Supplementary Information

Carba-sugars activate the glmS-riboswitch of *Staphylococcus aureus*

Christina E. Lünse[†], Magnus Schmidt[‡], Valentin Wittmann[‡,*], and Günter Mayer[†,*]

Materials and Methods

Primer:
S. aureus glmS:
5'+T7_glmS_Sau_long
TCG TAA TAC GAC TCA CTA TAG GTA ATG ATT AAT GGA AAG GGG G
5' Sau GlmS+T7
GAT AAT ACG ACT CAC TAT AGG GCA GTT AAA GCG CCT GTG CAA ATA
3' Sau glmS neu
ATC TTA TTA ACT TTG TCC ATT AAG TCA CCC

B. subtilis glmS:
glmS_40FL
TCG TAA TAC GAC TCA CTA TAG GTC TTG TTC TTA TTT TCT C
3'glmS_25FL
GTC CCC TTC CTA CAT GTT TTT TGG A

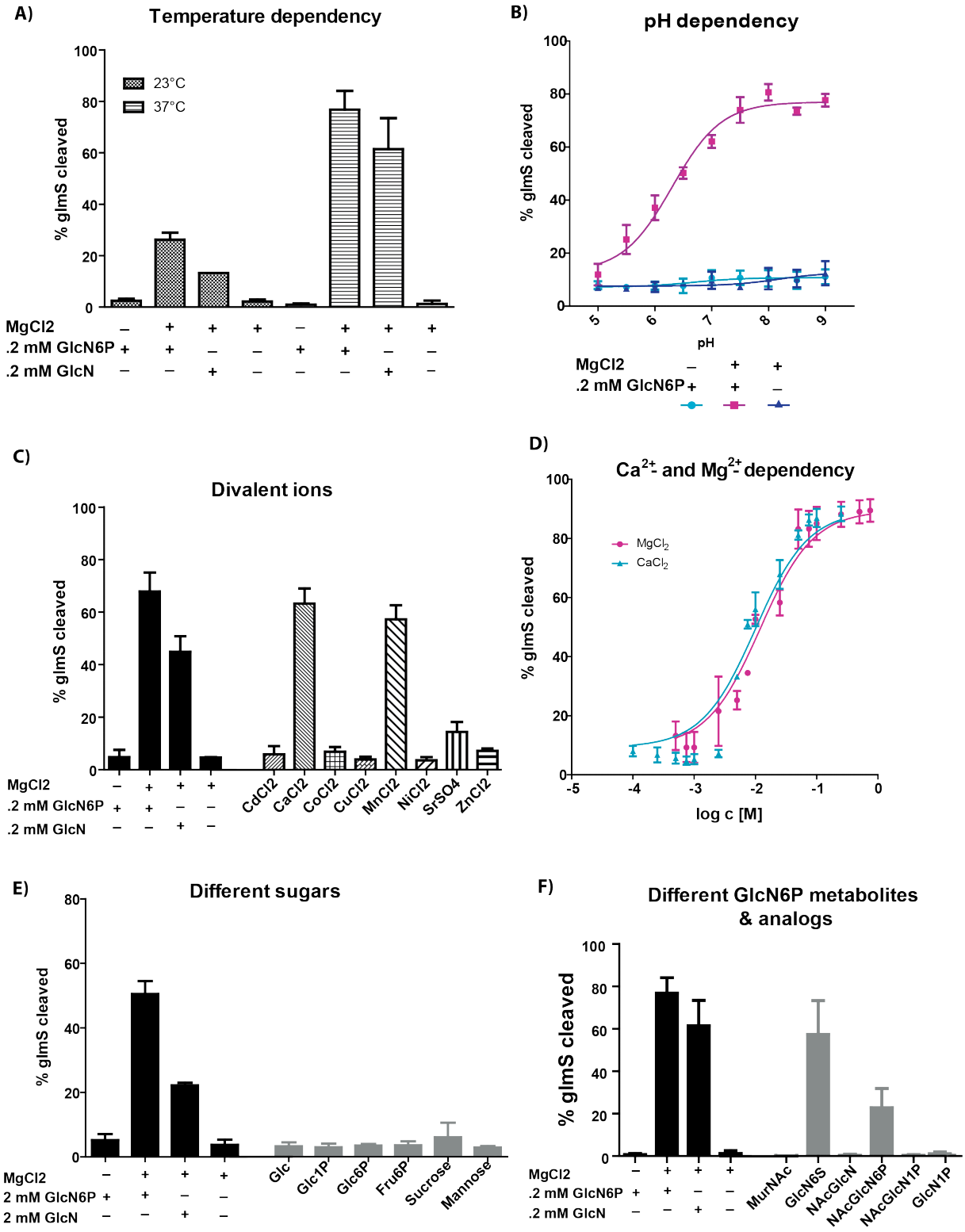


Figure S1: Biochemical characterization of the glmS-riboswitch of *Staphylococcus aureus* Mu50. A) Influence of reaction temperature on GlcN6P-induced glmS-riboswitch self-cleavage. Metabolite-induced self-cleavage (MISC) assay performed at 23°C and 37°C. **B)** pH-dependency of GlcN6P-induced glmS self-cleavage assayed by MISC. Cleavage occurred optimally at pH-value of ~7. At higher pH-values unspecific RNA cleavage occurs more frequently (controls w/o $MgCl_2$, or w/o GlcN6P). **C)** Influence of divalent metal-ions on

glmS-riboswitch cleavage. Ca^{2+}-, Mn^{2+}-, and Sr^{2+}-ions are capable of inducing glmS-riboswitch self-cleavage. **D)** GlcN6P-induced glmS-riboswitch self-cleavage is dependent on the concentration of divalent ions. Self-cleavage of the glmS-riboswitch in the presence of increasing concentrations of either $MgCl_2$ (magenta) or $CaCl_2$ (cyan). **E)** Influence of different carbohydrates on glmS-riboswitch self-cleavage. None of the tested compounds was able to induce cleavage of the glmS-riboswitch of *S. aureus* Mu50. **F)** MISC assay of GlcN6P-analogues [0.2 mM] and metabolites [0.2 mM] involved in GlcN6P biosynthesis. Glucosamin-6-sulfate and N-Acetyl-Glucosamin-6-phosphate are able to induce glmS-riboswitch self-cleavage.

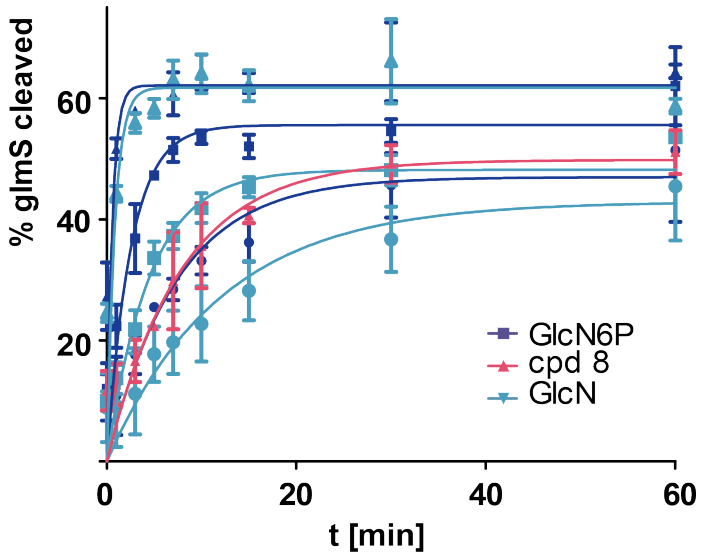


Figure S2 Cleavage rates of the glmS-riboswitch of *B. subtilis* at different concentrations [circles 2µM; squares 20µM; triangles 200µM] of GlcN6P (blue), compound 8 (cyan), and GlcN (magenta). Plots of the fraction cleaved as a function of time are shown. Experiments were done in triplicates.

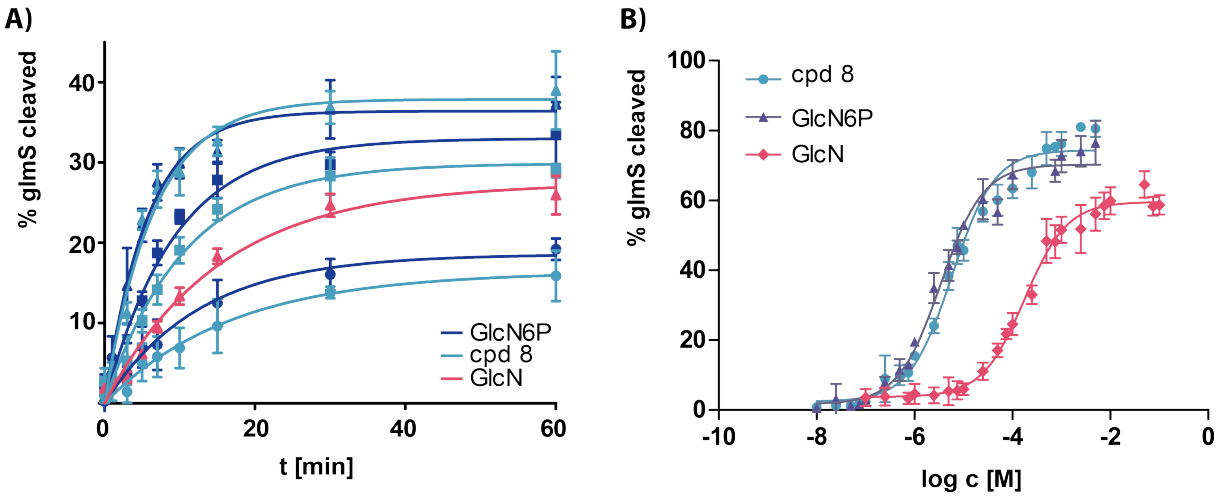


Figure S3: A) Rates of cleavage as shown in Fig. 3B including error bars. B) Concentration dependent cleavage of the *S. aureus* glmS-riboswitch induced by compound 8, glucosamine-6-phosphate (GlcN6P) and glucosamine (GlcN). All assays were performed in triplicates.

Synthesis of GlcN6P Analogs 1–8

General Methods

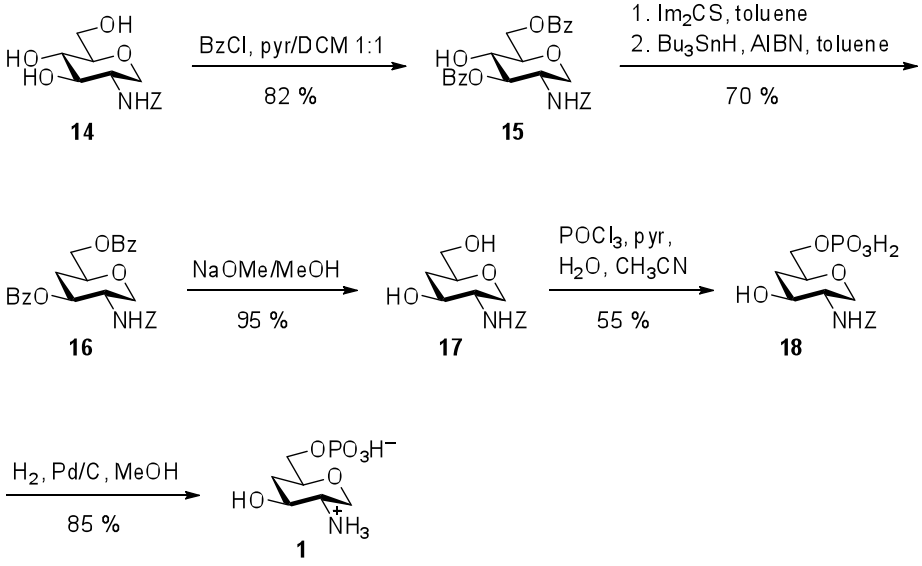
TLC was carried out on Silica Gel 60 F254 (Merck, layer thickness 0.2 mm) with detection by UV light (254 nm) and/or by charring with 15 % sulfuric acid in ethanol. Flash column chromatography (FC) was performed on Merck Silica Gel 60 (0.040–0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 and Bruker Avance DRX 600 instruments. Chemical shifts are reported in ppm relative to solvent signals ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; DMSO-d_6: δ_H = 2.49 ppm, δ_C = 39.7 ppm; CD_3OD: δ_H = 4.78 ppm, δ_C = 49.3 ppm). Signals were assigned by first-order analysis and, when feasible, assignments were supported by two-dimensional ^{1}H, ^{1}H and ^{1}H, ^{13}C correlation spectroscopy. $^{3}J_{H–H}$ and $^{1}J_{H–C}$ coupling constants are reported in Hz. ESI-IT mass spectra were recorded on a Bruker Esquire 3000 spectrometer. Elemental analysis was performed on an elementar CHNS vario EL instrument. RP-HPLC was performed on a LC-20A prominence system from Shimadzu. Used columns: Nucleosil 100-5 C-18 (analytical: 4 x 250 mm, flow 0.9 mL min^{-1}; semi-preparative: 16 x 250 mm, flow 9.6 mL min^{-1}) from Knauer. Eluent: gradient of water with 0.1 % TFA (eluent A) and acetonitrile with 0.1 % TFA (eluent B).

General Procedure 1: Radical Deoxygenation of Alcohols

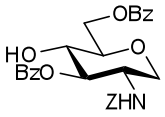
A) Under a nitrogen atmosphere the alcohol is dissolved together with 1.2-1.5 equiv. of 1,1-thiocarbonyl diimidazole in dry toluene (10 mL/mmol) and refluxed for 2-4 h. Bu_3SnH (2 equiv.) and AIBN (0.1 equiv.) are added an the mixture is stirred for additional 10-30 min under reflux. After evaporation of the solvent, the residue is dissolved in acetonitrile and washed with petroleum ether to remove the tin compounds. The product is purified by FC.

B) Under a nitrogen atmosphere the alcohol is dissolved together with 1.2-1.5 equiv. of phenyloxythiocarbonyl chloride in dry acetonitrile (10 mL/mmol) and refluxed for 8-16 h. The mixture is diluted with dichloromethane and washed with sat. $NaHCO_3$ solution and brine. The organic layer is dried (Na_2SO_4) and the solvent is evaporated. The remainder is dissolved in dry toluene (10 mL/mmol). Under a nitrogen atmosphere, Bu_3SnH (2 equiv.) and AIBN (0.1 equiv.) are added, and the mixture is stirred for 10-30 min under reflux. After evaporation of the solvent, the remainder is dissolved in acetonitrile and washed with petroleum ether to remove the tin compounds. The product is purified by FC.

Synthesis of GlcN6P analog 1



1,5-Anhydro-3,6-di-*O*-benzoyl-2-benzyloxycarbonylamino-2-deoxy-D-glucitol 15

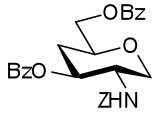


$C_{28}H_{27}NO_8$

505,52

Compound **14**[i] (800 mg, 2.7 mmol) was dissolved in dichloromethane/pyridine (1:1, 10 mL) under a nitrogen atmosphere and cooled to –20°C. Benzoyl chloride (690 µL, 5.9 mmol) was added slowly at –20 °C and the mixture was stirred for 1.5 h at rt. The reaction was quenched with MeOH (2 mL) and evaporated. Purification by FC (petroleum ether/EtOAc 5:2) yielded compound **15** (1.1 g, 82 %) as a colorless solid. R_f = 0.38 (petroleum ether-EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 8.09-8.04 (m, 5 H, Ph), 7.61-7.17 (m, 10 H, Ph),5.25(d, J = 8.4, 1 H, NH), 5.17 ('t', J = 9.6, 1 H, H-3), 4.98 (m, 2 H, $PhCH_2$), 4.72 (dd, J = 12.2, 4.1, 1 H, H-6a), 4.58 (dd, J = 12.2, 1.6, 1 H, H-6b), 4.19 (dd, J = 11.1, 5.0, 1 H, H-1a), 4.09 (m, 1 H, H-2), 3.79 ('t', J = 9.5, 1 H, H-4), 3.60 (ddd, J = 9.7, 4.3, 2.1, 1 H, H-5), 3.31 ('t', J = 11.1, 1 H, H-1b); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 167.9 (C(O)Ph), 167.2 (C(O)Ph), 155.9 ($PhCH_2OC(O)NH$), 136.1 (quaternary C), 133.5 (quaternary C), 133.3 (quaternary C), 130.1-127.8 (aromatic C), 79.0 (C-5), 77.5 (C-3), 69.1 (C-4), 68.8 (C-1), 66.8 ($PhCH_2$), 63.7 (C-6), 51.6 (C-2); (ESI-IT-MS): *m/z* $[M+Na]^+$: 528.2, $[M+K]^+$: 544.1; Anal. Calcd for $C_{28}H_{27}NO_8$: C, 66.53; H, 5.38; N, 2.77; Found: C, 66.47; H, 5.30; N, 2.78.

1,5-Anhydro-3,6-di-*O*-benzoyl-2-benzyloxycarbonylamino-2,4-dideoxy-D-*xylo*-hexitol 16



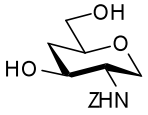
$C_{28}H_{27}NO_7$

489,52

Compound **15** was deoxygenated according to General Procedure 1A to yield **16** in 70 % yield. R_f = 0.49 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 8.07-8.02 (m, 5 H, Ph), 7.60-7.20 (m, 10 H, Ph), 5.11 (d't', J = 10.6, 4.6, 1 H, H-3), 5.02 (m, 2 H, $PhCH_2$), 4.94 (d, J = 7.4, 1 H, NH), 4.40 (m, 2 H, H-6a, H-6b), 4.27 (dd, J = 10.9, 4.4, 1 H, H-1a), 4.02 (m, 1H, H-2), 3.87 (m, 1 H-5), 3.25 ('t', J = 11.1, 1 H, H-1b), 2.28 (ddd, J = 12.7, 5.1, 2.1, 1 H, H-4a), 1.84 ('q', J = 11.7, 1 H, H-4b); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 166.8 (C(O)Ph), 166.3 (C(O)Ph), 155.9 ($PhCH_2OC(O)NH$), 136.2 (quaternary C), 133.4

(quaternary C), 133.2 (quaternary C), 130.0-127.8 (aromatic C), 74.1 (C-5), 72.3 (C-3), 68.9 (C-1), 66.6 (Ph<u>C</u>H$_2$), 66.2 (C-6), 52.0 (C-2), 33.3 (C-4); (ESI-IT-MS): *m/z* $[M+Na]^+$: 512.2, $[M+K]^+$: 528.1; Anal. Calcd for $C_{28}H_{27}NO_7$: C, 68.70; H, 5.56; N, 2.86; Found: C, 66.69; H, 5.60; N, 2.89.

1,5-Anhydro-2-benzyloxycarbonylamino-2,4-dideoxy-D-*xylo*-hexitol 17

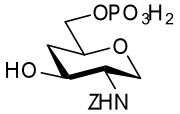


$C_{14}H_{19}NO_5$

281,3

To a solution of **16** (555 mg, 1.14 mmol) in MeOH (8 mL) was added a solution of sodium methylate (0.5 M in MeOH, 0.15 equiv). The mixture was stirred for 12 h at rt. After neutralization with acidic ion exchanger (DOWEX 50 W X8, H+ form), the mixture was filtered and lyophilized to yield **17** (300 mg, 95 %). R_f = 0.17 (EtOAc); ^{1}H NMR (600.1 MHz, DMSO-d$_6$): δ = 7.38-7.30 (m, 5 H, Ph), 7.09 (d, J = 8.0, 1 H, NH), 5.01 (m, 2 H, PhCH$_2$), 4.82 (br s, 1 H, OH), 4.63 (br. s, 1 H, OH), 3.76 (dd, J = 10.9, 5.0, 1 H, H-1a), 3.75 (m, 1 H, H-3), 3.35 (m, 1 H, H-6a), 3.29 (m, 1 H, H-6b), 3.24 (m, 1 H, H-5), 3.20 (m, 1 H, H-2), 2.92 ('t', J = 10.6, 1 H, H-1b), 1.86 (ddd, J = 12.8, 4.8, 1.6, 1 H, H-4a), 1.84 ('q', J = 11.9, 1 H, H-4b); ^{13}C NMR (150.9 MHz, DMSO-d$_6$): δ = 156.0 (PhCH$_2$O<u>C</u>(O)NH), 137.1 (quaternary C), 128.3-127.8 (aromatic C), 114.4 (C-5), 97.4 (C-3), 96.2 (C-1), 90.6 (<u>C</u>H$_2$), 88.0 (C-6), 69.2 (C-2), 33.7 (C-4); (ESI-IT-MS): *m/z* $[M+Na]^+$: 303.9, $[M+K]^+$: 319.9; Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98; Found: C, 59.68; H, 6.89; N, 4.93.

1,5-Anhydro-2-benzyloxycarbonylamino-2,4-dideoxy-D-*xylo*-hexitol 6-Phosphate 18

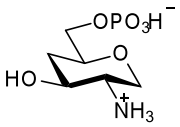


$C_{14}H_{20}NO_8P$

361,28

$POCl_3$ (186 μL, 2 mmol), H_2O (23 μL, 1.3 mmol), and pyridine (178 μL, 2.2 mmol) were dissolved in acetonitrile (2 mL) and stirred for 10 min at 0 °C. Compound **17** (130 mg, 0.46 mmol) was added and the mixture was stirred for 3 h at 0 °C. Water (3 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 5–65 % in 30 min) followed by lyophilization yielded compound **18** (90 mg, 55 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 14.9 min (5–65 % B in 30 min); ^{1}H NMR (600.1 MHz, D_2O): δ = 7.48-7.42 (m, 5 H, Ph), 5.19-5.12 (m, 2 H, CH_2), 3.99-3.96 (m, 2 H, H-1a, H-6a), 3.90 (m, 1 H, H-6b), 3.79-3.73 (m, 2 H, H-3, H-5), 3.50 (m, 1 H, H-2), 3.25 ('t', J = 11.3, 1 H, H-1b), 2.09 (dd, J = 12.8, 4.0, 1 H, H-4a), 1.51 ('q', J = 12.0, 1 H, H-4b); ^{13}C NMR (150.9 MHz, D_2O): δ = 159.3 (PhCH$_2$O<u>C</u>(O)NH), 137.4 (quaternary C), 129.8-128.7 (aromatic C), 76.6 (C-5), 70.4 (C-3), 68.7 (C-6), 68.5 (C-1), 68.1 (Ph<u>C</u>H$_2$), 54.7 (C-2), 35.7 (C-4); ^{31}P NMR (161.9 MHz, D_2O): δ = 3.75 (s, 1 P); (ESI-IT-MS): *m/z* $[M-H]^-$: 359.8; Anal. Calcd for $C_{14}H_{20}NO_8P$: C, 46.54; H, 5.58; N, 3.88; Found: C, 45.65; H, 5.47; N, 3.75.

1,5-Anhydro-2-amino-2,4-dideoxy-D-*xylo*-hexitol 6-Phosphate 1



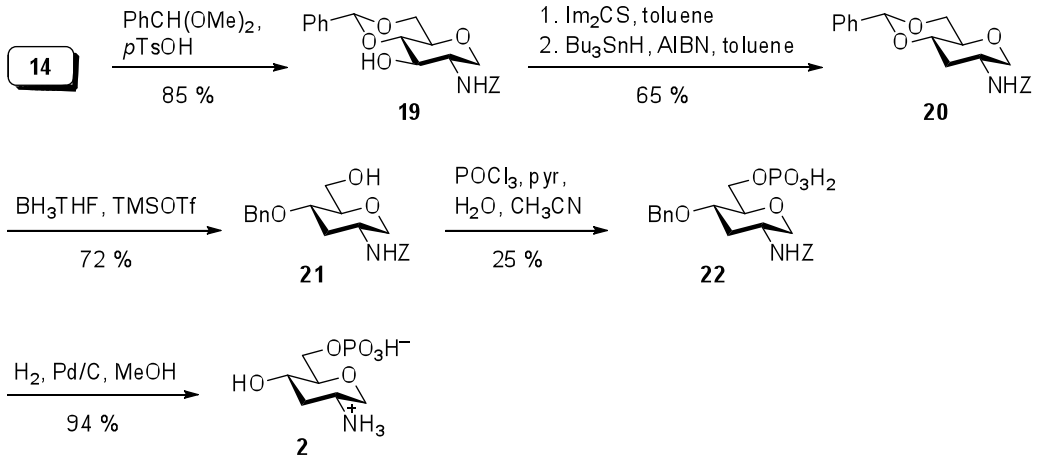
$C_6H_{14}NO_6P$

227,15

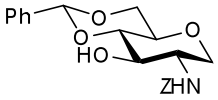
To a solution of **18** (60 mg, 0.17 mmol) in MeOH (3 mL) was added 10 % Pd on carbon (15 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, **1** was obtained as a colorless solid (34 mg, 85 %). ^{1}H

NMR (600.1 MHz, D_2O): δ = 4.23 (dd, J = 11.4, 5.0, 1 H, H-1a), 3.98-3.86 (m, 3 H, H-6a, H-6b, H-3), , 3.83 (ddd, J = 10.9, 5.8, 2.2, 1 H, H-5), 3.53 ('t', J = 11.6, 1 H, H-1b), 3.21 (d't', J = 10.5, 5.0, 1 H, H-2), 2.16 (ddd, J = 13.0, 4.9, 1.7, 1 H, H-4a), 1.59 ('q', J = 12.5, 1 H, H-4b); ^{13}C NMR (150.9 MHz, D_2O): δ = 76.9 (C-5), 79.0 (C-3), 67.8 (C-6), 66.6 (C-1), 53.6 (C-2), 35.5 (C-4); ^{31}P NMR (161.9 MHz, D_2O): δ = 2.55 (s, 1 P); (ESI-IT-MS): *m/z* $[M+H]^-$: 225.9; Anal. Calcd for $C_6H_{14}NO_6P$: C, 31.73; H, 6.21; N, 6.17; Found: C, 31.72; H, 6.01; N, 5.84.

Synthesis of GlcN6P analog 2



1,5-Anhydro-4,6-*O*-benzyliden-2-benzyloxycarbonylamino-2-deoxy-D-glucitol 19

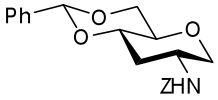


$C_{21}H_{23}NO_6$

385,41

Compound **14**[i] (1 g, 3.36 mmol) was dissolved in dioxane (20 mL) and benzaldehyde dimethyl acetal (1.23 mL, 8.4 mmol) and *p*TsOH (65 mg, 0.34 mmol) were added. The mixture was stirred for 4 h at rt and the solvent was evaporated. Crystallization from EtOAc gave **19** (1.1 g, 85 %) as a colorless solid. R_f = 0.46 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.48-7.29 (m, 10 H, Ph), 7.11 (d, J = 7.8, 1 H, NH), 5.71 (s, 1 H, PhCH), 5.28 (d, J = 4.9, 1 H, OH), 5.02 (m, 2 H, $PhCH_2$), 4.16 (dd, J = 10.3, 4.9, 1 H, H-6a), 3.80 (dd, J = 11.4, 5.1, 1 H, H-1a), 3.66 ('t', J = 10.2, 1 H, H-6b), 3.54-3.47 (m, 2 H, H-3, H-2), 3.42 ('t', J = 8.9, 1 H, H-4), 3.24 (d't', J = 9.6, 4.9, 1 H, H-5), 3.17 ('t', J = 11.0, 1 H, H-1b); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 156.0 ($PhCH_2O\underline{C}(O)NH$), 137.8 (quaternary C), 136.9 (quaternary C), 128.8-125.5 (aromatic C), 100.7 (Ph$\underline{C}$H), 81.8 (C-4), 71.2 (C-5), 70.9 (C-3), 68.5 (C-1), 67.8 (C-6), 65.4 (Ph$\underline{C}H_2$), 53.5 (C-2)**;** (ESI-IT-MS): *m/z* $[M+Na]^+$: 408.3, $[M+K]^+$: 424.2.

1,5-Anhydro-4,6-*O*-benzyliden-2-benzyloxycarbonylamino-2,3-dideoxy-D-*ribo*-hexitol 20



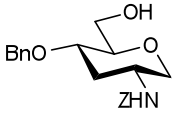
$C_{21}H_{23}NO_5$

369,41

Compound **19** was deoxygenated according to General Procedure 1B to yield **20** in 65 % yield. R_f = 0.21 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.39-7.32 (m, 10 H, Ph), 5.54 (s, 1 H, PhCH), 5.11 (m, 2 H, $PhCH_2$), 4.30 (dd, J = 10.8, 4.8, 1 H, H-6a), 4.14 (dd, J = 10.5, 4.9, 1 H, H-1a), 3.95 (m, 1 H, H-2), 3.70 ('t', 1 H, J = 10.0, H-6b), 3.61

(m, 1 H, H-4), 3.27 (d't', J = 9.4, 4.9, 1 H, H-5), 3.10 ('t', J = 10.8, 1 H, H-1b), 2.43 (m, 1 H, H-3a), 1.43 ('q', 1 H, J = 11.8, 1 H, H-3b); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 155.5 ($PhCH_2O\underline{C}(O)NH$), 137.3 (quaternary C), 136.1 (quaternary C), 129.1-126.1 (aromatic C), 101.7 (Ph<u>C</u>H), 77.2 (C-4), 73.5 (C-5), 71.0 (C-1), 69.2 (C-6), 67.0 (Ph<u>C</u>H_2), 46.7 (C-2), 35.7 (C-3); (ESI-IT-MS): *m/z* $[M+Na]^+$: 392.0, $[M+K]^+$: 407.9.

