Supporting Information

For

Chemical Synthesis of Natural and Azido-Modified UDP-Rhamnose and -Arabinofuranose for Glycan Array-Based Characterization of Plant Glycosyltransferases

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Abbreviations

ACN = Acetonitrile

AcOH = Acetic acid

AEX = Anion exchange

Anh. = Anhydrous

Aq. = Aqueous

DCM = Dichloromethane

DMAP = 4-Dimethylaminopyridine

DMF = Dimethylformamide

DMSO = Dimethylsulfoxide

EA = Ethyl acetate

Eq = Equivalents

Et_2O = Diethyl ether

EtOH = Ethanol

Hex = Hexane

Hilic = Hydrophilic interaction liquid chromatography

HPLC = High performance liquid chromatography

MeOH = Methanol

PTFE = Polytetrafluoroehtylene

R_f = Retention factor

rt = Room temperature

TBAF = Tetra-n-butylammonium fluoride

TBDPSCl = *tert*-Butyldiphenylsilyl chloride

TEA = Triethylamine

TEAB = Triethylammonium bicarbonate

THF = Tetrahydrofuran

TMSBr = Bromotrimethylsilane

Tol = Toluene

General methods

All purchased chemicals were used without further purification. Solvents were dried over activated 4 Å molecular sieves. Aqueous solutions of salts were saturated unless stated otherwise. The concentration of organic solutions was performed under reduced pressure at 40°C, unless stated otherwise. All reactions were monitored through thin layer chromatography (unless stated otherwise), which was performed on Merck pre-coated plates: generally, on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively, on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by a dipping reagent (anisaldehyde-H_2SO_4) and heating. Filtrations were performed using 25 mm syringe filters (PTFE, 0.45 µm) from Fischer Brand. Silica gel (0.040 – 0.063 mm) was used for direct phase column chromatography, and purifications were performed either by hand or on the automatic system Interchim puriFlash 4125 or Interchim puriFlash 5.250. AEX Chromatography was performed on an Amersham ÄKTA Explorer 100. Liquid chromatography-mass spectrometry (LC-MS) analysis were performed on a Shimadzu LC 10A combined with a Shimadzu LCMS-2020. NMR spectra were recorded on a Bruker Avance III 600 instrument (600.22 MHz for ^{1}H, 150.93 MHz for decoupled ^{13}C, 242.96 for decoupled ^{31}P), a Bruker AVIII-HD (300 MHz), a Varian Premium Compact (400 MHz) or a Bruker Ascend (400 MHz), using standard software provided by the manufacturer. ^{1}H spectra were referenced to 0 (external calibration to TMS) or 7.26 ppm for solutions in $CDCl_3$, 3.31 ppm for solutions in MeOD, 5.32 ppm for solutions in CD_2Cl_2 and 0 ppm (external calibration to DSS) for solutions in D_2O; ^{13}C spectra were referenced to 77.00 ppm for solutions in $CDCl_3$, 49.10 ppm for solutions in MeOD, 54.00 ppm for solutions in CD_2Cl_2 and 67.40 ppm (external calibration to 1,4-dioxane) for solutions in D_2O; ^{31}P NMR spectra where referenced to 0 ppm (external calibration to H_3PO_4) for solutions in MeOD and D_2O. Assignments were based on COSY, HSQC and HMBC spectra. ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

General procedures

General procedure A

A solution of anh. allyl alcohol (4.57 mL, 67.1 mmol) and anh. TEA (9.36 mL, 70.2 mmol) in anh. Et_2O (20 mL) was added dropwise to a stirred solution of $POCl_3$ (2.80 mL, 30.5 mmol) in anh. Et_2O (20 mL) within 45 minutes at 0°C under argon atmosphere. Formation of a white precipitate (Et_3NHCl) was observed as soon as the second solution was added to the reaction flask. The reaction mixture was stirred vigorously for additional 2 h, while allowed to slowly warm up to rt. The mixture was then cooled down to -18°C for 30 minutes to favour the precipitation of the salt, and filtered off under argon atmosphere. The solvent was removed under reduced pressure to afford crude diallyl chlorophosphate in a mixture with the by-product triallyl phosphate (diallyl chlorophosphate:triallyl phosphate = 5:1 from ^{1}H NMR integration. This ratio was used for future stoichiometric calculations). As triallyl phosphate does not react with hemiacetals, the mixture was used for the synthesis of glycosyl-1-phosphates without further purification. The hemiacetal (1.0 eq) and DMAP (15 eq) were dissolved in anh. DCM (25 mM solution of the hemiacetal) and a 1 M solution of the freshly prepared diallyl chlorophosphate (7.5 eq) in anh. DCM was added dropwise under argon atmosphere, while cooling down the system to 0°C. After removing the ice bath, a saturated solution of aq. $NaHCO_3$ was immediately added, unless stated otherwise. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Hex/EA: 8/2 to 3/7) afforded the desired diallyl-protected glycosyl-1-phosphates.

General procedure B

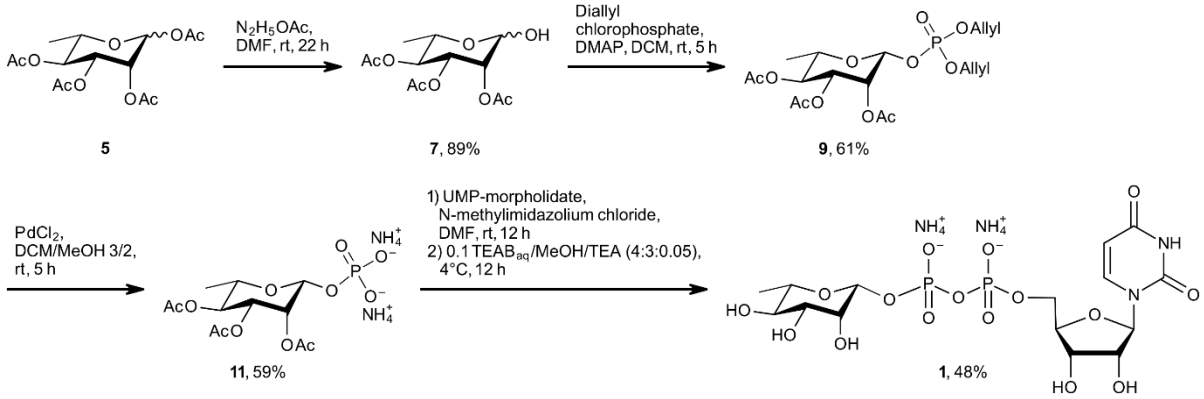
A solution of diallyl-protected glycosyl-1-phosphate (1.0 eq) and $PdCl_2$ (0.5 eq) in anh. DCM/MeOH: 3/2 (50 mM solution of diallyl-protected glycosyl-1-phosphate) was stirred at rt under argon atmosphere for 5 h, when triethylamine (2.0 eq) was added to the reaction mixture and the solution filtered over a PTFE syringe filter. The filtrate was then stirred for 2 h in presence of a palladium scavenger (QuadraPure® TU), filtered through cotton and concentrated under reduced pressure. The crude was then pre-purified by filtration over a 200 mg SeQuant ZIC-HILIC SPE column (pure ACN to ACN/milliQ water: 1/1). Final purification by Hilic-HPLC (SeQuant ZIC-HILIC, 5 µm, 200 Å, 250 x 10; pure ACN to ACN/NH_4COCH_3 buffer (aq., 13 mM): 6/4; flowrate = 5 mL/min) afforded the desired deprotected glycosyl-1-phosphates as di-ammonium salts.

General procedure C

UMP-morpholidate (4-morpholine-*N*,*N'*-dicyclohexylcarboxamidine salt, 1.6 eq) and *N*-methylimidazolium chloride (5.4 eq) were added to a solution of glycosyl-1-phosphate (1.0 eq) in anh. DMF (35 mM solution) under argon atmosphere and the mixture was stirred at rt for 16 h, when LC-MS analysis showed complete consumption of the starting material and formation of the product. The reaction was quenched by adding MeOH and the mixture concentrated under reduced pressure. After purification of the crude by flash reverse chromatography (C18 material, MeOH/NH_4OAc buffer (aq., 13 mM): 5/95 to 90/10) and concentration of the fractions under reduced pressure, the protected UDP-sugar was dissolved without further purification in a mixture of TEAB buffer (aq., 0.1 M)/MeOH/TEA: 4/3/0.05 (5 mM solution) and the solution was stirred at 4°C for 16 h. A small aliquot was dissolved in D_2O and analysed by ^{31}P NMR, indicating complete consumption of the starting material and formation of the product. Purification of the crude material by AEX chromatography (5 mL Bio-Rad EconoFit Macro-Prep High Q Column; aq. NH_4COCH_3 buffer: 0.05 to 0.5 M; flowrate = 5 mL/min; rt) afforded the desired UMP-sugars as di-ammonium salts.

Due to limited stability (hydrolysis, cyclic sugar-1,2-phosphate formation) of the products in solution, no ^{13}C NMR spectra were recorded for all UDP-sugars. Furthermore, in order to increase the stability of the products, NMR spectra were recorded in a 0.3 M solution of NH_4COCH_3 in D_2O. The main buffer peak is found at 1.9 ppm. A blank ^1H NMR spectrum of the buffered D_2O is reported at page 39.

Synthesis of UDP-Rha



Scheme S1. Synthesis of UDP-Rha (**1**).

2,3,4-Tri-*O*-acetyl-L-rhamnopyranose (**7**)

Hydrazine acetate (110 mg, 1.19 mmol) was added to a solution of **5**[1] (330 mg, 0.993 mmol) in DMF (5.0 mL) under argon atmosphere, and the mixture was stirred at 50°C until all the solid was dissolved (45 minutes). The mixture was then allowed to cool down to rt, diluted with EA and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Hex/EA: 6/4) afforded **7** (257 mg, 89%) as a white solid as a mixture of anomers (α:β = 6.7:1 from ^{1}H NMR integration); R_f 0.33 (Hex/EA: 6/4); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.38 (m_{br}, 1H, *H*-3 β), 5.36 (dd, *J* = 10.0, 3.4 Hz, 1H, *H*-3 α), 5.26-5.25 (m_{br}, 1H, *H*-2 α), 5.15 (s_{br}, 1H, *H*-1 α), 5.08 (t_{br}, *J* = 10.0 Hz, *H*-4 α), 5.02-5.01 (m, 2H, *H*-2 and *H*-4 β), 4.95 (s_{br}, 1H, *H*-1 β), 4.14-4.09 (m, 1H, *H*-5 α), 3.67 (s_{br}, 1H, 1-O*H* β), 3.59-3.54 (m, 1H, *H*-5 β), 3.38 (s_{br}, 1H, 1-O*H* α) 2.20 (s, 3H, -COC*H*$_3$ β), 2.14 (s, 3H, -COC*H*$_3$ α), 2.04 (s, 6H, -COC*H*$_3$ α and -COC*H*$_3$ β), 1.98 (s, 3H, -COC*H*$_3$ α), 1.97 (s, 3H, -COC*H*$_3$ β), 1.26 (d, *J* = 6.1 Hz, 3H, *H*-6 β), 1.21 (d, *J* = 6.3 Hz, 3H, *H*-6 α). The analytical data is in full agreement with literature data[2].

Diallyl(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)phosphate (**9**)

Starting from hemiacetal **7**, General procedure A afforded **9** (226 mg, 61%) as a colorless sticky oil. Addition of the diallyl chlorophosphate solution was performed within 5 h; R_f 0.31 (Hex/EA: 6/1); ^{1}H NMR (600 MHz, MeOD) δ 6.00-5.92 (m, 2H, *H*-2') 5.55 (d, $J_{P,H}$ = 7.0 Hz, 1H, *H*-1), 5.52 (d, *J* = 3.3 Hz, 1H, *H-2*), 5.40-5.36 (m, 1H, *H*-3'a), 5.28-5.25 (m, 1H, *H*-3'b), 5.18 (dd, *J* = 10.0, 3.3 Hz, 1H, *H*-3), 4.98 (t_{br}, J_1 = J_2 = 10.0 Hz, 1H, *H*-4), 4.59-4.54 (m, 4H, *H*-1'), 3.79-3.74 (m, 1H, H-5), 2.16 (s, 3H, -COC*H*$_3$), 2.05 (s, 3H, -COC*H*$_3$), 1.96 (s, 3H, -COC*H*$_3$), 1.25 (d, *J* = 6.2 Hz, 3H, *H*-6); ^{13}C NMR (151 MHz, MeOD) δ 171.8 (-C, -*C*OCH$_3$), 171.7 (-C, -*C*OCH$_3$), 171.4 (-C, -*C*OCH$_3$), 133.7 (-CH, d, $J_{C,P}$ = 7.3 Hz, *C*-2'), 133.6 (-CH, d, $J_{C,P}$ = 6.6 Hz, *C*-2'), 119.0 (2(-CH_2), *C*-3'), 95.6 (-CH, d, $J_{C,P}$ = 4.5 Hz, *C*-1), 72.4 (-CH, *C*-5), 72.0 (-CH, *C*-3), 71.7 (-CH, *C*-4), 70.5 (-CH, d, $J_{C,P}$ = 8.3 Hz, *C*-2), 70.2 (2(-CH_2), t_{br}, $J_{C,P}$ = 5.6 Hz, *C*-1'), 20.7 (-CH_3, -CO*C*H$_3$), 20.6 (-CH_3, -CO*C*H$_3$), 20.5 (-CH_3, -CO*C*H$_3$), 17.8 (-CH_3, *C*-6); ^{1}H-coupled ^{31}P NMR (243 MHz, MeOD) δ -3.41 (sx, *J* = 8.8 Hz); ESI-TOF HRMS m/z calcd for $C_{18}H_{27}O_{11}P$ $[M+H]^+$: 451.1364; found 451.1370.

1-*O*-Phosphoryl-2,3,4-tri-*O*-acetyl-β-L-rhamnopyranoside (**11**)

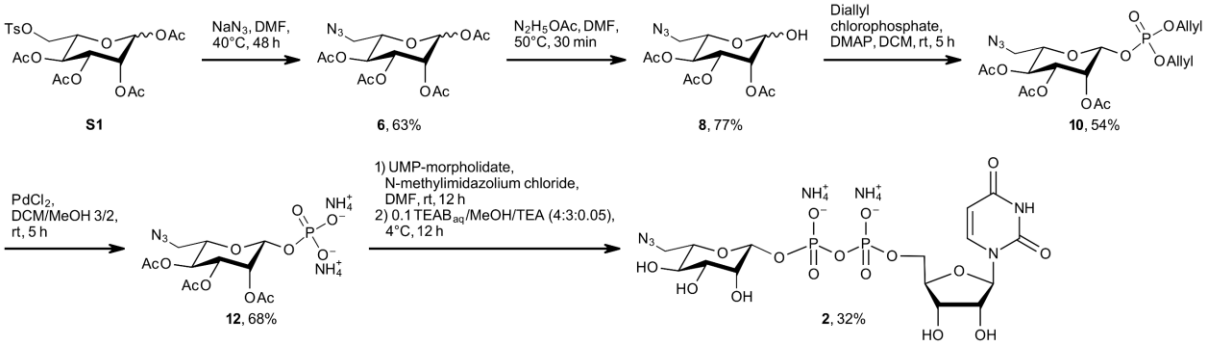
Starting from diallyl-protected phosphate **9**, General procedure B afforded **11** (30.0 mg, 59%) as a white solid; R_f 0.17 (DCM/MeOH/TEA: 1/1/0.1); ^{1}H NMR (600 MHz, MeOD) δ 5.48 (d, *J* = 3.3 Hz, 1H, *H*-2), 5.36 (d, J_{P-H} = 8.9 Hz, 1H, *H*-1), 5.10 (dd, *J* = 10.0, 3.3 Hz, 1H, *H*-3), 4.96 (t_{br}, J_1 = J_2 = 10.0 Hz, 1H, *H*-4), 3.71-3.67 (m, 1H, *H*-5), 2.14 (s, 3H, -COC*H*$_3$), 2.05 (s, 3H, -COC*H*$_3$), 1.94 (s, 3H, -COC*H*$_3$), 1.23 (d, *J* = 6.2 Hz, 3H, *H*-6). ^{13}C NMR (151 MHz, MeOD) δ 172.1 (-C, -*C*OCH$_3$), 171.9 (-C, -*C*OCH$_3$), 171.5 (-C, -*C*OCH$_3$), 94.9 (-CH, d, J_{C-P} = 3.5 Hz, *C*-1), 72.8 (-CH, *C*-3), 72.1 (-CH, *C*-5), 72.0 (-CH, *C*-4), 71.6 (d, -CH, J_{C-P} = 6.6 Hz, *C*-2), 20.9 (-CH_3, -CO*C*H$_3$), 20.8 (-CH_3, -CO*C*H$_3$), 20.7 (-CH_3, -CO*C*H$_3$), 17.9 (-CH_3, *C*-6); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -1.77; ESI-TOF HRMS m/z calcd for $C_{12}H_{19}O_{11}P$ $[M-H]^-$: 369.0592; found 369.0599.

UDP-β-L-Rhamnopyranose (**1**)

Starting from **11**, General procedure C afforded **1** (2.54 mg, 48% over two steps, product contains small amounts of NH_4COCH_3 buffer) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 8.02 (d, *J* = 8.2 Hz, 1H, *H*-6''), 6.06-6.02 (m, 2H, H-5'' and *H*-1'), 5.29 (d, *J* = 9.2 Hz, 1H, *H*-1), 4.45-4.43 (m, 2H, *H*-2' and *H*-3'), 4.35-4.34 (m, 1H, *H*-4'), 4.30 (ddd, *J* = 11.8, 4.7, 2.6 Hz, 1H, *H*-5'a), 4.25 (ddd, *J* = 11.8, 5.6, 2.9 Hz, *H*-5'b), 4.15 (d, *J* = 3.4 Hz, *H*-3), 3.71 (dd, *J* = 9.7, 3.4 Hz, *H*-4), 3.53-3.49 (m, 1H, *H*-5), 3.44 (t_{br}, *J* = 9.2-Hz, 1H, *H*-2), 1.38 (d, *J* = 6.1 Hz, 3H, C*H*$_3$-6); ^{1}H-decoupled ^{31}P NMR (243 MHz, D_2O) δ -11.39 (d, *J* = 21.0 Hz,

1P, -O*P*(O)(O$^-$)-UMP), -13.62 (d, *J* = 21.0 Hz, 1P, d, *J* = 20.6 Hz, -O*P*(O)(O$^-$)-Rha). The analytical data is in full agreement with literature data[3, 4].

Synthesis of UDP-N_3-Rha



Scheme S2. Synthesis of UDP-N_3-Rha (**2**).

6-Azido-6-deoxy-1,2,3,4-tetra-*O*-acetyl-L-mannopyranose (**6**)

Sodium azide (1.04 g, 15.9 mmol) was added to a solution of **S1**[5] (2.00 g, 3.98 mmol) under argon atmosphere. The solution was stirred at 40°C for 48 h, then concentrated under reduced pressure. The residue was dissolved in EA and washed with water, a saturated solution of aq. $NaHCO_3$ and brine. The organic layers were combined, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification of the crude by flash chromatography (1st purification, Hex/EA: 2/1; 2nd purification, Hex/Acetone: 8/2 with pyridine 0.1%) afforded **6** (932 mg, 63%) as a colourless sticky oil as a mixture of anomers (α:β = 1:10 from ^{1}H NMR integration); R_f 0.30 (Hex/EA : 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (d, *J* = 1.9 Hz, 1H, *H*-1 α), 5.86 (d, *J* = 1.0 Hz, 1H, *H*-1 β), 5.48 (dd, *J* = 3.2, 1.0 Hz, 1H, *H*-2 β), 5.37-5.31 (m, 2H, H-*3* and *H*-4 α), 5.27-5.23 (m, 2H, *H*-2 α and *H*-4 β), 5.11 (dd, *J* = 10.0, 3.2 Hz, *H*-3 β), 4.03-3.95 (m, 1H, *H*-5 α), 3.77-3.72 (m, 1H, *H*-5 β), 3.44-3.37 (m, 2H, *H*-6a α and *H*-6a β), 3.34-3.28 (m, 2H, *H*-6b α and *H*-6b β), 2.21 (s, 3H, -COC*H*$_3$ β), 2.18 (s, 3H, -COC*H*$_3$ α), 2.17 (s, 3H, -COC*H*$_3$ α), 2.10 (s, 3H, -COC*H*$_3$ β), 2.06 (s, 6H, -COC*H*$_3$ α and -COC*H*$_3$ β), 2.01 (s, 3H, -COC*H*$_3$ α), 2.00 (s, 3H, -COC*H*$_3$ β); The analytical data is in full agreement with literature data[5].

6-Azido-6-deoxy-2,3,4-tri-*O*-acetyl- L-mannopyranose (**8**)

Hydrazine acetate (23.4 mg, 0.254 mmol) was added to a solution of **6** (79.0 mg, 0.212 mmol) in DMF (1 mL) under argon atmosphere, and the mixture was stirred at 50°C until all the solid was dissolved (20 minutes), then allowed to cool down to rt, diluted with EA and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 8/2) afforded **8** (54.1 mg, 77%) as a colourless sticky oil as a mixture of anomers (α:β = 8:1 from ^{1}H NMR integration); R_f 0.33 (Hex/Acetone: 2/1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.42 (m, 2H, *H*-2 α and *H*-2 β), 5.29-5.25 (m, 3H, *H*-1, *H*-3 and *H*-4 α), 5.20 (t, *J* = 10.0 Hz, 1H, *H*-4 β), 5.08 (dd, *J* = 10.0, 3.2 Hz, 1H, *H*-3 β), 5.00 (s_{br}, 1H, *H*-1 β), 4.19 (dt, *J* = 9.7, 4.7 Hz, 1H, *H*-5 α), 3.66 (ddd, *J* = 10.0, 5.2 Hz, 1H, *H*-5 β), 3.40-3.39 (m, 2H, *H*-6 β), 3.36 (d_{br}, *J* = 4.7 Hz, 2H, *H*-6 α), 2.97 (d_{br}, *J* = 2.9 Hz, 1H, 1-O*H* α); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.3 (-C, -*C*OCH_3), 170.1 (-C, -*C*OCH_3), 170.0 (-C, -*C*OCH_3), 93.0 (-CH, *C*-1 β), 92.3 (-CH, *C*-1 α), 73.9 (-CH, *C*-5 β), 71.2 (-CH, *C*-3 β), 70.3 (-CH, *C*-2 β), 70.1 (-CH, *C*-5 α), 70.0 (-CH, *C*-3 α), 68.8 (-CH, *C*-2 α), 67.4 (-CH, *C*-4), 66.7 (-CH, *C*-4 β), 51.4 (-CH_2, *C*-6 α), 51.2 (-CH_2, *C*-6 β), 21.1 (-CH_3, -CO*C*H_3), 20.9 (-CH_3, -CO*C*H_3), 20.8 (-CH_3, -CO*C*H_3); ESI-TOF HRMS m/z calcd for $C_{12}H_{17}N_3O_8$ [M-H]$^-$: 330.0943; found 330.0943.

Diallyl(6-azido-6-deoxy-2,3,4-tri-*O*-acetyl-β-L-mannopyranosyl)phosphate (**10**)

Starting from hemiacetal **8**, General procedure A afforded **10** (24.1 mg, 54%) as a colorless sticky oil. Addition of the diallyl chlorophosphate solution was performed within 5 h; R_f 0.17 (Hex/EA: 1/1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.97-5.88 (m, 2H, *H*-2'), 5.52-5.50 (m, 2H, *H*-2 and *H*-1), 5.39-5.35 (m, 2H, 2(*H*-3'a)), 5.29-5.25 (m, 3H, 2(*H*-3'b) and *H*-4), 5.07 (dd, *J* = 10.0, 3.2 Hz, 1H, *H*-3), 4.63-4.49 (m, 4H, *H*-1'), 3.74-3.71 (m, 1H, *H*-5), 3.43 (dd, *J* = 13.5, 2.9 Hz, *H*-6a), 3.38 (dd, *J* = 13.5, 5.9 Hz, *H*-6b), 2.20 (s, 3H, -COC*H*$_3$), 2.06 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.1 (-C, -*C*OCH_3), 169.9 (-C, -*C*OCH_3), 169.8 (-C, -*C*OCH_3), 132.3 (d, $J_{C\text{-}P}$ = 8.1 Hz, *C*-2'), 132.1 (d, $J_{C\text{-}P}$ = 7.1 Hz, *C*-2'), 118.9 (-CH_2, *C*-3'), 118.8 (-CH_2, *C*-3'), 94.2 (d, $J_{C\text{-}P}$ = 4.2 Hz, *C*-1), 74.4 (-CH, *C*-5), 70.6 (-CH, *C*-3), 69.0 (t_{br}, $J_{C\text{-}P}$ = 4.3 Hz, *C*-1'), 68.7 (d, *J* = 8.3 Hz, *C*-2), 66.3 (-CH, *C*-4), 51.0 (-CH_2, *C*-6), 20.9 (2(-CH_3), -CO*C*H_3), 20.7 (-CH_3, -CO*C*H_3); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -2.88; ESI-TOF HRMS m/z calcd for $C_{18}H_{26}N_3O_{11}P$ $[M+K]^+$: 530.0936; found 530.0925.

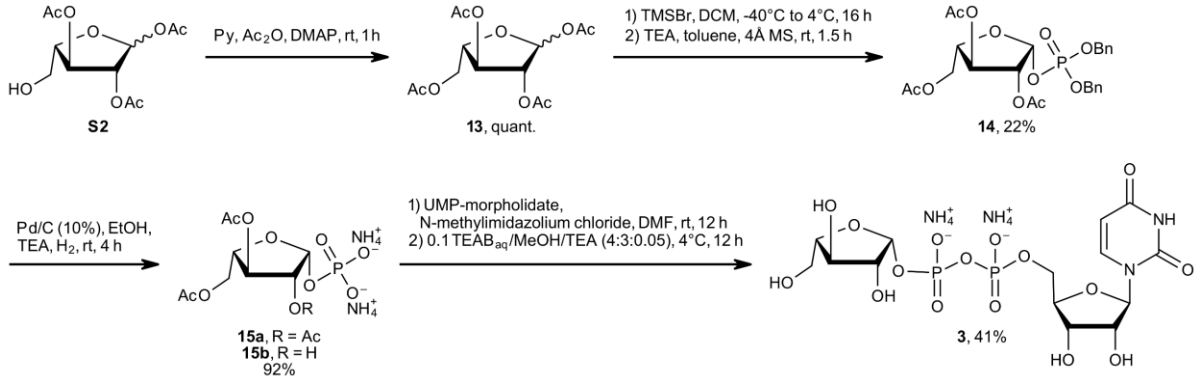
1-*O*-Phosphoryl-6-azido-6-deoxy-2,3,4-tri-*O*-acetyl-β-L-mannopyranoside (**12**)

Starting from diallyl-protected phosphate **10**, General procedure B afforded **12** (17.0 mg, 68%) as a white solid; R_f 0.20 (DCM/MeOH/TEA: 1/1/0.1); ^{1}H NMR (600 MHz, MeOD) δ 5.49 (d, $J_{P\text{-}H}$ = 3.2, $J_{1,2}$ = 1.0 Hz, 1H, *H*-2), 5.38 (dd, $J_{P\text{-}H}$ = 9.2, $J_{1,2}$ = 1.0 Hz, 1H, *H*-1), 5.23 (t_{br}, $J_1 = J_2$ = 9.9 Hz, 1H, *H*-4), 5.17 (dd, *J* = 3.2, 9.9 Hz, 1H, *H*-3), 3.80-3.77 (m, 1H, *H*-5), 3.59 (dd, *J* = 13.4, 3.0 Hz, 1H, *H*-6a), 3.35 (dd, *J* = 13.5, 4.6 Hz, 1H, *H*-6b), 2.15 (s, 3H, -COC*H*$_3$), 2.04 (s, 3H, -COC*H*$_3$), 1.94 (s, 3H, -COC*H*$_3$); ^{13}C NMR (151 MHz, MeOD) δ 172.1 (-C, -*C*OCH_3), 171.6 (-C, -*C*OCH_3), 171.4 (-C, -*C*OCH_3), 95.0 (-CH, d, $J_{C\text{-}P}$ = 3.7 Hz, *C*-1), 74.5 (-CH, *C*-5), 72.9 (-CH, *C*-3), 71.5 (d, -CH, $J_{C\text{-}P}$ = 6.3 Hz, *C*-2), 68.0 (-CH, *C*-4), 52.1 (-CH_2, *C*-6), 20.8 (-CH_3, -CO*C*H_3), 20.7 (-CH_3, -CO*C*H_3), 20.6 (-CH_3, -CO*C*H_3); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -0.85; ESI-TOF HRMS m/z calcd for $C_{12}H_{18}N_3O_{11}P$ $[M-H]^-$: 410.0606; found 410.0618.

UDP-6-N_3-β-L-Rhamnopyranose (**2**)

Starting from **12**, General procedure C afforded **2** (2.31 mg, 32% over two steps, product contains small amounts of NH_4COCH_3 buffer) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.2 Hz, 1H, *H*-6''), 6.02-5.99 (m, 2H, *H*-5'' and *H*-1'), 5.28 (d, *J* = 8.9 Hz, 1H, *H*-1), 4.41-4.39 (m, 2H, *H*-2' and *H*-3'), 4.32-30 (m, 1H, *H*-4'), 4.27 (ddd, *J* = 11.5, 4.6, 2.5 Hz, 1H, *H*-5'a), 4.22 (ddd, *J* = 11.5, 5.7, 2.8 Hz, 1H, *H*-5'b), 4.12 (d, *J* = 3.2 Hz, 1H, *H*-3), 3.76 (dd, *J* = 13.4, 2.4 Hz, *H*-6a), 3.72 (dd, *J* = 9.7, 3.2 Hz, 1H, *H*-4), 3.67-3.60 (m, 2H, *H*-2 and *H*-6b), 3.57-3.53 (m, 1H, *H*-5); ^{1}H-decoupled ^{31}P NMR (243 MHz, D_2O) δ -11.40 (d, *J* = 20.6 Hz, 1P, -O*P*(O)(O^-)-UMP), -13.73 (d, *J* = 20.6 Hz, -O*P*(O)(O^-)-Rha).

