Synthesis of Phosphatidylserine and Its Stereoisomers: Their Role in Activation of Blood Coagulation

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Supporting information

TABLE OF CONTENTS

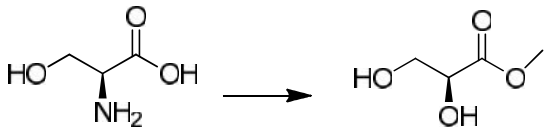
1. Experimental Procedure:

All moisture sensitive reactions were performed in oven dried glassware with Teflon coated magnetic stirring bar under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe and a stainless-steel needle. Reactions were monitored by thin layer chromatography (TLC, Silica gel 60 F254 Merck Made in Germany) plates by UV light, Cupric Acetate(5:1 Cupric Acetate-water) Bromocresol Green, Ninhydrin (0.2g ninhydrin in 100ml ethanol and heat to 110°C), Potassium permanganate as developing agents. All workup and purification procedures were carried out with reagent-grade solvents under room temperature atmosphere unless otherwise stated. Column chromatography was performed using silica gel 60-120 mesh, 100-200 mesh and 230-400 mesh. Yields mentioned as chromatographically and spectroscopically homogeneous materials unless otherwise stated. Optical rotations were measured using sodium (589, D line)

lamp and are reported as follows: $[\alpha]_D^{25}$ (c = mg/100 ml, solvent). HRMS were taken using QuadrupleTOF (Q-TOF) micro MS system using electron spray ionization (ESI) technique and MULDI-MS were measure MULDI-TOP matrix was taken saturated solution of 2,5-Dihydroxybenzoic acid (DHB). ^{1}H NMR spectra were recorded on 400 and 500 MHz spectrometers in $CDCl_3$ solvents and calibrated using residual undeuterated solvent as an internal reference, and the chemical shifts are shown in δ ppm scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t(triplet), q(quartet), br (broad), m (multiplet, for unresolved lines) etc. ^{13}C NMR spectra were recorded on 100 and 125 MHz and ^{31}P NMR spectra were recorded on 202 and 162 MHz. All the spectra was reported in $CDCl_3$ and relative to δ **7.26** ppm for ^{1}H NMR, δ **77.0** ppm for ^{13}C NMR and ^{31}P NMR spectra were taken ^{1}H decoupling.

2. Scheme of PL synthesis

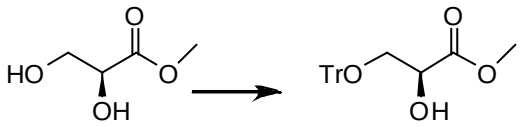
(S)-methyl 2,3-dihydroxypropanoate (9)[1]:



To make a solution of L-Serine (5.0 g 47.578 mmol), it was dissolved in 18M H_2SO_4 (5.28mL, 95.0 mmol), and a solution of sodium nitrite (65.9 g, 95.156 mmol, dissolved in 10mL H_2O) was added slowly (for over 30 min) to it at 0^0 C. The resulting solution was warmed to room temperature and stirred for 48h. The reaction mixture was concentrated under reduced pressure to get yellowish oil and diluted with 50ml EtOH. After that, insoluble inorganic salt is removed by Celite pad and concentrated under reduced pressure. The process was repeated 3 times to remove the residual H_2O and inorganic salt, resulting in the formation of crude S-2,3-dihydroxypropanoic acid as a colorless liquid. The crude material was dissolved in anhydrous MeOH (25mL) at 0^0 C with addition of thionly chloride (6.79 mL, 57.09 mmol) in a drop wise manner. The solution was stirred at 0^0 C for 30 min and at room temperature for 24 h. The solvent was removed under reduced pressure. The crude product was dissolved in methanol, followed by removal of the solvent under reduced pressure. This procedure was repeated three times. After that crude mixture was purified using flash column chromatography (SiO_2, 60-

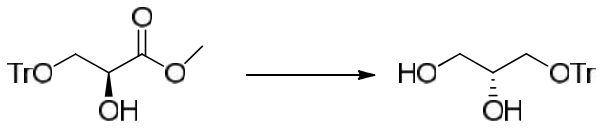
120mesh, 50% EtOAc in Hexane as elutant).The appearance of the purified compound ***9*** (4.45 g, 78 %) is like colorless oil. R_f = 0.5 (50% EtOAc in hexane). ^{1}H NMR(500 MHz, $CDCl_3$): δ 4.28-4.24(m, 1H), 3.89-3.86(m, 2H), 3.83-3.79(m, 1H), 3.78(s, 3H), 3.43(br,1H); ^{13}C NMR(100 MHz, $CDCl_3$)δ:173.5, 71.9, 64.1, 52.8; HRMS (ESI) m/z calculated for $C_4H_8O_4$ $[2M + Na]^+$ 263.074 found 263.074

(S)-methyl 2-hydroxy-3-(trityloxy)propanoate(10)[2]:



The purified product **9**, (4.2 g, 34.97 mmol) was dissolved in CH_2Cl_2 at 0°C in ice water bath after that TBAI (3.8g, 10.49 mmol) and Triethylamine(14.63ml, 104.9 mmol) were added to this solution. To this solution triphenylmethyl chloride (10.72 g, 38.47 mmol) was added at and whole solution was stirred for 18 h. After quenching the reaction with 0.5 M aqueous HCl, the resultant organic layer was washed with saturated $NaHCO_3$ and brine and the whole solution was dried over Na_2SO_4. The solvent was evaporated and the resultant residue was purified by chromatography (20% EtOAc in Hexane). The appearance of the purified compound ***10*** (10.89 g, 86%) is like a colorless oil. R_f = 0.5 (20% EtOAc in hexane). ^{1}H NMR (500 MHz, $CDCl_3$) : δ 7.41-7.40 (m, 6H), 7.31-7.28 (m, 6H) 7.25-7.21 (m, 3H), 4.26 (m, 1H), 3.76 (s, 3H) ,3.48-3.46 (dd, J = 4.5Hz, 3.5 Hz 1H), 3.37-3.34 (3.15 (br, 1H), 3.15(br, 1H) ; ^{13}C NMR (100 MHz) 173.64, 143.73, 128.76, 128.00, 127.28, 86.62, 70.92, 65.44, 52.58. Elemental Analysis calculated for ($C_{23}H_{22}O_4$): C, 76.22; H, 6.12; found: C,76.21; H, 6.11.

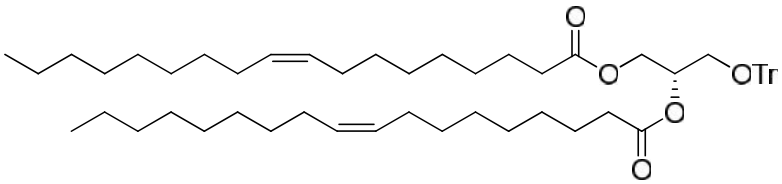
(R)-3-(trityloxy)propane-1,2-diol(11):



To the solution of (S)-2-hydroxy-3-(trityloxy) propanoic acid **10** (1.20 g, 3.31 mmol) in 50 ml of dry diethyl ether $LiAlH_4$ (0.314 g, 8.277 mmol) was added portion wisely at 0^{0}C. The reaction mixture was stirred at room temperature for another 1 hours. After that reaction was quenched carefully with 2ml of H_2O until no gas was released. 10ml 0.5 N HCl was added into the

reaction mixture. The product was filtrated by celite pad. The product was extracted with ethyl acetate (20mL x 3). The organic phase was washed with brine and dried over Na_2SO_4. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (50% EtOAc in Hexane) to yield the desired product **11**(1.040 g, 94% yield) as a colorless gummy substance, R_f = 0.4 (50% EtOAc in hexane).. $[\alpha]_D^{25}$ = -3.1 (c = 7, $CHCl_3$), ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.45-7.43(m, 6H), 7.34-7.31(m, 6H), 7.28-7.24(m, 3H), 3.88(dd, *J* = 5.5Hz, 4.4Hz, 1H), 3.60-3.72(m, 2H), 3.22-3.31(m, 2H), 2.50(br, 1H), 2.06 (br, 1H); ^{13}C NMR(100 MHz): δ 143.8, 128.8, 128.7, 128.1, 127.3, 87.1, 71.2, 65.2, 64.4; HRMS (ESI) m/z calculated for $C_{22}H_{22}O_3$ $[M + Na]^+$ 357.146 found 357.146

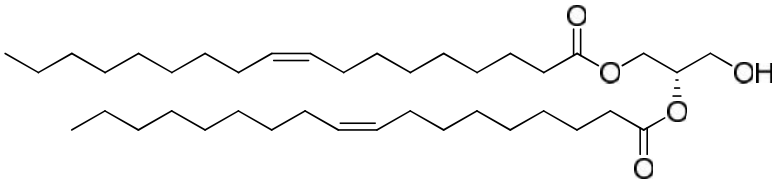
(Z)-(S)-3-(trityloxy)propane-1,2-diyl dioleate (12):



To a solution of **11**(5.5 g, 16.44 mmol), in 100mL CH_2Cl_2, then oleic acid(12.0, 37.82 mmol) was added at 0° C ice water bath in drop wise manner over 40 min. A solution of DCC(10.18 g, 49.34 mmol) in dry CH_2Cl_2(50 mL) was added to it followed with addition of DMAP(1 g, 8.22 mmol) and the resulting suspension was stirred for 18 h. The solid was removed by filtration through Celite, the filtrate was concentrated under reduced pressure, compound was purified by column chromatography (5 % EtOAc in Hexane) to yield the desired product **12** (13.63g, 96% yield) as a colorless oily substance, R_f = 0.5 (5% EtOAc in hexane). ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.42-7.21 (m, 6H) 7.30-7.21 (m, 9H), 5.37-5.32 (m, 4H), 5.28-5.23 (m, 1H), 4.35-4.32 (dd, *J* = 10Hz, 3.5Hz, 1H) 4.24-4.21 (dd, *J* = 6.5Hz, 5.5Hz, 1H), 3.25-3.20 (m, 2H), 2.34-2.31 (m, 2H), 2.29-2.20 (m, 2H), 2.06-1.98 (m, 8H), 1.64-1.60 (m, 2H), 1.56-1.53 (m, 2H), 1.26-1.25 (m, 40H), 0.92-0.83 (m, 6H); ^{13}C NMR (100 MHz): δ 173.5, 173.4, 143.7, 130.3, 130.1, 129.8, 129.0, 128.7, 128.5, 128.2, 128.0, 127.9, 127.4, 127.2, 125.9, 86.8, 70.5, 63.0, 62.3, 34.6, 34.6, 34.2, 32.7, 32.0, 31.6, 31.5, 30.3, 30.3, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.1, 27.3, 27.3, 27.3, 25.7, 25.1, 24.9, 22.8, 22.7, 14.2, 14.2;

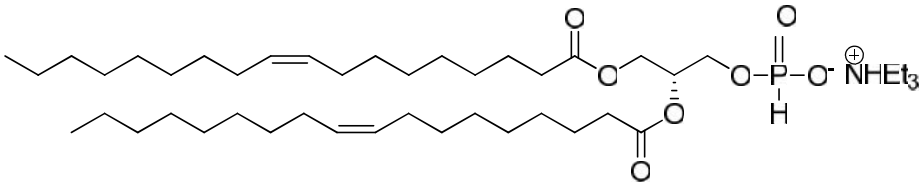
(MALDI-MS (ESI) m/z calculated for $C_{58}H_{86}O_5$ $[M + Na]^+$ 885.637 found 885.637. Elemental Analysis calculated for ($C_{58}H_{86}O_5$): C, 80.69; H, 10.04; found: C,80.65; H,10.06.

(Z)-(S)-3-hydroxypropane-1,2-diyl dioleate (13):



To a solution of **12**(13.00 g, 15.05 mmol) in 50 mL CH_2Cl_2, TFA (0.6mL, 8.09 mmol) was added drop wise at 0° C ice water bath over 5min and resulting solution was stirred for 4h. Solvent was removed under reduced pressure, compound was purified by column chromatography (5% EtOAc in Hexane) to yield the desired product **13** (9.16 g, 98% yield) as a colorless oily substance R_f = 0.5 (15% EtOAc in hexane). ^{1}H NMR(500 MHz, $CDCl_3$) : δ 5.39-5.32(m, 4H), 5.09-5.07(m, 1H), 4.33-4.30(m, 2H), 4.25-4.21(m, 1H), 3.73-3.72(m, 2H), 2.36-2.30(m, 4H), 2.07-1.99(m, 8H), 1.44-1.1.41(m, 4H), 1.37-1.25(m, 40), 0.89-0.83(m, 6H), ^{13}C NMR(100 MHz,$CDCl_3$) : δ 173.8, 173.5, 130.3, 130.1, 129.8, 72.3, 62.2, 61.7, 34.6, 34.4, 34.2, 33.9, 33.8, 32.0, 32.0, 31.7, 31.6, 31.5, 30.3, 29.9, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 29.2, 27.3, 27.3. (MALDI-MS (ESI) m/z calculated for $C_{39}H_{72}O_5$ $[M + Na]^+$ 643.527. found 643.527. Elemental Analysis calculated for ($C_{39}H_{72}O_5$): C, 75.43; H, 11.69; found: C, 75.42; H, 11.68.

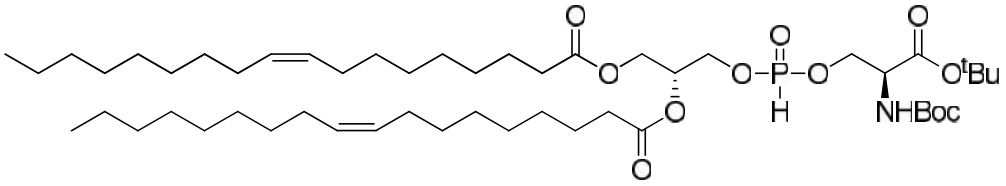
Triethylammonium (R)-2,3-bis (oleoyloxy)propyl phosphonate (6):



To a solution of **13**(3 g, 4.83 mmol) in dry pyridine Diphenyl phosphite(5.5mL, 28.98 mmol) was added drop wise over 2 min at 0° C ice water bath and resulting solution was stirred for 1h. After that 10mL of 1:1 triethyl amine–water mixture was added. The reaction mixture was stirred at room temperature for another 1hour, following removal of pyridine under reduced pressure. The crude product was dissolved in CH_2Cl_2; organic phase was washed with saturated solution of

$NaHCO_3$ (3 times), and next with brine finally dried over Na_2SO_4. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (95:5:0.5 $CHCl_3$-MeOH-NEt_3) to yield the desired product **6**(3.4 g, 90 % yield) as a colorless substance R_f = 0.4 (5% MeOH in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.59(br, 1H), 6.04(d, *J*=1.2Hz, 1H), 5.31(m, 4H), 5.30(m, 1H), 4.4-4.3(m, 1H), 4.2-4.1(m, 1H), 3.96-3.93(m, 2H), 3.03–2.98(m, 6H), 2.28-2.23(m, 4H), 2.02-2.00(m, 8H), 1.56(m, 4H), 1.30-1.23(m, 49H), 0.85-0.82(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 173.4, 173.0, 130.0, 129.8, 70.6, 62.7, 61.9, 45.6, 34.4,34.2,31.9,31.6, 29.8, 29.8, 29.7, 29.6, 29.4, 29.2, 27.3, 27.2, 25.7, 24.9, 22.7, 22.6, 14.1, 8.7; ^{31}P NMR(162 MHz, $CDCl_3$): δ 4.60; (MALDI-MS (ESI) m/z calculated for $C_{45}H_{88}NO_7P$ $[M + Na - NEt_3]^+$ 707.498 found 707.498 (In MALDI-MS counter ion triethyl amine is replaced by sodium ion) ; Elemental Analysis calculated for ($C_{45}H_{88}NO_7P$): C, 68.75; H, 11.28; N, 1.78; found: C, 68.78; H,11.26; N, 1.75.

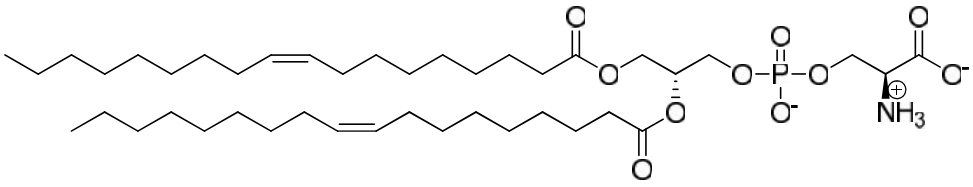
(R)-2,3-bis(oleoyloxy)popyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (20)[3]:



To a solution of compound **6**(150 mg, 0.187 mmol) and **(S)-tert-butyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate** (58 mg, 0.224 mmol) in dry pyridine, Pivaloyl chloride(0.150 mL, 1.12mmol) was added in drop wise manner at 0° C ice-water bath and resulting solution was stirred for 1h. Solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, organic phase was washed with saturated solution of $NaHCO_3$ (3 times), and brine and dried over Na_2SO_4. The resultant compound was purified by column chromatography (20% EtOAc in Hexane) to yield the desired product **20**(165mg, 95% yield) as a colorless oil, R_f = 0.5 (25% EtOAc in hexane). $[\alpha]_D^{25}$ = 7.56(c = 0.8, $CHCl_3$); ^{1}H NMR(500 MHz, $CDCl_3$): δ 6.19(d, *J* = 10Hz, 1H), 5.60-5.40(m, 1H), 5.36(m, 4H), 4.31-4.13(m, 8H), 2.33(m, 4H), 2.04-1.98(m, 8H), 1.61(m, 4H), 1.47-1.44(m, 18H), 1.29-1.18(m, 40), 1.87-1.85(m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.0, 174.0, 173.2, 130.3, 130.1, 129.8, 128.2, 83.2, 80.3, 73.0, 68.5, 65.1, 63.2, 63.0, 38.6, 34.2, 34.1, 34.0, 32.0, 31.6, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2,

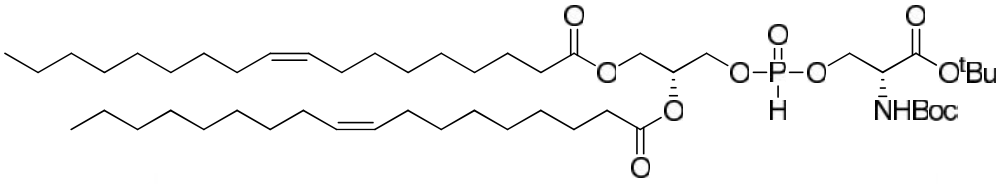
28.4, 28.1, 28.0, 27.3, 27.2, 27.1, 25.7, 25.0, 24.9, 22.8, 22.7, 18.7, 14.2, 14.2 ^{31}P NMR (202 MHz, $CDCl_3$) : δ 8.79. (MALDI-MS (ESI) m/z calculated for $C_{51}H_{94}NO_{11}P$ $[M + Na]^+$ 950.645 found 950.645; Elemental Analysis calculated for ($C_{51}H_{94}NO_{11}P$): C, 65.99; H, 10.21; N, 1.51; found: C, 65.65; H, 10.15; N, 1.50.

(S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1):



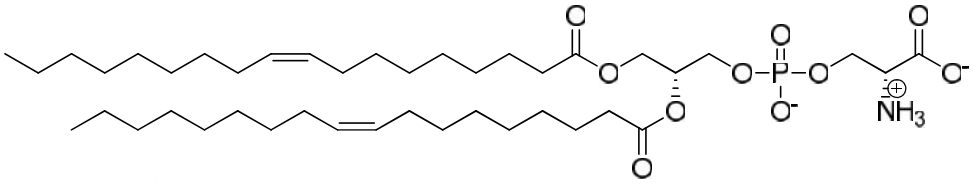
To a solution of **20**(150 mg, 0.161 mmol) in 95% pyridine-water (1.5 mL), iodine (10 mol%) was added at 0^0 C in ice-water bath.[4] Resulting solution was stirred for 3h. Solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2; organic phase was washed with saturated solution of Sodium thiosulfate (3 times). The solvent was removed under reduced pressure. Resultant compound was dissolved in 5mL CH_2Cl_2 at 0^0 C, 0.5mL TFA was added drop wise. The resulting solution was stirred at 0^0 C for 30min and subsequently at room temperature for 30 min. The solvent was removed under reduced pressure, washed with brine and dried over Na_2SO_4. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (9.5:0.5:0.5 = $CHCl_3$:MeOH:NEt_3) to yield the desired product **1**(122mg, 98% yield) as a colorless semisolid, R_f = 0.23 (5% MeOH in $CHCl_3$). $[\alpha]_D^{25}$ = 19.53 (c = 1.1, $CHCl_3$) which is similar to the natural PS + 20.04. (Optically purity of the compound 1 is 97.45%) ; ^{1}H NMR(500 MHz, $CDCl_3$): δ 8.28(br, 3H), 5.36-5.33(m, 4H), 4.9(m, 1H), 4.33-4.11(m, 7H), 2.35-2.30(m, 4H), 2.06-2.03(m, 8H), 1.60(m, 4H), 1.29-1.25(m, 40H), 0.88-0.85(m, 6H); ^{13}C NMR(125 MHz, $CDCl_3$): δ 173.5, 173.4, 173.1, 130.3, 130.1, 130.0, 129.8, 70.6, 69.6, 64.0, 62.7, 62.0, 45.6, 34.4, 34.2, 32.0, 31.6, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 27.3, 25.7, 25.0, 24.9, 22.7, 22.6, 14.1; ^{31}P NMR(162 MHz, $CDCl_3$): δ 0.50; (MALDI-MS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M + Na]^+$ 809.518 found 809.518; HRMS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M + K]^+$ 825.4920 found 825.4920; Elemental Analysis calculated for ($C_{42}H_{77}NO_{10}P$): C, 64.10; H, 9.86; N, 1.78; found: C, 64.08; H, 9.85; N, 1.77.

(R)-2,3-bis(oleoyloxy)popyl((R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (21) :



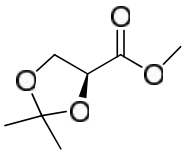
Procedure **20** was similarly followed again for the preparation of **21**. R_f = 0.5 (25% EtOAc in hexane) $[\alpha]_D^{25}$ = -9.21(c=1.1, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$): δ 6.15(d, 1H), 5.48-5.46(m, 1H), 5.35-5.3 (m, 4H) 4.39-4.09(m, 8H), 2.33-2.28(m, 4H), 2.06-1.97(m, 8H), 1.60-1.59(m, 4H), 1.53-1.50(m, 18H), 1.28-1.21(m, 40H), 0.94-0.93(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 184.1, 173.2, 173.1, 168.1, 130.3, 130.1, 129.8, 83.2, 83.1, 73.0, 63.2, 63.0, 60.5, 38.6, 34.2, 34.1, 34.0, 32.0, 31.6, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.4, 28.1, 28.0, 27.3, 27.2, 27.1,25.7, 25.0, 24.8, 22.8, 22.6, 14.2, 14.1; ^{31}P NMR(162 MHz, $CDCl_3$): δ 7.57. (MALDI-MS (ESI) m/z calculated for $C_{51}H_{94}NO_{11}P$ $[M+Na]^+$ 950.645 found 950.645; Elemental Analysis calculated for ($C_{51}H_{94}NO_{11}P$): C, 65.99; H, 10.21; N, 1.51; found: C, 65.65; H, 10.15; N, 1.50.

(R)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (3):



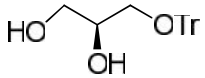
Procedure 1 was similarly followed again for the preparation of **3,** R_f = 0.23 (5% MeOH in $CHCl_3$). $[\alpha]_D^{25}$ = -11.23(c= 0.9, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$): δ 8.65(br, 3H), 5.34(m, 4H), 4.58-4.14(m, 8H), 2.34-2.31(m, 4H), 2.02-2.00(m, 8H), 1.61(m, 4H), 1.26-1.11(m, 40H), 0.88-0.86(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 178.1, 174.0, 130.3, 130.1, 129.8, 68.3, 65.0, 62.0, 53.2, 38.9, 34.1, 34.0, 33.9, 32.0, 31.9, 31.7, 31.6, 29.8, 29.7, 29.6, 29.4, 29.2, 29.1, 29.0, 28.3, 28.0, 27.9, 27.8, 27.7, 27.4, 27.3, 27.2, 27.1, 27.0, 26.9, 26.8, 24.9, 24.8, 22.7, 22.6, 14.1; ^{31}P NMR(202 MHz, $CDCl_3$): δ -1.34 (MALDI-MS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M+Na]^+$ 809.519 found 809.519; HRMS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M+K]^+$ 825.4920 found 825.4920; Elemental Analysis calculated for ($C_{42}H_{77}NO_{10}P$): C, 64.10; H, 9.86; N, 1.78; found: C, 64.08; H, 9.85; N, 1.77.

