

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

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## SUPPORTING INFORMATION

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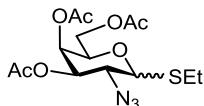
## Experimental part: general synthetic methods.

Reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Dichloromethane was distilled from  $\text{CaH}_2$  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  $\text{H}_2\text{SO}_4$ -*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 ( $^1\text{H}$  at 600.22 MHz;  $^{13}\text{C}$  at 150.92 MHz;  $^{31}\text{P}$  at 242.97 MHz) or on Bruker DPX 400 spectrometer ( $^1\text{H}$  at 400.13 MHz;  $^{13}\text{C}$  at 100.61 MHz;  $^{31}\text{P}$  at 161.68 MHz using standard Bruker NMR software. Chemical shifts are reported in ppm,  $^1\text{H}$ -NMR spectra in  $\text{CDCl}_3$  are referenced to internal TMS,  $^{13}\text{C}$ -spectra are referenced to the corresponding solvent signal (77.00 ppm for  $\text{CDCl}_3$ ). NMR spectra in other solvents are referenced to residual solvent signals (for MeOD: 3.31 ppm, 49.00 ppm,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, for  $\text{DMSO-d}_6$ : 3.31 ppm, 49.00 ppm,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, respectively).  $^{31}\text{P}$ -NMR Spectra in  $\text{CDCl}_3$  are referenced to external triphenylphosphine,  $^{31}\text{P}$ -Spectra in  $\text{D}_2\text{O}$  are referenced to external  $\text{H}_3\text{PO}_4$ . 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%  $\text{CH}_3\text{CN}$  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 $\mu$  C4 300A column using linear gradients  $\text{H}_2\text{O}$  (0.1% HCOOH)  $\rightarrow$   $\text{CH}_3\text{CN}$  (0.1% HCOOH). High resolution mass spectrometry (HRMS) was carried out from acetonitrile solutions (1–10 mg·l<sup>–1</sup>) 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 deg  $\text{dm}^{-1}\text{cm}^3\text{g}^{-1}$ .

## 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,<sup>1</sup> **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 trifluoromethanesulfonate<sup>2–4</sup> followed by deacetylation to provide **1**.<sup>5</sup>

### Synthesis of **S1**

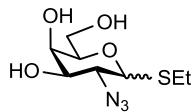


### Ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-D-galactopyranoside (**S1**)

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,  $\alpha/\beta$  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  $\mu$ l, 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  $\text{CH}_2\text{Cl}_2$  (200 ml), washed with satd. aq.  $\text{NaHCO}_3$  (100 ml) and brine (100 ml). The organic phase was dried over  $\text{MgSO}_4$ , 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.<sup>5</sup>

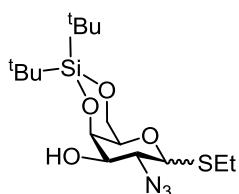
### Synthesis of 1



#### 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  $\mu\text{mol}$ ) 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  $\text{H}^+$  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.<sup>5</sup>

### Synthesis of 2



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

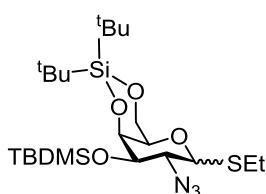
To a cooled (0°C) and stirred solution of **1** (1.338 g, 5.37 mmol) in dry pyridine (15 ml) di-*tert*-butylsilyl bis(trifluoromethanesulphonate) (1.36 ml, 7.51 mmol) was added *via* syringe under Ar. The reaction mixture was stirred for 5 min at 0°C, then gradually (10 min) warmed to r.t. and quenched with anhydrous MeOH (2 ml). The reaction mixture was diluted with toluene (50 ml) and concentrated to dryness. The residue was taken up in  $\text{CH}_2\text{Cl}_2$  (150 ml), washed with aq.  $\text{NaHCO}_3$  (150 ml),  $\text{H}_2\text{O}$  (50 ml) and brine (2×50 ml), dried over  $\text{Na}_2\text{SO}_4$  and concentrated. The residue was purified by MPLC to furnish  $\alpha$ -anomer **2 $\alpha$**  (1.33 g),  $R_f \alpha = 0.76$  (hexane/ethyl acetate, 5:1),  $[\alpha]_D^{20} = +124$  (*c* 0.74,  $\text{CHCl}_3$ ); and  $\beta$ -anomer **2 $\beta$**  (630 mg) as solids; combined yield 1.96 g (93.7%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\alpha$ -anomer):  $\delta = 5.35$  (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,  $\text{SCH}_2$ ), 1.22 (t,  $J = 7.4$  Hz, 3H,  $\text{CH}_3$ ), 1.00, 0.99 (2 s, 18H, 4× $\text{CH}_3$ , DTBS);

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta = 83.59$  (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 ( $\text{SCH}_2$ ), 14.71 ( $\text{CH}_3$ );

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

### Synthesis of 3



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

To a stirred solution of **2** (1.274 g, 3.27 mmol) in dry DMF (7 mL) *tert*-butyldimethylsilyl chloride (1.232 g, 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), NaHCO<sub>3</sub> (50 mL) and brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 5:1) to afford  $\alpha$ -anomer of **3 $\alpha$**  (1.1 g, 66.3%) as a syrup and  $\beta$ -anomer of **3 $\beta$**  (450 mg, 27.7%) as amorphous solid. Overall yield 1.531 g, 94%;  $\alpha/\beta = 2.4/1$ .

**3:  $\alpha$ -anomer:** R<sub>f</sub> = 0.77 (hexane/ethyl acetate, 5:1);  $[\alpha]_D^{20} = +6.9$  (c 0.94, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta = 4.36$  (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, SCH<sub>2</sub>), 1.27 (t,  $J = 7.4$  Hz, 3H, CH<sub>3</sub>), 1.04, 1.03, 0.93, (3 s, 27 H, DTBS, TBDMS), 0.16, 0.11 (2 s, 6 H, TBDMS).

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta = 84.03$  (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 (SCH<sub>2</sub>), 14.92 (CH<sub>3</sub>), -2.80, -4.23, -4.79 (CH<sub>3</sub>, TBDMS)

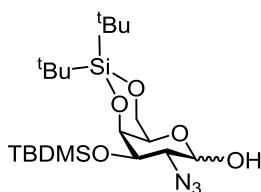
**3:  $\beta$ -anomer:** R<sub>f</sub> = 0.81 (hexane/ethyl acetate, 7:1);  $[\alpha]_D^{20} = +138.6$  (c 1.7, CHCl<sub>3</sub>);

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta = 4.27$  (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, H6b), 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, SCH<sub>2</sub>CH<sub>3</sub>), 1.29 (t,  $J_{CH_2,CH_3} = 7.4$  Hz, 3H, CH<sub>3</sub>), 1.07, 1.03, 0.94, (3 s, 27 H, DTBS, TBDMS), 0.16, 0.12 (2 s, 6 H, TBDMS).

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta = 84.37$  (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 (SCH<sub>2</sub>CH<sub>3</sub>), 14.98 (SCH<sub>2</sub>CH<sub>3</sub>), -2.80, -4.23, -4.79 (CH<sub>3</sub>, TBDMS);

HRMS (<sup>+</sup>ESI-TOF): for C<sub>22</sub>H<sub>45</sub>N<sub>3</sub>NaO<sub>4</sub>SSi<sub>2</sub> *m/z* [M+Na]<sup>+</sup>: 526.2562, found: 526.2567.

## Synthesis of 4



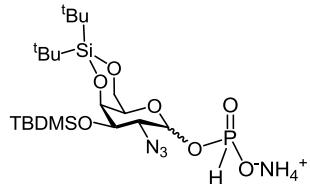
### 2-Azido-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-D-galactopyranose (4)

To a cooled (0°C) stirred solution of **3** (740 mg, 1.47 mmol) in acetone/water 10:1 (11 mL) aq satd. NaHCO<sub>3</sub> (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. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (50 mL), aq. NaHCO<sub>3</sub> (2×50 mL) and brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by MPLC (hexane/ethylacetate, 5:1 → 3:1) to give **4** ( $\alpha/\beta = 2:1$ , 571 mg, 79%); R<sub>f</sub> ( $\alpha$ ) = 0.51, R<sub>f</sub> ( $\beta$ ) = 0.37 (hexane/ethylacetate, 5:1),  $[\alpha]_D^{20}$  ( $\alpha/\beta = 2:1$ ) = +56 (c 0.95, CHCl<sub>3</sub>);

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

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 100MHz):  $\delta$  = 96.3 (C-1 $\beta$ ), 92.62 (C-1 $\alpha$ ), 74.1 (C-3 $\beta$ ), 73.8 (C-4 $\alpha$ ), 72.64 (C-4 $\beta$ ), 71.77 (C-5 $\beta$ ), 70.18 (C-3 $\alpha$ ), 67.72 (C-5 $\alpha$ ), 67.28 (C-6 $\alpha$ ), 67.12 (C-6 $\beta$ ), 65.62 (C-2 $\beta$ ), 61.37 (C-2 $\alpha$ ), 27.47, 27.33, 25.65, 23.44, 20.75, 17.97 57 (CH<sub>3</sub>, DTBS  $\alpha/\beta$ ), -4.29, -4.53, -4.84, -4.96 (CH<sub>3</sub>, TBDMS  $\alpha/\beta$ );  
HRMS (ESI-TOF): calcd. for C<sub>21</sub>H<sub>41</sub>N<sub>3</sub>O<sub>7</sub>Si *m/z* [M+HCOO]<sup>-</sup> 504.2567, found: 504.2565.

## Synthesis of 5



### 2-Azido-3-*O*-tert-butyldimethylsilyl-4,6-*O*-di-tert-butyldimethylsilylene-2-deoxy- $\alpha$ -D-galactopyranosyl-1-hydrogenphosphonate and 2-azido-3-*O*-tert-butyldimethylsilyl-4,6-*O*-di-tert-butyldimethylsilylene-2-deoxy- $\beta$ -D-galactopyranosyl-1-hydrogenphosphonate (ammonium salt) (5).

To a stirred solution of **4** ( $\alpha/\beta$  = 2:1, 421 mg, 915  $\mu$ mol) in CH<sub>3</sub>Cl (20 mL) a solution of Et<sub>3</sub>N 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 $\times$ 20 ml) and the reaction mixture was dried by repeated co-evaporation with dry toluene (4 $\times$ 20 ml). To a stirred solution of **4** (421 mg, 915  $\mu$ mol) in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) dry pyridine (200  $\mu$ l, 2.5 mmol) and a solution of 2-chloro-1,3,2-benzodioxaphosphorin-4-one (salicylchlorophosphite, SalPCI) (463 mg, 2.3 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (3 ml) were added successively under atmosphere of Ar. Reaction mixture was stirred for 5 h. at r.t., then aq Et<sub>3</sub>N (250  $\mu$ l, pH 10) was added and the mixture was stirred for additional 15 min, diluted with CHCl<sub>3</sub> (200 ml) and washed with 0.2 M TEAB (triethylammonium bicarbonate) buffer (3 $\times$ 50 ml). The combined aqueous phases were washed with CHCl<sub>3</sub> (3 $\times$ 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 (CHCl<sub>3</sub>/MeOH/25% aq.NH<sub>4</sub>OH, 6/1/0.07) to give **5** (390 mg, 78%,  $\alpha/\beta$  = 5:1) as a solid. R<sub>f</sub> = 0.13 (CHCl<sub>3</sub>/MeOH/25% aq.NH<sub>4</sub>OH, 6/1/0.07),  $[\alpha]_D^{20}$  = +75 (*c* 0.72, CHCl<sub>3</sub>);

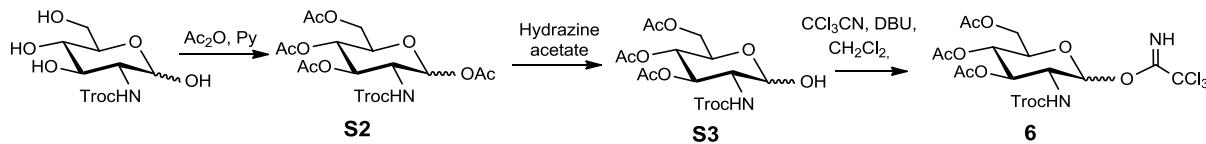
<sup>1</sup>H NMR (CDCl<sub>3</sub>:CD<sub>3</sub>OD, 4:1, 400 MHz):  $\delta$  = 6.91 (d,  $J_{\text{P},\text{H}} = 653$  Hz, 1 H, P-H $\beta$ ), 6.84 (d,  $J_{\text{P},\text{H}} = 646$  Hz, 1 H, P-H $\alpha$ ), 5.60 (dd,  $J_{1,2} = 3.4$  Hz,  $J_{1,\text{P}} = 8.2$  Hz, 1H, H-1- $\alpha$ ), 4.78 (dd,  $J_{1,2} = 8.3$  Hz,  $J_{\text{P},1} = 8.3$  Hz, 1H, H-1- $\beta$ ), 4.30 (d,  $J_{3,4} = J_{4,5} = 2.7$  Hz, 1 H, H-4- $\alpha$ ), 4.18 (dd,  $J_{6\text{a},6\text{b}} = 12.7$  Hz,  $J_{5,6\text{a}} = 2.0$  Hz, 1 H, H-6a- $\alpha$ ), 4.16 (m, 1H, H-6a- $\beta$ ), 4.12 (m, 1H, H-6b- $\beta$ ), 4.06 (dd,  $J_{6\text{a},6\text{b}} = 12.7$  Hz,  $J_{5,6\text{b}} = 1.8$  Hz, 1H, H-6b- $\alpha$ ), 3.97 (dd,  $J_{2,3} = 10$  Hz, 1H, H-3- $\alpha$ ), 3.88 (bs, 1H, H-5- $\alpha$ ), 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- $\beta$ ), 3.42 (dd,  $J_{2,3} = J_{3,4} = 9.7$  Hz, 1H, H-3- $\beta$ ), 3.38 (bs, 1H, H-5- $\beta$ ), 2.30 (NH<sub>4</sub><sup>+</sup>), 0.99 (s, 9H, 3 $\times$ CH<sub>3</sub>, DTBS- $\alpha$ ), 0.88 (s, 9H, 3 $\times$ CH<sub>3</sub>, DTBS- $\alpha$ ), 0.89 (s, 9H, 3 $\times$ CH<sub>3</sub>, TBDMS- $\alpha$ ), 0.11, 0.08 (2 s, 6H, 2 $\times$ CH<sub>3</sub>, TBDMS);

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

<sup>31</sup>P NMR (CDCl<sub>3</sub>:CD<sub>3</sub>OD, 4:1, 162 MHz):  $\delta$  = 2.99 ( $\alpha$ ,  $J_{\text{PH}} = 646$  Hz),  $\delta$  = 1.45, ( $\beta$ ,  $J_{\text{PH}} = 653$  Hz);  
HRMS (ESI-TOF): calcd. for C<sub>20</sub>H<sub>41</sub>N<sub>3</sub>O<sub>7</sub>PSi<sub>2</sub> *m/z* [M-H]<sup>-</sup> 522.2226, found: 522.2219.

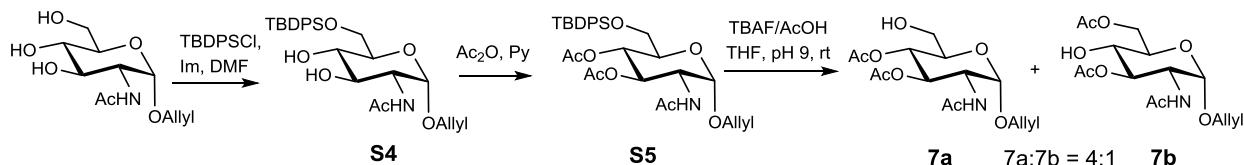
## Synthesis of 6

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



## Synthesis of 7

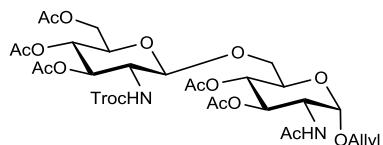
According to the reported procedure, allyl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside was treated with TBDPSCl /Im in DMF to provide **S4**, which was peracetylated to give **S5**, successive removal of the primary silyl group provided compounds **7a**<sup>9</sup> and **7b**. Since the 4-*O*-acetyl group in 6-*O*-unprotected glucopyranoses such as **7a** is prone to (4→6) acyl migration<sup>10,11</sup> to furnish **7b** under basic conditions<sup>12</sup> (TBAF, **7a**:**7b**, 2:1), the reaction mixture **S5**→**7a** was buffered with acetic acid (final pH = 9) which resulted in the substantial improvement of the **7a**:**7b** ratio (5:1).<sup>13</sup> Generally, the rate of the (4→6) acyl migration in glucopyranoses depends on the pH value and on the duration of the basic (or acidic) treatment.<sup>13,14</sup> 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→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- $\alpha$ -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 5min (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 Na<sub>2</sub>SO<sub>4</sub> 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.<sup>9</sup> Due to the known propensity of 4-*O*-acetyl group to undergo (4→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)- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -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 CH<sub>2</sub>Cl<sub>2</sub> (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 µl, 510 µmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (3 ml) was added dropwise under atmosphere of Ar. The reaction mixture was stirred for 1 h at -75°C, quenched by addition of Na<sub>2</sub>CO<sub>3</sub> (100 mg) and gradually warmed up to r.t. The mixture

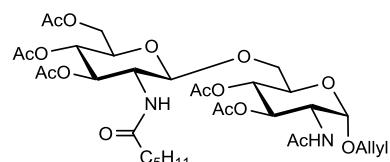
was diluted with  $\text{CH}_2\text{Cl}_2$  (150 ml), washed with aq.  $\text{NaHCO}_3$  ( $2 \times 50$  ml) and water ( $2 \times 50$  ml), dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The residue was purified by MPLC (hexane/ethyl acetate, 1:2) to furnish **8** (2.33 g, 63%) as a solid.  $R_f = 0.21$  (hexane/ethyl acetate, 1:2),  $[\alpha]_D^{20} = +43.0$  ( $c$  1.0,  $\text{CHCl}_3$ );

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 5.90 - 5.85$  (m, 1 H,  $\text{CH} =$ , 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,  $\text{CH}_2$ -*trans*, Allyl), 5.25 (dq,  $J = 10.5$  Hz,  $J = 1.3$  Hz, 1 H,  $\text{CH}_2$ -*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 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,  $\text{CH}_2$  Troc), 4.67 (bd,  $J = 12.8$  Hz, 1H,  $\text{CH}_2$  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,  $\text{OCH}_2$ , 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,  $\text{OCH}_2$ , 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,  $\text{CH}_3$ , Ac);

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta = 171.48, 170.8, 170.71, 169.94, 169.91, 169.54$  (6x CO, Ac, AcNH), 154.27 (CO, Troc), 133.39 (CH, Allyl), 118.61 ( $\text{CH}_2$ , Allyl), 101.69 (C-1'), 96.47 (C-1), 74.7 ( $\text{CH}_2$  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 ( $\text{OCH}_2$ , Allyl), 68.25 (C-6), 62.94 (C-6'), 56.11 (C-2'), 51.72 (C-2), 23.19, 20.73, 20.60 ( $\text{CH}_3$ , Acetyl);

HRMS ( $^{\dagger}\text{ESI-TOF}$ ): calcd. for  $\text{C}_{30}\text{H}_{42}\text{Cl}_3\text{N}_2\text{O}_{17}$   $m/z$  [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- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranoside (9)

To a stirred solution of **8** (440 mg, 545  $\mu\text{mol}$ ) in 8 ml hexanoic acid and 15 ml  $\text{CH}_2\text{Cl}_2$  was added Zn-dust (10  $\mu\text{m}$ , 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- $\beta$ -D-glucopyranosyl)-2-deoxy- $\alpha$ -D-glucopyranoside,  $R_f = 0.55$  (EtOAc/MeOH/AcOH, 5:1:0.05), diisopropylcarbodiimide (255  $\mu\text{l}$ , 1.64 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was diluted with  $\text{CHCl}_3$  (200 ml), solids were separated by filtration through the pad of Celite, the filtrate was washed with satd. aq.  $\text{NaHCO}_3$  ( $3 \times 100$  ml). The organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated. The residue was purified by MPLC on silica gel (hexane/ethyl acetate, 2:1  $\rightarrow$  0:1) to give **9** (487 mg, 76.8%) as a solid.  $R_f = 0.13$  (EtOAc/Hexane, 2:1),  $[\alpha]_D^{20} = +35$  ( $c$  0.68,  $\text{CHCl}_3$ );

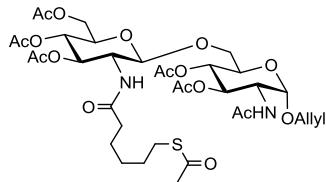
$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 5.88$  (m, 1 H,  $\text{CH} =$ , Allyl), 5.76 (d,  $J = 8.7$  Hz, 1 H, 2'-NHAc), 5.63 (d,  $J = 9.6$  Hz, 1 H, 2-NHAc), 5.31 (dq,  $J = 17.2$ ,  $J = 1.6$  Hz, 1 H,  $\text{CH}_2$ -*trans*, Allyl), 5.25 (dq,  $J = 10.4$  Hz,  $J = 1.3$  Hz, 1 H,  $\text{CH}_2$ -*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,  $\text{OCH}_2$ , 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, 1 H,  $\text{OCH}_2$ , 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,  $\alpha$ -CH<sub>2</sub>), 2.08, 2.06, 2.02, 2.01, 1.94 (6s, 18H,  $\text{CH}_3$ , 5 $\times$ Ac, NAc), 1.63 – 1.53 (m, 2H,  $\beta$ -CH<sub>2</sub>), 1.35 – 1.23 (m, 4 H,  $\gamma$ , $\delta$ -CH<sub>2</sub>), 0.88 (t,  $J = 7$  Hz, 3 H,  $\varepsilon$ -CH<sub>3</sub>);

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta = 173.42, 171.42, 170.96, 170.85, 170.06, 169.85, 169.50$  (CO, 6xAc, hexanoyl), 133.26 (CH, Allyl), 118.50 ( $\text{CH}_2$ , 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 ( $\text{OCH}_2$ , 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 ( $\alpha$ -

$\text{CH}_2$ ), 31.52 ( $\gamma\text{-CH}_2$ ), 25.41 ( $\beta\text{-CH}_2$ ), 23.37 ( $\text{CH}_3$ , Ac), 22.47 ( $\delta\text{-CH}_2$ ), 20.94, 20.91, 20.86, 20.77 ( $\text{CH}_3$ , Ac), 14.06 ( $\varepsilon\text{-CH}_3$ );

HRMS (ESI): calcd. for  $\text{C}_{33}\text{H}_{51}\text{N}_2\text{O}_{16}$   $m/z$  [M+H]<sup>+</sup>: 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)

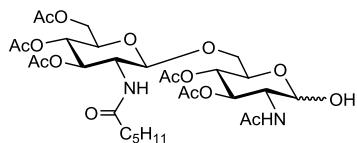
To a solution of **8** (2.0 g, 2.47 mmol) in AcOH (40 ml) was added Zn-dust (10  $\mu\text{m}$ , 2 g, 300 mol) at r.t. The Zn-dust was dispersed by ultrasonic treatment followed by stirring at r.t. for 5 h. The reaction mixture was diluted with  $\text{CHCl}_3$  (200 ml) and washed with aq. satd.  $\text{NaHCO}_3$ , dried with  $\text{Na}_2\text{SO}_4$ , 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- $\beta$ -D-glucopyranosyl)-2-deoxy- $\alpha$ -D-glucopyranoside] ( $R_f$  = 0.55 (EtOAc/MeOH/AcOH, 5:1:0.05), was dried *in vacuo* and dissolved in dry  $\text{CH}_2\text{Cl}_2$ . 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  $\text{CHCl}_3$  (200 ml), washed with water (100 ml), the organic phase was dried with  $\text{Na}_2\text{SO}_4$ , 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),  $[\alpha]_D^{20}$  = +35 ( $c$  1.0, MeOH);

<sup>1</sup>H NMR (MeOD, 600 MHz):  $\delta$  = 5.99-5.95 (m, 1H,  $\text{CH}=\text{Allyl}$ ), 5.37-5.34 (m, 2H,  $=\text{CH}_2$  *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,  $=\text{CH}_2$  *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  $\text{H}_2\text{O}$  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,  $\text{OCH}_2$ , Allyl), 4.12 (dd, 1H,  $J_{5',6'b} = 2.2$  Hz, H-6'b), 4.03-4.01 (m, 1H,  $\text{OCH}_2$ , Allyl), 3.97 (ddd,  $J_{5,6'a} = 2.0$  Hz,  $J_{5,6'b} = 5.7$  Hz, 1H, H-5), 3.91 (dd, 1H, H-2'), 3.89 (dd,  $J_{6'a,6'b} = 10.6$  Hz, 1H, H-6a), 3.78 (dd, 1H, H-5'), 3.57 (dd, 1H, H-6b), 2.86 (t,  $J = 7.2$  Hz, 2H,  $\varepsilon\text{-CH}_2\text{-SAC}$ ), 2.30 (s, 3H,  $\text{CH}_3$ , SAC), 2.19-2.16 (m, 2H,  $\alpha\text{-CH}_2$ , hexanoyl), 2.07, 2.03, 2.0, 1.98, 1.96, 1.92 (6 s, 18H,  $\text{CH}_3$ , 5×Ac, NHAc), 1.60-1.55 (m, 4H,  $\beta\text{-CH}_2$ ,  $\delta\text{-CH}_2$ ), 1.40-1.36 (m, 2H,  $\gamma\text{-CH}_2$ );

<sup>13</sup>C NMR (MeOD, 151 MHz):  $\delta$  = 198.0 (CO, SAC), 176.23 (CO, hexanoyl), 173.61, 172.50, 172.22, 172.97, 171.64, 171.42 (6xCO, Ac, NHAc), 135.17 (CH, Allyl), 118.54 (CH<sub>2</sub>, 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 ( $\text{OCH}_2$ , Allyl), 69.50 (C-6), 63.41 (C-6'), 55.29 (C-2'), 53.08 (C-2), 37.31 ( $\alpha\text{-CH}_2$ ), 30.67 ( $\text{CH}_3$ , SAC), 30.62 ( $\beta\text{-CH}_2$ ), 29.80 ( $\varepsilon\text{-CH}_2$ ), 29.47 ( $\gamma\text{-CH}_2$ ), 26.47 ( $\delta\text{-CH}_2$ ), 22.52, 20.90, 20.86, 20.83, 20.73 (6xCH<sub>3</sub>, Ac, NHAc).

HRMS (ESI-TOF): calcd for  $\text{C}_{35}\text{H}_{52}\text{N}_2\text{O}_{17}\text{S}$   $m/z$  [M+HCOO]<sup>+</sup> 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)

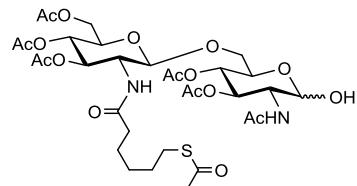
A solution of **9** (460 mg, 629  $\mu\text{mol}$ ) in freshly distilled dry THF (40 ml) was degassed by repeated evacuation and flushing the flask with helium. To this stirred solution  $\text{H}_2$ -activated (1,5-cyclooctadiene)

bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate  $[\text{Ir}^+(\text{COD})(\text{PMePh}_2)_2]\text{PF}_6^-$  (10.7 mg, 12.65  $\mu\text{mol}$ ) was added and the reaction mixture was stirred for 1 h. [Activation of the catalyst was performed as follows:  $\text{Ir}[(\text{COD})_2\text{bis}(\text{methyldiphenylphosphine})]\text{PF}_6$  (10.7 mg) 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  $\text{H}_2$ , which was kept for  $3 \times 15$  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  $4^\circ\text{C}$  and a solution of  $\text{I}_2$  (317 mg, 1.25 mmol) in  $\text{THF}/\text{H}_2\text{O}$  (1:1, 7 ml) was added. The stirring was continued for 1 h at  $4^\circ\text{C}$ , the mixture was diluted with cold ( $0^\circ\text{C}$ ) ethyl acetate (100 ml), ice cold aq. satd.  $\text{Na}_2\text{SO}_3$  and aq. satd.  $\text{NaHCO}_3$  (100 ml), followed by brine (50 ml). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , 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),  $[\alpha]_D^{20} = +24$  ( $c$  0.55,  $\text{CHCl}_3$ );

$^1\text{H}$  NMR ( $\text{DMSO-d}_6$ , 400 MHz,  $\alpha$ -anomer):  $\delta = 7.79$  (d,  $J = 9.2$  Hz, 1H,  $\text{NHAc}$ ), 7.68 (d,  $J = 9.2$  Hz, 1H,  $\text{NH}$ ), 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,  $\text{CH}_3$ , Ac), 1.45 (q, 2H,  $\beta\text{-CH}_2$ ), 1.31 – 1.14 (m, 4H,  $\gamma$ ,  $\delta\text{-CH}_2$ ), 0.85 (t,  $J = 7.1$  Hz, 3H,  $\varepsilon\text{-CH}_3$ );  $^{13}\text{C}$  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 ( $\text{CH}_3$ , Ac), 21.84 ( $\gamma\text{-CH}_2$ ), 20.49, 20.47, 20.43, 20.39, 20.34 (5x $\text{CH}_3$ , Ac), 14.76 ( $\varepsilon\text{-CH}_3$ ).

HRMS ( $^{\dagger}\text{ESI-TOF}$ ): 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- $\beta$ -D-glucopyranosyl]-2-deoxy-D-glucopyranose ( $\alpha/\beta = 10:1$ ) (**12**)

A solution of **10** (403 mg, 500  $\mu\text{mol}$ ) in dry THF (15 ml) was degassed by repeated evacuation and flushing the flask with helium. To this stirred solution the  $\text{H}_2$ -activated (1,5-cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate  $[\text{Ir}^+(\text{COD})(\text{PMePh}_2)_2]\text{PF}_6^-$  (42.3 mg, 50  $\mu\text{mol}$ ) 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  $-10^\circ\text{C}$  and a solution of  $\text{I}_2$  (125.5 mg, 600  $\mu\text{mol}$ ) in  $\text{THF}/\text{H}_2\text{O}$  (1:1, 1 ml) was added dropwise. After stirring for 1 hour at  $-10^\circ\text{C}$ , the reaction mixture was diluted with ethyl acetate (20 ml) 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  $\text{Na}_2\text{SO}_4$ , 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),  $[\alpha]_D^{20} = +23$  ( $c$  0.93, MeOH);

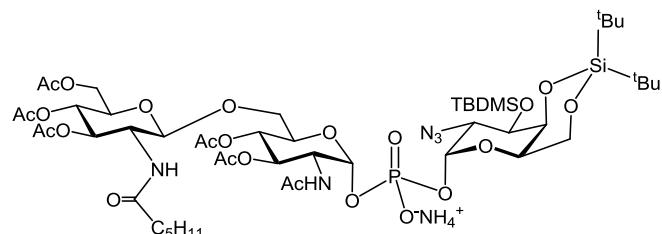
$^1\text{H}$  NMR ( $\text{MeOD}$ , 600 MHz,  $\alpha$ -anomer):  $\delta = 5.28$  (dd,  $J_{2,3} = 10.9$  Hz,  $J_{3,4} = 9.3$  Hz, 1H, H-3), 5.21 (dd,  $J_{2',3'} = 10.5$  Hz,  $J_{3',4'} = 9.3$  Hz, 1H, H-3'), 5.07 (d,  $J_{1,2} = 3.5$  Hz, 1H, H-1), 4.97 (t,  $J_{3',4'} = J_{4',5'} = 9.7$  Hz, 1H, 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,  $J_{5,6b} = 2.8$  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,  $\varepsilon\text{-CH}_2\text{-SAC}$ ), 2.30 (s, 3H,

CH<sub>3</sub>, SAc), 2.23-2.16 (m, 2H,  $\alpha$ -CH<sub>2</sub>, hexanoyl), 2.06, 2.03, 2.0, 1.98, 1.95, 1.91 (6 s, 18H, CH<sub>3</sub>, 5 $\times$ Ac, NHAc), 1.60-1.57 (m, 4H,  $\beta$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.39-1.36 (m, 2H,  $\gamma$ -CH<sub>2</sub>);

<sup>13</sup>C NMR (MeOD, 151 MHz,  $\alpha$ -anomer):  $\delta$  = 197.76 (CO, SAc), 176.48 (CO, hexanoyl), 173.56, 172.56, 172.38, 172.02, 171.75, 171.45 (6xCO, 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 ( $\alpha$ -CH<sub>2</sub>), 30.67 (CH<sub>3</sub>, SAc), 30.62 ( $\beta$ -CH<sub>2</sub>), 29.83 ( $\varepsilon$ -CH<sub>2</sub>), 29.48 ( $\gamma$ -CH<sub>2</sub>), 26.44 ( $\delta$ -CH<sub>2</sub>), 22.60, 20.96, 20.88, 20.86, 20.81, 20.73 (6xCH<sub>3</sub>, Ac, NHAc).

HRMS (ESI-TOF): calcd for C<sub>33</sub>H<sub>49</sub>N<sub>2</sub>O<sub>19</sub>S *m/z* [M+HCOO]<sup>+</sup> 809.2656, found: 809.2649.