Synthesis of UDP-Ara*f*



Scheme S2. Synthesis of UDP-Ara (**3**).

1,2,3,5-Tetra-*O*-acetyl-L-arabinofuranose (**13**)

A solution of **S2** (250 mg, 0.905 mmol, synthesis at page 10 in anh. pyridine/Ac_2O: 2/1 (9 mL) containing a catalytic amount of DMAP was stirred at rt under argon atmosphere for 1 h, when the reaction was quenched by adding MeOH at 0°C and the solvents were co-evaporated thrice with toluene under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 7/3) afforded **13** (287 mg, quant.) as a yellow sticky oil as a mixture of anomers (α:β = 1:1.3 from ^{1}H NMR integration); R_f 0.43 (Tol/EA: 7/3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.35 (d_{br}, 1H, *J* = 3.9 Hz, *H*-1 α), 6.17 (s, 1H, *H*-1 β), 5.33-5-32 (m, 2H, *H*-2 α and *H*-3 α), 5.19 (d_{br}, 1H, *J* = 1.7 Hz, *H*-2 β), 5.03-5.01 (m, 1H, *H*-3 β), 4.38-4.31 (m, 3H, *H*-4 and *H*-5a β and *H*-5a α), 4.25-4.16 (m, 3H, *H*-4 and *H*-5b α and *H*-5b β), 2.10 (s, 6H, 2(-COC*H*$_3$) β), 2.09 (s, 3H, -COC*H*$_3$ β), 2.08 (s, 3H, -COC*H*$_3$ α), 2.07 (s, 3H, -COC*H*$_3$ α), 2.07 (s, 3H, -COC*H*$_3$ β), 2.06 (s, 3H, -COC*H*$_3$ α), 2.05 (s, 3H, -COC*H*$_3$ α). The analytical data is in full agreement with literature data[6].

Dibenzyl(2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl)phosphate (**14**)

TMSBr (0.95 mL, 7.23 mmol) was added to a solution of **13** (230 mg, 0.723 mmol) in anh. DCM (3 mL) under argon atmosphere, while cooling the system to -40°C. The reaction was allowed to warm up to 0°C within 2 h while stirring. Additional additions of TMSBr (2 x 5.0 eq) were made after 8 and 16 h, each time after lowering the temperature to -40°C. After a total of 24 h, the solution was co-concentrated thrice with anh. toluene under reduced pressure at 30°C to afford the crude arabinosyl bromide (R_f 0.38 (Tol/EA: 1/1)) as an orange oil, which was re-dissolved in anh. toluene (3.6 mL) and transferred in an argon-flushed flask containing flame-dried 4Å MS (200 mg) and dibenzyl phosphate (603 mg, 2.17 mmol). After stirring for 15 minutes, anh. TEA (0.35 mL, 2.53 mmol) was added to the reaction mixture, which was stirred at rt for another hour, when ^{1}H NMR analysis of an aliquot showed complete consumption of the bromide. The reaction mixture was filtered through Celite and concentrated under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 6/4) afforded phosphate **14** (85.1 mg, 22% over two steps) as a yellowish sticky oil; R_f 0.39 (Tol/EA: 6/4); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.39-7.33 (m, 10H, Ph), 6.00 (dd, $J_{P,H}$ = 5.4, $J_{1,2}$ = 4.4 Hz, 1H, *H*-1), 5.39 (dd, *J* = 7.4, 5.9 Hz, 1H, *H*-3), 5.24 (ddd, $J_{2,3}$ = 7.4, $J_{2,1}$ = 4.4, $J_{P,H}$ = 2.0 Hz, 1H, *H*-2), 5.07-5.01 (m, 4H, 2(-C*H*$_2$Ph)), 4.43 (dd, *J* = 11.9, 3.8 Hz, 1H, *H*-5a), 4.22 (ddd, *J* = 5.9, 6.9, 3.8 Hz, 1H, *H*-4), 4.13 (dd, *J* = 11.9, 6.9 Hz, *H*-5b), 2.08 (s, 3H, -COC*H*$_3$), 1.97 (s, 3H, -COC*H*$_3$), 1.96 (s, 3H, -COC*H*$_3$); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 170.4 (-C, -*C*OCH$_3$), 170.2 (-C, -*C*OCH$_3$), 170.0 (-C, -*C*OCH$_3$), 135.9 (2(-C), Ph), 128.7, 128.6, 128.0, 127.9 (5(-CH), Ph), 97.8 (-CH, d, $J_{C,P}$ = 5.3 Hz, *C*-1), 80.0 (-CH, *C*-4), 76.0 (-CH, d, $J_{C,P}$ = 7.2 Hz, *C*-2), 74.0 (-CH, *C*-3), 69.5 (-CH_2, d, $J_{C,P}$ = 5.5 Hz, -*C*H$_2$Ph), 69.4 (-CH_2,

d, $J_{C,P}$ = 5.5 Hz, -*C*H_2Ph), 64.5 (-CH_2, *C*-5), 20.6, 20.5, 20.3 (3(-CH_3), 3(-CO*C*H_3)); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -2.87; ESI-TOF HRMS m/z calcd for $C_{25}H_{29}O_{11}P$ $[M+H]^+$: 537.1526; found 537.1523.

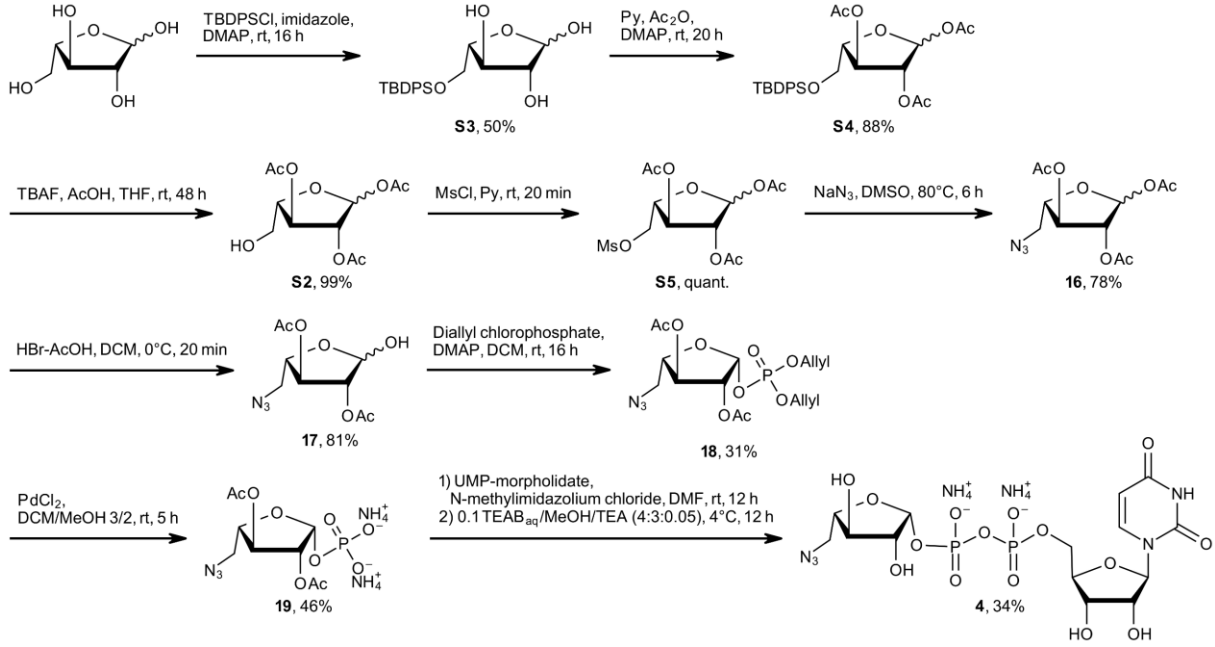
1-*O*-Phosphoryl-2,3,5-tri-*O*-acetyl-β-L-arabinofuranoside (**15a**) and 1-*O*-Phosphoryl-3,5-di-*O*-acetyl-β-L-arabinofuranoside (**15b**)

A flask containing dibenzyl-protected phosphate **14** (50.0 mg, 93.2 µmol) was flushed with argon thrice before the addition of EtOH (2 mL), TEA (162 µL, 1.17 mmol) and a catalytic amount of 10% Pd/C. The system was then flushed thrice with hydrogen and the mixture stirred at rt for 4 h, when the catalyst was filtered off over a PTFE syringe filter and the solvent removed under reduced pressure. The crude was then pre-purified by filtration over a 200 mg SeQuant ZIC-HILIC SPE column (pure ACN to ACN/milliQ water: 1/1). Final purification by Hilic-HPLC (SeQuant ZIC-HILIC, 5 µm, 200 Å, 250 x 10; pure ACN to ACN/NH_4COCH_3 buffer (aq., 13 mM): 6/4; flowrate = 5 mL/min) afforded a mixture of the desired phosphate **15a** and the 2-deacetylated phosphate **15b** (38.9 mg, 92%, a:b = 1:0.3 from ^{1}H NMR integration) as a colourless sticky oil; R_fa 0.27, R_fb 0.15 (DCM/MeOH/TEA: 1/1:0.1); ^{1}H NMR (600 MHz, MeOD) δ 5.81 (dd, $J_{P,H}$ = 6.9, $J_{1,2}$ = 4.3 Hz, 1H, *H*-1 a), 5.63 (dd, $J_{P,H}$ = 6.2, $J_{1,2}$ = 4.2 Hz, *H*-1 b), 5.40 (dd, *J* = 7.2, 5.6 Hz, 1H, *H*-3 a), 5.19-5.15 (m, 2H, H-2 a and *H*-3 b), 4.47 (dd, *J* = 11.5, 5.0 Hz, 1H, *H*-5a a), 4.44 (dd, *J* = 11.6, 4.7 Hz, 1H, *H*-5a b), 4.25-4.19 (m, 3H, *H*-5b a, *H*-2 b and *H*-5b b), 4.12 (ddd, *J* = 6.4, 5.6, 5.0 Hz, 1H, *H*-4 a), 4.05-4.11 (m, 1H, *H*-4 b), 2.10 (s, 3H, -COC*H*$_3$ b), 2.09 (s, 3H, -COC*H*$_3$ a), 2.07 (s, 3H, -COC*H*$_3$ a), 2.05 (s, 3H, -COC*H*$_3$ a), 2.04 (s, 3H, -COC*H*$_3$ b); ^{13}C NMR (151 MHz, MeOD) δ 173.2, 173.1, 172.8, 172.5 (5(-C), 5(-*C*OCH_3)), 99.3 (d, $J_{C,P}$ = 5.4 Hz, -CH, *C*-1 b), 97.4 (d, $J_{C,P}$ = 4.6 Hz, -CH, *C*-1 a), 80.6 (-CH, *C*-4 b), 80.1 (-CH, *C*-4 a), 79.6 (-CH, *C*-3 b), 77.7 (d, $J_{C,P}$ = 6.9 Hz, -CH, *C*-2 a), 77.5 (d, $J_{C,P}$ = 6.4 Hz, -CH, *C*-2 b), 77.0 (-CH, *C*-3 a), 67.1 (-CH_2, *C*-5 b), 66.9 (-CH_2, *C*-5 a), 20.8, 20.7 (5(-CH_3), 5(-CO*C*H_3)); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -0.65 (b), -1.39 (a); ESI-TOF HRMS m/z calcd for $C_{11}H_{17}O_{11}P$ $[M-H]^-$: 355.0430; found 355.0445; ESI-TOF HRMS m/z calcd for $C_9H_{15}O_{10}P$ $[M-H]^-$: 313.0325; found 355.0332.

UDP-β-L-Arabinofuranose (**3**)

Starting from a mixture of **15a** and **15b**, General procedure C afforded **3** (8.27 mg, 41% over two steps, 92% purity, product contains small amounts of NH_4COCH_3 buffer) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 8.01 (d, *J* = 8.2 Hz, 1H, *H*-6''), 6.05 (d, *J* = 3.4 Hz, 1H, *H*-1'), 6.04 (d, *J* = 8.2 Hz, 1H, *H*-5''), 5.71 (dd, *J* = 5.7, 3.9 Hz, 1H, *H*-1), 4.45-4.43 (m, 2H, *H*-2' and *H*-3'), 4.35 (t_{br}, J_1 = J_2 = 2.6 Hz, 1H, *H*-4'), 4.31 (ddd, *J* = 11.8, 4.4, 2.6 Hz, 1H, *H*-5'a), 4.26 (ddd, *J* = 11.8, 5.6, 2.6 Hz, 1H, *H*-5'b), 4.23-4.18 (m, 2H, *H*-2 and *H*-4), 3.98 (dt, *J* = 6.4, 3.1 Hz, 1H, *H*-3), 3.86 (dd, *J* = 12.7, 3.1 Hz, 1H, *H*-5a), 3.76 (d, *J* = 12.7, 6.1 Hz, 1H, *H*-5b); ^{1}H-decoupled ^{31}P NMR (243 MHz, D_2O) δ -11.38 (d, *J* = 20.3 Hz, 1P, -O*P*(O)(O^-)-UMP), -12.77 (d, *J* = 20.3 Hz, 1P, -O*P*(O)(O^-)-Ara).

Synthesis of UDP-N_3-Ara*f*



5-*O*-(*tert*-Butyldiphenylsilyl)-L-arabinofuranose (**S3**)

A mixture of L-arabinose (12.5 g, 83.3 mmol), imidazole (11.5 g, 168 mmol) and TBDPSCl (32.9 mL, 126 mmol) in anh. DMF (120 mL) was stirred at rt under argon atmosphere for 16 h. The mixture was then poured into a 1 M aq. solution of HCl and the product extracted thrice with DCM. The organic layers were combined and washed with a saturated solution of aq. $NaHCO_3$ and cold water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 9/1 to 1/1) afforded **S3** (16.2 g, 50%) as a colourless sticky oil and as mixture of anomers (α:β = 1:2.5 from ^{1}H NMR integration); R_f 0.45 (Tol/EA: 1/2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68-7.64 (m, 8H, *m*-H^{Ar} α and *m*-H^{Ar} β), 7.46-7.43 (m, 4H, *p*-H^{Ar} α and *p*-H^{Ar} β), 7.41-7.38 (m, 8H, *o*-H^{Ar} α and *o*-H^{Ar} β), 5.42 (s, 1H, *H*-1 β), 5.30 (dd, *J* = 8.0, 3.7 Hz, 1H, *H*-1 α), 4.29 (t, *J* = 4.5 Hz, 1H, *H*-3 α), 4.26 (dd, *J* = 4.0, 2.0 Hz, 1H, *H*-4 β), 4.21 (s_{br}, 1H, 3-O*H* β), 4.18 (d, *J* = 11.2 Hz, 1H, *H*-2 β), 4.04 (d, *J* = 11.2 Hz, 1H, *H*-3 β), 3.93-3.91 (m, 2H, *H*-2 α and *H*-4 α), 3.83 (d, *J* = 2.7 Hz, 1H, *H*-5a α), 3.81 (d, *J* = 2.0 Hz, 1H, *H*-5a β), 3.72 (dd, *J* = 11.5, 2.0 Hz, 1H, *H*-5b β), 3.69 (dd, *J* = 11.2, 2.7 Hz, 1H, *H*-5b α), 3.68 (s_{br}, 1H, 1-O*H* α), 3.29 (s_{br}, 1H, 3-O*H* α), 3.19 (s_{br}, 1H, 1-O*H* β), 2.82 (s_{br}, 1H, 2-O*H* β), 2.12 (s_{br}, 1H, 2-O*H* α), 1.07 (s, 9H, -C(C*H*$_3$)$_3$ α), 1.04 (s, 9H, -C(C*H*$_3$)$_3$ β). The analytical data is in full agreement with literature data[7].

1,2,3-Tri-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-L-arabinofuranose (**S4**)

Acetic anhydride (5.25 mL, 55.6 mmol) was slowly added to a solution of **S3** (2.16 g, 5.56 mmol) in anh. pyridine (15 mL) at 0°C under argon atmosphere and a catalytic amount of DMAP was added. The reaction mixture was stirred at rt for 20 h, when the solvent was co-evaporated thrice with toluene. Purification of the crude by flash chromatography (Tol/EA: 95/5) afforded **S4** (2.52 g, 88%) as a colourless sticky oil as a mixture of anomers (α:β = 1:0.6 from ^{1}H NMR integration); R_f 0.33 (Tol/EA : 95/5); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68-7.64 (m, 8H, *m*-H^{Ar} α and *m*-H^{Ar} β), 7.42-7.39 (m, 4H, *p*-H^{Ar} α and *p*-H^{Ar} β), 7.38-7.34 (m, 8H, *o*-H^{Ar} α and *o*-H^{Ar} β), 6.34 (d, *J* = 4.7 Hz, 1H, *H*-1 β), 6.17 (s, 1H, *H*-1 α), 5.60 (dd, *J* = 7.2, 5.7 Hz, 1H, *H*-3 β), 5.36 (dd, *J* = 4.5, 1.6 Hz, 1H, *H*-3 α), 5.33 (dd, *J* = 7.2, 4.7 Hz, 1H, *H*-2 β), 5.19 (d, *J* = 1.6 Hz, 1H, *H*-2 α), 4.23 (dd, *J* = 10.1, 4.5 Hz, 1H, *H*-4 α), 4.10 (dd, *J* = 11.1, 5.7 Hz, 1H, *H*-4 β), 3.87 (dd, *J* = 10.1, 4.1 Hz, 1H, *H*-5a α), 3.83 (dd, *J* = 10.1, 4.1 Hz, 1H, *H*-5b α), 3.81

(dd, *J* = 11.1, 5.3 Hz, 1H, *H*-5a β), 3.77 (dd, *J* = 11.1, 5.3 Hz, 1H, *H*-5b β), 2.09 (s, 6H, 2(-COC*H*$_3$) α), 2.05 (s, 3H, -COC*H*$_3$ β), 2.04 (s, 3H, -COC*H*$_3$ β), 2.02 (s, 3H, -COC*H*$_3$ α), 1.87 (s, 3H, -COC*H*$_3$ β), 1.04 (s, 18H, -C(C*H*$_3$)$_3$ α and -C(C*H*$_3$)$_3$ β); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.2 (-C, -*C*OCH$_3$ β), 170.1 (2(-C), -*C*OCH$_3$ α and -*C*OCH$_3$ β), 169.9 (-C, -*C*OCH$_3$ α), 169.8 (-C, -*C*OCH$_3$ β), 169.6 (-C, -*C*OCH$_3$ α), 135.9, 135.8 (8(-CH), *m*-C^{Ar} α and *m*-C^{Ar} β), 133.5, 133.3, 133.2 (4(-C), C^{Ar} α and C^{Ar} β), 130.0 (4(-CH), *p*-C^{Ar} α and *p*-C^{Ar} β), 128.0 (8(-CH), *o*-C^{Ar} α and *o*-C^{Ar} β), 99.8 (-CH, *C-1* α), 93.9 (-CH, *C-1* β), 85.1 (-CH, *C-4* α), 81.9 (-CH, *C-4* β), 81.6 (-CH, *C-2* α), 77.0 (-CH, *C-3* α), 75.9 (-CH, *C-2* β), 74.4 (-CH, *C-3* β), 64.6 (-CH$_2$, *C-5* β), 63.0 (-CH$_2$, *C-5* α), 27.0 (3(-CH$_3$), -C(*C*H$_3$)$_3$ β), 26.9 (3(-CH$_3$), -C(*C*H$_3$)$_3$ α), 21.3 (-CH$_3$, -CO*C*H$_3$ α), 21.2 (-CH$_3$, -CO*C*H$_3$ β), 21.0 (2(-CH$_3$), -CO*C*H$_3$ β and -CO*C*H$_3$ α), 20.9 (-CH$_3$, -CO*C*H$_3$ α), 20.7 (-CH$_3$, -CO*C*H$_3$ β), 19.5 (2(-C), -*C*(CH$_3$)$_3$ α and -*C*(CH$_3$)$_3$ β); ESI-TOF HRMS m/z calcd for $C_{27}H_{34}O_8Si$ [M+Na]$^+$: 537.1921; found 537.1924.

1,2,3-Tri-*O*-acetyl-L-arabinofuranose (S2)

Acetic acid (3.14 mL, 54.4 mmol) and a 1 M solution of TBAF in THF (27.2 mL, 27.2 mmol) were added to a solution of **S4** (14.0 g, 27.2 mmol) in THF (220 mL). The solution was stirred at rt for 48 h, when the solvent was evaporated under reduced pressure and the residue dissolved in EA, washed with a saturated solution of aq. $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 6/4) afforded **S2** (7.44 g, 99%) as a colourless sticky oil as a mixture of anomers (α:β = 1:0.3 from ^{1}H NMR integration); R_f 0.45 (Tol/EA: 4/6); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.36 (d, *J* = 4.0 Hz, 1H, *H*-1 β), 6.16 (s$_{br}$, 1H, *H*-1 α), 5.39-5.35 (m, 2H, *H*-2 and *H*-3 β), 5.23 (dd, *J* = 1.9, 0.6 Hz, 1H, *H*-2 α), 5.10 (ddd, *J* = 4.9, 1.9, 0.6 Hz, 1H, *H*-3 α), 4.21 (dd, *J* = 8.4, 4.9 Hz, 1H, *H*-4 α), 4.06 (dd, *J* = 11.5, 5.3 Hz, 1H, *H*-4 β), 3.87 (dd, *J* = 12.3, 3.6 Hz, 1H, *H*-5a α), 3.83 (dd, *J* = 11.5, 5.0 Hz, 1H, *H*-5a β), 3.78 (dd, *J* = 12.3, 3.6 Hz, 1H, *H*-5b α), 3.72 (dd, *J* = 11.5, 5.0 Hz, 1H, *H*-5b β), 2.20 (s, 3H, -COC*H*$_3$ α), 2.11 (s, 3H, -COC*H*$_3$ α), 2.10 (s, 3H, -COC*H*$_3$ α), 2.09 (s, 3H, -COC*H*$_3$ β), 2.08 (s, 3H, -COC*H*$_3$ β), 2.05 (s, 3H, -COC*H*$_3$ β); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1 (-C, -*C*OCH$_3$ β), 170.6 (-C, -*C*OCH$_3$ α), 169.8 (-C, -*C*OCH$_3$ α), 169.6 (-C, -*C*OCH$_3$ α), 169.4 (-C, -*C*OCH$_3$ β), 169.2 (-C, -*C*OCH$_3$ β), 99.5 (-CH, *C*-1 α), 93.6 (-CH, *C*-1 β), 85.2 (-CH, *C*-4 α), 82.7 (-CH, *C*-4 β), 81.2 (-CH, *C*-2 α), 76.9 (-CH, *C*-3 α), 75.6 (-CH, *C*-2 β), 74.7 (-CH, *C*-3 β), 63.6 (-CH$_2$, *C*-5 β), 62.0 (-CH$_2$, *C*-5 α), 21.3 (-CH$_3$, -CO*C*H$_3$ β), 21.2 (-CH$_3$, -CO*C*H$_3$ α), 21.0 (2(-CH$_3$), -CO*C*H$_3$ β and -CO*C*H$_3$ α), 20.9 (-CH$_3$, -CO*C*H$_3$ α), 20.6 (-CH$_3$, -CO*C*H$_3$ β); ESI-TOF HRMS m/z calcd for $C_{11}H_{16}O_8$ [M-HCOO]$^-$: 321.0827; found 321.0825.

1,2,3-Tri-*O*-acetyl-5-*O*-methylsulfonyl-L-arabinofuranose (S5)

Mesyl chloride (1.96 mL, 25.5 mmol) was added dropwise to a solution of **S2** (3.52 g, 12.8 mmol) in anh. pyridine (45 mL) under argon atmosphere. The solution was stirred at rt for 20 minutes, when the solvents were co-evaporated thrice with toluene under reduced pressure and the residue dissolved in DCM and washed with a saturated solution of aq. $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Hex/EA: 6/4) afforded **S5** (4.51 g, quant.) as a colourless sticky oil as a mixture of anomers (α:β = 1:0.5 from ^{1}H NMR integration); R_f 0.38 (Tol/EA: 6/4); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.39 (d, *J* = 4.5 Hz, 1H, *H*-1 β), 6.21 (s, 1H, *H*-1 α), 5.35 (dd, *J* = 7.1, 4.5 Hz, 1H, *H*-2 β), 5.30 (dd, *J* = 7.1, 5.5 Hz, 1H, *H*-3 β), 5.23 (d, *J* = 1.0 Hz, 1H, *H*-2 α), 5.05 (ddd, *J* = 4.7, 2.0, 1.0 Hz, 1H, *H*-3 α), 4.52-4.49 (m, 2H, *H*-5a α and *H*-5a β), 4.47-4.44 (m, 2H, *H*-5b α and *H*-5b β), 4.38 (td$_{br}$, *J* = 4.7, 3.3 Hz, 1H, *H*-4 α), 4.23 (ddd, *J* = 7.0, 5.5, 3.6 Hz, 1H, *H*-4 β), 3.07 (s, 6H, -SO$_3$C*H*$_3$ α and -SO$_3$C*H*$_3$ β), 2.14-2.13 (6s, 18H, 3(-COC*H*$_3$) α and 3(COC*H*$_3$) β); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6 (-C, -*C*OCH$_3$ β), 170.2 (-C, -*C*OCH$_3$ α), 169.8 (-C, -*C*OCH$_3$ β), 169.6 (-C, -*C*OCH$_3$ α), 169.5 (-C, -*C*OCH$_3$ β), 169.2 (-C, -*C*OCH$_3$ α), 99.4 (-CH, *C*-1 α), 93.4 (-CH, *C*-1 β), 82.7 (-CH, *C*-4 α), 80.4 (-CH, *C*-2 α), 79.9 (-CH, *C*-4 β), 76.7 (-CH, *C*-3 α), 75.2 (-CH, *C*-2

β), 74.5 (-CH, *C*-3 β), 69.8 (-CH_2, *C*-5 β), 68.1 (-CH_2, *C*-5 α), 38.0 (-CH_3, -SO_3CH_3 β), 37.8 (-CH_3, -SO_3CH_3 α), 21.1 (2(-CH_3), -CO*C*H_3 β and -CO*C*H_3 α), 20.8 (3(-CH_3), -CO*C*H_3 β and 2(-CO*C*H_3) α), 20.5 (-CH_3, -CO*C*H_3 β); ESI-TOF HRMS m/z calcd for $C_{12}H_{18}O_{10}S$ $[M+NH_4]^+$: 372.0964; found 372.0962.

5-Azido-5-deoxy-1,2,3-tri-*O*-acetyl-L-arabinofuranose (16)

NaN_3 (2.89 mg, 44.5 mmol) was added to a solution of **S5** (7.16 g, 20.2 mmol) in anh. DMSO (105 mL) under argon atmosphere. The mixture was stirred at 80°C for 6 h, when it was allowed to cool down to rt, diluted with EA and washed with cold water. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (Tol/EA: 8/2) afforded **16** (4.67 g, 78%) as a colourless sticky oil as a mixture of anomers (α:β = 1:0.2 from ^{1}H NMR integration); R_f 0.37 (Tol/EA: 95/5); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.40 (d, *J* = 4.0 Hz, 1H, *H*-1 β), 6.23 (s_{br}, 1H, *H*-1 α), 5.39-5.36 (m, 2H, *H*-2 and *H*-3 β), 5.22 (dd, *J* = 1.6, 0.4 Hz, 1H, *H*-2 α), 5.05 (ddd, *J* = 4.7, 1.6, 0.8 Hz, 1H, *H*-3 α), 4.29 (dt, *J* = 4.7, 3.3 Hz, 1H, *H*-4 α), 4.14-4.11 (m, 1H, *H*-4 β), 3.68 (dd, *J* = 13.4, 3.3 Hz, 1H, *H*-5a α), 3.60 (dd, *J* = 13.3, 3.7 Hz, 1H, *H*-5a β), 3.48-3.46 (m, 1H, *H*-5b β), 3.46 (dd, *J* = 13.4, 4.7 Hz, 1H, *H*-5b α), 2.14 (s, 3H, -COC*H*$_3$ α), 2.13 (s, 3H, -COC*H*$_3$ α), 2.12 (s, 6H, -COC*H*$_3$ α and -COC*H*$_3$ β), 2.10 (s, 3H, -COC*H*$_3$ β), 2.09 (s, 3H, -COC*H*$_3$ β); The analytical data is in full agreement with literature data[5].

5-Azido-5-deoxy-2,3-di-*O*-acetyl-L-arabinofuranose (17)

A solution of 33% HBr in AcOH (580 µL) was slowly added to an ice-cooled solution of **16** (315 mg, 1.05 mmol), in DCM (26 mL). The solution was stirred at 0°C for 20 minutes, when the reaction was quenched with cold water and the product extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (1st purification, Tol/ACN: 8/2; 2nd purification, Hex/EA: 7/3) afforded **17** (220 mg, 81%) as a colourless sticky oil as a mixture of anomers (α:β = 1:0.3 from ^{1}H NMR integration); R_f 0.27 (Hex/EA: 6/4); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.54 (d, *J* = 4.6 Hz, 1H, *H*-1 β), 5.42 (s_{br}, 1H, *H*-1 α), 5.23 (t_{br}, *J* = 5.3 Hz, 1H, *H*-3 β), 5.09 (d, *J* = 1.3 Hz, 1H, *H*-2 α), 5.08 (dd, *J* = 6.0, 4.7 Hz, 1H, *H*-2 β), 4.96 (d_{br}, *J* = 4.7 Hz, 1H, *H*-3 α), 4.34 (dt_{br}, *J* = 5.1, 3.1, Hz, 1H, *H*-4 α), 3.99 (dd, *J* = 9.7, 4.9 Hz, 1H, *H*-4 β), 3.64 (dd, *J* = 13.2, 3.1 Hz, 1H, *H*-5a α), 3.61-3.64 (m, 2H, *H*-5 β), 3.43 (dd, *J* = 13.2, 5.4 Hz, 1H, *H*-5b α), 2.12 (s, 3H, -COC*H*$_3$ β), 2.11 (s, 3H, -COC*H*$_3$ α), 2.10 (s, 3H, -COC*H*$_3$ α), 2.08 (s, 3H, -COC*H*$_3$ β); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7 (-C, -*C*OCH_3 β), 170.6 (-C, -*C*OCH_3 α), 170.3 (-C, -*C*OCH_3 β), 170.2 (-C, -*C*OCH_3 α), 100.8 (-CH, *C*-1 α), 95.3 (-CH, *C*-1 β), 82.8 (-CH, *C*-4 α), 81.9 (-CH, *C*-2 α), 80.3 (-CH, *C*-4 β), 78.0 (-CH, *C*-3 α), 77.0 (-CH, *C*-2 β), 76.6 (-CH, *C*-3 β), 53.8 (-CH_2, *C*-5 β), 51.9 (-CH_2, *C*-5 α), 21.0, 20.9, 20.8 (4(-CH_3), 2(-CO*C*H_3) β and 2(-CO*C*H_3) α); ESI-TOF HRMS m/z calcd for $C_9H_{13}N_3O_6$ $[M+HCOO]^-$: 304.0786; found 304.0790.