(S)-methyl 2,2-dioxolane-4-carboxylate(14):



To a solution of **9**(10 g, 83.26 mmol) and 2,2-dimethoxy propane(12.24 mL, 99.91 mmol) in acetone(50 mL), $HClO_4$ (0.522 ml, 8.32 mmol) was added at 0° C in ice bath. The mixture was stirred at room temperature for 2h, concentrated to remove the volatiles in vacuum, diluted with $NaHCO_3$(50 mL), and extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography (Hexane/EtOAc, 20:1) to yield compound **14** (12.26 g, 92%) as a colorless oil, R_f = 0.5 (10% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.54-4.51(m, 1H), 4.18-4.14(m, 1H),4.04-4.01(m, 1H), 3.70(s, 3H),1.42(s, 3H),1.33(s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 171.5, 111.3, 74.1, 67.2, 52.2, 25.5, 25.4; Elemental Analysis calculated for ($C_7H_{12}O_4$): C, 52.49; H, 7.55; found: C, 52.50; H, 7.53.

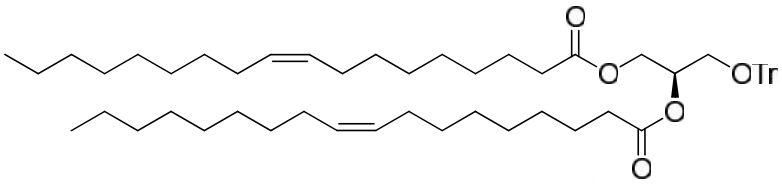
(S)-3-(trityloxy)propane-1,2-diol(17):



To the solution of **14** (5.00 g, 31.25 mmol) in 100 ml of dry diethyl ether, $LiAlH_4$ (3g, 78.12 mmol) was added portion wisely at 0° C. The reaction mixture was stirred at room temperature for another 1hour. After that, reaction was quenched carefully with 2ml of H_2O, until no gas was released. 10ml 0.5 N HCl was added into the reaction mixture. The product was filtered by celite pad. The product was extracted with ethyl acetate (50mL x 3). The organic phase was washed with brine and dried over Na_2SO_4. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (50% EtOAc in Hexane) to yield the desired product **15**(3.87 g, 94% yield) as a colorless substance. The crude product **15** (3.87 g, 29.28 mmol) was dissolved in CH_2Cl_2, TBAI (1.08g, 2.92 mmol) and Triethylamine(12.28 ml, 87.85 mmol) were added to this solution. To this solution triphenylmethyl chloride (8.97 g, 32.21 mmol) was added at 0^0 C in ice water bath and whole solution was stirred for 18h. After quenching the reaction with 0.5M aqueous HCl, the

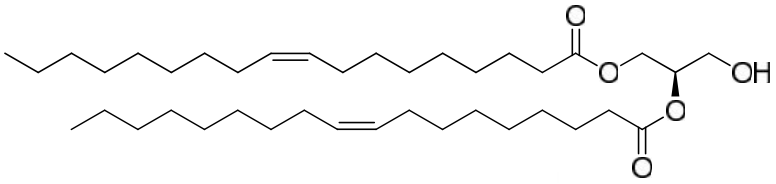
resultant organic layer was washed with saturated $NaHCO_3$ and brine. The whole solution was dried over Na_2SO_4. Solvent was evaporated and the resultant residue was purified by chromatography (20% EtOAc in Hexane) to yield product **16** (9.4 g, 86% yield). To a solution of compound **16**(3g, 8.01 mmol) in 50ml CH_3CN-H_2O (2:1), $SbCl_3$ (1.82g, 8.01 mmol) was added portion wisely at room temperature. Resultant solution was refluxed for 4h at 40-50^0 C. After quenching the reaction with saturated $NaHCO_3$ the product was concentrated by removing the solvent in vacuum. The product was extracted with ethyl acetated (50mL x 3). The organic phase was washed with brine and dried over Na_2SO_4. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography(50 % EtOAc in Hexane) to yield the desired product **17**(1.4g, 53% yield) as a colorless gummy substance, R_f = 0.5 (50% EtOAc in hexane); $[\alpha]_D^{25} = 3.8$(c = 2.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.15(m, 15H), 3.81(m, 1H), 3.64-3.61(m, 1H), 3.55-3.51(m, 1H), 3.22-3.16(m, 2H), 2.92(br, 1H), 2.60(br, 1H); ^{13}C NMR(125 MHz, $CDCl_3$): δ 143.7, 128.7, 127.9, 127.2, 86.9, 71.3, 65.0, 64.3; Elemental Analysis calculated for ($C_{22}H_{22}O_3$): C, 79.02; H, 6.63; found: C, 79.03; H, 6.61.

(Z)-(R)-3-(trityloxy)propane-1,2-diyl dioleate (18):



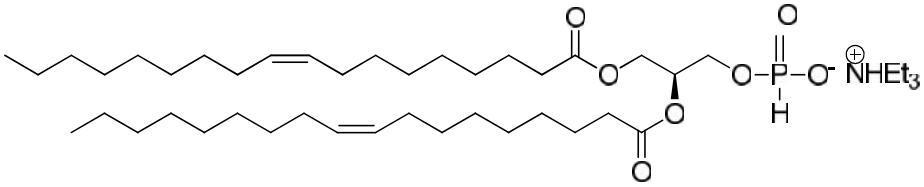
Procedure **12** was similarly followed again for the preparation of **18.** R_f = 0.5 (5% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCL_3$) : δ 7.44-7.23(m, 15H), 5.36(m, 4H), 5.27(m, 1H), 4.37-4.35(m, 1H), 4.26-4.25(m, 1H), 3.26-3.25(m, 2H), 2.35(m, 2H), 2.24-2.22(m, 2H), 2.05-2.03(m, 8H), 1.65-1.56(m, 2H), 1.28(m, 42H), 0.90-0.88(m, 6H); ^{13}C NMR (500 MHz, $CDCL_3$) : δ 173.42, 173.06, 143.76, 130.35, 130.14, 129.86, 128.77, 128.23,128.06,127.98, 127.25, 86.58, 70.60, 62.98, 622.41, 34.52, 34.23, 32.05, 31.67, 29.91, 29.87, 29.84, 29.80, 29.77, 29.67, 29.62, 29.46, 29.41, 29.36, 29.31, 29.26, 29.24, 27.37, 27.33, 25.80, 25.10, 24.97, 22.81, 22.71, 14.23; (MALDI-MS (ESI) m/z calculated for $C_{58}H_{86}O_5$ $[M + Na]^+$ 885.637 found 885.637; Elemental Analysis calculated for ($C_{58}H_{86}O_5$): C, 80.69; H, 10.04; found: C, 80.72; H, 10.05.

(Z)-(S)-3-hydroxypropane-1,2-diyl dioleate(19):



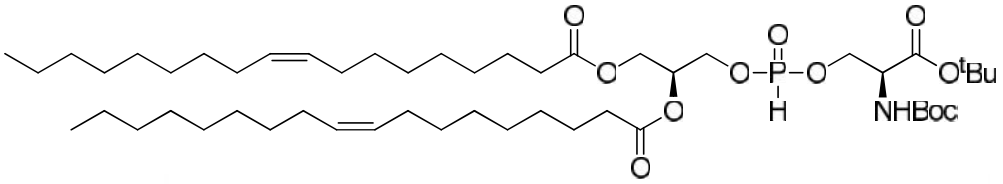
Procedure **13** was similarly followed again for the preparation of **19,** R_f = 0.5 (15% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36-5.31(m, 4H), 4.15-4.08(m, 4H), 3.73-3.72(m, 2H), 2.35-2.30(m, 4H), 2.05-1.98(m, 4H), 1.63-1.59(m, 4H), 1.29-1.25(m, 44H), 0.89-0.86(m, 6H); ^{13}C NMR(125 MHz, $CDCl_3$): δ 174.1, 130.3, 130.1, 129.8, 72.2, 68.5, 65.1, 34.4, 34.2, 32.0, 31.6, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.2, 27.3, 27.3, 25.7, 25.1, 24.8, 22.8, 22.7, 14.2, (MALDI-MS (ESI) m/z calculated for $C_{39}H_{72}O_5$ $[M + Na]^+$ 643.527 found 643.527.

(S)-2,3-bis(oleoyloxy)propyl phosphonate (8):



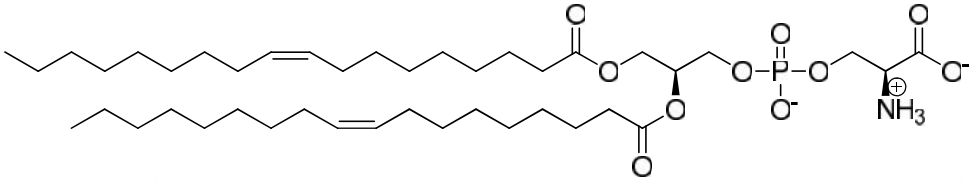
Procedure **6** was similarly followed again for the preparation of **8,** R_f = 0.4 (5% MeOH in $CHCl_3$); ^{1}H NMR(500 MHz, $CDCl_3$): δ 7.50(br, 1H), 6.04(s, 1H), 5.35-5.32(m, 4H), 5.31(m, 1H), 4.59-4.57(m, 1H), 4.23-4.16(m, 2H), 3.99-3.16(m, 1H), 3.05-3.01(m, 6H), 2.29-2.24(m, 4H), 2.04-1.94(m, 8H),1.57-1.56(m, 4H), 1.34-1.29(m, 49H), 0.89-0.86(m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.5, 130.3, 130.1, 129.8, 129.8, 69.8, 62.7, 62.2, 34.4, 34.2, 32.0, 31.6, 29.9, 29.8, 29.8, 29.7, 29.6, 29.4, 29.4, 29.3, 29.3, 27.3, 25.7, 24.9, 22.8, 22.6, 14.2, 14.1, 8.6. ^{31}P NMR(202 MHz, $CDCl_3$): δ 4.81, 4.13 (tautomerization),(MALDI-MS (ESI) m/z calculated for $C_{45}H_{88}NO_7P$ $[M + Na - NEt_3]^+$ 707.498 found 707.498 (In MALDI-MS counter ion triethyl amine is replaced by sodium ion); Elemental Analysis calculated for ($C_{45}H_{88}NO_7P$): C, 68.75; H, 11.28; N, 1.78; found: C, 68.78; H, 11.26; N, 1.75.

(S)-2,3-bis(oleoyloxy)propyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite(22):



Procedure 20 was similarly followed again for the preparation of 22, R_f = 0.5 (25% EtOAc in hexane); $[\alpha]_D^{25}$ = 9.53(c = 2.4, $CHCl_3$); ^{1}H NMR(500 MHz, $CDCl_3$) : δ 6.18(m, 1H), 5.48(m, 1H), 5.35-.5.29(m, 4H), 4.83(m, 1H), 4.40-4.10(m, 7H), 2.34-2.31(m, 4H), 2.11-1.94(m, 8H), 1.60(m, 4H), 1.46-1.33(m, 18H), 1.28-1.12(m, 40H) 0.87-0.85(m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.1, 173.2, 173.1, 168.1, 130.3, 130.1, 129.8, 83.2, 80.3, 73.0, 68.5, 66.1, 65.1, 63.2, 63.0, 61.6, 60.5, 54.5, 38.5, 34.2, 34.1, 34.0, 32.0, 31.6, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.4, 28.1, 28.0, 27.3, 27.1, 25.2, 24.9, 22.8, 22.69, 14.2, 14.1; ^{31}P NMR(202 MHz, $CDCl_3$): δ 8.43. (MALDI-MS (ESI) m/z calculated for $C_{51}H_{94}NO_{11}P$ $[M + Na]^+$ 950.645 found 950.645; Elemental Analysis calculated for ($C_{51}H_{94}NO_{11}P$): C, 65.99; H, 10.21; N, 1.51; found: C, 65.65; H,10.15; N, 1.50.

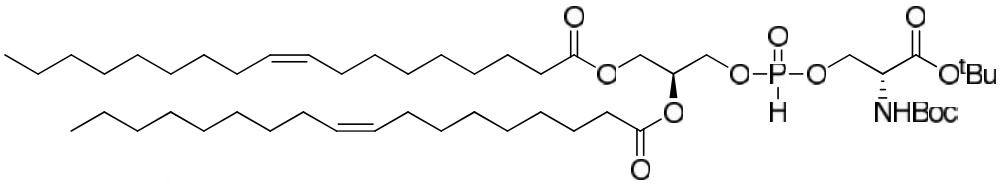
(S)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl) phosphate(2):



Procedure 1 was similarly followed again for the preparation of **2**,R_f = 0.23 (5% MeOH in $CHCl_3$); $[\alpha]_D^{25}$ = 11.59(c = 1.6, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$): δ 8.26-8.18(br, 3H), 5.35-5.32(m, 4H), 4.56-4.08(m, 8H), 2.34-2.31(m, 4H), 2.04-1.99(m, 8H), 1.59(m, 4H), 1.28-1.16(m, 40), 0.87-0.85(m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) : δ 178.1, 174.1, 130.3, 130.1, 129.8, 68.5, 65.1, 39.0, 34.2, 33.9, 32.0, 31.8, 31.6, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.7, 27.8, 27.3, 27.2, 27.1, 26.9, 26.8, 25.7, 24.9, 22.7, 22.6, 14.1. ^{31}P NMR (202 MHz, $CDCl_3$) : δ -1.55.; (MALDI-MS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M + Na]^+$ 809.518 found 809.518; HRMS (ESI) m/z

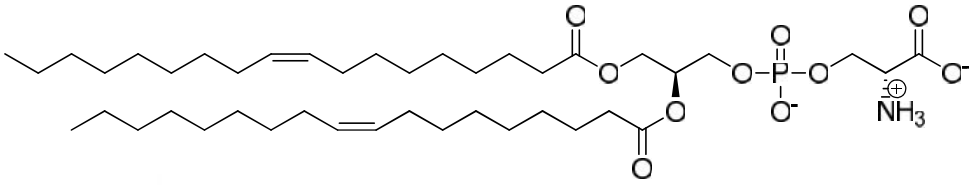
calculated for $C_{42}H_{77}NO_{10}P$ $[M+K]^+$ 825.4920 found 825.4920; Elemental Analysis calculated: C, 64.09; H, 9.86; N, 1.78; found: C, 64.08; H, 9.85; N, 1.77.

(S)-2,3-bis(oleoyloxy)propyl(R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (23):



Procedure **20** was similarly followed again for the preparation of **23**,R_f = 0.5 (25% EtOAc in hexane); $[\alpha]_D^{25}$= -7.29(c = 5.8, $CHCl_3$); ^{1}H NMR(500 MHz, $CDCl_3$) : δ 6.12(m, 1H), 5.33-5.31(m, 4H), 5.27(m, 1H), 4.40-4.23(m, 8H), 2.28(m, 4H), 1.97-1.94(m, 8H),1.62-1.61(m, 4H), 1.40-1.38(m, 18H), 1.19-1.12(m, 40H), 0.82-0.81(m, 6H); ^{13}C NMR(125 MHz, $CDCl_3$) : δ 185.2, 173.9, 174.0, 168.9, 129.9, 129.7, 82.6, 80.0, 64.9, 64.5, 53.7, 40.1, 38.8, 38.5, 34.9, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 28.2, 27.9, 27.2, 27.1, 26.9, 26.8, 26.6, 26.4, 24.8, 22.6, 14.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 8.17. (MALDI-MS (ESI) m/z calculated for $C_{51}H_{94}NO_{11}P$ $[M+Na]^+$ 950.645 found 950.645; Elemental Analysis calculated for ($C_{51}H_{94}NO_{11}P$): C, 65.99; H, 10.21; N, 1.51; found: C, 65.65; H, 10.15; N, 1.50.

(R)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl)phosphate(4):

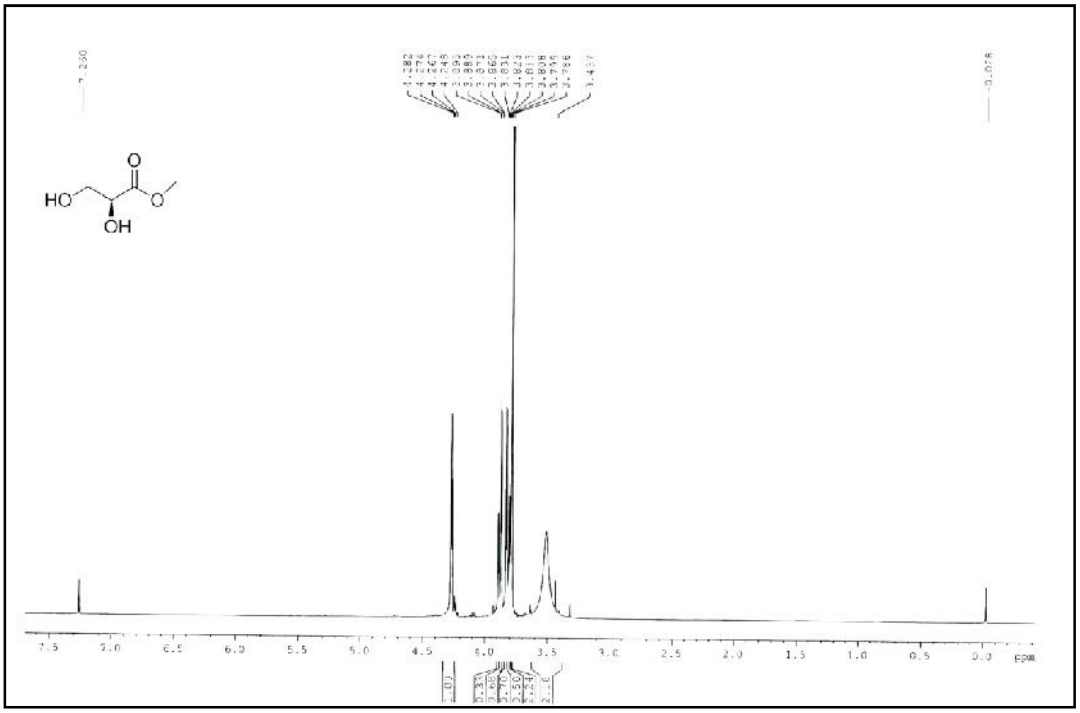


Procedure **1** was similarly followed again for the preparation of **4**, R_f = 0.23 (5% MeOH in $CHCl_3$); $[\alpha]_D^{25}$= -18.96 (c = 5.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) : δ 8.43(br, 3H), 5.31(m, 4H), 4.54-4.12(m, 8H), 2.33-2.27(m, 4H), 2.01-1.99(m, 8H), 1.57(m, 4H), 1.57-1.14(m, 40H), 0.86(m, 6H); ^{13}C NMR (125MHz, $CDCl_3$) : δ 173.5, 171.2, 130.3, 130.0, 129.7, 62.8, 60.4, 34.0, 31.9, 31.5, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 29.0, 27.7, 27.2, 25.6, 24.8, 22.7, 22.6, 21.0, 14.2, 14.1,; ^{31}P NMR (202 MHz, $CDCl_3$) : δ -1.18; (MALDI-MS (ESI) m/z calculated for $C_{42}H_{77}NO_{10}P$ $[M+Na]^+$ 809.518 found 809.518; HRMS (ESI) m/z calculated for

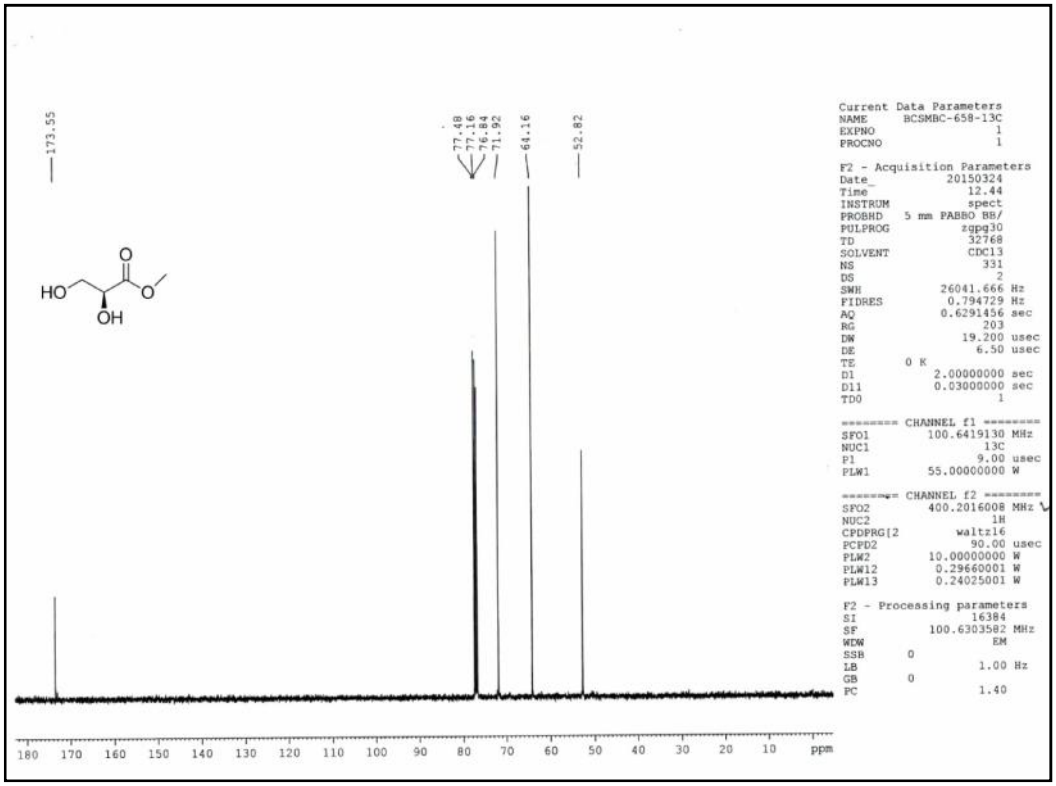
$C_{42}H_{77}NO_{10}P$ $[M + K]^+$ 825.4920 found 825.4920; Elemental Analysis calculated: C, 64.09; H, 9.86; N, 1.78; found: C, 64.08; H, 9.85; N, 1.77.

