Electronic Supplementary Information

for

ArnD is a deformylase involved in polymyxin resistance

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Materials and Methods

General Information

All non-aqueous reactions were performed in flame-dried glassware. All reagents were purchased either from Sigma-Aldrich, Toronto Research Chemicals Inc. (TRC), or Alfa Aesar and used without further purification unless otherwise stated. The formylation reagent NHS-formate was synthesized following a known procedure.[1] Dichloromethane, methanol, pyridine and triethylamine were distilled over CaH_2 under an atmosphere of Ar. All other solvents were used without further purification. DEAE cellulose resin was bought from BioPhoretics, Biogel P2 from Bio-Rad and Sep-Pak® C-18 columns from Waters. Silica gel chromatography was performed using Silica Gel SiliaFlash F60 (230-400 mesh, Silicycle). ^{1}H NMR spectra were recorded on a Bruker AV400 spectrometer at a field strength of 400 MHz. Proton-decoupled ^{31}P NMR spectra were recorded on Bruker AV400 spectrometer at a field strength of 162 MHz. ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer at a field strength of 100 MHz. Low resolution mass spectrometry was performed by electrospray ionization (ESI-MS) using a Waters ZQ LC mass spectrometer. High resolution mass spectrometry was performed by ESI-MS using an Esquire LC mass spectrometer at the UBC Mass spectrometry Facility. Neutral compounds were detected as positive ions, and negatively charged compounds were detected as negative ions.

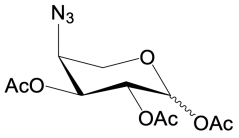
Protein Expression and Purification

A gene encoding for full-length *B. cenocepacia* ArnD (NCBI accession code WP_006482870.1) was codon optimized for expression in *Escherichia coli* and synthesized (GenScript). The gene was cloned into a pET28a expression vector such that a C-terminal His_8 tag and thrombin cleavage site would be in the expressed protein and confirmed by DNA sequencing. Recombinant ArnD was overexpressed in *E. coli* BL21 (λDE3) pLysS cells. A 1 L culture was grown in lysogeny-broth (LB) media supplemented with 40 μg/ml of kanamycin and 32 μg/ml of chloramphenicol at 37 °C to an OD_{600} of 0.7-0.9. The culture was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and grown overnight at 20 ºC. Cells were pelleted by centrifugation at 4 °C and resuspended in lysis buffer containing 50 mM Tris•HCl (pH 8), 300 mM NaCl, 10% (v/v) glycerol, 2 mM tris(2-carboxyethyl) phosphine (TCEP), and 1% (v/v) Triton X-100. DNAse and Halt™ EDTA-Free protease inhibitor cocktail (ThermoFisher Scientific) were added to the cell suspension prior to lysis at 4 °C using an EmulsiFlex-C5 homogenizer (Avestin). Insoluble material was removed by centrifugation and the soluble lysate was loaded onto HisTrap nickel affinity column (GE Healthcare) and eluted using a gradient to 0.5 M imidazole. The elution buffer was same as the loading buffer except the concentration of Triton X-100 was reduced to 0.02%. Fractions with the ArnD protein were pooled and dialyzed against 50 mM Tris•HCl (pH 8), 300 mM NaCl, 10% (v/v) glycerol, 2 mM TCEP overnight at 4 °C. Recombinant ArnD was concentrated to ~0.7 mg/ml, flash frozen in liquid nitrogen and stored at -70 °C. Protein purity was assessed to be > 90% by SDS-PAGE.

Test for ArnD Activity:

A) Mass spectrometry. ArnD (final concentration 0.03 mg/mL, diluted directly from storage buffer) was incubated for 20 h at 25 °C with either 4-formamido-4-deoxy-L-arabinose-α-phosphate (**1,** 5.5 mM) or 4-formamido-4-deoxy-L-arabinose-α-neryl-phosphate (**2,** 6.8 mM) in Tris•HCl buffer (pH 8.0, 50 mM) containing either $ZnCl_2$ (0.25 mM) or EDTA (0.5 mM). An incubation was also performed without added metal or EDTA. For ESI-MS analysis, sample solutions were made with 5 μL of reaction mixture and 15 μL H_2O, and the mass peaks were observed in the negative mode at 1 h and 20 h time points.
B) ^{1}H NMR spectroscopy. The sample was prepared as above with the exception that the substrate concentration was 7.0 mM and the enzyme concentration was 0.06 mg/mL (final concentrations, total volume 860 μL). No metals ions were added. The sample was incubated at 25 °C for 46 h. The enzyme was removed by centrifugal ultrafiltration with an Amicon® Ultra-15 Centrifugal filter. The filtrate was then purified using reversed phase silica gel chromatography (Sep-Pak C-18 column, size: 2 g/12 mL). After washing the column with MeOH and water (20 mL each), the aqueous phase (~2 mL) was applied to the column and eluted consecutively with aqueous solutions containing 0%, 5%, 10%, 20%, 30%, 50%, 70%, and 100% MeOH (20 mL each). The 50% MeOH-H_2O fraction was lyophilized to yield the product of the enzymatic reaction.

Synthetic Procedures

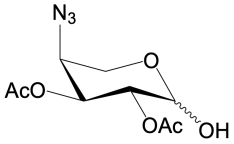


Acetyl 2,3-di-*O*- acetyl-4-azido-4-deoxy-α/β-L-arabinopyranoside (4): Compound **3** (647 mg, 3.42 mmol) was dissolved in a solution of acetic acid (6.40 mL, 112 mmol) and hydrochloric acid (2M, 3.16 mL, 6.33 mmol) was added. The clear solution was stirred at 65 °C for 15 h. The resulting brown solution was concentrated in vacuo and dried for 3 h to remove any remaining acid. The resulting brown residue was resuspended in distilled pyridine (6 mL). Acetic anhydride (1.16 mL, 12.3 mmol) was slowly added at 0 °C, along with a catalytic amount of DMAP (4 mg, 0.320 mmol), and the mixture was stirred for 15 h at rt under Ar. Volatiles were evaporated in vacuo to furnish a brown residue which was dissolved in 75 mL of ethyl acetate and washed successively with 25 mL each of 0.1 M HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and then evaporated in vacuo to afford a brown viscous oil which was purified by silica gel column chromatography using stepwise gradient elution, 1:9 (EtOAc: pet. ether) to 1:4 (EtOAc: pet. ether). Compound **4** was isolated as a colourless oil (641 mg, 62% yield, 3:5 mixture of α:β anomers).[2,3]

^{1}H NMR (400 MHz, $CDCl_3$) *3:5 mixture of* α:β *anomers*: δ ppm {6.28 (major, d, *J* = 2.6 Hz), 5.66 (minor, d, *J* = 5.8 Hz), 1H}, 5.37 (dd, *J* = 3.1, 2.5 Hz, 2H), 5.22 (dd, *J* = 7.8, 5.8 Hz, 1H), 5.13 (dd, *J* = 7.8, 3.5 Hz, 1H), 4.15 – 4.12 (m, 1H), 4.04 (dd, *J* = 12.1, 5.1 Hz, 2H), 4.02 – 3.96 (m, 1H), 3.82 (dd, *J* = 12.8, 2.2 Hz, 1H), 3.75 (dd, *J* = 12.0, 2.4 Hz, 1H), {2.13 (major, s), 2.12 (minor, s), 3H}, {2.12 (major, s), 2.09 (minor, s), 3H}, {2.06 (minor, s), 2.01 (major, s), 3H}.

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) 3*:5 mixture of* α:β *anomers* δ ppm [170.36, 169.99], [169.76, 169.18], 169.06, [91.58, 90.21], [70.95, 69.17], [67.78, 66.70], [62.70, 62.48], [59.40, 56.88], [20.95, 20.91], [20.76, 20.68], 20.65.

HRMS (ESI) m/z: $[M + H]^+$ calcd. for $C_{11}H_{15}N_3O_7$, 301.0902; found, 301.0901.

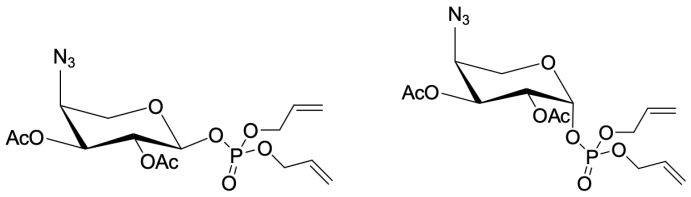


2,3-Di-*O*-acetyl-4-azido-4-deoxy-α/β-L-arabinopyranose (5): To a solution of compound **4** (372 mg, 1.24 mmol) in 5 mL DMF (de-gassed) was added recrystallized hydrazine acetate (125 mg, 1.36 mmol). The reaction was stirred at rt for 30 min. The reaction mixture was diluted with 60 mL EtOAc and washed successively with 20 mL each of H_2O, sat. $NaHCO_3$, H_2O and brine. The organic layer was dried over anhyd. Na_2SO_4 and concentrated in vacuo to furnish a colourless residue. The crude was purified using silica gel column chromatography with stepwise elution, 1:9 (EtOAc: pet. ether) to 1:2 (EtOAc: pet. ether), to afford compound **5** as colourless oil. Yield (219 mg, 68%, 3:10 mixture of α:β anomers).[4]

^1H NMR (400 MHz, $CDCl_3$) *3:10 mixture of* α:β *anomers*: δ ppm {5.44 - 5.40 (major, m), 5.12 - 5.06 (minor, m), 2H}, {5.18 (major, dd, *J* = 10.2, 3.4 Hz), 4.59 - 4.56 (minor, m), 1H}, {4.17 (major, dd, *J* = 12.6, 1.9 Hz), 4.03 (minor, dd, *J* = 17.2, 3.4 Hz), 1H}, {4.09 (major, ddd, *J* = 4.1, 2.2, 2.2 Hz), 4.03 - 4.00 (minor, m), 1H}, 3.82 (minor, d, *J* = 9.5 Hz), 3.24 (major, d, *J* = 3.9 Hz), 1H}, {3.69 (major, dd, *J* = 12.6, 2.5 Hz), 3.67 - 3.63 (minor, m), 1H}, {2.12 (minor, s), 2.12 (major, s), 3H}, {2.10 (minor, s), 2.10 (major, s), 3H}.

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) *3:10 mixture of* α:β *anomers*: δ ppm [171.07, 170.41, 170.33], [96.09, 91.08], [71.92, 69.05], [71.16, 68.70], [63.68, 60.18], [59.73, 58.62], [20.94, 20.93], [20.74, 20.66].

HRMS (ESI) *m/z*: $[M + Na]^+$ calcd. for $([C_9H_{13}N_3O_6]+Na)^+$, 282.0697; found, 282.0696.



Diallyl (2,3-di-*O*-acetyl-4-azido-4-deoxy-α/β-L-arabinopyranosyl) phosphotriester (6): To the dried compound **5** (297 mg, 1.14 mmol) was added a solution of 1*H*-tetrazole (0.45 M, 12.7 mL, 5.73 mmol) in MeCN. The volume was then reduced to ~0.3 mL in vacuo. Distilled DCM (7 mL) was then added to the resulting suspension and the mixture was placed under an atmosphere of Ar.

