Glycan Arrays Containing Synthetic *Clostridium difficile* Lipoteichoic Acid Oligomers as Tools Toward A Carbohydrate Vaccine

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Supporting Information

Contents

Reagents and general synthetic procedures

Commercial grade reagents and solvents were used without further purification, except as indicated below. All batch reactions were conducted under an Ar atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian 400-MR or Varian 600 spectrometer. The proton signal of residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$; δ 4.79 ppm for H_2O, 2.84 ppm for acetone-d_6) was used as an internal reference for ^{1}H spectra. For ^{13}C spectra, the chemical shifts are reported relative to the respective solvent (δ 77.16 ppm for $CDCl_3$, δ 29.84 ppm for acetone-d_6). For ^{13}C spectra in D_2O, MeOH (δ 49.50 ppm) was added as internal standard. Coupling constants are reported in Hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; m multiplet. Infrared (IR) spectra were recorded as thin films on a Perkin Elmer Spectrum 100 FTIR spectrophotometer. Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L 1000 at 589 nm and a concentration (c) expressed in g/100 ml. High-resolution mass spectra (HRMS) were recorded with an Agilent 6210 ESI-TOF mass spectrometer at the Freie Universität Berlin, Mass Spectrometry Core Facility. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed, using a 2,4,6-trihydroxyacetophenone (THAP) matrix.
Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or a 1:1 mixture of H_2SO_4 (2N) and resorcine monomethylether (0.2%) in ethanol. Column chromatography was performed using silica gel 60 (230–400 mesh). Size exclusion chromatography (SEC) was performed using Sephadex® LH-20 (GE Healthcare). Reversed phase solid phase extraction (RP SPE) was performed using Sep-Pak® C18 (Waters) cartridges.

General procedure (A) for removal of anomeric TBS.

Anomeric TBS-protected starting material (1.0 equiv) was dissolved in THF (reaction concentration at 0.15 M) and cooled to 0 °C. A solution of TBAF (1 M in THF, 1.2 equiv) and AcOH (1.4 equiv) was added and stirred for 1 h at 0 °C. The reaction mixture was diluted with EtOAc, washed with 0.1 N HCl, sat. aq. $NaHCO_3$ and brine. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded the corresponding lactol as a mixture of α and β anomers.

General procedure (B) for glycosyl-trichloroacetimidate synthesis.

To a solution of the lactol (1.0 equiv) in DCM (reaction concentration at 0.5 M) trichloroacetonitrile (10 equiv) and K_2CO_3 (1.7 equiv) were added and stirred for 3 h at room temperature. The crude product was concentrated and purified by column chromatography on silica gel (hexanes/EtOAc) to afford pure product as a mixture of α and β anomers.

General procedure (C) for TMSOTf-mediated glycosylation of glycosyl-imidates.

The acceptor (1.0 to 2.0 equiv) and glycosyl-trichloroacetimidate (1.0 to 1.3 equiv) were coevaporated with toluene three times and dried *in vacuo*. The residue was dissolved in DCM/Et_2O (1:1, reaction concentration at 80 to 120 mM) and freshly activated molecular sieves (4 Å) were added. The mixture was cooled to −20 °C and TMSOTf (0.1 equiv) was added. The reaction was brought to −10 °C over 1 h, then quenched by the addition of NEt_3 and concentrated under reduced pressure. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (D) for phosphoramidite synthesis.

The starting material (1.0 equiv) was dissolved in DCM/MeCN (1:1, reaction concentration at 25 mM) together with diisopropylammonium tetrazolide (1.0 equiv). Freshly activated molecular sieves (3 Å) were added and the mixture cooled to 0 °C. Then *N,N,N',N'*-tetraisopropylphosphordiamidite (1.2 equiv) was added and the reaction brought to room temperature over 3 h. The mixture was concentrated under reduced pressure on Isolute® (Biotage) and purified by column chromatography on silica gel to give the phosphoramidite as a mixture of two stereoisomers.

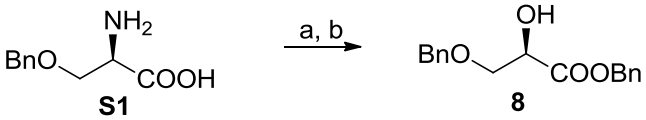
General procedure (E) for phosphoramidite coupling, oxidation and vinyl-ether deprotection.

Starting alcohol (1.0 equiv) was dissolved in 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL). Freshly activated molecular sieves (3 Å) and phosphoramidite (1.3 to 3.0 equiv, in 1.0 mL MeCN) were added. The reaction mixture was stirred for 1 h, then H_2O (0.5 mL) was added followed by I_2 (in 2.0 mL THF, 20 equiv) and stirred for further 3 h. The mixture was diluted with EtOAc and washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by SEC ($CHCl_3$/MeOH 1:1) to give the phosphotriester as a mixture of diastereomers.

General procedure (F) for final deprotection.

The phosphotriester (1.0 equiv) was dissolved in NEt_3 (1 mL), stirred for 16 h and concentrated. The residue was purified by SEC ($CHCl_3$/MeOH 1:1) to give the corresponding phosphodiester. The phosphodiester was dissolved in EtOH/H_2O/AcOH (50:50:1, reaction concentration 1 mM) and purged with Ar. After that Pd/C (10% Pd, w/w 2:1 with respect to phosphodiester) was added and the mixture purged with H_2, then transferred to a autoclave and treated with 4 bar H_2 for 20 h. The mixture was filtered, concentrated, the residue dissolved in H_2O/AcOH (100:1, reaction concentration 1 mM) and purged with Ar. After that Pd/C (10% Pd, w/w 2:1 with respect to residue) was added and the mixture purged with H_2, then transferred to a autoclave and treated with 4 bar H_2 for further 20 h. The mixture was filtered and concentrated. The crude product was dissolved in H_2O, subjected to reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) and lyophilized to give the target compound as a triethylammonium salt.

Synthesis of building blocks 8–10



Scheme S1: Synthesis of building block **8**. Reagents and conditions: (a) $NaNO_2$, H_2SO_4, H_2O, 0 °C to rt; (b) BnBr, K_2CO_3, DMF, 0 °C to rt, 63% over 2 steps.

Benzyl (2*R*)-2-hydroxy-3-benzyloxypropanoate (8)[1, 2]

To a solution of *O*-Benzyl-D-serine **S1** (1.0 g, 5.1 mmol) in 2 N H_2SO_4 (12 mL) at 0 °C a solution of $NaNO_2$ (2.0 g, 29.0 mmol) was added over 2 h. After complete addition the mixture was warmed to room temperature and stirred for additional 3 h. The reaction mixture was then diluted with brine, extracted with EtOAc and the organic layer dried over Na_2SO_4 and concentrated to

give the corresponding hydroxyacid. To a solution of crude hydroxyacid in DMF (20 mL) at 0 °C was added potassium carbonate (1.0 g, 7.6 mmol). Benzyl bromide (0.8 mL, 6.6 mmol) was added drop wise over 30 min, after complete addition the mixture was warmed to room temperature and let stir for 24 h. The reaction was then diluted with Et_2O and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with Et_2O and the combined organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded **8** (0.92 g, 3.2 mmol, 63%). $[\alpha]_D^{20}$ = + 20.0 º (c = 1.0, $CHCl_3$). NMR data is consistent with previously reported for the (2*S*)-enantiomer.[2] HRMS (ESI): Calcd for $C_{17}H_{18}O_4Na^+$ $[M+Na]^+$ 309.1097, found 309.1114.

tert-Butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-naphthyl-β-D-glucopyranoside (12)

To a solution of **11**[3] (1.04 g, 2.6 mmol) in DMF (10 mL) at 0 °C, NaH (0.073 g, 3.1 mmol) was added. Naphthyl bromide (0.85 g, 3.8 mmol) was added and the reaction mixture warmed to room temperature. After stirring for 1.5 h the reaction mixture was cooled to 0 °C, quenched by the slow addition of H_2O and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded **12** (1.20 g, 2.2 mmol, 87%). $[\alpha]_D^{20}$ = – 83.1 º (c = 1.7, $CHCl_3$); IR ν_{max} (film) 3059, 2930, 2959, 2110, 1463, 1372, 1256, 1177, 1096 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 4H), 7.55 – 7.37 (m, 8H), 5.59 (s, 1H), 5.06 (d, *J* = 11.6 Hz, 1H), 4.97 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 7.7 Hz, 1H), 4.30 (dd, *J* = 10.5, 5.0 Hz, 1H), 3.81 (t, *J* = 10.3 Hz, 1H), 3.75 (t, *J* = 9.3 Hz, 1H), 3.57 (t, *J* = 9.3 Hz, 1H), 3.44 – 3.35 (m, 2H), 0.95 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.3, 135.5, 133.4, 133.2, 129.2, 128.4, 128.2, 128.1, 127.8, 127.1, 126.3, 126.2, 126.1, 126.0, 101.6, 97.6, 81.8, 78.7, 74.9, 68.9, 68.8, 66.5, 25.7, 18.1, -4.2, -5.1; HRMS (MALDI-TOF): Calcd for $C_{30}H_{37}N_3O_5SiNa^+$ $[M+Na]^+$ 570.2395, found 570.2343.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-D-glucopyranoside (13)

A solution of **12** (1.20 g, 2.2 mmol) in DCM (18 mL) was cooled to 0 °C and borane tetrahydrofuran complex solution (1 M, 13.2 mL, 13.2 mmol) was added slowly. After complete addition TMSOTf (0.2 mL, 1.1 mmol) was added and the reaction mixture warmed to 10 °C over 16 h. After cooling to 0 °C, the reaction was quenched by the slow addition of sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated to give the 6-OH intermediate. The crude intermediate was dissolved in DCM (10 mL) together with DMAP (0.0027 g, 0.22 mmol) and levulinic acid (0.45 mL, 4.42 mmol). EDC (0.54 mL, 3.08 mmol) was added, the reaction mixture stirred for 1 h, concentrated and dissolved in EtOAc. The organic layer was washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$ and brine then dried over $MgSO_4$ and concentrated. The residue was reacted according to general procedure (A) to give lactol **13** (0.983 g, 1.84 mmol, 84%) as a mixture of α and β anomers. IR ν_{max} (film) 3418, 3060, 2919, 2109, 1737, 1717, 1358, 1259, 1209, 1159, 1075 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 3.8H), 7.60 – 7.40 (m, 2.8H), 7.35 – 7.23 (m, 5.5H), 5.33 (d, *J* = 3.2 Hz, 0.6H), 5.13 – 4.94 (m, 1.5H), 4.89 (dd, *J* = 11.0, 5.7 Hz, 1.0H), 4.66 – 4.59 (m, 1.4H), 4.43 – 4.34 (m, 1.0H), 4.27 – 4.07 (m, 2.2H), 3.58 – 3.40 (m, 3.1H), 2.80 – 2.71 (m, 1.9H), 2.60 – 2.54 (m, 1.9H), 2.18 (s, 2.7H).; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 207.4, 207.2, 172.6 (2C), 137.7, 137.6, 135.3 (2C), 133.4, 133.2, 128.7, 128.4, 128.2, 128.1 (2C), 128.0, 127.8 (2C), 127.1, 126.3, 126.2 (2C), 126.1, 96.3, 92.1, 83.1, 80.4, 78.5, 77.6, 77.4, 75.8, 75.3, 75.2, 73.4, 69.3, 67.6, 64.2, 63.2, 63.0, 38.2, 38.0,

30.1, 30.0, 28.1, 28.0; HRMS (MALDI-TOF): Calcd for $C_{29}H_{31}N_3O_7Na^+$ $[M+Na]^+$ 556.2054, found 556.2011.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-D-glucopyranosyl trichloroacetimidate (9)

According to general procedure (B), lactol **13** (0.983 g, 1.84 mmol) was reacted with trichloroacetonitrile (1.85 mL, 18.4 mmol) and K_2CO_3 (0.43 g, 3.13 mmol) in DCM (5 mL) to afford **9** (1.25 g, 1.84 mmol, 100%) as a mixture of α and β anomers. IR ν_{max} (film) 3337, 3059, 2921, 2112, 1739, 1719, 1677, 1358, 1284, 1156, 1064 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1.0H), 7.92 – 7.75 (m, 3.2H), 7.54 – 7.45 (m, 2.8H), 7.35 – 7.24 (m, 5.0H), 6.42 (d, *J* = 3.5 Hz, 0.3H), 5.64 (d, *J* = 8.3 Hz, 0.7H), 5.13 – 4.99 (m, 2.2H), 4.95 – 4.86 (m, 1.1H), 4.70 – 4.61 (m, 1.1H), 4.37 – 4.27 (m, 2.0H), 4.18 – 4.02 (m, 1.0H), 3.78 – 3.59 (m, 3.6H), 2.79 – 2.69 (m, 1.9H), 2.64 – 2.54 (m, 1.9H), 2.17 (s, 2.7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.4, 161.1, 160.8, 137.5, 137.4, 135.2, 135.1, 133.4, 133.2, 128.7 (2C), 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.1 (2C), 126.3 (2C), 126.2, 126.1, 96.8, 94.7, 83.1, 80.2, 77.6, 77.0, 75.9, 75.8, 75.6, 75.3, 74.1, 72.0, 65.9, 63.2, 62.6, 62.5, 38.0, 30.0 (2C), 28.0, 27.9; HRMS (MALDI-TOF): Calcd for $C_{31}H_{31}Cl_3N_4O_7Na^+$ $[M+Na]^+$ 699.1151, found 699.1136.

6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside (15)

To a solution of **14**[3] (2.00 g, 3.69 mmol) in MeOH (20 mL), 0.5 M NaOMe in MeOH (1.48 mL, 0.74 mmol) was added and stirred for 1.5 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. The residue was dissolved in THF (10 mL) together with TBAI (0.136 g, 0.37 mmol) and cooled to 0 °C. NaH (0.177 g, 7.38 mmol) and allyl bromide (0.64 mL, 7.38 mmol) were added and the reaction mixture warmed to room temperature. After stirring for 16 h the reaction was cooled to 0 °C and quenched by the slow addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was reacted according to general procedure (A) to give lactol **15** (1.18 g, 2.77 mmol, 75%) as a mixture of α and β anomers. IR ν_{max} (film) 3416, 2918, 2872, 2103, 1454, 1359, 1269, 1131, 1086, 1045 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.23 (m, 9.4H), 5.90 (ddt, *J* = 17.1, 10.4, 5.8 Hz, 0.9H), 5.36 – 5.15 (m, 2.5H), 4.97 – 4.78 (m, 3.0H), 4.68 – 4.55 (m, 1.4H), 4.18 – 3.92 (m, 3.6H), 3.69 – 3.35 (m, 5.3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.1, 137.9 (2C), 134.4, 134.3, 128.6 (3C), 128.3, 128.2, 128.1 (2C), 128.0, 127.9, 118.0 (2C), 96.3, 92.2, 83.2, 80.2, 78.7, 77.9, 75.7 (2C), 75.2 (2C), 74.9, 72.6 (2C), 70.6, 68.8, 68.7, 67.6, 64.1; HRMS (MALDI-TOF): Calcd for $C_{23}H_{27}N_3O_5Na^+$ $[M+Na]^+$ 448.1843, found 448.1825.

6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranosyl trichloroacetimidate (10)

According to general procedure (B), lactol **15** (0.68 mg, 1.60 mmol) was reacted with trichloroacetonitrile (1.60 mL, 15.98 mmol) and K_2CO_3 (0.38 g, 2.72 mmol) in DCM (3 mL) to afford **10** (0.75 g, 1.31 mmol, 82%) as a mixture of α and β anomers. IR ν_{max} (film) 3342, 2870, 2111, 1675, 1455, 1357, 1285, 1058 cm^{-1}; NMR data reported for the β anomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.40 – 7.28 (m, 10H), 5.89 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), 5.61 (d, *J* = 8.4 Hz, 1H), 5.27 (ddd, *J* = 17.2, 3.3, 1.6 Hz, 1H), 5.17 (ddd, *J* = 10.4, 3.0, 1.3 Hz, 1H), 4.95 – 4.84 (m, 3H), 4.71 – 4.67 (m, 1H), 4.09 – 3.96 (m, 2H), 3.81 – 3.66 (m, 4H), 3.60 – 3.52 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.2, 138.0, 137.9, 134.7, 128.6 (2C), 128.2, 128.1 (2C), 128.0, 117.4, 97.0, 83.2, 77.3, 76.2, 75.8, 75.2, 72.6, 68.1, 66.0; HRMS (MALDI-TOF): Calcd for $C_{25}H_{27}Cl_3N_4O_5Na^+$ $[M+Na]^+$ 591.0939, found 591.0918.

Synthesis of the repeating unit

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (16)

According to general procedure (C), glycosyl-imidate **9** (0.50 g, 0.74 mmol) and **8** (0.42 g, 1.48 mmol) were dissolved in DCM/Et_2O (1:1, 6 mL) and reacted to give **16** (0.48 g, 0.60 mmol, 81%). $[\alpha]_D^{20}$ = + 57.2 º (c = 3.2, $CHCl_3$); IR ν_{max} (film) 3032, 2920, 2106, 1737, 1719, 1455, 1361, 1261, 1208, 1155, 1100, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.75 (m, 4H), 7.54 – 7.44 (m, 3H), 7.38 – 7.23 (m, 15H), 5.26 (d, *J* = 12.1 Hz, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.14 – 5.06 (m, 2H), 5.02 (d, *J* = 10.8 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.63 – 4.49 (m, 4H), 4.23 – 4.09 (m, 4H), 3.89 – 3.78 (m, 2H), 3.64 – 3.55 (m, 1H), 3.45 (dd, *J* = 10.3, 3.6 Hz, 1H), 2.79 – 2.64 (m, 2H), 2.58 – 2.47 (m, 2H), 2.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.4, 172.5, 169.2, 137.9, 137.6, 135.4, 135.2, 133.4, 133.2, 128.7, 128.6 (3C), 128.5, 128.4, 128.1, 128.0 (2C), 127.9 (2C), 127.8, 127.0, 126.2, 126.1 (2C), 96.7, 80.3, 78.0, 75.7, 75.2, 74.4, 73.5, 70.1, 69.7, 67.4, 63.3, 62.7, 37.9, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{46}H_{47}N_3O_{10}Na^+$ $[M+Na]^+$ 824.3154, found 824.3115.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (17)

To a mixture of **16** (0.48 g, 0.60 mmol) in DCM (20 mL) and phosphate-buffer (7 mM, pH 7.4, 2 mL) at 0 °C DDQ (0.41 g, 1.80 mmol) was added portion wise over 1 h. The reaction mixture was warmed to room temperature and stirred for further 30 min. The mixture was diluted with sat. aq. $NaHCO_3$ solution, extracted with DCM and the organic layer dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) gave **17** (0.32 g, 0.48 mmol, 80%). $[\alpha]_D^{20}$ = + 2.0 º (c = 3.7, $CHCl_3$); IR ν_{max} (film) 3473, 2918, 2108, 1737, 1718, 1455, 1362, 1264, 1208, 1143, 1101, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 10H), 7.28 – 7.23 (m, 5H), 5.24 (d, *J* = 12.2 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 4.79 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 11.3 Hz, 1H), 4.56 – 4.51 (m, 2H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.21 – 4.07 (m, 4H), 3.86 – 3.75 (m, 2H), 3.45 (dd, *J* = 10.0, 8.7 Hz, 1H), 3.25 (dd, *J* = 10.4, 3.6 Hz, 1H), 2.80 – 2.68 (m, 2H), 2.58 – 2.50 (m, 2H), 2.16 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.5, 169.1, 138.0, 137.6, 135.2, 128.7, 128.6, 128.5 (2C), 128.2 (2C), 127.9, 127.8, 96.6, 78.0, 75.0, 74.4, 73.5, 72.0, 70.2, 69.3, 67.3, 62.9, 62.8, 37.9, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{35}H_{39}N_3O_{10}Na^+$ $[M+Na]^+$ 684.2528, found 684.2564.

6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (18)

According to general procedure (C), glycosyl-imidate **10** (0.36 g, 0.64 mmol) and **17** (0.32 g, 0.48 mmol) were dissolved in DCM/Et_2O (1:1, 6 mL) and reacted to give **18** (0.35 g, 0.33 mmol, 69%). $[\alpha]_D^{20}$ = + 92.4 º (c = 2.5, $CHCl_3$); IR ν_{max} (film) 3033, 2920, 2108, 1740, 1720, 1498, 1455, 1361, 1263, 1210, 1136, 1045 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.21 (m, 25H), 5.90 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), 5.57 (d, *J* = 3.8 Hz, 1H), 5.30 – 5.13 (m, 5H), 5.03 (d, *J* = 10.7 Hz, 1H), 4.95 (d, *J* = 10.7 Hz, 1H), 4.91 – 4.85 (m, 2H), 4.71 (d, *J* = 11.0 Hz, 1H), 4.59 – 4.50 (m, 4H), 4.28 – 4.12 (m, 4H), 4.09 – 4.01 (m, 3H), 3.99 – 3.90 (m, 1H), 3.90 – 3.85 (m, 2H), 3.84 – 3.72 (m, 3H), 3.66 (dd, *J* = 10.0, 8.8 Hz, 1H), 3.43 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.15 (dd, *J* = 10.5, 3.7 Hz, 1H), 2.83 – 2.69 (m, 2H), 2.63 – 2.52 (m, 2H), 2.17 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3, 172.5, 169.0, 138.4, 138.0, 137.7, 137.6, 135.2, 134.5, 128.8, 128.7, 128.6 (3C), 128.5, 128.1, 127.9 (3C), 127.8 (3C), 127.4, 117.6, 98.6, 97.0, 80.2, 79.2, 78.2, 75.6, 75.2, 75.1

(2C), 74.3 (2C), 73.6, 72.6, 71.7, 70.2, 69.2, 68.2, 67.4, 63.5, 62.5, 61.5, 38.0, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{58}H_{64}N_6O_{14}Na^+$ $[M+Na]^+$ 1091.4373, found 1091.4378.

2-*N*-Acetyl-6-*O*-allyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-*O*-levulinoyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (19)

To a solution of **18** (100 mg, 94 µmol) in pyridine (2 mL) thioacetic acid (0.8 mL) was added and stirred for 24 h. The reaction mixture was concentrated and purified by column chromatography on silica gel (hexanes/acetone) to afford **19** (69 mg, 63 µmol, 67%). $[\alpha]_D^{20}$ = + 70.0 º (c = 1.9, $CHCl_3$); IR ν_{max} (film) 3311, 3032, 2918, 2870, 1739, 1719, 1668, 1530, 1455, 1365, 1209, 1123, 1059 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 (dd, *J* = 16.9, 8.7 Hz, 25H), 6.75 (d, *J* = 9.5 Hz, 1H), 6.12 (d, *J* = 9.9 Hz, 1H), 5.86 (ddd, *J* = 22.8, 10.8, 5.6 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 1H), 5.27 – 5.19 (m, 3H), 5.13 – 5.07 (m, 1H), 4.88 – 4.80 (m, 3H), 4.69 – 4.59 (m, 3H), 4.53 – 4.38 (m, 5H), 4.28 (td, *J* = 10.1, 3.5 Hz, 1H), 4.16 – 3.96 (m, 7H), 3.80 – 3.52 (m, 7H), 2.86 – 2.71 (m, 2H), 2.65 – 2.51 (m, 2H), 2.19 (s, 3H), 2.02 (s, 3H), 1.78 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.4, 171.2, 169.9, 169.8, 138.7, 138.5, 137.4, 136.8, 135.1, 134.6, 128.8 (2C), 128.6, 128.5, 128.4 (2C), 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 117.4, 98.1, 98.0, 81.2, 79.1, 78.3, 75.3, 75.2, 74.9, 74.8, 74.6, 73.4, 72.6, 71.5, 70.2, 70.1, 69.1, 67.4, 62.3, 52.5, 51.9, 38.0, 30.0, 28.0, 23.4 (2C); HRMS (MALDI-TOF): Calcd for $C_{62}H_{72}N_2O_{16}Na^+$ $[M+Na]^+$ 1123.4774, found 1123.4747.

2-*N*-Acetyl-3,4-di-*O*-benzyl-6-*O*-prop-1-en-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (5)

A solution of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (5.3 mg, 6.3 µmol) in degassed THF (0.5 mL) was purged with H_2 for 15 min and added to a solution of **19** (69 mg, 63 µmol) in THF (2 mL). After stirring for 30 min, sat. aq. $NaHCO_3$ solution was added and the mixture extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give the corresponding vinyl ether. To a solution of crude vinyl in DCM (2 mL), hydrazine hydrate (6 µL, 123 µmol) dissolved in AcOH (100 µL) and pyridine (150 µL) was added and the solution stirred for 25 min. Sat. aq. $NaHCO_3$ solution was then added and the mixture extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (DCM/MeOH/acetone) afforded **5** (49 mg, 49 µmol, 78%). $[\alpha]_D^{20}$ = + 94.1 º (c = 1.3, $CHCl_3$); IR ν_{max} (film) 3329, 3064, 3032, 2925, 1744, 1667, 1525, 1455, 1366, 1312, 1124, 1063 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.14 (m, 25H), 6.76 (d, *J* = 9.9 Hz, 1H), 6.69 (d, *J* = 9.1 Hz, 1H), 6.17 (dd, *J* = 12.6, 1.5 Hz, 1H), 5.32 (d, *J* = 3.7 Hz, 1H), 5.24 (d, *J* = 12.2 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 4.85 – 4.78 (m, 3H), 4.76 – 4.61 (m, 4H), 4.57 – 4.47 (m, 4H), 4.34 (td, *J* = 10.2, 3.7 Hz, 1H), 4.30 – 4.21 (m, 1H), 4.18 – 4.10 (m, 1H), 3.95 (dd, *J* = 10.0, 4.3 Hz, 1H), 3.90 – 3.65 (m, 8H), 3.60 – 3.46 (m, 2H), 1.98 (s, 3H), 1.61 (s, 3H), 1.47 (dd, *J* = 6.7, 1.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 170.4, 169.8, 146.7, 138.8, 138.5, 137.5, 137.0, 135.1, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0 (2C), 127.9, 127.8, 127.7, 127.6, 99.0, 98.4, 98.0, 81.4, 78.8, 78.0, 77.4, 75.6, 75.4, 74.8, 74.5, 73.5, 72.8, 70.8, 70.3, 67.4, 60.5, 52.5 (2C), 23.5, 23.3, 12.6; HRMS (MALDI-TOF): Calcd for $C_{57}H_{66}N_2O_{14}Na^+$ $[M+Na]^+$ 1025.4406, found 1025.4404.

