

## Electronic Supplementary Information

# A traceless photocleavable linker for the automated glycan assembly of carbohydrates with free reducing ends

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## Abbreviations

AcOH: acetic acid; AcCl: acetic acid chloride; Ar: aryl; BB: building block; Bz: benzoyl; CbzCl: benzyl chloroformate; DCE: 1,2-dichloroethane; DCM: dichloromethane; DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone; DMF: dimethylformamide; DMAP: dimethylaminopyridine; EtOAc: ethyl acetate; ELSD: evaporative light scattering detector; Fmoc: fluorenylmethoxycarbonyl; hex: hexane; iPrOH: isopropanol; Lev: levinoyl; NaOMe: sodium methoxide; Nap: naphthyl, NIS: *N*-iodosuccinimide; NP: normal phase; MeCN: acetonitrile; MeOH: methanol; Ph: phenyl; Py: pyridine; RP: reversed phase; rt: room temperature; TfOH: trifluoromethanesulfonic acid; THF: tetrahydrofuran; TMSOTf: trimethylsilyl trifluoromethanesulfonate; UV: ultra violet.

## General Information

The automated syntheses were performed on a self-built synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Solvents and reagents were used as supplied without any further purification. Anhydrous solvents were taken from a dry solvent system (JC-Meyer Solvent Systems). Building blocks **8**, **13-18** were prepared as reported in the literature.<sup>[1]</sup> Column chromatography was carried out using Fluka Kieselgel 60 (230-400 mesh). NMR spectra were recorded on a Varian 400-MR (400 MHz) a Varian 600 (600 MHz) or a Bruker AVIII 700 (700 MHz) spectrometer using solutions of the respective compound in CDCl<sub>3</sub> or D<sub>2</sub>O. NMR chemical shifts ( $\delta$ ) are reported in ppm and coupling constants (*J*) in Hz. Spectra recorded in CDCl<sub>3</sub> used the solvent residual peak chemical shift as internal

standard (CDCl<sub>3</sub>: 7.26 ppm <sup>1</sup>H, 77.0 ppm <sup>13</sup>C). Spectra recorded in D<sub>2</sub>O used the solvent residual peak chemical shift as internal standard in <sup>1</sup>H NMR (D<sub>2</sub>O: 4.79 ppm <sup>1</sup>H). Optical rotations were measured using a UniPol L1000 polarimeter (Schmidt&Haensch) with concentrations expressed as g/100 mL. IR spectra were recorded on a Spectrum 100 FTIR spectrophotometer (Perkin-Elmer). High resolution mass spectra were obtained using a 6210 ESI-TOF mass spectrometer (Agilent). MALDI spectra were measured using a Bruker autoflex speed MALDI-TOF mass spectrometer. Analytical HPLC was performed on an Agilent 1200 series coupled to a quadrupole ESI LC/MS 6130 using a YMC-Diol-300-NP column (150 x 4.6 mm), a Phenomenex Luna C5 column (250 x 4.6 mm), or a Thermo Scientific Hypercarb column (150 x 4.6 mm). Preparative HPLC was performed on an Agilent 1200 series using a preparative YMC-Diol-300-NP column (150 x 20 mm), a semi-preparative Phenomenex Luna C5 column (250 x 10 mm) or a semi-preparative Thermo Scientific Hypercarb column (150 x 10 mm).

### **Synthesizer Modules and Conditions**

The linker-functionalized resin **2** (15.1 - 44.2 μmol of hydroxyl groups) was placed in the reaction vessel and swollen for at least 30 min in DCM. Before every synthesis the resin was washed with DMF, THF and DCM. Subsequently the glycosylation (module **A** and **B**) and deprotection (module **C**, **D** and **E**) steps were performed. Mixing of the components was accomplished by bubbling Argon through the reaction mixture.

#### **Module A: Glycosylation with Glycosyl Phosphates**

The resin (15.1 - 44.2 μmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 or -35 °C. Prior to the glycosylation reaction the resin was washed with TMSOTf in DCM and then DCM only. For the glycosylation reaction the DCM was drained and a solution of phosphate BB (1.8 – 4.0 equiv in 1 mL DCM) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of TMSOTf in DCM (1.8 – 4.0 equiv in 1 mL DCM). The glycosylation was performed for 5 min at -35 °C and then at -15 or -20 °C for 30 minutes. Subsequently the solution was drained and the resin was washed three times with DCM. The whole procedure was eventually performed twice to improve conversion of the acceptor sites. Afterwards the resin was washed three times with DCM at 25 °C.

*Activator solution: 62.5 mM solution of TMSOTf in dry DCM.*

Action	Cycles	Solvent	Reagent 1	Reagent 2	T (°C)	Incubation Time
Wash	1	DCM	TMSOTf		-30	2 min
Wash	1	DCM			-30	25 s
Glycosylation	2	DCM	BB (62.5 μmol)	TMSOTf (62.5 μmol)	-35	5 min
					-15/-20	30 min
Wash	3	DCM			-15/-20	15 s
Wash	3	DCM			25	15 s

## Module B: Glycosylation with Thioglycosides

The resin (15.1 - 44.2  $\mu\text{mol}$  of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to  $-30\text{ }^{\circ}\text{C}$ . Prior to the glycosylation reaction the resin was washed with TMSOTf in DCM and DCM. For the glycosylation reaction the DCM was drained and a solution of thioglycoside BB (1.8 – 4.0 equiv in 1 mL DCM) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of NIS (2.22 or 4.44 equiv based on resin loading) and TfOH (0.44 equiv) in DCM/dioxane (2:1). The glycosylation was performed for 5 min at  $-40\text{ }^{\circ}\text{C}$  and then for 40 min at  $-20\text{ }^{\circ}\text{C}$ . Subsequently the solution was drained and the resin was washed with DCM. The whole procedure was repeated once to ensure full conversion of all acceptor sites. Afterwards the resin was washed three times with DCM at  $25\text{ }^{\circ}\text{C}$ .

*Activator solution: solution of NIS (75 mM) and TfOH (7.5 mM) in DCM/dioxane.*

Action	Cycles	Solvent	Reagent 1	Reagent 2	T ( $^{\circ}\text{C}$ )	Incubation Time
Wash	1	DCM	TMSOTf		-30	2 min
Wash	1	DCM			-30	25 s
Glycosylation	1	DCM	BB (62.5 $\mu\text{mol}$ )	NIS (75 $\mu\text{mol}$ ) TfOH (7.5 $\mu\text{mol}$ )	-40	5 min
					-20	40 min
Wash	3	DCM			-15	15 s
Glycosylation	1	DCM	BB (62.5 $\mu\text{mol}$ )	NIS (75 $\mu\text{mol}$ ) TfOH (7.5 $\mu\text{mol}$ )	-40	5 min
					-20	40 min
Wash	3	DCM			-15	15 s
Wash	3	DCM			25	15 s

## Module C: Fmoc Deprotection

The resin was washed with DMF, swollen in 2 mL DMF and the temperature of the reaction vessel was adjusted to  $25\text{ }^{\circ}\text{C}$ . Prior to the deprotection step the DMF was drained and the resin was washed with DMF three times. For Fmoc deprotection 2 mL of a solution of 20%  $\text{Et}_3\text{N}$  in DMF was delivered to the reaction vessel. After 5 min the solution was drained and the whole procedure was repeated another two times. After Fmoc deprotection was complete the resin was washed with DMF, THF and DCM.

*Deprotection solution: 20% Et<sub>3</sub>N in dry DMF*

Action	Cycles	Solvent	Reagent	T (°C)	Incubation Time
Wash	3	DMF		25	15 s
Deprotection	3	DMF	Et <sub>3</sub> N	25	5 min
Wash	3	DMF		25	15 s
Wash	3	THF		25	15 s
Wash	3	DCM		25	15 s

#### **Module D: Lev Deprotection**

The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 1.3 mL DCM remained in the reaction vessel and 0.8 mL of a solution of 0.15 M hydrazine acetate in Py/AcOH/H<sub>2</sub>O (4:1:0.25) was delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After Lev deprotection was complete the resin was washed with DMF, THF and DCM.

*Deprotection solution: 0.15 M hydrazine acetate solution in Py/AcOH/H<sub>2</sub>O (4:1:0.25).*

Action	Cycles	Solvent	Reagent	Temperature	Incubation Time
Wash	3	DCM		25 °C	15 s
Deprotection	3	Py/AcOH/H <sub>2</sub> O (4:1:0.25)	hydrazine acetate	25°C	30 min
Wash	3	DMF		25 °C	15 s
Wash	3	THF		25 °C	15 s
Wash	3	DCM		25 °C	15 s

## Module E: Nap Deprotection

The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 40 °C. For Nap deprotection the DCM was drained and 1.5 mL of a solution of 0.1 M DDQ solution in DCE/MeOH/H<sub>2</sub>O (64:16:1) was delivered to the reaction vessel. After 20 min the reaction solution was drained and the whole procedure was repeated another six times. After Nap deprotection was complete the resin was washed with DMF, THF and DCM.

*Deprotection solution: 0.1 M DDQ solution in DCE/MeOH/H<sub>2</sub>O (64:16:1).*

Action	Cycles	Solvent	Reagent	Temperature	Incubation Time
Wash	3	DCM		25 °C	15 s
Deprotection	7	DCE/MeOH/H <sub>2</sub> O (64:16:1)	DDQ	40 °C	20 min
Wash	3	DMF		25 °C	15 s
Wash	3	THF		25 °C	15 s
Wash	3	DCM		25 °C	15 s

## Module F: Benzoylation

The temperature was adjusted to 25 °C and the resin washed with pyridine (2 mL) three times. For benzoylation the temperature was set to 40 °C and 2 mL pyridine and 1 mL of a solution containing 0.5 M benzoic anhydride and 0.25 M DMAP in DCE were delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After capping was complete the resin was washed with DCM.

*Benzoylation solution: solution of benzoic anhydride (0.5 M) and DMAP (0.25 M) in anhydrous DCE*

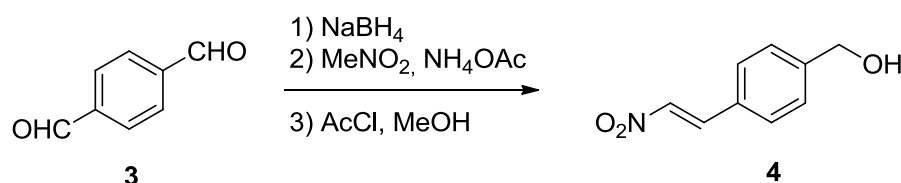
Action	Cycles	Solvent	Reagent	Temperature	Incubation Time
Wash	3	pyridine		25 °C	15 s
Benzoylation	3	pyridine	Bz <sub>2</sub> O (0.5 mmol) DMAP (0.25 mmol)	40 °C	30 min
Wash	3	DCM		25 °C	15 s

## Cleavage from the solid support

After assembly of the oligosaccharides cleavage from the solid support was accomplished using a continuous-flow photoreactor as described previously.<sup>[1a]</sup>

## Synthesis of the Traceless Photocleavable Linker

### (4-(2-Nitrovinyl)phenyl)methanol (**4**)<sup>[2]</sup>

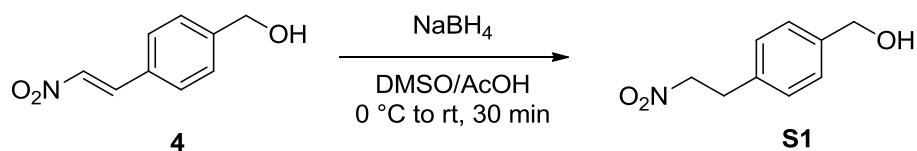


To a cooled (0 °C) solution of terephthalaldehyde (10.6 g, 79.0 mmol) in a mixture of non-anhydrous EtOH (100 mL) and THF (150 mL) was added NaBH<sub>4</sub> (0.900 g, 23.7 mmol) in one portion. The reaction mixture was allowed to warm up to 15 °C in a period of 3 h and quenched by the addition of 50 mL aq. HCl (1 M). After addition of sat. aq. NaCl-solution (200 mL) the mixture was extracted with Et<sub>2</sub>O twice. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*.

To the crude product (11.3 g, max. 79.0 mmol) in AcOH (90 mL) was added NH<sub>4</sub>OAc (17.0 g, 220 mmol) and MeNO<sub>2</sub> (20.0 mL, 375 mmol). The reaction mixture was gently refluxed at 95 °C for 5 h and cooled down to rt. After evaporation of AcOH and addition of EtOAc the organic phase was washed twice with sat. aq. NaCl-solution. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. After one time co-evaporation with toluene the brown residue was filtered through a short plug of silica gel (EtOAc/hex = 1:1) to give **4** (10.9 g) as a yellow solid.

The obtained product (10.9 g, max. 55.0 mmol) was dissolved in MeOH (55 mL) and AcCl (0.600 mL, 8.40 mmol) was added. After 3 hours the solvent was evaporated and (4-(2-nitrovinyl)phenyl)methanol (9.90 g, 55.2 mmol) was obtained as a fine yellow solid.

### (4-(2-Nitroethyl)phenyl)methanol (**S1**)

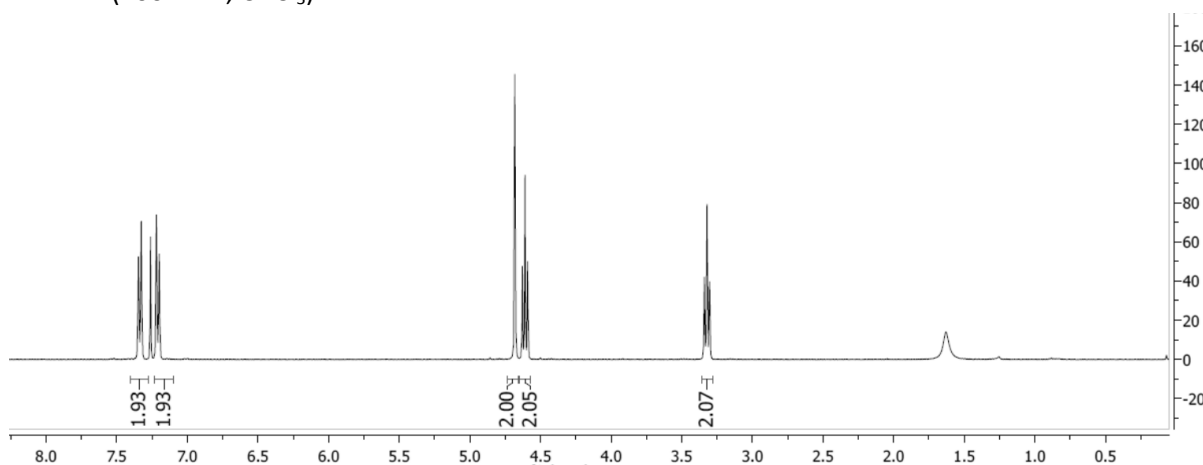


(4-(2-Nitrovinyl)phenyl)methanol (11.0 g, max. 61.4 mmol) was dissolved in 100 mL DMSO and 10 mL AcOH and cooled to 0 °C. Then NaBH<sub>4</sub> (3.80 g, 100 mmol) was added portionwise. The flask was lifted slightly above the cooling bath and reinserted to roughly maintain rt during addition. After 30 min the reaction mixture was cooled again to 0 °C and 100 mL EtOAc and 100 ml water were slowly and carefully added portionwise. After gas evolution has stopped the phases were separated and the aq. phase was extracted twice with EtOAc (2 x 200 mL). The organic layer was washed with sat. aq. NaCl-solution, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. Filtration of the crude product through a plug of Al<sub>2</sub>O<sub>3</sub> (EtOAc/hex = 1:1 -> EtOAc) gave **S1** (7.10 g, 64%) as a colorless solid.

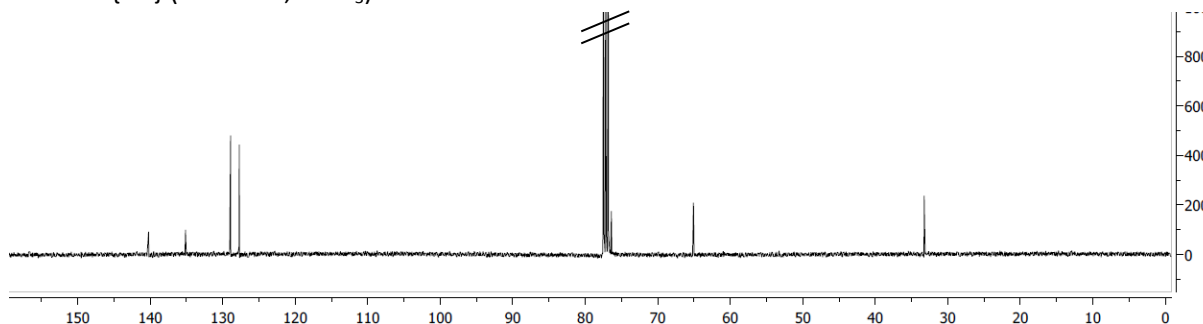
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.34 (d, *J* = 7.8 Hz, 2H, ArH), 7.21 (d, *J* = 7.9 Hz, 2H, ArH), 4.68 (s, 2H, CH<sub>2</sub>-OH), 4.61 (t, *J* = 7.3 Hz, 2H, CH<sub>2</sub>-NO<sub>2</sub>), 3.32 (t, *J* = 7.3 Hz, 2H, CH<sub>2</sub>-Ar) ppm. <sup>13</sup>C NMR{<sup>1</sup>H} (101 MHz, CDCl<sub>3</sub>): δ = 140.2, 135.1, 128.9, 127.7 (4C, Ar), 76.4 (CH<sub>2</sub>-NO<sub>2</sub>), 65.1 (CH<sub>2</sub>-OH), 33.3 (CH<sub>2</sub>-Ar) ppm. ESI-

HRMS:  $m/z$   $[M+Na]^+$  calcd. for  $C_9H_{11}NO_3$ : 204.0637; found: 204.0635. IR (neat)  $\nu_{max}$ : 726, 905, 1379, 1552  $cm^{-1}$ .

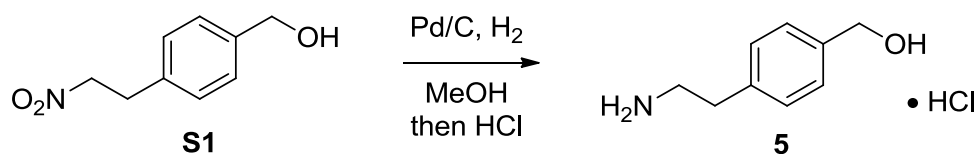
$^1H$  NMR (400 MHz,  $CDCl_3$ ):



$^{13}C$  NMR{ $^1H$ } (101 MHz,  $CDCl_3$ ):



#### (4-(2-Aminoethyl)phenyl)methanol hydrochloride (**5**)

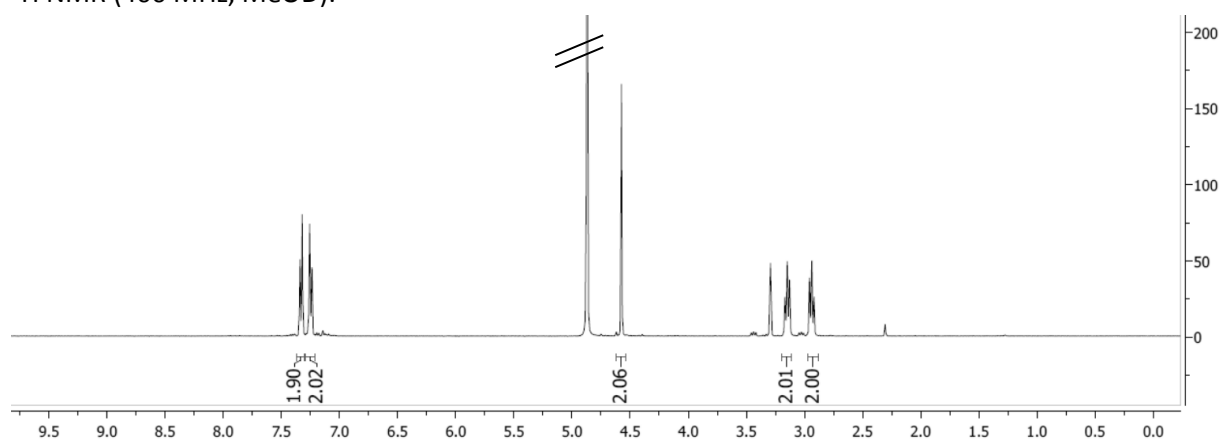


(4-(2-Nitroethyl)phenyl)methanol (6.60 g, 36.4 mmol) was dissolved in MeOH (100 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 330 mg). The suspension was flushed with Ar for 10 min then saturated with  $H_2$  for 20 min and stirred under a  $H_2$ -atmosphere for 8 h. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the crude product isolated as a colorless oil (5.20 g). 5.00 g of the crude product were dissolved in 20 ml iPrOH and 100 mL  $Et_2O$ . After cooling the solution to 0 °C conc. aq. HCl (37%, 5.3 mL) was added. After 10 min more  $Et_2O$  was added and the colorless precipitate was filtered off. After washing the solid with  $Et_2O$  (4-(2-aminoethyl)phenyl)methanol hydrochloride **5** (5.40 g, 28.8 mmol, 82%) was isolated as a beige solid.

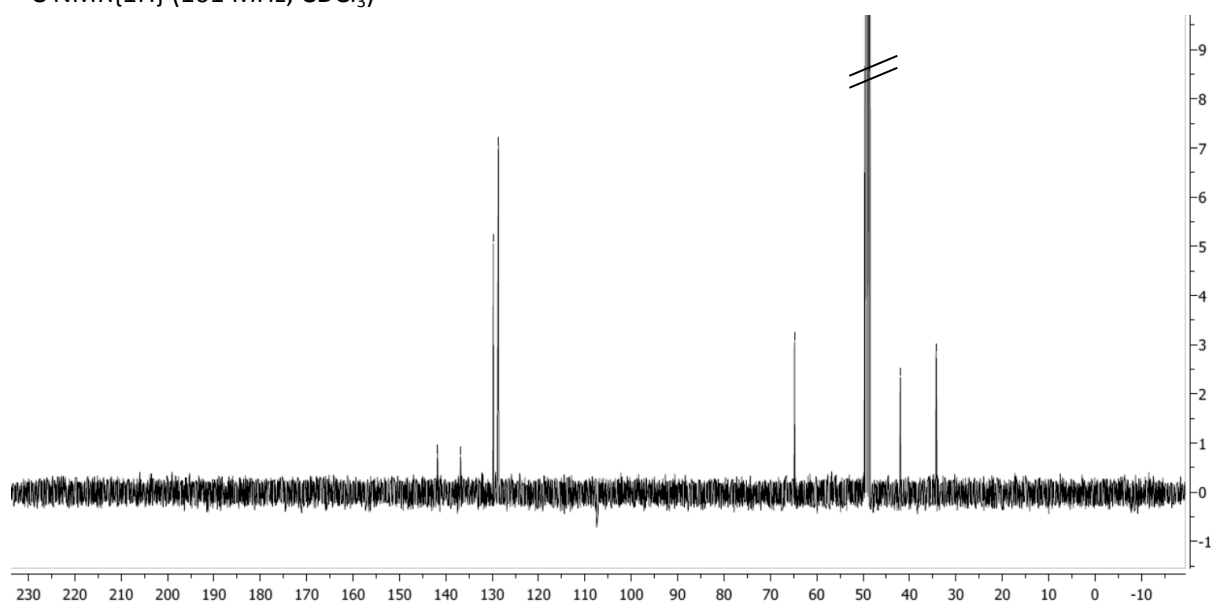
$^1H$  NMR (400 MHz, MeOD):  $\delta$  = 7.33 (d,  $J$  = 7.8 Hz, 2H, Ar), 7.24 (d,  $J$  = 7.9 Hz, 2H, Ar), 4.57 (s, 2H,  $CH_2$ -OH), 3.14 (t,  $J$  = 7.8 Hz, 2H,  $CH_2$ - $NH_2$ ), 2.93 (t,  $J$  = 7.8 Hz, 2H,  $CH_2$ -Ar) ppm.  $^{13}C$  NMR{ $^1H$ } (101 MHz, MeOD):  $\delta$  = 141.8, 136.8, 129.8, 128.6 (6C, Ar), 64.8 ( $CH_2$ OH), 42.0 ( $CH_2$ - $NH_2$ ), 34.3 ( $CH_2$ -Ar) ppm. ESI-

HRMS:  $m/z$   $[M+H-HCl]^+$  calcd. for  $C_9H_{14}NO$ : 152.1075; found: 152.1076. IR (neat)  $\nu_{max}$ : 832, 1013, 1464, 1603  $cm^{-1}$ .