### Synthesis of 13



**2-Azido-3-O-*tert*-butyldimethylsilyl-4,6-O-di-*tert*-butylsilylene-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-hexanoylamino- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranose (ammonium salt) (13)**

To a stirred solution of **5** (125.3 mg, 231  $\mu$ mol) and **11** (160 mg, 231  $\mu$ mol) {which were pre-dried by repeated co-evaporation with dry toluene (4 $\times$ 10 ml)} in dry pyridine (10 ml) a solution of pivaloyl chloride (PivCl, 300  $\mu$ mol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 ml) was added dropwise over 1 h. The reaction mixture was stirred for 2 h, cooled to -15°C and a solution of I<sub>2</sub> (44 mg) in Pyr/H<sub>2</sub>O (50:1, 2.5 ml) was added dropwise. The reaction mixture was stirred for 20 min at -15°C, diluted with CH<sub>2</sub>Cl<sub>2</sub> (150 ml) and washed with aq. satd. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (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 (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH, 7:1:0.1) to give **13** (150 mg, 53%) as a white solid; R<sub>f</sub> = 0.25 (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH, 7:1:0.08),  $[\alpha]_D^{20} = +83$  (*c* 0.7, MeOH);

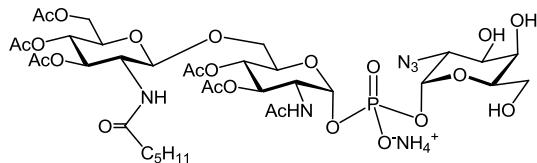
<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 7.26 (d, under CDCl<sub>3</sub> signal, 1H, 2'-NH), 6.42 (d, *J* = 9.6 Hz, 0.9H, 2-NHAc), 5.74 (dd, *J*<sub>1'',2''</sub> = 3.2 Hz, *J*<sub>1'',p</sub> = 7.9 Hz H-1''), 5.66 (dd, *J*<sub>1,2</sub> = 3.5 Hz, *J*<sub>1,p</sub> = 7.3 Hz, 1H, H-1), 5.17 (app. t, *J*<sub>2,3</sub> = 10.6 Hz, *J*<sub>3,4</sub> = 10.5 Hz, 1H, H-3), 5.10 (dd, *J*<sub>2',3'</sub> = *J*<sub>3',4'</sub> = 9.2 Hz, 1H, H-3'), 5.03 (dd, *J*<sub>3',4'</sub> = *J*<sub>4',5'</sub> = 9.5 Hz, 1H, H-4'), 4.92 (t, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 9.9 Hz, 1H, H-4), 4.74 (d, *J*<sub>1',2'</sub> = 8.7 Hz, 1H, H-1'), 4.40 (d, *J*<sub>3'',4''</sub> = 3.2 Hz, 1H, H-4''), 4.31 (dd, *J*<sub>5'',6''a</sub> = 1.3 Hz, *J*<sub>6''a,6''b</sub> = 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*<sub>5',6'b</sub> = 2.8 Hz, *J*<sub>6'a,6'b</sub> = 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*<sub>5a,6a</sub> = 1.8 Hz, *J*<sub>6a,6b</sub> = 12.3 Hz, 1H, H-6a), 3.78-3.73 (m, 2H, H-5', H-2''), 3.65 (dd, *J*<sub>5,6b</sub> = 6.4 Hz, 1H, H-6b), 2.30-2.17 (m, 2H,  $\alpha$ -CH<sub>2</sub>), 2.10 (s, 3H, CH<sub>3</sub>, NHAc), 2.04, 2.01, 2.008, 1.97 (4s, 15H, CH<sub>3</sub>, acetyl), 1.59 (q, 2H,  $\beta$ -CH<sub>2</sub>), 1.36-1.23 (m, 4H,  $\gamma$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.06 (s, 9H, 3 $\times$ CH<sub>3</sub>, DTBS), 1.02 (s, 9H, 3 $\times$ CH<sub>3</sub>, DTBS), 0.94 (s, 9H, 3 $\times$ CH<sub>3</sub>, TBDMS), 0.89 (t, *J* = 7 Hz, 3H,  $\varepsilon$ -CH<sub>3</sub>), 0.17, 0.15 (2s, 6H, 2 $\times$ CH<sub>3</sub>, TBDMS);

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  = 174.44 (CO, NHAc), 171.26, 171.20, 170.96, 170.79, 169.84, 169.38 (6 $\times$ CO, 5Ac, hexanoyl), 100.83 (d, *J*<sub>1,p</sub> = 5.9 Hz, C-1'), 95.50 (d, *J*<sub>1,p</sub> = 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*<sub>2,p</sub> = 8.1 Hz, C-2''), 53.08 (C-2'), 51.82 (d, *J*<sub>2,p</sub> = 6.7 Hz, C-2), 36.26 ( $\alpha$ -CH<sub>2</sub>), 31.30 ( $\gamma$ -CH<sub>2</sub>), 27.58, 27.30, 25.62, (DTBS, TBDMS), 25.54 ( $\beta$ -CH<sub>2</sub>), 23.07 (CH<sub>3</sub>, Ac), 22.46 ( $\delta$ -CH<sub>2</sub>), 20.75, 20.73, 20.65, 20.60, (CH<sub>3</sub>, Ac), 13.95 ( $\varepsilon$ -CH<sub>3</sub>), -4.12, -4.87 (CH<sub>3</sub>, TBDMS);

<sup>31</sup>P NMR (CDCl<sub>3</sub>, PPh<sub>3</sub> ext. standard, 162 MHz):  $\delta$  = -3.0

HRMS (<sup>+</sup>ESI-TOF): calcd for C<sub>50</sub>H<sub>87</sub>N<sub>5</sub>O<sub>23</sub>PSi<sub>2</sub> *m/z* [M+H]<sup>+</sup>: 1212.5063, found: 1212.5073.

## Synthesis of 14



### 2-Azido-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-hexanoylamino- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranose (ammonium salt) (14)

To a stirred solution of **13** (46 mg, 37.95  $\mu\text{mol}$ ) in dry THF (50 ml) in a PTFE flask was added a solution of pyridinium hydrofluoride (50  $\mu\text{l}$ ) 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),  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/25\%$  aq.  $\text{NH}_4\text{OH}$ , 50:25:3:0.2) to provide **14** (35.7 mg, 96.6%) as a solid,  $R_f = 0.55$  ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ , 50:25:4),  $[\alpha]_D^{20} = +72$  (*c* 0.6, MeOH);

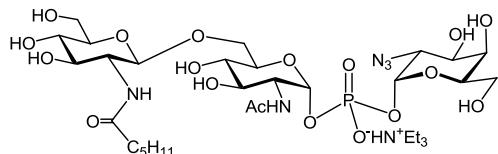
$^1\text{H}$  NMR (MeOD, 400 MHz):  $\delta = 5.64$  (dd,  $J_{1'',2''} = 3.3$  Hz,  $J_{1'',p} = 7.6$  Hz, 1H, H-1''), 5.60 (dd,  $J_{1,2} = 3.4$  Hz,  $J_{1,p} = 7.1$  Hz, 1H, H-1), 5.28 (dd,  $J_{3,4} = 10.7$  Hz,  $J_{2,3} = 9.1$  Hz, 1H, H-3), 5.23 (dd,  $J_{3',4'} = 9.9$  Hz,  $J_{2',3'} = 9.3$  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,  $J_{6'a,6'b} = 11.4$  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,  $\alpha$ -CH<sub>2</sub>, hexanoyl), 2.06, 2.03, 2.00, 1.97, 1.96, 1.95 (6s, 18H, CH<sub>3</sub>, Ac, NHAc), 1.61 (q, 2H,  $\beta$ -CH<sub>2</sub>), 1.38 - 1.29 (m, 4H,  $\gamma$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.16 (NH<sub>4</sub><sup>+</sup>, counter-ion), 0.92 (t,  $J = 7$  Hz, 3H,  $\varepsilon$ -CH<sub>3</sub>);

$^{13}\text{C}$  NMR (MeOD, 101 MHz):  $\delta = 177.06$  (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,  $J_{\text{C}1',\text{p}} = 5.9$  Hz, C-1''), 95.77 (d,  $J_{\text{C}1,\text{p}} = 6.1$  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,  $J_{\text{C}2',\text{p}} = 8.4$  Hz, C-2''), 55.29 (C-2), 53.06 (d,  $J_{\text{C}2,\text{p}} = 8.4$  Hz, C-2), 7.46 ( $\alpha$ -CH<sub>2</sub>), 32.61 ( $\gamma$ -CH<sub>2</sub>), 26.80 ( $\beta$ -CH<sub>2</sub>), 23.62 ( $\delta$ -CH<sub>2</sub>), 22.76, 20.89, 20.88, 20.84, 20.78, 20.76 (6 $\times$ CH<sub>3</sub>, Ac), 14.48 ( $\varepsilon$ -CH<sub>3</sub>);

$^{31}\text{P}$  NMR (MeOD, PPh<sub>3</sub> ext. standard, 162 MHz):  $\delta = -2.44$

HRMS (ESI-TOF): calcd for C<sub>36</sub>H<sub>55</sub>N<sub>5</sub>O<sub>23</sub>P *m/z* [M-H]<sup>-</sup> 956.3031, found: 956.3028

## Synthesis of 15



### 2-Azido-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-O-[2-deoxy-2-hexanoylamino- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranose (triethylammonium salt) (15)

A solution of **14** (35 mg, 35.92  $\mu\text{mol}$ ) in MeOH/H<sub>2</sub>O/Et<sub>3</sub>N (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 H<sub>2</sub>O, frozen and lyophilized to give **15** (30 mg, 98.4%) as a solid.  $R_f = 0.18$  ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ , 2:1:0.15),  $[\alpha]_D^{20} = +53$  (*c* 0.55, MeOH);

$^1\text{H}$  NMR (D<sub>2</sub>O, d-Acetone/D<sub>2</sub>O ext. standard, 600 MHz):  $\delta = 7.89$  (d,  $J = 9.2$  Hz, 0.6 H, NHAc), 5.47 (dd,  $J_{1'',2''} = 3.4$  Hz,  $J_{\text{p},\text{H}} = 7.5$  Hz, H-1''), 5.36 (dd,  $J_{1,2} = 3.4$  Hz,  $J_{\text{p},\text{H}} = 6.8$  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,

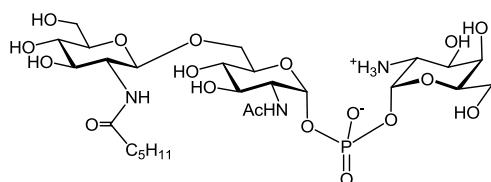
1 H, H-4''), 3.8 – 3.76 (m, 3 H, H-2, H-6'a, H-6'b), 3.63 (dd,  $J_{5'',6''a} = 4.4$  Hz,  $J_{6''a,6''b} = 11.6$  Hz, 1 H, H-6''a), 3.63 (dd,  $J_{5'',6''b} = 4.8$  Hz, 1 H, H-6''b), 3.62 (m, 1 H, H-3), 3.58 (dd,  $J_{2',3'} = 10.3$  Hz, 1 H, H-2'), 3.49 (dt,  $J_{2'',3''} = 10.2$  Hz, 1 H, H-2''), 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, CH<sub>2</sub>, Et<sub>3</sub>N, counter-ion), 2.18 (m, 2H,  $\alpha$ -CH<sub>2</sub>), 1.92 (s, 3H, CH<sub>3</sub>, NHAc), 1.48 (m, 2H,  $\beta$ -CH<sub>2</sub>), 1.19 – 1.15 (m, 4H,  $\gamma$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.13 (t, CH<sub>3</sub>, Et<sub>3</sub>N, counter-ion), 0.73 (t,  $J = 7$  Hz, 3 H,  $\varepsilon$ -CH<sub>3</sub>);

<sup>13</sup>C NMR (D<sub>2</sub>O, d-Acetone/D<sub>2</sub>O ext. standard, 150 MHz):  $\delta = 178.5$  (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 (CH<sub>2</sub>, Et<sub>3</sub>N, counter-ion), 36.71 ( $\alpha$ -CH<sub>2</sub>), 31.23 ( $\gamma$ -CH<sub>2</sub>), 25.76 ( $\beta$ -CH<sub>2</sub>), 22.66 (CH<sub>3</sub>, Ac), 22.34 ( $\delta$ -CH<sub>2</sub>), 13.90 ( $\varepsilon$ -CH<sub>3</sub>), 8.89 (CH<sub>3</sub>, Et<sub>3</sub>N, counter-ion);

<sup>31</sup>P NMR (D<sub>2</sub>O, H<sub>3</sub>PO<sub>4</sub> ext. standard, 243 MHz):  $\delta = -3.77$

HRMS (ESI-TOF): calcd for C<sub>26</sub>H<sub>45</sub>N<sub>5</sub>O<sub>18</sub>P m/z [M-H]<sup>-</sup> 746.2503; found: 746.2509.

## Synthesis of 16



### 2-Amino-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-O-[2-deoxy-2-hexanoylamino- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranose (16)

A solution of **15** (10 mg, 11.7  $\mu$ mol) in MeOH (10 ml) was applied to the H-Cube system (Pd/C cartridge, full H<sub>2</sub>-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.  $R_f = 0.11$  (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 2:1:0.15),  $[\alpha]_D^{20} = +68$  (*c* 0.68, MeOH);

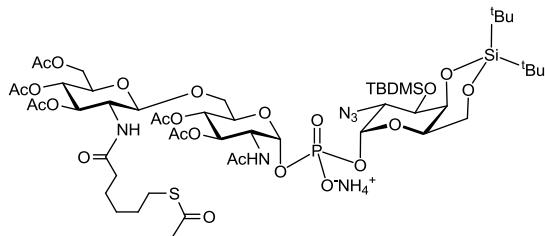
<sup>1</sup>H NMR (D<sub>2</sub>O, DSS ext. standard, 600 MHz, pH = 6.5):  $\delta = 5.76$  (dd,  $J_{1'',2''} = 3.5$  Hz,  $J_{P,H} = 6.4$  Hz, 1H, H-1''), 5.45 (dd,  $J_{1,2} = 3.5$  Hz,  $J_{P,H} = 7.4$  Hz, 1H, H-1), 4.58 ( $J_{1',2'} = 8.6$  Hz, 1H, H-1'), 4.11 (dd,  $J_{5'',4''} = 4.5$  Hz,  $J_{5'',6''a} = 7.8$  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, 1H, H-4), 3.43 (m, 1H, H-5'), 3.41 (dd,  $J_{4',5'} = 8.9$  Hz, 1H, H-4'), 2.29 (m, 2H,  $\alpha$ -CH<sub>2</sub>), 2.02 (s, 3H, CH<sub>3</sub>, NHAc), 1.60 (m, 2H,  $\beta$ -CH<sub>2</sub>), 1.32 – 1.26 (m, 4H,  $\gamma$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 0.85 (t,  $J = 7$  Hz, 3 H,  $\varepsilon$ -CH<sub>3</sub>);

<sup>13</sup>C NMR (D<sub>2</sub>O, dioxane ext. standard, 151 MHz):  $\delta = 178.75$  (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 ( $\alpha$ -CH<sub>2</sub>), 31.36 ( $\gamma$ -CH<sub>2</sub>), 25.96 ( $\beta$ -CH<sub>2</sub>), 22.83 (CH<sub>3</sub>, NHAc), 22.51 ( $\delta$ -CH<sub>2</sub>), 14.09 ( $\varepsilon$ -CH<sub>3</sub>);

<sup>31</sup>P NMR (D<sub>2</sub>O, H<sub>3</sub>PO<sub>4</sub> ext. standard, 243 MHz):  $\delta = -2.8$

HRMS (ESI-TOF): calcd for C<sub>26</sub>H<sub>47</sub>N<sub>3</sub>O<sub>18</sub>P m/z [M-H]<sup>-</sup> 720.2598, found: 720.2596.

## Synthesis of 17



**2-Azido-2-deoxy-3-*O*-*tert*-butyldimethylsilyl-4,6-*O*-di-*tert*-butyldimethylsilylene- $\alpha$ -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]- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranose (ammonium salt) (17)**

### Procedure via H-phosphonate coupling

17 was prepared from 5 (61.5 mg, 113  $\mu$ mol) and 12 (73.5 mg, 113  $\mu$ mol) in a way described for the preparation of 13. Purified by column chromatography on silica gel (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH, 70:10:0.5) afforded 17. Yield 74 mg (85%).  $R_f$  = 0.29 (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>3</sub>, 70:10:0.8),  $[\alpha]_D^{20}$  = +41 ( $c$  0.43, MeOH)

### Procedure via phosphoramidite coupling

To a stirred solution of 4 ( $\alpha/\beta = 2.6/1$ ; 38.8 mg, 100  $\mu$ mol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 ml) diisopropylethylamine (DIPEA) (106  $\mu$ l, 460  $\mu$ mol) and 2-cyanoethyl-*N,N*-diisopropylphosphorochloridite (66.4  $\mu$ l, 300  $\mu$ mol) (<sup>31</sup>P-NMR 180.0 ppm) were added successively under Ar. The reaction mixture was stirred for 1 h until <sup>31</sup>P-NMR analysis indicated the formation of a phosphoramidite 18 as diastereomeric mixture of (*R*) and (*S*) diastereomers on phosphorus: 4 signals corresponding of the  $\alpha/\beta = 2.6/1$  mixture, [<sup>31</sup>P-NMR, 162 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$  (ppm) = 151.48, 150.64, 150.39 and 149.51 (~ 2:1:1:2)]. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (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 (2×10 ml), evaporated and dried *in vacuo*. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and a solution of 12 (69.3 mg, 90  $\mu$ mol) in dry acetonitrile (10 ml) followed by a solution of 1*H*-tetrazole in dry acetonitrile (0.45 M, 1 ml, 450  $\mu$ 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  $\mu$ l, 178  $\mu$ mol) was added. The reaction mixture was stirred for 1 h at 4°C, then Et<sub>3</sub>N (0.2 ml, 717  $\mu$ mol) was added in portions over 30 min. Stirring was continued for 30 min, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (100 ml) and washed with 0.2 M TEAB buffer (3×20 ml). The organic phase was dried over cotton and concentrated. The residue was purified by column chromatography on silica gel (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH, 70:10:0.8) to provide 17 (28 mg, 24%) as a solid.

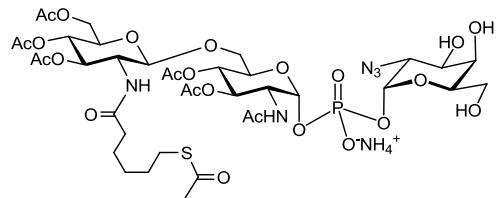
<sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta$  = 7.14 (bs, 0.8H, N'HCO), 6.34 (d,  $J$  = 8.9 Hz, 0.9H, NHAc), 5.76 (dd,  $J_{1'',2''}$  = 3.2 Hz,  $J_{1'',p}$  = 7.7 Hz, 1H, H-1''), 5.64 (dd,  $J_{1,2}$  = 3.3 Hz,  $J_{1,p}$  = 7.0 Hz, 1H, H-1), 5.16 (dd,  $J_{2,3}$  =  $J_{3,4}$  = 10.0 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,  $\epsilon$ -CH<sub>2</sub>-SAC), 2.32 (s, 3H, CH<sub>3</sub>, SAC), 2.24 (m, 2H,  $\alpha$ -CH<sub>2</sub>, hexanoyl), 2.10, 2.04, 2.02, 2.01, 1.97 (6 s, 18H, CH<sub>3</sub>, 5×Ac, NHAc), 1.59 (m, 4H,  $\beta$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.37 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 1.58 (NH<sub>4</sub><sup>+</sup>), 1.06, 1.02, (2s, 18H, CH<sub>3</sub>, DTBS), 0.95 (s, 9H, CH<sub>3</sub>, TBDMS), 0.17, 0.16 (2s, 6H, CH<sub>3</sub>, TBDMS);

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 151 MHz):  $\delta$  = 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 ( $\alpha$ -CH<sub>2</sub>), 30.62 (CH<sub>3</sub>, SAC), 29.31 ( $\beta$ -CH<sub>2</sub>), 28.75 ( $\epsilon$ -CH<sub>2</sub>), 28.09 ( $\gamma$ -CH<sub>2</sub>), 27.56, 27.33, 25.64 (CH<sub>3</sub>, DTBS, TBDMS), 25.12 ( $\delta$ -CH<sub>2</sub>), 23.38, 23.09, 20.76, 20.74, 20.68, 20.61 (CH<sub>3</sub>, Ac), -4.07, -4.81 (CH<sub>3</sub>, TBDMS);

<sup>31</sup>P NMR (CDCl<sub>3</sub>, PPh<sub>3</sub> external, 243 MHz):  $\delta$  = -0.89

HRMS (ESI-TOF): calcd for C<sub>52</sub>H<sub>87</sub>N<sub>5</sub>O<sub>24</sub>PSi<sub>2</sub> *m/z* [M-H]<sup>-</sup> 1284.4738, found: 1284.4726.

### Synthesis of 19



#### 2-Azido-2-deoxy- $\alpha$ -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- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranose (ammonium salt) (19)

To a cooled (4°C) stirred solution of **17** (60 mg, 46  $\mu$ mol) in dry THF (2.5 ml) in a PTFE flask was added a solution of HF•Py (150  $\mu$ l) 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 CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH (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 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>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 (CHCl<sub>3</sub>/MeOH/25% aq. NH<sub>4</sub>OH, 4:1:0.05);  $[\alpha]_D^{20}$  = +53 (*c* 0.85, MeOH);

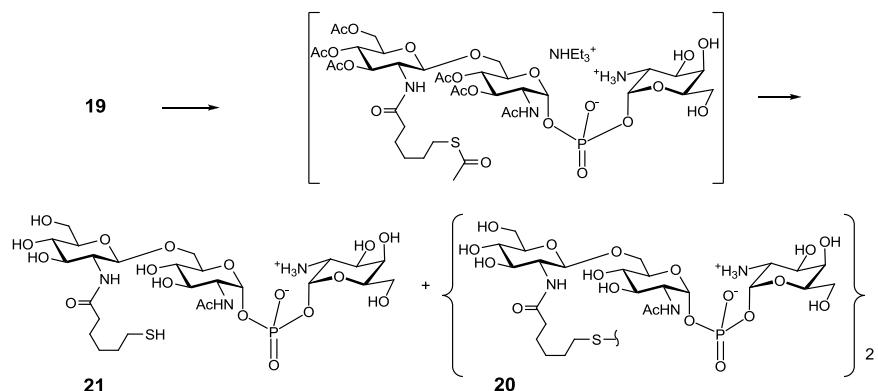
<sup>1</sup>H NMR (MeOD, 600 MHz):  $\delta$  = 5.62 (dd,  $J_{1'',2''}$  = 3.4 Hz,  $J_{1'',p}$  = 7.8 Hz, 1H, H-1''), 5.58 (dd,  $J_{1,2}$  = 3.5 Hz,  $J_{1,p}$  = 6.9 Hz, 1H, H-1), 5.28 (dd,  $J_{2,3}$  = 10.7 Hz,  $J_{3,4}$  = 9.3 Hz, 1H, H-3), 5.25 (dd,  $J_{3',4'}$  = 9.4 Hz,  $J_{2',3'}$  = 10.3 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,  $\epsilon$ -CH<sub>2</sub>-SAC), 2.30 (s, 3H, CH<sub>3</sub>, SAC), 2.29 (m, 1H,  $\alpha$ -CH<sub>2</sub>, hexanoyl), 2.24 (m, 1H,  $\alpha$ -CH<sub>2</sub>, hexanoyl), 2.06, 2.02, 2.00, 1.97, 1.96, 1.95 (6s, 18H, CH<sub>3</sub>, 5×Ac, NHAc), 1.60 (m, 4H,  $\beta$ -CH<sub>2</sub>,  $\delta$ -CH<sub>2</sub>), 1.40 (m, 2H,  $\gamma$ -CH<sub>2</sub>), 1.51 (NH<sub>4</sub><sup>+</sup>);

<sup>13</sup>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, 6xAc), 101.72 (C-1'), 95.93 (d,  $J_{C1'',p}$  = 5.8 Hz, C-1''), 95.73 (d,  $J_{C1,p}$  = 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,  $J_{C2'',p}$  = 8.5 Hz, C-2''), 55.41 (C-2), 53.09 (d,  $J_{C2,p}$  = 8.3 Hz, C-2), 37.23 ( $\alpha$ -CH<sub>2</sub>), 30.67 (CH<sub>3</sub>, SAC, under the signal of  $\beta$ -CH<sub>2</sub>), 30.67 ( $\beta$ -CH<sub>2</sub>), 29.89 ( $\epsilon$ -CH<sub>2</sub>), 29.49 ( $\gamma$ -CH<sub>2</sub>), 26.53 ( $\delta$ -CH<sub>2</sub>), 22.77, 20.90, 20.83, 20.81, 20.76 (6×CH<sub>3</sub>, Ac, NHAc);

<sup>31</sup>P NMR (MeOD, PPh<sub>3</sub> ext. standard, 243 MHz):  $\delta$  = -1.8;

HRMS (ESI-TOF): calcd for C<sub>38</sub>H<sub>57</sub>N<sub>5</sub>O<sub>24</sub>PS *m/z* [M-H]<sup>-</sup> 1030.2857, found: 1030.2870.

## Synthesis of 20 and 21



**2-Amino-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-2-deoxy-6-O-[2-deoxy-2-(6-mercaptop-hexanoyl)amino- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranose (21)**

To a stirred solution of **19** (17 mg, 16.2  $\mu$ 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  $\text{CHCl}_3$  (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  $\mu$ mol) (prepared by mixing of stoichiometric amounts of sodium thiophenolate and  $\text{SnCl}_2$  in acetonitrile, followed by separation of precipitate by filtration) in  $\text{CHCl}_3$  (3 ml),  $\text{Et}_3\text{N}$  (4.4  $\mu$ l, 32.4  $\mu$ mol) and thiophenole (3.4  $\mu$ l, 32.4  $\mu$ 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  $\text{CHCl}_3$  (2 $\times$ 5ml). The aqueous phase was separated, placed in a flask and stirred with disodium EDTA dihydrate (145 mg, 390  $\mu$ mol) at pH 5 (addition of satd. aq  $\text{NaHCO}_3$ ) for 30 min. The mixture was washed with hexane/diethylether (1/1, 2 $\times$ 10 ml) followed by  $\text{CHCl}_3$  (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- $\alpha$ -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- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranoside** (13.9 mg, 82%).  $R_f = 0.64$  ( $\text{CHCl}_3/\text{MeOH}/25\%$  aq.  $\text{NH}_4\text{OH}$ , 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]<sup>-</sup> 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  $\mu$ 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:

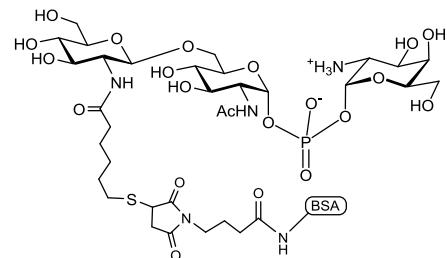
The free amine 2-amino-2-deoxy- $\alpha$ -D-galactopyranosyl-1-phosphoryl 2-acetamido-3,4-di-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(6-thioacetylhexanoyl)amino- $\beta$ -D-glucopyranosyl]-2-deoxy- $\alpha$ -D-glucopyranoside (9 mg, 8.9  $\mu$ mol) was dissolved in  $\text{MeOH}$  (1 ml) and a solution 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  $\alpha,\alpha$ -phosphodiester **20** was isolated as inner salt (pH 7) (3.0 mg, 3.9  $\mu$ 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:  $\beta\text{GlcN}(1\rightarrow 6)\alpha\text{GlcN}(1\rightarrow \text{P})$ .

<sup>1</sup>H NMR (D<sub>2</sub>O, DSS ext. standard, 600 MHz, pH = 7):  $\delta$  = 5.72 (dd,  $J_{1'',2''}$  = 3.5 Hz,  $^3J_{\text{P},\text{H-1}''}$  = 6.5 Hz, 1H, H-1''), 5.45 (dd,  $J_{1,2}$  = 3.4 Hz,  $^3J_{\text{P},\text{H-1}}$  = 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,  $^3J_{\text{P},\text{H-2}}$  = 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,  $J_{6''\text{a},6''\text{b}}$  = 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,  $\epsilon$ -CH<sub>2</sub>-SAC), 2.31 (m, 1H,  $\alpha$ -CH<sub>2</sub> hexanoyl), 2.03 (s, 3H, CH<sub>3</sub>, SAC), 1.70 (m, 2H,  $\beta$ -CH<sub>2</sub> hexanoyl), 1.63 (m, 2H,  $\delta$ -CH<sub>2</sub> hexanoyl), 1.42 (m, 2H,  $\gamma$ -CH<sub>2</sub> hexanoyl);

<sup>13</sup>C NMR (D<sub>2</sub>O, dioxane ext. standard, 151 MHz, pH = 7):  $\delta$  = 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,  $J_{\text{C},\text{P}}$  = 8.2 Hz, C-2), 51.46 (d,  $J_{\text{C},\text{P}}$  = 8.8 Hz, C-2''), 38.60 ( $\epsilon$ -CH<sub>2</sub>S-S-), 36.52 ( $\alpha$ -CH<sub>2</sub>), 28.62 ( $\beta$ -CH<sub>2</sub>), 27.71 (( $\gamma$ -CH<sub>2</sub>), 25.57 ( $\delta$ -CH<sub>2</sub>), 22.64 (CH<sub>3</sub>, NHAc);

<sup>31</sup>P NMR (D<sub>2</sub>O, H<sub>3</sub>PO<sub>4</sub> ext. standard, 243 MHz):  $\delta$  = -2.94

HRMS (ESI-TOF): calcd for C<sub>26</sub>H<sub>47</sub>N<sub>3</sub>O<sub>18</sub>PS *m/z* [M-H]<sup>-</sup> 752.2318; found: 752.2324.



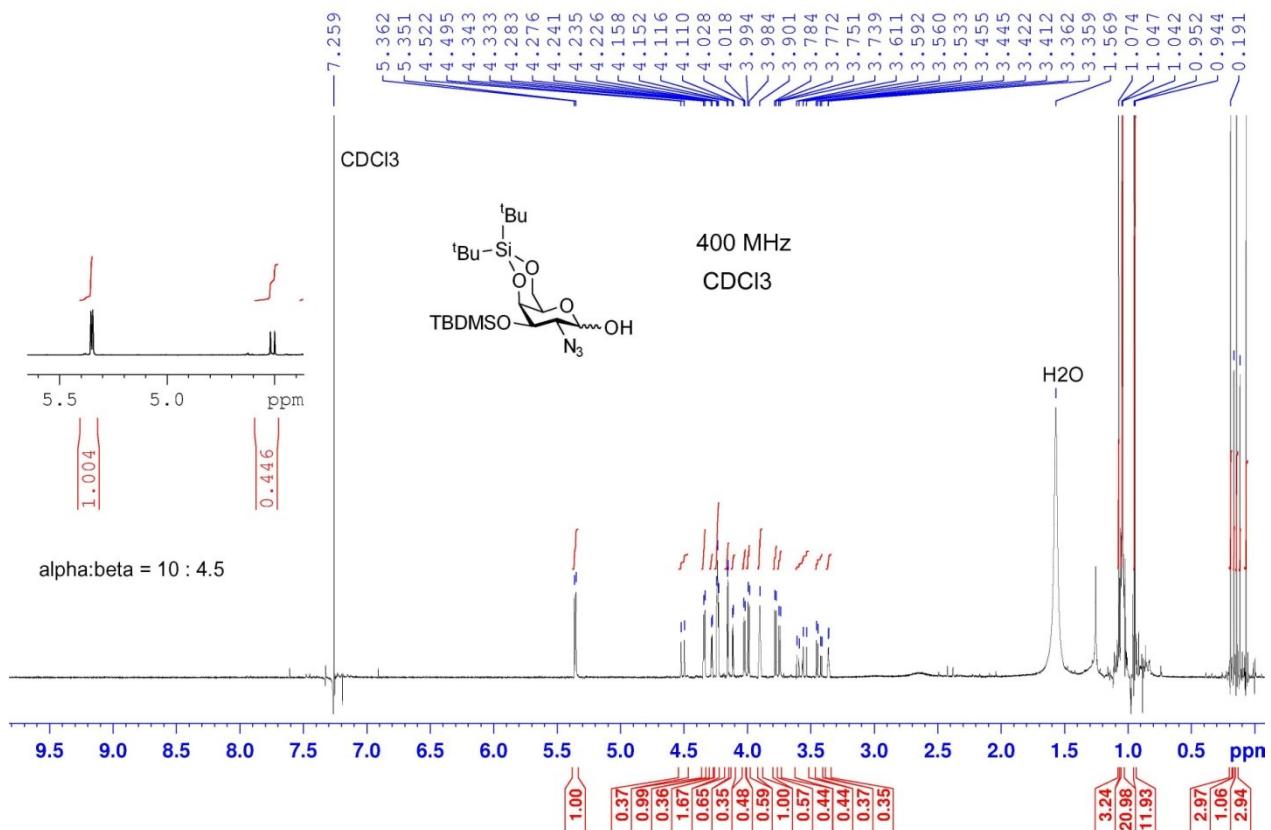
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl; 0.7 mg, 2.44  $\mu$ mol) was dissolved in 0.3 M ammonium bicarbonate buffer (2 ml, Ar-striped, pH = 5). **20** (1.5 mg, 1  $\mu$ mol) was added to this solution and the pH was again adjusted to 5. Ar was bubbled through the solution for 20 min and the solution was freeze-dried. After lyophilization, the reduction of the disulfide **20** to the corresponding thiol **21** was confirmed by <sup>1</sup>H- 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

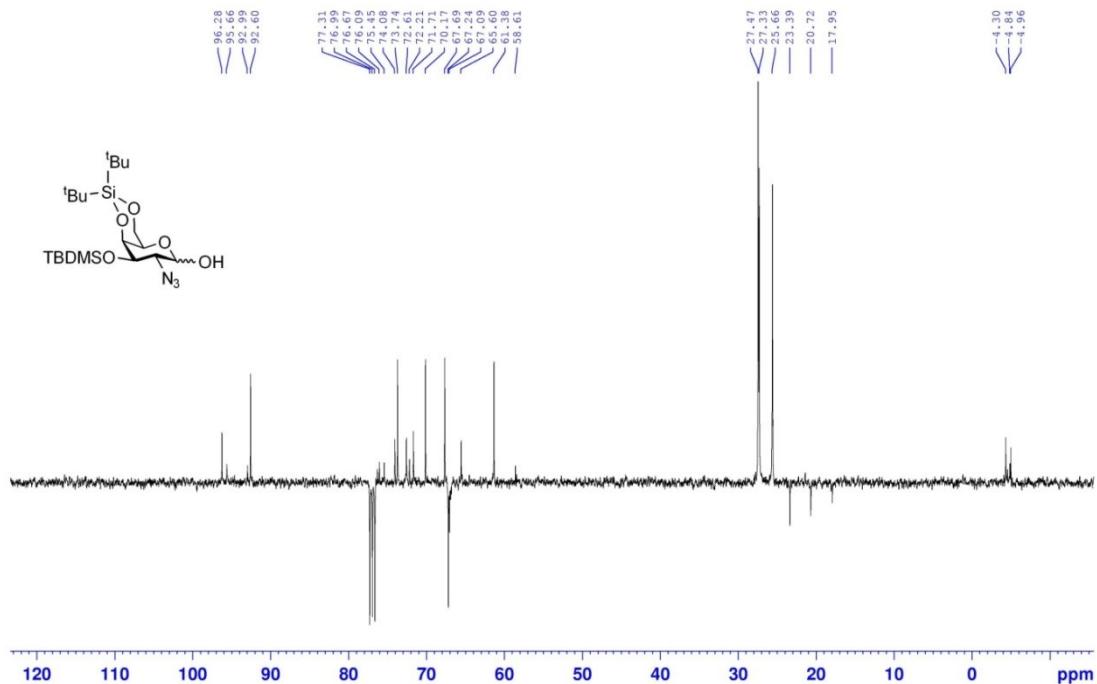
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## <sup>1</sup>H-, <sup>13</sup>C-, <sup>31</sup>P-NMR spectra, HSQC-NMR and MALDI-TOF spectra of the target compounds