2-*N*-Acetyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (S2)

To a solution of **5** (42 mg, 42 µmol) in THF (4 mL) and H_2O (1 mL), I_2 (106 mg, 0.42 mmol) was added and stirred for 3 h. The mixture was diluted with EtOAc and washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH/acetone) to afford **S2** (36 mg, 37 µmol, 89%). $[\alpha]_D^{20}$ = + 75.7 ° (c = 1.2, $CHCl_3$); IR ν_{max} (film) 3324, 3031, 2925, 1741, 1666, 1534, 1454, 1366, 1311, 1123, 1062, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 25H), 6.80 (dd, *J* = 14.8, 9.6 Hz, 2H), 5.27 – 5.11 (m, 3H), 4.85 – 4.74 (m, 4H), 4.67 (d, *J* = 10.6 Hz, 1H), 4.61 – 4.53 (m, 2H), 4.52 – 4.44 (m, 3H), 4.29 (ddd, *J* = 13.7, 9.6, 3.6 Hz, 2H), 4.07 – 3.98 (m, 1H), 3.91 – 3.67 (m, 10H), 3.48 – 3.40 (m, 1H), 1.97 (s, 3H), 1.57 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.8, 170.4, 170.0, 138.8, 138.3, 137.5, 136.9, 135.0, 128.9, 128.8 (2C), 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9 (2C), 127.8 (3C), 127.6 (2C), 98.5, 98.2, 81.3, 78.3, 75.7, 75.3, 74.9, 74.8, 73.4, 72.8, 70.3, 67.6, 62.5, 60.6, 53.0, 52.7, 23.5, 23.1; HRMS (MALDI-TOF): Calcd for $C_{54}H_{62}N_2O_{14}Na^+$ $[M+Na]^+$ 985.4093, found 985.4077.

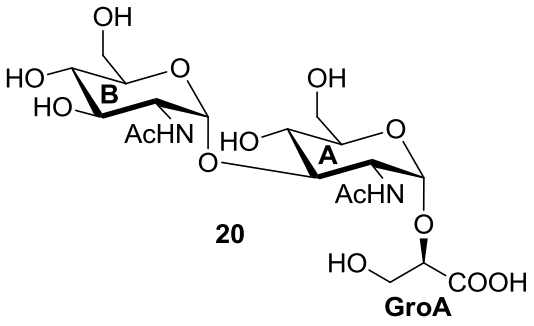
2-*N*-Acetyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-α-D-glucosaminopyranosyl-(1→2)-(2*R*)-3-hydroxypropanoate (20)

The protected repeating unit **S2** (12 mg, 12 µmol) was dissolved in a mixture of EtOH (2 mL), H_2O (2 mL) and AcOH (15 µL). The mixture was purged with Ar. Pd/C (10% Pd, 20 mg) was added and the mixture purged with H_2 and stirred for 16 h under H_2 atmosphere. The mixture was filtered and concentrated. The crude product was dissolved in H_2O and subjected to reversed phase solid phase extraction (RP SPE). Further purification by SEC (MeOH) gave **20** (5.0 mg, 9.8 µmol, 78%). HRMS (MALDI-TOF): Calcd for $C_{19}H_{32}N_2O_{14}Na^+$ $[M+Na]^+$ 535.1746, found 535.1774.

Table 1: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of repeating unit **20**.[a]

	α-GlcNAc **(A)**	*α-GlcNAc* **(B)**	*GroA*
H-1	5.01	5.37	
C-1	99.0	100.3	176.4
H-2	4.07	3.92	4.46
C-2	54.6	56.3	78.1
H-3	3.97	3.66	3.95/4.01
C-3	79.0	74.8	65.1
H-4	3.73	3.53	
C-4	73.4	72.0	
H-5	3.79	3.68	
C-5	75.1	73.3	
H-6 a/b	3.79/3.86	3.79/3.86	
C-6	62.8	62.8	
NAc	2.07/2.06; 24.8/24.6; 177 ppm		

[a] ^{1}H and ^{13}C NMR resonances were assigned based on HSQC and COSY experiments.



20

Synthesis of phosphoramidites and phosphate bridges oligomers

2-*N*-Acetyl-3,4-di-*O*-benzyl-6-*O*-prop-1-en-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-((*N*,*N*,-diisopropylamino)-2-cyanoethylphosphite)-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (6)

According to general procedure (D), **5** (50 mg, 50 µmol) was reacted with *N,N,N',N'*-tetraisopropylphosphordiamidite (18 mg, 60 µmol) in DCM/MeCN (2 mL). Column chromatography on silica gel (hexanes/acetone + 1% NEt_3) gave **6** (49 mg, 41 µmol, 82%) as a mixture of two diastereomers. ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.33 (s, 25H), 6.74 (dd, *J* = 9.1, 3.3 Hz, 1H), 6.27 (dd, *J* = 12.6, 1.6 Hz, 1H), 5.40 (dd, *J* = 9.2, 3.8 Hz, 1H), 5.24 (qd, *J* = 12.5, 1.3 Hz, 2H), 4.90 – 4.73 (m, 7H), 4.68 – 4.51 (m, 4H), 4.32 (ddt, *J* = 14.3, 9.8, 4.2 Hz, 1H), 4.24 – 4.07 (m, 3H), 3.56 – 3.47 (m, 14H), 3.56 – 3.47 (m, 1H), 2.78 – 2.72 (m, 1H), 2.70 (t, *J* = 6.0 Hz, 1H), 1.94 – 1.86 (m, 6H), 1.46 (ddd, *J* = 6.7, 1.5, 0.8 Hz, 3H), 1.24 – 1.12 (m, 12H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.6 (2C), 170.2 (2C), 170.0, 169.9, 147.8, 140.1, 139.7, 139.1 (2C), 139.0 (2C), 136.9, 129.4, 129.2 (2C), 129.1 (2C), 129.0 (2C), 128.9, 128.8, 128.6, 128.4 (3C), 128.3, 128.1, 99.2, 98.5, 82.0, 80.2, 79.3, 75.8 (2C), 75.5, 75.2, 74.6, 73.7, 71.2, 69.4, 67.4, 62.9, 59.7 (2C), 59.6, 59.5, 53.6 (2C), 52.7, 52.6, 44.0 (2C), 43.9, 43.8, 25.1 (2C), 25.0, 24.9 (3C), 24.8, 23.3, 23.2 (2C), 20.9, 20.8 (2C), 12.7; ^{31}P-NMR (162 MHz, acetone-d_6) δ 148.5, 148.3 (1P); HRMS (MALDI-TOF): Calcd for $C_{66}H_{83}N_4O_{15}PNa^+$ $[M+Na]^+$ 1225.5485, found 1225.5475.

***N*-Benzyl benzyl(5-((*N*,*N*,-diisopropylamino)-2-cyanoethylphosphite)pentyl)carbamate (7)**

According to general procedure (D), ***N***-benzyl benzyl(5-hydroxypentyl)carbamate (30 mg, 92 µmol) was reacted with *N,N,N',N'*-tetraisopropylphosphordiamidite (33 mg, 110 µmol) in DCM/MeCN (2 mL). Column chromatography on silica gel (hexanes/EtOAc + 1% NEt_3) gave **7** (42 mg, 80 µmol, 87%). IR ν_{max} (film) 2966, 2933, 2869, 1697, 1455, 1421, 1364, 1225, 1184, 1127, 1072, 1027, 975 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.56 – 7.15 (m, 10H), 5.17 (s, 2H), 4.54 (s, 2H), 3.89 – 3.75 (m, 2H), 3.63 (ddq, *J* = 13.4, 9.9, 6.7 Hz, 4H), 3.34 – 3.20 (m, 2H), 2.72 (t, *J* = 6.1 Hz, 2H), 1.67 – 1.50 (m, 4H), 1.44 – 1.29 (m, 2H), 1.18 (t, *J* = 6.5 Hz, 12H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 139.5, 129.3, 129.2, 128.6 (2C), 128.0, 67.4, 64.1, 64.0, 59.5, 59.3, 43.8, 43.7, 31.7, 31.6, 25.0, 24.9 (3C), 23.9, 20.8 (2C); ^{31}P-NMR (162 MHz, acetone-d_6) δ 147.1 (1P); HRMS (ESI): Calcd for $C_{29}H_{42}N_3O_4PNa^+$ $[M+Na]^+$ 550.2805, found 550.2817.

Protected monomer: 2-*N*-Acetyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-(5-(benzyl((benzyloxy)carbonyl)amino)pentyl (2-cyanoethyl) phosphoryl)-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (3)

According to general procedure (E), **5** (19 mg, 19 µmol) was reacted with **7** (30 mg, 57 µmol) in the presence of 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL, 0.38 mmol). Oxidation with I_2 (in 2.0 mL THF, 96 mg, 0.38 mmol) and purification by SEC afforded **3** (26 mg, 18 µmol, 98%) as a mixture of diastereomers. IR ν_{max} (film) 3324, 2929, 1695, 1671, 1531, 1455, 1422, 1367, 1260, 1125, 1068, 1029 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.45 – 7.19 (m, 35H), 6.98 (d, *J* = 9.4 Hz, 1H), 5.34 (d, *J* = 3.7 Hz, 1H), 5.24 (d, *J* = 12.4 Hz, 1H), 5.21 – 5.12 (m, 3H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.87 – 4.80 (m, 2H), 4.79 – 4.62 (m, 5H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 9.0 Hz, 3H), 4.34 – 4.16 (m, 6H), 4.14 – 4.00 (m, 4H), 3.90 – 3.63 (m, 6H), 3.52 (t, *J* = 9.4 Hz, 1H), 3.31 – 3.17 (m, 2H), 2.91 – 2.80 (m, 4H), 1.90 (s, 3H), 1.84 (s, 3H), 1.71 – 1.49 (m, 4H), 1.42 – 1.24 (m, 3H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.6, 170.4, 170.1, 170.0, 140.2, 139.9, 139.4, 139.1, 138.7, 138.6, 138.3, 136.8, 129.4, 129.3, 129.2, 129.1 (3C), 129.0 (2C), 128.9, 128.8, 128.6, 128.5, 128.4 (3C), 128.2, 128.1, 128.0, 118.2, 98.9, 98.4, 82.0, 79.5, 79.4, 76.0,

75.8 (2C), 75.4, 75.1, 74.9, 73.7, 73.4 (2C), 71.2, 71.1, 68.7, 67.5, 66.6, 63.3, 63.2 (2C), 63.1, 62.3, 53.7, 52.7, 50.8, 46.8, 30.6 (2C), 28.0, 23.4, 23.3, 23.2, 20.1, 20.0; ^{31}P-NMR (162 MHz, acetone-d_6) δ –1.4; HRMS (MALDI-TOF): Calcd for $C_{77}H_{89}N_4O_{19}PNa^+$ $[M+Na]^+$ 1427.5751, found 1427.5717.

Protected dimer (4)

According to general procedure (E), **3** (26 mg, 18 µmol) was reacted with **6** (30 mg, 25 µmol) in the presence of 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL, 0.38 mmol). Oxidation with I_2 (in 2.0 mL THF, 96 mg, 0.38 mmol) and purification by SEC afforded **4** (36 mg, 14 µmol, 78%) as a mixture of diastereomers. IR ν_{max} (film) 3328, 3033, 2923, 1745, 1672, 1525, 1455, 1367, 1280, 1126, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.43 – 7.17 (m, 60H), 6.93 (t, J = 9.9 Hz, 1H), 5.46 – 5.37 (m, 1H), 5.32 (d, J = 3.6 Hz, 1H), 5.26 – 5.10 (m, 6H), 5.00 – 4.92 (m, 1H), 4.90 – 4.61 (m, 14H), 4.60 – 4.43 (m, 8H), 4.31 – 4.20 (m, 9H), 4.16 – 3.96 (m, 10H), 3.92 – 3.72 (m, 10H), 3.71 – 3.61 (m, 2H), 3.55 – 3.48 (m, 1H), 3.29 – 3.17 (m, 2H), 2.87 – 2.80 (m, 6H), 2.77 – 2.67 (m, 2H), 1.94 – 1.75 (m, 12H), 1.69 – 1.46 (m, 4H), 1.39 – 1.22 (m, 2H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.5, 170.1, 140.2 (2C), 140.1, 140.0 (3C), 139.7 (3C), 139.5 (2C), 139.1, 139.0 (2C), 138.6 (3C), 138.3, 136.9 (2C), 136.8 (2C), 129.4 (2C), 129.3 (3C), 129.2 (2C), 129.1 (4C), 129.0 (3C), 128.9 (4C), 128.6 (3C), 128.5 (3C), 128.4, 128.3, 128.2, 128.0, 118.3, 118.2, 118.1, 110.9, 98.8 (2C), 98.7, 98.5, 82.0, 81.4 (2C), 79.8 (2C), 79.6 (2C), 79.4, 78.5, 78.4, 76.2, 76.1, 75.9, 75.7, 75.6, 75.5, 75.3, 75.2, 75.1, 75.0, 74.7, 73.7 (2C), 73.4 (2C), 71.4, 71.2 (2C), 71.1, 71.0, 70.9, 67.5 (4C), 67.4, 63.5 (2C), 63.3, 63.2, 63.1, 62.3, 62.2, 53.9, 53.7 (3C), 52.7 (2C), 52.5, 50.8, 47.6, 30.7, 30.6, 30.3, 30.1, 29.9, 29.7, 28.2, 23.5, 23.4 (2C), 23.2 (2C), 20.1, 20.0 (2C), 19.9; ^{31}P-NMR (162 MHz, acetone-d_6) δ –1.5 (2P), –1.6 (2P); HRMS (MALDI-TOF): Calcd for $C_{134}H_{153}N_7O_{35}P_2Na^+$ $[M+Na]^+$ 2504.9775, found 2504.9773.

Deprotected monomer: 2-*N*-Acetyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-6-(5-aminopentyl phosphoryl)-α-D-glucosaminopyranosyl-(1→2)-(2*R*)-3-hydroxypropanoate (1)

According to general procedure (F), protected monomer **3** (6 mg, 4.3 µmol) was first treated with NEt_3, purified by SEC and then subjected to Pd/C-catalyzed hydrogenolysis. Purification by RP SPE gave **1** (2.1 mg, 2.7 µmol, 63%) as a triethylammonium salt. ^{1}H-NMR (600 MHz, D_2O) δ 5.39 (d, J = 3.7 Hz, 1H), 4.94 (d, J = 3.6 Hz, 1H), 4.31 (dd, J = 4.6, 3.7 Hz, 1H), 4.13 – 4.07 (m, 3H), 4.03 – 3.98 (m, 1H), 3.96 – 3.89 (m, 6H), 3.87 – 3.80 (m, 3H), 3.69 – 3.62 (m, 2H), 3.58 – 3.51 (m, 1H), 3.03 (t, J = 7.5 Hz, 2H), 2.08 (s, 3H), 2.07 (s, 3H), 1.76 – 1.67 (m, 4H), 1.49 (dt, J = 15.3, 7.7 Hz, 2H); ^{13}C-NMR (150 MHz, D_2O) δ 177.0, 176.9, 176.4, 100.3, 99.1, 80.3, 78.8, 74.9, 74.0, 73.4, 73.1, 72.0, 68.5, 66.4, 65.4, 62.7, 56.2, 54.6, 42.0, 31.7, 28.9, 24.8, 24.6 (2C); ^{31}P-NMR (243 MHz, D_2O) δ -2.0; HRMS (MALDI-TOF): Calcd for $C_{24}H_{44}N_3O_{17}PNa^+$ $[M+Na]^+$ 700.2301, found 700.2300.

Deprotected dimer (2)

According to general procedure (F), protected dimer **4** (19 mg, 7.7 µmol) was first treated with NEt_3, purified by SEC and then subjected to Pd/C-catalyzed hydrogenolysis. Purification by RP SPE gave **2** (8.0 mg, 5.5 µmol, 72%) as a triethylammonium salt. ^{1}H-NMR (600 MHz, D_2O) δ 5.38 – 5.33 (m, 2H), 5.03 – 4.99 (m, 2H), 4.46 (dd, J = 6.5, 3.1 Hz, 2H), 4.22 – 4.08 (m, 8H), 4.02 – 3.83 (m, 15H), 3.82 – 3.77 (m, 1H), 3.75 – 3.71 (m, 1H), 3.70 – 3.61 (m, 4H), 3.56 – 3.52 (m, 1H), 3.03 (t, J = 7.5 Hz, 2H), 2.12 – 2.04 (m, 12H), 1.75 – 1.66 (m, 4H), 1.53 – 1.45 (m, 2H); ^{13}C-NMR (150 MHz, D_2O) δ 177.0 (2C), 176.9 (2C), 176.3 (2C), 100.6, 100.5, 99.2, 98.9, 79.3, 79.0, 78.3, 78.2, 74.9, 74.1 (2C), 74.0 (2C), 73.9, 73.4, 73.2, 73.0, 72.8, 72.0, 71.7, 68.6, 68.5,

66.7, 66.5, 65.1, 62.8, 56.3, 56.2, 54.6, 42.0, 31.8, 31.7, 28.9, 24.9, 24.8, 24.7, 24.6; ^{31}P-NMR (243 MHz, D_2O) δ -2.0; HRMS (MALDI-TOF): Calcd for $C_{43}H_{75}N_5O_{33}P_2Na^+$ $[M+Na]^+$ 1274.3712, found 1274.3764.

Table 2: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of dimer **2**.[a]

	α-GlcNAc **(A)**	*α-GlcNAc* **(B)**	*GroA*
H-1	5.01	5.36	
	4.98	*5.33*	
C-1	98.9, 99.1	100.5, 100.6	
	97.6	*99.3*	
H-2	4.10	3.93	4.46
	4.10	*3.94*	*4.41*
C-2	54.6	56.2, 56.3	78.3, 78.2
	53.1	*54.7*	*77.2*
H-3	3.97	3.65	3.92
	3.95	*3.66*	*3.92/3.96*
C-3	79.0, 79.3	74.9	68.5, 68.6
	78.0	*71.7*	*63.7*
H-4	3.72	3.54	
	3.83	*3.63*	
C-4	73.9, 74.0	72.0	
	71.3	*70.1*	
H-5	3.80	3.67	
	3.90	*3.71*	
C-5	73.0, 73.2	73.2	
	72.5	*72.5*	
H-6 a/b	4.10/4.15	3.96/3.98	
	4.12/4.17	*4.09/4.20*	
C-6	65.1	66.5	
	64.9	*65.2*	

[a] data of native LTA polymer[4] reported in italic.

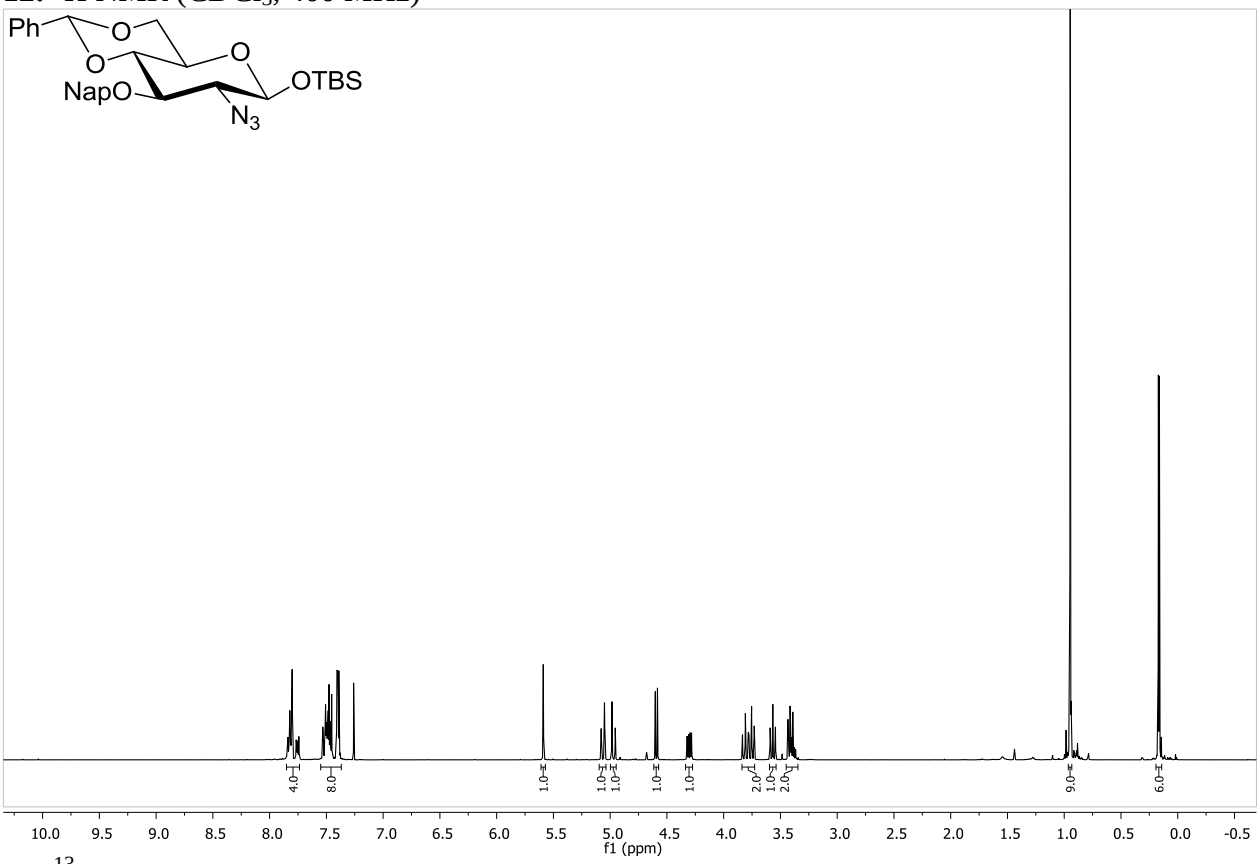
Preparation of microarrays

Oligosaccharides bearing an amine linker or proteins in coupling buffer (100 mM sodium phosphate, pH 8.5) were immobilized on CodeLink *N*-hydroxyl succinimide (NHS) ester activated glass slides (SurModics Inc., Eden Prairie, MN, USA) with a piezoelectric spotting device (S3; Scienion, Berlin, Germany). Microarray slides were incubated for 24 h in a humid chamber to complete coupling reactions, quenched with 50 mM aminoethanol solution, pH 9 for 1 h at 50°C, washed three times with deionized water, and stored desiccated until use.

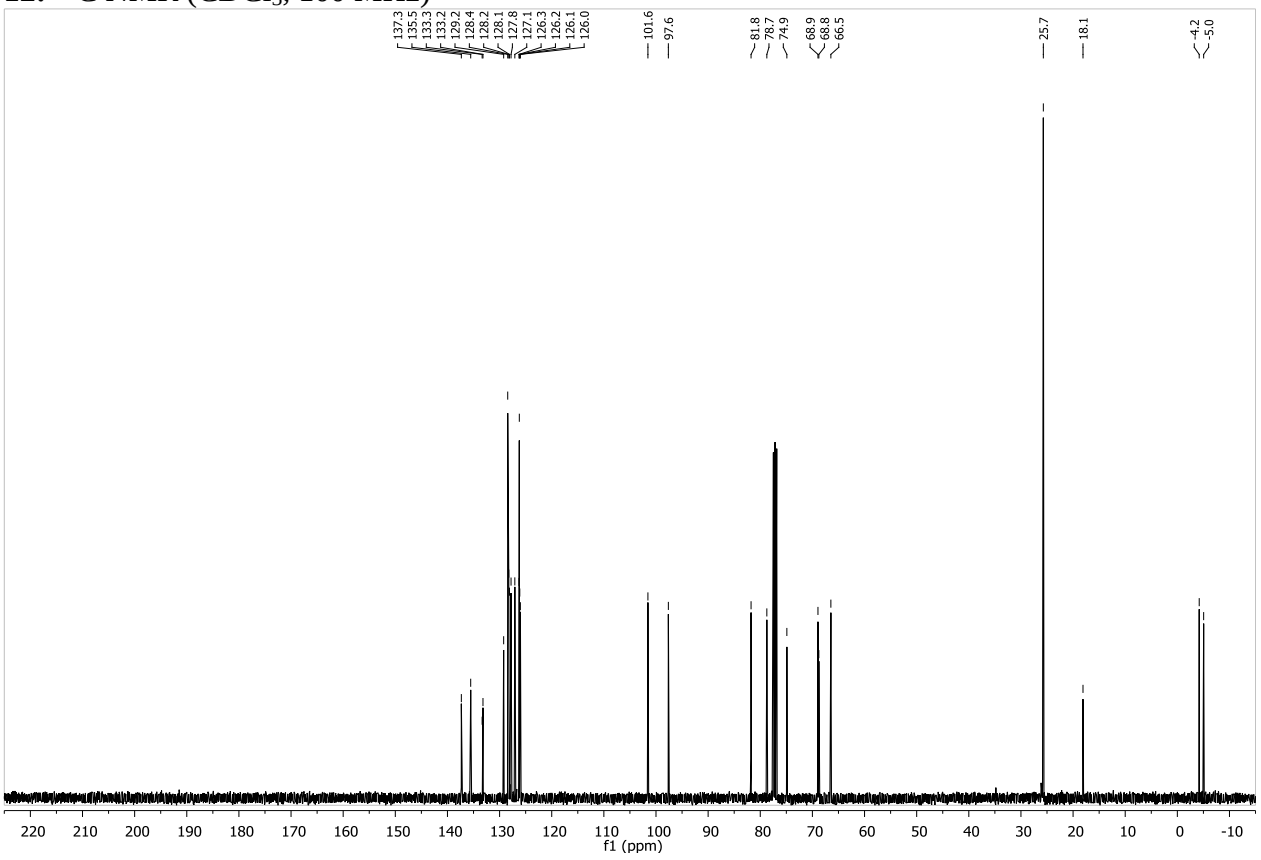
Microarray binding assay

Slides were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (w/v) for 1 h, washed three times with PBS and dried by centrifugation (300x g, 5 min.). A FlexWell 64 (Grace Bio-Labs, Bend, OR, USA) grid was applied to microarray slides. Resulting 64 wells were used for individual experiments. Slides were incubated with serum of *Clostridium difficile* patients, which was diluted 1:100 in PBS, in a humid chamber for 1 h, washed three times with 0.1% Tween-20 in PBS (v/v) and dried by centrifugation (300x g, 5 min.). Slides were incubated with fluorescence-labeled secondary antibody, goat anti-human IgG Alexa Fluor 647 (Life Technologies, Cat.# A-21445) diluted 1:400 in 1% BSA in PBS (w/v), in a humid chamber for 1 h, washed three times with 0.1% Tween-20 in PBS (v/v), rinsed once with deionized water and dried by centrifugation (300x g, 5 min.) prior to scanning with a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA). Image analysis was carried out with the GenePix Pro 7 software (Molecular Devices). The entire procedure was performed at room temperature.

