

Synthesis of Zwitterionic 1,1'-Glycosylphosphodiester, a Partial Structure of Galactosamine Modified *Francisella* Lipid A

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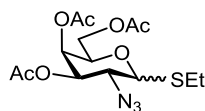
Experimental part: general synthetic methods.

Reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Dichloromethane was distilled from CaH_2 and stored over activated 4 Å molecular sieves (MS). THF was distilled over Na/benzophenone directly before use. Other solvents were dried by storage over activated MS for at least 48 h prior to use [toluene (4 Å), acetonitrile (3 Å) and DMF (3 Å)]. Residual moisture was determined by colorimetric titration on a Mitsubishi CA-21 Karl Fischer apparatus and did not exceed 20 ppm for dry solvents. Reactions were monitored by TLC performed on silica gel 60 F254 HPTLC pre-coated glass plates with a 25 mm concentration zone (Merck). Spots were visualized by UV light followed by dipping into a H_2SO_4 -*p*-anisaldehyde solution or a ninhydrin-EtOH solution and subsequent charring at 250°C. Solvents were removed under reduced pressure at < 40°C. Preparative MPLC was performed on silica gel 60 (230–400 mesh, Merck). Size exclusion chromatography was performed on BioRad Sephadex LH20 support. NMR spectra were recorded at 298 K on a Bruker Avance III 600 spectrometer (^1H at 600.22 MHz; ^{13}C at 150.92 MHz; ^{31}P at 242.97 MHz) or on Bruker DPX 400 spectrometer (^1H at 400.13 MHz; ^{13}C at 100.61 MHz; ^{31}P at 161.68 MHz) using standard Bruker NMR software. Chemical shifts are reported in ppm, ^1H -NMR spectra in CDCl_3 are referenced to internal TMS, ^{13}C -spectra are referenced to the corresponding solvent signal (77.00 ppm for CDCl_3). NMR spectra in other solvents are referenced to residual solvent signals (for MeOD: 3.31 ppm, 49.00 ppm, ^1H - and ^{13}C -NMR, for DMSO- d_6 : 3.31 ppm, 49.00 ppm, ^1H - and ^{13}C -NMR, respectively). ^{31}P -NMR Spectra in CDCl_3 are referenced to external triphenylphosphine, ^{31}P -Spectra in D_2O are referenced to external H_3PO_4 . For the trisaccharides the NMR signals of the distal GlcN moiety are indicated by primes, the signals of GalN moiety are indicated by double primes. HPLC-LRMS was performed by injections of 0.01-0.1% CH_3CN solutions into a Shimadzu LC-10AD VP system equipped with two gradient pumps, degasser, a Shimadzu LCMS 2020 detector and an AllTech 3300 ELSD detector. Analytes were eluted over a Phenomenex Jupiter 5 μ C4 300A column using linear gradients H_2O (0.1% HCOOH) \rightarrow CH_3CN (0.1% HCOOH). High resolution mass spectrometry (HRMS) was carried out from acetonitrile solutions (1–10 $\text{mg}\cdot\text{l}^{-1}$) on LC-TOF MS (Agilent 1200SL HPLC and Agilent 6210 ESI-TOF, Agilent Technologies). The mass spectrometer was tuned with Agilent tune mix to provide a mass accuracy below 2 ppm. The data were analyzed using Agilent Mass Hunter Software. MALDI-TOF was performed using a Bruker Autoflex Speed TOF-TOF instrument with 6-aza-2-thiothymine (ATT) as matrix. Optical rotation was measured on a Perkin Elmer 243 B polarimeter, equipped with a Haake water circulation bath and a Haake D1 immersion circulator for temperature control of the measuring cell. $[\alpha]_D^{20}$ Values are given in units of $\text{deg dm}^{-1}\text{cm}^3\text{g}^{-1}$.

Experimental part: synthetic procedures

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-galactopyranose was prepared from the corresponding galactal as reported,¹ **S1** was prepared from 1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-galactopyranose by reaction with ethylthiotrimethylsilane and catalytic amounts of trimethylsilyl trifluoromethanesulfonate²⁻⁴ followed by deacetylation to provide **1**.⁵

Synthesis of S1

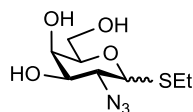


Ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-D-galactopyranoside (**S1**)

To a cooled (0°C) stirred solution of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-galactopyranose (3 g, 8.4 mmol, α/β 2/1) and (ethylthio)trimethylsilane (3.24 g, 24.11 mmol) in dry dichloroethane (50 ml) under atmosphere of Ar a solution of trimethylsilyl trifluoromethanesulfonate (357 μl , 1.61 mmol) in dry dichloroethane (2 ml) was added gradually over 10 min with a syringe. The reaction mixture was stirred for 3 h at r.t. and then for 3 days at 50°C. The

reaction mixture was diluted with CH_2Cl_2 (200 ml), washed with satd. aq. NaHCO_3 (100 ml) and brine (100 ml). The organic phase was dried over MgSO_4 , filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1) to give **S1** (2.1 g, 69%, $\alpha/\beta = 2/1$). NMR spectra were identical to the reported data.⁵

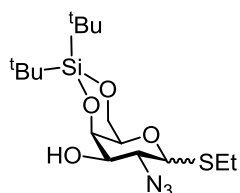
Synthesis of 1



Ethyl 2-azido-2-deoxy-1-thio-D-galactopyranoside (1)

To a stirred solution of ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-D-galactopyranoside (**S1**) (80 mg, 210 μmol) in dry methanol (6 ml) was added sodium methanoxide (0.1 N) until the pH = 10. After stirring at r.t. for 12 h (overnight) the pH was adjusted to 7 by adding Dowex W50 H^+ resin. The resin was removed by filtration, the filtrate was concentrated and dried. Yield: 53 mg (100%). NMR Spectra were identical with reported literature data.⁵

Synthesis of 2



Ethyl 2-azido-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-1-thio-D-galactopyranoside (2)

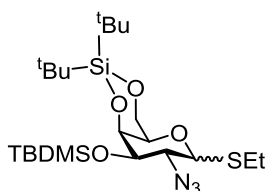
To a cooled (0°C) and stirred solution of **1** (1.338 g, 5.37 mmol) in dry pyridine (15 ml) di-*tert*-butylsilyl bis(trifluoromethanesulphonate) (1.36 ml, 7.51 mmol) was added *via* syringe under Ar. The reaction mixture was stirred for 5 min at 0°C , then gradually (10 min) warmed to r.t. and quenched with anhydrous MeOH (2 ml). The reaction mixture was diluted with toluene (50 ml) and concentrated to dryness. The residue was taken up in CH_2Cl_2 (150 ml), washed with aq. NaHCO_3 (150 ml), H_2O (50 ml) and brine (2×50 ml), dried over Na_2SO_4 and concentrated. The residue was purified by MPLC to furnish α -anomer **2a** (1.33 g), $R_f \alpha = 0.76$ (hexane/ethyl acetate, 5:1), $[\alpha]_D^{20} = +124$ (c 0.74, CHCl_3); and β -anomer **2b** (630 mg) as solids; combined yield 1.96 g (93.7%).

^1H NMR (CDCl_3 , 400 MHz, α -anomer): δ = 5.35 (d, $J_{1,2} = 5.5$ Hz, 1H, H-1), 4.37 (dd, $J_{3,4} = 3.4$ Hz, $J = 1$ Hz, 1H, H-4), 4.26 (dd, $J_{6a,6b} = 12.4$ Hz, $J_{6a,5} = 2.4$ Hz, 1H, H-6a), 4.08 (q, $J_{5,6b} = 1.8$ Hz, 1H, H-5), 4.06 (dd, 1H, H-6b), 3.92 (dd, $J_{2,3} = 10.3$ Hz, 1H, H-2), 3.72 (dd, 1H, H-3), 3.54 (s, 0.5 H, OH), 2.60 – 2.42 (bm, 2 H, SCH_2), 1.22 (t, $J = 7.4$ Hz, 3H, CH_3), 1.00, 0.99 (2 s, 18H, $4 \times \text{CH}_3$, DTBS);

^{13}C NMR (CDCl_3 , 101 MHz): δ = 83.59 (C-1), 72.56 (C-4), 70.76 (C-3), 67.51 (C-5), 66.92 (C-6), 61.26 (C-2), 27.65, 27.52, 27.37, 27.26, (DTBS), 24.68 (SCH_2), 14.71 (CH_3);

HRMS (ESI-TOF): calcd for $\text{C}_{17}\text{H}_{32}\text{N}_3\text{O}_6\text{SSi}$ m/z $[\text{M} + \text{COOH}]^-$ 434.1787; found: 434.1773

Synthesis of 3



Ethyl 2-azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-1-thio-D-galactopyranoside (**3**)

To a stirred solution of **2** (1.274 g, 3.27 mmol) in dry DMF (7 mL) *tert*-butyldimethylsilyl chloride (1.232g, 8.18 mmol) and imidazole (557 mg, 8.18 mmol) were added under Ar. The reaction mixture was stirred for 10 h at 50°C, cooled to r.t. and diluted with toluene (100 mL). The mixture was concentrated, the residue was dissolved in toluene (150 mL) and the solution was washed with water (50 mL), NaHCO₃ (50 mL) and brine (50 mL), dried over Na₂SO₄ and concentrated. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 5:1) to afford α -anomer of **3a** (1.1 g, 66.3%) as a syrup and β -anomer of **3b** (450 mg, 27.7%) as amorphous solid. Overall yield 1.531 g, 94%; α/β = 2.4/1.

3: α -anomer: R_f = 0.77 (hexane/ethyl acetate, 5:1); $[\alpha]_D^{20}$ = +6.9 (c 0.94, CHCl₃); ¹H NMR (CDCl₃, 400 MHz): δ = 4.36 (d, $J_{1,2}$ = 5.5 Hz, 1H, H-1), 4.29 (dd, $J_{3,4}$ = 3 Hz, $J_{4,5}$ = 1 Hz, 1H, H-4), 4.26 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.3 Hz, 1H, H-6a), 4.13 (dd, $J_{2,3}$ = 10 Hz, 1H, H-2), 4.08 (dd, $J_{6b,5}$ = 1.7 Hz, 1H, H-6b), 3.74 (dd, 1H, H-3), 4.00 (ddd, 1H, H-5), 2.57 (m, 2H, SCH₂), 1.27 (t, J = 7.4 Hz, 3H, CH₃), 1.04, 1.03, 0.93, (3 s, 27H, DTBS, TBDMS), 0.16, 0.11 (2 s, 6H, TBDMS).

¹³C NMR (CDCl₃, 101 MHz): δ = 84.03 (C-1), 73.74 (C-4), 72.24 (C-3), 68.19 (C-5), 67.44 (C-6), 61.28 (C-2), 27.65, 27.54, 25.86 (DTBS, TBDMS), 24.75 (SCH₂), 14.92 (CH₃), -2.80, -4.23, -4.79 (CH₃, TBDMS)

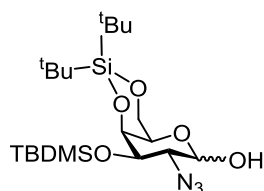
3: β -anomer: R_f = 0.81 (hexane/ethyl acetate, 7:1); $[\alpha]_D^{20}$ = +138.6 (c 1.7, CHCl₃);

¹H NMR (CDCl₃, 300 MHz): δ = 4.27 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 0.9 Hz, 1H, H-4), 4.23 (d, $J_{1,2}$ = 10.2 Hz, 1H, H-1), 4.21 (m, 2H, H-6a, H-6b), 3.61 (t, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 3.44 (dd, 1H, H-3), 4.00 (ddd, 1H, H-5), 2.74 (m, 2H, SCH₂CH₃), 1.29 (t, J_{CH_2,CH_3} = 7.4 Hz, 3H, CH₃), 1.07, 1.03, 0.94, (3 s, 27H, DTBS, TBDMS), 0.16, 0.12 (2 s, 6H, TBDMS).

¹³C NMR (CDCl₃, 75 MHz): δ = 84.37 (C-1), 75.82 (C-3), 75.26 (C-5), 72.71 (C-4), 67.35 (C-6), 64.01 (C-2), 27.55, 27.48, 25.63 (DTBS, TBDMS), 25.14 (SCH₂CH₃), 14.98 (SCH₂CH₃), -2.80, -4.23, -4.79 (CH₃, TBDMS);

HRMS (⁺ESI-TOF): for C₂₂H₄₅N₃NaO₄SSi₂ m/z [M+Na]⁺: 526.2562, found: 526.2567.

Synthesis of 4



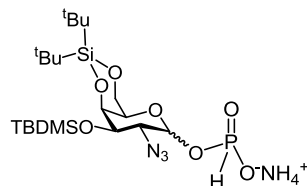
2-Azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-D-galactopyranose (**4**)

To a cooled (0°C) stirred solution of **3** (740 mg, 1.47 mmol) in acetone/water 10:1 (11 mL) aq satd. NaHCO₃ (1 mL) and *N*-bromosuccinimide (1.31 g, 7.35 mmol) were added successively. The reaction mixture was gradually (10 min) warmed up to r.t. and stirred for 20 min. The reaction mixture was diluted with ethylacetate (150 mL), washed with aq. Na₂S₂O₃ (50 mL), aq. NaHCO₃ (2×50 mL) and brine (50 mL), dried over Na₂SO₄ and concentrated. The residue was purified by MPLC (hexane/ethylacetate, 5:1 → 3:1) to give **4** (α/β = 2:1, 571 mg, 79%); R_f (α) = 0.51, R_f (β) = 0.37 (hexane/ethylacetate, 5:1), $[\alpha]_D^{20}$ (α/β = 2:1) = +56 (c 0.95, CHCl₃);

¹H NMR (CDCl₃, 400 MHz): δ = 5.35 (d, $J_{1\alpha,2\alpha}$ = 3.5 Hz, 1H, H-1 α), 4.51 (d, $J_{1\beta,2\beta}$ = 8 Hz, 0.4H, H-1 β), 4.34 (dd, $J_{3\alpha,4\alpha}$ = 3.5 Hz, $J_{4\alpha,5\alpha}$ = 0.8 Hz, 1H, H-4 α), 4.28 (d, 0.4H, H-6 β a), 4.26 (dd, $J_{6\alpha a,6\alpha b}$ = 12.5 Hz, $J_{5,6\alpha a}$ = 2.0 Hz, 1H, H-6 α a), 4.23 (m, 1.2H, H-4 β , H-6 β a, H-6 β b), 4.21 (dd, $J_{6\beta a,6\beta b}$ = 11.5 Hz, $J_{5\beta,6\beta b}$ = 1.9 Hz, 0.3H, H-6 β b), 4.13 (dd, $J_{5\alpha,6\alpha b}$ = 1.7 Hz, 0.7H, H-6 α b), 4.01 (dd, $J_{2\alpha,3\alpha}$ = 10.1 Hz, 1H, H-3 α), 3.9 (bs, 1H, H-5 α), 3.76 (dd, 1H, H-2 α), 3.56 (dd, $J_{2\beta,3\beta}$ = 9.8 Hz, 0.4H, H-2 β), 3.44 (dd, $J_{2\beta,3\beta}$ = 9.9 Hz, J = 3.3 Hz, 0.4H, H-3 β), 3.36 (ddd, $J_{5\beta,6\beta a}$ = 2.9 Hz, 0.4H, H-5 β), 1.05 (s, 9H, 3×CH₃, DTBS- α), 1.04 88 (s, 9H, 3×CH₃, DTBS- α), 0.96 (s, 9H, 3×CH₃, TBDMS- α), 0.19, 0.14 (2 s, 6H, 2×CH₃, TBDMS- α);

^{13}C NMR (CDCl_3 , 100MHz): δ = 96.3 (C-1 β), 92.62 (C-1 α), 74.1 (C-3 β), 73.8 (C-4 α), 72.64 (C-4 β), 71.77 (C-5 β), 70.18 (C-3 α), 67.72 (C-5 α), 67.28 (C-6 α), 67.12 (C-6 β), 65.62 (C-2 β), 61.37 (C-2 α), 27.47, 27.33, 25.65, 23.44, 20.75, 17.97 57 (CH_3 , DTBS α/β), -4.29, -4.53, -4.84, -4.96 (CH_3 , TBDMS α/β);
HRMS (ESI-TOF): calcd. for $\text{C}_{21}\text{H}_{41}\text{N}_3\text{O}_7\text{Si}$ m/z $[\text{M}+\text{HCOO}]^-$ 504.2567, found: 504.2565.

Synthesis of 5



2-Azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy- α -D-galactopyranosyl-1-hydrogenphosphonate and 2-azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy- β -D-galactopyranosyl-1-hydrogenphosphonate (ammonium salt) (5).

To a stirred solution of **4** (α/β = 2:1, 421 mg, 915 μmol) in CH_3Cl (20 mL) a solution of Et_3N and formic acid in chloroform (0.1 M, pH 5, 1 ml) was added, the pH value was adjusted to 5. After 12 h the ammonium formate – formic acid buffer was removed by co-evaporation with toluene (2×20 ml) and the reaction mixture was dried by repeated co-evaporation with dry toluene (4×20 ml). To a stirred solution of **4** (421 mg, 915 μmol) in dry CH_2Cl_2 (10 mL) dry pyridine (200 μl , 2.5 mmol) and a solution of 2-chloro-1,3,2-benzodioxaphosphorin-4-one (salicylchlorophosphite, SalPCl) (463 mg, 2.3 mmol) in dry CH_2Cl_2 (3 ml) were added successively under atmosphere of Ar. Reaction mixture was stirred for 5 h. at r.t., then aq Et_3N (250 μl , pH 10) was added and the mixture was stirred for additional 15 min, diluted with CHCl_3 (200 ml) and washed with 0.2 M TEAB (triethylammonium bicarbonate) buffer (3×50 ml). The combined aqueous phases were washed with CHCl_3 (3×50 ml). The combined organic phases were dried by passing through a pad of cotton and concentrated. The residue was purified by repeated MPLC on silica gel ($\text{CHCl}_3/\text{MeOH}/25\%$ aq. NH_4OH , 6/1/0.07) to give **5** (390 mg, 78%, α/β = 5:1) as a solid. R_f = 0.13 ($\text{CHCl}_3/\text{MeOH}/25\%$ aq. NH_4OH , 6/1/0.07), $[\alpha]_D^{20}$ = +75 (c 0.72, CHCl_3);

^1H NMR ($\text{CDCl}_3:\text{CD}_3\text{OD}$, 4:1, 400 MHz): δ = 6.91 (d, $^1J_{\text{P,H}}$ = 653 Hz, 1 H, P-H β), 6.84 (d, $^1J_{\text{P,H}}$ = 646 Hz, 1 H, P-H α), 5.60 (dd, $J_{1,2}$ = 3.4 Hz, $J_{1,\text{P}}$ = 8.2 Hz, 1H, H-1- α), 4.78 (dd, $J_{1,2}$ = 8.3 Hz, $^3J_{\text{P,1}}$ = 8.3 Hz, 1H, H-1- β), 4.30 (d, $J_{3,4} = J_{4,5}$ = 2.7 Hz, 1 H, H-4- α), 4.18 (dd, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 2.0 Hz, 1 H, H-6a- α), 4.16 (m, 1H, H-6a- β), 4.12 (m, 1H, H-6b- β), 4.06 (dd, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6b}$ = 1.8 Hz, 1H, H-6b- α), 3.97 (dd, $J_{2,3}$ = 10 Hz, 1H, H-3- α), 3.88 (bs, 1H, H-5- α), 3.69 (ddd, $J_{2,\text{P}}$ = 1.7 Hz, 1H, H-2), 3.58 (dd, $J_{2,3}$ = 9.8 Hz, 1H, H-2- β), 3.42 (dd, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H, H-3- β), 3.38 (bs, 1H, H-5- β), 2.30 (NH_4^+), 0.99 (s, 9H, $3 \times \text{CH}_3$, DTBS- α), 0.88 (s, 9H, $3 \times \text{CH}_3$, DTBS- α), 0.89 (s, 9H, $3 \times \text{CH}_3$, TBDMS- α), 0.11, 0.08 (2 s, 6H, $2 \times \text{CH}_3$, TBDMS);

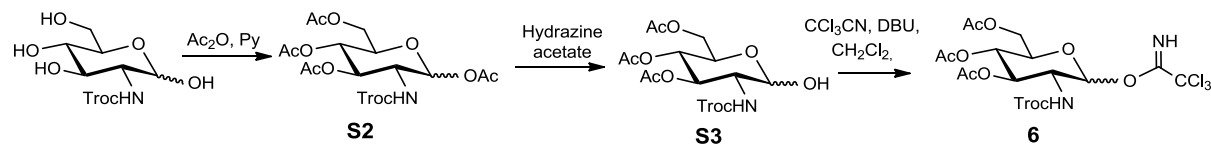
^{13}C NMR ($\text{CDCl}_3:\text{CD}_3\text{OD}$, 4:1, 101 MHz): δ = 96.23 (d, $J_{\text{C,P}}$ = 5.8 Hz, C-1- β), 93.92 (d, $J_{\text{C,P}}$ = 5.8 Hz, C-1- α), 73.82 (C-3- β), 73.51 (C-4- α), 72.48 (C-4- β), 71.66 (C-5- β), 70.08 (C-3- α), 68.62 (C-5- α), 66.79 (C-6- α), 64.53 (d, $J_{\text{C,P}}$ = 7.0 Hz, C-2- β), 60.72 (d, $J_{\text{C,P}}$ = 7.0 Hz, C-2- α), 27.82, 27.36, 27.25, 27.12, 25.49, 25.34, 23.26, 20.57 (CH_3 , DTBS α/β), -4.45, -4.72, -5.06, -5.17 (CH_3 , TBDMS α/β);

^{31}P NMR ($\text{CDCl}_3:\text{CD}_3\text{OD}$, 4:1, 162 MHz): δ = 2.99 (α , J_{PH} = 646 Hz), δ = 1.45, (β , J_{PH} = 653 Hz);

HRMS (ESI-TOF): calcd. for $\text{C}_{20}\text{H}_{41}\text{N}_3\text{O}_7\text{PSi}_2$ m/z $[\text{M}-\text{H}]^-$ 522.2226, found: 522.2219.

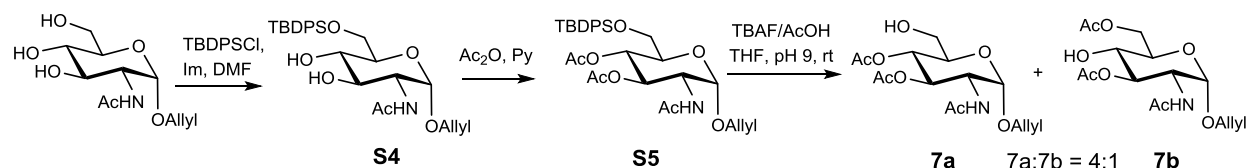
Synthesis of 6

S2 was obtained by peracetylation of 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose,⁶ followed by anomeric deprotection with hydrazine acetate to afford the known compound **S3**⁷ and reaction with trichloroacetonitrile/DBU which afforded the known trichloroacetimidate **6**.⁸



Synthesis of 7

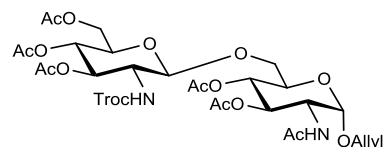
According to the reported procedure, allyl 2-acetamido-2-deoxy- α -D-glucopyranoside was treated with TBDPSCI/Im in DMF to provide **S4**, which was peracetylated to give **S5**, successive removal of the primary silyl group provided compounds **7a**⁹ and **7b**. Since the 4-*O*-acetyl group in 6-*O*-unprotected glucopyranoses such as **7a** is prone to (4 \rightarrow 6) acyl migration^{10,11} to furnish **7b** under basic conditions¹² (TBAF, **7a**:**7b**, 2:1), the reaction mixture **S5** \rightarrow **7a** was buffered with acetic acid (final pH = 9) which resulted in the substantial improvement of the **7a**:**7b** ratio (5:1).¹³ Generally, the rate of the (4 \rightarrow 6) acetyl migration in glucopyranoses depends on the pH value and on the duration of the basic (or acidic) treatment.^{13,14} Upon rapid isolation (flash chromatography on silica gel), the proportion of **7b** slightly increased (**7a**:**7b**, 4:1). For the synthesis of the disaccharide **8** a mixture of **7a** and **7b** (4:1) was successfully applied. Diminished reactivity of the migration product **7b** compared with the much higher reactivity of the acceptor **7a**, possessing free primary 6-OH group, under applied glycosylation conditions allowed for efficient glycosylation and uncomplicated isolation of the target (1 \rightarrow 6) disaccharide **8** in 63% yield.



7a + 7b

To a cooled (4°C) and stirred solution of allyl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-(tert-butyldiphenylsilyl)-2-deoxy- α -D-glucopyranoside (**S5**) (1.06 g, 1.8 mmol) in THF (20 ml) was added acetic acid until the pH reached 3.5. Then 1M *tetra*-butylammonium fluoride in THF (TBAF; 4 ml) was added dropwise over 5 min (the resulting pH = 9). The reaction was stirred at 4°C for 15 min and then warmed up to r.t. and stirred for another 90 min. Another portion 1M TBAF in THF was added (1 ml). The reaction mixture was stirred for 4h, then transferred into the mixture of water/brine (40 ml/40 ml) and washed with ethyl acetate (2x 40 ml). The combined organic phases were dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography on silica gel (ethyl acetate/hexane 3/1). Yield: 404 mg (67.6%). NMR spectra of the products were identical with the reported literature data.⁹ Due to the known propensity of 4-*O*-acetyl group to undergo (4 \rightarrow 6) acyl migration upon silica gel chromatography and storage, a 4:1 mixture of 6-OH (**7a**) and 4-OH (**7b**) products was used for the ensuing glycosylation.

Synthesis of 8



Allyl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranoside (8**).**

A solution of **6** (3.71 g, 6 mmol) and **7a**+**7b** (4:1, 1.6 g, 4.6 mmol) in dry CH₂Cl₂ (20 ml) was stirred with mol. sieves 4Å under Ar for 1. The mixture was cooled to -75°C and a solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 84 μ l, 510 μ mol) in dry CH₂Cl₂ (3 ml) was added dropwise under atmosphere of Ar. The reaction mixture was stirred for 1 h at -75°C, quenched by addition of Na₂CO₃ (100 mg) and gradually warmed up to r.t. The mixture

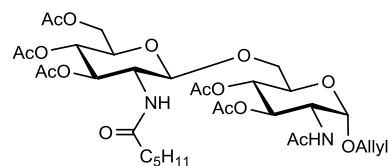
was diluted with CH₂Cl₂ (150 ml), washed with aq. NaHCO₃ (2×50 ml) and water (2×50 ml), dried over Na₂SO₄, and concentrated. The residue was purified by MPLC (hexane/ethyl acetate, 1:2) to furnish **8** (2.33 g, 63%) as a solid. *R*_f = 0.21 (hexane/ethyl acetate, 1:2), [α]_D²⁰ = +43.0 (*c* 1.0, CHCl₃);

¹H NMR (CDCl₃, 400 MHz): δ = 5.90 – 5.85 (m, 1 H, CH=, Allyl), 5.65 (d, *J* = 9.6 Hz, 0.9H, NH), 5.5 (d, *J* = 8.4 Hz, 0.8 H, N'H), 5.31 (dq, *J* = 17.2 Hz, *J* = 1.5 Hz, 1 H, CH₂-*trans*, Allyl), 5.25 (dq, *J* = 10.5 Hz, *J* = 1.3 Hz, 1 H, CH₂-*cis*, Allyl), 5.27 – 5.21 (m, 2 H, 2H, H-3, H-3'), 5.08 (dd, *J*_{3',4'} = *J*_{4',5'} = 10.1 Hz, 1 Hz, 1 H, H-4'), 5.06 (dd, *J*_{3,4} = *J*_{4,5} = 10.2 Hz, 1 H, H-4), 4.85 (d, *J*_{1,2} = 3.6 Hz, 1 H, H-1), 4.82 (bd, *J* = 12.8 Hz, 1H, CH₂ Troc), 4.67 (bd, *J* = 12.8 Hz, 1H, CH₂ Troc), 4.51 (d, *J*_{1',2'} = 8.5 Hz, 1 H, H-1'), 4.28 (dd, *J*_{2,3} = 9.6 Hz, 1H, H-2), 4.26 (dd, *J*_{6'a,6'b} = 12.3 Hz, *J*_{5',6'a} = 4.7 Hz, 1H, H-6'a), 4.18 (m, 1 H, OCH₂, Allyl), 4.14 (dd, *J*_{5',6'b} = 2.3 Hz, 1 H, H-6'b), 4.02 (dd, *J*_{6a,6b} = 11.2 Hz, *J*_{5,6a} = 2.1 Hz, 1H, H-6a), 3.97 (qt, *J* = 12.5 Hz, *J* = 6.3 Hz, *J* = 1.3 Hz, 1 H, OCH₂, Allyl), 3.89 (ddd, *J*_{5,6b} = 4.8 Hz, 1 H, H-5), 3.76 (m, 1H, H-2'), 3.68 (ddd, 1 H, H-5'), 3.44 (dd, 1 H, H-6b), 2.09, 2.05, 2.04, 2.02, 2.01, 1.94 (6s, 18H, CH₃, Ac);

¹³C NMR (CDCl₃, 101 MHz): δ = 171.48, 170.8, 170.71, 169.94, 169.91, 169.54 (6x CO, Ac, AcNH), 154.27 (CO, Troc), 133.39 (CH, Allyl), 118.61 (CH₂, Allyl), 101.69 (C-1'), 96.47 (C-1), 74.7 (CH₂ Troc), 72.15 (C-5'), 68.52 (C-4, C-4'), 68.09 (C-5), 68.73, 68.68 (C-3, C-3'), 68.66 (OCH₂, Allyl), 68.25 (C-6), 62.94 (C-6'), 56.11 (C-2'), 51.72 (C-2), 23.19, 20.73, 20.60 (CH₃, Acetyl);

HRMS (⁺ESI-TOF): calcd. for C₃₀H₄₂Cl₃N₂O₁₇ *m/z* [M+H]⁺: 807.1544, found: 807.1544.

Synthesis of 9



Allyl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-hexanoylamino-β-D-glucopyranosyl]-2-deoxy-α-D-glucopyranoside (**9**)

To a stirred solution of **8** (440 mg, 545 μmol) in 8 ml hexanoic acid and 15 ml CH₂Cl₂ was added Zn-dust (10 μm, 352 mg, 5.4 mmol) in two equal portions within 2 h. The dust was dispersed by ultrasonic treatment and the reaction mixture was stirred for 3 h at pH 4. As soon as TLC analysis indicated complete conversion of **8** into the intermediate amine allyl 2-acetamido-3,4-di-O-acetyl-6-O-(3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside, *R*_f = 0.55 (EtOAc/MeOH/AcOH, 5:1:0.05), diisopropylcarbodiimide (255 μl, 1.64 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was diluted with CHCl₃ (200 ml), solids were separated by filtration through the pad of Celite, the filtrate was washed with satd. aq. NaHCO₃ (3 × 100 ml). The organic phase was dried over MgSO₄, filtered and concentrated. The residue was purified by MPLC on silica gel (hexane/ethyl acetate, 2:1 → 0:1) to give **9** (487 mg, 76.8%) as a solid. *R*_f = 0.13 (EtOAc/Hexane, 2:1), [α]_D²⁰ = +35 (*c* 0.68, CHCl₃);

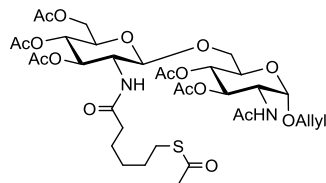
¹H NMR (CDCl₃, 400 MHz): δ = 5.88 (m, 1 H, CH=, Allyl), 5.76 (d, *J* = 8.7 Hz, 1 H, 2'-NHAc), 5.63 (d, *J* = 9.6 Hz, 1 H, 2-NHAc), 5.31 (dq, *J* = 17.2, *J* = 1.6 Hz, 1 H, CH₂-*trans*, Allyl), 5.25 (dq, *J* = 10.4 Hz, *J* = 1.3 Hz, 1 H, CH₂-*cis*, Allyl), 5.23 (dd, *J*_{2,3} = *J*_{3,4} = 9.2 Hz, 1H, H-3), 5.21 (dd, *J* = 10.1 Hz, *J* = 7.1 Hz, 1 H, H-3'), 5.09 (dd, *J*_{4,5} = 9.6 Hz, 1 H, H-4), 5.07 (dd, *J*_{4',5'} = 9.7 Hz, 1H, H-4'), 4.86 (d, *J*_{1,2} = 3.6 Hz, 1H, H-1), 4.50 (d, *J*_{1',2'} = 8.4 Hz, 1H, H-1'), 4.29 (ddd, 1H, H-2), 4.24 (dd, *J*_{6'a,6'b} = 12.3 Hz, *J*_{5',6'a} = 4.8 Hz, 1 H, H-6'a), 4.17 (qt, *J* = 12.8 Hz, *J* = 5.3 Hz, *J* = 1.5 Hz, 1 H, OCH₂, Allyl), 4.13 (dd, *J*_{5',6'b} = 2.5 Hz, 1H, H-6'b), 4.02 (ddd, 1H, H-2'), 4.03 (dd, *J*_{6a,6b} = 10.9 Hz, *J*_{5a,6a} = 2.0 Hz, 1H, H-6a), 3.96 (qt, *J* = 12.8 Hz, *J* = 6.2 Hz, *J* = 1.3 Hz, 1H, OCH₂, Allyl), 3.88 (ddd, 1H, H-5), 3.67 (qdd, 1H, H-5'), 3.40 (dd, *J*_{5a,6b} = 4.5 Hz, 1H, H-6b), 2.23 – 2.10 (m, 2H, α-CH₂), 2.08, 2.06, 2.02, 2.01, 2.00, 1.94 (6s, 18H, CH₃, 5×Ac, NAc), 1.63 – 1.53 (m, 2H, β-CH₂), 1.35 – 1.23 (m, 4 H, γ,δ-CH₂), 0.88 (t, *J* = 7 Hz, 3 H, ε-CH₃);

¹³C NMR (CDCl₃, 101 MHz): δ = 173.42, 171.42, 170.96, 170.85, 170.06, 169.85, 169.50 (CO, 6xAc, hexanoyl), 133.26 (CH, Allyl), 118.50 (CH₂, Allyl), 101.40 (C-1'), 96.24 (C-1), 72.83 (C-5'), 72.05, 71.50 (C-3, C-3'), 68.57 (C-5), 68.51 (OCH₂, Allyl), 68.47, 68.38 (C-4, C-4'), 67.66 (C-6), 62.06 (C-6'), 54.07 (C-2'), 51.78 (C-2), 36.80 (α-

CH₂), 31.52 (γ-CH₂), 25.41 (β-CH₂), 23.37 (CH₃, Ac), 22.47 (δ-CH₂), 20.94, 20.91, 20.86, 20.77 (CH₃, Ac), 14.06 (ε-CH₃);

HRMS (ESI): calcd. for C₃₃H₅₁N₂O₁₆ *m/z* [M+H]⁺: 731.3233, found: 733.3242.

Synthesis of 10



Allyl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino-β-D-glucopyranosyl]-2-deoxy-α-D-glucopyranoside (10)

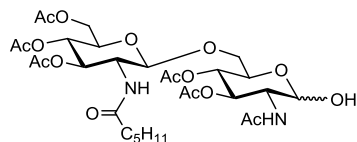
To a solution of **8** (2.0 g, 2.47 mmol) in AcOH (40 ml) was added Zn-dust (10 μm, 2 g, 300 mol) at r.t. The Zn-dust was dispersed by ultrasonic treatment followed by stirring at r.t. for 5 h. The reaction mixture was diluted with CHCl₃ (200 ml) and washed with aq. satd. NaHCO₃, dried with Na₂SO₄, filtered and concentrated. The intermediate amine [allyl 2-acetamido-3,4-di-O-acetyl-6-O-(3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside] (*R_f* = 0.55 (EtOAc/MeOH/AcOH, 5:1:0.05), was dried *in vacuo* and dissolved in dry CH₂Cl₂. To this solution 6-acetylthiohexanoic acid (1.23 ml, 7.4 mmol) and diisopropylcarbodiimide (1.1 ml, 7.4 mmol) were added successively. The reaction mixture was stirred for 8 h, diluted with CHCl₃ (200 ml), washed with water (100 ml), the organic phase was dried with Na₂SO₄, filtered and concentrated. The residue was purified by MPLC on silica gel (toluene/ethyl acetate, 2:1 → 0:1) to give **10** (1.8 g, 59.5%) as a solid. *R_f* = 0.39 (EtOAc), [α]_D²⁰ = +35 (*c* 1.0, MeOH);

¹H NMR (MeOD, 600 MHz): δ = 5.99-5.95 (m, 1H, CH=, Allyl), 5.37-5.34 (m, 2H, =CH₂ *trans*, Allyl), 5.26 (dd, *J*_{2,3} = 9.5 Hz, *J*_{3,4} = 10.8 Hz, 1H, H-3), 5.24-5.22 (m, 2H, =CH₂ *cis*, Allyl), 5.21 (dd, *J*_{2',3'} = 10.4 Hz, *J*_{3',4'} = 9.7 Hz, 1H, H-3'), 4.97 (t, *J*_{3',4'} = *J*_{4',5'} = 9.7 Hz, 1H, H-4'), 4.95 (t, *J*_{3,4} = *J*_{4,5} = 9.8 Hz, 1H, H-4), 4.81 (m, 1H, H-1 under H₂O signal), 4.62 (d, *J*_{1',2'} = 8.5 Hz, H-1'), 4.28 (dd, *J*_{6'a,6'b} = 12.2 Hz, *J*_{5,6'a} = 4.9 Hz, 1H, H-6'a), 4.23 (dd, *J*_{1,2} = 3.5 Hz, 1H, H-2), 4.23-4.19 (m, 2H, OCH₂, Allyl), 4.12 (dd, 1H, *J*_{5',6'b} = 2.2 Hz, H-6'b), 4.03-4.01 (m, 1H, OCH₂, Allyl), 3.97 (ddd, *J*_{5,6a} = 2.0 Hz, *J*_{5,6b} = 5.7 Hz, 1H, H-5), 3.91 (dd, 1H, H-2'), 3.89 (dd, *J*_{6a,6b} = 10.6 Hz, 1H, H-6a), 3.78 (dd, 1H, H-5'), 3.57 (dd, 1H, H-6b), 2.86 (t, *J* = 7.2 Hz, 2H, ε-CH₂-SAc), 2.30 (s, 3H, CH₃, SAc), 2.19-2.16 (m, 2H, α-CH₂, hexanoyl), 2.07, 2.03, 2.0, 1.98, 1.96, 1.92 (6 s, 18H, CH₃, 5×Ac, NHAc), 1.60-1.55 (m, 4H, β-CH₂, δ-CH₂), 1.40-1.36 (m, 2H, γ-CH₂);

¹³C NMR (MeOD, 151 MHz): δ = 198.0 (CO, SAc), 176.23 (CO, hexanoyl), 173.61, 172.50, 172.22, 172.97, 171.64, 171.42 (6×CO, Ac, NHAc), 135.17 (CH, Allyl), 118.54 (CH₂, Allyl), 102.47 (C-1'), 97.55 (C-1), 74.39 (C-3'), 73.17 (C-5'), 72.67 (C-3), 70.93 (C-4), 70.42 (C-4'), 70.14 (C-5), 69.71 (OCH₂, Allyl), 69.50 (C-6), 63.41 (C-6'), 55.29 (C-2'), 53.08 (C-2), 37.31 (α-CH₂), 30.67 (CH₃, SAc), 30.62 (β-CH₂), 29.80 (ε-CH₂), 29.47 (γ-CH₂), 26.47 (δ-CH₂), 22.52, 20.90, 20.86, 20.83, 20.73 (6×CH₃, Ac, NHAc).

