Traceless Photolabile Linker Expedites Chemical Synthesis of Complex Oligosaccharides by Automated Glycan Assembly

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1. General Information
All chemicals were reagent grade and were used as supplied unless otherwise noted. The automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a p-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04-0.063 mm). Analysis and purification by normal and reverse phase HPLC was performed by using an Agilent 1200 series. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. $^1$H, $^{13}$C, $^{19}$F, and HSQC NMR spectra were recorded in parts per million (δ) relative to the resonance of the solvent on a Varian 400-MR (400 MHz), Varian 600-MR (600MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. High resolution mass spectra were obtained using 6210 ESI-TOF mass spectrometer (Agilent) and MALDI-TOF autoflex™ (Bruker) instruments. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter, with concentrations expressed in g per 100ml. The loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 spectrometer.

2. Materials and conditions for automated synthesis

2.1 Materials and measurements
Solvents used for dissolving all building blocks and making of various solutions were taken from an anhydrous solvent system (jcmeyer phoenix solvent drying system). Wash solvents were HPLC grade. The building blocks were purchased from GlycoUniverse GmbH & CO KGaA, co-evaporated three times with anhydrous toluene and dried for 1 hour under high vacuum prior to use. All solution bottles were freshly prepared and kept under Argon during the automation process. Isolated yields of products were calculated on the basis of resin loading. Resin loading was determined following a reported protocol. Briefly, functionalized resin (40 mg) was treated with one glycosylation cycle using 65 mg of building block A, followed by DBU-promoted Fmoc cleavage and determination of dibenzofulvene concentration by measuring its UV absorbance. All automated syntheses were performed on 0.0125 mmol scale. Resin was placed in the reaction vessel and was swollen in CH$_2$Cl$_2$ for 20 min at room temperature before starting the first module. During this time, all reagent lines involved in the synthesis were washed and primed.

2.2 Preparation of stock solutions
- **Building Block**: 0.08 mmol of building block was dissolved in 1 mL of CH$_2$Cl$_2$.
- **Acidic Wash**: 0.45 mL of TMSOTf was added to 40 mL of CH$_2$Cl$_2$.
- **Activator Solution 1**: 1.35 g of recrystallized NIS was dissolved in 40 mL of a 2:1 mixture of anhydrous CH$_2$Cl$_2$ and dioxane, followed by addition of 55 µL of triflic acid. The solution was kept under ice-bath cooling for the duration of the automated run.
- **Activator Solution 2**: 0.9 mL of TMSOTf was added to 40 mL of CH$_2$Cl$_2$.
- **Pre-capping Solution**: 10 mL of pyridine was added to 90 mL of anhydrous DMF.
- **Capping Solution**: 1.2 mL of methansulfonic acid, 6 mL of acetic anhydride were added to 50 mL of anhydrous CH₂Cl₂.

- **Lev Deprotection Solution**: 725 mg of N₂H₄·HOAc was dissolved in 50 mL of a 4:1:0.25 mixture of pyridine/acetic acid/H₂O.

- **Fmoc Deprotection Solution 1**: 20 mL of piperidine was added to 80 mL of anhydrous DMF.

- **Fmoc Deprotection Solution 2**: 20 mL of Et₃N was added to 80 mL of anhydrous DMF.

### 2.3 Modules for automated synthesis

- **Module I - Acidic Wash** *(ca. 20 min)*: CH₂Cl₂ (2 mL) was added and the temperature was adjusted to -20 °C. Next, Acidic Wash solution (1 mL) was added dropwise to the reaction vessel. After bubbling for 3 min, the solution was drained and the resin was washed with CH₂Cl₂ for 25 s.

| Action          | Repeat | Solution         | Amount  | T (°C) | Bubbling |
|-----------------|--------|------------------|---------|--------|----------|
| Cooling         | -      |                  |         | -20    | 15 min   |
| Deliver         | 1      | CH₂Cl₂           | 2 mL    | -20    |          |
| Deliver         | 1      | Acidic Wash      | 1 mL    | -20    | 3 min    |
| Wash            | 1      | CH₂Cl₂           | 2 mL    | -20    | 25 s     |

- **Module IIA – Glycosylation with Thioglycoside** *(ca. 35 min)*: The building block solution (0.08 mmol of BB in 1mL of CH₂Cl₂) was delivered to the reaction vessel. After initiation temperature (T₁) was reached, Activator Solution 1 (1 mL) was added dropwise to the reaction vessel. After initiation time (t₁), an incubation temperature (T₂) was set and the incubation duration (t₂) was adjusted depending on the BB. The values for building block A were shown in the table below. The solution was drained and the resin was washed with CH₂Cl₂, CH₂Cl₂/dioxane (1:2, 3 mL for 20 s) and CH₂Cl₂ (twice, each with 2 mL for 25 s). The temperature was increased to 25 °C.

| Action          | Repeat | Solution            | Amount     | T (°C)       | Incubation          |
|-----------------|--------|---------------------|------------|--------------|---------------------|
| Cooling         | -      |                     |            | -20          |                     |
| Deliver         | 1      | Building Block A    | 1 mL       | -20          |                     |
| Deliver         | 1      | Activator Solution 1| 1 mL       | -20          |                     |
| Agitation (BB dependent) | 1 |                     |            | -20 (T₁)     | 5 min (t₁)           |
| Wash            | 1      | CH₂Cl₂              | 2 mL       | 0            | 5 s                 |
| Wash            | 1      | CH₂Cl₂/dioxane (1:2)| 3 mL       | 0            | 20 s                |
| Heating         | -      |                     |            | 25           |                     |
| Wash            | 2      | CH₂Cl₂              | 2 mL       | 25           | 25 s                |

- **Module IIb – Glycosylation with Glycosylphosphate** *(ca. 55 min)*: The building block solution (0.08 mmol of BB in 1mL of CH₂Cl₂) was delivered to the reaction vessel. After initiation temperature (T₁) was reached, Activator Solution 2 (1 mL) was added dropwise to the reaction vessel. Incubation temperature (T₂) was set and the incubation duration (t₂) was adjusted depending on the BB. The values for building block K were shown in the table below. The solution was drained and the resin was washed with CH₂Cl₂, CH₂Cl₂/dioxane (1:2, 3 mL for 20 s) and CH₂Cl₂ (twice, each with 2 mL for 25 s). The temperature was increased to 25 °C.
| Action             | Repeat | Solution                | Amount | T (°C) | Incubation |
|--------------------|--------|-------------------------|--------|--------|------------|
| Cooling            | -      |                         |        | -30    | -          |
| Deliver 1          | Building Block K | 1 mL                 | -30    | -      |
| Deliver 1          | Activator Solution 2 | 1 mL               | -30    | -      |
| Agitation (BB dependent) 1 | CH₂Cl₂ | 2 mL                  | 0      | 5 s    |
| Wash 1             | CH₂Cl₂/dioxane (1:2) | 3 mL                | 0      | 20 s   |
| Heating -          | -      | -                       | 25     | -      |
| Wash 2             | CH₂Cl₂ | 2 mL                  | 25     | 25 s   |

**Module III – Capping** *(ca. 30 min):* The resin was washed with DMF (twice with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. **Pre-capping Solution** (2 mL) was delivered. After 1 min, the reaction solution was drained and the resin washed with CH₂Cl₂ (three times with 3 mL for 25 s). **Capping Solution** (4 mL) was delivered to the reaction vessel. After 20 min, the solution was drained and the resin washed with CH₂Cl₂ (three times with 3 mL for 25 s).

| Action             | Repeat | Solution                | Amount | T (°C) | Incubation |
|--------------------|--------|-------------------------|--------|--------|------------|
| Heating            | -      | -                       | 25     | 5 min  |
| Wash 2             | DMF    | 2 mL                    | 25     | 25 s   |
| Deliver 1          | Pre-capping Solution | 2 mL          | 25     | 1 min  |
| Wash 3             | CH₂Cl₂ | 2 mL                    | 25     | 25 s   |
| Deliver 1          | Capping Solution | 4 mL          | 25     | 20 min |
| Wash 3             | CH₂Cl₂ | 2 mL                    | 25     | 25 s   |

**Module IVa – Fmoc Deprotection 1** *(ca. 15 min):* The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. 2 mL of **Fmoc Deprotection Solution 1** (2 mL) was delivered to the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 2 mL for 25 s) and CH₂Cl₂ (five times with 2 mL for 25 s). The temperature of the reaction was decreased to -20 °C for the next module.

| Action             | Repeat | Solution                | Amount | T (°C) | Incubation |
|--------------------|--------|-------------------------|--------|--------|------------|
| Heating            | -      | -                       | 25     | 5 min  |
| Wash 3             | DMF    | 2 mL                    | 25     | 25 s   |
| Deliver 1          | Fmoc Deprotection Solution 1 | 2 mL | 25 | 5 min |
| Wash 1             | DMF    | 2 mL                    | 25     | -      |
| Cooling            | -      | -                       | -20    | -      |
| Wash 3             | DMF    | 2 mL                    | -20    | 25 s   |
| Wash 5             | CH₂Cl₂ | 2 mL                    | -20    | 25 s   |

**Module IVb – Lev Deprotection** *(ca. 100 min):* The resin was washed with CH₂Cl₂ (three times with 2 mL for 15 s) and the temperature of the reaction vessel was adjusted to 25 °C. Fresh CH₂Cl₂ (1.3 mL) was delivered to the reaction vessel, followed by 0.8 mL of **Lev Deprotection Solution**. Bubbling continued for 30 min, after which the solution was drained and the whole process was repeated twice more. The solution was drained and the resin washed with DMF, THF and CH₂Cl₂ (three times each, with 2 mL for 15 s).
| Action          | Repeat | Solution       | Amount | T (°C) | Incubation |
|-----------------|--------|----------------|--------|--------|------------|
| Heating         | -      | -              | -      | 25     | 5 min      |
| Wash            | 3      | CH₂Cl₂         | 2 mL   | 25     | 15 s       |
| Deliver         | 1      | CH₂Cl₂         | 1.3 mL | 25     | -          |
| Deliver         | 1      | Lev Deprotection Solution | 0.8 mL | 25 | 30 min |
| Wash            | 3      | DMF            | 2 mL   | 25     | 15 s       |
| Wash            | 3      | THF            | 2 mL   | 25     | 15 s       |
| Wash            | 3      | CH₂Cl₂         | 2 mL   | 25     | 15 s       |

**Module IVc – Fmoc Deprotection 2** (ca. 45 min): The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. **Fmoc Deprotection Solution 2** (2 mL) was delivered to the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 2 mL for 25 s) and CH₂Cl₂ (five times with 2 mL for 25 s). The whole process was repeated twice more. The temperature of the reaction was decreased to -20 °C for the next module.

| Action          | Repeat | Solution       | Amount | T (°C) | Incubation |
|-----------------|--------|----------------|--------|--------|------------|
| Heating         | -      | -              | -      | 25     | 5 min      |
| Wash            | 3      | DMF            | 2 mL   | 25     | 25 s       |
| Deliver         | 1      | Fmoc Deprotection Solution 2 | 2 mL | 25 | 5 min |
| Wash            | 1      | DMF            | 2 mL   | 25     | -          |
| Cooling         | -      | -              | -      | -20    | -          |
| Wash            | 3      | DMF            | 2 mL   | -20    | 25 s       |
| Wash            | 5      | CH₂Cl₂         | 2 mL   | -20    | 25 s       |

**2.4 Modules for post-automated synthesis**

**Module V – Photocleavage:** The Vapourtec E-Series UV-150 Photoreactor Flow Chemistry System was employed. The medium pressure metal halide lamp was filtered using the commercial long-pass filter (No. 3, red). The resin, suspended in CH₂Cl₂, was loaded into a plastic syringe. The suspension was 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 temperature of the photoreactor was maintained at 20 °C and the lamp power was adjusted to 80%. Irradiation was set to 30 min, after which the solution was pushed out of the reactor. The existing flow was filtered and the filtrate was collected into a collection flask.

**Module VI – Methanolyis:** The resin was loaded into a filter syringe with a capped tip and was suspended in 4 mL of anhydrous THF. A solution of NaOMe in MeOH (0.4 mL, c 5.4M) was then added. The filter syringe was wrapped in aluminium foil and was attached to a rotavap. Spinning was continued for 1 hour, after which the solution was drained. The resin was then washed successively with MeOH, H₂O, 1M citric acid solution, DMF, THF, CH₂Cl₂.