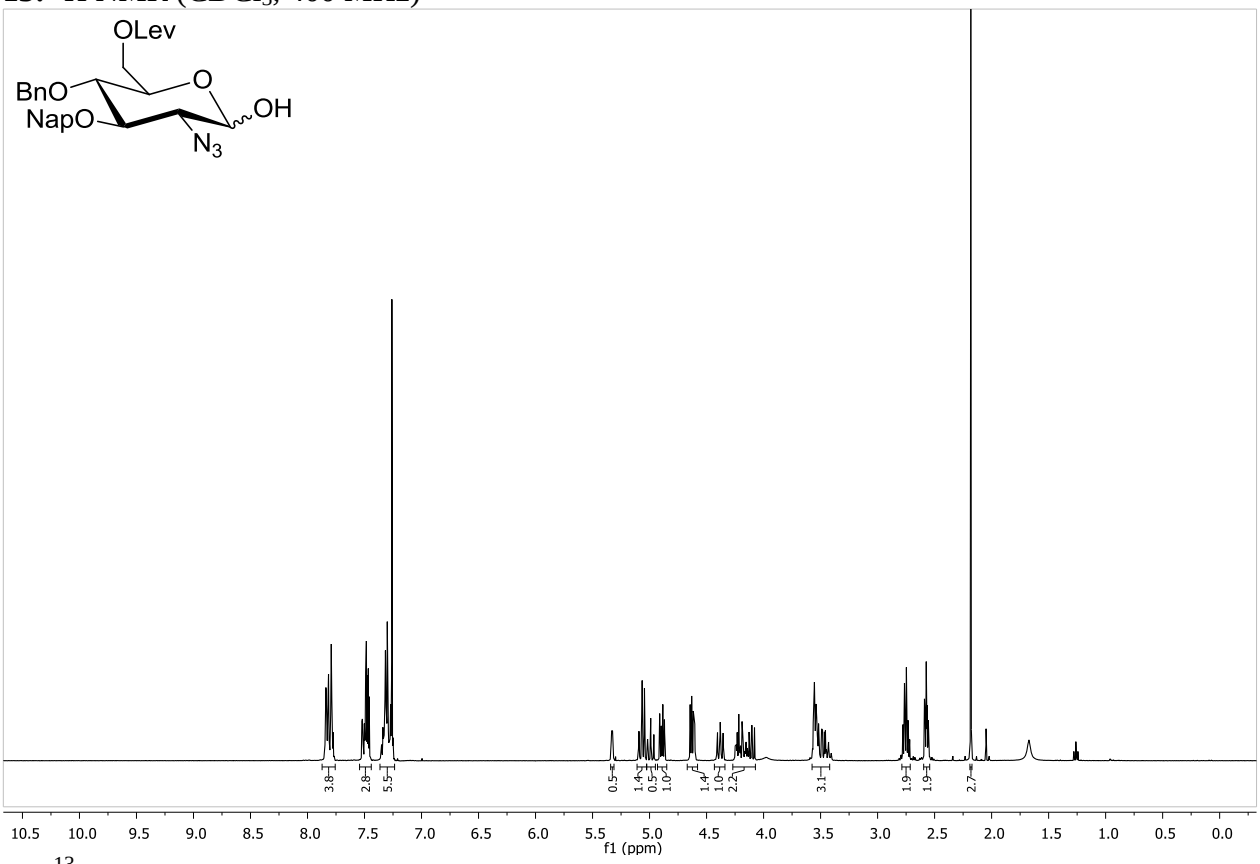
12: ^{1}H NMR ($CDCl_3$, 400 MHz)



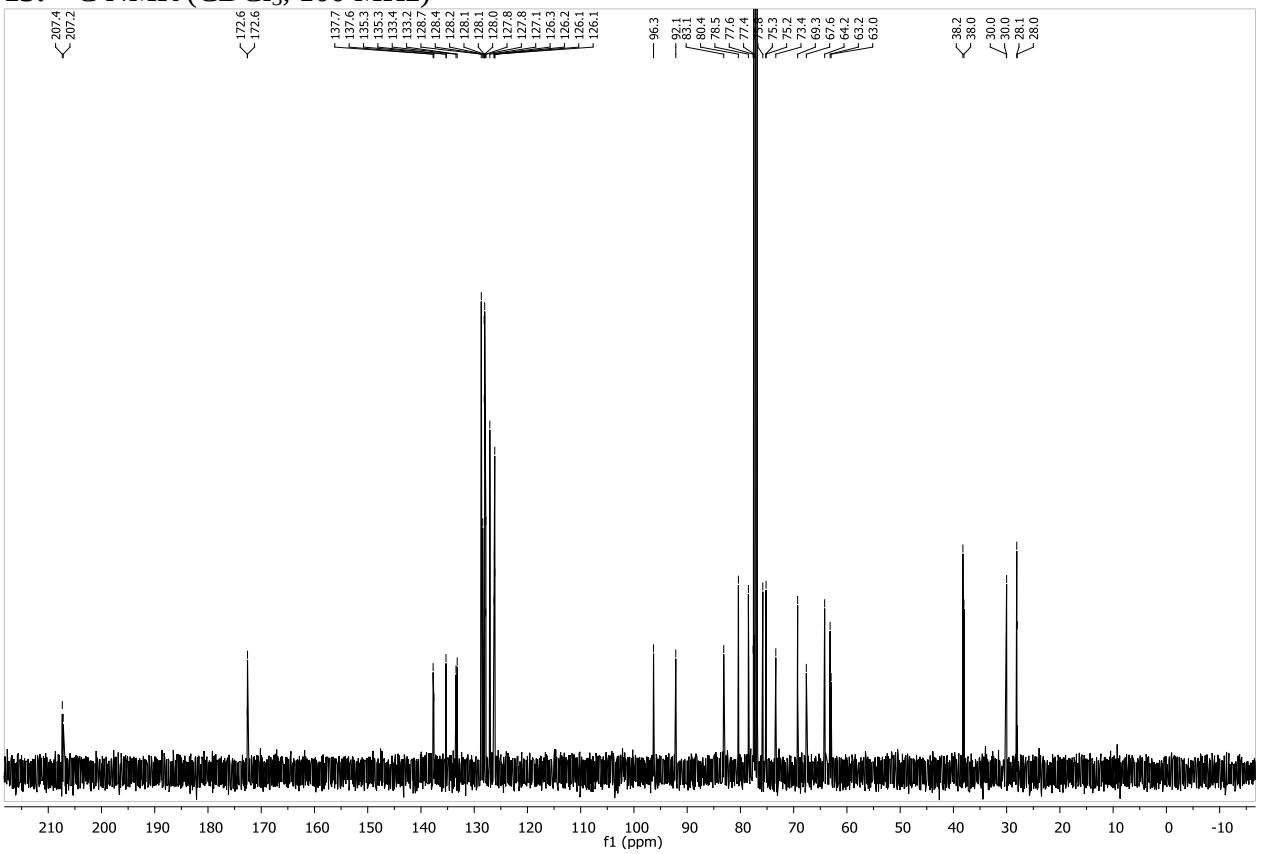
12: ^{13}C NMR ($CDCl_3$, 100 MHz)



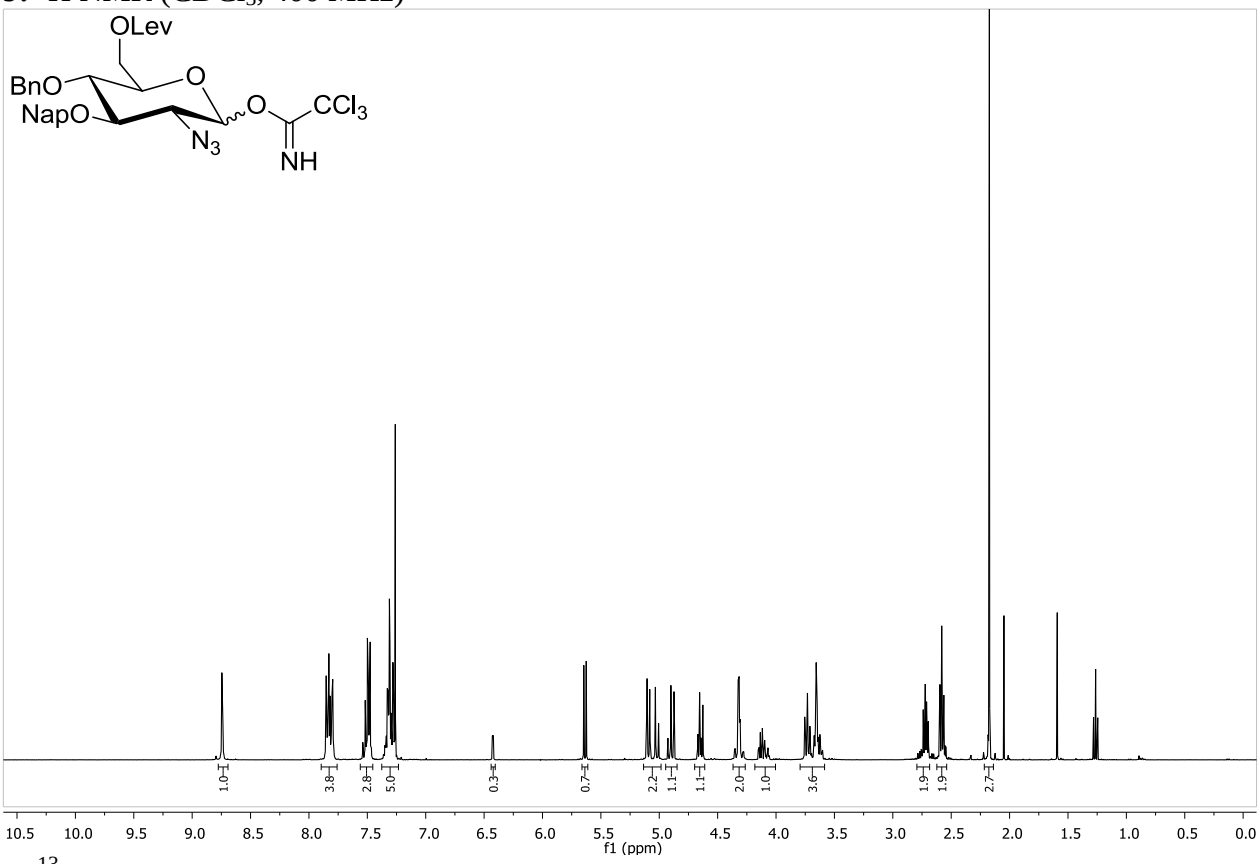
13: ^{1}H NMR ($CDCl_3$, 400 MHz)



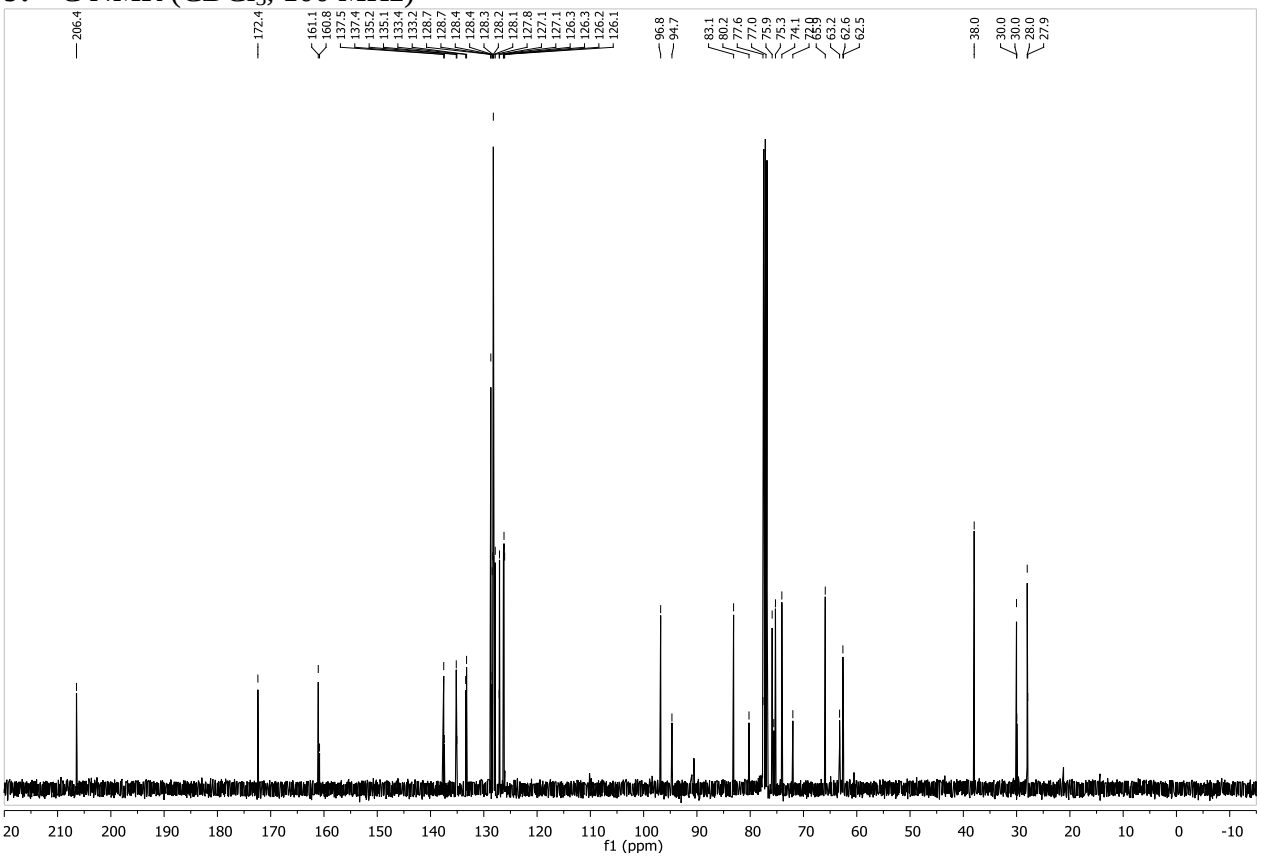
13: ^{13}C NMR ($CDCl_3$, 100 MHz)



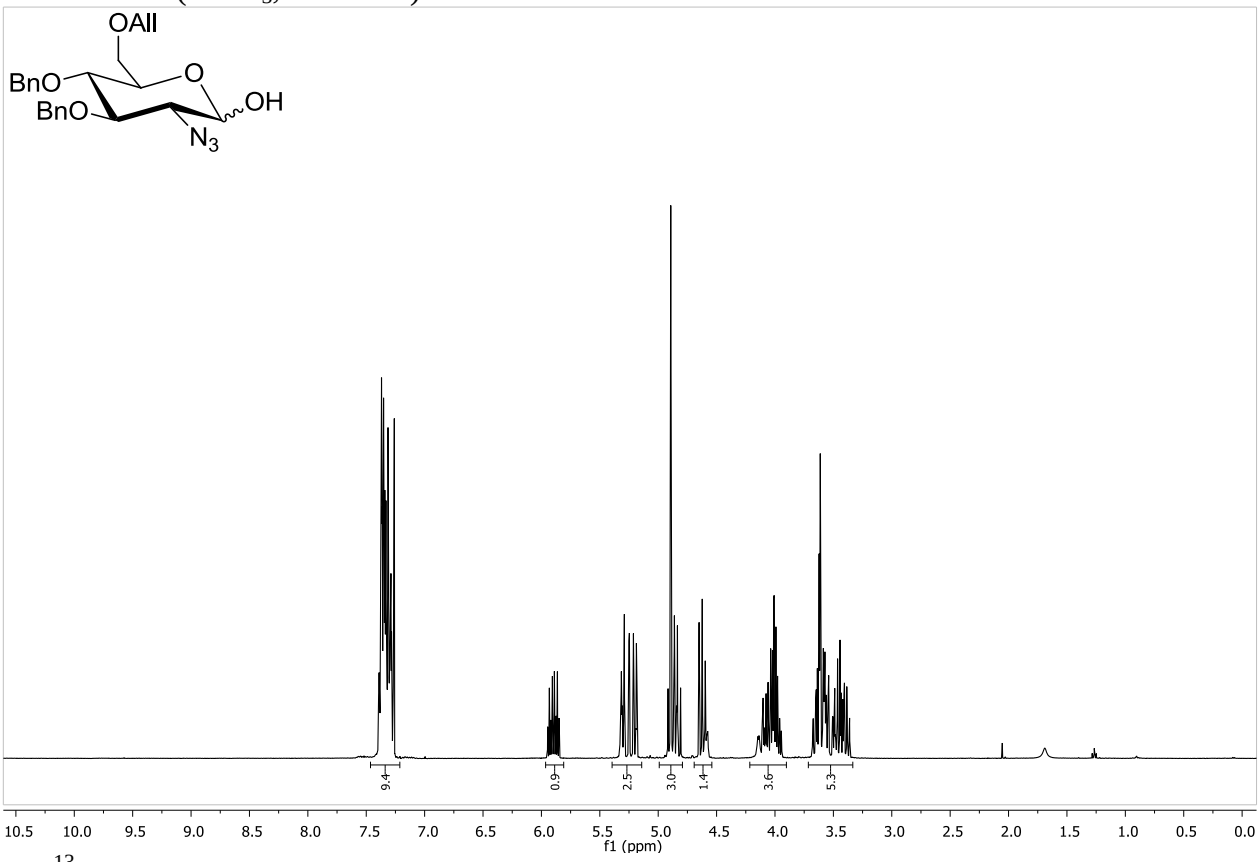
9: ^{1}H NMR ($CDCl_3$, 400 MHz)



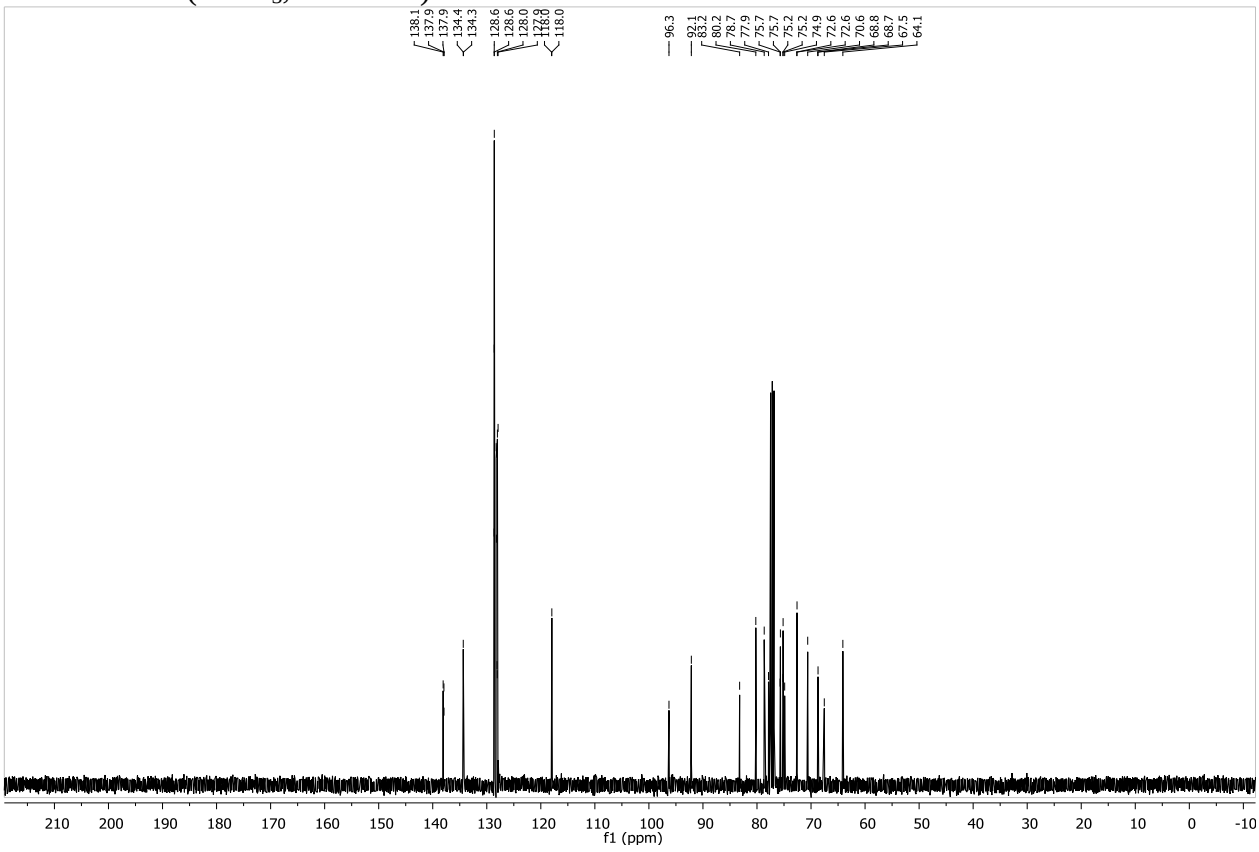
9: ^{13}C NMR ($CDCl_3$, 100 MHz)



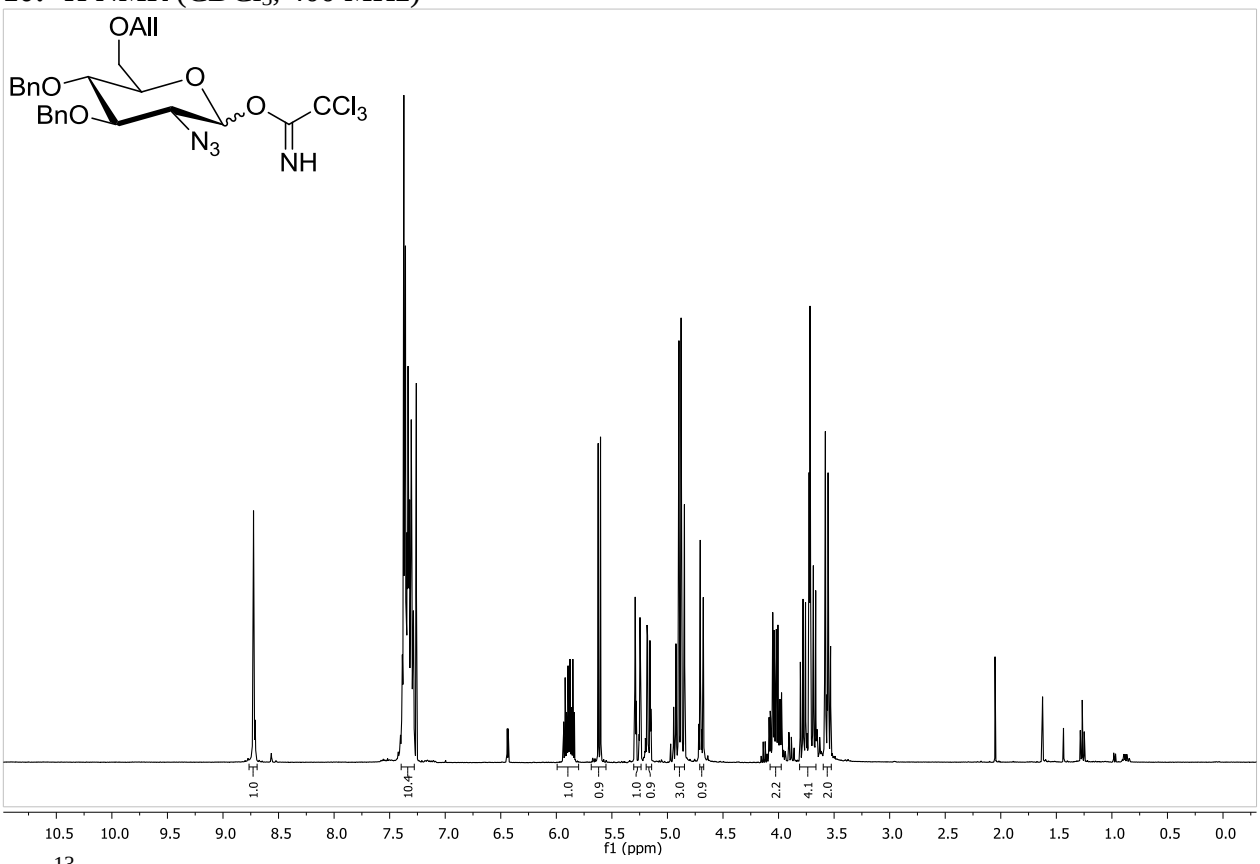
15: ^{1}H NMR ($CDCl_3$, 400 MHz)



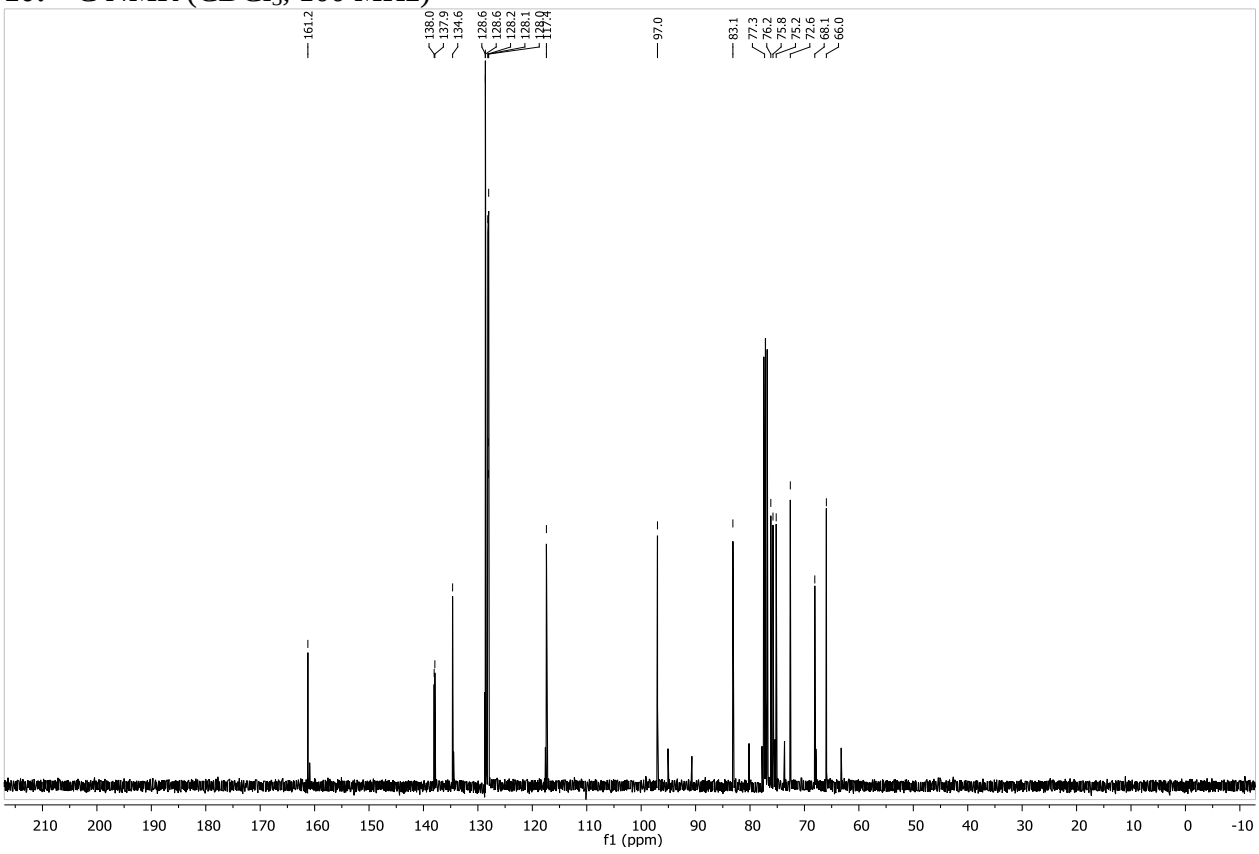
15: ^{13}C NMR ($CDCl_3$, 100 MHz)



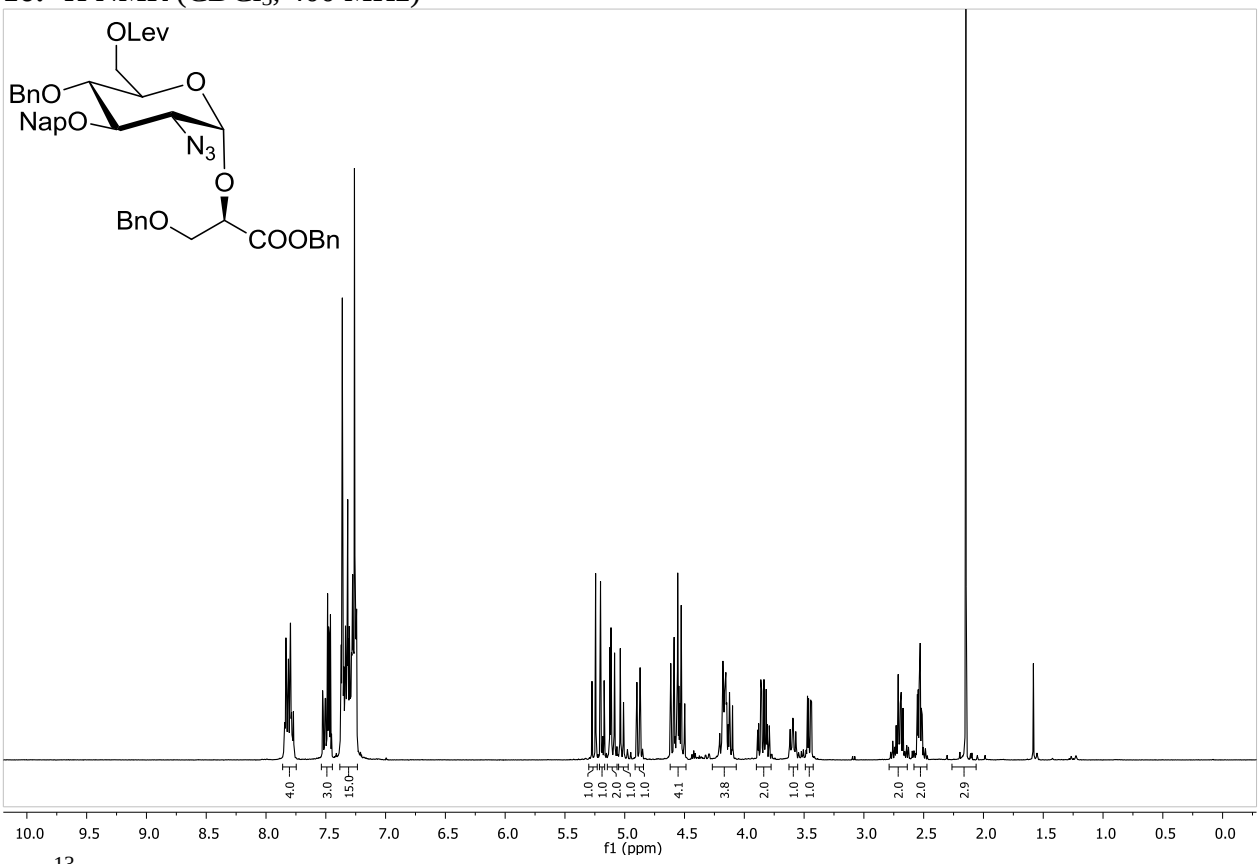
10: ^{1}H NMR ($CDCl_3$, 400 MHz)



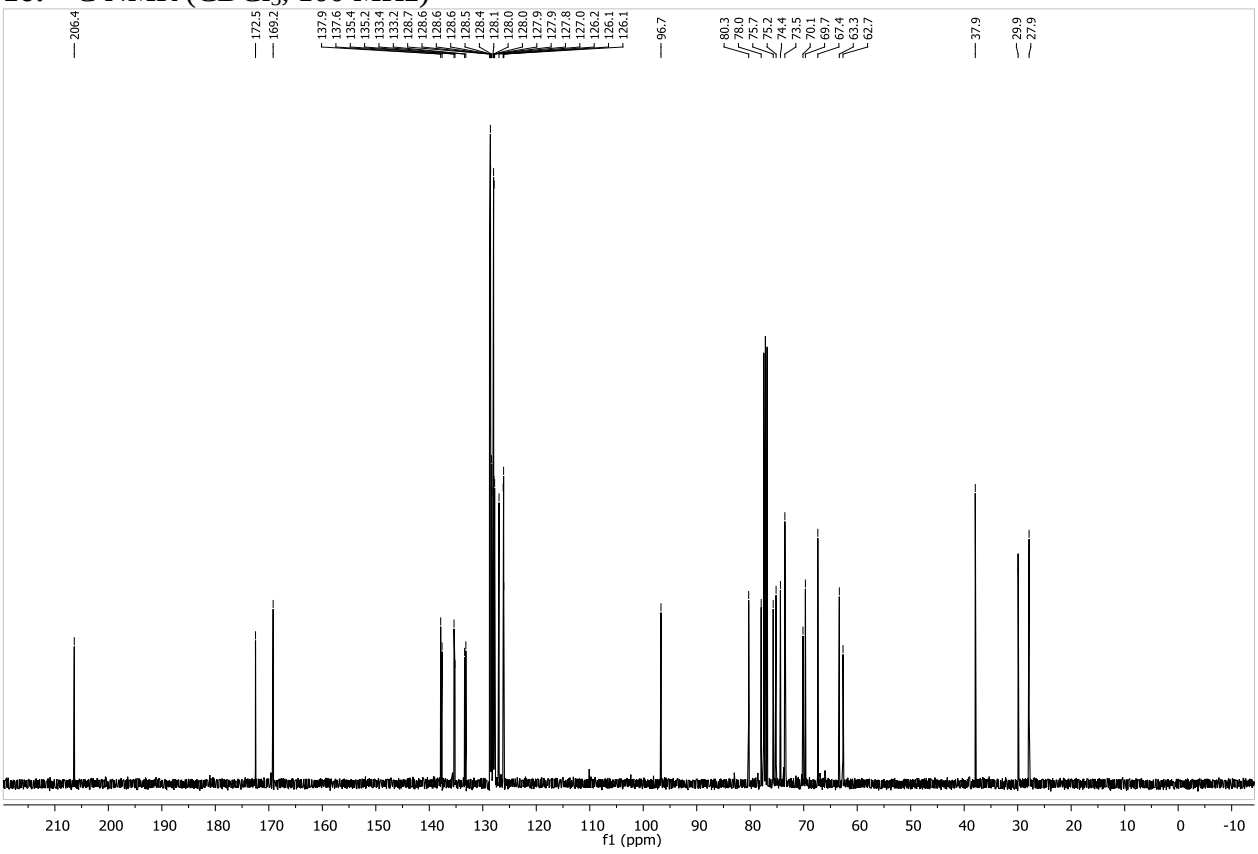
10: ^{13}C NMR ($CDCl_3$, 100 MHz)



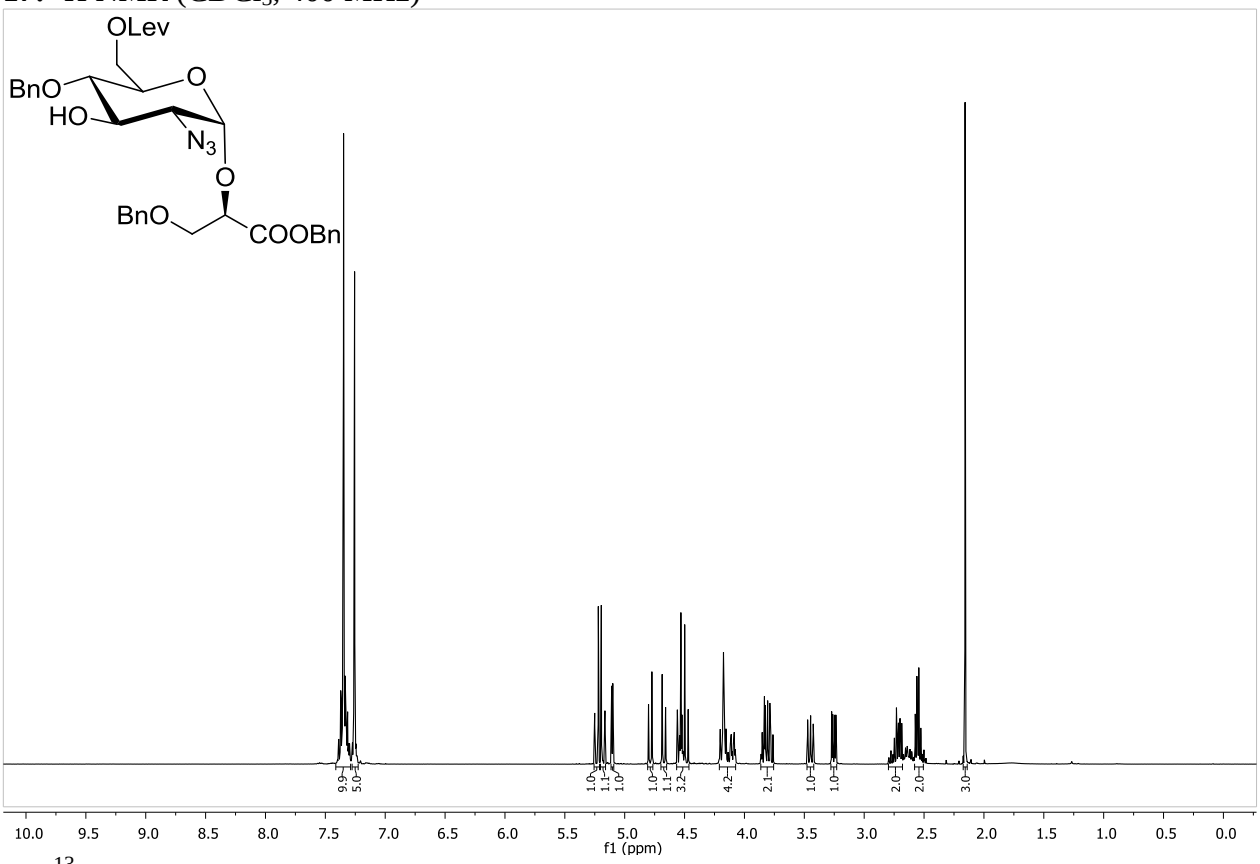
16: ^{1}H NMR ($CDCl_3$, 400 MHz)



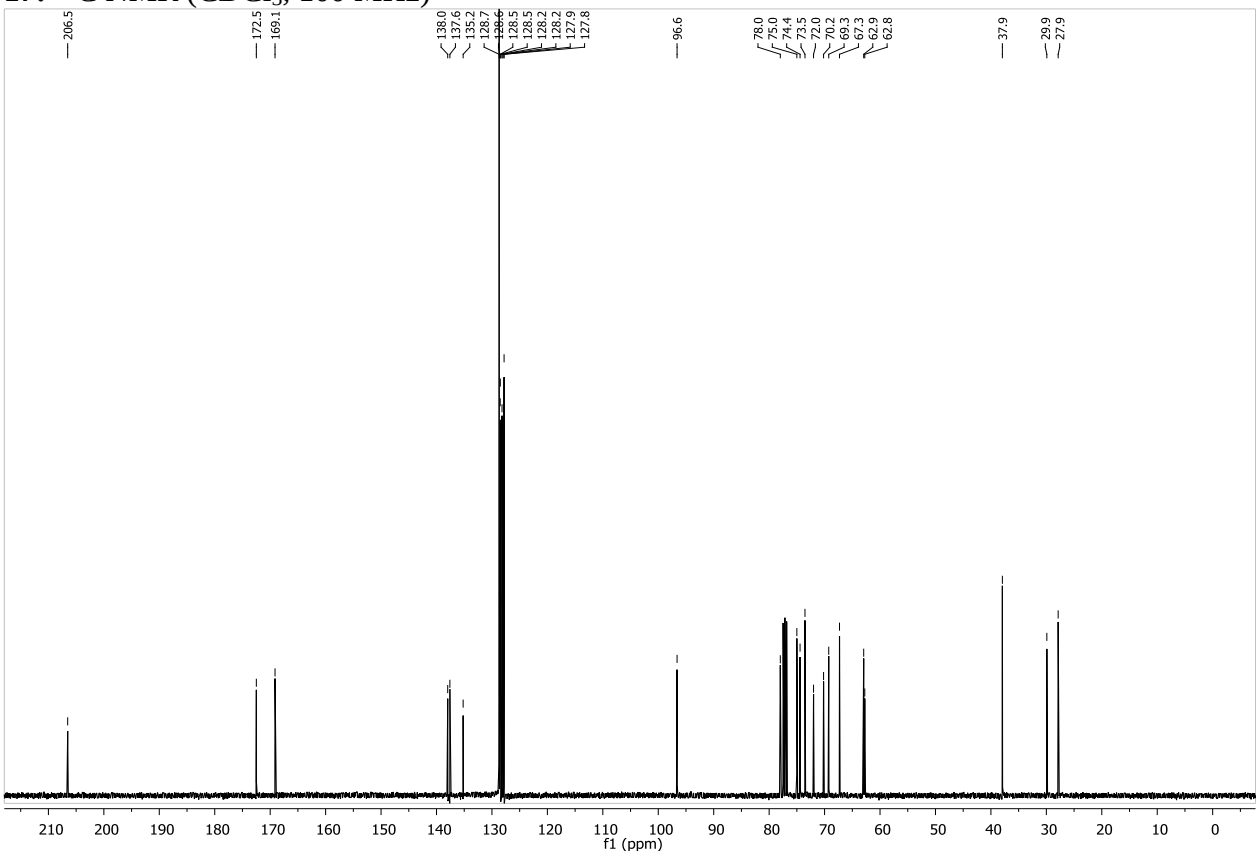
16: ^{13}C NMR ($CDCl_3$, 100 MHz)



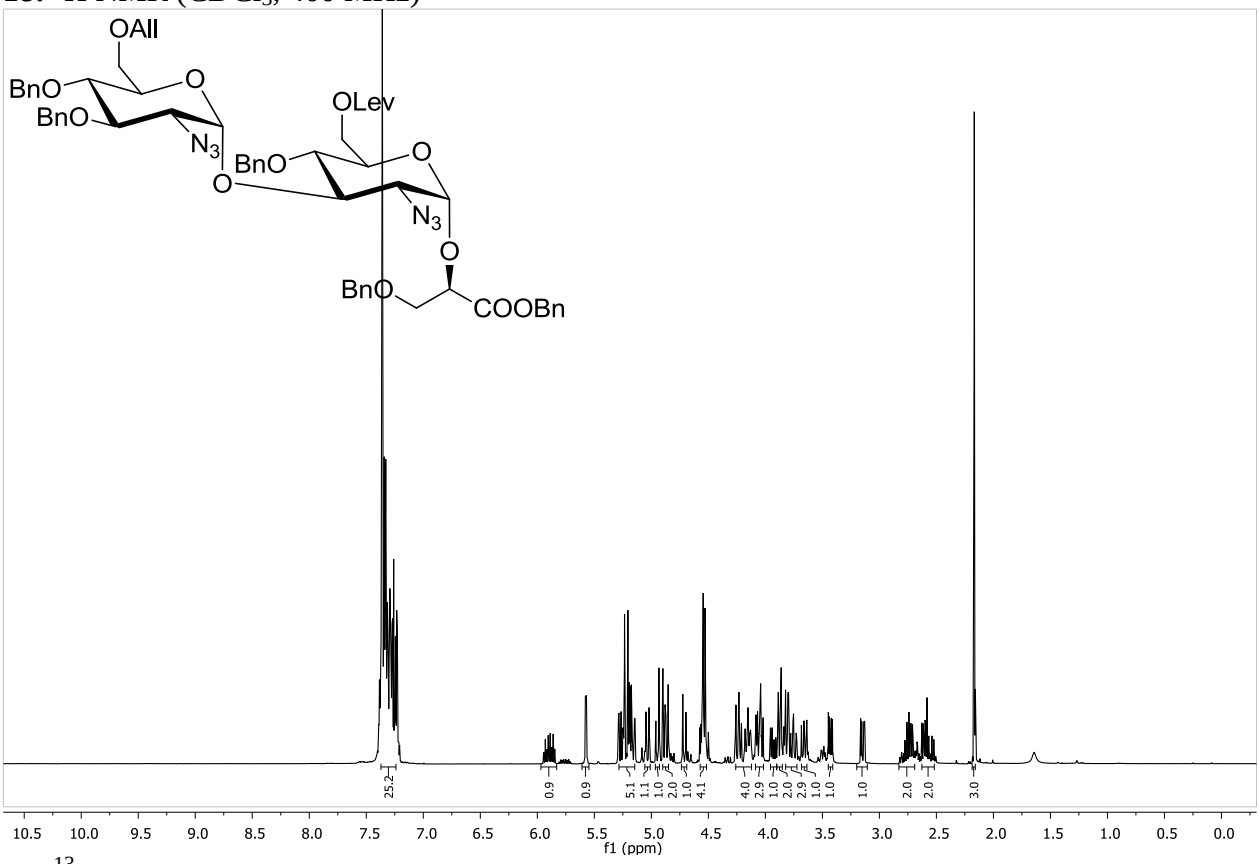
17: ^{1}H NMR ($CDCl_3$, 400 MHz)



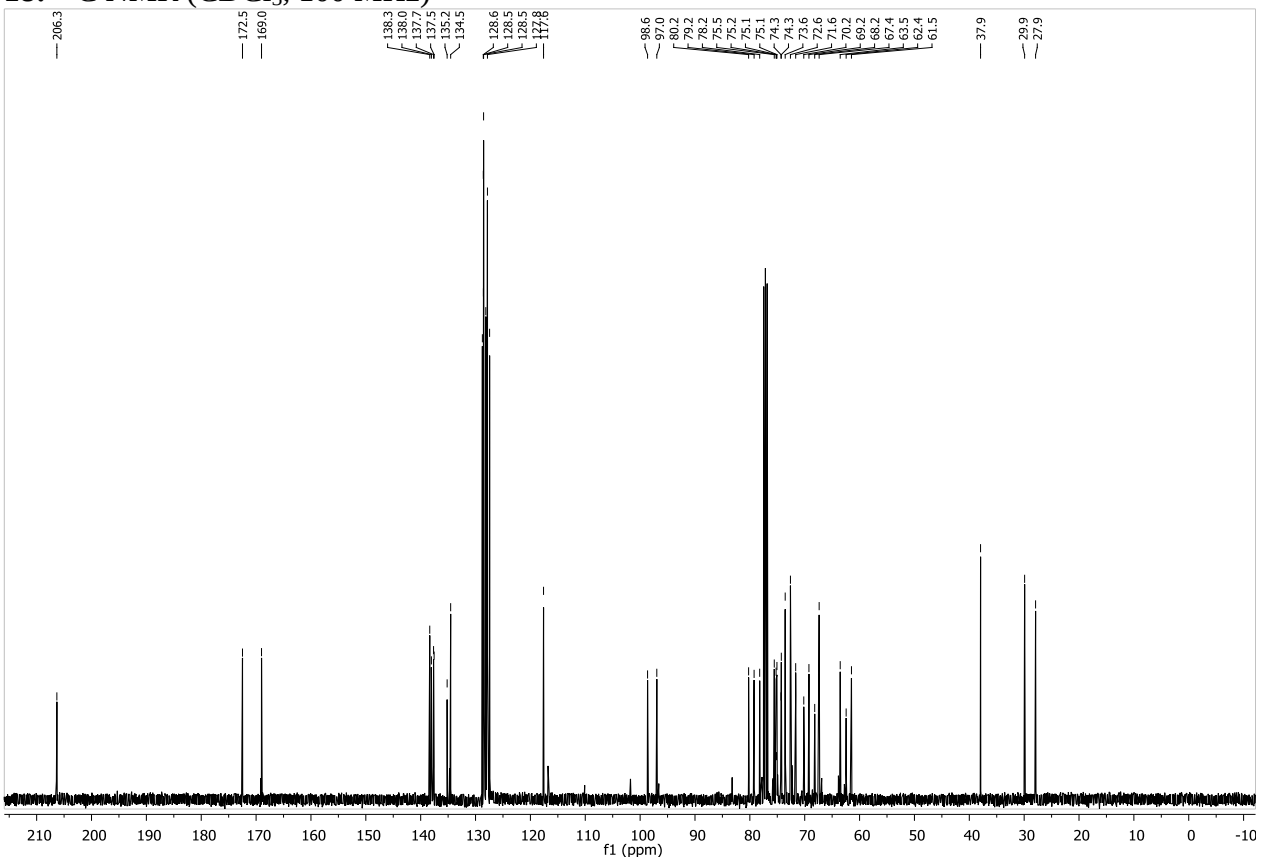
17: ^{13}C NMR ($CDCl_3$, 100 MHz)



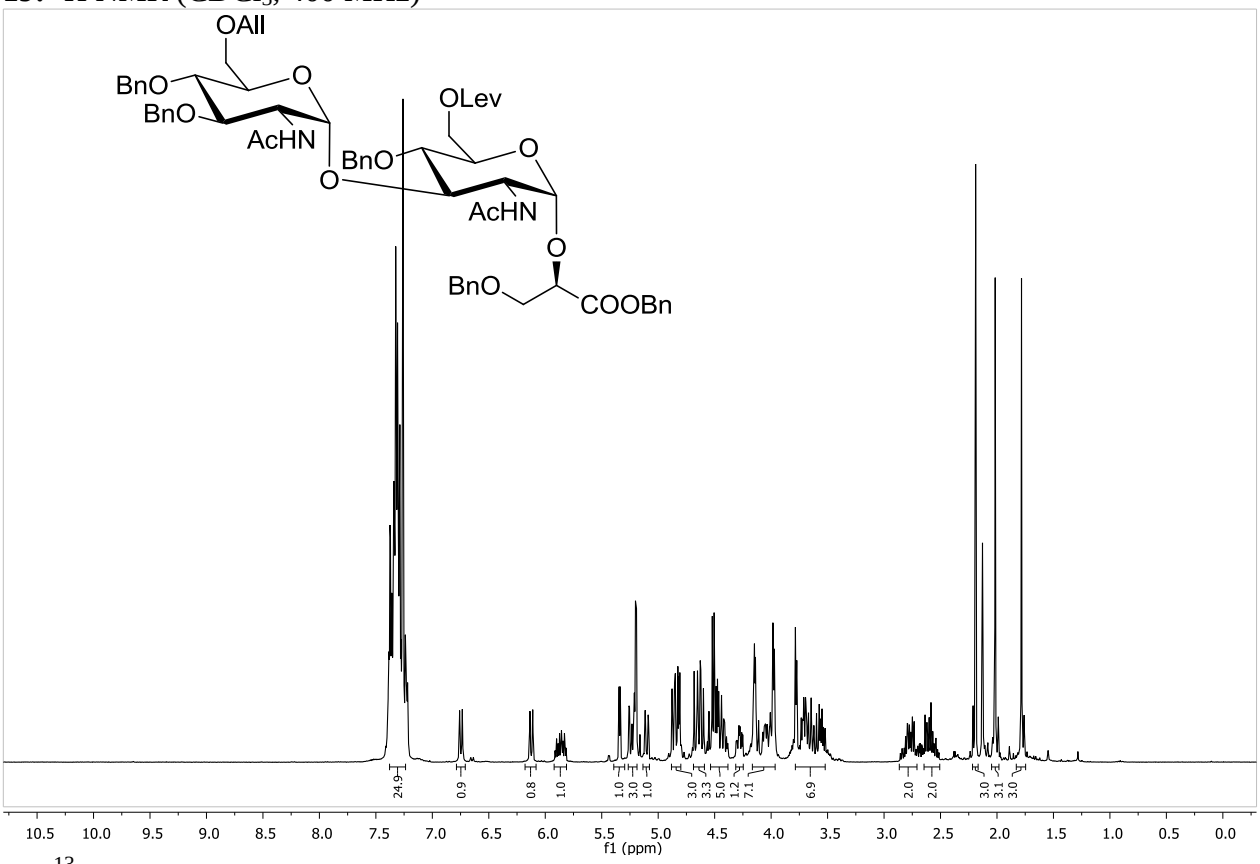
18: ^{1}H NMR ($CDCl_3$, 400 MHz)



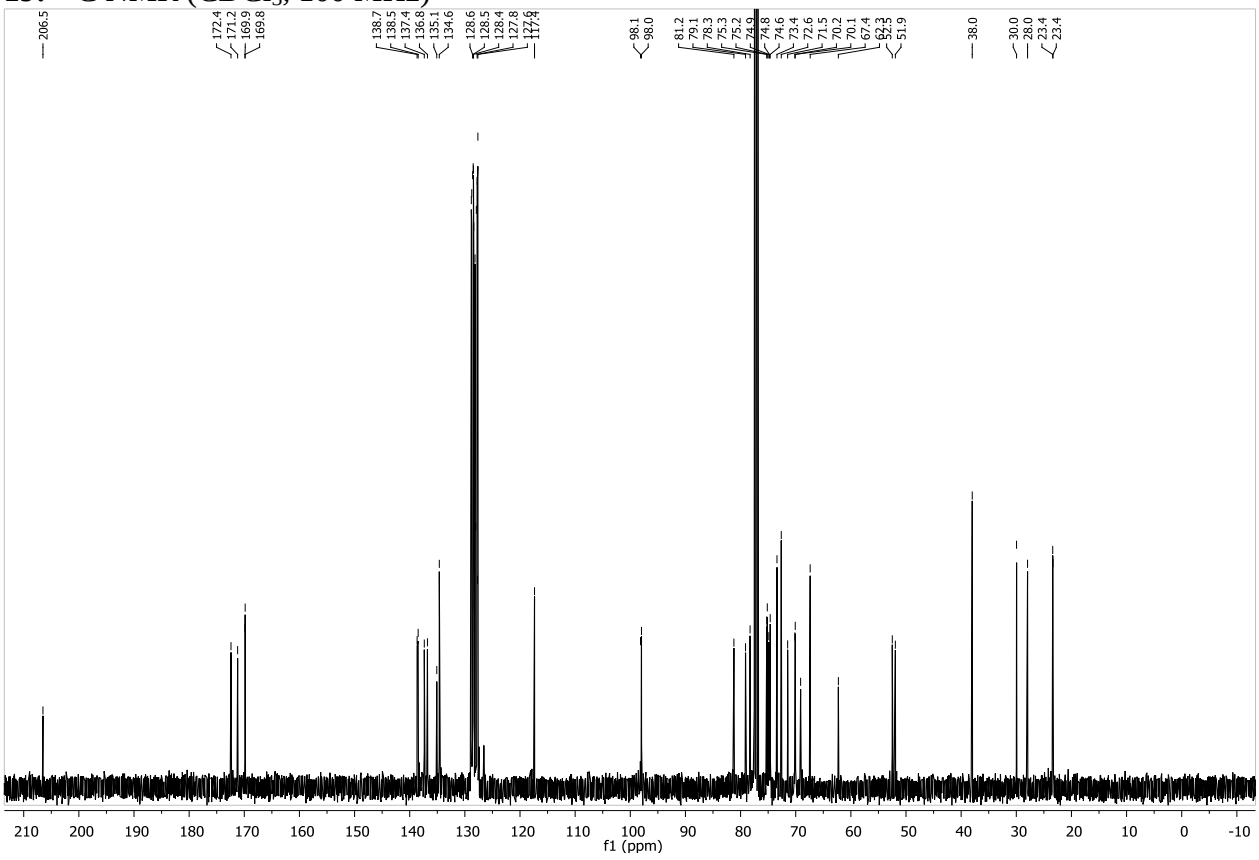
18: ^{13}C NMR ($CDCl_3$, 100 MHz)



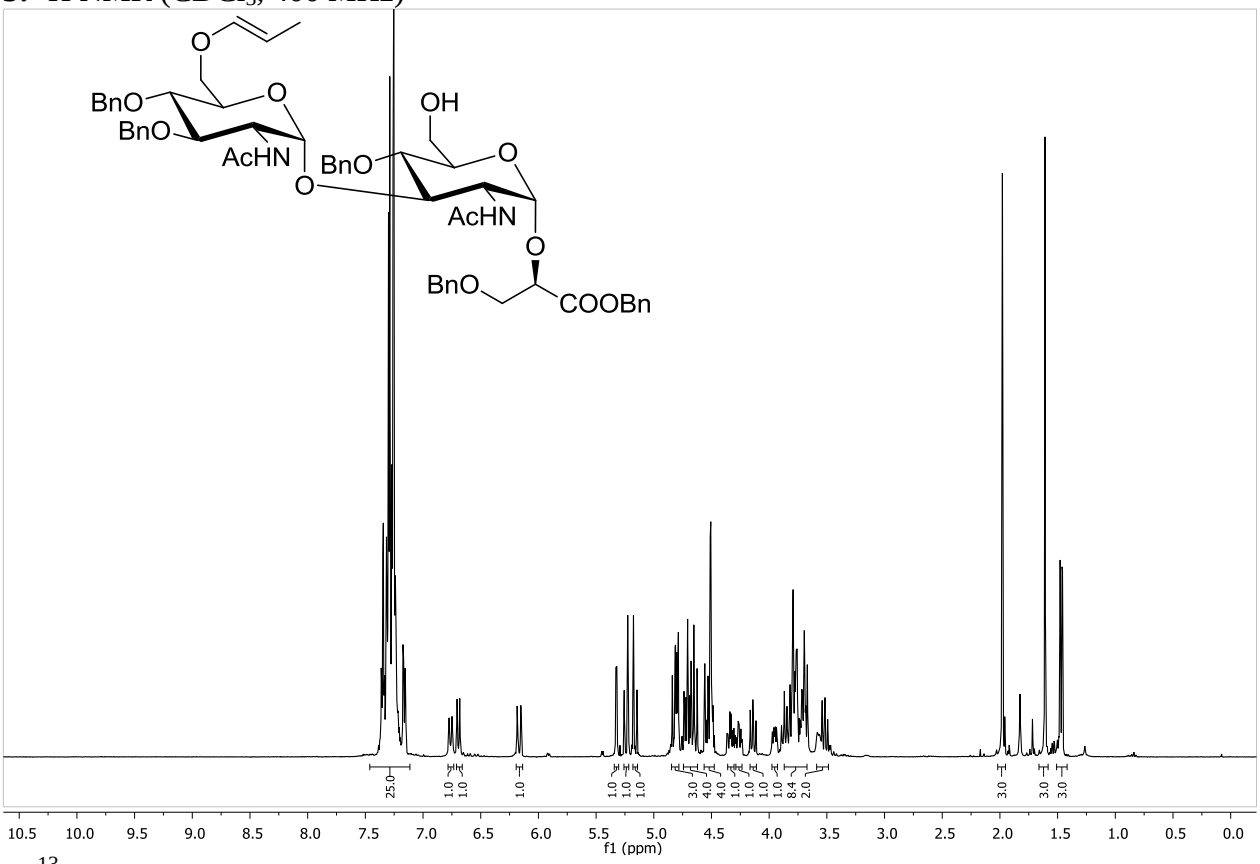
19: ^{1}H NMR ($CDCl_3$, 400 MHz)



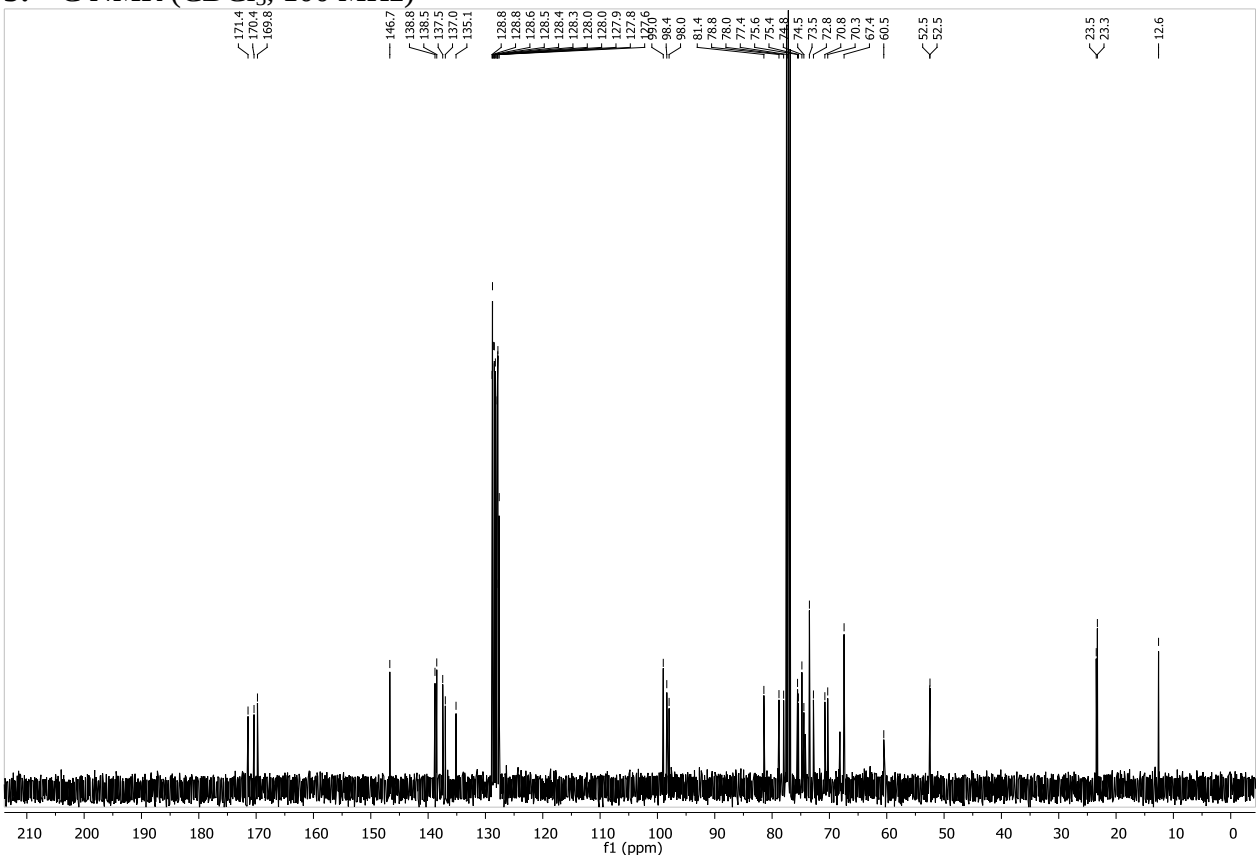
19: ^{13}C NMR ($CDCl_3$, 100 MHz)



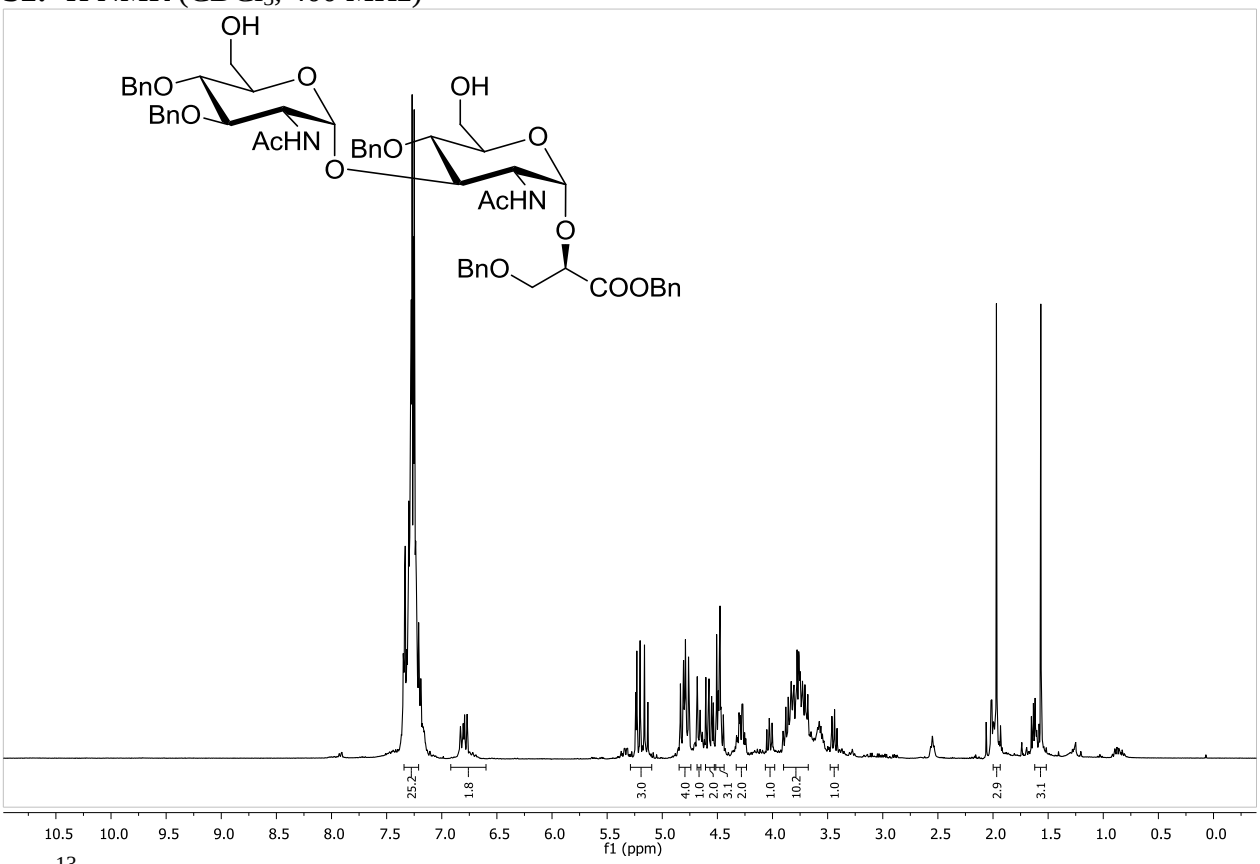
5: ^{1}H NMR ($CDCl_3$, 400 MHz)



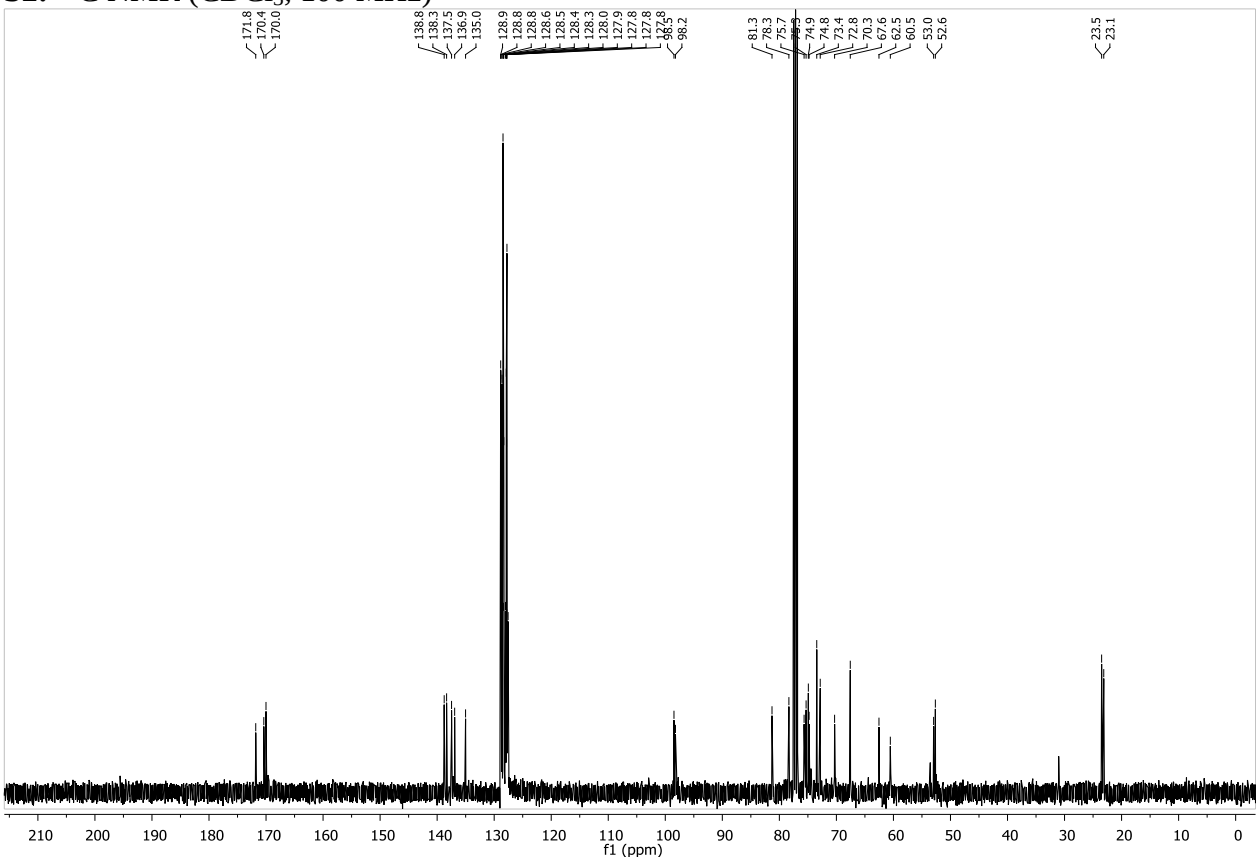
5: ^{13}C NMR ($CDCl_3$, 100 MHz)



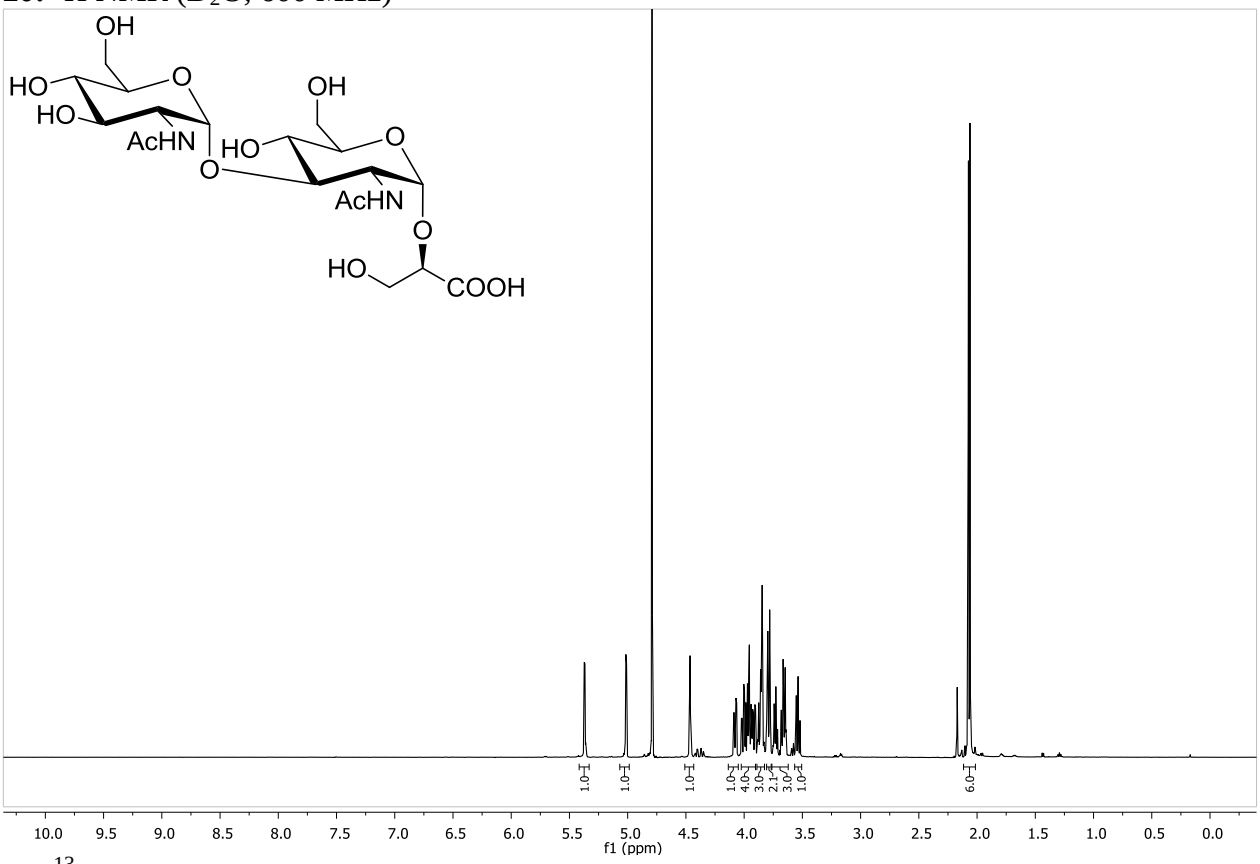
S2: ^{1}H NMR ($CDCl_3$, 400 MHz)



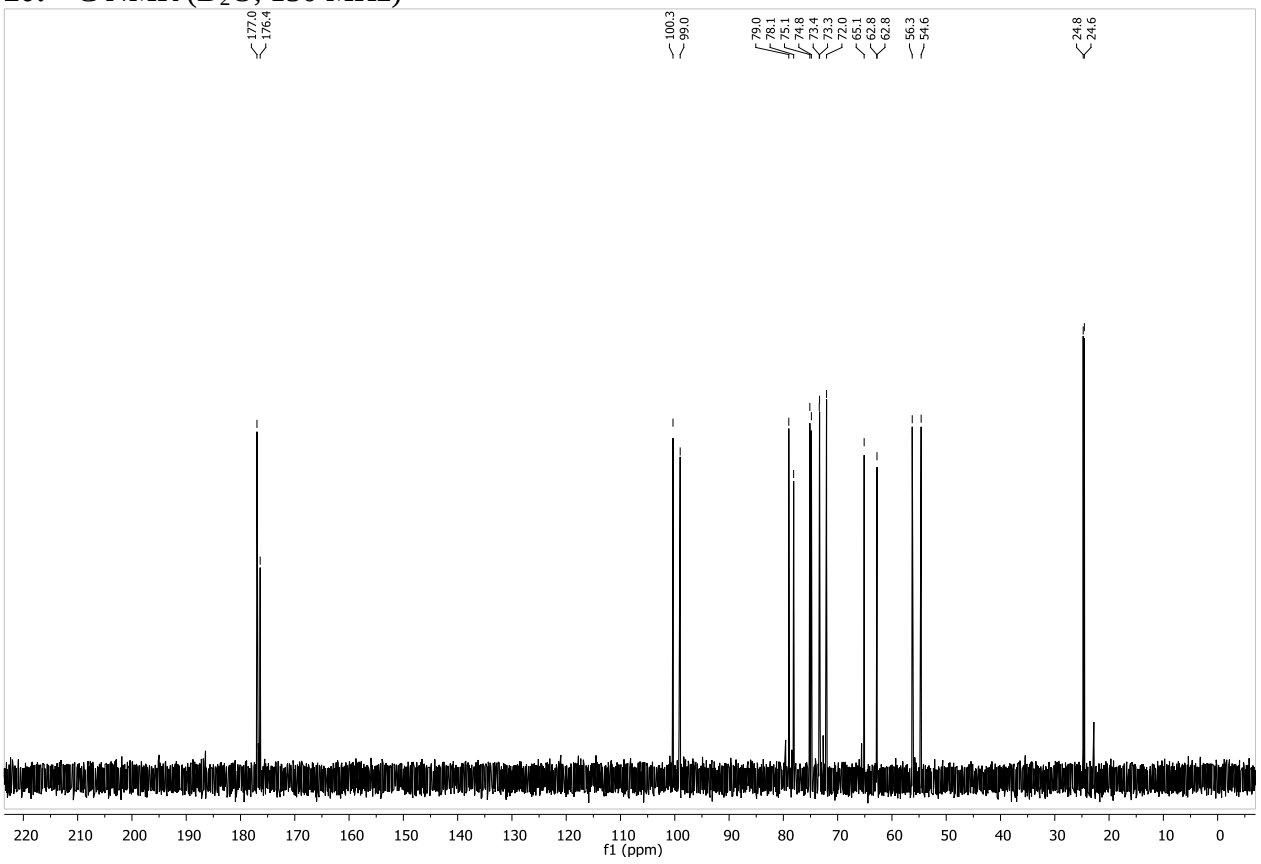
S2: ^{13}C NMR ($CDCl_3$, 100 MHz)