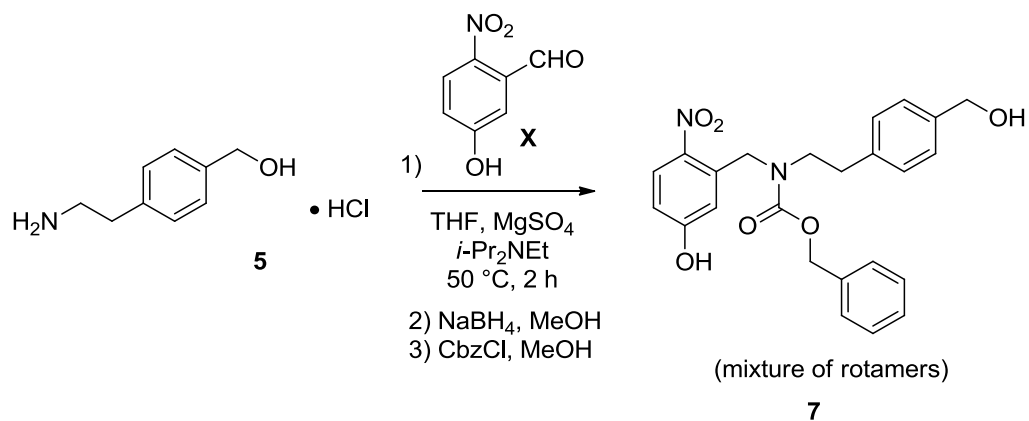
$^1H$  NMR (400 MHz, MeOD):



$^{13}C$  NMR{ $^1H$ } (101 MHz,  $CDCl_3$ )



### Benzyl 5-hydroxy-2-nitrobenzyl(4-(hydroxymethyl)phenethyl)carbamate (7)

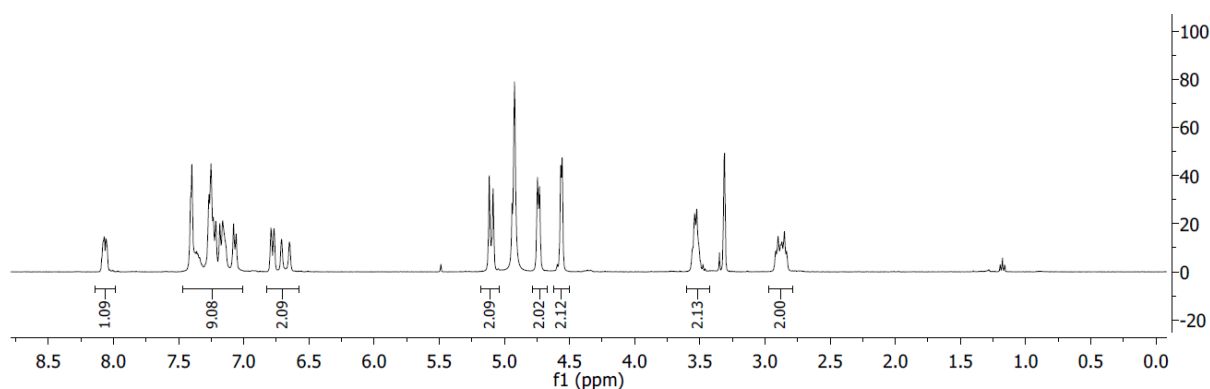




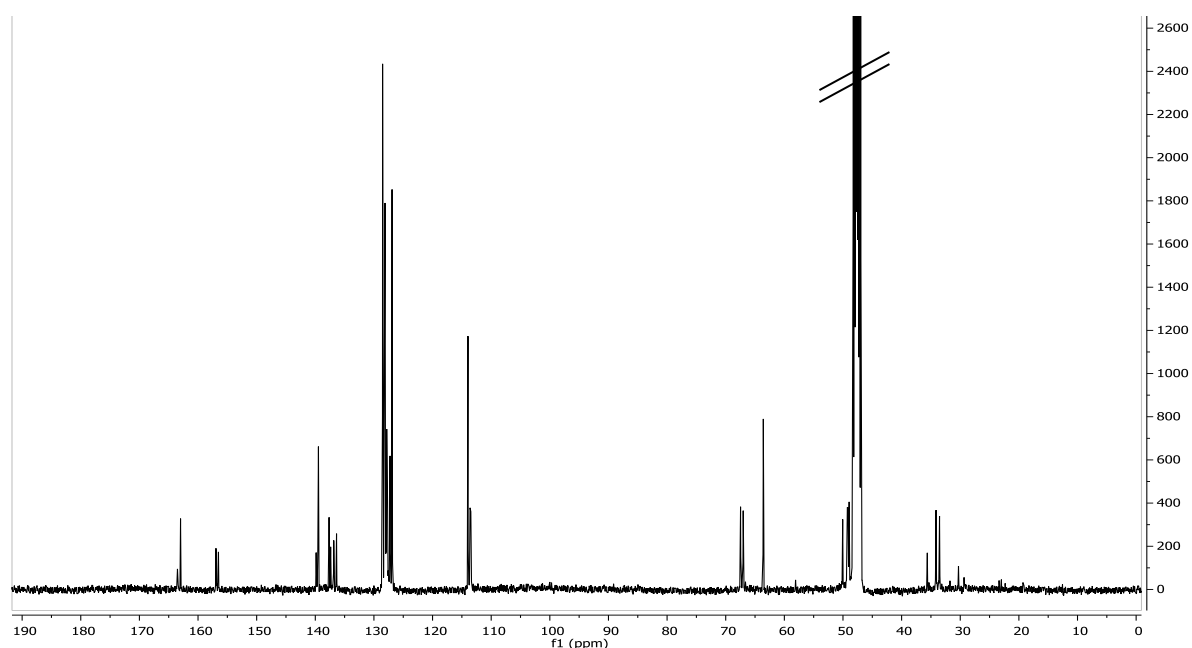
(4-(2-Aminoethyl)phenyl)methanol hydrochloride (4.41 g, 23.5 mmol) and 5-hydroxy-2-nitrobenzaldehyde (3.59 g, 21.5 mmol) were dissolved in THF (100 mL). After addition of  $\text{MgSO}_4$  (40 g) and  $i\text{-Pr}_2\text{NEt}$  (4.10 mL, 23.5 mmol) the reaction-mixture was heated to 50 °C and stirred for 2 hours. The  $\text{MgSO}_4$  was filtered off and washed with THF. After evaporation of the solvent a brown oil was obtained. The crude product was dissolved in MeOH (120 mL) and cooled to 0 °C. Then,  $\text{NaBH}_4$  (0.890 g, 23.5 mmol) was carefully added to this turbid solution. After the gas evolution has ceased (1-2 min), the resulting clear orange solution was stirred at rt under an atmosphere of argon for 1 h. Addition of acetone (30 mL) and evaporation of the solvents provided a crude product that was directly taken into the next step. The crude product was dissolved in 100 mL MeOH and  $\text{NEt}_3$  (6.50 mL, 47.0 mmol) and  $\text{CbzCl}$  (4.00 mL, 28.2 mmol) were added at rt under argon atmosphere. After stirring the turbid reaction mixture for two hours 1 N aq.  $\text{NaOH}$ -solution (50 mL) was added. After stirring for another 5 min, 100 mL 2 N aq.  $\text{HCl}$  solution was added and the aq. phase was extracted with  $\text{EtOAc}$  three times. The organic layer was washed with sat. aq.  $\text{NaCl}$ -solution, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography ( $\text{DCM}/\text{Et}_2\text{O} = 6:1 \rightarrow 2:1$ ) to afford benzyl 5-hydroxy-2-nitrobenzyl(4-(hydroxymethyl)phenethyl)carbamate **7** (6.97 g, 16.0 mmol, 74%).

$^1\text{H}$  NMR (400 MHz, MeOD, mixture of rotamers):  $\delta = 8.12\text{-}8.00$  (m, 1H, Ar), 7.43-7.03 (m, 9H, Ar), 6.81-6.63 (m, 2 H, Ar), 5.15-5.06 (m, 2H,  $\text{OCH}_2$ ), 4.75-4.70 (m, 2H,  $\text{OCH}_2$ ), 4.59-4.52 (m, 2H,  $\text{ArCH}_2\text{N}$ ), 3.56-3.48 (m, 2H,  $\text{NCH}_2$ ), 2.93-2.80 (m, 2H,  $\text{CH}_2$ ) ppm.  $^{13}\text{C}$  NMR{ $^1\text{H}$ } (100 MHz, MeOD, mixture of rotamers):  $\delta = 163.0, 139.4, 137.6, 128.5, 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 127.2, 126.8, 113.9, 113.5, 113.4, 110.0$  (18C, Ar), 67.4, 66.9 (1C,  $\text{OCH}_2$ ), 63.6, 60.9 (1C,  $\text{OCH}_2$ ), 50.0, 49.2, 49.1, 48.9 (2C,  $\text{ArCH}_2\text{N}$ ,  $\text{NCH}_2$ ), 34.1, 33.5 (1C,  $\text{CH}_2$ ) ppm. ESI-HRMS:  $m/z$   $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_6$ : 459.1532; found: 459.1535. IR (neat)  $\nu_{\text{max}}$ : 3214, 1674, 1303, 1251, 907, 725  $\text{cm}^{-1}$ .

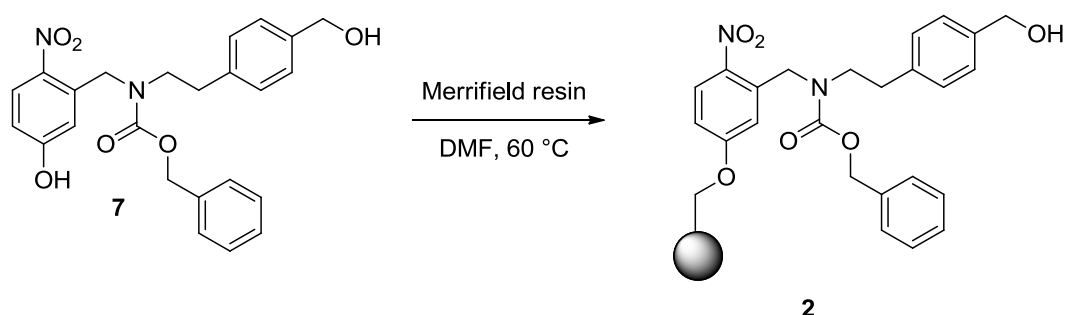
$^1\text{H}$  NMR (400 MHz, MeOD, mixture of rotamers):



$^{13}\text{C}$  NMR{1H} (100 MHz, MeOD, mixture of rotamers):



### Linker-functionalized Merrifield resin (**2**)

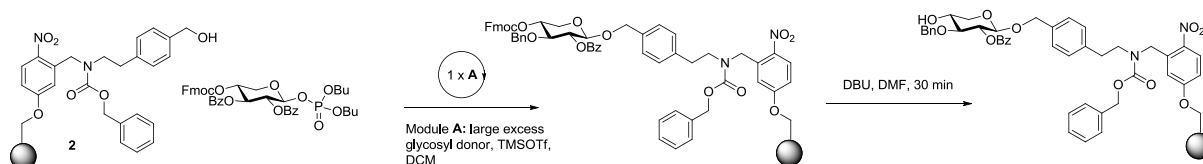


Merrifield resin LL (100-200 mesh, novabiochem, loading: 0.5 mmol/g, 2.00 g, 1.00 mmol) was swollen in anhydrous DMF and linker **7** (1.74 g, 4.00 mmol),  $\text{Cs}_2\text{CO}_3$  (1.30 g, 4.00 mmol) and TBAI (0.370 g, 1.00 mmol) were added. The mixture was gently shaken on a rotavap overnight at 60 °C and 900 mbar. Then water (10 mL) was added to the mixture and a yellow solid precipitated. The mixture was filtered through a syringe with filter and the residue washed with a mixture of THF and water (1:1, 40 mL). This water/THF/DMF phase was collected for reisolation of linker **7**. The resin was washed successively with THF, DMF, MeOH, DCM, MeOH, DCM (with 20 mL each of every solvent). The resin was dried under high vacuum for 2 h. Then the resin was suspended in anhydrous DMF (12 mL) and  $\text{CsOAc}$  (0.380 g, 2.00 mmol) was added. The mixture was gently shaken on a rotavap overnight at 60 °C and 900 mbar. Water (10 mL) was added to the mixture and a yellow solid precipitated. The mixture was filtered through a syringe with filter and the residue washed with a mixture of THF and water (1:1, 40 mL). The resin was washed successively with THF, DMF, MeOH, DCM, MeOH, DCM (with 20 mL each of every solvent). The resin **2** was dried under high vacuum overnight and protected from light with aluminum foil.

Reisolation of excess **7**: The water/THF/DMF phase was acidified with 2 N HCl-solution and extracted with EtOAc twice. The organic layer was washed with sat. aq. NaCl-solution, dried over  $\text{Na}_2\text{SO}_4$ ,

filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (DCM/Et<sub>2</sub>O = 4:1) to afford reisolated **7** (1.23 g, 2.82 mmol) as a yellow oil.

### Loading Determination<sup>[3]</sup>



For loading determination 57.7 mg of the resin were glycosylated in the automated oligosaccharide synthesizer. One glycosylation module [Module A: large excess of glycosyl donor (2 x 48.5 mg, 62.5  $\mu$ mol), TMSOTf, DCM, 2 x 35 min, -35  $^{\circ}$ C to -15  $^{\circ}$ C] was performed and the resin was dried under high vacuum overnight. It was swollen in 20 mL DMF for 30 min and subsequently stirred with 0.4 mL of DBU for another 30 min. Afterwards the solution was diluted with 80 mL with MeCN. Of this solution 2 mL were diluted to 25 mL with MeCN. A reference solution was prepared in the same manner but without addition of the resin. For both solutions the absorbance at 294 nm and 304 nm was measured and the loading was calculated using the following equations:

294 nm:

$$abs = 0.1056$$

$$Loading_{294 \text{ nm}} = \frac{abs \cdot 142.14}{m_{resin}} = \frac{0.1056 \cdot 142.14}{57.7 \text{ mg}} = 0.260 \frac{\text{mmol}}{\text{g}}$$

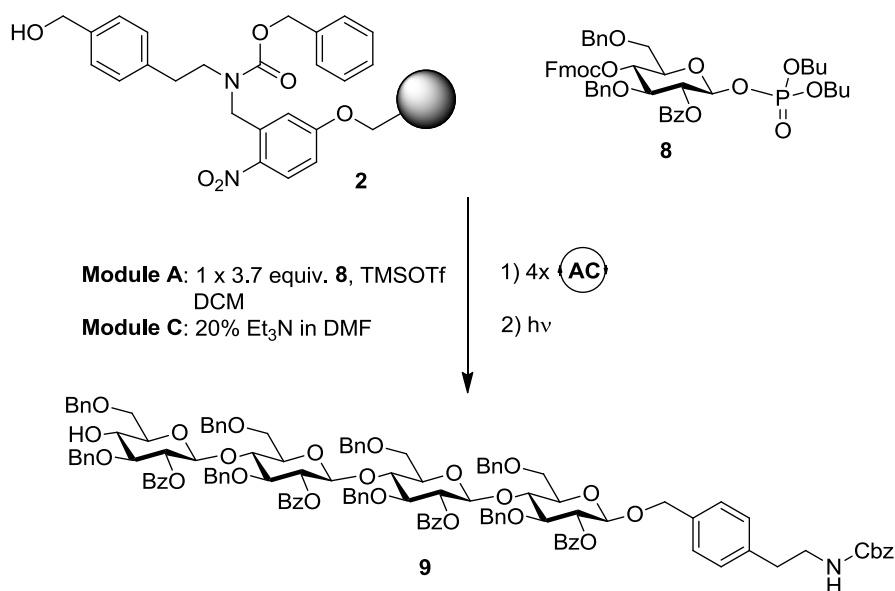
304 nm:

$$abs = 0.0906$$

$$Loading_{304 \text{ nm}} = \frac{abs \cdot 163.96}{m_{resin}} = \frac{0.0906 \cdot 163.96}{57.7 \text{ mg}} = 0.257 \frac{\text{mmol}}{\text{g}}$$

## Automated Glycan Assembly

**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3,6-O-dibenzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-benzoyl-3,6-O-dibenzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-benzoyl-3,6-O-dibenzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-benzoyl-3,6-O-dibenzyl- $\beta$ -D-glucopyranoside (9)**



Linker functionalized resin **2** (85 mg, 22.1  $\mu$ mol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30  $^{\circ}$ C to -15  $^{\circ}$ C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30  $^{\circ}$ C to -15  $^{\circ}$ C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30  $^{\circ}$ C to -15  $^{\circ}$ C)

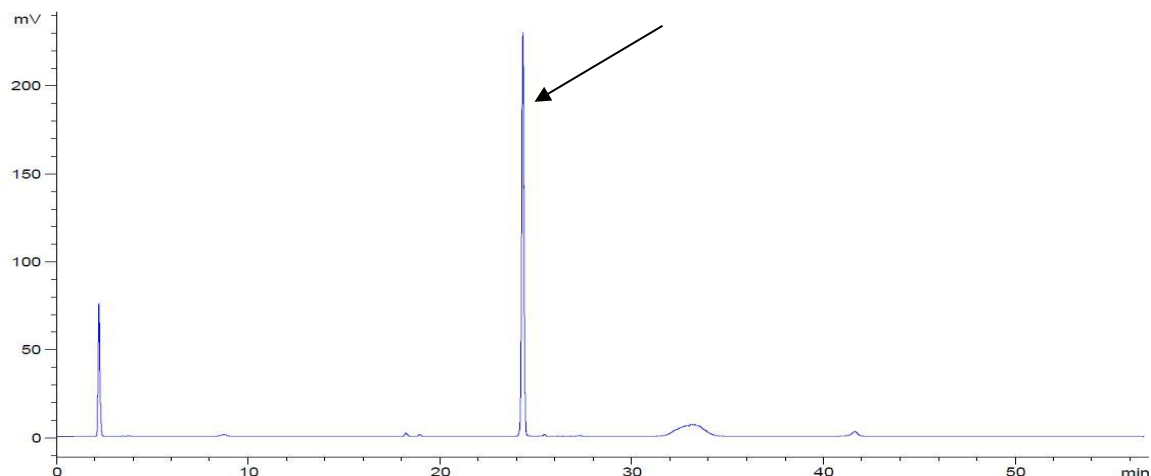
Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30  $^{\circ}$ C to -15  $^{\circ}$ C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide. The crude product was purified by normal phase HPLC using a preparative YMC Diol column affording the protected tetrasaccharide **9** (30.7 mg, 14.8  $\mu$ mol, 67% over 9 steps, based on resin loading).

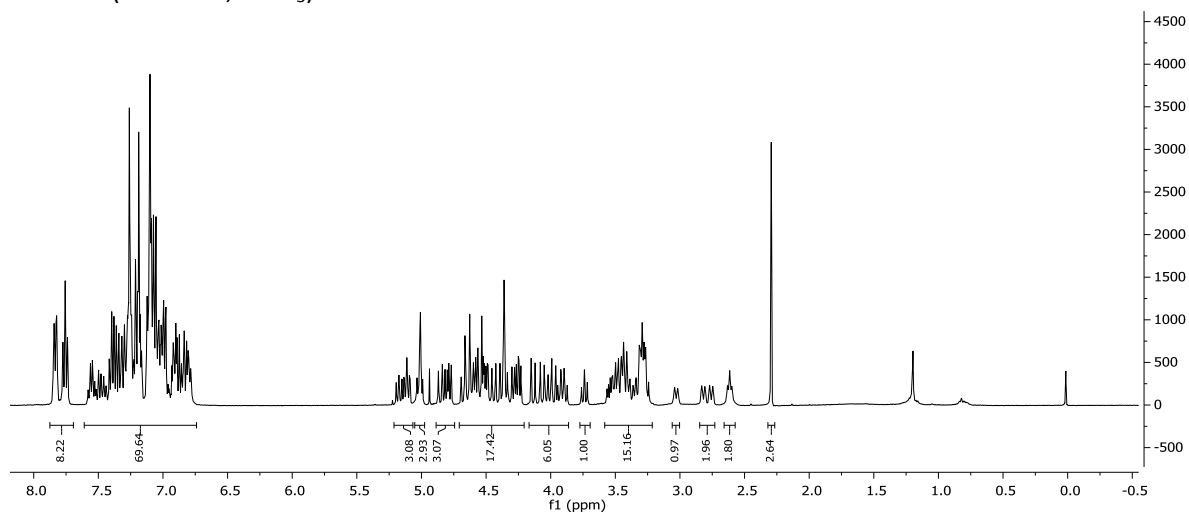
Crude NP-HPLC of tetrasaccharide **9** (ELSD trace):



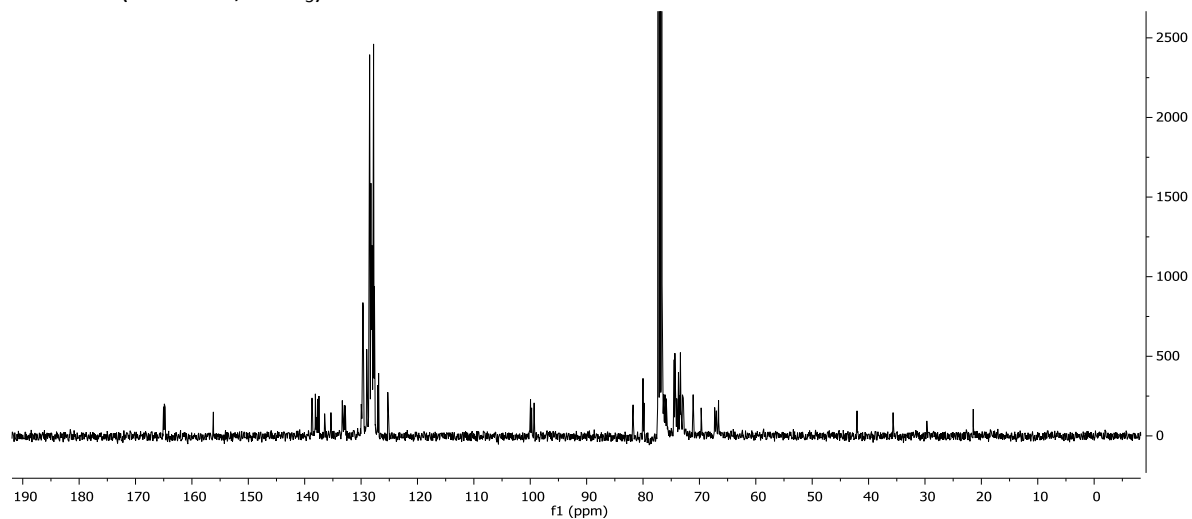
HPLC was performed using an YMC Diol column and a linear gradient from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.88-7.69 (m, 8H), 7.61-6.71 (m, 61H), 5.21-5.07 (m, 3H), 5.06-4.98 (m, 3H), 4.88-4.74 (m, 3H), 4.70-4.21 (m, 17H), 4.17-3.86 (m, 6H), 3.74 (t,  $J$  = 9.0 Hz, 1H), 3.58-3.22 (m, 15H), 3.03 (dt,  $J$  = 9.8, 2.6 Hz, 1H), 2.79 (ddt,  $J$  = 24.5, 9.8, 2.2 Hz, 1H), 2.62 (t,  $J$  = 6.9 Hz, 1H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 165.0, 164.9, 164.8, 164.7, 156.2, 138.7, 138.7, 138.6, 138.1, 138.0, 137.7, 137.6, 137.5, 136.5, 135.3, 133.3, 133.2, 133.0, 132.8, 130.0, 129.8, 129.7, 129.6, 129.5, 129.0, 128.9, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.0, 126.9, 125.3, 100.0, 99.9, 99.8, 99.3, 81.8, 80.0, 79.8, 76.3, 76.1, 75.8, 74.6, 74.5, 74.3, 74.2, 74.0, 73.7, 73.5, 73.4, 73.0, 72.8, 71.1, 70.0, 67.3, 67.0, 66.6, 42.0, 35.6, 29.7, 21.4 ppm. MALDI-TOF:  $m/z$   $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{125}\text{H}_{123}\text{NaNO}_{27}$ : 2094.294; found 2093.544.

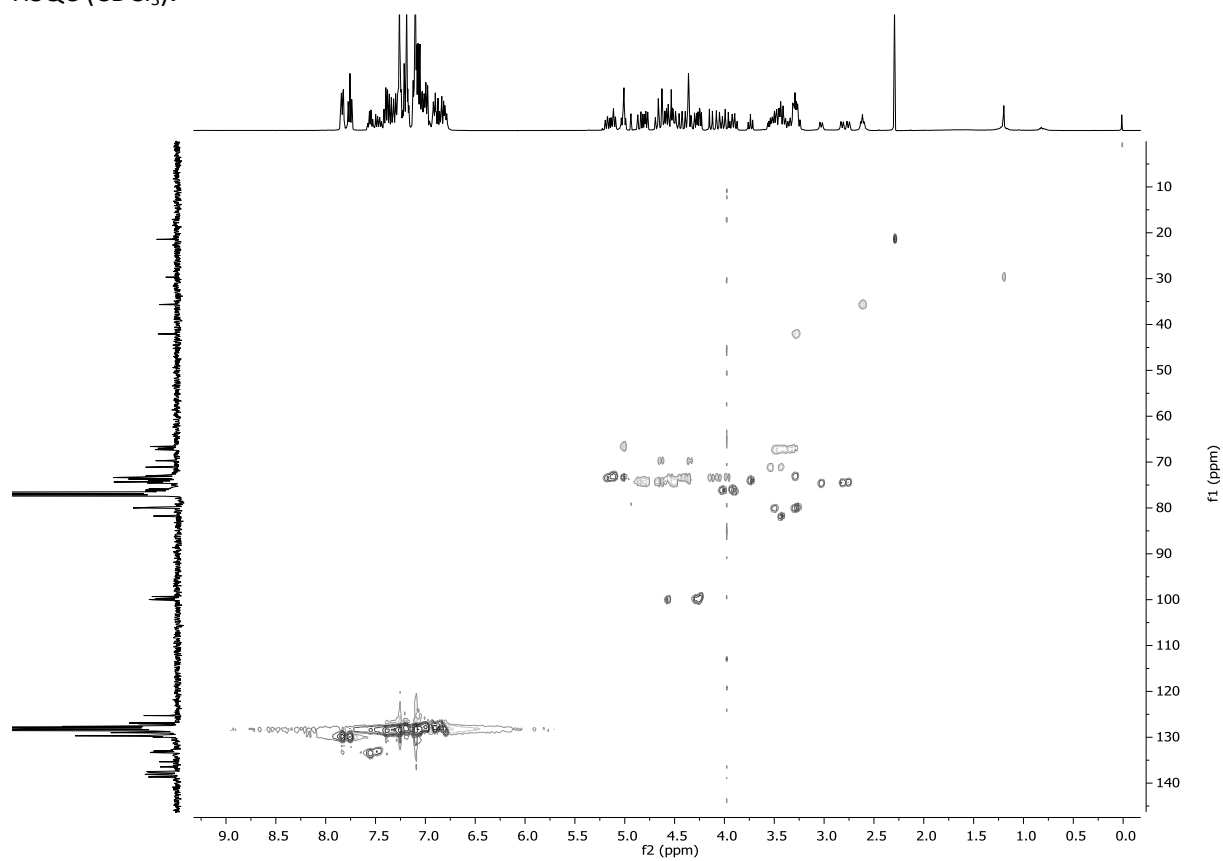
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):



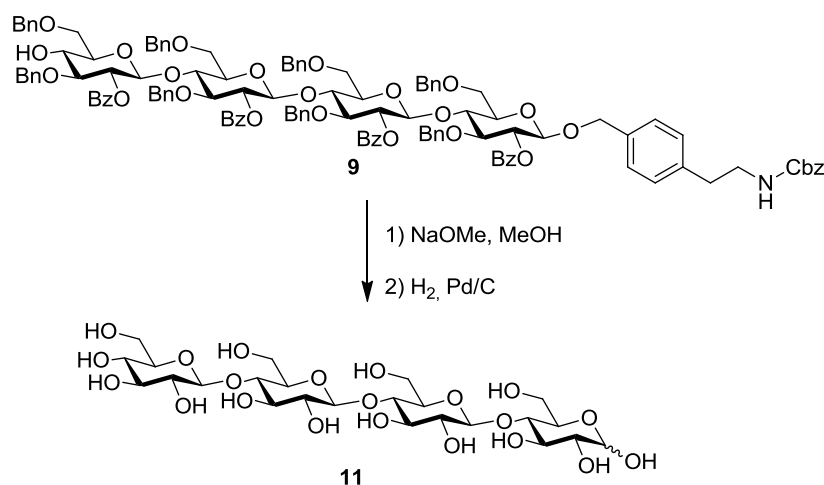
$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):



HSQC ( $\text{CDCl}_3$ ):

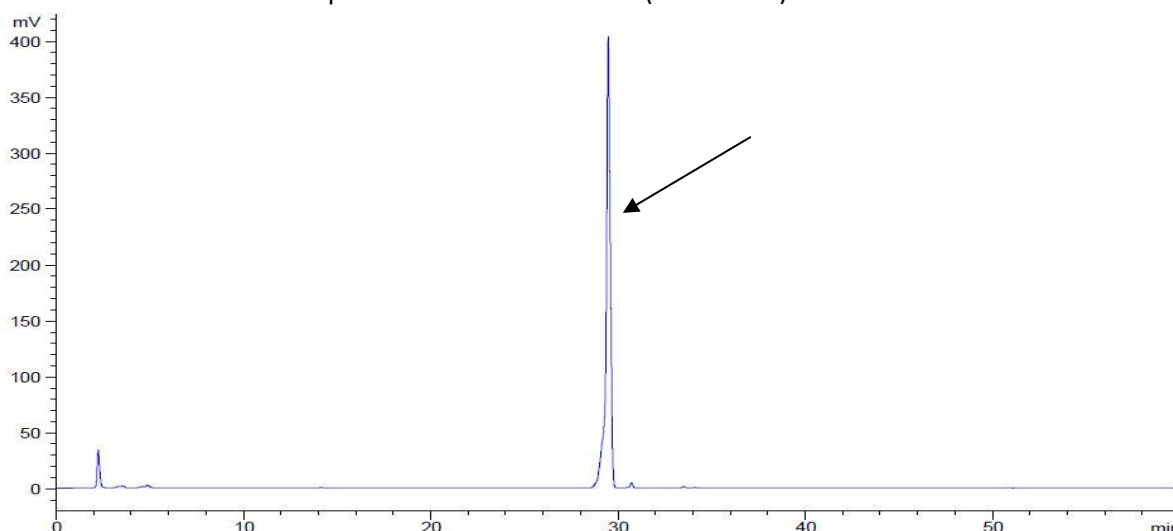


## $\beta$ -D-Glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose (**11**)



Tetrasaccharide **9** was dissolved in THF (3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of H<sup>+</sup>-Amberlite resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a preparative YMC Diol column affording the semi-protected disaccharide.