Diallyl(5-azido-5-deoxy-2,3-di-*O*-acetyl-β-L-arabinofuranosyl)phosphate (18)

Starting from hemiacetal **17**, General procedure A afforded **18** (73.0 mg, 31%) as a colorless sticky oil. Addition of the diallyl chlorophosphate solution was performed within 5 minutes and stirring was continued for additional 16 h after removing the ice bath; R_f 0.63 (Hex/EA: 1/1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.92 (dd, $J_{P,H}$ = 5.2, $J_{1,2}$ = 4.3 Hz, 1H, *H*-1), 5.90-5.84 (m, 2H, *H*-2'), 5.33-5.32 (m, 1H, *H*-3'a), 5.31-5.29 (m, 1H, *H*-3'b), 5.24 (dd, *J* = 7.4, 5.7 Hz, 1H, *H*-3), 5.22-5.21 (m, 1H, *H*-3'a), 5.20-5.17 (m, 2H, H-3'b and *H*-2), 4.53-4.49 (m, 4H, *H*-1'), 4.05 (ddd, *J* = 7.5, 5.7, 4.2 Hz, 1H, *H*-4), 3.56 (dd, *J* = 13.1, 7.5 Hz, 1H, *H*-5a), 3.53 (dd, *J* = 13.1, 4.2 Hz, 1H, *H*-5b), 2.04 (s, 3H, -COC*H*$_3$), 2.03 (s, 3H, -COC*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5 (-C, -*C*OCH_3), 170.0 (-C, -*C*OCH_3), 132.4 (2(-CH), d, $J_{C,P}$ = 7.4 Hz, *C*-2'), 118.7 (2(-CH_2), *C*-3'), 97.9 (-CH, d, $J_{C,P}$ = 4.8 Hz, *C*-1), 81.5 (-CH, *C*-4), 75.9 (-CH, d,

$J_{C,P}$ = 6.6 Hz, *C*-2), 75.0 (-CH, *C*-3), 68.6 (2(-CH_2), t_{br}, $J_{C,P}$ = 6.1 Hz, *C*-1'), 53.9 (-CH_2, *C*-5), 20.8 (-CH_3, -CO*C*H_3), 20.7 (-CH_3, -CO*C*H_3); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -2.97. ESI-TOF HRMS m/z calcd for $C_{15}H_{23}N_3O_9P$ $[M+H]^+$: 420.1177; found 420.1170.

1-*O*-Phosphoryl-5-azido-5-deoxy-2,3-di-*O*-acetyl-β-L-arabinofuranoside (**19**)

Starting from diallyl-protected phosphate **18**, General procedure B afforded **19** (30.0 mg, 46%) as a white solid; R_f 0.28 (DCM/MeOH/TEA: 1/1/0.1); ^{1}H NMR (600 MHz, MeOD) δ 5.81 (dd, $J_{P,H}$ = 7.0, $J_{1,2}$ = 4.2 Hz, 1H, *H*-1), 5.30 (dd, *J* = 7.1, 5.3 Hz, *H*-3), 5.17 (ddd, $J_{2,3}$ = 7.1, $J_{1,2}$ = 4.2, J_{P-H} = 1.5 Hz, 1H, *H*-2), 4.04 (ddd, *J* = 8.1, 5.3, 4.7 Hz, 1H, *H*-4), 3.75 (dd, *J* = 12.9, 8.1 Hz, 1H, *H*-5a), 3.58 (dd, *J* = 12.9, 4.7 Hz, 1H, *H*-5b), 2.09 (s, 3H, -COC*H*$_3$), 2.08 (s, 3H, -COC*H*$_3$); ^{13}C NMR (151 MHz, MeOD) δ 172.0 (2(-C), 2(-*C*OCH$_3$)), 97.6 (-CH, d, $J_{C,P}$ = 5.3 Hz, *C*-1), 81.2 (-CH, *C*-4), 77.8 (-CH, *C*-3), 77.7 (-CH, d, $J_{C,P}$ = 6.7 Hz, *C*-2), 55.6 (-CH_2, *C*-5), 20.7 (2(-CH_3), 2(-CO*C*H$_3$)); ^{1}H-decoupled ^{31}P NMR (243 MHz, $CDCl_3$) δ -1.47; ESI-TOF HRMS m/z calcd for $C_9H_{14}N_3O_9P$ $[M-H]^-$: 338.0389; found 338.0404.

UDP-5-N_3-β-L-Arabinofuranose (**4**)

Starting from **19**, General procedure C afforded **4** (1.51 mg, 34% over two steps, 97% purity, product contains small amounts of NH_4COCH_3 buffer) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.2 Hz, 1H, *H*-6''), 5.99-5.97 (m, 2H, *H*-5'' and *H*-1'), 5.67 (dd, *J* = 6.6, 4.0 Hz, *H*-1), 4.39-4.37 (m, 2H, *H*-2' and *H*-3'), 4.29-4.28 (m, 1H, *H*-4'), 4.25 (ddd, *J* = 11.8, 4.6, 2.6 Hz, 1H, *H*-5'a), 4.20 (ddd, *J* = 11.8, 5.2, 2.6 Hz, 1H, *H*-5'b), 4.17-4.12 (m, 2H, *H*-5a and *H*-3), 3.99 (td, *J* = 6.6, 4.5 Hz, 1H, *H*-2), 3.61-3.54 (m, 2H, *H*-4 and *H*-5b); ^{1}H-decoupled ^{31}P NMR (243 MHz, D_2O) δ -11.22 (d, *J* = 26.6 Hz, 1P, -O*P*(O)(O^-)-UMP), -13.07 (d, *J* = 26.6 Hz, 1P, -O*P*(O)(O^-)-Ara).

Enzyme analyses

Heterologous expression of *Pv*XAT3, *At*RRT4, *Pt*RRT5 and *At*RGGAT1

The cloning of the constructs of the catalytic domains of *Pv*XAT3, *At*RRT4, and *At*RGGAT1 into the pGEn2-DEST vector for transient expression in human embryonic kidney (HEK) 293 F cells has previously been described[8, 9]. The expression of these three proteins in HEK 293 F cells was performed as previously described[10]. The truncated version of *PtRRT5* (Pt005G059600), encoding amino acids 54-514, was synthesized and cloned into pDONR221 by Biocat (Heidelberg, Germany). Using LR clonase, the gene sequence was transferred to a pTT5 expression vector (https://www.addgene.org/vector-database/7108/), in which we had cloned the Gateway cassette of the pGEn2-DEST vector that includes on the N-terminus a His-tag, an Avi-tag, and a GFP-tag. Next, *Pt*RRT5 was expressed in HEK293 6E cells[11] as previously described[12]. The secreted protein was harvested from the supernatant of the cell suspension after centrifugation and was purified using immobilized metal ion affinity chromatography (IMAC). After dialysis and concentration using spin filters, the protein concentration was measured at 280 nm using a NanoDrop Microvolume Spectrophotometer.

Glycan array experiments

The GT glycan array experiment was performed as previously described[13]. In brief, 100 µl of GT assay solution was premixed in a reaction tube (16 µM *At*RRT4 or 6 µM *Pt*RRT5, 4 µM *At*RGGAT1, 0.1 mM UDP-N_3-rhamnose (omitted in the negative controls), 0.5 mM UDP-GalA, in 50 mM HEPES pH 6.8) and applied to the glycan microarray slide using a grid with 16 wells and incubated for 40 h at rt. For the arabinofuranosyl transferase *Pv*XAT3, 40 µM enzyme and 0.5 mM UDP-N_3-arabinofuranose in 50 mM HEPES, pH 6.8, was used and incubated for 16 h at rt. After the GT incubation, the slide was washed twice with PBS for 5 min, the grid was removed and the slide was further washed three times with PBS

containing 1% SDS for 15 min under continuous shaking. Next, the click reaction was carried out on the slide using a 16-well grid. For this, a DMF/water: 1/1 mixture, including the Sulfo-Cy5-Alkyne dye (Jena Biosciences, 2 mM final concentration), premixed $CuSO_4$ (1 mM) and tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, Roth, 1 mM), and sodium ascorbate (10 mM), was incubated for 1 h on the array. Next, the grid was removed and the slide was washed three times with 1% SDS in PBS for 20 min to remove unreacted dye, then two times with deionized water for 5 min to remove salts, and finally dried and scanned for fluorescence using a microarray scanner (Molecular Devices). Images were recorded at 500 PMT gain and processed using the GenePix Pro 7 software (Molecular Devices).

For the glycan array with natural UDP-arabinose, 20 µM *Pv*XAT3 was incubated with 0.5 mM UDP-arabinose in 50 mM HEPES pH 6.8 for 16 h at RT on the array. After incubation, the enzyme solution was removed and the slide was washed with 50 mM HEPES, pH 6.8, for 5 min. The detection using the CCRC-M154 antibody was performed as previously described[14]. Images were recorded at 500 PMT gain and fluorescence was analyzed using the GenePix Pro 7 software (Molecular Devices).

HPLC-based enzyme assay

To verify the activity of *Pt*RRT5, we used *At*RRT4 and *Pt*RRT5 for *in vitro* enzyme assays. The enzyme assay was carried out in a reaction mixture containing the enzyme, 2 mM UDP-Rha donor, 1 mM RG I heptasaccharide acceptor, and 5 mM $MnCl_2$ in a total reaction volume of 40 µl. The reactions were incubated for 16 h at rt and then terminated by heating to 85°C for 5 min. HPLC analyses were performed on a Shimadzu LC-10AD VP series with a Hypercarb™ Porous Graphitic Carbon column (100x4.6 mm, 5 µm particle size; Thermo Fisher Scientific) equipped with an Alltech 3000 Evaporative Light Scattering Detector (ELSD) and a Shimadzu LCMS-2020 mass spectrometer. The oligosaccharides were eluted at a flow rate of 0.7 mL/min, applying a water (with 0.25% formic acid, pH 3 adjusted with NH_3)/ACN gradient starting with 5% ACN for 5 minutes followed by a ramp up to 30% ACN for 35 minutes, a short increase to 100% ACN for 4 minutes, a decline back to 5% and an equilibration at 5% ACN for 5 minutes.

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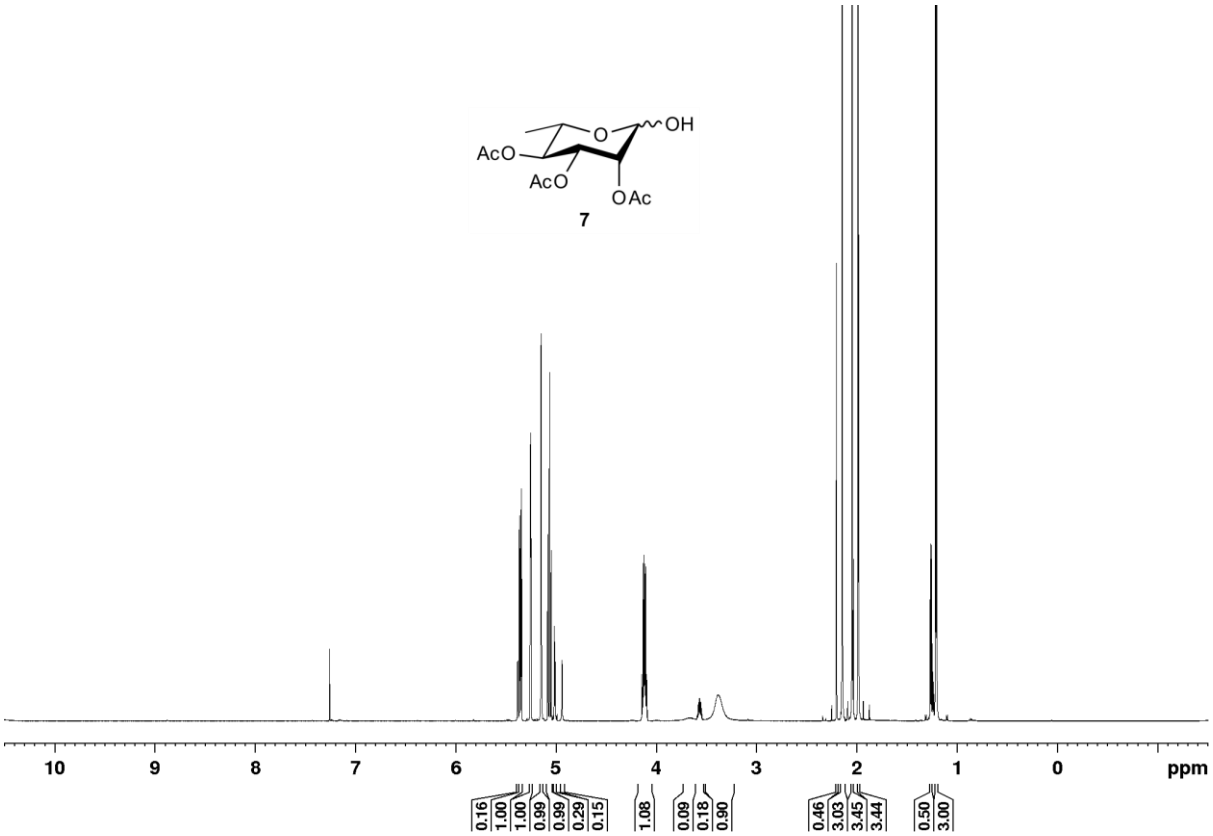
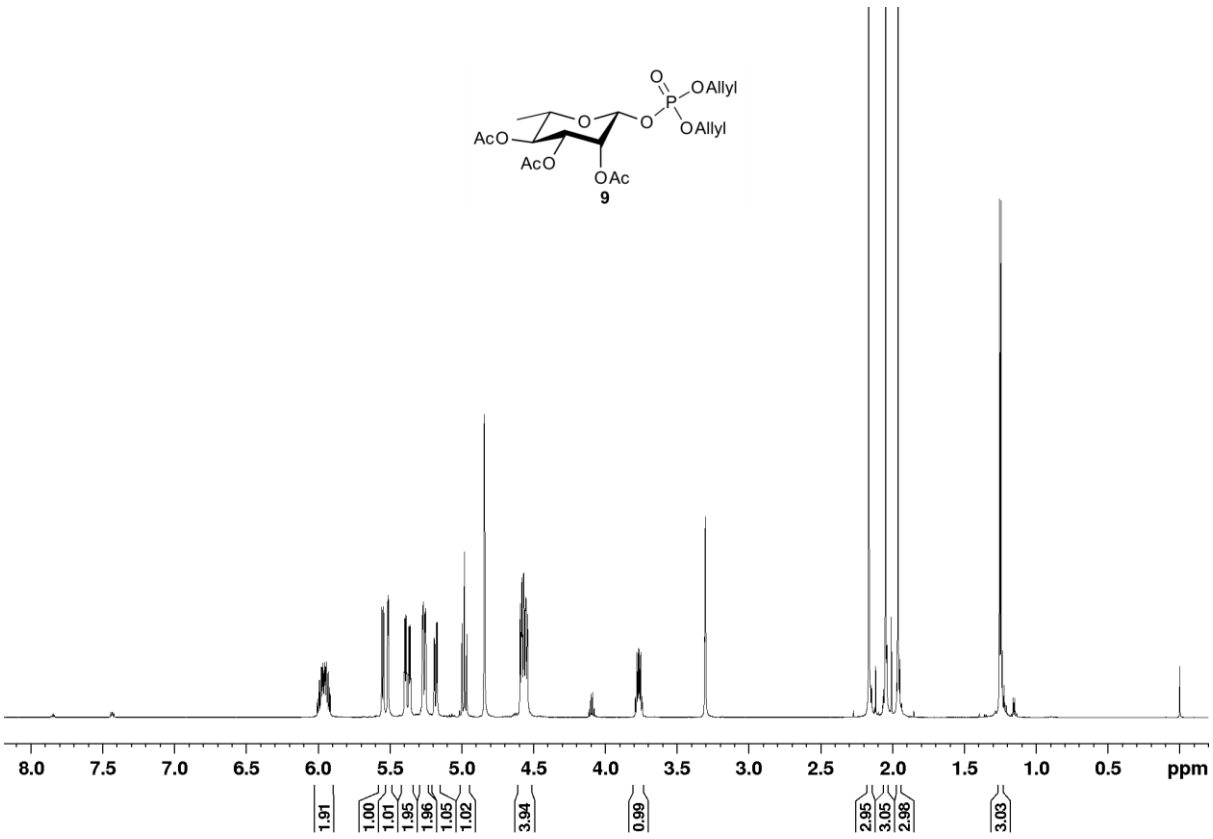
NMR Spectra

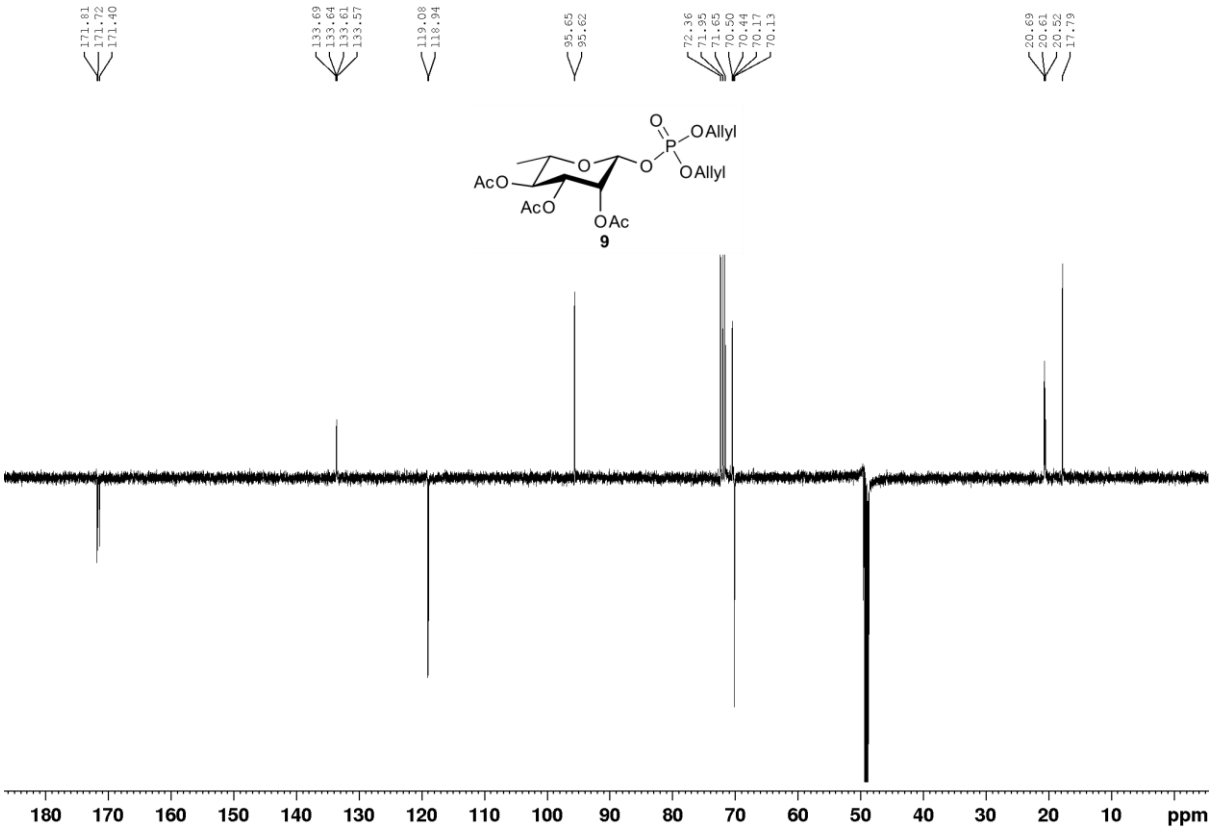
^{1}H NMR Spectrum of 7 in $CDCl_3$



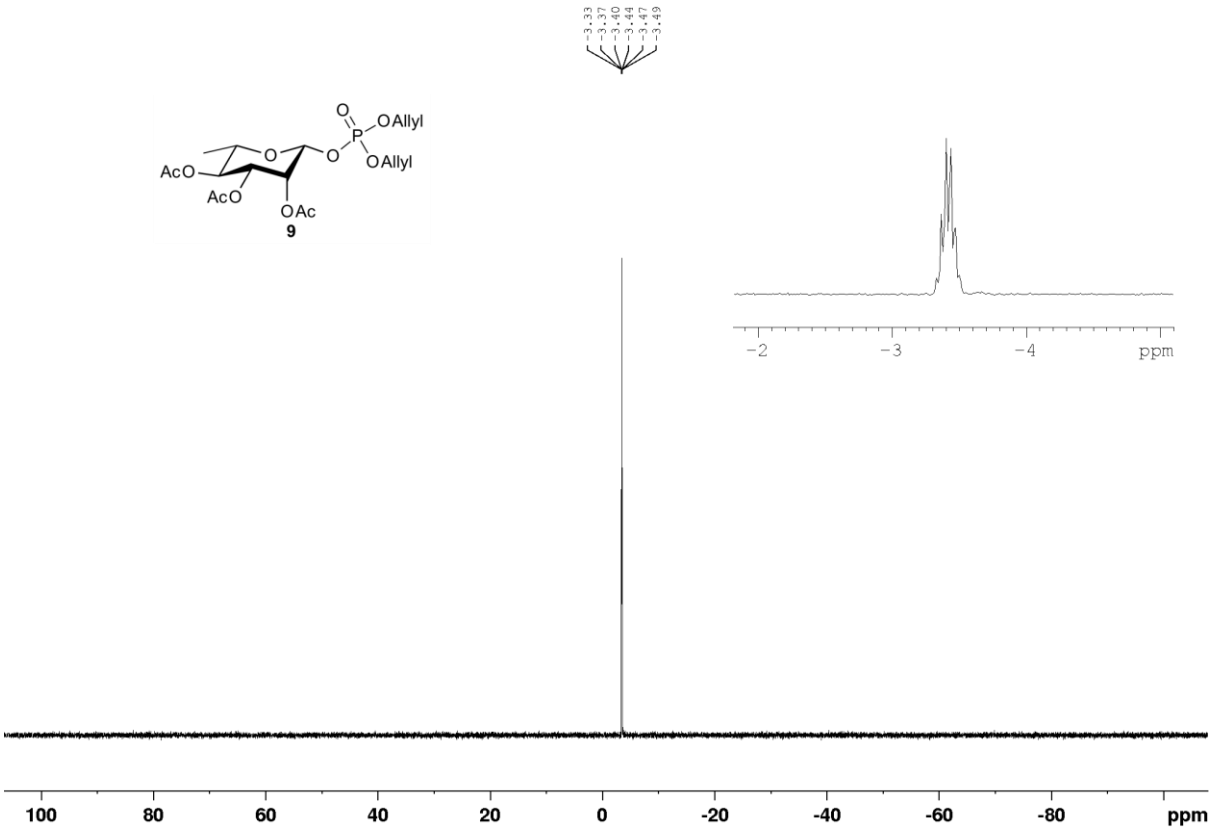
^{1}H NMR Spectrum of 9 in MeOD



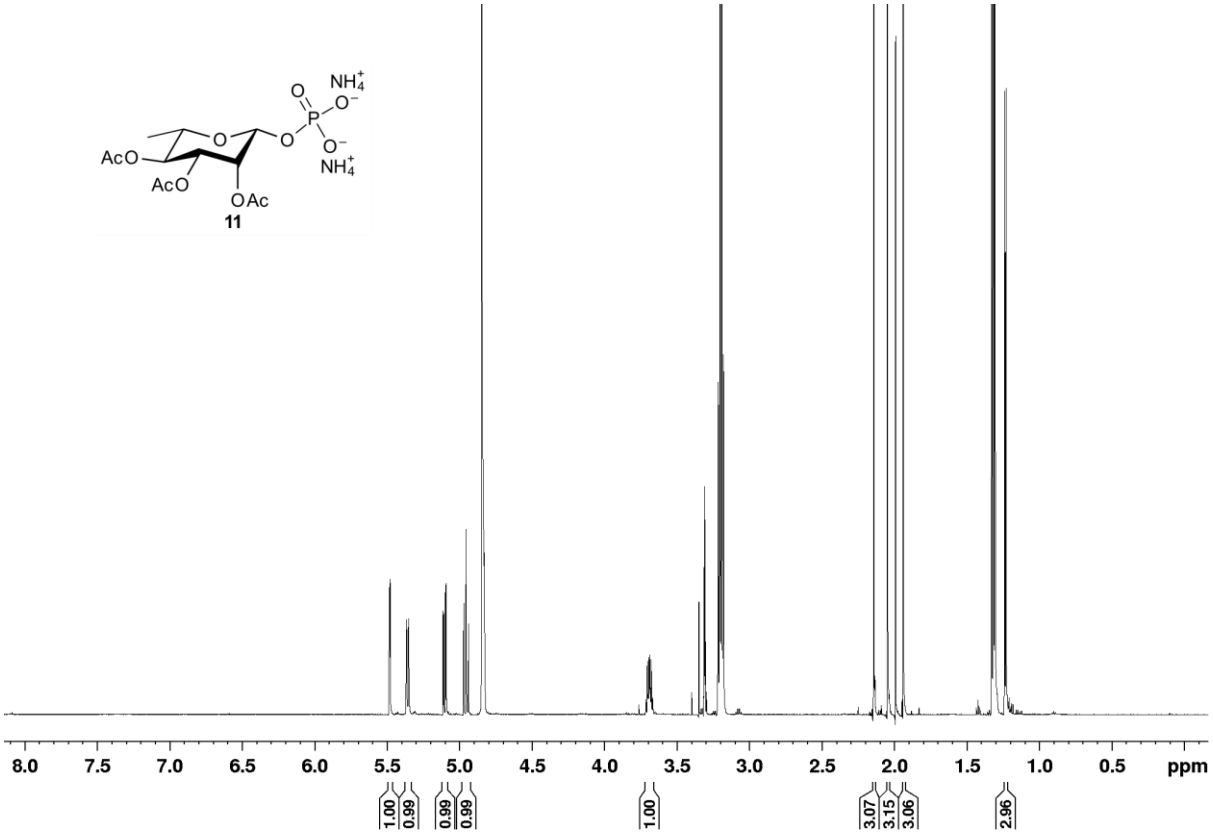
^{13}C APT NMR Spectrum of 9 in MeOD



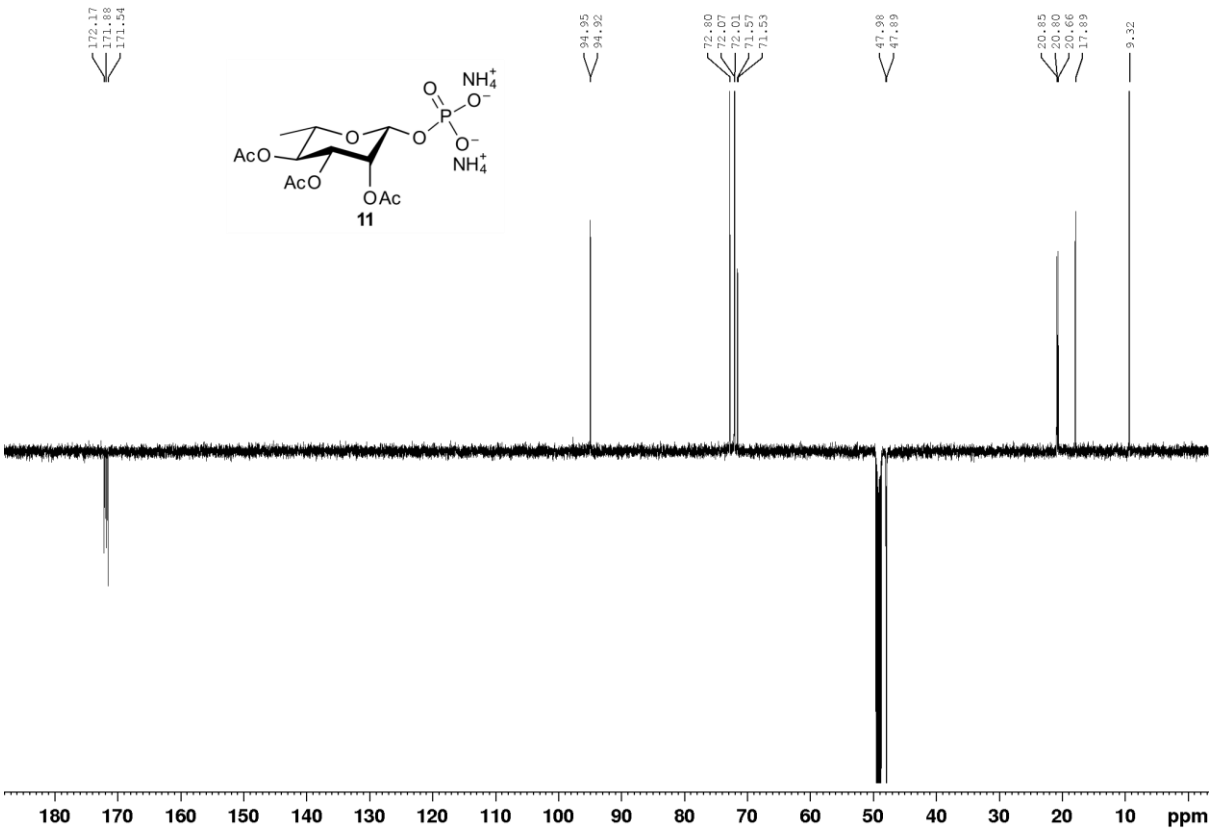
^{1}H-coupled ^{31}P NMR Spectrum 9 in MeOD