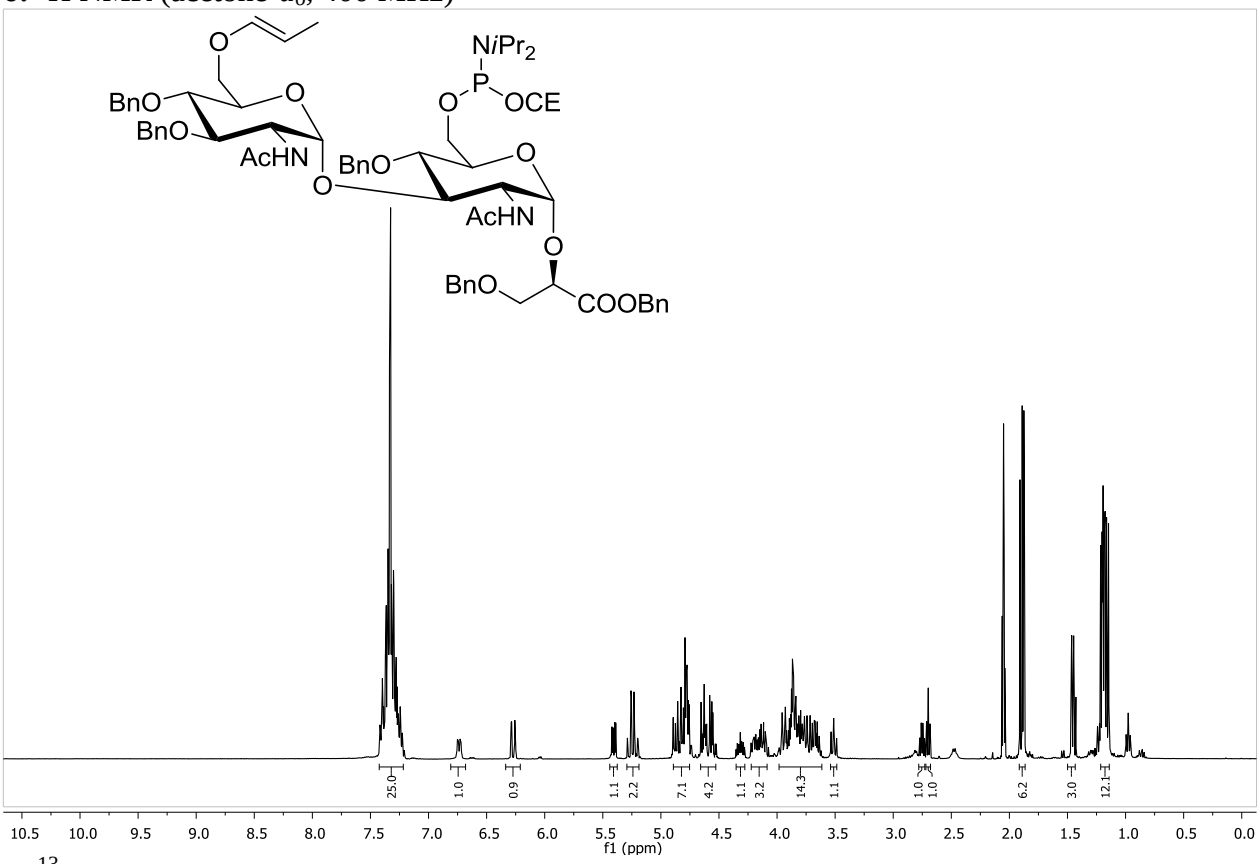
20: ^{1}H NMR (D_2O, 600 MHz)



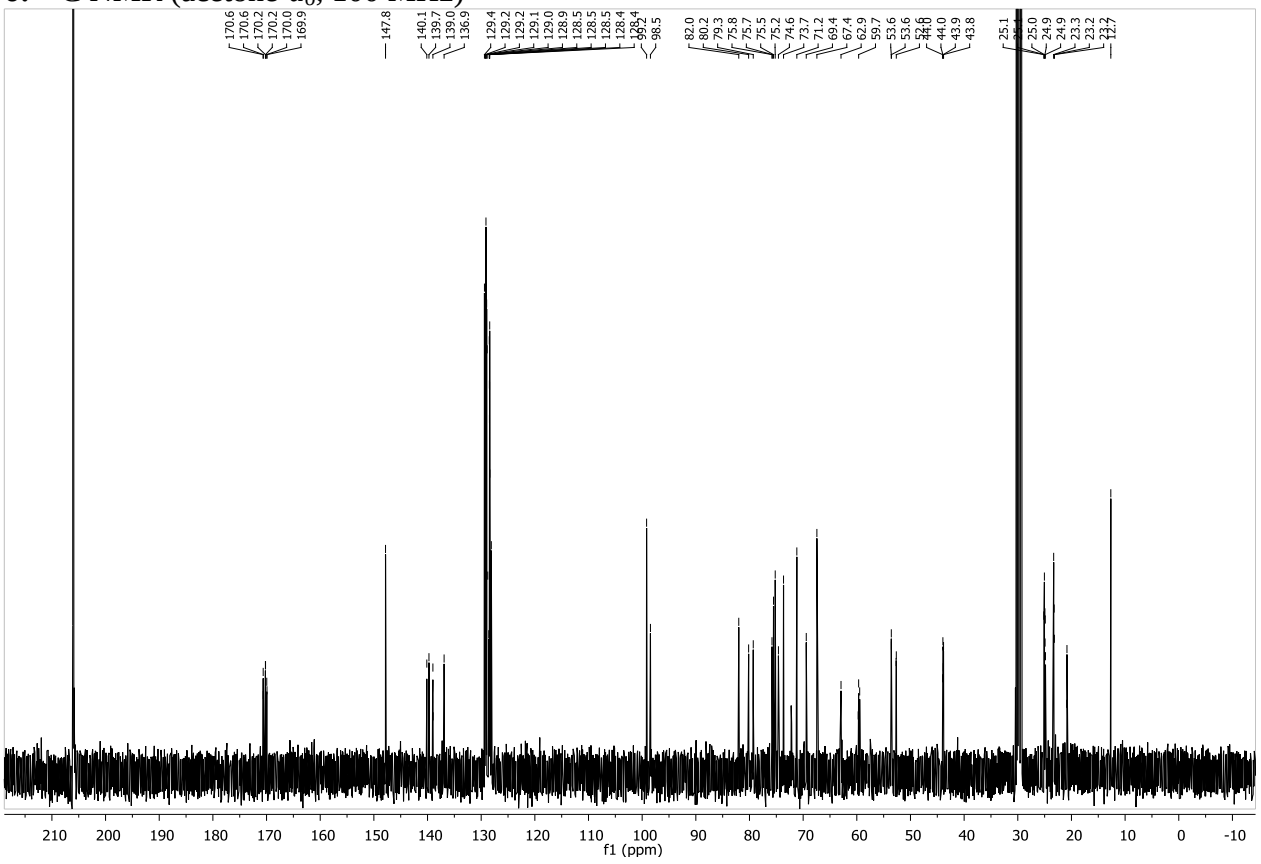
20: ^{13}C NMR (D_2O, 150 MHz)



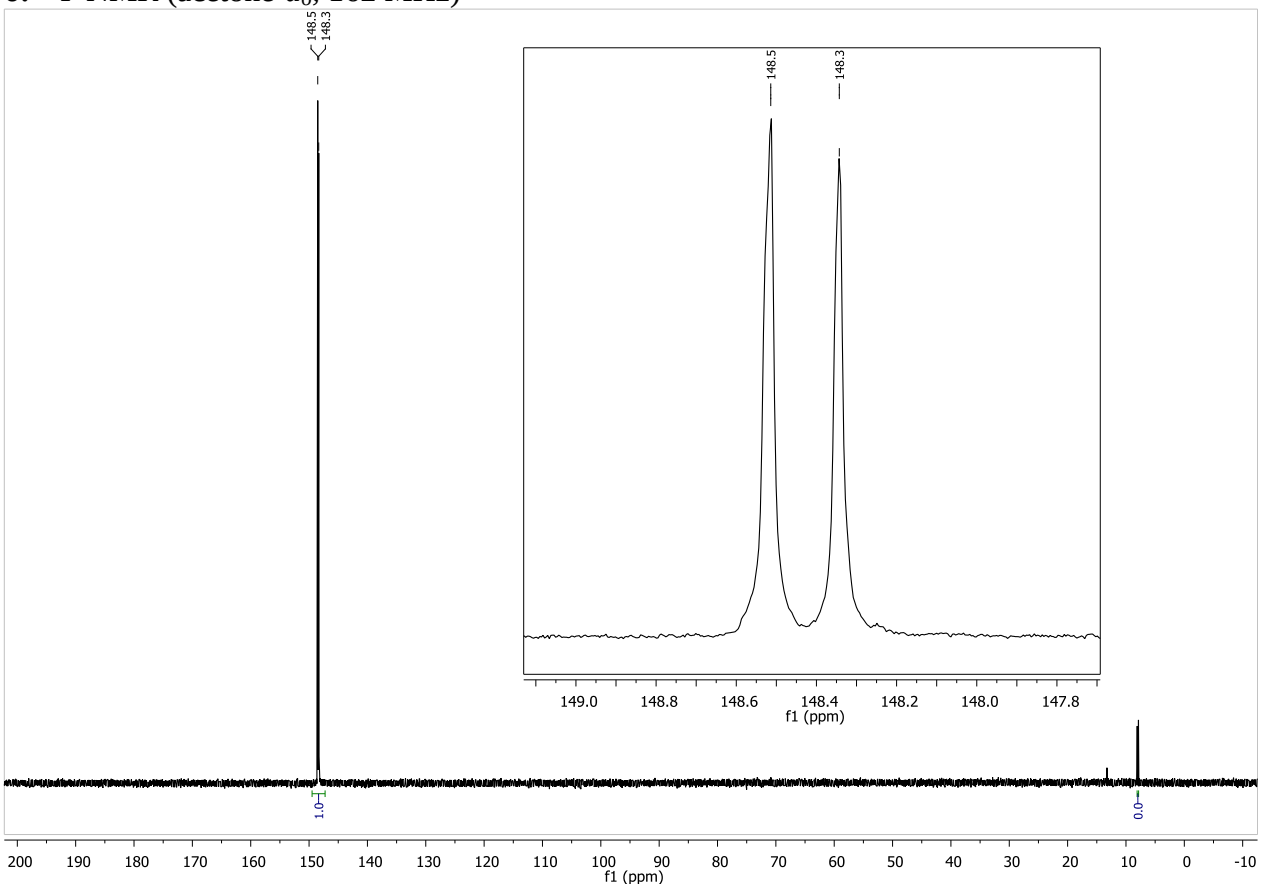
6: ^{1}H NMR (acetone-d_6, 400 MHz)



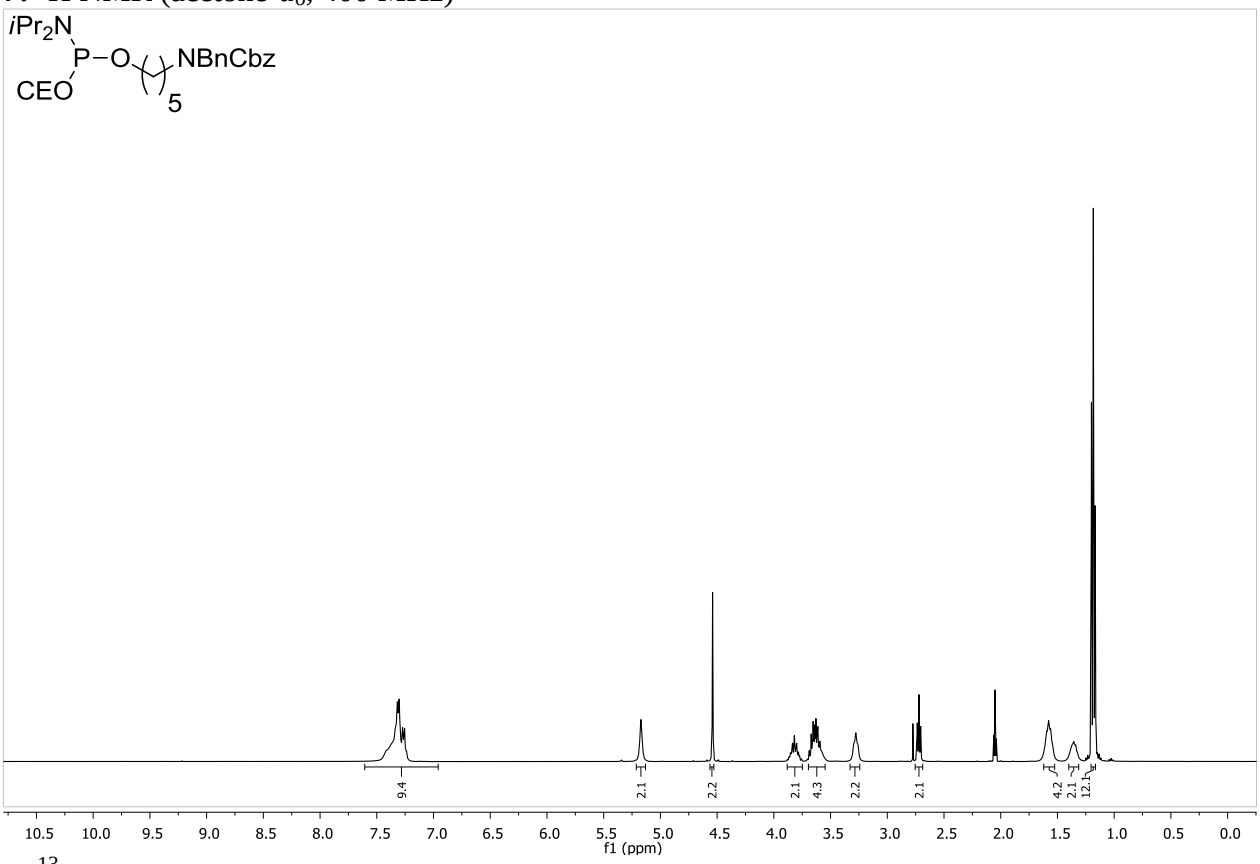
6: ^{13}C NMR (acetone-d_6, 100 MHz)



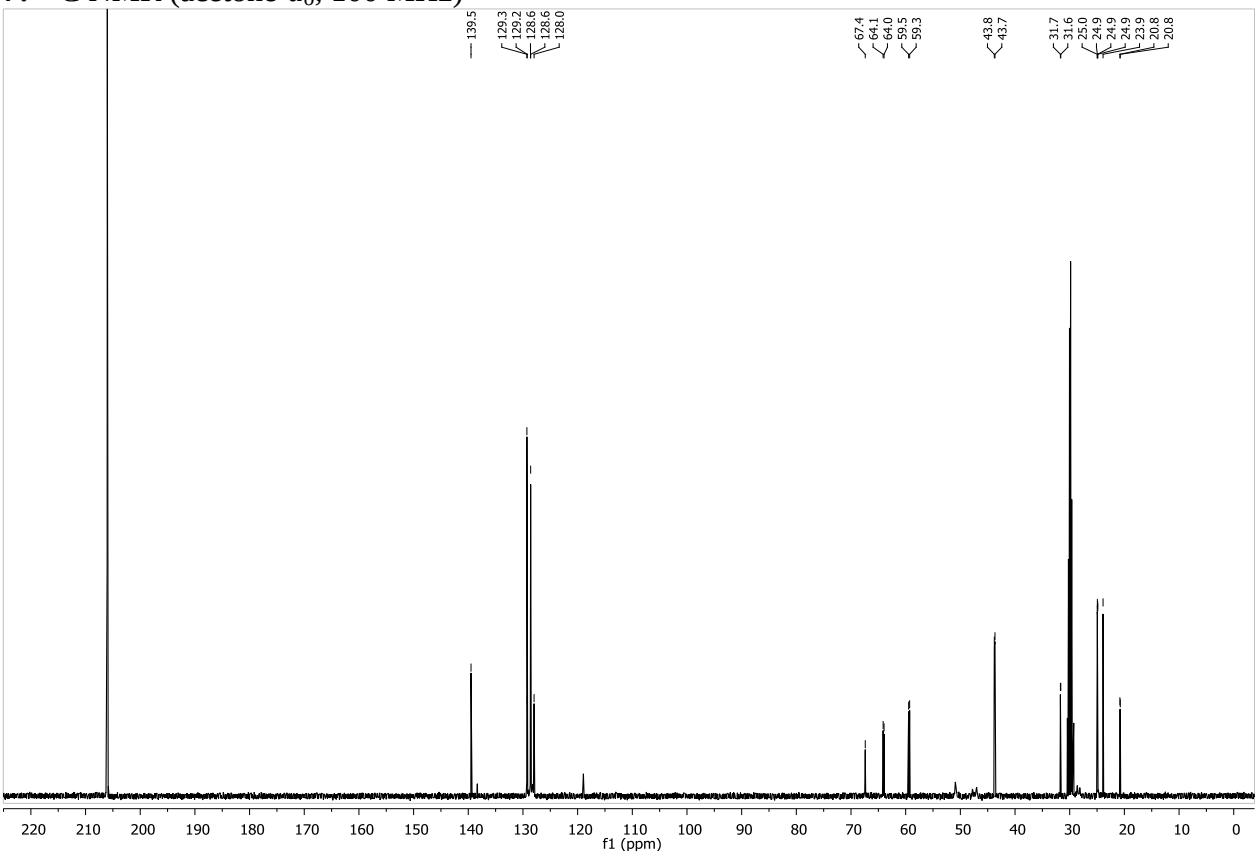
6: ^{31}P NMR (acetone-d_6, 162 MHz)



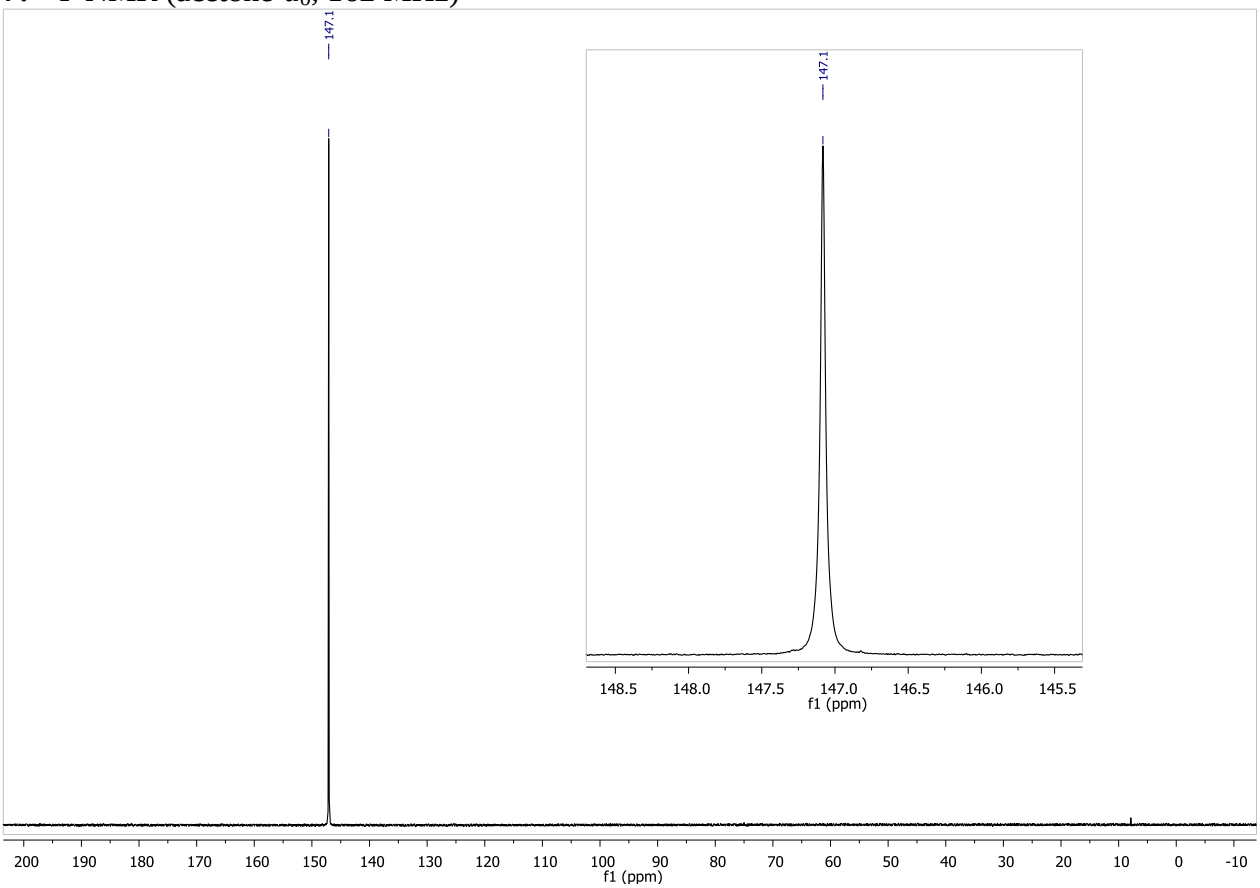
7: ^{1}H NMR (acetone-d_6, 400 MHz)



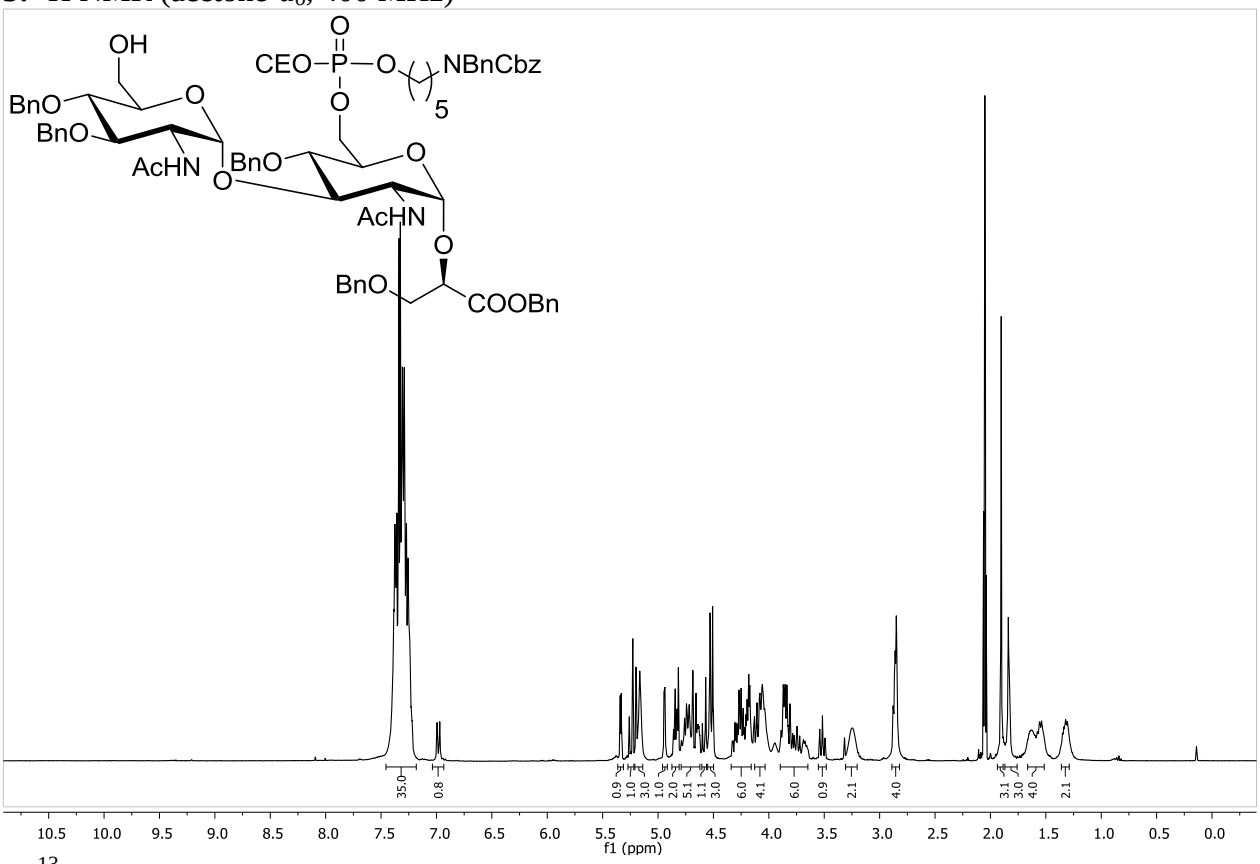
7: ^{13}C NMR (acetone-d_6, 100 MHz)