1,5-Anhydro-4-*O*-benzyl-2-benzyloxycarbonylamino-2,3-dideoxy-D-*ribo*-hexitol 21

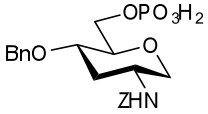


$C_{21}H_{25}NO_5$

371,43

Compound **20** (360 mg, 0.97 mmol) was dissolved in a 1 M solution of BH_3 in THF (10 mL) at 0 °C. TMSOTf (180 µL, 0.97 mmol) was added and the mixture was stirred for 45 min at rt. After addition of NEt_3 (0.5 mL) and MeOH (1 mL), the solvent was evaporated. Purification by FC yielded **21** (260 mg, 72 %) as a colorless solid. R_f = 0.26 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.38-7.29 (m, 10 H, Ph), 5.09 (m, 2 H, CH_2^Z), 4.62 (d, J = 11.3, 1 H, ½ CH_2^{Bn}), 4.49 (d, J = 11.3, 1 H, ½ CH_2^{Bn}), 4.07 (dd, J = 10.5, 4.3, 1 H, H-6a), 4.14 (ddd, J = 11.5, 6.0, 3.0, 1 H, H-1a), 3.78 (m, 1 H, H-2), 3.69 (m, 1 H, H-6b), 3.47 (d't', J = 9.7, 4.1, 1 H, H-4), 3.24 (m, 1 H, H-5), 3.076 ('t', J = 11.0, 1 H, H-1b), 2.54 (m, 1 H, H-3a), 1.33 ('q', 1 H, J = 11.0, 1 H, H-3b); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 155.5 ($PhCH_2O\underline{C}(O)NH$), 137.8 (quaternary C), 136.2 (quaternary C), 128.6-127.2 (aromatic C), 80.1 (C-5), 77.4 (C-4), 71.1 ($\underline{C}H_2$), 70.2 (C-1), 67.0 ($\underline{C}H_2$), 62.5 (C-6), 46.5 (C-2), 35.9 (C-3) (ESI-IT-MS): *m/z* $[M+Na]^+$: 394.1, $[M+K]^+$: 410.1; Anal. Calcd for $C_{21}H_{25}NO_5$: C, 57.91; H, 6.78; N, 3.77; Found: C, 57.96; H, 6.74; N, 3.79.

1,5-Anhydro-4-*O*-benzyl-2-benzyloxycarbonylamino-2,3-dideoxy-D-*ribo*-hexitol 6-Phosphate 22

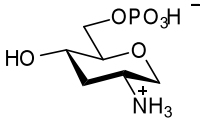


$C_{21}H_{26}NO_8P$

451,41

$POCl_3$ (270 µL, 2.9 mmol), water (41 µL, 2.3 mmol), and pyridine (300 µL, 3.2 mmol) were dissolved in acetonitrile (2.5 mL) and stirred for 10 min at 0 °C. Compound **21** (200 mg, 0.54 mmol) was added and the mixture was stirred for 24 h at rt. Water (4 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation of the solvent. Purification by RP-HPLC (semi-preparative column; 40–59 % in 30 min) followed by lyophilization yielded **22** (58 mg, 25 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 8.2 min (40–59 % B in 30 min); ^{1}H NMR (400.1 MHz, D_3COD): δ = 7.36-7.26 (m, 10 H, Ph), 5.06 (m, 2 H, CH_2^Z), 4.63 (d, J = 11.0, 1 H, ½ CH_2^{Bn}), 4.52 (d, J = 11.0, 1 H, ½ CH_2^{Bn}), 4.25 (dd, J = 10.8, 5.4, 1 H, H-6a), 4.10 (m, 1 H, H-6b), 3.94 (dd, J = 10.3, 4.0, 1 H, H-1a), 3.63 (m, 1 H, H-2), 3.49 (d't', J = 10.3, 4.4, 1 H, H-4), 3.31 (m, 1 H, H-5), 3.06 ('t', J = 10.7, 1 H, H-1b), 2.51 (m, H-3a), 1.38 ('q', J = 11.5, 1 H, H-3b); ^{13}C NMR (100.6 MHz, D_3COD): δ = 158.4 ($PhCH_2O\underline{C}(O)NH$), 139.8 (quaternary C), 138.4 (quaternary C), 129.6-128.9 (aromatic C), 80.6 (C-5), 73.5 (C-4), 72.2 ($\underline{C}H_2$), 71.3 (C-1), 67.5 ($\underline{C}H_2$), 67.0 (C-6), 47.8 (C-2), 36.6 (C-3); ^{31}P NMR (161.9 MHz, D_3COD): δ = 0.14 (s, 1 P); (ESI-IT-MS): *m/z* $[M-H]^-$: 450.3.

1,5-Anhydro-2-amino-2,3-dideoxy-D-*ribo*-hexitol 6-Phosphate 2

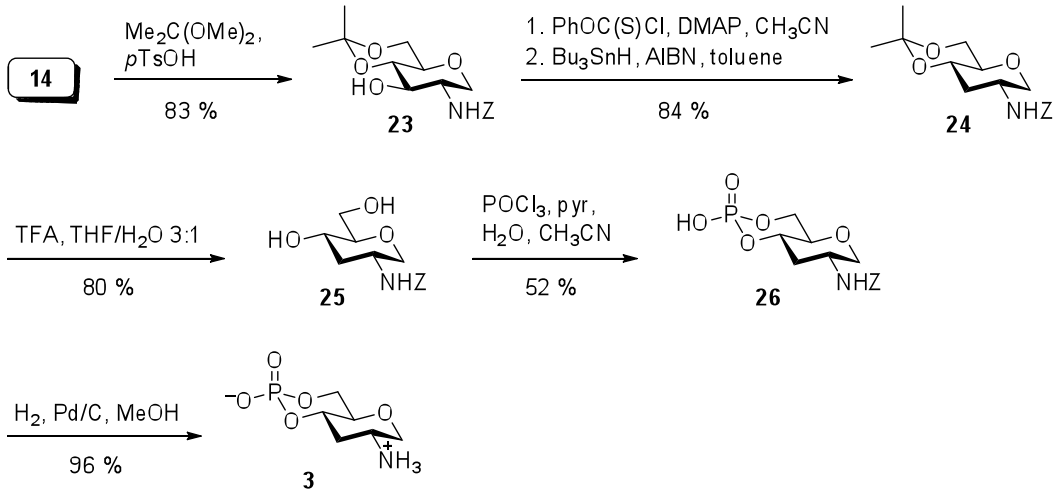


$C_6H_{14}NO_6P$

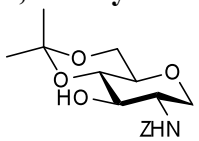
227,15

To a solution of compound **22** (51 mg, 0.11 mmol) in MeOH (3 mL) was added 10 % Pd on carbon (15 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 6 h. After filtration and lyophilization, compound **2** was obtained as a colorless solid (24 mg, 94 %). ^{1}H NMR (400.1 MHz, D_3COD): δ = 4.13-3.97 (m, 3 H, H-6a, H-1a, H-4) 4.01 (ddd, J = 10.5, 2.2, 1 H, H-6b), 3.48-3.38 (m, 3 H, H-5, H-1b, H-2), 2.48 (m, 1 H, H-3a), 1.62 ('q', J = 10.6, 1 H, H-3b); ^{13}C NMR (100.6 MHz, D_3COD): δ = 81.9 (C-5), 68.6 (C-1), 65.5 (C-6), 64.8 (C-4), 47.0 (C-2), 36.5 (C-3); ^{31}P NMR (161.9 MHz, D_3COD): δ = 1.10 (s, 1 P); (ESI-IT-MS): *m/z* $[M-H]^-$: 226.0; Anal. Calcd for $C_6H_{14}NO_6P$: C, 31.73; H, 6.21; N, 6.17; Found: C, 31.35; H, 6.55; N, 5.80.

Synthesis of GlcN6P analog 3



1,5-Anhydro-2-benzyloxycarbonylamino-2-deoxy-4,6-*O*-isopropyliden-D-glucitol 23



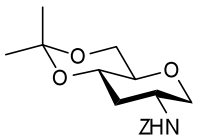
$C_{17}H_{23}NO_6$

337,37

Compound **14**[i] (600 mg, 2 mmol) was dissolved in a mixture of acetone (7.5 ml) and dichloromethane (5.5 ml). 2,2-Dimethoxypropane (1 ml) and *p*TsOH (16.5 mg) were added, and the mixture was stirred for 17 h at rt. After evaporation, purification by FC yielded **23** (565 mg, 83 %) as a colorless solid. R_f = 0.09 (petroleum ether/EtOAc 2:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.38-7.30 (m, 5 H, Ph),7.23 (d, J = 8.0, 1 H, NH), 5.07 (d, J = 5.8, 1 H, OH), 5.01 (m, 2 H, $PhCH_2$), 3.77-3.74 (m, 2 H, H-1a, H-6a), 3.61 ('t', J = 10.4, 1 H, H-6b), 3.49-3.43 (m, 1 H, H-2), 3.41 ('t', J = 9.2, 1 H, H-4), 3.35 (dd, J = 8.8, 5.9, 1 H, H-3), 3.09 ('t', J = 11.0, 1 H, H-1b), 3.03 (d't', J = 10.1, 5.5, 1 H, H-5), 1.43 (s, 3 H, CH_3), 1.31 (s, 3 H, CH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 156.0 ($PhCH_2O\underline{C}(O)NH$), 136.9 (quaternary C), 128.3-127.8 (aromatic C), 98.8 (quaternary C), 74.4 (C-4), 72.2 (C-5), 71.2 (C-3), 68.5 (C-1), 65.4 ($Ph\underline{C}H_2$), 61.4 (C-6), 53.6 (C-2), 29.1 ($\underline{C}H_3$), 19.1 ($\underline{C}H_3$); (ESI-IT-MS): *m/z* $[M+Na]^+$:

359.9, $[M+K]^+$: 375.9; Anal. Calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15; Found: C, 60.47; H, 6.92; N, 3.19.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3-dideox-4,6-*O*-isopropyliden-D-*ribo*-hexitol 24

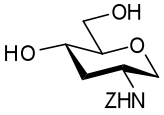


$C_{17}H_{23}NO_5$

321,37

Compound **23** was deoxygenated according to General Procedure 1B to yield **24** in 84 % yield. R_f = 0.31 (petroleum ether/EtOAc 2:1); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.39-7.31 (m, 5 H, Ph), 5.01 (m, 2 H, $PhCH_2$), 3.82 (dd, J = 10.6, 5.0, 1 H, H-1a), 3.73 (dd, J = 10.5, 5.1, 1 H, H-6a), 3.66-3.58 (m, 3 H, H-2, H-4, H-6b), 3.00 ('t', J = 10.7, 1 H, H-1b), 2.95 (d't', J = 10.0, 5.6, 1 H, H-5), 1.99 (m, 1 H, H-3a), 1.43 (s, 3 H, CH_3), 1.38 ('q', J = 11.9, 1 H, H-3b), (1.27 (s, 3 H, CH_3**)**; ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.4 ($PhCH_2O\underline{C}(O)NH$), 136.9 (quaternary C), 128.4-127.9 (aromatic C), 98.6 (quaternary C), 74.0 (C-5), 69.9 (C-1), 68.6 (C-4), 65.4 ($Ph\underline{C}H_2$), 61.8 (C-6), 46.3 (C-2), 35.0 (C-3), 29.0 ($\underline{C}H_3$), 19.1 ($\underline{C}H_3$); (ESI-IT-MS): *m/z* $[M+Na]^+$: 343.9, $[M+K]^+$: 359.9; Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36; Found: C, 63.50; H, 7.21; N, 4.36.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3-dideoxy-D-*ribo*-hexitol 25

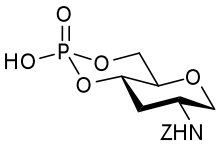


$C_{14}H_{19}NO_5$

281,3

Compound **24** (200 mg, 0.62 mmol) was dissolved in THF/water (4:1, 3 mL), TFA (115 µL) was added at 0 °C, and the mixture was stirred for 3 h at rt. After evaporation, **25** was crystallized from dichloromethane (140 mg, 80 %). R_f = 0.26 (EtOAc); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.38-7.30 (m, 5 H, Ph), 7.28 (d, J = 7.5, 1 H, NH), 5.00 (m, 2 H, $PhCH_2$), 4.85 (d, J = 5.2, 1 H, OH), 4.45 (m, 1 H, OH), 3.78 (ddd, J = 10.4, 4.6, 1.8, 1 H, H-1a), 3.66 (m, 1 H, H-6a), 3.45 (m, 1 H, H-2), 3.38 ('q', J = 10.6, 4.7, 1 H, H-6b), 3.26 (m, 1 H, H-4), 2.86 (m, 2 H, H-1b, H-5), 2.07 (m, 1 H, H-3a), 1.31 ('q', J = 11.7, 1 H, H-3b); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.5 ($PhCH_2O\underline{C}(O)NH$), 137.0 (quaternary C), 128.3-127.8 (aromatic C), 83.0 (C-5), 69.5 (C-1), 65.3 ($Ph\underline{C}H_2$),64.6 (C-4), 61.3 (C-6), 46.6 (C-2), 38.8 (C-3); (ESI-IT-MS): *m/z* $[M+Na]^+$: 304.0, $[M+K]^+$: 320.0; Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98; Found: C, 59.68; H, 6.88; N, 4.97.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3-dideoxy-D-*ribo*-hexitol 4,6-Cyclophosphate 26



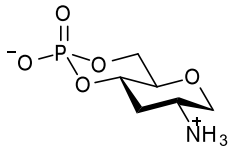
$C_{14}H_{18}NO_7P$

343,27

$POCl_3$ (169 µL, 1.85 mmol), H_2O (19 µL, 1.1 mmol), and pyridine (240 µL, 3.0 mmol) were dissolved in acetonitrile (1.5 mL) and stirred for 10 min at 0 °C. Compound **25** (100 mg, 0.36 mmol) was added, and the mixture was stirred for 2.5 h at 0 °C. Water (4 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 5–40 % in 30 min) followed by lyophilization yielded **26** (63 mg, 52 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 21.8 min (5–40 % B in 30 min); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.44 (d, J = 7.4, 1 H, NH), 7.38-7.30 (m, 5 H,

Ph), 5.01 (m, 2 H, $PhCH_2$), 4.21 (dd, J = 10.2, 4.9, 1 H, H-6a), 4.07 (d't', J = 10.4, 4.4, 1 H, H-4), 3.94 ('t', J = 10.4, 1 H, H-6b), 3.86 (ddd, J = 10.6, 4.9, 1.0, 1 H, H-1a), 3.64 (m, 1 H, H-2), 3.38 (d't', J = 9.8, 4.8, 1 H, H-5), 3.07 ('t', J = 11.0, 1 H, H-1b), 2.21 (m, H-3a), 1.59 ('q', J = 12.0, 1 H, H-3b); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.5 ($PhCH_2O\underline{C}(O)NH$), 136.8 (quaternary C), 128.4-127.9 (aromatic C), 74.7 (C-4), 72.8 (C-5), 69.8 (C-1), 67.7 (C-6) 65.7 ($Ph\underline{C}H_2$), 45.8 (C-2), 35.5 (C-3); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ = -6.35 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 341.9, Anal. Calcd for $C_{14}H_{18}NO_7P•0.5\ H_2O$: C, 47.73; H, 5.44; N, 3.98; Found: C, 47.78; H, 5.55; N, 4.10.

1,5-Anhydro-2-amino-2,3-dideoxy-D-*ribo*-hexitol 4,6-Cyclophosphate 3

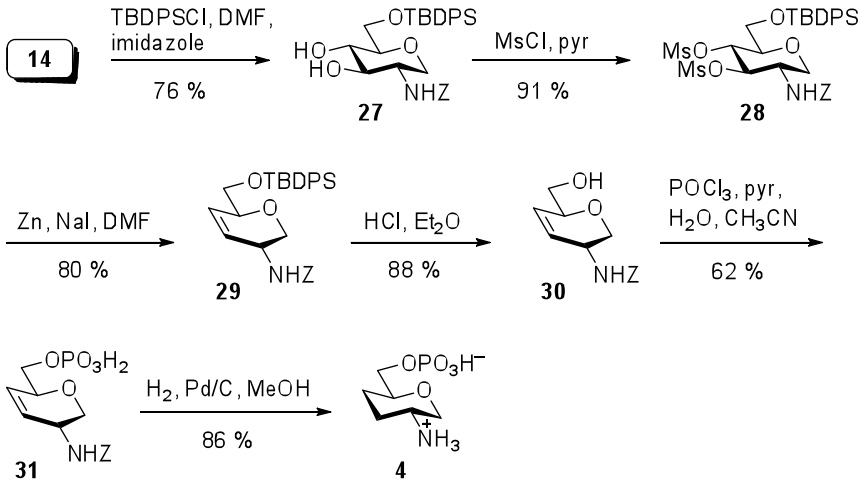


$C_6H_{12}NO_5P$

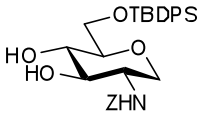
209,14

To a solution of compound **26** (66 mg, 0.19 mmol) in MeOH (4 mL) was added 10 % Pd on carbon (20 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, compound **3** was obtained as a colorless solid (38 mg, 96 %). ^{1}H NMR (400.1 MHz, D_2O): δ = 4.24 (dd, J = 10.5, 5.1, 1 H, H-6a), 4.19-4.10 (m, 2 H, H-1a, H-4), 4.01 (d't' J = 10.5, 2.2, 1 H, H-6b), 3.60-3.47 (m, 3 H, H-5, H-1b, H-2), 2.54 (m, 1 H, H-3a), 1.81 ('q', J = 11.4, 1 H, H-3b); ^{13}C NMR (100.6 MHz, D_2O): δ = 74.3 (C-5), 73.6 (C-4), 68.2 (C-1), 67.2 (C-6), 45.8 (C-2), 34.4 (C-3);^{31}P NMR (161.9 MHz, D_2O): δ = –2.39 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 207.9, Anal. Calcd for $C_6H_{12}NO_5P•H_2O$: C, 31.73; H, 6.21; N, 6.17; Found: C, 32.04; H, 6.54; N, 6.12.

Synthesis of GlcN6P analog 4



1,5-Anhydro-2-benzyloxycarbonylamino-2-deoxy-6-*O*-(*tert*-butyldiphenylsilyl)-D-glucitol 27

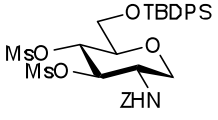


$C_{30}H_{37}NO_6Si$

535,70

To a solution of compound **14**[i] (330 mg, 1.1 mmol) in DMF (5 mL) was added TBDPSCl (330 mg, 1.1 mmol) and imidazole (220 mg, 3.3 mmol), and the mixture was stirred for 5 h at 0 °C. The mixture was diluted with dichloromethane and washed two times with 2 N HCl, sat. $NaHCO_3$ solution, and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. Purification by FC (petroleum ether/EtOAc 1:1) yielded **27** (450 mg, 76 %) as a colorless solid. R_f = 0.28 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.68-7.31 (m, 15 H, Ph), 5.10 (m, 2 H, $PhCH_2$), 4.89 (m, 1 H, NH), 4.09 (m 1 H, H-1a), 3.90 (dd, J = 10.6, 4.3, 1 H, H-6a), 3.84 (dd, J = 10.6, 5.4, 1 H, H-6b), 3.68 (m, 1 H, H-2), 3.63 ('t', J = 9.1, 1 H, H-4), 3.45 ('t', J = 9.4, 1 H, H-3), 3.27 (d't', J = 9.4, 4.9, 1 H, H-5), 3.09 ('t', J = 10.3, 1 H, H-1b), 1.06 (s, 9 H, CH_3); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 156.6 ($PhCH_2O\underline{C}(O)NH$), 136.0-135.5 (quaternary C), 130.0-127.8 (aromatic C), 78.5 (C-5), 76.4 (C-3), 73.5 (C-4), 68.3 (C-1), 67.2 ($Ph\underline{C}H_2$), 65.2 (C-6), 52.6 (C-2), 48.3 (quaternary C), 26.8 (3 x $\underline{C}H_3$); (ESI-IT-MS): *m/z* $[M+Na]^+$: 558.2, $[M+K]^+$: 574.2; Anal. Calcd for $C_{30}H_{37}NO_6Si$: C, 67.26; H, 6.96; N, 2.61; Found: C, 67.14; H, 6.90; N, 2.59.

1,5-Anhydro-2-benzyloxycarbonylamino-2-deoxy-3,4-di-*O*-mesyl-6-*O*-(*tert*-butyl-diphenylsilyl)-D-glucitol 28



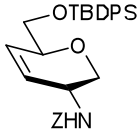
$C_{32}H_{41}NO_{10}S_2Si$

691,88

Compound **27** was dissolved in pyridine (10 mL), MsCl (390 µL, 5 mmol) was slowly added, and the mixture was stirred for 5 h at rt. The mixture was added to an ice/water mixture, the precipitate was filtered and dried to yield **28** (1.05 g, 91 %) as a colorless solid. R_f = 0.3 (petroleum ether/EtOAc 2:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.73-7.31 (m, 15 H, Ph), 5.22 (d, J = 6.7, 1 H, NH), 5.13 (m, 2 H, $PhCH_2$), 4.94 ('t', J = 9.4, 1 H, H-4), 4.79 ('t', J = 9.7, 1 H, H-3), 4.16 (dd, J = 11.4, 5.4, 1 H, H-1a), 3.95 (m, 1 H, H-2), 3.94 (dd, J = 12.0, 1.6, 1 H, H-6a), 3.88 (dd, J = 12.0, 3.8, 1 H, H-6b), 3.35 (m, 1 H, H-5), 3.18 ('t', J = 10.8, 1 H, H-

1b), 3.06 (s, 3 H, CH_3), 3.01(s, 3 H, CH_3), 1.08 (s, 9 H, CH_3); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 156.6 ($PhCH_2O\underline{C}(O)NH$), 135.9-135.5 (quaternary C), 130.0-127.7 (aromatic C), 80.3 (C-3), 79.3 (C-5), 74.0 (C-4), 68.6 (C-1), 67.5 ($Ph\underline{C}H_2$), 62.4 (C-6), 52.5 (C-2), 42.0 (quaternary C), 38.9 (s, $\underline{C}H_3$), 38.8 (s, $\underline{C}H_3$), 26.8 (3 x $\underline{C}H_3$); (ESI-IT-MS): *m/z* $[M+Na]^+$: 714.4, $[M+K]^+$: 730.3.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3,4-trideoxy-6-O-(*tert*-butyldiphenylsilyl)-D-*erythro*-hex-3-enitol 29

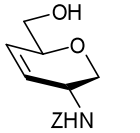


$C_{30}H_{35}NO_4Si$

501,69

Compound **28** was dissolved in DMF (25 mL), and zinc (1.1 g, 16.5 mmol) and NaI (18.4 g, 123 mmol) were added under a nitrogen atmosphere. The mixture was stirred for 4 h at 95 °C. The mixture was added to a 1:1 mixture of ice/chloroform and filtered. The organic layer was washed with brine, dried (Na_2SO_4), and the solvent was evaporated. Purification by FC (petroleum ether-EtOAc 4:1) yielded **29** (550 mg, 80 %) as a colorless solid. R_f = 0.39 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.68-7.36 (m, 15 H, Ph), 5.90 (m, 2 H, H-3, H-4), 5.12 (m, 2 H, $PhCH_2$), 4.87 (d, *J* = 9.2, 1 H, NH), 4.20 (m, 2 H, H-2, H-5), 4.02 (dd, *J* = 11.3, 4.1, 1 H, H-1a), 3.74 (dd, *J* = 10.4, 6.3, 1 H, H-6a), 3.65 (dd, *J* = 10.4, 5.5, 1 H, H-6b), 3.46 (dd, *J* = 11.4, 5.21, 1 H, H-1b), 1.06 (s, 9 H, CH_3); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.8 ($PhCH_2O\underline{C}(O)NH$), 135.9-135.5 (quaternary C), 136.4, (C-3), 126.6 (C-4), 128.5-127.5 (aromatic C), 86.7 (quaternary C), 74.1 (C-5), 66.7 ($Ph\underline{C}H_2$), 66.4 (C-1), 64.9 (C-6), 44.4 (C-2), 42.0 (quaternary C), 26.8 (3 x $\underline{C}H_3$); (ESI-IT-MS): *m/z* $[M+Na]^+$: 524.3, $[M+K]^+$: 540.2; Anal. Calcd for $C_{30}H_{35}NO_4Si$: C, 72.20; H, 7.23; N, 2.72; Found: C, 72.15; H, 7.59; N, 2.63.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3,4-trideoxy-D-*erythro*-hex-3-enitol 30

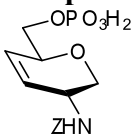


$C_{14}H_{17}NO_4$

263,29

Compound **29** (480 mg, 0.95 mmol) was dissolved in diethyl ether (10 mL) and 0.5 M HCl in MeOH (25 mL) was added. The mixture was stirred for 3 h at rt. Then, basic ion exchange resin was added. Filtration and evaporation yielded compound **30** (220 mg, 88 %) as a colorless solid. R_f = 0.22 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.38-7.31 (m, 5 H, Ph), 5.83 (m, 2 H, H-3, H-4), 5.11 (m, 2 H, $PhCH_2$), 4.77 (d, *J* = 8.3, 1 H, NH), 4.32 (m, 1 H, H-2), 4.20 (m, 1H, H-5) 4.13 (dd, *J* = 11.5, 4.0, 1 H, H-1a), 3.62 (m, H-6a, H-6b), 3.46 (dd, *J* = 11.2, 6.5, 1 H, H-1b); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.8 ($PhCH_2O\underline{C}(O)NH$), 136.2 (aromatic C), 128.8, (C-4), 128.1 (C-3), 128.9-128.3 (aromatic C), 74.4 (C-5), 66.7 ($Ph\underline{C}H_2$), 66.1 (C-1), 63.7 (C-6), 44.5 (C-2); (ESI-IT-MS): *m/z* $[M+Na]^+$: 285.9, $[M+K]^+$: 301.9; Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32; Found: C, 63.94; H, 6.54; N, 5.33.

1,5-Anhydro-2-benzyloxycarbonylamino-2,3,4-trideoxy-D-*erythro*-hex-3-enitol 6-Phosphate 31

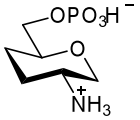


$C_{14}H_{18}NO_7P$

343,27

$POCl_3$ (380 µL, 4.2 mmol), H_2O (60 µL, 3.4 mmol), and pyridine (677 µL, 8.4 mmol) were dissolved in acetonitrile (3 mL) and stirred for 10 min at 0 °C. Compound **30** (220 mg, 0.54 mmol) was added, and the mixture was stirred for 20 h at rt. Water (4 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 20–65 % in 20 min) followed by lyophilization yielded **31** (180 mg, 62 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 11.3 min (20–65 % B in 20 min); ^{1}H NMR (600.1 MHz, CD_3OD): δ = 7.39-7.30 (m, 5 H, Ph), 5.86 (m, 2 H, H-3, H-4), 5.08 (m, 2 H, $PhCH_2$), 4.30 (m, 1 H, H-5), 4.20 (m, 1H, H-2) 4.13 (dd, J = 11.0, 4.9, 1 H, H-1a), 3.95 (m, H-6a, H-6b), 3.40 (dd, J = 11.0, 7.8, 1 H, H-1b); ^{13}C NMR (150.9 MHz, CD_3OD): δ = 158.5 ($PhCH_2O\underline{C}(O)NH$), 138.3 (quaternary C), 129.5, (C-4), 128.7 (C-3), 129.9-128.8 (aromatic C), 73.8 (C-5), 68.3 (C-6), 67.5 (C-1), 67.3 ($Ph\underline{C}H_2$), 45.7 (C-2); ^{31}P NMR (161.9 MHz, CD_3OD): δ = 3.73 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^–$: 342.9; Anal. Calcd for $C_{14}H_{18}NO_7P$: C, 48.07; H, 5.84; N, 4.06; Found: C, 48.00; H, 5.87; N, 3.98.

