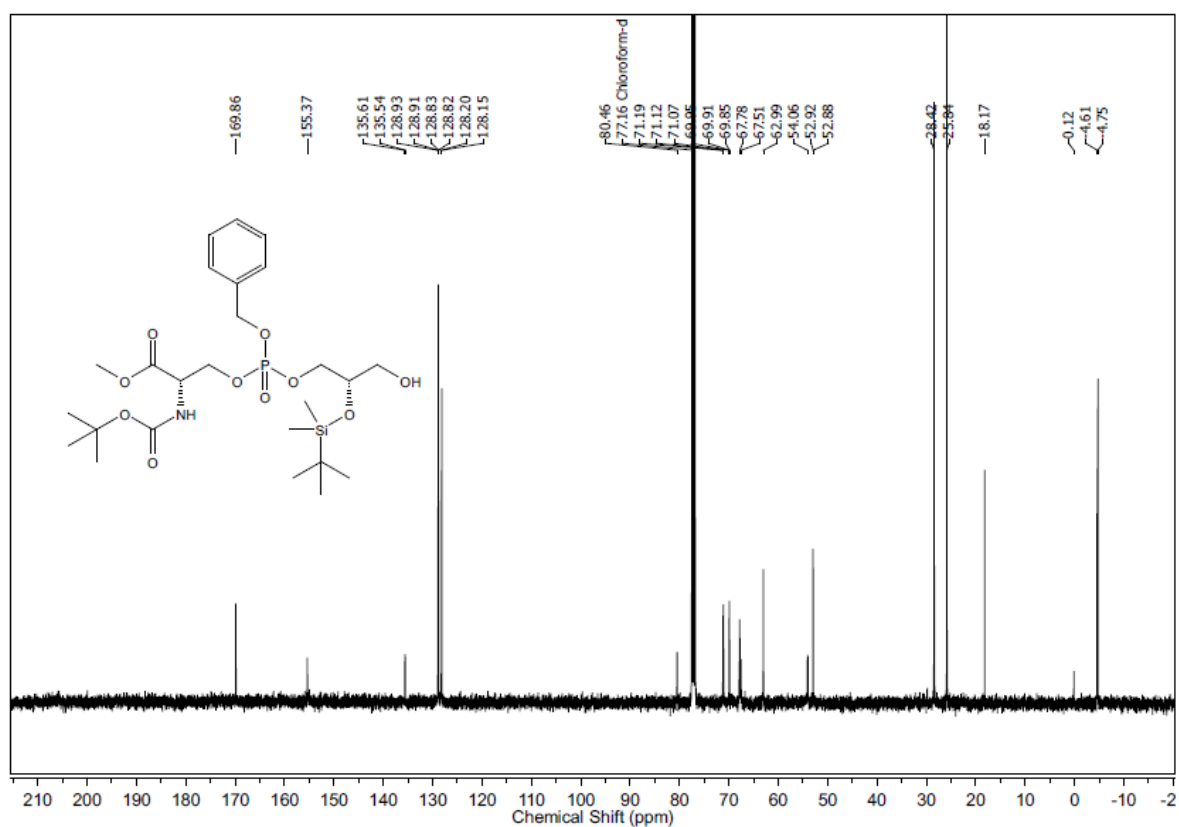
¹H NMR of compound (S)-6



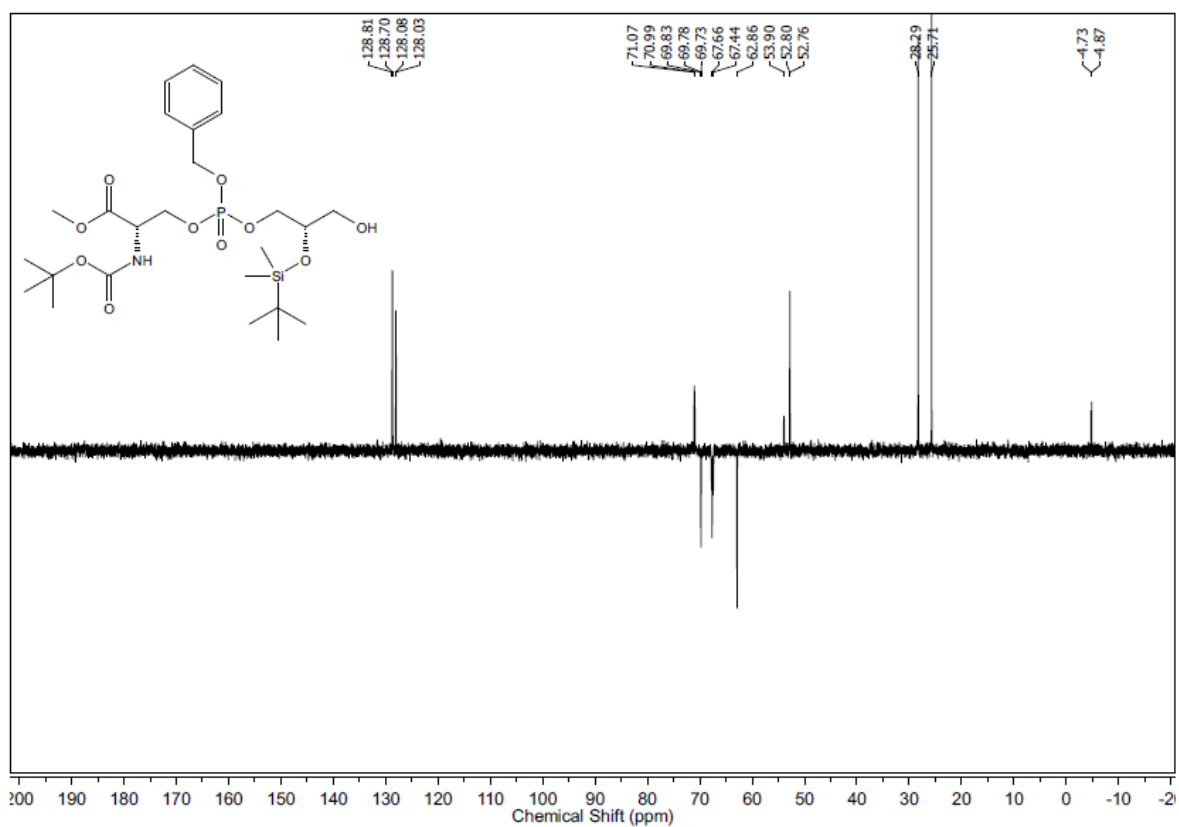
³¹P NMR of compound (S)-6



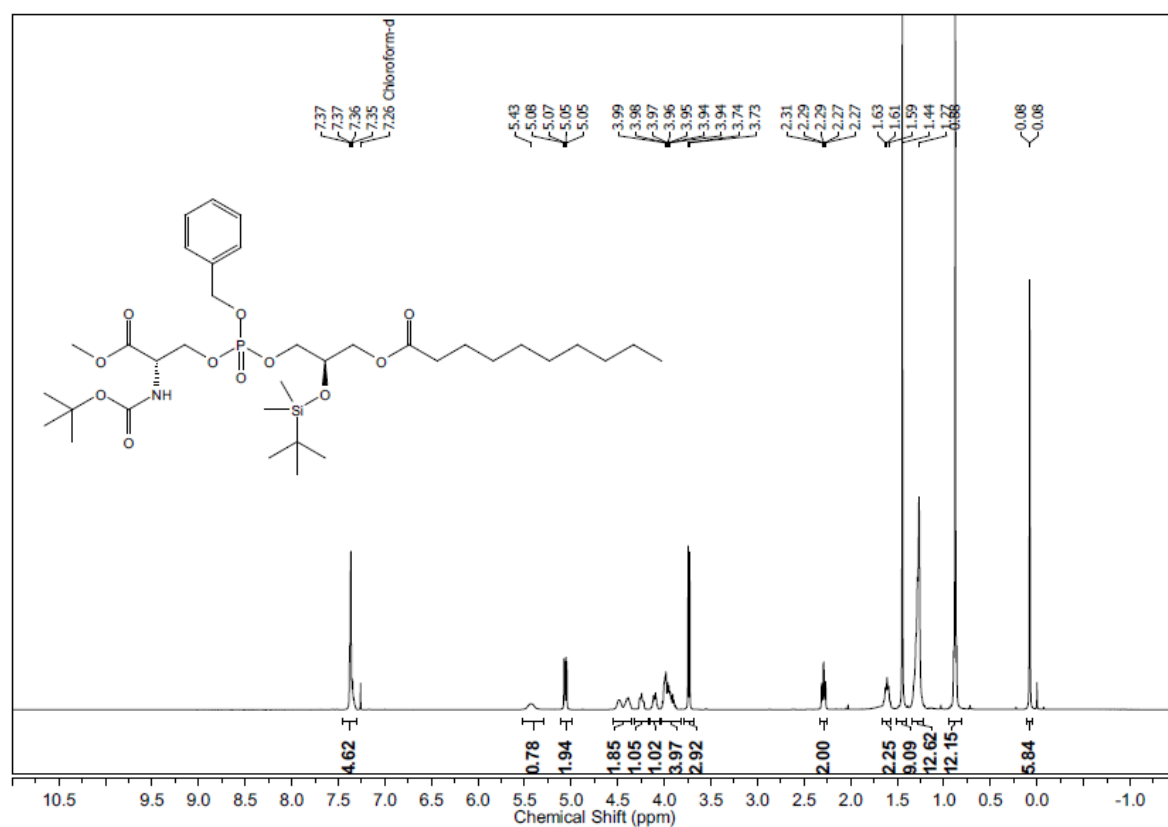
¹³C NMR of compound (S)-6



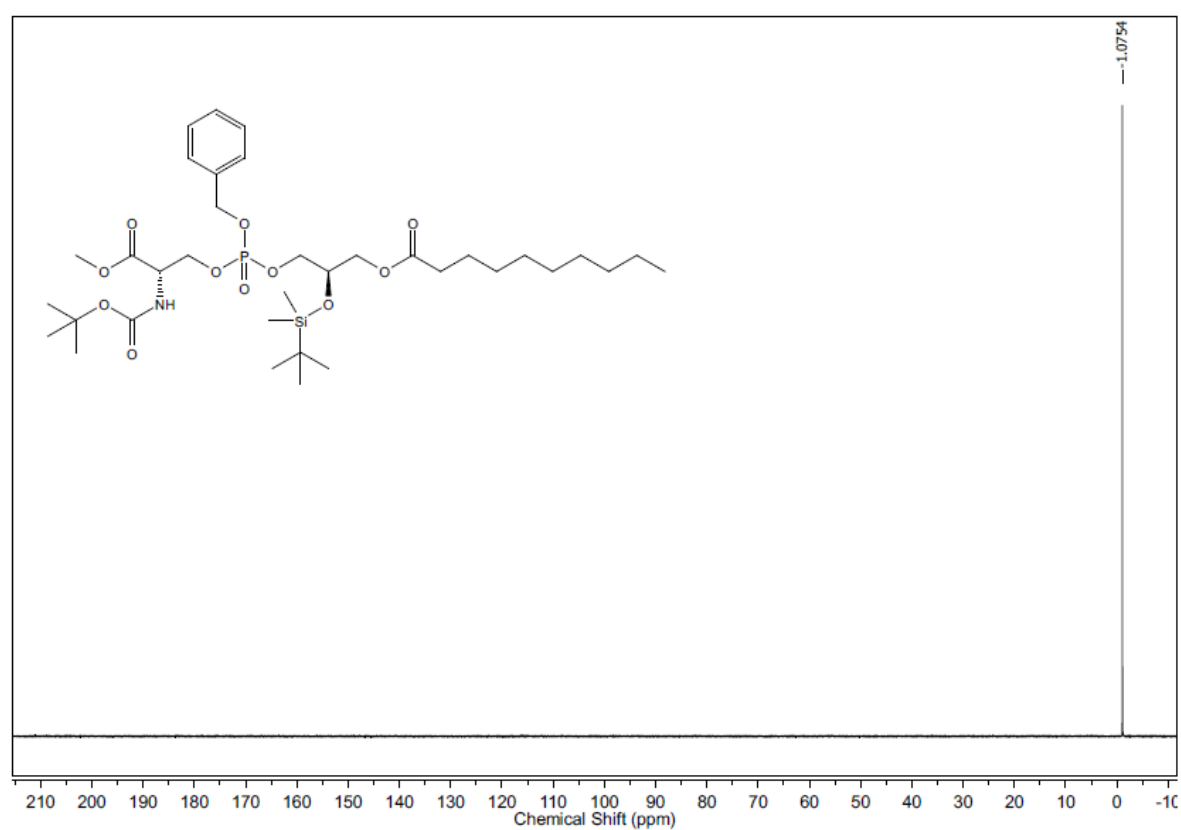
DEPT-135 NMR of compound (S)-6



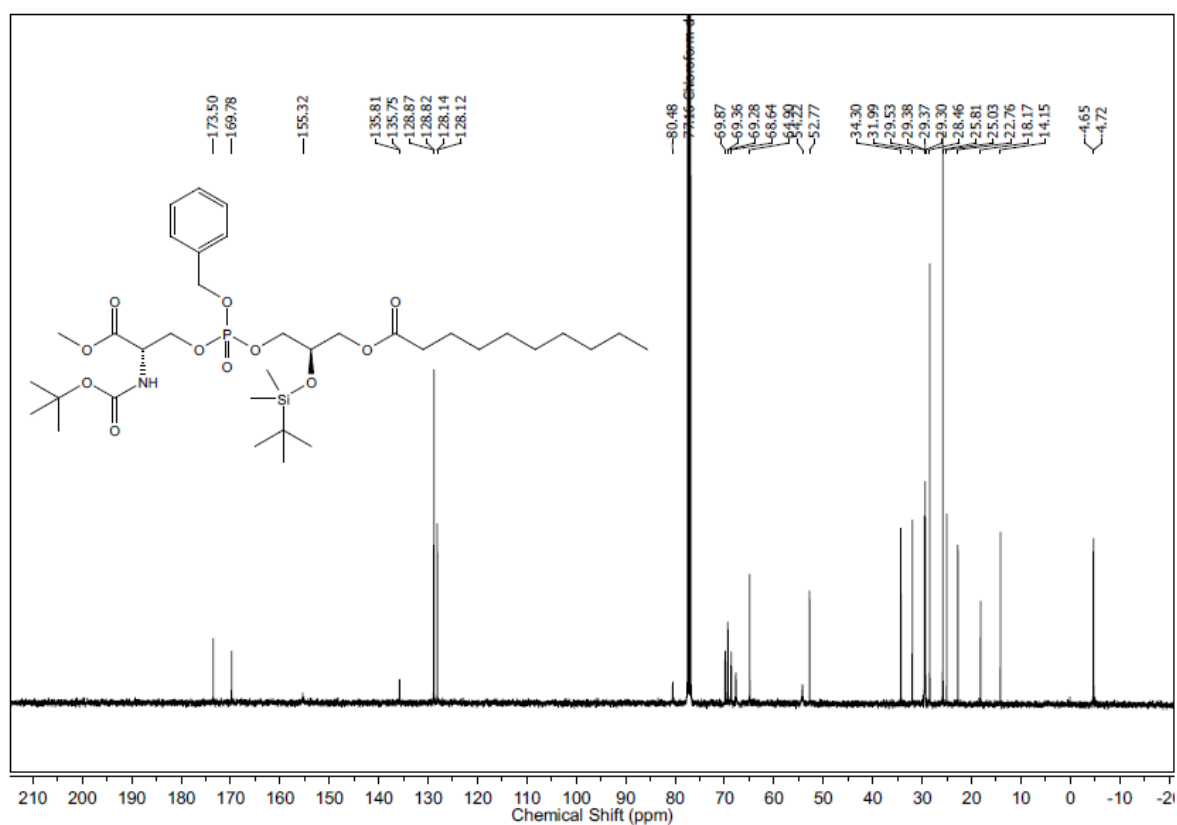
¹H NMR of compound (*R*)-**8a**



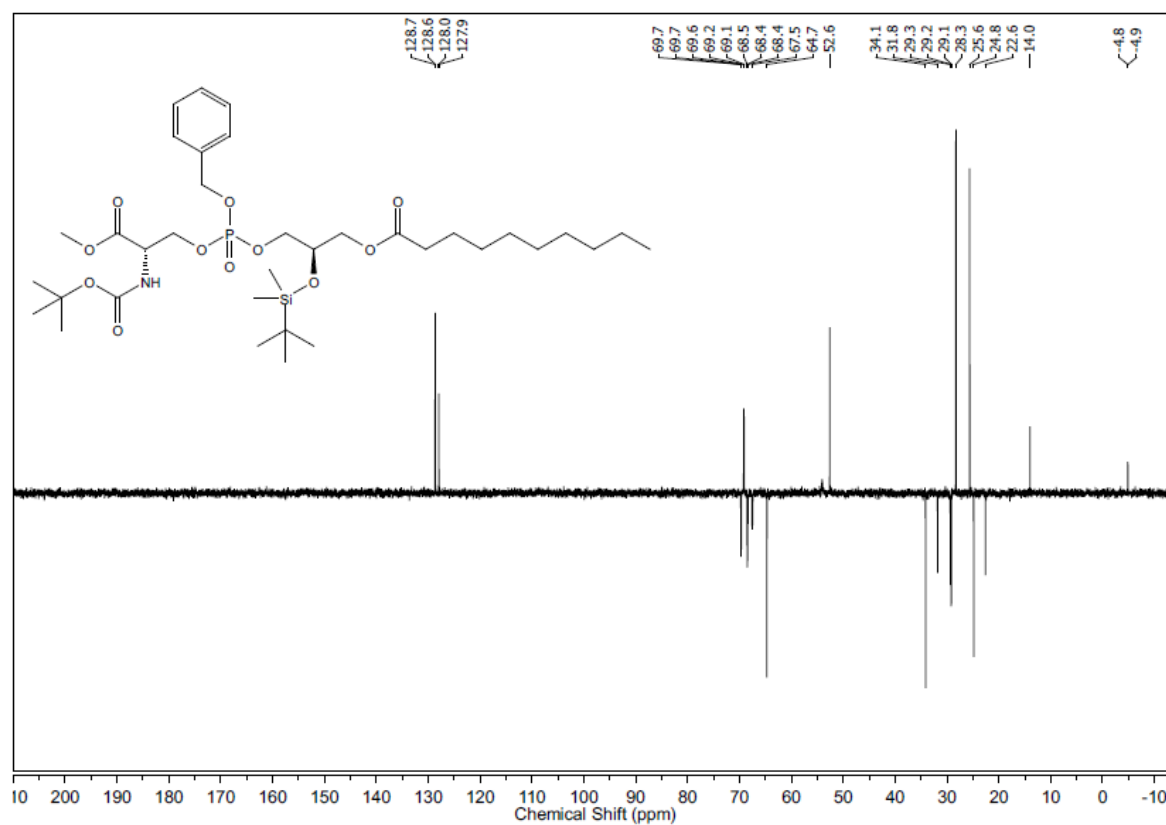
³¹P NMR of compound (*R*)-**8a**



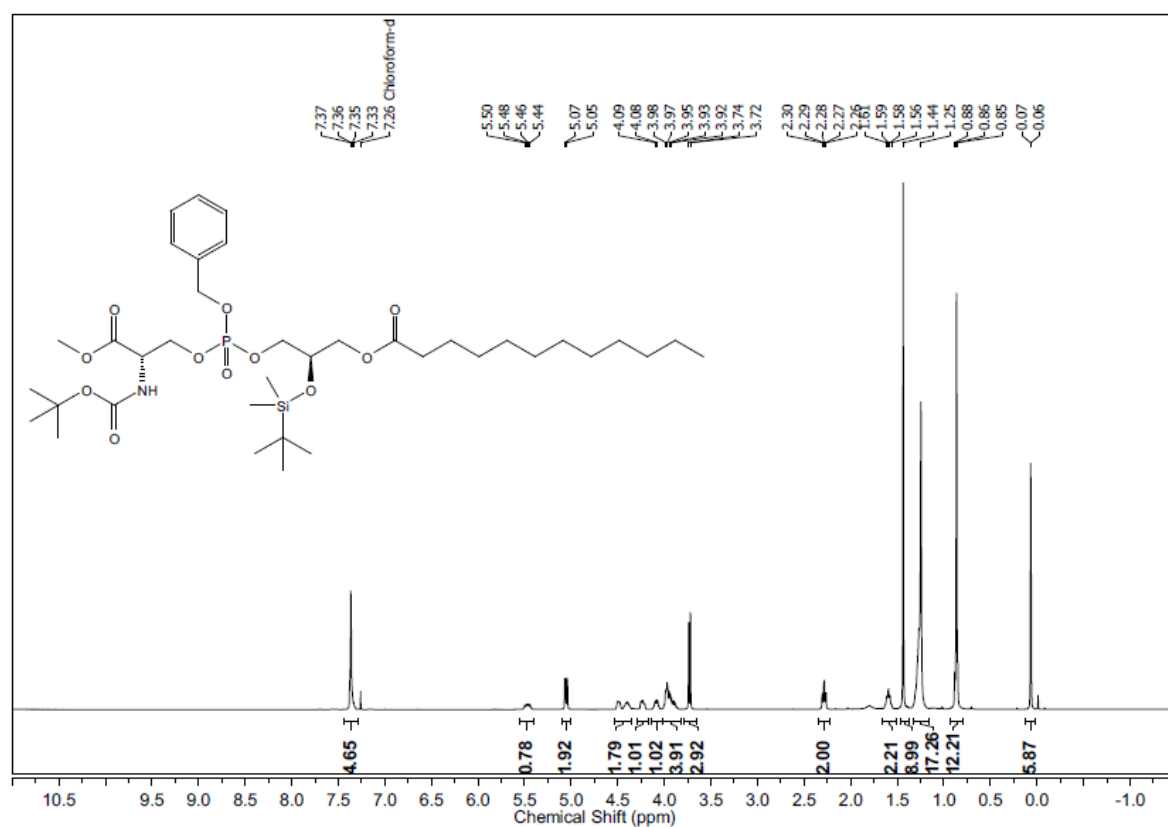
^{13}C NMR of compound (*R*)-**8a**



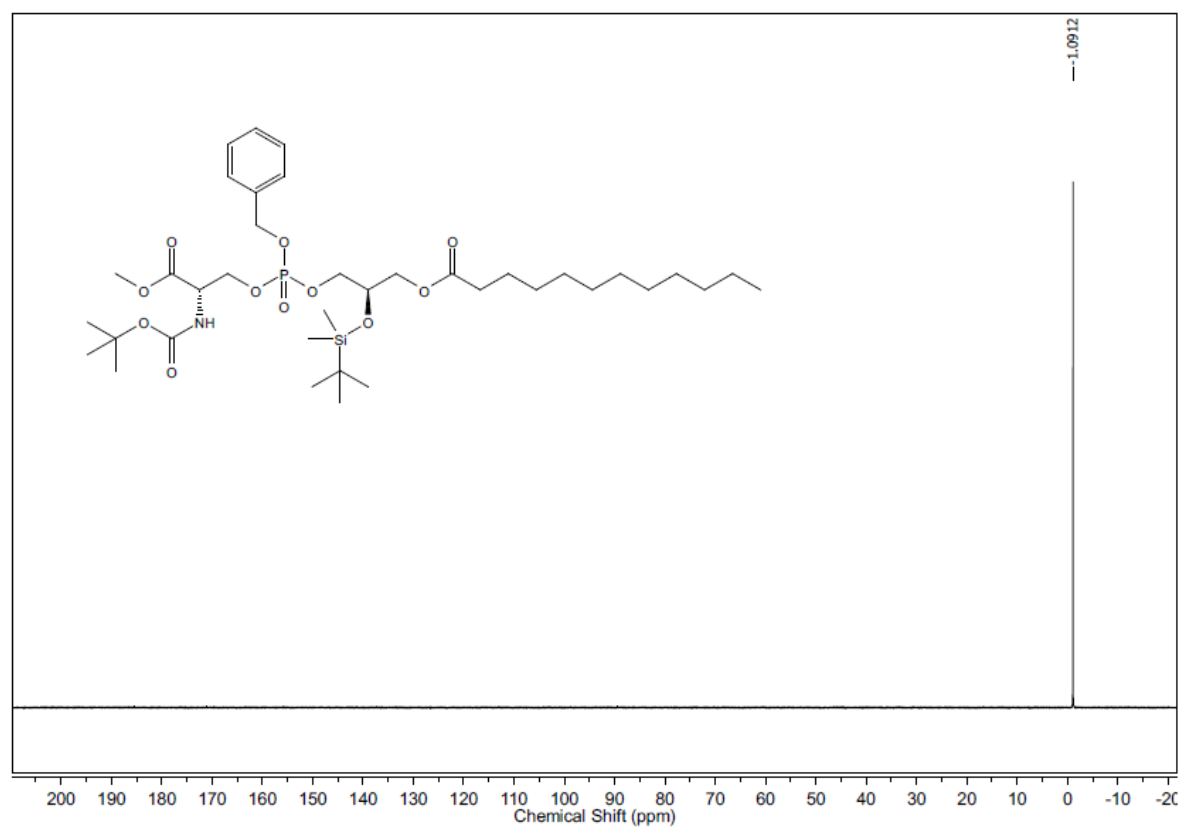
DEPT-135 NMR of compound (*R*)-**8a**



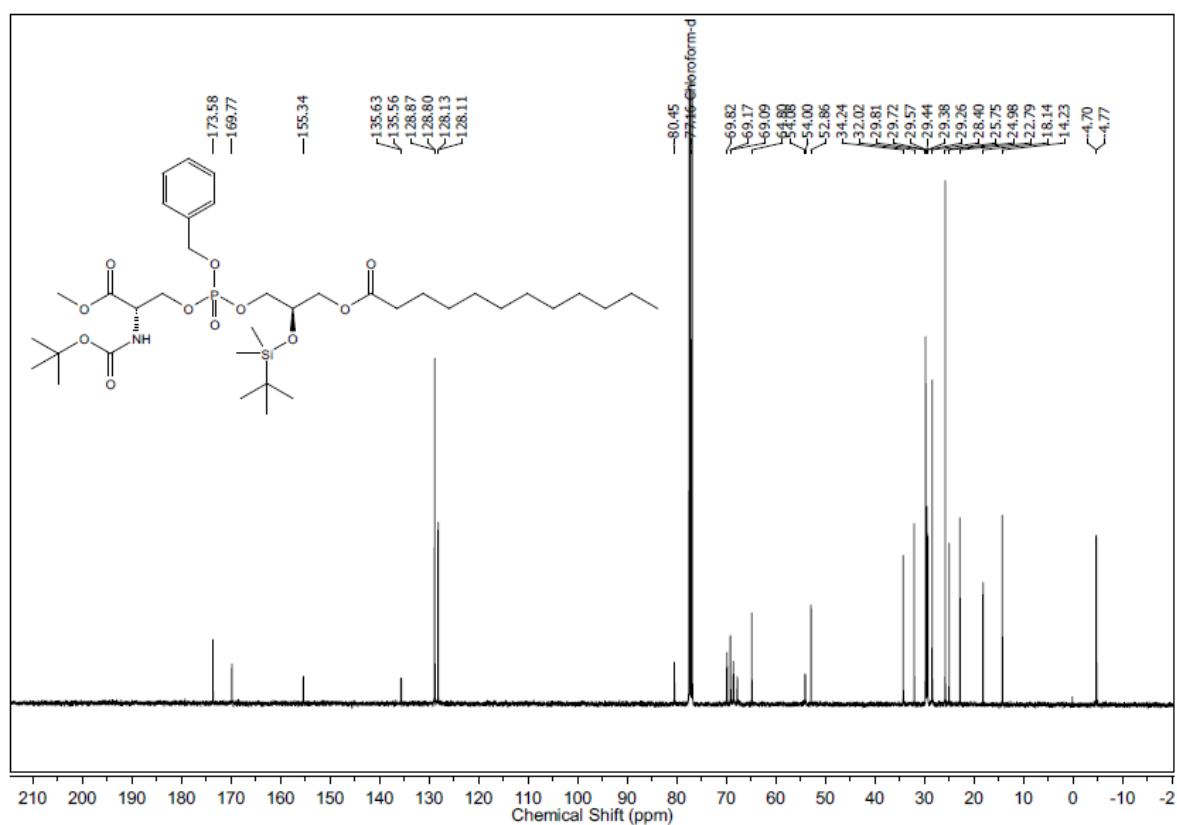
¹H NMR of compound (*R*)-**8b**



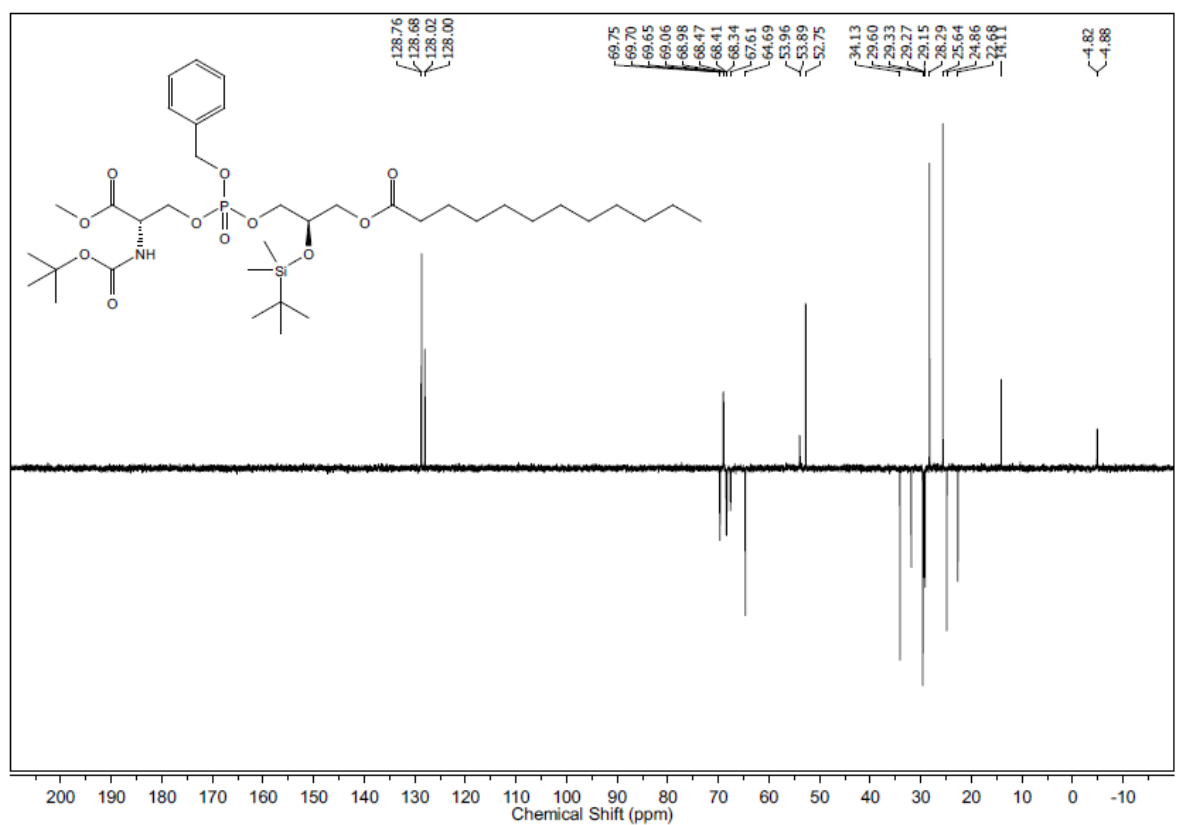
³¹P NMR of compound (*R*)-**8b**



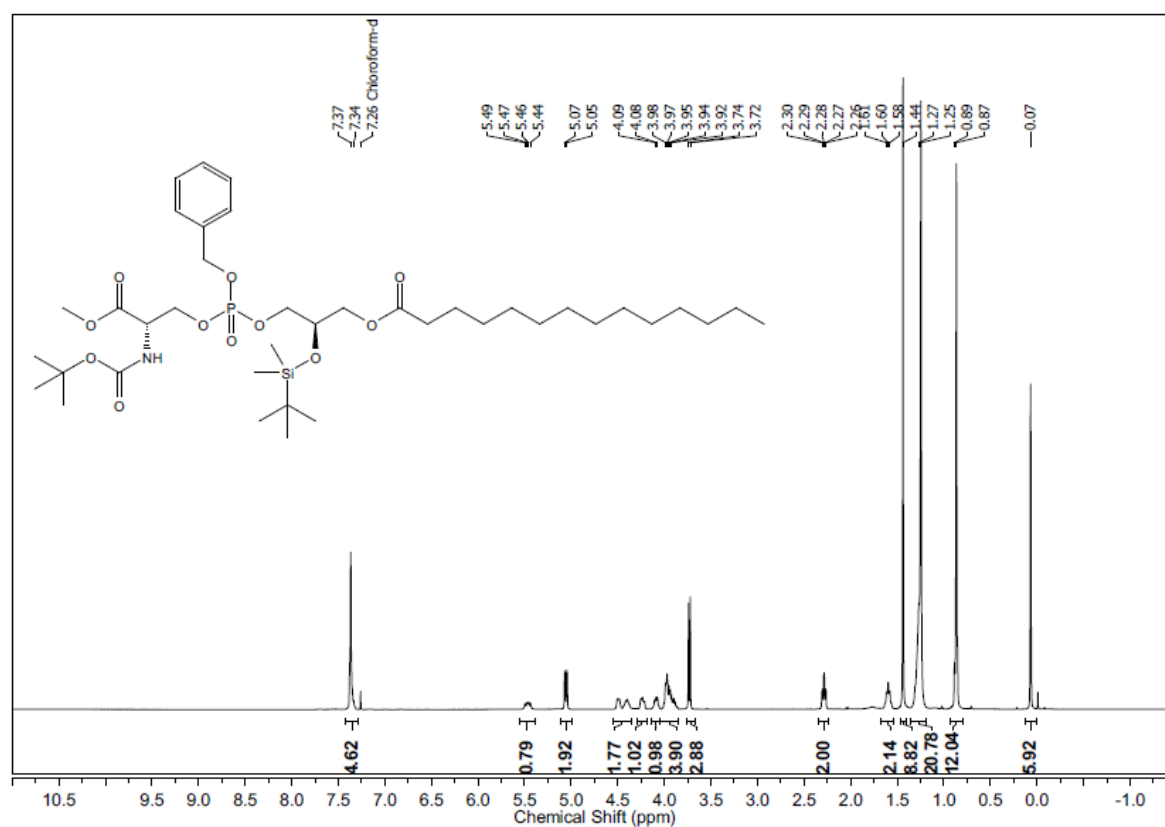
^{13}C NMR of compound (*R*)-**8b**



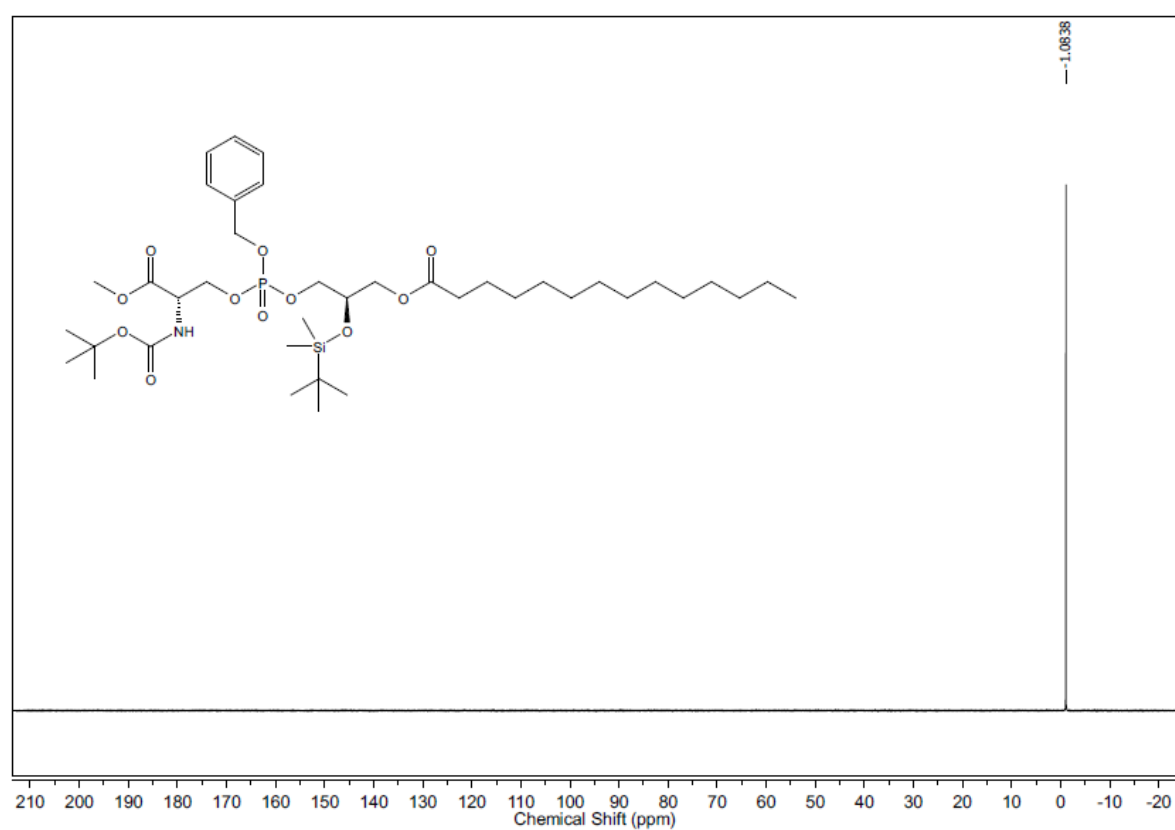
DEPT-135 NMR of compound (*R*)-**8b**



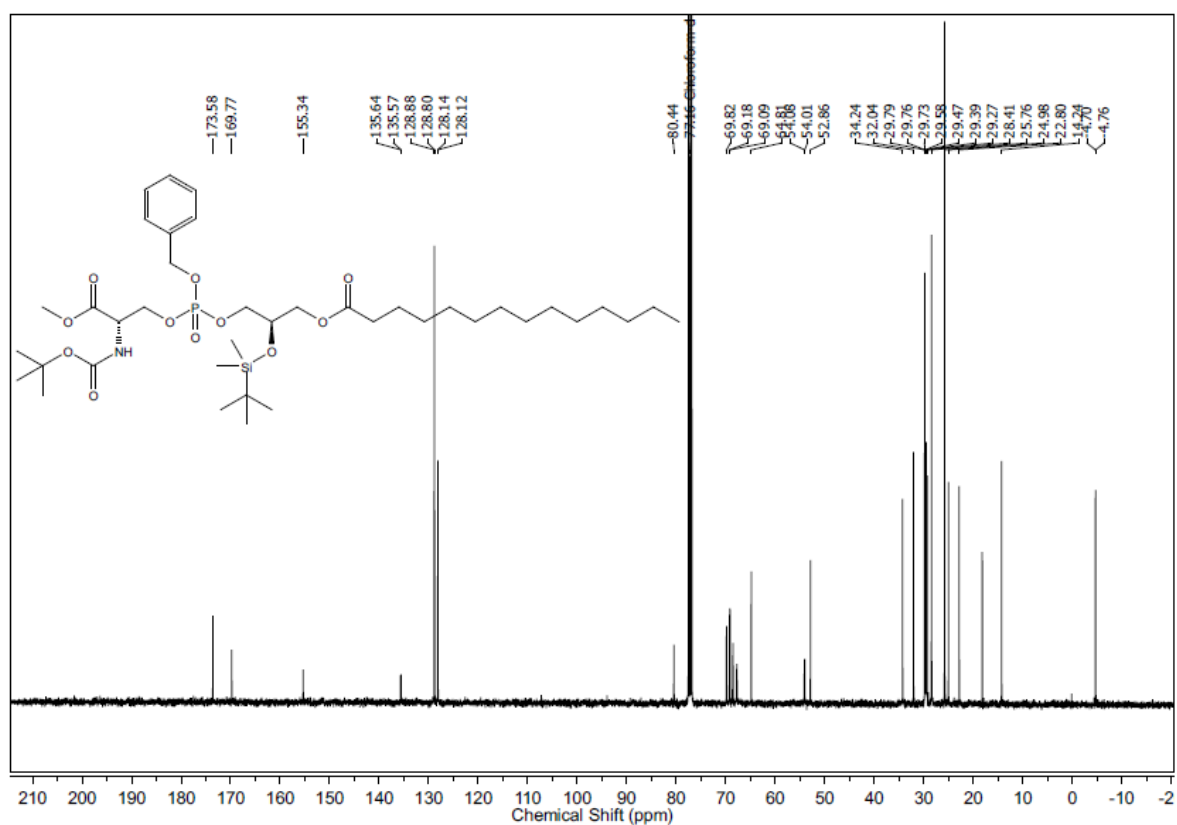
^1H NMR of compound (*R*)-**8c**



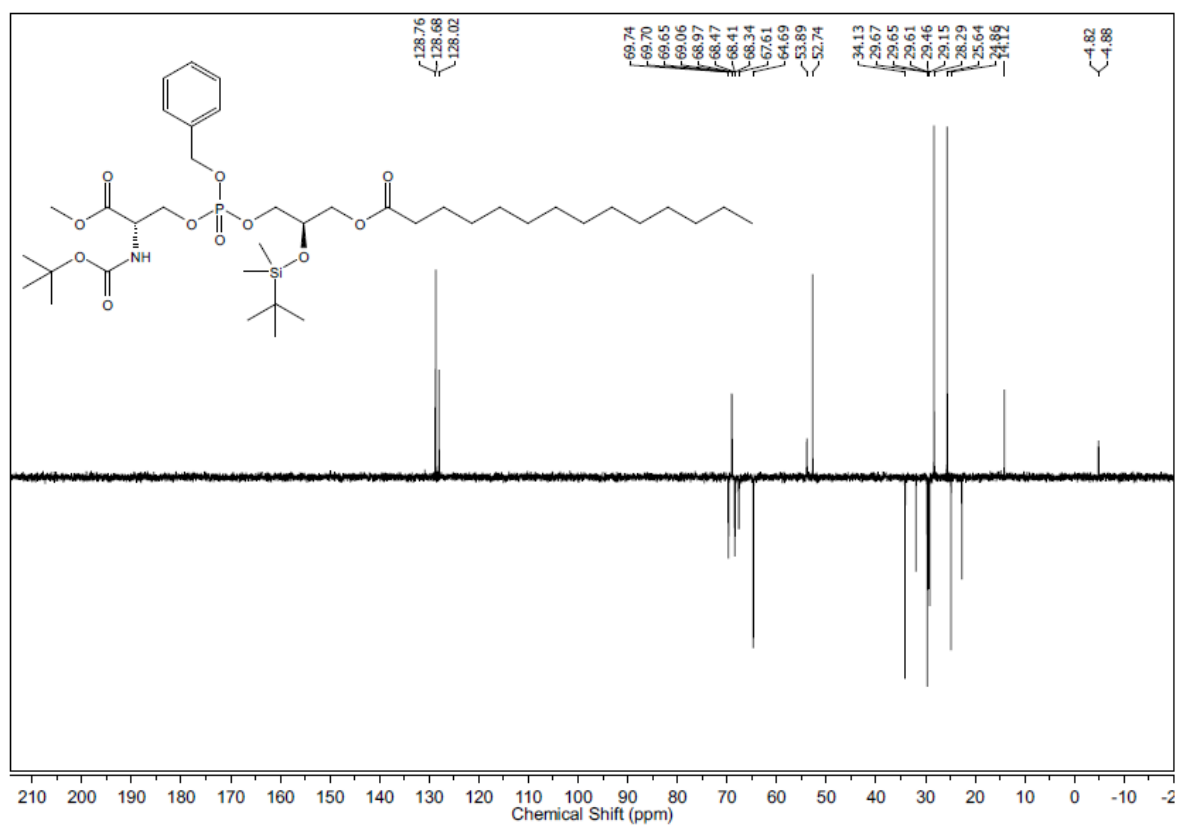
^{31}P NMR of compound (*R*)-**8c**



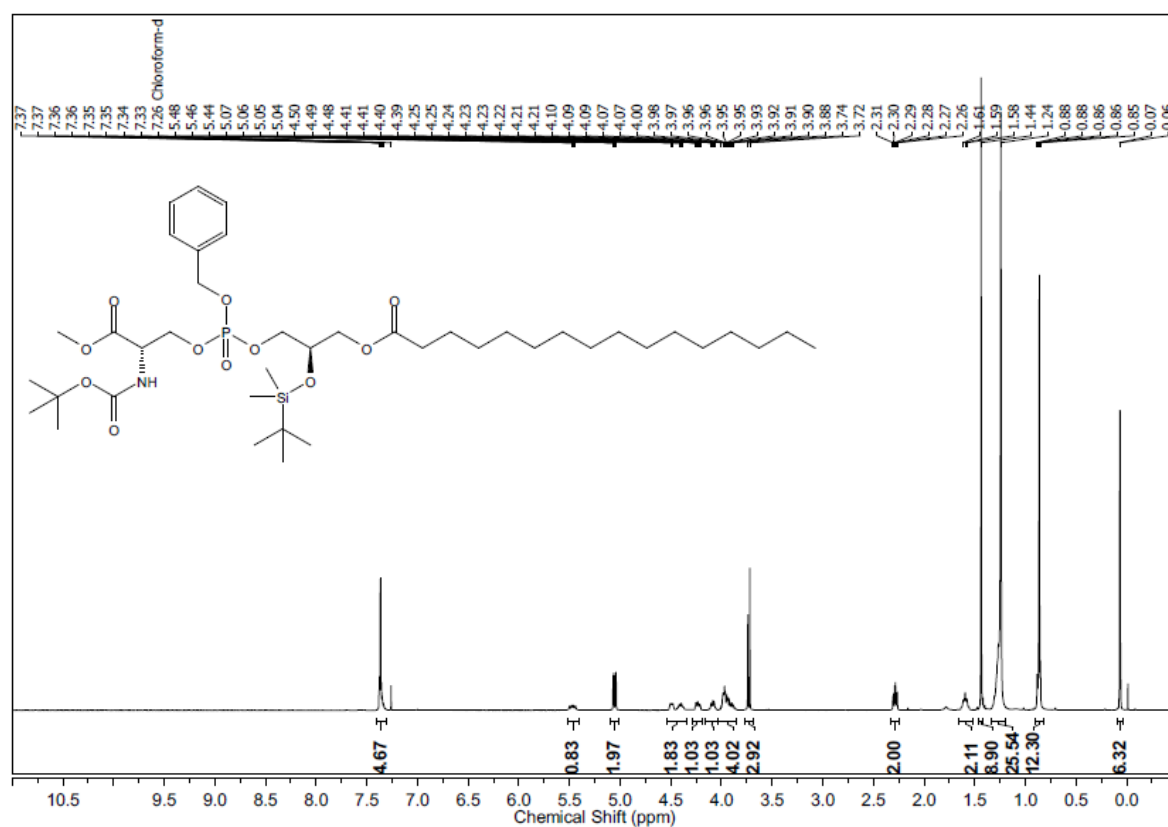
^{13}C NMR of compound (*R*)-**8c**



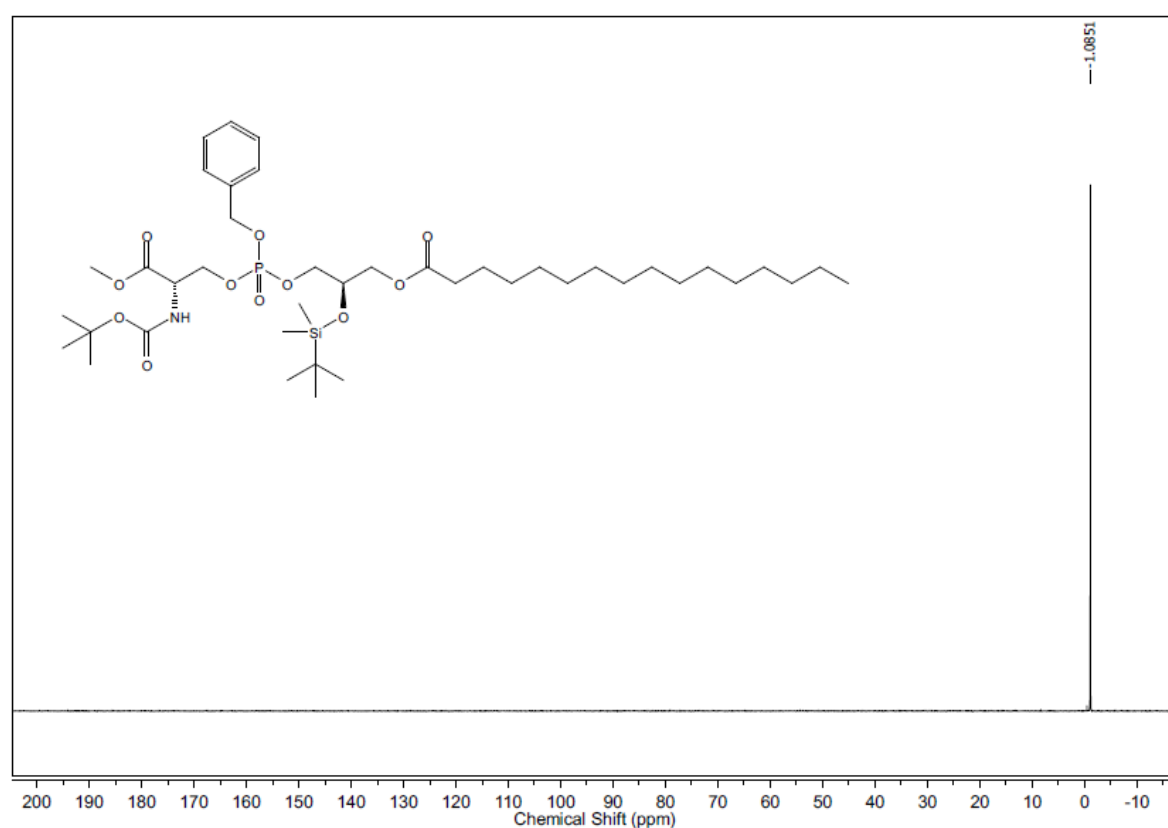