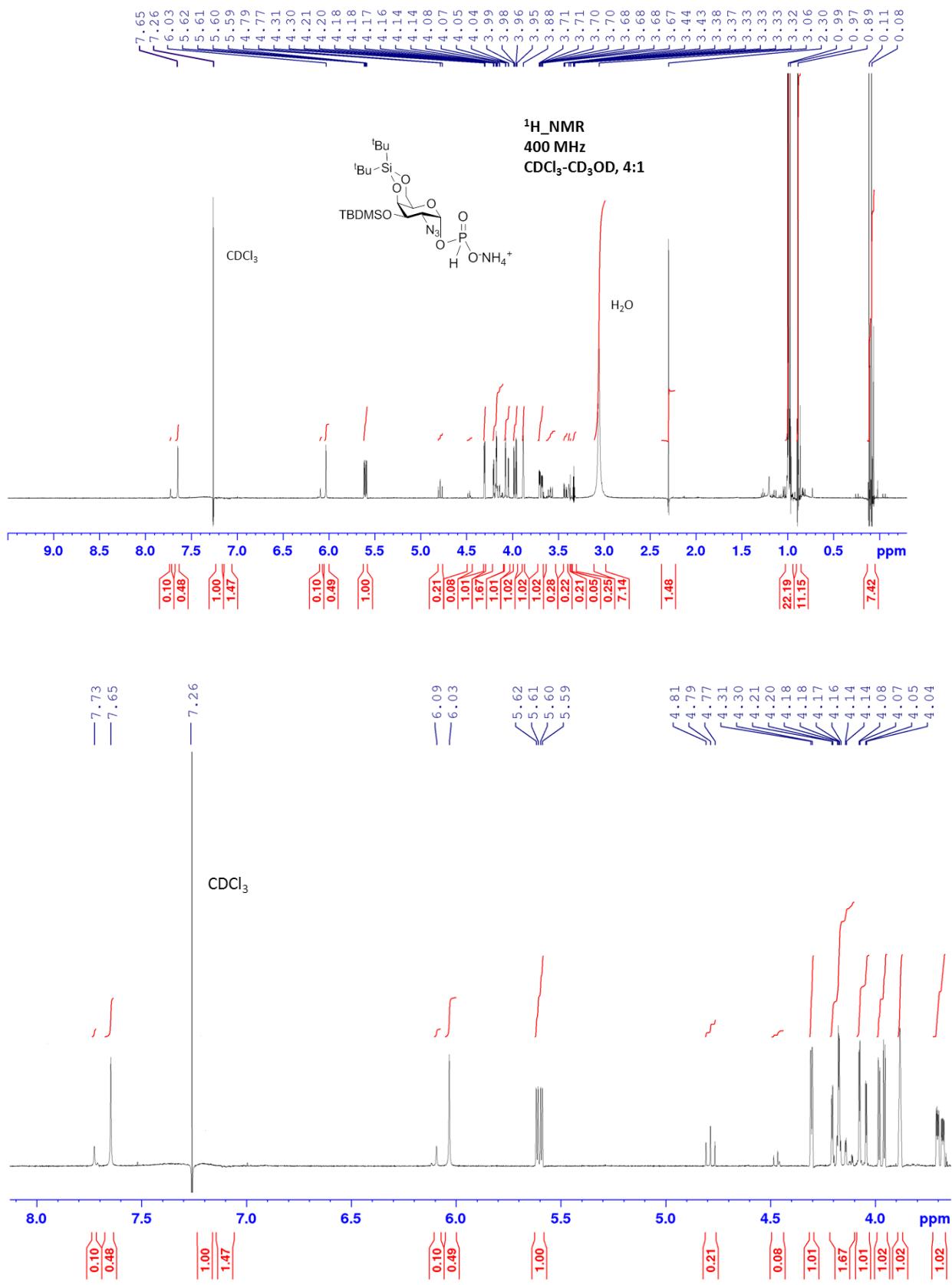
(4):  $^1\text{H-NMR}$ , 400 MHz,  $\text{CDCl}_3$



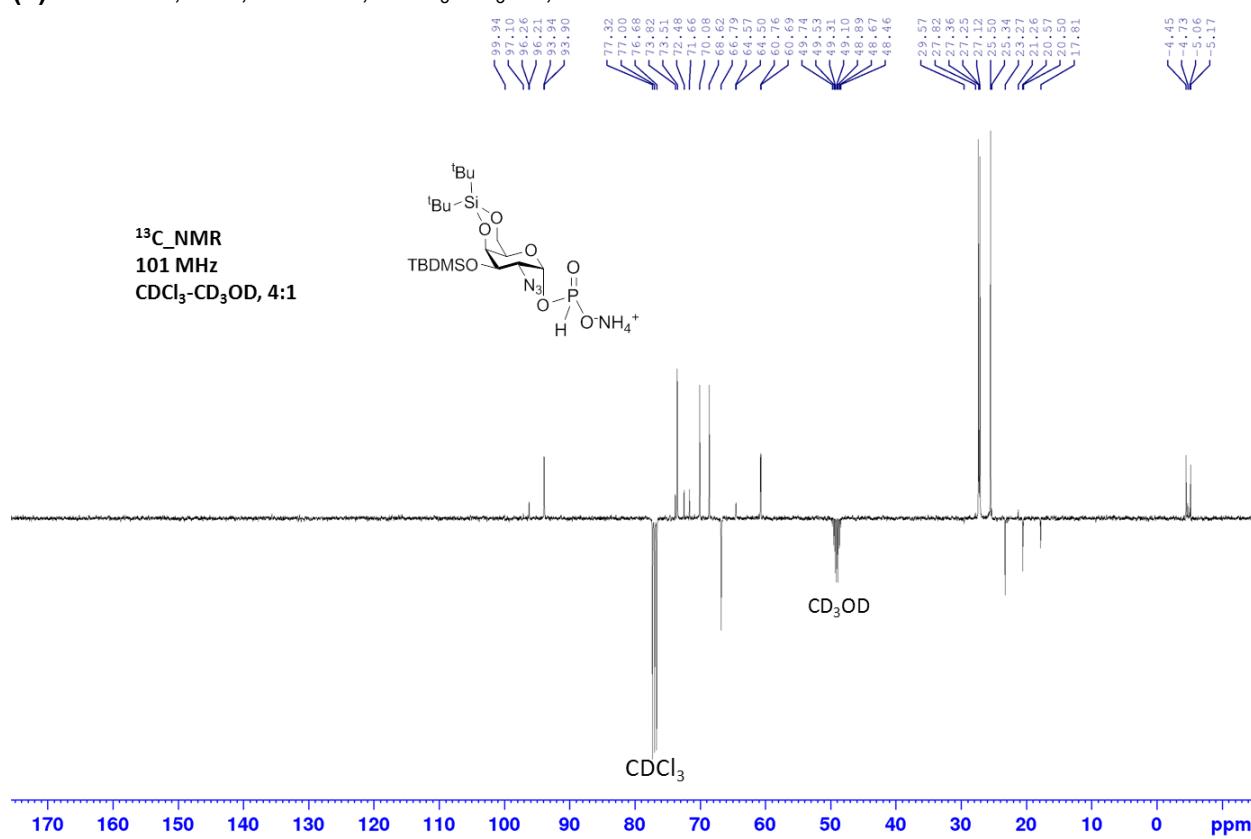
(4):  $^{13}\text{C}$ -NMR, APT, 101 MHz,  $\text{CDCl}_3$



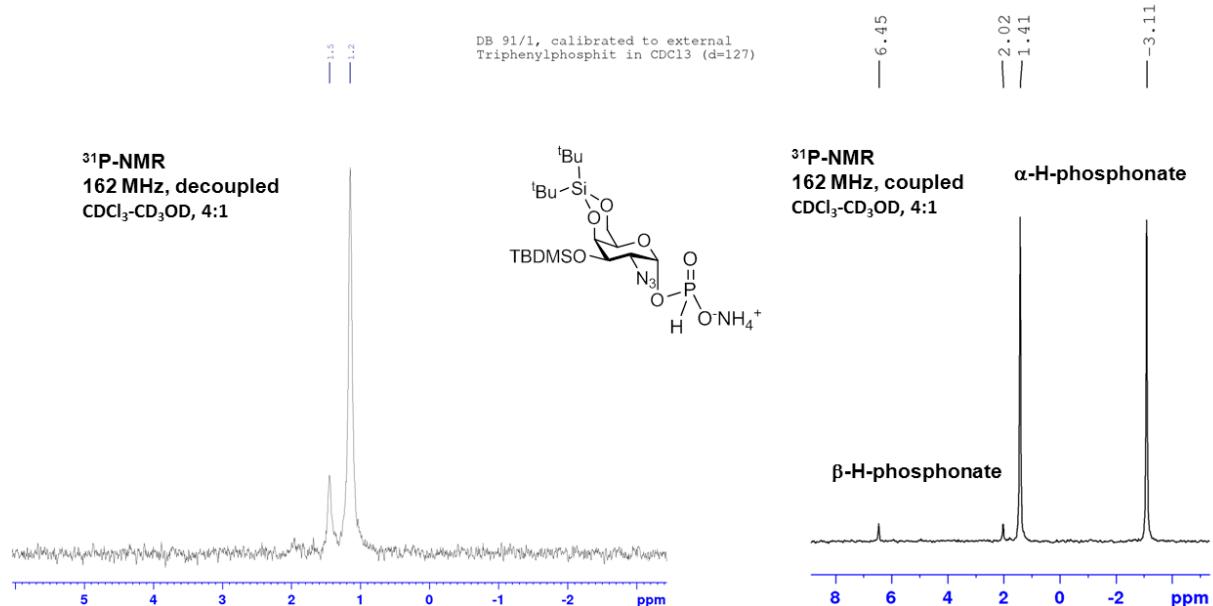
**(5):**  $\alpha/\beta = 5:1$ ,  $^1\text{H-NMR}$ , 400 MHz,  $\text{CDCl}_3:\text{CD}_3\text{OD}$ , 4:1



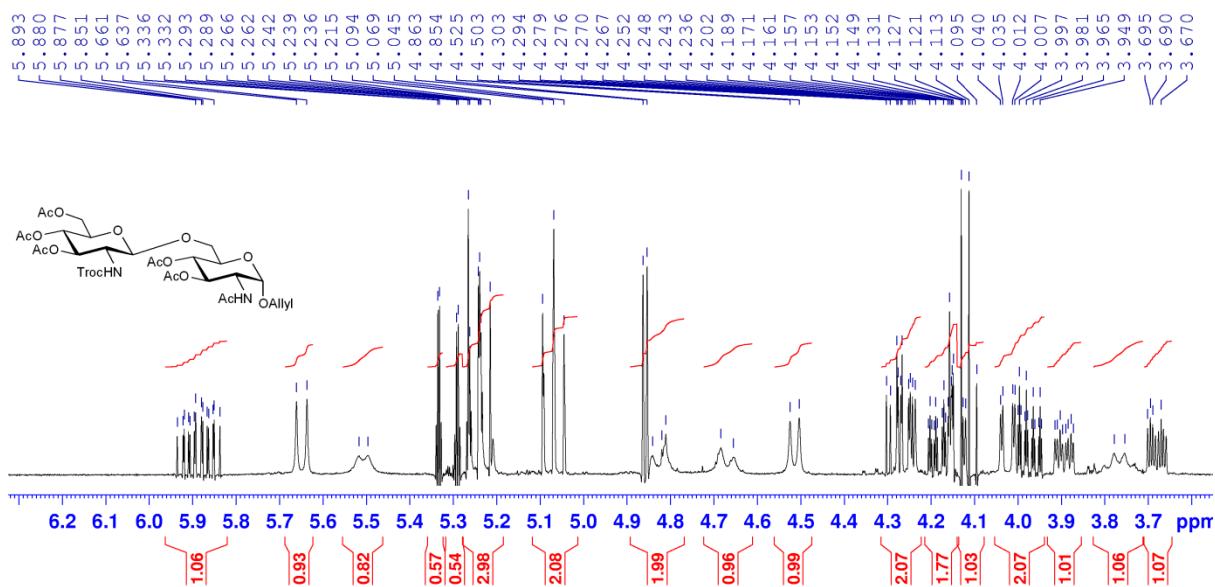
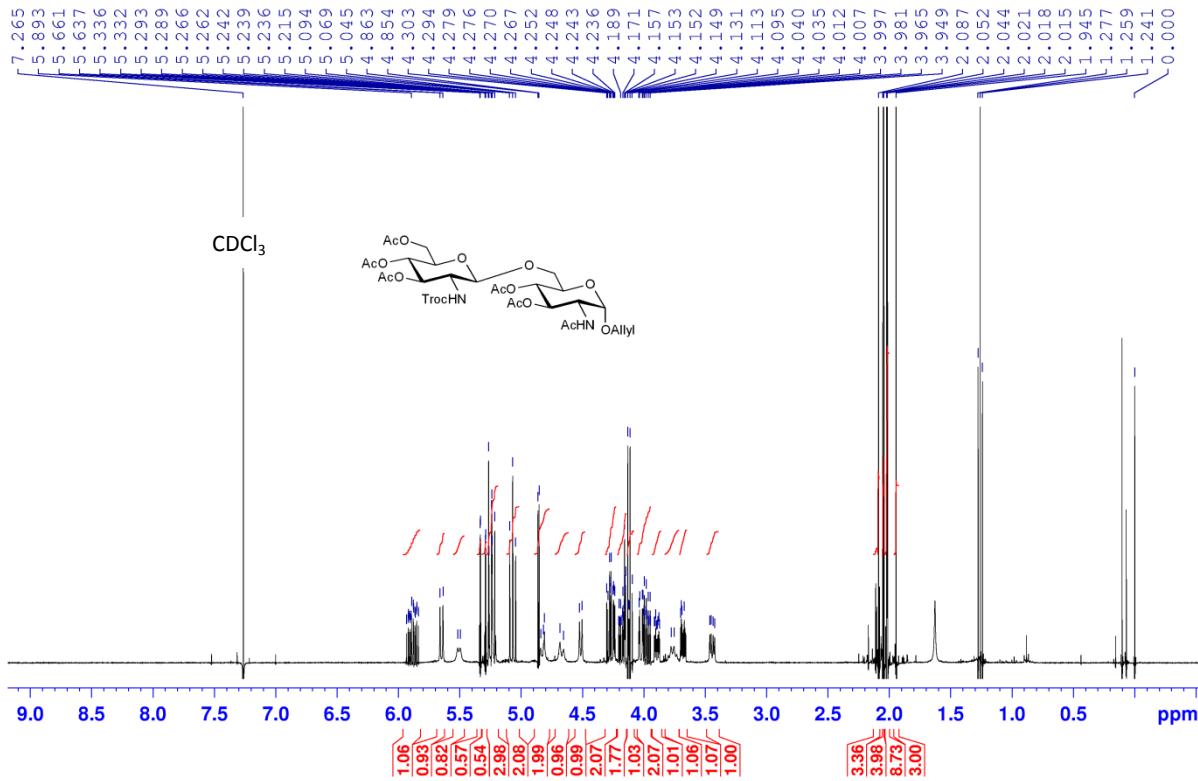
(5):  $^{13}\text{C}$ -NMR, APT, 101 MHz,  $\text{CDCl}_3\text{-CD}_3\text{OD}$ , 4:1



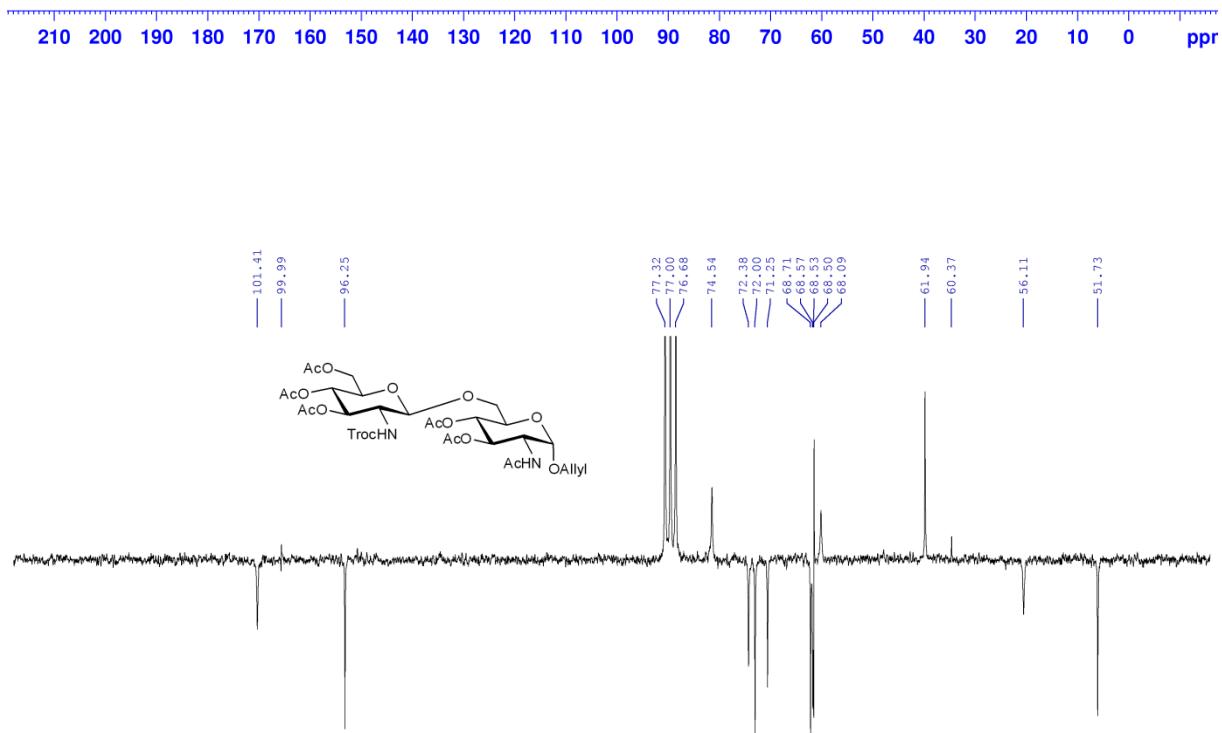
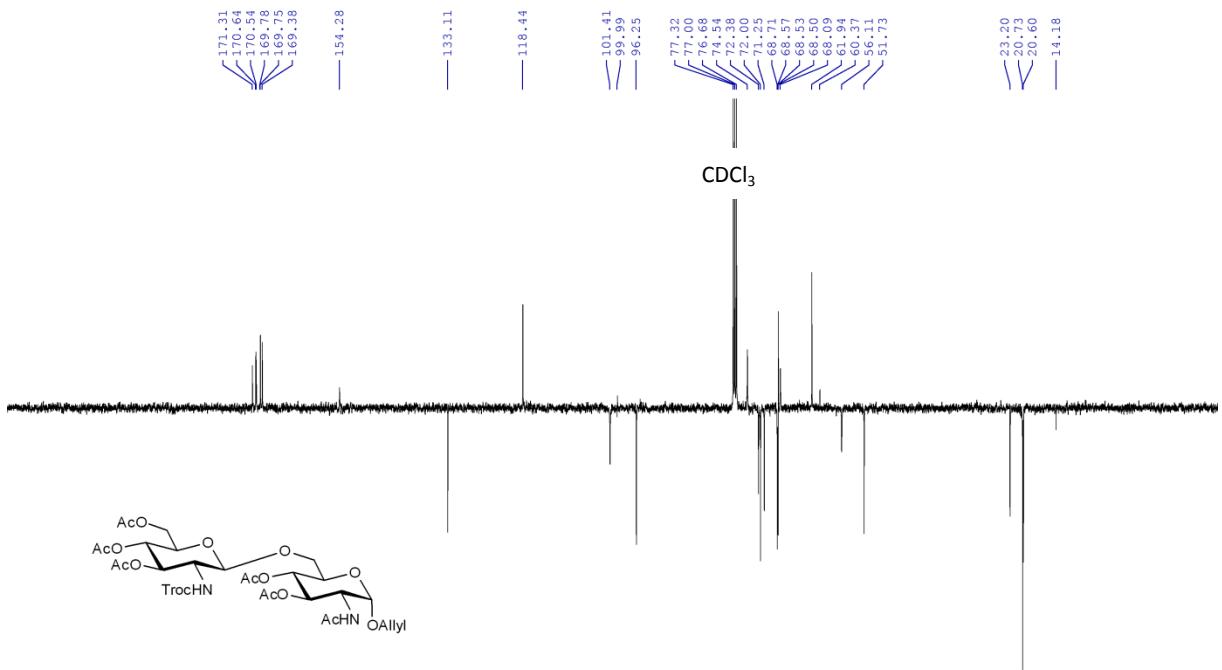
(5):  $\alpha/\beta = 5:1$



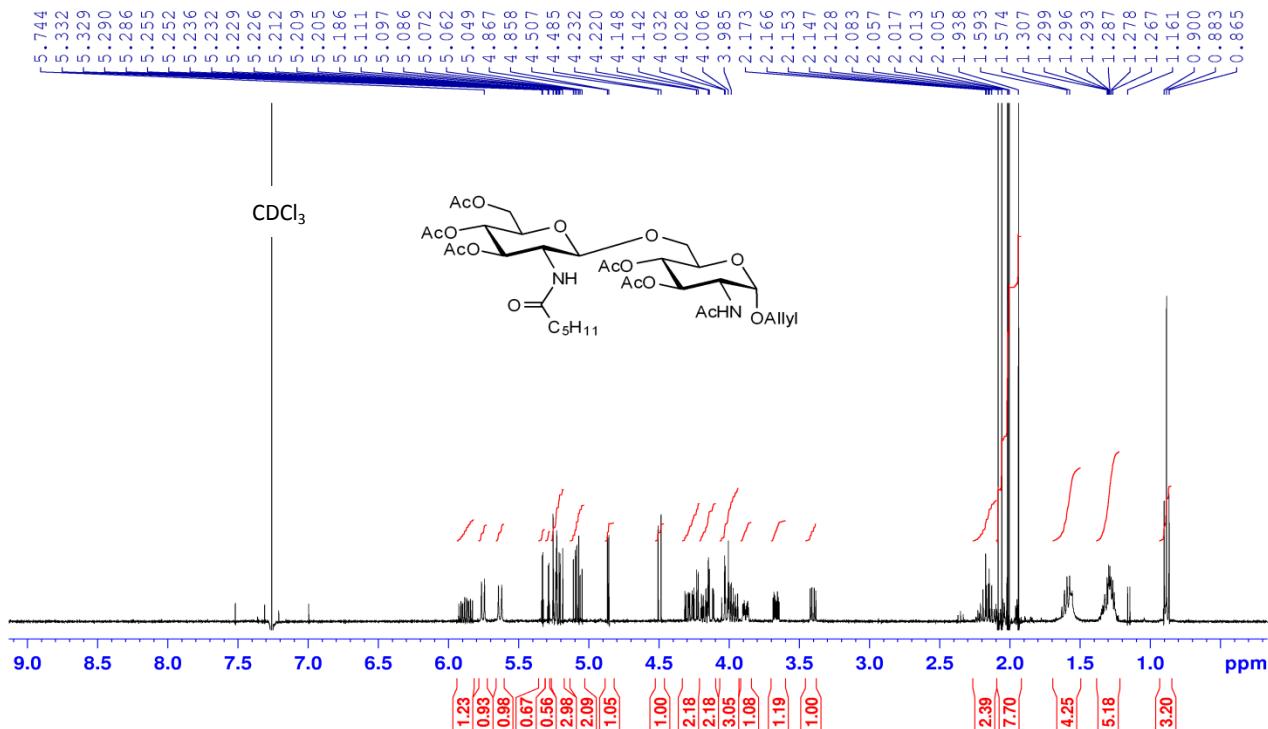
(8):  $^1\text{H}$ -NMR, 400 MHz,  $\text{CDCl}_3$



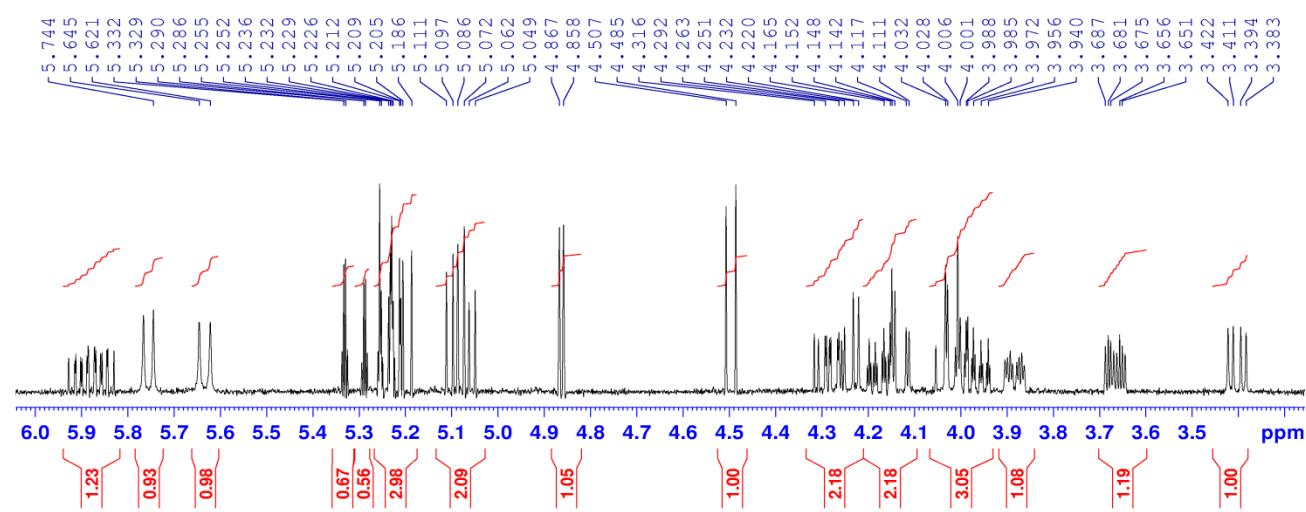
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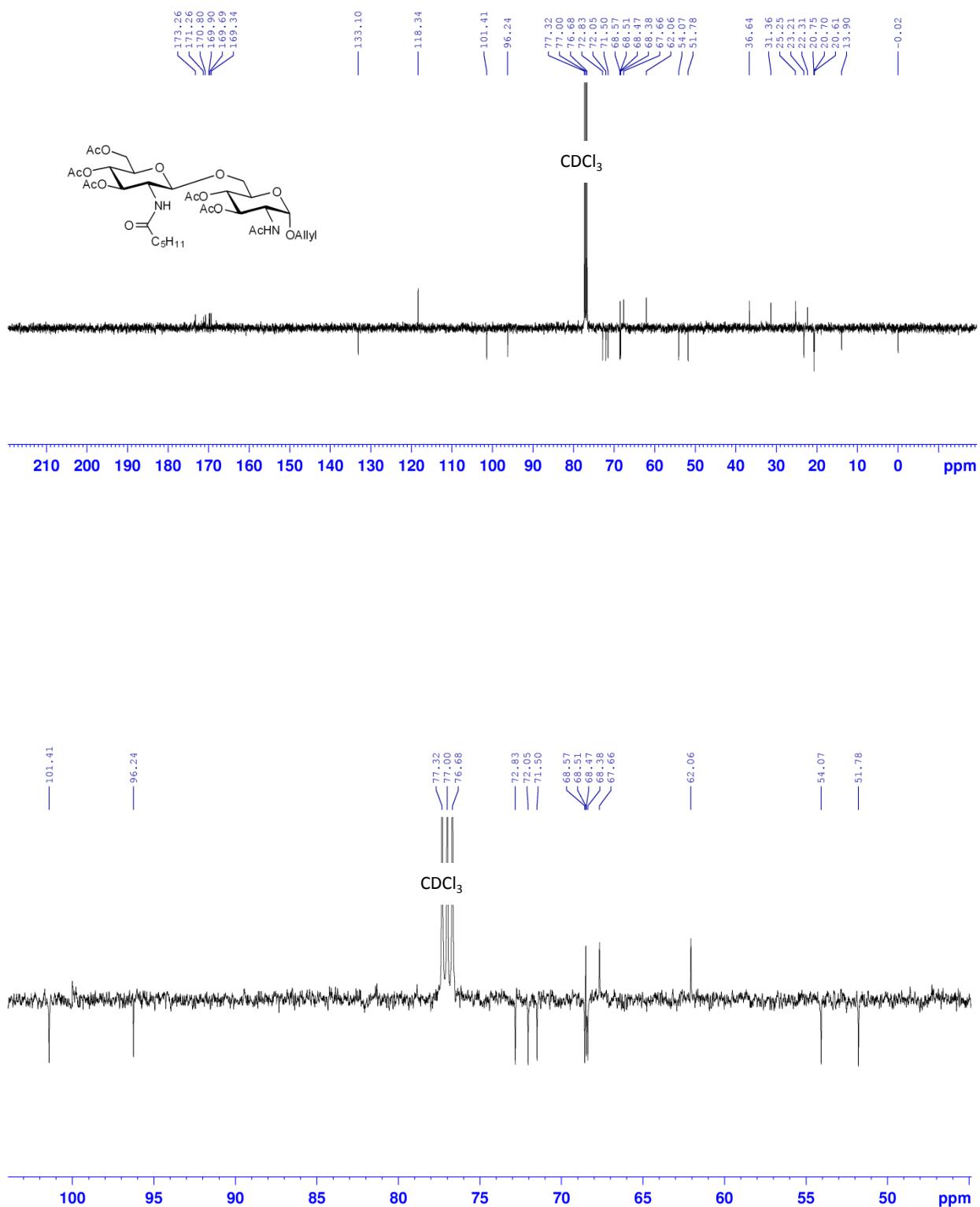
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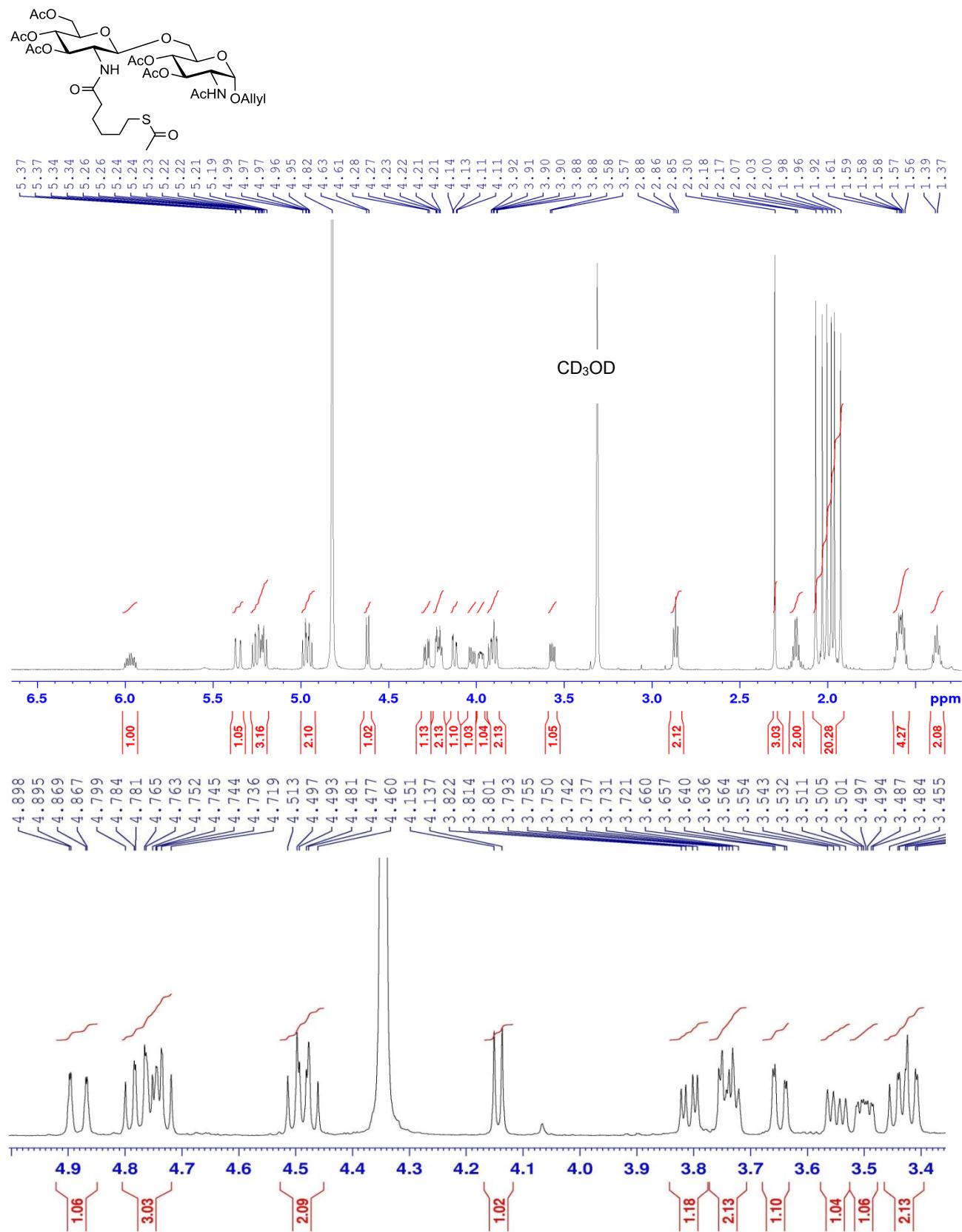
(9):  $^1\text{H}$ -NMR, 400 MHz,  $\text{CDCl}_3$