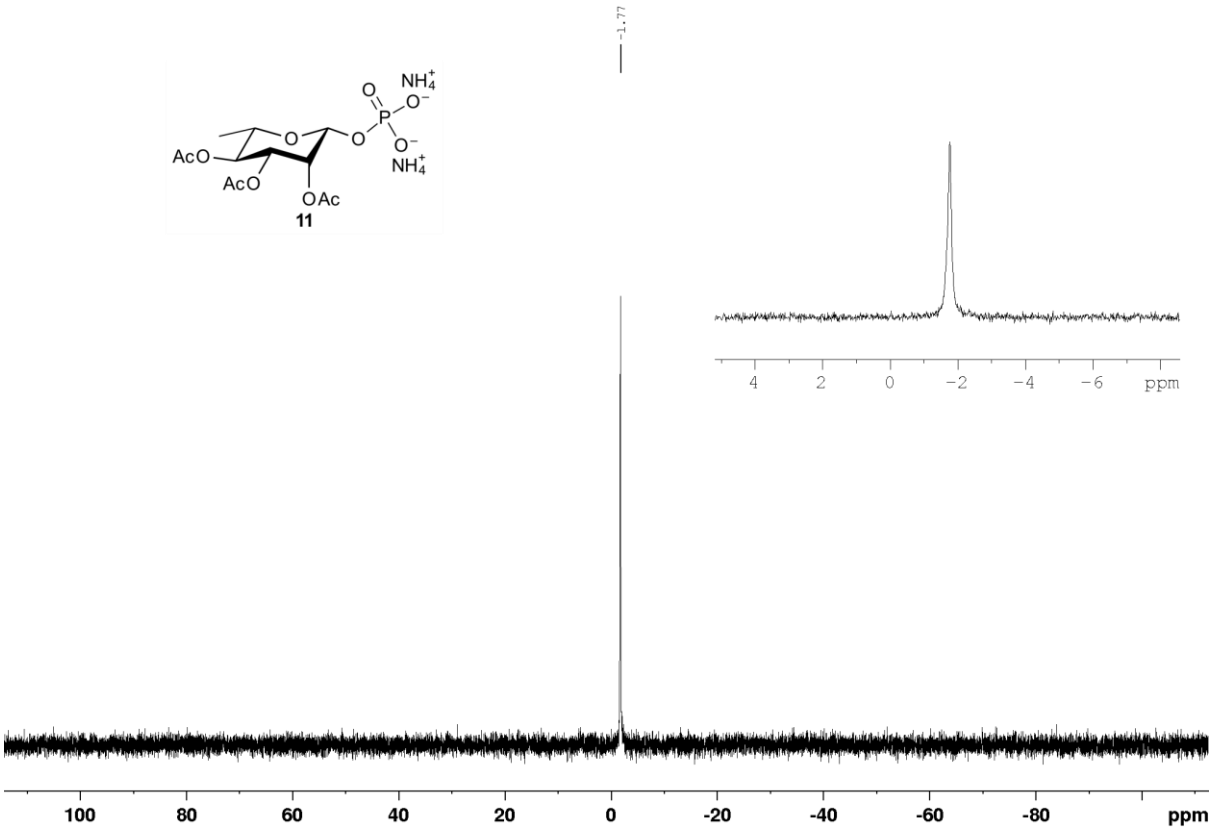
^{1}H NMR Spectrum of 11 in MeOD



^{13}C APT NMR Spectrum of 11 in MeOD

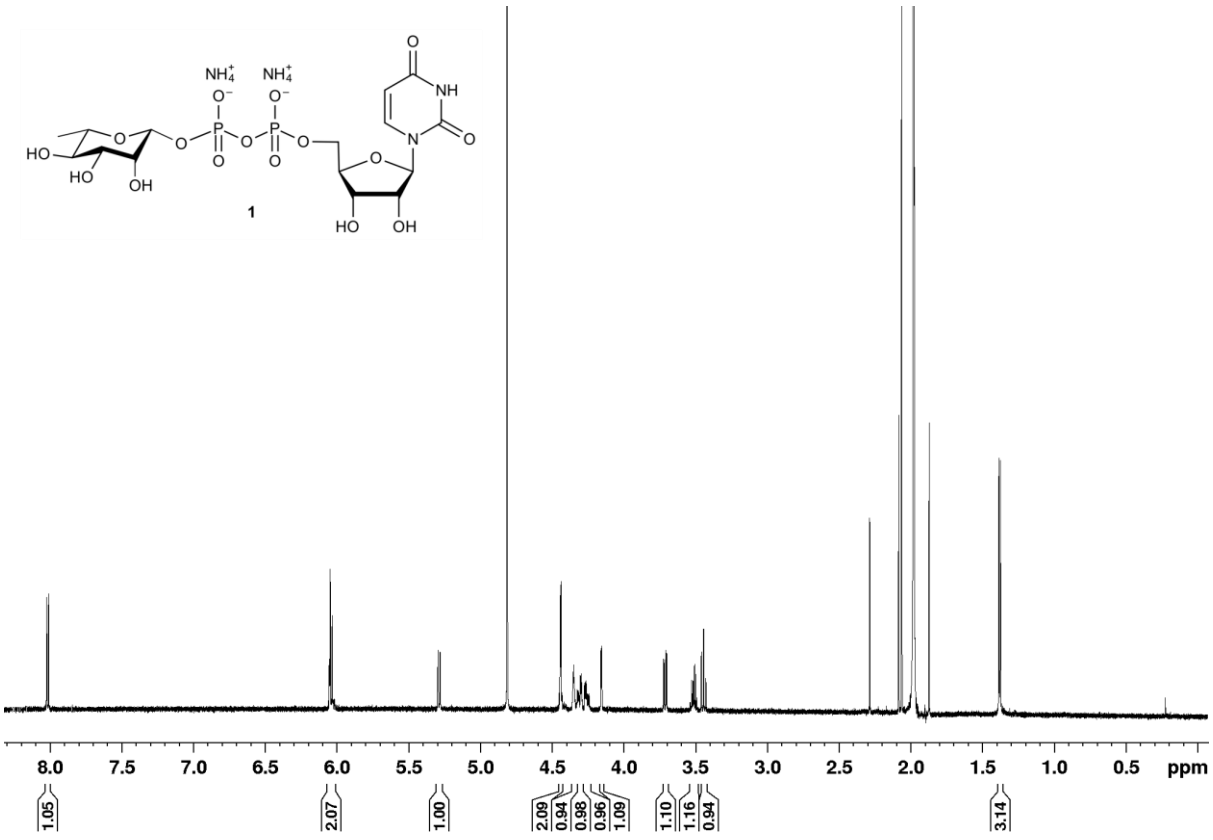


^{1}H-decoupled ^{31}P NMR Spectrum 11 in MeOD

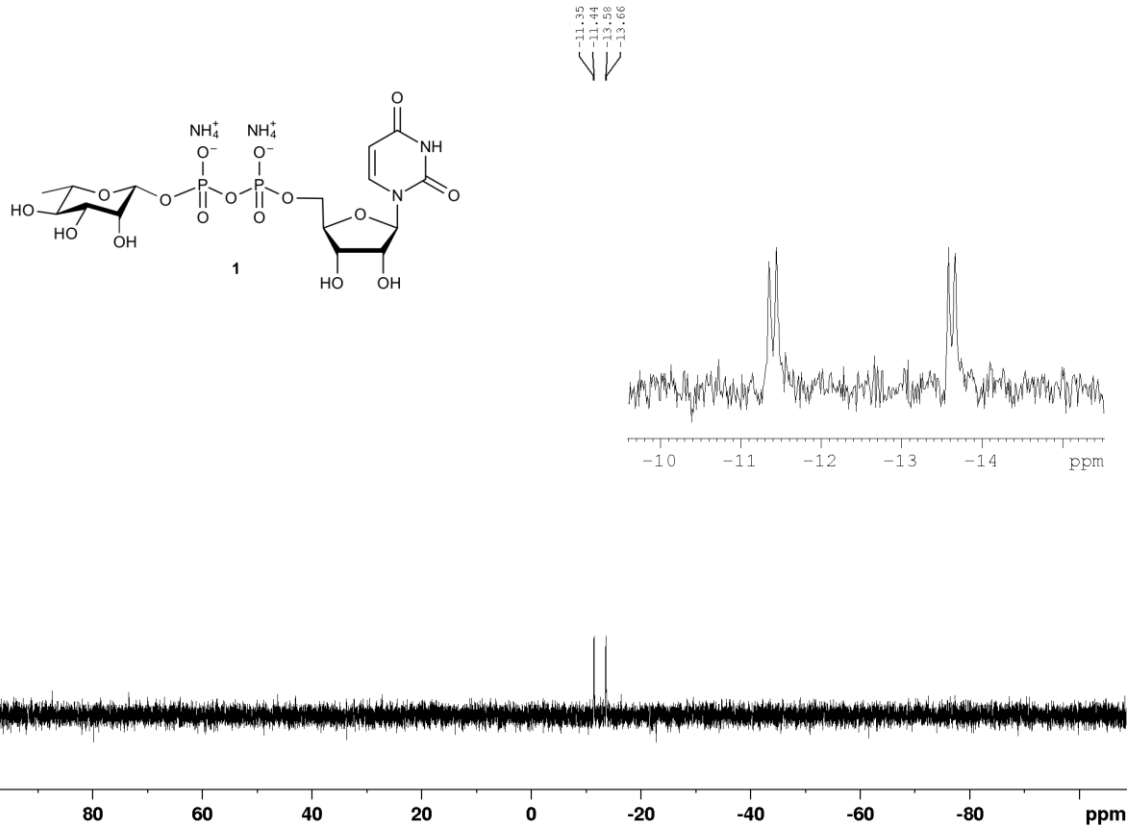


^{1}H NMR Spectrum of 1 in D_2O

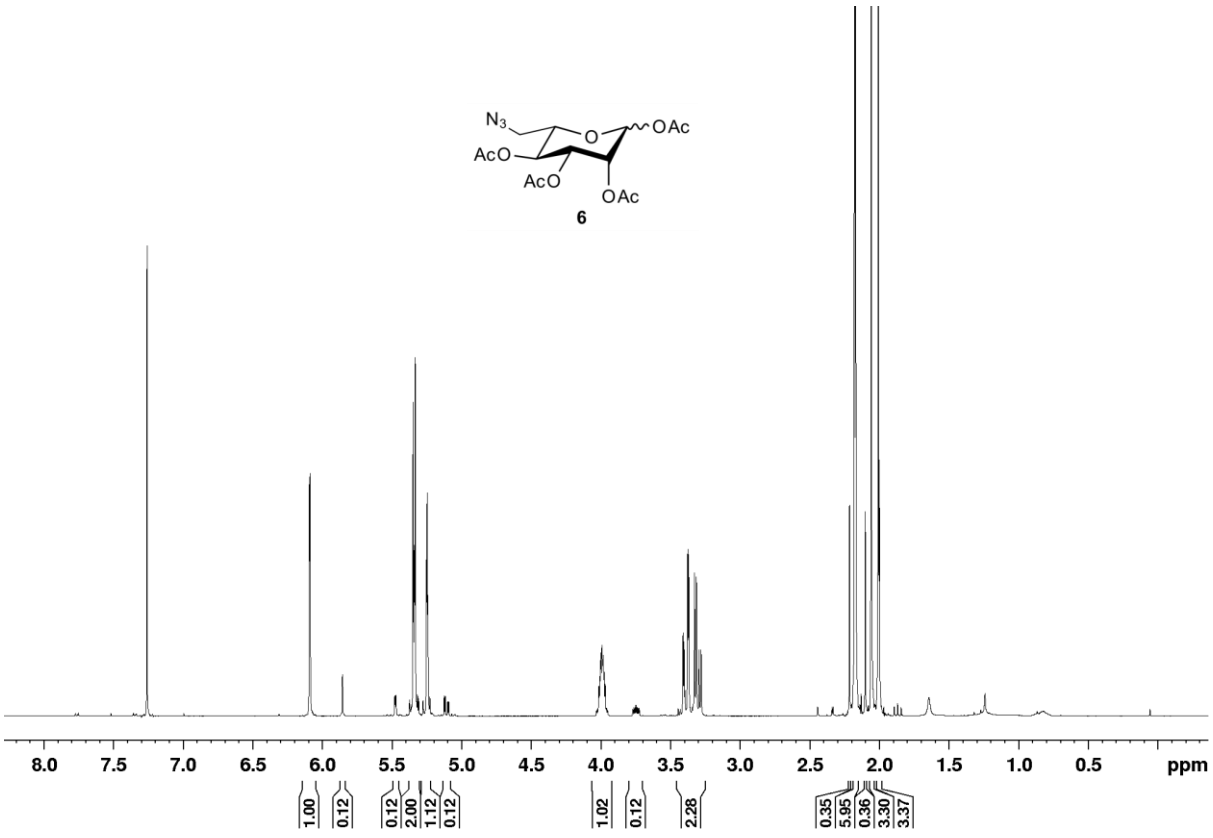
Contains NH_4COCH_3 (buffer)



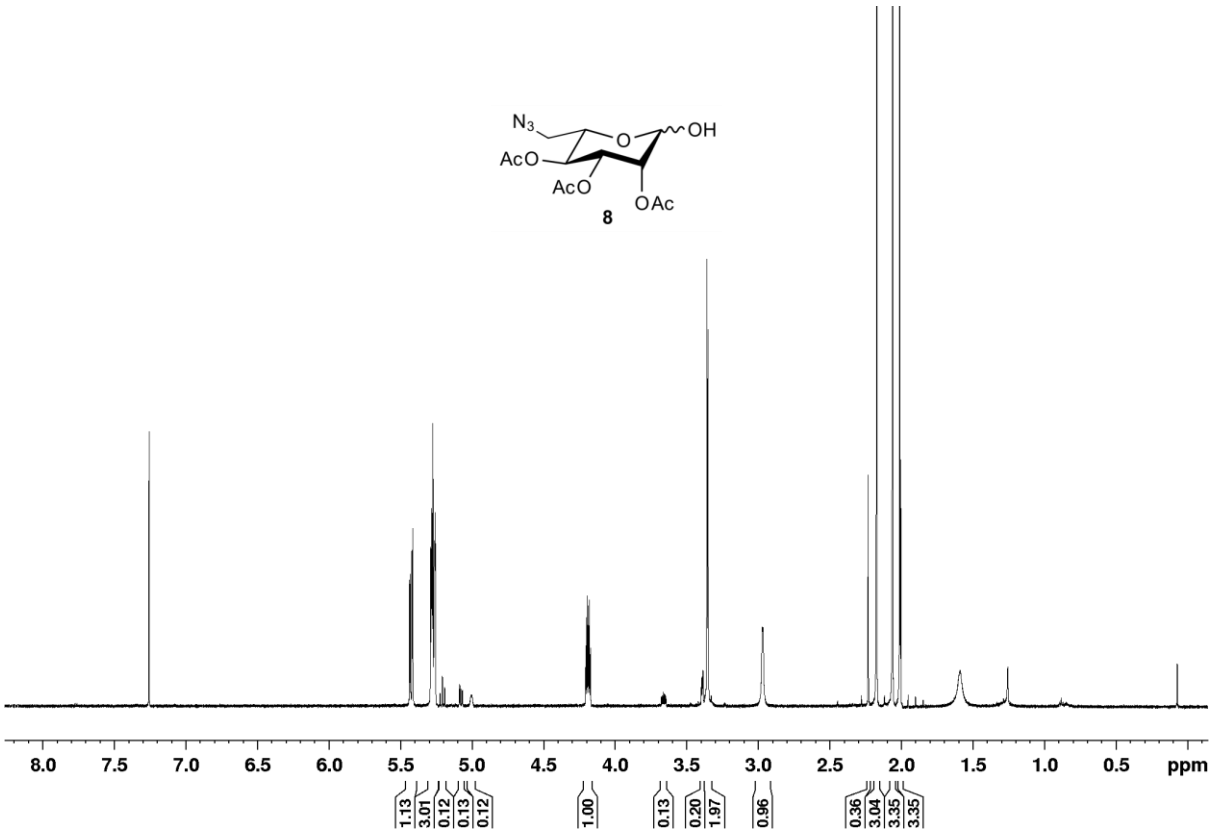
^{1}H-decoupled ^{31}P NMR Spectrum of 1 in D_2O



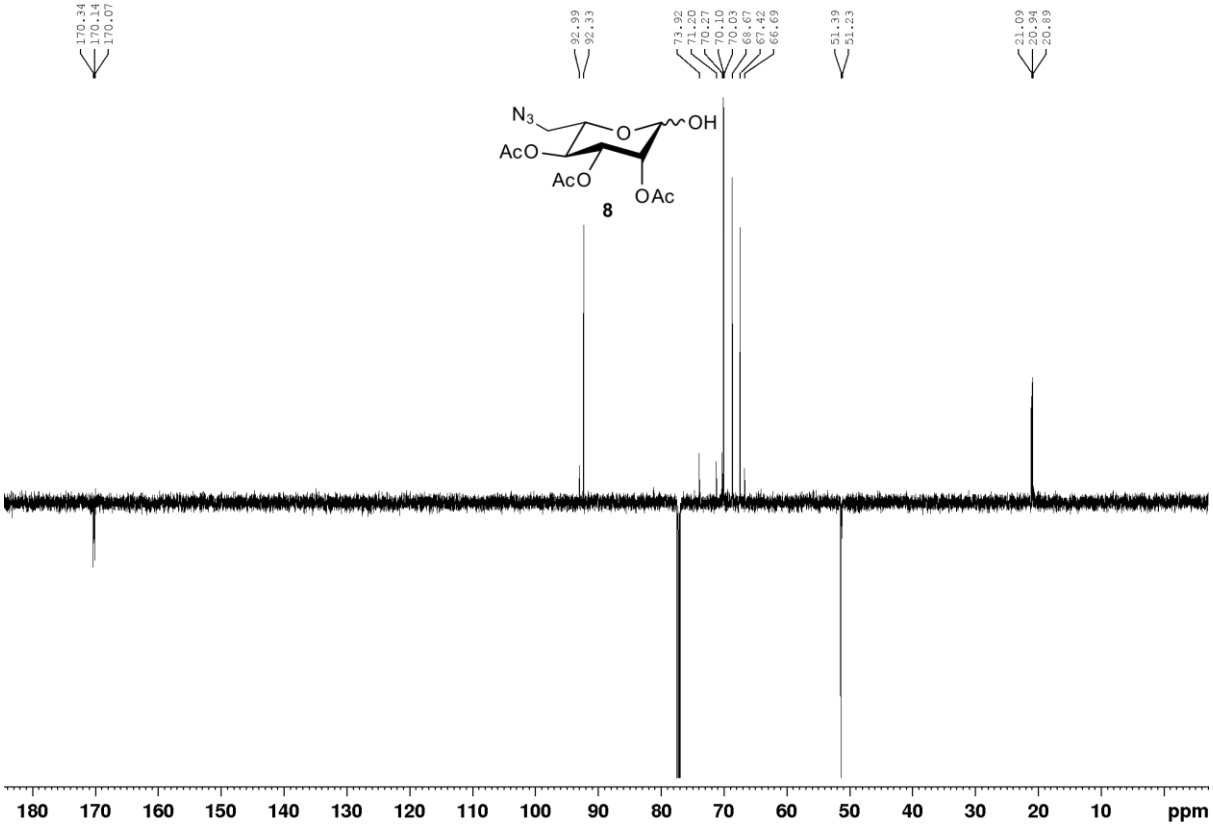
^{1}H NMR Spectrum of 6 in $CDCl_3$



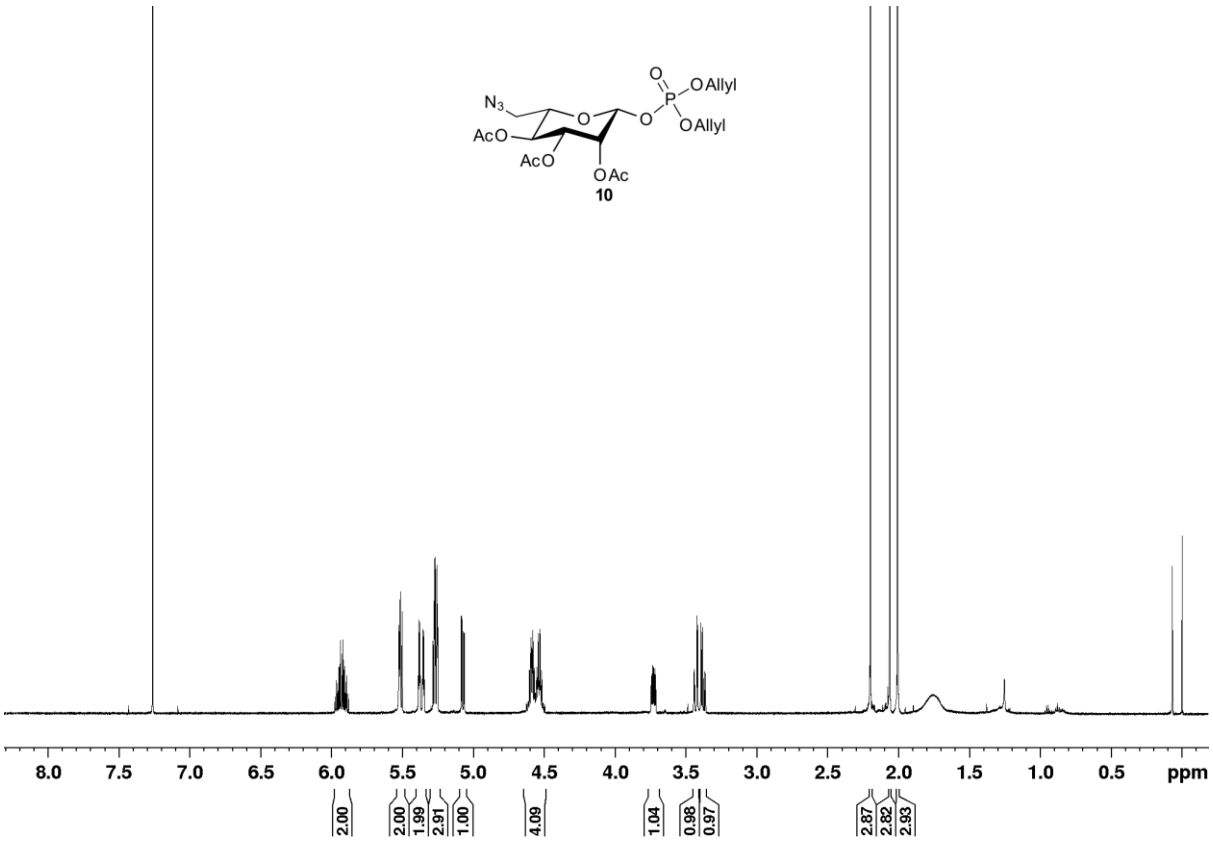
^{1}H NMR Spectrum of 8 in $CDCl_3$



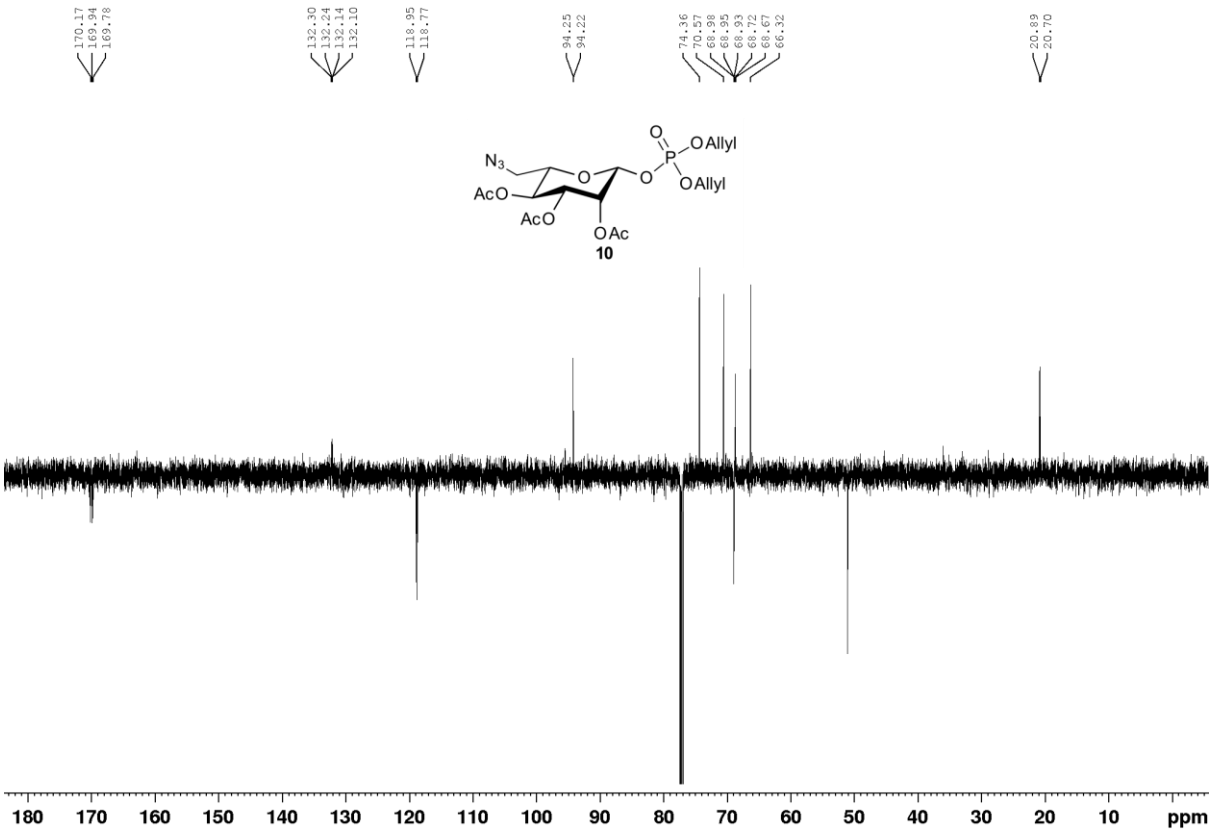
^{13}C APT NMR Spectrum of 8 in $CDCl_3$



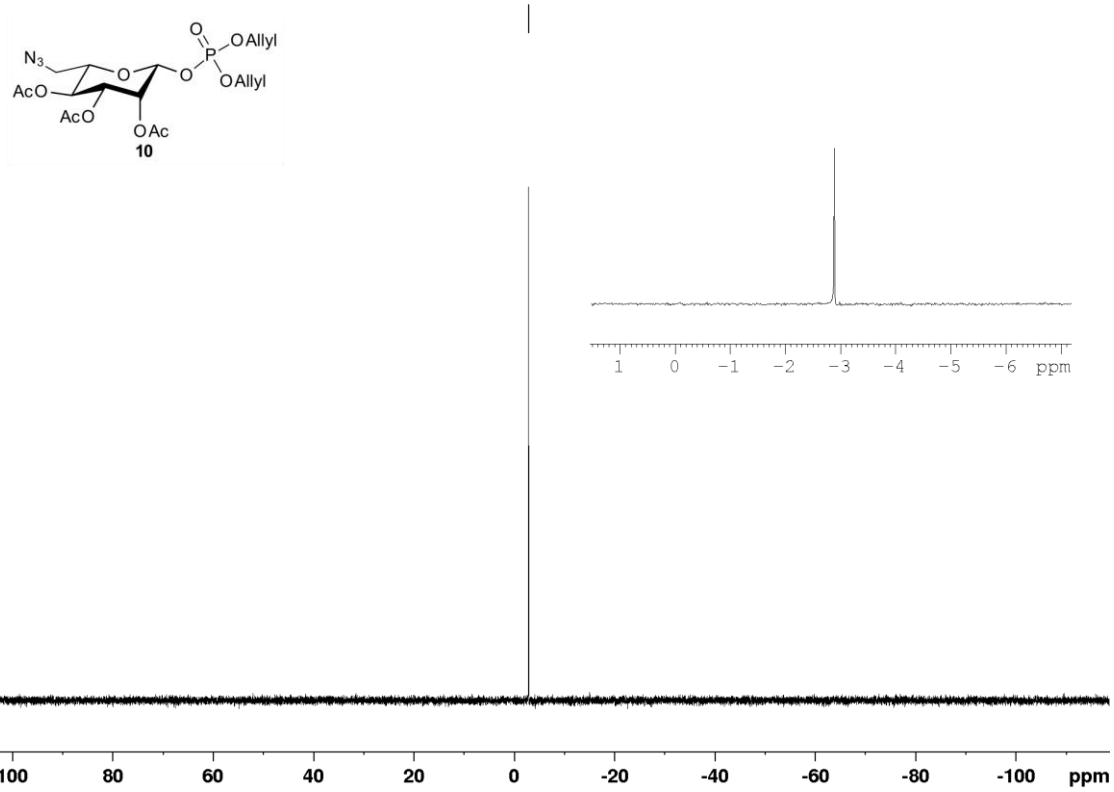
^{1}H NMR Spectrum of 10 in $CDCl_3$



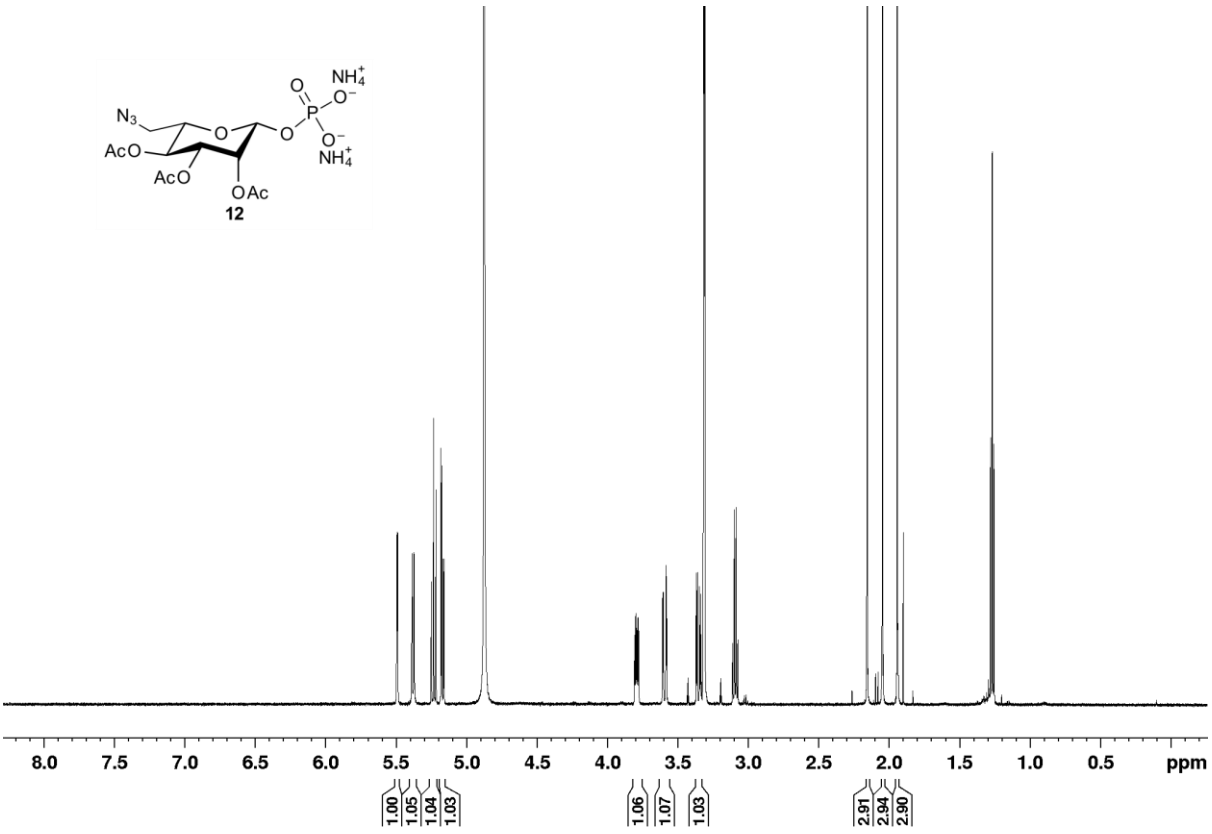
^{13}C APT NMR Spectrum of 10 in $CDCl_3$