DEPT-135 NMR of compound (*R*)-**8c**



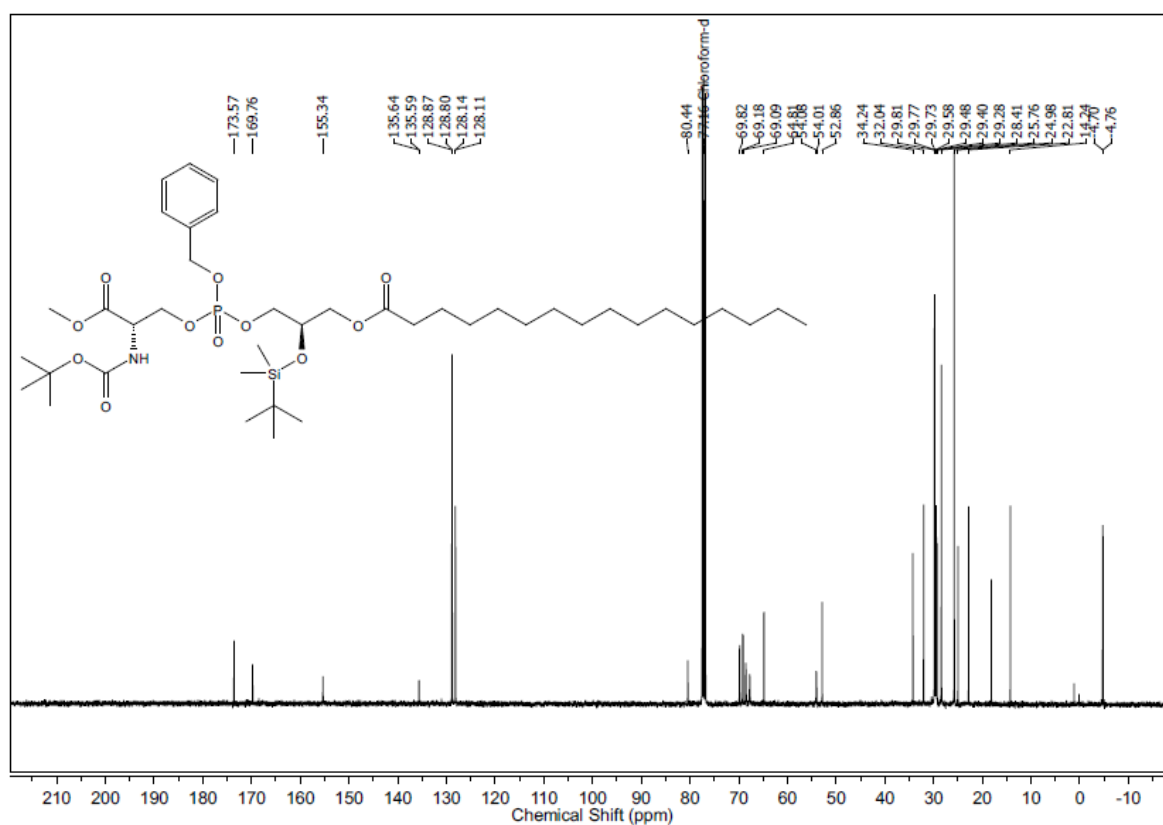
^1H NMR of compound (*R*)-**8d**



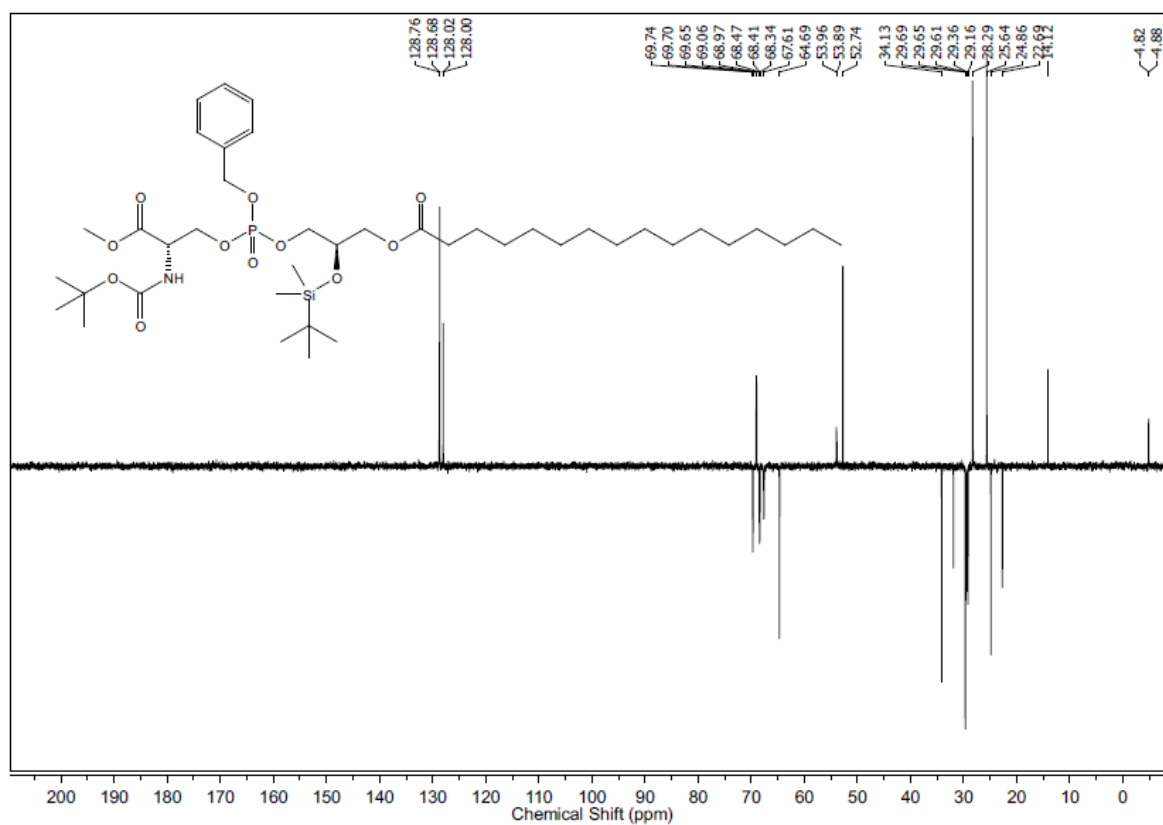
^{31}P NMR of compound (*R*)-**8d**



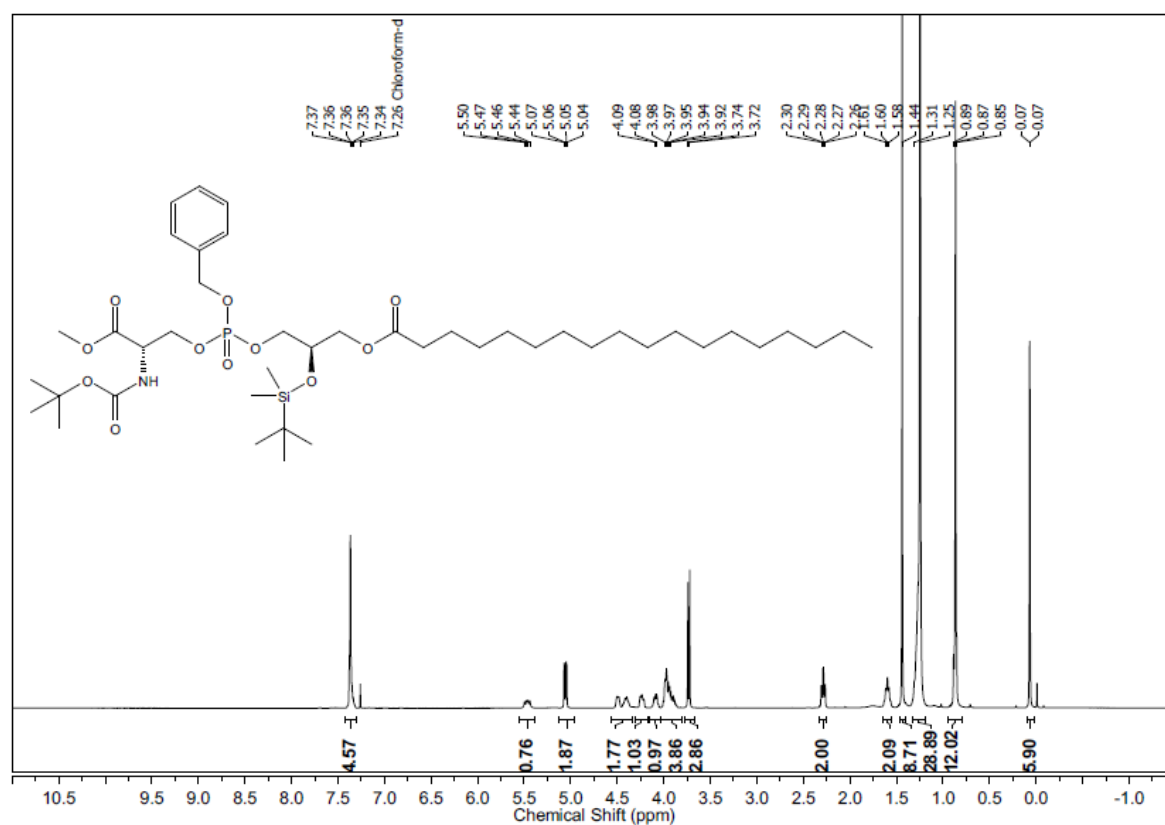
^{13}C NMR of compound (*R*)-**8d**



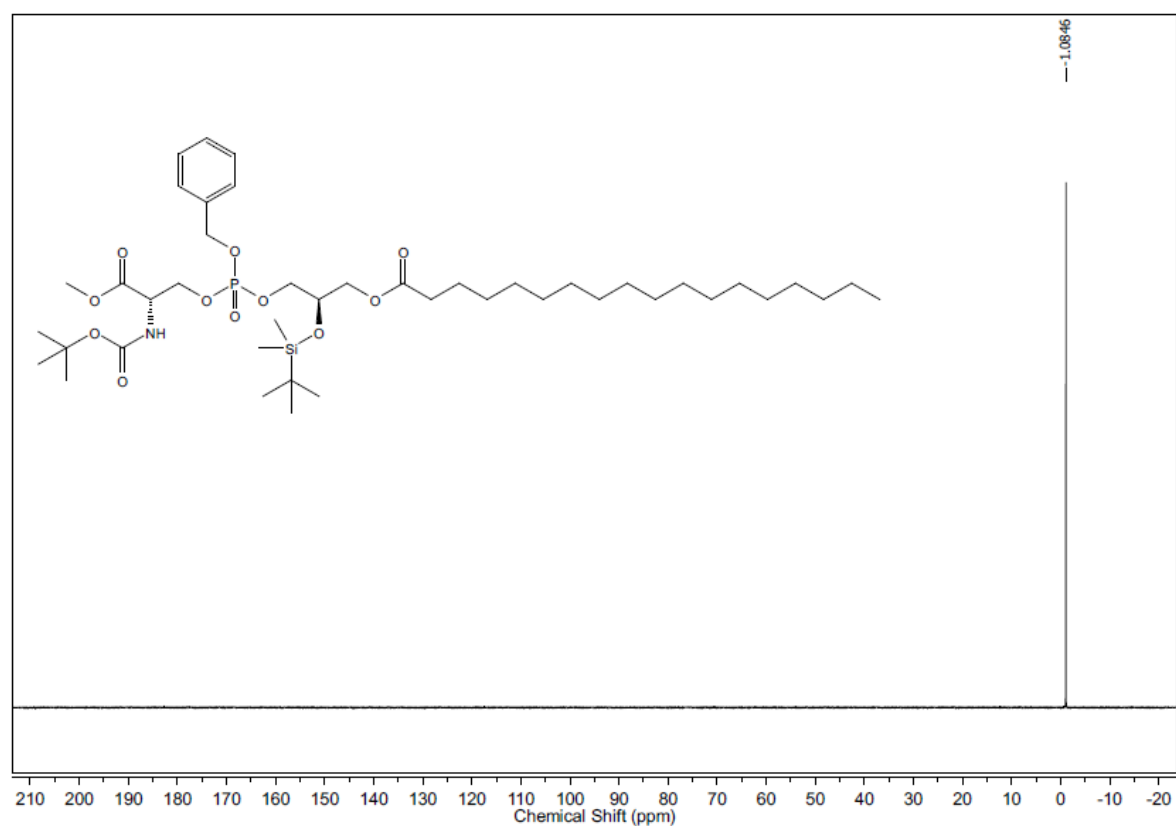
DEPT-135 NMR of compound (*R*)-**8d**



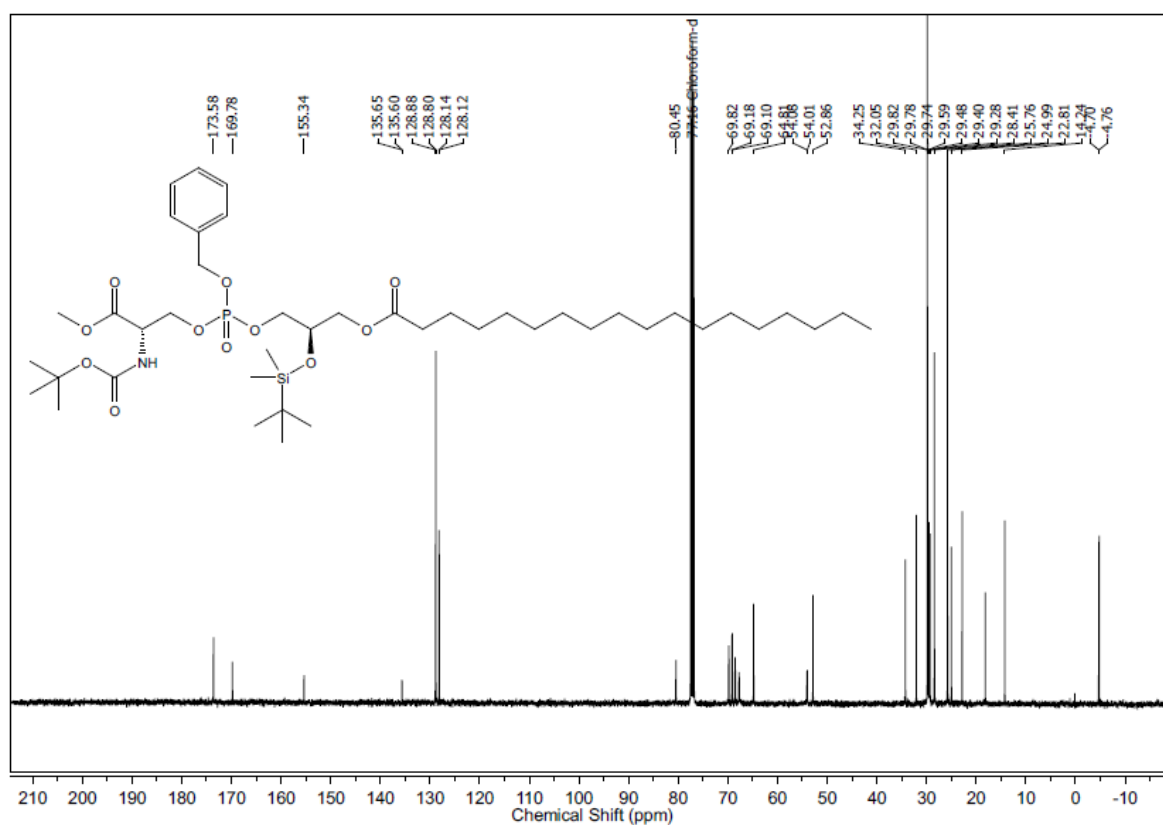
¹H NMR of compound (*R*)-**8e**



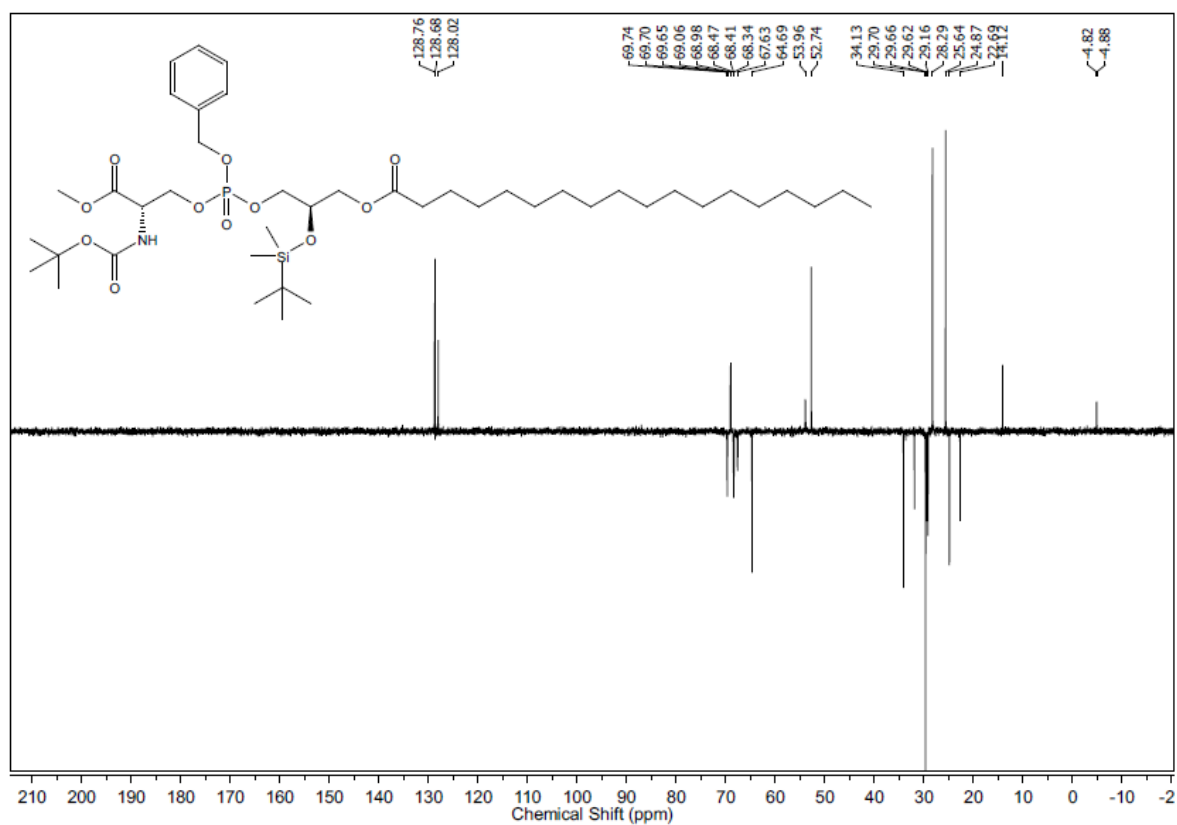
³¹P NMR of compound (*R*)-**8e**



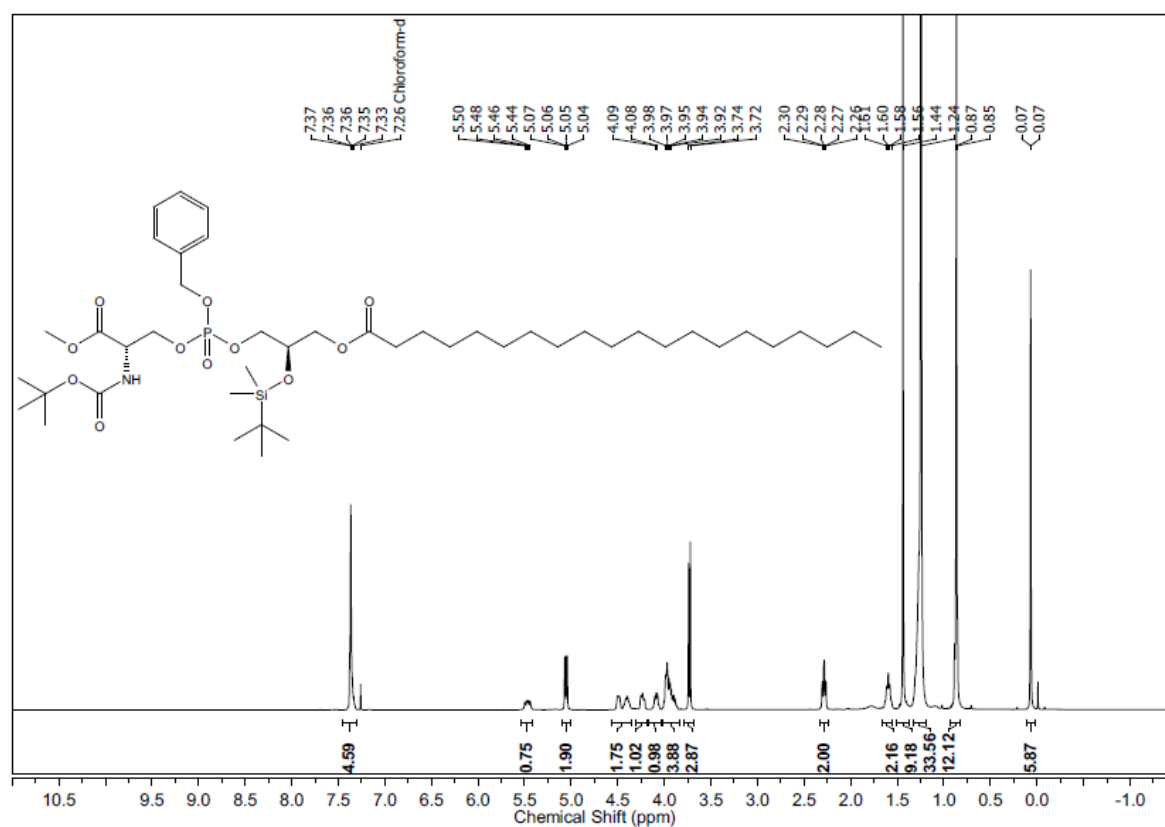
^{13}C NMR of compound (*R*)-**8e**



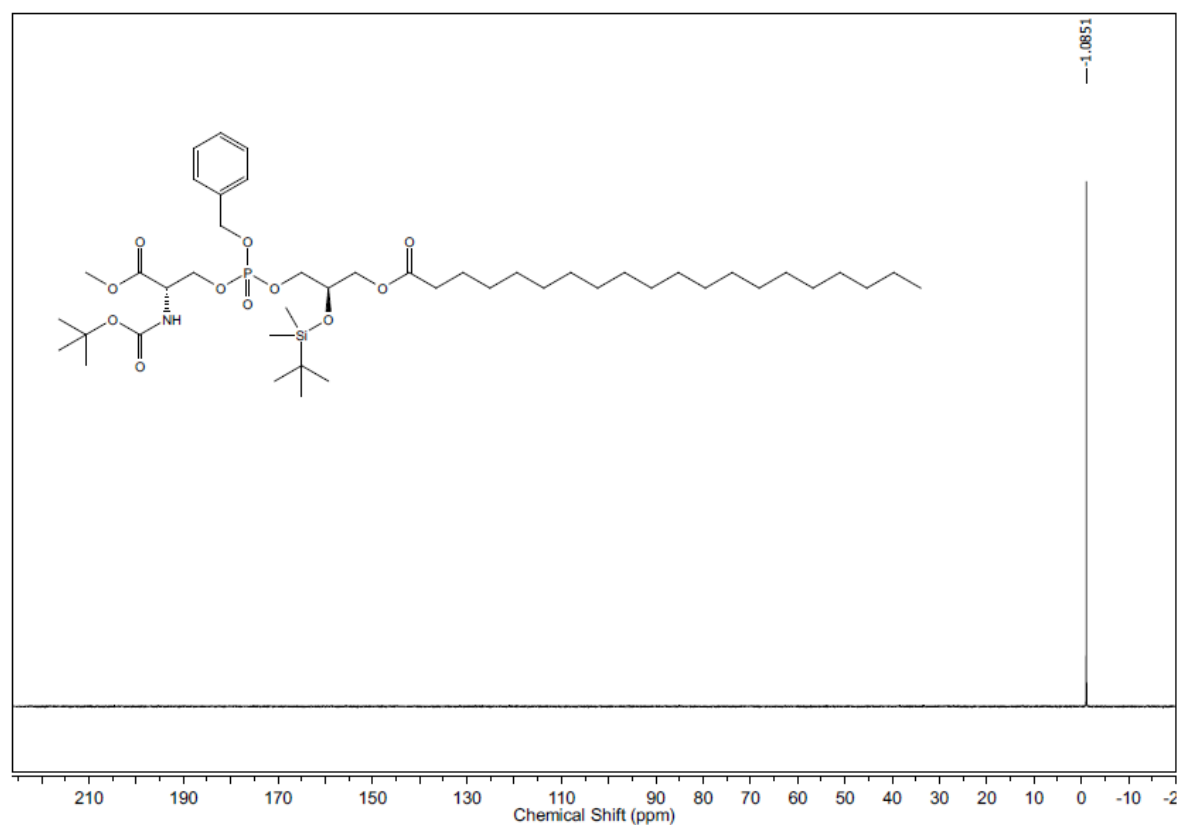
DEPT-135 NMR of compound (*R*)-**8e**



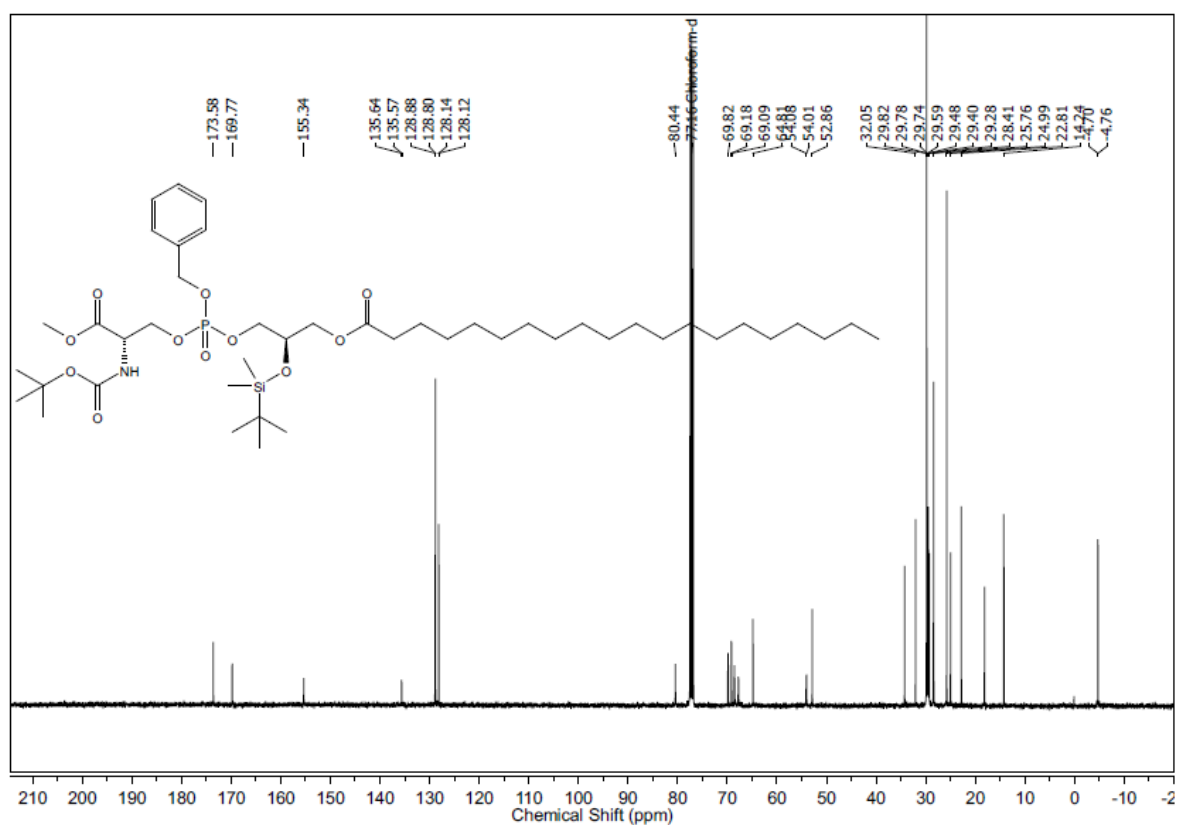
¹H NMR of compound (*R*)-**8f**



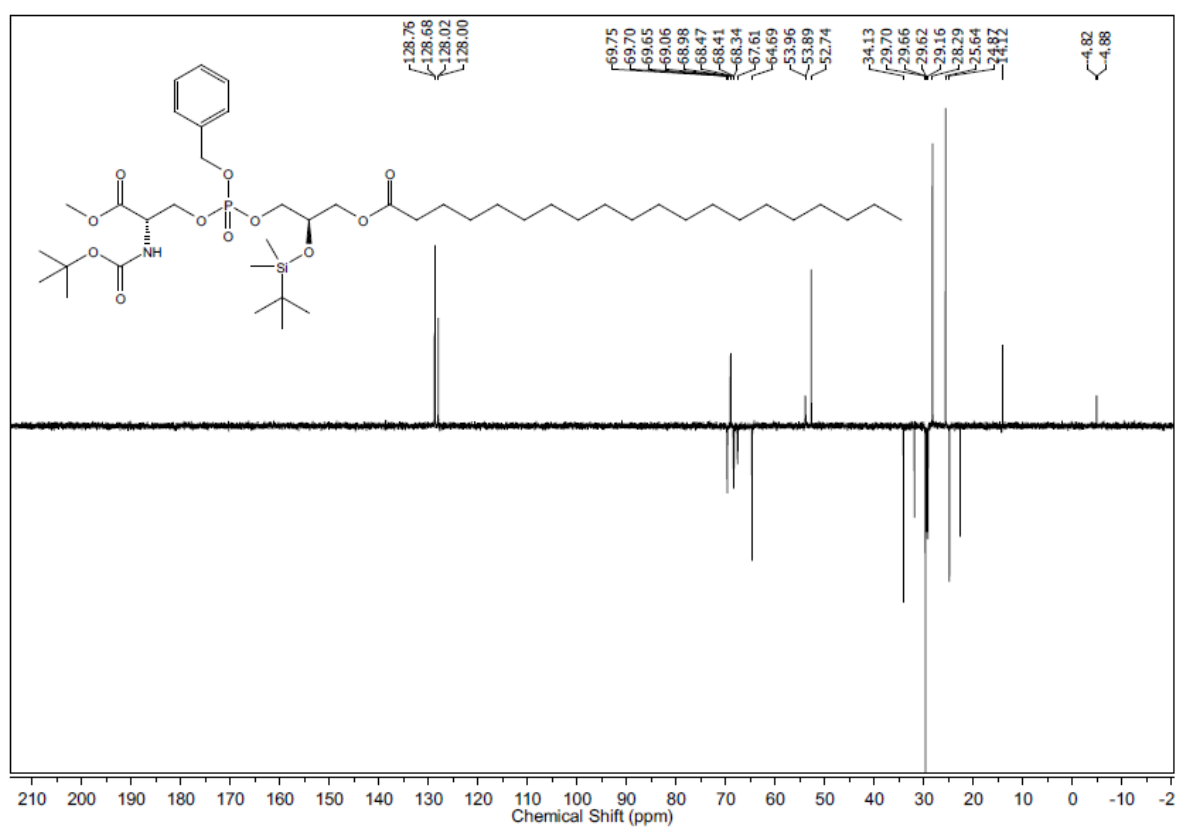
³¹P NMR of compound (*R*)-**8f**



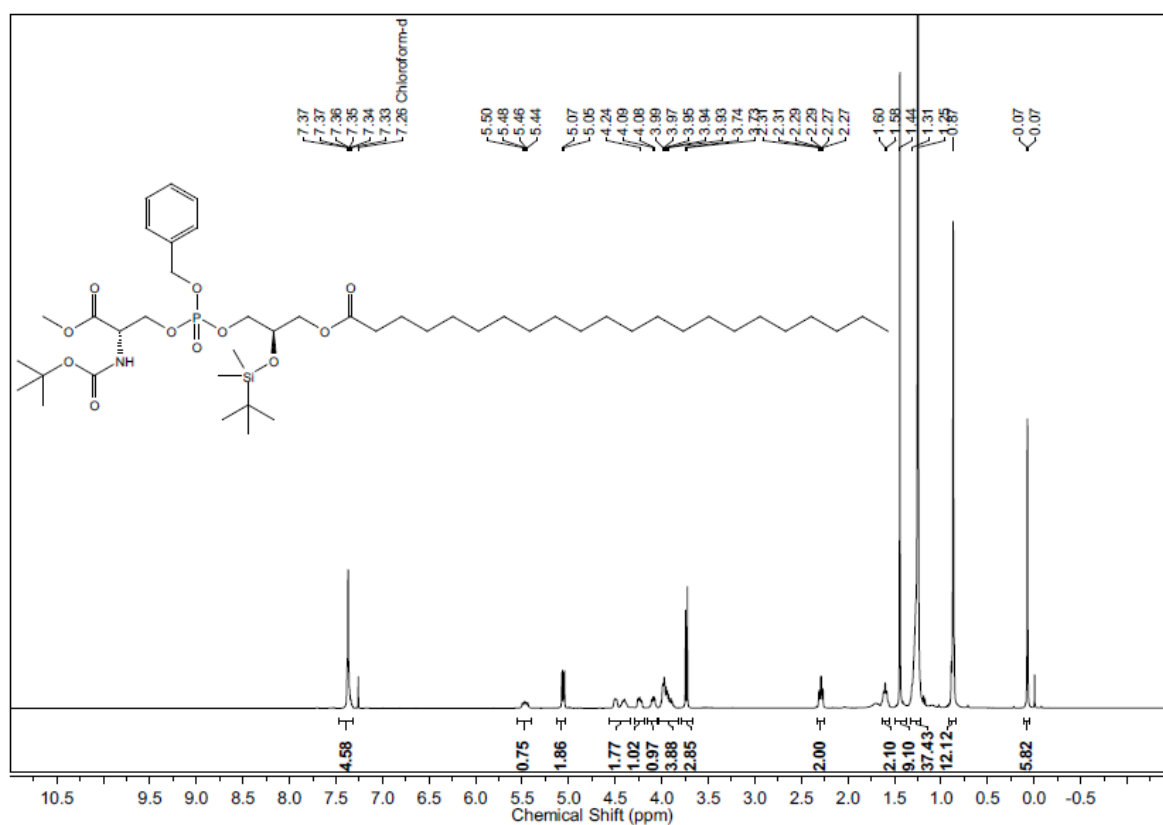
^{13}C NMR of compound (*R*)-**8f**



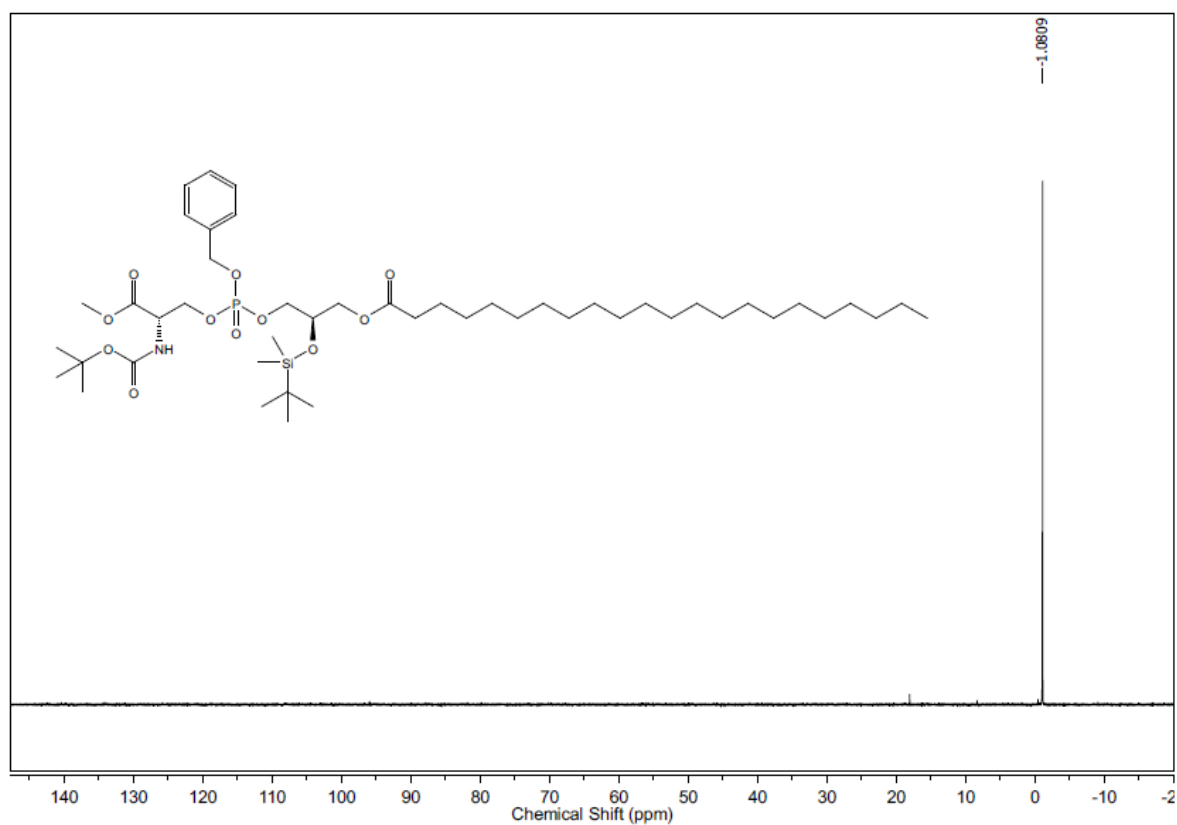
DEPT-135 NMR of compound (*R*)-**8f**



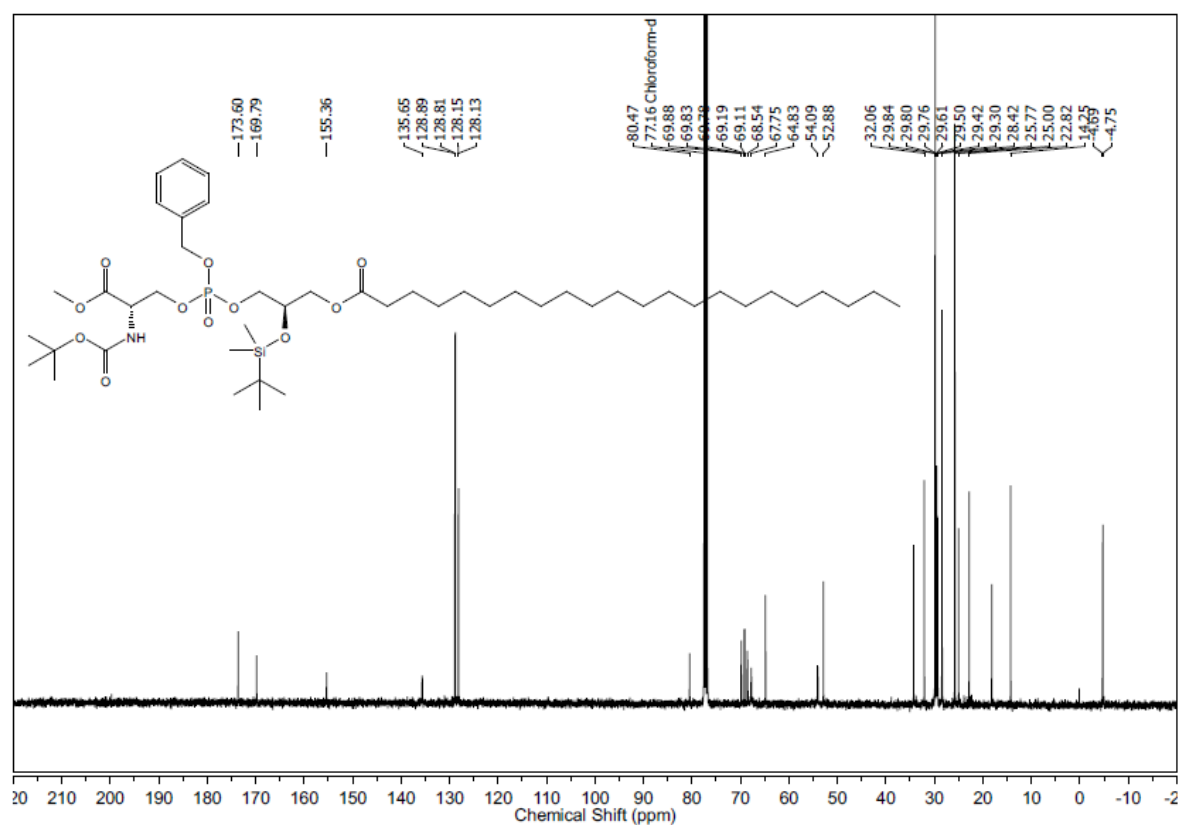
¹H NMR of compound (*R*)-**8g**



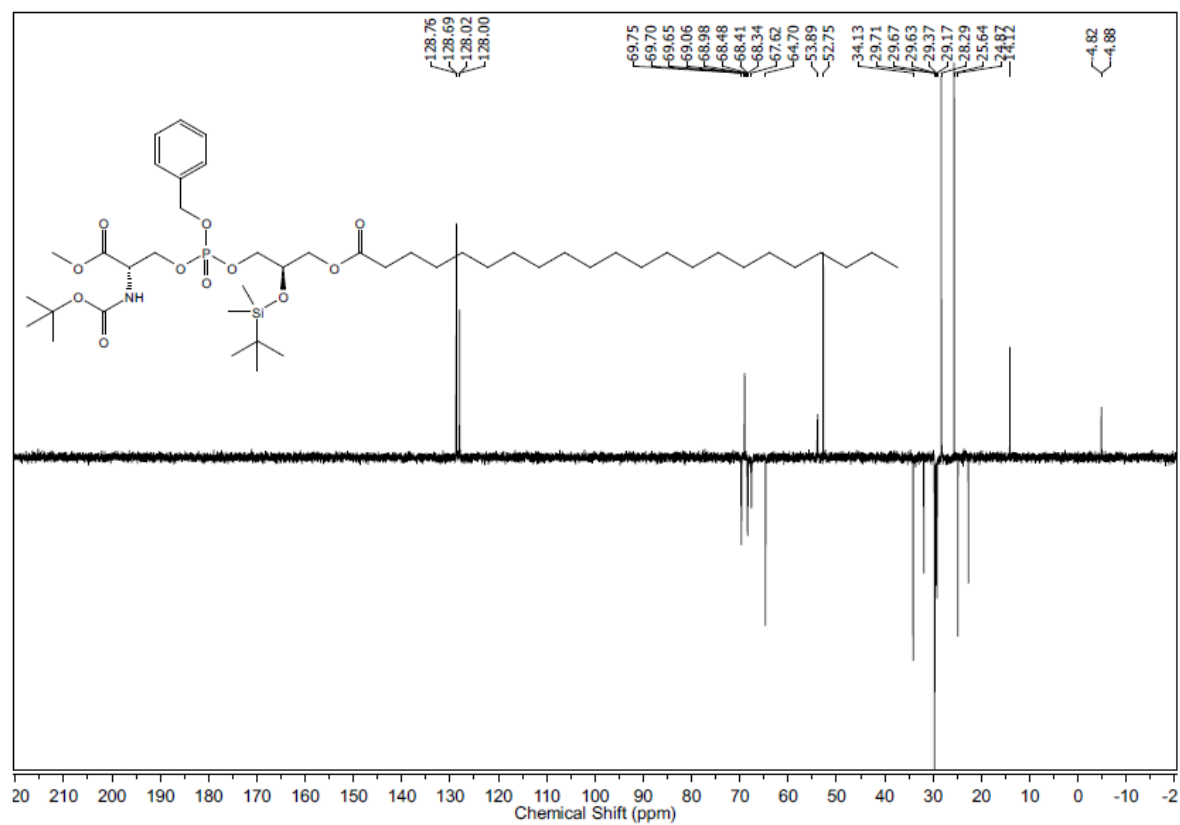
³¹P NMR of compound (*R*)-**8g**

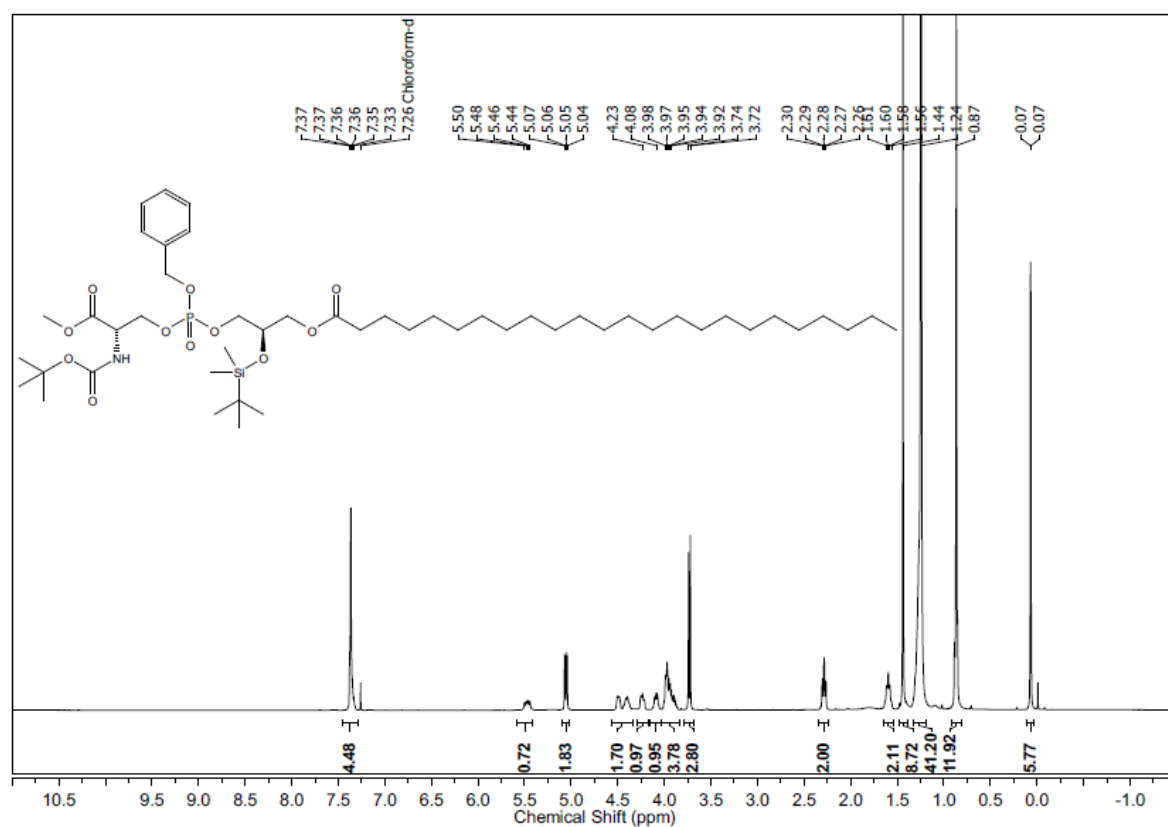
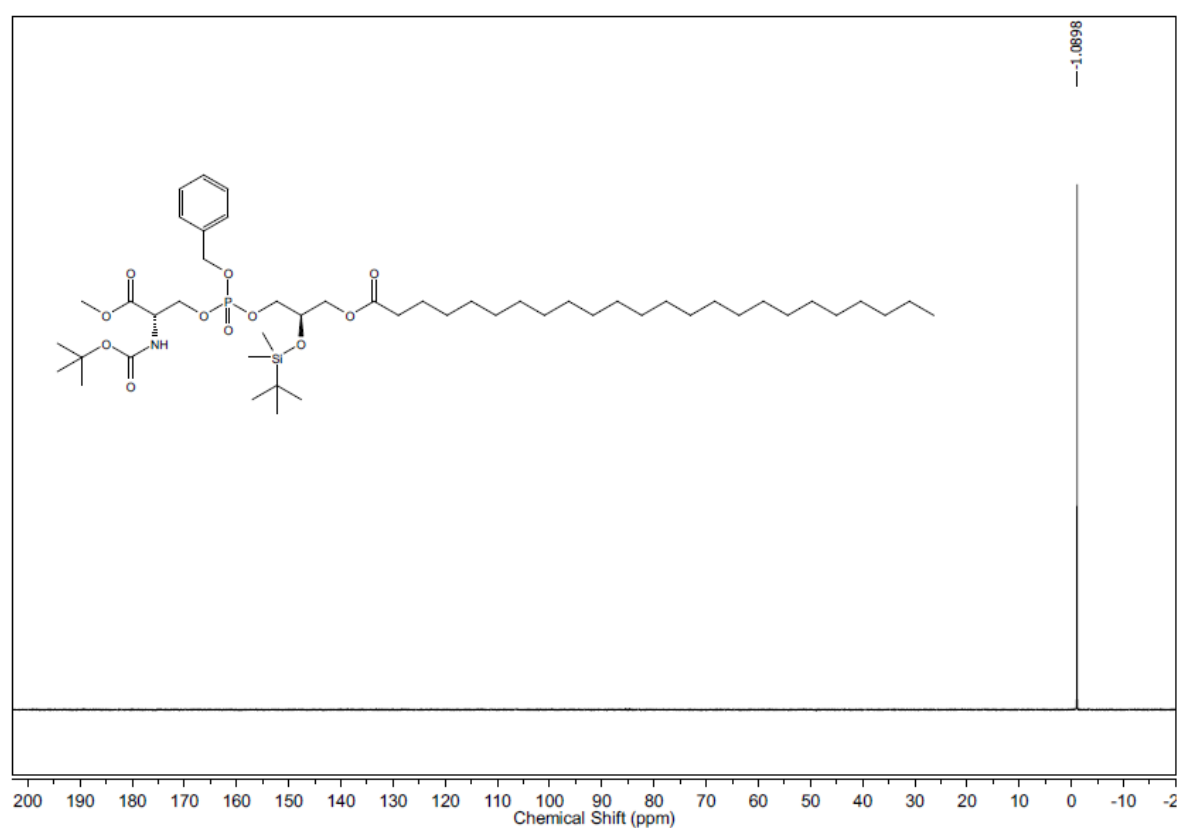