Crude NP-HPLC of the semi-protected tetrasaccharide (ELSD trace):

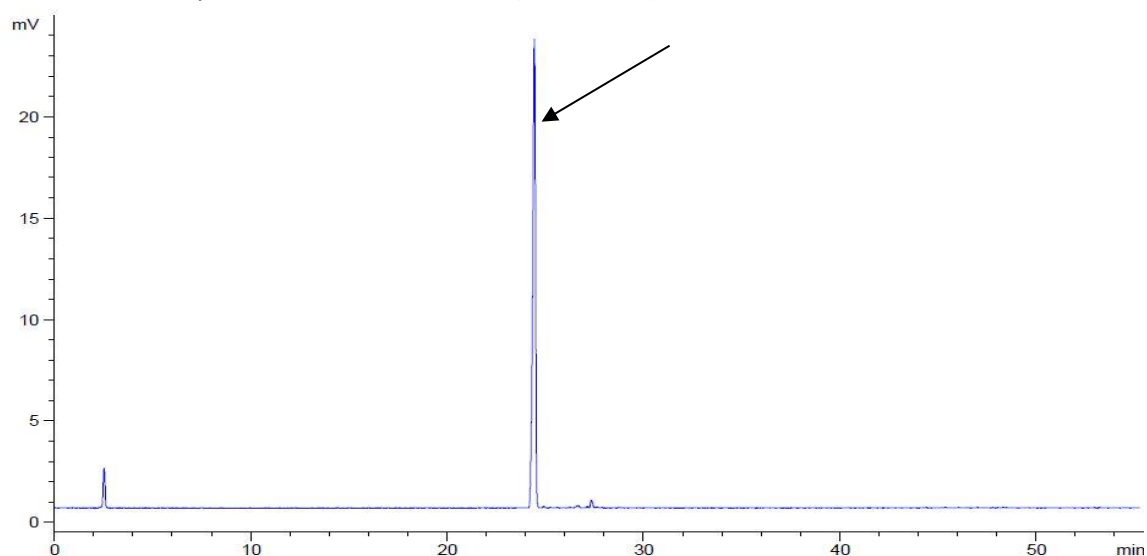


HPLC was performed using a YMC-Diol-300 column and a linear gradient from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 20 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under an H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter, the solvents were evaporated. The fully deprotected tetrasaccharide **11** was re-dissolved in 0.5 mL water and stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze a side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected tetrasaccharide **11** was directly

purified by reversed phase HPLC using a semi-preparative Hypercarb column to provide an  $\alpha/\beta$  mixture of the tetrasaccharide **11** (2.0 mg, 3.00  $\mu\text{mol}$ , 20% over 2 steps).

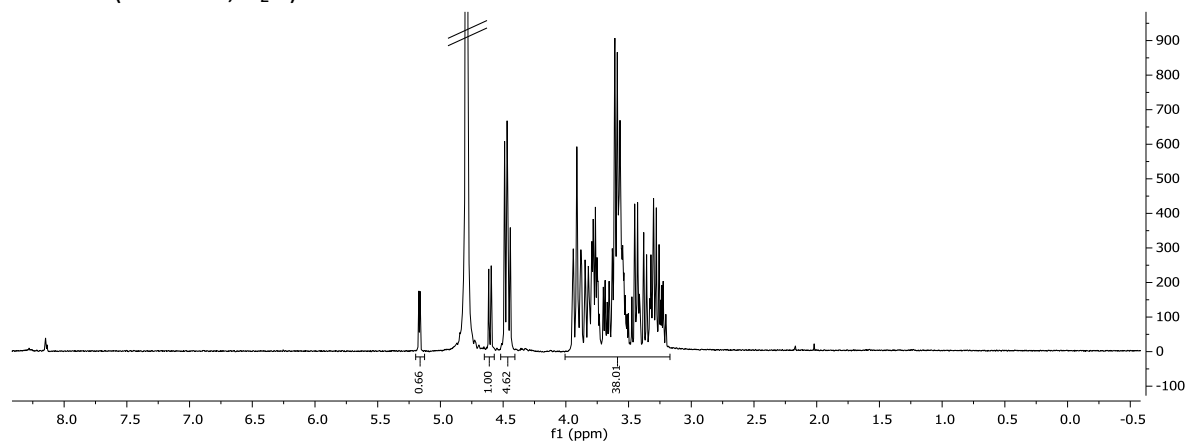
RP-HPLC of the deprotected disaccharide **11** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 62.5%  $\text{H}_2\text{O}$  (containing 0.1% of formic acid) in MeCN (26 min, flow rate 0.7 mL/min).

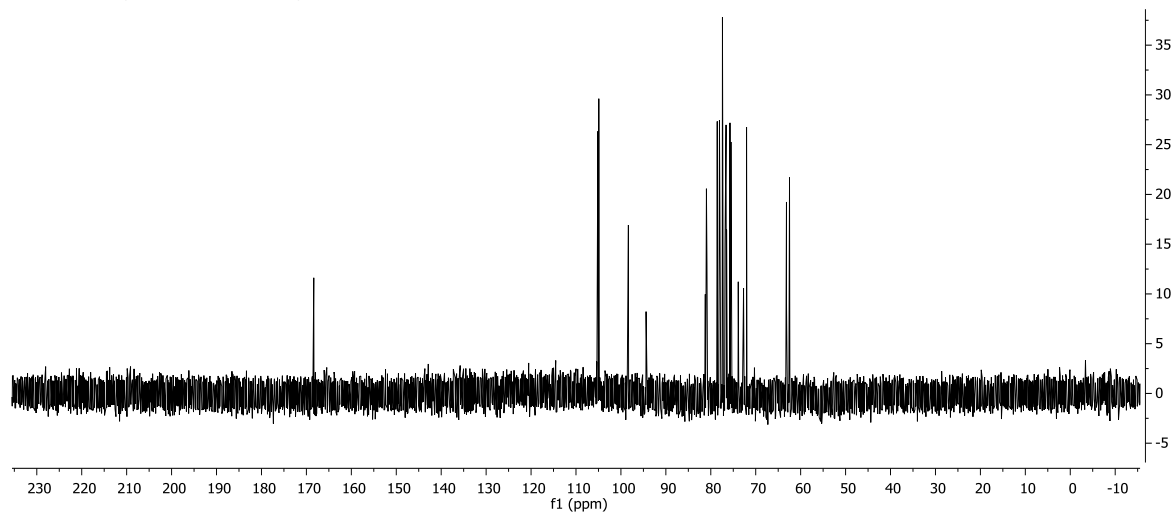
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 5.17 (d,  $J$  = 3.7 Hz, 1H), 4.61 (d,  $J$  = 8.0 Hz, 1H), 4.50-4.43 (m, 3H), 4.00-3.15 (m, 48H) ppm.  $^{13}\text{C}$  NMR (151 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 168.4, 105.1, 104.9, 98.3, 94.4, 81.2, 81.1, 81.0, 80.8, 78.6, 78.1, 77.4, 76.8, 76.6, 76.5, 75.7, 75.5, 73.9, 73.8, 72.7, 72.0, 63.2, 62.6, 62.5. ppm. ESI-HRMS:  $m/z$  =  $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{24}\text{H}_{42}\text{NaO}_{21}$ : 689.2116; found 689.2130

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):

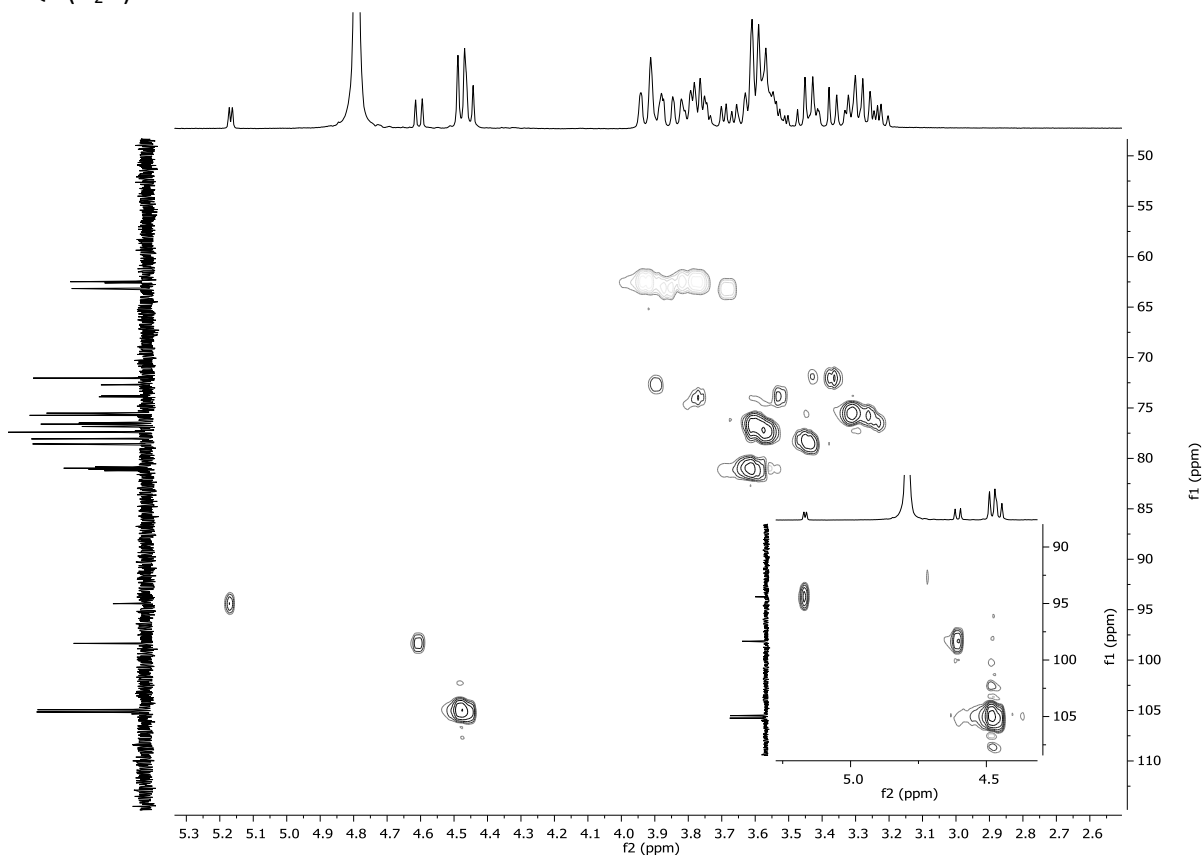




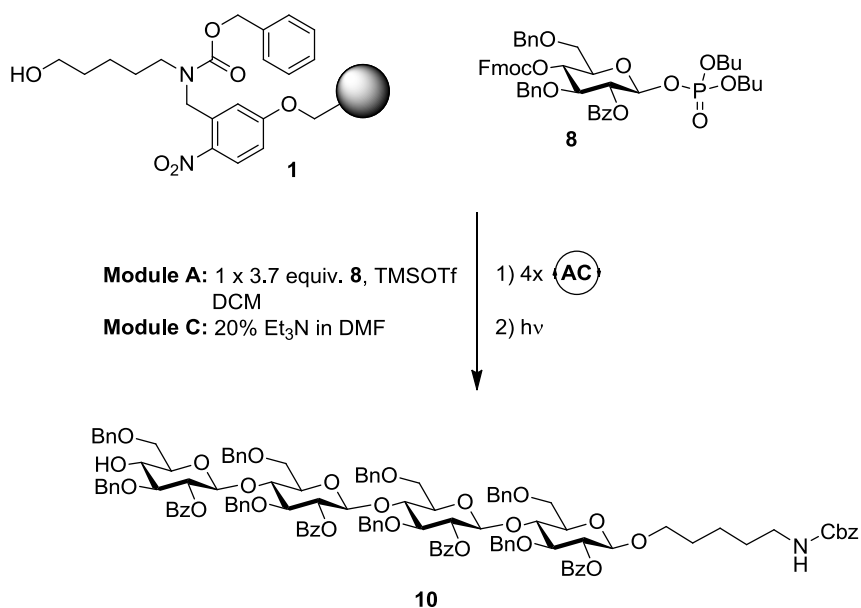
$^{13}\text{C}$  NMR (151 MHz,  $\text{D}_2\text{O}$ ):



HSQC ( $\text{D}_2\text{O}$ ):



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranoside (10)**



Linker functionalized resin **1** (52 mg, 16.9 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

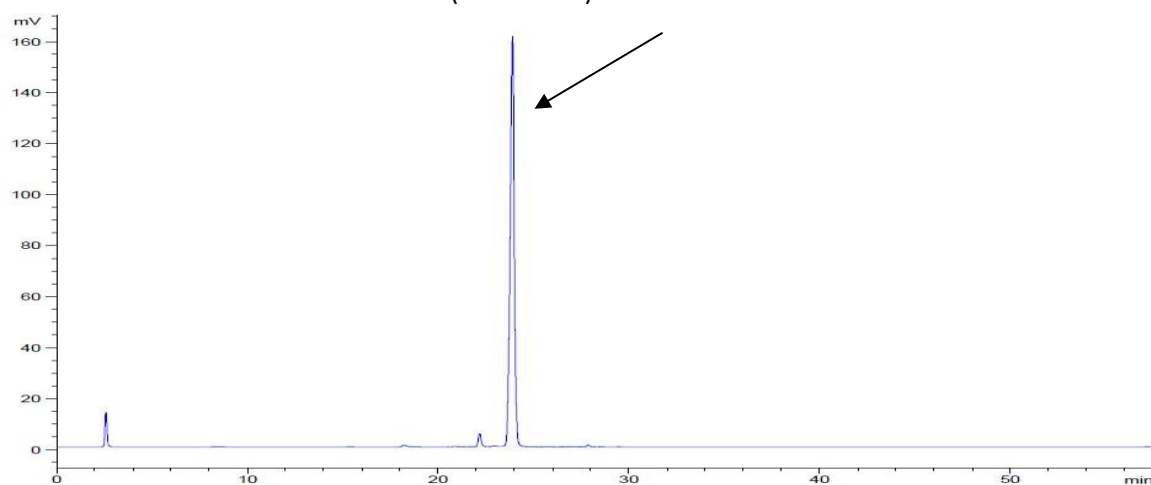
Module **A** (3.7 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **10**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column affording the protected tetrasaccharide **10** (22.2 mg, 11.0 μmol, 65% over 9 steps, based on resin loading).

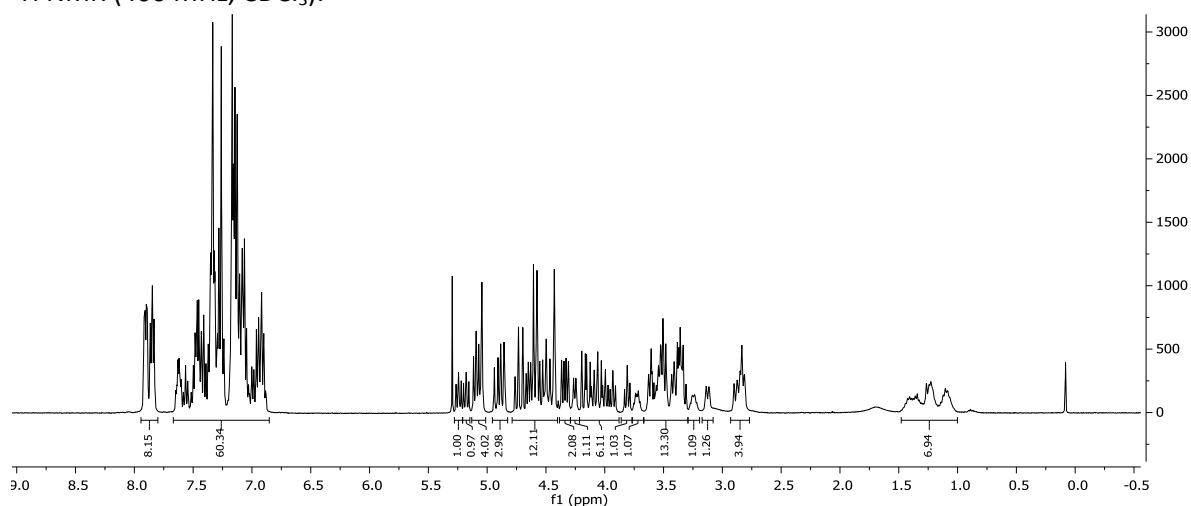
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.95-7.79 (m, 8H), 7.66-6.85 (m, 57H), 5.24 (dd,  $J$  = 9.6, 8.1 Hz, 1H), 5.18 (dd,  $J$  = 9.7, 8.2 Hz, 1H), 5.14-5.01 (m, 4H), 4.95-4.83 (m, 3H), 4.77-4.40 (m, 12H), 4.34 (dd,  $J$  = 15.2, 8.1 Hz, 2H), 4.26 (d,  $J$  = 8.0 Hz, 1H), 4.21-3.89 (m, 6H), 3.81 (t,  $J$  = 9.0 Hz, 1H), 3.76-3.69 (m, 1H), 3.65-3.29 (m, 13H), 3.28-3.20 (m, 1H), 3.15-3.09 (m, 1H), 2.92-2.79 (m, 4H), 1.47-1.01 (m, 6H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 165.0, 164.9, 164.8, 156.2, 138.7, 138.7, 138.6, 138.1, 138.0, 137.7, 137.6, 137.5, 136.7, 133.3, 133.2, 133.0, 132.8, 130.0, 129.7, 129.6, 129.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.0, 126.9, 101.1, 100.1, 99.9, 99.7, 81.8, 80.0, 79.8, 76.4, 76.1, 75.8, 74.6, 74.5, 74.3, 74.3, 74.1, 74.0, 73.7, 73.5, 73.4, 73.3, 73.0, 71.1, 69.3, 67.4, 67.0, 66.4, 53.4, 40.7, 29.3, 28.7, 23.0 ppm. MALDI-TOF:  $m/z$   $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{121}\text{H}_{123}\text{NaNO}_{27}$ : 2046.252; found 2045.909.

Crude NP-HPLC of tetrasaccharide **10** (ELSD trace):

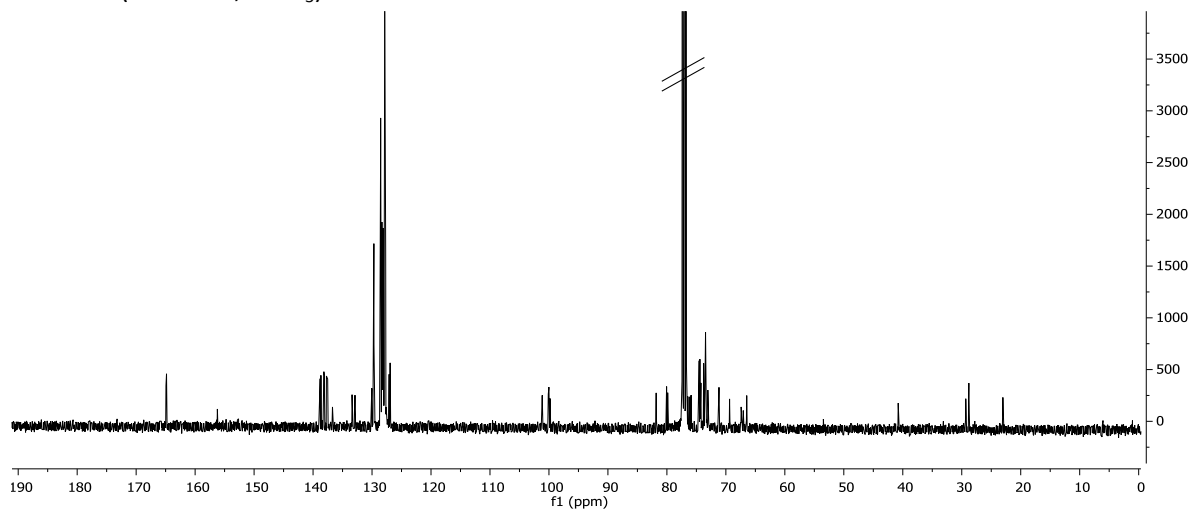


HPLC was performed using a YMC Diol column and a linear gradient from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

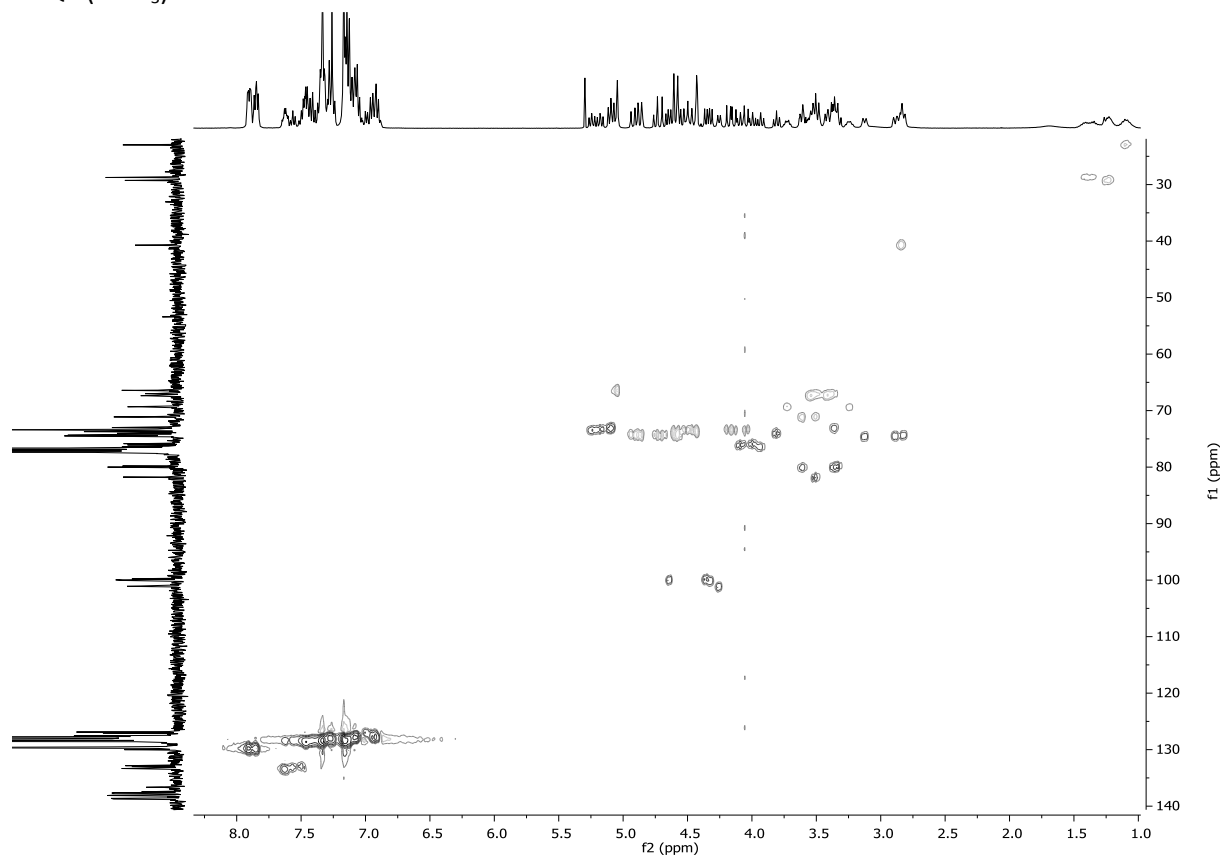
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):



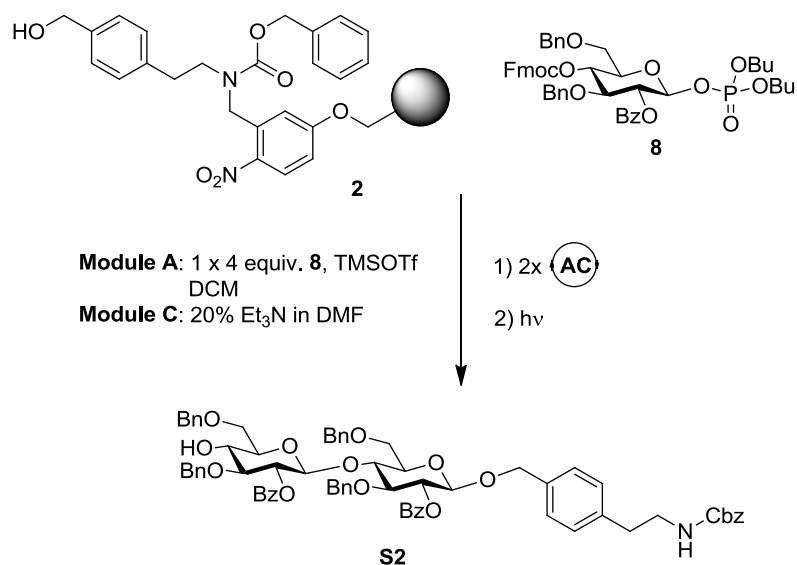
$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):



HSQC ( $\text{CDCl}_3$ ):



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranoside (S2)**



Linker functionalized resin **2** (60 mg, 15.1 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (4 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

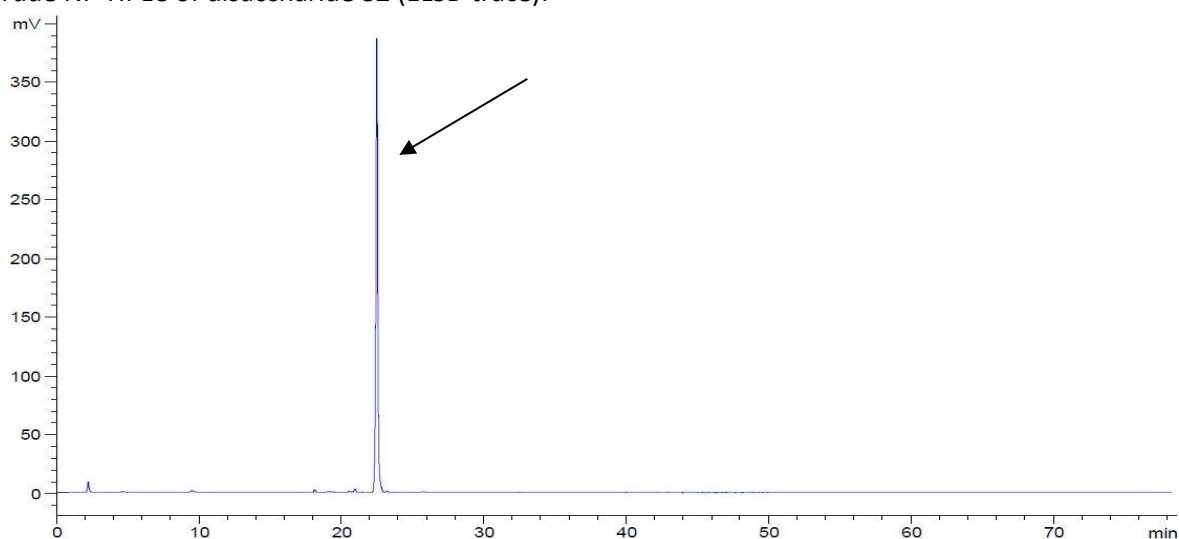
Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module **A** (4 equiv. **8**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

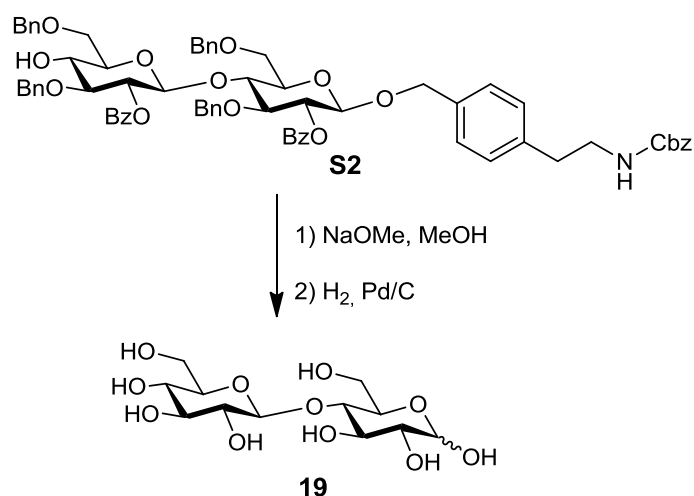
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected disaccharide. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of disaccharide **S2** (ELSD trace):



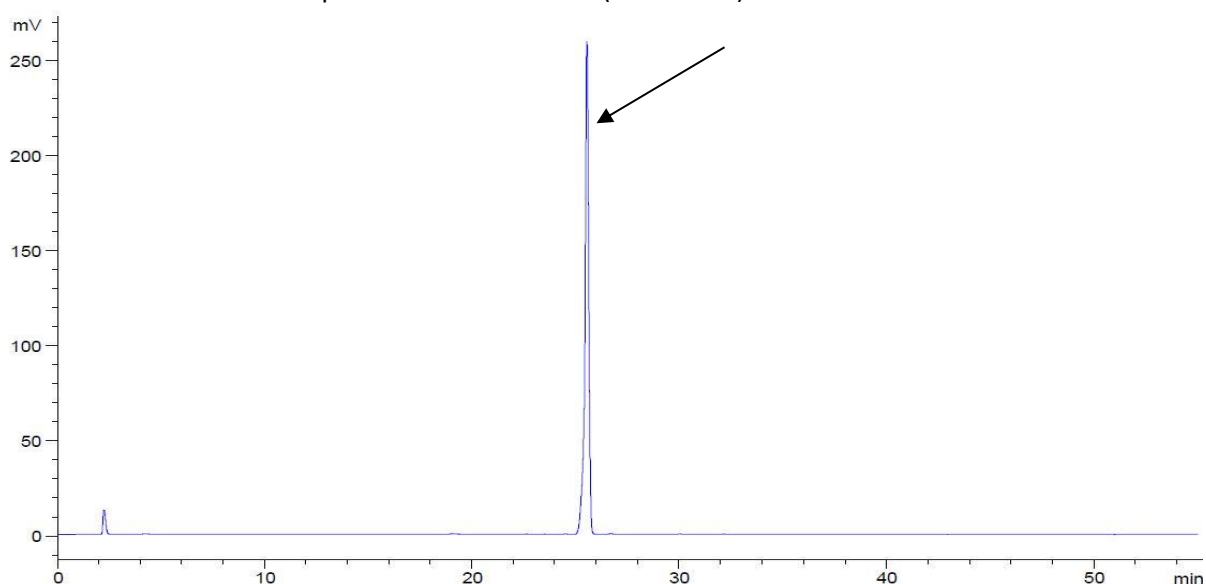
HPLC was performed using a YMC Diol column and a linear gradient from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

## $\beta$ -D-Glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose (**19**)



Disaccharide **S2** was dissolved in THF (3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of H<sup>+</sup>-Amberlite resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected disaccharide.