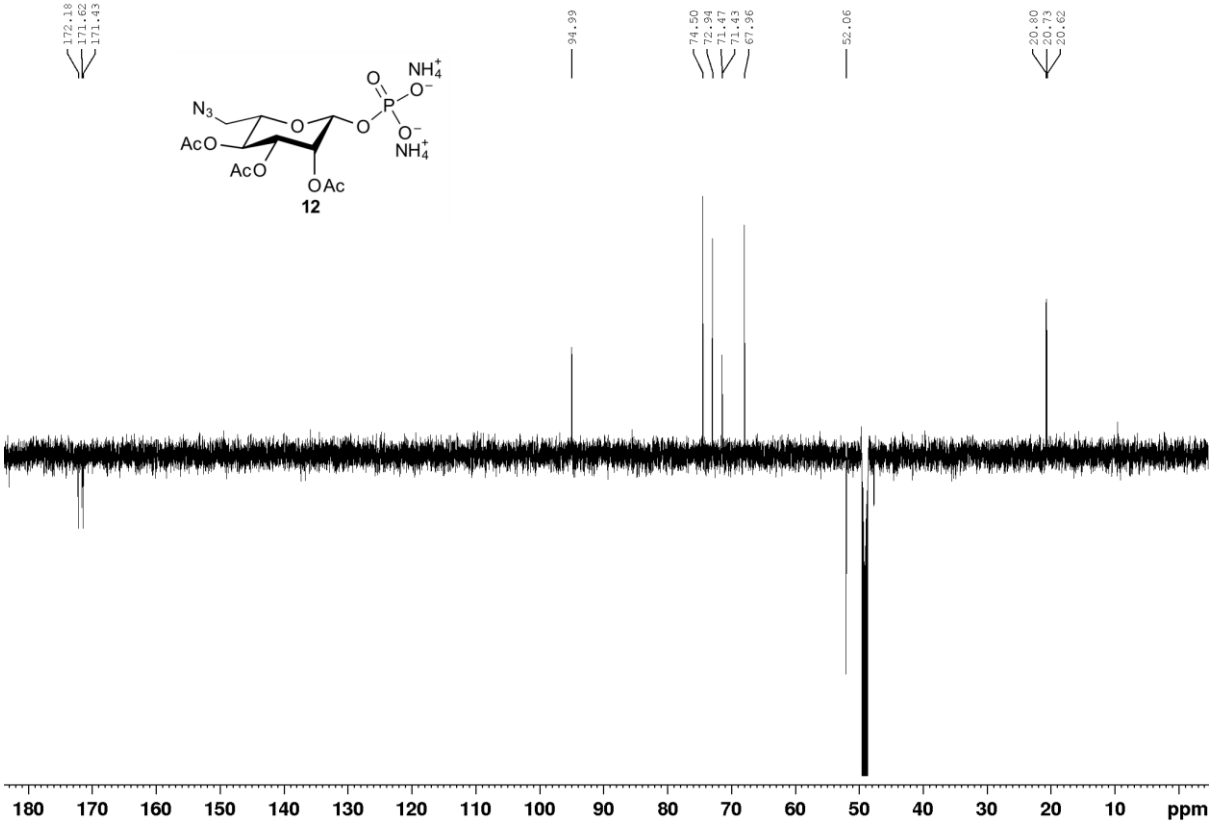
^{1}H-decoupled ^{31}P NMR Spectrum of 10 in $CDCl_3$



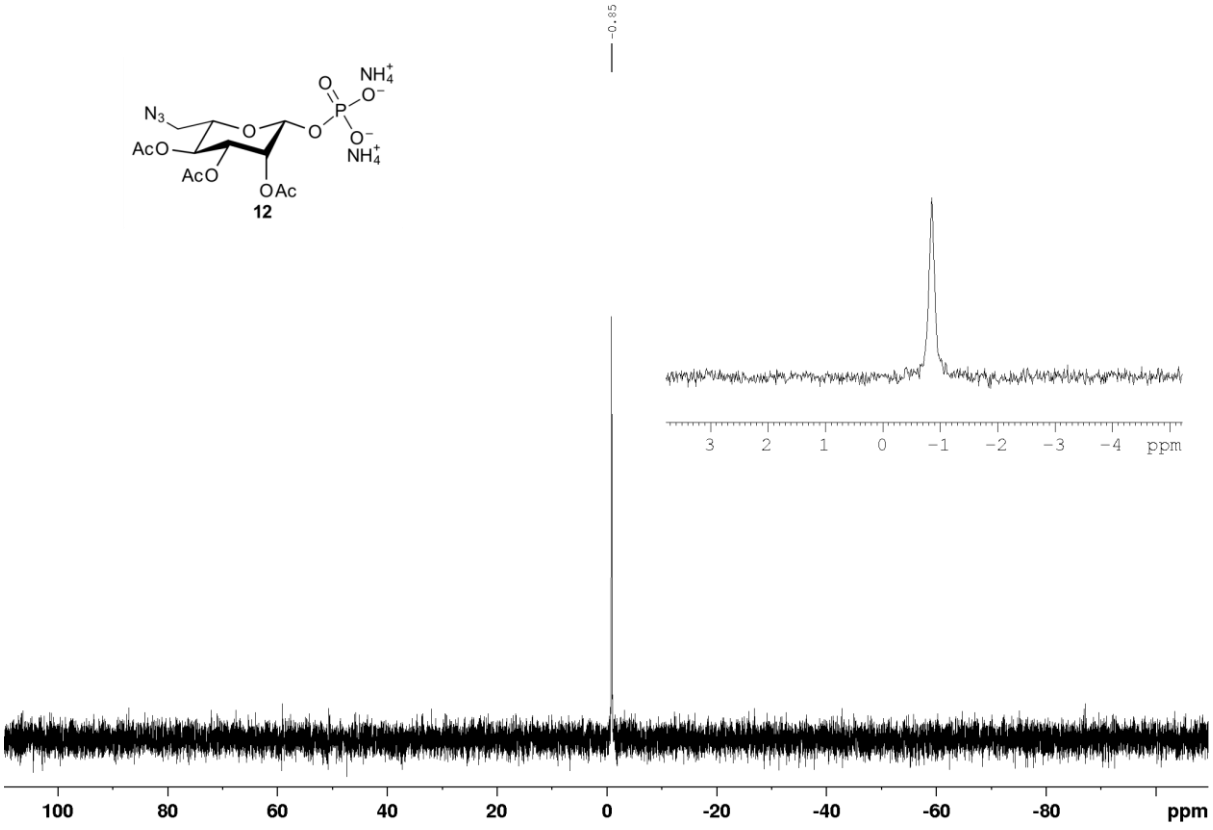
^{1}H NMR Spectrum of 12 in MeOD



^{13}C APT NMR Spectrum of 12 in MeOD

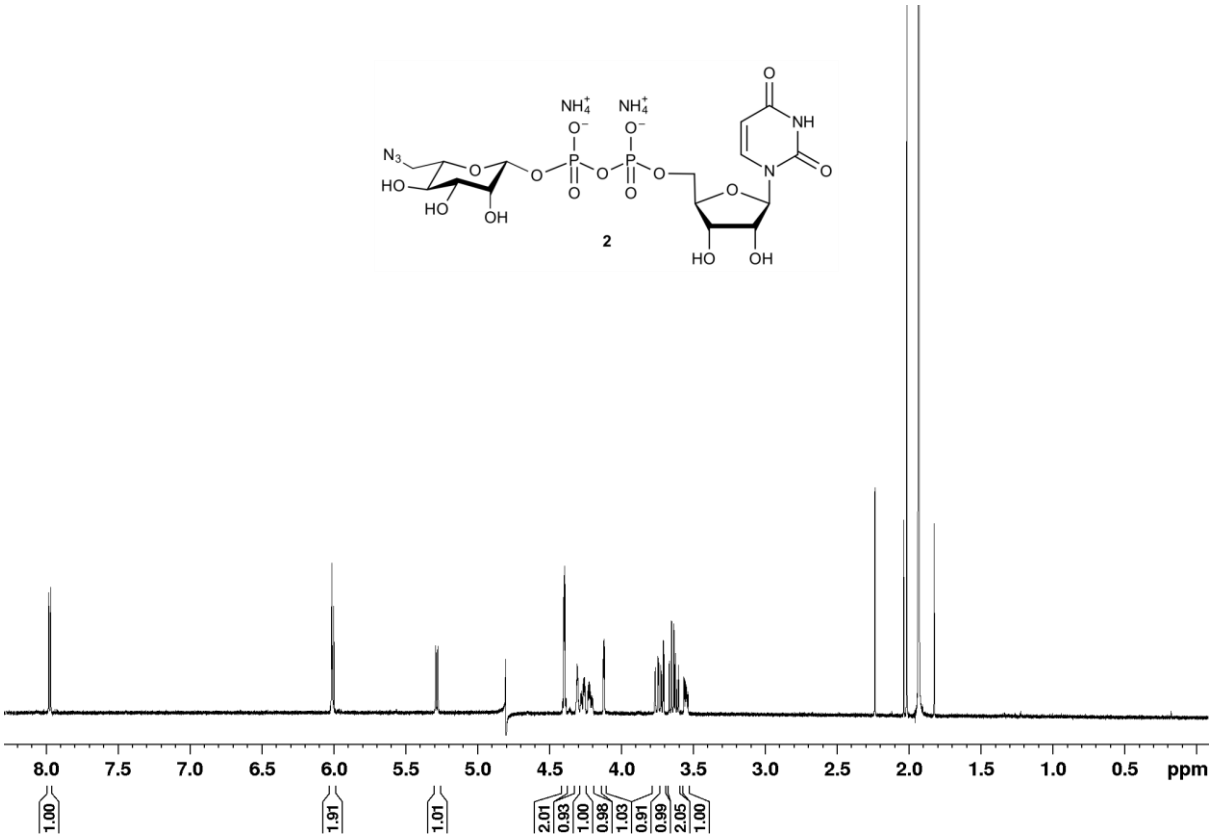


^{1}H-decoupled ^{31}P NMR Spectrum of 12 in MeOD

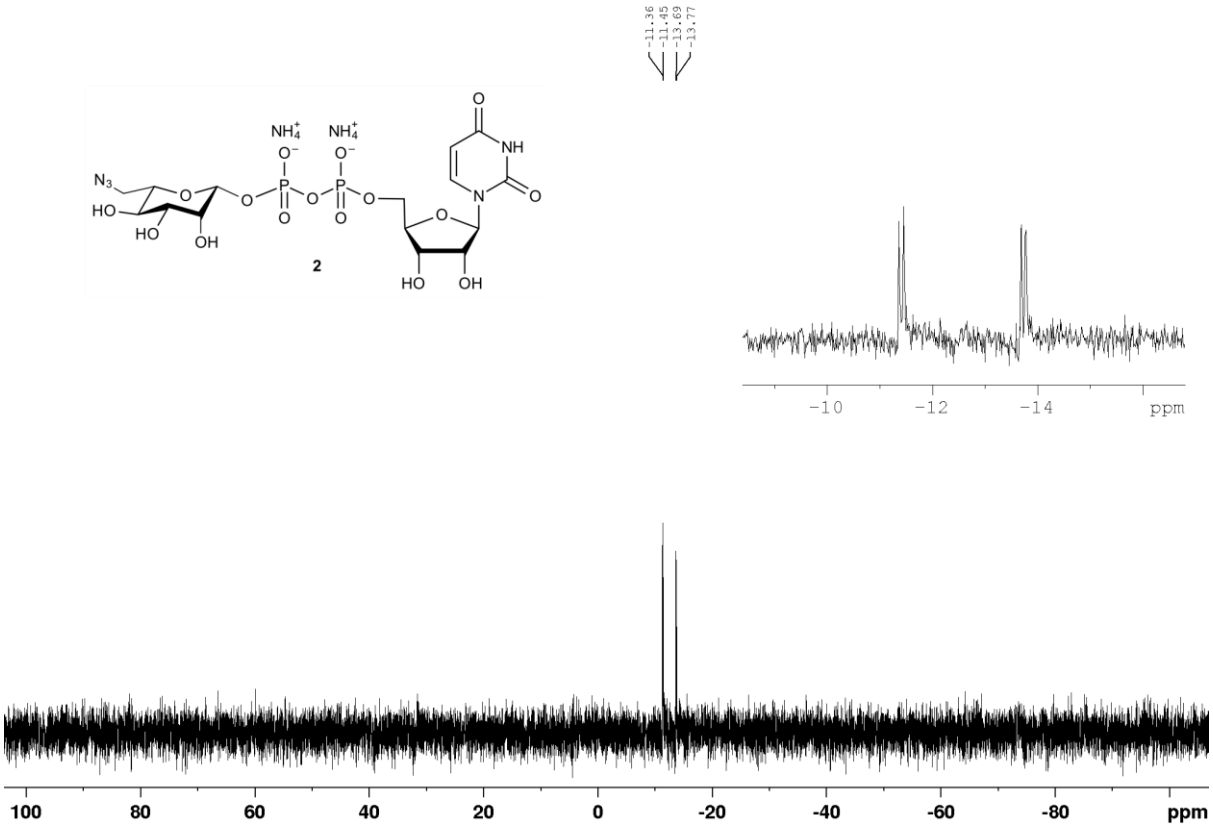


^{1}H NMR Spectrum of 2 in D_2O

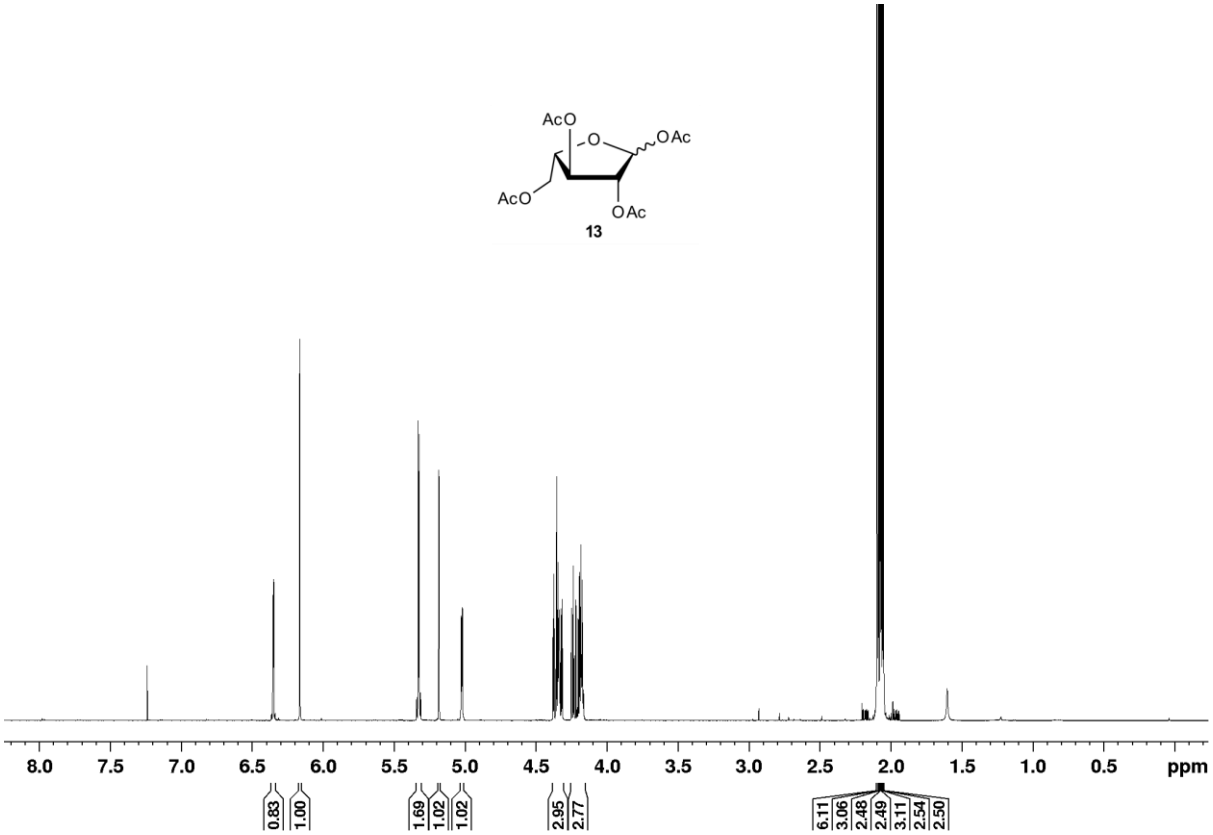
Contains NH_4COCH_3 (buffer)



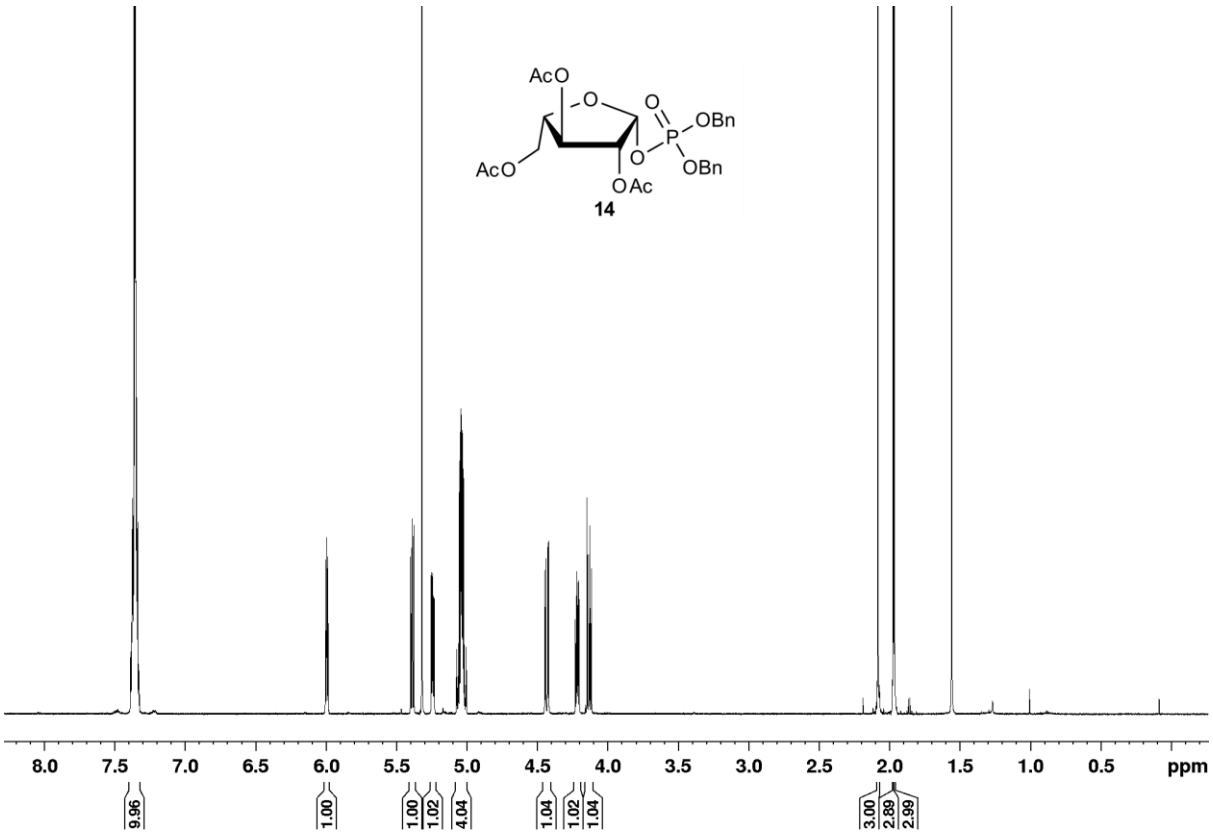
^{1}H-decoupled ^{31}P NMR Spectrum of 2 in D_2O



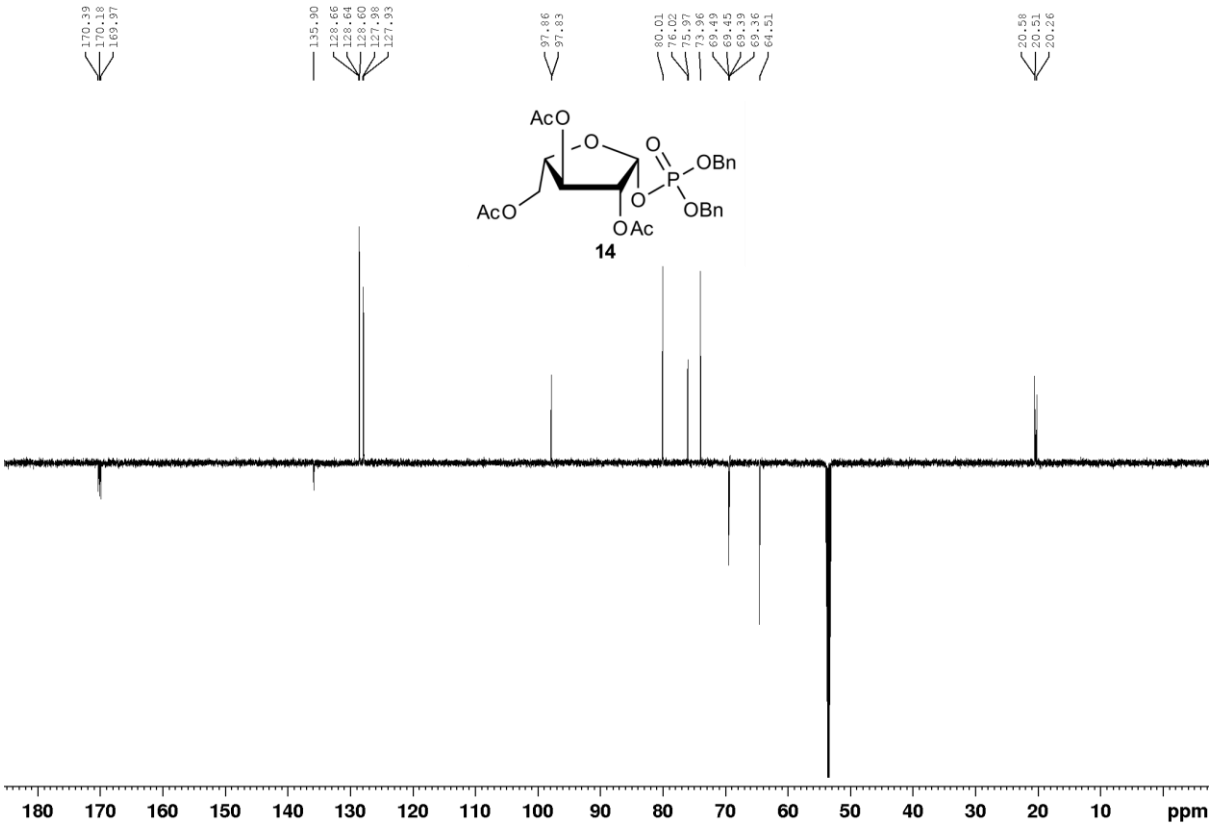
^{1}H NMR Spectrum of 13 in $CDCl_3$



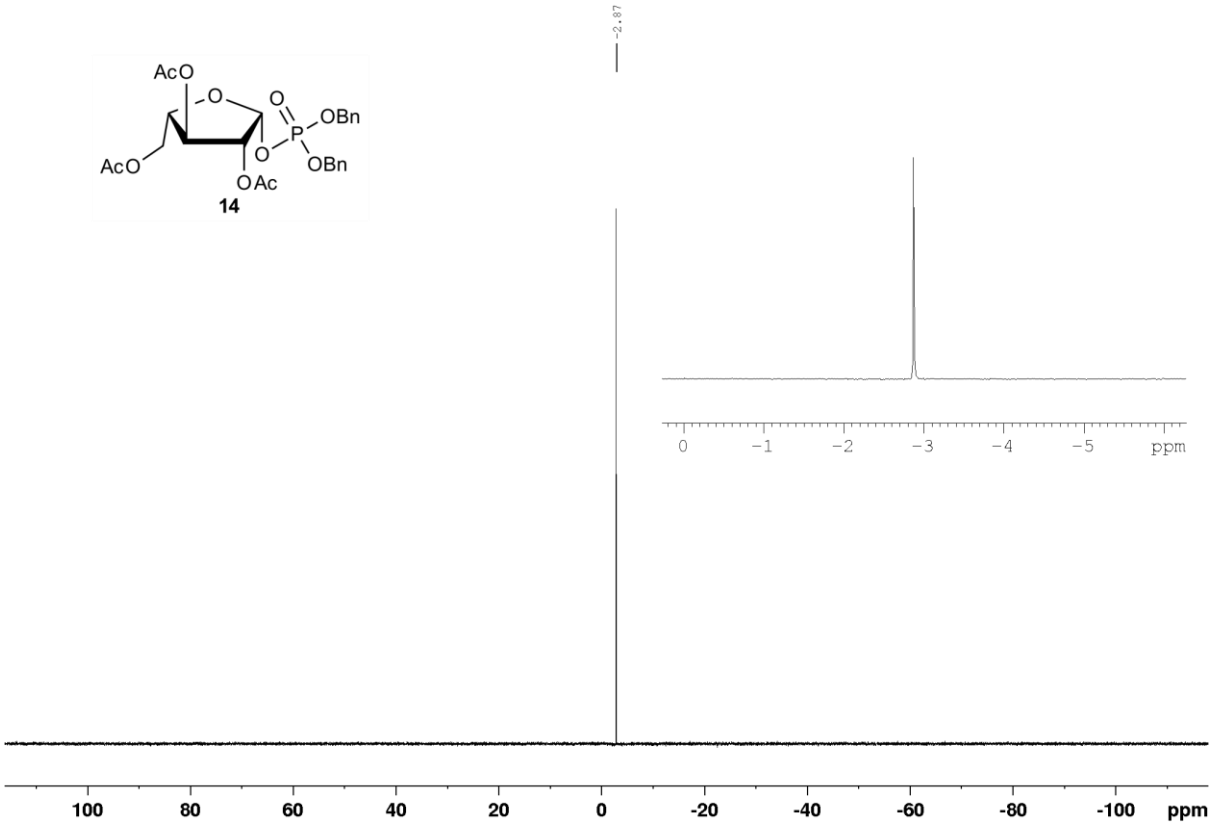
^{1}H NMR Spectrum of 14 in CD_2Cl_2



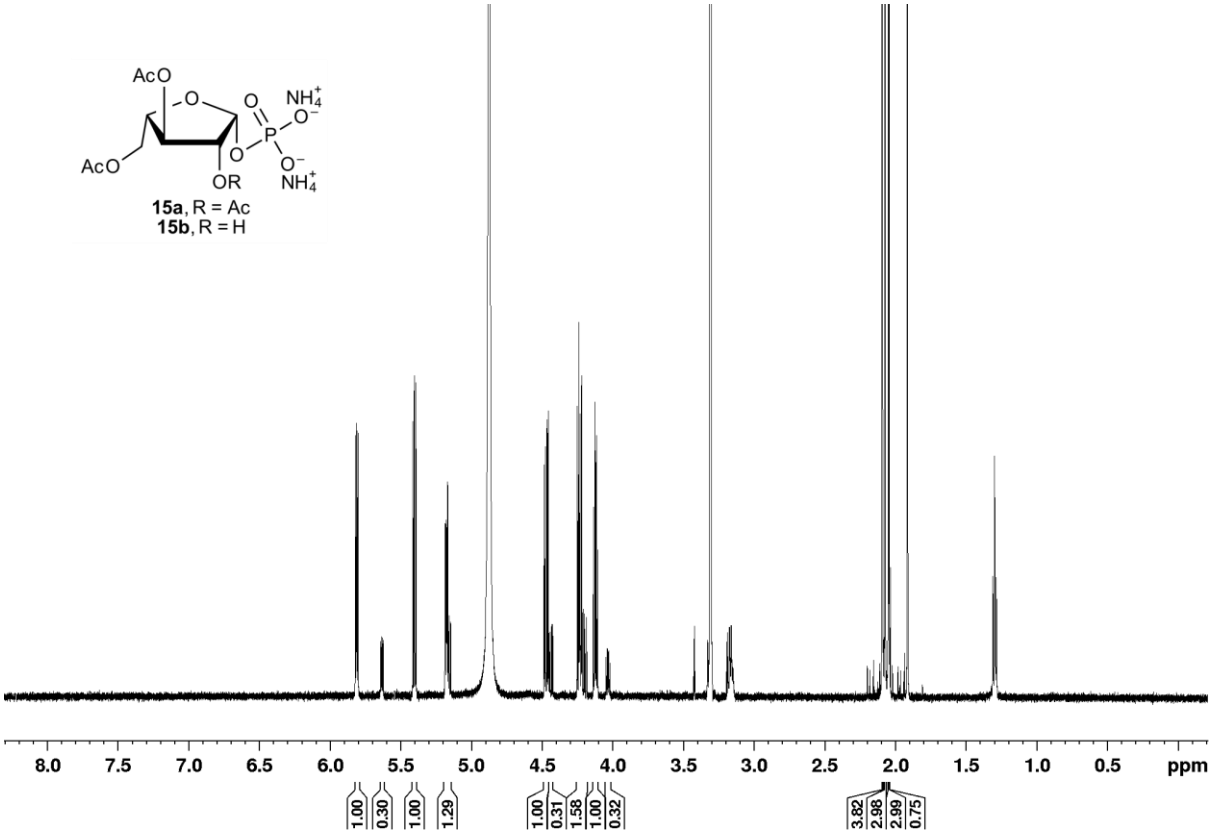
^{13}C APT NMR Spectrum of 14 in CD_2Cl_2