3. ^{1}H NMR, ^{31}P NMR and ^{13}C NMR,MALDI-MS Spectra:

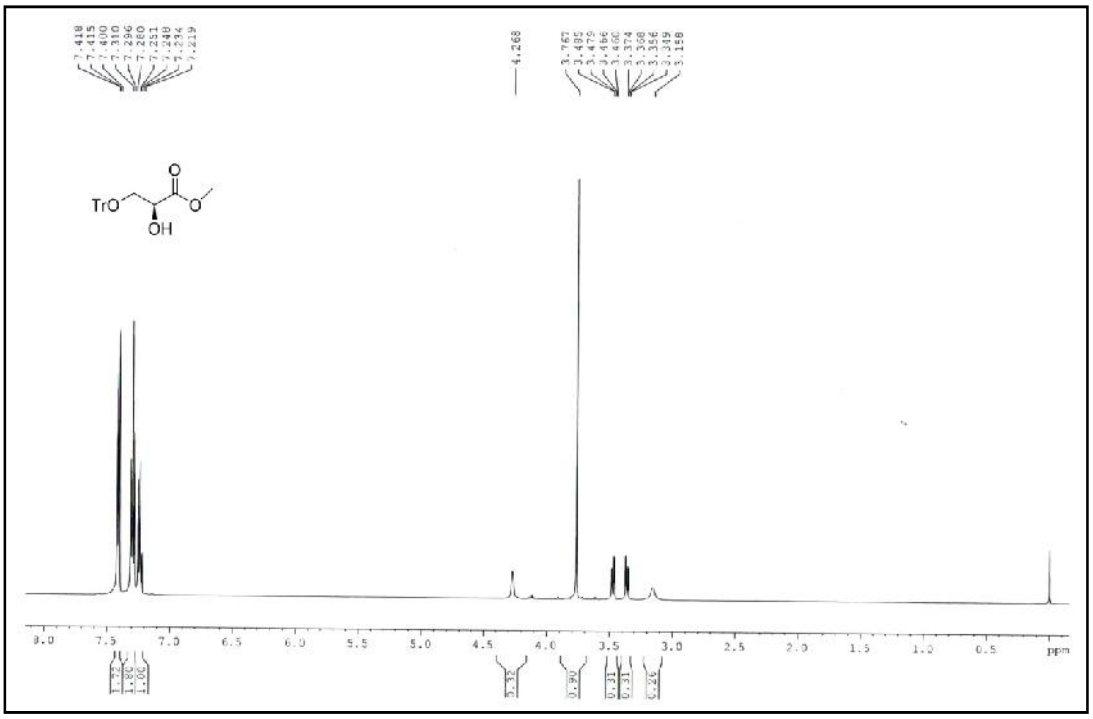
^{1}H NMR spectra of (S)-methyl 2,3-dihydroxypropanoate (9)



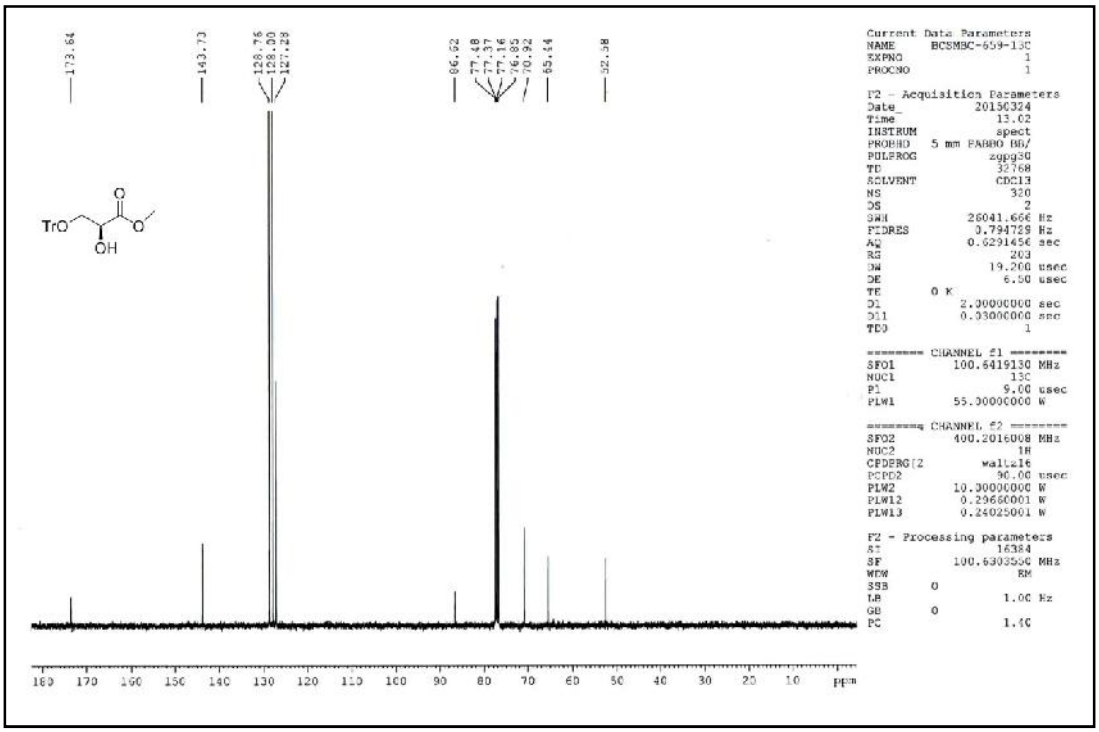
^{13}C NMR Spectra of (S)-methyl 2,3-dihydroxypropanoate (9)



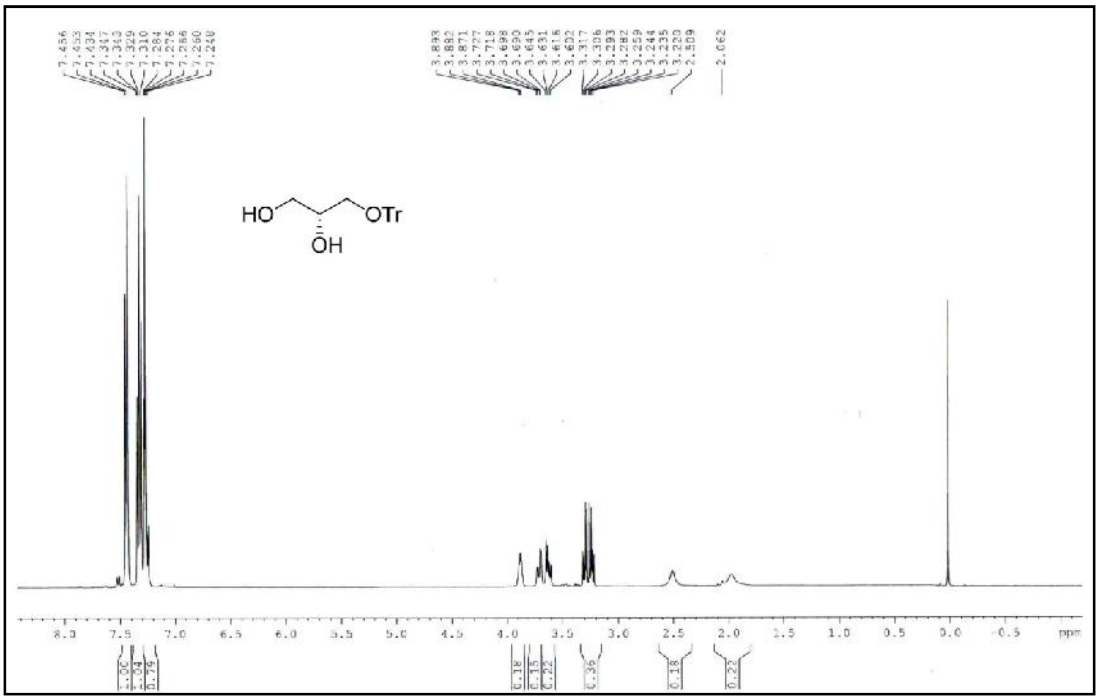
^{1}H NMR spectra of (S)-methyl 2-hydroxy-3-(trityloxy)propanoate (10)



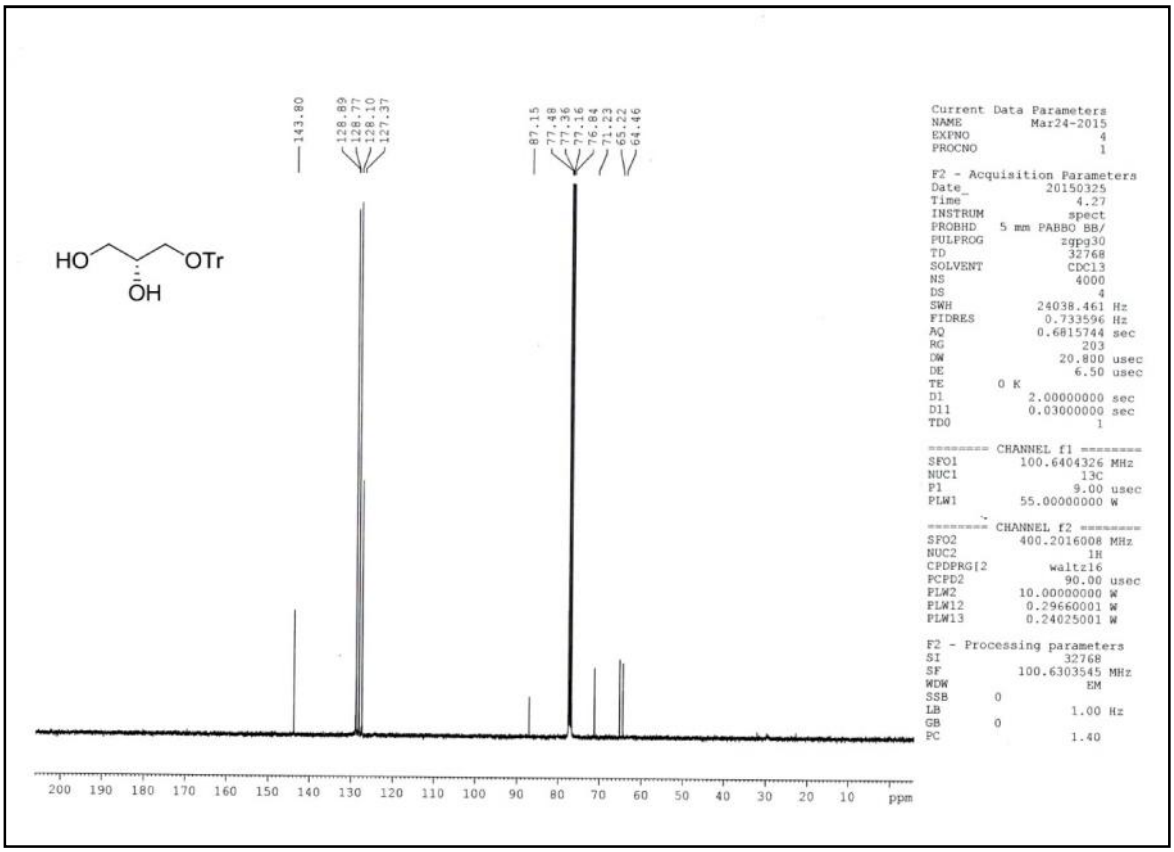
^{13}C NMR Spectra of compound (S)-methyl 2-hydroxy-3-(trityloxy)propanoate (10)



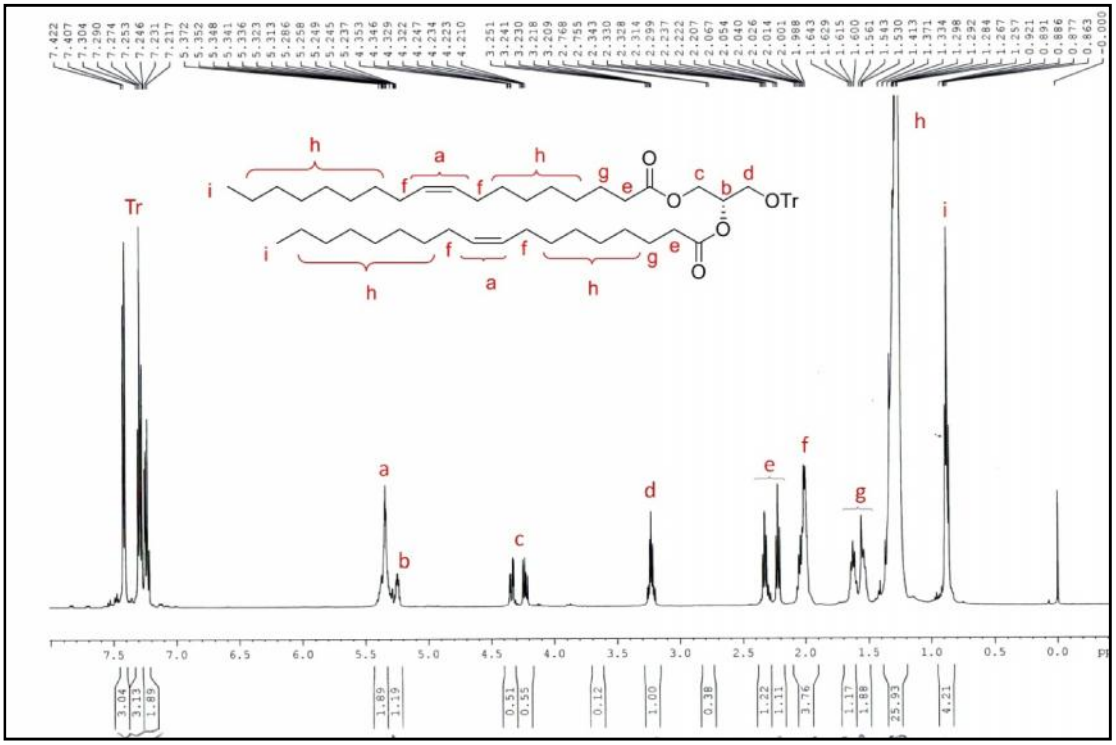
[1]H NMR spectra of (R)-3-(trityloxy)propane-1,2-diol(11)



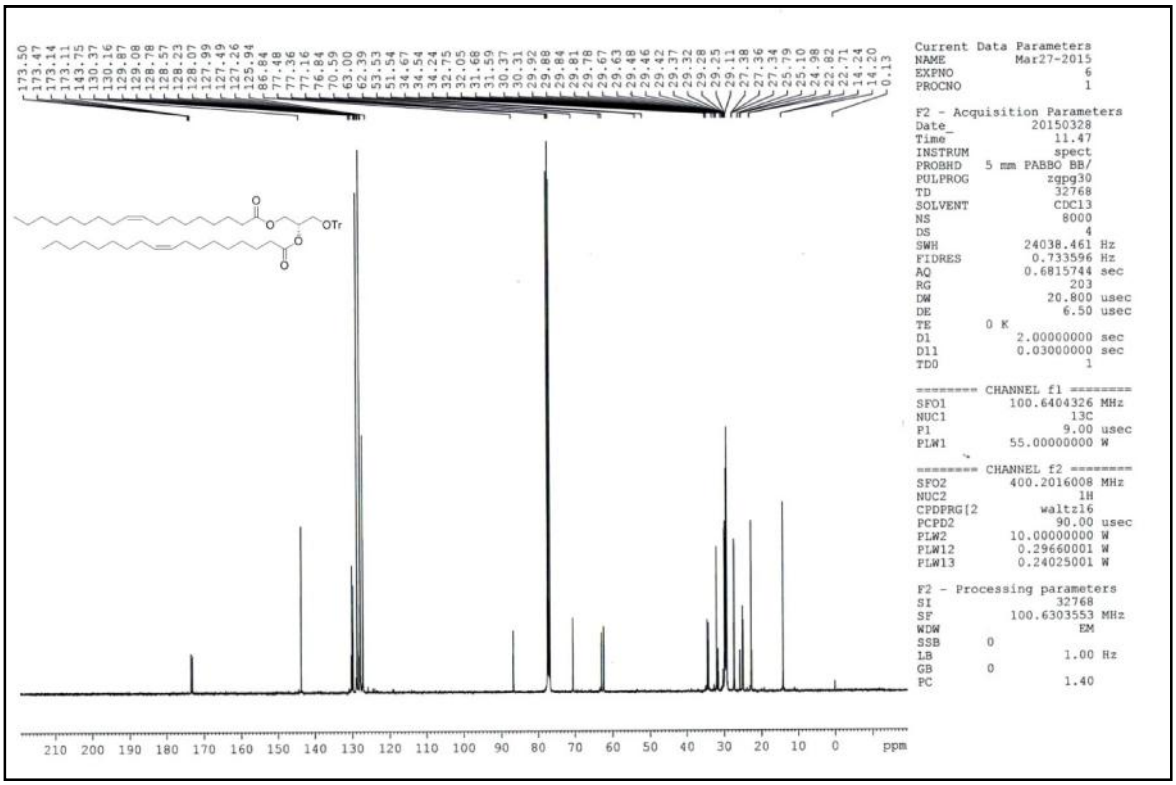
[13]C NMR Spectra of (R)-3-(trityloxy)propane-1,2-diol (11)



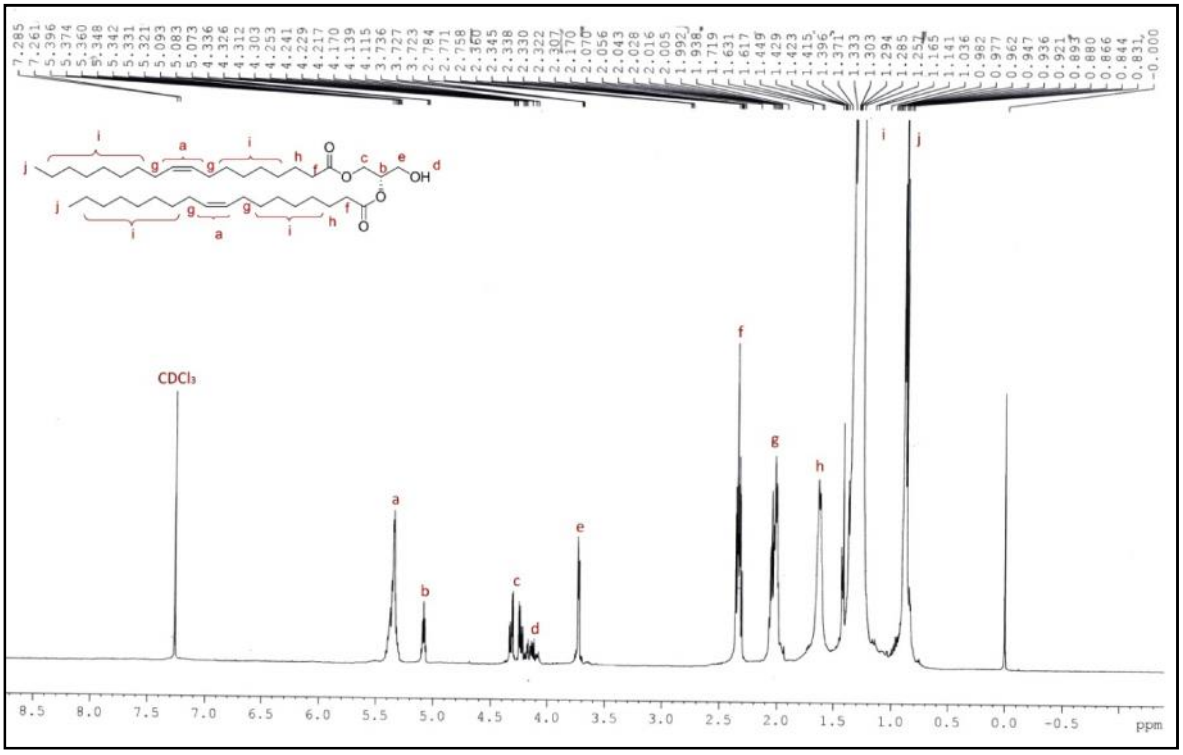
^{1}H NMR Spectra of (Z)-(S)-3-(trityloxy)propane-1,2-diyl dioleate (12)



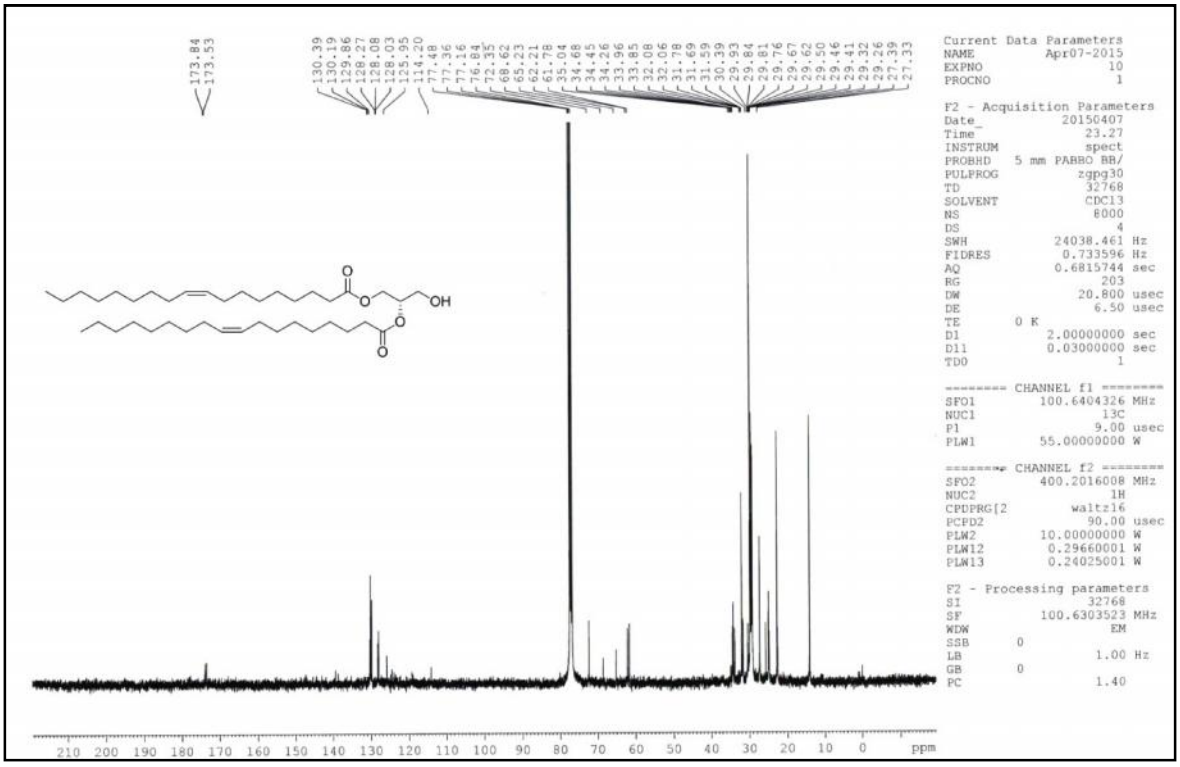
^{13}C NMR Spectra of (Z)-(S)-3-(trityloxy)propane-1,2-diyl dioleate (12)



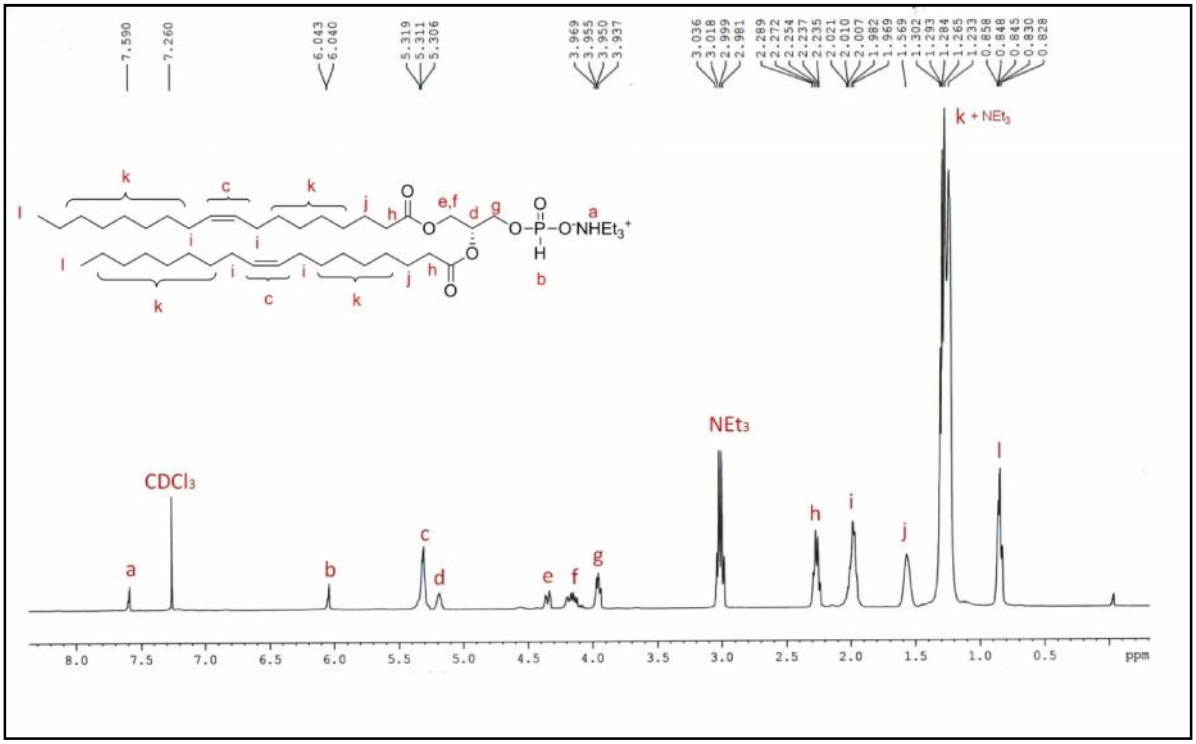
[1]H NMR Spectra of (Z)-(S)-3-hydroxypropane-1,2-diyl dioleate (13)