^{13}C NMR of compound (*R*)-**8g**

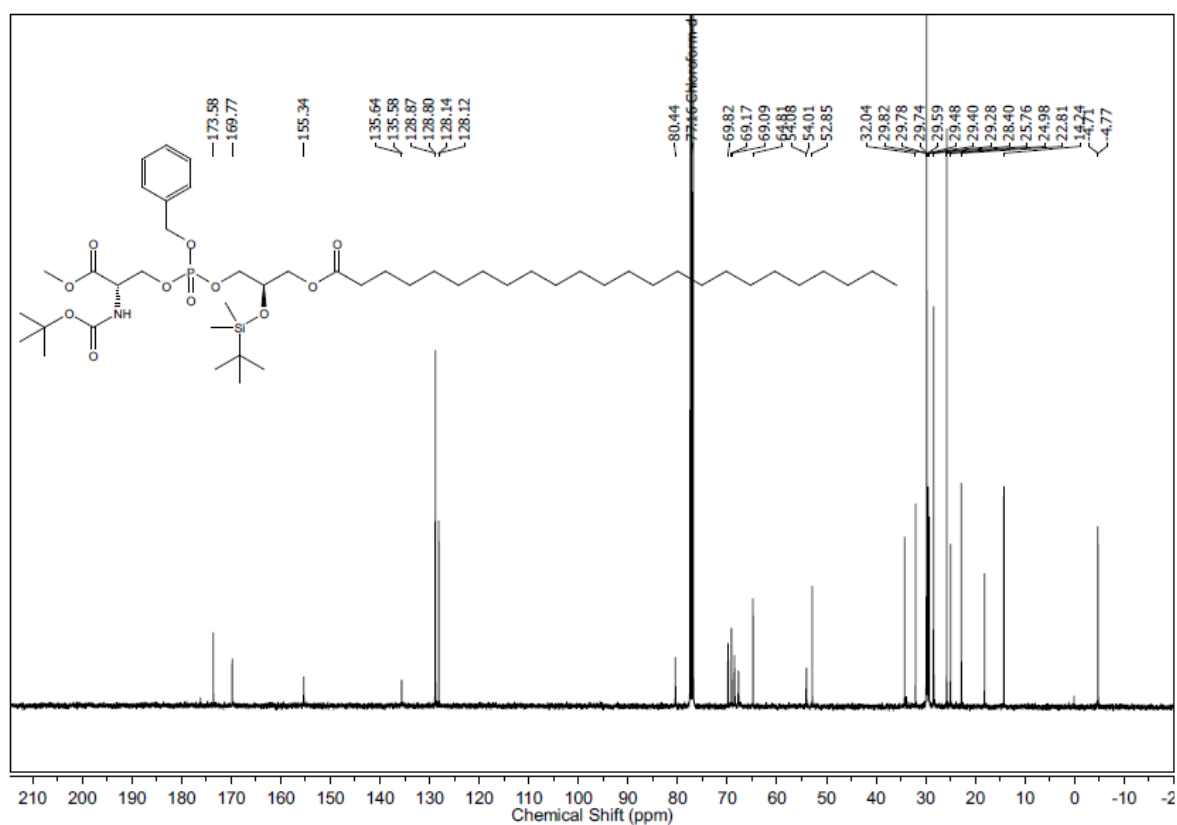


DEPT-135 NMR of compound (*R*)-**8g**

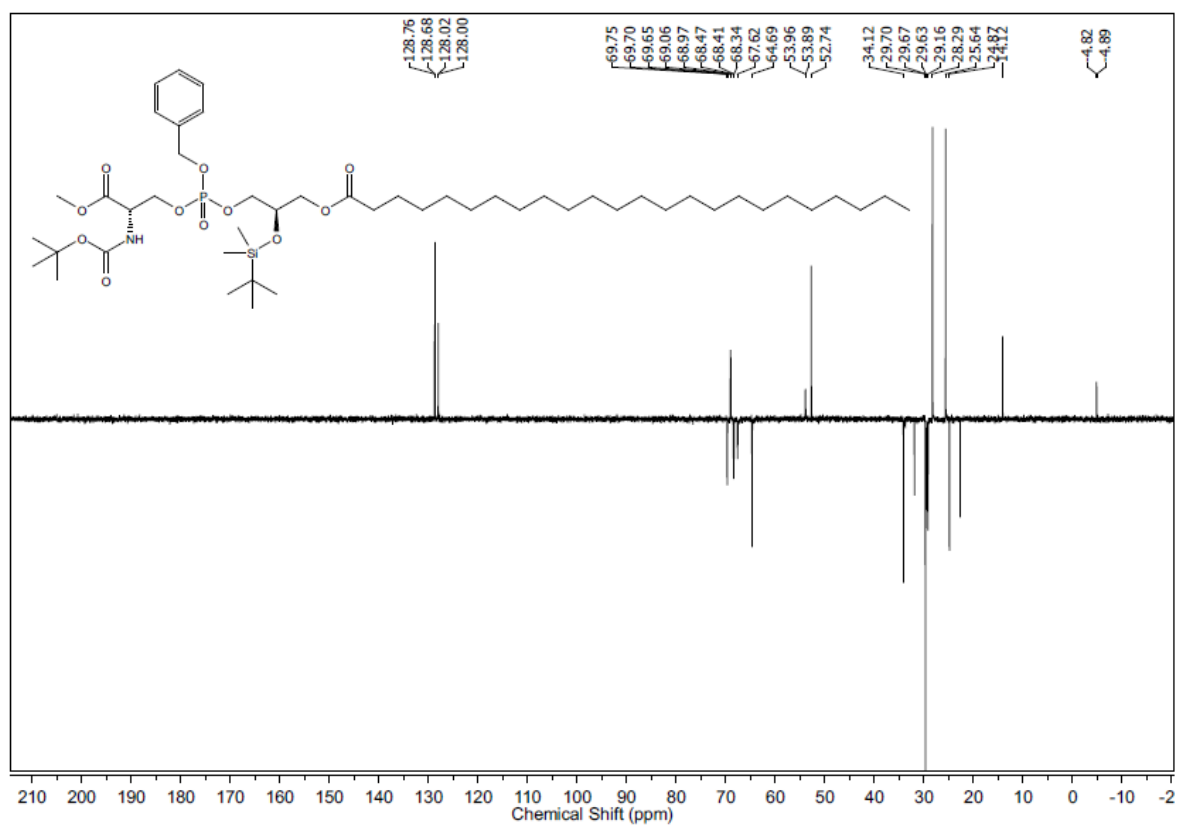


¹H NMR of compound (*R*)-**8h**³¹P NMR of compound (*R*)-**8h**

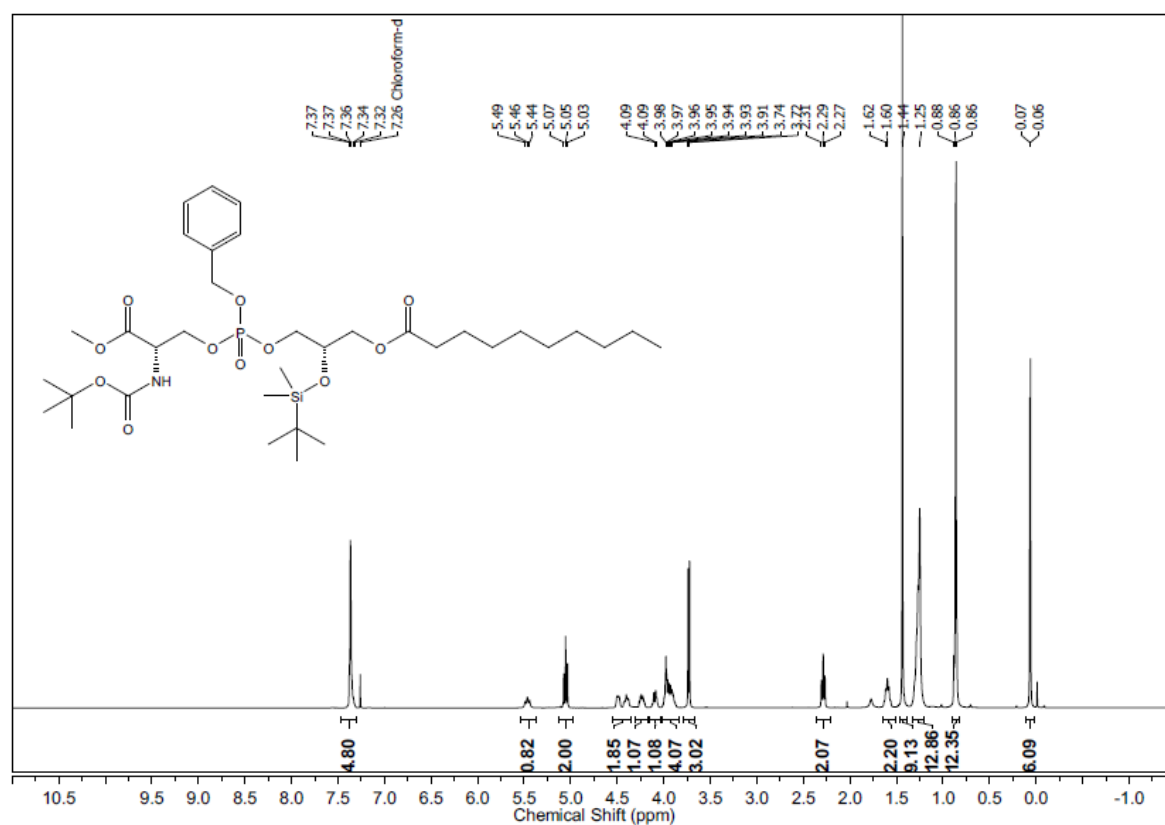
^{13}C NMR of compound (*R*)-**8h**



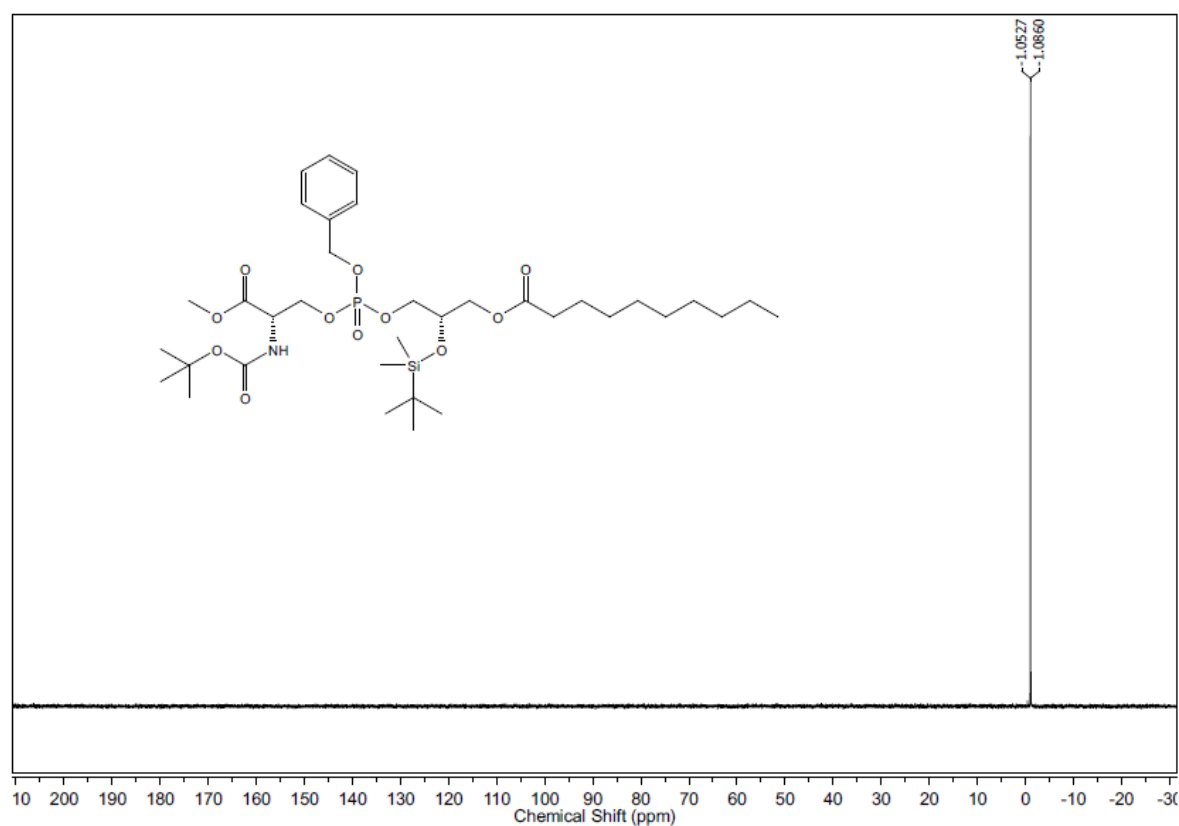
DEPT-135 NMR of compound (*R*)-**8h**



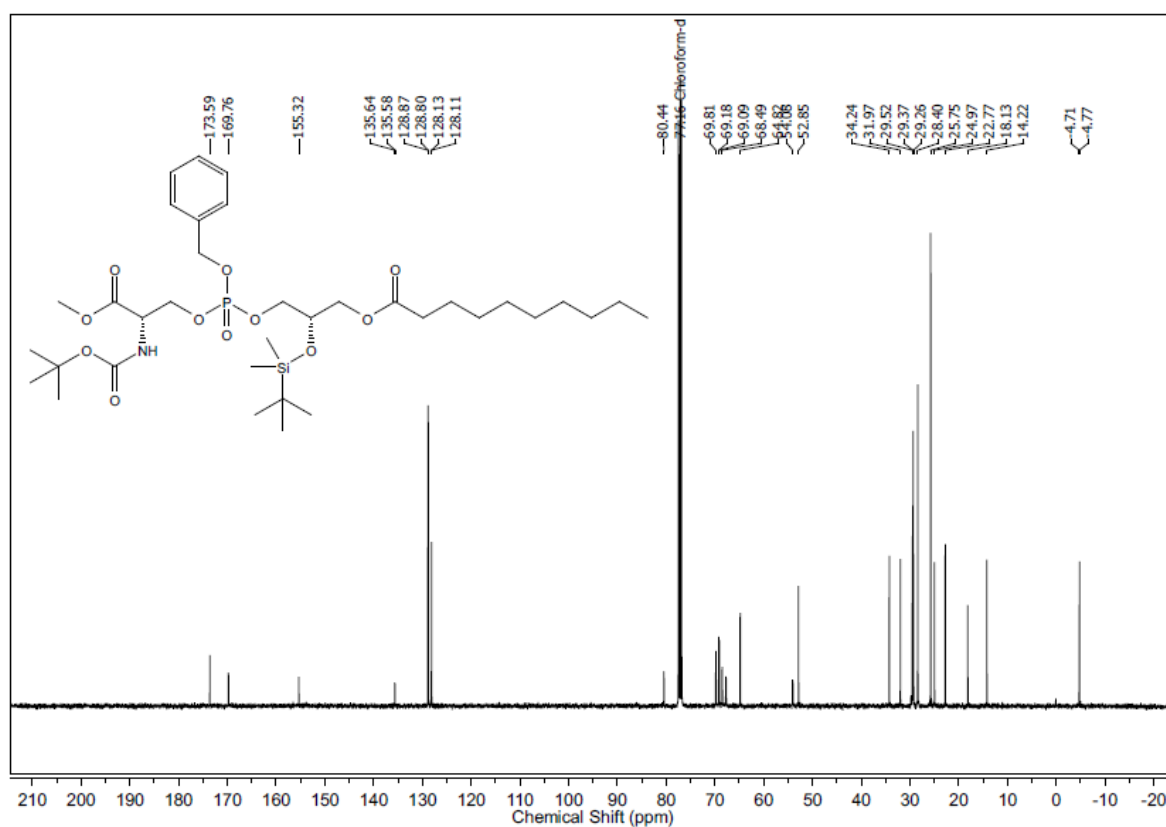
¹H NMR of compound (S)-8a



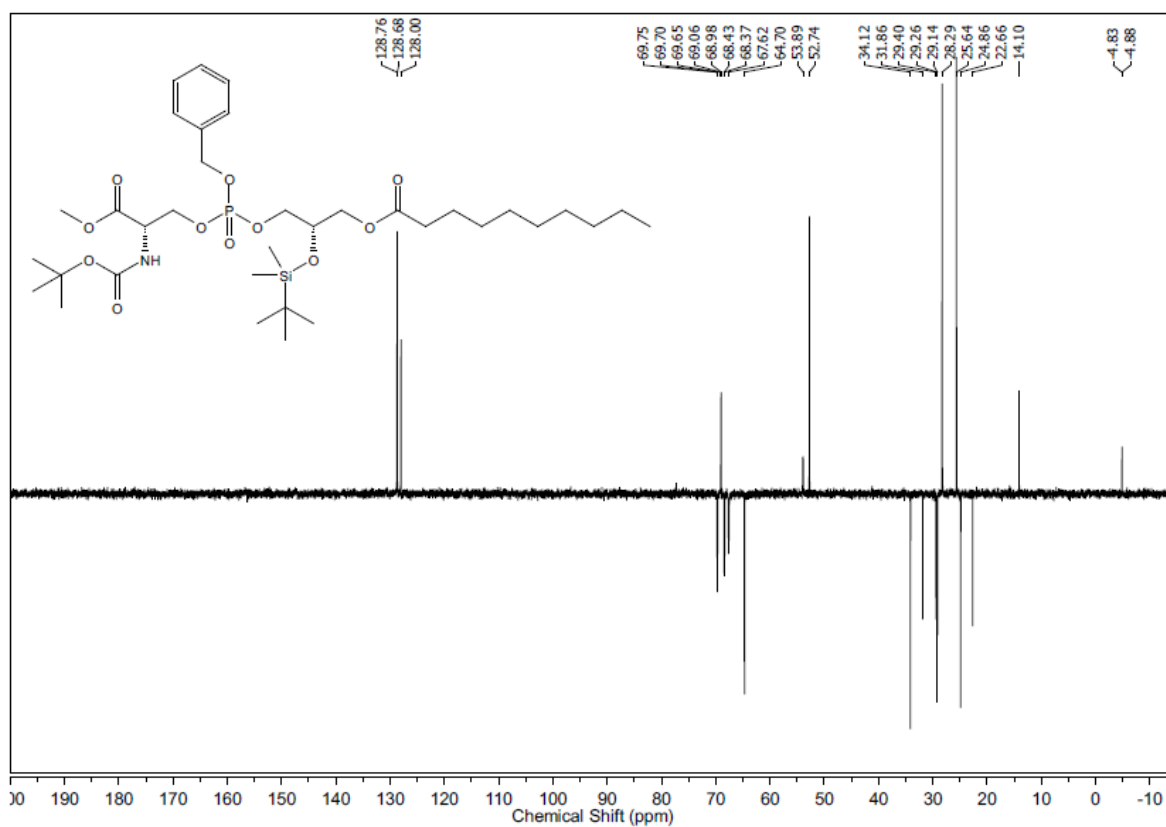
³¹P NMR of compound (S)-8a



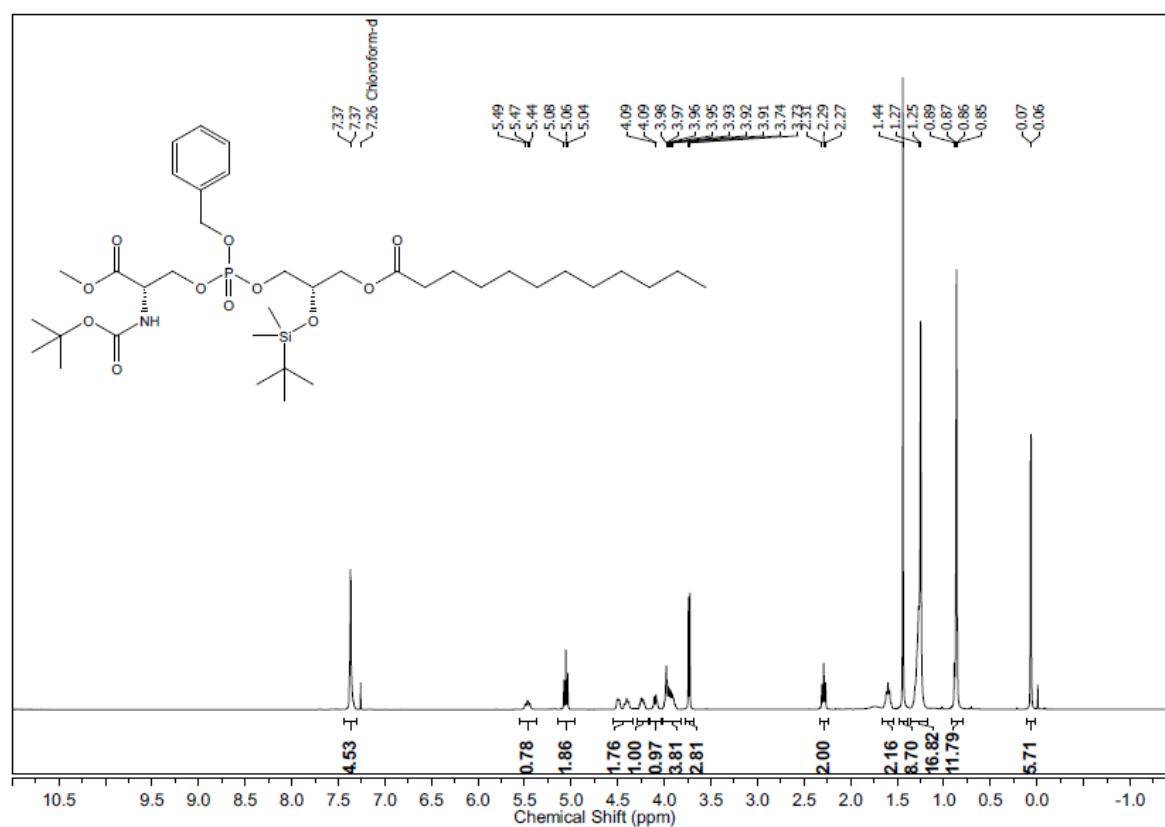
¹³C NMR of compound (S)-8a



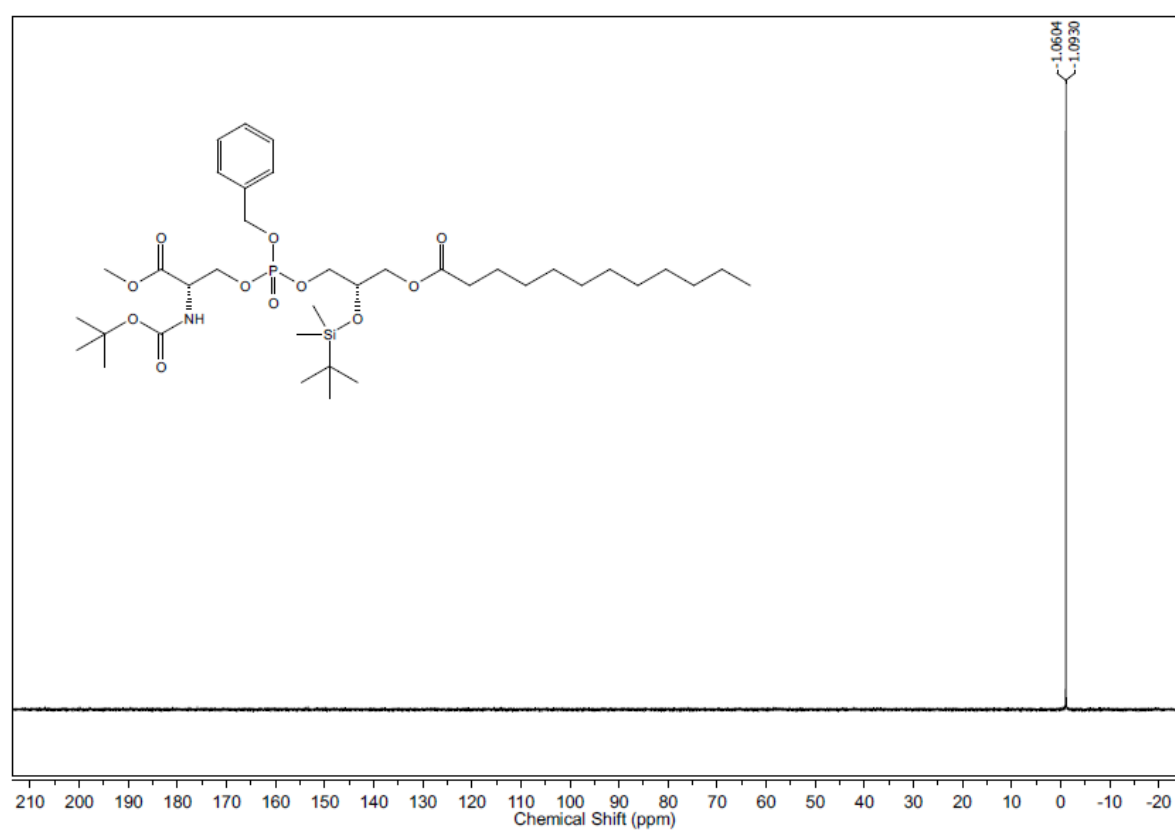
DEPT-135 NMR of compound (S)-8a



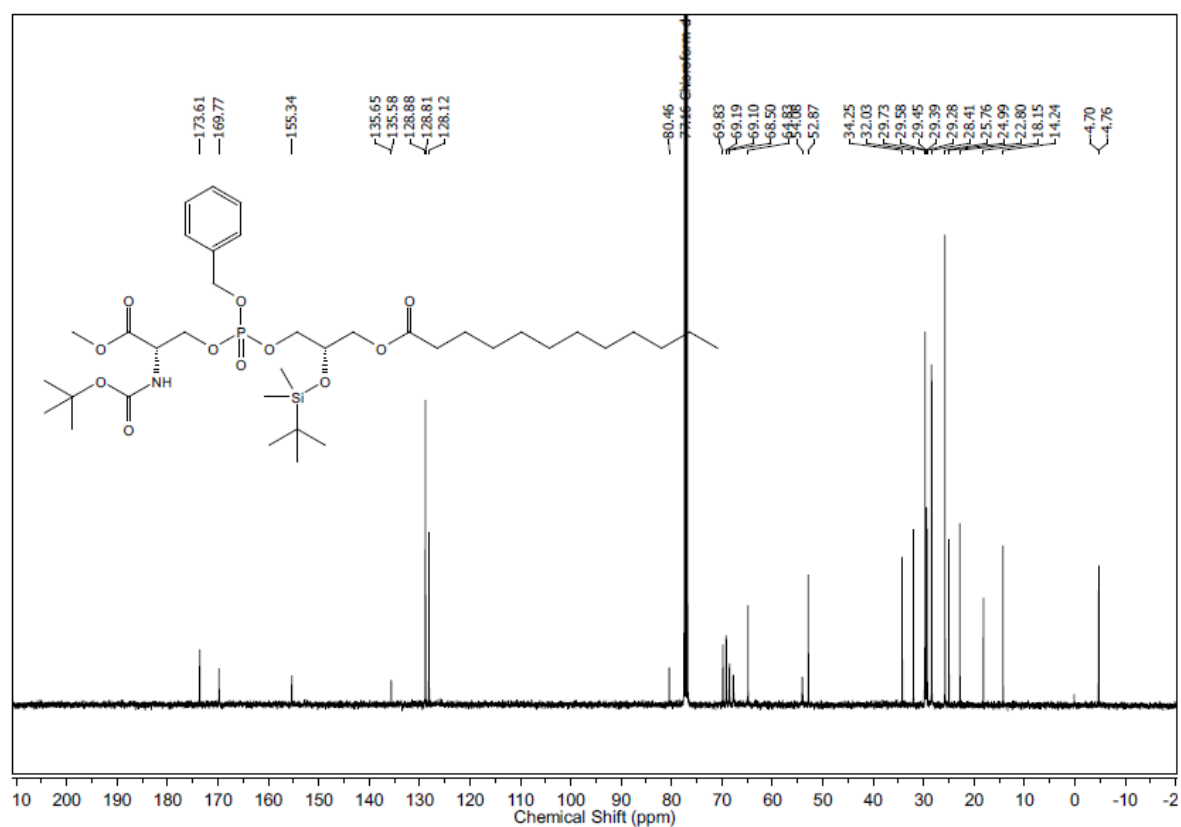
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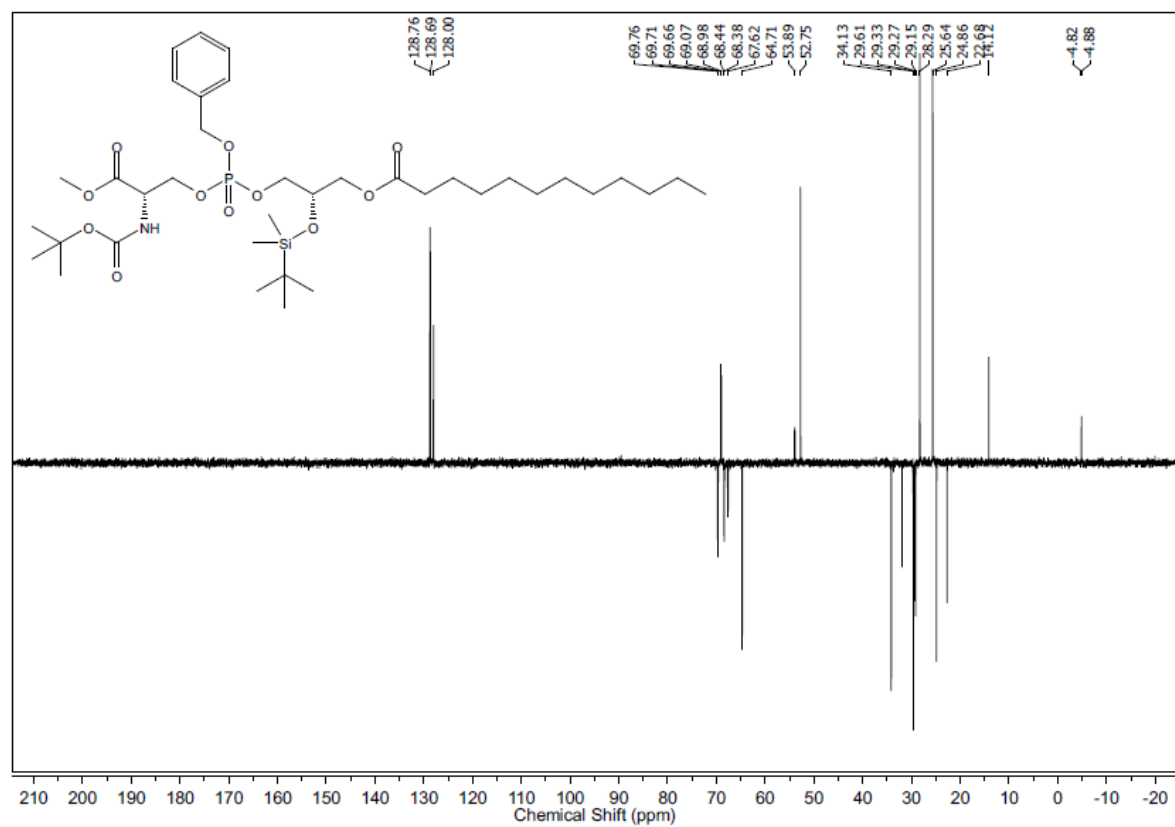
³¹P NMR of compound (S)-**8b**



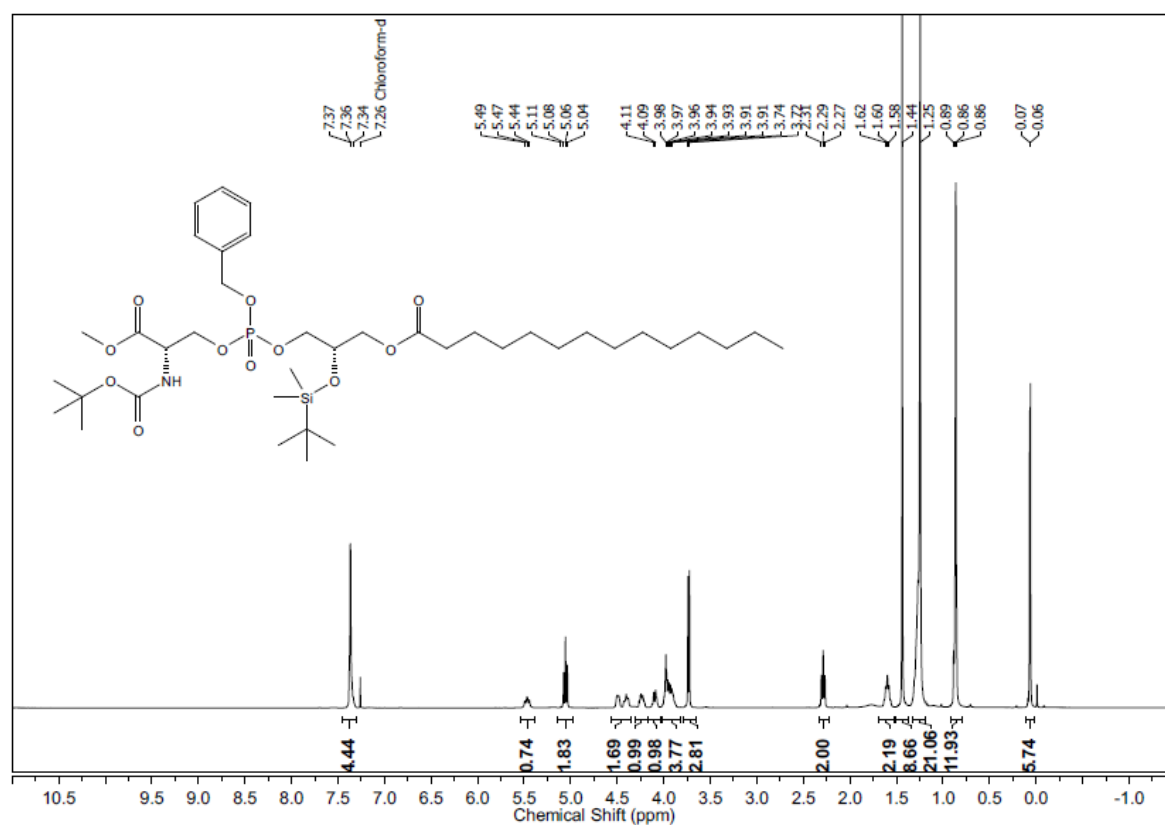
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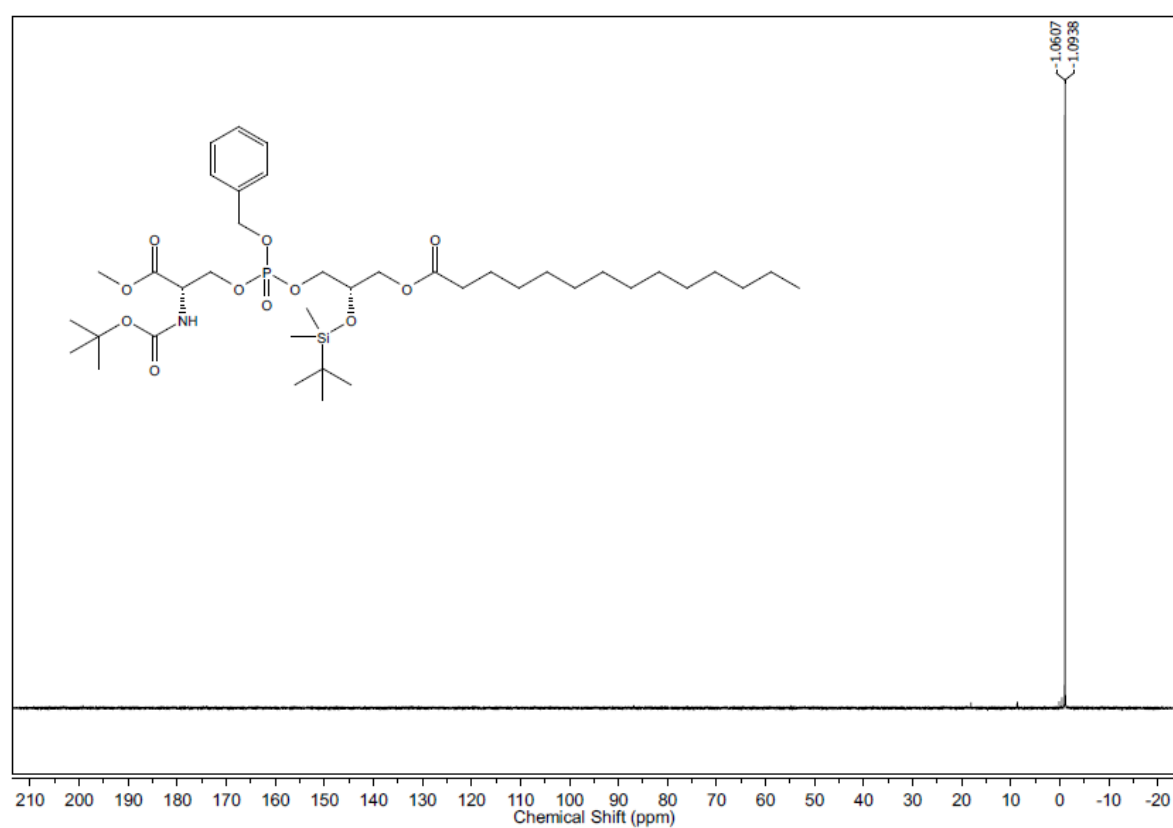
DEPT-135 NMR of compound (S)-**8b**



