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Automated synthesis of DNA, RNA, and peptides provides quickly and reliably important tools for biomedical research. Automated glycan assembly (AGA) is significantly more challenging as highly branched carbohydrates require strict regio- and stereocontrol during synthesis. A new AGA synthesizer enables rapid temperature adjustment from -40 °C to +100 °C to control glycosylations at low temperature and accelerates capping, protecting group removal, and glycan modifications by using elevated temperatures. Thereby, the temporary protecting group portfolio is extended from two to four orthogonal groups that give rise to oligosaccharides with up to four branches. In addition, sulfated glycans and unprotected glycans can be prepared. The new design reduces the typical coupling cycles from 100 min to 60 min while expanding the range of accessible glycans. The instrument drastically shorten and generalizes the synthesis of carbohydrates for use in biomedical and material science.

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Microwave-assisted Automated Glycan Assembly

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Abstract

Automated synthesis of DNA, RNA, and peptides provides quickly and reliably important tools for biomedical research. Automated glycan assembly (AGA) is significantly more challenging as highly branched carbohydrates require strict regio- and stereocontrol during synthesis. A new AGA synthesizer enables rapid temperature adjustment from -40 °C to +100 °C to control glycosylations at low temperature and accelerates capping, protecting group removal, and glycan modifications by using elevated temperatures. Thereby, the temporary protecting group portfolio is extended from two to four orthogonal groups that give rise to oligosaccharides with up to four branches. In addition, sulfated glycans and unprotected glycans can be prepared. The new design reduces the typical coupling cycles from 100 min to 60 min while expanding the range of accessible glycans. The instrument drastically shorten and generalizes the synthesis of carbohydrates for use in biomedical and material science.

Main

Automated chemical synthesis on solid support allows rapid access to homogeneous oligonucleotides^{1, 2}, peptides³, and oligosaccharides^{4, 5}. The interplay of technical advances and improved chemical methods has simplified and accelerated oligonucleotide and peptide synthesis. Automated glycan assembly (AGA) faces greater challenges considering the complexity of glycans that require stereo- and regiocontrol during the synthesis. Polysaccharides as long as 100-mers^{6, 7} as well as complex glycans containing different building blocks and linkages are accessible by AGA.⁴ Technical limitations of the current AGA synthesizer restrict assembly speed and the diversity of the glycan motifs can be prepared.

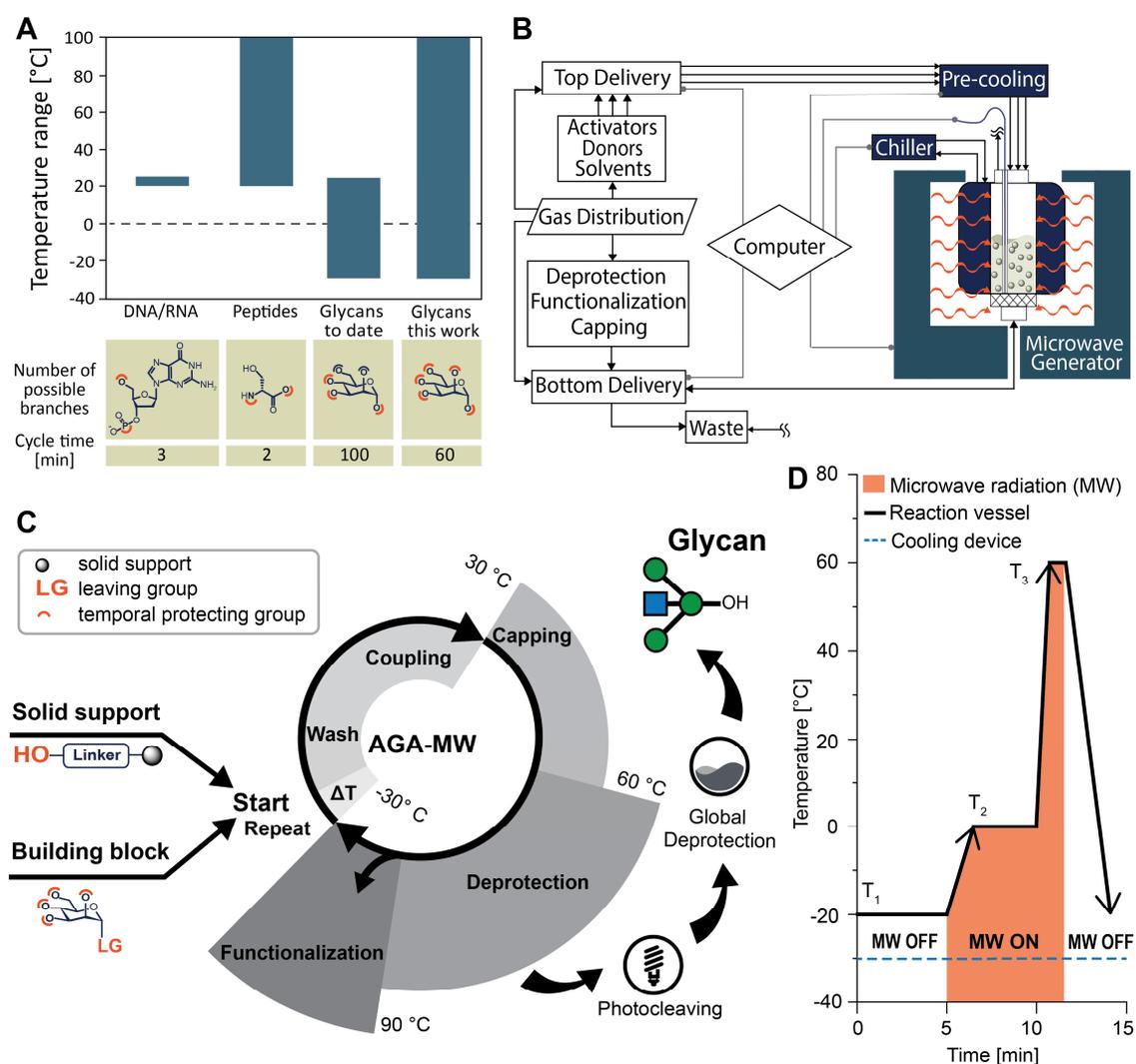


Figure 1 |(A) Automated solid phase synthesis of biomolecules. To date, AGA has been performed at low or ambient temperatures, this work now expands the temperature range from -40 °C to +100 °C. (B) Schematic representation of microwave-assisted AGA system. (C) Flowchart of standardized AGA-MW process: start (baseline temperature adjustment), acidic wash and coupling, capping, deprotection reaction, including automated post-assembly modifications. The radial axis indicates the

temperature range of the step. (D) Representation of the temperature profile regulated by the dual temperature regulation reactor (DTRR) system.

Peptides and oligonucleotides are linear and require no stereocontrol during bond formation (Figure 1A). Oligonucleotides are readily synthesized at room temperature (cycle time = 3 min),^{8,9} while peptide construction¹⁰ is drastically accelerated at elevated temperatures (cycle time = 2 min, +70 °C to 100 °C). In contrast, glycosylation reactions are very temperature sensitive, thus they require low temperatures for high yields and selectivities. In a typical AGA cycle, the low temperature glycosylations are followed by capping of deletion sequences and protecting group removal at room temperature (cycle time = 100 min). To date, the reaction temperature of AGA instruments⁴ is adjusted using a dynamic temperature control system connected to a jacketed reaction vessel. This thermoregulation system is inefficient when significant temperature differences are required during a synthesis cycle.¹¹ Most importantly, the synthesis of branched glycans requires several orthogonal temporary protecting groups, though the working temperature range of -30 °C to +25 °C restricts AGA to just two types of routinely used temporary protecting groups: 9-fluorenylmethyl carbonate (Fmoc) and levulinoyl esters (Lev) that can be cleaved in under 1.5 h.¹² Access to highly branched glycans has not yet been possible by AGA to date and the synthesis of regioselectively sulfated glycans required specialized instrumentation and very long reaction times.^{13, 14}

Here, we describe a technological advance that drastically impacts the chemical transformations amenable to AGA (Figure 1B). Dual thermoregulation through the integration of a microwave (MW) generator and a jacketed reaction vessel permits almost instant temperature changes over a wide range (-40 °C to +100 °C) with minimal energy consumption. This new instrument helps to significantly shorten the time required for the incorporation of each building block and expands the portfolio of orthogonal temporary protecting groups as well as glycan modifications.

Results

Instrument Design and Dual Temperature Regulation Reaction System

The key requirement for the new AGA instrument was quick and accurate adjustment of the reaction temperature over a wide range. The combination of the constant cooling action of a jacketed reaction with the heating power of microwave irradiation allows for rapid and accurate temperature adjustments over a broad range of temperatures during the entire AGA synthesis cycle (Figure 1C).¹⁵ With the dual temperature regulation reaction (DTRR) system, the fritted reaction vessel containing

the solid support is cooled to the lowest temperature required during the synthesis using a circulating coolant set at the baseline sub-zero temperature (Figure 1D). The temperature in the reaction vessel then is adjusted by microwave radiation. Throughout the heating phase, the temperature of the microwave-inert coolant in the jacket is unchanged and allows for rapid return to sub-zero temperatures. This design stands in stark contrast to coolable microwave reactors that operate at constant temperatures, and are not able of performing iterative/sequential multi-step syntheses.^{16,17}

The reaction vessel of the DTRR system is supplied with reagents by an automated delivery system capable of handling gas and liquids with a wide range of viscosities, vapor pressures, and pH (Figure 1B). Building blocks, activator, and washing solutions enter through the top of the reaction vessel. The temperature of these reagents is adjusted to the reaction temperature prior to addition using a pre-cooling heat exchanger to avoid temperature spikes due to warm reagents during glycosylations that tend to be very temperature sensitive. Capping and deprotection reagents are added from the bottom of the reaction chamber. In addition, gas to mix the reagents is added from the bottom and houses the effluent outlet. The activation, deprotection, and capping conditions are incompatible and use entirely different lines to avoid cross-contamination problems. The addition of the building blocks and activators are controlled via a syringe pump to ensure maximum accuracy ($1 \text{ mL} \pm 0.05 \text{ mL}$) while all other reagents are added to the reaction chamber by argon overpressure. The gas distribution system provides a separate inert atmosphere to each reagent group. The gas blanket prevents reagent degradation, cross-contamination by invasive reagents and drives several of the reagents through the system.

The delivery system and temperature regulation components are connected to a computer that controls the device operation (Figure 1B). The synthesis steps are programmed using a software developed for this purpose. Operative modules are selected and the process parameters are modified including building block position, temperature, incubation time, step iterations, reagent volume, and microwave power to prepare the desired glycan sequence. Importantly, the system contains a built-in fiber optic thermoprobe for the real-time temperature monitoring inside of the reaction vessel, to aid in optimizing the AGA cycle.

The dual temperature regulation system consumes less than half the energy when compared to the previous single device thermoregulation system (0.8 kW/h vs 1.8 kW/h). Moreover, to accelerate the regulation to sub-zero temperatures and suppress thermal spikes, a "pre-cooling" heat exchanger unit (Figure 1B and Figure 3A and B) cools the solvents (S), building block (BB), and activator solutions (Act) prior to reaching the reaction vessel (Figure 3A).

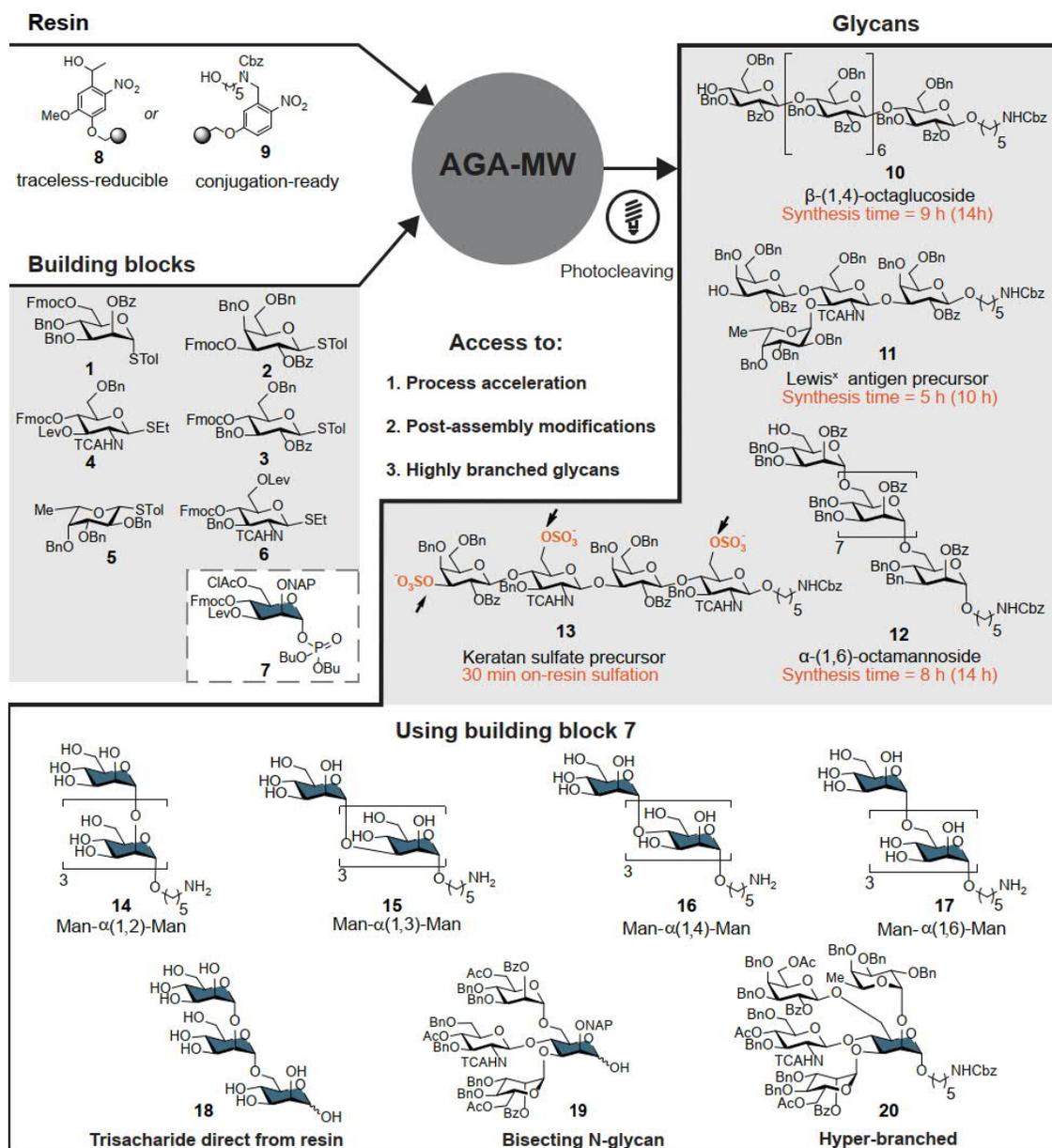


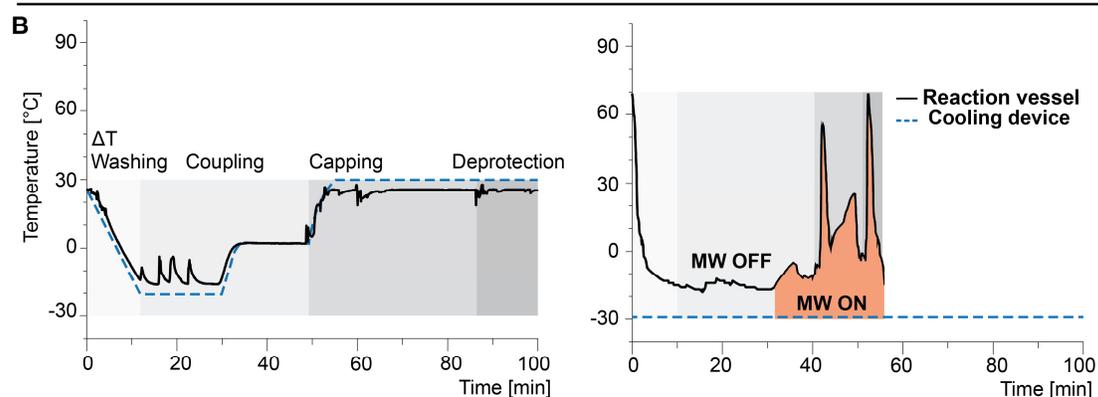
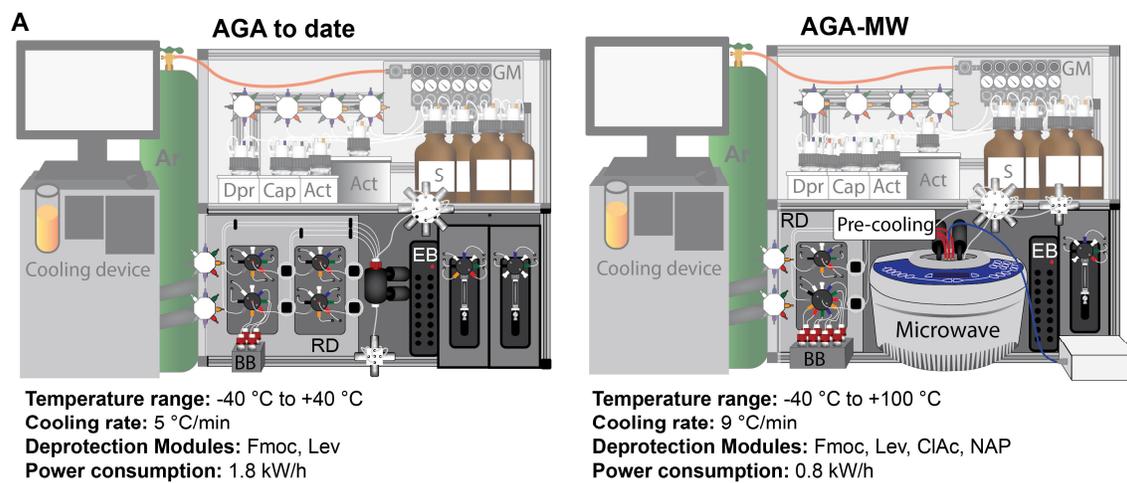
Figure 2 | Oligosaccharides 10-20 prepared to highlight the capabilities of an AGA-MW synthesizer. Time in parenthesis notes synthesis time using a standard AGA instrument.

Process Acceleration

The syntheses of linear β -(1,4)-octaglycoside 10, branched Lewis^x antigen¹⁸ tetrasaccharide precursor 11, and α -(1,6)-octamannoside 12 serve to illustrate the utility of the DTRR synthesizer (Figure 2). Solid support resin 9 is placed in the reaction vessel and the reaction temperature is adjusted to the baseline sub-zero temperature before washing with a series of solvents, and a neutralizing acidic (Figure 1C). This acidic wash is particular important during subsequent coupling cycles to ensure the removal of any remaining base. Glycan elongation begins with mixing the resin, the glycosyl building block (BB),

and activator at the baseline temperature (T_1) for a desired time (t_1), before the temperature of the reaction solution is linearly increased (ramp = 4 °C/min) to the next thermal stage (T_2) by microwave radiation and held for a desired period of time (t_2) (Figure 1D). Upon draining and washing, unreacted support bound hydroxyl nucleophiles are capped by acetylation at 25 °C. Removal of the temporary protecting group can then rapidly proceed at elevated temperatures. The DTRR system is not only effective at reaching temperatures above 25 °C, but also to promptly return to sub-zero temperatures for the next glycosylation (Figure 3B). During the synthesis of the linear α -(1,6)-octamannoside 12 and β -(1,4)-octagluco-side 10, Fmoc cleavage was achieved in one minute at 60 °C using 20% piperidine, with the temperature returning to -20 °C within 10 minutes (Figure 3B and 3C). The next coupling cycle then starts upon return to the baseline temperature and the process is repeated until the desired oligosaccharide sequence has been prepared. These octasaccharides were prepared in less than 9 h from the corresponding thioglycoside building blocks 1 and 3 (Figure 2, 12= 45% and 10= 41% yield), thereby drastically lowering the overall cycle time from the previous required 100 min for each monosaccharide incorporation with no reduction in yield (Figure 3B).¹¹ Further process acceleration resulted in reduced yields, as most of the remaining time is required for thorough resin washes between switching from acidic to basic conditions throughout the cycle.

The synthesis of branched Lewis^x antigen¹⁸ tetrasaccharide 11 required two temporary protecting groups, Fmoc and Lev ester (Figure 2). During the synthesis, the C-3 Lev ester group on the glucosamine 4 residue was first selectively removed with hydrazine acetate in 15 min at 35 °C (Figure 3C) in anticipation of fucosylation. Following the addition of 5, the C-4 Fmoc was removed to prepare for the subsequent galactosylation by 2 to afford 11 in 6 h and 53% yield. Levulinoyl ester cleavage was accelerated by 75 min (90 min \rightarrow 15 min) while unfortunately the unreactive glucosamine building block still required a double coupling to achieve high efficiencies.¹⁸



C

Module	T [°C]	Time [min]	
Glycosylation	-20 to 0	To date 25	
	-20 to -5	AGA-MW 13	
Capping	25	35	
	30		12
Deprotection	Fmoc	25	5
		60	1
	Lev	25	90
		35	
	NAP	40	240
60		60	
CIAC	-	Not available	
Post-Assembly	Sulfation	50	540
		90	
	Methanolysis	-	Not available
			480

Figure 3 | Comparison of the previous state of the art and DTRR synthesizer based on AGA-MW. (A) Pictorial representation and technical data (EB = electronic board, SP = syringe pump, GM = gas manifold, and RD = reagents distribution). (B) Temperature profile inside the reaction vessel during one coupling/capping/deprotection cycle of 12 (right) compared to that of one cycle in a standard AGA instrument (without microwave) and the DTRR system (with microwave).

Expansion of Orthogonal Temporary Protecting Group Portfolio

Carbohydrates, other than peptides and oligonucleotides, are branched. Therefore, the chemical synthesis of branched oligo- and polysaccharides requires more than one temporary protecting group. Orthogonal protecting groups allow for unmasking of one hydroxyl group in anticipation of elongation without affecting protecting groups at other positions. The ability to adjust the temperature in the DTRR system quickly to higher temperatures creates new opportunities for the use of orthogonal protecting groups that were previously not amenable to AGA. In order to create heavily branched glycans, ideally four orthogonal protecting groups are required. For this purpose, differentially protected mannose building block 7 was designed (Figure 2). Few other completely orthogonal building blocks have been reported¹⁹⁻²², yet none are amenable to AGA as many considerations have to be taken into account, such as tolerance of protecting groups to AGA conditions, total solubility of reagents and byproducts, fast and selective deprotection process as well as reactivity of the building block itself. Therefore, the protecting groups and their positions in 7 were chosen carefully for optimal performance in AGA. From the plethora of protecting groups that have been developed for solution phase chemistry, Fmoc, Lev, 2-naphthylmethyl ether (NAP), and chloroacetate ester (ClAc) were selected as orthogonal protecting groups^{12, 19} to be used for AGA.

Fmoc and Lev had already shown promise for rapid cleavage in the DTRR system, though it was found that repeated exposure of the Lev group to piperidine resulted in side reactions. The simple switch to triethylamine resolved the issue (Figure 3C). The Lev group was positioned at the C-3 as O-3 ester groups are reported to assist with strong α -selectivity.²³ It was found that the Fmoc was best positioned at the C-4 position, as a C-4 AcCl group tended to migrate to the primary C-6 hydroxy group upon a C-6 Fmoc cleavage. As a result, the AcCl was used to protect the primary C-6 position in mannose building block 7. Now capable of reaching 80 °C in the DTRR system – which was not achievable in the standard AGA system – the deprotection of the AcCl group using thiourea was facilitated in 45 minutes (Figure 3C).¹⁹ To balance the electron-withdrawing ester and carbamate groups (Fmoc, AcCl, and Lev), the electron-donating NAP group was chosen to protect the C-2 position to ensure proper reactivity of 7 when serving as both a glycosyl donor and as an acceptor. Even with the C-2 non-participating NAP ether group, complete α -stereoselectivity was observed for building block 7. To date, NAP ethers have been used in AGA²⁴ but their cleavage is very slow, sometimes incomplete and accompanied by side-products, arising from debenylation. While in the previous system the NAP group removal required unpractical 240 minutes using DDQ/MeOH/water in DCE at 40 °C, the DTRR system provides more practical and reliable NAP deprotections in 60 minutes at 60 °C using DDQ/MeOH in DCE (Figure 3C). Acidic conditions^{25, 26} failed to selectively cleave the NAP ether in the presence of Lev, AcCl, and Fmoc.

To illustrate the concept, α -(1,2), α -(1,3), α -(1,4) and α -(1,6)- tetramannosides 14-17 were assembled from building block 7 (Figure 2, yields: α -(1,2) 14 = 19%, α -(1,3) 15 = 12%, α -(1,4) 16, α -(1,6) 17 = 18%) with excellent regio- and stereo-selectivity. Encouraged by these results, *P. falciparum* GPI anchor mannose trisaccharide portion 18²⁷ containing both α -(1,2) and α -(1,6) linkages was achieved on polystyrene resin equipped with traceless-reducible photolabile linker 8. For that, the NAP group at the first sugar unit was removed and acetylated before extending at C-6 and then the C-2 position. After the assembly, solid support-bound unprotected oligosaccharide was prepared by removing the remaining NAP ether, followed by methanolysis using sodium methoxide (Figures 3C and S2). The global deprotection at solid support was confirmed by on-resin FT-IR (see SI). Photocleavage of the linker released the trisaccharide product 18 from the solid support (yield = 15%). This oligosaccharide is the first example of a natural, unmodified glycan produced by AGA without any manual protecting group manipulations otherwise known only from fully enzymatic or chemoenzymatic approaches. The syntheses of 14-17 as well as 18 also show, that a single building block such as 7 can give rise to many possible combinations of oligosaccharides.

Synthesis of Highly Branched Glycans

Naturally occurring glycans are frequently branched and in rare cases, such as of chorella viruses, three branches extend from a single monosaccharide.²⁸ For artificial systems, the creation of highly branched glycans where every hydroxyl group serves as a point of modulation are also desirable. Orthogonally protected mannose building block 7 served in the assembly of branched structures including a portion of bisecting *N*-glycan 19 (Figure 2, yield = 28%). Hyper-branched, unnatural tetrasaccharide 20 containing four branches bearing fucose, mannose, *N*-acetylglucosamine and galactose was assembled (Figure 2, yield = 32%). This highly branched tetrasaccharide illustrates the challenges associated with the analysis of such complex glycans. Characterization by NMR spectroscopy initially gave rise to concerns, as complex spectra seemed to suggest a failed synthesis. NMR analysis of the same samples at elevated temperature (see SI) revealed an entirely different picture and shows that such densely functionalized, protected oligosaccharides adopt structures containing different rotamers.

Post-assembly Modification

Many naturally occurring glycans are modified by sulfation, acetylation, or lipidation. Glycosaminoglycans and many marine glycans for example are heavily sulfated.^{29,30} The synthesis and purification of sulfated glycans is challenging due to the high polarity and sulfate lability. Solid support synthesis of sulfated glycans is advantageous as it minimizes the number of purification steps.^{13,14} The

automated construction of keratan sulfate tetrasaccharide 13 (Figure 2) served to illustrate microwave accelerated sulfation³¹ on solid support. Following the assembly of a tetrasaccharide employing building blocks 2 and 6, the levulinoyl esters were selectively cleaved to expose the C-6 hydroxy groups of the glucosamine residues as well as the C3-hydroxy group in the terminal galactose by Fmoc removal. The three unprotected hydroxy groups were sulfated within 30 minutes by a sulfur trioxide trimethyl amine complex in DMF at 90 °C (Figures 3C and S2).³¹ This sulfation process was a significant improvement over the previous sulfation protocol that required 9 h.¹³ Salts and excess sulfation reagents were simply washed away from the resin such that, following photocleavage from the resin, routine reverse-phase chromatography purification yielded 24% of desired trisulfated tetrasaccharide 13.

Discussion

Carbohydrates, the most abundant biomolecules in nature, are essential for structure, energy supply, and molecular interactions of living organisms.³² Rapid access to homogeneous glycans is essential for medical, biological, and material science investigations. Here, we report a technological advance, a new oligosaccharide synthesizer that broadens the range of possible synthetic transformations. Now, access to glycans with and without modifications is possible that were previously not accessible. The new AGA instrument combines microwave irradiation and constant cooling to allow for fast adjustments of temperatures from -40 °C up to +100 °C, with minimal energy consumption. The versatility of the new instrument concerning temperature range and control has no precedent in AGA or automated solid support synthesis in general. The reaction vessel is supplied with reagents via a delivery system that can handle a wide variety of gases, acids, bases, and high vapor pressure solvents.

Previously, some carbohydrate transformations were performed using microwave radiation^{16, 33} and even cooled microwave reactors.^{34, 35} However, microwave heating has not been implemented to regulate several steps during an oligosaccharide synthesis or for AGA.³⁴ Real-time monitoring of the rapid and accurate heating of the reaction mixture resulted in a significantly faster synthetic process as illustrated by the assembly of several linear and branched oligosaccharides. The ability to heat and cool reactions quickly and reliably, enables new chemical strategies for oligosaccharide assembly. A variety of temporary protecting groups can be cleaved on solid support such that fully deprotected oligosaccharides are released upon cleavage from the solid support. With four temporary protecting groups available, the construction of highly branched glycans is now possible. Glycan modifications such as sulfation found in glycosaminoglycans and marine glycans is now quickly possible.

Simultaneous cooling and microwave heating improves reactor temperature control by preventing run away temperatures, ease post-synthesis vessel handling, modify the nucleation and growth of solid products³⁶⁻³⁹, and to explore non-thermal microwave effects^{17, 34, 39}. Rapid adjustment to the optimal temperatures required during multistep syntheses is readily achieved in microwave-assisted reaction systems. By combining microwave dielectric heating and constant cooling, a wide temperature range for each reaction within a multi-step synthesis is available such that complex molecules can be prepared within a single device. The use of optimized monosaccharide building blocks and further improved coupling cycles will help the new AGA synthesizer to prepare ever more complex glycans even faster. The modular nature of the new device makes it ideally suited for expansion to the synthesis of other complex molecules and not just carbohydrates.⁴⁰

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Contributions

P.H.S. conceived the overall project and supervised the effort. J.D.F., S.L., and E.T.S. designed the experiments, ran the synthetic protocols, and characterized the products. The new system was constructed by J.D.F. with software help from K.B.. Building block 8 was designed by K.L.M.H. and S.L. and synthesized by A.A.J. and S.L.. P.H.S. wrote the paper together with J.D.F., S.L., and E.T.S. with help from all the authors.

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Ethics declarations

Conflict of interest statement: P.H.S. and K.L.M.H. declare a significant financial interest in GlycoUniverse GmbH & Co. KGaA, the company that commercialized the previous AGA synthesis instrument, building blocks, and other reagents.

METHODS

Glycan assembly.

Automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces. All details concerning pre-automation steps, building blocks, and modules used for the automated synthesis as well as post-assembly manipulations can be found in the SI.

Temperature regulation system.

A Discover microwave reactor (CEM) houses the reaction vessel. A jacket surrounding the reaction vessel provides constant cooling to the lowest target temperature during the synthesis ($> -40\text{ }^{\circ}\text{C}$). The cooling jacket is in fluid communication with a chiller that circulates a microwave transparent coolant working at a constant temperature. Any higher temperature during the synthesis cycle (up to $100\text{ }^{\circ}\text{C}$) is reached by microwave radiation. The reagent temperature is continuously monitored with an optic fiber probe inside the reaction vessel. The maximum microwave power used depends on the reagents, and is dynamically adjusted. The solvents, building block, and activator solutions are cooled to $-8\text{ }^{\circ}\text{C}$ before reach the reaction vessel by a precooling unit (see SI). The reaction vessel holds the solid support. While gas and liquid flow in and out the reaction vessel via the top and bottom inlets/outlets. A top outlet vents the exhaust gas.

Delivery systems.

The reagent containers are categorized into solvents, building blocks, activators, capping, and deprotection/functionalization. Each group has a separate pressurized inert atmosphere provided by a gas manifold. A syringe pump drives the building block and activator solutions from the reservoir to the upper part of the reaction vessel. Both reagents travel through separate handles by rotary valves. A buffering volume line between the pump and the reservoirs prevents the reagents from mixing. A third top inlet dispenses the solvents for washing and the gas for draining the reactor vessel. A top outlet vents the exhaust gas. The bottom inlet/outlet connects multiport valve. It serves to drain the liquid, delivering bubbling gas for mixing and post-coupling reagents (deprotection, capping, or post-modification reagent solutions). The washing solvents and bottom supplied reagents are gas-driven by differential pressure. The vent gases and drained liquid go to the waste container. Alternatively, the drained solutions can be collected for analysis or recovery of unreacted components.

Modular design.

A computer centralizes the control of delivery and temperature regulation systems. The entire device has a modular construction. All the components are accessible and replaceable. Capabilities can be expanded by adding elements or reorganizing fluid pathway. In a single working environment, the software allows the creation and storage of operational modules listing series of ground-level commands (on, off the device, and setting parameters). The modules execute generic process tasks such as system initialization, reactions, and the standby operation. The user builds a synthesis program by compiling modules. The settings of each module are adjustable.

AGA Cycle Modules.

This section describes the key modules during an AGA-MW cycle, for further modules refer to the SI.

