Automated Glycan Assembly of Xyloglucan Oligosaccharides

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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\(_3\): 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
The resin was washed with DMF, THF and TM. For the glycosylation reaction the DCM was drained and a removal of residual acetic acid. Intermediate glycosylation with Glycosyl Phosphates were performed. Mixing of the components was accomplished by bubbling Ar through the reaction mixture. 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.

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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% Et₃N 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% Et₃N in dry DMF

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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 N₂H₂·AcOH in Pyridine/AcOH/H₂O 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 $\text{N}_2\text{H}_4\cdot\text{AcOH}$ in Pyridine/AcOH/H$_2$O 4:1:0.25

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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.
Cleavage from the solid support

After assembly of the oligosaccharides cleavage from the solid support was accomplished using a continuous-flow photoreactor as described previously. 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-glucopyranoside3-5 (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. NaHCO₃ solution (100 mL) and extracted with DCM (100 mL). The organic layer was dried over Na₂SO₄, 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.

1H NMR (400 MHz, CDCl₃): 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.²

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 Na₂SO₄ 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.

[α]D²⁵ = +27.3 (c 1.0, CHCl₃). 1H NMR (400 MHz, CDCl₃): 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, CH₂Ph), 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, CH₃), ppm. 13C NMR (100 MHz, CDCl₃): 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 (CH$_2$Ph), 73.7 (CH$_3$Ph), 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$_8$S: 815.2655; found 815.2641. IR (neat) $\nu_{\text{max}}$ = 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-benzyol-3,6-O-dibenzyl-4-O-fluorenlycarboxymethyl-$\beta$-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 Na₂S₂O₃/NaHCO₃ (1:1, 100 mL) and extracted with DCM (100 mL). The organic layer was dried over Na₂SO₄ 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, CHCl₃). ¹H NMR (400 MHz, CDCl₃): 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, CH₂Ph), 4.56-4.46 (m, 3H, CH₂Ph), 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), 3.94-3.58 (m, 4H, 6-H, OBu), 3.78-3.58 (m, 4H, 6-H, OBu), 3.69-3.58 (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, CH₃), 0.66 (t, J = 7.4 Hz, 3H, CH₃) ppm. ¹³C NMR (125 MHz, CDCl₃): 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 (CH₂Ph), 73.6 (2C, CH₂Ph, 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, CH₃) ppm. ESI-HRMS: m/z [M+Na]⁺ calcd. for C₅₀H₅₅NaO₁₂P: 901.3329; found 901.3361. IR (neat) ν_max = 1753, 1733, 1248, 1027 cm⁻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\(^5\) 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.

\([\alpha]\)\(^D\) = +5.1 (c 1.0, CHCl\(_3\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 8.07\) (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\(_\text{H}_2\)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\(_3\)): \(\delta = 206.8, 173.2, 165.1\) (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 (CH\(_2\)Ph), 72.0 (C-2), 69.9 (C-4), 63.3 (C-6), 37.9 (Lev), 29.8 (O=CCH\(_3\)), 27.9 (Lev), 21.1 (CH\(_3\)) ppm. ESI-HRMS: m/z [M+Na]\(^+\) calcd. for C\(_{32}\)H\(_{34}\)NaO\(_8\)S: 601.1867; found 601.1910. IR (neat) \(\nu_{\text{max}}\): 3486, 1722, 1270, 1070 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) of S3:

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[Diagram of S3 molecule]

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S8
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 Na₂SO₄ and purified by silica gel chromatography (Tol/EtOAc 10:1) to give S4 (691 mg, 863 µmol, 74%) as a white solid.

$[\alpha]_D^{25} = +25.7 \text{ (c 1.0, CHCl₃)}$. $^1$H NMR (400 MHz, CDCl₃): $\delta = 8.05-8.00 \text{ (m, 2H, Ar), 7.77-7.71 \text{ (m, 2H, Ar), 7.64-7.54 \text{ (m, 3H, Ar), 7.47 \text{ (t, J = 7.8 Hz, 2H), 7.41-7.33 \text{ (m, 3H, Ar), 7.31-7.24 \text{ (m, 4H, Ar), 7.12-6.98 \text{ (m, 6H, Ar), 5.24 \text{ (t, J = 9.5 Hz, 1H, H-2), 4.93 \text{ (t, J = 9.6 Hz, 1H, H-4), 4.71 \text{ (d, J = 10 Hz, 1H, H-1), 4.56-4.44 \text{ (m, 3H, H-6a, CH₂Ph), 4.41-4.34 \text{ (m, 1H, H-6b), 4.29-4.24 \text{ (m, 2H, Fmoc), 4.19 \text{ (t, J = 7.1 Hz, 1H, Fmoc), 3.87 \text{ (t, J = 9.2 Hz, 1H, H-3), 3.77-3.70 \text{ (m, 1H, H-5), 2.84-2.71 \text{ (m, 2H, Lev), 2.68-2.58 \text{ (m, 2H, Lev), 2.33 \text{ (s, 3H, CH₃), 2.18 \text{ (s, 3H, CH₃) ppm}}}})$

