Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis of non-hydrolysable mimics of glycosylphosphatidylinositol (GPI) anchor

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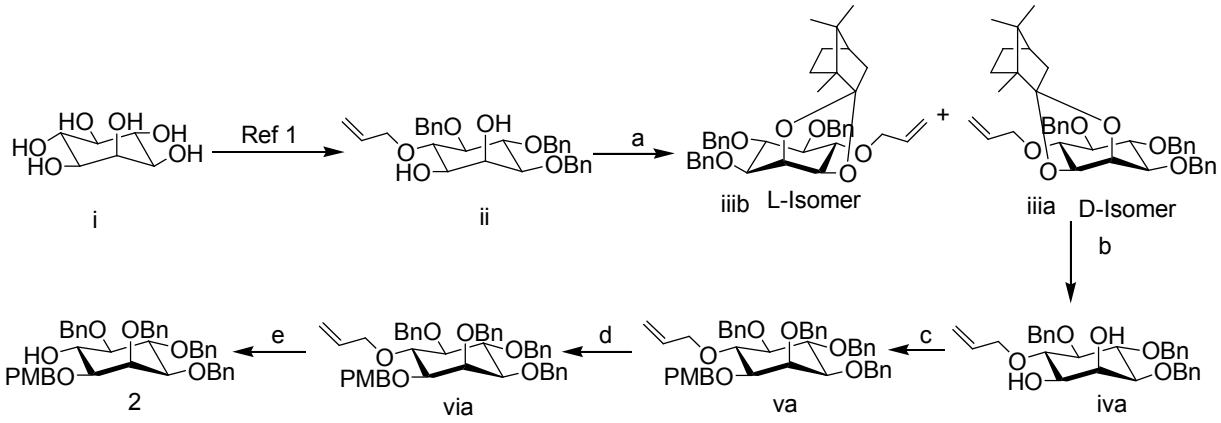
1. General:

All NMR measurements (^{1}H, ^{13}C and ^{31}P) were recorded on 400 or 500 MHz spectrometer fitted with pulse-field gradient probe, and tetramethylsilane (TMS) or residual resonance of deuterated solvent were used as internal reference. Chemical shifts are expressed in (δ) parts per million and coupling constants *J* in hertz. Mass spectra were recorded either with LCMS-QTOF instrument or with MALDI-TOF/TOF mass spectrophotometer using 2, 5-Dihydroxy benzoic acid/*α*-Cyano-4-hydroxy cinnamic acid as matrix in acetonitrile:water containing 0.01% TFA.

2. Synthesis of acceptor 2 (2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol):

This chiral intermediate was prepared in following steps (Scheme 1)[1]



Scheme S1: Synthesis of acceptor 2. Reagents and conditions: (a) camphor dimethyl ketal, dry CH_2Cl_2, *p*-TSA, reflux, 2 h; (b) PTSA, MeOH, 50 ^{o}C, 3 h; (c) i). $(Bu)_2SnO$, MeOH, reflux, 2h ii). PMBCl, $(Bu)_4NBr$, dry toluene, 4A^{o} MS, reflux 6 h; (d). BnBr, NaH, DMF, rt, 3 h; (e) i). *t*-BuOK, DMSO, 80 ^{o}C, 3 h; ii). 1M HCl:acetone (1:9), 80 ^{o}C, 30 min.

6-O-Allyl-3,4,5-tri-O-benzyl-1,2-O-{(+)-1,1,7-trimethyl[2,2,1]bicyclohepta-6-ylidene}-D-myo-inositol (iiia): To the solution of 6-O-Allyl-3,4,5-tri-O-benzyl-*myo*-inositol **ii** (10.00 g, 20.4 mmol) in a dry CH_2Cl_2 (120 mL), *p*-TSA (380 mg, 0.4 mmol) and camphordimethylacetal (10.10 g, 50.0 mmol) was added and the reaction mixture was heated to reflux for 2 h. After completion, reaction mixture was neutralized with triethylamine. Normal workup followed by flash chromatography (EOAc: hexane 1:9) provided the desired D-isomer **iiia** (5.00 g, 43.6%). TLC (EtOAc:hexane 1:9): R_f = 0.33; $[\alpha]_D$ = -7.1 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38-7.25 (m, 15H), 6.01-5.94 (m, 1H), 5.30 (dd, *J* = 17.3, 1.7 Hz, 1H), 5.17 (dd, *J* = 10.6 1.5 Hz,

1H), 4.81-4.69 (m, 6H), 4.40-4.36 (dtdt, *J* = 6.9, 5.7, 1.4 Hz, 1H), 4. 28-4.22 (m, 2H), 3.87 (dd, *J* = 7.1, 6.2 Hz, 1H), 3.89 (t, *J* = 8.5 Hz, 1H), 3.75-3.72 (m, 1H), 3.68 (dd, *J* = 9.8, 7.1 Hz, 1H), 3.36 (dd, *J* = 9.8, 8.2 Hz, 1H), 1.99-1.90 (m, 2H), 1.75-1.69 (m, 2H), 1.46 (d, *J* = 12.9 Hz, 1H), 1.43-1.37 (m, 1H), 1.26-1.24 (m, 1H), 1.07 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 138.70, 138.58, 138.51, 135.40, 128.39-127.37 (multiple peaks), 117.94, 116.23, 82.65, 82.29, 80.72, 78.20, 76.72, 74.76, 74.29, 72.70, 72.53, 72.40, 51.36, 47.98, 45.03, 44.83, 29.85, 26.95, 20.37, 20.18, 10.79; MALDI TOF MS: calcd for $C_{40}H_{48}O_6$ $(M+Na)^+$ 647.3349, found 647.3264.

***6-O-Allyl-3,4,5-tri-O-benzyl-D-myo-inositol (iva)*:** The compound **iiia** (2 g, 3.5 mmol) and *p*-TSA (0.72 g, 4.2 mmol) was dissolved in MeOH (33 mL) and stirred for 3 h at 50 ^{o}C. Reaction mixture was neutralized at room temperature with Et_3N and solvent was evaporated and diluted with EtOAc (100 mL), washed with water and brine solution. The organic layer was dried using Na_2SO_4 and concentrated. The residue was purified by flash chromatography to give 6-O-Allyl-3,4,5-tri-O-benzyl-D-*myo*-inositol **iva** (1.47 g, 86%) as a white solid. TLC (hexane/EtOAc, 1:1): R_f = 0.50; $[\alpha]_D$ = -10.5 (c 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.22 (m, 15H), 5.98-5.91 (m, 1H), 5.25 (dd, *J* = 17.1, 1.6 Hz, 1H), 5.15 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.93-4.79 (m, 4H), 4.70-4.69 (m, 2H), 4.43-4.37 (dtdt, *J* = 12.4, 5.9, 1.3 Hz, 1H), 4.28-4.23 (dtdt, *J* = 12.6, 5.6, 1.3Hz, 1H), 4.17 (t, *J* = 2.4 Hz, 1H), 3.92 (t, *J* = 9.6 Hz, 1H), 3.69 (t, *J* = 9.6 Hz, 1H), 3.44-3.38 (m, 3H), 2.73-2.70 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 138.66, 138.53, 137.79, 135.02, 128.51-127.60 (multiple peaks), 117.16, 83.19, 81.56, 80.93, 79.96, 75.91, 75.67, 74.33, 72.74, 71.69, 69.25; MALDI TOF MS: calcd for $C_{30}H_{34}O_6$ $(M+Na)^+$ 513.2253, found 513.2035.

6-O-Allyl-3,4,5-tri-O-benzyl-1-O-(4-methoxybenzyl)-D-myo-inositol (va): A solution of diol **iva** (2.00 g, 4.1 mmol) and dibutyltin oxide (1.00 g, 4.1 mmol) in toluene (140 mL) was heated to reflux for 3 h using Dean-Stark apparatus. After that PMBCl (0.71 mL, 5.3 mmol) and $(Bu)_4NBr$ (1.92 g, 6.0 mmol) was added and reflux for 1 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (9:1 to 8:2 hexane/EtOAc) to provide 6-*O*-Allyl-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol **va** (2.25 g, 90%) as white solid. TLC (hexane/EtOAc, 7:3): R_f = 0.56; $[\alpha]_D$ = -1.7 (c 0.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33-7.25 (m, 17H), 6.87 (d, *J* = 8.59, 2H), 6.03-5.93

(m, 1H), 5.20-5.17 (dd, *J* = 15.5, 1.2 Hz, 1H), 5.14 (dd, *J* = 11.7, 1.6 Hz, 1H), 4.89-4.81 (m, 4H), 4.69-4.60 (m, 4H), 4.45-4.38 (dtdt, *J* = 12.2, 5.6, 1.6 Hz, 1H), 4.37-4.30 (dtdt, *J* = 12.3, 5.9, 1.7 Hz, 1H), 4.15 (t, *J* = 2.3 Hz, 1H), 3.90 (t, *J* = 9.6 Hz, 1H), 3.81-3.77 (m, 4H), 3.41-3.35 (m, 2H), 3.30 (dd, *J* = 9.8, 2.0 Hz, 1H), 2.42 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 159.36, 138.80, 138.72, 137.99, 135.35, 130.14, 129.47, 128.63-127.54 (multiple peaks), 116.58, 113.96, 113.86, 83.16, 81.11, 80.89, 79.75, 79.27, 75.96, 75.91, 74.56, 72.70, 72.47, 67.71, 55.29; MALDI TOF MS: calcd for $C_{38}H_{42}O_7$ $(M+Na)^+$ 633.2828 found, 633.2833.

6-O-Allyl-2,3,4,5-tetra-O-benzyl-1-O-(4-methoxybenzyl)-D-myo-inositol (via): The compound **va** (2.15 g, 3.5 mmol) was dissolved in anhyd DMF (50 mL), solution was brought to 0 ^oC followed by addition of NaH (0.26 g, 11.1 mmol) and benzyl bromide (0.63 ml, 5.3 mmol). The reaction mixture stirred for 3 h at room temperature was subjected to brine treatment (500 ml) and extracted with EtOAc (80 ml). The organic extract was dried over anhyd Na_2SO_4, concentrated followed by column purification to provide 6-O-Allyl-2,3,4,5-tetra-O-benzyl-1-O-(4-methoxybenzyl)-D-*myo*-inositol **via** (2.42 g, 93%) as colorless syrup. TLC (hexane/EtOAc, 8:2): R_f = 0.45; $[\alpha]_D$ = -4.6 (c 0.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.23 (m, 22H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.98-5.95 (m, 1H), 5.27 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.13 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.90-4.80 (m, 6H), 4.64-4.56 (m, 3H), 4.53-4.50 (m, 1H), 4.41-4.37 (dtdt, *J* = 12.2, 5.8, 1.3Hz, 1H), 4.32-4.28 (dtdt, *J* = 12.2, 5.8, 1.3 Hz, 1H), 4.02 (t, *J* = 9.6 Hz, 1H), 3.97 (t, *J* = 2.3 Hz, 1H), 3.93 (t, *J* = 9.6 Hz, 1H), 3.81 (s, 3H), 3.40 (t, *J* = 9.2 Hz, 1H), 3.32 (dd, *J* = 9.8, 2.3 Hz, 1H), 3.26 (dd, *J* = 9.8, 2.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 159.36, 138.81, 138.72, 137.99, 135.36, 130.14, 129.48, 128.65-128.64 (multiple peaks), 116.59, 113.86, 83.17, 81.12, 80.91, 79.76, 79.29, 77.76, 77.24, 75.98, 74.57, 72.71, 72.48, 67.72, 55.30; MALDI TOF MS: calcd for $C_{45}H_{48}O_7$ $(M+Na)^+$ 723.3298, found 723.3182.

2,3,4,5-tetra-O-benzyl-1-O-(4-methoxybenzyl)-D-myo-inositol (2): The compound **via** (1.6 g, 2.14 mmol) was dissolved in anhyd DMSO (80 ml) and treated with potassium *tert*-butoxide (2.9 g, 11.42 mmol). The reaction mixture was heated at 80 ^oC after 3 h, poured into ice-cold water, and extracted with EtOAc. The organic layer was washed with brine and water, dried (Na_2SO_4), and concentrated. This compound was dissolved in a solution of 1 M HCl/acetone (1:9, 50 ml) and kept at 50 ^oC for 30 min, neutralized with TEA and concentrated. The residue on flash

chromatography (hexane/EtOAc, 3:1) resulted 2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol **2,** 1.24 g (82% yield) as white solid. TLC (hexane/EtOAc, 7:3): R_f = 0.5; $[\alpha]_D$ = -10.38 (c 0.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39-7.25 (m, 20H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.90 (d, *J* = 10.7 Hz, 1H), 4.89-4.83 (m, 3H), 4.82 (d, *J* = 10.7 Hz, 1H), 4.78 (d, *J* = 11.9 Hz, 1H), 4.67 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 11.3 Hz, 1H), 4.44 (d, *J* = 11.3 Hz, 1H), 4.14 (t, *J* = 9.7 Hz, 1H), 4.04 (t, *J* = 9.5 Hz, 1H), 4.02 (t, *J* = 2.2 Hz, 1H), 3.80 (s, 3H), 3.38-3.35 (m, 2H), 3.17-3.14 (dd, *J* = 9.9, 2.3 Hz, 1H), 2.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 159.35, 138.87, 138.81, 138.34, 129.97, 129.39, 128.40-127.38 (multiple peaks), 113.92, 83.47, 81.41, 81.11, 79.75, 75.79, 75.33, 74.06, 73.66, 72.86, 72.74, 71.92, 55.29; MALDI TOF MS: calcd for $C_{42}H_{44}O_7$ $(M+Na)^+$ 683.2985, found 683.2974.

2. Inhibition experiment data:

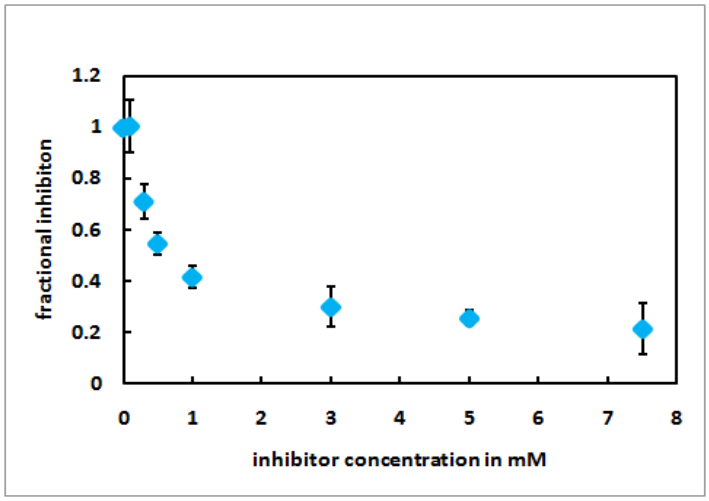


Fig 3: Dose response of **23a** on the % inhibition of GPI-NBD by PI-PLC. Fig represents the average data (n=4) for % inhibition of GPI-NBD cleavage by PI-PLC in presence of **23a**

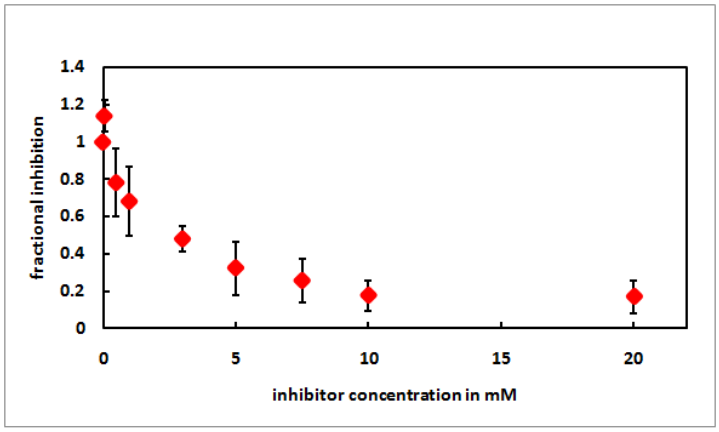
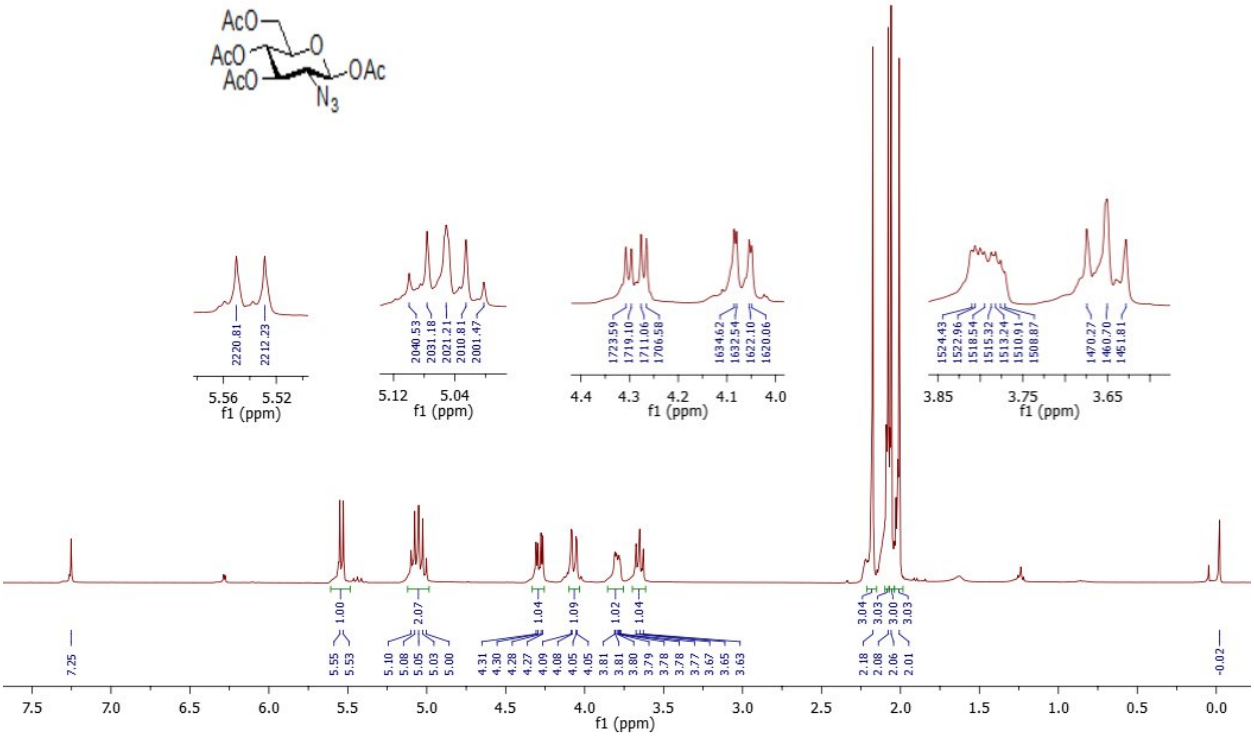