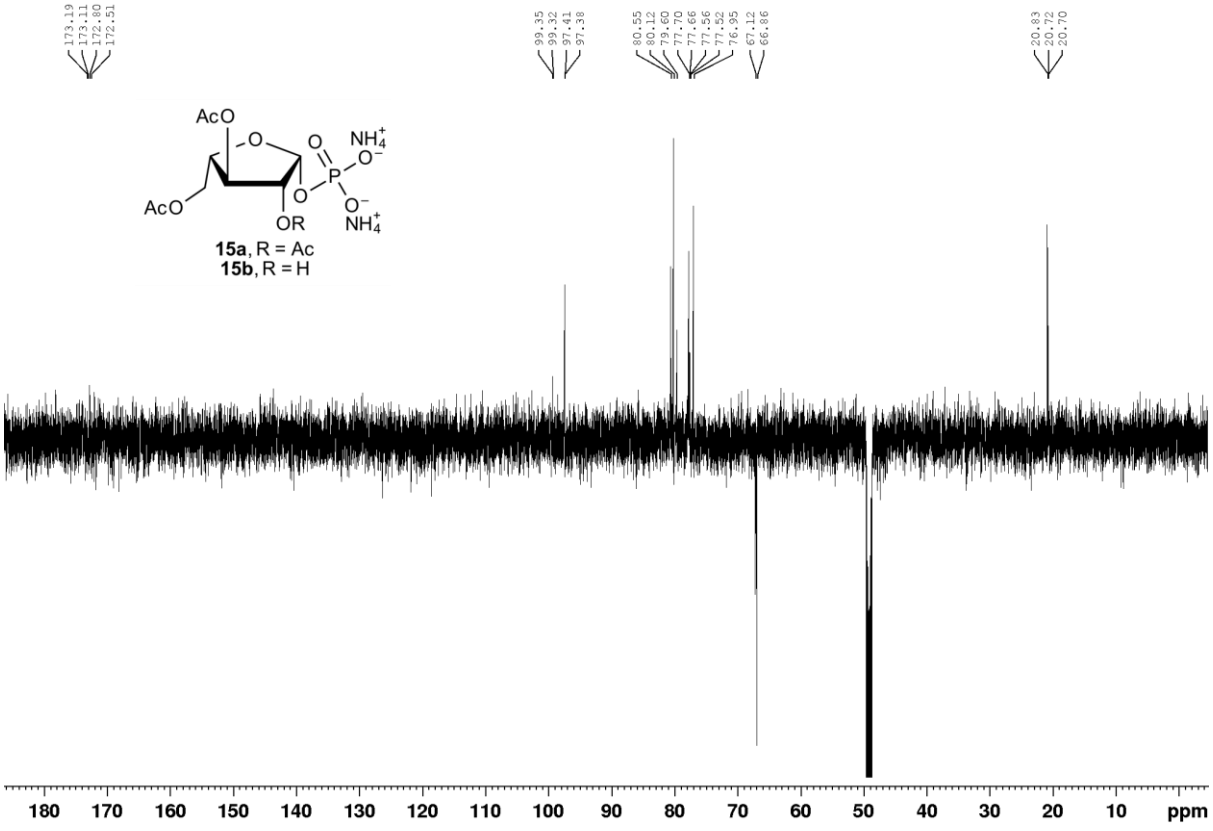
^{1}H-decoupled ^{31}P NMR Spectrum of 14 in CD_2Cl_2



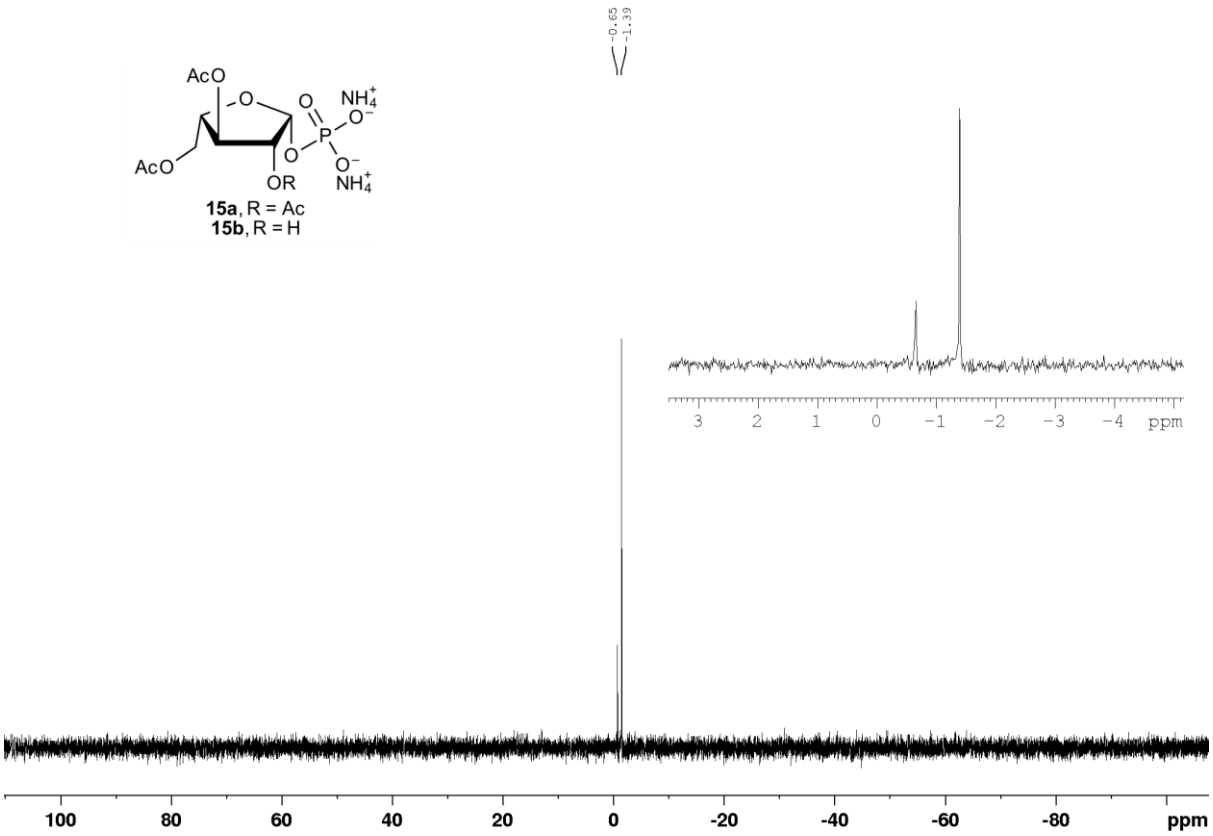
^{1}H NMR Spectrum of 15a and 15b in MeOD



^{13}C APT NMR Spectrum of 15a and 15b in MeOD

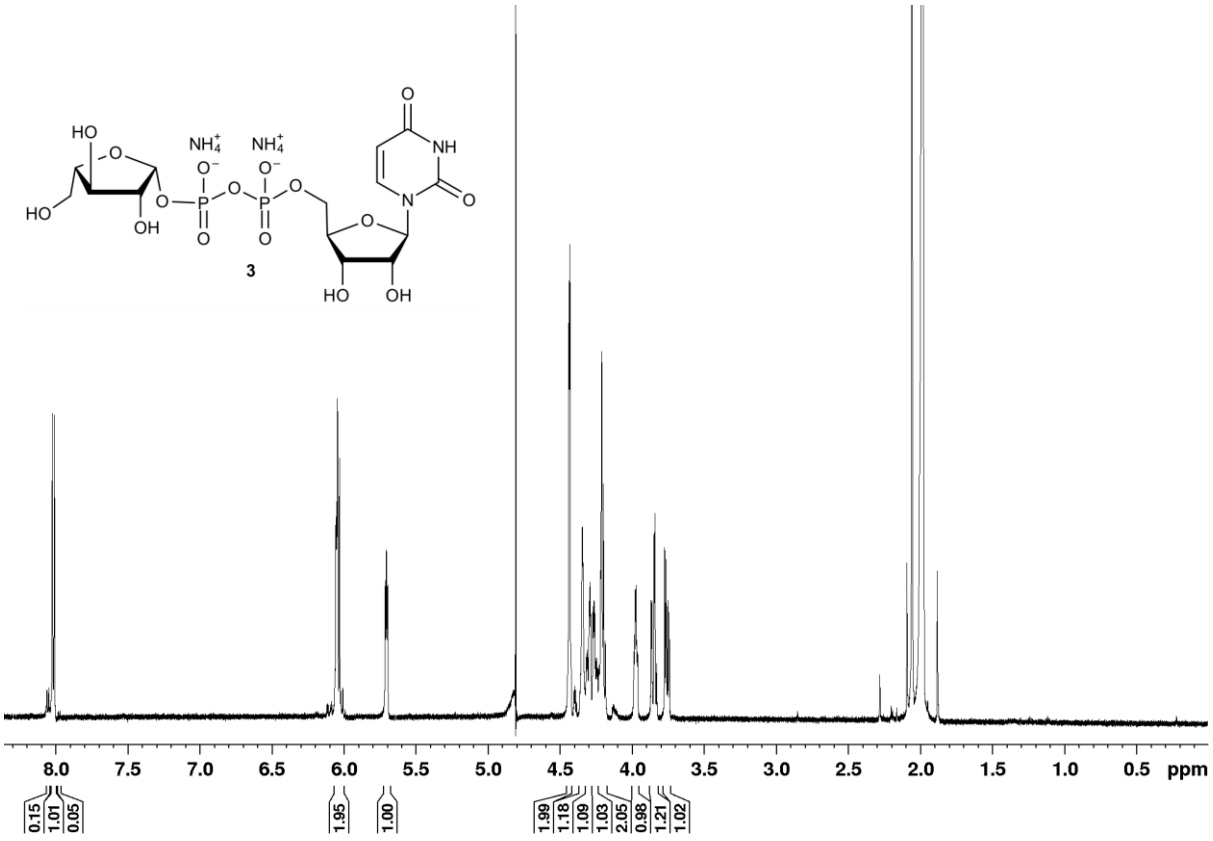


^{1}H-decoupled ^{31}P NMR Spectrum of 15a and 15b in MeOD

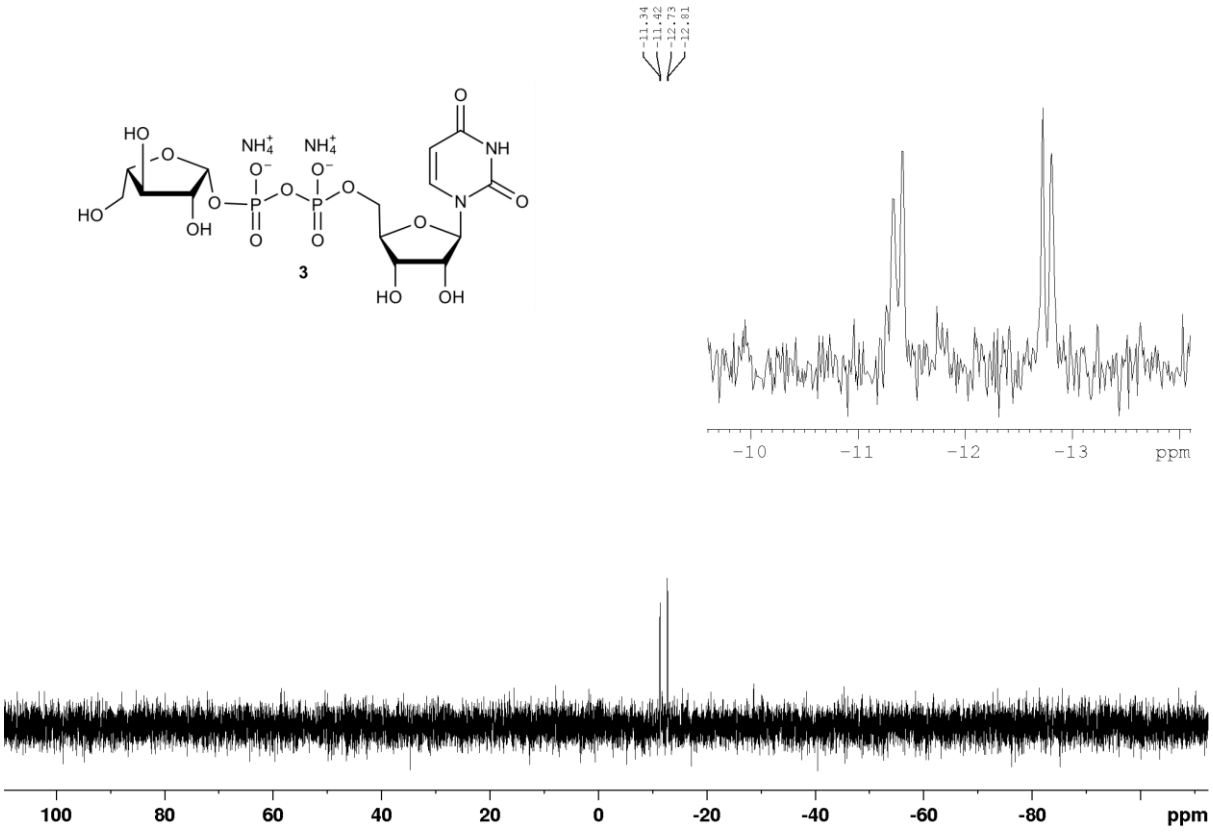


^{1}H NMR Spectrum of 3 in D_2O

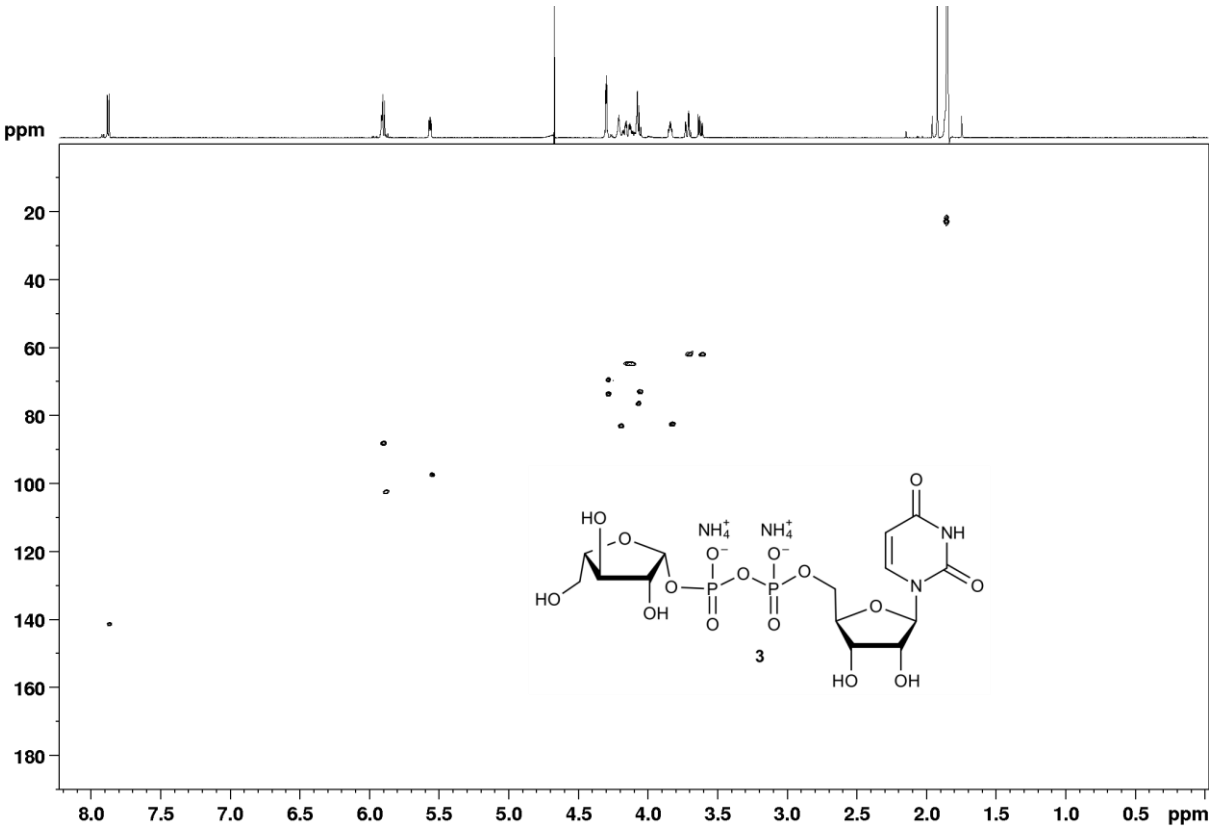
Contains NH_4COCH_3 (buffer)



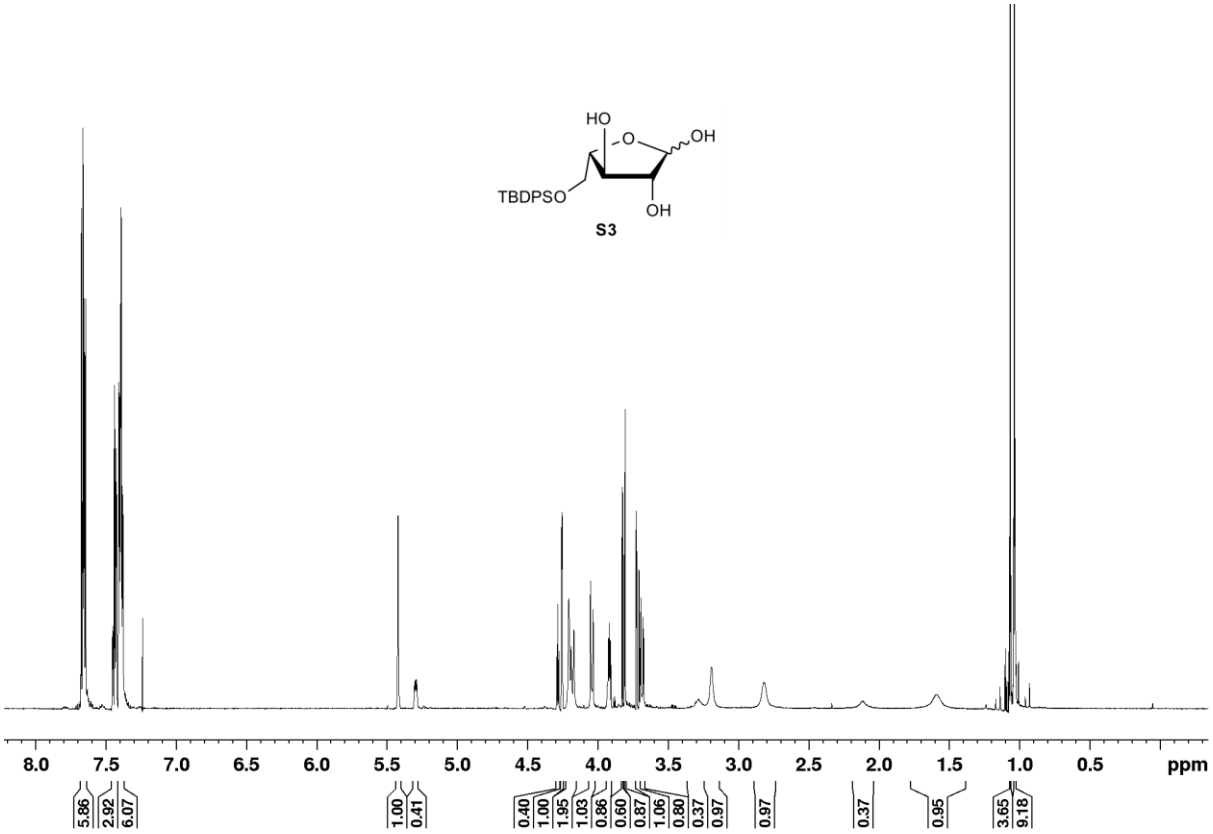
^{1}H-decoupled ^{31}P NMR Spectrum of 3 in D_2O