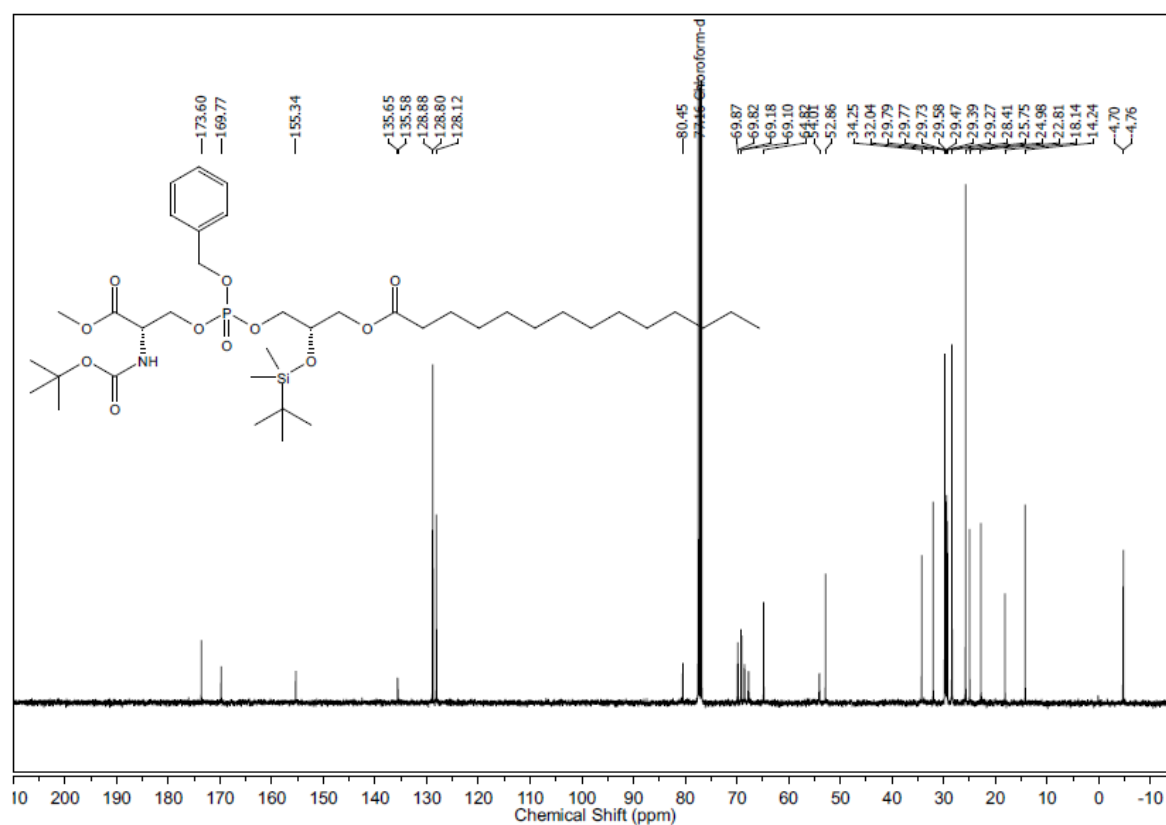
¹H NMR of compound (S)-**8c**



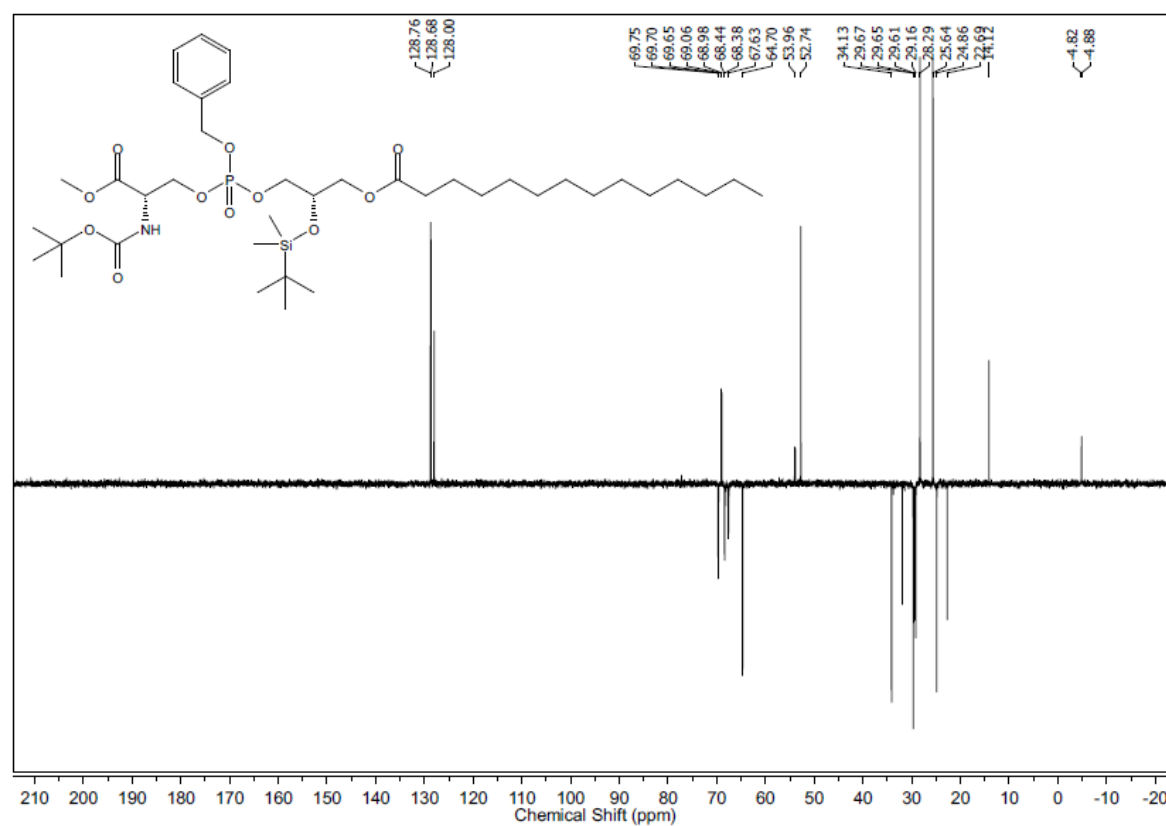
³¹P NMR of compound (S)-**8c**



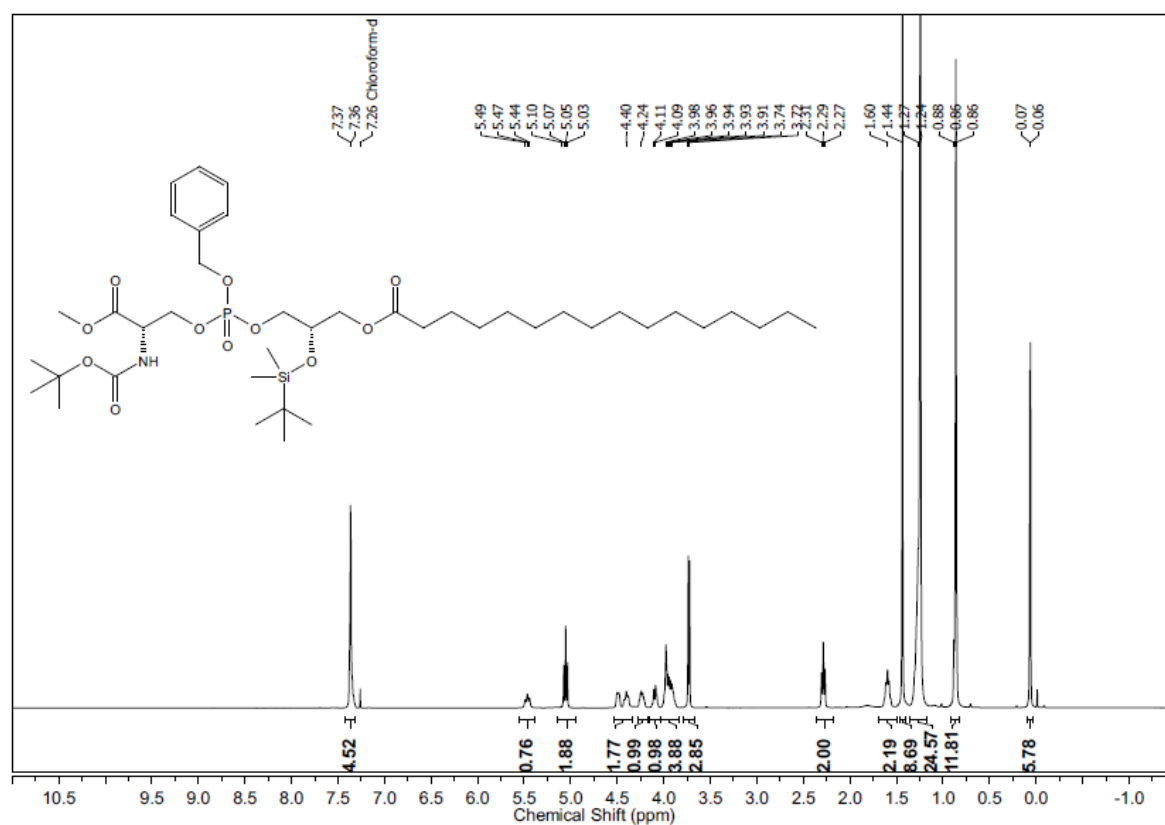
^{13}C NMR of compound (S)-**8c**



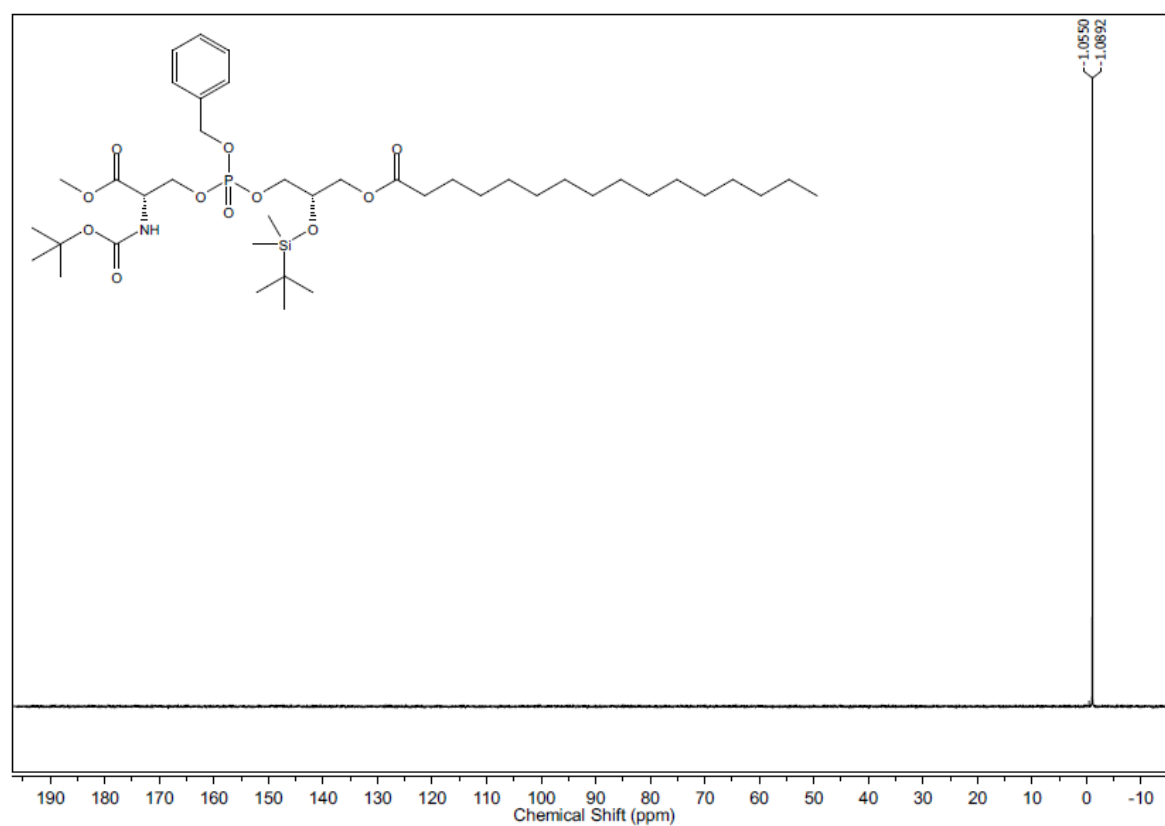
DEPT-135 NMR of compound (S)-**8c**



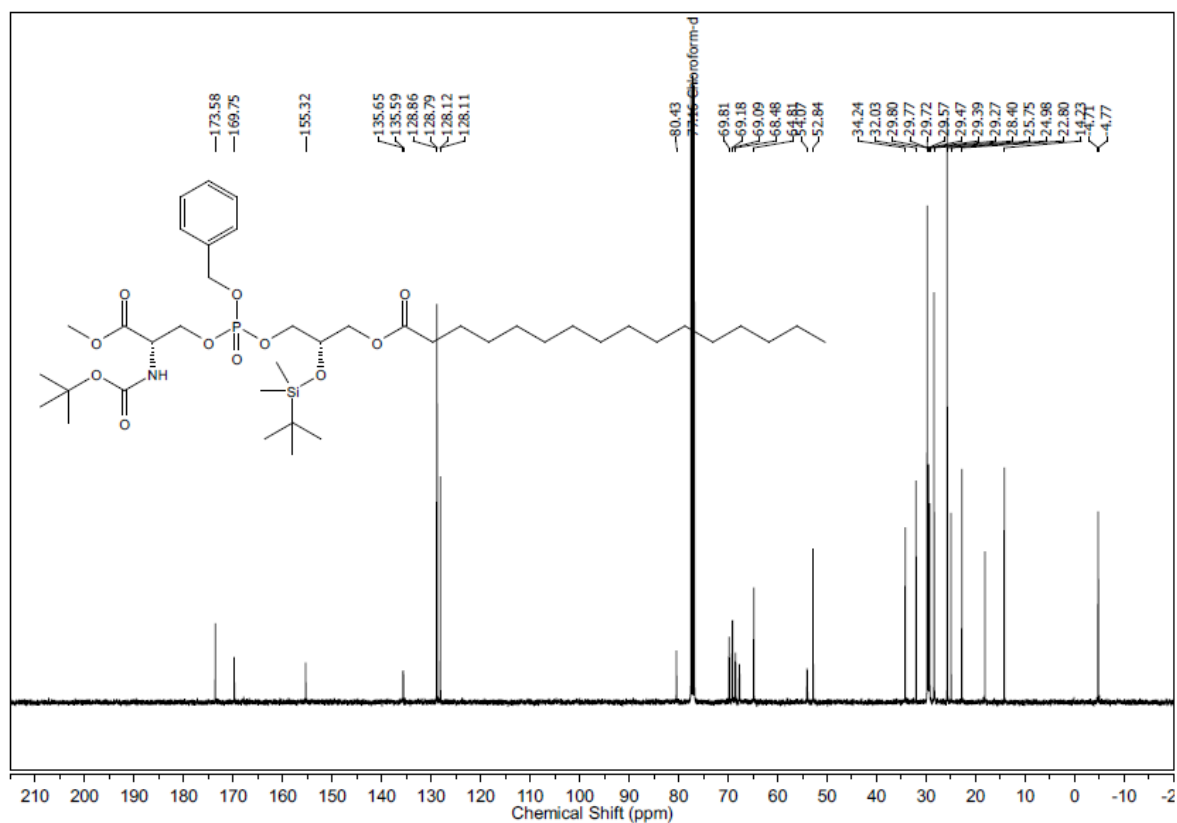
¹H NMR of compound (S)-8d



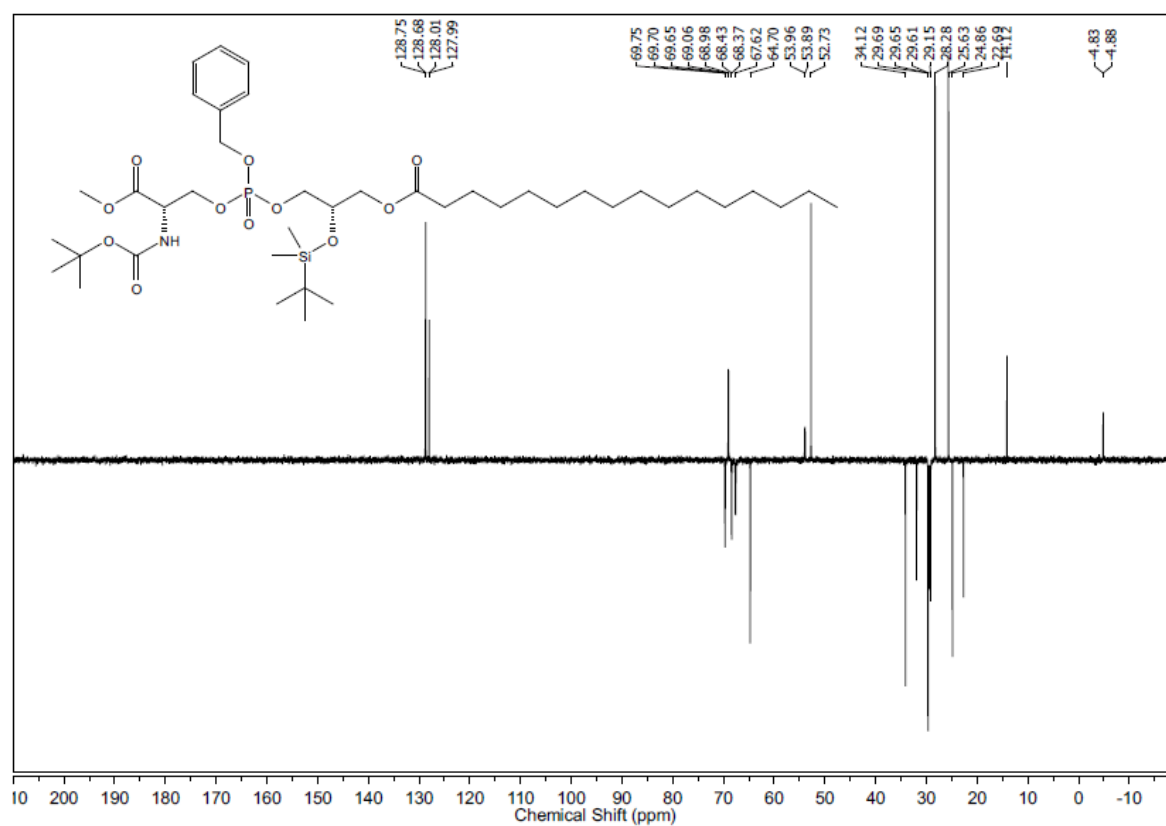
³¹P NMR of compound (S)-8d



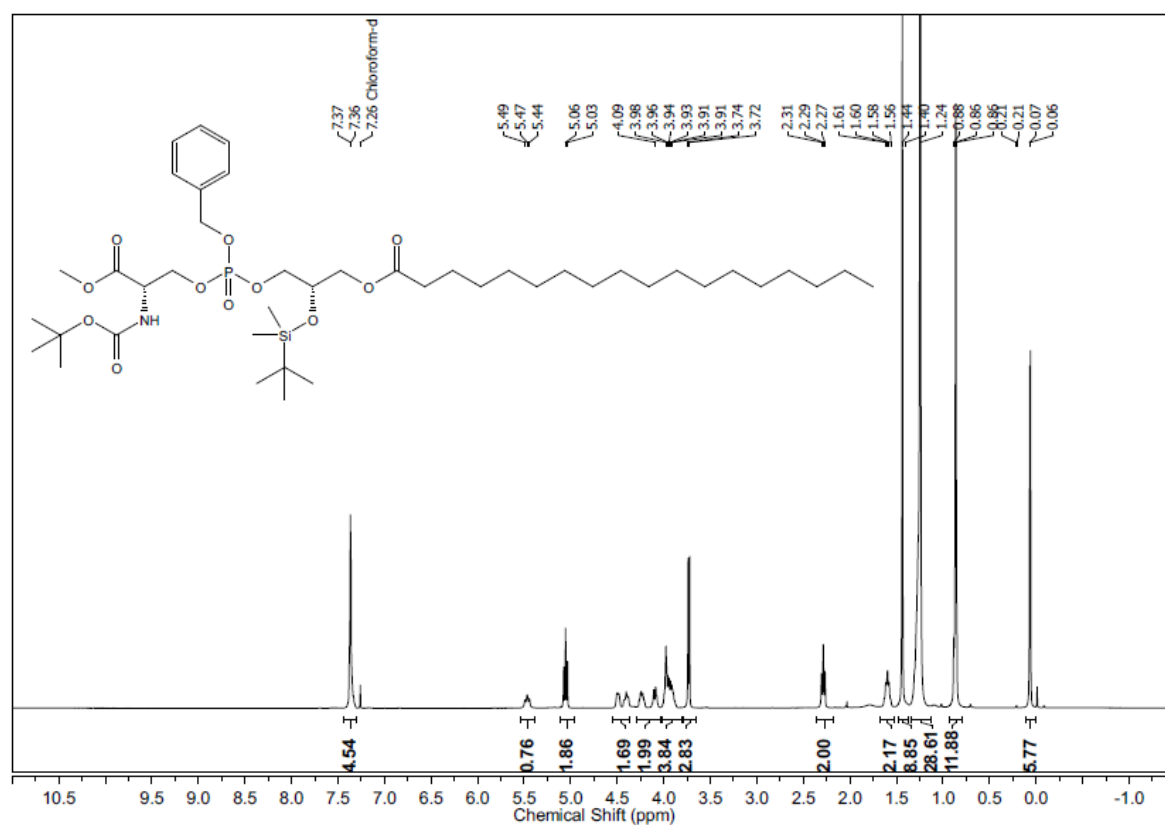
¹³C NMR of compound (S)-8d



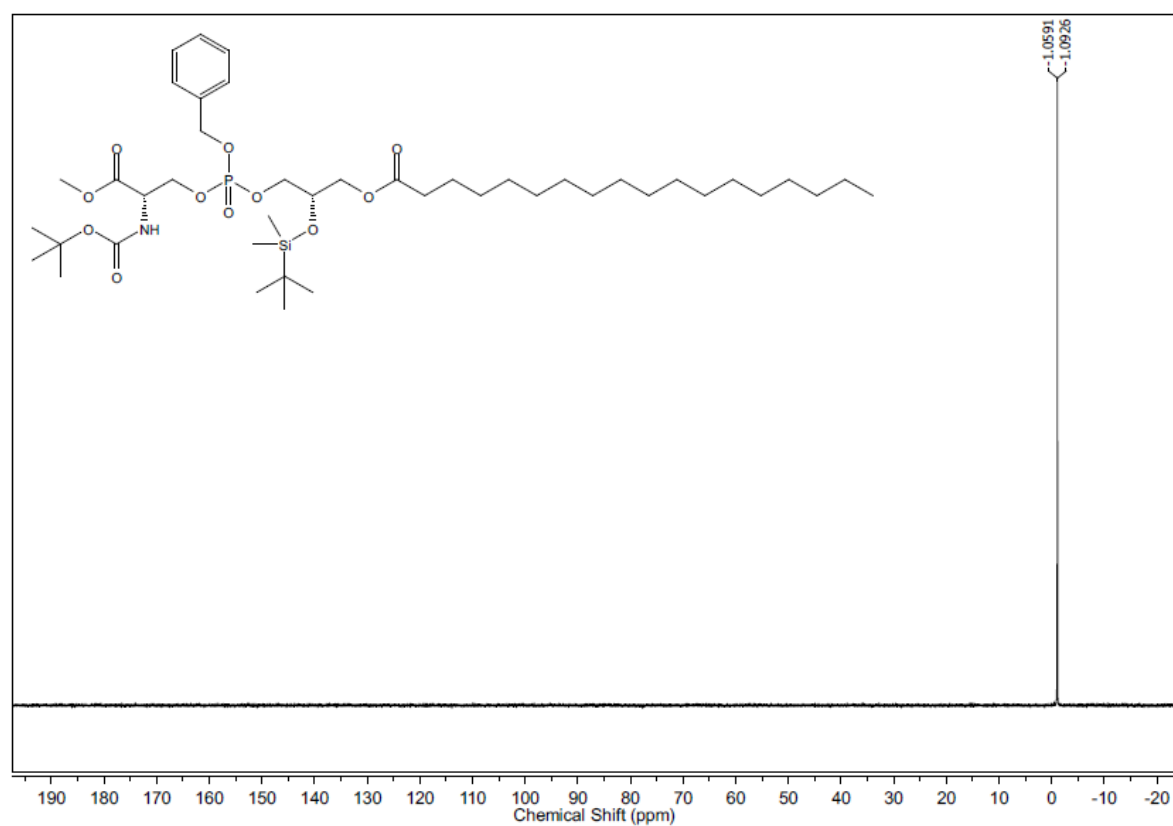
DEPT-135 NMR of compound (S)-8d



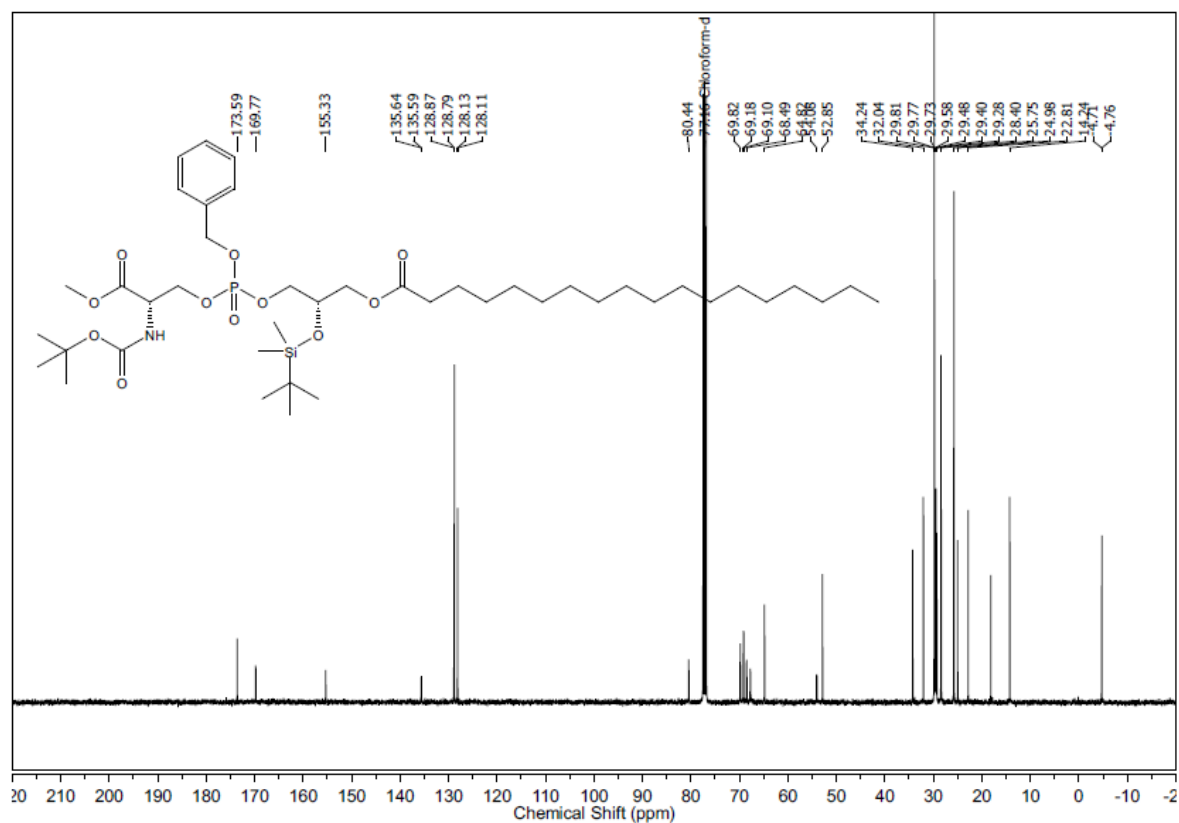
^1H NMR of compound (S)-**8e**



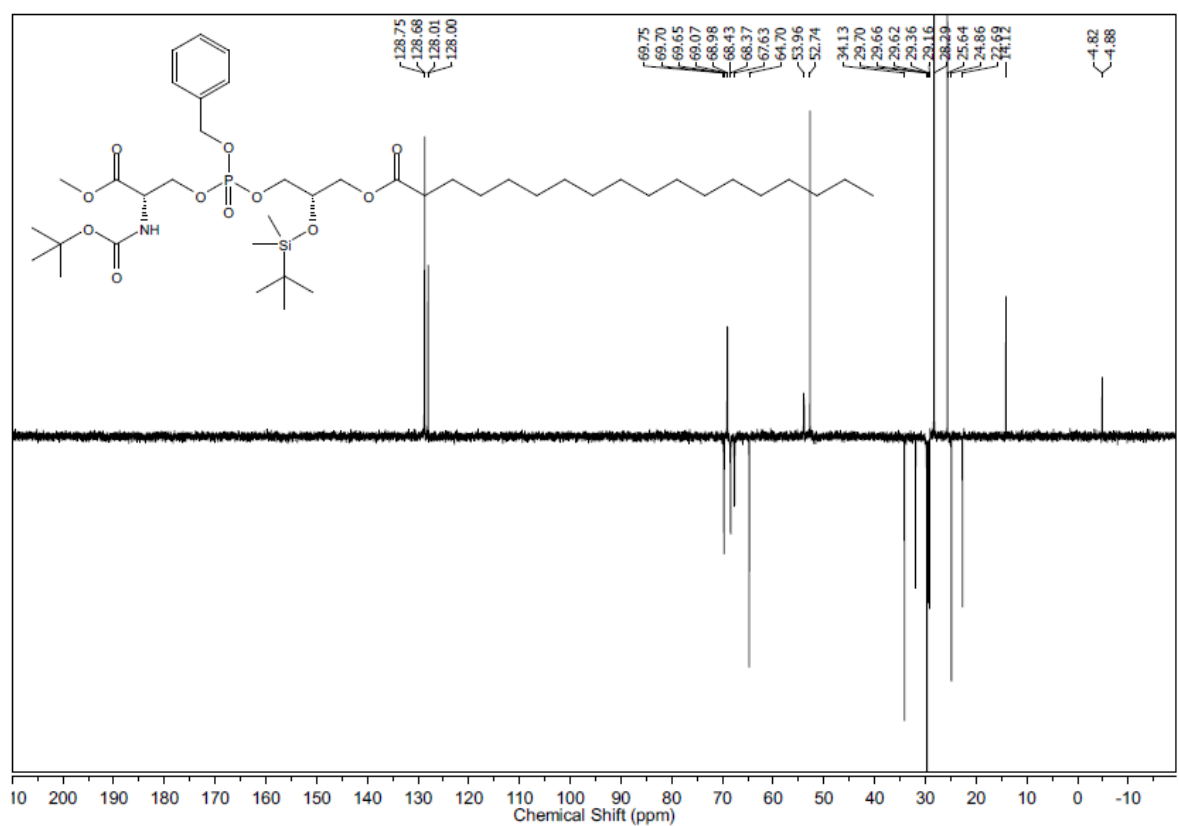
^{31}P NMR of compound (S)-**8e**



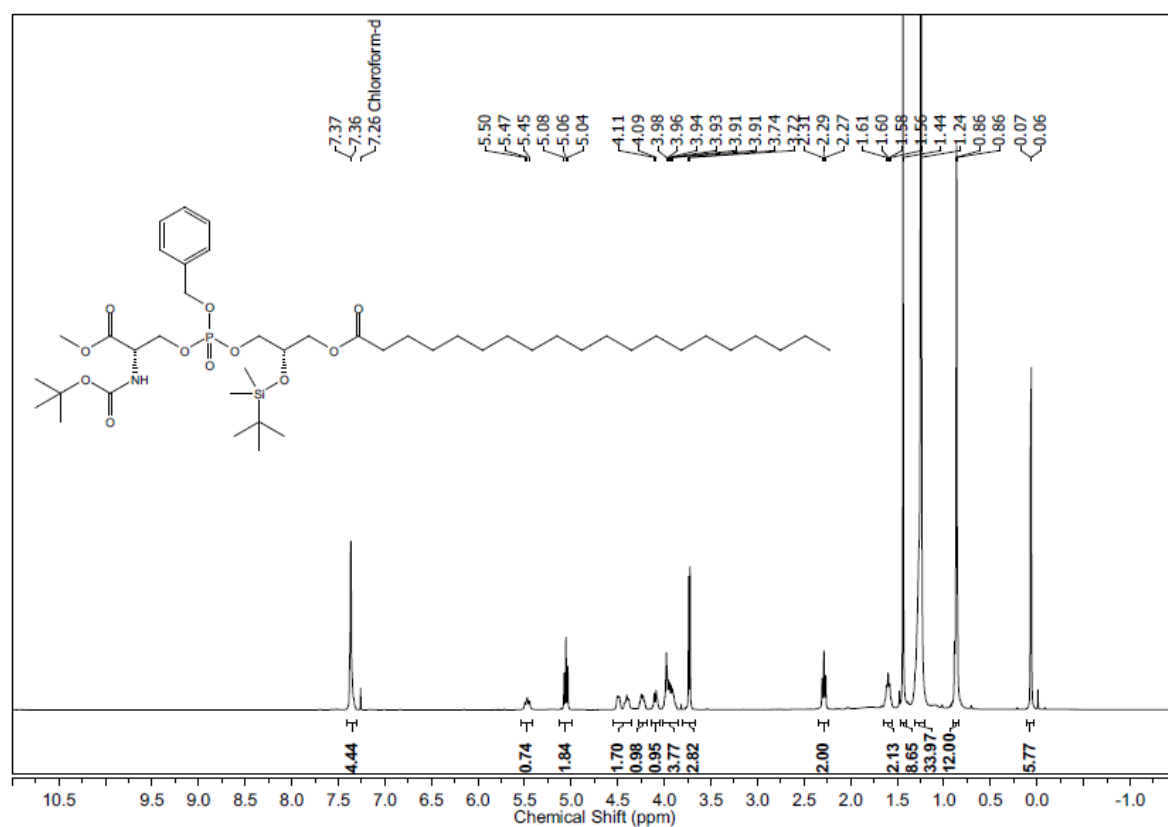
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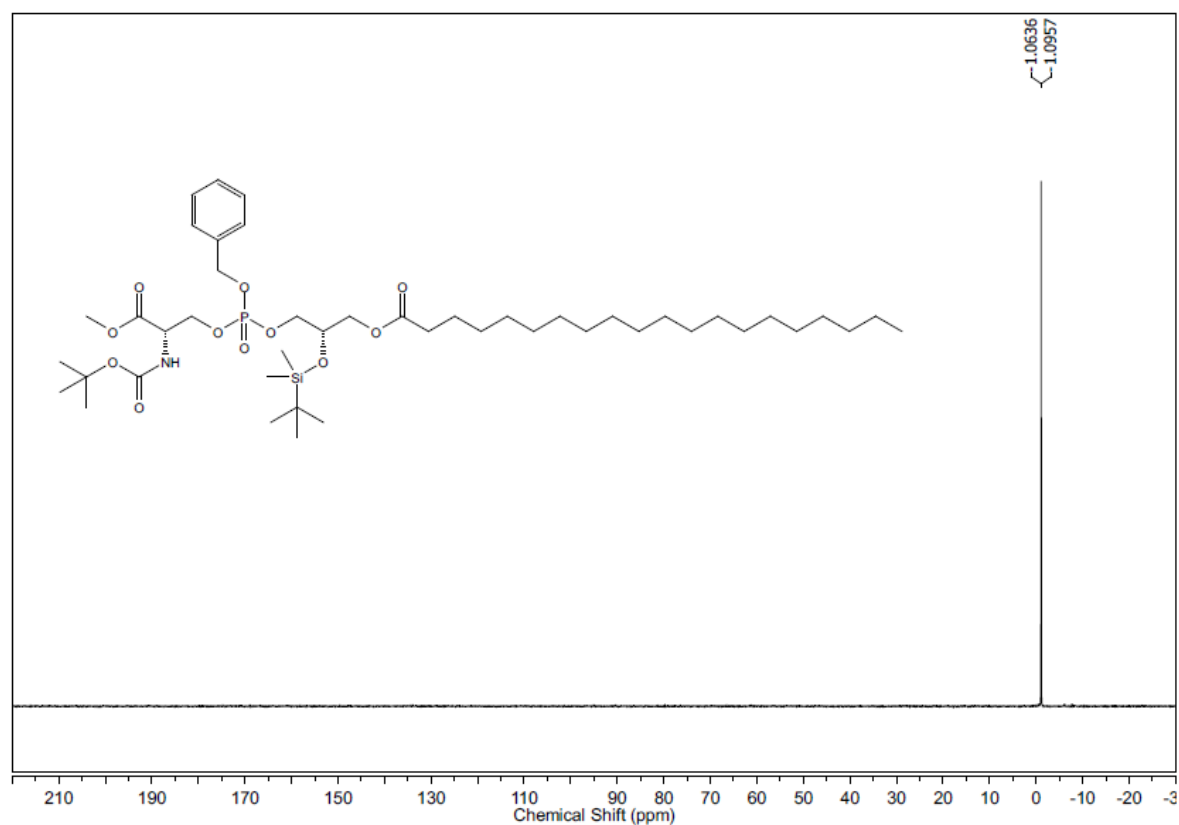
DEPT-135 NMR of compound (S)-**8e**



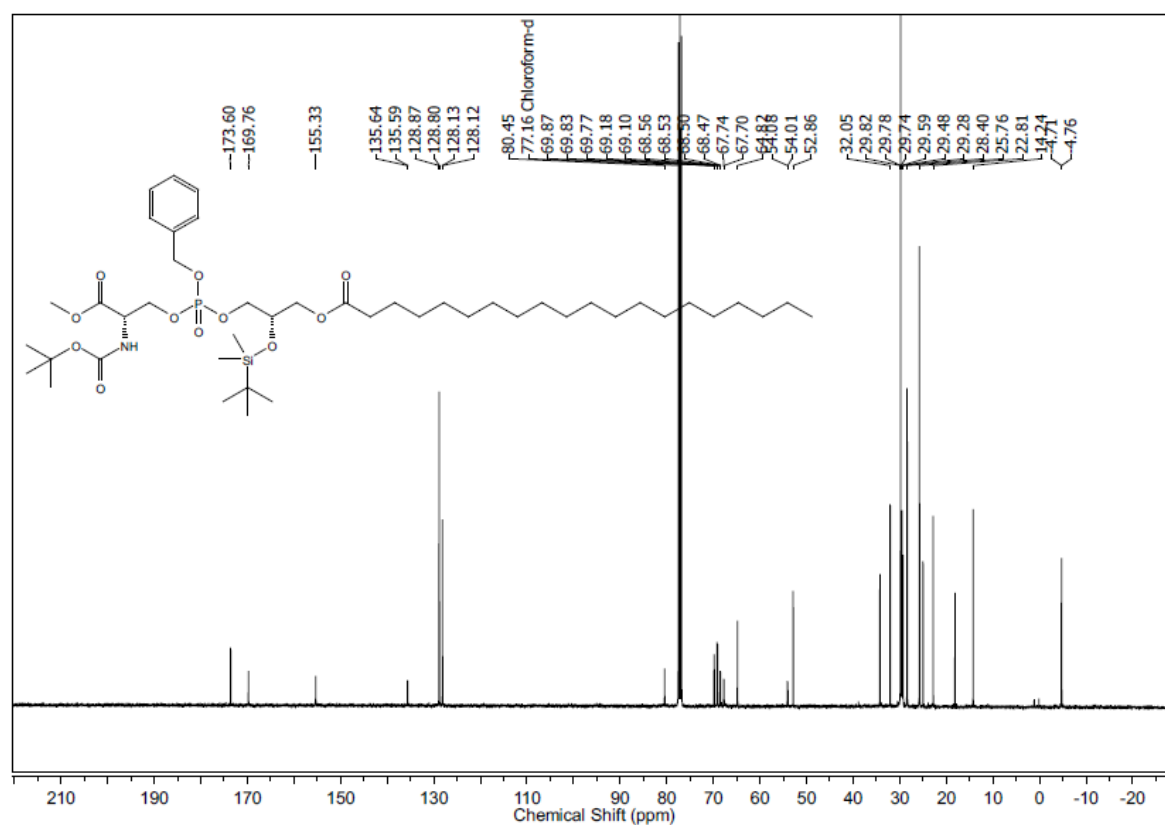
¹H NMR of compound (S)-**8f**



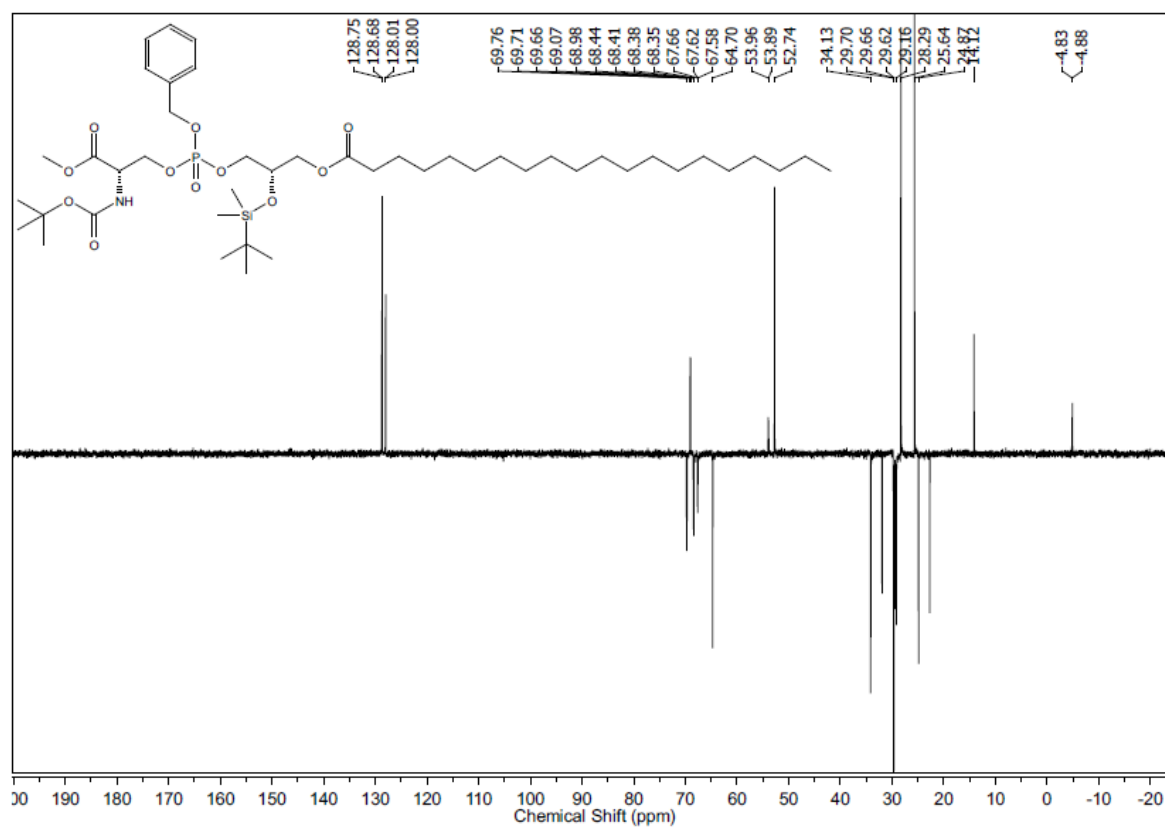
³¹P NMR of compound (S)-**8f**



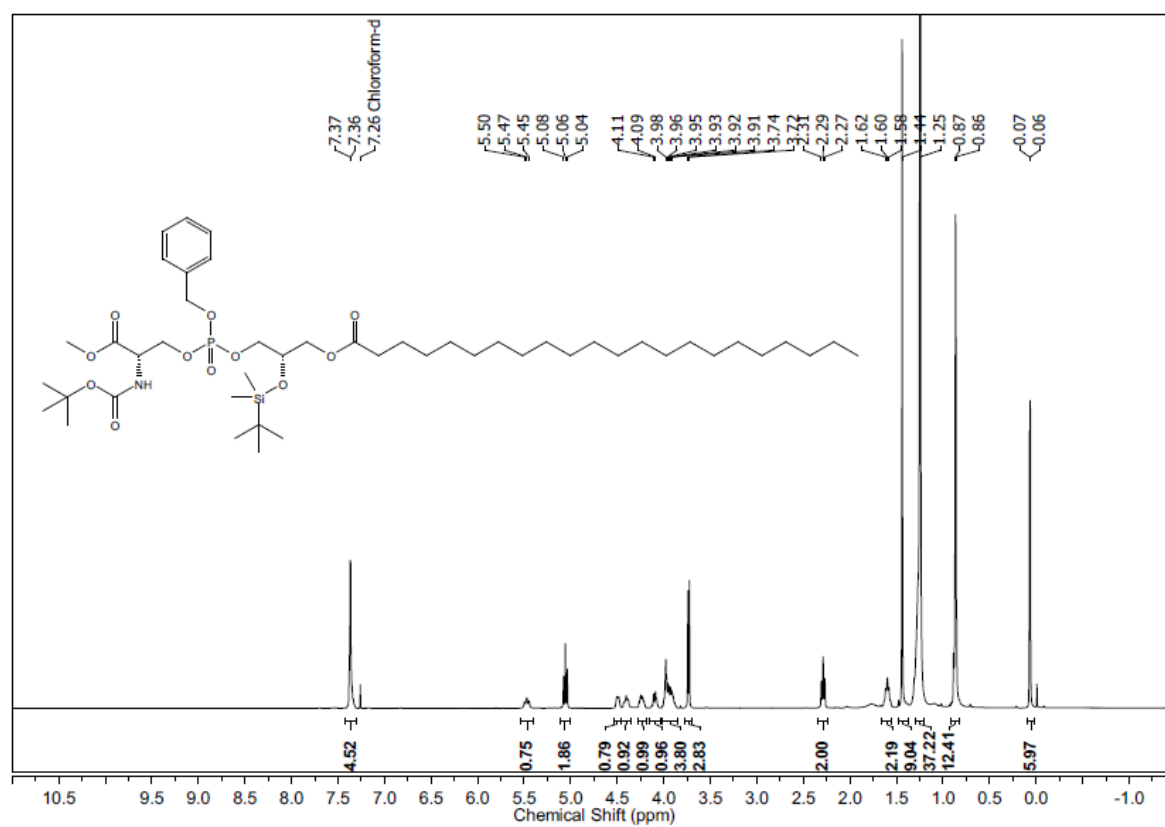
^{13}C NMR of compound (S)-8f



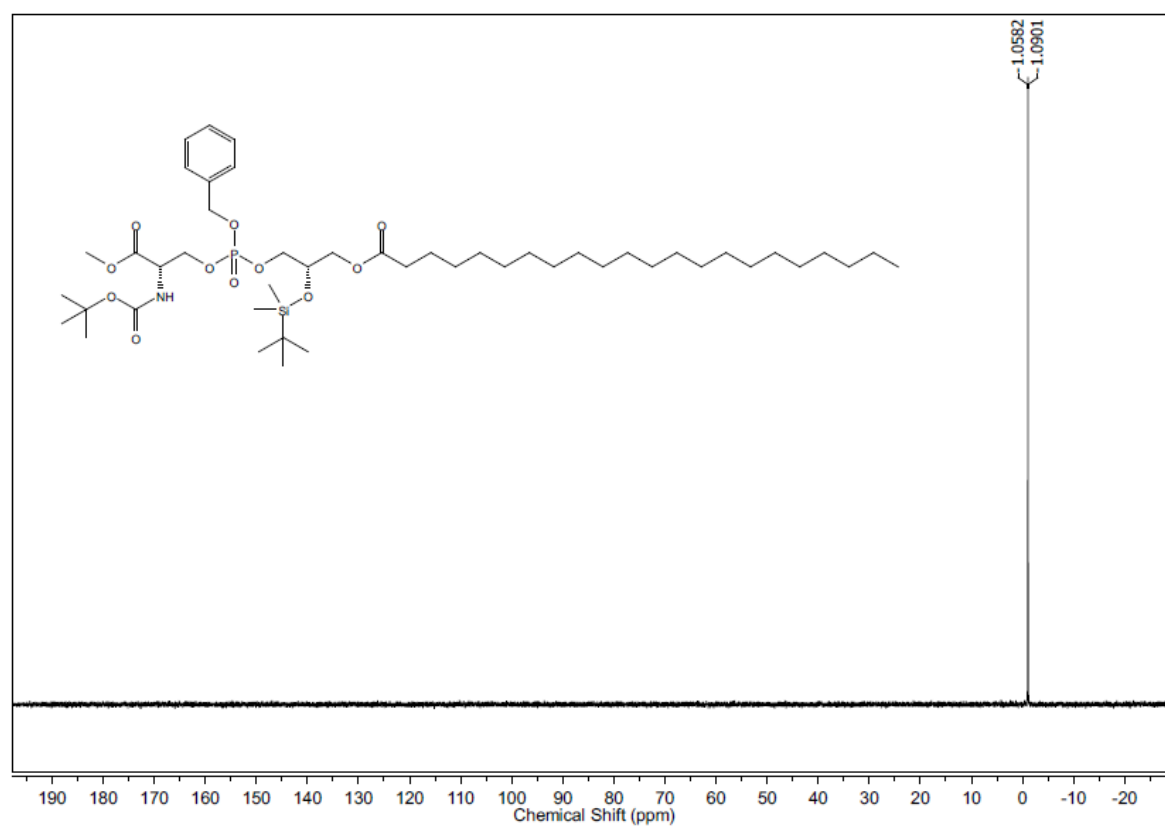
DEPT-135 NMR of compound (S)-8f



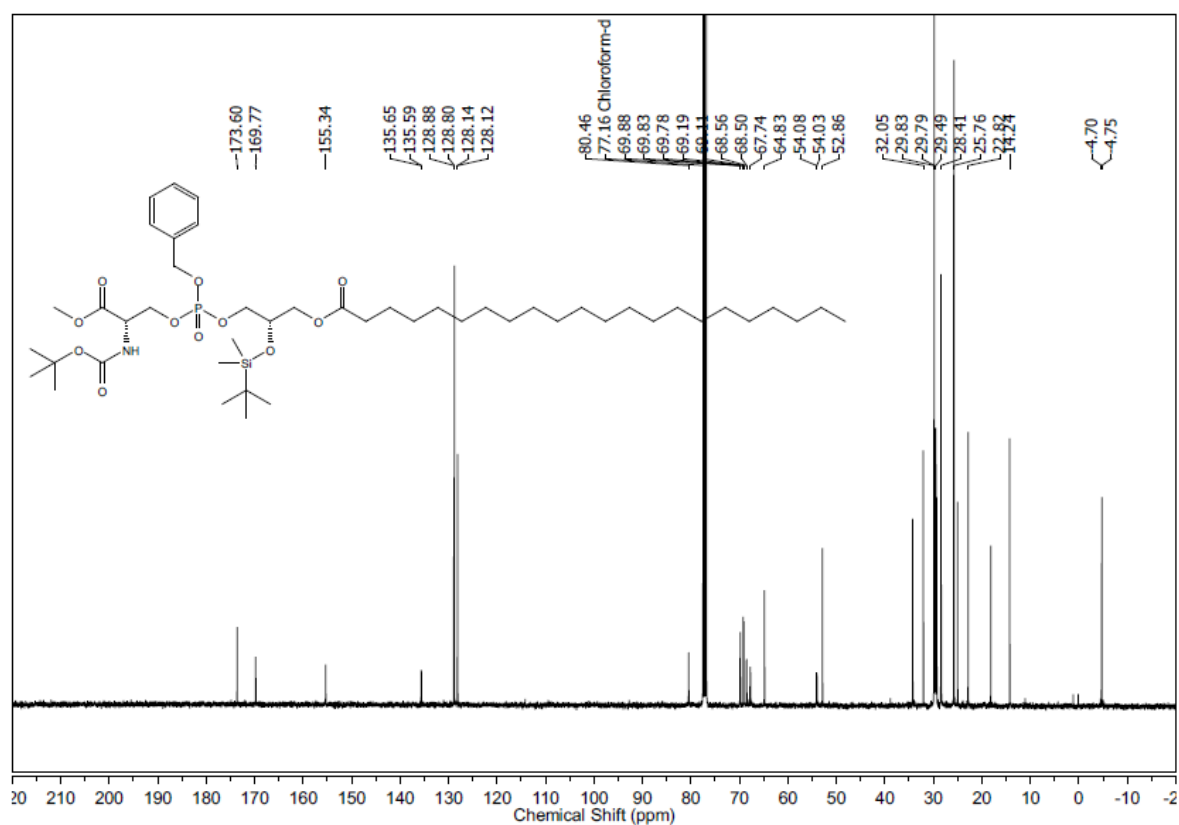
¹H NMR of compound (S)-**8g**



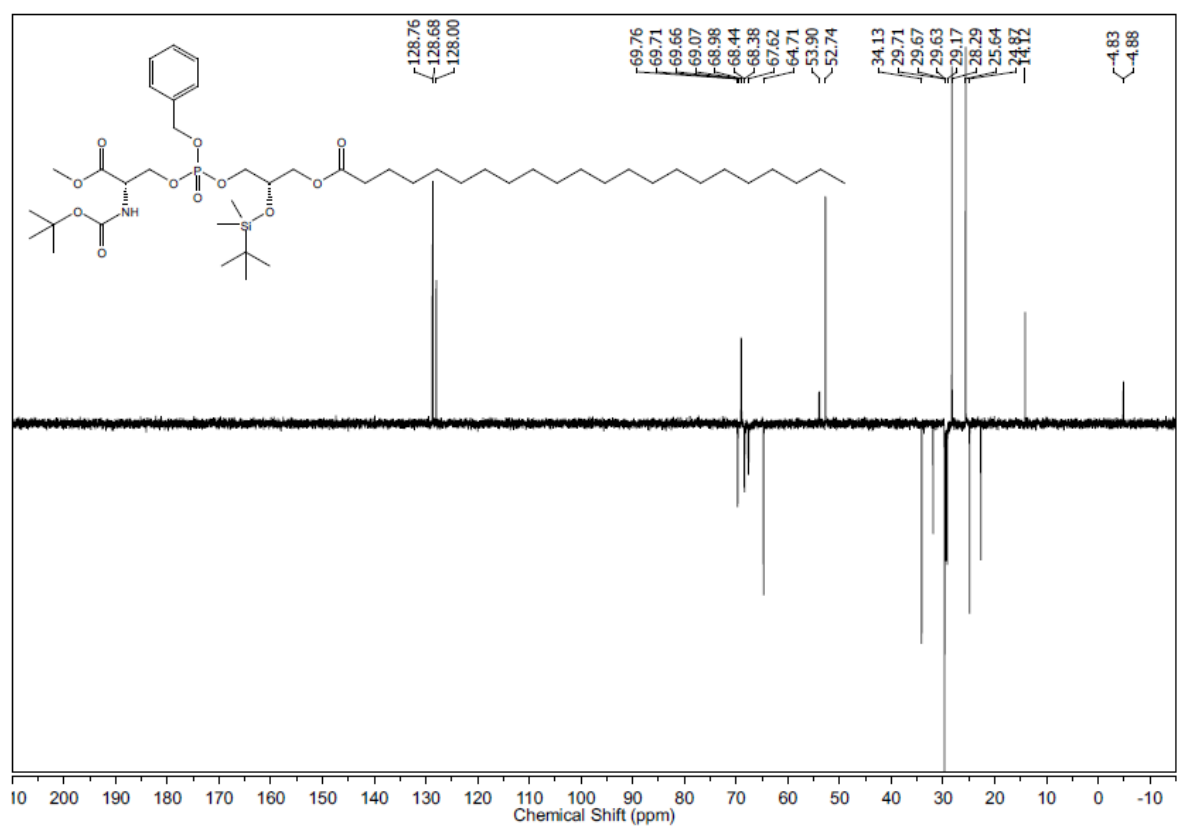
³¹P NMR of compound (S)-**8g**



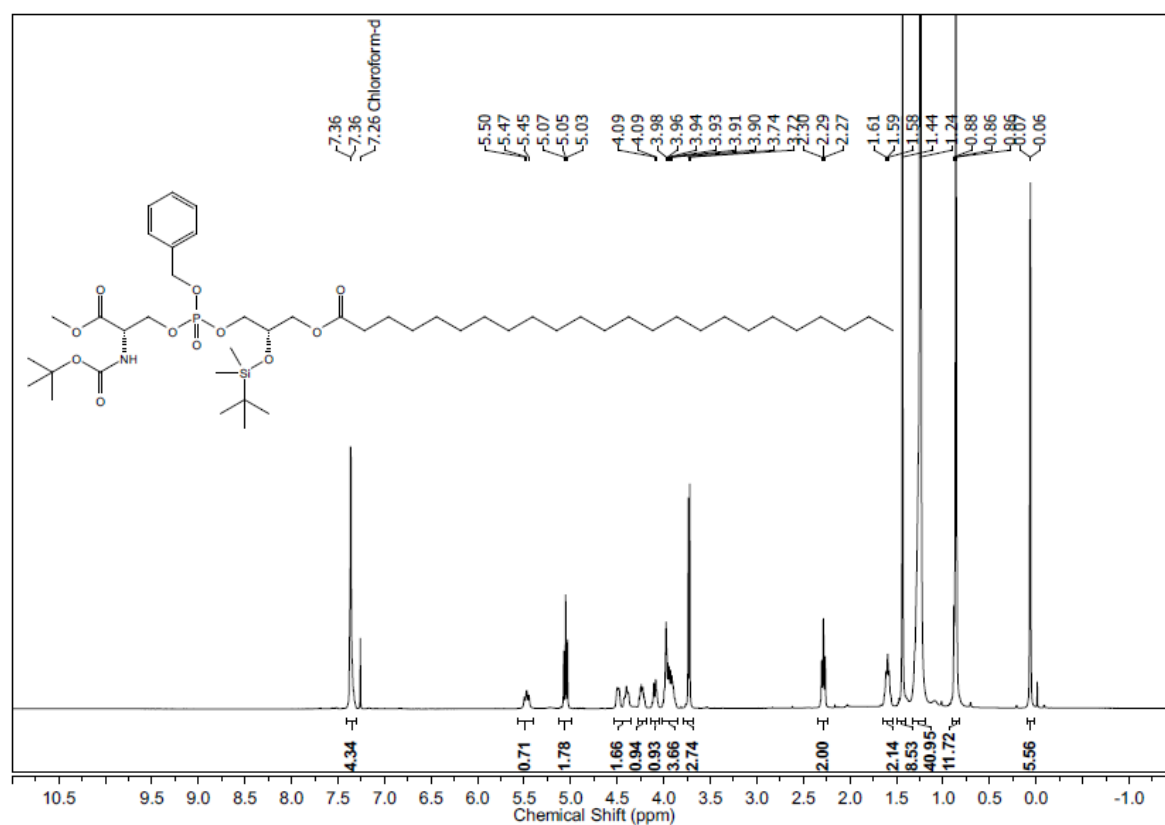
¹³C NMR of compound (S)-8g



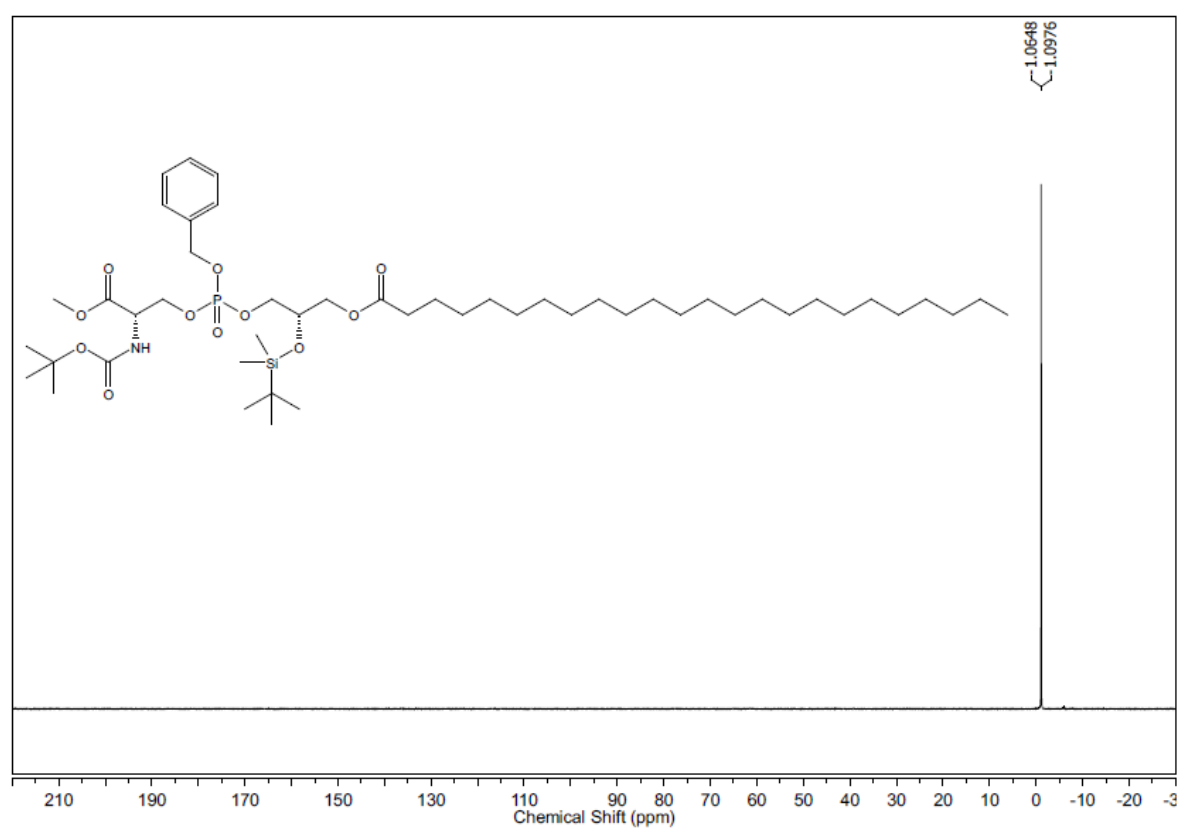
DEPT-135 NMR of compound (S)-8g



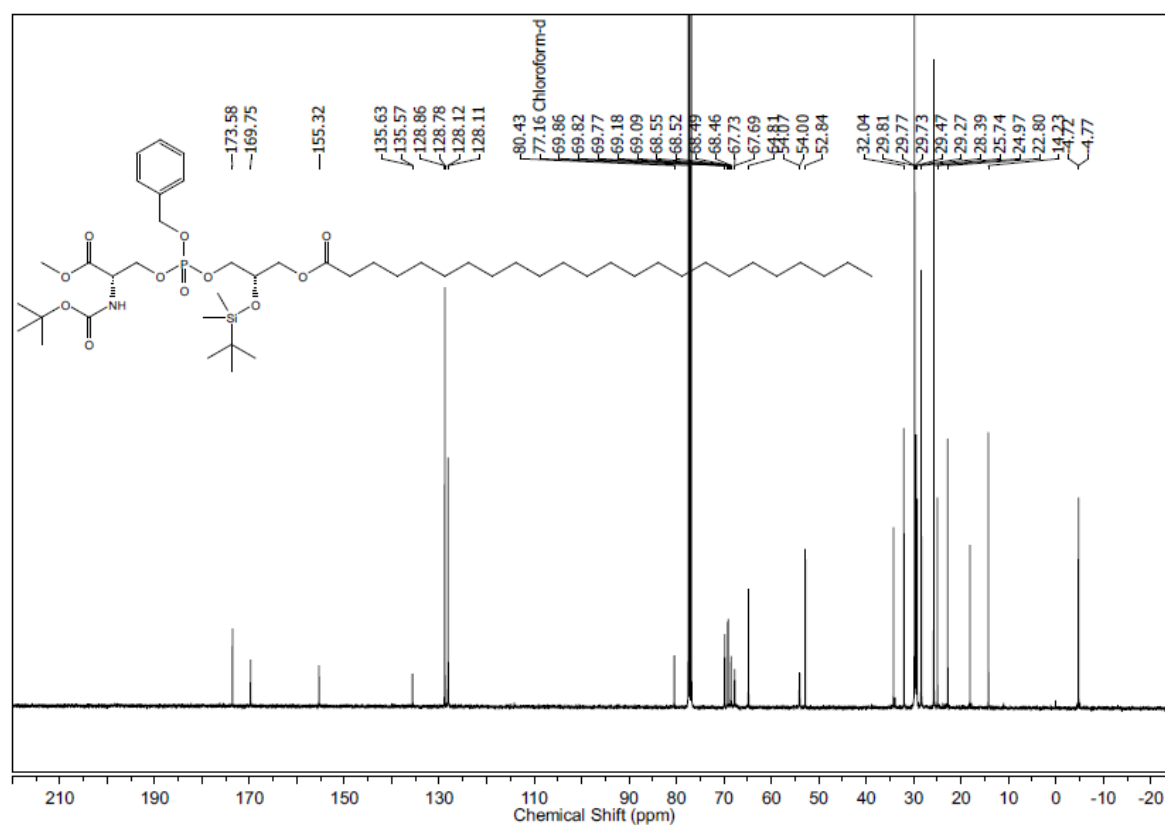
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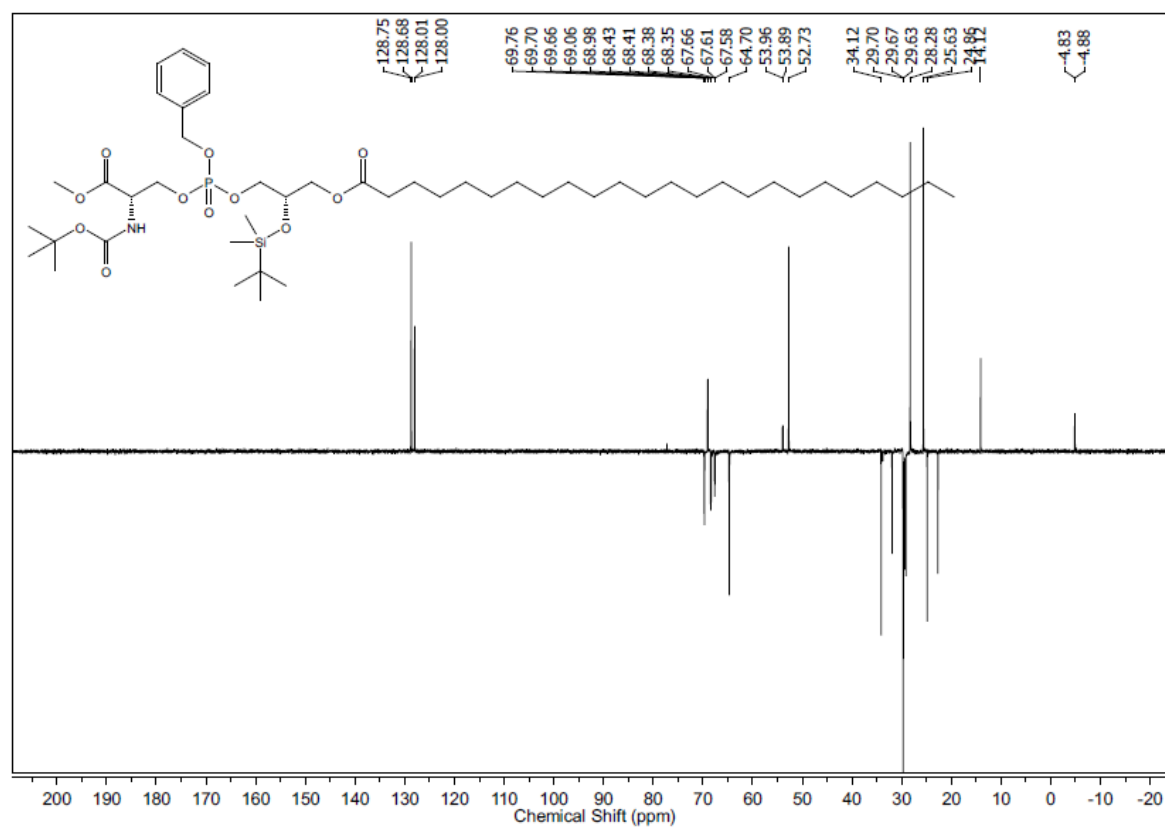
³¹P NMR of compound (S)-8h