Glycosylation: Once the temperature of the reaction vessel has adjusted to the desired temperature of the subsequent glycosylation by the cooling device, 1 mL of the Acidic Wash Solution is delivered to the reaction vessel. After three minutes, the solution is drained. Finally, the resin is washed with 3 mL CH_2Cl_2 . Upon draining the reaction vessel, 1 mL of Building Block Solution containing the appropriate building block is delivered from the building block storing component to the reaction vessel through the precooling device (set at $-20\text{ }^{\circ}\text{C}$). After the temperature again reaches the desired temperature (T_1), 1 mL of appropriated Activator Solution (see SI for specific cases) is delivered to the reaction vessel from the respective activator storing component to the reaction vessel through the precooling device (set at $-20\text{ }^{\circ}\text{C}$). The glycosylation mixture is incubated for the selected duration (t_1)

at the desired T_1 , then by microwave irradiation (max power = 120 W) the reaction temperature is linearly ramped to T_2 (rate = 4 °C/min). Once T_2 is reached, it is maintained by microwave irradiation and the reaction mixture is incubated for an additional time (t_2). Once the incubation time is finished, the reaction mixture is drained and the resin is washed with CH_2Cl_2 (1 x 2 mL for 15 s), then dioxane (1 x 2 mL for 15 s), and finally CH_2Cl_2 (2 x 2 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Capping: The resin is washed with DMF (2 x 3 mL for 15 s). Then 2 mL of Pre-capping Solution (10% v/v pyridine in DMF) are delivered and under microwave irradiation the reaction temperature is adjusted to and maintained at 50 °C for one minute (max power = 5 W). The resin is then washed with CH_2Cl_2 (3 x 2 mL for 15). Upon washing, 4 mL of Capping Solution (20% Ac_2O , 4% MsOH in CH_2Cl_2) are then delivered and the temperature is adjusted and maintained 25 °C by microwave irradiation (max power = 100 W). The resin and the reagents are incubated for 8 min. The solution is then drained from the reactor vessel and the resin is washed with CH_2Cl_2 (3 x 3 mL for 15 s). During the entire module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Fmoc Deprotection: The resin is first washed with DMF (3 x 3 mL for 15 s), and then 2 mL of Fmoc Deprotection Solution (20% v/v of piperidine or 20% v/v Et_3N in DMF, see SI for specific cases) is delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 60 °C by microwave irradiation (max power = 60 W). After 1 min the reaction solution is drained and the resin is washed with DMF (3 x 3 mL for 15 s) and CH_2Cl_2 (5 x 3 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Lev Deprotection: The resin is washed with CH_2Cl_2 (3 x 2 mL for 15 s), and then 2 mL of Lev Deprotection Solution, $\text{N}_2\text{H}_4\cdot\text{HOAc}$ in $\text{CH}_2\text{Cl}_2/\text{Pyr}/\text{HOAc}/\text{H}_2\text{O}$ (20:16:4:1) is delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 25 °C by microwave irradiation (max power = 180 W). After 5 min, the reaction solution is drained from the reactor vessel and the resin is washed with CH_2Cl_2 (3 x 2 mL for 15 s). Then, of fresh Lev Deprotection Solution (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF, THF, and CH_2Cl_2 (3 x 3 mL for 15 s, respectively). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

NAP Deprotection: The resin is first washed with CH_2Cl_2 (3 x 2 mL for 15 s) then 2 mL of NAP Deprotection Solution (0.022 m/v DDQ in a 4:1 mixture of DCE/methanol), was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 60 °C by microwave irradiation (max power = 180 W). After 30 min, the reaction solution is drained from the reactor vessel. The resin is washed with CH_2Cl_2 (3 x 2 mL for 15 s). Then, fresh Fmoc Deprotection Solution (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF, THF, and CH_2Cl_2 (3 x 3 mL for 120 s, respectively). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

CIAC Deprotection: The resin is first washed with CH_2Cl_2 (3 x 2 mL for 15 s) then CIAC Deprotection Solution (2 mL, 5% w/w Thiourea in a 10:1 mixture of 2-methoxyethanol/pyridine) was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 90 °C by microwave irradiation (max power = 180 W). After 22 min, the reaction solution

is drained from the reactor vessel. The resin is washed with DMF (3 x 2 mL for 15 s). Then fresh CIAC Deprotection Solution (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF (3 x 3 mL for 15 s) and CH₂Cl₂ (5 x 3 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Glycan Modification.

Sulfation serves as an example for a post-assembly modification reaction. The resin is first washed with CH₂Cl₂ (3 x 2 mL for 15 s) then 2 mL of sulfation solution, SO₃-TMA (20 equiv/OH) in DMF, was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 90 °C by microwave irradiation (max power = 90 W). After 15 min the reaction solution is drained from the reactor vessel. Again, 2 mL of fresh sulfation solution is added and the temperature is adjusted and maintained at 90 °C by microwave irradiation for 20 min (max power = 90 W). Upon completion, the resin is washed with DMF (3 x 2 mL for 15 s).

Methanolysis on resin was accomplished by washing the resin with CH₂Cl₂ (3 x 2 mL for 15 s) 2 mL of methanolysis solution (1:9 dissolution of sodium methoxide in methanol (0.5 M) in THF) was delivered to the reaction vessel at room temperature. After 1 h the reaction solution is drained from the reactor vessel. The incubation in methanolysis solution was repeated three more times. Then, the resin is washed with 10% aqueous citric acid, DMF, THF, and CH₂Cl₂ (3 x 3 mL for 120 s, respectively).

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Supporting Information

Microwave-assisted Automated Glycan Assembly

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1 Additional Figures

Module	Conditions	T[°C]	Time [min]	
Glycosylation	BB in CH ₂ Cl ₂ then NIS, TfOH in CH ₂ Cl ₂ /dioxane	-20 to 0 -20 to -5	To date 25 AGA-MW 13	
	Capping	10% Ac ₂ O, 2% MsOH in CH ₂ Cl ₂ ^a	25 30	35 12
Deprotection	Fmoc	20% piperidine or Et ₃ N in DMF	25 60	5 1
	Lev	7% N ₂ H ₄ ·HOAc in CH ₂ Cl ₂ /py/HOAc/H ₂ O	25 35	90 15
	NAP	2% DDQ in DCE/MeOH/H ₂ O ^b	40 60	240 60
	CIAC	5% thiourea in EGME/py	- 80	Not available 45
Post-Assembly	Sulfation	SO ₃ ·py (20 equiv/OH) or SO ₃ ·TMA in DMF ^c	50 90	540 30
	Methanolysis	CH ₃ ONa in MeOH (0.5 M)/THF 1:9	- 25	Not available 480

^aAGA-MW: double concentration. ^bAGA-MW: only DCE and MeOH. ^cTraditional AGA: SO₃·py in py/DMF, AGA-MW:SO₃·TMA in DMF.

Figure S-1. Comparison of modules in the standard system (without microwave) and the DTRR system (with microwave) and overview over the modules and conditions.

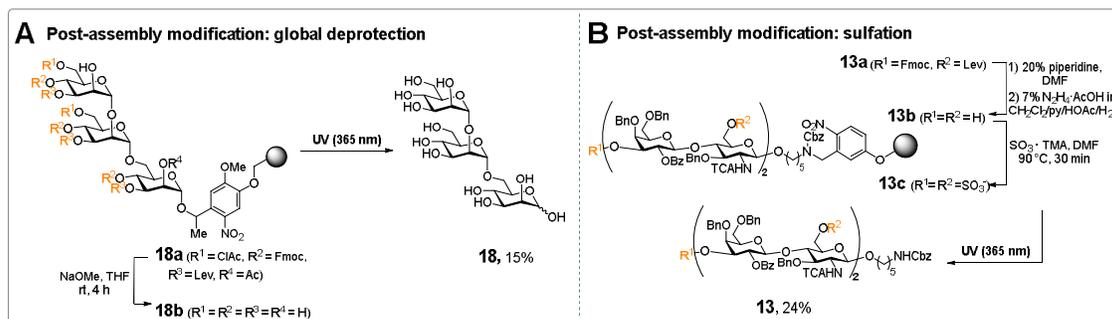


Figure S-1. Post-assembly modifications achieved by the DTRR system. (A) Trisaccharide **18** was assembled from building block **7** and fully deprotected on-resin. (B) Synthesis and on-resin sulfation of tetrasaccharide **13**.

2 General Information

All **chemicals** were reagent grade and used as supplied unless otherwise noted. All **solvents** for chemical reactions were commercially purchased in p.a. quality. If stated, they were dried in a Solvent Dispensing System (J.C. Meyer). For HPLC and MS spectrometry, solvents with corresponding quality were used. Water was used from a Milli Q-station from Millipore. **The automated syntheses** were performed on a TempDUO home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces described in section 4.

Reaction completion, identity, and purity of all compounds were determined by low resolution mass spectrometry (**ESI-LRMS**) or analytical thin-layer chromatography (**TLC**). TLC was performed on Merck silica gel 60 F₂₅₄ plates (0.25 mm). Compounds were visualized by UV irradiation (254 nm) or stained (*p*-Anisaldehyde Stain: 3.7 mL *p*-anisaldehyde, 135 mL ethanol, 5 mL sulfuric acid, 1.5 mL glacial acetic acid or Hanessian's Stain: 235 mL of distilled water, 12 g of ammonium molybdate, 0.5 g of ceric ammonium molybdate, and 15 mL sulfuric acid). **Flash column chromatography** was performed on Kieselgel 60 with 230-400 mesh (Sigma-Aldrich, St. Louis, USA) or C₁₈-reverse phased silica gel, fully endcapped (Sigma-Aldrich, St. Louis, USA). Analysis and purification by normal and reverse phase **HPLC** and ESI-LRMS was performed by using an Agilent 1200 series. Products were **lyophilized** using a Christ Alpha 2-4 LD plus freeze dryer. ¹H, ¹³C, COSY and HSQC **NMR spectra** were recorded in parts per million (δ) relative to the resonance of the solvent on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Assignments were supported by COSY and HSQC experiments. High resolution mass spectra (**HRMS**) were obtained using 6210 ESI-TOF mass spectrometer (Agilent) and **MALDI-TOF** autoflexTM (Bruker) instruments. **IR spectra** were recorded on a Perkin-Elmer 1600 FTIR spectrometer. **Optical rotations** were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter, with concentrations expressed in g/100 mL.

Amberlite IR-120 (Across Organics) protonic exchange resin was rinsed with THF, water, methanol and dichloromethane before use. Palladium on carbon was removed from reaction mixtures by filtration with Rotilabo syringe filters (Roth), PTFE filters (pore size: 0.45 μ m).

3 Materials and Conditions for Automated Synthesis

3.1 Materials and Measurements

Solvents used for dissolving all building blocks and making of various solutions were taken from Solvent Dispensing System (J.C. Meyer). Wash solvents were HPLC grade. The building blocks were purchased from GlycoUniverse GmbH & CO KGaA or synthesized if stated. Prior to automated synthesis, the building blocks were weighed and co-evaporated three times with anhydrous toluene and dried for at least 1 hour under high vacuum prior to use. All solutions were freshly prepared and kept under argon during the automation process. Isolated yields of products were calculated on the basis of resin loading. Functionalized resin **8** and **9** were synthesized as previously reported¹ and resin loading (0.3 mmol/g for **9** and 0.4 mmol/g for **8**) was determined following a published protocol.² Resin was placed in the reaction vessel and was swollen in dichloromethane for 20 min at room temperature before starting the first module. During this time, all reagent lines involved in the synthesis were washed and primed.

3.2 Preparation of Stock Solutions

Building Block Solution 1 (for thioglycosides): Thioglycoside building block (0.09 mmol, 6.5 equiv. per cycle) was dissolved in 1 mL (per cycle) of anhydrous CH₂Cl₂.

Building Block Solution 2 (for glycosyl phosphates): Glycosyl phosphate building block (0.06 mmol, 4.7 equiv. per cycle) was dissolved in 1 mL (per cycle) of anhydrous CH₂Cl₂.

Acidic Wash Solution: TMSOTf (0.45 mL, 0.31 mmol) was added to 40 mL of anhydrous CH₂Cl₂.

Activator Solution 1 (for thioglycosides): Recrystallized NIS (1.58 g, 0.16 mmol) was dissolved in 45 mL of a 2:1 mixture of anhydrous CH₂Cl₂/dioxane, followed by addition of triflic acid (55 μ L, 0.001 mmol). The solution was kept under ice-bath cooling for the duration of the automated run.

Activator Solution 2 (for glycosyl phosphates): TMSOTf (0.9 mL, 0.62 mmol) was added to 40 mL of anhydrous CH₂Cl₂.

Pre-capping Solution: Pyridine (10 mL) was added to 90 mL of DMF.

Capping Solution: Methanesulfonic acid (1.2 mL, 18.5 mmol), acetic anhydride (6 mL, 63.5 mmol) were added to 50 mL of anhydrous CH₂Cl₂.

Concentrated Capping Solution: Methanesulfonic acid (2.4 mL, 37 mmol), acetic anhydride (12 mL, 127 mmol) were added to 50 mL anhydrous CH₂Cl₂.

Lev Deprotection Solution: $\text{N}_2\text{H}_4 \cdot \text{HOAc}$ (725 mg, 7.87 mmol) was dissolved in 50 mL of a 4:1:0.25 mixture of pyridine/acetic acid/water.

Fmoc Deprotection Solution 1: Piperidine (20 mL) was added to 80 mL anhydrous DMF.

Fmoc Deprotection Solution 2: Et_3N (20 mL) was added to 80 mL anhydrous DMF.

NAP Deprotection Solution: DDQ (910 mg, 4.00 mmol) was dissolved in 40 mL of a 4:1 mixture of DCE/methanol. The solution was protected from light by aluminium foil for the duration of the automated run.

CIAC Deprotection Solution: Thiourea (2.5 g, 32.84 mmol) was dissolved in 55 mL of a 10:1 mixture of 2-methoxyethanol/pyridine.

Sulfation Solution: $\text{SO}_3 \cdot \text{TMA}$ (900 mg, 6.5 mmol) was added to 16 mL of DMF and sonicated until dissolved.

Methanolysis Solution: A solution of sodium methoxide in methanol (1 mL, 0.5 M) was added to 9 mL THF.

3.3 Modules for Automated Synthesis

Initiation: The resin loaded in the reaction vessel is washed with DMF, THF, and CH₂Cl₂ (3 x 3 mL for 15 s, respectively). The resin is then swollen in 2 mL CH₂Cl₂ for 20 minutes while the temperature of the reaction vessel is cooled to the lowest temperature required throughout the synthesis.

Module I - Acidic Washing: Once the temperature of the reaction vessel has adjusted to the desired temperature of the subsequent glycosylation by the cooling device, 1 mL of the **Acidic Wash Solution** is delivered to the reaction vessel through the precooling device (set at -20 °C). After three minutes, the solution is drained. Finally, the resin is washed with 3 mL CH₂Cl₂ (bubbling = 15 s) and drained.

Module IIa – Glycosylation (for thioglycosides): Upon draining the CH₂Cl₂ in the reaction vessel, 1 mL of **Building Block Solution 1** containing the appropriate building block is delivered from the building block storing component to the reaction vessel through the precooling device (set at -20 °C). After the temperature reaches the desired temperature (T₁), **Activator Solution 1** (1 mL) is delivered to the reaction vessel from the respective activator storing component to the reaction vessel through the precooling device (set at -20 °C). The glycosylation mixture is incubated for the selected duration (t₁) at the desired T₁, then by microwave irradiation (max power = 120 W) the reaction temperature is linearly ramped to T₂ (rate = 4 °C/min). Once T₂ is reached, it is maintained by microwave irradiation and the reaction mixture is incubated for an additional time (t₂). Once the incubation time is finished, the reaction mixture is drained and the resin is washed with CH₂Cl₂ (1 x 2 mL for 15 s), then dioxane (1 x 2 mL for 15 s), and finally CH₂Cl₂ (2 x 2 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Module IIb – Glycosylation (for glycosyl phosphate): Upon draining the CH₂Cl₂ in the reaction vessel, **Building Block Solution 1** (1 mL) containing the appropriate building block is delivered from the building block storing component to the reaction vessel through the precooling device (set at -20 °C). After the temperature again reaches the desired temperature (T₁), **Activator Solution 1** (1 mL) is delivered to the reaction vessel from the respective activator storing component to the reaction vessel through the precooling device (set at -20 °C). The glycosylation mixture is incubated for the selected duration (t₁) at the desired T₁, then by microwave irradiation (max power = 180 W) the reaction temperature is linearly ramped to T₂ (rate = 4 °C/min). Once T₂ is reached, it is maintained by microwave irradiation and the reaction mixture is incubated for an additional time (t₂). Once the incubation time is finished, the reaction mixture is drained and the resin is washed with DCE (1 x 2 mL for 5 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Module IIIa - Capping: The resin is washed with DMF (2 x 3 mL for 15 s). Then **Pre-capping Solution** (2 mL) is delivered and under microwave irradiation the reaction temperature is adjusted to and maintained at 50 °C for one minute (max power = 5 W). The resin is then washed with CH₂Cl₂ (3 x 2 mL for 15). Upon washing, **Capping Solution** (4 mL) is delivered and the temperature is adjusted and maintained 25 °C by microwave irradiation (max power = 100 W). The resin and the reagents are incubated for 8 min. The solution is then drained from the reactor vessel and the resin is washed with CH₂Cl₂ (3 x 3 mL for 15 s). During the entire module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Module IIIb – Capping (Sterically hindered hydroxyls): The resin is initially washed with DMF (2 x 3 mL for 15 s). Then **Pre-capping Solution** (2 mL) is delivered and under microwave irradiation the reaction temperature is adjusted to and maintained at 50 °C for one minute (max power = 5 W). The resin is then washed with CH₂Cl₂ (3 x 2 mL for 15). Upon washing, of **Concentrated Capping Solution** (4 mL) is then delivered and the temperature is adjusted to and maintained at 25 °C by microwave irradiation (max power = 100 W). The resin and the reagents are incubated for 8 min. The solution is then drained from the reactor vessel and the resin is washed with CH₂Cl₂ (2 x 3 mL for 15 s). Then, DMF (4 mL) is delivered and irradiated with microwaves (max power = 5 W) for 10 s and the solution is allowed to incubate for an additional 50 s before draining. During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis.

Module IVa - Fmoc Deprotection 1: The resin is first washed with DMF (3 x 3 mL for 15 s), and then **Fmoc Deprotection Solution 1** (2 mL) is delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 60 °C by microwave irradiation (max power = 60 W). After 1 min the reaction solution is drained and the resin is washed with DMF (3 x 3 mL for 15 s) and CH₂Cl₂ (5 x 3 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Module IVb - Lev Deprotection: The resin is washed with CH₂Cl₂ (3 x 2 mL for 15 s), and then **Lev Deprotection Solution** (2 mL) is delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 25 °C by microwave irradiation (max power = 180 W). After 5 min, the reaction solution is drained from the reactor vessel and the resin is washed with CH₂Cl₂ (3 x 2 mL for 15 s). Then, of fresh **Lev Deprotection Solution** (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF, THF, and CH₂Cl₂ (3 x 3 mL for 15 s, respectively). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Module IVc - Fmoc Deprotection 2: The resin is first washed with DMF (3 x 3 mL for 15 s), and then **Fmoc Deprotection Solution 2** (2 mL) is delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 60 °C by microwave irradiation (max power = 60 W). After 5 min the reaction solution is drained and the resin is washed with DMF (3 x 2 mL for 15 s). Then, fresh **Fmoc Deprotection Solution 2** (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF (3 x 3 mL) and CH₂Cl₂ (3 x 3 mL) for 15 s each time. During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Module IVd – NAP Deprotection: The resin is first washed with CH₂Cl₂ (3 x 2 mL for 15 s) then **NAP Deprotection Solution** (2 mL) was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 60 °C by microwave irradiation (max power = 180 W). After 30 min, the reaction solution is drained from the reactor vessel. The resin is washed with CH₂Cl₂ (3 x 2 mL for 15 s). Then, fresh **Fmoc Deprotection Solution 2** (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF, THF, and CH₂Cl₂ (3 x 3 mL for 120 s, respectively). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Module IVe – CIAC Deprotection: The resin is first washed with CH₂Cl₂ (3 x 2 mL for 15 s) then **CIAC Deprotection Solution** (2 mL) was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 90 °C by microwave irradiation (max power = 180 W). After 22 min, the reaction solution is drained from the reactor vessel. The resin is washed with DMF (3 x 2 mL for 15 s). Then fresh **CIAC Deprotection Solution 2** (2 mL) is delivered and the process is repeated twice more. Then, the resin is washed with DMF (3 x 3 mL for 15 s) and CH₂Cl₂ (5 x 3 mL for 15 s). During the module, the active cooling element is maintained at the lowest temperature required throughout the synthesis. After this module the resin is ready for the next glycosylation cycle.

Module V – Sulfation: The resin is first washed with CH₂Cl₂ (3 x 2 mL for 15 s) then **Sulfation Solution** (2 mL) was delivered to the reaction vessel. The temperature of the reagents inside the reactor vessel is then adjusted to and maintained at 90 °C by microwave irradiation (max power = 90 W). After 15 min the reaction solution is drained from the reactor vessel. Again, fresh **Sulfation Solution** (2 mL) is added and the temperature is adjusted and maintained at 90 °C by microwave irradiation for 20 min (90 W). Upon completion, the resin is washed with DMF (3 x 2 mL for 15 s).

Module VI – Methanolysis: The resin was washed with CH₂Cl₂ (3 x 2 mL for 15 s) then **Methanolysis Solution** (2 mL) was delivered to the reaction vessel at room temperature. After

1 h the reaction solution is drained from the reactor vessel. The incubation in **Methanolysis Solution** was repeated three more times. Then, the resin is washed with 10% aqueous citric acid, DMF, THF, and CH₂Cl₂ (3 x 3 mL for 120 s, respectively).

3.4 Post-synthesizer Manipulation

Cleavage from Solid Support (Method A-1): Protected Oligosaccharides

After automated synthesis, the resin was removed from the reaction vessel, suspended in CH₂Cl₂ (20 mL), and photocleaved in a continuous-flow photoreactor. A Vapourtec E-Series easy-MedCHem, equipped with a UV-150 Photochemical reactor having a UV-150 Medium-Pressure Mercury Lamp (arc length 27.9 cm, 450 W) surrounded by a long-pass UV filter (Pyrex, 50% transmittance at 305 nm) was used. A Pump 11 Elite Series (Harvard Apparatus syringe pump at a flow rate of 0.8 mL/min) was used to pump the mixture through a FEP tubing (i.d. 3.0 inch, volume: 12 mL) at 20 °C. The reactor was washed with 20 mL CH₂Cl₂ at a flow rate of 2.0 mL/min. The output solution was filtered to remove the resin and the solvent was evaporated *in vacuo*. Crude was then analyzed by MALDI.

Cleavage from Solid Support (Method A-2): Deprotected Oligosaccharides

After automated synthesis, the resin was removed from the reaction vessel, suspended in a 10:1 mixture of THF/water (20 mL), and photocleaved in a continuous-flow photoreactor. A Vapourtec E-Series easy-MedCHem, equipped with a UV-150 Photochemical reactor having a UV-150 LED lamp (365 nm) was used. A Pump 11 Elite Series (Harvard Apparatus syringe pump at a flow rate of 2 mL/min) was used to pump the mixture through a FEP tubing (i.d. 3.0 inch, volume: 12 mL) at 20 °C. The reactor was washed with 20 mL of a 10:1 mixture of THF/water at a flow rate of 2.0 mL/min, followed by water, acetonitrile and CH₂Cl₂. The output solution was filtered to remove the resin and the solvent was evaporated *in vacuo*. Crude was then analyzed by MALDI.

Cleavage from Solid Support (Method A-3): Sulfated Oligosaccharides

After automated synthesis, the resin was removed from the reaction vessel, suspended in a 9:1 mixture of CH₂Cl₂/MeOH (20 mL) and photocleaved in a continuous-flow photoreactor. A Vapourtec E-Series easy-MedCHem, equipped with a UV-150 Photochemical reactor having a UV-150 Medium-Pressure Mercury Lamp (arc length 27.9 cm, 450 W) surrounded by a long-pass UV filter (Pyrex, 50% transmittance at 305 nm) was used. A Pump 11 Elite Series (Harvard Apparatus syringe pump at a flow rate of 0.7 mL/min) was used to pump the mixture through a FEP tubing (i.d. 3.0 inch, volume: 12 mL) at 20 °C. An addition 20 mL of a 9:1 mixture of CH₂Cl₂/MeOH at a flow rate of 0.7 mL/min was then passed through the reactor. The reactor

was then washed with CH₂Cl₂ at rate of 2.0 mL/min. The output solution was filtered to remove the resin and the solvent was evaporated *in vacuo*. Crude was then analyzed by QToF (negative mode).

Deprotection of Oligosaccharides

AGA-synthesized and photocleaved product was subjected to methanolysis and hydrogenolysis. The hydrogenolysis product was purified by RP-HPLC and lyophilized on a Christ Alpha 2-4 LD plus freeze dryer to afford the final deprotected compound.

- **Methanolysis (Method C):** To a solution of protected oligosaccharide in MeOH:CH₂Cl₂ (2 mL, 1:1), sodium methoxide (0.5 M solution in MeOH, 2.2 equiv. per ester group) was added. The mixture was stirred at room temperature for 2 h. Then Amberlite IR-120 (H⁺ form) was added to quench. After neutralization, the reaction mixture was filtered and the solvent was removed *in vacuo*. The crude compound was used for hydrogenolysis without further purification.
- **Hydrogenolysis (Method D):** The crude compound obtained after methanolysis was dissolved in 4 mL of EtOAc:*t*-BuOH:H₂O (2:1:1). Pd/C (10%) was added to the solution and the suspension was stirred in a H₂ bomb with 60 psi pressure over night. The insoluble material was removed by a CHROMAFIL ®Xtra, RC 0.45 syringe filter. The solid was washed once with *t*-BuOH and several times with water. The filtrate was collected and concentrated *in vacuo*.

3.5 Analytical NP/RP-HPLC and purification

Analytical NP-HPLC of Crude Material (Method B-1)

Analytical NP-HPLC was conducted on an Agilent 1200 Series system. A YMC-Diol-300-NP column (150 mm x 4.60 mm I.D.) was used with a flow rate of 1.00 mL/min and hexane/EtOAc as eluent (20% EtOAc in hexane for 5 min, 20 → 100% EtOAc in hexane over 35 min, 100% EtOAc for 10 min).

Analytical NP-HPLC of Crude Material (Method B-2)

Analytical NP-HPLC was conducted on an Agilent 1200 Series system. A YMC-Diol-300-NP column (150 mm x 4.60 mm I.D.) was used at a flow rate of 1.00 mL/min with hexane/EtOAc as eluent (20% EtOAc in hexane for 5 min, 20 → 55% EtOAc in hexane over 35 min, 55 → 100% EtOAc in hexane over 35 min, 100% EtOAc for 10 min).

Preparative NP-HPLC of Crude Material (Method B-3)

Analytical NP-HPLC was conducted on an Agilent 1200 Series system. A YMC-Diol-300-NP column (150 mm x 20 mm I.D.) was used at a flow rate of 15.00 mL/min with hexane/EtOAc as eluent (20% EtOAc in hexane for 5 min, 20 → 55% EtOAc in hexane over 35 min, 100% EtOAc for 10 min).

Preparative NP-HPLC of Crude Material (Method B-4)

Analytical NP-HPLC was conducted on an Agilent 1200 Series system. A YMC-Diol-300-NP column (150 mm x 20 mm I.D.) was used at a flow rate of 15.00 mL/min with hexane/EtOAc as eluent (20% EtOAc in hexane for 5 min, 20 → 100% EtOAc in hexane over 35 min, 100% EtOAc for 10 min).

Analytical/preparative RP-HPLC of Crude Material (Method E-1)

Crude products were dissolved in water and analyzed/purified using analytical/preparative HPLC. A Thermo-Scientific Hypercarb column (150 mm x 4.60 mm I.D.) was used for analytical RP-HPLC with a flow rate of 0.70 mL/min with water (0.1% HCO₂H)/acetonitrile as eluents (100% H₂O (0.1% HCO₂H) for 5 min, 0 → 30% acetonitrile in H₂O (0.1% HCO₂H) over 30 min, 30 → 100% acetonitrile in H₂O (0.1% HCO₂H) over 5 min, 100% acetonitrile for 5 min).

Analytical/preparative RP-HPLC of Crude Material (Method E-2)

Crude products were dissolved in water and analyzed/purified using analytical/preparative HPLC. A Thermo-Scientific Hypercarb column (150 mm x 4.60 mm I.D.) was used for analytical RP-HPLC with a flow rate of 0.70 mL/min with H₂O (0.1% HCO₂H)/acetonitrile as eluents (100% H₂O (0.1% HCO₂H) for 5 min, 0 → 10% acetonitrile in H₂O (0.1% HCO₂H) over 30 min, 10 → 100% acetonitrile in H₂O (0.1% HCO₂H) over 5 min, 100% acetonitrile for 5 min).

Analytical RP-HPLC of Crude Material (Method E-3)

Crude product was dissolved in 50% water/acetonitrile and analyzed using a Synergi column (250 mm x 4.60 mm I.D.) for analytical RP-HPLC with a flow rate of 1.0 mL/min with aqueous 0.01 M NH_4HCO_3 /acetonitrile as eluents (95% 0.01 M NH_4HCO_3 for 5 min, 5 → 100% acetonitrile over 30 min, 100% acetonitrile for 5 min).

4 Microwave-assisted Automated Glycan Assembly System (DTRR System)

4.1 Dual Temperature Regulation Reaction System

A microwave reactor Discover from CEM accommodates the reaction vessel. A jacket surrounding the reaction vessel provides constant cooling to the lowest target temperature during the synthesis (up to $-40\text{ }^\circ\text{C}$). The cooling jacket is in fluid communication with a Unistat 705w chiller from Huber that circulates a microwave transparent coolant working at a constant temperature. We adjust any higher temperature during the cycle (up to $100\text{ }^\circ\text{C}$) by microwave irradiation. The reagent temperature is continuously monitored with an optic fiber probe in the reaction vessel. While the maximum microwave power irradiated depends on the reagents, and it is dynamically adjusted. The solvents, donor, and activator solutions are cooled to $-8\text{ }^\circ\text{C}$ before reach the reaction vessel. The cooling power, in this case, is provided by Peltier Elements supported with circulating cooling water at $15\text{ }^\circ\text{C}$, in aluminum constructions thermally isolate.

4.2 Reagents storage

The reagents are separately loaded under argon in glass vessels categorized into solvents, donors, activators, capping, and deprotection/functionalization. Three 2.5 L bottles supply CH_2Cl_2 , DMF and THF for washing, one 2.5 L bottle containing DCE as driving liquid for the syringe pump. Up to eight tubes (capacity = 10 mL) can accommodate the building block solutions. Two 100 mL vessels are available for capping reagents. There are positions for three activator reagents (100 mL or optional 250 mL each), with one container placed in an ice bath to preserve sensitive reagents (e.g. NIS/TfOH). Simultaneously, up to four reagents are loaded for deprotection and/or functionalization. Each group of reagents has a separate pressurized inert atmosphere. A tailor-made Swagelok manifold provides argon (grade = 5.0) throughout the system.

4.3 Delivery Systems

A Kloehn V6 syringe pump with 0.05 mL precision delivers the building block and activator solutions from their reservoirs through the top of the reaction vessel. Both reagents travel through separate lines via Kloehn rotary valves, a 12-way (glycosyl donor building block) and a 6-way (activators) valve. A buffering volume line between the pump and the reservoirs prevents the reagents from mixing. A third top inlet is connected to a Bio-Chem 4-way solenoid valve which provides the solvents for washing and the gas for draining the reactor vessel. A top outlet vents the exhaust gas. The bottom inlet/outlet connects to a Bio-Chem 8-way valve. This valve serves to drain the liquid, provides gas for bubbling/mixing, and delivery post-coupling reagents (deprotections, capping, or post glycan synthesis modification reagent solutions). The washing solvents and bottom supplied reagents are gas driven by differential pressure. The vent gases and drained liquid go to a waste container and are controlled by solenoid valves (Biochem). Alternatively, the drained solutions could be collected for analysis or recovery of the unreacted components. The tubing is made of PTEF and the wetted surface on the valves is Teflon.

4.4 Automated Control System

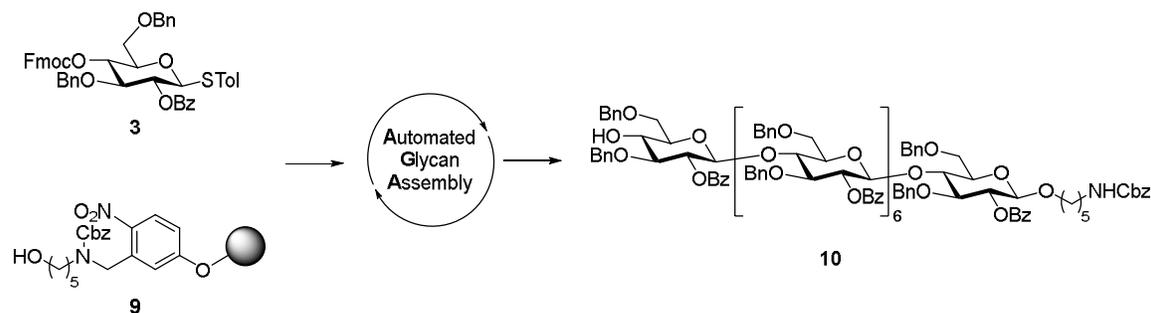
A computer centralizes the control of the system by a National Instruments PCI-6519 Digital I/O Device connected to the delivery and temperature regulation system. The entire device has a modular construction. All components are accessible and replaceable. The capabilities are expandable by adding elements or reorganizing the pathway of the fluids.

In a single working environment, the software allows for the creation and storage of operational modules by listing a series of ground-level commands (on/off of the devices, and setting parameters). The modules execute generic process tasks such as system initialization, reactions, and the standby operation. The user builds a synthesis program by compiling modules. The settings of each module are adjustable. Once saved the synthesis program can be loaded and run. The temperature at the reaction vessel and the chiller is monitored and registered. A Voltcraft energy check 3000 reads online the power consumption of the chiller.

5 Automated Glycan Assembly of Oligosaccharides

5.1 Glucose Octamer 10

N-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,6-di-*O*-benzyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3,6-di-*O*-benzyl- β -D-glucopyranoside (**10**)



Repeat	Building Blocks	Modules	Notes
4x	3 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-20 °C (T ₁) 8 min (t ₁) -5 °C (T ₂) 8 min (t ₂)
		III – Capping (Conc Capping) IVa – Fmoc Deprotection	
4x	3 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-20 °C (T ₁) 8 min (t ₁) -5 °C (T ₂) 10 min (t ₂)
		III – Capping (Conc Capping) IVa – Fmoc Deprotection	

Protected **10** was obtained after photocleavage from solid support following **Method A-1**. The crude residue was then purified by normal phase flash chromatography (SiO₂, Hex/EtOAc) or preparative HPLC (**Method B-4**) to afford compound **10** (21 mg, 0.0055 mmol, 41%) as a colorless oil.