$^{13}$C NMR (100 MHz, CDCl₃): $\delta = 206.4, 172.3, 164.8, 154.1 \text{ (4C, C=O), 143.2, 143.0, 141.3, 141.2, 138.5, 137.0, 133.5, 133.3, 129.9, 129.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.6, 127.2, 125.0, 124.9, 120.0 \text{ (30C, Ar), 86.4 \text{ (C-1), 80.9 \text{ (C-3), 75.6 \text{ (C-5), 74.4 \text{ (CH₂Ph), 74.2 \text{ (C-4), 71.8 \text{ (C-2), 70.2 \text{ (C-6), 62.6 \text{ (Fmoc), 46.7 \text{ (Fmoc), 37.8 \text{ (Lev), 29.9 \text{ (O=CCH₃), 27.9 \text{ (Lev), 21.1 \text{ (CH₃) ppm. ESI-HRMS: m/z [M+Na]^+ calcd. for C₄₇H₄₄NaO₁₁S: 823.2548; found 823.2588. IR (neat) } ν_{max}}: 1751, 1258 \text{ cm}^{-1}}.}$
$^1$H NMR (400 MHz, CDCl$_3$) of S4:

![H NMR spectrum](image)

$^{13}$C NMR (100 MHz, CDCl$_3$) of S4:

![C NMR spectrum](image)

Dibutoxyphosphoryloxy-2-O-benzoyl-3-O-benzyl-4-O-fluorenlycarboxymethyl-6-O-levulinoyl-thio-$\beta$-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$_2$S$_2$O$_3$/NaHCO$_3$ (1:1, 30 mL) and extracted with DCM (30 mL). The organic layer was dried over Na$_2$SO$_4$ 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.
$[a]_D^{25} = +26.4$ (c 1.0, CHCl$_3$). $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 8.00$ (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, CH$_2$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, Bu), 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$_3$): $\delta = 206.3$, 172.2, 164.7, 154.0 (4C, C=O), 143.2, 142.9, 141.3, 141.2, 136.8, 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 (CH$_2$Ph), 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=CCH$_3$), 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$_{49}$H$_{55}$NaO$_{14}$P: 909.3222; found 909.3276. IR (neat) $\nu_{\text{max}}$: 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 (8.50 g, 16.1 mmol) in acetone/H₂O (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 Na₂S₂O₃ (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, CDCl₃): 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, CH₂Ph), 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-xylopyanoside (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, CDCl₃): 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, CH₂Ph), 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 (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 NaHCO₃ (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 \( \mu \)mol, 39%) as a pale-yellow oil and the respective \( \beta \) isomer (31.4 mg, 36.0 \( \mu \)mol, 20%) as a white solid.

\([\alpha]^{25}_D = +35.5 \text{ (c 1.0, CHCl}_3\text{)}\). \(^1\)H NMR (400 MHz, CDCl\(_3\): \( \delta = 8.07 \text{ (d, } J = 7.3 \text{ Hz, 2H, Ar), 7.61 \text{ (t, } J = 7.4 \text{ Hz, 1H, Ar), 7.48 \text{ (t, } J = 7.7 \text{ Hz, 2H, Ar), 7.40-7.20 \text{ (m, 17H, Ar), 7.16 \text{ (s, 5H, Ar), 7.06 \text{ (d, } J = 7.9 \text{ Hz, 2H, Ar), 5.22 \text{ (t, } J = 9.5 \text{ Hz, 1H, H-2 Glc), 4.89 \text{ (s, 2H, CH}_2\text{Ph), 4.80-4.60 \text{ (m, 8H, 3 X CH}_2\text{Ph, H-1 Xyl, H-1 Glc), 4.01 \text{ (dd, } J = 9.4 \text{ Hz, 4.5 Hz, 1H, H-6a Glc), 3.88 \text{ (t, } J = 8.8 \text{ Hz, 1H, H-3 Xyl), 3.76 \text{ (t, } J = 8.7 \text{ Hz, 1H, H-4 Glc), 3.72-3.54 \text{ (m, 6H, H-3 Glc, H-4 Xyl, H-5a Xyl, H-5b Xyl, H-5 Glc, H-6b Glc), 3.47 \text{ (dd, } J = 9.5 \text{ Hz, 3.6 Hz, 1H, H-2 Xyl), 3.24 \text{ (s, 1H, OH), 2.24 \text{ (s, 3H, CH}_3\text{ ppm.} \(^{13}\)C NMR (100 MHz, CDCl\(_3\): 165.2 \text{ (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 \text{ (36C, Ar), 97.8 \text{ (C-1 Xyl), 86.7 \text{ (C-1 Glc), 83.4 \text{ (C-3 Glc), 81.3 \text{ (C-3 Xyl), 79.5 \text{ (C-2 Xyl), 77.9 \text{ (C-5 Glc), 75.8, 74.7, 73.7, 73.4, (4C, CH}_2\text{Ph), 73.0 \text{ (C-4 Glc), 71.9 \text{ (C-2 Glc), 68.8 \text{ (C-6 Glc), 60.1 \text{ (C-5 Xyl), 21.1 \text{ (CH}_3\text{ ppm. ESI-HRMS: m/z [M+H]^+ calcd. for C}_{53}\text{H}_{54}\text{NaO}_{10}\text{S: 905.3330; found 905.3503. IR (neat) } \nu_{\text{max}}\text{: 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.