**Module VII – Hydrogenation:** The compound was dissolved in 4 mL of EA/tBuOH/H₂O (2:1:1). Pd-C (10%) was added to the solution (100% by weight) and the suspension was stirred in a H₂ bomb with 60 psi pressure for 1 hour. The insoluble material was removed by a CHROMAFIL®Xtra, RC 0.45 syringe filter. The solid was washed once with tBuOH and several times with H₂O. The filtrate was collected, dried and purified by reverse-phase HPLC.
2.5 Modules for HPLC purification

Crude products were analyzed and purified using analytical or preparative HPLC (Agilent 1200 Series System). All unprotected products were isolated as formate salt.

- **Module VIIIa (YMC-Diol-300 column, 150 x 4.6 mm):** flow rate of 1.0 mL/min with 20% EtOAc/Hexane as eluents [isocratic (5 min), linear gradient to 55% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)].

- **Module VIIIb (YMC-Diol-300 column, 150 x 20 mm):** flow rate of 4.6 mL/min with 20% EtOAc/Hexane as eluents [isocratic (5 min), linear gradient to 55% EtOAc (30 min), linear gradient to 100% EtOAc (5 min)].

- **Module VIIlc (Synergi Hydro RP18 column, 250 x 2.6 mm):** flow rate of 0.7 mL/min with H$_2$O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 10% ACN (30 min), linear gradient to 100% ACN (5 min)].

- **Module VIIIId (Synergi Hydro RP18 column, 250 x 10 mm):** flow rate of 3.7 mL/min with H$_2$O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 10% ACN (30 min), linear gradient to 100% ACN (5 min)].

3. Preparation of Linker-functionalized Merrifield Resins

### 3.1 O-linked oNB-type resin 8

![Chemical structure of oNB-type resin 8](image)

Merrifield resin LL (100-200 mesh, initial loading 0.5 mmol/g, 2.00 g, 1 mmol) was pre-washed three times with CH$_2$Cl$_2$ and was swollen in anhydrous DMF before 3-(hydroxymethyl)-4-nitropheno l 10 (0.68 g, 4 mmol), Cs$_2$CO$_3$ (1.63 g, 5 mmol) and TBAI (0.370 g, 1 mmol) were added. The suspension was gently shaken on a rotavap at 60 °C for 24 hours at 600 mbar. The mixture was filtered and the resin was washed successively with 20 mL each of THF, H$_2$O, THF, DMF, MeOH, CH$_2$Cl$_2$. The resin was suspended in anhydrous DMF and CsOAc (0.96 g, 5 mmol) was added. The suspension was gently shaken on a rotavap at 60 °C for 24 hours at 600 mbar. The mixture was filtered and the resin was washed successively with 20 mL each of THF, H$_2$O, THF, DMF, MeOH, CH$_2$Cl$_2$. The resin 8 was dried under high vacuum overnight and protected from light with aluminum foil. Loading (ca. 0.34 mmol/g) was determined according to reported protocol.¹
5-Aminopentanol (10 g, 97.0 mmol), 5-hydroxy-2-nitrobenzaldehyde 9 (15.4 g, 92 mmol) and Na$_2$SO$_4$ (22.7 g, 160 mmol) were stirred in anhydrous THF (250 mL) at room temperature. After 16 hours, the suspension was filtered and concentrated _in vacuo_. The crude product was dissolved in ethanol (250 mL) and cooled to 0 °C. NaBH$_4$ (3.67 g, 97 mmol) was added portion-wise to the mixture and allowed to warm to room temperature. After 4 hours, the mixture was quenched by addition of acetone (15 mL) and the solvents were evaporated to yield crude compound 11. To a solution of 11 in MeOH (250 mL) was added trimethylamine (40 mL, 291 mmol) and benzyl chloroformate (CbzCl, 34.6 mL, 243 mmol) at room temperature. After 5 hours, K$_2$CO$_3$ (40 g) was added to the reaction mixture and stirred for an hour. The crude mixture was filtered through Celite and the solvents evaporated _in vacuo_. The crude was extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$, filtered, concentrated and purified by flash column chromatography to obtain photocleavable linker S1 (17 g, 73%, yellow solid). $^1$H-NMR (400 MHz, CDCl$_3$, mixture of rotamers) δ 8.20 (d, $J = 8.9$ Hz, 1H), 7.43 – 7.32 (m, 2H), 7.29 – 7.23 (m, 3H), 7.10 (dd, $J = 6.6$, 3.0 Hz, 2H), 6.87 (dd, $J = 9.0$, 2.6 Hz, 1H), 6.82 (d, $J = 2.7$ Hz, 1H), 6.75 (s, 1H), 5.19 (s, 1H), 5.07 (s, 2H), 4.93 (s, 2H), 3.65 (q, $J = 6.9$ Hz, 3H), 3.39 (t, $J = 6.4$ Hz, 5H), 1.65 – 1.57 (m, 1H), 1.54 (dt, $J = 12.4$, 6.4 Hz, 5H), 1.44 (ddt, $J = 13.9$, 8.7, 4.7 Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) δ 162.64, 157.14, 139.69, 137.82, 135.86, 128.96, 128.58, 128.52, 128.33, 128.16, 127.56, 114.98, 113.01, 67.65, 62.24, 50.12, 49.13, 31.83, 27.79, 22.76 ppm. m/z (HRMS+) for C$_{20}$H$_{24}$N$_2$O$_4$Na [M+Na]$^+$ calcd. 411.1527, found: 411.1518.
\(^1\)H-NMR (400 MHz, CDCl\(_3\)) of S1

\(^{13}\)C-NMR: (100 MHz, CDCl\(_3\)) of S1

The N-linked oNB-type resin 3 was prepared following the protocol of resin 8. Loading (ca. 0.33 mmol/g) was measured.
3.3 O-linked MeNV-type resin S

To a suspension of apocynin 12 in ethanol (15 g, 90 mmol) was added K$_2$CO$_3$ (13.7 g, 99 mmol) and allyl bromide (8.6 mL, 99 mmol). The solution was refluxed overnight at 70 °C or until complete consumption of starting material was indicated by TLC spotting. The solution was filtered to remove solid material and the filtrate was evaporated. The crude was extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$, filtered, concentrated and purified by flash column chromatography (Hex/EA 3:1) to obtain compound S2 (18.3 g, 98%, yellow oil). IR (thin film) $\nu_{\text{max}} = 3083, 3003, 2938, 1673, 1587, 1509, 1464, 1417, 1358, 1333, 1267, 1217, 1179.26, 1150, 1136, 1079$ cm$^{-1}$; $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.52 (d, $J = 7.2$ Hz, 2H), 6.94 – 6.75 (m, 1H), 6.06 (ddt, $J = 17.3$ Hz, 10.7 Hz, 5.4 Hz, 1H), 5.41 (dd, $J = 17.4$ Hz, 1.6 Hz, 1H), 5.31 (dd, $J = 10.6$ Hz, 1.4 Hz, 1H), 4.66 (dt, $J = 5.5$ Hz, 1.5 Hz, 2H), 3.91 (s, 3H), 2.54 (s, 3H) ppm; $^{13}$C-NMR: (100 MHz, CDCl$_3$) $\delta$ 200.26, 154.35, 148.44, 138.25, 132.98, 131.57, 119.55, 108.76, 108.39, 70.30, 56.76, 30.51 ppm. m/z (HRMS+) for C$_{12}$H$_{15}$O$_3$ [M+H]$^+$ calcd. 207.1016, found: 207.1039.

$^1$H-NMR (400 MHz, CDCl$_3$) of S2

$^{13}$C-NMR: (100 MHz, CDCl$_3$) of S2
To a solution of compound S2 (18.3 g, 88 mmol) in trifluoroacetiac acid (60 mL) was added KNO₃ (9.86 g, 98 mmol) portion-wise at 10 °C. The resulting black solution was heated at 60 °C for 4 hours under argon. Upon complete consumption of starting material was indicated by TLC spotting, CH₂Cl₂ and cold water were added and the organic layer was extracted. The aqueous layer was extracted once with CH₂Cl₂. The combined organic layer was neutralized with saturated NaHCO₃ solution until off-gassing ceased. The organic layer was dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography (Hex/EA 3:1) to provide compound 13 (13.4 g, 60%, yellow solid). IR (thin film) ν_max = 3675, 2982, 2310, 1706, 1576, 1519, 1335, 1282, 1212, 1037, 814 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.59 (s, 1H), 6.75 (s, 1H), 6.05 (ddt, J = 17.3 Hz, 10.7 Hz, 5.5 Hz, 1H), 5.45 (dd, J = 17.2 Hz, 1.5 Hz, 1H), 5.36 (dd, J = 10.6 Hz, 1.3 Hz, 1H), 4.68 (dt, J = 5.5 Hz, 1.5 Hz, 2H), 3.96 (s, 3H), 2.47 (s, 3H) ppm; ¹³C-NMR: (100 MHz, CDCl₃) δ 200.26, 154.35, 148.44, 138.25, 132.98, 131.57, 119.55, 108.76, 108.39, 70.30, 56.76, 30.51 ppm. m/z (HRMS+) for C₁₂H₁₄NO₅ [M+H]+ calcd. 252.0866, found: 252.0875.

¹H-NMR: (400 MHz, CDCl₃) of 13:

¹³C-NMR: (100 MHz, CDCl₃) of 13:
To a solution of compound 13 (13.4 g, 53 mmol) in ethanol (125 mL) was added NaBH₄ (4.0 g, 106 mmol) portion-wise at 0°C. The reaction mixture was kept at 25°C for 4 hours under argon. Acetone was added to quench the reaction in an ice-bath and the excess solvent was removed under reduced pressure. 50 mL of EtOAc was added to the crude mixture and the organic phase was extracted with aqueous citric acid. The organic layer was dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography (Hex/EA 2:1) to provide compound 14 (12.0 g, 90%, yellow solid). IR (thin film) ʋ_max = 2983, 1517, 1332, 1271, 1214, 1103, 1018 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (s, 1H), 7.29 (s, 1H), 6.05 (ddt, J = 17.2, 10.7, 5.5 Hz, 1H), 5.53 (q, J = 6.2 Hz, 1H), 5.46 (dq, J = 17.2, 1.5 Hz, 1H), 5.34 (dq, J = 10.6, 1.3 Hz, 1H), 4.64 (dt, J = 5.5, 1.5 Hz, 2H), 3.97 (s, 3H), 1.53 (d, J = 6.3 Hz, 3H) ppm; ¹³C NMR (101 MHz, Chloroform-d) δ 154.13, 146.53, 139.46, 137.16, 132.04, 119.25, 109.34, 108.69, 70.17, 65.83, 56.50, 24.36 ppm. m/z (HRMS+) for C₁₂H₁₅NO₅ [M+H]+ calcd. 254.1023, found: 254.1019.

¹H-NMR: (400 MHz, CDCl₃) of 14:

¹³C-NMR: (100 MHz, CDCl₃) of 14:
To a stirred solution of compound 14 (3.79 g, 15 mmol) in anhydrous MeOH was added a catalytic amount of Pd(PPh\(_3\))\(_4\) (346 mg, 0.30 mmol) under a nitrogen atmosphere. The solution was stirred for 5 min, and K\(_2\)CO\(_3\) (6.25 g, 45 mmol) was added. The reaction was run overnight. The reaction mixture was dried, and the residue was treated with citric acid (1M). The aqueous phase was extracted with CH\(_2\)Cl\(_2\). The combined organic layer was dried over Na\(_2\)SO\(_4\), filtered, concentrated and purified by flash column chromatography (CH\(_2\)Cl\(_2\)/EA = 2:1) to give compound 15 (2.85 g, 89%, yellow solid).