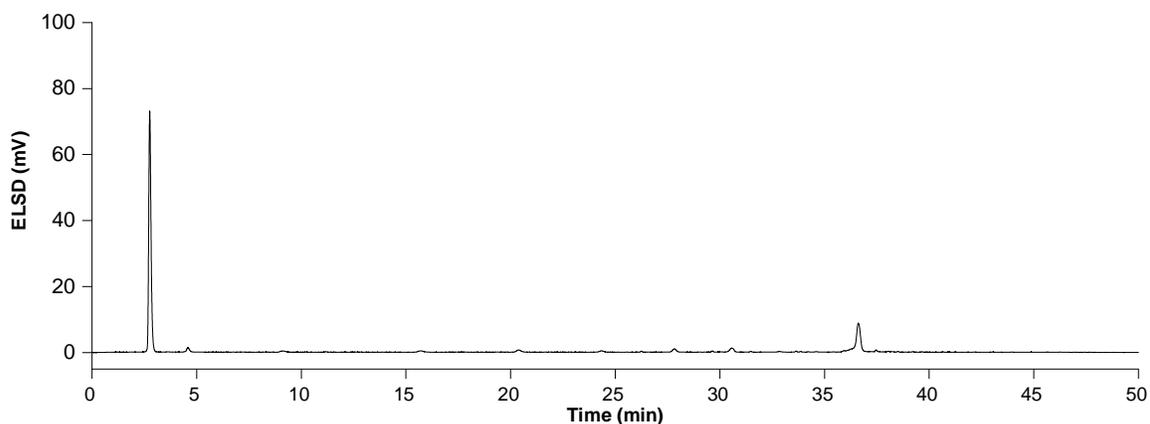
¹H NMR (700 MHz, CDCl₃) δ 7.90 (d, *J* = 7.0 Hz, 2H), 7.87 (d, *J* = 7.4 Hz, 2H), 7.85 – 7.79 (m, 12H), 7.63 – 7.52 (m, 7H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.45 – 7.36 (m, 15H), 7.38 – 7.29 (m, 13H), 7.23 (d, *J* = 7.0 Hz, 2H), 7.18 – 6.84 (m, 54H), 6.99 – 6.84 (m, 19H), 5.23 (dd, *J* = 9.5, 8.1 Hz, 1H), 5.17 (dd, *J* = 9.6, 8.1 Hz, 1H), 5.14 – 5.01 (m, 8H), 4.94 – 4.81 (m, 7H), 4.73 (d, *J* = 11.7

Hz, 1H), 4.67 (d, $J = 11.6$ Hz, 1H), 4.63 (d, $J = 8.1$ Hz, 1H), 4.59 – 4.45 (m, 10H), 4.44 – 4.33 (m, 8H), 4.31 – 4.21 (m, 6H), 4.14 (dd, $J = 18.7, 12.1$ Hz, 2H), 4.08 (t, $J = 9.3$ Hz, 1H), 4.04 – 3.86 (m, 11H), 3.82 – 3.77 (m, 1H), 3.74 – 3.67 (m, 1H), 3.62 – 3.55 (m, 2H), 3.54 – 3.18 (m, 24fH), 3.10 (d, $J = 9.5$ Hz, 1H), 3.04 (d, $J = 1.9$ Hz, 1H), 2.90 – 2.71 (m, 8H), 1.44 – 1.37 (m, 4H), 1.13 – 1.01 (m, 2H) ppm.

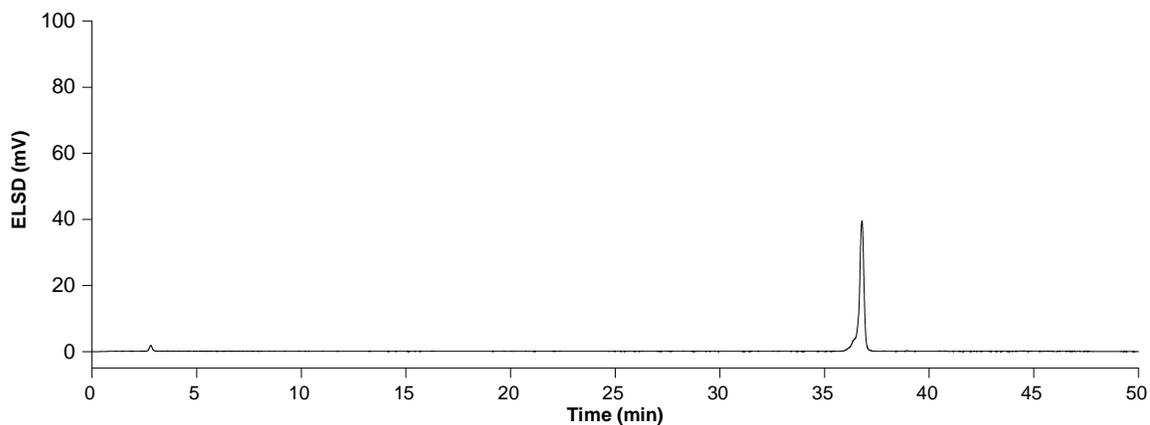
^{13}C NMR (176 MHz, CDCl_3): δ 165.2, 165.1, 164.9, 156.4, 139.0, 138.9, 138.8, 138.3, 138.3, 137.9, 137.8, 137.7, 136.9, 133.5, 133.3, 133.0, 129.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 101.3, 100.2, 100.2, 100.0, 82.0, 80.2, 80.1, 76.6, 76.3, 76.2, 76.0, 74.8, 74.7, 74.6, 74.5, 74.4, 74.3, 74.2, 73.9, 73.7, 73.6, 73.4, 73.2, 71.3, 69.5, 67.6, 67.5, 67.3, 66.6, 40.9, 32.1, 29.8, 29.4, 28.9, 23.2, 22.8, 14.3 ppm.

HRMS (QToF): Calcd for $\text{C}_{229}\text{H}_{227}\text{NO}_{51}\text{Na}_2$ [$\text{M} + 2\text{Na}$] $^{2+}$ 1926.2492; found 1926.2581.

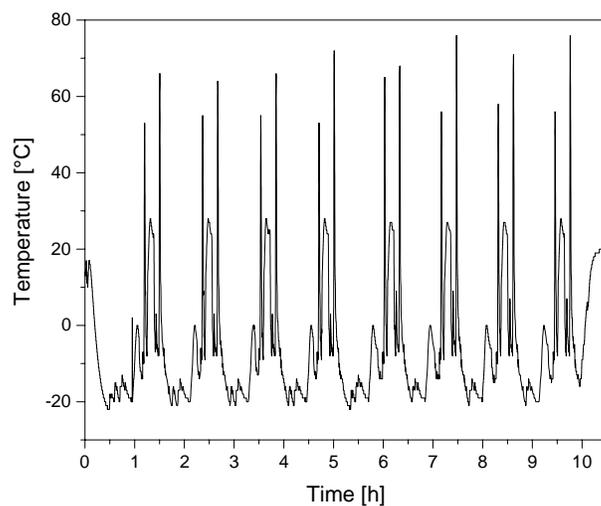
NP-HPLC of crude **10** (ELSD trace, **Method B-3**, $t_{\text{R}} = 36.6$ min):



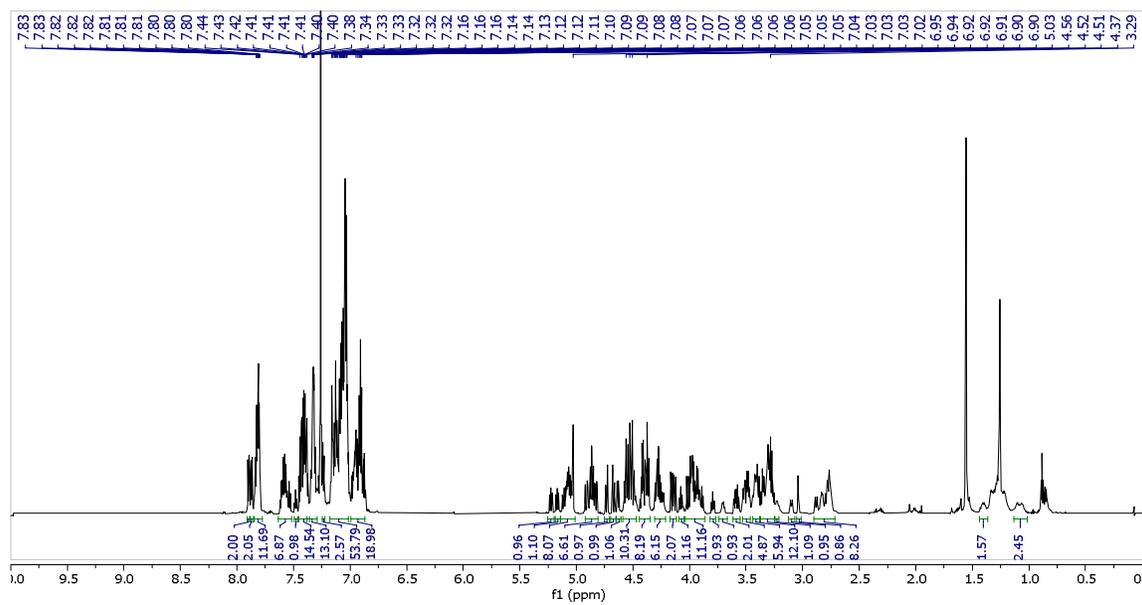
NP-HPLC of purified **10** (ELSD trace, **Method B-3**, $t_{\text{R}} = 36.7$ min):



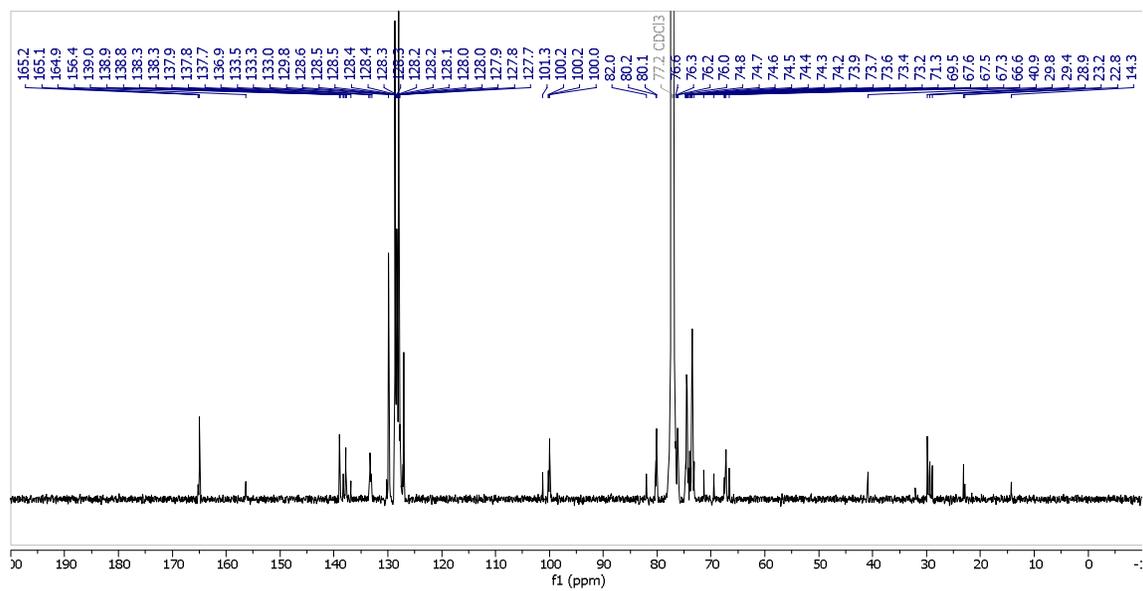
Temperature profile inside the reaction vessel during the synthesis of **10**:



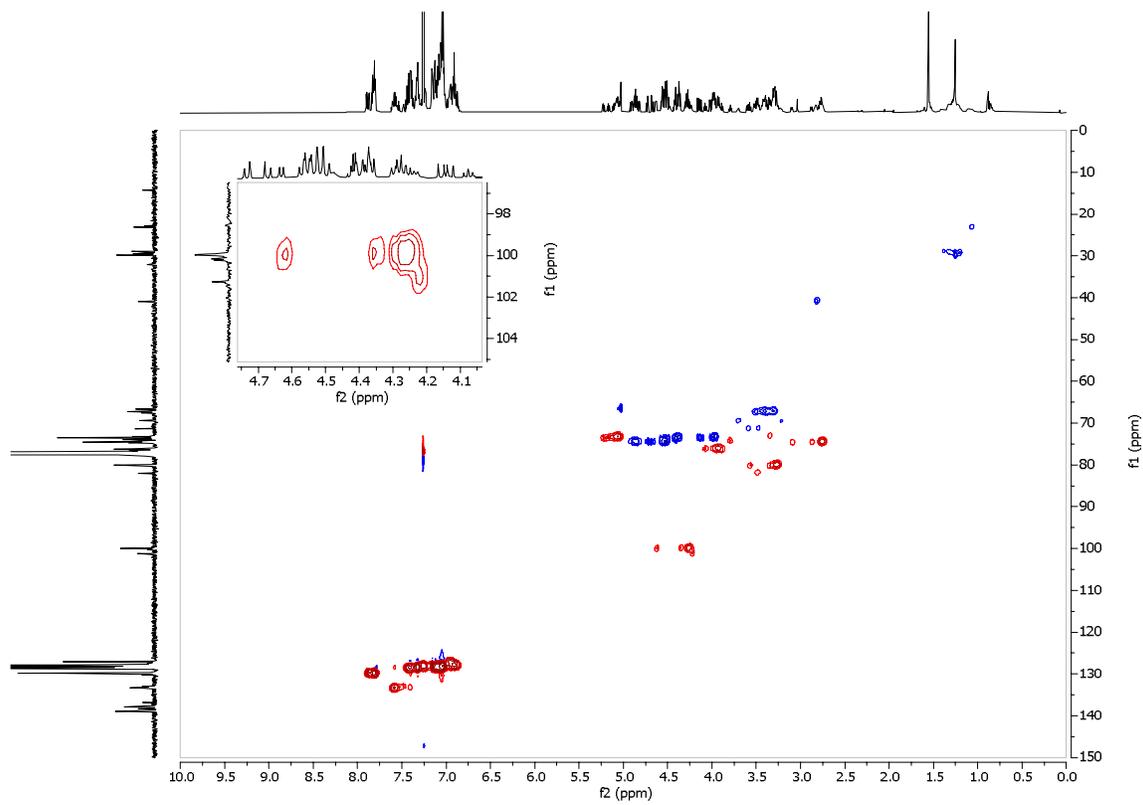
^1H NMR (700 MHz, CDCl_3) of **10**:



^{13}C NMR (176 MHz, CDCl_3) of **10**:

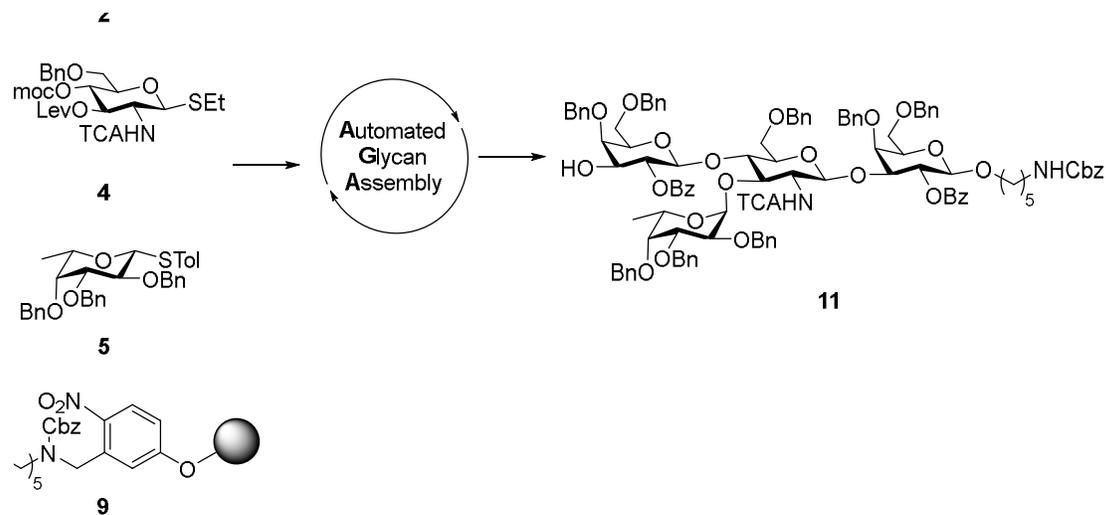


^{13}C , ^1H HSQC of **10**:



5.2 Lewis Antigen 11

N-Benzyloxycarbonyl-5-amino-pentyl 4,6-di-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-6-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl]-2-deoxy-2-*N*-trichloroacetyl- β -D-glucopyranosyl-(1 \rightarrow 3)-2-*O*-benzoyl-4,6-di-*O*-benzyl- β -D-galactopyranoside (**11**)



Repeat	Building Blocks	Modules	Notes
1x	2 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-30 °C (T ₁) 8 min (t ₁) -10 °C (T ₂) 10 min (t ₂)
		IVa – Fmoc Deprotection	
1x	4 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside (2 cycles)	-30 °C (T ₁) 10 min (t ₁) -5 °C (T ₂) 30 min (t ₂)
		IVb – Lev Deprotection	
1x	5 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-30 °C (T ₁) 8 min (t ₁) -5 °C (T ₂) 10 min (t ₂)
		IVa – Fmoc Deprotection	
1x	2 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-30 °C (T ₁) 8 min (t ₁) -5 °C (T ₂) 10 min (t ₂)
		IVa – Fmoc Deprotection	

Protected **11** was obtained after photocleavage from solid support following **Method A-1**. The crude residue was then purified by normal phase flash chromatography (SiO₂, Hex/EtOAc) or

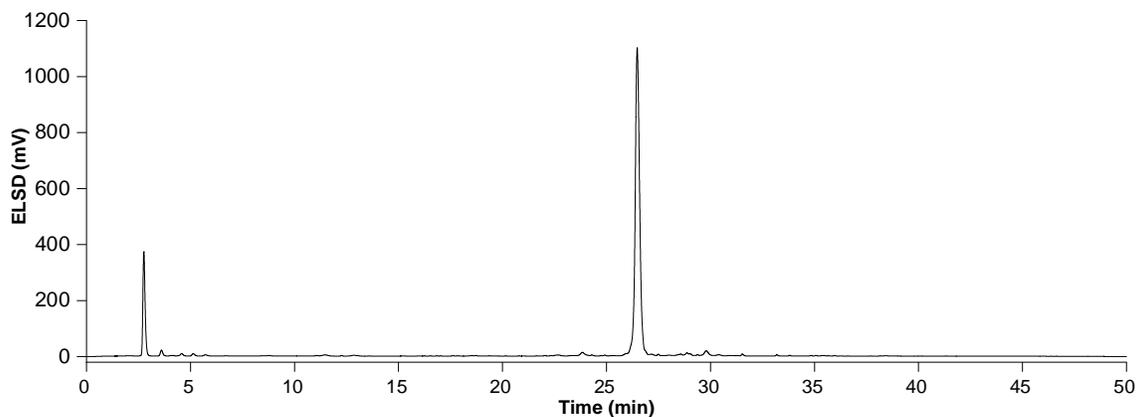
preparative HPLC (**Method B-4**) to afford compound **11** (14 mg, 0.0072 mmol, 53%) as a colorless oil.

¹H NMR (700 MHz, CDCl₃): δ 7.96 (d, *J* = 6.9 Hz, 2H), 7.93 (d, *J* = 7.0 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.49 – 7.43 (m, 3H), 7.37 – 7.16 (m, 46H), 6.50 (d, *J* = 8.5 Hz, 1H), 5.46 (dd, *J* = 10.2, 7.8 Hz, 1H), 5.14 (d, *J* = 3.8 Hz, 1H), 5.10 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.05 (s, 2H), 4.89 (d, *J* = 11.8 Hz, 1H), 4.76 – 4.61 (m, 8H), 4.58 – 4.52 (m, 5H), 4.46 (d, *J* = 6.5 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.39 – 4.30 (m, 6H), 4.25 (d, *J* = 11.5 Hz, 1H), 4.13 (t, *J* = 8.0 Hz, 1H), 4.00 (d, *J* = 2.9 Hz, 1H), 3.96 – 3.92 (m, 3H), 3.90 (dd, *J* = 10.3, 3.0 Hz, 2H), 3.83 – 3.78 (m, 3H), 3.69 – 3.59 (m, 4H), 3.55 – 3.52 (m, 2H), 3.48 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.45 – 3.41 (m, 1H), 3.39 (s, 1H), 3.30 – 3.24 (m, 2H), 2.83 (q, *J* = 6.7 Hz, 2H), 2.20 (d, *J* = 9.5 Hz, 1H), 1.36 – 1.17 (m, 6H), 1.13 (d, *J* = 6.5 Hz, 3H) ppm.

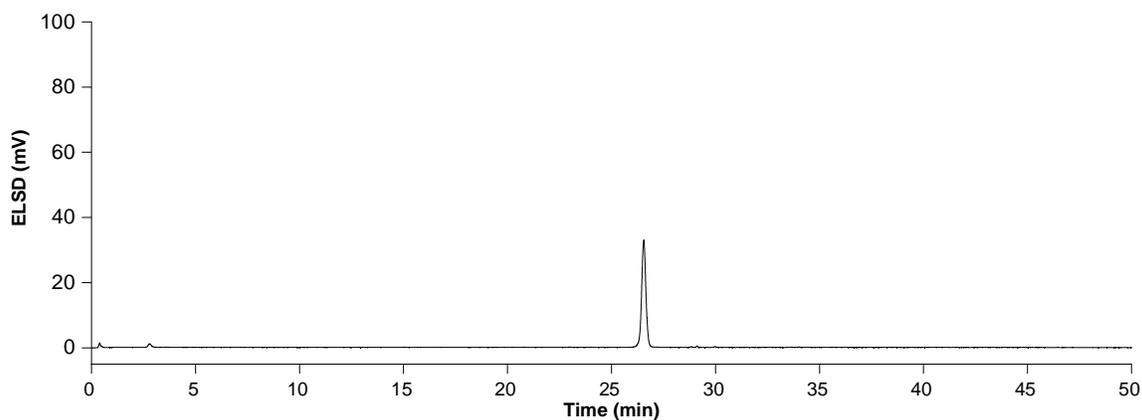
¹³C NMR (176 MHz, CDCl₃): δ 166.3, 165.2, 161.4, 156.4, 139.2, 139.1, 138.9, 138.6, 138.1, 138.1, 137.9, 137.7, 136.9, 133.5, 133.3, 130.2, 129.9, 129.8, 129.1, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.5, 127.1, 101.9, 100.5, 99.5, 97.3, 92.1, 79.7, 78.9, 78.6, 76.4, 76.3, 76.1, 75.8, 75.4, 75.1, 74.7, 74.2, 74.1, 74.0, 73.7, 73.7, 73.5, 73.5, 73.3, 73.2, 73.1, 73.0, 72.6, 69.4, 69.2, 68.2, 67.6, 66.7, 66.6, 40.9, 29.9, 29.5, 29.0, 23.2, 16.6 ppm.

HRMS (QToF): Calcd for C₁₀₉H₁₁₅Cl₃N₂O₂₄Na [M + Na]⁺ 1963.6797; found 1963.6837.

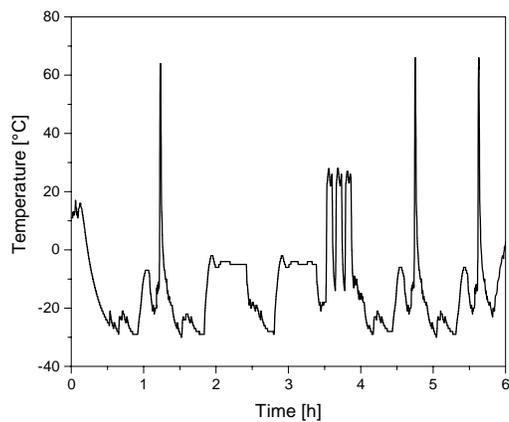
NP-HPLC of crude **11** (ELSD trace, **Method B-3**, *t_R* = 26.5 min):



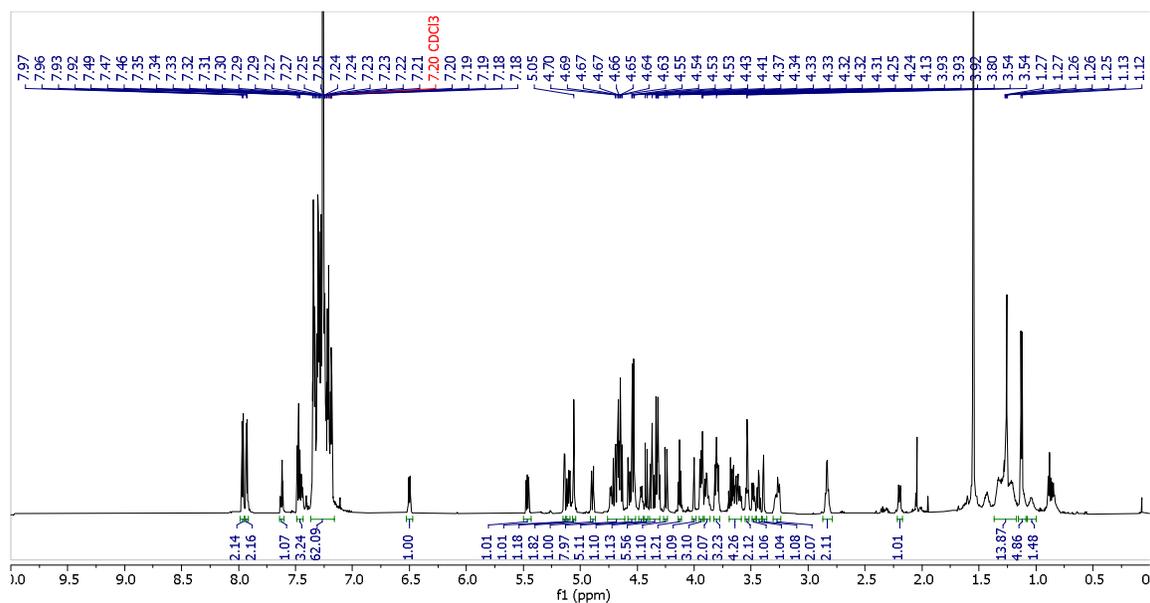
NP-HPLC of purified **11** (ELSD trace, **Method B-3**, $t_R = 26.5$ min):



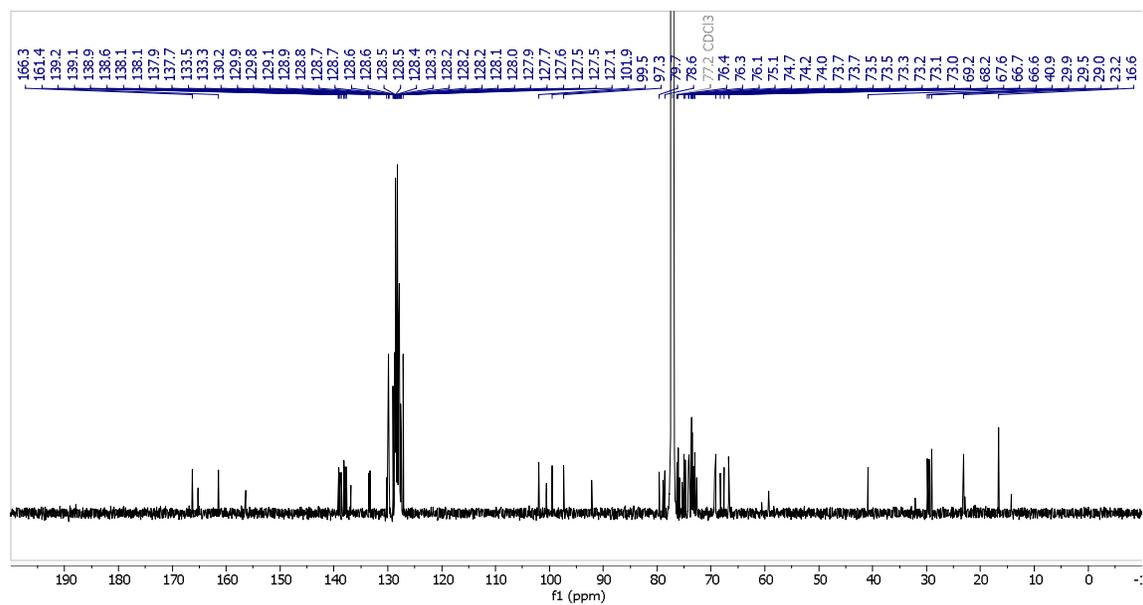
Temperature profile inside the reaction vessel during the synthesis of **11**:



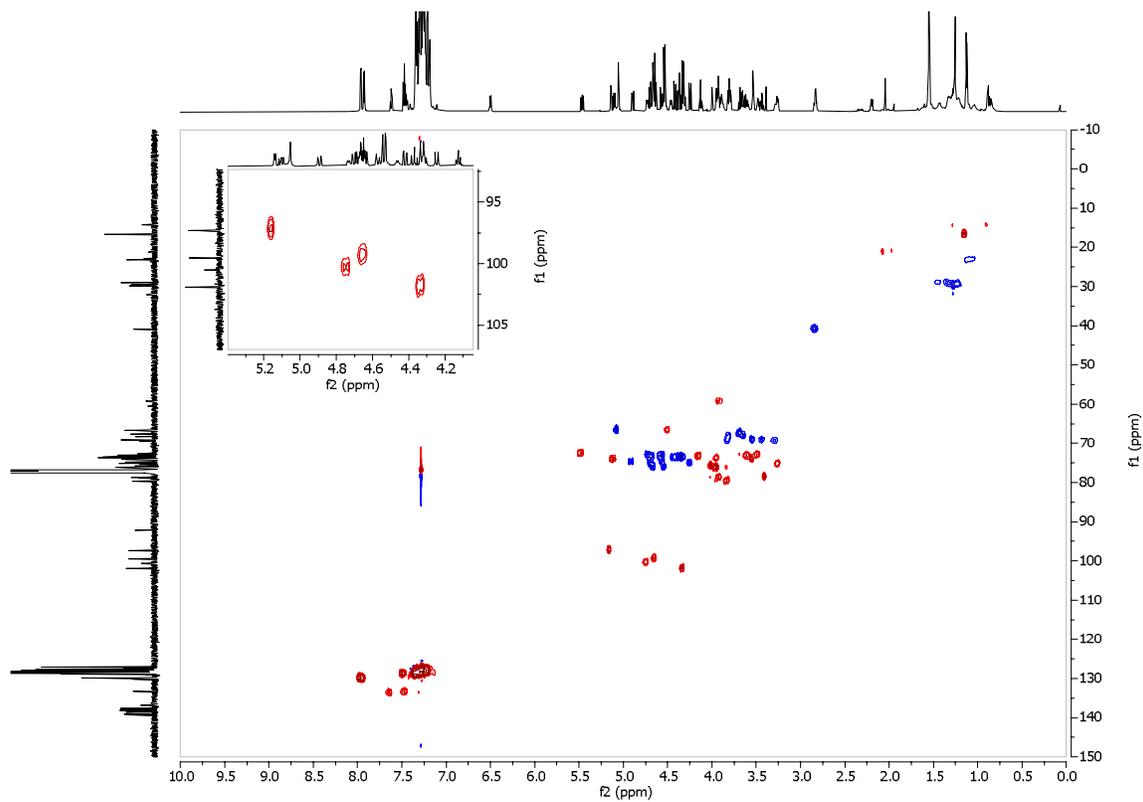
^1H NMR (700 MHz, CDCl_3) of **11**:



^{13}C NMR (176 MHz, CDCl_3) of 11:

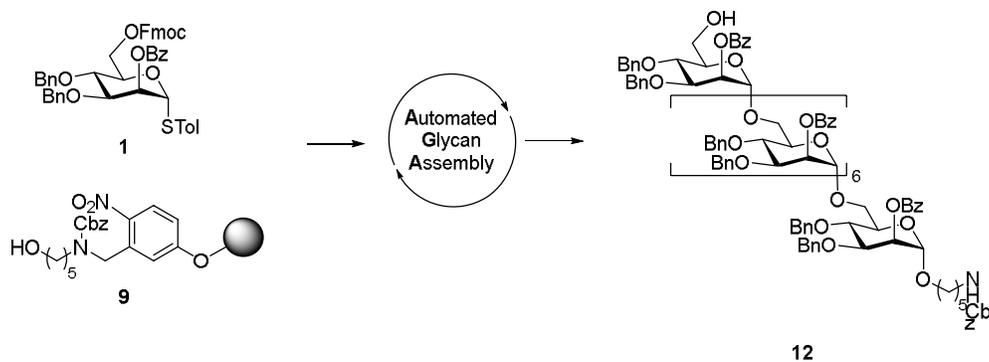


$^{13}\text{C}, ^1\text{H}$ HSQC of 11:



5.3 Mannose Octamer 12

N-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,4-di-*O*-benzyl- α -D-mannopyranosyl-(1 \rightarrow 6)-2-*O*-benzoyl-3,4-di-*O*-benzyl- α -D-mannopyranoside (**12**)



Repeat	Building Blocks	Modules	Notes
4x	1 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-20 °C (T ₁) 8 min (t ₁) 0 °C (T ₂) 5 min (t ₂)
		III – Capping IVa – Fmoc Deprotection	
4x	1 (6.5 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with thioglycoside	-20 °C (T ₁) 8 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVa – Fmoc Deprotection	

Protected **12** was obtained after photocleavage from solid support following **Method A-1**. The crude residue was then purified by normal phase flash chromatography (SiO₂, Hex/EtOAc) or preparative HPLC (**Method B-4**) to afford compound **12** (23 mg, 0.006 mmol, 45%) as a colorless oil.

