Electronic Supplementary Information

Automated Glycan Assembly of Xyloglucan Oligosaccharides

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Contents

Abbreviations

Ac: acetyl; Ar: aryl; BB: building block; Bu: Butyl; Bz: Benzoyl; DABCO: 1,4-Diazabicyclo[2.2.2]octane; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM: dichloromethane; DIC: *N,N'*-diisopropylcarbodiimide; DMF: dimethylformamide; DMAP: dimethylaminopyridine; EtN_3, TEA: triethylamine; EtOAc: ethyl acetate; Fmoc: fluorenylmethoxycarbonyl; Hex: hexane; Lev: levulinic; NIS: N-iodosuccinimide; NP: normal phase; Ph: phenyl; RP: reverse phase; rt: room temperature; THF: tetrahydrofuran; TFA: trifluoroacetic acid; TFAA: trifluoroacetic anhydride; TfOH: trifluoromethanesulfonic acid; TMSOTf: trimethylsilyl trifluoromethanesulfonate; Tol: toluene.

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 **4** was prepared according to

literature.[1] The Resin loading was determined as described previously.[2] Intermediate 4-methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-thio-β-D-glucopyranoside,[3-5] intermediate 4-methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-thio-β-D-glucopyranoside (**7**)[5] and intermediate 4-methylphenyl 2,3,4-*O*-tribenzyl-β-D-xylopyranoside (**3**)[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 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 internal standard in ^1H NMR (D_2O: 4.79 ppm ^1H) and acetic acid as 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 Luna 5u Silica 100A column (250 x 4.6 mm), a Phenomenex Luna C5 column (250 x 4.6 mm), 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 semi-preparative Luna 5u Silica 100A column, a semi-preparative Phenomenex Luna C5 column (250 x 10 mm) or a preparative YMC-Diol-300 column (150 x 20 mm).

Synthesizer Modules and Conditions

The linker-functionalized resin **4** (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 **D**) and deprotection (Module **B** and **C**) 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 -15 °C for 30 or 35 minutes. Subsequently the solution was drained and the resin was washed three times with DCM. The whole procedure was performed once, twice or three times 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
					-15	30 / 35 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 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 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	DMF	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: 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 DCM. 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 -55 °C and then for 40 min at -30 °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 °C.

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

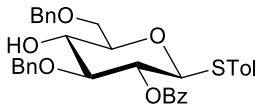
Action	Cycles	Solvent	Reagent 1	Reagent 2	T (°C)	Incubation Time
Wash	1	DCM	TMSOTf		-30	2 min
Wash	1	DCM			-30	25 s
Glycosylation	1	DCM	BB (62.5 µmol)	NIS (75 µmol) TfOH (7.5 µmol)	-55	5 min
					-30	40 min
Wash	3	DCM			-15	15 s
Glycosylation	1	DCM	BB (62.5 µmol)	NIS (75 µmol) TfOH (7.5 µmol)	-55	5 min
					-30	40 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

4-Methylphenyl 2-*O*-benzoyl-3,6-*O*-dibenzyl-thio-β-D-glucopyranoside (S1)

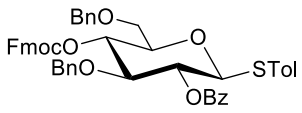


4-Methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-thio-β-D-glucopyranoside[3-5] (15.0 g, 26.4 mmol) was dissolved in DCM (200 mL) and triethylsilane (25.3 mL, 158 mmol) was added. Then the reaction was cooled to 0 °C and TFAA (3.73 mL, 26.4 mmol) was slowly added. The reaction was stirred at 0 °C for 20 min, TFA (10.2 mL, 132 mmol) was added dropwise and the reaction was gradually warmed to rt. After 2 h the reaction was quenched with sat. aq. $NaHCO_3$ solution (100 mL) and extracted with DCM (100 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by silica gel chromatography (Hex/EtOAc 4:1) to give compound **S1** (10.5 g, 18.4 mmol, 70%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 7.8 Hz, 2H, Ar), 7.60 (t, *J* = 7.4 Hz, 1H, Ar), 7.51-7.44 (m, 2H, Ar), 7.40-7.29 (m, 7H, Ar), 7.17 (s, 5H, Ar), 7.02 (d, *J* = 7.7 Hz, 2H, Ar), 5.23 (t, *J* = 9.5 Hz, 1H), 4.80-4.49 (m, 5H), 3.83-3.73 (m, 3H), 3.68 (t, *J* = 8.9 Hz, 1H), 3.63-3.54 (m, 1H), 2.29 (s, 3H) ppm.

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

4-Methylphenyl 2-*O*-benzoyl-3,6-*O*-dibenzyl-4-*O*-fluorenylcarboxymethyl-thio-β-D-glucopyranoside (S2)

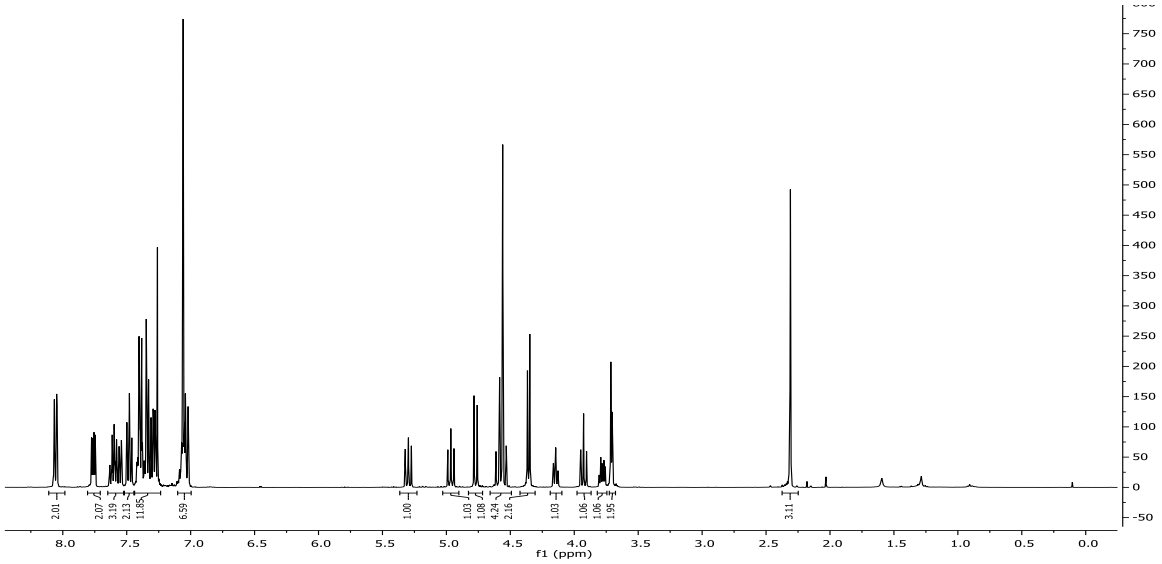


To a solution of **S1** (2.30 g, 4.03 mmol) in DCM (30 mL) and pyridine (5 mL), FmocCl (1.34 g, 5.18 mmol) was added. After 5.5 h more pyridine (1 mL) and FmocCl (1.04 g, 4.02 mmol) were added. The reaction mixture was stirred for another 2 h and then diluted with DCM (100 mL) and washed with a 1 M HCl solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and purified by silica gel chromatography (Tol/EtOAc 8:1) to give compound **S2** (2.89 g, 3.64 mmol, 90%) as a white solid.

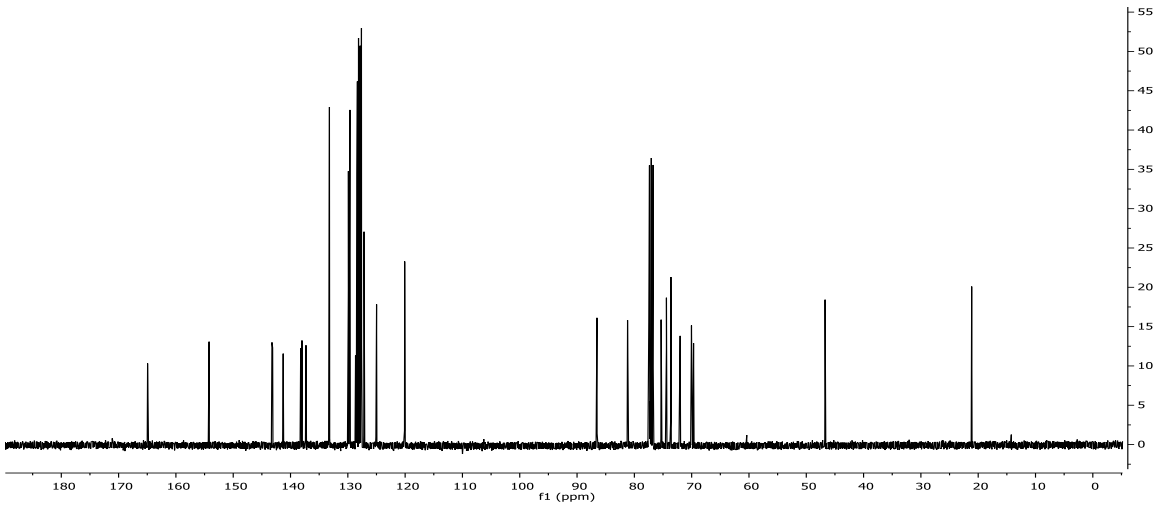
$[\alpha]_D^{25}$ = +27.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09-8.03 (m, 2H, Ar), 7.76 (dd, *J* = 7.5, 3.9 Hz, 2H, Ar), 7.65-7.53 (m, 3H, Ar), 7.48 (t, *J* = 7.7 Hz, 2H, Ar), 7.44-7.27 (m, 12H, Ar), 7.11-6.99 (m, 6H, Ar), 5.30 (t, *J* = 9.5 Hz, 1H, H-2), 4.97 (t, *J* = 9.6 Hz, 1H, H-4), 4.77 (d, *J* = 10.0 Hz, 1H, H-1), 4.63-4.51 (m, 4H, C*H*$_2$Ph), 4.36 (d, *J* = 7.0 Hz, 2H, Fmoc), 4.15 (t, *J* = 6.6 Hz, 1H, Fmoc), 3.93 (t, *J* = 9.1 Hz, 1H, H-3), 3.78 (m, 1H, H-5), 3.71 (m, 2H, H-6), 2.31 (s, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 165.1,

154.3 (2C, C=O), 143.4, 143.2, 141.42, 141.39, 138.4, 138.1, 137.4, 133.4, 133.3, 130.0, 129.9, 129.8, 128.8, 128.6, 128.5, 128.3, 128.0, 127.7, 127.3, 125.2, 125.1, 120.2 (36C, Ar), 86.6 (C-1), 81.3 (C-3), 77.6 (C-5), 75.5 (C-4), 74.5 (*C*H_2Ph), 73.7 (*C*H_2Ph), 72.1 (C-2), 70.2 (Fmoc), 69.8 (C-6), 46.8 (Fmoc), 21.3 (CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{49}H_{44}NaO_8S$: 815.2655; found 815.2641. IR (neat) ν_{max} = 1753, 1318, 1248, 1069 cm^{-1}.

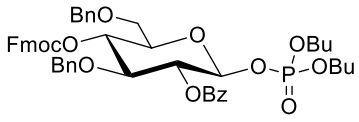
^{1}H NMR (400 MHz, $CDCl_3$) of thioglycoside **S2**:



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



Dibutoxyphosphoryloxy 2-*O*-benzoyl-3,6-*O*-dibenzyl-4-*O*-fluorenylcarboxymethyl-β-D-glucopyranoside (1)

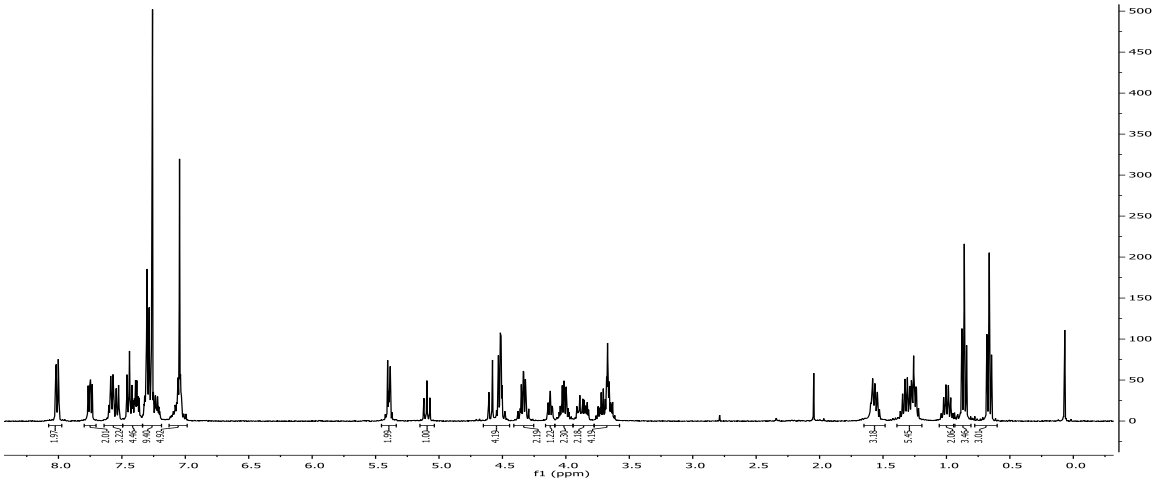


A solution of dibutyl phosphate (5.00 mL, 25.2 mmol) in DCM (15 mL) was dried over molecular sieves. After 1 h the supernatant (4.1 mL) was added to a solution of **S2** (2.72 g, 3.43 mmol) in DCM

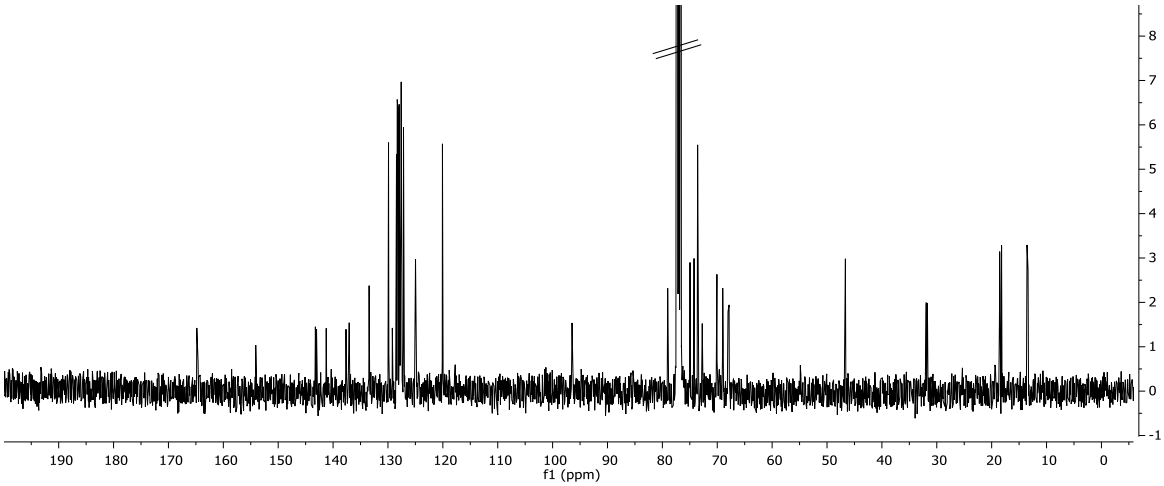
(20 mL) and cooled to 0 °C. Then NIS (926 mg, 4.12 mmol) and TfOH (90.0 µL, 1.03 mmol) were added. The reaction was stirred for 2 h, quenched with an aqueous solution of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 100 mL) and extracted with DCM (100 mL). The organic layer was dried over Na_2SO_4 and purified by silica gel chromatography (Hex/EtOAc 4:1) to give **1** (2.78 g, 3.16 mmol, 92%) as a yellow oil.

$[\alpha]_D^{25}$ = +35.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04-7.99 (m, 2H, Ar), 7.78-7.72 (m, 2H, Ar), 7.62-7.51 (m, 3H, Ar), 7.47-7.35 (m, 4H, Ar), 7.33-7.18 (m, 7H, Ar), 7.10-6.99 (m, 5H, Ar), 5.44-5.33 (m, 2H, H-1, H-2), 5.10 (t, *J* = 9.6 Hz, 1H, H-4), 4.59 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph), 4.56-4.46 (m, 3H, C*H*$_2$Ph), 4.40-4.26 (m, 2H, Fmoc), 4.12 (dd, *J* = 9.5, 4.9 Hz, 1H, Fmoc), 4.08-3.95 (m, 2H, OBu), 3.94-3.79 (m, 2H, H-5, H-3), 3.78-3.58 (m, 4H, 6-H, OBu), 1.65-1.46 (m, 2H, Bu), 1.39-1.19 (m, 4H, Bu), 0.99 (dd, *J* = 15.1, 7.5 Hz, 2H, Bu), 0.86 (t, *J* = 7.4 Hz, 3H, CH_3), 0.66 (t, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): 164.9, 154.1 (2C, C=O), 143.2, 143.0, 141.3, 137.1, 133.4, 129.9, 128.5, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.2, 125.1, 125.0, 120.0 (30C, Ar), 96.5 (C-1), 79.0 (C-3), 75.0 (C-4), 74.2 (*C*H$_2$Ph), 73.6 (2C, *C*H$_2$Ph, C-5), 72.7 (1C, C-2), 70.0 (Fmoc), 69.0 (C-6), 68.0 (OBu), 67.8 (OBu), 46.7 (Fmoc), 32.0, 31.8 (2C, Bu), 18.5, 18.2 (2C, Bu), 13.6, 13.4 (2C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{50}H_{55}NaO_{12}P$: 901.3329; found 901.3361. IR (neat) ν_{max} = 1753, 1733, 1248, 1027 cm^{-1}.

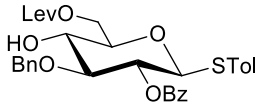
^{1}H NMR (400 MHz, $CDCl_3$) of glycosyl phosphate **1**:



^{13}C NMR (100 MHz, $CDCl_3$) of glycosyl phosphate **1**:



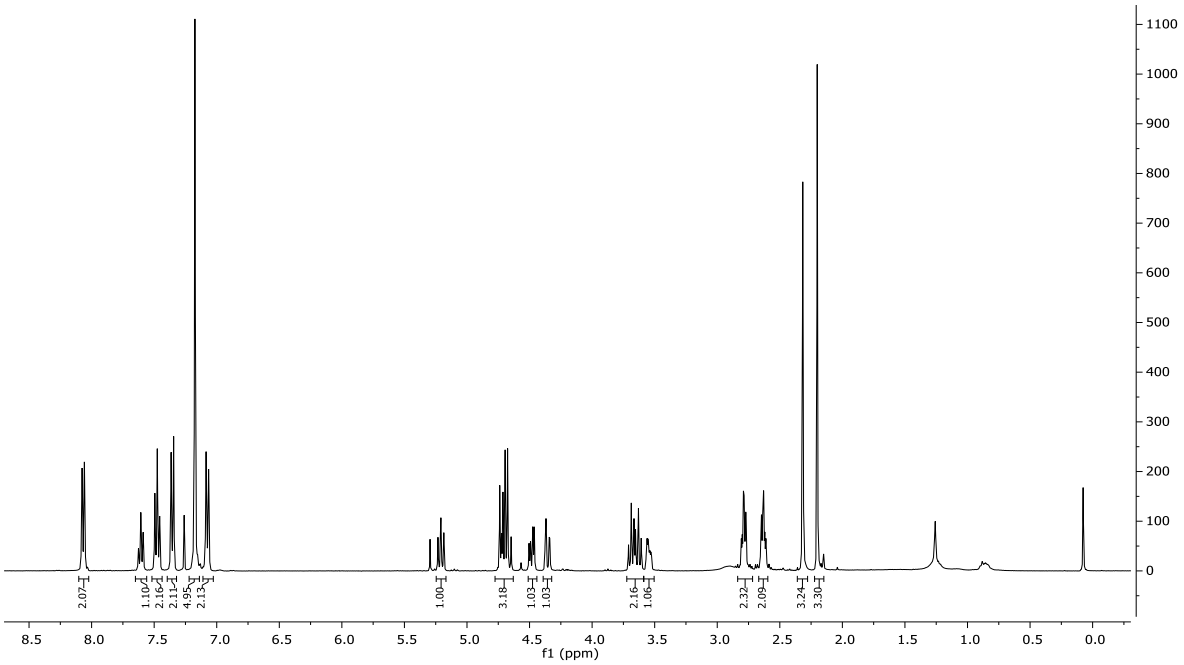
4-Methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-thio-β-D-glucopyranoside (S3)



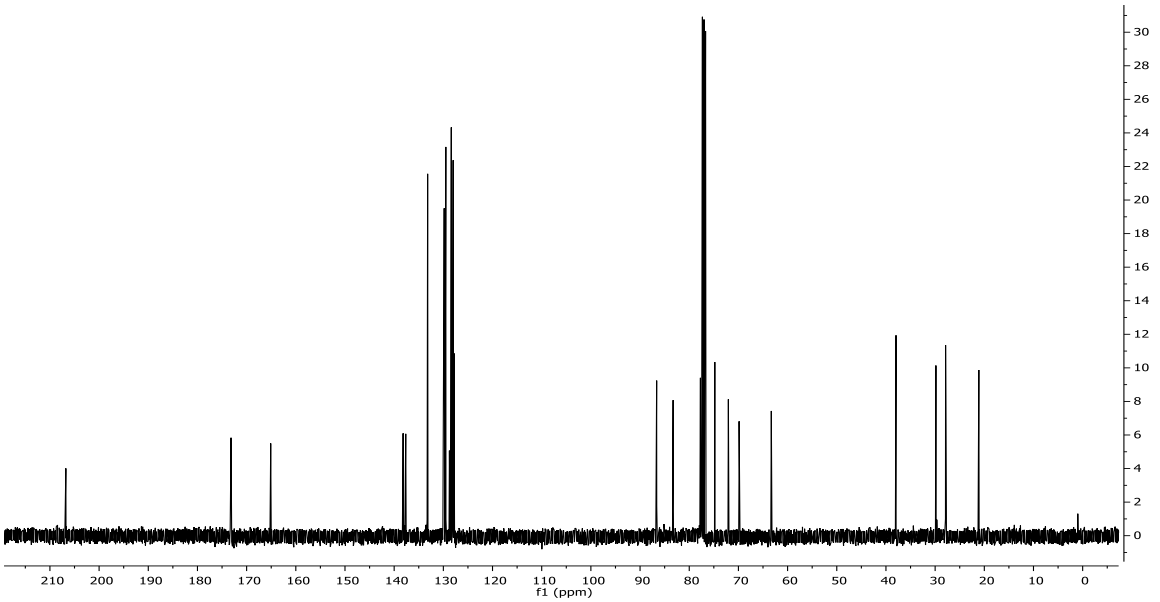
4-Methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-thio-β-D-glucopyranoside[5] (728 mg, 1.51 mmol) was dissolved in DCM (10 mL), levulinic acid (352 mg, 3.03 mmol) and 2-chloro-1-methylpyridinium iodide (774 mg, 3.03 mmol) were added. The reaction was stirred for 15 min and then cooled to -15 °C. At this temperature DABCO (680 mg, 6.06 mmol) was added. The reaction mixture was stirred for 40 min, filtered over a plug of celite© and concentrated. The crude product was purified by silica gel chromatography (Hex/EtOAc 3:1) to yield **S3** (671 mg, 1.16 mmol, 77%) as a white solid.

$[\alpha]_D^{25}$ = +5.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 7.4 Hz, 2H, Ar), 7.61 (t, *J* = 7.4 Hz, 1H, Ar), 7.48 (t, *J* = 7.7 Hz, 2H, Ar), 7.35 (d, *J* = 8.0 Hz, 2H, Ar), 7.17 (s, 5H, Ar), 7.07 (d, *J* = 8.0 Hz, 2H, Ar), 5.21 (t, *J* = 9.4 Hz, 1H, H-2), 4.77-4.63 (m, 3H, H-1, C*H*$_2$Ph), 4.48 (dd, *J* = 12.1 Hz, 4.6 Hz, 1H, H-6a), 4.36 (dd, *J* = 12.1 Hz, 1.7 Hz, 1H, H-6b), 3.73-3.59 (m, 2H, H-3, H-4), 3.58-3.58 (m, 1H, H-5), 2.83-2.75 (m, 2H, Lev), 2.67-2.61 (m, 2H, Lev), 2.32 (s, 3H, CH_3), 2.20 (s, 3H, O=CCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.8, 173.2, 165.1 (3C, *C*=O), 138.2, 137.6, 133.2, 129.8, 129.7, 129.5, 128.8, 128.4, 128.0, 127.8 (13C, Ar), 86.6 (C-1), 83.3 (C-3), 77.7 (C-5), 74.8 (*C*H$_2$Ph), 72.0 (C-2), 69.9 (C-4), 63.3 (C-6), 37.9 (Lev), 29.8 (O=C*C*H$_3$), 27.9 (Lev), 21.1 (CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{32}H_{34}NaO_8S$: 601.1867; found 601.1910. IR (neat) ν_{max}: 3486, 1722, 1270, 1070 cm^{-1}.