Crude RP-HPLC of the semi-protected disaccharide (ELSD trace):



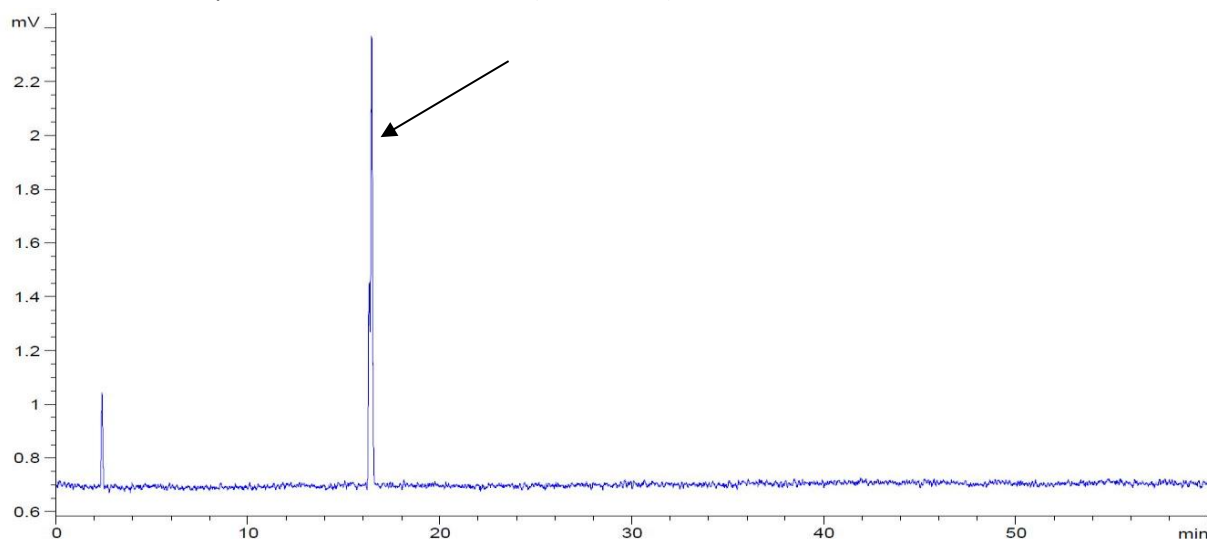
HPLC was performed using a semi-preparative C5 column and a linear gradient from 20% to 100% ACN in H<sub>2</sub>O (45 min, flow rate 1 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 20 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under an H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter, the solvents were evaporated. The fully deprotected disaccharide **19** was re-dissolved in 0.5 mL water and stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze a side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected disaccharide **19** was directly purified by

reversed phase HPLC using a semi-preparative Hypercarb column to provide an  $\alpha/\beta$  mixture of the disaccharide **19** (0.8 mg, 2.34  $\mu$ mol, 15% over 9 steps, based on resin loading).

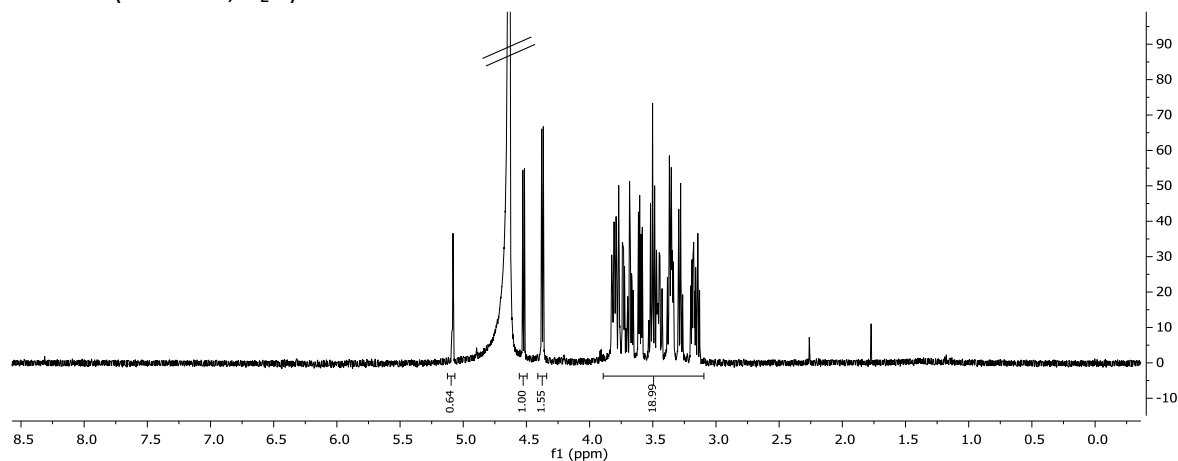
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 5.11 (d,  $J$  = 3.8 Hz, 1H), 4.55 (d,  $J$  = 8.0 Hz, 1H), 4.40 (d,  $J$  = 7.9 Hz, 2H), 3.88-3.13 (m, 24H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 102.5, 95.7, 91.7, 78.6, 78.5, 75.9, 75.4, 74.7, 74.2, 73.8, 73.1, 71.3, 71.1, 70.0, 69.4, 60.5, 60.0, 59.8 ppm. ESI-HRMS:  $m/z$  =  $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{12}\text{H}_{22}\text{NaO}_{11}^+$ : 365.2862; found 365.1012.

RP-HPLC of the deprotected disaccharide **19** (ELSD trace):

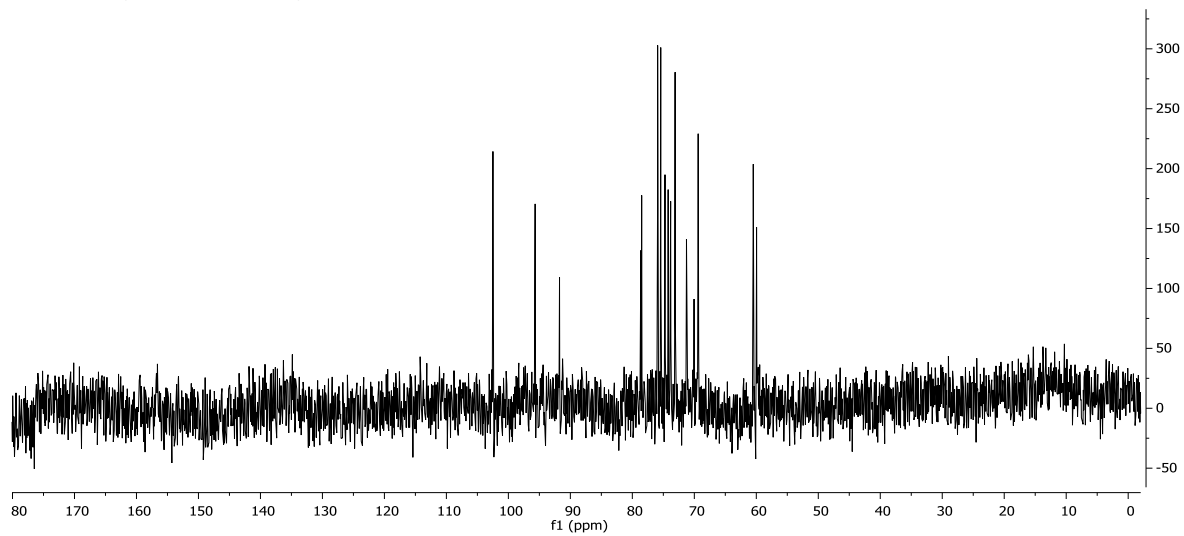


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30%  $\text{H}_2\text{O}$  (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

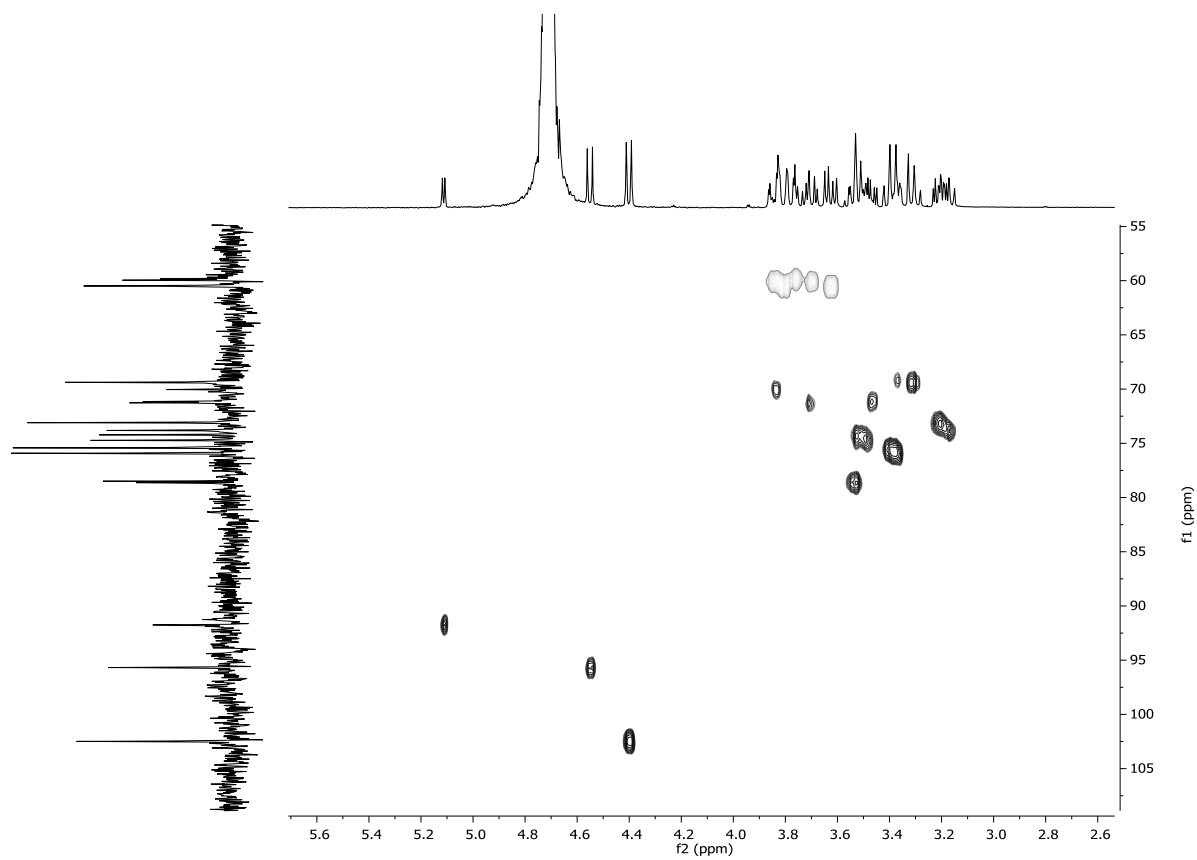
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):



$^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):

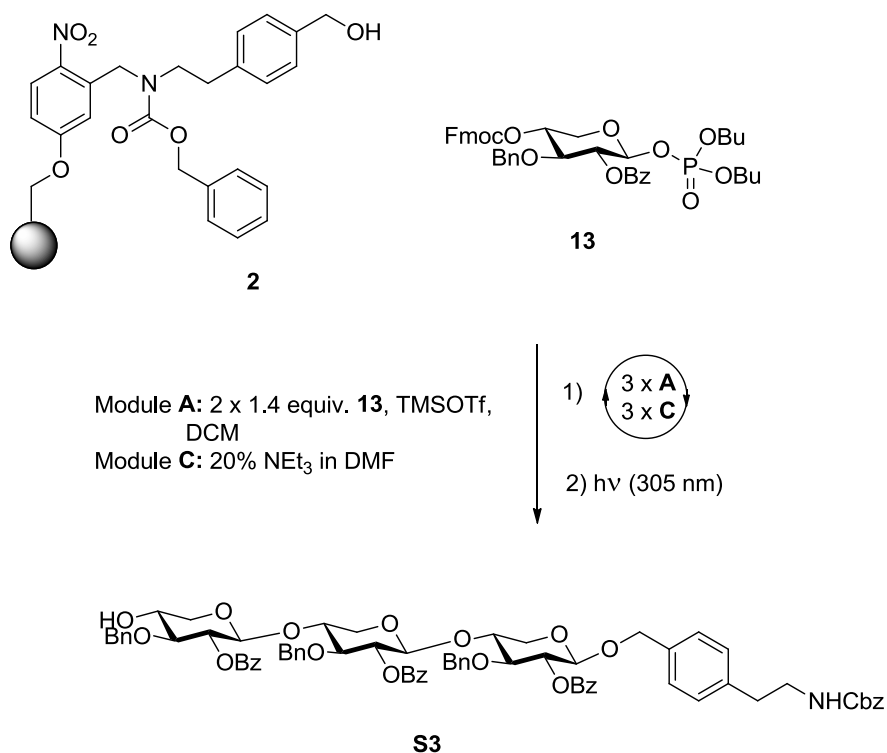


HSQC ( $\text{D}_2\text{O}$ ):





**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3-O-benzyl-β-D-xylopyranosyl-(1→4)-2-O-benzoyl-3-O-benzyl-β-D-xylopyranosyl-(1→4)-2-O-benzoyl-3-O-benzyl-β-D-xylopyranoside (S3)**



Linker-functionalized resin **2** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)

Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)

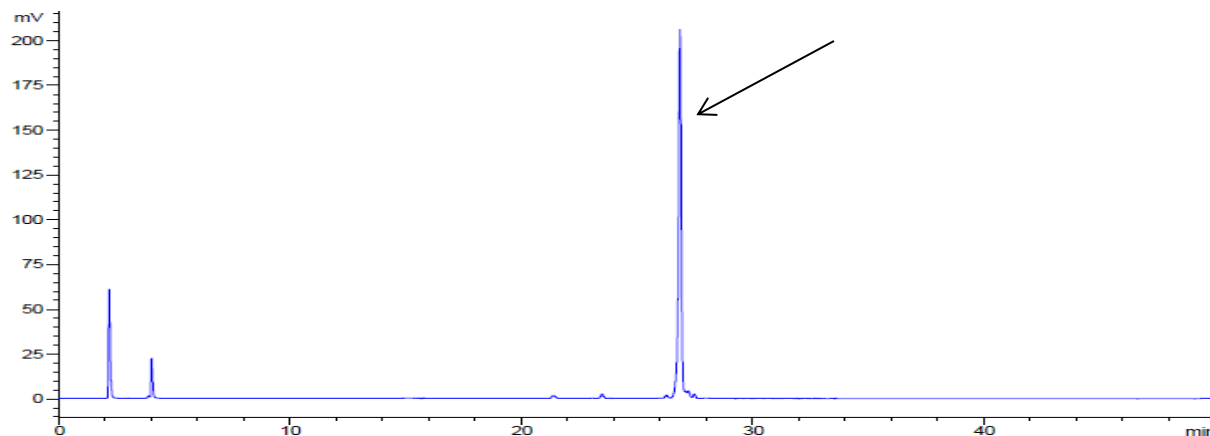
Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)

Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)

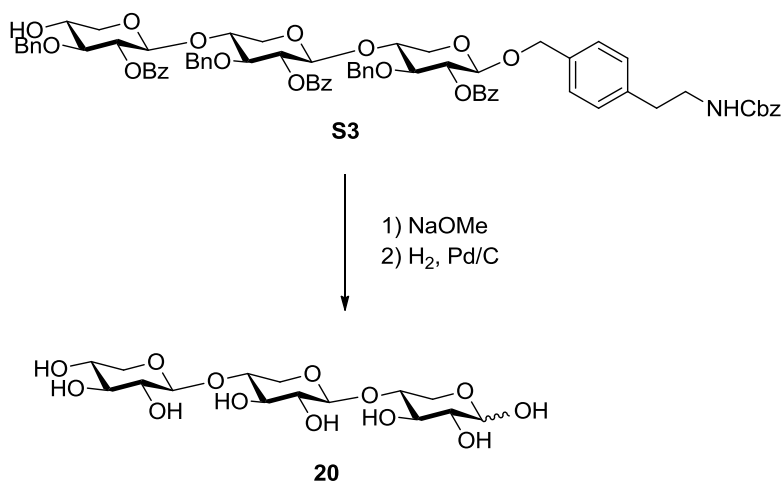
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected trisaccharide **S3** (30.1 mg, 23.8 μmol, 54%)

Crude NP-HPLC of trisaccharide **S3** (ELSD trace):



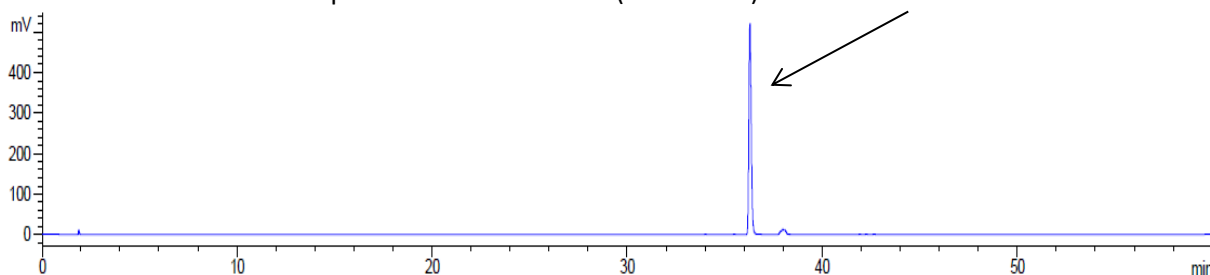
HPLC was performed using a YMC diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

### $\beta$ -D-Xylopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose (**20**)



Trisaccharide **S3** was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide.

Crude RP-HPLC of the semi-protected trisaccharide (ELSD trace):

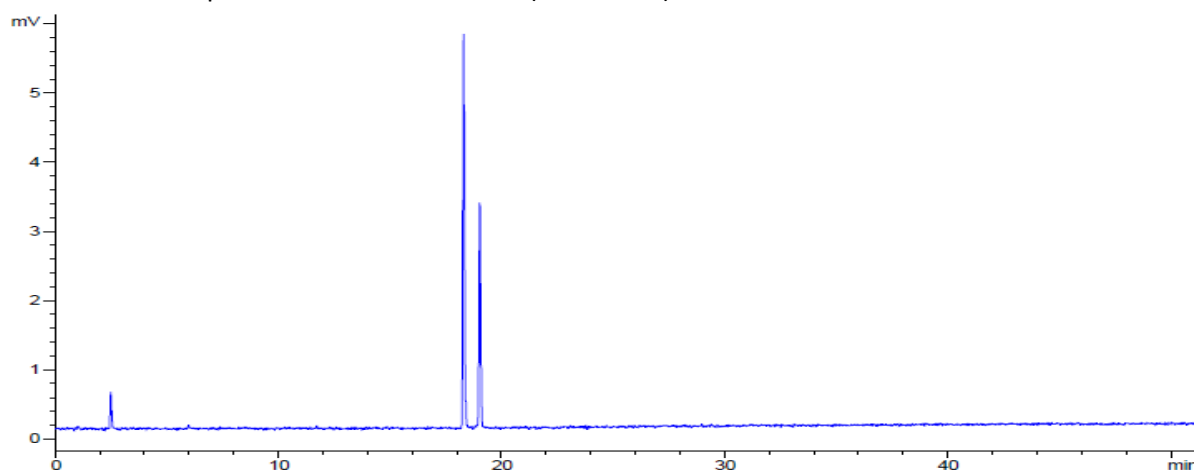


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 1.0 ml/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture was left. The fully deprotected trisaccharide **20** was stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **20** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an  $\alpha/\beta$ -mixture of the trisaccharide **20** (1.9 mg, 4.64  $\mu$ mol, 10% over 9 steps, based on resin loading).

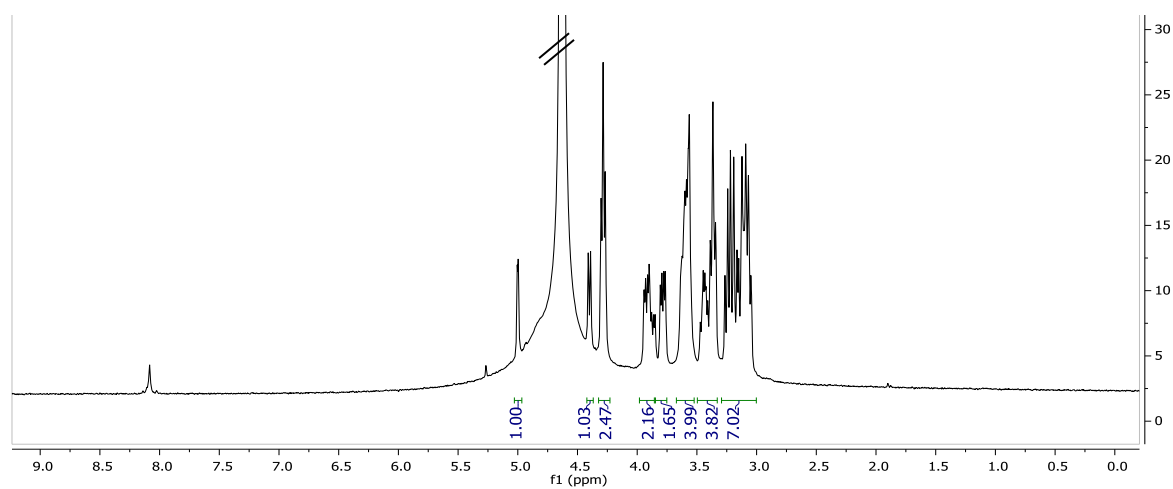
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 5.04 (d,  $J$  = 3.7 Hz,  $\alpha$ -1H), 4.44 (d,  $J$  = 7.9 Hz,  $\beta$ -1H), 4.32 (t,  $J$  = 7.2 Hz, 5H), 3.96 (dd,  $J$  = 11.8, 5.3 Hz, 2H), 3.95 – 3.86 (m, 2H), 3.82 (dd,  $J$  = 11.6, 5.5 Hz, 2H), 3.70 – 3.56 (m, 9H), 3.52 – 3.36 (m, 9H), 3.31 – 3.19 (m, 6H), 3.19 – 3.07 (m, 9H) ppm. <sup>13</sup>C NMR{<sup>1</sup>H} (101 MHz, D<sub>2</sub>O):  $\delta$  = 101.8, 101.6, 96.4, 91.9, 76.5, 76.3, 75.5, 73.9, 73.8, 73.6, 72.7, 72.6, 71.3, 70.9, 69.1, 65.1, 62.9 ppm. ESI-HRMS:  $m/z$  = [M+Na]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>26</sub>NaO<sub>13</sub><sup>+</sup>: 437.1271; found 437.1254.

RP-HPLC of the deprotected trisaccharide **20** (ELSD trace):

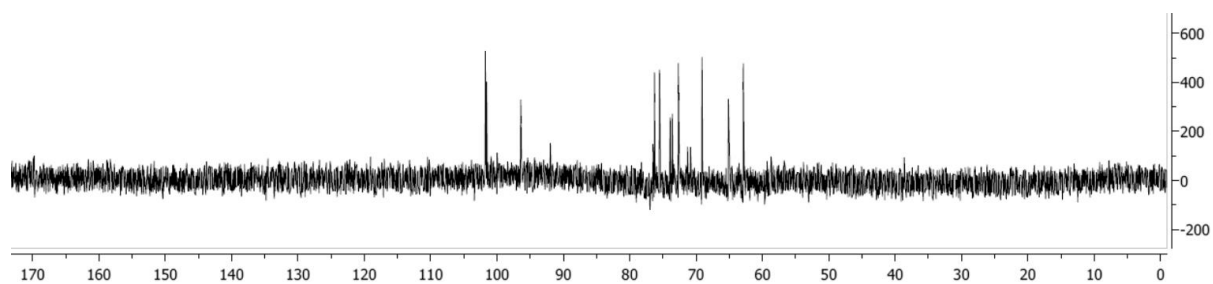


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).