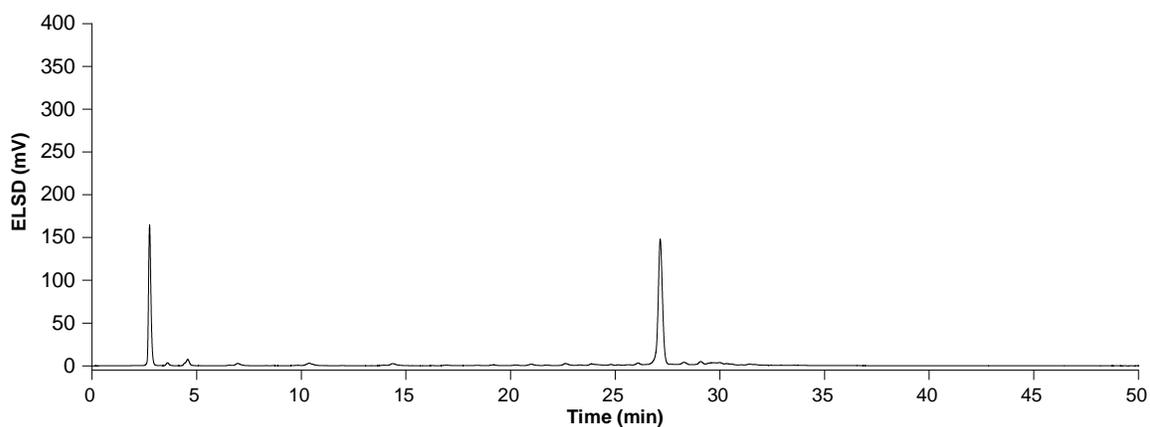
¹H NMR (700 MHz, CDCl₃): δ 8.18 (t, *J* = 8.4 Hz, 12H), 8.13 (d, *J* = 8.0 Hz, 2H), 8.10 (d, *J* = 8.2 Hz, 2H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.55 – 7.46 (m, 23H), 7.37 – 7.29 (m, 12H), 7.27 – 7.05 (m, 72H), 5.87 – 5.80 (m, 6H), 5.79 (s, 1H), 5.64 (s, 1H), 5.13 – 5.03 (m, 9H), 4.94 – 4.77 (m, 18H), 4.73 (d, *J* = 11.3 Hz, 1H), 4.61 (d, *J* = 11.1 Hz, 1H), 4.57 (d, *J* = 11.0 Hz, 1H), 4.49 (dd, *J* = 11.3, 5.6 Hz, 2H), 4.44 (dd, *J* = 15.1, 11.0 Hz, 7H), 4.39 – 4.33 (m, 5H), 4.12 – 4.08 (m,

2H), 4.07 – 4.02 (m, 6H), 4.01 – 3.83 (m, 12H), 3.82 – 3.71 (m, 7H), 3.71 – 3.57 (m, 9H), 3.56 – 3.39 (m, 7H), 3.19 (d, $J = 8.0$ Hz, 2H), 1.52 (m, 4H), 1.37 (m, 2H) ppm.

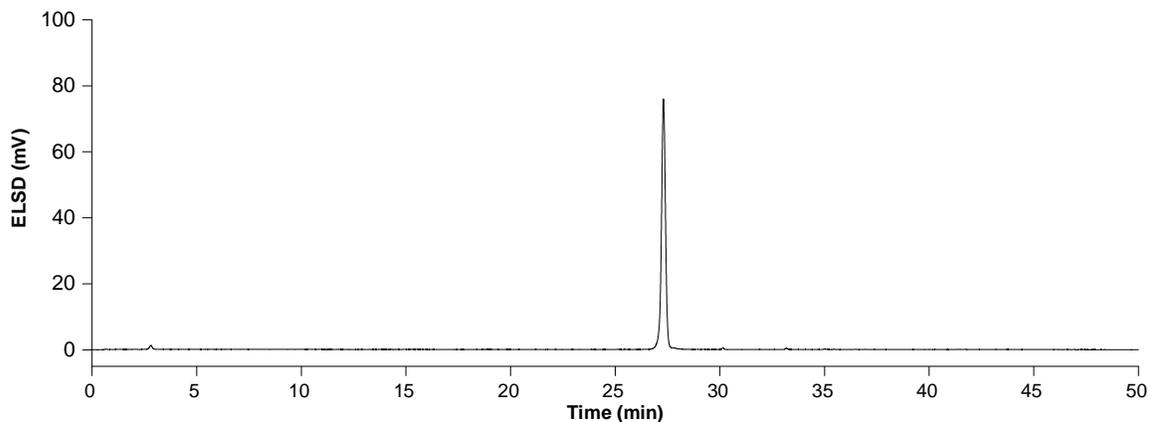
^{13}C NMR (101 MHz, CDCl_3): δ 166.0, 165.7, 165.6, 165.5, 156.5, 138.6, 138.6, 138.6, 138.5, 138.4, 138.4, 138.0, 137.7, 137.6, 137.6, 136.8, 133.5, 130.0, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.8, 127.8, 127.8, 127.7, 127.5, 127.3, 127.2, 98.6, 98.5, 98.2, 97.6, 78.7, 78.3, 77.8, 77.4, 75.3, 75.1, 74.3, 74.0, 73.8, 72.2, 71.7, 71.5, 71.4, 71.3, 71.1, 71.0, 70.8, 69.1, 68.6, 68.4, 67.9, 66.7, 66.2, 65.8, 61.9, 41.1, 29.9, 29.2, 23.5 ppm.

HRMS (QToF): Calcd for $\text{C}_{229}\text{H}_{227}\text{NO}_{51}\text{Na}_2$ [$\text{M} + 2\text{Na}$] $^{2+}$ 1926.2492; found 1926.2581.

NP-HPLC of crude **12** (ELSD trace, **Method B-3**, $t_{\text{R}} = 27.0$ min):

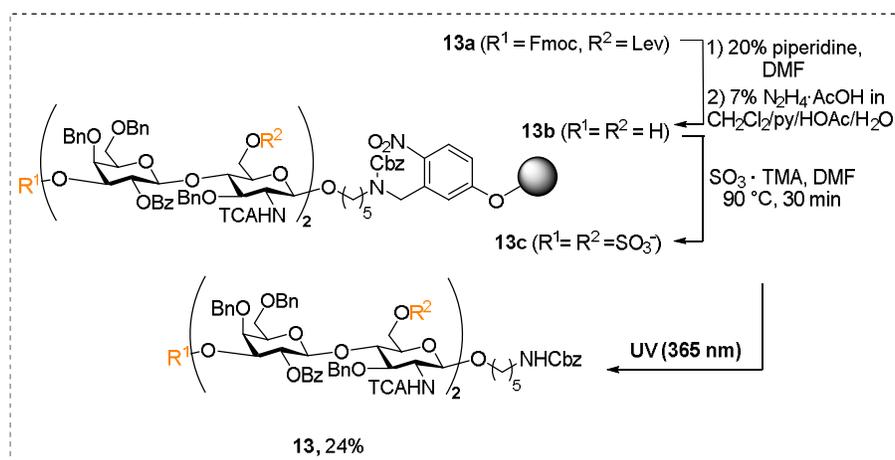
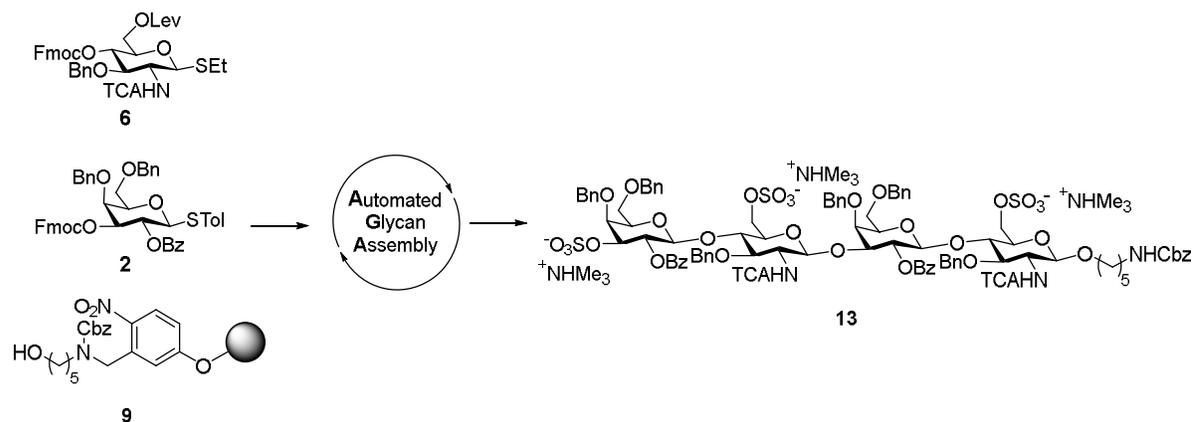


NP-HPLC of purified **12** (ELSD trace, **Method B-3**, $t_{\text{R}} = 27.1$ min):



5.4 Keratan Sulfate 13

N-Benzyloxycarbonyl-5-amino-pentyl 4,6-di-*O*-benzyl-3-*O*-sulfo- β -D-galactopyranosyl-(1 \rightarrow 4)-6-*O*-sulfo-3-*O*-benzyl-2-deoxy-2-*N*-trichloroacetyl- β -D-glucopyranosyl-(1 \rightarrow 3)-4,6-di-*O*-benzyl-3- β -D-galactopyranosyl-(1 \rightarrow 4)-6-*O*-sulfo-3-*O*-benzyl-2-deoxy-2-*N*-trichloroacetyl- β -D-glucopyranoside Trrimethylammonium Salt (13)



Repeat	Building Blocks	Modules	Notes
2x		I – Acidic Wash	
	6 (6.5 equiv.)	IIb – Glycosylation with thioglycoside (2 cycles)	-30 °C (T_1) 10 min (t_1) -5 °C (T_2) 30 min (t_2)
		III – Capping IVa – Fmoc Deprotection	
	2 (6.5 equiv.)	I – Acidic Wash IIb – Glycosylation with thioglycoside (2 cycles)	-30 °C (T_1) 8 min (t_1) -5 °C (T_2) 10 min (t_2)
		IVa – Fmoc Deprotection	
1x		IVb – Lev Deprotection	
		V – Sulfation	

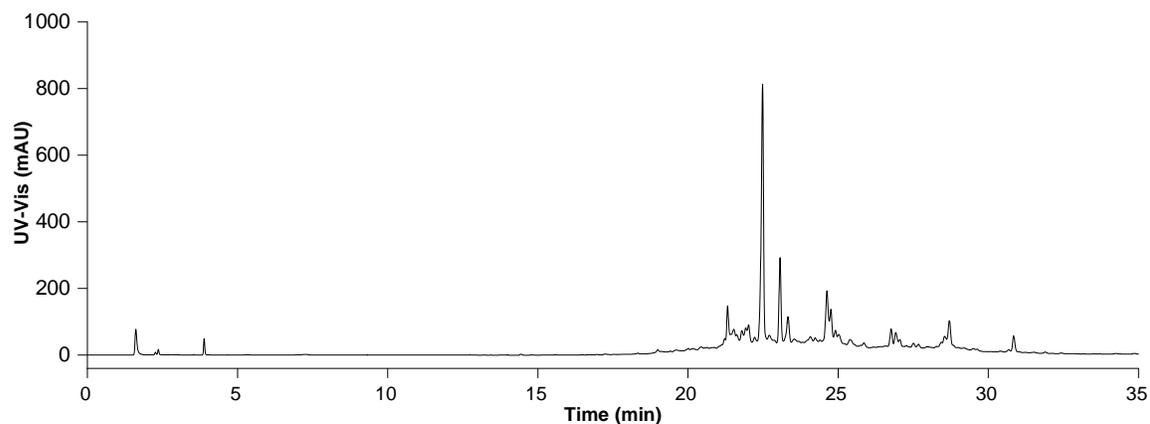
Protected **13** was obtained after photocleavage from solid support following **Method A-3**. The crude residue was then purified by reverse phase flash chromatography (C-18 silica gel, water/acetonitrile) to afford compound **13** (7 mg, 0.0072 mmol, 24%) as a tan oil upon concentration. Note: Fractions were analyzed by mass spectrometry (negative mode) for purity before combination.

¹H NMR (700 MHz, MeOD): δ 8.23 (d, J = 6.9 Hz, 2H), 8.13 (d, J = 6.8 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.58 (t, J = 7.3 Hz, 1H), 7.54 (t, J = 7.8 Hz, 2H), 7.50 – 7.44 (m, 4H), 7.42 – 7.37 (m, 2H), 7.34 – 7.16 (m, 35H), 7.14 (t, J = 7.1 Hz, 2H), 7.10 (dt, J = 9.0, 7.3 Hz, 3H), 7.02 (dt, J = 14.9, 7.6 Hz, 4H), 5.56 (dd, J = 10.2, 7.9 Hz, 1H), 5.49 (dd, J = 10.2, 7.9 Hz, 1H), 5.14 (d, J = 9.8 Hz, 2H), 5.09 (d, J = 11.4 Hz, 1H), 5.05 – 5.01 (m, 4H), 4.90 (d, J = 8.0 Hz, 1H), 4.80 (dd, J = 10.2, 3.1 Hz, 1H), 4.68 (d, J = 8.3 Hz, 1H), 4.59 (dd, J = 21.4, 11.4 Hz, 2H), 4.48 (dd, J = 16.7, 10.0 Hz, 2H), 4.42 – 4.35 (m, 3H), 4.31 – 4.18 (m, 4H), 4.13 – 4.01 (m, 3H), 3.92 – 3.84 (m, 3H), 3.78 (dd, J = 7.1, 4.9 Hz, 1H), 3.73 – 3.66 (m, 3H), 3.61 (dd, J = 10.4, 8.6 Hz, 1H), 3.54 (dd, J = 10.6, 4.5 Hz, 1H), 3.47 – 3.38 (m, 3H), 3.35 (dd, J = 6.2, 3.6 Hz, 1H), 3.06 – 3.02 (m, 2H), 2.82 (s, 27H), 1.45 (d, J = 16.9 Hz, 4H), 1.35 – 1.25 (m, 2H) ppm.

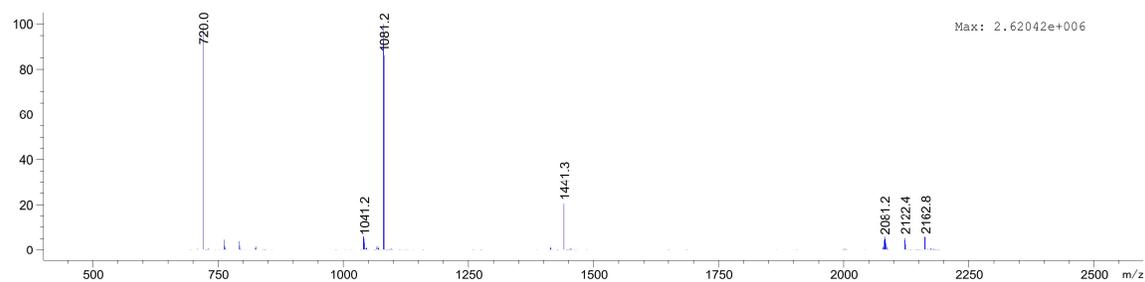
¹³C NMR (176 MHz, MeOD): δ 167.4, 167.1, 164.2, 158.9, 140.8, 140.6, 140.1, 140.0, 139.7, 138.5, 134.4, 131.6, 131.3, 131.1, 129.3, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 103.2, 102.0, 101.8, 101.4, 94.2, 93.7, 81.2, 80.8, 79.7, 79.2, 76.9, 76.7, 76.4, 76.3, 76.0, 75.6, 74.8, 74.7, 74.5, 74.3, 73.7, 72.9, 70.8, 70.4, 69.6, 67.3, 66.4, 66.1, 58.3, 58.0, 57.6, 57.5, 57.4, 45.6, 41.7, 30.5, 30.2, 24.3, 17.4, 17.3, 17.2 ppm.

HRMS (QTOF): Calcd for C₉₇H₁₀₀Cl₆N₃O₃₄S₃ [M]³⁻ 718.7833; found 718.7853.

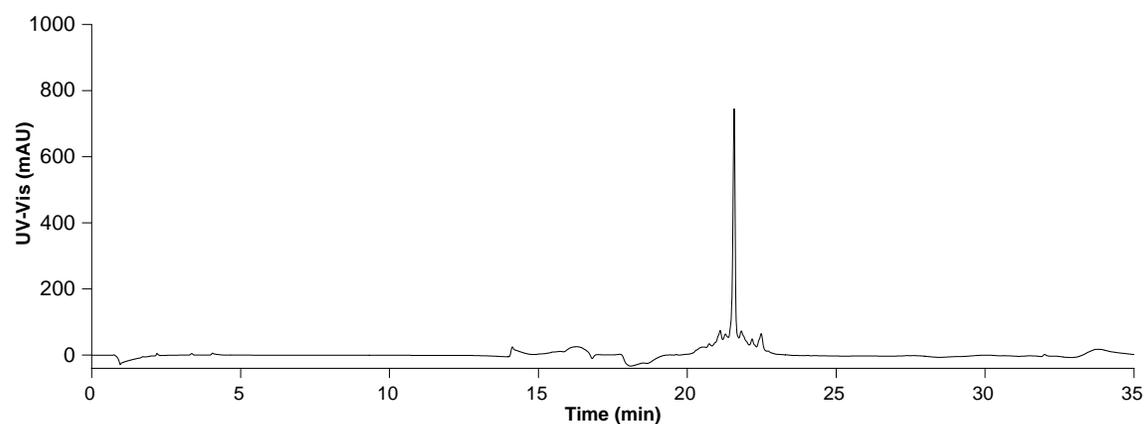
RP-HPLC of crude **13** (UV-Vis trace (230 nm), **Method E-3**, t_R = 22.5 min):



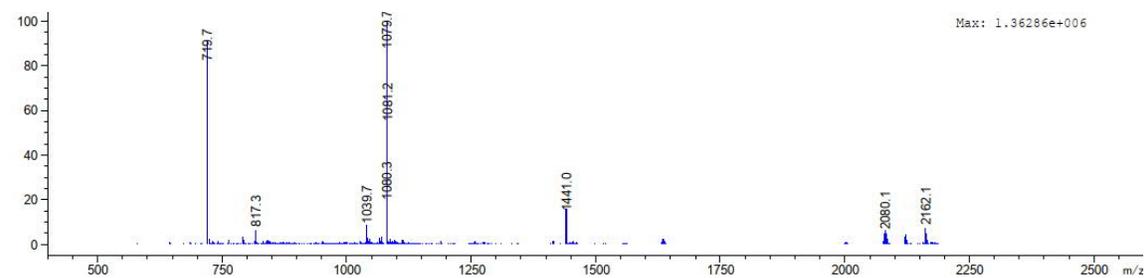
Mass spectrum at 22.5 min:



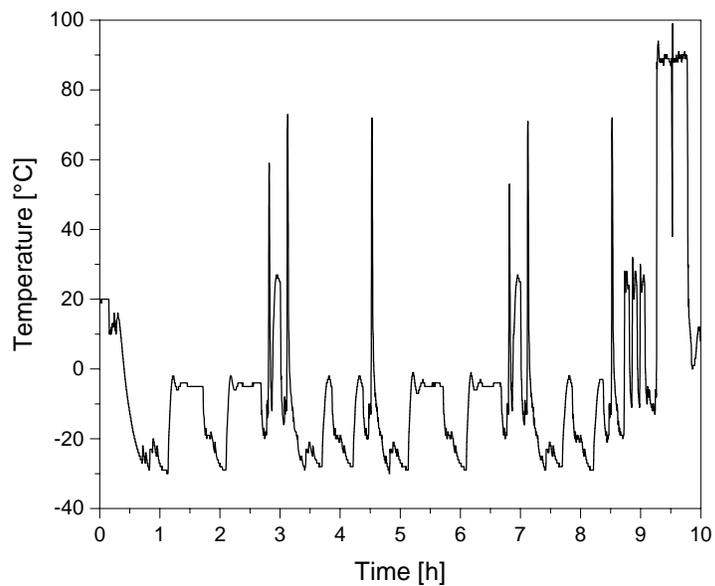
RP-HPLC of purified **13** (UV-Vis trace (230 nm), **Method E-3**, $t_R = 21.6$ min):



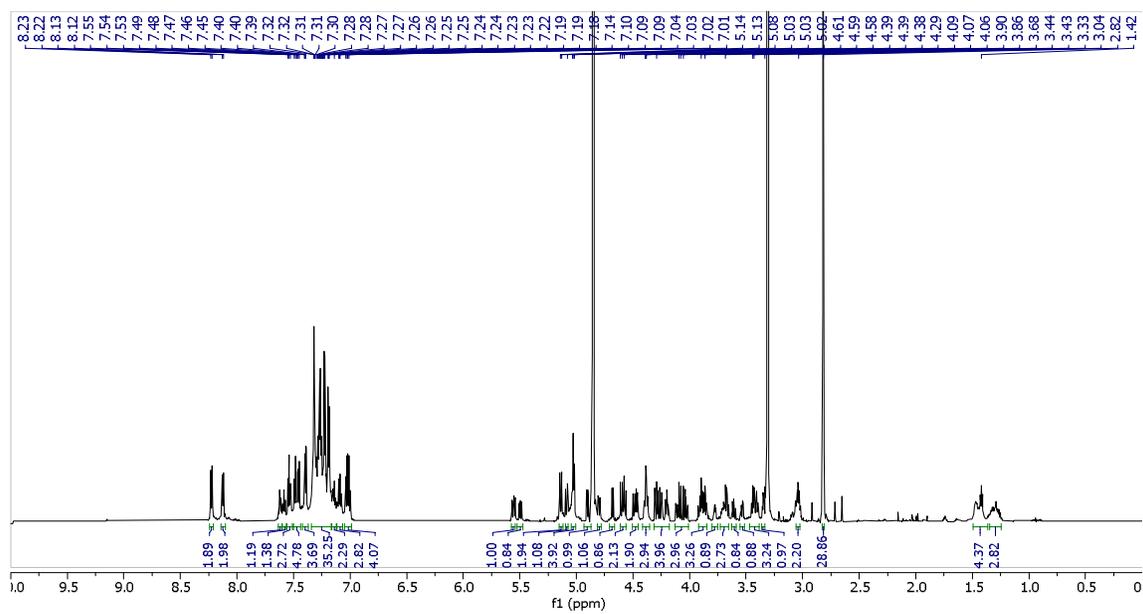
Mass spectrum at 21.6 min



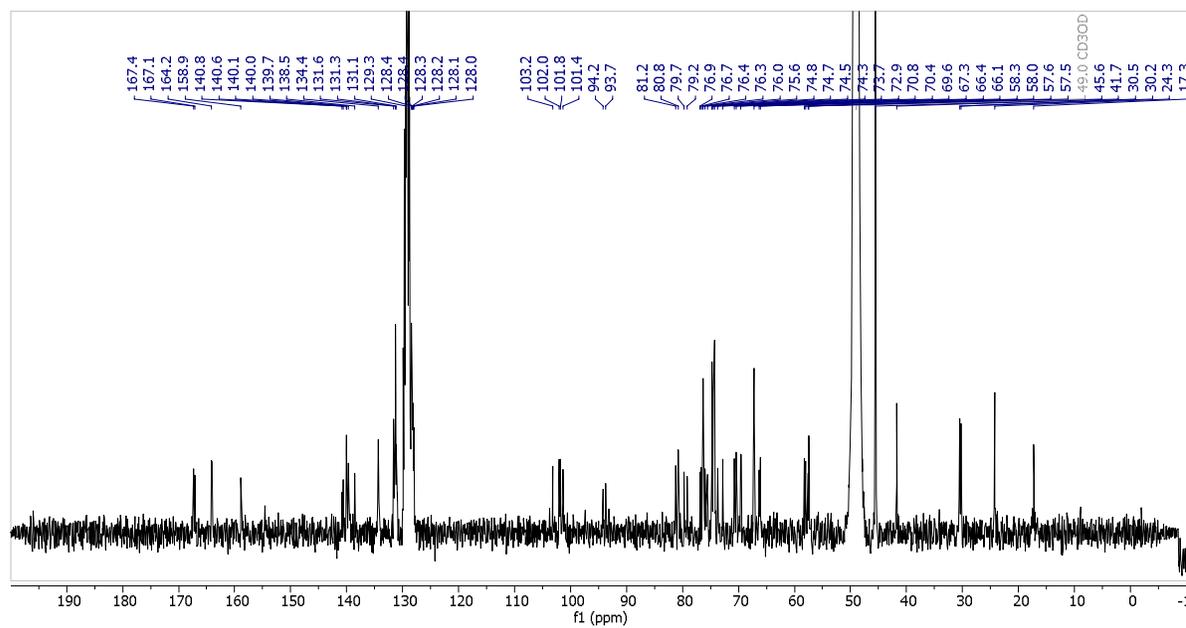
Temperature profile inside the reaction vessel during the synthesis of **13**:



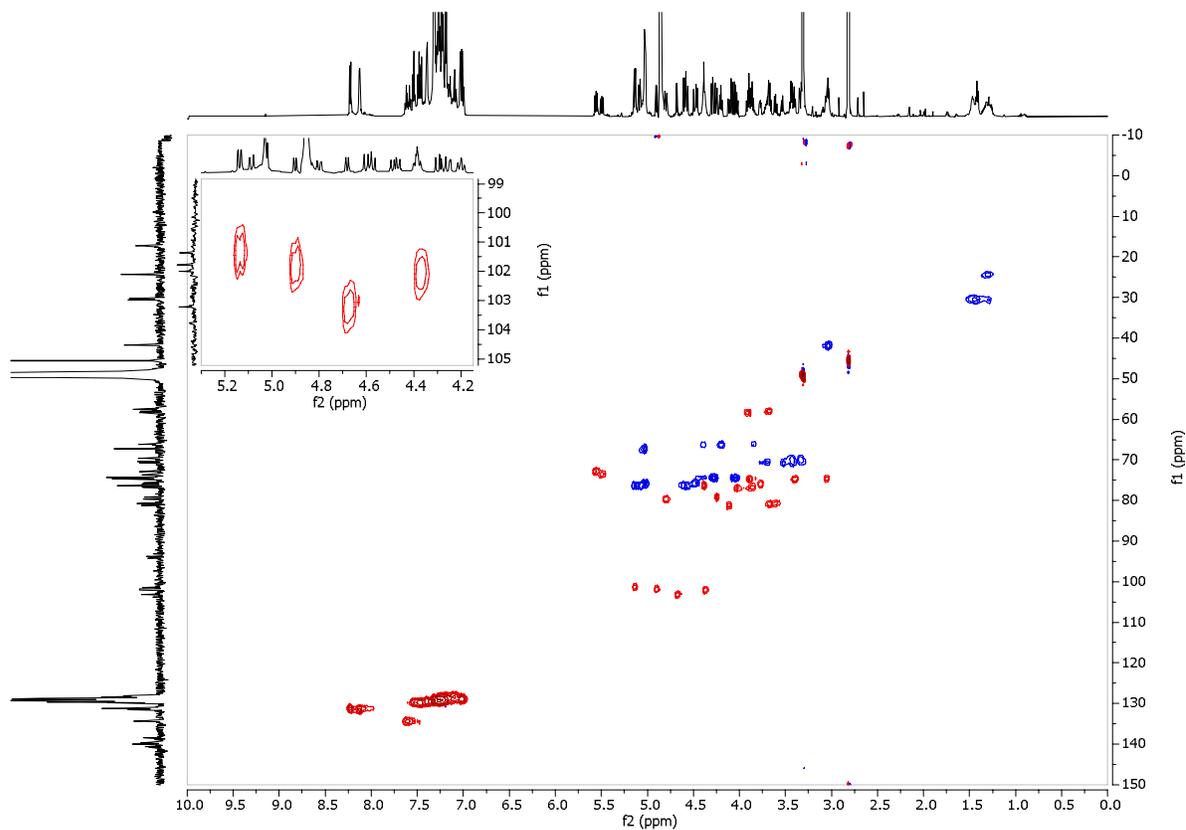
^1H NMR (700 MHz, CDCl_3) of **13**:



^{13}C NMR (176 MHz, CDCl_3) of **13**:



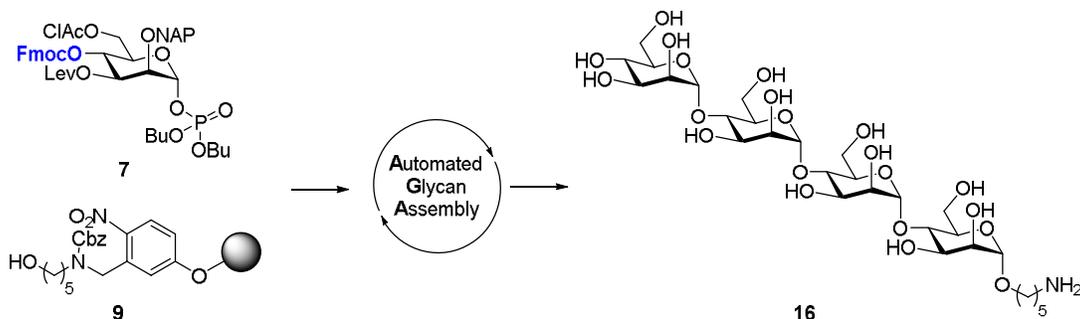
$^{13}\text{C}, ^1\text{H}$ HSQC of **13**:



5.5 Automated Glycan Assembly Using an Orthogonal Building Block

5.5.1 Linear Mannose Tetramers (14-17)

5-Amino-pentyl α -(1 \rightarrow 4)-D-tetramannopyranoside (**16**)



Repeat	Building Blocks	Modules	Notes
1x	7 (2 x 4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate – 2 cycles	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVc – Fmoc Deprotection	
3x	7 (4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVc – Fmoc Deprotection	

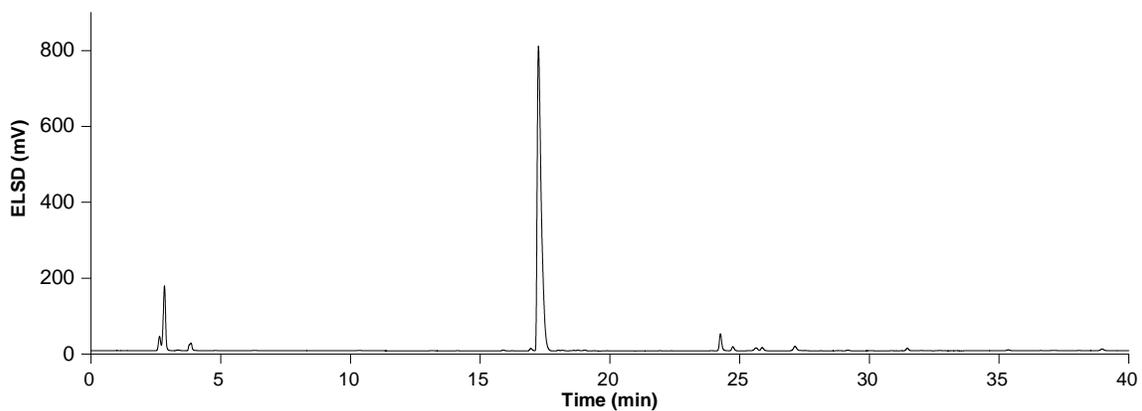
Protected **16** (15 mg, 0.007 mmol, crude yield: 52%) was obtained as a colorless oil after photocleavage from solid support following **Method A-1**. Deprotection of **16** following **Method C** and **D** and purification by reverse-phase HPLC (**Method E-1**, $t_R = 17.3$ min) afforded deprotected compound **16** (1.8 mg, 0.002 mmol, 18%) as a white solid after lyophilization.

¹H NMR (700 MHz, D₂O): δ 5.28 – 5.24 (m, 3H), 4.88 (d, $J = 1.8$ Hz, 1H), 4.07 (dd, $J = 3.3, 1.9$ Hz, 1H), 4.03 (dd, $J = 3.4, 1.9$ Hz, 2H), 3.99 – 3.66 (m, 22H), 3.57 (dt, $J = 10.0, 6.2$ Hz, 1H), 3.04 – 3.00 (m, 2H), 1.75 – 1.62 (m, 4H), 1.52 – 1.41 (m, 2H) ppm.

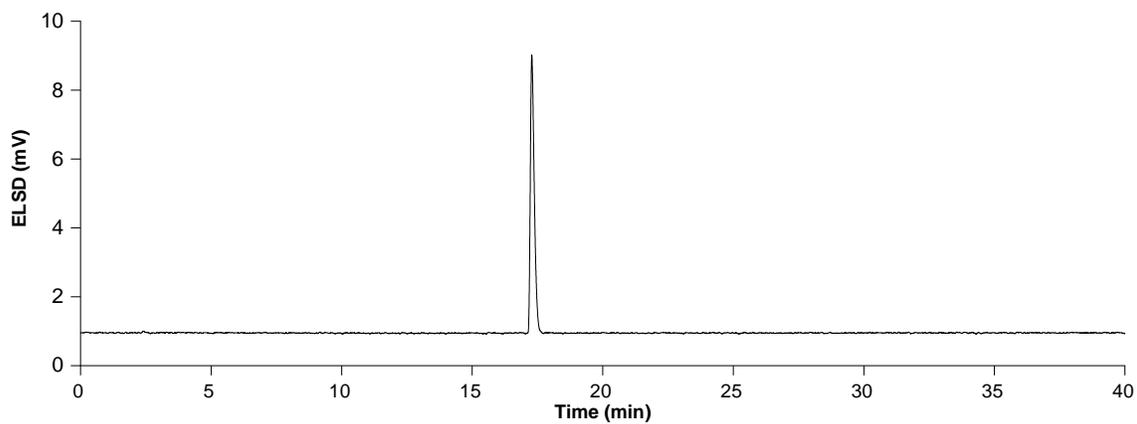
¹³C NMR (176 MHz, D₂O): δ 101.4, 101.3, 99.6, 74.3, 74.1, 74.1, 73.7, 72.3, 72.2, 71.2, 71.1, 70.8, 70.8, 70.7, 70.7, 70.6, 70.4, 70.3, 67.6, 66.5, 61.0, 61.0, 60.9, 39.4, 28.0, 26.8, 22.4 ppm.

HRMS (QToF): Calcd for C₂₉H₅₃NO₂₁ [M + H]⁺ 752.3183; found 752.3205.