1,5-Anhydro-2-amino-2,3,4-trideoxy-D-*erythro*-hexitol 6-Phosphate 4

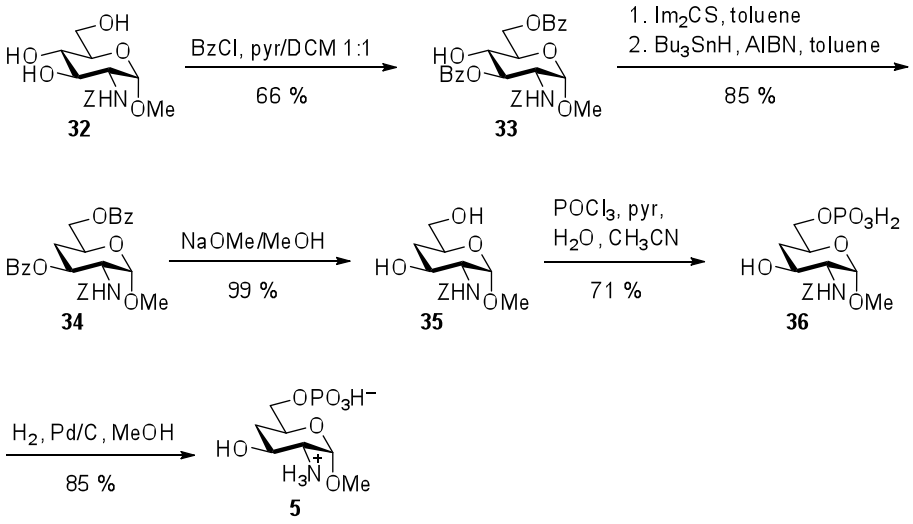


$C_6H_{14}NO_5P$

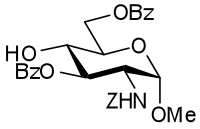
211,15

To a solution of compound **31** (72 mg, 0.21 mmol) in MeOH (6 mL) was added 10 % Pd on carbon (25 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, compound **4** was obtained as a colorless solid (28 mg, 86 %). ^{1}H NMR (600.1 MHz, D_2O): δ = 4.19 (d‘q’, J = 10.9, 4.3, 2.3, 1 H, H-1a), 3.94 (ddd, J = 11.3, 5.8, 3.3, 1 H, H-6a), 3.85 (d‘t’ J = 11.3, 6.7, 1 H, H-6b), 3.73 (m, 1 H, H-5), 3.50 (‘t’, J = 10.8, 1 H, H-6b), 3.38 (d‘t’, J = 11.2, 4.3, 1 H, H-2), 2.27 (m, 1 H, H-3a), 1.87 (d‘q’, J = 13.6, 3.2, 1 H, H-4a), 1.72 (d‘q’, J = 12.5, 4.3, 1 H, H-3b), 1.58 (d‘q’, J = 13.0, 4.0, 1 H, H-4b); ^{13}C NMR (150.9 MHz, D_2O): δ = 77.4 (C-5), 68.4 (C-1), 68.0 (C-6), 47.2 (C-2), 27.6 (C-3), 25.9 (C-4); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ = 2.38 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^–$: 210.0; Anal. Calcd for $C_6H_{14}NO_5P$: C, 34.13; H, 6.68; N, 6.63; Found: C, 34.00; H, 6.41; N, 6.58.

Synthesis of GlcN6P analog 5



Methyl 3,6-Di-*O*-benzoyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside 33

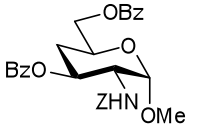


$C_{29}H_{29}NO_9$

535,54

Compound **32**[ii] (1 g, 3.1 mmol) was dissolved in dichloromethane/pyridine (1:1, 10 mL) under a nitrogen atmosphere and cooled to –20°C. Benzoyl chloride (780 µL, 6.7 mmol) was added slowly at –20 °C, and the mixture was stirred for 2.5 h at rt. The reaction was quenched with MeOH (2 mL), and the solvent was evaporated. Purification by FC (petroleum ether/EtOAc 3:1) yielded **33** (1.1 g, 66 %) as a colorless solid. R_f = 0.44 (petroleum ether/EtOAc 3:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 8.08-8.03 (m, 5 H, Ph), 7.60-7.12 (m, 10 H, Ph), 5.33 ('t', J = 10.1, 1 H, H-3), 5.18 (d, J = 10.2, 1 H, NH), 4.96 (m, 2 H, $PhCH_2$), 4.81 (d, J = 3.2, 1 H, H-1), 4.73 (dd, J = 12.1, 4.5, 1 H, H-6a), 4.59 (dd, J = 12.1, 2.2, 1 H, H-6b), 4.09 (d't', J = 10.1, 3.4, 1 H, H-2), 4.01 (ddd, J = 9.9, 4.6, 2.3, 1 H, H-5), 3.83 ('t', J = 9.4, 1 H, H-4), 3.42 (s, 3 H, CH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 167.9 (C(O)Ph), 166.8 (C(O)Ph), 155.9 ($PhCH_2OC(O)NH$), 136.0 (quaternary C), 133.3 (quaternary C), 133.3 (quaternary C), 130.1-127.8 (aromatoc C), 98.9 (C-1), 75.6 (C-3), 70.6 (C-5), 69.6 (C-4), 67.0 ($PhCH_2$), 63.7 (C-6), 55.6 (CH_3) 53.8 (C-2); (ESI-IT-MS): *m/z* $[M+Na]^+$: 557.9, $[M+K]^+$: 574.0; Anal. Calcd for $C_{29}H_{29}NO_9$: C, 65.04; H, 5.46; N, 2.62; Found: C, 65.11; H, 5.50; N, 2.62.

Methyl 3,6-Di-*O*-benzoyl-2-benzyloxycarbonylamino-2,4-dideoxy-α-D-*xylo*-hexopyranoside 34



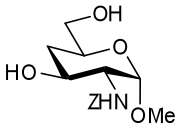
$C_{29}H_{29}NO_8$

519,54

Compound **33** was deoxygenated according to General Procedure 1A to yield **34** in 85 % yield. R_f = 0.17 (petroleum ether/EtOAc 2:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 8.06-8.03 (m, 5 H, Ph), 7.59-7.19 (m, 10 H, Ph), 5.32 (d't', J = 11.0, 4.9, 1 H, H-3), 5.06 (d, J = 9.8, 1 H, NH), 5.02 (m, 2 H, $PhCH_2$), 4.87 (d, J = 3.5, 1 H, H-1), 4.43 (m, 2 H, H-6a, H-6b), 4.27 (m, 1 H, H-5), 4.17 (d't', J = 10.3, 3.3, 1 H, H-2), 3.42 (s, 3 H, CH_3), 2.24 (ddd, J = 12.5, 4.9,

2.2, 1 H, H-4a), 1.81 (‘q’, J = 12.0, 1 H, H-4b); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 166.4 (C(O)Ph), 166.3 (C(O)Ph), 156.1 ($PhCH_2OC(O)NH$), 136.2 (quaternary C), 133.2 (quaternary C), 133.1 (quaternary C), 129.9-127.6 (aromatic C), 99.5 (C-1), 70.0 (C-3), 66.9 ($PhCH_2$), 66.0 (C-6), 65.8 (C-5), 3.43 (CH_3), 54.0(C-2), 33.1 (C-4); (ESI-IT-MS): *m/z* $[M+Na]^+$: 542.2, $[M+K]^+$: 558.2; Anal. Calcd for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63; N, 2.70; Found: C, 67.02; H, 5.48; N, 2.87.

Methyl 2-Benzyloxycarbonylamino-2,4-dideoxy-α-D-*xylo*-hexopyranoside 35

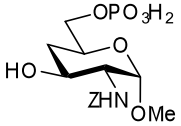


$C_{15}H_{21}NO_6$

311,33

To a solution of **34** (400 mg, 0.77 mmol) in MeOH (4 mL) was added a solution of sodium methylate (0.5 M in MeOH, 0.15 equiv). The mixture was stirred for 12 h at rt. After neutralization with acidic ion exchanger (DOWEX 50 W X8, H+ form), the mixture was filtered and lyophilized to yield **35** (240 mg, 99 %). R_f = 0.21 (EtOAc); ^{1}H NMR (600.1 MHz, CD_3OD): δ = 7.37-7.28 (m, 5 H, Ph), 5.09 (m, 2 H, $PhCH_2$), 4.72 (d, J = 3.5, 1 H, H-1), 3.80-3.78 (m, 2 H, H-3, H-5), 3.55-3.50 (m, 3 H, H-2, H-6a, H-6b), 3.33 (s, 3 H, CH_3), 1.95 (ddd, J = 12.7, 4.9, 1.9, 1 H, H-4a), 1.39 (m, 1 H, H-4b);^{13}C NMR (150.9 MHz, CD_3OD): δ = 159.1 ($PhCH_2OC(O)NH$), 138.4 (quaternary C), 129.7-129.0 (aromatic C), 100.8 (C-1), 70.1 (C-5), 67.8 ($PhCH_2$), 67.2 (C-3), 65.7 (C-6), 58.7 (C-2), 55.6 (CH_3), 37.0 (C-4); (ESI-IT-MS): *m/z* $[M+Na]^+$: 334.1, $[M+K]^+$: 350.1; Anal. Calcd for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N, 4.50; Found: C, 58.11; H, 6.90; N, 4.27.

Methyl 2-Benzyloxycarbonylamino-2,4-dideoxy-α-D-*xylo*-hexopyranoside 6-Phosphate 36

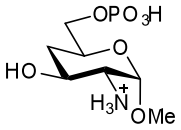


$C_{15}H_{22}NO_9P$

391,31

$POCl_3$ (215 µL, 2.3 mmol), H_2O (32 µL, 1.8 mmol), and pyridine (204 µL, 2.5 mmol) were dissolved in acetonitrile (2 mL) and stirred for 10 min at 0 °C. Compound **35** (140 mg, 0.45 mmol) was added, and the mixture was stirred for 6 h at 0 °C. Water (4 mL) was added, and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 15–30 % in 20 min) followed by lyophilization yielded **36** (125 mg, 71 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 13.9 min (15–30 % B in 20 min); ^{1}H NMR (600.1 MHz, DMSO-d_6): δ = 7.37-7.29 (m, 5 H, Ph), 7.04 (d, J = 7.9, 1 H, NH), 5.02 (m, 2 H, $PhCH_2$), 4.65 (d, J = 3.5, 1 H, H-1), 3.81-3.68 (m, 3 H, H-5, H-6a, H-6b), 3.66 (d‘t’, J = 10.7, 5.0, 1 H, H-3), 3.31 (ddd, J = 10.9, 8.5, 3.5, 1 H, H-2), 3.21 (s, 3 H, CH_3), 1.87 (ddd, J = 12.6, 4.9, 1.3, 1 H, H-4a), 1.29 (‘q’, J = 11.8, 1 H, H-4b); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 156.1 ($PhCH_2OC(O)NH$), 137.1 (quaternary C), 128.3-127.8 (aromatic C), 98.8 (C-1), 67.4 (C-6), 66.0 (C-5), 65.1 ($PhCH_2$), 64.1 (C-3), 57.4 (C-2), 54.6 (CH_3), 36.2 (C-4); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ = –0.35 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^–$: 389.8; Anal. Calcd for $C_{15}H_{22}NO_9P$: C, 46.04; H, 5.67; N, 3.58; Found: C, 45.92; H, 5.33; N, 3.55.

Methyl 2-Amino-2,4-dideoxy-α-D-*xylo*-hexopyranoside 6-Phosphate 5

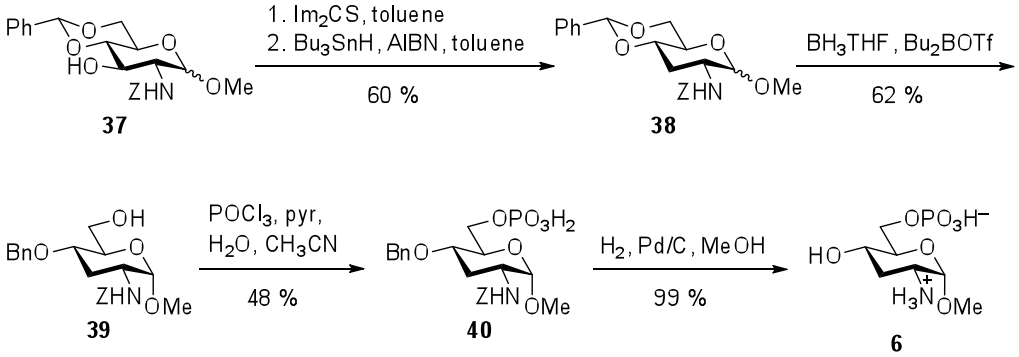


$C_7H_{16}NO_7P$

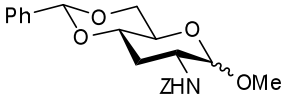
257,18

To a solution of compound **36** (70 mg, 0.18 mmol) in MeOH (6 mL) was added 10 % Pd on carbon (20 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, compound **5** was obtained as a colorless solid (39 mg, 85 %). ^{1}H NMR (600.1 MHz, D_2O): δ = 5.08 (d, J = 3.5, 1 H, H-1), 4.16-4.11 (m, 2 H, H-3, H-5), 4.01-3.90 (m, 2 H, H-6a, H-6b), 3.48 (s, 3 H, CH_3), 3.27 (dd, J = 10.5, 3.5, 1 H, H-2), 2.14 (ddd, J = 12.8, 5.0, 2.0, 1 H, H-4a), 1.65 ('q', J = 12.0, 1 H, H-4b); ^{13}C NMR (150.9 MHz, D_2O): δ = 97.7 (C-1), 68.7 (C-5), 67.5 (C-6), 65.3 (C-3), 56.2 (CH_3), 56.1 (C-2), 35.1 (C-4); ^{31}P NMR (161.9 MHz, D_2O): δ = –2.18 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 255.8; Anal. Calcd for $C_7H_{16}NO_7P•H_2O$: C, 30.55; H, 6.59; N, 5.09; Found: C, 30.75; H, 6.46; N, 5.26.

Synthesis of GlcN6P analog 6



Methyl 4,6-*O*-Benzylidene-2-benzyloxycarbonylamino-2,3-dideoxy-α,β-D-*ribo*-hexo-pyranoside 38

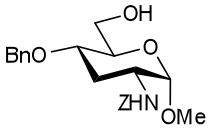


$C_{22}H_{25}NO_6$

399,44

Compound **37**[iii,iv] was deoxygenated according to the General Procedure 1B to yield **38** in 60 % yield (α/β = 1:4). R_f = 0.43 (petroleum ether/EtOAc 2:1); ^{1}H NMR β-anomer (400.1 MHz, DMSO-d_6): δ = 7.47 (d, J = 8.2, 1 H, NH), 7.41-7.31 (m, 10 H, Ph), 5.61 (s, 1 H, PhCH), 5.02 (m, 2 H, $PhCH_2$), 4.32 (d, J = 8.0, 1 H, H-1), 4.32 (dd, J = 10.2, 4.0, 1 H, H-6a), 3.72-3.70 (m, 2 H, H-4, H-6b), 3.50 (m, 1 H, H-2), 3.36 (s, 3 H, CH_3), 3.33 (m, 1 H, H-5), 2.13 (d't', J = 11.6, 4.2, 1 H, H-3a), 1.43 ('q', 1 H, J = 12.2, 1 H, H-3b); ^{13}C NMR β-anomer (100.6 MHz, DMSO-d_6): δ = 155.5 ($PhCH_2O\underline{C}(O)NH$), 137.7 (quaternary C), 137.6 (quaternary C), 128.9-126.1 (aromatic C), 104.1 (C-1), 100.8 (Ph$\underline{C}$H), 75.6 (C-4), 70.1 (C-5), 68.4 (C-6), 65.6 (Ph$\underline{C}H_2$), 50.7 (C-2), 34.6 (C-3); (ESI-IT-MS): *m/z* $[M+Na]^+$: 422.3, $[M+K]^+$: 438.3; Anal. Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51; Found: C, 66.14; H, 6.35; N, 3.65.

Methyl 4-*O*-Benzyl-2-benzyloxycarbonylamino-2,3-dideoxy-α-D-*ribo*-hexopyranoside 39

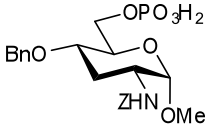


$C_{22}H_{27}NO_6$

401,45

Compound **38** (490 mg, 1.2 mmol) was dissolved in a 1 M solution of BH_3 in THF (12.5 mL) at 0 °C. Bu_2BOTf (1.24 mL) was added and the mixture was stirred for 90 min at 0 °C. After addition of NEt_3 (1 mL) and MeOH (1 mL), the solvent was evaporated. Purification by FC (petroleum ether/EtOAc 2:1) yielded **39** (300 mg, 62 %) as a colorless solid. R_f = 0.11 (petroleum ether/EtOAc 2:1); ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 7.38-7.29 (m, 10 H, Ph), 5.10 (m, 2 H, CH_2^Z), 5.02 (d, J = 9.7, 1 H, NH), 4.64 (d, J = 11.5, 1 H, ½ CH_2^{Bn}), 4.59 (d, 4.49 J = 3.3, 1 H, H-1), 4.45 (d, J = 11.5, 1 H, ½ CH_2^{Bn}), 3.86-3.81 (m, 2 H, H-2, H-6a), 3.73 (ddd, J = 11.8, 7.4, 4.3, 1 H, H-6b), 3.61 (m, 1 H, H-5), 3.52 (d‘t’, J = 10.5, 4.3, 1 H, H-4), 3.37 (s, 3 H, CH_3), 2.37 (d‘t’, J = 11.7, 4.5, 1 H, H-3a), 1.88 (dd, J = 7.4, 5.9, 1 H, OH), 1.65 (‘q’, 1 H, J = 11.5, 1 H, H-3b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 155.6 ($PhCH_2O\underline{C}(O)NH$), 137.9 (quaternary C), 136.2 (quaternary C), 128.6-127.7 (aromatic C), 97.4 (C-1), 72.3 (C-4), 70.9 (C-5), 70.8 ($\underline{C}H_2$), 67.2 ($\underline{C}H_2$), 62.6 (C-6), 52.1 ($\underline{C}H_3$), 49.2 (C-2), 30.9 (C-3); (ESI-IT-MS): *m/z* $[M+Na]^+$: 424.2, $[M+K]^+$: 440.2.

Methyl 4-*O*-Benzyl-2-benzyloxycarbonylamino-2,3-dideoxy-α-D-*ribo*-hexopyranoside 6-Phosphate 40

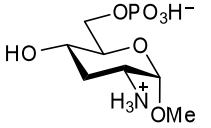


$C_{22}H_{28}NO_9P$

481,43

$POCl_3$ (178 μL, 1.9 mmol), H_2O (27 μL, 1.5 mmol), and pyridine (166 μL, 2.1 mmol) were dissolved in acetonitrile (2 mL) and stirred for 10 min at 0 °C. Compound **39** (150 mg, 0.37 mmol) was added and the mixture was stirred for 6 h at 0 °C. Water (4 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 20–45 % in 20 min) followed by lyophilization yielded **40** (86 mg, 48 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 11.0 min (20–45 % B in 20 min); ^{1}H NMR (400.1 MHz, D_3COD): δ = 7.36-7.26 (m, 10 H, Ph), 5.07 (m, 2 H, CH_2^Z), 4.63 (d, J = 11.3, 1 H, ½ CH_2^{Bn}), 4.60 (d, J = 3.3, 1 H, H-1), 4.51 (d, J = 11.3, 1 H, ½ CH_2^{Bn}), 4.22 (ddd, J = 10.9, 5.4, 1.5, 1 H, H-6a), 4.13 (m, 1 H, H-6b), 3.75-3.67 (m, 2 H, H-2, H-5), 3.59 (d‘t’, J = 10.7, 4.7, 1 H, H-4), 3.39 (s, 3 H, CH_3), 2.23 (d‘t’, J = 11.6, 4.6, H-3a), 1.38 (‘q’, J = 11.6, 1 H, H-3b); ^{13}C NMR (100.6 MHz, D_3COD): δ = 158.3 ($PhCH_2O\underline{C}(O)NH$), 139.7 (quaternary C), 138.4 (quaternary C), 129.6-128.9 (aromatic C), 98.9 (C-1), 73.2 (C-4), 72.1 ($\underline{C}H_2$), 71.5 (C-5), 67.7 ($\underline{C}H_2$), 66.7 (C-6), 55.5 ($\underline{C}H_3$), 51.0 (C-2), 31.5 (C-3); ^{31}P NMR (161.9 MHz, D_3COD): δ = 0.10 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^–$: 480.2.

Methyl 2-Amino-2,3-dideoxy-α-D-*ribo*-hexopyranoside 6-Phosphate 6



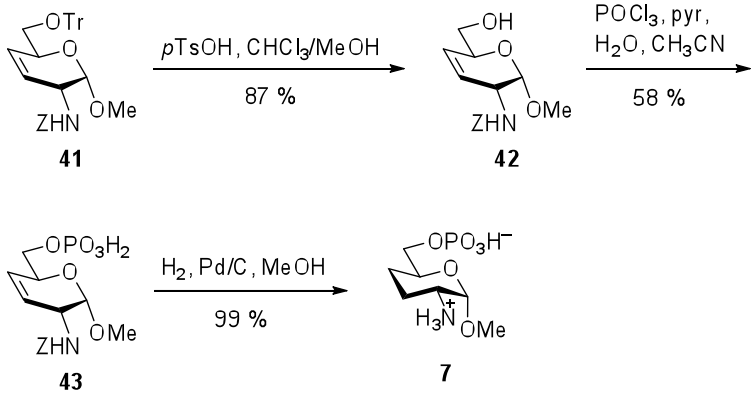
$C_7H_{16}NO_7P$

257,18

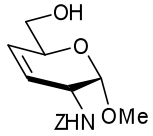
To a solution of compound **40** (78 mg, 0.16 mmol) in MeOH (5 mL) was added 10 % Pd on carbon (20 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, compound **6** (40 mg, 99 %) was obtained as a colorless solid. ^{1}H NMR (400.1 MHz, D_2O): δ = 4.89 (d, J = 3.5, 1 H, H-1), 4.09 (m, 2 H, H-6a, H-6b), 3.80 (ddd, J = 10.9, 9.8, 4.6, 1 H, H-4), 3.73 (d‘t’, J = 9.8, 2.8, 1 H, H-5), 3.56

(ddd, J = 12.0, 9.8, 3.6, 1 H, H-2), 2.26 (d't', J = 11.6, 4.7, 1 H, H-3a), 1.62 ('q', J = 11.4, 1 H, H-3b); ^{13}C NMR (100.6 MHz, D_2O): δ = 94.4 (C-1), 71.4 (C-5), 63.3 (C-6), 62.5 (C-4), 54.9 ($\underline{C}H_3$), 47.8 (C-2), 30.0 (C-3); ^{31}P NMR (161.9 MHz, D_2O): δ = 1.04 (s, 1 P); (ESI-IT-MS): *m/z* $[M-H]^-$: 255.8; Anal. Calcd for $C_7H_{16}NO_7P \bullet H_2O$: C, 30.55; H, 6.59; N, 5.09; Found: C, 30.32; H, 6.77; N, 4.90.

Synthesis of GlcN6P analog 7



Methyl 2-Benzyloxycarbonylamino-2,3,4-trideoxy-α-D-*erythro*-hex-3-enopyranoside 42

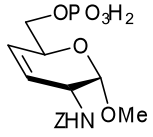


$C_{15}H_{19}NO_5$

293,32

Compound **41**[v] (650 mg, 1.21 mmol) was dissolved in chloroform/MeOH (2:1, 12 mL), and *p*TsOH (104 mg, 0.54 mmol) was added. The mixture was stirred for 10 h at rt, washed with brine, dried (Na_2SO_4), and the solvent was evaporated. Purification by FC (petroleum ether/EtOAc 1:1) yielded **42** (310 mg, 87 %) as a colorless solid. R_f = 0.16 (petroleum ether/EtOAc 1:1); ^{1}H NMR (600.1 MHz, $CDCl_3$): δ = 7.38-7.32 (m, 5 H, Ph), 5.70 (m, 2 H, H-3, H-4), 5.12 (m, 2 H, $PhCH_2$), 4.88 (d, J = 4.3, 1 H, H-1), 4.50 (m, 1 H, H-2), 4.20 (m, 1H, H-5) 3.73 (ddd, J = 11.1, 7.1, 3.5, 1 H, H-6a), 3.62 (m, 1 H, H-6b), 3.46 (s, 3 H, CH_3), 1.92 (t, J = 6.6, 1 H, OH); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 155.9 ($PhCH_2O\underline{C}(O)NH$), 136.3 (quaternary C), 128.6-128.2 (aromatic C), 126.7, (C-4), 126.6 (C-3), 97.2 (C-1), 68.7 (C-5), 66.9 ($Ph\underline{C}H_2$), 64.9 (C-6), 55.8 ($\underline{C}H_3$), 47.2 (C-2); (ESI-IT-MS): *m/z* $[M+Na]^+$: 316.0, $[M+K]^+$: 332.1; Anal. Calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78; Found: C, 61.62; H, 6.60; N, 4.89.

Methyl 2-Benzyloxycarbonylamino-2,3,4-trideoxy-α-D-*erythro*-hex-3-enopyranoside 6-Phosphate 43



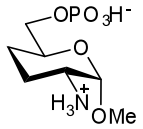
$C_{15}H_{20}NO_8P$

373,29

$POCl_3$ (437 µL, 4.8 mmol), H_2O (69 µL, 3.8 mmol), and pyridine (770 µL, 9.6 mmol) were dissolved in acetonitrile (4 mL) and stirred for 10 min at 0 °C. Compound **42** (280 mg, 0.95 mmol) was added and the mixture was stirred for 20 h at rt. Water (10 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-

preparative column; 20–65 % in 20 min) followed by lyophilization yielded **43** (205 mg, 58 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 12.5 min (20–65 % B in 20 min); ^{1}H NMR (600.1 MHz, CD_3OD): δ = 7.37-7.28 (m, 5 H, Ph), 5.82 (d‘t’, J = 10.5, 2.0, 1 H, H-3), 5.64 (d‘q’, J = 10.5, 2.2, 1 H, H-4), 5.09 (m, 2 H, $PhCH_2$), 4.86 (m, 1 H, H-1), 4.37-4.32 (m, 2 H, H-2, H-5), 3.99 (m, 2 H, H-6a, H-6b), 3.45 (s, 3 H, CH_3); ^{13}C NMR (150.9 MHz, CD_3OD): δ = 158.6 ($PhCH_2O\underline{C}(O)NH$), 138.4 (quaternary C), 129.6-129.0 (aromatic C), 127.8, (C-4), 127.3 (C-3), 98.8 (C-1), 69.2 (C-6), 68.8 (C-5), 67.2 ($Ph\underline{C}H_2$), 56.5 (CH_3), 49.2 (C-2); (ESI-IT-MS): *m/z* $[M–H]^-$: 372.1; Anal. Calcd for $C_{15}H_{20}NO_8P$: C, 48.76; H, 5.40; N, 3.75; Found: C, 48.73; H, 5.50; N, 3.77.

Methyl 2-Amino-2,3,4-trideoxy-α-D-*erythro*-hexopyranoside 6-Phosphate 7

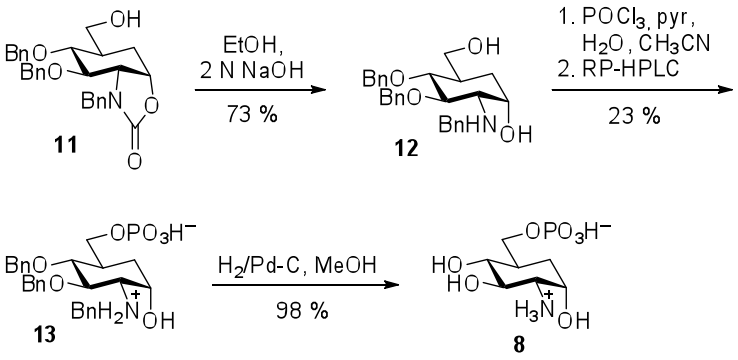


$C_7H_{16}NO_6P$

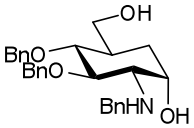
241,18

To a solution of compound **43** (78 mg, 0.16 mmol) in MeOH (5 mL) was added 10 % Pd on carbon (20 mg), and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 2 h. After filtration and lyophilization, **7** was obtained as a colorless solid (40 mg, 99 %). ^{1}H NMR (400.1 MHz, D_2O): δ = 4.89 (d, J = 3.5, 1 H, H-1), 3.96 (m, 1 H, H-5), 3.86 (m *2* H, H-6a, H-6b), 3.41 (s, 3 H, CH_3), 3.36 (ddd, J = 12.2, 5.0, 3.5, 1 H, H-2), 1.92 (m, 1 H, H-3a), 1.86 (d‘q’, J = 12.5, 4.1, 1 H, H-3b), 1.73 (d‘q’, J = 13.3, 3.1, 1 H, H-4a), 1.58 (d‘q’, J = 13.3, 4.2, 1 H, H-4b); ^{13}C NMR (100.6 MHz, D_2O): δ = 96.7 (C-1), 69.0 (C-5), 68.0 (C-6), 57.1 (CH_3), 49.9 (C-2), 25.6 (C-4), 22.8 (C-3); ^{31}P NMR (161.9 MHz, D_2O): δ = 1.20 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 239.9; Anal. Calcd for $C_7H_{16}NO_6P$: C, 34.86; H, 6.0; N, 5.81; Found: C, 34.40; H, 6.52; N, 6.82.