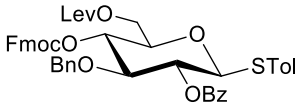
^{1}H NMR (400 MHz, $CDCl_3$) of **S3**:



^{13}C NMR (100 MHz, $CDCl_3$) of **S3**:



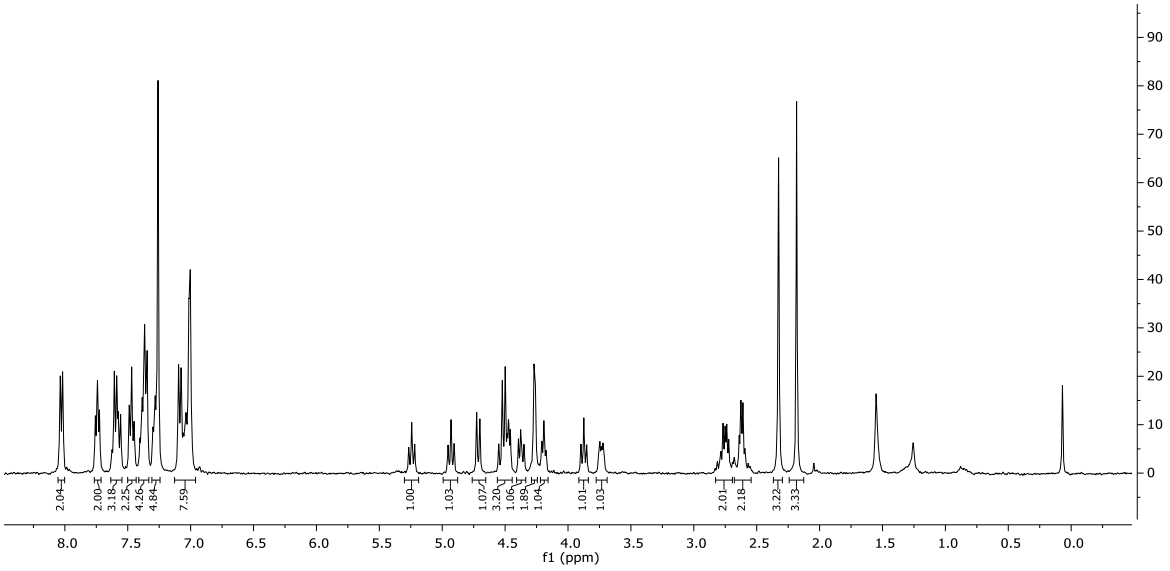
4-Methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylcarboxymethyl-6-*O*-levulinoyl-thio-β-D-glucopyranoside (S4)



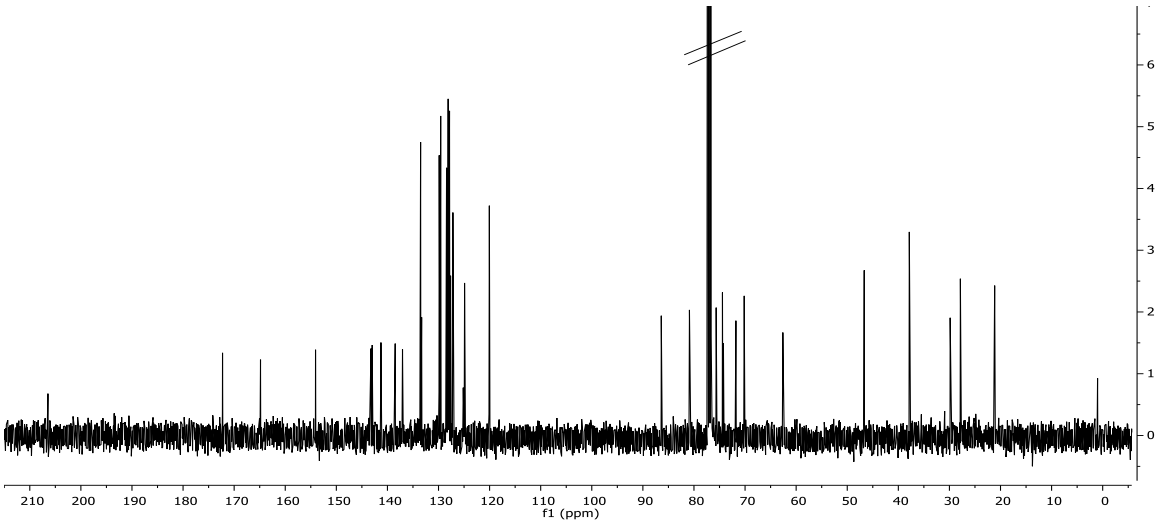
To a solution of **S3** (671 mg, 1.16 mmol) in DCM (10 mL) and pyridine (2.3 mL), FmocCl (403 mg, 1.56 mmol) was added. The reaction mixture was stirred overnight, then diluted with DCM (100 mL) and washed with a 1 M HCl solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and purified by silica gel chromatography (Tol/EtOAc 10:1) to give **S4** (691 mg, 863 µmol, 74%) as a white solid.

$[\alpha]_D^{25}$ = +25.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05-8.00 (m, 2H, Ar), 7.77-7.71 (m, 2H, Ar), 7.64-7.54 (m, 3H, Ar), 7.47 (t, *J* = 7.8 Hz, 2H), 7.41-7.33 (m, 3H, Ar), 7.31-7.24 (m, 4H, Ar), 7.12-6.98 (m, 6H, Ar), 5.24 (t, *J* = 9.5 Hz, 1H, H-2), 4.93 (t, *J* = 9.6 Hz, 1H, H-4), 4.71 (d, *J* = 10 Hz, 1H, H-1), 4.56-4.44 (m, 3H, H-6a, C*H*$_2$Ph), 4.41-4.34 (m, 1H, H-6b), 4.29-4.24 (m, 2H, Fmoc), 4.19 (t, *J* = 7.1 Hz, 1H, Fmoc), 3.87 (t, *J* = 9.2 Hz, 1H, H-3), 3.77-3.70 (m, 1H, H-5), 2.84-2.71 (m, 2H, Lev), 2.68-2.58 (m, 2H, Lev), 2.33 (s, 3H, CH_3), 2.18 (s, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.4, 172.3, 164.8, 154.1 (4C, C=O), 143.2, 143.0, 141.3, 141.2, 138.5, 137.0, 133.5, 133.3, 129.9, 129.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.6, 127.2, 125.0, 124.9, 120.0 (30C, Ar), 86.4 (C-1), 80.9 (C-3), 75.6 (C-5), 74.4 (*C*H$_2$Ph), 74.2 (C-4), 71.8 (C-2), 70.2 (C-6), 62.6 (Fmoc), 46.7 (Fmoc), 37.8 (Lev), 29.9 (O=C*C*H$_3$), 27.9 (Lev), 21.1 (CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{47}H_{44}NaO_{10}S$: 823.2548; found 823.2588. IR (neat) ν_{max}: 1751, 1258 cm^{-1}.

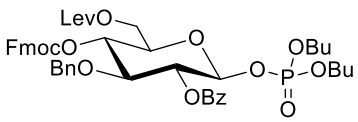
^{1}H NMR (400 MHz, $CDCl_3$) of **S4**:



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



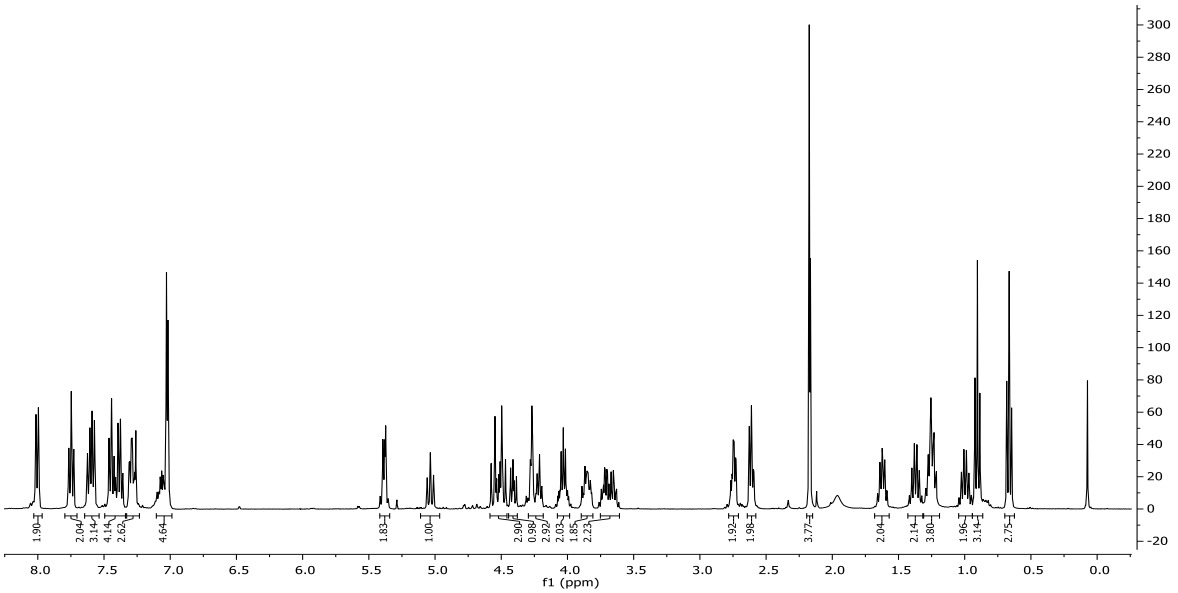
Dibutoxyphosphoryloxy-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylcarboxymethyl-6-*O*-levulinoyl-thio-β-D-glucopyranoside (2)



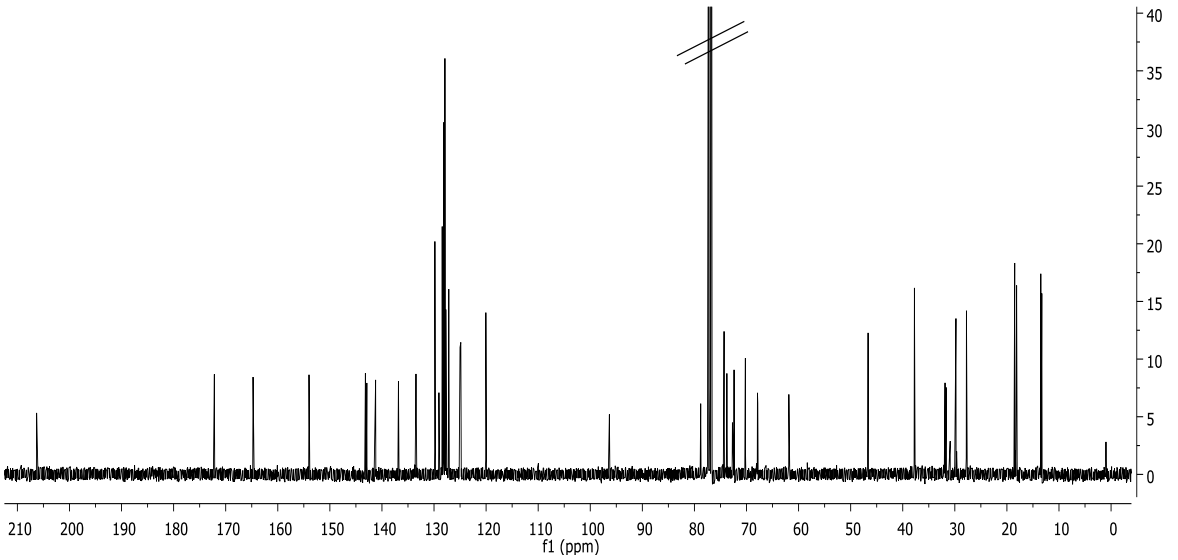
A solution of dibutyl phosphate (850 µL, 4.29 mmol) in DCM (12 mL) was dried over molecular sieves. After 1 h the supernatant of this mixture (1.40 mL) was added to **S4** (126 mg, 157 µmol) and cooled to -15 °C. Then a solution of NIS in DCM (1 mL, 178 µmol) and TfOH (10.0 µL, 114 µmol) were added. The reaction was stirred for 2.5 h, quenched with an aqueous solution of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 30 mL) and extracted with DCM (30 mL). The organic layer was dried over Na_2SO_4 and purified by silica gel chromatography (EtOAc/Hex/DCM 1:3:2 → 1:2:2) to yield compound **2** (105 mg, 118 µmol, 75%) as a yellow oil.

$[\alpha]_D^{25}$ = +26.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, *J* = 7.3 Hz, 2H, Ar), 7.75 (t, *J* = 7.4 Hz, 2H, Ar), 7.64-7.55 (m, 3H, Ar), 7.48-7.34 (m, 4H, Ar), 7.32-7.24 (m, 3H, Ar), 7.12-6.98 (m, 4H, Ar), 5.42-5.35 (m, 2H, H-1, H-2), 5.04 (t, *J* = 9.6 Hz, 1H, H-4), 4.59-4.46 (m, 3H, H-6a, C*H*$_2$Ph), 4.44-4.37 (m, 1H, H-6b), 4.30-4.18 (m, 3H, Fmoc), 4.07-3.98 (m, 2H, OBu), 3.90-3.81 (m, 2H, H-5, H-3), 3.75-3.60 (m, 2H, OBu), 2.78-2.70 (m, 2H, Lev), 2.64-2.58 (m, 2H, Lev), 2.17 (s, 3H, Lev), 1.66-1.57 (m, 2H, Bu), 1.43-1.32 (m, 2H, Bu), 1.30-1.20 (m, 2H, Bu), 1.05-0.94 (m, 2H, Bu), 0.90 (t, *J* = 7.4 Hz, 3H, Bu), 0.66 (t, *J* = 7.4 Hz, 3H, Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.3, 172.2, 164.7, 154.0 (4C, C=O), 143.2, 142.9, 141.3, 141.2, 136.8, 133.5, 129.8, 129.0, 128.4, 128.1, 127.9, 127.9, 127.7, 127.2, 125.0, 124.8, 120.0 (24C, Ar), 96.4 (C-1), 78.8 (C-3), 74.31 (*C*H_2Ph), 73.77 (C-4), 72.7 (C-2), 72.4 (C-5), 70.2 (C-6), 68.0, 67.9 (2C, OBu), 61.86 (Fmoc), 46.6 (Fmoc), 37.8 (Lev), 31.9 , 31.6 (2C, Bu), 29.8 (O=C*C*H_3), 27.77 (Lev), 18.5, 18.1 (2C, Bu), 13.49, 13.30 (2C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{48}H_{55}NaO_{14}P$: 909.3222; found 909.3276. IR (neat) ν_{max}: 1739, 1252, 1029 cm^{-1}.

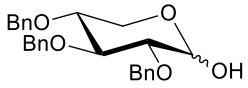
^{1}H NMR (400 MHz, $CDCl_3$) of **2**:



^{13}C NMR (100 MHz, $CDCl_3$) of **2**:



2,3,4-*O*-Tribenzyl-D-xylopyranose (S5)



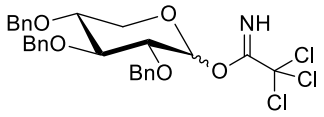
NIS (5.45 g, 24.2 mmol) was added to a stirred solution of **3**[6] (8.50 g, 16.1 mmol) in acetone/H_2O (9:1, 100 mL). The reaction was stirred for 5 min at rt. Then the reaction mixture was diluted with EtOAc (100 mL) and washed with an aqueous solution of $Na_2S_2O_3$ (100 mL). The crude compound was purified through a short plug of silica gel (EtOAc/Hex 1:4) to yield **S5** (5.50 g, 13.1 mmol, 81% yield) as a mixture of α/β-isomers.

α isomer:

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37-7.29 (m, 15H, Ar), 5.11 (t, d, *J* = 3.2 Hz, 1H, H-1), 4.90-4.63 (m, 6H, C*H*$_2$Ph), 3.89-3.77 (m, 2H, H-3, H-5a), 3.67 (dd, *J* = 11.2 Hz, 5.3 Hz, 1H, H-5b), 3.59-3.52 (m, 1H, H-4), 3.48 (dd, *J* = 8.8 Hz, 3.5 Hz, 1H H-2), 2.88 (d, *J* = 3.0 Hz, OH) ppm.

The analytical data is in agreement with literature data.[8]

***O*-Trichloroacetimidoyl 2,3,4-*O*-tribenzyl-D-xylopyranoside (7)**



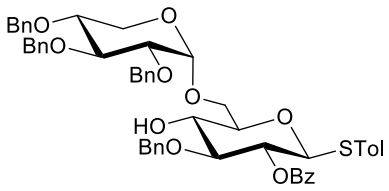
To a cooled (0 °C) solution of compound **S5** (1.80 g, 4.28 mmol) in DCM, DBU (128 µL, 856 µmol) and trichlorocetonitrile (4.30 mL, 42.9 mmol) were added. After 3 h the solvent was evaporated and the product was purified by silica gel chromatography (EtOAc/Hex 1:5 and 5% TEA) to yield **9** (2.33 g, 4.12 mmol, 96%, α/β = 2:1) as a yellow oil.

α isomer:

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.59 (s, 1H, NH), 7.41-7.24 (m, 15H, Ar), 6.37 (d, *J* = 3.4 Hz, 1H, H-1), 4.97-4.61 (m, 6H, C*H*$_2$Ph), 3.99 (t, *J* = 9.2 Hz, 1H), 3.84-3.62 (m, 4H) ppm.

The analytical data is in agreement with literature data.[9]

4-Methylphenyl 2,3,4-*O*-tribenzyl-α-D-xylopyranosyl-(1→6)-2-*O*-benzoyl-3-*O*-benzyl-thio-β-D-glucopyranoside (8).

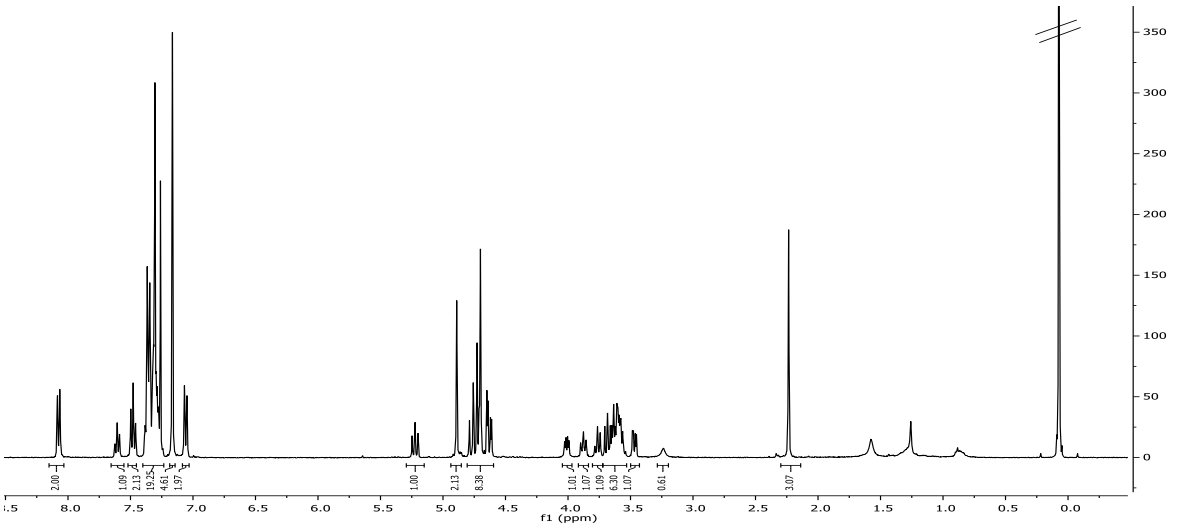


To a solution of **6** (102 mg, 181 µmol) and **7**[5] (105 mg, 218 µmol) in DCM (1 mL) at -78 °C a TMSOTf solution in DCM (30 µL, 17.0 µmol) was added. The reaction was gradually warmed to -10 °C, then quenched with sat. aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL). The

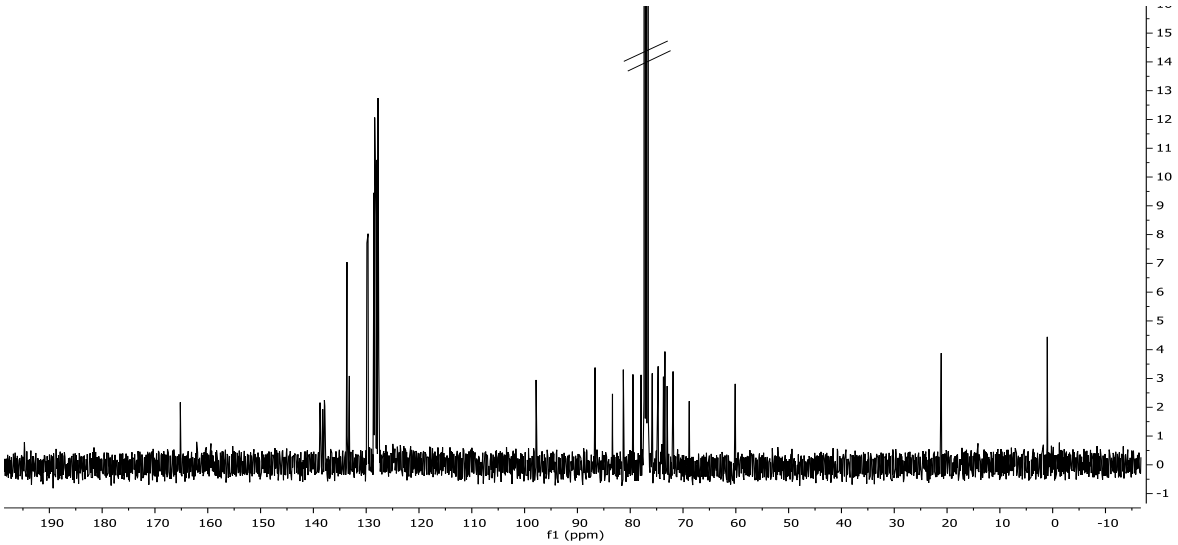
crude compound was purified by silica gel chromatography (EtOAc/Hex 1:4) to give **8** (62.3 mg, 71.0 µmol, 39%) as a pale-yellow oil and the respective β isomer (31.4 mg, 36.0 µmol, 20%) as a white solid.

$[a]_D^{25}$ = +35.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 7.3 Hz, 2H, Ar), 7.61 (t, *J* = 7.4 Hz, 1H, Ar), 7.48 (t, *J* = 7.7 Hz, 2H, Ar), 7.40-7.20 (m, 17H, Ar), 7.16 (s, 5H, Ar), 7.06 (d, *J* = 7.9 Hz, 2H, Ar), 5.22 (t, *J* = 9.5 Hz, 1H, H-2 Glc), 4.89 (s, 2H, C*H*$_2$Ph), 4.80-4.60 (m, 8H, 3 X C*H*$_2$Ph, H-1 Xyl, H-1 Glc), 4.01 (dd, *J* = 9.4 Hz, 4.5 Hz, 1H, H-6a Glc), 3.88 (t, *J* = 8.8 Hz, 1H, H-3 Xyl), 3.76 (t, *J* = 8.7 Hz, 1H, H-4 Glc), 3.72-3.54 (m, 6H, H-3 Glc, H-4 Xyl, H-5a Xyl, H-5b Xyl, H-5 Glc, H-6b Glc), 3.47 (dd, *J* = 9.5 Hz, 3.6 Hz, 1H, H-2 Xyl), 3.24 (s, 1H, OH), 2.24 (s, 3H, CH3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 165.2 (1C, C=O), 138.7, 138.3, 138.2, 137.9, 137.8, 133.6, 133.2, 129.9, 129.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.6 (36C, Ar), 97.8 (C-1 Xyl), 86.7 (C-1 Glc), 83.4 (C-3 Glc), 81.3 (C-3 Xyl), 79.5 (C-2 Xyl), 77.9 (C-5 Glc), 75.8, 74.7, 73.7, 73.4, (4C, *C*H_2Ph), 73.0 (C-4 Glc), 71.9 (C-2 Glc), 68.8 (C-6 Glc), 60.1 (C-5 Xyl), 21.1 (CH_3) ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{53}H_{54}NaO_{10}S$: 905.3330; found 905.3503. IR (neat) ν_{max}: 3480, 1729, 1269, 1072, 1028 cm^{-1}.

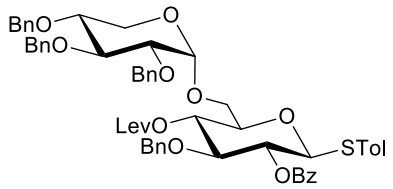
^{1}H NMR (400 MHz, $CDCl_3$) of **8**:



^{13}C NMR (100 MHz, $CDCl_3$) of **8**:



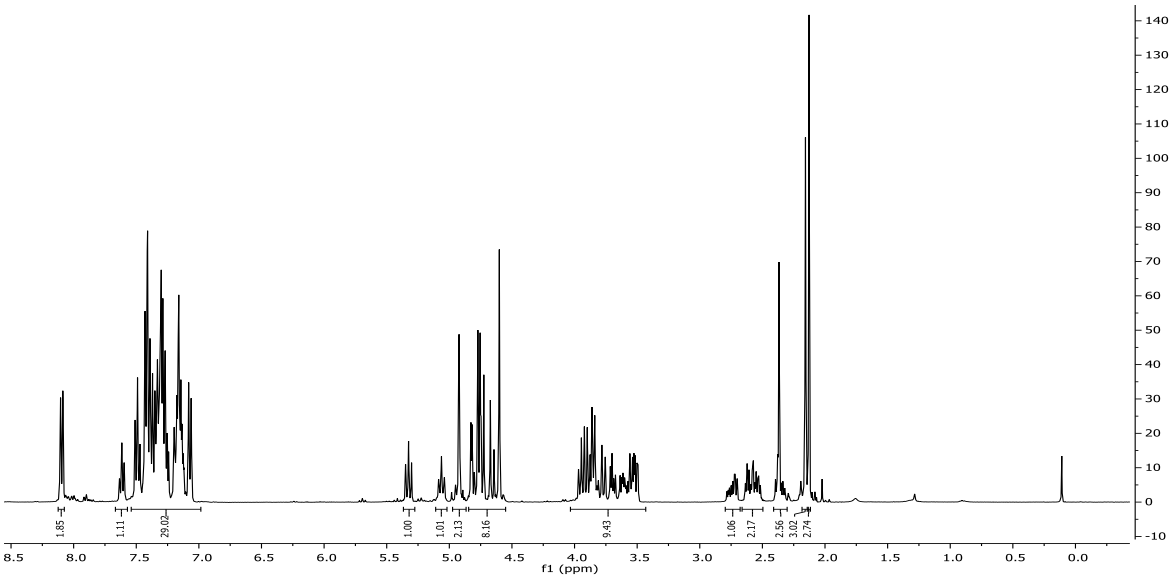
4-Methylphenyl 2,3,4-*O*-tribenzyl-α-D-xylopyranosyl-(1→6)-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-thio-β-D-glucopyranoside (9).



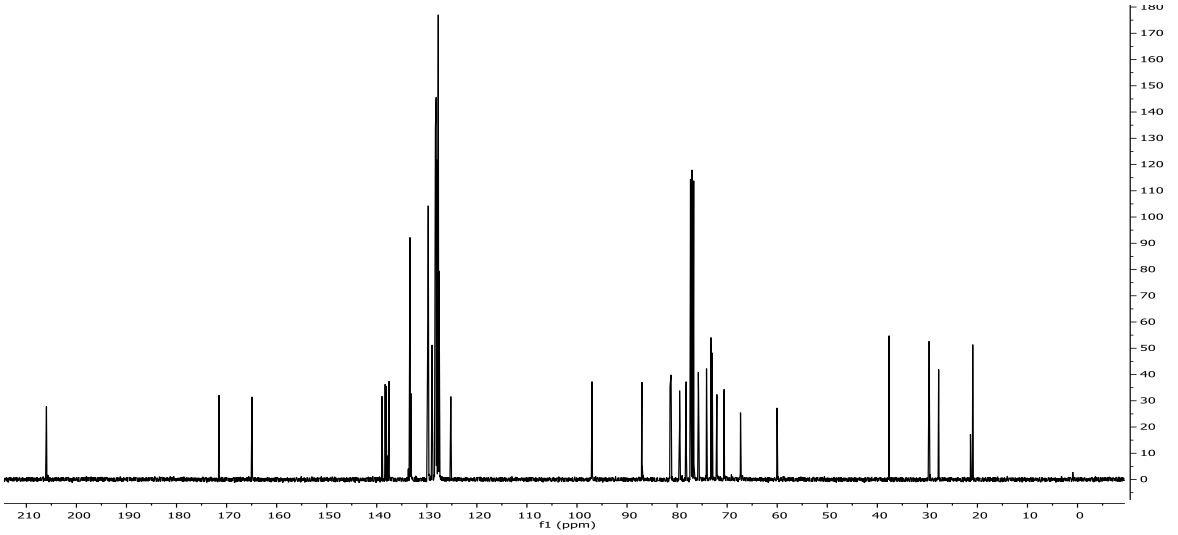
Compound **8** (2.00 g, 2.27 mmol) was dissolved in DCM (15 mL) and cooled to 0 °C. Then DMAP (139 μg, 1.13 mmol), DIC (420 μL, 2.72 mmol) and LevOH (527 mg, 4.54 mmol) were added. After 5 min the ice bath was removed. After the reaction was stirred for 6 h at rt, another portion of DMAP (139 μg, 1.13 mmol) was added and the reaction was left stirred overnight. The day after the reaction mixture was filtered through a plug of celite© and concentrated. The compound was purified by silica gel chromatography (Hex/EtOAc 4:1) to yield the intermediate **9** (1.87 g, 1.91 mmol, 84%) as a white solid.