HRMS (ESI-TOF): calcd for C₃₅H₅₂N₂O₁₇S *m/z* [M+HCOO]⁻ 849.2969, found: 849.2952.

Synthesis of 11



2-Acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-hexanoylamino-β-D-glucopyranosyl]-2-deoxy-D-glucopyranose (11)

A solution of **9** (460 mg, 629 μmol) in freshly distilled dry THF (40 ml) was degassed by repeated evacuation and flushing the flask with helium. To this stirred solution H₂-activated (1,5-cyclooctadiene)

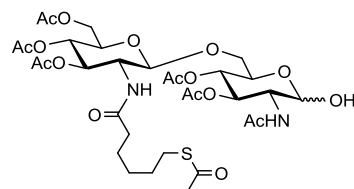
bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate $[\text{Ir}^+(\text{COD})(\text{PMePh}_2)_2]\text{PF}_6^-$ (10.7 mg, 12.65 μmol) was added and the reaction mixture was stirred for 1 h. [Activation of the catalyst was performed as follows: $\text{Ir}[(\text{COD})_2\text{bis(methyldiphenylphosphine)}]\text{PF}_6$ (10.7mg) was placed in a three necked flask and dissolved in freshly distilled dry THF (25 ml). The solution was first degassed by repeated evacuation and filling the flask with Ar, then Ar was exchanged to H_2 , which was kept for 3×15 sec in the flask so that the solution turned colourless. The gaseous phase was exchanged to Ar and a solution of the activated catalyst was transferred to the reaction mixture.] The reaction mixture was cooled to 4°C and a solution of I_2 (317 mg, 1.25 mmol) in THF/ H_2O (1:1, 7 ml) was added. The stirring was continued for 1 h at 4°C , the mixture was diluted with cold (0°C) ethyl acetate (100 ml), ice cold aq. satd. Na_2SO_3 and aq. satd. NaHCO_3 (100 ml), followed by brine (50 ml). The organic phase was dried over Na_2SO_4 , filtered and concentrated. The residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{Acetone}$, 2:1) to give **11** (300 mg, 69%, $\alpha/\beta = 2.4:1$) as white amorphous solid. $R_f = 0.21$ ($\text{CH}_2\text{Cl}_2/\text{Acetone}$, 2:1), $[\alpha]_{\text{D}}^{20} = +24$ (c 0.55, CHCl_3);

^1H NMR ($\text{DMSO}-d_6$, 400 MHz, α -anomer): $\delta = 7.79$ (d, $J = 9.2$ Hz, 1H, NHAc), 7.68 (d, $J = 9.2$ Hz, 1H, N'H), 7.05 (dd, 1H, OH), 5.13 (dd, $J_{2,3} = 9.2$ Hz, $J_{3,4} = 9.3$ Hz, 1H, H-3), 5.07 (dd, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 9.5$ Hz, 1H, H-3'), 4.96 (d, $J_{1,2} = 3.4$ Hz, $J_{1,\text{OH}} = 4.5$ Hz, 1H, H-1), 4.81 (dd, $J_{4',5'} = 9.7$ Hz, 1H, H-4'), 4.74 (dd, $J_{4,5} = 9.9$ Hz, 1H, H-4), 4.63 (d, $J_{1',2'} = 8.6$ Hz, 1H, H-1'), 4.17 (dd, $J_{6'a,6'b} = 12.3$ Hz, $J_{5',6'a} = 5.1$ Hz, 1H, H-6'a), 4.05 (ddd, $J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 5.4$ Hz, 1H, H-5), 3.99 (dd, $J_{5',6'b} = 2.4$ Hz, 1H, H-6'b), 3.96 (ddd, 1H, H-2), 3.81 (ddd, 1H, H-5'), 3.77 (ddd, 1H, H-2'), 3.69 (dd, $J_{6a,6b} = 11$ Hz, 1H, H-6a), 3.53 (dd, 1H, H-6b), 2.07 – 1.99 (m, 5H, $\alpha\text{-CH}_2$, $\text{CH}_3\text{-Ac}$), 1.97, 1.96, 1.89, 1.88, 1.80 (5s, 15H, CH_3 , Ac), 1.45 (q, 2H, $\beta\text{-CH}_2$), 1.31 – 1.14 (m, 4H, γ , $\delta\text{-CH}_2$), 0.85 (t, $J = 7.1$ Hz, 3H, $\epsilon\text{-CH}_3$);

^{13}C NMR ($\text{DMSO}-d_6$, 101 MHz): $\delta = 172.21$ (CO, hexanoyl), 170.06, 169.74, 169.54, 169.52, 169.34, 169.27 (CO, 6xAc), 100.14 (C-1'), 90.62 (C-1), 72.73 (C-3'), 70.79 (C-3), 70.52 (C-5'), 69.45 (C-4), 68.58 (C-4'), 67.68 (C-5), 67.29 (C-6), 61.88 (C-6'), 52.40 (C-2'), 51.35 (C-2), 35.51 ($\alpha\text{-CH}_2$), 30.64 ($\delta\text{-CH}_2$), 24.86 ($\beta\text{-CH}_2$), 22.28 (CH_3 , Ac), 21.84 ($\gamma\text{-CH}_2$), 20.49, 20.47, 20.43, 20.39, 20.34 (5x CH_3 , Ac), 14.76 ($\epsilon\text{-CH}_3$).

HRMS ($^+\text{ESI-TOF}$): calcd. for $\text{C}_{30}\text{H}_{47}\text{N}_2\text{O}_{16}$ m/z $[\text{M}+\text{H}]^+$: 691.2921, found: 691.2921.

Synthesis of 12



2-Acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- β -D-glucopyranosyl]-2-deoxy-D-glucopyranose ($\alpha/\beta = 10:1$) (**12**)

A solution of **10** (403 mg, 500 μmol) in dry THF (15 ml) was degassed by repeated evacuation and flushing the flask with helium. To this stirred solution the H_2 -activated (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate $[\text{Ir}^+(\text{COD})(\text{PMePh}_2)_2]\text{PF}_6^-$ (42.3 mg, 50 μmol) in dry THF (25 ml) was added and the reaction mixture was stirred for 1 h. The mixture was stirred for 90 min, cooled to -10°C and a solution of I_2 (125.5 mg, 600 μmol) in THF/water (1:1, 1 ml) was added dropwise. After stirring for 1 hour at -10°C , the reaction mixture was diluted with ethyl acetate (20ml) and washed with ice cold aq. satd. $\text{Na}_2\text{SO}_3/\text{NaHCO}_3$ (1:1, 10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4 , filtered and concentrated. The residue was purified by column chromatography (EtOAc) to give **12** (267.6 mg, 70%, $\alpha/\beta = 10:1$) as a white solid. $R_f = 0.15$ (EtOAc), $[\alpha]_{\text{D}}^{20} = +23$ (c 0.93, MeOH);

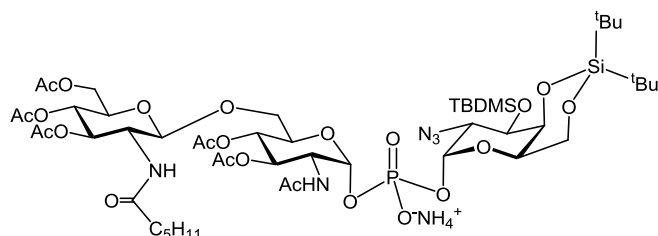
^1H NMR (MeOD, 600 MHz, α -anomer): $\delta = 5.28$ (dd, $J_{2,3} = 10.9$ Hz, $J_{3,4} = 9.3$ Hz, 1H, H-3), 5.21 (dd, $J_{2',3'} = 10.5$ Hz, $J_{3',4'} = 9.3$ Hz, 1H, H-3'), 5.07 (d, $J_{1,2} = 3.5$ Hz, 1H, H-1), 4.97 (t, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, 1H, H-4'), 4.96 (t, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 4.62 (d, $J_{1',2'} = 8.5$ Hz, 1H, H-1'), 4.27 (dd, $J_{6'a,6'b} = 12.3$ Hz, $J = 4.8$ Hz, 1H, H-6'a), 4.16 (ddd, $J_{5,6a} = 2.5$ Hz, $J_{5,6b} = 4.7$ Hz, 1H, H-5), 4.13 (dd, 1H, H-2), 4.12 (dd, 1H, $J_{5,6'b} = 2.8$ Hz, H-6'b), 3.88 (dd, 1H, H-2'), 3.89 (dd, $J_{6a,6b} = 10.8$ Hz, 1H, H-6a), 3.57 (dd, 1H, H-6b), 2.87 (t, $J = 7.2$ Hz, 2H, $\epsilon\text{-CH}_2\text{-SAc}$), 2.30 (s, 3H,

CH₃, SAc), 2.23-2.16 (m, 2H, α-CH₂, hexanoyl), 2.06, 2.03, 2.0, 1.98, 1.95, 1.91 (6 s, 18H, CH₃, 5×Ac, NHAc), 1.60-1.57 (m, 4H, β-CH₂, δ-CH₂), 1.39-1.36 (m, 2H, γ-CH₂);

¹³C NMR (MeOD, 151 MHz, α-anomer): δ = 197.76 (CO, SAc), 176.48 (CO, hexanoyl), 173.56, 172.56, 172.38, 172.02, 171.75, 171.45 (6×CO, Ac, NHAc), 102.23 (C-1'), 92.68 (C-1), 74.40 (C-3'), 73.15 (C-5'), 72.83 (C-3), 71.08 (C-4), 70.40 (C-4'), 69.45 (C-5), 69.32 (C-6), 63.43 (C-6'), 55.30 (C-2'), 53.69 (C-2), 37.29 (α-CH₂), 30.67 (CH₃, SAc), 30.62 (β-CH₂), 29.83 (ε-CH₂), 29.48 (γ-CH₂), 26.44 (δ-CH₂), 22.60, 20.96, 20.88, 20.86, 20.81, 20.73 (6×CH₃, Ac, NHAc).

HRMS (ESI-TOF): calcd for C₃₃H₄₉N₂O₁₉S *m/z* [M+HCOO]⁻ 809.2656, found: 809.2649.

Synthesis of 13



2-Azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butyldimethylsilylene-2-deoxy-α-D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-hexanoylamino-β-D-glucopyranosyl]-2-deoxy-α-D-glucopyranose (ammonium salt) (13)

To a stirred solution of **5** (125.3 mg, 231 μmol) and **11** (160 mg, 231 μmol) {which were pre-dried by repeated co-evaporation with dry toluene (4×10 ml)} in dry pyridine (10 ml) a solution of pivaloyl chloride (PivCl, 300 μmol) in dry CH₂Cl₂ (1 ml) was added dropwise over 1 h. The reaction mixture was stirred for 2 h, cooled to -15°C and a solution of I₂ (44 mg) in Pyr/H₂O (50:1, 2.5 ml) was added dropwise. The reaction mixture was stirred for 20 min at -15°C, diluted with CH₂Cl₂ (150 ml) and washed with aq. satd. Na₂S₂O₃ (25 ml) and 0.5 M triethylammonium bicarbonate buffer (TEAB; 50 ml). The organic phase was filtered through cotton and concentrated at 20°C. The residue was purified by column chromatography (CHCl₃/MeOH/25% aq. NH₄OH, 7:1:0.1) to give **13** (150 mg, 53%) as a white solid; R_f = 0.25 (CHCl₃/MeOH/25% aq. NH₄OH, 7:1:0.08), [α]_D²⁰ = +83 (*c* 0.7, MeOH);

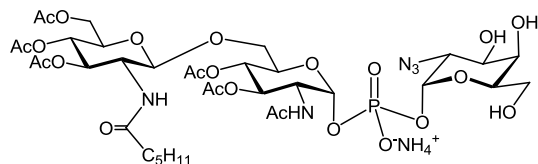
¹H NMR (CDCl₃, 400 MHz): δ = 7.26 (d, under CDCl₃ signal, 1H, 2'-NH), 6.42 (d, *J* = 9.6 Hz, 0.9H, 2-NHAc), 5.74 (dd, *J*_{1',2''} = 3.2 Hz, *J*_{1',P} = 7.9 Hz H-1'), 5.66 (dd, *J*_{1,2} = 3.5 Hz, *J*_{1,P} = 7.3 Hz, 1H, H-1), 5.17 (app. t, *J*_{2,3} = 10.6 Hz, *J*_{3,4} = 10.5 Hz, 1H, H-3), 5.10 (dd, *J*_{2,3'} = *J*_{3',4'} = 9.2 Hz, 1H, H-3'), 5.03 (dd, *J*_{3',4'} = *J*_{4',5'} = 9.5 Hz, 1H, H-4'), 4.92 (t, *J*_{3,4} = *J*_{4,5} = 9.9 Hz, 1H, H-4), 4.74 (d, *J*_{1',2'} = 8.7 Hz, 1H, H-1'), 4.40 (d, *J*_{3'',4''} = 3.2 Hz, 1H, H-4''), 4.31 (dd, *J*_{5'',6''a} = 1.3 Hz, *J*_{6''a,6''b} = 13.1 Hz, 1H, H-6''a), 4.28 – 4.21 (m, 4H, H-2, H-6'a, H-5, H-6''b), 4.14 (dd, *J*_{5',6'b} = 2.8 Hz, *J*_{6'a,6'b} = 12.6 Hz, 1H, H-6'b), 4.10-4.06 (m, 2H, H-2', H-3''), 4.03 (bs, 1H, H-5''), 3.84 (dd, *J*_{5a,6a} = 1.8 Hz, *J*_{6a,6b} = 12.3 Hz, 1H, H-6a), 3.78-3.73 (m, 2H, H-5', H-2''), 3.65 (dd, *J*_{5,6b} = 6.4 Hz, 1H, H-6b), 2.30 – 2.17 (m, 2H, α-CH₂), 2.10 (s, 3H, CH₃, NHAc), 2.04, 2.01, 2.008, 1.97 (4s, 15H, CH₃, acetyl), 1.59 (q, 2H, β-CH₂), 1.36 – 1.23 (m, 4 H, γ-CH₂, δ-CH₂), 1.06 (s, 9H, 3×CH₃, DTBS), 1.02 (s, 9H, 3×CH₃, DTBS), 0.94 (s, 9H, 3×CH₃, TBDMS), 0.89 (t, *J* = 7 Hz, 3H, ε-CH₃), 0.17, 0.15 (2 s, 6 H, 2×CH₃, TBDMS);

¹³C NMR (CDCl₃, 101 MHz): δ = 174.44 (CO, NHAc), 171.26, 171, 20, 170.96, 170.79, 169.84, 169.38 (6×CO, 5Ac, hexanoyl), 100.83 (d, *J*_{1,P} = 5.9 Hz, C-1'), 95.50 (d, *J*_{1,P} = 4.7 Hz, C-1''), 93.57 (d, C-1), 73.67 (C-4''), 72.95 (C-3'), 71.79 (C-5'), 71.22 (C-5), 70.84 (C-3), 69.86 (C-3''), 69.14 (C-5''), 68.74 (C-4'), 68.28 (C-4), 66.98, 66.84 (C-6, C-6''), 62.06 (C-6'), 60.62 (d, *J*_{2,P} = 8.1 Hz, C-2''), 53.08 (C-2'), 51.82 (d, *J*_{2,P} = 6.7 Hz, C-2), 36.26 (α-CH₂), 31.30 (γ-CH₂), 27.58, 27.30, 25.62, (DTBS, TBDMS), 25.54 (β-CH₂), 23.07 (CH₃, Ac), 22.46 (δ-CH₂), 20.75, 20.73, 20.65, 20.60, (CH₃, Ac), 13.95 (ε-CH₃), -4.12, -4.87 (CH₃, TBDMS);

³¹P NMR (CDCl₃, PPh₃ ext. standard, 162 MHz): δ = -3.0

HRMS (⁺ESI-TOF): calcd for C₅₀H₈₇N₅O₂₃PSi₂ *m/z* [M+H]⁺: 1212.5063, found: 1212.5073.

Synthesis of 14



2-Azido-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-hexanoylamino- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranose (ammonium salt) (**14**)

To a stirred solution of **13** (46 mg, 37.95 μ mol) in dry THF (50 ml) in a PTFE flask was added a solution of pyridinium hydrofluoride (50 μ l) in dry pyridine (1 ml) *via* syringe. The reaction mixture (pH = 6) was stirred for 1 h, diluted with toluene (10 ml) and concentrated to dryness. The residue was purified by flash chromatography on Isolute cartridge (Biotage Isolute Flash Si II 2g (6 ml) SPE column (Part No. 440-0200-C), CHCl₃/MeOH/H₂O/25% aq. NH₄OH, 50:25:3:0.2) to provide **14** (35.7mg, 96.6%) as a solid, R_f = 0.55 (CHCl₃/MeOH/H₂O, 50:25:4), $[\alpha]_D^{20}$ = +72 (*c* 0.6, MeOH);

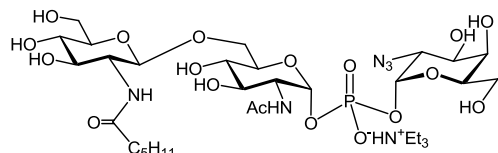
¹H NMR (MeOD, 400 MHz): δ = 5.64 (dd, $J_{1'',2''}$ = 3.3 Hz, $J_{1'',P}$ = 7.6 Hz, 1H, H-1''), 5.60 (dd, $J_{1,2}$ = 3.4 Hz, $J_{1,P}$ = 7.1 Hz, 1H, H-1), 5.28 (dd, $J_{3,4}$ = 10.7 Hz, $J_{2,3}$ = 9.1 Hz, 1H, H-3), 5.23 (dd, $J_{3',4'}$ = 9.9 Hz, $J_{2',3'}$ = 9.3 Hz, 1H, H-3'), 5.02 (dd, $J_{4,5}$ = 10.2 Hz, 1H, H-4), 5.0 (dd, $J_{4',5'}$ = 9.6 Hz, 1H, H-4'), 4.72 (d, $J_{1',2'}$ = 8.6 Hz, 1H, H-1'), 4.32 (ddd, J = 2.0 Hz, 1H, H-5), 4.29 (dd, $J_{6'a,6'b}$ = 12.2 Hz, $J_{5',6'a}$ = 4.7 Hz, 1H, H-6a'), 4.26 (ddd, 1H, H-2), 4.11 (dd, 1H, $J_{5',6'b}$ = 2.4 Hz, H-6'b), 4.10 (m, 1H, H-5''), 4.0 (dd, $J_{2'',3''}$ = 10.5 Hz, $J_{3'',4''}$ = 3.2 Hz, 1H, H-3''), 4.01 - 3.93 (m, 3H, H-2', H-6a, H-4''), 3.83 (dd, $J_{6'',6'b}$ = 11.4 Hz, $J_{5'',6''a}$ = 7.9 Hz, 1H, H-6a''), 3.82 (m, 1H, H-5'), 3.74 (m, 1H, H-2''), 3.73 (dd, $J_{5'',6''b}$ = 4.1 Hz, 1H, H-6''b), 3.63 (dd, $J_{6a,6b}$ = 11.2 Hz, $J_{6b,5}$ = 4.9 Hz, 1H, H-6b), 2.34 - 2.22 (m, 2H, α -CH₂, hexanoyl), 2.06, 2.03, 2.00, 1.97, 1.96, 1.95 (6s, 18H, CH₃, Ac, NHAc), 1.61 (q, 2H, β -CH₂), 1.38 - 1.29 (m, 4H, γ -CH₂, δ -CH₂), 1.16 (NH₄⁺, counter-ion), 0.92 (t, J = 7 Hz, 3H, ϵ -CH₃);

¹³C NMR (MeOD, 101 MHz): δ = 177.06 (CO, NHAc), 173.74, 172.66, 172.16, 171.94, 171.64, 171.58 (6 \times CO, 5Ac, Hexanoyl), 101.73 (C-1'), 95.94 (d, $J_{C1'',P}$ = 5.9 Hz, C-1''), 95.77 (d, $J_{C1,P}$ = 6.1 Hz, C-1), 74.62 (C-5''), 74.14 (C-3'), 72.99 (C-5'), 72.66 (C-3), 71.51 (C-4''), 71.02 (C-5), 70.46, 70.43 (C-4, C-4'), 69.81 (C-3''), 68.52 (C-6), 63.41 (C-6'), 63.13 (C-6''), 62.01 (d, $J_{C2'',P}$ = 8.4 Hz, C-2''), 55.29 (C-2), 53.06 (d, $J_{C2,P}$ = 8.4 Hz, C-2), 7.46 (α -CH₂), 32.61 (γ -CH₂), 26.80 (β -CH₂), 23.62 (δ -CH₂), 22.76, 20.89, 20.88, 20.84, 20.78, 20.76 (6 \times CH₃, Ac), 14.48 (ϵ -CH₃);

³¹P NMR (MeOD, PPh₃ ext. standard, 162 MHz): δ = -2.44

HRMS (ESI-TOF): calcd for C₃₆H₅₅N₅O₂₃P m/z [M-H]⁻ 956.3031, found: 956.3028

Synthesis of 15



2-Azido-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-O-[2-deoxy-2-hexanoylamino- β -D-glucopyranosyl]- α -D-glucopyranose (triethylammonium salt) (**15**)

A solution of **14** (35 mg, 35.92 μ mol) in MeOH/H₂O/Et₃N (7:3:1, 2 ml, pH = 11.5) was stirred for 20 h, the reaction mixture was diluted with toluene (5 ml) and concentrated. The residue was dissolved in 5 ml H₂O, frozen and lyophilized to give **15** (30 mg, 98.4%) as a solid. R_f = 0.18 (CHCl₃/MeOH/H₂O, 2:1:0.15), $[\alpha]_D^{20}$ = +53 (*c* 0.55, MeOH);

¹H NMR (D₂O, d-Acetone/D₂O ext. standard, 600 MHz): δ = 7.89 (d, J = 9.2 Hz, 0.6 H, NHAc), 5.47 (dd, $J_{1'',2''}$ = 3.4 Hz, $J_{P,H}$ = 7.5 Hz, H-1''), 5.36 (dd, $J_{1,2}$ = 3.4 Hz, $J_{P,H}$ = 6.8 Hz, H-1), 4.51 ($J_{1',2'}$ = 8.6 Hz, 1 H, H-1'), 3.96 (dd, J = 4.9 Hz, J = 7.4 Hz, 1 H, H-5), 3.94 - 3.90 (m, 3 H, H-6a, H-3'', H-5''), 3.82 (ddd, J = 3.4 Hz, J = 4.5 Hz, J = 10.4 Hz,

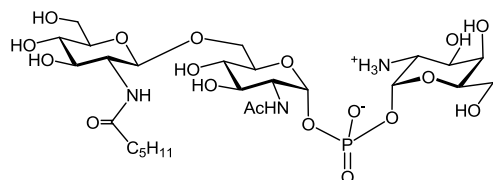
1 H, H-4''), 3.8 – 3.76 (m, 3 H, H-2, H-6'a, H-6'b), 3.63 (dd, $J_{5'',6''a} = 4.4$ Hz, $J_{6''a,6''b} = 11.6$ Hz, 1 H, H-6''a), 3.63 (dd, $J_{5'',6''b} = 4.8$ Hz, 1 H, H-6''b), 3.62 (m, 1 H, H-3), 3.58 (dd, $J_{2',3'} = 10.3$ Hz, 1 H, H-2'), 3.49 (dt, $J_{2'',3''} = 10.2$ Hz, 1 H, H-2''), 3.44 – 3.88 (m, 2 H, H-3', H-4), 3.31 (dd, $J = 1.9$ Hz, $J = 3.5$ Hz, 1 H, H-4'), 3.30 (dd, $J = 16.7$ Hz, $J_{5',6'b} = 9.1$ Hz, 1 H, H-5'), 3.05 (q, CH₂, Et₃N, counter-ion), 2.18 (m, 2H, α-CH₂), 1.92 (s, 3H, CH₃, NHAc), 1.48 (m, 2H, β-CH₂), 1.19 – 1.15 (m, 4H, γ-CH₂, δ-CH₂), 1.13 (t, CH₃, Et₃N, counter-ion), 0.73 (t, $J = 7$ Hz, 3 H, ε-CH₃);

¹³C NMR (D₂O, d-Acetone/D₂O ext. standard, 150 MHz): δ = 178.5 (Acyl), 175.1 (Acetyl), 101.84 (C-1'), 94.95 (d, C-1'', C-1'), 76.64 (C-5'), 74.48 (C-3'), 73.07 (C-5''), 72.90 (C-5), 71.30 (C-3), 70.70 (C-4'), 70.13 (C-4), 69.44 (C-4''), 68.23 (C-3''), 68.21 (C-6), 61.80 (C-6''), 61.40 (C-6'), 60.41 (d, C-2''), 56.09 (C-2'), 54.22 (d, C-2), 47.36 (CH₂, Et₃N, counter-ion), 36.71 (α-CH₂), 31.23 (γ-CH₂), 25.76 (β-CH₂), 22.66 (CH₃,Ac), 22.34 (δ -CH₂), 13.90 (ε-CH₃), 8.89 (CH₃, Et₃N, counter-ion);

³¹P NMR (D₂O, H₃PO₄ ext. standard, 243 MHz): δ = -3.77

HRMS (ESI-TOF): calcd for C₂₆H₄₅N₅O₁₈P m/z [M-H]⁻ 746.2503; found: 746.2509.

Synthesis of 16



2-Amino-2-deoxy-α-D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-O-[2-deoxy-2-hexanoylamino-β-D-glucopyranosyl]-α-D-glucopyranose (16)

A solution of **15** (10 mg, 11.7 μmol) in MeOH (10 ml) was applied to the H-Cube system (Pd/C cartridge, full H₂-mode, 25°C, flow rate of 0.5 ml/min, 4 h). After washing the system with MeOH (2x 4 ml), the reaction mixture was concentrated and purified by size exclusion chromatography on Sephadex LH20 (4 g, MeOH, flow rate 0.6 ml/min) to provide **16** (8.4 mg, 98.8%) as a solid. $R_f = 0.11$ (CHCl₃/MeOH/H₂O, 2:1:0.15), $[\alpha]_D^{20} = +68$ (c 0.68, MeOH);

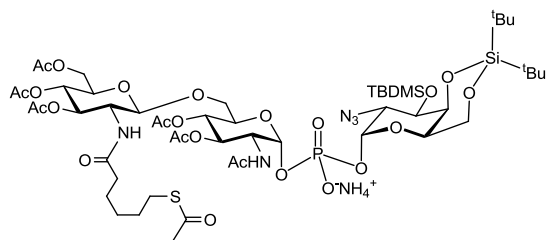
¹H NMR (D₂O, DSS ext. standard, 600 MHz, pH = 6.5): δ = 5.76 (dd, $J_{1'',2''} = 3.5$ Hz, $J_{P,H} = 6.4$ Hz, 1H, H-1''), 5.45 (dd, $J_{1,2} = 3.5$ Hz, $J_{P,H} = 7.4$ Hz, 1H, H-1), 4.58 ($J_{1',2'} = 8.6$ Hz, 1H, H-1'), 4.11 (dd, $J_{5'',4''} = 4.5$ Hz, $J_{5'',6''a} = 7.8$ Hz, $J_{5'',6''b} = 3.2$ Hz, 1H, H-5''), 4.09 (dd, $J_{2'',3''} = 10.8$ Hz, $J_{3'',4''} = 3.9$ Hz, 1H, H-3''), 4.05 (dd, $J_{5,6a} = 2.1$ Hz, $J_{6a,6b} = 11.9$ Hz, 1H, H-6a), 4.04 (d, 1H, H-4''), 3.99 (ddd, $J_{5,6b} = 4.6$ Hz, $J_{5,4} = 10.1$ Hz, 1H, H-5), 3.92 (dd, $J_{2,3} = 7.9$ Hz, 1H, H-2), 3.91 (dd, $J_{6'a,6'b} = 12.3$ Hz, $J_{5',6'a} = 2.0$ Hz, 1H, H-6'a), 3.90 (dd, 1H, H-6b), 3.79 (dd, $J_{6'a,6''b} = 11.9$ Hz, 1H, H-6''a), 3.77 (dd, 1H, H-6''b), 3.75 (d, $J_{2',3'} = 10.5$ Hz, 1H, H-2'), 3.76 (dd, $J_{3,4} = 9.5$ Hz, 1H, H-3), 3.73 (dd, $J_{5',6'b} = 5.5$ Hz, 1H, H-6'b), 3.56 (ddd, 1H, H-2''), 3.52 (dd, $J_{3',4'} = 8.3$ Hz, 1H, H-3'), 3.46 (dd, 1 H, H-4), 3.43 (m, 1H, H-5'), 3.41 (dd, $J_{4',5'} = 8.9$ Hz, 1H, H-4'), 2.29 (m, 2H, α-CH₂), 2.02 (s, 3H, CH₃, NHAc), 1.60 (m, 2H, β-CH₂), 1.32 – 1.26 (m, 4H, γ-CH₂, δ-CH₂), 0.85 (t, $J = 7$ Hz, 3 H, ε-CH₃);

¹³C NMR (D₂O, dioxane ext. standard, 151 MHz): δ = 178.75 (CO, 2-NHAc), 175.40 (CO, 2'-NH-hexanoyl), 102.65 (C-1'), 95.10 (d, $J_{C,P} = 6.0$ Hz, C-1), 93.43 (d, $J_{C,P} = 5.5$ Hz, C-1''), 76.78 (C-5), 74.67 (C-3'), 73.43 (C-5''), 72.24 (C-5), 71.11 (C-3), 70.80 (C-4'), 70.51 (C-4), 69.48 (C-6), 68.81 (C-4''), 67.06 (C-3''), 61.92 (C-6''), 61.52 (C-6'), 56.05 (C-2'), 54.24 (d, $J_{C,P} = 8.3$ Hz, C-2), 51.81 (d, $J_{C,P} = 8.6$ Hz, C-2''), 36.90 (α-CH₂), 31.36 (γ-CH₂), 25.96 (β-CH₂), 22.83 (CH₃, NHAc), 22.51 (δ-CH₂), 14.09 (ε-CH₃);

³¹P NMR (D₂O, H₃PO₄ ext. standard, 243 MHz): δ = -2.8

HRMS (ESI-TOF): calcd for C₂₆H₄₇N₃O₁₈P m/z [M-H]⁻ 720.2598, found: 720.2596.

Synthesis of 17



2-Azido-2-deoxy-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranose (ammonium salt) (17)

Procedure via H-phosphonate coupling

17 was prepared from **5** (61.5 mg, 113 μ mol) and **12** (73.5 mg, 113 μ mol) in a way described for the preparation of **13**. Purified by column chromatography on silica gel (CHCl₃/MeOH/25% aq. NH₄OH, 70:10:0.5) afforded **17**. Yield 74 mg (85%). R_f = 0.29 (CHCl₃/MeOH/25% aq. NH₃, 70:10:0.8), $[\alpha]_D^{20}$ = +41 (c 0.43, MeOH)

Procedure via phosphoramidite coupling

To a stirred solution of **4** (α/β = 2.6/1; 38.8 mg, 100 μ mol) in dry CH₂Cl₂ (2 ml) diisopropylethylamine (DIPEA) (106 μ l, 460 μ mol) and 2-cyanoethyl-*N,N*-diisopropylphosphorochloridite (66.4 μ l, 300 μ mol) (³¹P-NMR 180.0 ppm) were added successively under Ar. The reaction mixture was stirred for 1 h until ³¹P-NMR analysis indicated the formation of a phosphoramidite **18** as diastereomeric mixture of (*R*) and (*S*) diastereomers on phosphorus: 4 signals corresponding of the α/β = 2.6:1 mixture, [³¹P-NMR, 162 MHz, CD₂Cl₂, δ (ppm) = 151.48, 150.64, 150.39 and 149.51 (~2:1:1:2)]. The reaction mixture was diluted with CH₂Cl₂ (50 ml) and washed with 0.2 M TEAB buffer (20 ml). The organic phase was dried over cotton, concentrated to dryness, then dissolved in dry toluene (2 \times 10 ml), evaporated and dried *in vacuo*. The residue was dissolved in dry CH₂Cl₂ (2 ml) and a solution of **12** (69.3 mg, 90 μ mol) in dry acetonitrile (10 ml) followed by a solution of 1*H*-tetrazole in dry acetonitrile (0.45 M, 1 ml, 450 μ mol) were added under Ar. The stirring was continued for 2 h, the mixture was cooled to 4°C and a solution of *tert*-butylhydroperoxide in *tert*-BuOH (80%, 20 μ l, 178 μ mol) was added. The reaction mixture was stirred for 1 h at 4°C, then Et₃N (0.2 ml, 717 μ mol) was added in portions over 30 min. Stirring was continued for 30 min, the reaction mixture was diluted with CH₂Cl₂ (100 ml) and washed with 0.2 M TEAB buffer (3 \times 20 ml). The organic phase was dried over cotton and concentrated. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH/25% aq. NH₄OH, 70:10:0.8) to provide **17** (28 mg, 24%) as a solid.

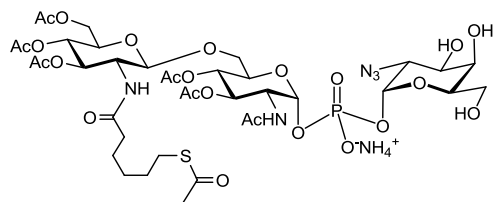
¹H NMR (CDCl₃, 600 MHz): δ = 7.14 (bs, 0.8H, N'HCO), 6.34 (d, J = 8.9 Hz, 0.9H, NHAc), 5.76 (dd, $J_{1'',2''}$ = 3.2 Hz, $J_{1'',P}$ = 7.7 Hz, 1H, H-1''), 5.64 (dd, $J_{1,2}$ = 3.3 Hz, $J_{1,P}$ = 7.0 Hz, 1H, H-1), 5.16 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1H, H-3), 5.12 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.9 Hz, 1H, H-3'), 5.04 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.6 Hz, 1H, H-4'), 4.93 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.9 Hz, 1H, H-4), 4.75 (d, $J_{1',2'}$ = 8.3 Hz, 1H, H-1'), 4.43 (d, $J_{3'',4''}$ = 2.9 Hz, 1H, H-4''), 4.33 (d, $J_{6'',a,5''}$ = 1.8 Hz, $J_{6'',a,6''b}$ = 13.7 Hz, 1H, H-6''a), 4.28 – 4.21 (m, 4H, H-2, H-6'a, H-5, H-6''b), 4.15 (d, $J_{6'a,5'}$ = 2.0 Hz, $J_{6'a,6'b}$ = 12.2 Hz, 1H, H-6'b), 4.10 – 4.06 (m, 2H, H-2', H-3''), 4.04 (bs, 1H, H-5''), 3.85 – 3.74 (m, 3H, H-6a, H-2'', H-5'), 3.66 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 6.2 Hz, 1H, H-6'b), 2.84 (t, J = 7.4 Hz, 2H, ϵ -CH₂-SAc), 2.32 (s, 3H, CH₃, SAc), 2.24 (m, 2H, α -CH₂, hexanoyl), 2.10, 2.04, 2.02, 2.01, 1.97 (6 s, 18H, CH₃, 5 \times Ac, NHAc), 1.59 (m, 4H, β -CH₂, δ -CH₂), 1.37 (m, 2H, γ -CH₂), 1.58 (NH₄⁺), 1.06, 1.02, (2s, 18H, CH₃, DTBS), 0.95 (s, 9H, CH₃, TBDMS), 0.17, 0.16 (2s, 6H, CH₃, TBDMS);

¹³C NMR (CDCl₃, 151 MHz): δ = 196.51 (CO, SAc), 174.26 (CO, hexanoyl), 171.25, 170.95, 170.87, 170.75, 169.77, 169.39 (CO, 6xAc), 100.73 (C-1'), 95.74 (C-1''), 93.87 (C-1), 73.61 (C-4''), 72.94 (C-3'), 71.92 (C-5'), 71.21 (C-5), 70.78 (C-3), 69.86 (C-3''), 69.30 (C-5''), 68.71 (C-4'), 68.30 (C-4), 66.99 (C-6), 66.82 (C-6''), 62.06 (C-6'), 60.59 (d, $J_{C2'',P}$ = 8.3 Hz, C-2''), 53.21 (C-2'), 51.71 (d, $J_{C2,P}$ = 8.2 Hz, C-2), 35.91 (α -CH₂), 30.62 (CH₃, SAc), 29.31 (β -CH₂), 28.75 (ϵ -CH₂), 28.09 (γ -CH₂), 27.56, 27.33, 25.64 (CH₃, DTBS, TBDMS), 25.12 (δ -CH₂), 23.38, 23.09, 20.76, 20.74, 20.68, 20.61 (CH₃, Ac), -4.07, -4.81 (CH₃, TBDMS);

^{31}P NMR (CDCl_3 , PPh_3 external, 243 MHz): $\delta = -0.89$

HRMS (ESI-TOF): calcd for $\text{C}_{52}\text{H}_{87}\text{N}_5\text{O}_{24}\text{PSSi}_2$ m/z $[\text{M-H}]^-$ 1284.4738, found: 1284.4726.