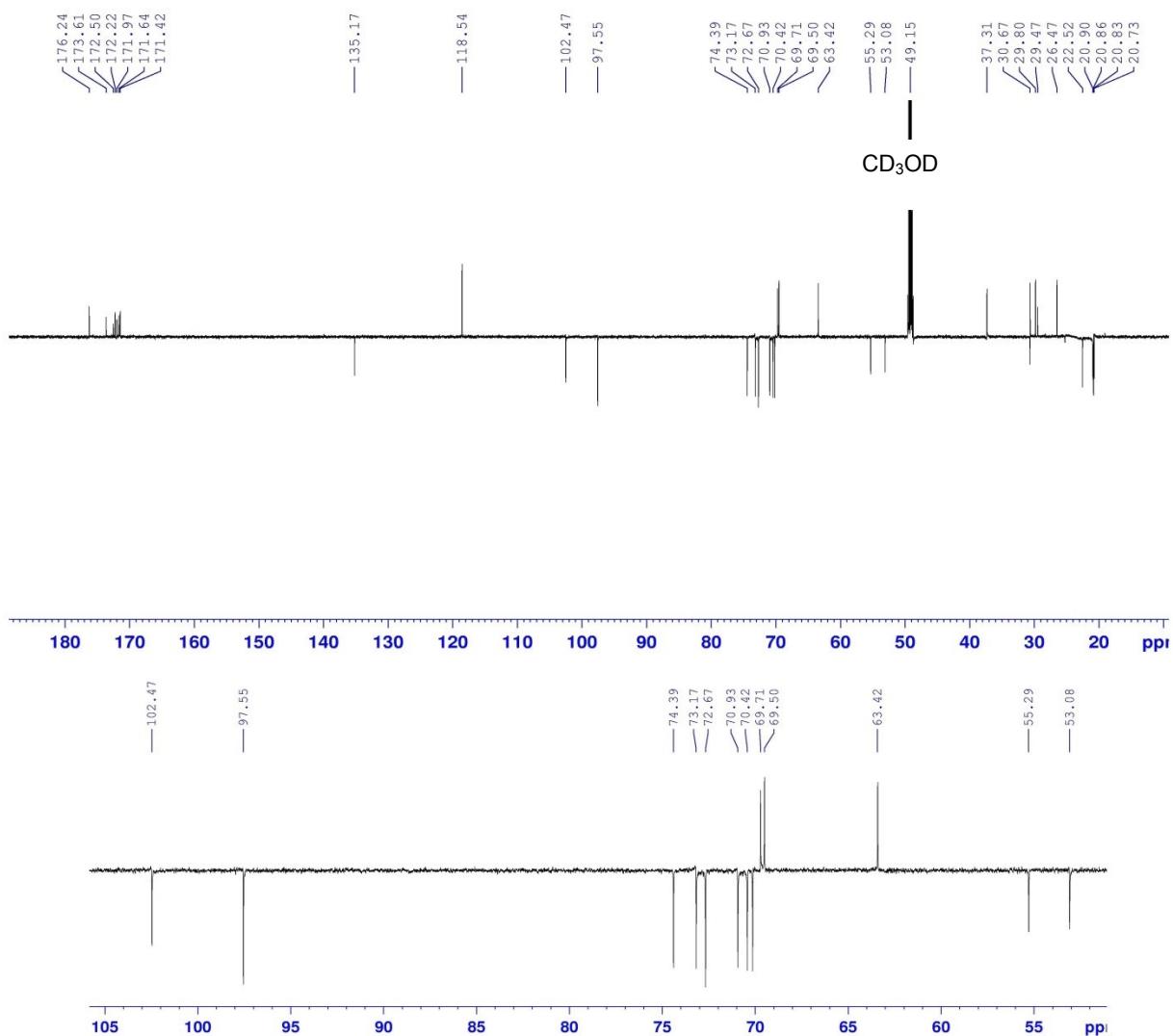
(9):  $^{13}\text{C}$ -NMR, 101 MHz,  $\text{CDCl}_3$



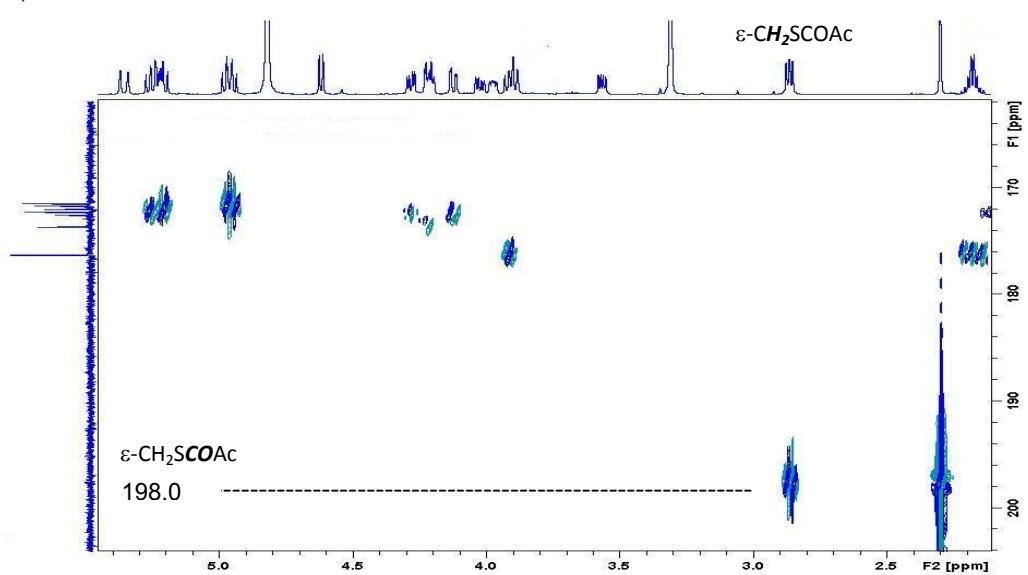
**(10):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$**



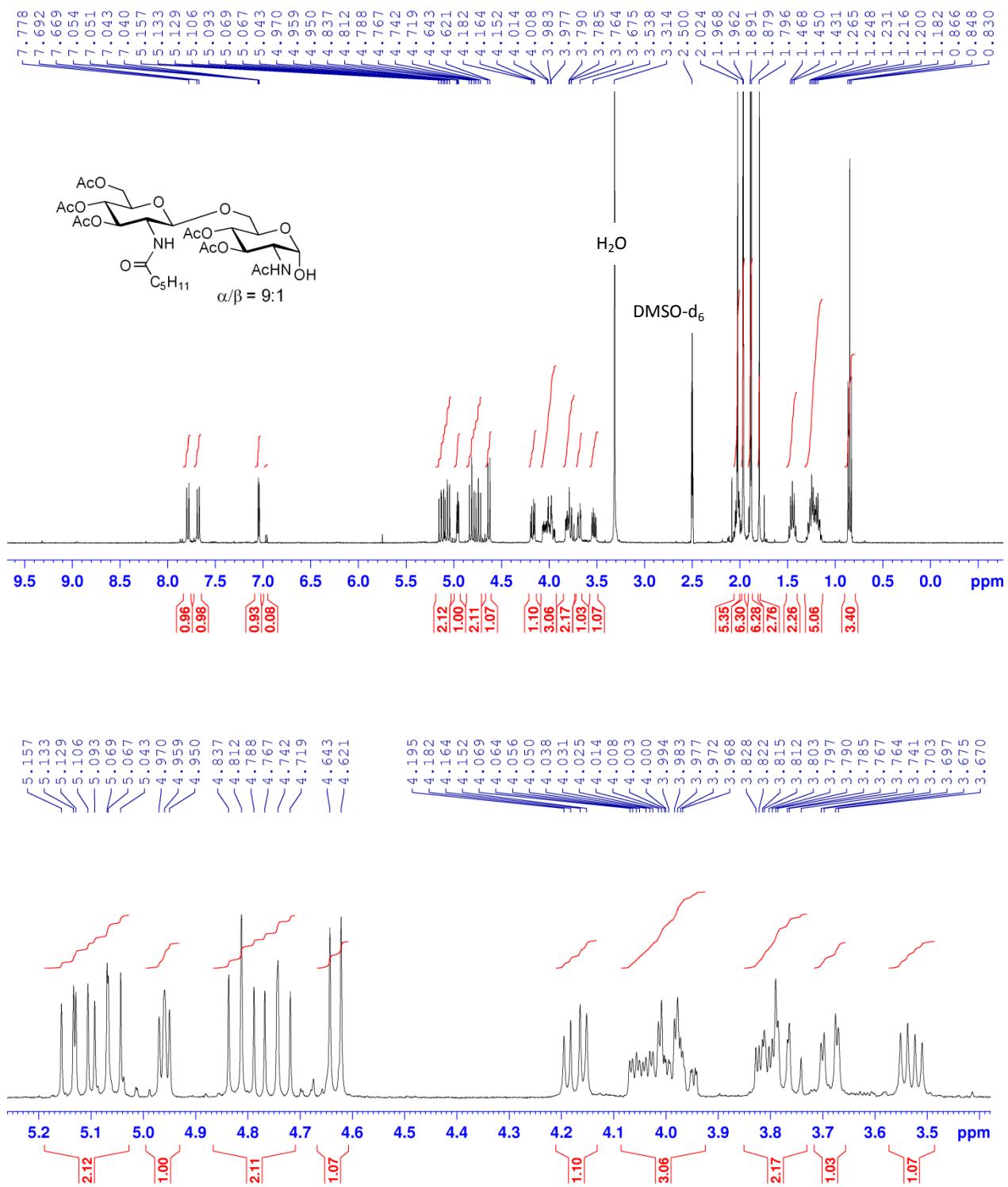
(10):  $^{13}\text{C}$ -NMR, 151 MHz,  $\text{CD}_3\text{OD}$



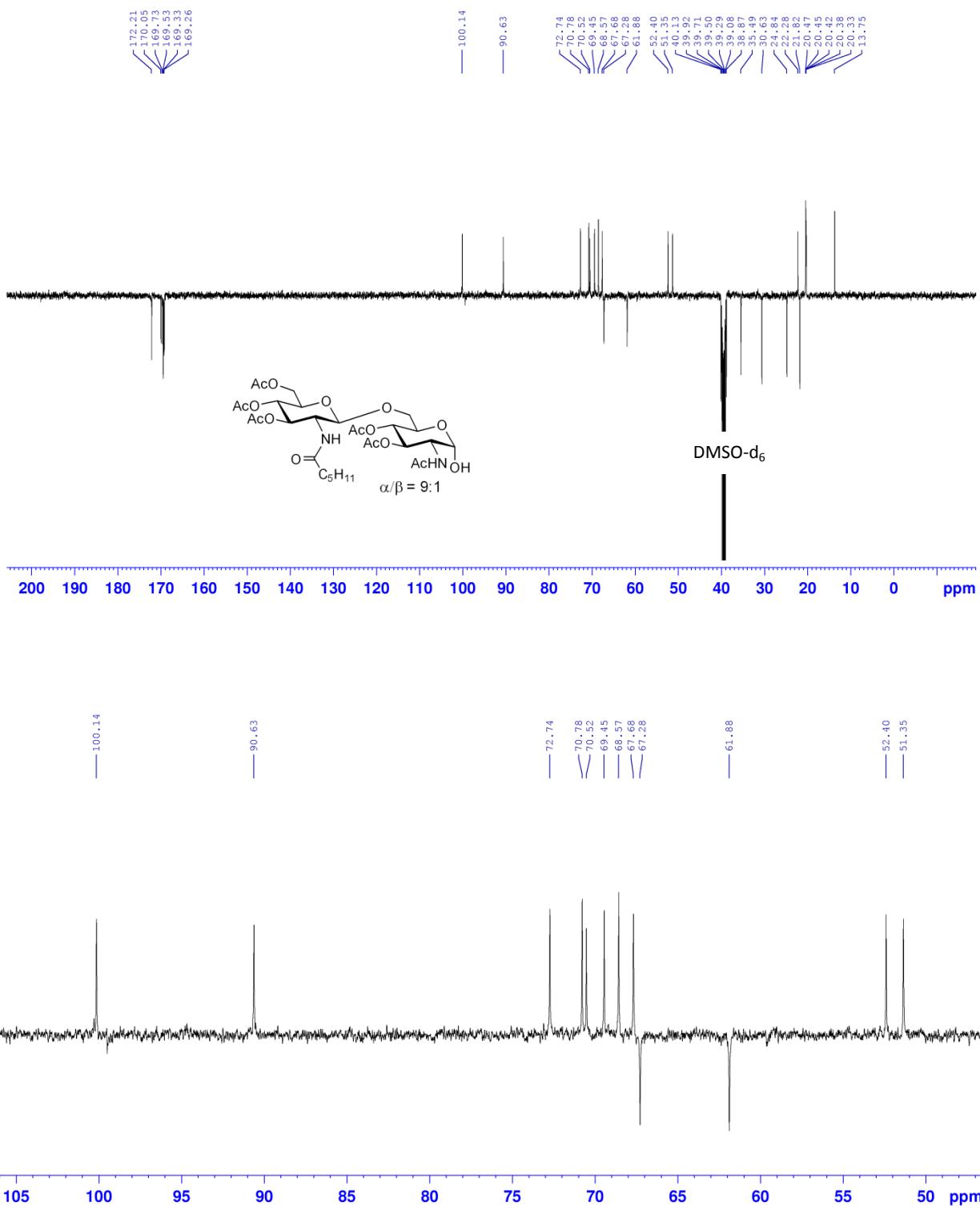
(10): HMBC,  $\text{MeOD}$



(11):  $^1\text{H-NMR}$ , 400 MHz,  $\text{DMSO-d}_6$ ; **11-}\alpha** was isolated for characterisation

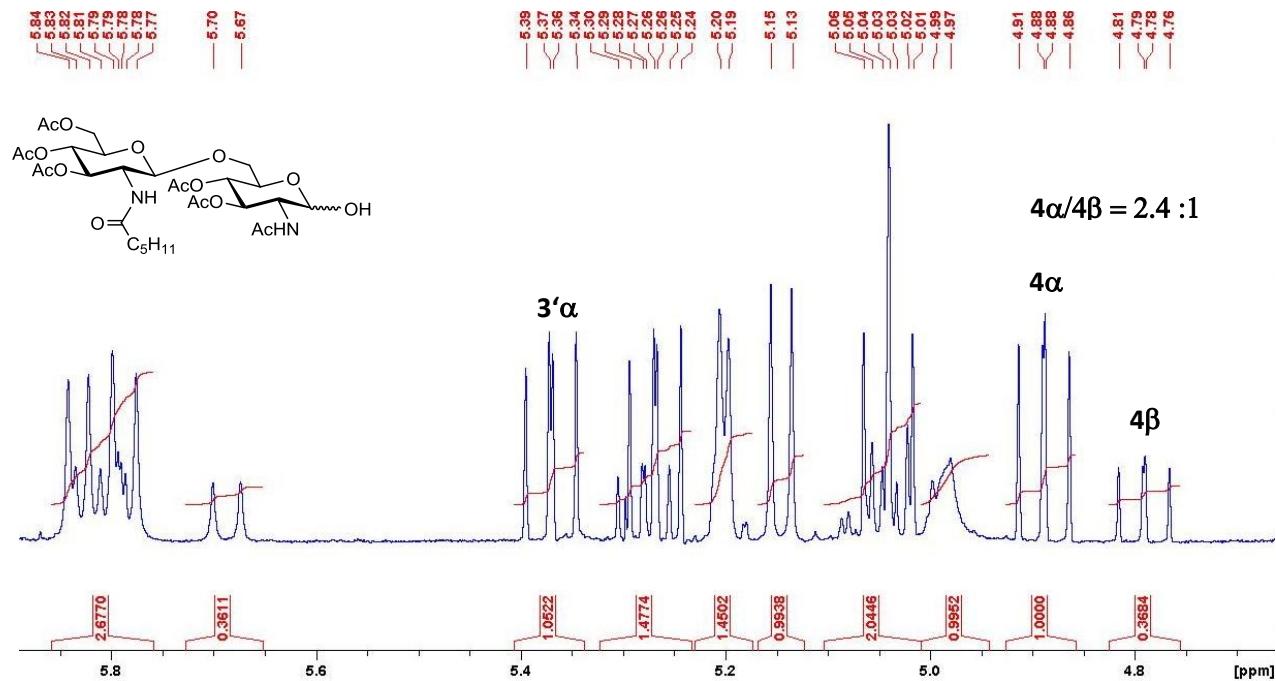


(11):  $^{13}\text{C}$ -NMR, APT, 101 MHz,  $\text{DMSO-d}_6$

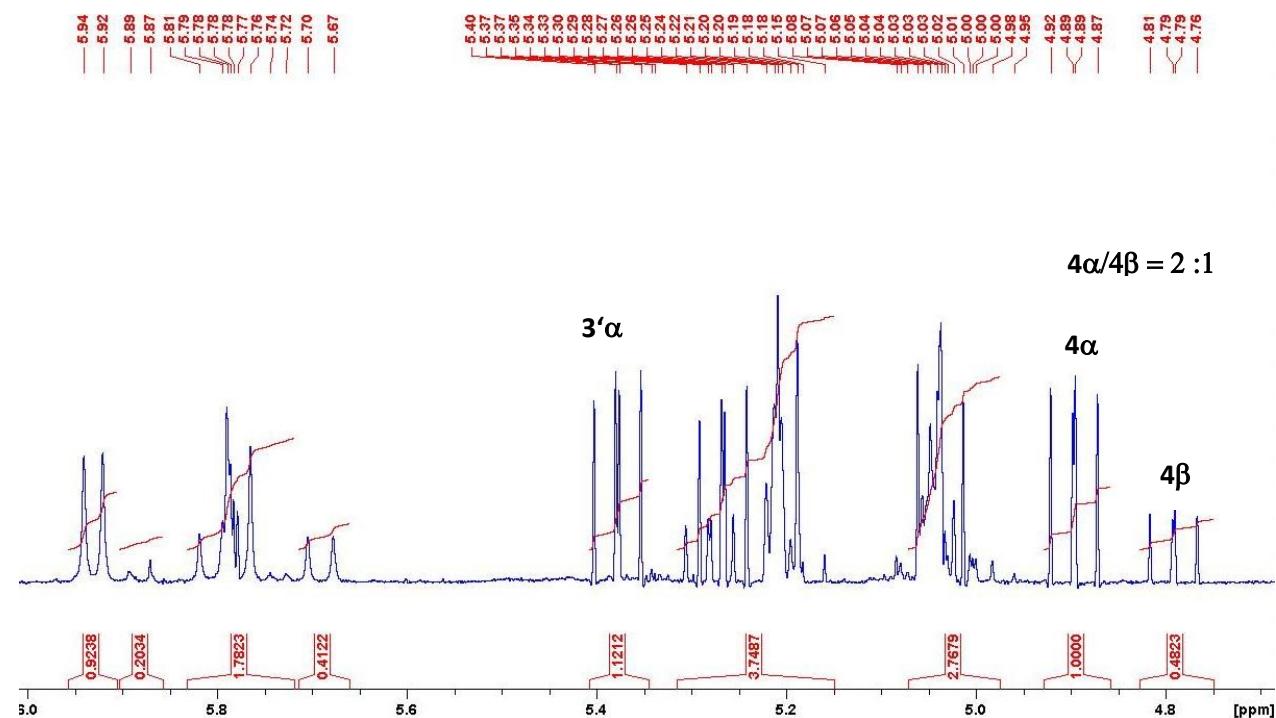


(11) was commonly isolated as anomeric mixture with the  $\alpha/\beta$  ratio ranging from 2:1 to 2.4:1

(11):  $^1\text{H-NMR}$ , 400 MHz,  $\text{CDCl}_3$        $\alpha/\beta = 2.4:1$

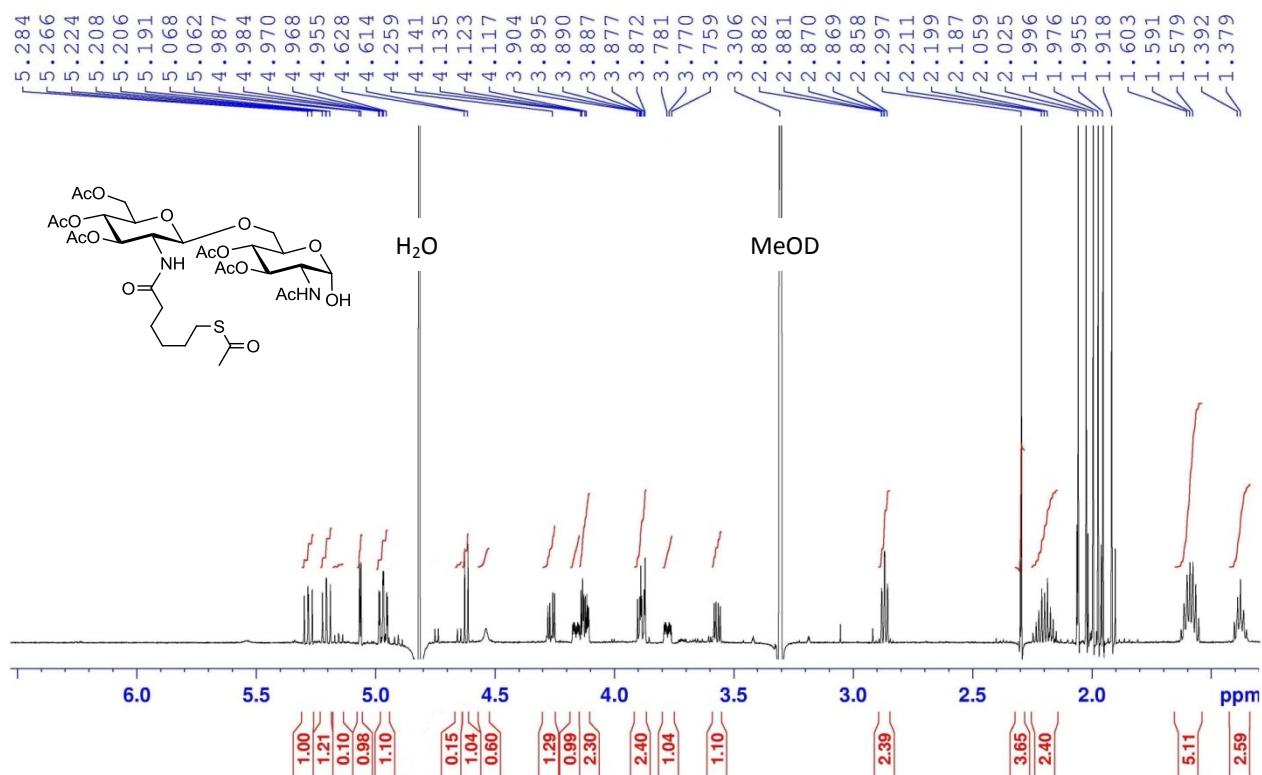


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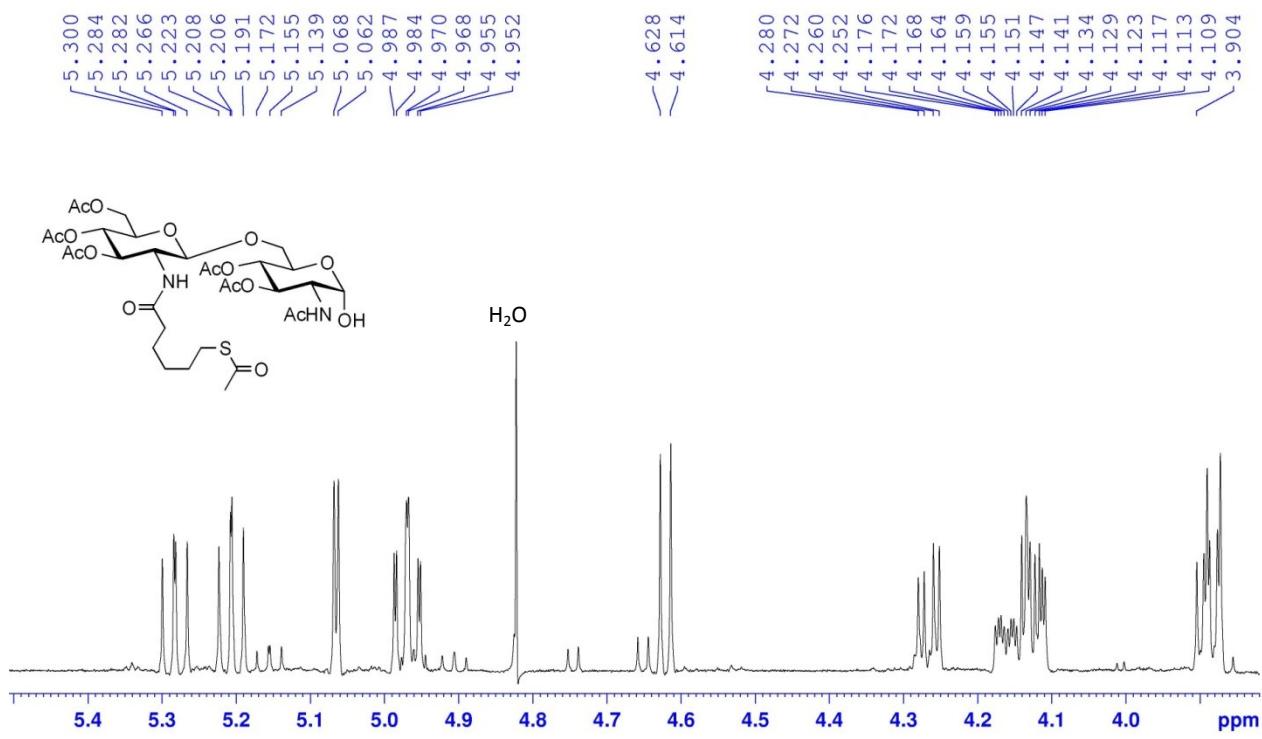


(12):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$

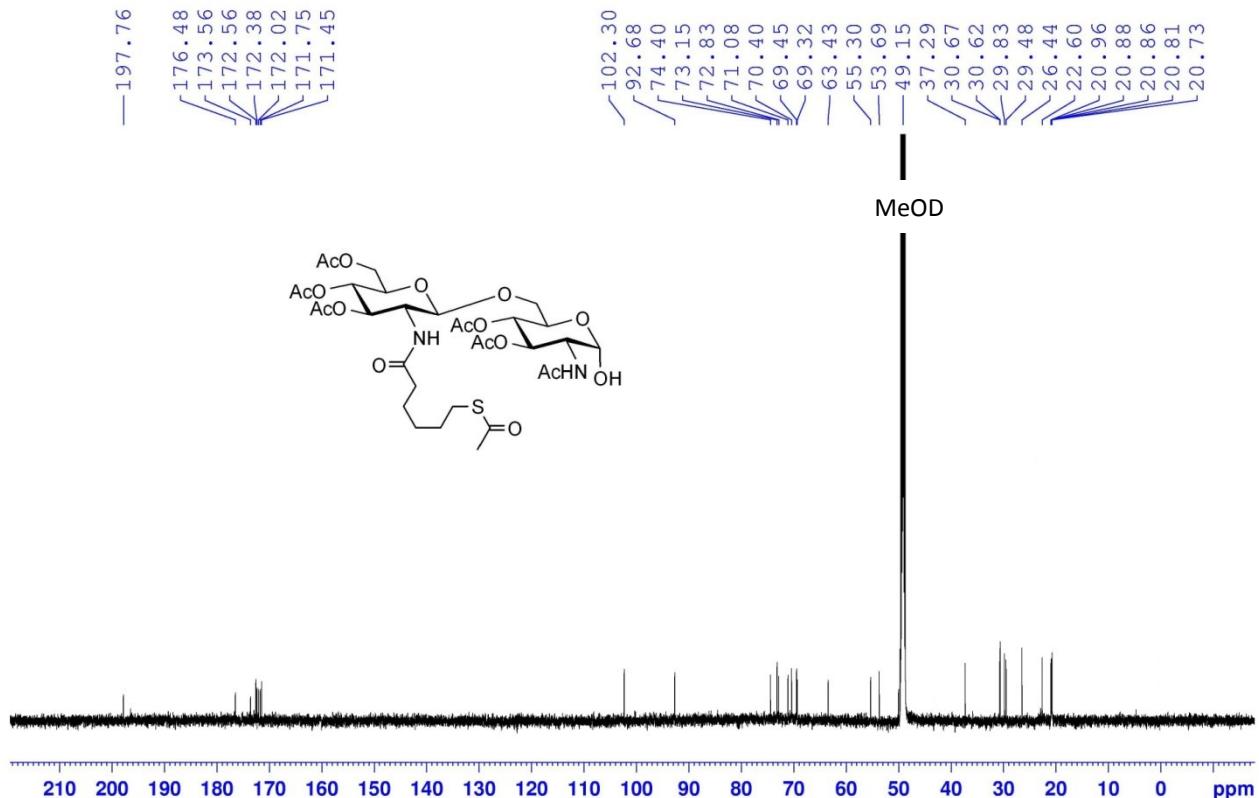
$\alpha/\beta = 10:1$



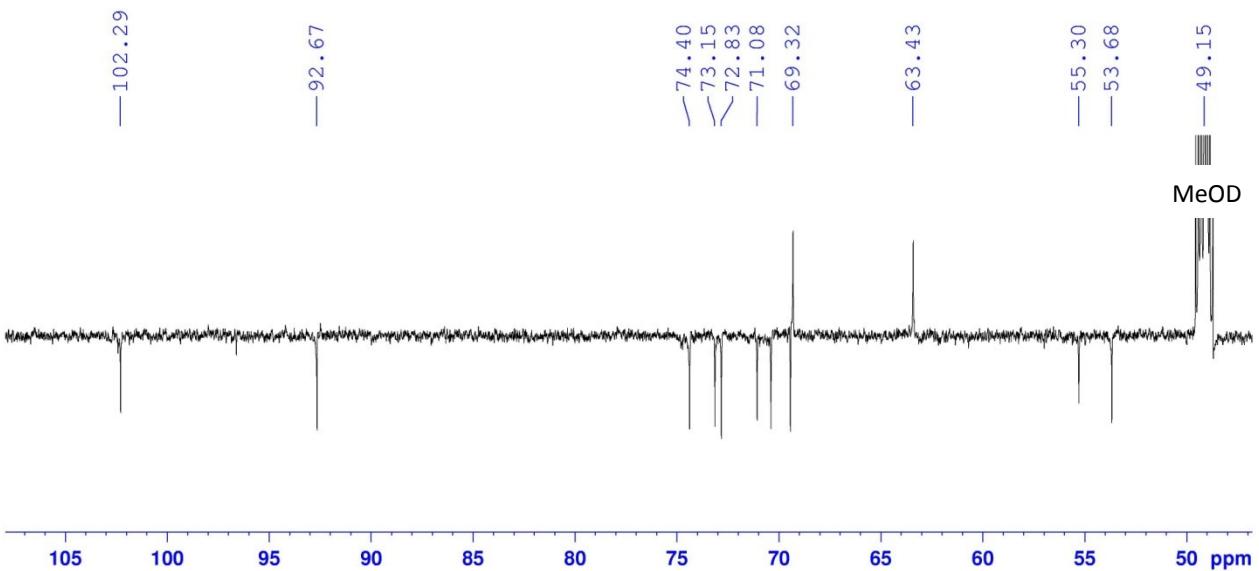
(12):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$  (3.5 - 5.5 ppm)  $\alpha/\beta = 10:1$



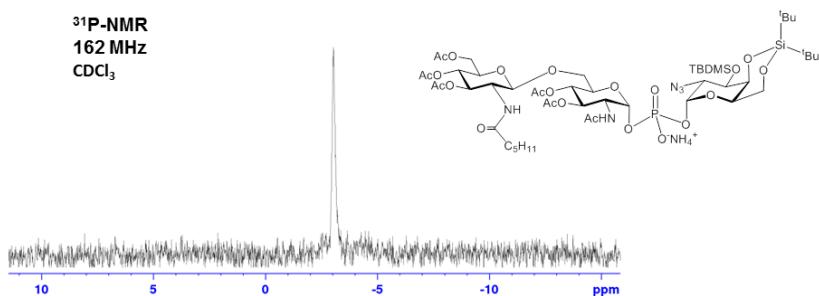
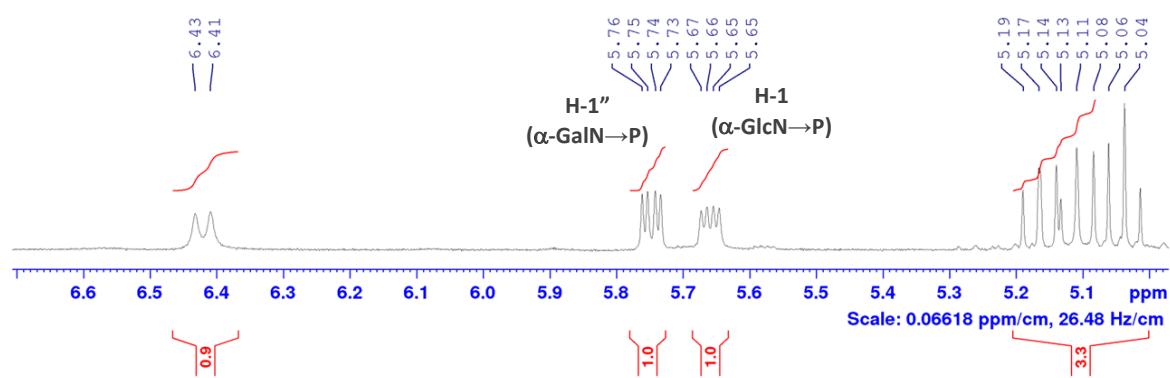
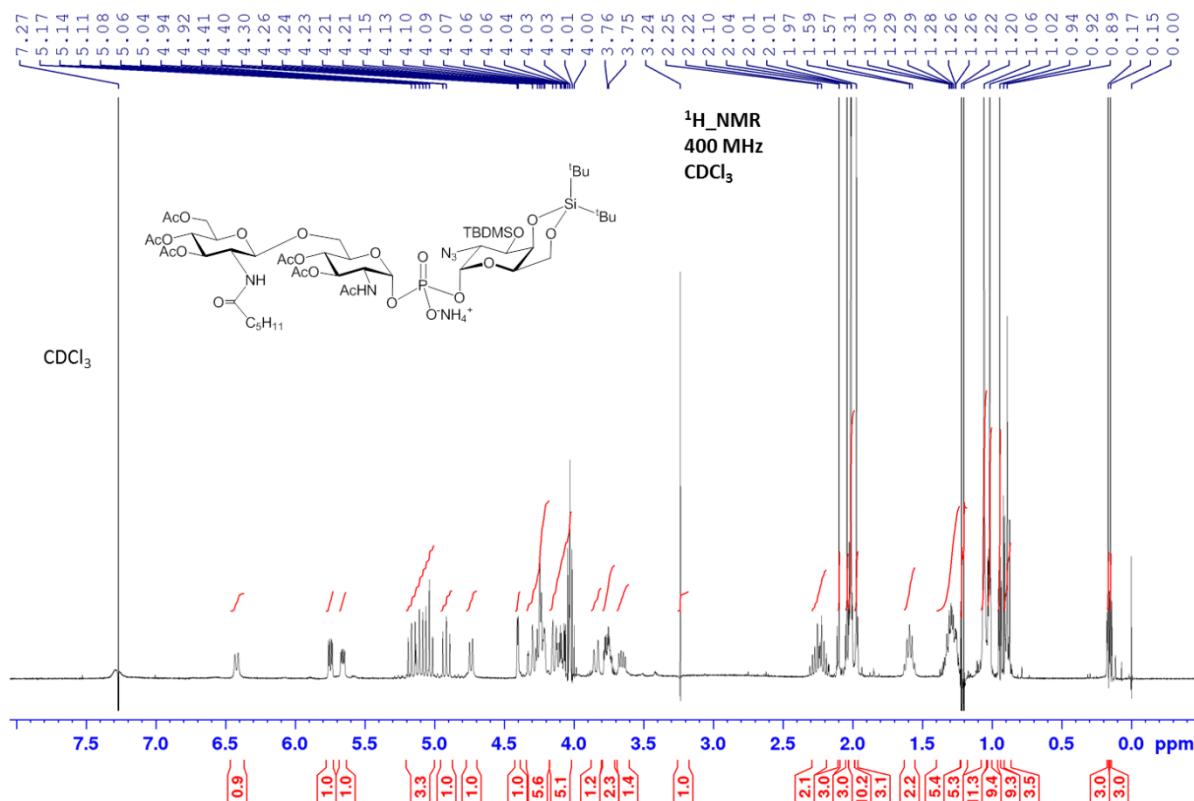
(12):  $^{13}\text{C}$ -NMR, 151 MHz,  $\text{CD}_3\text{OD}$     $\alpha/\beta = 10:1$  (low concentration because of pure solubility)