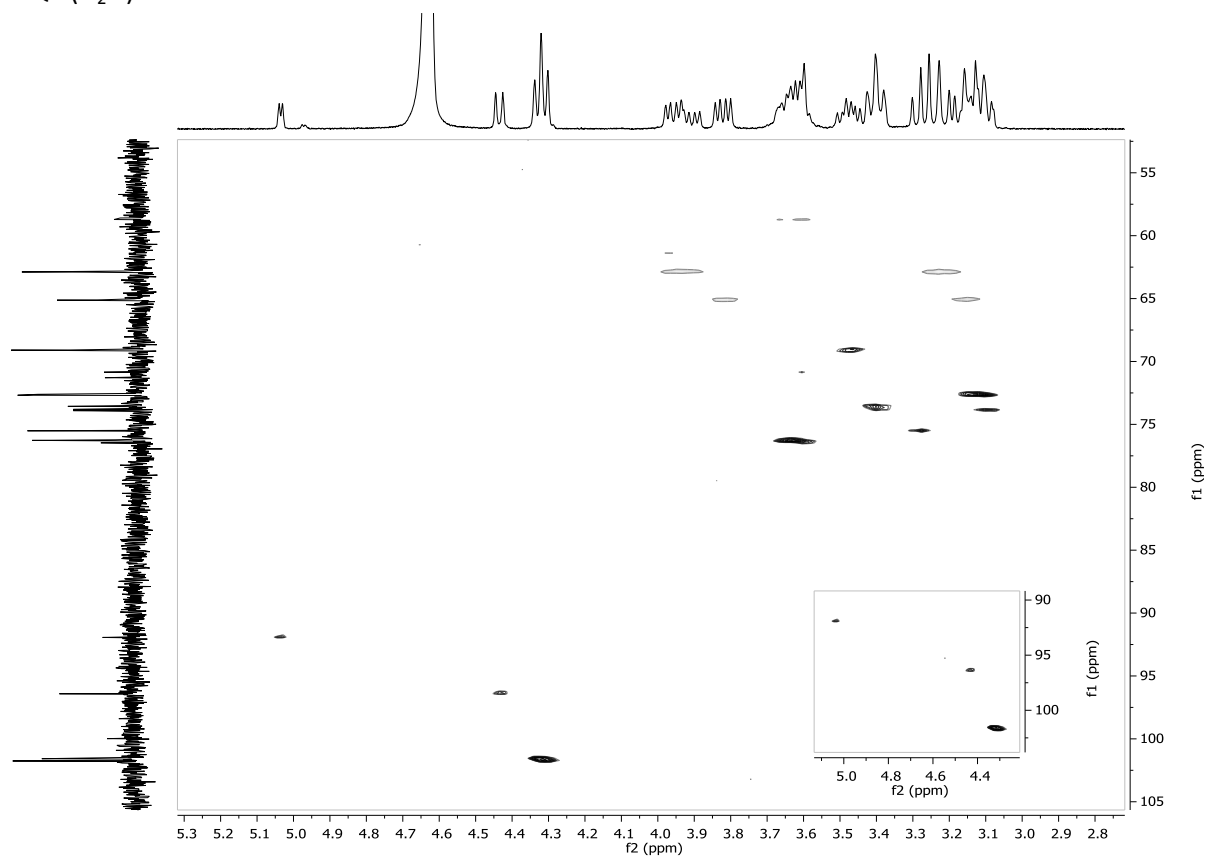
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):



$^{13}\text{C}$  NMR{ $^1\text{H}$ } (101 MHz,  $\text{D}_2\text{O}$ ):

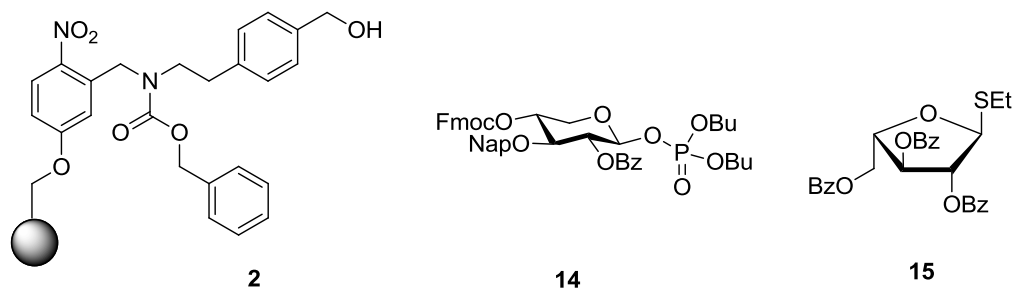


HSQC ( $\text{D}_2\text{O}$ ):



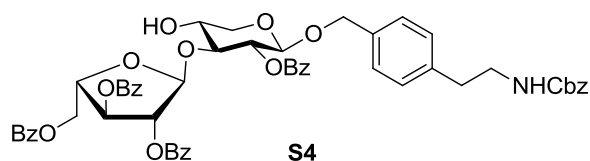
**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl)  
arabinofuranosyl]-3-O-benzyl-β-D-xylopyranoside (S4)**

**2-O-[2,3,5-tri-O-benzoyl-α-L-**



Module **A**: 2 x 1.4 equiv. **14**,  
TMSOTf, DCM  
Module **B**: 2 x 1.4 equiv. **15**, NIS,  
DCM/Dioxane  
Module **C**: 20% NEt<sub>3</sub> in DMF  
Module **E**: DDQ, DCE/MeOH/H<sub>2</sub>O  
(64:16:1)

1) **A**  
2) **E**  
3) **B**  
4) **C**  
5) hv (305 nm)



Linker-functionalized resin **2** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 1.4 equiv **14**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)

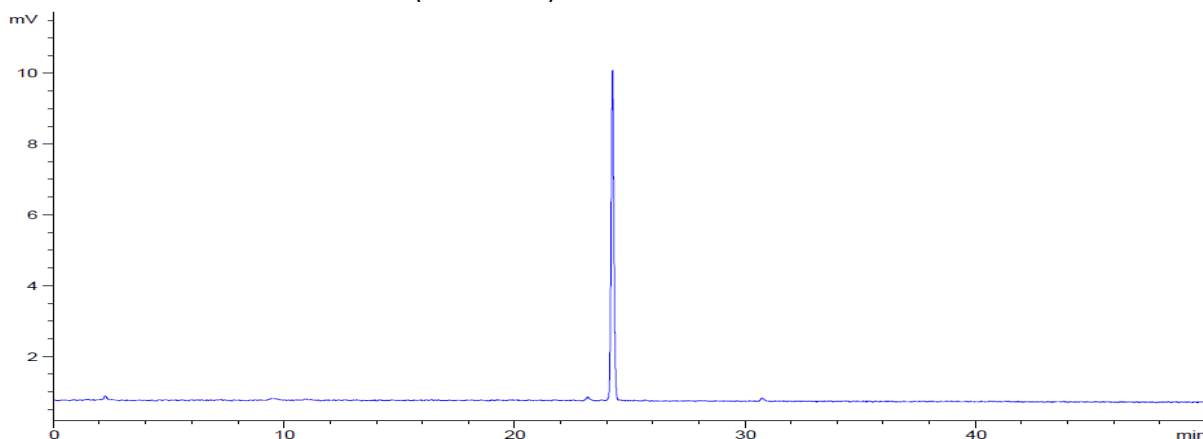
Module **E** (0.1 M DDQ in DCE/MeOH/H<sub>2</sub>O (64:16:1))

Module **B** (2 x 1.4 equiv **15**, NIS, 2 x 35 min, -40 °C to -20 °C)

Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)

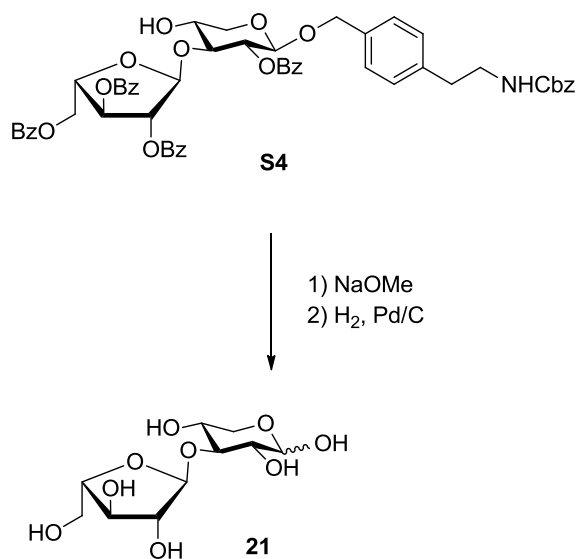
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected disaccharide **S4** (10.2 mg, 10.6 μmol, 24%)

Crude NP-HPLC of trisaccharide **S4** (ELSD trace):



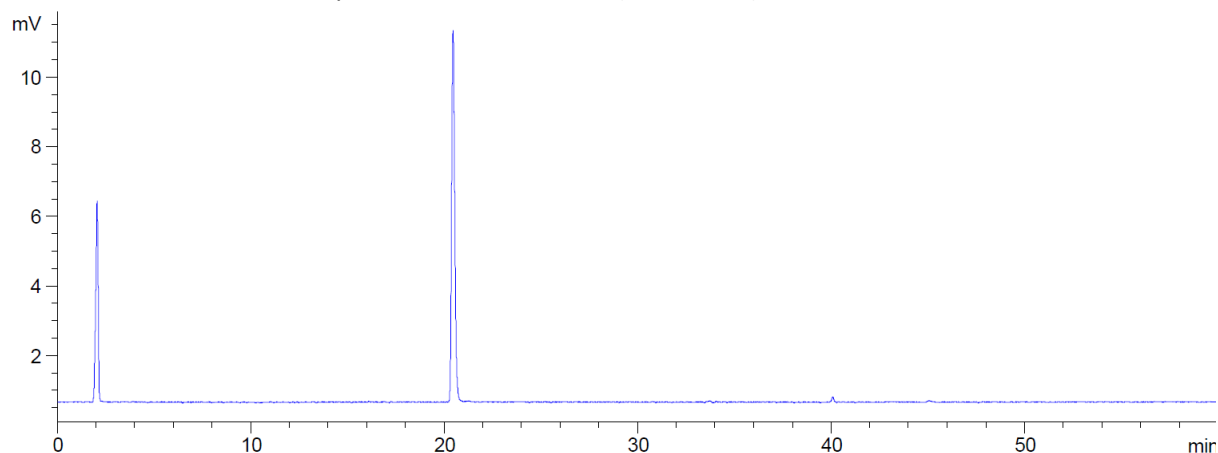
HPLC was performed using a YMC diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

### 2-O-[ $\alpha$ -L-Arabinofuranosyl]-D-xylopyranose (**21**)



Disaccharide **S4** was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected disaccharide.

Crude RP-HPLC of the semi-protected disaccharide (ELSD trace):

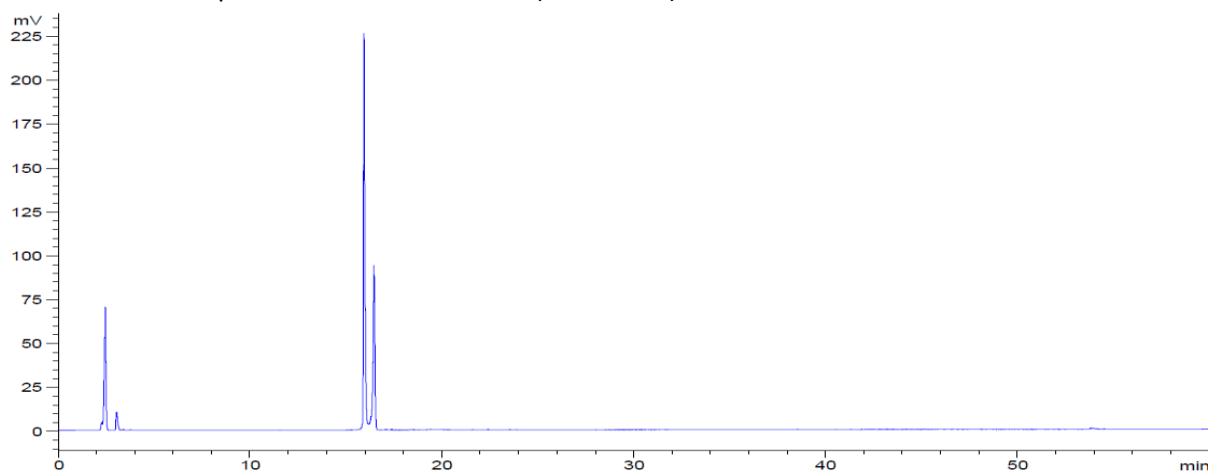


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 1.0 ml/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture was left. The fully deprotected disaccharide **21** was stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected disaccharide **21** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an  $\alpha/\beta$ -mixture of the disaccharide **21** (2.2 mg, 7.72  $\mu$ mol, 18% over 7 steps, based on resin loading).

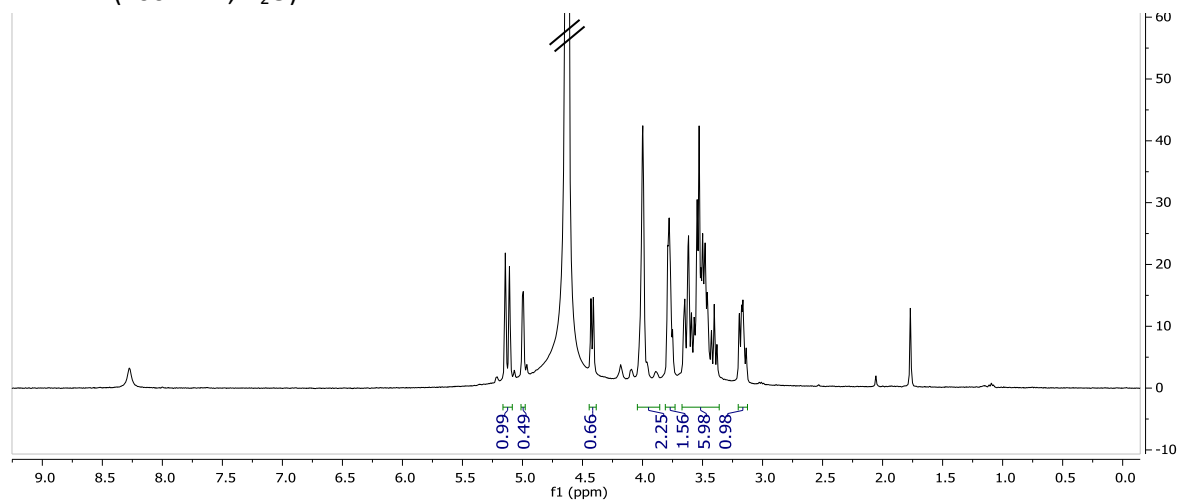
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 5.13 (d,  $J$  = 13.8 Hz, 1H), 5.00 (d,  $J$  = 3.4 Hz, 1H,  $\alpha$ -1H), 4.42 (d,  $J$  = 8.0 Hz, 1H,  $\beta$ -1H), 4.08 – 3.95 (m, 2H), 3.85 – 3.70 (m, 2H), 3.68 – 3.36 (m, 6H), 3.25 – 3.10 (m, 1H) ppm. <sup>13</sup>C NMR{<sup>1</sup>H} (101 MHz, D<sub>2</sub>O):  $\delta$  = 108.1, 108.0, 96.3, 92.1, 83.8, 83.7, 81.8, 81.0, 79.2, 76.3, 73.8, 71.1, 67.8, 67.7, 64.8, 61.0, 60.8 ppm. ESI-HRMS:  $m/z$  = [M+Na]<sup>+</sup> calcd. for C<sub>10</sub>H<sub>18</sub>NaO<sub>9</sub><sup>+</sup>: 305.0849; found 305.0848.

RP-HPLC of the deprotected disaccharide **21** (ELSD trace):

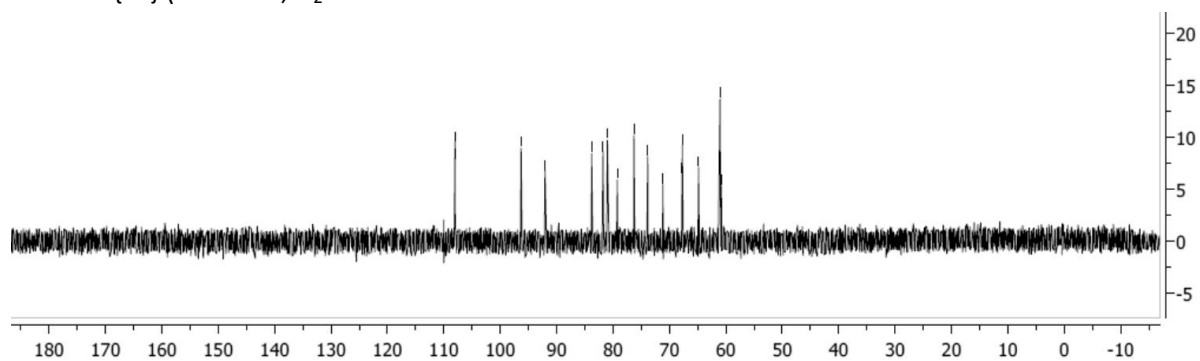


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):

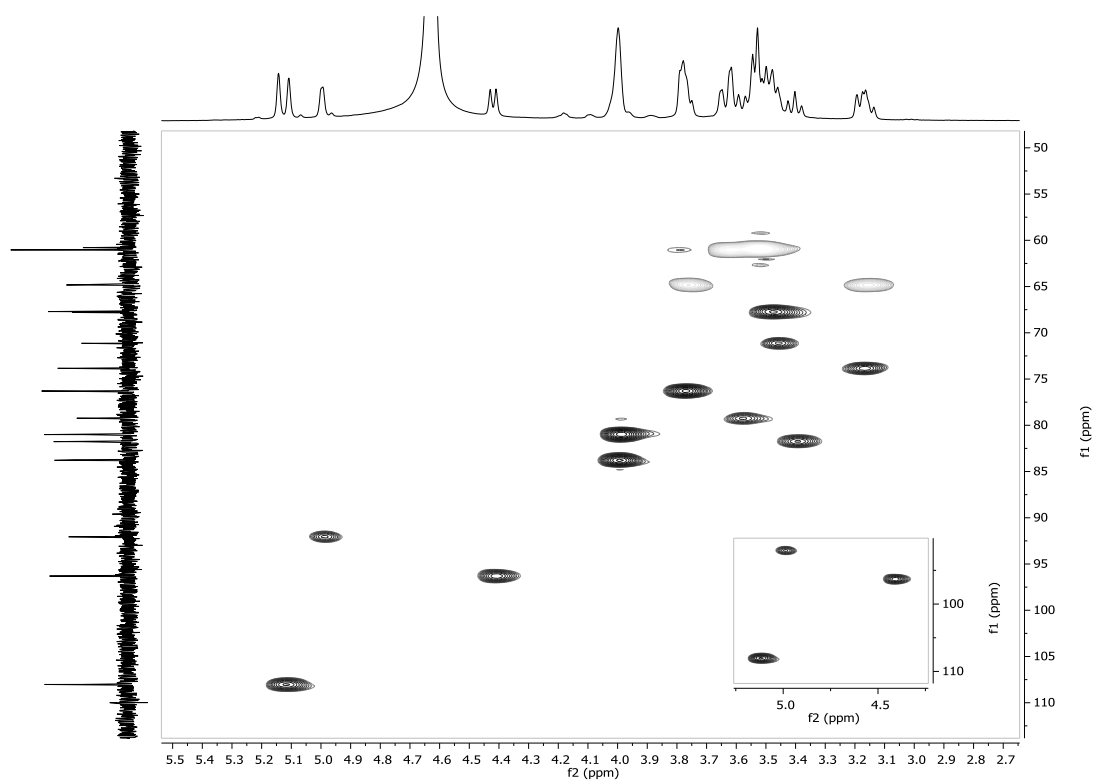


<sup>13</sup>C NMR{<sup>1</sup>H} (101 MHz, D<sub>2</sub>O):

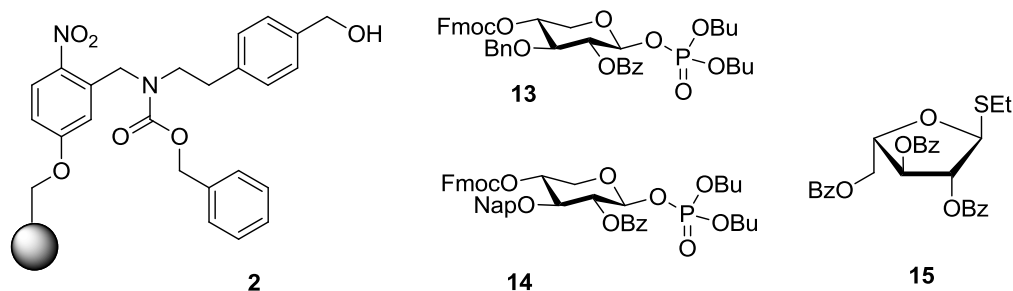




HSQC (D<sub>2</sub>O):

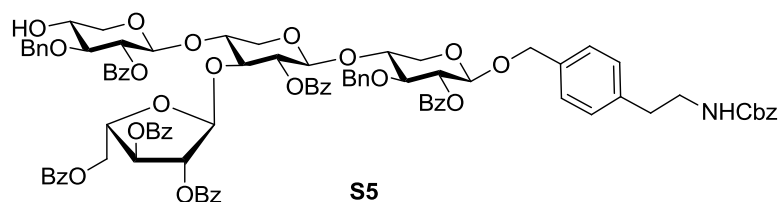


**Benzylloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3-O-benzyl  $\beta$ -D-xylopyranosyl-2-O-[2,3,5-tri-O-benzoyl- $\alpha$ -L-arabinofuranosyl]-3-O-benzoyl- $\beta$ -D-xylopyranosyl-2-O-benzoyl-3-O-benzyl  $\beta$ -D-xylopyranoside (S5)**



Module **A**: 2 x 1.4 equiv. **13** or  
**15**, TMSOTf, DCM  
Module **B**: 2 x 1.4 equiv. **15**, NIS,  
DCM/Dioxane  
Module **C**: 20%  $\text{NEt}_3$  in DMF  
Module **E**: DDQ, DCE/MeOH/ $\text{H}_2\text{O}$   
(64:16:1)

1)  $\begin{matrix} 3 \times \mathbf{A} \\ 2 \times \mathbf{C} \end{matrix}$   
2) **E**  
3) **B**  
4) **C**  
5)  $h\nu$  (305 nm)

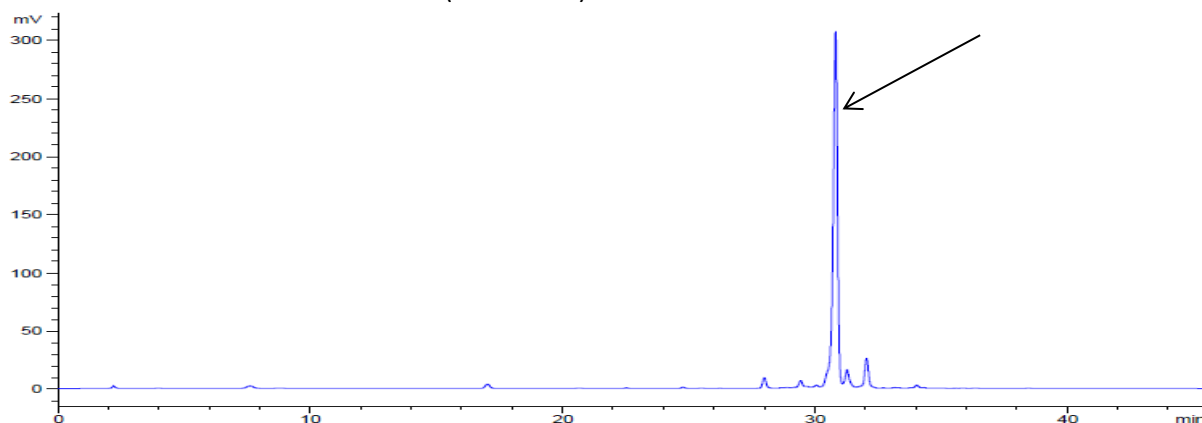


Linker-functionalized resin **2** (170 mg, 44.2  $\mu\text{mol}$ ) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35  $^\circ\text{C}$  to -15  $^\circ\text{C}$ )  
Module **C** (20%  $\text{NEt}_3$  in DMF, 3 x 5 min, r.t.)  
Module **A** (2 x 1.4 equiv **14**, TMSOTf, DCM, 2 x 35 min, -35  $^\circ\text{C}$  to -15  $^\circ\text{C}$ )  
Module **C** (20%  $\text{NEt}_3$  in DMF, 3 x 5 min, r.t.)  
Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35  $^\circ\text{C}$  to -15  $^\circ\text{C}$ )  
Module **E** (0.1 M DDQ in DCE/MeOH/ $\text{H}_2\text{O}$  (64:16:1))  
Module **B** (2 x 1.4 equiv **15**, NIS, 2 x 35 min, -40  $^\circ\text{C}$  to -20  $^\circ\text{C}$ )  
Module **C** (20%  $\text{NEt}_3$  in DMF, 3 x 5 min, r.t.)

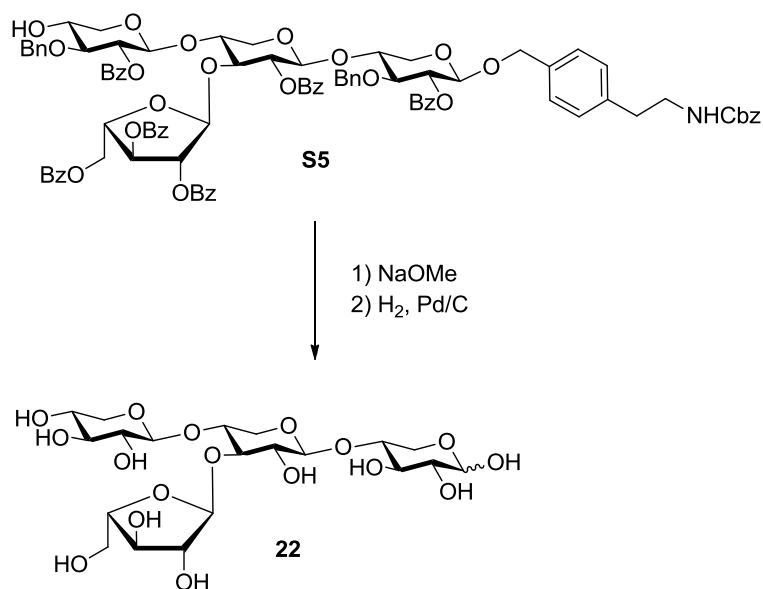
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected tetrasaccharide **S5** (28.1 mg, 17.4  $\mu\text{mol}$ , 40%).

Crude NP-HPLC of tetrasaccharide **S5** (ELSD trace):



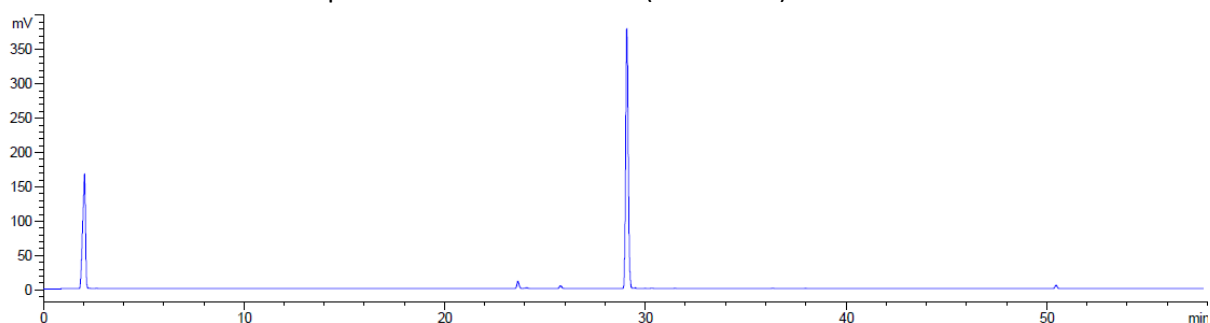
HPLC was performed using a YMC diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

### $\beta$ -D-Xylopyranosyl-2-O-[ $\alpha$ -L-arabinofuranosyl]- $\beta$ -D-xylopyranosyl-D-xylopyranose (**22**)



Tetrasaccharide **S5** was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide.

Crude RP-HPLC of the semi-protected tetrasaccharide (ELSD trace):

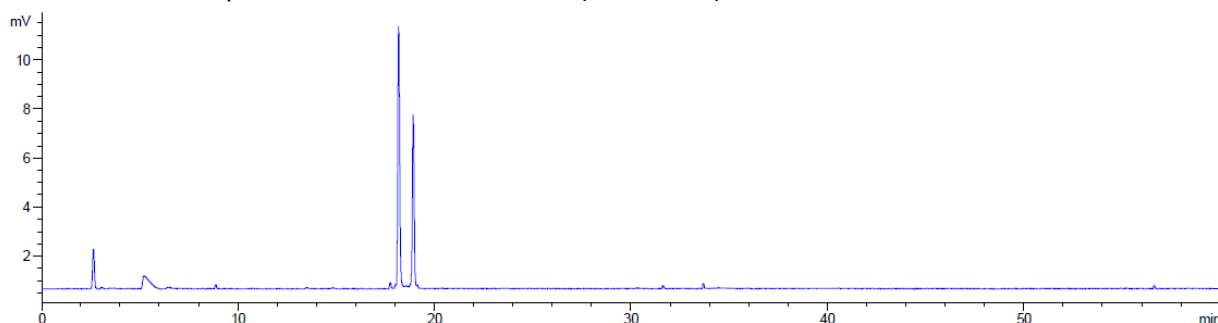


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 1.0 ml/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture was left. The fully deprotected tetrasaccharide **22** was stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **22** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an  $\alpha/\beta$ -mixture of the tetrasaccharide **22** (2.6 mg, 4.68  $\mu$ mol, 11% over 11 steps, based on resin loading).