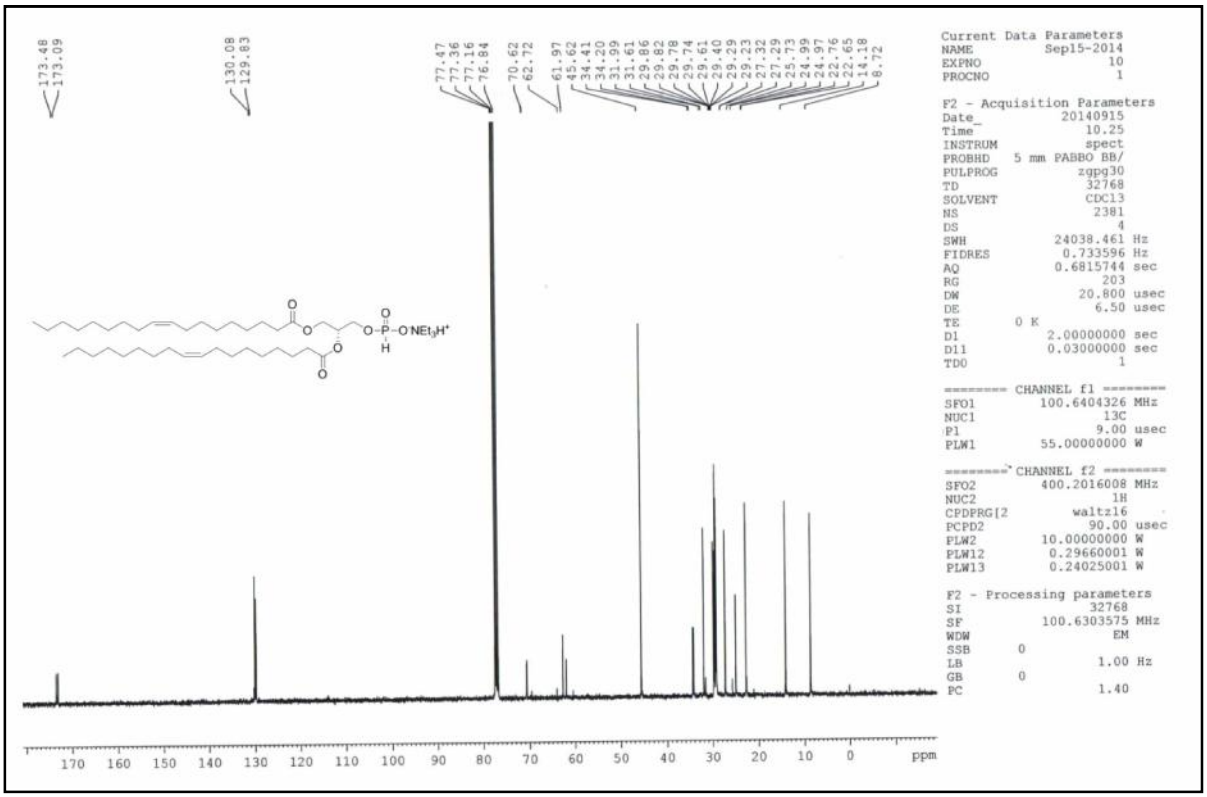
[13]C NMR Spectra of (Z)-(S)-3-hydroxypropane-1,2-diyl dioleate (13)



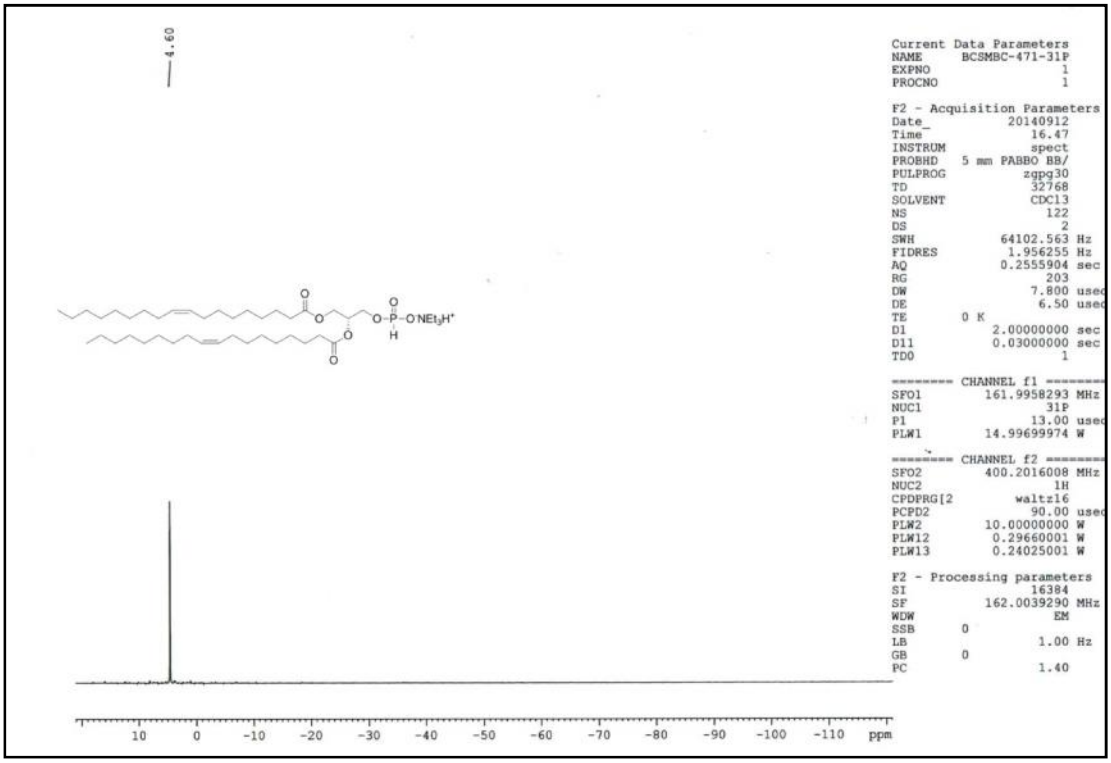
[1]H NMR Spectra of Triethylammonium (R)-2,3-bis (oleoyloxy)propyl phosphonate (6)



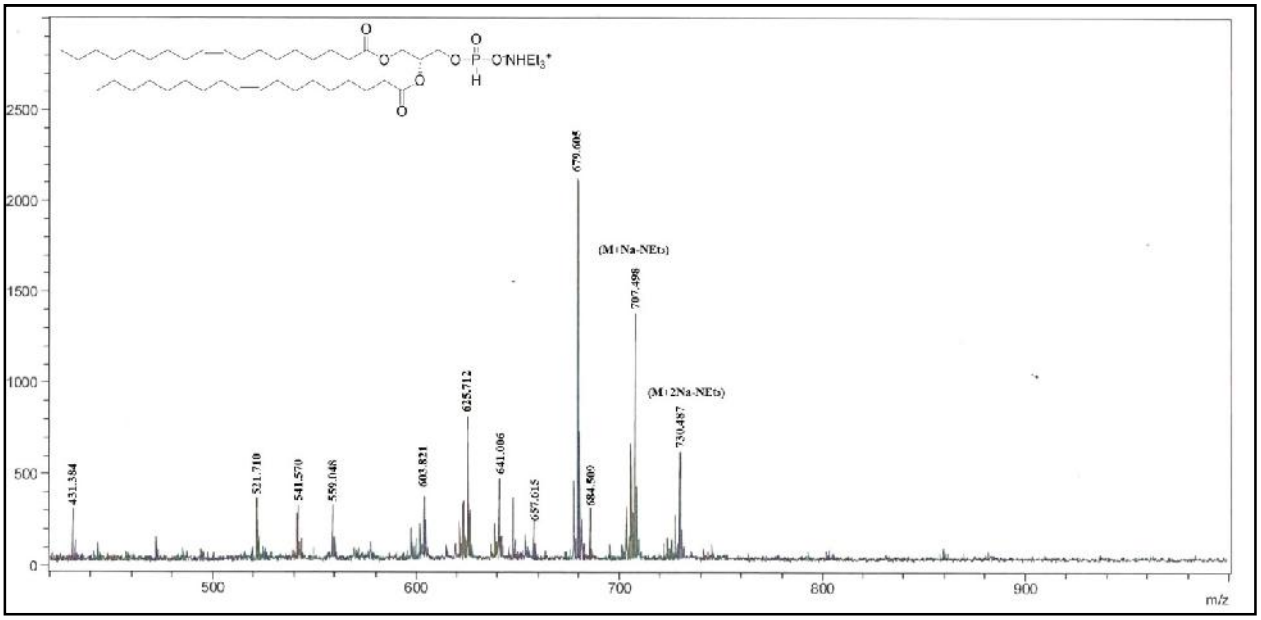
[13]C NMR Spectra of Triethylammonium (R)-2,3-bis (oleoyloxy)propyl phosphonate (6)



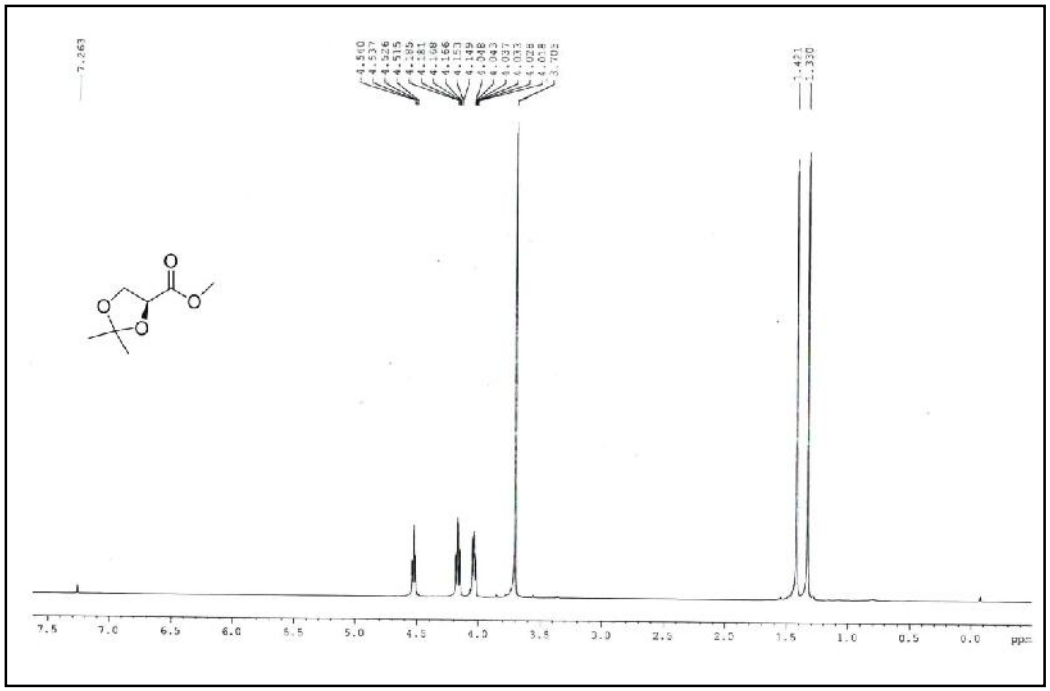
^{31}P NMR Spectra of Triethylammonium (R)-2,3-bis (oleoyloxy)propyl phosphonate (6)



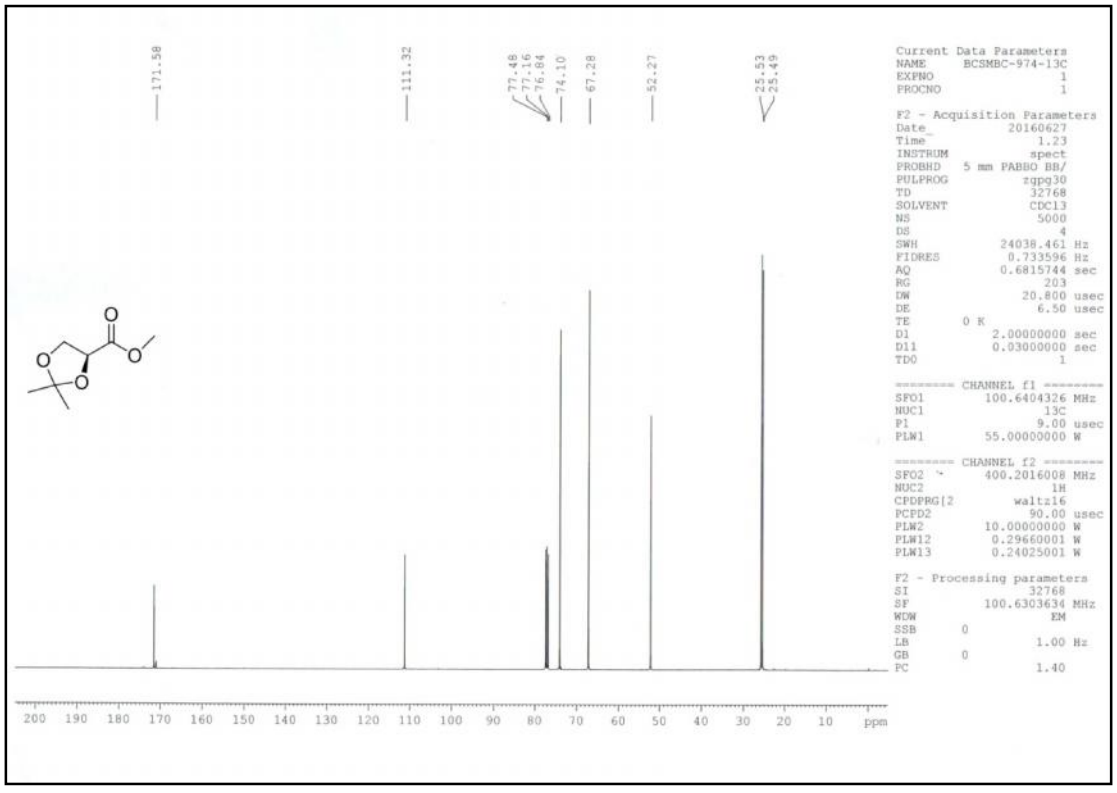
MALDI-MS of Triethylammonium (R)-2,3-bis (oleoyloxy)propyl phosphonate (6)



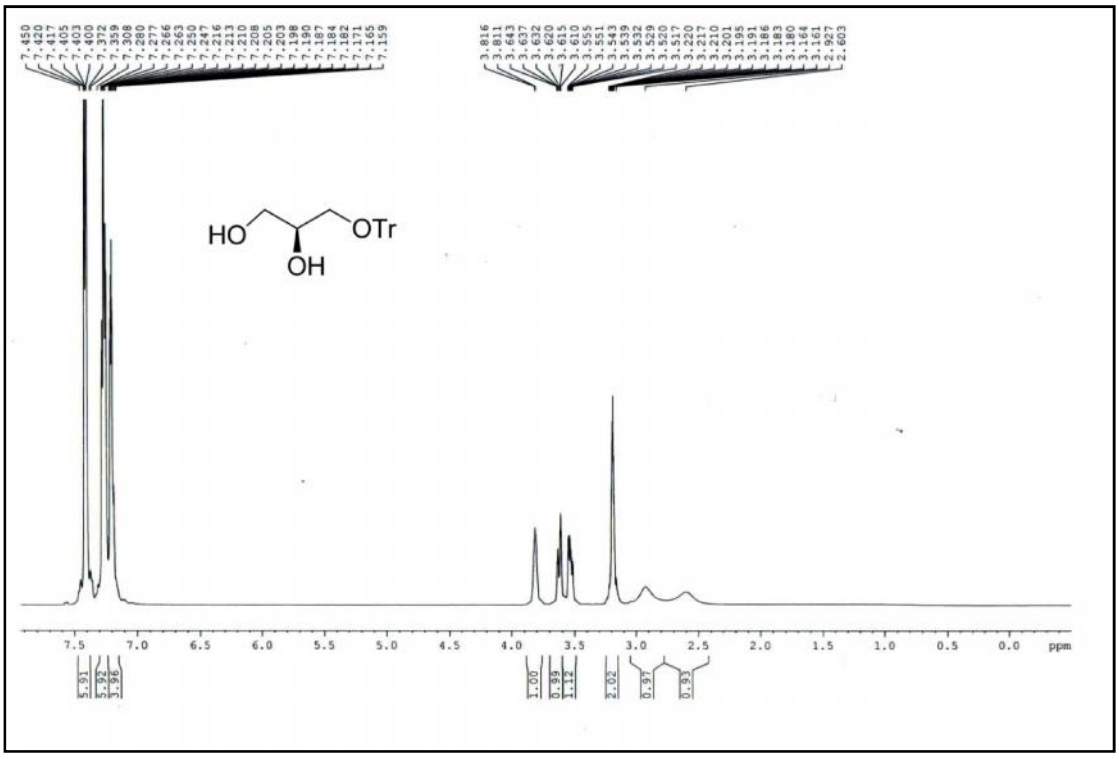
^{1}H NMR Spectra of (S)-methyl 2,2-dioxolane-4-carboxylate(14)



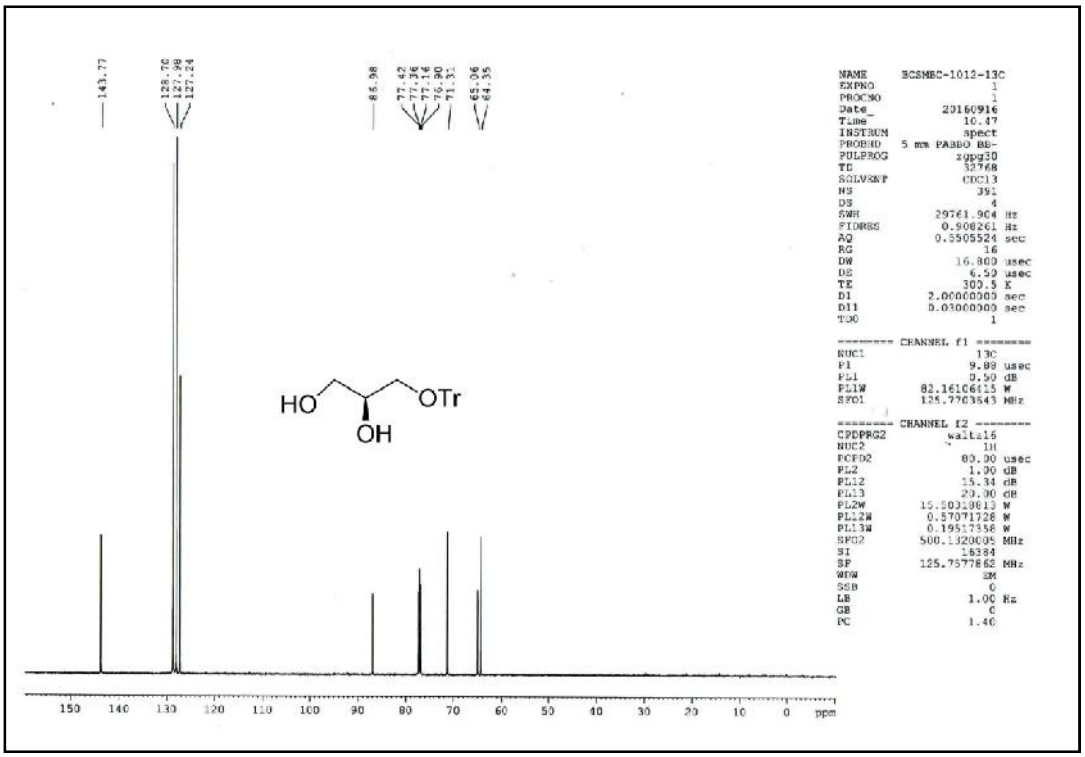
^{13}C NMR Spectra of (S)-methyl 2,2-dioxolane-4-carboxylate(14)



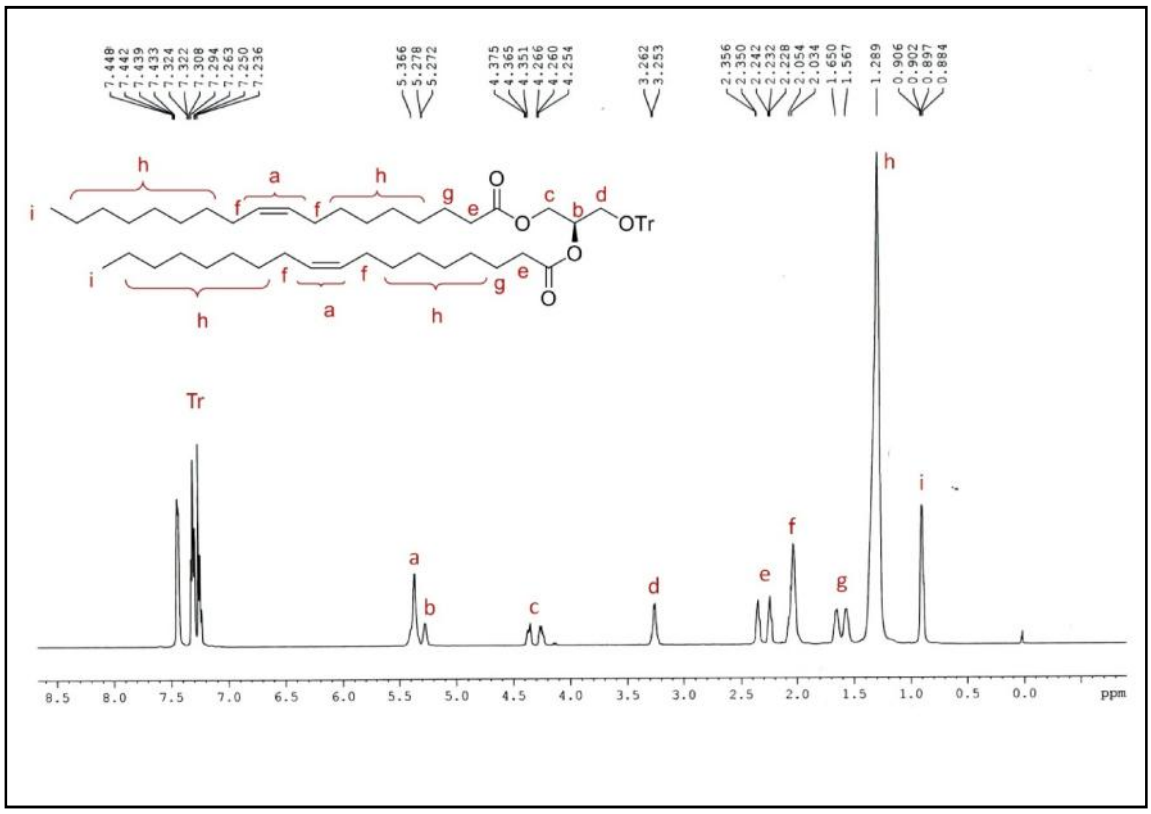
^{1}H NMR Spectra of (S)-3-(trityloxy)propane-1,2-diol (17)



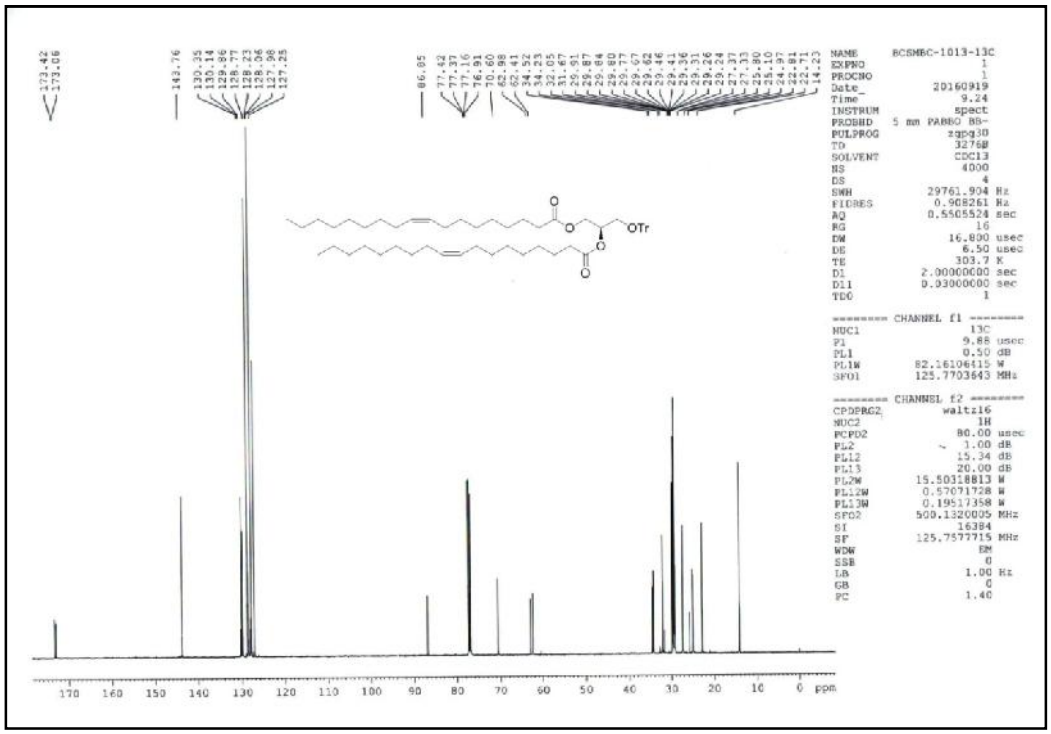
^{13}C NMR Spectra of (S)-3-(trityloxy)propane-1,2-diol(17):