Synthesis of GlcN6P analog 8



(1*S*,2*S*,3*R*,4*R*,5*R*)-3,4-Di-O-benzyl-2-benzylamino-5-hydroxymethyl-cyclohexane-1,3,4-triole 12

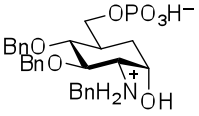


$C_{28}H_{33}NO_4$

447,57

Compound **11**[iii] (580 mg, 1.23 mmol) was dissolved in EtOH/2N NaOH (1:1, 30 mL) and stirred for 14 h under reflux. The mixture was diluted with water and extracted with EtOAc (2 x 50 mL). The organic layer was dried (Na_2SO_4) and the solvent was evaporated. Purification by FC (petroleum ether/EtOAc 1:1) yielded **12** (400 mg, 73 %) as a colorless solid. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 7.37-7.18 (m, 15 H, Ph), 4.99 (d, J = 11.7, 1 H, CH_2), 4.93 (d, J = 11.1, 1 H, CH_2), 4.72 (d, J = 11.1, 1 H, CH_2), 4.64 (d, J = 11.7, 1 H, CH_2), 4.02 (d, J = 2.9, 1 H, H-1), 3.82 (d, J = 13.1, 1 H, CH_2), 3.75 (dd, J = 10.9, 3.6, 1 H, H-7a), 3.69 ('t', J = 9.4, 1 H, H-3), 3.63 (d, J = 13.1, 1 H, CH_2), 3.57 (dd, J = 10.9, 4.5, 1 H, H-7b), 3.46 (dd, J = 10.6, 9.4, 1 H, H-4), 2.61 (dd, J = 9.9, 2.9, 1 H, H-2), 2.14 (m, 1 H, H-5), 1.94 (d't', J = 14.3, 3.6, 1 H, H-6a), 1.94 (ddd, J = 14.3, 13.0, 2.8, 1 H, H-6b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ =140.6 (quaternary C), 138.3 (quaternary C), 138.2 (quaternary C), 128.7-127.7 (aromatic C), 83.3 (C-4), 82.1 (C-3), 75.3 ($\underline{C}H_2$), 74.7 ($\underline{C}H_2$), 64.2 (C-1), 64.0 (C-7), 63.3 (C-2), 38.6 (C-5), 30.4 (C-6); (ESI-IT-MS): *m/z* $[M+H]^+$: 448.3, $[M+Na]^+$: 470.3 Anal. Calcd for $C_{28}H_{33}NO_4$: C, 75.14; H, 7.43; N, 3.13; Found C, 74.54; H, 7.66; N, 3.10.

(1*S*,2*S*,3*R*,4*R*,5*R*)-3,4-Di-*O*-benzyl-2-benzylamino-5-((phosphonooxy)methyl)-cyclo-hexane-1,3,4-triol 13



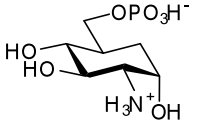
$C_{28}H_{34}NO_7P$

527,55

$POCl_3$ (160 µL, 1.74 mmol), H_2O (24 µL, 1.34 mmol), and pyridine (149 µL, 1.85 mmol) were dissolved in acetonitrile (2 mL) and stirred for 10 min at 0 °C. Compound **12** (150 mg, 0.34 mmol) was added and the mixture was stirred for 8.5 h at 0 °C. Water (4 mL) was added and the mixture was stirred for 1 h at rt followed by evaporation. Purification by RP-HPLC (semi-preparative column; 30–55 % in 10 min) followed by lyophilization yielded compound **13** (40 mg, 23 %) as a colorless solid. RP-HPLC: (semi-preparative column): t_r = 7.5 min (30–55 % B in 10 min); ^{1}H NMR (400.1 MHz, MeOD): δ = 7.44-7.23 (m, 15 H, Ph), 5.12 (d, J = 11.5, 1 H, CH_2), 4.82-4.75 (m, 2 H, CH_2), 4.75 (d, J = 11.5, 1 H, CH_2), 4.39 (d, J = 2.7, 1 H, H-1), 4.33-4.25 (m, 3 H, CH_2, H-7a), 4.06 (dd, J = 10.5, 9.1, 1 H, H-7b), 4.03 (ddd, J = 10.3, 4.9, 2.4, 1 H, H-3), 3.61 (dd, J = 10.7, 9.2, 1 H, H-4), 3.24 (dd, J = 10.4, 2.8, 1 H, H-2),

2.23 (br. ‘t’, *J* = 11.9, 1 H, H-5), 1.99 (d‘t’, *J* = 14.8, 3.5, 1 H, H-6a), 1.73 (ddd, *J* = 14.9, 13.2, 2.0, 1 H, H-6b); ^{13}C NMR (100.6 MHz, MeOD): δ =139.7 (quaternary C), 139.7 (quaternary C), 130.8 (quaternary C), 131.3-128.3 (aromatic C), 83.2 (C-4), 80.9 (C-3), 76.3 ($\underline{C}H_2$), 75.9 ($\underline{C}H_2$), 66.4 (C-7), 64.9 (C-1), 63.5 (C-2), 50.9 ($\underline{C}H_2$), 38.8 (C-5), 33.4 (C-6); ^{31}P NMR (161.9 MHz, DMSO-d_6): δ = 0.34 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 526.1; Anal. Calcd for $C_{28}H_{34}NO_7P$•TFA: C, 56.16; H: 5.50; N, 2.18; Found: C, 56.71; H, 5.81; N, 2.27.

(1*S*,2*S*,3*R*,4*R*,5*R*)-2-Amino-5-((phosphonooxy)methyl)-cyclohexane-1,3,4-triol 8



$C_7H_{16}NO_7P$

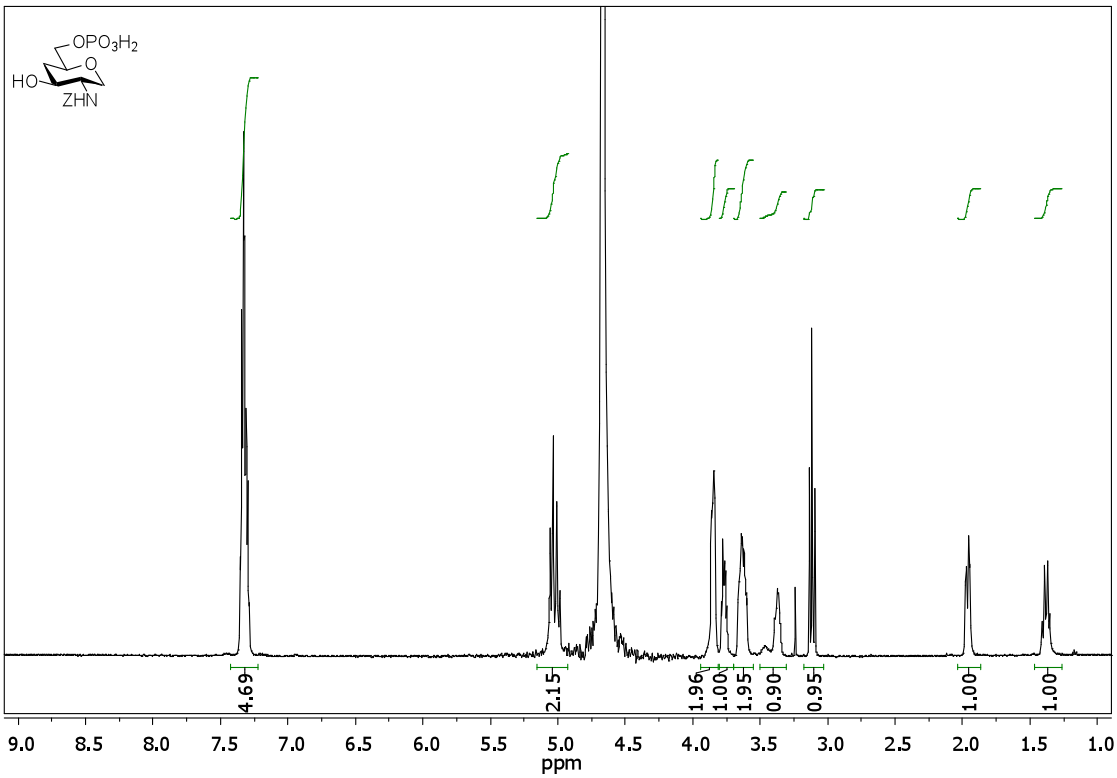
257,18

To a solution of compound **13** (40 mg, 0.068 mmol) in MeOH (2 mL) was added 10 % Pd on carbon catalyst (10 mg) and the mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at rt for 4 h. After filtration and lyophilization, compound **8** was obtained as a colorless solid (16 mg, 98 %). ^{1}H NMR (400.1 MHz, D_2O): δ = 4.34 (‘q’, *J* = 3.03, 1 H, H-1), 4.11 (d‘t’, *J* = 10.5, 5.1, 1 H, H-7a), 4.00 (ddd, *J* = 10.2, 5.6, 2.7, 1 H, H-7b), 3.80 (dd, *J* = 10.7, 9.1, 1 H, H-3), 3.51 (dd, *J* = 10.7, 9.4, 1 H, H-4), 3.28 (dd, *J* = 10.7, 2.9, 1 H, H-2), 2.21.2.07 (m 1 H, H-5), 2.04 (d‘t’, *J* = 14.9, 3.7, 1 H, H-6a), 1.75 (ddd, *J* = 15.3, 13.1, 2.4, 1 H, H-6b); ^{13}C NMR (100.6 MHz, D_2O): δ = 72.3 (C-4), 71.2 (C-3), 65.3 (C-7), 65.2 (C-1), 56.5 (C-2), 37.1 (C-5), 31.3 (C-6); ^{31}P NMR (161.9 MHz, D_2O): δ = -0.74 (s, 1 P); (ESI-IT-MS): *m/z* $[M–H]^-$: 256.0; Anal. Calcd for $C_7H_{16}NO_7P$: C, 32.69; H: 6.27; N, 5.45; Found: C, 32.68; H, 5.81; N, 5.33.

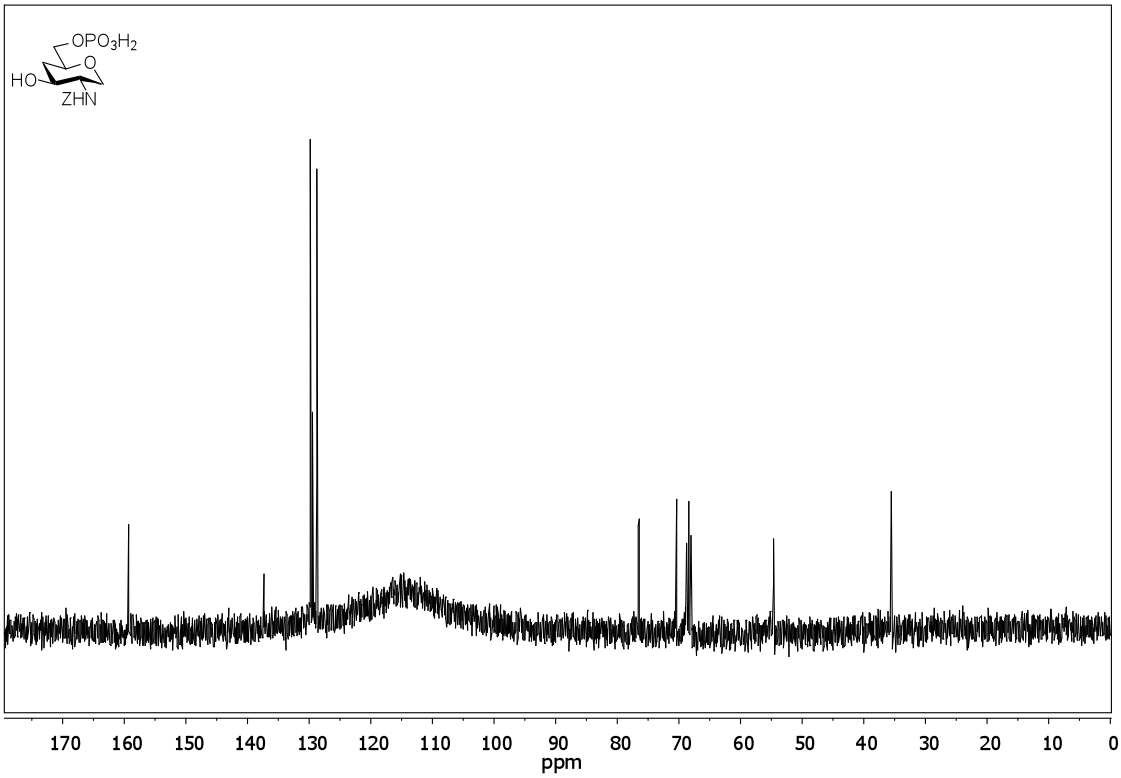
Synthesis of GlcN6P analog 9

GlcN6P analog **9** was prepared according to a published procedure.[vi]

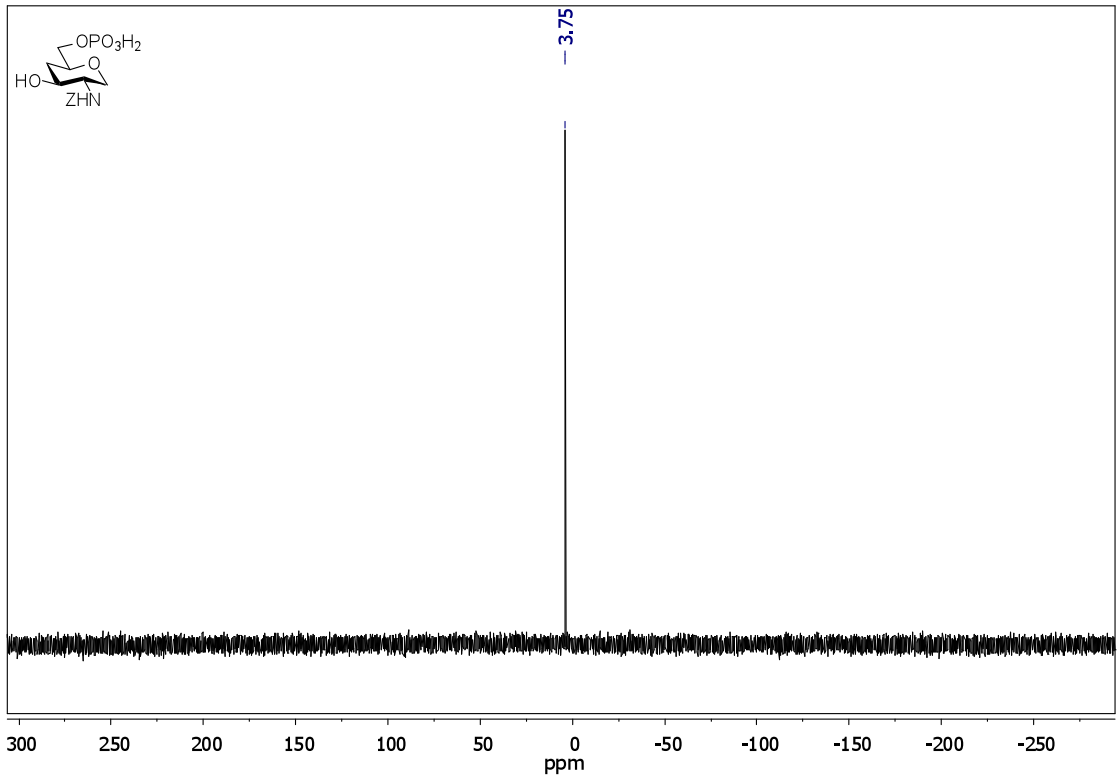
(i) Kozono, T.; N-Alkyl-1-deoxyglucosamine derivative, *Patent JP61257980*, **1986**.
(ii) van den Berg, R. J. B. H. N.; Noort, D.; van der Marel, G. A.; van Boom, J. H.; Benschop, H. P. *J. Carbohydr. Chem.* **2002**, *21*, 167-188.
(iii) Barton, D. H. R.; Augy-Dorey, S.; Camara, J.; Dalko, P.; Delaumény, J. M.; Géro, S. D.; Quiclet-Sire, B.; Stütz, P. *Tetrahedron* **1990**, *46*, 215-230.
(iv) Leder, I. G. *J. Carbohydr. Chem.* **1988**, *7*, 583-592.
(v) Yasuda, N.; Matsuda, K.; Tsutsumi, H.; Takaya, T. *Carbohydr. Res.* **1986**, *146*, 51-61.
(vi) Miyamoto, M.; Baker, M. L.; Lewis, M. D. *Tetrahedron Lett.* **1992**, *33*, 3725-3728.



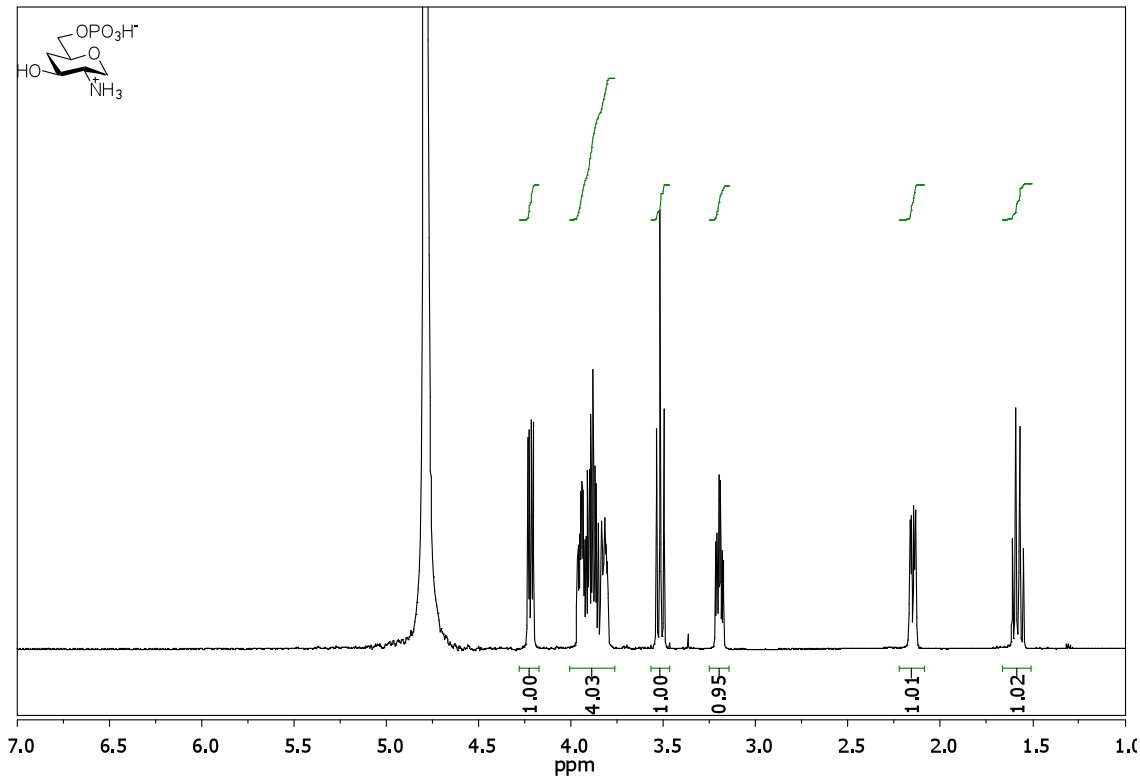
^{1}H NMR (600.1 MHz, D_2O) of compound **18**



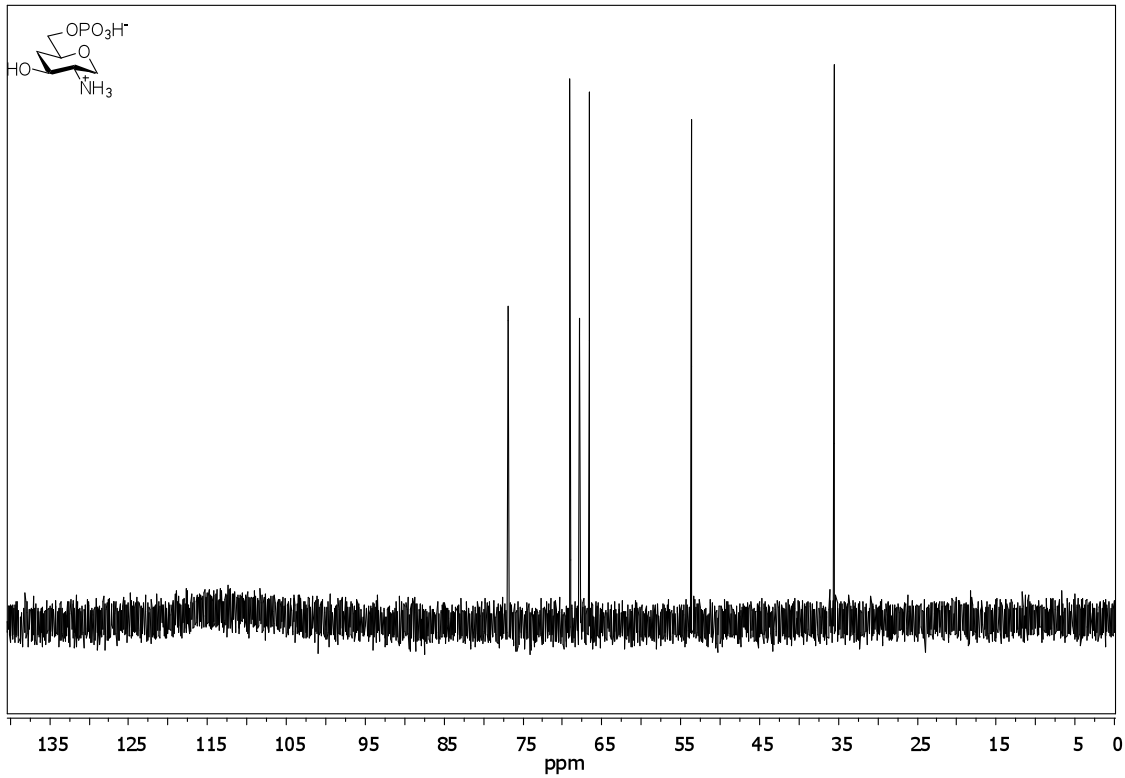
^{13}C NMR (150.9 MHz, D_2O) of compound **18**



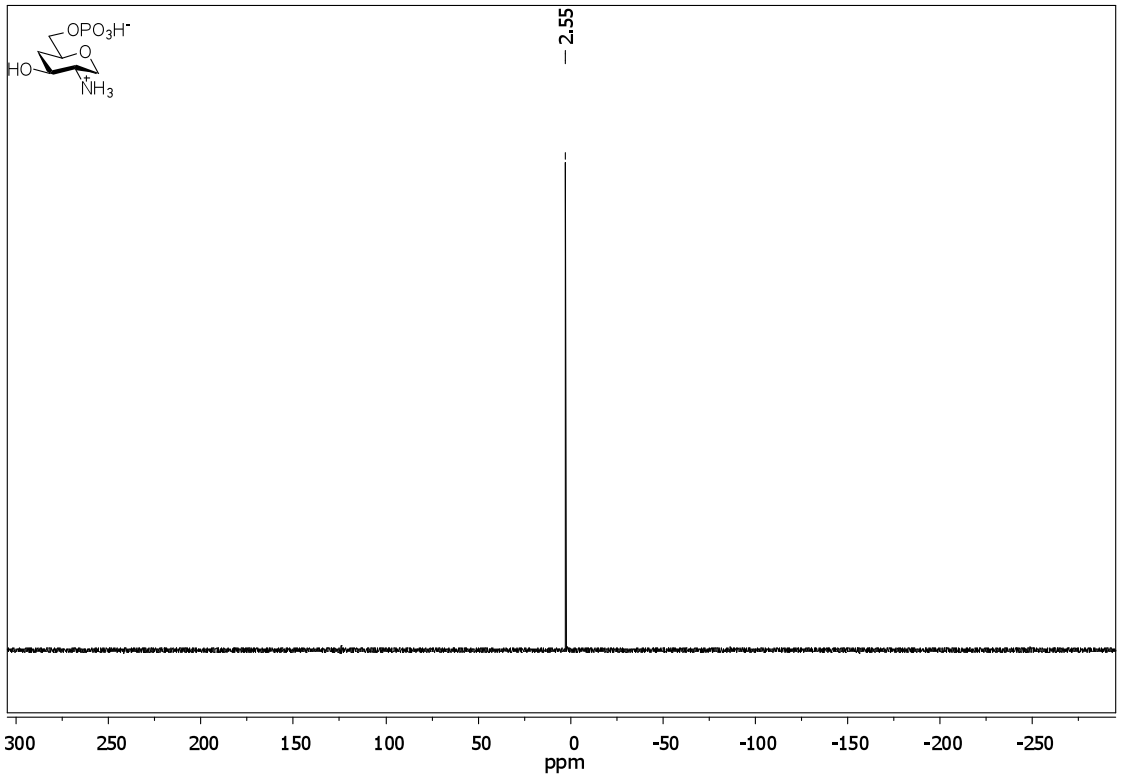
^{31}P NMR (161.9 MHz, D_2O) of compound **18**



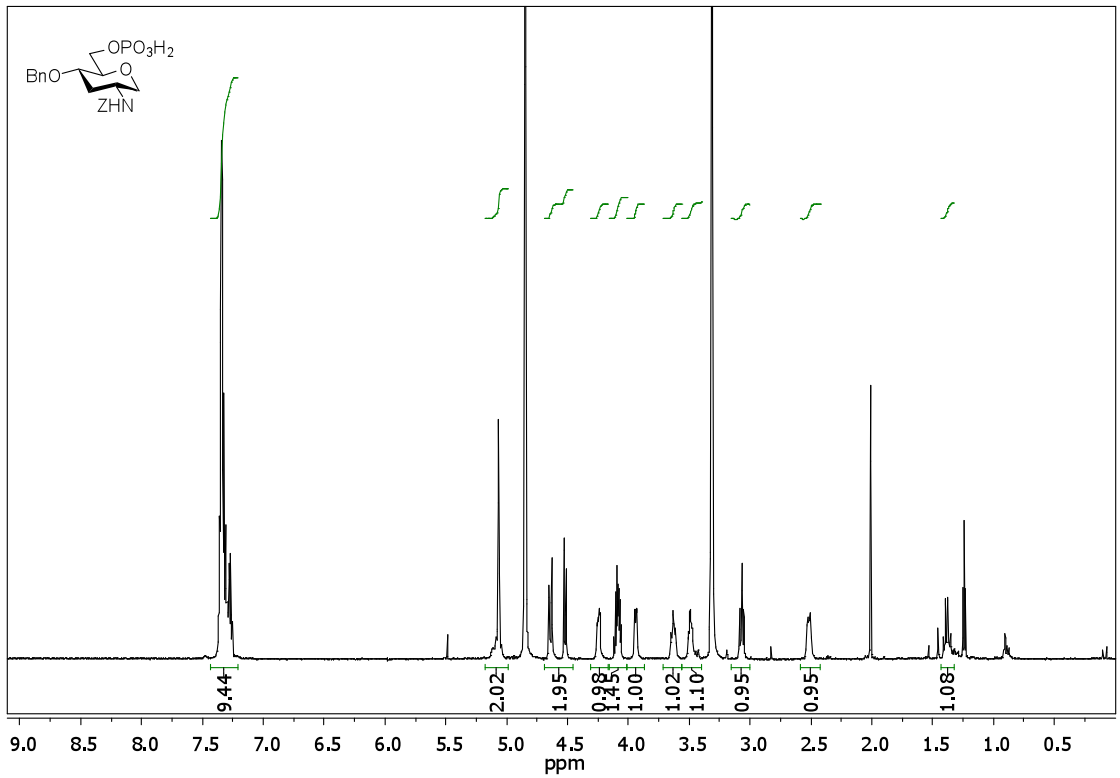
^{1}H NMR (600.1 MHz, D_2O) of compound **1**



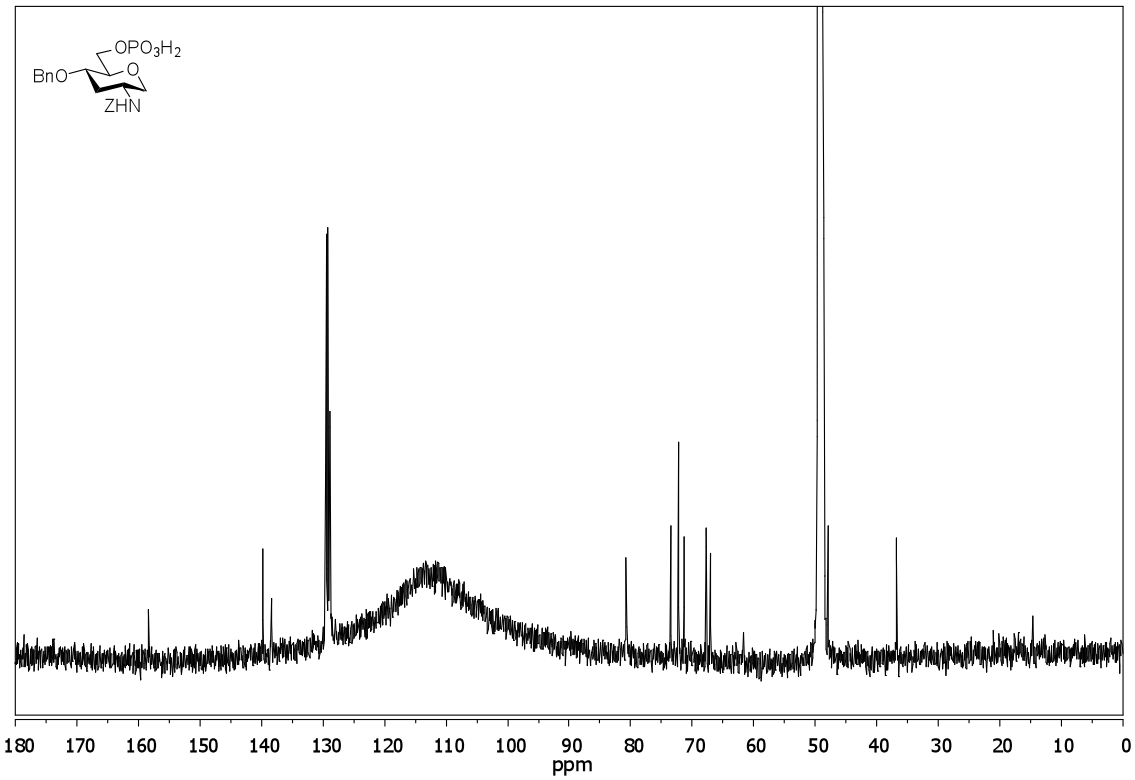
^{13}C NMR (150.9 MHz, D_2O) of compound **1**