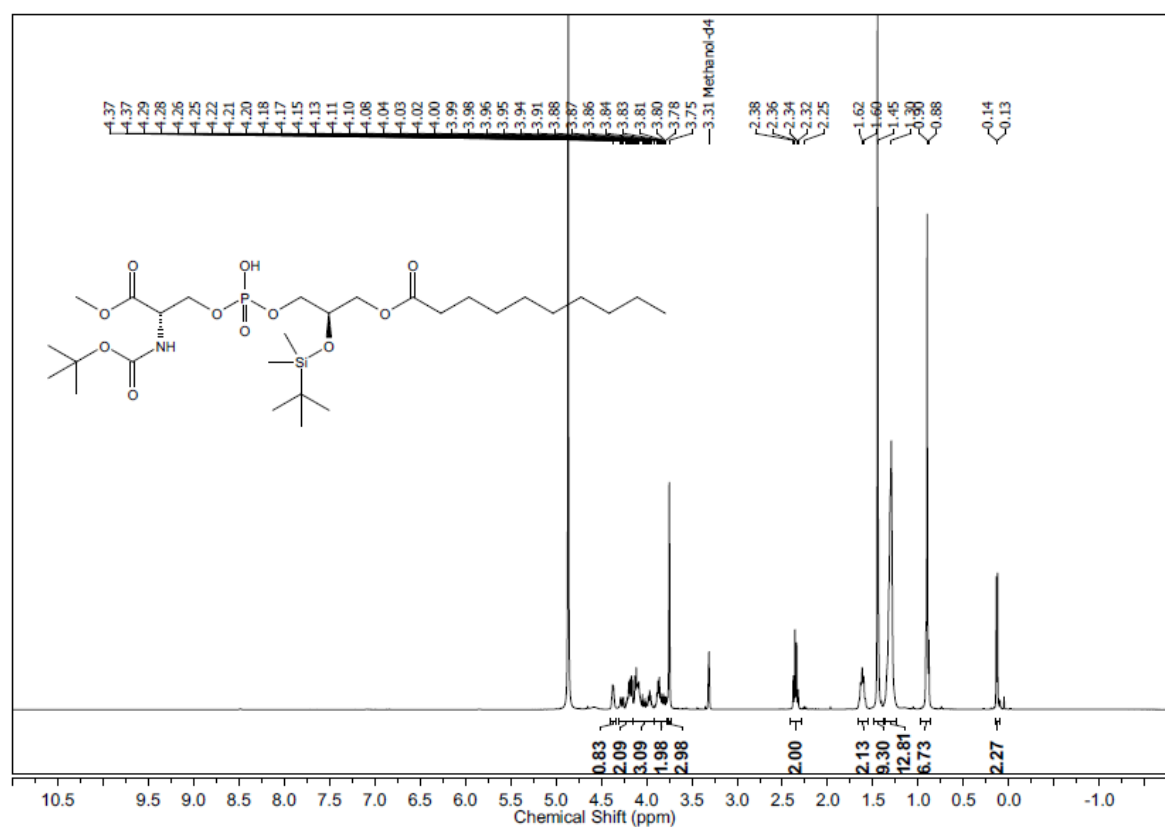
^{13}C NMR of compound (S)-8h



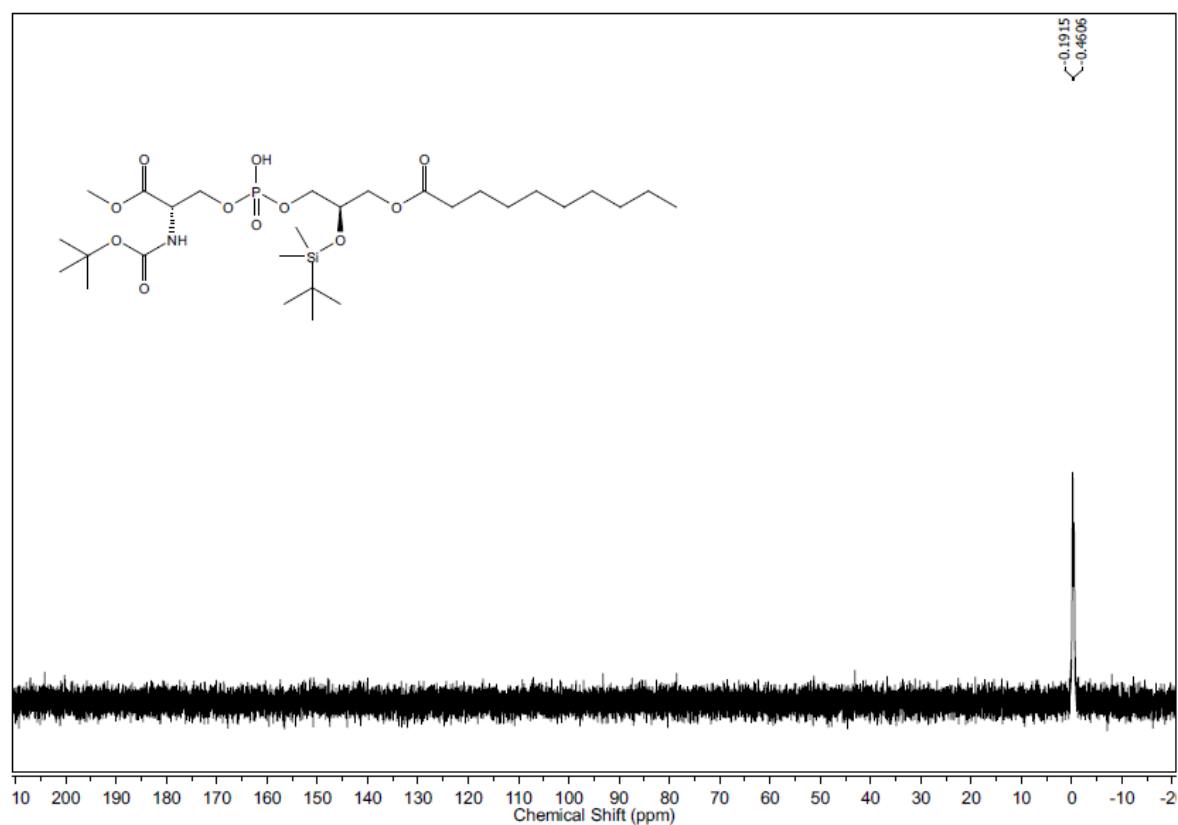
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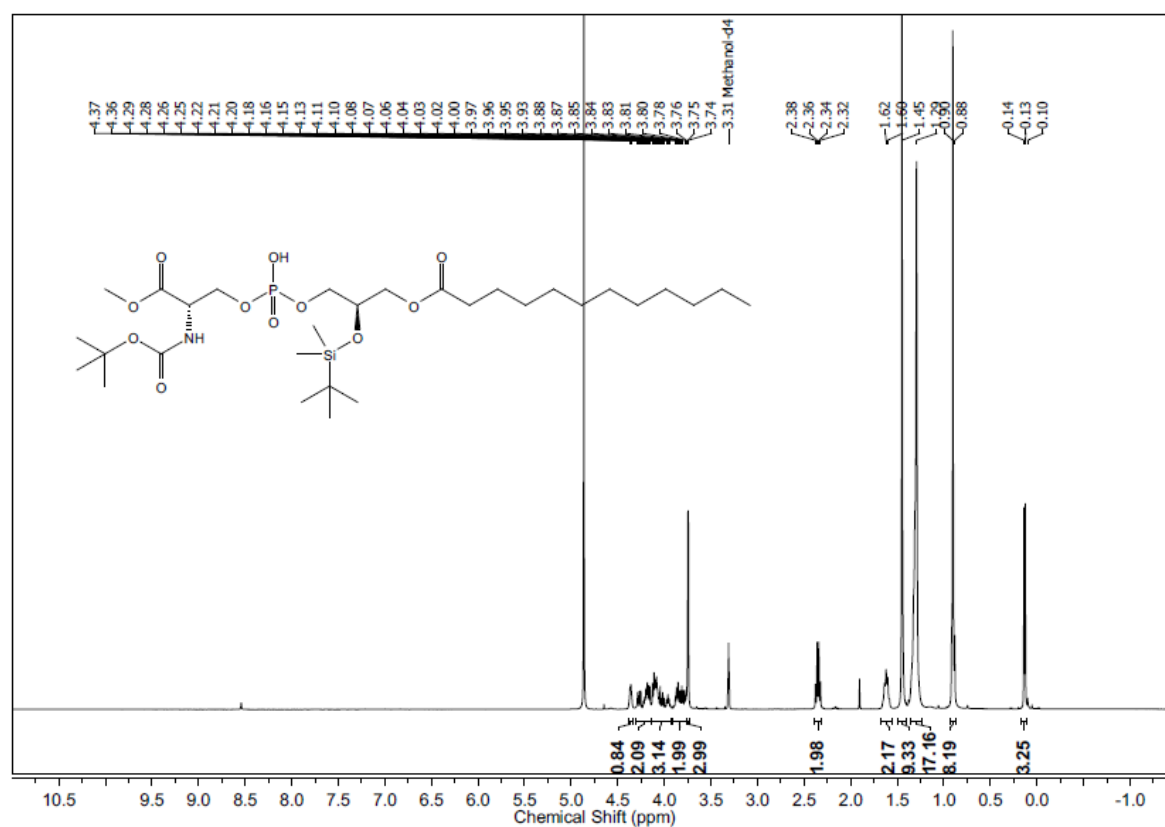
^1H NMR of intermediate (*R*)-**9a**



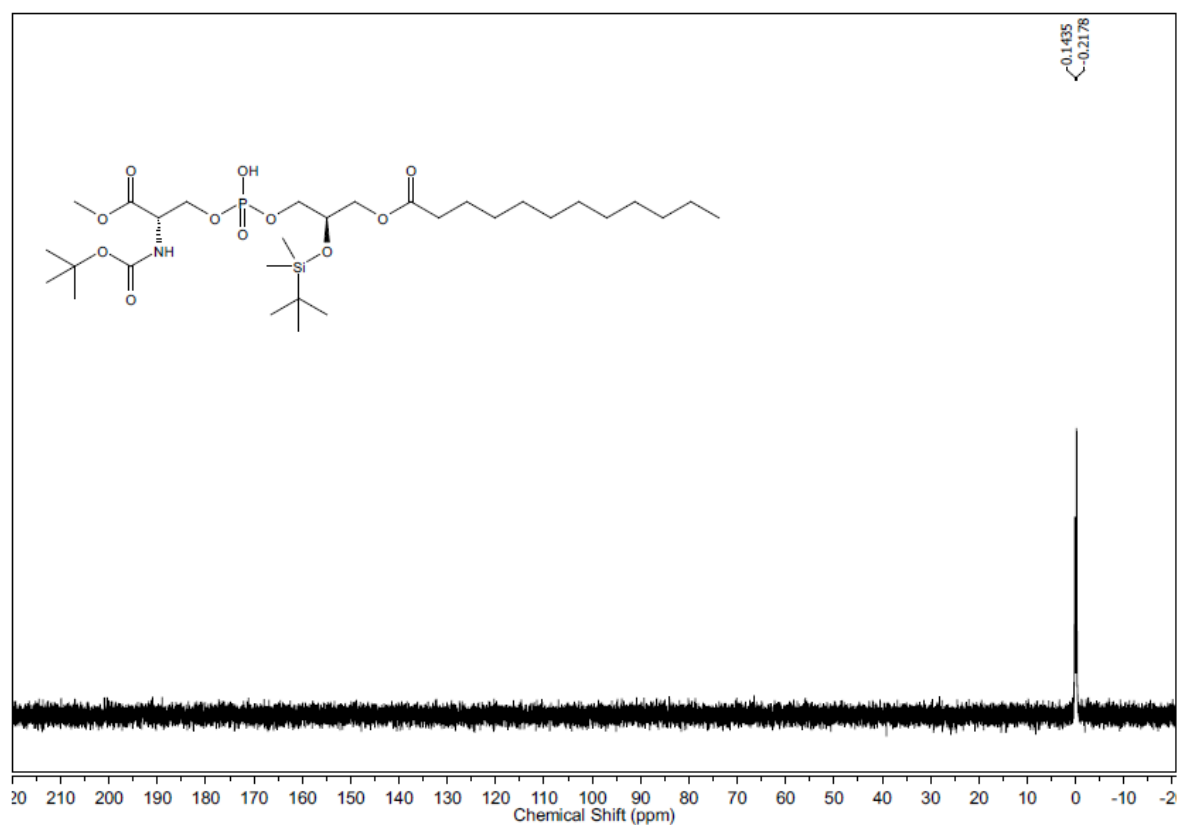
^{31}P NMR of intermediate (*R*)-**9a**



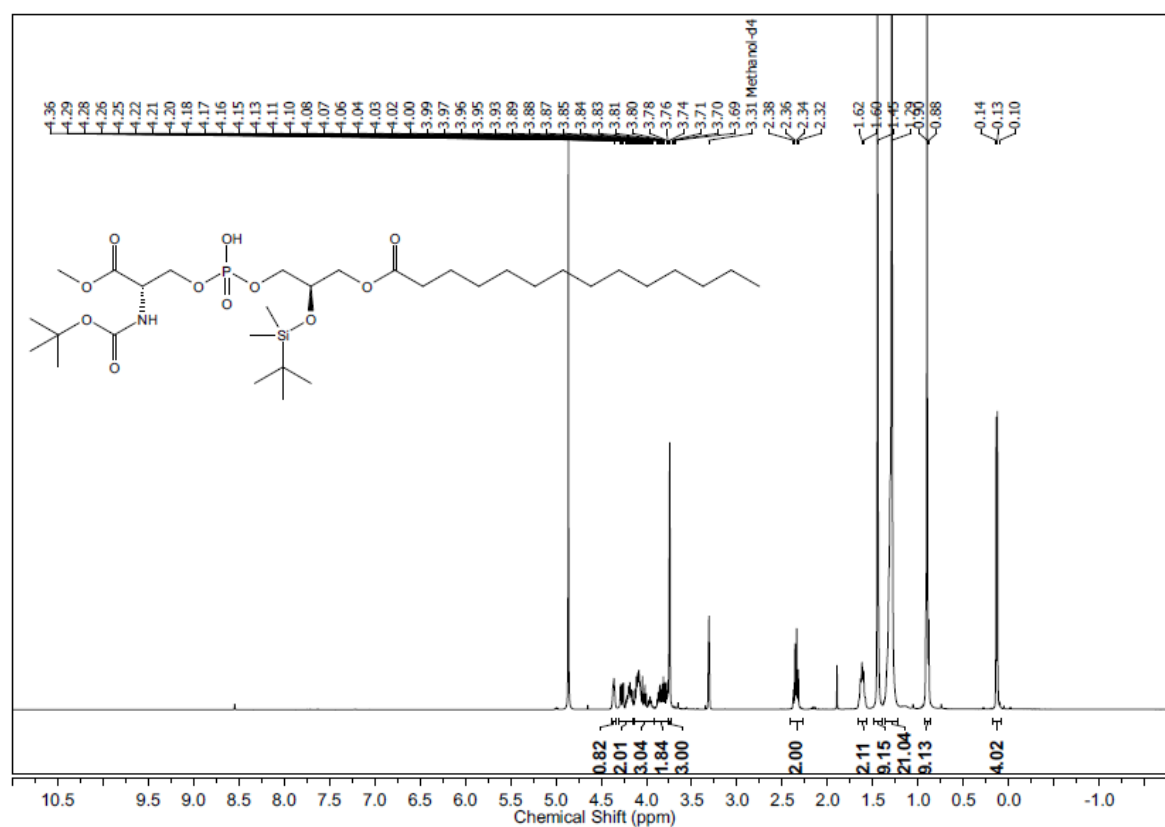
^1H NMR of intermediate (*R*)-**9b**



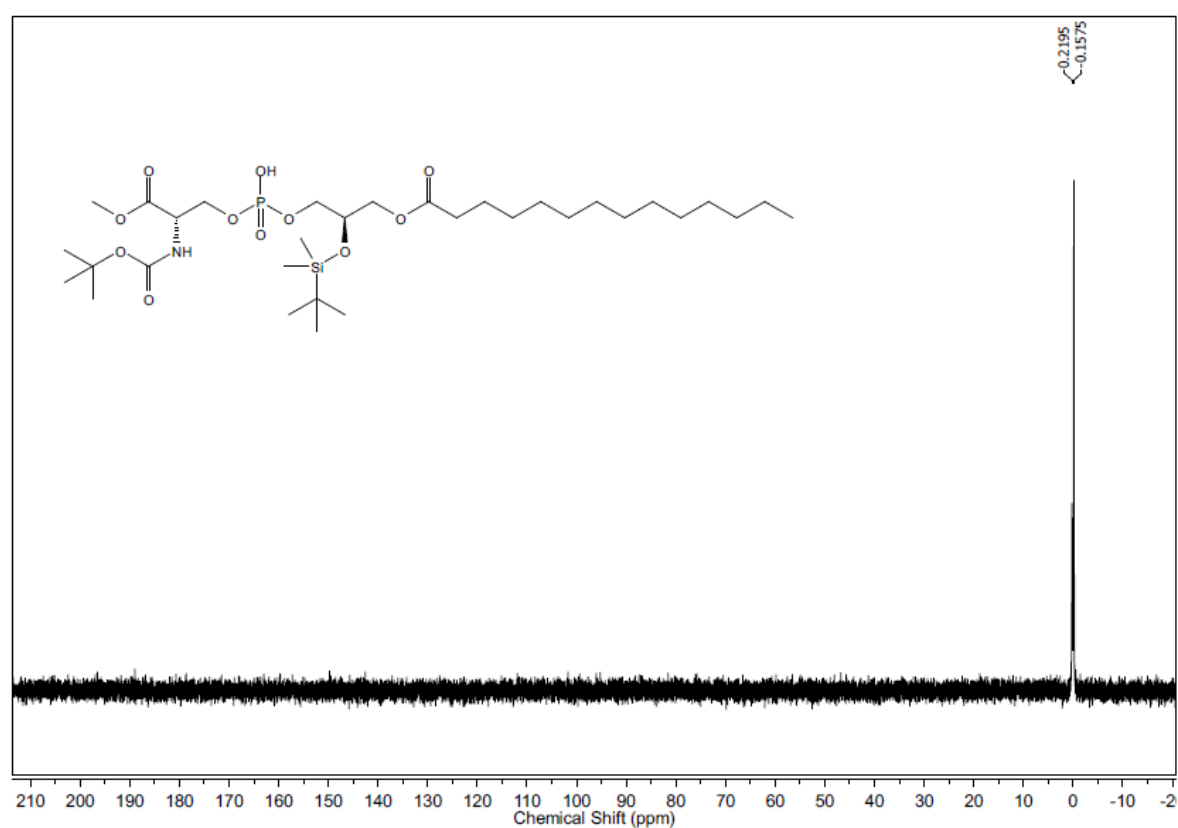
^{31}P NMR of intermediate (*R*)-**9b**



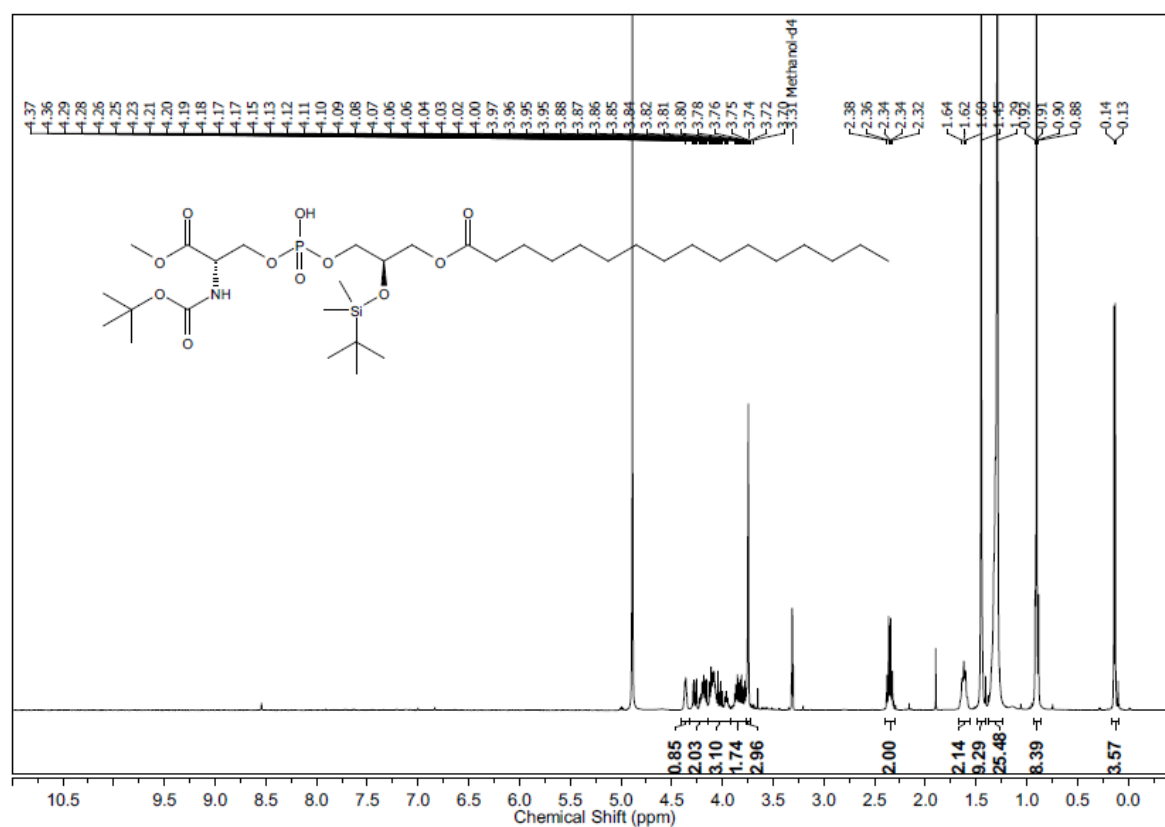
¹H NMR of intermediate (*R*)-**9c**



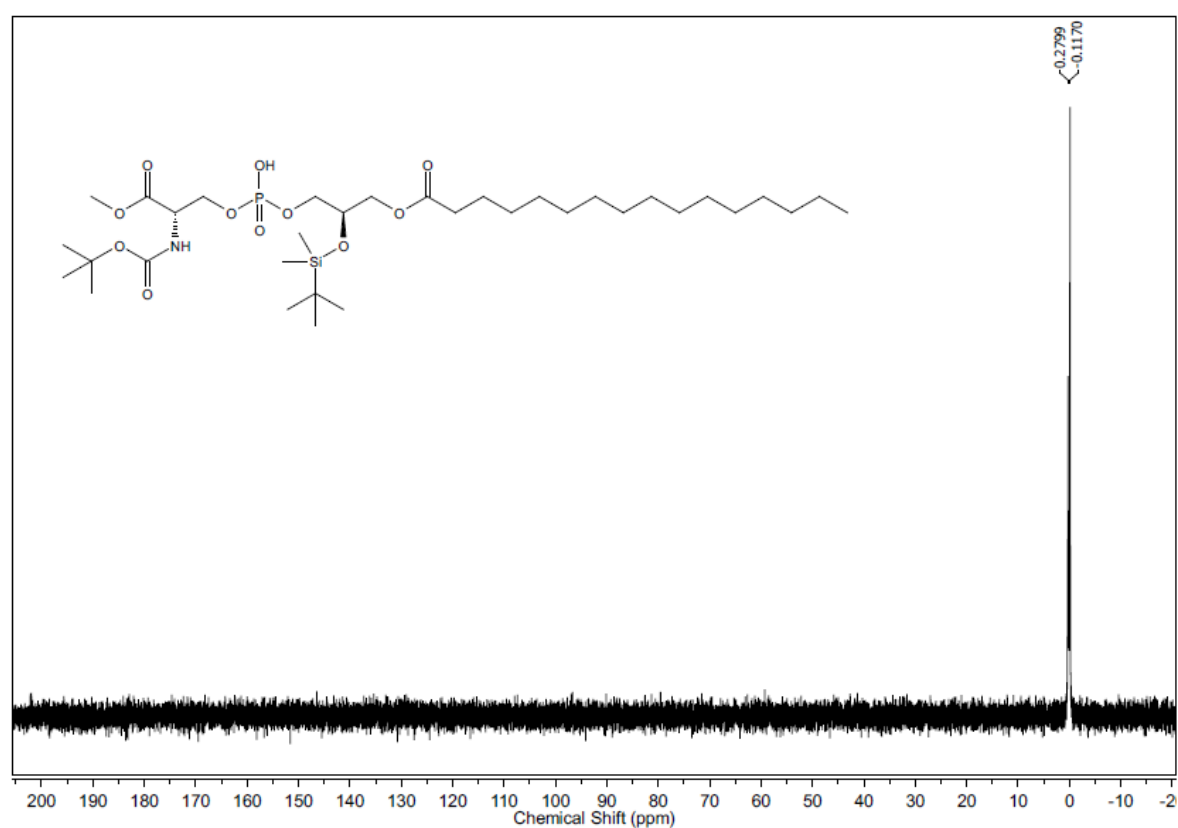
³¹P NMR of intermediate (*R*)-**9c**



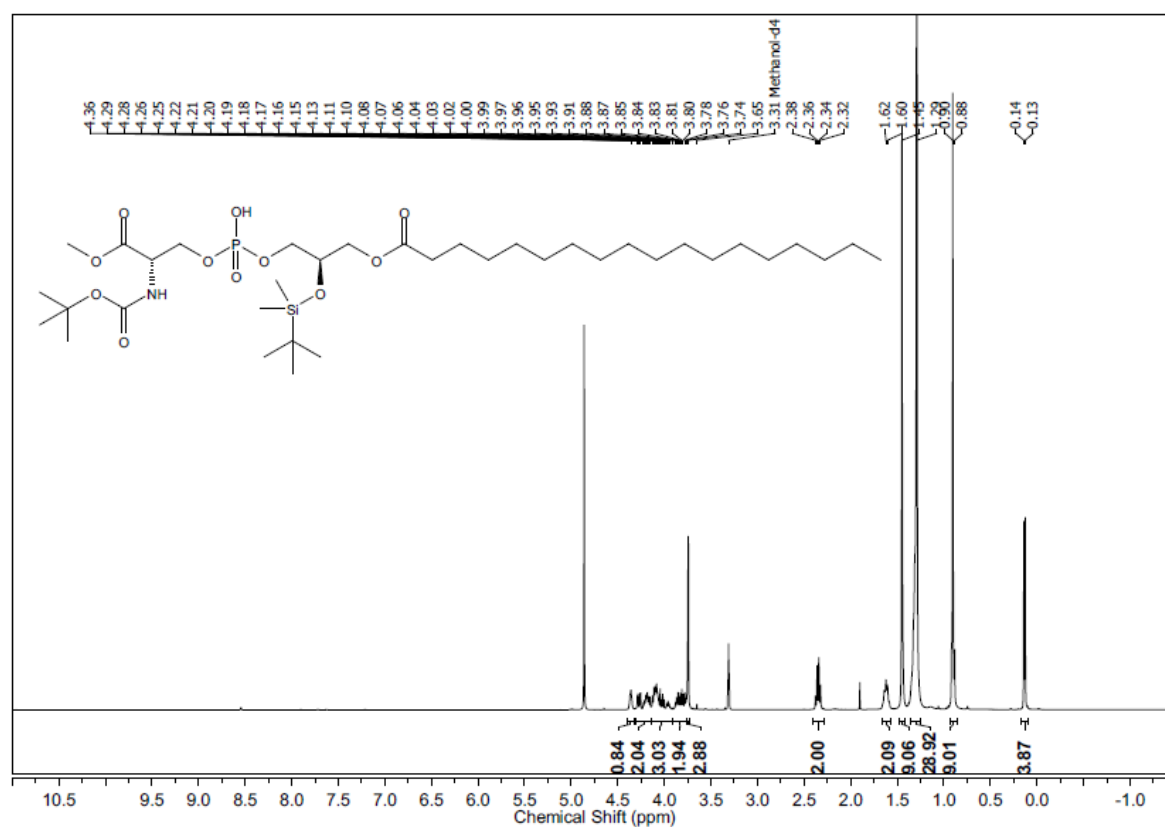
¹H NMR of intermediate (*R*)-**9d**



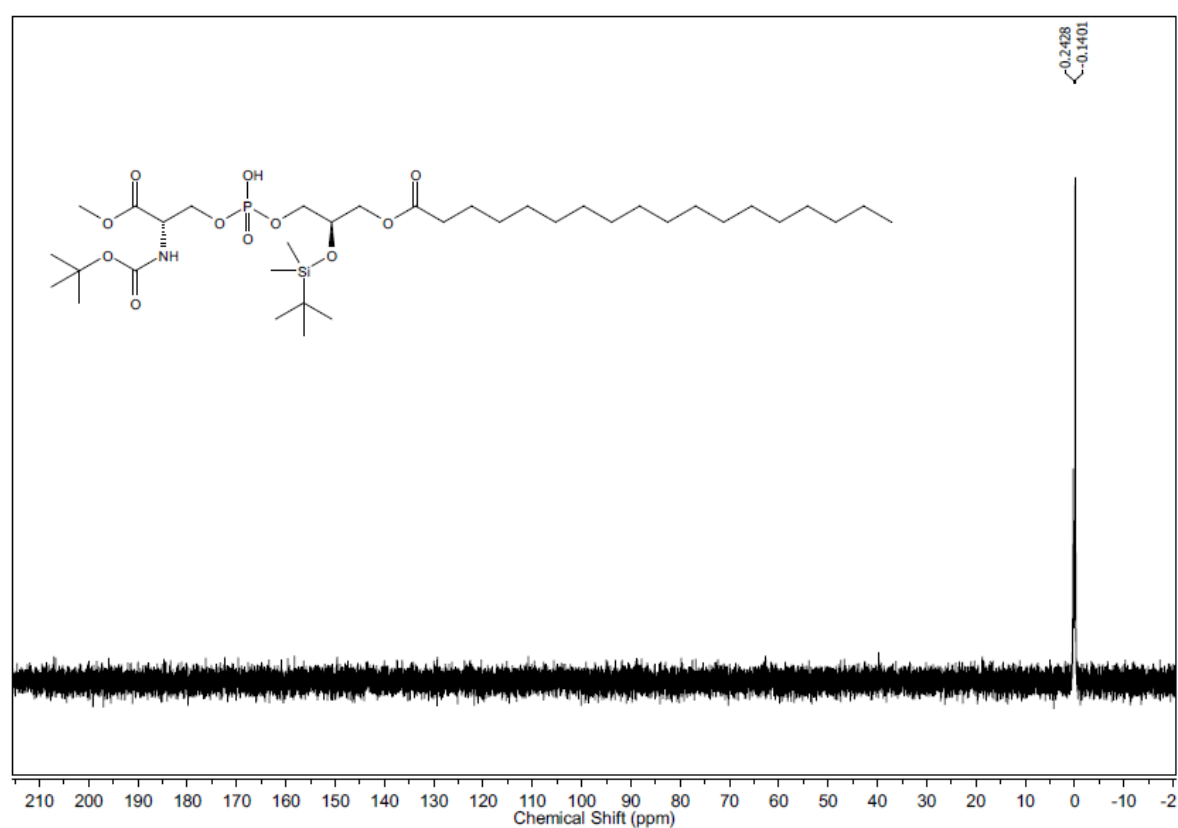
³¹P NMR of intermediate (*R*)-**9d**



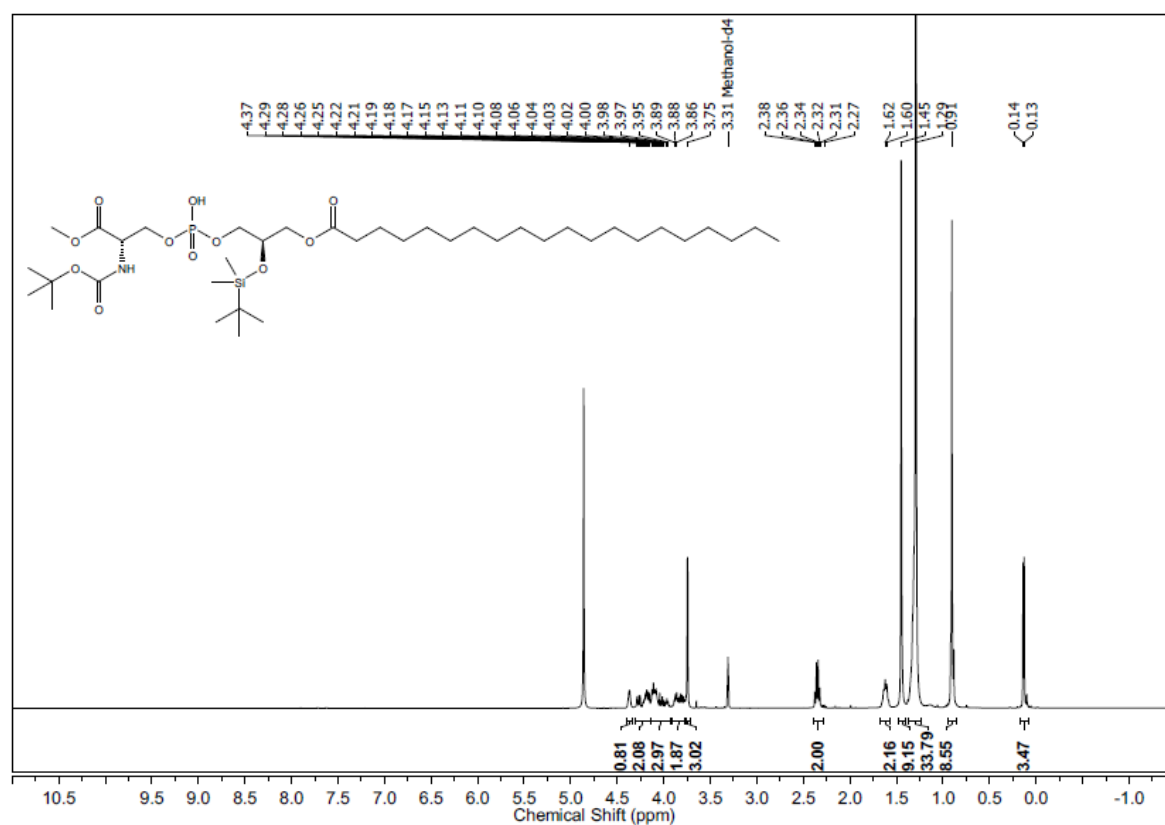
^1H NMR of intermediate (*R*)-**9e**



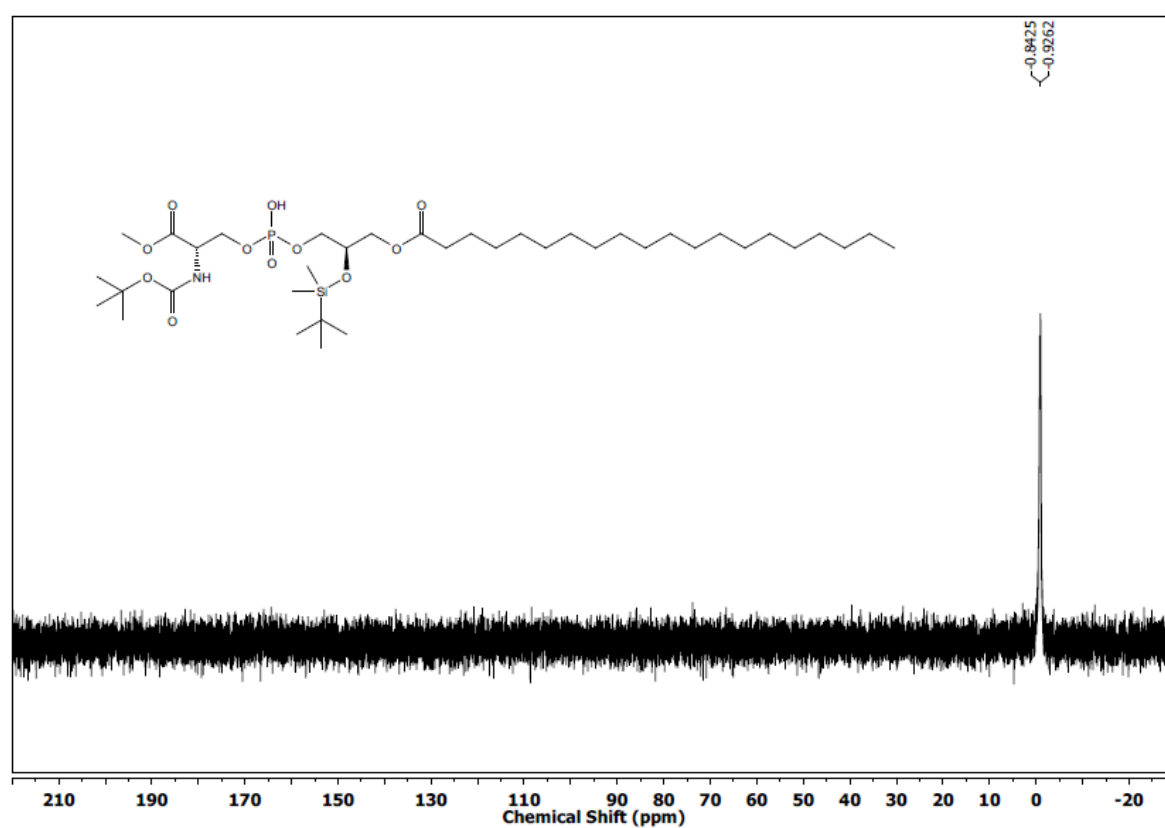
^{31}P NMR of intermediate (*R*)-**9e**



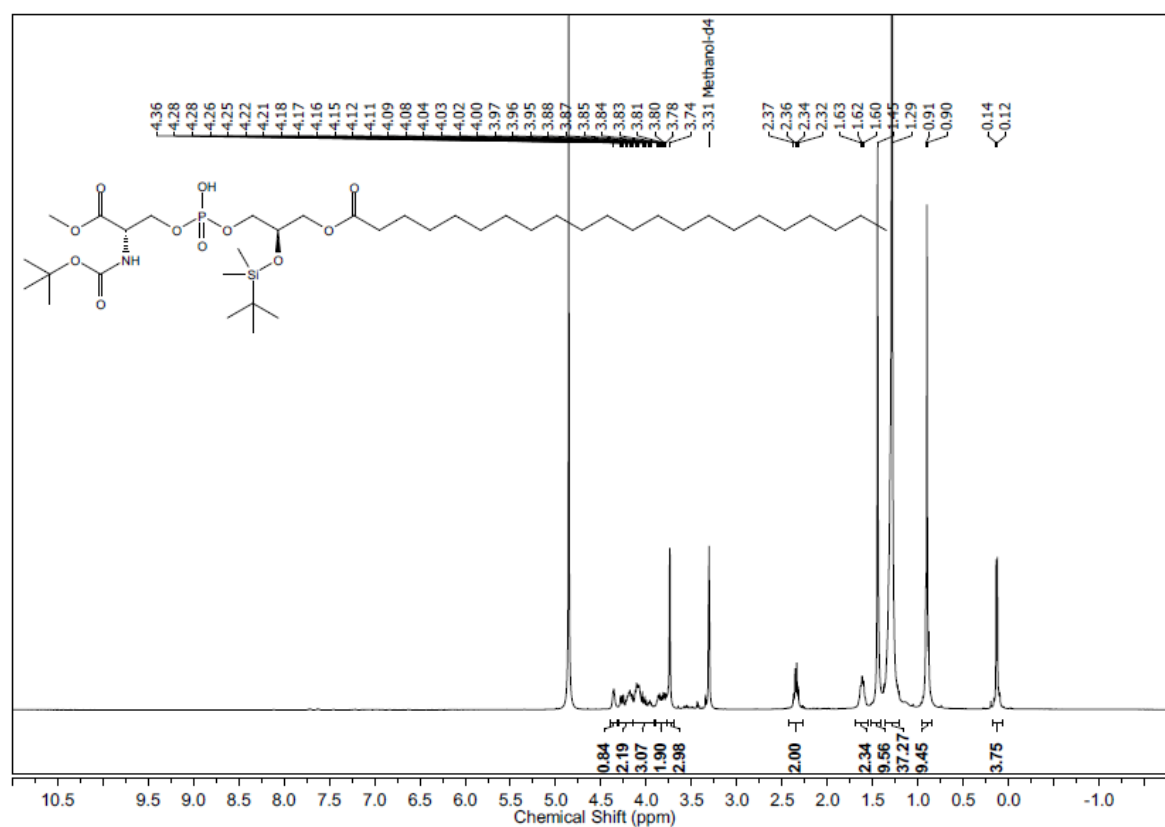
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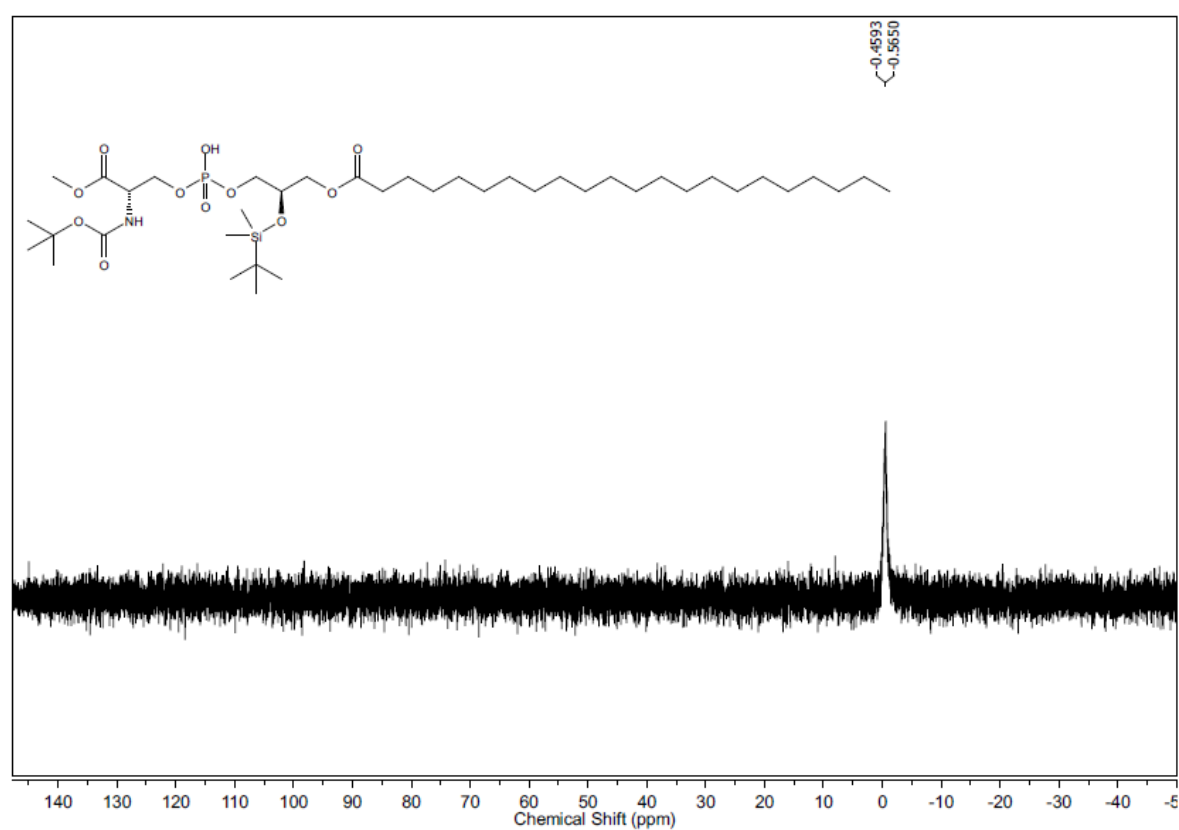
^{31}P NMR of intermediate (*R*)-**9f**