Synthesis of 19



2-Azido-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranose (ammonium salt) (19)

To a cooled (4°C) stirred solution of **17** (60 mg, 46 μmol) in dry THF (2.5 ml) in a PTFE flask was added a solution of $\text{HF}\cdot\text{Py}$ (150 μl) in pyridine (1 ml) dropwise *via* syringe. The reaction mixture was allowed to warm to r.t. and stirred for 12 h. The reaction mixture was diluted with $\text{CHCl}_3/\text{MeOH}/25\%$ aq. NH_4OH (4:1:0.05, 10 ml) and mixed with S60 silica gel (2 g). The solids (Si-F precipitate + silica gel) were removed by filtration, the filtrate was concentrated, and the residue was purified by column chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 8:2:0.1 \rightarrow 8:4:0.6) to give **19** (45.3 mg, 94%) as a colorless solid. $R_f = 0.11$ ($\text{CHCl}_3/\text{MeOH}/25\%$ aq. NH_4OH , 4:1:0.05); $[\alpha]_{\text{D}}^{20} = +53$ (c 0.85, MeOH);

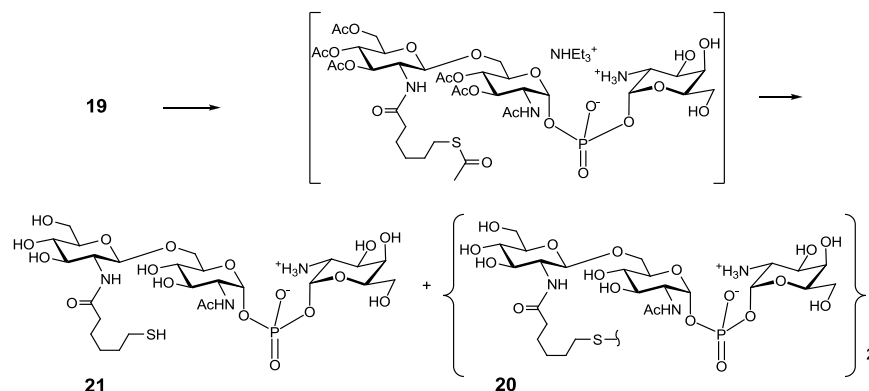
^1H NMR (MeOD, 600 MHz): $\delta = 5.62$ (dd, $J_{1',2''} = 3.4$ Hz, $J_{1',\text{P}} = 7.8$ Hz, 1H, H-1''), 5.58 (dd, $J_{1,2} = 3.5$ Hz, $J_{1,\text{P}} = 6.9$ Hz, 1H, H-1), 5.28 (dd, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 9.3$ Hz, 1H, H-3), 5.25 (dd, $J_{3',4'} = 9.4$ Hz, $J_{2',3'} = 10.3$ Hz, 1H, H-3'), 5.03 (dd, $J_{4,5} = 10.1$ Hz, 1H, H-4), 4.99 (dd, $J_{4',5'} = 9.7$ Hz, 1H, H-4'), 4.77 (d, $J_{1',2'} = 8.5$ Hz, 1H, H-1'), 4.31 (m, 1H, H-5), 4.29 (dd, $J_{6a',6b'} = 12.2$ Hz, $J_{6a',5'} = 4.7$ Hz, 1H, H-6a'), 4.28 (m, 1H, H-2), 4.11 (dd, 1H, $J_{5'',6b''} = 2.5$ Hz, H-6''b), 4.10 (m, 1H, H-5''), 4.01 (dd, $J_{2'',3''} = 10.4$ Hz, $J_{3'',4''} = 3.3$ Hz, 1H, H-3''), 3.94 (d, 1H, $J_{4'',5''} = 3.5$ Hz, H-4''), 3.92 (m, 1H, H-2'), 3.91 (dd, $J_{6a,6b} = 11.3$ Hz, $J_{6a,5} = 5.6$ Hz, 1H, H-6a), 3.814 (m, 1H, H-5), 3.810 (dd, $J_{6'a,6''b} = 11.5$ Hz, $J_{5'',6''a} = 7.6$ Hz, 1H, H-6''a), 3.71 (dd, $J_{5'',6b''} = 4.2$ Hz, 1H, H-6''b), 3.68 (m, 1H, H-2''), 3.65 (dd, $J_{6b,5} = 4.4$ Hz, 1H, H-6b), 2.88 (t, $J = 7.3$ Hz, 2H, $\epsilon\text{-CH}_2\text{-SAC}$), 2.30 (s, 3H, CH_3 , SAC), 2.29 (m, 1H, $\alpha\text{-CH}_2$, hexanoyl), 2.24 (m, 1H, $\alpha\text{-CH}_2$, hexanoyl), 2.06, 2.02, 2.00, 1.97, 1.96, 1.95 (6s, 18H, CH_3 , $5\times\text{Ac}$, NHAc), 1.60 (m, 4H, $\beta\text{-CH}_2$, $\delta\text{-CH}_2$), 1.40 (m, 2H, $\gamma\text{-CH}_2$), 1.51 (NH_4^+);

^{13}C NMR (MeOD, 151 MHz): $\delta = 197.82$ (CO, SAC), 176.77 (CO, hexanoyl), 173.72, 172.63, 172.14, 171.99, 171.67, 171.57 (CO, $6\times\text{Ac}$), 101.72 (C-1'), 95.93 (d, $J_{\text{C}1',\text{P}} = 5.8$ Hz, C-1''), 95.73 (d, $J_{\text{C}1,\text{P}} = 6.1$ Hz, C-1), 74.64 (C-3'), 74.15 (C-5''), 73.01 (C-5'), 72.68 (C-3), 71.61 (C-5), 71.05 (C-4''), 70.51 (C-4, C-4'), 69.82 (C-3''), 68.58 (C-6), 63.45 (C-6'), 63.16 (C-6''), 62.04 (d, $J_{\text{C}2'',\text{P}} = 8.5$ Hz, C-2''), 55.41 (C-2), 53.09 (d, $J_{\text{C}2,\text{P}} = 8.3$ Hz, C-2), 37.23 ($\alpha\text{-CH}_2$), 30.67 (CH_3 , SAC, under the signal of $\beta\text{-CH}_2$), 30.67 ($\beta\text{-CH}_2$), 29.89 ($\epsilon\text{-CH}_2$), 29.49 ($\gamma\text{-CH}_2$), 26.53 ($\delta\text{-CH}_2$), 22.77, 20.90, 20.83, 20.81, 20.76 ($6\times\text{CH}_3$, Ac, NHAc);

^{31}P NMR (MeOD, PPh_3 ext. standard, 243 MHz): $\delta = -1.8$;

HRMS (ESI-TOF): calcd for $\text{C}_{38}\text{H}_{57}\text{N}_5\text{O}_{24}\text{PS}$ m/z $[\text{M-H}]^-$ 1030.2857, found: 1030.2870.

Synthesis of 20 and 21



2-Amino-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-*O*-[2-deoxy-2-(6-mercapto-hexanoyl)amino- β -D-glucopyranosyl]- α -D-glucopyranose (**21**)

To a stirred solution of **19** (17 mg, 16.2 μ mol) in $\text{CHCl}_3/\text{MeOH}$ (2:1, 3 ml) was added a solution of $\text{Sn}(\text{SPh})_3 \cdot \text{Et}_3\text{N}$ complex in CHCl_3 (3 ml). [$\text{Sn}(\text{SPh})_3 \cdot \text{Et}_3\text{N}$ complex was prepared as follows: to a suspension of $\text{Sn}(\text{SPh})_2$ (10.9 mg, 32.4 μ mol) (prepared by mixing of stoichiometric amounts of sodium thiophenolate and SnCl_2 in acetonitrile, followed by separation of precipitate by filtration) in CHCl_3 (3 ml), Et_3N (4.4 μ l, 32.4 μ mol) and thiophenole (3.4 μ l, 32.4 μ l) were added successively. Complete disappearance of solids indicated a formation of a soluble $\text{Sn}(\text{SPh})_3 \cdot \text{Et}_3\text{N}$ complex]. The reaction mixture was stirred for 6 h, then diluted with toluene (5 ml) and concentrated to dryness. The residue was dissolved in water (10 ml) and washed with hexane/diethylether (1:1, 3 \times 5 ml) and CHCl_3 (2 \times 5 ml). The aqueous phase was separated, placed in a flask and stirred with disodium EDTA dihydrate (145 mg, 390 μ mol) at pH 5 (addition of satd. aq NaHCO_3) for 30 min. The mixture was washed with hexane/diethylether (1/1, 2 \times 10 ml) followed by CHCl_3 (2 \times 10 ml). The aqueous phase was lyophilized and the residue was purified by size exclusion chromatography on Sephadex LH-20 using methanol as mobile phase. Appropriate fractions were pooled and concentrated to provide free amine **2-amino-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranoside** (13.9 mg, 82%). R_f = 0.64 ($\text{CHCl}_3/\text{MeOH}/25\%$ aq. NH_4OH , 20:10:0.3); HRMS (ESI-TOF): calcd. for $\text{C}_{38}\text{H}_{57}\text{N}_5\text{O}_{24}\text{PS}$ m/z [M-H] $^-$ 1004.2952; found: 1004.2948. The free amine was kept frozen at -30°C or immediately subjected to deacetylation according to one of the following procedures:

Deacetylation procedure A:

The crude free amine (5.5 mg, 5.47 μ mol) was dissolved in a degassed and Ar-stripped mixture of $\text{MeOH}/\text{H}_2\text{O}/\text{Et}_3\text{N}$ (7/3/1, pH 11.5, 2 ml). After stirring for 16 h under exclusion of light, water (8 ml) was added and the mixture was frozen and lyophilized. The residue was purified by size exclusion chromatography on a BioGel P2 (BioRad) column (10 \times 250 cm, 5% EtOH in water) to provide **21** (as a mixture of a 1:1 thiol (**21**)/disulfide (**20**) which was contaminated with 20 % of hydrolysis product resulting from the cleavage of GalN). Yield (20% hydrolysis product): 3.9 mg (~76% from **19**).

Deacetylation procedure B:

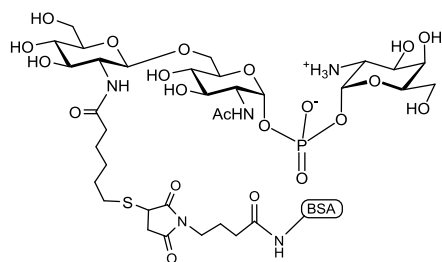
The free amine 2-amino-2-deoxy- α -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- β -D-glucopyranosyl]-2-deoxy- α -D-glucopyranoside (9 mg, 8.9 μ mol) was dissolved in MeOH (1 ml) and a solution of 50% of hydroxylamine in water (1 ml) was added. After stirring for 12 h under exclusion of light the mixture was diluted with water (10 ml) and lyophilized. The residue was purified by column chromatography on a Superdex Peptide (BioRad) column (10/300) which was equilibrated with 50 ml of an ammonium bicarbonate buffer (0.3 M) in water/ethanol (95/5, v/v) at a flow rate of 0.7 ml/min and a pressure of 12-14 bar. The α,α -phosphodiester **20** was isolated as inner salt (pH 7) (3.0 mg, 3.9 μ mol, 44% from **19**), R_f = 0.16 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 1/1/0.3); and 4 mg of **20** containing 30% of hydrolysis product resulting from the loss of GalN: $\beta\text{GlcN}(1\rightarrow6)\alpha\text{GlcN}(1\rightarrow\text{P})$.

^1H NMR (D_2O , DSS ext. standard, 600 MHz, pH = 7): δ = 5.72 (dd, $J_{1'',2''} = 3.5$ Hz, $^3J_{\text{P,H-1''}} = 6.5$ Hz, 1H, H-1''), 5.45 (dd, $J_{1,2} = 3.4$ Hz, $^3J_{\text{P,H-1}} = 7.3$ Hz, 1H, H-1), 4.59 ($J_{1',2'} = 8.5$ Hz, 1H, H-1'), 4.11 (dd, $J_{5'',6''\text{b}} = 4.7$ Hz, $J_{4'',5''} = 7.6$ Hz, 1H, H-5''), 4.05 (dd, $J_{5,6\text{a}} = 1.8$ Hz, $J_{6\text{a},6\text{b}} = 11.9$ Hz, 1H, H-6a), 4.03 (d, $J_{5'',4''} = J_{3'',4''} = 2.9$ Hz, 1H, H-4''), 4.02 (dd, $J_{2'',3''} = 10.9$ Hz, 1H, H-3''), 3.99 (ddd, $J_{5,6\text{a}} = 1.8$ Hz, $J_{5,6\text{b}} = 4.6$ Hz, $J_{4,5} = 10.1$ Hz, 1H, H-5), 3.92 (ddd, $J_{2,3} = 10.1$ Hz, $^3J_{\text{P,H-2}} = 2.7$ Hz, 1H, H-2), 3.91 (dd, $J_{5',6'\text{a}} = 1.7$ Hz, $J_{6'\text{a},6'\text{b}} = 10.8$ Hz, 1H, H-6'a), 3.90 (dd, $J_{5,6\text{b}} = 4.7$ Hz, 1H, H-6b), 3.78 (dd, $J_{6'',6''\text{b}} = 12.1$ Hz, 1H, H-6''a), 3.76 (dd, 1H, H-6''b), 3.75 (d, $J_{2',3'} = 10.2$ Hz, 1H, H-2'), 3.74 (dd, $J_{3,4} = 8.5$ Hz, 1H, H-3), 3.73 (dd, $J_{5',6'\text{b}} = 5.2$ Hz, 1H, H-6'b), 3.54 (dd, $J_{3',4'} = 8.4$ Hz, 1H, H-3'), 3.47 (dd, 1H, H-4), 3.46 (ddd, 1H, H-2''), 3.43 (m, 1H, H-5), 3.42 (dd, $J_{4',5'} = 9.5$ Hz, 1H, H-4'), 2.75 (t, $J = 7.2$ Hz, 2H, $\epsilon\text{-CH}_2\text{-SAc}$), 2.31 (m, 1H, $\alpha\text{-CH}_2$, hexanoyl), 2.03 (s, 3H, CH_3 , SAc), 1.70 (m, 2H, $\beta\text{-CH}_2$, hexanoyl), 1.63 (m, 2H, $\delta\text{-CH}_2$, hexanoyl), 1.42 (m, 2H, $\gamma\text{-CH}_2$, hexanoyl);

^{13}C NMR (D_2O , dioxane ext. standard, 151 MHz, pH = 7): δ = 180.69 (CO, 2-NHAc), 175.06 (CO, 2'-NH-hexanoyl), 102.23 (C-1'), 94.82, 94.78 (C-1, C-1''), 76.51 (C-5'), 74.41 (C-3'), 73.0 (C-5''), 72.96 (C-5), 70.99 (C-3), 70.59 (C-4'), 70.20 (C-4), 68.96 (C-6), 68.81 (C-4''), 68.83 (C-3''), 61.71 (C-6''), 61.30 (C-6'), 55.93 (C-2'), 54.09 (d, $J_{\text{C,P}} = 8.2$ Hz, C-2), 51.46 (d, $J_{\text{C,P}} = 8.8$ Hz, C-2''), 38.60 ($\epsilon\text{-CH}_2\text{S-S-}$), 36.52 ($\alpha\text{-CH}_2$), 28.62 ($\beta\text{-CH}_2$), 27.71 ($\gamma\text{-CH}_2$), 25.57 ($\delta\text{-CH}_2$), 22.64 (CH_3 , NHAc);

^{31}P NMR (D_2O , H_3PO_4 ext. standard, 243 MHz): δ = -2.94

HRMS (ESI-TOF): calcd for $\text{C}_{26}\text{H}_{47}\text{N}_3\text{O}_{18}\text{PS}$ m/z $[\text{M-H}]^-$ 752.2318; found: 752.2324.



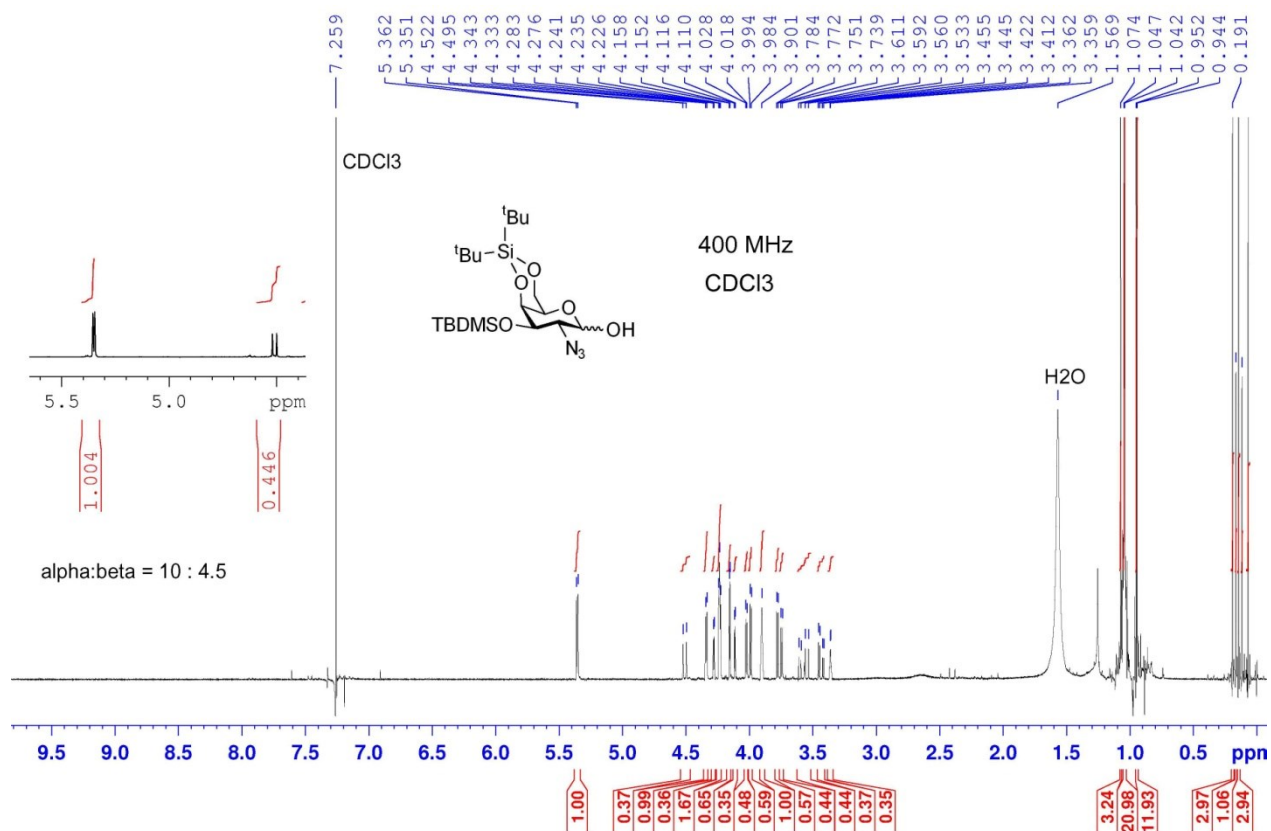
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl; 0.7 mg, 2.44 μmol) was dissolved in 0.3 M ammonium bicarbonate buffer (2 ml, Ar-stripped, pH = 5). **20** (1.5 mg, 1 μmol) was added to this solution and the pH was again adjusted to 5. Ar was bubbled through the solution for 20 min and the solution was freeze-dried. After lyophilization, the reduction of the disulfide **20** to the corresponding thiol **21** was confirmed by ^1H - and HSQC – NMR. The resulting thiol **21** was directly coupled to maleimide-activated BSA according to the manufacturer (Sigma-Aldrich) instructions. The BSA-conjugate was purified by size exclusion chromatography on a pre-packed Sephadex G25 column and on a Bio-Gel P2 (BioRad) column (60 cm) using ultrapure water as eluent. After lyophilization the BSA-conjugate was further purified by dialysis against ultra-pure water (2 l). Yield 4.5 mg. Sugar-loading was analyzed by MALDI-TOF mass spectrometry (BSA-**20**: 78 kDa vs. BSA: 66.6 kDa) and comprised 13 pseudo-trisaccharide units per BSA molecule.

References

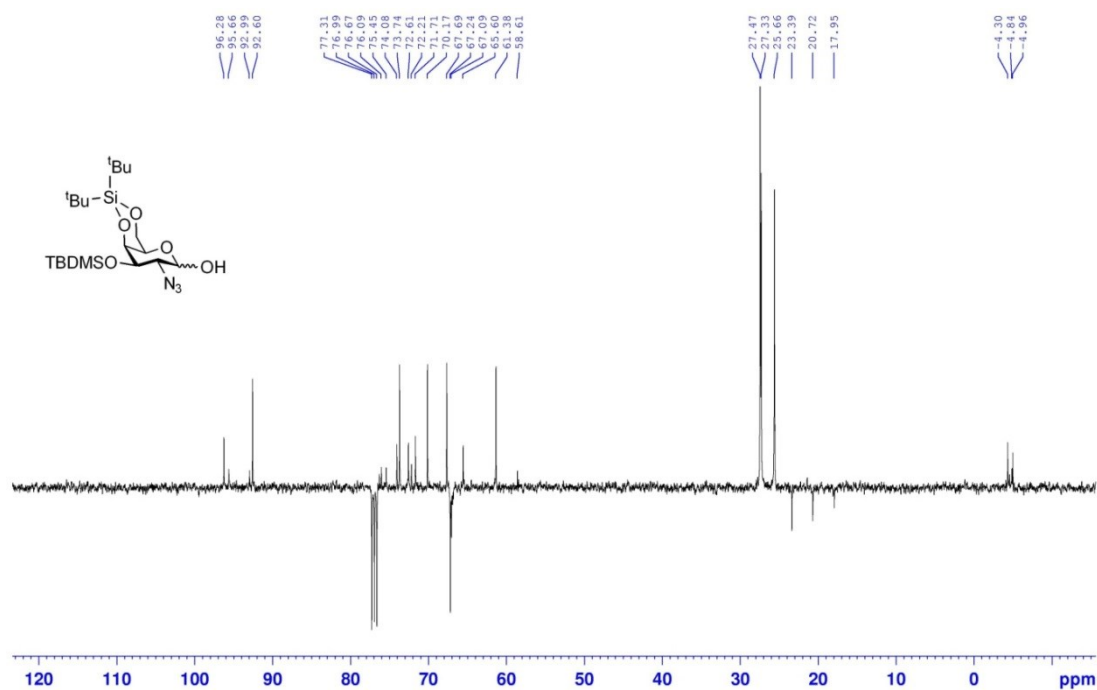
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^1H -, ^{13}C -, ^{31}P -NMR spectra, HSQC-NMR and MALDI-TOF spectra of the target compounds

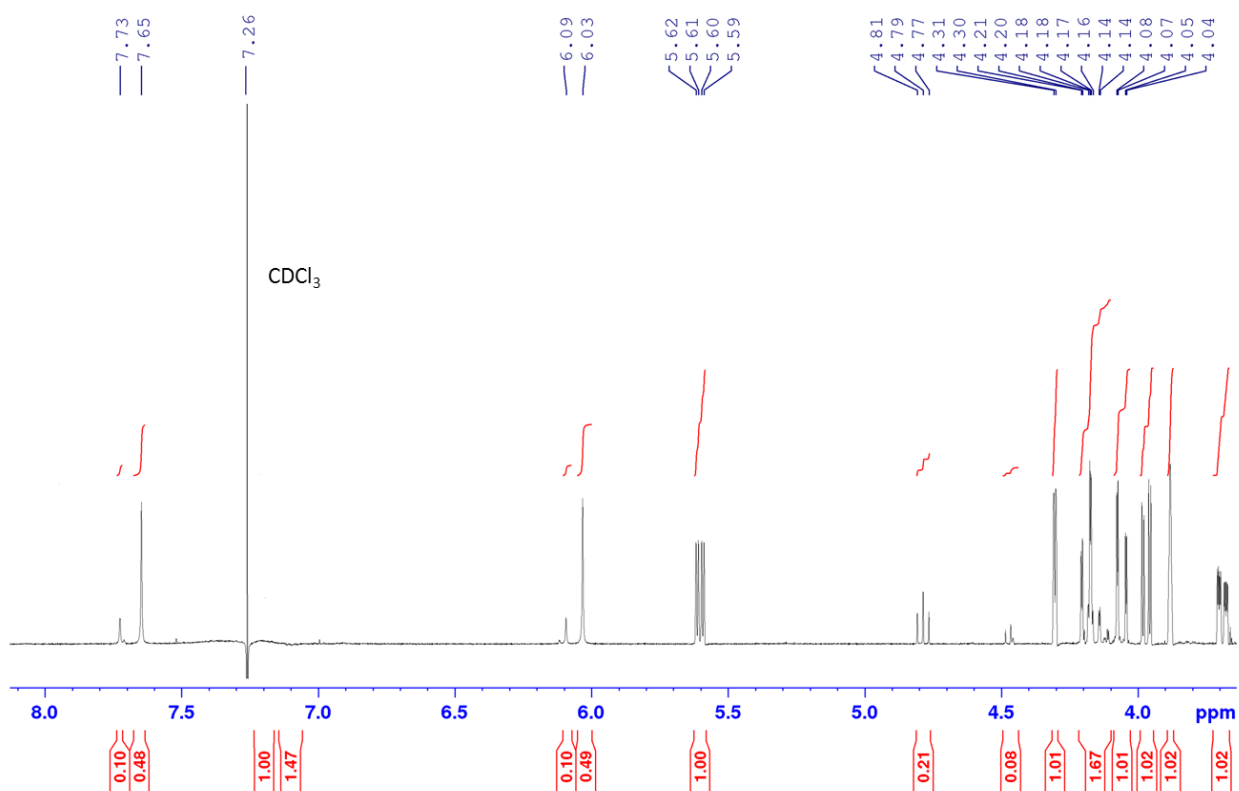
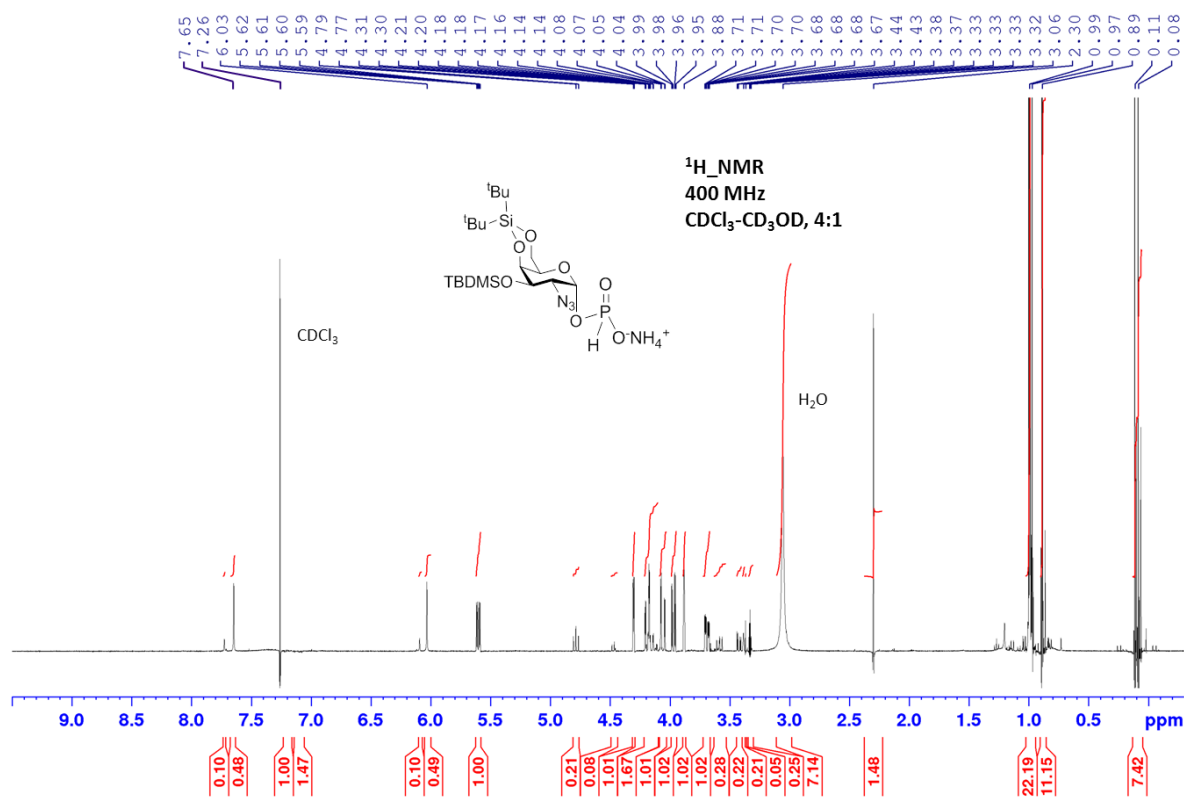
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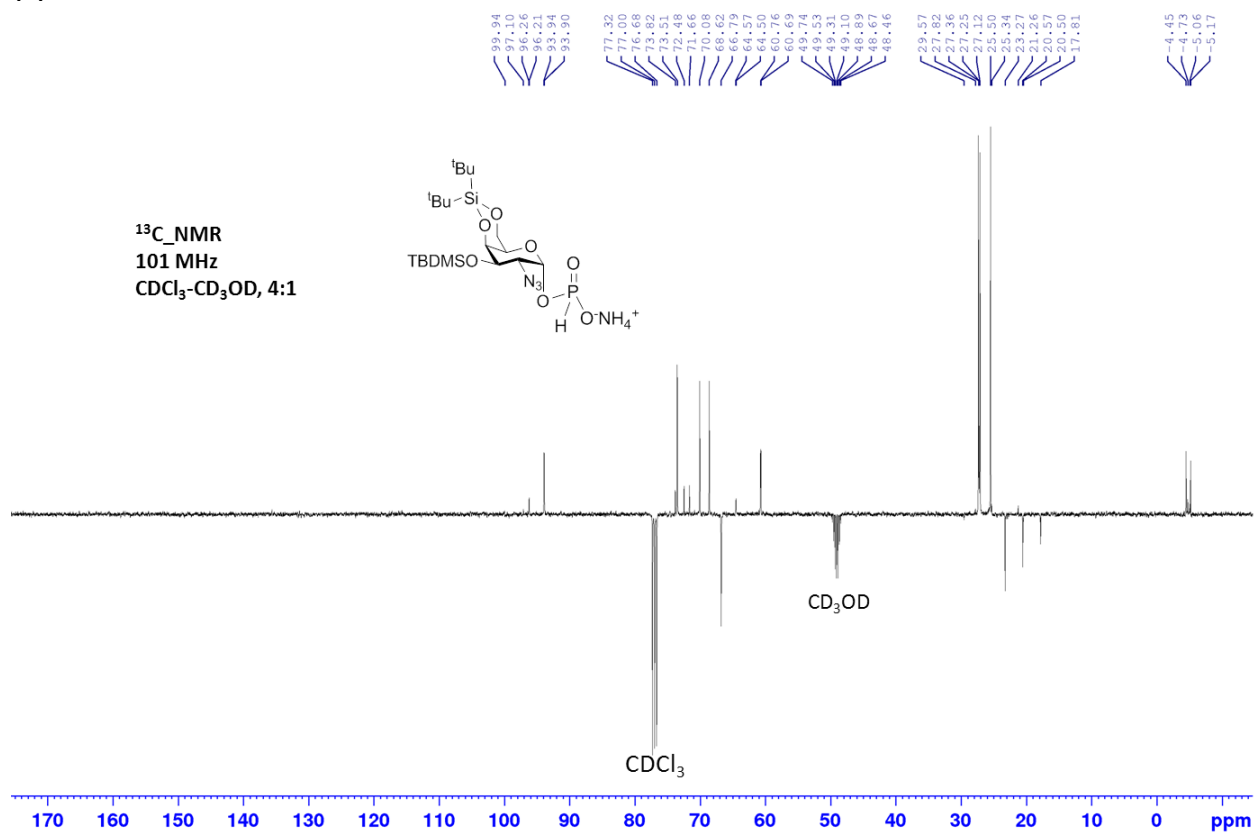
(4): ^{13}C -NMR, APT, 101 MHz, CDCl_3



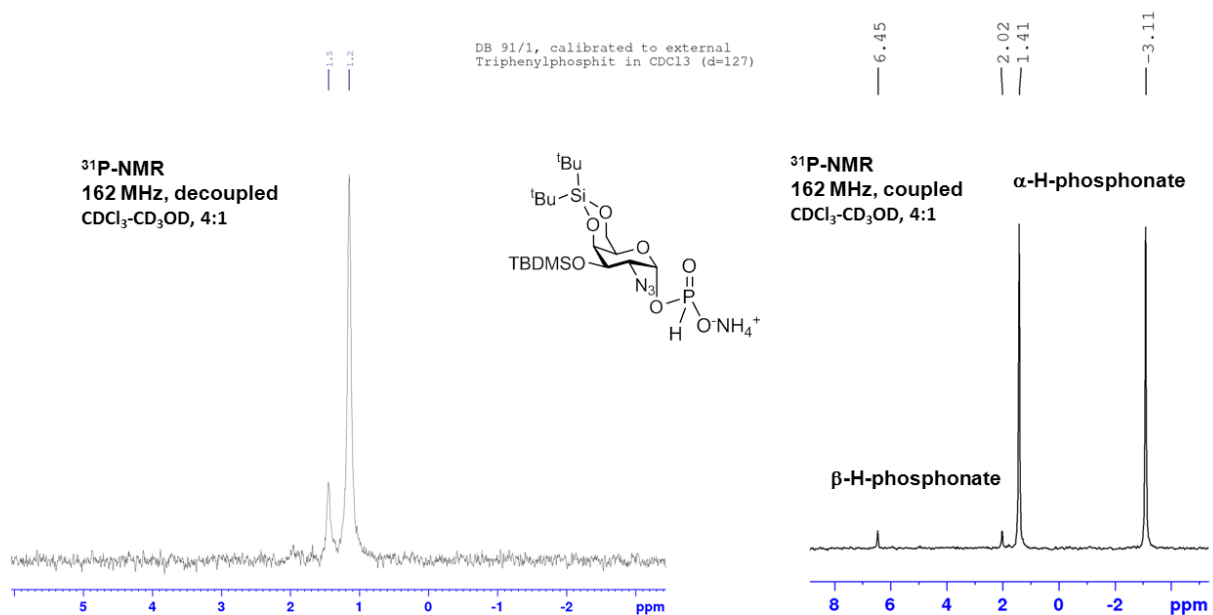
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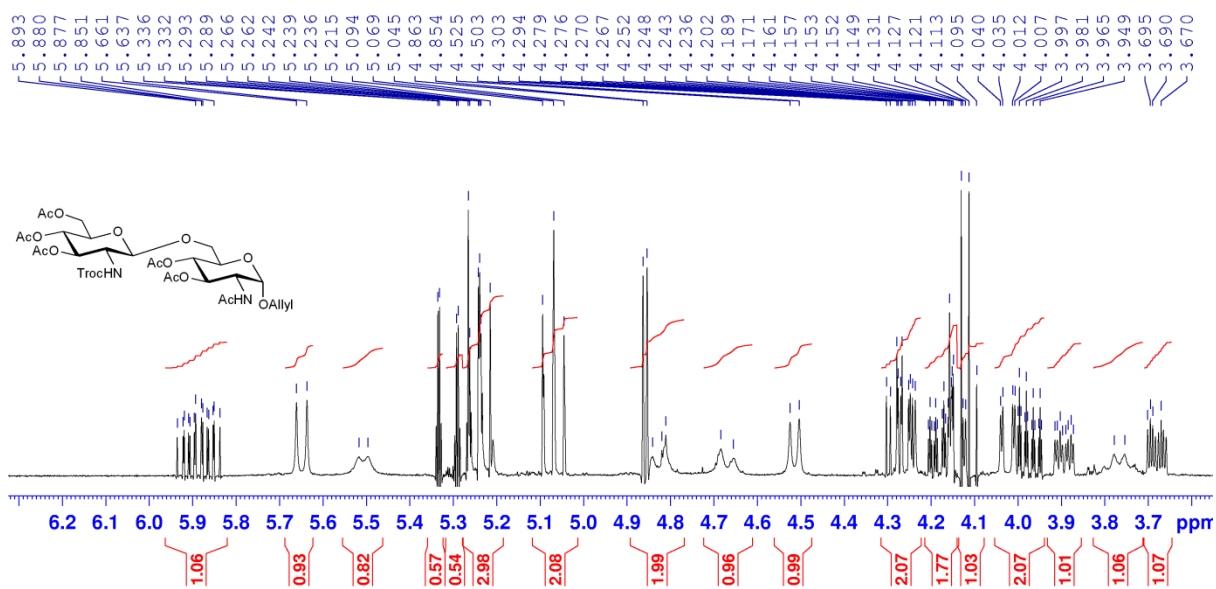
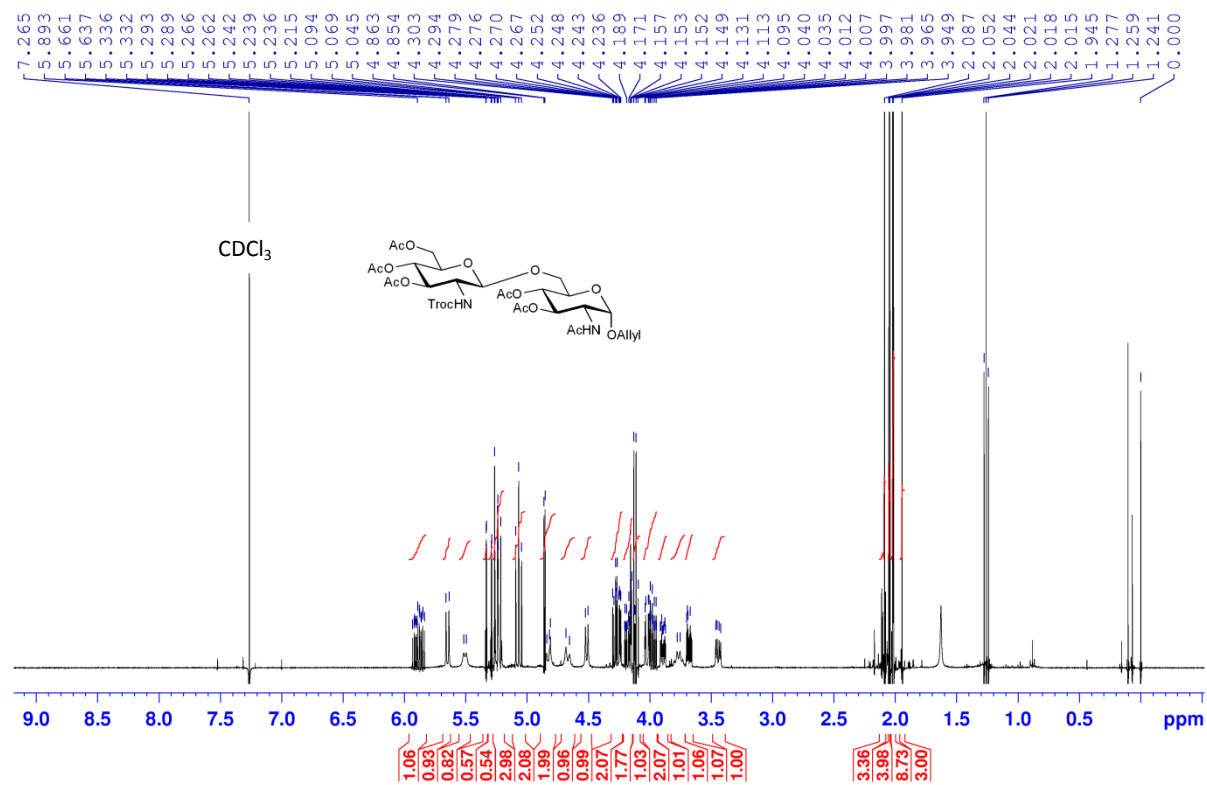
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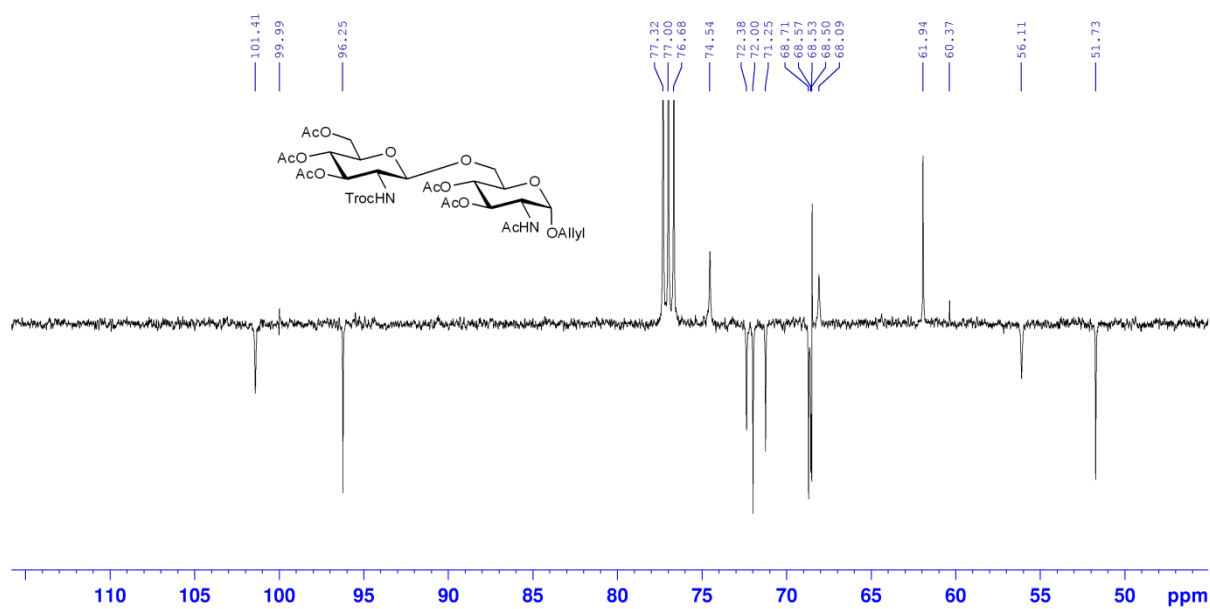
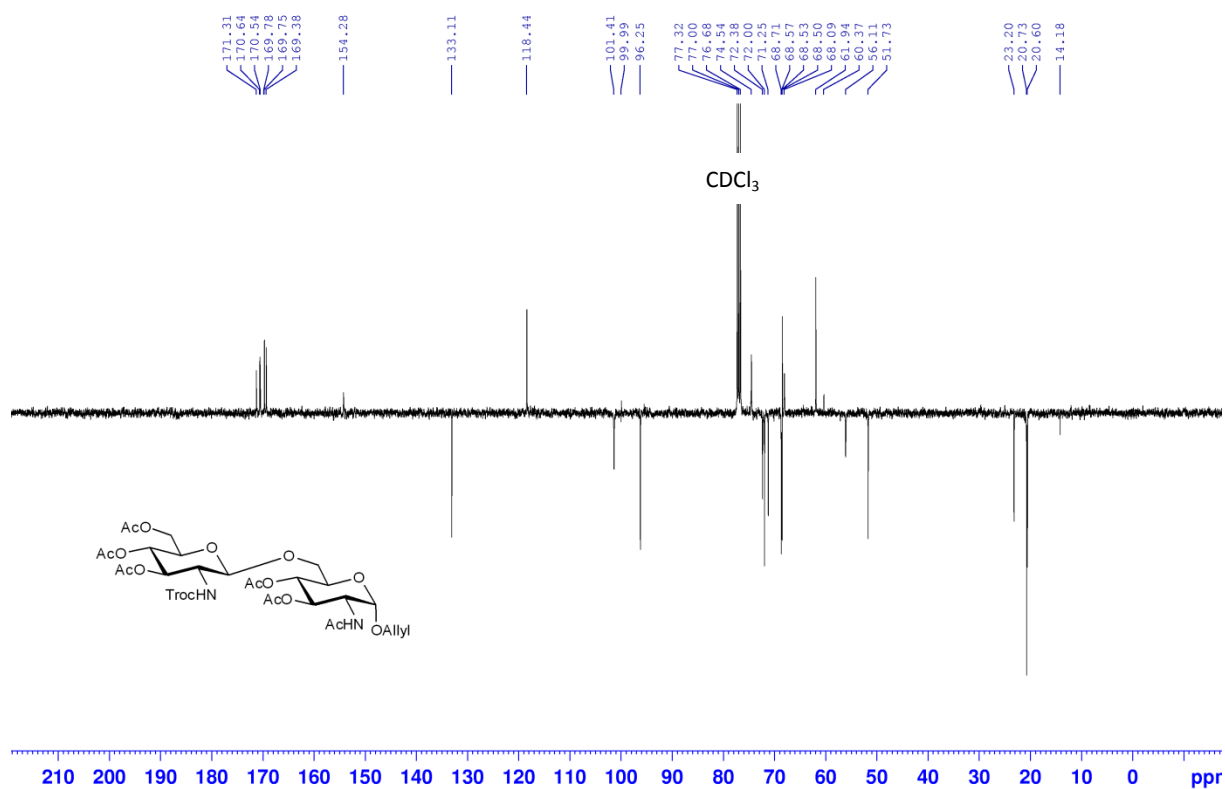
(5): $\alpha/\beta = 5:1$