$[\alpha]_D^{25}$ = +26.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (d, *J* = 7.2 Hz, 2H, Ar), 7.62 (t, *J* = 7.4 Hz, 1H, Ar), 7.53-7.00 (m, 26H, Ar), 5.33 (t, *J* = 9.6 Hz, 1H, H-2 Glc), 5.06 (t, *J* = 9.3 Hz, 1H, H-4 Glc), 4.92 (s, 2H, C*H*$_2$Ph), 4.87-4.56 (m, 8H, 3 x C*H*$_2$Ph, H-1 Glc, H-1 Xyl), 4.03-3.44 (m, 9H, H-3 Glc, H-5 Glc, H-6a Glc, H-6b Glc, H-2 Xyl, H-3 Xyl, H-4 Xyl, H-5a Xyl, H-5b Xyl), 2.74-2.69 (m, 1H, Lev), 2.66-2.49 (m, 2H, Lev), 2.41-2.31 (m, 1H, Lev), 2.16 (s, 3H, CH_3), 2.13 (s, 3H, O=CCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 206.0, 171.5, 164.9 (3C, C=O), 139.0, 138.3, 138.1, 137.7, 137.5, 133.7, 133.4, 133.2, 129.8, 129.7, 128.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.5, 125.1 (36C, Ar), 97.0, (C-1 Xyl), 87.0 (C-1 Glc), 81.4 (C-3 Glc), 81.2 (C-3 Xyl), 79.5 (C-2 Xyl), 78.2 (C-5 Glc), 77.3 (C-4 Xyl), 75.7, 74.1, 73.2, 73.0 (4C, *C*H$_2$Ph), 72.0 (C-4 Glc), 70.6 (C-2 Glc), 67.3 (C-6 Glc), 60.0 (C-5 Xyl), 37.6 (Lev), 29.6 (O=C*C*H$_3$), 27.77 (Lev), 20.9 (CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{58}H_{60}NaO_{12}S$: 1003.3698; found 1003.3839. IR (neat) ν_{max}: 1722, 1089, 1072, 1042, 1029 cm^{-1}.

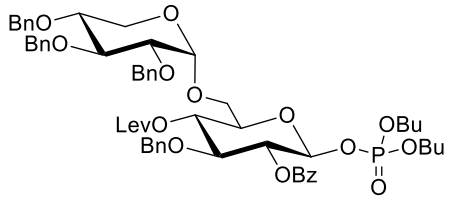
^{1}H NMR (400 MHz, $CDCl_3$) of **9**:



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



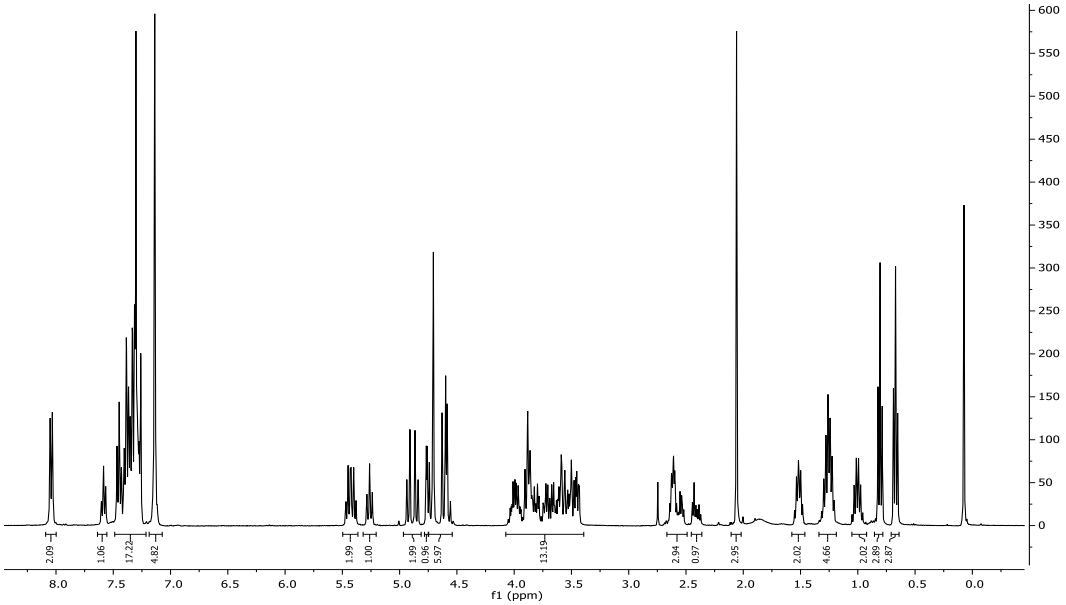
Dibutoxyphosphoryloxy 2,3,4-*O*-tri-benzyl-α-D-xylopyranosyl-(1→6)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-β-D-glucopyranoside (10).



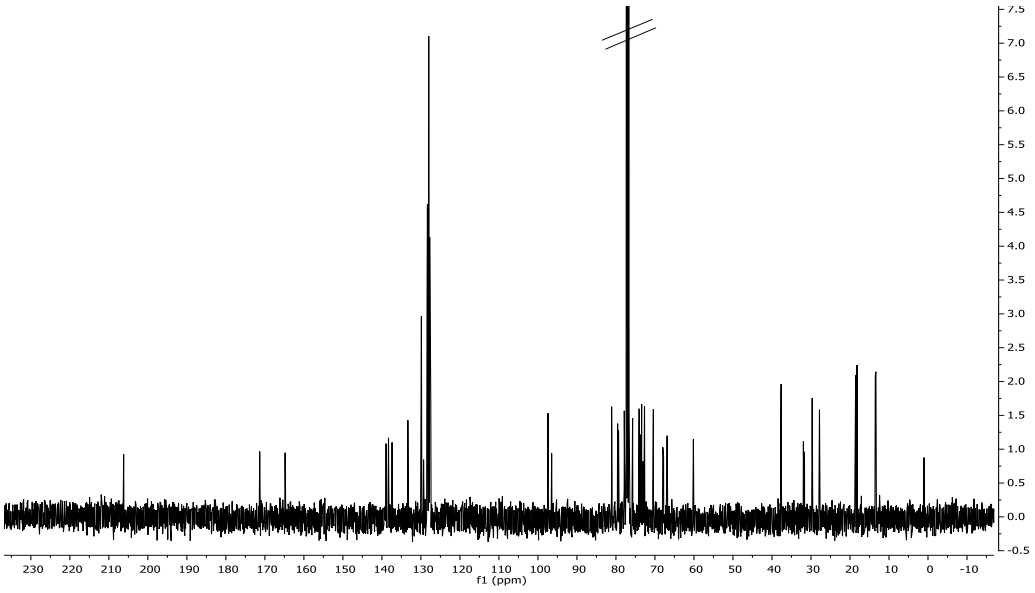
A solution of dibutyl phosphate (2.00 mL, 10.1 mmol) in DCM (10 mL) was dried over molecular sieves. After 1 h the supernatant of this mixture (5.40 mL) was added to **9** (1.78 g, 1.81 mmol) and cooled to 0 °C. Then NIS (490 mg, 2.18 mmol) and TfOH (50.0 µL, 563 µmol) were added. The reaction was stirred 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 4:1) to give compound **10** (1.10 g, 1.03 mmol, 57%) as a yellow oil.

$[\alpha]_D^{25}$ = +41.2 (*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), 7.48-7.25 (m, 17H, Ar), 7.14 (s, 5H, Ar), 5.48-5.37 (m, 2H, H-2 Glc, H-1 Glc), 5.26 (t, *J* = 9.5 Hz, 1H, H-4 Glc), 4.96-4.83 (m, 2H, C*H*$_2$Ph), 4.76 (d, *J* = 3.4 Hz, 1H, H-1 Xyl), 4.75-4.55 (m, 6H, 3 x C*H*$_2$Ph), 4.06-3.41 (m, 13H, 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), 2.65-2.51 (m, 3H, Lev), 2.41 (m, 1H, Lev), 2.06 (s, 3H, Lev), 1.56-1.47 (m, 2H, Bu), 1.32-1.20 (m, 4H, Bu), 1.06-0.95 (m, 2H, Bu), 0.81 (t, 3H, *J* = 7.4 Hz, CH_3), 0.67 (t, 3H, *J* = 7.4 Hz, CH_3) ppm. ^{13}C NMR (100 MHz, CDCl3): 206.2, 171.3, 164.8 (3C, C=O), 138.9, 138.4, 138.3, 137.4, 133.3, 129.9, 129.3, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.5 (30C, Ar), 97.4, (C-1 Xyl), 96.3 (C-1 Glc), 81.1 (C-3), 79.6 (C-2 Xyl), 79.3 (C-3), 77.9 (C-5 Glc), 75.7 (*C*H$_2$Ph), 73.8 (C-4 Xyl), 73.4 (2C, *C*H$_2$Ph), 73 (C-2 Glc), 72.7 (*C*H$_2$Ph), 70.4 (C-4 Glc), 67.9, 67.7 (2C, OBu), 66.9 (C-6 Glc), 60.1 (C-5 Xyl), 37.7 (Lev), 31.9, 31.7 (2C, Bu), 29.7, 27.8 (2C, Lev), 18.5, 18.2, 13.5, 13.3 (4C, Bu) ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C_{59}H_{71}NaO_{16}P$: 1089.4377; found 1089.4384. IR (neat) ν_{max}: 1734, 1267, 1095, 1072, 1029 cm^{-1}.

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

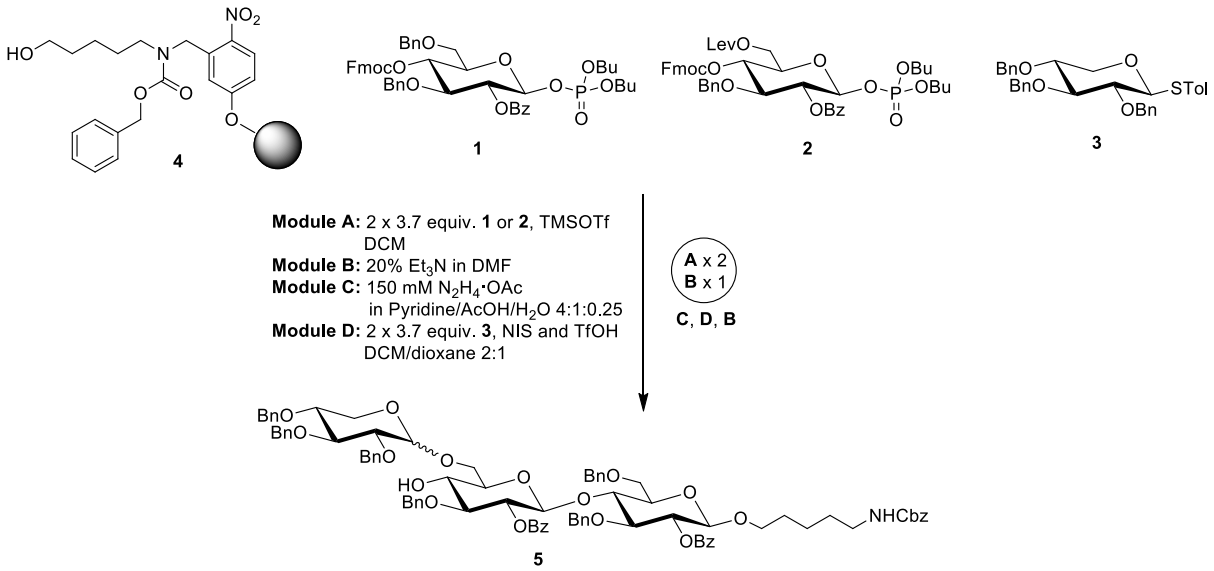


^{13}C NMR (100 MHz, $CDCl_3$) of **10**:



Automated synthesis of xyloglucan and cellulose fragmets

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-glucopyranoside (5)

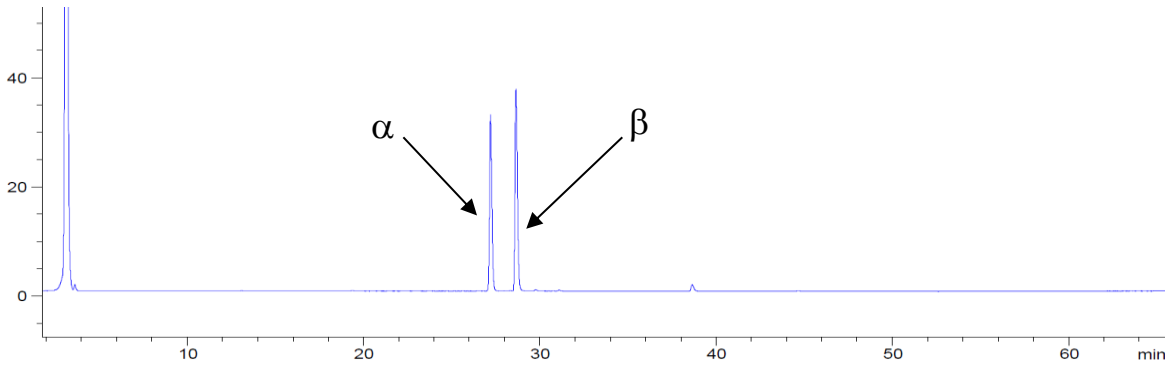


Linker functionalized resin **4** (40 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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** (2x 3.7 equiv. **2**, TMSOTf, DCM, 2 x 35 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 **D** (2 x 3.7 equiv. **3**, NIS and TfOH, DCM/dioxane 2:1, 2 x 35 min, -55 °C to -35 °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 trisaccharide **5** as a mixture of α/β-isomers. The crude product was purified by normal phase HPLC using a semi-preparative Luna 5u Silica 100A column.

NP-HPLC of the crude fully protected α/β-mixture of trisaccharides **5**:



HPLC was performed using a Luna 5u Silica 100A column and linear gradients from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

The two isomers were separated by normal phase HPLC using a semi-preparative Luna 5u Silica 100A column affording the isomer **5α** (4.3 mg, 2.98 µmol, 18% over 7 steps, based on resin loading) and the isomer **5β** (2.8 mg, 1.94 µmol, 11% over 7 steps, based on resin loading).

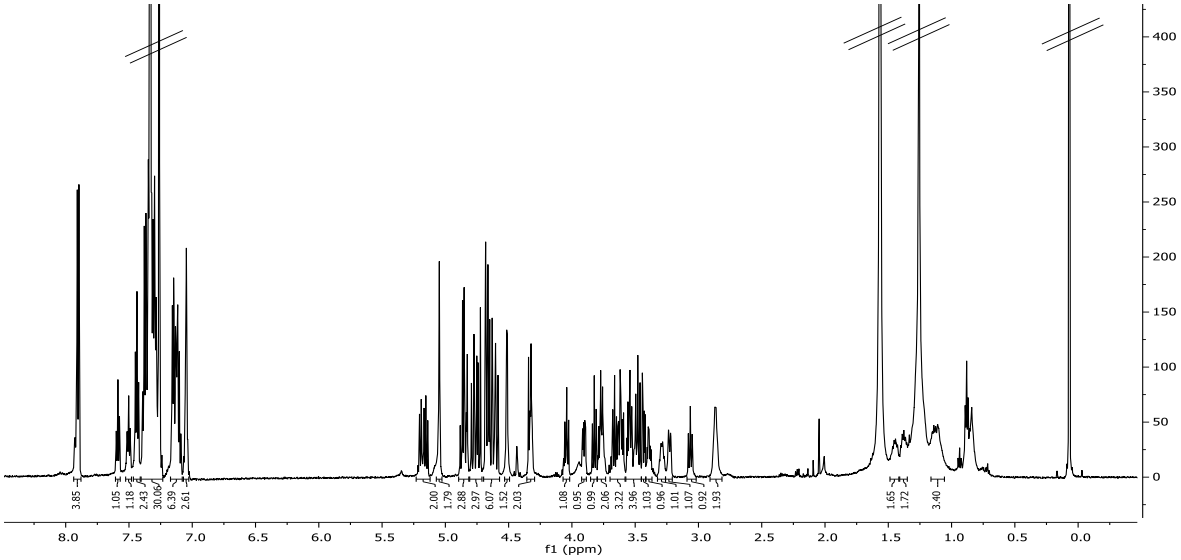
Isomer **5α**:

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.90 (d, *J* = 7.5 Hz, 4H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.39-7.23 (m, 28H), 7.18-7.08 (m, 6H), 7.07-7.03 (m, 3H), 5.22-5.13 (m, 2H), 5.05 (s, 2H), 4.89-4.81 (m, 3H), 4.79-4.71 (m, 3H), 4.70-4.57 (m, 6H), 4.51 (d, *J* = 3.6 Hz, 1H), 4.37-4.29 (m, 2H), 4.04 (t, *J* = 9.1 Hz, 1H), 3.90 (dd, *J* = 9.7, 4.2 Hz, 1H), 3.82 (t, *J* = 9.1 Hz, 1H), 3.80-3.73 (m, 2H), 3.70-3.58 (m, 3H), 3.58-3.45 (m, 4H), 3.43 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.39 (td, *J* = 8.8, 4.2 Hz, 1H), 3.32-3.26 (m, 1H), 3.26-3.21 (m, 1H), 3.06 (t, *J* = 9.1 Hz, 1H), 2.90-2.83 (m, 2H), 1.49-1.04 (m, 6H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ = 165.0, 156.2, 138.7, 138.5, 138.2, 138.0, 137.8, 136.7, 133.1, 132.9, 130.0, 129.8, 129.7, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.1, 101.1, 100.2, 98.4, 81.4, 80.1, 79.4, 75.7, 74.7, 74.4, 74.0, 73.6, 73.4, 73.2, 72.1, 71.0, 69.4, 67.6, 66.5, 60.2, 40.8, 28.8, 23.0 ppm. MALDI-TOF**:** m/z $[M+Na]^+$ calcd. for $C_{86}H_{91}NaNO_{19}$: 1464.608; found 1464.739.

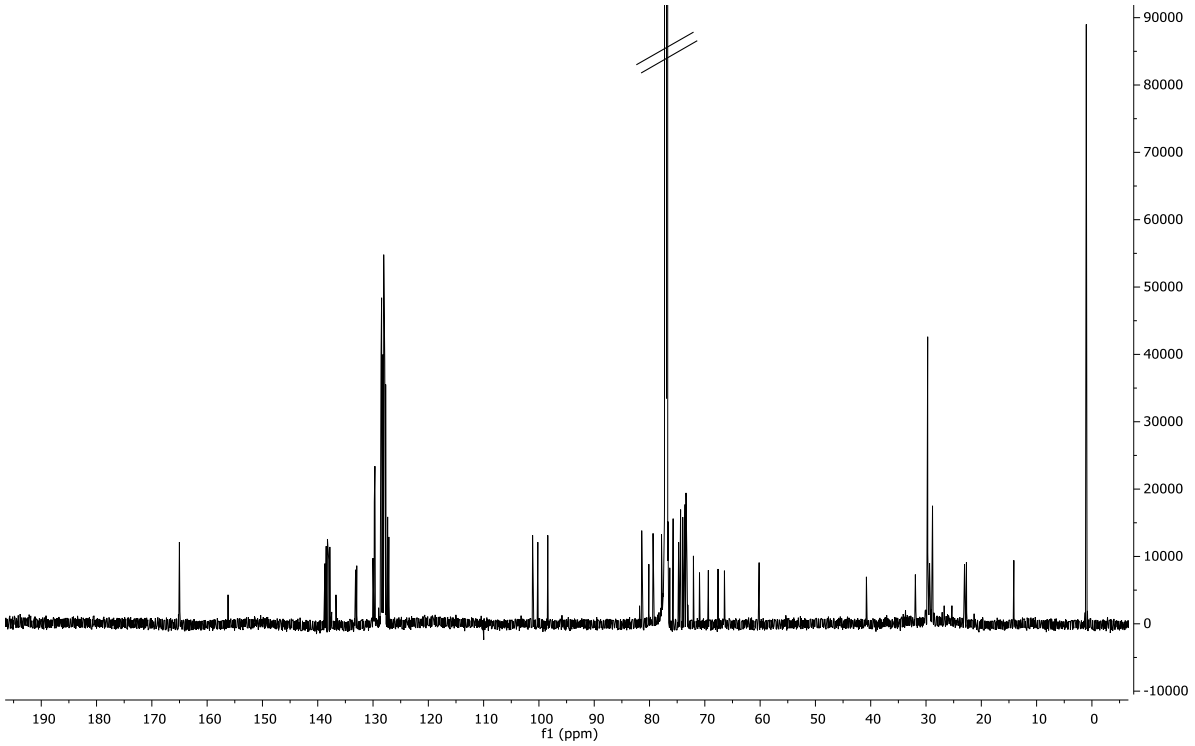
Isomer **5β**:

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.92 (d, *J* = 7.2 Hz, 2H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.38- 7.21 (m, 28H), 7.19-7.11 (m, 6H), 7.08-7.02 (m, 3H), 5.23-5.14 (m, 2H), 5.04 (s, 2H), 4.89-4.80 (m, 4H), 4.75-4.63 (m, 4H), 4.63-4.56 (m, 3H), 4.35-4.27 (m, 3H), 4.05 (t, *J* = 9.1 Hz, 1H), 3.84 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.80 (dd, *J* = 10.7, 5.5 Hz, 1H), 3.78-3.63 (m, 5H), 3.62-3.57 (m, 2H), 3.54-3.45 (m, 3H), 3.38-3.32 (m, 1H), 3.32-3.27 (m, 2H), 3.26-3.21 (m, 1H), 3.12 (dd, *J* = 11.6, 9.9 Hz, 1H), 2.89-2.81 (m, 2H), 1.49-1.04 (m, 6H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ = 165.0, 156.2, 138.7, 138.2, 138.1, 136.7, 133.3, 132.9, 130.1, 129.7, 129.7, 128.5, 128.3, 128.1, 127.9, 127.8, 127.5, 127.1, 103.6, 101.1, 100.4, 83.1, 81.7, 81.2, 80.5, 75.4, 74.8, 74.5, 74.4, 73.7, 73.5, 73.4, 73.3, 73.2, 73.1, 69.6, 69.2, 67.7, 66.4, 63.5, 40.8, 28.9, 23.0, 22.7 ppm. MALDI-TOF**:** m/z $[M+Na]^+$ calcd. for $C_{86}H_{91}NaNO_{19}$: 1464.608; found 1464.639.

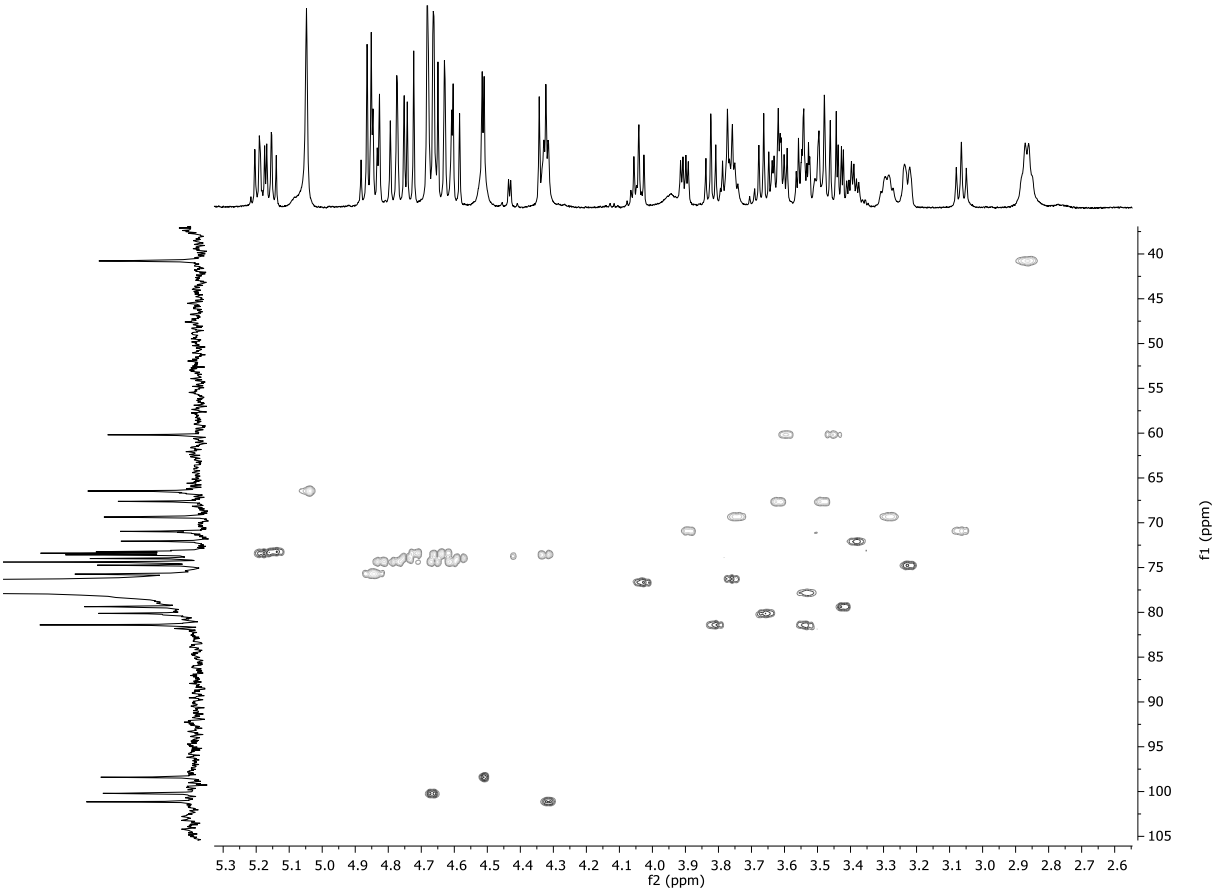
^{1}H NMR (600 MHz, $CDCl_3$) of isomer **5α**:



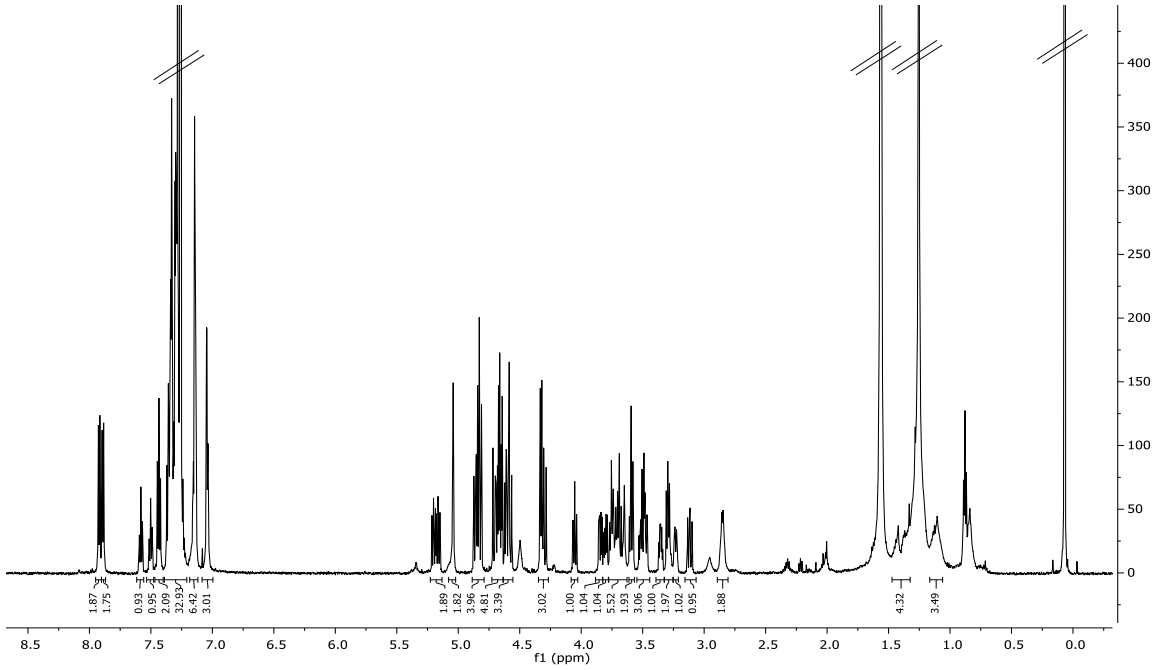
^{13}C NMR (176 MHz, $CDCl_3$) of isomer **5α**:



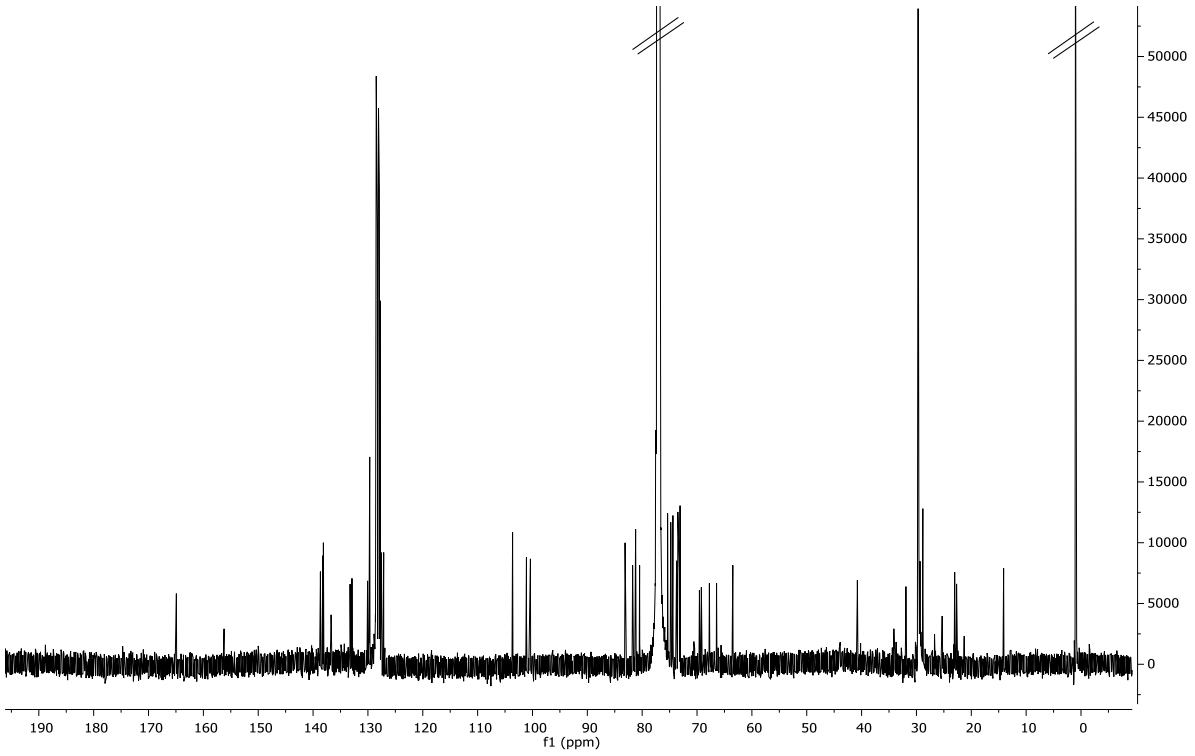
HSQC ($CDCl_3$) of isomer **5α**:



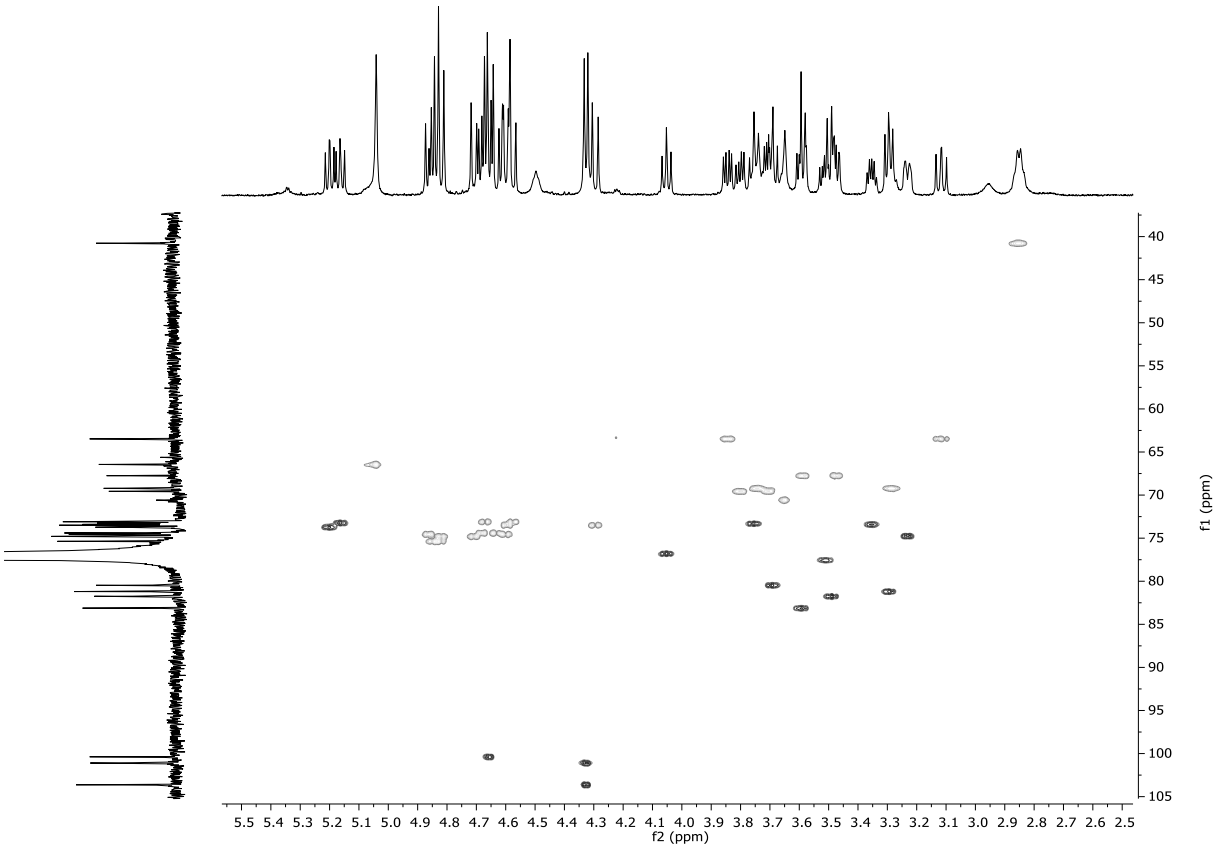
^{1}H NMR (600 MHz, $CDCl_3$) of isomer **5β**:



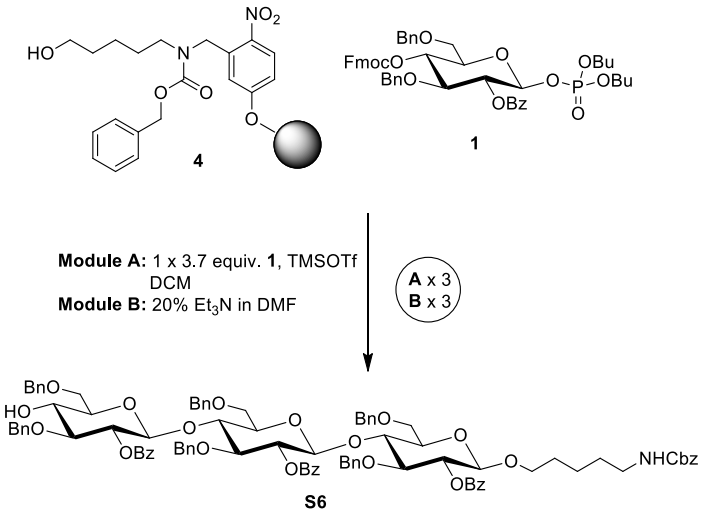
^{13}C NMR (176 MHz, $CDCl_3$) of isomer **5β**:



HSQC ($CDCl_3$) of isomer **5β**:



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-glucopyranoside (S6)

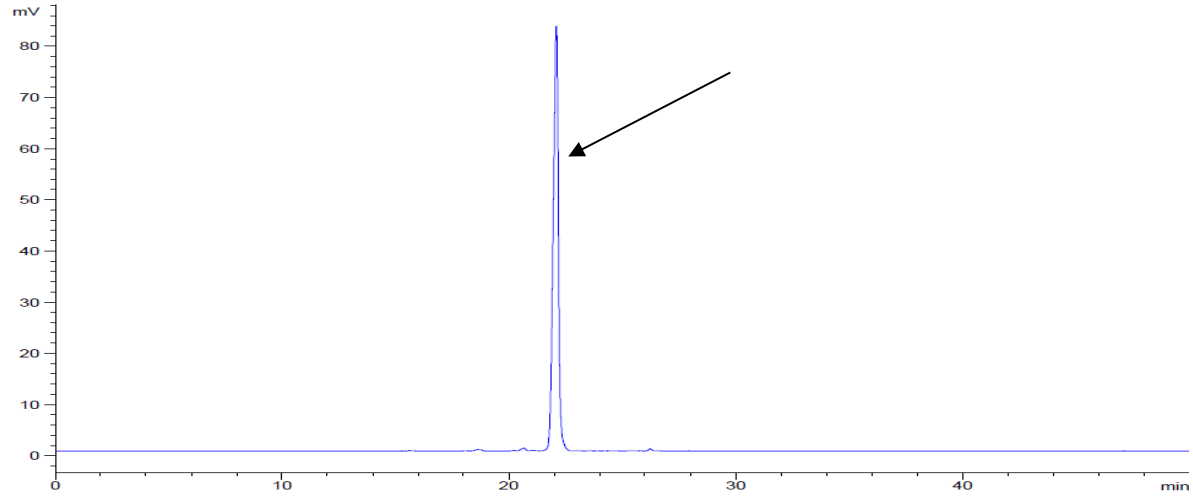


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (3.7 equiv. **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 trisaccharide **S6**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

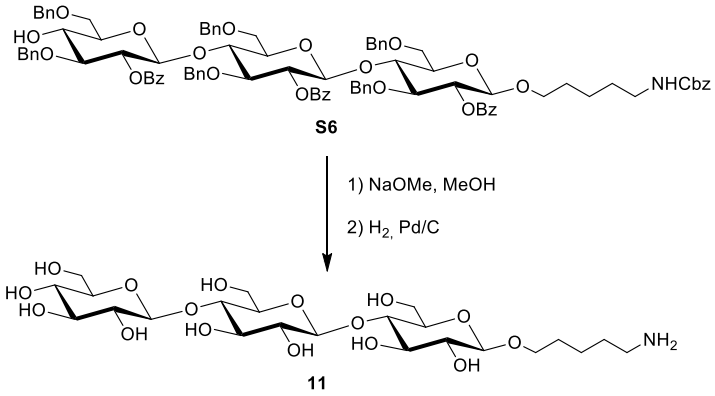
Crude NP-HPLC of trisaccharide **S6** (ELSD trace):



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 trisaccharide.

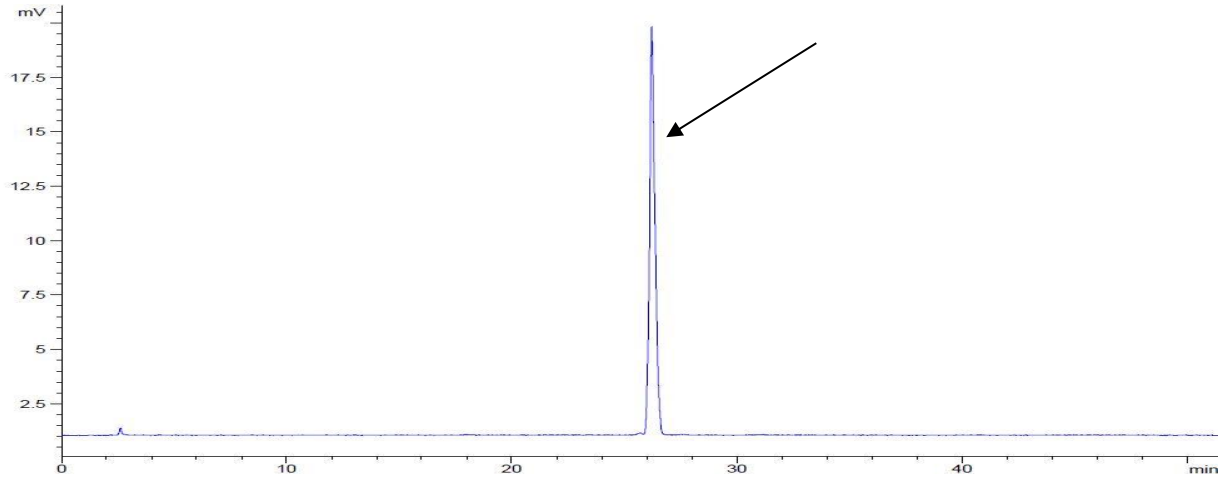
Aminopentyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (11)



Trisaccharide **S6** 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 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 Phenomenex Luna C5 column affording the semi-protected tetrasaccharide.

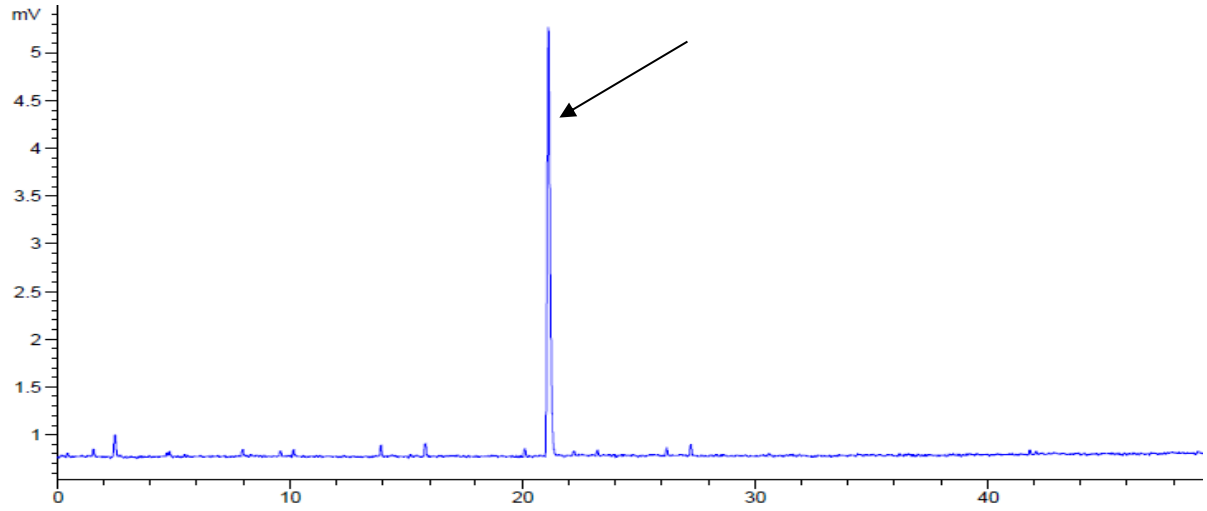
Crude RP-HPLC of the semi-protected trisaccharide (ELSD trace):



HPLC was performed using a Phenomenex Luna C5 column and linear gradients from 20% to 100% ACN in H_2O (45 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, 10 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 trisaccharide **11** (2.8 mg, 4.75 µmol, 28% over 9 steps, based on resin loading).

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

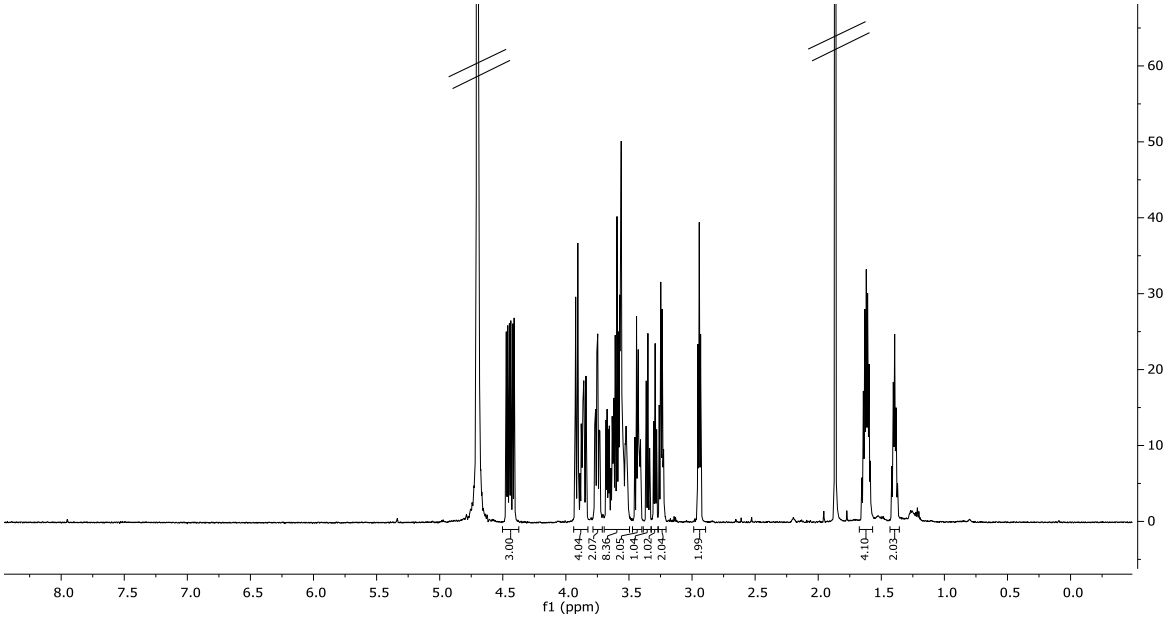


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

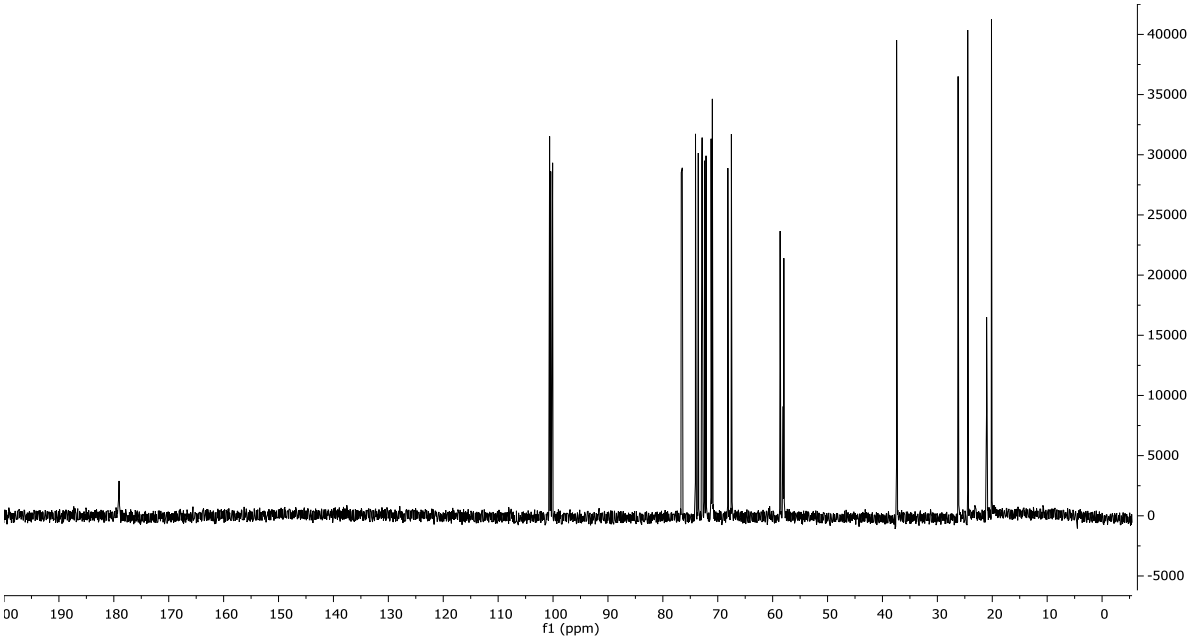
^{1}H NMR (700 MHz, D_2O): δ = 4.57-4.59 (m, 3H), 4.03-3.92 (m, 4H), 3.84 (td, *J* = 11.9, 5.0 Hz, 2H), 3.78-3.59 (m, 8H), 3.55-3.49 (m, 2H), 3.44 (t, *J* = 9.4 Hz, 1H), 3.38 (t, *J* = 8.3 Hz, 1H), 3.36-3.31 (m, 2H), 3.03 (t, *J* = 7.5 Hz, 2H), 1.75-1.67 (m, 4H), 1.51-1.45 (m, 2H) ppm. ^{13}C NMR (175 MHz, D_2O): 100.6, 100.4,

100.1, 76.6, 76.5, 74.0, 73.5, 72.9, 72.8, 72.4, 72.1, 71.2, 71.0, 71.0, 68.2, 67.5, 58.6, 58.1, 58.0, 37.4, 26.2, 24.5, 20.1 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{23}H_{44}NO_{16}$: 590.2655; found 590.2723.

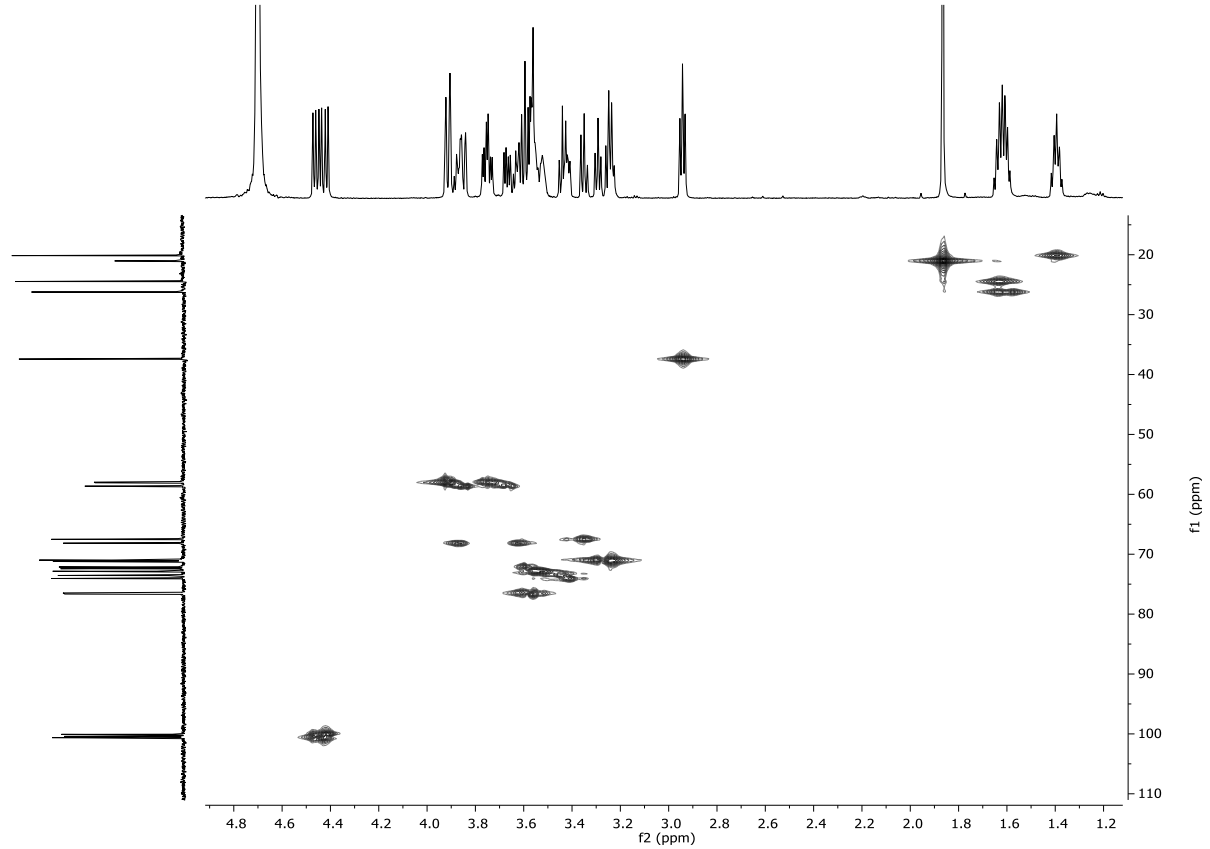
^{1}H NMR (700 MHz, D_2O) of **11**:



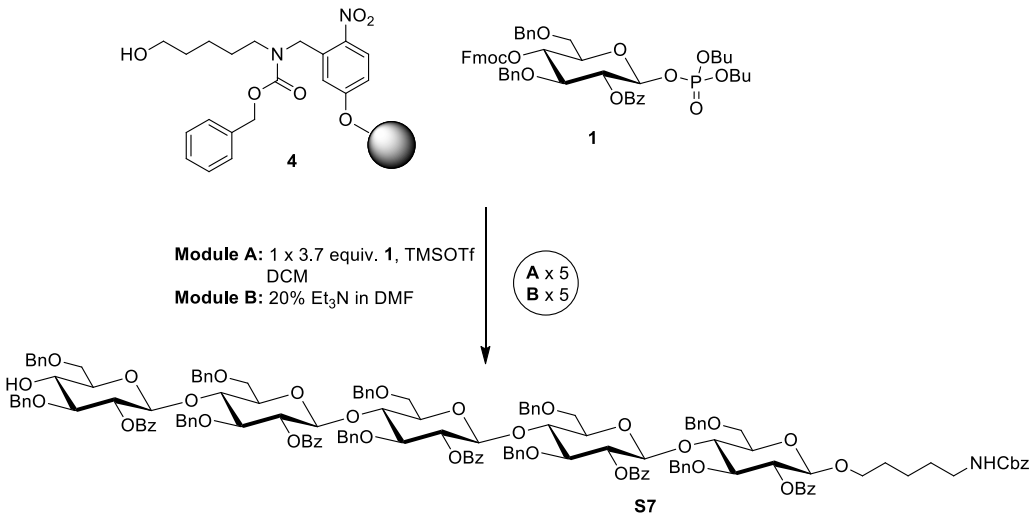
^{13}C NMR (176 MHz, D_2O) of **11**:



HMQC (D_2O) of **11**:



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-glucopyranosyl-(1→4)-2-*O*-benzoyl-3,6-*O*-dibenzyl-β-D-glucopyranoside (S7)



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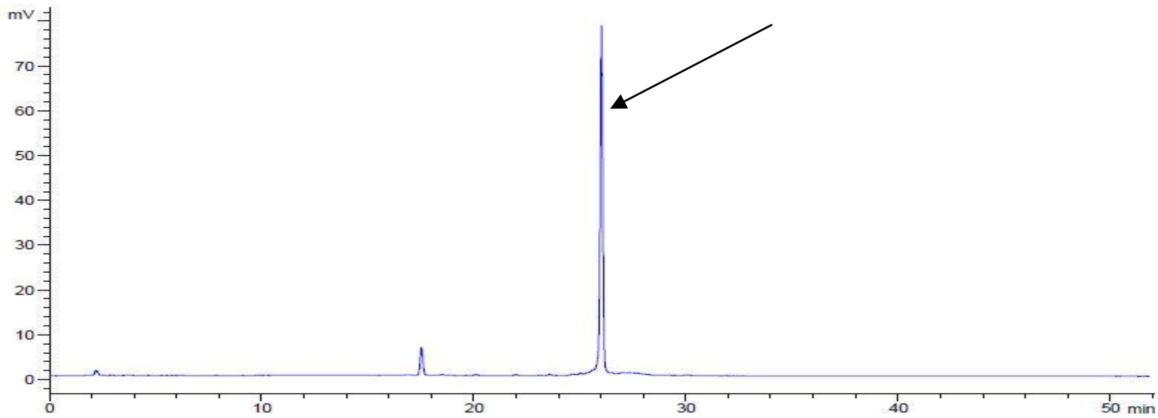
Module **A** (3.7 equiv. **1**, TMSOTf, DCM, 35 min, -30 °C to -15 °C)

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)
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 pentasaccharide **S7**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

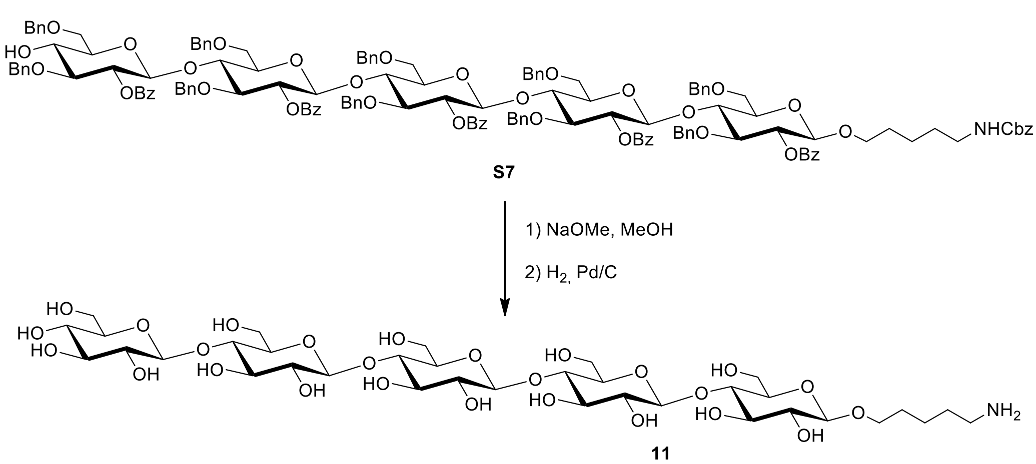
Crude NP-HPLC of pentasaccharide **S7** (ELSD trace):



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).