\[ [\alpha]_D^{25} = +26.7 \ (c \ 1.0, \ \text{CHCl}_3) \]

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 8.10 \) (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, CH\(_2\)Ph), 4.87-4.56 (m, 8H, 3 x CH\(_2\)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), 2.16 (s, 3H, CH\(_3\)), 2.13 (s, 3H, O=CC\(_3\)) 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, 128.4, 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, CH\(_2\)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=CC\(_3\)), 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) \( \nu_{\max} \): 1722, 1089, 1072, 1042, 1029 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) of 9:
13C NMR (100 MHz, CDCl3) 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 Na2S2O3/NaHCO3 (1:1, 50 mL) and brine (50 mL). The organic layer was dried over Na2SO4 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.

[α]D25 = +41.2 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3): 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, CH2Ph), 4.76 (d, J = 3.4 Hz, 1H, H-1 Xyl), 4.75-4.55 (m, 6H, 3 x CH2Ph), 4.06-3.41 (m, 13H, 4 x OBu, 6Ha Glc, 6Hb Glc, 5Ha Xyl, 5Hb Xyl, H-2 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, J = 7.4 Hz, CH3), 0.67 (t, J = 7.4 Hz, CH3) ppm.

13C 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 (CH3Ph), 73.8 (C-4 Xyl), 73.4 (2C, CH2Ph), 73 (C-2 Glc), 72.7 (CH2Ph), 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 C56H71NaO16P: 1089.4377; found 1089.4384. IR (neat) νmax: 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 fragments

Benzyloxy carbonylamino pentyl 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% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.7 equiv. 2, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C)
Module C (150 mM N2H4·AcOH in pyridine/AcOH/H2O 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% NEt3 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), 3.43 (dd, J = 9.5, 3.7 Hz, 1H), 3.39 (dd, 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, 138.2, 138.1, 136.7, 133.3, 132.9, 130.0, 129.8, 129.7, 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, 128.5, 128.3, 128.1, 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.

$^{1}$H NMR (600 MHz, CDCl$_3$) of isomer 5α:
$^{13}$C NMR (176 MHz, CDCl$_3$) of isomer $5\alpha$: 

![Carbon NMR spectrum of isomer $5\alpha$.](image)

HSQC (CDCl$_3$) of isomer $5\alpha$: 

![HSQC spectrum of isomer $5\alpha$.](image)
$^1$H NMR (600 MHz, CDCl$_3$) of isomer 5β:

$^{13}$C NMR (176 MHz, CDCl$_3$) of isomer 5β:
HSQC (CDCl₃) 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 H₂O (45 min, flow rate 1 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H₂O (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 H₂ for 30 min and stirred under an H₂-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% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

1H NMR (700 MHz, D₂O): 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. 13C NMR (175 MHz, D₂O): 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, 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$_2$O) of 11:

$^{13}$C NMR (176 MHz, D$_2$O) of 11:
S25

HMQC (D$_2$O) 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-glucopyranoside (S7)

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)
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 57 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/H₂O (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 H₂ for 30 min and stirred under an H₂-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% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O): $\delta$ = 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$_2$O): 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$_2$O) of 12:

$^{13}$C NMR (151 MHz, D$_2$O) of 12:
HSQC (D₂O) 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% NEt₃ 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 N₂H₄·AcOH in pyridine/AcOH/H₂O 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% NEt₃ 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.