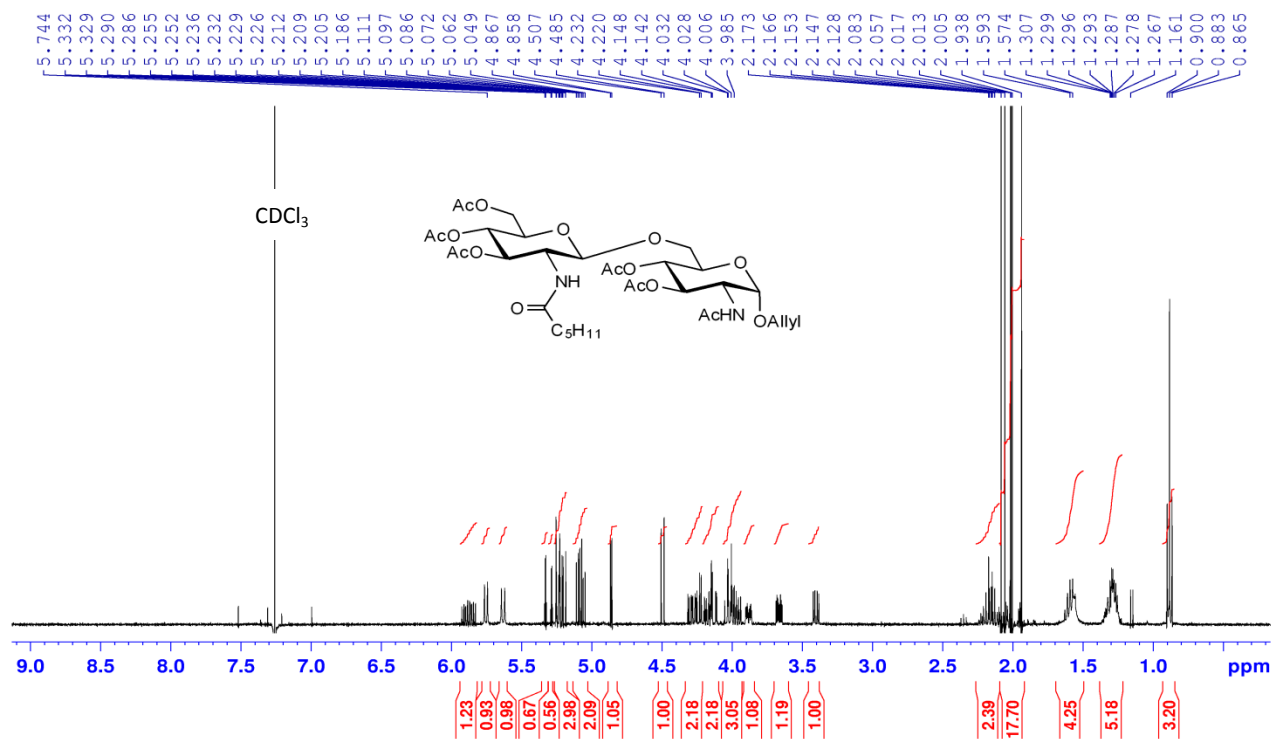
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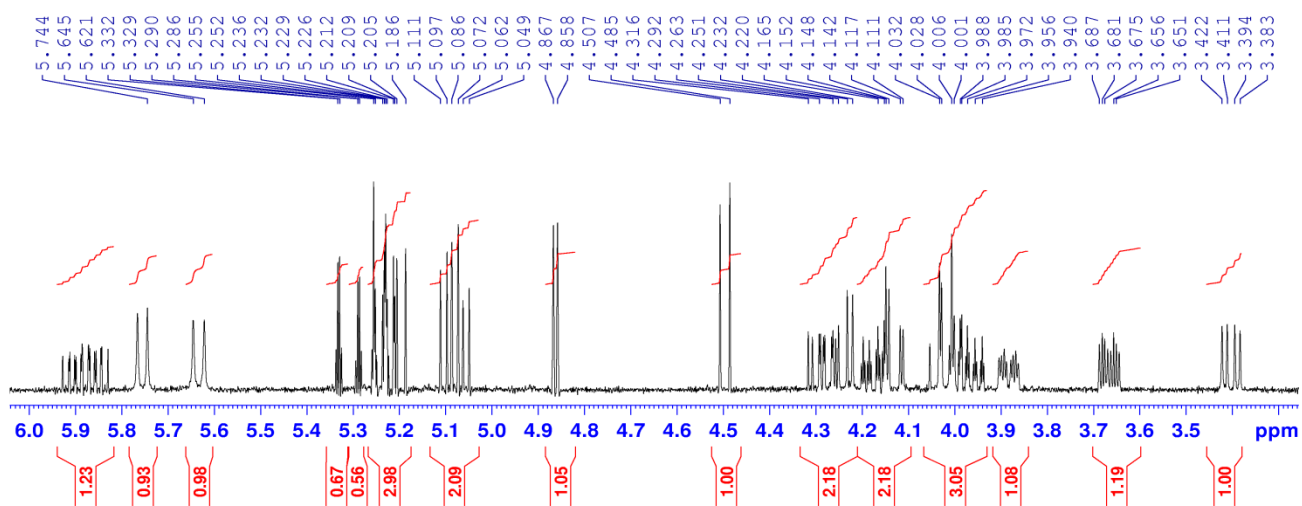
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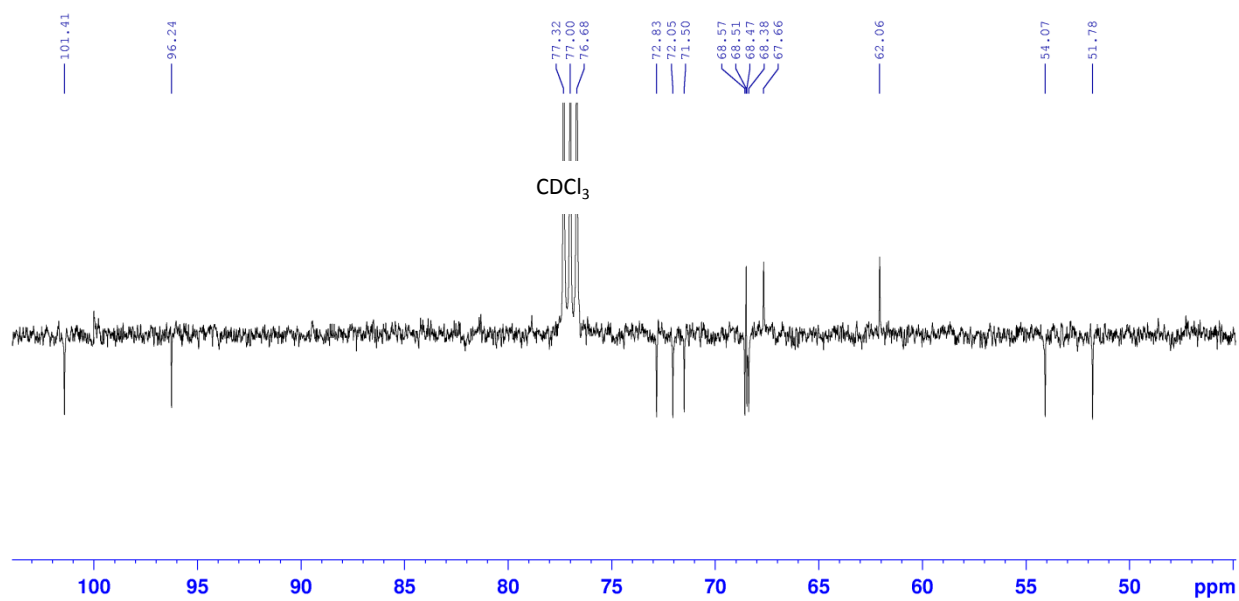
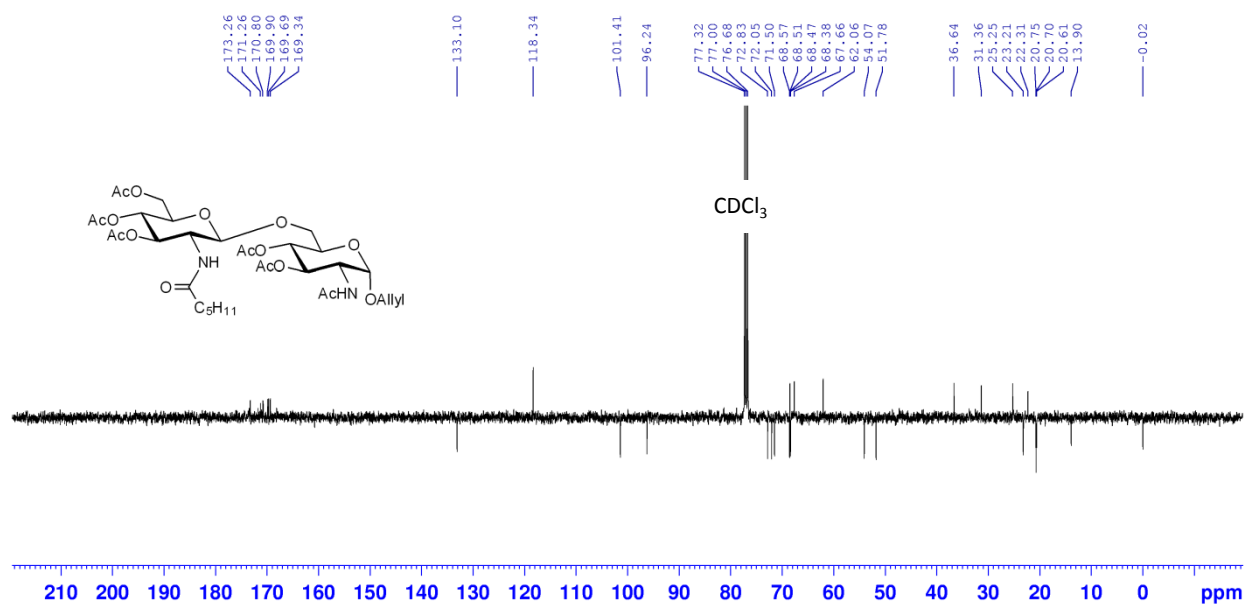
(9): ^1H -NMR, 400 MHz, CDCl_3



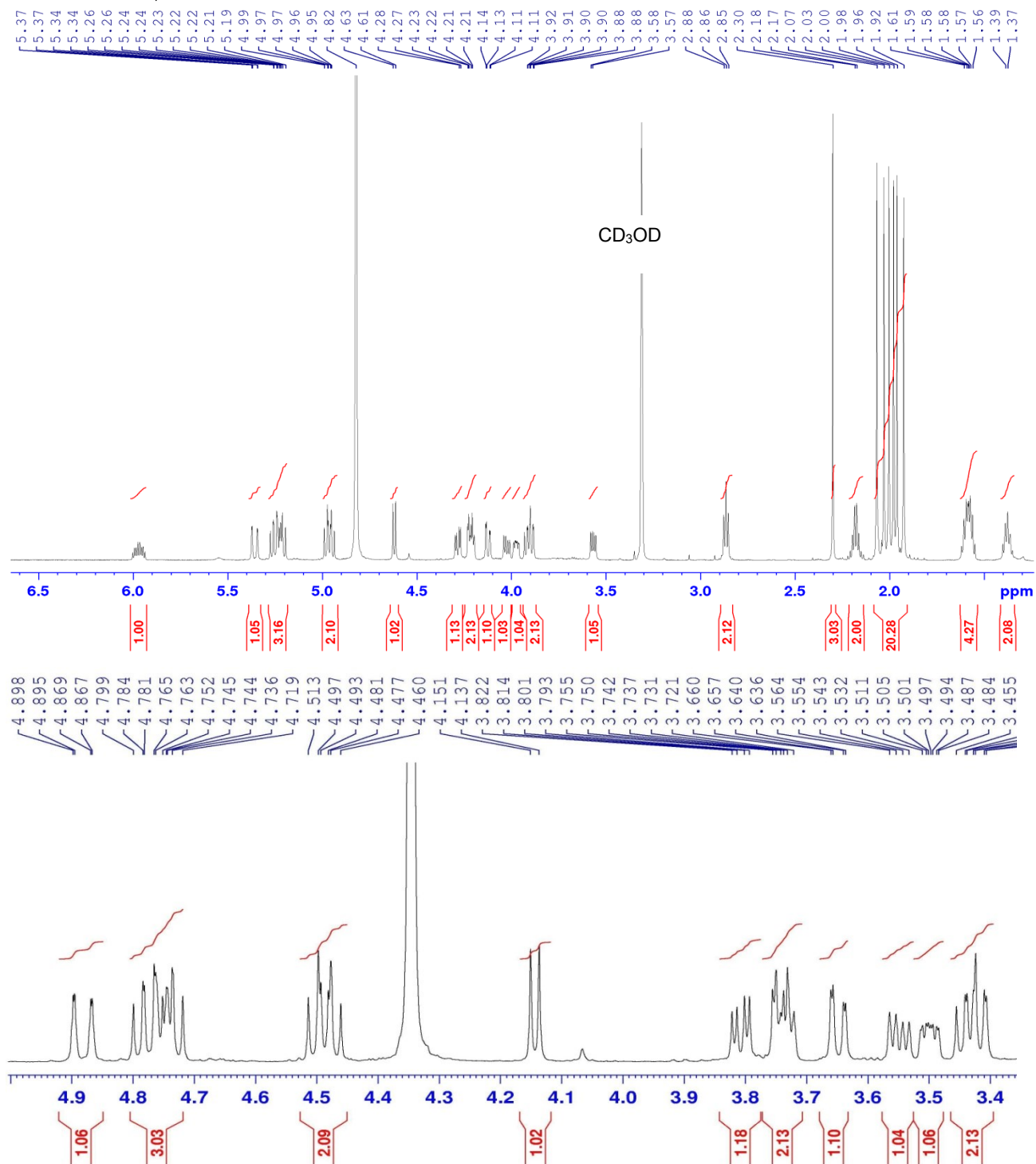
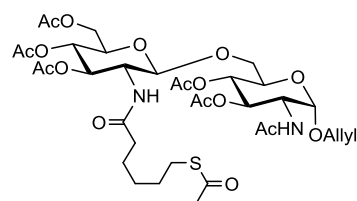
(9): ^1H -NMR, 400 MHz, CDCl_3



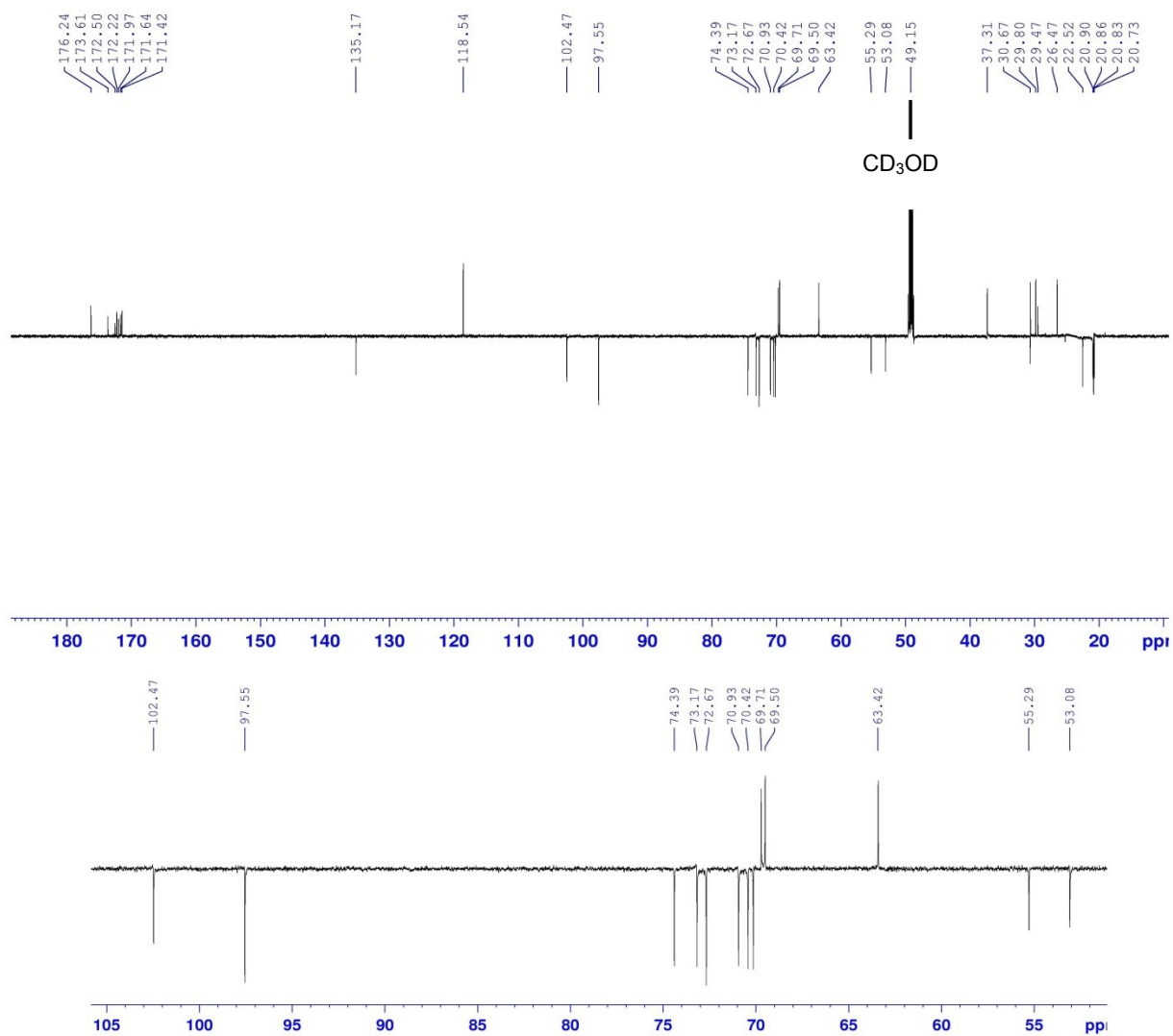
(9): ^{13}C -NMR, 101 MHz, CDCl_3



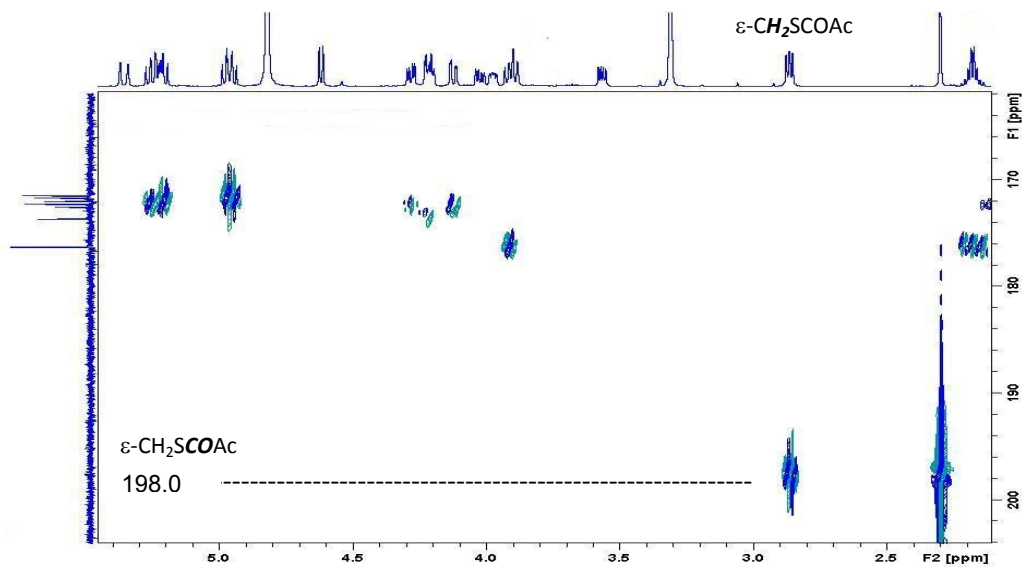
(10): ^1H -NMR, 600 MHz, CD_3OD



(10): ^{13}C -NMR, 151 MHz, CD_3OD



(10): HMBC, MeOD

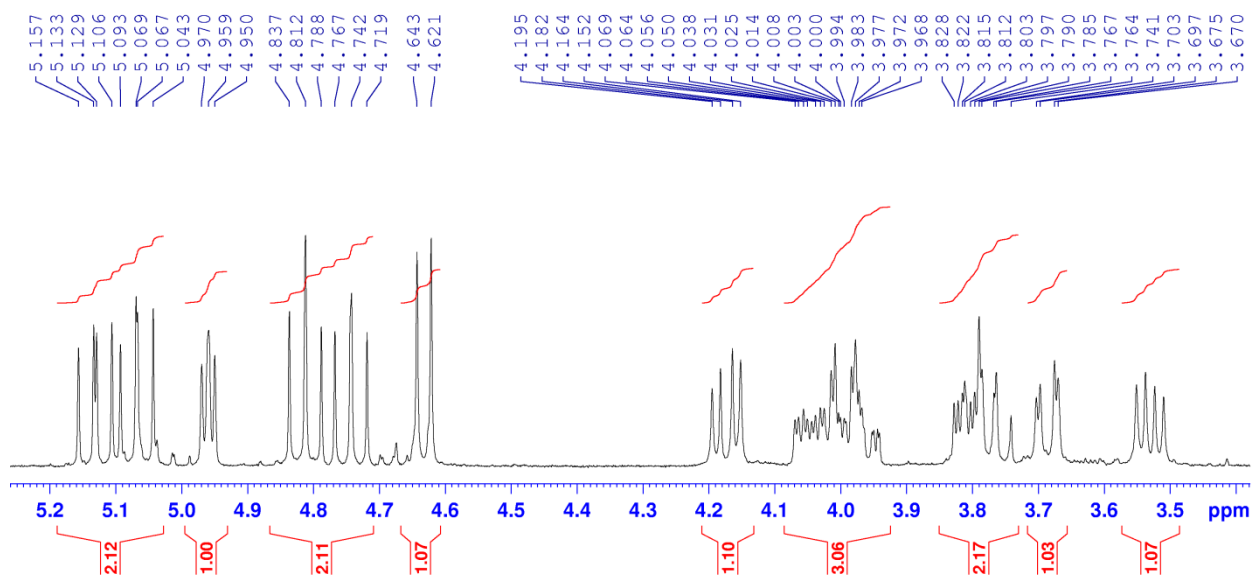


Chemical structure of compound 1 is shown above the spectrum. The structure is a disaccharide derivative with an acetate group and a C₅H₁₁ group. The ratio $\alpha/\beta = 9:1$.

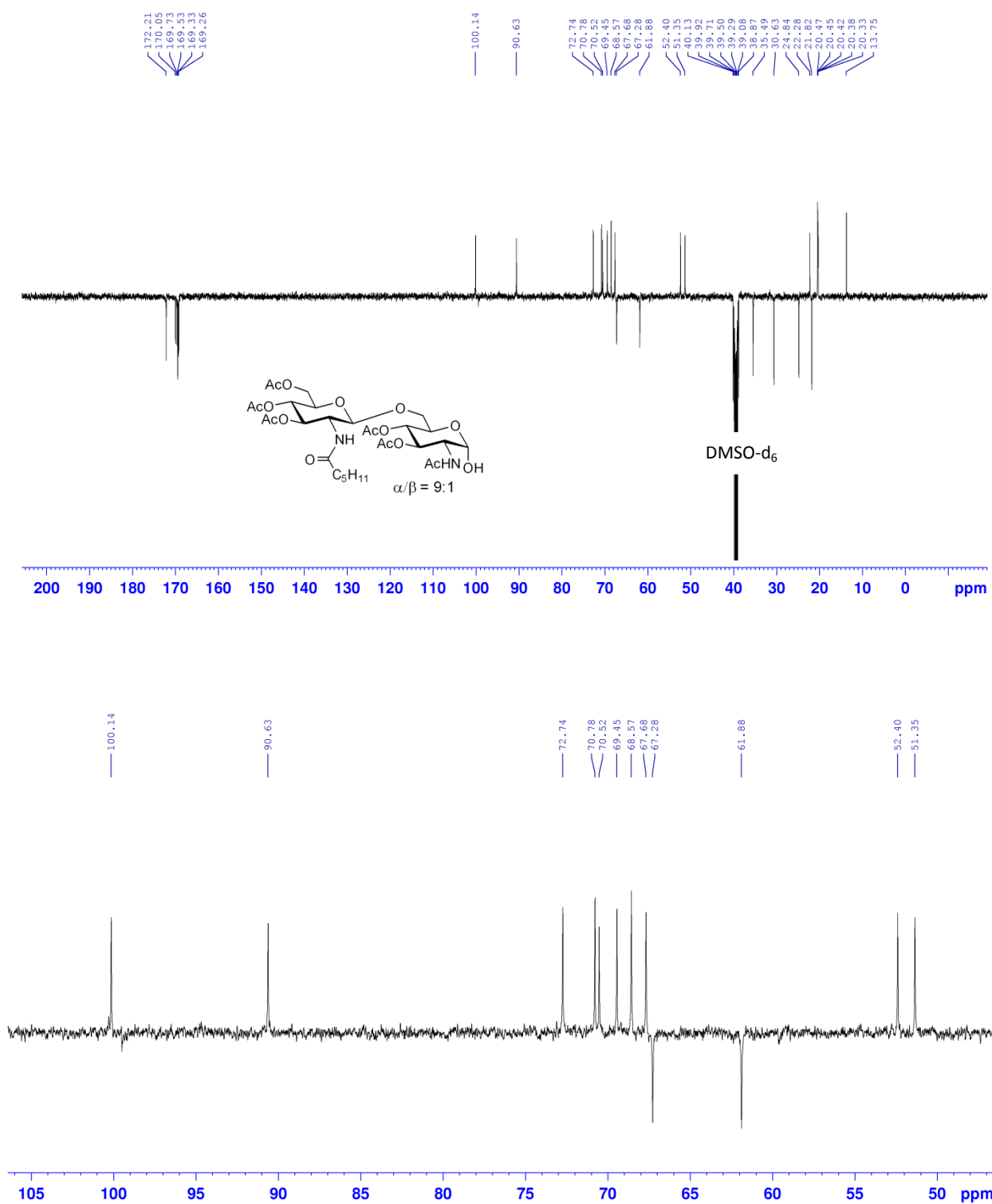
The spectrum shows peaks from 0.8 to 7.8 ppm. The chemical shift values (ppm) are listed on the right side of the spectrum. The integration values for each peak group are listed below the spectrum.

Chemical shift values (ppm): 7.778, 7.692, 7.669, 7.054, 7.051, 7.043, 7.040, 5.157, 5.133, 5.129, 5.106, 5.093, 5.069, 5.067, 5.043, 4.970, 4.959, 4.950, 4.837, 4.812, 4.788, 4.767, 4.742, 4.719, 4.643, 4.621, 4.182, 4.164, 4.152, 4.014, 4.008, 3.983, 3.977, 3.790, 3.785, 3.764, 3.675, 3.538, 3.314, 2.500, 2.024, 1.968, 1.962, 1.891, 1.879, 1.796, 1.468, 1.450, 1.431, 1.265, 1.248, 1.231, 1.216, 1.200, 1.182, 0.866, 0.848, 0.830.

Integration values: 0.96, 0.98, 0.93, 0.06, 2.12, 1.00, 2.11, 1.07, 1.10, 3.06, 2.17, 1.93, 1.07, 5.35, 6.30, 6.28, 2.76, 2.26, 5.06, 3.40.

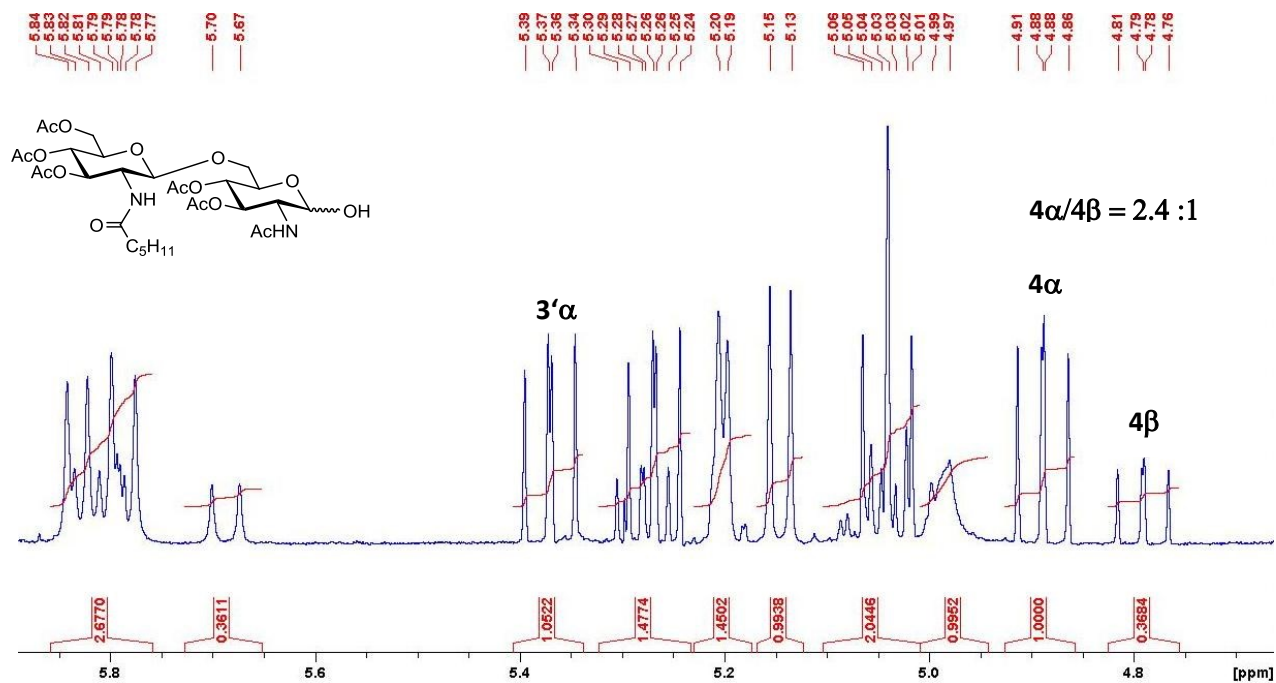


(11): ^{13}C -NMR, APT, 101 MHz, DMSO- d_6

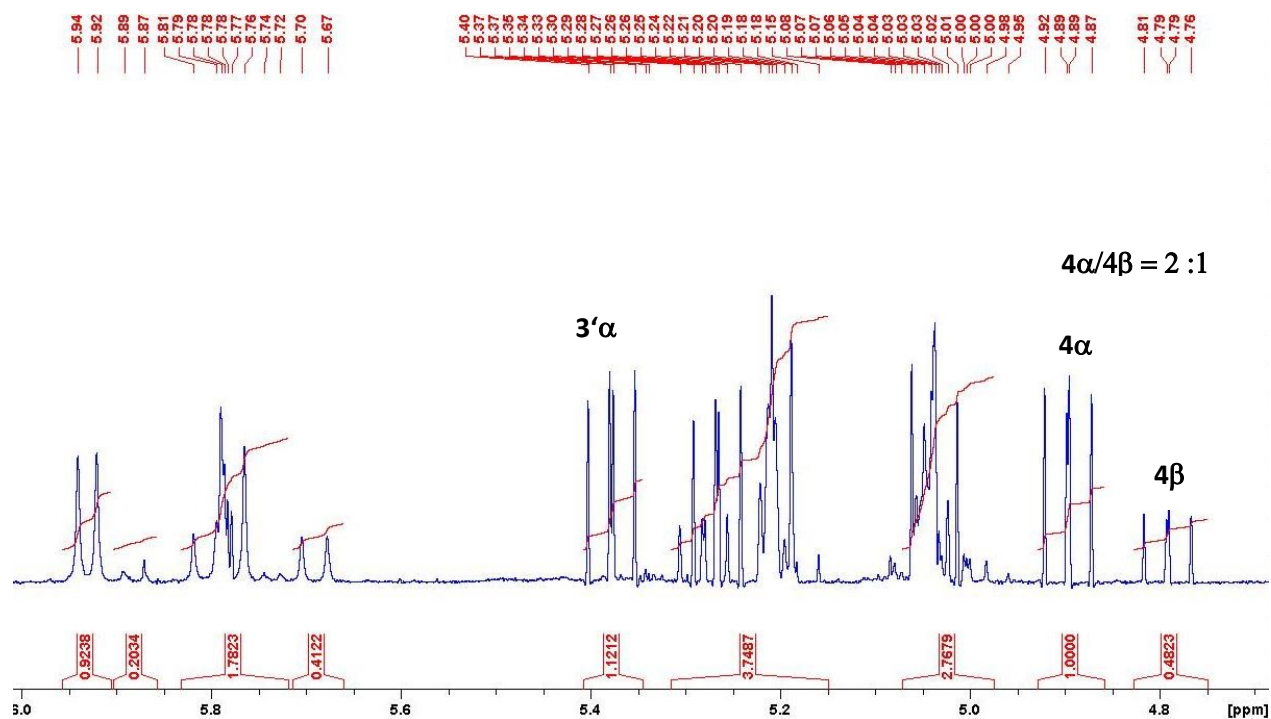


(11) was commonly isolated as anomeric mixture with the α/β ratio ranging from 2:1 to 2.4:1