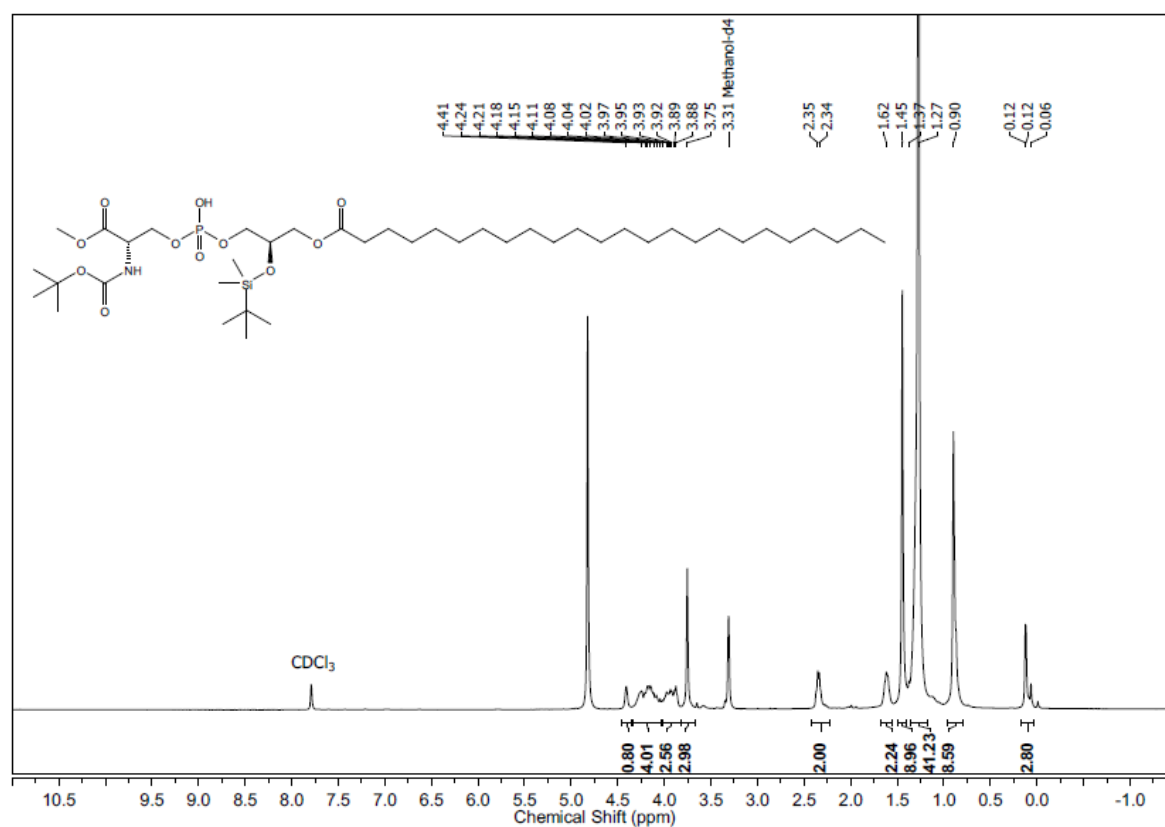
^1H NMR of intermediate (*R*)-**9g**



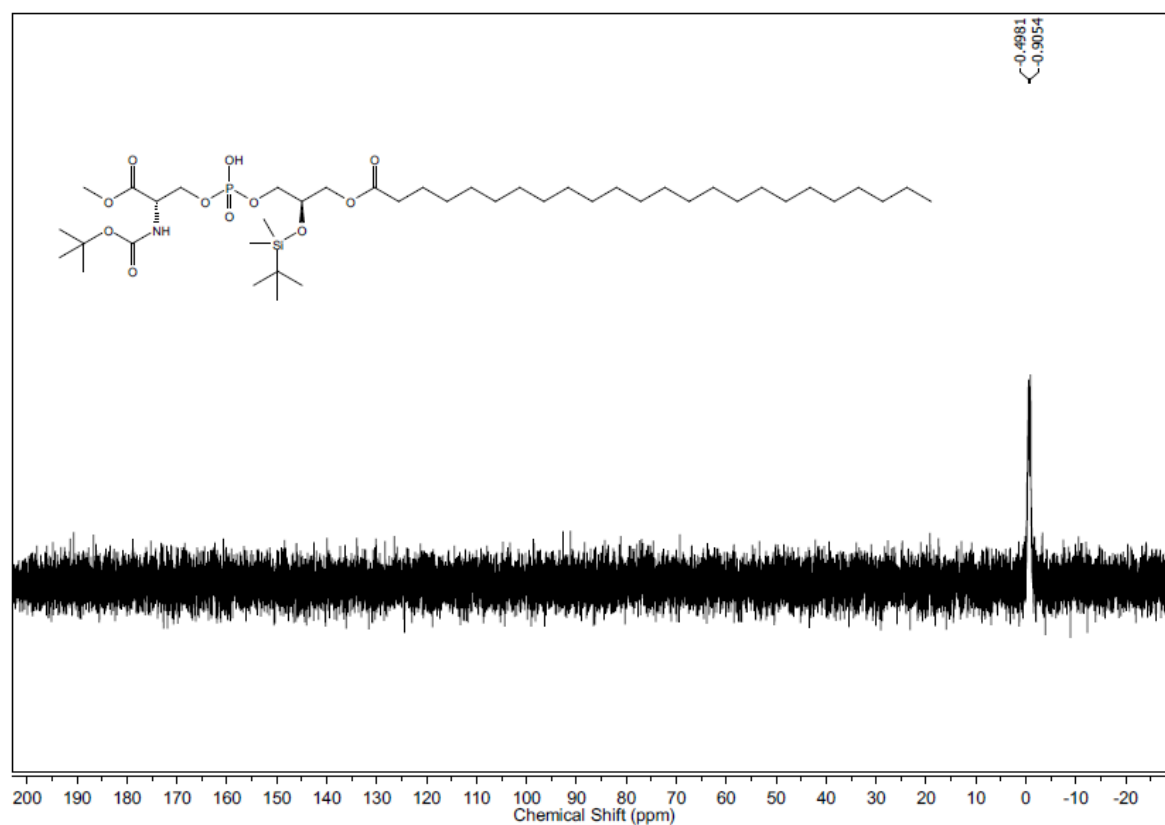
^{31}P NMR of intermediate (*R*)-**9g**



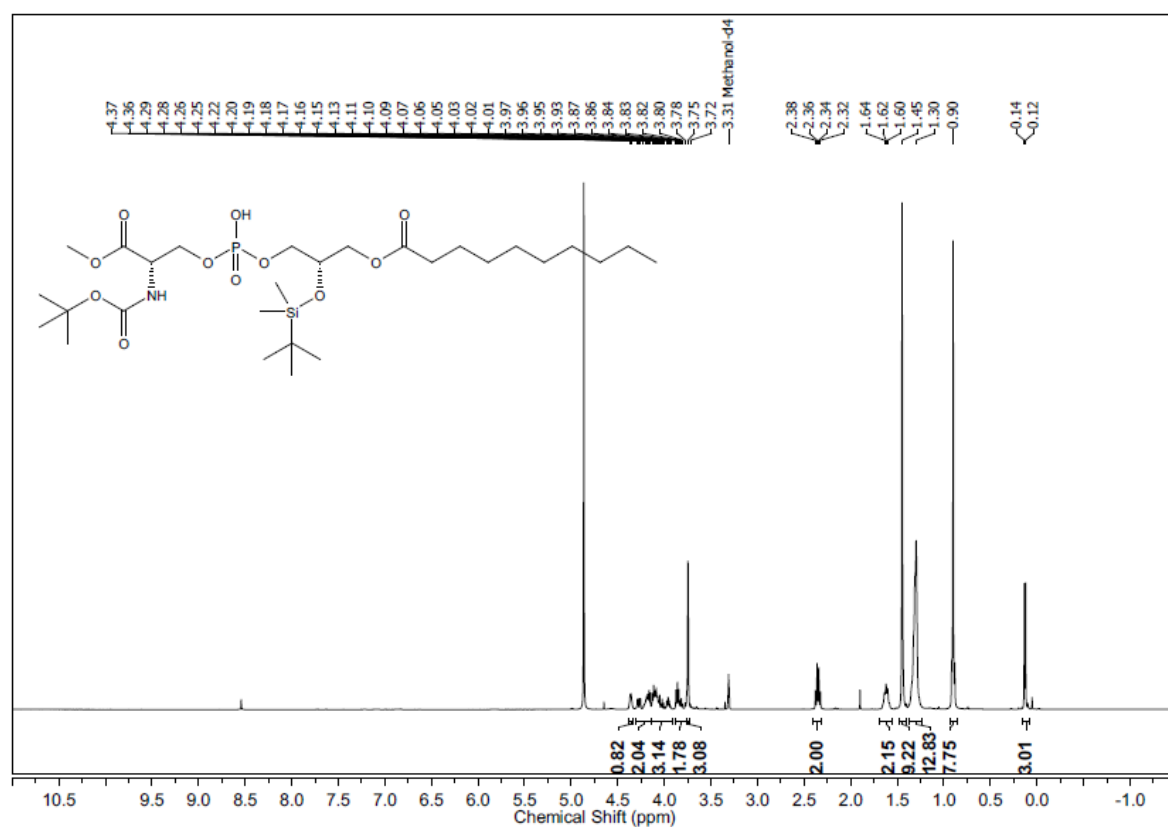
^1H NMR of intermediate (*R*)-**9h**



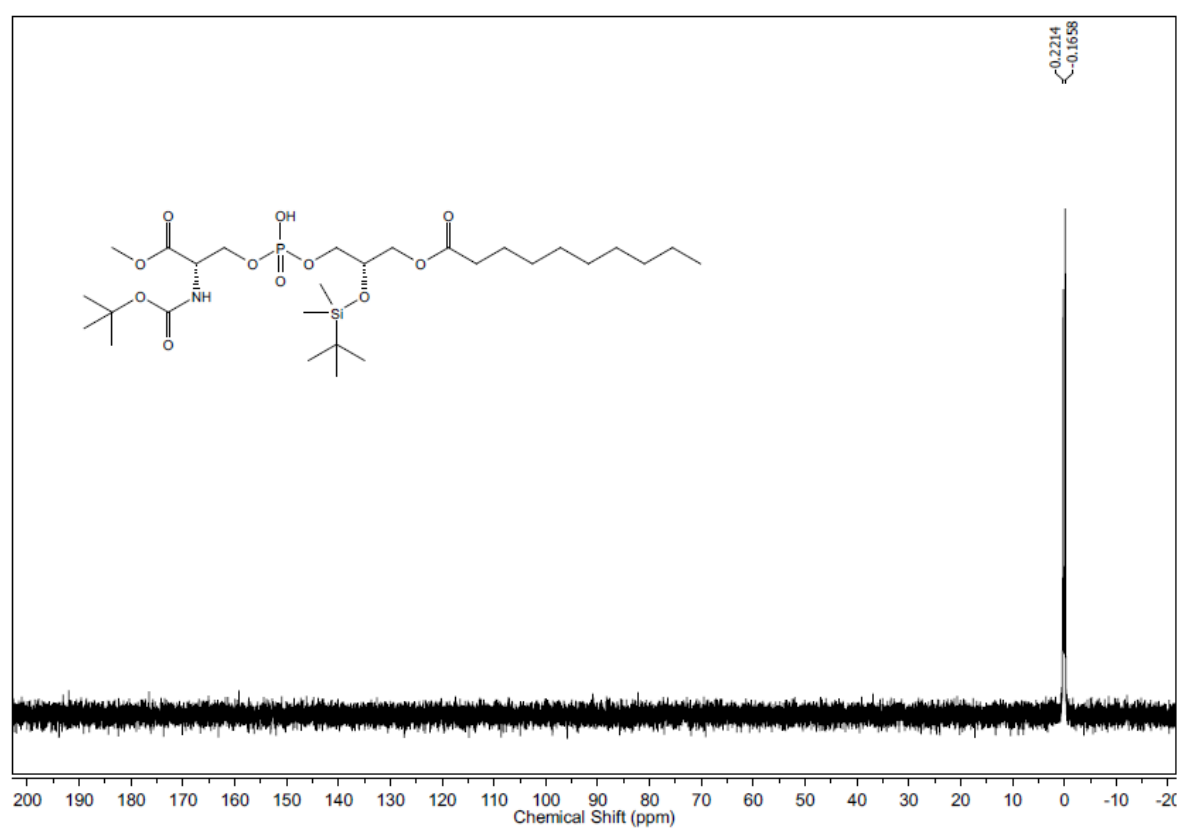
^{31}P NMR of intermediate (*R*)-**9h**



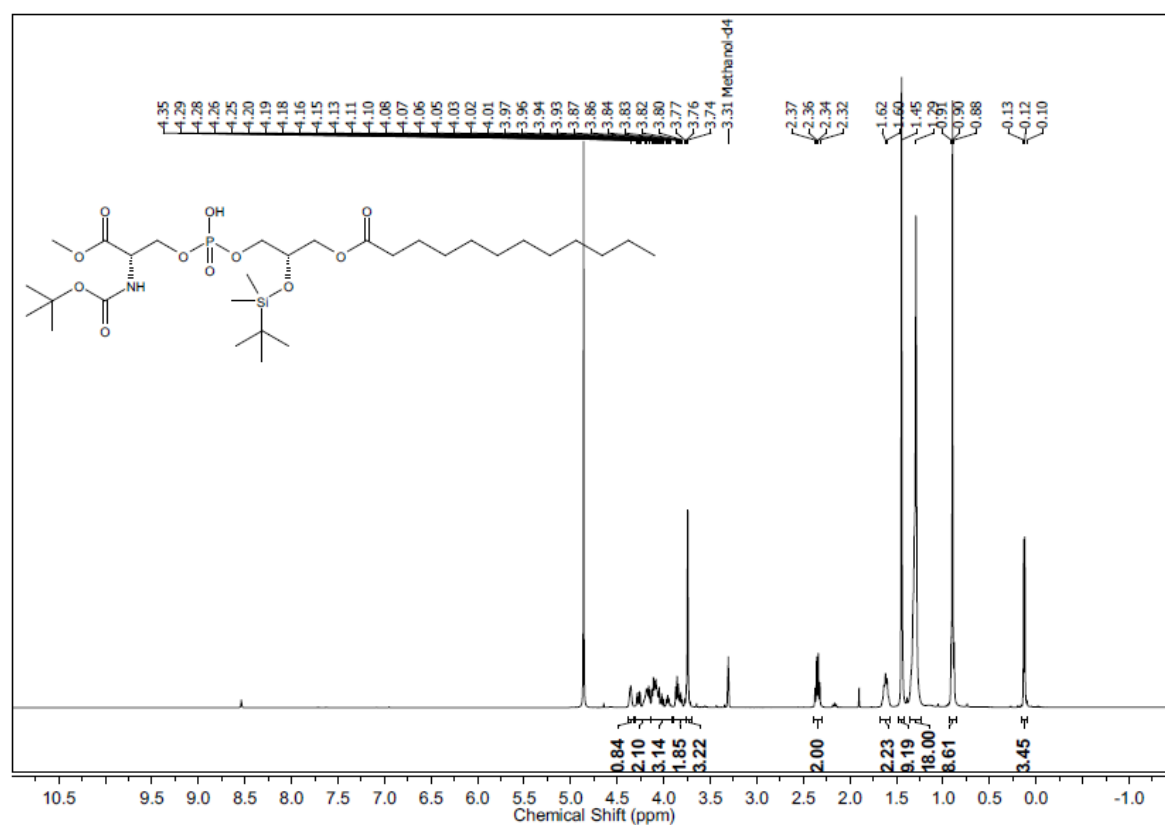
^1H NMR of intermediate (S)-**9a**



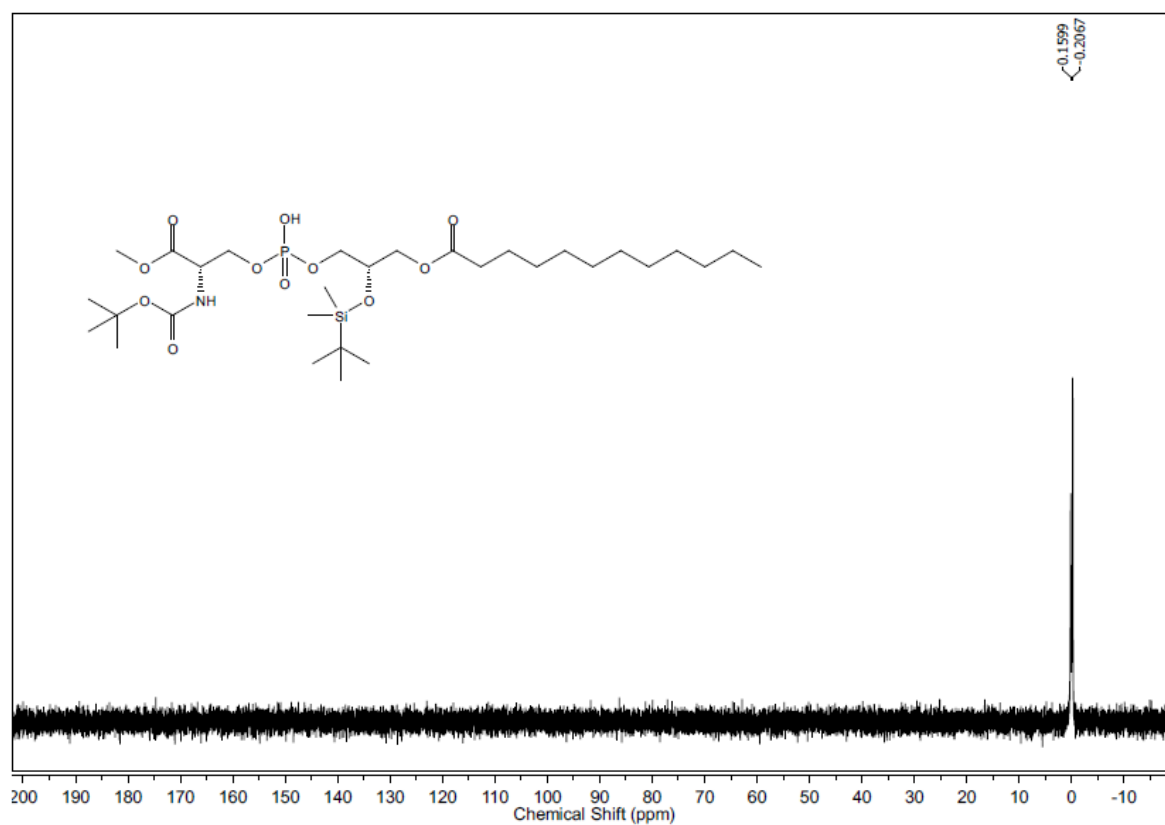
^{31}P NMR of intermediate (S)-**9a**



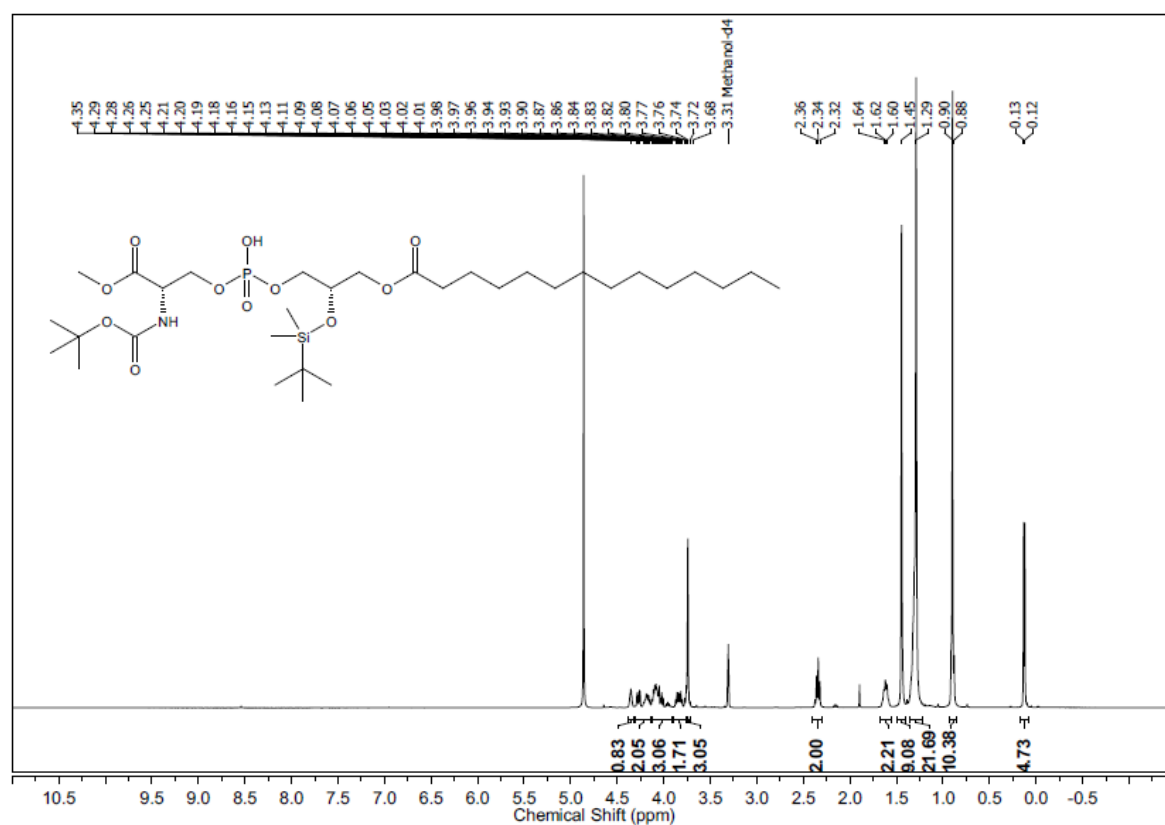
^1H NMR of intermediate (S)-**9b**



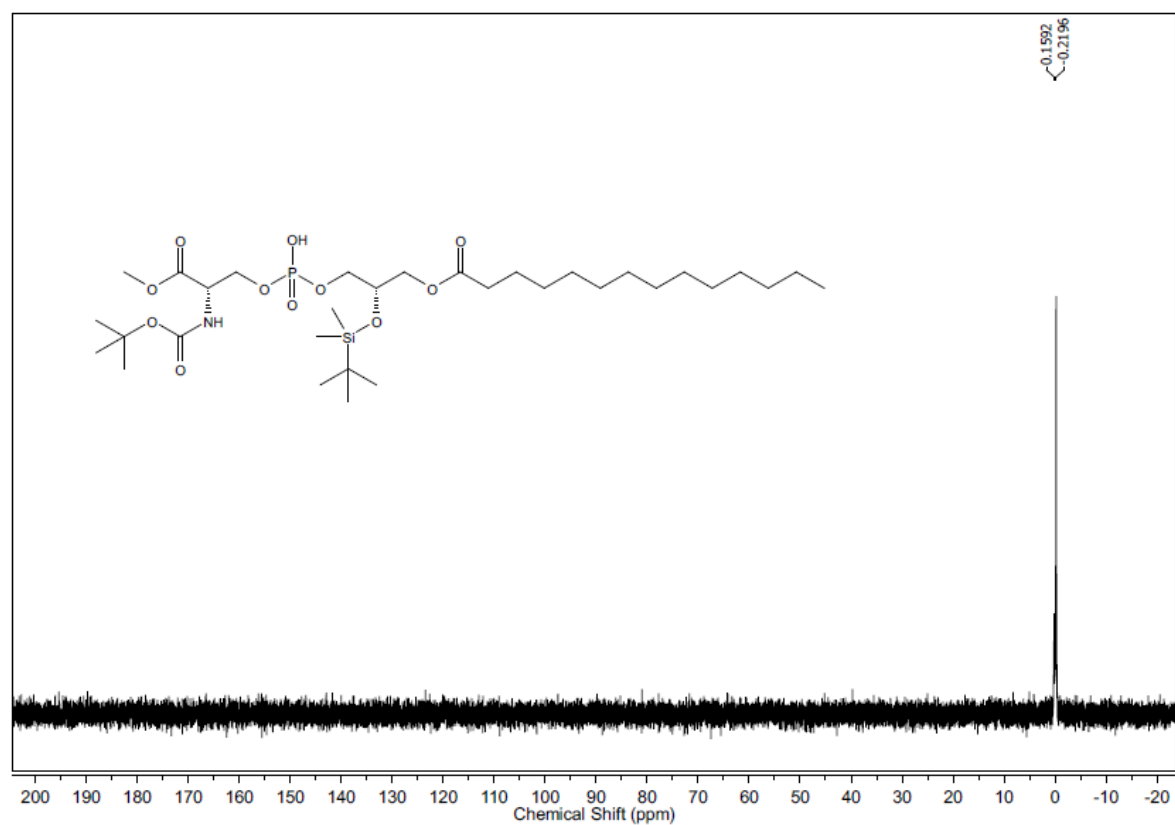
^{31}P NMR of intermediate (S)-**9b**



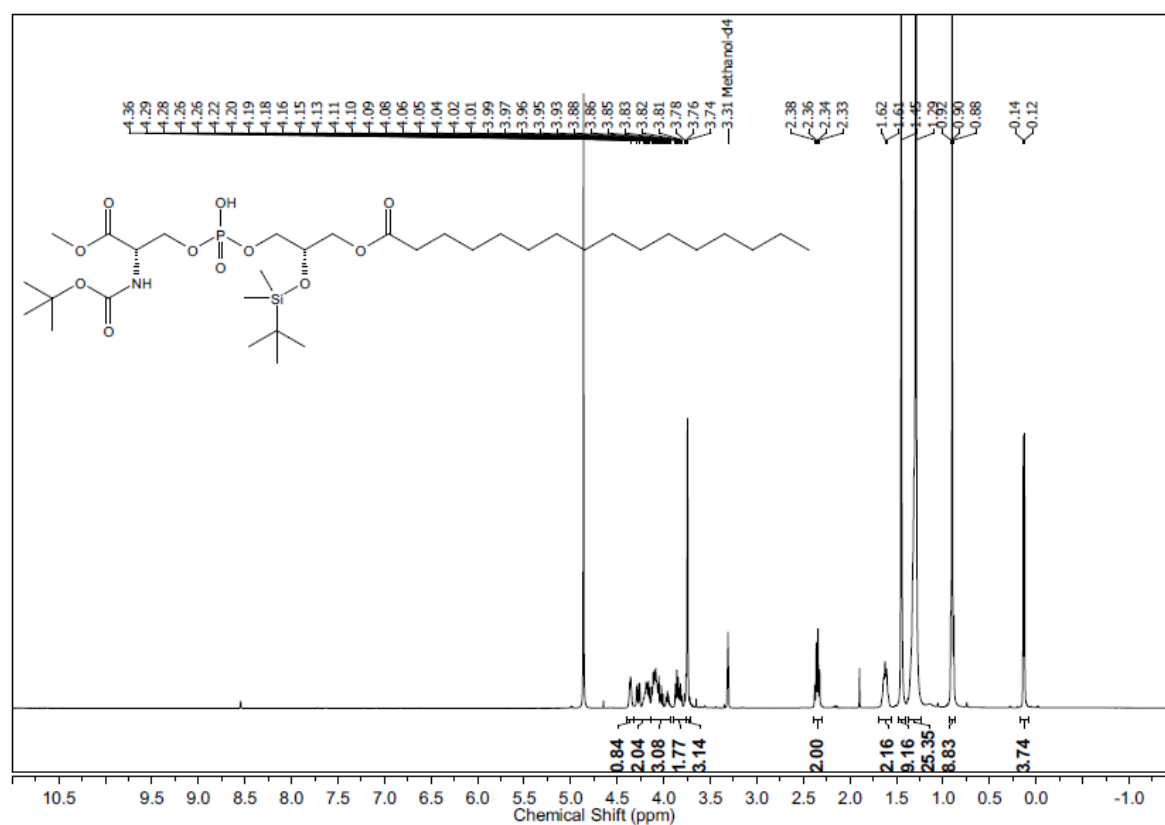
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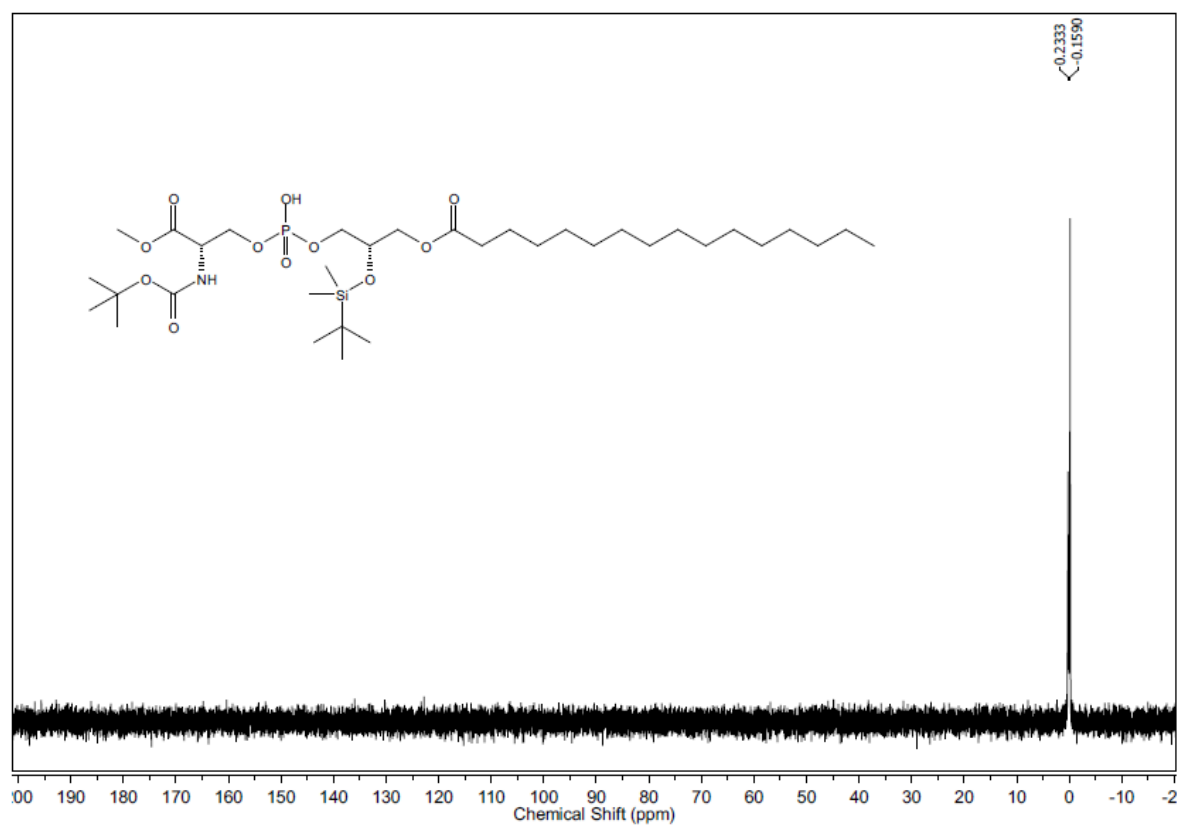
^{31}P NMR of intermediate (S)-**9c**



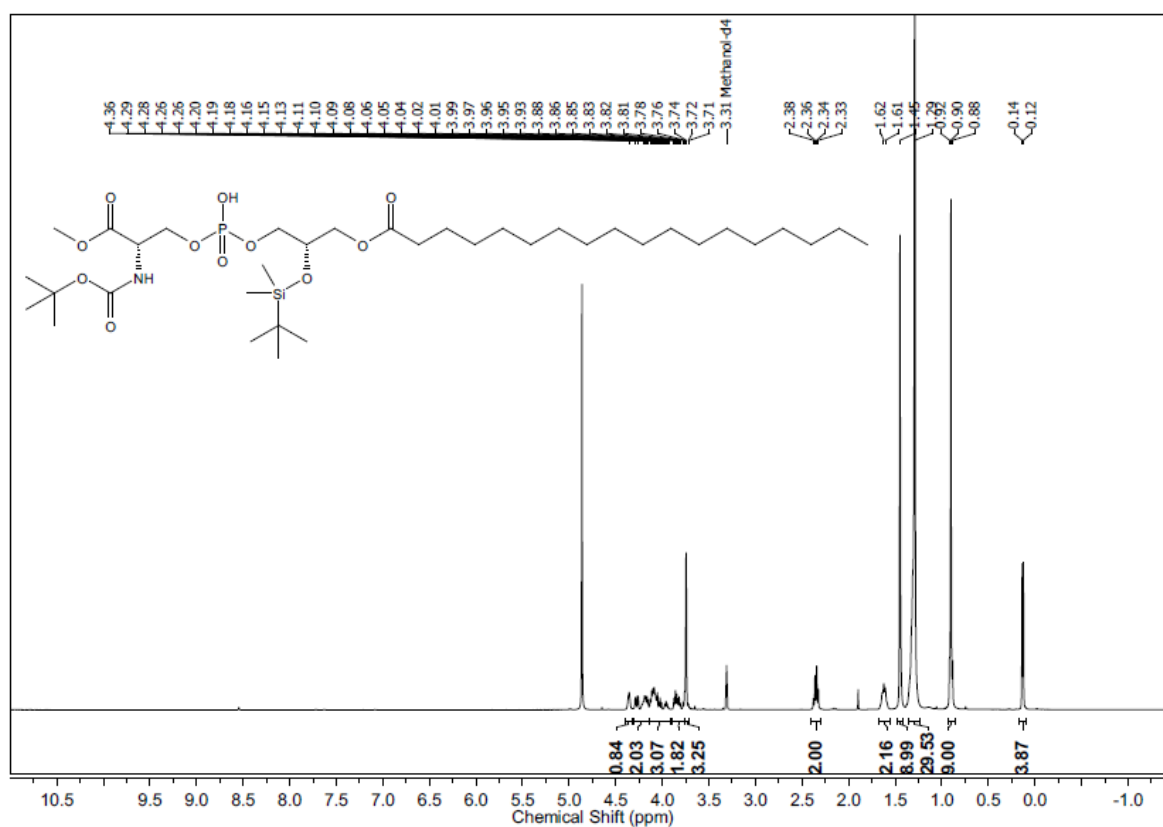
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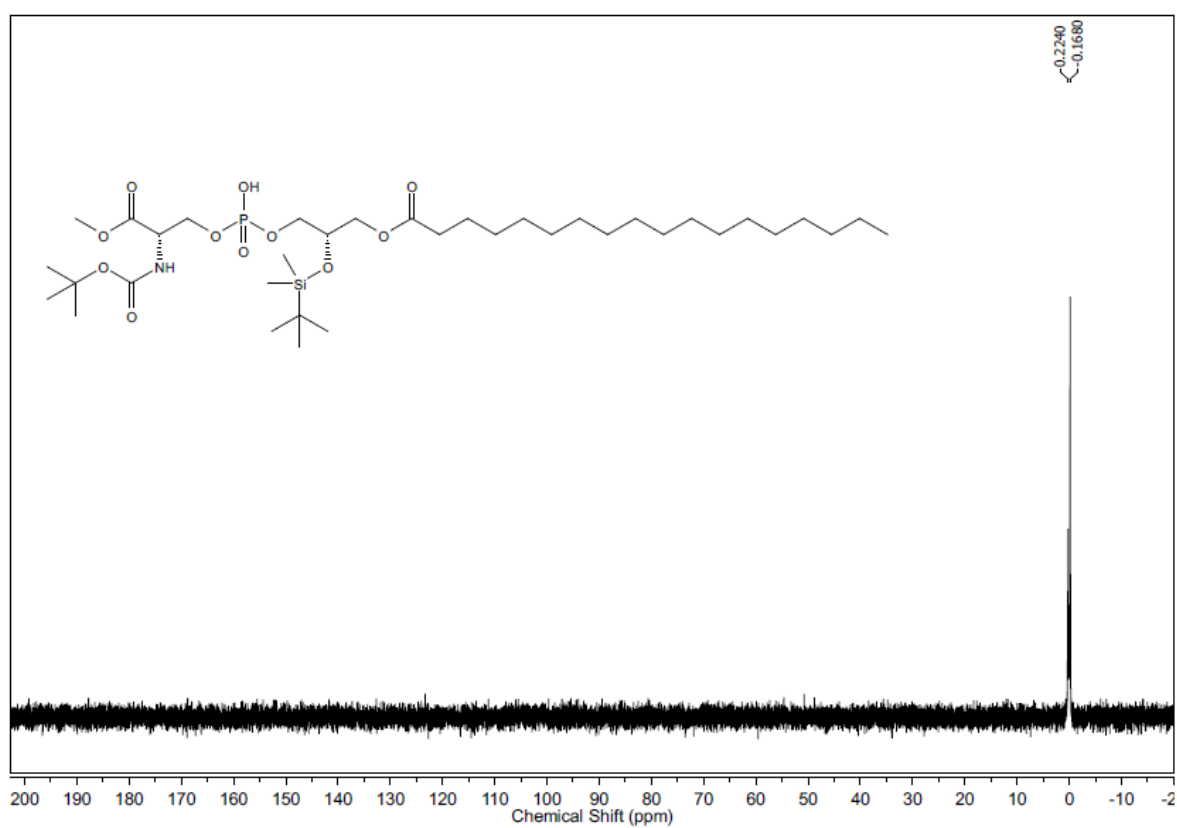
^{31}P NMR of intermediate (S)-**9d**