IR (neat) \(\nu_{\text{max}}\) = 3661, 2983, 2891, 2307, 1463, 1275, 1152 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, acetone-\(d_6\)) \(\delta\) 8.48 (s, 1H), 7.45 (d, \(J = 5.3\) Hz, 2H), 5.43 (qd, \(J = 6.2, 4.1\) Hz, 1H), 4.54 (d, \(J = 4.1\) Hz, 1H), 3.97 (s, 3H), 2.05 (p, \(J = 2.2\) Hz, 1H), 1.44 (d, \(J = 6.2\) Hz, 3H) ppm; \(^{13}\)C NMR (100 MHz, acetone-\(d_6\)) \(\delta\) 206.26, 152.88, 145.78, 137.34, 111.40, 109.54, 65.37, 56.36, 25.34 ppm. m/z (HRMS+) for C\(_9\)H\(_{11}\)NO\(_5\)Na [M+Na]\(^{+}\) calcd. 236.0529, found 236.0926.

\(^1\)H-NMR (400 MHz, acetone-\(d_6\)) of 15:

\(^{13}\)C-NMR (100 MHz, acetone-\(d_6\)) of 15:
The N-linked oNB-type resin 5 was prepared following the protocol of resin 8. Loading (ca. 0.35 mmol/g) was measured.

### 3.4 N-linked MeNV-type resin 6

To a solution of compound 13 (7.50 g, 30 mmol) and 5-aminopentanol (9.23 g, 90 mmol) in absolute ethanol (70 mL) was added titanium(IV)-isopropoxide (13.25 mL, 45 mmol). The reaction was stirred under argon at room temperature overnight. Sodium borohydride (6.59 g, 180 mmol) was then added at 0 °C and the resulting mixture was stirred for an additional 2 hours. The reaction was quenched by addition of water and stirring was continued for additional 20 minutes. The solvent was removed under reduced pressure, and 1M citric acid (50 mL) was added. The crude mixture was filtered over Celite and extracted with CH₂Cl₂. The acidic aqueous phase was neutralized with sodium hydroxide NaOH to pH 10-12 and extracted with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated in vacuo to afford the crude amine S₃, which was used immediately in the next step.

To a solution of the crude amine S₃ in anhydrous DMF (50 mL) was added dropwise DIEA (26 mL, 150 mmol) and benzyl chloroformate (19.2 mL, 135 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight, after which water (100 mL) was added and the crude product was extracted with EtOAc twice. The combined organic layer was extracted with water three times. The extracted organic layers were dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography (Hex/EA = 2:3) to afford compound 16 (mixture of rotamers, 5.64 g, 39%, yellow oil). IR (neat) νmax = 3459, 3018, 3006, 2972, 2949, 2576, 2245, 2126, 1980, 1738, 1436, 1367, 1229 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.45 (s, 0H), 7.32 – 7.17 (m, 2H), 6.04 (ddt, J = 17.4, 10.7, 5.5 Hz, 0H), 5.75 (s, 1H), 5.59 – 5.24 (m, 1H), 5.04 (q, J = 12.3 Hz, 1H), 4.61 (dq, J = 5.6, 1.4 Hz, 1H), 3.79 (s, 1H), 3.55 (t, J = 6.5 Hz, 1H), 3.25 (s, 1H), 1.59 (d, J = 7.0 Hz, 1H), 1.45 (s, 3H), 1.33 – 1.16 (m, 1H) ppm; ¹³C-NMR (101 MHz, CDCl₃) δ 156.18, 153.33, 146.60, 132.08, 128.51, 128.07, 128.06, 119.30, 109.74, 70.21, 67.26, 62.77, 56.34, 32.31, 29.75, 23.24, 19.28 ppm. m/z (HRMS+) for C₂₅H₂₃N₂O₇Na [M+Na]⁺ calcd. 495.2102, found 495.2105.
To a stirred solution of compound 16 (5.64 g, 12 mmol) in anhydrous MeOH was added a catalytic amount of Pd(PPh₃)₄ (277 mg, 0.24 mmol) under a nitrogen atmosphere. The solution was stirred for 5 min, and K₂CO₃ (5 g, 36 mmol) was added. The reaction was run overnight. The reaction mixture was dried, and the residue was treated with citric acid 1M. The aqueous phase was extracted with CH₂Cl₂. The combined organic layer was dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography (CH₂Cl₂/EA = 3:1) to give compound S4 (mixture of rotamers, 2.1 g, 40%, yellow solid). IR (neat) ν max = 3459, 3018, 2972, 2576, 2245, 2126, 1738, 1436, 1366, 1217 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, mixture of rotamers) δ 7.50 (s, 1H), 7.27 (q, J = 15.7, 10.9 Hz, 5H), 5.86 (d, J = 103.3 Hz, 2H), 5.07 (q, J = 12.3 Hz, 2H), 3.83 (s, 3H), 3.57 (t, J = 6.4 Hz, 2H), 3.26 (s, 2H), 1.71 – 1.32 (m, 8H), 1.32 – 1.14 (m, 2H) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ 156.20, 150.35, 144.57, 128.51, 128.12, 128.07, 111.64, 67.30, 62.76, 56.32, 32.24, 29.61, 23.21, 19.29 ppm. m/z (HRMS+) for C₂₃H₂₇N₂O₇Na [M+Na]^+ calcd. 455.1789, found 436.1792.
$^{1}$H-NMR (400 MHz, CDCl$_3$) of compound S4:

13C-NMR (100 MHz, CDCl$_3$) of compound S4:

The OBn-linked MeNV-type resin 6 was prepared following the protocol of resin 8. Loading (ca. 0.32 mmol/g) was measured.
To a solution of 4-allyloxymethylbenzyl alcohol $^2$ (1.14 g, 6.3 mmol) in anhydrous CH$_2$Cl$_2$ was added dropwise phosphorus tribromide (1.2 mL, 12.6 mmol). The solution was stirred at room temperature for an hour. The reaction mixture was diluted with CH$_2$Cl$_2$ (40 mL) and washed twice with saturated aqueous NaHCO$_3$. The combined organic layer was dried over Na$_2$SO$_4$, filtered, concentrated and purified by flash column chromatography (CH$_2$Cl$_2$) to give 4-allyloxymethylbenzyl bromide S4, which was used immediately for the next step.

To a solution of compound 14 (1.00 g, 3.9 mmol) in anhydrous THF (40 mL) was added NaH (60% in mineral oil, 0.78 g, 19.5 mmol). After one hour at room temperature, the freshly prepared compound S4 (1.5 g, 6.3 mmol) and TBAI (2.51 g, 7.8 mmol) was added. The reaction was heated to 60 °C and the reaction was monitored by TLC. More NaH and TBAI were added after 10 hours. The reaction was cooled to room temperature and THF was removed under reduced pressure. EtOAc (50 mL) was added to the crude and an ice-water solution (50 mL) was slowly quench the residual NaH. The extracted organic layers were dried over Na$_2$SO$_4$, filtered, concentrated and purified by flash column chromatography (Hex/EA = 5:1 to 3:1) to afford compound 17 (1.34 g, 83%, yellow oil). IR (neat) $\nu_{\text{max}}$ = 2981, 1581, 1516, 1336, 1272, 1215, 1094 cm$^{-1}$; $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.60 (d, $J = 1.4$ Hz, 1H), 7.36 – 7.22 (m, 5H), 6.08 (ddt, $J = 16.2$, 10.7, 5.3 Hz, 1H), 5.95 (ddt, $J = 16.1$, 10.5, 5.4 Hz, 1H), 5.47 (d, $J = 17.3$ Hz, 1H), 5.36 (t, $J = 10.6$ Hz, 1H), 5.33 – 5.24 (m, 2H), 5.21 (d, $J = 10.1$ Hz, 1H), 4.67 (d, $J = 5.5$ Hz, 2H), 4.51 (s, 2H), 4.34 (s, 2H), 4.02 (d, $J = 5.6$ Hz, 2H), 3.93 (s, 3H), 1.55 (d, $J = 6.1$ Hz, 3H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 154.29, 146.61, 140.36, 137.94, 137.26, 135.76, 134.69, 132.03, 127.90, 127.80, 119.18, 117.23, 109.15, 108.59, 73.29, 71.79, 71.10, 70.12, 56.46, 23.67 ppm. m/z (HRMS+) for C$_{23}$H$_{27}$NO$_6$Na [M+Na]$^+$ calcd. 436.1731, found 436.1741.
$^1$H-NMR (400 MHz, CDCl$_3$) of compound 17:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of compound 17:
To a solution of compound 17 (1.16 g, 2.8 mmol) in MeOH (35 mL) was added 1,3-dimethylbarbituric acid (1.80 g, 11.5 mmol). Tetrakis(triphenylphosphine)palladium(0) (488 mg, 0.42 mmol) was added and the system was charged with nitrogen. The reaction was stirred at room temperature for 18 hours, after which the solvent was removed under reduced pressure. CH₂Cl₂ (50 mL) was added to the crude and the organic phase was washed with aqueous citric acid (50 mL). After extracting the aqueous phase with CH₂Cl₂ (50 mL), the organic layers were combined and dried with Na₂SO₄. The extracted organic layers were dried over Na₂SO₄, filtered, concentrated and purified by flash column chromatography (Hex/EA = 2:1 to 1:2, then Hex/CH₂Cl₂/EA = 1:1:2) to give compound S5 (710 mg, 76%, yellow oil). IR (neat) νmax = 3377, 1674, 1519, 1341, 1279, 1215, 1092, 1015 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.65 (s, 1H), 7.37 – 7.26 (m, 5H), 5.24 (q, J = 6.3 Hz, 1H), 4.71 (s, 2H), 4.37 (d, J = 1.8 Hz, 2H), 3.99 (s, 3H), 1.98 – 1.64 (br, 1H), 1.57 (d, J = 6.3 Hz, 3H) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ 151.31, 144.51, 141.29, 140.43, 137.35, 134.47, 127.99, 127.19, 111.03, 108.08, 73.12, 70.99, 65.12, 56.49, 23.81 ppm. m/z (HRMS+) for C₁₇H₁₉NO₆Na [M+Na]^+ calcd. 356.1105, found 356.1117.

¹H-NMR (400 MHz, CDCl₃) of compound S5:

¹³C-NMR (100 MHz, CDCl₃) of compound S5:
The OBn-linked MeNV-type resin 7 was prepared following the protocol of resin 8. Loading (ca. 0.36 mmol/g) was measured.
4. Synthesis of Oligosaccharides

4.1 Synthesis of monosaccharide 18

Automation sequence: A

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 1x     | A (6.5 eq.)    | I – Acidic Wash | -20 °C (T1) 5 min (t1) |
|        |                 | IIa – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2) |
|        |                 | III – Capping |       |
|        |                 | IVa – Fmoc Deprotection |       |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 18 (2.1 mg, 34% from resin 8, or 4.2 mg, 65% from resin 5).

Analytical Data for 18: $^1$H-NMR (400 MHz, CDCl$_3$) δ 8.13 – 8.02 (m, 2H), 7.58 (td, $J = 7.2, 1.3$ Hz, 1H), 7.51 – 7.42 (m, 2H), 7.37 – 7.18 (m, 8H), 5.60 (dd, $J = 3.2, 1.9$ Hz, 1H), 5.31 (d, $J = 1.9$ Hz, 1H), 4.98 – 4.85 (m, 1H), 4.79 (dd, $J = 16.7, 11.4$ Hz, 1H), 4.63 (dd, $J = 10.9, 6.6$ Hz, 1H), 4.61 – 4.52 (m, 1H), 4.20 – 4.12 (m, 1H), 4.01 – 3.87 (m, 2H), 3.82 (qd, $J = 11.8, 2.6$ Hz, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 138.11, 137.88, 133.32, 130.05, 129.89, 129.73, 128.59, 128.49, 128.45, 128.42, 128.32, 128.17, 127.96, 127.83, 127.65, 92.62, 77.53, 75.23, 74.02, 71.89, 71.54, 69.33, 62.19. m/z (HRMS+) for C$_{27}$H$_{28}$O$_7$Na [M+Na]$^+$ calcd. 487.1727, found: 487.1743.

Crude NP-HPLC of 18 (ELSD trace, Method Villa, $t_R$ = 18.7 min)
$^1$H-NMR (400 MHz, CDCl$_3$) of 18:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of 18:
4.2 Synthesis of monosaccharide 19

**Automation sequence: A**

| Repeat | Building Blocks       | Modules                                      | Notes                      |
|--------|-----------------------|----------------------------------------------|----------------------------|
| 1x     | A (6.5 eq.)          | I – Acidic Wash                              | -20 °C (T1) 5 min (t1)     |
|        |                       | IIa – Glycosylation with Thioglycoside        | 0 °C (T2) 20 min (t2)      |
|        |                       | III – Capping                                |                            |
|        |                       | IVa – Fmoc Deprotection                       |                            |

Cleavage from solid support as described in **Module V – Photocleavage**, followed by purification using preparative HPLC (Method VIIIb) afforded compound 19 (5.4 mg, 65% from resin 3, or 3.5 mg, 44% from resin 6).

