Streamlined Access to Conjugation-Ready Glycans by Automated Synthesis

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1. General Experimental Methods

General Information for Chemical Synthesis: All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in
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oven-dried glassware under an inert atmosphere unless noted otherwise. Reagent grade N,N-dimethylformamide (DMF) was dried over activated molecular sieves prior to use. Pyridine, triethylamine (NEt₃) and acetonitrile (MeCN) were distilled over CaH₂ prior to use. Dichloromethane (DCM, CH₂Cl₂), toluene and tetrahydrofuran (THF) were purified by a Cycle-Tainer Solvent Delivery System unless noted otherwise. All solvents used on the automated synthesizer were extra dry grade without molecular sieves, purchased from Acros in sure-seal bottles, except DCM and THF, which were dried using a dry still. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25mm). Compounds were visualized by UV-irradiation or by dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Purification by size exclusion recycling HPLC was carried out using JAI LC 9101 equipped with JAIGEL-1H and -2H column in a series (CHCl₃). Purification by reverse phase HPLC was performed using Agilent 1200 series equipped with a Macherey-Nagel Nucleodur Pyramid C-18 column (length 250 mm, 40 mm i.d., flow 10 mL/min) unless noted otherwise. ¹H, ¹³C spectra were recorded on a Varian Mercury 300 (300 MHz), Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), Bruker ECX (400 MHz), Bruker DRX500 (500 MHz), or Bruker DRX700 (700 MHz) spectrometer in CDCl₃ with chemical shifts referenced to internal standards using CDCl₃ (7.26 ppm ¹H, 77.0 ppm ¹³C) unless otherwise stated. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ¹H NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service at the Laboratory for Organic Chemistry (LOC) at ETH Zürich and the MS-service at Department of Organic Chemistry at Free University Berlin. High-resolution MALDI and ESI mass spectra were conducted on an IonSpec Ultra instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR
spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

2. Automated Oligosaccharide Synthesis Instrument

The controller, syringe pump, rotary ports, solenoid valve driver and fraction collector were custom made by J-KEM Scientific. The cryostat LH-85 was acquired from Julabo. Custom-made Teflon parts were produced by the mechanics shop at the ETH Zurich. The machine housing was obtained from Maytec. The reactor and the building block storage vessels were custom made. The argon manifold was acquired from Lüedi (Switzerland), check valves and in-line filters were acquired from Swagelok (www.swagelok.com). PTFE tubing, ferrules, fittings, argon distribution manifolds, solvent and reagent bottle caps were from Omnifit.

Instrument Setup

The instrument consists of a syringe pump-driven part and a solenoid valve-driven part (Scheme S1, Figures S1 and S2). A double-jacketed glass reaction vessel, a cryostat, reagent, solvent and waste vessels, a fraction collector and an argon manifold complete the parts list.
Scheme S1. Setup of the fully automated oligosaccharide synthesizer.
Figure S1. Picture of the fully automated synthesizer: 1. dual syringe pump unit, 2. rotary valve unit, 3. controller, 4. personal computer, 5. cryostat, 6. solenoid unit, 7. fraction collector, 8. argon manifold, 9. solvent reservoirs, and 10. reagent reservoirs.
Figure S2. Picture of synthesizer: 11. building block vessels, 12. reaction vessel, 14. loops, and 15. argon distribution manifold.

The controller (component 3, Figure S1) coordinates the syringe pump-driven part, the solenoid driven-part, the cryostat and the fraction collector. It serves as mediator between the electro-mechanical parts and the computer. The computer program sends commands pre-defined by J-KEM to the controller that translates them into the command structure of the electro-mechanical devices. The controller collects data from the attached devices, such as the temperature of the cryogenic fluid in the cryostat.

The argon manifold (component 8, Figure S1) was built by Lüedi (Switzerland). This manifold can be replaced by a Swagelok manifold (www.swagelok.com) as demonstrated in an upgraded version of the machine. Five pressure regulators with pressure indicators are connected to the main line, and throughout this manuscript are referred to as positions I through V. These positions were implemented to allow for stable and independently adjustable pressure in the...
various compartments. Positions I-III are connected to the syringe pump-driven part of the system via distribution manifolds (component 15, Figure S2), while positions IV-V are connected to the solenoid valve-driven part of the system. Position IV, in addition to the pressure regulator, also features a flow regulator.

The reaction vessel (Scheme S2) is a double-jacketed cylinder. The double jacket allows for circulation of cryogenic fluid and is connected to the cryostat. A porous glass filter at the bottom the reaction vessel retains the resin. Below this filter a threaded joint is installed. This joint holds a Teflon plug through which a PTFE line is connected to the bottom of the reaction vessel. This bottom PTFE line is connected to a 4-way solenoid valve manifold. The reaction vessel top also features a Teflon plug fitted into a threaded joint. This top plug holds 6 PTFE tubes. The tube in the center is connected to an 8-way a solenoid manifold. The solvent inlet into the vessel is directed towards the walls of the vessel. This shower-like setup washes resins off the walls and prevents splashing when solvents are introduced into the vessel. One of the tubes on the outer perimeter of the reaction vessel is connected to a single solenoid valve and serves as an exhaust (referred to as Position VI). The exhaust is only open when the reaction vessel is under positive argon pressure. This setup guarantees an inert atmosphere in the reaction vessel, as the reaction vessel, apart from the exhaust, is completely sealed from the atmosphere. The remaining four tubes on the outer perimeter of the reaction vessel, termed "reaction vessel lines" (rv 3-6), are each connected to one of the rotary valves (valves 3-6) of the syringe pump system.
**Scheme S2.** Schematic drawing of the reaction vessel featuring the cryostat inlets, the reaction vessel bottom and the reaction vessel top with all connected lines and solenoid manifolds.

**Syringe Pump**

The syringe pump-driven part (Figure S1, components 1 and 2; Figure S2, component number 14) of the system consists of a dual syringe pump unit and the four eight-way rotary valve units. Only one syringe pump is used. The syringe pump part serves in the delivery of reagents (building blocks, activating and deprotecting reagents).
The rotary valves and syringe pump rotary valves are referred to as "PORT" (Figure S3). The number X identifies the PORT, while the number Y (as indicated on the periphery of the circles in Figure S3) identifies the position of the PORT inlet. When identifying a particular PORT inlet the following the description "PORT(X,Y)" is used. Every PORT has a central outlet and eight peripheral inlets. There are two syringe pump rotary valves, PORT 1 and 2, and four rotary valves, PORT 3 to 6. PORT 1 and PORT 2 have a stepper motor driven syringe on the central outlet and eight threaded inlets. PORT 3 to 6 have threads on both outlets and inlets.

**Figure S3.** Rotary valve referred to as PORTs. In green the Teflon rotor including a line depicting the channel in the rotor.

A scheme of the complete PORT setup is shown in Figure S4. Syringe pump 1 (also referred to as PORT 1) is not used. Syringe 2 (PORT 2) is exclusively filled with 1,2-dichloroethane, no other solvents enter into this syringe. This precludes solvent cross contamination. The size of the loops installed between the rotary valves and the syringe pump rotary valve is 4 mL for the rotary valves holding the
deprotecting reagents (PORT 4), activating reagents (PORT 6) and the two rotary valves (PORT 3 and 5) holding the building blocks. Resting positions i.e. inlets plugged with a Teflon plug were introduced. These positions are used to secure the syringe pump system during the operation of the solenoid-driven part of the system. In adopting the resting position upon completion of every syringe pump-driven delivery operation, any connection of the valve to the reaction vessel is cut. The rotary valve inlets PORT(X,6) are connected to the reaction vessel via reaction vessel lines with the dead volume of 0.15 mL. PORT(X,7) are connected to the waste and PORT(X,8) are connected to the argon manifold.

**Figure S4.** The PORT setup. (RV = reaction vessel, DCE = 1,2-Dichloroethane, BB = building block all lines 1/16” PTFE tube except line to DCE bottle which is 1/8” PTFE tube)

**List of Solvents and Stock Solutions Connected to PORTs**

**TMSOTf:** TMSOTf (364 μl) in DCM (40 mL)
**Piperidine**: 20% in DMF (v/v)

**Hydrazine**: hydrazine monohydrate (680 μl, 0.56 M) in pyridine (15 mL) and HOAc (10 mL).

**NaOME**: 0.25 M solution of NaOMe in MeOH.

**Bu₃SnH**: Bu₃SnH (135 μl) and AIBN (4 mg) in xylene (1.9 mL degassed)

**NIS**: N-Iodosuccinimide (1.48 g, 6.66 mmol) and TfOH (60 μl, 0.66 mmol) in DCM (20 mL) and dioxane (20 mL).

**Tf₂O**: Tf₂O (0.84 mL) in DCM (19.2 mL).

**TBAF**: TBAF (5 mL, 1 M in THF, 5 mmol), HOAc (0.43 mL, 7.5 mmol) and THF (14.6 mL)

**BH₃**: BH₃ (2.5 mL, 1 M solution in THF) in DCM (2.5 mL)

**Bu₂BOTf**: Bu₂BOTf (0.5 mL, 1 M solution in DCM) in DCM (9.5 mL)

**PORT Setup**

PORT(1,Y): not used

PORT 2 central outlet: connected to 10 mL syringe (syringe pump)

PORT(2,1): connected to 1,2-dichloroethane (5 L bottle) via PTFE line (1/8" inner diameter)

PORT(2,2): Teflon plug, resting position
PORT(2,3): connected to PORT 3 central outlet via PTFE loop (1/16" inner diameter, volume 2 mL)

PORT(2,4): connected to PORT 4 central outlet via PTFE loop (1/16" inner diameter, volume 4 mL)

PORT(2,5): connected to PORT 5 central outlet via PTFE loop (1/16" inner diameter, volume 2 mL)

PORT(2,6): connected to PORT 6 central outlet via PTFE loop (1/16" inner diameter, volume 4 mL)

PORT(2,7): connected to waste container via PTFE line (1/16" inner diameter)

PORT(2,8): connected to Argon manifold (position II, Ar-pressure 0.3 bar) via PTFE line (1/16" inner diameter)

PORT 3 central outlet: connected to PORT(2,3) via PTFE loop (1/16" inner diameter, volume 2 mL)

PORT(3,1): Teflon plug, resting position

PORT(3,2): connected to building block storage vessel 1 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL)

PORT(3,3): connected to building block storage vessel 2 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL)

PORT(3,4): connected to building block storage vessel 3 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL)
PORT(3,5): connected to building block storage vessel 4 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL)

PORT(3,6): connected to reaction vessel inlet top via PTFE line (1/16" inner diameter, volume 0.15 mL)

PORT(3,7): connected to waste container via PTFE line (1/16" inner diameter)

PORT(3,8): connected to Argon manifold (position II, Ar-pressure 0.3 bar) via PTFE line (1/16" inner diameter)

PORT 4 central outlet: connected to PORT(2,4) via PTFE loop (1/16" inner diameter, volume 4 mL)

PORT(4,1): Teflon plug, resting position

PORT(4,2) : connected to piperidine in DMF storage vessel (20% piperidine in DMF in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(4,3): connected to DMF storage vessel (100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(4,4): connected to hydrazine storage vessel (hydrazine in pyridine and HOAc, in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(4,5): connected to NaOMe storage vessel (NaOMe in MeOH, in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)
PORT(4,6): connected to reaction vessel inlet top via PTFE line (1/16" inner diameter, volume 0.15 mL)

PORT(4,7): connected to waste container via PTFE line (1/16" inner diameter)

PORT(4,8): connected to Argon manifold (position 1, Ar-pressure 0.3 bar) via PTFE line (1/16" inner diameter)

PORT 5 central outlet: connected to PORT(2,5) via PTFE loop (1/16" inner diameter, volume 2 mL)

PORT(5,1): Teflon plug, resting position

PORT(5,2): connected to building block storage vessel 5 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL). For custom programs building block vessel 5 is also used for storage of BH₃ in THF.

PORT(5,3): connected to building block storage vessel 6 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL). For custom programs building block vessel 6 is also used for storage of TBAF.

PORT(5,4): connected to building block storage vessel 7 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL). For custom programs building block vessel 7 is also used for storage of Bu₃SnH, AIBN or Bu₂BOTf.

PORT(5,5): connected to building block storage vessel 8 (10 mL glass tube, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume 0.1 mL). For custom programs building block vessel 7 is also used for storage of Bu₃SnH.
PORT(5,6): connected to reaction vessel inlet top via PTFE line (1/16" inner diameter, volume 0.15 mL)

PORT(5,7): connected to waste container via PTFE line (1/16" inner diameter)

PORT(5,8): connected to Argon manifold (position II, Ar-pressure 0.3 bar) via PTFE line (1/16" inner diameter)

PORT 6 central outlet: connected to PORT(2,6) via PTFE loop (1/16" inner diameter, volume 4 mL)

PORT(6,1): Teflon plug, resting position

PORT(6,2): connected to TMSOTf storage vessel (TMSOTf in DCM, in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(6,3): connected to NIS storage vessel (NIS and TfOH in dioxane DCM, 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(6,4): connected to dioxane storage vessel (in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(6,5): connected to Tf$_2$O storage vessel (Tf$_2$O in DCM, in 100 mL Schott glass bottle, connected to Ar manifold position 1, Ar-pressure 0.3 bar) via PTFE tube (1/16" inner diameter, volume ca. 0.5 mL)

PORT(6,6): connected to reaction vessel inlet top via PTFE line (1/16" inner diameter, volume 0.15 mL)

PORT(6,7): connected to waste container via PTFE line (1/16" inner diameter)
PORT(6,8): connected to Argon manifold (position II, Ar-pressure 0.3 bar) via PTFE line (1/16" inner diameter)

**Solenoid Valves**

The solenoid setup delivers solvents, mixes reaction solutions and removes waste from the reaction vessel. No solenoid valves were installed between the gas manifold and solvent containers as these vessels are always kept under inert gas pressure. Check valves were installed *in lieu* of the solenoid valves between the gas manifold and solvent storage vessels to prevent cross contamination. In-line filters were installed upstream from any solenoid valve as particles entering the valve lead to damage and leakage. Solenoid valves in Scheme S3 are labeled 1 through 16.
Scheme S3. Overview of the automated synthesizer plumbing that unifies the liquid and gas connections of the syringe pump system (left) and solenoid valve system (right) to the reaction vessel (center). For simplicity, the gas connections to the argon manifold are represented by the Roman numerals I-V.

Programming and Operation

The programming language of the terminal program uses commands that are grouped into modules. The combination of commands into modules allows for operations such as washes or glycosylations or deprotections.
Washing Module

Washing with a solvent involves the following steps: delivery of the solvent to the reaction vessel, mixing, and subsequent delivery of the soiled washing solvent to waste. Delivery of solvents takes place by opening a solenoid valve on the 8-way manifold, as well as the guard solenoid valve, and simultaneous opening of the single solenoid valve at the reaction vessel top connected to the exhaust. Opening one of the eight valves connected to the reaction vessel results in delivery of one of the solvents. The exhaust has to be opened to prevent overpressure in the reaction vessel.

Mixing takes place by simultaneously opening of the argon valve on the 4-way solenoid manifold at the bottom of the reaction vessel and the exhaust line. Thereby a flow of argon passes via the porous glass filter through the reaction or washing solution. Mixing by gas flow avoids mechanical shaking.

Waste removal is achieved by opening the inlet on the 8-way manifold connected to argon and the guard valve. Argon pressurizes the reaction vessel. Upon pressurizing the vessel, a valve on the 4-way solenoid manifold at the bottom of the vessel is opened. The argon pressure expels the solution from the vessel via the glass filter that retains the resin in the vessel. The expelled solutions can be directed to waste or to a fraction collector by selecting the appropriate solenoid valve either leading to the waste container or to the fraction collector.

Reaction Modules

Glycosylation, deprotection and linker cleavage are performed automatically. The corresponding modules require the combination of syringe pump reagent delivery, solenoid mixing, and delivery to waste as well as cryostat temperature control.
Delivery of a reagent is preceded by setting the appropriate cryostat temperature and priming the loops utilized for reagent delivery with dichloroethane. Once the cryostat has reached the appropriate temperature, the reagent is taken up into the loop. If the reagent is a building block it is delivered directly via the respective reaction vessel line to the reaction vessel. For stock solutions, the loop is first primed with the respective solution to be dispensed before a second aliquot of stock solution is taken up into the loop and delivered to the reaction vessel. The dead volumes of the lines (the volume of the tubing itself) that lead from from the rotary valve to the respective reagent storage vessel, as well as the dead volume of the reaction vessel line have been accounted for in the programming. Upon delivery, the reaction vessel line is emptied and the respective loop is primed. Once the reaction time is exceeded the reaction mixture is delivered either to waste or to a fraction collector via the respective outlet. Finally, the reaction vessel lines are cleaned by priming with solvent from the loop.

For general glycosylations: "Glycosylation (type of leaving group, number of building block equivalents used, number of activator equivalents used, reaction time, temperature, send to (W)aste or (F)raction collector)" e.g. "Glycosylation (Imidate, 5 eq bb X, 0.5 eq TMSOTf, 60 min., -10 °C, F)".

