Electronic Supplementary Information

Automated Glycan Assembly of Oligosaccharides Related to Arabinogalactan Proteins

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Abbreviations

AcOH: acetic acid; Ar: aryl; BB: building block; Bu: butane; DCE: 1,2-dichloroethane; DCM: dichloromethane; DIC: N,N'-diisopropylcarbodiimide; DMF: dimethylformamide; DMAP: dimethylaminopyridine; EE: ethyl acetate; ELSD: evaporative light scattering detector; Fmoc: fluorenylmethoxycarbonyl; hex: hexane; Lev: levinoyl; NaOMe: sodium methoxide; NIS: N-iodosuccinimide; NP: normal phase; MeCN: acetonitrile; MeOH: methanol; Ph: phenyl; Py: pyridine; RP: reversed phase; rt: room temperature; TBS: tert-butyldimethylsilyl; TFOH: trifluoromethanesulfonic acid; THF: tetrahydrofuran; TMSOTf: trimethylsilyl trifluoromethanesulfonate; UV: ultra violet.

General Information

The automated syntheses were performed on a self-built synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Linker-functionalized resin L was synthesized according to literature procedures 1. Resin loading was determined by performing one glycosylation (Module A) with BB-1 followed by DBU promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance. 2 Advanced intermediates p-tolyl 4,6-O-benzylidene-1-thio-β-D-galactopyranosides (BB1-d), p-tolyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (BB1-d), and ethyl 2-O-benzoyl-3,4-O-dibenzyl-6-O-fluorenylcarbonylmethoxy-1-thio-β-D-galacto-
pyranoside (BB3-a)\textsuperscript{4} and building block ethyl 2,3,5-tri-O-benzoyl-1-thio-\(\alpha\)-L-arabinofuranoside (BB4)\textsuperscript{5} were synthesized according to literature procedures. Phosphate BBs (BB-1 to BB-3) were used in the automated synthesis as mixtures of \(\alpha/\beta\)-anomers. 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\(_2\)O. NMR chemical shifts (\(\delta\)) are reported in ppm and coupling constants (\(J\)) in Hz. Spectra recorded in CDCl\(_3\) or D\(_2\)O used the solvent residual peak chemical shift as internal standard (CDCl\(_3\): 7.26 ppm \(^1\text{H}\), 77.1 ppm \(^{13}\text{C}\)). Spectra recorded in D\(_2\)O used an acetic acid peak as internal standard in \(^1\text{H}\) NMR (D\(_2\)O: 2.08 ppm) and \(^{13}\text{C}\) NMR (acetic acid in D\(_2\)O: 21.03 ppm). 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). Analytical HPLC was performed on an Agilent 1200 series coupled to a quadrupole ESI LC/MS 6130 using a YMC-Pack DIOL-300-NP column (150 x 4.6 mm), a Phenomenex Luna C5 column (250 x 4.6 mm), or a Thermo Scientific Hypercarb column (150 x 4.6 mm). Preparative HPLC was performed on an Agilent 1200 series using a preparative YMC-Pack-Diol-300-NP (150 x 20 mm) or a semi-preparative Phenomenex Luna C5 column (250 x 10 mm).

**Synthesizer Modules and Conditions**

After placement of the linker-functionalized resin L (16.9 \(\mu\)mol of hydroxyl groups) in the reaction vessel the resin was washed with DMF, THF, and DCM. Subsequently the glycosylation (Module A and D), deprotection (Module B and C) and capping (Module E) steps were performed. Mixing of the components was accomplished by bubbling Argon through the reaction mixture.

**Module A: Glycosylation with Glycosyl Phosphates**

The resin (16.9 \(\mu\)mol 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 phosphate BB (3.75 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 (3.75 equiv. in 1 mL DCM). The glycosylation was performed for 5 min at -35 °C and for 30 min at -20 °C. Subsequently the solution was drained and the resin was washed twice with DCM. The whole procedure was repeated once to ensure full conversion of all acceptor sites.

**Activator solution: 63.5 mM solution of TMSOTf in dry DCM.**

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<thead>
<tr>
<th>Action</th>
<th>Cycles</th>
<th>Solvent</th>
<th>Reagent 1</th>
<th>Reagent 2</th>
<th>Temperature</th>
<th>Incubation Time</th>
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<td>5 min</td>
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<td></td>
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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% Et$_3$N in DMF was delivered to the reaction vessel. After 5 min the solution was drained and the whole procedure was repeated another two times. After Fmoc deprotection was complete the resin was washed with DMF, THF and DCM.

**Deprotection solution: 20% NEt$_3$ in dry DMF.**

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Module C: Lev Deprotection

The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 1.3 mL DCM remained in the reaction vessel and 0.8 mL of a solution of 0.15 M hydrazine acetate in Py/AcOH/H$_2$O (4:1:0.25) was delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After Lev deprotection was complete the resin was washed with DMF, THF and DCM.

**Deprotection solution: 0.15 M hydrazine acetate solution in Py/AcOH/H$_2$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 (3.75 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 (4.4 equiv.) and TfOH (0.4 equiv.) in DCM/dioxane (3:1). The glycosylation was performed for 5 min at -40 °C and for 40 min at -20 °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 anhydrous DCM.

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<td>DCM</td>
<td>BB (63.5 µmol)</td>
<td>NIS (75 µmol), TfOH (7.5 µmol)</td>
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<td>NIS (75 µmol), TfOH (7.5 µmol)</td>
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Module E: Benzoylation

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

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

<table>
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<td>B$_2$O (0.5 mmol)</td>
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<td>DMAP (0.25 mmol)</td>
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<td>3</td>
<td>DCM</td>
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<td>25 °C</td>
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</table>
Cleavage from the solid support

After assembly of the oligosaccharides cleavage from the solid support was accomplished by modification of a previously published protocol, using the Vapourtec E-Series UV-150 photoreactor Flow Chemistry System. The medium pressure metal halide lamp is filtered using the commercially available red filter. The resin, suspended in DCM, was loaded into a plastic syringe. The suspension was then pumped using a syringe pump (PHD2000, Harvard Apparatus) at 1 mL/min through a 10 mL reactor, constructed of 1/8 inch o.d. FEP tubing. The total volume within the photoreactor was 9 mL. The temperature of the photoreactor was maintained at 20 °C and the lamp power was 80%. The exiting flow was deposited in a 10 mL syringe containing a filter, with a collection flask beneath the syringe.

Synthesis of Building Blocks

Building Block 1 (BB1)

\[
\begin{align*}
\text{BB1-a} & \quad \xrightarrow{\text{HSTol, BF$_3$OEt$_2$, DCM, rt, 18 h}} \quad \text{BB1-b} \\
\text{BB1-b} & \quad \xrightarrow{\text{NaOMe, MeOH, rt, 1 h}} \quad \text{BB1-c} \\
\text{BB1-b} & \quad \xrightarrow{\text{TBSO, Bz$_2$O, DMAP, NET$_3$, 0°C to rt, 18 h}} \quad \text{BB1-e} \\
\text{BB1-e} & \quad \xrightarrow{\text{TBSO, Imidazole, 0°C to rt, 18 h}} \quad \text{BB1-d} \\
\text{BB1-g} & \quad \xrightarrow{\text{BH$_3$·TMSOTf, DCM, 0°C, 1.5 h}} \quad \text{BB1-h} \\
\text{BB1-h} & \quad \xrightarrow{\text{BnBr, NaH, DMF/DCM (1:1), 0°C to rt, 4 h}} \quad \text{BB1-i} \\
\text{BB1-i} & \quad \xrightarrow{\text{BF$_3$OEt$_2$, ACN, 0°C, 5 min}} \quad \text{BB1-j} \\
\text{BB1-j} & \quad \xrightarrow{\text{HOP(O)(OBu)$_2$, NIS, TIOH, DCM, 0°C, 1 h}} \quad \text{BB1-k} \\
\end{align*}
\]

\text{p-Tolyl 3-O-tert-butylmethylsilyl-4,6-O-benzylidene-1-thio-\(\beta\)-D-galactopyranoside (BB1-e)}

In 200 mL anhydrous DCM 15.5 g (41.5 mmol) of p-tolyl 4,6-O-benzylidene-1-thio-\(\beta\)-D-galactopyranoside (BB1-d) were dissolved. Upon addition of 9.5 g (63.0 mmol) tert-butyl-chloro(dimethyl)silane and 4.8 g (70.5 mmol) imidazole at 0°C under Ar atmosphere a colorless precipitate
was formed immediately. The temperature was gradually allowed to reach rt and the reaction mixture was stirred at rt overnight. The solution was diluted with DCM and the organic layer was washed with sat. aq. NaHCO₃-solution twice. The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was subjected to silica gel column chromatography (EE/hex = 1:1) to give the silylether BB₁-e (16.3 g, 33.3 mmol, 80% yield) as a colorless solid.