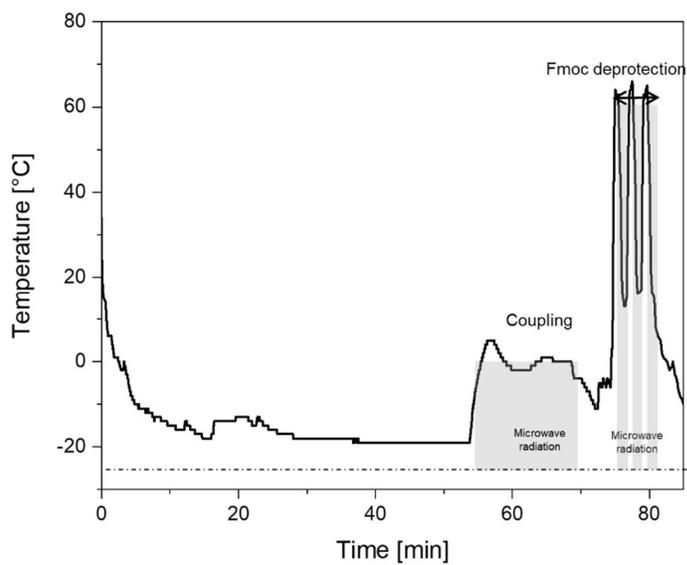
RP-HPLC of crude deprotected **16** (ELSD trace, **Method E-1**, $t_R = 17.3$ min):



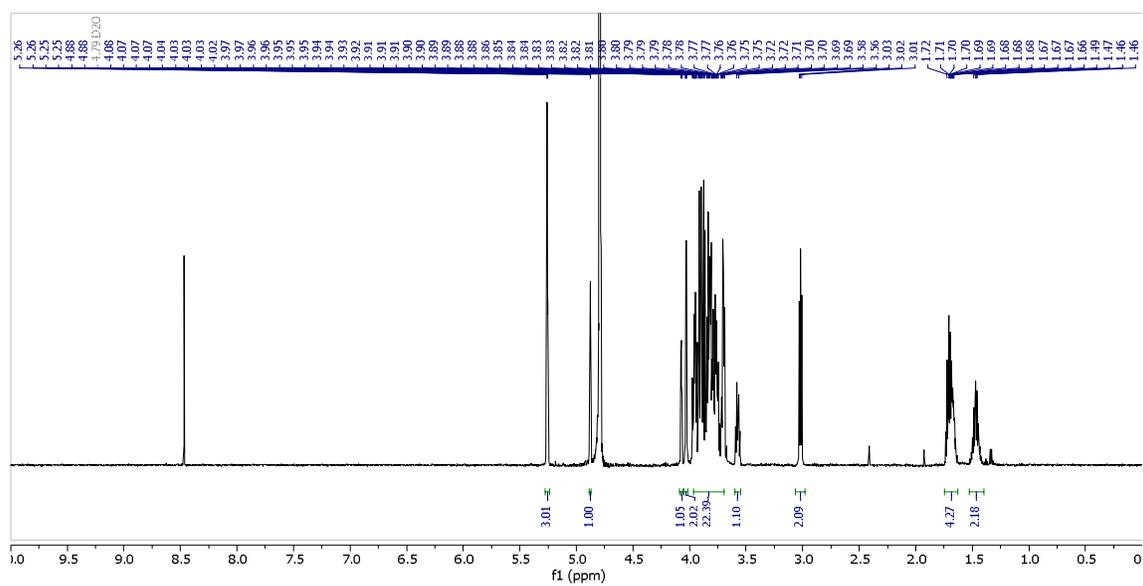
RP-HPLC of purified deprotected **16** (ELSD trace, **Method E-1**, $t_R = 17.3$ min):



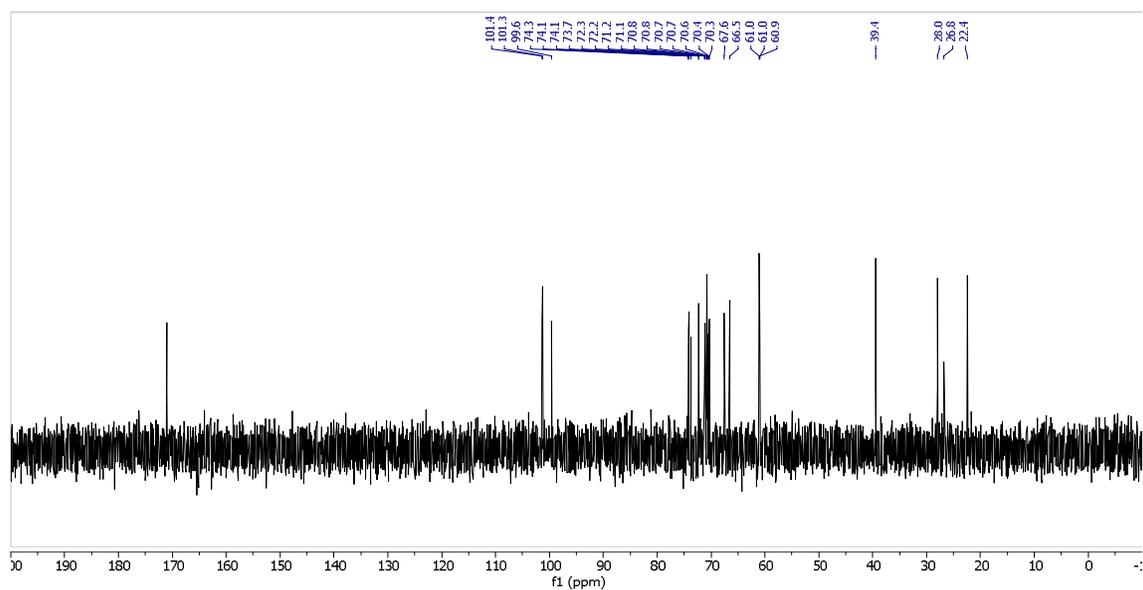
Temperature profile inside the reaction vessel during one synthesis cycle of **16**:



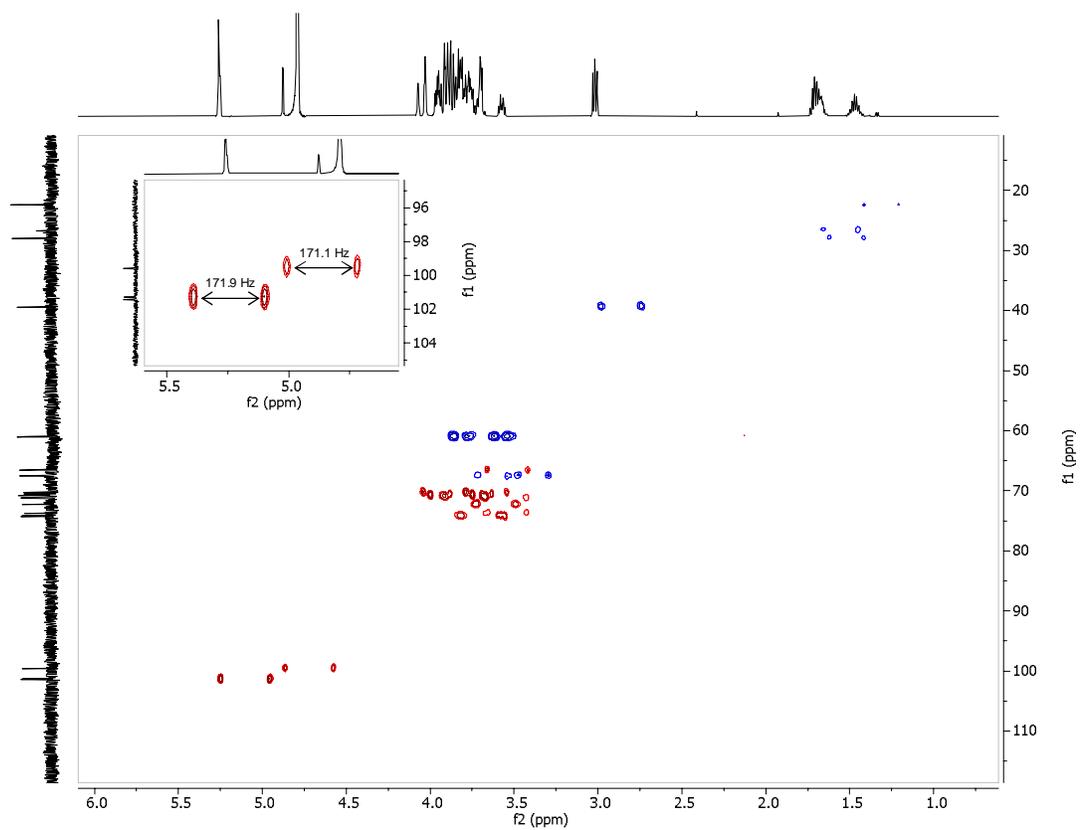
¹H NMR (700 MHz, D₂O) of **16**:



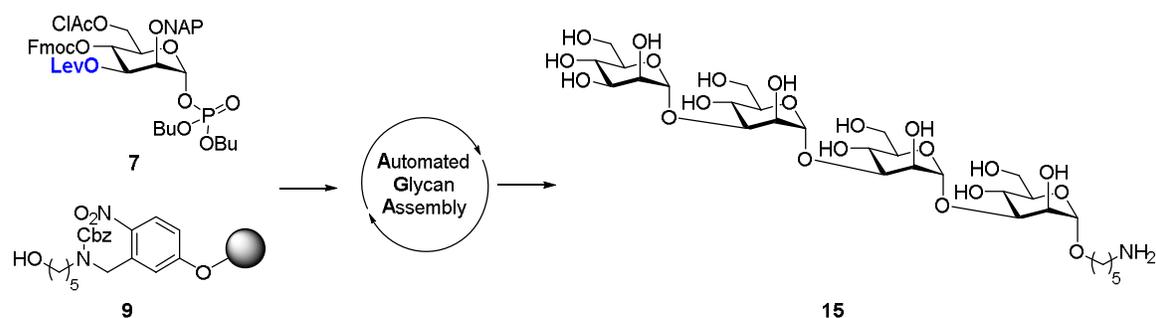
¹³C NMR (176 MHz, D₂O) of **16**:



Coupled ^{13}C , ^1H HSQC of **16**:



5-Amino-pentyl α -(1 \rightarrow 3)-D-tetramannopyranoside (**15**)



Repeat	Building Blocks	Modules	Notes
1x	7 (2 x 4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate – 2 cycles	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVb – Lev Deprotection	
3x	7 (4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVb – Lev Deprotection	

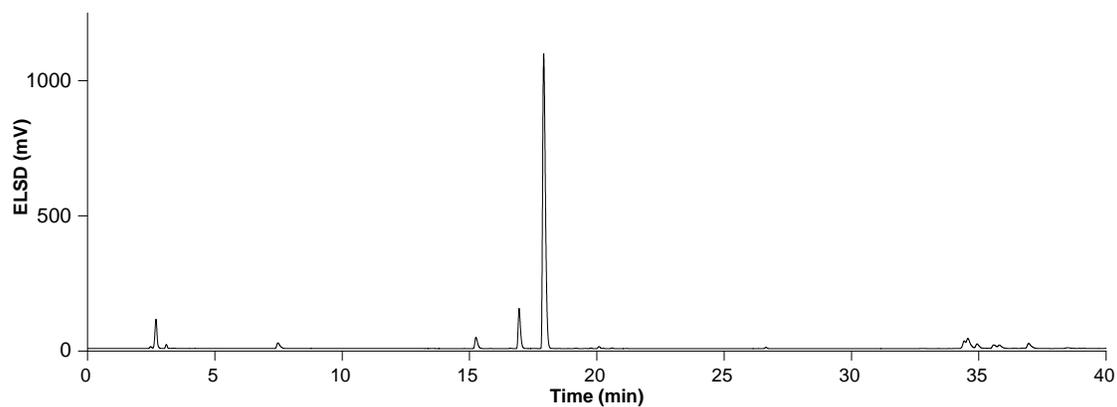
Protected **15** (26 mg, 0.010 mmol, crude yield: 74%) was obtained as a colorless oil after photocleavage from solid support following **Method A-1**. Deprotection of **15** following **Method C** and **D** and purification by reverse-phase HPLC (**Method E-1**, $t_R = 18.0$ min) afforded deprotected compound **15** (1.2 mg, 0.002 mmol, 12%) as a white solid after lyophilization.

¹H NMR (700 MHz, D₂O): δ 5.15 (d, $J = 1.8$ Hz, 1H), 5.13 (d, $J = 1.7$ Hz, 1H), 5.12 (d, $J = 1.9$ Hz, 1H), 4.86 (d, $J = 1.4$ Hz, 1H), 4.26 – 4.23 (m, 1H), 4.12 – 4.07 (m, 2H), 4.06 – 4.00 (m, 2H), 3.96 – 3.73 (m, 12H), 3.71 – 3.62 (m, 2H), 3.57 (dt, $J = 9.4, 5.9$ Hz, 1H), 3.02 (t, $J = 7.6$ Hz, 2H), 1.75 – 1.62 (m, 4H), 1.54 – 1.40 (m, 2H) ppm.

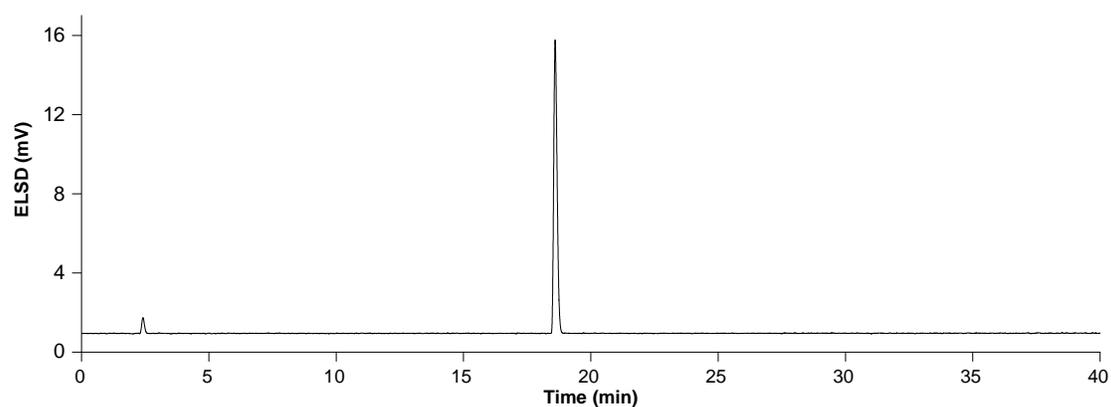
¹³C NMR (176 MHz, D₂O): δ 102.3, 102.2, 102.2, 99.6, 78.3, 78.1, 78.1, 73.6, 73.5, 73.4, 72.9, 70.4, 70.0, 69.7, 69.7, 67.5, 66.9, 66.2, 66.1, 66.1, 61.1, 61.0, 61.0, 60.9, 39.4, 28.0, 26.6, 22.5 ppm.

HRMS (QToF): Calcd for C₂₉H₅₃NO₂₁ [M + H]⁺ 752.3183; found 752.3218.

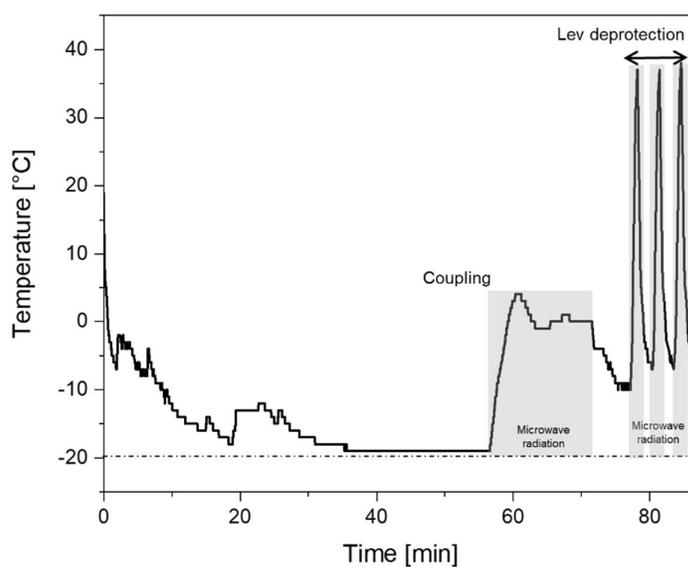
RP-HPLC of crude deprotected **15** (ELSD trace, **Method E-1**, $t_R = 18.0$ min):



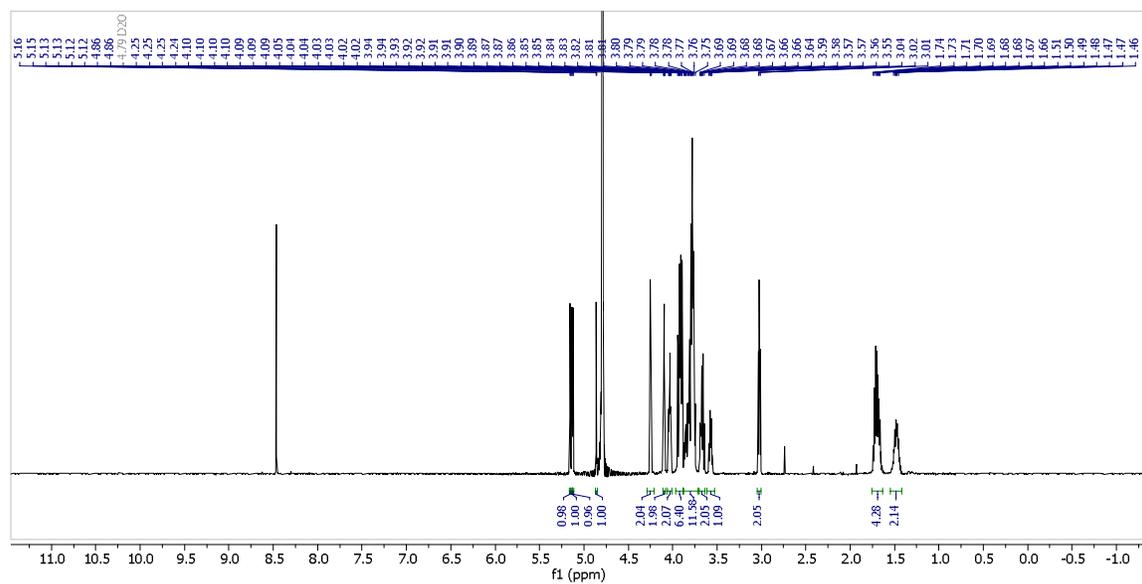
RP-HPLC of purified deprotected **15** (ELSD trace, **Method E-1**, $t_R = 18.0$ min):



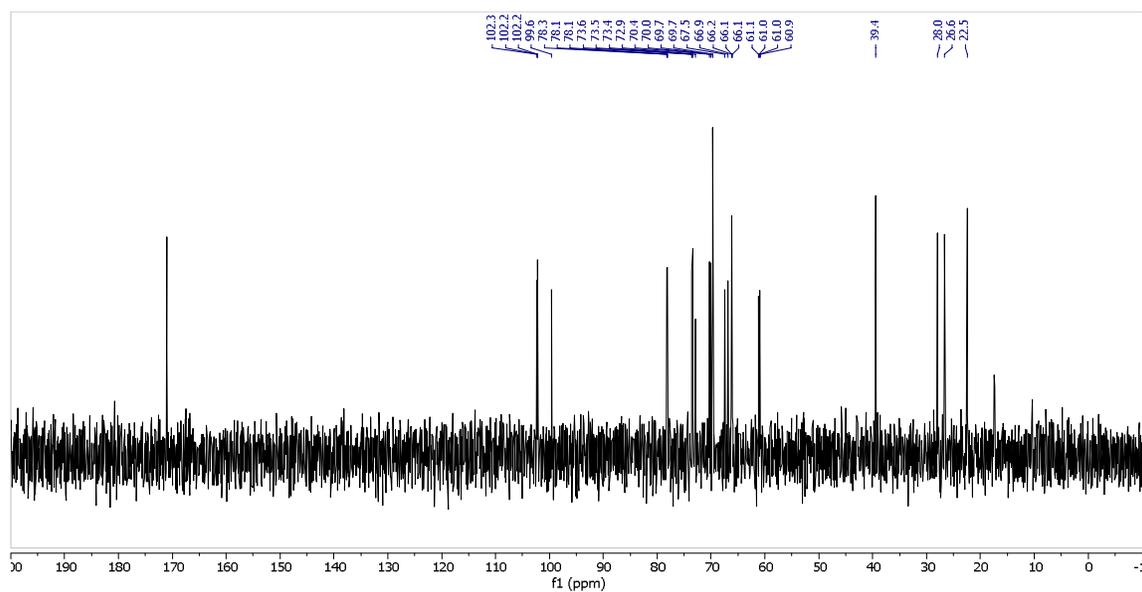
Temperature profile inside the reaction vessel during one synthesis cycle of **15**:



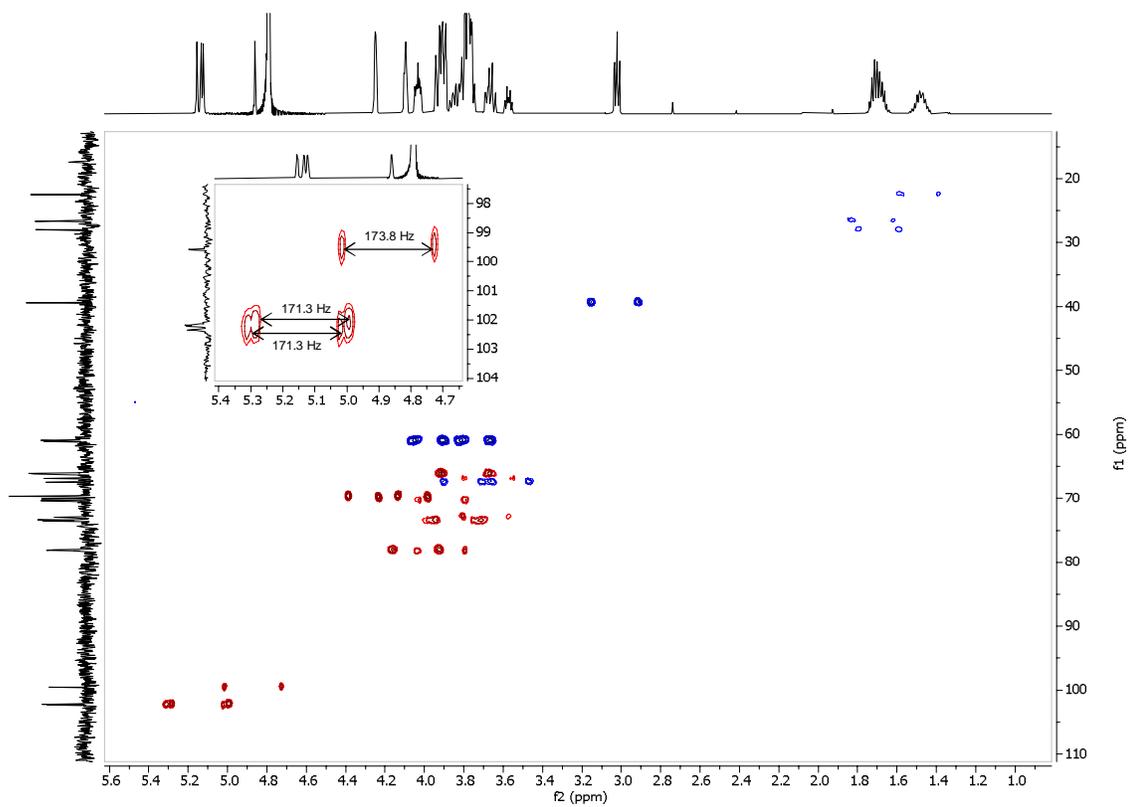
¹H NMR (700 MHz, D₂O) of **15**:



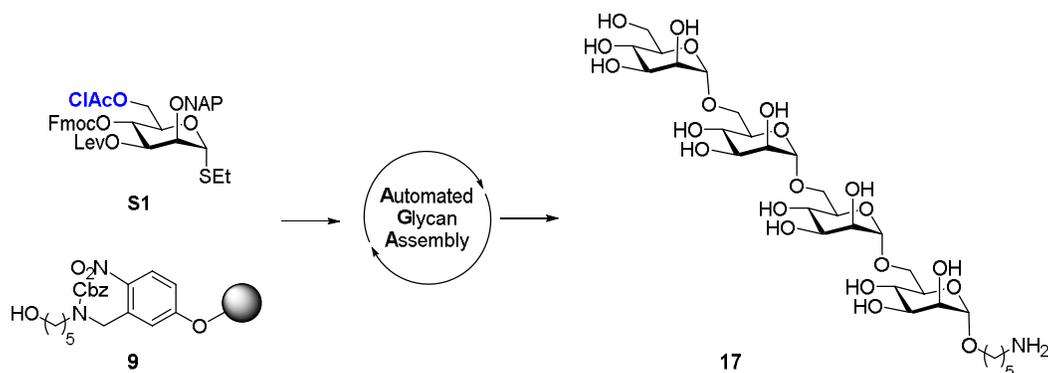
¹³C NMR (176 MHz, D₂O) of **15**:



Coupled ^{13}C , ^1H HSQC of 15:



5-Amino-pentyl α -(1 \rightarrow 6)-D-tetramannopyranoside (**17**)



Repeat	Building Blocks	Modules	Notes
1x	S1 (2 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 35 min (t ₂)
		III – Capping	
		IVe – ClAc Deprotection	
3x	S1 (6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 35 min (t ₂)
		III – Capping	
		IVe – ClAc Deprotection	

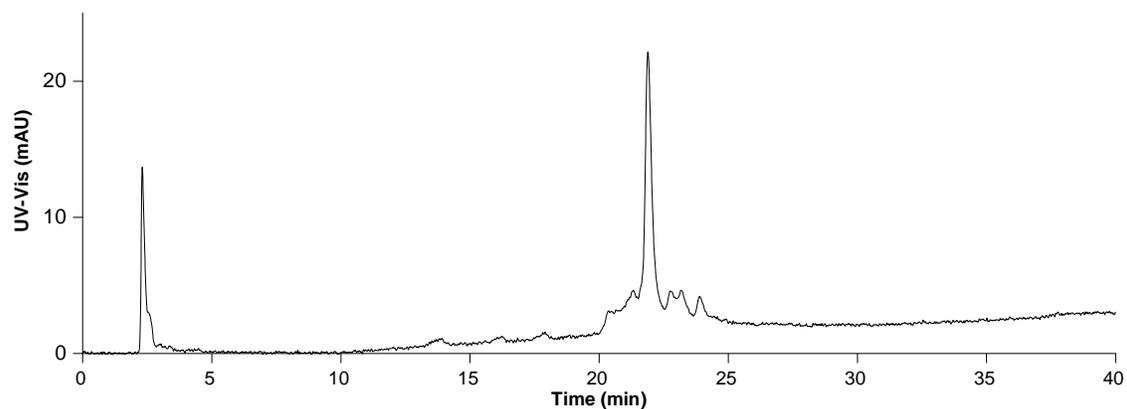
Protected **17** (22 mg, 0.008 mmol, crude yield: 58%) was obtained as a colorless oil after photocleavage from solid support following **Method A-1**. Deprotection of **17** following **Method C** and **D** and purification by reverse-phase HPLC (**Method E-1**, $t_R = 18.8$ min) afforded deprotected compound **17** (1.8 mg, 0.002 mmol, 18%) as a white solid after lyophilization.

¹H NMR (600 MHz, D₂O): δ 4.88 (d, $J = 1.8$ Hz, 1H), 4.86 (d, $J = 1.9$ Hz, 1H), 4.86 (d, $J = 1.8$ Hz, 1H), 4.83 (d, $J = 1.8$ Hz, 1H), 4.00 – 3.65 (m, 24H), 3.62 (t, $J = 9.7$ Hz, 1H), 3.54 (dt, $J = 9.9, 6.1$ Hz, 1H), 3.00 – 2.93 (m, 2H), 1.72 – 1.61 (m, 4H), 1.49 – 1.35 (m, 2H) ppm.

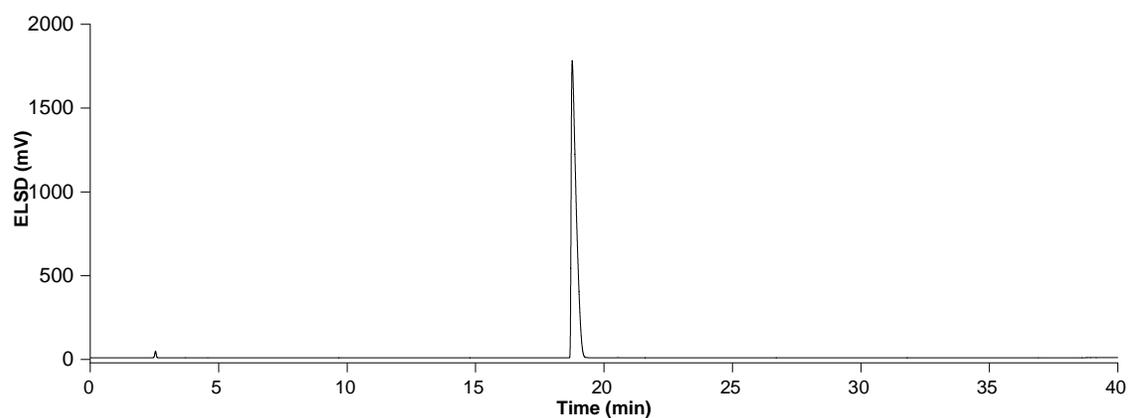
¹³C NMR (151 MHz, D₂O): δ 99.8, 99.3, 99.2, 72.6, 70.8, 70.7, 70.6, 70.6, 70.5, 70.0, 69.9, 69.9, 67.6, 66.7, 66.5, 66.5, 66.5, 65.5, 65.5, 65.4, 60.8, 39.3, 28.0, 26.6, 22.5 ppm.

HRMS (QToF): Calcd for C₂₉H₅₃NO₂₁ [M + H]⁺ 752.3183; found 752.3209.