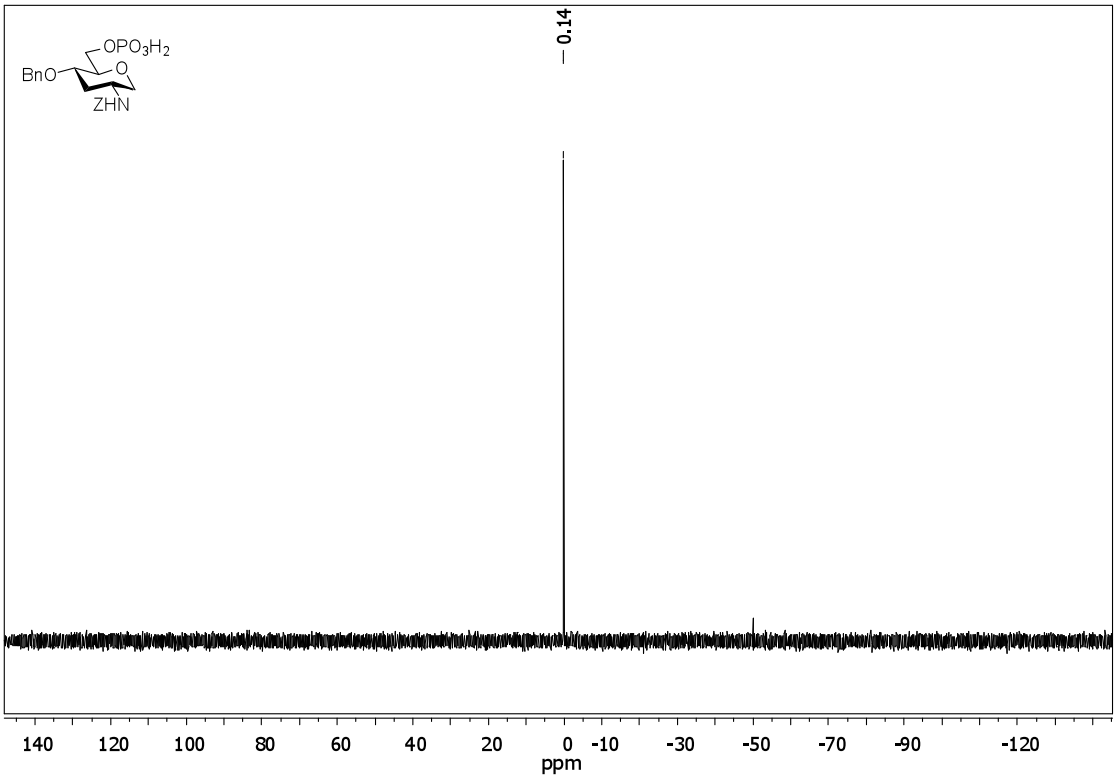
^{31}P NMR (161.9 MHz, D_2O) of compound **1**



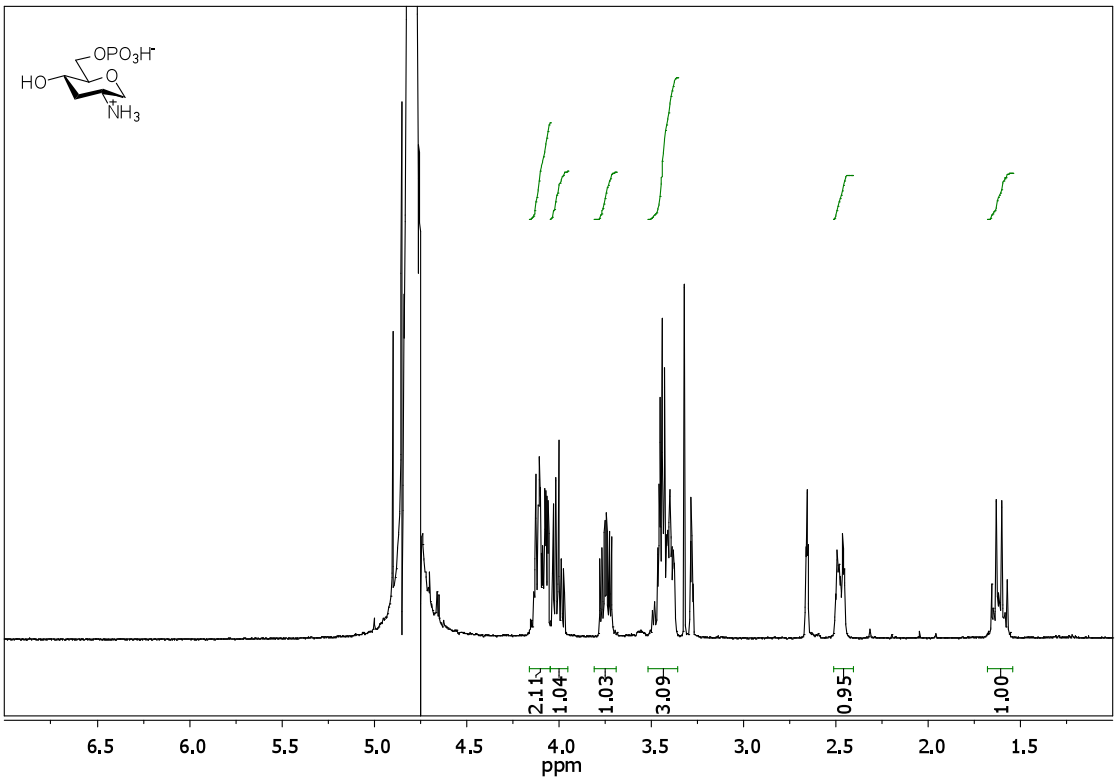
^{1}H NMR (400.1 MHz, D_3COD) of compound **22**



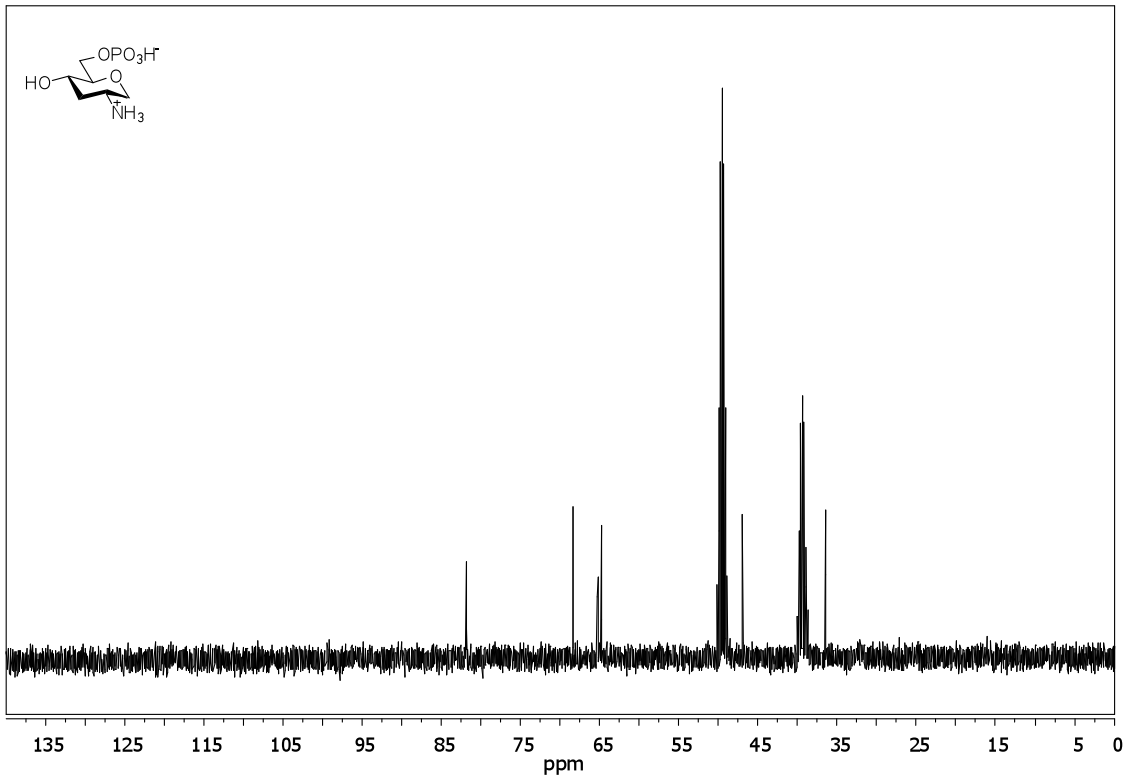
^{13}C NMR (100.6 MHz, D_3COD) of compound **22**



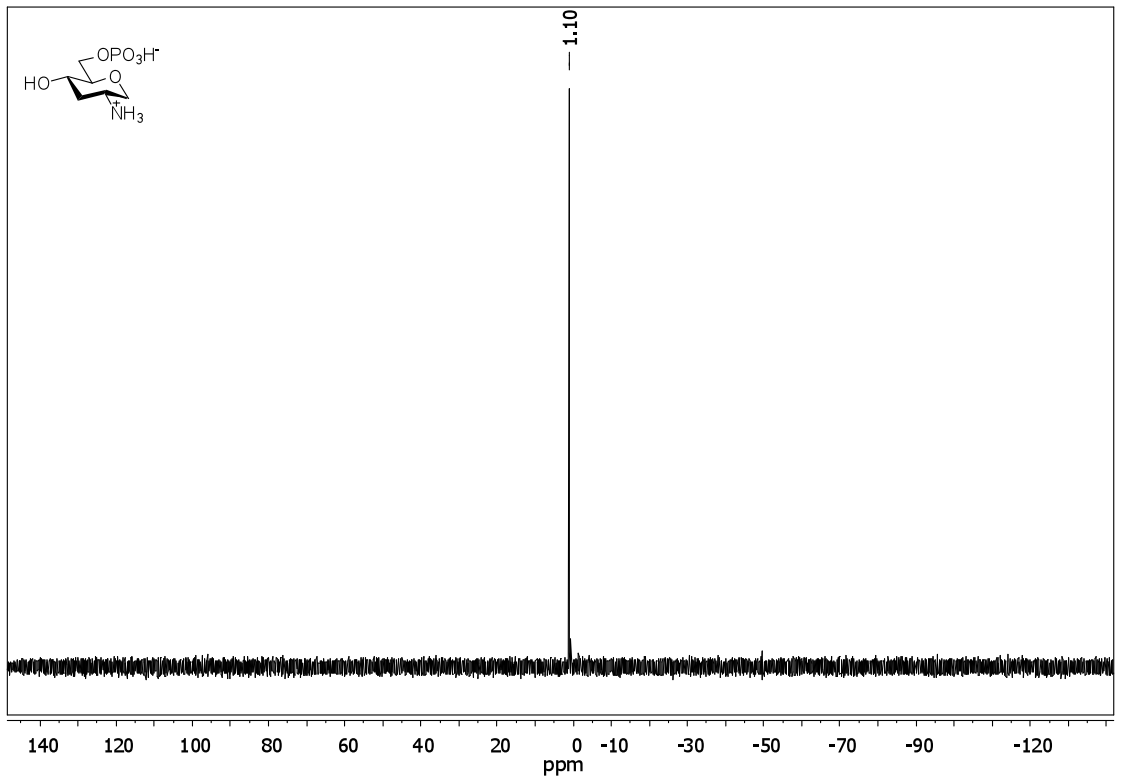
^{31}P NMR (161.9 MHz, D_3COD) of compound **22**



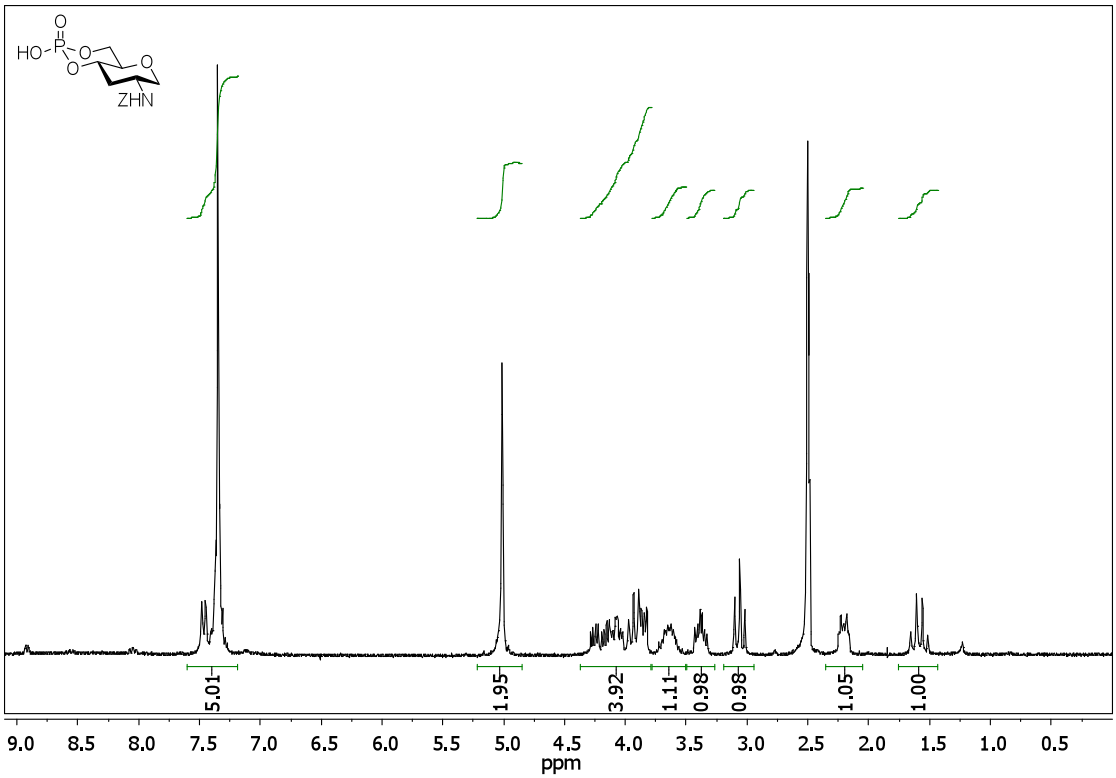
^{1}H NMR (400.1 MHz, D_3COD) of compound **2**



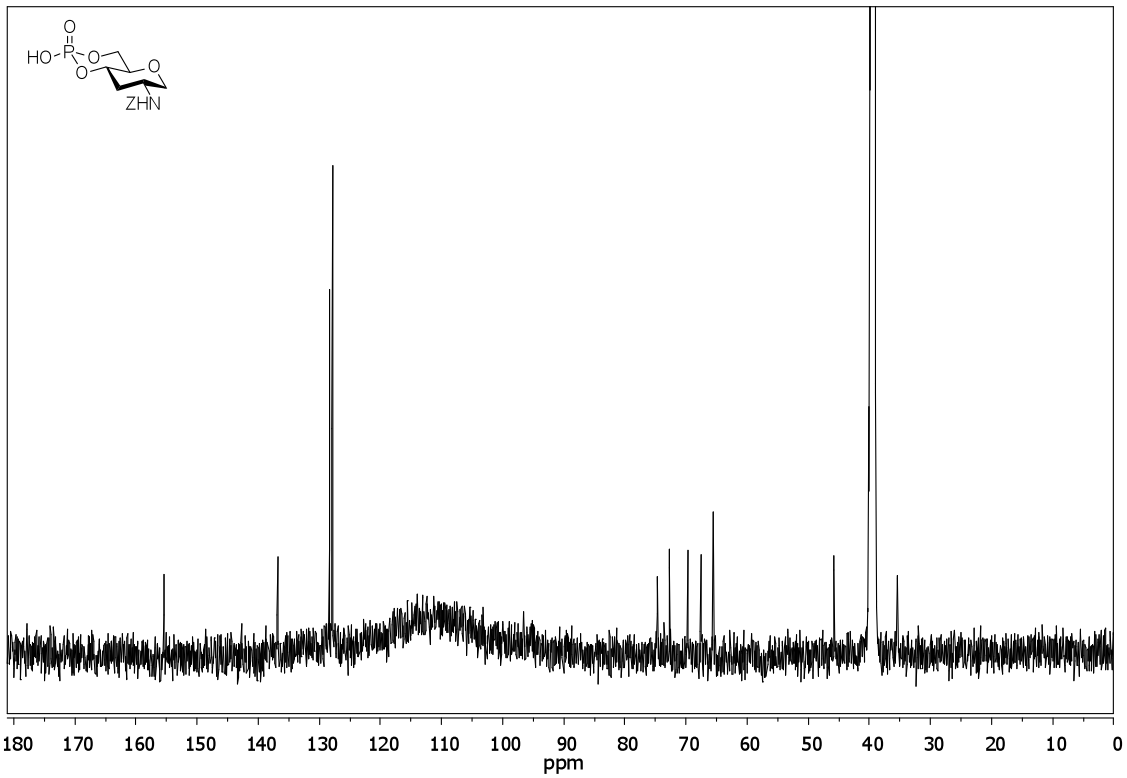
^{13}C NMR (100.6 MHz, D_3COD) of compound **2**



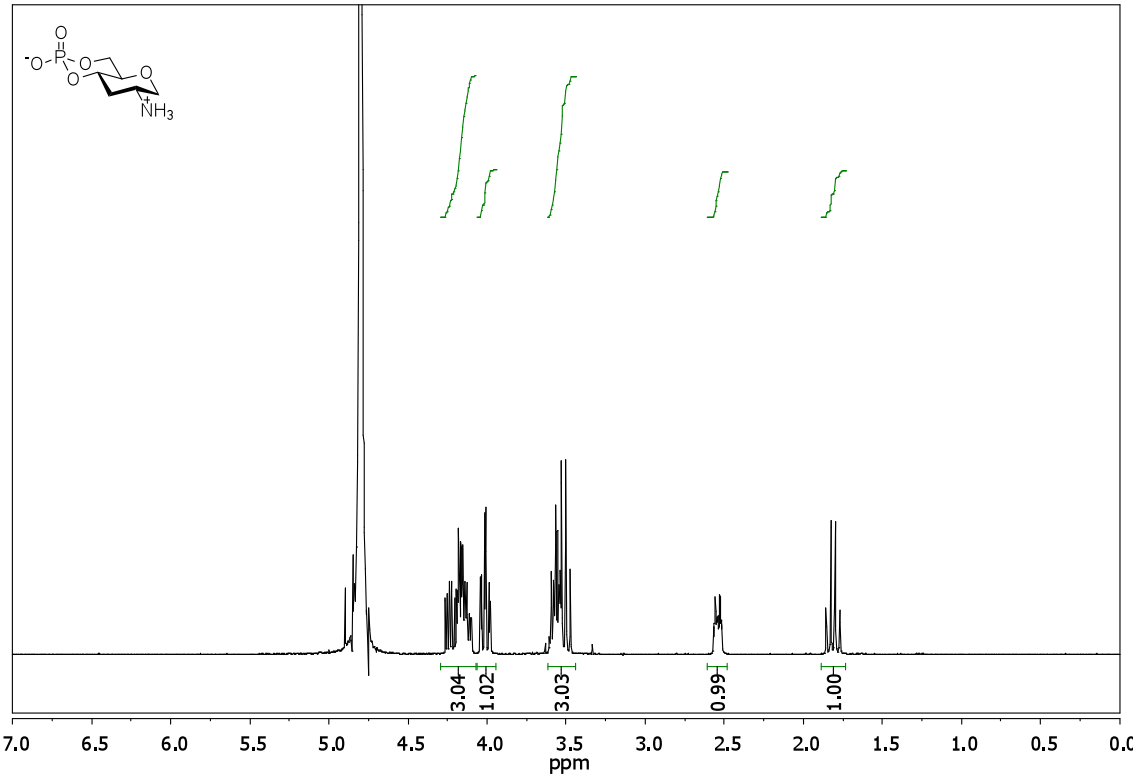
^{31}P NMR (161.9 MHz, D_3COD) of compound **2**



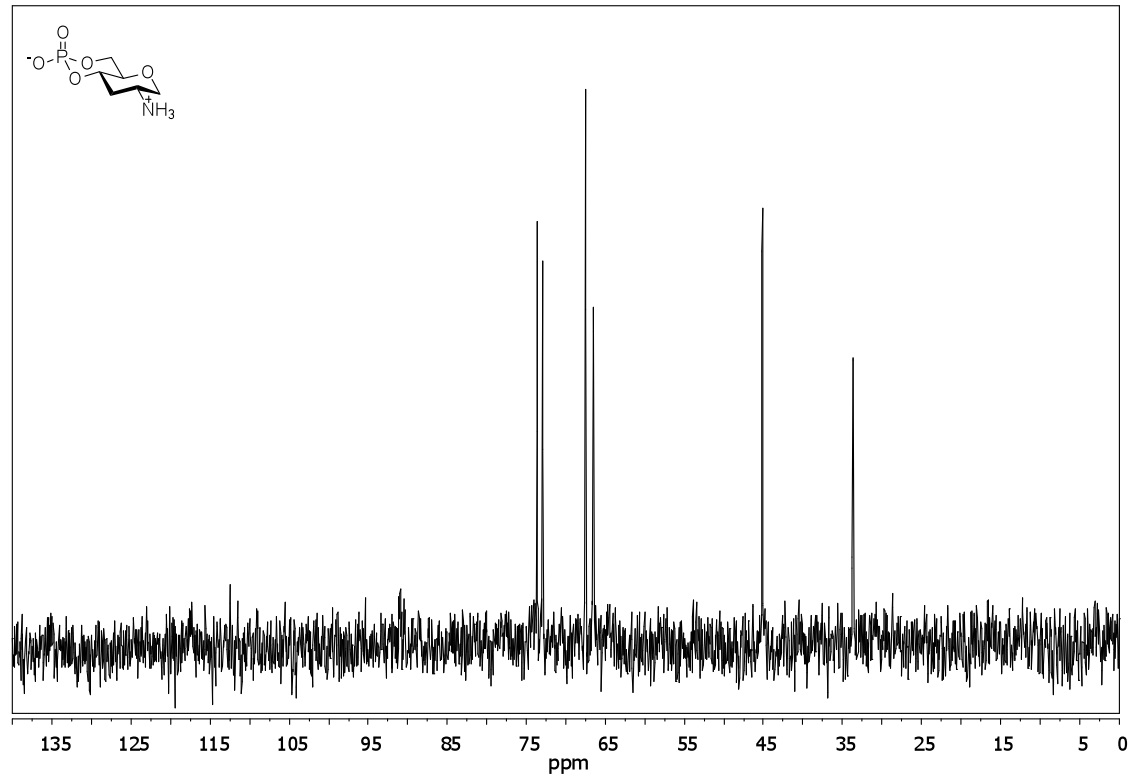
^{1}H NMR (600.1 MHz, DMSO-d_6) of compound **26**



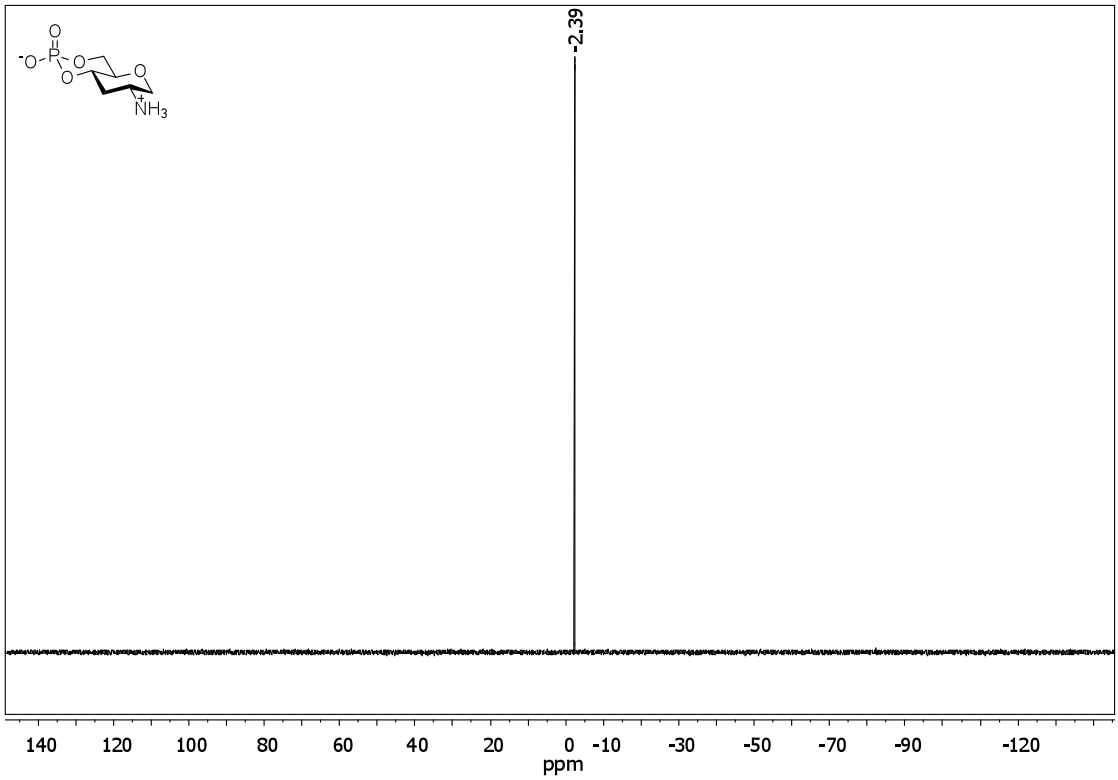
^{13}C NMR (150.9 MHz, DMSO-d_6) of compound **26**



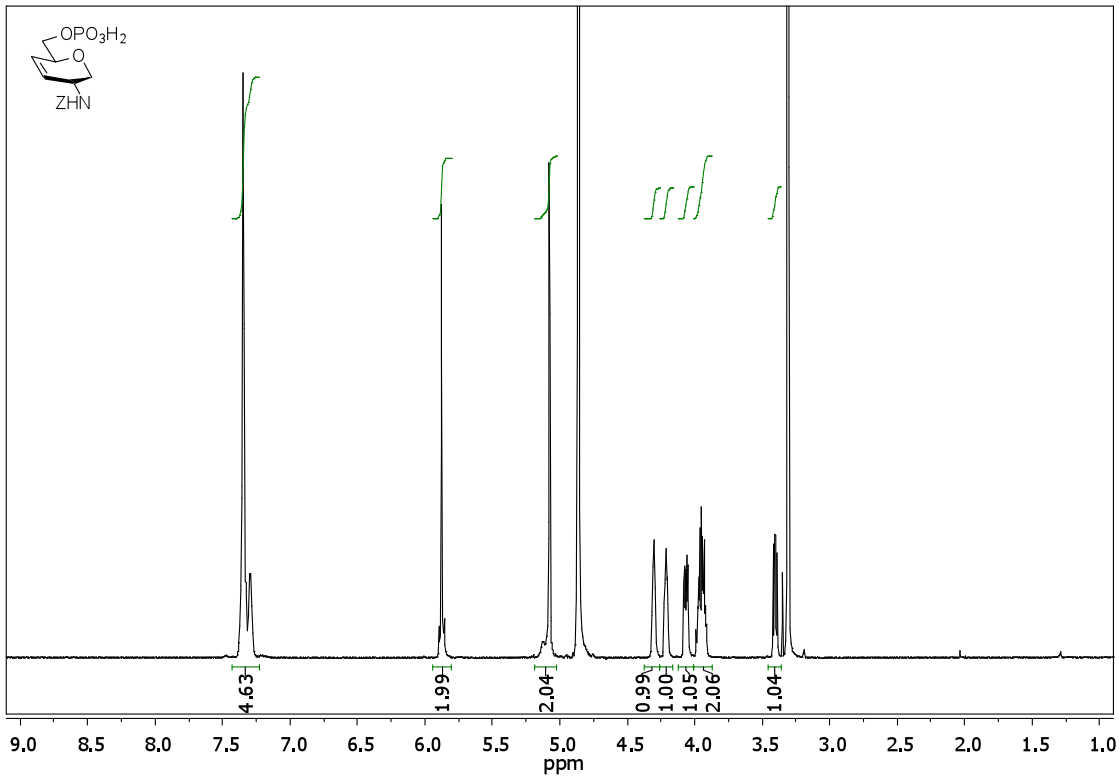
^{1}H NMR (400.1 MHz, D_2O) of compound **3**