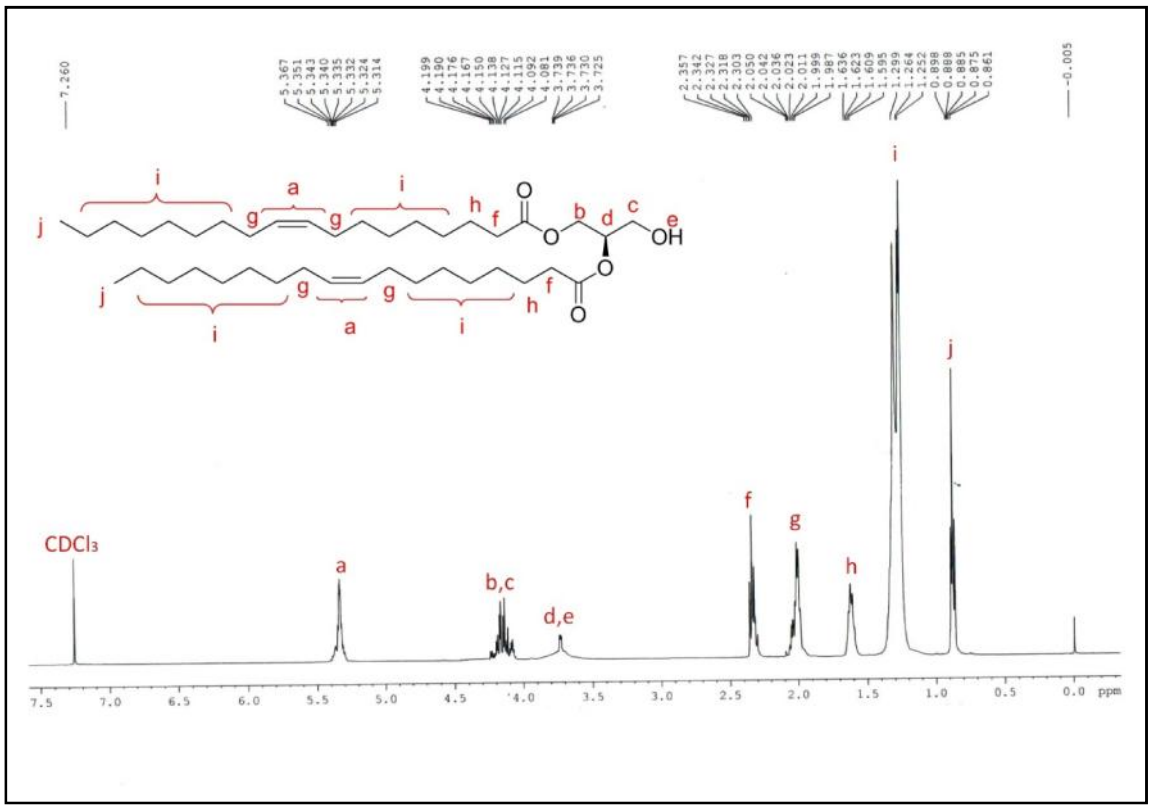
^{1}H NMR Spectra of (Z)-(R)-3-(trityloxy)propane-1,2-diyl dioleate (18)



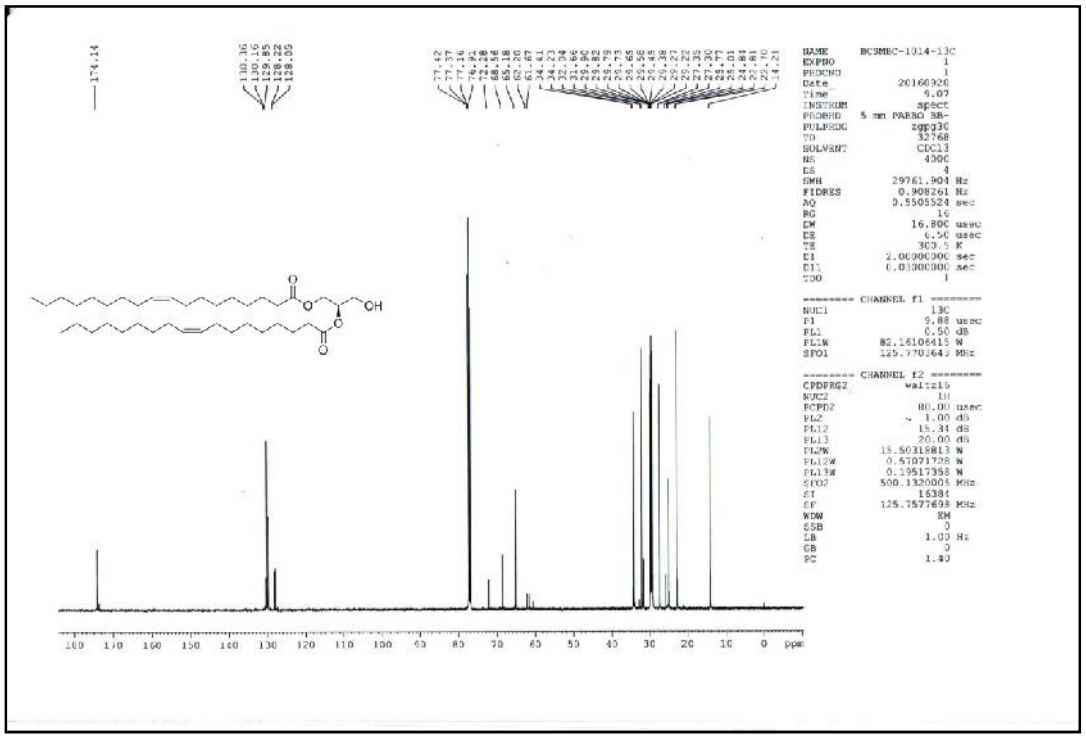
^{13}C NMR Spectra of (Z)-(R)-3-(trityloxy)propane-1,2-diyl dioleate (18)



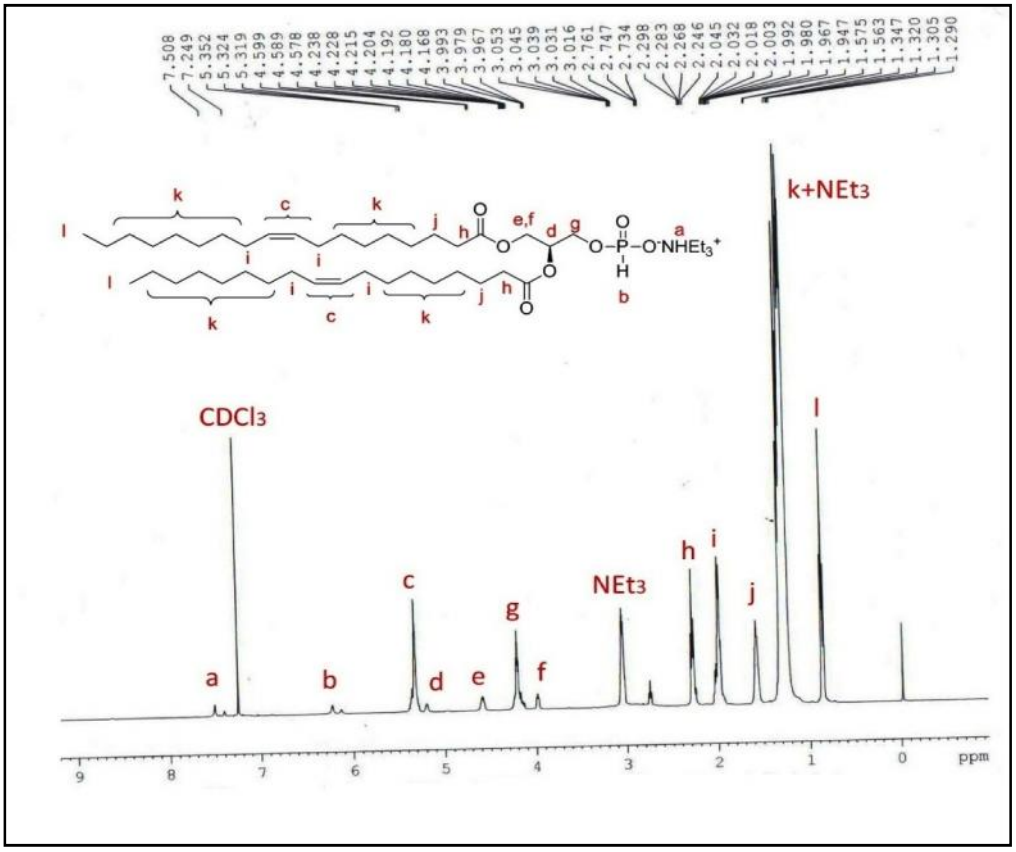
^{1}H NMR Spectra of (Z)-(S)-3-hydroxypropane-1,2-diyl dioleate(19)



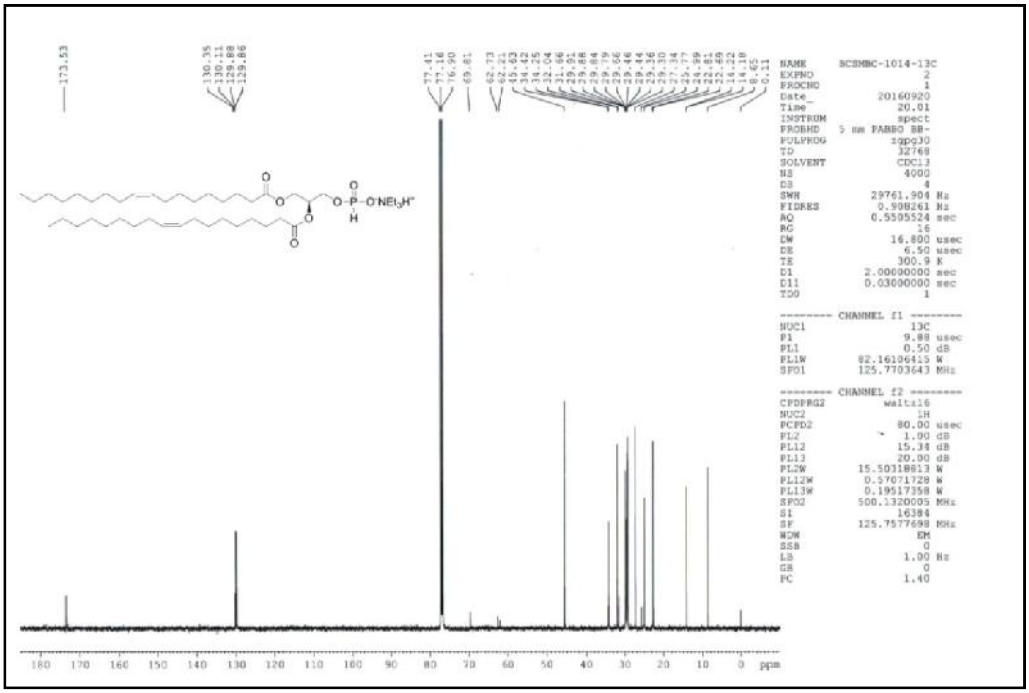
^{13}C NMR Spectra of (Z)-(S)-3-hydroxypropane-1,2-diyl dioleate(19)



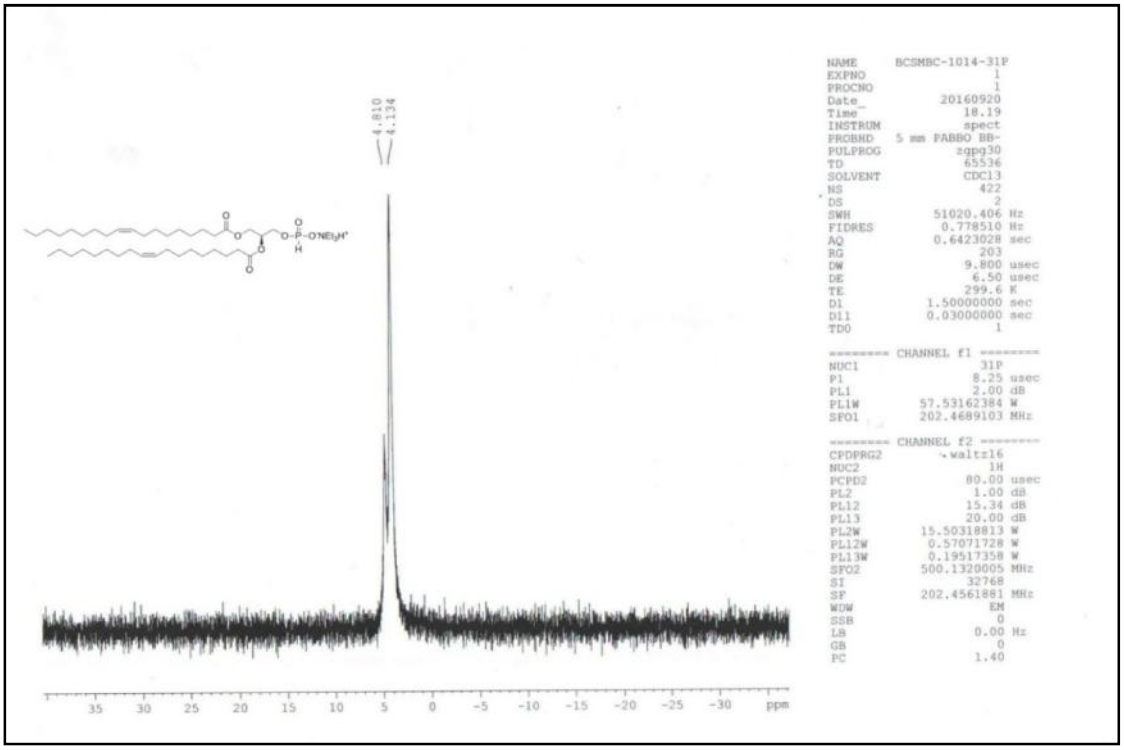
^{1}H NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl phosphonate (8)



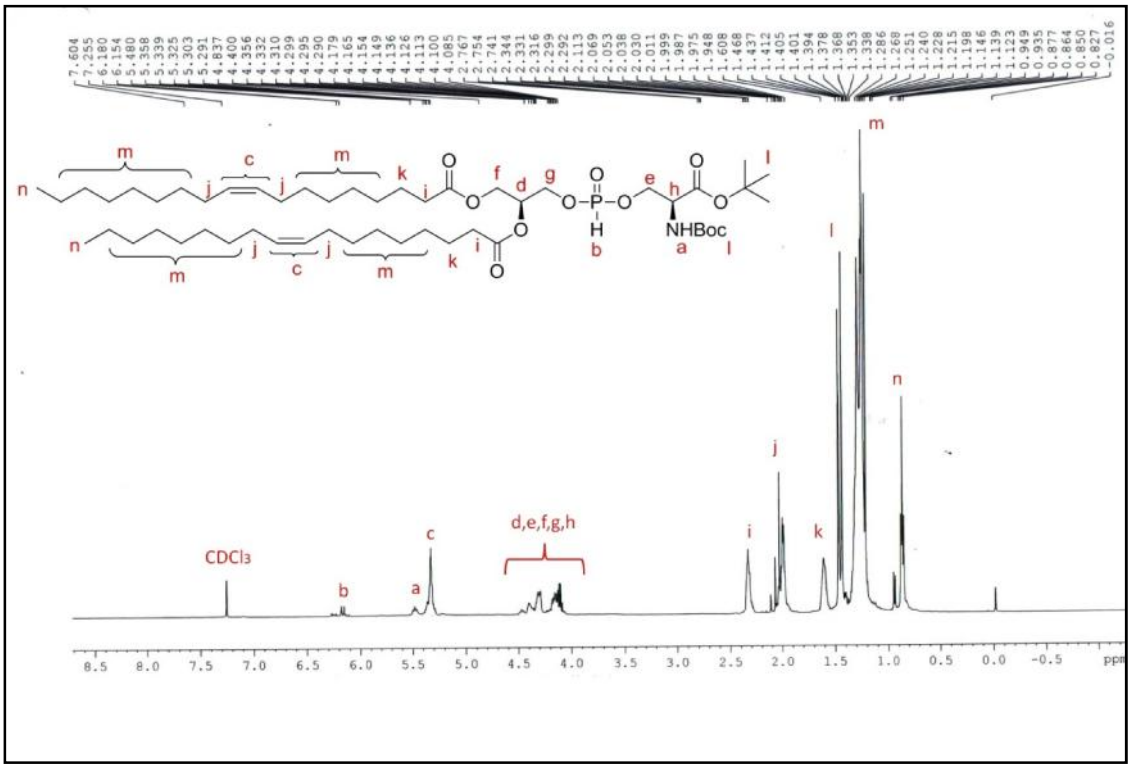
^{13}C NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl phosphonate (8)



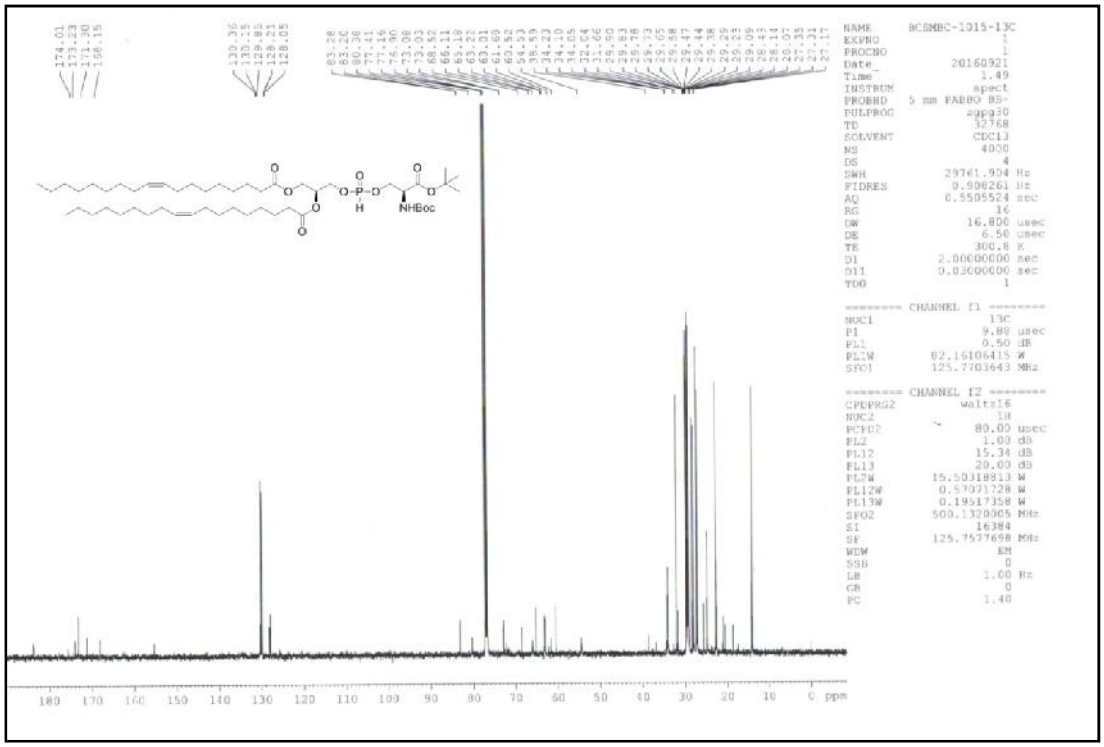
^{31}P NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl phosphonate (8)



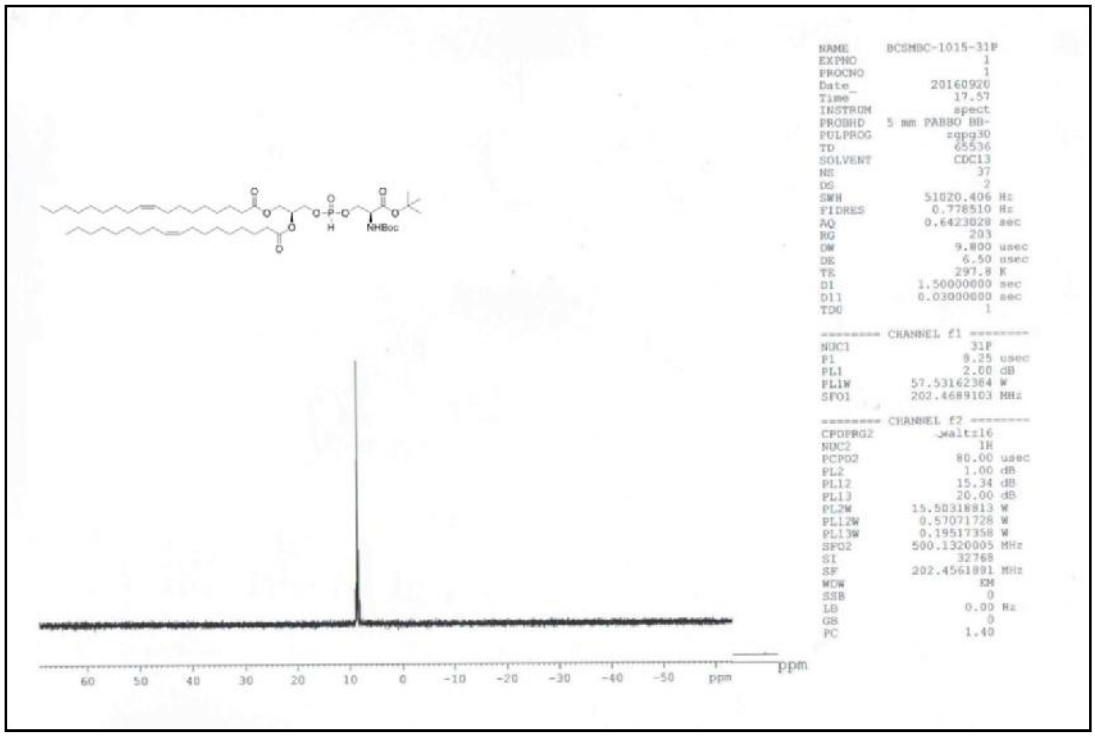
^{1}H NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite(22)



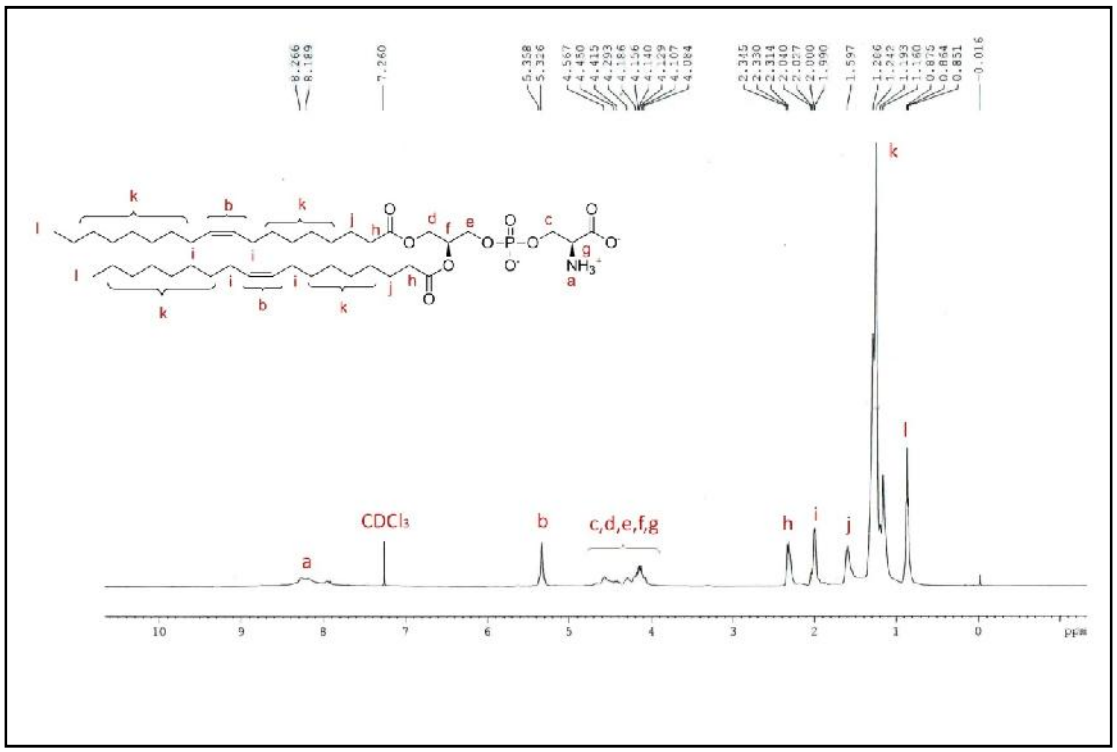
^{13}C NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite(22)