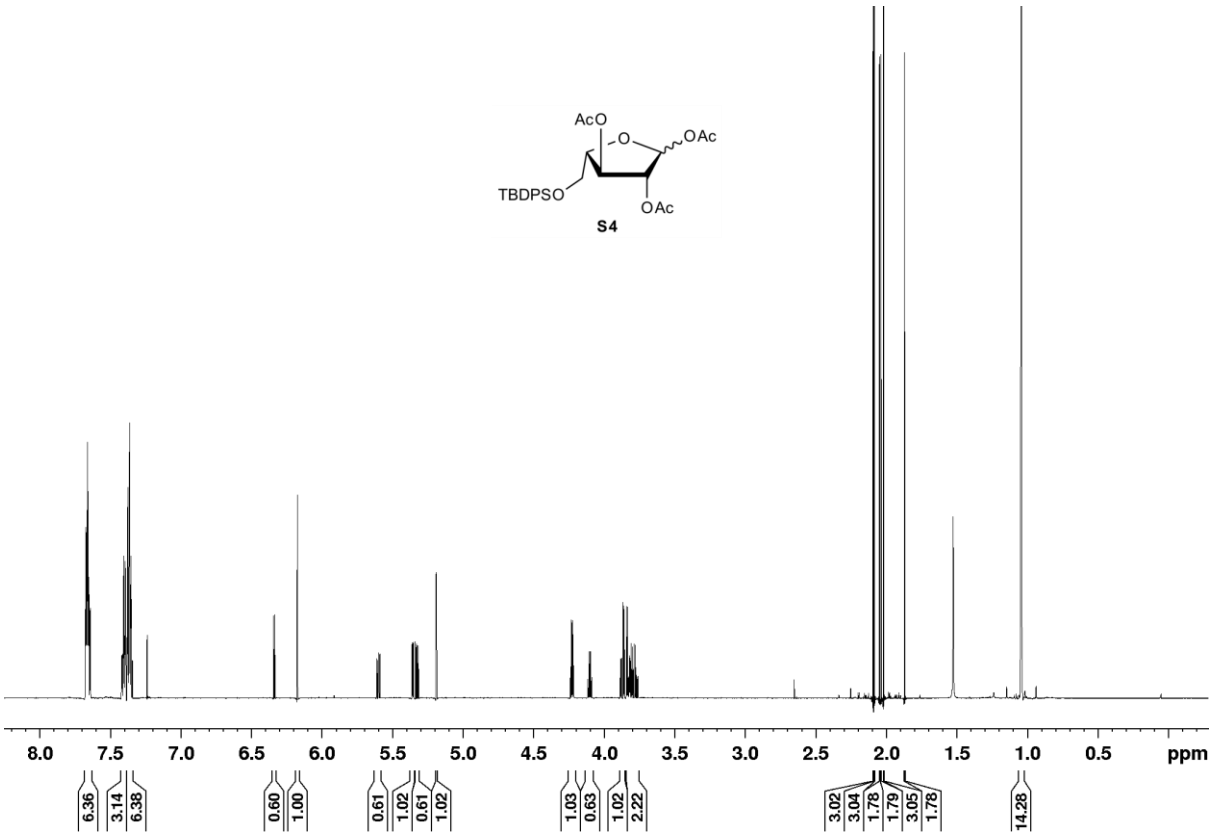
^{1}H-^{13}C HSQC Spectrum of 3 in D_2O



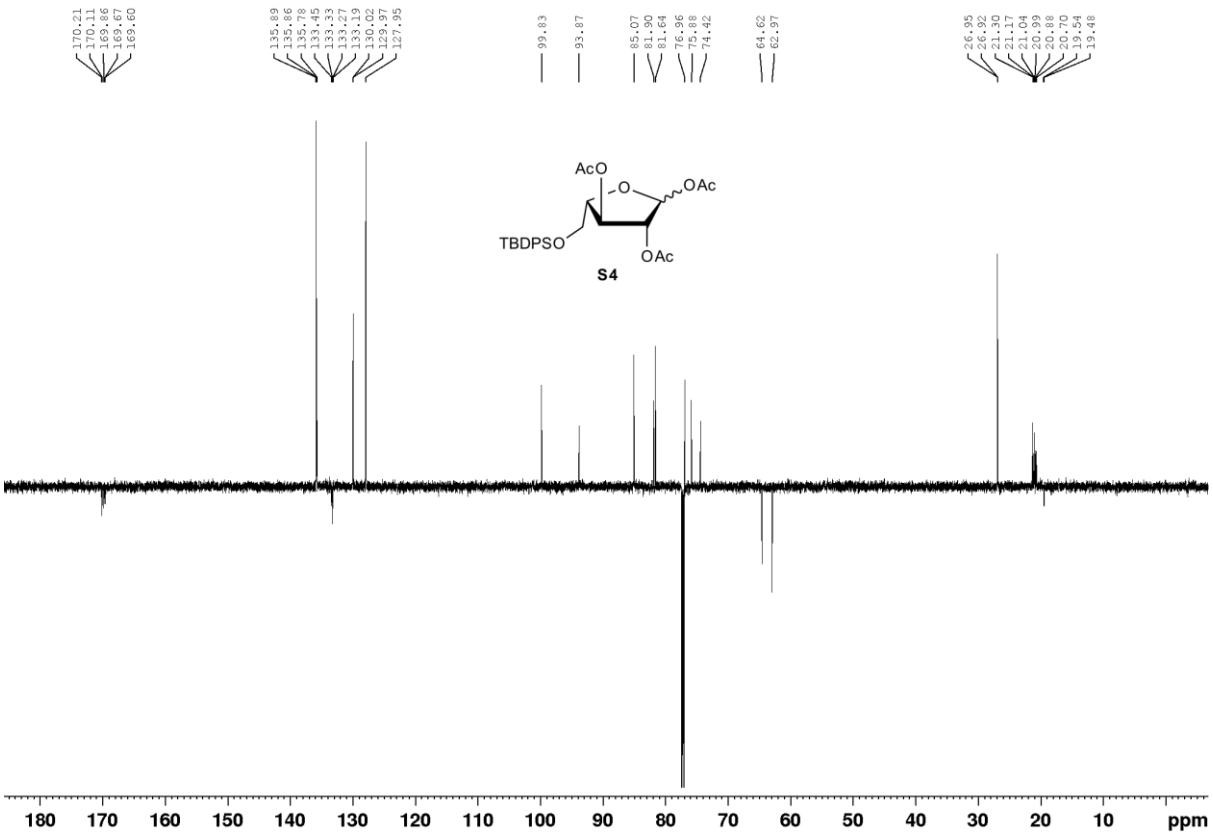
^{1}H NMR Spectrum of S3 in $CDCl_3$



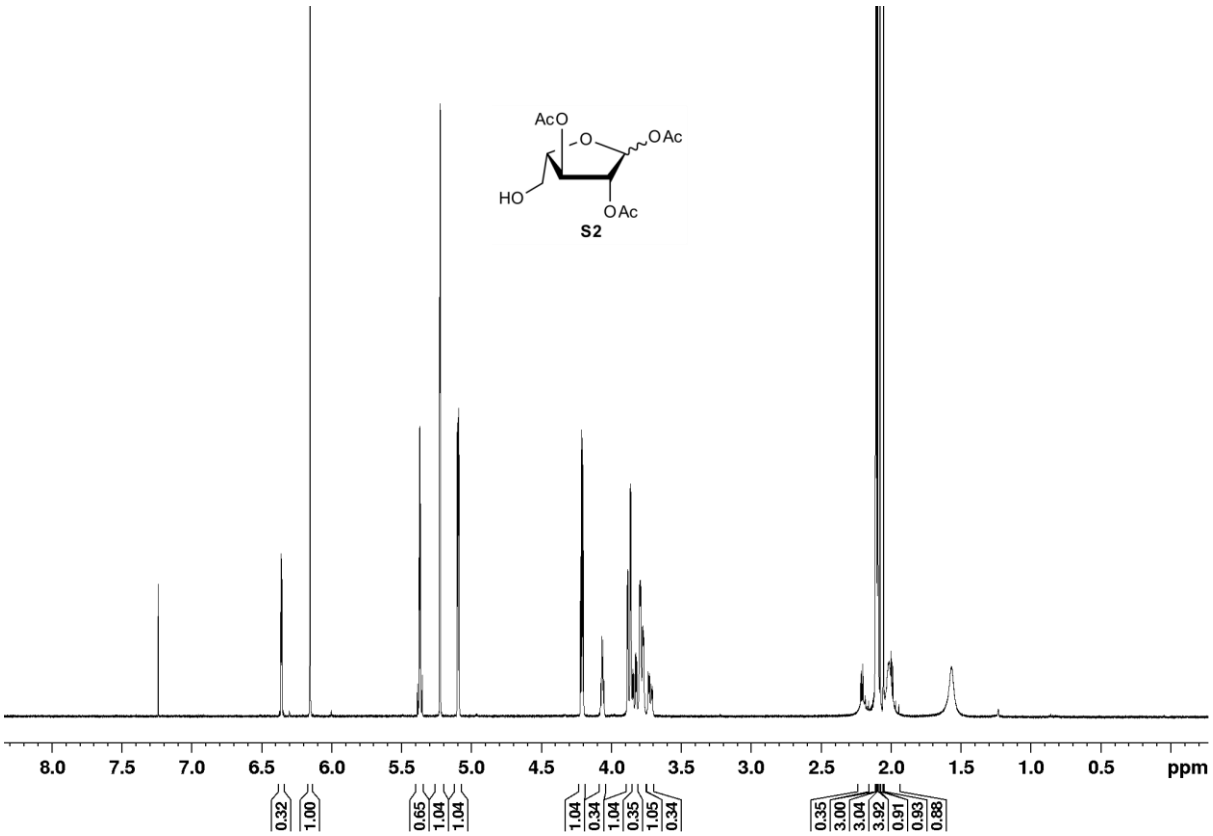
^{1}H NMR Spectrum of S4 in $CDCl_3$



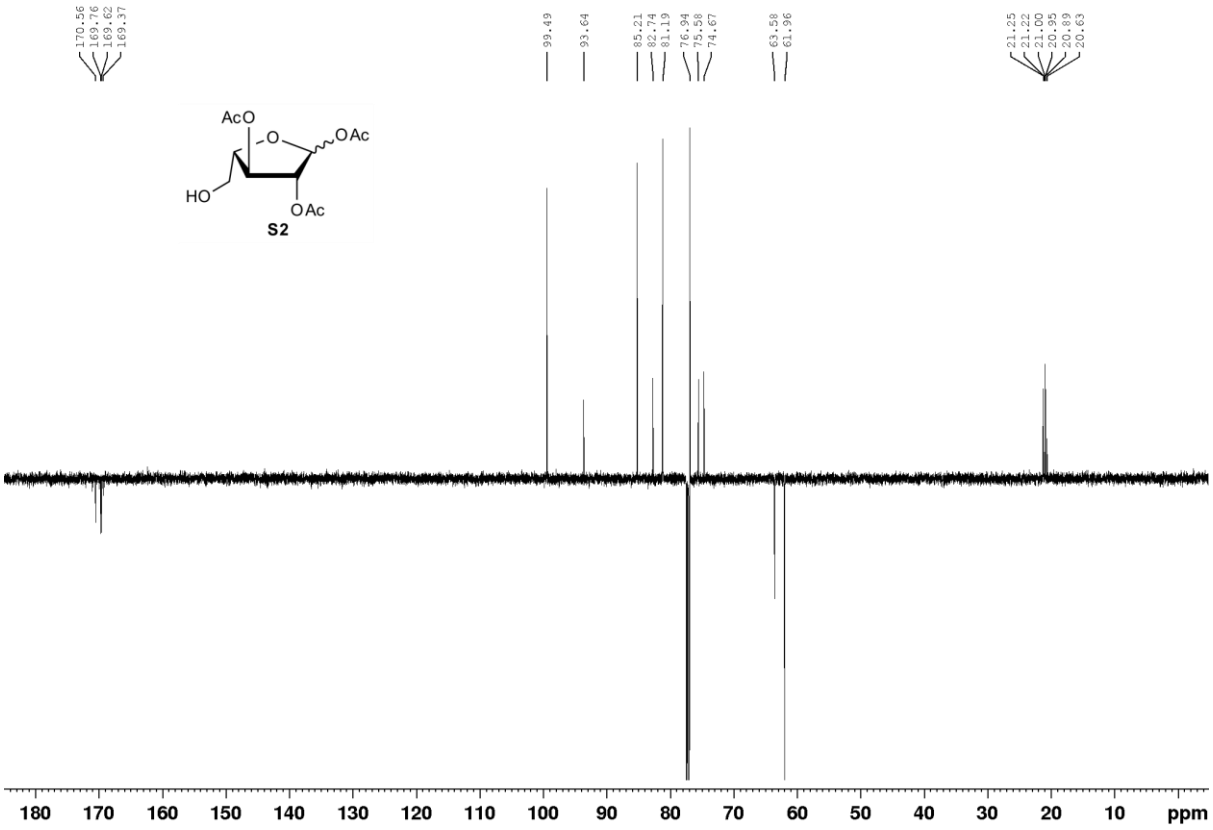
^{13}C APT NMR Spectrum of S4 in $CDCl_3$



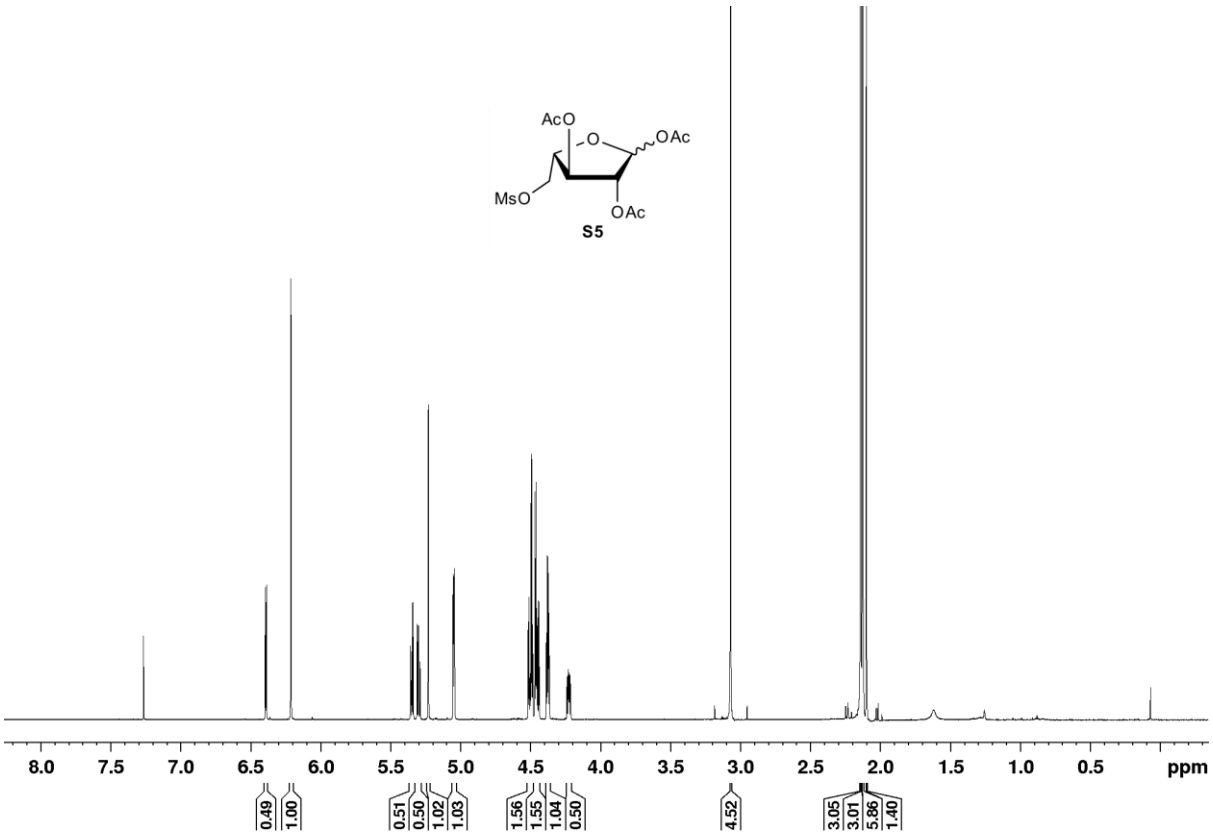
^{1}H NMR Spectrum of S2 in $CDCl_3$



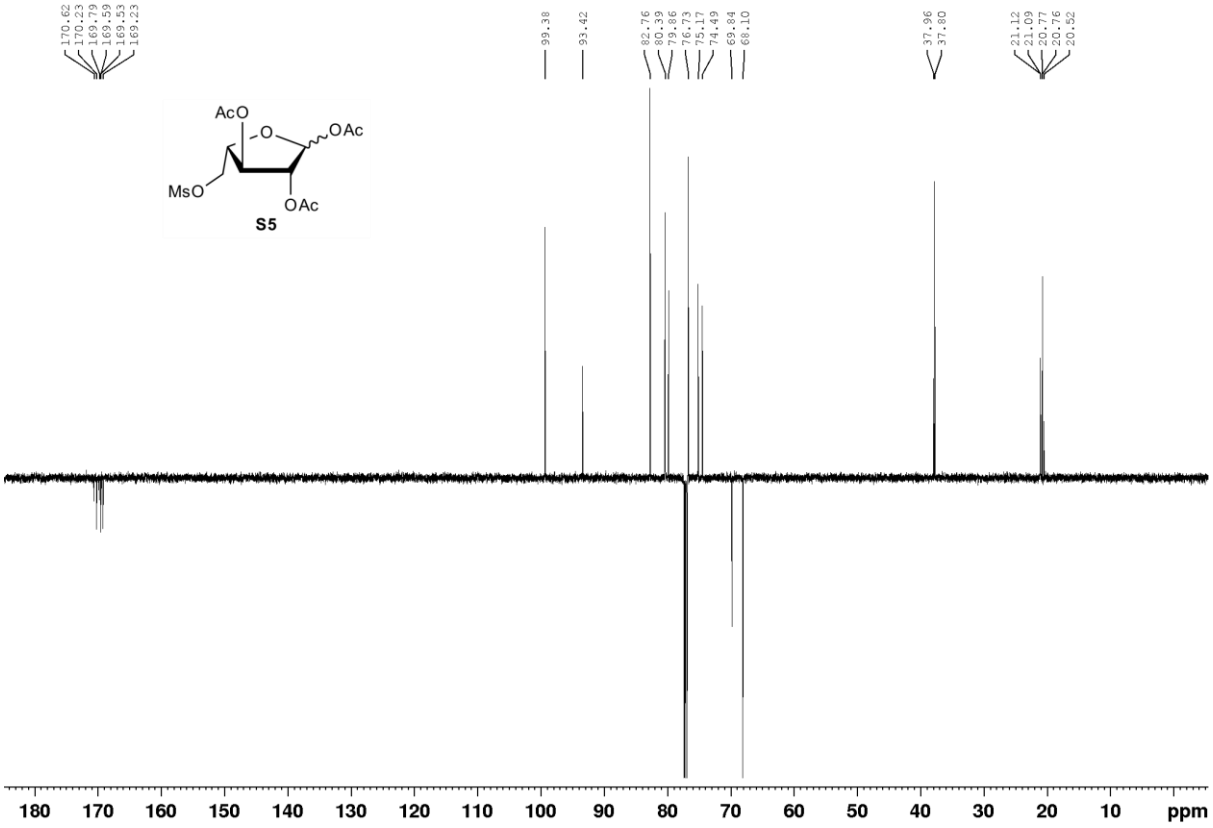
^{13}C APT NMR Spectrum of S2 in $CDCl_3$



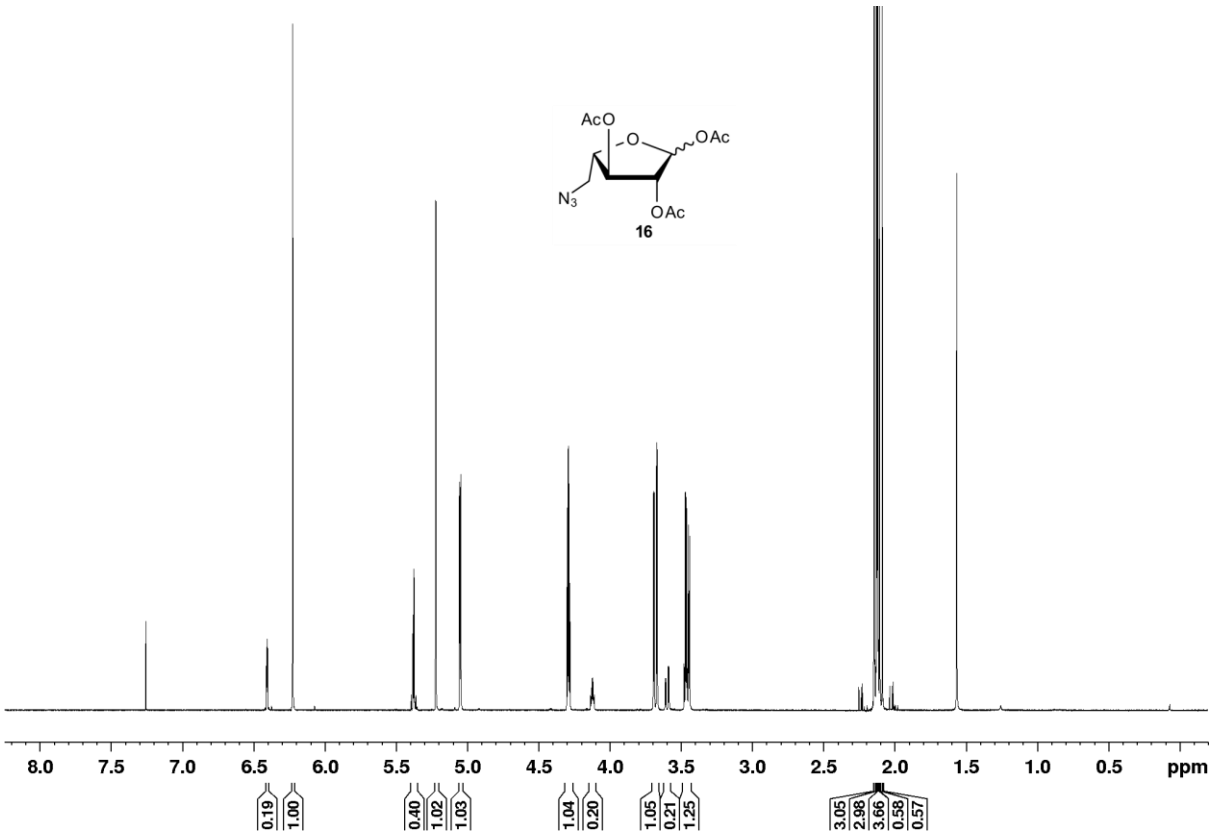
^{1}H NMR Spectrum of S5 in $CDCl_3$



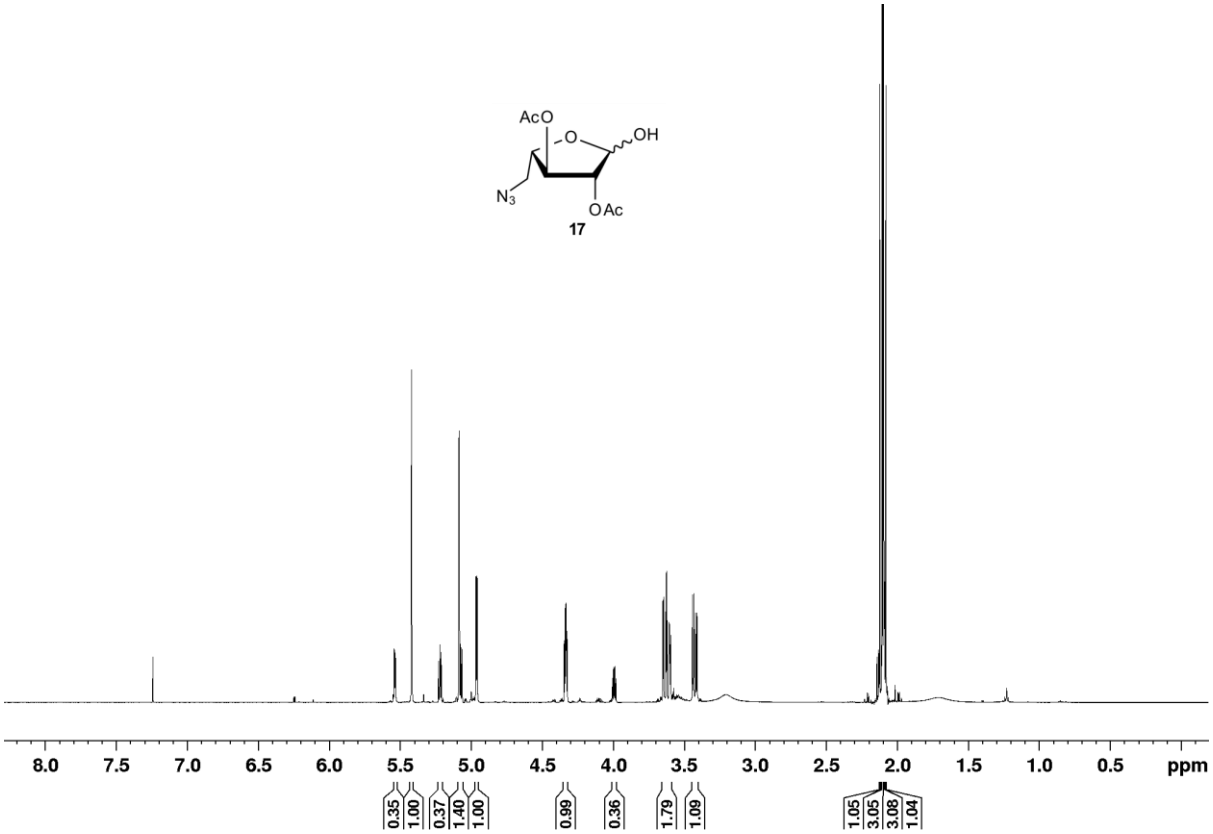
^{13}C APT NMR Spectrum of S5 in $CDCl_3$



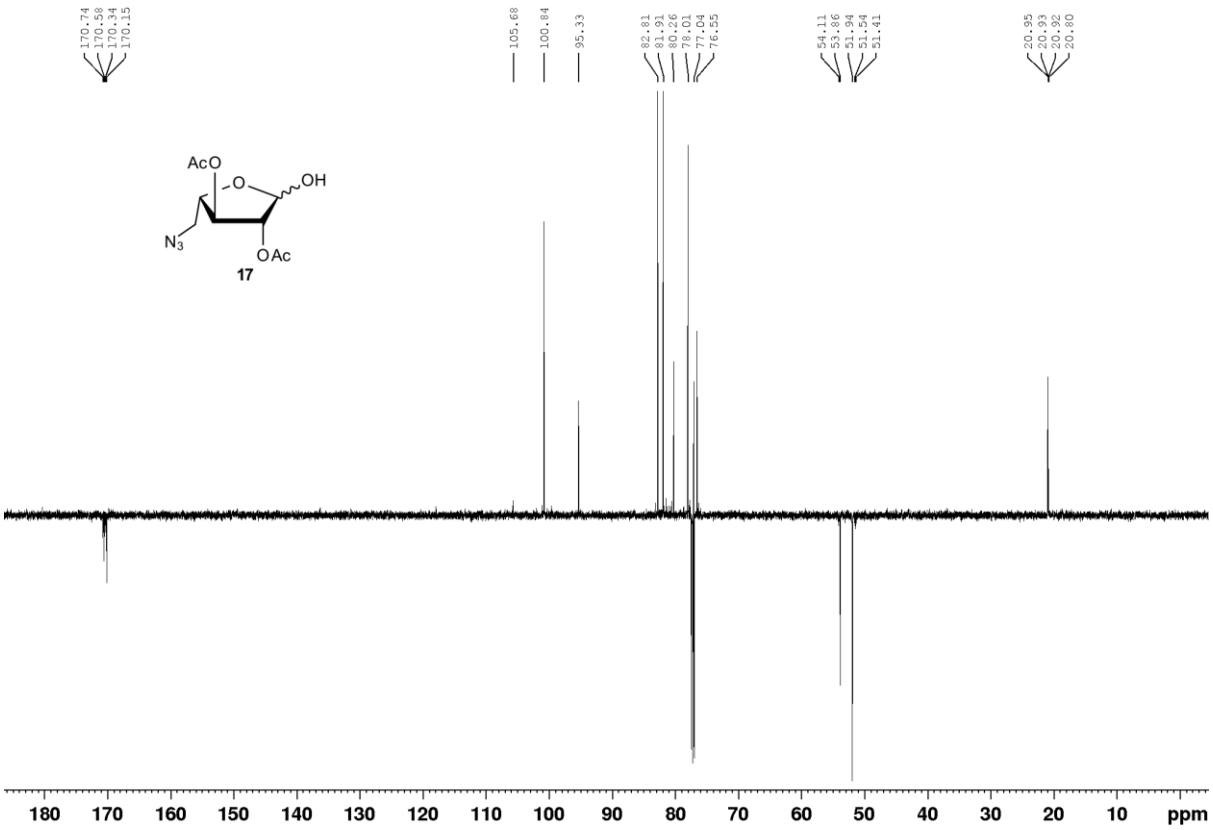
^{1}H NMR Spectrum of 16 in $CDCl_3$



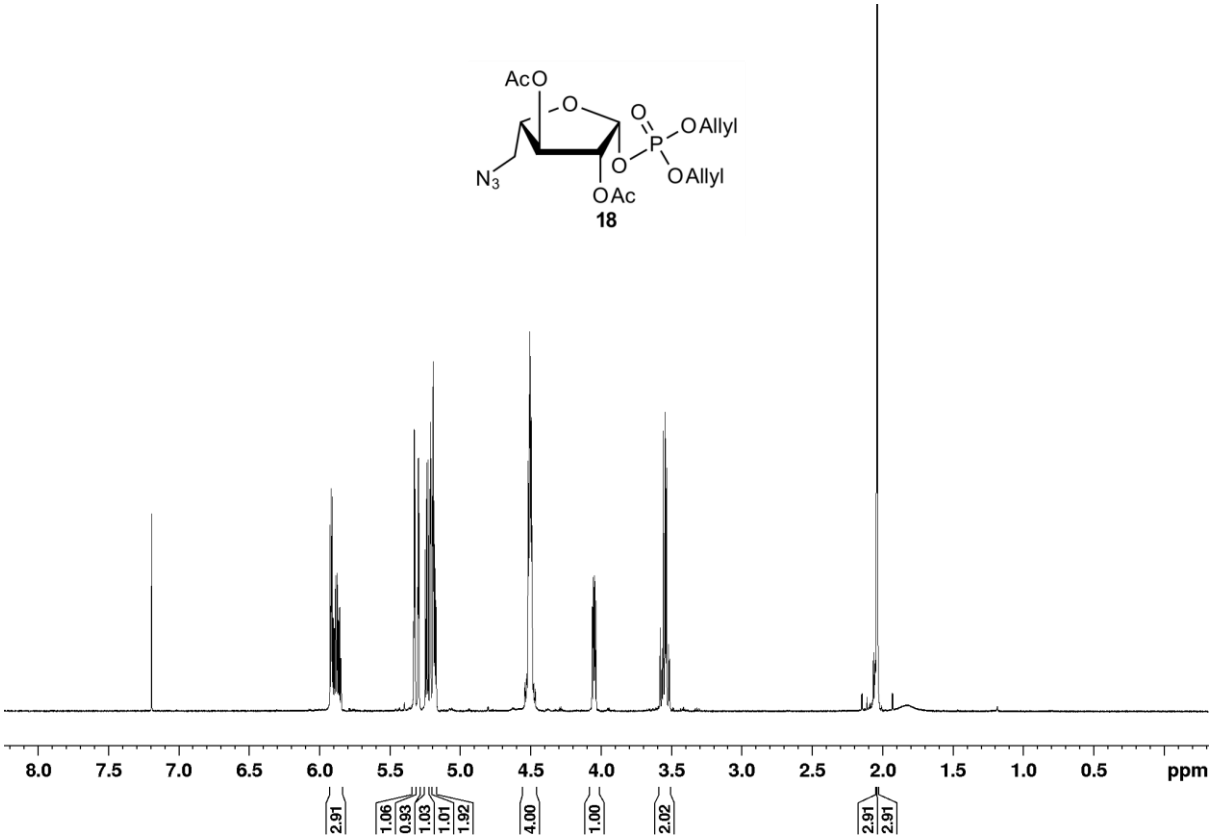
^{1}H NMR Spectrum of 17 in $CDCl_3$



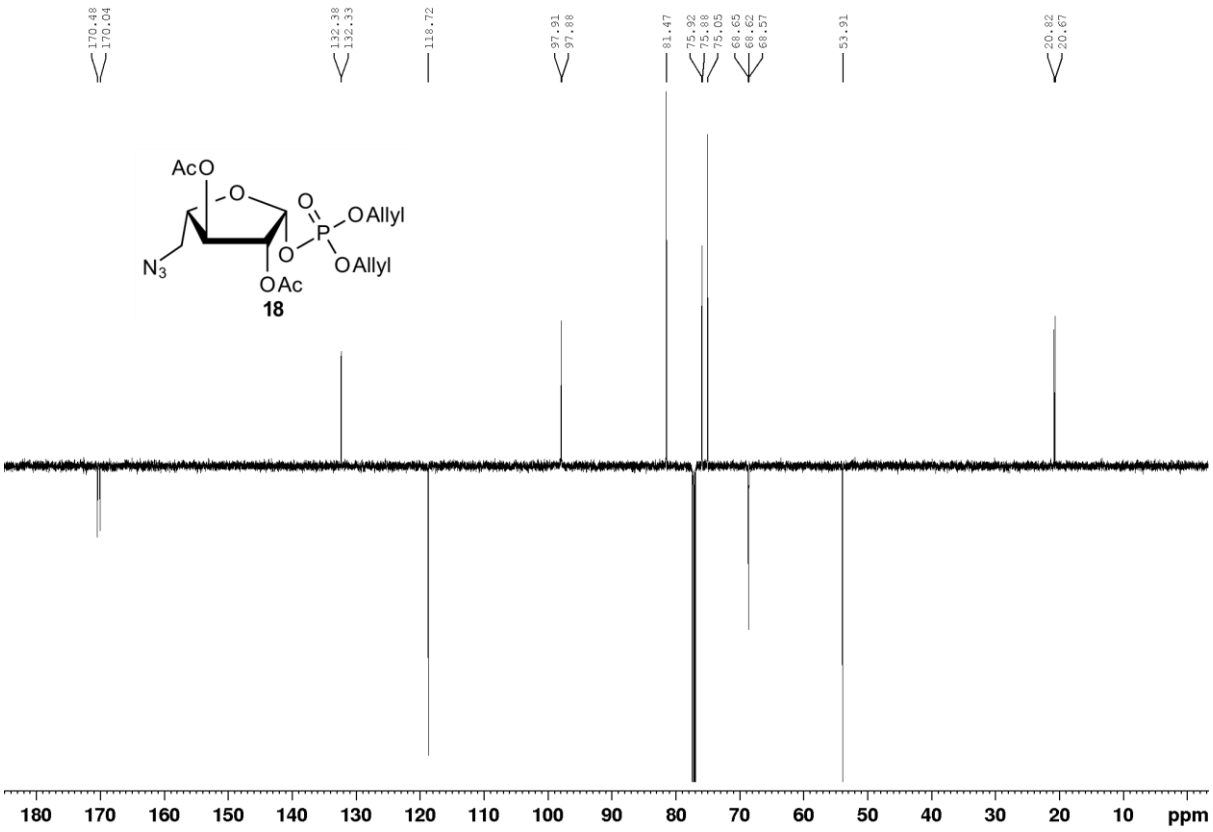
^{13}C APT NMR Spectrum of 17 in $CDCl_3$