[α]D₂₅ = -11.9 (c 1.8, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ = 7.57 – 7.54 (m, 2H, Ar), 7.46 – 7.41 (m, 2H, Ar), 7.37 – 7.33 (m, 3H, Ar), 7.03 (d, J = 7.9, 2H, Ar), 5.48 (s, 1H, CHPh), 4.49 (d, J = 9.0, 1H, H-1), 4.38 (dd, J = 12.3, 1.5, 1H, H-6), 4.05 – 3.99 (m, 2H, H-5, H-6), 3.77 (td, J = 9.0, 1.9, 1H, H-2), 3.72 (dd, J = 9.1, 3.2, 1H, H-3) 3.50 (d, J = 1.0, 1H, H-4), 2.31 (s, 3H, SPhCH₃), 0.87 (s, 9H, TBS ), 0.09 (s, 3H, TBS), 0.08 (s, 3H, TBS) ppm. ¹³C NMR (¹H) (101 MHz, CDCl₃) δ = 138.1, 138.1, 133.7, 129.7, 128.9, 128.1, 126.4 (12 C, Ar), 101.0 (CHPh), 87.6 (C-1), 76.7 (C-5), 75.6 (C-3), 70.2 (C-4), 69.5 (C-6), 68.1 (C-2), 25.8 (TBS), 21.3 (SPhCH₃), 18.3 (TBS), -4.1 (TBS), -4.5 (TBS) ppm. ESI-HRMS: m/z [M+Na]⁺ calcd. for C₂₆H₃₆NaO₅Si: 511.1950; found 511.1979. IR (neat). νmax = 1240, 1131, 1095, 1040 cm⁻¹

¹H NMR (400 MHz, CDCl₃) of BB₁-e:

¹³C NMR (101 MHz, CDCl₃) of BB₁-e:
To 11.8 g (24.2 mmol) of BB1-e in 150 mL anhydrous DCM, 1.48 g (12.0 mmol) of DMAP and 16.4 g (72.4 mmol) of benzoic anhydride were added under Ar atmosphere. The solution was cooled to 0 °C and 20 mL (145 mmol) of NEt3 were slowly added. After stirring overnight at rt the reaction mixture was diluted with DCM followed by washes with sat. aq. NaHCO3-solution and brine. The organic phase was subsequently dried over Na2SO4 and concentrated in vacuo. Recrystallization from ethanol gave the benzoyl protected galactose BB1-f as colorless crystals in 73% yield (10.5 g, 17.7 mmol).

1H NMR (400 MHz, CDCl3) δ = 8.06 – 8.02 (m, 2H, Ar), 7.57 (J = 7.4, 1H, Ar), 7.47-7.43 (m, 6H, Ar), 7.39 – 7.34 (m, 3H, Ar), 7.01 (d, J = 7.9, 2H, Ar), 5.50 (s, 1H, CHPh), 5.44 (t, J = 9.5, 1H, H-2), 4.76 (d, J = 9.9, 1H, H-1), 4.42 (dd, J = 12.2, 1.2, 1H, H-4), 4.10 (d, J = 3.0, 1H, H-6), 4.05 (dd, J = 12.3, 1.4, 1H, H-6), 4.01 (dd, J = 9.3, 3.0, 1H, H-3), 3.56 (s, 1H, H-5), 2.30 (s, 3H, SPhC6H3), 0.71 (s, 9H, TBS), 0.00 (s, 3H, TBS), -0.16 (s, 3H, TBS) ppm.

The analytical data is in agreement with literature data.6

A 1 M solution of borane in THF (70.7 mL, 70.7 mmol) and TMSOTf (1.97 g, 8.84 mmol) were sequentially added to a solution of BB1-f (10.5 g, 17.7 mmol) in anhydrous dichloromethane (50 mL) at 0 °C under Ar atmosphere. After stirring for 1.5 h the reaction was quenched by portion-wise addition of Et3N/MeOH (1:10) solution until no further gas evolved. The resulting mixture was concentrated under reduced pressure and the remains were taken up in DCM and washed with sat. aq. NaHCO3-solution and brine. After drying the organic phase over Na2SO4 and removal of the solvent in vacuo, 10.1 g (17.0 mmol, 96%) of BB1-g were obtained as colorless foam.

1H NMR (400 MHz, CDCl3) δ = 8.05 (d, J = 7.2, 2H, Ar), 7.58 (t, J = 7.4, 1H, Ar), 7.46 (t, J = 7.7, 2H, Ar), 7.40 – 7.29 (m, 7H, Ar), 7.02 (d, J = 8.1, 2H, Ar), 5.63 (t, J = 9.2, 1H, H-2), 5.08 (d, J = 11.6, 1H, CH2Ph), 4.72 (d, J = 9.7, 1H, H-1), 4.58 (d, J = 11.6, 1H, CH2Ph), 3.96 (d, J = 8.3, 1H, H-3), 3.92 – 3.85 (m, 1H, H-3), 3.76 (d, J = 2.4, 1H, H-6), 3.61 (ddd, J = 16.8, 14.2, 8.2, 2H, H-6, H-5), 2.29 (s, 3H, SPhCH3), 0.78 (s, 9H, TBS), 0.10 (s, 3H, TBS), -0.10 (s, 3H, TBS) ppm.

The analytical data is in agreement with literature data.6
**p-Tolyl 2-O-benzoyl-3-O-tert-butyldimethylsilyl-4,6-O-dibenzyl-1-thio-β-D-galactopyranoside (BB1-h)**

BB1-g (6 g, 10.1 mmol) and 1.0 g (25.2 mmol) of NaH (60% dispersion in mineral oil) were dissolved in 50 mL anhydrous THF under Ar atmosphere, resulting in a gray suspension, and 3.6 mL (30.3 mmol) of benzylbromide were added drop-wise at 0 °C. The suspension was stirred at rt for 4h. The reaction was stopped by the addition of aq. NH₄Cl-solution. When no more gas evolved, the organic layer was separated and washed with water. The solution was dried over Na₂SO₄ and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (0:1 to 1:9 EE/hex) to afford BB1-h (5.08 g, 7.42 mmol) in 73% yield.

1H NMR (400 MHz, CDCl₃) δ = 8.10 – 7.99 (m, 2H, Ar), 7.57 (t, J = 7.4, 1H, Ar), 7.45 (t, J = 7.7, 2H, Ar), 7.40 – 7.26 (m, 12H, Ar), 6.98 (d, J = 8.1, 2H, Ar), 5.61 (t, J = 9.0, 1H, H-2), 5.08 (d, J = 11.4, 1H, CH₂Ph), 4.71 (d, J = 9.4, 1H, H-1), 4.56 – 4.41 (m, 3H, CH₂Ph), 3.96 (d, J = 8.4, 1H, H-3), 3.83 (d, J = 2.4, 1H, H-4), 3.77 – 3.61 (m, 3H, H-6, H-5), 2.28 (s, 3H, SPhCH₃), 0.76 (s, 9H, TBS), 0.09 (s, 3H, TBS), -0.11 (s, 3H, TBS) ppm.

The analytical data is in agreement with literature data.⁶

**p-Tolyl 2-O-benzoyl-4,6-O-dibenzyl-1-thio-β-D-galactopyranoside (BB1-i)**

BB1-h (2.52 g, 3.68 mmol) was dissolved in 20 mL anhydrous MeCN under Ar atmosphere at 0 °C. When 0.5 mL (4.05 mmol) boron trifluoride etherate were added the solution turned yellow immediately. The reaction was stopped after 5 min by neutralization with sat., aq. NaHCO₃-solution. During the neutralization process a colorless precipitate formed, which was taken up in DCM. The organic phase was subsequently washed with brine, dried over Na₂SO₄ and the solvent was removed in vacuo, yielding 1.76 g (3.09 mmol, 84%) of BB1-i as a colorless oil that was used without further purification.

1H NMR (400 MHz, CDCl₃) δ = 8.06 (d, J = 7.8 Hz, 2H, Ar), 7.58 (t, J = 7.3 Hz, 2H, Ar), 7.46 (t, J = 7.6 Hz, 2H, Ar), 7.39 – 7.26 (m, 11H, Ar), 7.03 (d, J = 7.8 Hz, 2H, Ar), 5.22 (t, J = 9.7 Hz, 1H, H-2), 4.74 (d, J = 9.6 Hz, 3H, H-1, CH₂Ph), 4.52 (q, J = 11.6 Hz, 2H, CH₂Ph), 3.98 (d, J = 2.6 Hz, 1H, H-4), 3.84 – 3.71 (m, 4H, H-3, H-5, H-6), 2.49 (d, J = 9.5 Hz, 1H, OH), 2.31 (s, 3H, SPhCH₃) ppm.

The analytical data is in agreement with literature data.³
**p-Tolyl 2-O-benzoyl-3-O-fluorenylcarbonylmethoxy-4,6-O-dibenzyl-1-thio-β-D-galacto-pyranoside (BB1-j)**

Pyridine (1.2 mL, 15.0 mmol) was added to a solution of 2.24 g (3.93 mmol) of BB1-i in 50 mL anhydrous DCM. The mixture was stirred for 15 min and 2.59 g (10.0 mmol) FmocCl were added. After stirring for additional 6 h at rt the solvent was removed *in vacuo* and the residue was co-evaporated with toluene twice. The remaining oil was taken up in DCM, washed with brine twice, dried over Na₂SO₄ and the solvent was removed *in vacuo*. The remaining oil was taken up and crystallization from EtOH yielded BB1-j as colorless crystals in 92% yield (2.86 g, 3.61 mmol).