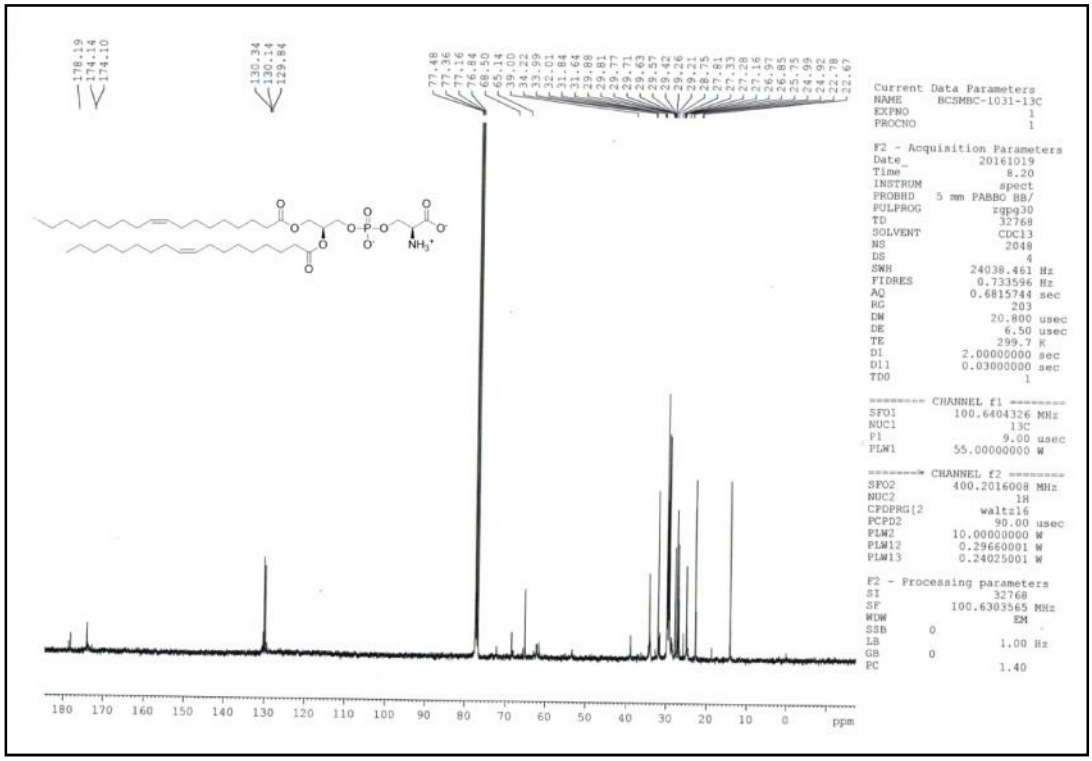
^{31}P NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite(22)



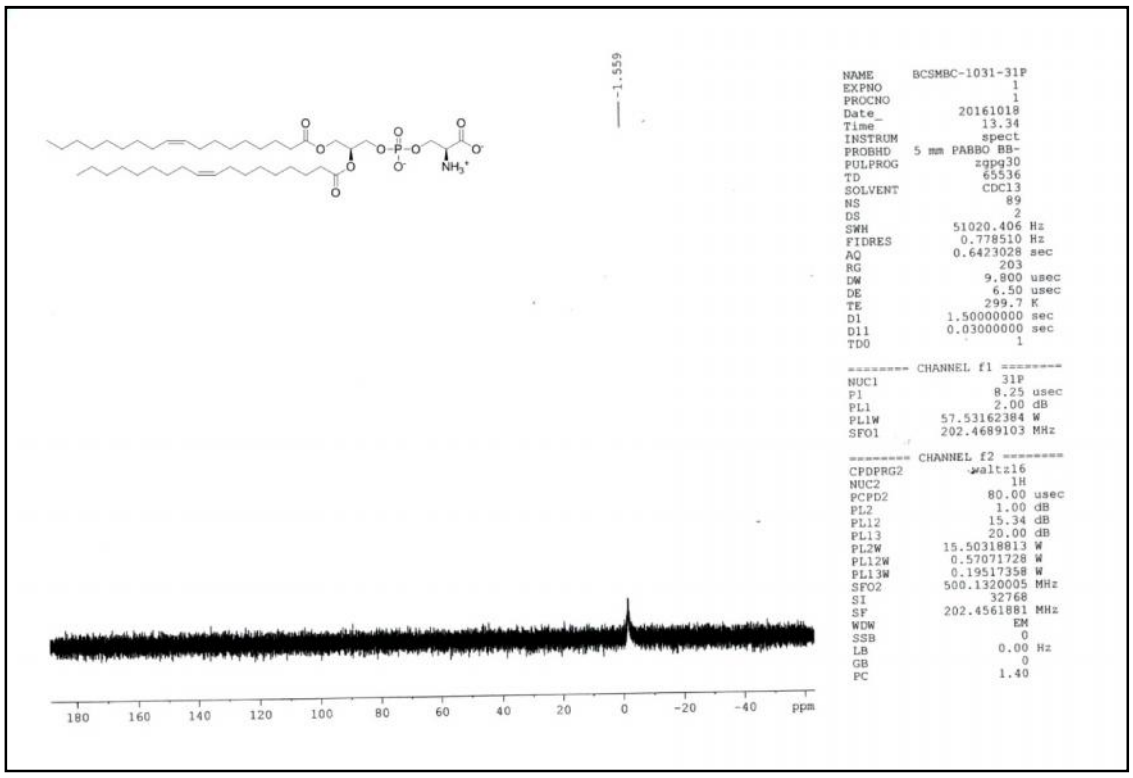
^{1}H NMR Spectra of (S)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl) phosphate(2)



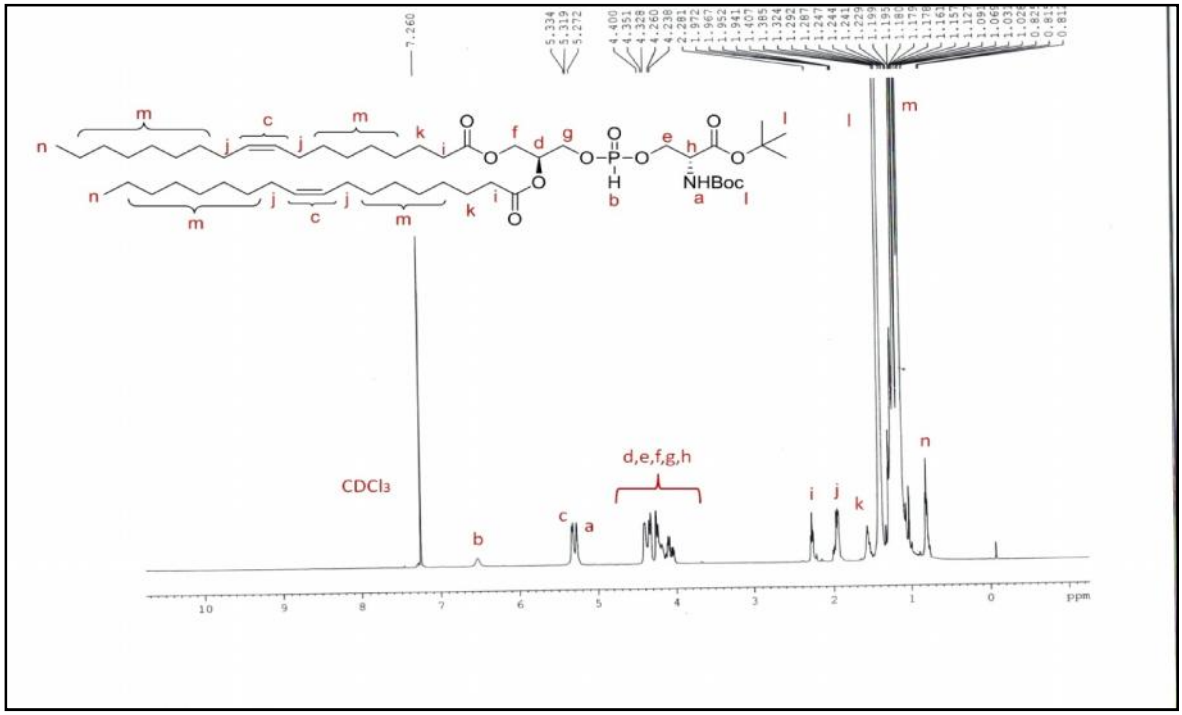
^{13}C NMR Spectra of (S)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl) phosphate(2)



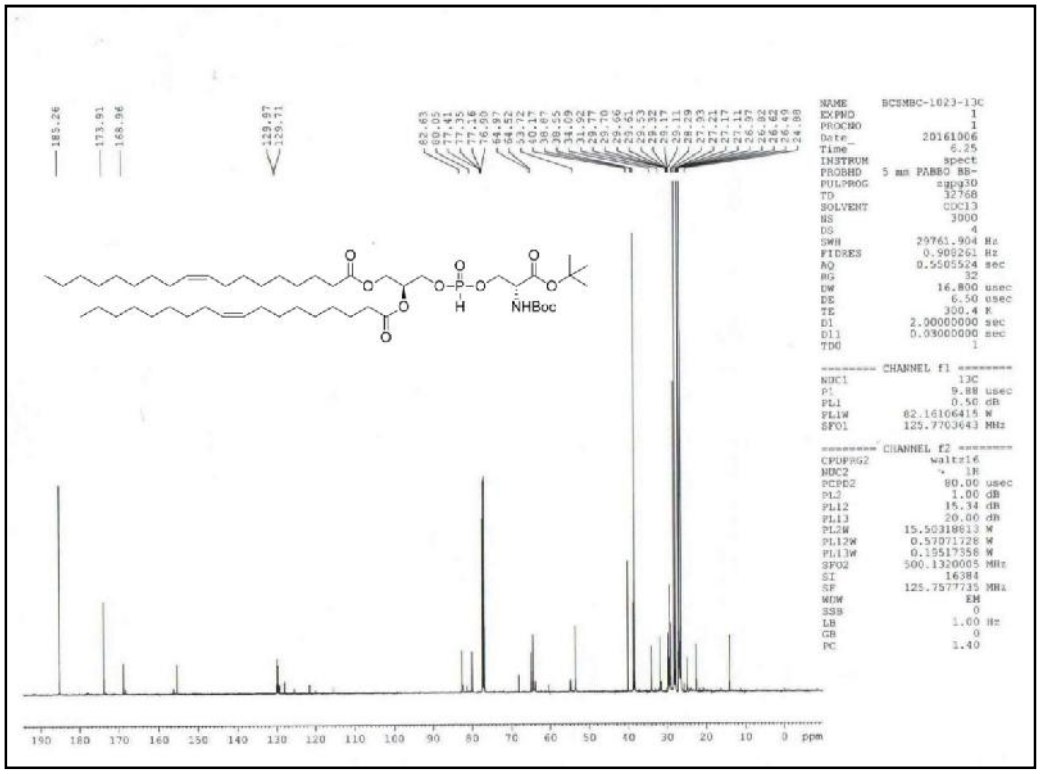
^{31}P NMR Spectra of (S)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl) phosphate(2)



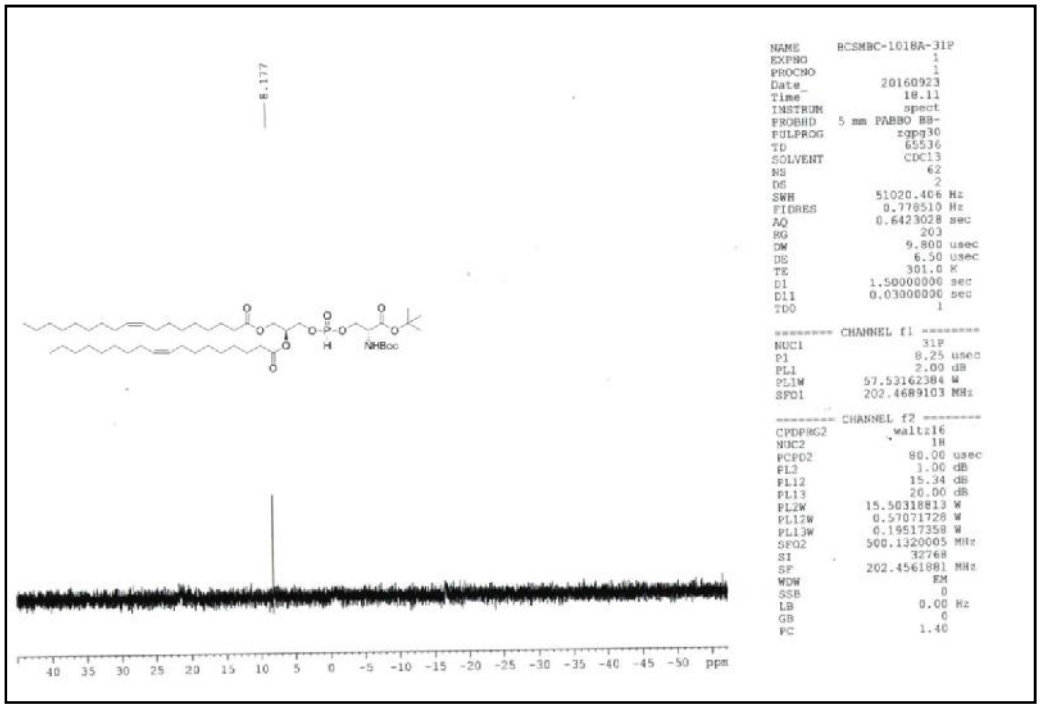
^{1}H NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl(R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (23)



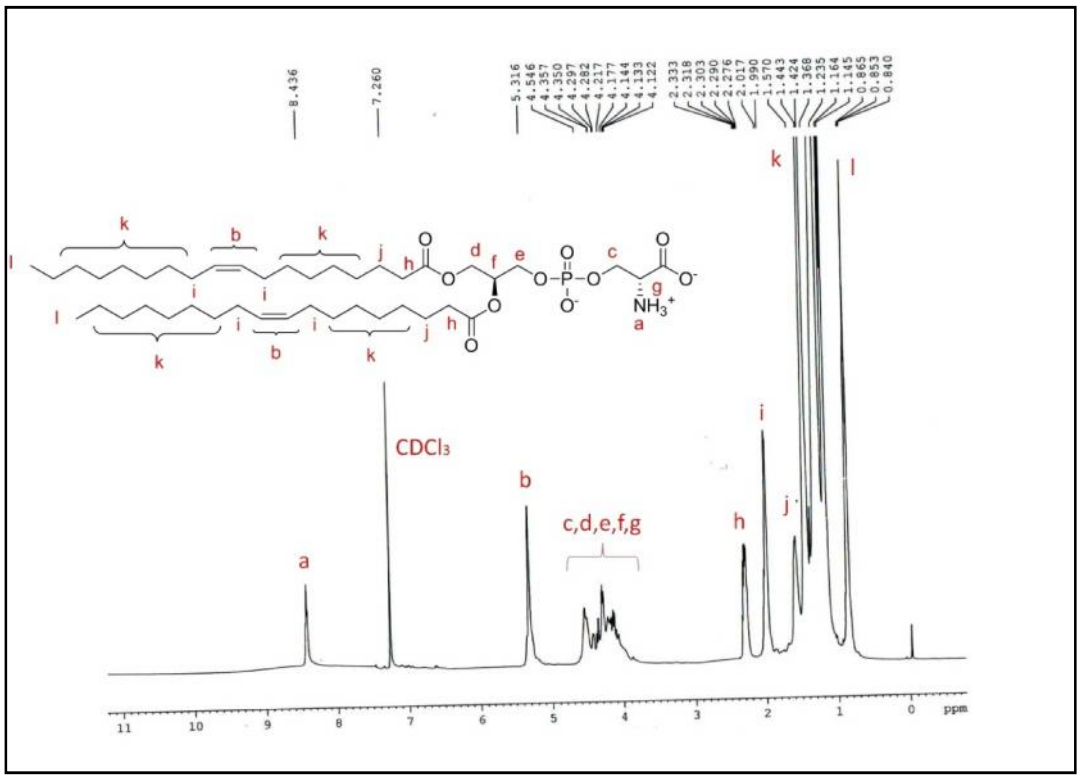
^{13}C NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl(R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (23)



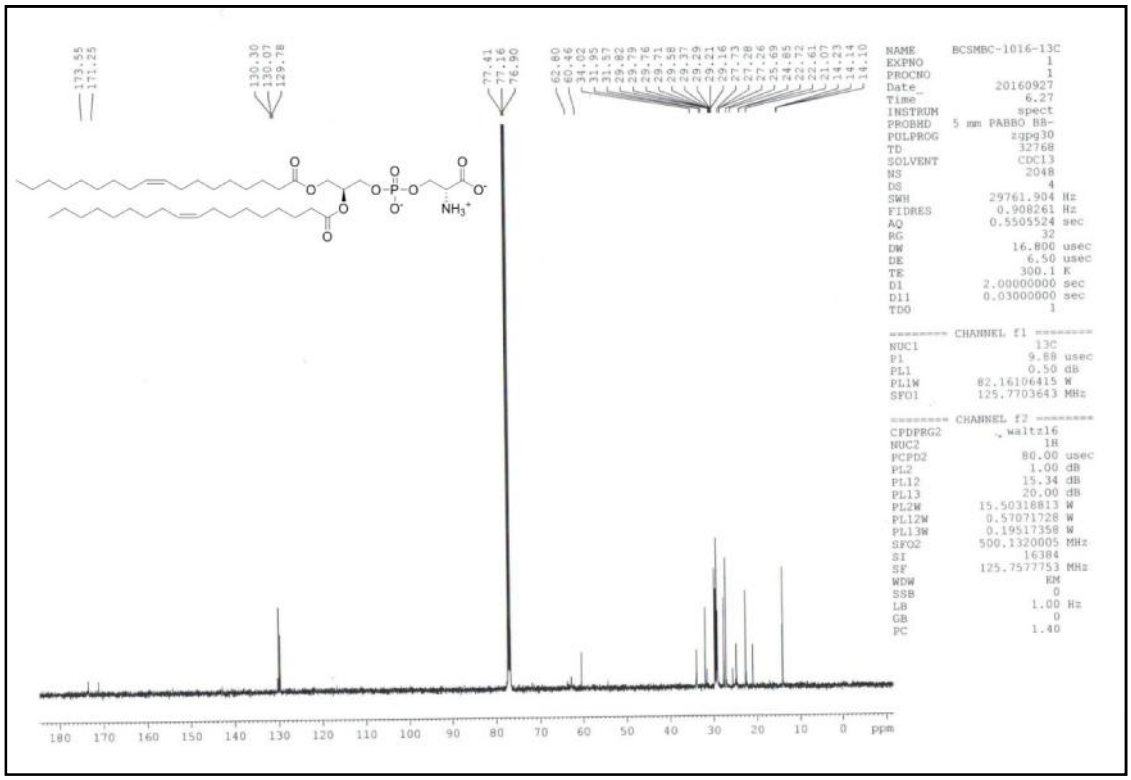
^{31}P NMR Spectra of (S)-2,3-bis(oleoyloxy)propyl(R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (23)



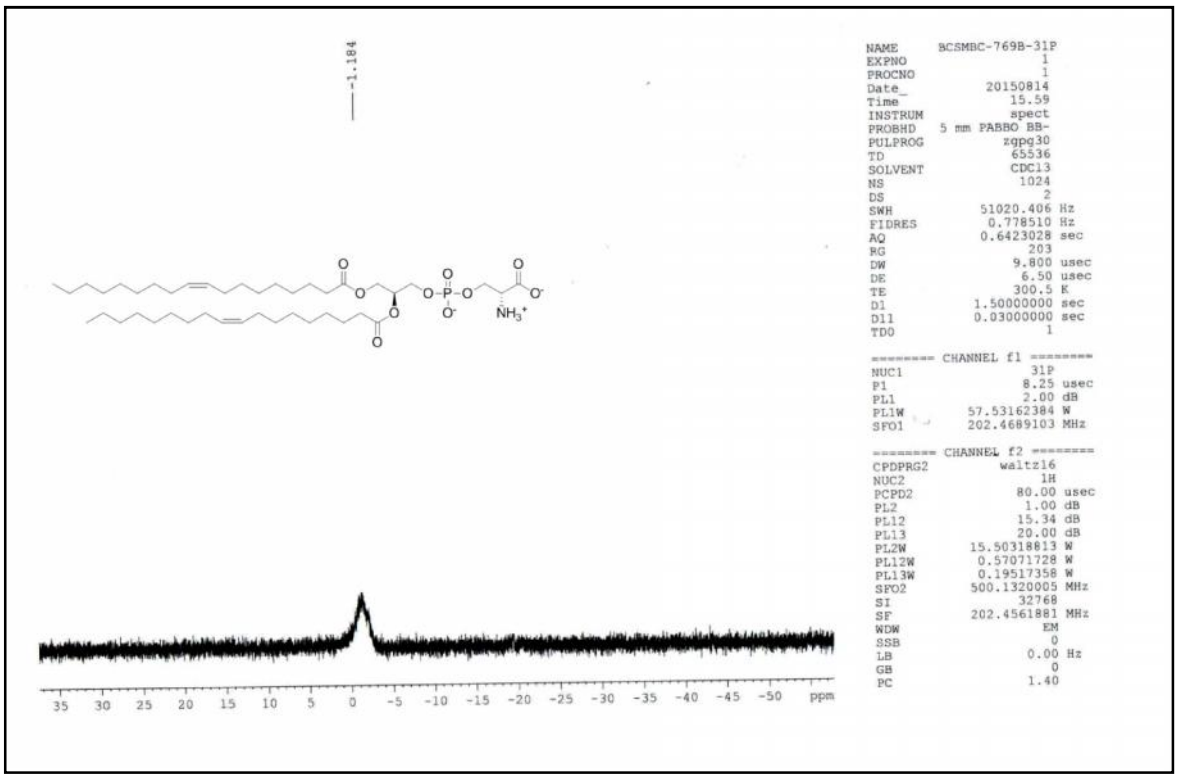
[1]H NMR Spectra of (R)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl)phosphate(4)



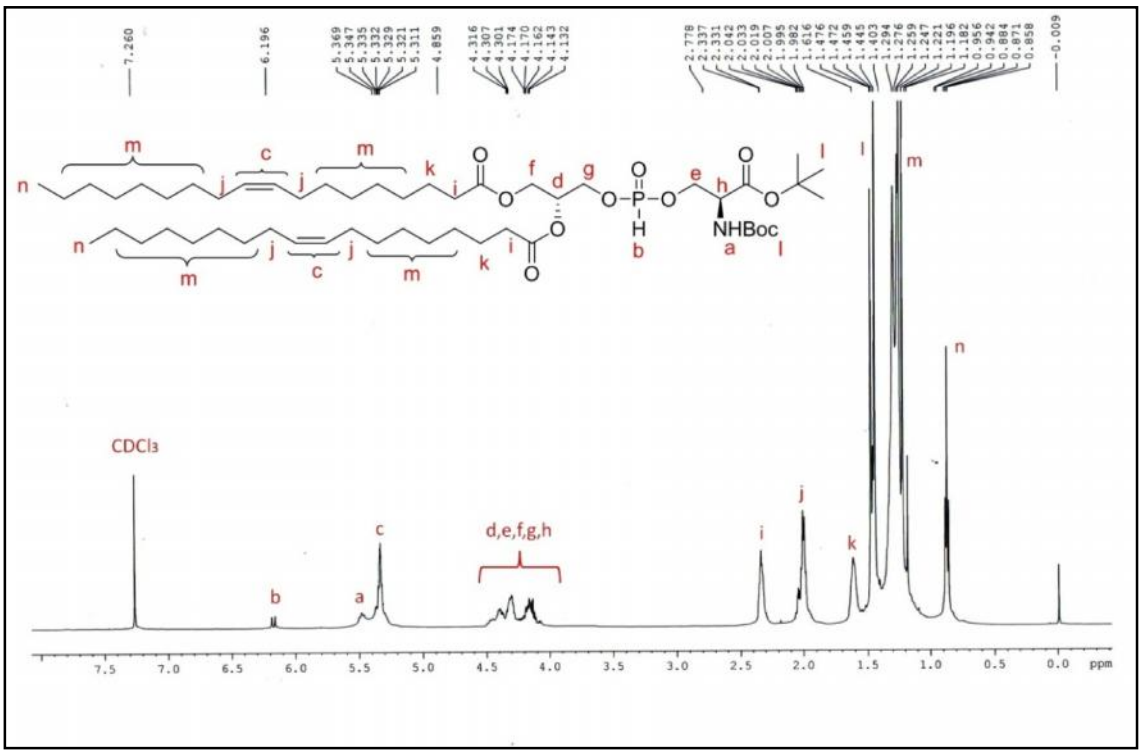
[13]C NMR Spectra of 4(R)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl)phosphate(4)