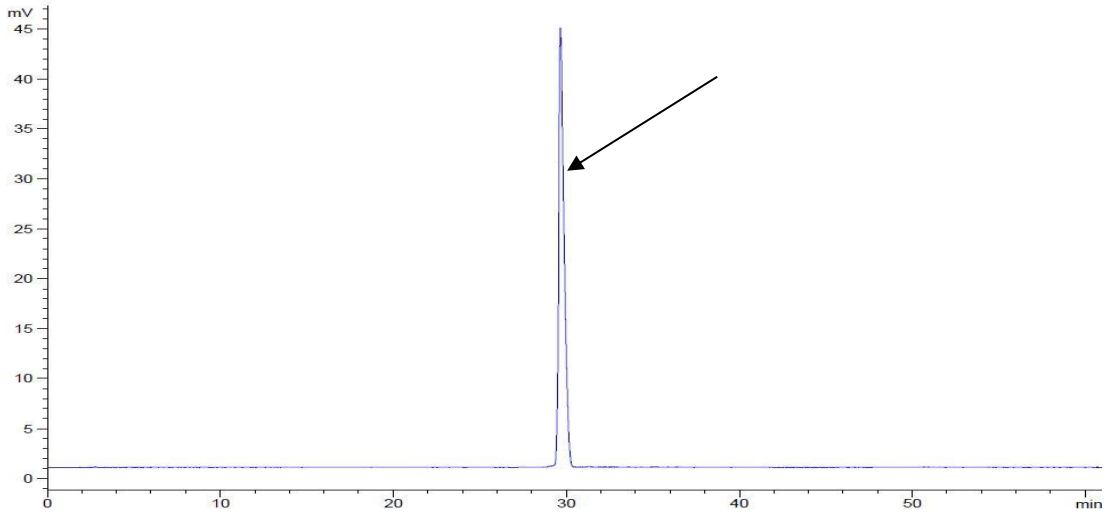
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected pentasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (12)



Pentasaccharide **S7** 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 prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected pentasaccharide.

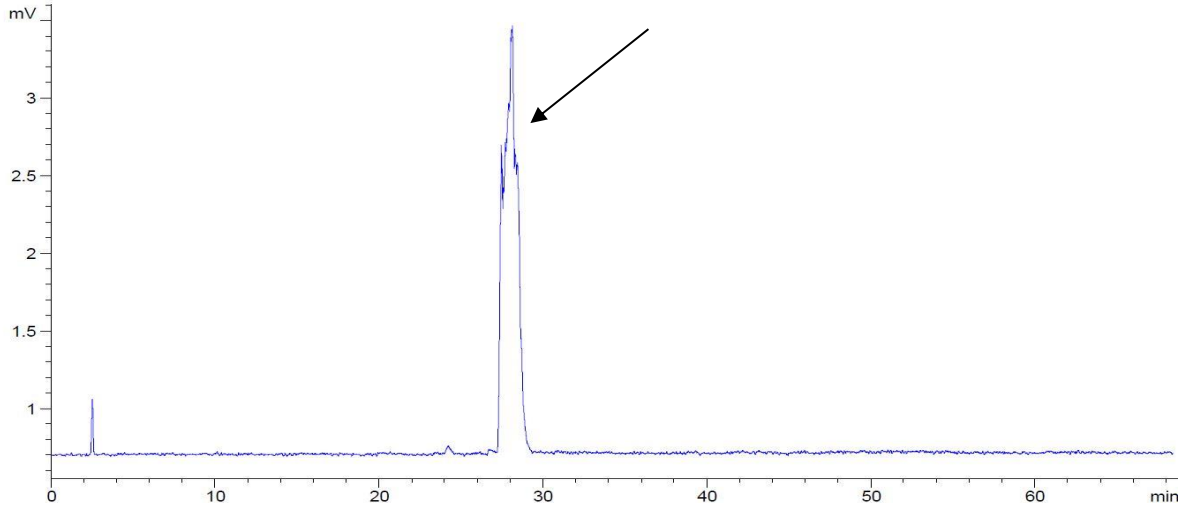
Crude NP-HPLC of the semi-protected pentaccharide (ELSD trace):



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, 10 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 **12** (3.5 mg, 3.83 µmol, 23% over 13 steps, based on resin loading).

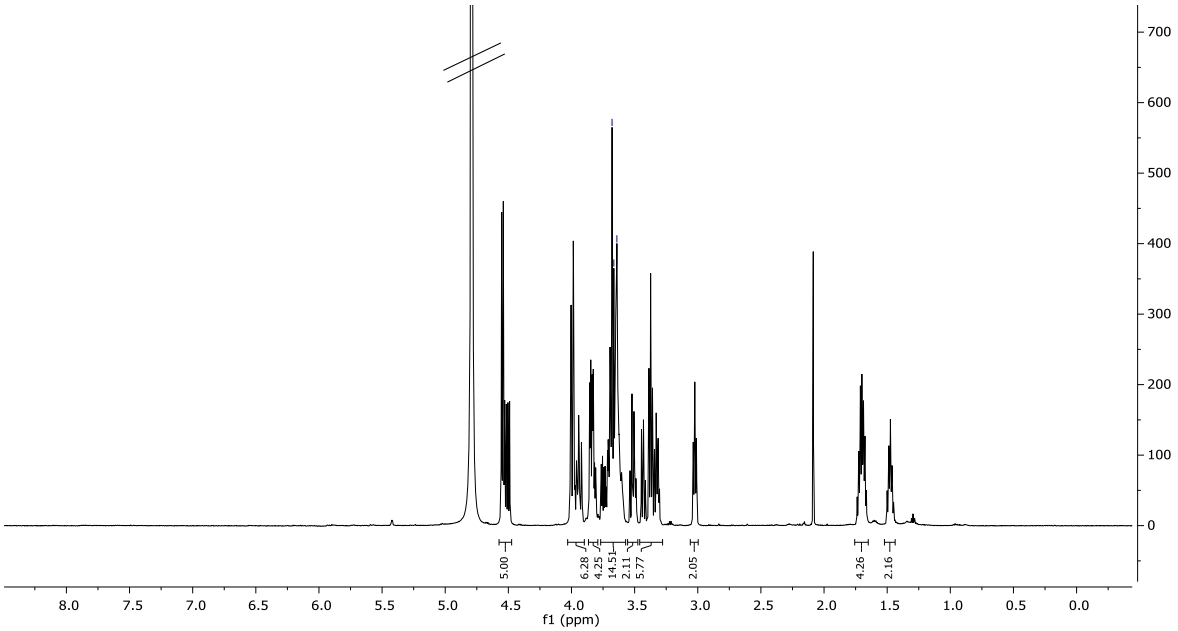
RP-HPLC of the deprotected pentasaccharide **12** (ELSD trace):



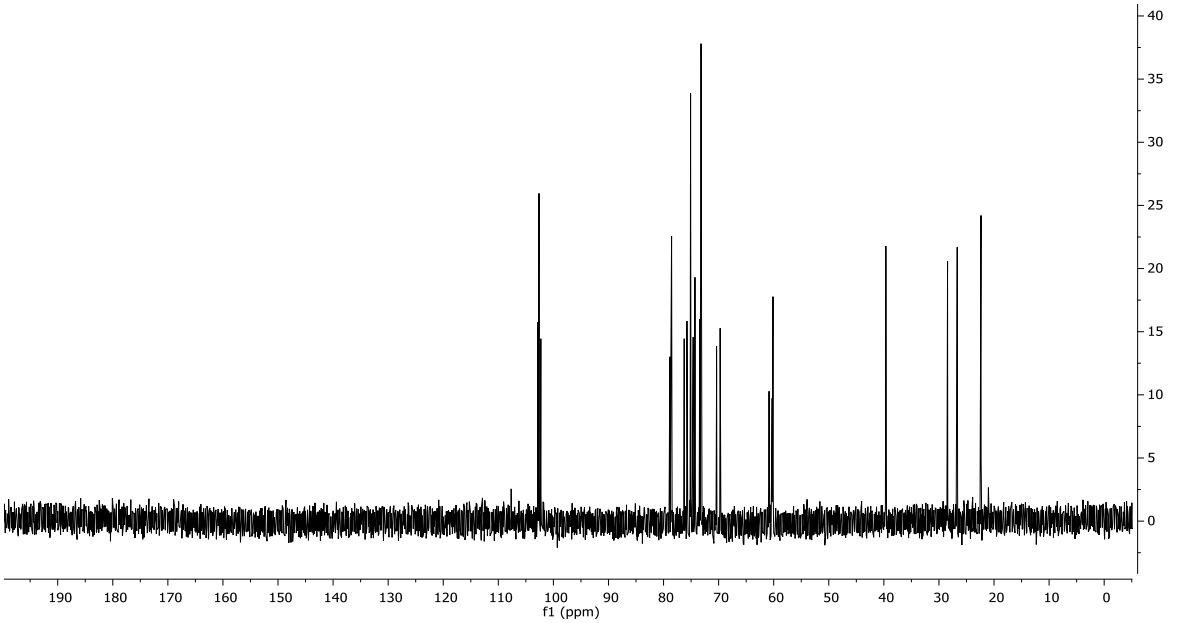
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.61-4.45 (m, 5H), 4.05-3.90 (m, 6H), 3.88-3.79 (m, 4H), 3.78-3.57 (m, 14H), 3.55-3.47 (m, 2H), 3.45-3.28 (m, 6H), 3.02 (t, *J* = 7.5 Hz, 2H), 1.75-1.65 (m, 4H), 1.53-1.44 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): 102.8, 102.6, 102.3, 78.9, 78.7, 78.5, 76.2, 75.7, 75.1, 75.0, 74.6, 74.3, 74.3, 73.4, 73.2, 70.4, 69.7, 60.8, 60.3, 60.1, 39.6, 28.4, 26.7, 22.4 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{35}H_{64}NO_{26}$: 914.3711; found 914.3747.

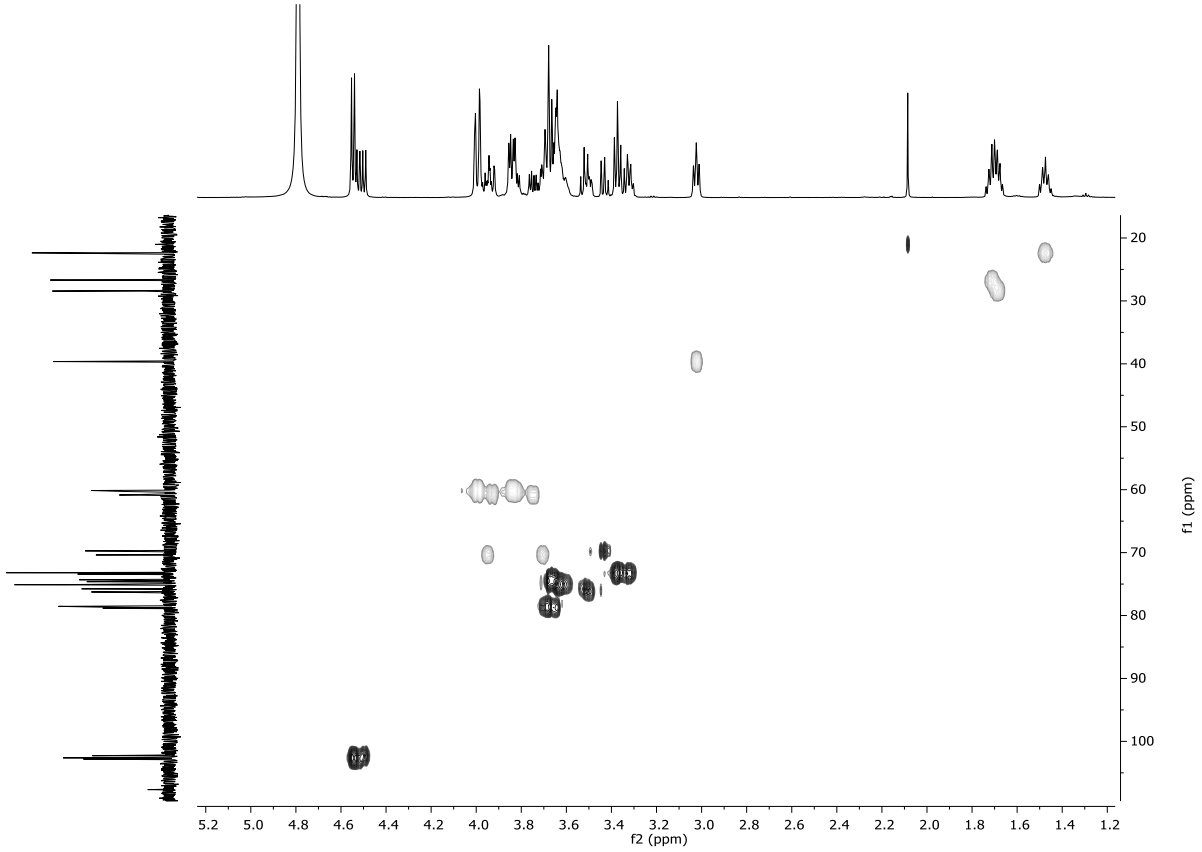
^{1}H NMR (600 MHz, D_2O) of **12**:



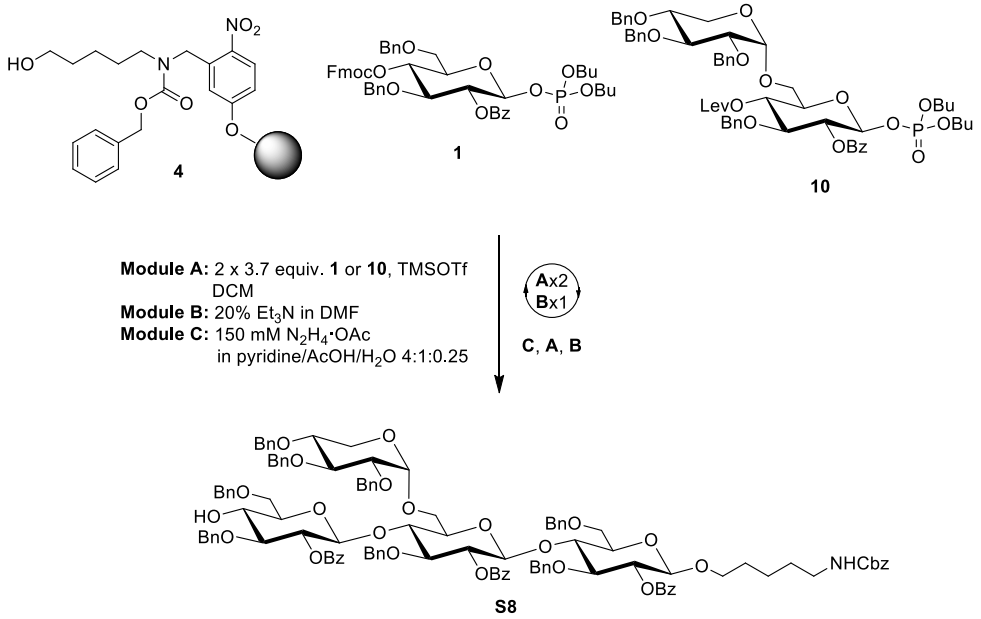
^{13}C NMR (151 MHz, D_2O) of **12**:



HSQC (D_2O) of **12**:



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-glucopyranoside (S8)

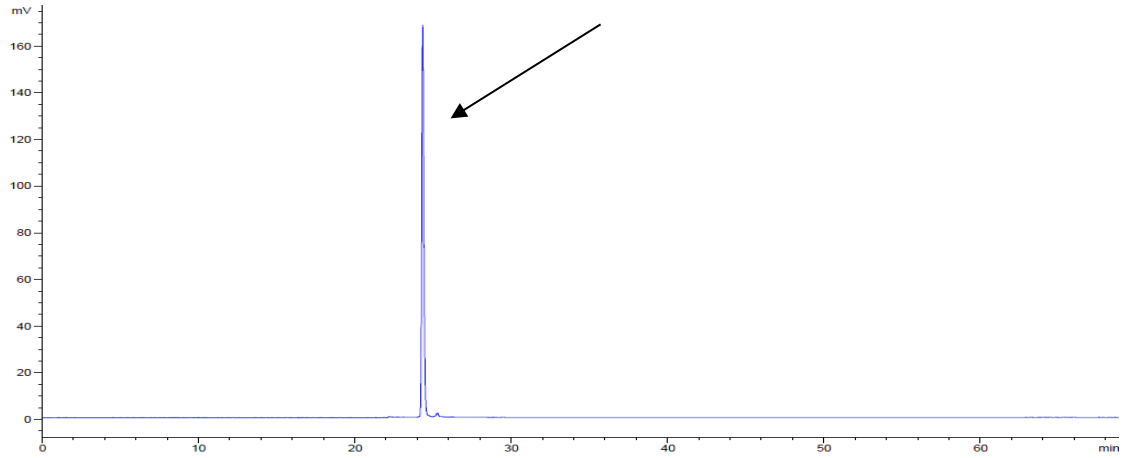


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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. **10**, 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 tetrasaccharide **S8**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

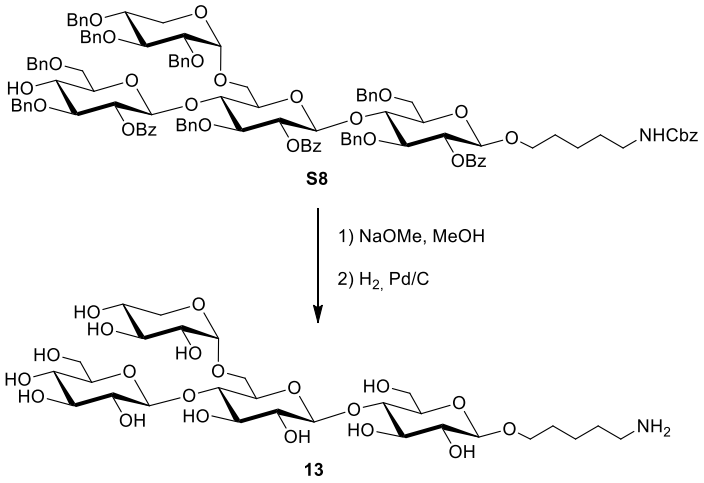
Crude NP-HPLC of tetrasaccharide **S8** (ELSD trace):



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).

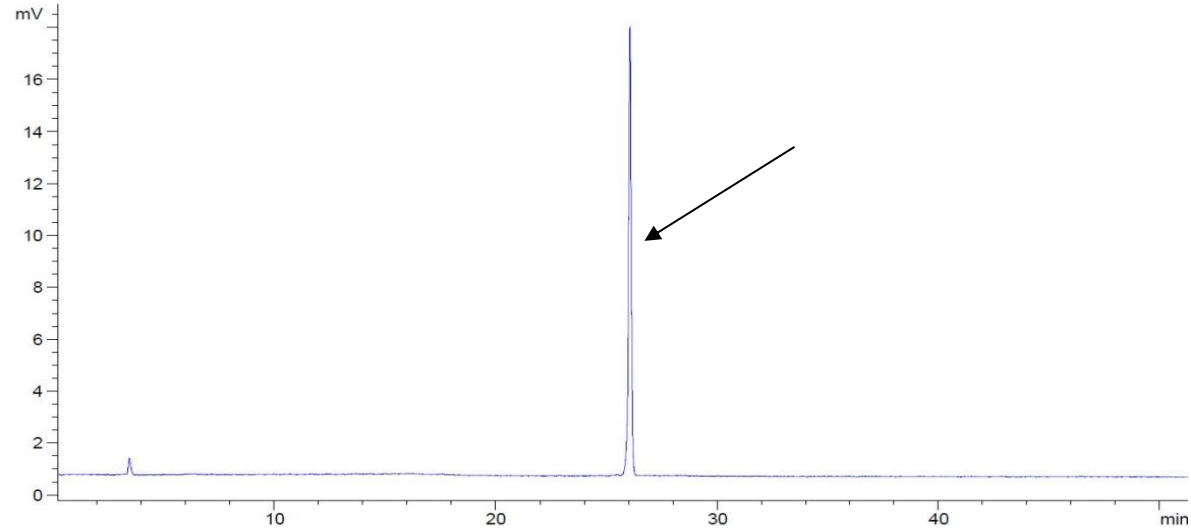
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected tetrasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (13)



Tetrasaccharide **S8** 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 solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected tetrasaccharide.

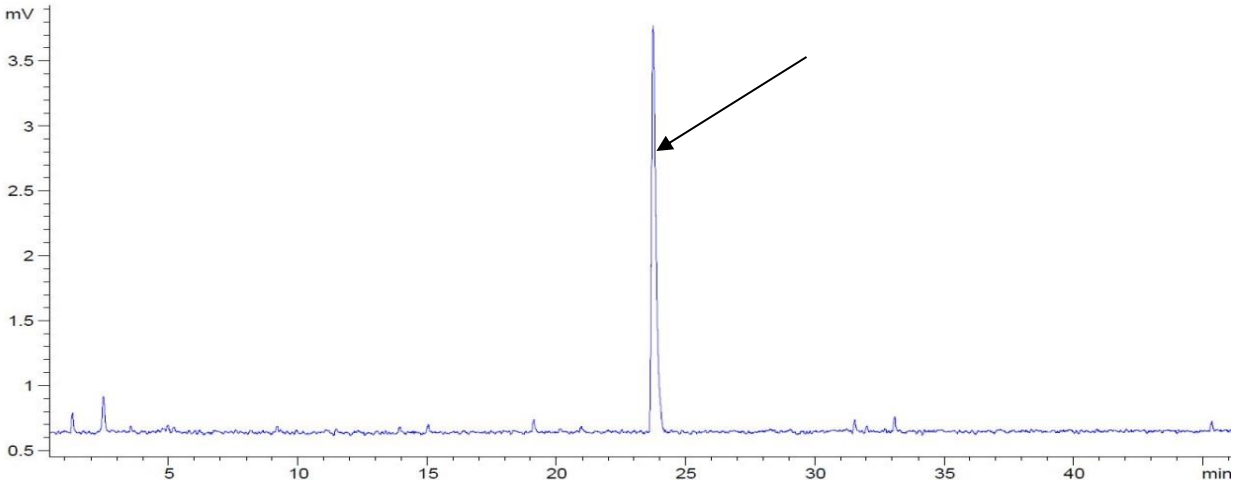
Crude NP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



HPLC was performed using 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, 13 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 **13** (1.8 mg, 2.49 µmol, 15% over 9 steps, based on resin loading).

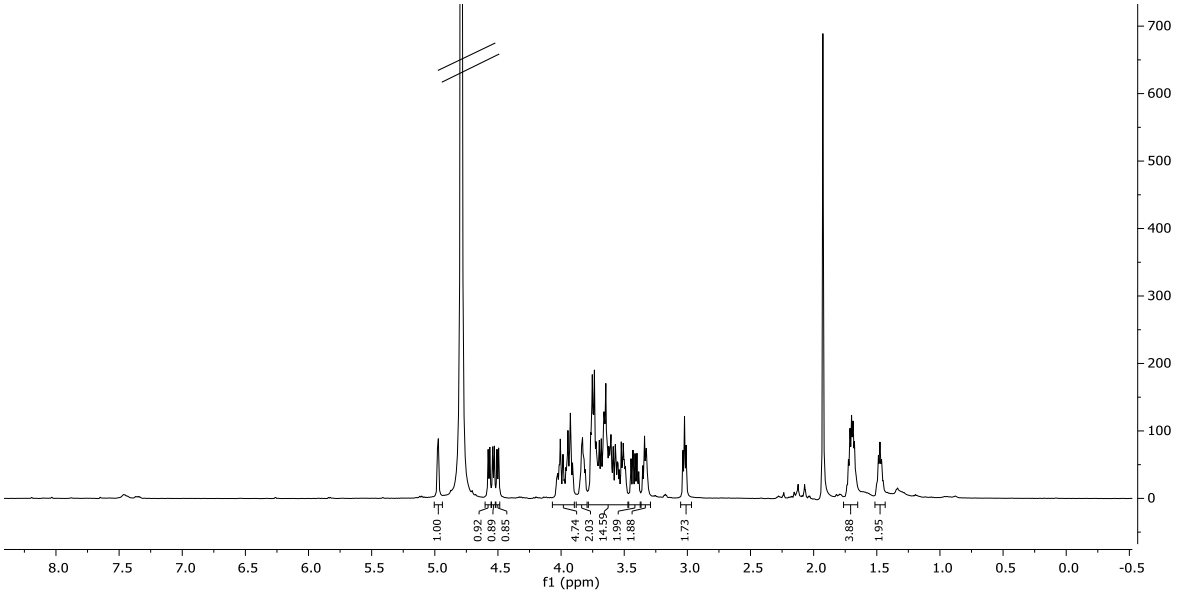
RP-HPLC of the deprotected tetrasaccharide **13** (ELSD trace):



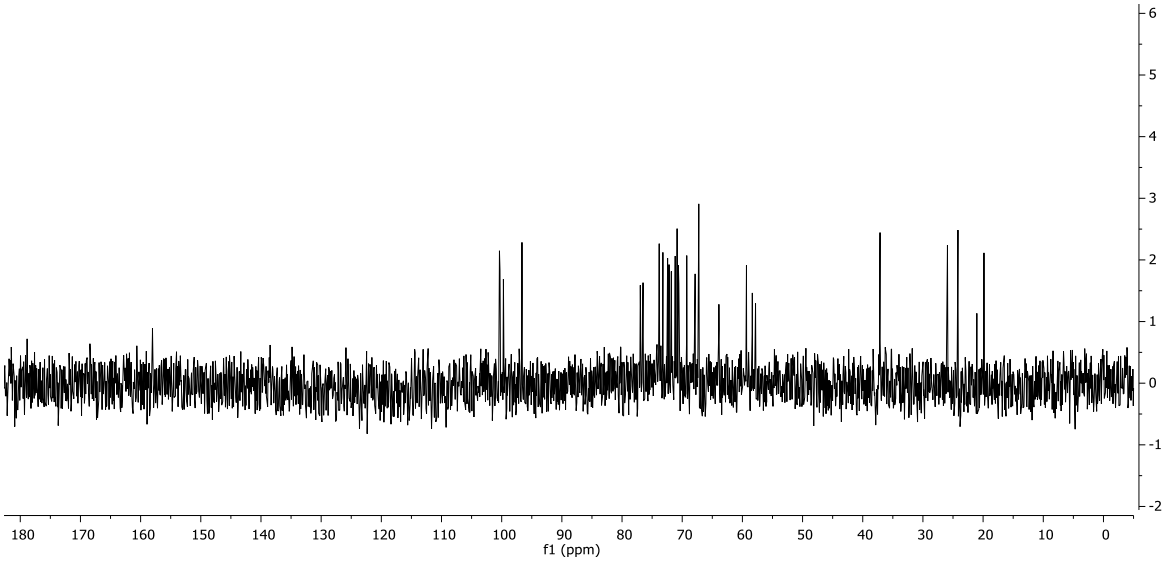
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.97 (d, *J* = 3.6 Hz, 1H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.05-3.90 (m, 5H), 3.86-3.81 (m, 2H), 3.78-3.48 (m, 14H), 3.45-3.38 (m, 2H), 3.36-3.31 (m, 2H), 3.02 (t, *J* = 7.5 Hz 2H), 1.75-1.66 (m, 4H), 1.51-1.44 (m, 2H) ppm.^{13}C NMR (151 MHz, D_2O): 100.4, 100.3, 99.7, 96.6, 77.0, 76.5, 73.8, 73.2, 72.4, 72.2, 71.8, 71.2, 70.8, 70.8, 70.6, 70.6, 69.2, 67.8, 67.2, 63.9, 59.3, 58.3, 57.8, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{28}H_{52}NO_{20}$: 722.3078; found 722.3108

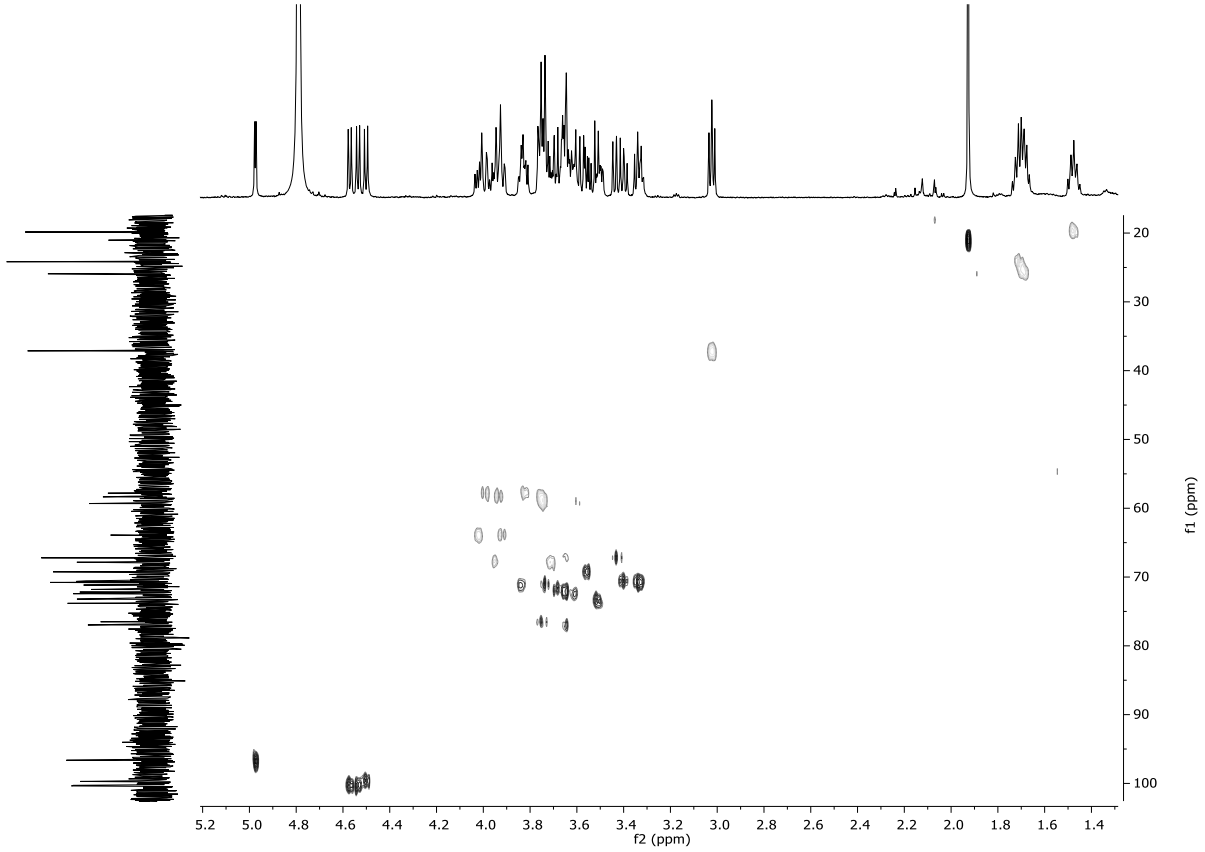
^{1}H NMR (600 MHz, D_2O) of **13**:



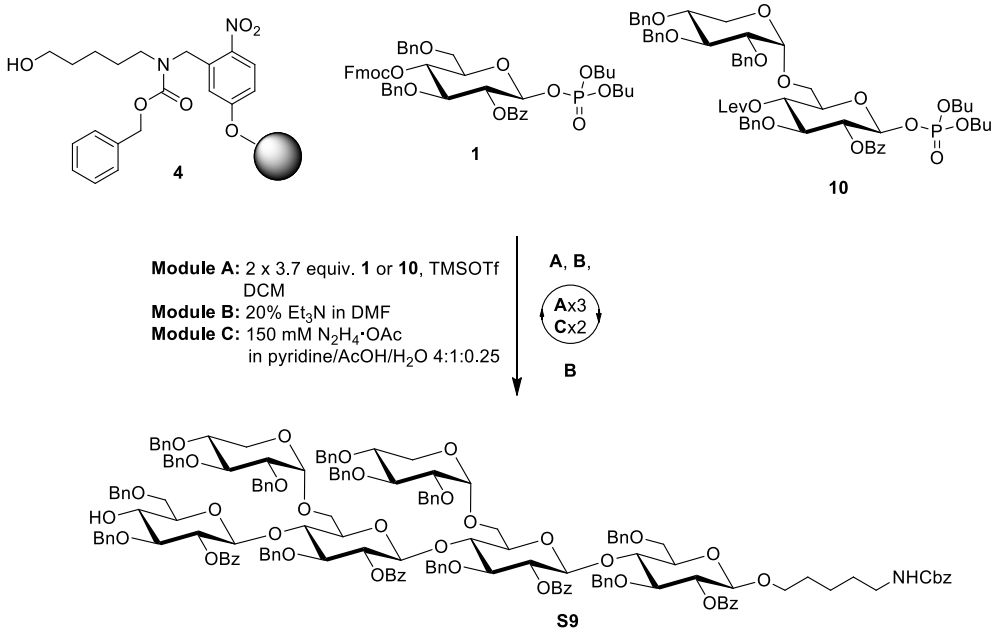
^{13}C NMR (151 MHz, D_2O) of **13**:



HSQC (D_2O) of **13**:



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-*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 (S9)

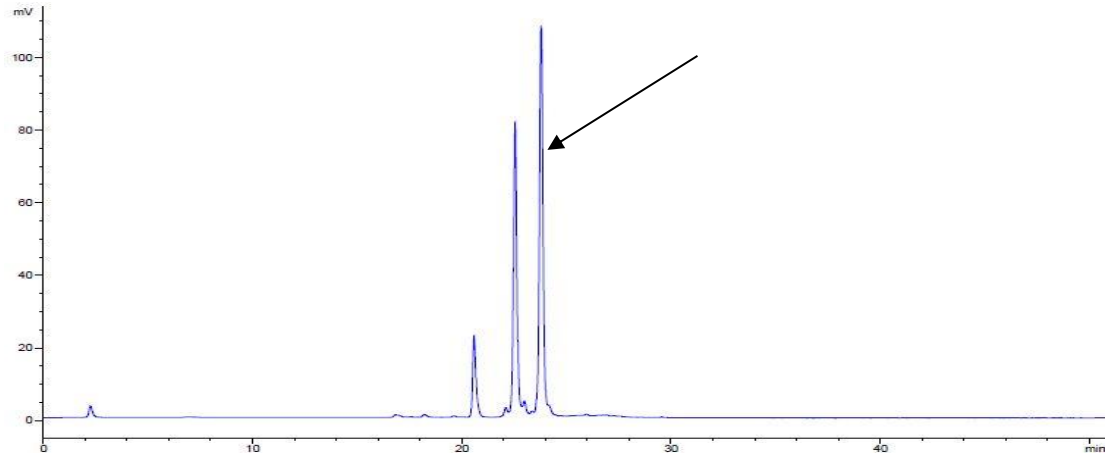


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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. **10**, 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. **10**, 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 tetrasaccharide **S9**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

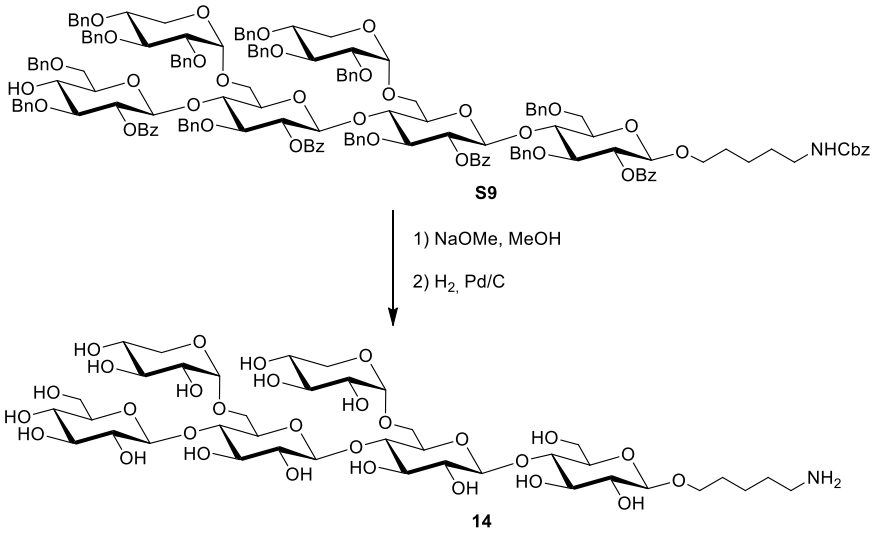
Crude NP-HPLC of hexasaccharide **S9** (ELSD trace):



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

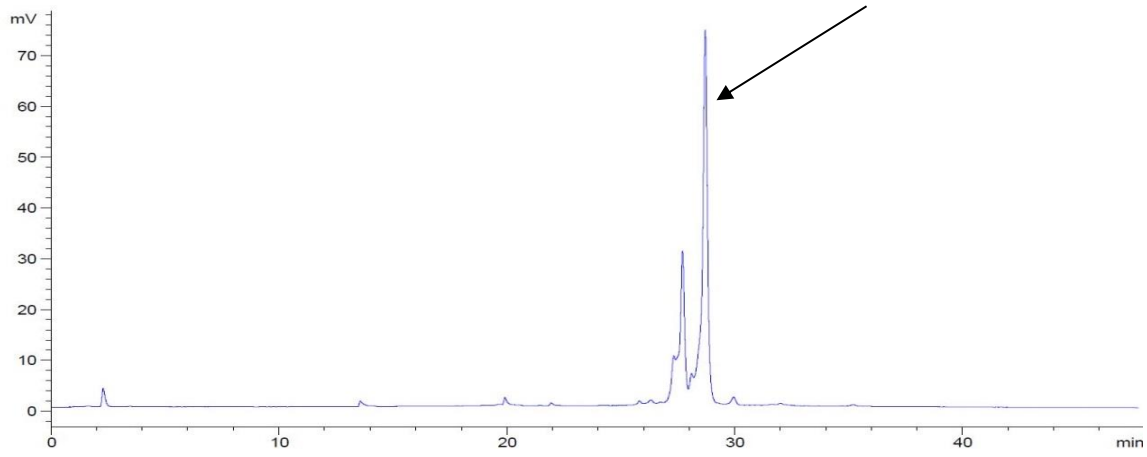
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected hexasaccharide (13.9 mg, 5.25 µmol, 31% over 9 steps, based on resin loading)

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (14)



Hexasaccharide **S9** (26.8 mg, 10.1 µmol) 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 solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected hexasaccharide.

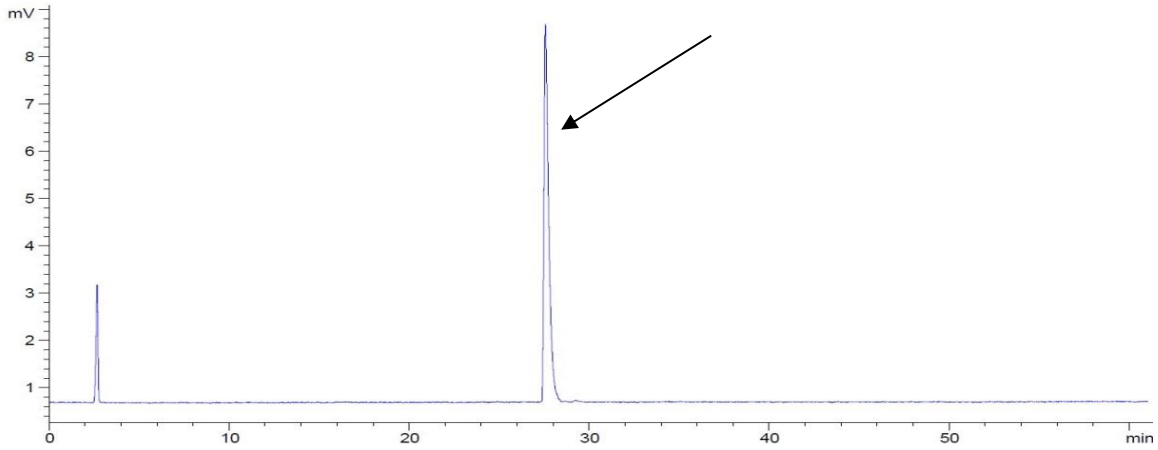
Crude NP-HPLC of the semi-protected hexasaccharide (ELSD trace):



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, 20 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 **14** (5.6 mg, 5.51 µmol, 55% over 2 steps).

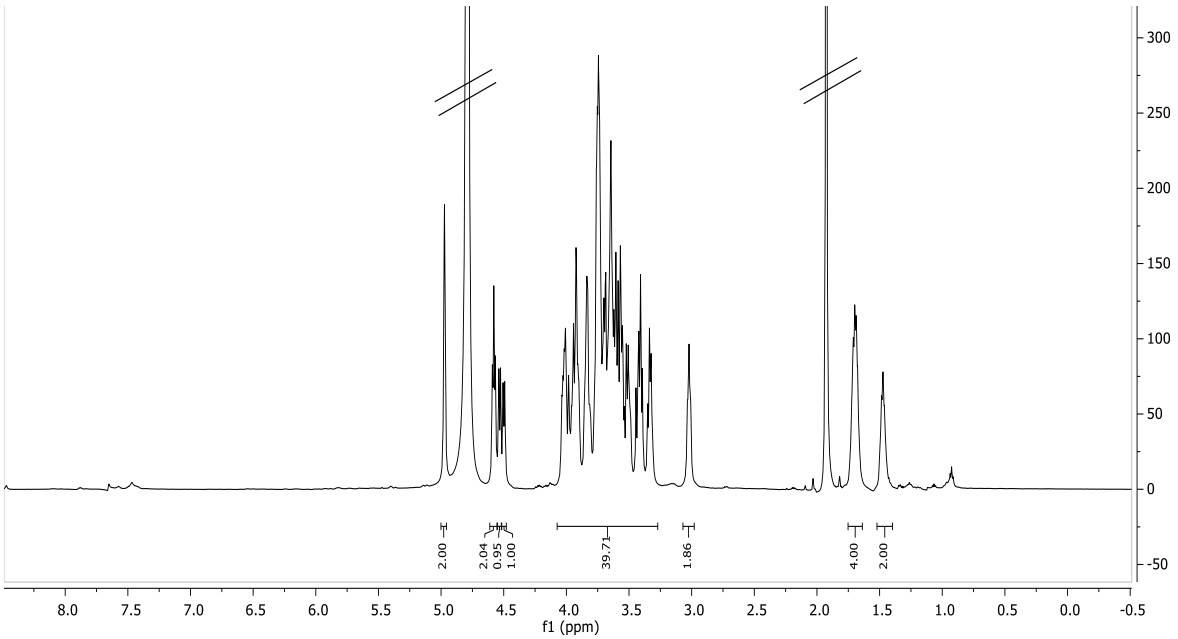
RP-HPLC of the deprotected hexasaccharide **14** (ELSD trace):



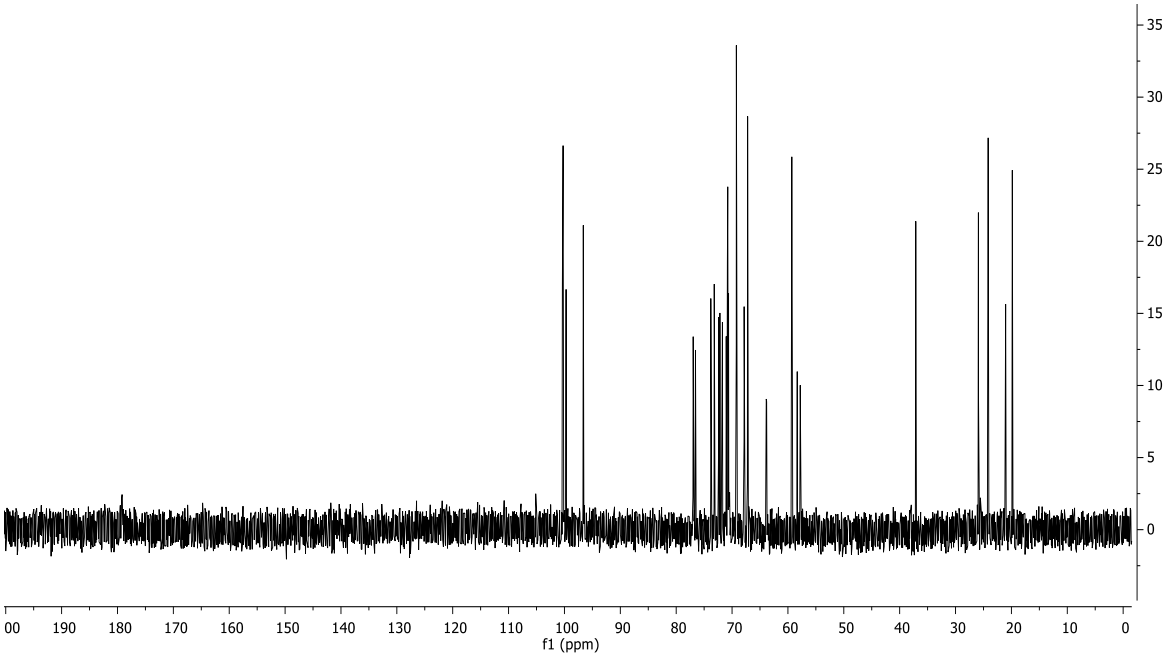
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (600 MHz, D_2O): δ = 4.97 (s, 2H), 4.58 (t, *J* = 7.2 Hz, 2H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.06-3.29 (m, 36H), 3.02 (t, *J* = 7.3 Hz, 2H), 1.73-1.66 (m, 4H), 1.52-1.43 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): 100.4, 100.2, 99.7, 96.6, 96.6, 77.0, 76.9, 76.5, 73.8, 73.2, 72.4, 72.2, 71.8, 71.7, 71.2, 71.0, 70.8, 70.8, 70.8, 70.6, 70.5, 70.5, 69.2, 67.8, 67.2, 63.9, 59.3, 58.3, 57.8, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{39}H_{70}NO_{29}$: 1016.4029; found 1016.4116.

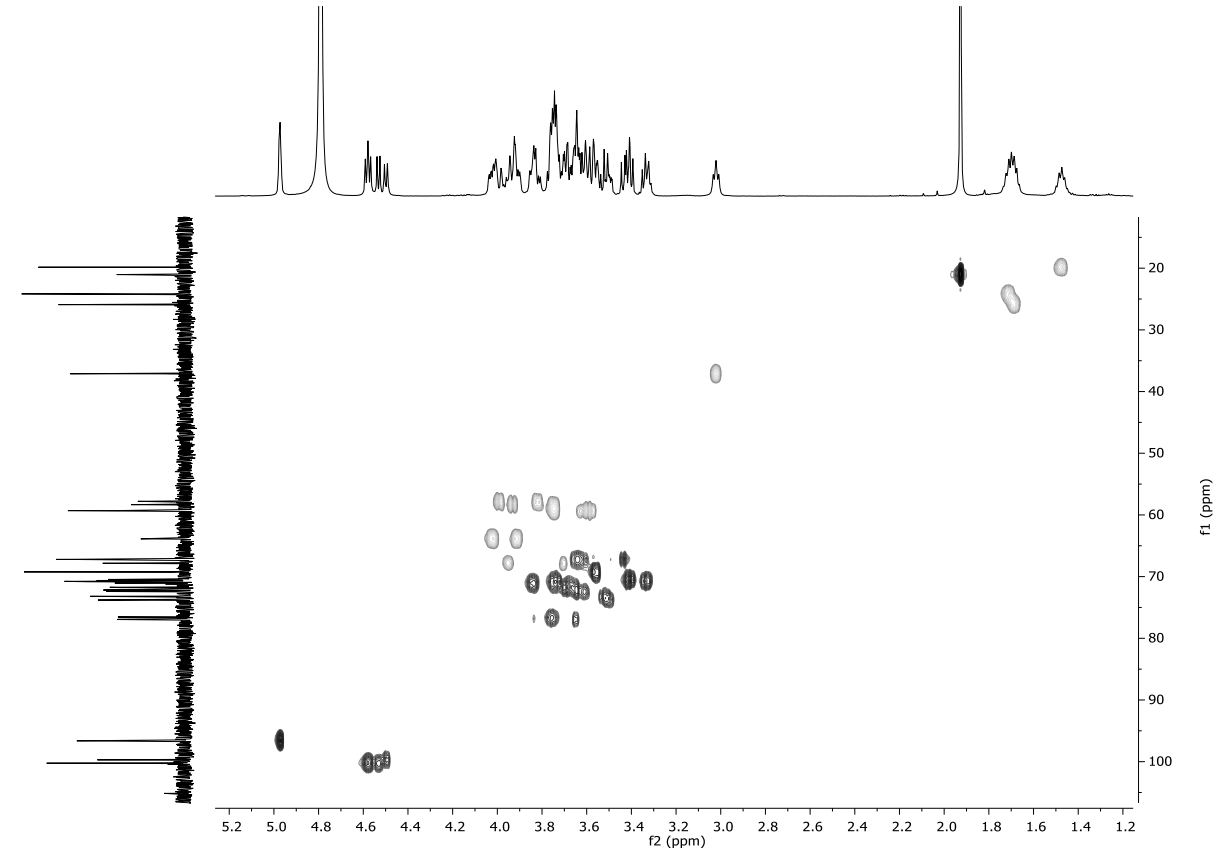
^{1}H NMR (600 MHz, D_2O) of **14**:



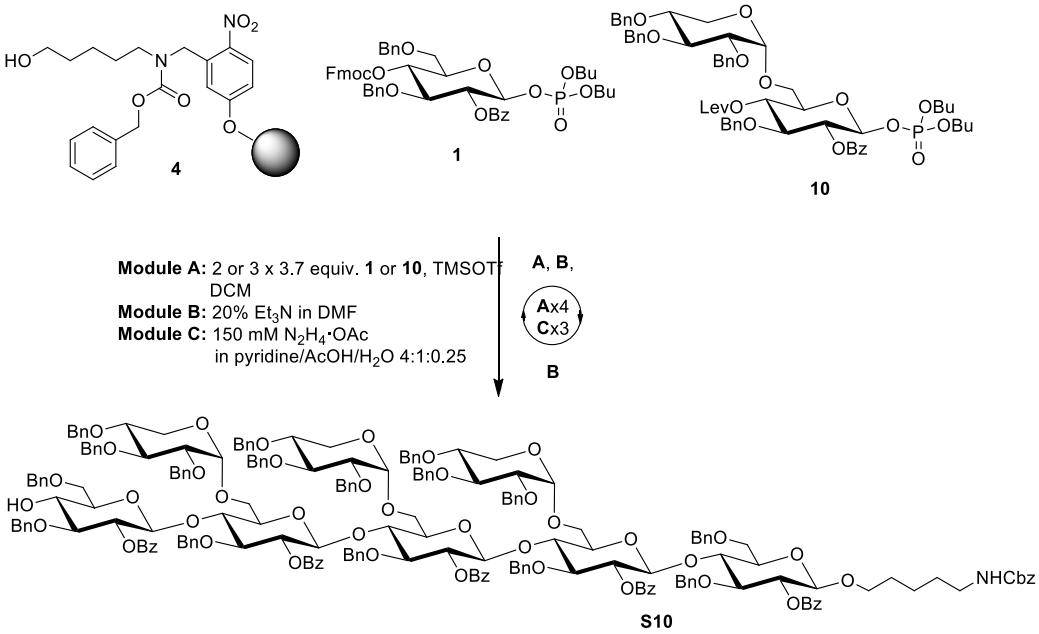
^{13}C NMR (151 MHz, D_2O) of **14**:



HSQC (D_2O) of **14**:



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-*O*-benzyl-6-*O*-[2,3,4-*O*-tribenzyl-α-D-xylopyranosyl]-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 (S9)

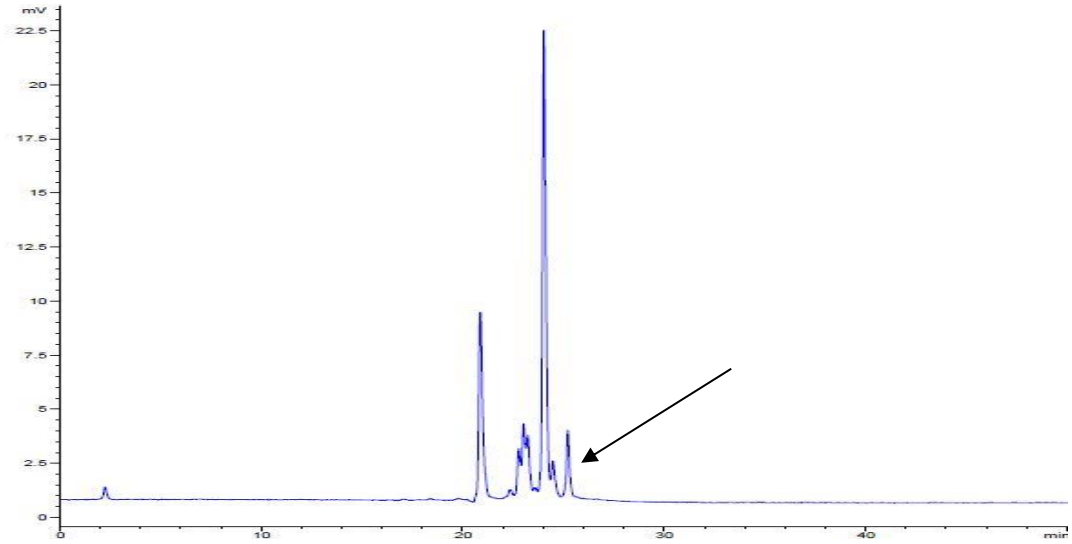


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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. **10**, 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** (3 x 3.7 equiv. **10**, TMSOTf, DCM, 3 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** (3 x 3.7 equiv. **10**, TMSOTf, DCM, 3 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 octasaccharide **S10**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

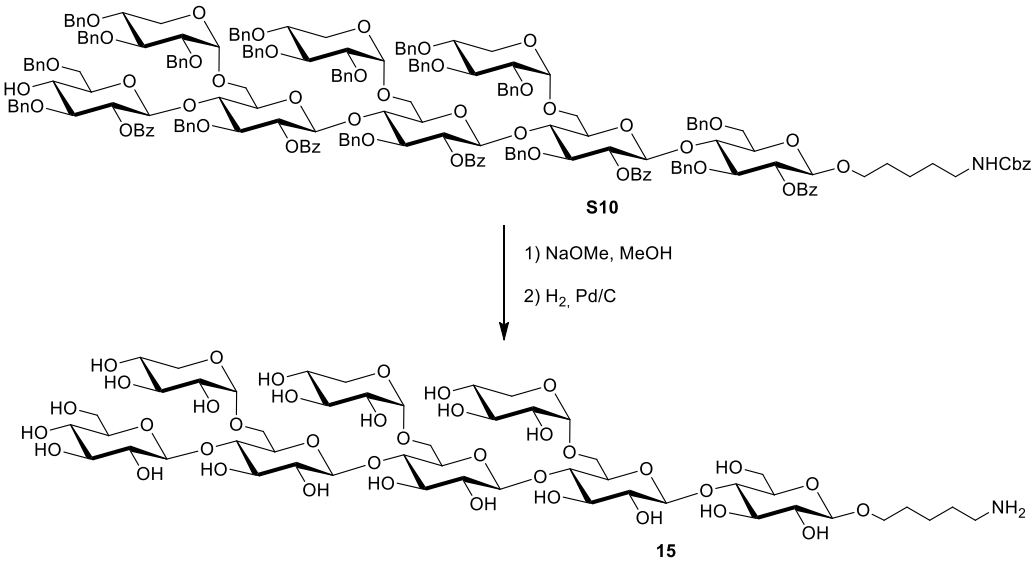
Crude NP-HPLC of octasaccharide **S10** (ELSD trace):



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

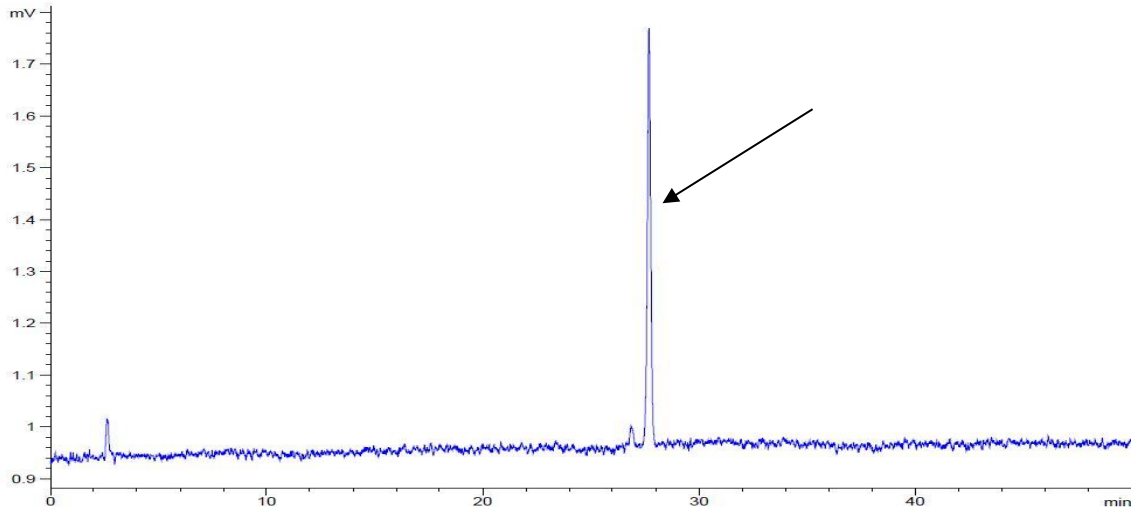
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected octasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (15)



Octasaccharide **S10** 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 prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected octasaccharide.

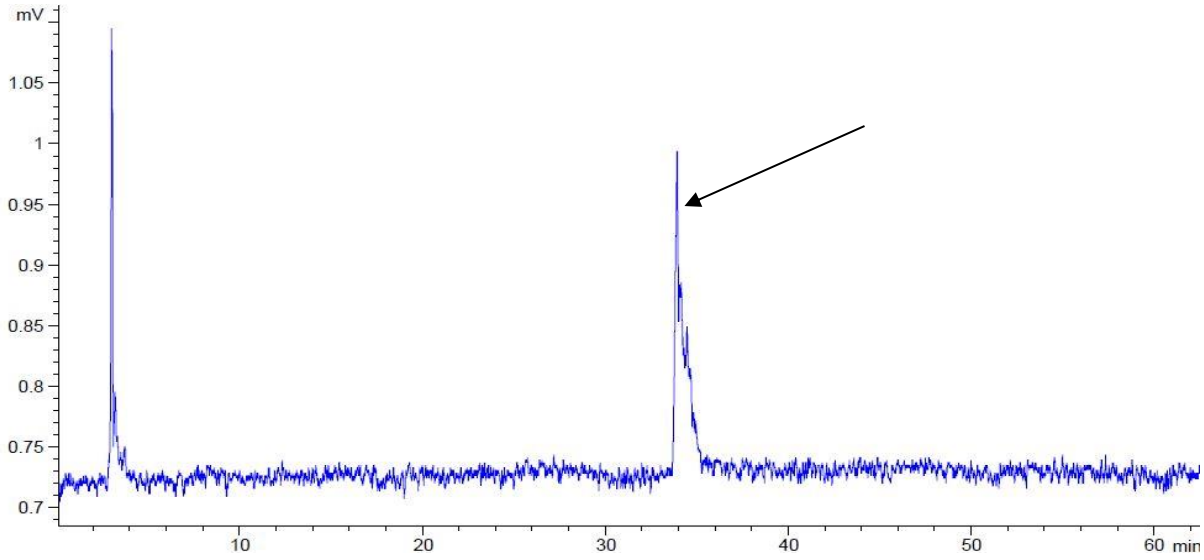
Crude NP-HPLC of the semi-protected octasaccharide (ELSD trace):



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, 10 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 octasaccharide **15** (0.5 mg, 0.38 µmol, 2% over 13 steps).

RP-HPLC of the deprotected octasaccharide **15** (ELSD trace):

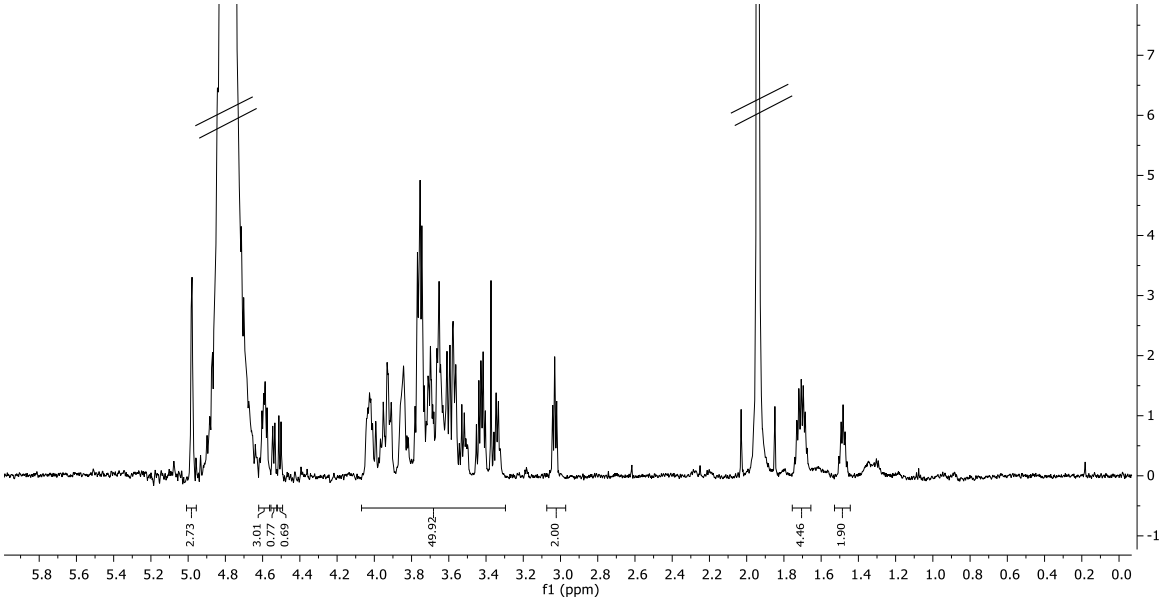


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

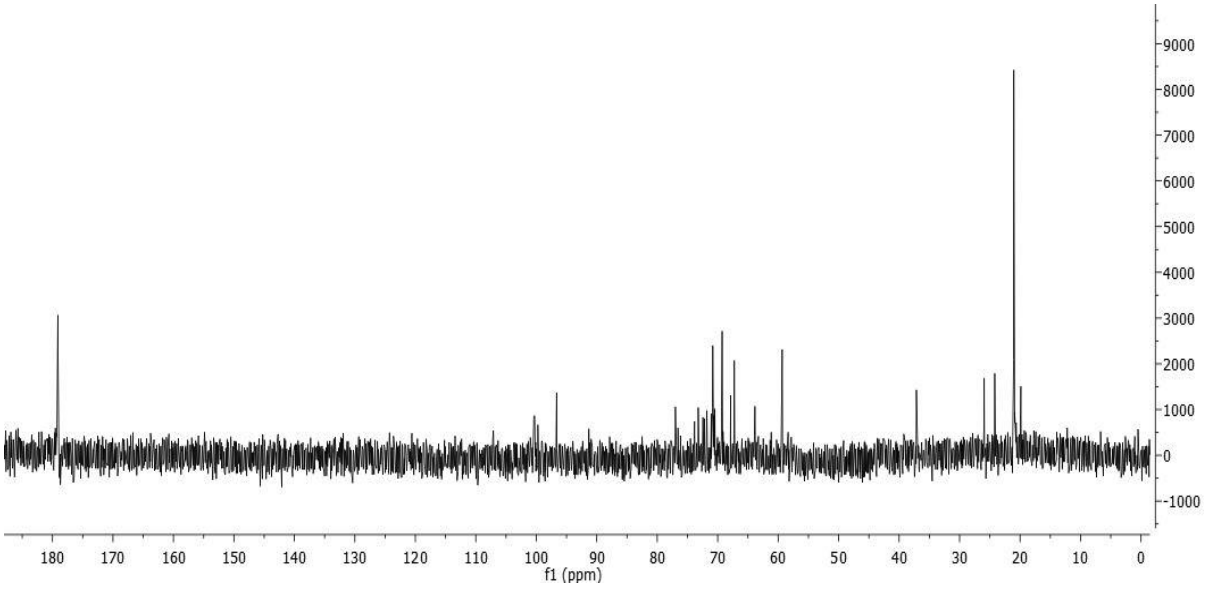
^{1}H NMR (700 MHz, D_2O): δ = 4.98 (m, 3H), 4.62-4.56 (m, 3H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.06-3.49 (m, 44H), 3.47-3.40 (m, 3H), 3.03 (t, *J* = 7.5 Hz, 2H), 1.75-1.67 (m, 4H), 1.51-1.46 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): 100.4, 100.3, 99.7, 96.6, 77.0, 73.8, 73.2, 72.4, 72.2, 71.8,

70.8, 69.3, 67.9, 67.2, 63.8, 59.3, 37.1, 25.9, 24.2, 19.9 ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C_{50}H_{87}NaNO_{38}$: 1332.4799; found 1333.4848.

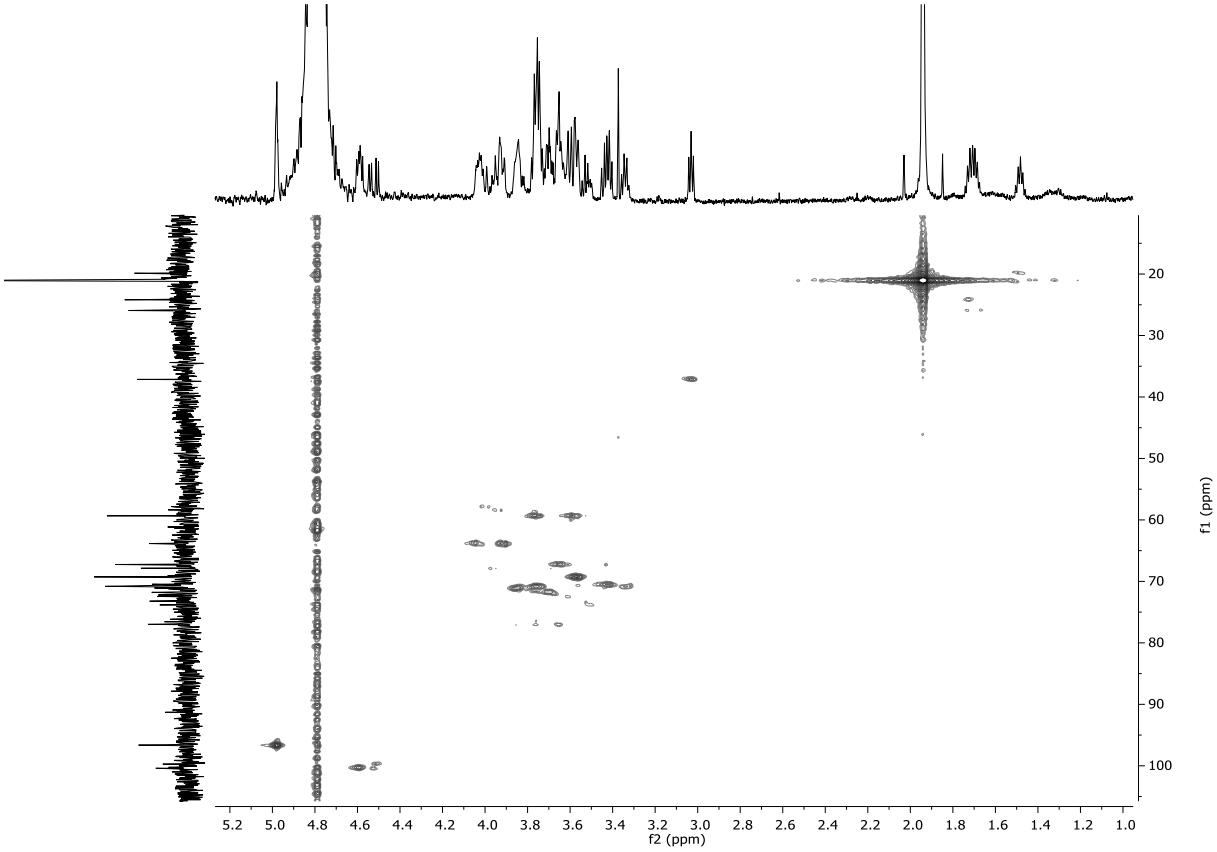
^{1}H NMR (700 MHz, D_2O) of **15**:



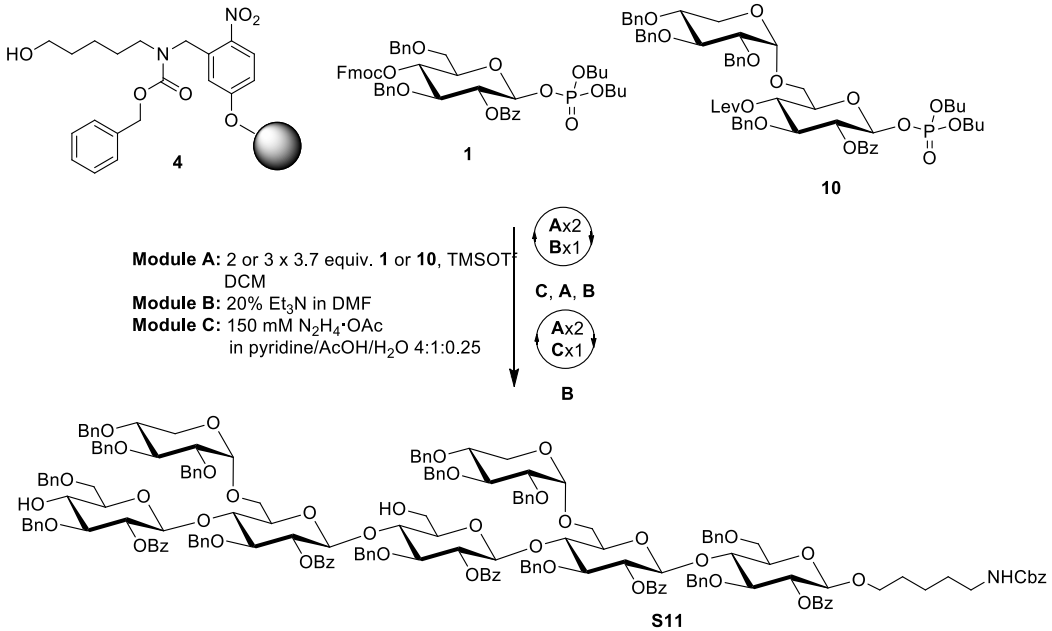
^{13}C NMR (176 MHz, D_2O) of **15**:



HMQC (D_2O) of **15**:



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-*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 (S11)

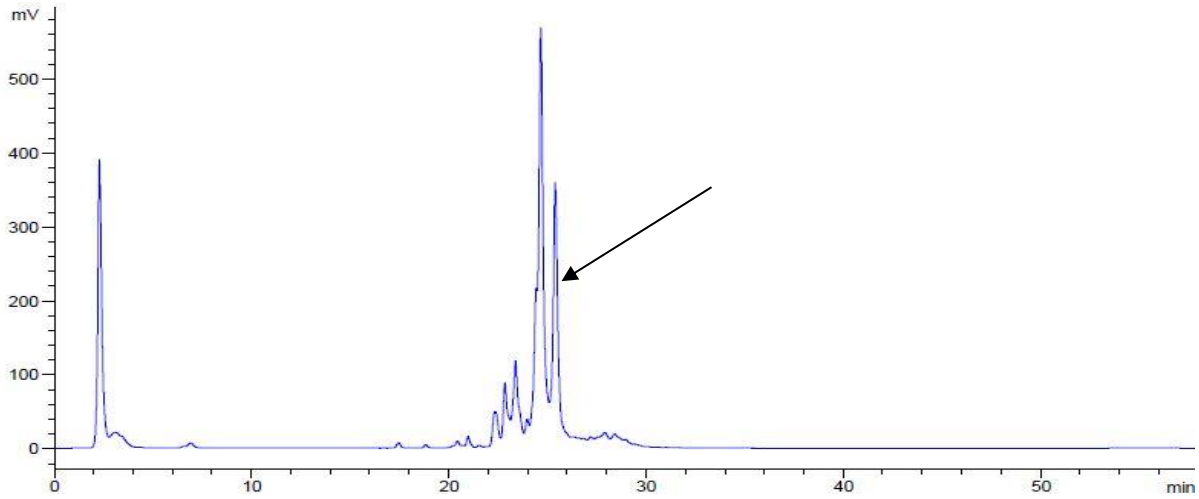


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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 **10**, TMSOTf, DCM, 3 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** (3 x 3.7 equiv **10**, TMSOTf, DCM, 3 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 heptasaccharide **S11**. The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

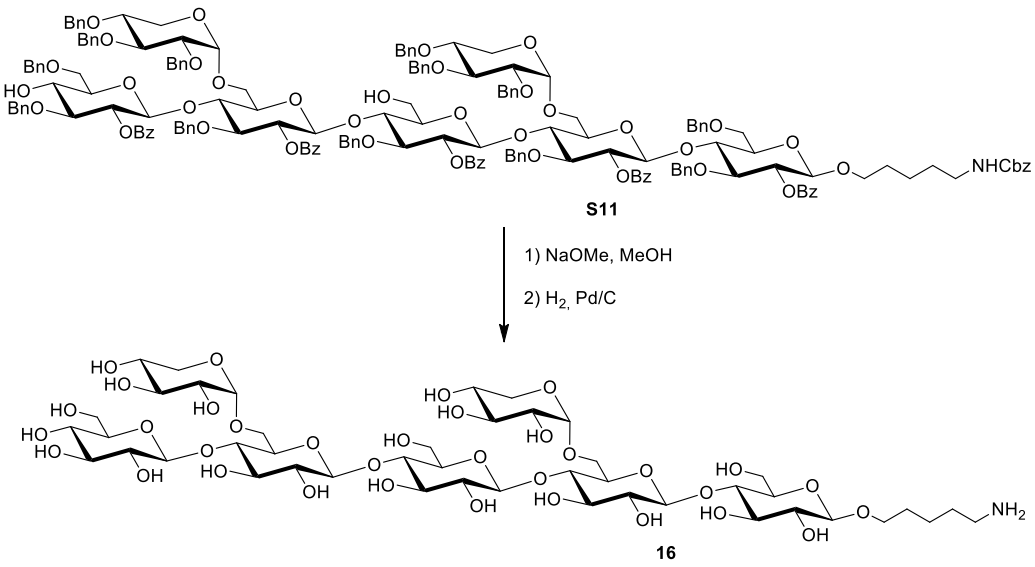
Crude NP-HPLC of heptasaccharide **S11** (ELSD trace):



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

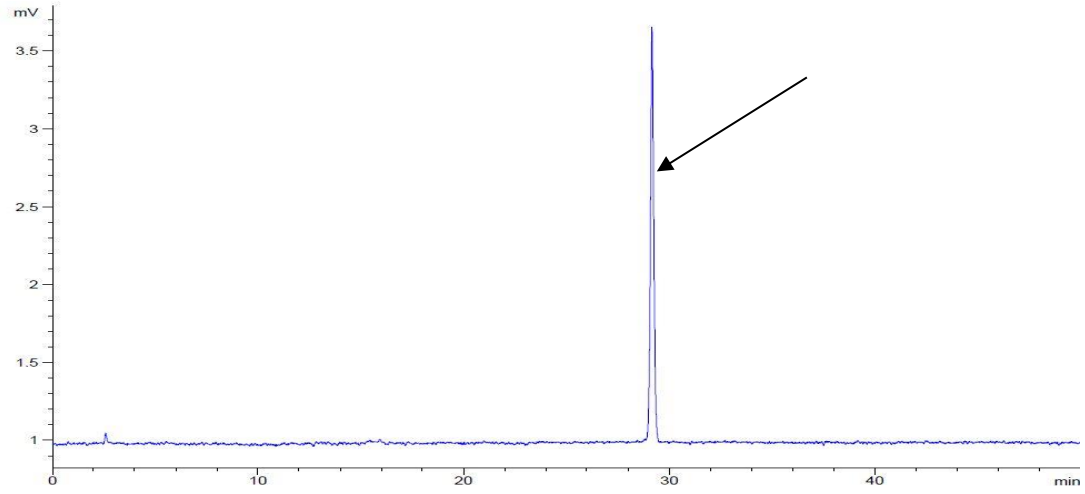
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected heptasaccharide.

Aminopentyl β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (16)



Heptasaccharide **S11** 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 prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected heptasaccharide.

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

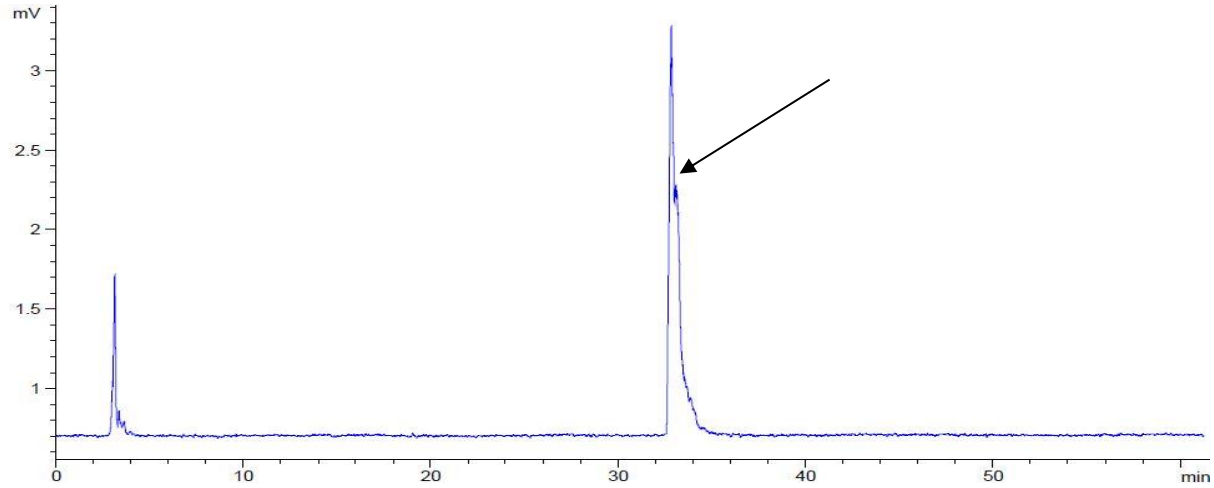


HPLC was performed using a YMC-Diol-300 column and linear gradients from 10% to 100% ethyl acetate in hexane (40 min, flow rate 1 mL/min).

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, 10 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 heptasaccharide **16** (2.0 mg, 1.7 µmol, 10% over 13 steps).