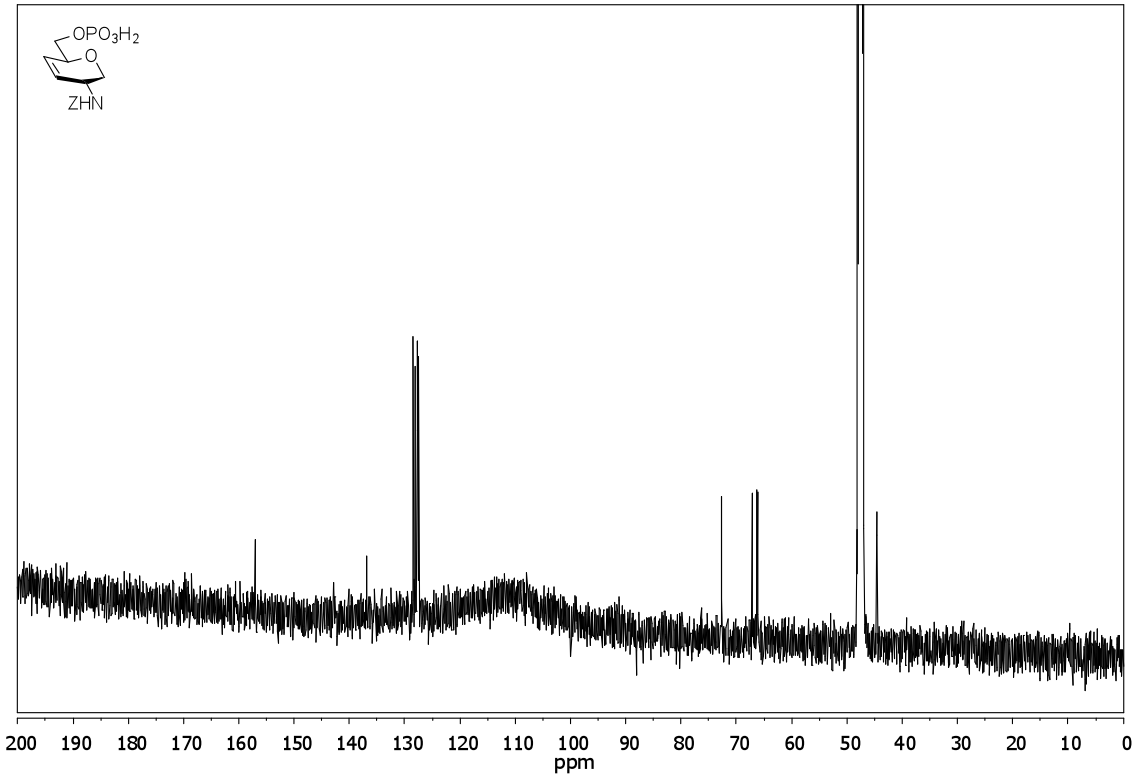
^{13}C NMR (100.6 MHz, D_2O) of compound **3**



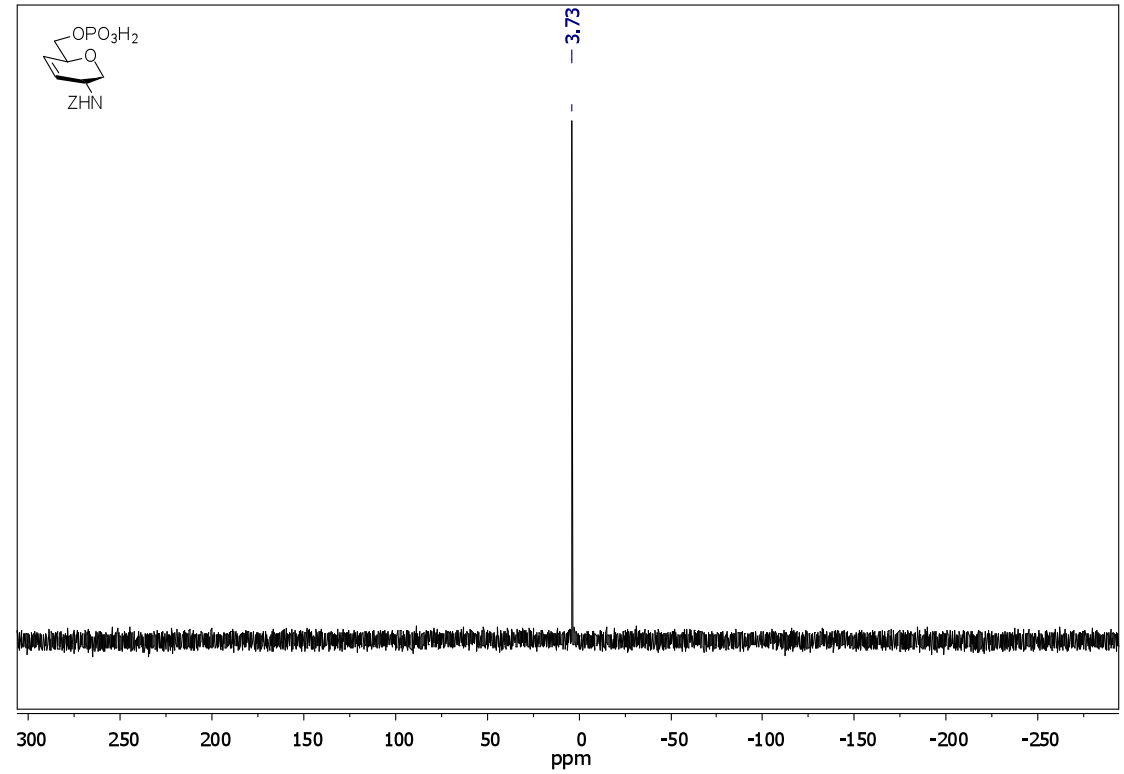
^{31}P NMR (161.9 MHz, D_2O) of compound **3**



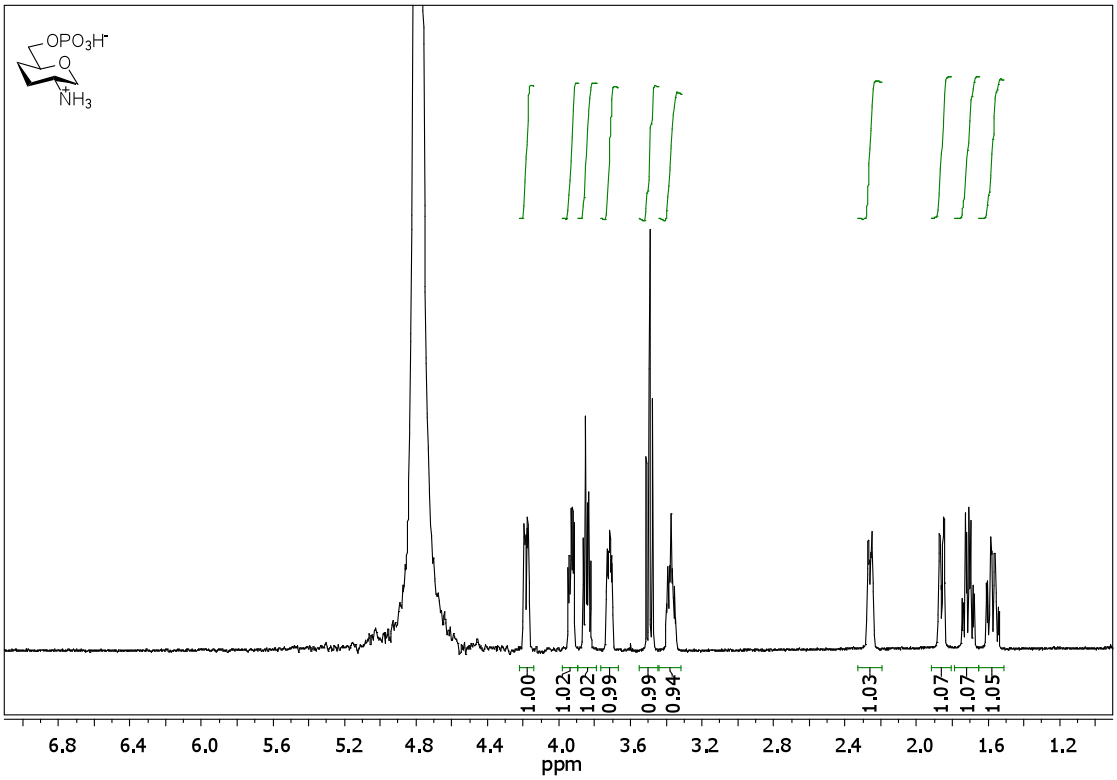
^{1}H NMR (600.1 MHz, CD_3OD) of compound **31**



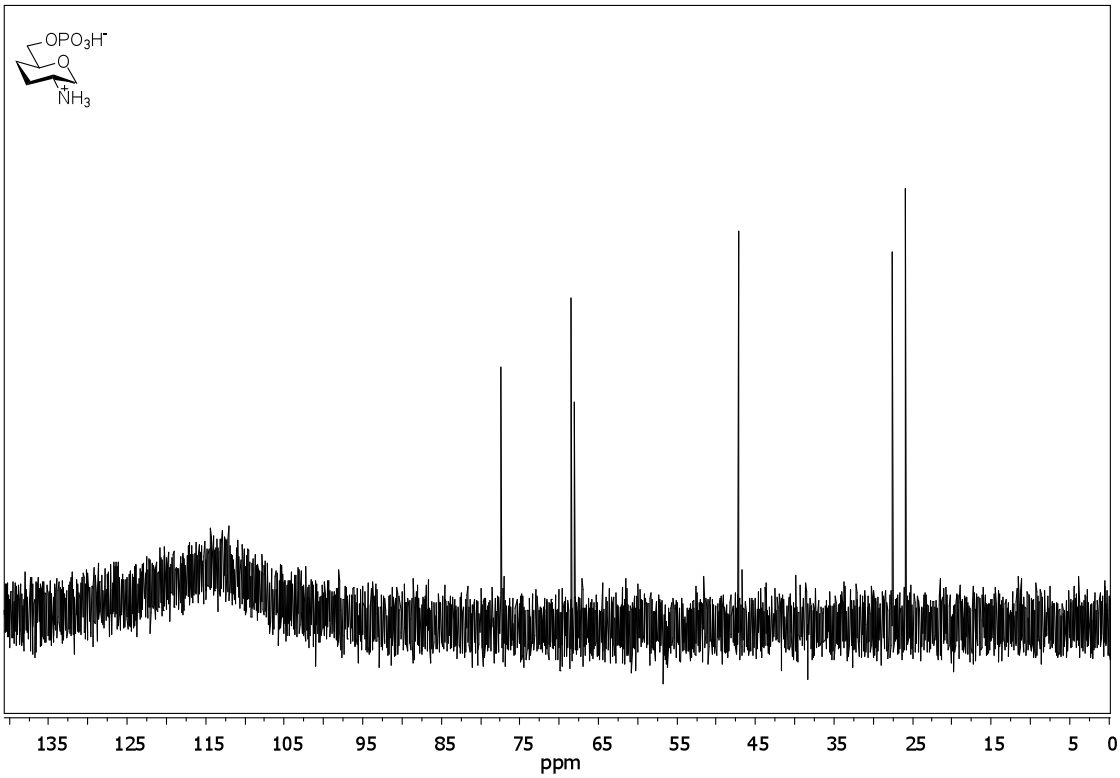
^{13}C NMR (150.9 MHz, CD_3OD) of compound **31**



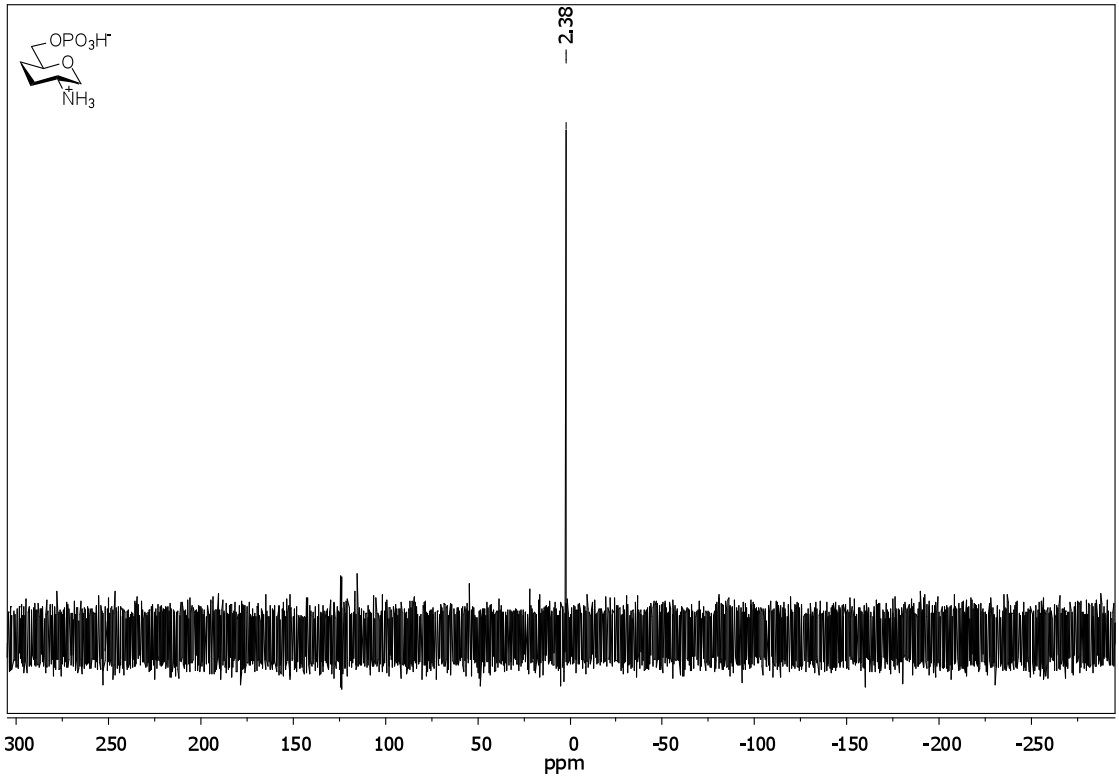
^{31}P NMR (161.9 MHz, CD_3OD) of compound **31**



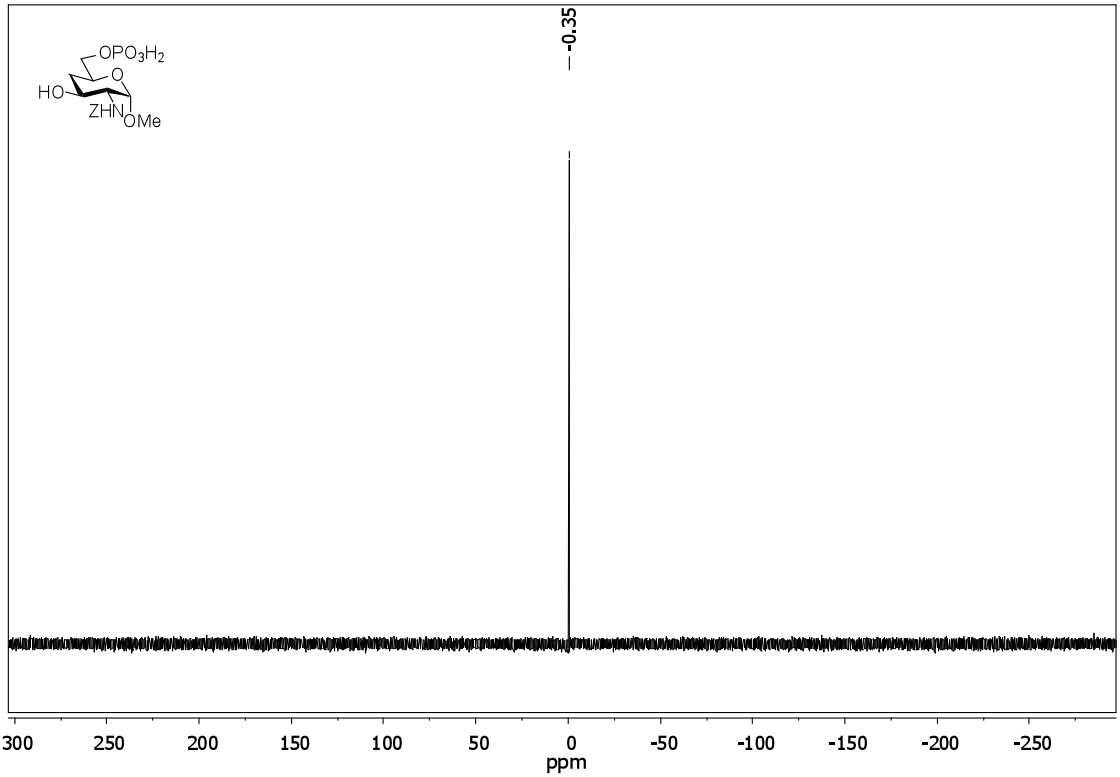
^{1}H NMR (600.1 MHz, D_2O) of compound **4**



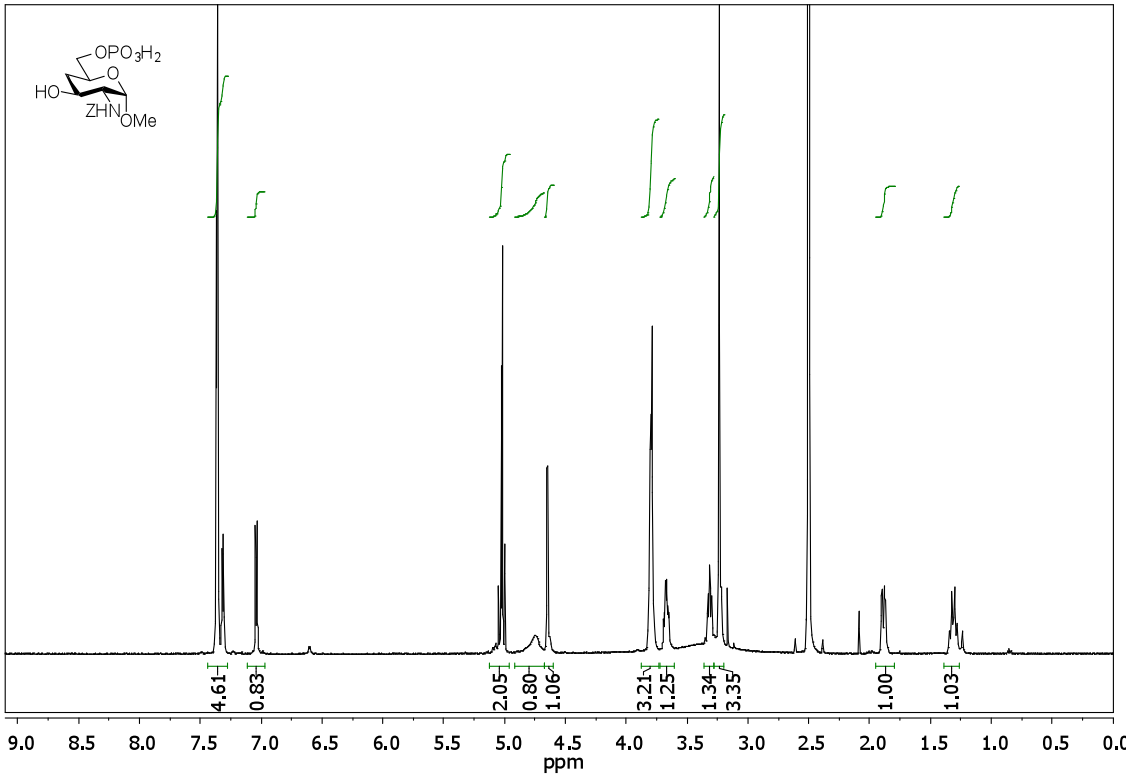
^{13}C NMR (150.9 MHz, D_2O) of compound **4**



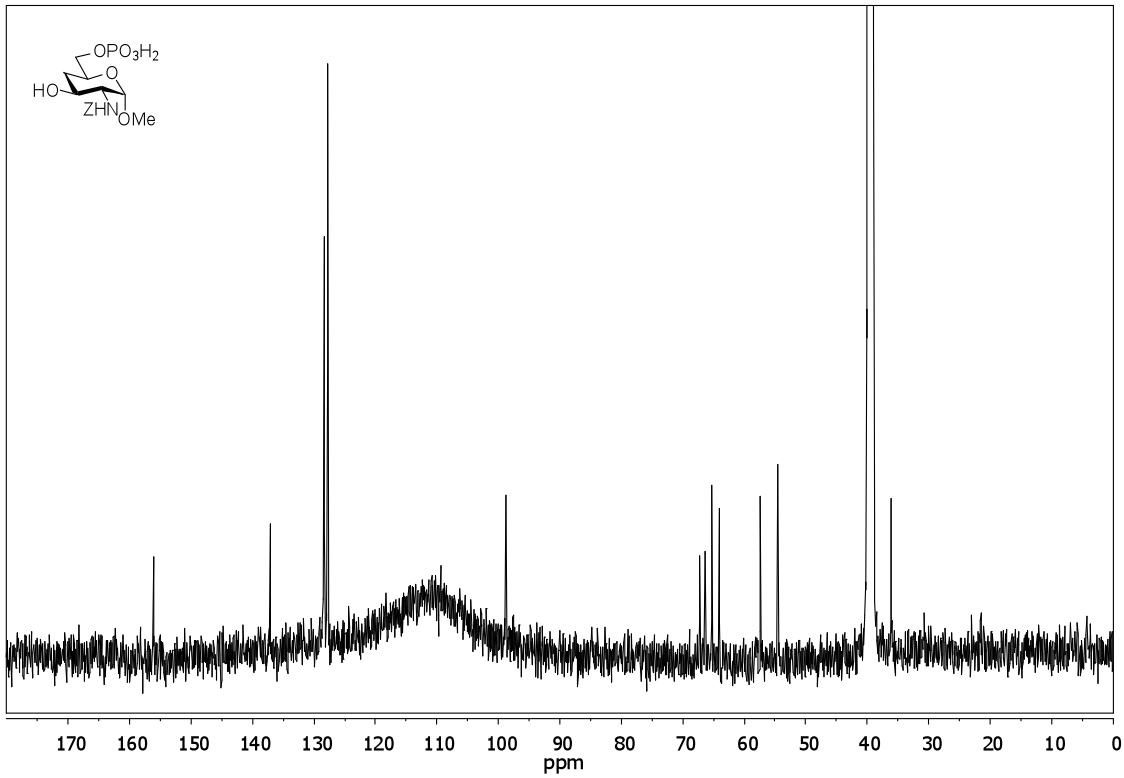
^{31}P NMR (161.9 MHz, DMSO-d_6) of compound **4**



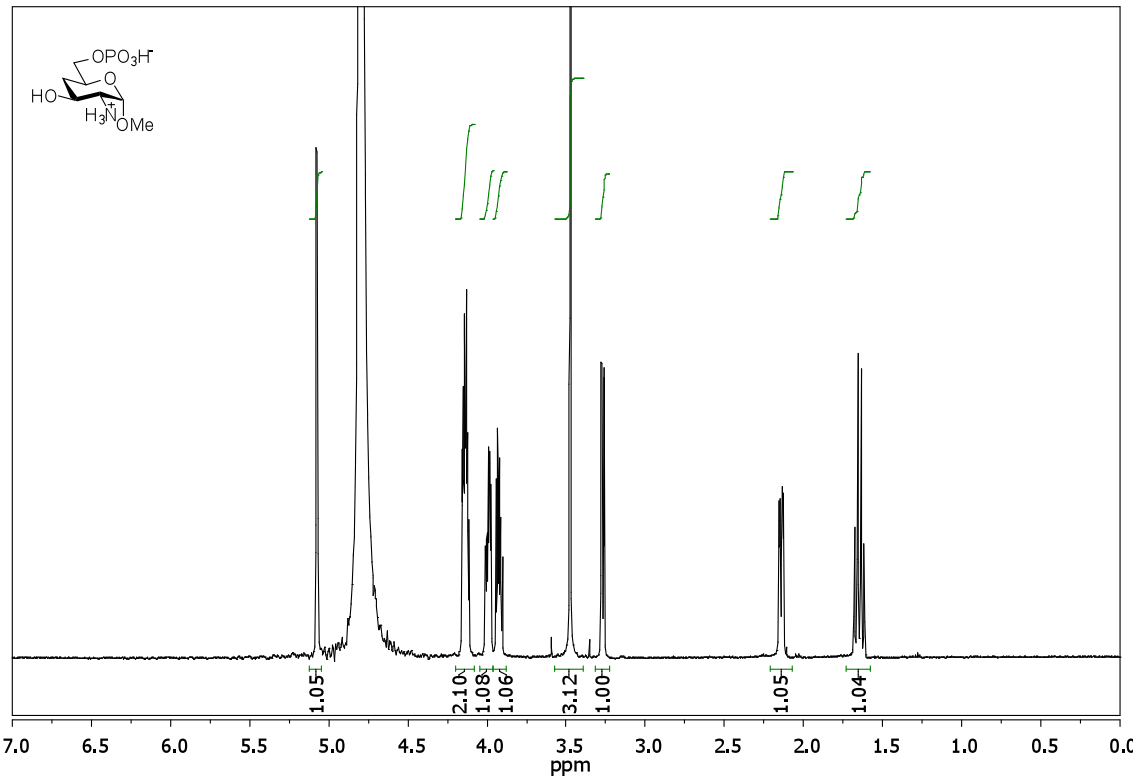
^{31}P NMR (161.9 MHz, DMSO-d_6) of compound **36**



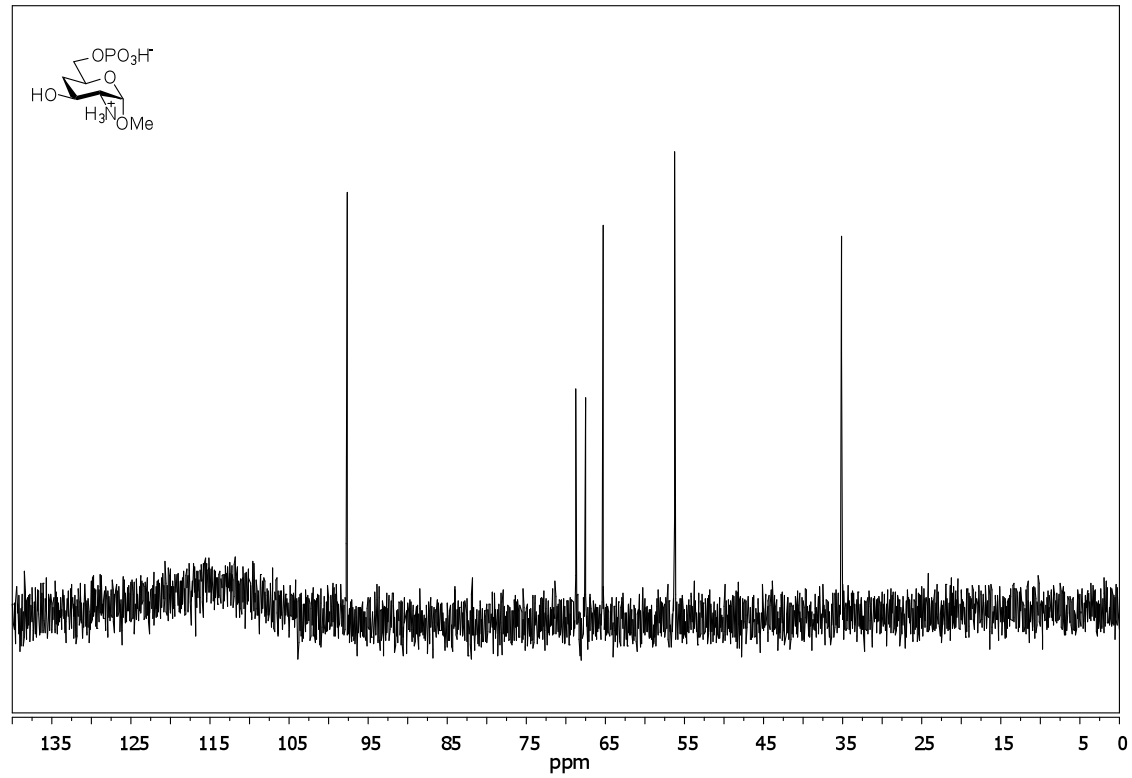
^{1}H NMR (600.1 MHz, DMSO-d_6) of compound **36**