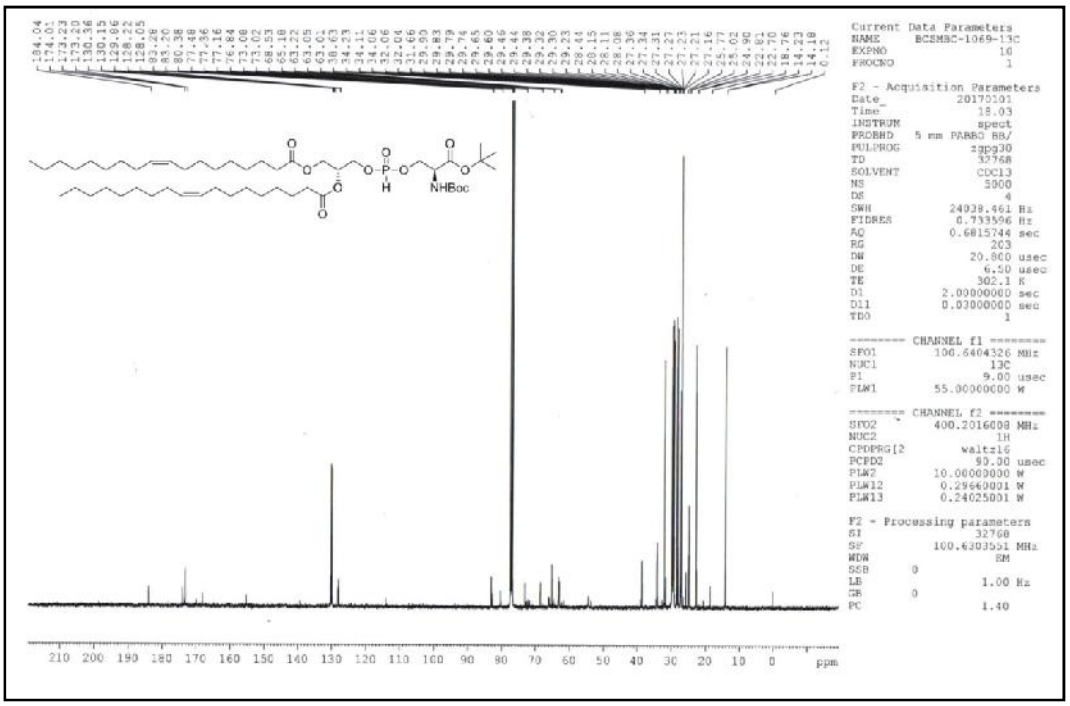
^{31}P NMR Spectra of (R)-2-amino-2-carboxyethyl ((S)-2,3-bis(oleoyloxy)propyl)phosphate(4)



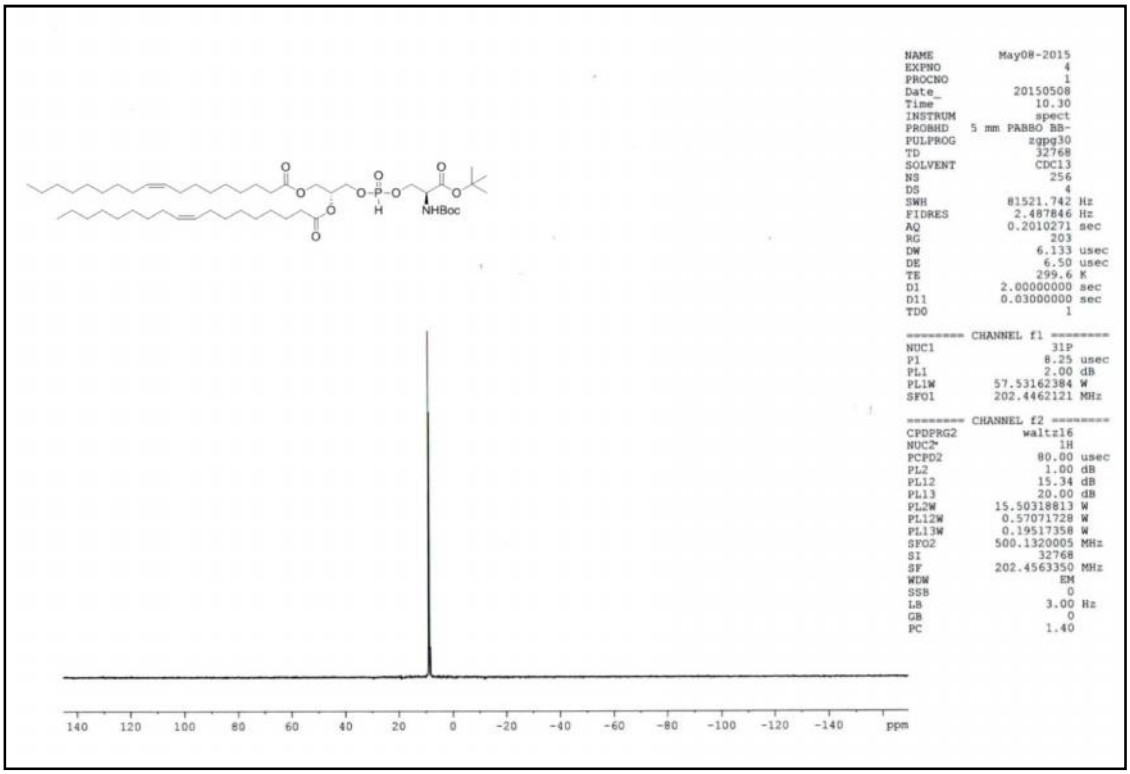
^{1}H NMR Spectra of (R)-2,3-bis(oleoyloxy)popyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (20)



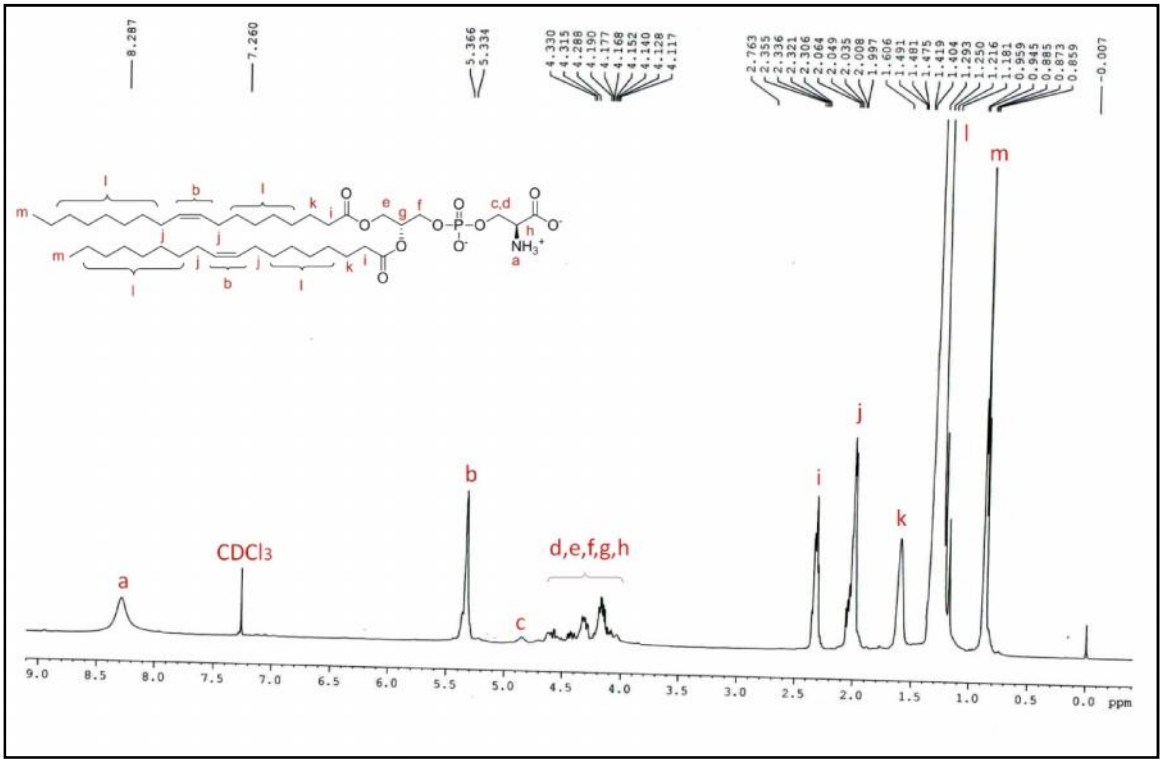
^{13}C NMR Spectra of (R)-2,3-bis(oleoyloxy)popyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (20)



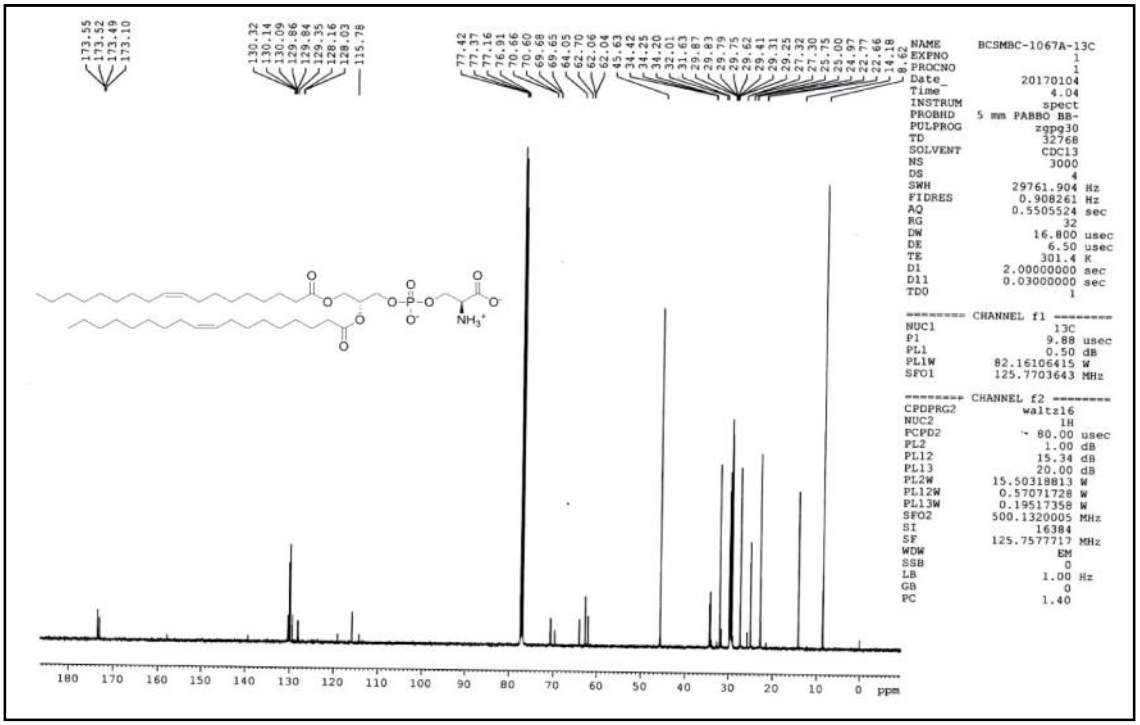
^{31}P NMR Spectra of (R)-2,3-bis(oleoyloxy)popyl((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (20)



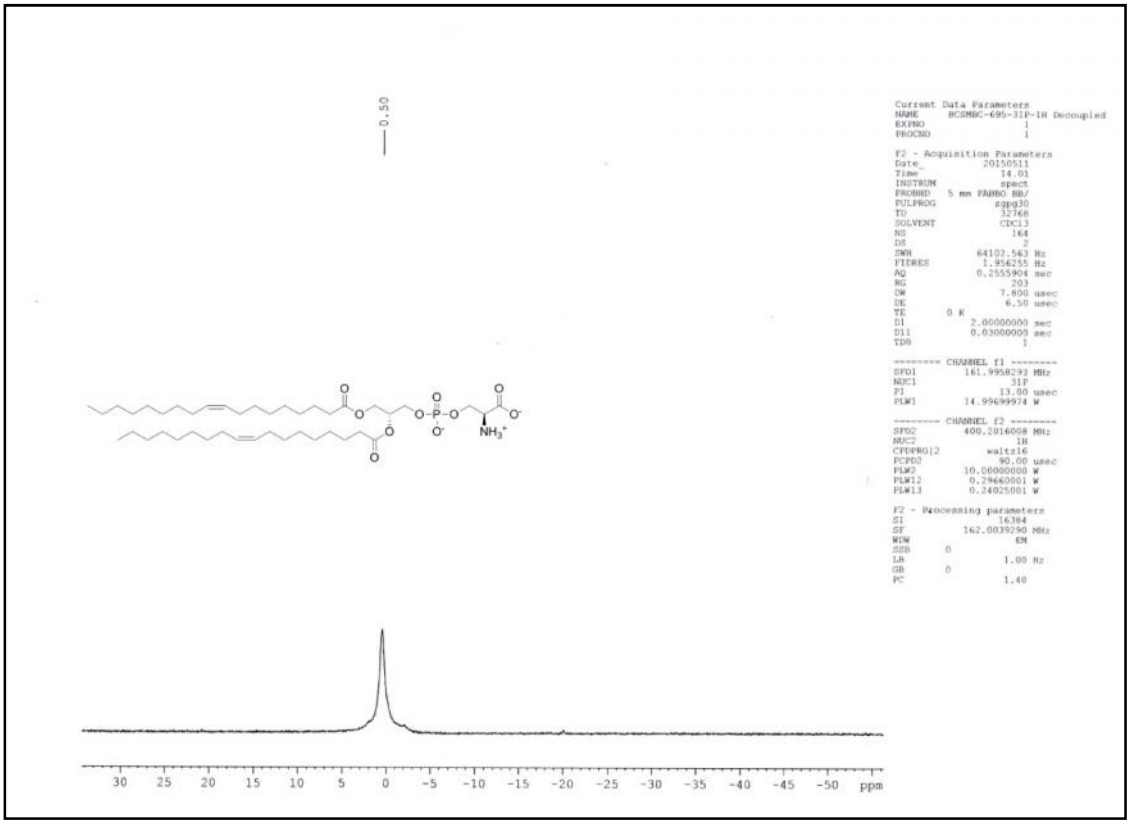
[1]H NMR Spectra of (S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1)



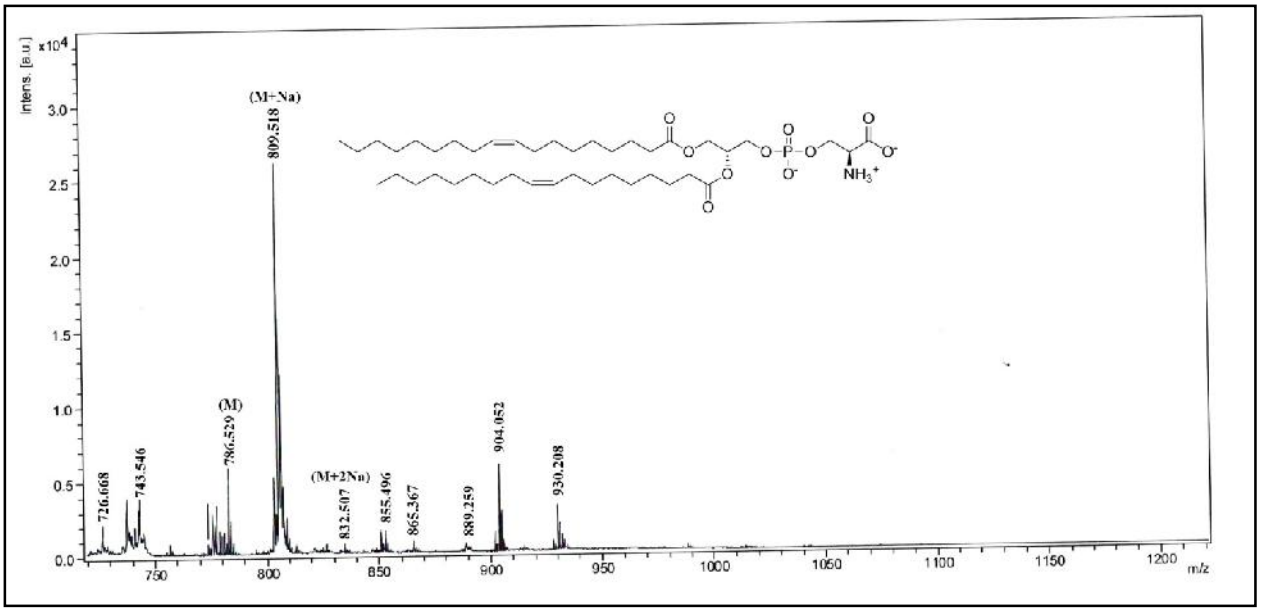
[13]C NMR Spectra of (S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1)



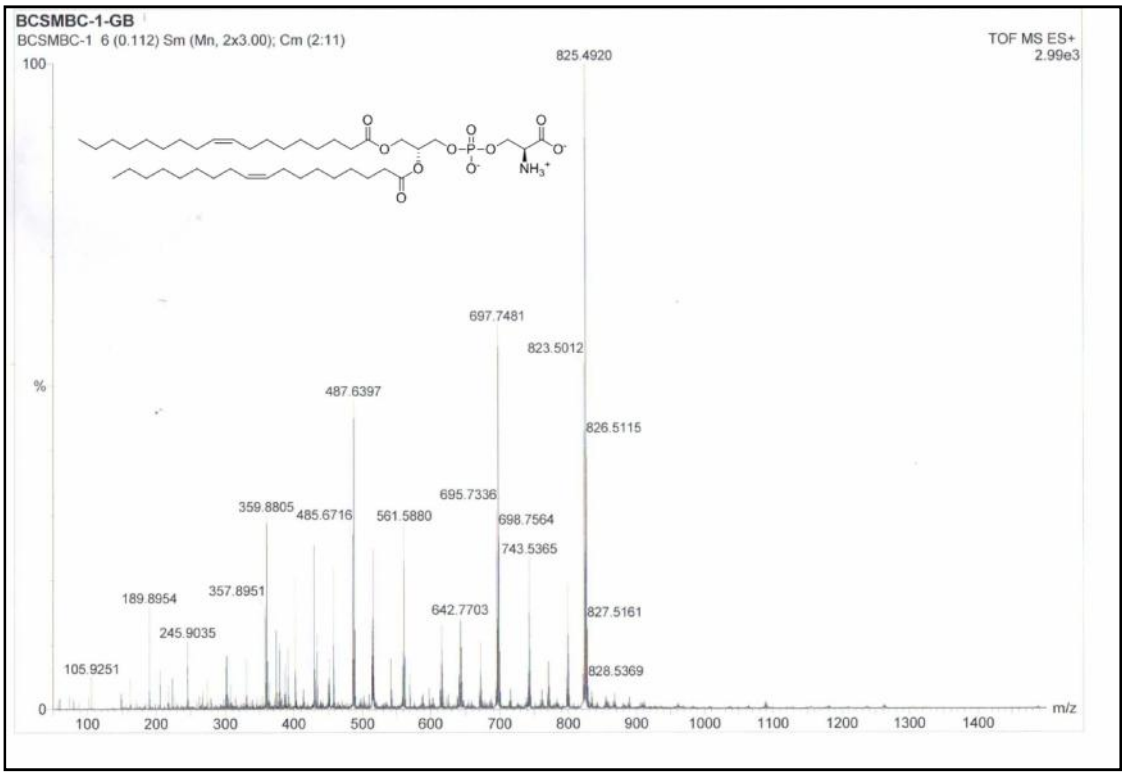
^{31}P NMR Spectra of (S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1)

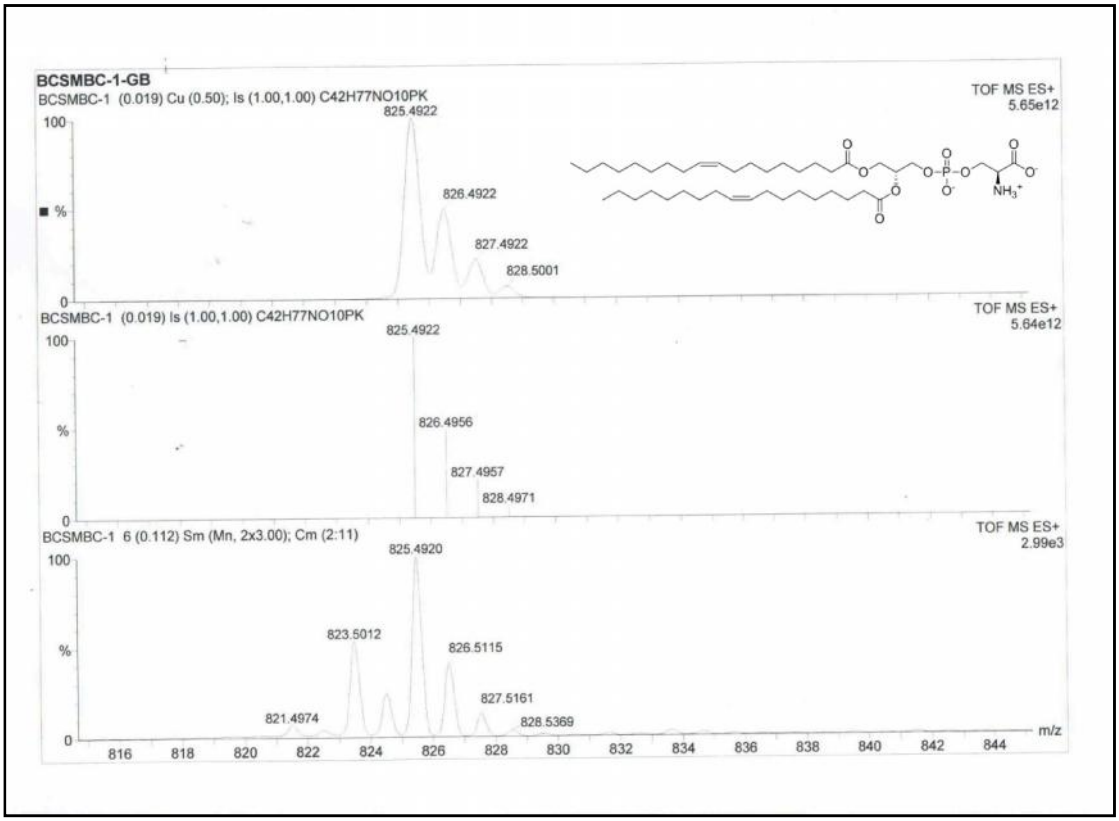


MALDI-TOP-MS of (S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1)