[α]D²⁵ = +31.8 (c 0.16, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ = 8.05 (d, J = 7.4 Hz, 2H, Ar), 7.67 (dd, J = 7.0, 5.5 Hz, 2H, Ar), 7.54 (t, J = 7.4 Hz, 1H, Ar), 7.47 – 7.28 (m, 18H, Ar), 7.11 (dt, J = 19.3, 7.5 Hz, 3H, Ar), 7.02 (d, J = 7.9 Hz, 2H, Ar), 5.71 (t, J = 10.0 Hz, 1H, H-2), 5.06 (dd, J = 9.9, 2.8 Hz, 1H, H-3), 4.83 – 4.75 (m, 2H, H-1, PhCH₂), 4.52-4.43 (m, 3H, PhCH₂), 4.29 (dd, J = 10.3, 7.2 Hz, 1H, Fmoc), 4.20 (dd, J = 10.2, 8.0 Hz, 1H, Fmoc), 4.12 (d, J = 2.1 Hz, 1H, H-4), 4.05 (t, J = 7.4 Hz, 1H, Fmoc), 3.81 (t, J = 6.2 Hz, 1H, H-5), 3.74 – 3.63 (m, 2H, H-6), 2.30 (s, 3H, SPhCH₃) ppm. ¹³C NMR (¹H) (101 MHz, CDCl₃) δ = 165.2, 154.6 (2C, C=O), 143.3, 142.9, 141.3, 141.2, 138.0, 137.9, 137.9, 133.3, 133.0, 130.0, 129.7, 129.3, 128.5, 128.4, 128.2, 127.9, 127.9, 127.8, 127.2, 127.2, 125.3, 125.0, 120.0 (36C, Ar), 87.2 (C-1), 79.2 (C-3), 75.1 (PhCH₂), 74.0 (C-4), 73.7 (PhCH₃), 70.2 (Fmoc), 68.8 (C-2), 68.4 (C-6), 46.5 Fmoc, 21.31 (SPhCH₃) ppm. ESI-HRMS: m/z [M+Na]+ calcd. for C₅₉H₄₃NaO₈S: 815.2654; found 815.2610. IR (neat) ν_max = 1722, 1276, 1255, 1110 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) of BB1-j:
To a suspension of 5 g dry molecular sieves in 50 mL anhydrous DCM dibutyl phosphate (5 mL, 25.2 mmol) was added and the mixture was stirred for 1.5 h. Subsequently the molecular sieves were allowed to settle for 30 min and the supernatant was added to a solution of BB1-j (10.0 g, 12.6 mmol) in 100 mL anhydrous DCM, cooled to 0 °C under Ar atmosphere. NIS (3.69 g, 16.4 mmol) and TfOH (0.03 mL, 0.34 mmol) were added and the resulting purple reaction mixture was stirred for 1 h. The reaction was quenched by addition of sat. aq. NaHCO₃-solution and washed with sat. aq. Na₂S₂O₃-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na₂SO₄ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography (EE/hex = 1:8 to 1:1) to give the phosphate BB1 (9.19 g, 10.2 mmol, 81% yield) as a mixture of α/β anomers (α/β~ 4:3) as a highly viscous and sticky oil.

Analytical data for β-anomer:

\[ [\alpha]_{D}^{25} = +22.8 (c 0.23, CHCl₃) \]

H NMR (400 MHz, CDCl₃) δ = 8.06 (d, J = 7.8, 2H, Ar), 7.66 (t, J = 7.2, 2H, Ar), 7.54 (t, J = 7.4, 1H, Ar), 7.49 – 7.39 (m, 4H, Ar), 7.38 – 7.27 (m, 12H, Ar), 7.15 – 7.04 (m, 2H, Ar), 5.78 (dd, J = 10.4, 8.1, 1H, H-2), 5.41 (t, J = 7.7, 1H, H-1), 5.01 (dd, J = 10.5, 2.9, 1H, H-3), 4.74 (d, J = 11.3, 1H, PhCH₂), 4.54 – 4.43 (m, 3H, PhCH₂), 4.29 (ddd, J = 28.4, 10.5, 7.6 Hz, 2H, Fmoc), 4.13 (d, J = 1.6, 1H, H-4), 4.08 (t, J = 7.2, 1H, Fmoc), 4.05 – 3.95 (m, 2H, OBu), 3.92 (t, J = 6.6, 1H, H-5), 3.78 – 3.61 (m, 4H, H-6, OBu), 1.61 – 1.54 (m, 2H, OBu), 1.38 – 1.23 (m, 4H, OBu), 1.07 – 0.96 (m, 2H, OBu), 0.87 (t, J = 7.4, 3H, CH₃), 0.67 (t, J = 7.4, 3H, CH₃) ppm. ¹³C NMR (1H) (101 MHz, CDCl₃) δ = 165.1, 154.5 (2C, C=O), 143.3, 142.9, 141.3, 141.2, 137.8, 137.7, 133.5, 130.0, 129.3, 128.6, 128.4, 128.4, 128.0, 127.9, 127.2, 127.2, 125.2, 125.0, 120.1 (23C, Ar), 96.9 (d, J = 4.9 Hz, 1C C-1), 77.3 (C-3), 75.4 (PhCH₂), 73.9 (C-5), 73.6 (PhCH₂), 73.5 (C-4), 70.2 (Fmoc), 70.0 (C-2), 68.1 (d, J = 6.3 Hz, 1C OBu), 67.9 (d, J = 6.4 Hz, 1C, OBu), 67.5 (C-6), 46.6 (Fmoc), 32.1 (d, J = 7.5 Hz, 1C, OBu), 31.89 (d, J = 7.3 Hz, OBu), 18.6, 18.3 (2C, OBu), 13.70, 13.51 (2C, OBu(CH₃)) ppm. ESI-HRMS: m/z [M+Na]⁺ calcd. for C₅₆H₅₁NaO₁₁₄P: 901.3328; found 901.3339. IR (neat) ν max = 1735, 1270, 1095, 1027 cm⁻¹.
$^1$H NMR (400 MHz, CDCl$_3$) of BB1:

$^{13}$C NMR (101 MHz, CDCl$_3$) of BB1:
**Building Block 2 (BB2)**

A solution of 6.07 g (10.2 mmol) **BB1-g** in 100 mL DCM at 0 °C was treated with DMAP (0.75 g 6.13 mmol), DIC (1.93 g, 15.3 mmol) and levulinic acid (1.7 mL, 16.4 mmol) was added. The solution was stirred for 3 h at rt. A white precipitate slowly formed and the solution became pink. After complete conversion, the solvent was removed in vacuo and the product was purified by silica gel column chromatography (hex/EE = 3:2) to give BB2-a as a colorless foam in 91% yield (6.42 g, 9.27 mmol)

\[ \alpha \] D 25 = +23.1 (c 1.4, CHCl3).

1H NMR (400 MHz, CDCl3) δ = 8.06 – 8.02 (m, 2H, Ar), 7.58 (t, J = 7.4 Hz, 1H, Ar), 7.45 (t, J = 7.7 Hz, 2H, Ar), 7.38 – 7.26 (m, 7H, Ar), 7.01 (d, J = 7.9 Hz, 2H, Ar), 5.64 – 5.59 (m, 1H, H-2), 5.10 (d, J = 11.4 Hz, 1H, PhCH3), 4.71 (d, J = 9.6 Hz, 1H, H-1), 4.56 (d, J = 11.4 Hz, 1H, PhCH3), 4.32 (dd, J = 11.2, 6.9 Hz, 1H, H-6), 4.21 (dd, J = 11.2, 5.5 Hz, 1H, H-6), 3.97 (d, J = 8.8 Hz, 1H, H-3), 3.81 – 3.73 (m, 2H, H-4, H-5 ), 2.74 (t, J = 6.5 Hz, 2H, Lev(CH2)), 2.53 (t, J = 6.6 Hz, 2H, Lev(CH2)), 2.29 (s, 3H, SPhC3H3), 2.19 (s, 3H, LevCH3), 0.77 (s, 9H, TBS), 0.10 (d, J = 3.5 Hz, 3H, TBS), -0.10 (s, 3H, TBS) ppm. 13C NMR (101 MHz, CDCl3) \{1H\} δ = 206.6, 172.6, 165.3 (3 C, C=O), 138.5, 137.7, 133.1, 132.6, 130.4, 129.9, 129.5, 128.4, 128.3, 127.9, 127.6 (18 C, Ar), 87.2 (C-1), 76.9, 76.1, 75.7 (C-3), 75.0 (PhCH3), 71.0 (C-2), 63.6 (C-6), 38.0 (Lev), 29.9 (Lev), 27.9 (Lev), 25.6 (TBS), 21.2 (SPhCH3), 17.9 (TBS), -3.92 (TBS), -4.98 (TBS). ESI-HRMS: m/z [M+Na]+ calcd. for C38H48NaO8SSi: 715.2736, found: 715.2652

IR (neat) ν max = 1719, 1260, 1088, 1069 cm⁻¹.
$^1$H NMR (400 MHz, CDCl$_3$) of BB2-a:

$^{13}$C NMR (101 MHz, CDCl$_3$) of BB2-a:

$p$-Tolyl 2-O-benzoyl-4-O-dibenzyl-6-O-levulinoyl-1-thio-β-D-galactopyranoside (BB2-b)

BB2-a (6.37 g, 9.27 mmol) was dissolved in 80 mL MeCN, the solution was cooled to 0 °C and 1.3 mL (10.1 mmol) of boron trifluoride etherate were added. The solution was stirred at 0 °C for ten min and subsequently quenched by the addition of sat. aq. NaHCO$_3$-solution. The product was extracted from the aq. solution with DCM and the organic layer was washed with water twice. The organic layer was dried over MgSO$_4$. The product BB2-b was obtained in quantitative yields (5.32 g, 9.27 mmol) as a transparent oil after removal of the solvent in vacuo.