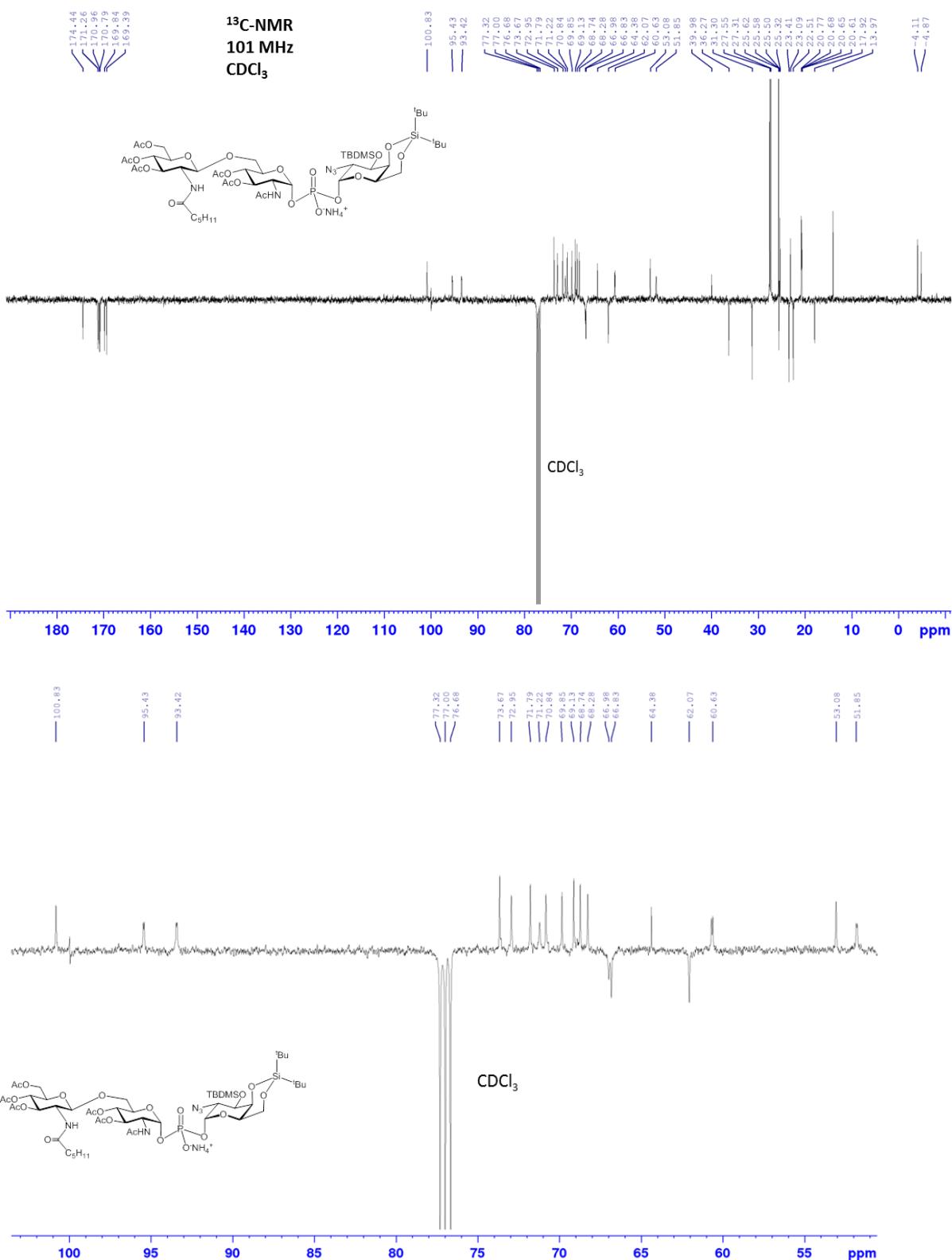
(12):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{CD}_3\text{OD}$ , (50 – 110 ppm)    $\alpha/\beta = 10:1$



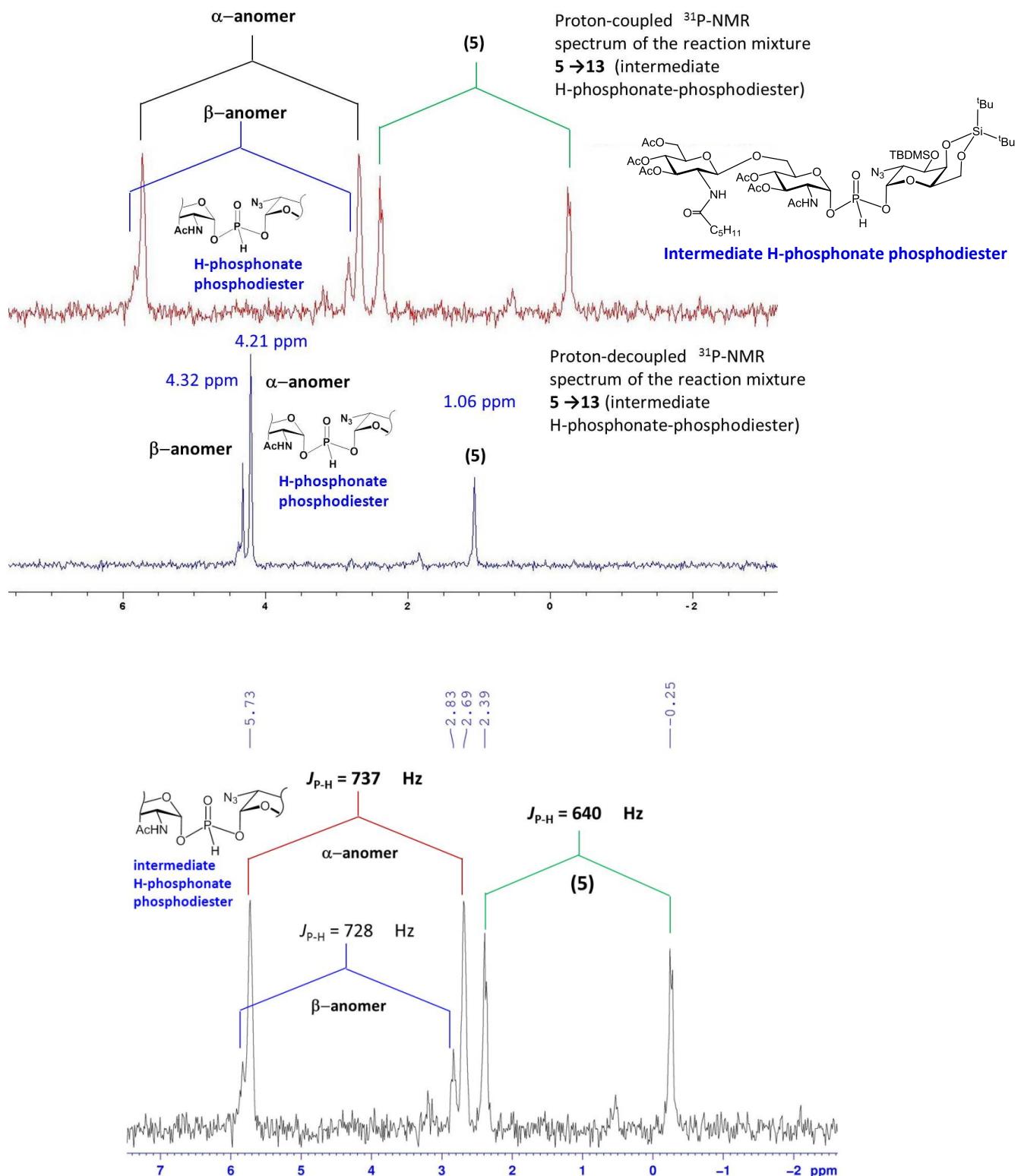
**(13):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$**



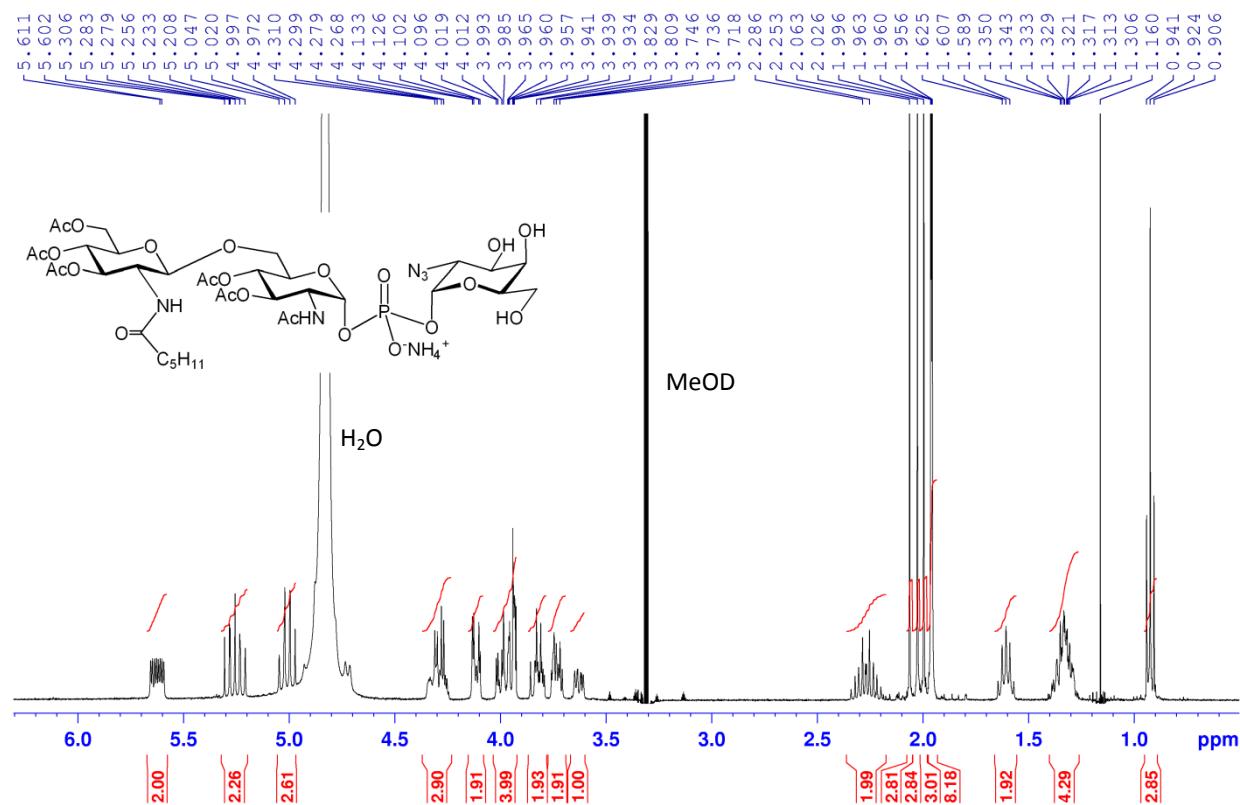
(13):  $^{13}\text{C}$ -NMR, APT, 101 MHz,  $\text{CDCl}_3$



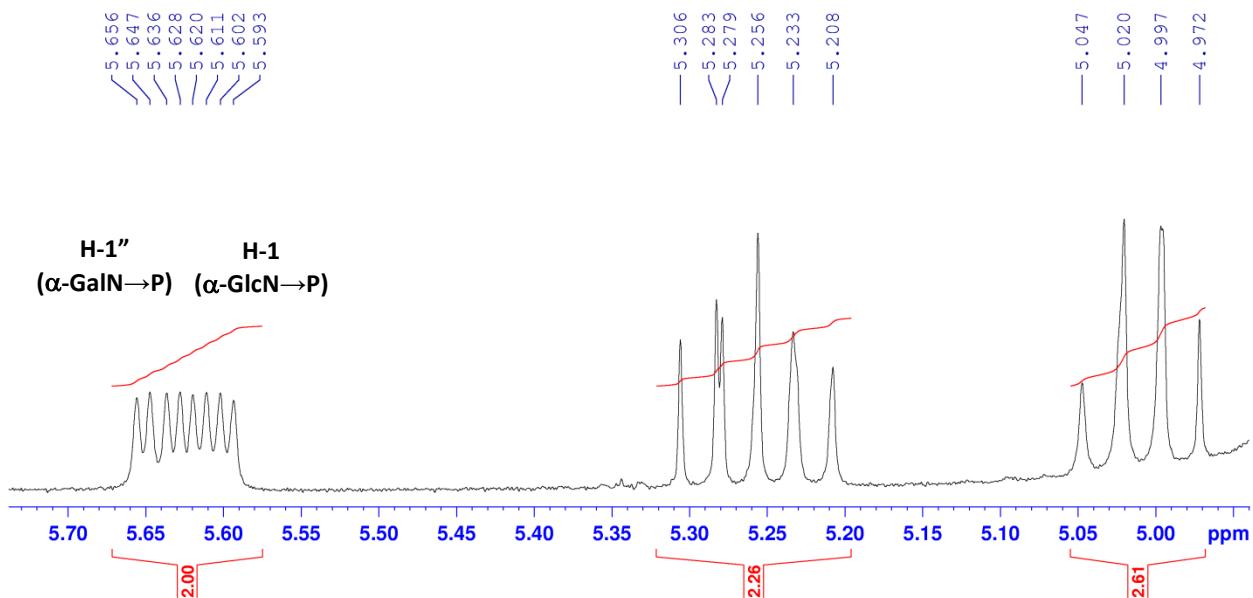
5 → 13:  $^{31}\text{P}$ -NMR, 243 MHz,  $\text{CDCl}_3$ , reaction controlled by  $^{31}\text{P}$ -NMR



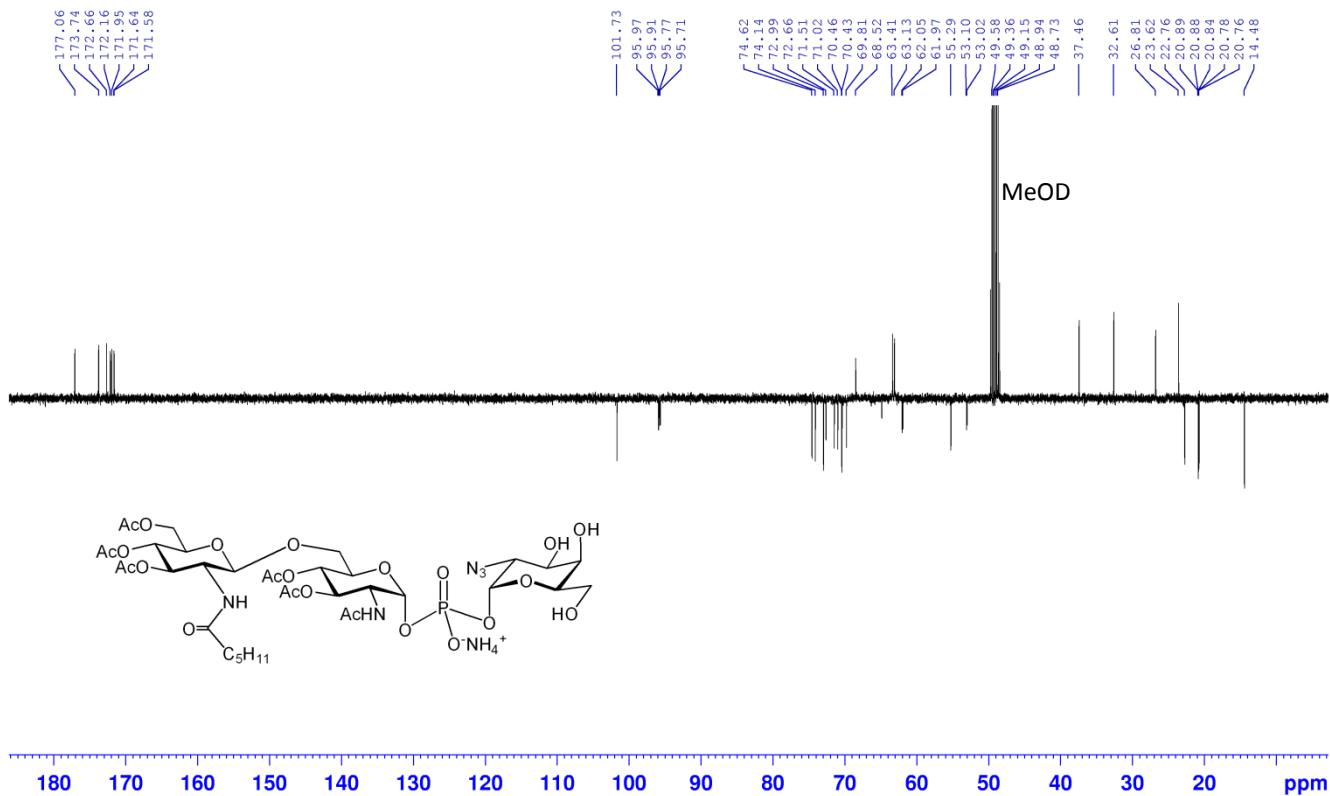
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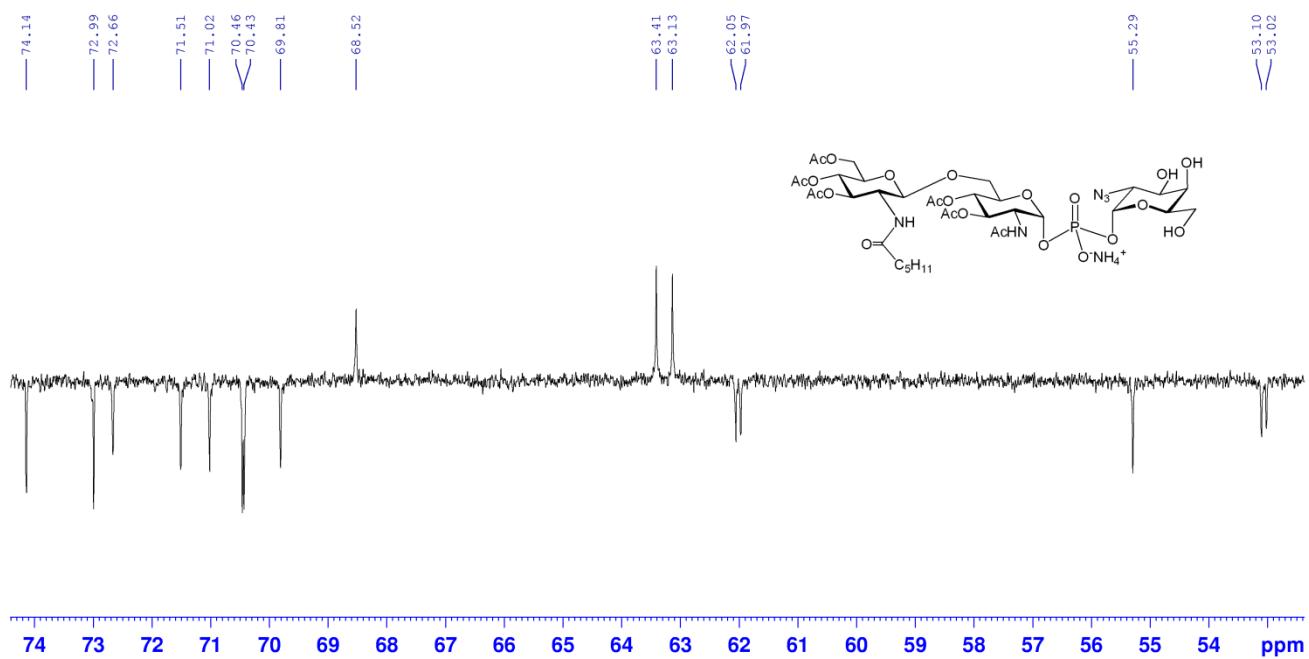
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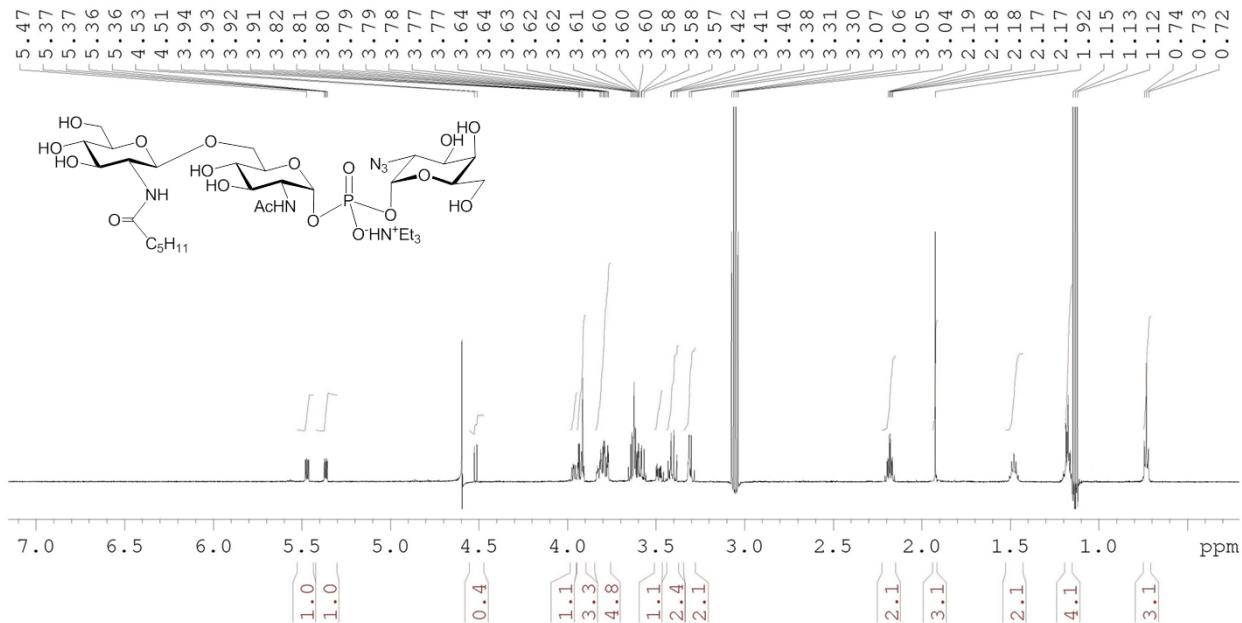
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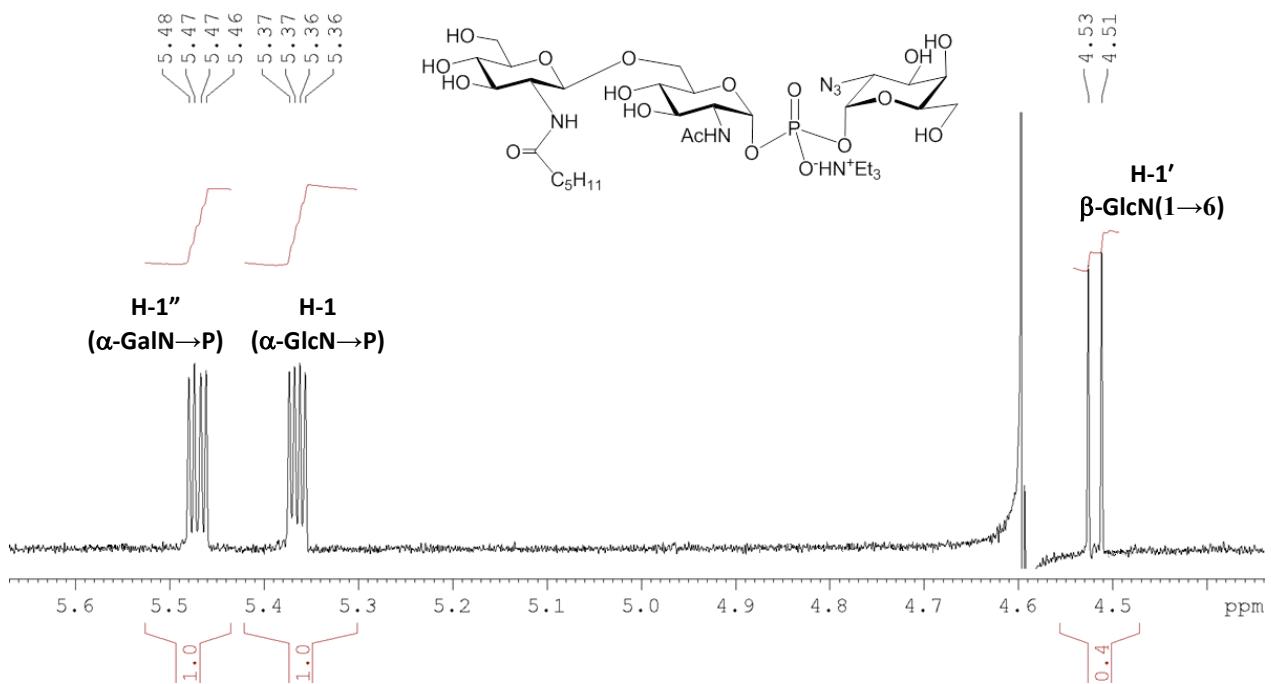
(14):  $^{13}\text{C}$ -NMR, APT, 101 MHz,  $\text{CD}_3\text{OD}$



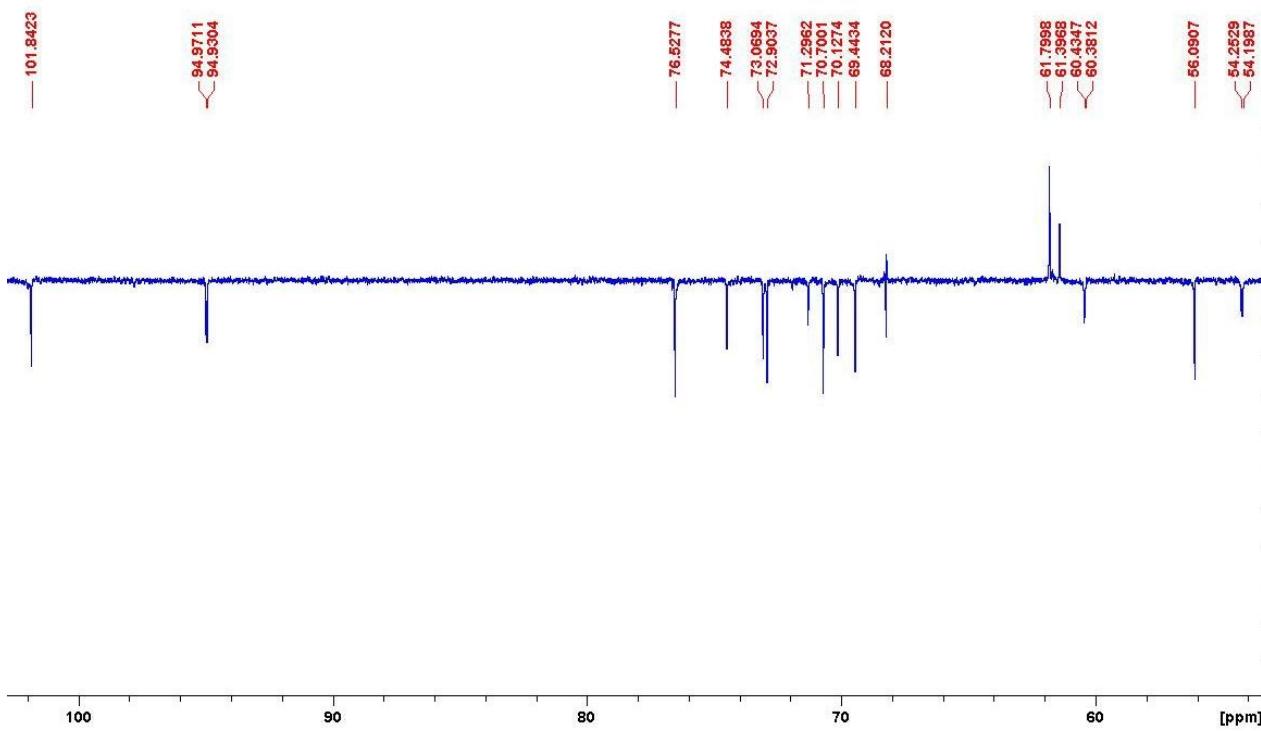
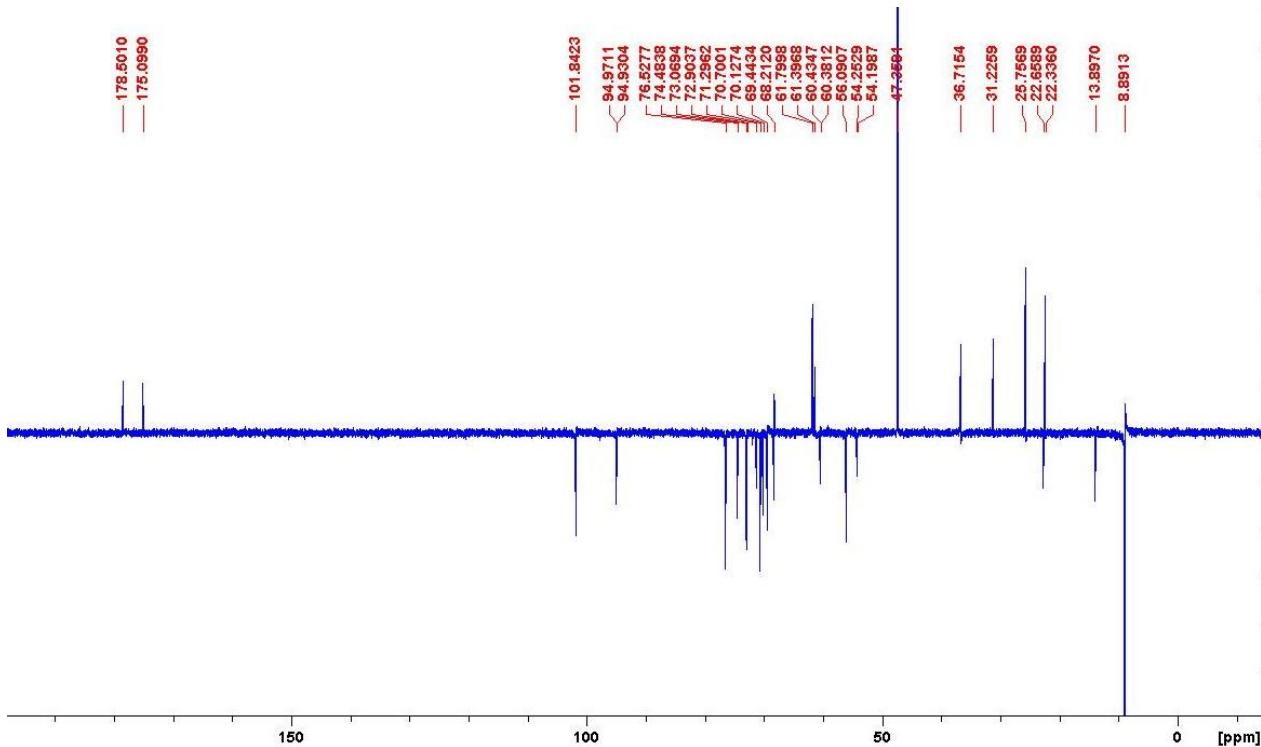
(15):  $^1\text{H}$ -NMR, 600 MHz,  $\text{D}_2\text{O}$



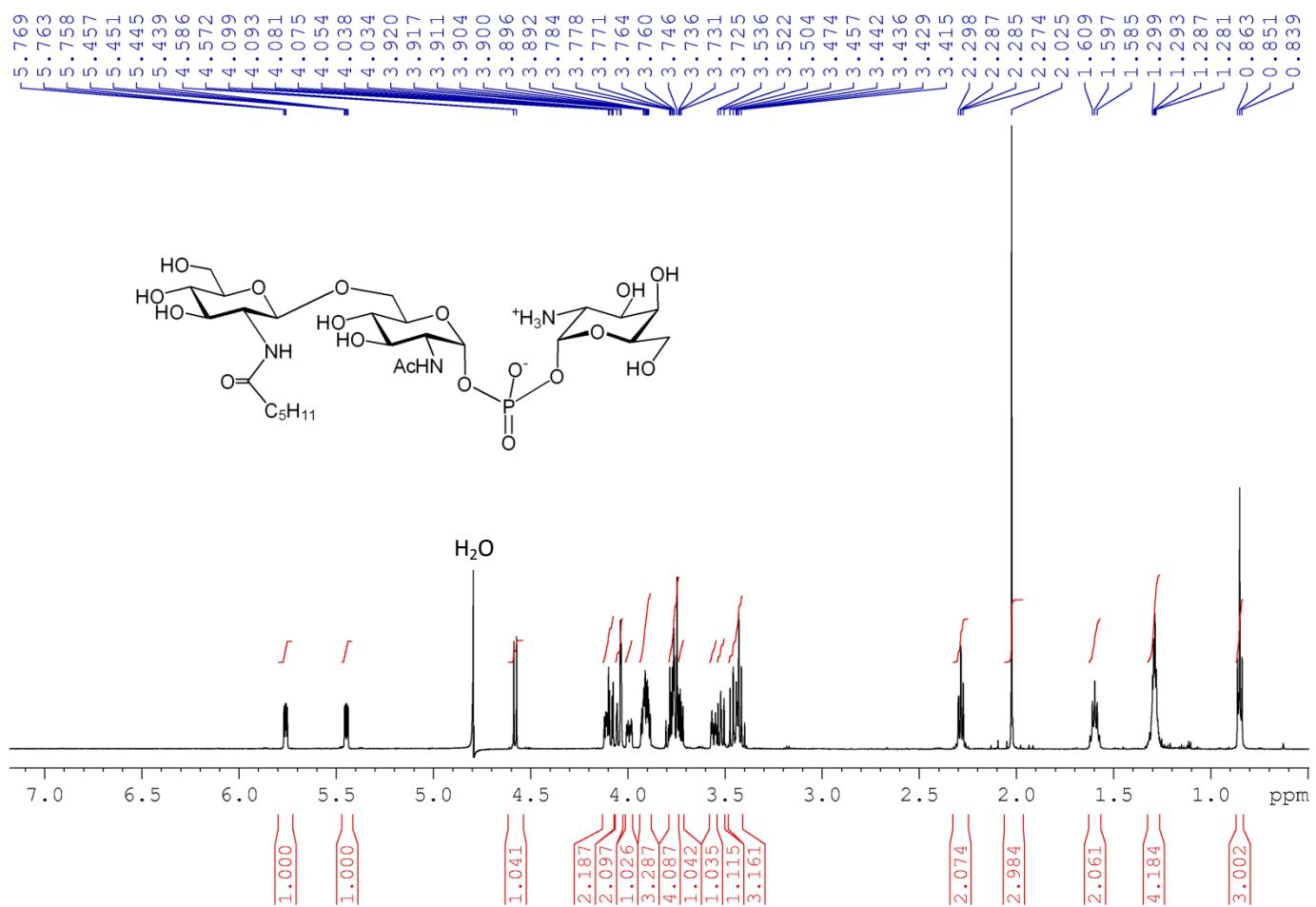
(15):  $^1\text{H}$ -NMR, 600 MHz,  $\text{D}_2\text{O}$ , 4.0 – 6.0 ppm