(11): $^1\text{H-NMR}$, 400 MHz, CDCl_3 $\alpha/\beta = 2.4:1$

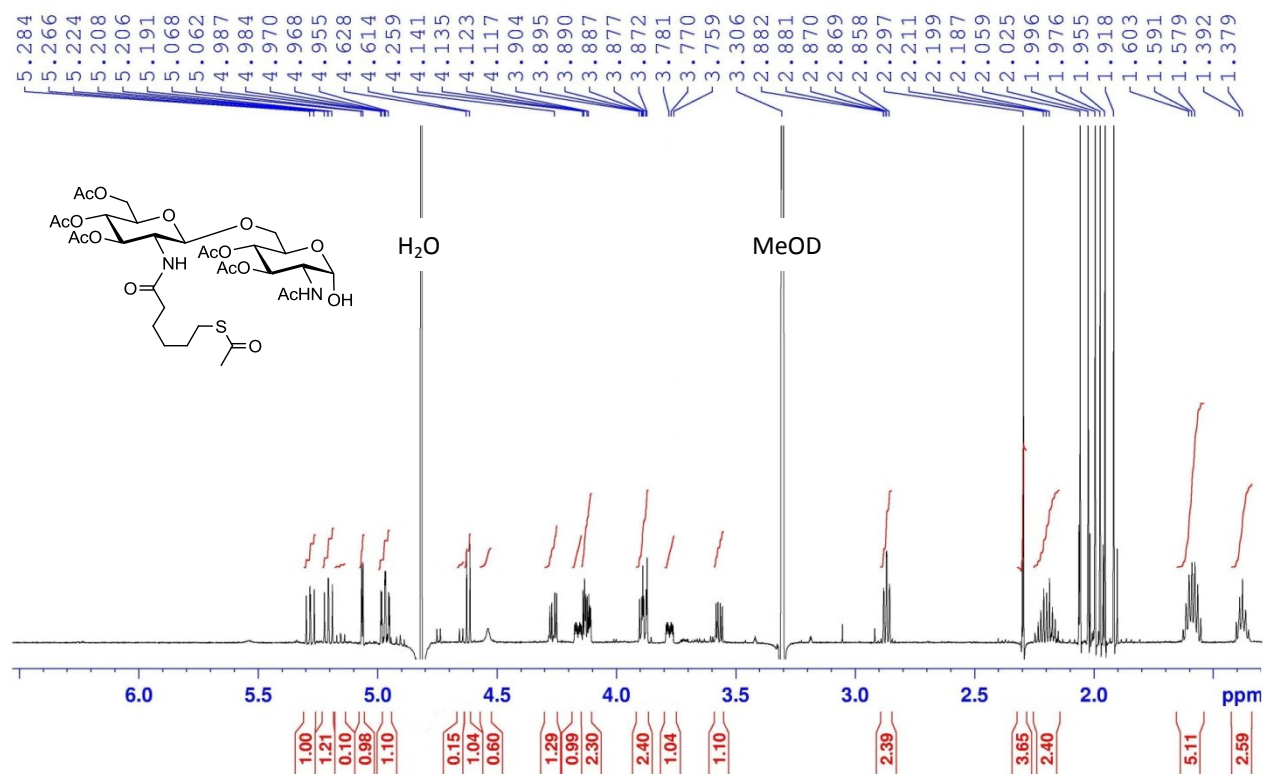


(11): $^1\text{H-NMR}$, 400 MHz, CDCl_3 $\alpha/\beta = 2:1$



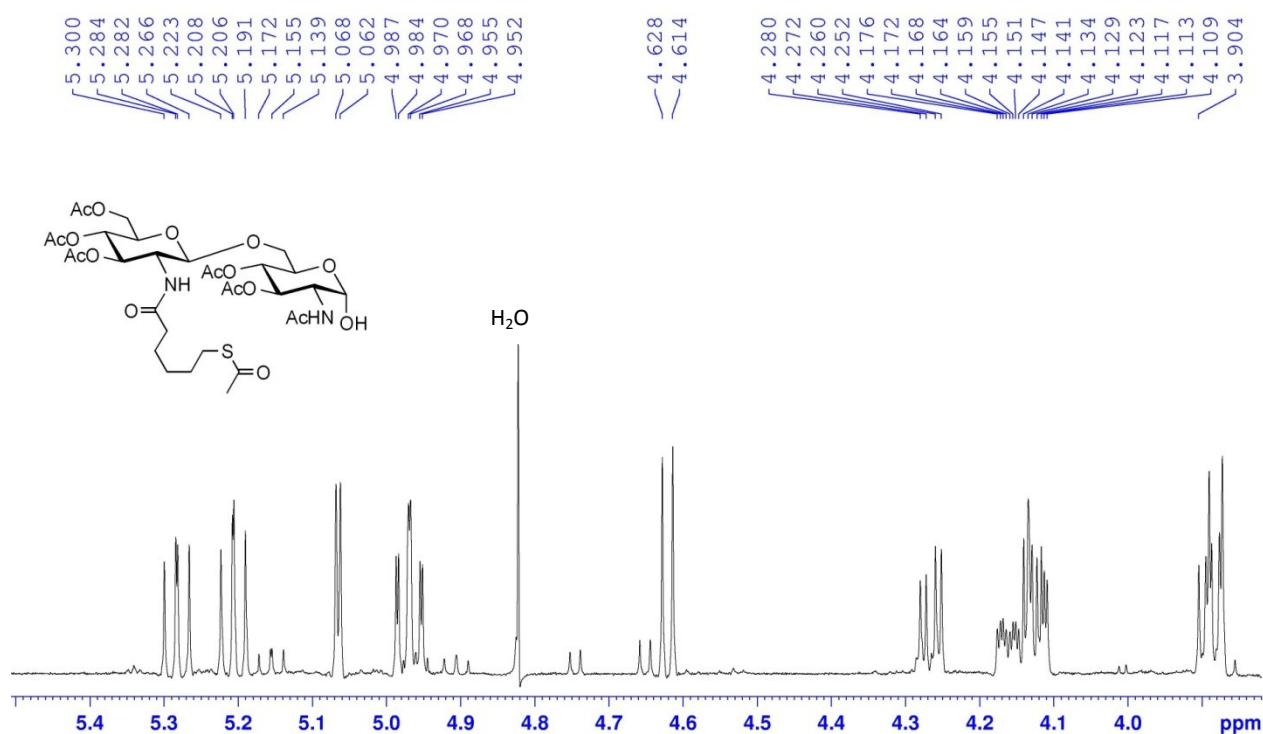
(12): ^1H -NMR, 600 MHz, CD_3OD

$\alpha/\beta = 10:1$

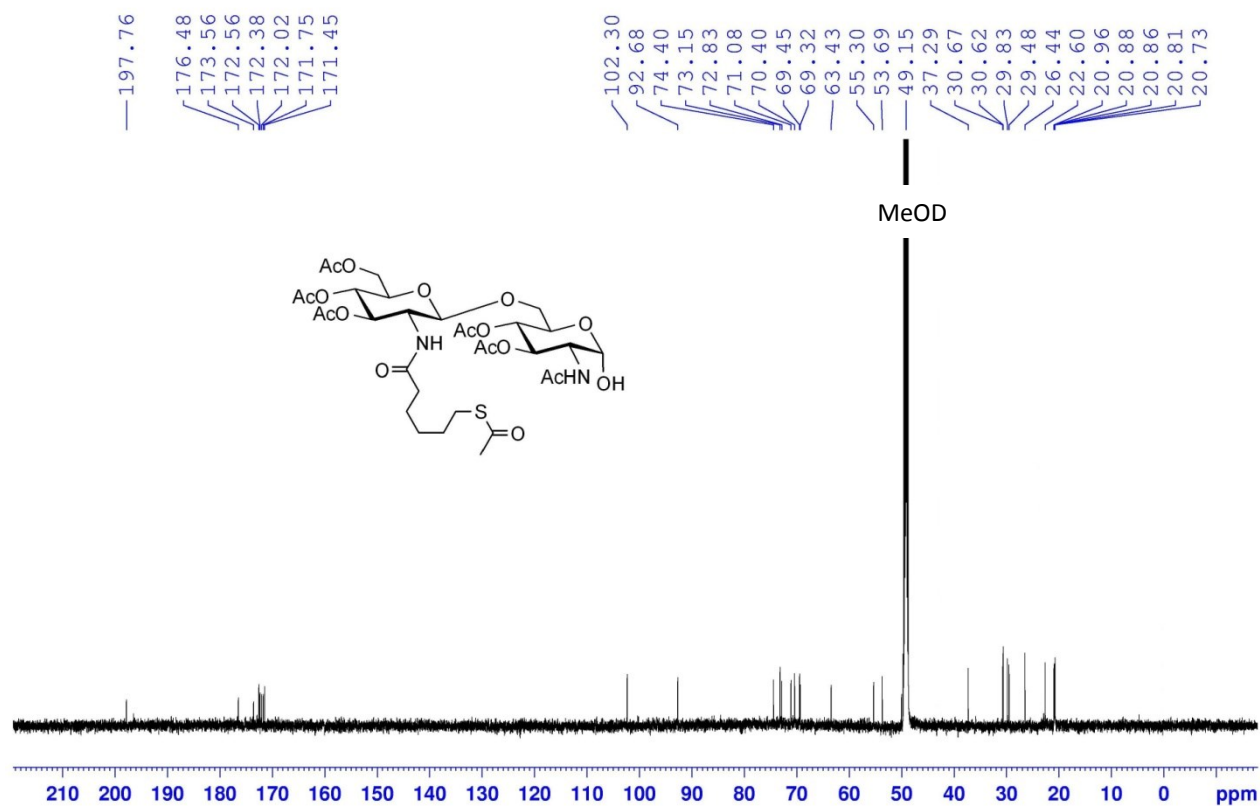


(12): ^1H -NMR, 600 MHz, CD_3OD (3.5 - 5.5 ppm)

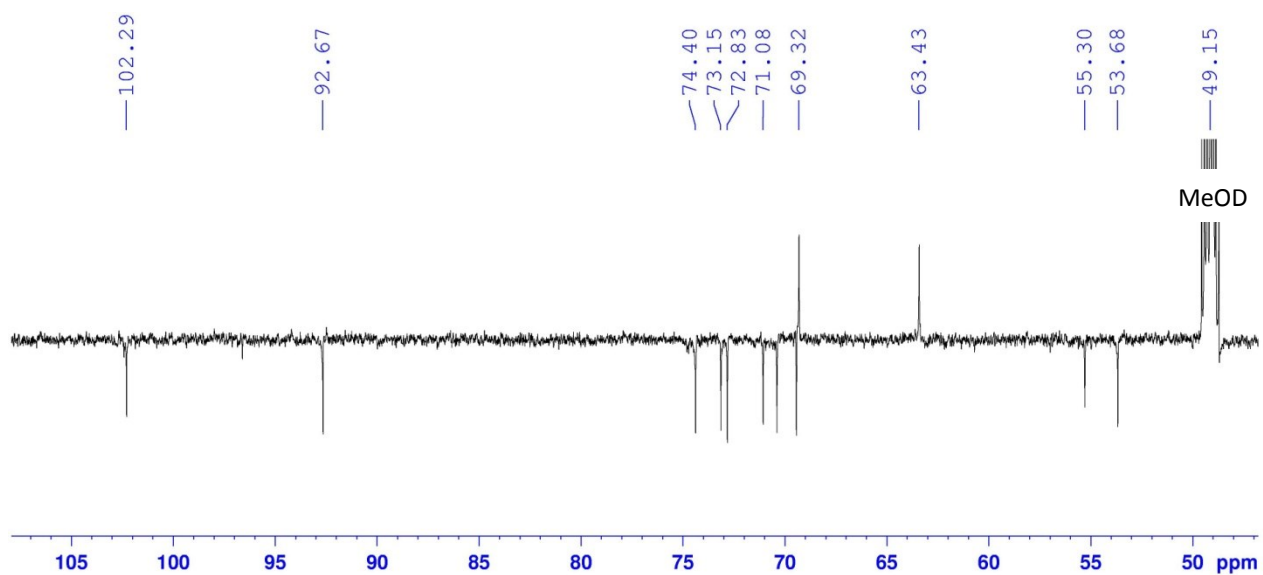
$\alpha/\beta = 10:1$



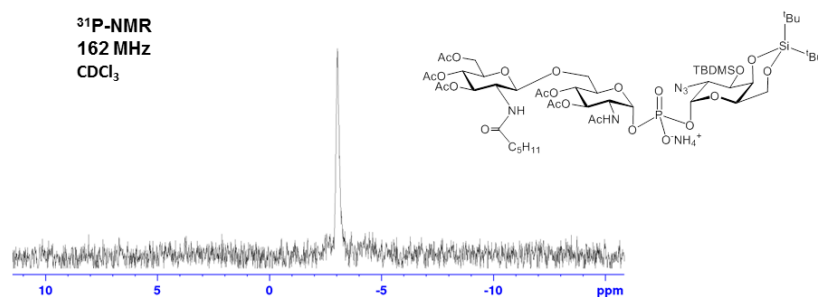
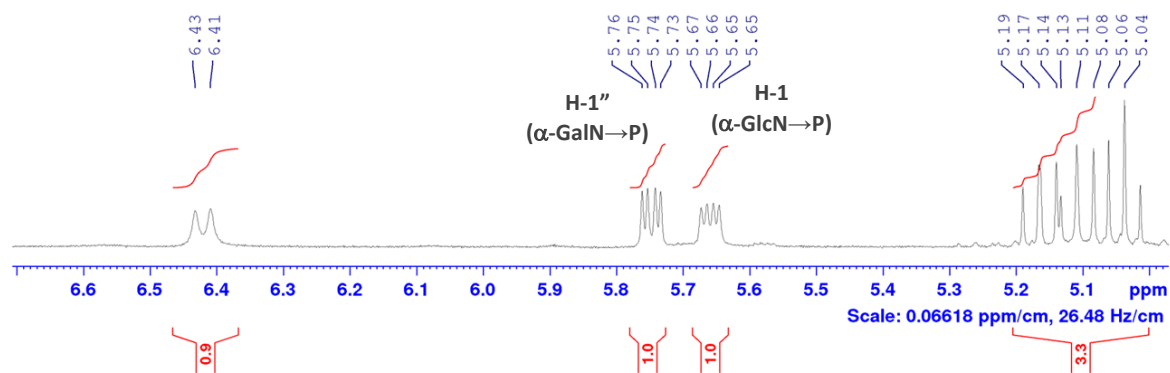
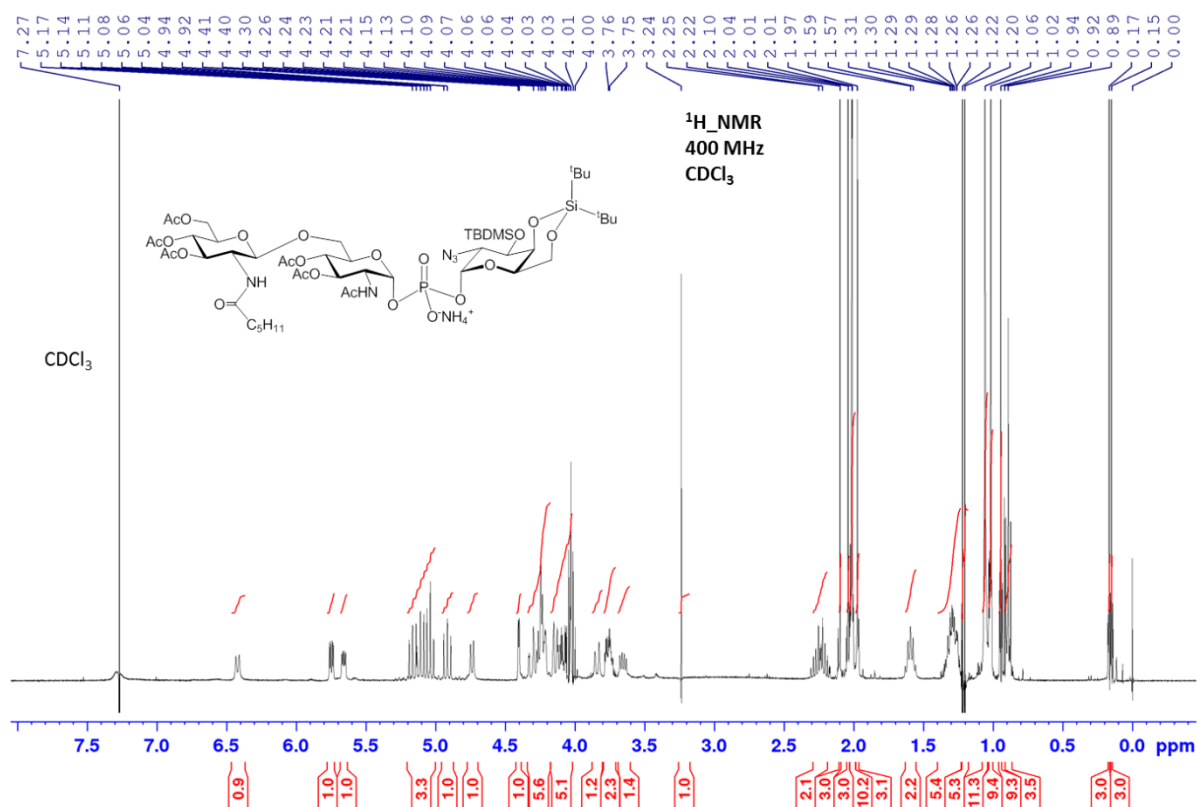
(12): ^{13}C -NMR, 151 MHz, CD_3OD $\alpha/\beta = 10:1$ (low concentration because of pure solubility)



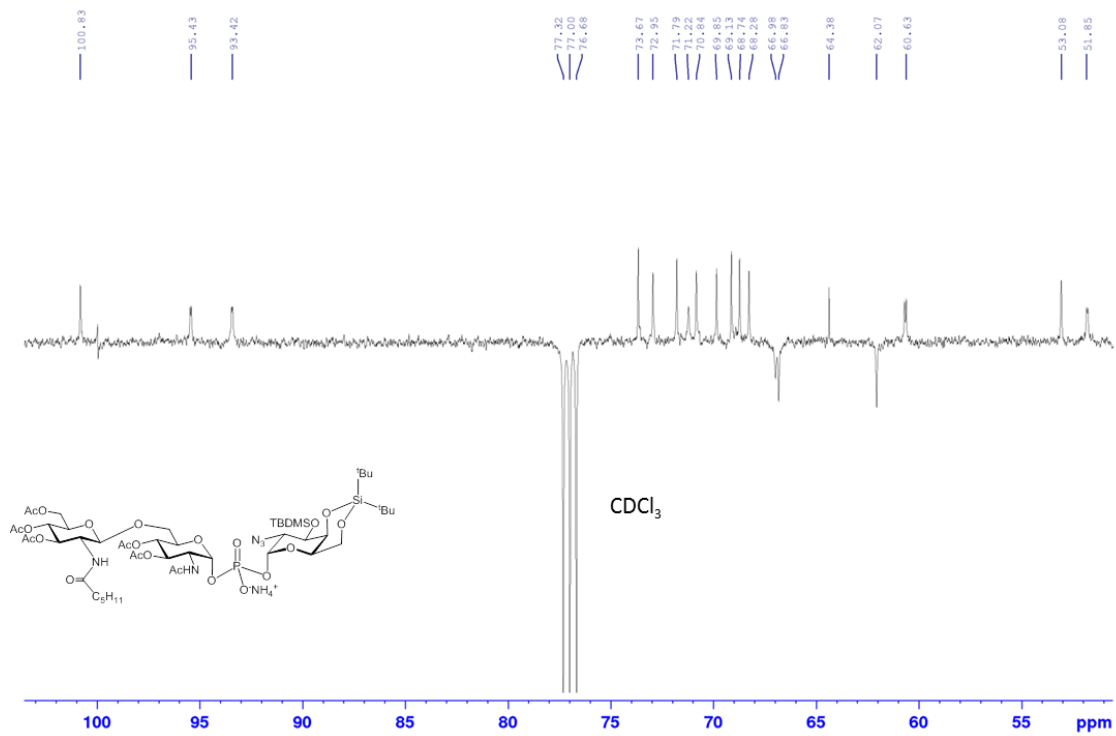
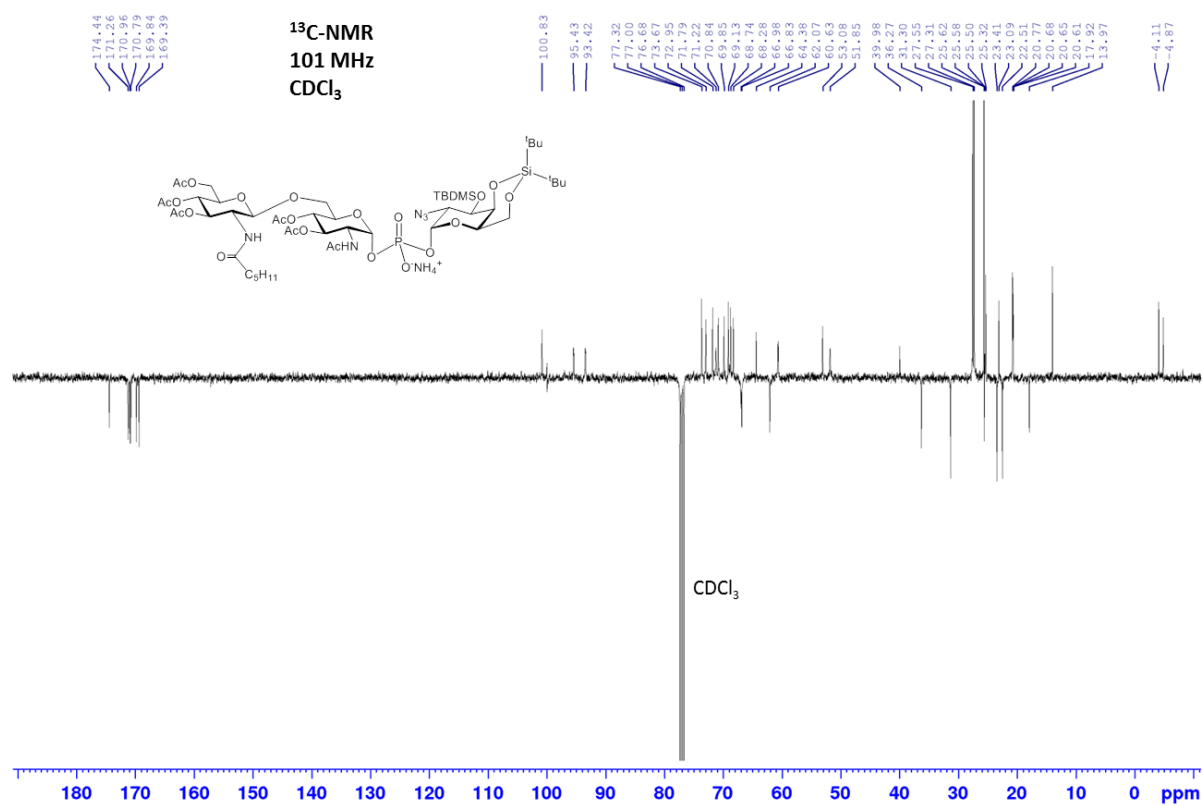
(12): ^{13}C -NMR, APT, 151 MHz, CD_3OD , (50 – 110 ppm) $\alpha/\beta = 10:1$



(13): ^1H -NMR, 600 MHz, CD_3OD



(13): ^{13}C -NMR, APT, 101 MHz, CDCl_3



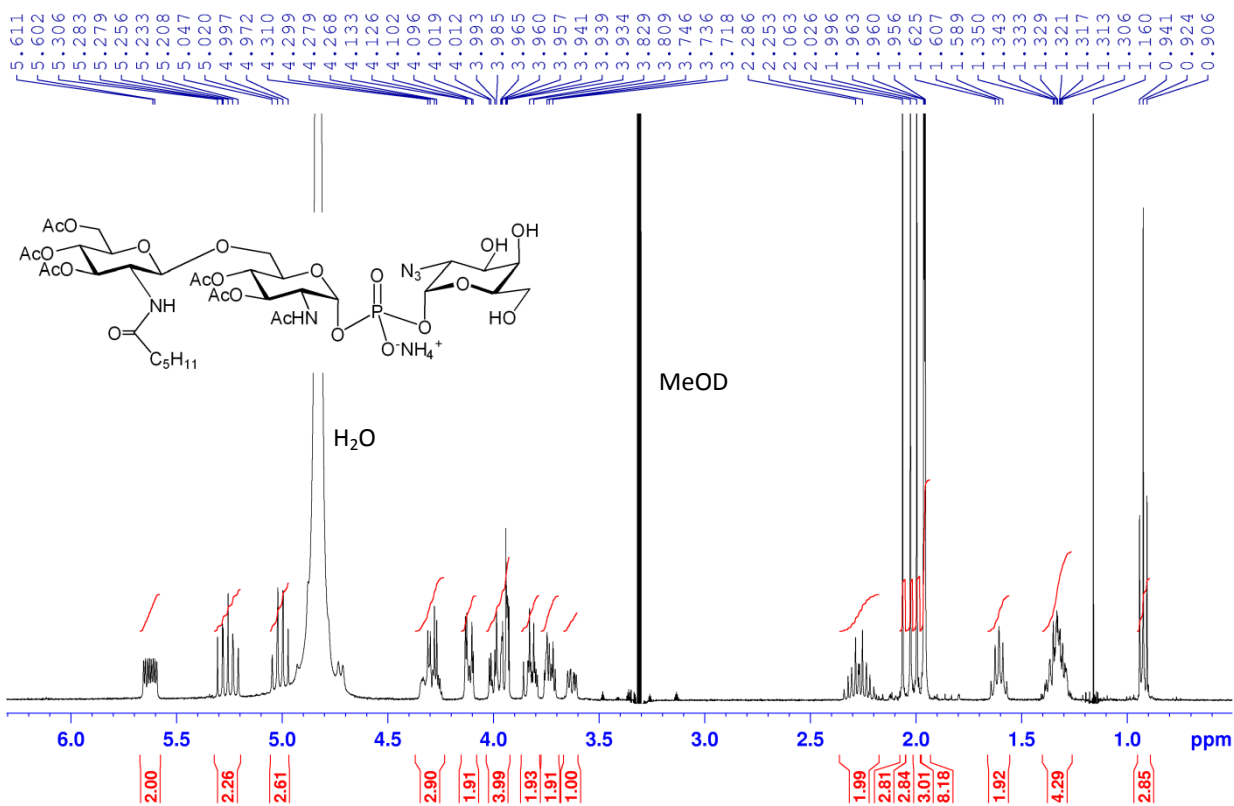
Proton-coupled ^{31}P -NMR spectrum of the reaction mixture $5 \rightarrow 13$ (intermediate H-phosphonate-phosphodiester)

Proton-decoupled ^{31}P -NMR spectrum of the reaction mixture $5 \rightarrow 13$ (intermediate H-phosphonate-phosphodiester)

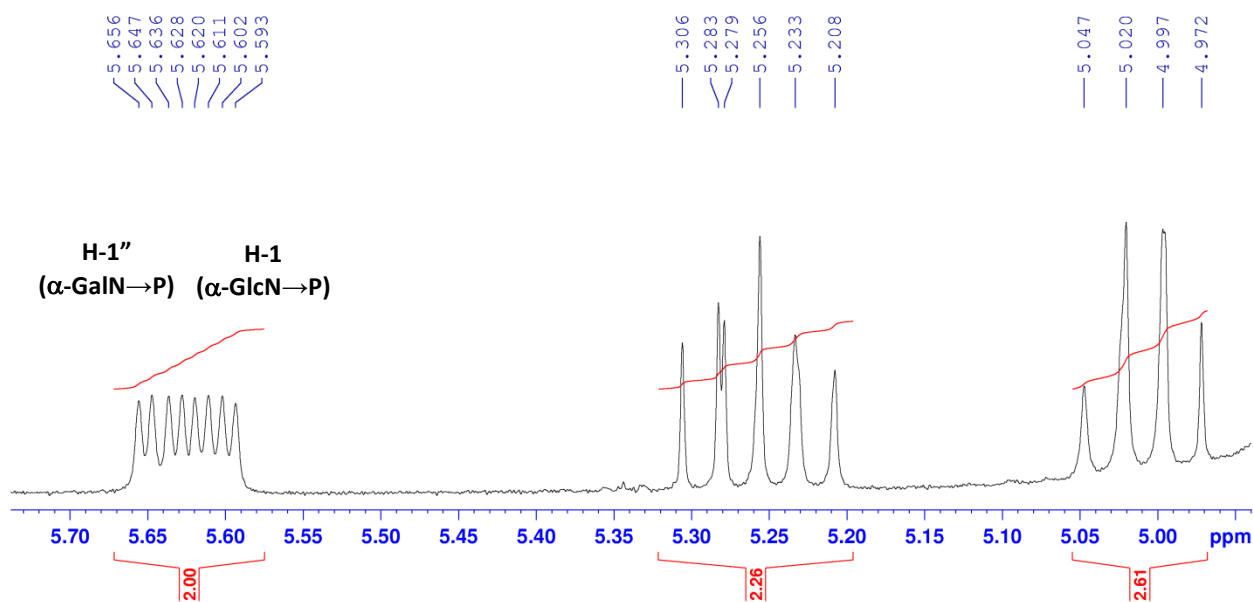
Chemical structures:

- H-phosphonate phosphodiester:** A sugar molecule with an H-phosphonate group and an azide group.
- Intermediate H-phosphonate phosphodiester:** A sugar molecule with an H-phosphonate group, an azide group, and a TBDMSO group.