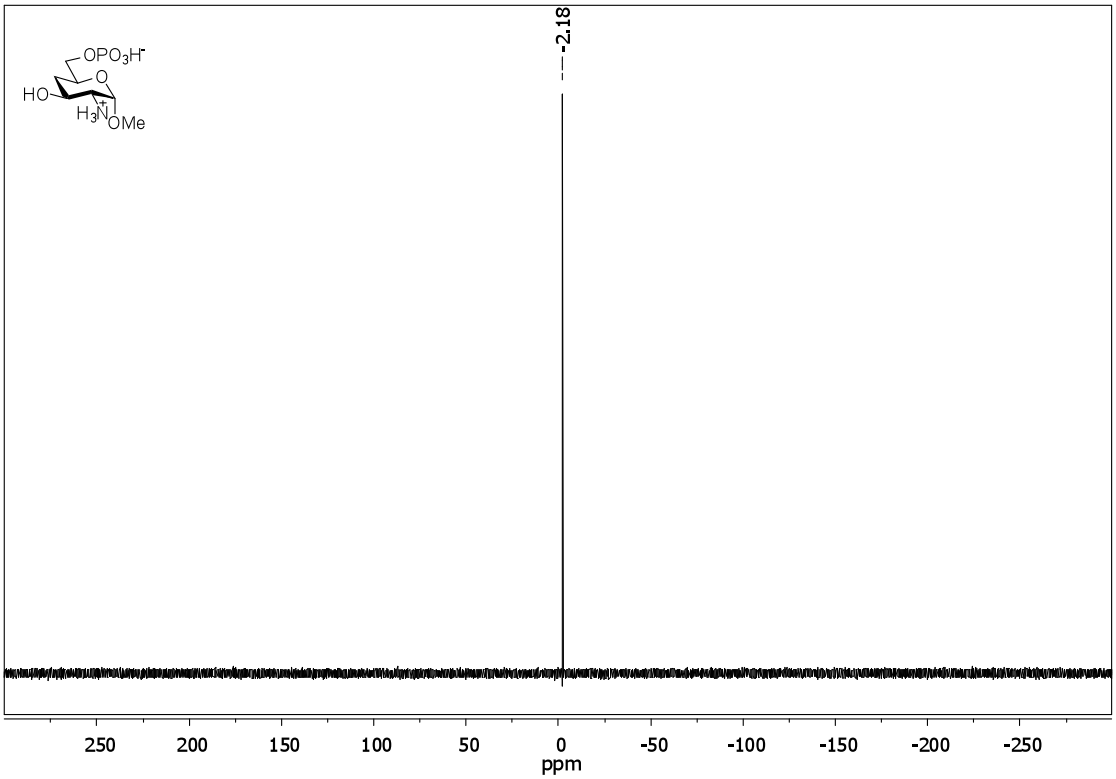
^{13}C NMR (150.9 MHz, DMSO-d_6) of compound **36**



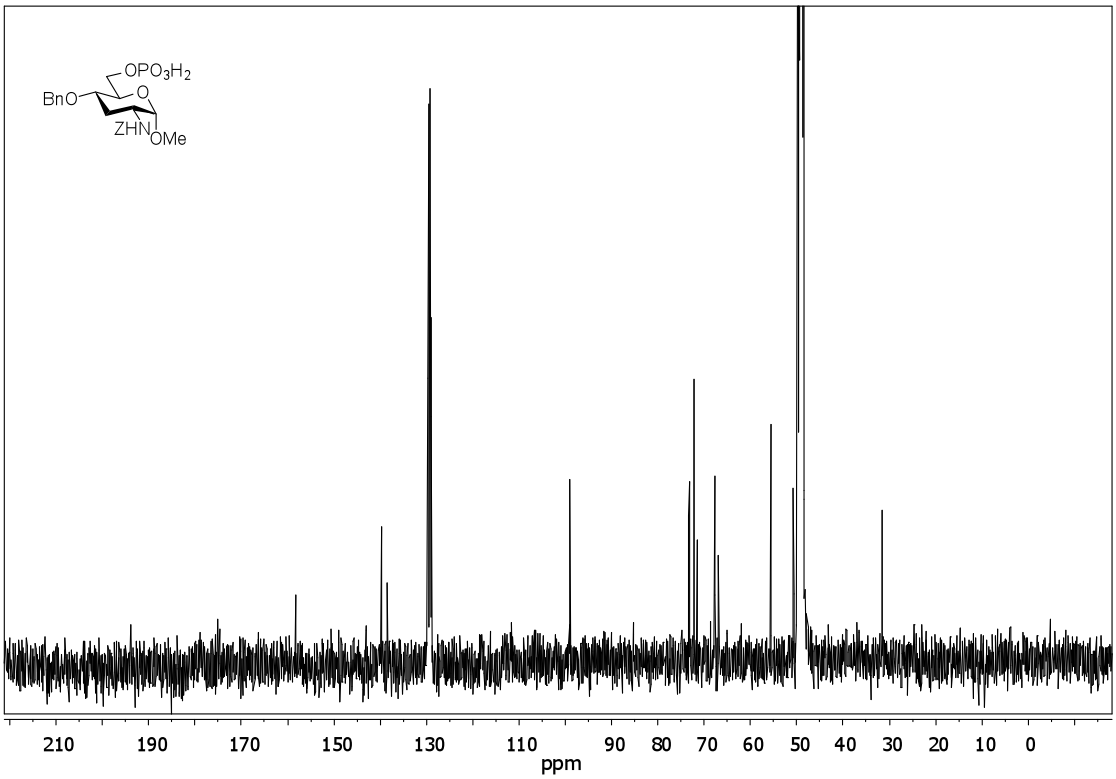
^{1}H NMR (600.1 MHz, D_2O) of compound **5**



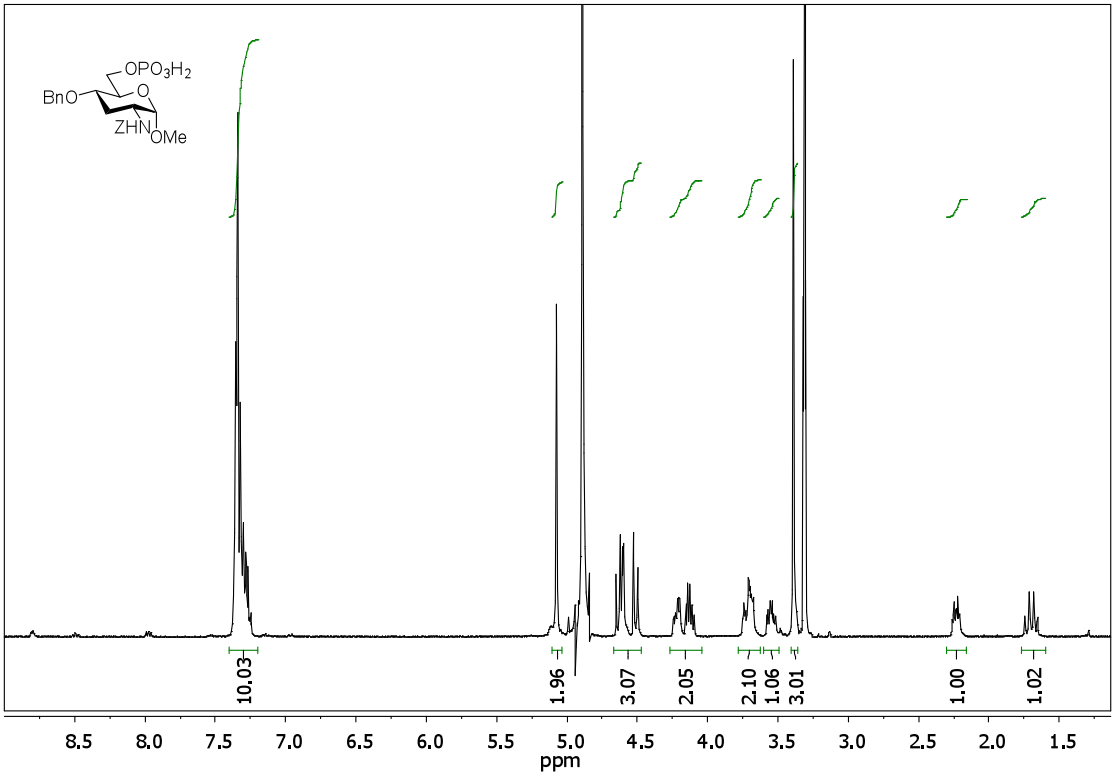
^{13}C NMR (150.9 MHz, D_2O) of compound **5**



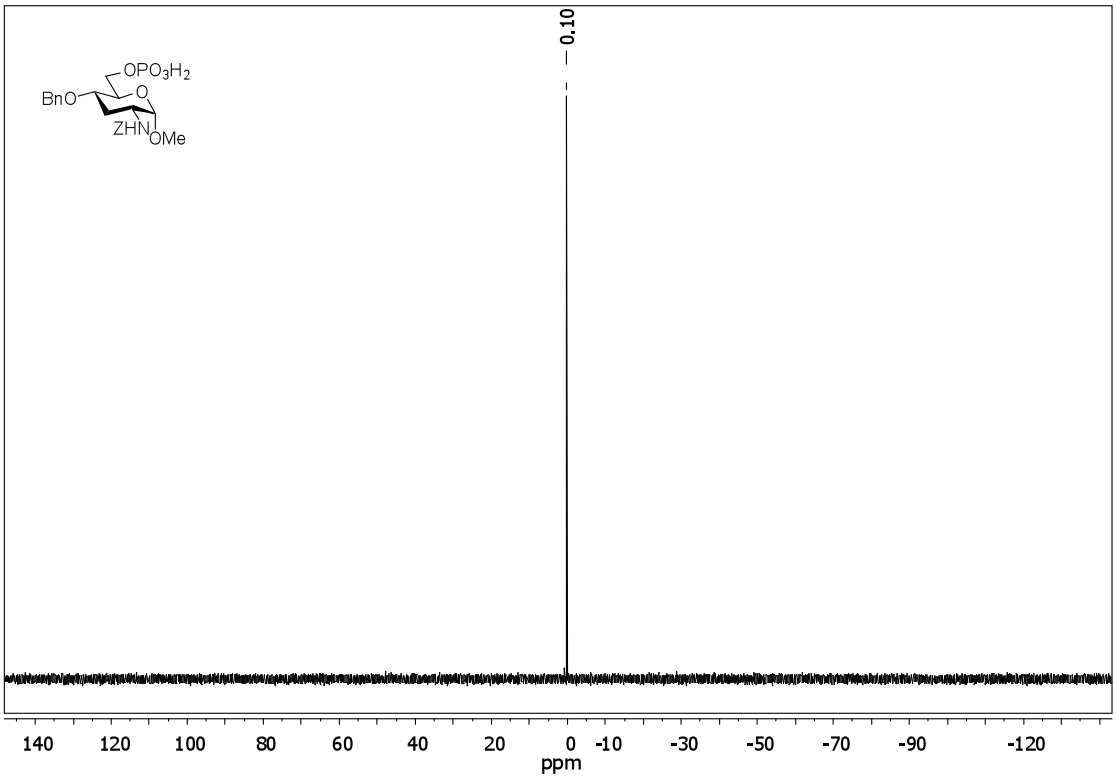
^{31}P NMR (161.9 MHz, D_2O) of compound **5**



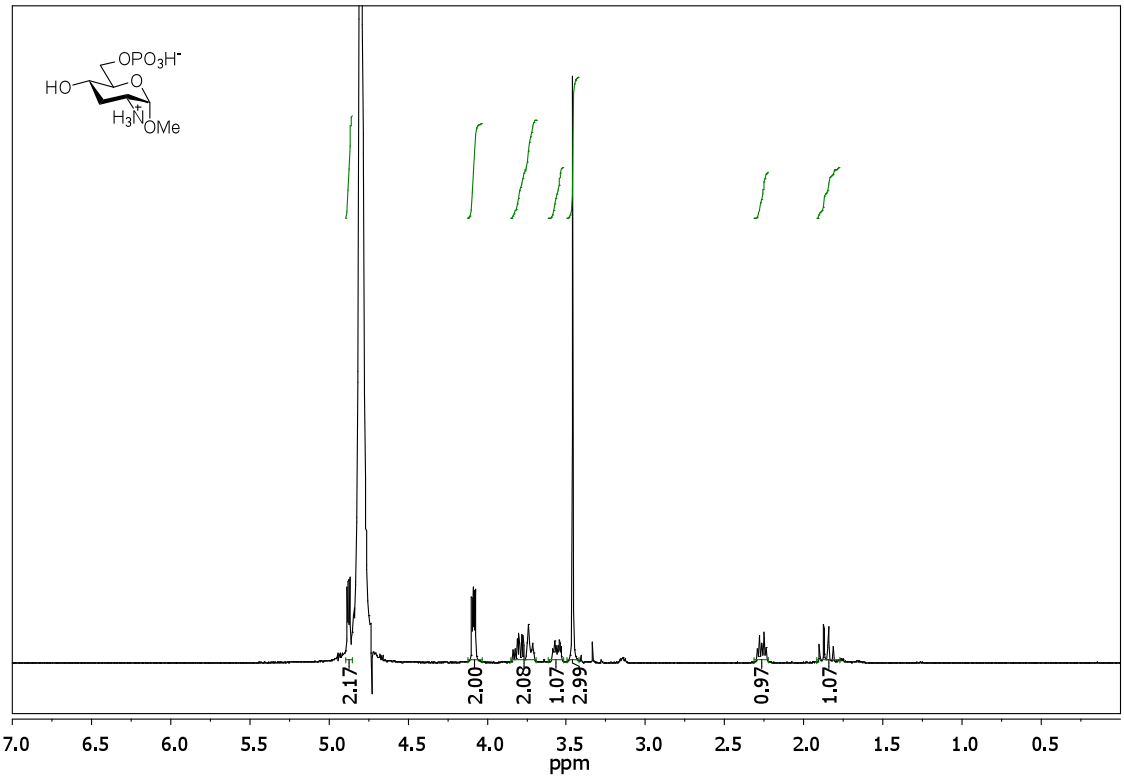
^{13}C NMR (100.6 MHz, D_3COD) of compound **40**



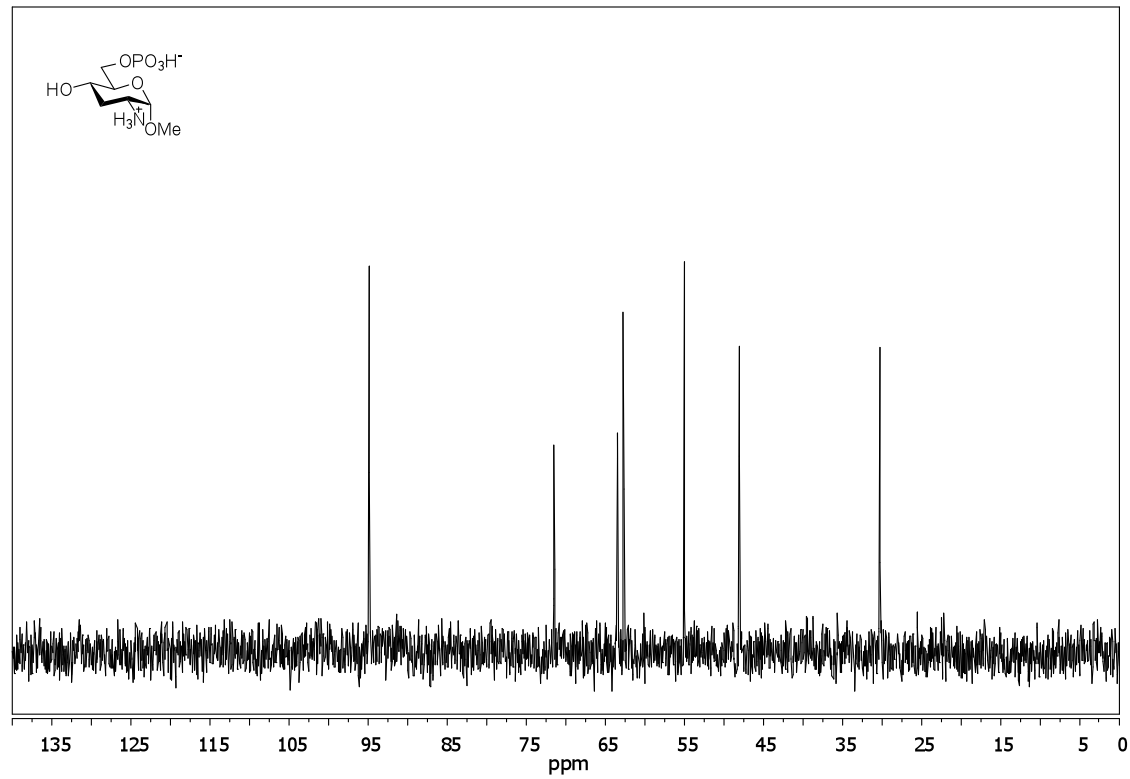
^{1}H NMR (400.1 MHz, D_3COD) of compound **40**



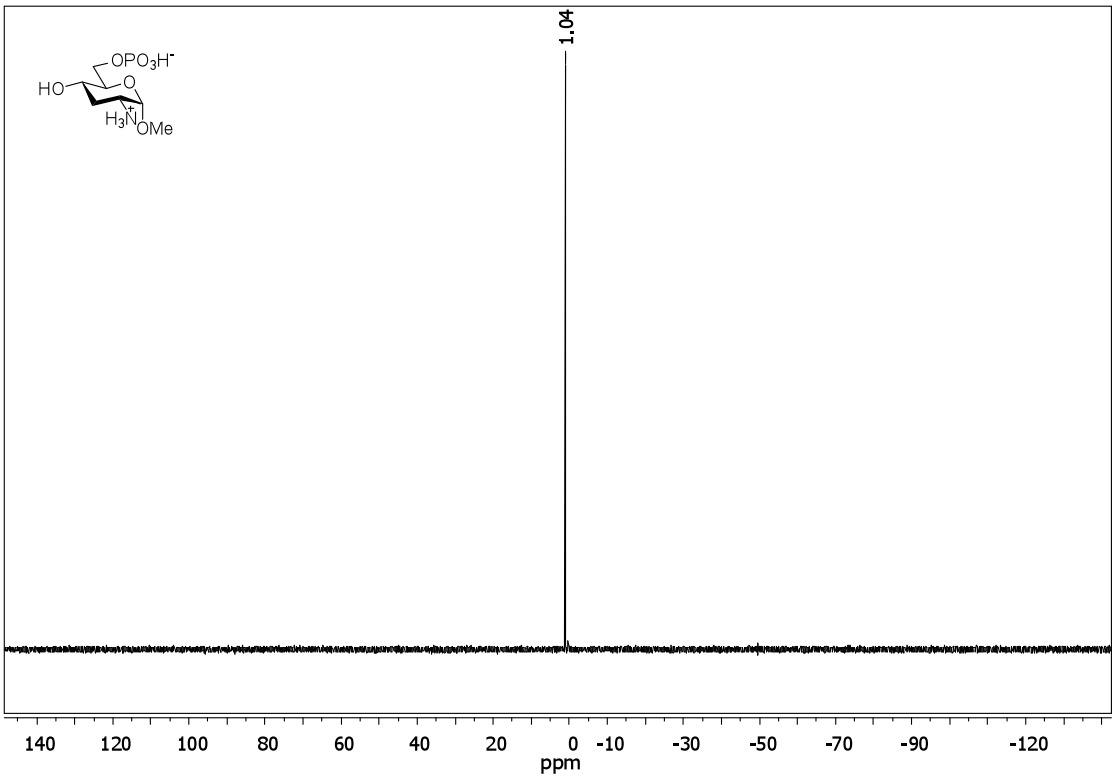
^{31}P NMR (161.9 MHz, D_3COD) of compound **40**



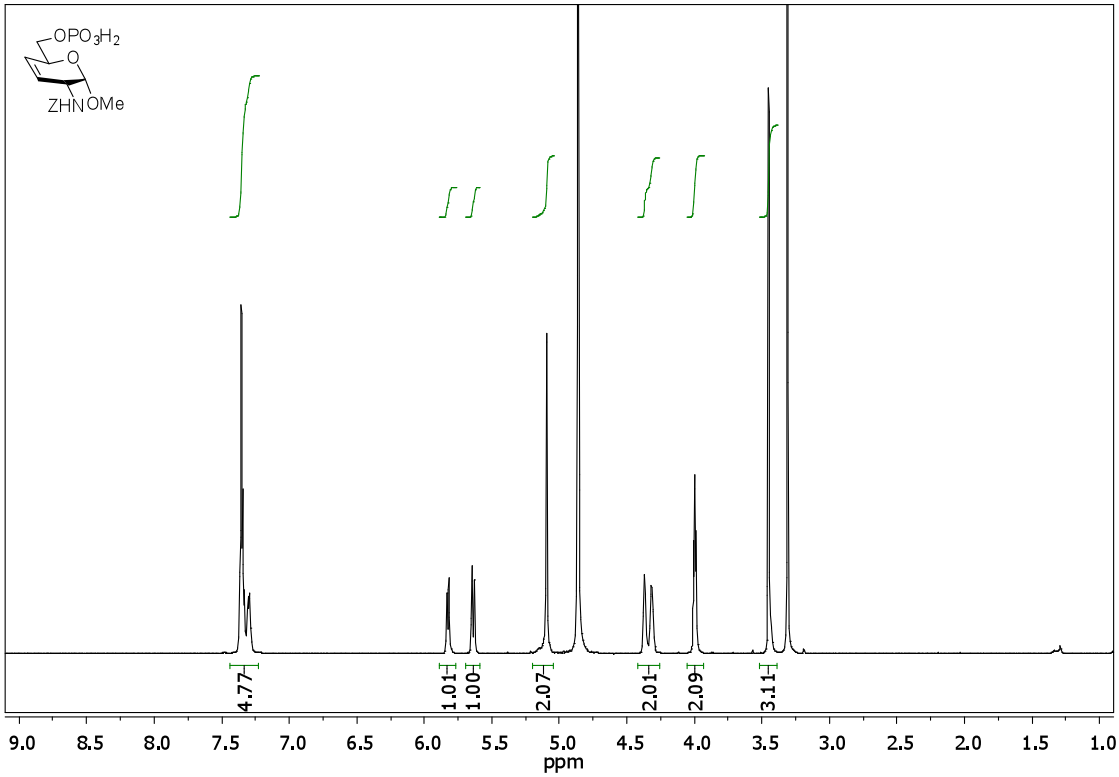
^{1}H NMR (400.1 MHz, D_2O) of compound **6**



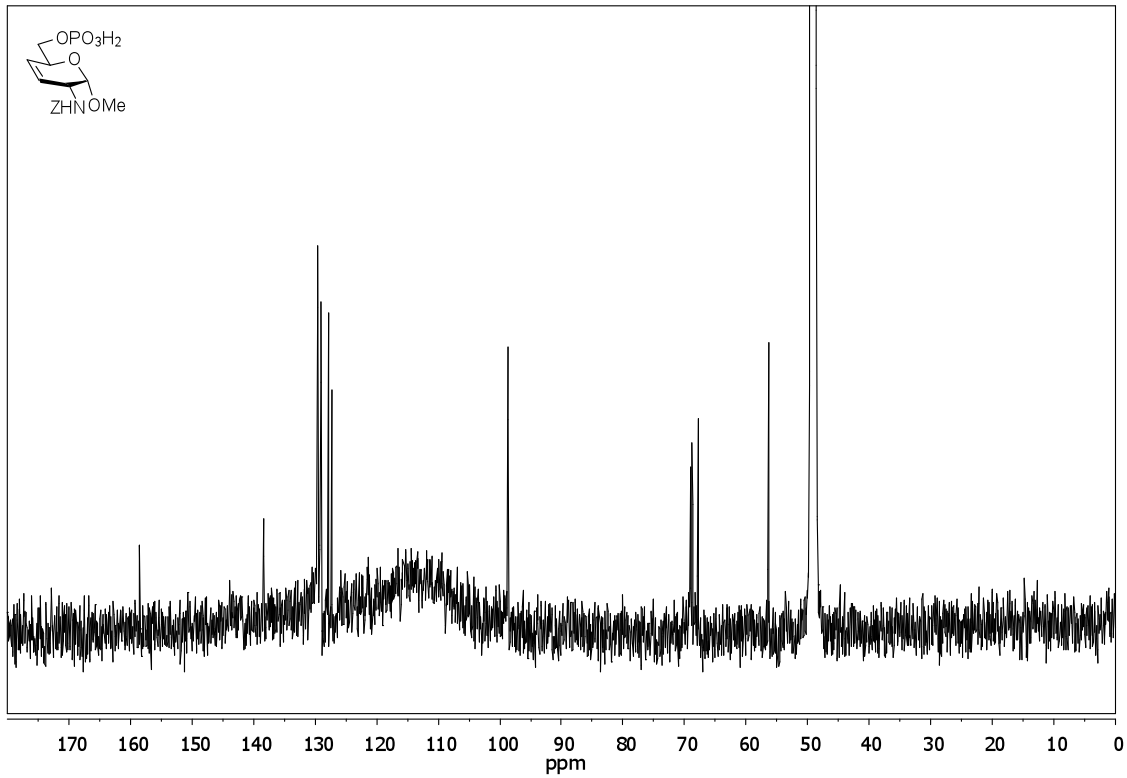
^{13}C NMR (100.6 MHz, D_2O) of compound **6**



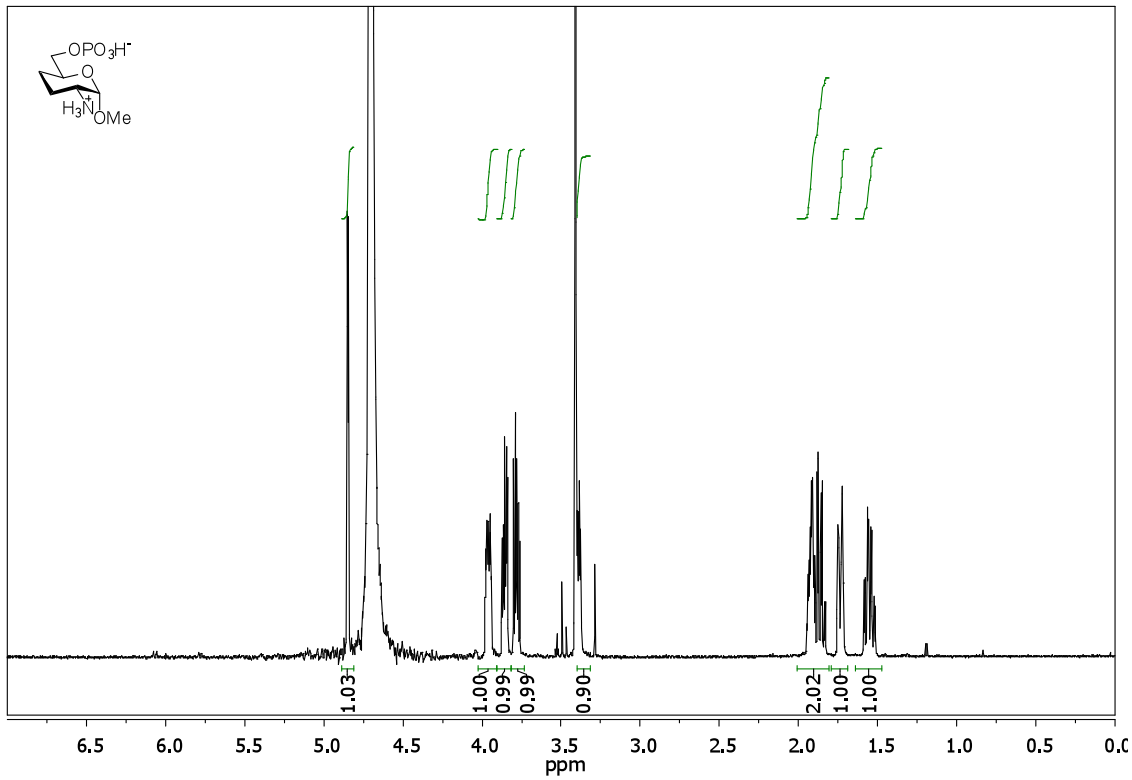
^{31}P NMR (161.9 MHz, D_2O) of compound **6**



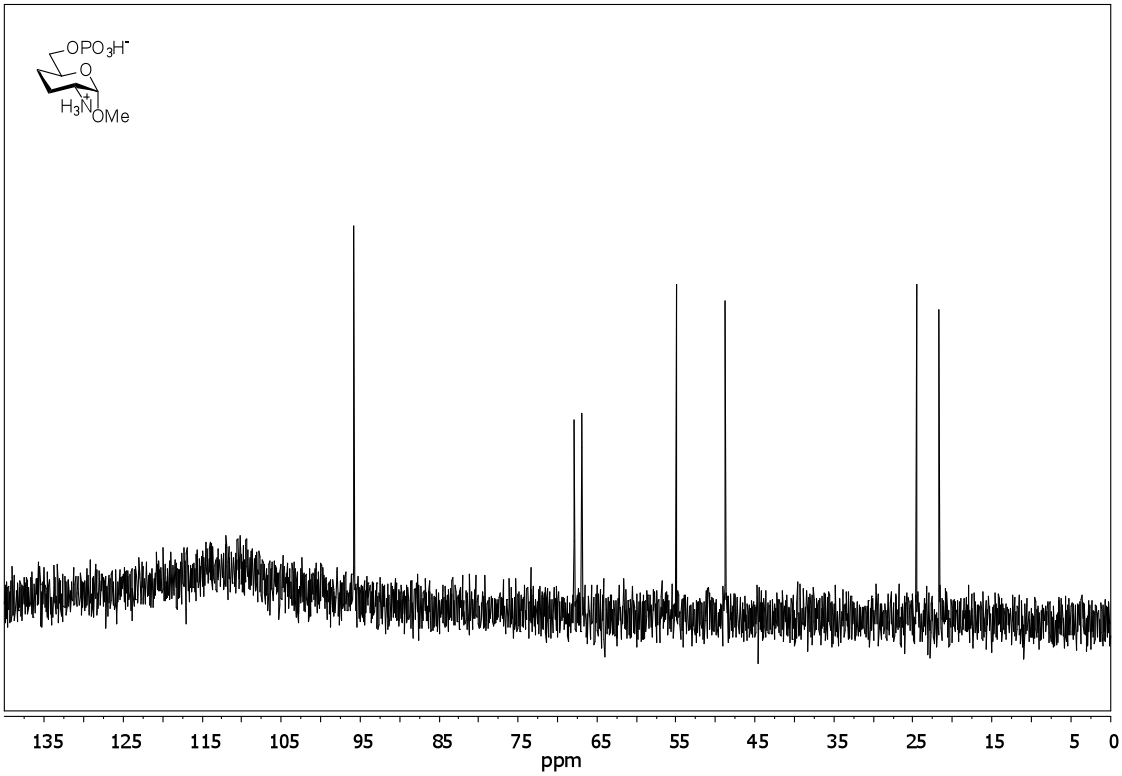
^{1}H NMR (600.1 MHz, CD_3OD) of compound **43**