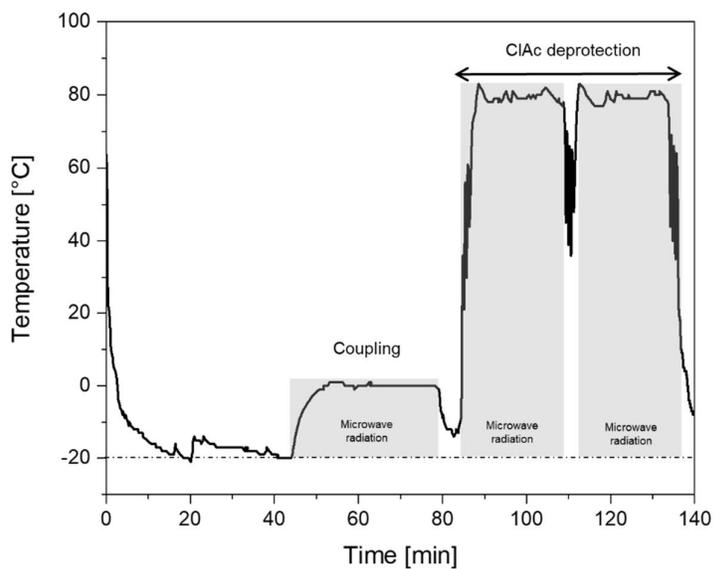
NP-HPLC of crude protected **17** (ELSD trace, **Method B-1**, $t_R = 21.9$ min):



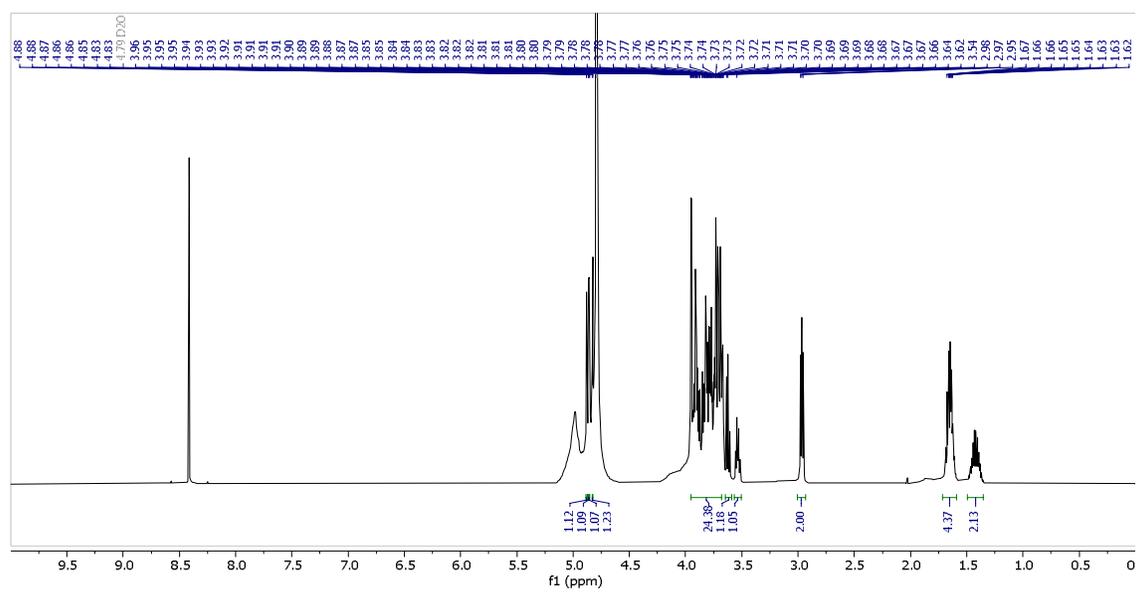
RP-HPLC of purified deprotected **17** (ELSD trace, **Method E-1**, $t_R = 18.8$ min):



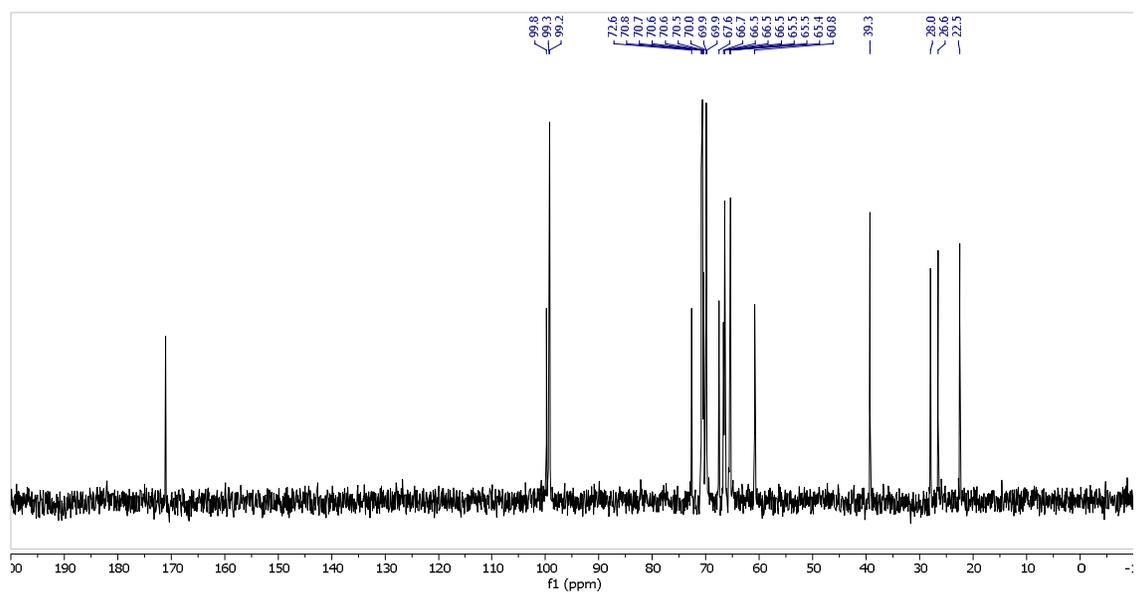
Temperature profile inside the reaction vessel during one synthesis cycle of **17**:



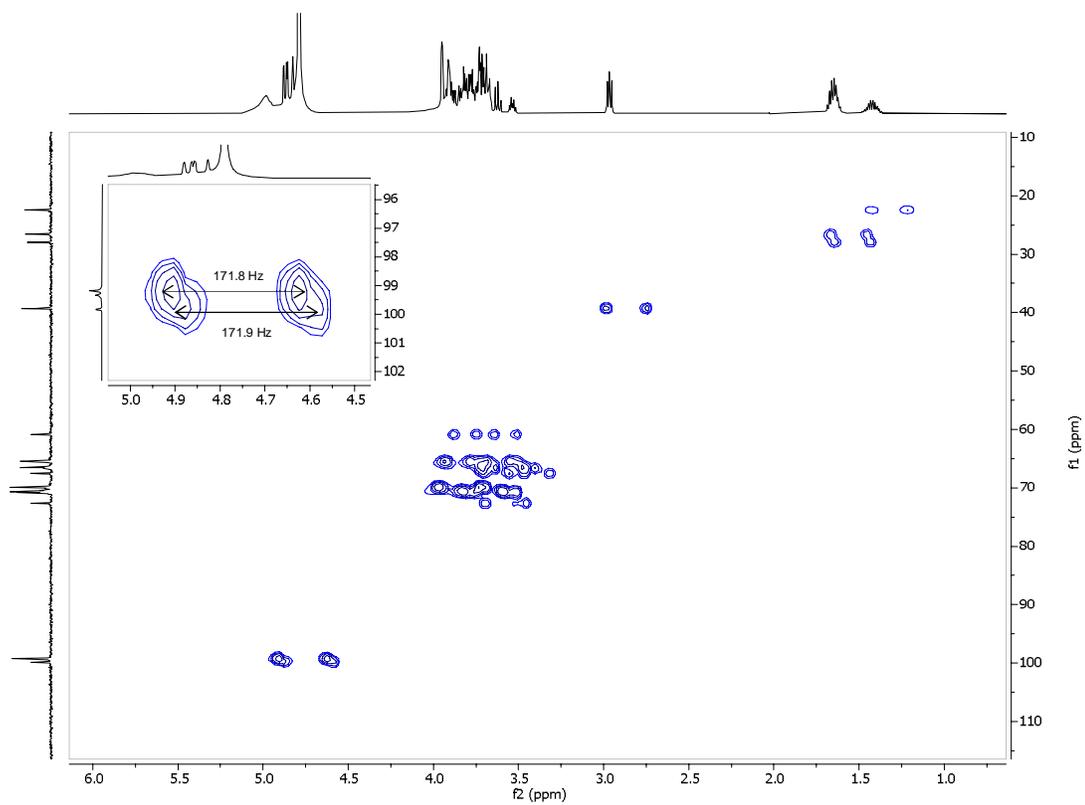
^1H NMR (600 MHz, D_2O) of **17**:



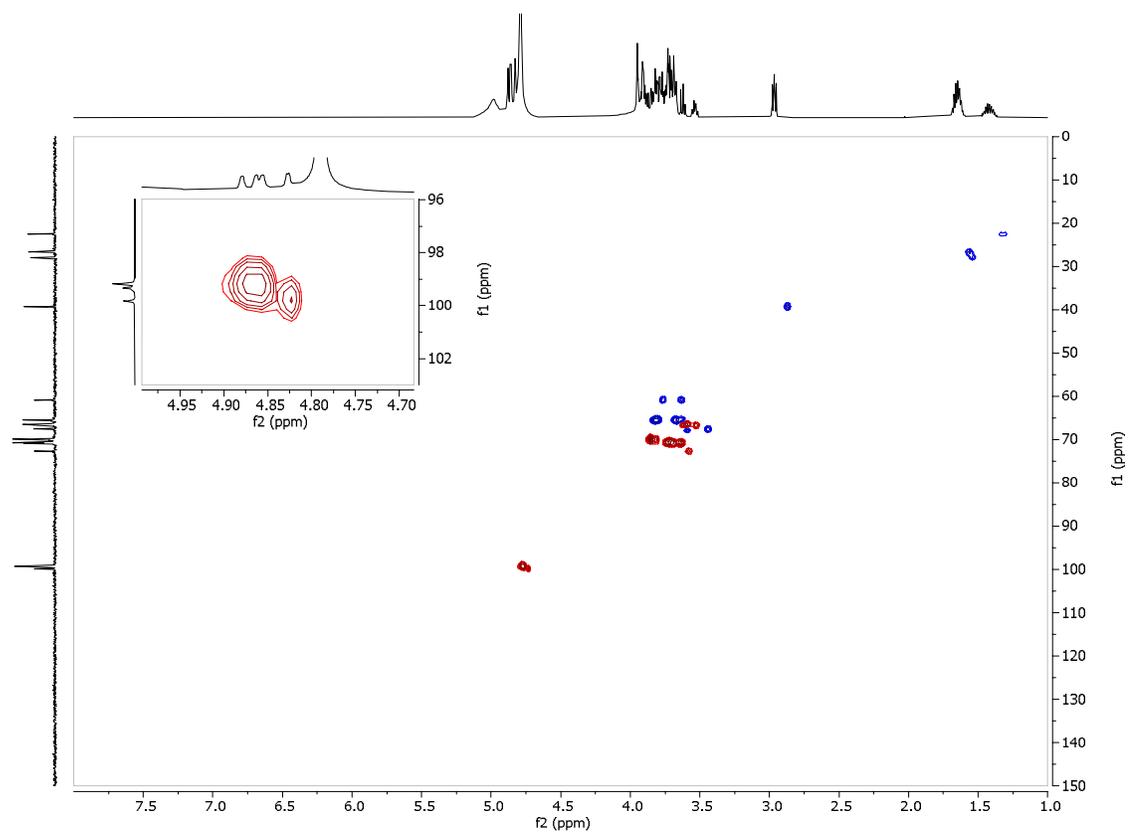
^{13}C NMR (151 MHz, D_2O) of **17**:



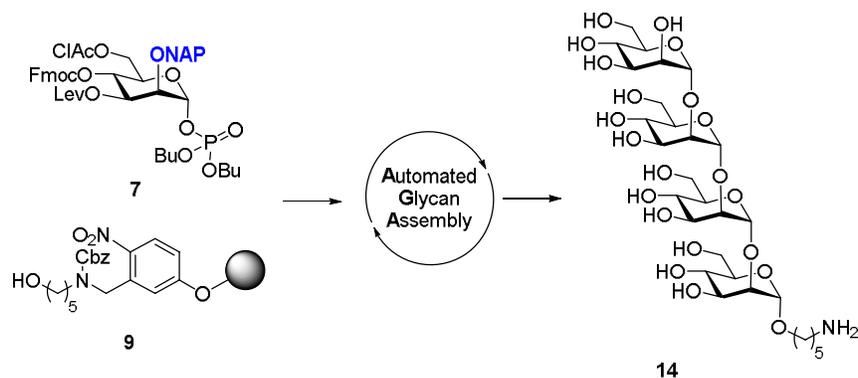
Coupled $^{13}\text{C}, ^1\text{H}$ HSQC of 17:



$^{13}\text{C}, ^1\text{H}$ HSQC of 17:



5-Amino-pentyl α -(1 \rightarrow 2)-D-tetramannopyranoside (**14**)



Repeat	Building Blocks	Modules	Notes
4x		I – Acidic Wash	
	7 (2 x 4.7 equiv.)	IIb – Glycosylation with glycosyl phosphate – 2 cycles	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping	
		IVd – NAP Deprotection	

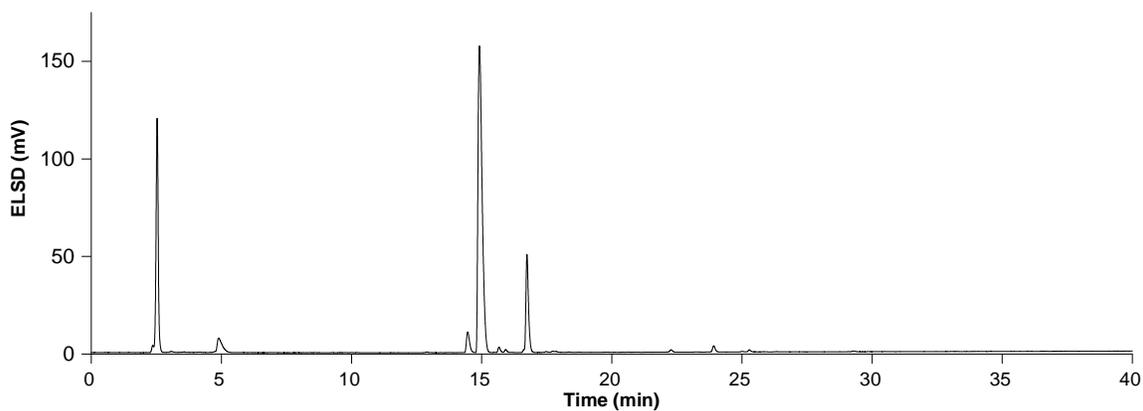
Protected **14** (17 mg, 0.007 mmol, crude yield: 51%) was obtained as a colorless oil after photocleavage from solid support following **Method A-1**. Deprotection of **14** following **Method C** and **D** and purification by reverse-phase HPLC (**Method E-1**, $t_R = 15.0$ min) afforded deprotected compound **14** (1.9 mg, 0.003 mmol, 19%) as a white solid after lyophilization.

¹H NMR (600 MHz, D₂O): δ 5.27 (d, $J = 1.9$ Hz, 1H), 5.25 (d, $J = 1.9$ Hz, 1H), 5.06 (d, $J = 1.9$ Hz, 1H), 5.01 (d, $J = 1.9$ Hz, 1H), 4.10 – 4.01 (m, 3H), 3.96 – 3.78 (m, 9H), 3.78 – 3.56 (m, 13H), 3.55 – 3.47 (m, 1H), 3.00 – 2.94 (m, 2H), 1.71 – 1.58 (m, 4H), 1.46 – 1.36 (m, 2H) ppm.

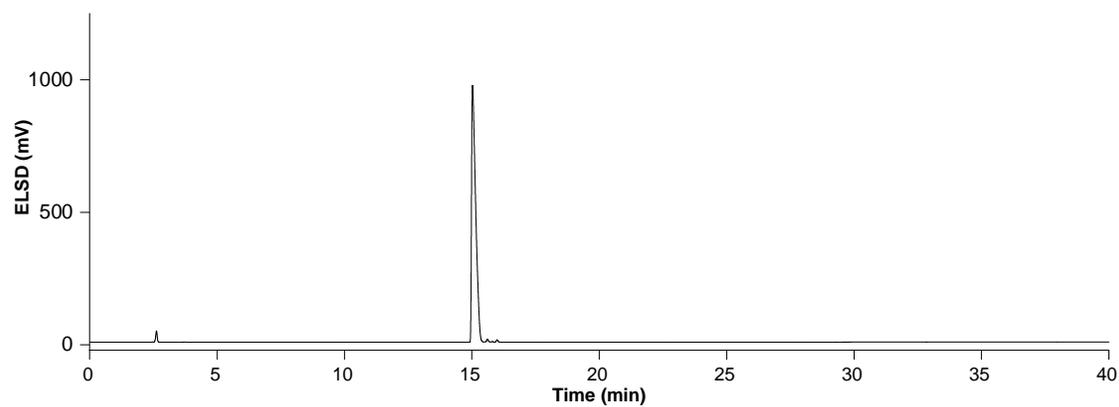
¹³C NMR (151 MHz, D₂O): δ 102.1, 100.6, 100.5, 97.9, 85.8, 79.5, 78.8, 78.5, 73.2, 73.1, 72.7, 70.2, 70.1, 69.9, 69.8, 67.5, 67.0, 66.9, 66.9, 66.7, 61.8, 61.0, 60.9, 38.7, 27.9, 26.4, 22.4 ppm.

HRMS (QToF): Calcd for C₂₉H₅₃NO₂₁ [M + H]⁺ 752.3183; found 752.3214.

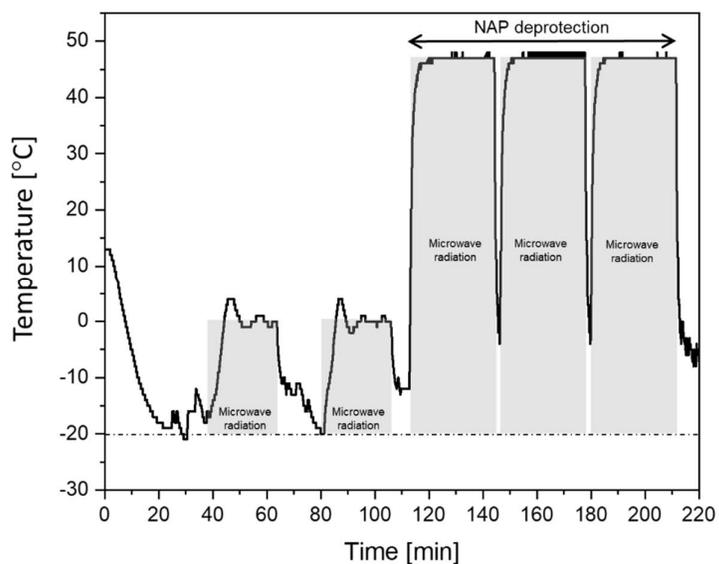
RP-HPLC of crude deprotected **14** (ELSD trace, **Method E-1**, $t_R = 15.0$ min):



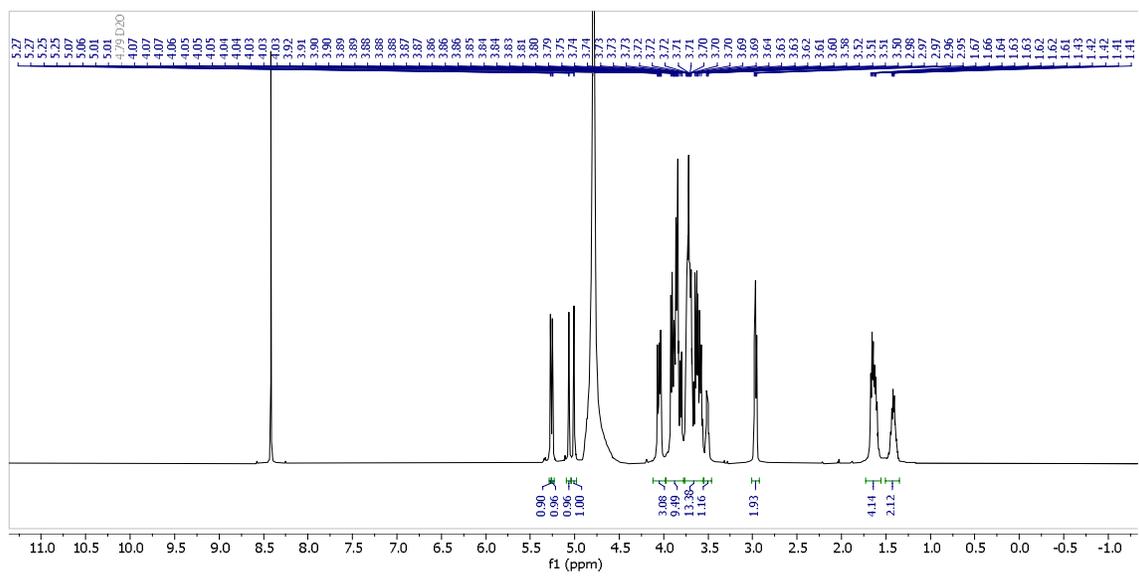
RP-HPLC of purified deprotected **14** (ELSD trace, **Method E-1**, $t_R = 15.0$ min):



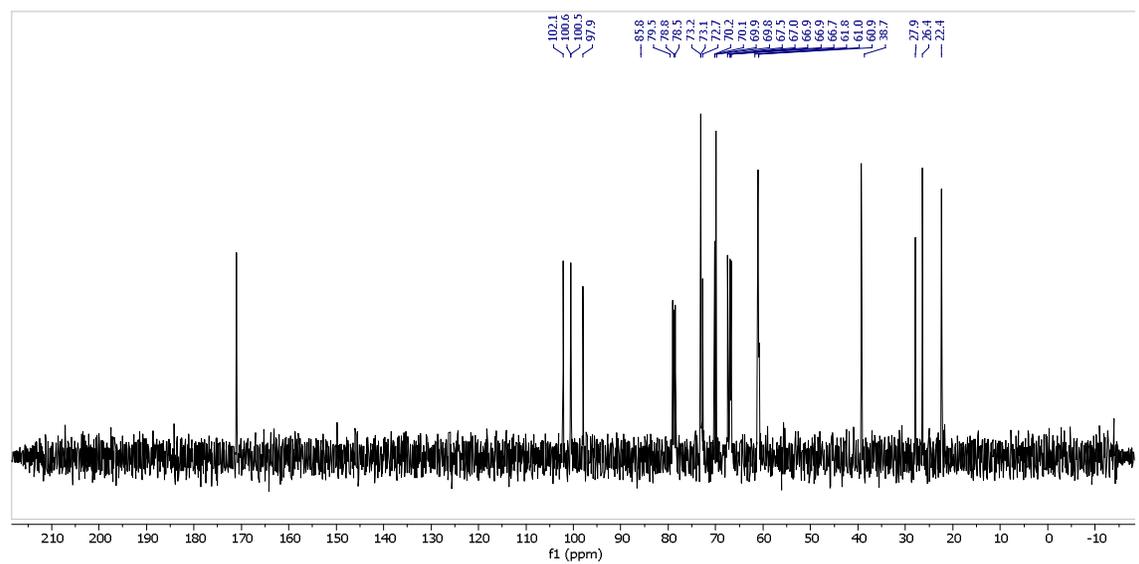
Temperature profile inside the reaction vessel during one synthesis cycle of **14**:



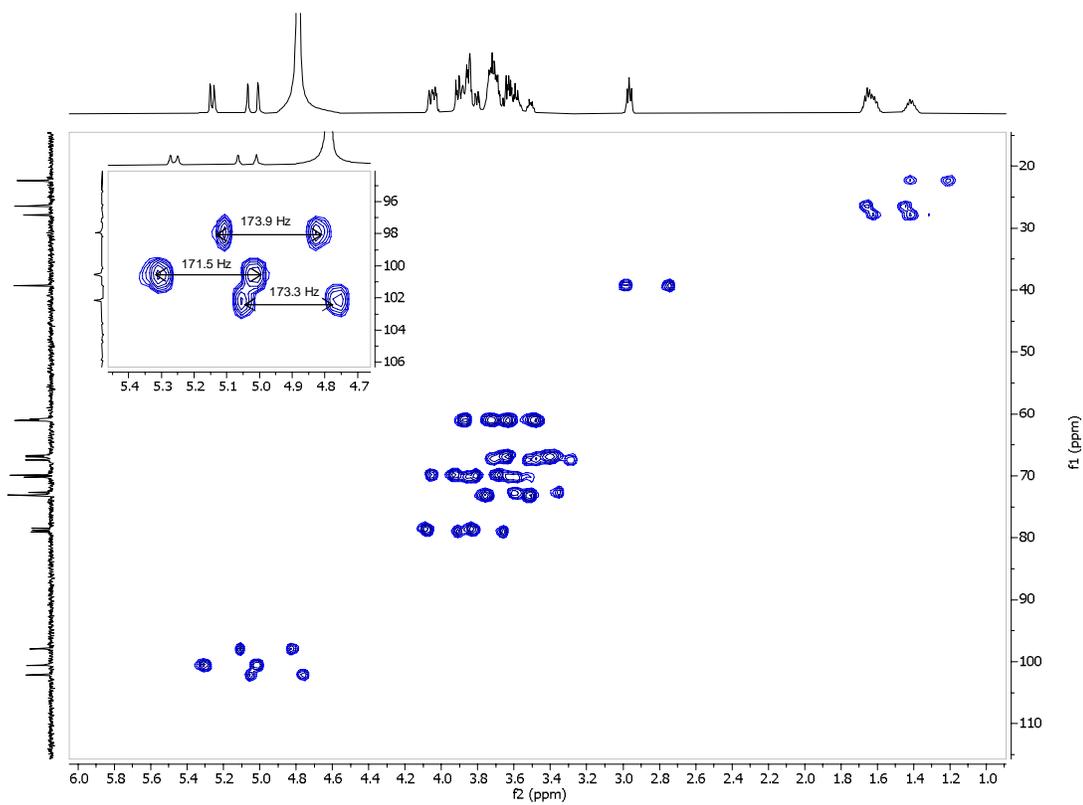
¹H NMR (600 MHz, D₂O) of **14**:



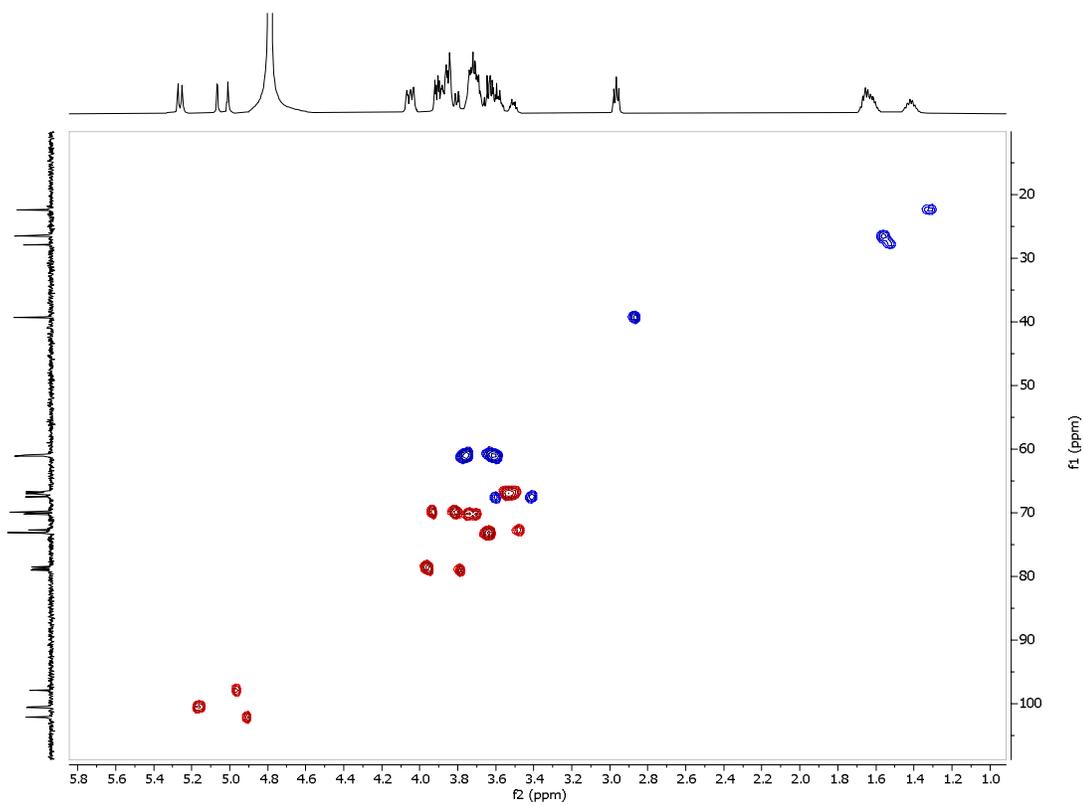
¹³C NMR (151 MHz, D₂O) of **14**:



Coupled ^{13}C , ^1H HSQC of **14**:

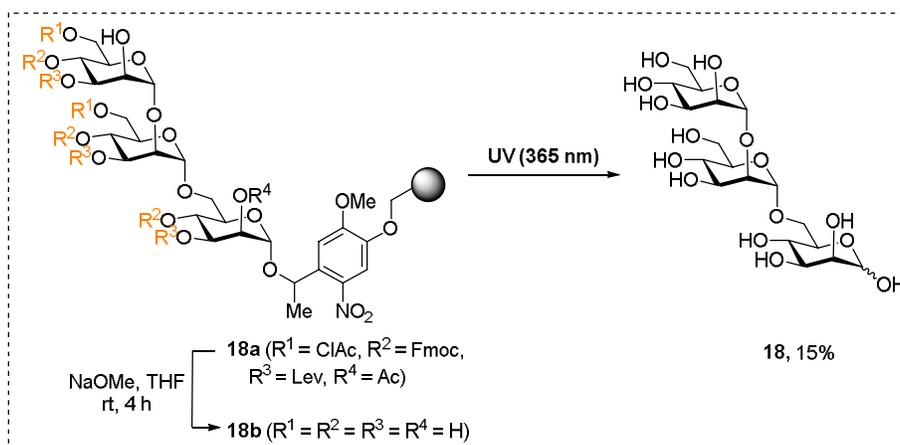
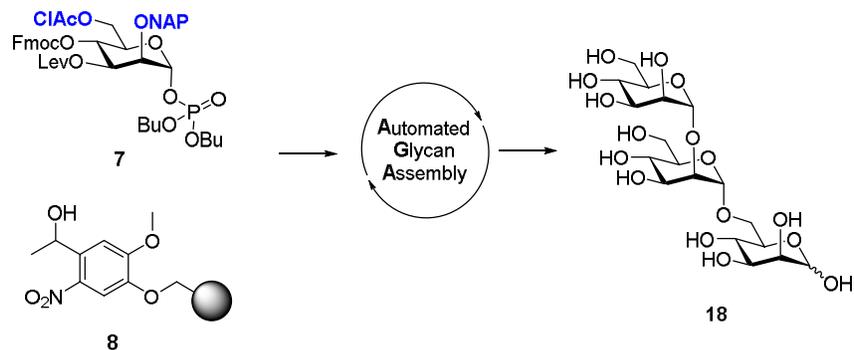


^{13}C , ^1H HSQC of **14**:



5.5.2 Non-Linear On-Resin Deprotected Trimer (18)

α -D-Mannopyranosyl-(1 \rightarrow 2)- α -D-mannopyranosyl-(1 \rightarrow 6)- α/β -D-mannopyranoside (18)



Repeat	Building Blocks	Modules	Notes
1x	7 (2 x 4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate – 2 cycles	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		IVd – NAP Deprotection III – Capping IVe – ClAc Deprotection	
1x	7 (4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVd – NAP Deprotection	
1x	7 (2 x 4.7 equiv.)	I – Acidic Wash	
		IIb – Glycosylation with glycosyl phosphate – 2 cycles	-20 °C (T ₁) 30 min (t ₁) 0 °C (T ₂) 10 min (t ₂)
		III – Capping IVd – NAP Deprotection VI - Methanolysis	

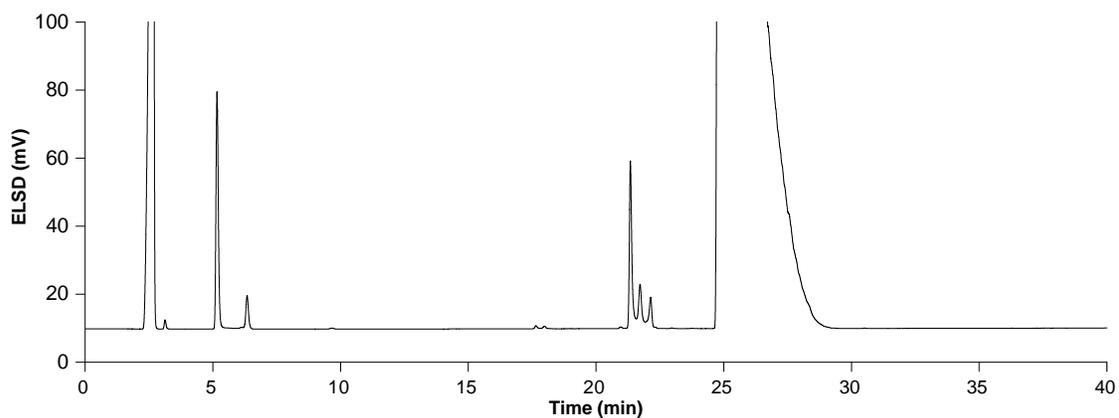
Photocleavage from solid support using **Method A-2** followed by purification by reverse-phase HPLC (**Method E-2**, $t_R = 21.6$ min) afforded deprotected compound **18** (1.2 mg, 0.002 mmol, 15%) as a white solid after lyophilization.

^1H NMR (700 MHz, D_2O , α -isomer): δ 5.17 (d, $J = 1.9$ Hz, 1H), 5.14 (d, $J = 1.9$ Hz, 1H), 5.05 (d, $J = 2.2$ Hz, 1H), 4.08 (dd, $J = 3.4, 1.8$ Hz, 1H), 4.03 (dd, $J = 3.5, 1.8$ Hz, 1H), 4.00 – 3.69 (m, 14H), 3.66 – 3.62 (m, 2H) ppm.

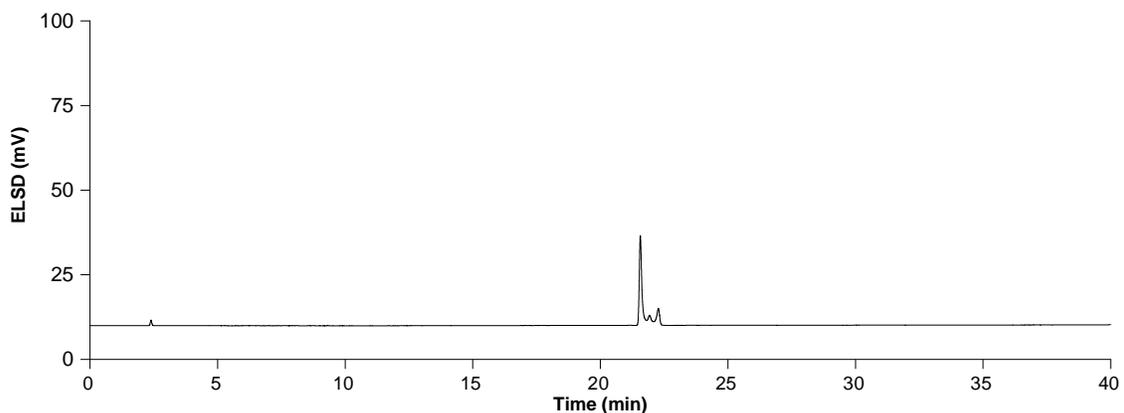
^{13}C NMR (176 MHz, D_2O , α -isomer): δ 102.3, 98.7, 94.2, 78.5, 73.2, 72.7, 71.1, 70.7, 70.6, 70.4, 70.3, 70.2, 69.9, 66.9, 66.7, 66.0, 61.1, 60.9 ppm.

HRMS (QToF): Calcd for $\text{C}_{18}\text{H}_{32}\text{O}_{16}\text{Na}$ $[\text{M} + \text{Na}]^+$ 527.1583; found 527.1586.

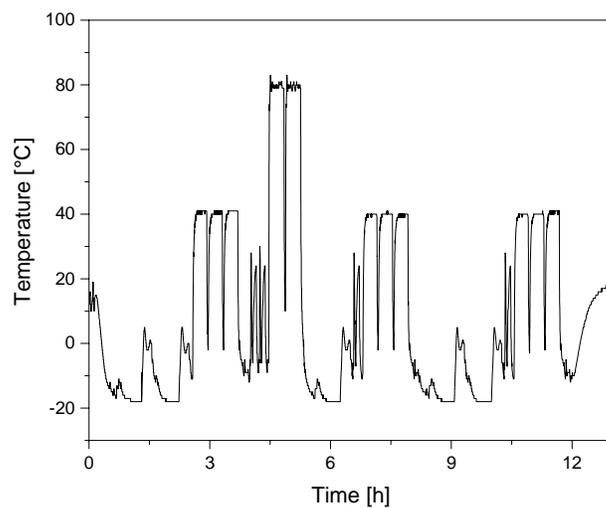
RP-HPLC of crude deprotected **18** (ELSD trace, **Method E-2**, $t_R = 21.6$ min):



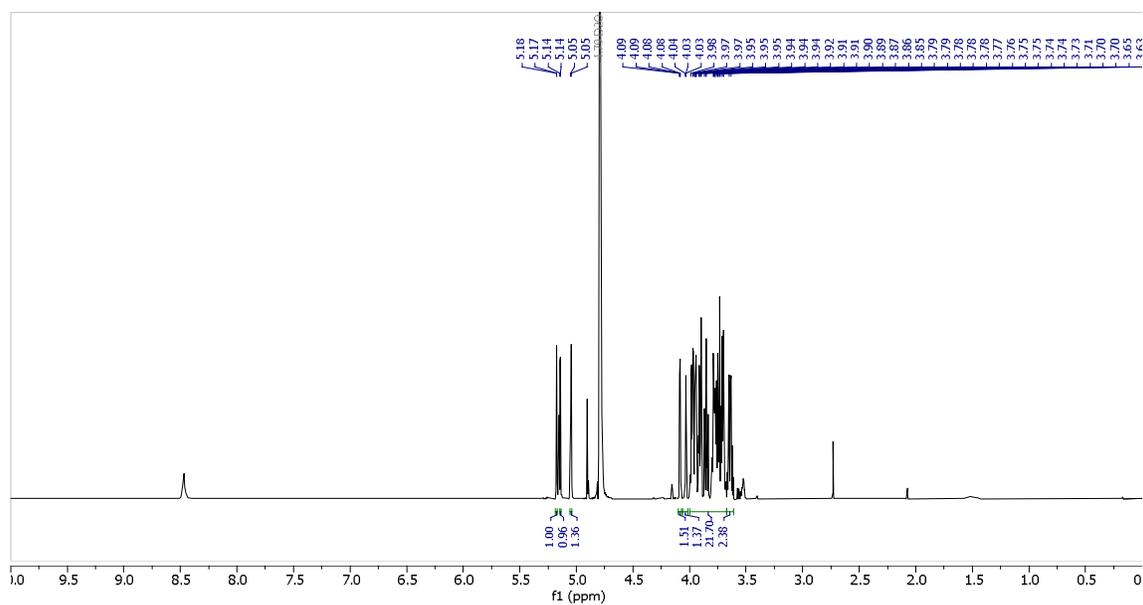
RP-HPLC of purified deprotected **18** (ELSD trace, **Method E-2**, $t_R = 21.6$ min):



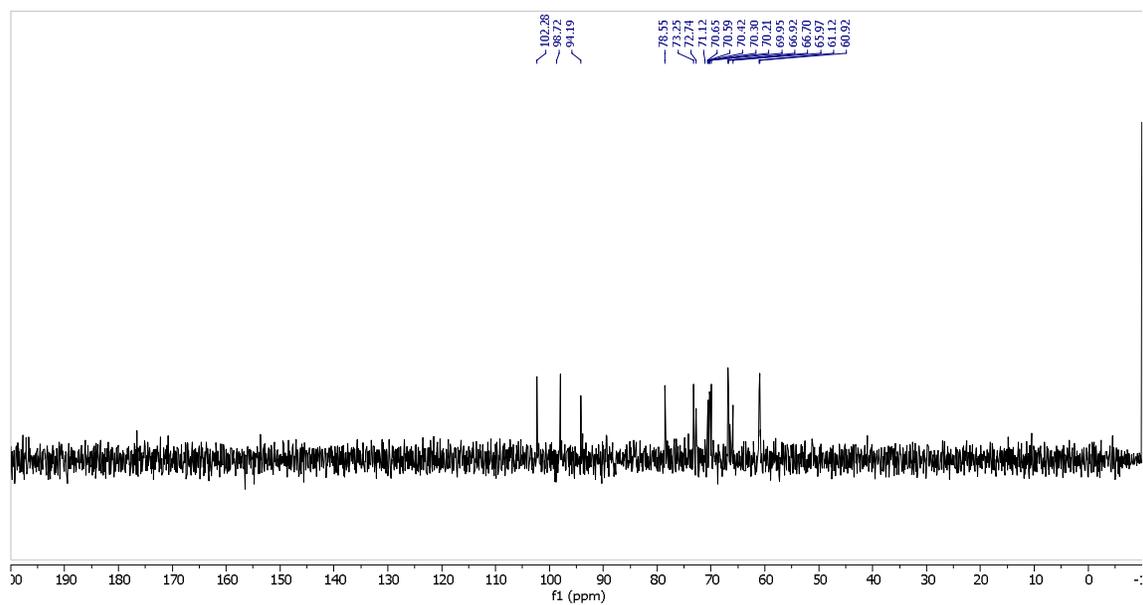
Temperature profile inside the reaction vessel during the synthesis of **18**:



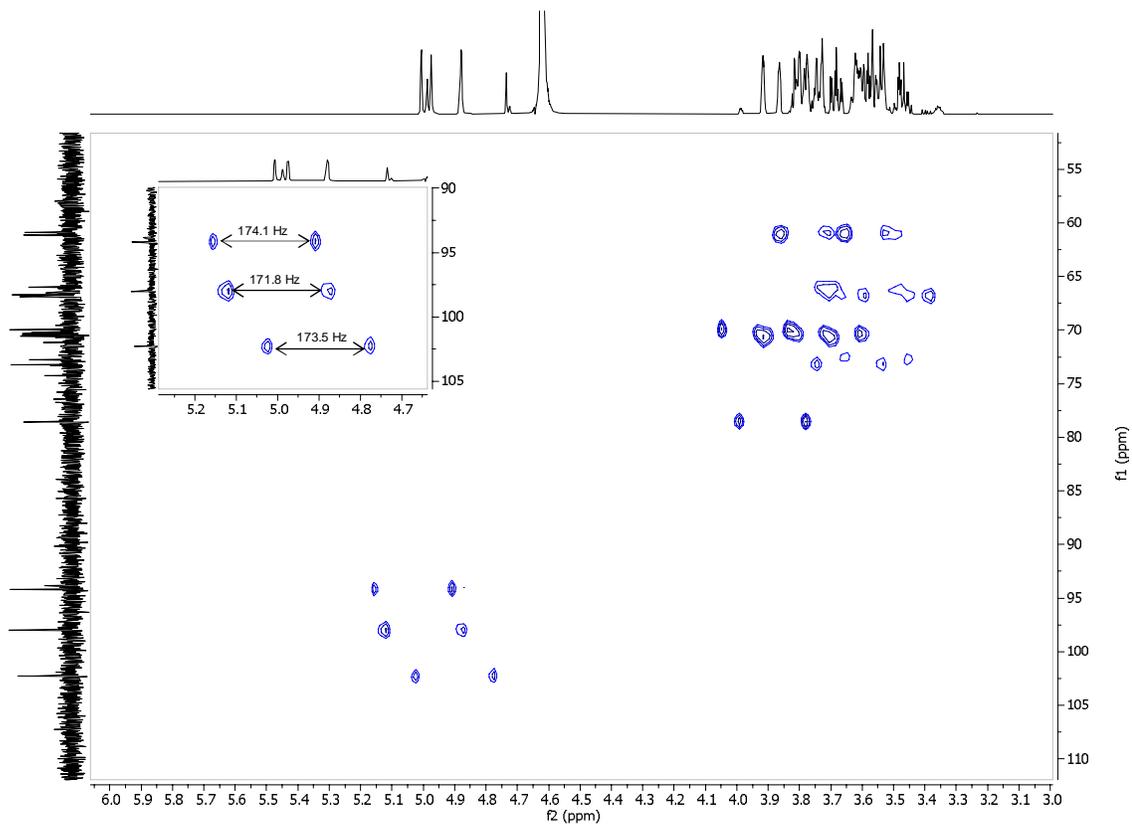
^1H NMR (700 MHz, D_2O) of **18**:



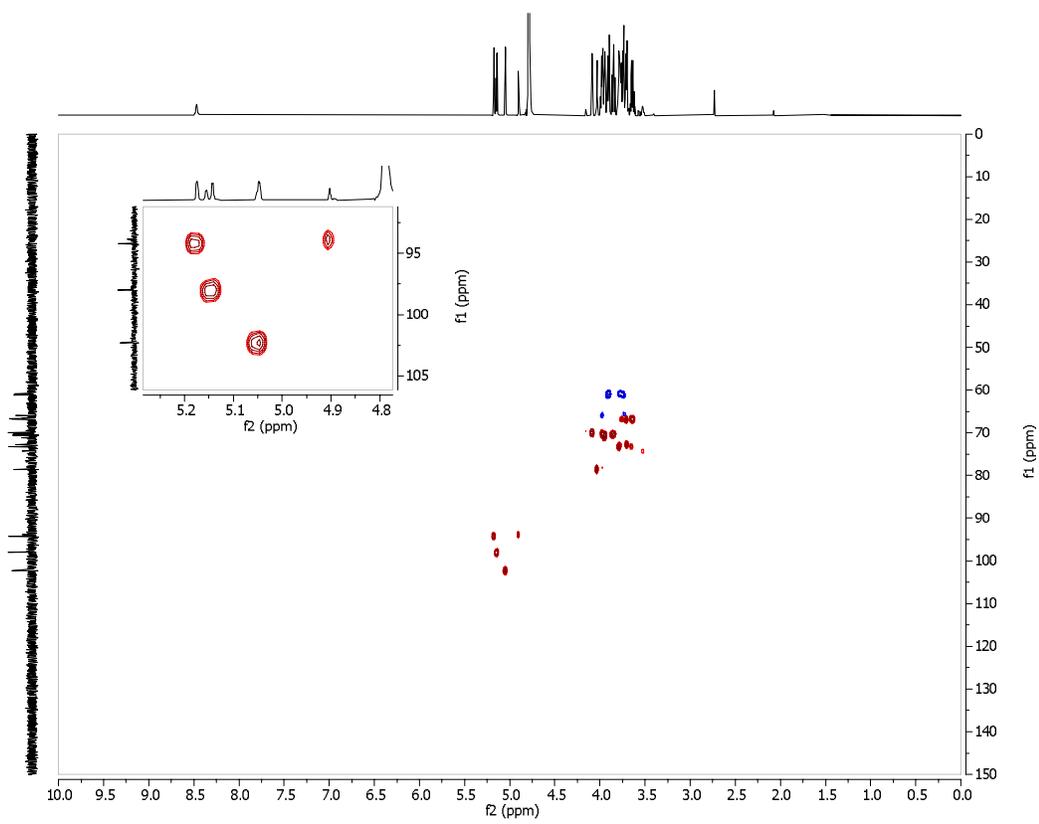
¹³C NMR (151 MHz, D₂O) of **18**:



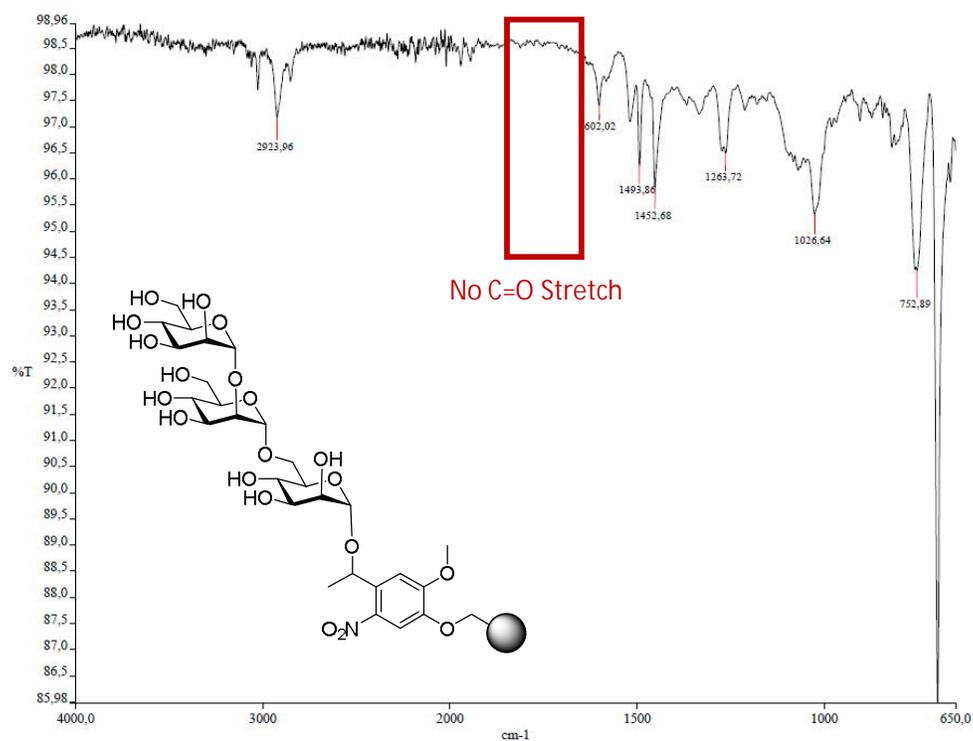
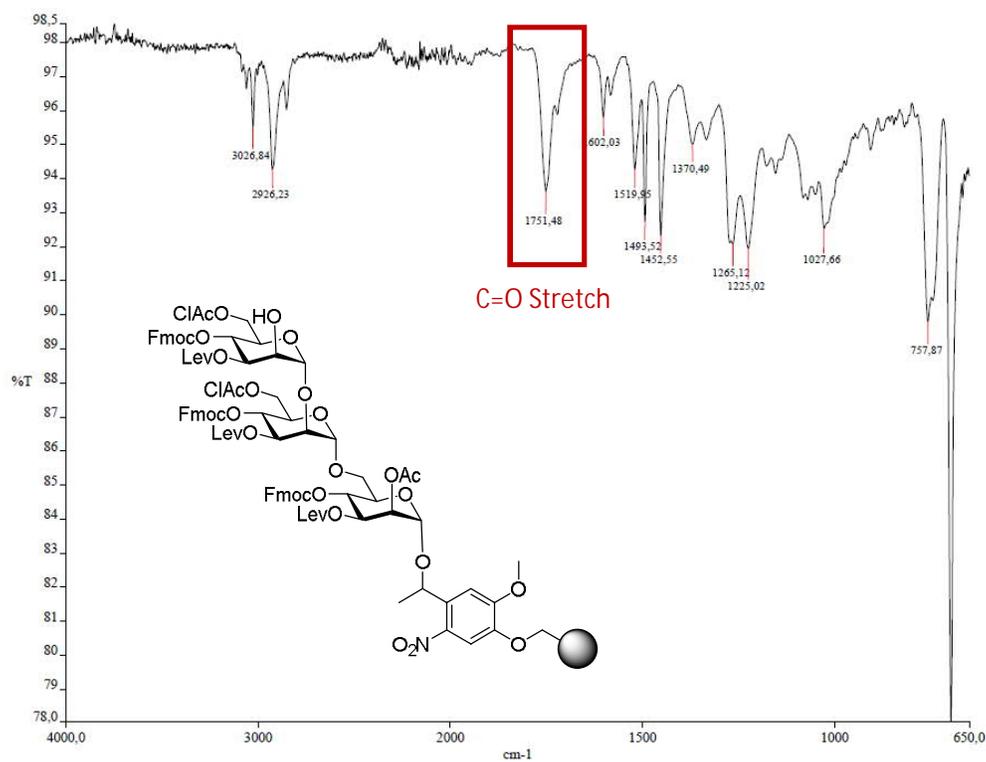
Coupled ¹³C, ¹H HSQC of **18**:



$^{13}\text{C}, ^1\text{H}$ HSQC of **18**:



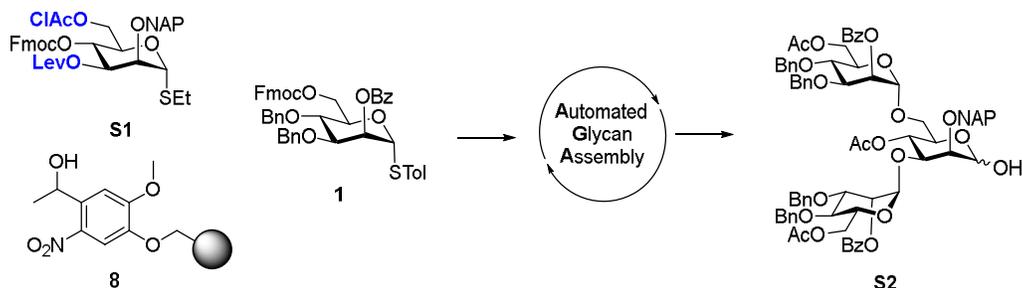
FT-IR spectrum¹ of the resin containing **18** before and after **Module VI**:



¹ 20-30 beads of resin were ground using a mortar. The ground beads were suspended in CH_2Cl_2 and applied on a Perkin-Elmer 1600 FTIR spectrometer. Spectra were taken after evaporation of the CH_2Cl_2 .

5.5.3 Double Branched Trimer (S2)

6-O-Acetyl-2-O-benzoyl-3,4-di-O-benzyl- α -D-mannopyranosyl-(1 \rightarrow 3)-[6-O-acetyl-2-O-benzoyl-3,4-di-O-benzyl- α -D-mannopyranosyl-(1 \rightarrow 6)]-2-O-acetyl-2-O-(2-naphthalenylmethyl)- α/β -D-mannopyranoside (S2)



Repeat	Building Blocks	Modules	Notes
1x	S1 (2 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 35 min (t ₂)
		IVc – Fmoc Deprotection III – Capping IVb – Lev Deprotection IVe – ClAc Deprotection	
1x	1 (3 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 3 cycles	-20 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 25 min (t ₂)
		IVa – Fmoc Deprotection (6 cycles) III – Capping (4 cycles)	

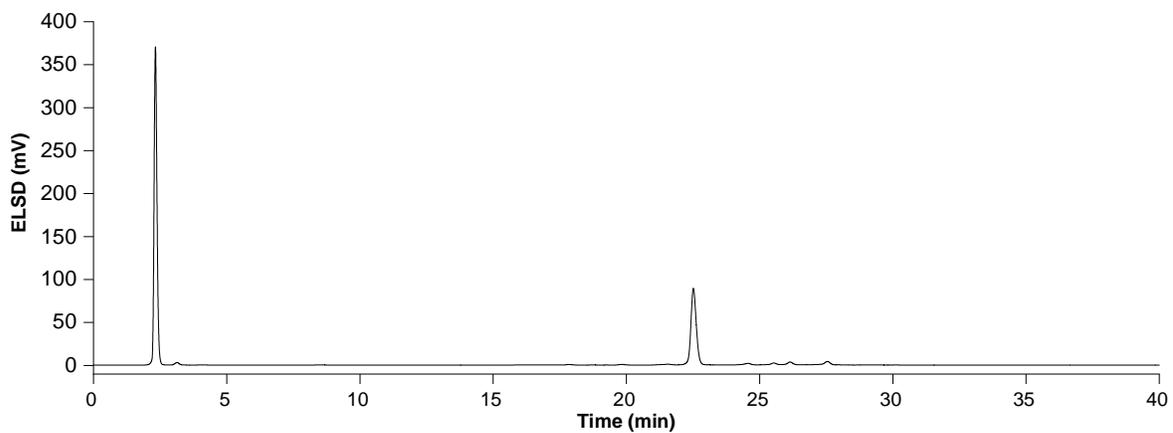
Photocleavage from solid support using **Method A-1** followed by purification by normal-phase HPLC (**Method B-3**, $t_R = 22.7$ min) afforded compound **S2** (14 mg, 0.011 mmol, **65%**) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 8.09 – 8.01 (m, 5H), 7.75 – 7.56 (m, 7H), 7.52 – 7.39 (m, 6H), 7.36 – 7.16 (m, 19H), 5.60 (s, 1H), 5.48 (s, 1H), 5.39 (t, $J = 10.0$ Hz, 1H), 5.24 (s, 1H), 5.11 (s, 1H), 5.01 – 4.67 (m, 7H), 4.65 – 4.42 (m, 6H), 4.37 – 3.95 (m, 8H), 3.92 – 3.78 (m, 5H), 2.18 (s, 3H), 2.05 (s, 3H), 1.91 (s, 3H) ppm.

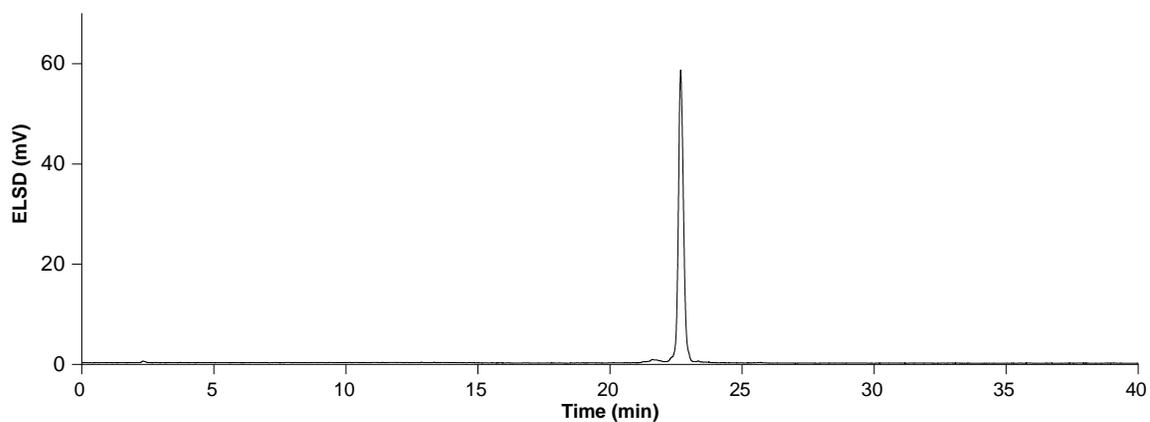
¹³C NMR (101 MHz, CDCl₃): δ 171.2, 170.8, 170.4, 165.8, 165.6, 138.2, 138.0, 137.7, 135.2, 133.5, 133.5, 133.2, 133.1, 130.0, 130.0, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.2, 128.0, 127.9, 127.8, 127.8, 127.0, 126.3, 126.1, 125.8, 99.5, 97.6, 92.7, 78.2, 77.8, 77.4, 76.0, 75.3, 75.2, 73.8, 73.7, 73.2, 71.6, 71.5, 70.5, 70.1, 70.0, 69.3, 69.0, 68.9, 68.0, 63.4, 63.3, 21.1, 21.0, 20.7 ppm.

HRMS (QToF): Calcd for $C_{77}H_{78}O_{21}Na$ $[M + Na]^+$ 1361.4928; found 1361.4954.

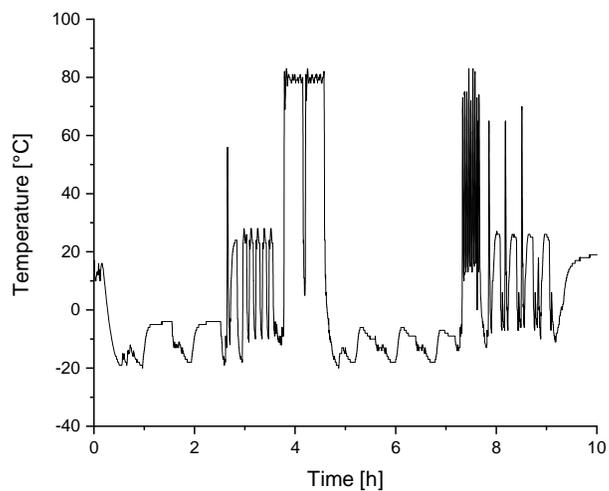
NP-HPLC of crude **S2** (ELSD trace, **Method B-2**, $t_R = 22.7$ min):

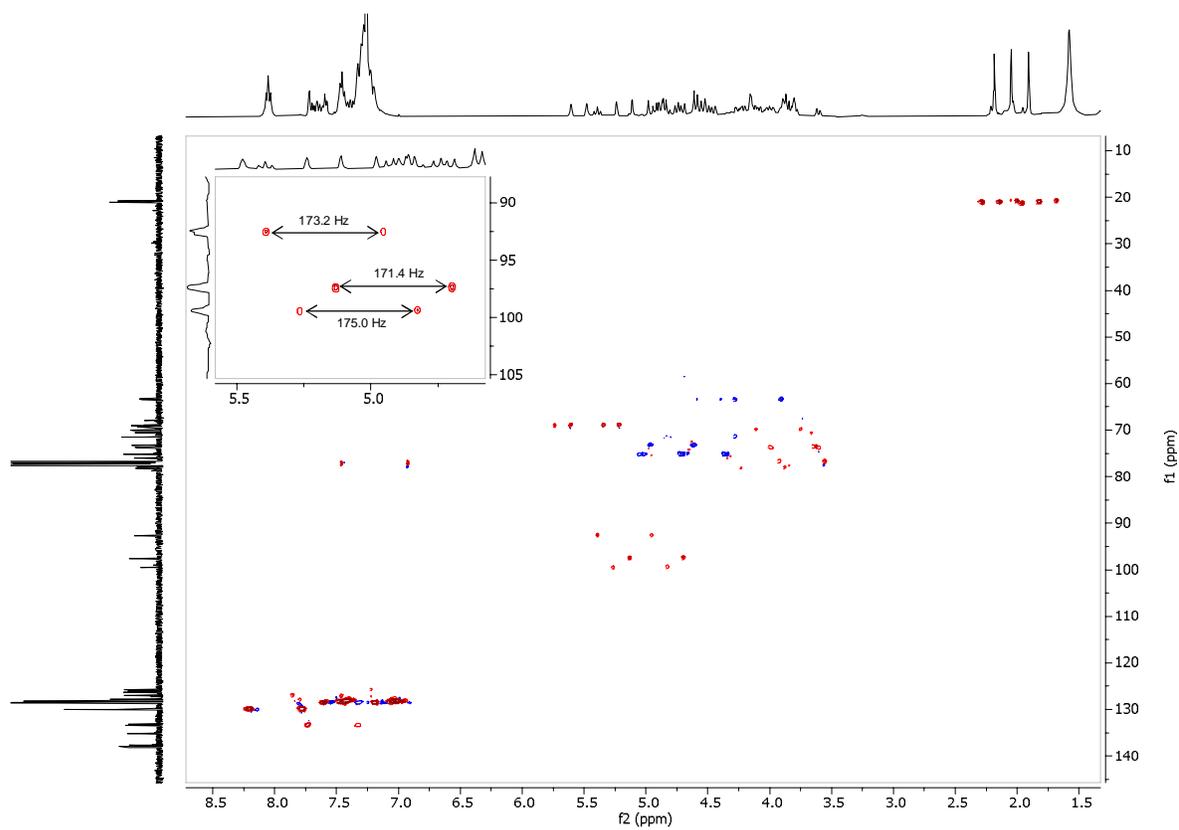


NP-HPLC of purified **S2** (ELSD trace, **Method B-2** $t_R = 22.7$ min):



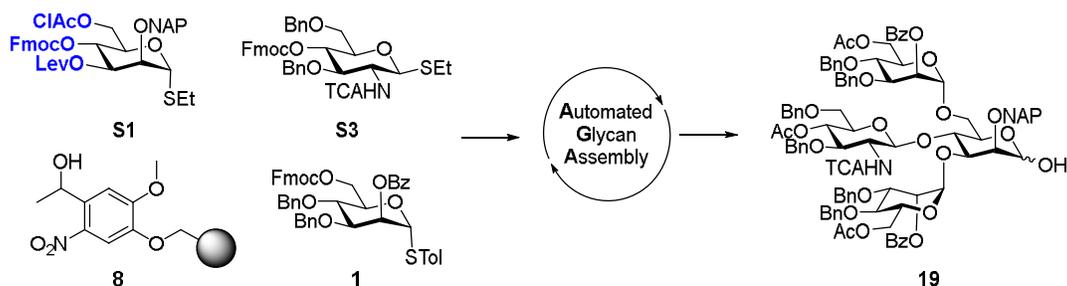
Temperature profile inside the reaction vessel during one synthesis cycle of **S2**:





5.5.4 Bisecting GlcNAc Tetramer (Triple-Branched Mannose 19)

6-O-Acetyl-2-O-benzoyl-3,4-di-O-benzyl- α -D-mannopyranosyl-(1 \rightarrow 3)-[4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-N-trichloroacetyl- β -D-glucopyranosyl-(1 \rightarrow 4)]-[6-O-acetyl-2-O-benzoyl-3,4-di-O-benzyl- α -D-mannopyranosyl-(1 \rightarrow 6)]-2-O-(2-naphthalenylmethyl)- α / β -D-mannopyranoside (19)



Repeat	Building Blocks	Modules	Notes
1x	S1 (2 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 35 min (t ₂)
		III – Capping IVc – Fmoc Deprotection	
1x	S3 (6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 45 min (t ₂)
		III – Capping IVb – Lev Deprotection IVe – ClAc Deprotection	
1x	1 (3 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 3 cycles	-20 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 25 min (t ₂)
		IVa – Fmoc Deprotection (6 cycles) III – Capping (4 cycles)	

Photocleavage from solid support using **Method A-1** followed by purification by normal-phase HPLC (**Method B-3**, $t_R = 21.6$ min) afforded compound **19** (8 mg, 0.004 mmol, **28%**) as a colorless oil.

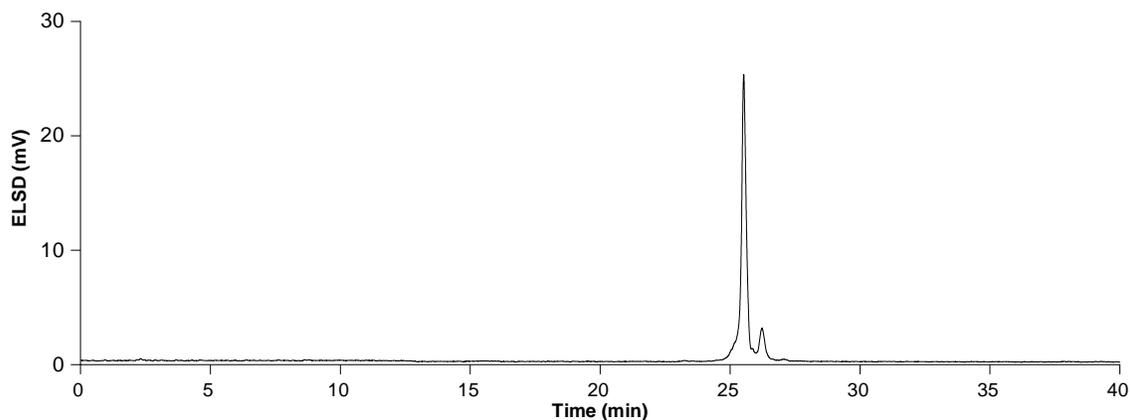
¹H NMR (600 MHz, CDCl₃, α -isomer): δ 8.11 – 8.06 (m, 4H), 7.65 – 7.56 (m, 5H), 7.47 – 7.39 (m, 7H), 7.36 – 7.11 (m, 31H), 5.86 (s, 1H), 5.74 – 5.70 (m, 1H), 5.39 (d, $J = 2.0$ Hz, 1H), 5.34 (t, $J = 9.4$ Hz, 1H), 5.16 – 5.06 (m, 3H), 4.94 (d, $J = 10.7$ Hz, 1H), 4.87 (d, $J = 11.2$ Hz, 1H), 4.81 – 4.70 (m, 5H), 4.65 – 4.58 (m, 6H), 4.55 – 4.49 (m, 4H), 4.41 (dd, $J = 11.4, 5.0$ Hz, 2H),

4.37 – 4.31 (m, 3H), 4.25 – 4.09 (m, 7H), 3.92 – 3.85 (m, 6H), 2.06 (s, 3H), 1.91 (s, 3H), 1.80 (s, 3H).

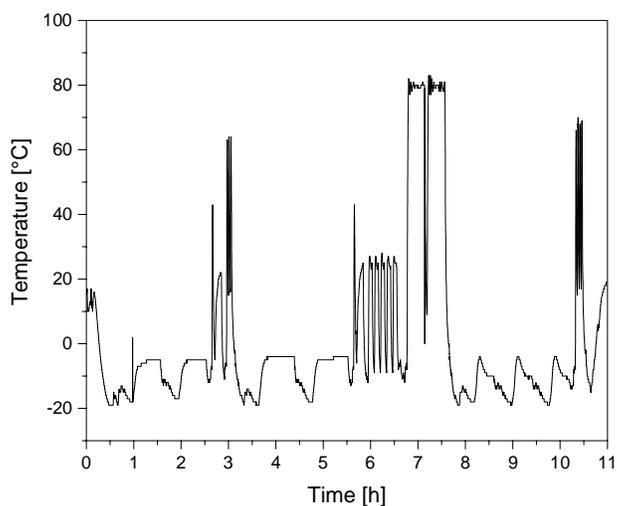
¹³C NMR (176 MHz, CDCl₃, α-isomer): δ 169.9, 169.6, 169.2, 165.0, 164.5, 137.1, 136.9, 136.9, 136.6, 134.1, 132.1, 132.0, 128.9, 128.9, 128.9, 128.8, 128.8, 127.6, 127.6, 127.4, 127.4, 127.4, 127.3, 127.3, 127.3, 127.2, 127.1, 127.0, 127.0, 126.9, 126.9, 126.9, 126.8, 126.8, 126.7, 126.7, 126.6, 98.4, 97.6, 96.5, 96.5, 91.6, 77.1, 76.7, 74.9, 74.1, 72.7, 72.6, 72.1, 70.5, 70.3, 69.4, 69.0, 68.9, 68.2, 68.0, 67.9, 66.8, 62.3, 62.2, 19.9, 19.8, 19.6 ppm.

HRMS (QToF): Calcd for C₉₉H₁₀₀Cl₃NO₂₆ [M + Na]⁺ 1846.5491; found 1846.5565.