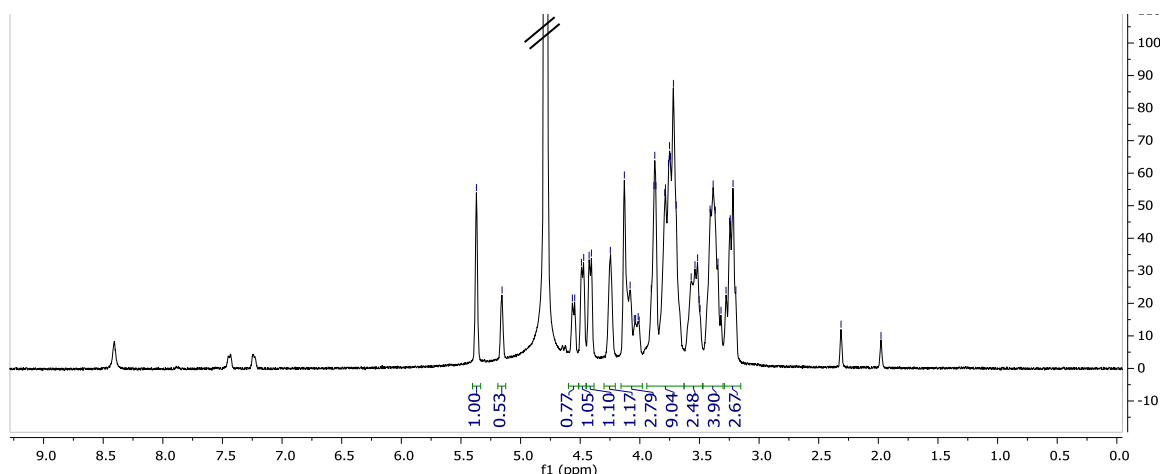
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 5.37 (s, 1H), 5.16 (s, 1H,  $\alpha$ -1H), 4.56 (d,  $J$  = 7.9 Hz, 1H,  $\beta$ -1H), 4.48 (d,  $J$  = 7.6 Hz, 1H), 4.42 (d,  $J$  = 7.8 Hz, 1H), 4.29 – 4.21 (m, 1H), 4.16 – 4.00 (m, 3H), 3.95 – 3.64 (m, 9H), 3.61 – 3.47 (m, 3H), 3.45 – 3.31 (m, 4H), 3.30 – 3.16 (m, 3H) ppm. <sup>13</sup>C NMR {<sup>1</sup>H} (101 MHz, D<sub>2</sub>O):  $\delta$  = 107.5, 101.6, 101.4, 96.4, 92.0, 84.7, 80.6, 77.2, 77.1, 76.6, 76.4, 75.5, 73.9, 73.8, 73.5, 73.2, 72.9, 71.3, 69.1, 65.0, 62.9, 62.7, 61.2, 58.7 ppm. ESI-HRMS:  $m/z$  = [M+Na]<sup>+</sup> calcd. for C<sub>20</sub>H<sub>34</sub>NaO<sub>17</sub><sup>+</sup>: 569.1694; found 569.1697.

RP-HPLC of the deprotected tetrasaccharide **22** (ELSD trace):

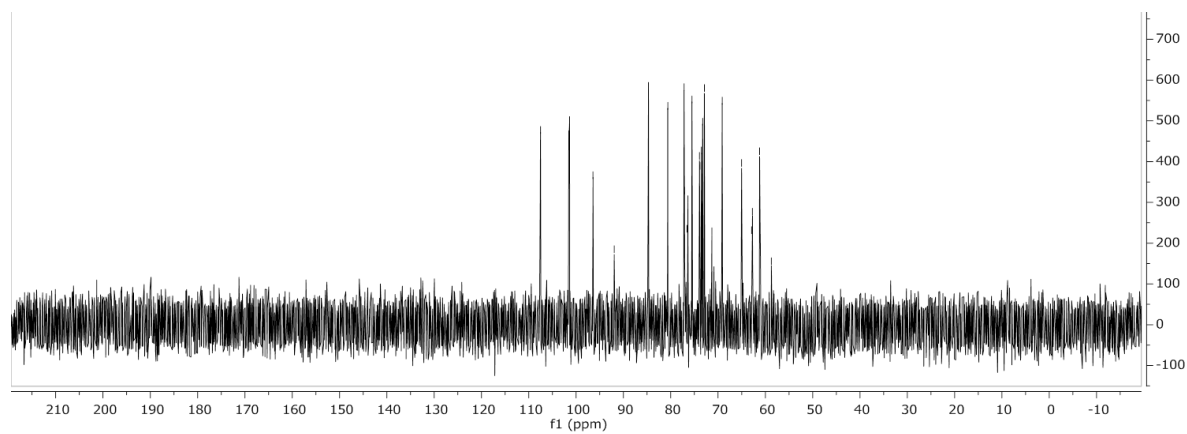


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).

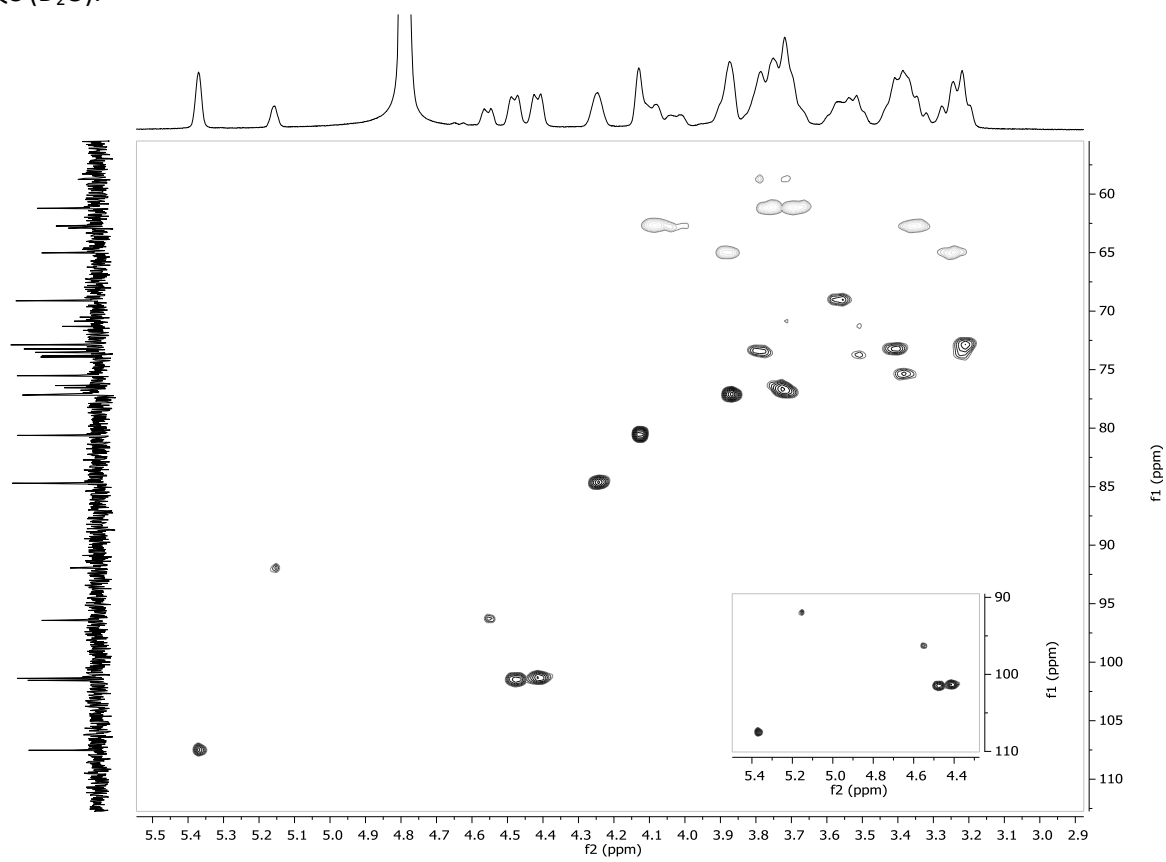
<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):



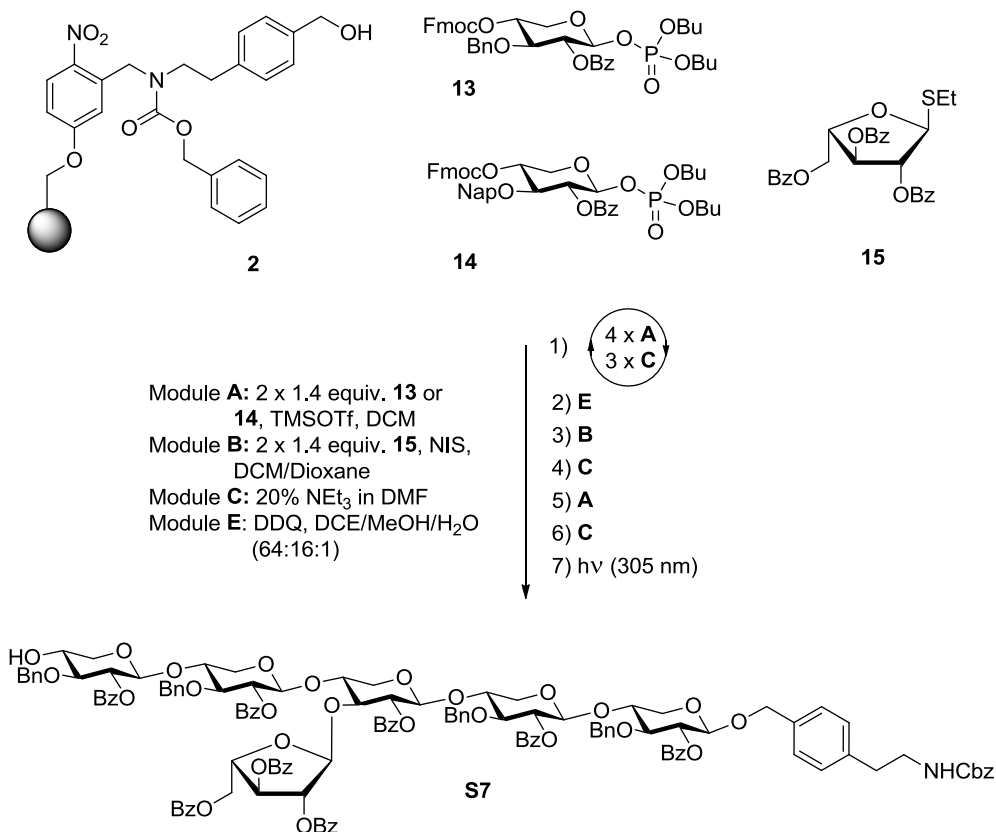
$^{13}\text{C}$  NMR  $\{^1\text{H}\}$  (101 MHz,  $\text{D}_2\text{O}$ ):



HSQC ( $\text{D}_2\text{O}$ ):



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3-O-benzyl-β-D-xylopyranosyl-2-O-benzoyl-3-O-benzyl-β-D-xylopyranosyl-2-O-[2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl]-3-O-benzoyl-β-D-xylopyranosyl-2-O-benzoyl-3-O-benzyl-β-D-xylopyranosyl-2-O-benzoyl-3-O-benzyl β-D-xylopyranoside (S7)**

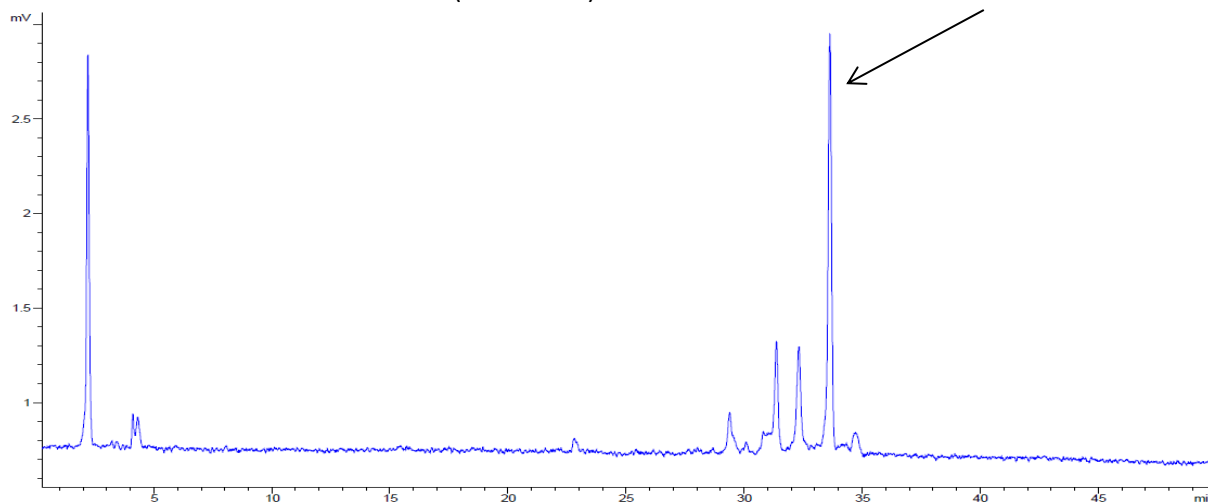


Linker-functionalized resin **2** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

- Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)
- Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)
- Module **A** (2 x 1.4 equiv **14**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)
- Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
- Module **E** (0.1 M DDQ in DCE/MeOH/H<sub>2</sub>O (64:16:1))
- Module **B** (2 x 1.4 equiv **15**, NIS, 2 x 35 min, -40 °C to -20 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)
- Module **A** (2 x 1.4 equiv **13**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, r.t.)

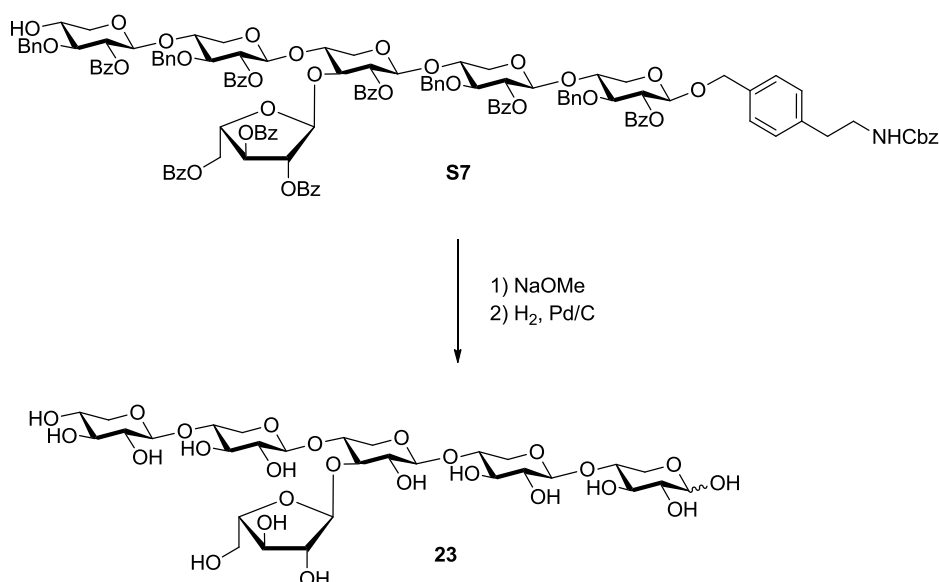
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected hexasaccharide **S7** (22.1 mg, 9.73 μmol, 22%).

Crude NP-HPLC of hexasaccharide **57** (ELSD trace):



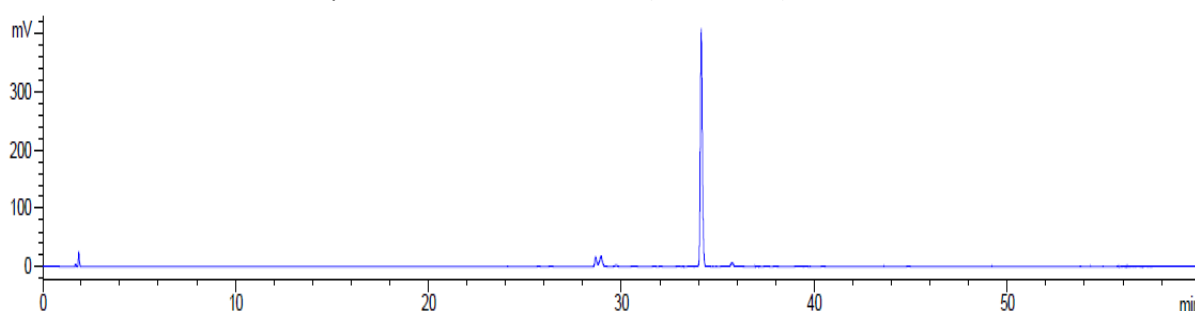
HPLC was performed using a YMC diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

### $\beta$ -D-Xylopyranosyl-2-O-[ $\alpha$ -L-arabinofuranosyl]- $\beta$ -D-xylopyranosyl-D-xylopyranose (**23**)



Hexasaccharide **57** was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected hexasaccharide.

Crude RP-HPLC of the semi-protected hexasaccharide (ELSD trace):

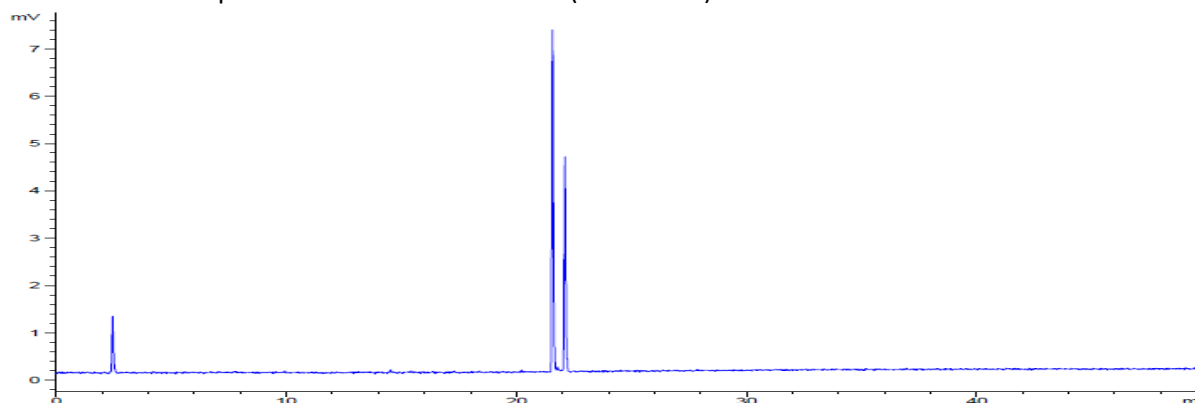


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 1.0 ml/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H<sub>2</sub>O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was saturated with H<sub>2</sub> for 30 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture was left. The fully deprotected hexasaccharide **23** was stirred with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **23** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an  $\alpha/\beta$ -mixture of the hexasaccharide **23** (1.0 mg, 1.25  $\mu$ mol, 3% over 15 steps, based on resin loading).

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 5.20 (s, 1H), 4.99 (d,  $J$  = 3.6 Hz, 1H,  $\alpha$ -1H), 4.39 (dd,  $J$  = 7.7, 1.7 Hz, 1H), 4.34 – 4.22 (m, 4H), 4.09 (q,  $J$  = 5.1 Hz, 1H), 3.98 – 3.83 (m, 4H), 3.80 – 3.69 (m, 3H), 3.68 – 3.30 (m, 15H), 3.29 – 3.02 (m, 10H) ppm. <sup>13</sup>C NMR {<sup>1</sup>H} (151 MHz, D<sub>2</sub>O):  $\delta$  = 110.2, 110.0, 104.9, 104.4, 104.2, 103.9, 87.3, 83.3, 82.6, 82.5, 79.9, 79.8, 79.1, 79.0, 78.9, 78.2, 76.2, 76.2, 75.9, 75.6, 75.5, 75.3, 75.3, 74.0, 72.5, 71.8, 67.8, 65.5, 65.5, 65.4, 65.2, 63.9, 63.9, 63.2 ppm. ESI-HRMS:  $m/z$  = [M+Na]<sup>+</sup> calcd. for C<sub>20</sub>H<sub>34</sub>NaO<sub>17</sub><sup>+</sup>: 833.2539; found 833.2526.

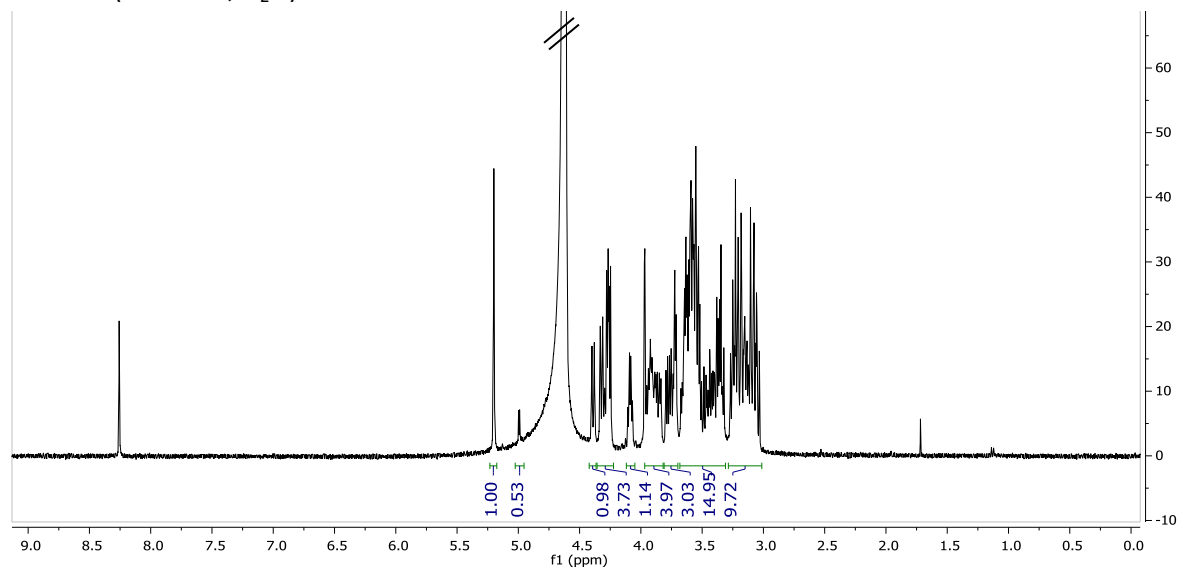
RP-HPLC of the deprotected hexasaccharide **23** (ELSD trace):



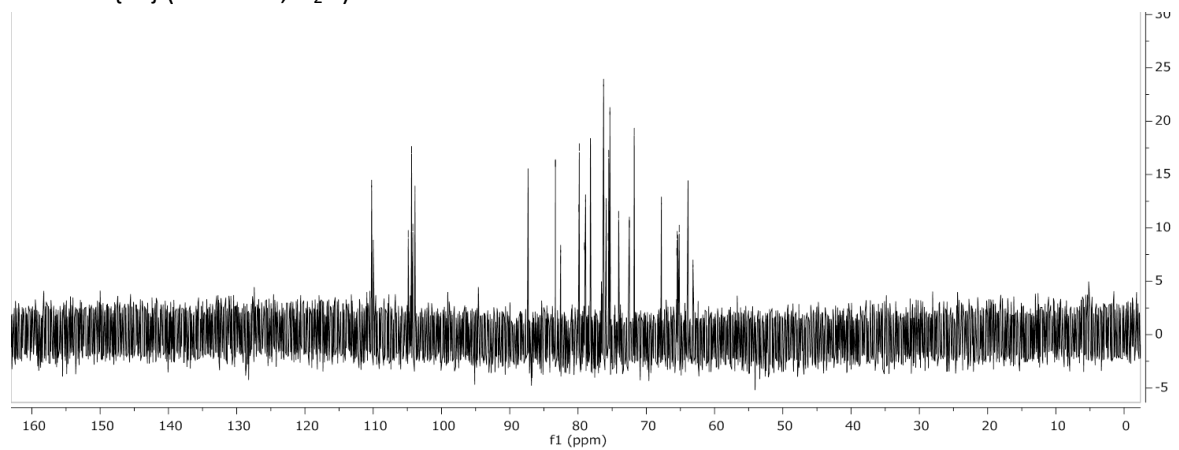
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).



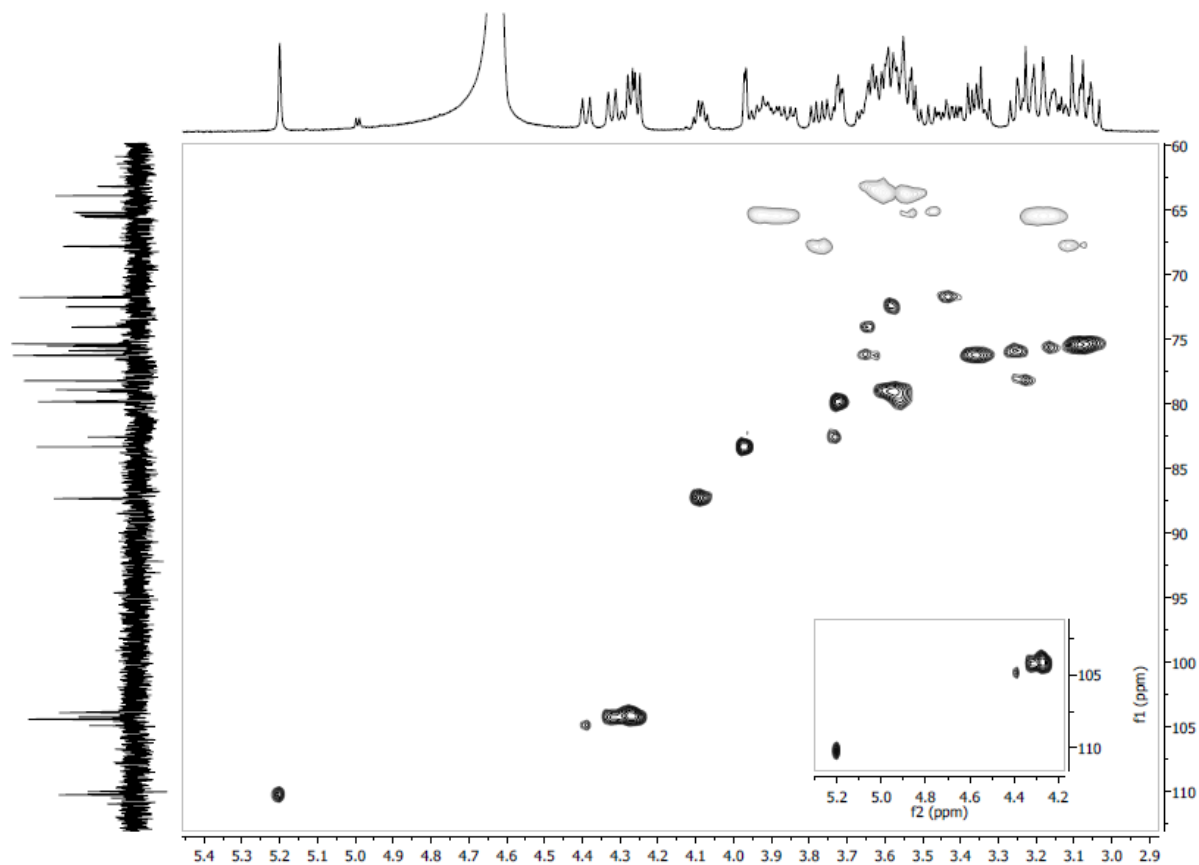
$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):



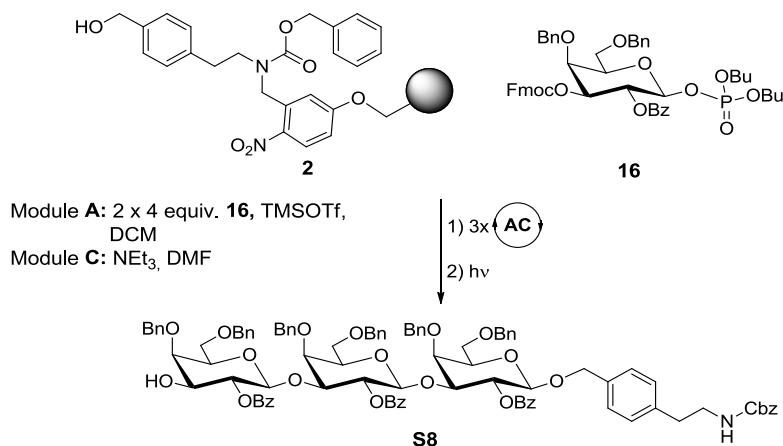
$^{13}\text{C}$  NMR{ $^1\text{H}$ } (151 MHz,  $\text{D}_2\text{O}$ ):



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



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside (S8)**



Linker functionalized resin **2** (60 mg, 15.1 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module A (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module A (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

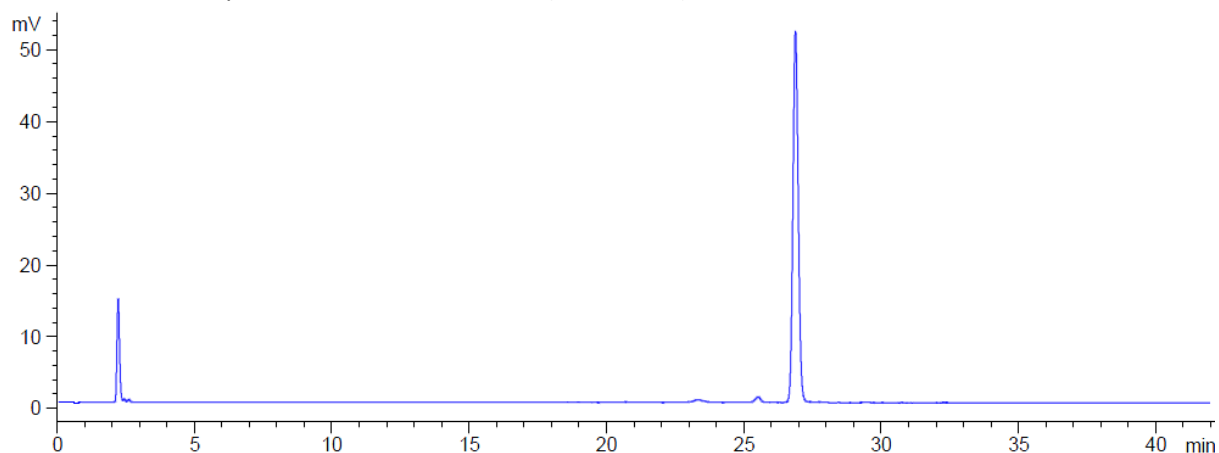
Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

Module A (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

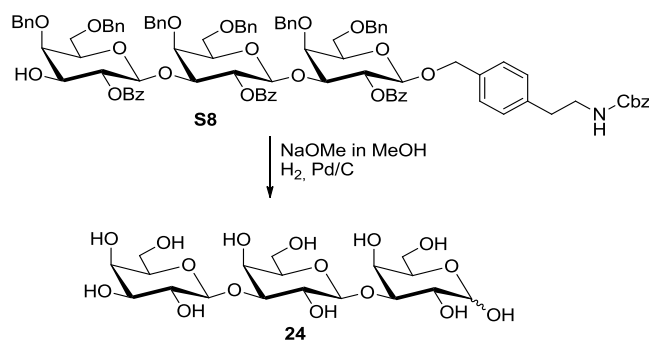
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected trisaccharide **S8**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (12 mg, 7.39 μmol, 49%).