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/H2O (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 H2 for 30 min and stirred under an H2-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% H2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O): $\delta = 4.97$ (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$_2$O): 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$_{10}$: 722.3078; found 722.3108

$^1$H NMR (600 MHz, D$_2$O) of 13:

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

Benzylxycarbonylaminopentyl 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)

Module A: 2 x 3.7 equiv. 1 or 10, TMSOTf
DCM
Module B: 20% Et$_3$N in DMF
Module C: 150 mM N$_2$H$_4$·OAc in pyridine/AcOH/H$_2$O 4:1:0.25
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% NEt₃ 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 N₂H₄·AcOH in pyridine/AcOH/H₂O 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 N₂H₄·AcOH in pyridine/AcOH/H₂O 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% NEt₃ 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/H₂O (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 H₂ for 30 min and stirred under an H₂-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% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

¹H NMR (600 MHz, D₂O): 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. ¹³C NMR (151 MHz, D₂O): 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, 70.6, 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 C₃₉H₇₀NO₂₉: 1016.4029; found 1016.4116.

¹H NMR (600 MHz, D₂O) of 14:
$^{13}$C NMR (151 MHz, D$_2$O) of 14:

HSQC (D$_2$O) of 14:
Benzylxycarbonylaminopentyl 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 ºC to -15 ºC)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module A (2 x 3.7 equiv. 10, TMSOTf, DCM, 2 x 40 min, -35 ºC to -15 ºC)
Module C (150 mM N₂H₄·AcOH in pyridine/AcOH/H₂O 4:1:0.25, 3 x 30 min, rt)
Module A (3 x 3.7 equiv. 10, TMSOTf, DCM, 3 x 40 min, -35 ºC to -15 ºC)
Module C (150 mM N₂H₄·AcOH in pyridine/AcOH/H₂O 4:1:0.25, 3 x 30 min, rt)
Module A (3 x 3.7 equiv. 10, TMSOTf, DCM, 3 x 40 min, -35 ºC to -15 ºC)
Module C (150 mM N₂H₄·AcOH in pyridine/AcOH/H₂O 4:1:0.25, 3 x 30 min, rt)
Module A (2 x 3.7 equiv. 1, TMSOTf, DCM, 2 x 35 min, -30 ºC to -15 ºC)
Module B (20% NEt₃ 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$_2$O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was saturated with H$_2$ 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$_2$O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

$^1$H NMR (700 MHz, D$_2$O): $\delta$ = 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$_2$O): 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$_2$O) of 15:

$^{13}$C NMR (176 MHz, D$_2$O) of 15:
HMOC (D$_2$O) of 15:

Benzyloxycarbonylamino pentyl 2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3-O-benzyl-6-O-[2,3,4-O-tribenzyl-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3-O-benzyl-6-O-[2,3,4-O-tribenzyl-α-D-xylopyranosyl]-β-D-glucopyranosyl-(1\(\rightarrow\)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% NEt₃ 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 N₂H₄·AcOH in pyridine/AcOH/H₂O 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% NEt₃ 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 N₂H₄·AcOH in pyridine/AcOH/H₂O 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% NEt₃ 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/H₂O (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 H₂ for 30 min and stirred under an H₂-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% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

¹H NMR (700 MHz, D₂O): 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. ¹³C NMR (176 MHz, D₂O): 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, 71.1, 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 C₄₅H₉₀NO₄: 1178.4557; found 1178.4575.

¹H NMR (700 MHz, D₂O) of 16:
$^{13}$C NMR (176 MHz, D$_2$O) of 16:

HMOC (D$_2$O) of 16:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-3-O-benzyl-6-O-[2,3,4-O-tribenzyl-\(\alpha\)-D-xylopyranosyl]-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3-O-benzyl-6-O-[2,3,4-O-tribenzyl-\(\alpha\)-D-xylopyranosyl]-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3,6-O-dibenzyl-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)4)-2-O-benzoyl-3,6-O-dibenzyl-\(\beta\)-D-glucopyranoside (S11)

Linker functionalized resin 4 (53 mg, 16.9 \(\mu\)mol) 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, \(-30^\circ\)C to \(-15^\circ\)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, \(-30^\circ\)C to \(-15^\circ\)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, \(-35^\circ\)C to \(-15^\circ\)C)

**Module C** (150 mM \(\text{N}_2\text{H}_4\cdot\text{AcOH}\) in pyridine/\(\text{AcOH}/\text{H}_2\text{O}\) 4:1:0.25, 3 x 30 min, rt)

**Module A** (3 x 3.7 equiv 10, TMSOTf, DCM, 3 x 40 min, \(-35^\circ\)C to \(-15^\circ\)C)

**Module C** (150 mM \(\text{N}_2\text{H}_4\cdot\text{AcOH}\) in pyridine/\(\text{AcOH}/\text{H}_2\text{O}\) 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.
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/H₂O (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 H₂ for 30 min and stirred under an H₂-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% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

¹H NMR (700 MHz, D₂O): 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. ¹³C NMR (176 MHz, D₂O): 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, 71.4, 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.

^1H NMR (600 MHz, D_{2}O) of 17:

13C NMR (151 MHz, D_{2}O) of 17:
HMOC (D<sub>2</sub>O) of 17:

References