$[\alpha]_D^{25} = +4.7$ (c 1.1, CHCl$_3$). $^1$H NMR (400 MHz, CDCl$_3$) δ = 8.05 (d, J = 8.1 Hz, 2H, Ar), 7.57 (dd, J = 10.6, 4.2 Hz, 1H, Ar), 7.45 (t, J = 7.7 Hz, 2H, Ar), 7.37 – 7.28 (m, 7H, Ar), 7.04 (d, J = 7.9 Hz, 2H, Ar), 5.23 (t, J = 9.7 Hz, 1H, H-2), 4.82 (d, J = 11.6 Hz, 1H, PhCH$_2$), 4.76 – 4.66 (m, 2H, PhCH$_2$, H-1), 4.35 (dd, J = 11.1, 6.9 Hz, 1H, H-6), 4.17 (dd, J = 11.2, 6.0 Hz, 1H, H-6), 3.88 (d, J = 2.3 Hz, 1H, H-3), 3.83 (dd, J = 9.5, 3.2 Hz, 1H, H-4), 3.75 (t, J = 6.4 Hz, 1H, H-5), 2.74 (t, J = 6.5 Hz, 2H, Lev(CH$_3$)), 2.54 (t, J = 6.4 Hz, 2H, Lev(CH$_3$)), 2.30 (s, 3H, SPhCH$_3$), 2.17 (s, 3H, LevCH$_3$) ppm. $^{13}$C NMR$^1$(H) (101 MHz, CDCl$_3$) δ = 206.9,
172.5, 166.8 (3 C, C=O), 138.1, 137.8, 133.4, 133.1, 130.0, 129.6, 128.8, 128.5, 128.5, 127.9, 127.9 (18 C, Ar), 86.3 (C-1), 76.5 (C-3), 76.1 (C-5), 75.5 (PhCH$_3$), 74.4(C-4), 72.1 (C-2), 63.0 (C-6), 37.9 (Lev), 29.9 (Lev), 27.8(Lev), 21.2 (SPhCH$_3$) ppm. [M+Na]$^+$ calcd. for C$_{32}$H$_{34}$NaO$_8$S: 601.1872; found 601.1911. IR (neat) $\nu_{max}$ =1723, 1713, 1271, 1250 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) of BB2-b:

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.08 – 8.03 (m, 2H, Ar), 7.69 (dd, $J$ = 7.5, 4.2 Hz, 2H, Ar), 7.56 (t, $J$ = 7.4 Hz, 1H, Ar), 7.49 – 7.29 (m, 13H, Ar), 7.20 – 7.03 (m, 4H, Ar), 5.72 (t, $J$ = 4.2 Hz, 2H, Ar).

$^{13}$C NMR (101 MHz, CDCl$_3$) of BB2-b:

$p$-Tolyl 2-O-benzoyl-3-O-fluorenycarbonylmethoxy-4-O-dibenzyl-6-O-levulinoyl-1-thio-$\beta$-D-galactopyranoside (BB2-c)

To a solution of 5.32 g (9.27 mmol) BB2-b in 100 mL DCM and 2.5 mL (30.9 mmol) pyridine 4.83 g (18.6 mmol) Fmoc-Cl were added and the solution was stirred at rt for 6 h. Subsequently the mixture was evaporated in vacuo and the resulting oil re-dissolved in 50 mL DCM. The organic layer was washed with brine three times, dried over Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. Recrystallization from ethanol gave the pure product BB2-c in 75% yield (5.60 g, 6.99 mmol) as a colorless solid.

$[\alpha]_D^{25}$ = +35.7 (c 1.1, CHCl$_3$).$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.08 – 8.03 (m, 2H, Ar), 7.69 (dd, $J$ = 7.5, 4.2 Hz, 2H, Ar), 7.56 (t, $J$ = 7.4 Hz, 1H, Ar), 7.49 – 7.29 (m, 13H, Ar), 7.20 – 7.03 (m, 4H, Ar), 5.72 (t, $J$ = 4.2 Hz, 2H, Ar).
10.0 Hz, 1H, H-2), 5.07 (dd, J = 10.0 Hz, 2H, H-3), 4.83 (d, J = 11.5 Hz, 1H, PhCH$_2$), 4.80 (d, J = 10.0 Hz, 1H, H-1), 4.53 (d, J = 11.4 Hz, 1H, PhCH$_2$), 4.37 – 4.29 (m, 2H, H-6, Fmoc), 4.27 – 4.14 (m, 2H, H-6, Fmoc), 4.07 (t, J = 7.5 Hz, 1H, Fmoc), 4.04 (d, J = 2.4 Hz, 1H, H-4), 3.81 (t, J = 6.4 Hz, 1H, H-5), 2.74 (t, J = 6.1 Hz, 2H, Lev(CH$_2$)), 2.53 (t, J = 6.5 Hz, 2H, Lev(CH$_2$)), 2.31 (s, 3H, SPhCH$_3$), 2.18 (s, 3H, Lev(CH$_3$)).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ = 206.5, 172.4, 165.1, 154.5 (4 C, C=O), 143.3, 142.8, 141.3, 141.2, 138.2, 137.5, 133.4, 133.0, 130.6, 129.6, 129.6, 129.1, 129.1, 128.5, 128.5, 128.4, 128.0, 127.9, 127.2, 127.2, 125.2, 125.0, 120.1 (26 C, Ar), 87.1 (C-1), 79.1 (C-3), 75.9 (C-5), 75.0 (PhCH$_3$), 73.5 (C-4), 70.3 (Fmoc), 68.6 (C-2), 62.7 (C-6), 46.5 (Fmoc), 37.9 (Lev), 29.9 (Lev), 27.8 (Lev), 21.3 (SPhCH$_3$) ppm. [M+Na]$^+$ calcld. for C$_{47}$H$_{44}$NaO$_{10}$S: 823.2552, found: 823.2558 IR (neat) $\nu_{\text{max}}$ = 1742, 1723, 1273, 1249 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) of BB2-c:

$^{13}$C NMR (101 MHz, CDCl$_3$) of BB2-c:

Dibutoxyphosphoryloxy 2-O-benzoyl-3-O-fluorenlycarbonylmethoxy-4-O-benzyl-6-O-levulinoyl-$\alpha$/$\beta$-D-galactopyranoside (BB2)
To a suspension of 1 g dry molecular sieves in 20 mL anhydrous DCM was added dibutyl phosphate (1.0 mL, 5.01 mmol) and the mixture was stirred for 1.5 h. Subsequently the molecular sieves were allowed to settle for 30 min, the supernatant was added to a solution of BB2-c (1.85 g, 2.50 mmol) in 50 mL anhydrous DCM and cooled to 0 °C under Ar atmosphere. NiS (0.73 g, 3.26 mmol) and TfOH (0.07 mL, 0.79 mmol) were added and the purple reaction mixture was stirred for 1 h. The reaction was quenched by addition of sat. aq. NaHCO$_3$-solution and washed with sat. aq. Na$_2$S$_2$O$_3$-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na$_2$SO$_4$ and the solvent removed in vacuo. The product was purified by silica gel column chromatograph (EE/hex = 1:8 to 1:1) to give the phosphate BB2 (2.04 g, 2.30 mmol, 92% yield) as a mixture of α/β anomers (α/β ~ 1:2) as a highly viscous and sticky oil.

Analytical data for β-anomer:

[$\alpha$]$_D$$^{25}$ = +28.8 (c 0.5, CHCl$_3$) $^1$H NMR (400 MHz, CDCl$_3$) δ = 8.10 – 8.02 (m, 2H, Ar), 7.70 – 7.65 (m, 2H, Ar), 7.55 (t, $J$ = 7.4 Hz, 1H, Ar), 7.48 (d, $J$ = 7.5 Hz, 1H, Ar), 7.45 – 7.40 (m, 1H, Ar), 7.40 – 7.28 (m, 6H, Ar), 7.16 – 7.06 (m, 2H, Ar), 5.81 (dd, $J$ = 10.5, 8.0 Hz, 1H, H-2), 5.42 (t, $J$ = 7.7 Hz, 1H, H-1), 5.02 (dd, $J$ = 10.5, 2.9 Hz, 1H, H-3), 4.80 (d, $J$ = 11.2 Hz, 1H, PhCH$_2$), 4.52 (d, $J$ = 11.2 Hz, 1H, PhCH$_2$), 4.40 – 4.18 (m, 4H, H-6, Fmoc), 4.15 – 3.97 (m, 5H, H-4, Fmoc, OBu), 3.91 (t, $J$ = 6.4 Hz, 1H, H-5), 3.71 (ddq, $J$ = 27.5, 9.8, 6.7 Hz, 2H, OBu), 2.75 (t, $J$ = 6.5 Hz, 2H, Lev), 2.52 (t, $J$ = 6.5 Hz, 2H, Lev), 2.19 (s, 3H, CH$_3$(Lev)), 1.65 – 1.57 (m, 2H, OBu), 1.42 – 1.21 (m, 4H, OBu), 1.02 (dq, $J$ = 14.7, 7.4 Hz, 2H, OBu), 0.90 (t, $J$ = 7.4 Hz, 3H, CH$_3$(OBu)), 0.68 (t, $J$ = 7.4 Hz, 3H, CH$_3$(OBu)). $^{13}$C NMR ($^1$H) (101 MHz, CDCl$_3$) δ 206.4, 172.3, 165.1, 154.4 (4C, C=O), 143.2, 142.8, 141.3, 141.2, 137.2, 133.5, 130.0, 129.2, 128.6, 128.6, 128.2, 127.9, 127.2, 127.1, 125.2, 125.0, 120.1 (18 C, Ar), 96.8 (d, $J$ = 5.0 Hz), 77.4 (C-3), 75.4 (PhCH$_2$), 73.1 (C-4), 72.9 (C-5), 70.3 (Fmoc), 69.8 (C-2), 68.1 (d, $J$ = 6.3 Hz, 1C OBu), 68.0 (d, $J$ = 6.3 Hz, OBu), 62.2 (C-6), 46.5 (Fmoc), 37.9 (Lev), 32.1 (d, $J$ = 7.5 Hz, 1C, OBu), 31.8 (d, $J$ = 7.4 Hz, 1C, OBu) 29.9 (Lev), 27.8 (Lev), 18.6, 18.3, 13.6, 13.4 (4C, OBu) ppm. ESI-HRMS: m/z [M+Na]$^+$ calcd. for C$_{48}$H$_{55}$O$_{14}$NaP: 909.3227, found: 909.3265. IR (neat) $\nu_{\text{max}}$ = 1738, 1272, 1250, 1028 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$) of BB2:
Building Block 3 (BB3)