Analytical Data for 19: $^1$H-NMR (400 MHz, CDCl$_3$) δ 8.13 – 8.06 (m, 2H), 7.67 – 7.58 (m, 1H), 7.50 (t, J = 7.7 Hz, 2H), 7.41 – 7.22 (m, 14H), 5.60 (dd, J = 3.2, 1.9 Hz, 1H), 5.12 (s, 2H), 4.99 – 4.90 (m, 2H), 4.87 – 4.76 (m, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.61 (d, J = 11.3 Hz, 1H), 4.18 – 4.09 (m, 1H), 4.02 – 3.65 (m, 5H), 3.44 (dt, J = 9.7, 6.3 Hz, 1H), 3.23 (q, J = 6.7 Hz, 2H), 1.70 – 1.49 (m, 4H), 1.28 (q, J = 4.4, 3.4 Hz, 2H)ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 165.78, 138.15, 137.98, 136.61, 133.33, 129.92, 129.82, 128.55, 128.53, 128.47, 128.34, 128.27, 128.17, 128.13, 127.98, 127.89, 127.66, 97.83, 78.20, 74.13, 71.82, 71.56, 69.20, 67.82, 66.65, 62.22, 40.93, 29.76, 29.36, 29.01, 23.35 ppm. m/z (HRMS+) for C$_{40}$H$_{45}$NO$_9$Na [M+Na]$^+$ calc'd. 706.2986, found: 706.2997.

Crude NP-HPLC of 19 (ELSD trace, Method VIIIa, $t_R$= 11.8 min)
$^1$H-NMR (400 MHz, CDCl$_3$) of 19:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of 19:
4.3 Synthesis of monosaccharide 20

Automation sequence: A

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 1x     | A (6.5 eq.)    | I – Acidic Wash | -20 °C (T1) 5 min (t1) |
|        | IIA – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2) |
|        | III – Capping |         |       |
|        | IVA – Fmoc Deprotection |         |       |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 20 (3.7 mg, 44% from resin 7).

Analytical Data for 20: $^1$H-NMR $^2$H NMR (600 MHz, CDCl$_3$) δ 8.05 (dd, $J = 8.2$, 1.4 Hz, 2H), 7.61 – 7.55 (m, 1H), 7.46 (t, $J = 7.6$ Hz, 2H), 7.35 (d, $J = 7.9$ Hz, 2H), 7.34 – 7.29 (m, 4H), 7.31 – 7.26 (m, 4H), 7.28 – 7.19 (m, 3H), 5.62 (dd, $J = 3.2$, 1.9 Hz, 1H), 5.00 (d, $J = 1.9$ Hz, 1H), 4.90 (d, $J = 10.9$ Hz, 1H), 4.76 (d, $J = 11.3$ Hz, 1H), 4.72 – 4.66 (m, 3H), 4.64 (d, $J = 10.9$ Hz, 1H), 4.54 (dd, $J = 23.7$, 11.6 Hz, 2H), 4.14 (dd, $J = 9.4$, 3.1 Hz, 1H), 3.97 (t, $J = 9.5$ Hz, 1H), 3.84 (dd, $J = 11.7$, 2.8 Hz, 1H), 3.82 – 3.74 (m, 2H) ppm; $^{13}$C-NMR (150 MHz, CDCl$_3$) δ 165.62, 133.25, 129.85, 128.45, 128.40, 128.32, 128.27, 128.17, 127.92, 127.81, 127.58, 127.14, 97.21, 78.18, 75.30, 74.03, 72.03, 71.58, 69.30, 69.14, 65.07, 62.11 ppm. m/z (HRMS+) for C$_{35}$H$_{36}$O$_8$Na [M+Na]$^+$ calcd. 607.2302, found: 607.2311.

Crude NP-HPLC of 20 (ELSD trace, Method VIIa, $t_R=21.7$ min)
\(^1\)H-NMR (600 MHz, CDCl\(_3\)) of 20:

\[^{13}\)C-NMR (150 MHz, CDCl\(_3\)) of 20:
### 4.4 Synthesis of tetrasaccharide 21

**Resin:**

- 8
- 5

**Building Block:**

- FmocO
- BnO
- Br
- Stil
- Obz

**Automated Glycan Assembly**

**Repeat** | **Building Blocks** | **Modules** | **Notes**
--- | --- | --- | ---
4x | A (6.5 eq.) | I – Acidic Wash | -20 °C (T1) 5 min (t1)
 | IIa – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2)
 | III – Capping | 0 °C (T2) 20 min (t2)
 | IVa – Fmoc Deprotection |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 21 (5.4 mg, 22% from resin 8, or 17.4 mg, 69% from resin 5).

**Analytical Data for 21:**

- $^1$H-NMR (400 MHz, CDCl$_3$) δ 8.19 – 8.07 (m, 9H), 7.62 (t, $J$ = 7.4 Hz, 1H), 7.55 – 7.44 (m, 12H), 7.35 – 7.08 (m, 40H), 5.80 (t, $J$ = 2.5 Hz, 1H), 5.76 (d, $J$ = 3.0 Hz, 1H), 5.67 – 5.57 (m, 1H), 5.23 (d, $J$ = 1.8 Hz, 1H), 5.15 (d, $J$ = 1.8 Hz, 1H), 5.06 (dd, $J$ = 10.3, 1.8 Hz, 2H), 4.94 – 4.86 (m, 4H), 4.86 – 4.78 (m, 4H), 4.73 (d, $J$ = 11.4 Hz, 1H), 4.16 (dd, $J$ = 9.3, 3.3 Hz, 1H), 4.13 – 4.03 (m, 4H), 3.89 (ddt, $J$ = 19.8, 13.9, 10.2 Hz, 11H), 3.76 – 3.56 (m, 5H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 165.67, 137.60, 133.37, 133.33, 133.22, 129.94, 129.89, 129.86, 128.66, 128.59, 128.55, 128.38, 128.37, 128.35, 128.33, 128.29, 128.26, 128.23, 128.13, 128.05, 128.00, 127.87, 127.78, 127.72, 127.67, 127.64, 127.55, 127.50, 98.04, 97.78, 97.72, 92.59, 78.43, 78.08, 75.28, 75.20, 75.11, 74.43, 74.23, 73.69, 72.05, 71.58, 71.37, 71.23, 71.01, 70.93, 70.56, 69.26, 68.74, 68.62, 68.51, 66.37, 66.11, 65.53, 61.86 ppm. m/z (HRMS+) for C$_{108}$H$_{106}$O$_{25}$Na [M+Na]$^+$ calcd. 1826.6949, found: 1826.7011.

Crude NP-HPLC of 21 (ELSD trace, Method VIIIa, $t_R$= 25 min)
$^1$H-NMR (400 MHz, CDCl$_3$) of 21:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of 21:

$^{13}$C-$^1$H HSQC of 21:
4.5 Synthesis of tetrascarhide 22

Automation sequence: AAAA

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 4x     | A (6.5 eq.)   | I – Acidic Wash | -20 °C (T1) 5 min (t1) |
|        |                | IIa – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2) |
|        |                | III – Capping | |
|        |                | IVa – Fmoc Deprotection | |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 22 (14.5 mg, 55% from resin 3, or 11.8 mg, 46% from resin 6).

Analytical data for 22: \(^1\)H-NMR (600 MHz, CDCl₃) δ 8.14 (dtd, J = 6.2, 4.2, 2.0 Hz, 3H), 8.12 – 8.09 (m, 2H), 8.09 – 8.04 (m, 2H), 7.63 – 7.55 (m, 1H), 7.53 – 7.44 (m, 10H), 7.50 (d, J = 1.9 Hz, 1H), 7.49 (s, 1H), 4.90 (s, 1H), 4.88 (s, 2H), 4.85 (d, J = 8.9 Hz, 1H), 4.83 (d, J = 2.3 Hz, 1H), 4.79 (d, J = 11.9 Hz, 2H), 4.77 (s, 1H), 4.70 (d, J = 11.4 Hz, 1H), 4.59 (d, J = 11.1 Hz, 1H), 4.55 (d, J = 11.0 Hz, 1H), 4.48 (d, J = 4.8 Hz, 1H), 4.46 (d, J = 4.6 Hz, 1H), 4.43 (d, J = 3.8 Hz, 1H), 4.41 (d, J = 3.4 Hz, 1H), 4.40 (d, J = 11.5 Hz, 1H), 4.35 (d, J = 11.4 Hz, 1H), 4.08 (ddd, J = 9.4, 6.1, 3.2 Hz, 2H), 4.02 (dt, J = 9.4, 3.2 Hz, 2H), 3.98 – 3.94 (m, 2H), 3.93 – 3.89 (m, 2H), 3.90 – 3.86 (m, 1H), 3.86 – 3.82 (m, 1H), 3.80 (dq, J = 10.0, 1.7 Hz, 1H), 3.76 (ddd, J = 11.5, 5.2, 2.5 Hz, 2H), 3.69 – 3.63 (m, 4H), 3.60 (dd, J = 11.5, 1.7 Hz, 1H), 3.55 (dt, J = 9.8, 3.2 Hz, 1H), 3.51 (dd, J = 11.6, 1.8 Hz, 1H), 3.40 (dt, J = 10.0, 6.5 Hz, 1H), 3.16 (q, J = 7.3 Hz, 2H), 1.57 (t, J = 7.5 Hz, 2H), 1.49 (q, J = 7.4 Hz, 2H), 1.35 (dt, J = 9.6, 4.5 Hz, 2H) ppm; \(^{13}\)C-NMR (150 MHz, CDCl₃) δ 137.57, 133.26, 133.22, 129.93, 129.87, 129.85, 129.83, 129.81, 128.59, 128.54, 128.45, 128.31, 128.29, 128.27, 128.23, 128.16, 128.12, 128.06, 128.00, 127.98, 127.62, 127.58, 127.35, 127.31, 127.21, 98.34, 98.11, 98.07, 97.85, 78.57, 78.31, 78.19, 77.68, 75.14, 75.06, 74.98, 74.19, 73.89, 73.82, 73.71, 72.06, 71.61, 71.38, 71.32, 71.18, 70.88, 70.72, 69.04, 68.57, 68.53, 68.40, 67.75, 66.52, 66.12, 65.84, 61.82, 40.92, 29.75, 29.01, 23.40 ppm. m/z (HRMS+) for C₁₂₁H₁₂₂O₂₇N₁₁Na [M+Na]^+ calcd. 2045.8208, found: 2045.8221.
Crude NP-HPLC of 22 (ELSD trace, Method VIIIa, $t_R=22$ min)

$^1$H-NMR (600 MHz, CDCl$_3$) of 22:

$^{13}$C-NMR (150 MHz, CDCl$_3$) of 22:
4.6 Synthesis of tetrasaccharide 23

Automation sequence: AAAA

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 4x     | A (6.5 eq.)    | I – Acidic Wash | -20 °C (T1) | 5 min (t1) |
|        |                | IIa – Glycosylation with Thioglycoside | 0 °C (T2) | 20 min (t2) |
|        |                | III – Capping |       |       |
|        |                | IVa – Fmoc Deprotection |       |       |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 23 (15.2 mg, 55% from resin 7).