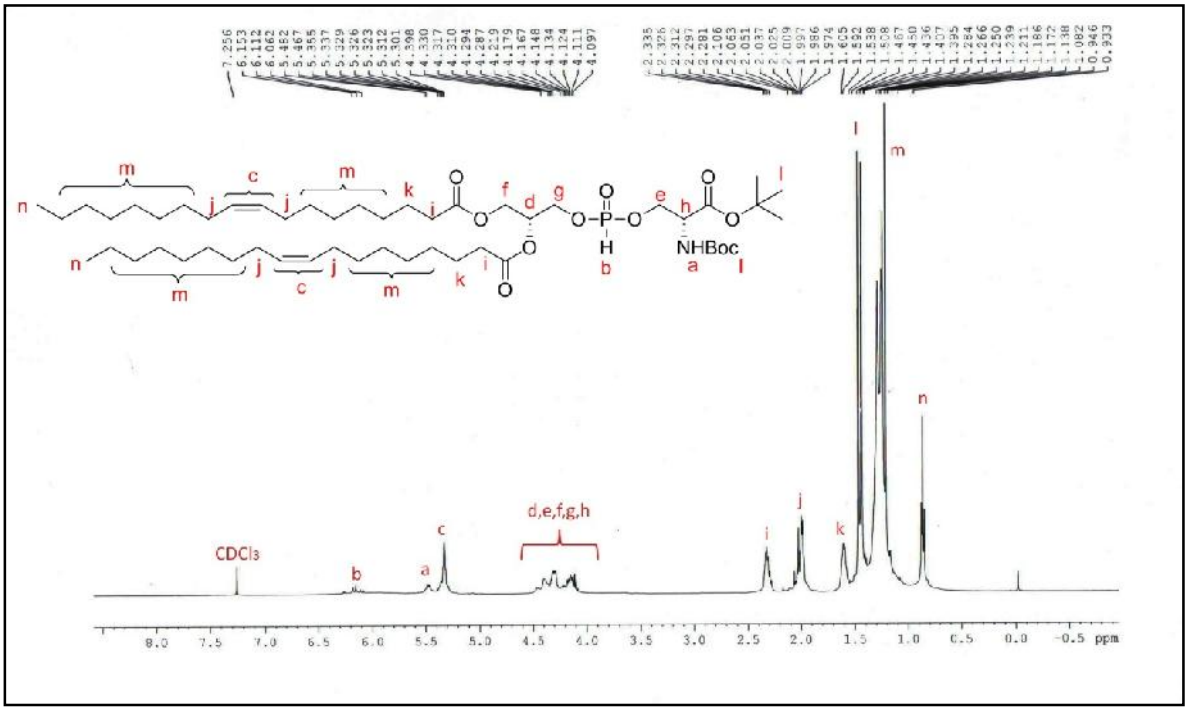


HRMS (ESI) **of (S)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (1)**

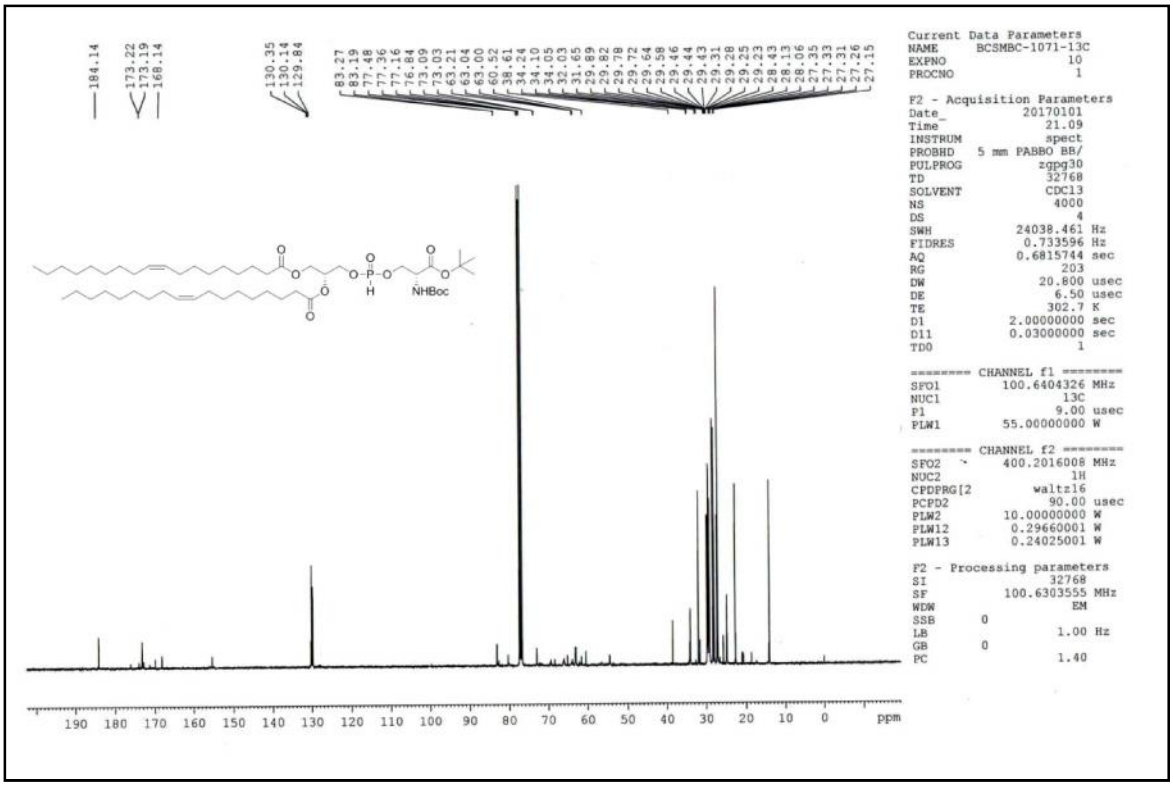




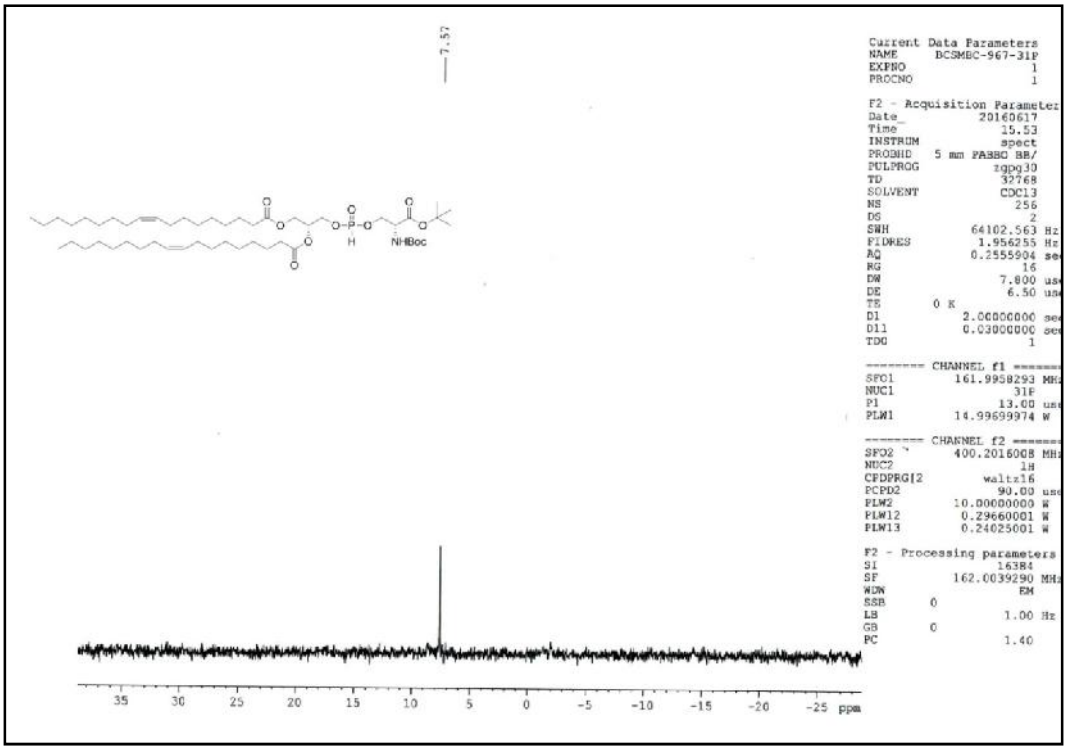
^{1}H NMR Spectra of(R)-2,3-bis(oleoyloxy)popyl((R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (21)



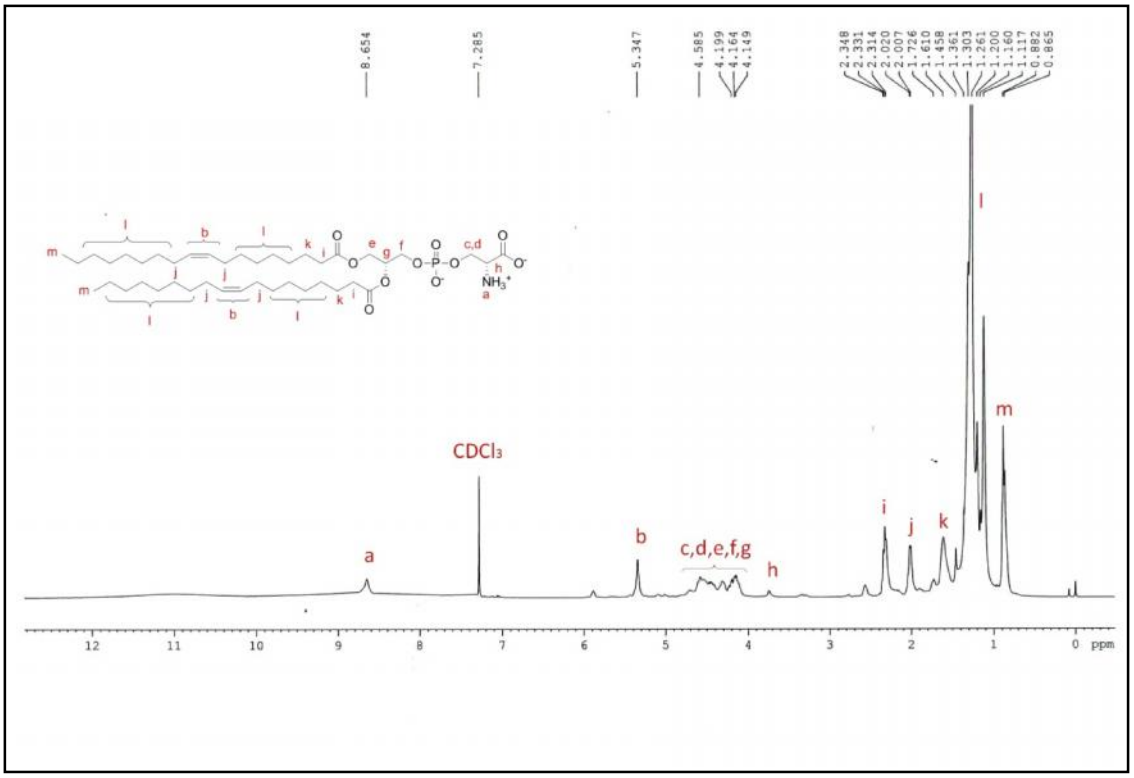
^{13}C NMR Spectra of (R)-2,3-bis(oleoyloxy)popyl((R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (21)



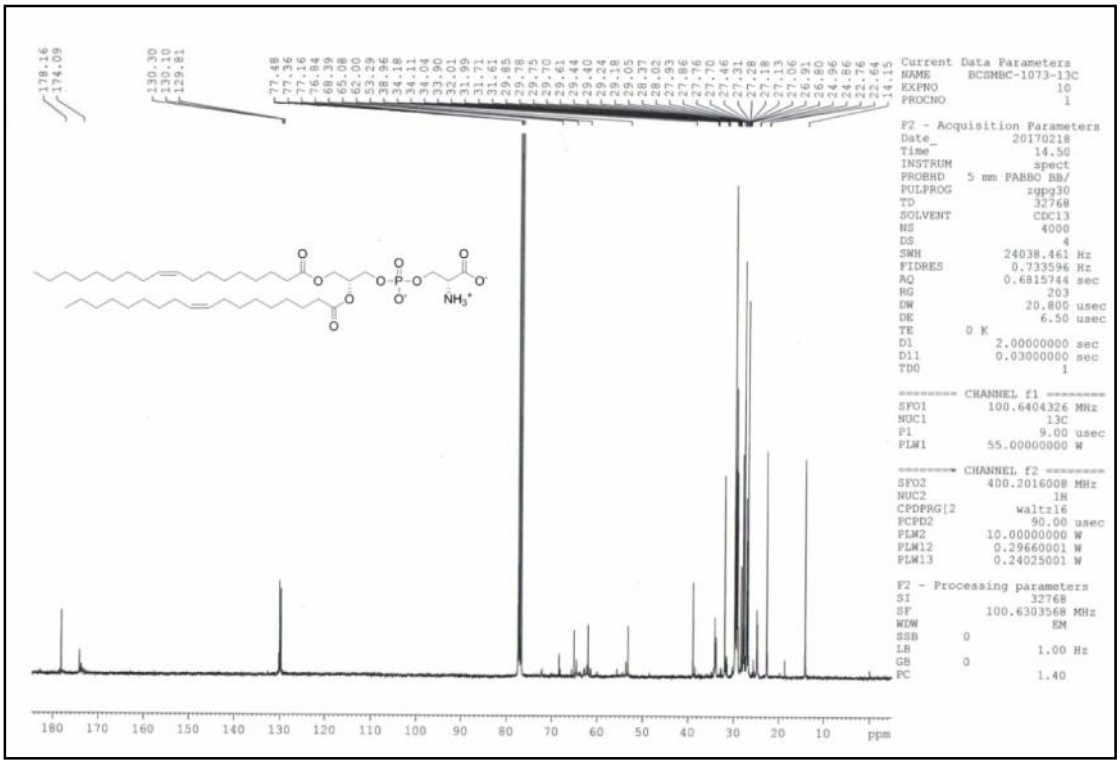
^{31}P NMR Spectra of (R)-2,3-bis(oleoyloxy)popyl((R)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl) phosphite (21)



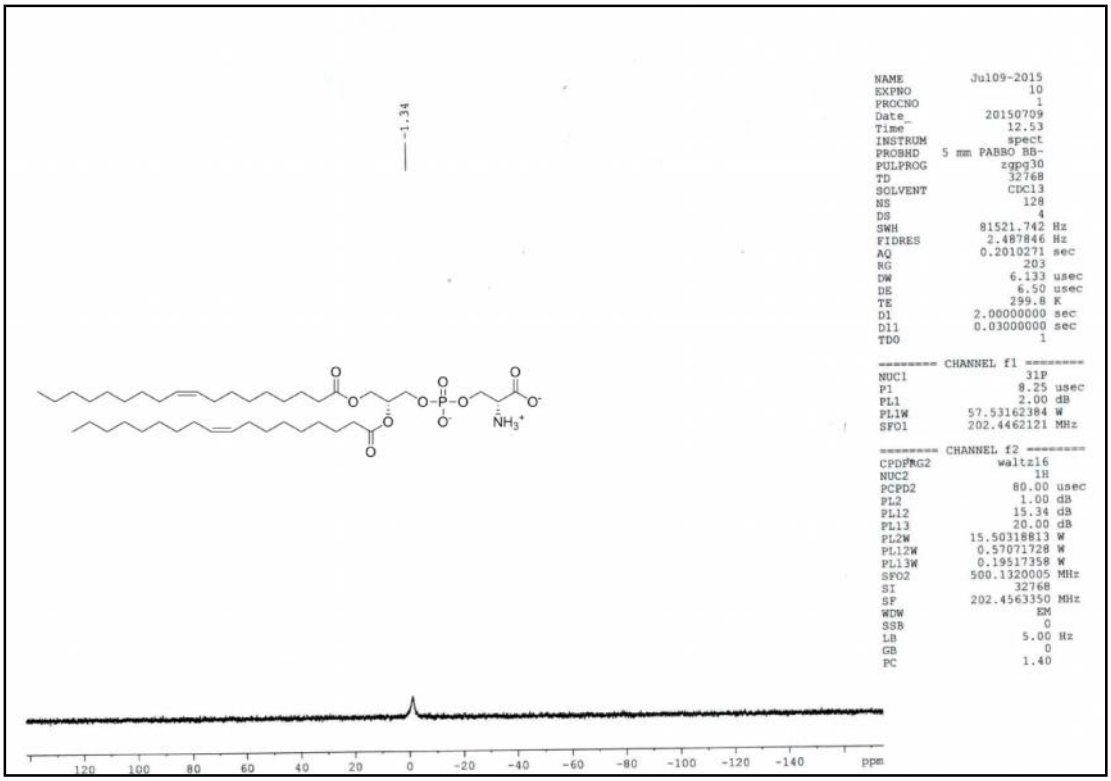
^{1}H NMR Spectra of (R)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (3)



^{13}C NMR Spectra of (R)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (3)



^{31}P NMR Spectra of (R)-2 amino-2-carboxyethyl-((R)-2,3-bis(oleoyloxy)propyl) phosphate (3)



4. DLS Analysis, Preperation of relipidated Tissue Factor and FXa procoagulant Assay

4.1 DLS Analysis: 25µl of Liposome containing TF was diluted with deionized water to get final volume of 1mL and the DLS measurement was taken with it to get the overall idea about the diameter of the prepared liposome.

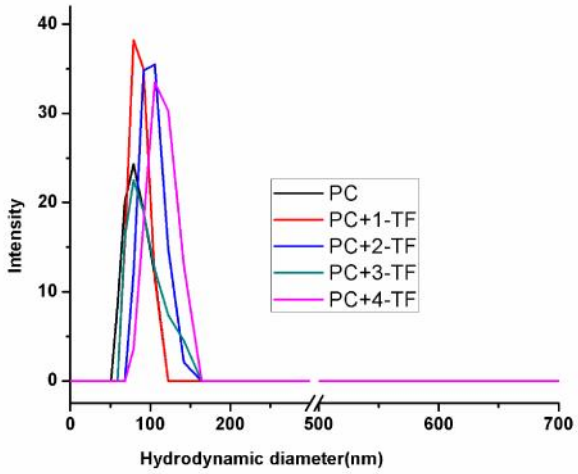


Fig S1: DLS analysis with different types of TF containing liposome.

4.2 Preparation of relipidated Tissue-Factor: In Pyrex glass tube 80% (W/W) PC and 20% PS Pyrex were taken and evaporated to dry by passing N_2 gas with constant rolling the tube, which makes lipid film at the bottom of the tube. These lipids were suspended into Hepes buffer having the detergent β-octyl- glucopyranoside. For reconstitution of TF, purified TF (1:10000, mole ratio of TF:PL) was incubated with suspended PL in buffer for half an hour, and the mixture was dialyzed against Hepes buffer for 72 hr to form the TF embedded liposomes.

4.3 FXa procoagulant Assay: With these liposomes pro-coagulant activity was measured by FXa generation assay. Saturated concentration of lipidated TF (1nM) was incubated with limited concentration of recombinant FVIIa (10pM) for 5 min followed by incubate with saturated concentration of FX (175nM) for 10 min in Hepes buffer saline (HBSA) having 5mM $CaCl_2$. Reaction was stopped with 20mM EDTA solution. Generated FXa was estimated using substantial amount of chromogenic substrate S-2765 by colorimetric method, measuring 2 min kinetics at 405nm. Data presented here are as mean +/- SEM (n=3). Differences are statistically significant at $p < 0.05$ by student's t-test.

5. Computational Section

5.1 Supplemental Material and Methods:

Computational modeling of flTF-FVIIa in membrane bilayer:

The solvated-equilibrated full-length TF-FVIIa in POPC:POPS in a 4:1 ratio was taken from our previously MD simulation work.[5] Here, different forms of phosphatidylserine viz. 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine and 2,3-dioleoyl-*sn*-glycero-1-phospho-D-serine are denoted by molecule 1 and 4, respectively. In the POPC:POPS lipid bilayer, POPS is molecule 1. To construct molecule 4 lipid bilayer, molecule 1 was extracted from this bilayer. Since the molecules are identical in all respects except for their chirality, viz. R- and S-type. Therefore, molecule 4 was created from 1 using discovery studio software. Parameter and topology file for molecule 4 was created using the existing topology file molecule 1. After that, PC:4 lipid bilayer were constructed. In addition to these systems, we have also constructed POPC (100%) bilayer using CHARMM membrane builder server and subjected to equilibration run (8-10 ns) as similar with the previously mentioned protocol.[5] We have made three individual flTF-FVIIa binary complex systems imbedded in three independent lipid bilayer, POPC, POPC:1 and POPC:4 bilayer for MD simulation study. Bad lipid and water molecules were removed from TF-FVIIa complex system in order to prevent steric clashes among them.

Molecular dynamic simulation protocol:

Molecular dynamic (MD) simulations were performed using NAMD2.9 tools along with CHARMM36 force field parameter.[6,7] All three systems, flTF-FVIIa in PC, PC:1 and PC:4 membrane lipid were solvated using TIP3P water model in a periodic truncated with box size

128(x) x 128(y) x 182(z) $Å^3$ at least 12 Å from any given atom. 5mM calcium ionic concentration was maintained by replacing several water molecules with Ca^{2+} and Cl^- ions in the systems. Temperature of the system was kept constant at 310 K using 1 ps^{-1} dampling coefficient (γ) by Langevin dynamics. The Particle Mesh Ewald (PME) method was used for long-range electrostatic interaction calculation.[8] The direct non-bonded potential cutoff was set to 12 Å with pair-list distance cutoff of 2 Å. During the simulation, 1-4 scaling factor was used. The constant pressure was maintained at 1 atm using the *Langevin piston Nosé-Hoover* method using the periodic boundary conditions.[9,10] For the proper equilibration of the system, the model complex in lipid bilayer was subjected to several cycles of equilibration followed by energy minimization. Initially, dynamics were performed on all lipids, water molecules and counterions with 4000 steps of energy minimization for the first 0.5 ns simulation with 1 fs time interval using a short "constant-pressure" in NPT condition to pack lipid and water molecules against the protein and the counterions to relax their positions, however the modeled protein was kept fixed in this equilibration run. In the next step, 2 ns short equilibration was performed with 2 fs timestep using NPT with protein constrained, in which SHAKE algorithm was applied for all hydrogen atoms. Protein atoms were constrained with a small harmonic potential (1.0 kcal/mol/$Å^2$ spring constant). In this last run, the whole system was switched to NPnAT ensemble (constant membrane-normal pressure, Pn, temperature and membrane area) with 2 fs timestep interval without any constraint on the proteins for 40 ns production run. Detailed of the system sizes and simulation setup are summarized in Table S1.

5.2 Supplemental Tables:

Table S1. Summary of the system size and NAMD simulation setup.

System	flTF-FVIIa complex in PC lipid bilayer model	flTF-FVIIa complex in PC:1 lipid bilayer model	flTF-FVIIa complex in PC:4 lipid bilayer model
Lipids composition	100POPC:100POPC [100 leaflets in each leaflets]	160POPC:40POPS (1) [100 leaflets in each leaflets]	160POPC:40POPS(4) [100 leaflets in each leaflets]
Initial box size ($Å^3$)	124(x) x 124(y) x 175(z)= 2,690,800 $Å^3$	128(x) x 128(y) x 182(z)= 2,981,888 $Å^3$	128(x) x 128(y) x 182(z)= 2,981,888 $Å^3$
Number of Ca^{2+} ions	15 (9 bound + 6 additional ions)	16 (9 bound + 7 additional ions)	16 (9 bound + 7 additional ions)
Number of Cl- ions	13 (1bound + 12 additional ions)	17 (1bound + 16 additional ions)	17 (1bound + 16 additional ions)
Water model	TIP3P	TIP3P	TIP3P
Number of water molecules	63644	72,342	69,392
Total number of atoms	253310	279,869	278,056
Time step integration	2 fs	2 fs	2 fs
Simulation setup schemes	Minimization (4000 steps) → Heating at 310 K → 0.5 ns NPT simulation under restraints on proteins → 2 ns with 2fs timestep using NPT ensemble with protein constrained → proteins simulations without any restraint on protein using *NPnAT* (production run)	Minimization (4000 steps) → Heating at 310 K → 0.5 ns NPT simulation under restraints on proteins → 2 ns with 2fs timestep using NPT ensemble with protein constrained → proteins simulations without any restraint on protein using *NPnAT* (production run)	Minimization (4000 steps) → Heating at 310 K → 0.5 ns NPT simulation under restraints on proteins → 2 ns with 2fs timestep using NPT ensemble with protein constrained → proteins simulations without any restraint on protein using *NPnAT* (production run)
Total simulation time in production run (*NPnAT* ensemble)	40 ns	40 ns	40 ns

Table S2. Comparative analysis of interacting residues of light chain of FVIIa and TF with two distinct phosphatidylserine moiety (molecule 1 and 4) for H-bond formation with a distance cut-off of 3.5 Å obtained from 40 ns simulation study of flTF-FVIIa binary complex in two lipid environment.

Protein	**flTF-FVIIa complex in molecule 1 lipid environment**	**flTF-FVIIa complex in molecule 4 lipid environment**
Tissue Factor	Glu183-mainN/1-sideO13 Asp180-mainN/1-sideO13A Tyr185-sideOH/1-sideO13A PS1-mainN/Glu183-sideOE1 PS1-mainN/Asp180-sideOD1 Gly182-mainN/1-sideO32 Lys159-sideNZ/1-sideO13B PS1-mainN/Asp180-sideOD2 Lys181-sideNZ/1-sideO32 Glu183-mainN/1-sideO11 PS1-mainN/Asn184-mainO Asn184-mainN/1-sideO21	Lys159-sideNZ/4-sideO14 Ser161-sideOG/4-sideO14 Ser162-sideOG/4-sideO13 Ser163-sideOG/4-sideO14 Gly251-mainN/4-sideO14
Factor VIIa	PS1-mainN/Gla7-sideOE11 Gla29-sideCB/1-sideO11 PS1-mainN/Arg9-mainO PS1-mainN/Gla16-sideOE21 PS1-mainN/Gla16-sideOE22 PS1-mainN/Gla6-sideOE12 PS1-mainN/Asn2-sideOD1	Gly11-mainN/4-sideO13 Gly11-mainN/4-sideO14 Gly11-mainN/4-sideP Arg28-sideNH1/4-sideO13A Arg28-sideNH2/4-sideO13B Lys32-sideNZ/4-sideO13A

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