Dibutoxyphosphoryloxy 2-0-benzoyl-3,4-O-dibenzyl-6-O-fluorenlycarbonylmethoxy-α/β-D-galactopyranoside (BB3)

To a suspension of 1 g dry molecular sieves in 50 mL anhydrous DCM was added dibutyl phosphate (0.6 mL, 3.0 mmol) and the mixture was stirred for 1.5 h. Subsequently the molecular sieves were allowed to settle for 30 min, the supernatant was added to a solution of ethyl 2-0-benzoyl-3-O-fluorenlycarbonylmethoxy-4,6-O-dibenzyl-1-thio-β-D-galactopyranoside (0.98g, 1.38 mmol) in 100 mL anhydrous DCM and cooled to 0 °C under Ar atmosphere. NIS (0.41 g, 1.80 mmol) and TfOH (0.04 mL, 0.45 mmol) were added and the purple reaction mixture was stirred for 1 h. The reaction was quenched by addition of sat. aq. NaHCO₃-solution and washed with sat. aq. Na₂S₂O₃-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na₂SO₄ and the solvent removed in vacuo. The product was purified by silica gel column chromatography (EE/hex = 1:8 to 1:1) to give the phosphate BB3 (0.98 g, 1.11 mmol, 80% yield) as a mixture of α/β anomers (α/β~ 2:1) as a highly viscous and sticky oil.

Characterization for α-anomer:

$^1$H NMR (400 MHz, CDCl₃) δ = 8.05 (d, J = 7.5 Hz, 2H, Ar), 7.78 (d, J = 7.5 Hz, 2H, Ar), 7.63 – 7.55 (m, 3H, Ar), 7.43 (dt, J = 10.8, 7.6 Hz, 4H, Ar), 7.37 – 7.23 (m, 12H, Ar), 5.94 (dd, J = 6.7, 3.5 Hz, 1H, H-1), 5.66 (dt, J = 10.4, 3.2 Hz, 1H, H-2), 5.02 (d, J = 11.4 Hz, 1H, PhCH₃), 4.74 (s, 2H, PhCH₃), 4.64 (d, J =
11.4 Hz, 1H, PhCH₂), 4.41 (m, 2H, Fmoc, H-5), 4.33 – 4.26 (m, 2H, Fmoc, H-6), 4.25 – 4.19 (m, 2H, H-6, Fmoc), 4.16 (dd, J = 10.4, 2.4 Hz, 1H, H-3), 4.06 – 3.95 (m, 3H, H-4, OBU), 3.94 – 3.75 (m, 2H, OBU), 1.58 – 1.48 (m, 2H, OBU), 1.38 – 1.23 (m, 4H, OBU), 1.14 (dq, J = 15.1, 7.4 Hz, 2H, OBU), 0.84 (t, J = 7.4 Hz, 3H, OBU), 0.74 (t, J = 7.4 Hz, 3H, OBU) ppm. ¹³C NMR (¹H) (101 MHz, CDCl₃) δ = 165.6, 154.7 (2 C, C=O), 143.1, 141.2, 137.6, 137.6, 133.2, 129.8, 129.5, 128.4, 128.4, 128.3, 128.3, 127.9, 127.8, 127.6, 127.1, 125.0, 120.0 (26 C, Ar), 94.8 (d, J = 6.4 Hz, C-1), 75.7 (C-5), 74.8 (PhCH₂), 73.6 (C-4), 72.9 (PhCH₂), 70.3 (Fmoc), 70.3 (C-2), 69.9 (C-5), 67.75 (d, J = 5.6 Hz, OBU), 67.69 (d, J = 5.7 Hz, OBU), 66.4 (C-6), 46.6 (Fmoc), 32.07 (d, J = 7.3 Hz, OBU), 31.89 (d, J = 6.8 Hz, OBU), 18.5 (OBU), 18.3 (OBU), 13.5 (OBU), 13.4 (OBU) ppm. ESI-HRMS: m/z [M+Na]⁺ calcd. for C₅₀H₅₅NaO₁₂P: 901.3328, found: 901.3199. IR (neat) ν_max = 1743, 1724, 1251, 1027 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) of BB3:

¹³C NMR (101 MHz, CDCl₃) of BB3:
Automated Synthesis of Arabinogalactan Fragments

Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)

Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)

Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 1. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected trisaccharide 1 (ELSD trace):

HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).
Aminopentyl-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (1)

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

Crude RP-HPLC of the semi-protected trisaccharide 1 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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, 8 mg). The suspension was flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected trisaccharide 1 (1.0 mg, 1.76 µmol, 11% over 9 steps, based on resin loading).

¹H NMR (600 MHz, D₂O) δ = 4.84 (d, J = 7.7 Hz, 1H), 4.79 (d, J = 7.6 Hz, 1H), 4.62 (d, J = 8.0 Hz, 1H), 4.37 (t, J = 3.0 Hz, 2H), 4.14 – 4.08 (m, 2H), 4.03 – 3.82 (m, 15H), 3.80 – 3.76 (m, 1H), 3.17 (t, J = 7.5 Hz, 2H), 1.90 – 1.80 (m, 4H), 1.68 – 1.59 (m, 2H) ppm. ¹³C NMR {¹H} (151 MHz, D₂O) δ = 102.1, 101.8, 100.1, 80.2, 79.8, 72.9, 72.5, 72.5, 70.3, 68.8, 68.0, 67.7, 67.7, 66.3, 66.3, 66.1, 58.7, 58.7, 37.2, 25.9, 24.3, 19.9 ppm. ESI-HRMS: m/z [M+H]⁺ calcd. for C₂₃H₄₄NO₁₆: 590.2660, found: 590.2677
RP-HPLC of the deprotected trisaccharide 1 (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) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

¹H NMR (600 MHz, D₂O) of trisaccharide 1:

¹³C NMR (151 MHz, D₂O) of trisaccharide 1:
HSQC (D$_2$O) of trisaccharide 1, enlargement of anomeric peak region

Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-benzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-3-O-hydroxyl-4,6-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)

Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)

Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide 2. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected tetrasaccharide 2 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (2)

The protected tetrasaccharide 2 was dissolved in THF (5 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 over a short silica column (DCM/MeOH 1:0 to 4:1).
Crude RP-HPLC of the semi-protected tetrasaccharide 2 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/MeOH/AcOH/H₂O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetragalactoside 2 (1.3 mg, 1.73 µmol, 10% over 11 steps, based on resin loading).

¹H NMR (600 MHz, D₂O) δ = 4.86 – 4.83 (m, 2H), 4.79 (d, J = 7.6 Hz, 1H), 4.62 (d, J = 8.0 Hz, 1H), 4.37 (t, J = 3.1 Hz, 3H), 4.15 – 4.08 (m, 2H), 4.04 – 3.82 (m, 20H), 3.78 (dd, J = 9.9, 7.7 Hz, 1H), 3.19 – 3.15 (m, 2H), 1.90 – 1.82 (m, 4H), 1.67 – 1.60 (m, 2H) ppm. ¹³C NMR (¹H) (151 MHz, D₂O) δ = 102.05, 101.77, 101.73, 100.14, 80.18, 79.81, 79.75, 72.84, 72.53, 72.48, 70.29, 68.80, 68.00, 67.99, 67.72, 67.65, 66.33, 66.25, 66.16, 66.10, 58.73, 58.68, 37.16, 25.94, 24.33, 19.88 ppm. ESI-HRMS: m/z [M+H]⁺ calcd. for C₂₉H₅₄NO₂₁: 752.3188, found: 752.3331

RP-HPLC of the deprotected tetrasaccharide 2 (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) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O) of tetrasaccharide 2:

$^{13}$C NMR (151 MHz, D$_2$O) of tetrasaccharide 2:
HSQC (D$_2$O) of tetrasaccharide 2, enlargement of anomeric peak region:

Benzyloxycarbonylaminopentyl 2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB3, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B: NEt$_3$, DMF

1) 3 x AB
2) hv (305 nm)
Module A (2 x 3.75 equiv. BB3, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB3, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 3. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected trisaccharide 3 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (3)

The protected trisaccharide 3 was dissolved in THF (5 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 \textit{in vacuo}. The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide.
Crude RP-HPLC of semi-protected trisaccharide 3 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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, 8 mg). The suspension was flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tri saccharide 3 (4.0 mg, 6.72 µmol, 39% over 9 steps, based on resin loading).

\(^1\)H NMR (700 MHz, D₂O) \(\delta = 4.64\) (d, \(J = 7.9\) Hz, 1H), 4.62 (d, \(J = 7.8\) Hz, 1H), 4.58 (d, \(J = 7.9\) Hz, 1H), 4.23 – 4.21 (m, 2H), 4.15 – 4.05 (m, 8H), 3.98 – 3.91 (m, 2H), 3.88 – 3.81 (m, 5H), 3.72 – 3.66 (m, 3H), 3.18 (t, \(J = 7.3\) Hz, 2H), 1.89 – 1.82 (m, 4H), 1.65 – 1.60 (m, 2H) ppm. \(^{13}\)C NMR \(^{1}\)H (176 MHz, D₂O) \(\delta = 101.1, 101.1, 100.5, 72.9, 71.5, 70.5, 70.4, 70.4, 68.5, 68.5, 67.9, 67.0, 66.9, 66.4, 66.4, 58.8, 37.1, 25.9, 24.2, 19.8 \)ppm. HRMS: m/z [M+H]⁺ calcd. for \(C_{23}H_{43}NO_{16}\): 590.2660, found: 590.2710

RP-HPLC of deprotected trigalactoside 3 (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) and from 30% to 0% H₂O in MeCN (10 min, 0.7 mL/min).
$^1$H NMR (700 MHz, D$_2$O) of trigalactoside 3:

$^{13}$C NMR (176 MHz, D$_2$O) of trigalactoside 3:
HSQC ($\text{D}_2\text{O}$) of trigalactoside 3, enlargement of anomeric peak region:

Benzyloxycarbonylaminopentyl
$2\text{-}O\text{-}\text{benzoyl-4,6-O-dibenzyl-\text{\textbeta}{\text{-D-galactopyranosyl-(1\rightarrow3)}-2\text{-}O\text{-}\text{benzoyl-4-O-benzyl-6-O-}[2\text{-}O\text{-}\text{benzoyl-4,6-O-dibenzyl-\text{\textbeta}{\text{-D-galactopyranosyl)]-\text{\textbeta}{\text{-D-galactopyranosyl-(1\rightarrow3)}-2\text{-}O\text{-}\text{benzoyl-4,6-O-dibenzyl-\text{\textbeta}{\text{-D-galactopyranoside}}}}$}

Linker functionalized resin L (43 mg, 13.7 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module A (2 x 5 equiv. BB1 or BB2, TMSOTf, DCM)
Module B: NEt$_3$, DMF
Module C: 150 mM $\text{N}_2\text{H}_4\text{AcOH}$, 25 °C, 30 min

1) 3 x A
2) C
3) AB
4) hv (305 nm)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)
Module A (2 x 5 equiv. BB$_2$, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)
Module A (2 x 5 equiv. BB$_1$, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 5 equiv. BB$_1$, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide 4. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

**Crude NP-HPLC of protected tetrasaccharide 4 (ELSD trace):**

![HPLC graph](image)

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

**Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (4)**

The protected, branched tetrasaccharide 4 was dissolved in 5 mL THF and 0.5 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected trisaccharide 4 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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, 8 mg). The suspension was flushed with Ar for 10 min then saturated with H2 for 10 min and stirred under a H2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide 4 (1.5 mg, 2.00 µmol, 15% over 11 steps, based on resin loading).

1H NMR (600 MHz, D2O) δ = 4.79 (d, J = 7.8 Hz, 1H), 4.72 (d, J = 7.6 Hz, 1H), 4.57 (d, J = 8.0 Hz, 1H), 4.54 (d, J = 7.9 Hz, 1H), 4.34 (dd, J = 7.2, 3.2 Hz, 2H), 4.18 – 4.12 (m, 1H), 4.09 – 3.98 (m, 5H), 3.97 – 3.92 (m, 2H), 3.92 – 3.70 (m, 15H), 3.62 (dd, J = 9.8, 8.0 Hz, 1H), 3.12 (t, J = 7.5 Hz, 2H), 1.80 (tt, J = 14.0, 7.2 Hz, 4H), 1.61 – 1.55 (m, 2H) ppm. 13C NMR(1H) (151 MHz, D2O) δ = 102.9, 102.5, 102.1, 101.0, 81.0, 80.6, 73.7, 73.7, 73.4, 72.0, 71.3, 71.1, 69.6, 69.4, 68.8, 68.5, 68.5, 67.8, 67.2, 67.2, 67.1, 67.0, 59.6, 59.6, 59.5, 38.0, 26.8, 25.0, 20.7 ppm. HRMS: m/z [M+Na]+ calcd. for C29H53NNaO21: 774.3007, found 774.3030

RP-HPLC of deprotected tetrasaccharide 4 (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) and from 30% to 0% H2O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O) of tetrasaccharide 4:

$^{13}$C NMR (151 MHz, D$_2$O) of tetrasaccharide 4:

HSQC (D$_2$O) of tetrasaccharide 4, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranosyl]-β-D-galacto-pyranosyl]-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1 or BB2, TMSOTf, DCM)
Module B: NEt3, DMF
Module C: 150 mM N₂H₄·AcOH, 25 °C, 30 min

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide 5. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.
Crude NP-HC of protected pentasaccharide 5 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (5)

The protected, branched pentasaccharide 5 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected pentasaccharide 5 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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, 8 mg). The suspension was flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide 5 (1.6 mg, 1.73 µmol, 10% over 13 steps, based on resin loading).

¹H NMR (700 MHz, D₂O) δ = 4.82 (d, J = 7.8 Hz, 1H), 4.75 (d, J = 7.7 Hz, 1H), 4.61 – 4.56 (m, 3H), 4.37 (dd, J = 8.1, 2.9 Hz, 2H), 4.19 – 4.14 (m, 2H), 4.11 – 3.98 (m, 9H), 3.97 – 3.73 (m, 17H), 3.68 – 3.63 (m, 2H), 3.14 (t, J = 7.5 Hz, 2H), 1.82 (tt, J = 14.3, 7.4 Hz, 4H), 1.63 – 1.56 (m, 2H) ppm. ¹³C NMR¹H (176 MHz, D₂O) δ = 102.0, 101.5, 101.2, 101.1, 100.0, 80.1, 79.7, 72.8, 72.8, 72.5, 71.4, 71.9, 70.9, 70.4, 70.3, 70.2, 68.7, 68.4, 68.4, 67.9, 67.6, 67.5, 67.6, 67.1, 67.0, 66.3, 66.1, 66.0, 58.7, 58.7, 58.6, 37.0, 25.8, 24.1, 19.8 ppm. HRMS: m/z [M+H]⁺ calcd. for C₃₅H₆₄NO₂₆: 914.3716, found: 914.3783

RP-HPLC of the deprotected pentasaccharide 5 (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) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (700 MHz, D$_2$O) of pentagalactoside 5:

$^{13}$C NMR (176 MHz, D$_2$O) of pentagalactoside 5:

HMQC (D$_2$O) of pentagalactoside 5, enlargement of anomeric region:
Benzyloxycarbonylaminopentyl

2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galacto-pyranosyl]-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1 or BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1 or BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 hexasaccharide 6. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.
Crude NP-HPLC of protected hexasaccharide 6 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[6-O-[6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (6)

The protected, branched hexasaccharide 6 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of the semi-protected hexasaccharide 6 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H$_2$O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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, 8 mg). The suspension was flushed with Ar for 10 min then saturated with H$_2$ for 10 min and stirred under a H$_2$-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide 6 (0.9 mg, 0.84 µmol, 7% over 15 steps, based on resin loading).

$^1$H NMR (700 MHz, D$_2$O) $\delta = 4.86$ (d, $J = 7.9$ Hz, 1H), 4.79 (d, $J = 7.7$ Hz, 1H), 4.66 – 4.60 (m, 4H), 4.43 – 4.39 (m, 2H), 4.24 – 4.18 (m, 3H), 4.15 – 4.06 (m, 11H), 4.04 (dd, $J = 9.9$, 3.3 Hz, 1H), 4.01 – 3.81 (m, 17H), 3.78 (dd, $J = 9.8$, 7.7 Hz, 1H), 3.73 – 3.67 (m, 3H), 3.19 – 3.16 (m, 2H), 1.90 – 1.83 (m, 4H), 1.66 – 1.61 (m, 2H) ppm. $^{13}$C NMR($^1$H) (176 MHz, D$_2$O) $\delta = 102.1, 101.6, 101.2$ (3 C), 100.1, 80.2, 79.7, 72.9, 72.8, 72.5, 71.5, 71.4, 71.0, 70.5, 70.3, 68.8, 68.5, 67.9, 67.7, 67.6, 67.2, 67.1, 67.0, 66.4, 66.2, 66.1, 58.8, 58.7, 37.1, 25.9, 24.2, 19.8 ppm. HRMS: m/z [M+Na]$^+$ calcd. for C$_{41}$H$_{73}$NNaO$_{31}$: 1098.4064 found: 1098.4163

RP-HPLC of deprotected hexasaccharide 6 (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) and from 30% to 0% H$_2$O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (700 MHz, D$_2$O) of hexasaccharide 6:

$^{13}$C NMR (176 MHz, D$_2$O) of hexasaccharide 6:
HMOC (D$_2$O) of hexasaccharide 6, enlargement of anomeric peak region:

Benzyloxycarbonylaminopentyl 2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-benzyl-β-D-galactopyranoside,
Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 hexasaccharide 7. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected hexasaccharide 7 (ELSD trace):

HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)
Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (7)

The protected branched hexasaccharide 7 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of the semi-protected hexasaccharide 7 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide 7 (0.9 mg, 0.84 µmol, 5% over 15 steps, based on resin loading).

¹H NMR (600 MHz, D₂O) δ = 4.87 – 4.84 (m, 2H), 4.79 (d, J = 7.6 Hz, 1H), 4.63 – 4.60 (m, 3H), 4.42 – 4.35 (m, 3H), 4.23 – 4.18 (m, 2H), 4.13 – 4.03 (m, 8H), 4.02 – 3.76 (m, 23H), 3.73 – 3.66 (m, 2H), 3.20 – 3.16 (m, 2H), 1.90 – 1.82 (m, 4H), 1.67 – 1.61 (m, 2H) ppm. ¹³C NMR (151 MHz, D₂O) δ = 102.0, 101.7, 101.6, 101.2, 100.1, 80.2, 79.8, 79.7, 72.9, 72.8, 72.5, 72.4, 71.4, 71.0, 70.5, 70.4, 70.3,
68.8, 68.5, 68.5, 67.9, 67.7, 67.6, 67.0, 66.4, 66.3, 66.2, 66.1, 66.1, 58.8, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z [M+Na]^+ calcd. for C_{41}H_{73}NNaO_{31}: 1098.4064, found: 1098.4173

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

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

^1H NMR (600 MHz, D_2O) of hexasaccharide 7:

^13C NMR (151 MHz, D_2O) of hexasaccharide 7:
HSQC (D$_2$O) of hexasaccharide 7, enlargement of anomic peak region:

Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2,3,5-O-tribenzyl-α-L-arabinofuranoside

Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt$_3$ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module D (2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide 8. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected tetrasaccharide 8 (ELSD trace):

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

Aminopentyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-α-L-arabinofuranoside (8)

The protected tetrasaccharide 8 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of the semi-protected tetrasaccharide 8 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H$_2$O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H$_2$ for 10 min and stirred under a H$_2$-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide 8 (2.0 mg, 2.81 µmol, 17% over 10 steps, based on resin loading).

$^1$H NMR (700 MHz, D$_2$O) δ = 6.13 (s, 1H), 5.59 – 5.54 (m, 2H), 5.34 (d, J = 8.0 Hz, 1H), 5.11 – 5.08 (m, 3H), 5.03 – 4.99 (m, 2H), 4.86 – 4.82 (m, 2H), 4.75 – 4.70 (m, 3H), 4.69 – 4.55 (m, 15H), 3.90 (t, J = 7.5 Hz, 2H), 2.62 – 2.54 (m, 4H), 2.38 – 2.33 (m, 2H) ppm. $^{13}$C NMR($^1$H) (176 MHz, D$_2$O) δ = 107.0, 101.8, 101.8, 100.1, 81.6, 80.2, 79.8, 79.0, 77.8, 74.3, 72.7, 72.5, 72.5, 68.0, 67.7, 67.6, 66.2, 66.1, 59.0, 58.6, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z [M+H]$^+$ calcd. for C$_{28}$H$_{51}$NO$_{20}$: 722.3082, found: 722.3036.

RP-HPLC of the deprotected tetrasaccharide 8 (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) and from 30% to 0% H$_2$O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (700 MHz, D$_2$O) of tetrasaccharide 8:

$^{13}$C NMR (176 MHz, D$_2$O) of tetrasaccharide 8:
HMOC (D₂O) of tetrasaccharide 8, enlargement of anomeric peak region:

Benzyloxycarbonylaminoethyl 2-0-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside

Module A: 2 x 3.75 equiv. BB1 or BB2, TMSOTf, DCM
Module B: NEt₃, DMF
Module C: 150 mM N₂H₂, AcOH, 25 °C, 30 min
Module D: 2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane

1) 3 x A
2) 2 x B
3) C
4) D
5) B
6) hv (305 nm)
Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module C (3 cycles)
Module D (2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °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 9. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

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

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

Aminopentyl β-D-galactopyranosyl-(1→3)-6-O-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (9)
The protected tetrasaccharide 9 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected terasaccharide 9 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H2 for 10 min and stirred under a H2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide 9 (2.0 mg, 2.72 µmol, 17% over 11 steps, based on resin loading).

1H NMR (600 MHz, D2O) δ = 5.15 (d, J = 1.4 Hz, 1H), 4.77 (d, J = 7.7 Hz, 1H), 4.70 (d, J = 7.6 Hz, 1H), 4.54 (d, J = 8.0 Hz, 1H), 4.29 (dd, J = 12.5, 3.2 Hz, 2H), 4.16 (dd, J = 3.4, 1.7 Hz, 1H), 4.13 (td, J = 6.0, 3.3 Hz, 1H), 4.07 – 4.00 (m, 3H), 3.99 – 3.95 (m, 2H), 3.94 – 3.83 (m, 9H), 3.82 – 3.73 (m, 6H), 3.70 (dd, J = 9.9, 7.7 Hz, 1H), 3.13 – 3.07 (m, 2H), 1.82 – 1.73 (m, 4H), 1.55 (dt, J = 15.4, 7.6 Hz, 2H) ppm.

13C NMR(1H) (151 MHz, D2O) δ = 106.9, 103.3, 102.9, 101.4, 82.9, 81.6, 81.0, 80.1, 75.5, 74.1, 73.8, 72.3, 71.5, 70.0, 69.1, 68.9, 68.8, 67.6, 67.5, 67.5, 66.4, 60.2, 60.0, 60.0, 38.4, 27.2, 25.4, 21.1 ppm.


RP-HPLC of the deprotected tetrasaccharide 9 (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) and from 30% to 0% H2O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O) of tetrasaccharide 9:

$^{13}$C NMR (151 MHz, D$_2$O) of tetrasaccharide 9:

HSQC (D$_2$O) of tetrasaccharide 9, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-4-O-benzyl-β-D-galactopyranosyl-(1→6)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB3 or BB2, TMSOTf, DCM)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module D (2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB3, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 10. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.
Crude NP-HPLC of protected tetrasaccharide 10 (ELSD trace):

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

Aminopentyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-3-O-[α-L-arabinofuranosyl]-(1→6)-β-D-galactopyranoside (10)

The protected tetrasaccharide 10 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected tetrasaccharide 10 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide 10 (2.0 mg, 2.77 µmol, 16% over 11 steps, based on resin loading).

¹H NMR (700 MHz, D₂O) δ = 5.39 (s, 1H), 4.70 (d, J = 7.8 Hz, 1H), 4.62 (d, J = 7.9 Hz, 1H), 4.59 (d, J = 7.8 Hz, 1H), 4.38 – 4.37 (m, 1H), 4.32 (d, J = 2.3 Hz, 1H), 4.31 – 4.28 (m, 1H), 4.24 – 4.19 (m, 2H), 4.15 – 4.05 (m, 8H), 4.00 (d, J = 12.5 Hz, 1H), 3.98 – 3.80 (m, 9H), 3.72 – 3.66 (m, 2H), 3.18 (t, J = 7.4 Hz, 2H), 1.90 – 1.82 (m, 4H), 1.66 – 1.60 (m, 2H) ppm. ¹³C NMR(¹H) (176 MHz, D₂O) δ = 107.0, 101.1, 100.8, 100.4, 81.6, 79.0, 77.9, 74.3, 72.9, 71.5, 71.3, 70.4, 70.4, 68.5, 68.4, 67.9, 67.5, 67.0, 66.8, 66.4, 66.4, 66.2, 58.9, 58.7, 37.1, 25.9, 24.2, 19.8 ppm. HRMS: m/z [M+Na]⁺ calcd. for C₂₈H₅₁NO₂₀: 744.2902, found: 744.2903.

RP-HPLC of deprotected tetrasaccharide 10 (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) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (700 MHz, D$_2$O) of tetrasaccharide 10:

$^{13}$C NMR (176 MHz, D$_2$O) of tetrasaccharide 10:

HMQC (D$_2$O) of tetrasaccharide 10, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-3-O-fluorenylcarboxymethyl-4,6-O-dibenzyl-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-2,3,5-O-tribenzoyl-α-L-arabinofuranoside

Linker functionalized resin L (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 3.75 equiv. BB1 or BB2, TMSOTf, DCM)
Module B: NEt₃, DMF
Module C: 150 mM N₂H₄, AcOH, 25 °C, 30 min
Module D: 2 x 3.75 equiv BB4, NIS, DCM/Dioxane

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide 11. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.
Crude NP-HPLC of protected tetrasaccharide 11 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-α-L-arabinofuranoside (11)

The Tetrasaccharide 11 was dissolved in 5 mL THF and 1 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected terasaccharide 11 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/MeOH/AcOH/H₂O (4:2:1:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide 11 (1.2 mg, 1.66 µmol, 10% over 11 steps, based on resin loading).