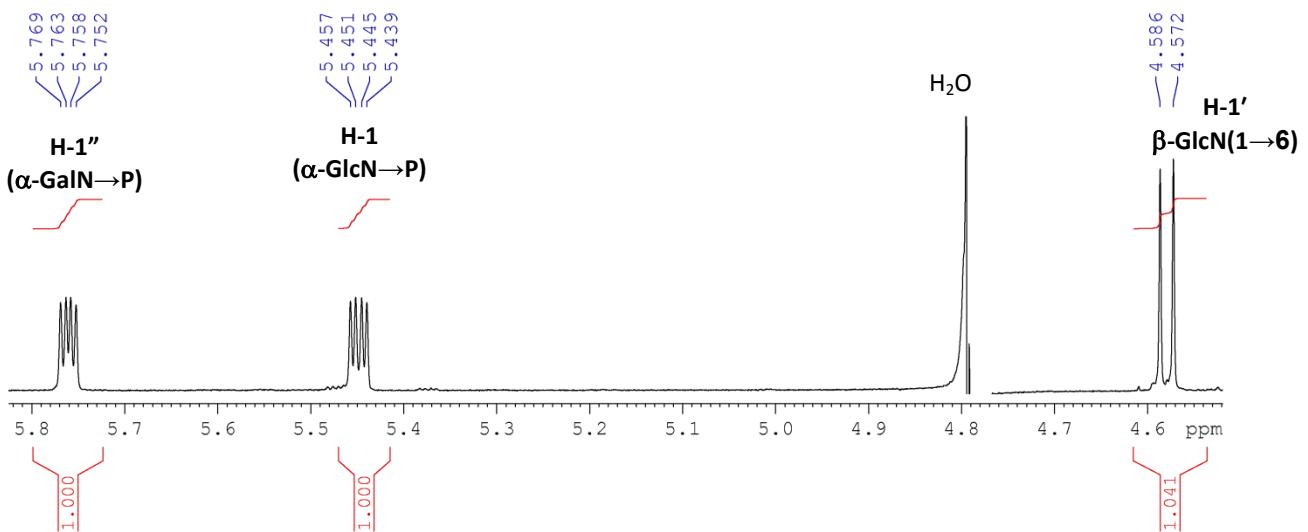
(15):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{D}_2\text{O}$



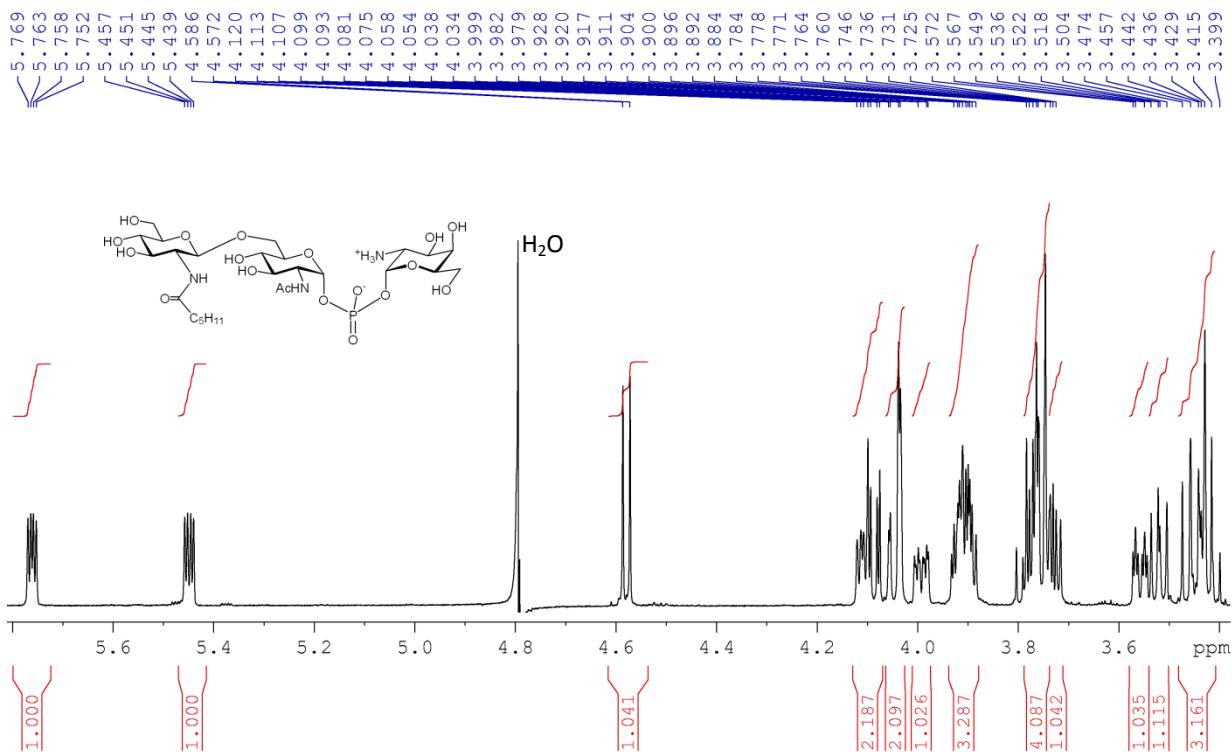
**(16):  $^1\text{H-NMR}$ , 600 MHz,  $\text{D}_2\text{O}$**



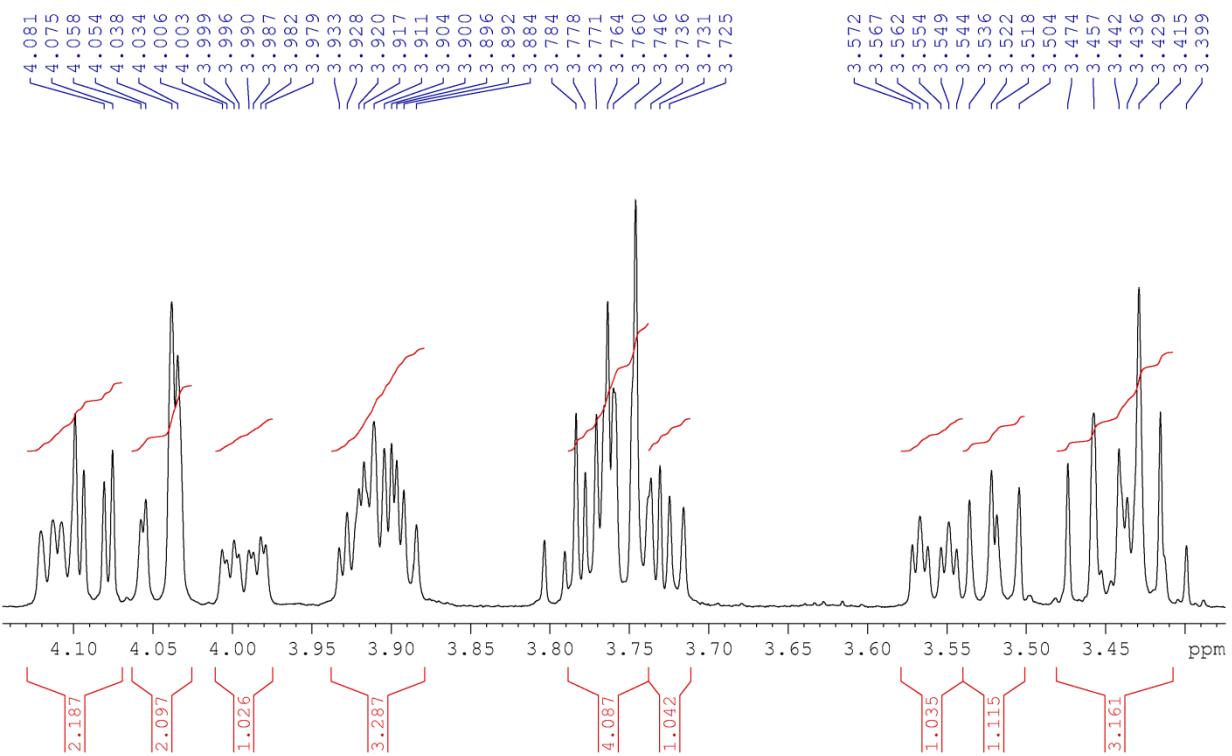
**(16):  $^1\text{H-NMR}$ , 600 MHz,  $\text{D}_2\text{O}$ , 4.5 – 6.0 ppm**



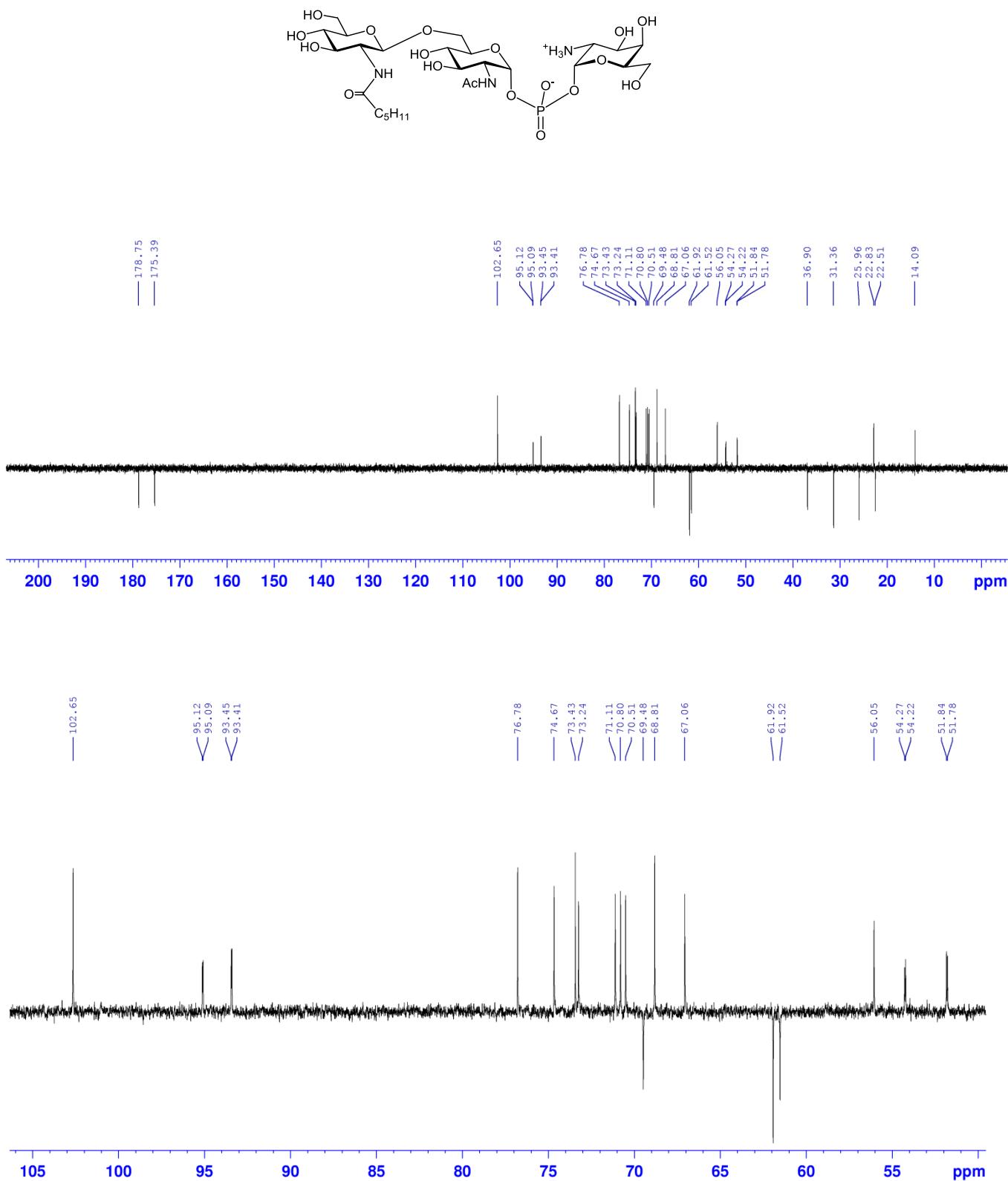
(16):  $^1\text{H-NMR}$ , 600 MHz,  $\text{D}_2\text{O}$ , 6.0 – 3.0 ppm



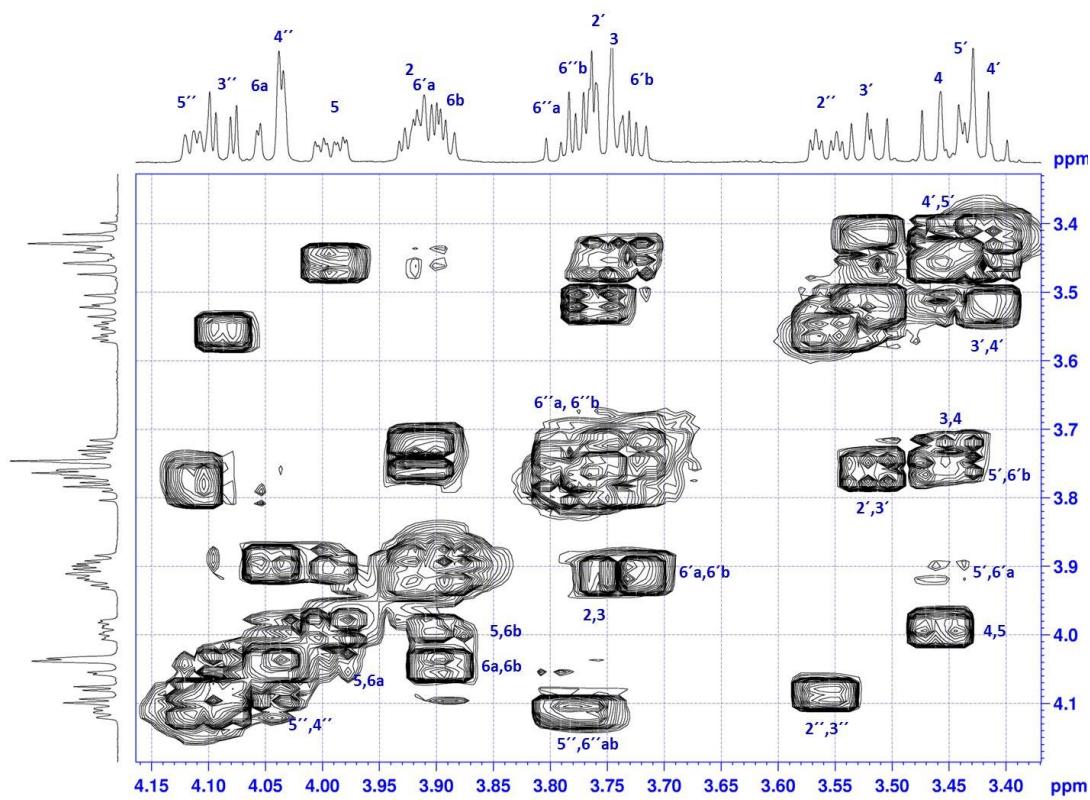
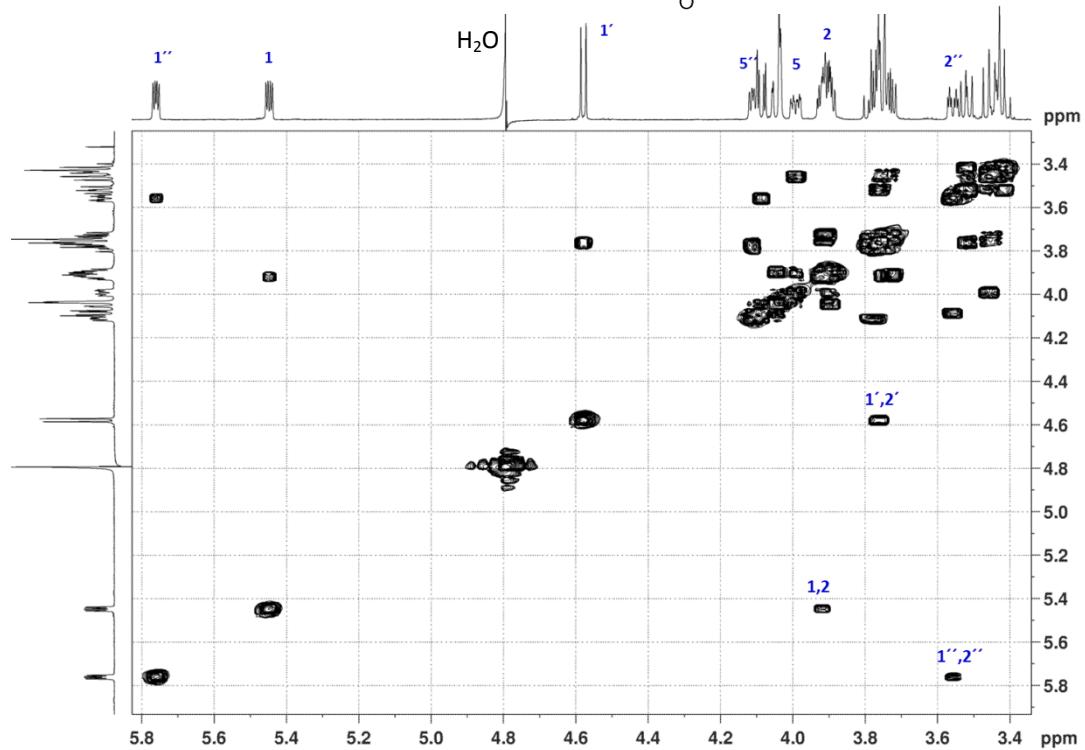
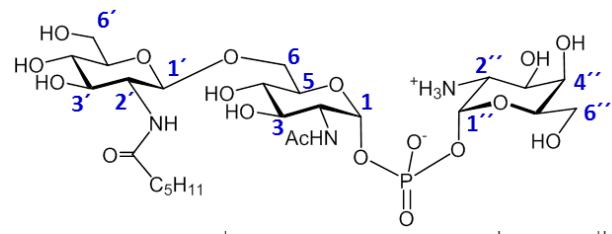
(16):  $^1\text{H-NMR}$ , 600 MHz,  $\text{D}_2\text{O}$ , 4.5 – 3.0 ppm



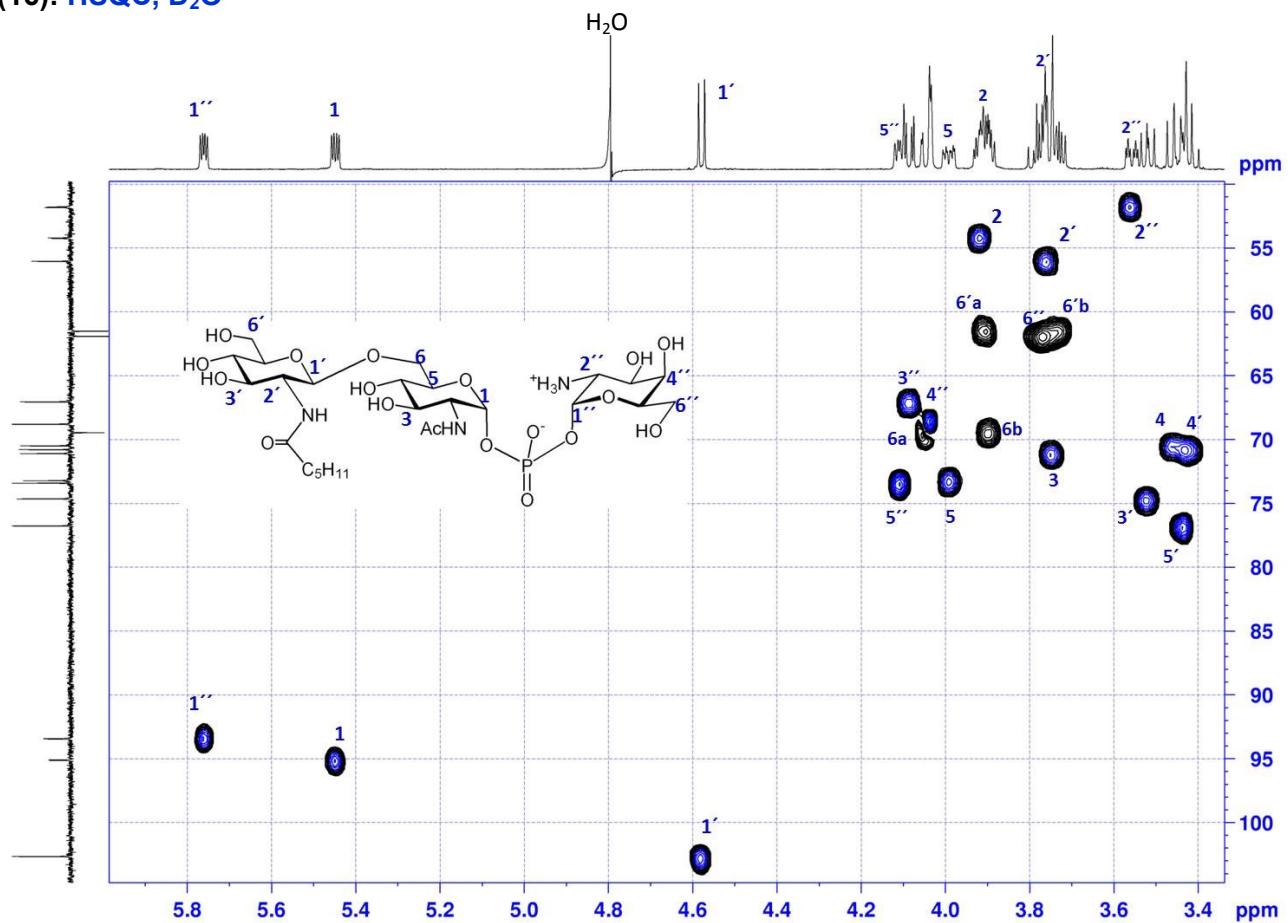
(16):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{D}_2\text{O}$



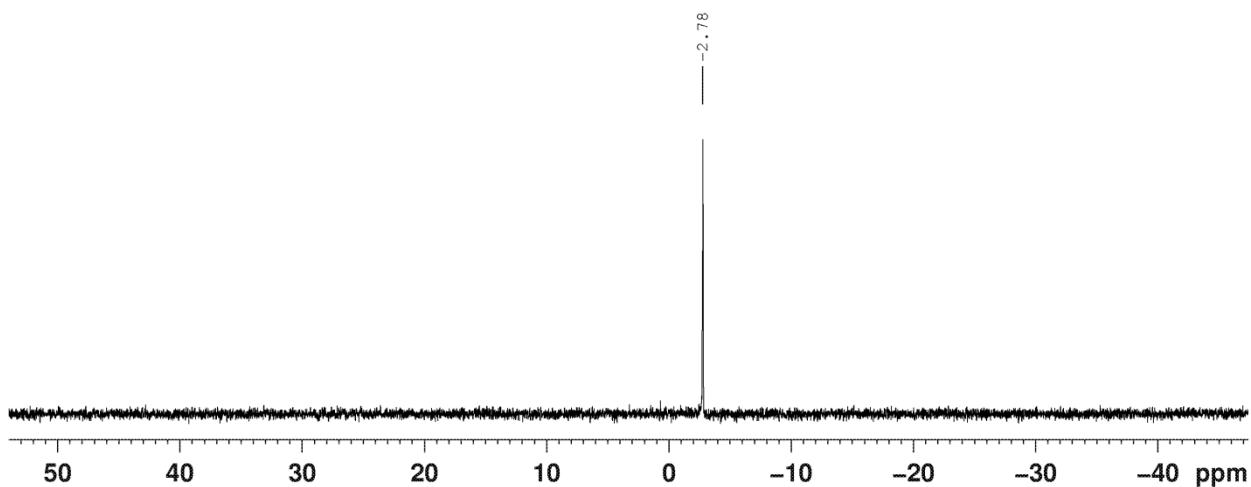
**(16): COSY, D<sub>2</sub>O**



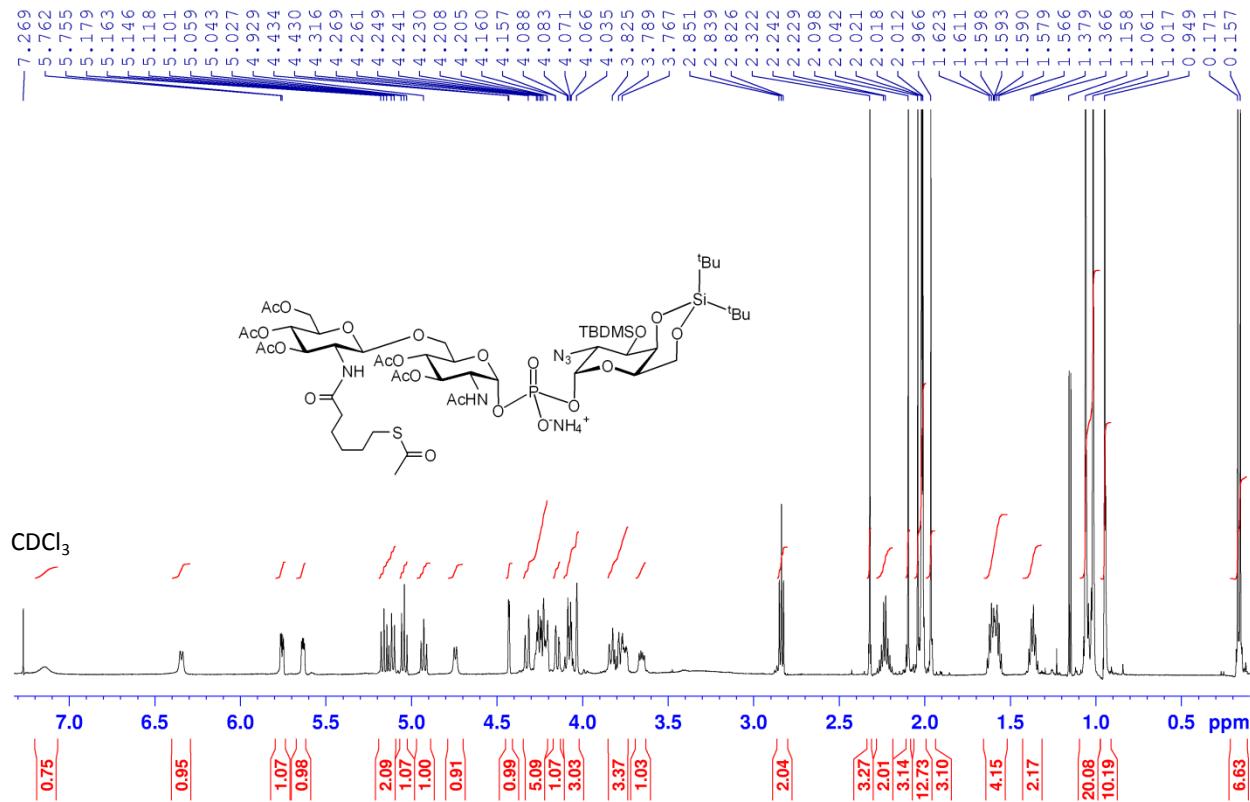
(16): HSQC, D<sub>2</sub>O



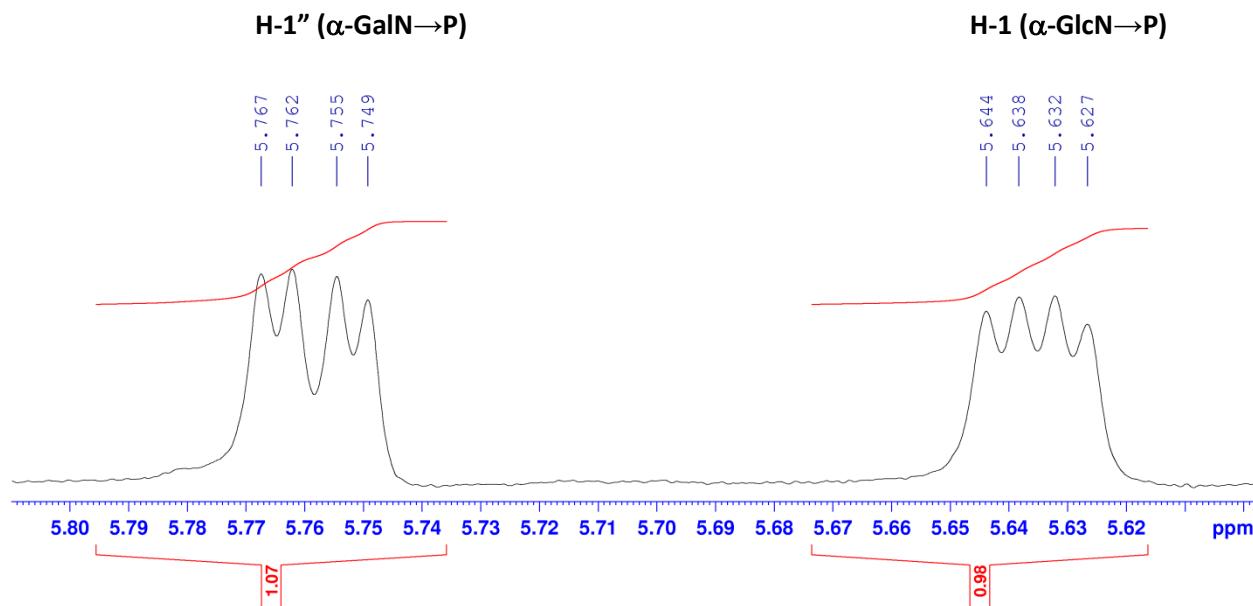
(16): <sup>31</sup>P-NMR, 243 MHz, D<sub>2</sub>O



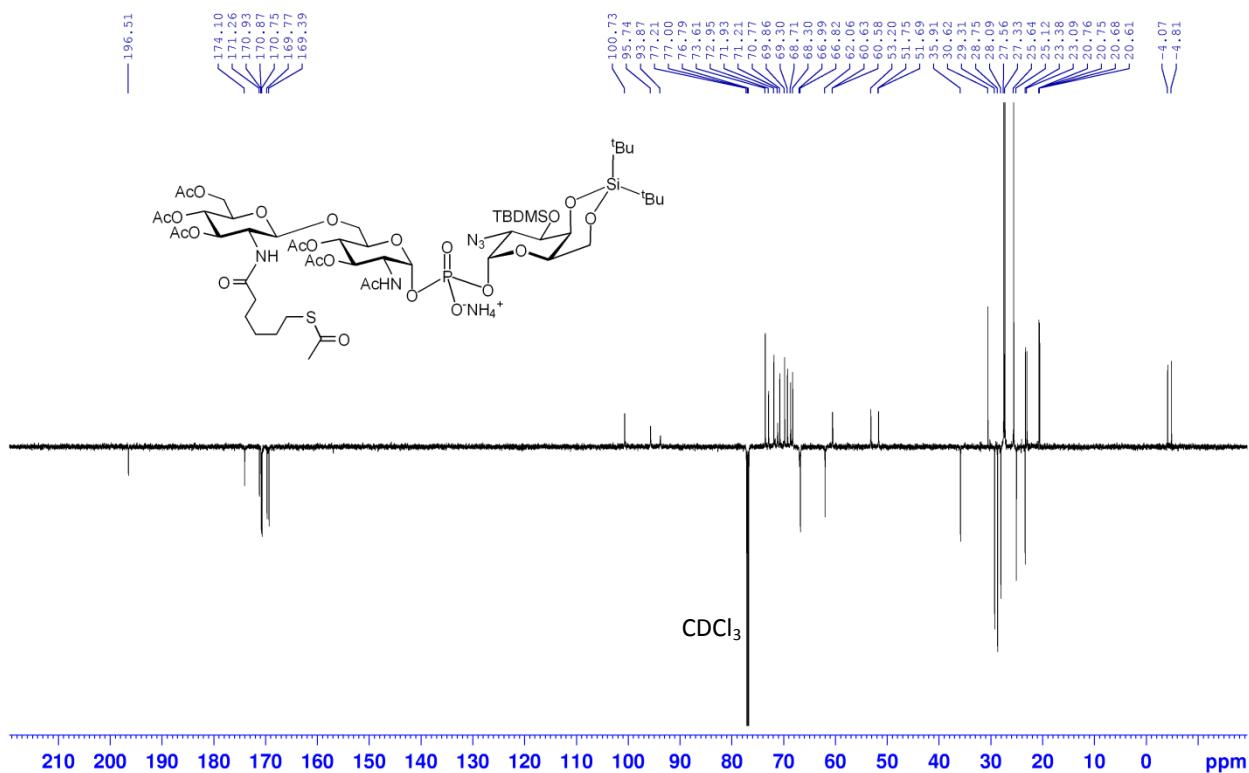
(17):  $^1\text{H}$ -NMR, 600 MHz,  $\text{CDCl}_3$