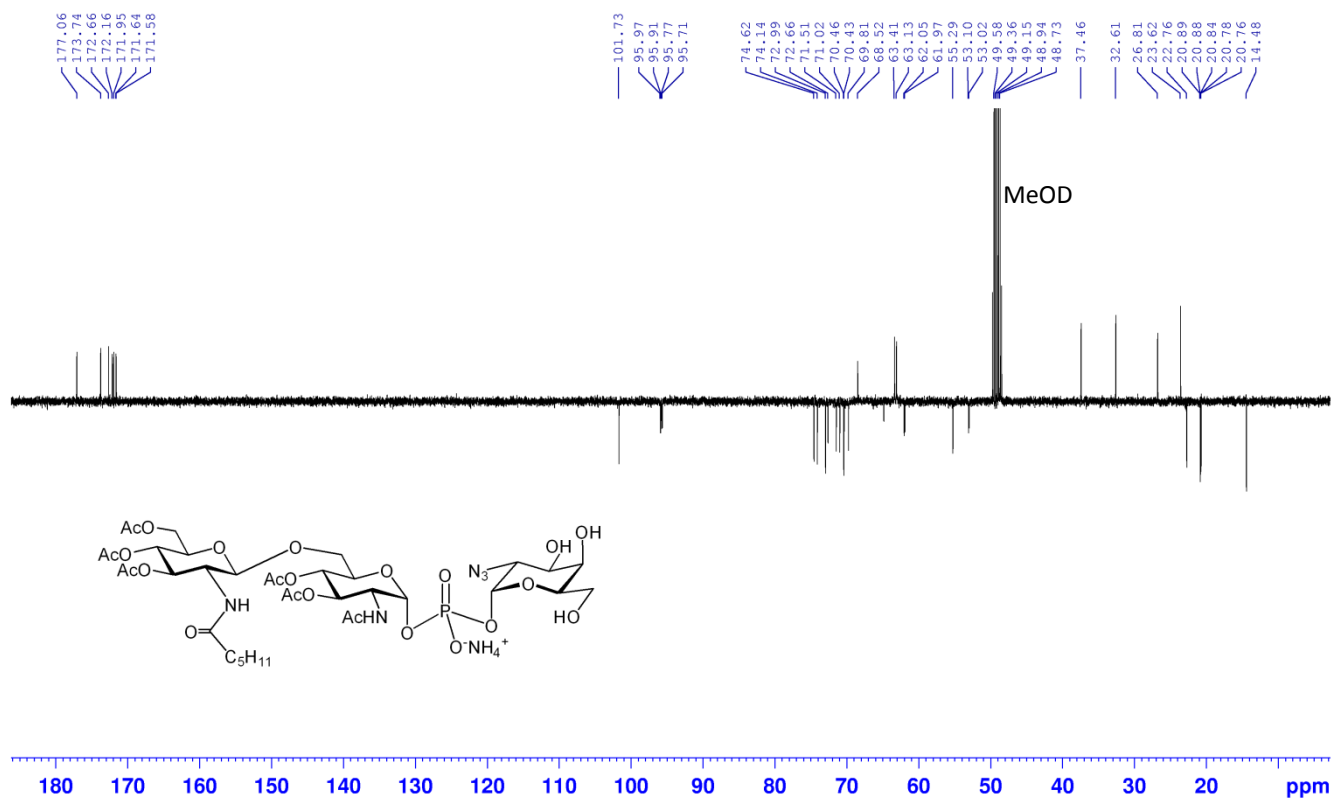
(14): ^1H -NMR, 400 MHz, CD_3OD



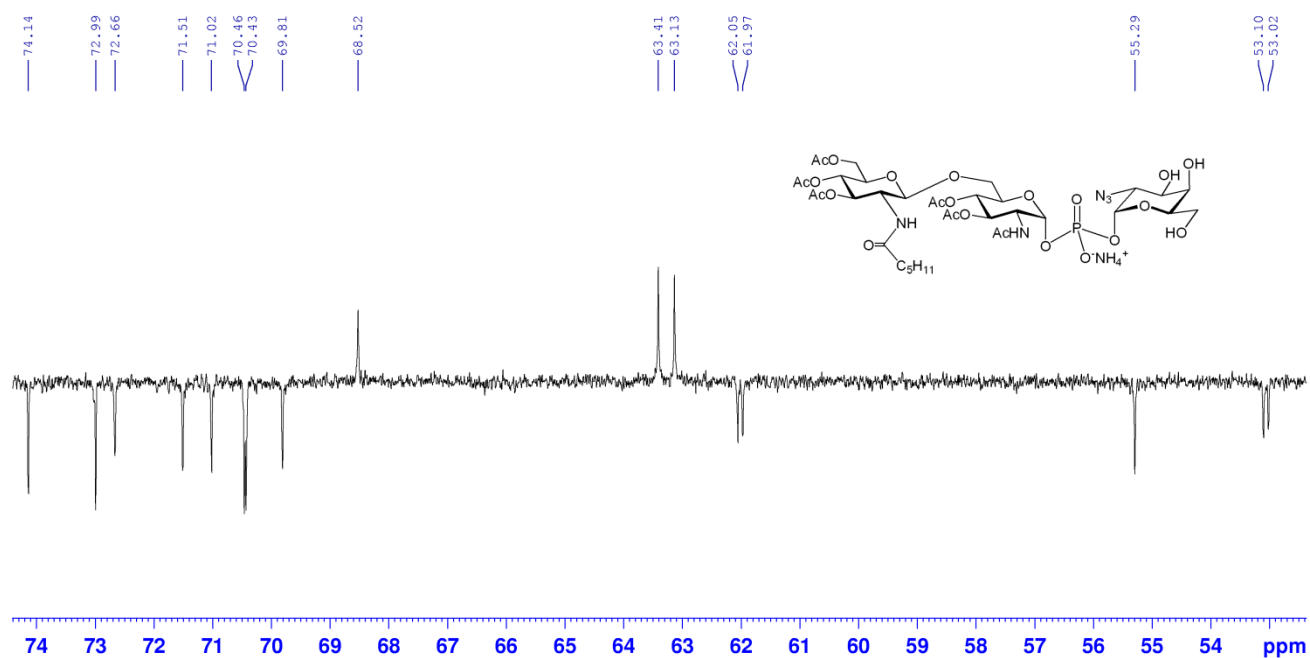
(14): ^1H -NMR, 400 MHz, CD_3OD



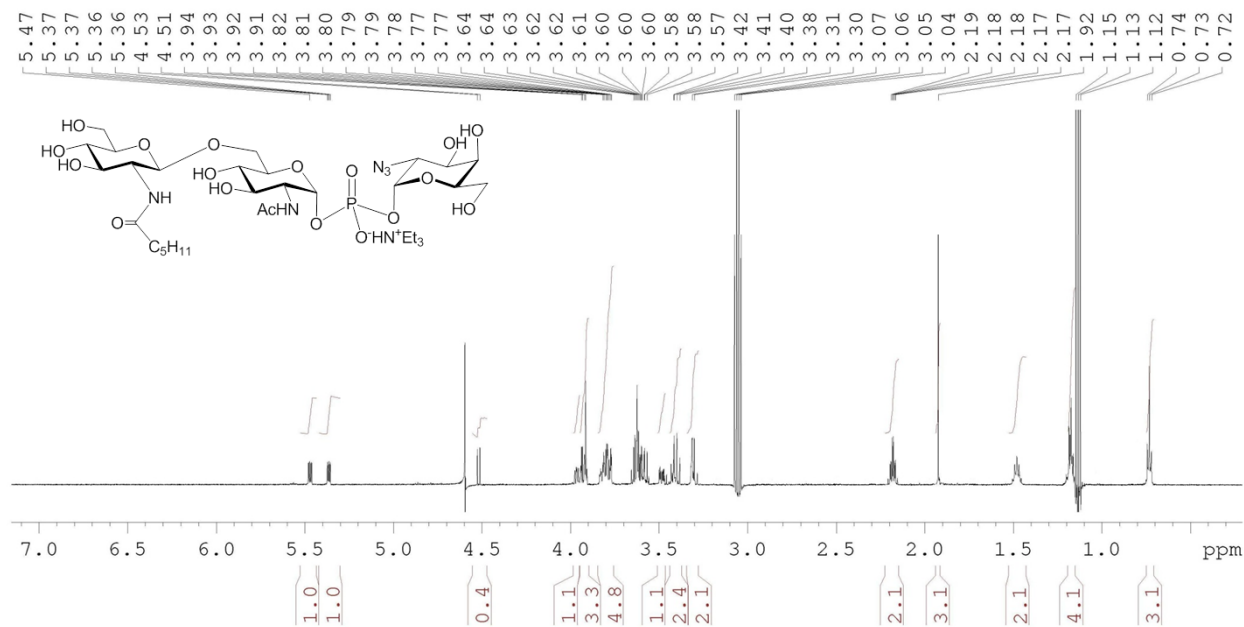
(14): ^{13}C -NMR, APT, 101 MHz, CD_3OD



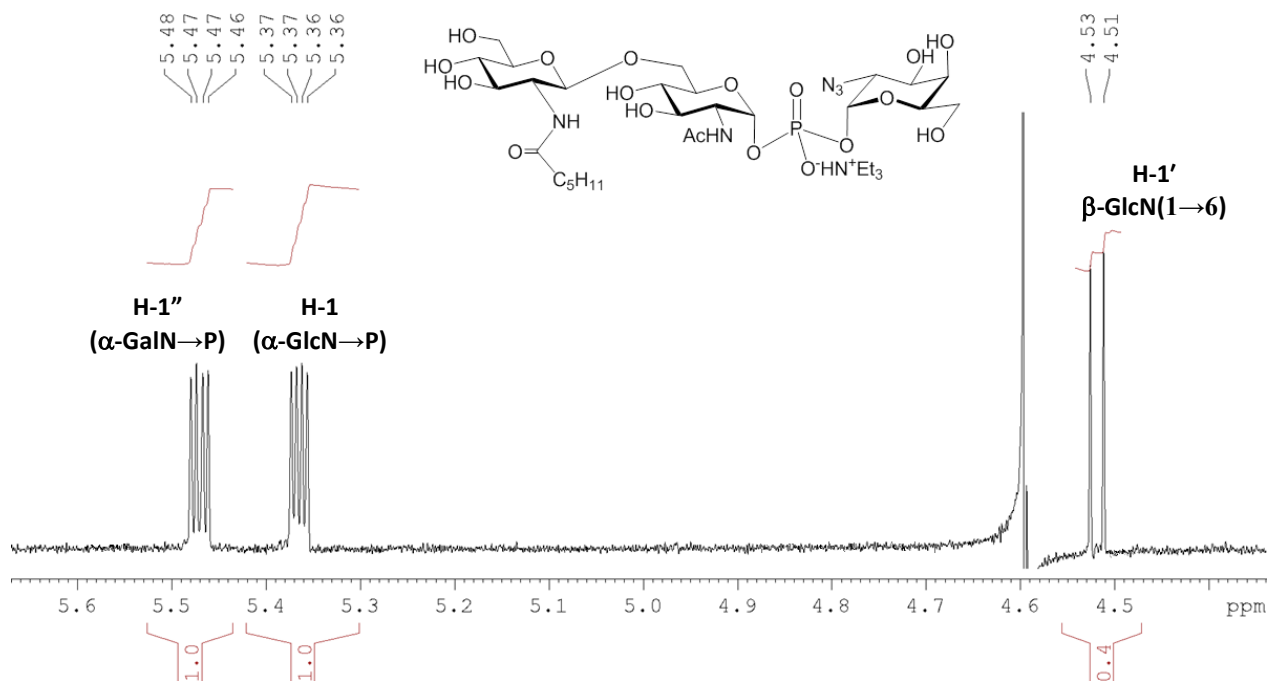
(14): ^{13}C -NMR, APT, 101 MHz, CD_3OD



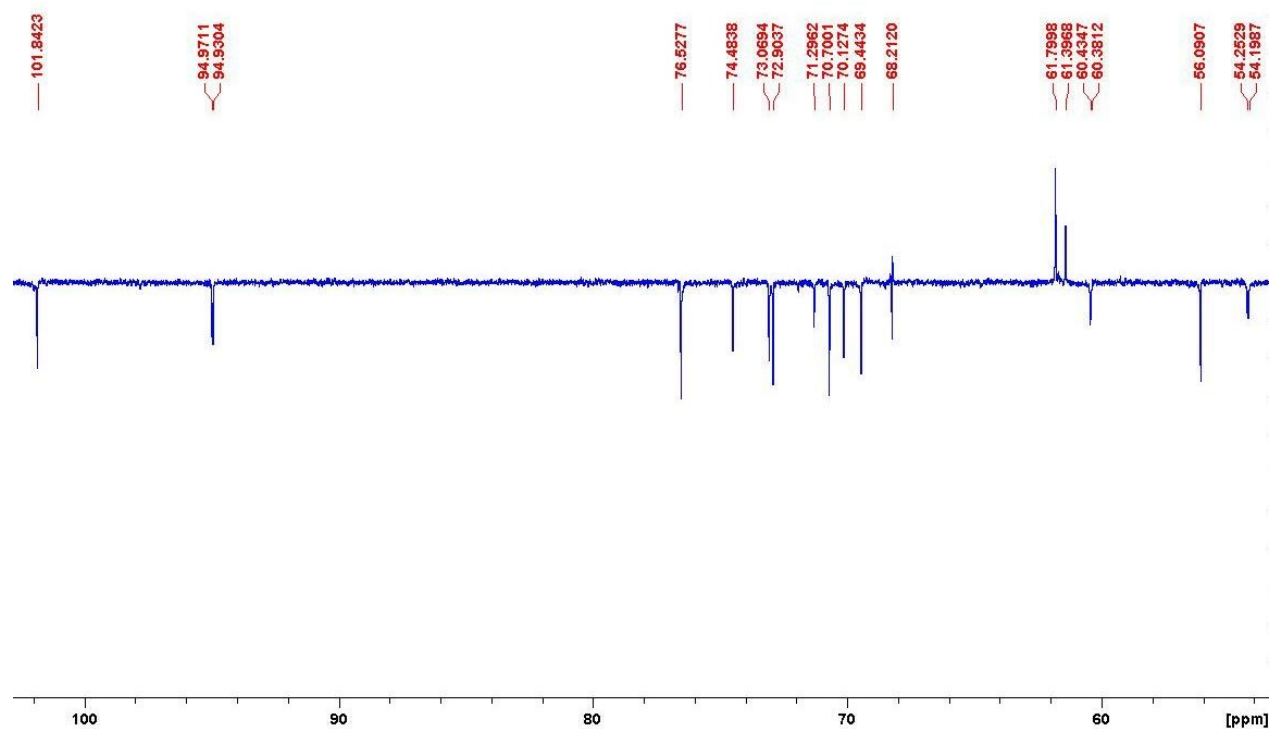
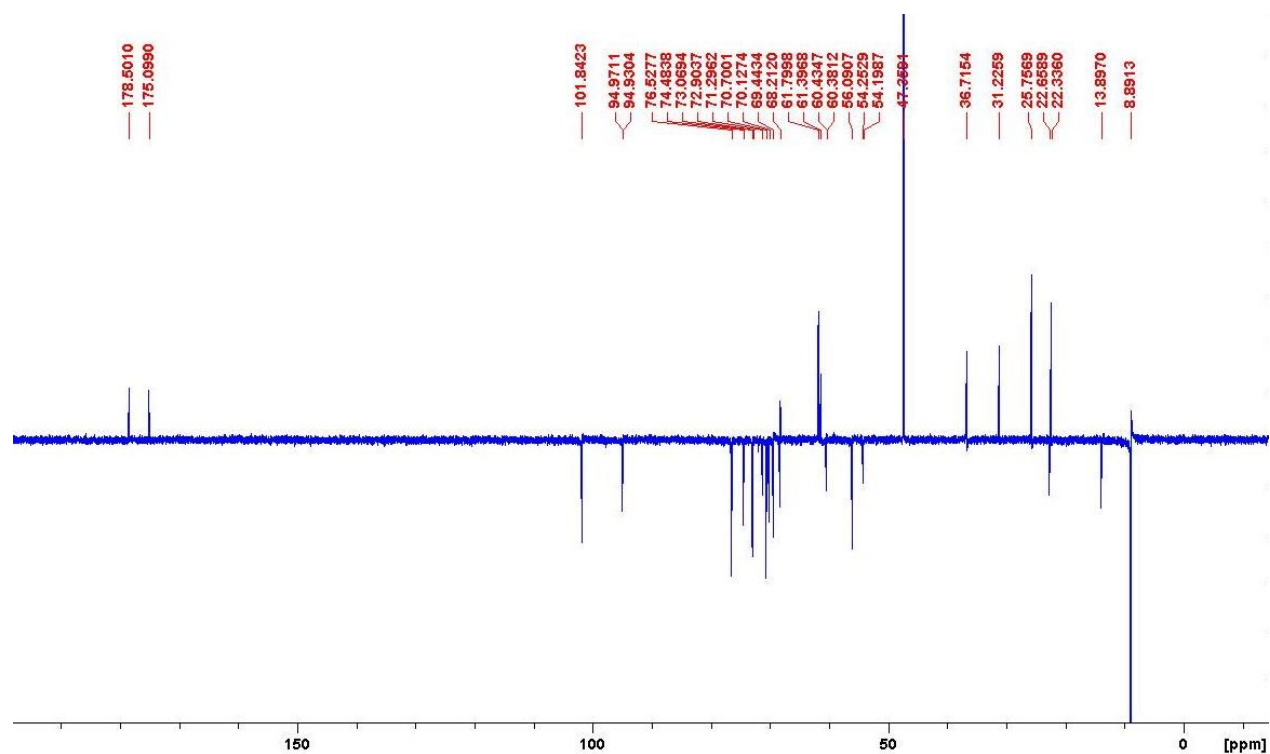
(15): ^1H -NMR, 600 MHz, D_2O



(15): ^1H -NMR, 600 MHz, D_2O , 4.0 – 6.0 ppm



(15): ^{13}C -NMR, APT, 151 MHz, D_2O



Chemical structure of compound 10 is shown above the spectrum. The structure is a complex molecule with a central phosphate group linked to three sugar-like rings. The leftmost ring is a pyranose with a C₅H₁₁ group. The middle ring is a pyranose with an AcHN group. The rightmost ring is a pyranose with a C₅H₁₁ group.

¹H NMR spectrum (DMSO-d₆) of compound 10. The x-axis represents chemical shift in ppm, ranging from 0 to 7.0. The spectrum shows several peaks, with integration values indicated below the baseline and peak positions listed above the spectrum.

Integration values (from left to right): 1.000, 1.000, 1.041, 2.187, 2.097, 1.026, 3.287, 4.087, 1.042, 1.035, 1.115, 3.161, 2.074, 2.984, 2.061, 4.184, 3.002.

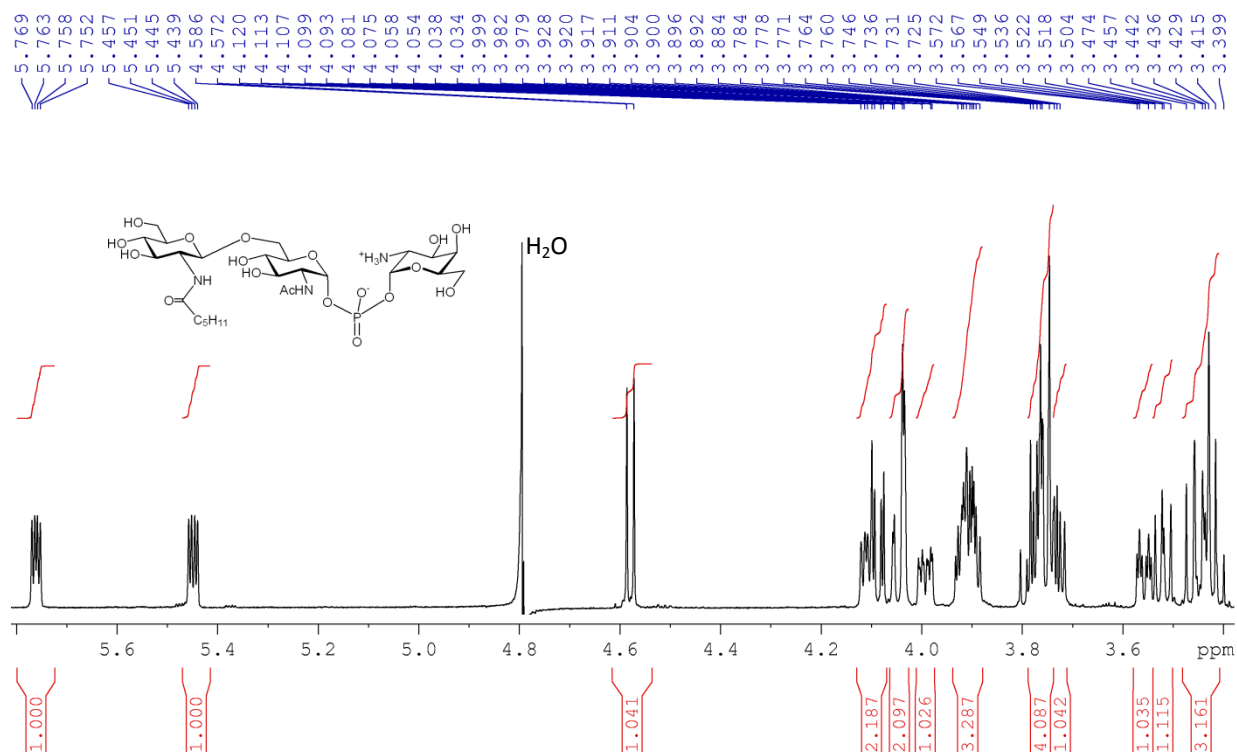
Peak positions (ppm): 5.769, 5.763, 5.758, 5.457, 5.451, 5.445, 5.439, 4.586, 4.572, 4.099, 4.093, 4.081, 4.075, 4.054, 4.038, 4.034, 3.920, 3.917, 3.911, 3.904, 3.900, 3.896, 3.892, 3.784, 3.778, 3.771, 3.764, 3.760, 3.746, 3.736, 3.731, 3.725, 3.536, 3.522, 3.504, 3.474, 3.457, 3.442, 3.436, 3.429, 3.415, 2.298, 2.287, 2.285, 2.274, 2.025, 1.609, 1.597, 1.585, 1.299, 1.293, 1.287, 1.281, 0.863, 0.851, 0.839.

^1H NMR spectrum of the product in $\text{DMSO}-d_6$.

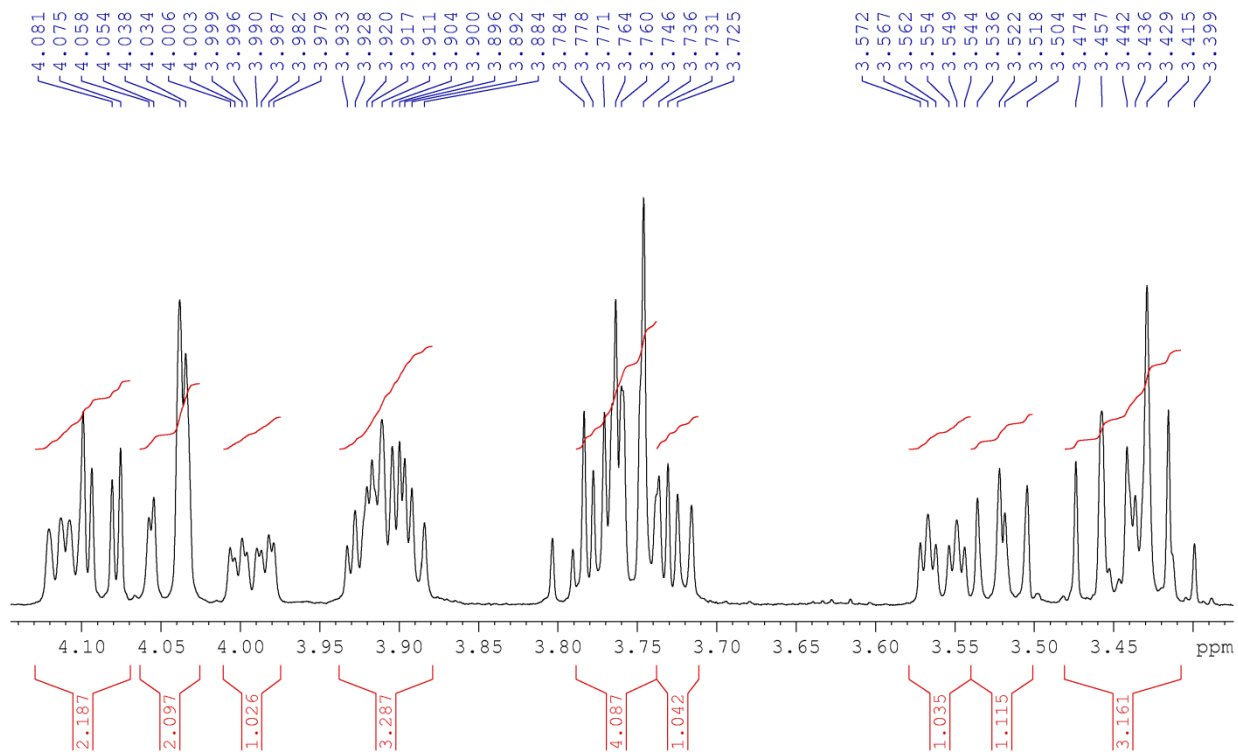
Chemical structure of the product is shown above the spectrum:

CC1=CC=C(C=C1)C2=CC=CC=C2C3=CC=CC=C3C4=CC=CC=C4C5=CC=CC=C5C6=CC=CC=C6C7=CC=CC=C7C8=CC=CC=C8C9=CC=CC=C9C10=CC=CC=C10C11=CC=CC=C11C12=CC=CC=C12C13=CC=CC=C13C14=CC=CC=C14C15=CC=CC=C15C16=CC=CC=C16C17=CC=CC=C17C18=CC=CC=C18C19=CC=CC=C19C20=CC=CC=C20C21=CC=CC=C21C22=CC=CC=C22C23=CC=CC=C23C24=CC=CC=C24C25=CC=CC=C25C26=CC=CC=C26C27=CC=CC=C27C28=CC=CC=C28C29=CC=CC=C29C30=CC=CC=C30C31=CC=CC=C31C32=CC=CC=C32C33=CC=CC=C33C34=CC=CC=C34C35=CC=CC=C35C36=CC=CC=C36C37=CC=CC=C37C38=CC=CC=C38C39=CC=CC=C39C40=CC=CC=C40C41=CC=CC=C41C42=CC=CC=C42C43=CC=CC=C43C44=CC=CC=C44C45=CC=CC=C45C46=CC=CC=C46C47=CC=CC=C47C48=CC=CC=C48C49=CC=CC=C49C50=CC=CC=C50C51=CC=CC=C51C52=CC=CC=C52C53=CC=CC=C53C54=CC=CC=C54C55=CC=CC=C55C56=CC=CC=C56C57=CC=CC=C57C58=CC=CC=C58C59=CC=CC=C59C60=CC=CC=C60C61=CC=CC=C61C62=CC=CC=C62C63=CC=CC=C63C64=CC=CC=C64C65=CC=CC=C65C66=CC=CC=C66C67=CC=CC=C67C68=CC=CC=C68C69=CC=CC=C69C70=CC=CC=C70C71=CC=CC=C71C72=CC=CC=C72C73=CC=CC=C73C74=CC=CC=C74C75=CC=CC=C75C76=CC=CC=C76C77=CC=CC=C77C78=CC=CC=C78C79=CC=CC=C79C80=CC=CC=C80C81=CC=CC=C81C82=CC=CC=C82C83=CC=CC=C83C84=CC=CC=C84C85=CC=CC=C85C86=CC=CC=C86C87=CC=CC=C87C88=CC=CC=C88C89=CC=CC=C89C90=CC=CC=C90C91=CC=CC=C91C92=CC=CC=C92C93=CC=CC=C93C94=CC=CC=C94C95=CC=CC=C95C96=CC=CC=C96C97=CC=CC=C97C98=CC=CC=C98C99=CC=CC=C99C100=CC=CC=C100C101=CC=CC=C101C102=CC=CC=C102C103=CC=CC=C103C104=CC=CC=C104C105=CC=CC=C105C106=CC=CC=C106C107=CC=CC=C107C108=CC=CC=C108C109=CC=CC=C109C110=CC=CC=C110C111=CC=CC=C111C112=CC=CC=C112C113=CC=CC=C113C114=CC=CC=C114C115=CC=CC=C115C116=CC=CC=C116C117=CC=CC=C117C118=CC=CC=C118C119=CC=CC=C119C120=CC=CC=C120C121=CC=CC=C121C122=CC=CC=C122C123=CC=CC=C123C124=CC=CC=C124C125=CC=CC=C125C126=CC=CC=C126C127=CC=CC=C127C128=CC=CC=C128C129=CC=CC=C129C130=CC=CC=C130C131=CC=CC=C131C132=CC=CC=C132C133=CC=CC=C133C134=CC=CC=C134C135=CC=CC=C135C136=CC=CC=C136C137=CC=CC=C137C138=CC=CC=C138C139=CC=CC=C139C140=CC=CC=C140C141=CC=CC=C141C142=CC=CC=C142C143=CC=CC=C143C144=CC=CC=C144C145=CC=CC=C145C146=CC=CC=C146C147=CC=CC=C147C148=CC=CC=C148C149=CC=CC=C149C150=CC=CC=C150C151=CC=CC=C151C152=CC=CC=C152C153=CC=CC=C153C154=CC=CC=C154C155=CC=CC=C155C156=CC=CC=C156C157=CC=CC=C157C158=CC=CC=C158C159=CC=CC=C159C160=CC=CC=C160C161=CC=CC=C161C162=CC=CC=C162C163=CC=CC=C163C164=CC=CC=C164C165=CC=CC=C165C166=CC=CC=C166C167=CC=CC=C167C168=CC=CC=C168C169=CC=CC=C169C170=CC=CC=C170C171=CC=CC=C171C172=CC=CC=C172C173=CC=CC=C173C174=CC=CC=C174C175=CC=CC=C175C176=CC=CC=C176C177=CC=CC=C177C178=CC=CC=C178C179=CC=CC=C179C180=CC=CC=C180C181=CC=CC=C181C182=CC=CC=C182C183=CC=CC=C183C184=CC=CC=C184C185=CC=CC=C185C186=CC=CC=C186C187=CC=CC=C187C188=CC=CC=C188C189=CC=CC=C189C190=CC=CC=C190C191=CC=CC=C191C192=CC=CC=C192C193=CC=CC=C193C194=CC=CC=C194C195=CC=CC=C195C196=CC=CC=C196C197=CC=CC=C197C198=CC=CC=C198C199=CC=CC=C199C200=CC=CC=C200C201=CC=CC=C201C202=CC=CC=C202C203=CC=CC=C203C204=CC=CC=C204C205=CC=CC=C205C206=CC=CC=C206C207=CC=CC=C207C208=CC=CC=C208C209=CC=CC=C209C210=CC=CC=C210C211=CC=CC=C211C212=CC=CC=C212C213=CC=CC=C213C214=CC=CC=C214C215=CC=CC=C215C216=CC=CC=C216C217=CC=CC=C217C218=CC=CC=C218C219=CC=CC=C219C220=CC=CC=C220C221=CC=CC=C221C222=CC=CC=C222C223=CC=CC=C223C224=CC=CC=C224C225=CC=CC=C225C226=CC=CC=C226C227=CC=CC=C227C228=CC=CC=C228C229=CC=CC=C229C230=CC=CC=C230C231=CC=CC=C231C232=CC=CC=C232C233=CC=CC=C233C234=CC=CC=C234C235=CC=CC=C235C236=CC=CC=C236C237=CC=CC=C237C238=CC=CC=C238C239=CC=CC=C239C240=CC=CC=C240C241=CC=CC=C241C242=CC=CC=C242C243=CC=CC=C243C244=CC=CC=C244C245=CC=CC=C245C246=CC=CC=C246C247=CC=CC=C247C248=CC=CC=C248C249=CC=CC=C249C250=CC=CC=C250C251=CC=CC=C251C252=CC=CC=C252C253=CC=CC=C253C254=CC=CC=C254C255=CC=CC=C255C256=CC=CC=C256C257=CC=CC=C257C258=CC=CC=C258C259=CC=CC=C259C260=CC=CC=C260C261=CC=CC=C261C262=CC=CC=C262C263=CC=CC=C263C264=CC=CC=C264C265=CC=CC=C265C266=CC=CC=C266C267=CC=CC=C267C268=CC=CC=C268C269=CC=CC=C269C270=CC=CC=C270C271=CC=CC=C271C272=CC=CC=C272C273=CC=CC=C273C274=CC=CC=C274C275=CC=CC=C275C276=CC=CC=C276C277=CC=CC=C277C278=CC=CC=C278C279=CC=CC=C279C280=CC=CC=C280C281=CC=CC=C281C282=CC=CC=C282C283=CC=CC=C283C284=CC=CC=C284C285=CC=CC=C285C286=CC=CC=C286C287=CC=CC=C287C288=CC=CC=C288C289=CC=CC=C289C290=CC=CC=C290C291=CC=CC=C291C292=CC=CC=C292C293=CC=CC=C293C294=CC=CC=C294C295=CC=CC=C295C296=CC=CC=C296C297=CC=CC=C297C298=CC=CC=C298C299=CC=CC=C299C300=CC=CC=C300C301=CC=CC=C301C302=CC=CC=C302C303=CC=CC=C303C304=CC=CC=C304C305=CC=CC=C305C306=CC=CC=C306C307=CC=CC=C307C308=CC=CC=C308C309=CC=CC=C309C310=CC=CC=C310C311=CC=CC=C311C312=CC=CC=C312C313=CC=CC=C313C314=CC=CC=C314C315=CC=CC=C315C316=CC=CC=C316C317=CC=CC=C317C318=CC=CC=C318C319=CC=CC=C319C320=CC=CC=C320C321=CC=CC=C321C322=CC=CC=C322C323=CC=CC=C323C324=CC=CC=C324C325=CC=CC=C325C326=CC=CC=C326C327=CC=CC=C327C328=CC=CC=C328C329=CC=CC=C329C330=CC=CC=C330C331=CC=CC=C331C332=CC=CC=C332C333=CC=CC=C333C334=CC=CC=C334C335=CC=CC=C335C336=CC=CC=C336C337=CC=CC=C337C338=CC=CC=C338C339=CC=CC=C339C340=CC=CC=C340C341=CC=CC=C341C342=CC=CC=C342C343=CC=CC=C343C

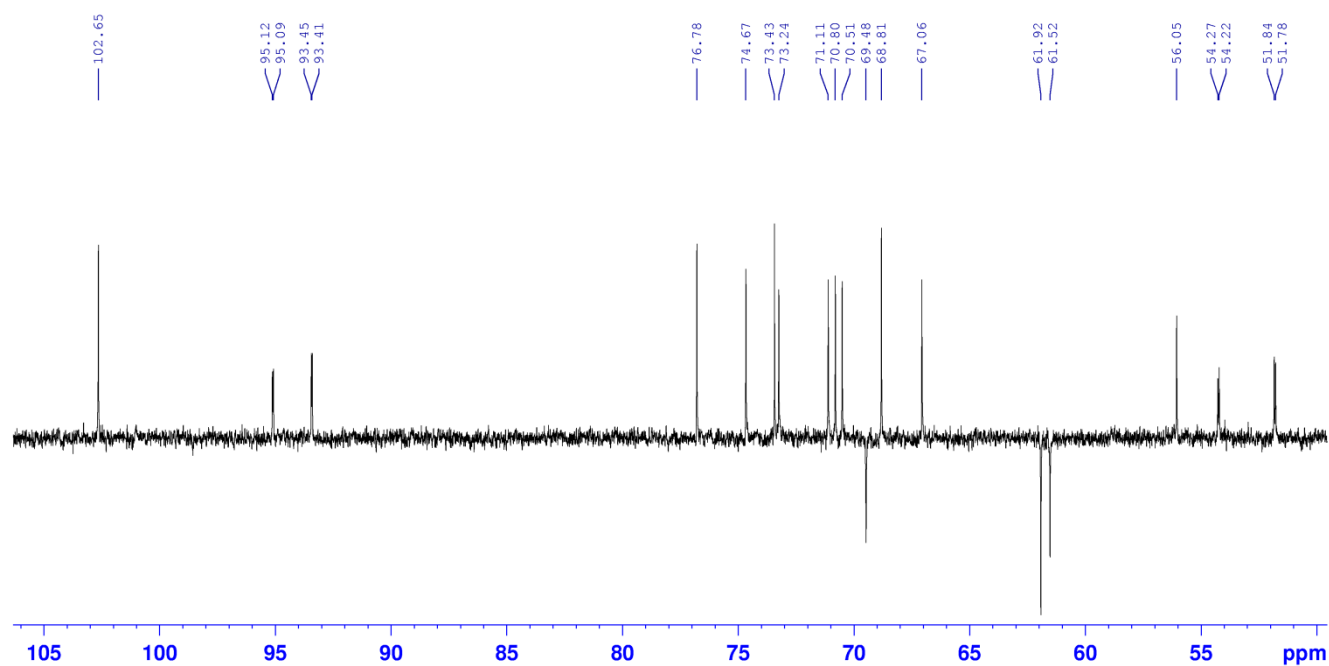
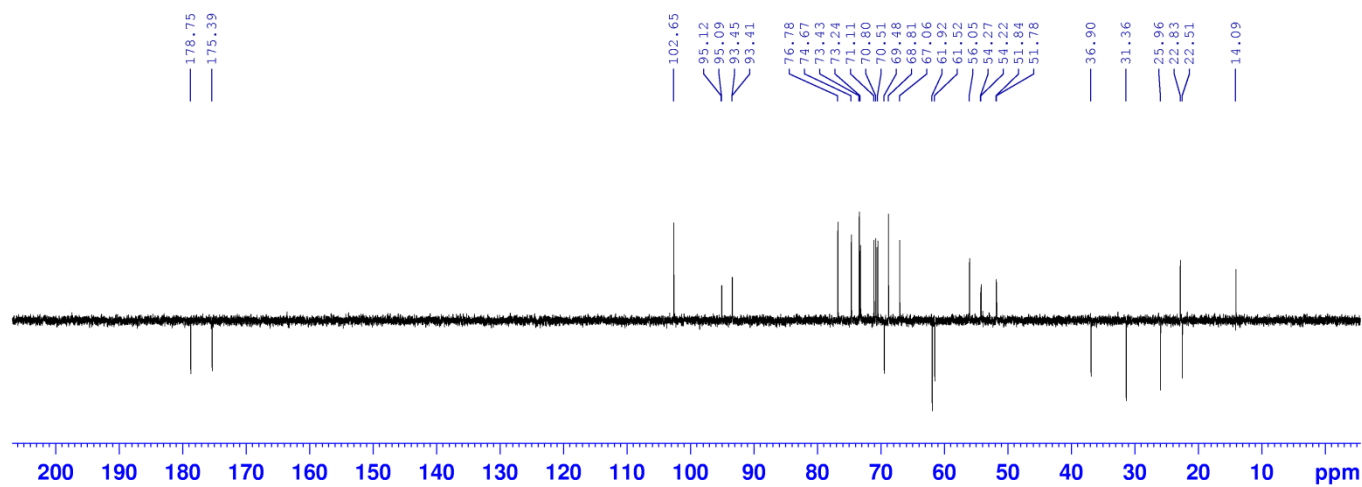
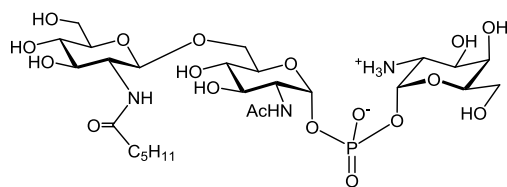
(16): ^1H -NMR, 600 MHz, D_2O , 6.0 – 3.0 ppm



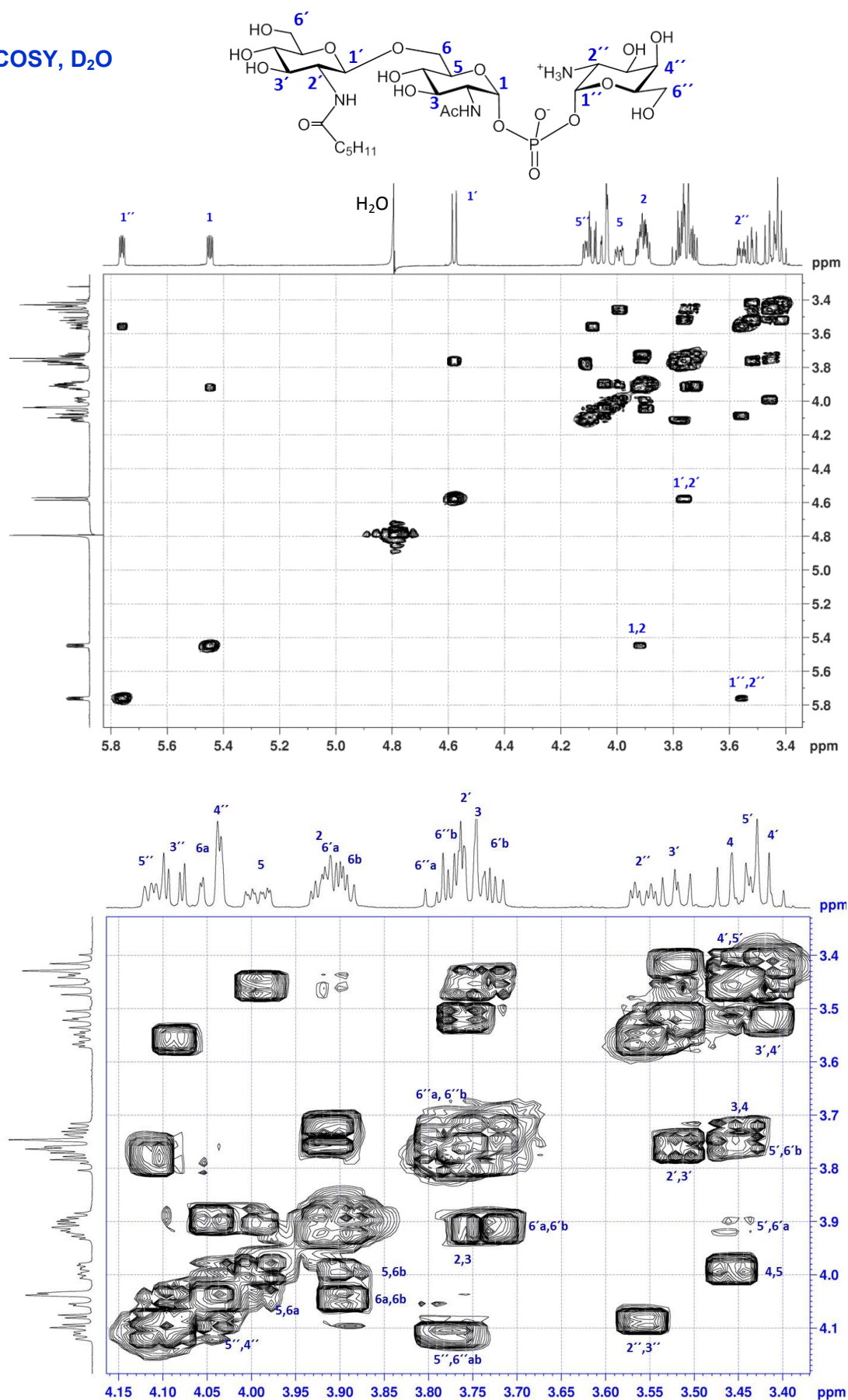
(16): ^1H -NMR, 600 MHz, D_2O , 4.5 – 3.0 ppm