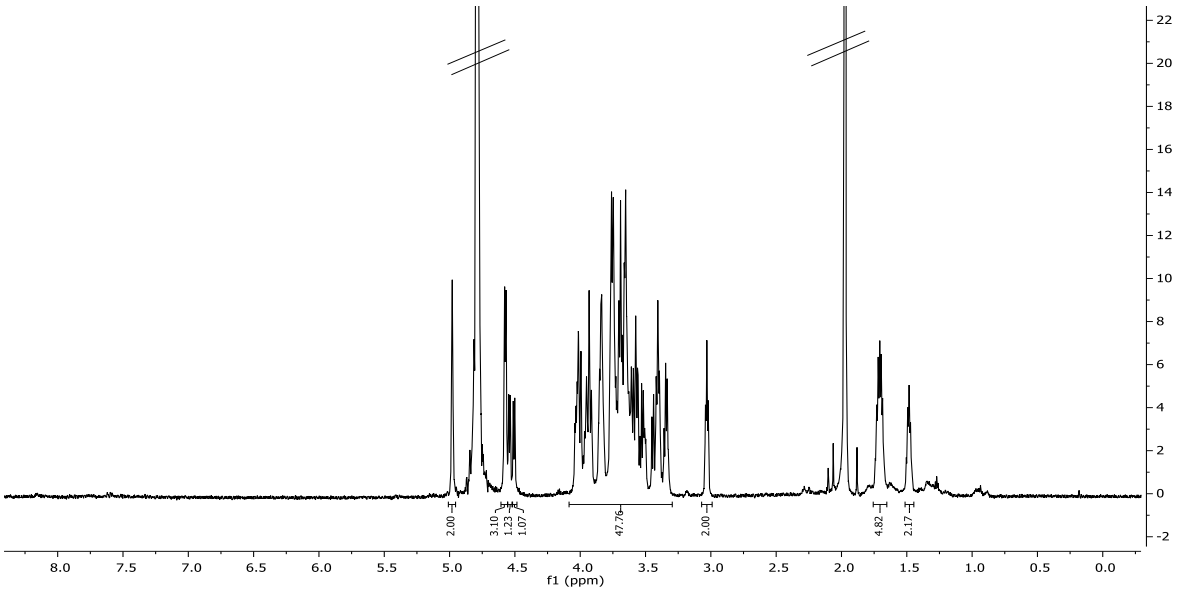
RP-HPLC of the deprotected heptasaccharide **16** (ELSD trace):



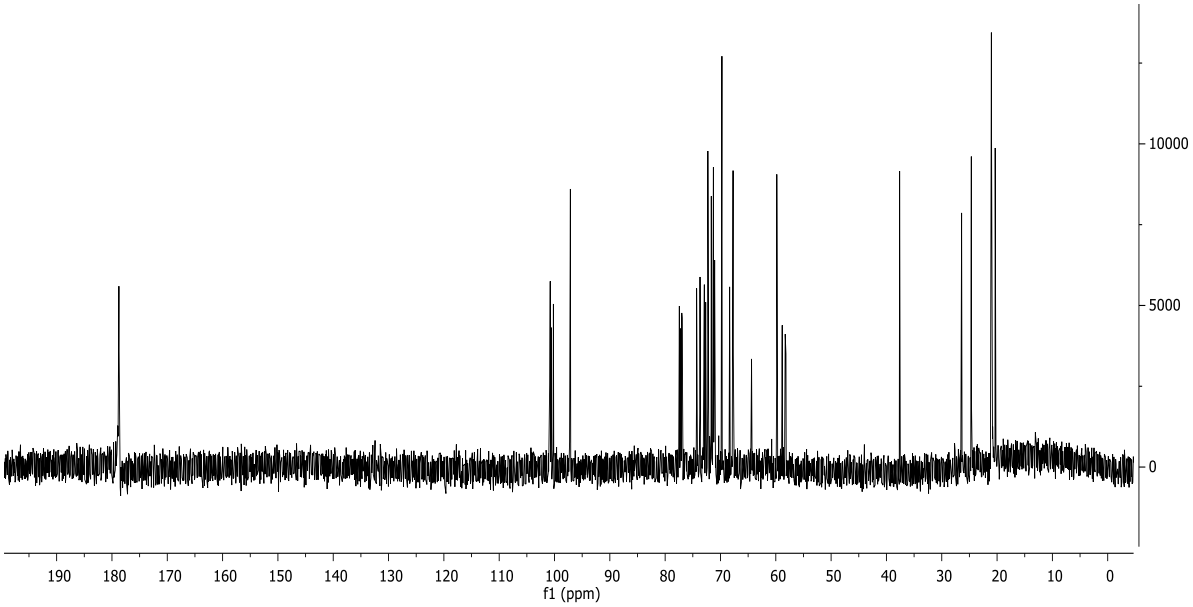
HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

^{1}H NMR (700 MHz, D_2O): δ = 4.98 (s, 2H), 4.57 (d, *J* = 7.8 Hz, 3H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.51 (d, *J* = 7.9 Hz, 1H), 4.05-3.31 (m, 42H), 3.03 (t, *J* = 7.3 Hz, 2H), 1.75-1.65 (m, 4H), 1.52-1.44 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): 100.9, 100.8, 100.6, 100.2, 97.1, 77.4, 77.1, 77.0, 76.9, 74.3, 73.7, 73.0, 72.9, 72.7, 72.3, 71.7, 71.3, 71.3, 71.1, 71.1, 71.0, 69.7, 68.3, 67.7, 64.4, 60.5, 59.8, 59.5, 58.8, 58.5, 58.3, 58.2, 57.6, 37.6, 26.4, 24.7, 20.3 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{45}H_{80}NO_{34}$: 1178.4557; found 1178.4575.

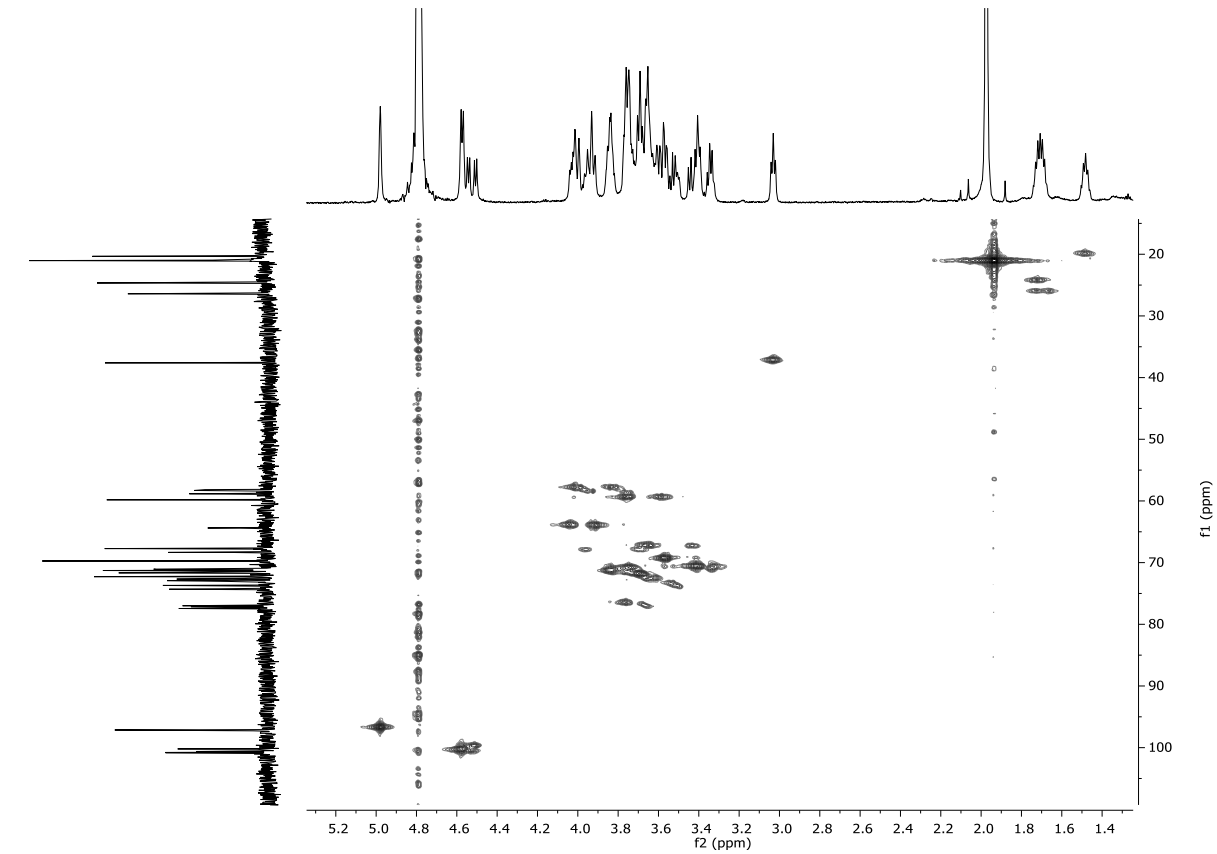
^{1}H NMR (700 MHz, D_2O) of **16**:



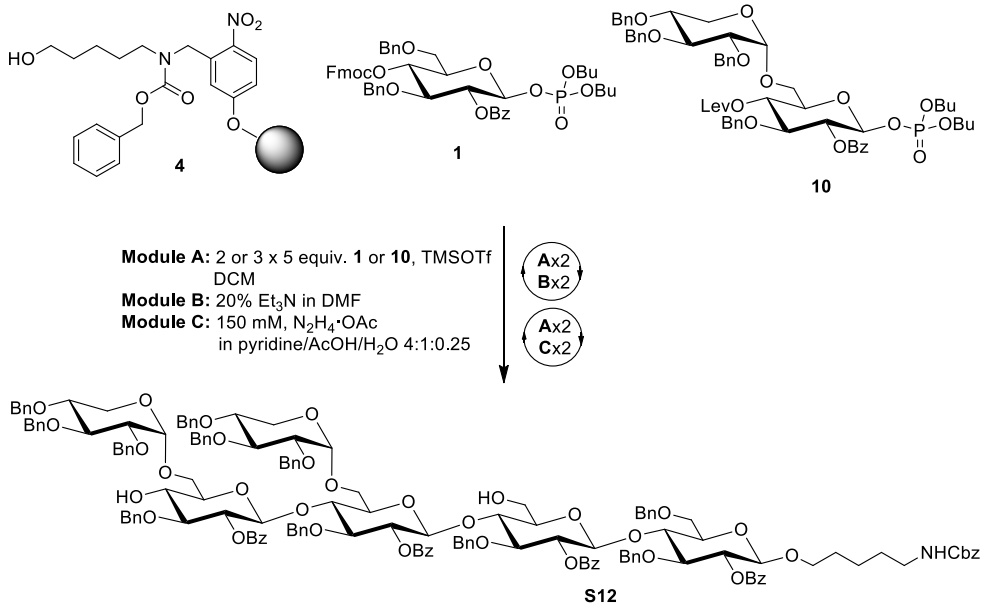
^{13}C NMR (176 MHz, D_2O) of **16**:



HMQC (D_2O) of **16**:



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-*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 (S11)

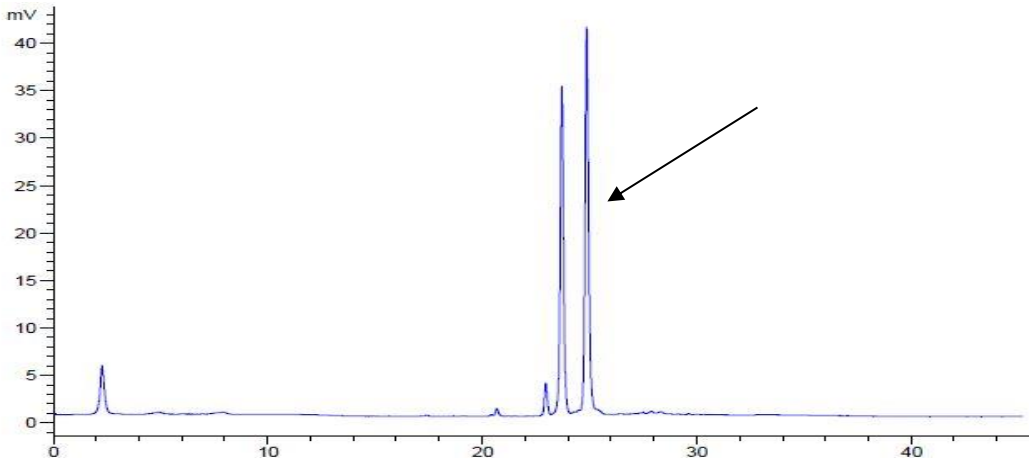


Linker functionalized resin **4** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

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)
Module **A** (2 x 3.7 equiv **10**, 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** (3 x 3.7 equiv **10**, TMSOTf, DCM, 3 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 hexasaccharide **S12**, The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column.

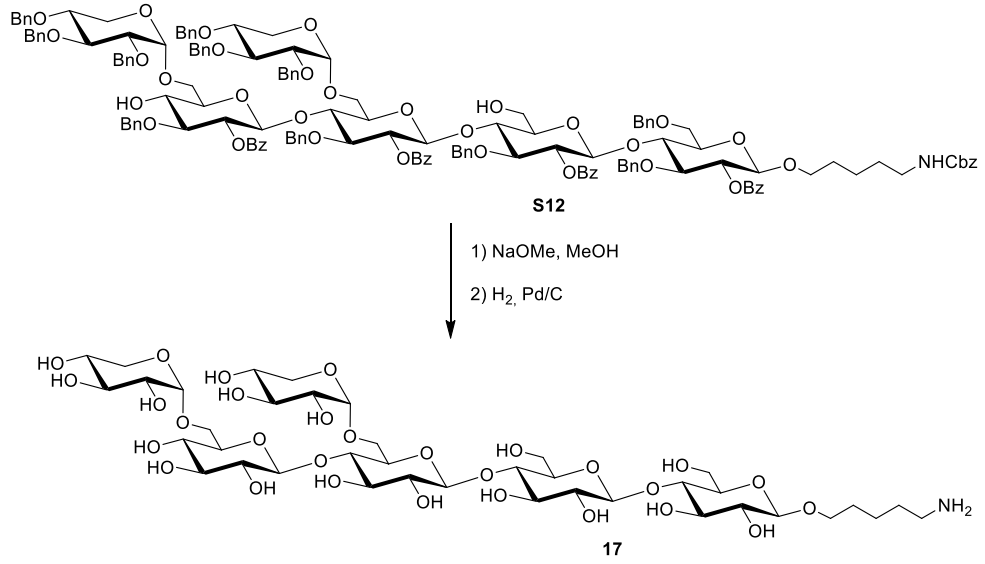
Crude NP-HPLC of hexasaccharide **S12** (ELSD trace):



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

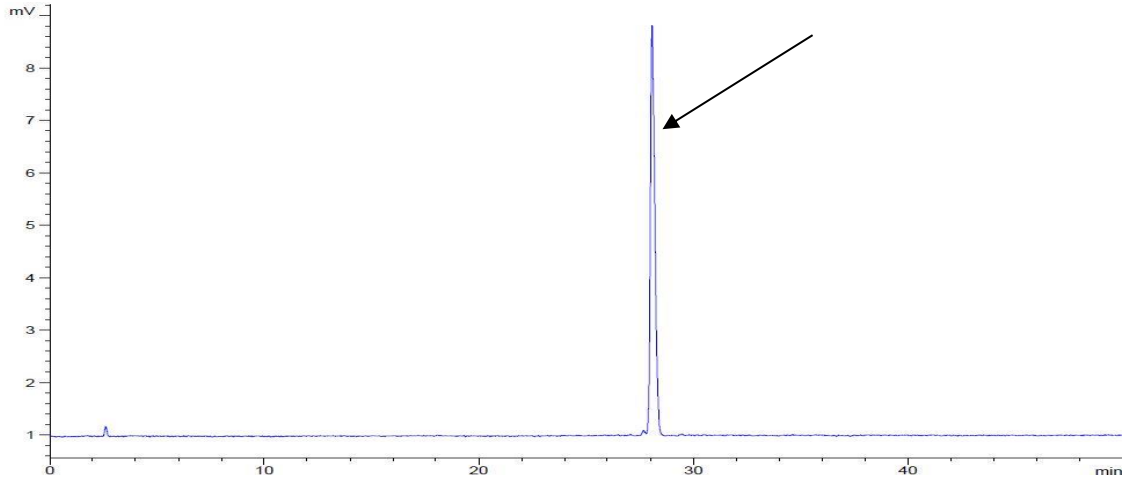
The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the protected hexasaccharide.

Aminopentyl 6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-6-*O*-[α-D-xylopyranosyl]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (17)



Hexasaccharide **S11** 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 prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by normal phase HPLC using a preparative YMC-Diol-300 column affording the semi-protected hexasaccharide.

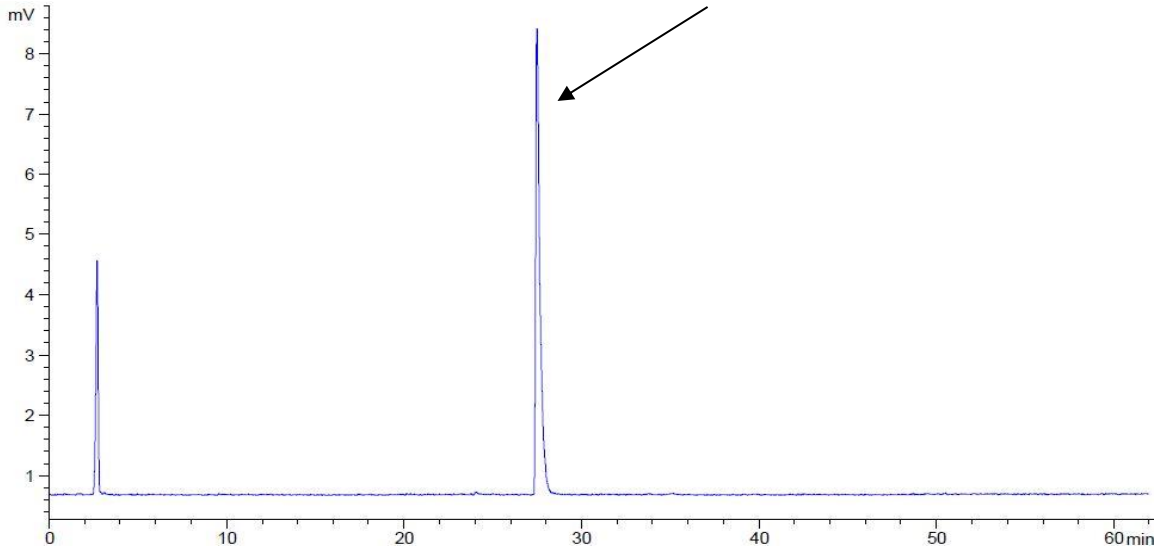
Crude NP-HPLC of the semi-protected hexasaccharide (ELSD trace):



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, 10 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 **17** (1.5 mg, 1.48 µmol, 9% over 11 steps).

RP-HPLC of the deprotected hexasaccharide **17** (ELSD trace):

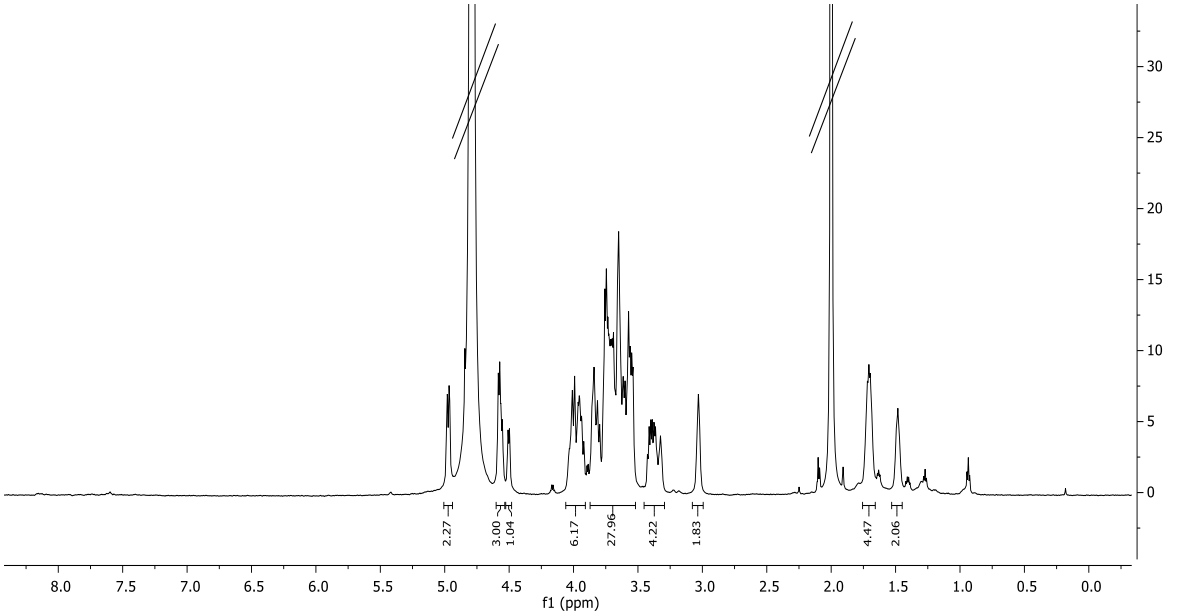


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

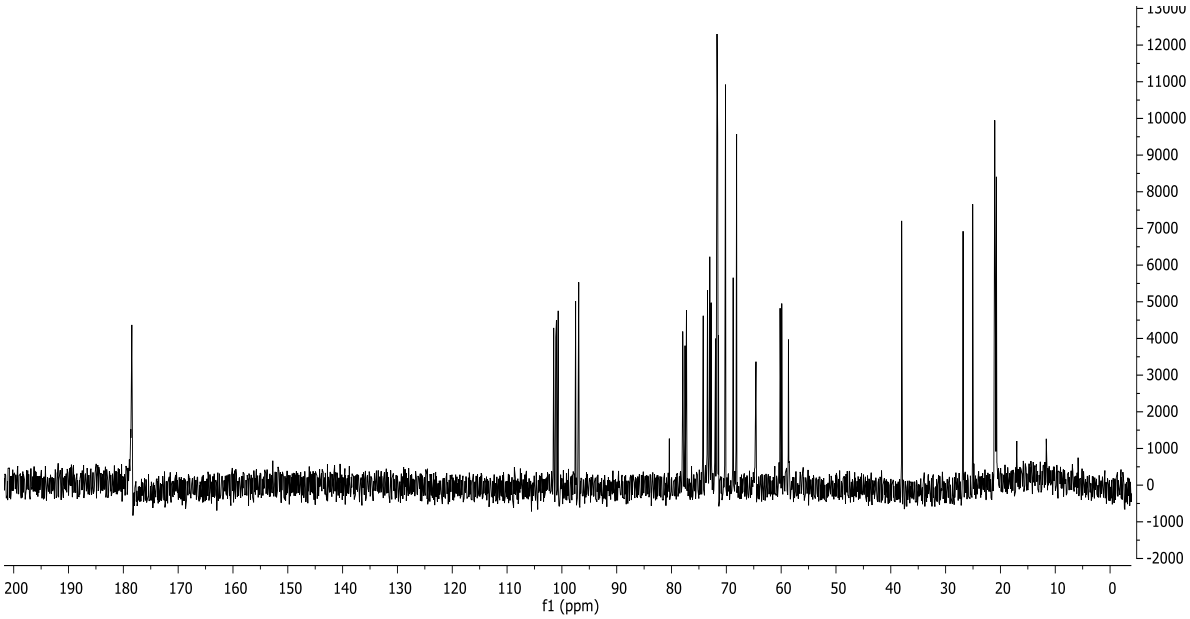
^{1}H NMR (700 MHz, D_2O): δ = 5.01-4.94 (m, 2H), 4.59-4.54 (m, 3H), 4.50 (d, *J* = 8.4 Hz, 1H), 4.05-3.91 (m, 6H), 3.87-3.52 (m, 26H), 3.44-3.31 (m, 4H), 3.06-3.01 (m, 2H), 1.74-1.66 (m, 4H), 1.52-1.44 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ = 178.5, 178.4, 101.5, 101.1, 101.0, 100.7, 97.5, 96.9, 77.9, 77.5, 77.3, 74.2, 73.4, 73.0, 73.0, 72.8, 72.7, 72.0, 71.7, 71.6, 71.6, 71.4, 70.1, 68.8, 68.2, 68.1, 64.7, 64.6,

60.2, 59.9, 58.7, 58.5, 38.0, 26.8, 25.1, 20.7 ppm. ESI-HRMS**:** m/z $[M+H]^+$ calcd. for $C_{39}H_{70}NO_{29}$: 1016.4029; found 1016.4093.

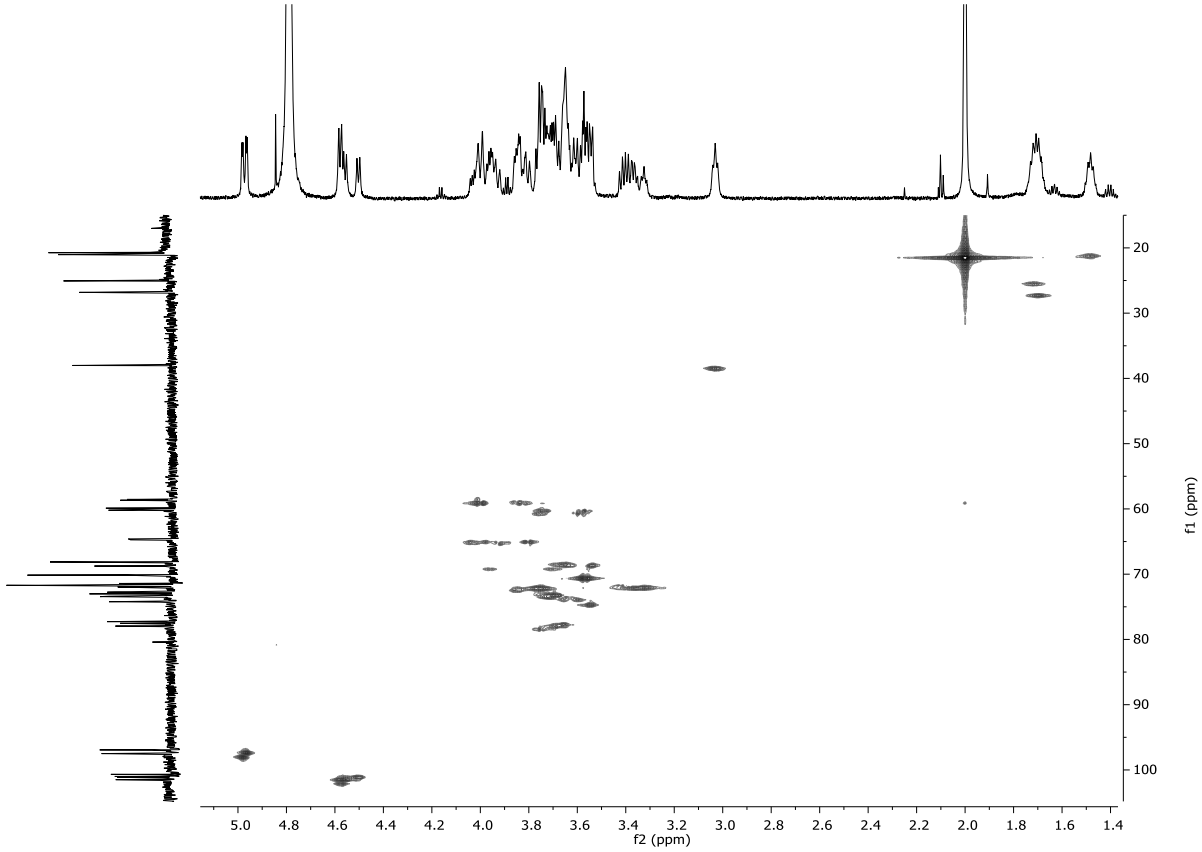
^{1}H NMR (600 MHz, D_2O) of **17**:



^{13}C NMR (151 MHz, D_2O) of **17**:



HMQC (D_2O) of **17**:



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