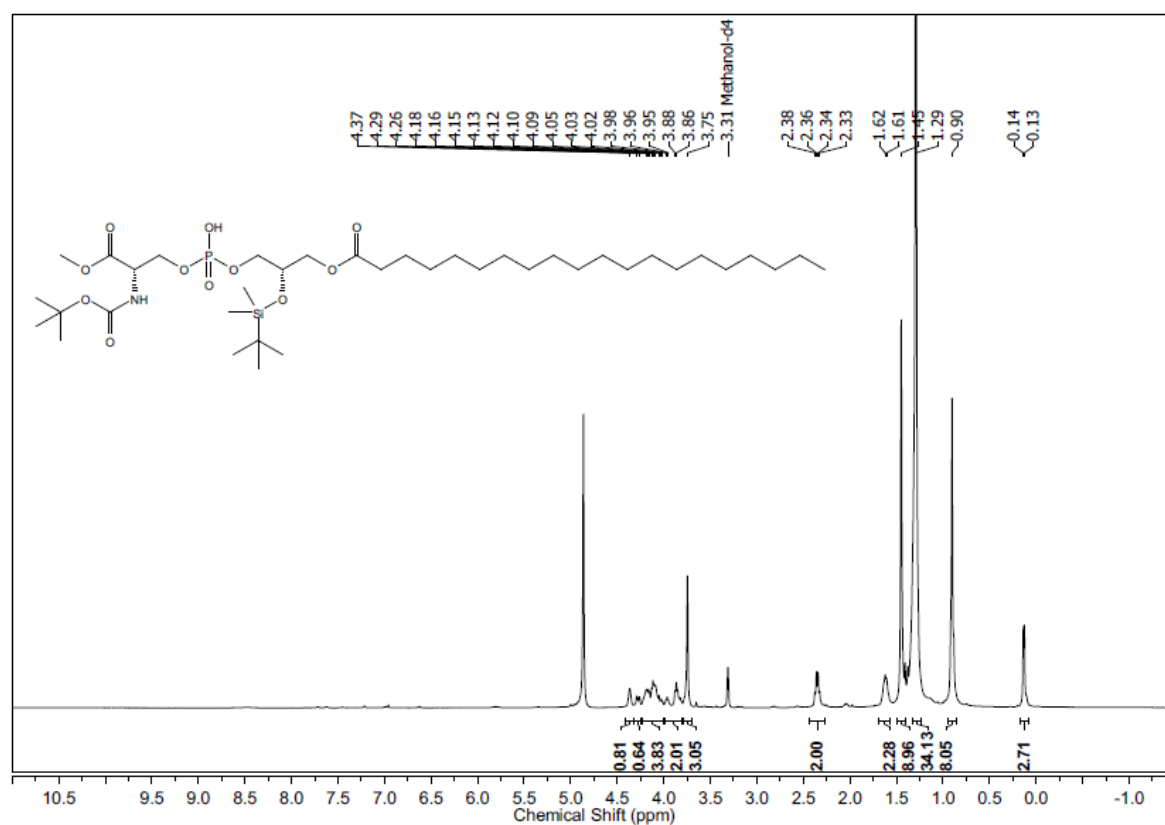
¹H NMR of intermediate (S)-**9e**



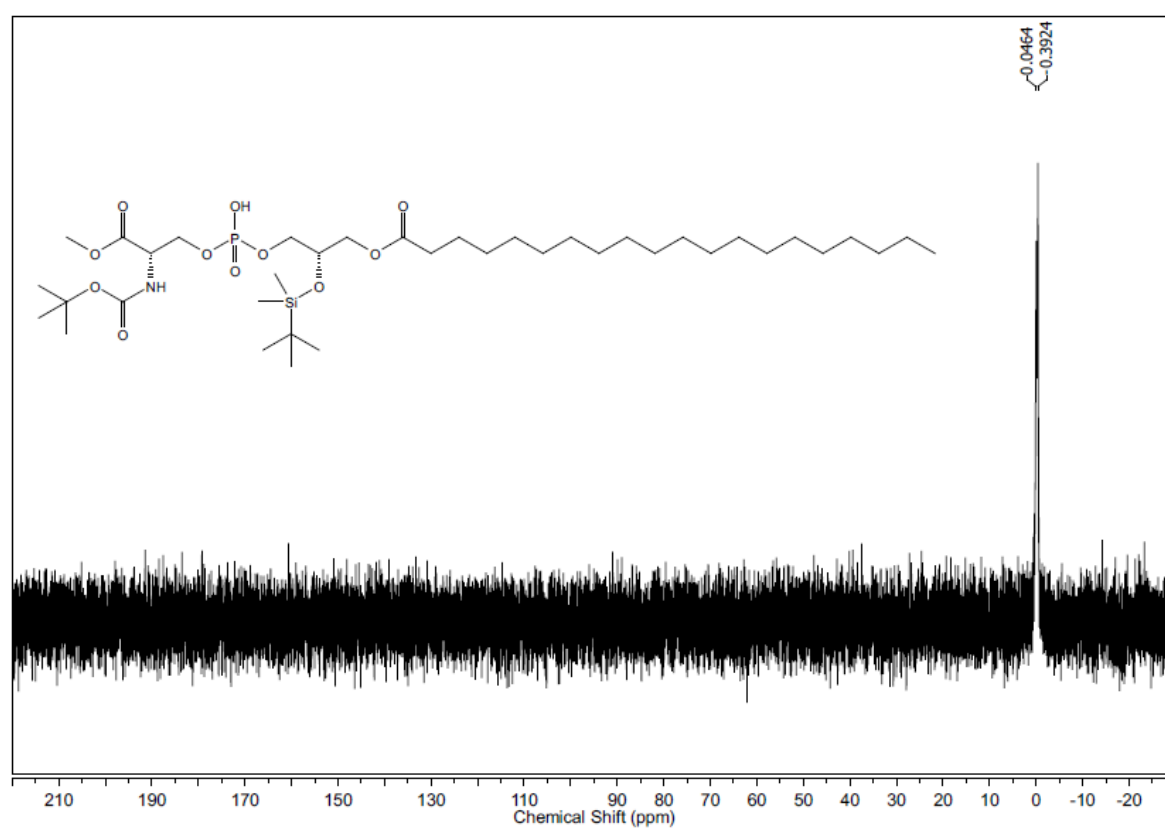
³¹P NMR of intermediate (S)-**9e**



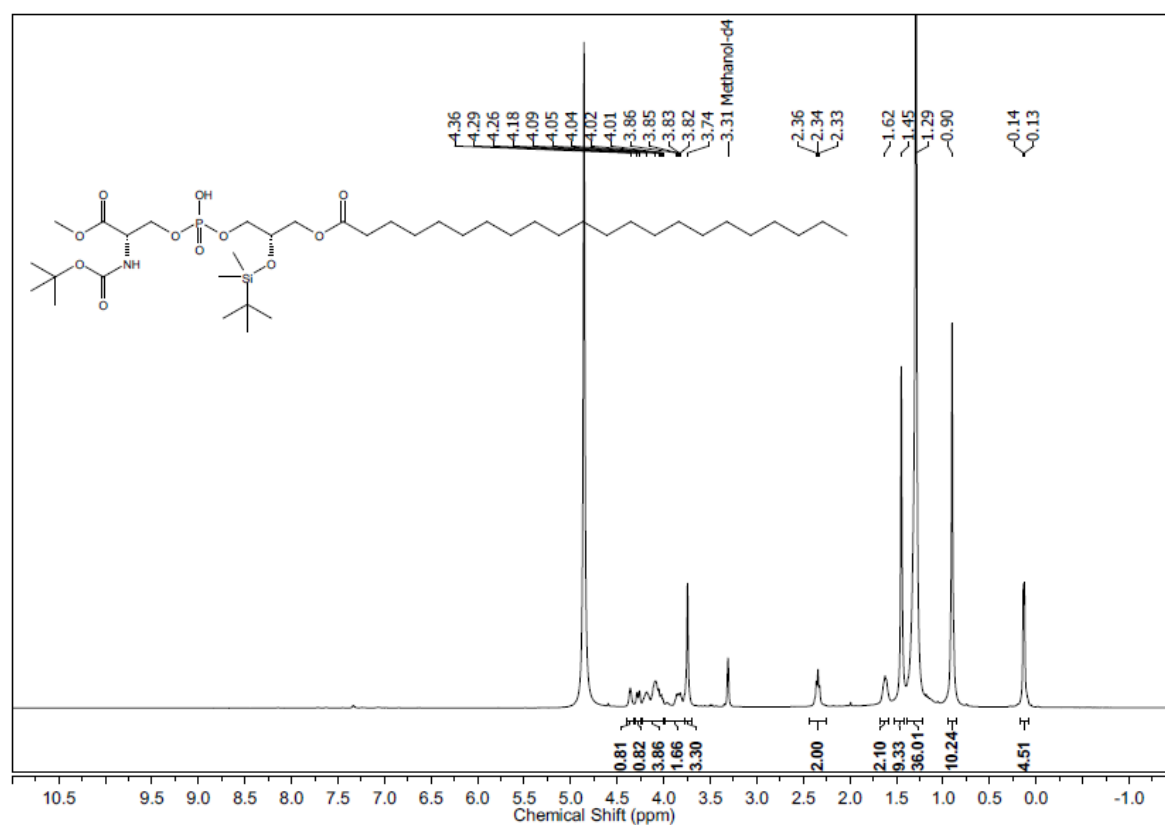
^1H NMR of intermediate (S)-**9f**



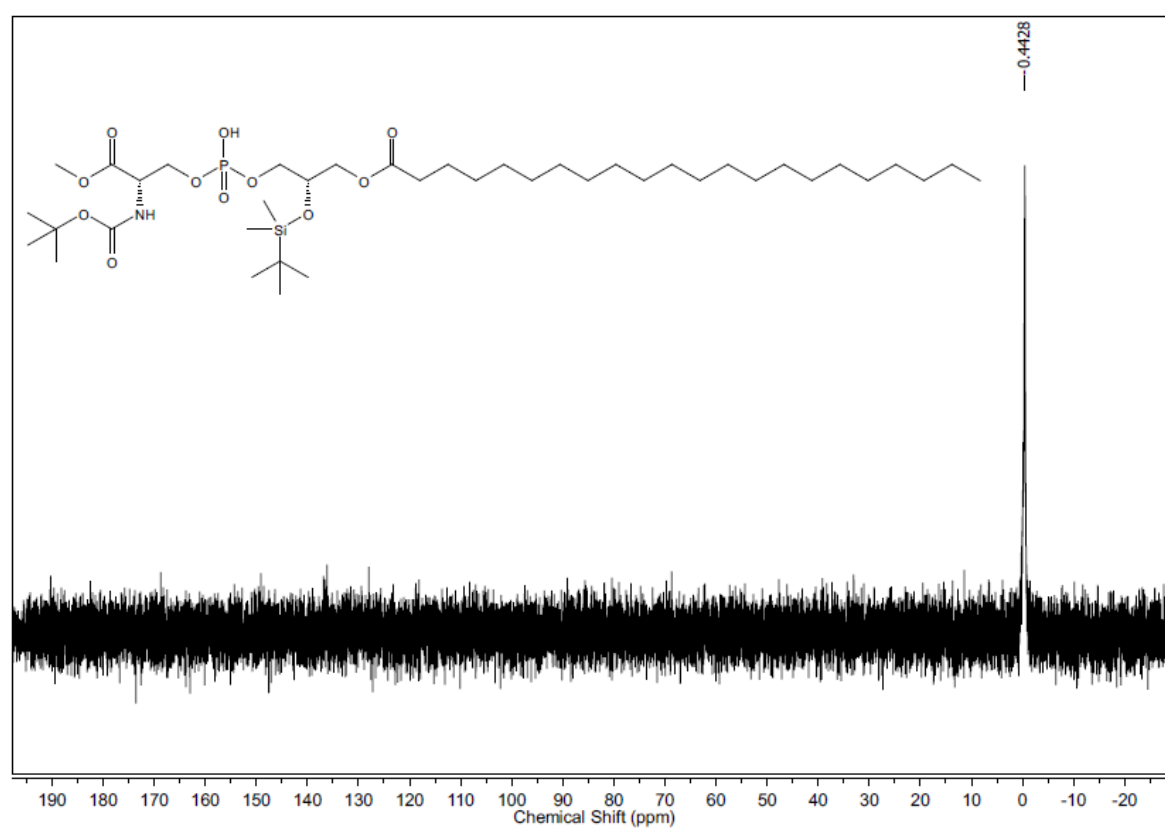
^{31}P NMR of intermediate (S)-**9f**



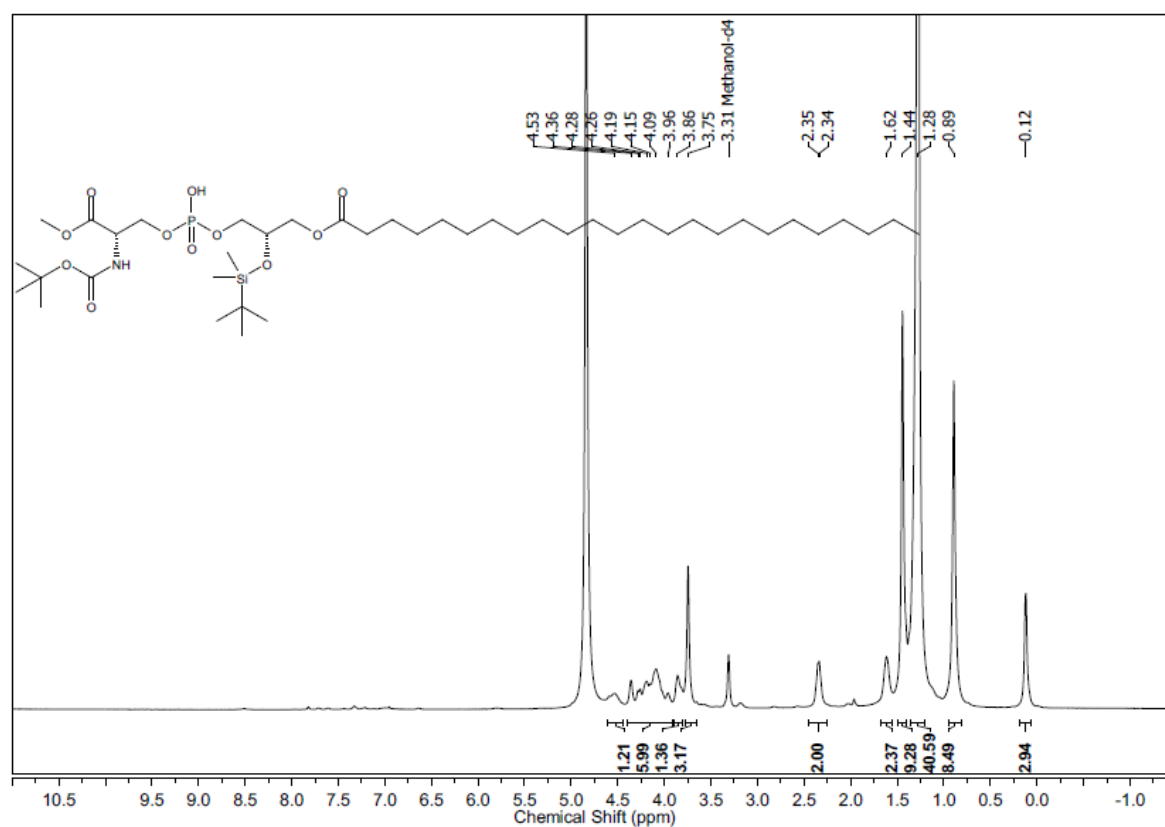
¹H NMR of intermediate (S)-**9g**



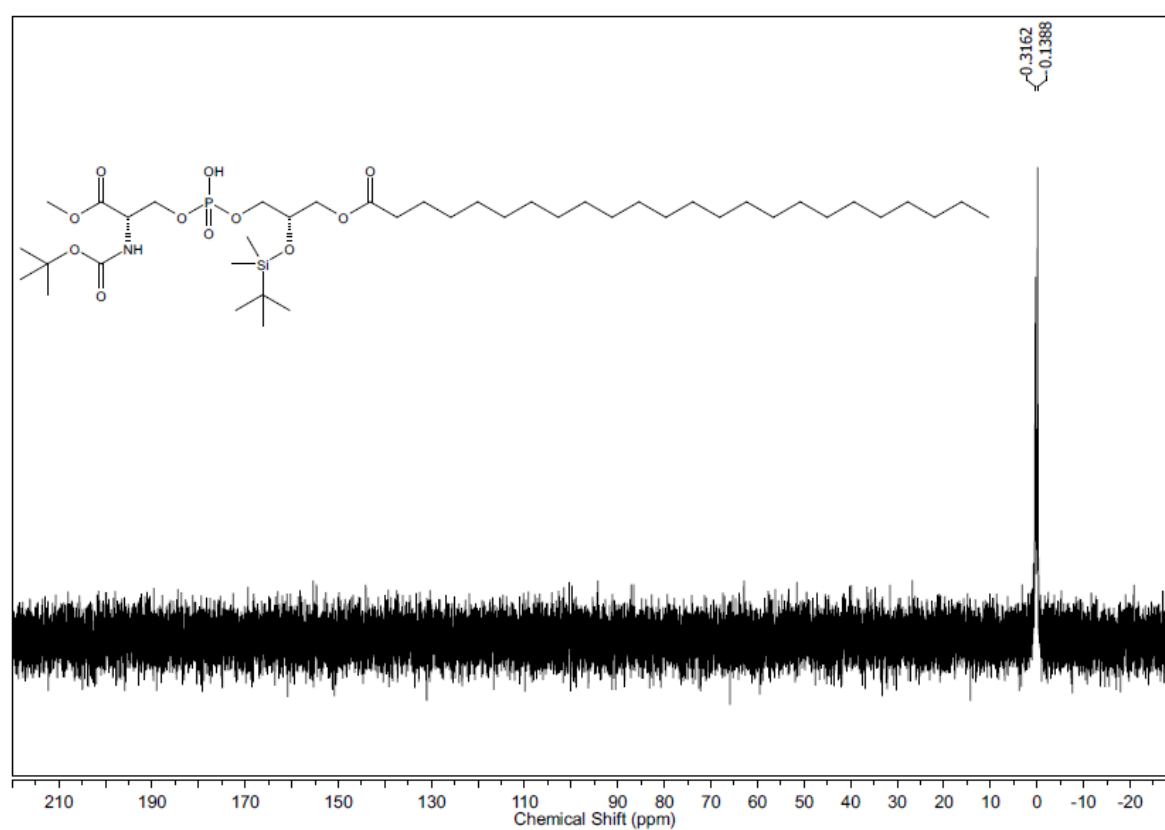
³¹P NMR of intermediate (S)-**9g**



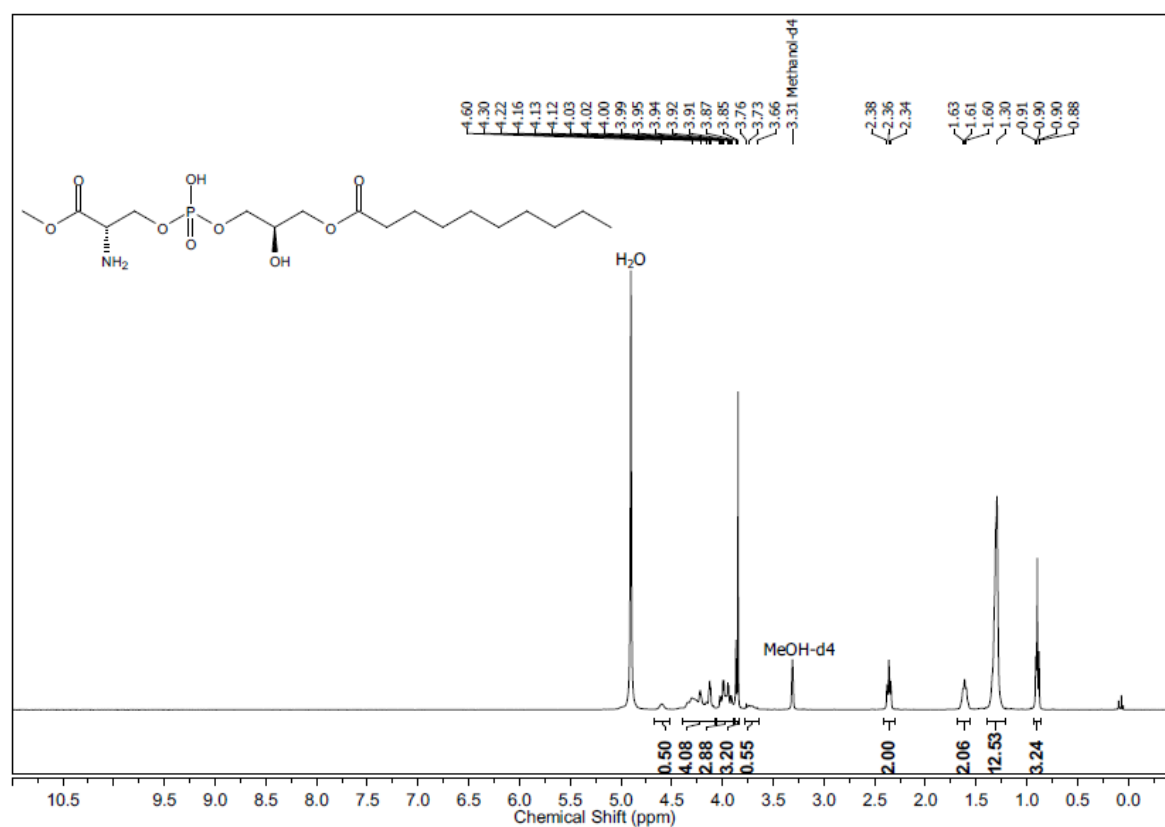
¹H NMR of intermediate (S)-**9h**



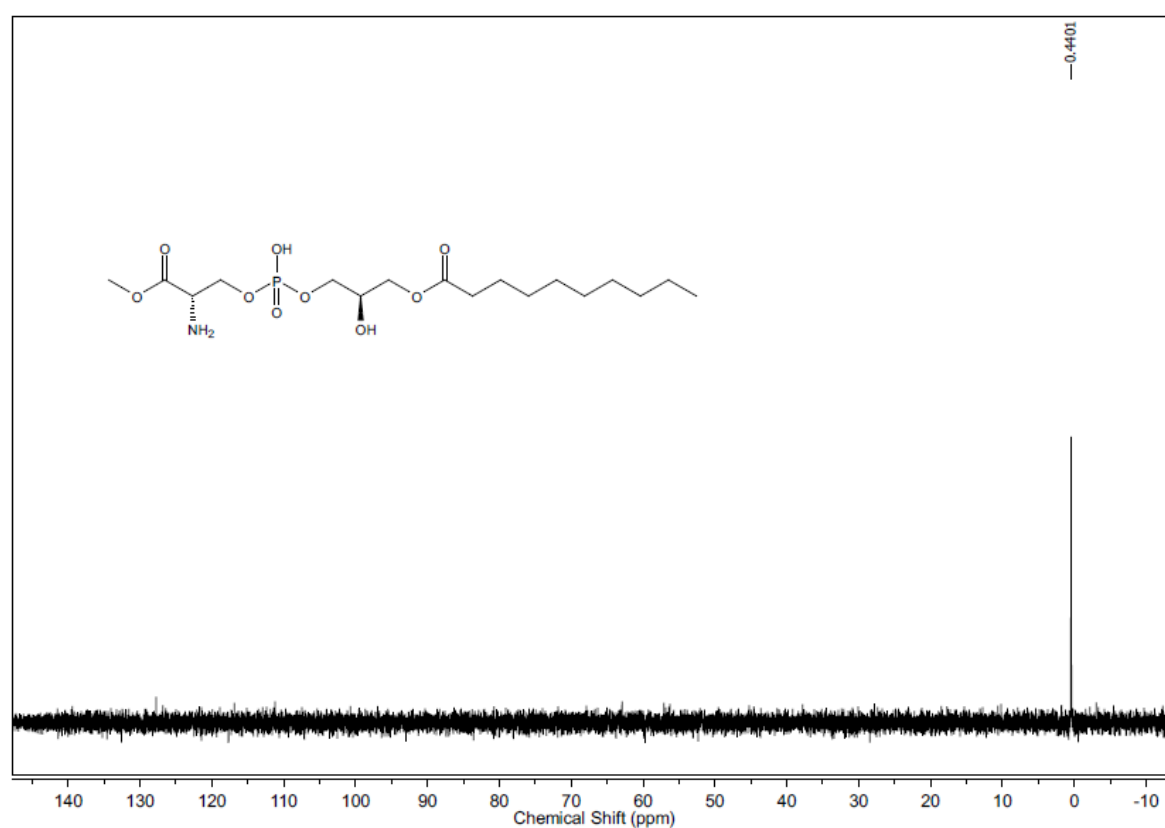
³¹P NMR of intermediate (S)-**9h**



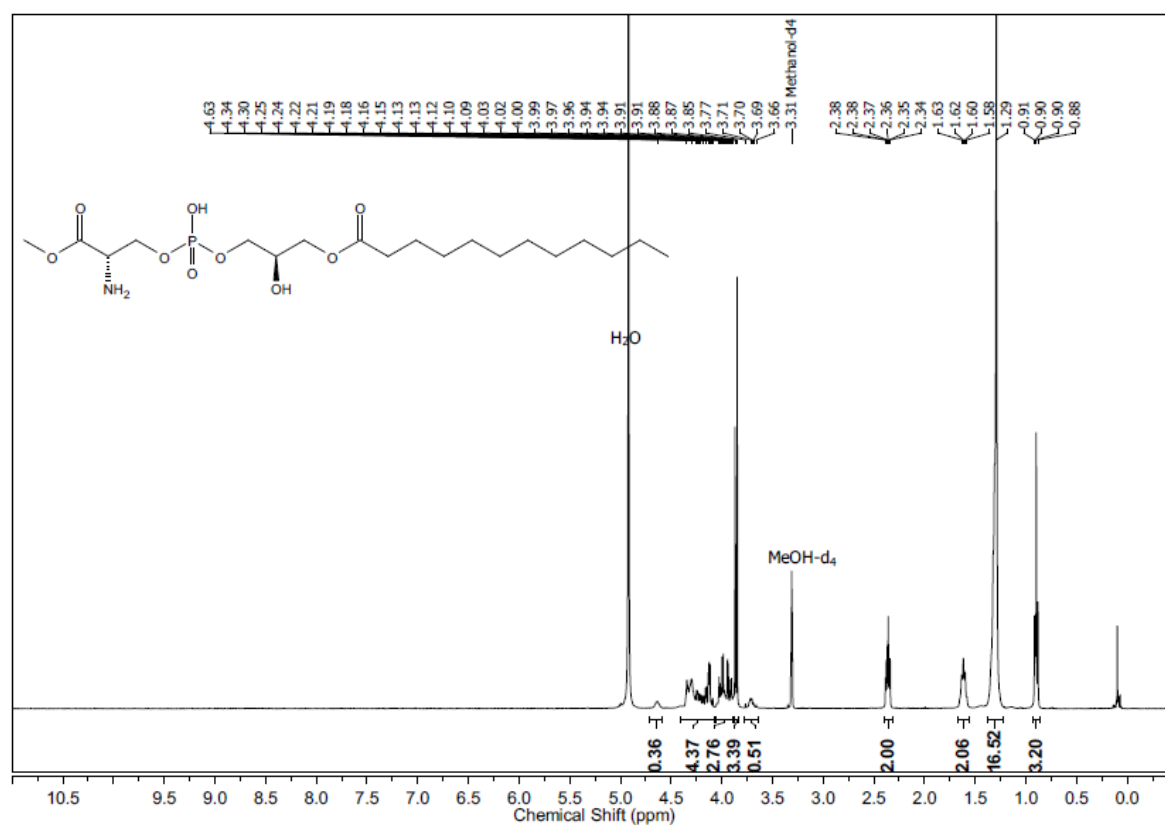
^1H NMR of final compound (*R*)-**2a**



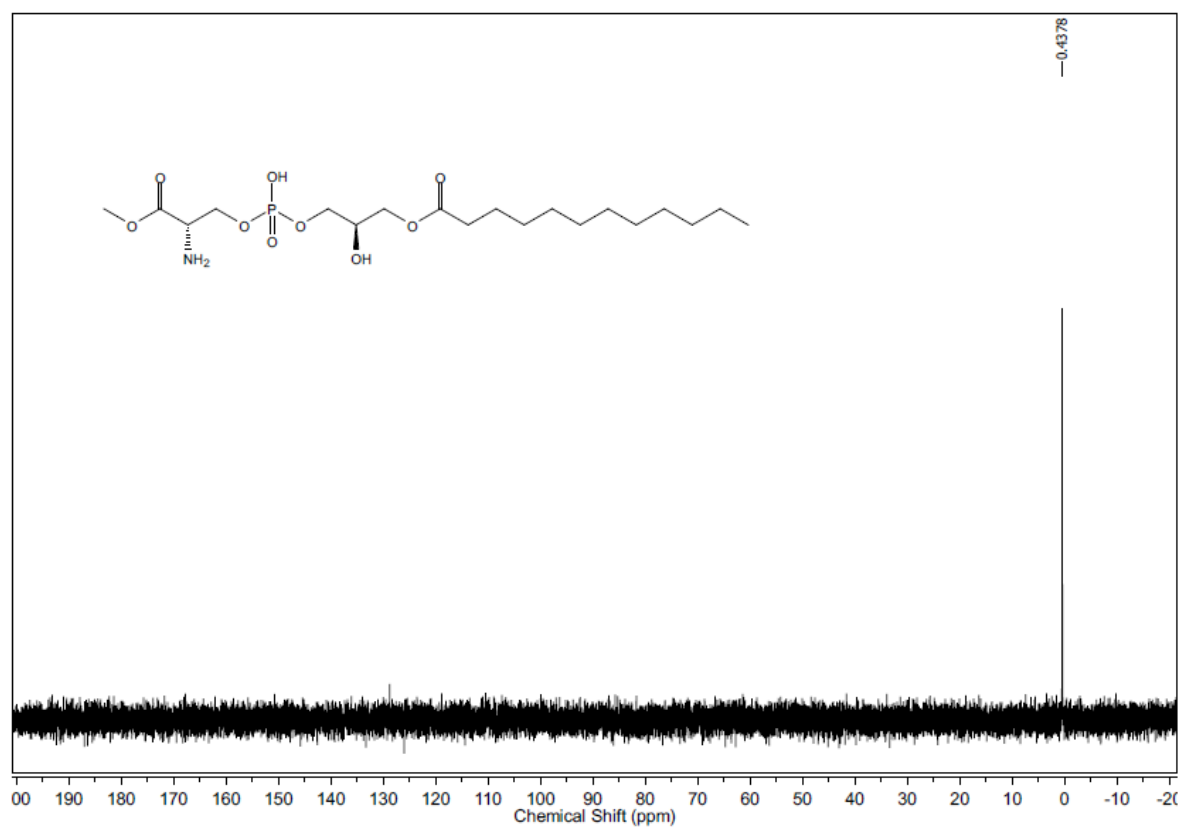
^{31}P NMR of final compound (*R*)-**2a**



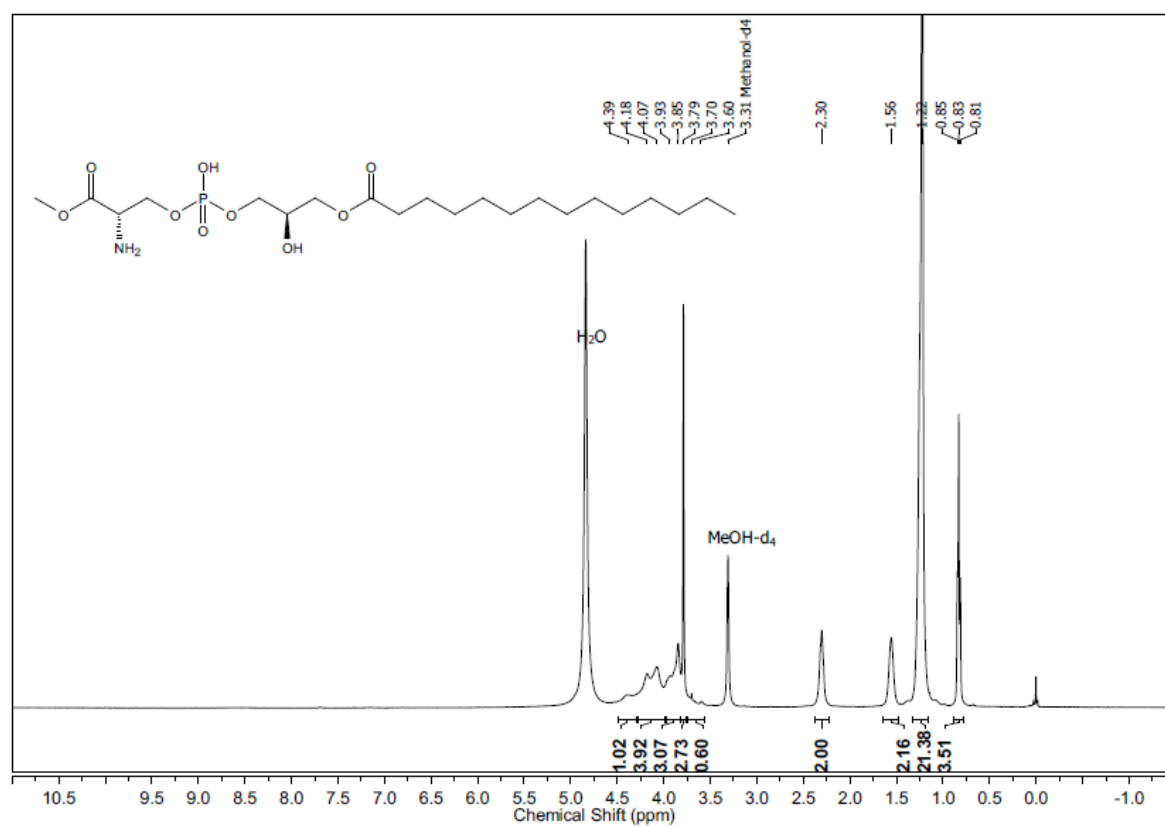
¹H NMR of final compound (*R*)-**2b**



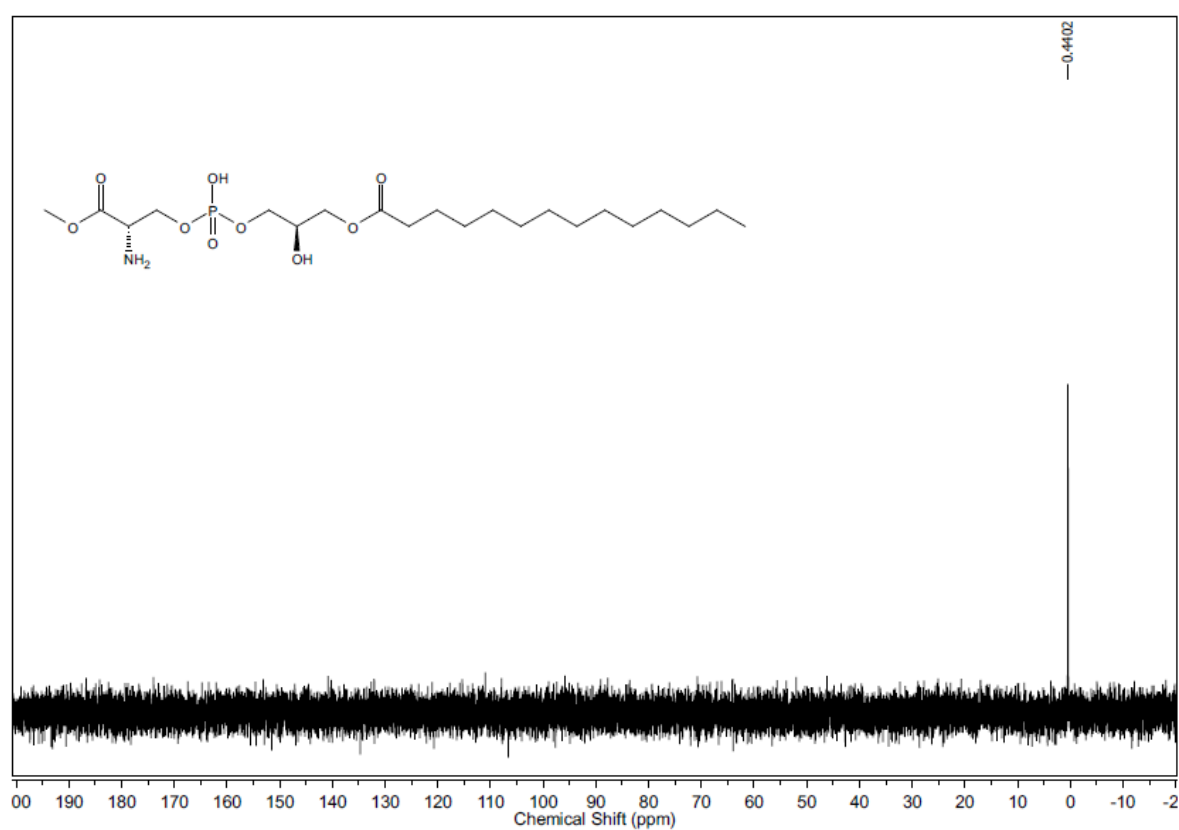
³¹P NMR of final compound (*R*)-**2b**



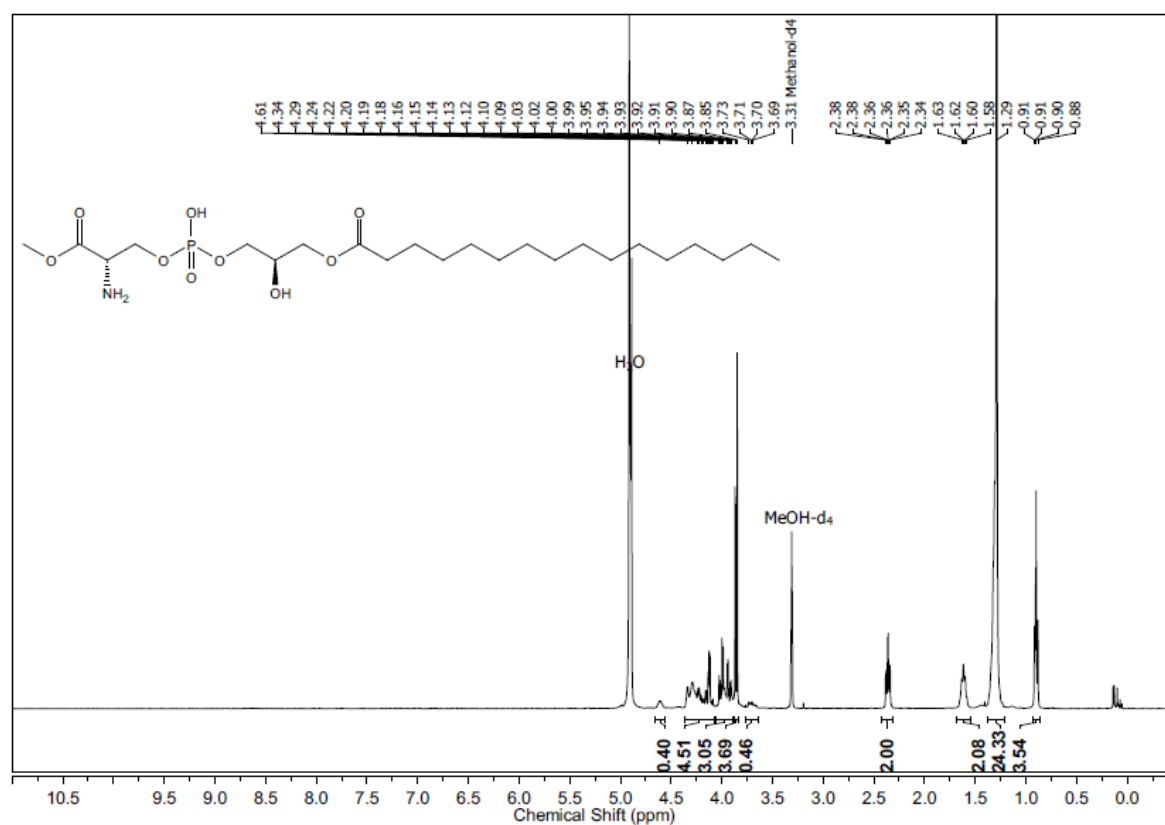
^1H NMR of final compound (*R*)-**2c**



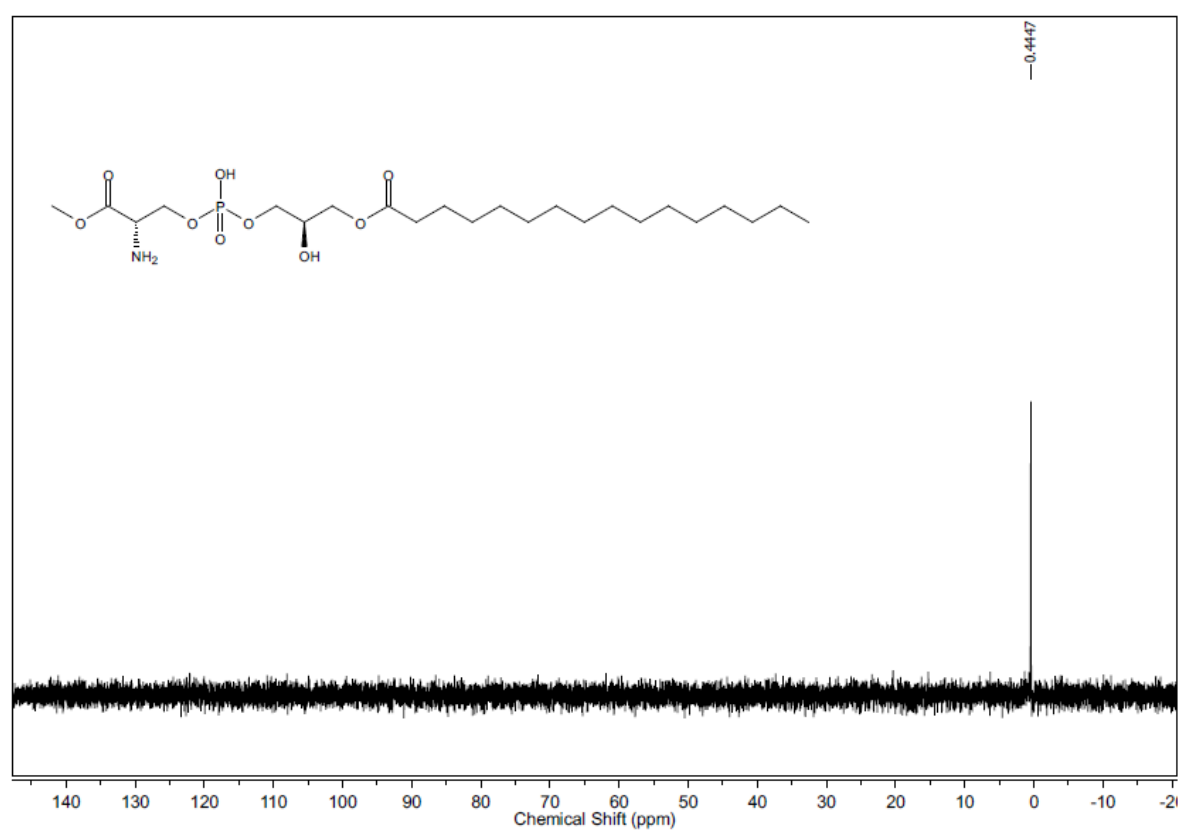
^{31}P NMR of final compound (*R*)-**2c**



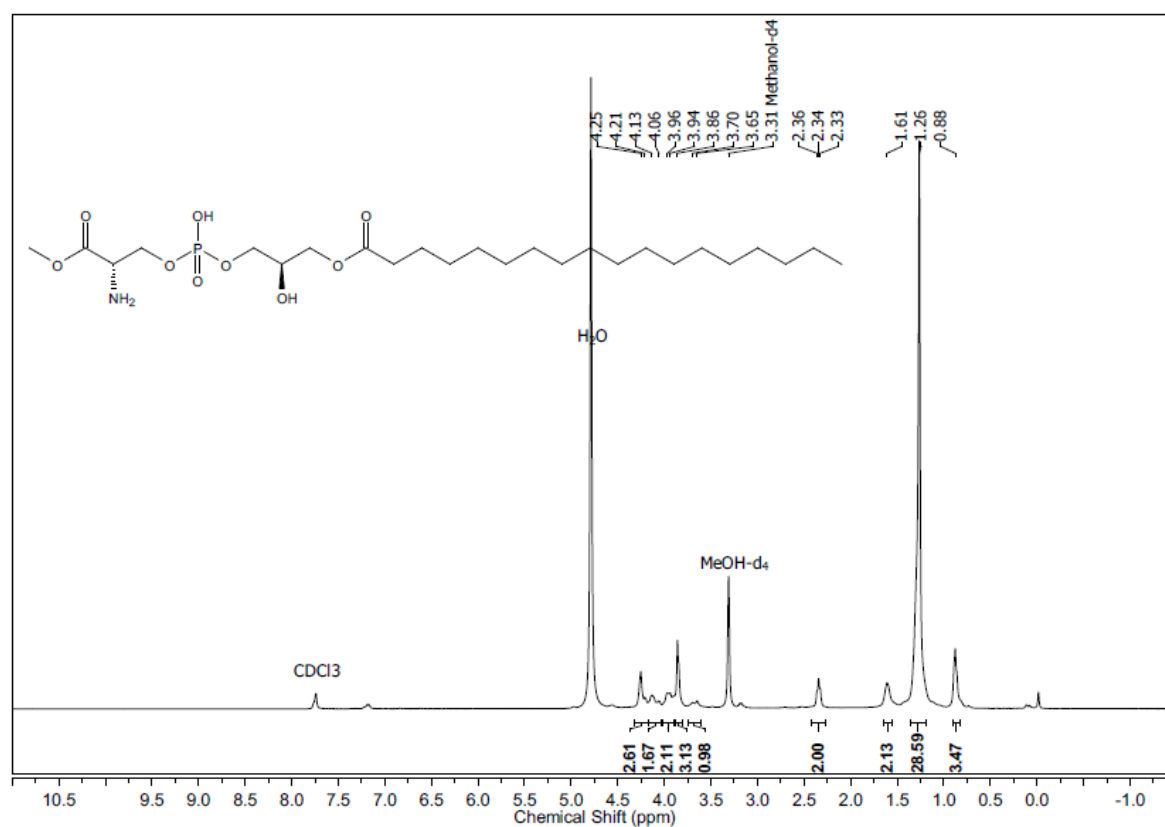
¹H NMR of final compound (*R*)-**2d**



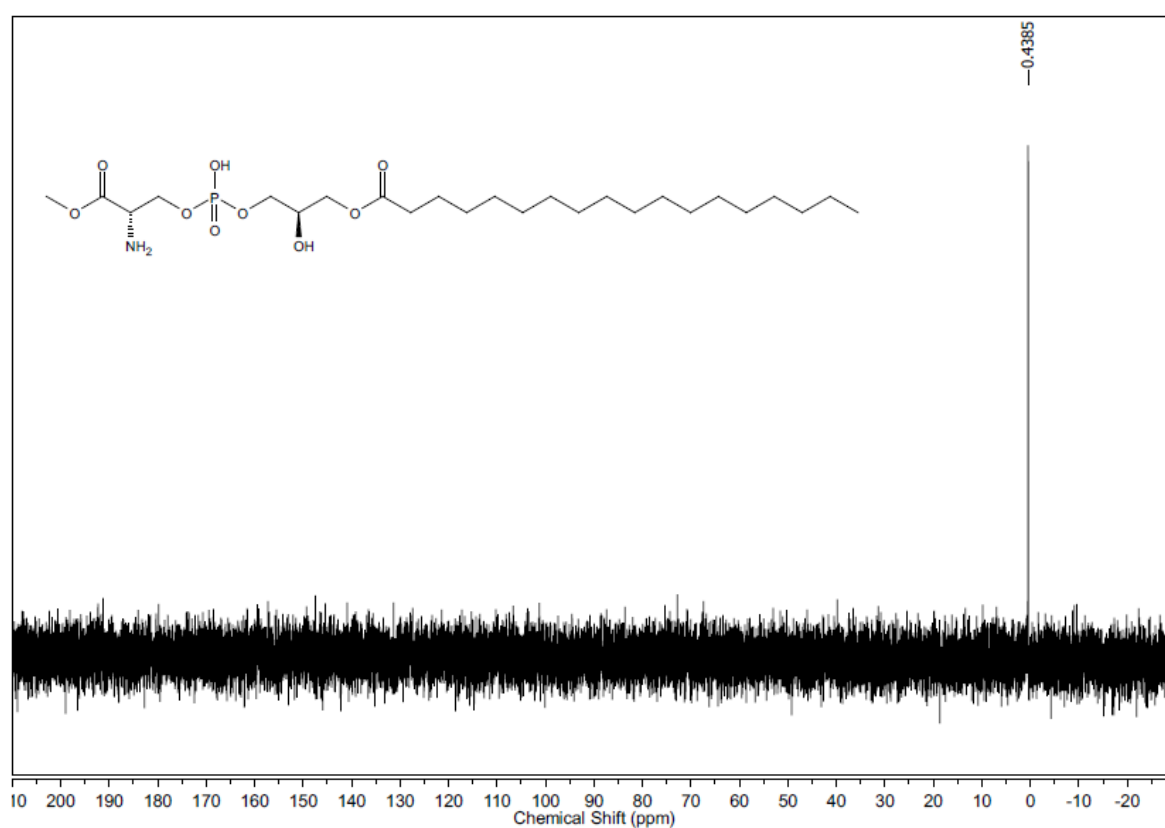
³¹P NMR of final compound (*R*)-**2d**



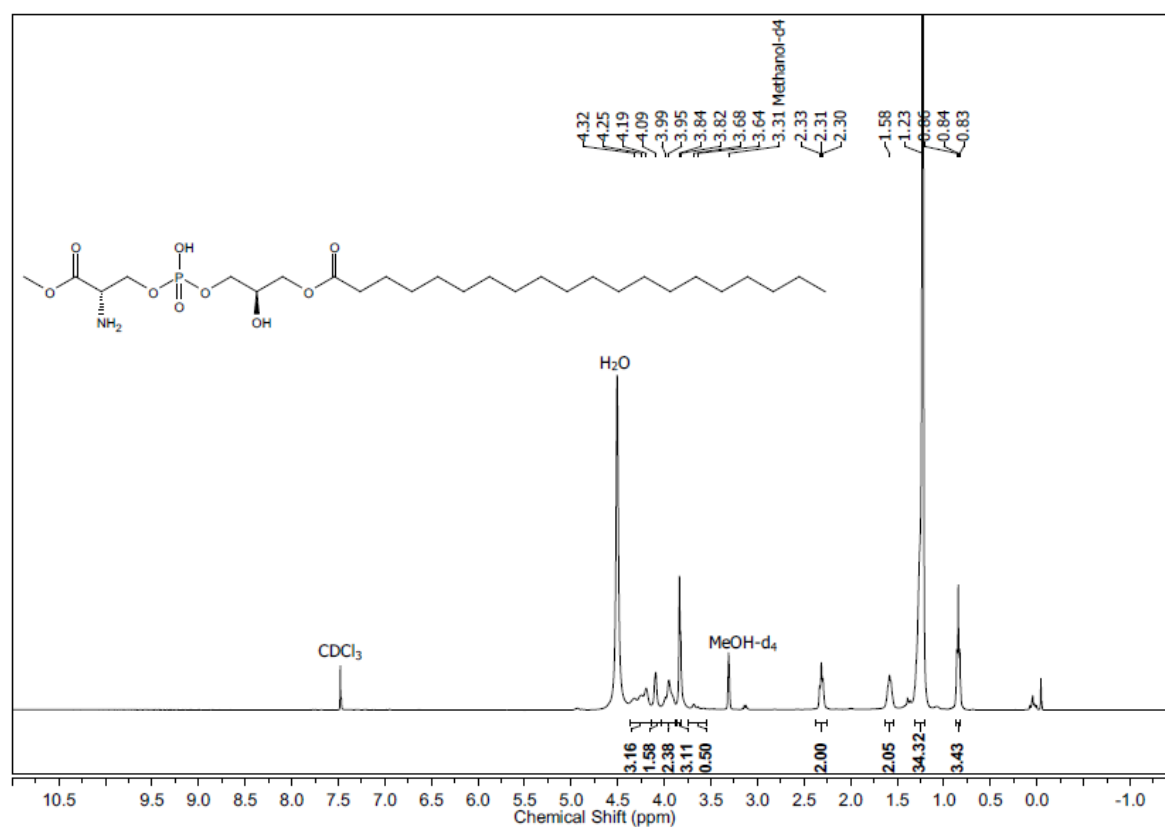
^1H NMR of final compound (*R*)-**2e**



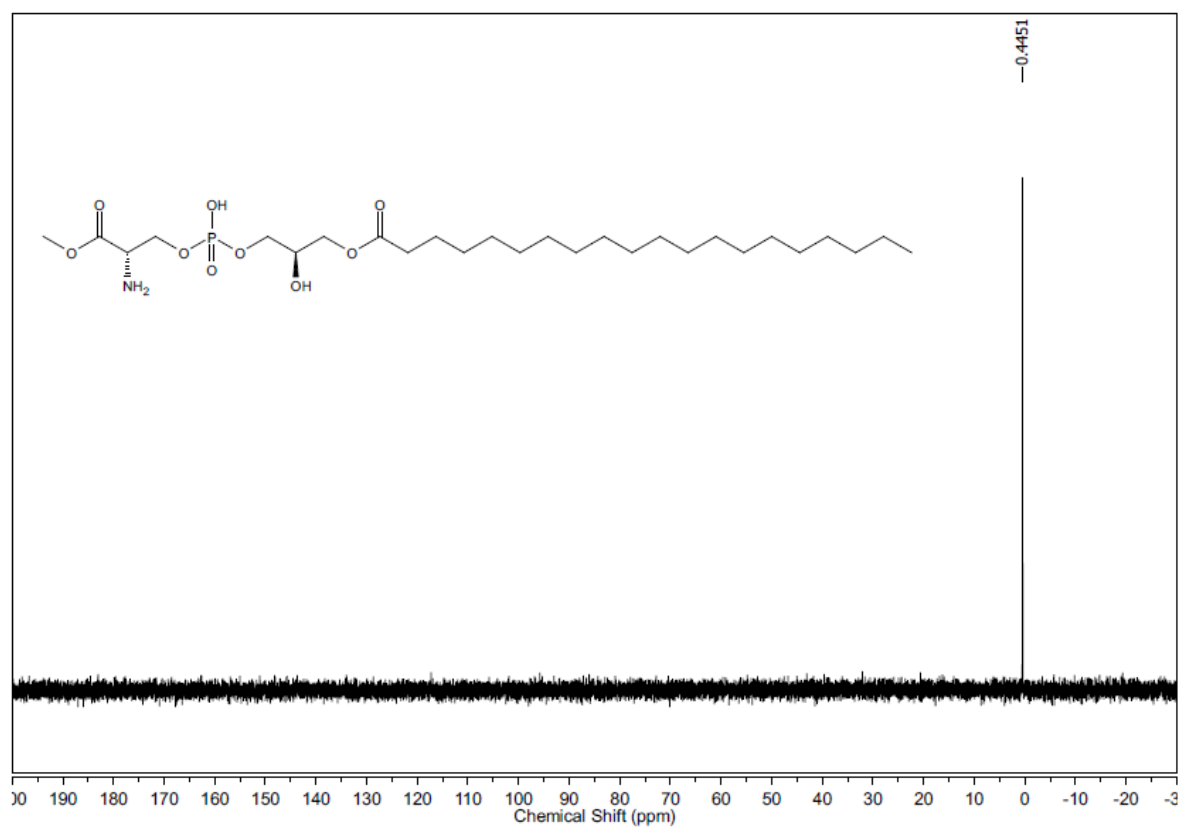
^{31}P NMR of final compound (*R*)-**2e**



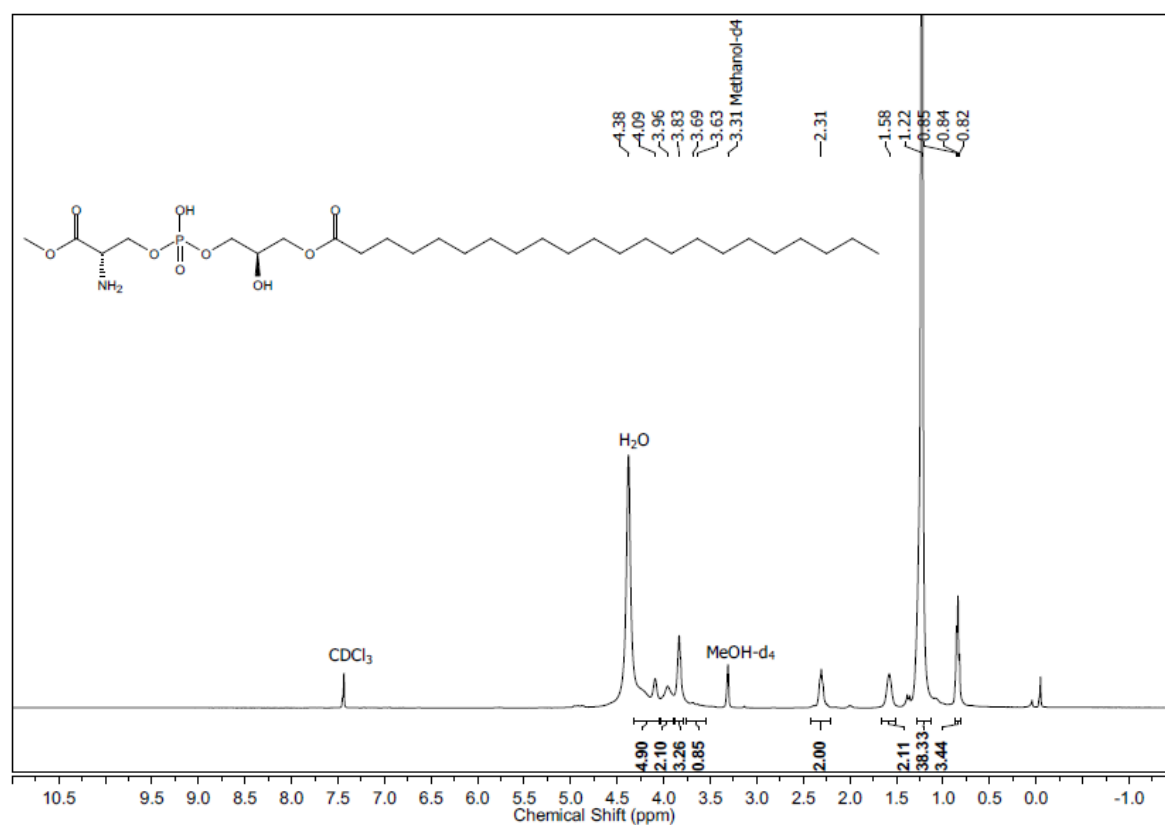
¹H NMR of final compound (*R*)-**2f**



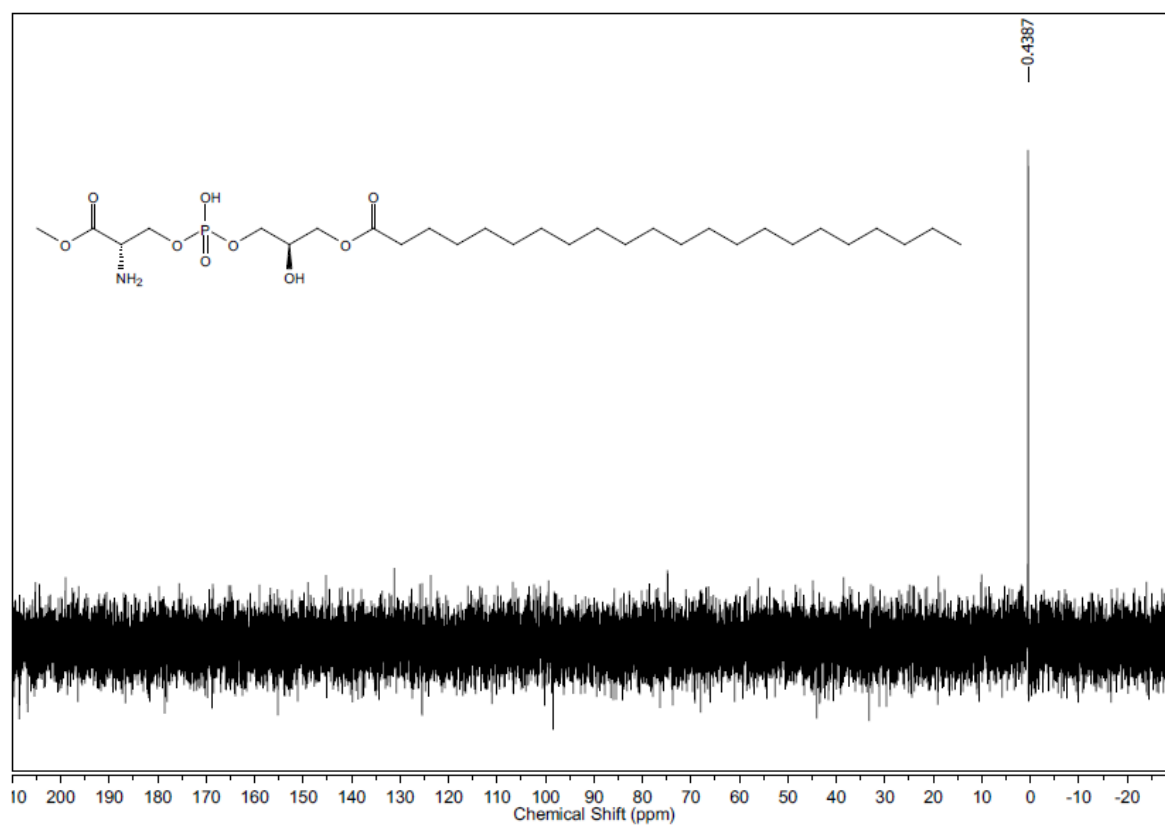
³¹P NMR of final compound (*R*)-**2f**



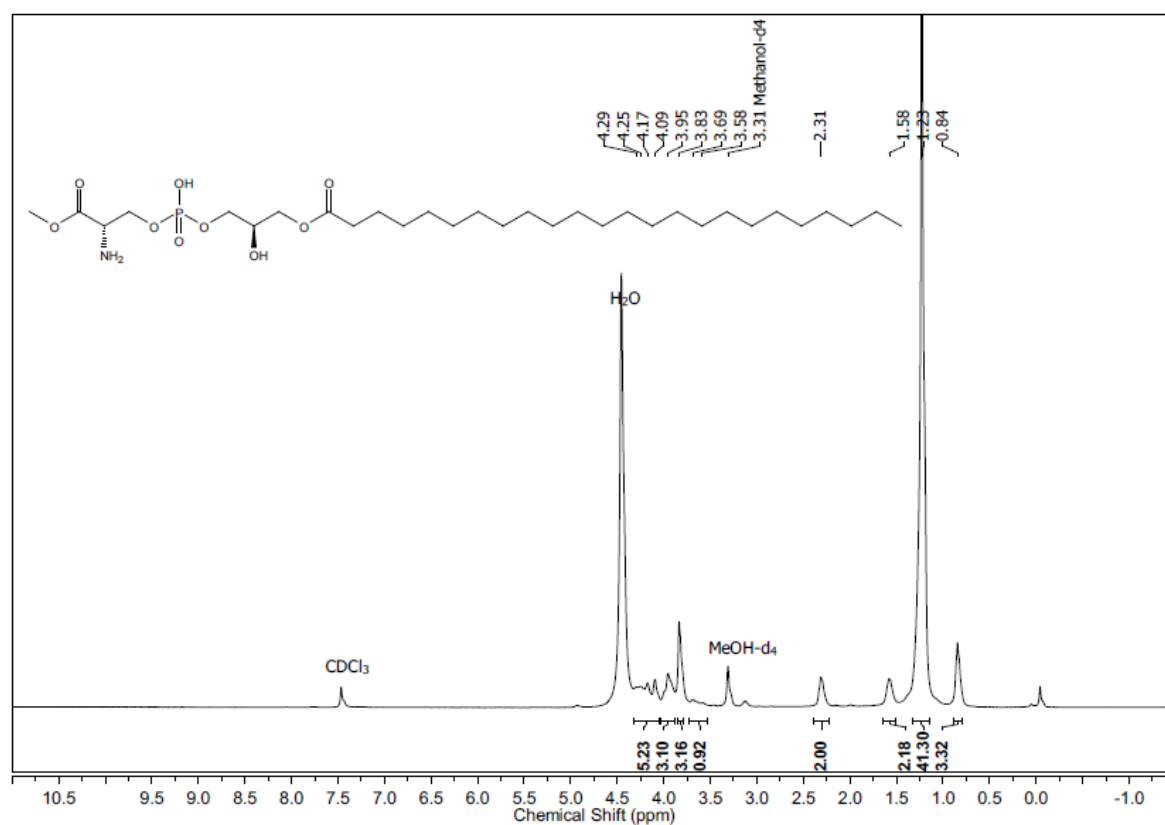
^1H NMR of final compound (*R*)-**2g**



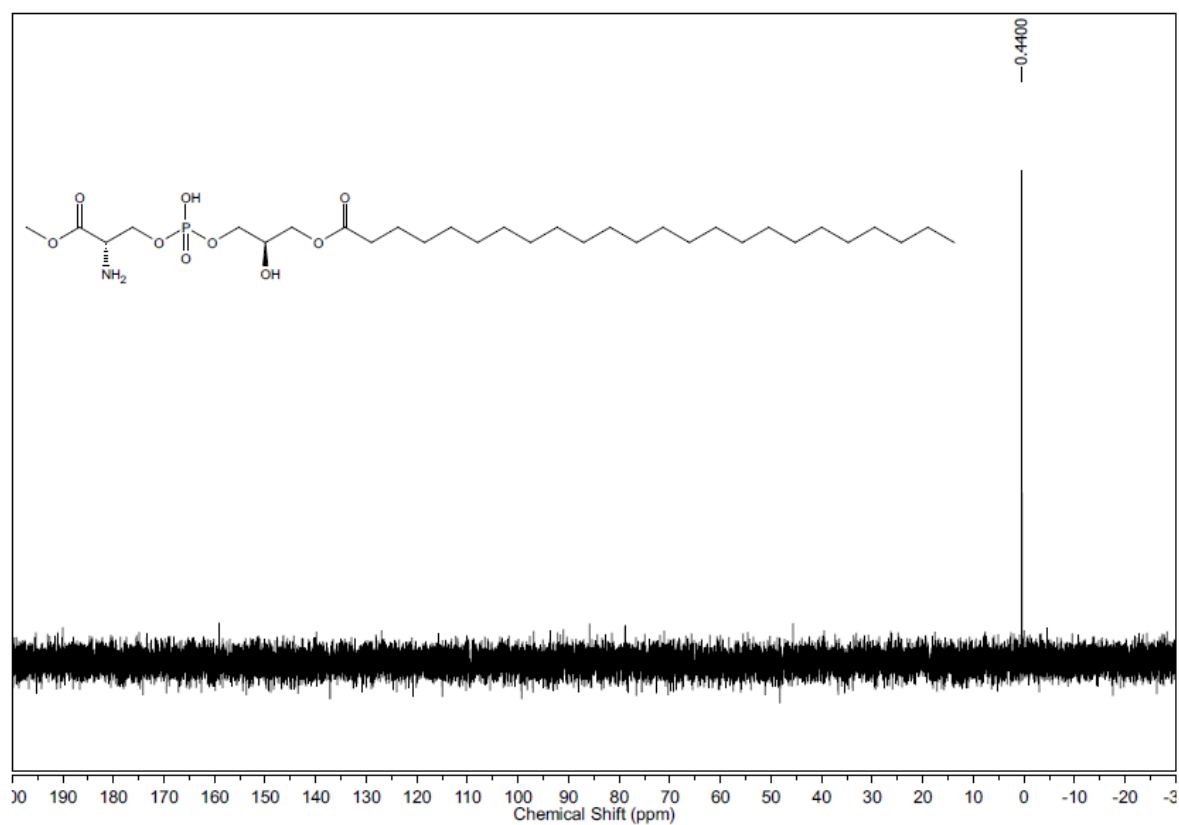
^{31}P NMR of final compound (*R*)-**2g**



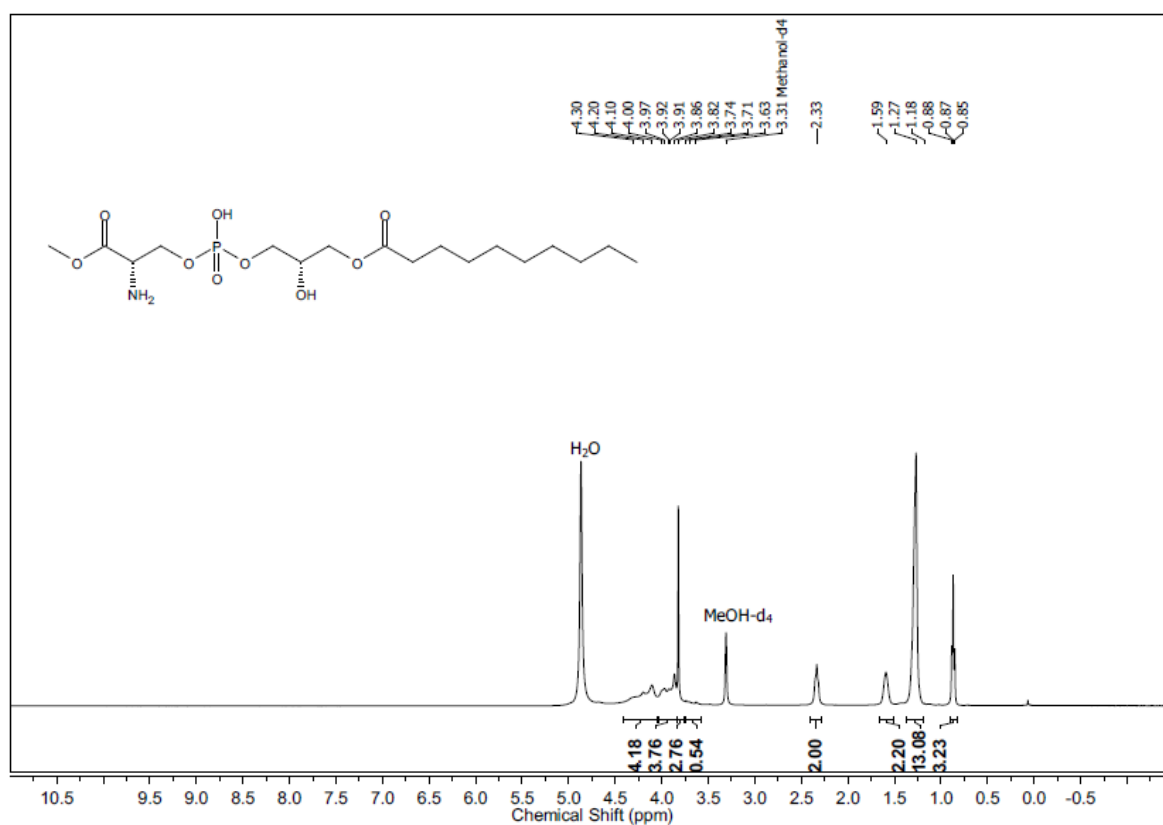
^1H NMR of final compound (*R*)-**2h**



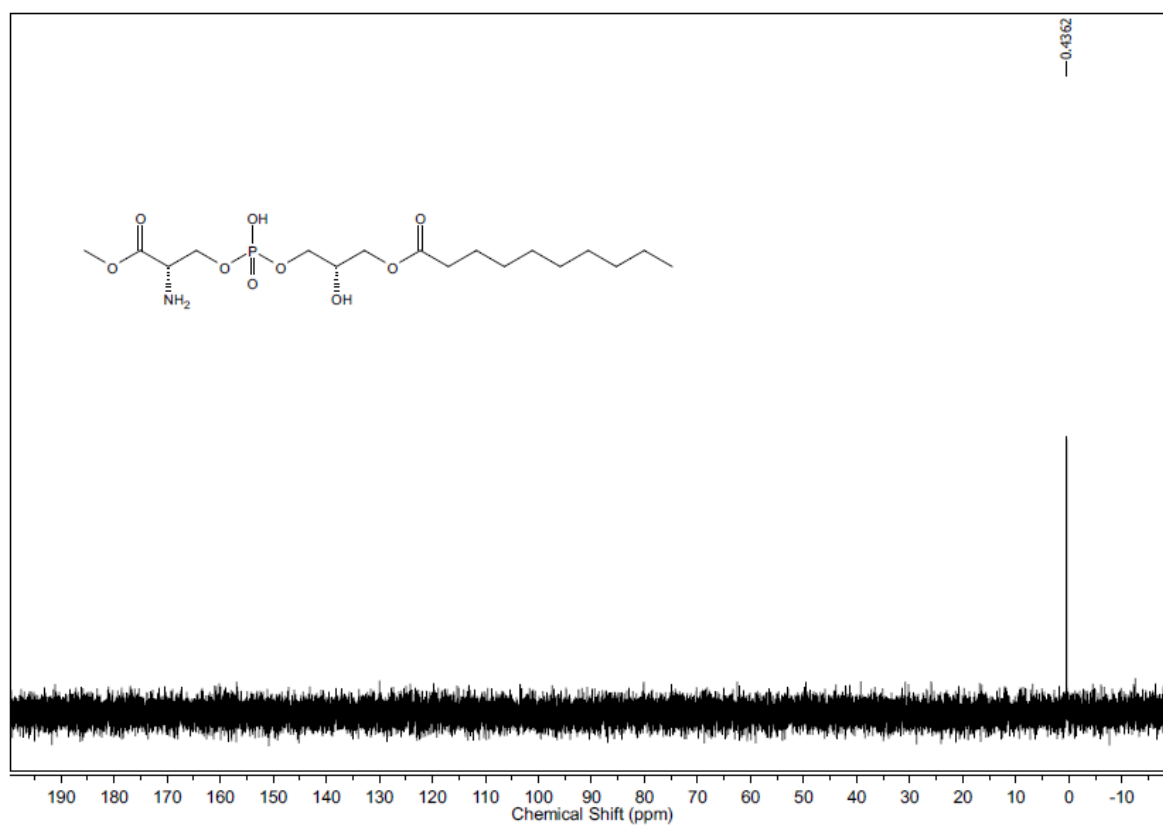
^{31}P NMR of final compound (*R*)-**2h**