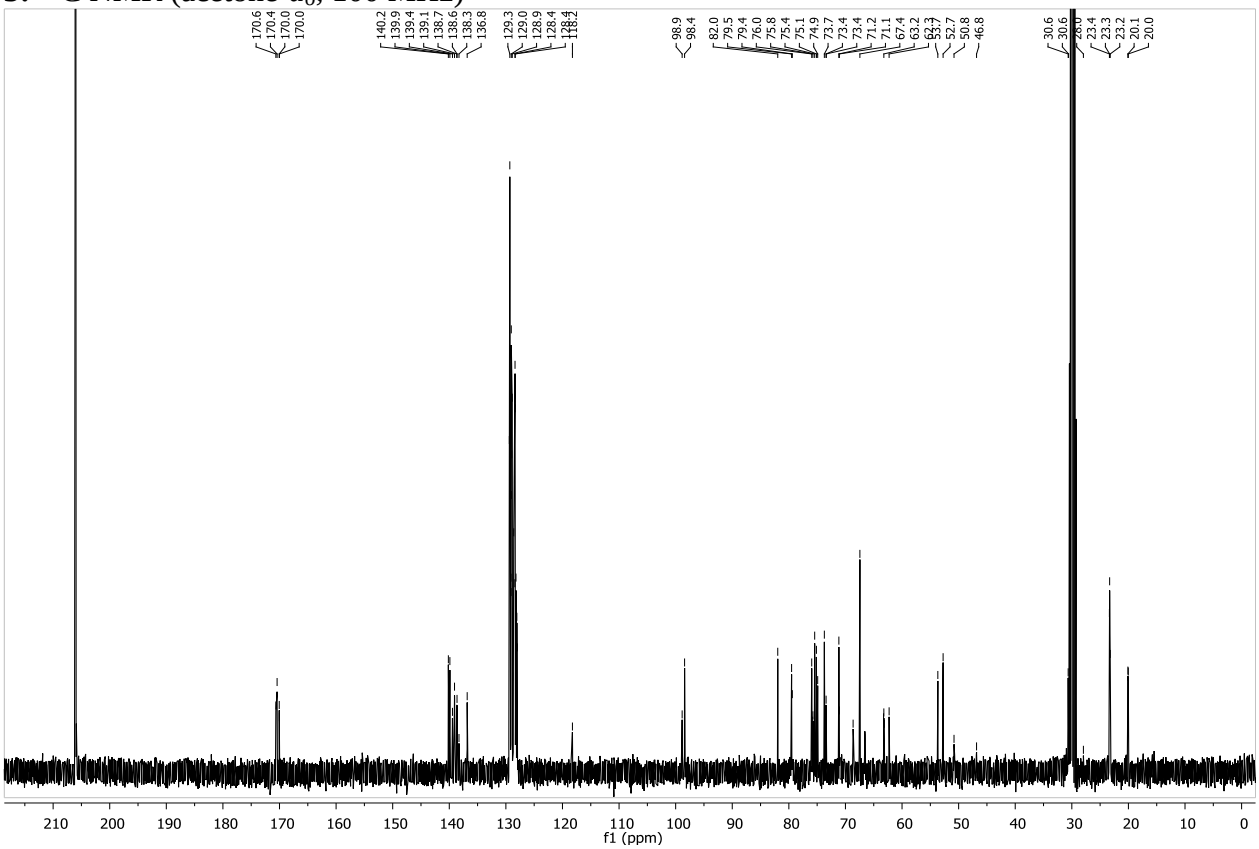
7: ^{31}P NMR (acetone-d_6, 162 MHz)



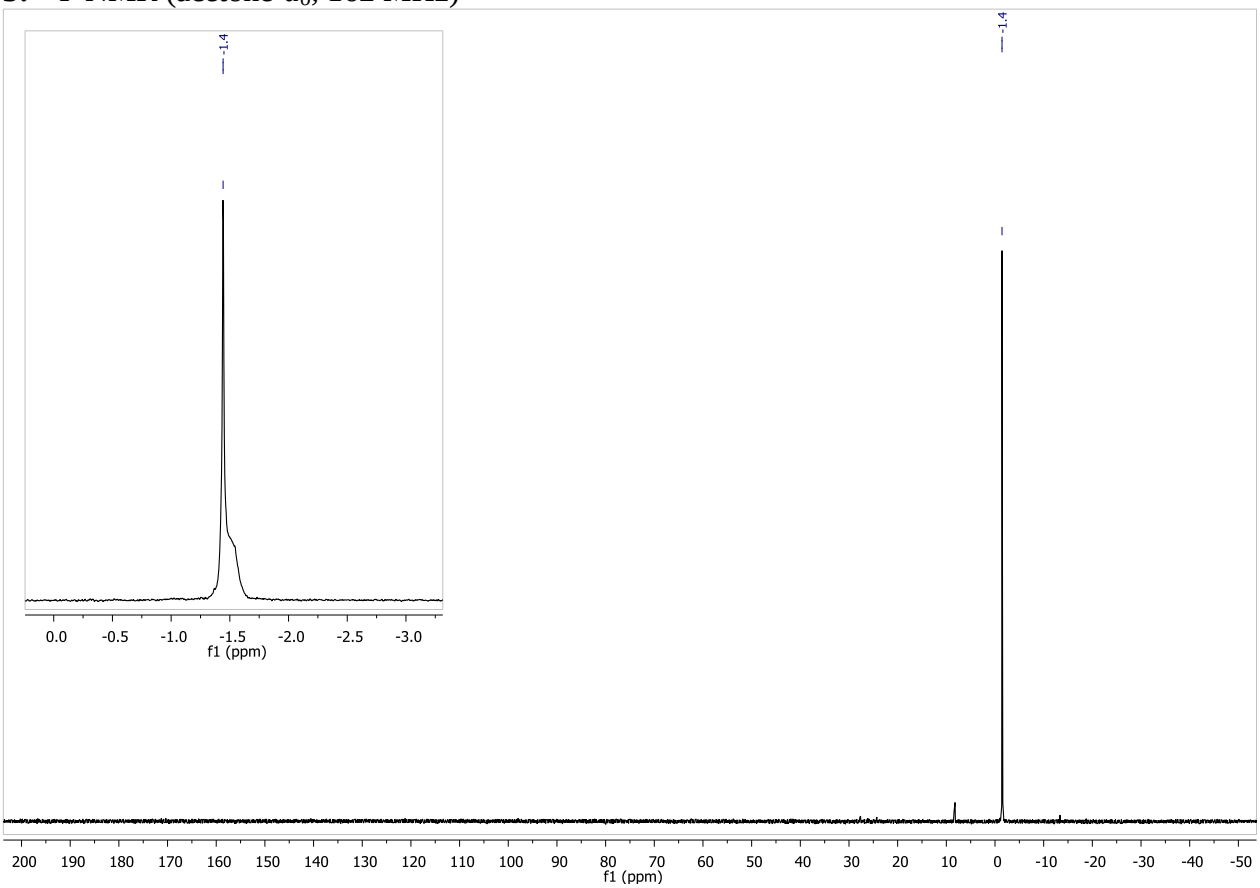
3: ^{1}H NMR (acetone-d_6, 400 MHz)



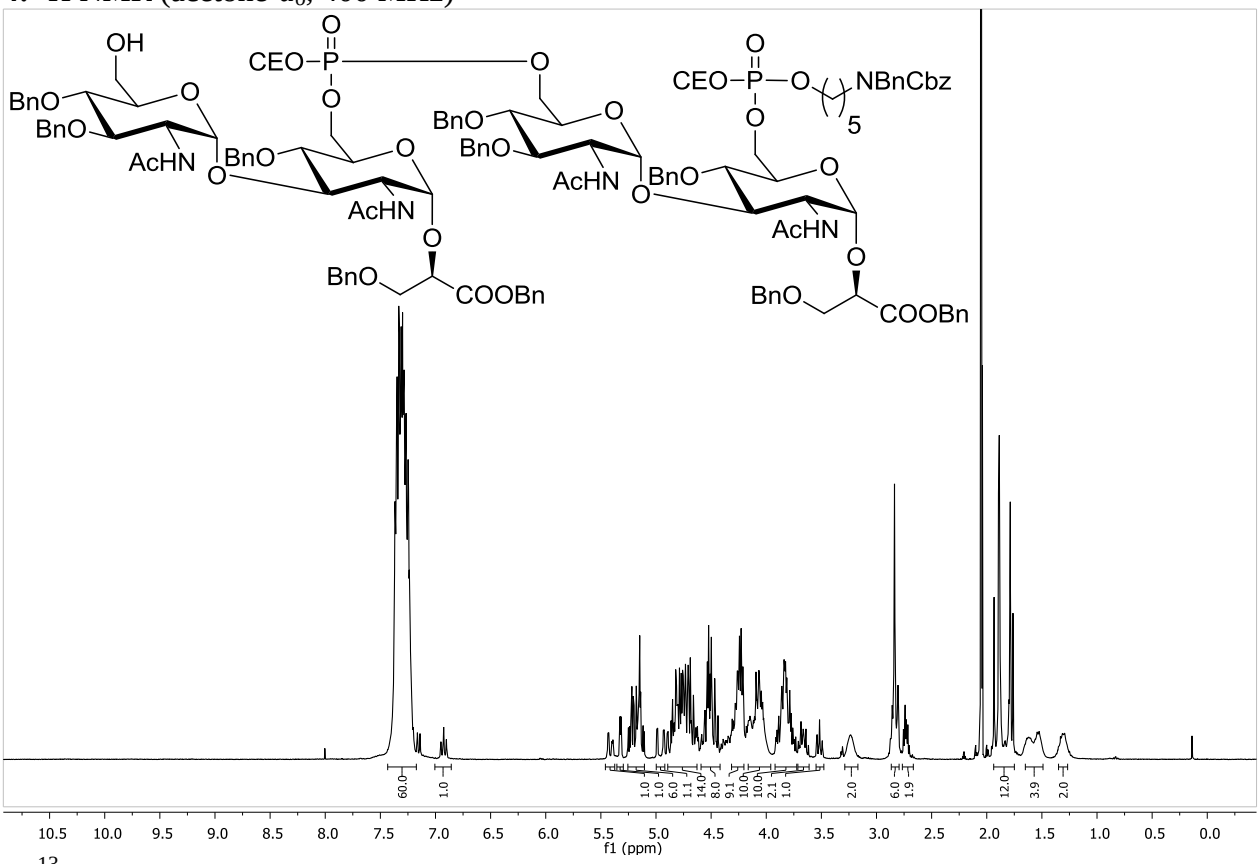
3: ^{13}C NMR (acetone-d_6, 100 MHz)



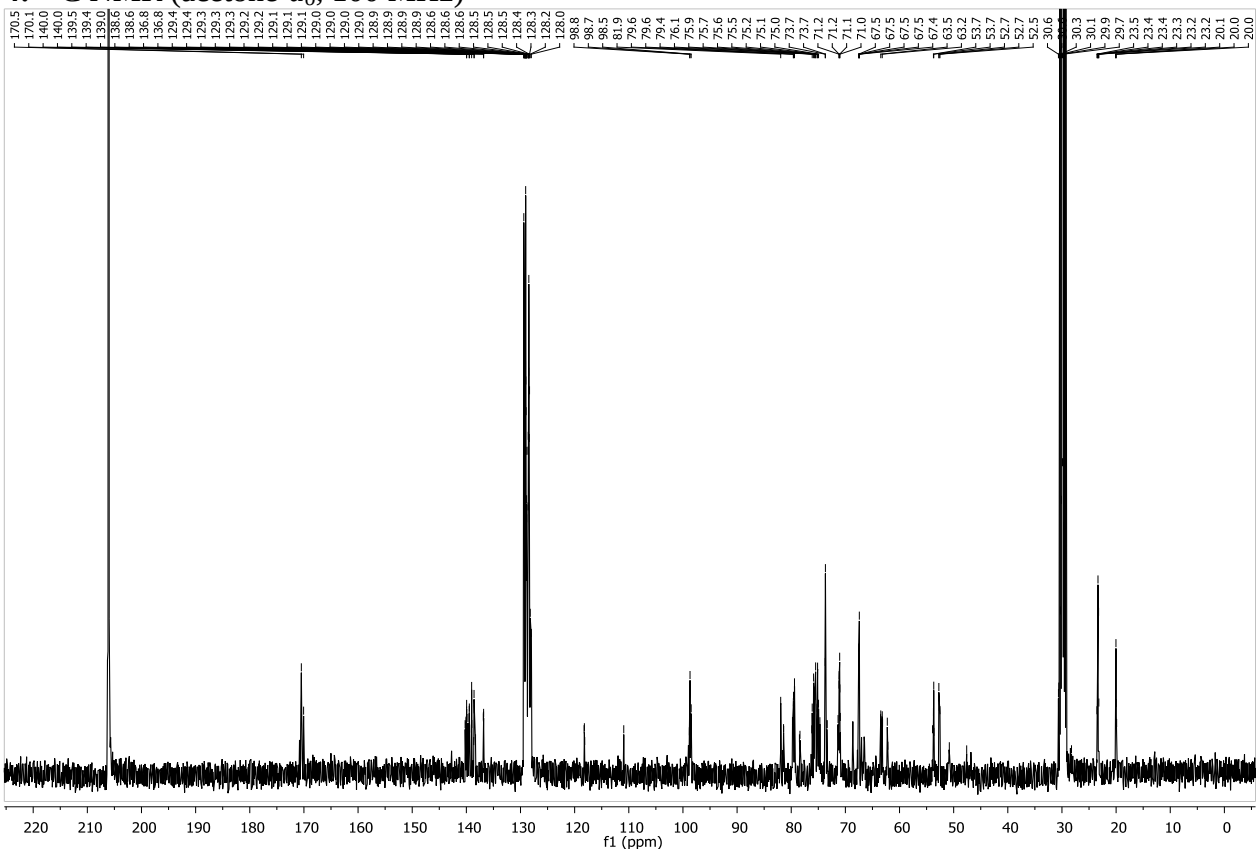
3: ^{31}P NMR (acetone-d_6, 162 MHz)



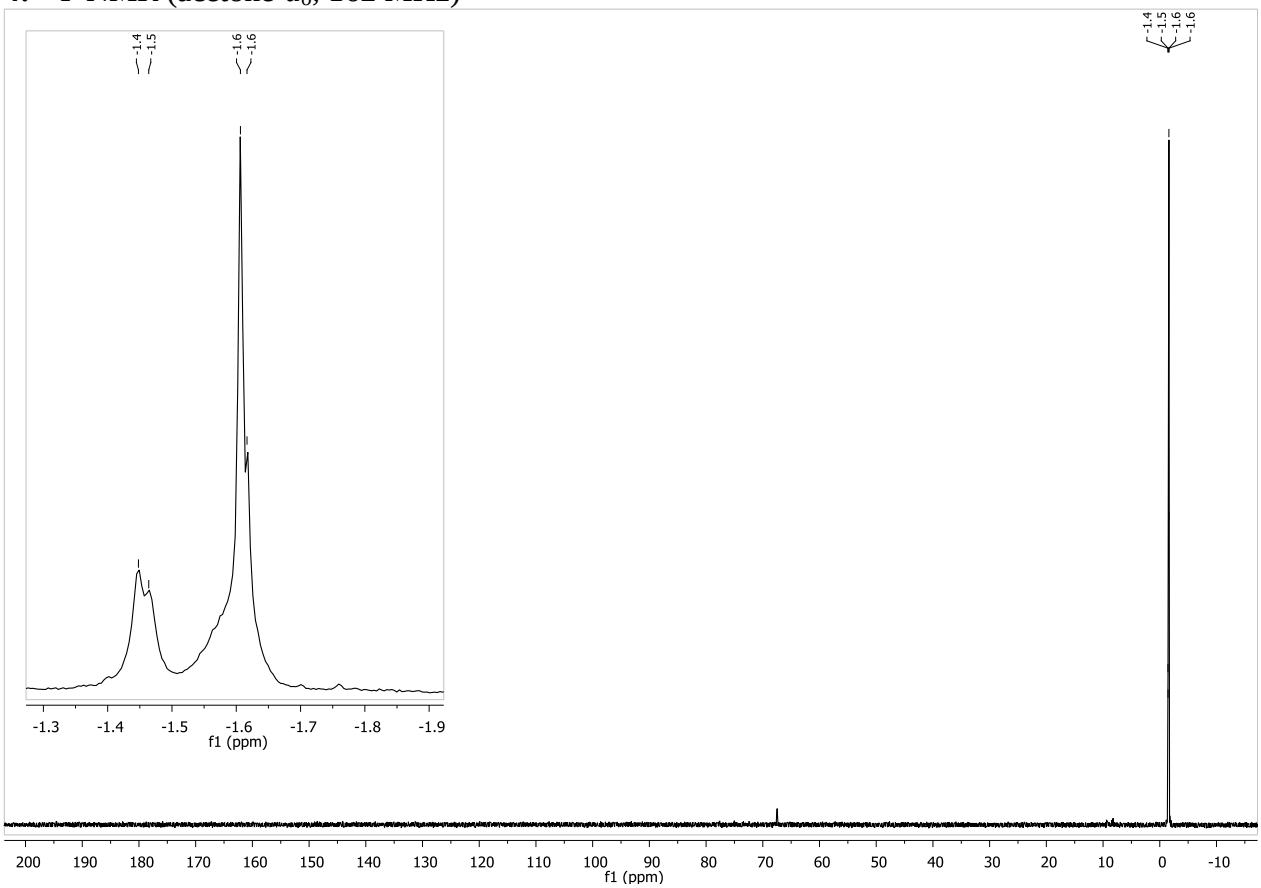
4: ^{1}H NMR (acetone-d_6, 400 MHz)



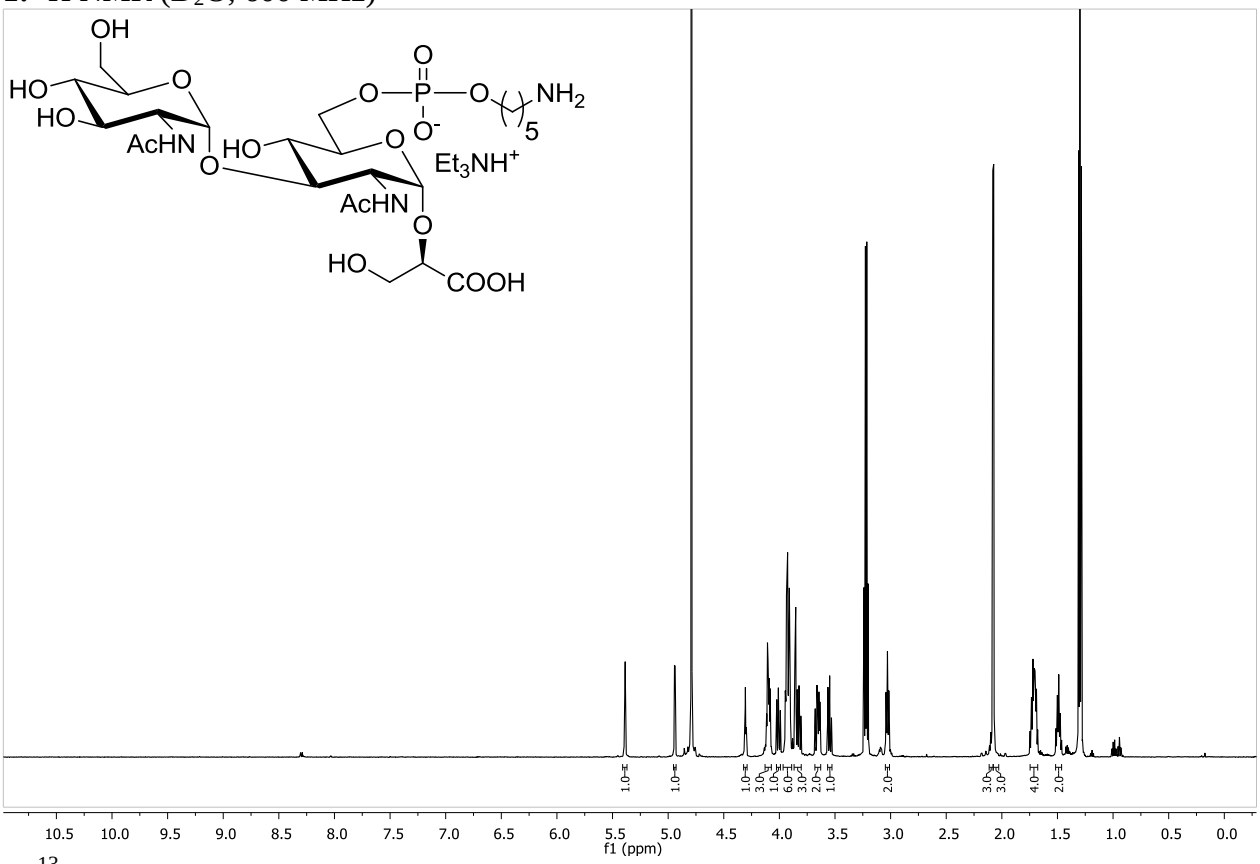
4: ^{13}C NMR (acetone-d_6, 100 MHz)



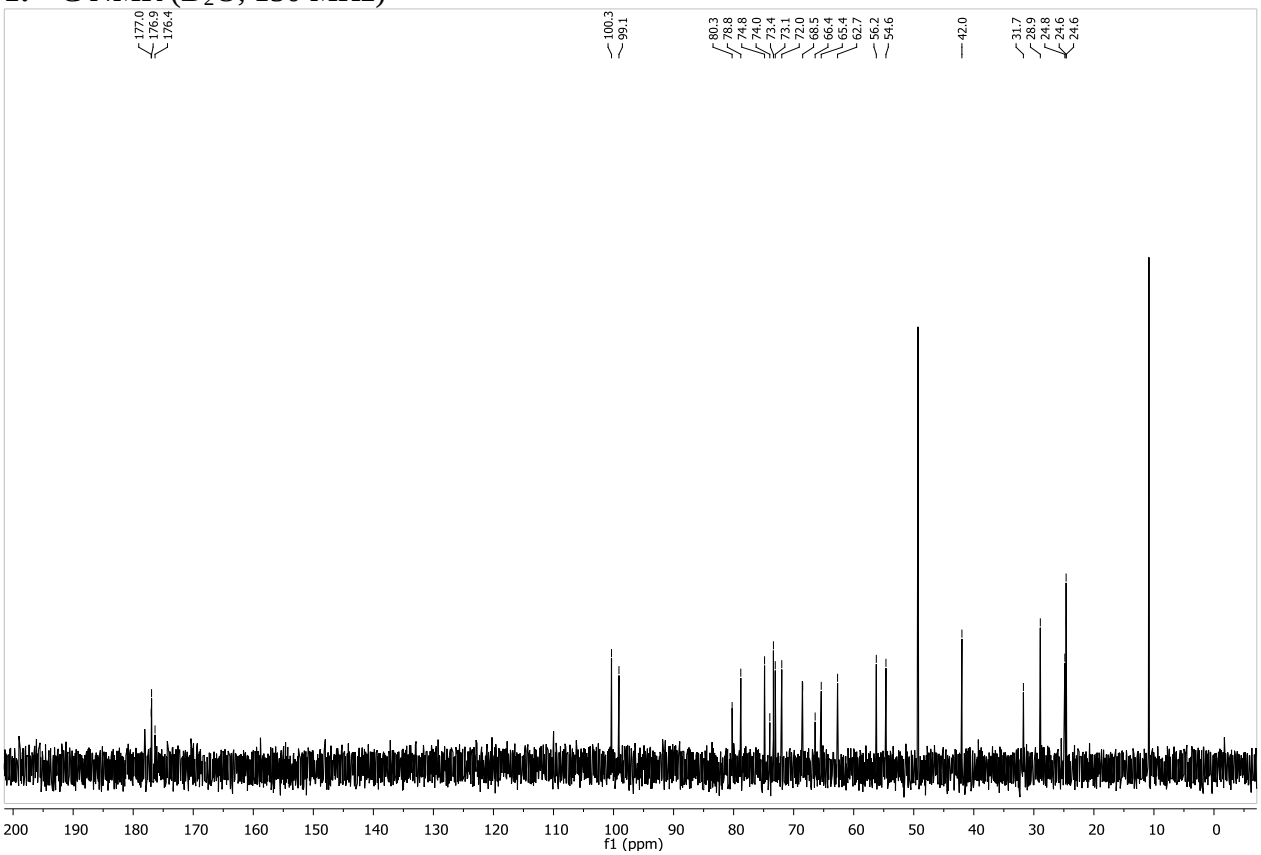
4: ^{31}P NMR (acetone-d_6, 162 MHz)



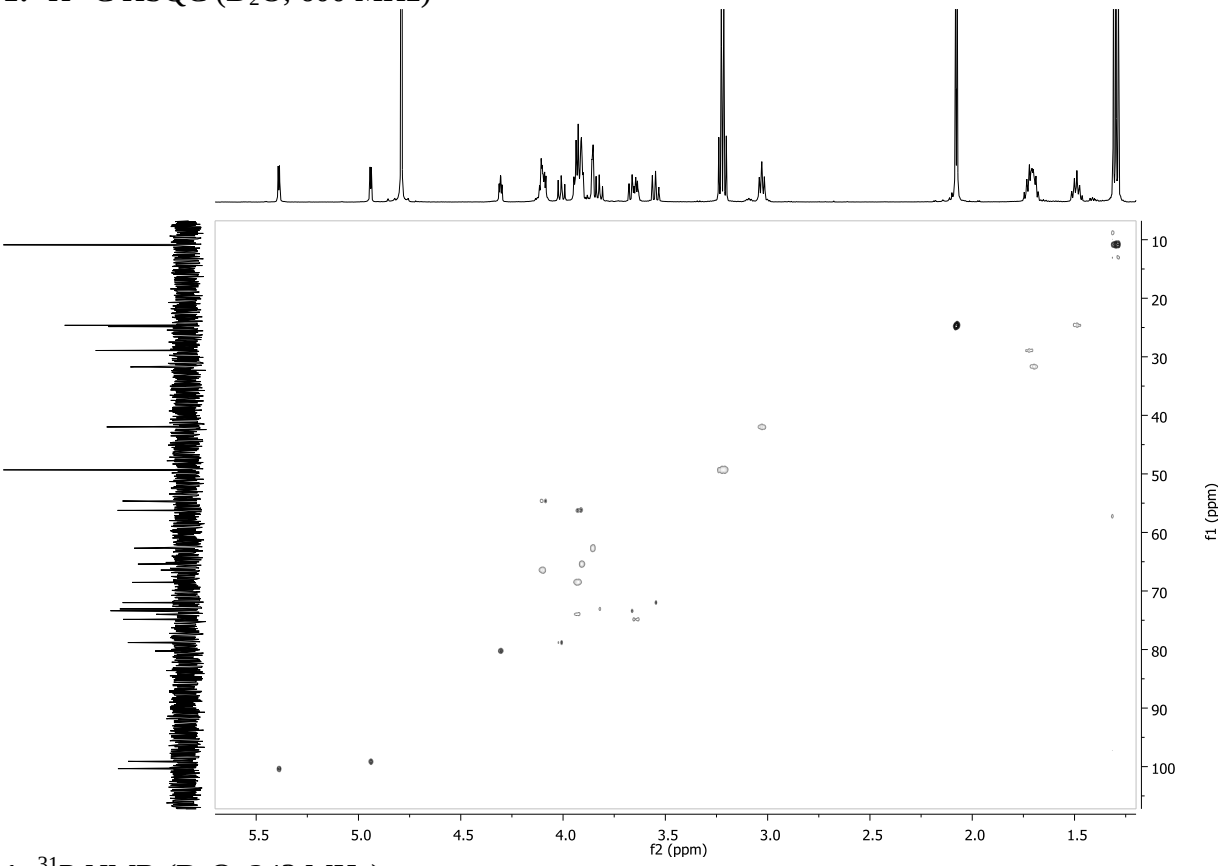
1: ^{1}H NMR (D_2O, 600 MHz)