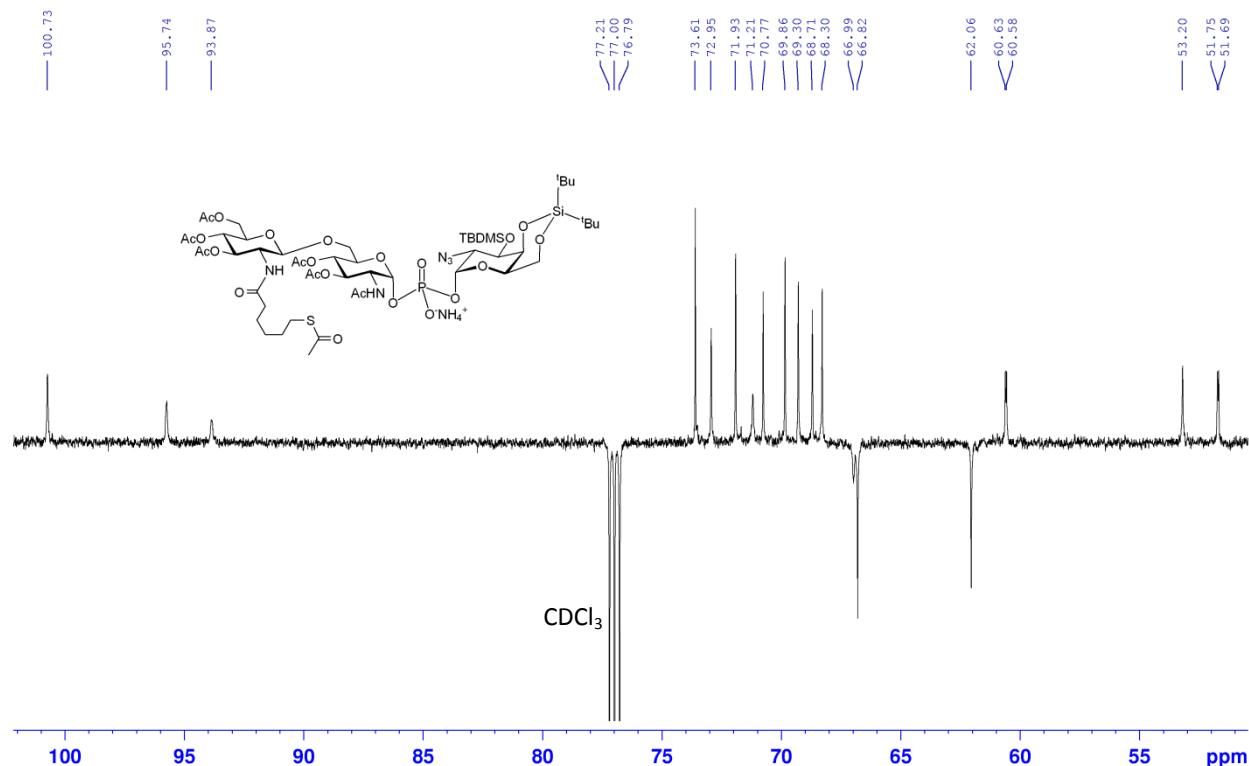
(17):  $^1\text{H}$ -NMR, 600 MHz,  $\text{CDCl}_3$ , 6.0 – 5.5 ppm



(17):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{CDCl}_3$

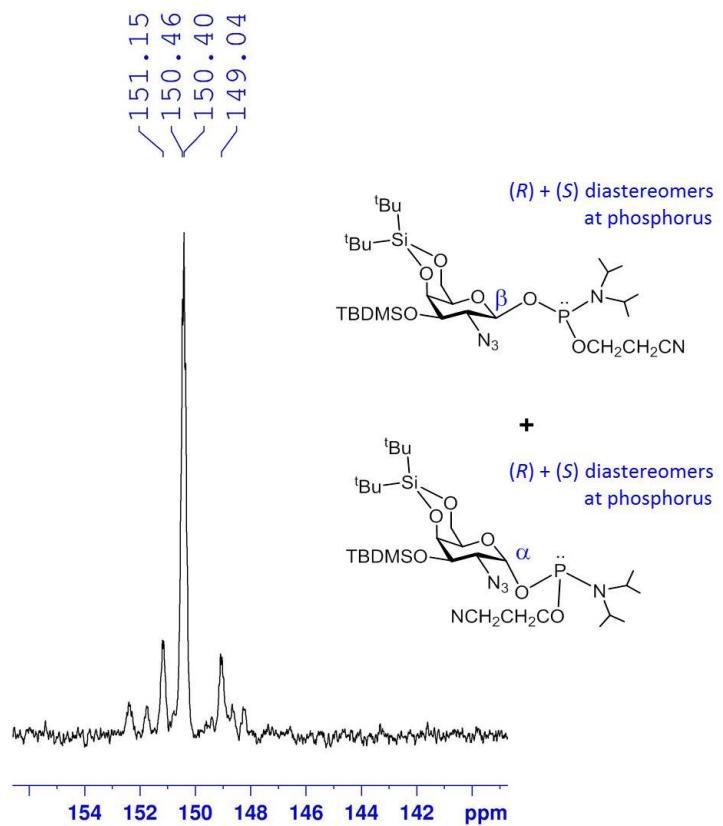
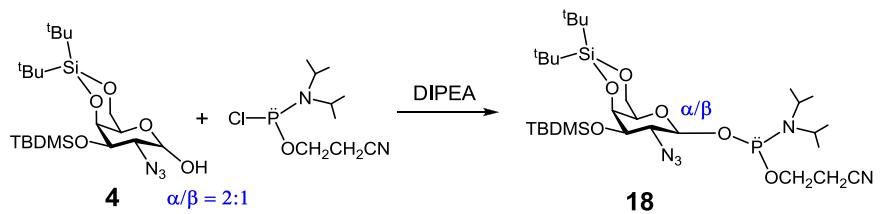


(17):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{CDCl}_3$ , 110 – 50 ppm

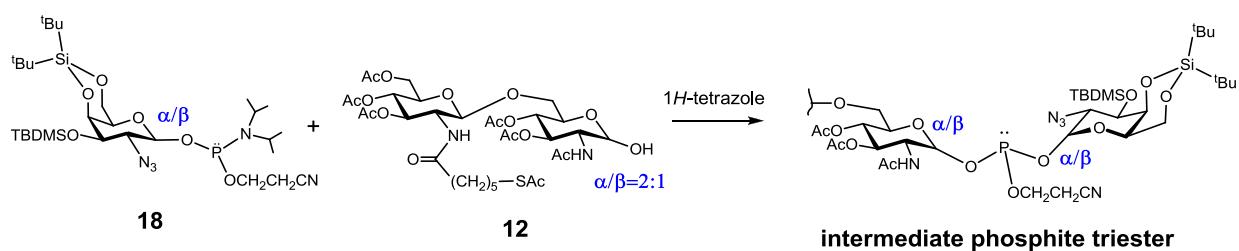


(18):  $^{31}\text{P}$ -NMR, 162 MHz,  $\text{CH}_2\text{Cl}_2 + \text{CD}_2\text{Cl}_2$

Preparation of 18 (*in situ*)

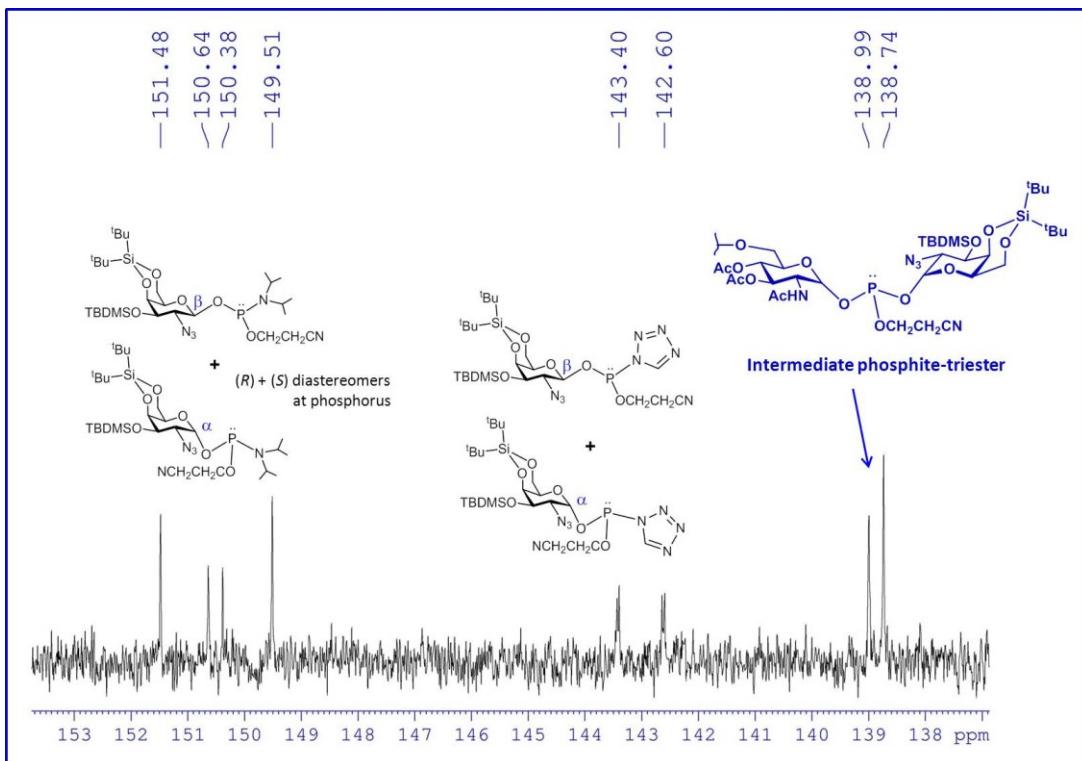
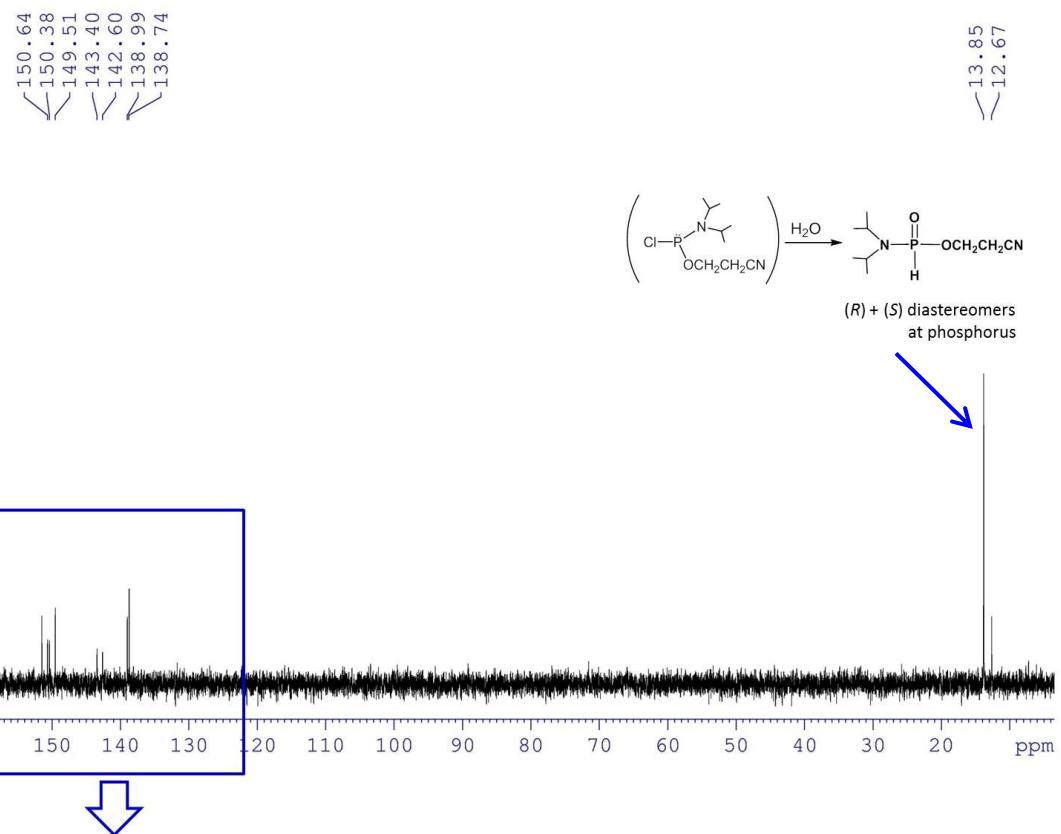


Preparation of 17 via 18 followed by  $^{31}\text{P}$ -NMR

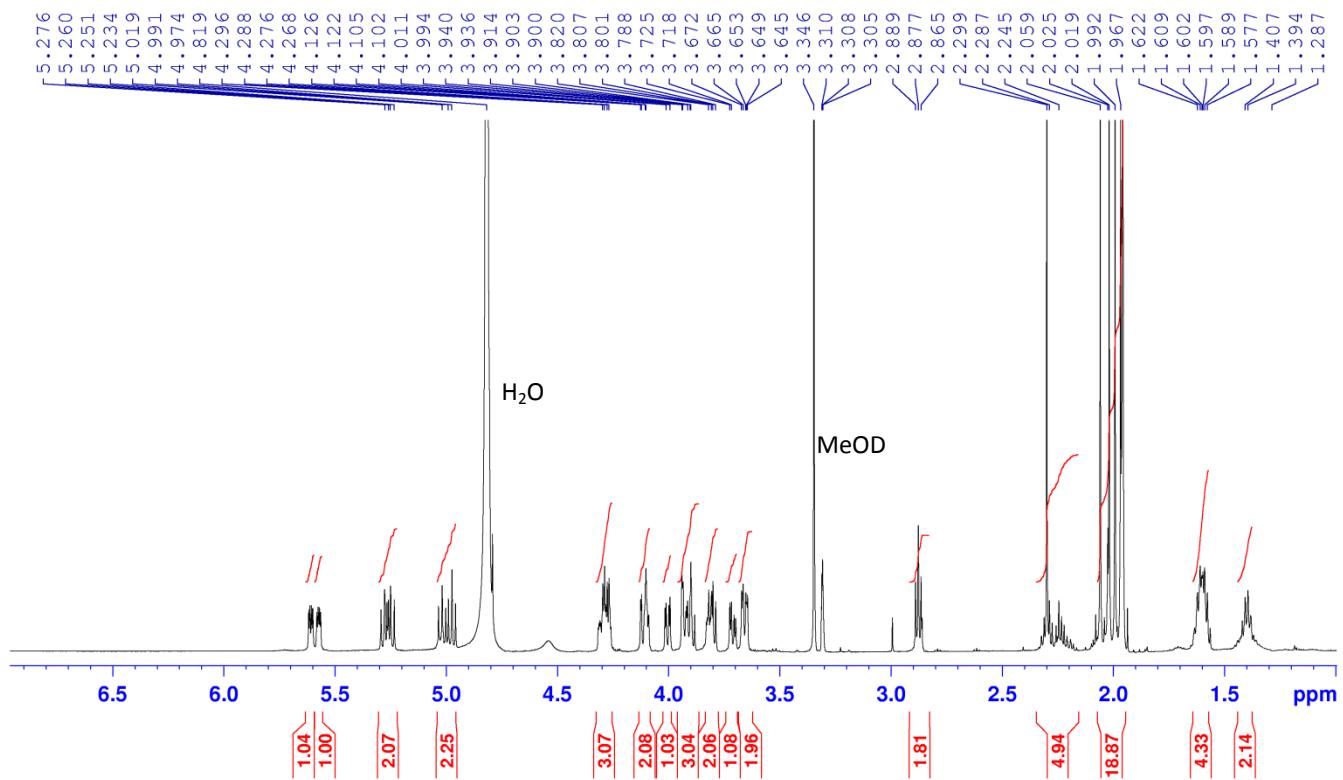


**<sup>31</sup>P-NMR**, 162 MHz, [CH<sub>2</sub>Cl<sub>2</sub> + CH<sub>3</sub>CN] + CD<sub>2</sub>Cl<sub>2</sub>

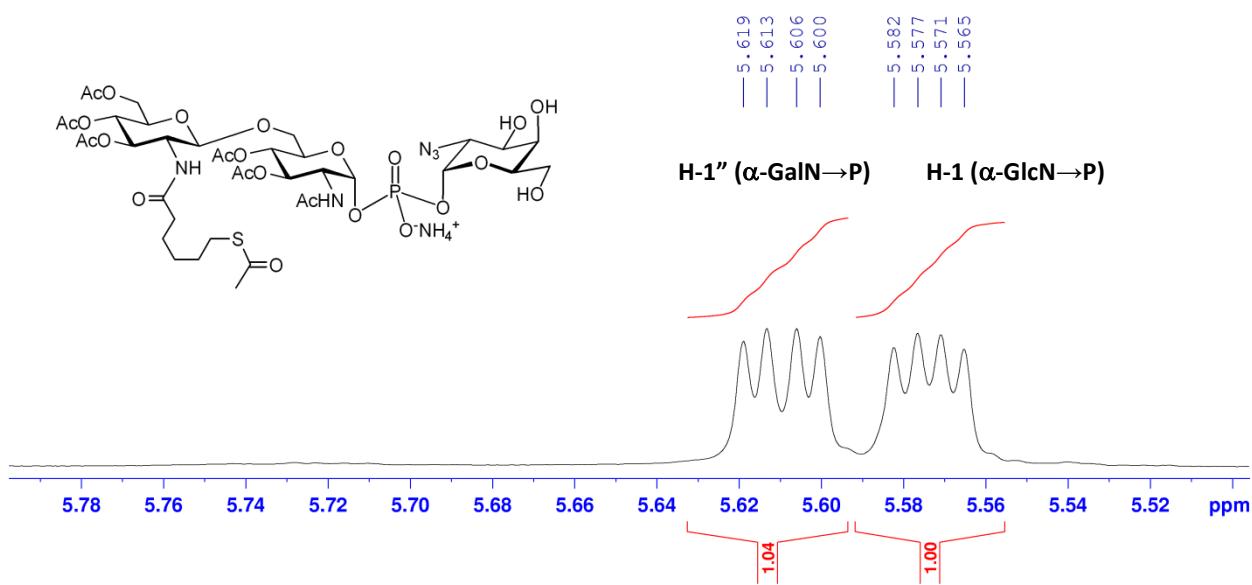
### Reaction 18 + 12 → 17 in the NMR tube



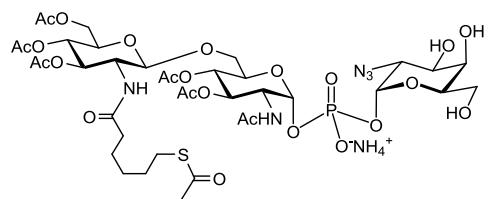
(19):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$ ,



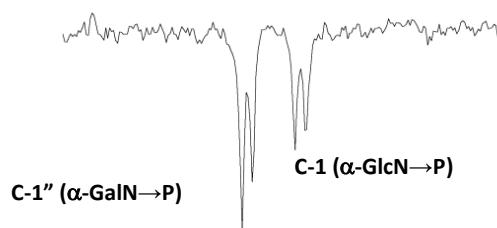
(19):  $^1\text{H-NMR}$ , 600 MHz,  $\text{CD}_3\text{OD}$ , 6.0 – 5.5 ppm



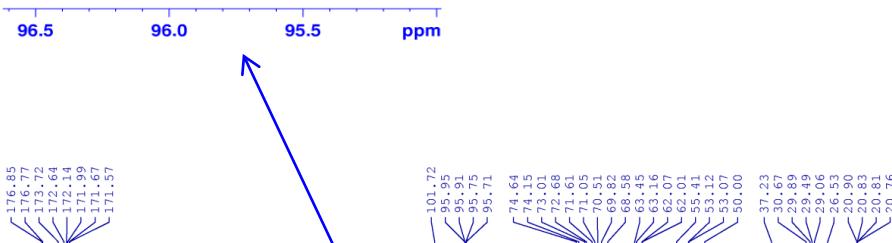
(19):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{CD}_3\text{OD}$



95.95  
95.91  
95.75  
95.71



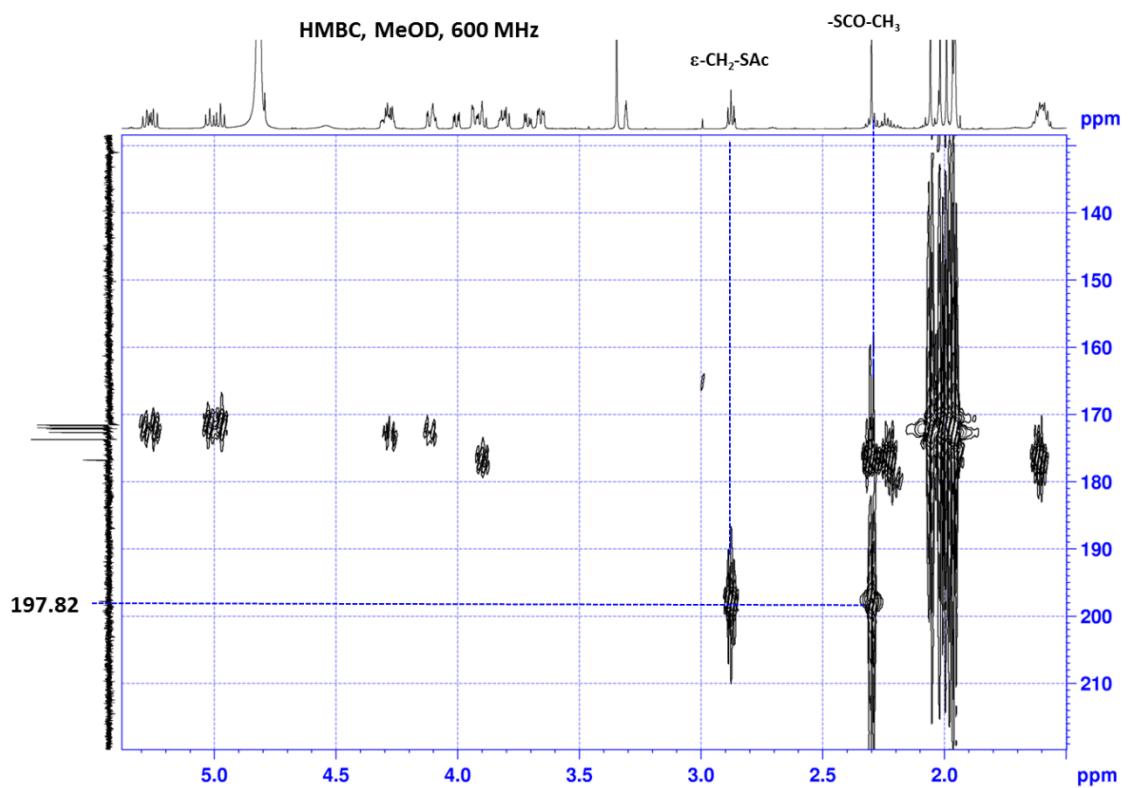
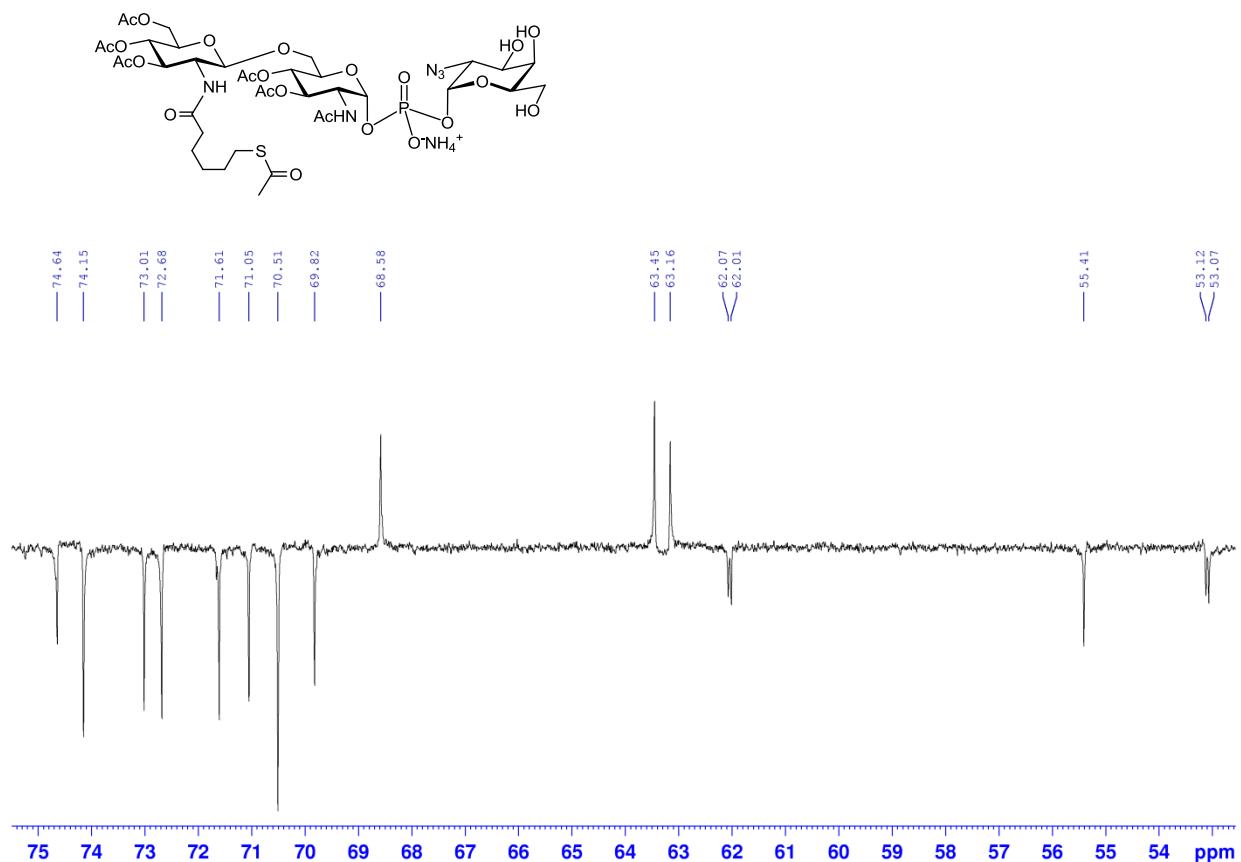
197.62



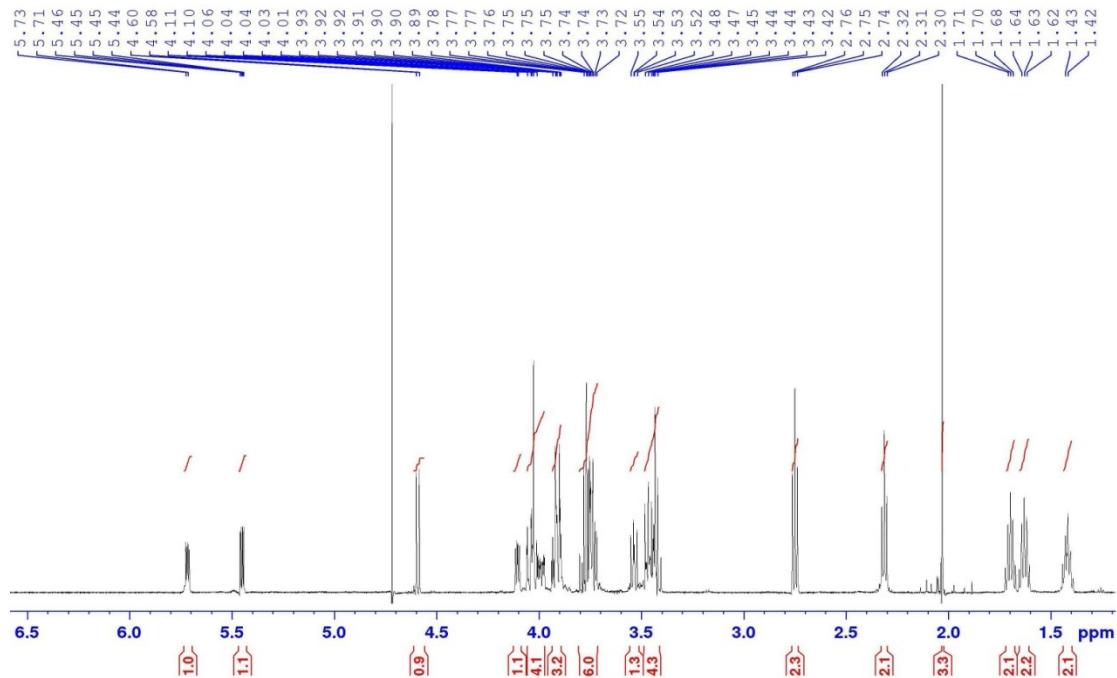
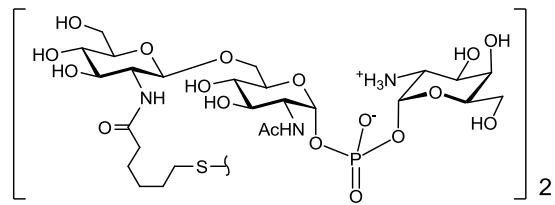
MeOD

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

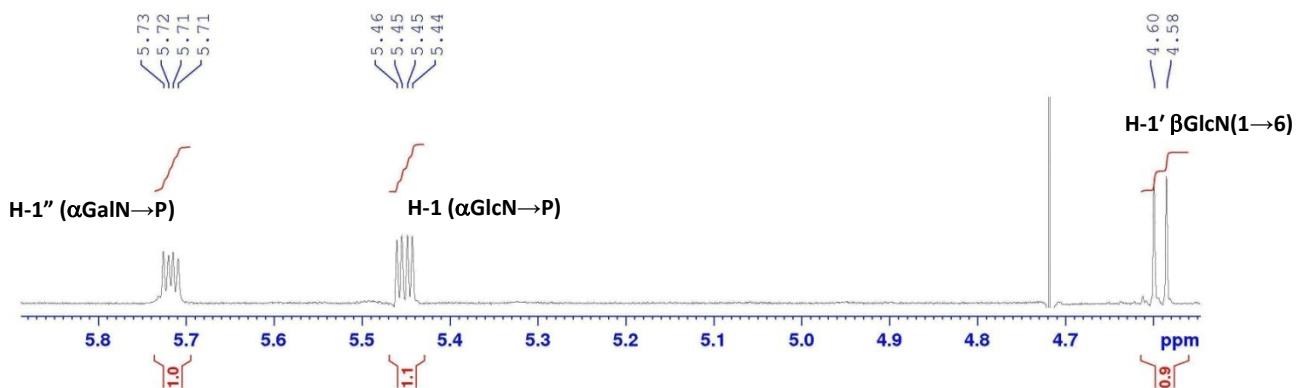
(19):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{CD}_3\text{OD}$



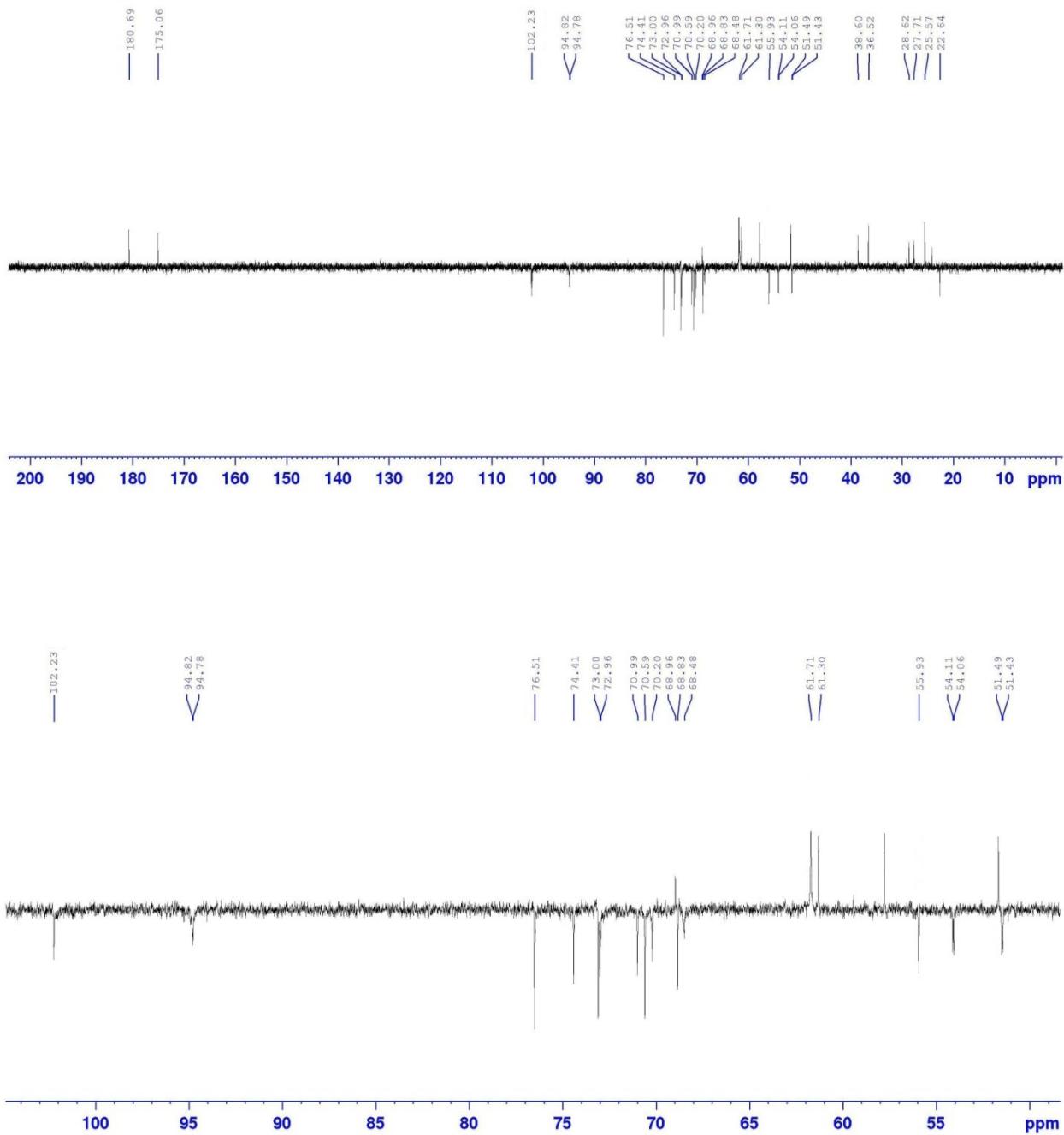
(20):  $^1\text{H}$ -NMR, 600 MHz,  $\text{D}_2\text{O}$