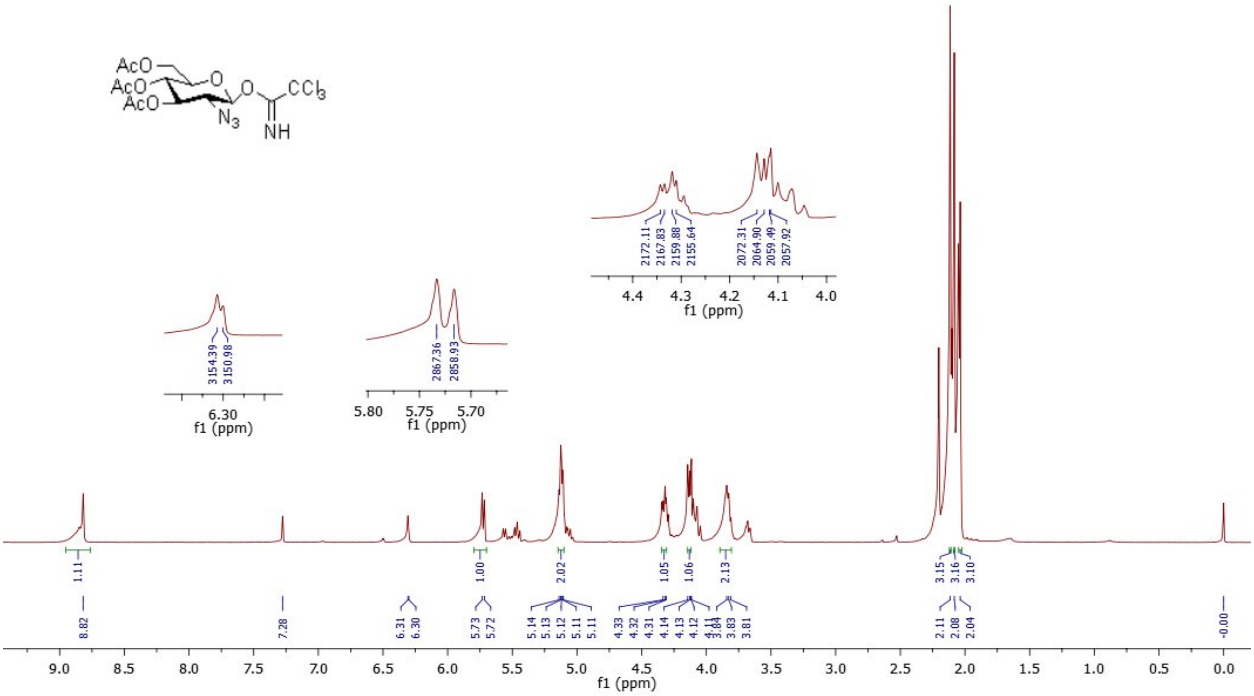
Fig 4: Dose response of **23b** on the % inhibition of GPI-NBD by PI-PLC. Fig represents the average data (n=5) for % inhibition of GPI-NBD cleavage by PI-PLC in presence of **23b**

3. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and Mass Spectrogram:

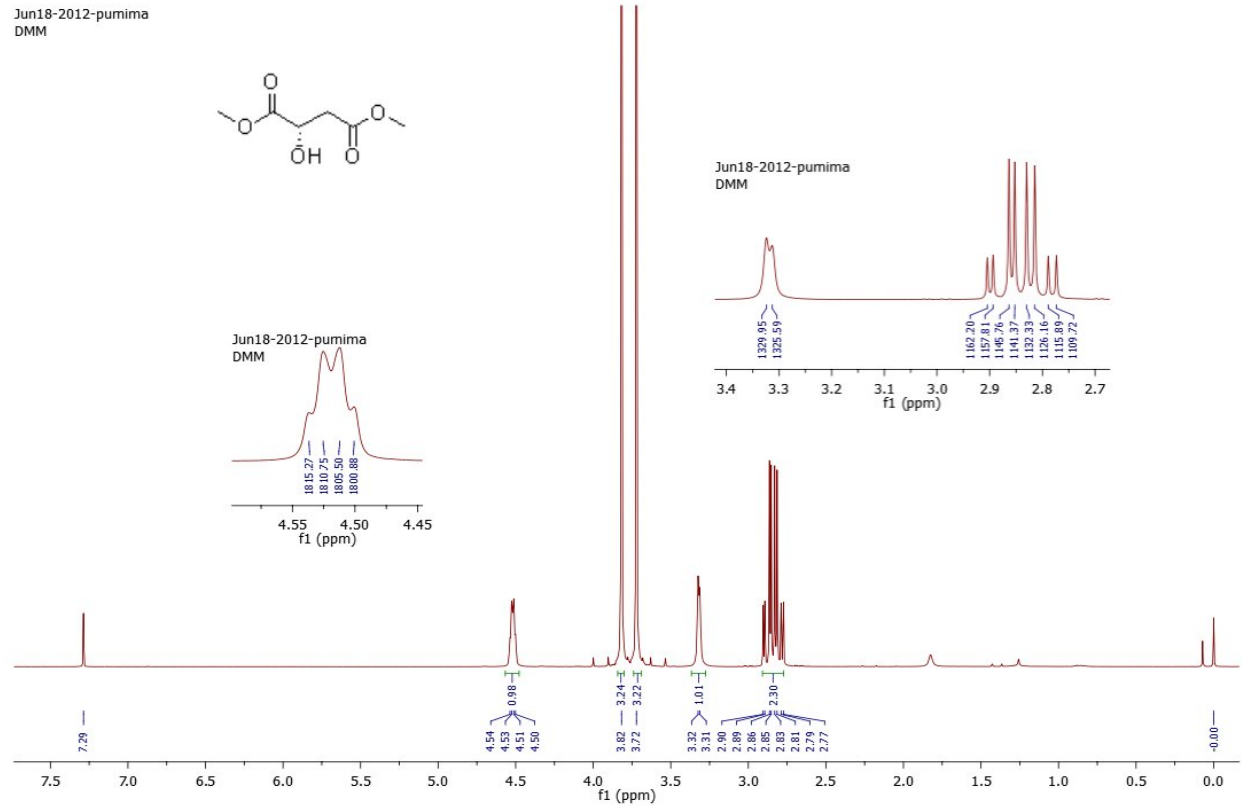
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 4:



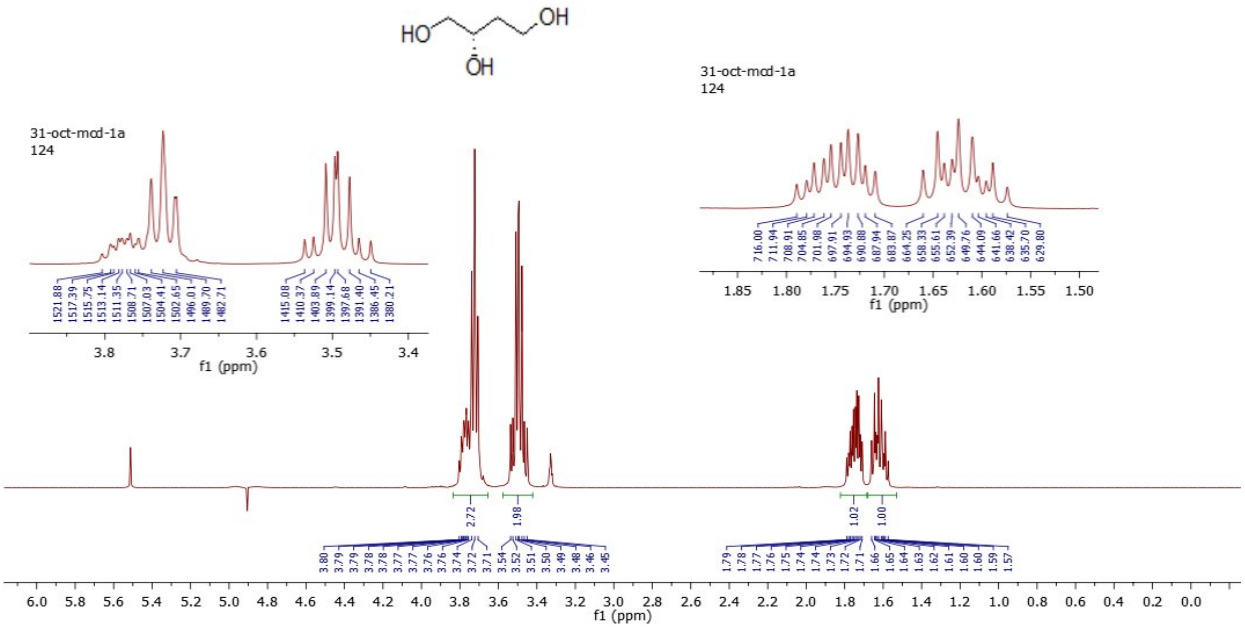
^{1}H NMR spectrogram ($CDCl_3$, 500 MHz) of compound 6:



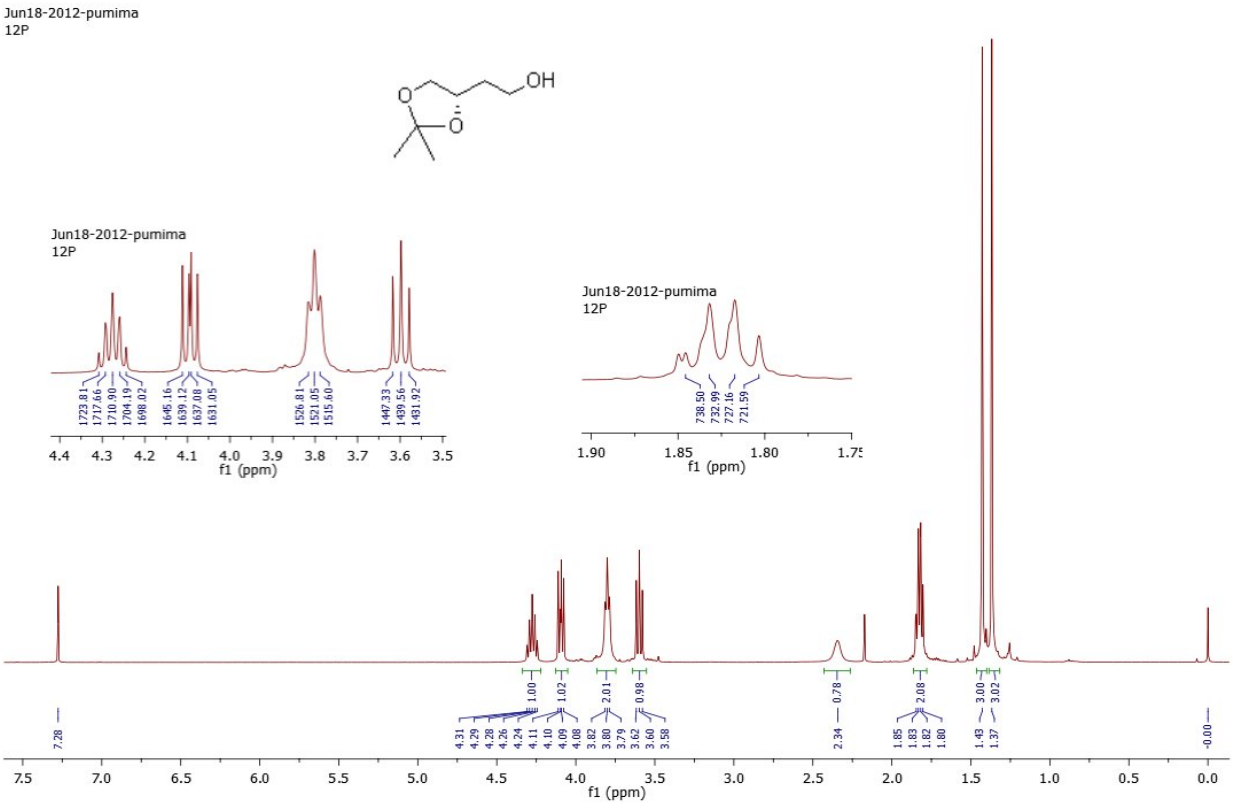
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 11:



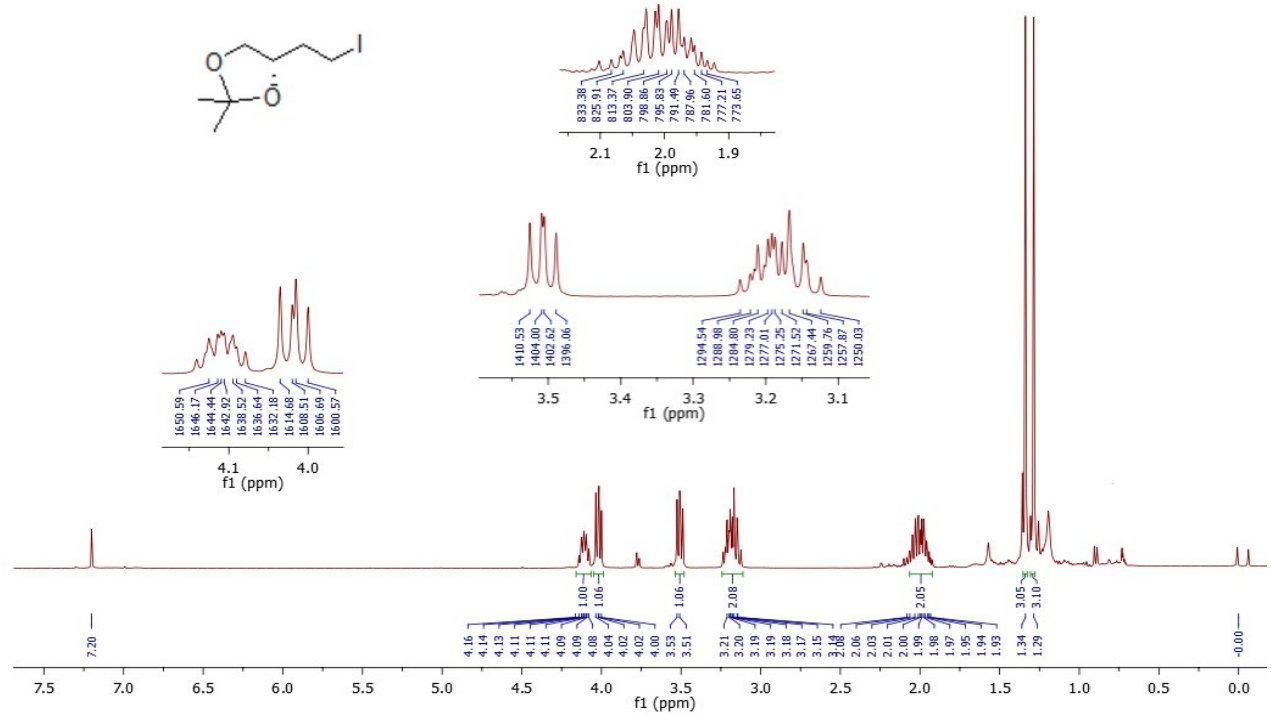
^{1}H NMR spectrogram (CD_3OD, 400 MHz) of compound 12:



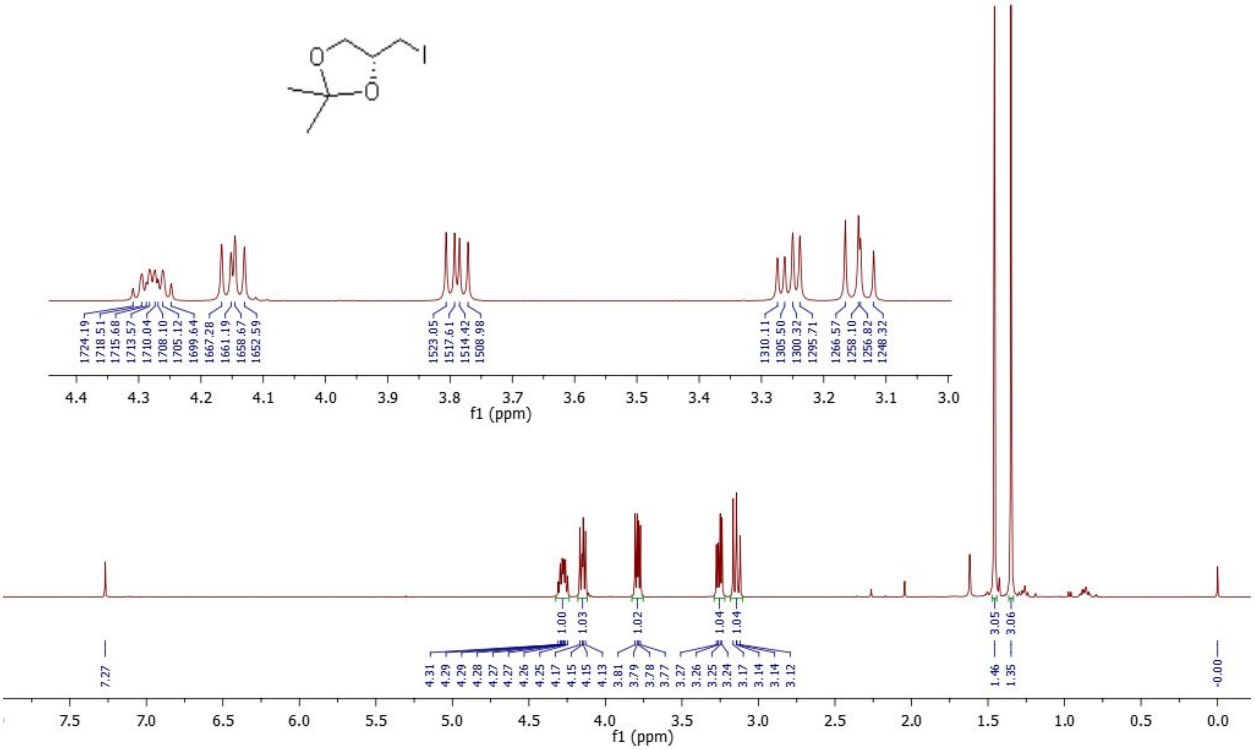
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 13:



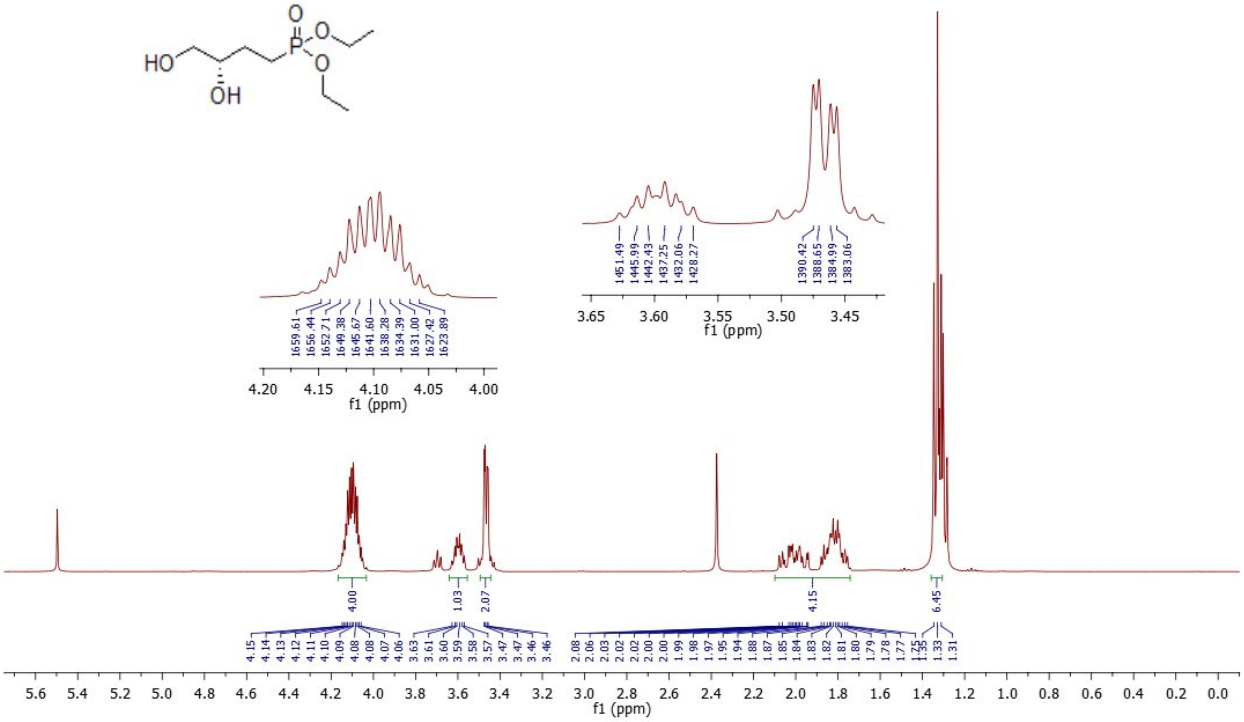
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 14a:



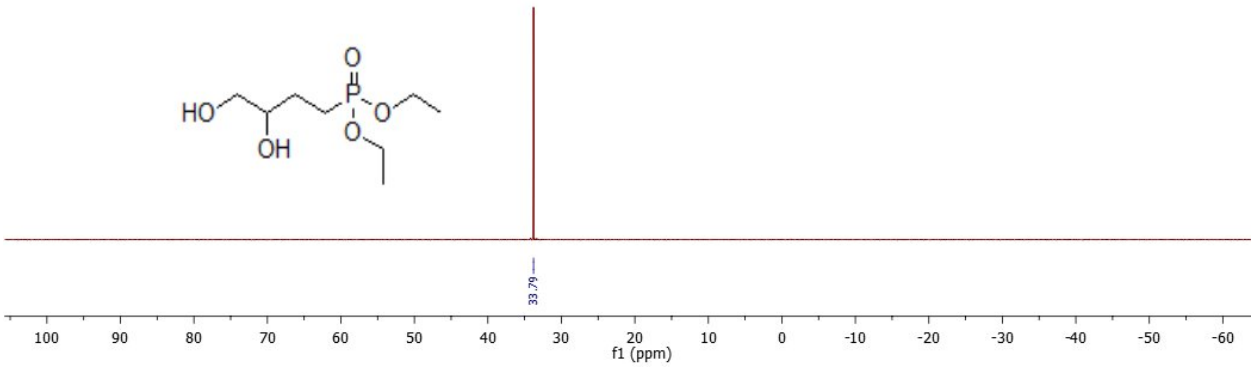
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 14b:



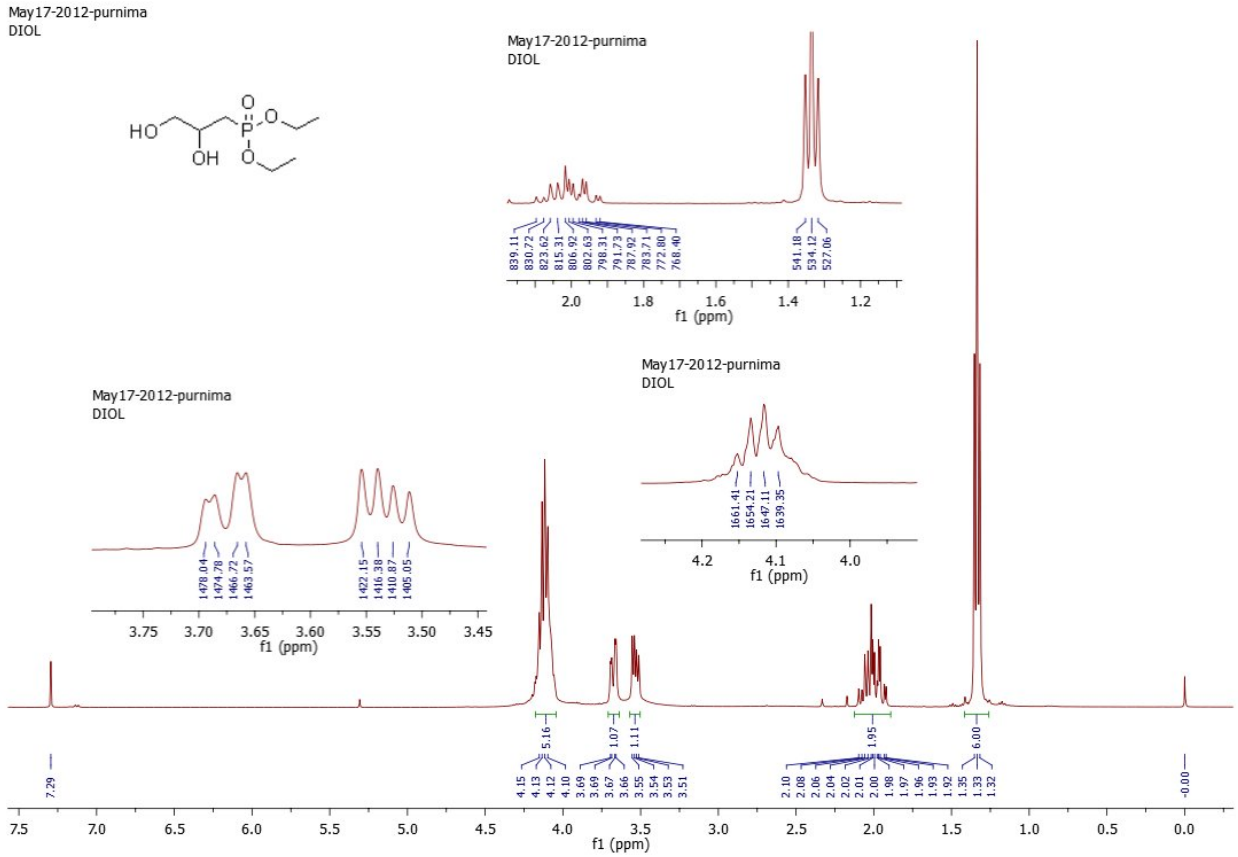
^{1}H NMR spectrogram (CD_3OD, 400 MHz) of compound 16a:



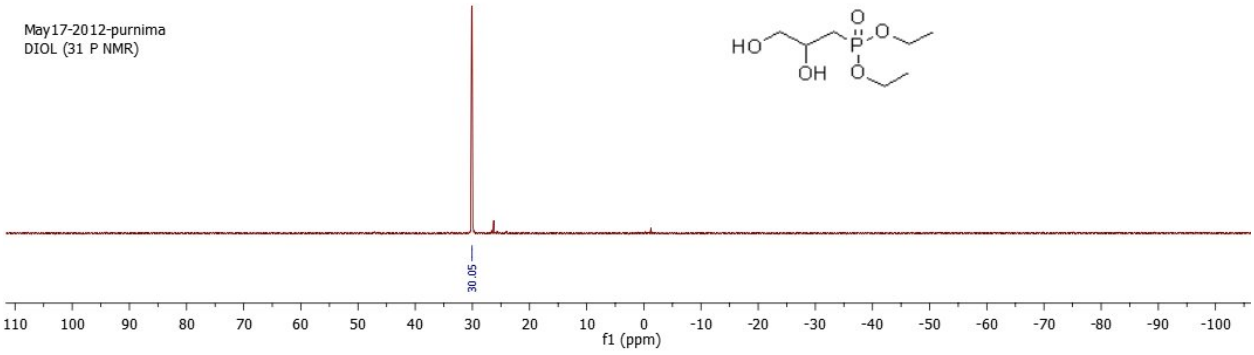
^{31}P NMR spectrogram (CD_3OD, 161 MHz) of compound 16a:



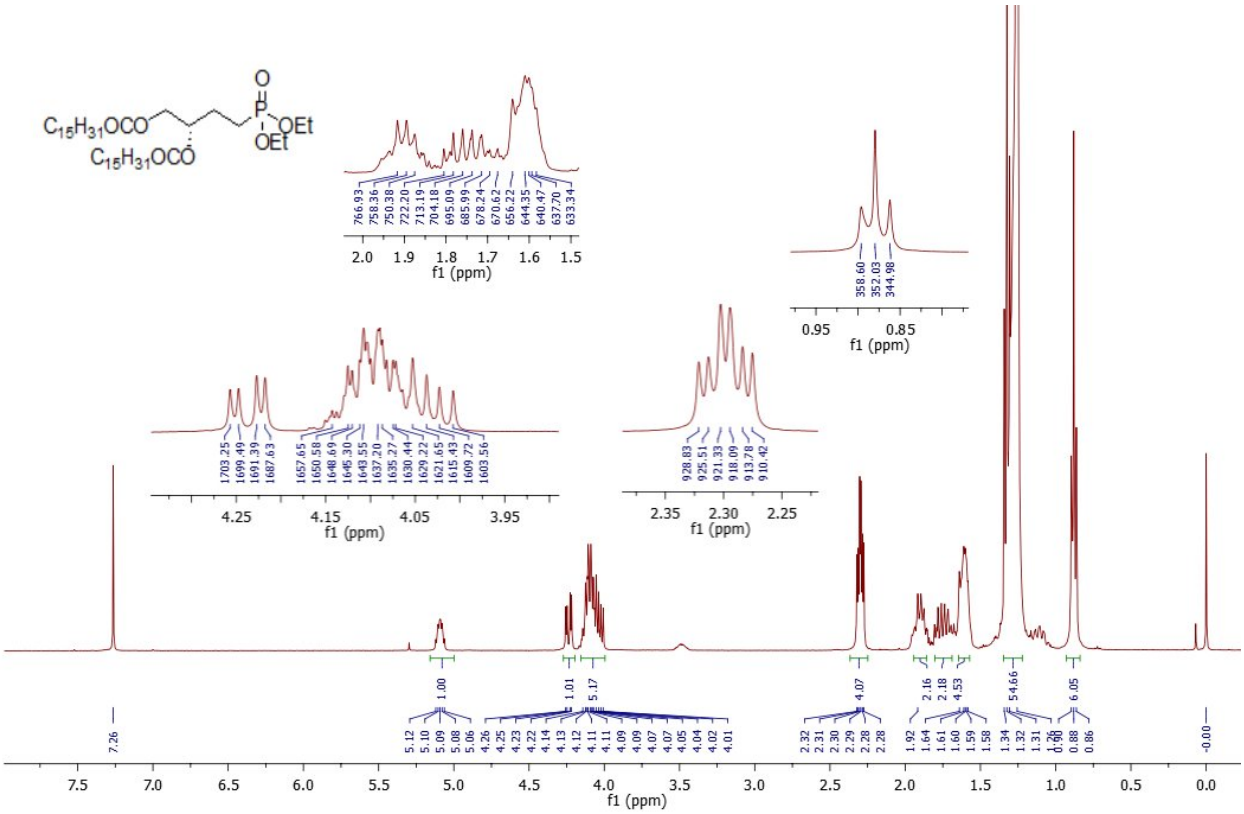
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 16b:



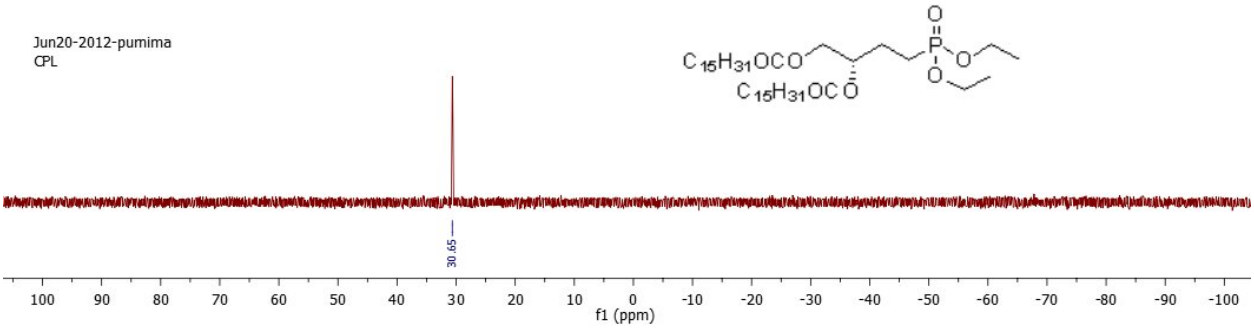
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 16b:



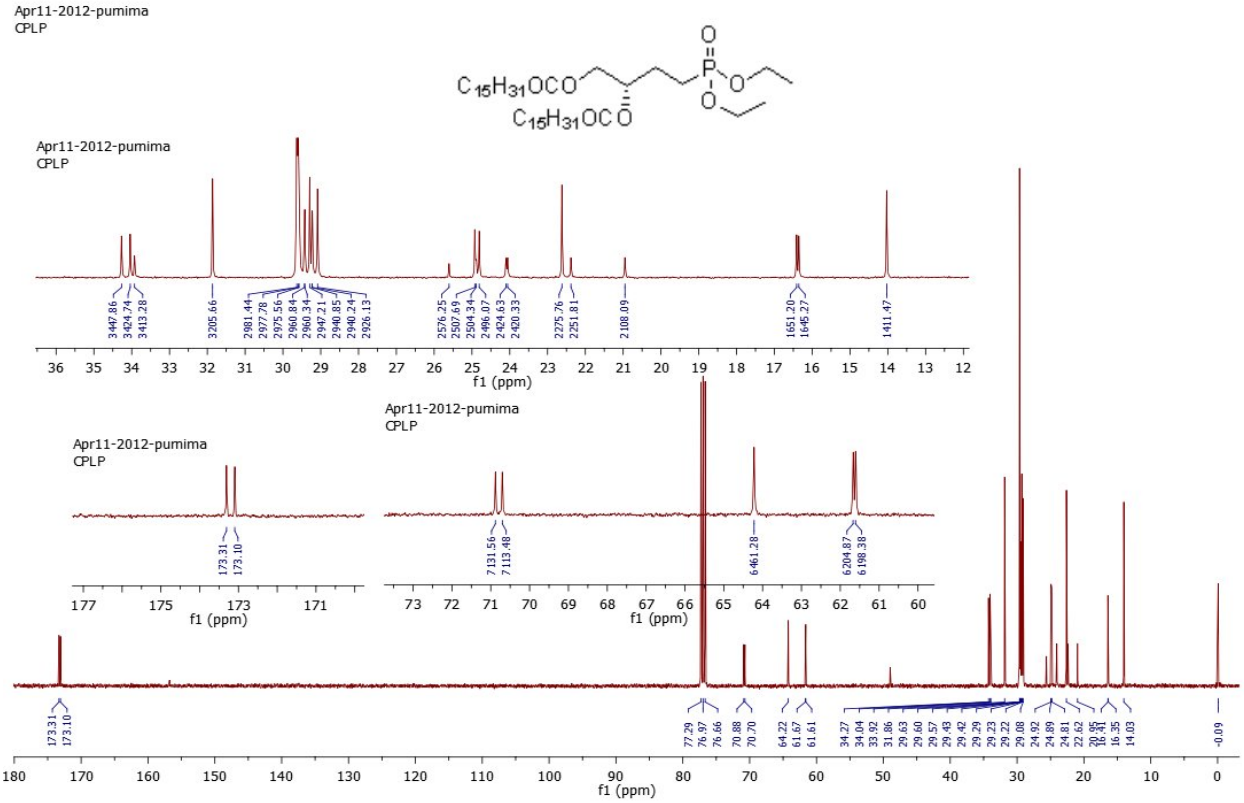
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 17a:



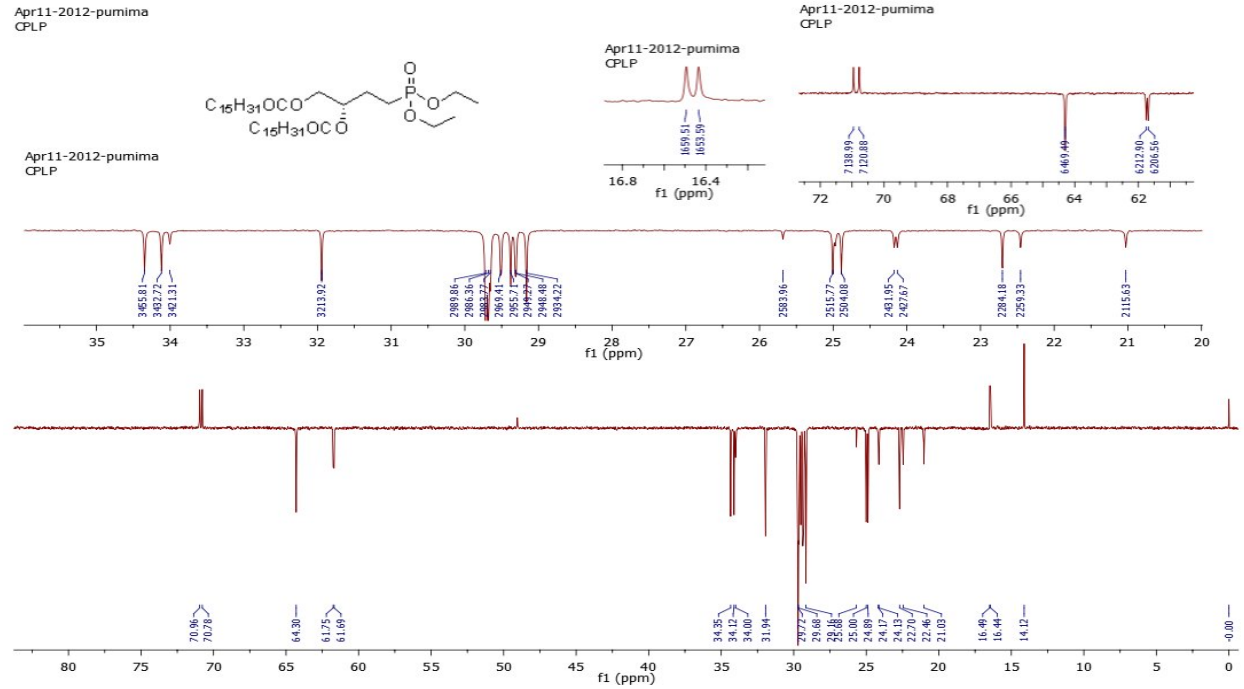
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 17a:



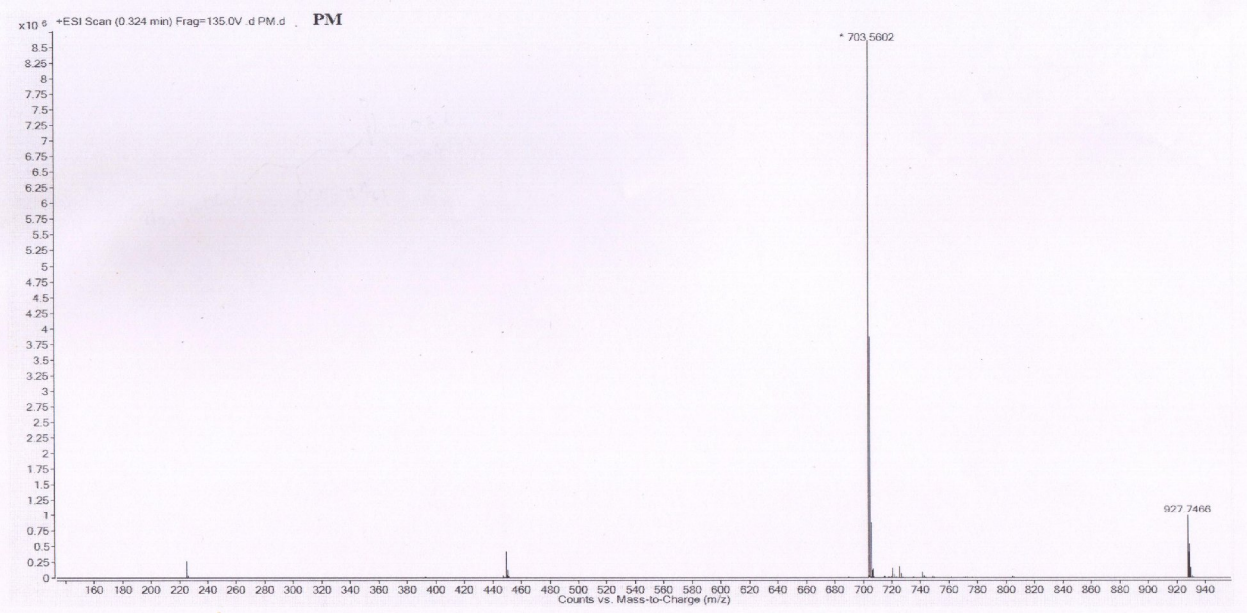
^{13}C NMR spectrogram ($CDCl_3$, 100 MHz) of compound 17a:



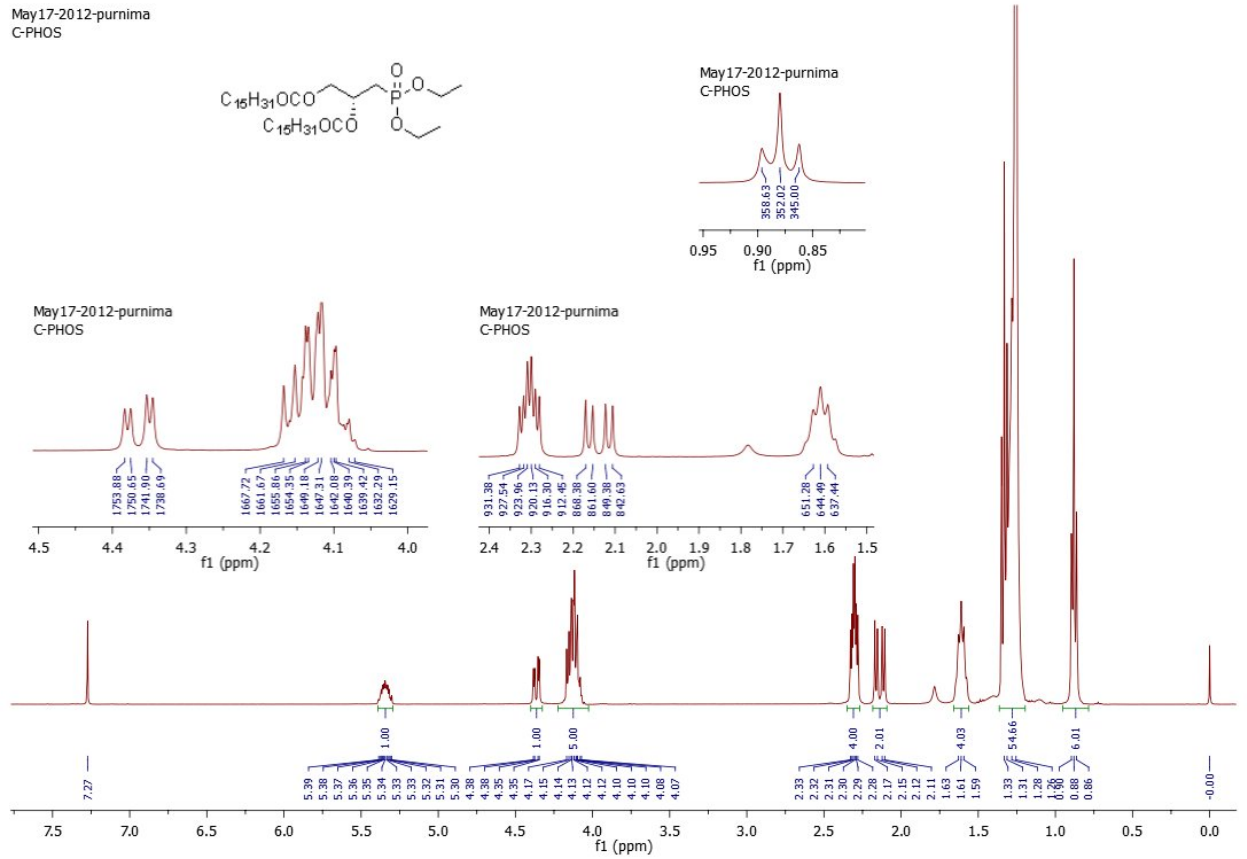
DEPT spectrogram ($CDCl_3$, 100 MHz) of compound 17a:



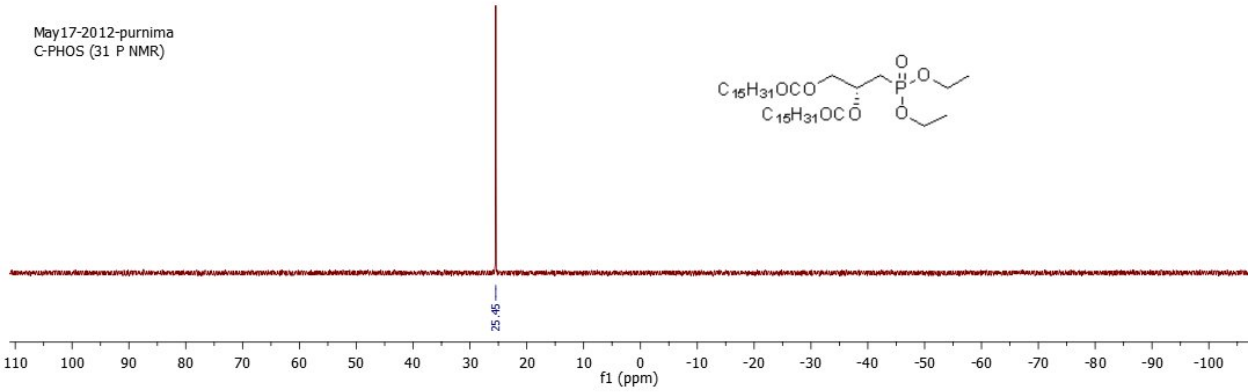
HRMS (ESI) spectrogram of compound 17a:



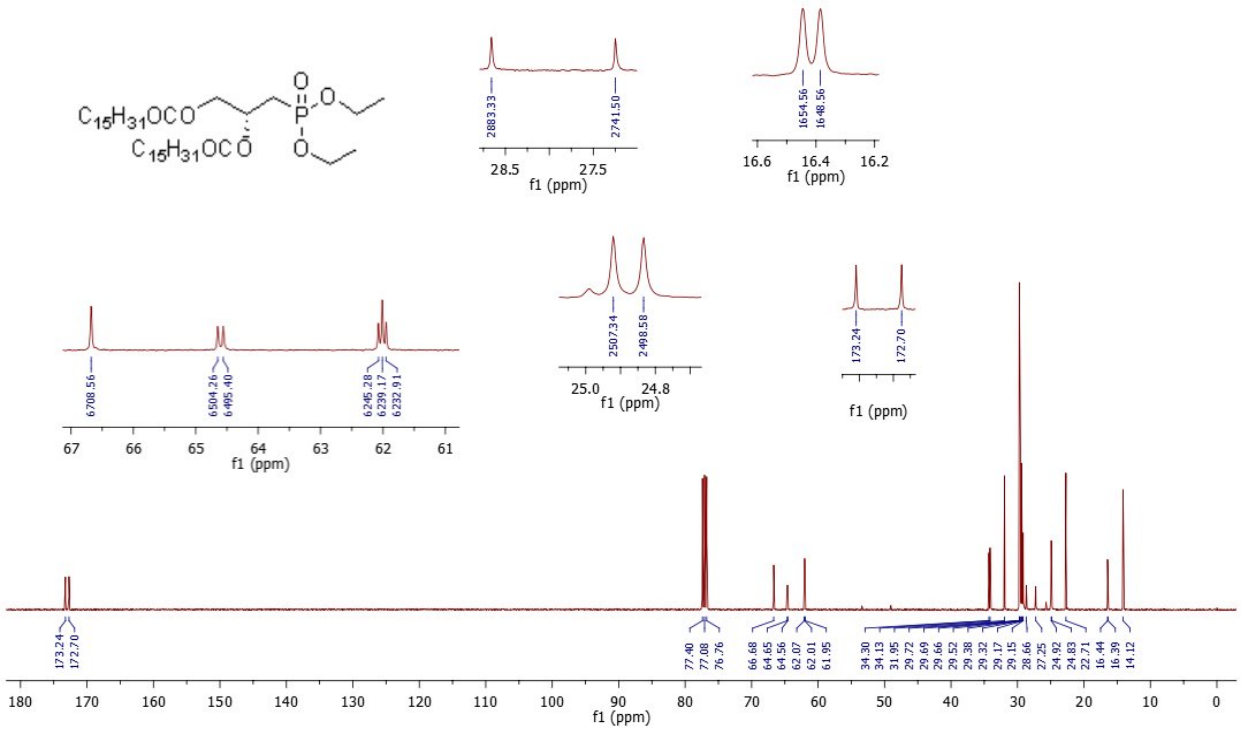
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 17b:



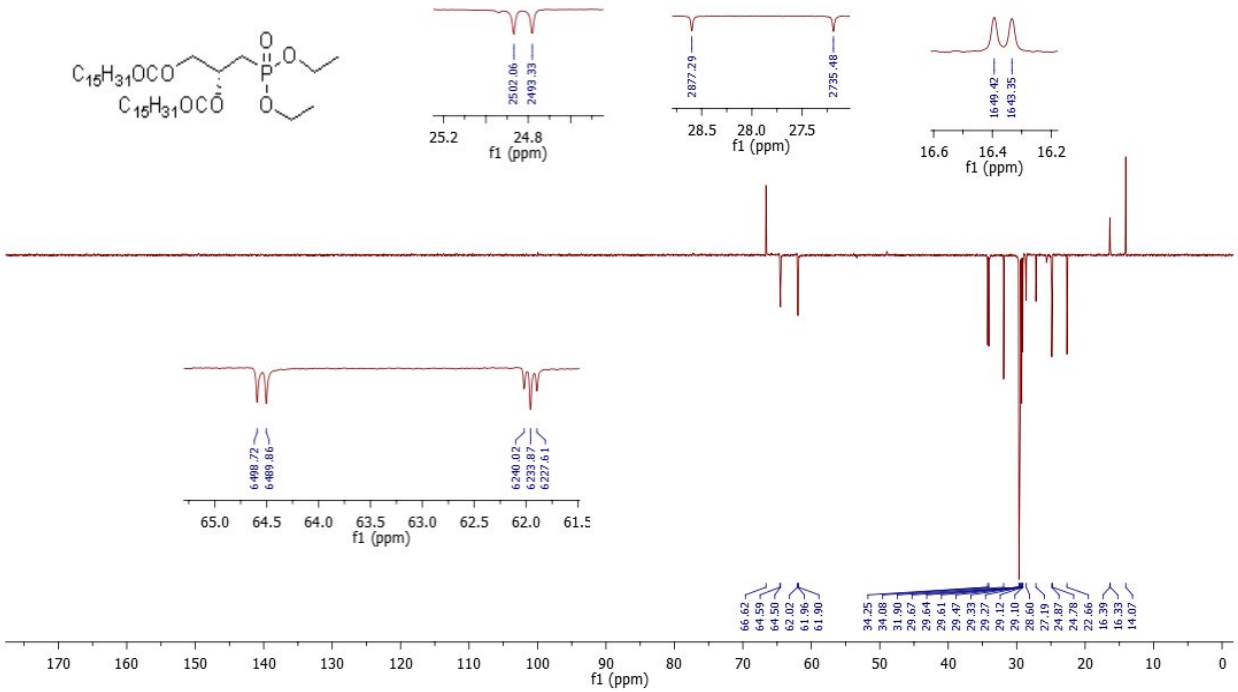
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 17b:



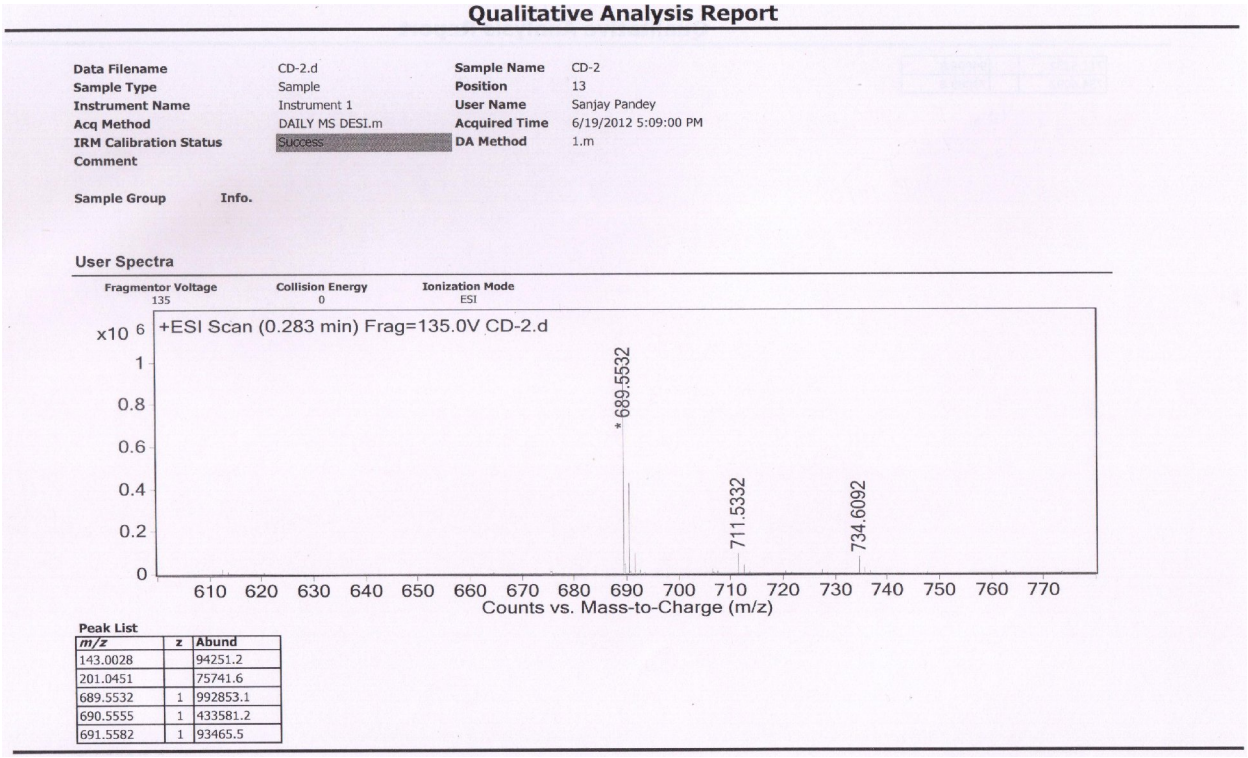
^{13}C NMR spectrogram ($CDCl_3$, 100 MHz) of compound 17b:



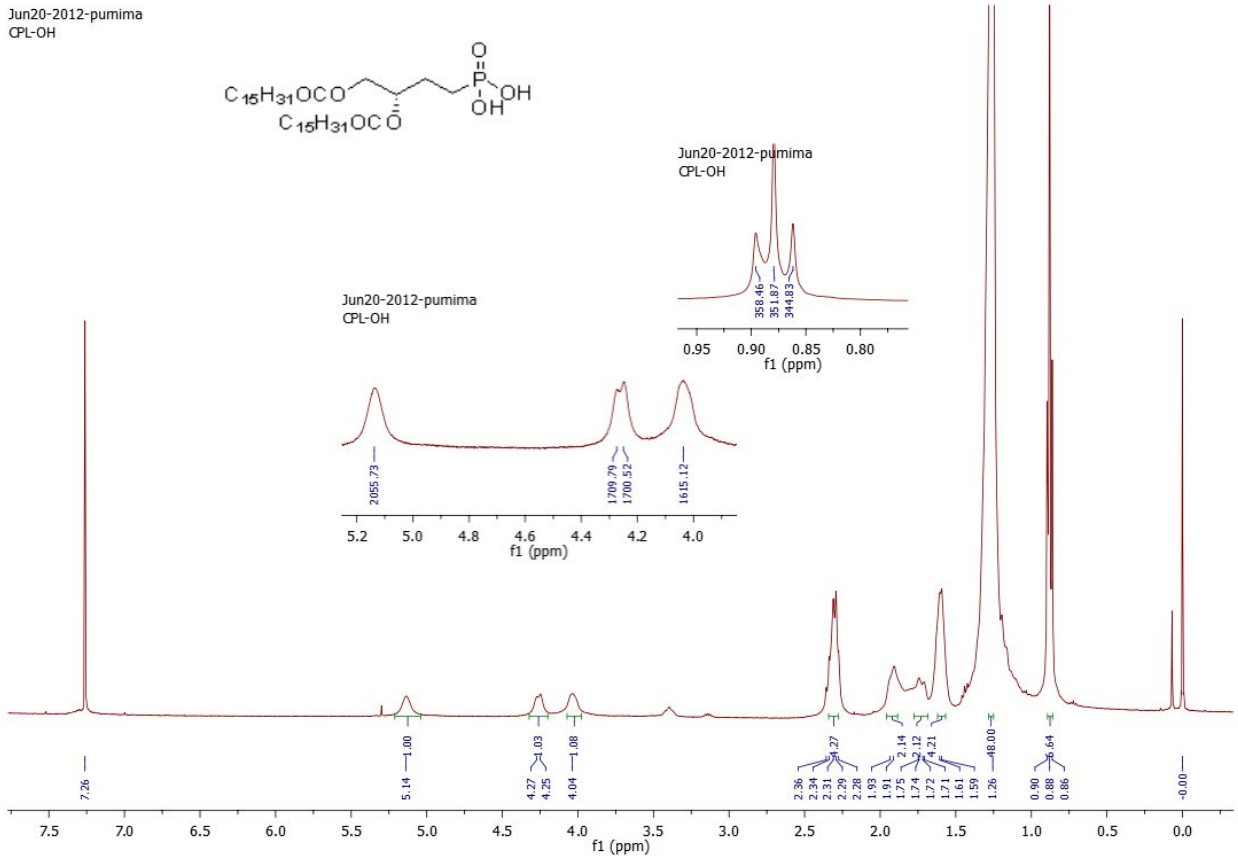
DEPT spectrogram ($CDCl_3$, 100 MHz) of compound 17b:



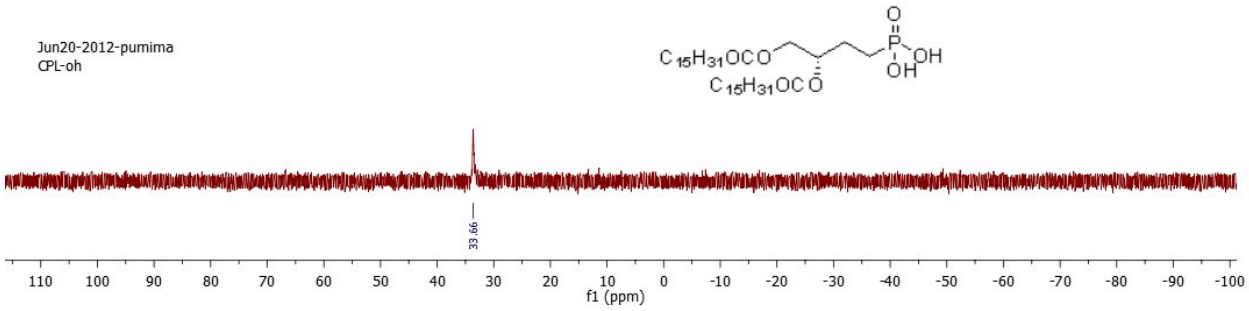
HRMS (ESI) spectrogram of compound 17b:



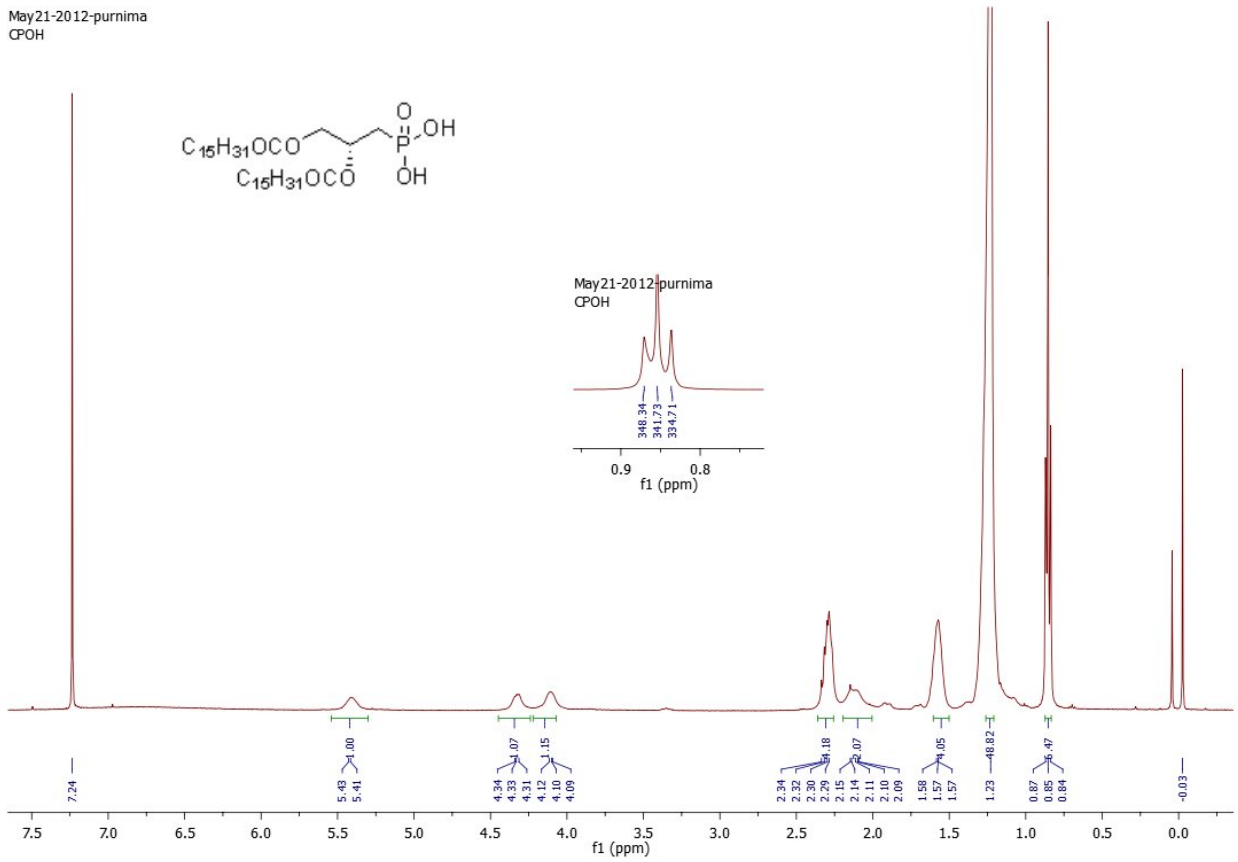
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 18a:



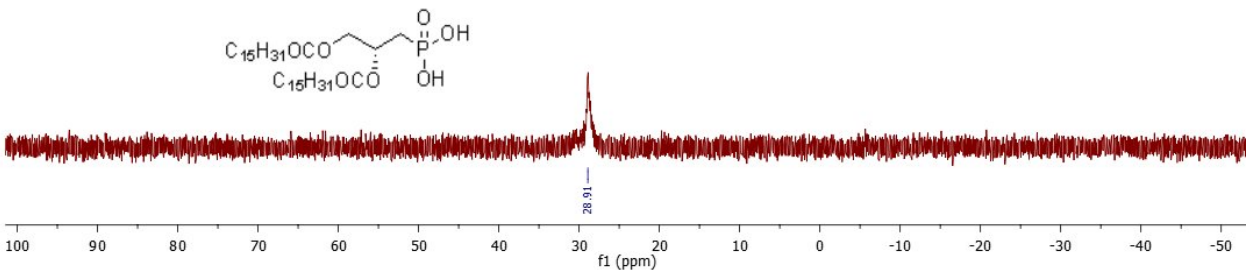
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 18a:



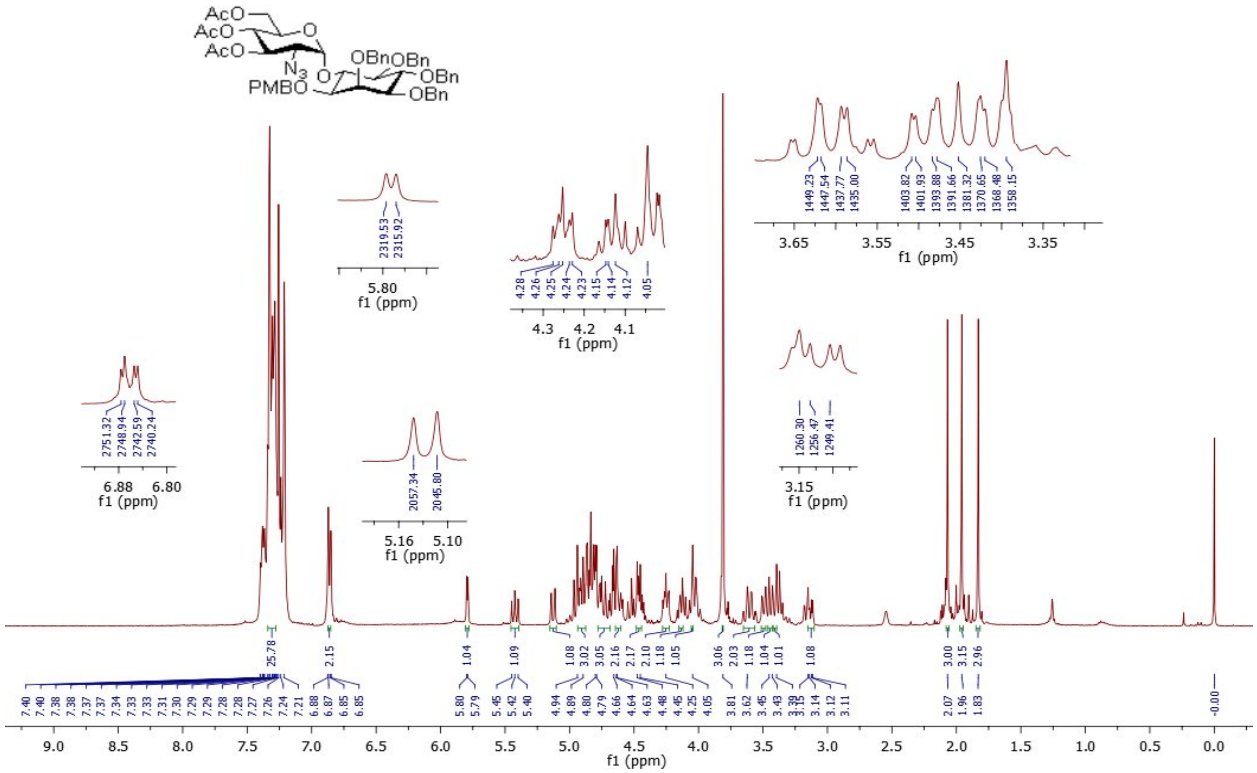
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 18b:



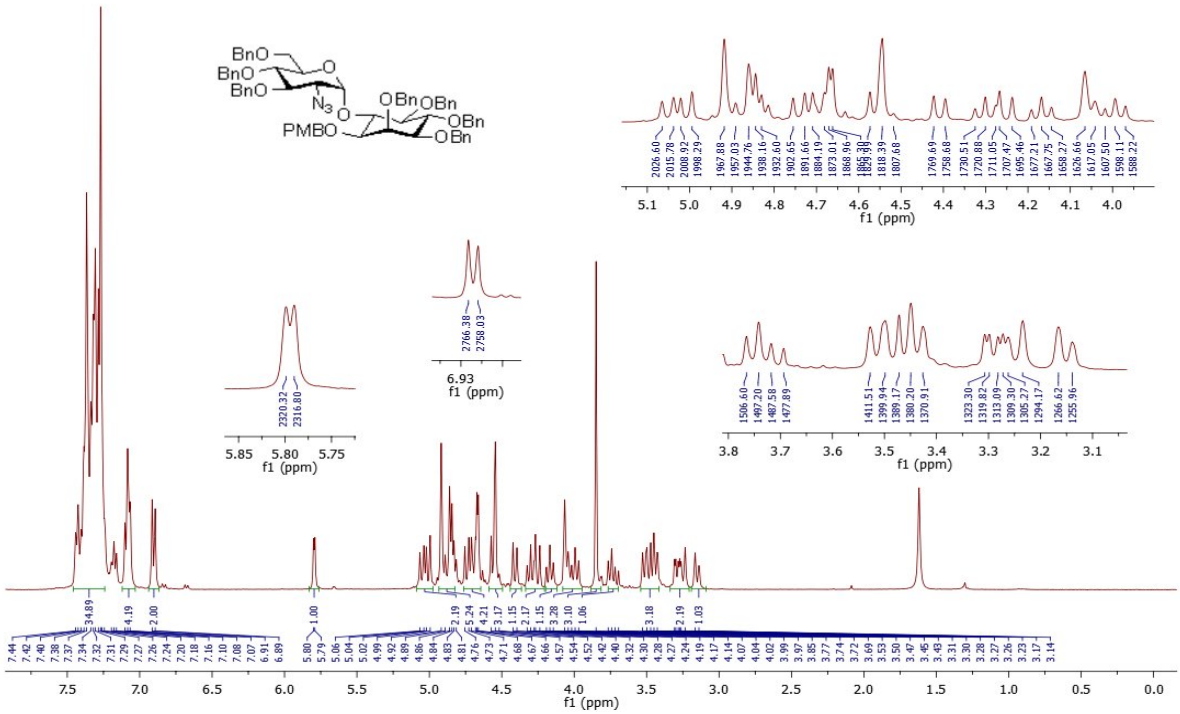
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 18b:



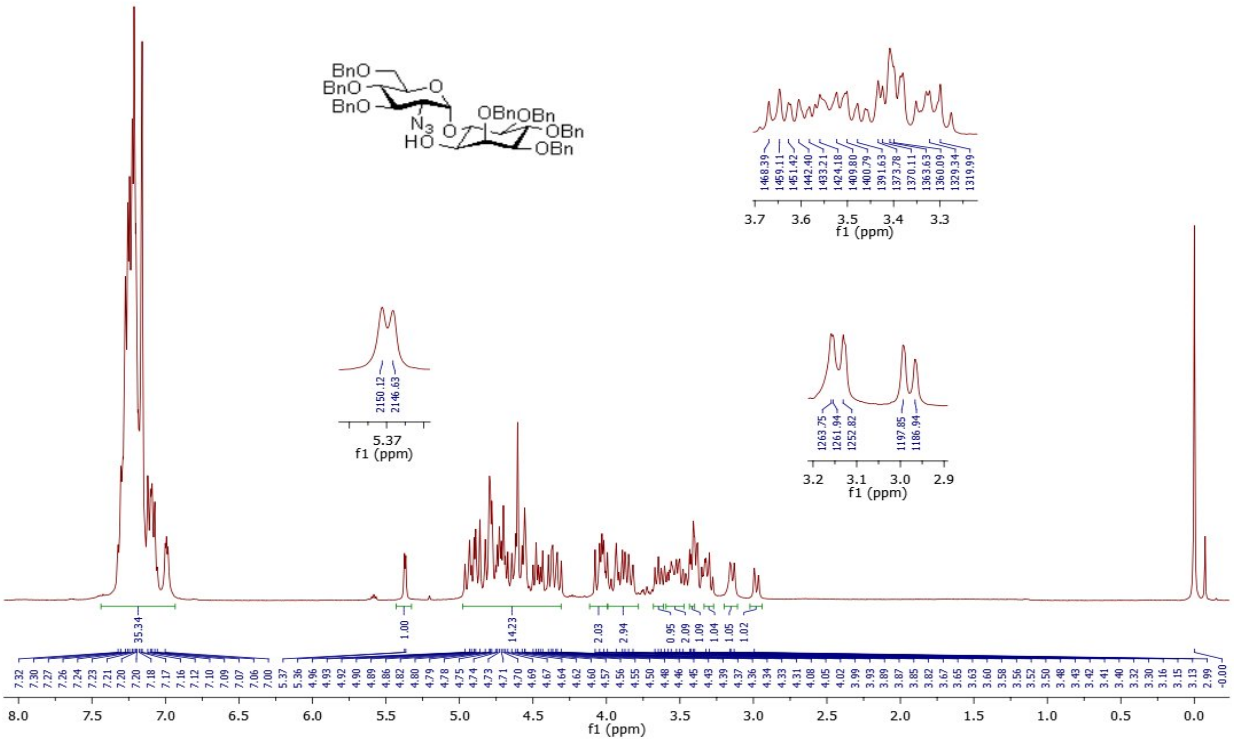
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 19:



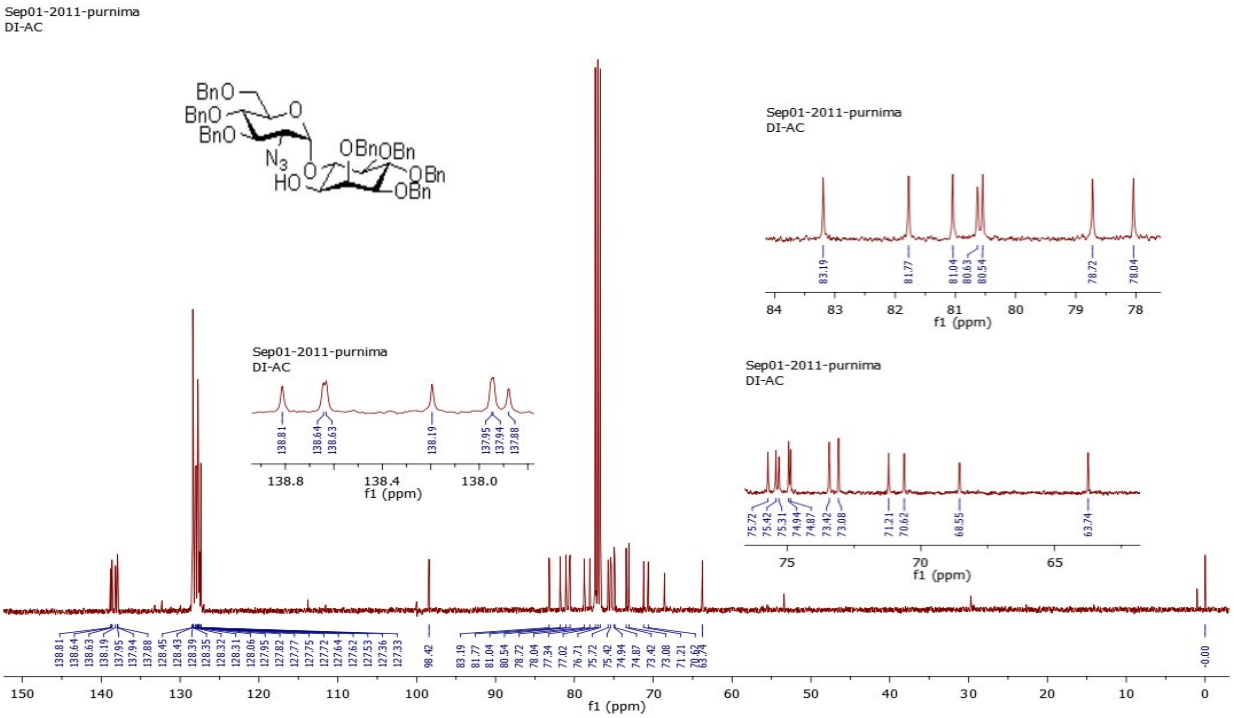
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 20:



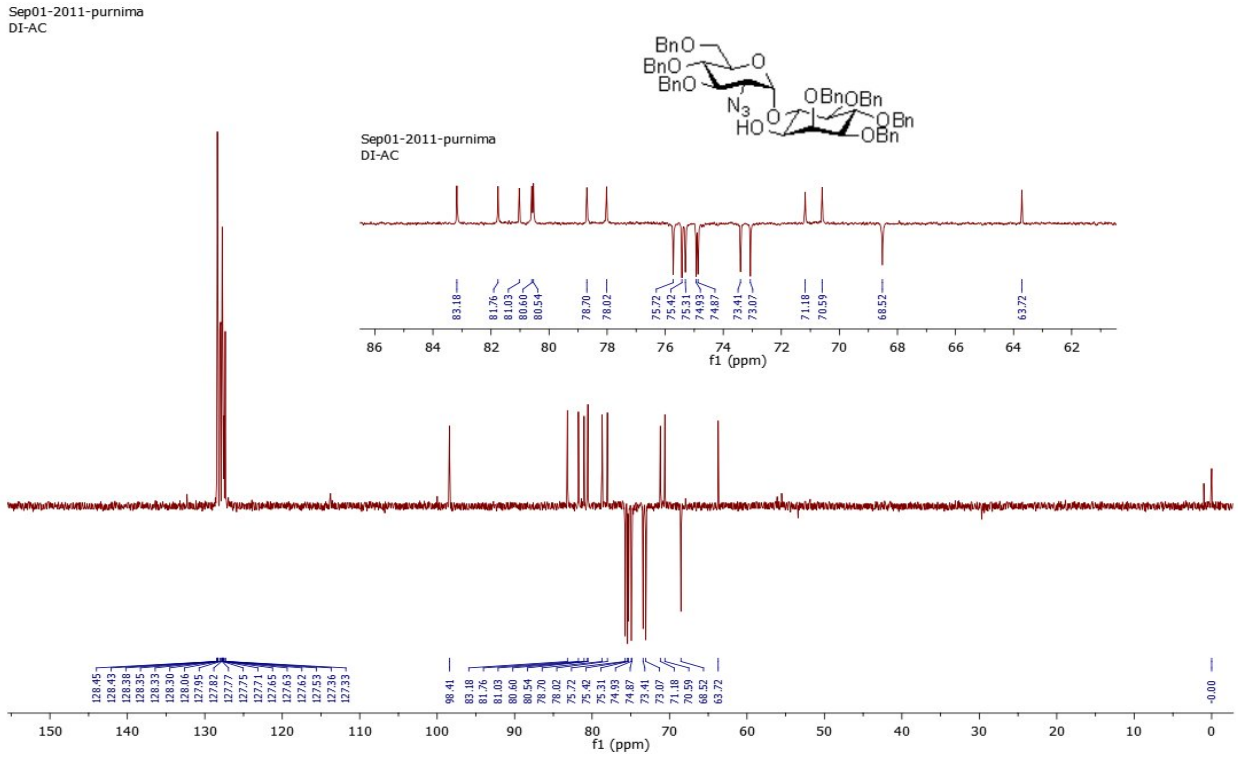
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 21:



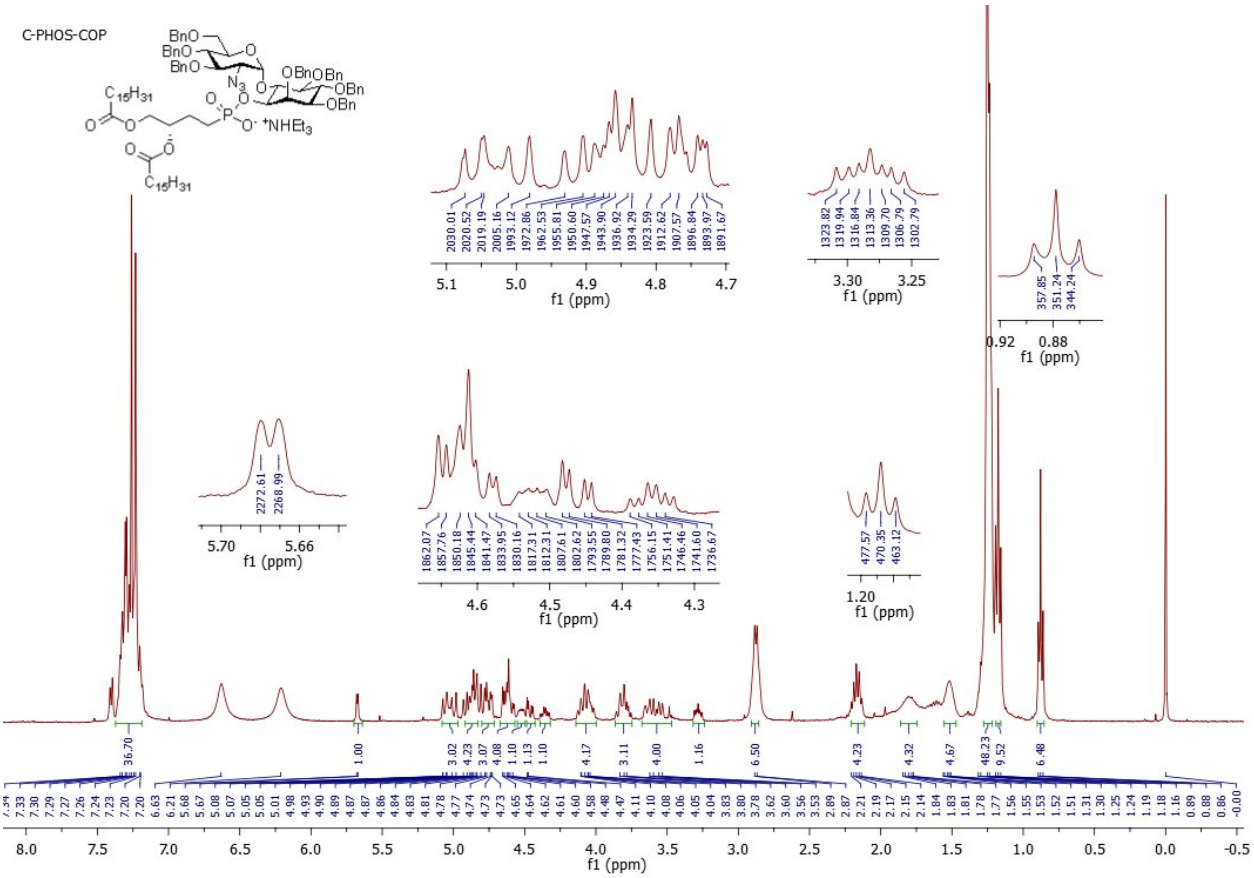
^{13}CNMR spectrogram ($CDCl_3$, 100 MHz) of compound 21:



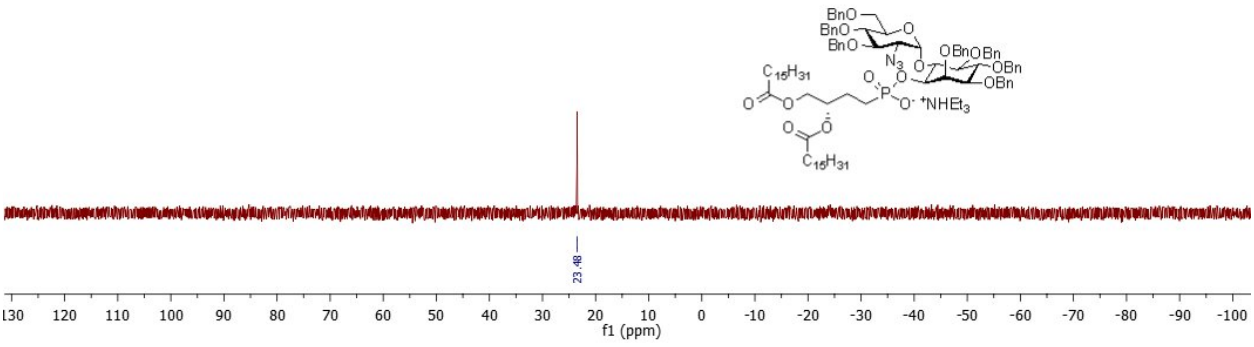
DEPT spectrogram ($CDCl_3$, 100 MHz) of compound 21:



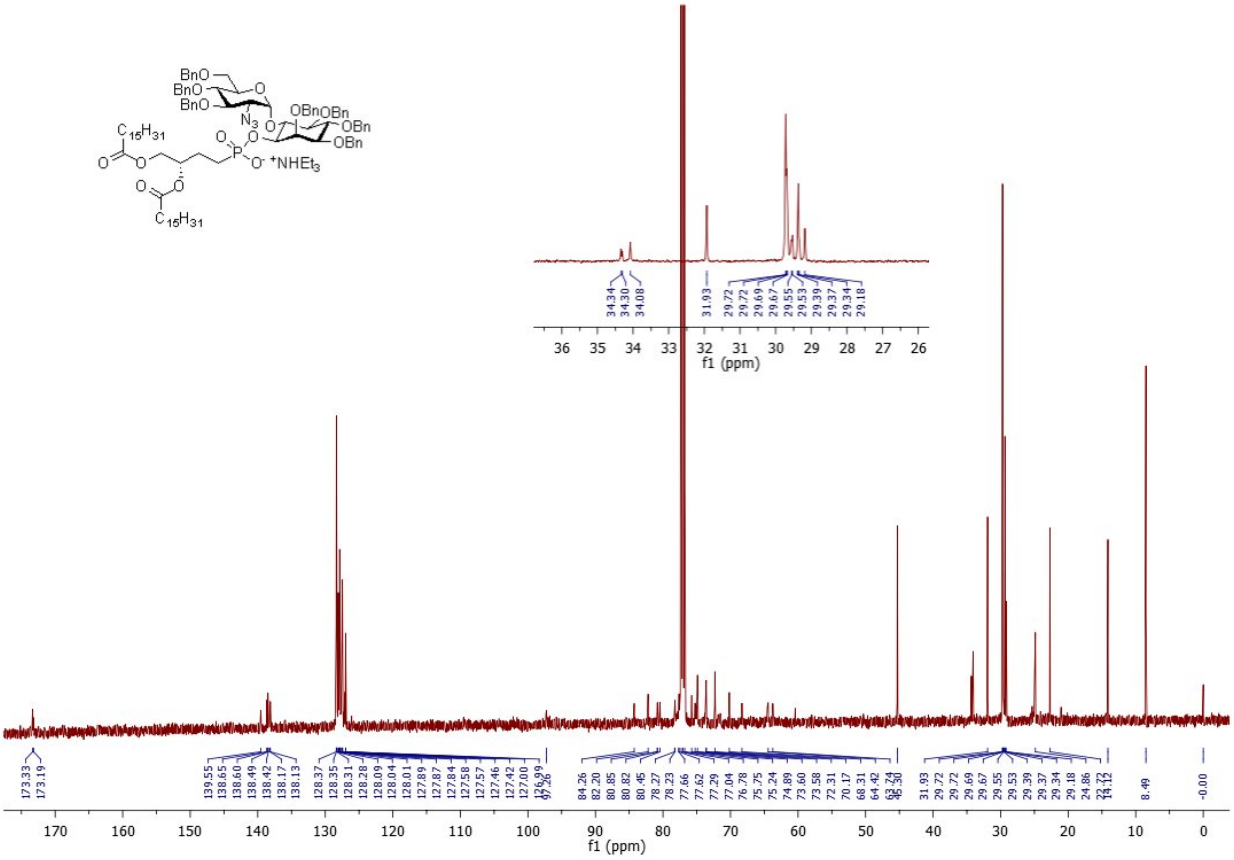
^{1}H NMR spectrogram ($CDCl_3$, 400 MHz) of compound 22a:



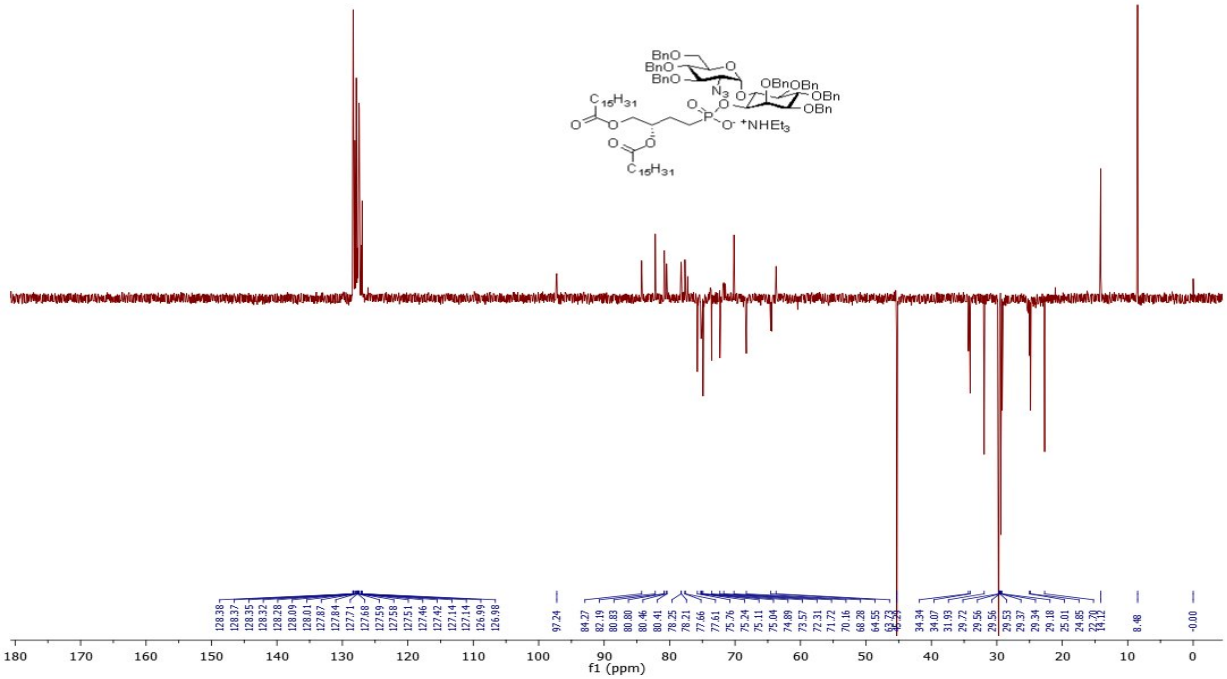
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 22a:



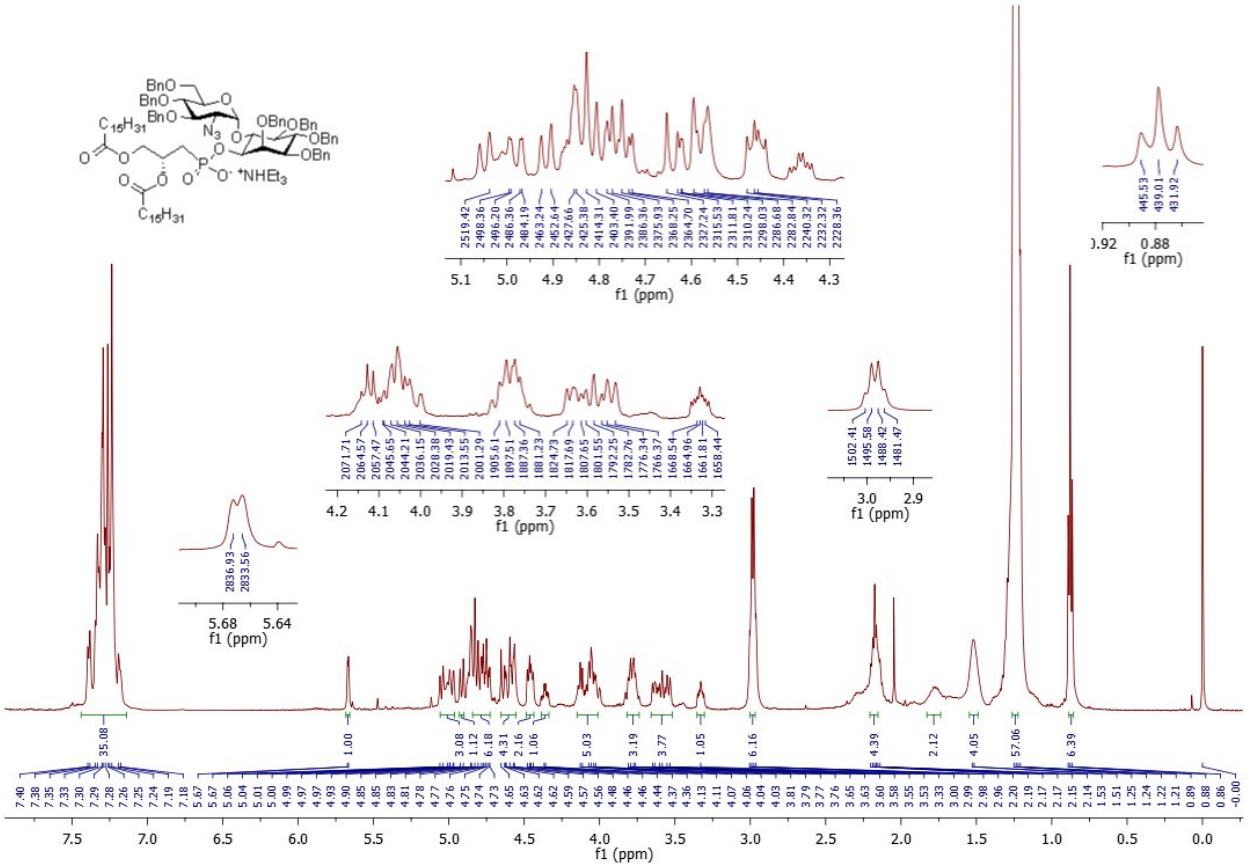
^{13}C NMR spectrogram ($CDCl_3$, 100 MHz) of compound 22a:



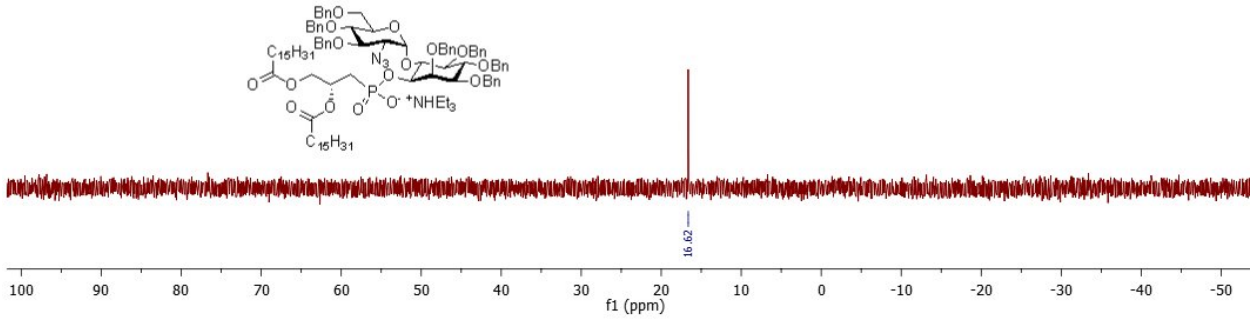
DEPT spectrogram ($CDCl_3$, 100 MHz) of compound 22a:



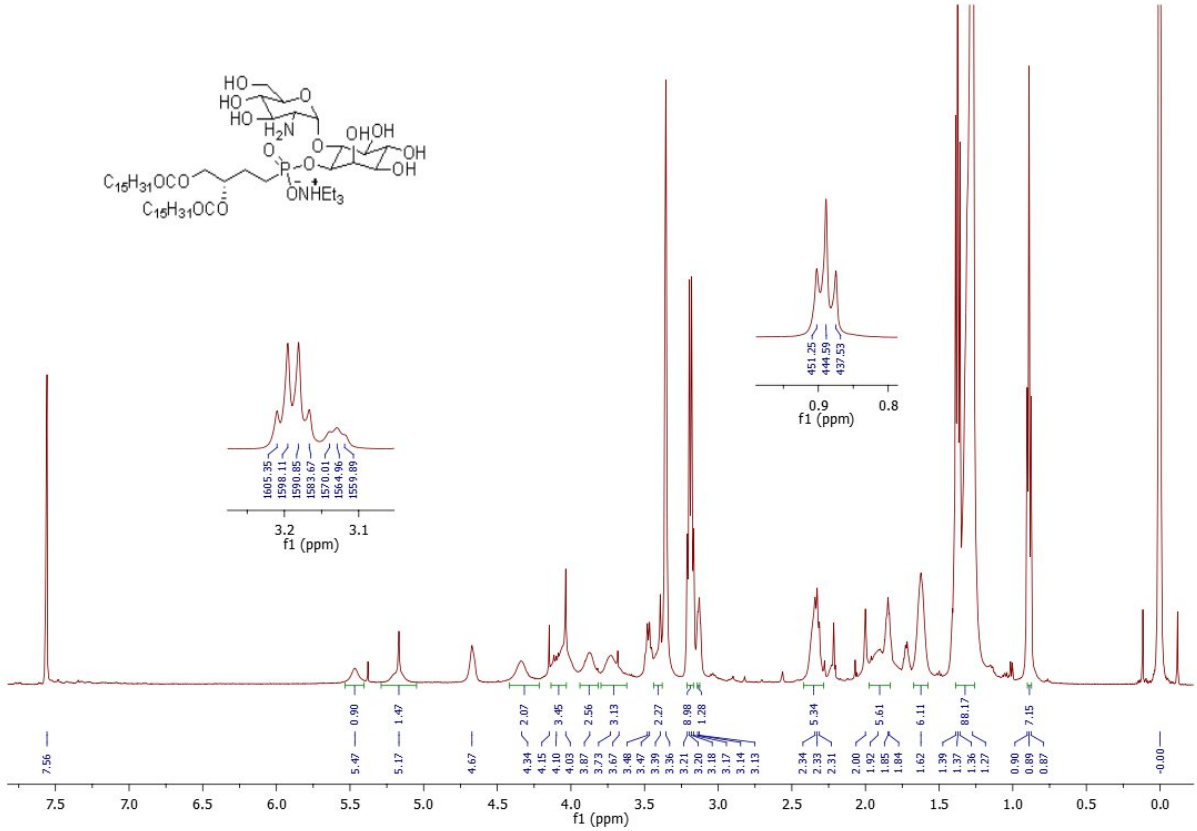
^{1}H NMR spectrogram ($CDCl_3$, 500 MHz) of compound 22b:



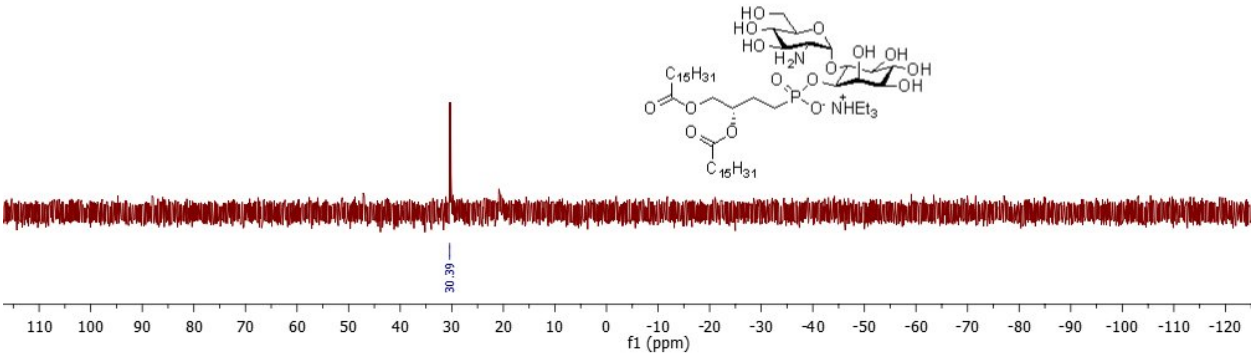
^{31}P NMR spectrogram ($CDCl_3$, 161 MHz) of compound 22b:



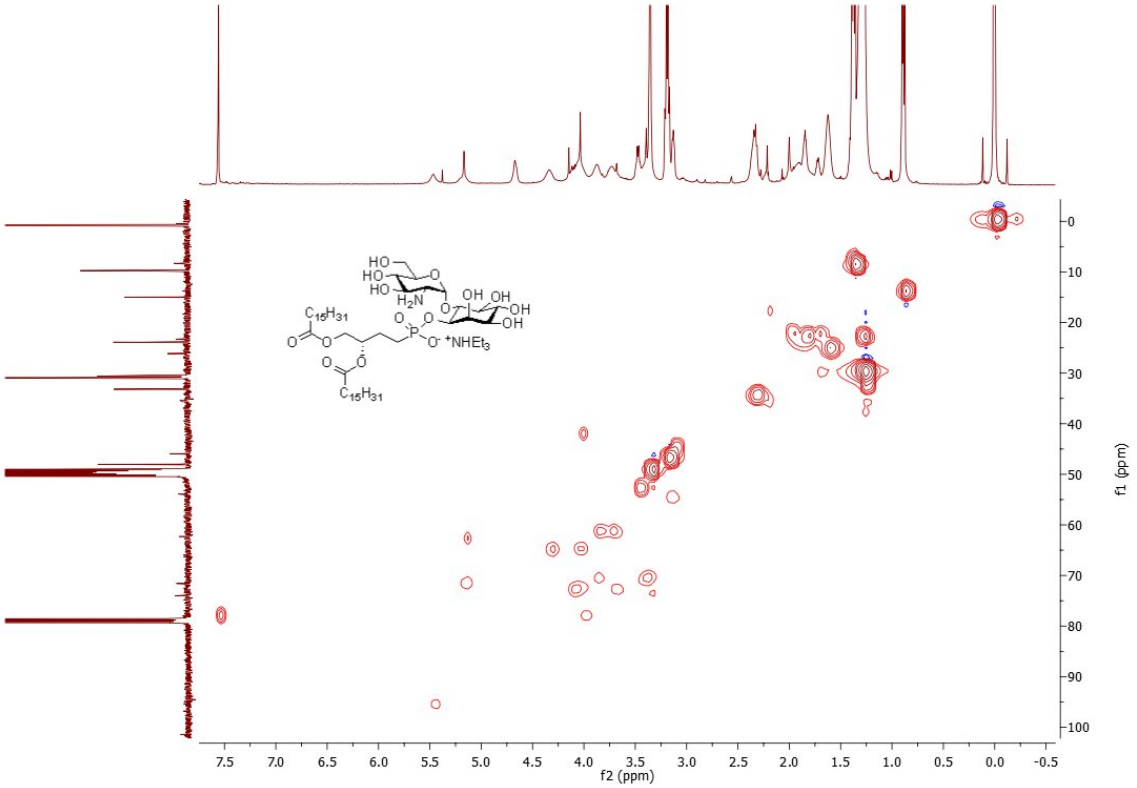
^{1}H NMR spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 400 MHz) of compound 23a:



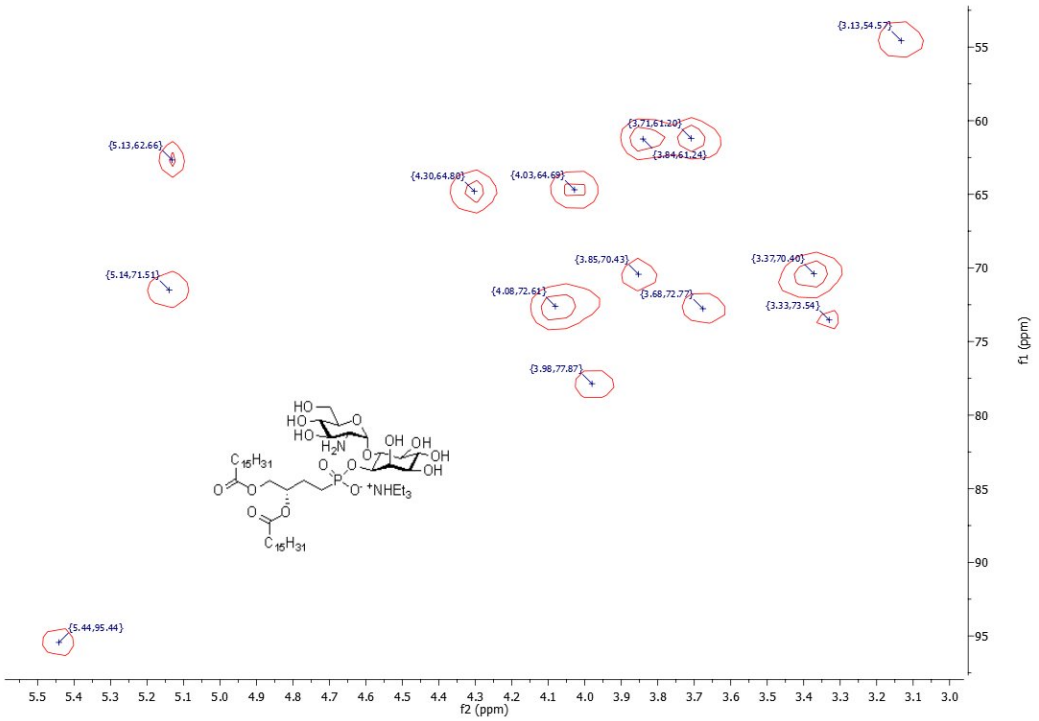
^{31}P NMR spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 161 MHz) of compound 23a:



HSQC spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 400 MHz) of compound 23a:



HSQC spectrogram (expansion) of compound 23a:



HRMS (ESI) spectrogram of compound 23a:

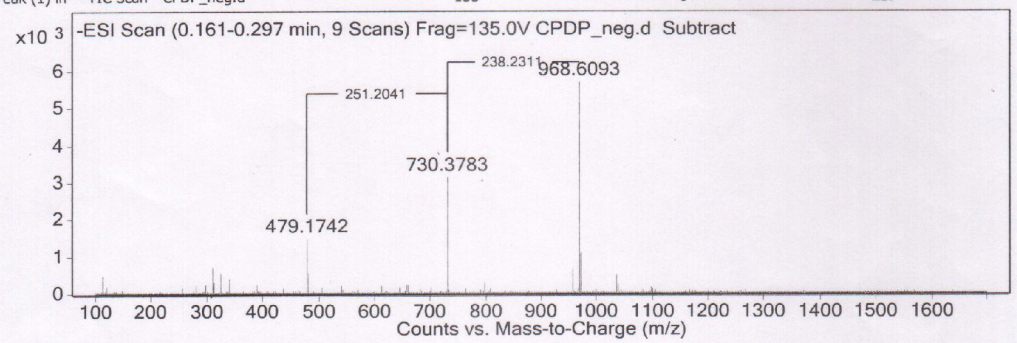
Qualitative Analysis Report

Data Filename	CPDP_neg.d	**Sample Name**	CPDP_neg
Sample Type	Sample	**Position**	Vial 1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	23-05-2012 11:03:41
IRM Calibration Status	Success	**DA Method**	23_may_2012.m
Comment			

Sample Group	**Info.**
User Defined 1	**User Defined 2**

User Spectra

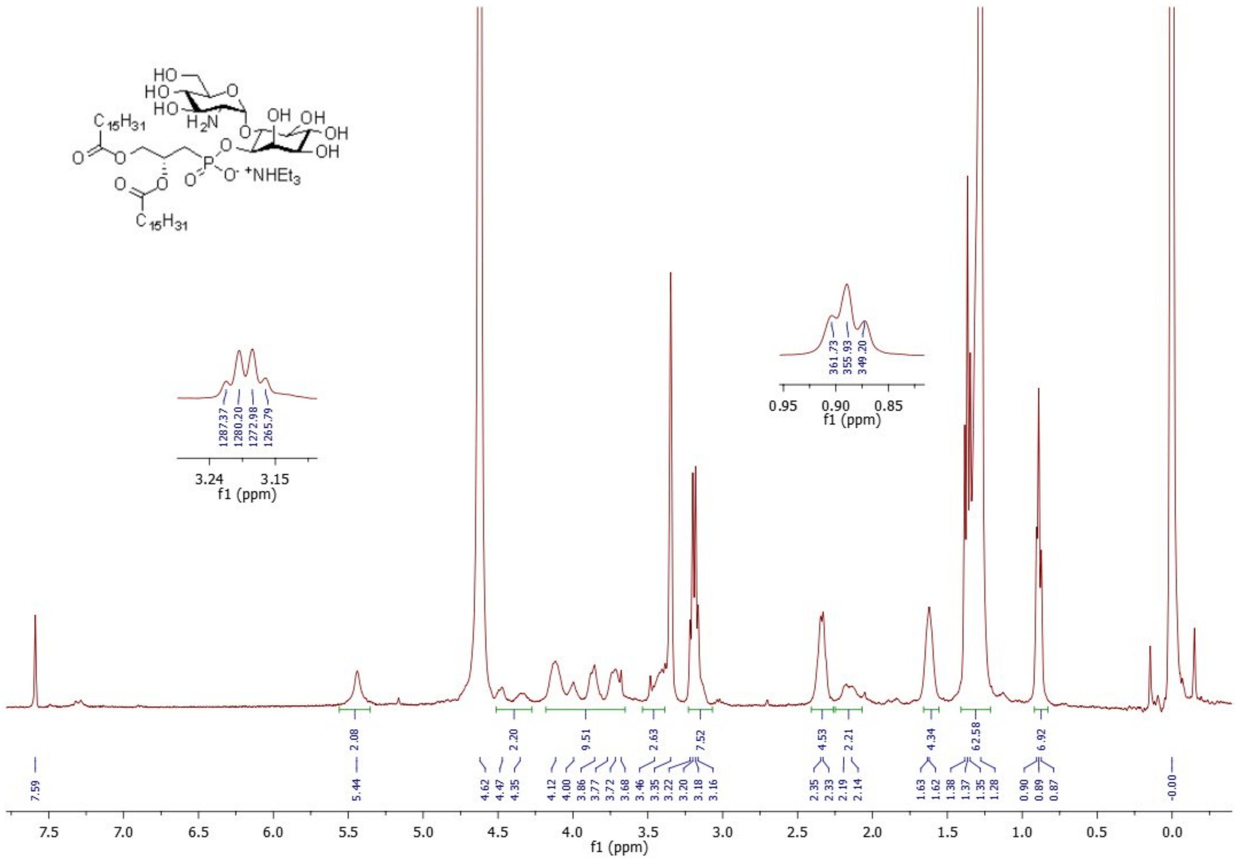
Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
Peak (1) in "- TIC Scan - CPDP_neg.d"	135	0	ESI



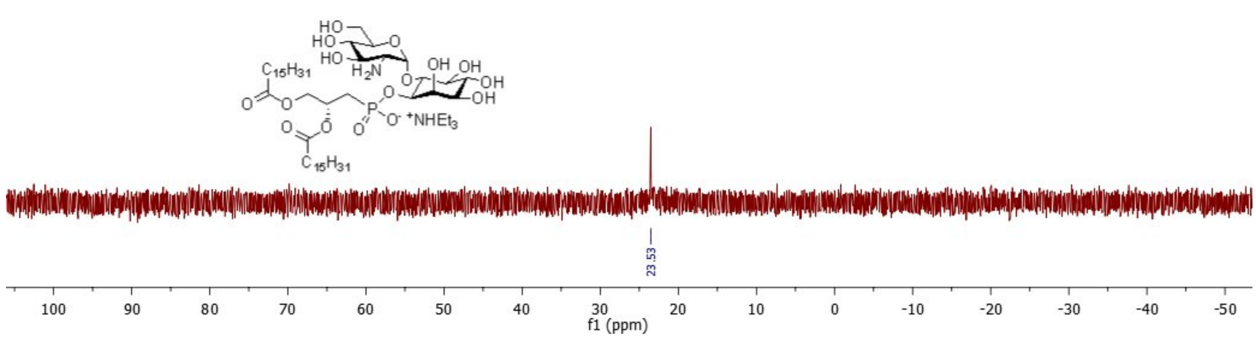
Peak List

m/z	z	Abund
479.1742		1479.8
730.3783		3145.1
968.6093	1	5709.3
969.6126	1	3309.3
970.6161	1	1078.3

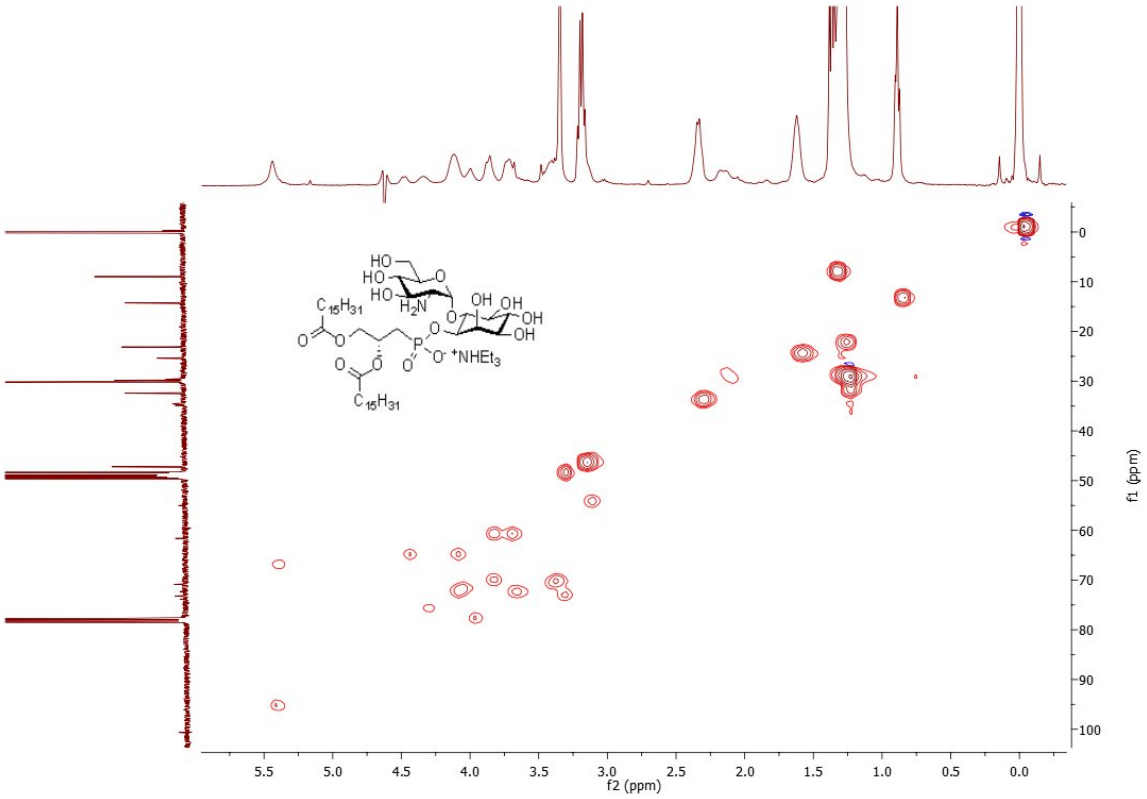
[1]H NMR spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 400 MHz) of compound 23b:



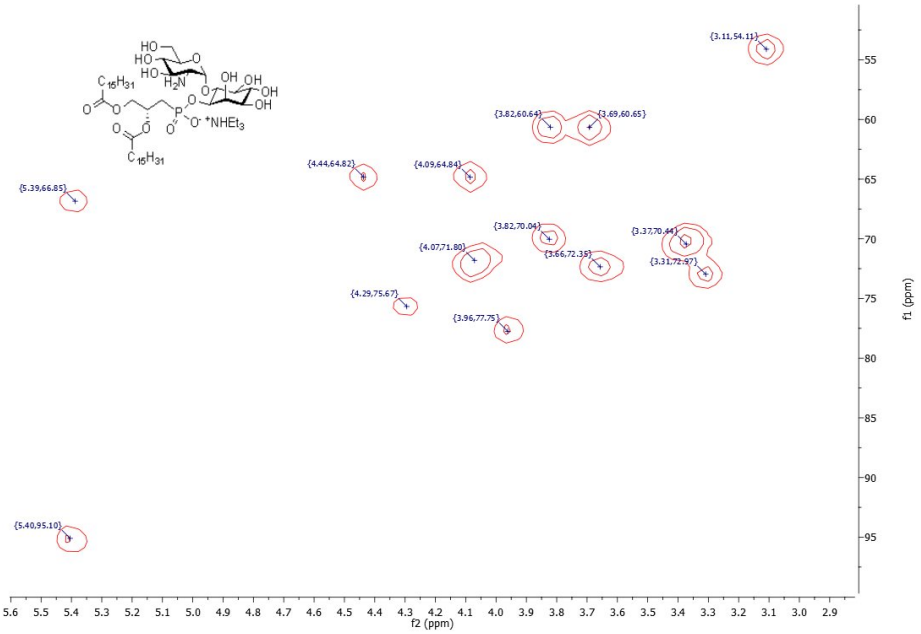
[31]P NMR spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 161 MHz) of compound 23b:



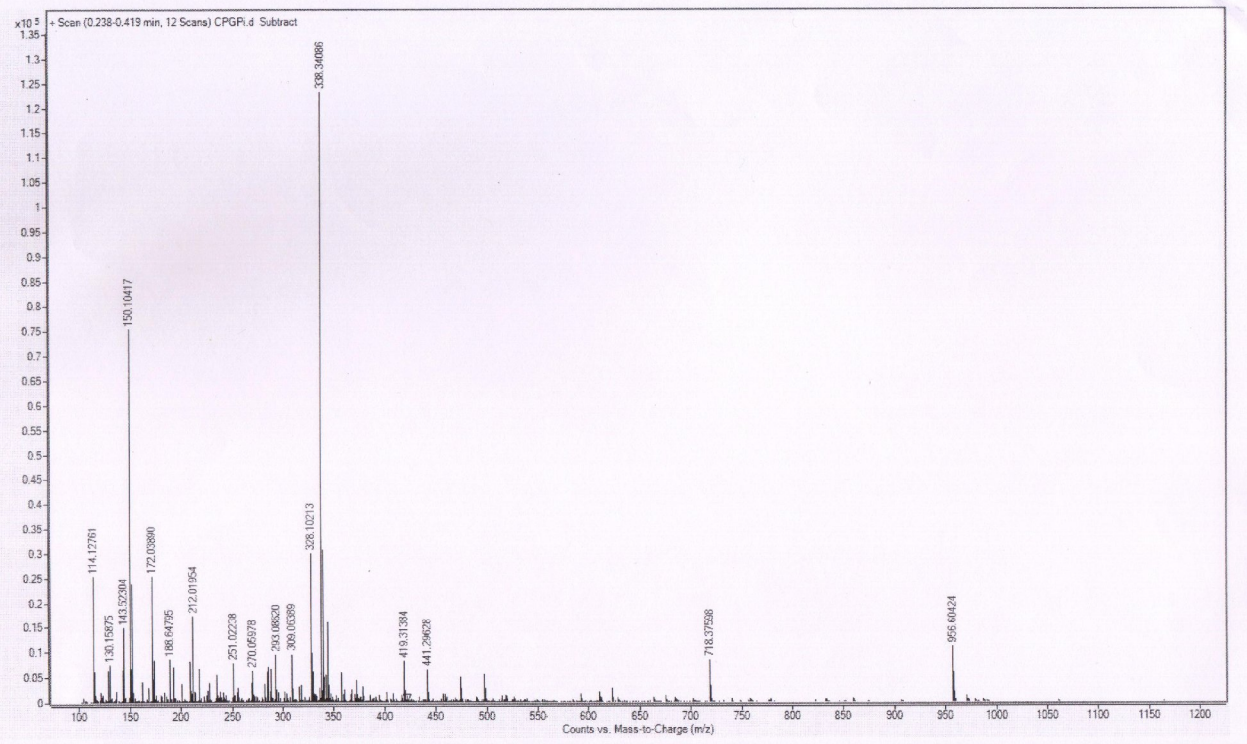
HSQC spectrogram ($CDCl_3$:CD_3OD:D_2O = 4:4:1, 400 MHz) of compound 23b:



HSQC spectrogram (expansion) of compound 23b:



HRMS (ESI) spectrogram of compound 23b:



References:

1. a) V. Saikam, R. Raghupathy, M. Yadav, V. Gannedi, P. P. Singh, N. A. Qazi, S. D. Sawant and R. A. Vishwakarma, *Tetrahedron Lett*., 2011, **52**, 4277; b) S. Cottaz, J. S. Brimacombe, M. A. J. Ferguson, *J. Chem. Soc., Perkin Trans. 1* 1993, 2945; c) R. A. Vishwakarma and D. Ruhela, *Enzymes* 2009, **26**, 181.