Crude NP-HPLC of protected trisaccharide **S8** (ELSD trace):



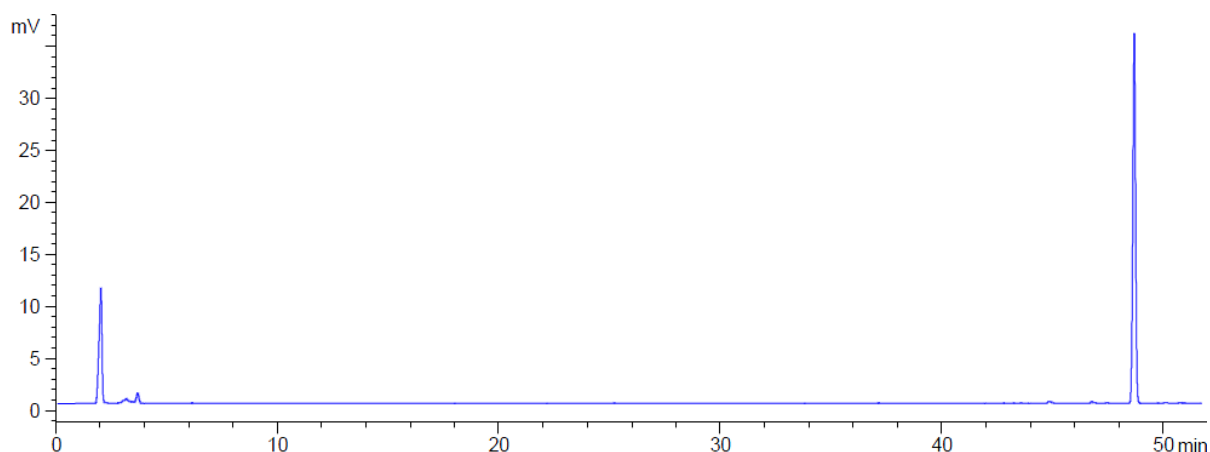
HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

## $\beta$ -D-Galactopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)-D-galactopyranose (**24**)



The protected trisaccharide **58** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide.

Crude RP-HPLC of the semi-protected trisaccharide (ELSD trace):



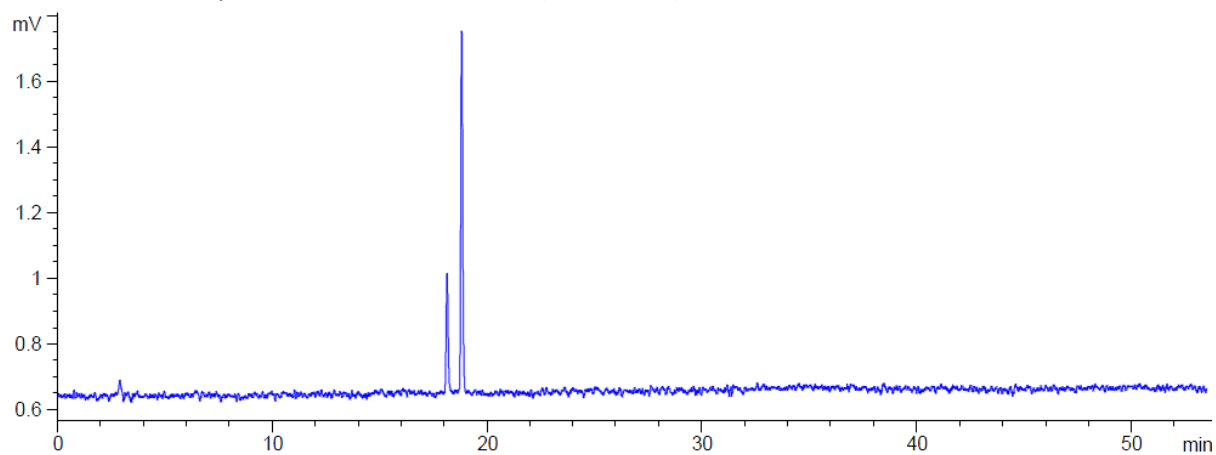
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H<sub>2</sub>O/AcOH (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with Ar for 5 min then saturated with H<sub>2</sub> for 10 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated. The fully deprotected trisaccharide **24** was re-dissolved in 0.5 mL water and stirred for one hour with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze a side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **24** was directly purified by reversed phase HPLC using a semi-preparative Hypercarb column to give an  $\alpha/\beta$ -mixture of the trisaccharide **24** (0.6 mg, 1.19  $\mu$ mol, 8% over 9 steps, based on resin loading).

<sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  = 5.30 (d,  $J$  = 3.4 Hz, 1H), 4.70 (t,  $J$  = 7.4 Hz, 1H), 4.65 (t,  $J$  = 8.1 Hz, 2H), 4.28 (d,  $J$  = 2.0 Hz, 1H), 4.22 (b, 2H), 4.14 (t,  $J$  = 6.1 Hz, 1H), 4.01 (qd,  $J$  = 10.3, 3.2 Hz, 1H), 3.94 (d,  $J$  = 3.2 Hz, 1H), 3.88 – 3.82 (m, 2H), 3.81 – 3.61 (m, 13H) ppm. <sup>13</sup>C NMR {<sup>1</sup>H} (151 MHz, D<sub>2</sub>O)  $\delta$  = 106.6, 98.7,

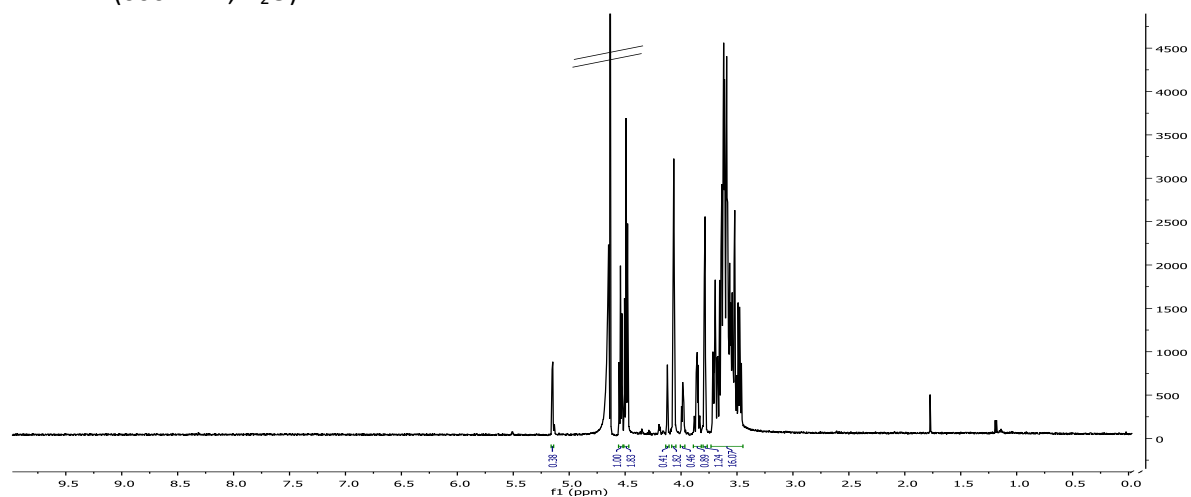
85.0, 84.6, 81.9, 77.3, 77.2, 75.1, 73.6, 73.5, 72.8, 71.1, 70.9, 63.7, 63.5, 63.5, 63.4, 63.4 ppm. ESI-HRMS:  $m/z$   $[M+Na]^+$  calcd. for  $C_{18}H_{32}O_{16}$ : 527.1588, found: 527.1534

RP-HPLC of the deprotected trisaccharide **24** (ELSD trace):

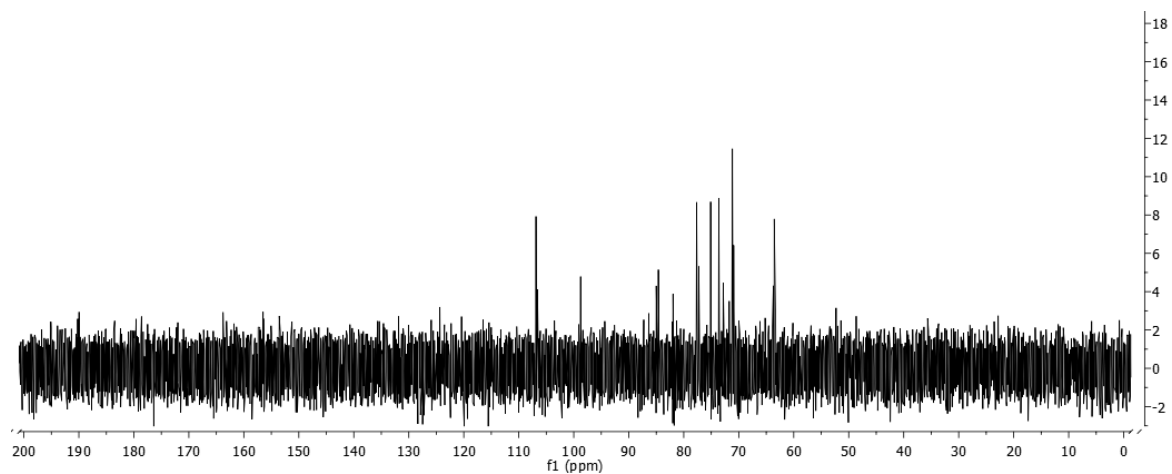


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30%  $H_2O$  (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0%  $H_2O$  in MeCN (10 min, flow rate 0.7 mL/min).

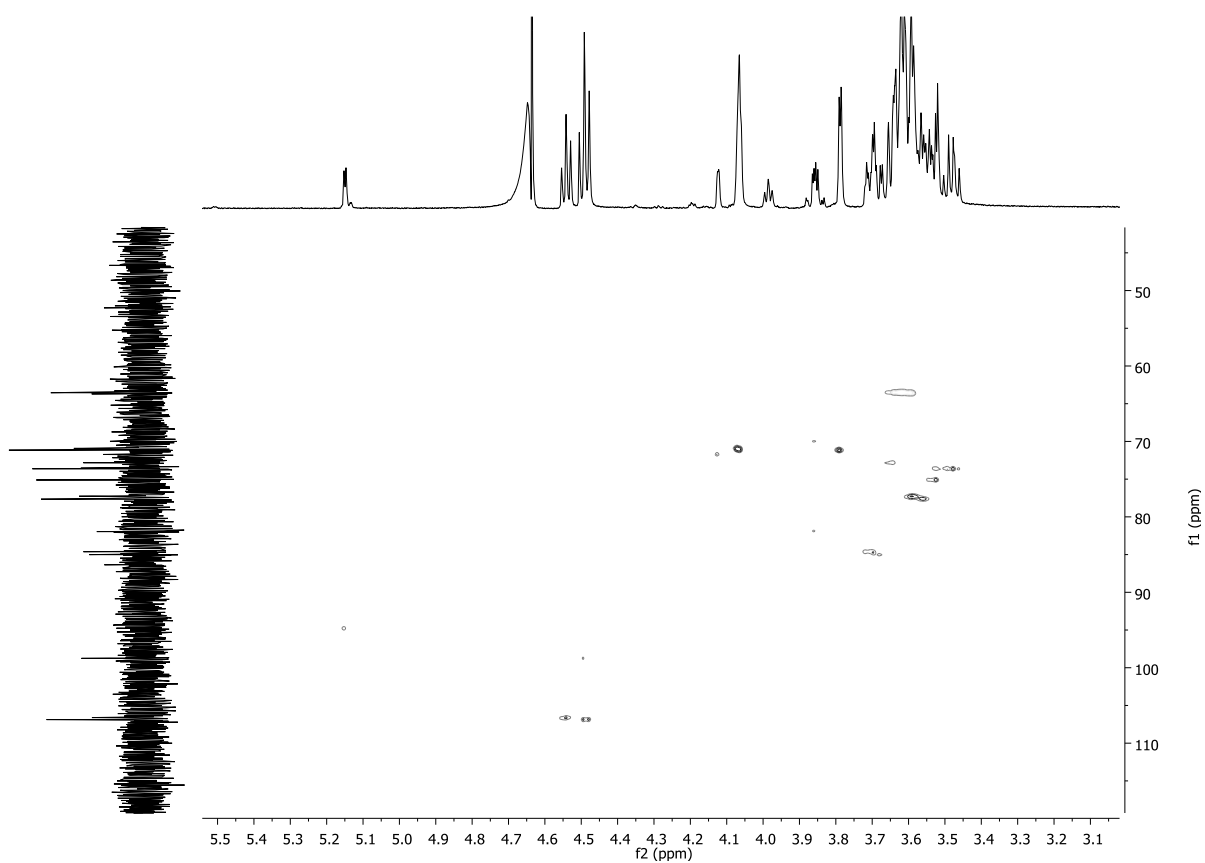
$^1H$  NMR (600 MHz,  $D_2O$ ):



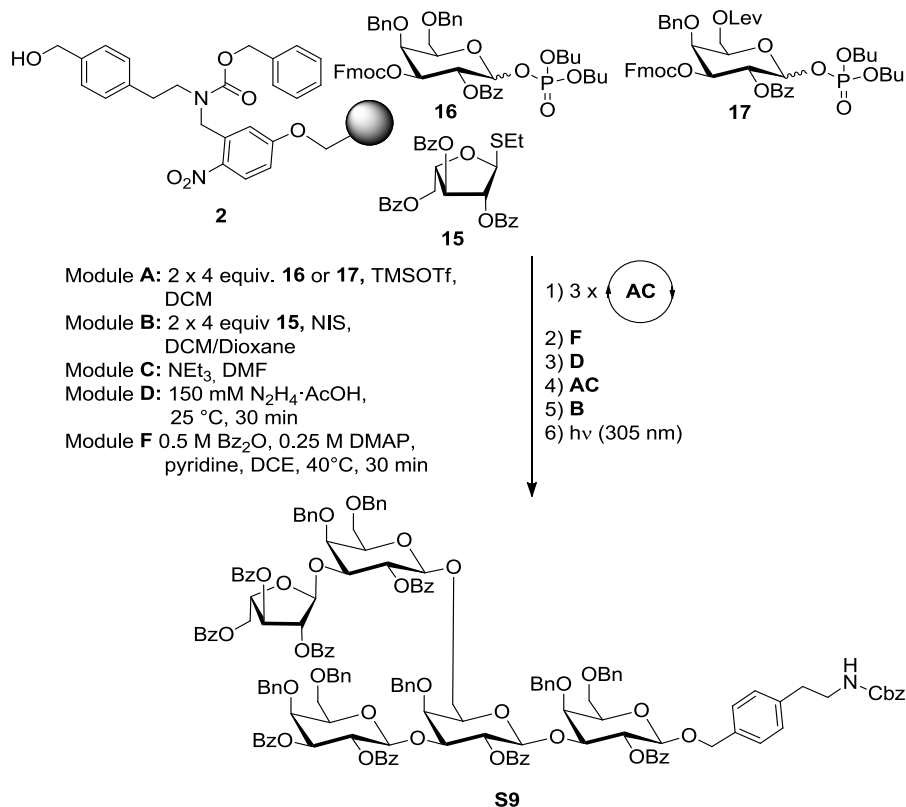
$^{13}C$  NMR (151 MHz,  $D_2O$ ):



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



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-4-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-4,6-O-dibenzyl-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside (**S9**)**

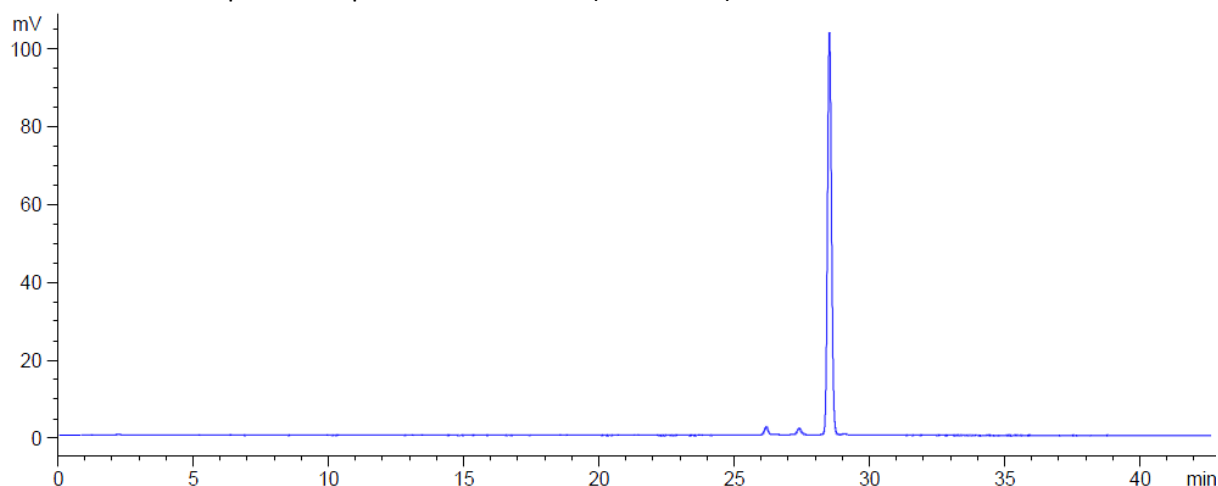


Linker functionalized resin **2** (60 mg, 15.1 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

- Module **A** (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module **A** (2 x 4 equiv. **17**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module **A** (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module **F** (0.5 M Bz<sub>2</sub>O, 0.25 M DMAP, Py, DCE, 3 x 30 min, 40 °C)
- Module **D** (0.15 M N<sub>2</sub>H<sub>4</sub> in Py/AcOH/H<sub>2</sub>O (4:1:0.25), 3 x 30 min, 25 °C)
- Module **A** (2 x 4 equiv. **16**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module **C** (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module **B** (2 x 4 equiv. **15**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

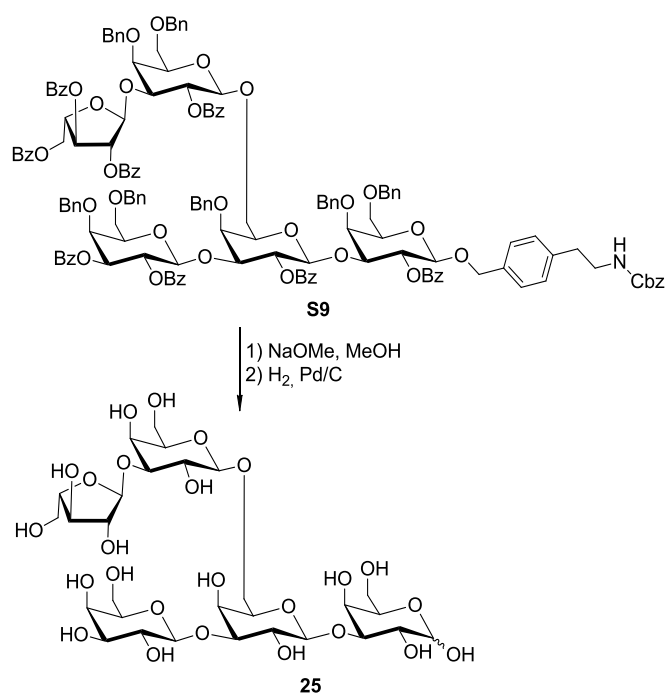
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **S9**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (17 mg, 6.72 μmol, 45%).

Crude NP-HPLC of protected pentasaccharide **S9** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

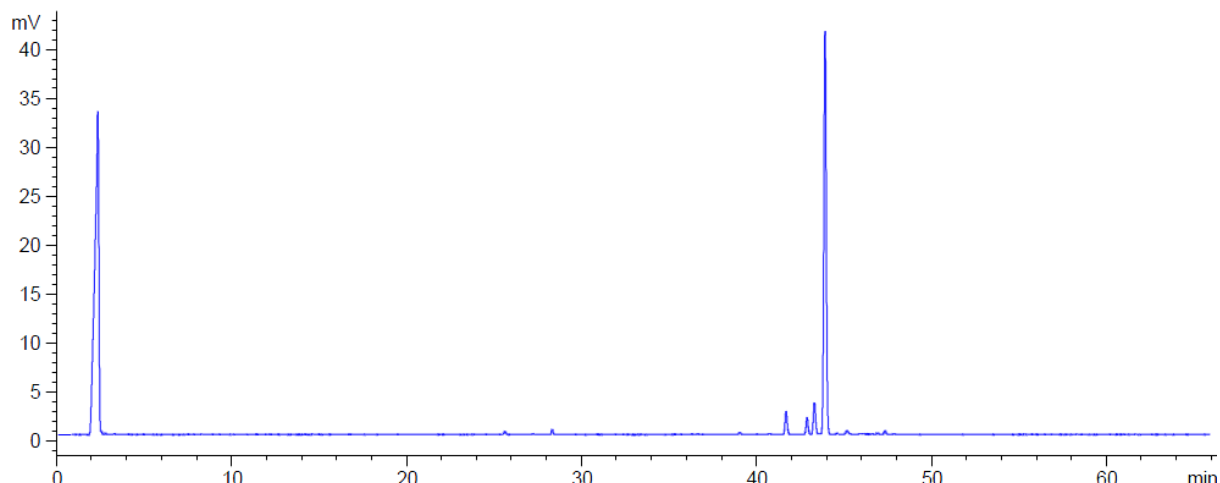
**$\beta$ -D-Galactopyranosyl-(1 $\rightarrow$ 3)-6-O-[3-O-[ $\alpha$ -L-arabinofuranosyl]- $\beta$ -D-galactopyranosyl]- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)-D-galactopyranose (**25**)**



The protected pentasaccharide **S9** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected hexasaccharide.



Crude RP-HPLC of the semi-protected pentasaccharide (ELSD trace):

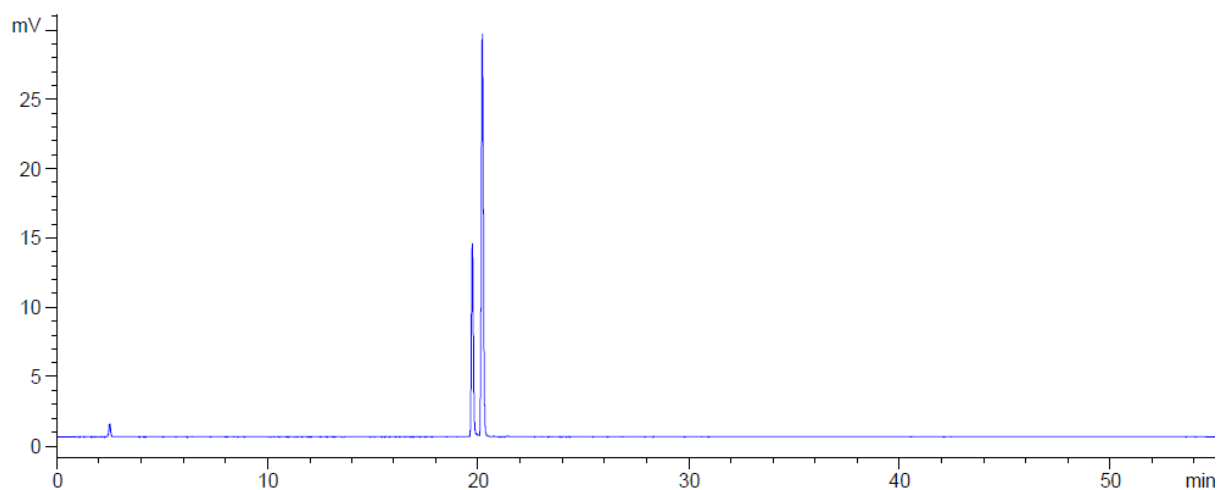


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H<sub>2</sub>O/AcOH (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with Ar for 10 min then saturated with H<sub>2</sub> for 10 min and stirred under a H<sub>2</sub>-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture remained and the solvents were removed at a lyophilizer. The fully deprotected pentasaccharide **25** was re-dissolved in 0.5 mL water and purified by reversed phase HPLC using a semi-preparative Hypercarb column to give an  $\alpha/\beta$ -mixture of the pentasaccharide **25** (1.2 mg, 1.48  $\mu$ mol, 10% over 14 steps, based on resin loading).