For thioglycoside glycosylations two reaction temperatures and times are indicated, as the reagent addition generally takes place at -40 °C, and after 5 minutes the temperature is raised to -20 °C and maintained there for further 40 minutes: "Glycosylation (Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)".

For deprotections: "Deprotection (temporary protecting group cleaved, reaction time, reaction temperature, send to (W)aste or (F)raction collector)" e.g. Deprotection (Fmoc, 3x, 5 min., 25 °C, F). For linker cleavage "Cleavage (linker, 1x, 25 °C, F)".
Temperature Module

Resetting the temperature after a glycosylation is generally preceded by washing steps at low temperature, which are represented by distinct modules. A temperature module is therefore required to reset the temperature. This module sets a temperature (i.e. a set temperature command is sent to the cryostat) and then the program monitors the actual cryostat temperature (i.e. the temperature of the cryostat cooling fluid) until a preset threshold temperature is exceeded (PTEMPHIGH command), or under-run (PTEMPLOW command). The resulting intermission can take place either in the presence ("Temperature (25 °C, 24 °C, DCM)") or absence ("Temperature (25 °C, 20 °C, none)") of solvents in the reaction vessel. The first temperature indicates the temperature the chiller is set to, while the second temperature indicates the temperature the controller waits for before continuing with the program execution.

Module Combinations

Combining the wash and reaction modules results in a coupling cycle. A detailed description of the sequence of modules that are used can be found in the following section.

Module Sequences

Glycosyl Imidate Double coupling - Fmoc deprotection

Temperature (25 °C, 20 °C, none)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)
Wash (DCM, 6x, 25 °C, F)

Glycosylation (Imidate, 5 eq BB X, 0.5 eq TMSOTf, 60 min., -10 °C, F)

Add DCM (2 mL), set temperature (-10 °C).

Prime Loop 3 and empty reaction vessel Line 3, wait until set temperature is reached, DCM to waste, delivery of bb in DCM (0.75 mL), empty RV Line 3, prime loop 3, push back bb to storage vessel.

Prime Loop 6 and empty RV Line 6, delivery of TMSOTf (0.25 mL) stock solution empty RV Line 6, prime Loop 6, push back TMSOTf to storage vessel.

Glycosylation reaction (60 min.), while continuously purging with high Ar flow; collect glycosylation mix to fraction collector, clean RV Lines 3 and 6 and prime Loops 3 and 6.

Wash (DCM, 6x, -10 °C, F)

Glycosylation (Imidate, 5 eq BB X, 0.5 eq TMSOTf, 60 min., -10 °C, F)

Wash (DCM, 6x, -10 °C, F)

Temperature (25 °C, 24 °C, DCM)

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DMF, 3x, 25 °C, W)

Deprotection (Fmoc, 3x, 5 min., 25 °C, F)

Add DMF, prime Loop 4 and empty RV Line 4, repeat 3x: delivery of piperidine stock solution, deprotection reaction 5 min, while continuously purging with high
Ar flow; collect deprotection mix to fraction collector, empty and clean RV Line 4, prime Loop 4, push back.

Wash (DMF, 3x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (HOAc, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**Thioglycoside Double Coupling - Fmoc Deprotection**

Temperature (25 °C, 20 °C, none)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Glycosylation (Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)

Add DCM (2 mL), set temperature(-40 °C). Prime Loop 3 and empty RV Line 3

Wait till set temperature is reached, DCM to waste, delivery of bb in DCM (1 mL), empty RV Line 3, prime Loop 3, push back bb to storage vessel. Prime Loop 6,
and empty RV Line 6, delivery of NIS stock solution (0.75 mL). Empty RV Line 6, prime Loop 6, push back NIS to storage vessel. Glycosylation reaction 5 min at -40 °C followed by 40 min at -20 °C, while continuously purging with high Ar flow. Clean RV Lines 3 and 6 and prime Loops 3 and 6.

Wash (THF, 6x, -20 °C, W)

Wash (DCM, 6x, -20 °C, W)

Glycosylation (Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)

Wash (THF, 6x, -20 °C, W)

Wash (DCM, 6x, -20 °C, W)

Temperature (25 °C, 24 °C, DCM)

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DMF, 3x, 25 °C, F)

Deprotection (Fmoc, 3x, 5 min., 25 °C, F)

Wash (DMF, 3x, 25 °C, F)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (HOAc, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)
Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**Beta-Man tom Deprotection**

Temperature (25 °C, 20 °C, none)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Glycosylation (Carboxybenzyl, 5 eq BB X, 5 eq Tf₂O, 5 eq, DTBMP, -30 °C, 120 min, F), add DCM (2 mL), set temperature(-30 °C, -29 °C, DCM). Prime Loop 3 and empty RV Line 3, wait till set temperature is reached, send DCM to waste, empty RV Line 3, prime Loop 3, push back reagent. Delivery of BB in 1 mL DCM. Prime Loop 6 and empty RV Line 6, delivery of Tf₂O stock solution (0.5 mL), empty RV Line 6, prime Loop 6, push back Tf₂O to storage. Glycosylation reaction 120 min at -30 °C, while continuously purging with high Ar flow. Collect glycosylation mix to fraction collector. Clean RV Lines 3 and 6 and prime Loops 3 and 6.

Wash (DCM, 6x, -30 °C, W)

Wash (DCM, 6x, -30 °C, F)

Glycosylation (Carboxybenzyl, 5 eq BB X, 5 eq Tf₂O, 5 eq, DTBMP, -30 °C, 120 min, F)

Wash (DCM, 6x, -30 °C, F)
Addition (Piperidine, 1x, -30 °C, W), add DMF (2 mL). Prime Loop 4 and empty RV Line 4, remove DMF, 1x delivery of piperidine stock solution, empty and clean RV Line 4 and prime Loop 4, push back piperidine.

Wash (DMF, 3x, -30 °C, W)

Temperature (25 °C, 24 °C, DCM)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Deprotection (tom, 1x, 120 min, 25 °C, W), empty RV Line 5, prime Loop 5, deliver TBAF stock solution, empty RV Line 4, push back TBAF stock solution to storage vessel, prime Loop 4, deprotection time 2 h while purging with Ar every 2 min. for 5 s.

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)

Deprotection (tom, 1x, 120 min, 25 °C, W)

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)
TCA-Reduction 1 Cycle (Treatment With 10 eq Bu$_3$SnH and cat. AIBN)

Temperature (25 °C, 20 °C, dry)

Wash (THF, 6x, 25 °C, W)

Deprotection (TCA, 1x, 120 min., 90 °C, W)

Prime Loop 5 and empty RV Line 5, deliver Bu$_3$SnH, AIBN stock solution (2 mL), empty RV Line 5, prime Loop 5, push back Bu$_3$SnH, AIBN, degass at 25 °C for 9 min. by high Ar flow. Set temperature 90 °C, deprotection reaction for 2 h while purging with Ar every 2 min for 5 s.

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Linker Cleavage by Treatment With 10 eq NaOMe)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Idle until depaused
Cleavage (linker, 1x, 25 °C, F), prime Loop 4 and empty RV Line 4. Deliver DCM (3 mL) to reaction vessel, deliver NaOMe stock solution (1 mL), empty RV Line 4, prime Loop 4, push back stock NaOMe solution to storage vessel, cleavage reaction 1.5 h, collect mix to fraction collector. Wash resin with 2 x DCM: MeOH (2:1), 5 x DCM, collect all washes to fraction collector clean NaOMe delivery line.

**Alpha Man Double Coupling**

Temperature (25 °C, 20 °C, dry)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Glycosylation (Mannose-Imidate, 3eq X, 0.3 eq TMSOTf, 10 °C, 15 min, F), addition of DCM (2 mL), set temperature 10 °C. Prime Loop 3 and empty RV Line 3, check temperature, deliver BB in DCM (1 mL). Empty RV Line 3, prime Loop 3. Empty RV Line 6 and prime Loop 6. Delivery of TMSOTf stock solution (0.15 mL). Empty RV Line 6 and prime Loop 6, push back TMSOTf to storage. Glycosylation reaction for 15 min, deliver reaction mix to fraction collector. Prime Loops 3 and 6 and clean RV Lines 3 and 6.

Wash( DCM, 6x, 10 °C, F)

Glycosylation (Mannose-Imidate, 3eq X, 0.3 eq TMSOTf, 10 °C, 15 min, F)

Wash (DCM, 6x, 10 °C, F)

Temperature (25 °C, 20 °C, DCM)
Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (piperidine, 1x, 25 °C, W)

Addition of DMF, fill loop with piperidine stock solution (2.5 mL), DMF to waste. Delivery of piperidine stock solution (2.5 mL), empty Rv Line 4, prime Loop 4, push back piperidine stock solution.

Wash (DMF, 3x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**Benzylidene Opening**

Temperature (25 °C, 20 °C, dry)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)
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Deprotection (Benzylidene-opening, 1x, 0 °C, 360 min., F), addition of DCM (2 mL), set temperature to 0 °C. Empty RV Line 5, prime Loop 5, check temperature, deliver BH\textsubscript{3} stock solution (1.5 mL, 30 eq.). Empty RV Line 5, prime Loop 5, push back to storage. Empty RV Line 5, prime Loop 5, deliver Bu\textsubscript{2}BOTf stock solution (0.5 mL, 1 eq.). Empty RV Line 5, prime Loop 5, push back to storage, deprotection reaction (5 h), every 2 min bubbling for 5 s. Collect to fraction collector, clean RV Line 5.

Wash (DCM, 6x, 0 °C, F)

Wash (THF, 6x, 0 °C, W)

Wash (piperidine, 1x, 0 °C, W)

Addition of DMF, fill loop with piperidine stock solution (2.5 mL), DMF to waste, delivery of piperidine stock solution (2.5 mL). Prime Loop 4, clean RV Line 4, empty RV Line 4, push back piperidine stock solution.

Wash (DMF, 3x, 0 °C, W)

Temperature (25 °C, 24 °C, DCM)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (HOAc, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)
Wash (DCM/MeOH, 36x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C)

3. Solid Support and Building Block Synthesis

Linker Synthesis 1

Synthesis of the linker 1 is outlined in Scheme S4, and described in detail below. To summarize, acid SI-1 was converted to hydroxymethylated acid SI-2 by chloromethylation and hydrolysis\textsuperscript{1,2}. Methyl ester SI-5 was synthesized by treatment of SI-2 with diazomethane, followed by carbamate formation with 5-(benzylamino)penten-1-ol SI-4\textsuperscript{3}. The ester of resulting linker construct SI-5 was hydrolyzed with aqueous potassium hydroxide. Acid SI-6 was installed on solid support by reaction of the corresponding cesium-salt with Merrifield resin\textsuperscript{4} and the resin was capped with excess acetic acid cesium-salt under the same conditions. Subsequently, the loading of resin 1 was conducted by reaction with fluorenlymethoxycarbonyl chloride (Fmoc), washing and Fmoc cleavage. Loading was determined by UV-quantification of the cleavage mixture.
Scheme S4. Synthesis of resin 1.

**Methyl 3-(4-(hydroxymethyl)phenyl)propanoate (SI-3).** *Caution:* The generation and handling of diazomethane requires special precautions. A solution of 3-(4-(hydroxymethyl)phenyl)propionic acid **SI-2** (11.7 g, 65 mmol) (1) in THF (150 mL) was treated at room temperature with a solution of diazomethane in diethyl ether until a strong yellow color persisted. Then, acetic acid was added dropwise until the solution turned colorless. The solution was diluted with ethyl acetate, and washed with saturated aqueous NaHCO₃ solution and brine. The aqueous layers were sequentially re-extracted once with ethyl acetate. The combined organic phases were dried over MgSO₄, filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc = 7:3) afforded **SI-3** (11.1 g, 88%). $^1$H NMR (CDCl₃, 300 MHz) δ 7.29-7.26 (m, 2H), 7.20-7.17 (m, 2H), 4.64 (d, $J$ = 5.9 Hz, 2H), 3.66 (s, 3H), 2.94 (t, $J$ = 7.8 Hz, 2H),
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2.62 (t, $J = 7.8$ Hz, 2H), 1.93 (t, $J = 5.9$ Hz, 1H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 173.2, 139.8, 138.8, 128.4, 127.2, 65.1, 51.7, 35.8, 30.7; EI-HRMS: $m/z$ calcd for C$_{11}$H$_{14}$O$_3$ [M$^+$] 194.0938, found 194.0937. IR (thin film) $\nu$ = 3607, 3446, 3008, 2953, 2872, 1732, 1514, 1438, 1366, 1292, 1036, 1003 cm$^{-1}$.

**Methyl 3-((benzyl(5-hydroxypentyl)-carbamoyloxy)methyl)phenyl)propanoate (SI-5).** A solution of SI-3 (8.0 g, 41 mmol) in acetonitrile (210 mL) was treated at room temperature with triethylamine (17.4 mL, 124 mmol) and cooled to 0 °C. At 0 °C, N,N'-disuccinimidyl carbonate (DSC) (15.9 g, 62 mmol) was added. The solution was warmed to 18°C over 1 h, diluted with ethyl acetate (800 mL), and washed with saturated aqueous NaHCO$_3$ solution. The aqueous layer was re-extracted once with ethyl acetate. The combined organic phases were dried over MgSO$_4$, filtered and concentrated *in vacuo* to afford the mixed carbonate that was used without further purification. A solution of the crude carbonate in CH$_2$Cl$_2$ (82 mL) was added at 0 °C to a solution of SI-4 (16.0 g, 83 mmol)$^3$ and triethylamine (14.4 mL, 103 mmol) in CH$_2$Cl$_2$ (210 mL). The solution was stirred for 50 min at room temperature, diluted with CH$_2$Cl$_2$ and washed with saturated aqueous NaHCO$_3$ solution. The aqueous layer was re-extracted twice with CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over MgSO$_4$, filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc = 2:1 to 1:1) afforded SI-5 (15.1 g, 88%) as a colorless oil. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.38-7.09 (m, 9H), 5.13 (s, 2H), 4.49 (s, 2H), 3.67 (s, 3H), 3.67-3.46 (m, 2H), 3.34-3.13 (m, 2H), 2.95 (s, 2H), 2.63 (s, 2H), 1.64-1.40 (m, 4H), 1.40-1.19 (m, 2H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 173.2, 156.7, 156.2, 140.2, 137.8, 134.7, 134.6, 128.4, 128.3, 128.1, 127.7, 127.2, 66.9, 62.5, 51.6, 50.4, 50.1, 46.9, 46.0, 35.5, 32.2, 30.5, 27.8, 27.3, 22.8; MALDI-HRMS: $m/z$ calcd for C$_{24}$H$_{31}$NO$_5$Na [M+Na$^+$] 436.2094, found 436.2097. IR (thin film) $\nu$ = 3610, 3467, 3008, 2938, 1732, 1690, 1468, 1438, 1364, 1303, 1072 cm$^{-1}$. 
3-(4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propionic acid (SI-6). SI-5 (3 g) was dissolved in THF (30 mL), aqueous KOH (1 M, 30 mL) was added and the solution was stirred for 13 h. The pH was adjusted to 7 by addition of acidic Amberlite IR-120. The Amberlite was filtered off and rinsed with THF/H₂O (1:1). The filtrate was concentrated, the residue was coevaporated with toluene purified by flash column chromatography (CH₂Cl₂/MeOH = 99:1 to 9:1 with 1% HOAc) and dried under high vacuum to afford SI-6 (3 g, quant.) as a colorless oil. ¹H NMR (CDCl₃, 300 MHz) δ 7.38-7.10 (m, 9H), 5.21 (s, 2H), 4.56 (s, 2H), 3.68-3.52 (m, 2H), 3.3-3.15 (m, 2H), 3.03 (s, 2H), 2.74 (s, 2H), 1.70-1.15 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 177.5, 177.1, 156.8, 156.4, 140.2, 137.8, 134.8, 128.6, 128.55, 128.4, 128.2, 127.8, 127.4, 127.2, 67.1, 62.6, 50.6, 50.2, 46.9, 46.2, 30.4, 27.4, 22.8; MALDI-HRMS: m/z calcd for C₂₄H₃₁NO₅Na [M+Na⁺] 422.1938, found: 422.1940. IR (thin film) ν = 3685, 3602, 3436, 3008, 2936, 1690, 1602, 1515, 1474, 1453, 1426, 1364, 1248, 1127, 1074, 1042, 908, 816 cm⁻¹.

3-(4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propionic ester resin (1).

**Functionalization.** Merrifield resin (3g, 1.14 mmol, copolymerized, Polymer Labs, 0.38 mmol/g free sites) was swollen in CH₂Cl₂ (30 mL). Acid SI-6 (690 mg, 1.71 mmol) was dissolved in EtOH (6 mL) and H₂O (1.5 mL) before aqueous Cs₂CO₃ (0.85 mL, 1 M) was added to adjust to pH 7. The solution was concentrated and coevaporated three times with dioxane. The resin was drained and washed twice with DMF (10 mL). The Cs-salt of the acid was dissolved in DMF (10 mL) and added to the resin followed by a catalytic amount of sodium iodide. The resulting mixture was heated to 50 °C for 13 h. Upon cooling to room temperature, the resin was washed consecutively three times each with DMF, H₂O/DMF (1:1), DMF, DCM, MeOH, DCM. This procedure was repeated twice.
Capping of Unreacted Sites. The functionalized resin was preswollen in CH$_2$Cl$_2$ (30 mL). Acetic acid (652 µL, 11.4 mmol) was dissolved in EtOH (6 mL) and H$_2$O (1.5 mL) before aqueous Cs$_2$CO$_3$ (5.7 mL, 1 M) was added to adjust to pH 7. The solution was concentrated and coevaporated three times with dioxane. The resin was drained and washed twice with DMF (10 mL). The Cs-salt of the acid was dissolved in DMF (17 mL) and added to the resin followed by a catalytic amount of sodium iodide. The resulting mixture was heated to 50°C for 24 h. Upon cooling to room temperature the resin was washed consecutively three times each with DMF, H$_2$O/DMF (1:1), DMF, DCM, MeOH, DCM. The resin was dried under high vacuum.

Determination of Loading. An aliquot of functionalized and capped resin (29.8 mg) was swollen in CH$_2$Cl$_2$ (1 mL) for 1 h. Pyridine (100 µL) and fluorenlymethoxycarbonyl chloride (Fmoc) (100 mg) were added and the mixture was shaken overnight. The resin was drained, washed with CH$_2$Cl$_2$ and MeOH (six alternating washes), swollen in CH$_2$Cl$_2$, drained and dried. A solution of piperidine in DMF (20%, 2 mL) was added and the resin was shaken for 4 h. The solution was drained and a 100 µL aliquot was taken. This aliquot was diluted to 10 mL and the UV absorption at 301 nm was determined: A$_{301} = 0.206$. The loading was calculated according to the following formula:

$$\text{Loading } \left[ \frac{\text{mmol}}{g} \right] = \frac{\text{Absorption} \times \text{Dilution} \times \text{Volume} \times 1000}{\text{Extinction Coefficient} \times \text{Resin Weight}} = \frac{0.206 \times 100 \times 0.002 \times 1000}{7800 \times 0.0298} = 0.177 \frac{\text{mmol}}{g}$$
Synthesis of Building Blocks

Synthesis of Building Block 1,6-Thioethylglucosamine (2).

Scheme S5. Synthesis of building blocks 2 and 3.

Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside (SI-7). To a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-N-trichloroacetamido-D-glucopyranose\(^5\) (16.0 g, 32.45 mmol) in CH\(_2\)Cl\(_2\) (35 mL) was added ethanethiol (2.7 mL, 35.70 mmol) and boron trifluoride etherate (3.1 mL, 24.34 mmol) at 0 °C. The reaction was allowed to warm to room temperature and was stirred overnight. The mixture was diluted with EtOAc (100 mL) and washed with water (150 mL), aqueous saturated NaHCO\(_3\) solution (2 x 75 mL) and brine (50 mL). The organic layer was dried over MgSO\(_4\), filtered and concentrated. Crystallization from EtOAc/hexanes gave SI-7 (13.6 g, 85%). [\(\alpha\)]\(_D\)\(^{24}\) = 65.8 (c 2.0, CHCl\(_3\)); mp. 133-134 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 6.98 (d, J = 9.3 Hz, 1H), 5.35 (dd, J = 9.5, 10.3 Hz, 1H), 5.12 (t, J = 9.7 Hz, 1H), 4.69 (d, J = 10.3 Hz, 1H), 4.25 (dd, J = 5.2, 12.4 Hz, 1H), 4.22-4.03 (m, 2H), 3.77 (ddd, J =
2.4, 5.2, 10.0 Hz, 1H), 2.86-2.61 (m, 2H), 2.08 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.27 (t, J = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 171.0, 170.6, 169.2, 161.9, 92.2, 83.8, 76.1, 73.1, 68.5, 62.3, 54.7, 24.3, 20.7, 20.6, 20.5, 14.9; HR-MALDI-MS: $m/z$ [M+Na$^+$] calcd for C$_{16}$H$_{22}$Cl$_3$NO$_8$SNa 516.0024, found 516.0018. IR (thin film) $\nu = 3336, 2968, 1749, 1529, 1368, 1229, 1039, 821$ cm$^{-1}$.

**Ethyl 2-deoxy-2-N-trichloroacetamido-3,4,6-tri-O-trimethylsilyl-thio-$\beta$-D-glucopyranoside (SI-8).** To a solution of SI-7 (13.5 g, 27.4 mmol) in MeOH (130 mL) sodium methoxide (148 mg, 2.74 mmol) was added at room temperature. The solution was stirred overnight, and was neutralized using acidic Amberlite resin IR-120. The resin was filtered off and washed with MeOH (50 mL). The filtrate was evaporated in vacuo to afford the triol intermediate (10.1 g, quant.). The triol (10.1 g, 27.4 mmol) was suspended in CH$_2$Cl$_2$ (50 mL) and Et$_3$N (76.2 mL, 548 mmol). The mixture was cooled to 0 °C and chlorotrimethylsilane (14.0 mL, 109.6 mmol) was added dropwise at the same temperature. The reaction was allowed to warm to room temperature and was stirred overnight. The solvent was evaporated in vacuo. The solid residue was suspended in hexanes/EtOAc (9/1, 200 mL) and filtered. The filtrate was evaporated and purified by flash column chromatography on silica gel (hexanes/EtOAc = 19:1 with 0.1% triethylamine) to provide SI-8 (14.5 g, 90%). $[\alpha]_D^{25} = -18.3$ (c 1.0, CHCl$_3$); mp. 142-143 °C; $^1$H NMR (400 MHz, CDCl$_3$) δ 6.94 (d, J = 7.8 Hz, 1H), 4.54 (d, J = 8.2 Hz, 1H), 3.76 (dd, J = 4.9, 10.9 Hz, 1H), 3.71-3.63 (m, 2H), 3.63-3.53 (m, 2H), 3.30 (dd, J = 4.9, 10.9 Hz, 1H), 2.68-2.38 (m, 2H), 1.09 (t, J = 7.4 Hz, 3H), 0.00 (s, 9H), −0.00 (s, 9H), −0.05 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 160.9, 92.2, 81.7, 80.8, 74.3, 70.3, 61.93, 56.5, 24.4, 14.6, 0.1, −0.0, −0.8; HR-MALDI-MS: $m/z$ [M+2+Na$^+$] calcd for C$_{19}$H$_{40}$Cl$_3$NO$_5$S$i_3$Na 608.0865, found 608.0858. IR (thin film) $\nu = 3309, 2966, 1694, 1532, 1248, 1157, 837$ cm$^{-1}$. 

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Ethyl 3,4-di-O-benzyl-2-deoxy-2-N-trichloracetamido-thio-β-D-glucopyranoside (SI-9). A mixture of compound SI-8 (15 g, 25.6 mmol), benzaldehyde (6.25 mL, 61.5 mmol), and freshly dried 4Å molecular sieves (10 g) in CH₂Cl₂ (300 mL) was stirred using an over-head stirrer at 0 °C for 1 h. TMSOTf (695 μL, 3.84 mmol) was added to the solution, and the mixture was continuously stirred at the same temperature for another 1 h. The reaction was cooled to −78 °C, and triethylsilane (4.5 mL, 28.2 mmol) and TMSOTf (347 μL, 1.92 mmol) were sequentially added to the reaction solution. The mixture was stirred for an additional 16 h at −78 °C. Additional triethylsilane (2.25 mL, 14.1 mmol) and TMSOTf (347 μL, 1.92 mmol) were added to the reaction solution and the mixture was stirred at −78 °C for another 8 h. The reaction was warmed up to 0 °C. A solution of borane in THF (1 M, 120.0 mL, 120.0 mmol) and TMSOTf (2.3 mL, 12.8 mmol) were sequentially added to the reaction at the same temperature, and the mixture was stirred for another 6 h. The reaction was slowly quenched by MeOH (50 mL) at 0 °C. The mixture was filtered through a pad of celite, and the filtrate was coevaporated with MeOH (200 mL) in vacuo. The residue was dissolved in EtOAc (200 mL), washed with water (100 mL) and the aqueous layer was extracted three times with EtOAc (100 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered and concentrated in vacuo. Purification with flash column chromatography on silica gel (cyclohexane/EtOAc = 2:1 to 3:2 to 1:1) gave SI-9 (13.40 g, 95%). [α]D₂⁴ −3.3 (c 1.0, CHCl₃); mp. 147-148 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.23-7.11 (m, 10H), 6.70 (d, J = 8.2 Hz, 1H), 4.78 (d, J = 10.2 Hz, 1H), 4.68 (d, J = 10.9 Hz, 2H), 4.58 (d, J = 10.8 Hz, 1H), 4.53 (d, J = 11.1 Hz, 1H), 3.90 (dd, J = 8.6, 9.7 Hz, 1H), 3.74 (dd, J = 2.4, 12.1 Hz, 1H), 3.63-3.45 (m, 3H), 3.34 (ddd, J = 2.7, 4.5, 9.5 Hz, 1H), 2.67-2.46 (m, 2H), 1.77 (s, 1H), 1.12 (t, J = 7.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 161.7, 137.7, 137.6, 128.5, 127.92, 127.86, 127.82, 92.4, 82.9, 81.8, 79.7, 78.2, 75.3, 74.9, 61.9, 57.3, 24.6, 15.1; HR-MALDI-MS: m/z [M+Na⁺] calcd for C₂₄H₂₈Cl₃NO₅SNa 570.0646, found 570.0655. IR (thin film) ν = 3324, 1687, 1527, 1084, 823 cm⁻¹.
Ethyl 3,4-di-O-benzyl-6-O-fluorenylmethoxycarbonyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside (Building Block 2). Compound SI-9 (13.40 g, 24.41 mmol) was coevaporated three times with toluene (100 mL), and was dissolved in pyridine (44 mL) and CH$_2$Cl$_2$ (67 mL). FmocCl (7.58 g, 29.3 mmol) was added at room temperature and the reaction mixture was stirred for 24 h. Methanol (50 mL) was added, and the mixture was poured into water (100 mL). The aqueous layer was extracted three times with EtOAc (100 mL). The organic layers were combined, dried (MgSO$_4$), filtered, and evaporated. Crystallization from EtOAc/EtOH gave 2 (14.2 g, 75%). Purification of the mother liquor by flash column chromatography on silica gel (hexane/EtOAc = 10:1 to 3:1 to 2:1 to 1:1) provided additional 2 (2.27 g, 12%) as well as recovered starting material SI-9 (1.13 g, 8%). $[\alpha]_D^{24} = -15.0$ (c 2.0, CHCl$_3$); mp. 172-173 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.75 (dd, $J = 0.7, 7.6$ Hz, 2H), 7.63-7.55 (m, 2H), 7.39 (td, $J = 0.6, 7.5$ Hz, 2H), 7.35-7.25 (m, 11H), 6.85 (d, $J = 8.2$ Hz, 1H), 4.91 (d, $J = 10.1$ Hz, 1H), 4.87-4.78 (m, 2H), 4.74 (d, $J = 10.8$ Hz, 1H), 4.60 (d, $J = 11.0$ Hz, 1H), 4.45 (dd, $J = 2.3, 11.6$ Hz, 1H), 4.43-4.33 (m, 2H), 4.30 (dd, $J = 5.3, 11.6$ Hz, 1H), 4.23 (t, $J = 7.4$ Hz, 1H), 4.06 (dd, $J = 8.2, 9.6$ Hz, 1H), 3.80-3.65 (m, 2H), 3.61 (dd, $J = 8.2, 9.4$ Hz, 1H), 2.70 (m, 2H), 1.24 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.6, 154.9, 143.4, 143.3, 141.30, 141.29, 137.5, 137.4, 128.6, 128.1, 128.0, 128.0, 127.90, 127.86, 127.2, 125.2, 125.1, 120.1, 92.4, 82.6, 81.7, 78.2, 75.4, 75.0, 70.0, 66.6, 57.4, 46.7, 24.7, 15.1; HR-MALDI-MS: m/z [M+2+Na$^+$] calcd. for C$_{39}$H$_{38}$Cl$_3$NO$_7$SNa 794.1303, found 794.1291. IR (thin film) $\nu$ = 3309, 3031, 1749, 1689, 1534, 1258, 1085, 824 cm$^{-1}$. 
Synthesis of Building Block 1,4-Thioethylglucosamine (3).

**Ethyl 3,6-di-O-benzyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside (SI-11).** A mixture of SI-8 (15 g, 25.6 mmol), benzaldehyde (6.25 mL, 61.5 mmol), and freshly dried 4Å molecular sieves (10 g) in CH$_2$Cl$_2$ (300 mL) was stirred using an over-head stirrer at 0 °C for 1 h. TMSOTf (695 μL, 3.84 mmol) was added to the solution, and the mixture was continuously stirred at the same temperature for another hour. The reaction was cooled to −78 °C, and triethylsilane (4.5 mL, 28.2 mmol) and TMSOTf (347 μL, 1.92 mmol) were sequentially added to the reaction solution. The mixture was stirred for an additional 16 h at −78 °C. Additional triethylsilane (2.25 mL, 14.1 mmol) and TMSOTf (347 μL, 1.92 mmol) were added to the reaction solution, and the mixture was stirred at −78 °C for 8 h. Triethylsilane (49.1 mL, 307.2 mmol) and trifluoroacetic acid (9.8 mL, 128.0 mmol) were then sequentially added to the reaction at the same temperature, and the mixture was stirred for 4 h. The reaction flask was slowly warmed to room temperature and the mixture was filtered through a pad of celite. The filtrate was carefully washed twice with saturated aqueous NaHCO$_3$ (150 mL). The aqueous layer was extracted three times with EtOAc (150 mL), and the combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. Purification by flash column chromatography on silica gel (cyclohexane/EtOAc = 4:1) provided SI-10 (12.3 g, 87%). [α]$_D^{23}$ = −16.0 (c 1.0, CHCl$_3$); mp. 55-56 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.22-7.08 (m, 10H), 6.70 (d, $J = 8.3$ Hz, 1H), 4.69 (d, $J = 10.2$ Hz, 1H), 4.60 (ABq, $J = 11.2$ Hz, 2H), 4.39 (ABq, $J = 12.0$, 2H), 3.69 (dd, $J = 8.5$, 9.9 Hz, 1H), 3.63-3.46 (m, 4H), 3.37 (dt, $J = 4.9$, 9.7 Hz, 1H), 2.70 (d, $J = 2.6$ Hz, 1H), 2.62-2.42 (m, 2H), 1.07 (t, $J = 7.4$, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.6, 137.9, 137.54, 128.5, 128.5, 128.0, 127.9, 127.9, 127.7, 92.4, 82.8, 81.4, 77.9, 74.7, 73.7, 73.3, 70.6, 56.8, 24.4, 15.1; HR-MALDI-MS: m/z [M+Na$^+$] calcd for C$_{24}$H$_{28}$Cl$_3$NO$_5$SNa 570.0646, found 570.0653. IR (thin film) $\nu = 3323$, 2870, 1690, 1528, 1060, 821 cm$^{-1}$.
Ethyl 3,6-di-O-benzyl-4-O-fluorenymethoxycarbonyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside (Building Block 3). Compound SI-10 (4.92 g, 8.96 mmol) was coevaporated three times with toluene (30 mL) and dissolved in pyridine (49 mL). To the mixture was added FmocCl (4.64 g, 17.93 mmol) at room temperature and stirred for 5 h. Methanol (20 mL) was added and the mixture was poured into water (50 mL). The aqueous layer was extracted three times with EtOAc (50 mL). The organic layers were combined, dried (MgSO\textsubscript{4}), filtered, and evaporated. Purification by flash column chromatography on silica gel (cyclohexane/EtOAc = 9:1 to 3:1 to 2:1 to 1:1) provided 3 (5.50 g, 80%) and recovered starting material (0.42 g, 9%). [\textalpha]\textsubscript{D}\textsuperscript{27} = -5.2 (c 1.0, CHCl\textsubscript{3}); mp. 126-127 °C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 7.67 (dt, J = 1.0, 7.6 Hz, 2H), 7.48 (dd, J = 0.7, 7.2 Hz, 1H), 7.43 (d, J = 7.5 Hz, 1H), 7.30 (t, J = 7.5 Hz, 2H), 7.26-7.18 (m, 5H), 7.18-7.09 (m, 7H), 6.82 (d, J = 7.9 Hz, 1H), 4.95 (d, J = 10.3 Hz, 1H), 4.86 (dd, J = 9.1, 9.8 Hz, 1H), 4.56 (s, 2H), 4.45 (s, 2H), 4.29-4.19 (m, 2H), 4.15 (t, J = 9.8 Hz, 1H), 4.03 (t, J = 7.2 Hz, 1H), 3.74-3.63 (m, 1H), 3.64-3.50 (m, 3H), 2.77-2.53 (m, 2H), 1.21 (t, J = 7.4 Hz, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \( \delta \) 161.6, 154.3, 143.3, 143.1, 141.3, 141.3, 137.9, 137.3, 128.41, 128.3, 127.9, 127.9, 127.8, 127.6, 127.2, 127.2, 125.1, 125.0, 120.07, 120.06, 92.3, 82.5, 78.8, 76.2, 74.7, 73.6, 70.1, 69.6, 57.7, 46.7, 24.7, 15.2; HR-MALDI-MS: \( m/z \) [M+2+Na\textsuperscript{+}] calcd for C\textsubscript{39}H\textsubscript{38}Cl\textsubscript{3}NO\textsubscript{7}SNa 794.1303, found 794.1297. IR (thin film) \( \nu \) = 3316, 2929, 1754, 1690, 1531, 1257, 738 cm\textsuperscript{-1}. 


Synthesis of Building Block 1,4-N-phenyltrifluoroacetimidoyl glucosamine (4).

**Scheme S6.** Synthesis of Glucosamine building block 4.

**tert-Butyldimethylsilyl 3,6-Di-O-benzyl-4-O-fluorenylmethoxycarbonyl-2-N-trichloroacetamido-β-D-glucopyranoside (SI-11).** \textit{tert-Butyldimethylsilyl} 3,6-di-O-benzyl-2-N-trichloroacetamido-β-D-glucopyranoside\(^6\) (2.24 g, 3.63 mmol, 1 eq.) was coevaporated three times with toluene, dried under high vacuum and dissolved in pyridine (20 mL). Fmoc-Cl was added (1.88 g, 7.26 mmol, 2 eq) and the solution was stirred for 1 h. Methanol (5 mL) was added, the mixture was stirred for 30 min and poured into water (40 mL). The aqueous phase was extracted with CH\(_2\)Cl\(_2\) (350 mL). The organic extracts were washed with HCl (1 M, 120 mL), brine (80 mL), dried over MgSO\(_4\) and evaporated. Silica gel flash column chromatography (hexane/EtOAc = 5:1) afforded SI-11 (2.15 g, 70%). \(R_f\) (SiO\(_2\), hexanes/EA = 4:1) = 0.39; [\(\alpha\)]\(_D\)\(^{25}\) 5.8 (c 1, CDCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.75 (d, \(J = 7.6\) Hz, 2H), 7.58-7.50 (m, 2H), 7.41-7.20 (14 H), 6.96 (d, \(J = 7.6\) Hz, 1H), 5.19 (d, \(J = 7.8\) Hz, 1H), 4.93 (t, \(J = 9.2\) Hz, 1H), 4.64 (s, 2H), 4.54 (s, 2H), 4.36-4.29 (m, 1H), 4.14-4.09 (m, 1H), 3.80-3.73 (m, 1H), 3.65-3.62 (m, 2H), 3.53-3.43 (m, 1H), 0.90 (s, 9H), 0.16-0.13 (m, 6H); \(^1\)C NMR (75.4 MHz, CDCl\(_3\)) \(\delta\) 161.5, 154.1, 143.1, 143.0, 141.1, 137.8, 137.3, 128.2, 127.8, 127.7, 127.4, 127.1, 125.0, 94.2, 94.0, 92.3, 76.3, 74.4, 73.5, 73.0, 70.1, 70.0, 60.7, 60.6, 46.6, 28.8, 25.7, 18.0, −4.1, −5.1; HR-MALDI-MS: \(m/z\) [M + Na\(^+\)] calc. 862.2215, found 862.2116. IR (thin film) \(\nu\) = 3424, 3020, 2937, 2654, 1749, 1718, 1521, 1453, 1386, 1360, 1257, 1122, 1065, 972, 842 cm\(^{-1}\).
3,6-Di-O-benzyl-4-O-fluorenylmethoxycarbonyl-2-deoxy-2-N-trichloroacetamido-\(\alpha\)-d-glucopyranosyl N-phenyl-trifluoroacetimidate (Building Block 4). In a plastic vessel, monosaccharide SI-11 (1.51 g, 1.79 mmol) was dissolved in THF (9 mL) and HF pyridine (0.9 mL, ca 70% HF) was slowly added. The mixture was stirred overnight. Saturated aqueous NaHCO\(_3\) was added and the mixture was extracted three times with EtOAc. The combined organic extracts were dried over MgSO\(_4\), filtered and concentrated. The residue was coevaporated three times with toluene, dissolved in CH\(_2\)Cl\(_2\) (7 mL), N-phenyl-2,2,2-trifluoroacetimidoyl chloride\(^7\) (0.91 g, 4.37 mmol, 2.5 eq.) and Cs\(_2\)CO\(_3\) (1.14 g, 3.49 mmol, 2 eq.) were added. The mixture was stirred at room temperature for 3 h, filtered over Celite and concentrated. The residue was purified by silica gel flash column chromatography (cyclohexane to cyclohexane/EtOAc = 4:1). The product 4 was obtained as a colorless foam (1.21 g, 76% over 2 steps). \(R_t\) (cyclohexane/EtOAc = 4:1) = 0.24; \([\alpha]_D^{20}\) 56.6 (c 1, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.73-7.80 (m, 2H), 7.55-7.64 (m, 2H), 7.83-7.10 (m, 17H), 6.56-6.54 (m, 1H), 6.56-6.38 (m, 2H), 5.23-5.04 (m, 1H), 4.90-3.84 (m, 11H), 3.71-3.64 (m, 2H); \(^{13}\)C NMR (75.4 MHz, CDCl\(_3\)) \(\delta\) 162.0, 154.3, 154.2, 143.3, 143.2, 143.0, 141.5, 141.5, 137.8, 137.0, 129.0, 128.9, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.4, 125.5, 125.5, 125.2, 124.9, 120.4, 120.3, 119.4, 104.2, 93.4, 93.3, 92.2, 76.0, 74.8, 74.1, 74.0, 73.9, 72.0, 71.9, 71.4, 70.6, 70.5, 70.1, 70.0, 68.7, 66.0, 53.8, 47.1, 47.0; HR-MALDI-MS: \(m/z\) [M + Na\(^+\)] calc. 919.1538 found 919.1544. IR (thin film) \(\nu\) = 3425, 3008, 2927, 1752, 1724, 1656, 1594, 1514, 1452, 1386, 1312, 1259, 1166, 1117, 1027, 980, 881, 820 cm\(^{-1}\).
Synthesis of Building Block 1,4-Thioethylglucose (5).

Scheme S7. Synthesis of Glucose building block 5.

Ethyl 2,3,4,6-tetra-O-trimethylsilyl-thio-β-D-glucopyranoside (SI-12). To a solution of ethyl 2,3,4,6-tetra-O-acetyl-thio-β-D-glucopyranoside (9.5 g, 24.2 mmol) in MeOH (90 mL) was added sodium methoxide (131 mg, 2.42 mmol) at room temperature. The solution was stirred overnight, and was neutralized using acidic Amberlite resin IR-120. The resin was filtered off and washed with MeOH (50 mL). The filtrate was evaporated in vacuo to afford the tetraol intermediate (5.41 g, quant.). The tetraol (5.41 g, 24.2 mmol) was suspended in CH₂Cl₂ (30 mL) and Et₃N (70.0 mL, 506 mmol), and the mixture was cooled to 0 °C. Chlorotrimethylsilane (16.2 mL, 126.5 mmol) was added dropwise at the same temperature, and the reaction was allowed to warm up to room temperature and was stirred overnight. The solvent was evaporated in vacuo. The solid residue was suspended with 10% EtOAc/hexane (200 mL) and filtered. The filtrate was evaporated, and purified by flash column chromatography on silica gel (EtOAc/hexanes = 5:95 with 0.1% triethylamine) to provide the desired product SI-12 (12.1 g, 93%) as colorless syrup. [α]D²⁰ = −22.3 (c 1.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.35 (d, J = 9.0 Hz, 1H), 3.77 (dd, J = 2.2, 11.3 Hz, 1H), 3.62 (dd, J = 5.8, 11.6 Hz, 1H), 3.43-3.30 (m, 3H), 3.23-3.19 (m, 1H), 1.27 (t, J = 7.5 Hz, 3H), 0.19 (s, 9H), 0.15 (s, 9H), 0.14 (s, 9H), 0.09 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 86.2, 81.2, 79.8, 75.4, 71.7, 62.6, 26.9, 25.1, 15.0, 1.6, 1.4, 0.9, −0.2; HR-ESI-MS: m/z [M+Na⁺] calcd for C₃₀H₄₆O₅SSi₄Na 535.2197, found 535.2195. IR (thin film) ν = 2957, 2900, 1249, 1161, 1090, 839 cm⁻¹.
**Ethyl 2-O-benzoyl-3,6-di-O-benzyl-thio-β-D-glucopyranoside (SI-13).** To a solution of **SI-12** (500 mg, 0.975 mmol), PhCHO (217 μL, 2.144 mmol), and freshly dried 4Å molecular sieves (250 mg) in CH₂Cl₂ (7.5 mL) was added TMSOTf (27 μL, 0.146 mmol) at -78 °C under argon atmosphere. After stirring at -78 °C for 2 h, Et₃SiH (171 μL, 1.072 mmol) and TMSOTf (14 μL, 0.073 mmol) were sequentially added to the solution, and the mixture was continuously stirred for another 16 h. Benzoic anhydride (478 mg, 2.144 mmol), benzoic acid (244 mg, 0.195 mmol) and TMSOTf (90 μL, 0.487 mmol) were consecutively added to the solution. The reaction flask was warmed up to 0 °C and the mixture was stirred at the same temperature for 3 days. The mixture was then cooled to -78 °C, and Et₃SiH (1.86 mL, 11.69 mmol) followed by TFA (0.375 mL, 4.873 mmol) were added under argon at -78 °C and the mixture was kept stirring for 3 h. The reaction was warmed up to 0 °C and slowly quenched by saturated aqueous NaHCO₃ (10 mL). The mixture was filtered through a pad of celite, and the filtrate was extracted three times with EtOAc (20 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered, evaporated, and purified by flash column chromatography on silica gel (hexane/EtOAc = 3:1) to give **SI-13** (255 mg, 51%). [α]D²° 13.1 (c 0.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.04-8.01 (m, 2H), 7.58-7.54 (m, 1H), 7.44 (t, J = 7.9 Hz, 2H), 7.35-7.28 (m, 5H), 7.17 (s, 5H), 5.26 (dd, J = 9.2, 10.0 Hz, 1H), 4.70 (ABq, J = 11.5 Hz, 2H), 4.59 (ABq, J = 12.0 Hz, 2H), 4.54 (d, J = 10.0 Hz, 1H), 3.82-3.77 (m, 3H), 3.68 (t, J = 8.9 Hz, 1H), 3.56 (dt, J = 9.5, 4.6 Hz, 1H), 2.80 (s, 1H), 2.76 (m, 2H), 1.21 (t, J = 7.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.2, 137.9, 137.7, 133.2, 129.8, 128.45, 128.40, 128.36, 128.0, 127.82, 127.76, 127.74, 83.6, 83.5, 78.2, 74.6, 73.7, 72.3, 72.0, 70.5, 23.9, 14.9; HR-ESI-MS: m/z [M+Na⁺] calcd for C₂₉H₃₂O₆SNa 531.1817, found 531.1807. IR (thin film) ν = 3463, 3031, 2870, 1723, 1451, 1266, 1066 cm⁻¹.
Ethyl 2-O-benzoyl-3,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-thio-β-D-glucopyranoside (Building Block 5). Compound SI-13 (795 mg, 1.563 mmol) was coevaporated three times with toluene (50 mL) and was dissolved in CH₂Cl₂ (6 mL) and pyridine (4 mL). To the mixture was added FmocCl (485 mg, 1.876 mmol) at room temperature under argon atmosphere and the mixture was stirred for 24 h. Methanol (5 mL) was added before and the mixture was poured into water (20 mL). The aqueous layer was extracted three times with EtOAc (30 mL). The organic layers were combined, washed with brine, dried (MgSO₄), filtered, and evaporated. Flash column chromatography on silica gel (hexanes/EtOAc = 10:1 to 6:1 to 2:1) provided 5 (872 mg, 76%) and recovered SI-13 (93 mg, 12%).

[α]D²⁰ 32.3 (c 0.75, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.00 (dd, J = 1.2, 8.3 Hz, 2H), 7.73 (dd, J = 4.0, 7.6 Hz, 2H), 7.59-7.50 (m, 3H), 7.43 (t, J = 8.0 Hz, 2H), 7.37 (td, J = 2.7, 7.5 Hz, 2H), 7.32-7.18 (m, 7H), 7.03 (s, 5H), 5.32 (t, J = 9.9 Hz, 1H), 4.97 (t, J = 9.8 Hz, 1H), 4.60-4.53 (m, 5H), 4.31 (d, J = 6.9 Hz, 2H), 4.10 (t, J = 7.1 Hz, 1H), 3.89 (t, J = 9.2 Hz, 1H), 3.76-3.71 (m, 1H), 3.66-3.65 (m, 2H), 2.77-2.63 (m, 2H), 1.22 (t, J = 7.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.0, 154.2, 143.3, 143.1, 141.29, 141.26, 137.9, 137.4, 133.2, 129.9, 129.7, 128.40, 128.36, 128.32, 128.13, 128.10, 128.0, 127.8, 127.7, 127.6, 127.5, 127.2, 125.1, 125.0, 120.1, 83.7, 81.1, 77.4, 75.6, 74.4, 73.6, 71.9, 70.0, 69.7, 46.7, 24.1, 14.9; HR-ESI-MS: m/z [M+Na⁺] calcd for C₄₄H₄₂O₈SNa 753.2498, found 753.2479. IR (thin film) ν = 3031, 2871, 1753, 1728, 1451, 1252, 1070, 739 cm⁻¹.

Synthesis of Building Block 1,3-Thioethylgalactose (6).

![Scheme S8. Synthesis of Galactose building block 6.](image-url)
**Supporting Information**

**Seeberger et al.**

**Ethyl 4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-thio-\(\beta\)-D-galactopyranoside (SI-14).** Ethyl 4,6-di-O-benzyl-thio-\(\beta\)-D-galactopyranoside\(^8\) (2.92 g, 7.24 mmol) was coevaporated three times with toluene (30 mL), then dissolved in toluene (91 mL) in a round bottom flask equipped with a dean stark apparatus. Dibutyltin oxide (2.07 g, 8.33 mmol) was added and the solution was heated to 150 °C. Toluene (20 mL) was collected over a period of 1 h when fresh toluene was added and the reaction was cooled to room temperature. FmocCl (2.06 g, 7.97 mmol) was added and the reaction was stirred for another 2 h. Dichloromethane was added (100 mL) and the organic layer was extracted twice with HCl (0.5 M, 100 mL), dried over MgSO\(_4\), filtered and concentrated under reduced pressure. Silica column chromatography (cyclohexane/EtOAc = 19:1 to 3:1) afforded SI-14 with a yield of 87% (3.94 g). \([\alpha]_D^{20}\) 3.24 (c 1, CHCl\(_3\)); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.73 (t, \(J = 7.4\) Hz, 2H), 7.61 (dd, \(J = 7.4, 13.1\) Hz, 2H), 7.33-7.12 (m, 14H), 4.68 (dd, \(J = 3.1, 9.7\) Hz, 1H), 4.57 (d, \(J = 11.3\) Hz, 1H), 4.46-4.39 (m, 2H), 4.36 (dt, \(J = 4.7, 7.6\) Hz, 3H), 4.29 (d, \(J = 9.7\) Hz, 1H), 4.19 (t, \(J = 7.2\) Hz, 1H), 3.94 (ddd, \(J = 2.8, 7.5, 9.7\) Hz, 2H), 3.66 (dd, \(J = 3.7, 10.0\) Hz, 1H), 3.56-3.50 (m, 2H), 2.65 (qq, \(J = 7.4, 12.7\) Hz, 2H), 2.33 (d, \(J = 2.5\) Hz, 1H), 1.22 (dd, \(J = 5.2, 9.7\) Hz, 3H); \(^13\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 154.7, 143.5, 142.9, 141.3, 141.2, 138.0, 137.7, 128.4, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.1, 127.1, 125.2, 125.0, 120.0, 86.5, 80.7, 77.2, 75.1, 74.1, 73.5, 69.9, 68.2, 68.0, 46.7, 24.4, 15.3; HR-ESI: \(m/z\) [M+Na\(^+\)] calcd. for C\(_{37}\)H\(_{38}\)NaO\(_7\)S 649.2236, found 649.2233. IR (thin film) \(\nu = 3320, 2943, 2832, 1449, 1113, 1021\) cm\(^{-1}\).

**Ethyl 4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-2-O-pivaloyl-thio-\(\beta\)-D-galactopyranoside (Building Block 6).** Compound SI-14 (950 mg, 1.51 mmol) was dissolved in dichloromethane (15mL) under an argon atmosphere and the solution was cooled to −15 °C. Pivaloyl chloride (373 \(\mu L\), 3.04 mmol) was added and the reaction was stirred for 40 min. The reaction was diluted with hexane/ethyl acetate (1/1 v/v, 100 mL) and the formed precipitate was filtered...
through a silica pad. The organic layer was washed with HCl (0.3 M, 3 x 50 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Silica column chromatography (cyclohexane/EtOAc = 19:1 to 4:1) afforded 6 with a yield of 90% (964 mg). [α]D²⁰ 1.81 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.80-7.68 (m, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.41-7.10 (m, 16H), 5.46 (t, J = 10.0 Hz, 1H), 4.92 (dd, J = 3.0, 10.0, Hz, 1H), 4.76 (d, J = 11.5 Hz, 1H), 4.55-4.40 (m, 4H), 4.37-4.27 (m, 2H), 4.21 (t, J = 7.3 Hz, 1H), 4.07 (d, J = 2.6 Hz, 1H), 3.75 (t, J = 6.5 Hz, 1H), 3.63 (d, J = 6.6 Hz, 2H), 2.81-2.61 (m, 2H), 1.28-1.21 (m, 3H), 1.16 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 176.9, 154.6, 143.3, 143.2, 141.40, 141.39, 138.1, 137.9, 128.6, 128.4, 128.2, 128.08, 128.06, 127.98, 127.96, 127.8, 127.33, 127.32, 125.30, 125.26, 120.21, 120.18, 83.8, 79.1, 77.3, 75.1, 74.1, 73.7, 70.3, 68.2, 67.5, 46.8, 38.8, 27.1, 23.8, 15.0; ESI-MS: m/z [M+NH₄⁺] calcd for C₄₂H₆₀N_O₈S⁺: 728.32, found 728.3. IR (thin film) ν = 3511, 3035, 1746, 1259, 1153, 1073, 1027, 740, 698 cm⁻¹.

**Synthesis of Building Block N-Phenyl-trifluoroacetimidoyl-(α-2-3-sialyl)galactose (9).**

![Scheme S9. Synthesis of Sialic Acid-Galactose disaccharide building block 9.](image)

**(N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-α-neuraminosyl)-(2→3)-O-1,5-anhydro-4,6-O-benzyl-2-deoxy-D-lyxo-hex-1-enopyranose** (SI-15). To a solution of 4,6-O-benzyl-galactal (3 g, 9.2 mmol) and methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-(dibenzylphosphityl)-3,5-dideoxy-β-glycero-D-galacto-2-nonulopyranosonate⁹ (4.2 g, 5.6 mmol) in anhydrous acetonitrile (40 mL) were added 4Å-AW MS (ca. 100 rods). The mixture was stirred for 30 min at room temperature under an argon atmosphere. The solution was then cooled to −42 °C.
and TMSOTf (198 μL, 1.1 mmol) was added. After 2 h the mixture was neutralized with a few drops of Et₃N. Molecular sieves were removed by filtration through celite. The filtrate was concentrated and purified by silica gel chromatography (cyclohexane/EtOAc = 4:1 to 0:1) to afford SI-15 in 64% yield (4.7 g). [α]D²⁰

1H NMR (500 MHz, CDCl₃) δ 7.38-7.23 (m, 10H), 6.38 (dd, J = 1.4, 6.2 Hz, 1H), 5.40 (ddd, J = 2.6, 5.7, 8.4, Hz, 1H), 5.31 (dd, J = 1.6, 8.4, Hz, 1H), 5.18 (d, J = 9.0 Hz, 1H), 4.94 (ddd, J = 4.7, 9.9, 12.2, Hz, 1H), 4.83 (m, 2H), 4.62 (s, 1H), 4.57 (d, J = 12.0 Hz, 1H), 4.48(d, J = 11.8 Hz, 1H), 4.38 (d, J = 11.8 Hz, 1H), 4.31 (dd, J = 2.6, 12.5 Hz, 1H), 4.14 (m, 1H), 4.07 (m, 3H), 3.73 (s, 3H), 3.68 (t, J = 8.4 Hz, 1H), 3.55 (dd, J = 5.6, 9.9 Hz, 1H), 2.64 (dd, J = 4.7, 12.9 Hz, 1H), 2.14 (s, 6H), 2.09-1.99 (m, 7H), 1.90 (s, 3H); 13C NMR (126 MHz, CDCl₃) δ 171.2, 170.8, 170.4, 170.3, 170.0, 168.9, 144.4, 138.7, 138.2, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 102.3, 99.4, 75.8, 74.1, 73.6, 72.8, 71.2, 69.2, 68.9, 68.8, 67.6, 62.6, 53.0, 49.8, 38.3, 23.4, 21.3, 21.1, 21.00, 20.97; HR-MALDI MS: m/z [M+Na⁺] calcd for C₄₀H₄₉NO₁₆Na: 822.2949, found 822.2961. IR (thin film) ν = 2927, 1744, 1664, 1540, 1454, 1369, 1223, 1125, 1038, 752 cm⁻¹.

Acetyl (N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-α-neuraminosyl)-(2→3)-α-2-O-acetyl-4,6-di-O-benzyl-D-galactopyranoside (SI-16). A solution of acetoxyiodobenzene in dichloromethane (18.5 mL, 1.93 g, 5.99 mmol) was added at −40 °C to a cold solution of SI-15 (2.4 g, 3.00 mmol) in CH₂Cl₂ (18.5 mL) under an argon atmosphere followed by BF₃⋅Et₂O (280 μL, 2.2 mmol). After stirring for 2 h, pyridine (2 mL) and Ac₂O (2 mL) were added and the mixture was stirred at room temperature for 12 h and concentrated. The crude residue was dissolved in EtOAc and washed with 10% citric acid, water, NaHCO₃ solution and brine. The combined organic phase was dried over MgSO₄, filtered and concentrated. Purification with flash column silica gel chromatography (cyclohexane/EtOAc = 3:2 to 1:4) gave SI-16 (2.31 g, 2.52 mmol, 84%). [α]D²⁰

1H NMR (600 MHz, CDCl₃) δ 7.32-7.16 (m, 10H), 5.72 (dd, J = 1.9, 8.2, Hz, 1H), 5.53 (t, J = 7.9 Hz, 1H), 5.34 (t, J = 9.2 Hz, 1H), 5.32-5.28
(m, 2H), 4.82-4.76 (m, 2H), 4.46-4.41 (m, 3H), 4.41-4.34 (q, 2H), 4.11-3.99 (m, 1H), 3.94 (dd, J = 6.8, 12.3 Hz, 1H), 3.78 (m, J = 4.7, 7.8, 13.2 Hz, 2H), 3.65 (s, 3H), 3.63-3.58 (m, 1H), 3.53 (dd, J = 5.4, 9.1 Hz, 1H), 3.48 (s, 1H), 2.62 (dd, J = 4.5, 12.8 Hz, 1H), 2.17 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (m, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.82 (s, 3H); 13C NMR (151 MHz, CDCl₃) δ 170.9, 170.7, 170.4, 170.2, 169.8, 169.8, 169.3, 168.2, 138.5, 138.1, 128.4, 128.2, 127.8, 127.7, 127.5, 97.6, 92.4, 74.8, 74.7, 74.4, 73.4, 73.2, 72.5, 69.2, 69.0, 68.6, 68.6, 67.6, 67.5, 62.7, 49.2, 37.8, 23.2, 21.4, 21.1, 21.0, 20.9, 20.8, 20.8, 20.8, 20.7; HR-MALDI MS: m/z [M+Na⁺] calcd for C₄₄H₅₅NO₂₀Na: 940.3215, found 940.3227. IR (thin film) ν = 2934, 1744, 1370, 1217 cm⁻¹.

(\textit{N}-acetyl-4,7,8,9-tetra-\textit{O}-acetyl-1-methyl-\textit{α}-neuraminosyl)-(2→3)-\textit{α}-2-\textit{O}-acetyl-4,6-di-\textit{O}-benzyl-\textit{d}-galactopyranosyl-N-phenyl trifluoroacetimidate (Building Block 9). To a solution of \textit{SI}-16 (2.6 g, 2.8 mmol) in DMF (44 mL) was added hydrazine acetate (580 mg, 6.30 mmol). After stirring for 15 h at room temperature under argon atmosphere, the mixture was diluted with EtOAc and washed twice with a 10% citric acid solution (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated. Purification by flash column silica gel chromatography (cyclohexane/EtOAc = 5:1 to 0:1) gave the corresponding hemiacetal (2.25 g, 92%). The latter (1.95 g, 2.23 mmol) was dissolved in dichloromethane (30 mL) and Cs₂CO₃ (2.18 g, 6.67 mmol) and CF₃C(NPh)Cl (1.45 g, 7.05 mmol) were added. After stirring overnight at room temperature under argon atmosphere, the mixture was filtered through celite and concentrated. Silica column chromatography (cyclohexane/EtOAc = 7:3 to 9:1) afforded the desired product 9 (2.06 g, 86%). $^1$H NMR (600 MHz, CDCl₃) δ 7.34-7.23 (m, 12H), 7.08 (t, J = 7.5 Hz, 1H), 6.84 (d, J = 7.2 Hz, 2H), 5.59 (ddd, J = 2.6, 6.9, 8.2 Hz, 1H), 5.54-5.48 (m, 1H), 5.37 (dd, J = 2.6, 8.1 Hz, 1H), 5.32 (d, J = 10.2 Hz, 1H), 4.90-4.83 (m, 2H), 4.52-4.40 (m, 6H), 4.11-4.05 (m, 1H), 4.02 (dd, J = 6.7, 12.4 Hz, 2H), 3.84 (dd, J = 6.0, 8.5 Hz, 1H), 3.71 (s, 3H), 3.67 (d, J =
6.2 Hz, 1H), 3.53 (s, 1H), 2.68 (dd, $J = 4.6$, 12.7 Hz, 1H), 2.25 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (m, 1H), 2.03 (s, 3H), 1.95 (s, 3H), 1.87 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 170.9, 170.6, 170.4, 170.2, 169.8, 169.4, 168.2, 138.4, 138.1, 128.7, 128.4, 128.4, 128.3, 127.8, 127.7, 127.7, 127.7, 127.6, 125.5, 124.2, 119.5, 97.7, 74.8, 74.5, 74.5, 73.8, 73.3, 72.6, 69.1, 69.1, 68.6, 67.7, 62.7, 60.5, 53.0, 49.2, 37.7, 23.2, 21.4, 20.8, 20.8, 20.7, 14.2; HR-MALDI MS: $m/z$ [M+Na$^+$] calcd for C$_{50}$H$_{57}$F$_3$N$_2$O$_{19}$Na: 1069.3405, found 1069.3420. IR (thin film) $\nu$ = 3328, 1745, 1666, 1551, 1453, 1371, 1040, 699 cm$^{-1}$.

**Synthesis of Branching Building Block (1-3)-(1-4) Thioethylglucosamine (11).**

![Scheme S10](image)

**Scheme S10.** Synthesis of building block Glucosamine Thioglycoside 11.

**Ethyl 4,6-O-benzylidene-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamidothio-$\beta$-d-glucopyranoside (SI-17).** A mixture of SI-8 (500 mg, 0.854 mmol), benzaldehyde (91 mL, 0.897 mmol), and freshly dried 4Å molecular sieves (500 mg) in CH$_2$Cl$_2$ (7.5 mL) was stirred at 0 °C for 1 h. TMSOTf (23 $\mu$L, 0.128 mmol) was added to the solution and the mixture was kept stirring at 0 °C for another 2 h. A TBAF solution (1 M in THF, 0.95 mL, 0.95 mmol) was added to the mixture, the reaction was warmed up to room temperature and was kept stirring for another 6 h. DMAP (104.3 mg, 0.854 mmol), LevOH (281 $\mu$L, 2.734 mmol) and DIC (319 $\mu$L, 2.050 mmol) were consecutively added and the mixture was stirred at room temperature overnight before being filtered through a pad of celite. The
filtrate was washed with water (20 mL) and the aqueous layer was extracted with EtOAc (40 mL). The organic layers were combined, dried over MgSO₄, filtered and evaporated. Flash column chromatography on silica gel (hexanes/EtOAc = 5:2) gave product SI-17 (408 mg, 86%). [α]D²⁵ −0.1 (c 0.7, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.48-7.46 (m, 2H), 7.39-7.35 (m, 3H), 7.16 (d, J = 9.5 Hz, 1H), 5.53 (s, 1H), 5.47 (t, J = 10.0 Hz, 1H), 4.66 (d, J = 10.5 Hz, 1H), 4.20 (dd, J = 5.0, 10.5 Hz, 1H), 4.11 (q, J = 9.5 Hz, 1H), 3.73 (td, J = 4.9, 9.5 Hz, 1H), 3.65 (td, J = 5.0, 9.5 Hz, 1H), 2.74-2.55 (m, 6H), 2.13 (s, 3H), 1.24 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 205.5, 173.2, 162.0, 137.0, 129.0, 128.24, 128.21, 128.18, 126.0, 101.12, 101.10, 92.4, 84.6, 78.7, 72.7, 70.6, 68.3, 55.1, 37.9, 29.6, 28.1, 24.5, 14.9; HR-ESI-MS: m/z [M+2+Na⁺] calcd for C₂₂H₂₆Cl₃NO₇SNa 578.0360, found 578.0391. IR (thin film) ν = 3342, 2924, 1717, 1529, 1082, 820 cm⁻¹.

Ethyl 6-O-benzyl-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside (SI-18). To a solution of SI-17 (8.36 g, 15.07 mmol) and Et₃SiH (14.4 mL, 90 mmol) in CH₂Cl₂ (80 mL) was added trifluoroacetic anhydride (0.63 mL, 4.52 mmol) and trifluoroacetic acid (5.8 mL, 75 mmol) at 0 °C. The mixture was stirred at the same temperature for 6 h. The reaction was quenched by pouring it into saturated aqueous NaHCO₃ (300 mL). The mixture was extracted three times with EtOAc (200 mL). The organic layers were combined, washed with brine (100 mL), dried over MgSO₄, filtered and evaporated. Purification with flash column chromatography on silica gel (hexanes/EtOAc = 3:2 to 1:1) gave SI-18 (7.89 g, 94%). [α]D²⁰ −33.4 (c 4.9, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.27 (m, 5H), 6.90 (d, J = 9.4 Hz, 1H), 5.18 (dd, J = 9.0, 10.4 Hz, 1H), 4.57 (ABq, J = 12.1 Hz, 2H), 4.57 (d, J = 10.3 Hz, 1H), 4.03 (q, J = 9.4 Hz, 1H), 3.81-3.76 (m, 3H), 3.63-3.59 (m, 1H), 2.79-2.47 (m, 6H), 2.15 (s, 3H), 1.24 (t, J = 7.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 207.6, 173.5, 161.9, 137.8, 128.4, 127.8, 127.7, 92.4, 83.6, 78.4, 76.6, 73.6, 70.1, 69.8, 54.3, 38.3, 29.7, 28.2, 24.0, 14.0; HR-ESI-MS: m/z [M+Na⁺] calcd for C₂₂H₂₆Cl₃NO₇SNa 578.0550, found
578.0534. IR (thin film) ν = 3341, 2927, 1705, 1704, 1527, 1069, 820 cm⁻¹.

**Ethyl 6-O-benzyl-4-O-fluorenylmethoxycarbonyl-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside** (Building Block 11). Compound **SI-18** (7.83 g, 14.06 mmol) was coevaporated three times with toluene (100 mL) and was dissolved in pyridine (40 mL). To the mixture was added FmocCl (4.36 g, 16.87 mmol) at room temperature, and stirred for 24 h. Methanol (50 mL) was added, and the mixture was poured into water (100 mL). The aqueous layer was extracted three times with EtOAc (100 mL). The organic layers were combined, dried over MgSO₄, filtered and evaporated. Flash column chromatography on silica gel (hexane/EtOAc = 9:1 to 2:1 to 1:1) provided the desired product **11** (6.78 g, 62%) along with recovered **SI-18** (2.65 g, 34%). [α]D²⁰ −6.9 (c 3.0, CHCl₃); mp. 139-140 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.3 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.30-7.19 (m, 7H), 6.81 (d, J = 8.3 Hz, 1H), 5.40 (t, J = 9.6 Hz, 1H), 5.01 (t, J = 9.6 Hz, 1H), 4.71 (d, J = 10.4 Hz, 1H), 4.51 (ABq, J = 12.3 Hz, 2H), 4.44-4.40 (m, 1H), 4.27 (t, J = 1.2, 8.8 Hz, 1H), 4.20 (t, J = 7.2 Hz, 1H), 4.02 (q, J = 10.2 Hz, 1H), 3.81-3.76 (m, 1H), 3.65-3.63 (m, 2H), 2.80-2.45 (m, 6H), 2.04 (s, 3H), 1.28 (t, J = 6.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 205.7, 172.9, 161.9, 154.0, 143.5, 143.2, 141.3, 141.2, 137.7, 128.3, 127.9, 127.62, 127.56, 127.2, 125.2, 125.1, 120.0, 92.3, 83.6, 77.1, 73.6, 73.4, 73.1, 70.5, 69.0, 54.8, 46.9, 37.7, 29.5, 28.1, 24.1, 14.9; HR-ESI-MS: m/z [M+1+Na⁺] calcd for C₃₇H₃₈Cl₃NO₉SNa 802.1201, found 802.1157. IR (thin film) ν = 3344, 2927, 1755, 1718, 1528, 1257, 1159, 819 cm⁻¹.
4. Automated Synthesis of Oligosaccharides

4.1. Synthesis of β-(1→4)-glucosamine hexasaccharide 17

Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl 2-acetamido-2-deoxy-3,6-dibenzyl-β-D-glucopyranoside (SI-19)

The following module sequences were performed on 25 μmol scale with respect to resin 1:

1) Glycosyl Imidate Double coupling - Fmoc deprotection using building block 4
2) TCA-reduction one cycle (treatment with 10 eq Bu₃SnH and cat. AIBN)
3) Linker cleavage (treatment with 10 eq NaOMe)

The eluted solution was neutralized with acidic Amberlite IR-120 and concentrated under vacuum. The crude residue (16.9 mg, 85%) was purified by preparative HPLC over 40 min using a gradient of 60 to 95% of acetonitrile (0.1% TFA) in water (0.05% TFA) to yield SI-19 (7 mg, 35% yield based on resin). $[\alpha]_D^{25}$ –9.0 (c 0.75, CHCl₃); $^{1}$H NMR (300 MHz, CDCl₃) δ 7.60-7.08 (m, 19H), 6.12-5.05 (m, 1H), 5.18-5.06 (m, 2H), 4.88-4.41 (m, 7H), 4.06-3.10 (m, 13H), 3.01-2.86 (m, 2H), 2.72-2.57 (m, 2H), 2.90-1.73 (m, 3H), 1.68-1.10 (m, 6H); $^{13}$C NMR (75 MHz, CDCl₃) δ 140.3, 138.6, 137.9, 137.8, 128.5, 128.6, 128.4, 128.1, 127.9, 127.8, 127.3, 127.2, 100.0, 80.8, 76.6, 74.3, 73.7, 73.6, 70.8, 69.5, 67.0, 57.3, 51.7, 50.3, 35.6, 30.6, 28.8, 27.3, 23.5, 23.4; MALDI-HRMS: m/z calcd for [M+Na$^+$], 819.3827 found 819.3811. IR (thin film) ν = 3458, 3007, 2940, 2868, 1731, 1683, 1517, 1473, 1453, 1437, 1366, 1248, 1073, 986, 906 cm$^{-1}$. 
Figure S5. LC-MS of the crude monoglucosamine SI-19. Conditions: 40 to 100% A in B, A = CH₃CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA from 2 to 32 min.


To a solution of SI-19 (17.0 mg, 23.9 µmol) in THF/MeOH/water/AcOH (2 mL/1 mL/1 mL/0.05 mL) was added 10% Pd/C (20 mg). The mixture was stirred under an atmosphere of hydrogen for 66 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated and the residue was purified by solid phase extraction (Sep-pak C-18, water) to give SI-20 (6.8 mg, 93%) The purity of SI-20 was assessed by analytical HPLC using a 0 to 10% gradient (acetonitrile in water, Pyramid nucleodur C-18 column, Figure S6). $^1$H NMR (400 MHz, D₂O) δ 4.45 (d, J = 8.4 Hz, 1H), 3.94-3.80 (m, 2H), 3.74-3.44 (m, 4H), 3.44-3.33 (m, 2H), 3.00-2.88 (m, 2H), 1.98 (s, 3H), 1.70-1.48 (m, 4H), 1.34 (m, 2H); $^{13}$C NMR (100 MHz, D₂O) δ 174.5, 101.2, 75.9, 73.9, 70.1, 70.0, 60.8, 55.7, 39.4, 28.1, 26.5, 23.3, 22.20, 22.16; ESI-HRMS: m/z calcd for C₁₃H₂₆N₂O₆Na [M+Na⁺], 329.1683 found 329.1679.
Figure S6. LC-MS of SI-20. Conditions: 0 to 10% A in B, A = CH$_3$CN, B = water, over 20 min.

Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoxyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside(1→4)]₅-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside (SI-21) Using Glycosyl N-Phenyl Trifluoroacetimidate Building Block 4

The following modules were performed on 25 μmol scale based on resin 1:

1) Six cycles: Glycosyl Imidate Double coupling - Fmoc deprotection using building block 4

2) Six cycles TCA-reduction (treatment with 10 eq Bu₃SnH and cat. AIBN)

3) Linker Cleavage (treatment with 10 eq NaOMe)

The eluate was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a gradient of 60 to 70% over 40 min MeCN/i-PrOH (4:1) in water/i-PrOH (4:1) and recycling HPLC to yield SI-21 (4.9 mg, 7% yield from resin). The LC-MS chromatogram of product SI-21 is shown in Figure S7.

**Figure S7.** LC-MS of crude protected (1→4)-β-N-acetyl-hexaglucosamine SI-21 synthesized from glycosyl imidate 4. Conditions: 40 to 100% A in B, A = CH₃CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA over 31 min.
Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside(1→4)]5-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside (SI-21) Using Glycosyl N-Phenyl Trifluoroacetimidate Building Block 3

The following module sequences were performed on 25 μmol scale based on resin 1:

1) Six cycles Thioglycoside Double Coupling - Fmoc deprotection with building block 3

2) Eight cycles TCA-reduction (treatment with 10 eq Bu3SnH and cat. AIBN)

3) Linker cleavage (treatment with 10 eq NaOMe)

The eluate was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a gradient of 60 to 70% over 40 min of MeCN + 20% i-PrOH in water + 20% i-PrOH to yield SI-21 (21.1 mg, 31% overall yield from resin). [α]D25 –16.9 (c 1.0, CHCl3);

1H NMR (600 MHz, CDCl3) δ 7.42-7.10 (m, 69H), 6.70-6.60 (m, 1H), 6.59-6.47 (m, 1H), 5.89-5.75 (m, 1H), 5.60-5.47 (m, 1H), 5.44-5.34 (m, 1H), 5.31-5.17 (m, 1H), 5.12 (m, 2H), 4.83-4.31 (m, 25H), 4.31-4.09 (m, 7H), 4.09-3.81 (m, 10H), 3.80-3.67 (m, 4H), 3.65 (s, 3H), 3.63-3.07 (m, 23H), 2.93 (t, J = 7.7 Hz, 2H), 2.61 (t, J = 7.8 Hz, 2H), 1.95 (s, 3H), 1.84 (s, 3H), 1.78 (s, 3H), 1.77 (s, 3H), 1.76 (s, 3H), 1.72 (s, 3H), 1. 59-1.40 (m, 4H), 1.35-1.12 (m, 6H); 13C NMR (150 MHz, CDCl3) δ 173.4, 173.3, 171.2, 171.8, 170.8, 156.7, 156.2, 140.2, 139.3, 138.8, 138.7, 138.6, 138.5, 138.4, 138.1, 138.0, 137.9, 137.8, 137.8, 137.3, 134.9, 134.7, 128.8, 128.6, 128.6, 128.5, 128.49, 128.47, 128.4, 128.32, 128.29,
128.27, 128.20, 128.17, 128.07, 127.99, 127.92, 127.85, 127.81, 127.77, 127.70, 127.66, 127.5, 127.4, 127.3, 127.2, 114.0, 100.27, 100.21, 99.4, 80.8, 80.1, 79.7, 79.5, 79.3, 75.5, 75.4, 75.2, 75.0, 74.8, 74.4, 74.1, 73.8, 73.7, 73.5, 73.43, 73.37, 73.2, 72.9, 72.7, 70.9, 69.9, 69.8, 69.0, 68.9, 66.9, 54.8, 54.3, 54.1, 54.0, 53.8, 51.6, 50.4, 50.2, 47.2, 46.1, 45.9, 35.6, 33.8, 31.9, 30.6, 29.7, 29.6, 29.5, 29.3, 29.1, 28.9, 27.9, 27.3, 23.4, 23.2, 23.1, 23.0, 22.8, 22.6, 14.1, 8.5; HR-MALDI-MS: m/z [M+Na⁺] calcd for C₁₅₆H₁₈₁N₇O₃₅Na 2735.2491, found 2735.254. IR (thin film) ν = 3351, 3081, 1664, 1550, 1063, 736 cm⁻¹.

Figure S8. LC-MS of crude protected (1→4)-β-N-acetyl-hexaglucosamine SI-21 synthesized from thioglycoside 3. Conditions: 60 to 80% A in B, A = CH₃CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA from 2 to 32 min.


To a solution of SI-21 (9.8 mg, 3.6 µmol) in THF/MeOH/water/AcOH (2 mL/1 mL/1 mL/0.05 mL) was added 10% Pd/C (15 mg). The mixture was stirred under an atmosphere of hydrogen for 70 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by solid phase extraction (Sep-pak 1g cartridge, C-18, water) to give 17
(3.8 mg, 79%). The purity was assessed by analytical HPLC using a 0 to 10% gradient (acetonitril in water, Pyramid nucleodur C-18 column) as shown in Figure S9. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 4.48 (m, 5H), 4.40 (d, $J = 8.0$ Hz, 1H), 3.90-3.72 (m, 9H), 3.72-3.32 (m, 30H), 2.95-2.84 (m, 2H), 1.99-1.95 (m, 15H), 1.94 (s, 3H), 1.65-1.54 (m, 2H), 1.53-1.47 (m, 2H), 1.33-1.27 (m, 2H); $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 174.6, 174.4, 101.4, 101.2, 101.0, 79.2, 79.1, 78.9, 75.9, 74.5, 73.4, 72.4, 72.1, 72.0, 70.1, 69.7, 39.3, 28.0, 26.3, 22.1, 22.1; HR-MALDI-MS: $m/z$ [M+H$^+$] calcd for C$_{53}$H$_{91}$N$_7$O$_{31}$ 1322.5832, found 1322.581.

**Figure S9.** LC-MS of unprotected 1,4-hexa-glucosamine 17. Conditions: 0 to 10% A in B, A = CH$_3$CN, B = water, over 20 min.
4.2 Synthesis of 4.1 β-(1→6)-glucosamine hexamer 15


Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,4-dibenzy1-β-D-glucopyranoside(1→6)]-2-acetamido-2-deoxy-3,4-di-O-benzyl-β-D-glucopyranoside (SI-22).

The following modules were performed on 25 μmol scale based on resin 1:

1) Six cycles Thioglycoside Double Coupling - Fmoc deprotection using building block 2

2) Six cycles TCA-reduction (treatment with 10 eq Bu₃SnH and cat. AIBN)

3) Linker Cleavage (treatment with 10 eq NaOMe)

The eluate was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a gradient of 60 to 70% over 40 min of MeCN/i-PrOH (4:1) in water/i-PrOH (4:1) to yield SI-22 (39.4 mg, 58% overall yield from resin). \([\alpha]_D^{25} -33.4 \ (c \ 1.0, \ CHCl_3)\); \(^1\)H...
NMR (600 MHz, CDCl₃) δ 8.93 (s, 2H), 8.73 (s, 1H), 8.45 (s, 1H), 8.34-8.14 (m, 1H), 7.35-6.79 (m, 69H), 6.78-6.62 (m, 2H), 5.65-2.93 (m, 73H), 2.93-2.82 (m, 2H), 2.63-2.49 (m, 2H), 2.40-1.86 (m, 18H), 1.66-0.98 (m, 10H); ¹³C NMR (150 MHz, CDCl₃) δ 173.2, 172.9, 171.5, 171.4, 171.2, 170.5, 159.9, 159.6, 156.8, 156.2, 140.2, 139.0, 138.8, 138.4, 138.1, 138.0, 137.5, 135.0, 134.6, 129.5, 129.2, 128.6, 128.3, 128.2, 128.1, 128.0, 127.7, 127.5, 127.3, 127.1, 126.9, 126.6, 103.3, 102.6, 102.0, 100.2, 85.2, 83.4, 83.2, 82.3, 81.8, 79.9, 76.5, 76.1, 75.6, 74.8, 74.4, 72.6, 72.1, 66.7, 56.8, 56.1, 51.6, 50.3, 47.6, 46.6, 35.6, 33.9, 32.0, 30.6, 29.70, 29.67, 29.63, 29.5, 29.4, 29.2, 29.0, 28.7, 28.2, 27.7, 23.6, 23.5, 23.3, 23.2, 22.7, 22.3, 14.1; HR-MALDI-MS: m/z [M+1+Na⁺] calcd for C₁₅₆H₁₈₁N₇O₃₅Na: 2736.2524, found: 2736.259. IR (thin film) ν = 3281, 1654, 1556, 1071, 699 cm⁻¹.

**Figure S10.** LC-MS of crude (1→6)-β-N-acetyl-hexaglucosamine SI-22 synthesized from thioglycoside 3. Conditions: 60 to 80% A in B, A = CH₃CN + 20% isopropanol + 0.1% TFA, B = water + 20% isopropanol + 0.1% TFA from 2 to 32 min.

To a solution of SI-22 (15 mg, 5.5 µmol) in THF/MeOH/water/AcOH (2 mL/1 mL/1 mL/0.05 mL) was added 10% Pd/C (20 mg). The mixture was stirred under a hydrogen atmosphere for 36 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by size exclusion chromatography (Superdex, 5% EtOH in water) and solid phase extraction (Sep-Pak 1g cartridge, C-18, water) to give 15 (4.2 mg, 58%). The purity of the compound was assessed by analytical HPLC using a 0 to 10% gradient (acetonitrile in water, Pyramid nucleodur C-18 column) as shown in Figure S11. 

$^1$H NMR (600 MHz, D$_2$O) δ 4.56-4.39 (m, 6H), 4.21-4.08 (m, 5H), 3.93-3.85 (m, 1H), 3.84-3.78 (m, 1H), 3.77-3.62 (m, 1H), 3.61-3.45 (m, 1H), 3.44-3.29 (m, 1H), 2.97-2.91 (m, 2H), 2.03-1.99 (m, 1H), 1.99 (s, 3H), 1.97 (s, 3H), 1.62 (m, 2H), 1.58-1.50 (m, 2H), 1.36 (m, 2H); $^{13}$C NMR (150 MHz, D$_2$O) δ 174.48, 174.45, 101.61, 101.57, 101.1, 75.9, 74.6, 74.51, 74.48, 74.47, 74.41, 73.9, 73.7, 69.99, 69.97, 69.91, 69.86, 68.6, 68.5, 68.3, 60.7, 55.6, 55.5, 55.4, 39.4, 28.0, 26.4, 22.30, 22.28, 22.25, 22.14, 22.13; HR-MALDI-MS: m/z [M+H$^+$] calcd for C$_{53}$H$_{91}$N$_7$O$_{31}$ 1322.5832, found 1322.584.

Figure S11. LC-MS of unprotected 1,6-hexaglucosamine 15. Conditions: 0 to 10% A in B, A = CH$_3$CN, B = water, over 20 min.
4.3 Synthesis of Dodecaglucosamine

Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,4-dibenzyl-β-D-glucopyranoside(1→6)]_{11}-2-acetamido-2-deoxy-3,4-dibenzyl-β-D-glucopyranoside (SI-23).

The following modules were performed on 25 μmol scale based on resin 1.

1) Twelve cycles Thioglycoside double coupling - Fmoc deprotection using building block 2

2) Sixteen cycles TCA-reduction (treatment with 20 eq Bu₃SnH and cat. AIBN)

3) Cleavage of linker (treatment with 10 eq NaOMe)

The eluate solution was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a 0 to 10% gradient (MeOH in DCM, YMC-pack silica column) over 35 min to afford SI-23 (54.4 mg, 43% overall yield from resin). ¹H NMR (700 MHz, CDCl₃) δ 7.48-6.67 (m, 129H), 5.18-4.65 (m, 49H), 4.55 (s, 11H), 4.30 (s, 19H), 4.06 (s, 19H), 3.84 (s, 21H), 3.66 (s, 12H), 3.24 (s, 33H), 2.94 (t, J = 7.7 Hz, 4H), 2.62 (t, 4H), 2.51-1.97 (m, 36H), 1.58 (s, 3H), 1.38-1.09 (m, 22H), 0.95-0.86 (m, 6H); ¹³C NMR (176 MHz, CDCl₃) δ 171.2 (m), 140.2, 139.5 (m), 138.8, 137.6 (m), 129.7, 129.0, 128.5 (m), 128.3 (m), 128.8 (m), 127.4 (m), 127.2 (m), 126.9 (m), 101.9 (m), 85.1 (m), 81.9 (m), 71.1 (m), 66.9, 56.9 (m), 51.5, 35.6, 31.9, 30.6, 29.7, 29.3, 23.2, 22.7, 14.0; MALDI-MS: m/z [M+Na⁺] calcd for C_{288}H_{331}N_{13}NaO_{65} 5034.29, found 5034.80.
Figure S12. HPLC of the crude protected 1,6-\textit{N}-acetyl-glucosamine dodecasaccharide SI-23. Conditions: 0 to 10% A in B, A = MeOH, B = DCM, over 35 min.

**Aminopentyl (acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)- (acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1 \(\rightarrow\) 6)- (acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)(1 \(\rightarrow\) 6)-acetamido-2-deoxy-2-\(\beta\)-D-glucopyranoside (dodecaglucosamine, 16).

To a solution of SI-23 (9 mg, 1.7 \(\mu\)mol) in t-BuOH/THF/1 M HCl (0.75 mL/ 1.25 mL/ 0.02 mL) was added 20\% Pd(OH)$_2$/C (10 mg). The mixture was stirred under an atmosphere of hydrogen for 36 h. Pd(OH)$_2$/C (15 mg) was added followed by several drops of water and the mixture was stirred for additional 3 days. The catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by reverse phase HPLC using a 0 to 25\% gradient (acetonitrile in water with 1\% formic acid, Pyramid nucleodur C-18 column) to give 16 (2.3 mg, 53\%) as shown in Figure S13. $^1$H NMR (600 MHz, D$_2$O) \(\delta\) 4.60-4.48 (m, 12H), 4.21-4.08 (m, 12H), 3.96 (m, 1H), 3.87 (m, 1H), 3.81-3.70 (m, 26H), 3.64-3.52 (m, 34H), 3.49-3.34 (m, 21H), 3.07-2.99 (m, 2H), 2.14-
2.04 (m, 36H), 1.70 (m, 2H), 1.62 (m, 2H), 1.43 (m, 2H); \(^{13}\)C NMR (extracted from HSQC; 176 MHz, \(\text{D}_2\text{O}\)) \(\delta\) 101.6, 101.1, 75.9, 74.5, 73.73, 73.70, 70.0, 69.92, 69.90, 69.8, 68.4, 60.68, 60.67, 60.67, 55.5, 55.4, 39.3, 28.0, 26.3, 22.2, 22.0; HR-MALDI-MS: \(m/z\) [M+Na\(^+\)] calcd for C\(_{101}\)H\(_{169}\)N\(_{13}\)O\(_{61}\) 2563.042, found 2563.306.

Figure S13. LC-MS of the unprotected 1,6-dodecaglucosamine 16. Conditions: 0 to 25% A in B, A = CH\(_3\)CN + 1% Formic Acid, B = water 1% Formic Acid, over 25 min.

4.4 Synthesis of iso-Gb3 trisaccharide 18

Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1→3)-4,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-β-D-glucopyranoside (SI-24).

The following modules were performed on 25 μmol scale based on resin 1:

1) Thioglycoside Double coupling–Fmoc deprotection using building block 5
2) Thioglycoside Double coupling–Fmoc deprotection using building block 6
3) Thioglycoside Double coupling–Fmoc deprotection using perbenzylated thioethyl galactose 8^{10}
4) Linker Cleavage (treatment with 10eq NaOMe)

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1 mL), THF (1 mL) and aqueous KOH (3N, 200 μL, ca. 24 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. The crude residue was purified by preparative HPLC using a 40 to 100% gradient of A in B (A= acetonitrile + 0.1% TFA, B= water + 0.1% TFA, Pyramid nucleodur C-18 column) to afford SI-24 (32 mg, 80% overall yield from resin). {superscript}1H NMR (600 MHz, CDCl{subscript}3) δ 7.35-7.11 (m, 49H), 5.12 (s, 2H), 5.01-4.90 (m, 4H), 4.85 (d, J = 11.5 Hz, 1H), 4.76 (t, J = 13.3 Hz, 1H), 4.67 (dd, J = 11.8, 20.6 Hz, 2H), 4.61-4.52 (m, 3H), 4.50-4.35 (m, 7H), 4.31-4.26 (m, 1H), 4.23-4.14 (m, 3H), 4.11 (dd, J = 3.7, 10.0 Hz, 1H), 3.92 (dd, J = 6.6, 16.1 Hz, 1H), 3.89-3.83 (m, 3H), 3.81-3.70 (m, 4H), 3.66 (d, J = 5.8 Hz, 1H), 3.58-3.49 (m, 2H), 3.47-3.37 (m, 5H), 3.36-3.25 (m, 4H), 3.16 (s, 3H), 2.97-2.88 (m, 2H), 2.68-2.55 (m, 2H), 1.65-1.53 (m, 2H), 1.50-1.39 (m, 2H), 1.15 (m, 2H); {superscript}13C NMR (151 MHz, CDCl{subscript}3) δ 130.1, 128.6, 128.50, 128.48, 128.3, 128.2, 127.98, 127.97, 127.9, 127.7, 127.4, 103.4, 102.9, 99.8, 85.5, 83.4, 78.7, 75.2, 75.0, 74.9, 74.8, 74.6, 74.1, 74.0, 73.6, 73.5, 73.3, 73.1,
71.7, 70.4, 69.74, 69.72, 69.2, 68.7, 68.4, 67.2, 51.8, 50.6, 50.4, 50.3, 47.2, 46.9, 46.2, 35.8, 35.1, 33.6, 32.1, 30.8, 30.5, 29.9, 29.3, 27.9, 27.3, 24.9, 23.4, 23.1, 22.9, 20.0, 14.3; HR-ESI MS: $m/z$ [M+Na$^+$] calcd for C$_{97}$H$_{107}$NNaO$_{20}$: 1628.7284; Found: 1628.7333.

**Figure S14.** LC-MS of crude SI-24. Conditions 40 to 100% A in B; A = CH$_3$CN + 0.1% TFA, B = water + 0.1% TFA over 31 min.

**Aminopentyl (α-D-galactopyranosyl)-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside** (iso-Gb3, 18). To a solution of SI-24 (4 mg, 2.49 µmol) in methanol (1.5 mL), THF (1 mL), water (200 µL), toluene (200 µL) and acetic acid (100 µL) was added Pd/C (10 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by solid phase extraction (Sep-pak C-18, water + 0.1% TFA) to give 18 (1.2 mg, 80%). $^1$H NMR (600 MHz, D$_2$O) δ 5.16 (d, $J = 3.7$ Hz, 1H), 4.54 (d, $J = 7.8$ Hz, 1H), 4.51 (d, $J = 8.1$ Hz, 1H), 4.23-4.19 (m, 2H), 4.04 (s, 1H), 4.01 (d, $J = 12.2$ Hz, 1H), 3.99-3.93 (m, 2H), 3.88 (dd, $J = 3.8$, 10.4 Hz, 1H), 3.86-3.78 (m, 4H), 3.78-3.73 (m, 3H), 3.72-3.65 (m, 4H), 3.64-3.59 (m,
1H), 3.33 (t, J = 7.8 Hz, 1H), 3.03 (t, J = 7.5 Hz, 2H), 1.75-1.65 (m, 4H), 1.52-1.44 (m, 2H); 13C NMR (176 MHz, D2O) δ 102.9, 102.1, 95.5, 78.8, 77.3, 75.1, 74.8, 74.6, 72.7, 70.9, 70.1, 69.6, 69.3, 69.2, 68.2, 64.9, 61.03, 60.98, 60.2, 39.4, 28.2, 26.4, 22.1; HR-MALDI-MS: m/z [M+H+] calcd for C23H44NO16 590.266, found 590.454.

4.5 Synthesis of Sialyl Lactose 19

![Scheme S14. Automated synthesis of sialyl lactose 19.](image)

Automated Synthesis of Protected Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoiloxy)methyl)phenyl)propanoyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)-4,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-β-D-glucopyranoside (SI-25).

The following modules sequences were performed on 25 μmol scale with respect to resin 1:

1) Thioglycoside Double Coupling - Fmoc deprotection with building block 5
2) Glycosyl Imidate Double coupling (0 °C) - Fmoc deprotection with building block 9
3) Linker Cleavage (treatment with 10eq NaOMe)
The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1.6 mL), water (0.4 mL), THF (0.5 mL) and aqueous KOH (1N, 300 μL, ca. 14 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. Preparative HPLC using 60% MeCN in water (with 0.1% TFA, C-8, 10 mL/min) as eluent afforded 13.7 mg of **SI-25** (40% overall yield from resin) (Figure S15).

**1H NMR** (600 MHz, CD$_3$OD) $\delta$ 7.42-7.07 (m, 29H), 5.12 (d, $J = 17.3$ Hz, 2H), 4.99 (dd, $J = 11.0$, 13.1 Hz, 2H), 4.69 (d, $J = 10.6$ Hz, 1H), 4.63 (d, $J = 11.5$ Hz, 1H), 4.57-4.48 (m, 4H), 4.43 (d, $J = 7.7$ Hz, 1H), 4.35 (d, $J = 11.8$ Hz, 1H), 4.29-4.22 (m, 1H), 4.16 (d, $J = 11.8$ Hz, 1H), 4.05 (dd, $J = 3.1$, 9.7 Hz, 1H), 4.00 (dd, $J = 3.4$, 10.9 Hz, 1H), 3.95-3.91 (m, 2H), 3.88 (t, $J = 10.1$ Hz, 2H), 3.83-3.75 (m, 4H), 3.73-3.65 (m, 3H), 3.62 (dd, $J = 1.3$, 9.0 Hz, 1H), 3.56-3.42 (m, 4H), 3.36-3.30 (m, 2H), 3.25-3.20 (m, 1H), 2.91 (t, $J = 7.1$ Hz, 2H), 2.82 (dd, $J = 4.6$, 13.0 Hz, 1H), 2.60 (t, $J = 7.6$ Hz, 2H), 2.03 (s, 3H), 1.99 (t, $J = 12.6$ Hz, 1H), 1.64-1.47 (m, 4H), 1.41-1.27 (m, 2H); **13C NMR** (151 MHz, CD$_3$OD) $\delta$ 177.4, 176.1, 173.0, 141.32, 141.30, 140.7, 140.5, 130.5, 130.3, 130.23, 130.17, 130.04, 129.98, 129.8, 129.8, 129.5, 129.4, 129.3, 129.0, 105.5, 104.9, 100. 9, 85.4, 79.6, 78.2, 78.0, 77.3, 77.0, 76.6, 76.4, 75.8, 75.3, 75.2, 75.1, 73.7, 72.5, 71.5, 70.6, 70.4, 69.6, 69.1, 65.3, 54.6, 42.0, 37.5, 32.5, 31.2, 25.2, 23.5; HR-ESI MS: m/z: [M+Na$^+$] calcd for C$_{74}$H$_{90}$N$_2$NaO$_{23}$ 1397.5832, found 1397.5814.

**Figure S15.** LC-MS of crude **SI-25**. Conditions: 60% A in B; A = CH$_3$CN + 0.1% TFA, B = water + 0.1%TFA over 30 min.
Aminopentyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (sialyl lactose, 19). Acetic acid (50 μL) was added to a solution of SI-25 (13 mg, 9.09 μmol) in methanol/water (2 mL, 8/2) followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by preparative HPLC using a 0 to 20% gradient (CH₃CN in water + 0.1% TFA, Pyramid nucleodur C-18 column) over 35 min to afford oligosaccharide 19 (6 mg) with a 91% yield. The purity of 19 was assessed by analytical HPLC using a 95 to 5% gradient (CH₃CN in water, TSKgel amide-80 TOSOH) as shown in Figure S16. ^1H NMR (600 MHz, D₂O) δ 4.53 (dd, J = 7.9, 25.1 Hz, 2H), 4.14 (dd, J = 2.9, 9.8 Hz, 1H), 4.03-3.93 (m, 3H), 3.92-3.81 (m, 4H), 3.80-3.64 (m, 9H), 3.63-3.55 (m, 3H), 3.32 (t, J = 12.0 Hz, 1H), 3.03 (t, J = 7.4 Hz, 2H), 2.78 (dd, J = 4.5, 12.4 Hz, 1H), 2.05 (s, 3H), 1.83 (t, J = 12.2 Hz, 1H), 1.75-1.65 (m, 4H), 1.51-1.44 (m, 2H); ^13C NMR (151 MHz, D₂O) δ 174.8, 173.4, 102.6, 102.0, 99.6, 78.3, 75.5, 75.1, 74.7, 74.4, 72.9, 72.8, 71.6, 70.0, 69.3, 68.2, 68.1, 67.4, 62.6, 61.0, 60.0, 51.6, 39.5, 39.3, 28.1, 26.3, 22.00, 21.98; MALDI-MS: m/z [M+Na⁺] calcd for C₂₈H₅₀N₂NaO₁₉ 741.290, found 741.383.

Figure S16. LC-MS of 19. Conditions: 95 to 5% A in B; A = CH₃CN, B = water, over 30 min.
4.6 Synthesis of Sialyl Lactosamine 20


Automated Synthesis of Protected Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)-β-D-4,6-di-O-benzyl-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside (SI-26).

The following modules sequences were performed on 25 μmol scale with respect to resin 1:

1) Thioglycoside Double coupling - Fmoc deprotection with building block 3
2) Glycosyl Imidate Double coupling (0 °C) – Fmoc deprotection with building block 9
3) TCA-reduction (treatment with 20 eq Bu₃SnH and cat. AIBN)
4) Linker Cleavage (treatment with 10 eq NaOMe)

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was purified by preparative HPLC using a 30 to 90% gradient over 90 min (CH₃CN + 0.05% TFA in water + 0.1% TFA; 10 mL/min, C8 30mm) affording SI-26 (Figure S17)
33% (overall yield from resin): $^1$H NMR (500 MHz, MeOD) δ 7.49-7.10 (m, 29H), 5.15 (d, $J = 9.6$ Hz, 2H), 5.01 (d, $J = 11.4$ Hz, 2H), 4.63 (dd, $J = 11.7$, 43.9 Hz, 2H), 4.55 (d, $J = 2.3$ Hz, 1H), 4.53 (s, 2H), 4.48-4.43 (m, 2H), 4.26 (ABq, $J = 11.8$, 87.9, Hz, 2H), 4.11 (dd, $J = 3.1$, 9.7 Hz, 1H), 4.04 (dd, $J = 4.4$, 11.5 Hz, 1H), 3.99 (t, $J = 9.2$ Hz, 1H), 3.97-3.95 (m, 1H), 3.93-3.87 (m, 2H), 3.87-3.77 (m, 4H), 3.76-3.69 (m, 3H), 3.67 (s, 3H), 3.66 (s, 1H), 3.64 (m, 2H), 3.55 (d, $J = 9.3$ Hz, 1H), 3.46 (dd, $J = 6.6$, 9.0 Hz, 1H), 3.41-3.37 (m, 1H), 3.34-3.31 (m, 1H), 3.31-3.22 (m, 2H), 2.96 (t, $J = 7.6$ Hz, 2H), 2.87 (dd, $J = 4.6$, 12.9 Hz, 1H), 2.67 (t, $J = 7.6$ Hz, 2H), 2.07 (s, 3H), 1.99 (t, $J = 12.8$ Hz, 1H), 1.90 (s, 3H), 1.55 (m, 4H), 1.32 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 175.3, 175.0, 173.1, 172.4, 159.2, 158.5, 158.0, 156.9, 154.5, 152.0, 142.0, 140.50, 140.46, 139.8, 139.6, 139.3, 136.0, 129.6, 129.5, 129.3, 129.2, 129.1, 129.00, 128.95, 128.7, 128.5, 128.2, 111.4, 106.8, 104.0, 102.7, 100.2, 82.0, 78.8, 77.5, 77.4, 76.4, 76.3, 75.5, 75.1, 74.6, 74.3, 74.3, 72.8, 71.7, 70.3, 69.8, 69.7, 69.7, 68.8, 68.30, 68.25, 68.23, 68.18, 68.17, 68.16, 68.1, 64.5, 56.3, 53.8, 52.1, 49.6, 49.5, 49.3, 49.1, 41.3, 36.5, 31.6, 30.2, 28.8, 28.4, 24.2, 23.0, 22.7; HR-MALDI MS: $m/z$: [M+Na$^+$] calcd for C$_{77}$H$_{95}$N$_3$NaO$_{23}$: 1452.6254, found: 1452.6249.

Figure S17. LC-MS of crude SI-26. Conditions 40-100% A in B, A = CH$_3$CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1 % TFA over 31 min.
Aminopentyl (5-Acetamido-3,5-dideoxy-d-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (sialyl lactosamine, 20). To a solution of SI-26 (13 mg, 9.09 μmol) in methanol/water (2 mL, 8/2) acetic acid (50 μL) was added followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by solid phase extraction (Sep-Pak C-18, water + 0.1% TFA) to give 20 (5.37 mg, 7.09 μmol, 78% yield). The purity of the compound was assessed by analytical HPLC using a 95 to 5% gradient (CH$_3$CN in water, TSKgel amide-80 TOSOH) as shown in Figure S18. $^1$H NMR (500 MHz, D$_2$O) δ 4.57 (d, J = 7.9 Hz, 1H), 4.54 (d, J = 7.9 Hz, 1H), 4.15 (dd, J = 3.1, 9.9 Hz, 1H), 4.03-3.99 (m, 1H), 3.98 (d, J = 3.2 Hz, 1H), 3.95-3.84 (m, 5H), 3.78-3.72 (m, 6H), 3.71-3.68 (m, 2H), 3.65 (dd, J = 6.3, 12.8 Hz, 1H), 3.62-3.56 (m, 4H), 3.01 (t, J = 7.6 Hz, 2H), 2.78 (dd, J = 4.6, 12.6 Hz, 1H), 2.05 (d, J = 1.5 Hz, 6H), 1.85 (t, J = 12.1 Hz, 1H), 1.69 (m, 2H), 1.61 (m, 2H), 1.42 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 175.0, 174.4, 173.2, 102.6, 101.1, 99.5, 78.4, 75.5, 75.1, 74.8, 73.0, 72.4, 71.5, 70.1, 69.4, 68.1, 68.1, 67.5, 62.7, 61.0, 60.0, 55.1, 51.7, 48.8, 39.3, 28.1, 26.4, 22.1, 22.1, 22.0; MALDI-MS: m/z [M+Na$^+$] calcd for C$_{30}$H$_{53}$N$_3$NaO$_{19}$ 782.317, found 782.329.

Figure S18. LC-MS of 20. Conditions: 95 to 5% A in B; A = CH$_3$CN, B = water, over 30 min.
4.7 Synthesis of Sialyl Lewis\textsuperscript{x} 21

\begin{center}
\includegraphics[width=\textwidth]{scheme_s16.png}
\end{center}

Scheme S16. Automated Synthesis of Sialyl Lewis\textsuperscript{x} 21.

**Automated Synthesis of Protected Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (5-Aacetamido-3,5-dideoxy-d-glycero-a-D-galacto-non-2-ulopyranosylonic-acid)-(2\textrightarrow{\textbullet}\textrightarrow{\textbullet})-4,6-di-O-benzyl-\beta-D-galactopyranosyl-(1\textrightarrow{\textbullet})-[2-O-benzyl-\alpha-L-fucopyranosyl-(1\textrightarrow{\textbullet})]-2-acetamido-2-deoxy-3,6-di-O-benzyl-\beta-D-glucopyranoside (SI-27).**

The following modules sequences were performed on 25 \textmu mol scale with respect to resin 1:

1) Thioglycoside Double coupling–Fmoc deprotection with building block 11
2) Glycosyl Imidate Double coupling (0 °C)-Lev deprotection with building block 9
3) Glycosyl Imidate Double coupling (-10 °C) with building block 10\textsuperscript{11}
4) TCA Reduction by Treatment with 10 eq of Bu\textsubscript{3}Sn/H/AIBN
5) Linker Cleavage by Treatment with 10 eq NaOMe

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1.6 mL), water (0.4 mL), THF (0.5 mL) and aqueous KOH
(1N, 300 μL, ca. 14 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. The crude residue was purified by preparative HPLC using a 35 to 60% gradient (CH₃CN in water + 20% i-PrOH, + 0.1% TFA) over 35 min to afford the desired compound (20 mg) in 51% yield from the resin. ¹H NMR (600 MHz, CD₃OD) δ 7.48-7.16 (m, 29H), 5.32 (s, 1H), 5.12 (d, J = 18.3 Hz, 2H), 4.75 (d, J = 11.7 Hz, 1H), 4.68-4.63 (m, 2H), 4.54-4.46 (m, 5H), 4.42-4.32 (m, 2H), 4.09 (d, J = 10.4 Hz, 1H), 4.04-3.97 (m, 3H), 3.95-3.90 (m, 2H), 3.88-3.84 (m, 2H), 3.83-3.80 (m, 1H), 3.77 (m, 4H), 3.71 (dd, J = 5.9, 10.7 Hz, 2H), 3.62 (dd, J = 4.1, 9.7 Hz, 2H), 3.55 (m, 4H), 3.48 (d, J = 2.8 Hz, 1H), 3.41 (s, 1H), 3.27-3.19 (m, 3H), 2.92 (t, J = 7.6 Hz, 2H), 2.83 (dd, J = 4.5, 12.9 Hz, 1H), 2.60 (t, J = 7.6 Hz, 2H), 2.03 (s, 3H), 1.94 (t, J = 15.2 Hz, 1H), 1.90 (d, J = 22.6 Hz, 3H), 1.50-1.38 (m, 4H), 1.35-1.19 (m, 2H), 0.94 (d, J = 6.6 Hz, 3H); ¹³C NMR (151 MHz, CD₃OD) δ 177.4, 176.1, 173.84, 173.83, 141.0, 140.8, 140.7, 140.6, 130.7, 130.5, 130.29, 130.27, 130.2, 130.1, 129.9, 129.7, 129.5, 129.39, 129.37, 104.4, 103.8, 97.7, 79.7, 78.2, 77.4, 77.2, 77.1, 76.4, 76.3, 75.9, 75.1, 75.0, 74.9, 74.6, 73.6, 73.3, 72.2, 71.1, 70.62, 70.59, 70.5, 70.4, 69.6, 69.1, 67.9, 65.3, 62.4, 58.6, 54.6, 50.4, 42.3, 37.5, 32.5, 31.1, 25.1, 24.3, 23.5, 21.7; HR-ESI MS: m/z [M-H] calcd for C₈₂H₁₀₂N₃O₂₇ 1560.6706, found 1560.6757.

Figure S19. LC-MS of crude SI-27. Conditions: 20 to 60% A in B; A = CH₃CN + 0.1% TFA + 20% i-PrOH, B = water + 0.1% TFA + 20% i-PrOH over 20 min.
Aminopentyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (sialyl lewis^x, 21). To a solution of SI-27 (13 mg, 14.3 μmol) in methanol/water (2 mL, 8/2) acetic acid (30 μL) was added followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by HPLC using a 0 to 20% gradient (CH3CN in water + 0.1% TFA, Pyramid nucleodur C-18 column) over 35 min to afford the desired compound 21 (Figure S20) (3.3 mg) in 30% yield. ¹H NMR (600 MHz, D₂O) δ 5.11 (d, J = 3.7 Hz, 1H), 4.83 (d, J = 6.5 Hz, 1H), 4.53 (d, J = 7.8 Hz, 2H), 4.10 (d, J = 9.9 Hz, 1H), 4.03 (d, J = 12.3 Hz, 1H), 3.97-3.83 (m, 10H), 3.79 (s, 1H), 3.73-3.63 (m, 6H), 3.63-3.57 (m, 4H), 3.54 (m, 1H), 3.00 (t, J = 7.5 Hz, 2H), 2.78 (dd, J = 4.9, 12.6 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.81 (t, J = 12.0 Hz, 1H), 1.72-1.65 (m, 2H), 1.64-1.57 (m, 2H), 1.45-1.37 (m, 2H), 1.18 (d, J = 6.5 Hz, 3H); ¹³C NMR (151 MHz, D₂O) δ 175.0, 174.1, 173.8, 101.6, 101.0, 99.6, 98.5, 75.6, 75.2, 74.9, 74.8, 73.3, 72.9, 71.8, 71.8, 70.1, 69.2, 69.1, 68.2, 68.1, 68.1, 67.7, 67.3, 66.6, 62.6, 61.4, 59.6, 55.8, 51.6, 39.7, 39.3, 28.0, 26.3, 22.1, 22.0, 15.2; HR-ESI MS: m/z [M+Na]^+ calcd for C₃₆H₆₃N₃NaO₂₃: 928.3750; Found: 928.3762.

Figure S20. LC-MS of 21. Conditions: 0 to 10% A in B; A = CH₃CN, B = water, over 20 min.
4.8 Synthesis of the N-Glycan Core Pentasaccharide 22

Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-(1→3)-(3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3-di-O-benzyl-β-D-mannopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside (23).

The following modules were performed on 25 µmol scale based on resin 1:

1) Two cycles Thioglycoside Double Coupling - Fmoc deprotection using building block 3
2) Beta-Man tom Deprotection using building block 13
3) Benzylidene Opening
4) Alpha Man Double Coupling using building block 14
5) Two cycles TCA-reduction (treatment with 20 eq Bu₃SnH and cat. AIBN)
6) Cleavage of linker (treatment with 10 eq NaOMe)

The eluate solution was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. Purification by preparative HPLC (C18 Nucleosil, 4.6x 250 mm, 60 to 95% acetonitrile (+0.05% TFA) in water (+0.05% TFA) over 51 min gave pure 23 as a mixture of two isomers (2 mg, 3.5% overall yield based on 1). The isomeric mixture 23 was separated by a second purification step using a 20 to 90% gradient (ethylacetate in hexane, YMC-pack diol column) over 30 min to afford 23α and 23β (β/α = 3/1) (Figure S21). 23β: ¹H NMR (700 MHz, CDCl₃) δ 7.45-7.09 (m, 69H), 6.39 (m, 1H), 5.12 (m, 3H), 4.94 (d, J = 13.1 Hz, 3H), 4.88 (d, J = 12.6 Hz, 1H), 4.82-4.74 (m, 3H), 4.70 (d, J = 12.3 Hz, 1H), 4.65-4.57 (m, 3H), 4.55 (d, J = 15.9 Hz, 4H), 4.52-4.39 (m, 12H), 4.39-4.30 (m, 3H), 4.06-3.91 (m, 4H), 3.87 (s, 3H), 3.84-3.71 (m, 10H), 3.69-3.58 (m, 10H), 3.55 (d,
$J = 9.9$ Hz, 2H), 3.48 (m, 2H), 3.30 (m, 1H), 3.22-3.12 (m, 4H), 2.93 (t, $J = 7.8$ Hz, 2H), 2.61 (t, $J = 7.9$ Hz, 2H), 2.04 (d, $J = 7.1$ Hz, 1H), 1.88 (s, 3H), 1.63 (s, 3H), 1.50 (m, 4H), 1.26 (m, 2H); $^{13}$C NMR (176 MHz, CDCl$_3$) $\delta$ 173.5, 170.6, 170.4, 156.4, 140.4, 139.2, 139.1, 138.7, 138.6, 138.5, 138.3, 138.1, 138.0, 128.7, 128.5, 128.4, 128.3, 128.1, 128.04, 127.97, 127.93, 127.87, 127.7, 127.6, 127.4, 101.5, 101.2, 100.6, 100.1, 100.0, 81.4, 80.2, 79.9, 78.6, 78.4, 75.3, 75.2, 75.1, 75.0, 74.8, 74.6, 74.4, 73.6, 73.4, 73.5, 73.3, 72.3, 71.6, 70.1, 69.5, 69.1, 68.8, 68.1, 67.1, 66.7, 51.8, 35.8, 30.8, 29.9, 29.7, 29.4, 29.3, 23.5, 23.3; HR-MALDI-MS: m/z [M+Na]$^+$ for C$_{142}$H$_{159}$N$_3$O$_{30}$ calc. 2410.0940, found 2410.083.

**Figure S21.** HPLC of the resolved $23\alpha/\beta$ mixture. Conditions: 20 to 90% A in B, A = EtOAc, B = hexanes, over 30 min.

**Aminopentyl (α-d-mannopyranosyl)-(1→3)-[α-d-mannopyranosyl-(1→6)]-(β-d-mannopyranosyl)-(1→3)-β-D-2-acetamido-2-deoxy-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside** (core pentasaccharide, 22). To a solution of $23\beta$ (1.5 mg, 0.55 μmol) in MeOH/water/THF/AcOH (1 mL/250 μL/125 μL/50 μL) was added 10% Pd/C (8 mg). The mixture was stirred under an atmosphere of hydrogen for 36 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by solid phase extraction (Sep-Pak C-18, 0 to 40% MeOH in water + 0.1% TFA) to give 22 (0.54 mg, 78% (corrected for the presence of methanol)).

$^1$H NMR (700 MHz, D$_2$O) $\delta$ 5.12 (s, 1H), 4.94 (s, 1H), 4.79-4.77 (m, 1H), 4.62 (d, $J = 8.2$ Hz, 1H), 4.51 (d, $J = 7.3$ Hz, 1H), 4.27 (s, 1H), 4.08 (s, 1H), 3.99 (s, 1H),
3.96-3.88 (m, 8H), 3.85-3.71 (m, 12H), 3.70-3.57 (m, 9H), 3.01 (t, J = 7.5 Hz, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 1.69 (m, 2H), 1.64-1.59 (m, 2H), 1.44-1.29 (m, 2H); $^{13}$C NMR (176 MHz, D$_2$O) δ 174.6, 102.5, 101.4, 101.1, 100.3, 99.6, 80.5, 79.6, 79.4, 74.5, 74.4, 74.2, 73.4, 72.6, 72.4, 71.9, 70.4, 70.1, 70.01, 69.96, 69.9, 66.8, 65.8, 65.81, 65.79, 61.10, 61.05, 60.07, 60.06, 59.98, 59.97, 54.8, 54.7, 39.3, 28.0, 26.3, 22.13, 22.12; HR-MALDI-MS: m/z [M+Na$^+$] for C$_{39}$H$_{69}$N$_3$O$_{26}$ calc. 1018.41, found 1018.54.
5. Glycobiological Tools

5.1 Neo-Glycoconjugate: 1,4-Hexaglucosamine-BSA-Conjugate

Compound 17 (0.9 mg, 0.66 mmol) was dissolved in 100 mL 50 mM sodium phosphate buffer pH7.2. Diethyl squarate (0.335 mg, 2.0 mmol) dissolved in 100 mL ethanol was added. The mixture was agitated overnight at room temperature. The mixture was then purified by reverse phase HPLC (several injections) on a Merck KGaA LiChroCART 250 mm x 4 mm column with LiChrospher 100 RP-18 (5 mm) solid phase using a gradient of 0 to 50% methanol/water.

The isolated fraction of squarate-sugar conjugate (0.2 mg, 0.14 mmol) and BSA (0.2 mg, 3.0 mmol) were dissolved in sodium bicarbonate buffer. The mixture was shaken overnight at room temperature. SDS-PAGE on a 12.5% acrylamide gel shows a diffuse band at approximately 80 kDa (Figure S22b). The mixture was agitated for an additional day. The conjugate was purified using a Microcon centrifugal filter device by Millipore with a molecular weight cutoff of 30kDa. The carbohydrate-protein conjugate was centrifuged twice against 320 mL of ultrapure water (14000 g at 25 °C for 12 min) and lyophilized. The percentage of loading was determined by MALDI-TOF (Figure S22a).
Figure S22. a). MALDI-TOF analysis of the BSA-Hexasaccharide Conjugate. b) SDS-PAGE of the BSA-hexasaccharide conjugate.
5.2 Glycan Microarray

Printing

Oligosaccharide solutions (10 mM solutions were prepared in sodium phosphate buffer, pH 9) were printed on N-hydroxysuccinimide (NHS)-activated CodeLink glass slides, using a piezoelectric spotting device\textsuperscript{13} (Sciennon) to place 1 nL of solution at each spot. Each compound was spotted in six replicas of three different concentrations (1 mM, 0.25 mM, 0.1 mM). After printing, the slides were sealed in a humidity chamber and incubated overnight to covalently couple.

Screening

The slides were washed three times with water in a 50 mL centrifugation tube, quenched with 45 mL of quenching solution for 1 h at 50 °C. Three washes with water were used to clean the surface of the slide, thus the residual water was removed by centrifugation (2500 rpm for 5 min, r.t.). The slides were incubated with BSA (1% in PBS, 100 µL) in a closed, moisturized chamber for 1 h, to block non-specific interactions. Then, the slides were washed (2 x 10 min in 20 mL PBS buffer) and dried by centrifugation (200 rpm for 5 min, r.t.). The slides were incubated with biotinylated wheat germ agglutinin (WGA) (Vector Laboratories) solution (84.5 µL water, 0.5 µL 2% Tween20, 10 µL PBS, 5 µg lectin, 100 µL) in a sealed humidity chamber for 1 h, washed with PBS and dried by centrifugation. WGA is a lectin that specifically interacts with the glycans glucosamine and sialic acid, which are components of the synthetic oligosaccharides in the microarrays. A last incubation step was performed with
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an alexa fluor 594 labeled streptavidin solution (Invitrogen) (77.5 µL water, 0.5 µL 2% Tween20, 10 µL PBS, 10 µL 5% BSA, 2 µg streptavidin marker, 100 µL) under exclusion of light. The slides were washed (PBS), rapidly rewashed with water and dried by centrifugation. A fluorescent microarray scanner (Tecan LS400 scanner analysis by Array-Pro Analyzer) was used for detection (Figure S23 b). Alternatively, after blocking with BSA, slides were incubated with alexa fluor (594) labeled wheat germ agglutinin (Sigma) solutions (87.5 µL water, 0.5 µL 2% Tween20, 10 µL PBS, 2 µg lectin, 100 µL) in a sealed humidity chamber for 1 h, washed (PBS), dried (centrifugation) and scanned (Figure S23a).
Figure S23. Incubation of a glycan microarray with the lectin WGA which specifically recognizes N-acetyl-glucosamine and sialic acid glycans.
6. References


7. **NMR of protected and deprotected glycans**
SI-22

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SI-26-HSQC
SI-28-HSQC-coupled