Electronic Supplementary Information

Automated glycan assembly of galactosylated xyloglucan oligosaccharides and their recognition by plant cell wall glycan-directed antibodies

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Contents

Abbreviations

Ac: acetyl; AcOH: acetic acid; AGA: automated glycan assembly; Ar: aryl; BB: building block; Bn: benzyl; BSA: bovine serum albumin; Bu: Butyl; Bz: Benzoyl; Cbz: carboxybenzyl; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DCE: 1,2-dichloroethane; DCM: dichloromethane; DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone; DIC: *N,N'*-diisopropylcarbodiimide; DMF: dimethylformamide; DMAP: dimethylaminopyridine; EtN_3: triethylamine; Et: ethyl; EtOAc: ethyl acetate; Et_2O: diethyl ether; Fmoc: fluorenylmethyloxycarbonyl; Glc: glucose; Hex: hexane; Lev: levulinoyl; LevOH: levulinic acid; mAbs: monoclonal antibodies; MeCN: acetonitrile; MeOH: methanol; NaOMe: sodium methoxide; NHS: *N*-hydroxyl succinimide; NIS: *N*-iodosuccinimide; NP: normal-phase; PBS: phosphate-buffered saline; Ph: phenyl; PMB: p-methoxybenzyl; RP: reverse-phase; rt: room temperature; THF: tetrahydrofuran; TfOH: trifluoromethanesulfonic acid; TMSOTf: trimethylsilyl trifluoromethanesulfonate; Tol: tolyl; Xyl: xylose.

General information

The automated syntheses were performed on a self-built synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Linker-functionalized resin **5** was prepared according to literature.[1] The Resin loading was determined as described previously.[2] Compounds **1**[3], **4**[4] and intermediates **S9**[5] and **S2**[6] were prepared as reported in the literature. Solvents and reagents were used as supplied without any further purification. Anhydrous solvents were taken from a dry solvent system (JC-Meyer Solvent Systems). 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 $CDCl_3$ or D_2O. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Spectra recorded in $CDCl_3$ used the solvent residual peak chemical shift as an internal standard ($CDCl_3$: 7.26 ppm ^1H, 77.0 ppm ^{13}C). Spectra recorded in D_2O used the solvent residual peak chemical shift as an internal standard in ^1H NMR (D_2O: 4.79 ppm ^1H) and acetic acid as the internal standard in ^{13}C NMR (acetic acid in D_2O: 21.03 ppm ^{13}C). Yields of final deprotected oligosaccharides were determined after removal of residual acetic acid. 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) and a MALDI-TOF autoflex™ (Bruker). Analytical HPLC was performed on an Agilent 1200 series coupled to a quadrupole ESI LC/MS 6130 using a YMC-Diol-300 column (150 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 column (150 x 20 mm) or a semi-preparative Thermo Scientific Hypercarb column (150 x 10 mm).

Synthesizer modules and conditions

The linker-functionalized resin **5** (16.9 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 deprotection (Module **B**, **C** and **D**) steps were performed. Mixing of the components was accomplished by bubbling Argon through the reaction mixture.

Module A: Glycosylation with glycosyl phosphates

The resin (16.9 µmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 °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 (3.7 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 (3.7 equiv in 1 mL DCM). The glycosylation was performed for 5 min at -30 °C or -35 °C and then at -20 °C, -15 °C or -10 °C for 40, 30 or 35 minutes, respectively. Subsequently the solution was drained and the resin was washed three times with DCM. The whole procedure was performed once or 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	1-3	DCM	BB (62.5 µmol)	TMSOTf (62.5 µmol)	-35/-30	5 min
					-20/-15/-10	40 / 35/ 30 min
Wash	3	DCM			-15	15 s
Wash	3	DCM			25	15 s

Module B: Fmoc deprotection.

The resin was washed with DMF, swollen in 2 mL DMF, and then the temperature of the reaction vessel was adjusted to 25 °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% Et_3N 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_3N in dry DMF

Action	Cycles	Solvent	Reagent	T (°C)	Incubation Time
Wash	3	DMF		25	15 s
Deprotection	3	DMF	Et_3N	25	5 min
Wash	3	DMF		25	15 s
Wash	3	THF		25	15 s
Wash	3	DCM		25	15 s

Module C: Lev deprotection

Prior to the deprotection step the resin was washed with DCM three times, swollen in 1.3 mL DCM, and then the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 0.8 mL of a solution of 150 mM N_2H_4·AcOH in Pyridine/AcOH/H_2O 4:1:0.25 was delivered to the reaction vessel. After 30 min, the solution was drained and the deprotection step was repeated two times. After Lev deprotection was complete the resin was washed with DCM, DMF, THF and again DCM three times each.

Deprotection solution: 150 mM N_2H_4·AcOH in Pyridine/AcOH/H_2O 4:1:0.25

Action	Cycles	Solvent	Reagent	T (°C)	Incubation Time
Wash	3	DCM		25	15 s
Deprotection	3	*Pyridine/AcOH/H_2O*	N_2H_4·AcOH	25	30 min
Wash	3	DMF		25	15 s
Wash	3	THF		25	15 s
Wash	3	DCM		25	15 s

Module D: 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 0.1 M DDQ solution in DCE/MeOH/H_2O (64:16:1) was delivered to the reaction vessel. After 20 min the reaction solution was drained and the resin was washed with DMF, THF and DCM.

Deprotection solution: 0.1 M DDQ solution in DCE/MeOH/H_2O (64:16:1).

Action	Cycles	Solvent	Reagent	T (°C)	Incubation Time
Wash	3	DCM		25	15 s
Deprotection	1	DCE/MeOH/H_2O	DDQ	40	20 min
Wash	3	DMF		25	15 s
Wash	3	THF		25	15 s
Wash	3	DCM		25	15 s

Module E: Glycosylation with thioglycosides

The resin (16.9 µmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 °C. Prior to the glycosylation reaction, the resin was washed with TMSOTf in DCM and then DCM alone. For the glycosylation reaction, the DCM was drained and a solution of thioglycoside BB (3.7 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 (4.44 equiv) and TfOH (0.44 equiv) in DCM/dioxane (2:1). The glycosylation was performed for 5 min at -35 °C and then for 35 min at -10 °C. Subsequently, the solution was drained and the resin was washed with DCM. Afterwards the resin was washed three times with DCM at 25 °C.

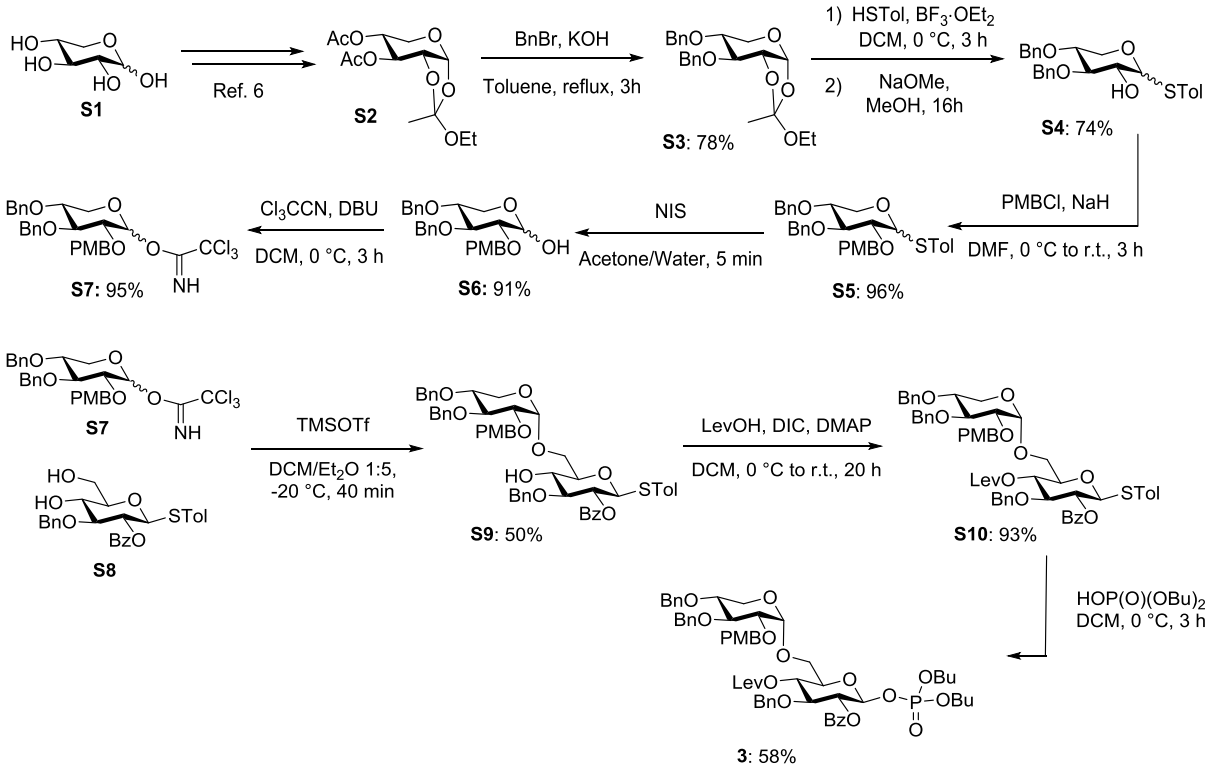
Activator solution: solution of NIS (75 mM) and TfOH (7.5 mM) in DCM/dioxane.

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	1	DCM	BB (62.5 µmol)	NIS (75 µmol) TfOH (7.5 µmol)	-35	5 min
					-10	35 min
Wash	3	DCM			-15	15 s
Wash	3	DCM			25	15 s

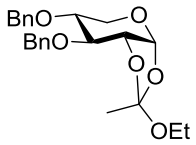
Cleavage from the solid support

After assembly of the oligosaccharides cleavage from the solid support was accomplished using a continuos-flow photoreactor as described previously. [2]

Synthesis of building blocks



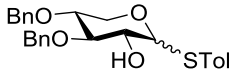
3,4-*O*-Dibenzyl-1,2-*O*-(1-ethoxyethylidene)-D-xylopyranose (S3)



KOH (18.3 g, 327 mmol) was added to a solution of **S2**[6] (12.4 g, 40.8 mmol) in toluene (200 mL) under vigorous stirring. Then, the reaction was heated until reflux and BnBr (19.4 mL 163 mmol) was added drop-wise. The reaction was vigorously stirred for 3 h and then cooled to RT. The reaction mixture was divided in half and each one was diluted with DCM (300 mL) and washed with water (300 mL). The organic layers were combined and concentrated, and the crude product was purified by silica gel chromatography (EtOAc/Hex 1:6) giving **S3** (9.80 g, 24.5 mmol, 78 % yield) as a yellow oil.

The analytical data is in agreement with literature data.[7]

4-Methylphenyl 3,4-*O*-dibenzyl-1-thio-D-xylopyranoside (S4)

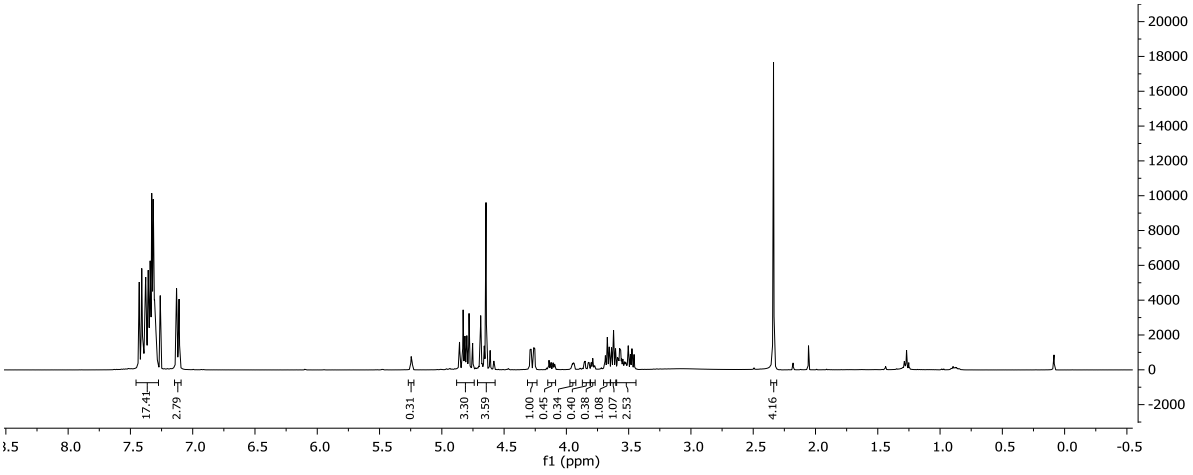


S3 (12.3 g, 30.7 mmol) was dissolved in anhydrous DCM (250 mL), and then 4-methylbenzenethiol (5.72 g, 46.1 mmol) was added under argon atmosphere. The reaction mixture was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (5.84 mL, 46.1 mmol) was added drop-wise. After 2 h the reaction was quenched with Et_3N (6.42 mL, 46.1 mmol) and washed with water (200 mL). The organic layer was concentrated in vacuum. The crude product was dissolved in MeOH (100 mL) and NaOMe (5.00 g, 93.0 mmol) was

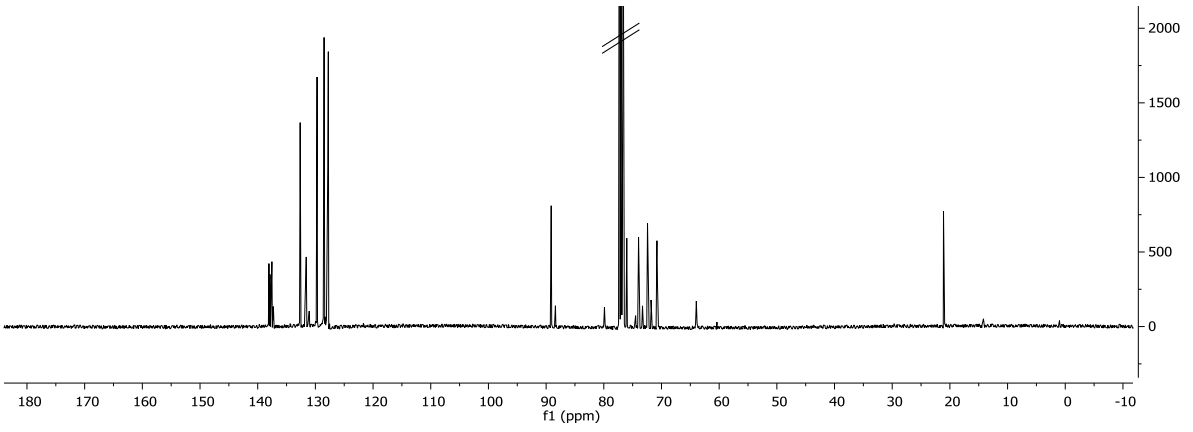
added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by silica gel chromatography (Hex/EtOAc 6:1) to give **S4** (9.90 g, 22.7 mmol, 74 % yield, α/β = 1:3) as a white solid.

$[\alpha]_D^{25}$ = -46.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47-7.28 (m, 20H, Ar), 7.12 (d, *J* = 7.9 Hz, 4H, Ar), 5.24 (d, *J* = 2.6 Hz, 1H, H-1α), 4.87-4.74 (m, 4H, C*H*$_2$Ph-β, CH*H*Ph-α, H-1-β), 4.72-4.57 (m, 4H, C*H*$_2$Ph-β, C*H*$_2$Ph-α, C*H*HPh-α), 4.27 (dd, *J* = 11.9, 3.5 Hz, 1H, H-5a-β), 4.16-4.08 (m, 1H, H-5a-α), 3.95 (dd, *J* = 5.5, 2.7 Hz, 1H, H-2-α), 3.84 (dd, *J* = 12.4, 2.8 Hz, 1H, H-5b-α), 3.79 (t, *J* = 5.1 Hz, 1H, H-3-α), 3.67 (t, *J* = 6.2 Hz, 1H, H-2-β), 3.62 (t, *J* = 6.4 Hz, 1H, H-3-β), 3.59-3.44 (m, 3H, H-4-β, H-4-α, H-5b-β), 2.34 (s, 6H, C*H*$_3$-α, C*H*$_3$-β). ^{13}C NMR (101 MHz, $CDCl_3$): 138.0, 137.9, 137.7, 137.5, 137.3, 137.2, 132.6, 131.6, 131.1, 129.8, 129.7, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5 (36 C, Ar), 89.1 (1C, C-1-β), 88.4 (1C, C-1-α), 79.9 (1C, C-3-β), 77.2 (1C, C-3-α), 76.0 (1C, C-4-β), 74.5 (1C, C-4-α), 74.0, 73.3, 72.4, 71.8 (4C, *C*H$_2$Ph), 70.8 (1C, C-2-β), 70.7 (1C, C-2-α), 64.0 (2C, C-5-β, C-5-α), 21.1 (2C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{26}H_{28}NaO_4S$: 459.1606; found 459.1604. IR (neat) ν_{max}: 3493, 2870, 1495, 1475, 1065 cm^{-1}.

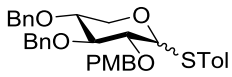
^{1}H NMR (400 MHz, $CDCl_3$) of **S4**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S4**:



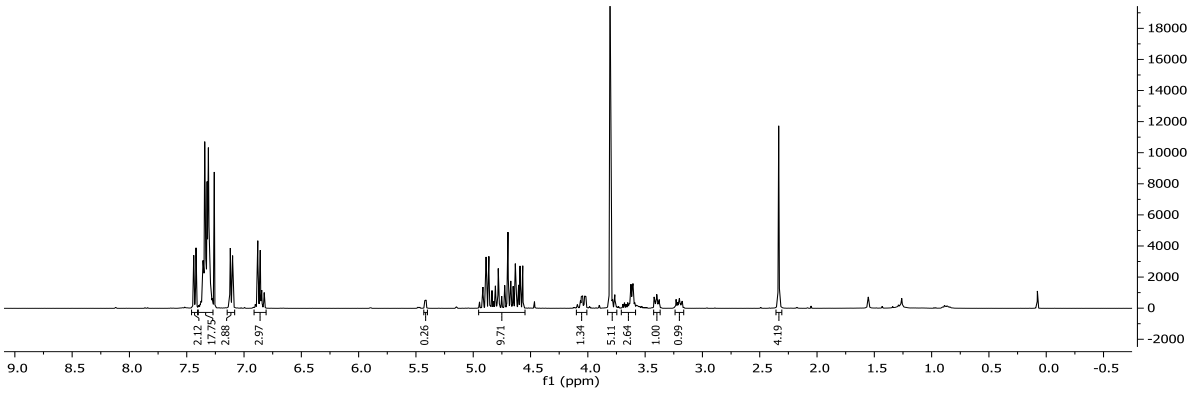
4-Methylphenyl 3,4-*O*-dibenzyl-2-*O*-4-methoxybezyl-1-thio-D-xylopyranoside (S5)



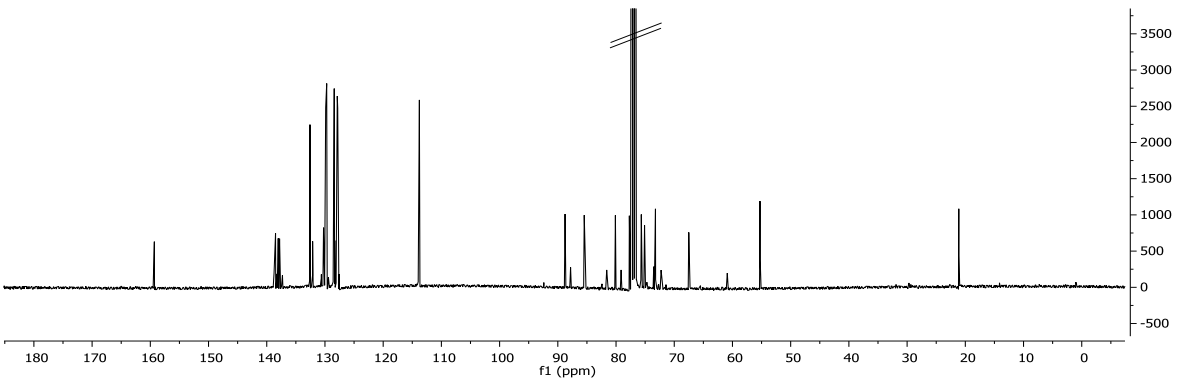
To a cooled (0 °C) solution of **S4** (2.94 g, 6.52 mmol) and PMBCl (1.77 mL, 13.0 mmol) in DMF (60 mL), NaH (323 mg, 8.08 mmol) was added. The reaction was allowed to warm up to room temperature and stirred for an additional 3 h. The reaction was quenched with NH_4Cl, diluted with DCM (250 mL), and washed with NH_4Cl (250 mL), H_2O (250 mL), and brine (250 mL). The crude compound was purified by silica gel chromatography (EtOAc/Hex 1:6) to give **S5** (3.47 g, 6.23 mmol, 96% yield, α/β = 1:3) as a white solid.

$[\alpha]_D^{25}$ = +29.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, *J* = 8.2 Hz, 2H, Ar), 7.38-7.29 (m, 26H), 7.11 (d, *J* = 7.6 Hz, 4H, Ar), 6.90-6.81 (m, 4H, Ar), 5.42 (d, *J* = 4.5 Hz, 1H, H-1α), 4.99-4.60 (m, 12H, 3xCH_2Ph-β, 3xCH_2Ph-α), 4.58 (d, *J* = 9.4 Hz, 1H, H-1β), 4.10-4.01 (m, 2H, H-5a-α, H-5a-β), 3.83-3.75 (m, 8H, OCH_3-β, OCH_3-α, H-3-α, H-2-α), 3.70-3.57 (m, 4H, H-3-β, H-5b-α, H-4-α, H-4-β), 3.40 (t, *J* = 9.0 Hz, 1H, H-2β), 3.24-3.16 (m, 1H, H-5a-β), 2.33 (s, 6H, CH_3-α, CH_3-β). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.3, 138.7, 138.5, 138.2, 138.0, 137.8, 137.3, 132.6, 132.1, 130.2, 129.9, 129.7, 129.4, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 113.8 (48C, Ar), 88.8 (1C, C-1-β), 87.8 (1C, C-1-α), 85.5 (1C, C-3-β), 81.6 (1C, C-3-α), 80.1 (1C, C-2-β), 79.12 (1C, C-2-α), 77.7 (1C, C-4-β), 77.6 (1C, C-4-α), 75.7, 75.1, 73.5, 73.2, 72.2 (6C, *C*H_2Ph), 67.5 (1C, C-5-β), 60.9 (1C, C-5-α), 55.3 (2C, O*C*H_3), 21.1 (2C, *C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{34}H_{36}NaO_5S$: 579.2181; found 579.2175. IR (neat) ν_{max}: 2865, 1515, 1250, 1075 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) of **S5**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S5**:

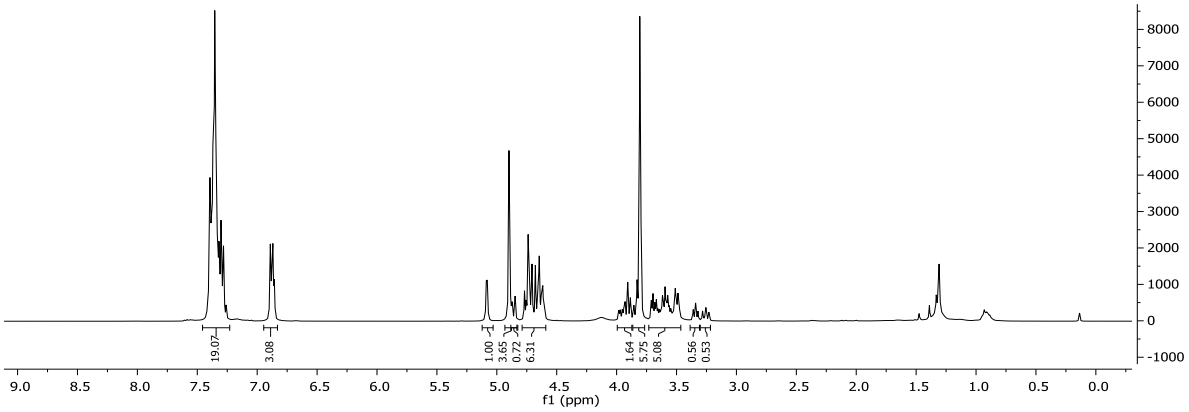


3,4-*O*-dibenzyl-2-*O*-4-methoxybezyl-D-xylopyranose (S6)

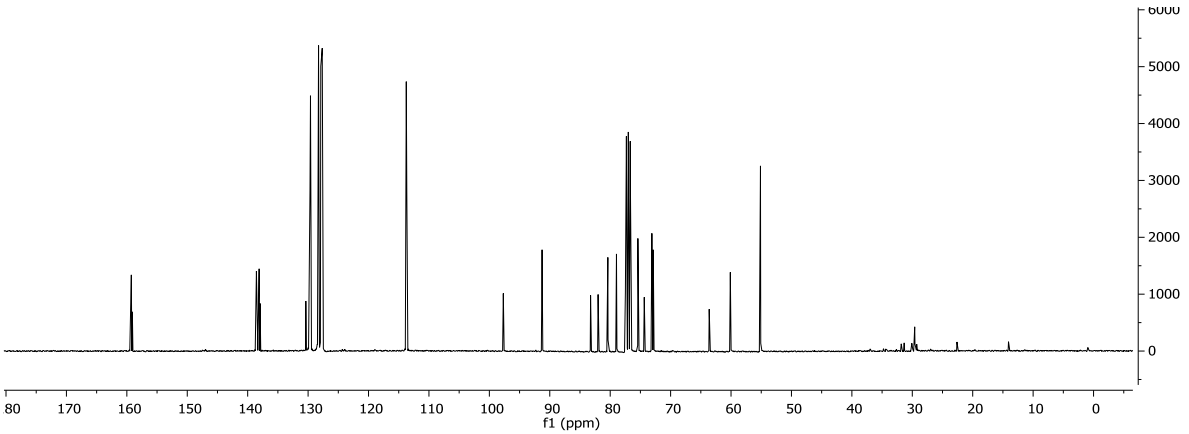
NIS (10.5 g, 18.7 mmol) was added to a stirred solution of **S5** (6.37 g, 28.3 mmol) in acetone/H_2O (9:1, 140 mL). The reaction was stirred for an additional 5 min at RT. Then, the reaction mixture was diluted with EtOAc (100 mL) and washed with an aqueous solution of $Na_2S_2O_3$ (200 mL), water (200 mL), and brine (200 mL). The crude compound was passed through a short plug of silica gel (EtOAc/Hex 1:3) to yield **S6** (7.70 g, 17.1 mmol, 91% yield, α/β = 2:1).

$[a]_D^{25}$ = +15.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48-7.21 (m, 24H, Ar), 7.01-6.77 (m, 4H, Ar), 5.08 (d, *J* = 3.5 Hz, H-1-α), 4.95- 4.83 (m, 5H, C*H*$_2$Ph-α, C*H*$_2$Ph-β, CH*H*Ph-β), 4.79-4.57 (m, 8H, 2xC*H*$_2$Ph-α, C*H*$_2$Ph-β, CH*H*Ph-β,H-1-β), 3.99-3.87 (m, 2H, H-5a-β, H-3-α), 3.86-3.78 (m, 7H, H-5a-α, OC*H*$_3$-β, OC*H*$_3$-α), 3.72-3.46 (m, 5H, H-3-β, H-5b-α, H-4-α, H-4-β, H-2-α), 3.34 (t, *J* = 7.9 Hz, 1H, H-2β), 3.31-3.20 (dd, *J* = 11.6, 9.6 Hz, 1H, H-5a-β). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.3, 159.1, 138.5, 138.4, 138.1, 137.9, 130.4, 129.8, 129.7, 129.6, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.7, 127.5, 113.7 (36C, Ar), 97.7 (1C, C-1-β), 91.3 (1C, C-1-α), 83.2 (1C, C-3-β), 82.0 (1C, C-2-β), 80.4 (1C, C-3-α), 79.0 (1C, C-2-α), 77.5 (1C, C-4-β), 77.4 (1C, C-4-α), 75.4 , 75.4, 74.4, 73.2, 73.1, 72.9 (6C, *C*H$_2$Ph), 63.6 (1C, C-5-β), 60.1 (1C, C-5-α), 55.1 (2C, O*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{27}H_{30}NaO_6$: 473.1940; found 473.1928. IR (neat) ν_{max}: 3364, 2931, 1516, 1251, 1088, 1072, 1030 cm^{-1}.

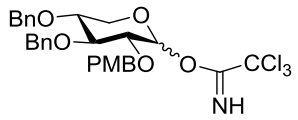
^{1}H NMR (400 MHz, $CDCl_3$) of **S6**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S6**:



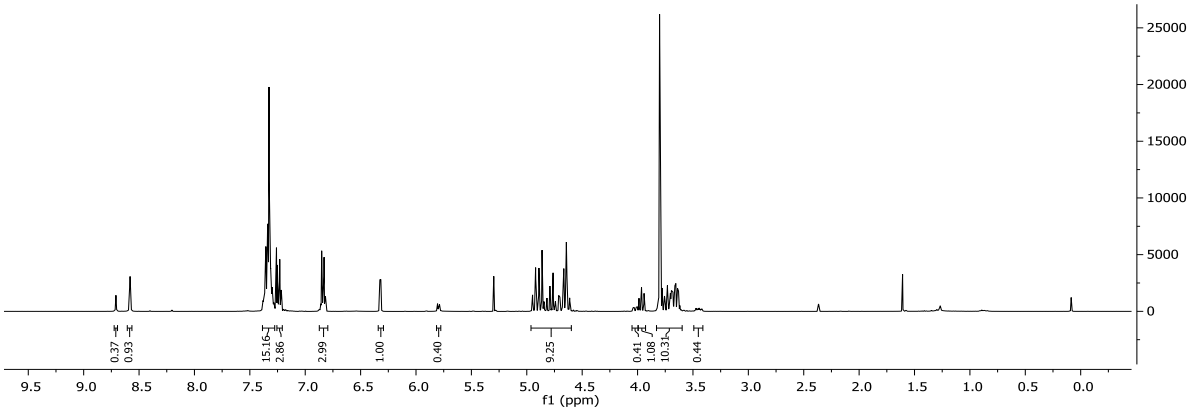
***O*-Trichloroacetimidoyl 3,4-*O*-dibenzyl-2-*O*-4-methoxybezyl-D-xylopyranoside (S7)**



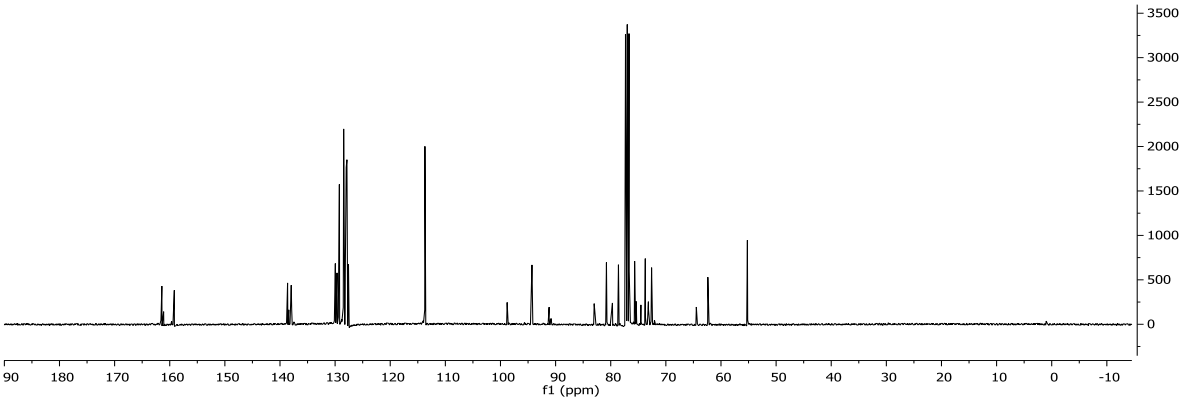
To a cooled (0 °C) solution of compound **S6** (3.83 g, 8.50 mmol) in DCM (30 mL), DBU (127 µL, 850 µmol) and trichloroacetonitrile (3.41 mL, 34.0 mmol) were added. After 3 h the solvent was evaporated and the product was purified by silica gel chromatography (EtOAc/Hex 1:5, 5% Et_3N) to yield **S7** (4.78 g, 8.03 mmol, 95%, α/β = 2:1) as a yellow oil.

$[a]_D^{25}$ = +39.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.71 (s, 1H, CN*H*-β), 8.58 (s, 1H, CN*H*-α), 7.40-7.29 (m, 20H), 7.28-7.21 (m, 4H, Ar), 6.88-6.80 (m, 4H, Ar), 6.32 (d, *J* = 3.5 Hz, 1H, H-1-α), 5.84-5.77 (m, 1H, H-1-β), 4.97-4.58 (m, 12H, 3xCH_2Ph-α, 3xCH_2Ph-β), 4.04-4.00 (m, 1H, H-5a-β), 3.96 (t, *J* = 9.1 Hz, 1H, H-3-α), 3.83-3.60 (m, 13H, H-5a-α, OCH_3-β, OCH_3-α, H-3-β, H-5b-α, H-4-α, H-4-β, H-2-α, H-2β), 3.49-3.42 (m, 1H, H-5b-β) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.4, 161.1 (2C, *C*=NH), 159.2, 138.6, 138.4, 138.0, 137.9, 130.0, 129.6, 129.4, 129.2, 128.4, 128.3, 128.0, 127.9, 127.8, 127.6, 113.7 (36C, Ar), 98.8 (1C, C-1-β), 94.3 (1C, C-1-α), 91.2 (1C, *C*Cl_3-α), 90.8 (1C, *C*Cl_3-β), 83.0 (1C, C-3-β), 80.8 (1C, C-3-α), 79.7 (1C, C-2-β), 78.6 (1C, C-2-α), 77.1 (2C, C-4-β, C-4-α), 75.7, 75.4, 74.5, 73.7, 73.2, 72.6 (6C, *C*H_2Ph), 64.5 (1C, C-5-β), 62.4 (1C, C-5-α), 55.2 (2C, O*C*H_3). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{29}H_{30}Cl_3NNaO_6$: 616.1036; found 616.1027. IR (neat) ν_{max}: 3342, 2903, 1672, 1515, 1249, 1072, 1030 cm^{-1}.

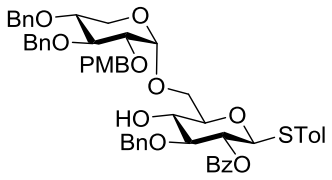
^{1}H NMR (400 MHz, $CDCl_3$) of **S7**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S7**:



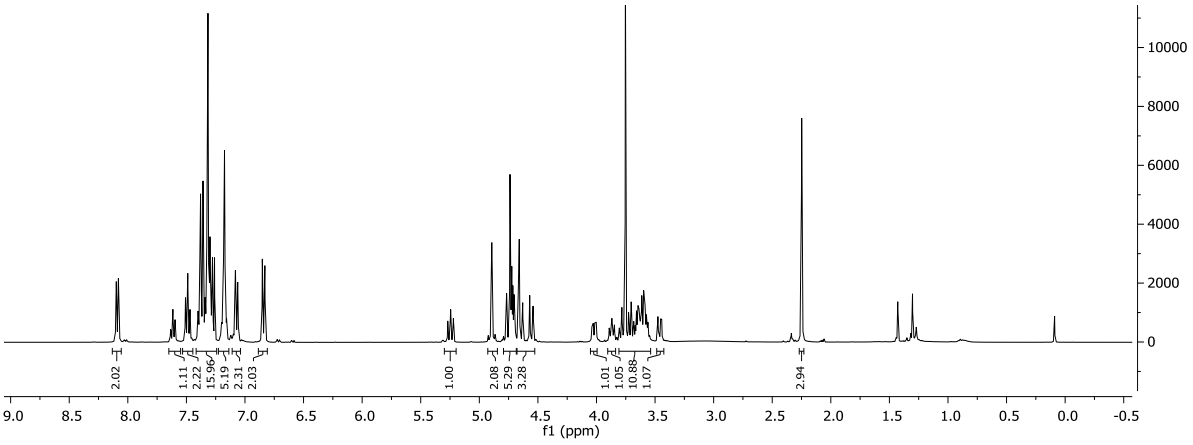
3,4-*O*-dibenzyl-2-*O*-4-methoxybezyl-D-xylopyranosyl -(1→6)-2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-glucopyranoside (S9).



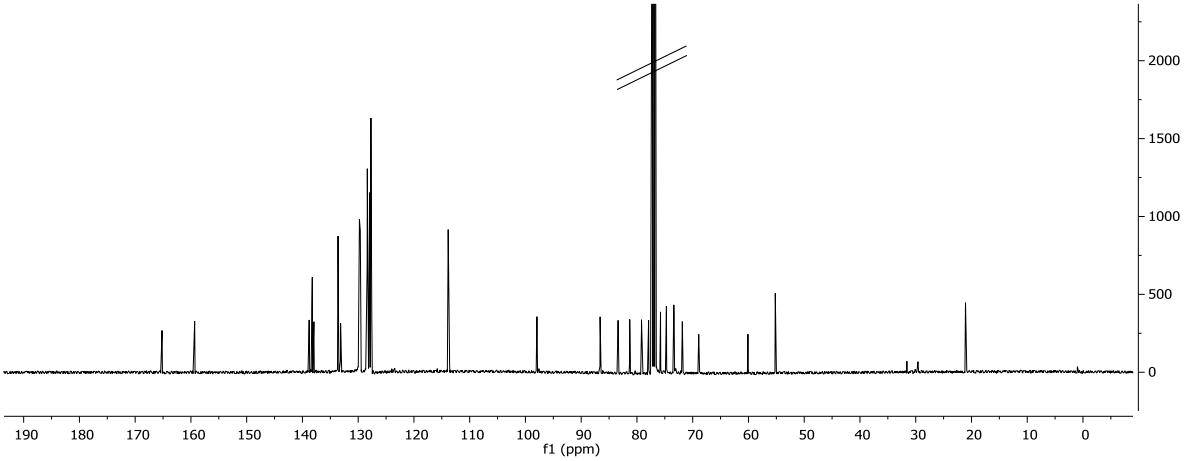
To a solution of **S7** (1.48 g, 2.49 mmol) and **S8** (1.31 g, 2.74 mmol) in a mixture of DCM/Et_2O 1:5 (50 mL) at -15 °C, TMSOTf (45 µL, 24.9 µmol) was added. After 40 minutes the reaction was diluted with DCM (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (100 mL), water (100 mL) and brine (100 mL). The crude compound was purified by silica gel chromatography (EtOAc/Hex 1:3) to give **S9** (1.14 g, 1.25 mmol, 50%) as a pale-yellow oil.

$[\alpha]_D^{25}$ = +47.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, *J* = 7.0 Hz, 2H, Ar), 7.62 (t, *J* = 7.4 Hz, 1H, Ar), 7.49 (t, *J* = 7.6 Hz, 2H, Ar), 7.42-7.23 (m, 14H, Ar), 7.22-7.14 (m, 5H, Ar), 7.07 (d, *J* = 7.9 Hz, 2H, Ar), 6.84 (d, *J* = 8.6 Hz, 2H, Ar), 5.24 (t, *J* = 9.5 Hz, 1H, H-2-Glc), 4.89 (s, 2H, C*H*$_2$Ph), 4.79-4.69 (m, 5H, H-1-Glc, 2xC*H*$_2$Ph), 4.68-4.52 (m, 3H, C*H*$_2$Ph, H-1-Xyl), 4.02 (dd, *J* = 9.9, 4.8 Hz, 1H, H-6a-Glc), 3.87 (t, *J* = 8.9 Hz, 1H, H-3-Xyl), 3.81-3.54 (m, 10H, H-4-Xyl, H-5a-Xyl, H-5b-Xyl, H-3-Glc, H-5-Glc, H-6b-Glc, H-4-Glc, OC*H*$_3$), 3.46 (dd, *J* = 9.5, 3.6 Hz, 1H, H-2-Xyl), 2.25 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.2 (1C, *C*=O), 159.3, 138.8, 138.2, 137.9, 133.6, 133.2, 129.9, 129.9, 129.8, 129.7, 129.6, 128.5, 128.4, 128.3, 128.3, 127.9, 127.9, 127.7, 127.6, 113.8 (36C, Ar), 97.9 (1C, C-1-Xyl), 86.61 (1C, C-1-Glc), 83.4 (1C, C-3-Glc), 81.3 (1C, C-3-Xyl), 79.2 (1C, C-2-Xyl), 77.9 (1C, C-4-Xyl), 77.2 (1C, C-5-Glc), 75.8, 74.7, 73.4, 73.3 (5C, *C*H$_2$Ph) , 73.1 (1C, C-4-Glc), 71.9 (1C, C-2-Glc), 68.9 (1C, C-6-Glc), 60.1 (1C, C-5-Xyl), 55.2 (1C, O*C*H$_3$), 21.1 (1C, *C*H$_3$) ppm. ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{54}H_{56}NaO_{11}S$: 935.3341; found 935.3349. IR (neat) ν_{max}: 3493, 2883, 1729, 1515, 1269, 1251, 1071, 1028 cm^{-1}.

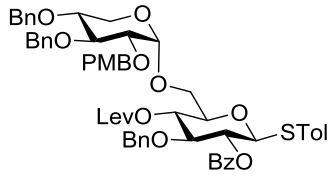
^{1}H NMR (400 MHz, $CDCl_3$) of **S9**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S9**:



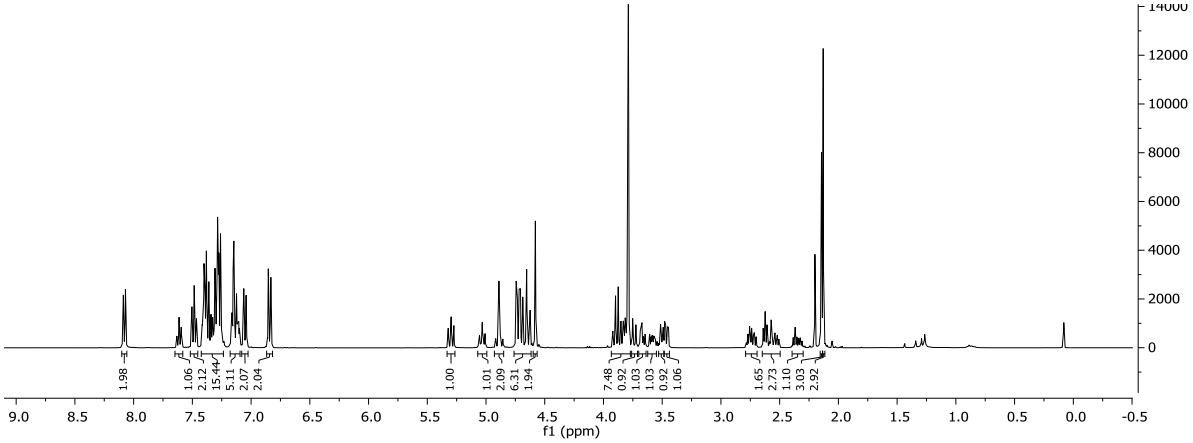
4-Methylphenyl 2,3,4-*O*-tribenzyl-α-D-xylopyranosyl-(1→6)-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (S10).



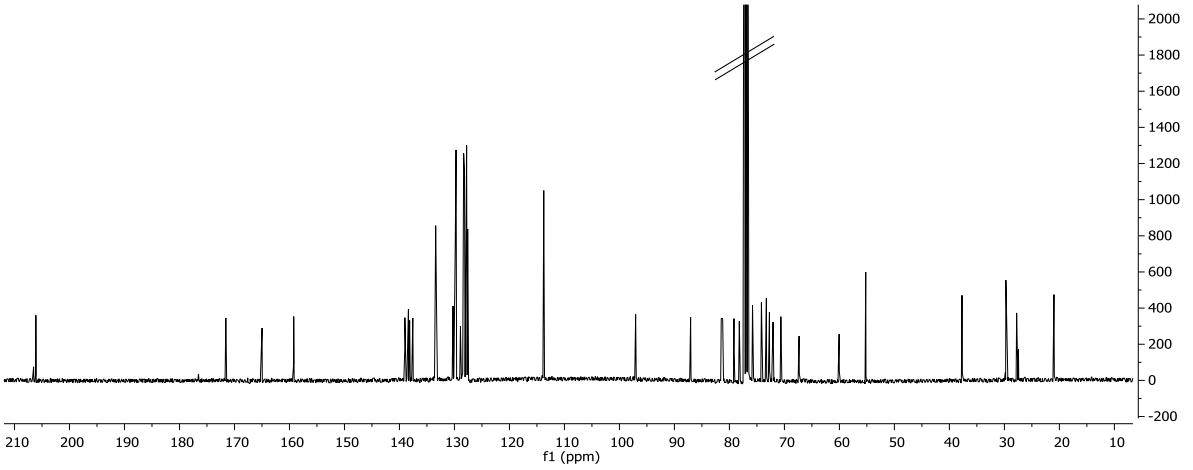
Compound **S9** (1.63 g, 1.87 mmol) was dissolved in DCM (30 mL) and cooled to 0 °C. Then DMAP (218 μg, 200 μmol), DIC (417 μL, 2.68 mmol), and LevOH (283 mL, 2.68 mmol) were added. After 5 min the ice bath was removed and the reaction was left stirring overnight. The next day, the reaction mixture was filtered through a plug of celite© and concentrated. The compound was purified by silica gel chromatography (Hex/EtOAc 2:1) to yield **S10** (1.68 g, 1.61 mmol, 93%) as a pale-yellow oil.

$[\alpha]_D^{25}$ = +30.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10-8.06 (m, 2H, Ar), 7.61 (t, *J* = 7.4 Hz, 1H, Ar), 7.48 (t, *J* = 7.7 Hz, 2H, Ar), 7.42-7.23 (m, 14H, Ar), 7.18-7.10 (m, 5H, Ar), 7.05 (d, *J* = 7.9 Hz, 2H, Ar), 6.84 (d, *J* = 8.6 Hz, 2H, Ar), 5.30 (dd, *J* = 10.1, 9.1 Hz, 1H, H-2-Glc), 5.03 (t, *J* = 9.5 Hz, 1H, H-4-Glc), 4.94-4.85 (d, *J* = 2.8 Hz, 2H, C*H*$_2$Ph), 4.75-4.61 (m, 6H, 2 x C*H*$_2$Ph, H-1-Glc, H-1-Xyl), 4.58 (s, 1H, C*H*$_2$Ph), 3.93-3.77 (m, 7H, H-3-Xyl, H-3-Glc, H-5-Glc, H-6a-Glc, OC*H*$_3$), 3.74 (d, *J* = 10.9 Hz, H-5a-Xyl), 3.67 (dd, *J* = 11.0, 5.6 Hz, 1H, H-5b-Xyl), 3.62-3.55 (m, 1H, H-4-Xyl), 3.50 (d, *J* = 9.2 Hz, 1H, H-6a-Glc), 3.46 (dd, *J* = 9.6, 3.5 Hz, 1H, H-2-Xyl), 2.80-2.69 (m, 1H, Lev), 2.65-2.50 (m, 2H, Lev), 2.40-2.30 (m, 1H, Lev), 2.14 (s, 3H, CH_3), 2.13 (s, 3H, O=CC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 206.1, 171.6, 165.0 (3C, C=O), 159.2, 139.0, 138.4, 138.1, 137.6, 133.4, 133.2, 130.3, 129.8, 129.8, 129.7, 128.9, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 113.8 (36C, Ar), 97.0 (1C, C-1-Xyl), 87.0 (1C, C-1-Glc), 81.5, 81.2 (2C, C-3-Glc, C-3-Xyl), 79.2 (1C, C-2-Xyl), 78.2 (1C, C-4-Xyl), 77.2 (1C, C-5-Glc), 75.8, 74.2, 73.4, 72.7 (4C, *C*H$_2$Ph), 72.0 (1C, C-2-Glc), 70.6 (1C, C-4-Glc), 67.3 (1C, C-6-Glc), 60.0 (1C, C-5-Xyl), 55.2 (1C, O*C*H$_3$), 37.7 (1C, Lev), 29.7 (1C, O=C*C*H$_3$), 27.8 (1C, Lev), 21.0 (1C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{59}H_{62}NaO_{13}S$: 1033.3809; found 1033.3807. IR (neat) ν_{max}: 2922, 1722, 1515, 1250, 1072 cm^{-1}.

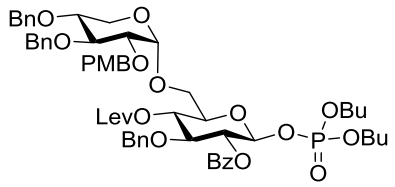
^{1}H NMR (400 MHz, $CDCl_3$) of **S10**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S10**:



Dibutoxyphosphoryloxy 2,3,4-*O*-tri-benzyl-α-D-xylopyranosyl-(1→6)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-β-D-glucopyranoside (3).

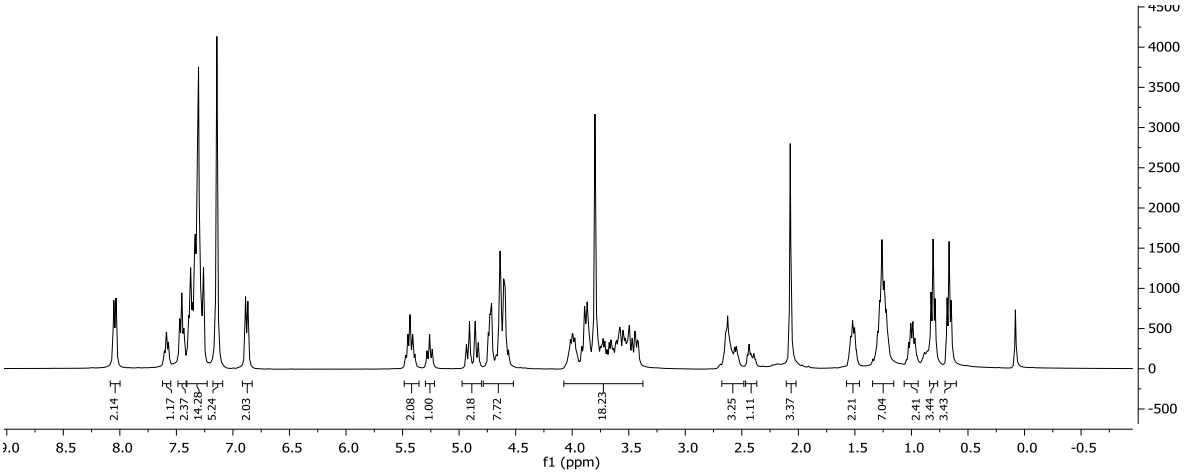


A solution of dibutyl phosphate (2.00 mL, 10.1 mmol) in DCM (20 mL) was dried over molecular sieves. After 1 h the supernatant of this mixture (7.72 mL) was added to **S10** (749 mg, 740 µmol) and cooled to -15 °C. Then NIS (200 mg, 888 µmol) and TfOH (20.0 µL, 222 µmol) were added. The reaction was stirred for 2 h, diluted with DCM (50 mL) and washed with an aqueous solution of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and purified by silica gel chromatography (Hex/EtOAc 2:1) to give compound **3** (470 mg, 428 µmol, 58%) as a yellow oil.

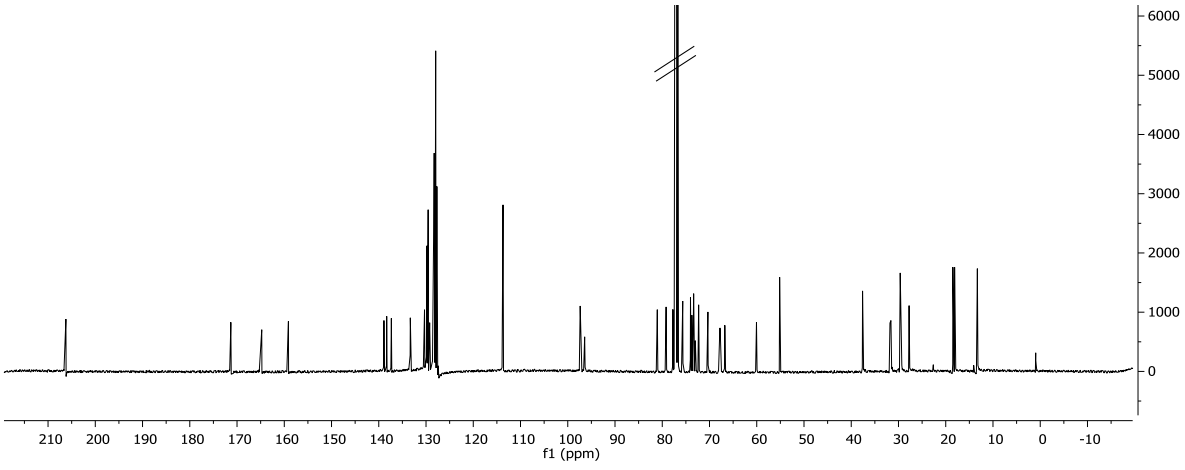
$[\alpha]_D^{25}$ = +40.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, *J* = 7.5 Hz, 2H, Ar), 7.58 (t, *J* = 7.4 Hz, 1H, Ar), 7.45 (t, *J* = 7.6 Hz, 2H, Ar), 7.41-7.24 (m, 12H, Ar), 7.14 (s, 5H, Ar), 6.87 (d, *J* = 8.2 Hz, 2H, Ar), 5.48-5.36 (m, 2H, H-2-Glc, H-1-Glc), 5.26 (t, *J* = 9.4 Hz, 1H, H-4-Glc), 4.94-4.80 (m, 2H, C*H*$_2$Ph),

4.75-4.54 (m, 7H, H-1-Xyl, 3 x CH_2Ph), 4.05-3.38 (m, 16H, OCH_3, 4 x OBu, 6Ha-Glc, 6Hb-Glc, 5Ha-Xyl, 5Hb-Xyl, H-2-Xyl, H-3-Glc, H-4 Xyl, H-3-Xyl, H-5-Glc), 2.66-2.51 (m, 3H, Lev), 2.46-2.36 (m, 1H), 2.06 (s, 3H, Lev), 1.57-1.47 (m, 2H, Bu), 1.31-1.19 (m, 4H, Bu), 1.04-0.96 (m, 2H, Bu), 0.80 (t, J = 7.4 Hz, 3H, CH_3), 0.66 (t, J = 7.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, CDCl3): δ = 206.2, 171.3, 164.8 (3C, C=O), 159.1, 138.9, 138.3, 137.3, 133.3, 130.3, 129.8, 129.5, 129.2, 128.4, 128.3, 128.2, 127.9, 127.7, 127.6, 127.4, 113.7 (25C, Ar), 97.4 (1C, C-1-Xyl), 96.4 (1C, C-1Glc), 81.0 (1C, C-3-Xyl), 79.3 (1C, C-3-Glc), 79.2 (1C, C-2-Xyl), 77.8 (1C, C-4-Xyl), 75.7, 74.0 (2C, CH$_2$Ph), 73.7 (1C, C-5-Glc), 73.3 (1C, CH$_2$Ph), 73.0 (1C, C-2-Glc), 72.3 (1C, CH$_2$Ph), 70.3 (1C, C-4-Glc), 67.9, 67.7 (2C, OBu), 66.8 (1C, C-6-Glc), 60.0 (1C, C-5-Xyl), 55.1 (1C, OCH$_3$), 37.6 (1C, Lev), 31.8, 31.6 (2C, Bu), 29.6 (1C, O=CCH$_3$), 27.7 (1C, Lev), 18.5, 18.1, 13.5, 13.3 (4C, Bu). ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{60}H_{73}NaO_{17}S$: 1119.4483; found 1119.4469. IR (neat) ν_{max}: 2963, 1734, 1267, 1091, 1072, 1030 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) of **3**:

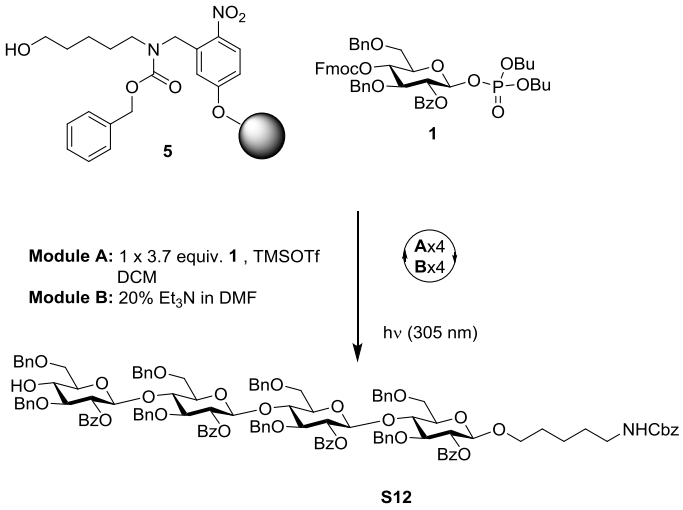


^{13}C NMR (100 MHz, $CDCl_3$) of **3**:



Automated Glycan Assembly

Benzyloxycarbonylaminopentyl 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 (S12)

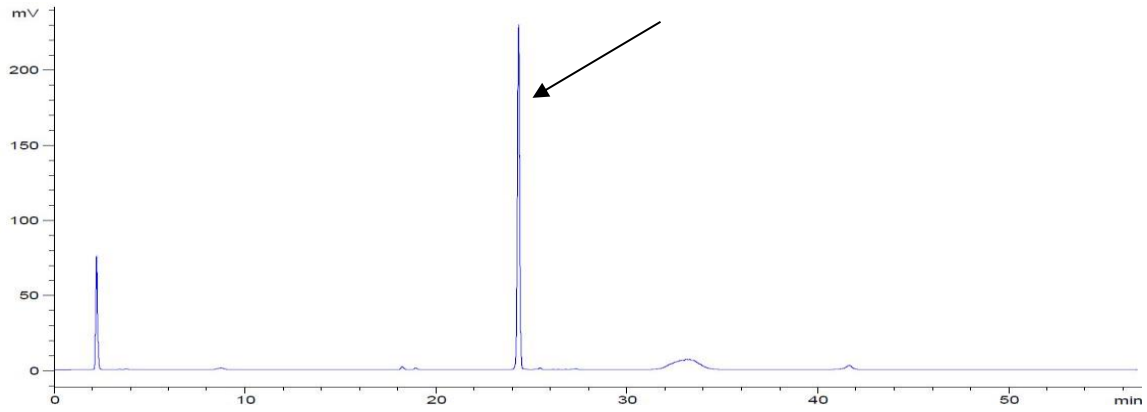


Linker-functionalized resin **5** (85 mg, 22.1 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (3.7 equiv **1**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (3.7 equiv **1**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (3.7 equiv **1**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (3.7 equiv **1**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

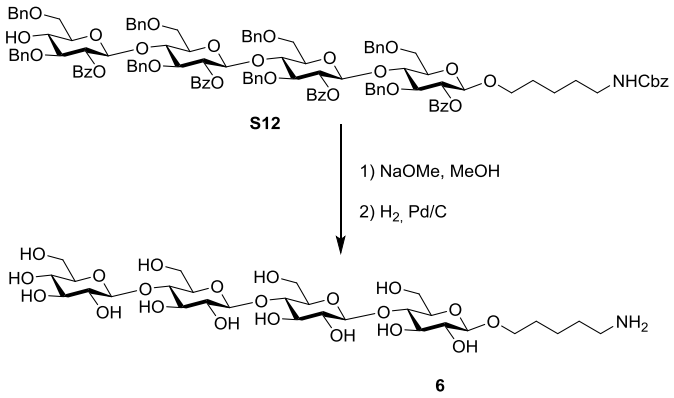
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide. The crude product was purified by normal-phase HPLC using a preparative YMC Diol column affording the protected tetrasaccharide **S12**.

Crude NP-HPLC of tetrasaccharide **S12** (ELSD trace):



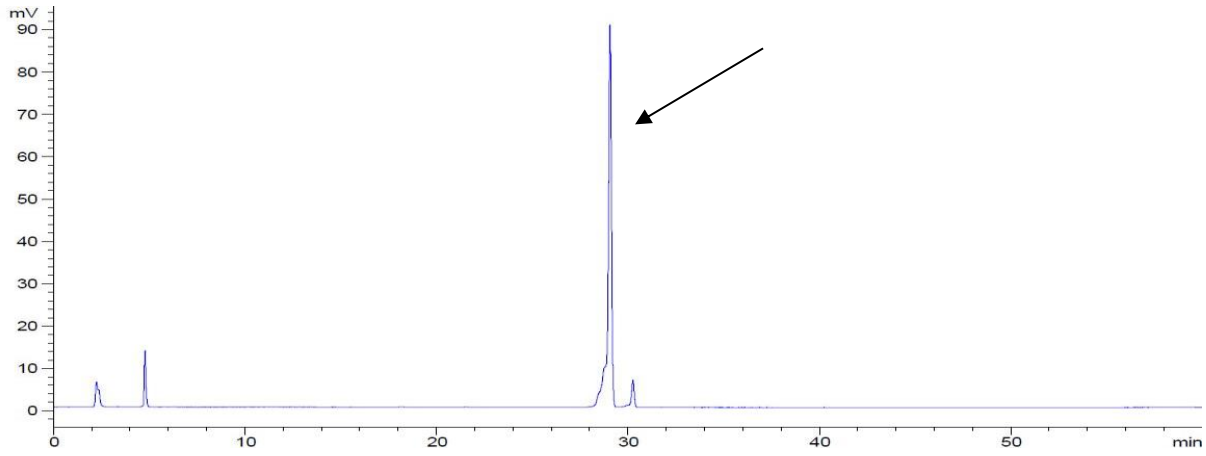
HPLC was performed using an YMC Diol column and a linear gradient from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

Aminopentyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (6)



Tetrasaccharide **S12** was dissolved in THF (3 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 solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected tetrasaccharide.

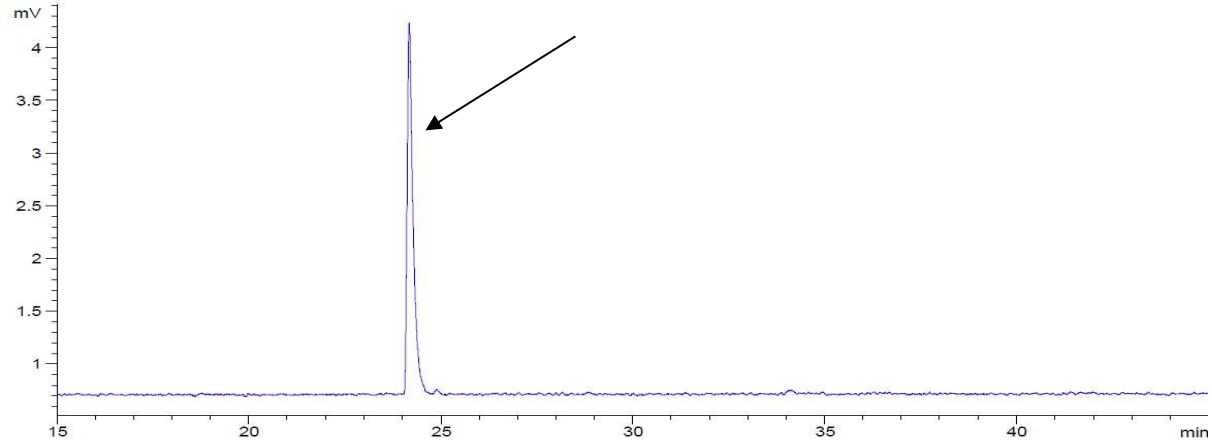
Crude NP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



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).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 11 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter, the solvents were evaporated and the product was purified by reversed-phase HPLC using a semi-preparative hypercarb column to provide the fully deprotected tetrasaccharide **6** (2.3 mg, 3.06 µmol, 14% over 11 steps, based on resin loading).

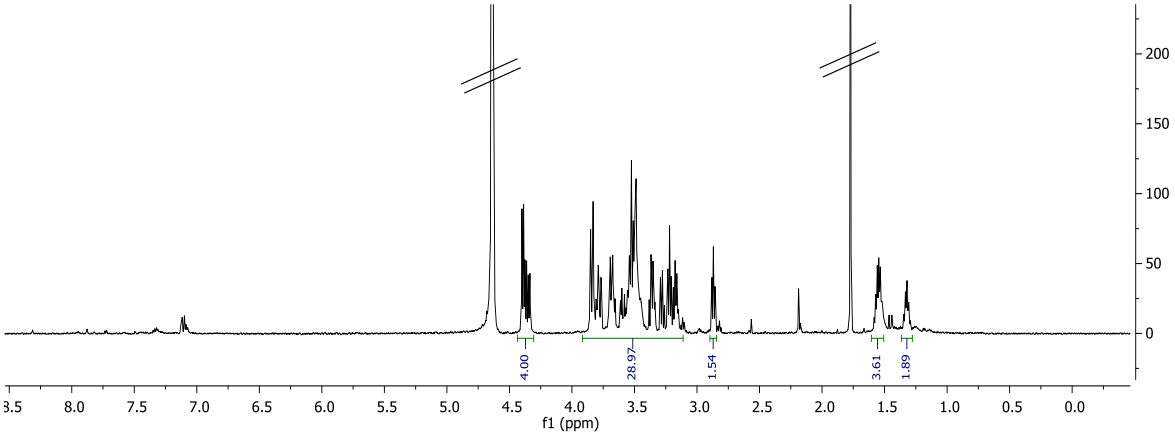
RP-HPLC of the deprotected tetrasaccharide **6** (ELSD trace):



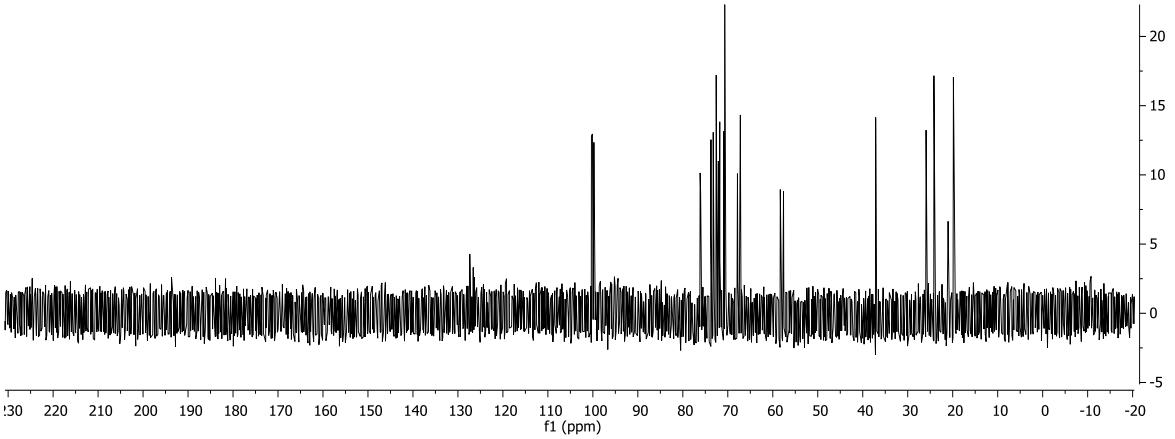
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.61-4.45 (m, 4H), 4.06-3.28 (m, 26H), 3.02 (t, *J* = 7.6 Hz, 2H), 1.76-1.64 (m, 4H), 1.51-1.44 (m, 2H) ppm.^{13}C NMR (151 MHz, D_2O): δ = 100.3, 100.1, 99.8, 76.3, 76.1, 76.0, 73.7, 73.2, 72.6, 72.5, 72.1, 71.8, 70.9, 70.7, 67.9, 67.2, 58.3, 57.8, 57.6, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C_{29}H_{53}NNaO_{21}$: 774.3008; found 774.3036.

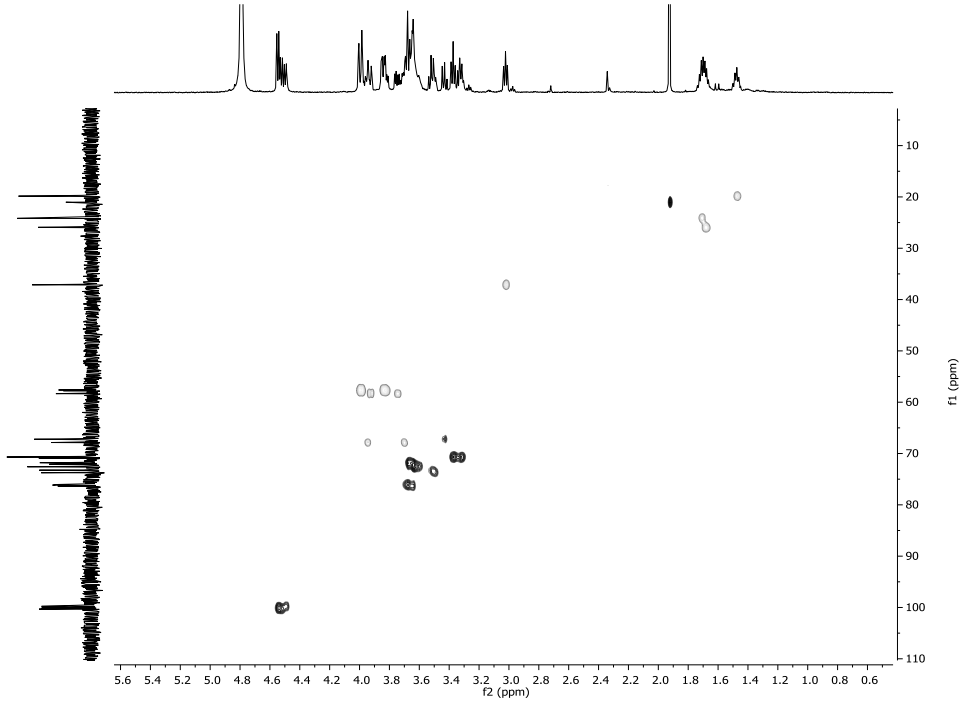
^{1}H NMR (600 MHz, D_2O) of **6**:



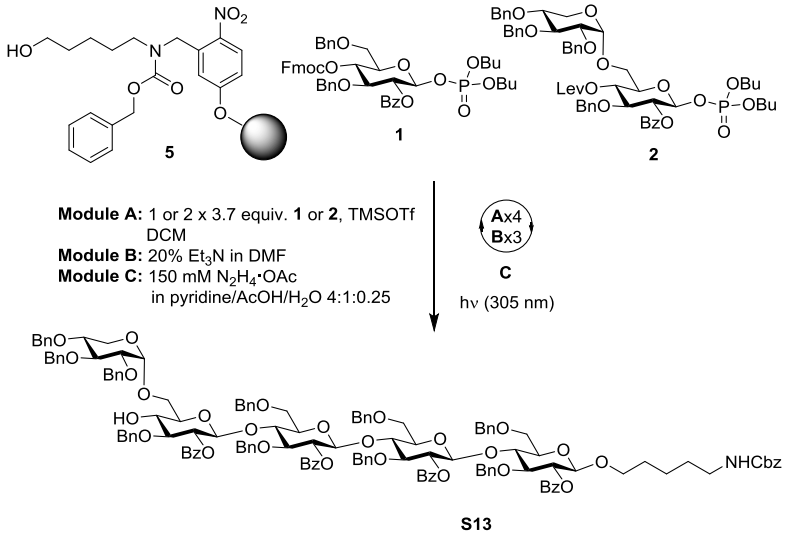
^{13}C NMR (151 MHz, D_2O) of **6**:



HSQC (D_2O) of **6**:



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-[2,3,4-*O*-tribenzyl-α-D-xylopyranosyl]-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 (S13)

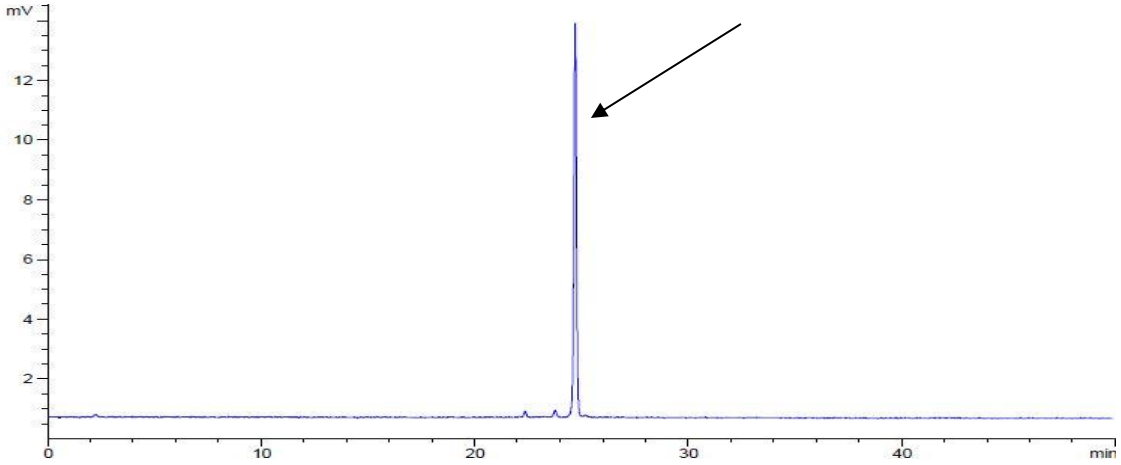


Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **2**, TMSOTf, DCM, 2 x 40 min, -35 °C to -15 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **S13**. The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column.

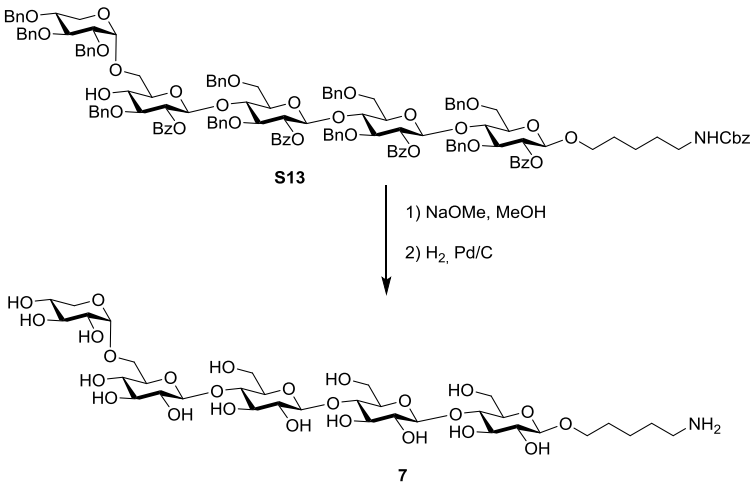
Crude NP-HPLC of pentasaccharide **S13** (ELSD trace):



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

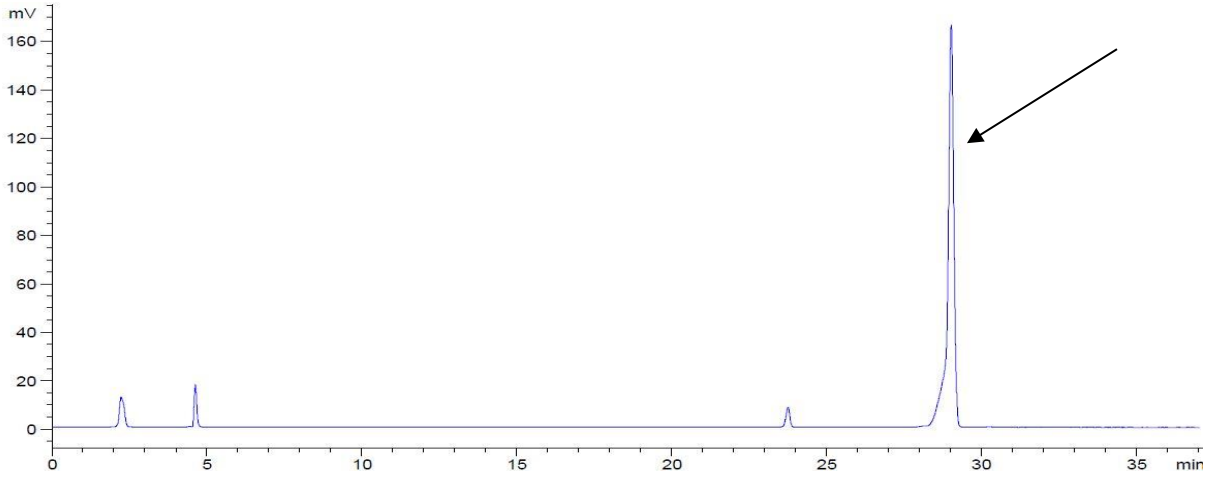
The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the protected pentasaccharide.

Aminopentyl 6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (7)



Pentasaccharide **S13** was dissolved in THF (3 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 solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected pentasaccharide.

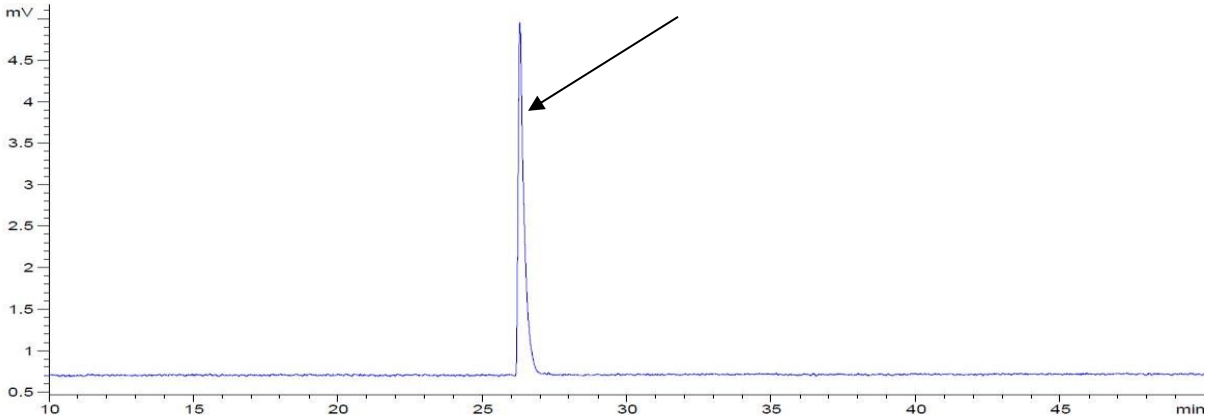
Crude NP-HPLC of the semi-protected pentasaccharide (ELSD trace):



HPLC was performed using a YMC-Diol-300 column and linear gradients from 10% to 70% ethyl acetate in hexane (29 min, flow rate 1 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 11 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated and the pentasaccharide was purified by reversed-phase HPLC using a semi-preparative hypercarb column to provide the fully deprotected pentasaccharide **7** (1.5 mg, 1.70 µmol, 10% over 11 steps, based on resin loading).

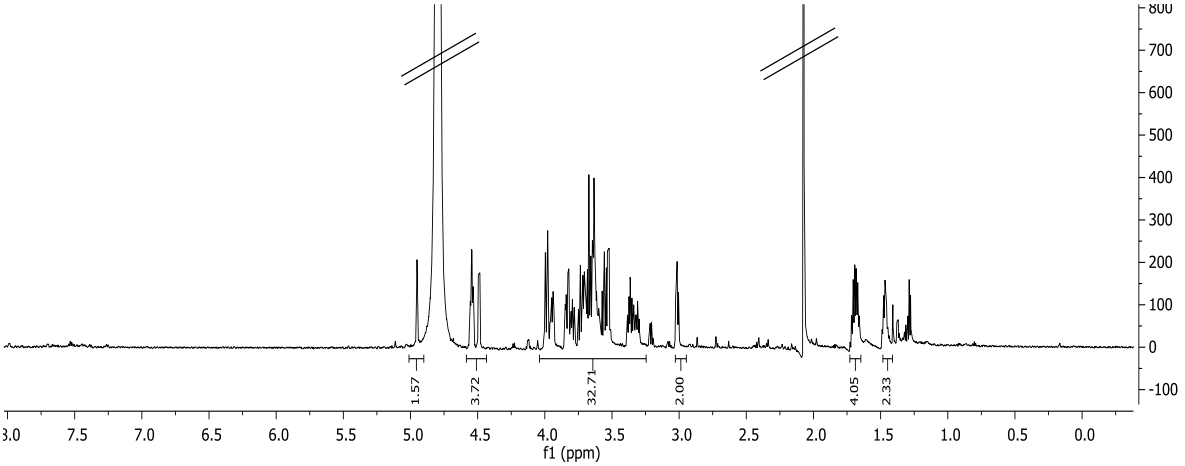
RP-HPLC of the deprotected pentasaccharide **7** (ELSD trace):



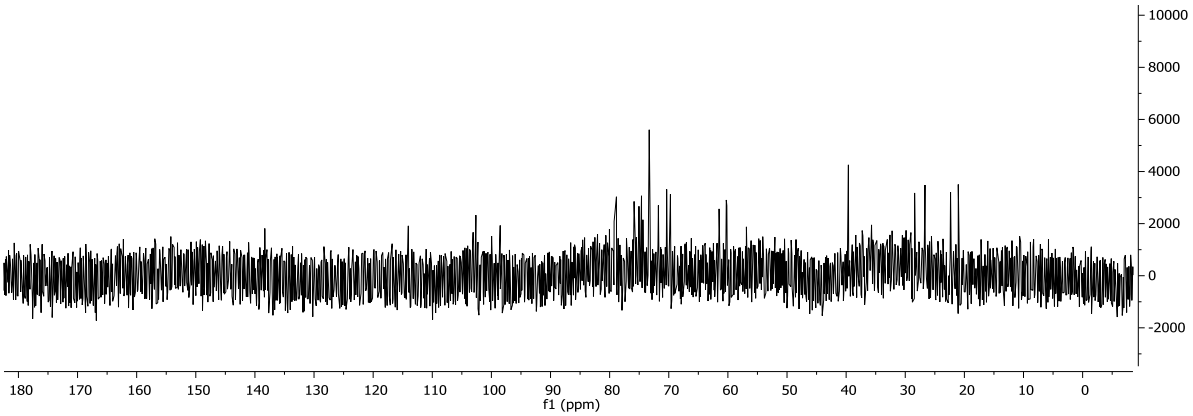
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (700 MHz, D_2O): δ = 4.95 (d, *J* = 3.7 Hz, 1H), 4.59-4.45 (m, 4H), 4.01-3.26 (m, 31H), 3.02 (t, *J* = 7.6 Hz, 2H), 1.73-1.62 (m, 4H), 1.49-1.43 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): 103.1, 102.6, 102.3, 98.5, 78.8, 75.9, 75.1, 75.0, 74.6, 74.5, 74.4, 74.3, 73.2, 73.1, 71.8, 70.4, 69.7, 61.5, 60.3, 60.2, 60.1, 39.6, 28.4, 26.7, 22.3. ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{34}H_{62}NO_{25}$: 884.3611; found 884.3640.

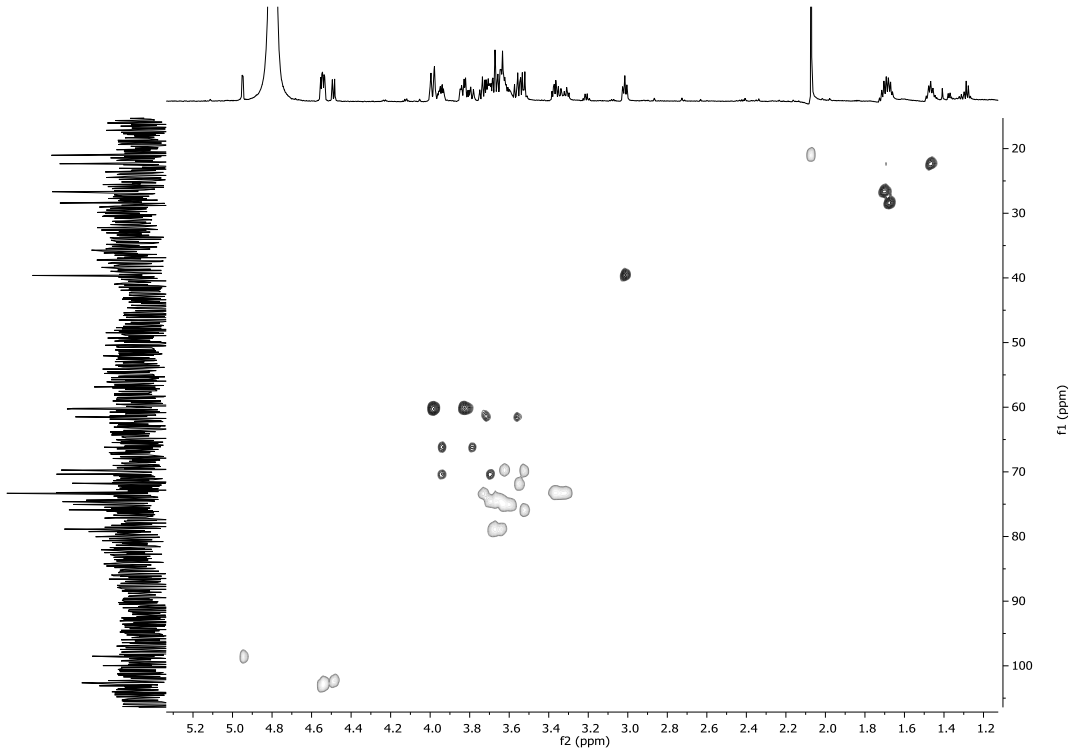
^{1}H NMR (600 MHz, D_2O) of **7**:



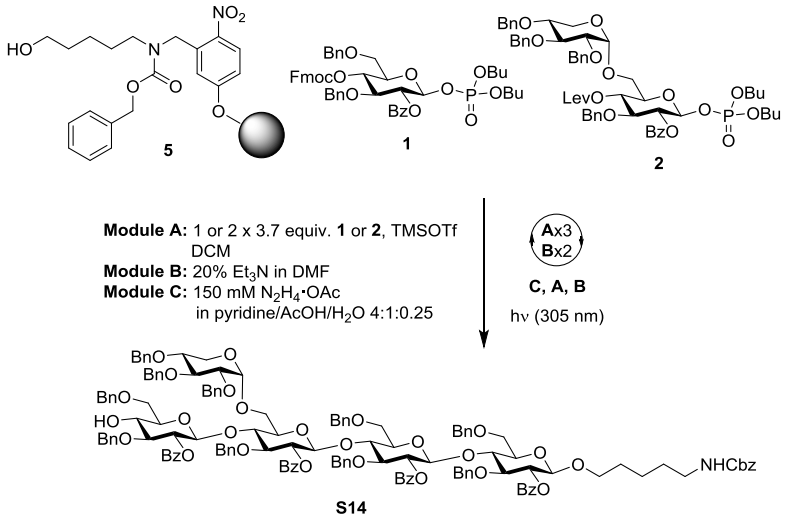
^{13}C NMR (176 MHz, D_2O) of **7**:



HSQC (D_2O) of **7**:



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-[2,3,4-*O*-tribenzyl-α-D-xylopyranosyl]-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 (S14)

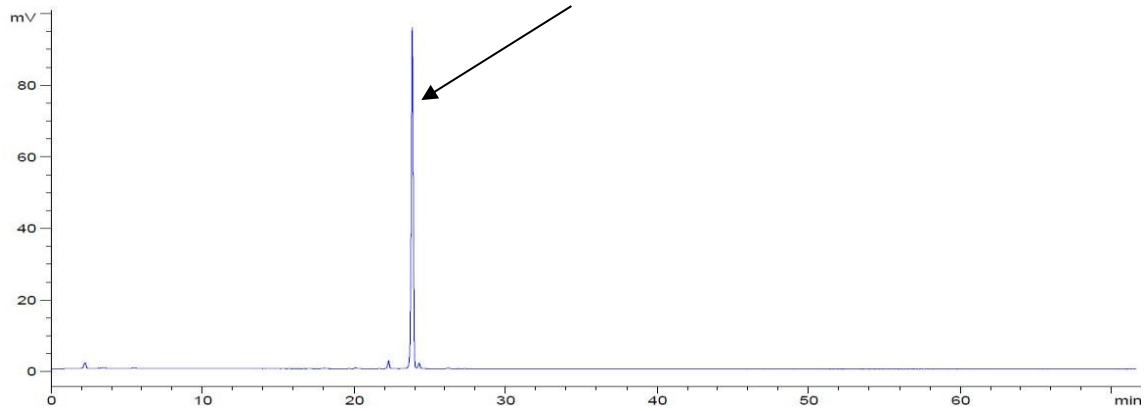


Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **2**, TMSOTf, DCM, 2 x 40 min, -35 °C to -15 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)
Module **A** (2 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 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 pentasaccharide **S14**. The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column.

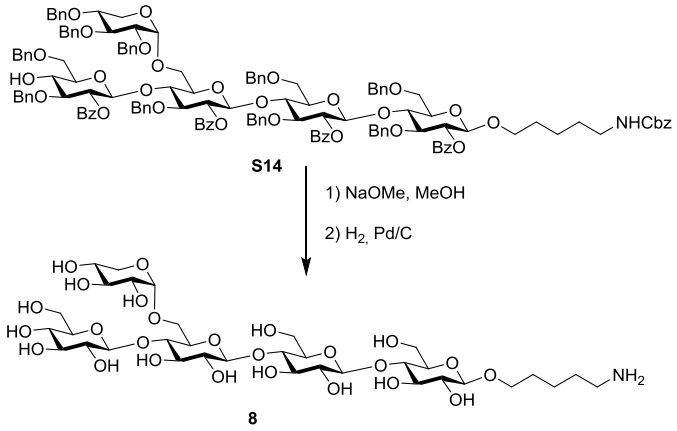
Crude NP-HPLC of pentasaccharide **S14** (ELSD trace):



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

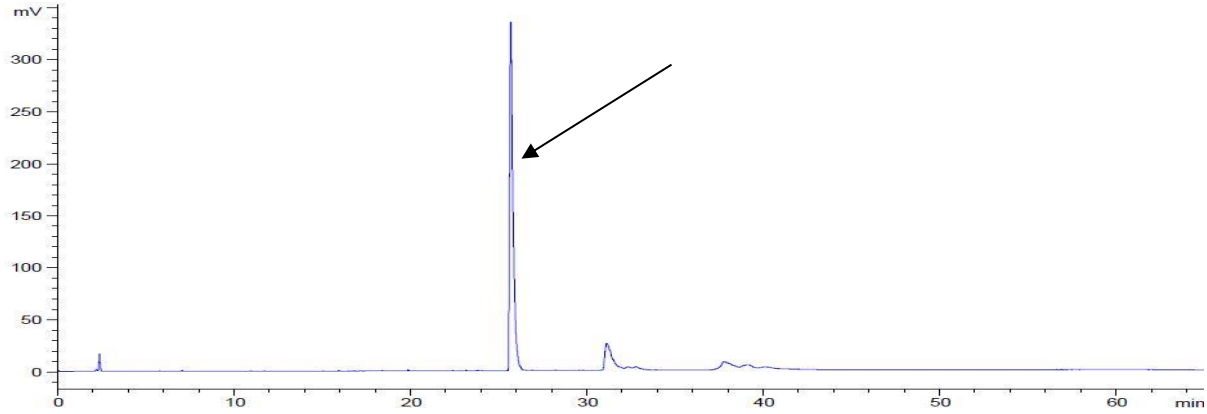
The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the protected pentasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (8)



Pentasaccharide **S14** was dissolved in THF (3 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 solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected pentasaccharide.

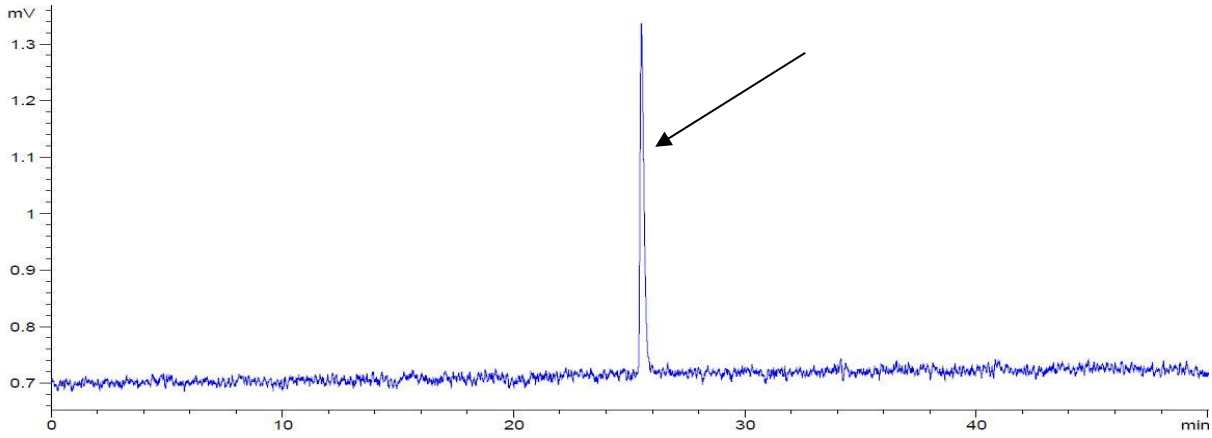
Crude NP-HPLC of the semi-protected pentasaccharide (ELSD trace):



HPLC was performed using a YMC-Diol-300 column and linear gradients 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_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 11 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide **8** (5.3 mg, 6.00 µmol, 36% over 11 steps, based on resin loading).

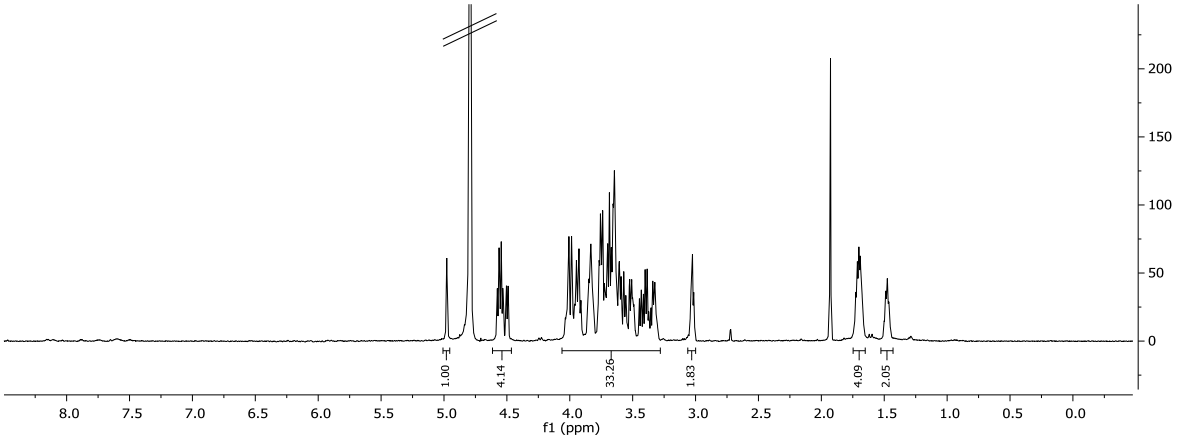
RP-HPLC of the deprotected pentasaccharide **8** (ELSD trace):



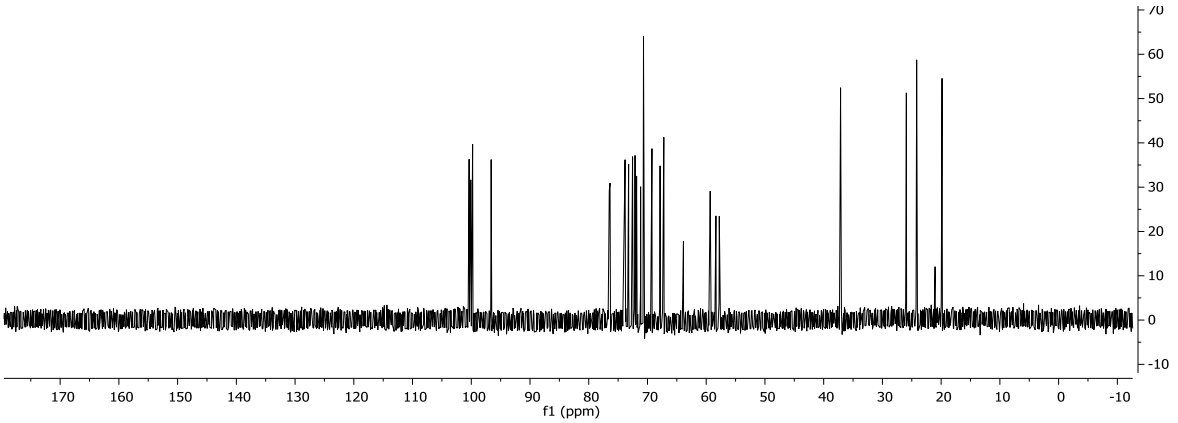
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.98 (d, *J* = 3.2 Hz, 1H), 4.60-4.46 (m, 4H), 4.05-3.28 (m, 31H), 3.05-3.00 (m, 2H), 1.76-1.62 (m, 4H), 1.56-1.42 (m, 2H).ppm.^{13}C NMR (151 MHz, D_2O) δ = 100.4, 100.2, 100.1, 99.8, 96.6, 76.6, 76.5, 76.4, 73.8, 73.2, 72.5, 72.1, 71.8, 71.2, 70.8, 70.7, 70.5, 69.2, 67.9, 67.2, 63.9, 59.3, 58.3, 57.8, 57.6, 37.1, 25.9, 24.2, 19.8. ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{34}H_{62}NO_{25}$: 884.3647; found 884.3611.

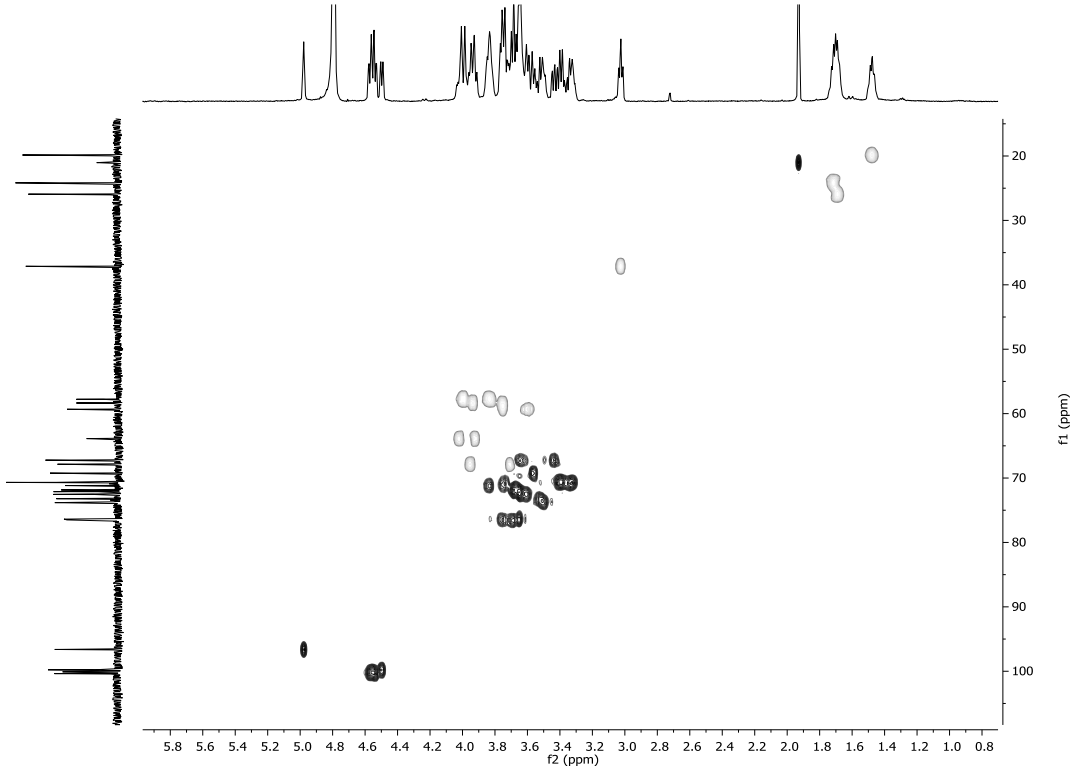
^{1}H NMR (600 MHz, D_2O) of **8**:



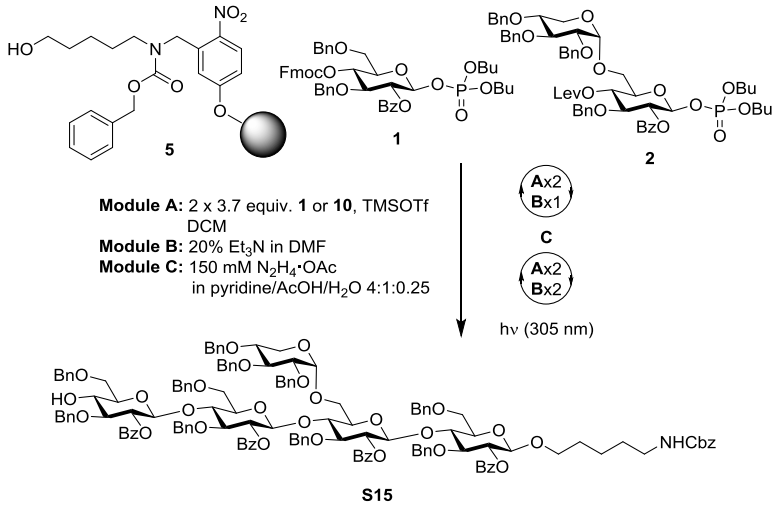
^{13}C NMR (151 MHz, D_2O) of **8**:



HSQC (D_2O) of **8**:



Benzyloxycarbonylaminopentyl 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-*O*-benzyl-6-*O*-[2,3,4-*O*-tribenzyl-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-glucopyranoside (S15)

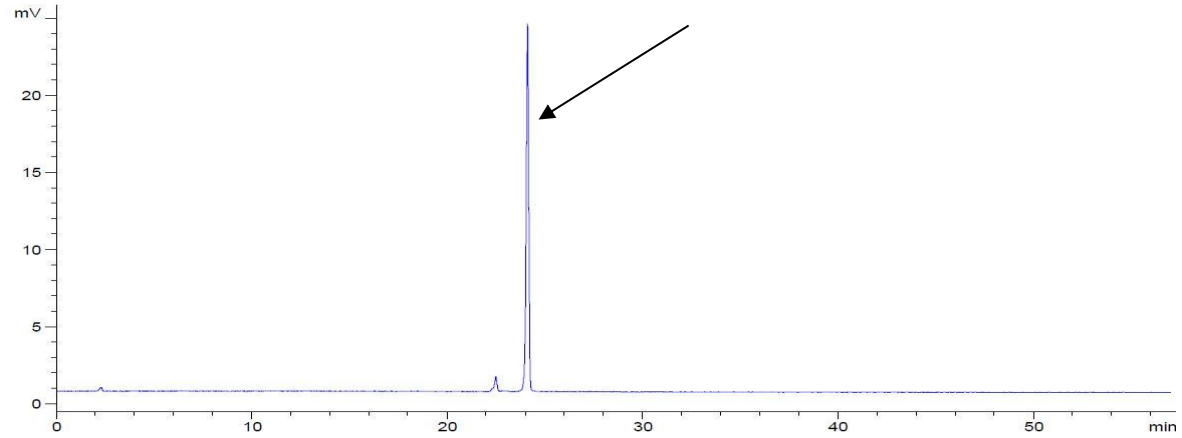


Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **2**, TMSOTf, DCM, 2 x 40 min, -35 °C to -15 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)
Module **A** (2 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 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 pentasaccharide **S15**. The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column.

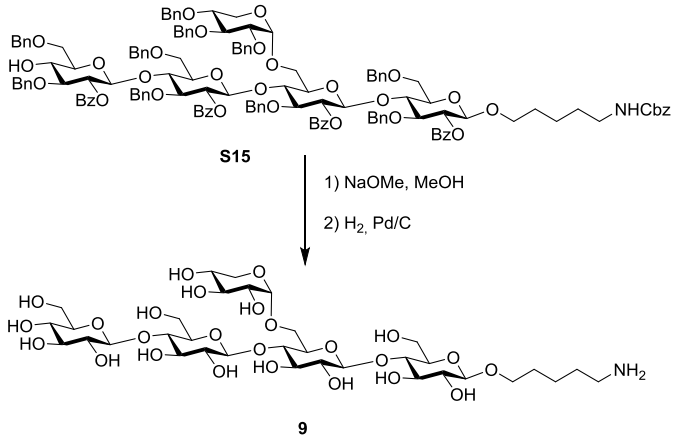
Crude NP-HPLC of pentasaccharide **S15** (ELSD trace):



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

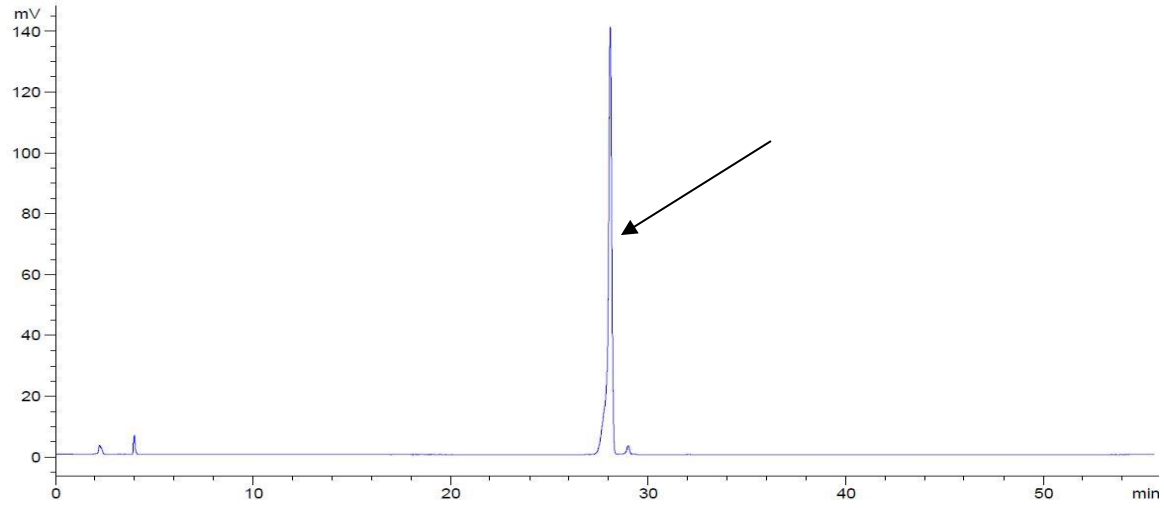
The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the protected pentasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (9)



Pentasaccharide **S15** was dissolved in THF (3 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 solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected pentasaccharide.

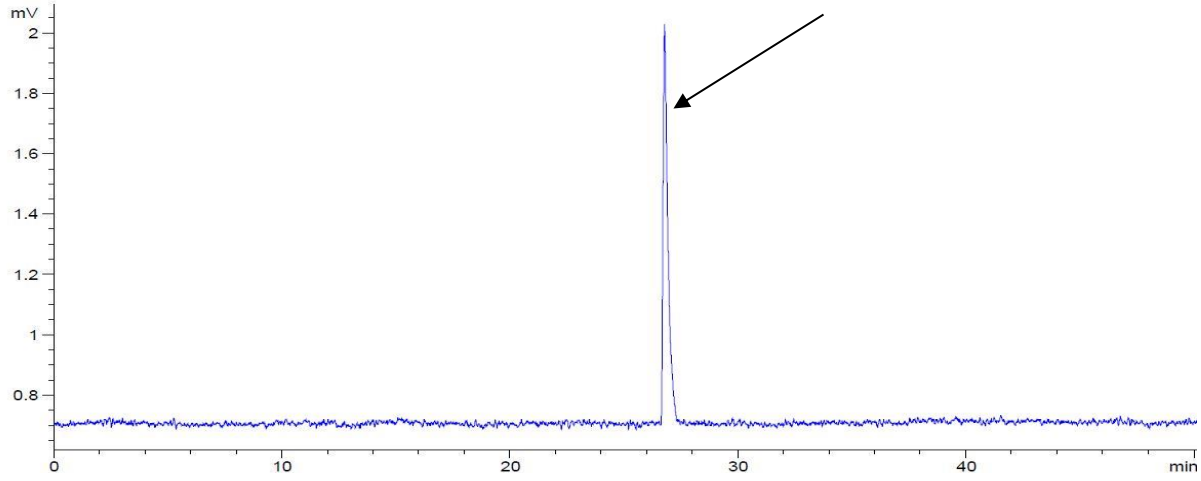
Crude NP-HPLC of the semi-protected pentasaccharide (ELSD trace):



HPLC was performed using a YMC-Diol-300 column and linear gradients 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_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 14 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide **9** (6.3 mg, 7.13 µmol, 42% over 11 steps, based on resin loading).

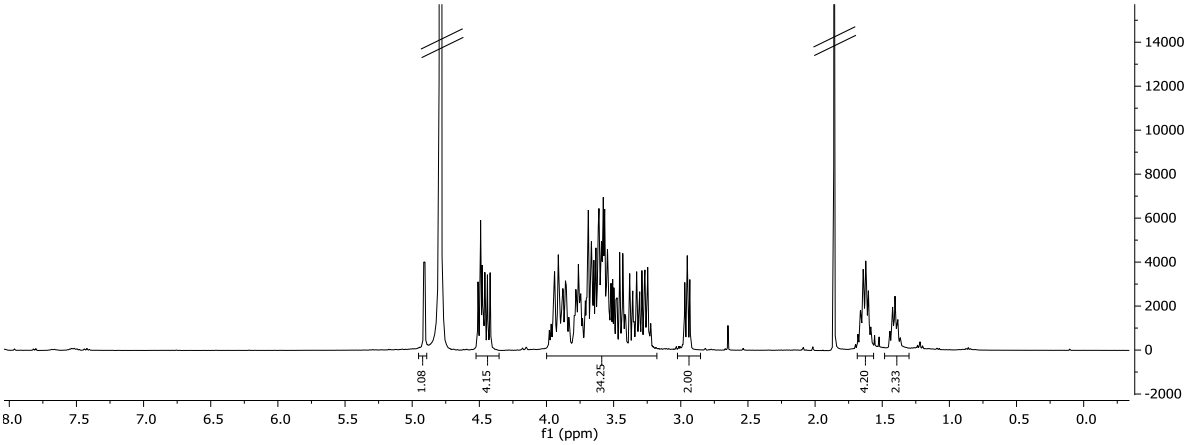
RP-HPLC of the deprotected pentasaccharide **9** (ELSD trace):



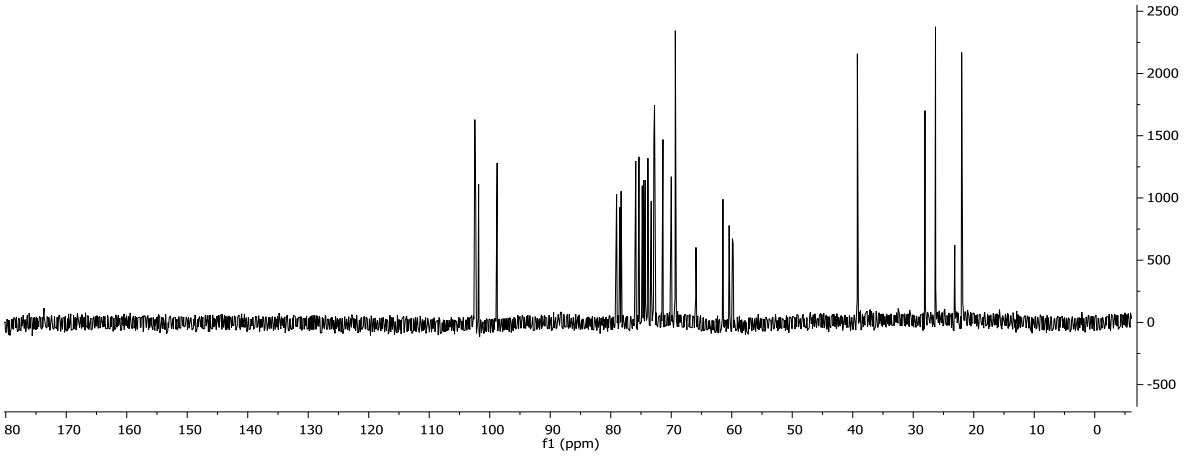
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.91 (d, *J* = 3.7 Hz, 1H), 4.53-4.40 (m, 4H), 4.01-3.22 (m, 31H), 2.93 (t, *J* = 7.6 Hz, 2H), 1.68-1.58 (m, 4H), 1.45-1.36 (m, 2H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 100.4, 100.2, 99.7, 96.7, 76.9, 76.4, 76.2, 73.8, 73.2, 72.7, 72.5, 72.2, 71.8, 71.7, 71.2, 70.6, 69.3, 67.9, 67.2, 63.8, 59.3, 58.3, 57.8, 57.7, 37.1, 25.9, 24.2, 19.9 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{34}H_{62}NO_{25}$: 884.3647; found 884.3641.

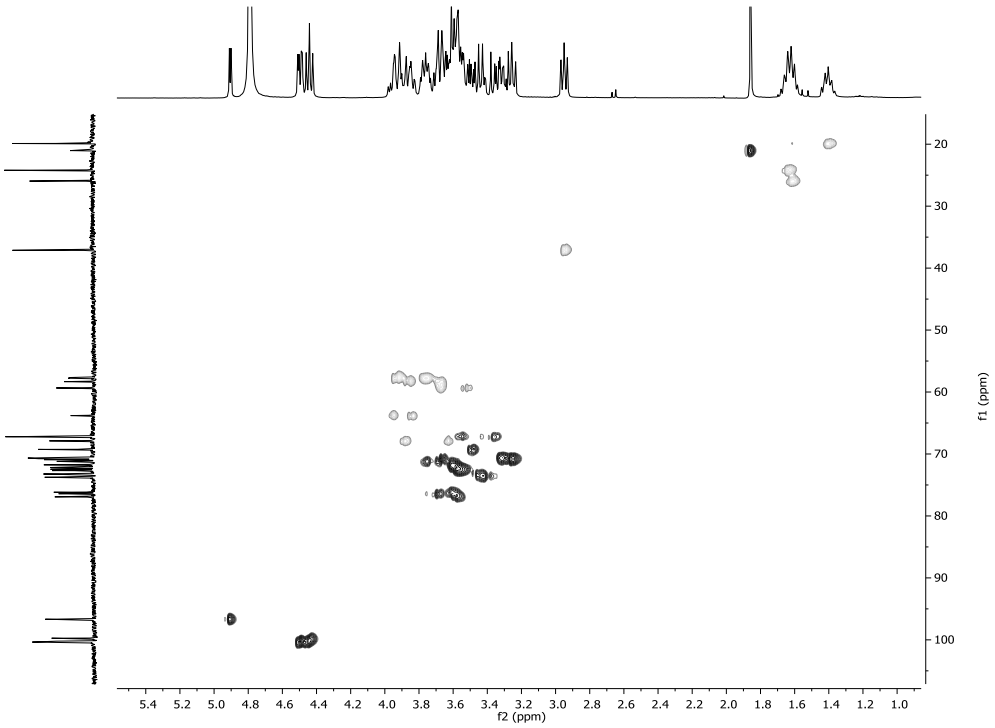
^{1}H NMR (400 MHz, D_2O) of **9**:



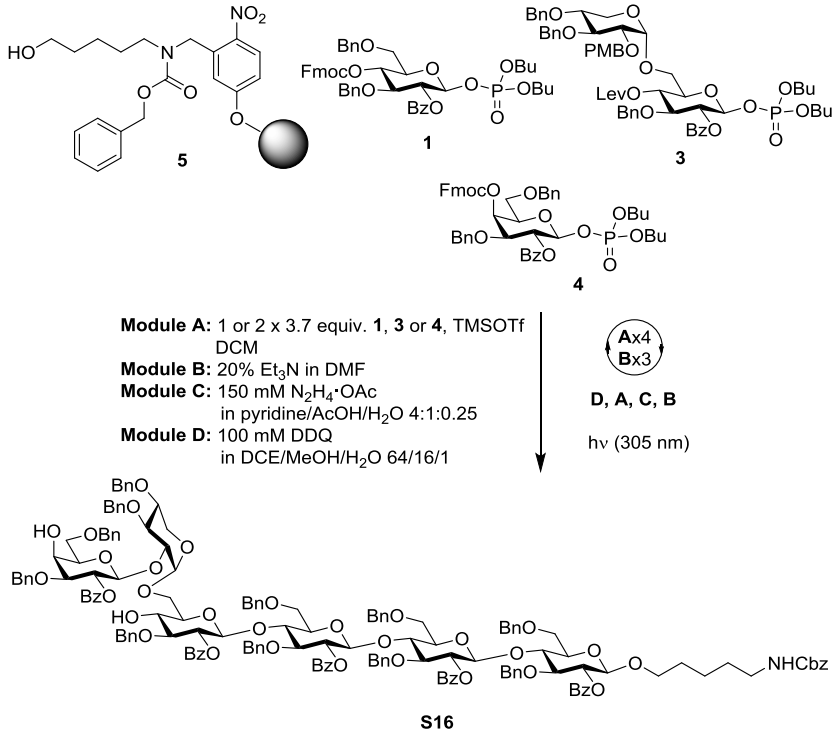
^{13}C NMR (151 MHz, D_2O) of **9**:



HSQC (D_2O) of **9**:



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-[2-*O*-[2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-galactopyranosyl]-3,4-*O*-dibenzyl-α-D-xylopyranosyl]-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 (S16)



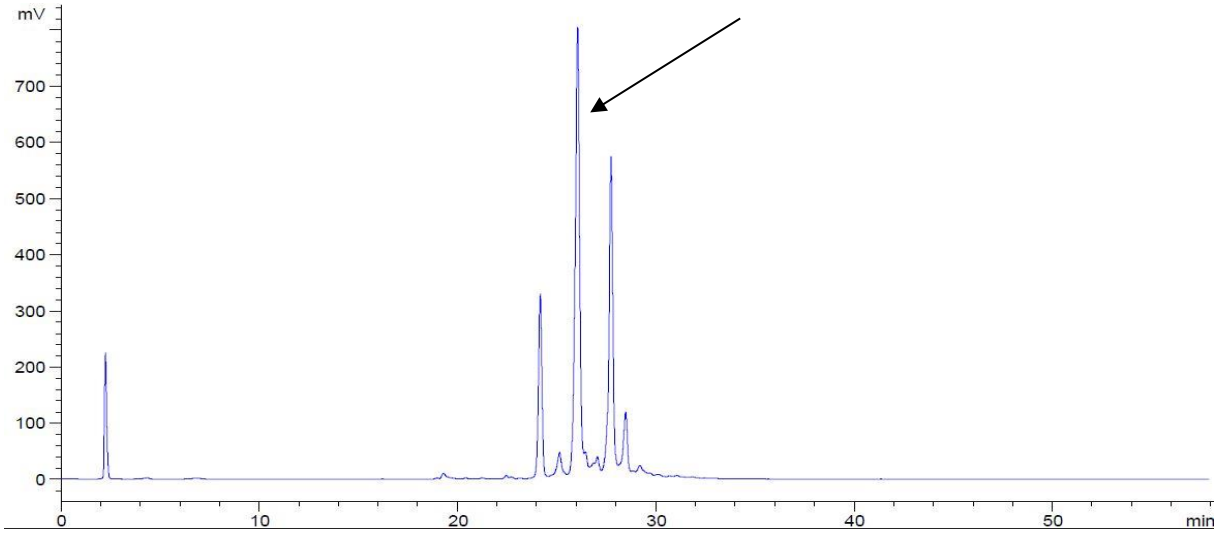
S16

Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **3**, TMSOTf, DCM, 2 x 40 min, -35 °C to -10 °C)
Module **D** (100 mM DDQ in DCE/MeOH/H_2O 64:16:1, 1 x 20 min, 40 °C)
Module **A** (2 x 3.7 equiv **4**, TMSOTf, DCM, 2 x 45 min, -35 °C to -20 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)
Module **B** (20% NEt_3 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 **S16**. The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column.

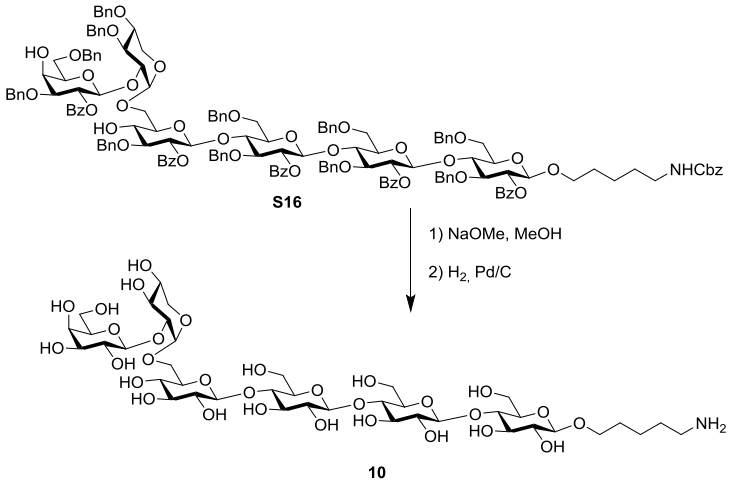
Crude NP-HPLC of hexasaccharide **S16** (ELSD trace):



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

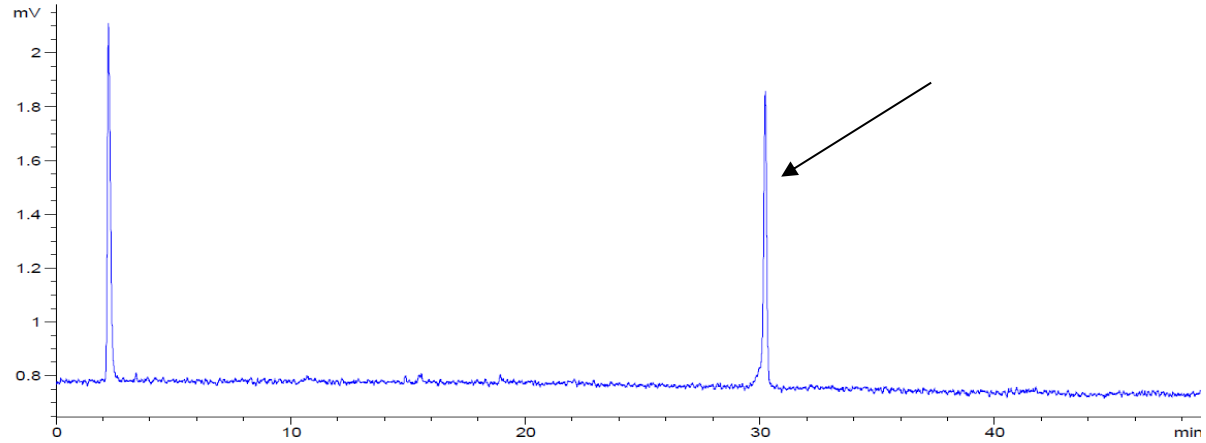
The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the protected hexasaccharide.

Aminopentyl 6-*O*-[2-*O*-[β-D-galactocopyranosyl]-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (10)



Hexasaccharide **S16** was dissolved in THF (3 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred for 24 h and then other NaOMe (0.5 M in MeOH, 0.5 mL). The reaction was stirred other 24 h and then NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred 6 h and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected hexasaccharide.

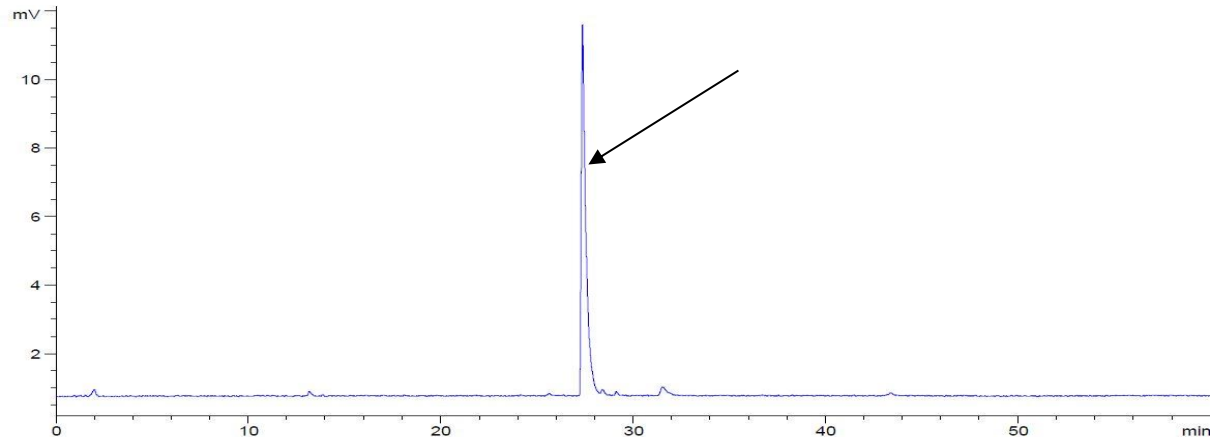
Crude NP-HPLC of the semi-protected hexasaccharide (ELSD trace):



HPLC was performed using a YMC-Diol-300 column and linear gradients 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_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 11 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide **10** (1.3 mg, 1.24 µmol, 7% over 13 steps, based on resin loading).

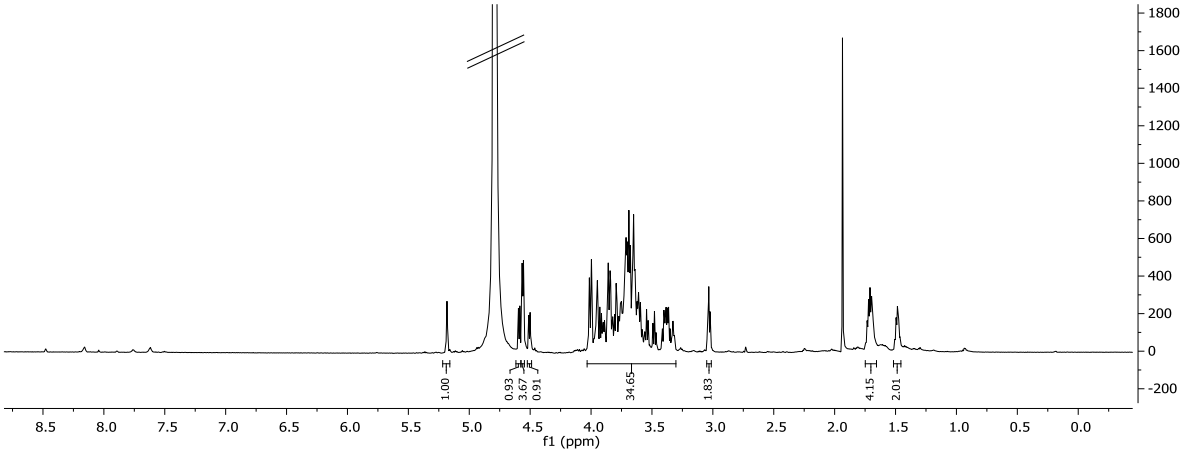
RP-HPLC of the deprotected hexasaccharide **10** (ELSD trace):



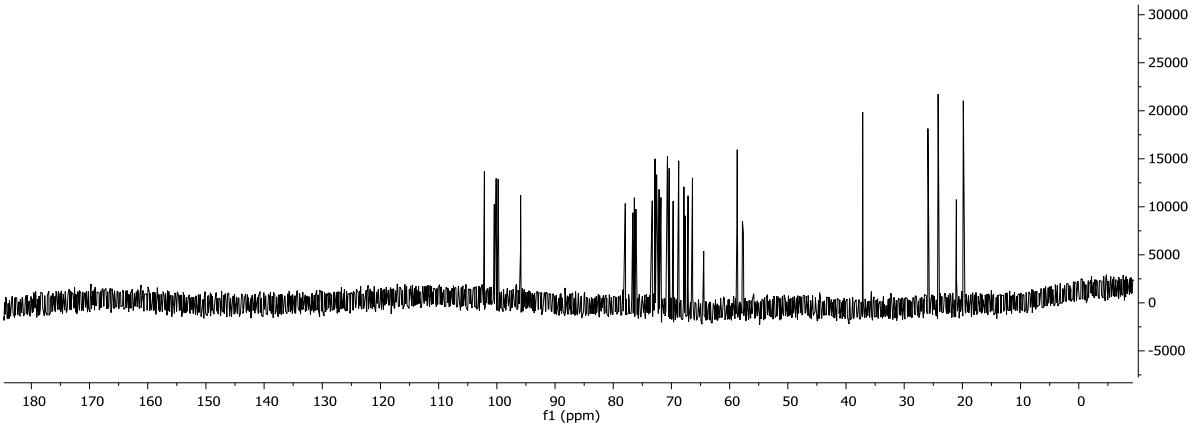
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (700 MHz, D_2O): δ = 5.18 (s, 1H), 4.59 (d, *J* = 7.6 Hz, 1H), 4.56 (d, *J* = 7.5 Hz, 3H), 4.51 (d, *J* = 8.0 Hz, 1H), 4.03-3.30 (m, 37H), 3.03 (t, *J* = 7.7 Hz, 2H), 1.76-1.65 (m, 4H), 1.53-1.45 (m, 2H) ppm.^{13}C NMR (176 MHz, D_2O): δ = 102.1, 100.4, 100.1, 99.8, 95.9, 77.9, 76.6, 76.4, 76.1, 73.3, 72.8, 72.6, 72.5, 72.2, 72.1, 71.9, 71.8, 70.8, 70.7, 70.4, 69.7, 68.7, 67.9, 67.6, 67.1, 66.4, 64.5, 58.8, 58.7, 57.8, 57.7, 57.6, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{40}H_{72}NO_{30}$: 1046.4139; found 1046.4166.

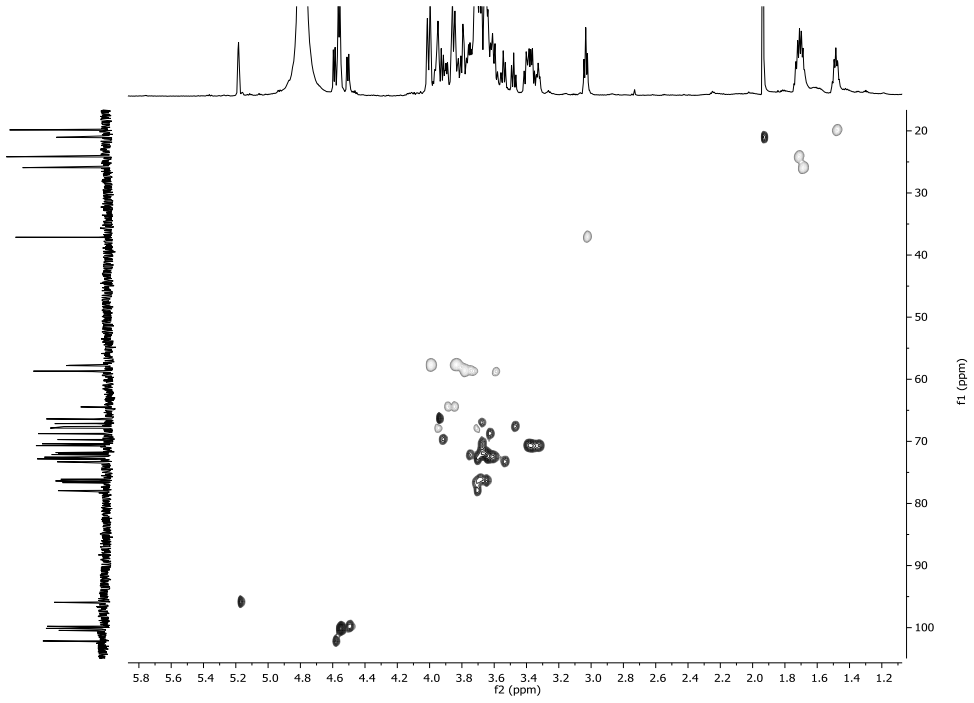
^{1}H NMR (700 MHz, D_2O) of **10**:



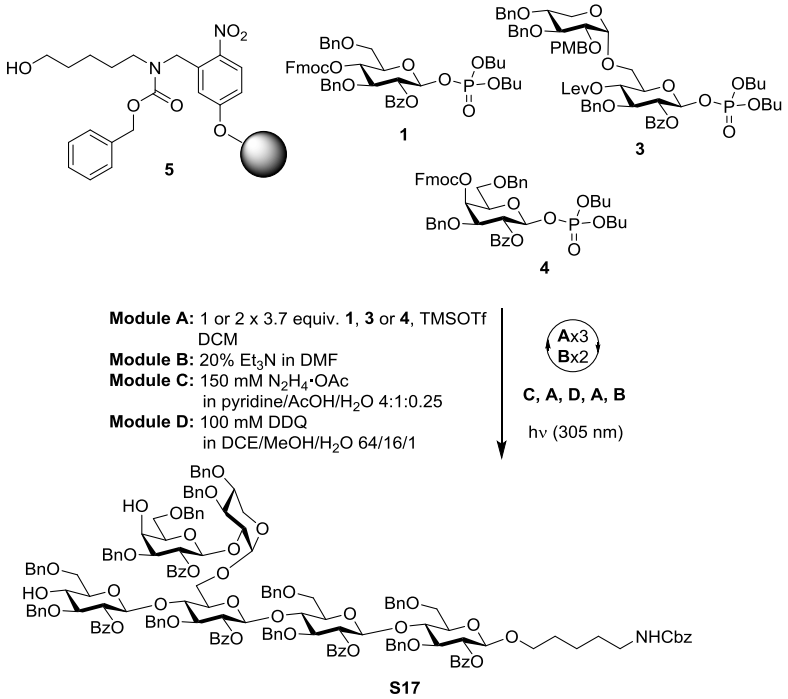
^{13}C NMR (151 MHz, D_2O) of **10**:



HSQC (D_2O) of **10:**



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-[2-*O*-[2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-galactopyranosyl]-3,4-*O*-dibenzyl-α-D-xylopyranosyl]-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 (S17)

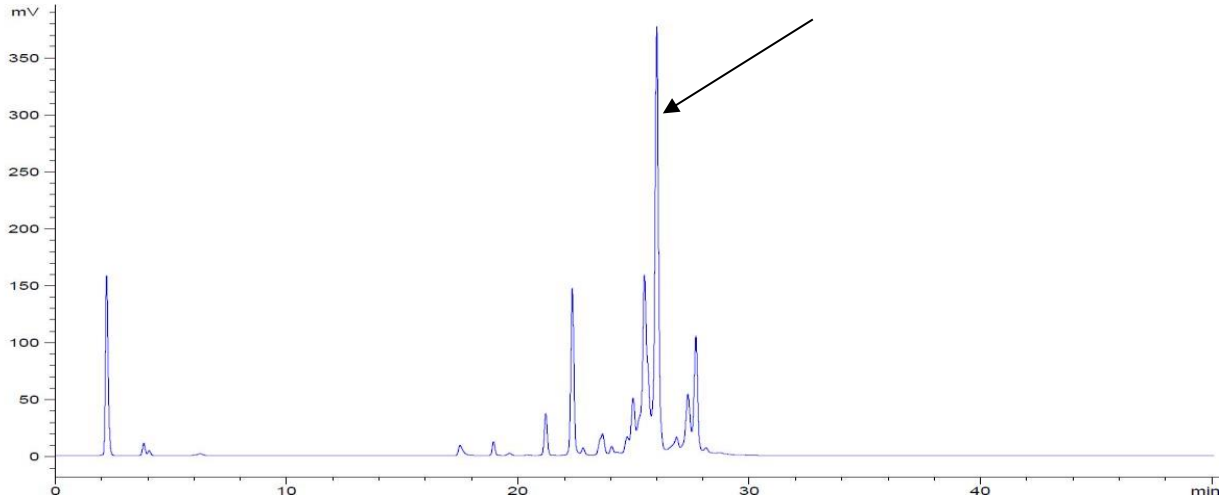


Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.7 equiv **3**, TMSOTf, DCM, 2 x 40 min, -35 °C to -10 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)
Module **A** (2 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **D** (100 mM DDQ in DCE/MeOH/H_2O 64:16:1, 1 x 20 min, 40 °C)
Module **A** (2 x 3.7 equiv **4**, TMSOTf, DCM, 2 x 45 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 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 **S17**. The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column.

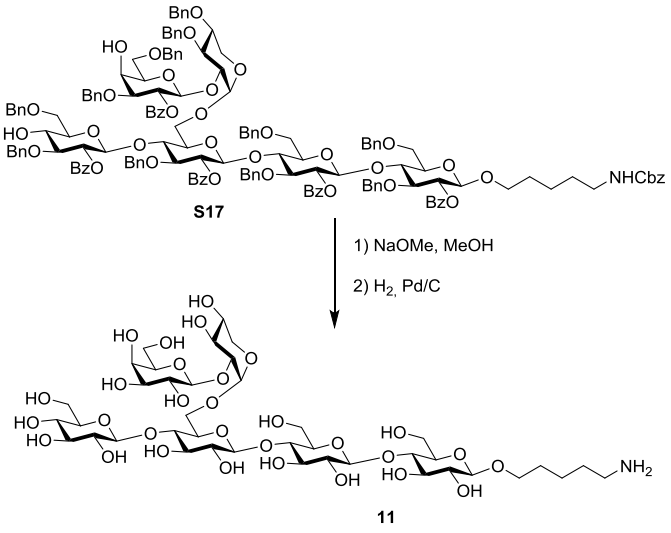
Crude NP-HPLC of hexasaccharide **S17** (ELSD trace):



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

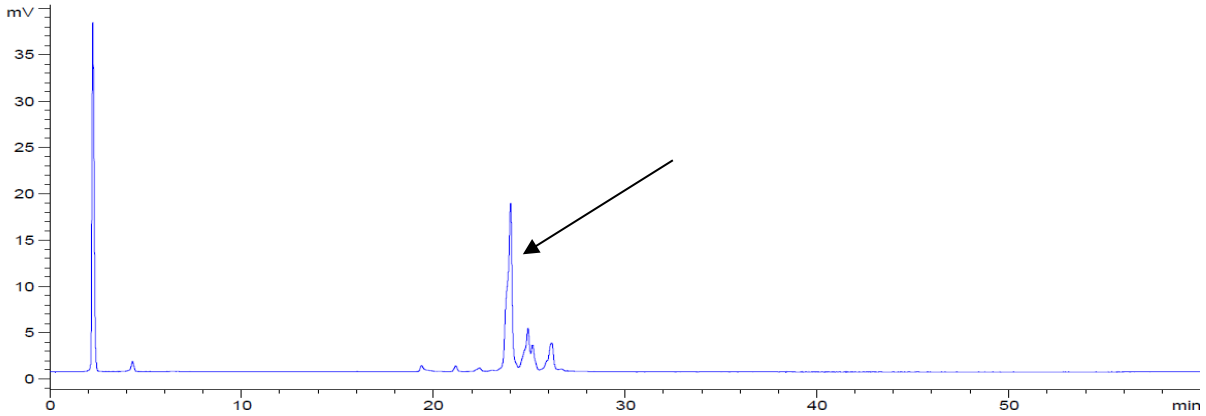
The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the protected hexasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[2-*O*-[β-D-galactocopyranosyl]-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (11)



Hexasaccharide **S17** 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 then other NaOMe (0.5 M in MeOH, 0.5 mL). The reaction was stirred other 48 h and then 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 solvent was removed *in vacuo.* The crude product was purified by normal-phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected hexasaccharide.

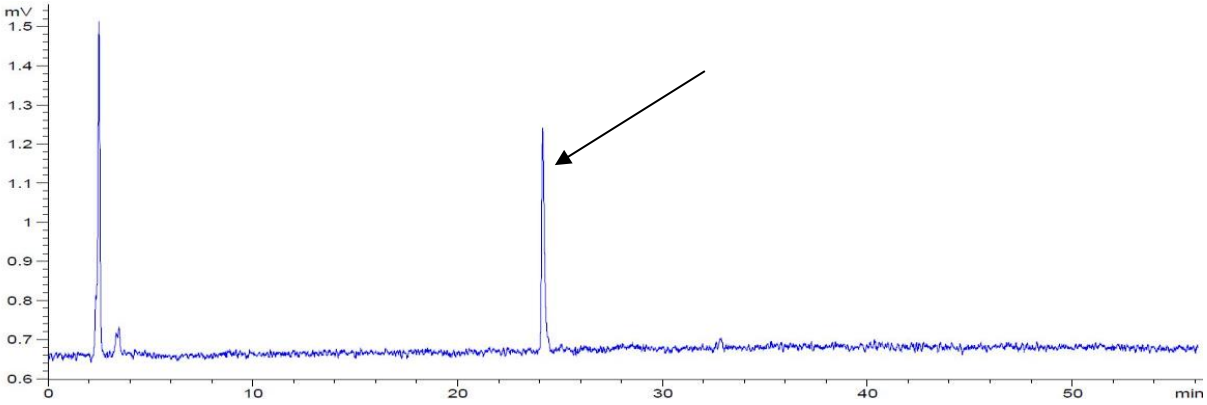
Crude NP-HPLC of the semi-protected hexasaccharide (ELSD trace):



HPLC was performed using a YMC-Diol-300 column and linear gradients 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_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 11 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide **11** (1.3 mg, 1.24 µmol, 7% over 13 steps, based on resin loading).

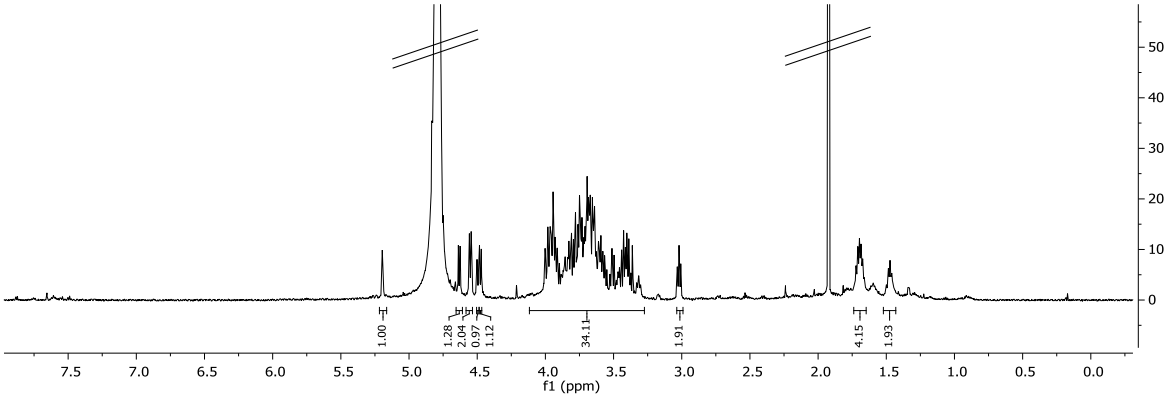
RP-HPLC of the deprotected hexasaccharide **11** (ELSD trace):



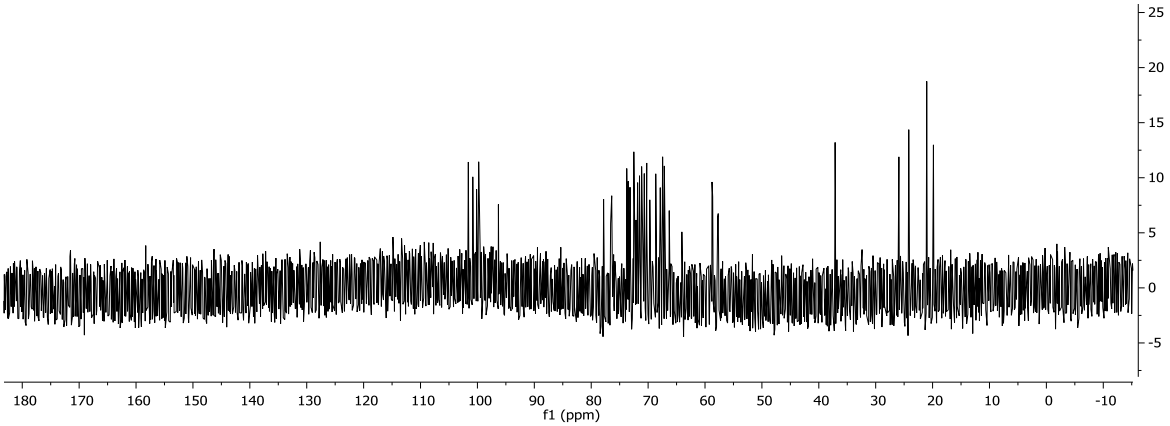
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 5.19 (d, *J* = 3.6 Hz, 1H), 4.63 (d, *J* = 7.8 Hz, 1H), 4.55 (d, *J* = 7.9 Hz, 2H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.12-3.27 (m, 37H), 3.02 (t, *J* = 7.5 Hz, 2H), 1.74-1.65 (m, 4H), 1.51-1.44(m, 2H) ppm.^{13}C NMR (151 MHz, D_2O): δ = 101.6, 100.8, 100.1, 99.8, 96.3, 77.8, 76.6, 76.4, 73.8, 73.5, 73.2, 72.5, 72.1, 71.8, 71.5, 71.1, 70.7, 70.5, 70.2, 69.7, 68.7, 67.9, 67.4, 67.1, 66.3, 64.1, 58.8, 58.6, 37.1, 25.9, 24.2, 19.8. ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{40}H_{72}NO_{30}$: 1046.4139; found 1046.4188.

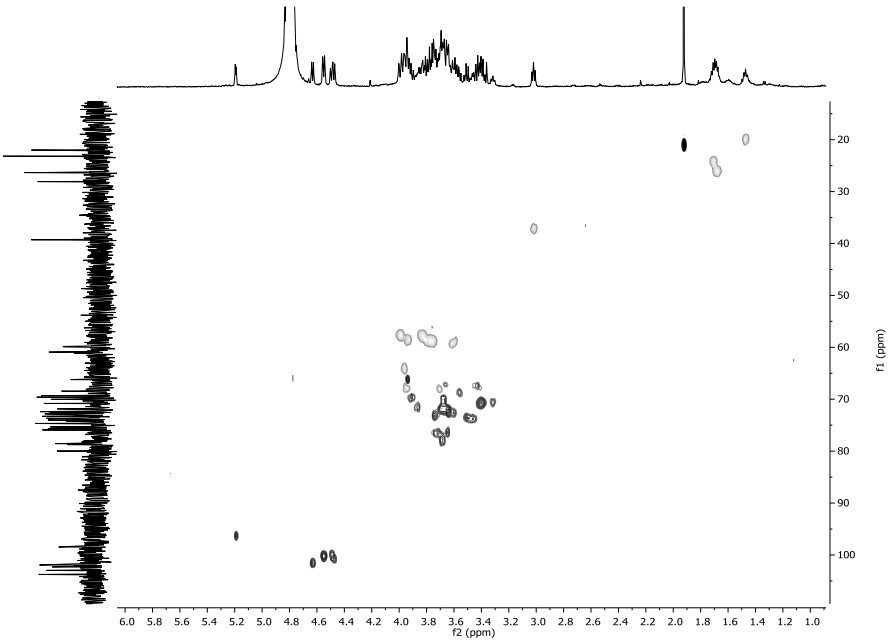
^{1}H NMR (600 MHz, D_2O) of **11**:



^{13}C NMR (151 MHz, D_2O) of **11**:

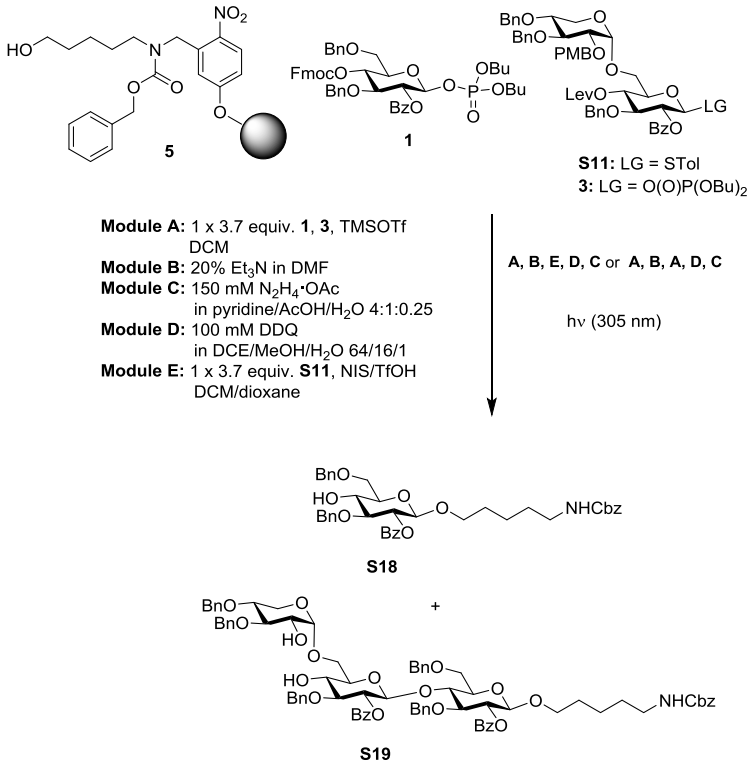


HSQC (D_2O) of **11**:



Glycosylation with thioglycoside BB vs phosphate BB

Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-[3,4-*O*-dibenzyl-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-glucopyranoside (S19)

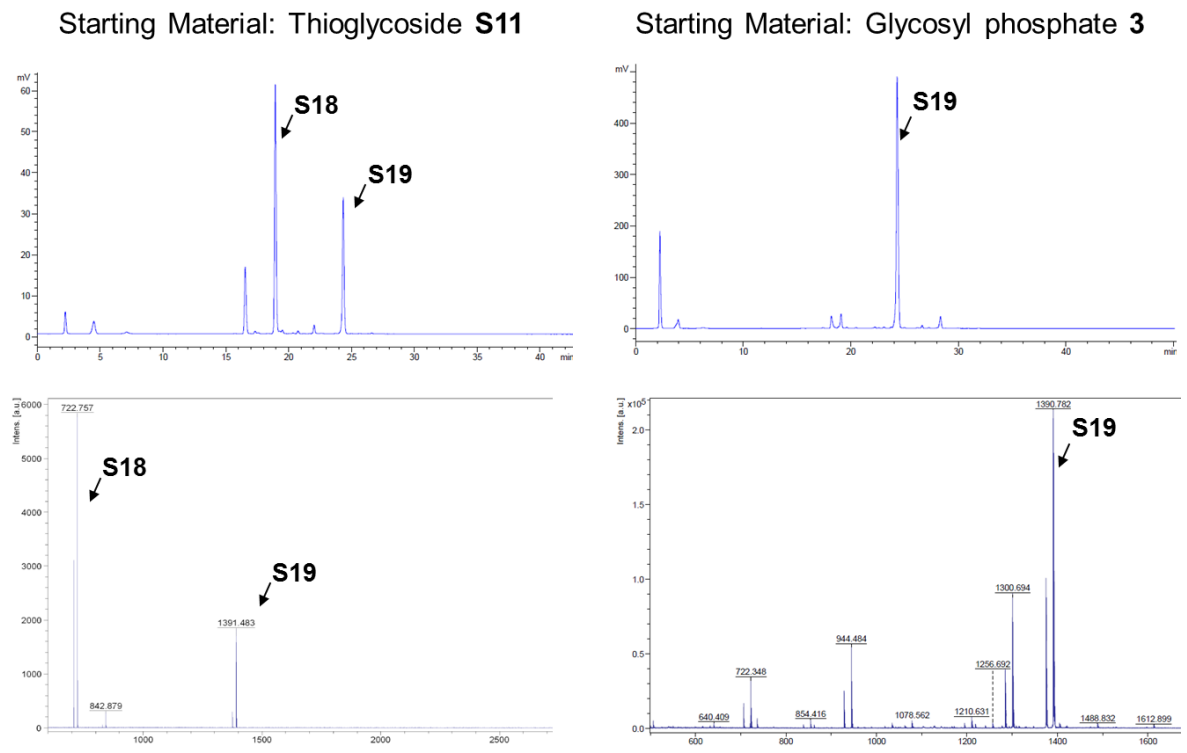


Linker-functionalized resin **5** (52 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 3.7 equiv **1**, TMSOTf, DCM, 2 x 35 min, -30 °C to -15 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 3.7 equiv **4**, TMSOTf, DCM, 1 x 40 min, -35 °C to -10 °C) or Module **E** (1 x 3.7 equiv **S11**, NIS and TfOH, DCM/dioxane 2:1, 1 x 40 min, -35 °C to -10 °C)
Module **D** (100 mM DDQ in DCE/MeOH/H_2O 64:16:1, 3 or 2 x 20 min, 40 °C)
Module **C** (150 mM N_2H_4·AcOH in pyridine/AcOH/H_2O 4:1:0.25, 3 x 30 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected trisaccharide **S19**.

Crude NP-HPLC (ELSD trace) and MALDI-TOF spectra of the reaction mixtures after using either thioglycoside BB **S11** or glycosyl phosphate BB **3** in the AGA of trisaccharide **S19**:



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).

Microarray printing

The xyloglucan oligosaccharides were printed on CodeLink *N*-hydroxyl succinimide (NHS) ester-activated glass slides (SurModics Inc., Eden Prairie, MN, USA) using a non-contact piezoelectric spotting device (S3; Scienion, Berlin, Germany). To assess the binding strength of the mAbs we used four different printing concentrations (200 µM, 50 µM, 12.5 µM, and 3.1 µM) diluted in coupling buffer (80% 50 mM sodium phosphate, pH 8.5, 0.005% CHAPS, and 20% PEG400). The printing was performed at room temperature and 40% humidity. After printing, the microarray slides were quenched for 1 h at room temperature in 100 mM ethanolamine, 50 mM sodium phosphate, pH 9, and washed twice with deionized water.

Assessing the binding specificities of xyloglucan-directed mAbs

To incubate different antibodies per microarray slide, we applied a FlexWell 64 grid (Grace Bio-Labs, Bend, OR, USA) to the slide. The slides were blocked with 1% (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 h at room temperature. Then, hybridoma supernatants of the antibodies were diluted 1:10 in PBS containing 1% (w/v) BSA, added to the slides and incubated for 1 h. After three washes with PBS, the slides were incubated with the respective secondary antibodies for 1 h. Goat anti-mouse IgG AF647 (Thermo Fisher) was used for CCRC-M48, CCRC-M49, CCRC-M50, CCRC-M51, CCRC-M52, CCRC-M53, CCRC-M54, CCRC-M55, CCRC-M57, CCRC-M58, CCRC-M87, CCRC-M88, CCRC-M89, CCRC-M93, CCRC-M95, CCRC-M96, CCRC-M99, CCRC-M101, and CCRC-M104. Goat

anti-mouse IgM AF594 (Thermo Fisher) was used as a secondary antibody for detection of CCRC-M86, CCRC-M90, CCRC-M100, and CCRC-M103. Unbound secondary antibodies were removed using consecutive washes with 0.1% (v/v) Tween-20 in PBS, PBS, and deionized water. Slides were dried by centrifugation (300 x g, 2 min), then the fluorescent signal on the slides was scanned with a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA). Image analysis and quantification of the fluorescent signal was carried out with GenePix Pro 7 software (Molecular Devices) using the same settings for each antibody. To obtain relative signal intensities, the average of the fluorescence values for the four concentrations were calculated and normalized to the highest recorded value for each of the two respective secondary antibodies.

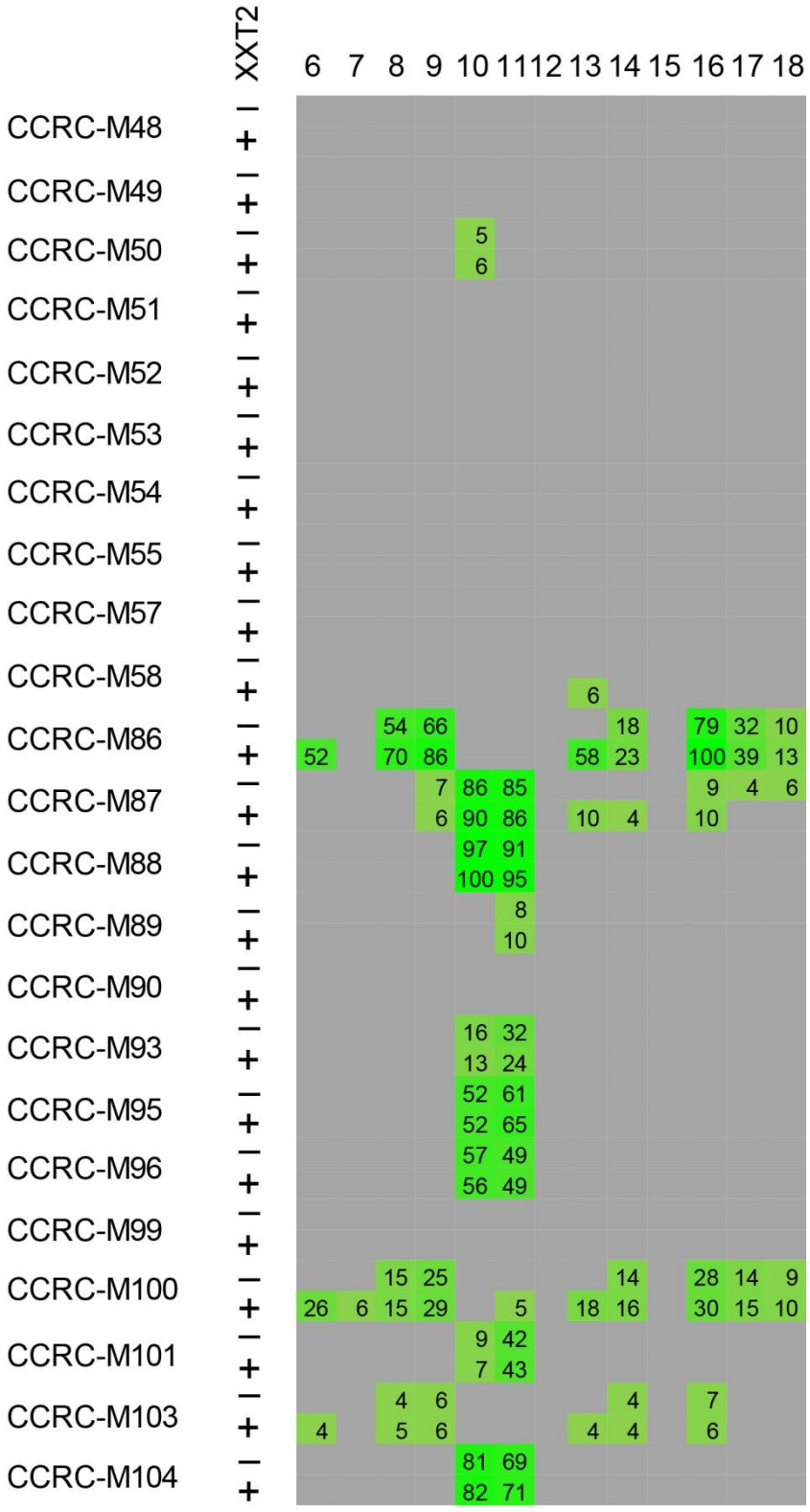
Expression of the soluble catalytic domain of AtXXT2 in HEK293 cells

*At*XXT2 was cloned in a manner similar to the previously described procedure.[8] Briefly, to create Gateway entry clones the truncated coding region of *At*XXT2 (amino acids 48-461) was PCR amplified (*At*XXT2_48F, 5'-AACTTGTACTTTCAAGGCGAGCAAGATCTTGACGAG-3' and *At*XXT2_461R, 5'-ACAAGAAAGCTGGGTCCTAAACTTGATTGGTTTGTAC-3') using Phusion High-Fidelity DNA Polymerase (Thermo Scientific) from cDNA prepared from leaves of wild-type *A. thaliana* (Col-0). A second set of universal primers (attB_Adapter-F, 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGAAAACTTGTACTTTCAAGGC-3' and attB_Adapter-R, 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTC-3') was used to complete the attB recombination sites and add a tobacco etch virus (TEV) protease cleavage site.[9] The *att*B-PCR product was cloned into the pDONR221 plasmid vector (Life technologies) to create an entry clone, which was then recombined into a Gateway-adapted version of the pGEn2 mammalian expression vector (pGEn2-DEST)(Meng et al., 2013) using Gateway BP Clonase II Enzyme Mix and Gateway LR Clonase II Enzyme Mix (Life Technologies), respectively. The resulting expression construct encodes a fusion protein comprised of an amino-terminal signal sequence, an 8xHis tag, an AviTag, the "superfolder" GFP (sfGFP) coding region, the tobacco etch virus (TEV) protease recognition sequence, then residues 48 to 461 of *At*XXT2.

Recombinant expression was performed by transient transfection of suspension culture HEK293-F cells as previously described.[9,10] Small scale purification of 8xHis-tagged enzyme secreted into the culture medium was performed using a 1 ml HisTrap HP column (GE Healthcare) as previously described.[8] Purified His-GFP-XXT2 was concentrated to 1 mg/ml using an Amicon Ultra Centrifugal Filter Device (30,000 MWCO, Merck Millipore) and dialyzed (3,500 MWCO) into HEPES sodium salt-HCl (75mM, pH 6.8) and used directly for reactions, or stored at 4 °C or -80 °C in aliquots.

Application of a glycosyltransferase on the glycan microarray

The glycosyltransferase assays (20 µl) were carried out using a FlexWell 64 grid applied to the glycan array. Similar to previous studies[11,12] we used 1 mM UDP-xylose as a donor with 5 µg of xyloglucan xylosyltransferase (XXT) 2 for each enzymatic reaction in 50 mM HEPES buffer, pH 7.4, including 1 mM $MgCl_2$ and 1 mM $MnCl_2$. The reactions were incubated overnight at room temperature and terminated by two washes with PBS. Incubation with buffer only was used as a negative control. The modification of the xyloglucan oligosaccharides was assessed using antibody staining as described above.



Supplementary Figure 1. Binding affinities of 23 xyloglucan-related mAbs[13] to the glycan array with and without XXT2 treatment. The relative fluorescent signal intensities for the different antibodies are displayed in percent of the highest value. Enzymatic treatment is indicated in the "XXT2"column with (+) or (-) for buffer control.

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