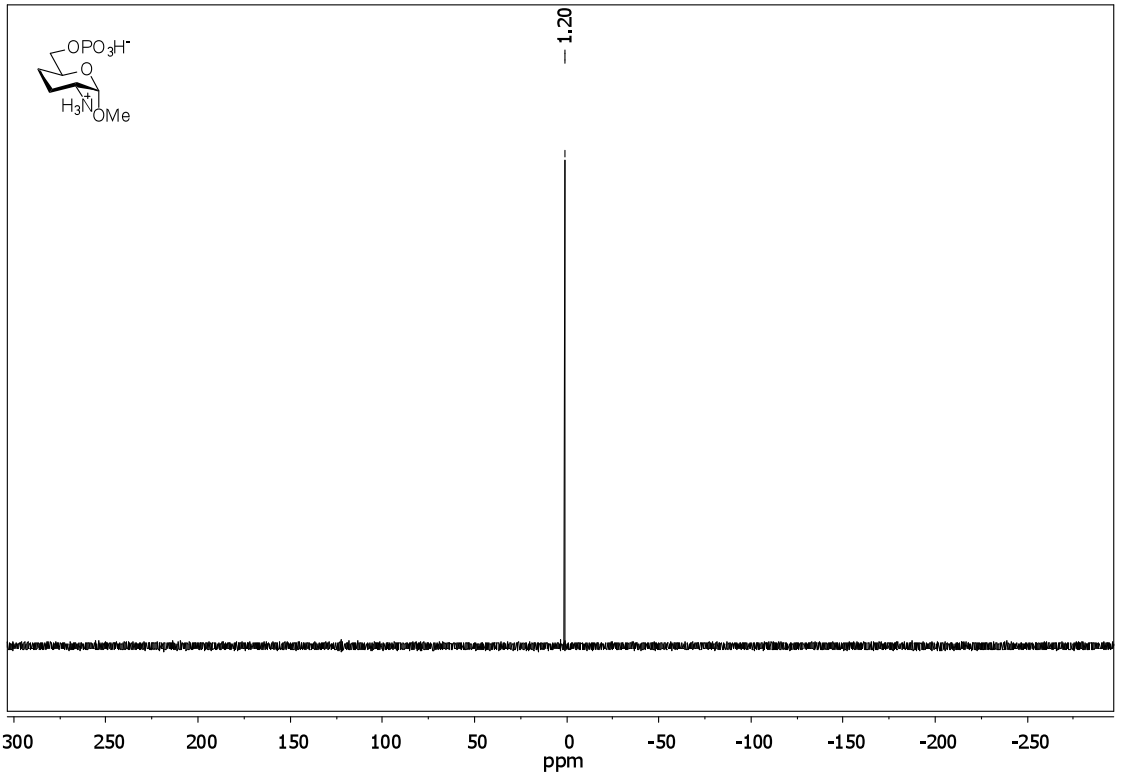
^{13}C NMR (150.9 MHz, CD_3OD) of compound **43**



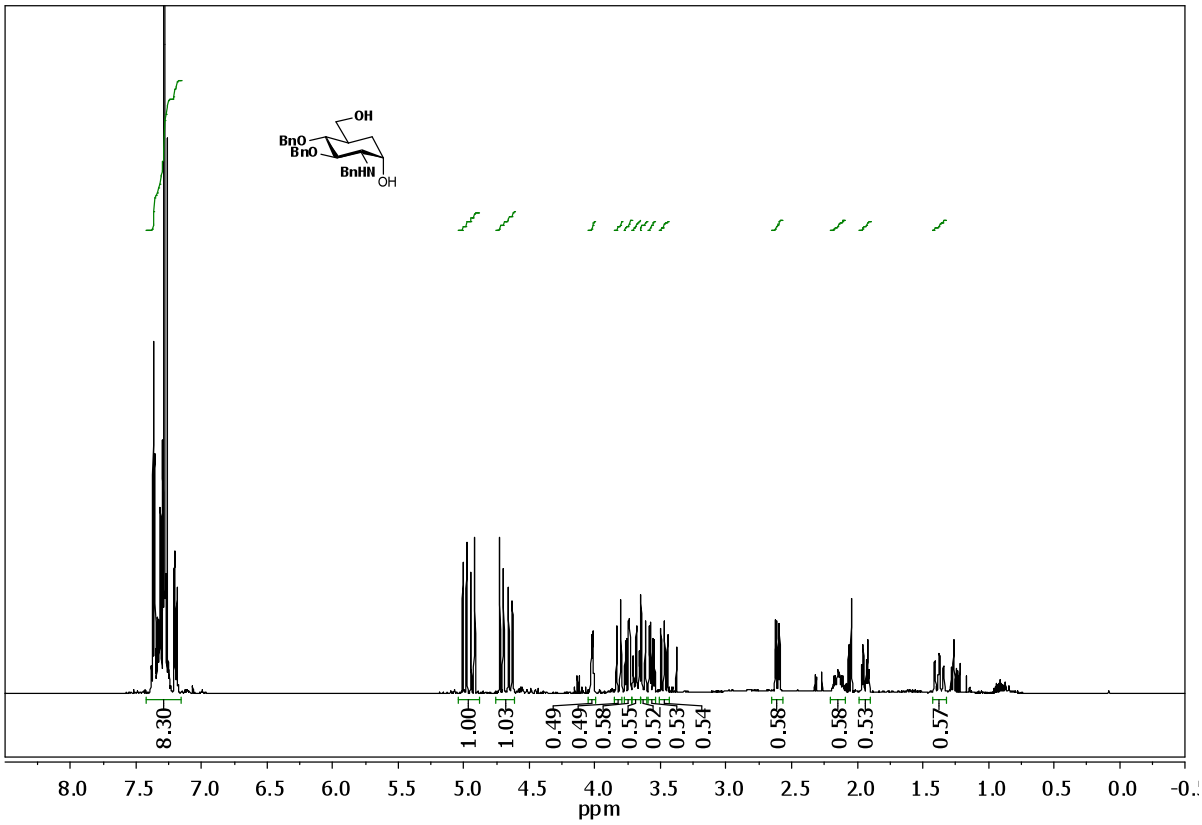
^{1}H NMR (400.1 MHz, D_2O) of compound **7**



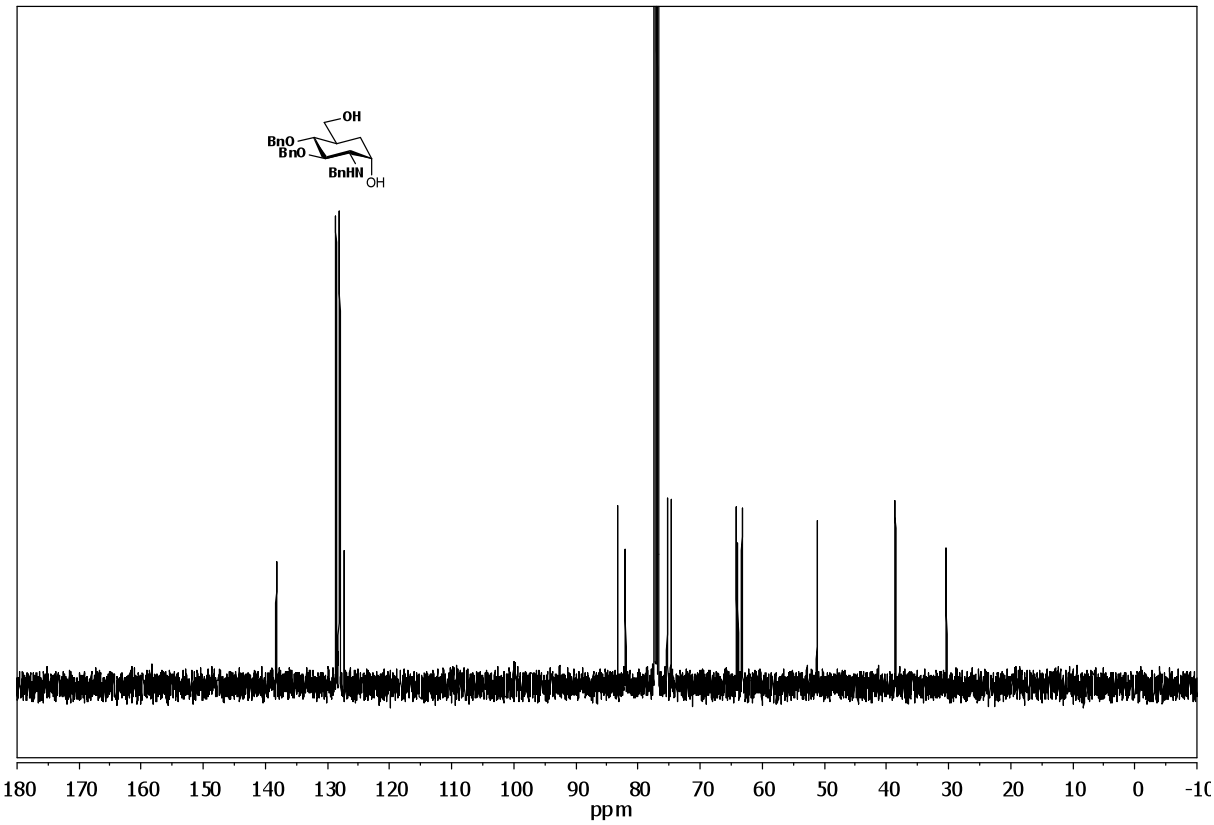
^{13}C NMR (100.6 MHz, D_2O) of compound **7**



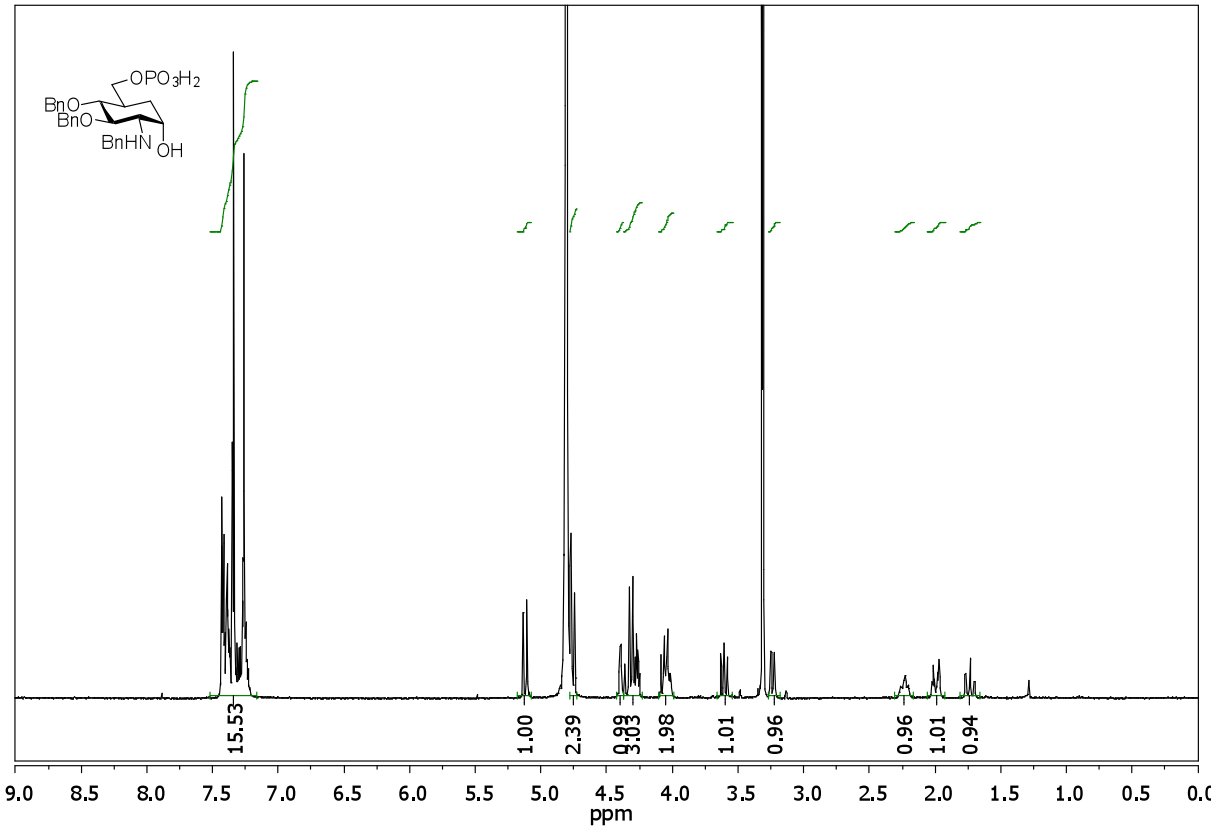
^{31}P NMR (161.9 MHz, D_2O) of compound **7**



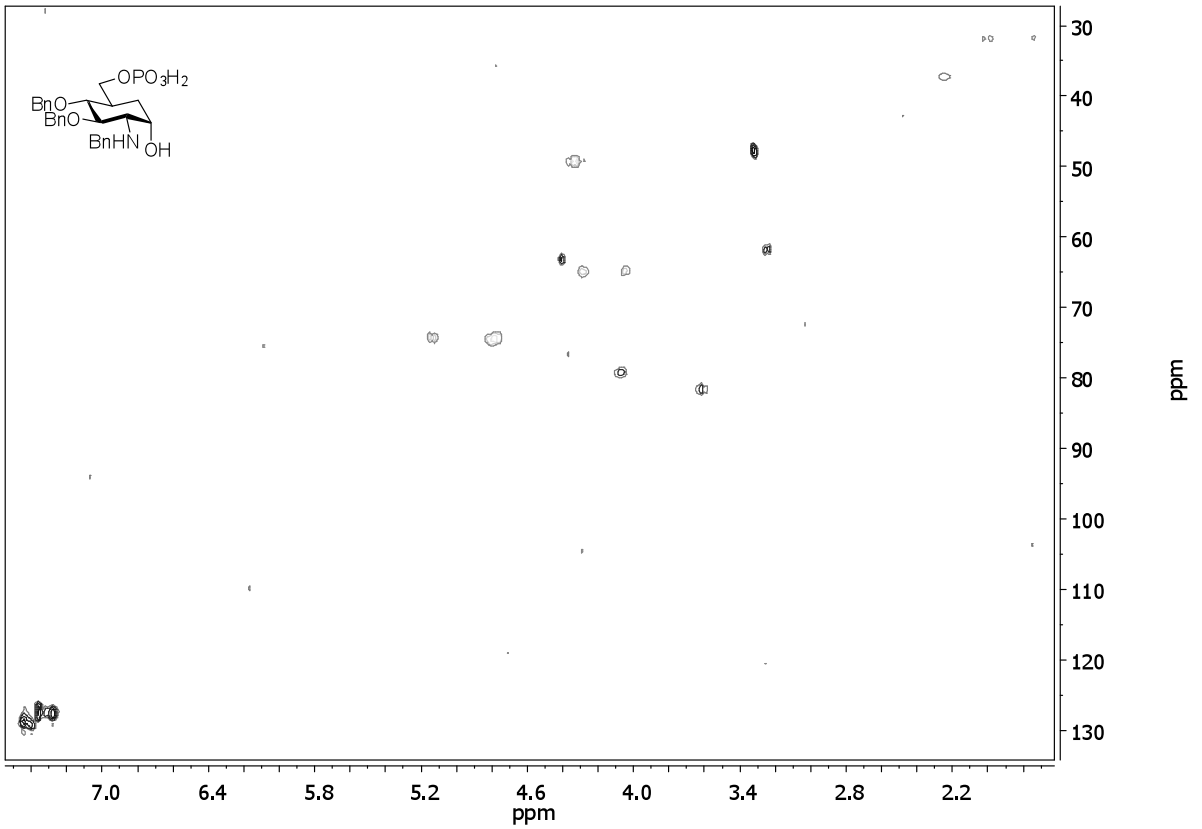
^{1}H NMR (400.1 MHz, $CDCl_3$) of compound **12**



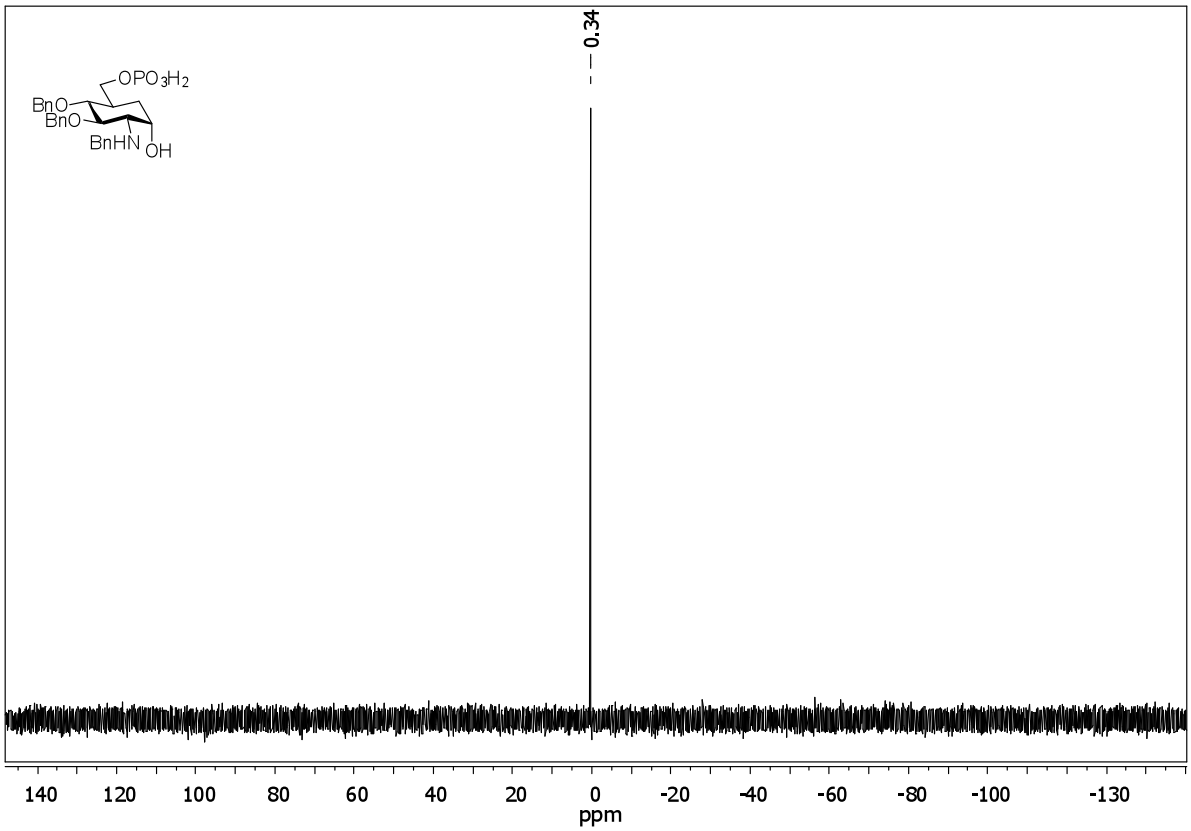
^{13}C NMR (100.6 MHz, $CDCl_3$) of compound **12**



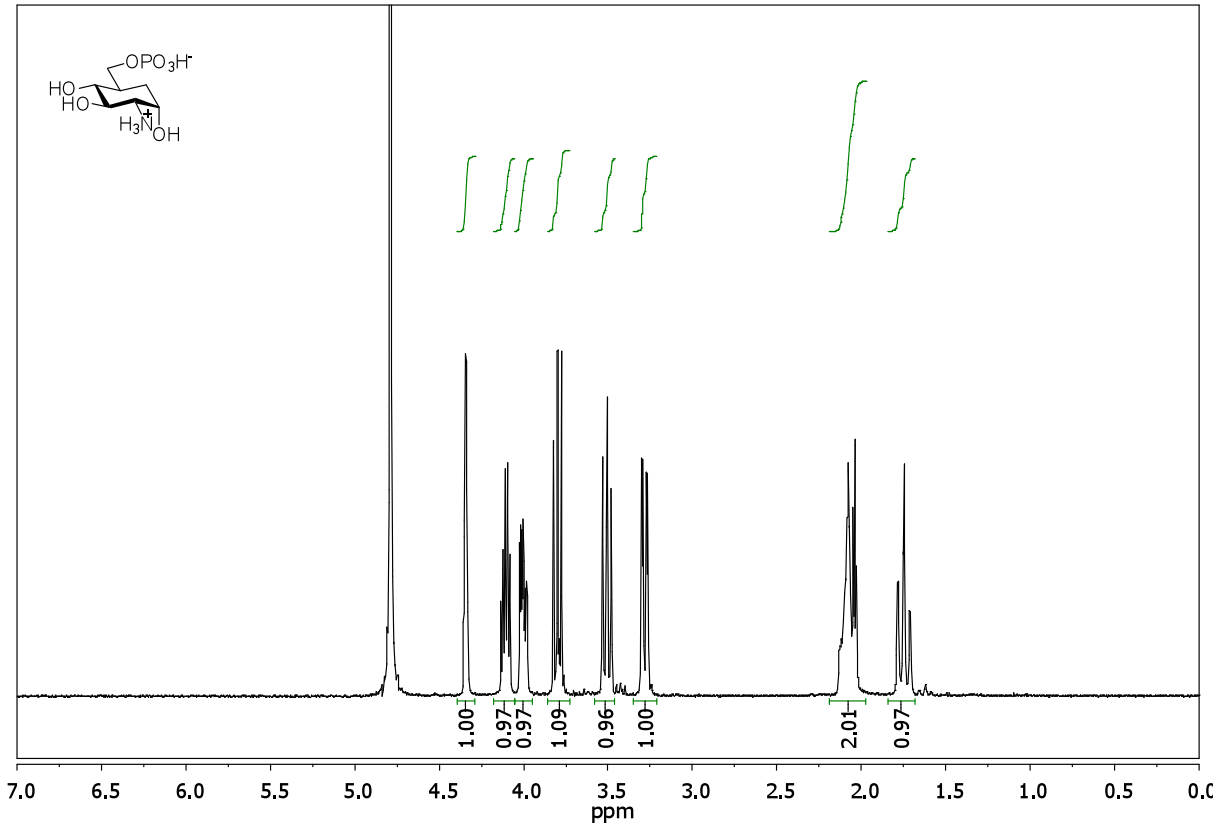
^{1}H NMR (400.1 MHz, MeOD) of compound **13**



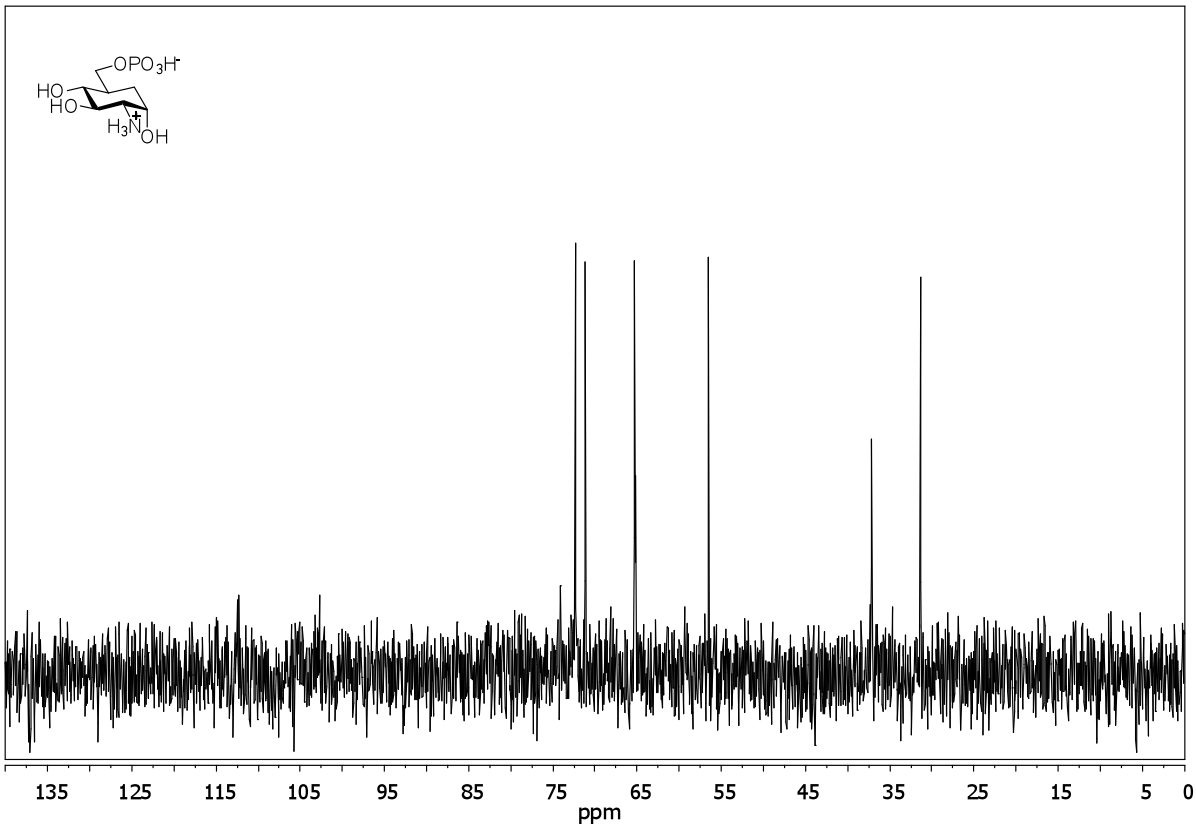
^{1}H, ^{13}C correlation (HSQC, MeOD) of compound **13**



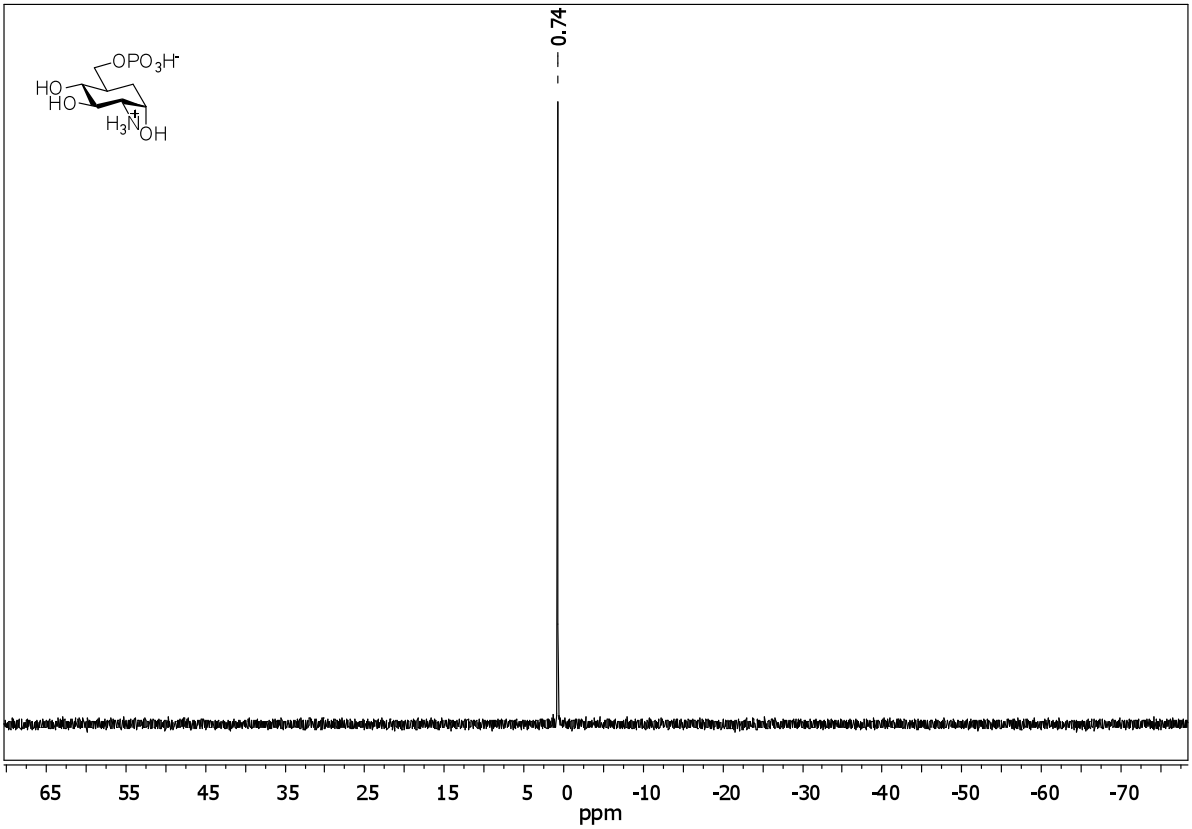
^{31}P NMR (161.9 MHz, DMSO-d_6) of compound **13**



^{1}H NMR (400.1 MHz, D_2O) of compound **8**



^{13}C NMR (100.6 MHz, D_2O) of compound **8**



^{31}P NMR (161.9 MHz, D_2O) of compound **8**