Analytical Data for 23: ^1^H-NMR (400 MHz, CDCl₃) δ 8.19-8.14 (m, 4H), 8.10 (s, 3H), 8.08 (d, J = 2.0 Hz, 2H), 8.06 (d, J = 1.5 Hz, 1H), 7.64 – 7.57 (m, 1H), 7.48 (ddd, J = 13.9, 6.9, 4.7 Hz, 11H), 7.33 – 7.18 (m, 28H), 7.18 – 7.09 (m, 9H), 5.82 (dd, J = 3.1, 1.9 Hz, 1H), 5.80 (dd, J = 3.1, 1.9 Hz, 1H), 5.77 – 5.70 (m, 1H), 5.68 (dd, J = 3.3, 1.8 Hz, 1H), 5.09 (d, J = 1.8 Hz, 1H), 5.06 (d, J = 1.7 Hz, 1H), 5.03 (d, J = 1.8 Hz, 1H), 4.96 (d, J = 1.8 Hz, 1H), 4.90 (d, J = 2.1 Hz, 1H), 4.87 (t, J = 1.8 Hz, 1H), 4.83 (s, 1H), 4.81 (s, 1H), 4.78 (s, 1H), 4.69 (dd, J = 14.5, 11.7 Hz, 2H), 4.63 (s, 2H), 4.59 (d, J = 11.1 Hz, 1H), 4.55-4.50 (m, 2H), 4.50 – 4.47 (m, 1H), 4.45 (d, J = 2.5 Hz, 2H), 4.42 (s, 1H), 4.41 – 4.32 (m, 1H) ppm; ^13^C-NMR (100 MHz, CDCl₃) δ 137.58, 133.36, 133.30, 129.92, 129.89, 129.85, 128.66, 128.57, 128.53, 128.52, 128.38, 128.35, 128.32, 128.29, 128.22, 128.19, 128.06, 128.04, 127.72, 127.68, 127.64, 127.42, 127.38, 127.33, 127.13, 98.36, 98.18, 98.09, 96.86, 78.66, 78.27, 78.22, 75.21, 75.12, 74.21, 73.92, 73.81, 73.68, 72.10, 71.68, 71.38, 71.35, 71.19, 71.06, 70.92, 69.04, 68.96, 68.55, 68.39, 66.22, 65.85, 65.51, 65.03, 61.83. m/z [HRMS+] for C₁₁₅H₁₁₄O₃₂Na [M+Na]^+ calcd. 1945.7524, found: 1945.7549.

Crude NP-HPLC of 23 (ELSD trace, Method Vllla, tᵣ = 27.8 min)
$^1$H-NMR (400 MHz, CDCl$_3$) of 23:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of 23:

$^{13}$C-$^1$H HSQC of 23:
## 4.7 Synthesis of hexasaccharide 24

Resin:

![Resin Diagram]

Building Block:

![Building Block Diagram]

Automation sequence: BBBBBBB

| Repeat | Building Blocks | Modules | Notes |
|--------|-----------------|---------|-------|
| 6x     | B (6.5 eq.)     | I – Acidic Wash | -20 °C (T1) 5 min (t1) |
|        |                 | IIa – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2) |
|        |                 | III – Capping |       |
|        |                 | IVa – Fmoc Deprotection |       |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 24 (6.1 mg, 17% from resin 8, or 25.5 mg, 70% from resin 5).

Analytical Data for 24: $^1$H-NMR (400 MHz, CDCl$_3$) δ 8.17 (dq, $J = 5.2$, 3.5, 2.7 Hz, 6H), 8.14 – 8.05 (m, 6H), 7.61 (t, $J = 7.4$ Hz, 1H), 7.50 (ddd, $J = 13.7$, 6.2, 4.1 Hz, 18H), 7.35 – 7.07 (m, 46H), 5.84 (q, $J = 2.8$ Hz, 2H), 5.78 (dt, $J = 7.5$, 2.4 Hz, 3H), 5.68 – 5.59 (m, 1H), 5.22 (d, $J = 1.8$ Hz, 1H), 5.15 (d, $J = 1.7$ Hz, 1H), 5.09 (d, $J = 1.7$ Hz, 1H), 5.07 (d, $J = 1.7$ Hz, 1H), 5.05 (d, $J = 1.8$ Hz, 2H), 4.90 (dq, $J = 10.8$, 3.8, 3.0 Hz, 6H), 4.85 – 4.77 (m, 6H), 4.74 (d, $J = 11.4$ Hz, 1H), 4.61 (d, $J = 11.2$ Hz, 1H), 4.58 – 4.49 (m, 3H), 4.48 – 4.40 (m, 6H), 4.35 (dd, $J = 11.5$, 7.5 Hz, 2H), 4.15 (dd, $J = 9.2$, 3.2 Hz, 1H), 4.06 (tdd, $J = 9.2$, 6.9, 4.1 Hz, 5H), 4.00 – 3.90 (m, 4H), 3.84 (ddd, $J = 14.2$, 11.3, 8.6, 2.8 Hz, 6H), 3.77 – 3.59 (m, 7H), 3.57 – 3.43 (m, 3H) ppm. $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 138.44, 138.22, 137.54, 133.38, 133.32, 133.22, 129.93, 129.84, 128.67, 128.59, 128.56, 128.53, 128.36, 128.33, 128.31, 128.27, 128.17, 128.13, 128.09, 128.04, 127.82, 127.77, 127.71, 127.66, 127.62, 127.59, 127.55, 127.44, 127.39, 127.29, 127.09, 98.44, 98.07, 97.62, 78.34, 78.16, 78.10, 75.20, 75.11, 74.39, 73.96, 73.72, 73.64, 72.05, 71.58, 71.48, 71.32, 71.18, 71.00, 70.96, 70.86, 69.24, 68.70, 68.49, 68.33, 66.34, 65.73, 65.37, 61.81 ppm. m/z (HRMS+) for C$_{162}$H$_{158}$O$_{37}$Na [M+Na]$^+$ calcld. 2719.0408, found: 2179.0471.

Crude NP-HPLC of 24 (ELSD trace, Method VIIIa, $t_R$ = 27.7 min)
$^1$H-NMR (400 MHz, CDCl$_3$) of 24:

$^{13}$C-NMR (100 MHz, CDCl$_3$) of 24:

$^{13}$C-$^1$H HSQC of 24:
4.8 Synthesis of hexasaccharide 25

Automation sequence: BBBBBB

| Repeat | Building Blocks | Modules | Notes |
|--------|-----------------|---------|-------|
| 6x     | B (6.5 eq.)     | Ia – Glycosylation with Thioglycoside | -20 °C (T1) 5 min (t1) |
|        |                  | IIa – Glycosylation with Thioglycoside | 0 °C (T2) 20 min (t2) |
|        |                  | III – Capping |  |
|        |                  | IVa – Fmoc Deprotection |  |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 25 (18.5 mg, 48% from resin 3, or 16.0 mg, 43% from resin 6).

Analytical Data for compound 25 are consistent with previous report.³

Crude NP-HPLC of 25 (ELSD trace, Method VIIa, t_R = 30.6 min)

³H-NMR (700 MHz, CDCl₃) of 25:
4.9 Synthesis of hexasaccharide 26

Resin: 

Automation sequence: BBBBBB

| Repeat | Building Blocks | I – Acidic Wash | Notes |
|--------|-----------------|-----------------|-------|
| 6x     | B (6.5 eq.)     | IIa – Glycosylation with Thioglycoside | -20 °C (T1) 5 min (t1) 0 °C (T2) 20 min (t2) |

-20 °C (T1) 5 min (t1)
-20 °C (T2) 20 min (t2)

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 26 (18.6 mg, 46% from resin 7).

Analytical Data for 26: 
1H-NMR (600 MHz, CDCl3) δ 7.88 (dd, J = 15.5, 7.7 Hz, 4H), 7.85 – 7.76 (m, 8H), 7.66 (dq, J = 35.0, 14.2, 7.3 Hz, 7H), 7.46 – 7.29 (m, 17H), 7.17 – 6.98 (m, 39H), 6.98 – 6.85 (m, 10H), 5.22 (t, J = 8.9 Hz, 1H), 5.16 (td, J = 8.8, 4.0 Hz, 2H), 5.09 (dt, J = 17.2, 8.8 Hz, 2H), 5.03 (t, J = 8.8 Hz, 1H), 4.95 – 4.89 (m, 2H), 4.86 – 4.83 (m, 1H), 4.81 (d, J = 12.3 Hz, 1H), 4.72 (dd, J = 12.2, 8.8 Hz, 2H), 4.66 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 8.1 Hz, 1H), 4.59 – 4.47 (m, 10H), 4.43 (d, J = 7.7 Hz, 1H), 4.40 – 4.34 (m, 3H), 4.32 – 4.25 (m, 4H), 4.18 (d, J = 12.1 Hz, 1H), 4.12 (d, J = 12.2 Hz, 1H), 4.07 (t, J = 9.3 Hz, 1H), 4.03 – 3.97 (m, 7H), 3.79 (t, J = 9.0 Hz, 1H), 3.59 (dd, J = 9.9, 4.6 Hz, 1H), 3.55 – 3.45 (m, 5H), 3.41 (q, J = 5.8 Hz, 3H), 3.37 (d, J = 7.4 Hz, 1H), 3.30 (ddt, J = 18.3, 13.2, 9.0 Hz, 7H), 3.06 (dt, J = 9.7, 2.7 Hz, 1H), 2.90 – 2.84 (m, 1H), 2.82 – 2.72 (m, 3H) ppm; 13C-NMR (150 MHz, CDCl3) δ 164.80, 138.79, 137.69, 133.32, 133.17, 129.78, 129.68, 129.64, 129.54, 128.53, 128.51, 128.48, 128.46, 128.43, 128.36, 128.25, 128.19, 128.17, 128.12, 128.06, 128.02, 127.88, 127.84, 127.80, 127.78, 127.74, 127.73, 127.63, 127.56, 127.03, 126.88, 126.86, 126.79, 99.97, 99.80, 99.33, 81.81, 80.00, 79.89, 76.09, 75.97, 75.86, 74.52, 74.47, 74.36, 74.29, 74.23, 74.15, 74.02, 73.73, 73.54, 73.41, 73.39, 73.31, 73.26, 73.03, 72.86, 71.11, 69.74, 67.31, 67.00, 65.03 ppm. m/z (HRMS+) for C170H166O38Na [M+Na]+ calcd. 2839.0983, found: 2839.1030.

Crude NP-HPLC of 26 (ELSD trace, Method VIIIa, tR= 38 min)
$^1$H-NMR (600 MHz, CDCl$_3$) of 26:

$^{13}$C-NMR (150 MHz, CDCl$_3$) of 26:

$^{13}$C-$^1$H HSQC of 26:
4.10 Synthesis of octasaccharide 27

To a solution of recrystallized silver triflate (6.6 mg, 25.7 µmol) and bis (cyclopentadienyl) hafnium dichloride (7.3 mg, 19.3 µmol) in anhydrous toluene (1 mL) were added activated 3Å molecular sieves (10 mg) at room temperature for 15 min protected from light. The reaction was cooled to -40 °C. A solution of glycosyl donor 28 (8.5 mg, 6.4 µmol) and glycosyl acceptor 29 (10.2 mg, 4.3 µmol) in anhydrous CH₂Cl₂ (1 mL) was introduced. The mixture was stirred at -20 °C for 4 hours, quenched with Et₃N, diluted with EtOAc, and filtered through Celite. The filtrate was extracted with aqueous NaHCO₃ (10 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄, filtered, concentrated and purified by preparative HPLC (Method VIIIb) to afford compound 27 (3.33 mg, 21% isolated yield).