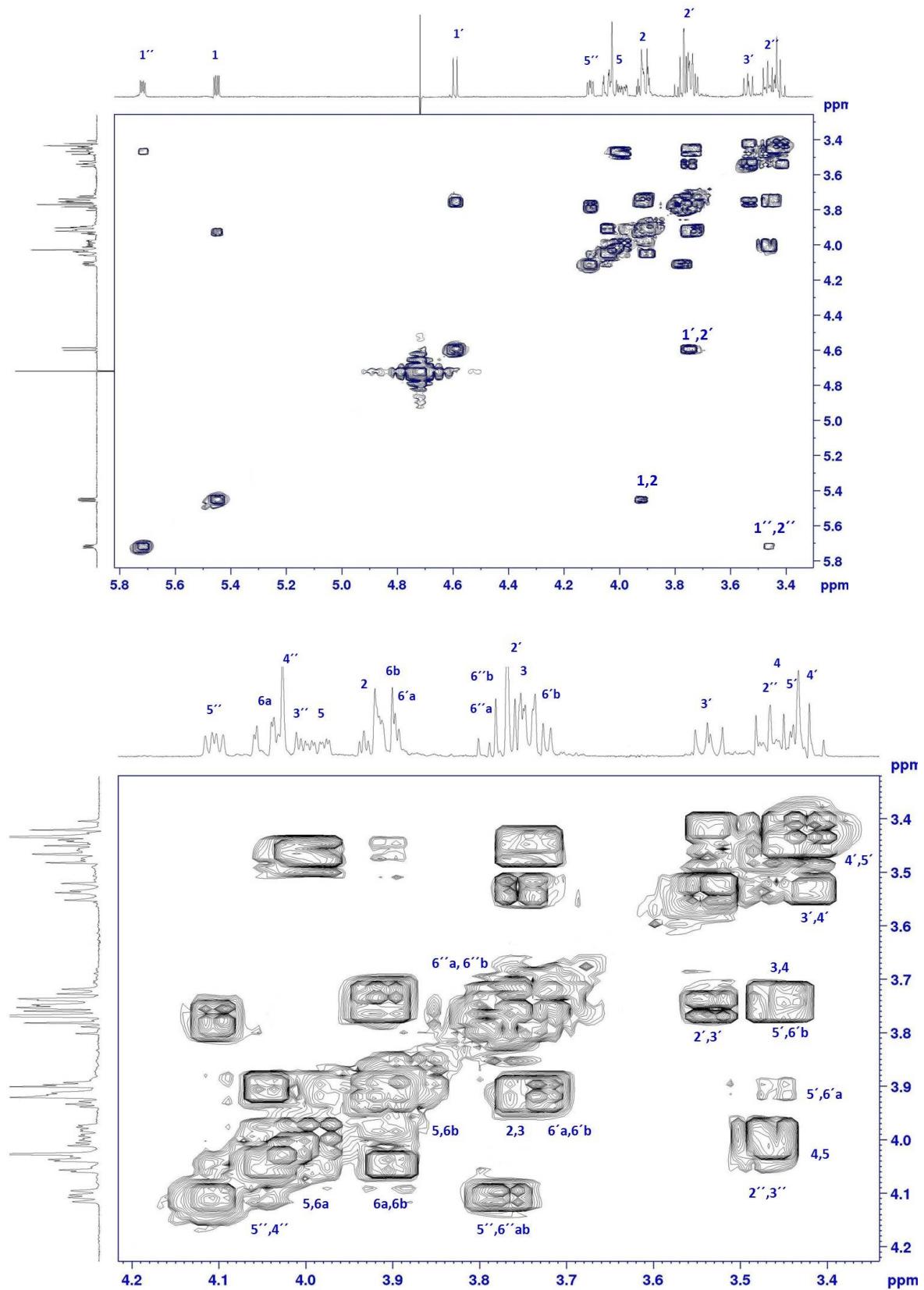
**(20):**  $^1\text{H}$ -NMR, 600 MHz,  $\text{D}_2\text{O}$ , 6.0 – 4.5 ppm



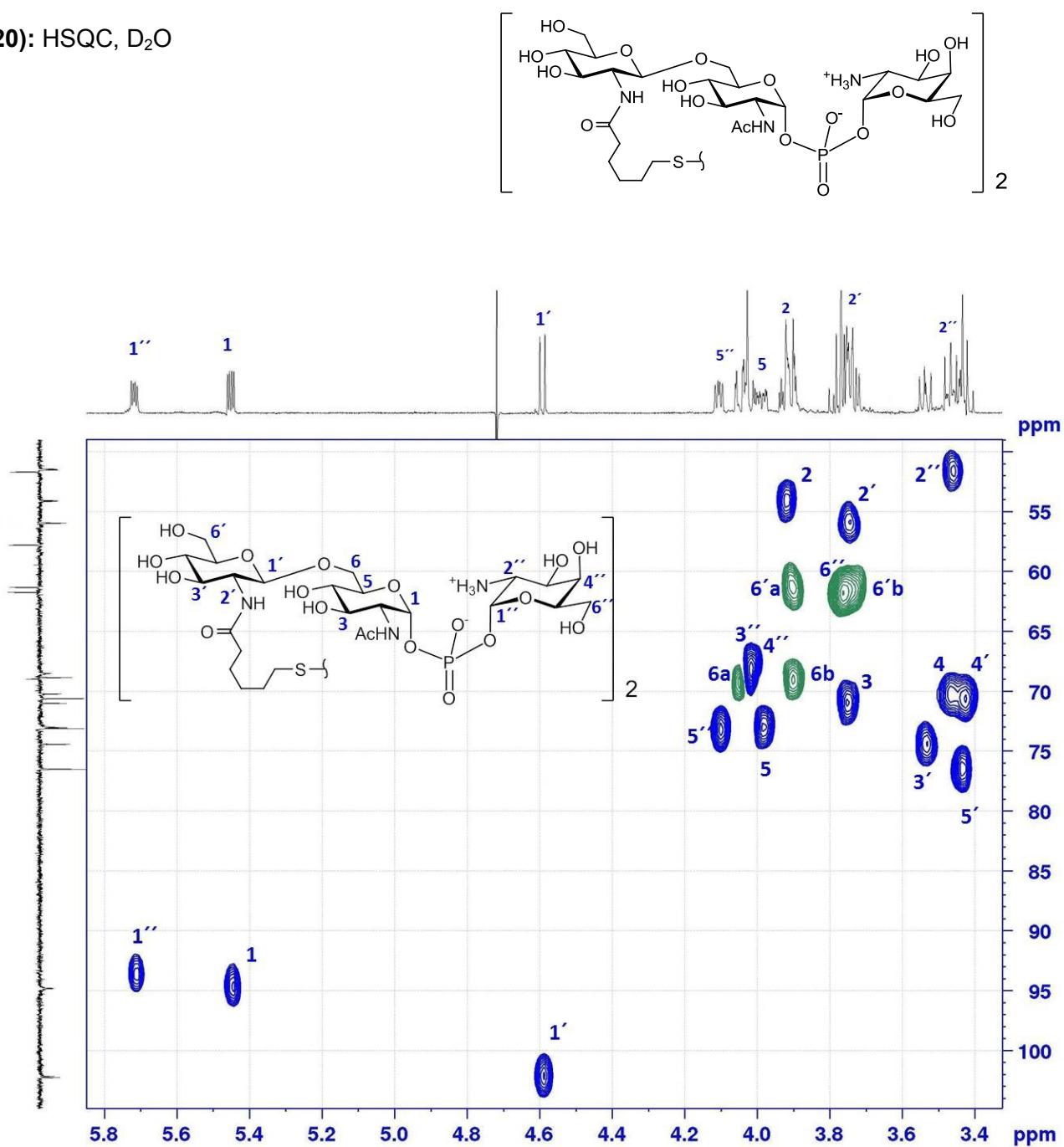
(20):  $^{13}\text{C}$ -NMR, APT, 151 MHz,  $\text{D}_2\text{O}$



(20): COSY, 600 MHz, D<sub>2</sub>O

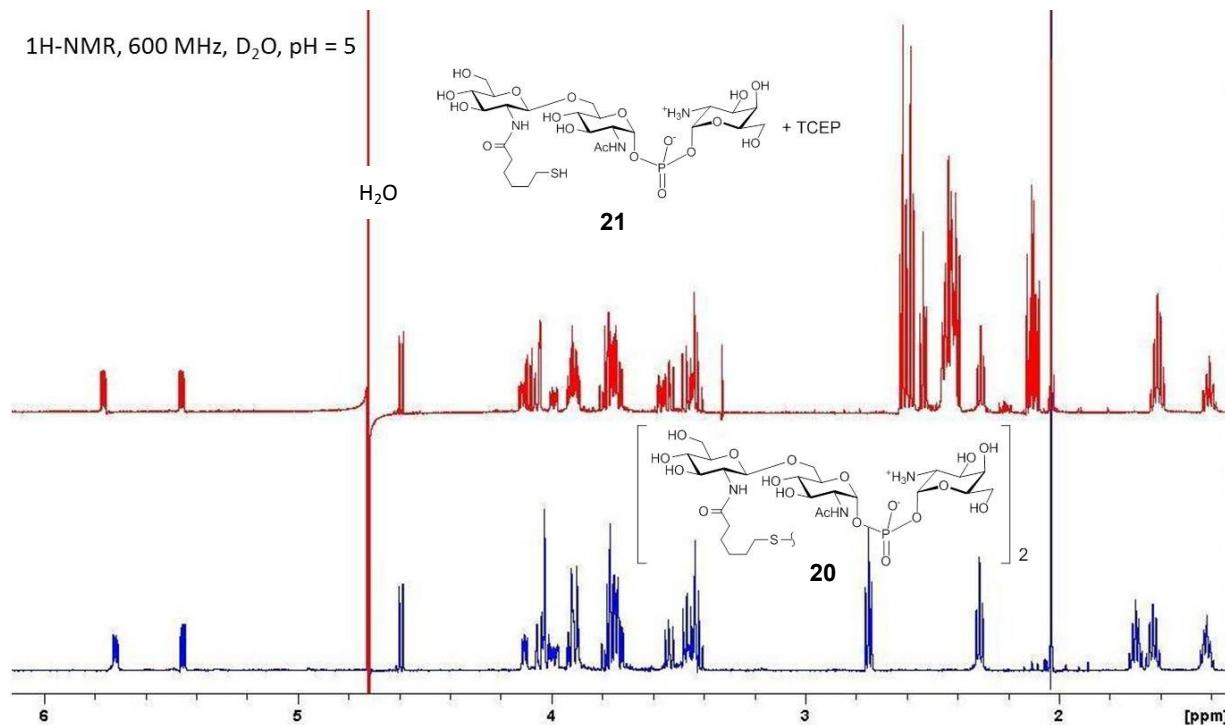


(20): HSQC, D<sub>2</sub>O

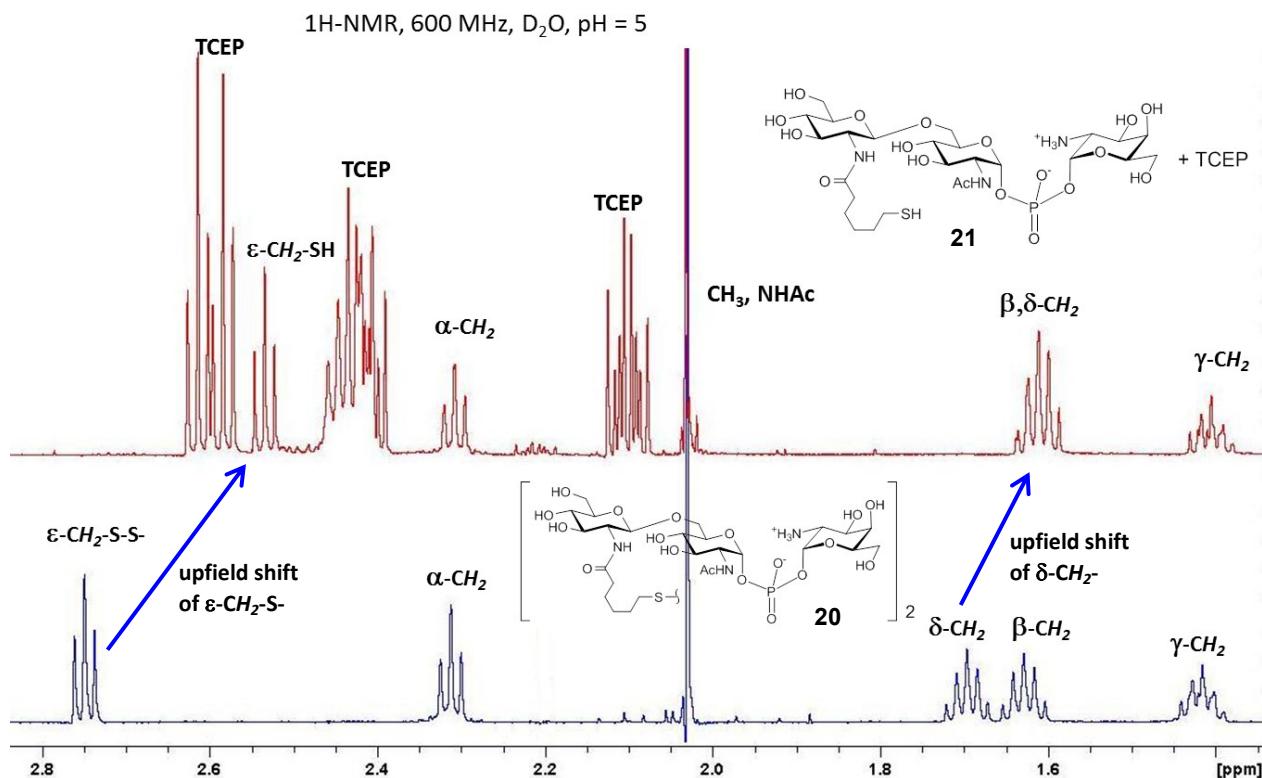


Reduction of the disulfide bond in **20** using TCEP (Tris(2-carboxyethyl)phosphine)

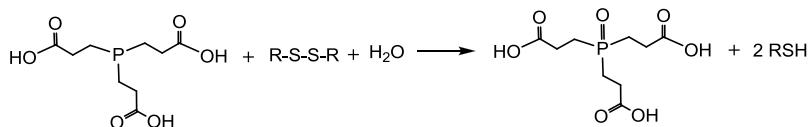
Stacked  $^1\text{H}$ -NMR spectra of **21** +TCEP vs. **20** (0 - 6 ppm)



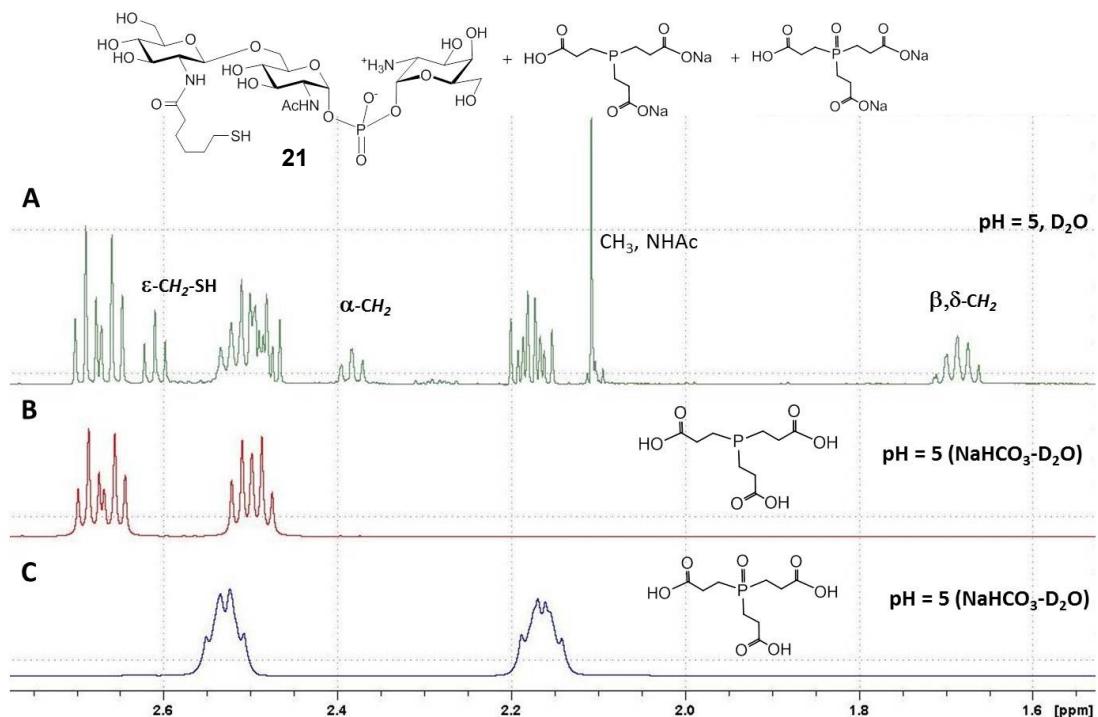
Stacked  $^1\text{H}$ -NMR spectra of **20+TCEP** ( $\epsilon$ -sulphydryl group - spacer) vs. **21** ( $\epsilon$ -disulphide) (1.2 – 2.8 ppm)



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

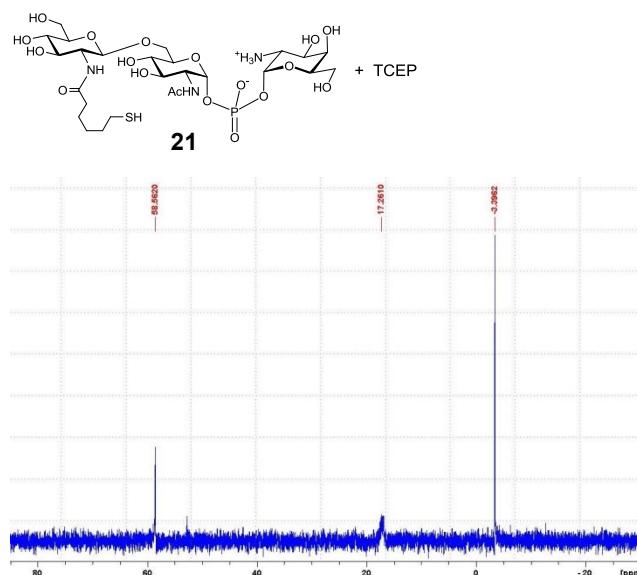


Stacked  $^1\text{H}$ -NMR spectra of (A) **21+TCEP** vs. (B) **TCEP** and (C) **TCEP oxide** (1.5 – 2.8 ppm)

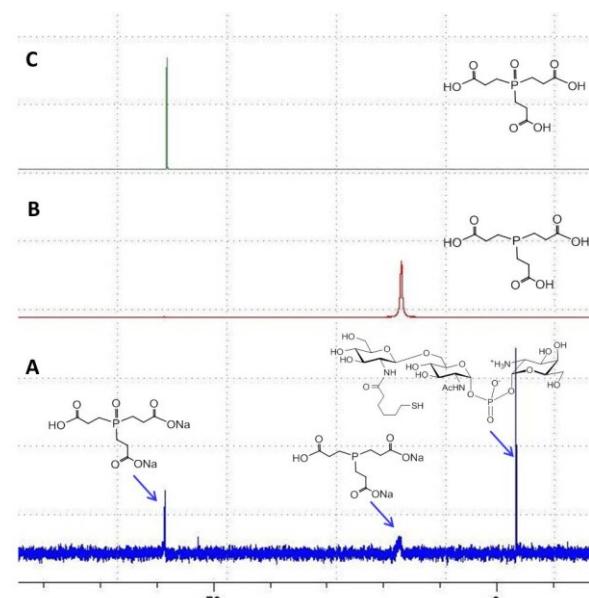


$^{31}\text{P}$ -NMR, 243 MHz,  $\text{D}_2\text{O}$  **21+TCEP**

( $\epsilon$ -sulfhydryl group - spacer)

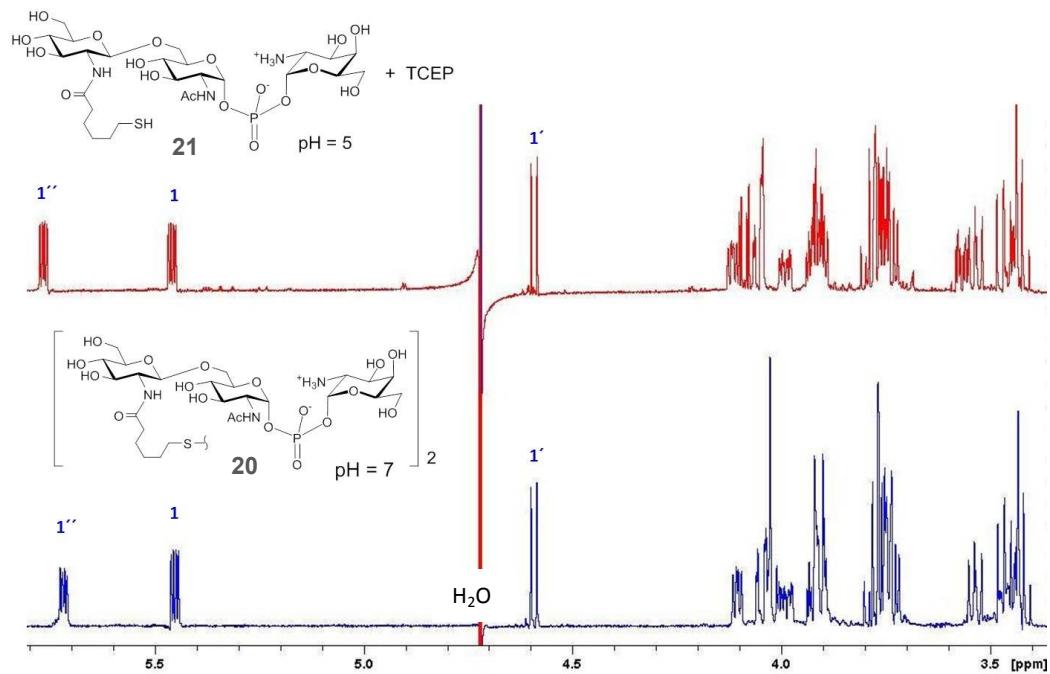


Stacked  $^{31}\text{P}$ -NMR spectra of (A) **21+TCEP** vs. (B) **TCEP** and (C) **TCEP oxide**

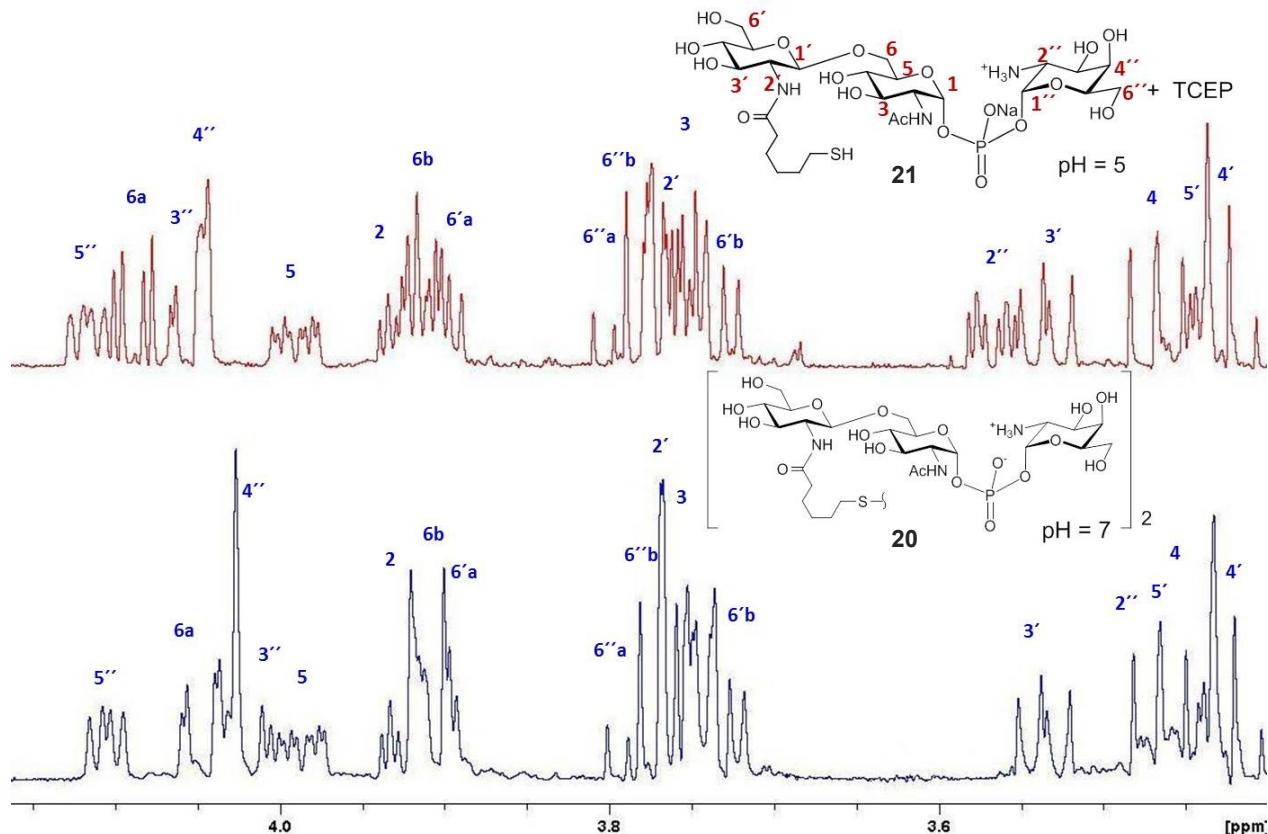


<sup>1</sup>H-NMR, 600 MHz, D<sub>2</sub>O

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

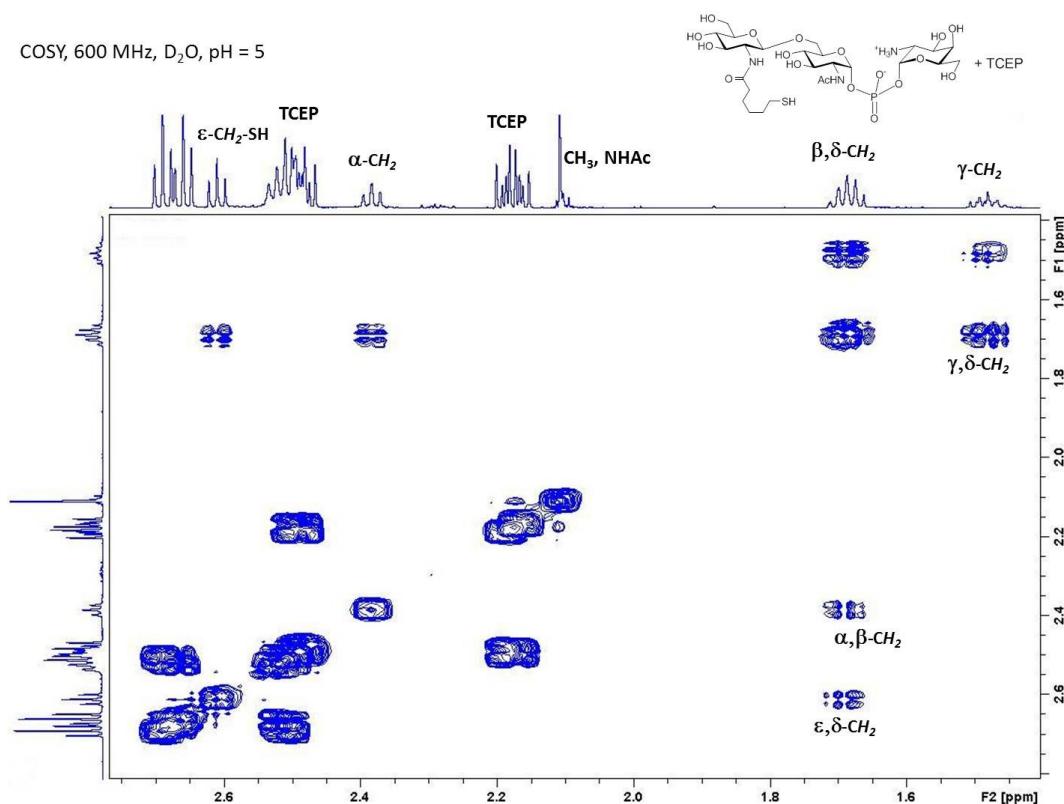


Stacked  $^1\text{H}$ -NMR spectra of **21+TCEP** ( $\varepsilon$ -sulphydryl group - spacer) vs. **20** (disulphide) (3.4 - 4.2 ppm)

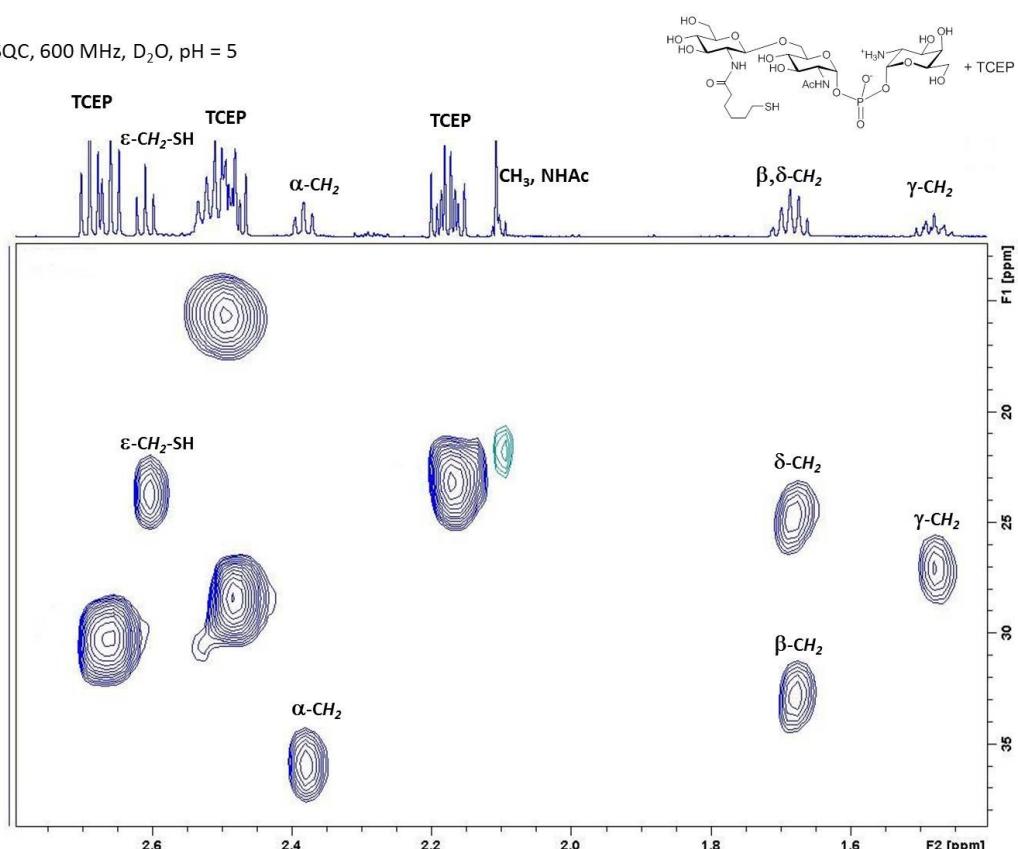


## 21+TCEP

COSY, 600 MHz,  $D_2O$ , pH = 5

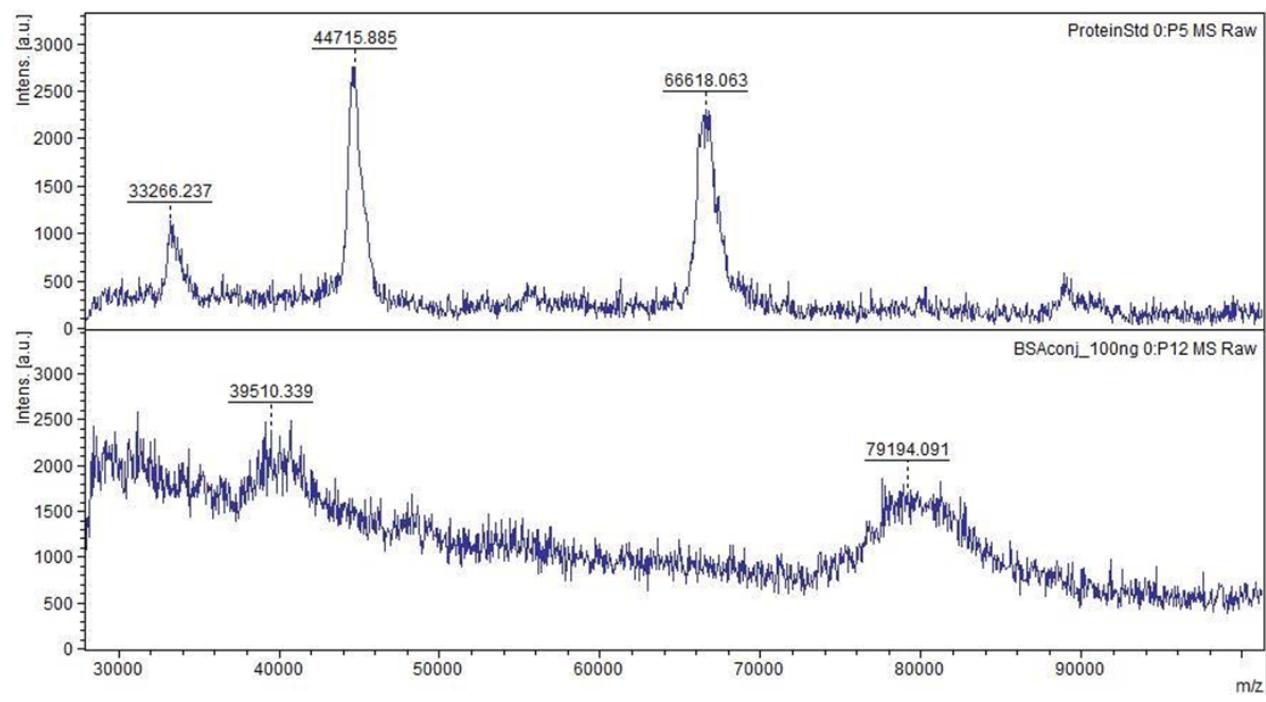


HSQC, 600 MHz,  $D_2O$ , pH = 5



## 21-BSA, MALDI-TOF

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



### 21-BSA after purification and dialysis