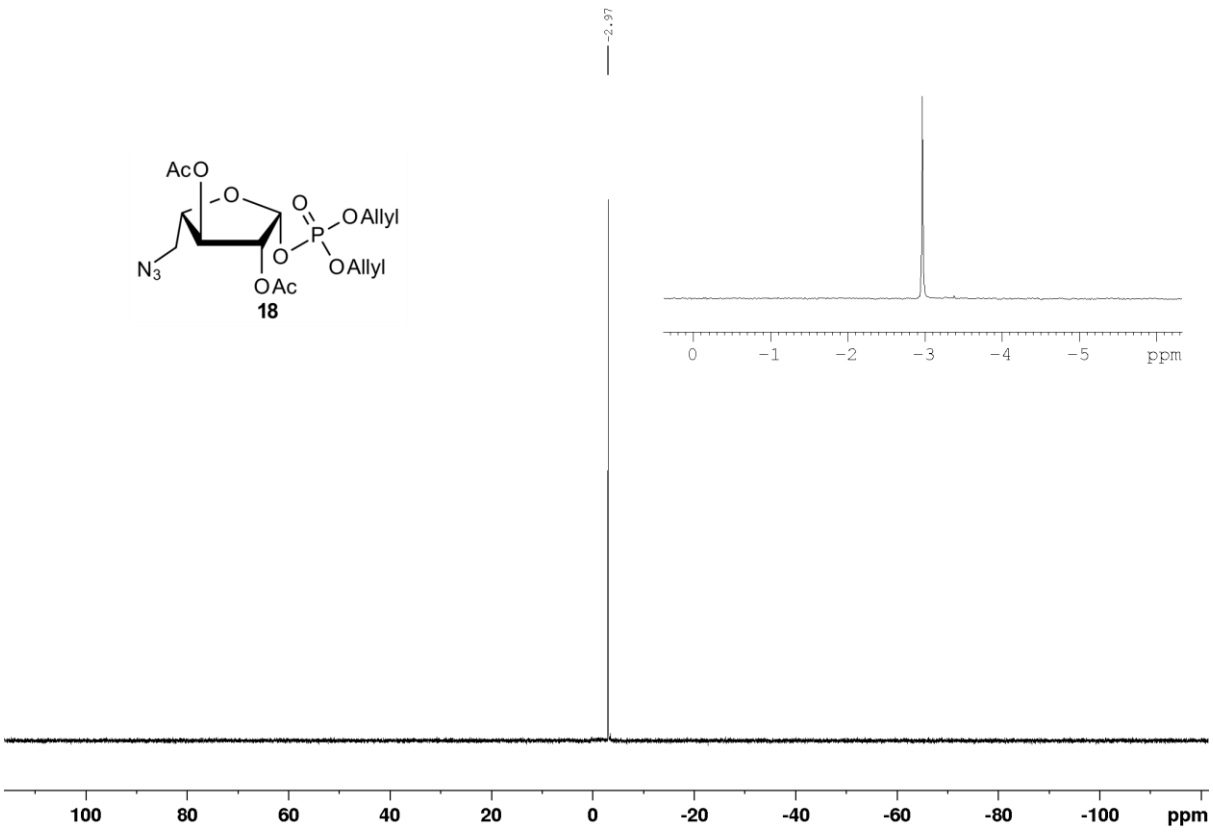
^{1}H NMR Spectrum of 18 in $CDCl_3$



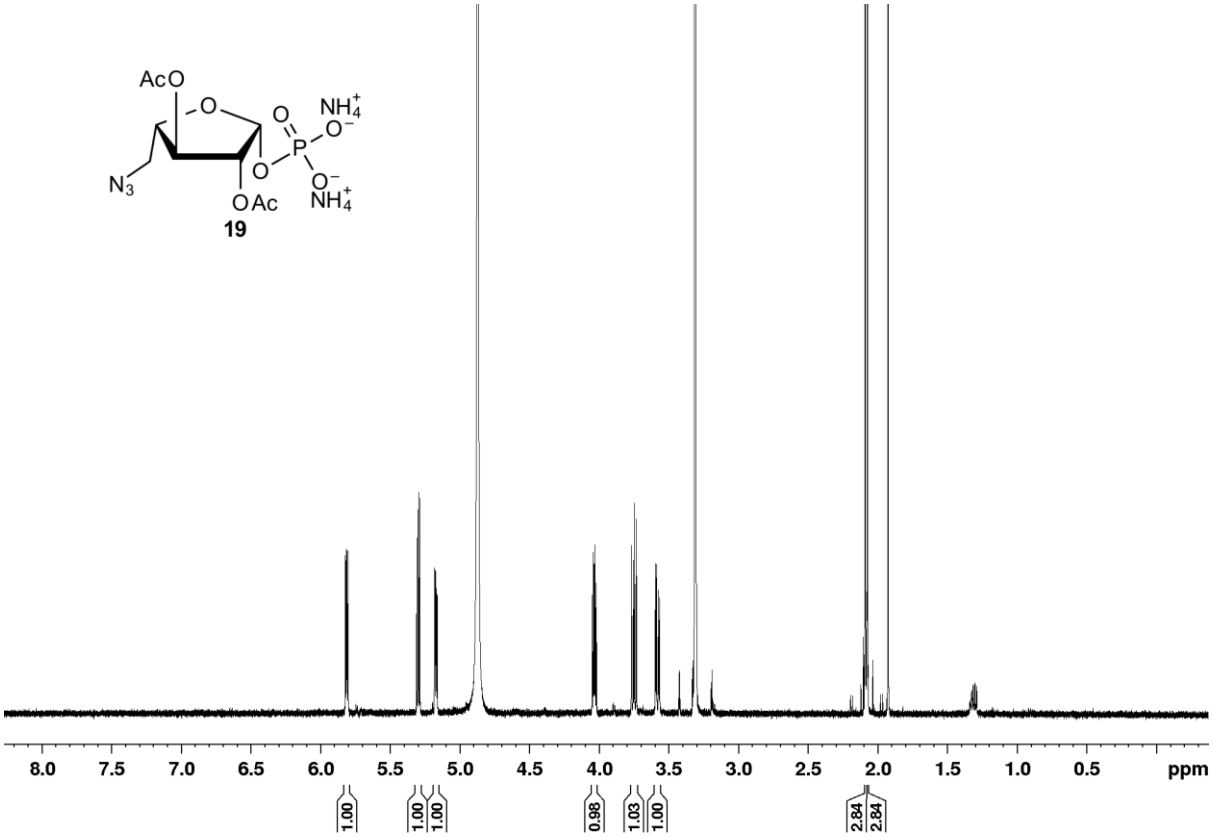
^{13}C APT NMR Spectrum of 18 in $CDCl_3$



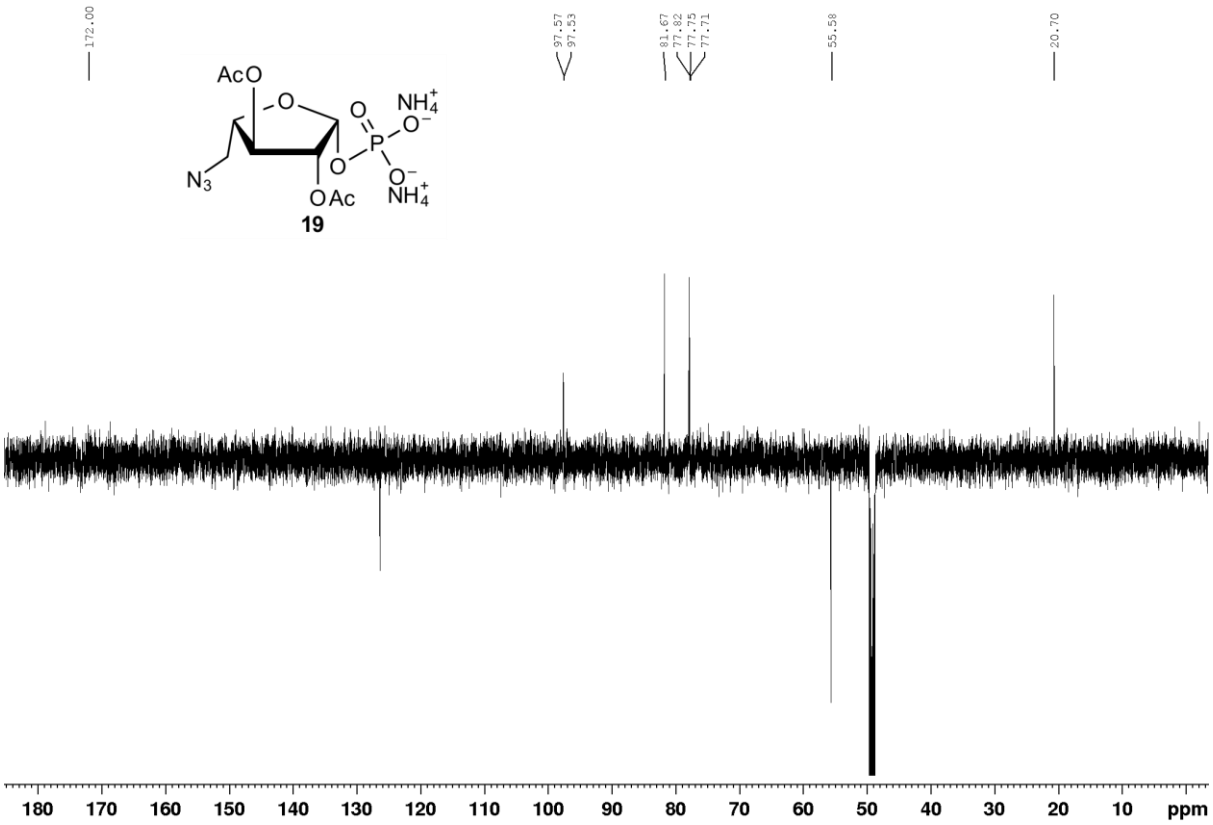
^{1}H-decoupled ^{31}P NMR Spectrum of 18 in $CDCl_3$



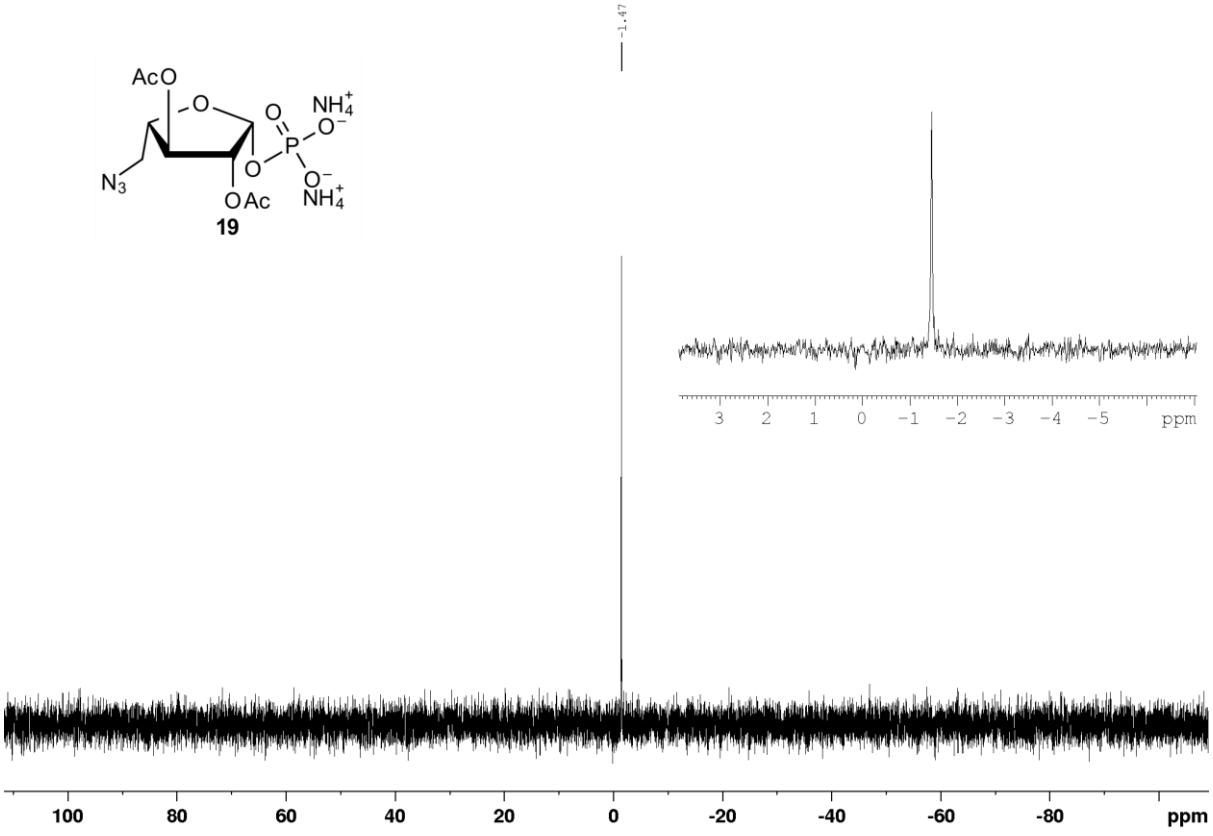
^{1}H NMR Spectrum of 19 in MeOD



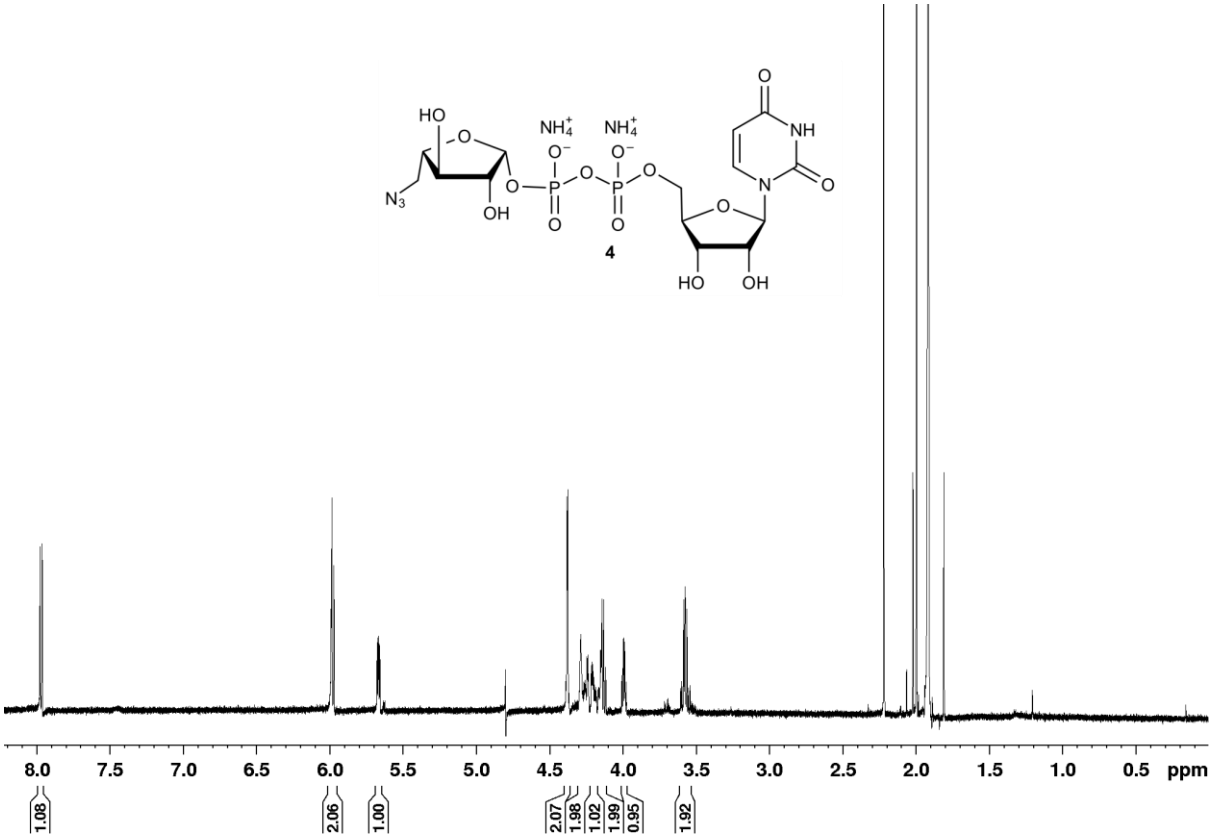
^{13}C APT NMR Spectrum of 19 in MeOD



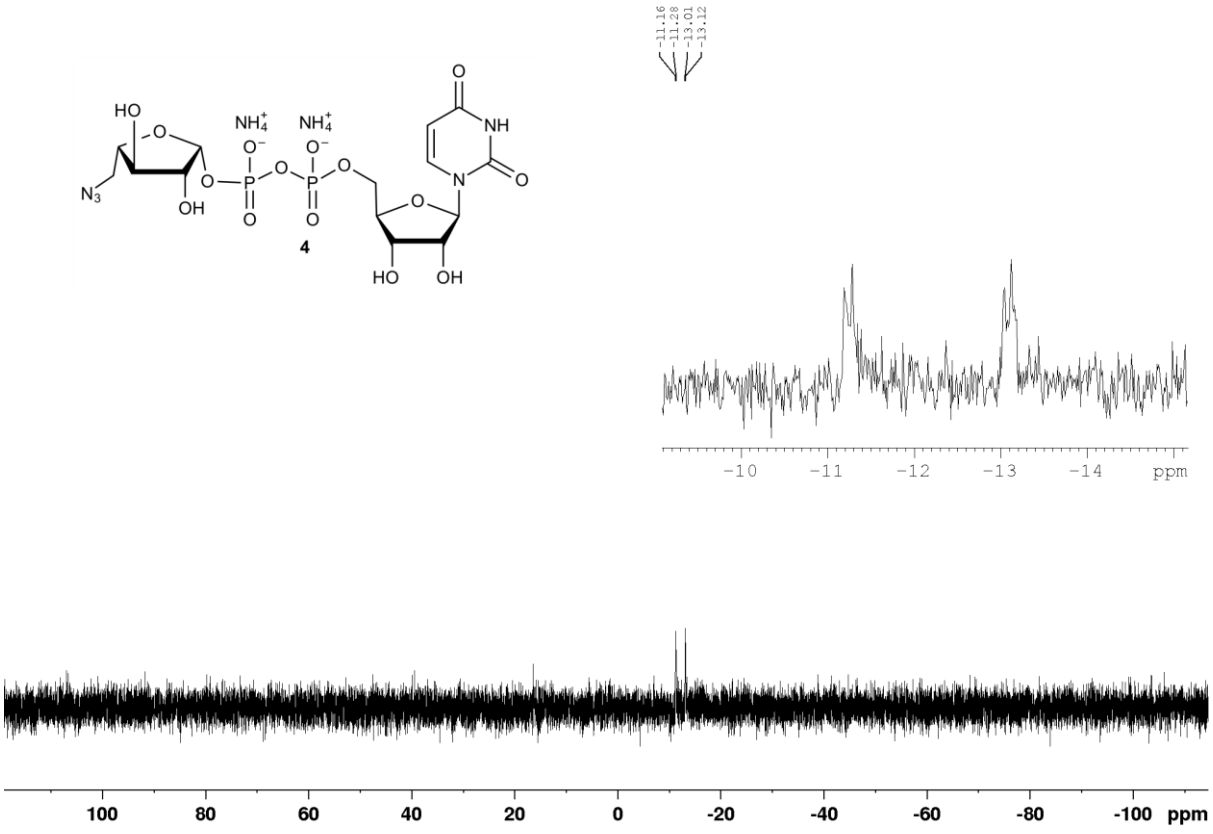
^{1}H-decoupled ^{31}P NMR Spectrum of 19 in MeOD



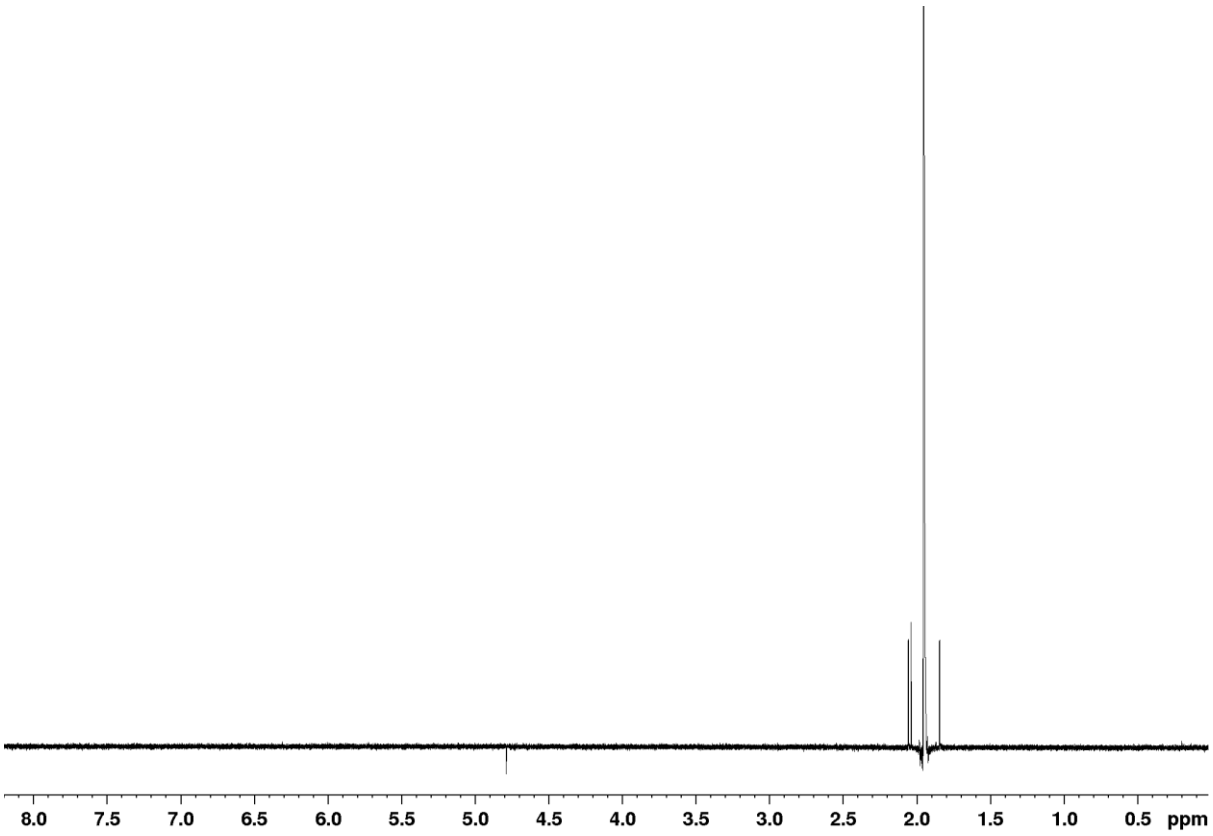
^{1}H NMR Spectrum of 4 in D_2O



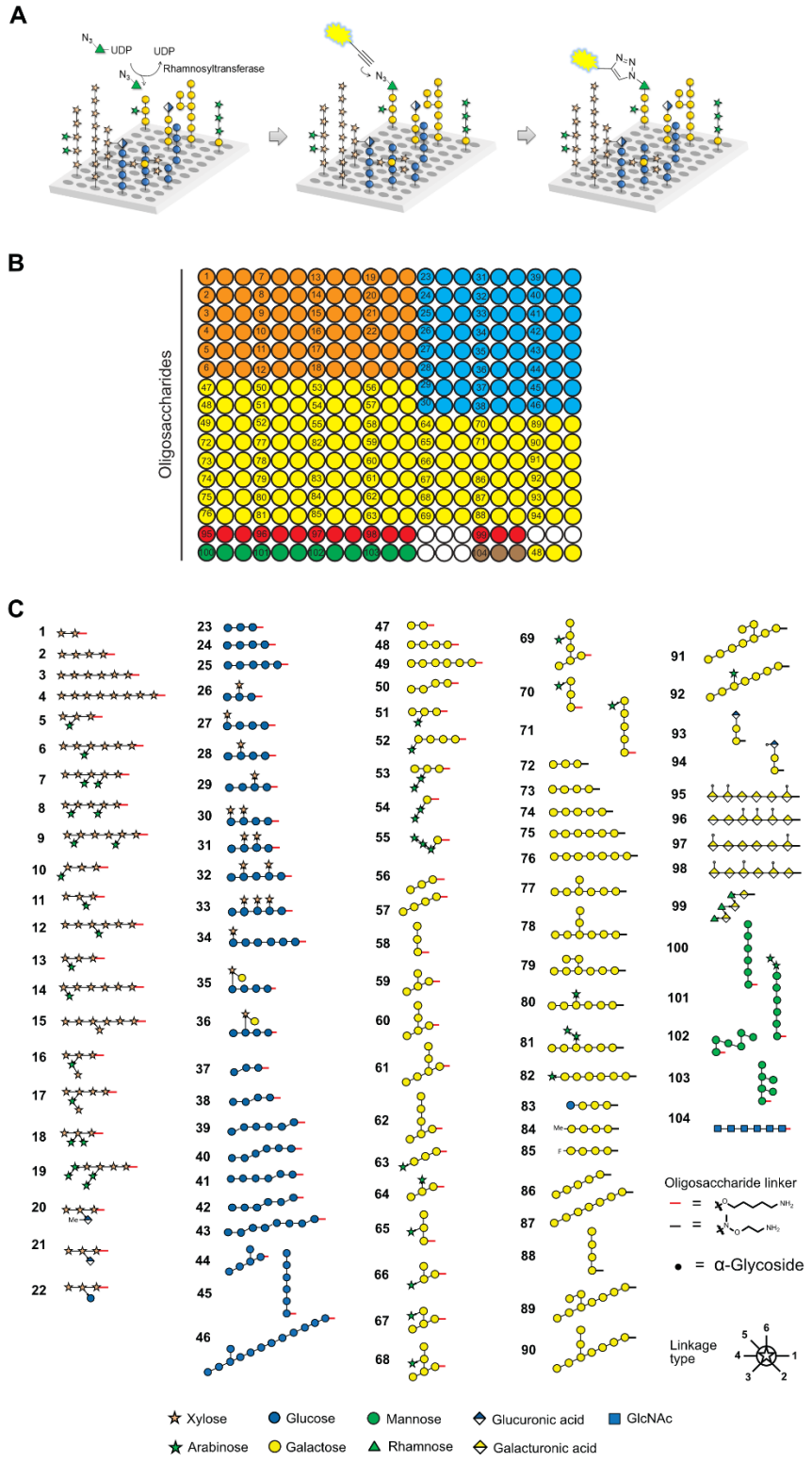
^{1}H-decoupled ^{31}P NMR Spectrum of 4 in D_2O



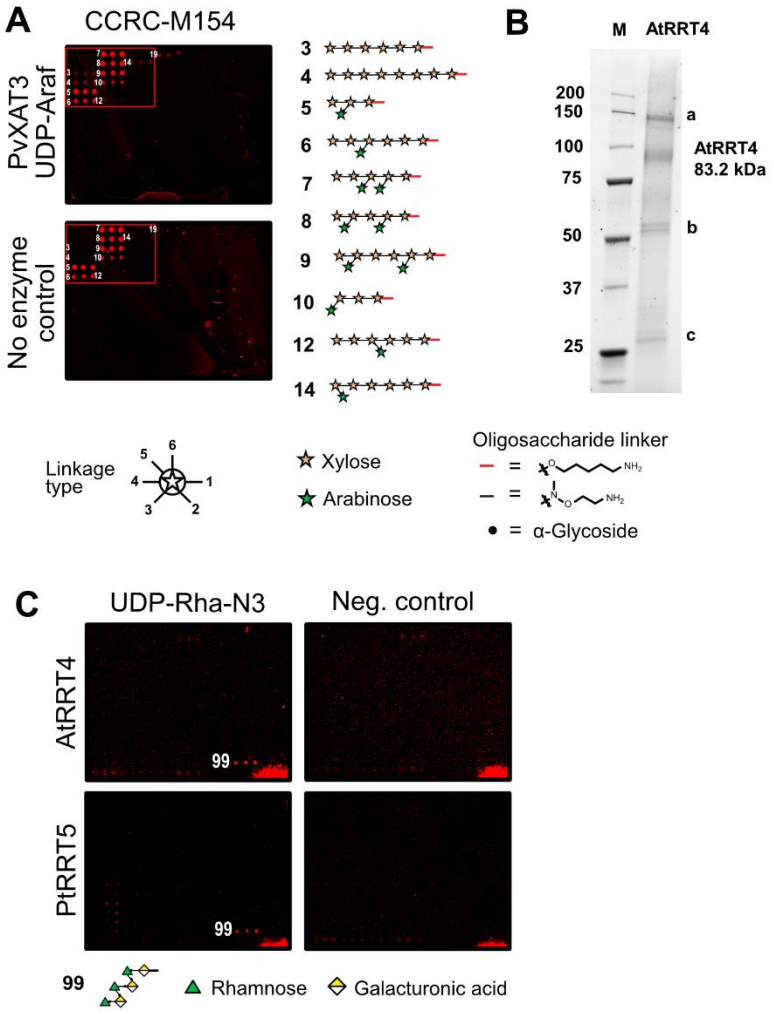
^{1}H spectrum of NH_4COCH_3 buffer



Supplementary Figures



Supplementary Figure 1. Plant GT glycan array platform; **A**. General principle of the GT array platform; **B**. Printing pattern of the array; **C**. Numbering of the oligosaccharides on the array.



Supplementary Figure 2. Glycan array-based analysis of plant cell wall-biosynthetic GTs; **A**. Activity of the arabinofuranosyltransferase *Pv*XAT3 on a glycan microarray using UDP-Ara*f*. Product detection was performed with the monoclonal antibody CCRC-M154. The two enlarged images shown in Figure 3 of the manuscript are indicated with a red box; **B**. SDS-PAGE analysis of purified *At*RRT4 enzyme. The enzyme is expressed as fusion proteins with a His-tag and a green fluorescent protein (GFP) at the N-terminus. The additional bands probably correspond to (a) aggregated *At*RRT4 protein as previously described for this enzyme (b) the *At*RRT4 alone (expected mass 51.8 kDa) and (c) the cleaved GFP-tag (expected mass 27.6 kDa). The linker region between *At*RRT4 and GFP is 3 kDa and might explain the double bands in (b) and (c); **C**. Activity of the rhamnosyltransferases *At*RRT4 and *Pt*RRT5 on a glycan microarray transferring azido-modified UDP-Rha. Note that the hexasaccharide 99 was elongated by a GalA on-chip using the galacturonic acid transferase RGGAT1 that was co-incubated in all four experiments. In the negative control the UDP-N3-Rha was omitted.

Protein sequences

The transmembrane domain was omitted for the expression of the glycosyltransferases. The protein sequences used for expression are given below.

PvXAT3_59-541

ptsilikqkvdsppatsrktatdalpggdprvvddeadvrpkgtkreeeesrvlsepdptsgmteltankdgggrksdeetlggegkgkqdg
eerghaaekhkvtlptvsnytihdsedtengkqddgtgsdlqgskplcdfsnfranvcemrgdvrvhpnatsimfmepagsqrdelwkik
pyprkgdefclshiteltvksskvapectkyhdvpavifsltgytgnlfhdftdvmvplfttasefngevqflitdmalwwtikyhtvlqklskypl
idfskdeqvhcfkhvivglhaymeftidsskaphnysmvdfnrfmrgayslprdtvtvlgeypkvkprlliikrhrtrmflnldeiiamaeelgf
evvtdeanvssdiskfarlvntvdvmmgvhgagltncvflpqnatliqivpwgglewvsrtdfgnpaelmgmhykqysisvdesslteqyp
rdheifknpiafhkhgfdfirqtfmdkqnvkldckrfrpillealdnlnp

AtRRT4_51-391

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DKTSFWADPSGFEDIFDVRHFIDSLRDEVRILRRLPKRFSRKYGYQMFEMPPVSWSDEKYYLKQVLPLFSKHKVVHF
NRTDTRLANNGLPLSLQWLRCRVNFQGLKFTPQLEALGSKLVRILQQRGPFVALHLRYEMDMLAFSGCTHGCTEE
EAEELKKMRYTYPWWREKEIVSEERRAQGLCPLTPEEVALVLKALGFEKNTQIYIAAGEIYGSEHRLSVLREAFPRIVK
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PtRRT5_54-514

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RGGAT1_78-581

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