Analytical Data for 27: ¹H-NMR (600 MHz, CDCl₃) δ 7.99 (dd, J = 19.3, 7.5 Hz, 1H), 7.94 – 7.89 (m, 1H), 7.53 (dt, J = 26.9, 7.3 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.30 (td, J = 14.9, 14.4, 7.3 Hz, 15H), 7.28 – 7.21 (m, 4H), 7.22 (d, J = 6.3 Hz, 5H), 7.15 (qd, J = 17.1, 16.2, 10.8 Hz, 8H), 7.08 (s, 1H), 7.11 – 7.03 (m, 1H), 6.91 (s, 1H), 5.62 – 5.49 (m, 1H), 5.05 – 4.97 (m, 2H), 4.92 – 4.86 (m, 1H), 4.85 – 4.72 (m, 2H), 4.67 (d, J = 15.3 Hz, 1H), 4.59 (ddd, J = 24.9, 18.7, 10.5 Hz, 3H), 4.55 (s, 1H), 4.56 – 4.44 (m, 2H), 4.40 (dt, J = 20.6, 7.7 Hz, 1H), 4.22 – 3.89 (m, 4H), 3.81 (dd, J = 13.6, 2.8 Hz, 1H), 3.74 (dt, J = 20.9, 8.0 Hz, 2H), 3.69 – 3.60 (m, 2H), 3.50 (s, 0H), 3.56 – 3.42 (m, 1H), 3.39 (t, J = 6.3 Hz, 1H), 3.34 (s, 1H), 3.39 – 3.29 (m, 1H), 3.08 (dd, J = 16.8, 9.8 Hz, 1H), 2.03 (d, J = 4.1 Hz, 1H), 1.95 (d, J = 4.8 Hz, 1H), 1.93 (s, 1H), 1.80 (t, J = 6.7 Hz, 2H), 1.44 (s, 2H), 1.47 – 1.39 (m, 1H), 1.37 (d, J = 6.2 Hz, 1H), 1.31 – 1.18 (m, 3H) ppm; ¹³C-NMR (150 MHz, CDCl₃) δ 130.15, 129.74, 129.00, 128.84, 128.58, 128.46, 128.43, 128.38, 128.35, 128.31, 128.29, 128.26, 128.21, 128.17, 128.10, 128.05, 127.89, 127.84, 127.82, 127.77, 127.71, 127.58, 127.55, 127.26, 126.98, 99.44, 78.64, 75.98, 75.05, 74.92, 73.38, 72.34, 71.98, 71.83, 71.60, 69.67, 66.46, 62.77, 62.45, 40.87, 29.67, 28.88, 20.81, 16.61 ppm. m/z (HRMS+) for C₁₉₉H₂₀₉Cl₆N₃O₄₉Na[M+Na]⁺ calcd. 3661.2015, found: 3661.3992.

Purified NP-HPLC of 27 (ELSD trace, Method Vilia, tᵣ= 31.1 min)
$\text{H-NMR (600 MHz, CDCl}_3\text{) of 27:}$

$\text{C-NMR (150 MHz, CDCl}_3\text{) of 27:}$

$\text{C-H HSQC of 27:}$
4.11 Synthesis of glycosyl donor 28

To a solution of compound 30 (9.6 mg, 7.28 µmol) in CH₂Cl₂ (2 mL) was added a solution of Deoxo-Fluor (11 µL, 15 µmol) at -30 °C. The reaction was stirred for 1 hour, after which the temperature was raised to 0 °C. The reaction was quenched with 2 mL of NaHCO₃. The organic phase was extracted with aqueous citric acid, dried over Na₂SO₄, filtered, concentrated and purified by preparative HPLC (Method VIIIb) to provide compound 28 (8.5 mg, 89% yield).

Analytical Data for 28: ¹H-NMR (600 MHz, CDCl₃) δ 7.95 – 7.84 (m, 2H), 7.63 – 7.57 (m, 1H), 7.49 – 7.41 (m, 2H), 7.38 – 7.16 (m, 39H), 6.76 (d, J = 9.3 Hz, 1H), 5.57 (d, J = 2.9 Hz, 0H), 5.51 – 5.44 (m, 2H), 5.26 (d, J = 3.8 Hz, 1H), 4.82 (d, J = 10.9 Hz, 1H), 4.78 (dd, J = 11.8, 8.5 Hz, 2H), 4.69 (d, J = 11.5 Hz, 1H), 4.67 – 4.64 (m, 2H), 4.63 – 4.60 (m, 1H), 4.57 (d, J = 20.7 Hz, 1H), 4.53 (d, J = 6.2 Hz, 1H), 4.51 (d, J = 2.6 Hz, 1H), 4.50 – 4.46 (m, 1H), 4.33 (d, J = 12.2 Hz, 1H), 4.27 (dd, J = 10.7, 7.5 Hz, 1H), 4.22 – 4.14 (m, 2H), 4.12 (d, J = 11.3 Hz, 1H), 4.07 (dd, J = 10.2, 8.9 Hz, 1H), 4.00 (dd, J = 10.2, 3.8 Hz, 1H), 3.90 (dd, J = 10.2, 2.8 Hz, 1H), 3.77 (dd, J = 11.3, 2.3 Hz, 1H), 3.60 (dt, J = 10.1, 2.1 Hz, 1H), 3.45 (dd, J = 11.4, 1.8 Hz, 1H) ppm; ¹³C-NMR (150 MHz, CDCl₃) δ 137.96, 137.47, 133.24, 129.62, 128.87, 128.60, 128.51, 128.41, 128.25, 128.22, 128.20, 128.14, 128.07, 128.05, 128.03, 127.82, 127.43, 127.29, 127.25, 126.96, 99.72, 97.43, 80.09, 78.93, 78.15, 78.01, 74.98, 73.74, 73.03, 72.34, 72.09, 72.01, 71.77, 71.43, 66.89, 62.26, 20.78, 16.34 ppm. ¹⁹F-NMR (564 MHz, CDCl₃) δ -146.18 (dd, J = 53.3, 26.6 Hz) ppm. m/z (HRMS+) for C₇₁H₁₇₂Cl₁₆FNO₁₆Na [M+Na]⁺ calcd. 1342.3871, found 1342.3888.

Purified NP-HPLC of 28 (ELSD trace, Method VIIia, tᵣ= 10.6 min)
$^{1}H$-NMR (600 MHz, CDCl$_3$) of 28:

$^{13}C$-NMR (150 MHz, CDCl$_3$) of 28:

$^{13}$C($^{1}H$)-$^{1}H$ (coupled) HSQC of 28:

$^{19}F$-NMR (564 MHz, CDCl$_3$) of 28:
4.12 Synthesis of glycosyl acceptor 29

Cleavage from solid support as described in **Module V – Photocleavage**, followed by purification using preparative HPLC (**Method VIIIb**) afforded compound 29 (16.9 mg, 55% from resin 3, both isomers, from which 10.2 mg of **29-α**-isomer and 6.6 mg of **29-β**-isomer were separated).
Analytical data for 29 (α-isomer): $^1$H-NMR (600 MHz, CDCl$_3$) δ 8.02 – 7.97 (m, 2H), 7.95 – 7.89 (m, 2H), 7.60 – 7.54 (m, 1H), 7.46 – 7.41 (m, 3H), 7.34 – 7.26 (m, 19H), 7.24 – 7.16 (m, 20H), 7.12 – 7.04 (m, 4H), 5.67 (t, $J$ = 9.9 Hz, 1H), 5.60 (dd, $J$ = 10.1, 7.9 Hz, 1H), 5.06 (s, 2H), 5.03 – 4.98 (m, 3H), 4.85 (d, $J$ = 8.2 Hz, 1H), 4.83 – 4.74 (m, 3H), 4.70 – 4.63 (m, 4H), 4.61 (d, $J$ = 7.9 Hz, 1H), 4.58 (d, $J$ = 11.6 Hz, 1H), 4.54 (d, $J$ = 3.7 Hz, 1H), 4.52 (d, $J$ = 2.1 Hz, 1H), 4.49 (dd, $J$ = 11.7, 7.9 Hz, 2H), 4.44 – 4.39 (m, 3H), 4.37 (d, $J$ = 10.9 Hz, 1H), 4.24 – 4.19 (m, 2H), 4.14 (td, $J$ = 12.0, 8.3 Hz, 4H), 4.10 – 4.05 (m, 2H), 4.01 (td, $J$ = 11.9, 5.7 Hz, 3H), 3.94 (t, $J$ = 2.6 Hz, 1H), 3.83 (dd, $J$ = 9.4, 3.8 Hz, 2H), 3.75 (ddd, $J$ = 18.8, 11.8, 9.7, 4.2 Hz, 5H), 3.65 (td, $J$ = 5.0, 1.8 Hz, 1H), 3.61 – 3.57 (m, 1H), 3.55 (dd, $J$ = 10.9, 3.4 Hz, 1H), 3.48 (ddd, $J$ = 12.7, 10.5, 3.3 Hz, 2H), 3.40 (t, $J$ = 6.5 Hz, 2H), 3.34 (dt, $J$ = 10.7, 3.1 Hz, 2H), 3.19 – 3.07 (m, 4H), 1.94 (s, 3H), 1.93 (s, 3H), 1.83 (s, 3H), 1.57 – 1.50 (m, 2H), 1.47 (p, $J$ = 7.3 Hz, 2H), 1.34 – 1.27 (m, 3H) ppm; $^{13}$C-NMR (150 MHz, CDCl$_3$) δ 161.69, 138.38, 133.41, 133.18, 129.84, 129.76, 129.73, 129.44, 128.64, 128.50, 128.48, 128.45, 128.38, 128.37, 128.33, 128.31, 128.27, 128.15, 128.08, 128.05, 128.04, 127.90, 127.89, 127.86, 127.82, 127.79, 127.78, 127.73, 127.69, 127.64, 127.60, 127.54, 127.50, 127.33, 101.14, 100.24, 97.98, 97.68, 80.00, 79.75, 74.80, 74.57, 74.44, 73.69, 73.42, 72.79, 72.38, 72.28, 71.96, 71.76, 71.68, 70.01, 69.89, 69.74, 69.56, 67.96, 67.78, 66.81, 66.52, 63.44, 63.29, 62.83, 57.76, 40.91, 29.75, 28.94, 23.36, 20.80, 20.76, 20.69 ppm. m/z (HRMS+) for C$_{128}$H$_{136}$O$_{37}$NaCl$_3$ [M+Na]$^+$ calcld. 2359.7906, found: 2359.8015.

Purified NP-HPLC of 29 (α-isomer) (ELSD trace, Method Villa, t$_R$ = 31.9 min)
$^1$H-NMR (600 MHz, CDCl$_3$) of 29 (α-isomer):

$^{13}$C-NMR (150 MHz, CDCl$_3$) of 29 (α-isomer):

$^{13}$C($^1$H)$^{1}$H (coupled) HSQC of 29 (α-isomer):
Analytical data for 29 (β-isomer): $^1$H-NMR (600 MHz, CDCl$_3$) δ 7.93 (ddd, $J$ = 8.5, 5.6, 1.4 Hz, 4H), 7.60 – 7.54 (m, 1H), 7.44 (t, $J$ = 7.8 Hz, 2H), 7.36 – 7.25 (m, 24H), 7.19 (ddt, $J$ = 22.5, 8.8, 2.3 Hz, 14H), 7.11 – 7.07 (m, 2H), 5.60 (dd, $J$ = 10.1, 7.9 Hz, 1H), 5.54 (t, $J$ = 9.8 Hz, 1H), 5.06 (d, $J$ = 5.4 Hz, 3H), 5.00 (dd, $J$ = 11.1, 7.1 Hz, 2H), 4.90 – 4.86 (m, 2H), 4.84 – 4.75 (m, 3H), 4.73 – 4.70 (m, 3H), 4.69 – 4.63 (m, 3H), 4.61 (d, $J$ = 7.9 Hz, 1H), 4.59 (s, 1H), 4.57 (s, 1H), 4.54 (dd, $J$ = 6.2, 4.6 Hz, 2H), 4.53 (d, $J$ = 2.2 Hz, 1H), 4.51 (d, $J$ = 3.0 Hz, 1H), 4.48 (s, 1H), 4.40 (d, $J$ = 11.1 Hz, 1H), 4.35 (d, $J$ = 11.1 Hz, 1H), 4.31 (d, $J$ = 11.0 Hz, 1H), 4.21 (dd, $J$ = 9.6, 3.0 Hz, 1H), 4.13 (s, 1H), 4.11 (s, 1H), 4.09 – 4.05 (m, 1H), 4.04 (q, $J$ = 2.4, 1.8 Hz, 1H), 4.01 (d, $J$ = 8.3 Hz, 1H), 3.98 (d, $J$ = 3.4 Hz, 1H), 3.90 (dt, $J$ = 13.3, 4.8 Hz, 3H), 3.82 (d, $J$ = 2.8 Hz, 1H), 3.79 (dt, $J$ = 11.8, 3.8 Hz, 4H), 3.69 (dd, $J$ = 3.4, 1.8 Hz, 1H), 3.64 – 3.53 (m, 6H), 3.48 (dd, $J$ = 10.2, 2.7 Hz, 1H), 3.42 – 3.38 (m, 2H), 3.32 (dd, $J$ = 9.1, 3.0 Hz, 1H), 3.19 (tq, $J$ = 7.8, 2.6 Hz, 2H), 3.13 (q, $J$ = 7.1 Hz, 3H), 1.94 (s, 3H), 1.81 (s, 3H), 1.77 (s, 3H), 1.55 (q, $J$ = 5.6, 4.3 Hz, 2H), 1.47 (t, $J$ = 7.6 Hz, 2H), 1.30 (d, $J$ = 6.3 Hz, 2H) ppm. $^{13}$C-NMR (150 MHz, CDCl$_3$) δ 138.11, 137.91, 133.16, 133.03, 129.75, 128.49, 128.42, 128.38, 128.31, 128.24, 128.17, 128.07, 127.91, 127.80, 127.77, 127.70, 127.54, 100.31, 98.00, 97.61, 95.84, 80.02, 75.07, 74.80, 74.63, 74.43, 74.32, 73.86, 73.39, 72.70, 72.38, 72.29, 71.97, 70.91, 68.21, 68.01, 66.57, 66.14, 63.38, 63.28, 62.84, 40.88, 29.72, 28.88, 23.24, 20.81, 20.67, 20.61 ppm. m/z (HRMS+) for C$_{128}$H$_{130}$O$_{37}$NNaCl$_3$ [M+Na]$^+$ calcd. 2359.7906, found: 2359.8130.