1H NMR (600 MHz, D₂O) δ = 5.40 (d, J = 1.3 Hz, 1H), 4.62 (d, J = 8.0 Hz, 1H), 4.60 (d, J = 7.9 Hz, 1H), 4.40 – 4.37 (m, 2H), 4.30 (ddd, J = 12.3, 6.1, 3.0 Hz, 2H), 4.20 (dd, J = 10.3, 3.0 Hz, 1H), 4.15 – 4.03 (m, 5H), 4.02 – 3.97 (m, 3H), 3.96 – 3.83 (m, 11H), 3.83 – 3.79 (m, 1H), 3.69 (dd, J = 9.9, 7.9 Hz, 1H), 3.20 – 3.16 (m, 2H), 1.86 (tt, J = 14.2, 7.1 Hz, 4H), 1.67 – 1.60 (m, 2H) ppm. 13C NMR (1H) (151 MHz, D₂O) δ = 107.0, 101.7, 101.2, 100.1, 81.6, 80.1, 79.0, 77.6, 74.3, 72.9, 72.5, 71.2, 70.5, 68.5, 67.9, 67.7, 67.6, 66.9, 66.3, 66.2, 66.2, 58.9, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z [M+H]+ calcd. for C₂₈H₅₁NO₂₀: 722.3082, found: 722.3125.

RP-HPLC of 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).
$^1$H NMR (600 MHz, D$_2$O) of tetrasaccharide 11:

$^{13}$C NMR (151 MHz, D$_2$O) of tetrasaccharide 11:

HSQC (D$_2$O) of tetrasaccharide 11, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-3-O-2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-4,6-O-dibenzyl-β-D-galactopyranosyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-3-O-acetyl-4,6-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module E (3 cycles)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt₃ in DMF, 3 x 5 min, rt)
Module D (2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide 12. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.
Crude NP-HPLC of protected pentasaccharide 12 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[3-O-[α-L-arabinofuranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (12)

The protected branched pentasaccharide 12 was dissolved in 5 mL THF and 0.5 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected pentasaccharide 12 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H2 for 10 min and stirred under a H2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide 12 (2.7 mg, 3.05 µmol, 16% over 14 steps, based on resin loading).

1H NMR (600 MHz, D2O) δ = 5.40 (s, 1H), 4.85 (d, J = 7.7 Hz, 1H), 4.78 (d, J = 7.6 Hz, 1H), 4.65 (d, J = 7.9 Hz, 1H), 4.62 (d, J = 8.0 Hz, 1H), 4.42 – 4.37 (m, 3H), 4.31 – 4.25 (m, 2H), 4.21 (q, J = 8.7 Hz, 1H), 4.14 – 4.04 (m, 6H), 4.03 – 3.76 (m, 24H), 3.18 (t, J = 7.4 Hz, 2H), 1.90 – 1.81 (m, 4H), 1.63 (dt, J = 15.0, 7.7 Hz, 2H) ppm. 13C NMR (151 MHz, D2O) δ = 106.9, 102.1, 101.6, 101.0, 100.1, 81.6, 80.1, 79.7, 79.0, 78.0, 74.2, 72.8, 72.7, 72.5, 71.1, 70.3, 68.7, 67.9, 67.7, 67.6, 67.0, 66.3, 66.2, 66.1, 58.9, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z [M+H]+ calcd. for C34H61NO25: 884.3610, found: 884.3550.

RP-HPLC of 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) and from 30% to 0% H2O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O) of pentasaccharide 12:

$^{13}$C NMR (151 MHz, D$_2$O) of pentasaccharide 12:

HSQC (D$_2$O) of pentasaccharide 12, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1\(\rightarrow\)3)-2-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-4-O-benzyl-6-O-[2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl]-β-D-galactopyranosyl]-(1\(\rightarrow\)3)-2-O-benzoyl-3-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB2, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module B (20% NEt3 in DMF, 3 x 5 min, rt)
Module D (2 x 3.75 equiv. BB4, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 hexasaccharide 13. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected hexasaccharide 13 (ELSD trace):

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

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[3-O-[α-L-arabinofuranosyl]-6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (13)

The protected hexasaccharide 13 was dissolved in 5 mL THF and 0.5 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed in vacuo. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).
Crude RP-HPLC of semi-protected hexasaccharide 13 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H2 for 10 min and stirred under a H2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide 13 (1.9 mg, 1.82 µmol, 11% over 17 steps, based on resin loading).

1H NMR (600 MHz, D2O) δ = 5.33 (s, 1H), 4.79 (d, J = 7.8 Hz, 1H), 4.72 (d, J = 7.6 Hz, 1H), 4.60 (d, J = 7.9 Hz, 1H), 4.56 - 4.54 (m, 2H), 4.35 - 4.30 (m, 3H), 4.24 - 4.21 (m, 2H), 4.15 - 4.09 (m, 2H), 4.07 - 3.99 (m, 8H), 3.96 (dd, J = 9.9, 3.1 Hz, 1H), 3.94 - 3.90 (m, 2H), 3.90 - 3.84 (m, 7H), 3.84 - 3.69 (m, 11H), 3.63 (dd, J = 9.7, 8.0 Hz, 1H), 3.11 (t, J = 7.4 Hz, 2H), 1.83 - 1.75 (m, 4H), 1.59 - 1.53 (m, 2H) ppm. 13C NMR(1H) (151 MHz, D2O) δ = 108.0, 103.1, 102.7, 102.3, 102.1, 101.2, 82.7, 81.3, 80.8, 80.1, 79.0, 75.3, 74.0, 73.9, 73.6, 72.3, 72.0, 71.6, 71.4, 69.8, 69.6, 69.0, 68.8, 68.7, 68.7, 68.3, 68.0, 67.4, 67.4, 67.3, 67.3, 67.2, 60.0, 59.9, 59.8, 59.8, 38.2, 27.0, 25.2, 20.9 ppm. HRMS: m/z [M+Na]+ calcd. for C40H71NO30: 1068.3958, found: 1068.4056.

RP-HPLC of deprotected hexaasaccharide 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) and from 30% to 0% H2O in MeCN (10 min, flow rate 0.7 mL/min).
$^1$H NMR (600 MHz, D$_2$O) of hexasaccharide 13:

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

HSQC (D$_2$O) of hexasaccharide 13, enlargement of anomeric peak region:
Benzyloxycarbonylaminopentyl 2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-benzoyl-4-O-benzyl-6-O-[2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-4-O-benzyl-6-O-[2-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranosyl]β-D-galactopyranosyl]-(1→3)-2-O-benzoyl-3-O-benzoyl-4,6-O-dibenzyl-β-D-galactopyranoside

Linker functionalized resin L (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 3.75 equiv. BB1 or BB2, TMSOTf, DCM)
Module B: NEt3, DMF
Module C: 50 mM N2H4, AcOH, 25 °C, 30 min
Module D: 2 x 3.75 equiv BB4, NIS, DCM/Dioxane
Module E: 0.5 M Bz2O, 0.25 M DMAP, pyridine, DCE, 40°C, 30 min

1) 3 x AB
2) 2 x ECAB
3) DC
4) AB
5) hv (305 nm)
Module D (2 x 3.75 equiv. BB4, NIS, TFOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module C (3 cycles)
Module A (2 x 3.75 equiv. BB1, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °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 14. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

Crude NP-HPLC of protected heptasaccharide 14 (ELSD trace):

HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-O-[6-O-[3-O-[α-L-arabinofuranosyl]-6-O-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (14)
The protected heptasaccharide 14 was dissolved in 5 mL THF and 0.5 mL of 0.5 M NaOMe was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected heptasaccharide 14 (ELSD trace):

The product was dissolved in a mixture of EE/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 flushed with Ar for 10 min then saturated with H₂ for 10 min and stirred under a H₂-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected heptasaccharide 14 (1.9 mg, 1.55 µmol, 9% over 21 steps, based on resin loading).

¹H NMR (600 MHz, D₂O) δ = 5.40 (d, J = 0.8 Hz, 1H), 4.86 (d, J = 7.7 Hz, 1H), 4.78 (d, J = 7.6 Hz, 1H), 4.69 (d, J = 7.9 Hz, 1H), 4.62 (dd, J = 7.9, 4.6 Hz, 3H), 4.41 (dd, J = 8.1, 3.2 Hz, 2H), 4.38 (dd, J = 3.4, 1.5 Hz, 1H), 4.32 – 4.28 (m, 2H), 4.23 – 4.18 (m, 3H), 4.14 – 4.05 (m, 11H), 4.03 (dd, J = 9.9, 3.2 Hz, 1H), 4.01 – 3.97 (m, 2H), 3.96 – 3.90 (m, 7H), 3.90 – 3.80 (m, 11H), 3.78 (dd, J = 9.8, 7.7 Hz, 1H), 3.72 – 3.66 (m, 2H), 3.20 – 3.16 (m, 2H), 1.90 – 1.82 (m, 4H), 1.63 (dt, J = 15.3, 7.7 Hz, 2H) ppm. ¹³C NMR (151 MHz, D₂O) δ = 107.0, 102.1, 101.6, 101.2, 101.2, 100.9, 100.1, 81.6, 80.3, 79.7, 79.1, 77.9, 74.4, 72.9, 72.9, 72.6, 71.4, 71.4, 71.0, 70.5, 70.4, 70.3, 68.8, 68.5, 68.5, 68.0, 67.7, 67.6, 67.3, 67.2, 67.2, 66.9, 66.4, 66.4, 66.2, 66.2, 66.1, 59.0, 58.8, 58.8, 58.7, 37.1, 25.9, 24.2, 19.9 ppm. HRMS: m/z [M+Na]⁺ calcd. for C₄₆H₸₁NNaO₃₅: 1230.4486, found: 1230.4396.

RP-HPLC of deprotected heptasaccharide 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).
$^1$H NMR (600 MHz, D$_2$O) of heptasaccharide 14:

$^{13}$C NMR (151 MHz, D$_2$O) of heptasaccharide 14:

HSQC (D$_2$O) of heptasaccharide 14, enlargement of anomeric peak region:
References