Diallyl *N,N*-diisopropylphosphoramidite (1.0 mL, 3.78 mmol) was added drop-wise and the mixture was allowed to stir at rt for 45 min. The mixture was then cooled to -78 ^{o}C and m-CPBA (989 mg, 5.73 mmol) was added in one portion. The mixture was allowed to stir for 30 min and then was slowly warmed to rt. It was diluted with 45 mL DCM and successively washed with 2 x 15 mL 10% $Na_2S_2O_3$, 2 x 15 mL $NaHCO_3$ and 15 mL H_2O. The organic phase was dried over anhyd. Na_2SO_4 and then concentrated in vacuo. The faint yellow oil was purified by silica gel column chromatography using step wise elution, 3:1 (pet. ether: EtOAc) to 1:1 (EtOAc: pet. ether) to yield **6**-α anomer (103 mg, 25 %) and **6**-β anomer (305 mg, 64 %). It was noted that the **6**-β anomer elutes before the **6**-α anomer.[5]

β *anomer* **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 6.00 – 5.88 (m, 2H), 5.86 (dd, *J* = 6.6, 3.3 Hz, 1H), 5.41 – 5.33 (m, 3H), 5.30 – 5.20 (m, 3 H), 4.60 – 4.54 (m, 4H), 4.16 – 4.11 (m, 2H), 3.82 (dd, *J* = 12.6, 1.8 Hz, 1H), 2.13 (s, 3H), 2.08 (s, 3H).

^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$,): δ ppm – 1.74 (s).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ ppm 170.26, 169.97, 132.26, 132.19, 118.92, 118.84, 95.03 (d, *J* = 5.3 Hz), 68.73 (d, *J* = 5.4 Hz), 68.65 (d, *J* = 5.4 Hz), 67.36 (d, *J* = 7.1 Hz), 62.04, 59.44, 20.83, 20.70.

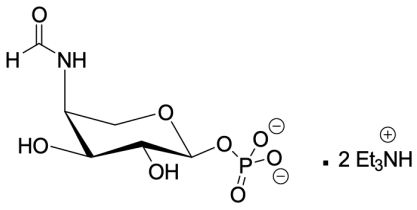
HRMS (ESI) *m/z*: [M + Na]$^{+}$ calcd. for ([$C_{15}H_{22}N_3O_9P$ + Na]$^{+}$), 442.0986; found, 442.0996.

α *anomer* **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 5.92 (ddt, *J* = 16.4, 10.2, 4.8 Hz, 2H), 5.36 (ddd, *J* = 17.2, 2.8, 1.4 Hz, 1H), 5.30 (dd, *J* = 6.9, 5.0 Hz, 1H), 5.28-5.23 (bm, 2H), 5.20 (dd, *J* = 7.2, 5.0 Hz, 1H), 5.11 (dd, *J* = 7.2, 3.5 Hz, 1H), 2.13 (s, 3H), 2.09 (s, 3H).

^{31}P{^{1}H} NMR ($CDCl_3$, 162 MHz): δ ppm – 2.45 (s).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ ppm 170.04, 169.15, 132.26, 132.08, 118.80, 118.72, 95.78 (d, *J* = 5.3 Hz), 70.30, 68.75 (d, *J* = 2.9 Hz), 68.70 (d, *J* = 2.7 Hz), 68.39 (d, *J* = 10.0 Hz), 61.88, 56.12, 20.84, 20.74.

HRMS (ESI) *m/z*: [M + Na]$^{+}$ calcd. for ([$C_{15}H_{22}N_3O_9P$ + Na]$^{+}$), 442.0986; found, 442.0993.



4-Deoxy-4-*N*-formamido-α-L-arabinopyranosyl phosphate, triethylammonium salt, (1): Compound **6**-α (93 mg, 0.222 mmol) was dissolved in 3 mL THF and 4 mL MeOH under Ar. To the stirring solution was added p-toluenesulphinic acid sodium salt (79 mg, 0.444 mmol) and tetrakistriphenylphosphine palladium (20 mg, 0.018 mmol). The mixture was stirred for 17 h at rt after which it was concentrated in vacuo and co-evaporated with toluene. The crude was

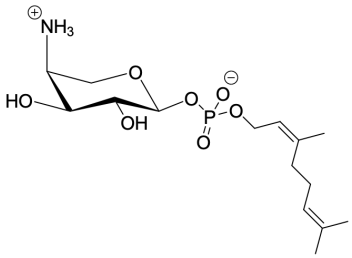
resuspended in a mixture of MeOH-H_2O-Et_3N (5:2:1, 8 mL), and stirred at rt for 22h. The solution was filtered to remove precipitation and the filtrate was concentrated in vacuo. The resulting syrup was dissolved in water and washed with DCM (3 x 15 mL). The aqueous layer was concentrated and co-evaporated with toluene in vacuo to afford crude azide as a pale-yellow solid. This crude was resuspended in H_2O-MeOH (1:1, 10 mL) and 10% Pd/C (3 mg) was added. The mixture was stirred at rt for 15 h under an atmosphere of H_2. The suspension was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in H_2O-MeOH (4:1, 5 mL) to which NHS-formate (28 mg, 0.195 mmol) was added. The mixture was stirred for 4 h at rt. The H_2O was lyophilized to furnish a crude which was purified by anion-exchange chromatography using DEAE-cellulose (100 mL) and stepwise elution with triethyl ammonium bicarbonate (TEAB) buffer (pH = 7.5). The column was equilibrated with 1 mM TEAB and was washed with 50 mL each of 2 mM, 5 mM, 10 mM, 20 mM, 30 mM, 40 mM and 50 mM, and each of the fractions was analyzed by ESI-MS for the presence of 4-deoxy-4-*N*-formamido-α-L-arabinopyranosyl phosphate. The fraction found to contain product (40 mM) was lyophilized to afford compound **1**•2 Et_3NH^+ as a white solid (11 mg, 20% over 4 steps).[6]

^{1}H NMR (400 MHz, D_2O) 5:1 mixture of rotamers: δ ppm {8.18 (major), 8.08 (minor)} (s, 1H), 4.90 (dd, *J* = 7.6, 7.6 Hz, 1H), 4.38 - 4.34 (bm, 1H), 3.94 – 3.87 (m, 2H), 3.75 (dd, *J* = 12.6, 2.1 Hz, 1H), 3.55 (dd, *J* = 8.9, 6.9 Hz, 1H), 3.22 (q, *J* = 7.3 Hz, 12H), 1.29 (t, *J* = 7.3 Hz, 18H).

^{31}P{^{1}H} NMR (162 MHz, D_2O): δ ppm - 0.34 (s).

^{13}C{^{1}H} NMR (100 MHz, D_2O) δ ppm 164.64, 98.09 (d, *J* = 9.4 Hz), 71.58 (d, *J* = 8.2 Hz)., 70.52, 64.26, 48.02, 46.88, 8.42.

HRMS (ESI) m/z: $[M - H]^-$ calcd. for $C_6H_{11}NO_8P^-$, 256.0228; found, 256.0224.



2(Z)-2,6-Dien-3,7-dimethyl-octa-1-yl (4-amino-4-deoxy-α-L-arabinopyranosyl) phosphate (7): To a solution of compound **6**- α (53 mg, 0.126 mmol) in a mixture of DCM-MeOH (5 mL, 3:2) was added $PdCl_2$ (11 mg, 0.0630 mmol). The brown suspension was stirred for 5 h at rt under Ar or until ESI-MS confirmed disappearance of starting material. The resulting black suspension was filtered through celite to furnish a yellow solution which was concentrated in vacuo. Freshly distilled pyridine and pulverized activated molecular sieves were added to the crude under Ar. To the stirring suspension, nerol (0.09 mL, 0.508 mmol) and trichloroacetonitrile (0.13 mL, 1.25 mmol) were added. The mixture was then stirred for 15 h at 65 °C under Ar. The resulting brown

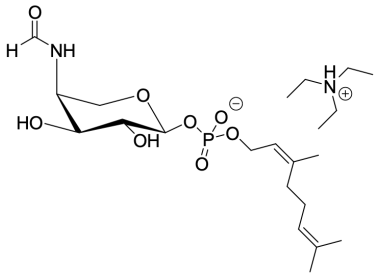
solution was filtered and concentrated in vacuo. The crude was partially purified by silica gel column chromatography using stepwise elution (hexane/ethyl acetate/ MeOH 1:1:0 to 0:1:0 to 0:4:1) to afford neryl-(2,3-*O*-diacetyl-4-azido-4-deoxy-α-L-arabinopyranosyl) phosphate along with 6 equivalents of a triethyl ammonium salt as a colourless syrup. To this material (34 mg) was added a mixture of distilled MeOH-H_2O-Et_3N (4 mL, 5:2:1) and the resulting clear solution was stirred for 22 h at rt. The solvents were volatilized in vacuo to furnish a colourless oil. The oil was dissolved in THF: H_2O (5 mL, 3:2) and trimethyphosphine (1 M in THF, 130 μL, 0.129 mmol) was added. The solution was heated to 40 °C for 1 h. The mixture was diluted with 2 mL of H_2O and then washed with 5 x 2 mL $CHCl_3$. The combined organic phases were re-extracted with 2 x 20 mL H_2O. The combined aqueous fractions were lyophilized to yield the off-white crude which was purified on Biogel P-2 with H_2O as the eluent. The aqueous fractions containing the compound (as determined by ESI-MS) were lyophilized to afford compound **7** (21 mg, 41% yield) as off-white solid.[7,8]

^{1}H NMR (400 MHz, D_2O,) 5.44 (m, 1H), 5.20 (bm, 1H), 4.87 (dd, *J* = 7.1, 7.1 Hz, 1H), 4.44 (dd, *J* = 6.9, 6.9 Hz, 1H), 3.95 (dd, *J* = , 1H), 3.97 (dd, *J* = 12.9, 3.7 Hz, 1H), 3.90 (dd, *J* = 8.5, 4.3 Hz, 1H), 3.76 (dd, *J* = 12.9, 2.5 Hz, 1H), 3.63 (dd, *J* = 8.5, 6.4 Hz, 1H), 3.37 – 3.34 (bm, 1H), 2.21 – 2.15 (bm, 4H), 1.79 (s, 3H), 1.71 (s, 3H), 1.64 (s, 3H).

^{31}P{^{1}H} NMR (162 MHz, D_2O): δ ppm -0.69 (s).

^{13}C{^{1}H} NMR (100 MHz, D_2O) δ ppm 134.20, 124.03, 120.41 (d, *J* = 7.4 Hz), 100.10, 98.15 (d, *J* = 7.1 Hz), 70.78 (d, *J* = 9.0 Hz), 70.44, 63.88, 62.94 (d, *J* = 5.5 Hz), 50.02, 31.47, 26.16, 25.08, 22.84, 17.18.