Purified NP-HPLC of 29 (β-isomer) (ELSD trace, Method VIIIa, $t_R$= 30.5 min)
$^1$H-NMR (600 MHz, CDCl$_3$) of 29 (β-isomer):

$^{13}$C-NMR (150 MHz, CDCl$_3$) of 29 (β-isomer):

$^{13}$C($^1$H)$_{-}^1$H (coupled) HSQC of 29 (β-isomer):
### 4.13 Synthesis of tetrasaccharide S6

Resin: [OH]

#### Building Blocks:

| Automation sequence: F₂GH₂E |
|-----------------------------|
| **Repeat** | **Building Blocks** | **Modules** | **Notes** |
| 1x | I – Acidic Wash | IIa – Glycosylation with Thioglycoside | \(-20^\circ \text{C} (T1)\) 5 min (t1) | 
|  | [run twice] | 0 \(^\circ \text{C} (T2)\) 20 min (t2) |  
| 1x | I – Acidic Wash | IIa – Glycosylation with Thioglycoside | \(-20^\circ \text{C} (T1)\) 5 min (t1) | 
|  | G (6.5 eq.) | 0 \(^\circ \text{C} (T2)\) 20 min (t2) |  
| 1x | I – Acidic Wash | IIa – Glycosylation with Thioglycoside | \(-20^\circ \text{C} (T1)\) 5 min (t1) | 
|  | H (6.5 eq.) | 0 \(^\circ \text{C} (T2)\) 40 min (t2) |  
| 1x | I – Acidic Wash | IIa – Glycosylation with Thioglycoside | \(-20^\circ \text{C} (T1)\) 5 min (t1) | 
|  | E (6.5 eq.) | 0 \(^\circ \text{C} (T2)\) 40 min (t2) |  
|  | IVc – Fmoc Deprotection |  |  
|  | III – Capping |  |  
|  | III – Capping |  |  
|  | IVc – Fmoc Deprotection |  |  
|  | IVc – Fmoc Deprotection |  |  
|  | II – LeV Deprotection |  |  
|  | II – LeV Deprotection |  |  

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound S6 (14.1 mg, 55% from resin 3).

#### Analytical Data for S6

\(^1\text{H}-\text{NMR} (600 \text{ MHz, CDCl}_3) \delta 7.97 (\text{dd}, J = 8.2, 1.4 \text{ Hz, 2H}), 7.95 – 7.92 (m, 2H), 7.61 – 7.56 (m, 1H), 7.46 – 7.41 (m, 3H), 7.34 – 7.31 (m, 3H), 7.31 – 7.26 (m, 11H), 7.25 – 7.19 (m, 7H), 7.19 – 7.15 (m, 7H), 7.10 – 7.06 (m, 3H), 5.60 (dd, J = 10.1, 7.9 \text{ Hz, 1H}), 5.35 (t, J = 9.9 \text{ Hz, 1H}), 5.07 (s, 3H), 5.00 (dd, J = 11.0, 8.8 \text{ Hz, 2H}), 4.88 (d, J = 8.1 \text{ Hz, 1H}), 4.83 (d, J = 1.5 \text{ Hz, 1H}), 4.76 (dd, J = 10.6, 6.3 \text{ Hz, 2H}), 4.72 (d, J = 2.7 \text{ Hz, 1H}), 4.69 (d, J = 2.0 \text{ Hz, 1H}), 4.65 (d, J = 12.3 \text{ Hz, 1H}), 4.61 (d, J = 8.0 \text{ Hz, 1H}), 4.59 (d, J = 1.5 \text{ Hz, 1H}), 4.57 (d, J = 1.2 \text{ Hz, 1H}), 4.54 (d, J = 2.7 \text{ Hz, 1H}), 4.53 – 4.50 (m, 2H), 4.49 (d, J = 4.5 \text{ Hz, 1H}), 4.34 (d, J = 11.0 \text{ Hz, 1H}), 4.29 (d, J = 10.7 \text{ Hz, 1H}), 4.15 (s, 1H), 4.07 (dd, J = 11.2, 6.4 \text{ Hz, 1H}), 4.02 (dd, J = 10.2, 5.4 \text{ Hz, 2H}), 4.00 – 3.99 (m, 2H), 3.98 – 3.96 (m, 1H), 3.91 (dd, J
\( \delta = 11.8, 5.3 \text{ Hz, 1H}), 3.86 \text{ (dt, } J = 9.8, 3.8 \text{ Hz, 1H}), 3.83 \text{ (dd, } J = 3.0, 1.1 \text{ Hz, 1H}), 3.77 \text{ (dd, } J = 3.8, 2.2 \text{ Hz, 1H}), 3.75 \text{ (d, } J = 3.7 \text{ Hz, 1H}), 3.73 \text{ (dd, } J = 3.7, 1.5 \text{ Hz, 1H}), 3.62 \text{ (s, } 0 \text{H}), 3.60 \text{ (d, } J = 8.9 \text{ Hz, 1H}), 3.57 \text{ (dt, } J = 8.3, 3.4 \text{ Hz, 1H}), 3.48 \text{ (dt, } J = 10.0, 2.6 \text{ Hz, 2H}), 3.42 - 3.38 \text{ (m, 3H}), 3.19 \text{ (dt, } J = 9.2, 3.1 \text{ Hz, 1H}), 3.15 \text{ (q, } J = 6.6 \text{ Hz, 2H}), 1.93 \text{ (s, 3H)}, 1.82 \text{ (s, 3H)}, 1.54 \text{ (q, } J = 7.6 \text{ Hz, 2H}), 1.48 \text{ (t, } J = 7.6 \text{ Hz, 2H}), 1.34 - 1.30 \text{ (m, 2H) ppm}; ^{13}C\text{-NMR (150 MHz, CDCl}_3) \delta 170.71, 170.42, 166.24, 164.92, 161.70, 138.50, 138.41, 138.14, 137.81, 137.53, 133.17, 129.85, 129.76, 128.55, 128.51, 128.49, 128.38, 128.34, 128.31, 128.25, 128.15, 128.09, 128.06, 128.00, 127.84, 127.82, 127.78, 127.73, 127.69, 127.64, 127.54, 127.34, 100.27, 98.10, 97.94, 97.03, 80.00, 78.55, 78.30, 75.98, 74.82, 74.69, 74.50, 74.43, 73.42, 73.11, 72.38, 72.29, 71.97, 71.78, 70.78, 69.95, 69.69, 68.84, 67.68, 66.59, 66.38, 63.33, 62.84, 57.74, 40.91, 29.72, 28.93, 23.34, 20.80, 20.69 \text{ ppm. m/z (HRMS+) for } C_{106}H_{112}O_{28}NaCl_3 \text{ [M+Na]}^{+}\text{ calcd. 1975.6362, found: 1975.6545.}

Crude NP-HPLC of S6 (ELSD trace, Method IIla, \( t_s = 27.5 \text{ min})

\[^{1}H\text{-NMR (600 MHz, CDCl}_3\) of S6:
$^{13}$C-NMR (150 MHz, CDCl$_3$) of S6:

$^{13}$C-$^1$H-$^1$H (coupled) HSQC of S6:
4.14 Synthesis of trisaccharide 30

**Resin:**

**Building Blocks:**

| Automation sequence: CD₂E |
|---------------------------|
| Repeat | Building Blocks | Modules | Notes |
|--------|-----------------|---------|-------|
| 1x     | I – Acidic Wash  | IIa – Glycosylation with Thioglycoside | -20 °C (T1) 5 min (t1) |
|        |                 | III – Capping | 0 °C (T2) 20 min (t2) |
|        |                 | IVb – Lev Deprotection | |
| 1x     | I – Acidic Wash  | IIa – Glycosylation with Thioglycoside | -40 °C (T1) 5 min (t1) |
|        |                 | IIa – Glycosylation with Thioglycoside | -20 °C (T2) 20 min (t2) |
|        |                 | III – Capping | |
|        |                 | IVa – Fmoc Deprotection | |
| 1x     | I – Acidic Wash  | IIa – Glycosylation with Thioglycoside | -20 °C (T1) 5 min (t1) |
|        |                 | III – Capping | 0 °C (T2) 20 min (t2) |
|        |                 | III – Capping | |

Cleavage from solid support as described in Module V – Photocleavage, followed by purification using preparative HPLC (Method VIIIb) afforded compound 30 (9.6 mg, 52% from resin 5).

Analytical Data for 30: ¹H-NMR (600 MHz, CDCl₃, both isomers) δ 7.91 (dd, J = 8.4, 1.3 Hz, 2H), 7.62 – 7.58 (m, 1H), 7.49 – 7.43 (m, 3H), 7.37 – 7.33 (m, 6H), 7.24 – 7.20 (m, 6H), 7.19 – 7.15 (m, 11H), 6.82 (d, J = 9.6 Hz, 1H), 5.48 (dd, J = 10.1, 8.0 Hz, 1H), 5.27 (d, J = 3.7 Hz, 1H), 5.16 (d, J = 3.7 Hz, 1H), 4.83 (d, J = 10.9 Hz, 1H), 4.79 (d, J = 11.5 Hz, 1H), 4.75 (d, J = 12.1 Hz, 1H), 4.69 (d, J = 2.2 Hz, 2H), 4.67 (t, J = 2.1 Hz, 1H), 4.65 (s, 1H), 4.59 (d, J = 11.4 Hz, 1H), 4.53 (s, 1H), 4.51 (d, J = 2.1 Hz, 1H), 4.50 (s, 1H), 4.49 (s, 1H), 4.48 (d, J = 4.1 Hz, 1H), 4.31 (d, J = 12.2 Hz, 1H), 4.28 – 4.25 (m, 1H), 4.19 – 4.15 (m, 1H), 4.11 (d, J = 5.7 Hz, 1H), 4.10 – 4.08 (m, 2H), 3.98 (dd, J = 10.3, 3.9 Hz, 2H), 3.90 (dd, J = 10.1, 2.8 Hz, 1H), 3.83 – 3.81 (m, 1H), 3.78 (dd, J = 10.9, 2.9 Hz, 1H), 3.70 (dt, J = 10.0, 2.3 Hz, 2H), 3.54 – 3.48 (m, 1H), 3.43 – 3.38 (m, 2H), 3.38 – 3.36 (m, 3H), 2.00 (s, 3H), 1.24 (d, J = 6.6 Hz, 3H) ppm; ¹³C-NMR (150 MHz, CDCl₃) δ 170.24, 164.56, 161.58, 139.40, 139.16, 138.38, 137.99, 137.89, 137.49, 133.14, 129.74, 129.68, 128.87, 128.61, 128.54, 128.50, 128.48, 128.46, 128.44, 128.39, 128.38, 128.26, 128.20, 128.16, 128.11, 128.02, 128.00, 127.85, 127.80, 127.78, 127.60, 127.59, 127.53, 127.45, 127.19, 127.16, 126.98, 126.94, 99.87, 97.18, 90.83, 80.16, 79.81, 78.85, 78.29, 76.02, 74.95, 74.94, 73.64, 73.22, 73.16, 72.80, 72.51, 72.36, 72.18, 72.16, 72.11, 72.05, 71.94, 71.89, 71.57, 70.45, 67.69, 66.65, 62.57, 62.31, 55.99, 20.79, 16.37 ppm. m/z (HRMS+) for C₇₁H₇₄O₁₇NNaCl₃ [M+Na]⁺ calcd. 1340.3915, found: 1340.4072.
Crude NP-HPLC of 30 (ELSD trace, Method VIIIa, t_R= 23.1 min, t_R= 26 min)

\[ \text{H-NMR (600 MHz, CDCl}_3\text{) of 30:} \]

\[ \text{C-NMR (150 MHz, CDCl}_3\text{) of 30:} \]
$^{13}\text{C}^{{}\text{H}}}-{^1}\text{H}$ (coupled) HSQC of 30:
4.15 Synthesis of heptasaccharide 31

Resin: 5

Building Blocks:

Automation sequence: $\text{K}_2\text{K}_2\text{K}_2\text{K}_2\text{K}_2$

| Repeat | Building Blocks       | Modules                                      | Notes                  |
|--------|-----------------------|----------------------------------------------|------------------------|
| 7x     | K (4 eq.)             | I – Acidic Wash                              | -30 °C (T1) 10 min (t1) |
|        |                       | IIb – Glycosylation with Glycosylphosphate  | -10 °C (T2) 40 min (t2) |
|        |                       | III – Capping                                |                        |
|        |                       | IVa – Fmoc Deprotection                      |                        |

Post-Automation: **Module VI – Methanolation**, then **Module V – Photocleavage**, then **Module VII – Hydrogenation**. The crude unprotected oligosaccharide was purified using reverse-phase preparative HPLC (**Method VIIId**) to afford compound 31 (5.9 mg, 30% from resin 5).