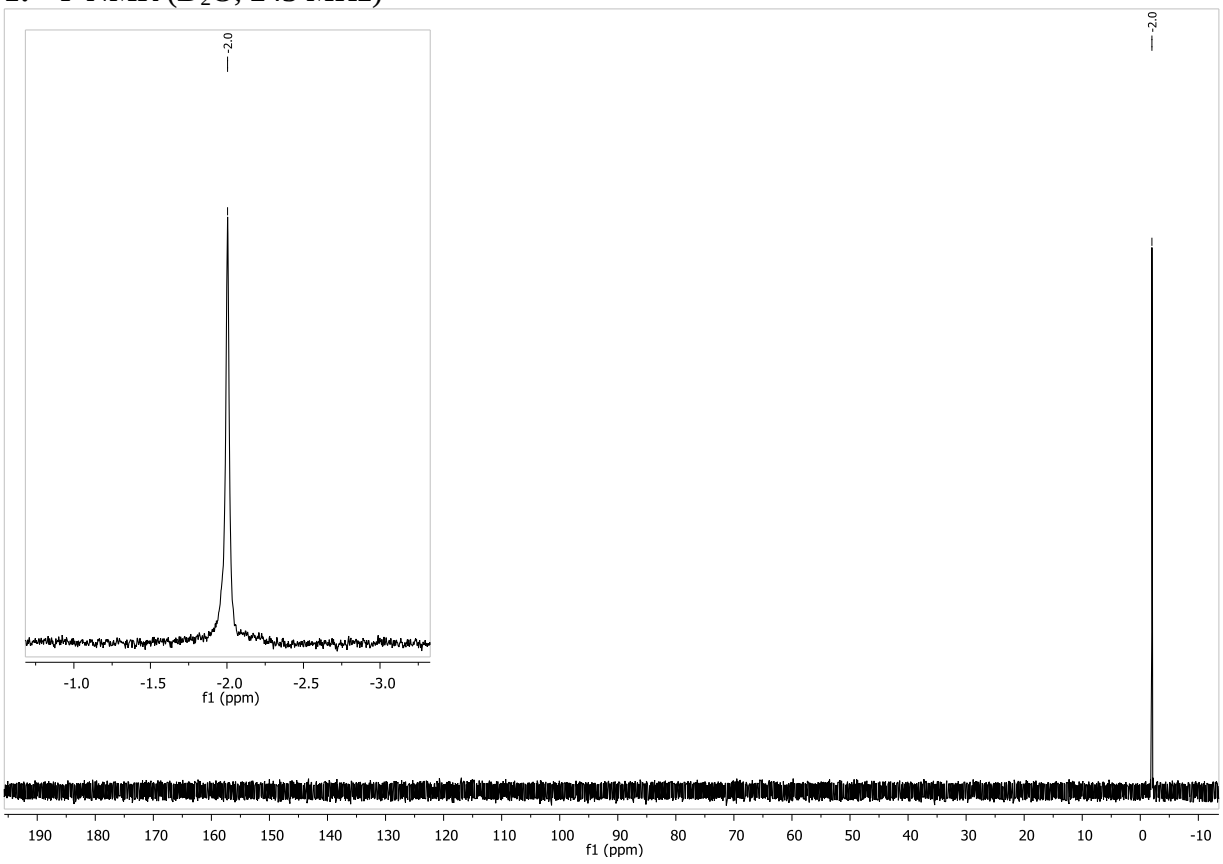
1: ^{13}C NMR (D_2O, 150 MHz)



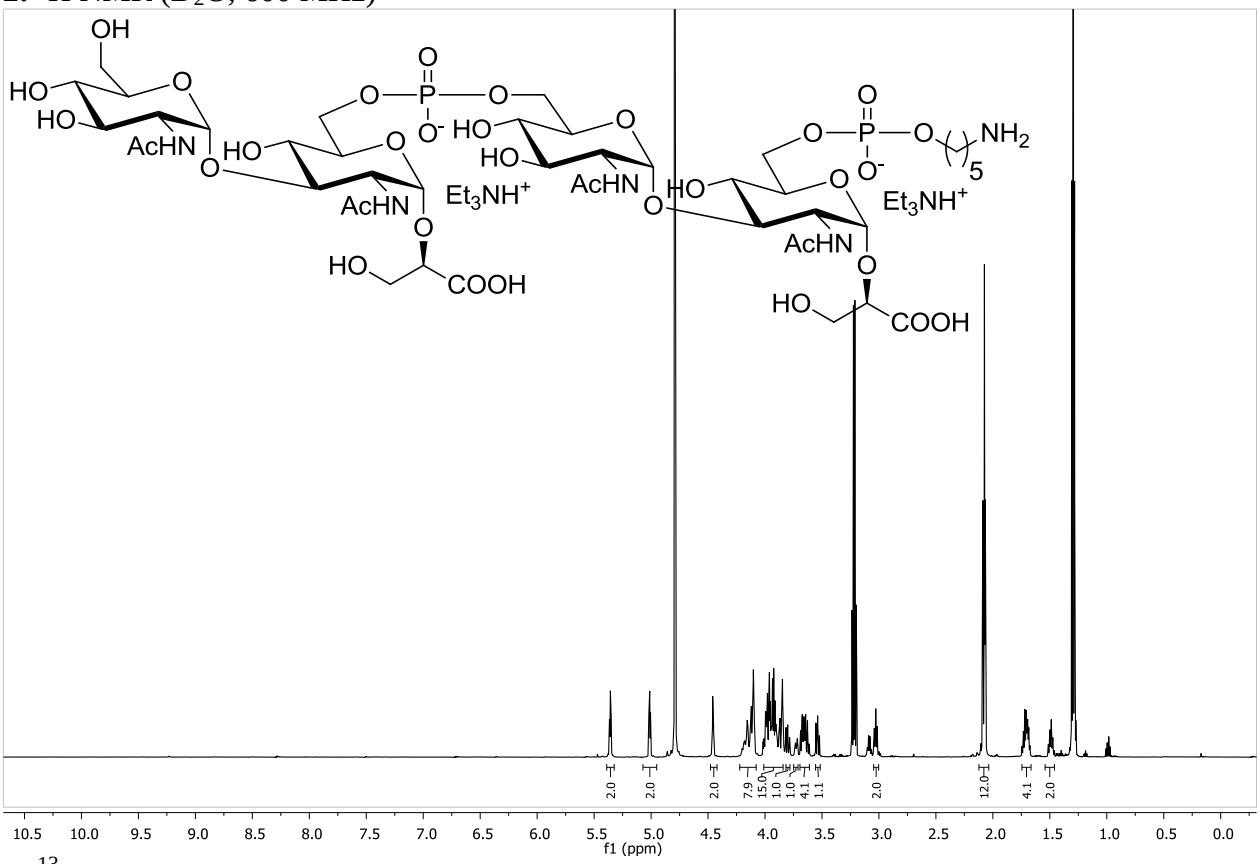
1: $^{1}H^{13}C$ HSQC (D_2O, 600 MHz)



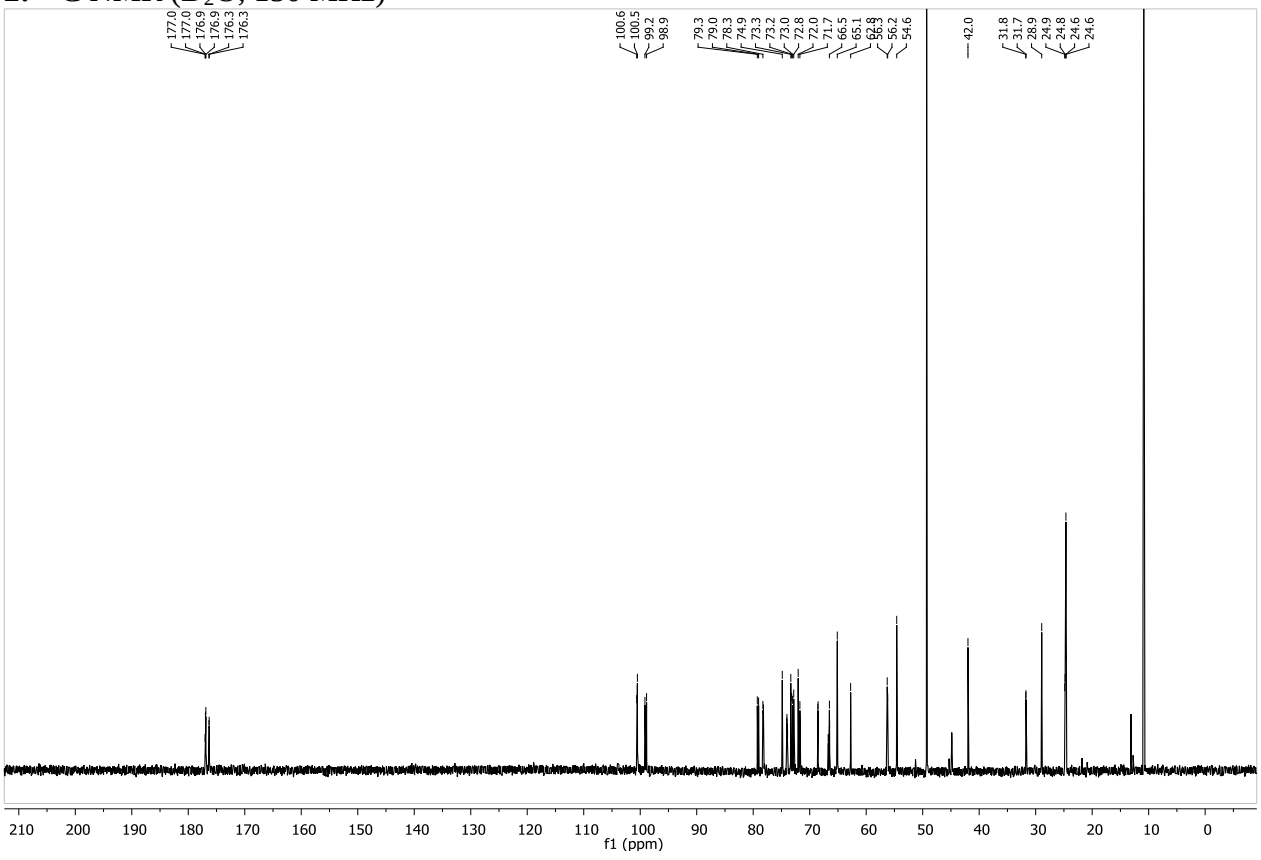
1: ^{31}P NMR (D_2O, 243 MHz)



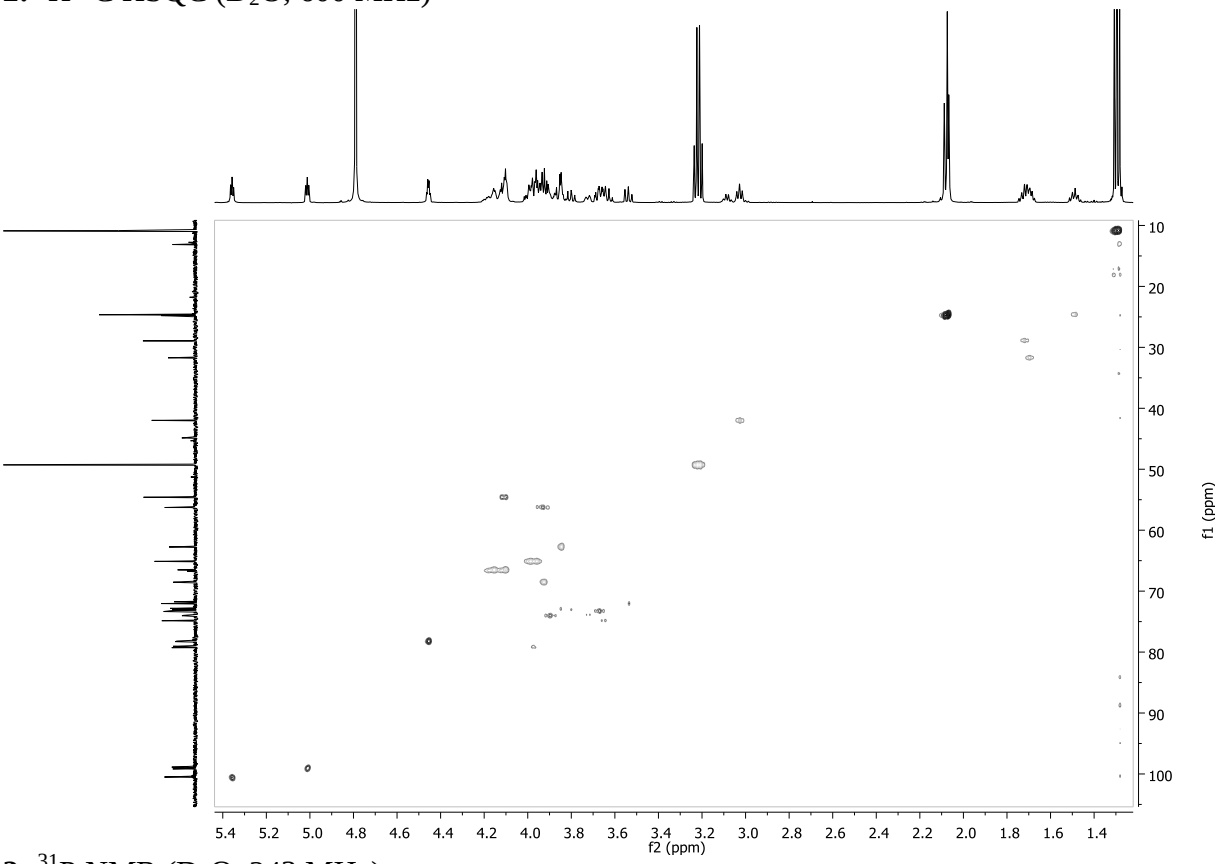
2: ^{1}H NMR (D_2O, 600 MHz)



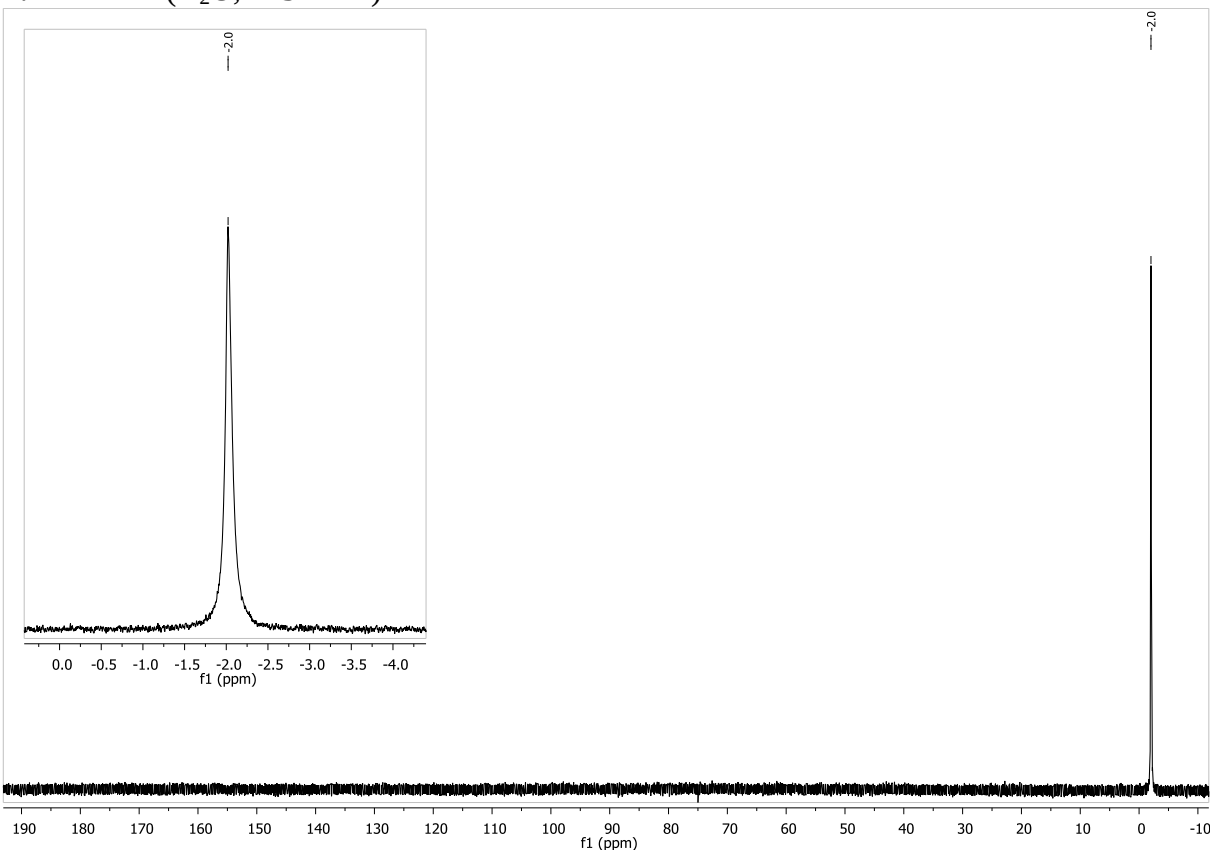
2: ^{13}C NMR (D_2O, 150 MHz)



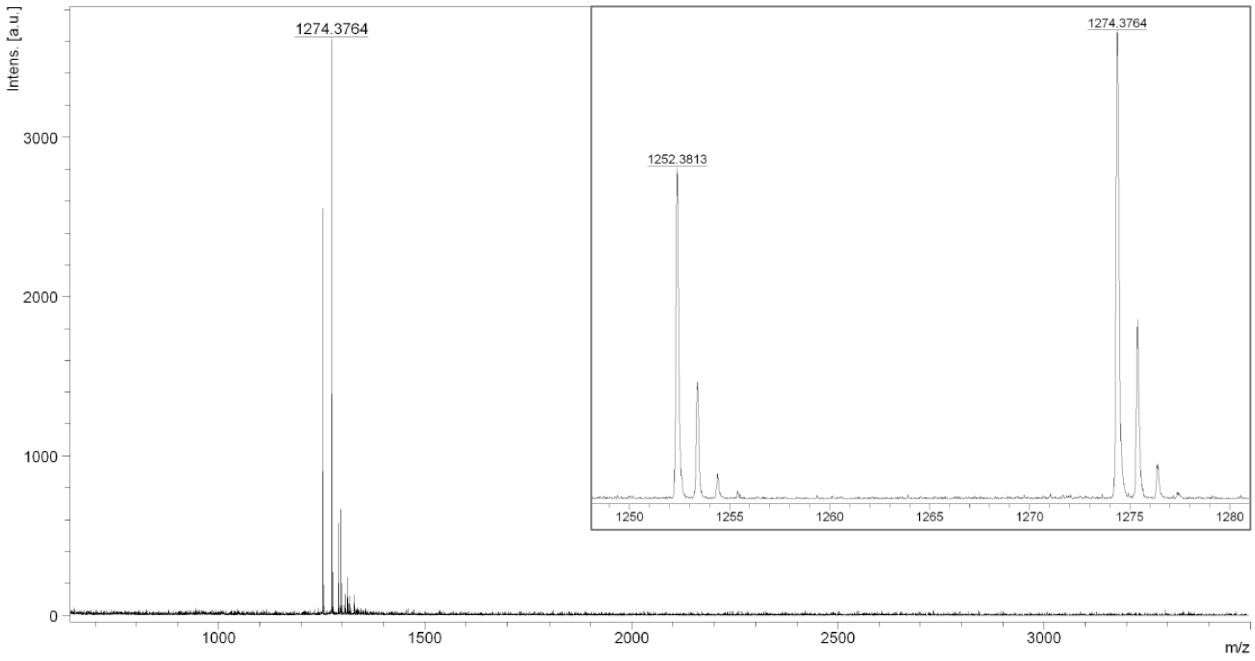
2: $^{1}H^{13}C$ HSQC (D_2O, 600 MHz)

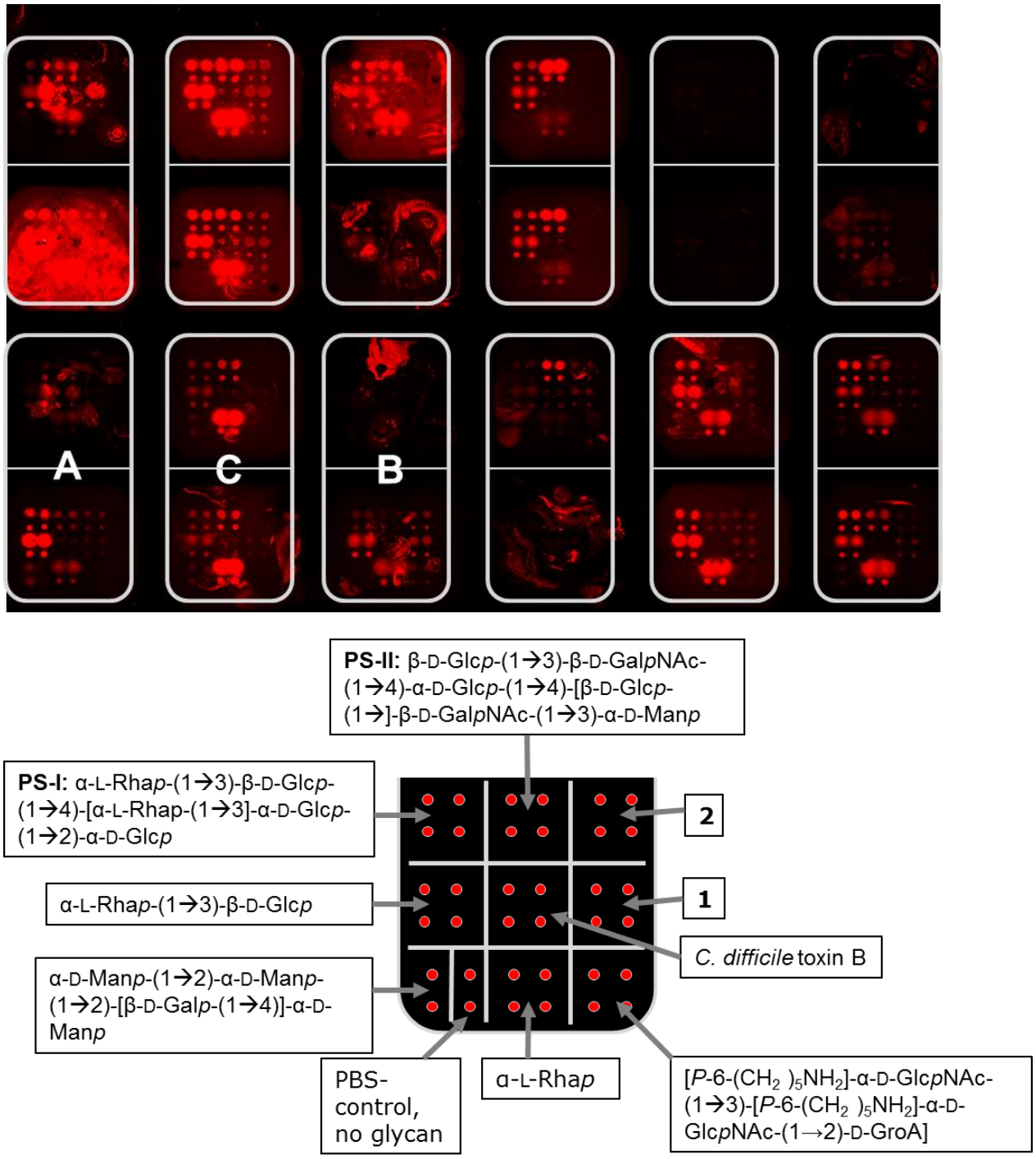


2: ^{31}P NMR (D_2O, 243 MHz)



2: MALDI-TOF MS





Supplementary Figure 1: Screening of human sera for IgG antibodies by glycan microarray. Twelve serum samples (diluted 1:100 in PBS) of CDI patients were screened in duplicate. Fluorescence excited at 635 nm is shown. Representative samples A, B and C are designated as such. The printing pattern is indicated below the scan. The presence of anti-Rhamnose IgG antibodies in human sera has been reported previously[5, 6]. α-L-Rha*p*-(1→3)-β-D-Glc*p* is a substructure of the PS-I repeating unit antigen. α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* is a *Leishmania* lipophosphoglycan capping tetrasaccharide (LT)[7, 8]. Oligosaccharides were printed at a concentration of 1 mM (upper two spots of each oligosaccharide) and 0.1 mM (lower two spots). LT was printed exclusively at 1 mM.

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