HRMS (ESI) m/z: $[M + Na]^+$ calcd. for $([C_{15}H_{28}NO_7P]+Na)^+$, 388.1496; found, 388.1495.



2(Z)-2,6-Dien-3,7-dimethyl-octa-1-yl (4-deoxy-4-*N*-formamido-α-L-arabinopyranosyl) phosphate, triethylammonium salt (2): Compound **7** (3 mg, 0.0061 mmol) was dissolved in H_2O-MeOH (4 mL, 4:1) and NHS-formate (4 mg, 0.0280 mmol) was added followed by triethylamine (10 μL, 0.0721 mmol). The mixture was stirred at rt under Ar for 2.5 h or until ESI-MS confirmed disappearance of the amine. The solution was concentrated under reduced pressure and lyophilized. The resulting solid was resuspended in 2 mL H_2O and purified using reversed phase silica gel chromatography (Sep-Pak C-18 columns, size: 2 g/12 mL). After washing the column with MeOH and water (20 mL each), the aqueous phase (~2 mL) was applied to the column and eluted consecutively with aqueous solutions containing 0%, 5%, 10%, 20%, 30%, 50%, 70%,

and 100% MeOH (20 mL each). The 50% MeOH-H_2O fraction was lyophilized to yield compound **2**. Et_3NH^+ as a white solid (3 mg, 83%).

^{1}H NMR (D_2O, 400 MHz) 5:1 mixture of rotamers: δ ppm {8.19 (major), 8.09 (minor)} (s, ^{1}H), 5.45 (t, *J* = 7.3 Hz, 1H), 5.20 (bt, 1H), 4.88 (dd, *J* = 7.2, 7.2 Hz, 1H), 4.44 (dd, *J* = 6.7, 6.7 Hz, 2H), 4.37 (bm, 1H), 3.95 – 3.86 (m, 2H), 3.74 (dd, *J* = 12.5, 1.8 Hz, 2H), 3.56 (dd, *J* = 8.6, 7.0 Hz, 1H), 3.22 (q, *J* = 7.3 Hz, 6H), 2.23 – 2.09 (m, 4H), 1.79 (s, 1H), 1.71 (s, 1H), 1.64 (s, 1H), 1.30 (t, *J* = 7.3 Hz, 9H).

^{31}P{^{1}H} NMR (D_2O, 162 MHz): δ ppm - 0.71 (s).

^{13}C{^{1}H} NMR (100 MHz, D_2O) δ ppm 164.61, 143.56, 134.14, 124.04, 120.36 (d, *J* = 10.8 Hz), 100.11, 98.21 (d, *J* = 6.3 Hz), 71.46 (d, *J* = 8.5 Hz), 70.41, 64.18, 62.90 (d, *J* = 5.9 Hz), 47.99, 46.88, 31.45, 26.15, 25.06, 22.81, 17.17, 8.42.

HRMS (ESI) m/z: $[M - H]^-$ calcd. for $C_{16}H_{27}NO_8P^-$, 392.1480; found, 392.1484.

References:

1. Müller, B.; Blaukopf, M.; Kosma, P. et al. *Synthesis* **2010**, 18, 3143-3151.
2. Blaukopf, M.; Müller, B.; Hofinger, A.; Kosma, P. *Eur. J. Org. Chem.* **2012**, 119 – 131.
3. Beahm, B. J.; Dehnert, K. W.; Derr, N. L.; Kuhn, J.; Eberhart, J. K.; Spillmann, D.; Amacher, S. L.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2014**, 53, 3347 – 3352.
4. Polakova, M.; Roslund, M. U.; Ekholm, F. S.; Saloranta, T.; Leino, R. *Eur. J. Org. Chem.* **2009**, 870 – 888.
5. Marchesan, S.; MacMillan, D. *Chem. Commun.* **2008**, 4321 – 4323
6. Barre, A.; Tintas, M. L.; Alix, F.; Gembus, V.; Papamicael, C.; Levacher, V. *J. Org. Chem.*, **2015**, 80 (13), 6537 – 6544.
7. Li, T. L.; Tikad, A.; Pan, W. D.; Vinent, S. P. *Org. Lett.,* **2014**, 16, 21, 5628 – 5631.
8. Olagnon, C.; Feria, J. M.; Grunwald-Gruber, C.; Blaukopf, M.; Valvano, M. A.; Kosma, P. *ChemBioChem,* **2019**, 20, 2936 – 2948.

Figure S1. ^{1}H NMR ($CDCl_3$, 400 MHz) of compound **4**:

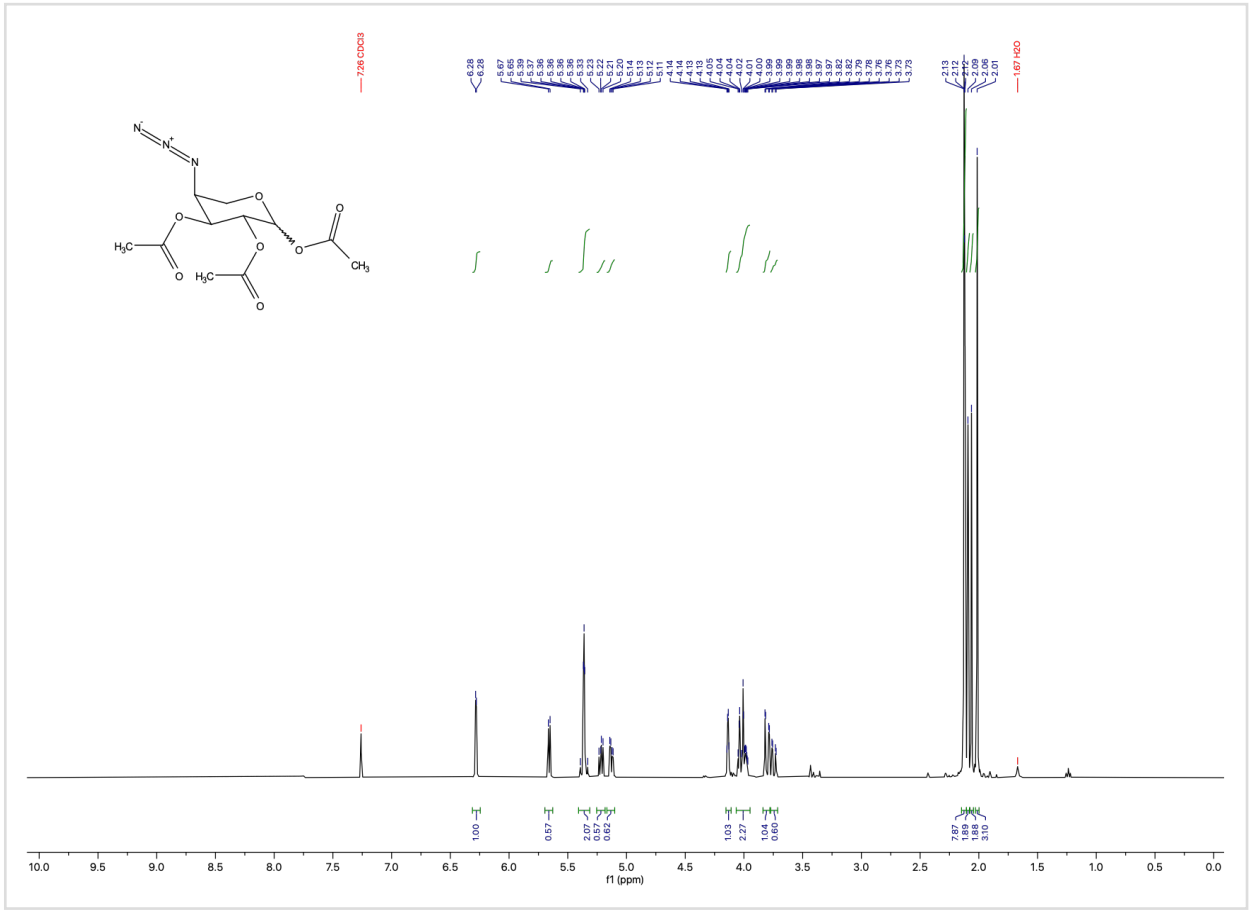


Figure S2. ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **4**:

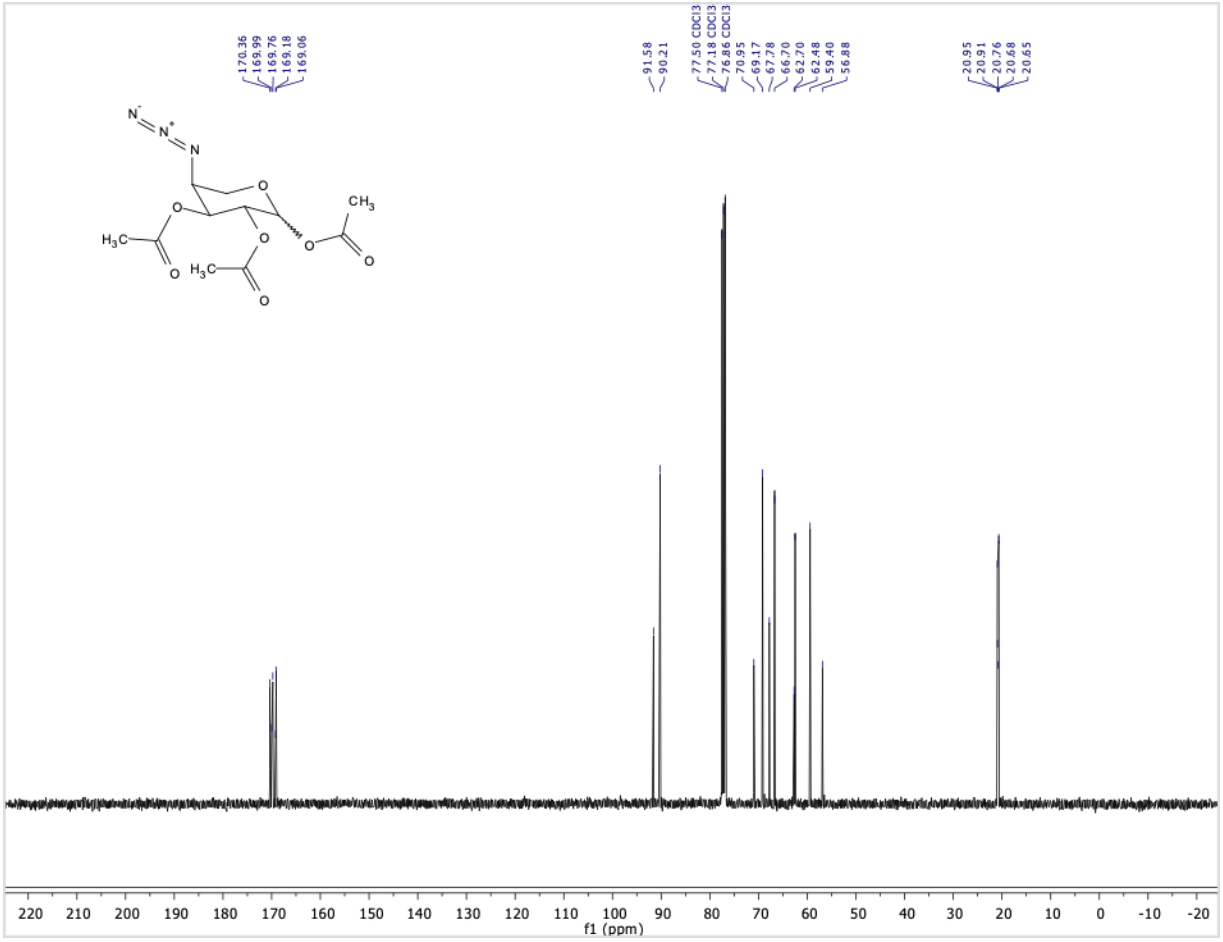


Figure S3. ^{1}H NMR ($CDCl_3$, 400 MHz) of compound **5**:

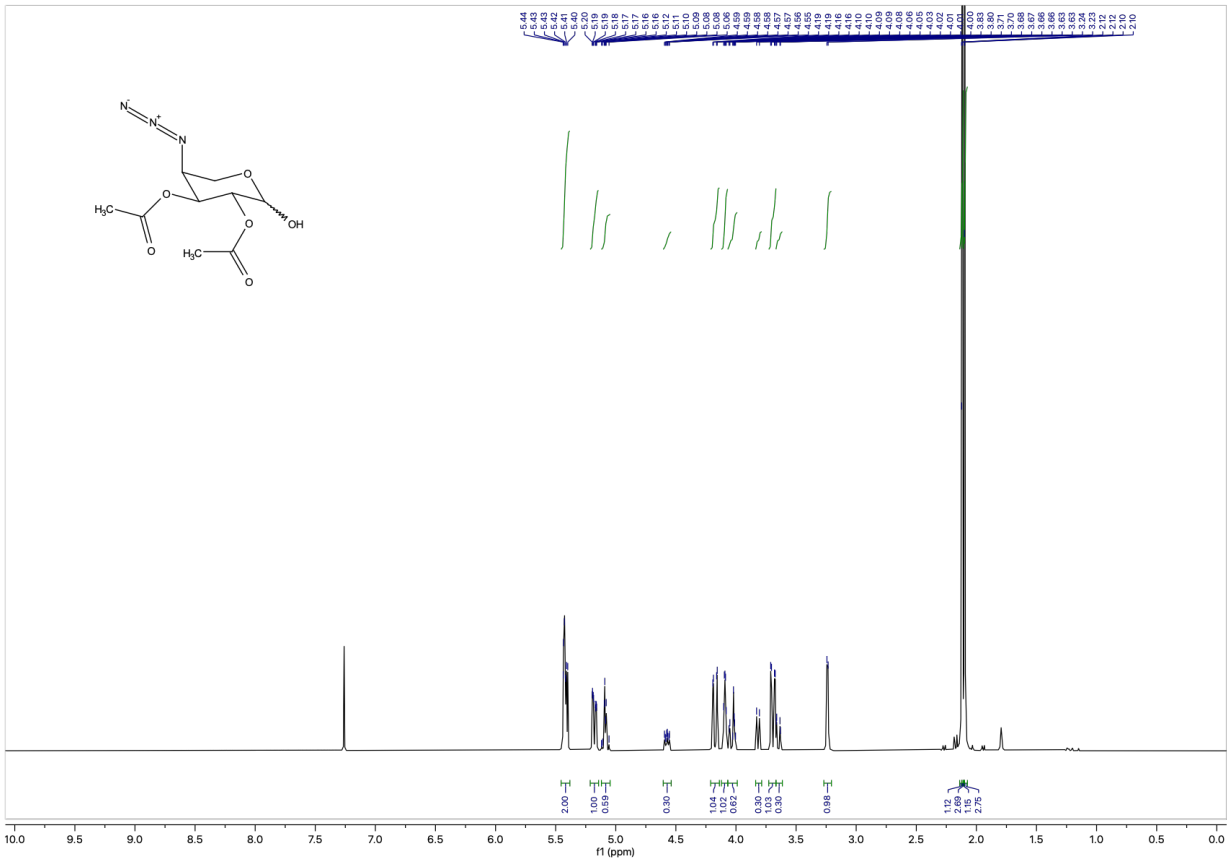


Figure S4. ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **5**:

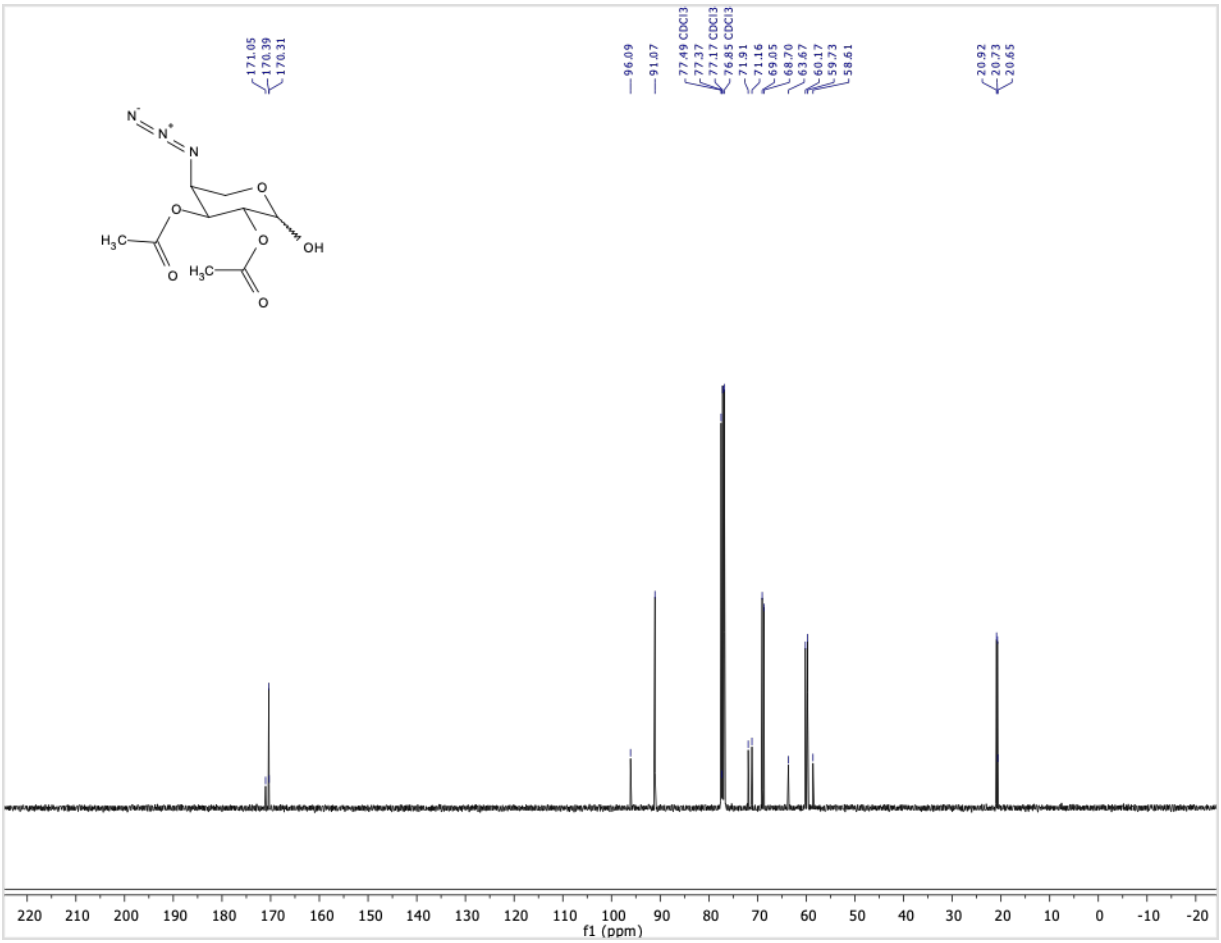


Figure S5. ^{1}H NMR ($CDCl_3$, 400 MHz) of Compound **6**α:

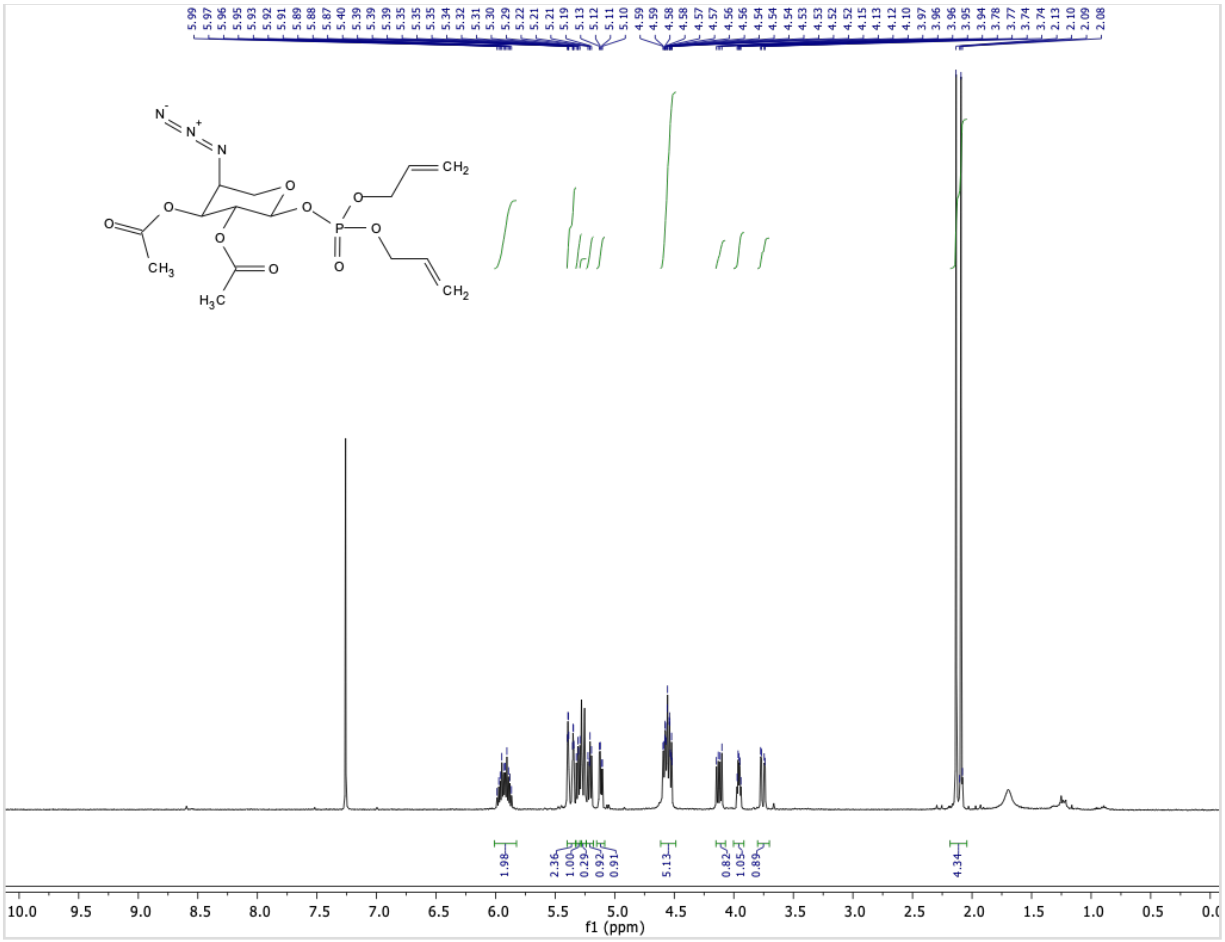


Figure S6. ^{31}P $\{^{1}H\}$ NMR ($CDCl_3$, 162 MHz) of compound **6**α:

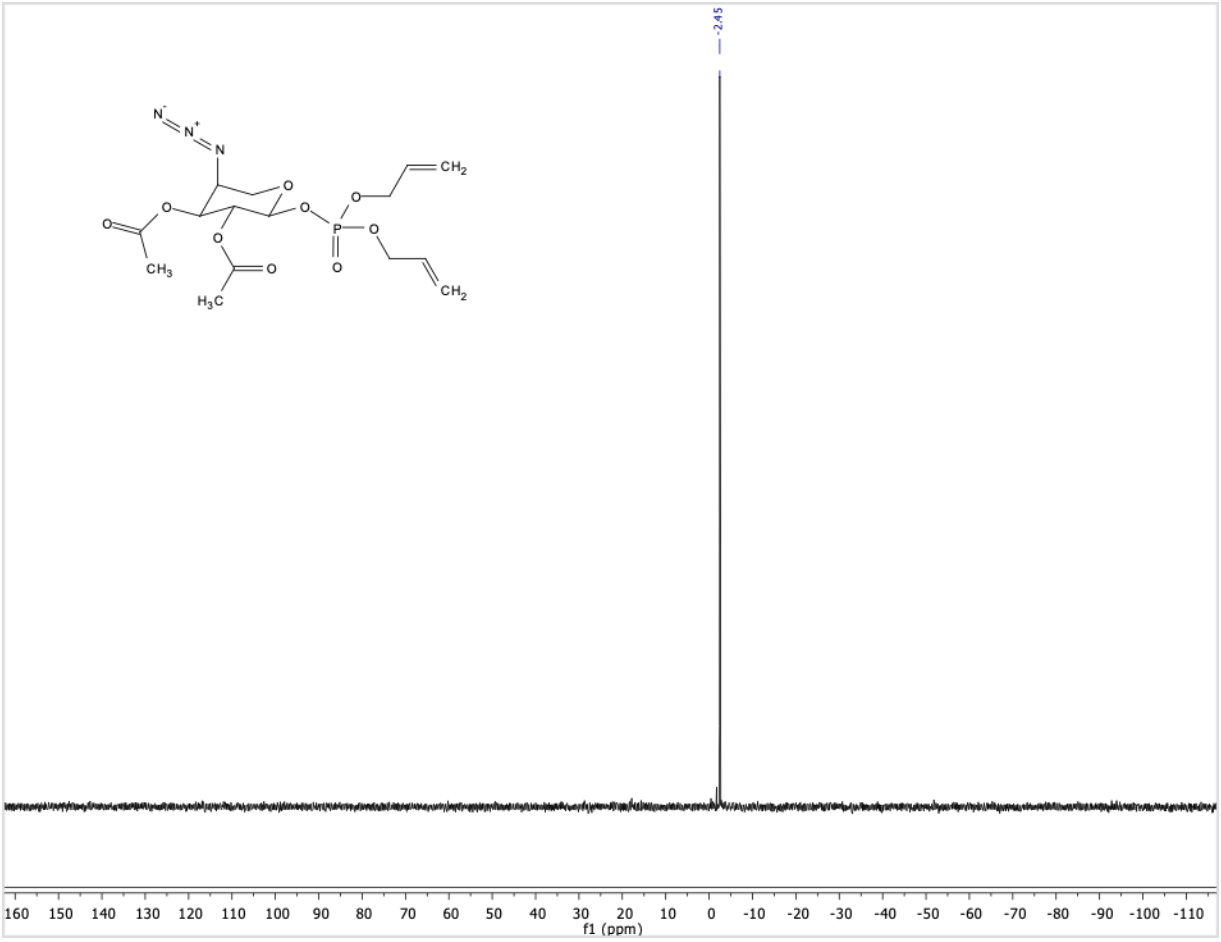


Figure S7. ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **6**α:

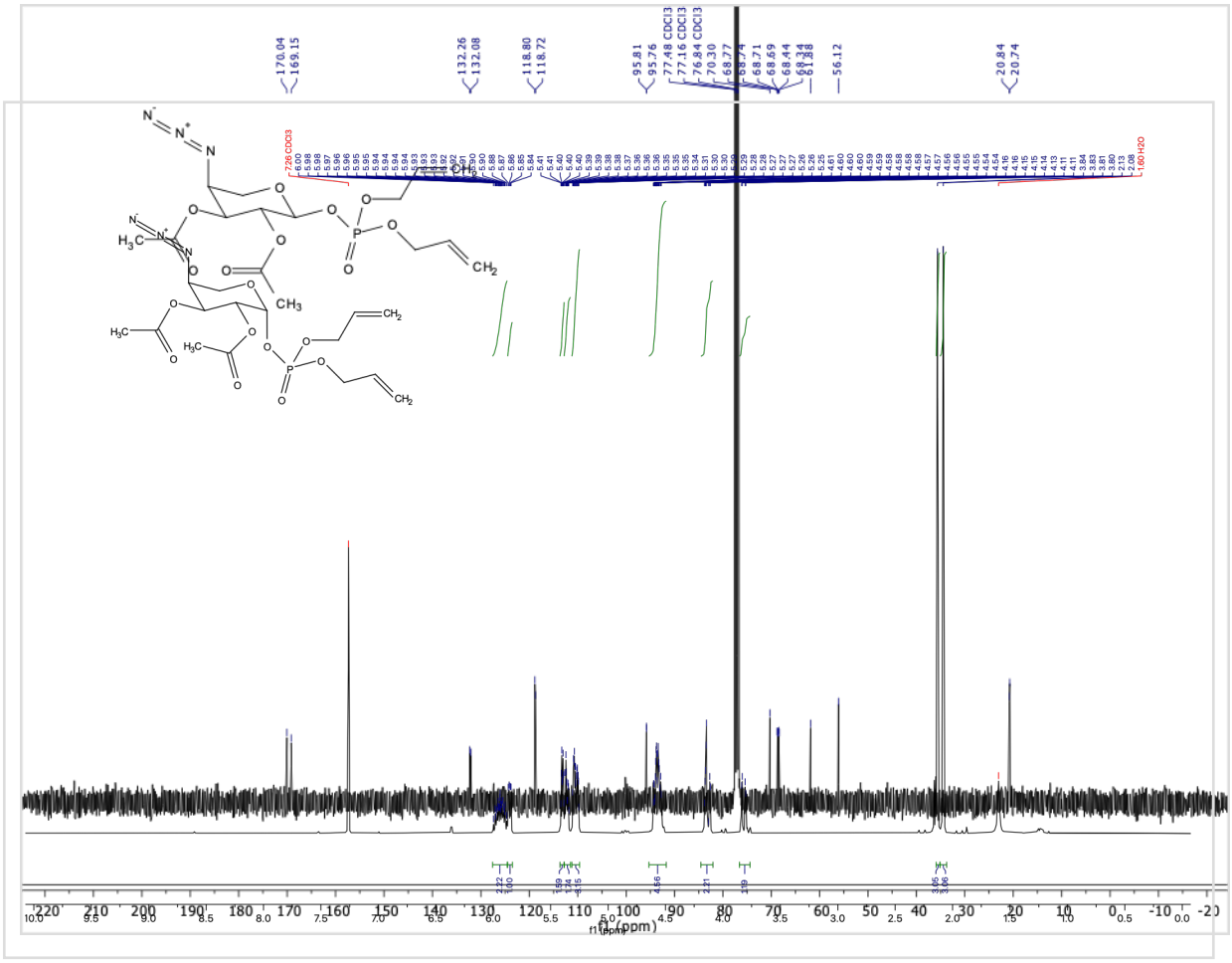


Figure S8. ^{1}H NMR ($CDCl_3$, 400 MHz) of compound **6**β:

Figure S9. ^{31}P $\{^{1}H\}$ NMR ($CDCl_3$, 162 MHz) of compound **6**β:

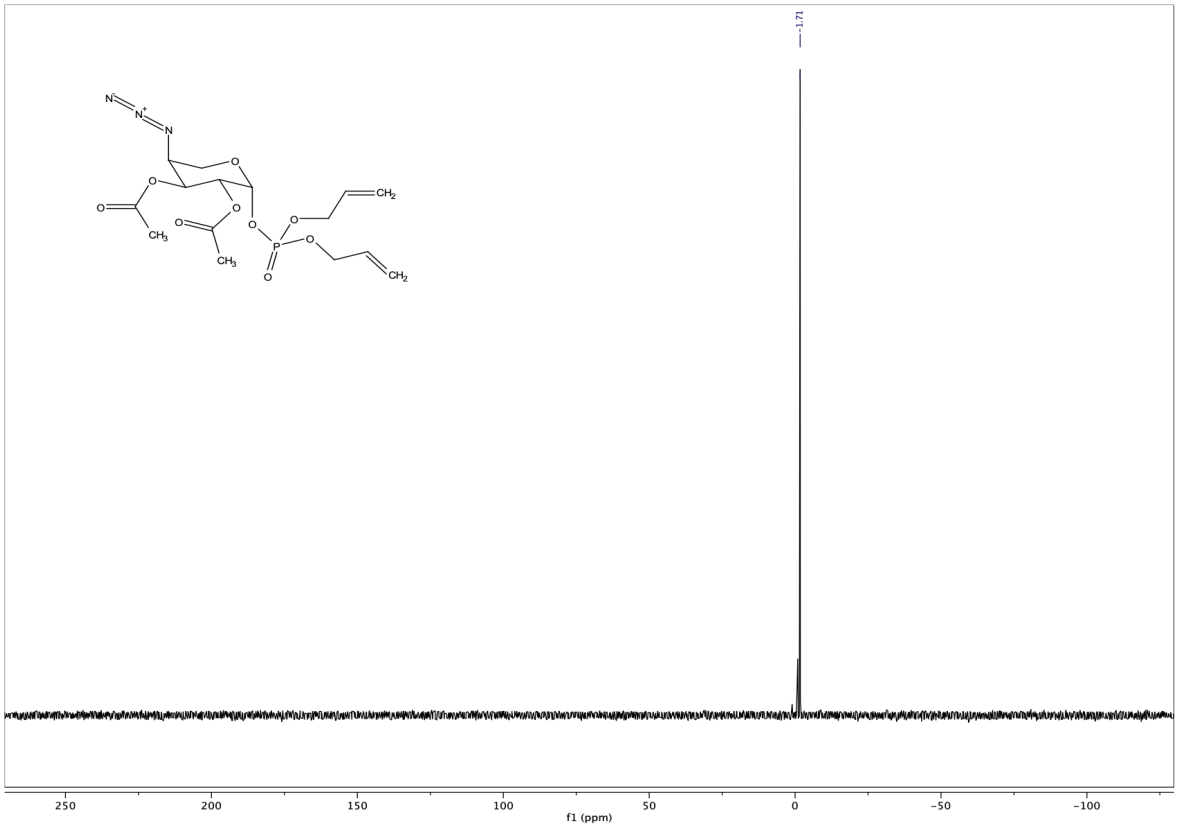


Figure S10. ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **6**β:

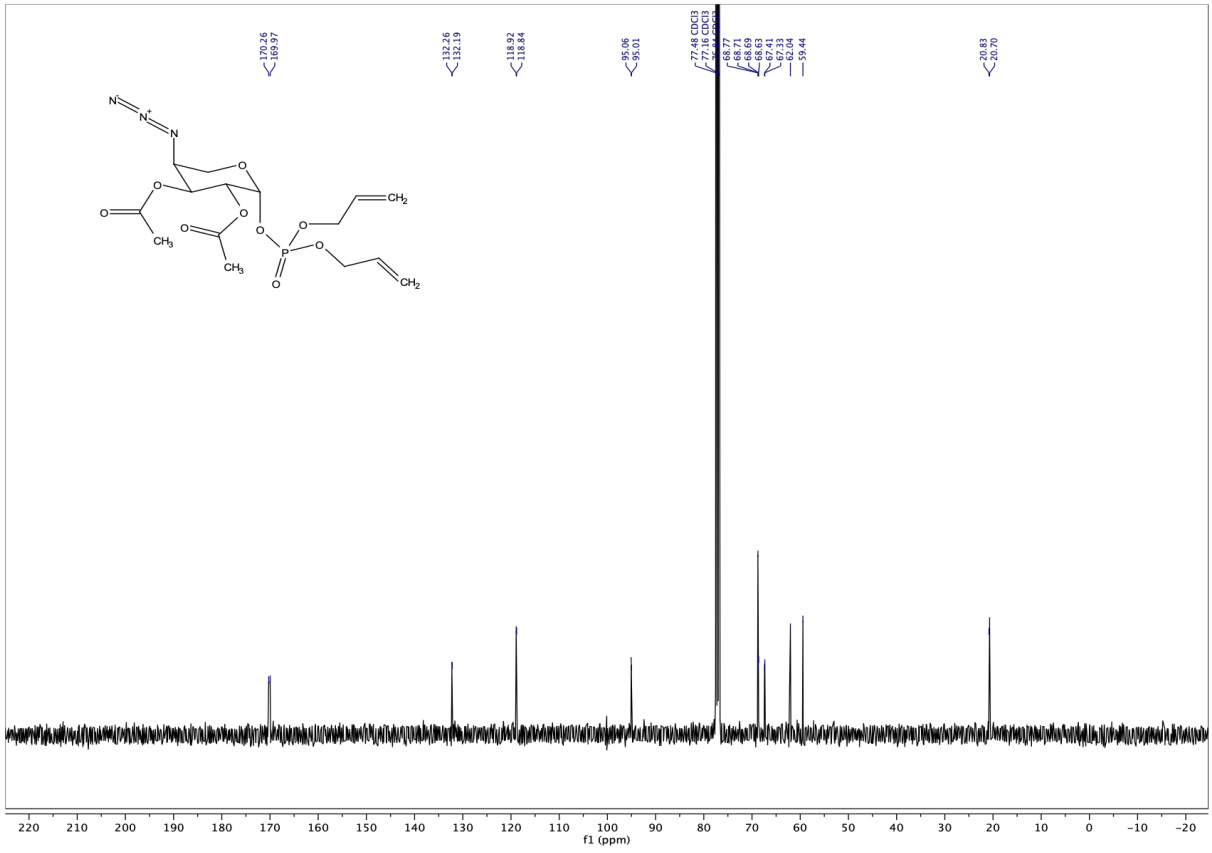


Figure S11. ^{1}H NMR (D_2O, 400 MHz) of compound **1**:

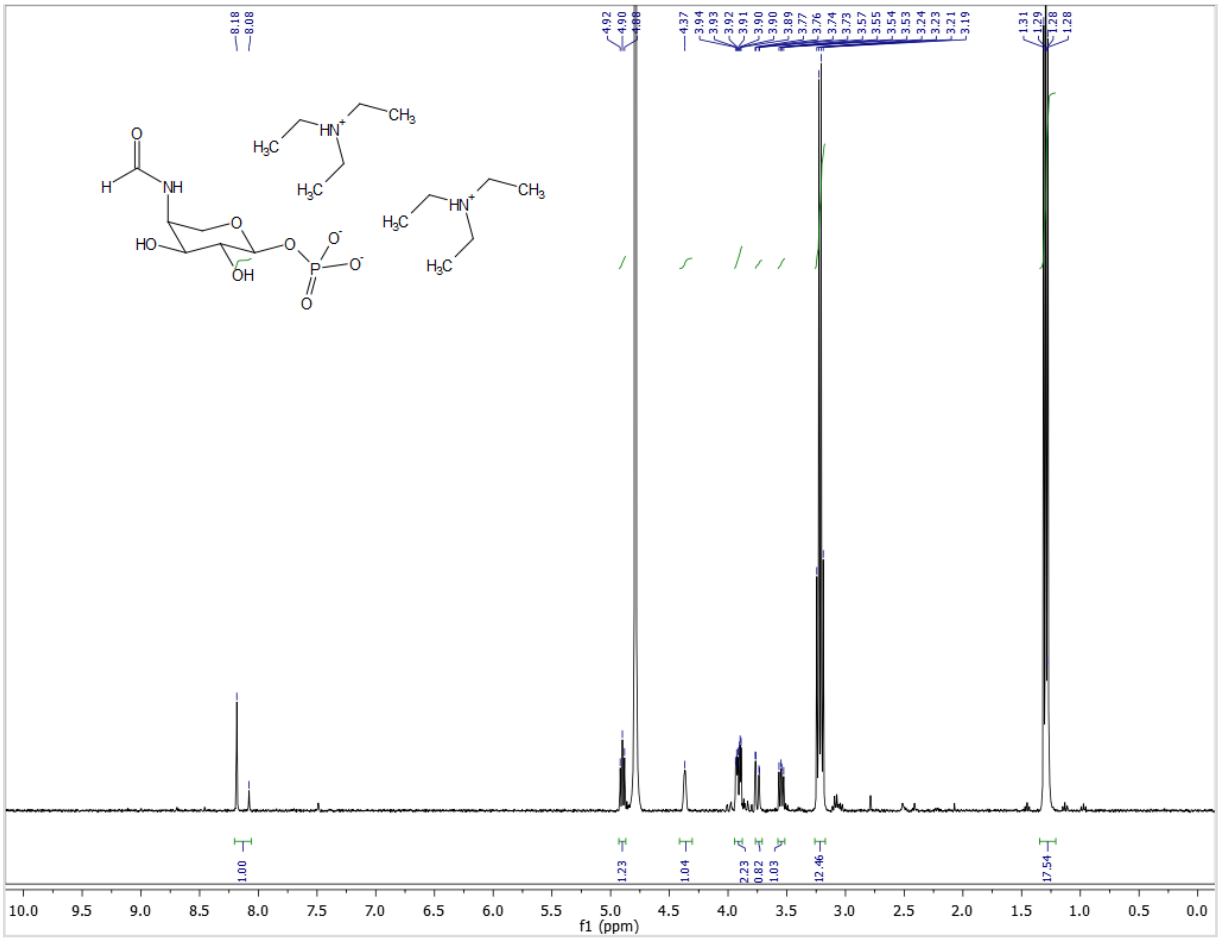

8.18
8.08
4.92
4.90
4.88
4.37
3.94
3.93
3.92
3.91
3.90
3.90
3.89
3.77
3.76
3.74
3.73
3.57
3.55
3.54
3.53
3.24
3.23
3.21
3.19
1.31
1.29
1.28
1.28
1.00
1.23
1.04
2.23
0.82
1.03
12.46
17.54
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure S12. ^{13}P $\{^{1}H\}$ NMR (D_2O, 162 MHz) of compound **1**:

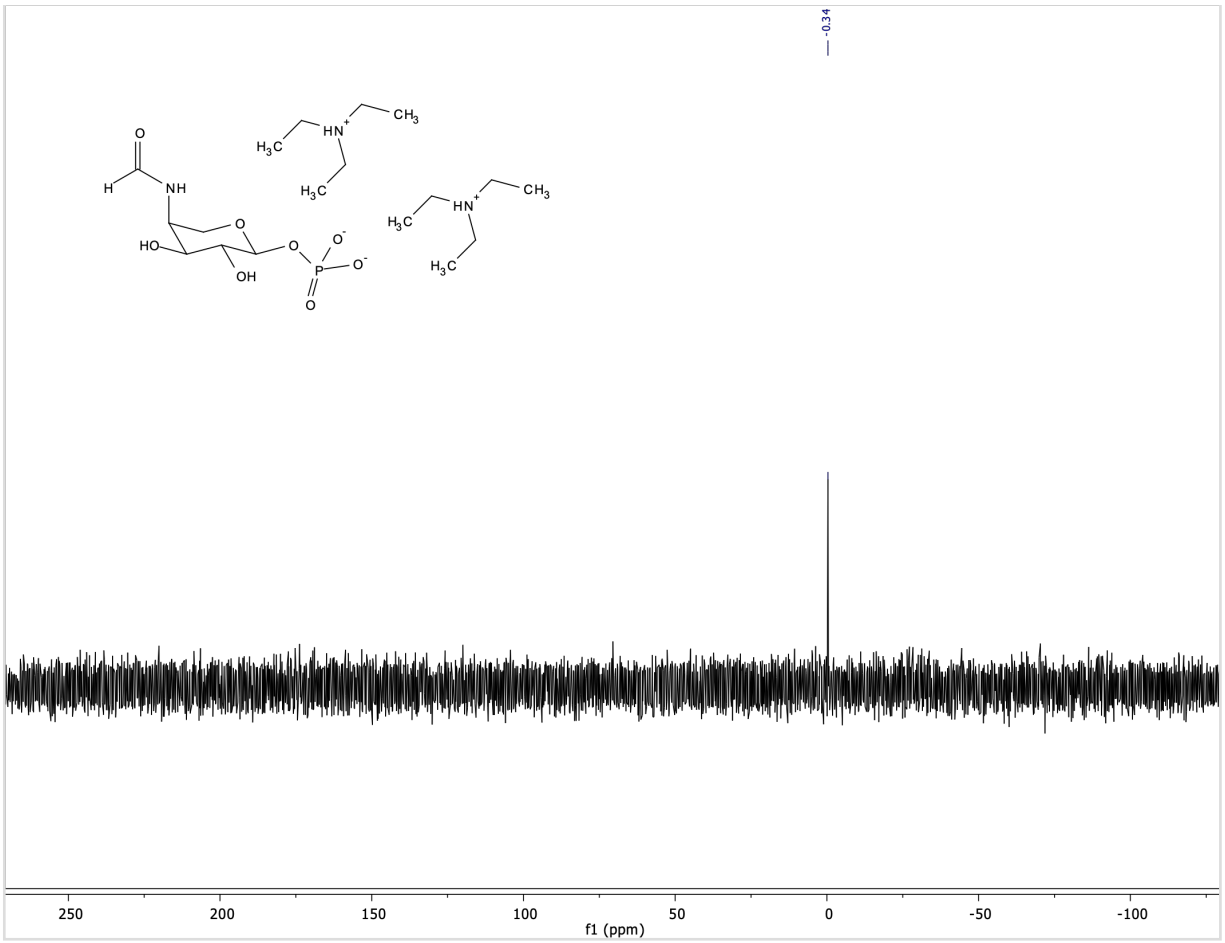


Figure S13. ^{13}C NMR (D_2O, 100 MHz) of compound **1**:

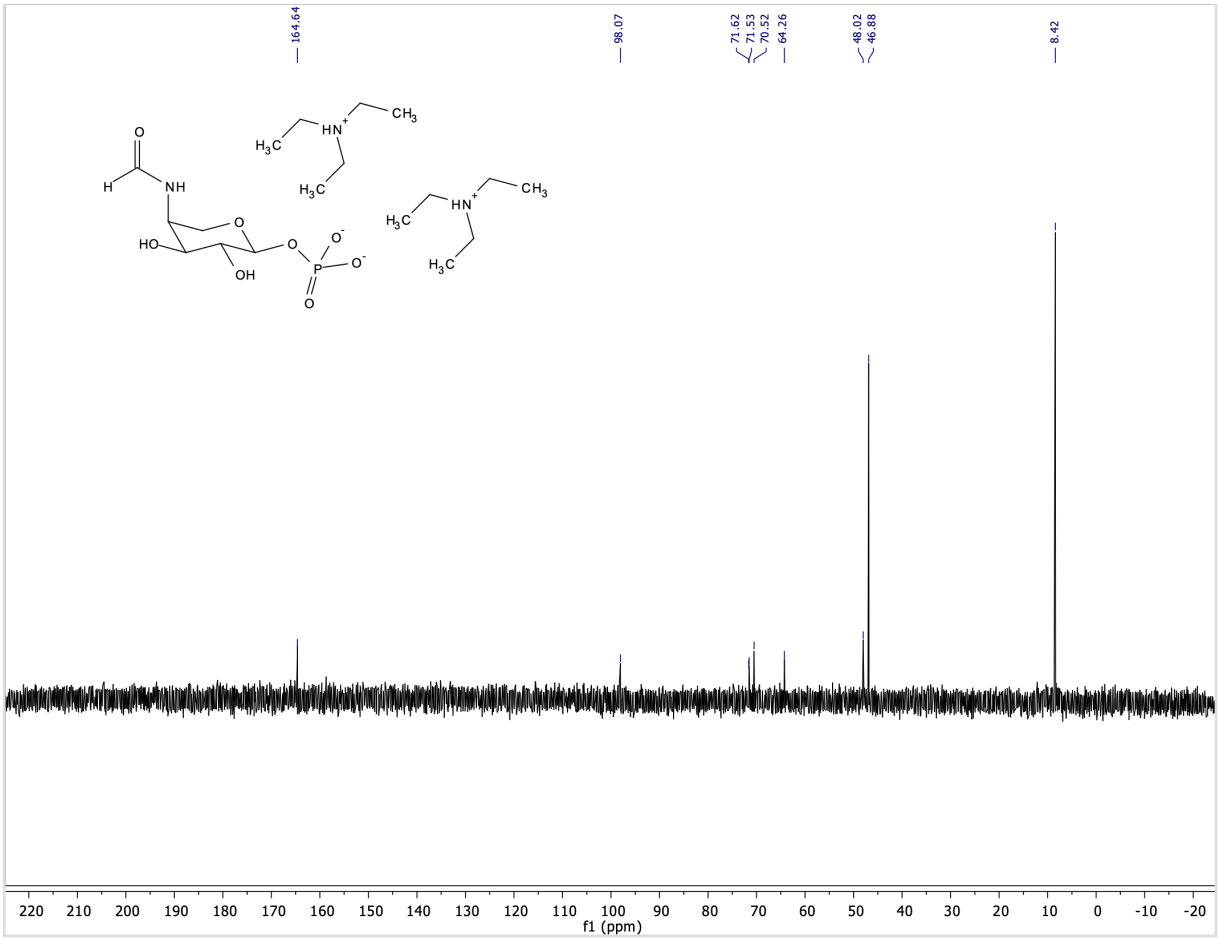


Figure S14. ^{1}H NMR (D_2O, 400 MHz) of compound **7**:

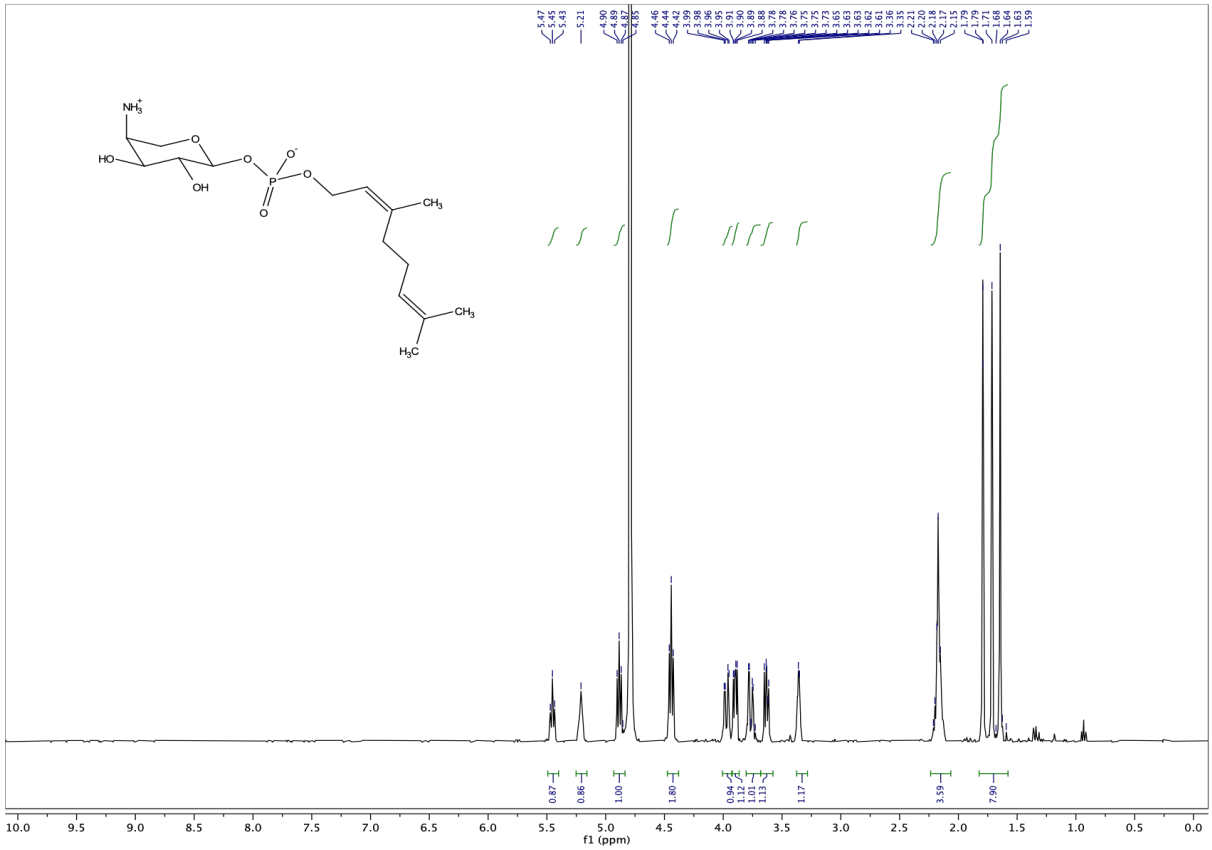


Figure S15. ^{31}P $\{^{1}H\}$ NMR (D_2O, 162 MHz) of compound **7**:

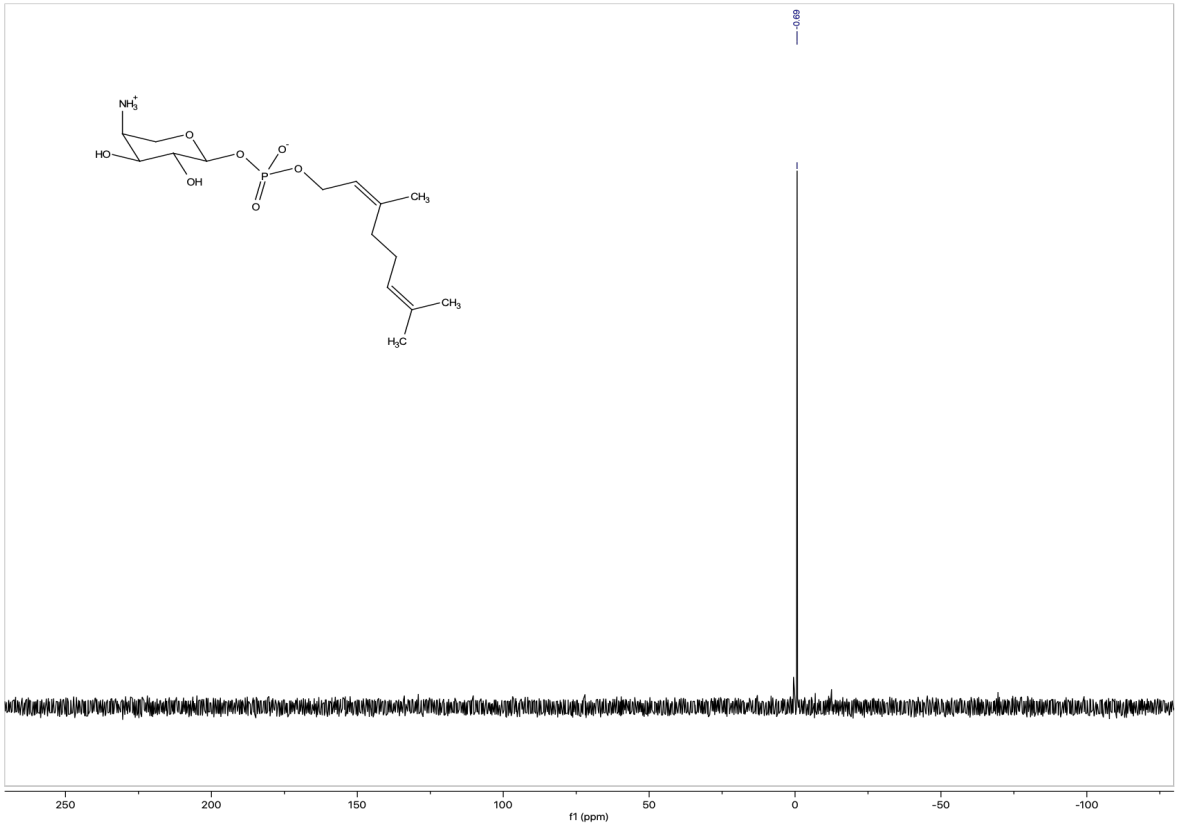


Figure S16. ^{13}C NMR (D_2O, 100 MHz) of compound **7**:

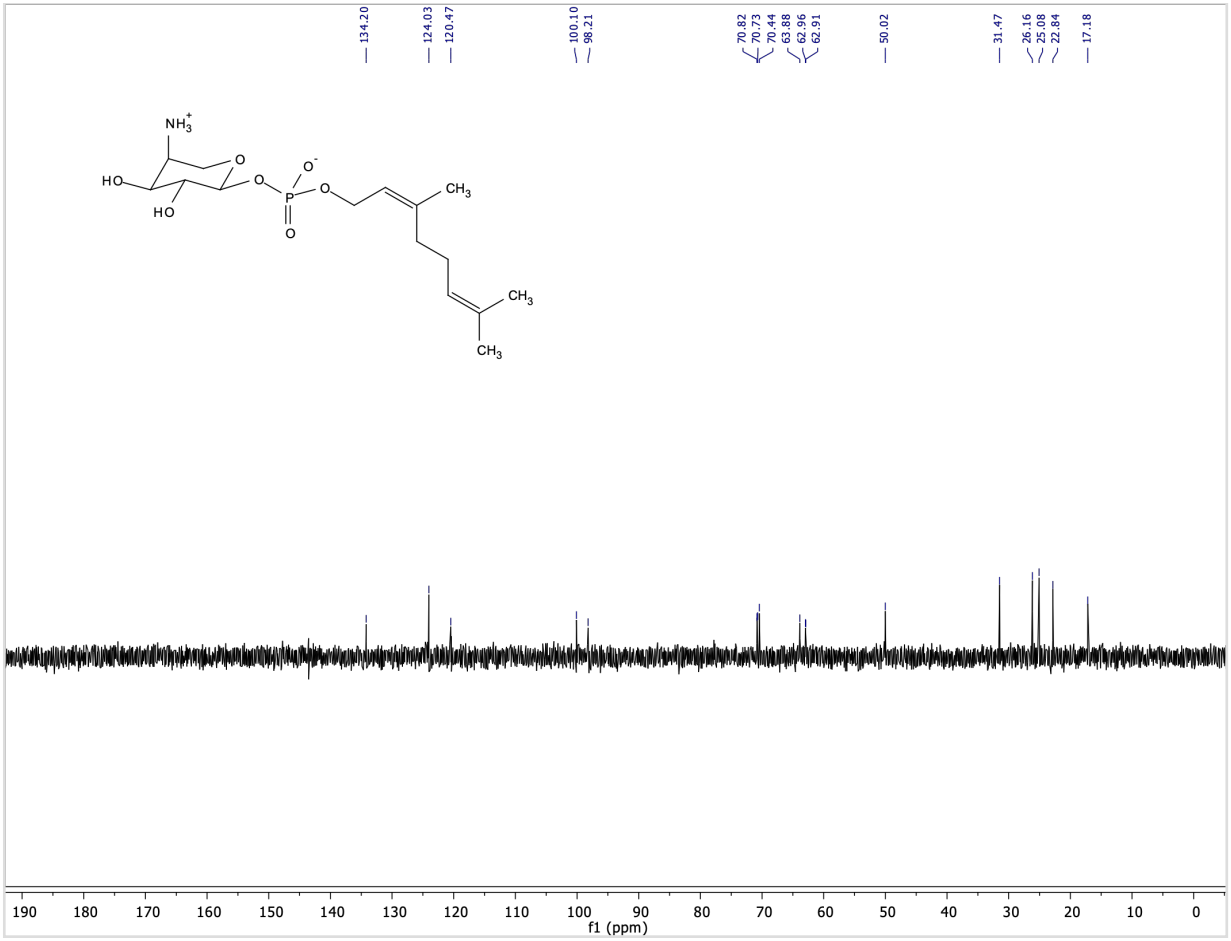


Figure S17. ^{1}H NMR (D_2O, 400 MHz) of compound **2**:

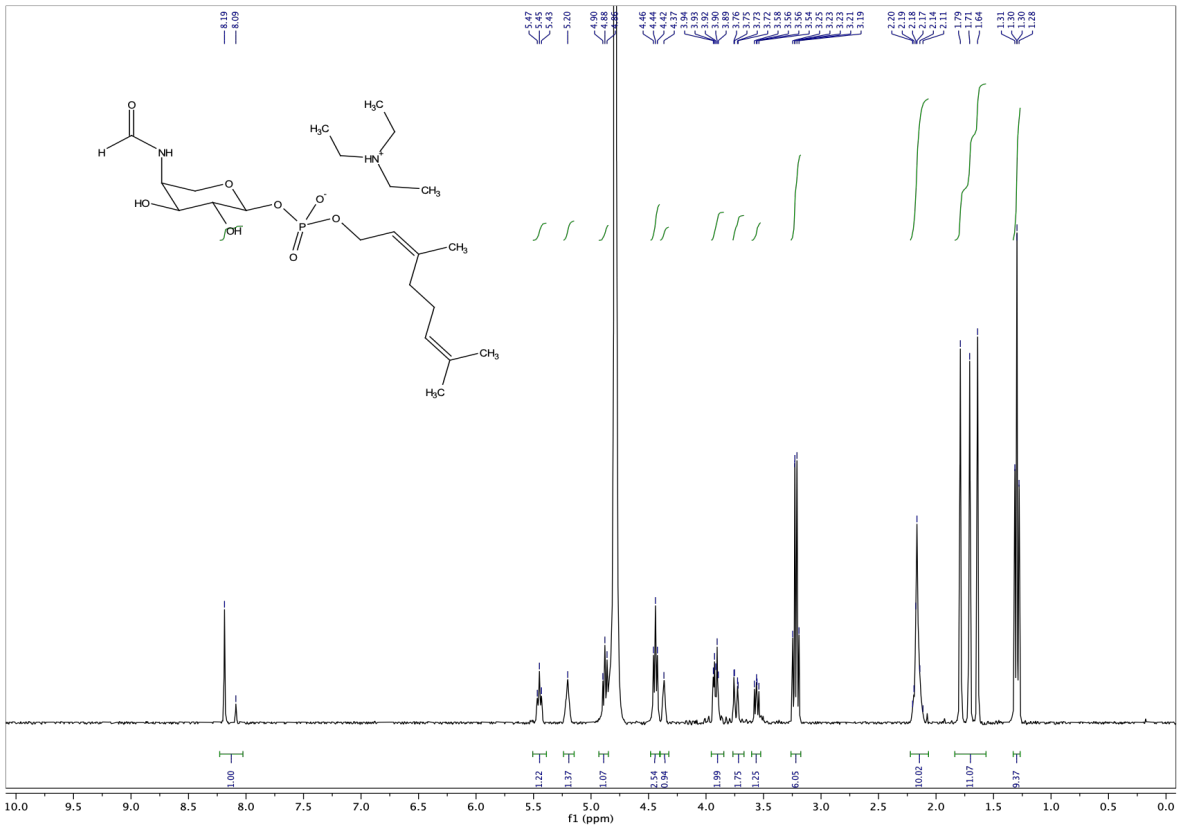


Figure S18. ^{31}P $\{^{1}H\}$ NMR (D_2O, 162 MHz) of compound **2**:

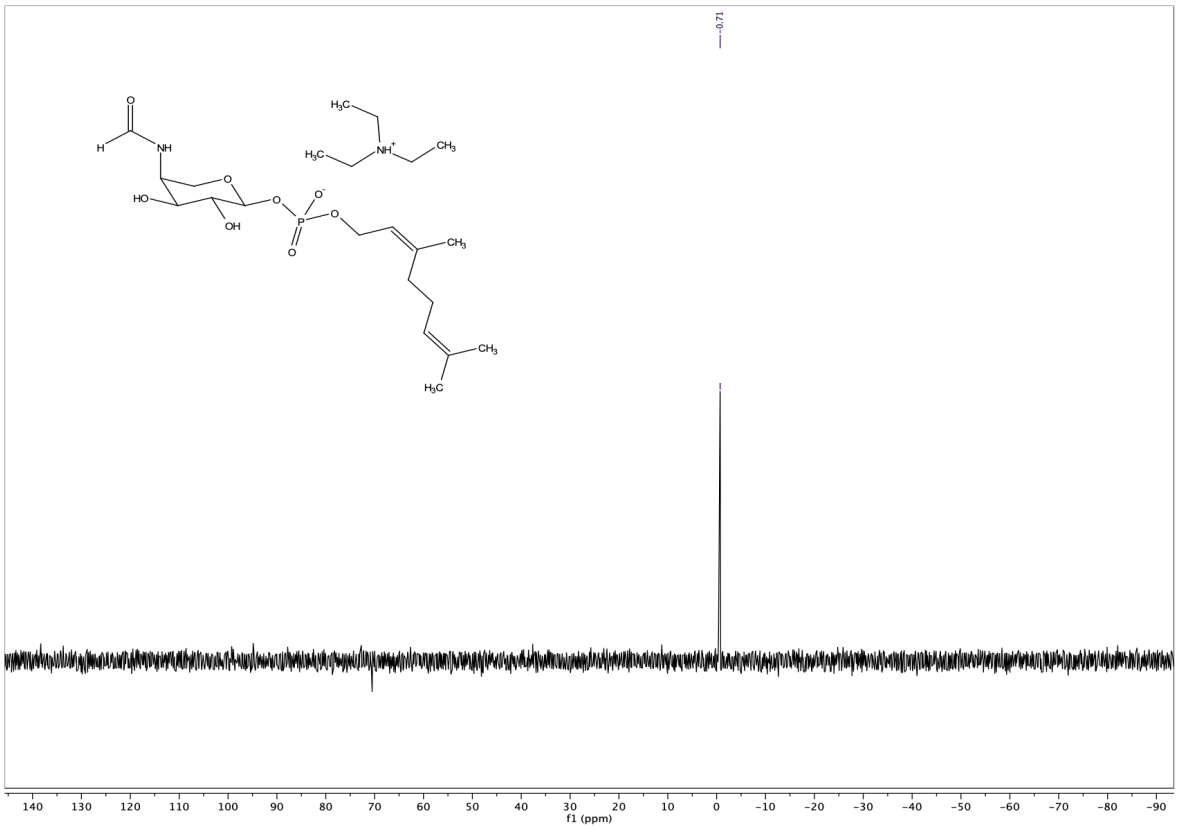


Figure S19. ^{13}C NMR (D_2O, 100 MHz) of compound **2**: