Supporting Information

Chemoenzymatic Synthesis of GDP-Azidodeoxymannoses: Probes for Mannosyltransferase Activity and Tools for Glycoprotein Remodelling

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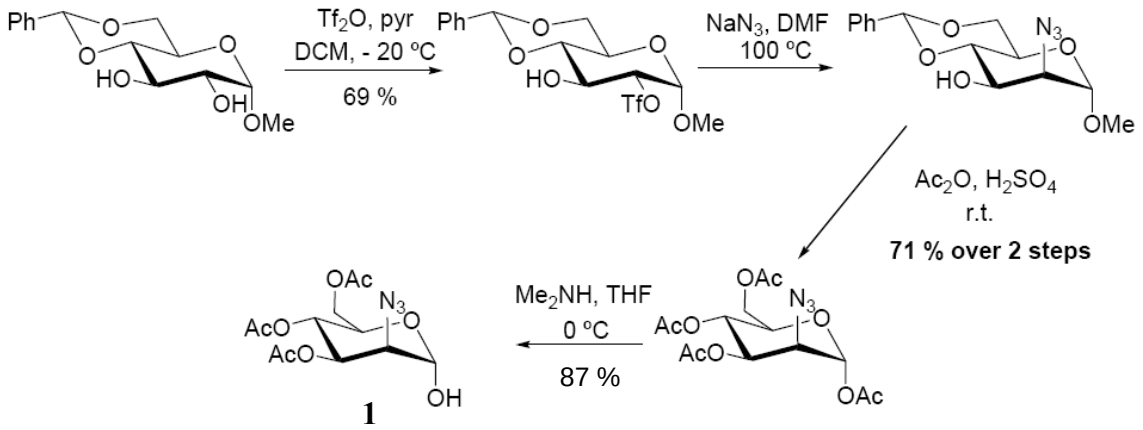
General Methods

^{1}H NMR (300, or 500 MHz), ^{13}C NMR (75 or 125 MHz), and ^{31}P NMR (101 or 121 MHz) spectra were recorded on a Bruker 250Y spectrometer. Chemical shifts (δ) are reported in ppm relative to $Si(CH_3)_4$ (δ=0) and coupling constants (J) in Hz, signals are sharp unless stated as broad (br), s: singlet, d: doublet, t: triplet, m: multiplet and q: quaternary. Residual protic solvent, $CDCl_3$ (δ H: 7.26, s) was used as the internal standard in ^{1}H-NMR spectra unless otherwise stated. Low resolution electrospray mass spectroscopy was carried out on a Micromass Quattro LC electrospray mass spectrometer with an applied voltage of 35-50 V. High resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility at University College London. Analytical TLC was carried out on Merck aluminium backed plates coated with silica gel $60F_{254}$. Flash chromatography was carried out over Fisher silica gel 60 Å particle size 35-70 μ. Components were visualized using *p*-anisaldehyde dip and UV light (254 nm).

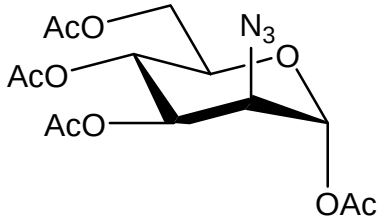
Summary scheme for the synthesis of **1**:



Synthesis of 2-azido-4,6-*O*-benzylidene-2-deoxy-α-methyl-D-mannopyranoside:

Synthesis of 2-azido-4,6-*O*-benzylidene-2-deoxy-α-methyl-D-mannopyranoside was carried out as previously reported.[1]

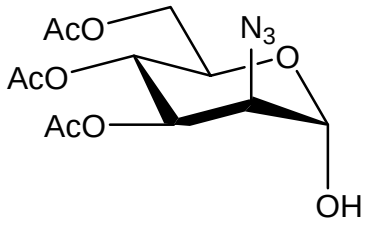
Synthesis of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside:



A solution of 2-azido-4,6-*O*-benzylidene-2-deoxy-α-methyl-D-mannopyranoside (532 mg, 1.7 mmol) in Ac_2O (9.9 ml) and sulfuric acid (310 μL) was stirred at room temperature for 2.5 h. The reaction mixture was then diluted with EtOAc and with sat. aq.

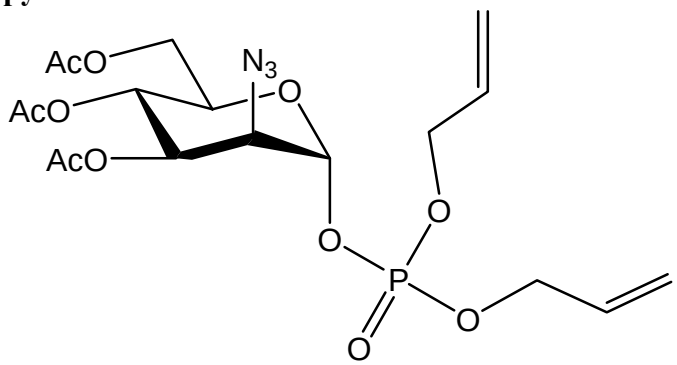
$NaHCO_3$ which was slowly added. The organic layer was washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by flash chromatography over silica (3/1 petroleum ether/EtOAc) to afford a pale yellow oil (388 mg, 78%). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 6.07 (1H, d, J = 1.8 Hz, H1), 5.33 (2H, m, H3, H4), 4.21 (1H, dd, J = 12.4 Hz, J = 4.4 Hz, H6a), 4.07-3.94 (3H, m, H6b, H2, H5), 2.12 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), and 2.01 (3H, s, $COCH_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 170.5, 169.9, 169.2, 168.1 (4qC, 4 x $COCH_3$), 91.1 (1CH, C1), 70.5, 70.4, 65.1, 60.3 (4CH, C2-C5), 60.8 ($1CH_2$, C6), 20.7, 20.5, 20.4, and 20.3 ($4CH_3$, 4 x COCH3). MS (ESI): *m/z* 396.1 $(M+Na)^+$, $C_{14}H_{19}N_3O_9Na$ requires 396.3.

Synthesis of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (1).



The peracetylated 2-azido-2-deoxy-α-D-mannose (334 mg, 0.90 mmol) was dissolved in dry THF (2.0 ml) with stirring, and cooled to 0 °C. A solution of Me_2NH in THF (0.963 ml, 2.0 M) was then added dropwise, and the reaction was allowed to reach r.t. After 2 h, analysis by TLC (2/1 hexane/EtOAc) indicated that the reaction was complete, and thus it was concentrated *in vacuo*. The product was purified by flash chromatography over silica (2/1 petroleum ether/EtOAc) to afford a colourless syrup (258 mg, 87 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.40 (1H, dd, J = 9.9, J = 3.6 Hz, H3), 5.28 (1H, dd, J = 9.9, J = 9.9 Hz, H4), 5.21 (1H, d, J = 1.6 Hz, H1), 4.58 (1H, br s, OH), 4.12 (3H, m, H5, H6a, H6b), 4.00 (1H, dd, J = 3.6, J = 1.6 Hz, H2), 2.05 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), and 1.99 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 171.2, 170.2, 169.8 (3qC, 3 x $COCH_3$), 92.6 (1CH, C1), 70.8, 68.2, 66.1 (3CH, C3, C4, C5), 62.2 ($1CH_2$, C6), 62.0 (1CH, C2), 20.7, 20.5, and 20.4 ($3CH_3$, 3 x COCH3). MS (ESI): *m/z* 354.1 $(M+Na)^+$, $C_{12}H_{17}N_3O_8Na$ requires 354.1.

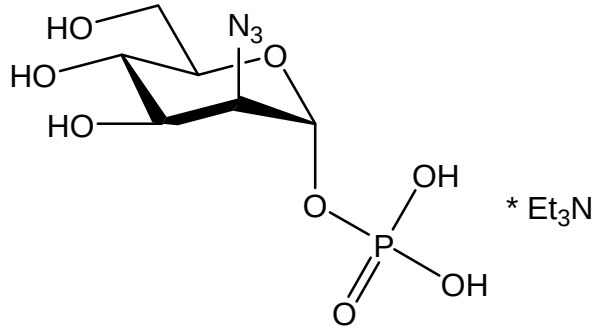
Synthesis of diallylmonophosphate-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-manno-pyranoside:



A solution of the 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (456 mg, 1.38 mmol) in dry DCM (15.0 ml) was added to a flask with 1-*H*-tetrazole (482 mg, 6.88 mmol) under Ar and stirred at room temperature for 10 minutes. Diallyl-*N,N*-diisopropyl-

phosphoramidite (169 μL, 0.63 mmol) was then added dropwise and stirring was continued for further 4 h at room temperature. The reaction mixture was then cooled to -40 °C and *m*-chloroperbenzoic acid (MCPBA, 1.19 g, 6.88 mmol) was added. The reaction was allowed to come slowly to room temperature over approximately 1 h, then it was diluted with DCM (15.0 ml) and washed with 10 % $NaHSO_3$ (2 x 15.0 ml), sat. aq. $NaHCO_3$ (2 x 15.0 ml), and water (2 x 15.0 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated to afford the crude product as an off-white solid that was purified by flash chromatography over silica (1:1 – 2:1 EtOAc/petroleum ether). NMR and ES-MS analysis revealed contamination by $PHO(OAll)_2$ as well as the β isomer (34 mg, 5%) of the desired product; both contaminants were successfully removed by further column chromatography over silica (7:1 EtOAc/petroleum ether). The purified α-monophosphate was isolated as a colourless syrup (310 mg, 46 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.93-5.78 (2H, m, 2 x CH vinyl), 5.58 (1H, dd, J = 1.7, 6.4 Hz, H1), 5.29 (4H, m, 2 x CH_2 vinyl), 5.26 (2H, m, H3, H4), 4.50 (4H, m, 2 x CH_2 allyl), 4.14 (1H, dd, J = 4.3, -12.1 Hz, H6a), 4.04 (1H, m, H5), 3.98 (1H, dd, J = 2.2, - 13.5 Hz, H6b), 2.00, 1.97, and 1.95 (3 x 3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 170.4, 169.7, 169.3 (3qC, 3 x $COCH_3$), 131.9, 131.8 (2CH, 2 x CH vinyl, $J_{C\text{-}P}$ = 5 Hz), 119.0, 118.8 ($2CH_2$, 2 x CH_2 vinyl), 95.4 (1CH, C1, $J_{C\text{-}P}$ = 5 Hz), 77.4, 70.2, 65.1, 61.2 (4CH, C2-C5), 68.8, 68.6 ($2CH_2$, 2 x CH_2 allyl, $J_{C\text{-}P}$ = 5 Hz), 61.6 ($1CH_2$, C6), 20.6, 20.5, and 20.4 ($3CH_3$, 3 x $COCH_3$). ^{31}P NMR (121 MHz, $CDCl_3$): δ (ppm) = -2.1. *m/z*. IR (ν_{max}, thin film): 3018, 2961, 2856, 2113, 1749, 1649, 1455, 1425, 1413, 1368, 1242, 1224, 1160, 1070, 1042, 1028, 972, 958, 879, 833, 822, 759, 700 cm^{-1}. HR-MS (ESI): *m/z* 514.12132 $(M+Na)^+$, $C_{18}H_{26}N_3O_{11}PNa$ requires 514.12026 (error: 2.06 ppm).

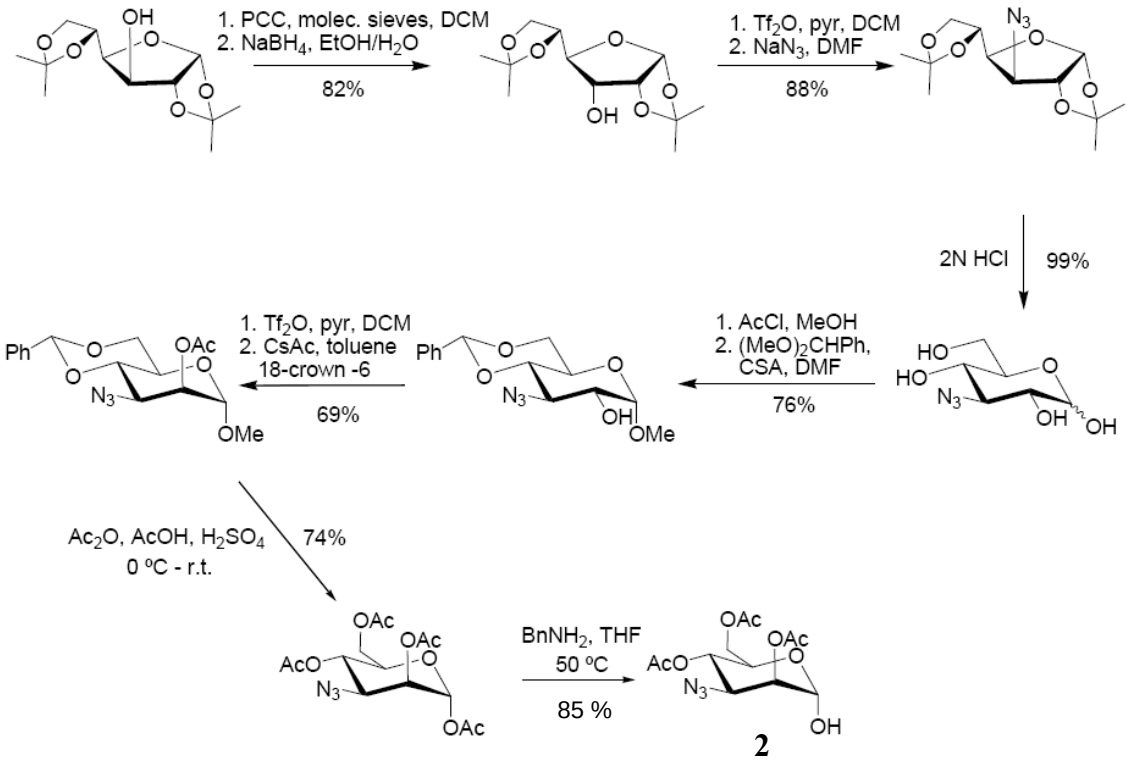
Monophosphate-2-azido-2-deoxy-α-D-mannopyranoside (5)



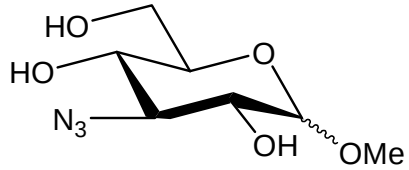
To a 100 ml round bottom flask containing the diallylmonophosphate-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (293 mg, 0.60 mmol), under Ar, were added: a solution of dry THF/MeOH (24.0 ml), *p*-toluensulphinic acid sodium salt (213 mg, 1.20 mmol), and tetrakis-triphenylphosphine palladium (51.7 mg, 0.05 mmol). The reaction mixture was stirred under Ar for 17 h, concentrated *in vacuo* and coevaporated with toluene (3 x 20.0 ml). The crude was resuspended in a mixture of 5:2:1 $MeOH/H_2O/Et_3N$ (30.0 ml), and stirred at room temperature for 22 h. The solution was filtered to remove a black precipitate, then concentrated *in vacuo*. The resulting syrup was dissolved in water and washed with DCM (3 x 35.0 ml) to remove organic products. The aq. layer was then concentrated and coevaporated with toluene (3 x 15.0 ml) to afford the product as a pale yellow foam (220 mg, 86 % considering the bis-triethylammonium salt). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.49 (1H, dd, J = 6.9, 1.7 Hz, H1), 4.24 (1H, dd, J = 9.6, 3.5

Hz, H3), 4.12 (1H, dd, J = 3.5, 1.7 Hz, H2), 3.94 (1H, dd, J = 2.2 Hz, H6a), 3.94 (1H, m, H5), 3.78 (1H, dd, J = 6.4, - 12.6 Hz, H6b), 3.68 (1H, dd, J = 9.6, 9.6 Hz, H4), 3.06 (6H, s, $N(CH_2CH_3)_3$), and 1.14 (9H, s, $N(CH_2CH_3)_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)= 93.6 (1CH, d, C1, $J_{C\text{-}P}$ = 4 Hz), 73.4, 70.5, 67.2, 65.2 (4CH, C2-C5), 61.2 ($1CH_2$, C6), 46.5 ($N(\underline{C}H_2CH_3)_3$), and 8.2 ($N(CH_2\underline{C}H_3)_3$). ^{31}P NMR (121 MHz, $CDCl_3$): δ (ppm) = +2.7. MS (ESI): m/z 284.2 $(M–H)^-$, $C_{12}H_{17}N_3O_{11}P$ requires 284.1.

Summary scheme for the synthesis of **2**: [2]



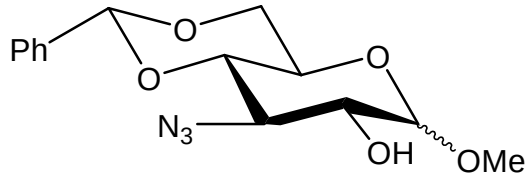
Synthesis of methyl 3-azido-3-deoxy-α-D-glucopyranoside



Acetyl chloride (150 µl) was added to a solution of the 3-azido-3-deoxy-D-glucose (1.00 g, 4.88 mmol) in dry MeOH (15 ml) and refluxed for 24h under nitrogen. The reaction solution was cooled down to room temperature, neutralised with sodium bicarbonate, and the solvent was removed *in vacuo*. The crude was then dissolved in ethyl acetate, passed through a plug of silica (eluant ethyl acetate), and the solvent was removed *in vacuo* to afford a quantitative yield (1.06 g) of the crude product as a white solid and as a mixture of the two anomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.85 (0.6H, d, J = 3.6 Hz, H1α), 4.49 (0.4H, d, J = 7.9 Hz, H1β), 3.96 (0.4H, dd, J = 2.1, -12.2 Hz, H6aβ), 3.91

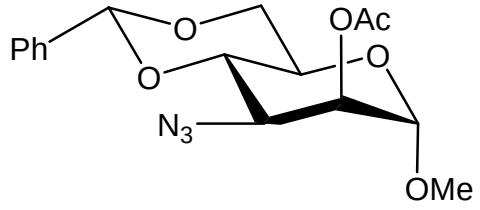
(0.6H, dd, *J* = 2.1, -12.2 Hz, H6aα), 3.83-3.47 (4.6H, m), 3.62 (1.8H, s, OCH_3α), 3.48 (1.2H, s, OCH_3β), 3.32 (0.4H, dd, *J* = 7.9, 9.8 Hz, H2β). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 103.6 (C1β), 99.0 (C1α), 76.9, 72.1, 71.8, 70.5, 70.4, 69.1, 68.8, 66.7, (8CH, C2-C5), 60.8, 60.7 ($2CH_2$, C6), 57.6, 55.4 ($2CH_3$, 2 x OCH_3). MS (ESI): *m/z* 242.2 $(M+Na)^+$, $C_7H_{13}N_3O_5Na$ requires 242.1.

Synthesis of methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-D-glucopyranoside



Benzaldehyde dimethylacetal (2.74 ml, 17.8 mmol) was added dropwise to a solution of the methyl 3-azido-3-deoxy-α-D-glucopyranoside (1,300 mg, 5.94 mmol) in dry DMF (35 ml). The resulting solution was adjusted to pH 4 using camphorsulfonic acid, and stirring was continued at room temperature under nitrogen. After 3h, pH was neutralised by addition of Et_3N, and the solvent was removed *in vacuo*. The crude product is purified by flash chromatography (hexane/ethyl acetate 1/1) to give the product as a colourless oil (1.39 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* (ppm) = 7.51 (2H, m, Ar), 7.39 (3H, m, Ar), 5.81 (1H, s, CHAr), 4.74 (1H, d, *J* = 3.8 Hz, H1), 4.35 (1H, dd, *J* = 4.6 Hz, *J* = - 10.7 Hz, H6a), 4.29 (1H, dd, *J* = 4.6 Hz, *J* = - 10.5 Hz, H6b), 3.81 (1H, m, H5), 3.76 (1H, dd, *J* = 10.1 Hz, *J* = 10.1 Hz, H4), 3.72 (1H, dd, *J* = 10.1 Hz, *J* = 10.1 Hz, H3), 3.55 (1H, m, H2), 3.47 (3H, s, OCH_3), and 2.71 (1H, s (br), OH). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* (ppm) = 136.8 (1qC, Ar), 129.1, 128.3, 126.1 (3CH, 3 x ArH), 101.5 (1CH, ArH), 99.3 (1CH, C1), 79.6, 71.7, 63.5, 62.7 (4CH, C2, C3, C4, C5), 68.9 ($1CH_2$, C6), and 55.6 (OCH_3). MS (ESI): *m/z* 330.4 $(M+Na)^+$, $C_{14}H_{17}N_3O_5Na$ requires 330.1.

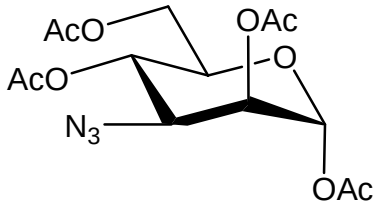
Synthesis of methyl 2-*O*-acetyl-3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-manno-pyranoside



The methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-D-glucopyranoside (257 mg, 0.837 mmol) was dissolved in dry DCM (5 ml) under nitrogen. Pyridine (135 µl, 1.67 mmol) was added and the solution was stirred at – 35 ºC for 30 minutes. Triflic anhydride (162 µl, 0.963 mmol) was added dropwise with stirring and the mixture allowed to reach -10 ºC. After 2h, methanol (1 ml) was added to quench the reaction and the resulting solution warmed to room temperature. The solution was washed with water (5 ml), pH 7 PBS solution (5 ml), and the aqueous layer re-extracted with chloroform (5 ml x 2). The organic fractions were combined, dried with magnesium sulphate, filtered, and the solvent was evaporated *in vacuo*. The residue was dissolved in toluene (5 ml); cesium acetate (241 mg, 1.26 mmol), and 18-crown-6 (332 mg, 1.26 mmol) were added, and the

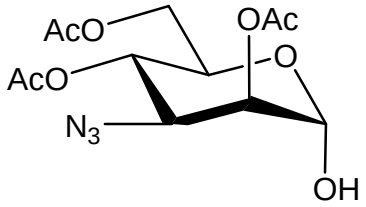
resulting solution was refluxed for 2 h. After cooling down to room temperature, the solvent was removed *in vacuo*, the residue redissolved in DCM (10 ml), and washed with water (2 x 10 ml). The organic fractions were combined, dried (magnesium sulphate), and evaporated to dryness. Column chromatography (pet. ether/ethyl acetate 3/2) afforded the product as an off-white solid (201 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.50 (2H, m, ArH), 7.38 (3H, m, ArH), 5.67 (1H, s, CHAr), 5.17 (1H, dd, J = 3.1 Hz, J = 1.6 Hz, H2), 4.66 (1H, d, J = 1.6 Hz, H1), 4.31 (1H, dd, J = 2.9 Hz, J = - 8.5 Hz, H6a), 4.07 (1H, m, H5), 4.00 (1H, dd, J = 10.3 Hz, J = 3.2 Hz, H3), 3.91 (1H, m, H6b), 3.86 (1H, dd, J = 10.3 Hz, J = 10.3 Hz, H4), 3.40 (3H, s, OCH_3), and 2.18 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 169.8 (1qC, $COCH_3$), 136.9 (1qC, Ar), 129.1, 128.3, 126.0 (3CH, 3 x ArH), 101.8 (1CH, ArH), 98.8 (1CH, C1), 77.2 (1CH, C2), 70.9 (1CH, C5), 68.8 (CH_2), 63.8 (1CH, C4), 57.9 (1CH, C3), 55.3 ($1CH_3$, OCH_3), and 20.9 ($1CH_3$, $COCH_3$). IR (ν_{max}, thin film): 2995, 2978, 2926, 2948, 2105, 1740, 1470, 1447, 1425, 1368, 1261, 1227, 1128, 1086, 1041, 1010, 974, 909, 884, 747 cm^{-1}. HR-MS (ESI): *m/z* 350.13405 $(M+H)^+$, $C_{14}H_{21}N_3O_9^+$ requires 350.13521 (error: 3.31 ppm).

Synthesis of 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranoside



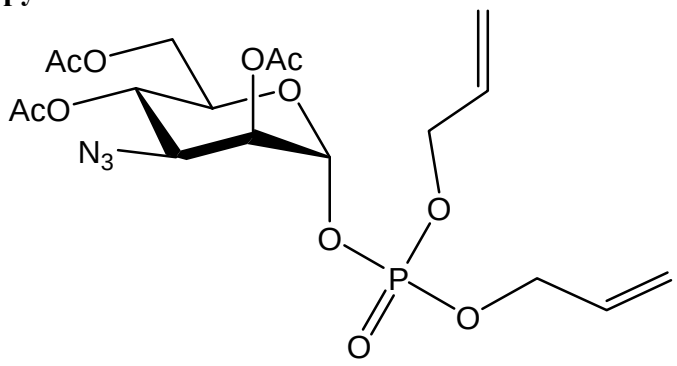
The methyl 2-*O*-acetyl-3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-mannopyranoside (302 mg, 0.865 mmol) was dissolved in acetic anhydride (6.0 ml), and the solution was cooled down to 0 ºC. Concentrated H_2SO_4 (175 µl) was added dropwise and the reaction was allowed to come to room temperature by removal of the ice bath. Stirring was continued overnight. The reaction mixture was then diluted with chloroform (5 ml), cooled to 0 ºC with an ice bath, and neutralised with sat. aq. $NaHCO_3$, which was slowly added. The organic layer was washed with distilled water (5 ml x 2), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash chromatography over silica (3/2 petroleum ether/EtOAc, Rf 0.3) to afford a pale yellow oil (242 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 6.01 (1H, d, J = 1.8 Hz, H1), 5.27 (1H, dd, J = 10.4 Hz, J = 10.4 Hz, H4), 5.14 (1H, dd, J = 3.3 Hz, J = 1.9 Hz, H2), 4.20 (1H, dd, J = 5.0 Hz, J = - 12.4 Hz, H6a), 4.04 (1H, dd, J = 2.6 Hz, J = - 12.4 Hz, H6b), 3.95 (1H, ddd, J = 10.4 Hz, J = 5.0 Hz, J = 2.5 Hz, H4), 3.81 (1H, dd, J = 10.4 Hz, J = 3.3 Hz, H3), 2.13 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), and 2.04 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 170.6, 169.5, 169.4, 167.8 (4qC, 4 x $COCH_3$), 89.8 (1CH, C1), 70.4, 69.4, 66.2 (3CH, C2, C4, C5), 62.1 ($1CH_2$, C6), 58.6 (1CH, C3), 20.7, 20.7, 20.6, and 20.6 ($4CH_3$, 4 x COCH3). IR (ν_{max}, thin film): 3027, 2110, 1749, 1435, 1372, 1214, 1148, 1048, 1026, 973, 754 cm^{-1}. MS (ESI): *m/z* 396. $(M+Na)^+$, $C_{14}H_{19}N_3O_9Na$ requires 396.3.

Synthesis of 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranoside (2)



The peracetylated 3-azido-3-deoxy-α-D-mannose (247 mg, 0.66 mmol) in dry THF (3.5 ml) was treated with benzylamine (86 µl) under nitrogen. The reaction mixture was warmed to 50 ºC and stirring continued for 24 h under nitrogen. The solvent was then removed under reduced pressure and the crude product purified by flash chromatography over silica (3/2 pet. ether/EtOAc) to afford the product as a colourless oil (186 mg, 85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.22 (1H, dd, J = 10.3, J = 10.3, H4), 5.15 (1H, d, J = 1.7, H1), 5.10 (1H, dd, J = 1.7, J = 3.2, H2), 4.39 (1H, s (br), OH), 4.19-4.05 (3H, m, H6a, H6b, H5), 3.85 (1H, dd, J = 10.3, J = 3.2, H3), 2.10 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), and 2.06 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 171.0, 170.3, 169.9 (3qC, 3 x $COCH_3$), 91.3 (1CH, C1), 71.4, 68.3, 66.9 (3CH, C2, C4, C5), 62.6 (1CH_2, C6), 58.3 (1CH, C3), 21.0, 20.8 and 20.7 (3CH_3, 3 x $COCH_3$). IR (ν_{max}, thin film): 3430, 3024, 2960, 2111, 1749, 1651, 1432, 1373, 1225, 1121, 1048, 974, 756 cm^{-1}. HR-MS (ESI): *m/z* 354.09178 $(M+Na)^+$, $C_{12}H_{17}N_3O_8Na$ requires 354.09133 (error 1.27 ppm).

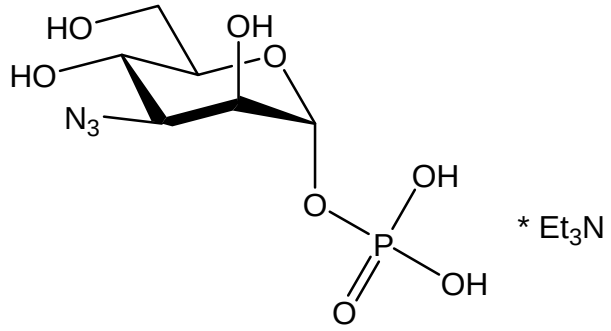
Synthesis of diallylmonophosphate-2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-α-D-manno-pyranoside



A solution of the 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranoside (170 mg, 0.51 mmol) in dry DCM (11.0 ml) was added to a flask with 1-*H*-tetrazole (179 mg, 2.55 mmol) under nitrogen and stirred at room temperature for 10 minutes. Diallyl-*N,N*-diisopropyl-phosphoramidite (325 µL, 1.21 mmol) was then added dropwise and stirring was continued overnight at room temperature. The reaction mixture was then cooled at -40 °C and *m*-chloroperbenzoic acid (MCPBA, 440 mg, 2.55 mmol) was added. The reaction was allowed to come slowly to room temperature over approximately 1 h, then it was diluted with DCM (10.0 ml) and washed with 10 % $NaHSO_3$ (2 x 10.0 ml), sat. aq. $NaHCO_3$ (2 x 10.0 ml), and water (2 x 10.0 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated to afford the crude as an off-white solid that was purified by flash chromatography over silica (1:1 – 2:1 EtOAc/petroleum ether). NMR and ES-MS

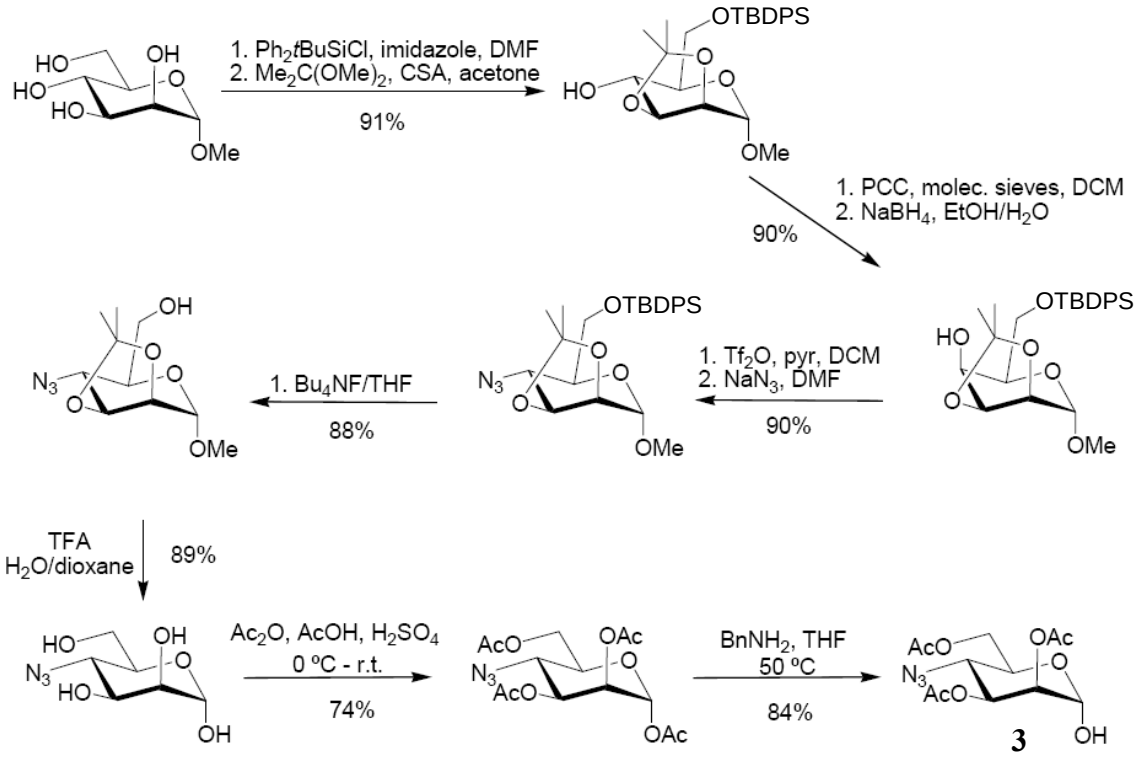
analysis revealed contamination by $PHO(OAll)_2$ which was successfully removed by further column chromatography over silica (7:1 EtOAc/petroleum ether). The purified α-monophosphate was isolated as a colourless syrup (123 mg, 49 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.93-5.82 (2H, m, 2 x CH allyl), 5.54 (1H, dd, J = 1.6, 6.4 Hz, H1), 5.35-5.21 (5H, m, H4, 2 x CH_2 allyl), 5.17 (1H, dd, J = 1.6, 2.8 Hz, H2), 4.56-4.51 (4H, m, 2 x PO-CH_2), 4.18 (1H, dd, J = 5.0, -12.3 H6a), 4.08 (1H, ddd, J = 10.3, 5.0, 2.2, H5), 4.04 (1H, dd, J = 2.2, J = -12.3, H6b), 3.81 (1H, d, J = 10.4, 2.8 Hz, H3), 2.10, 2.06, and 2.01 (3 x 3H, s, 3 x $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 170.4, 169.4, 169.4 (3qC, 3 x $COCH_3$), 131.9 (2CH, 2 x CH allyl, $J_{C\text{-}P}$ = 7 Hz), 119.0 ($2CH_2$, 2 x CH_2 allyl, $J_{C\text{-}P}$ = 7 Hz), 94.3 (1CH, C1, $J_{C\text{-}P}$ = 6 Hz), 70.2, 68.6, 66.0 (3CH, C2, C4, C5), 68.8, 68.7 ($2CH_2$, 2 x CH_2 vinyl), 61.9 ($1CH_2$, C6), 20.6, 20.6, and 20.6 ($3CH_3$, 3 x $COCH_3$). ^{31}P NMR (101 MHz, $CDCl_3$): δ (ppm) = -2.2. IR (ν_{max}, thin film): 3088, 2984, 2110, 1739, 1649, 1456, 1426, 1373, 1215, 1161, 1071, 1040, 1020, 970, 958, 887, 757 cm^{-1}. HR-ESMS: *m/z* 514.12092 $(M+Na)^+$, $C_{18}H_{26}N_3O_{11}PNa$ requires 514.12026 (error: 1.28 ppm).

Synthesis of monophosphate-3-azido-3-deoxy-α-D-mannopyranoside (6)



To a 25 ml round bottom flask containing diallylmonophosphate-2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranoside (116 mg, 0.24 mmol), under Ar, were added: a solution of dry THF/MeOH (10.0 ml), *p*-toluensulfinic acid sodium salt (84 mg, 0.47 mmol), and tetrakistriphenylphosphine palladium (20.4 mg, 0.02 mmol). The reaction mixture was stirred under Ar for 24 h, concentrated *in vacuo* and coevaporated with toluene (3 x 5.0 ml). The crude product was resuspended in a mixture of 5:2:1 $MeOH/H_2O/Et_3N$ (11.5 ml), and stirred at room temperature for 24 h. The solution was filtered to remove a black precipitate, then concentrated *in vacuo*. The resulting syrup was dissolved in water and washed with DCM (3 x 15.0 ml) to remove organic products. The aq. layer was then concentrated and coevaporated with toluene (3 x 5.0 ml) to afford the product as a pale yellow foam (120 mg, quantitative based on isolation of the bis-triethylammonium salt). ^{1}H NMR (500 MHz, D_2O): δ (ppm) = 5.30 (1H, d, J = 8.5, 2.1 Hz, H1), 4.05 (1H, dd, J = 3.2, 2.1 Hz, H2), 3.93 (1H, ddd, J = 9.9, 6.1, 2.3 Hz, H5), 3.89 (1H, dd, J = 2.3, - 12.1 Hz, H6a), 3.85 (1H, dd, J = 10.3, 3.1 Hz, H3), 3.76 (1H, dd, J = 10.3, 9.9 Hz, H4), 3.73 (1H, dd, J = 6.1, -12.3 Hz, H6b), 3.16 (6H, q, $N(CH_2CH_3)_3$), and 1.28 (9H, s, $N(CH_2CH_3)_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ (ppm)= 95.0 (1CH, d, C1, $J_{C\text{-}P}$ = 4 Hz), 73.5, 70.7, 66.3, 63.0 (4CH, C2-C5), 61.5 ($1CH_2$, C6), 46.5 ($N(\underline{C}H_2CH_3)_3$), and 8.2 ($N(CH_2\underline{C}H_3)_3$). ^{31}P NMR (101 MHz, $CDCl_3$): δ (ppm) = +2.9. MS (ESI): *m/z* 284.0 $(M–H)^-$, $C_{12}H_{17}N_3O_{11}P$ requires 284.14.

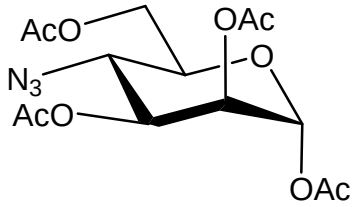
Summary scheme for the synthesis of **3**: [3]



Methyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside

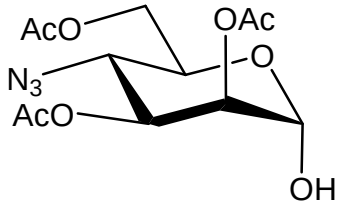
Synthesis of 4-azido-4-deoxy-2,3-O-isopropylidene-α-D-mannopyranoside was carried out as previously reported.[3]

Synthesis of 1, 2, 3, 6-tetra-*O*-acetyl-4-azido-4-deoxy-α-D-mannopyranoside



The methyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside (750 mg, 2.90 mmol) was dissolved in 0.3 ml 1,4-dioxane and aqueous TFA (TFA/water 1/1, 10 ml), and stirring was continued at room temperature. After 1h TLC (ether/hexane 3/1) showed complete conversion of the starting material (Rf 0.1) to a single product (Rf ~0). The solvent was co-evaporated with toluene (5 ml x 2) to dryness *in vacuo*. The residue was dissolved in acetic anhydride (16.5 ml), and the solution was cooled down to 0 ºC. Concentrated H_2SO_4 (0.5 ml) was added dropwise and the reaction was allowed to come to room temperature by removal of the ice bath. Stirring was continued overnight. The reaction mixture was then diluted with chloroform (10 ml), cooled to 0 ºC with an ice bath, and neutralised with sat. aq. $NaHCO_3$, which was slowly added. The organic layer was washed with distilled water (20 ml x 2), dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by flash chromatography over silica (3/2 petroleum ether/EtOAc, Rf 0.3) to afford a pale yellow oil (702 mg, 65% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.98 (1H, d, J = 1.8 Hz, H1), 5.19 (1H, dd, J = 9.7, 3.4 Hz, H3), 5.16 (1H, dd, J = 3.4, 1.8 Hz, H2), 4.28 (1H, dd, J = 2.5 - 12.3 Hz, H6a), 4.23 (1H, dd, J = 4.0, - 12.3 Hz, H6b), 3.84 (1H, dd, J = 10.5, 9.7 Hz, H4), 3.75 (1H, ddd, J = 10.5, 4.0, 2.5 Hz, H5), 2.10, 2.08, 2.05, and 2.02 (4 x 3H, s, $COCH_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 169.8, 169.6, 169.5, 168.1 (4qC, 4 x $COCH_3$), 90.5 (1CH, C1), 68.4, 67.7, 67.5 (3CH, C2, C3, C5), 62.8 ($1CH_2$, C6), 56.5 (1CH, C4), 20.8, 20.7, 20.7, and 20.6 ($4CH_3$, 4 x COCH3). IR(ν_{max}, thin film): 3022, 2958, 2113, 1749, 1434, 1371, 1235, 1154, 1077, 1022, 977, 903, 756, 700 cm^{-1}. HR-MS (ESI): *m/z* 396.10098 $(M+Na)^+$, $C_{14}H_{19}N_3O_9Na$ requires 396.10190 (error: 2.32 ppm).

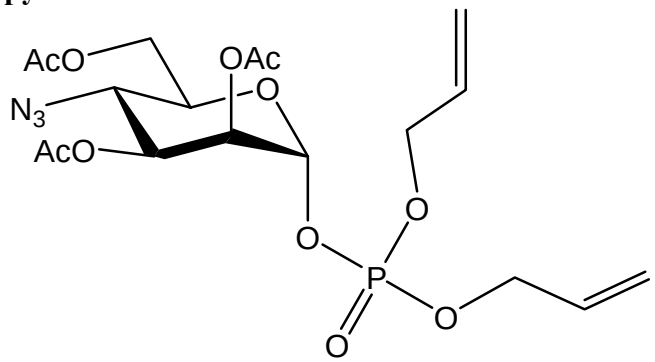
Synthesis of 2,3,6-tri-*O*-acetyl-4-azido-4-deoxy-α-D-mannopyranoside (3)



The peracetylated 4-azido-4-deoxy-α-D-mannose (700 mg, 1.88 mmol) in dry THF (10.0 ml) was treated with benzylamine (246 µl) under nitrogen. The reaction mixture was warmed to 50 ºC and stirring continued for 24 h under nitrogen. The solvent was then removed under reduced pressure and the crude product purified by flash chromatography over silica (3/2 pet. ether/EtOAc) to afford the product as a colourless oil (523 mg, 84 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.34 (1H, dd, J = 10.3, 3.2 Hz, H3), 5.23 (1H, dd, J = 3.1, 1.8 Hz, H2), 5.19 (1H, d, J = 1.6, H1), 4.39 (1H, dd, J = 2.2, - 12.3 Hz, H6a), 4.26 (1H, dd, J = 4.6, - 12.3 Hz, H6b), 3.99 (1H, ddd, J = 10.5, 4.5, 2.2 Hz, H5), 3.93 (1H, br s, OH), 3.82 (1H, dd, J = 10.3, 10.3 Hz, H4), 2.14, 2.12, and 2.07 (3 x 3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 170.9, 170.0, 169.8 (3 x qC, $COCH_3$),

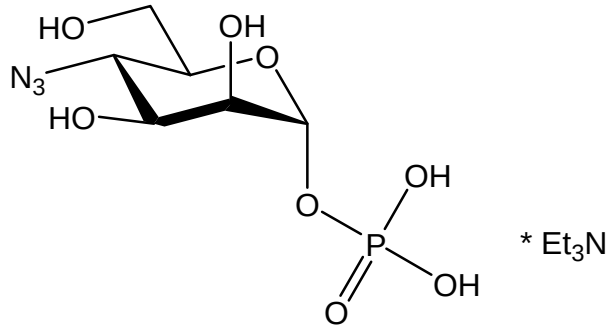
92.2 (1CH, C1), 70.2, 69.3, 68.7 (3CH, C2, C3, C5), 63.4 (1CH_2, C6), 57.0 (1CH, C4), 20.8 (3CH_3, 3 x $COCH_3$). IR (ν_{max}, thin film): 3443, 3022, 2958, 2113, 1748, 1646, 1434, 1371, 1235, 1154, 1121, 1075, 903, 796, 756, 700 cm^{-1}. HR-MS (ESI): *m/z* 354.09047 $(M+Na)^+$, $C_{12}H_{17}N_3O_8Na$ requires 354.09133 (error: 2.43 ppm).

Synthesis of diallylmonophosphate-2,3,4-tri-*O*-acetyl-4-azido-4-deoxy-α-D-manno-pyranoside



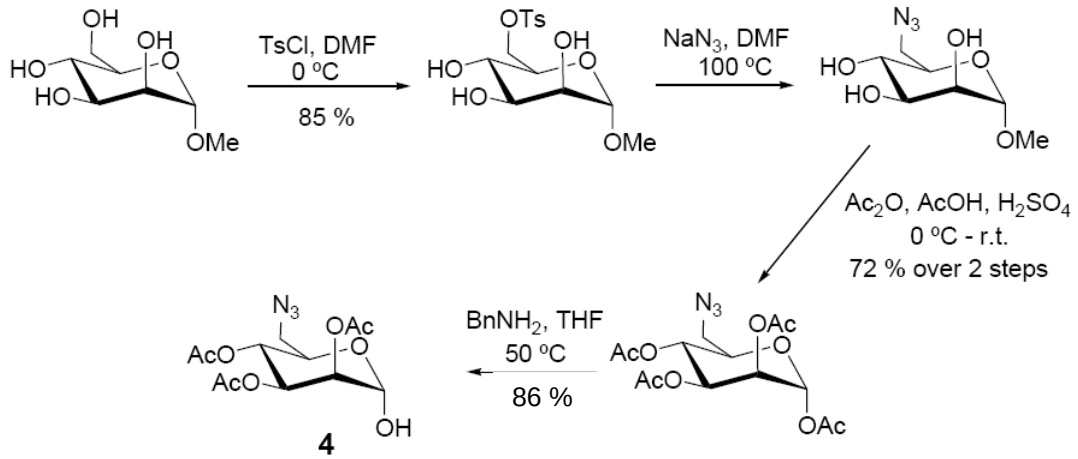
A solution of the 2,3,6-tri-*O*-acetyl-4-azido-4-deoxy-α-D-mannopyranoside (440 mg, 1.33 mmol) in dry DCM (30.0 ml) was added to a flask with 1-*H*-tetrazole (465 mg, 6.64 mmol) under nitrogen and stirred at room temperature for 10 minutes. Diallyl-*N,N*-diisopropyl-phosphoramidite (846 μL, 3.15 mmol) was then added dropwise and stirring was continued overnight at room temperature. The reaction mixture was then cooled at -40 °C and *m*-chloroperbenzoic acid (MCPBA, 1145 mg, 6.64 mmol) was added. The reaction was allowed to come slowly to room temperature over approximately 1 h, then it was diluted with DCM (30.0 ml) and washed with 10 % $NaHSO_3$ (2 x 30.0 ml), sat. aq. $NaHCO_3$ (2 x 30.0 ml), and water (2 x 30.0 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated to afford the crude as an off-white solid that was purified by flash chromatography over silica (1:1 – 2:1 EtOAc/petroleum ether). NMR and ES-MS analysis revealed contamination by $PHO(OAll)_2$ which was successfully removed by further column chromatography over silica (7:1 EtOAc/petroleum ether). The purified α-monophosphate was isolated as a colourless syrup (313 mg, 48 %). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.98-5.80 (2H, m, 2 x CH vinyl), 5.58 (1H, dd, J = 1.6, 6.7 Hz, H1), 5.35-5.15 (6H, m, H2, H3, 2 x CH_2 vinyl), 4.56-4.51 (4H, m, 2 x CH_2 allyl), 4.28 (1H, dd, J = 2.1, - 12.3 Hz, H6a), 4.21 (1H, dd, J = 4.1, - 12.2 H6b), 3.81 (1H, m, H5), 2.08 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), and 1.99 (3H, s, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 170.4, 169.5, 169.4 (3qC, 3 x $COCH_3$), 131.9, 131.8 (2CH, 2 x CH vinyl), 118.9, 118.7 (2CH_2, CH_2 vinyl), 95.0 (1CH, d, C1, $J_{C\text{-}P}$ = 5 Hz), 70.5, 69.6, 68.7, (3CH, C2, C3, C5), 67.9 (1CH_2, C6), 62.8 (2CH_2, CH_2 allyl), 56.3 (1CH, C4), 20.7, 20.6, and 20.5 (3CH_3, 3 x $COCH_3$). ^{31}P NMR (121 MHz, $CDCl_3$): δ (ppm) = -2.0. IR (ν_{max}, thin film): 3087, 3018, 2968, 2958, 2114, 1755, 1650, 1455, 1426, 1372, 1232, 1163, 1081, 1023, 974, 964, 756 cm^{-1}. HR-MS (ESI): *m/z* 514.12026 $(M+Na)^+$, $C_{18}H_{26}N_3O_{11}PNa$ requires 514.12132 (error 2.06 ppm).

Synthesis of monophosphate-4-azido-4-deoxy-α-D-mannopyranoside (7)

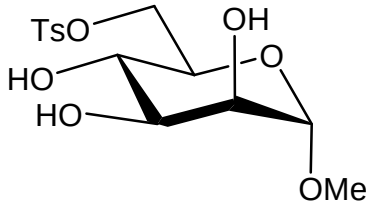


To a 50 ml round bottom flask containing the diallylmonophosphate-2,3,4-tri-*O*-acetyl-4-azido-4-deoxy-α-D-mannopyranoside (174 mg, 0.37 mmol), under Ar, were added: a solution of dry THF/MeOH (26 ml), *p*-toluensulfinic acid sodium salt (131 mg, 0.74 mmol), and tetrakis-triphenylphosphine palladium (26 mg, 0.025 mmol). The reaction mixture was stirred under Ar for 17 h, concentrated *in vacuo* and coevaporated with toluene (3 x 10.0 ml). The crude was resuspended in a mixture of 5:2:1 MeOH/H_2O/TEA (15.0 ml), and stirred at room temperature for 22 h. The solution was filtered to remove a black precipitate, then concentrated *in vacuo*. The resulting syrup was dissolved in water and washed with DCM (3 x 18.0 ml) to remove organic products. The aq. layer was then concentrated and coevaporated with toluene (3 x 10.0 ml) to afford the product as a pale yellow foam (133 mg, 88 % based on isolation of the bis-triethylammonium salt). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 5.36 (1H, dd, J = 8.4, 2.0 Hz, H1), 4.05 (1H, dd, J = 10.2, 3.3 Hz, H3), 3.94 (1H, dd, J = 3.3, 2.1 Hz, H2), 3.87 (1H, dd, J = 1.7, -11.9 Hz, H6a), 3.80 (1H, ddd, J = 10.2, 5.0, 1.7 Hz, H5), 3.76 (1H, dd, J = 5.0, - 11.9 Hz, H6b), 3.62 (1H, dd, J = 10.2, 10.2 Hz, H4), 3.06 (6H, s, $N(CH_2CH_3)_3$), and 1.14 (9H, s, $N(CH_2CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)= 95.7 (1CH, d, C1, $J_{C\text{-}P}$ = 5 Hz), 71.9, 70.9, 70.0, 59.6 (4CH, C2-C5), 61.8 ($1CH_2$, C6), 46.5 ($N(\underline{C}H_2CH_3)_3$), and 8.2 ($N(CH_2\underline{C}H_3)_3$). ^{31}P NMR (121 MHz, $CDCl_3$): δ (ppm) = +2.8. MS (ESI): m/z 284.1 $(M-H)^-$, $C_{12}H_{17}N_3O_{11}P$ requires 284.1.

Summary scheme for the synthesis of **4**:

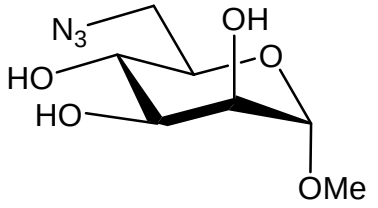


Synthesis of 6-*O*-toluensulfonyl-α-methyl-D-mannopyranoside



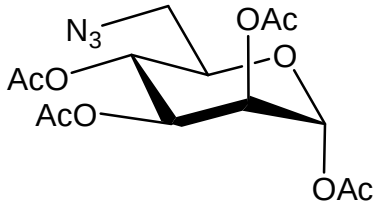
A solution of the α-methyl-D-mannopyranoside (3.36 g, 17.3 mmol) in dry pyridine (20.0 ml) was cooled to 0 ºC in an ice bath with stirring. A solution of *p*-toluensulfonyl chloride (3.61 g, 19.0 mmol) in dry pyridine (15.0 ml) was then added dropwise and stirring was continued at 0 ºC until the reaction seemed complete by TLC. The solvent was then removed *in vacuo* to afford a crude product, which was purified by flash chromatography over silica (15:2 DCM/MeOH) to afford 6-*O*-toluensulphonyl-α-methyl-D-mannopyranoside (4.99 g, 85%) as a colourless oil**.** ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 7.77 (2H, d, J = 8.3 Hz, 2 × ArH), 7.29 (2H, d, J = 8.1 Hz, 2 × ArH), 4.62 (1H, br s, H1), 4.28 (2H, br s, H4, H5), 3.88 (1H, br s, H2), 3.68 (3H, m, H3, CH_2), 3.24 (3H, s, OCH_3), and 2.38 (3H, s, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 144.6 (qC, Ts), 132.3, 129.6, 127.7 (4CH, Ts), 100.7 (1CH, C1), 71.3, 70.1, 69.8, 69.6, 66.7, 54.5 (4CH, C2-C5), 30.4 ($1CH_3$, OCH_3), and 21.3 ($1CH_3$). MS (ESI): *m/z* 370.8 $(M+Na)^+$, $C_{18}H_{20}O_8NSNa$ requires 371.4.

Synthesis of 6-azido-6-deoxy-α-methyl-D-mannopyranoside



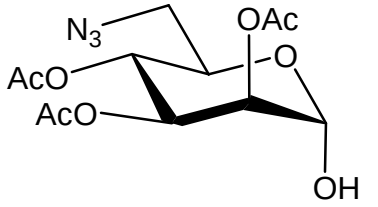
To a solution of dry DMF (20.0 ml) and NaN_3 (4.5 g, 69.0 mmol), was added a solution of the 6-*O*-toluensulfonyl-α-methyl-D-mannopyranoside (3.45 g, 9.90 mmol) in dry DMF. The solvent had been dried over molecular sieves under Argon for 1 h prior to use. The reaction was heated to 100 ºC with stirring behind a blast shield and heating continued at 100 ºC for 6 h. Upon cooling to r.t., the colourless precipitate was removed by filtration through a plug of silica under suction and the solvent was evaporated to dryness. The solid was dissolved in DCM, filtered and evaporated under vacuum. The product was purified by flash chromatography over silica (15:2 DCM/MeOH) to afford the 6-azido-6-deoxy-α-methyl-D-mannopyranoside as a colourless syrup (2.17 g, quantitative). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 4.38 (1H, br s, H1), 3.59 (1H, m), 3.35 (2H, m), 3.24 (2H, m, CH_2), and 3.12 (3H, s, OCH_3). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 100.3 (1CH, C1), 71.1, 70.7, 69.7, 67.5 (4CH, C2-C5), 53.9 ($1CH_3$, OCH_3), and 50.8 ($1CH_2$, C6). MS (ESI): *m/z* 241.7 $(M+Na)^+$, $C_7H_{13}N_3O_5Na$ requires 242.2.

Synthesis of 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside



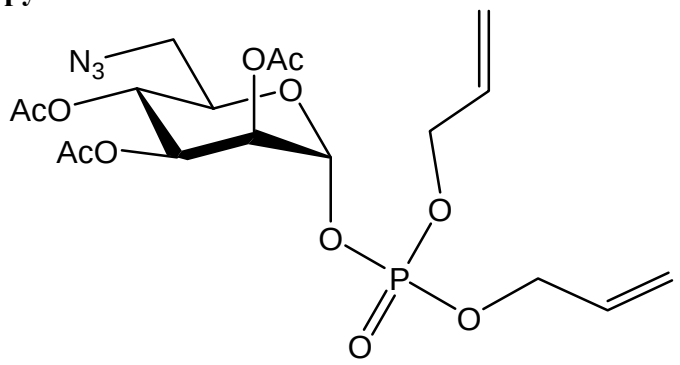
The 6-azido-6-deoxy-α-methyl-D-mannopyranoside (2.17 g, 9.9 mmol) was dissolved in 1:1 Ac_2O/AcOH (206 ml) and cooled to 0 ºC. Concentrated H_2SO_4 (2.2 ml) was added dropwise over 30 min. with stirring. The reaction was then allowed to come to room temperature by removal of the ice bath and stirring was continued overnight. After 26 h the reaction mixture was poured into ice water (250 ml) and the aqueous phase was extracted with DCM (3 × 100 ml). The combined organic extracts were washed with cold water (200 ml), sat. aq. $NaHCO_3$ (200 ml) and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the crude product which was purified by flash chromatography over silica (60:40 pet. ether/EtOAc) to afford the peracetylated product 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside as a colourless oil (2.93 g, 72 % over 2 steps). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 5.97 (1H, d, J = 1.5 Hz, H1), 5.24-5.21 (2H, m, H3, H4), 5.13 (1H, m, J = 2.09, H2), 3.90 (1H, m, H5), 3.30 (1H, dd, J = 13.5 Hz, J = 2.8 Hz, H6a), 3.20 (1H, dd, J = 13.5, J = 5.6, H6b), 2.07 (6H, s, 2 x $COCH_3$), 1.99 (3H, s, $COCH_3$), and 1.90 (3H, s, $COCH_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 169.6, 169.4, 169.2, 167.6 (4qC, 4 x $COCH_3$), 89.9 (1CH, C1), 71.4, 68.2, 67.9, 66.0 (4CH, C2-C5), 50.3 ($1CH_2$, C6), 20.4, 20.3, 20.3, and 20.2 ($4CH_3$, 4 × $COCH_3$). MS (ESI): *m/z* 395.9 $(M+Na)^+$, $C_{14}H_{19}N_3O_9Na$ requires 396.3.

Synthesis of 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranose (4)



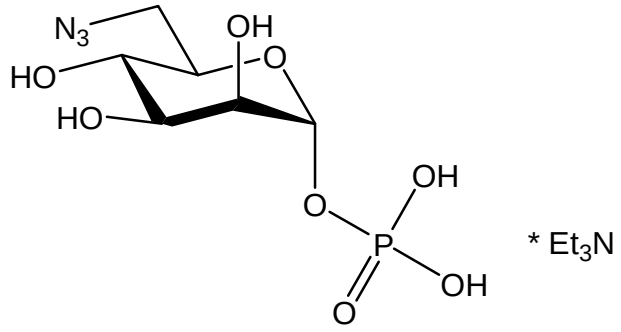
The peracetylated 6-azido-6-deoxy-α-D-mannose (2.93 g, 7.90 mmol) in dry THF (40.0 ml) was treated with benzylamine (1036 µl) under Ar. The solvent was further dried over molecular sieves under Ar for 1 h prior to use. The reaction mixture was warmed to 50 ºC and stirring continued for 24 h under Ar. The solvent was then removed under reduced pressure and the crude product purified by flash chromatography over silica (60:40 pet. ether/EtOAc) to afford the product as a colourless oil (2.10 g, 86 %). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 5.34 (1H, dd, J = 10.0, J = 3.0, H3), 5.22-5.14 (3H, m, H1, H2, H4), 4.67 (1H, br s, OH), 4.14 (1H, m, H5), 3.30-3.27 (2H, m, H6a, H6b), 2.10 (3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), and 1.93 (3H, s, $COCH_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 170.1, 169.9, 169.8 (3qC, 3 x $COCH_3$), 91.3 (1CH, C1), 69.8, 68.9, 68.4, 66.8 (4CH, C2-C5), 50.5 ($1CH_2$, C6), 20.3, 20.1, and 20.1 ($3CH_3$, 3 x $COCH_3$). MS (ESI): *m/z* 354.1 $(M+Na)^+$, $C_{12}H_{17}N_3O_8Na$ requires 354.3.

Synthesis of diallylmonophosphate-2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-manno-pyranoside



A solution of the 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranose (266 mg, 0.80 mmol) in dry DCM (20.0 ml) was added to a flask with 1-*H*-tetrazole (280 mg, 4.0 mmol) under Ar and stirred at room temperature for 10 minutes. Diallyl-*N,N*-diisopropyl-phosphoramidite (510 μL, 1.9 mmol) was then added dropwise and stirring was continued for further 4.5 h at room temperature. The reaction mixture was then cooled at -40 °C and *m*-chloroperbenzoic acid (MCPBA, 690 mg, 4.0 mmol) was added. The reaction was allowed to come slowly to room temperature over approximately 1 h, then it was diluted with DCM (15.0 ml) and washed with 10 % $NaHSO_3$ (2 x 15.0 ml), sat. aq. $NaHCO_3$ (2 x 15.0 ml), and water (2 x 15.0 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated to afford the crude as an off-white solid that was purified by flash chromatography over silica (1:1 – 2:1 EtOAc/petroleum ether). NMR and ES-MS analysis revealed contamination by $PHO(OAll)_2$ which was successfully removed by further column chromatography over silica (7:1 EtOAc/petroleum ether). The purified α-monophosphate was isolated as a colourless syrup (240 mg, 72 %). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 5.96-5.80 (2H, m, 2 x CH allyl), 5.57 (1H, dd, J = 1.0, 5.6 Hz, H1), 5.35 (1H, d, J = 1.0 Hz, H3), 5.28-5.19 (5H, m, H2, 2 x CH_2 allyl), 4.56-4.50 (5H, m, H4, 2 x PO-CH_2), 4.06 (1H, m, H5), 3.36 (1H, dd, J = 13.5, J = 2.8, H6a), 3.28 (1H, dd, J = 13.5, J = 5.5, H6b), 2.08 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), and 1.96 (3H, s, $COCH_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm) = 169.7, 169.5, 169.4 (3qC, 3 x $COCH_3$), 131.9, 131.8 (2CH, 2 x CH allyl, $J_{C\text{-}P}$ = 6 Hz), 118.9, 118.7 (2CH_2, 2 x CH_2 allyl), 94.6 (1CH, d, C1, $J_{C\text{-}P}$ = 6 Hz), 71.5, 68.6, 67.9, 66.1 (4CH, C2-C5), 68.8, 68.5 (2CH_2, 2 x CH_2 vinyl, $J_{C\text{-}P}$ = 5 Hz), 50.6 (1CH_2, C6), 20.6, 20.5, and 20.4 (3CH_3, 3 x $COCH_3$). ^{31}P NMR (101 MHz, $CDCl_3$): δ (ppm) = -1.8. IR (ν_{max}, thin film): 2960, 2950, 2856, 2103, 1751, 1632, 1454, 1425, 1370, 1242, 1224, 1159, 1071, 1040, 1020, 980, 971, 960, 882, 820, 746 cm^{-1}. HR-MS (ESI): *m/z* 492.13834 $(M+H)^+$, $C_{18}H_{27}N_3O_{11}P$ requires 492.139492 (error: 2.34 ppm).

Synthesis of monophosphate-6-azido-6-deoxy-α-D-mannopyranoside (8)



To a 100 ml round bottom flask containing diallylmonophosphate-2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (384 mg, 0.78 mmol), under Ar, were added: a solution of dry THF/MeOH (32.0 ml), *p*-toluensulfinic acid sodium salt (278 mg, 1.56 mmol), and tetrakis-triphenylphosphine palladium (68 mg, 0.06 mmol). The reaction mixture was stirred under Ar for 6.5 h, concentrated *in vacuo* and coevaporated with toluene (3 x 20.0 ml). The crude was resuspended in a mixture of 5:2:1 MeOH/H_2O/TEA (40.0 ml), and stirred at room temperature for 18 h. The solution was filtered to remove a black precipitate, then concentrated *in vacuo*. The resulting syrup was dissolved in water and washed with DCM (3 x 50.0 ml) to remove organic products. The aq. layer was then concentrated and coevaporated with toluene (3 x 25.0 ml) to afford the product as a pale yellow foam (339 mg, 86 % based on isolation of the bis-triethylammonium salt). ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) = 5.21 (1H, d, J = 1.7 Hz, H1), 3.86-3.78 (3H, m, H2-4), 3.64-3.45 (3H, m, H5, $H6_{a-b}$), 3.06 (6H, s, $N(CH_2CH_3)_3$), and 1.14 (9H, s, $N(CH_2CH_3)_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ (ppm)= 95.1 (1CH, d, C1, $J_{C\text{-}P}$ = 4 Hz), 71.4, 70.8, 69.7, 67.3 (4CH, C2-C5), 51.1 ($1CH_2$, C6), 46.5 ($N(\underline{C}H_2CH_3)_3$), and 8.2 ($N(CH_2\underline{C}H_3)_3$). ^{31}P NMR (101 MHz, $CDCl_3$): δ (ppm) = +1.9. MS (ESI): *m*/*z* 284.0 $(M-H)^-$, $C_{12}H_{17}N_3O_{11}P$ requires 284.14.

Expression of His_{10}-GDPManPP

Expression of His_{10}-tagged proteins was carried out according to the manufacturers-instructions (pET system manual, Novagen). A single colony of E. coli (strain ER2566) transformed with the cDNA encoding GDPManPP (NdeI/BamHI inserted into pET16b) was used to inoculate a 10 ml culture of L.B. medium containing 100 μg/ml ampicillin. The culture was incubated at 37 °C with shaking (250 rpm) overnight. This culture was then used to inoculate 2 x 500 ml of fresh L.B. medium containing the required antibiotic and incubated at 37 °C with shaking until A_{650} = 0.6. IPTG was added to a concentration of 1 mM and the incubation was continued with shaking at 30 °C for 5h. Cells were harvested by centrifugation at 8000 rpm (10,000 G) at 4 °C for 10 minutes. The pellet could then be stored at -20 °C prior to purification.

Preparation of a cell-free extract (CFE)

A pellet derived from a 1 l induced culture was thawed on ice and resuspended in 35 ml binding buffer (5mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 8) containing 10 mM PMSF. The cells were sonicated on ice for 15 periods of 30 seconds separated by 30 seconds intervals. Triton X-100 was added to a final concentration of 0.1% v/v and the resulting solution was rocked on ice for 10 minutes. The cells were then sonicated again on ice for 15 periods of 30 seconds separated by 30 seconds intervals. Inclusion bodies and cellular debris were collected by centrifugation at 10,000 rpm for 15 min. at 4 °C. The resulting pellet was discarded and the CFE supernatant was stored at -20 °C prior to affinity chromatography.

Column chromatography of His_{10}-GDPManPP

The CFE supernatants were purified under non-denaturing conditions using Ni^{2+} immobilised on a metal chelation resin as described in the manufacturer's instructions (Sigma-Aldrich). The column (2.5 ml bed volume) was charged with 7 ml aqueous $NiSO_4$ and equilibrated with 12.5 ml binding buffer. The CFE was then loaded onto the column and unbound proteins were washed with binding buffer containing 1mM PMSF, and then wash buffer (50 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, 1 mM PMSF, pH 7.8) until $A_{280} < 0.1$. The target protein was then eluted in 1.5 ml fractions using eluting buffer (500 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7) until $A_{280} < 0.1$. The fractions were then analysed by SDS-PAGE: 10 µl aliquots were mixed with 10 µl of SDS loading buffer. The fractions containing the desired protein were combined, exchanged 3 times against fresh buffer (50 mM Tris pH 7.6), and concentrated to a final volume of 1 ml using a Vivaspin (MWCO 30 kDa) concentrator, which was previously passivated with 5% Tween 20 according to the manufacturers instructions.

Preparation and validation of the malachite reagent

To a 100 ml volumetric flask were added: 34.0 mg of malachite green oxalate salt (Sigma), 1235.9 mg of ammonium molybdate, 3.4 ml of absolute ethanol, about 80 ml of deionised water, 8.59 ml of concentrated HCl (37%), 1 ml of Tween 20 and deionised water up to 100 ml total volume. The resulting mixture was stirred at room temperature for 1h, then it was filtered (0.2 µm) and stored at 4 °C for at least 7 days prior to use.
Prior to use, the reagent was calibrated by using a standard series of KH_2PO_4 solutions (in deionised water) at variable concentrations. On a 96-well plate, 25 µl of each standard was added to a well containing 100 µl of malachite reagent, in triplicate. The plate was incubated at 37 °C with mixing (SpectraMax Plus 384, Molecular Devices), and the absorbance was read at 650 nm over 30 minutes. Typically, the absorbance maximum was reached within the first 15 minutes. The absorbance values at 15 minutes were plotted against the phosphate concentration, and from linear fitting an equation was obtained, where the slope gave the conversion factor.

GDPManPP activity assay
M1P / AzM1P-TEA salt / GTP 100 μM
GDPManPP 10 μg
iPPase 0.5 U
$MgCl_2$ 8 mM
DTT 1 mM
TRIS 50 mM pH 7.6 up to 500 μL (final volume).

The reaction was carried out in triplicate in 1.5 mL Eppendorf tubes in an Eppendorf Thermomixer, at 37 °C, 300 rpm. Every 10 minutes, 25 μL samples were taken and added to a 96-wells plate with 100 μL of malachite reagent (validated the same day). The malachite assay was carried out in a plate reader (SpectraMax Plus 384, Molecular Devices) at 37 °C, with mixing, for 15 minutes, then the absorbance at 650 nm was read. Values were plotted over time.

Semipreparative GDPManPP reaction
M1P / AzM1P-TEA salt / GTP 20 mM
GDP-ManPP 1.6 mg
iPPase 40 U
$MgCl_2$ 8 mM
DTT 5 mM
BSA 4 mg
TRIS 50 mM pH 7.6 up to 4 ml (final volume).

The reaction was heated at 37 ºC with shaking (250 rpm). After 60 h, the reaction was centrifuged at 3000 pm for 15 minutes at 4 °C, and the supernatant was filtered using a Pall device (MWCO 10 kDa) prior to purification by HPLC.
HPLC: (Sphereclone SAX 5 μ, 250 mm x 10 mm, flow = 5mL/min; solvent A = dH_2O; solvent B = 0.6 M ammonium formate). Gradient: from t=0 to t=5', B increases from 0% to 10%; from t=5' to t=25' B increases from 10% to 25%; from t=25' to t=30' B increases from 25% to 100%; then B is kept at 100% until t=35', when it changes at once to 0% and is kept 0% until t=40'. Absorbance is monitored at 230 and 254 nm. The product has t_R = 12' (m/z = 629.3). Note: On the semi-preparative column GDP has t_R = 18', and GTP has t_R = 22'.
The fractions containing the product are freeze-dried and then stored at – 20 °C.

GDP-2-azido-2-deoxy-α-D-mannopyranoside (9)
^{1}H NMR (500 MHz, D_2O): δ (ppm) = 8.11 (1H, s, H8), 5.93 (1H, d, J = 6.2 Hz, H1'), 5.57 (1H, dd, J = 6.0, 1.8 Hz, H1"), 4.77 (1H, dd, J = 5.9, 5.1 Hz, H2'), 4.51 (1H, dd, J = 5.1, 3.6 Hz, H3'), 4.35 (1H, m, H4'), 4.21 (2H, m, H5'a, H5'b), 4.14 (1H, dd, J = 9.5, 3.9 Hz, H3"), 4.11 (1H, dd, J = 3.8, 1.8 Hz, H2"), 3.84 (2H, m, H5", H6a"), 3.73 (1H, dd, J = 5.7, -13.1 Hz, H6b"), and 3.66 (1H, dd, J = 9.7, 9.7 Hz, H4"). ^{13}C NMR (125 MHz, D_2O): δ (ppm)= 159.7, 154.6, 152.5, (3Cq, C2, C4, C6), 138.3 (1CH, C8), 116.9 (1Cq, C5), 95.2 (1CH, d, J_{C-P} = 5 Hz, C1"), 87.5 (1CH, C1'), 84.4 (1CH, d, J_{C-P} = 9 Hz, C4'), 74.3 (1CH, C2'), 74.2 (1CH, C5"), 71.0 (1CH, C3'), 70.5 (1CH, C3"), 66.9 (1CH, C4"), 65.9 ($1CH_2$, d, J_{C-P} = 5 Hz, C5'), 64.6 (1CH, d, J = 9 Hz, C2") and 61.0 ($1CH_2$, C6"). ^{31}P NMR (101 MHz, D_2O): δ (ppm) = -10.2, -12.9. HR-MS (ESI): m/z 629.0745 $(M–H)^-$, $C_{16}H_{23}N_8O_{15}P_2$ requires 629.0758 (error: 2.1 ppm).

GDP-3-azido-3-deoxy-α-D-mannopyranoside (10)
^{1}H NMR (500 MHz, D_2O): δ (ppm) = 8.10 (1H, s, H8), 5.92 (1H, d, J = 5.8 Hz, H1'), 5.47 (1H, dd, J = 6.0, 1.8 Hz, H1"), 4.72 (1H, dd, J = 6.0, 5.1 Hz, H2'), 4.50 (1H, dd, J = 5.1, 3.6 Hz, H3'), 4.35 (1H, m, H4'), 4.22 (2H, m, H5'a, H5'b), 3.99 (1H, dd, J = 3.2, 1.9 Hz, H2"), 3.91 (1H, dd, J = 9.5, 3.2 Hz, H3"), and 3.88-3.68 (4H, m, H4", H5", H6a", H6b"). ^{13}C NMR (125 MHz, D_2O): δ (ppm)= 159.6, 154.5, 152.4, (3Cq, C2, C4, C6), 138.1 (1CH, C8), 116.9 (1Cq, C5), 97.1 (1CH, d, J_{C-P} = 6 Hz, C1"), 87.5 (1CH, C1'), 84.2 (1CH, d, J_{C-P} = 8 Hz, C4'), 74.4 (1CH, C2'), 72.7 (1CH, C5"), 70.9 (1CH, C3'), 70.1 (1CH, C2"), 69.8 (1CH, C3"), 65.9 ($1CH_2$, d, J_{C-P} = 5 Hz, C5'), 61.5 ($1CH_2$, C6"), 59.1 (1CH, C4"). ^{31}P NMR (101 MHz, D_2O): δ (ppm) = -10.1, -12.7 HR-MS (ESI): m/z 629.0745 $(M–H)^-$, $C_{16}H_{23}N_8O_{15}P_2$ requires 629.0758 (error 2.1 ppm).

GDP-4-azido-4-deoxy-α-D-mannopyranoside (11)
^{1}H NMR (500 MHz, D_2O): δ (ppm) = 8.10 (1H, s, H8), 5.92 (1H, d, J = 5.8 Hz, H1'), 5.49 (1H, dd, J = 6.0, 1.9 Hz, H1"), 4.72 (1H, dd, J = 5.9, 5.1 Hz, H2'), 4.48 (1H, dd, J = 5.1, 3.8 Hz, H3'), 4.33 (1H, m, H4'), 4.19 (2H, m, H5'a, H5'b), 3.99 (1H, dd, J = 3.2, 1.9 Hz, H2"), 3.91 (1H, dd, J = 9.5, 3.3 Hz, H3"), 3.80 (1H, dd, J = 11 Hz, H6a"), and 3.68 (3H, m, H4", H5", H6b"). ^{13}C NMR (125 MHz, D_2O): δ (ppm)= 159.7, 154.6, 152.6, (3Cq, C2, C4, C6), 138.2 (1CH, C8), 116.9 (1Cq, C5), 97.1 (1CH, d, J_{C-P} = 6 Hz, C1"), 87.5 (1CH, C1'), 84.2 (1CH, d, J_{C-P} = 8 Hz, C4'), 74.4 (1CH, C2'), 72.7 (1CH, C5"), 70.9 (1CH, C3'), 70.1 (1CH, C2"), 69.8 (1CH, C3"), 65.9 ($1CH_2$, d, J_{C-P} = 5 Hz, C5'), 61.5 ($1CH_2$, C6"), 59.1 (1CH, C4"). ^{31}P NMR (101 MHz, D_2O): δ (ppm) = -10.1, -12.7. HR-MS (ESI): m/z 629.0745 $(M–H)^-$, $C_{16}H_{23}N_8O_{15}P_2$ requires 629.0758 (error: 2.1 ppm).

GDP-6-azido-6-deoxy-α-D-mannopyuranoside (12)
^{1}H NMR (500 MHz, D_2O): δ (ppm) = 8.10 (1H, s, H8), 5.82 (1H, d, J = 5.9 Hz, H1'), 5.47 (1H, dd, J = 6.9, 1.5 Hz, H1"), 4.75 (1H, dd, J = 5.9, 5.1 Hz, H2'), 4.49 (1H, dd, J = 5.1, 3.6 Hz, H3'), 4.33 (1H, m, H4'), 4.19 (2H, m, H5a', H5b'), 4.02 (1H, dd, J = 3.2, 1.5 Hz, H2"), 3.91 (1H, ddd, J = 9.9, 4.4, 2.5 Hz, H5"), 3.89 (1H, dd, J = 9.8, 3.4 Hz, H3"), 3.70 (1H, dd, J = 9.9, 9.9 Hz, H4"), 3.63 (1H, dd, J = 2.5, -13.6 Hz, H6a"), and 3.55 (1H, dd, J= 4.6, -13.6 Hz, H6b"). ^{13}C NMR (125 MHz, D_2O): δ (ppm)= 159.6, 154.5, 152.4, (3Cq, C2, C4, C6), 138.3 (1CH, C8), 116.9 (1Cq, C5), 97.1 (1CH, d, J_{C-P} = 6 Hz, C1"), 87.6 (1CH, C1'), 84.4 (1CH, d, J_{C-P} = 8 Hz, C4'), 74.3 (1CH, C2'), 72.8 (1CH, C5"), 71.0 (1CH, C3'), 70.8 (1CH, C3"), 70.3 (1CH, C2"), 67.6 (1CH, C4"), 65.9 ($1CH_2$, d, J_{C-P} = 5 Hz, C5'), and 51.4 ($1CH_2$, C6"). ^{31}P NMR (101 MHz, D_2O): δ (ppm) = -10.3, -12.8. HR-MS (ESI): m/z 629.0745 $(M–H)^-$, $C_{16}H_{23}N_8O_{15}P_2$ requires 629.0758 (error: 2.1 ppm).

ManT expression
A single colony of pManFlag20 in XL-1 Blue cells was used to seed 10 ml of fresh L.B. medium containing Ampicillin (100 μg/ml). The culture was incubated at 37 °C overnight with shaking at 250 rpm. The overnight culture was then spun down (3000 rpm, 4 °C, 15 min), the supernatant was discarded, and the cells pellet was resuspended in 10 ml of fresh M9-CA modified medium (M9 salts with 20 g/l casamino acids, 0.4% glycerol, 2 mM $MgSO_4$) containing Ampicillin (100 μg/ml). This was grown as above until OD_{600}=0.5, then protein expression was induced with 5 μM IPTG at 30 °C for 12h. Cells were harvested by centrifugation at 8000 rpm (10,000 G) at 4 °C for 15 minutes. The pellet could then be stored at -20 °C prior to purification.

ManT Purification
The cells pellet from 1l culture was thawed on ice and resuspended in 20mL of ice-cold 20% sucrose, 10mM tris pH 7.6, containing 2 mM PMSF 2 mM. 0.4 mL of 0.5M EDTA was slowly added. Cells were incubated on ice for 30 minutes, and then collected by centrifugation at 8000 rpm for 20 minutes. The supernatant was dialysed against 0.1M ammonium formate, pH 7.5, and then concentrated down to 1 ml on a Vivaspin concentrator (MWCO 10 kDa).

ManT activity assay

One 1.5 ml Eppendorf tube containing ManT (107.2 μL), the GDP-mannose donor (1.25 mM of GDPMan, GDP-2-AzMan, GDP-3-AzMan, GDP-4-AzMan, or GDP-6-AzMan) and the α-methyl mannoside acceptor (7.5 mM), in a total volume of 160 μl of 50 mM ammonium formate buffer pH 7.5, 10 mM $MnCl_2$, was incubated at 30 °C for 24 h. The proteins were then separated by membrane filtration (MWCO 10 kDa), and the filtrate was analyzed by ESI-MS.

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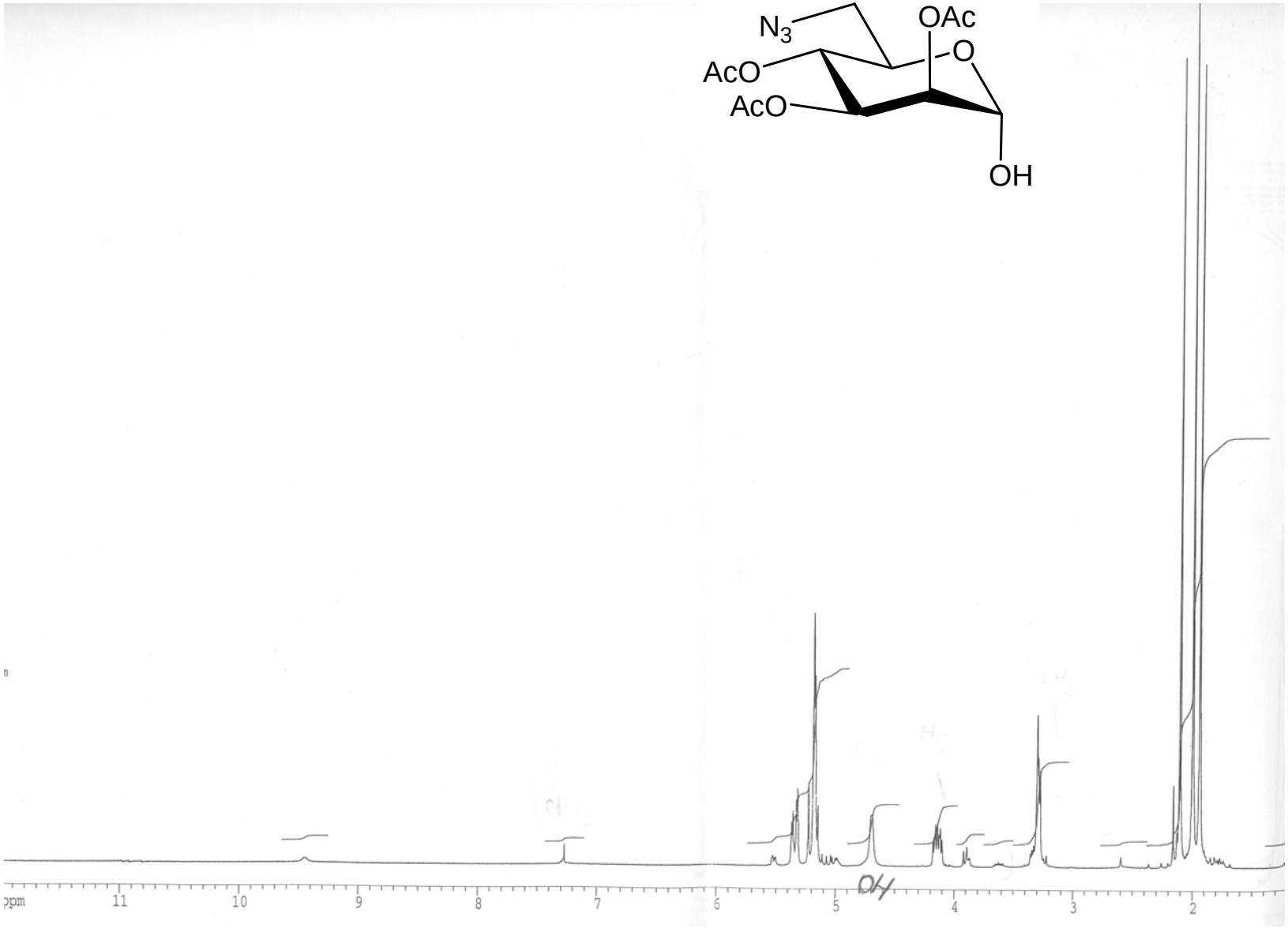
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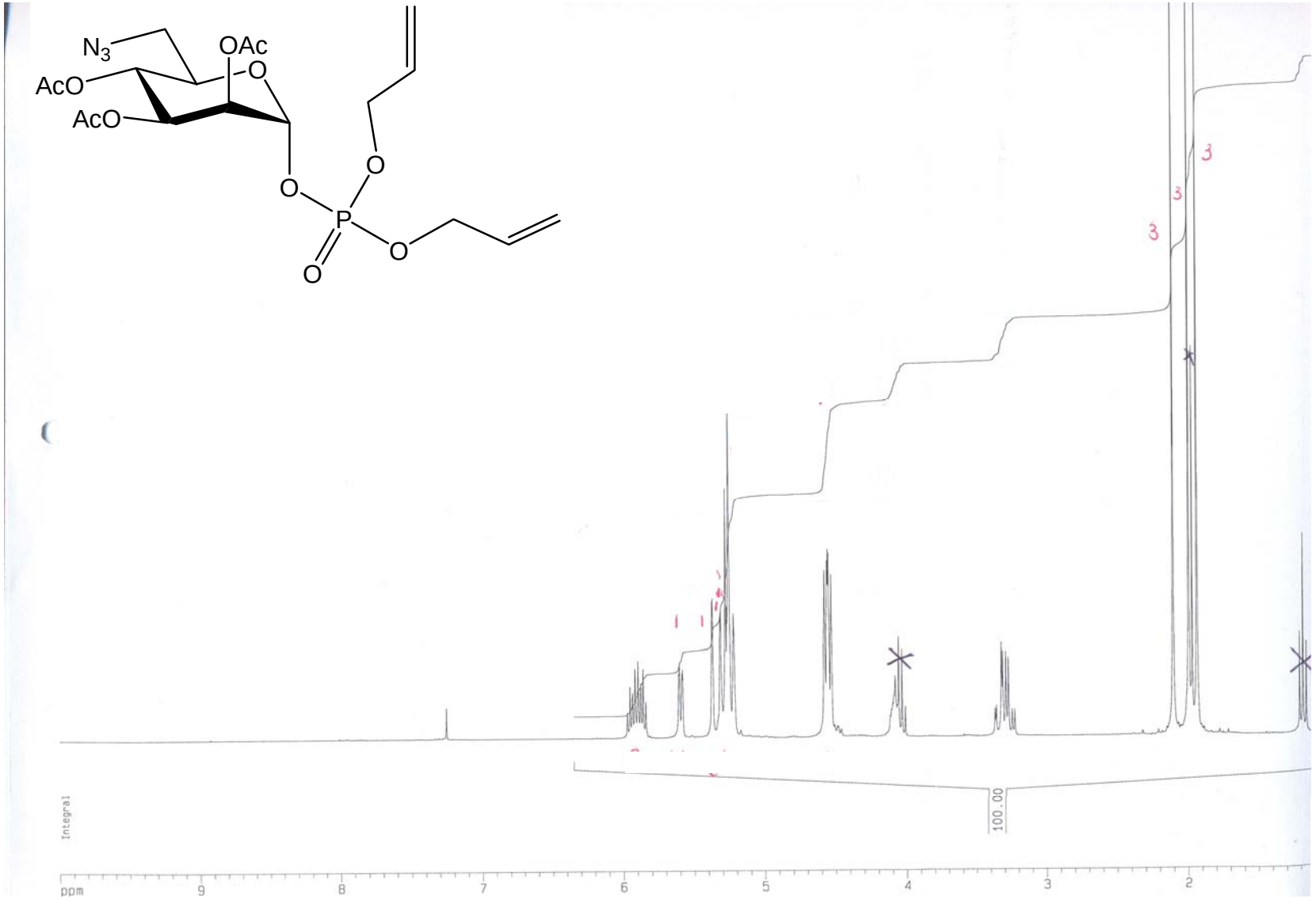
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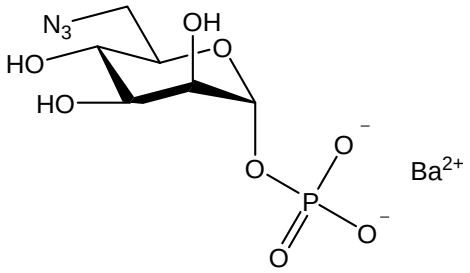
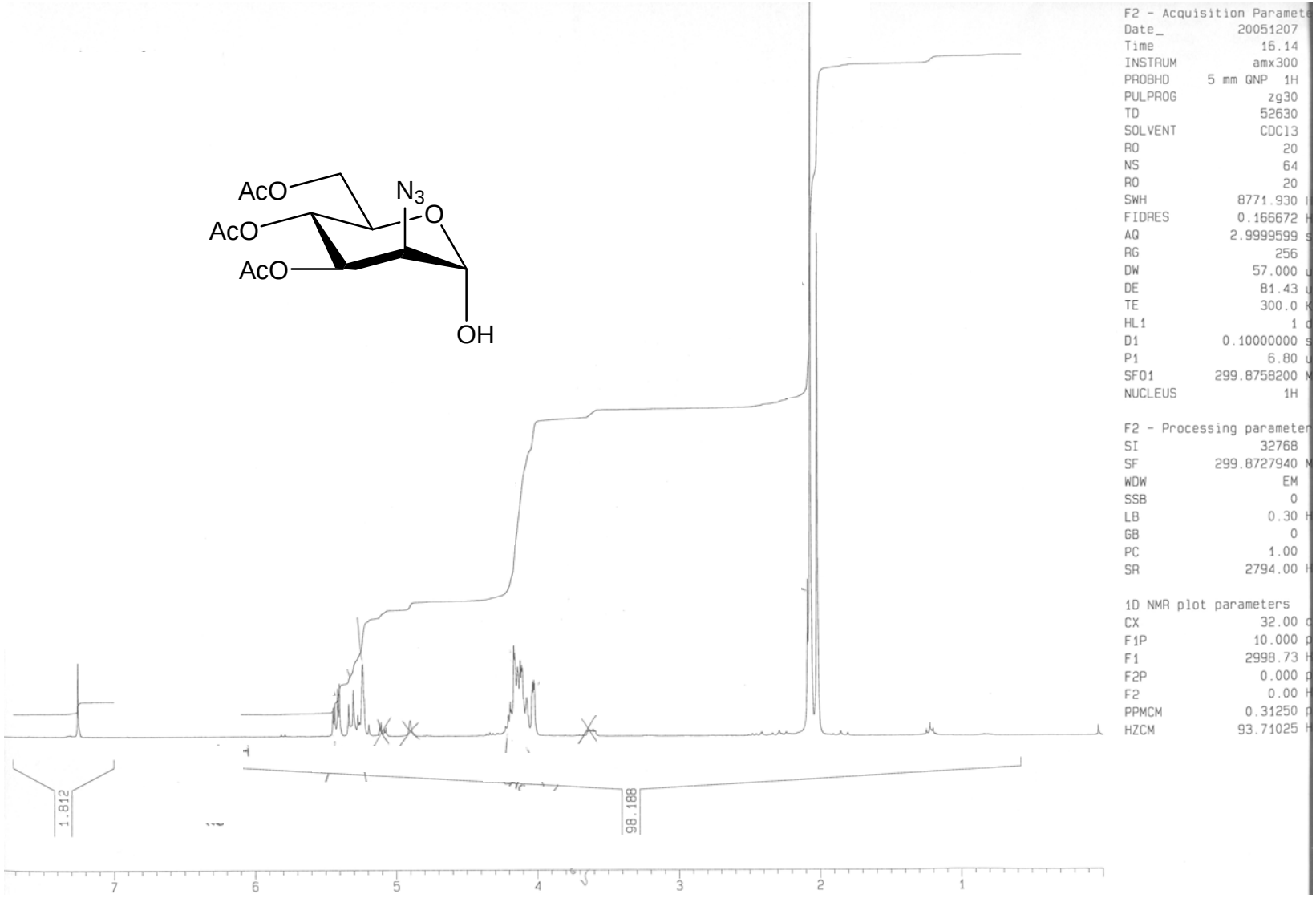
6
5
4
3
2



While the phosphates are obtained and used as triethylammonium salts, conversion to Barium salts through ion exchange (though not routinely employed) allowed for simpler NMR spectra


AcO
AcO
AcO
N3
O
OH
1.812
98.188
7
6
5
4
3
2
1
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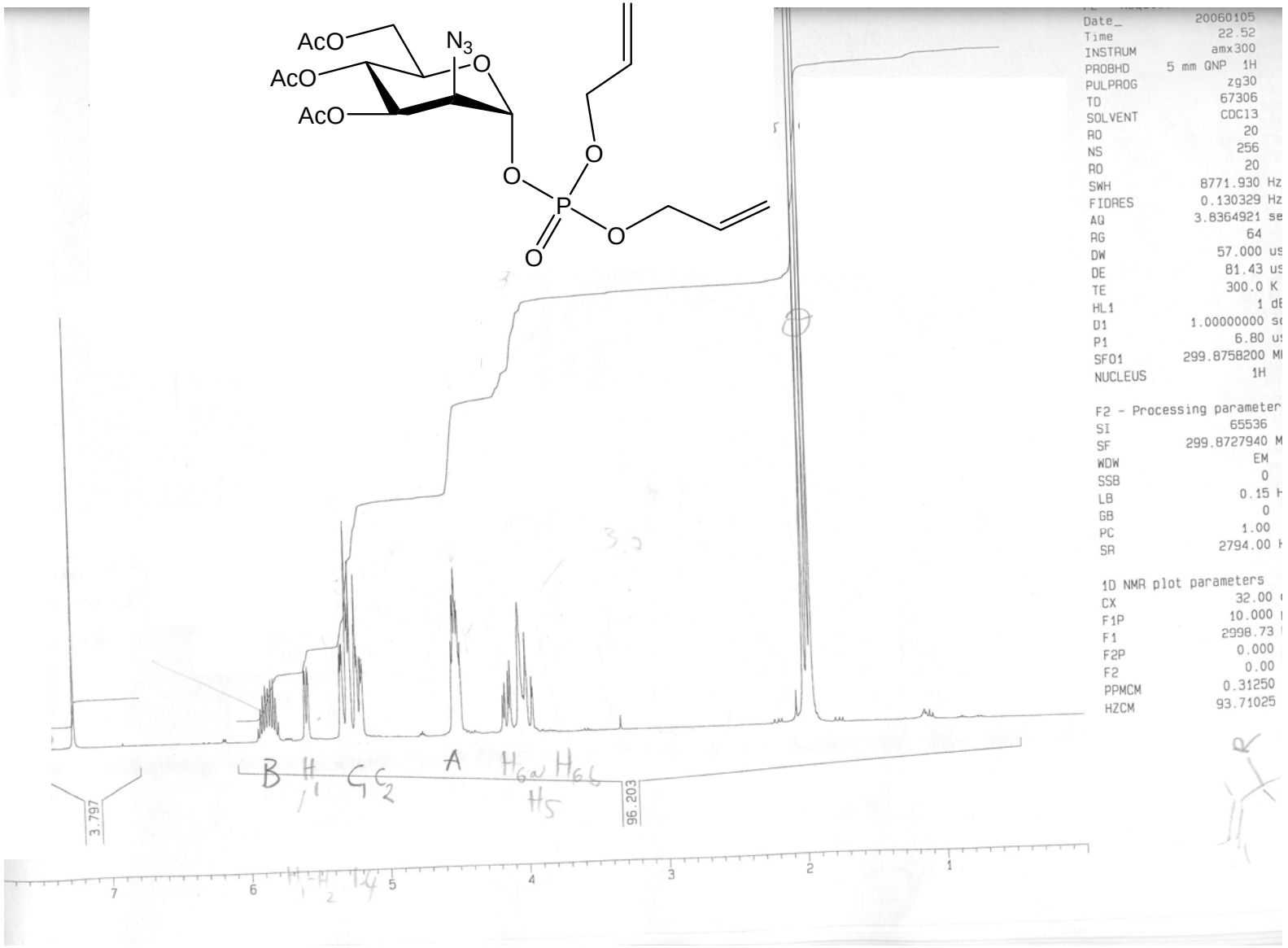
Time 16.14
INSTRUM amx300
PROBHD 5 mm GNP 1H
PULPROG zg30
TD 52630
SOLVENT CDCl3
RO 20
NS 64
RO 20
SWH 8771.930 H
FIDRES 0.166672 H
AQ 2.9999599 s
RG 256
DW 57.000 u
DE 81.43 u
TE 300.0 K
HL1 1 c
D1 0.10000000 s
P1 6.80 u
SFO1 299.8758200 M
NUCLEUS 1H
F2 - Processing parameter
SI 32768
SF 299.8727940 M
WDW EM
SSB 0
LB 0.30 H
GB 0
PC 1.00
SR 2794.00 H
1D NMR plot parameters
CX 32.00 c
F1P 10.000 p
F1 2998.73 H
F2P 0.000 p
F2 0.00 H
PPMCM 0.31250 p
HZCM 93.71025 H


AcO
AcO
AcO
N3
O
O
O
P
O
O
O
Date_ 20060105
Time 22.52
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PULPROG zg30
TD 67306
SOLVENT CDCl3
RO 20
NS 256
RO 20
SWH 8771.930 Hz
FIDRES 0.130329 Hz
AQ 3.8364921 se
RG 64
DW 57.000 us
DE 81.43 us
TE 300.0 K
HL1 1 dB
D1 1.00000000 s
P1 6.80 us
SFO1 299.8758200 M
NUCLEUS 1H
F2 - Processing parameter
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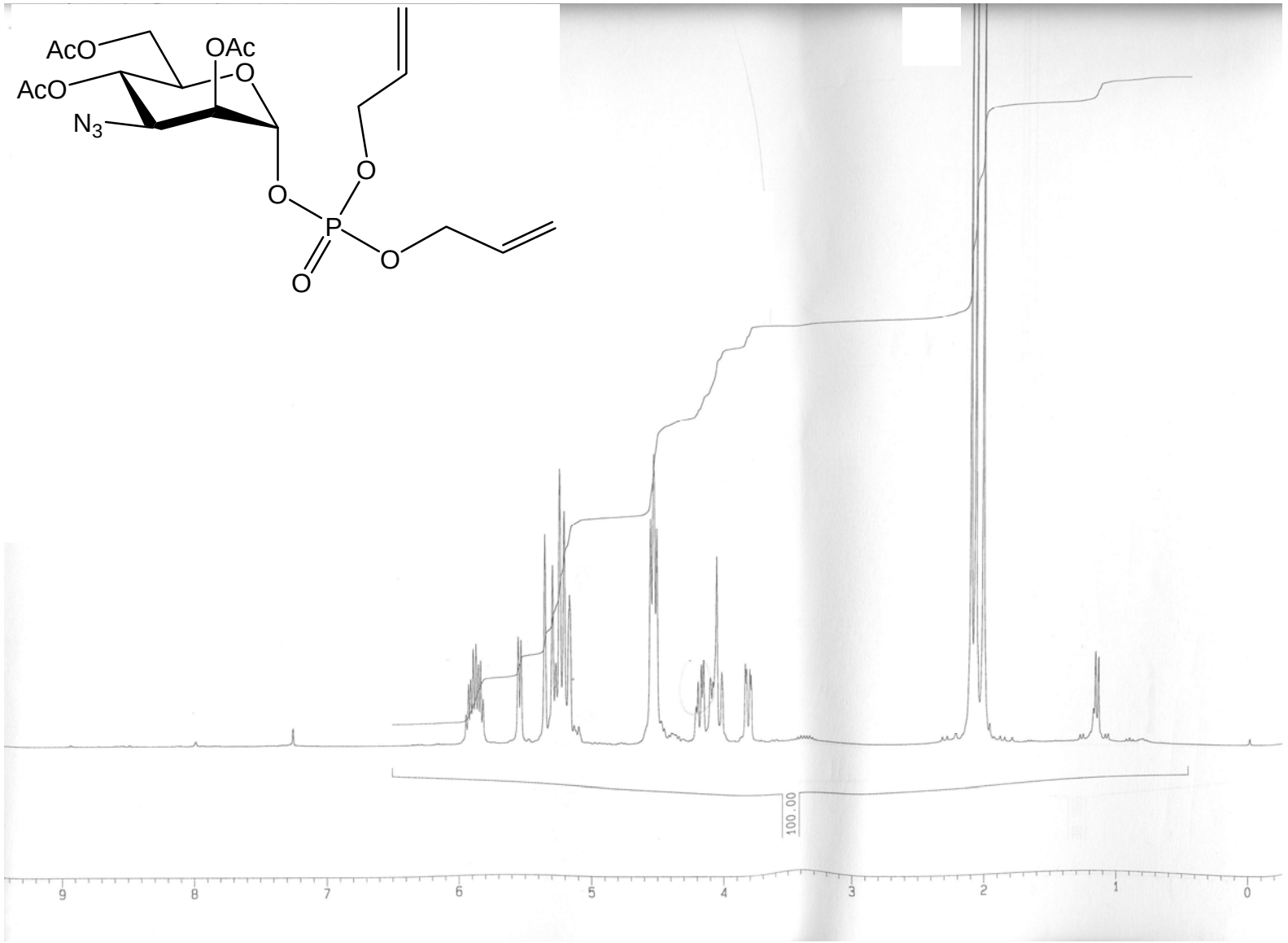
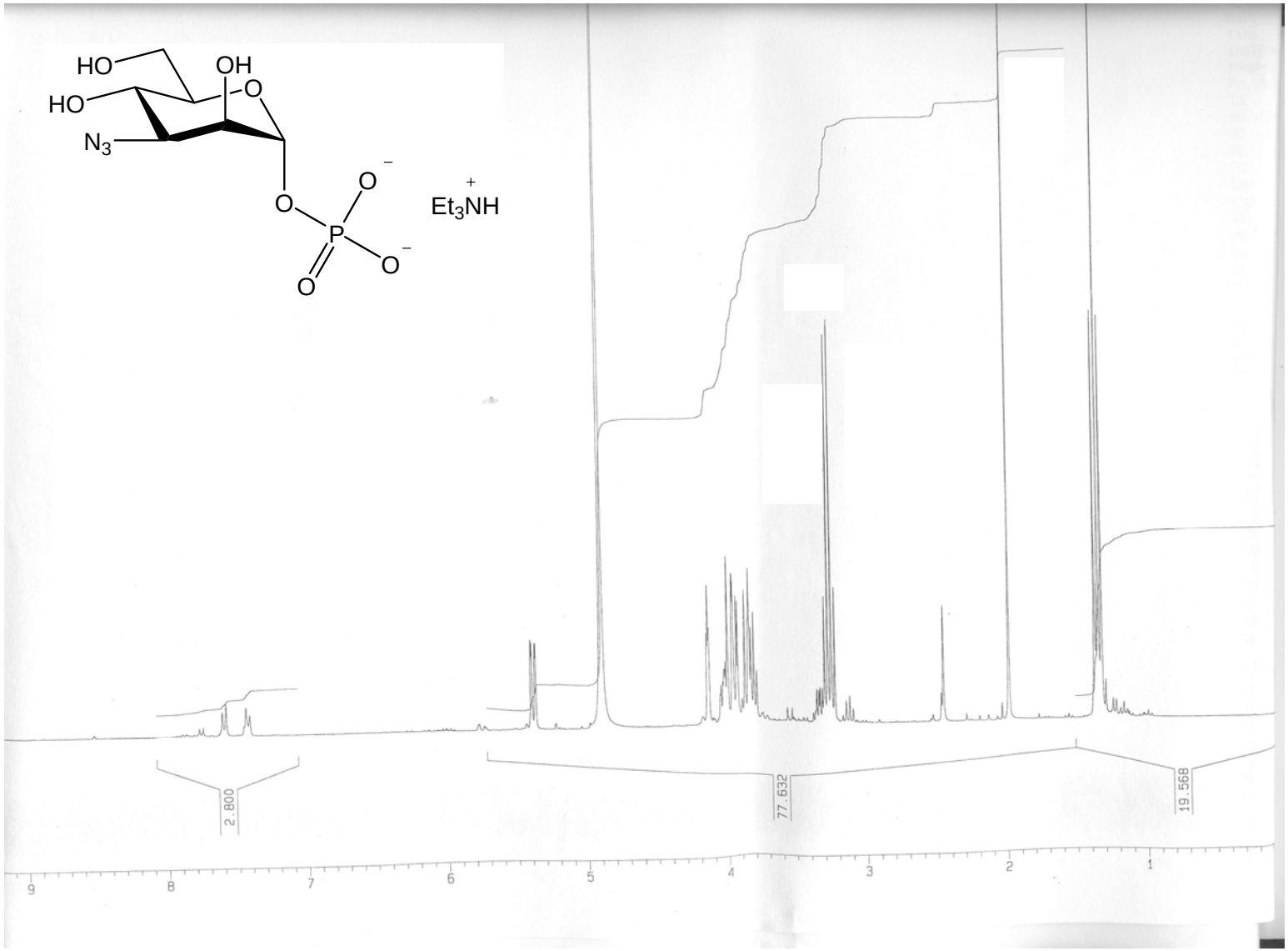
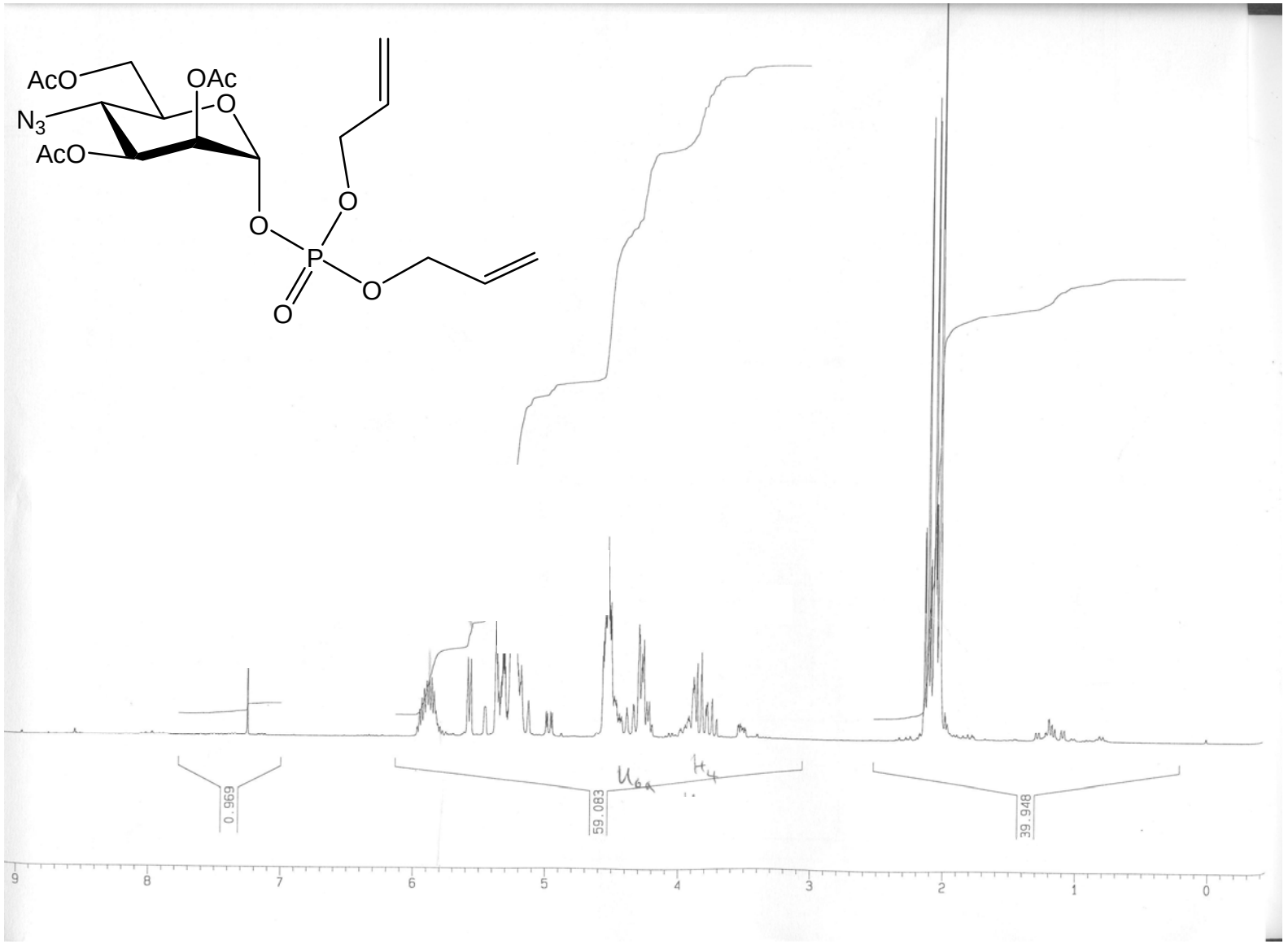
SF 299.8727940 M
WDW EM
SSB 0
LB 0.15 H
GB 0
PC 1.00
SR 2794.00 H
1D NMR plot parameters
CX 32.00
F1P 10.000
F1 2998.73
F2P 0.000
F2 0.00
PPMCM 0.31250
HZCM 93.71025
B
A
H6a H6b
H5
3.797
96.203
7
6
5
4
3
2
1


AcO
OAc
AcO
O
N3
O
O
P
O
O
O
100.00
9
8
7
6
5
4
3
2
1
0


HO
OH
HO
O
N3
O
O
P
O
O
Et3NH
2.800
77.632
19.568
9
8
7
6
5
4
3
2
1


AcO
OAc
O
N3
AcO
O
O
P
O
O
0.969
59.083
39.948
9
8
7
6
5
4
3
2
1
0