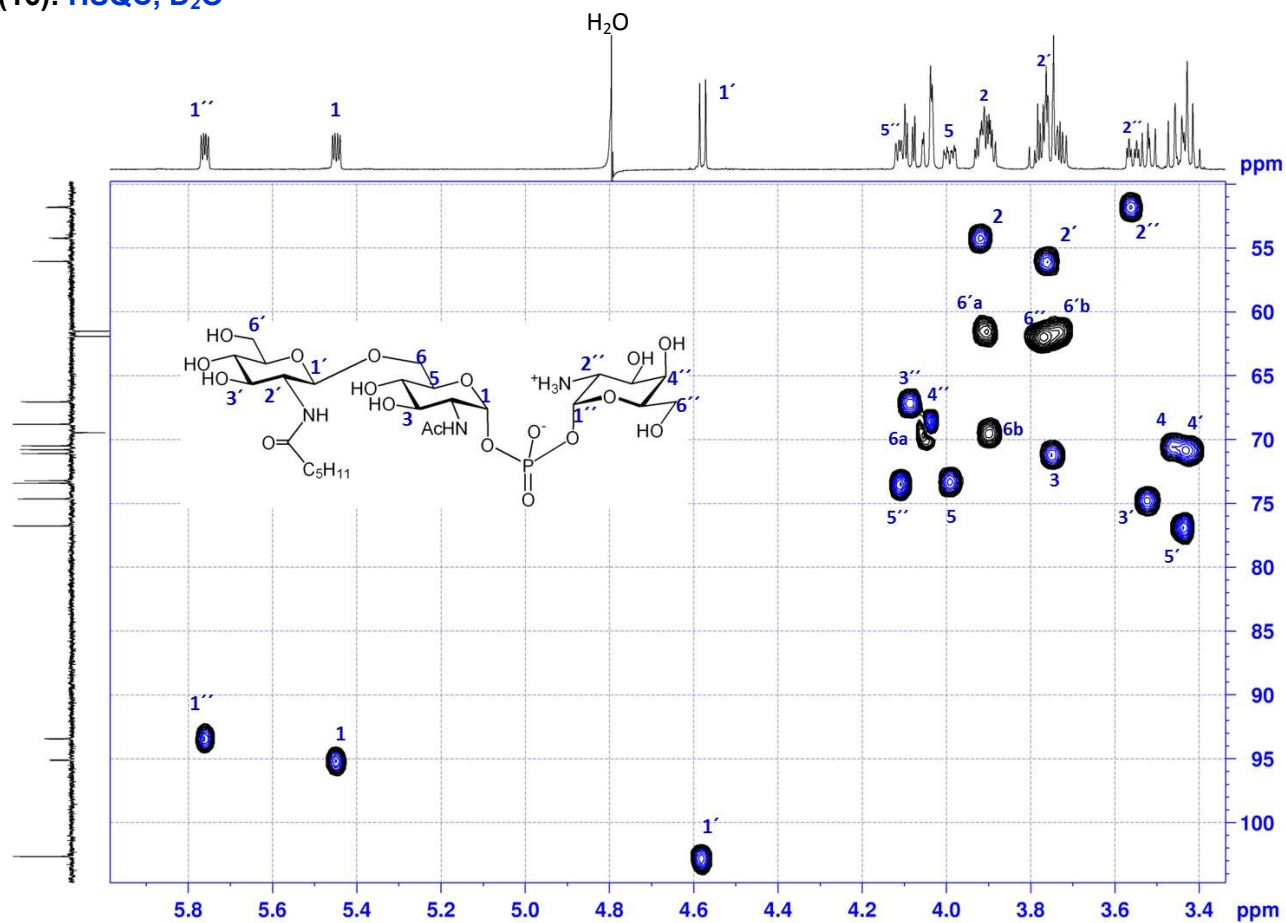
(16): ^{13}C -NMR, APT, 151 MHz, D_2O



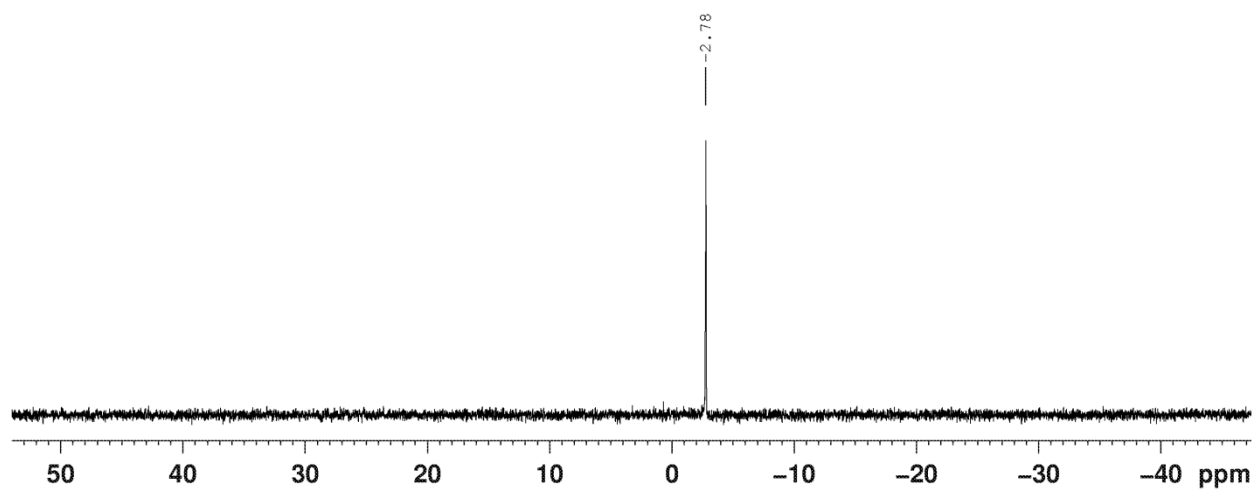
(16): COSY, D₂O



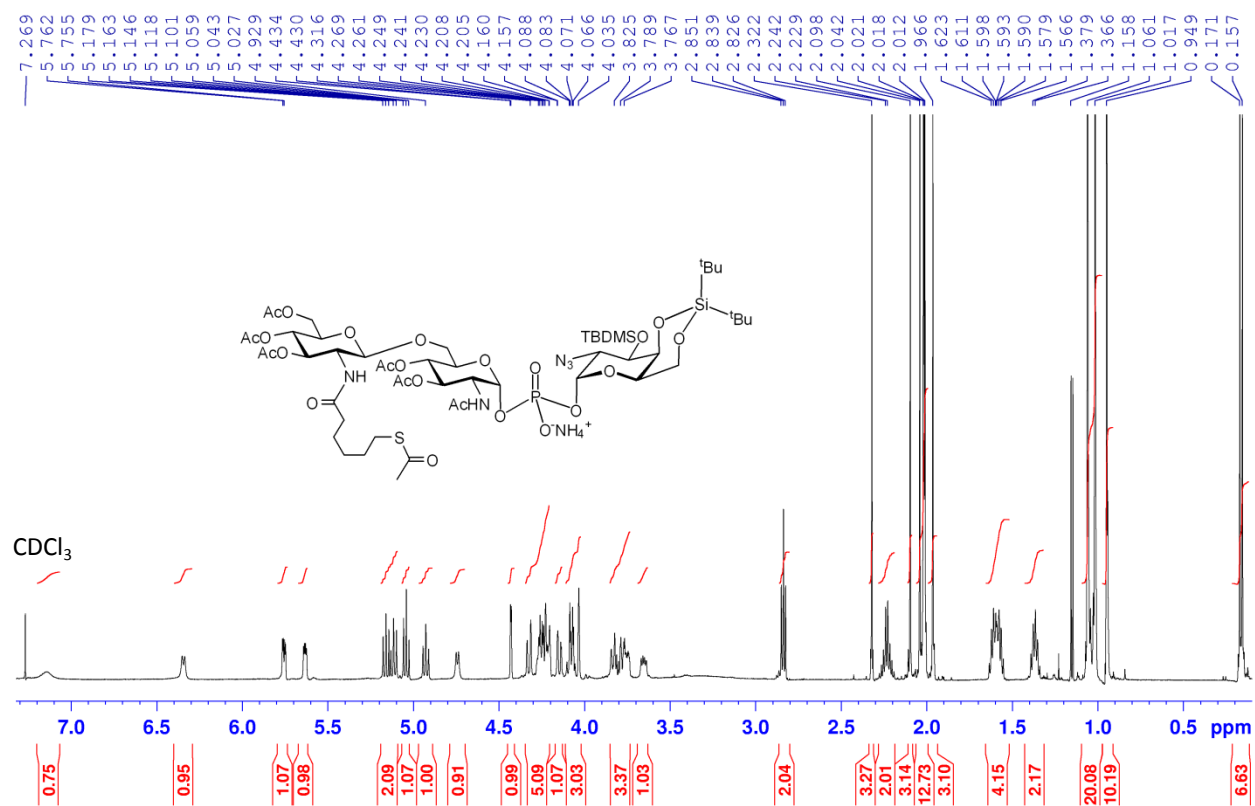
(16): HSQC, D₂O



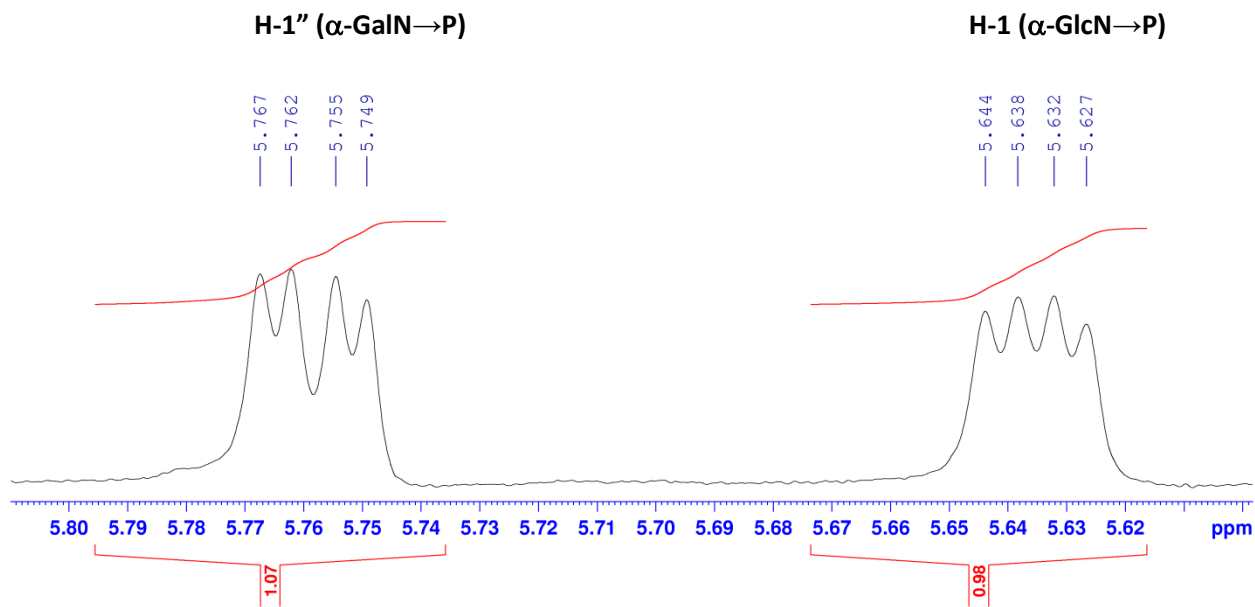
(16): ³¹P-NMR, 243 MHz, D₂O



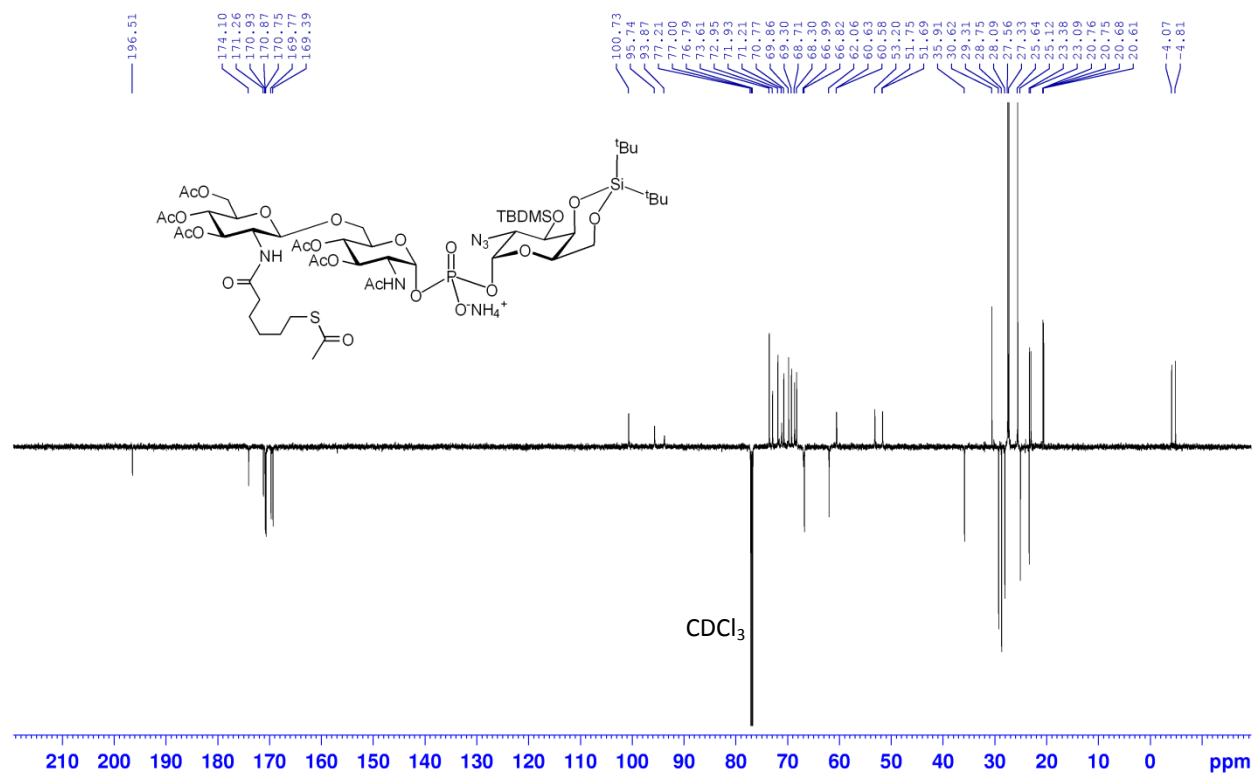
(17): ^1H -NMR, 600 MHz, CDCl_3



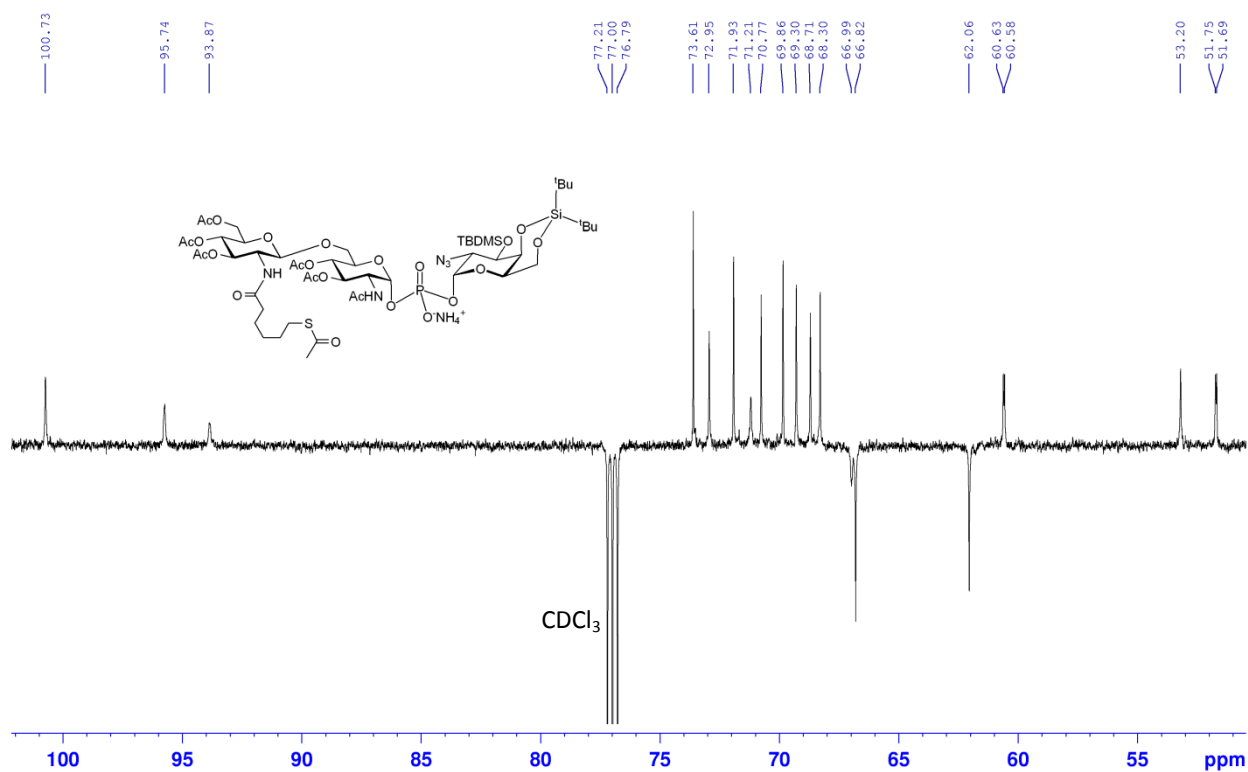
(17): ^1H -NMR, 600 MHz, CDCl_3 , 6.0 – 5.5 ppm



(17): ^{13}C -NMR, APT, 151 MHz, CDCl_3

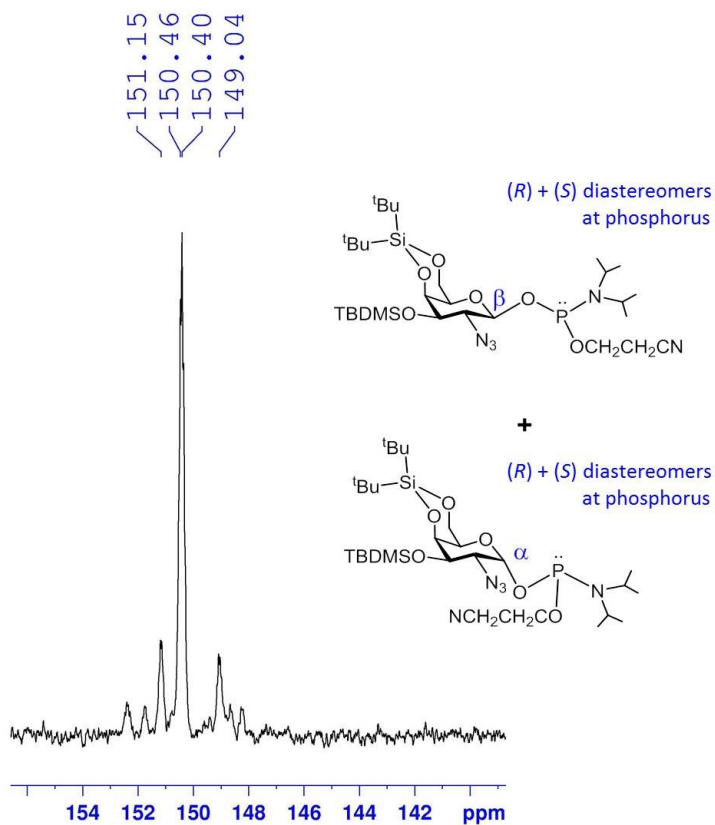
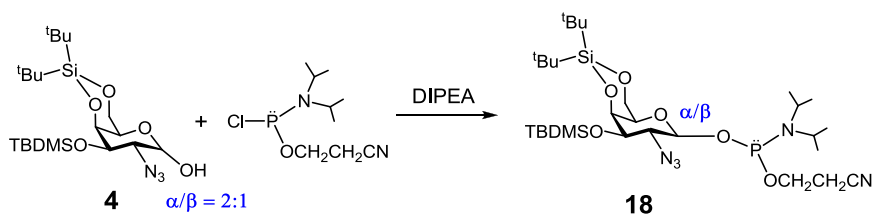


(17): ^{13}C -NMR, APT, 151 MHz, CDCl_3 , 110 – 50 ppm

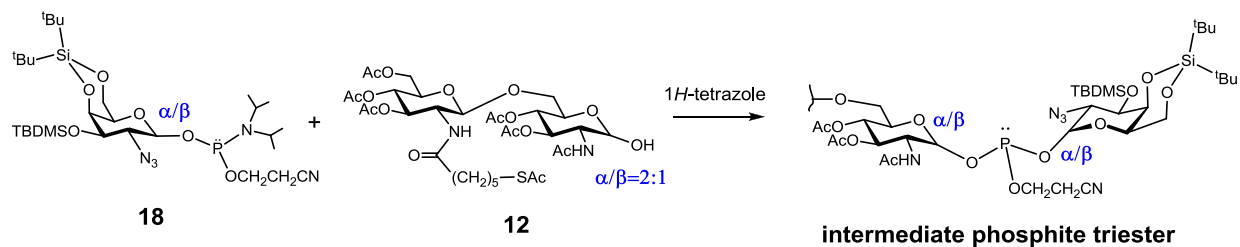


(18): ^{31}P -NMR, 162 MHz, $\text{CH}_2\text{Cl}_2 + \text{CD}_2\text{Cl}_2$

Preparation of **18** (*in situ*)

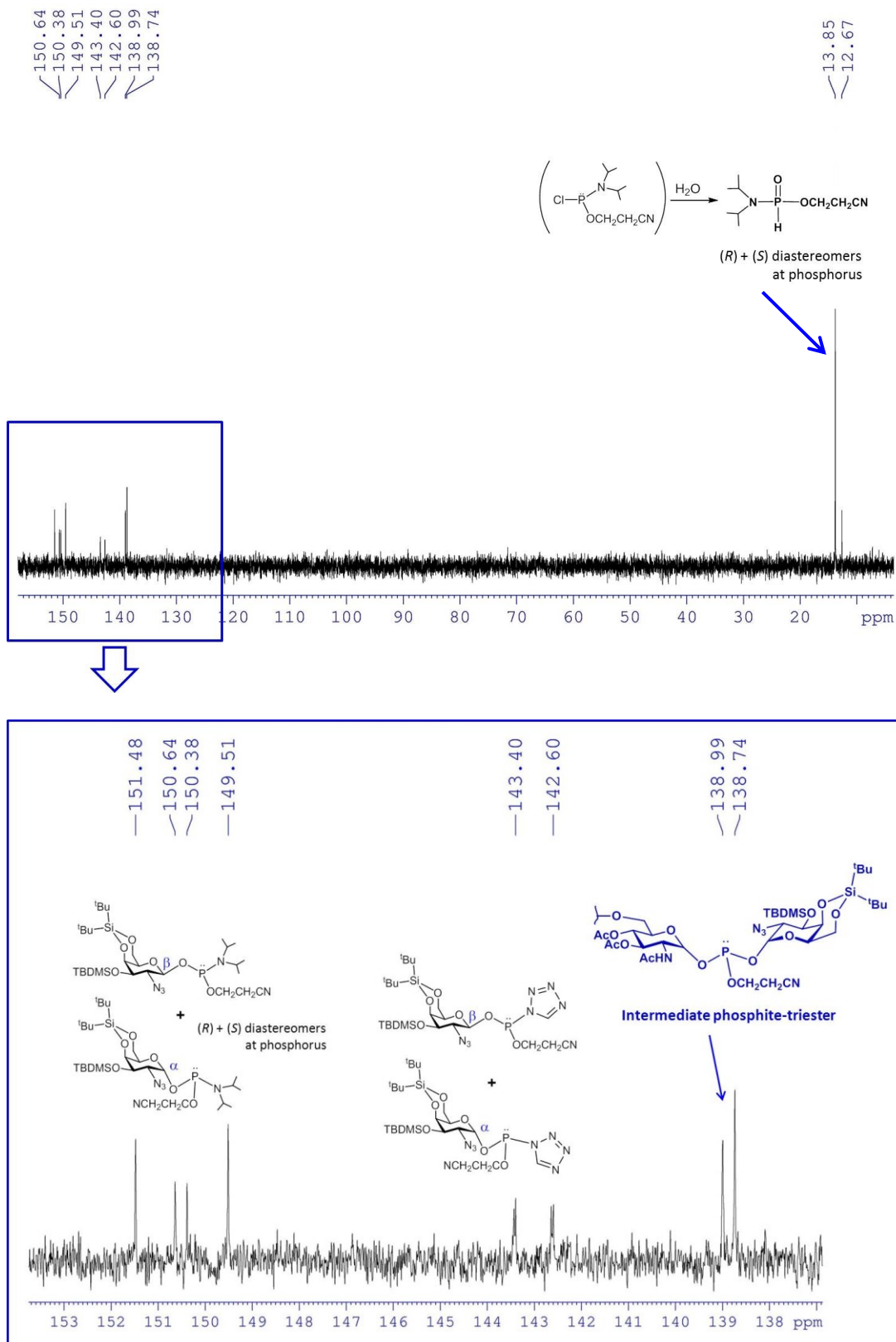


Preparation of **17** *via* **18** followed by ^{31}P -NMR

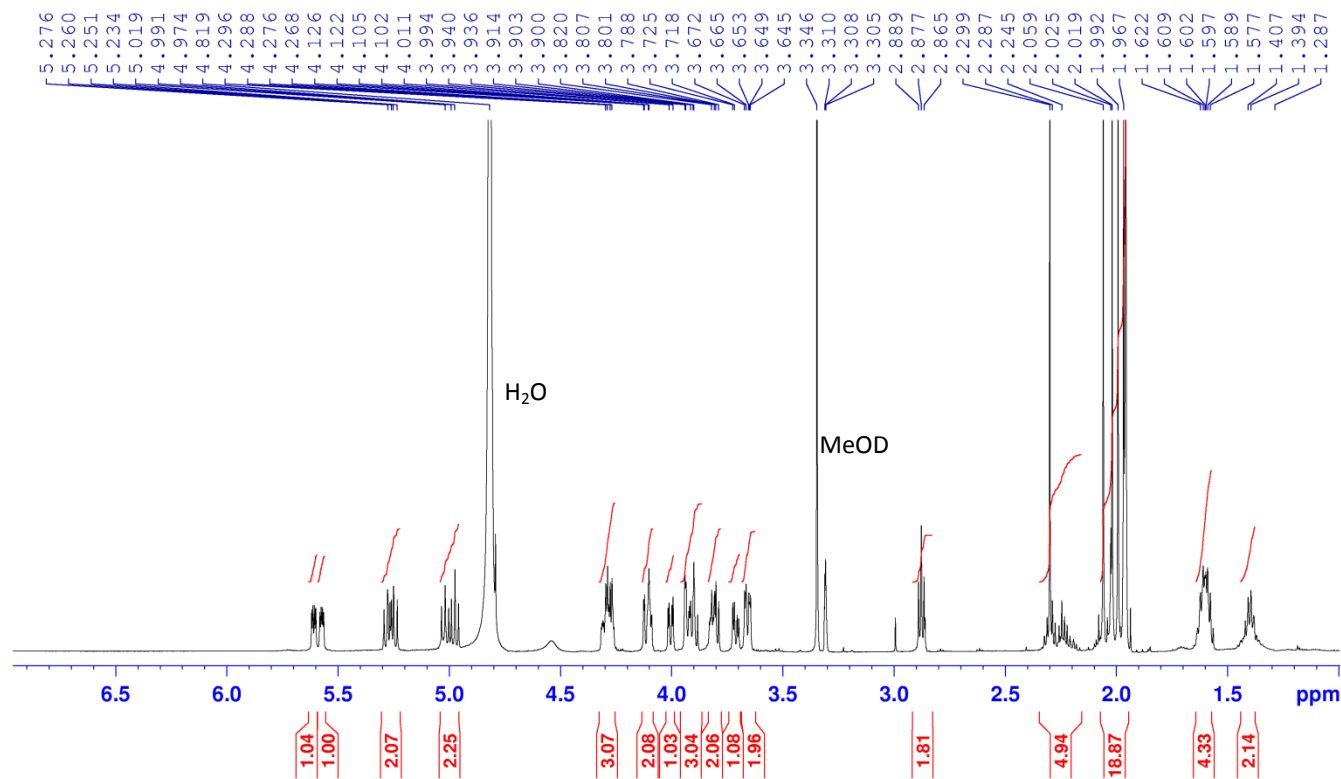


³¹P-NMR, 162 MHz, [CH₂Cl₂ + CH₃CN] + CD₂Cl₂

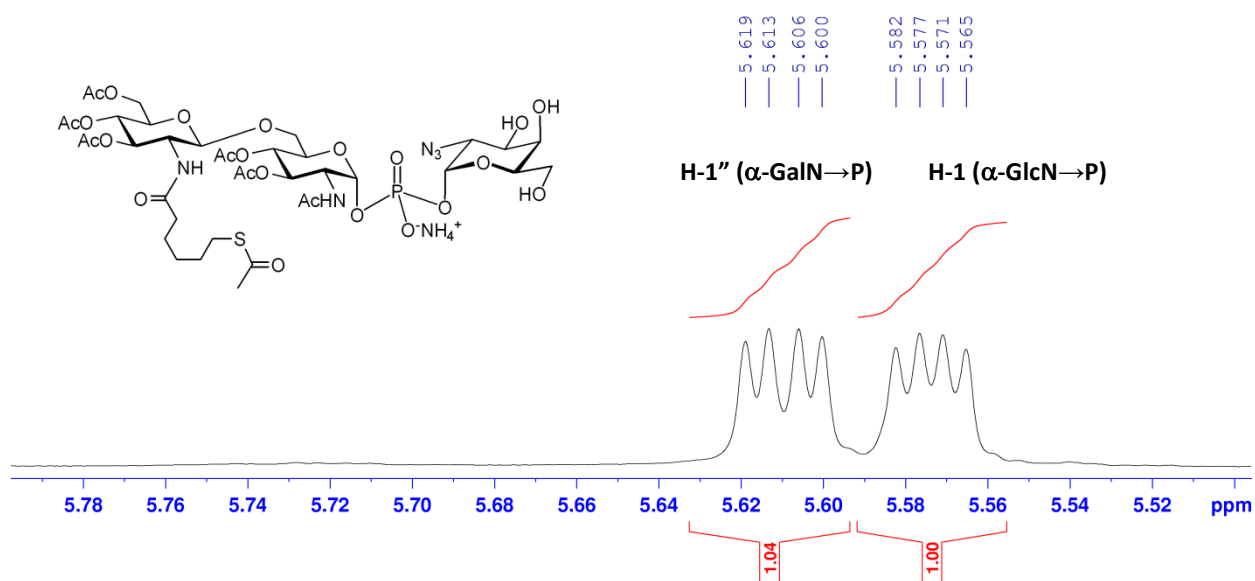
Reaction **18** + **12** → **17** in the NMR tube



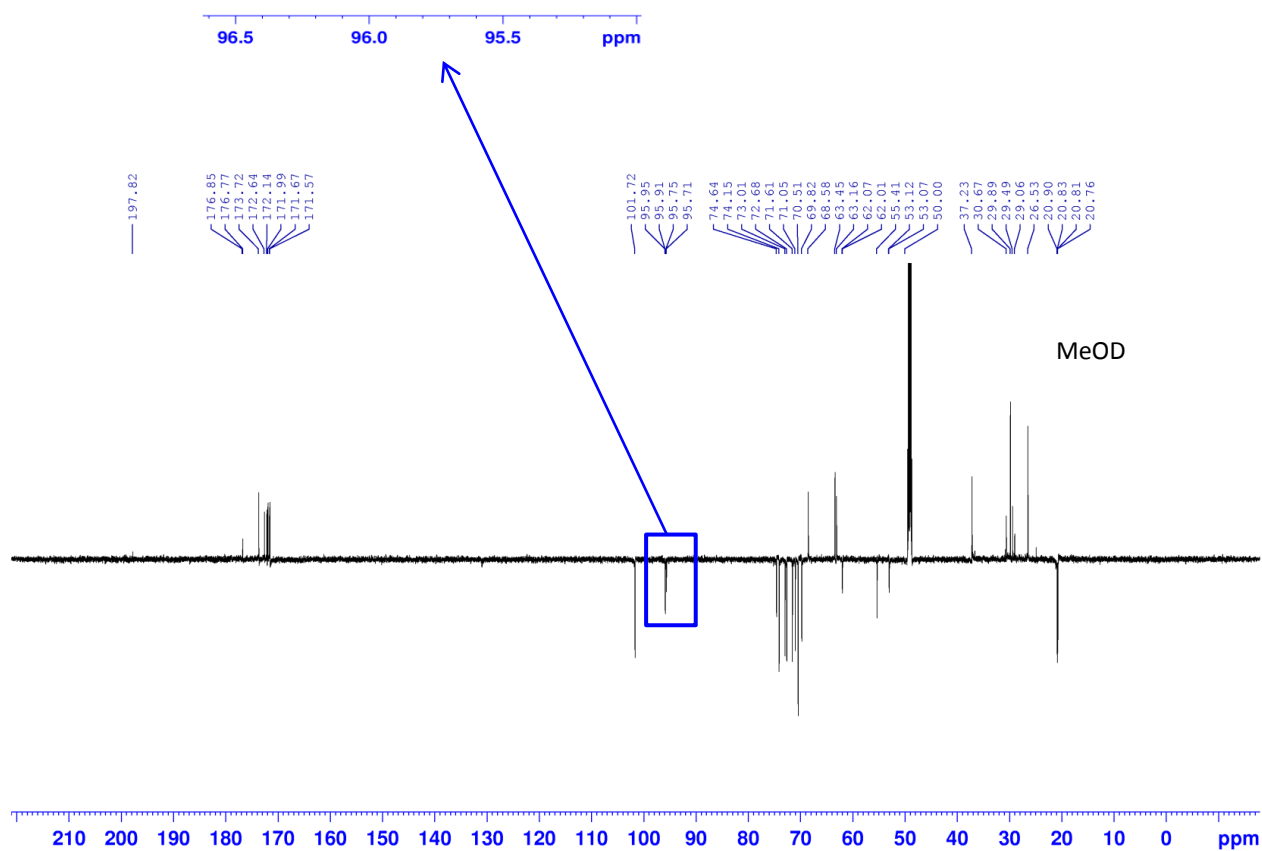
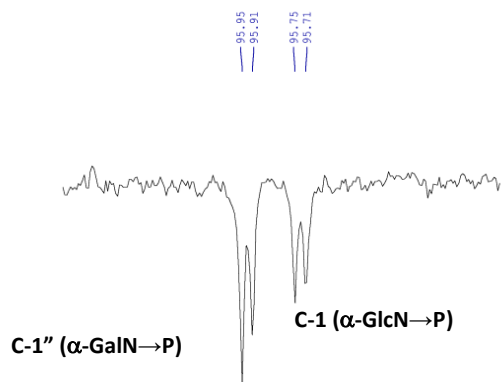
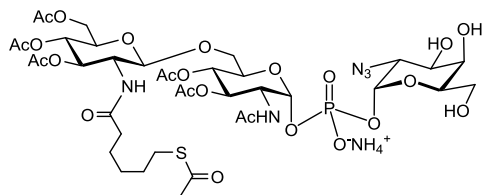
(19): ^1H -NMR, 600 MHz, CD_3OD ,



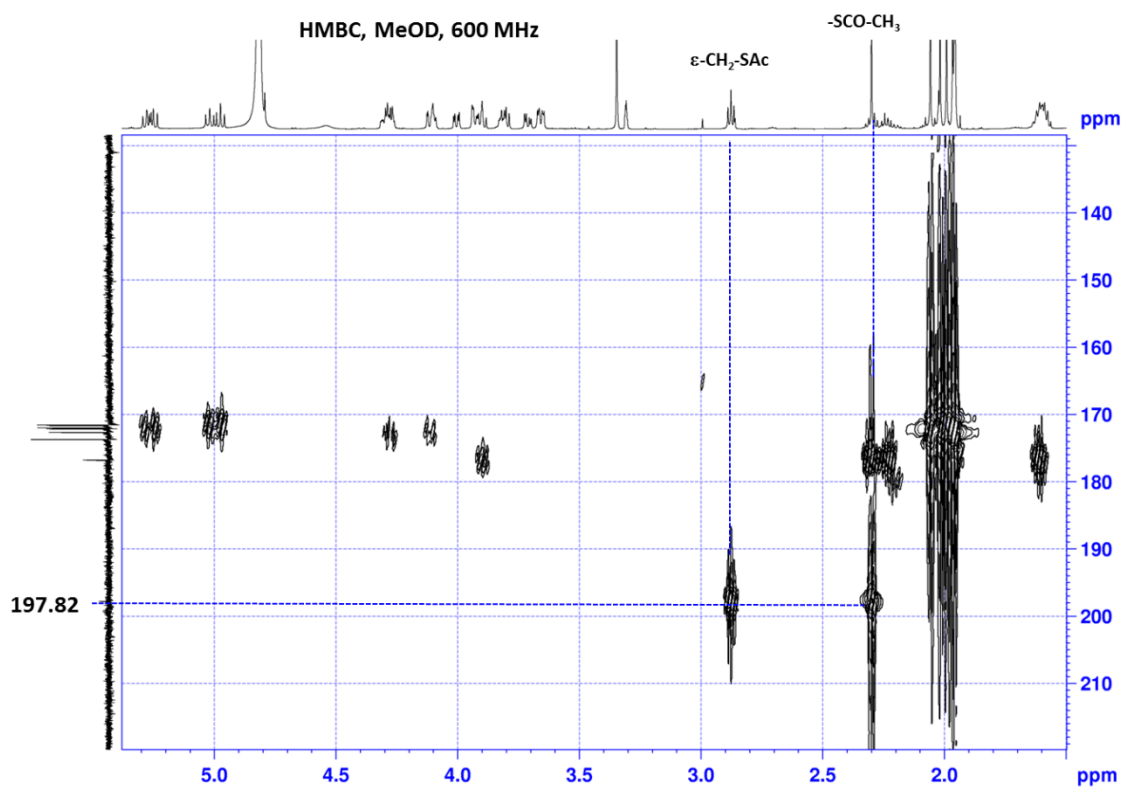
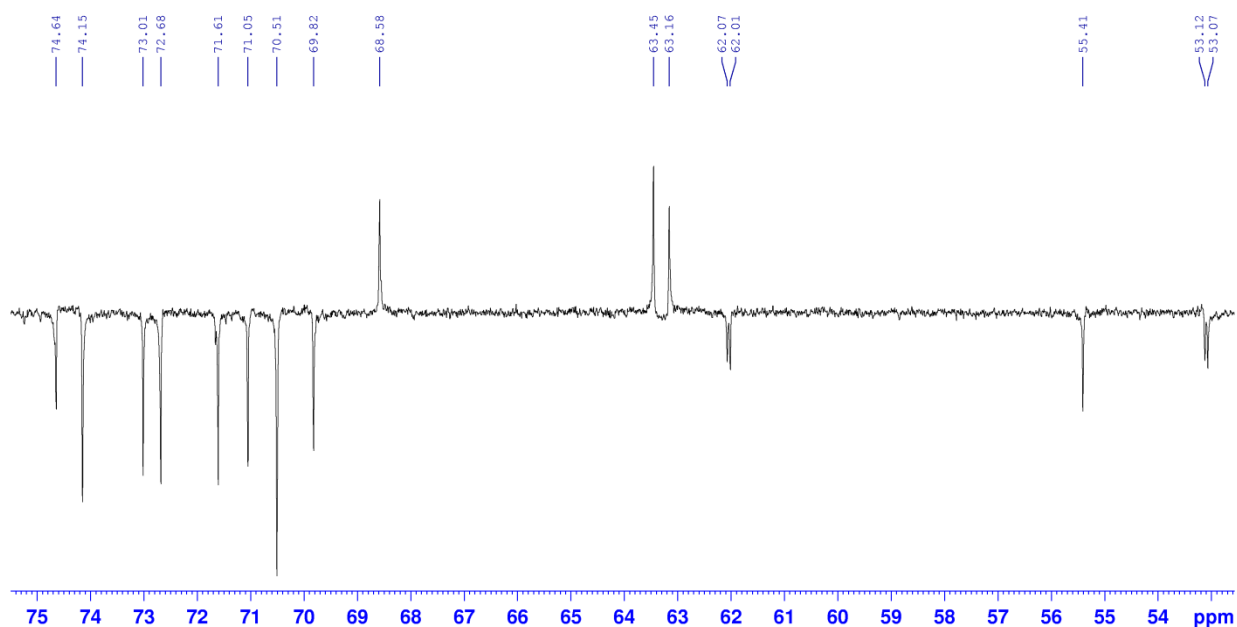
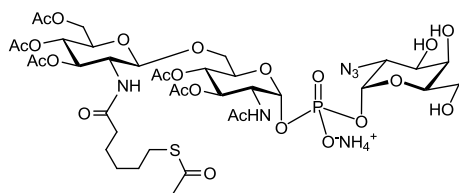
(19): ^1H -NMR, 600 MHz, CD_3OD , 6.0 – 5.5 ppm



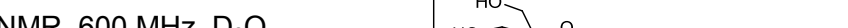
(19): ^{13}C -NMR, APT, 151 MHz, CD_3OD



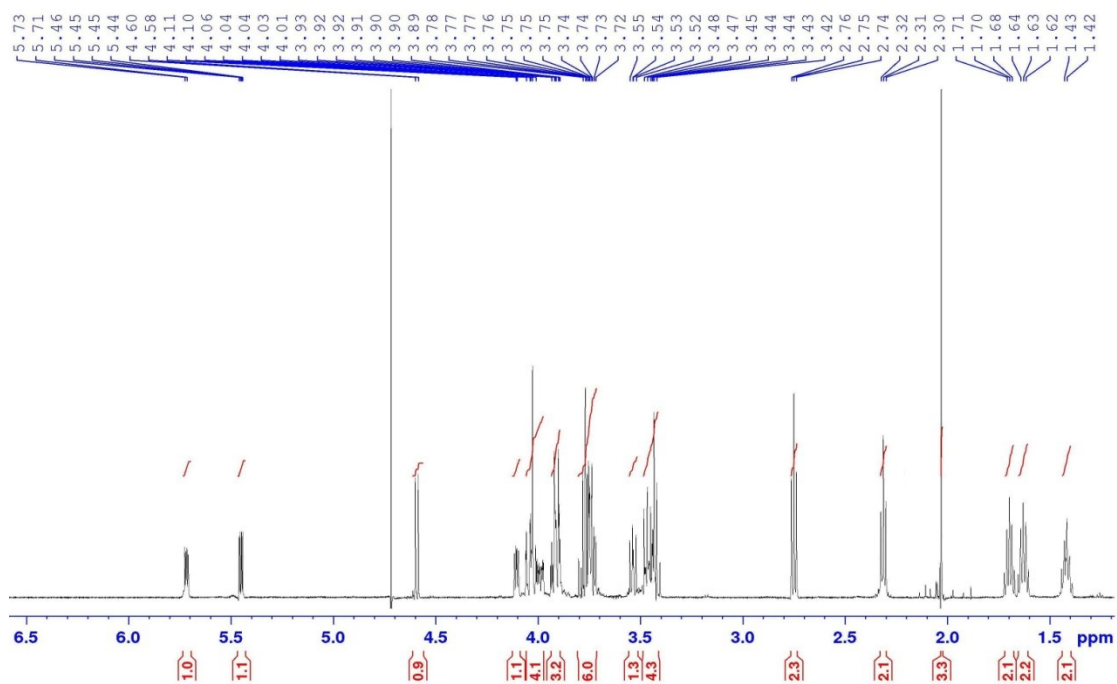
(19): ^{13}C -NMR, APT, 151 MHz, CD_3OD



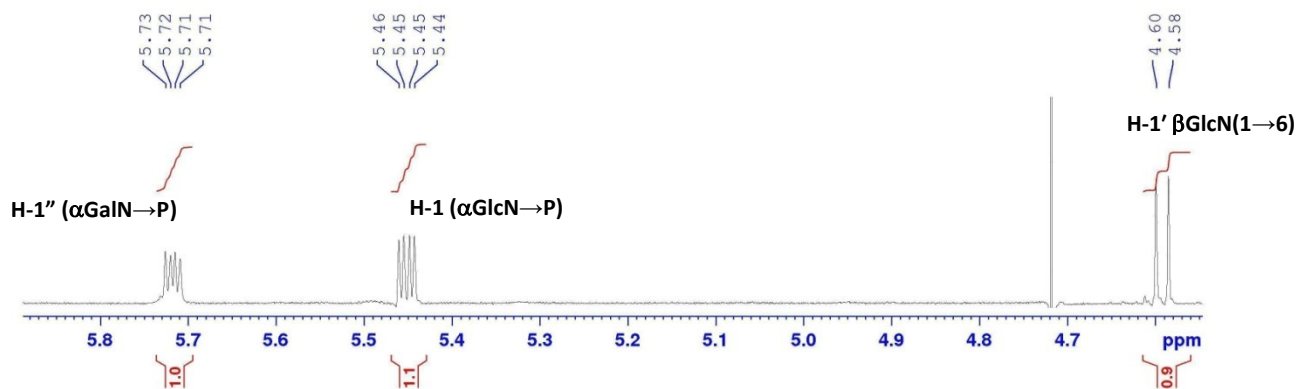
(20): $^1\text{H-NMR}$, 600 MHz, D_2O



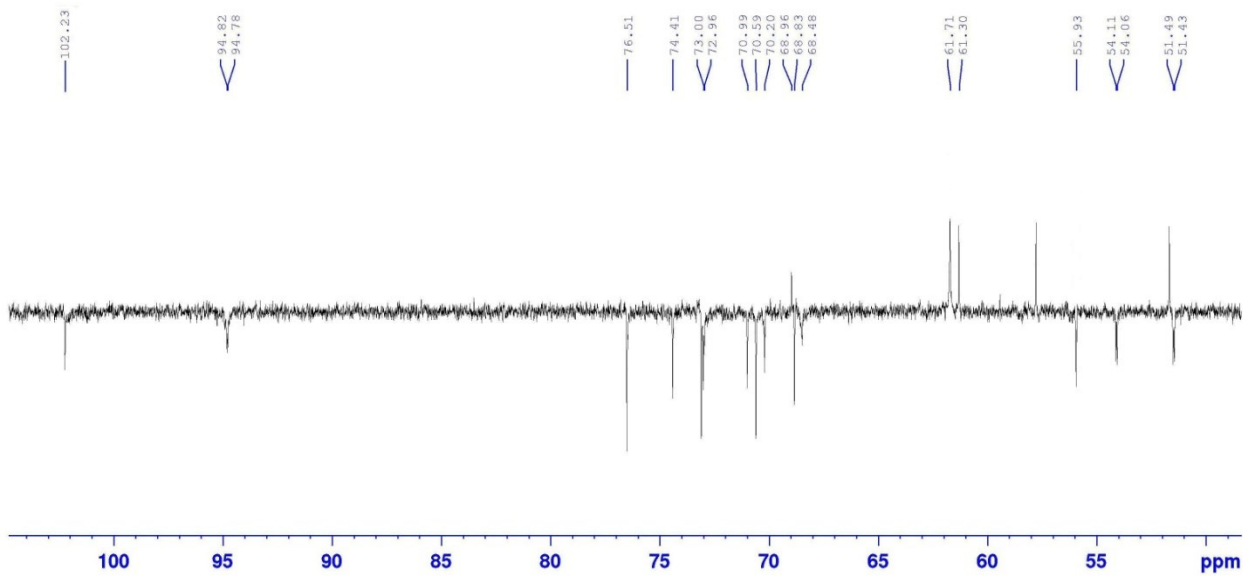
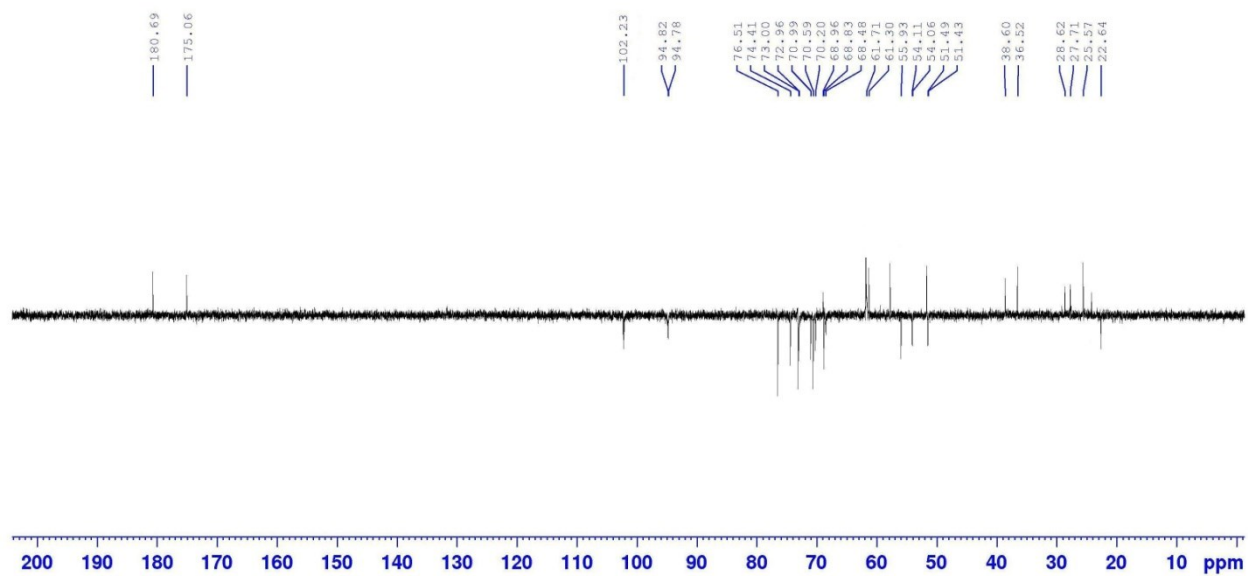
Chemical structure of compound (20) is shown, enclosed in brackets with a subscript 2. The structure represents a dicationic disaccharide derivative, specifically a 2,3,6-tri-O-acetyl-4-O-(phosphoryl)-α-D-glucopyranoside-1,4:6,1'-bisphosphate-2,3,6-tri-O-acetyl-β-D-galactopyranoside. The structure includes a glucose derivative (left) and a galactose derivative (right), both with acetamido (AcHN) groups and hydroxyl (HO) groups. The phosphate groups are linked by a pyrophosphate bridge, and the entire structure is enclosed in brackets with a subscript 2.



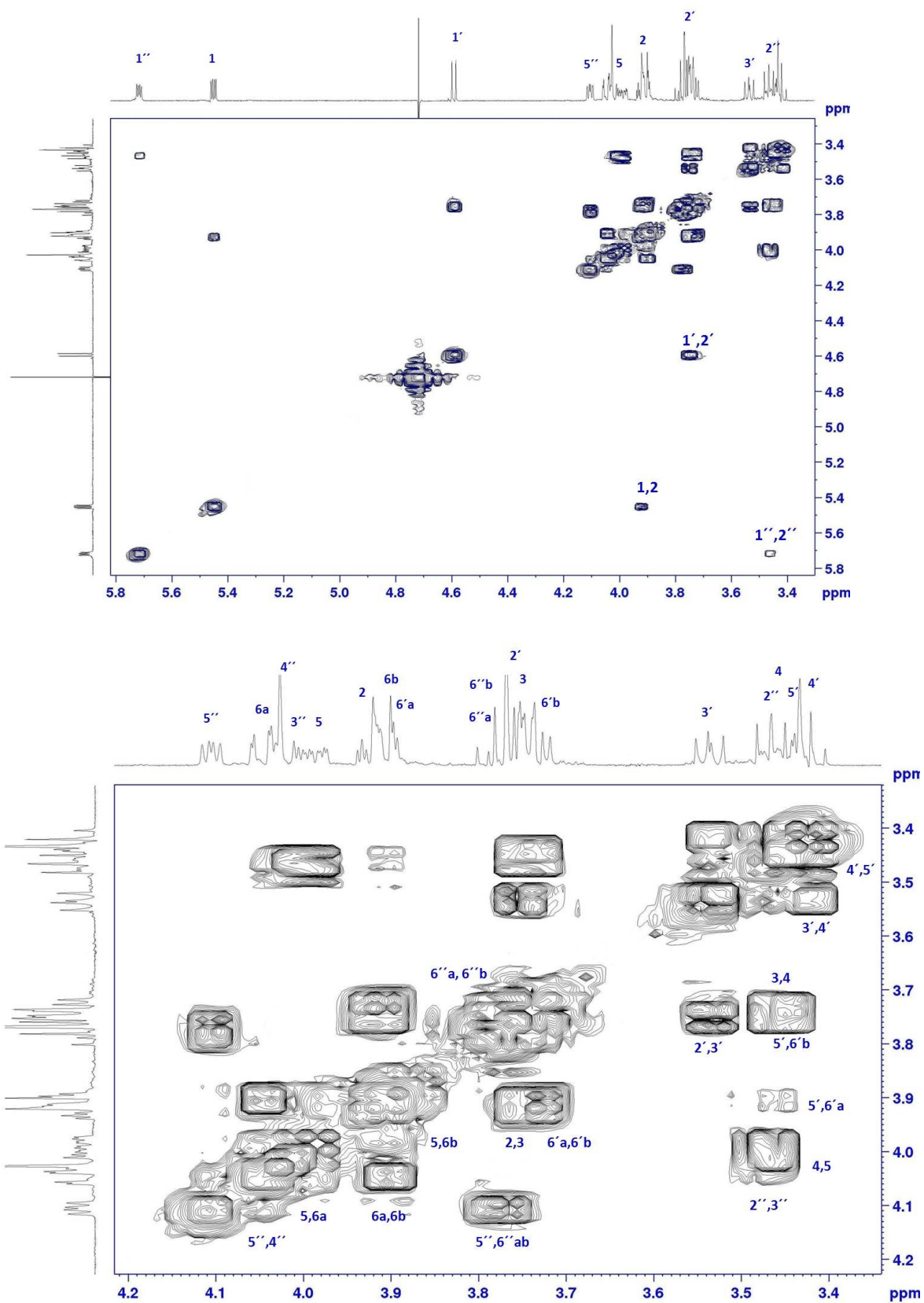
(20): ^1H -NMR, 600 MHz, D_2O , 6.0 – 4.5 ppm



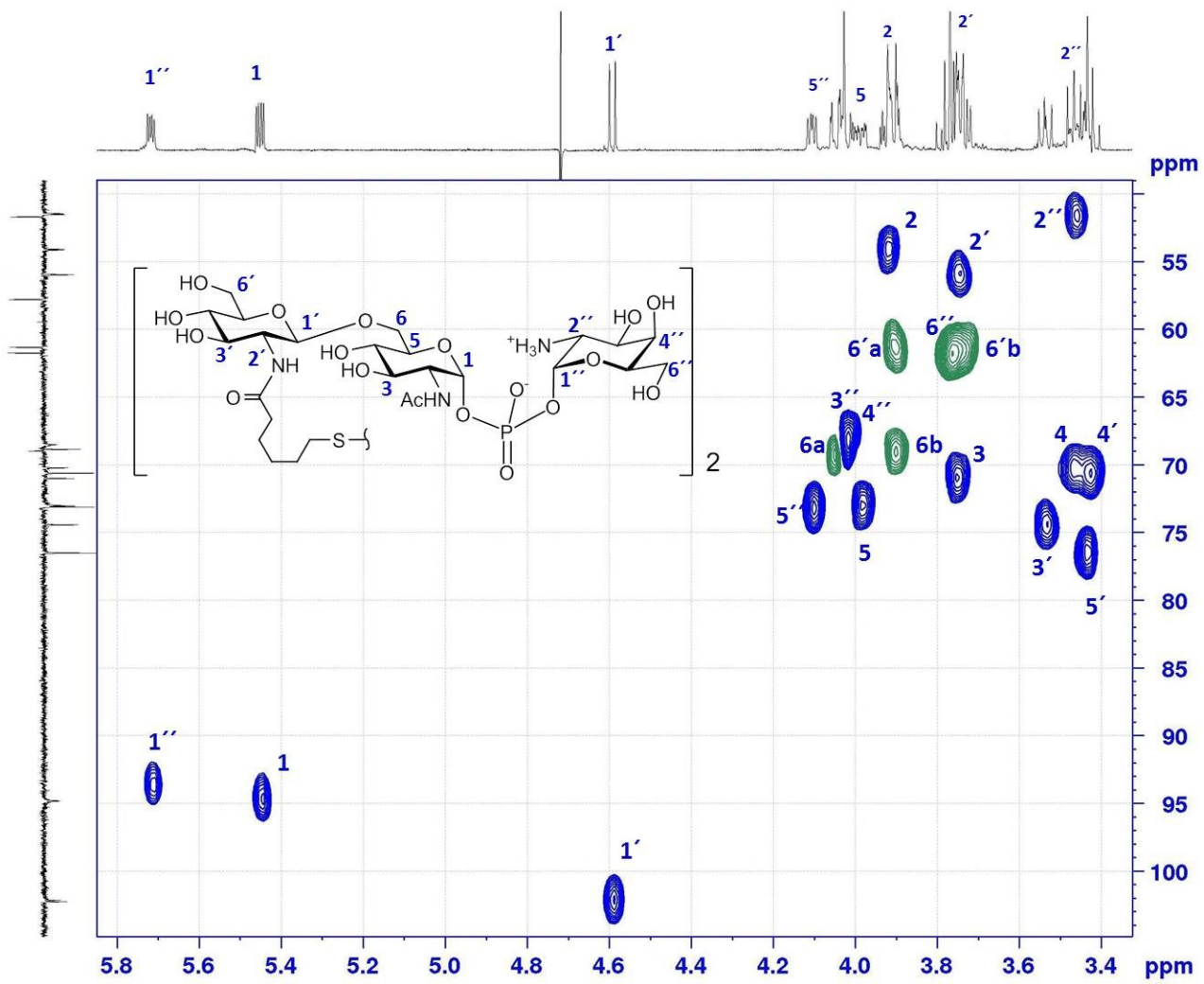
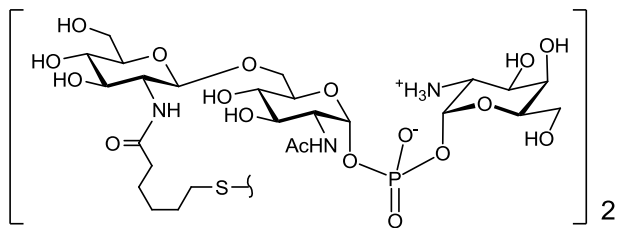
(20): ^{13}C -NMR, APT, 151 MHz, D_2O



(20): COSY, 600 MHz, D₂O

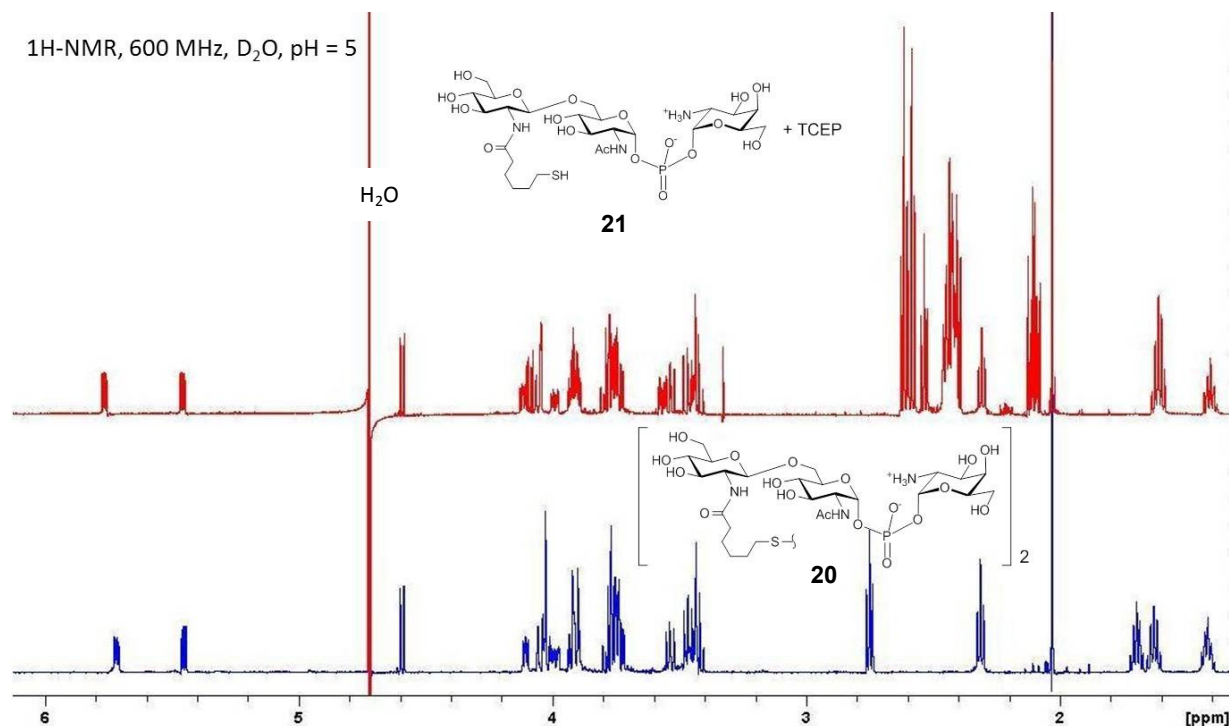


(20): HSQC, D₂O

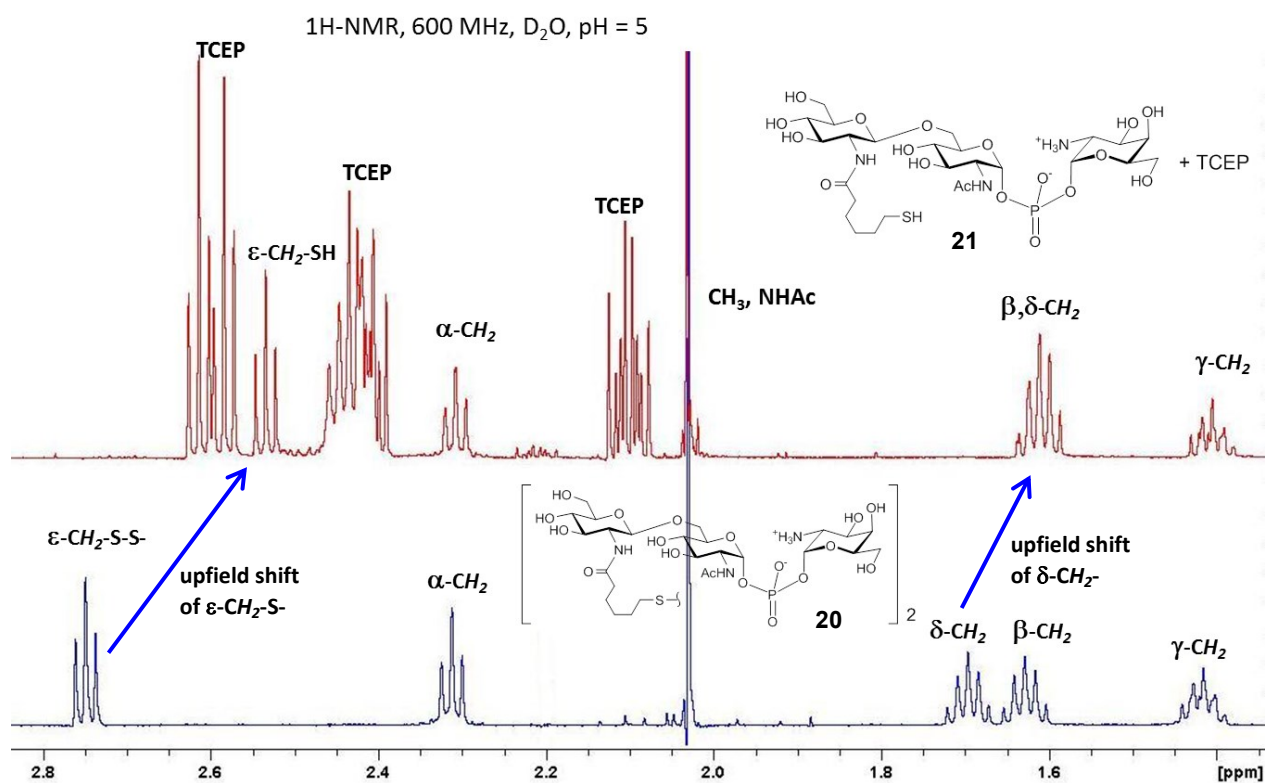


Reduction of the disulfide bond in **20** using TCEP (Tris(2-carboxyethyl)phosphine)

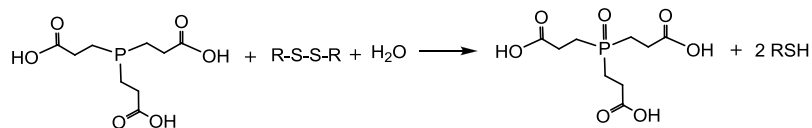
Stacked ^1H -NMR spectra of **21** + TCEP vs. **20** (0 - 6 ppm)



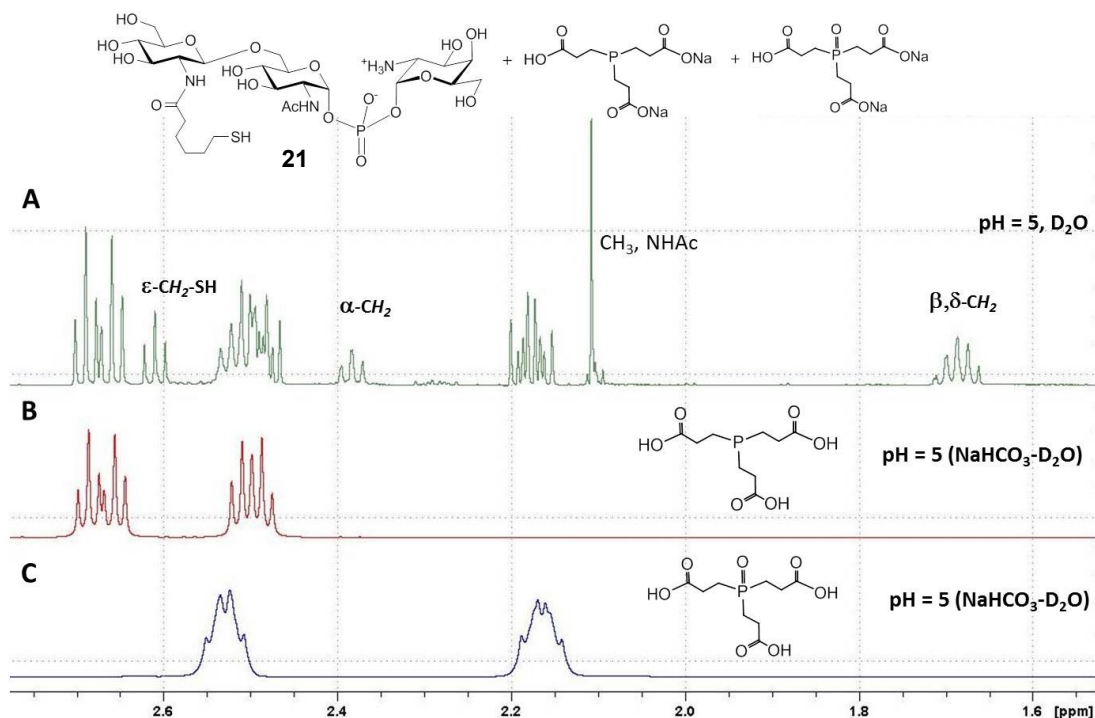
Stacked ^1H -NMR spectra of **20** + TCEP (ϵ -sulfhydryl group - spacer) vs. **21** (ϵ -disulphide) (1.2 – 2.8 ppm)



Reduction of disulfide bond in **20** using TCEP (Tris(2-carboxyethyl)phosphine)

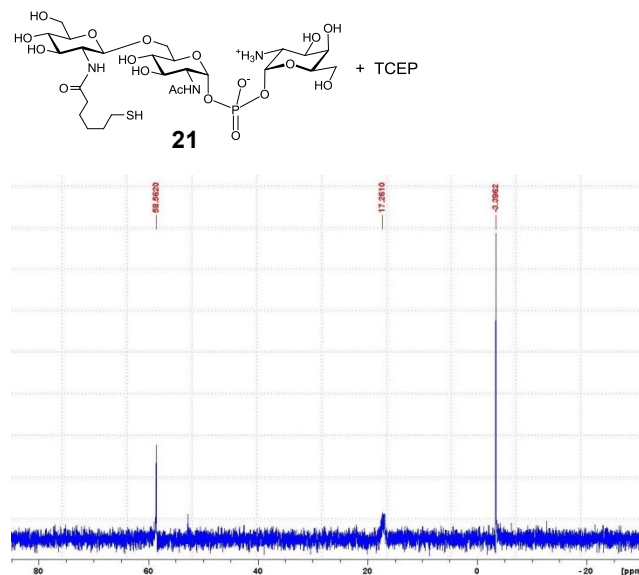


Stacked $^1\text{H-NMR}$ spectra of (A) **21**+TCEP vs. (B) TCEP and (C) TCEP oxide (1.5 – 2.8 ppm)

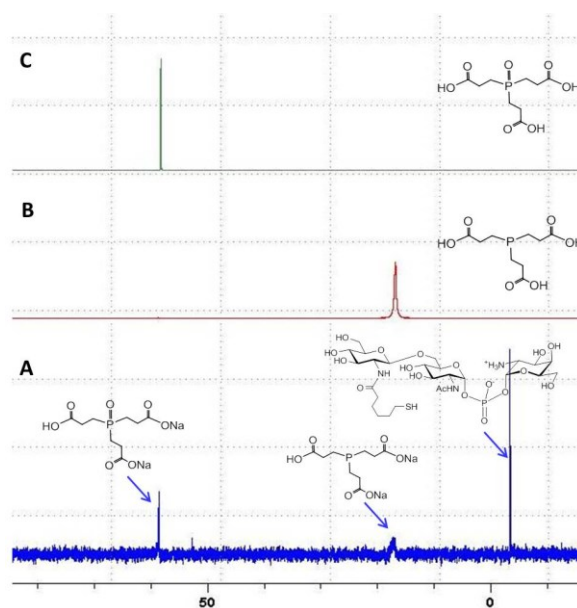


$^{31}\text{P-NMR}$, 243 MHz, D_2O **21**+TCEP

(ϵ -sulfhydryl group - spacer)

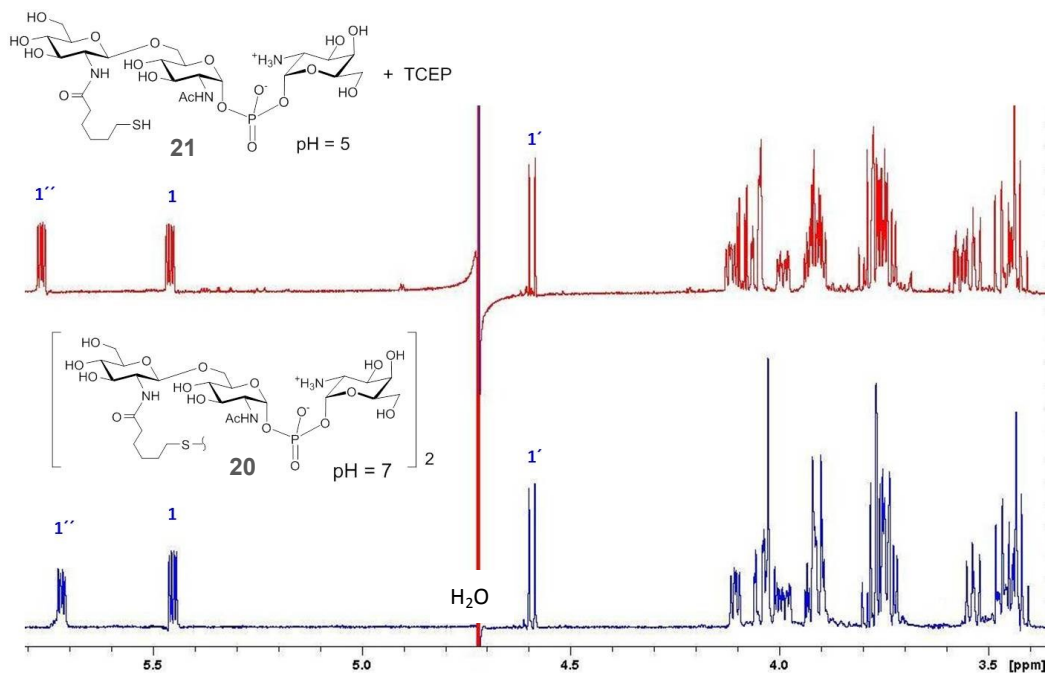


Stacked $^{31}\text{P-NMR}$ spectra of (A) **21**+TCEP vs. (B) TCEP and (C) TCEP oxide

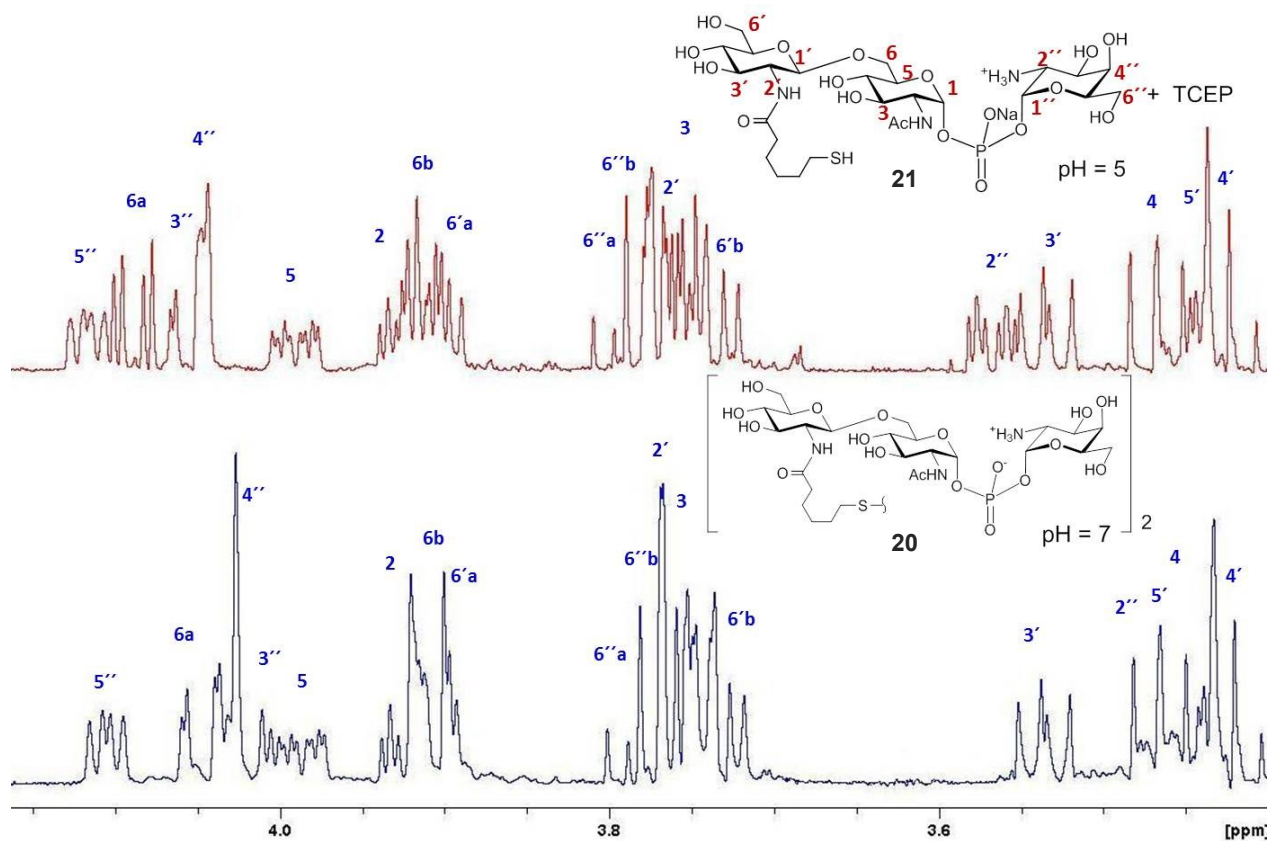


$^1\text{H-NMR}$, 600 MHz, D_2O

Stacked $^1\text{H-NMR}$ spectra of **21+TCEP** (ϵ -sulfhydryl group - spacer) vs. **20** (disulphide) (3.2 - 6.0 ppm)

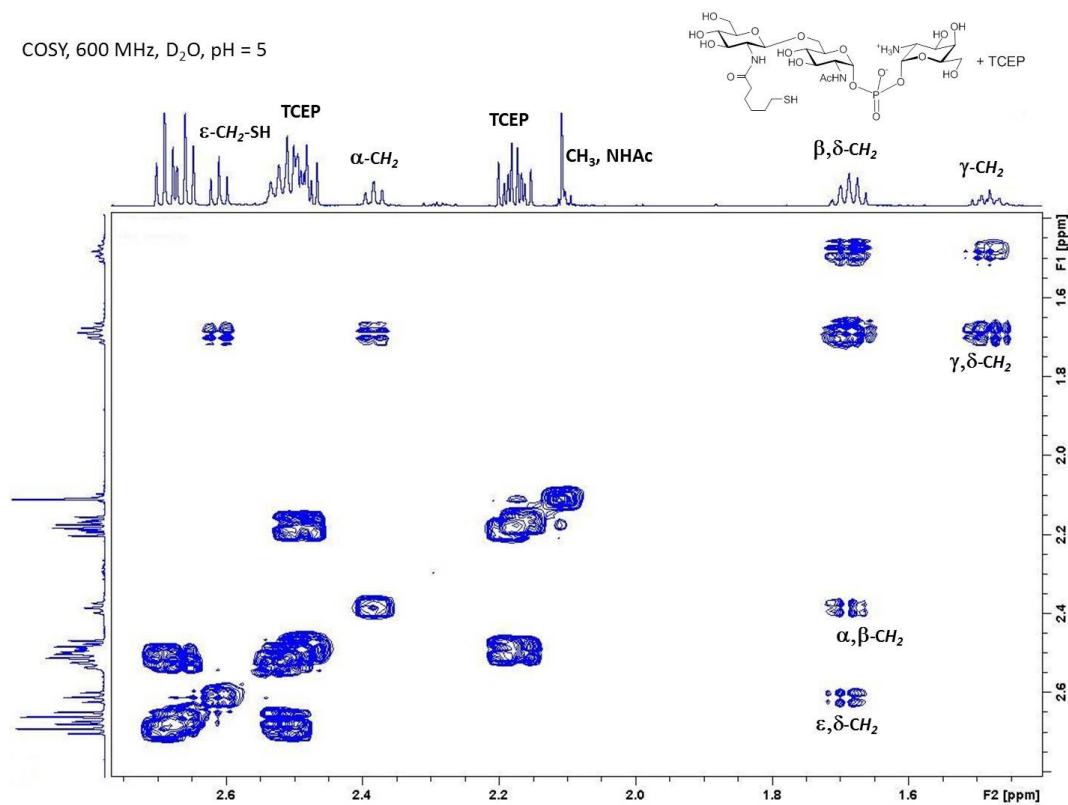


Stacked $^1\text{H-NMR}$ spectra of **21+TCEP** (ϵ -sulfhydryl group - spacer) vs. **20** (disulphide) (3.4 - 4.2 ppm)

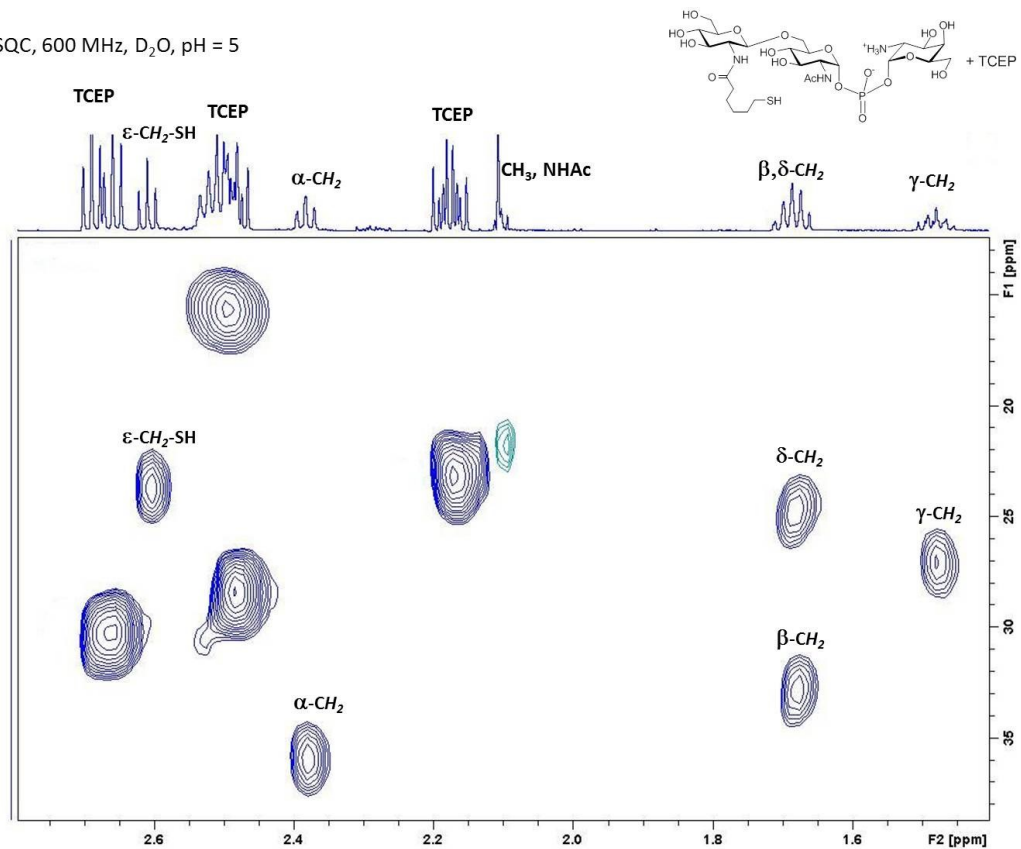


21+TCEP

COSY, 600 MHz, D₂O, pH = 5

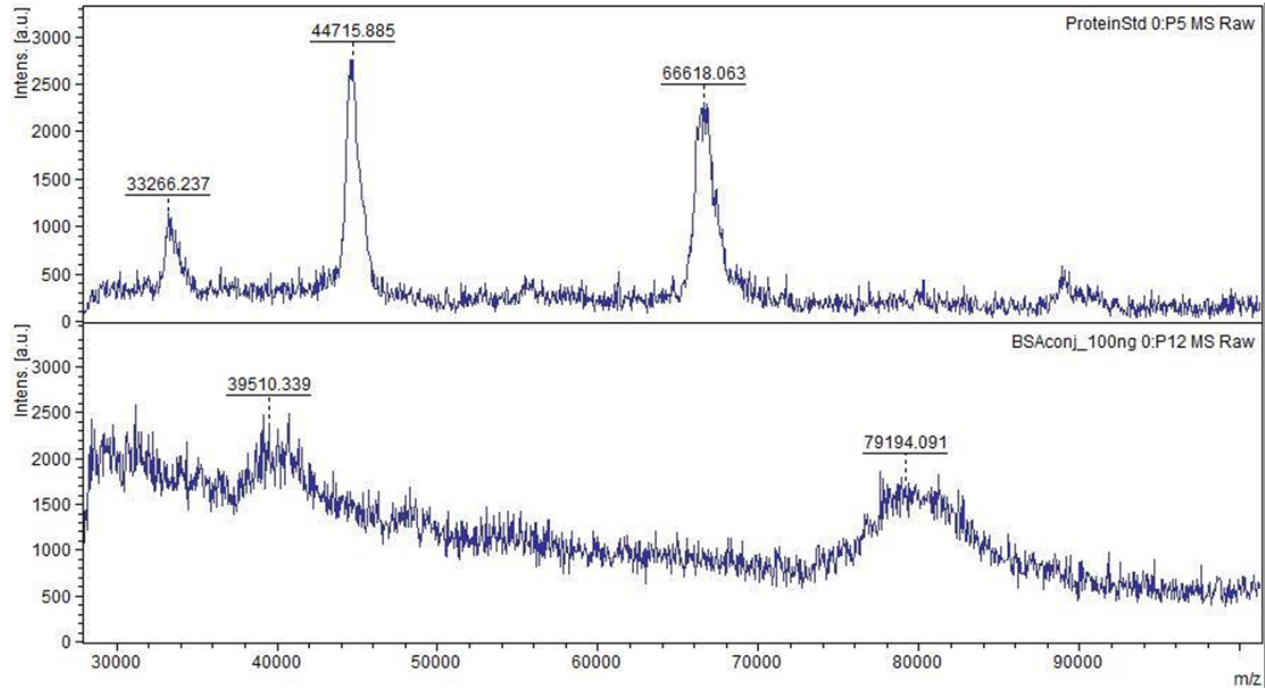


HSQC, 600 MHz, D₂O, pH = 5



21-BSA, MALDI-TOF

21-BSA after purification on Sephadex G-25 and on Bio-Gel P2



21-BSA after purification and dialysis