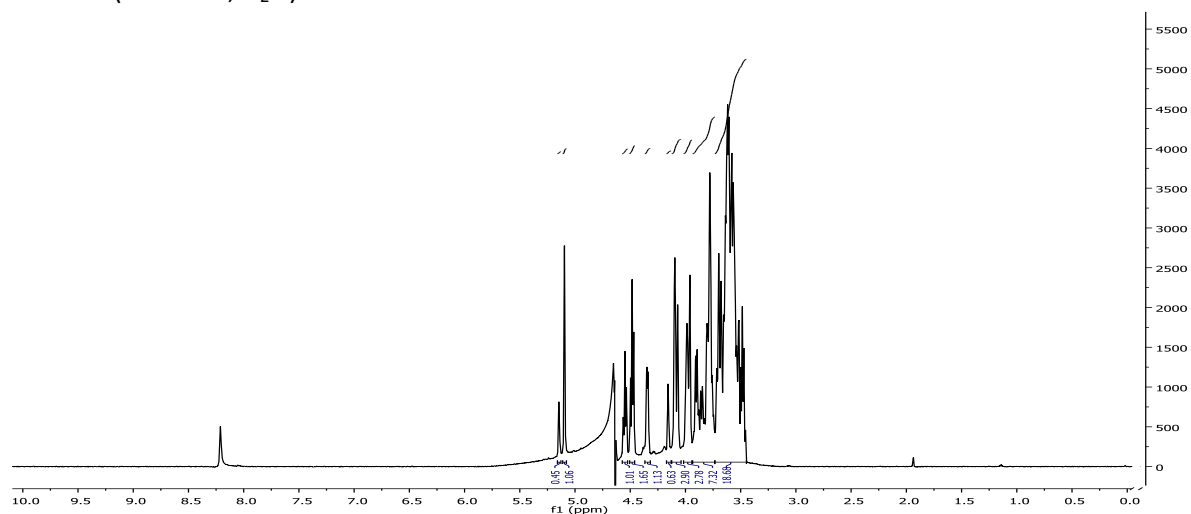
<sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta$  = 5.15 (s, 1H), 5.10 (s, 1H), 4.55 (t,  $J$  = 8.0 Hz, 1H), 4.48 (t,  $J$  = 8.6 Hz, 1H), 4.34 (d,  $J$  = 6.2 Hz, 1H), 4.16 (s, 1H), 4.08 (d,  $J$  = 15.4 Hz, 3H), 3.97 (d,  $J$  = 15.5 Hz, 2H), 3.93 – 3.74 (m, 7H), 3.73 – 3.45 (m, 17H) ppm. <sup>13</sup>C NMR {<sup>1</sup>H} (151 MHz, D<sub>2</sub>O):  $\delta$  = 111.8, 111.8, 106.9, 106.4, 106.4, 105.8, 98.7, 98.7, 94.7, 86.4, 84.9, 84.9, 84.5, 83.8, 83.8, 82.8, 82.8, 81.9, 79.0, 79.0, 77.6, 77.5, 77.3, 75.8, 75.8, 75.1, 73.6, 73.5, 72.7, 72.6, 72.5, 71.8, 71.7, 71.7, 71.7, 71.1, 71.0, 71.0, 71.0, 70.9, 69.9, 63.8, 63.7, 63.5 ppm. [M+Na]<sup>+</sup> calcd. for C<sub>29</sub>H<sub>50</sub>O<sub>25</sub>: 821.2538, found: 821.2538

RP-HPLC of the deprotected pentasaccharide **25** (ELSD trace):

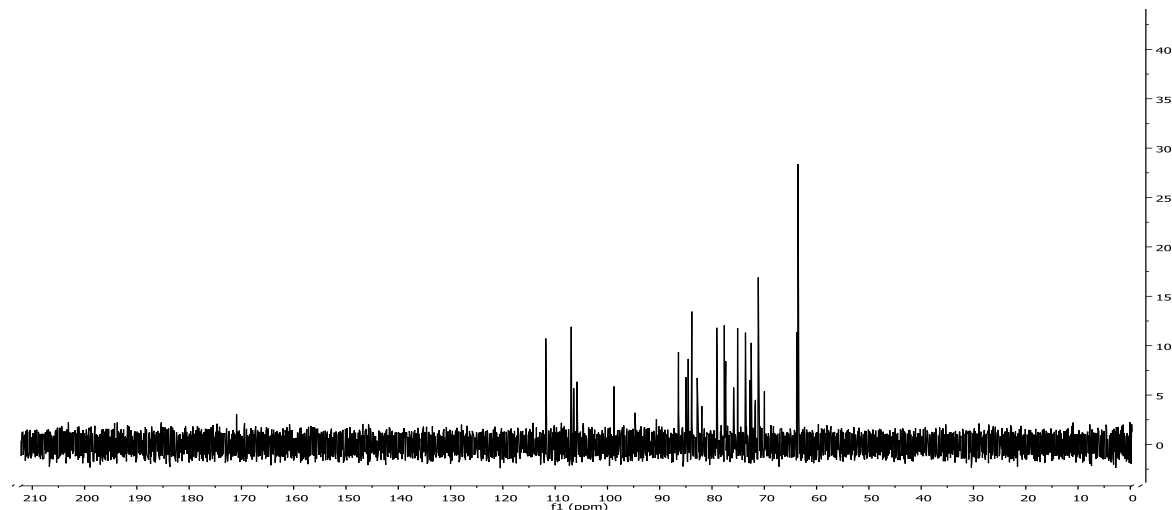


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).

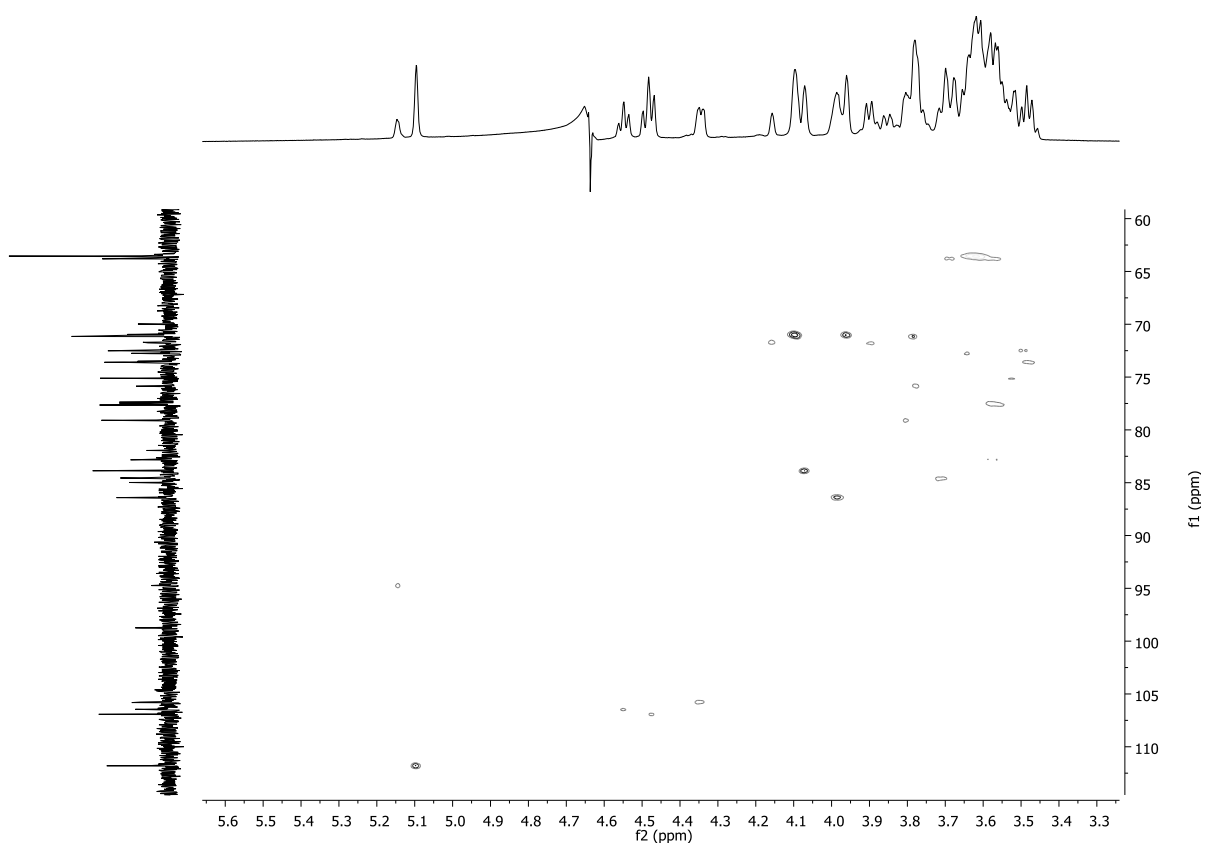
<sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):



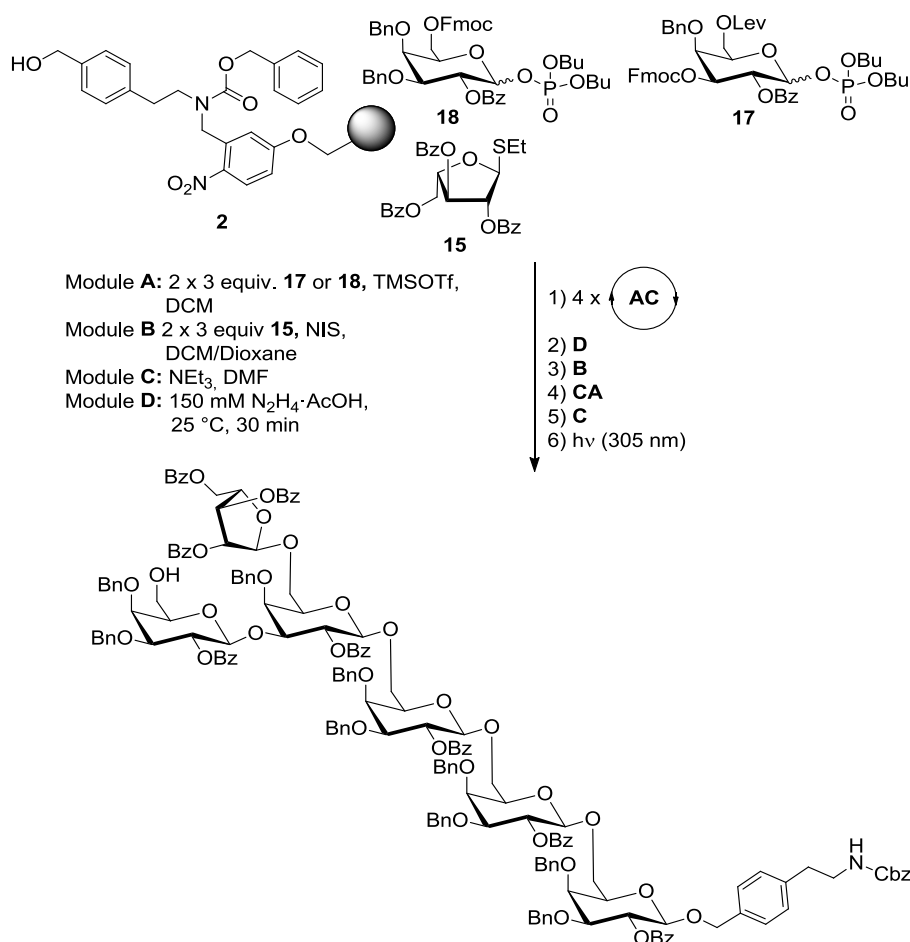
<sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O):



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



**Benzyloxycarbonyl-(4-(2-aminoethyl)benzyl) 2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranoside (S10)**

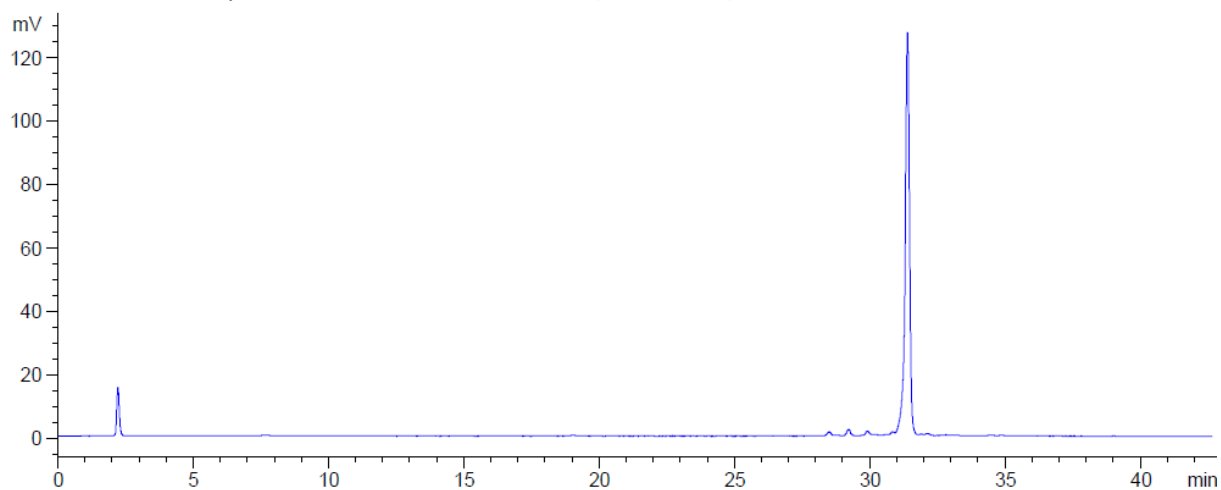


Linker functionalized resin **2** (85 mg, 22.1 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

- Module A (2 x 3 equiv. **18**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module A (2 x 3 equiv. **18**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module A (2 x 3 equiv. **18**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module A (2 x 3 equiv. **17**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module D (0.15 M N<sub>2</sub>H<sub>4</sub> in Py/AcOH/H<sub>2</sub>O (4:1:0.25), 3 x 30 min, 25 °C)
- Module B (2 x 3 equiv. **15**, NIS, TFOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
- Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)
- Module A (2 x 3 equiv. **18**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
- Module C (20% NEt<sub>3</sub> in DMF, 3 x 5 min, rt)

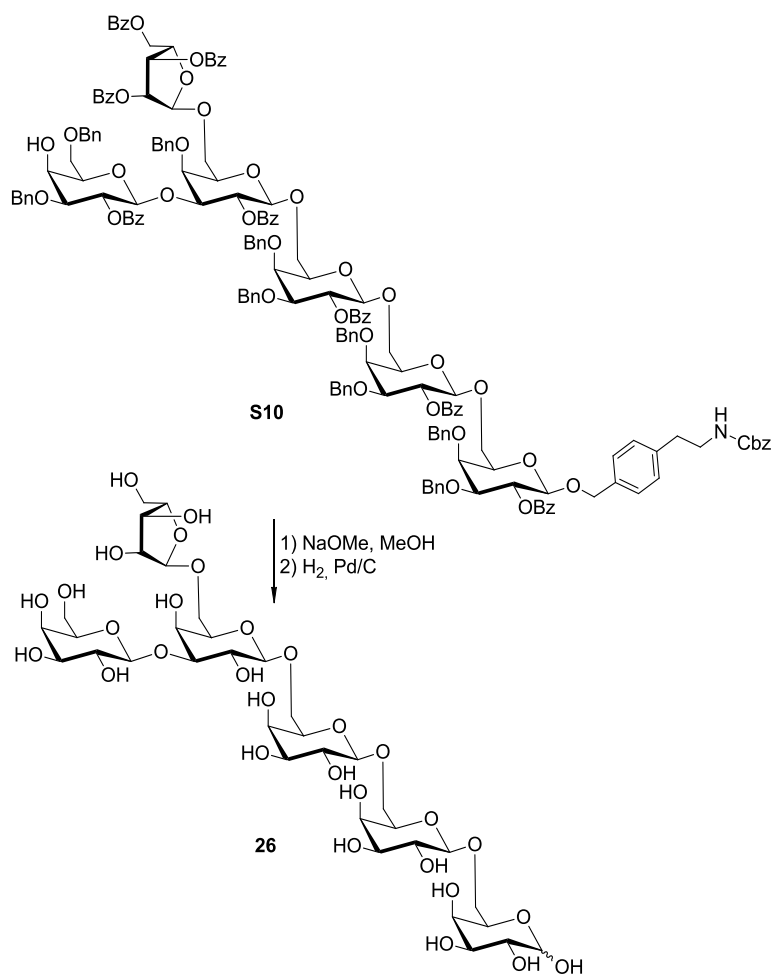
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected hexasaccharide **S10**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (23 mg, 8.01  $\mu$ mol, 36%).

Crude NP-HPLC of protected hexasaccharide **S10** (ELSD trace):



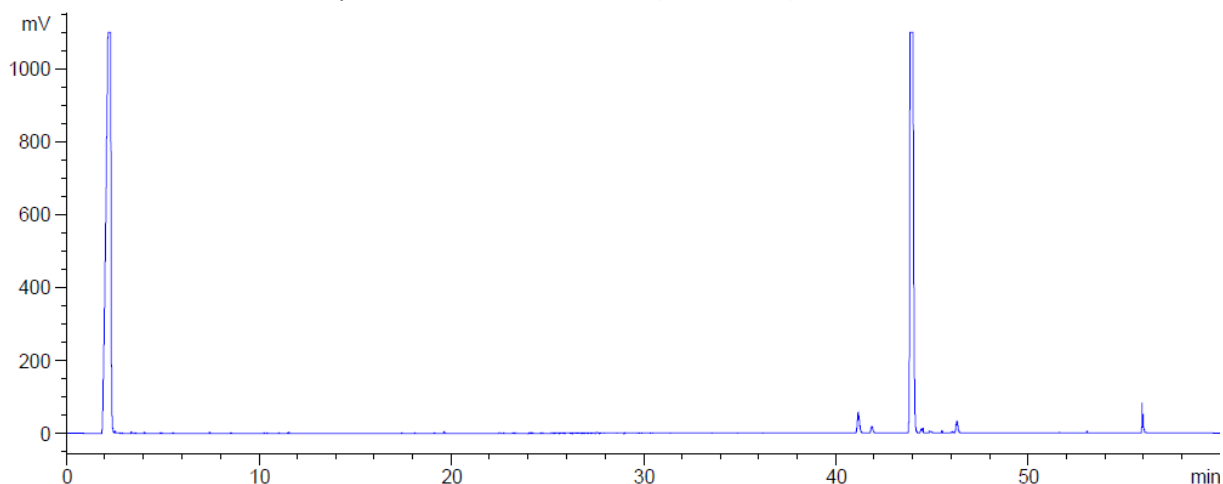
HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

**$\beta$ -D-Galactopyranosyl-(1 $\rightarrow$ 3)-[6-O-[ $\alpha$ -L-arabinofuranosyl]- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-D-galactopyranose (**26**)**



The protected hexasaccharide **S10** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo* to afford the semi-protected hexasaccharid.

Crude RP-HPLC of the semi-protected hexasaccharide (ELSD trace):

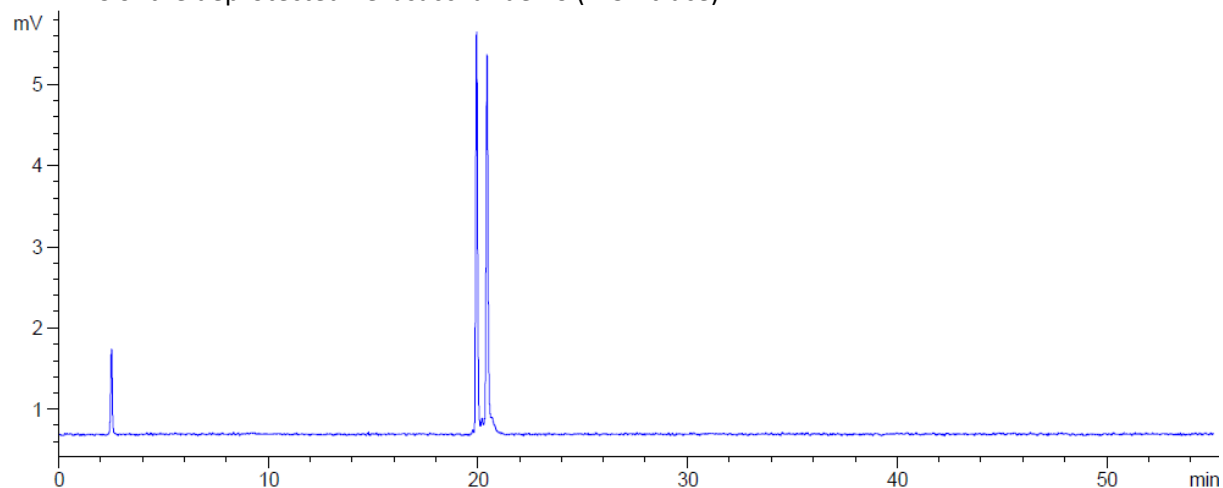


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H<sub>2</sub>O/AcOH (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with Ar for 10 min, then saturated with H<sub>2</sub> for 10 min and stirred under a H<sub>2</sub>-atmosphere overnight. The hydrogenation procedure was repeated two times until all protecting groups were removed. After filtration of the reaction mixture through a syringe filter the solvents were evaporated. The fully deprotected hexasaccharide **26** was re-dissolved in 0.5 mL water and stirred for one hour with a spatula tip of H<sup>+</sup>-Amberlite resin to hydrolyze a side product that has been formed by condensation of the oligosaccharide with the cleaved linker. The fully deprotected hexasaccharide **26** was purified by reversed phase HPLC using a semi-preparative Hypercarb column to give an  $\alpha/\beta$ -mixture of the hexasaccharide **26** (0.8 mg, 0.85  $\mu$ mol, 4% over 15 steps, based on resin loading).

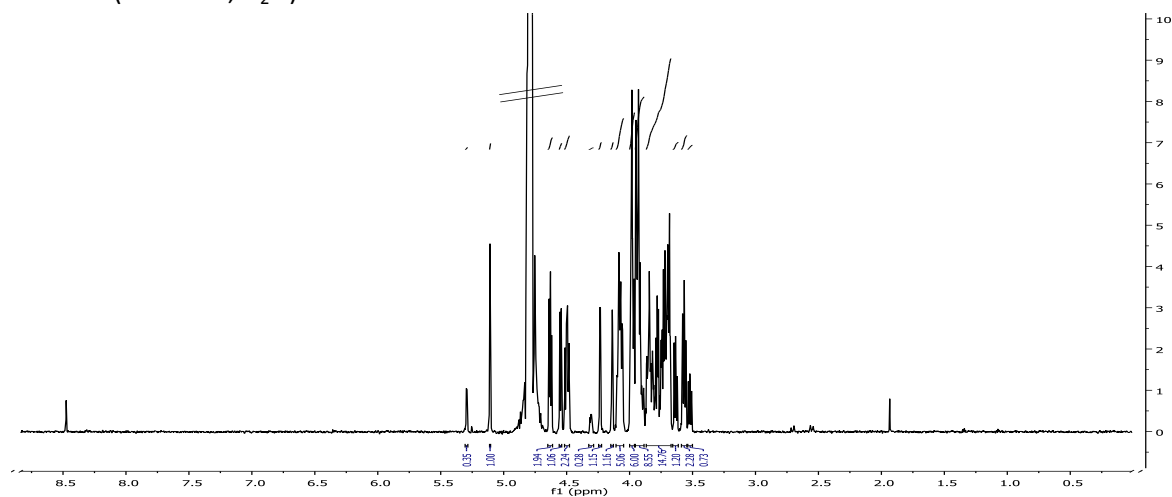
<sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O)  $\delta$  = 5.29 (d,  $J$  = 3.8 Hz, 1H), 5.11 (s, 1H), 4.65 – 4.61 (m, 2H), 4.55 (d,  $J$  = 7.9 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.31 (dd,  $J$  = 7.8, 4.0 Hz, 1H), 4.23 (d,  $J$  = 3.0 Hz, 1H), 4.14 – 4.13 (m, 1H), 4.10 – 4.05 (m, 5H), 4.00 – 3.67 (m, 24H), 3.65 – 3.62 (m, 1H), 3.58 – 3.55 (m, 2H), 3.52 (dd,  $J$  = 9.8, 8.1 Hz, 1H) ppm. <sup>13</sup>C NMR {<sup>1</sup>H} (176 MHz, D<sub>2</sub>O)  $\delta$  = 107.8, 104.3, 103.4, 103.2, 103.1, 96.4, 92.3, 83.8, 82.2, 81.0, 76.4, 75.0, 73.7, 73.5, 72.6, 72.5, 71.7, 71.0, 70.7, 70.6, 69.7, 69.5, 69.4, 69.3, 69.2, 68.9, 68.7, 68.6, 68.5, 68.2, 67.3, 61.1, 60.9 ppm. [M+Na]<sup>+</sup> calcd. for C<sub>35</sub>H<sub>60</sub>O<sub>30</sub>: 983.3067, found: 983.3007

RP-HPLC of the deprotected hexasaccharide **26** (ELSD trace):

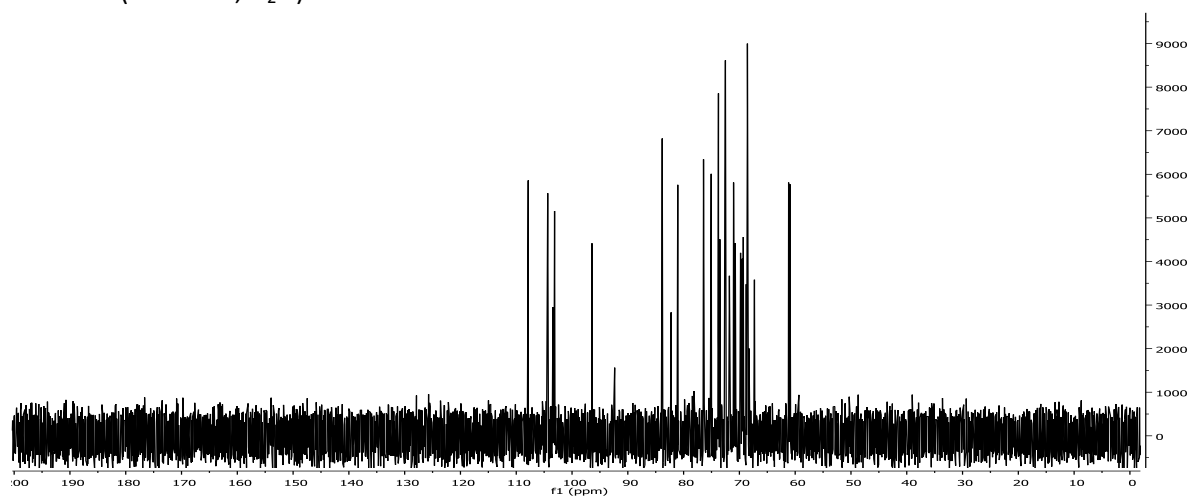


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H<sub>2</sub>O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H<sub>2</sub>O in MeCN (10 min, flow rate 0.7 mL/min).

<sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O):

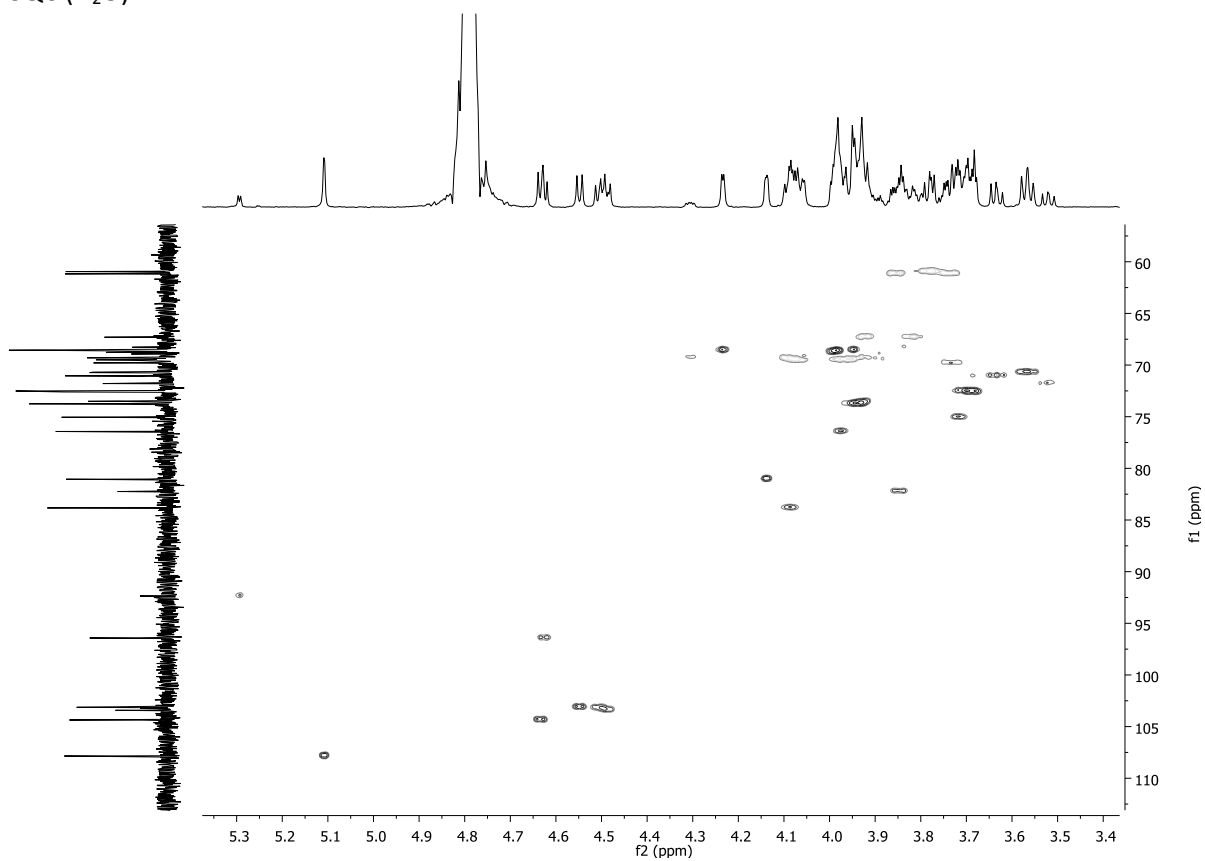


<sup>13</sup>C NMR (176 MHz, D<sub>2</sub>O):





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



## References

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