Analytical Data for 31: $^1$H-NMR (600 MHz, D$_2$O, both isomers) δ 5.10 (d, $J = 3.7$ Hz, 1H), 4.67 – 4.65 (m, 5H), 4.63 – 4.60 (m, 3H), 4.54 (d, $J = 8.0$ Hz, 1H), 3.80 (d, $J = 2.0$ Hz, 6H), 3.79 – 3.76 (m, 7H), 3.76 – 3.71 (m, 2H), 3.70 – 3.67 (m, 1H), 3.67 – 3.64 (m, 9H), 3.63 – 3.56 (m, 9H), 3.44 – 3.40 (m, 8H), 3.40 – 3.34 (m, 14H), 3.32 – 3.25 (m, 3H), 3.22 (dd, $J = 9.4$, 7.9 Hz, 2H) ppm; $^{13}$C-NMR (150 MHz, D$_2$O) δ 102.74, 102.57, 102.46, 102.44, 95.61, 91.95, 84.41, 84.16, 83.99, 82.18, 75.93, 75.55, 75.51, 75.48, 73.77, 73.38, 73.23, 73.17, 71.16, 70.99, 69.50, 68.05, 68.01, 60.60, 60.46 ppm. m/z (HRMS+) for $\text{C}_{42}\text{H}_{72}\text{O}_{36}\text{Na}$ [M+Na]$^+$ calcd. 1175.3695, found: 1175.3660.

Crude RP-HPLC of 31 (ELSD trace, **Method VIIic**, $t_R$ = 31.2 min)
$^1$H-NMR (600 MHz, D$_2$O) of 31:

$^{13}$C-NMR (150 MHz, D$_2$O) of 31:

$^{13}$C-$^1$H HSQC of 31:
4.16 Synthesis of pentasaccharide 32

Resin: 5

Building Blocks:

Automation sequence: K_B2K3K_2K2

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 1x     | K (4 eq.) [run twice] | I – Acidic Wash | |
|        |                 | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        |                 | III – Capping | -10 °C (T2) 40 min (t2) |
|        |                 | IVa – Fmoc Deprotection | |
| 1x     | B (6.5 eq.) [run twice] | I – Acidic Wash | |
|        |                 | IIa – Glycosylation with Thioglycoside | -20 °C (T1) 5 min (t1) |
|        |                 | III – Capping | 0 °C (T2) 20 min (t2) |
|        |                 | IVa – Fmoc Deprotection | |
| 3x     | K (4 eq.) [run twice] | I – Acidic Wash | |
|        |                 | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        |                 | III – Capping | -10 °C (T2) 40 min (t2) |
|        |                 | IVa – Fmoc Deprotection | |

Post-Automation: Module VI – Methanolysis, then Module V – Photocleavage, then Module VII – Hydrogenation. The crude unprotected oligosaccharide was purified using reverse-phase preparative HPLC (Method VIIId) to afford compound 32 (2.6 mg, 22% from resin 5).

Analytical Data for 32: 1H-NMR (700 MHz, D2O, both isomers) δ 5.16 (d, J = 3.7 Hz, 1H), 4.73 – 4.65 (m, 2H), 4.59 (dd, J = 8.1, 1.6 Hz, 1H), 4.46 (d, J = 8.0 Hz, 2H), 3.92 (d, J = 2.2 Hz, 1H), 3.91 – 3.89 (m, 2H), 3.86 – 3.85 (m, 2H), 3.83 (m, 4H), 3.82 – 3.77 (m, 1H), 3.76 – 3.72 (m, 2H), 3.69 (dd, J = 8.3, 3.2 Hz, 2H), 3.65 (ddd, J = 16.3, 9.8, 4.0 Hz, 4H), 3.59 (dd, J = 6.5, 2.7 Hz, 4H), 3.57 – 3.53 (m, 4H), 3.49 – 3.45 (m, 3H), 3.45 – 3.42 (m, 8H), 3.40 (dd, J = 9.1, 6.9 Hz, 1H), 3.37 (d, J = 8.2 Hz, 1H), 3.34 (s, 1H), 3.30 – 3.25 (m, 2H) ppm; 13C-NMR (176 MHz, D2O) δ 102.81, 102.68, 102.59, 102.48, 102.35, 95.69, 84.50, 84.22, 83.77, 82.24, 78.54, 76.01, 75.61, 75.57, 75.56, 74.85, 74.15, 74.13, 73.82, 73.46, 73.23, 73.05, 71.04, 69.58, 68.13, 68.08, 67.97, 60.70, 60.68, 60.56, 60.00 ppm. m/z (HRMS+) for C30H52O26Na [M+Na]+ calcd. 851.2639, found: 851.2628.
Crude RP-HPLC of 32 (ELSD trace, Method VIIIc, $t_R = 25.8$ min)

$^1$H-NMR (700 MHz, D$_2$O) of 32:

$^{13}$C-NMR (176 MHz, D$_2$O) of 32:
$^{13}$C-H HSQC of 32:
### 4.17 Synthesis of octasaccharide 33

**Resin:**

[Image of resin structure]

**Building Blocks:**

[Diagram of building blocks]

**Automation sequence:** $K_2L_2K_2L_2K_2K_6$

| Repeat | Building Blocks | Modules | Notes |
|--------|----------------|---------|-------|
| 2x     | K (4 eq.)      | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) -10 °C (T2) 40 min (t2) |
|        | [run twice]    |         |       |
| 1x     | K (4 eq.)      | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) -10 °C (T2) 40 min (t2) |
|        | [run twice]    |         |       |
| 6x     | K (4 eq.)      | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) -10 °C (T2) 40 min (t2) |

**Post-Automation:** Module VI – Methanolyis, then Module V – Photocleavage, then Module VII – Hydrogenation. The crude unprotected oligosaccharide was purified using reverse-phase preparative HPLC (Method VIIId) to afford compound 33 (1.7 mg, 8% from resin 5).

**Analytical Data for 33:**

$^1$H-NMR (700 MHz, D$_2$O, both isomers) $\delta$ 5.15 (d, $J = 3.7$ Hz, 1H), 4.75 – 4.64 (m, 6H), 4.59 (d, $J = 8.1$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 2H), 4.43 (d, $J = 7.9$ Hz, 2H), 4.14 (dd, $J = 18.1, 11.5$ Hz, 5H), 3.86 – 3.82 (m, 14H), 3.81 – 3.76 (m, 2H), 3.75 – 3.68 (m, 9H), 3.68 – 3.60 (m, 13H), 3.55 – 3.36 (m, 32H), 3.35 – 3.31 (m, 4H), 3.28 (t, $J = 8.7$ Hz, 2H), 3.24 (t, $J = 8.6$ Hz, 4H) ppm; $^{13}$C-NMR (176 MHz, D$_2$O) $\delta$ 102.95, 102.90, 102.81, 102.64, 102.55, 102.52, 95.69, 92.03, 84.69, 84.49, 84.24, 84.14, 84.10, 83.90, 76.01, 75.93, 75.66, 75.64, 75.62, 75.58, 75.56, 75.52, 74.93, 74.58, 73.84, 73.46, 73.33, 73.30, 73.26, 73.22, 73.14, 73.09, 71.23, 71.07, 69.64, 69.58, 69.42, 68.98, 68.62, 68.15, 68.13, 68.09, 60.70, 60.54 ppm. m/z (HRMS+) for C$_{48}$H$_{82}$O$_{41}$Na [M+Na]$^+$ calcd. 1337.4224, found: 1337.4276.
Repurified RP-HPLC of 33 (ELSD trace, Method VIIIc, $t_R = 33.7$ min)

$^1$H-NMR (700 MHz, D$_2$O) of 33:

$^{13}$C-NMR (176 MHz, D$_2$O) of 33:
$^{13}$C-H HSQC of 33:
4.18 Synthesis of octasaccharide 34

Resin:

Building Blocks:

Automation sequence: $K_3K_2K_2L_2K_2K_2K_2$

| Repeat | Building Blocks | Modules | Notes |
|--------|-----------------|---------|-------|
| 3x     | I – Acidic Wash | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        | $K$ (4 eq.) [run twice] | | -10 °C (T2) 40 min (t2) |
|        | III – Capping | | |
|        | IVc – Fmoc Deprotection | | |
| 1x     | I – Acidic Wash | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        | $L$ (4 eq.) [run twice] | | -10 °C (T2) 40 min (t2) |
|        | III – Capping | | |
|        | IVc – Fmoc Deprotection | | |
| 2x     | I – Acidic Wash | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        | $K$ (4 eq.) [run twice] | | -10 °C (T2) 40 min (t2) |
|        | III – Capping | | |
|        | IVc – Fmoc Deprotection | | |
| 1x     | III – Capping | | |
| 1x     | I – Acidic Wash | IIb – Glycosylation with Glycosylphosphate | -30 °C (T1) 10 min (t1) |
|        | $K$ (4 eq.) [run twice] | | -10 °C (T2) 40 min (t2) |
|        | III – Capping | | |
|        | IVc – Fmoc Deprotection | | |

Post-Automation: Module VI – Methanolysis, then Module V – Photocleavage, then Module VII – Hydrogenation. The crude unprotected oligosaccharide was purified using reverse-phase preparative HPLC (Method VIIIId) to afford compound 34 (3.5 mg, 16% from resin 5).

Analytical Data for 34: $^1$H-NMR (600 MHz, D$_2$O, both isomers) δ 5.11 (d, $J = 3.7$ Hz, 1H), 4.67 (d, $J = 8.0$ Hz, 5H), 4.64 – 4.59 (m, 8H), 4.55 (d, $J = 8.0$ Hz, 1H), 4.41 – 4.36 (m, 5H), 4.11 – 4.07 (m, 5H), 3.81 – 3.77 (m, 26H), 3.73 – 3.30 (m, 32H), 3.30 – 3.16 (m, 14H) ppm; $^{13}$C-NMR (150 MHz, D$_2$O) δ 102.74, 102.72, 102.52, 102.43, 95.61, 91.91, 85.14, 84.63, 84.17, 83.75, 82.99, 75.93, 75.80, 75.53, 75.48, 75.45, 74.45, 74.38, 73.53, 73.38, 73.18, 73.14, 73.09, 72.99, 71.08, 70.75, 69.51, 68.67, 68.17, 68.15, 68.09, 68.06, 67.98, 60.63, 60.47 ppm. m/z (HRMS+) for $C_{48}H_{82}O_{41}Na$ [M+Na]$^+$ calcd. 1337.4224, found: 1337.4276.
Repurified RP-HPLC of 34 (ELSD trace, Method VIIIc, $t_R = 33.5$ min)

$^1$H-NMR (600 MHz, D$_2$O) of 34:

$^{13}$C-NMR (150 MHz, D$_2$O) of 34:
$^{13}$C-$^1$H HSQC of 34:
5. References

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