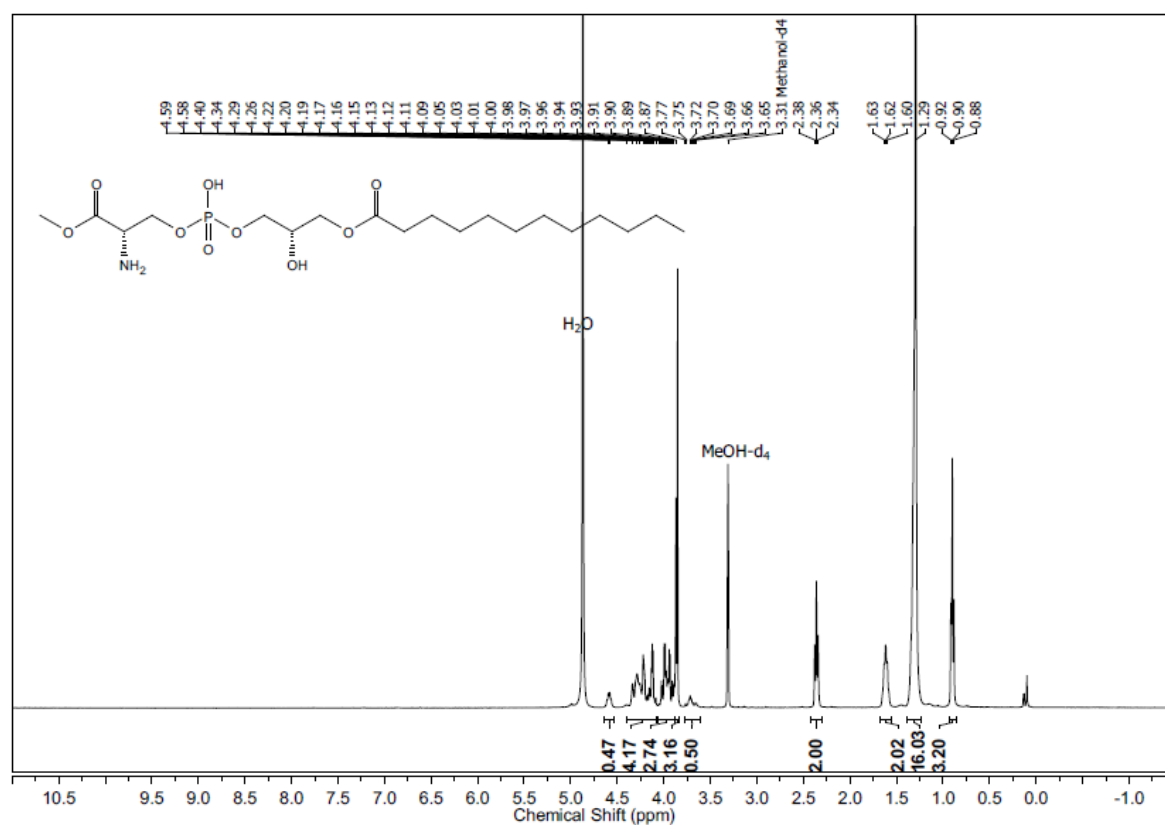
¹H NMR of final compound (S)-2a



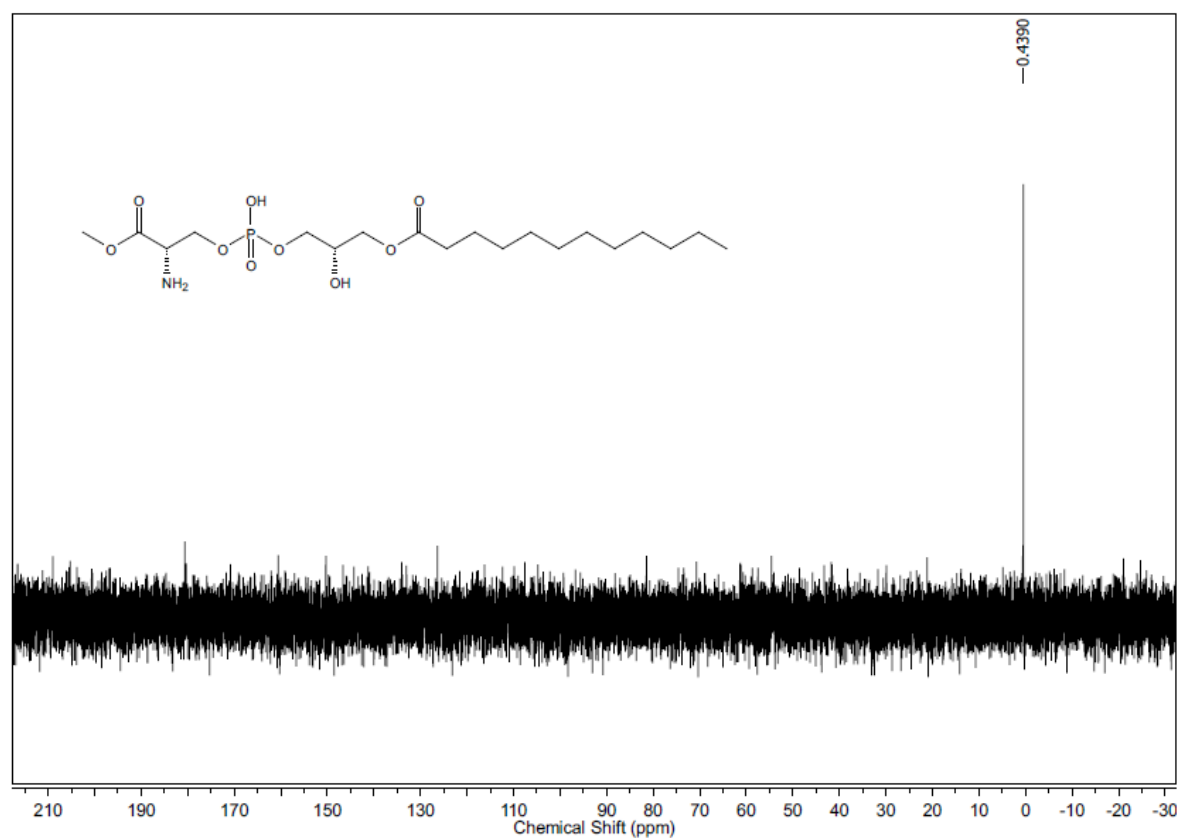
³¹P NMR of final compound (S)-2a



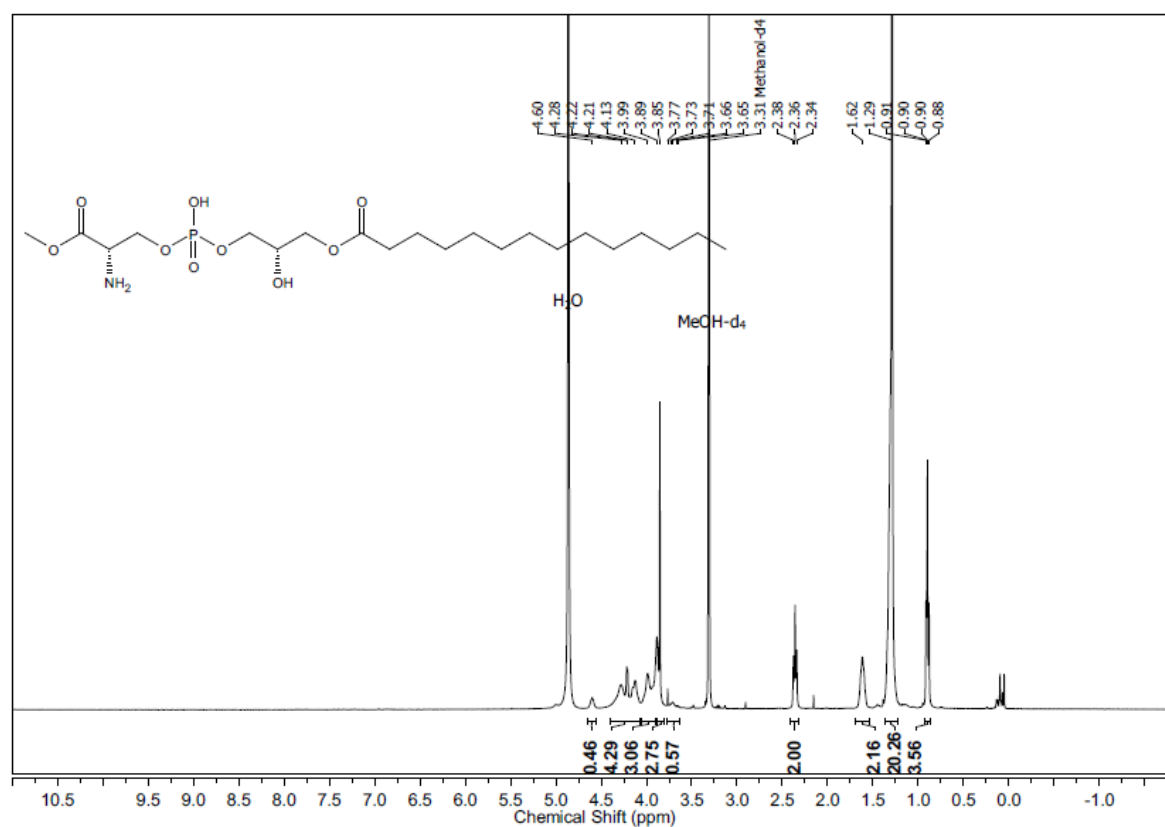
¹H NMR of final compound (S)-2b



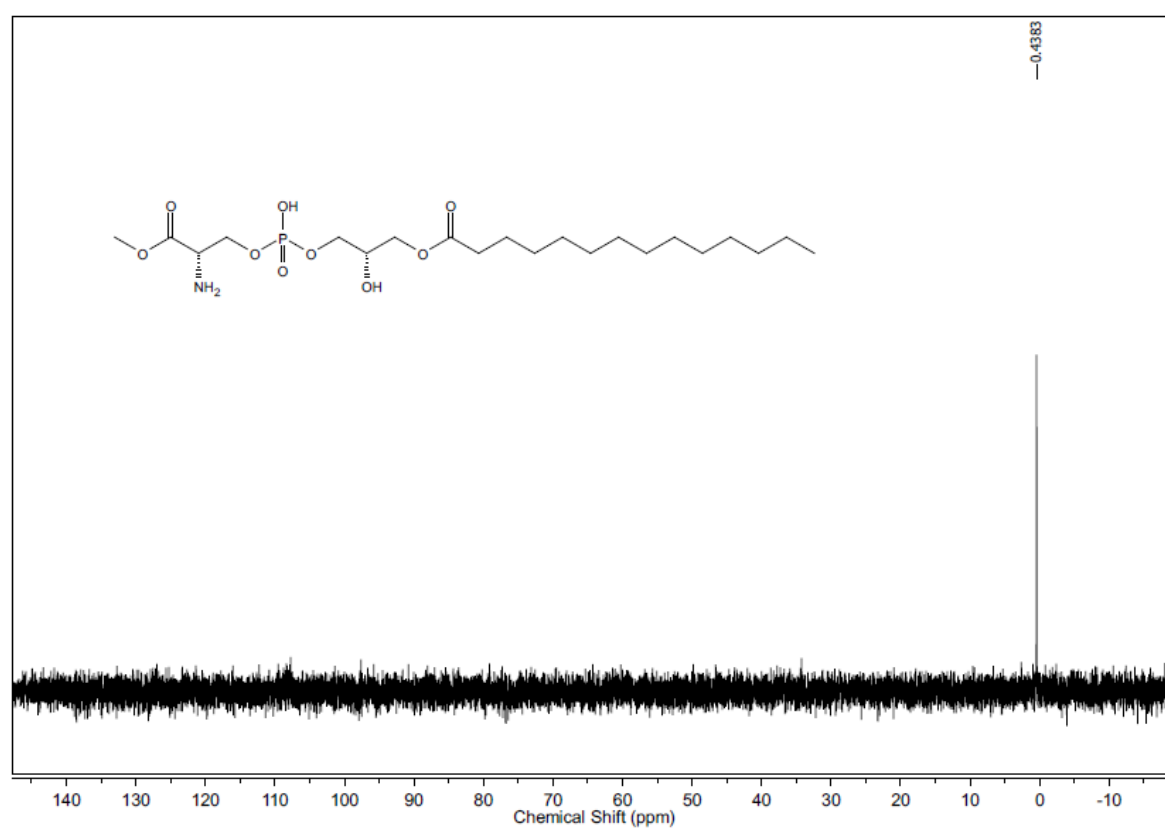
³¹P NMR of final compound (S)-2b



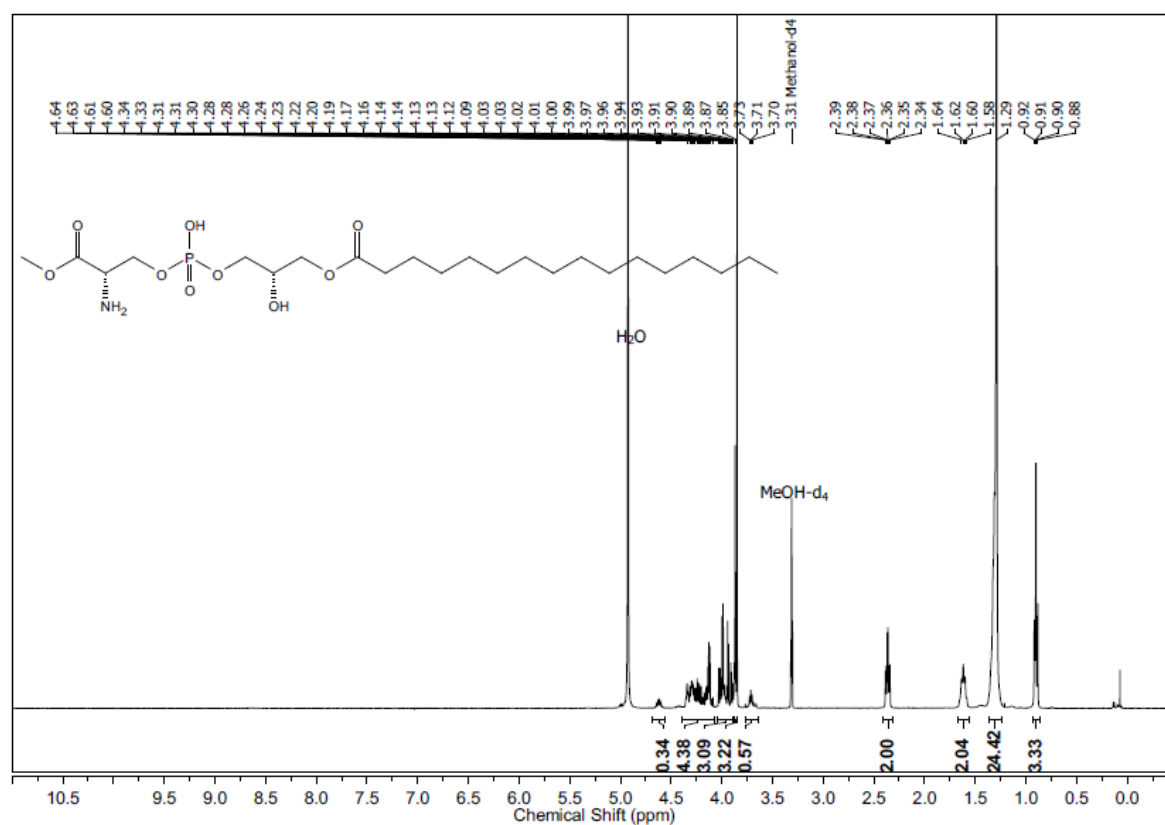
^1H NMR of final compound (S)-**2c**



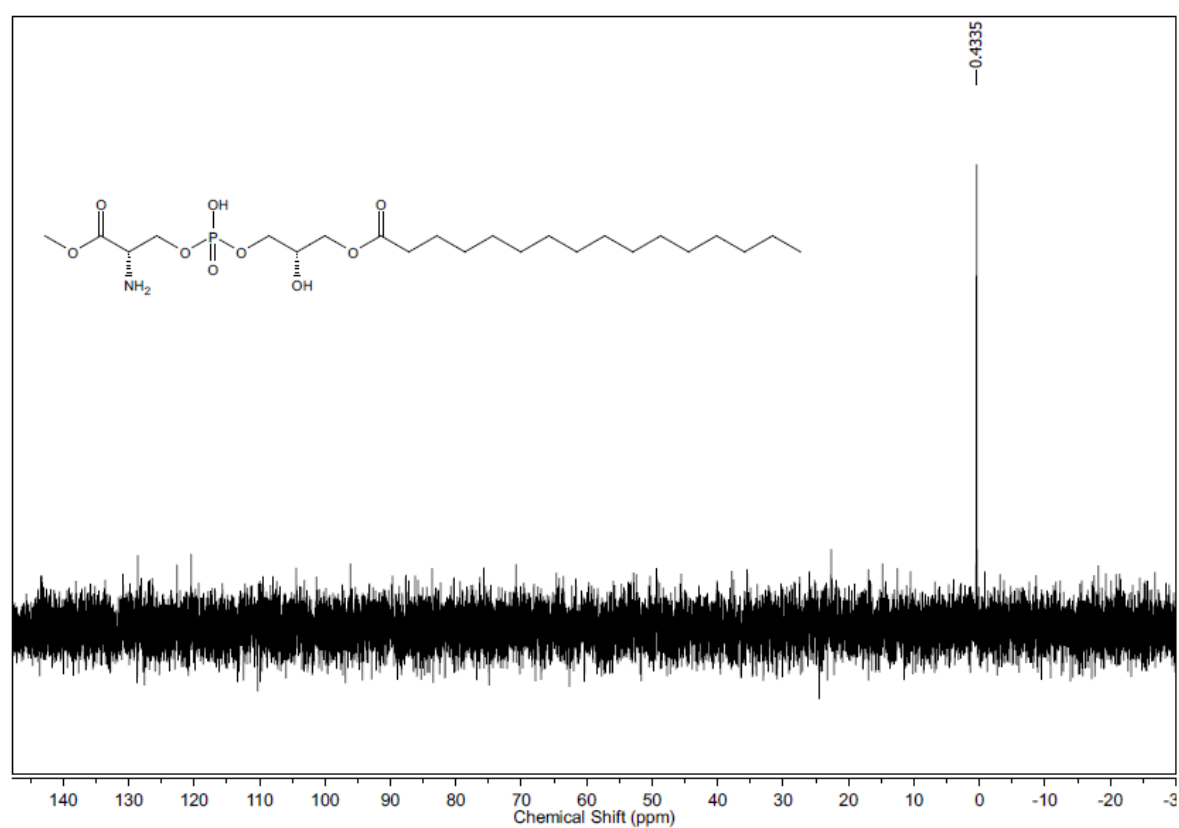
^{31}P NMR of final compound (S)-**2c**



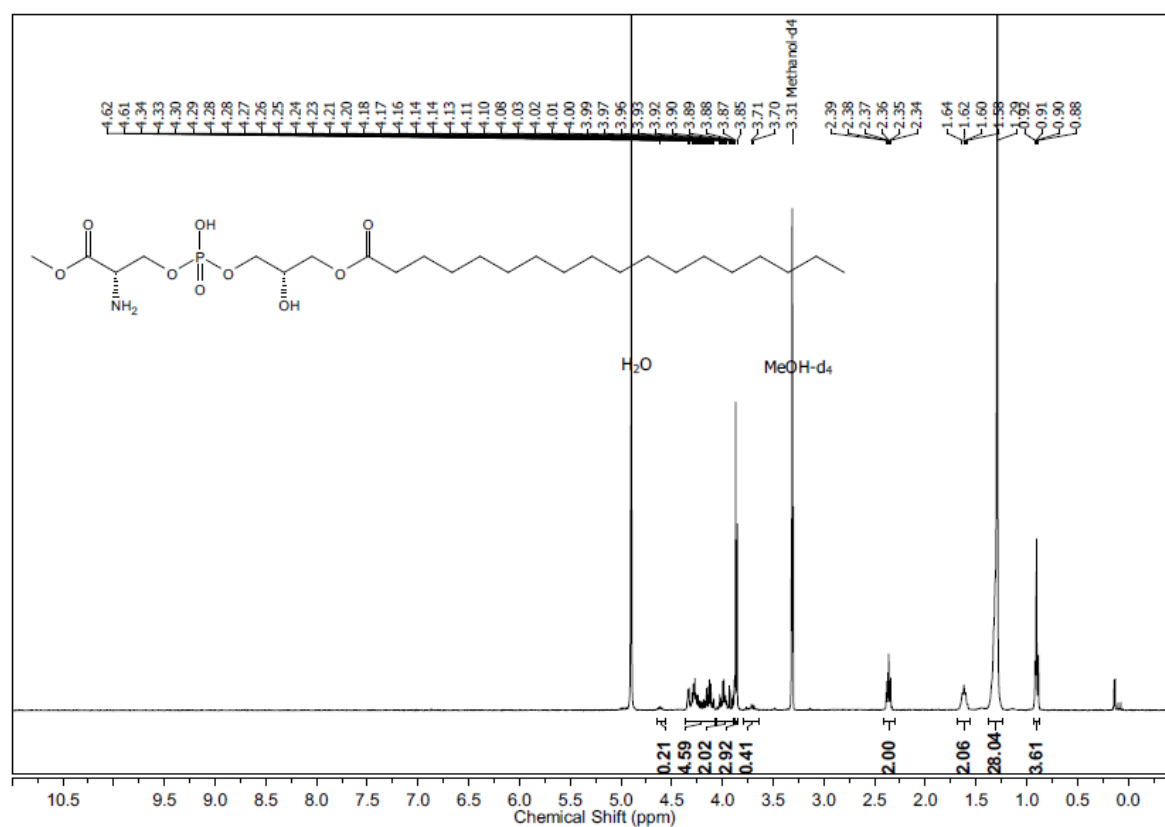
¹H NMR of final compound (S)-2d



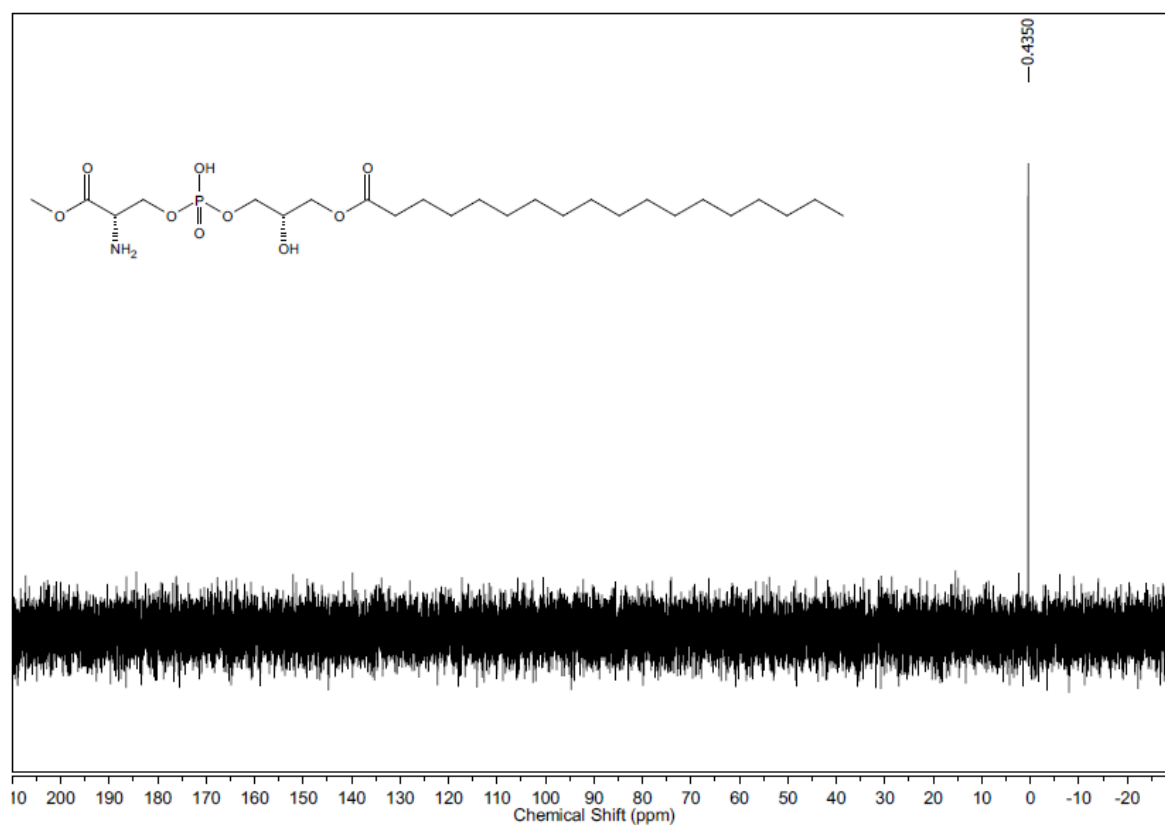
³¹P NMR of final compound (S)-2d



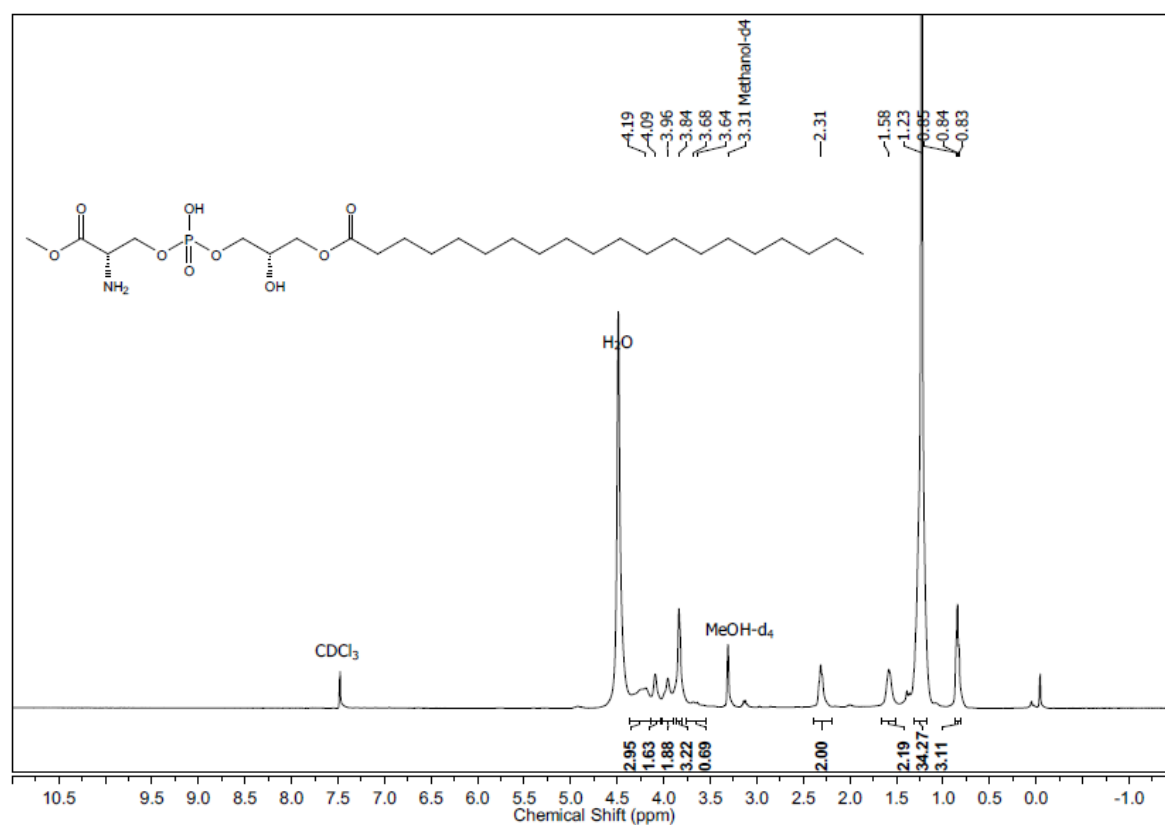
¹H NMR of final compound (S)-**2e**



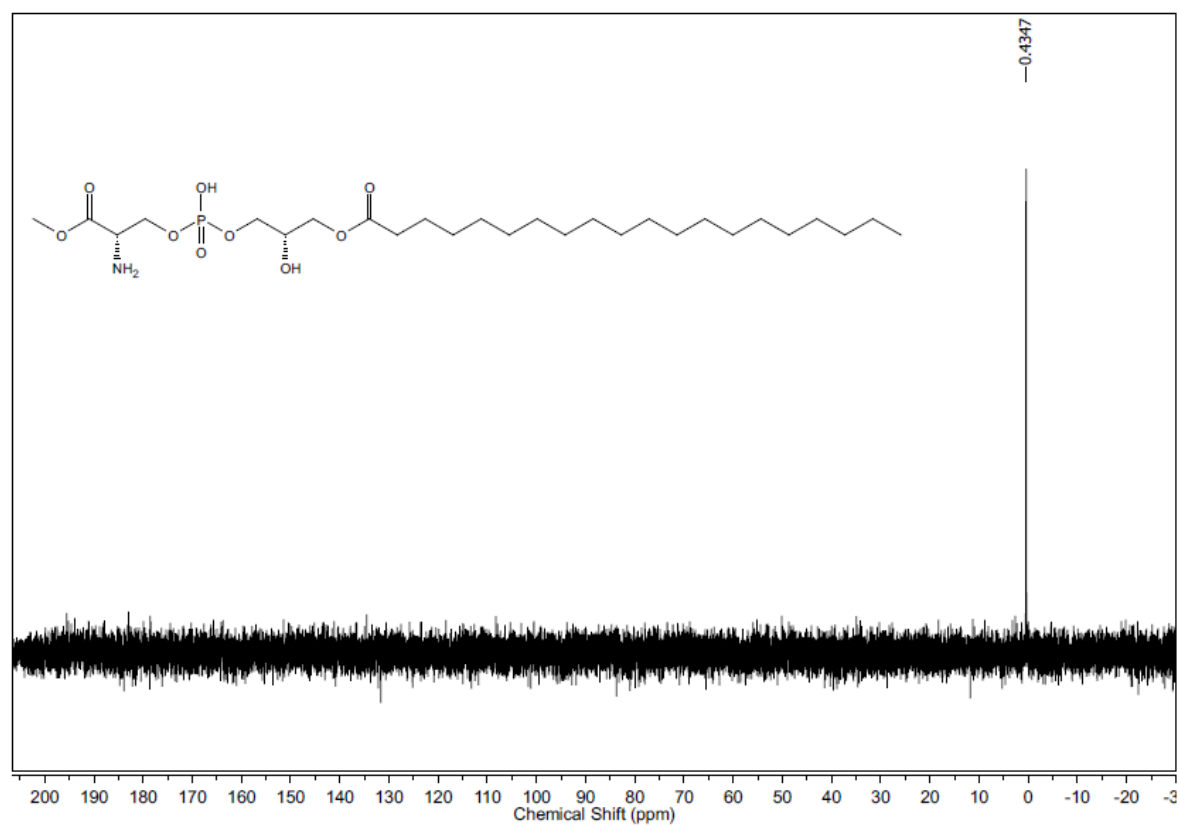
³¹P NMR of final compound (S)-**2e**



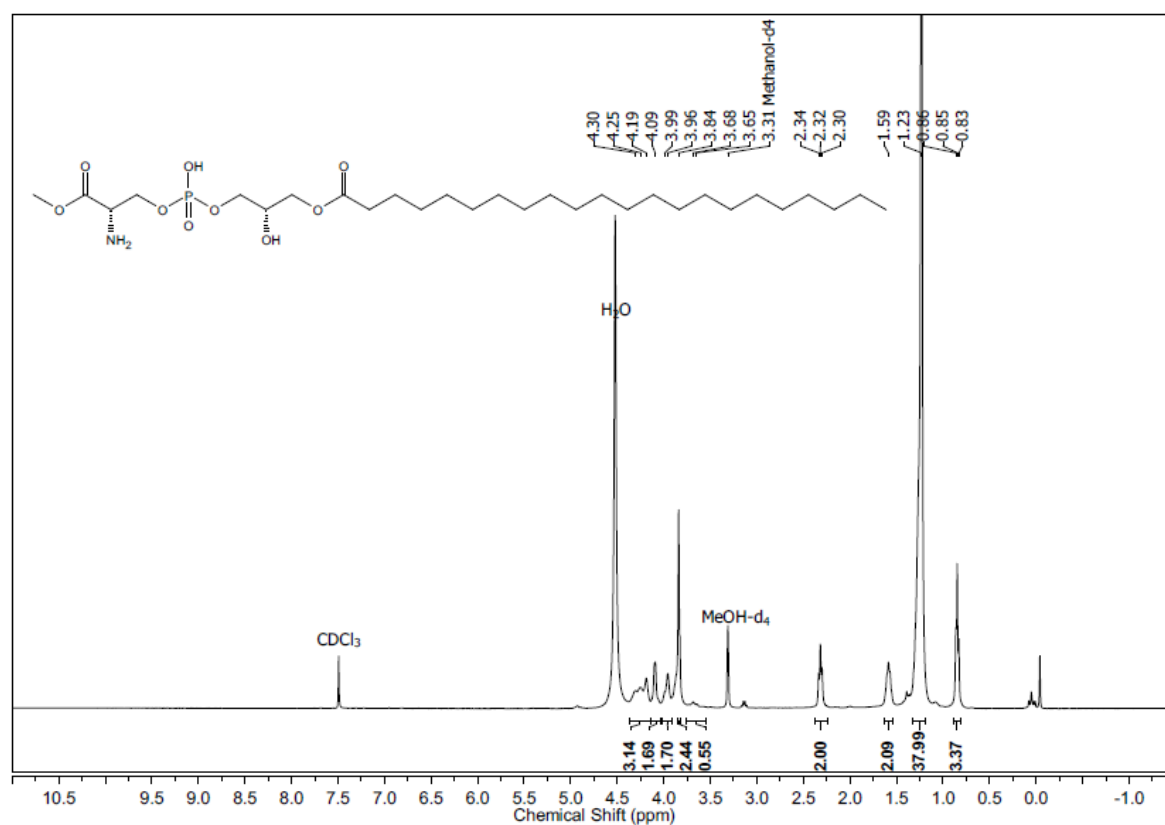
¹H NMR of final compound (S)-**2f**



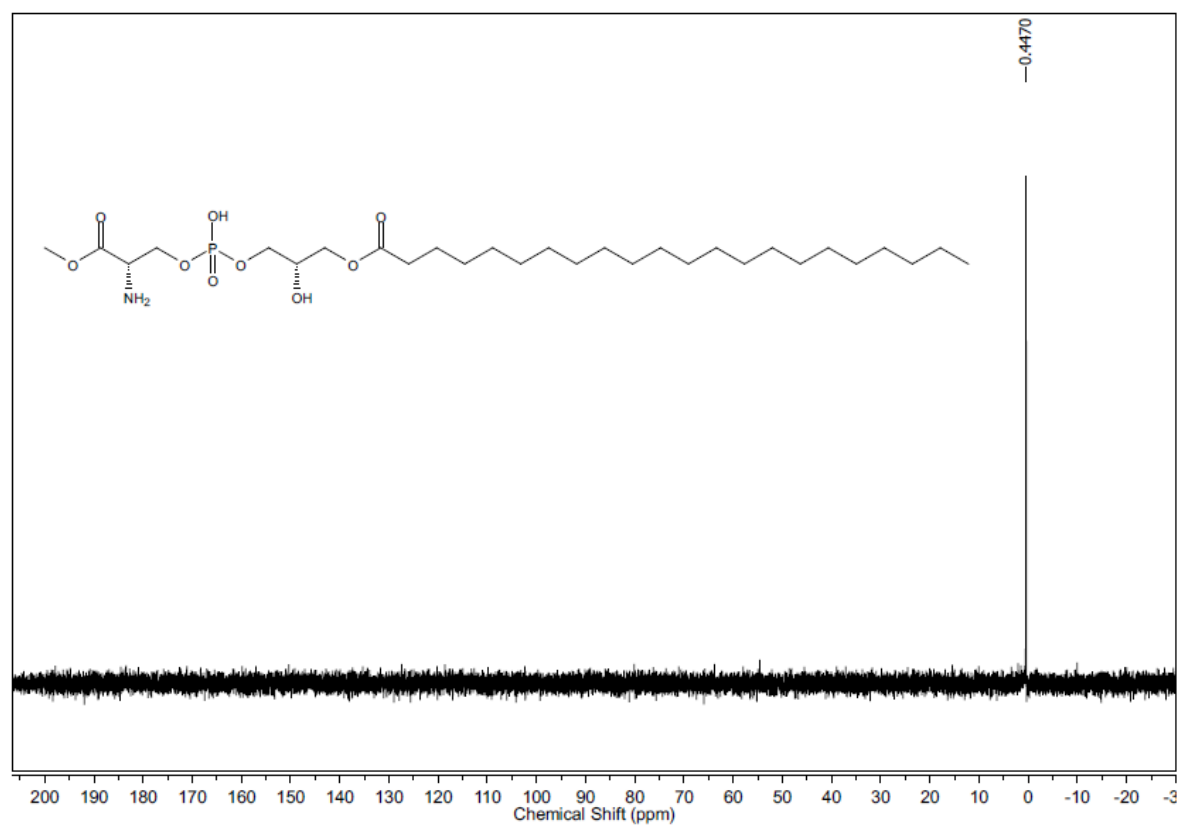
³¹P NMR of final compound (S)-**2f**



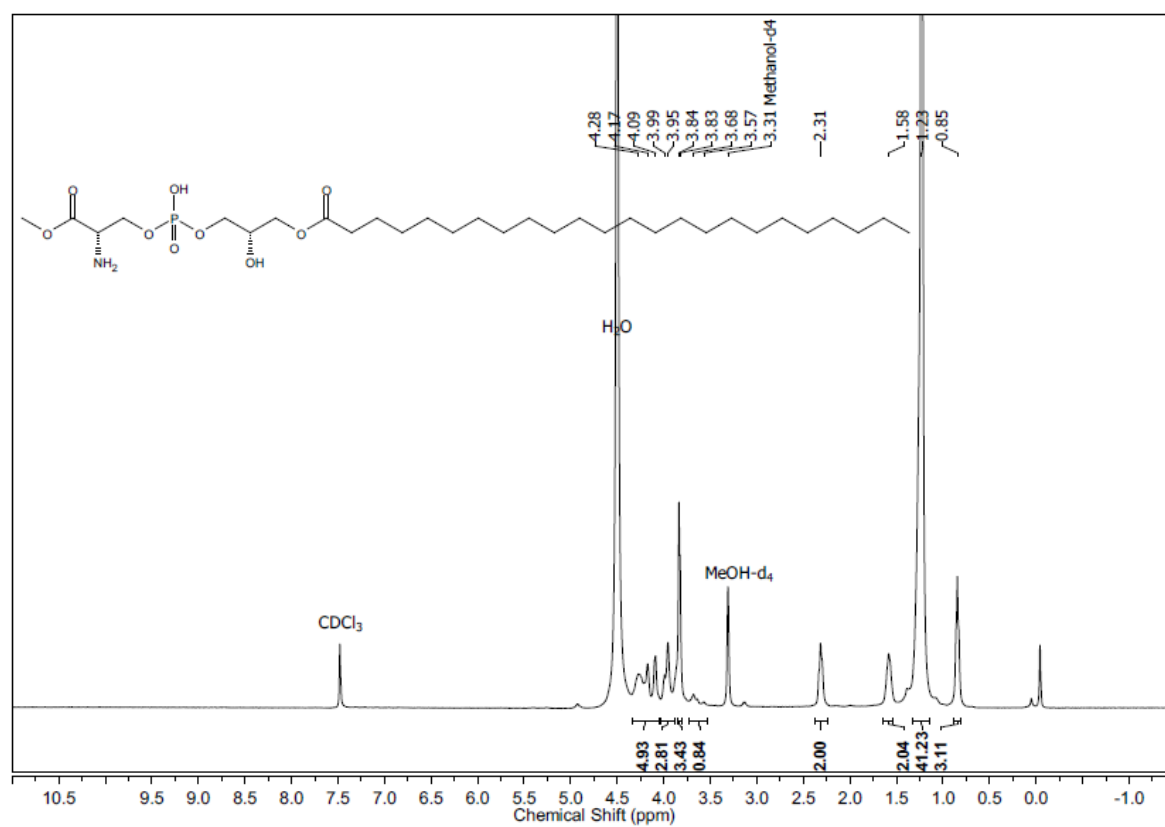
¹H NMR of final compound (S)-**2g**



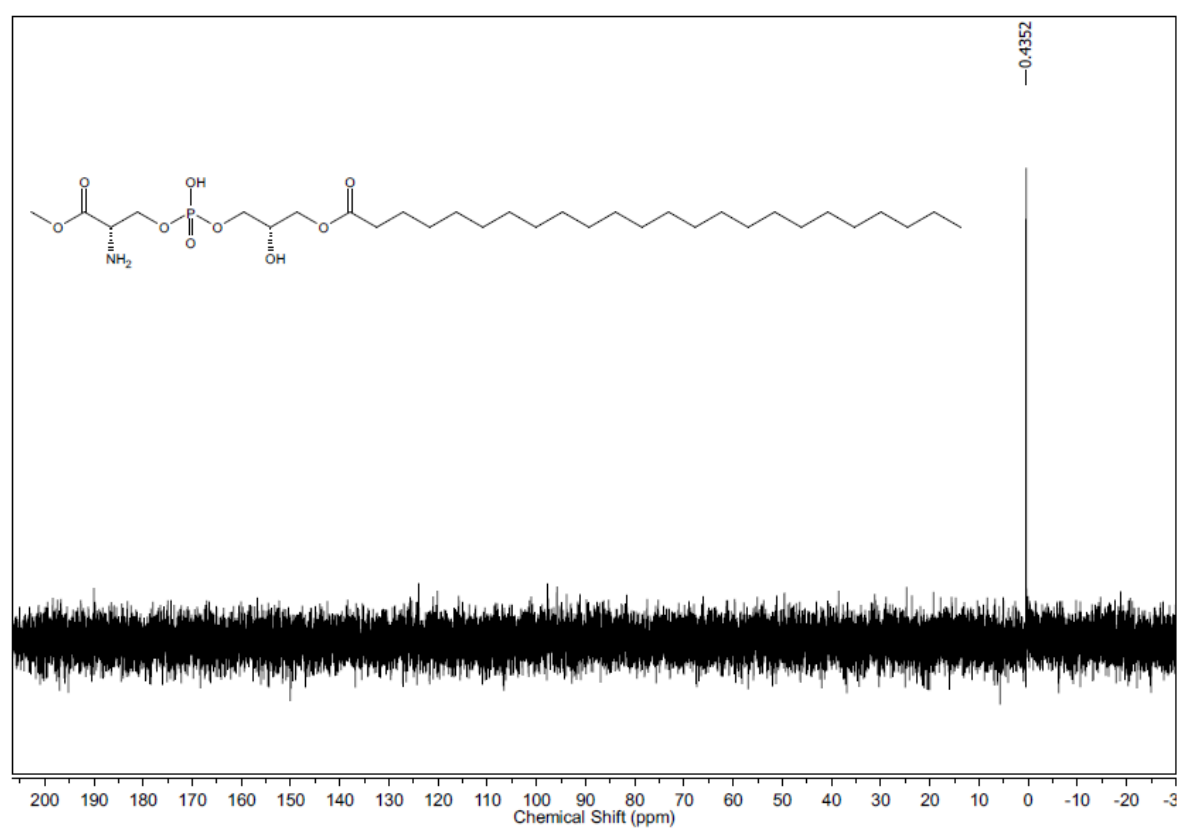
³¹P NMR of final compound (S)-**2g**

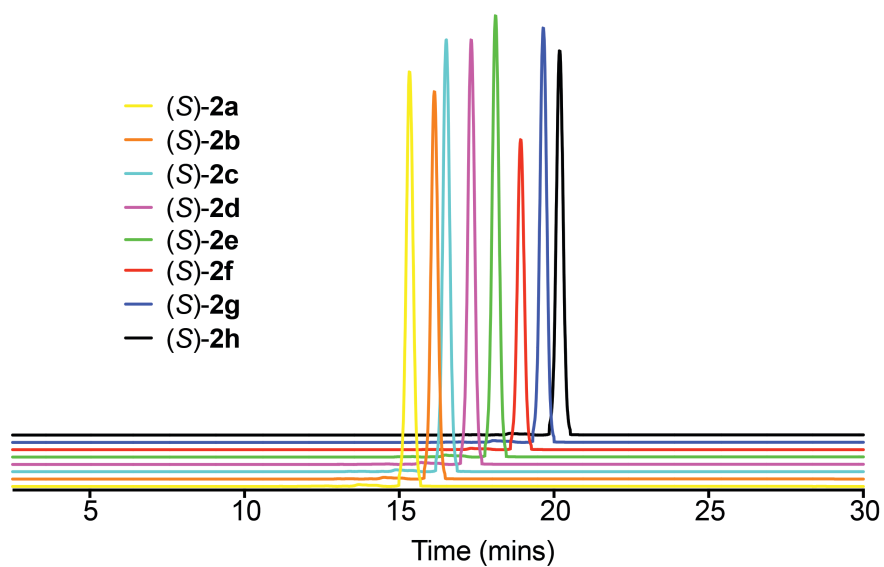
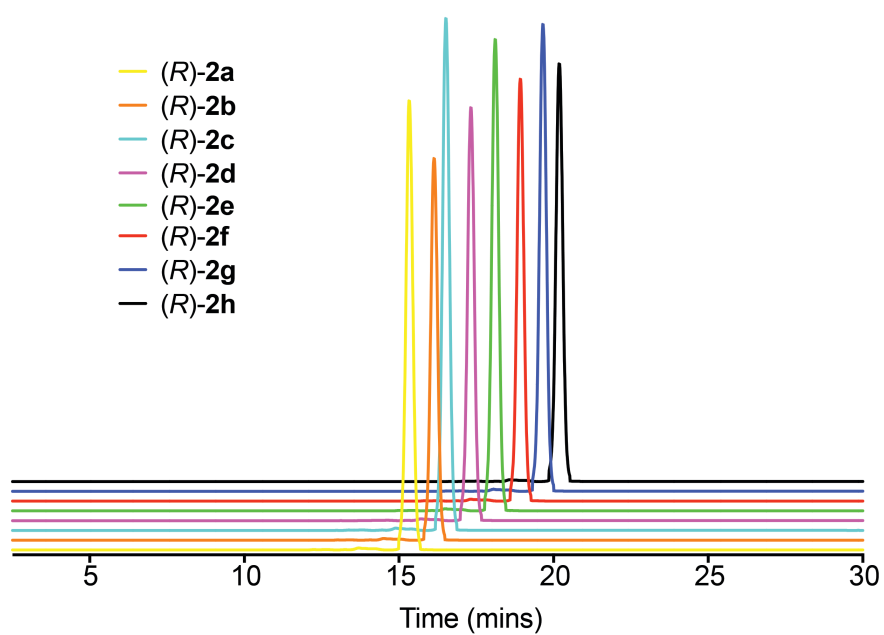


^1H NMR of final compound (S)-2h



^{31}P NMR of final compound (S)-2h





LC-MS trace analysis: showing purity of final compounds (*R*)-2a-h and (*S*)-2a-h described in this study, and tested in the biological assays.

Table S1. RT-PCR primers, Related to Figures 3, and S3

Gene/Primer Name	Primer Sequences
GPR34 Fwd/Rev (422-bp)	5'-GAGCACTTCGGCTTACTTGG-3' 5'-TTCCATGAGAGGAGCAAAGC-3'
GPR174 Fwd/Rev (479-bp)	5'-CTGCATCAGTGTGCGAAGAT-3' 5'-TCACTCTTCTGGCAAAGCAA-3'
P2Y10 Fwd/Rev (439-bp)	5'-AAGAGCCCAGCTGACACAAC-3' 5'-AAGAGCCCAGCTGACACAAC-3'
TLR2 Fwd/Rev (451-bp)	5'-GACTCACAGCAGCCATGAAA-3' 5'-TCGCGGATCGACTTTAGACT-3'
ACTB Fwd/Rev (454-bp)	5'-GGGAATGGGTCAGAAGGACT-3' 5'-ACGCTCGGTCAGGATCTTC-3'
GAPDH Fwd/Rev (403-bp)	5'-ACTTGAAGGGTGGAGCCAAA-3' 5'-AGATCCACGACGGACACATT-3'