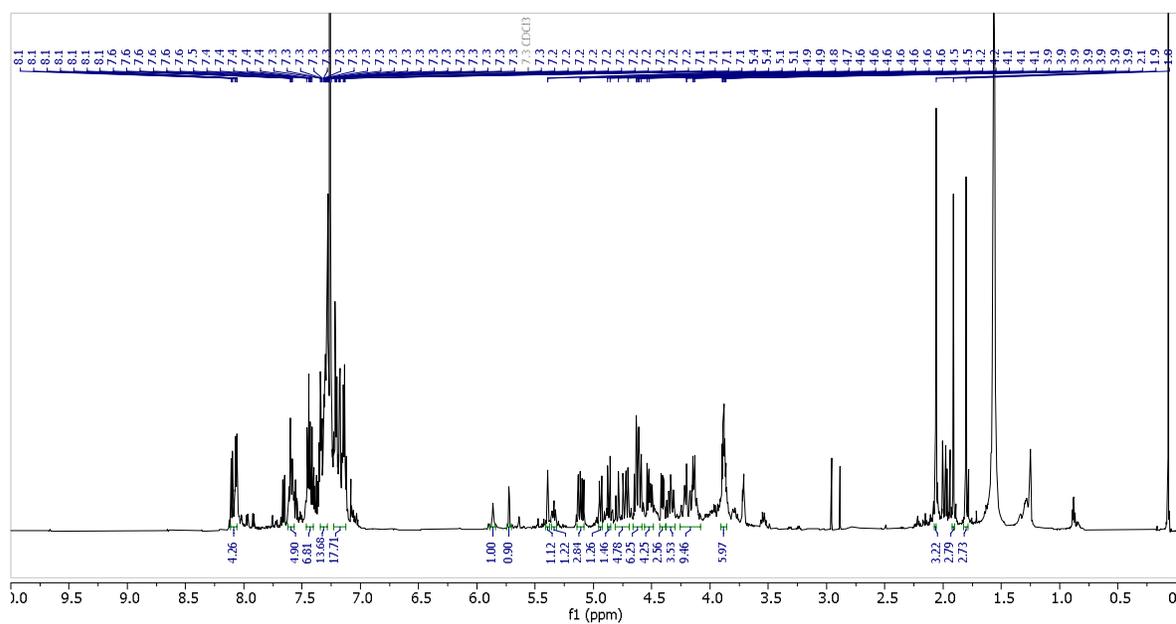
NP-HPLC of purified **19** (ELSD trace, **Method B-2** t_R = 25.5 min):



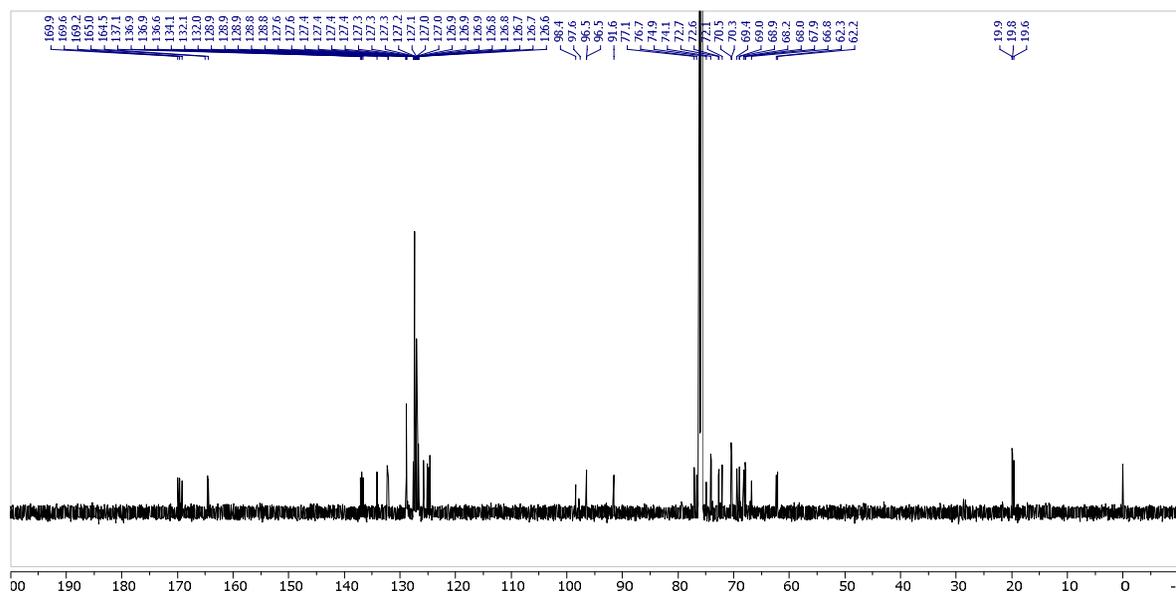
Temperature profile inside the reaction vessel during one synthesis cycle of **19**:



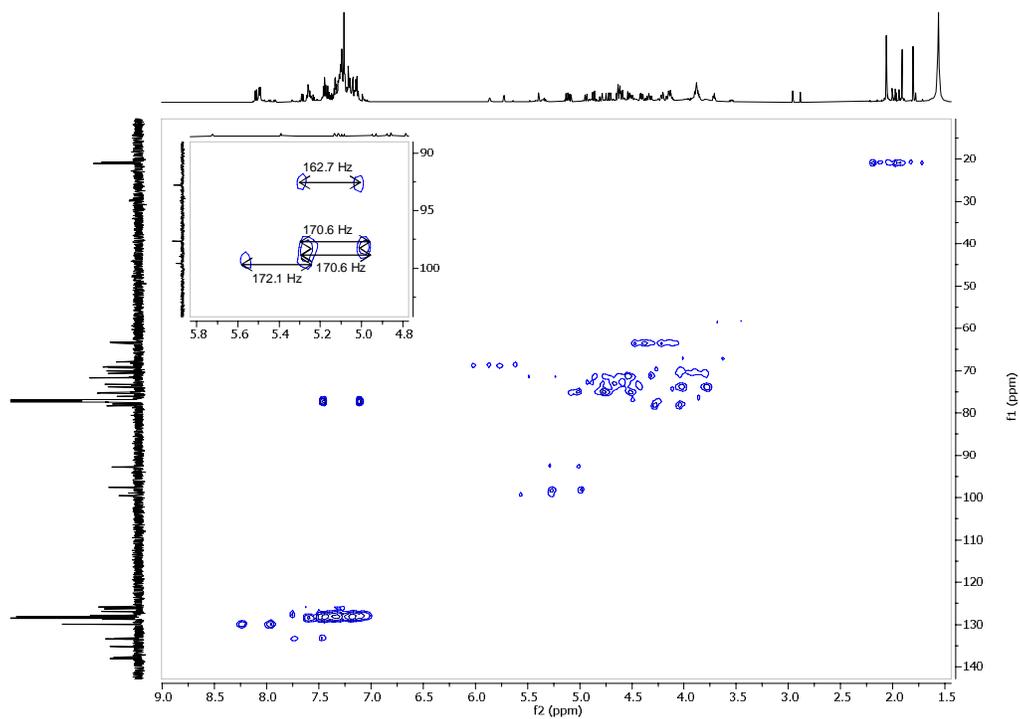
^1H NMR (600 MHz, CDCl_3) of **19**:



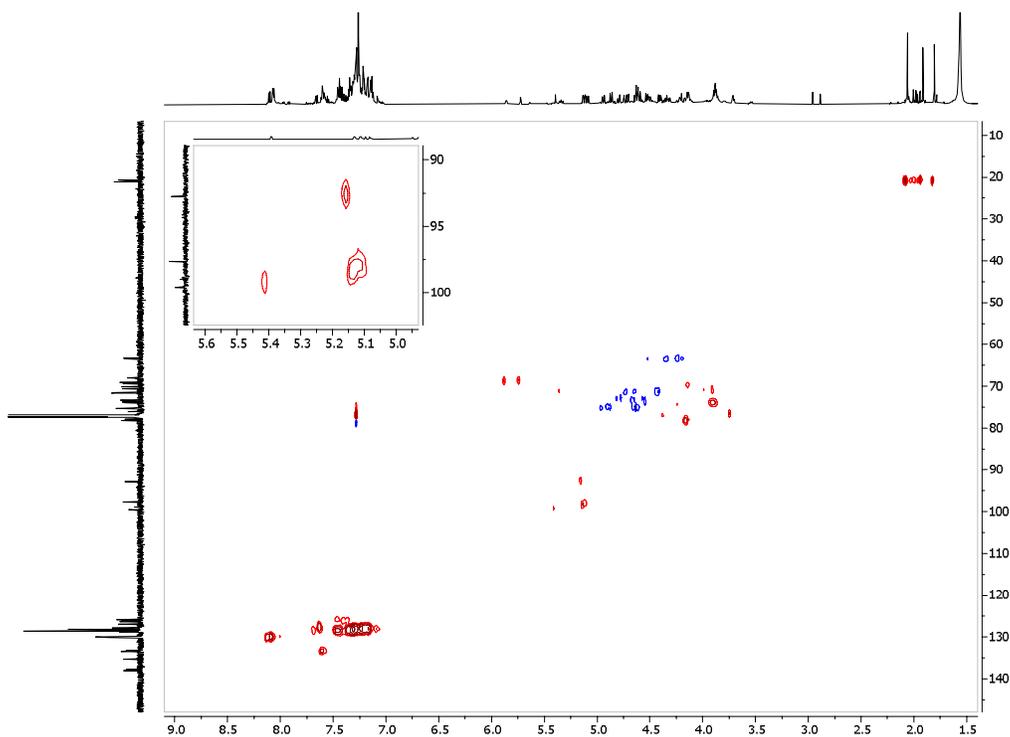
^{13}C NMR (176 MHz, CDCl_3) of **19**:



Coupled ^{13}C , ^1H HSQC of **19**:

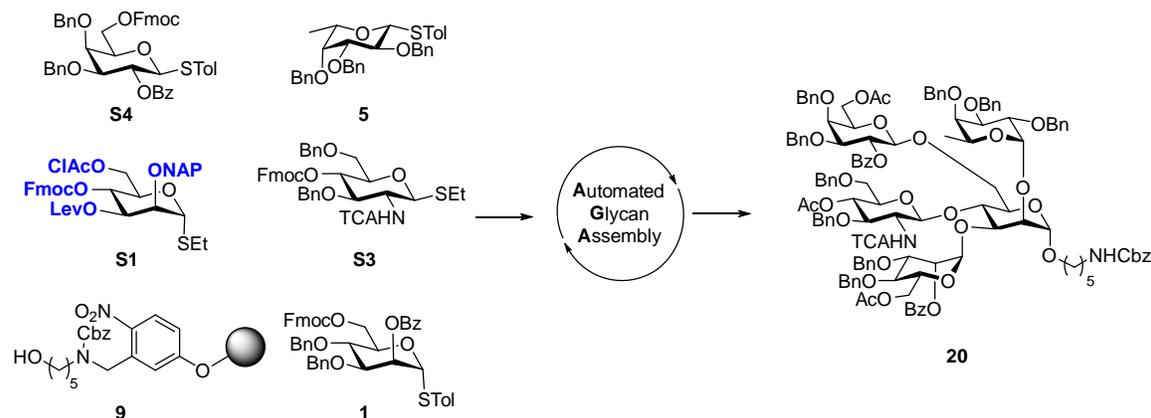


^{13}C , ^1H HSQC of **19**:



5.5.5 Hyperbranched Mannose Pentamer (20)

***N*-Benzyloxycarbonyl-5-amino-pentyl 2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)-[6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl- α -D-mannopyranosyl-(1 \rightarrow 3)]-[4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetyl- β -D-glucopyranosyl-(1 \rightarrow 4)]-[6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 6)]- α -D-mannopyranoside (20)**



Repeat	Building Blocks	Modules	Notes
1x	S1 (2 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 35 min (t ₂)
		III – Capping	
		IVc – Fmoc Deprotection	
1x	S3 (6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 2 cycles	-20 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 45 min (t ₂)
		III – Capping	
		IVb – Lev Deprotection	
1x	1 (3 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside	-20 °C (T ₁) 10 min (t ₁) 0 °C (T ₂) 25 min (t ₂)
		IVe – ClAc Deprotection	
1x	S4 (3 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 3 cycles	-30 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 25 min (t ₂)
		IVd – NAP Deprotection	
1x	5 (3 x 6.5 equiv.)	I – Acidic Wash	
		IIa – Glycosylation with thioglycoside – 3 cycles	-30 °C (T ₁) 10 min (t ₁) -10 °C (T ₂) 25 min (t ₂)
		IVa – Fmoc Deprotection (6 cycles)	
		III – Capping (4 cycles)	

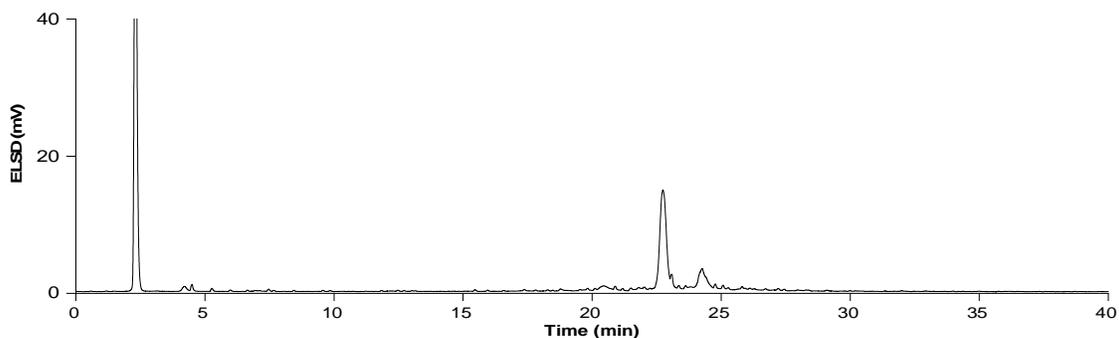
Photocleavage from solid support using **Method A-1** followed by purification by normal-phase HPLC (**Method B-3**, $t_R = 22.8$ min) afforded compound **20** (12 mg, 0.005 mmol, **32%**) as a colorless oil.

^1H NMR (600 MHz, $[\text{D}_6]\text{-DMSO}$): δ 8.02 (dd, $J = 8.2, 1.6$ Hz, 2H), 7.91 (dd, $J = 8.2, 1.3$ Hz, 2H), 7.66 – 7.62 (m, 1H), 7.61 – 7.55 (m, 1H), 7.48 (dt, $J = 17.4, 7.8$ Hz, 5H), 7.35 – 7.31 (m, 12H), 7.30 – 7.19 (m, 27H), 7.19 – 7.13 (m, 10H), 5.33 (dd, $J = 10.2, 8.0$ Hz, 1H), 5.22 (d, $J = 2.2$ Hz, 1H), 5.04 – 5.00 (m, 3H), 4.87 (d, $J = 11.6$ Hz, 1H), 4.80 – 4.41 (m, 22H), 4.37 – 4.19 (m, 5H), 4.17 – 4.09 (m, 3H), 4.05 – 3.81 (m, 12H), 3.78 – 3.71 (m, 2H), 3.63 – 3.54 (m, 3H), 2.94 – 2.88 (m, 2H), 1.98 (s, 3H), 1.96 (s, 3H), 1.83 (s, 3H), 1.30 – 1.24 (m, 8H), 1.03 (d, $J = 6.2$ Hz, 3H) ppm.

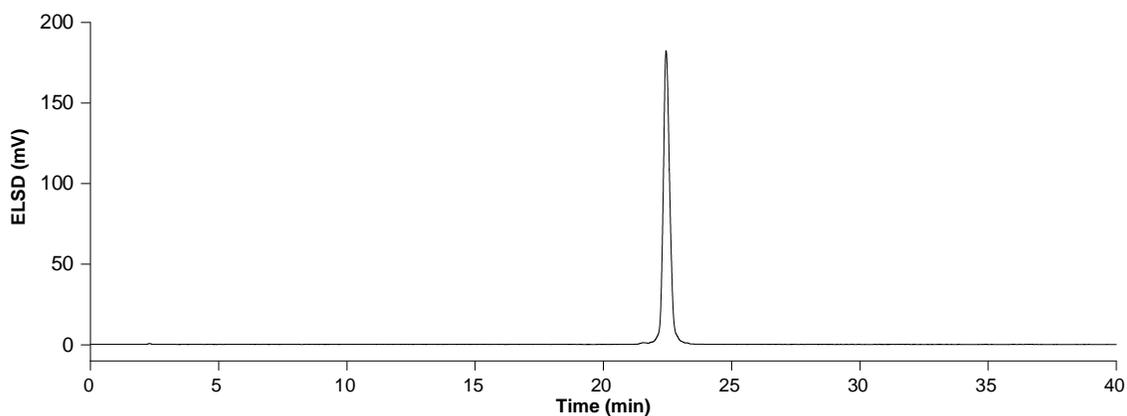
^{13}C NMR (151 MHz, $[\text{D}_6]\text{-DMSO}$, as per HSQC): δ 128.6, 128.5, 132.7, 132.3, 127.8, 127.1, 127.1, 126.7, 126.7, 71.2, 98.3, 95.2, 64.5, 73.3, 100.1, 73.6, 70.5, 100.2, 99.1, 69.5, 73.2, 71.6, 70.7, 70.4, 73.5, 72.0, 72.0, 62.3, 61.8, 61.7, 72.6, 69.3, 77.4, 65.9, 73.4, 79.0, 75.0, 77.9, 71.3, 55.9, 71.1, 77.2, 71.5, 39.7, 20.2, 19.8, 28.2, 27.8, 15.8 ppm.

HRMS (QToF): Calcd for $\text{C}_{128}\text{H}_{137}\text{Cl}_3\text{N}_2\text{O}_{32}\text{Na}$ $[\text{M} + \text{Na}]^+$ 2341.8112; found 2341.8408.

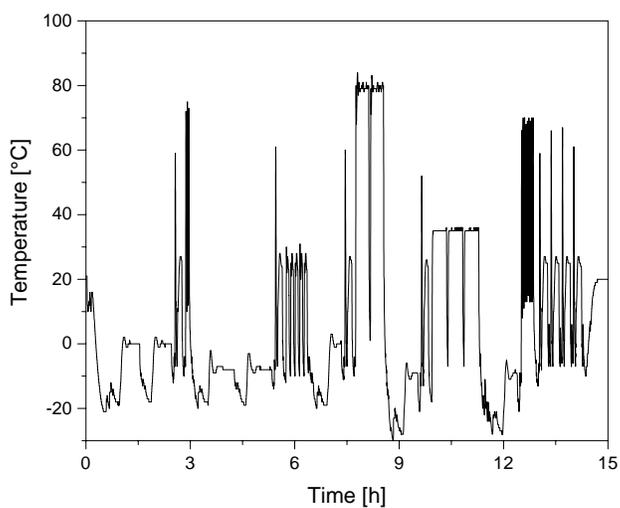
NP-HPLC of crude **20** (ELSD trace, **Method B-2**, $t_R = 22.5$ min):



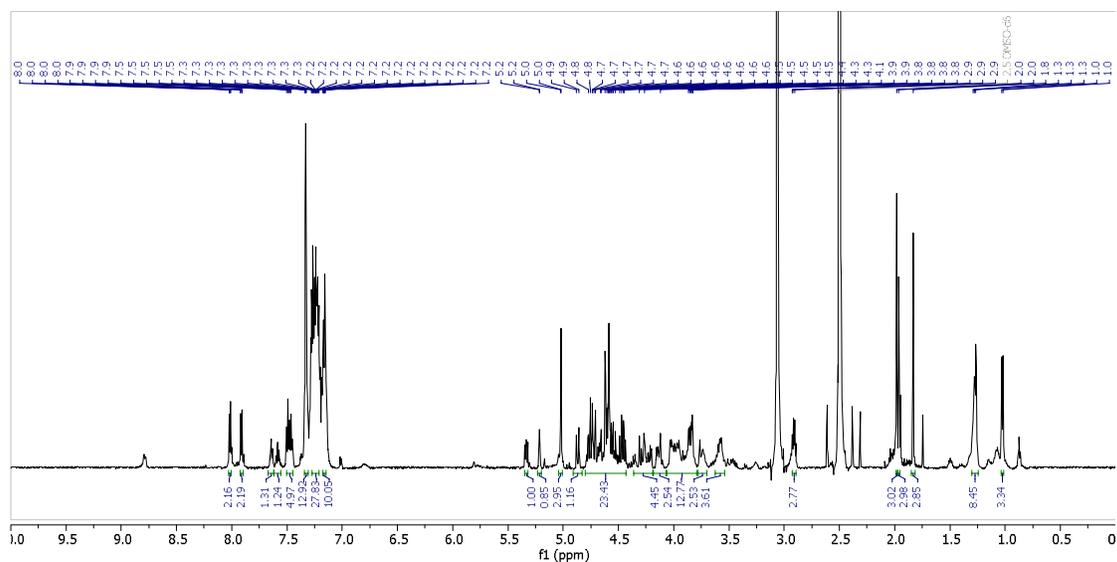
NP-HPLC of purified **20** (ELSD trace, **Method B-2**, $t_R = 22.5$ min):



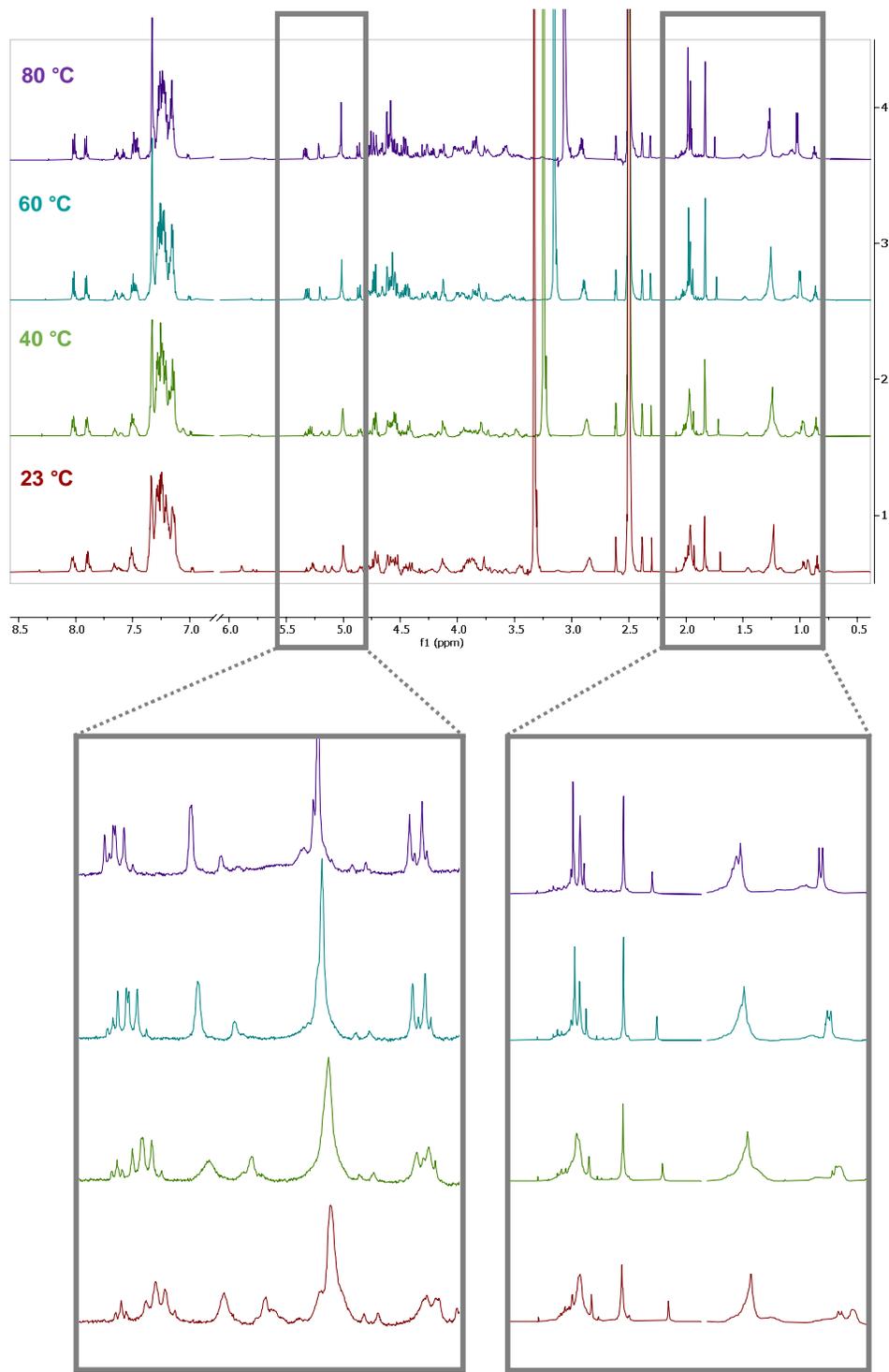
Temperature profile inside the reaction vessel during the synthesis of **20**:



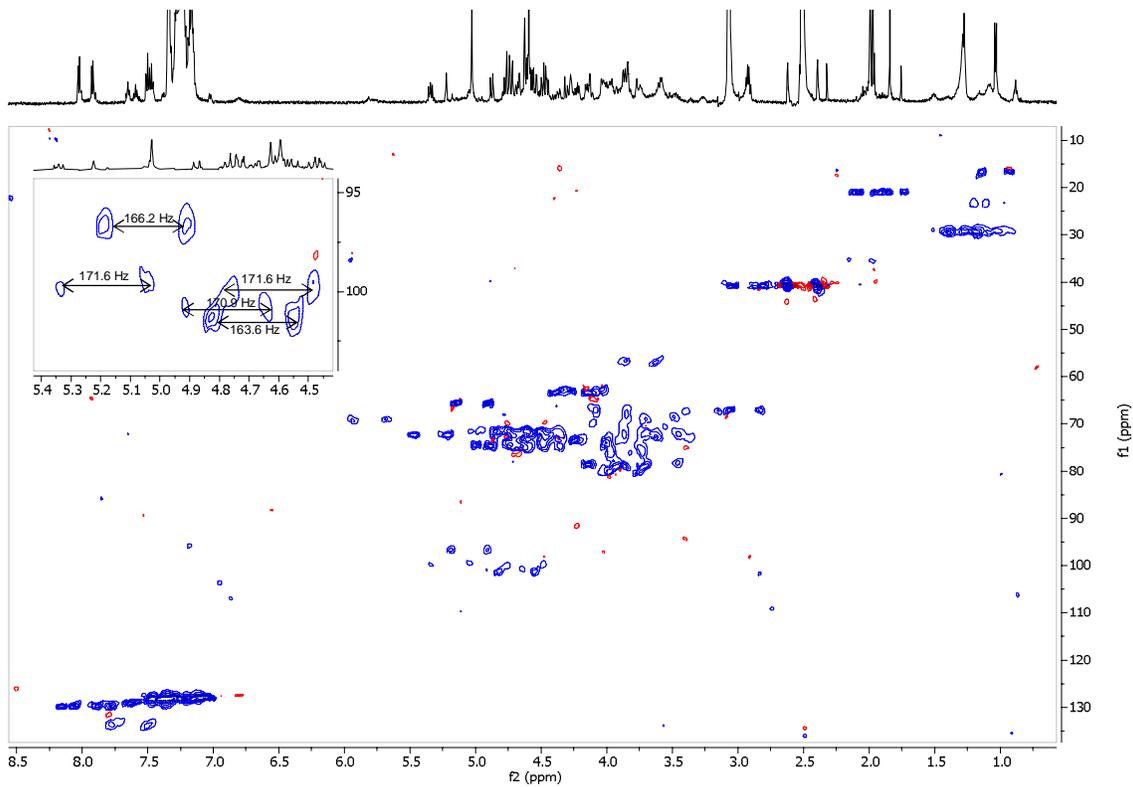
^1H NMR (600 MHz, $[\text{D}_6]$ -DMSO, 80 °C) of **20**:



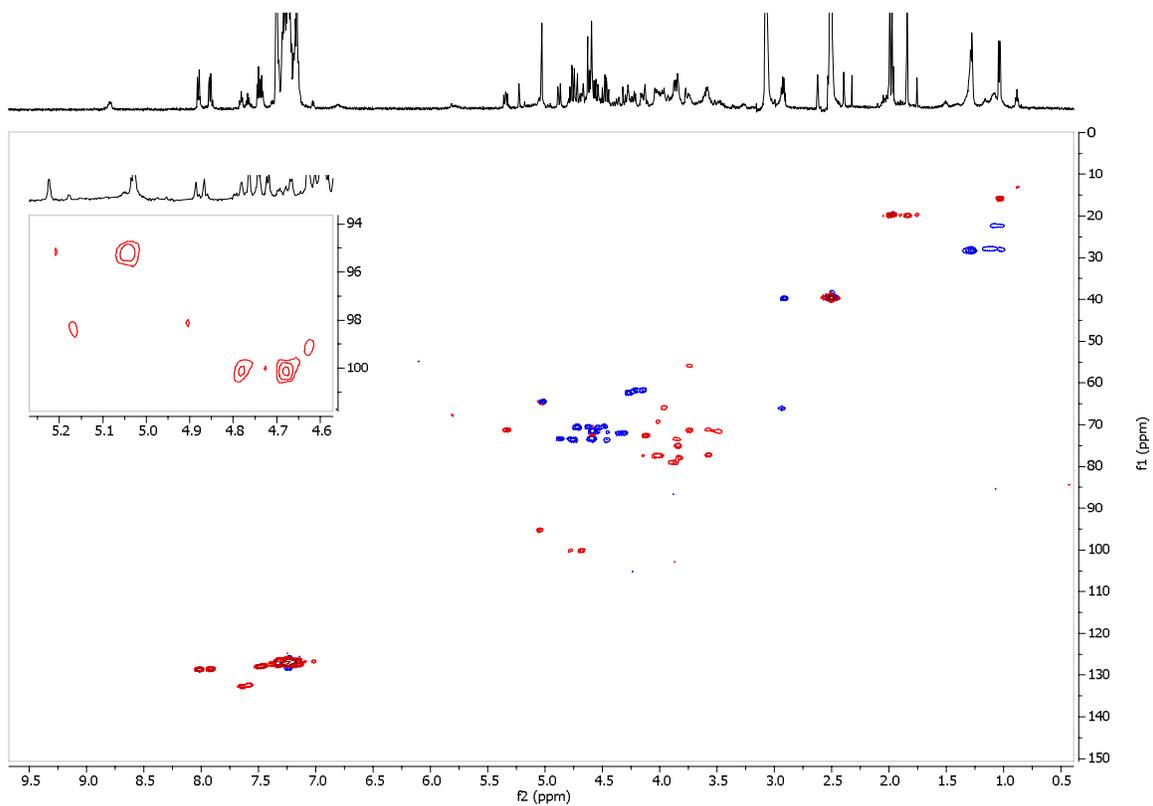
^1H NMR (600 MHz, $[\text{D}_6]$ -DMSO, 23 to 80 °C) of **20**:



Coupled ^{13}C , ^1H HSQC of **20** (80 °C):



^{13}C , ^1H HSQC of **20** (80 °C):



6 Preparation of Orthogonal Building Block 7

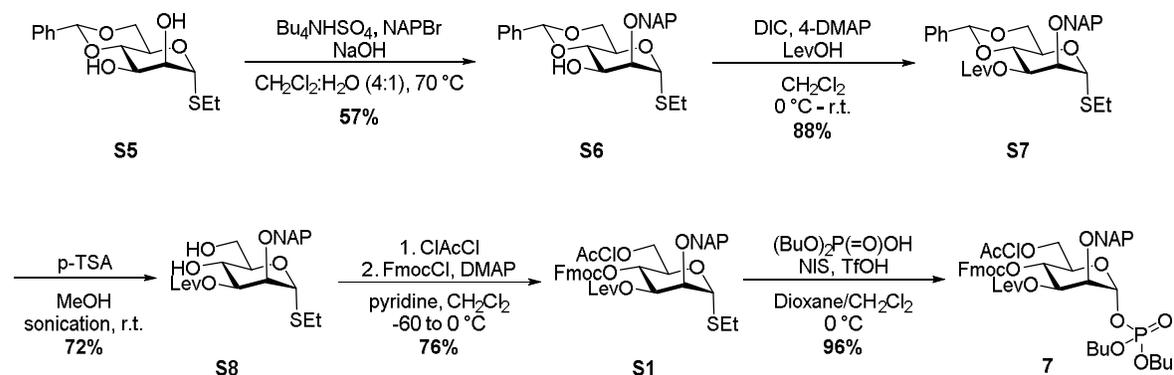
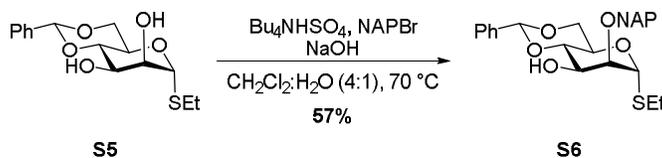


Figure S-3. Synthesis of orthogonal building block 7.

Ethyl 4,6-O-benzylidene-2-O-(2-naphthalenylmethyl)-1-thio- α -D-mannopyranoside (**S6**)



To a suspension of diol **S5** (20.0 g, 64.03 mmol, 1.0 equiv.) in CH_2Cl_2 (300 mL) was added Bu_4NHSO_4 (3.0 g, 12.17 mmol, 0.2 equiv.), followed by addition of 2-(bromomethyl)naphthalene (15.6 g, 70.43 mmol, 1.1 equiv.). An aqueous solution of NaOH (4.0 g in 100 mL) was added and the biphasic solution was refluxed at 70 °C overnight. The mixture was allowed to cool down to room temperature and the aqueous phase was extracted with CH_2Cl_2 . The combined organic phase was washed with citric acid solution, dried over Na_2SO_4 , filtered and concentrated. The title compound **S6** (16.8 g, 36.32 mmol, 57%) was obtained as a white solid after purification by column chromatography (SiO_2 , Hex/EtOAc 3:1).

$R_f = 0.44$ (Hex/EtOAc 3:1).

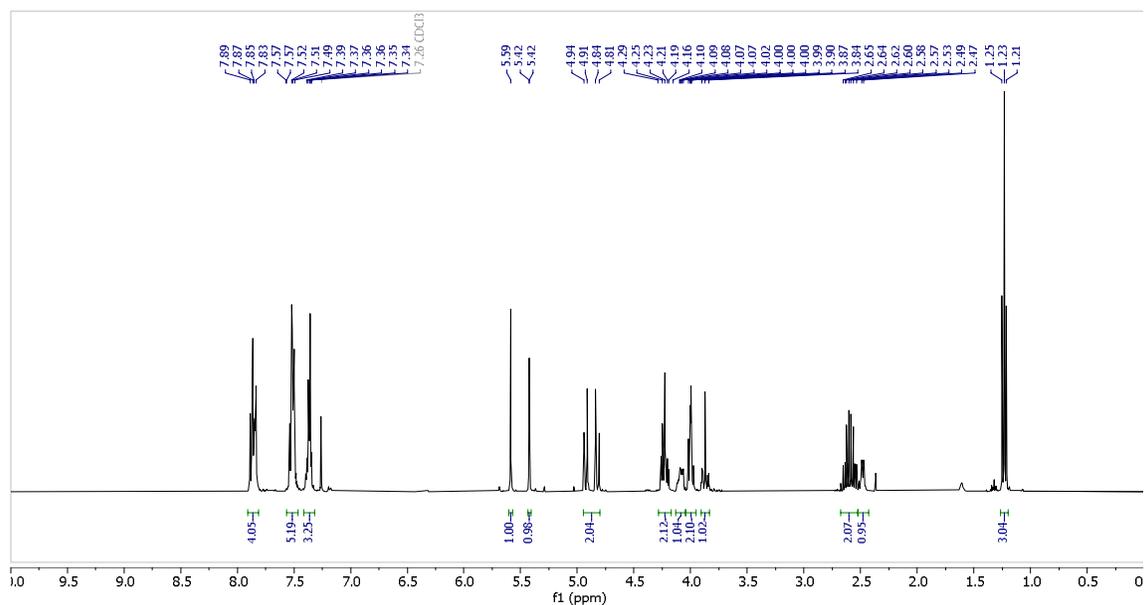
$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.91 – 7.79 (m, 4H), 7.59 – 7.44 (m, 5H), 7.44 – 7.31 (m, 3H), 5.59 (s, 1H), 5.42 (d, $J = 1.2$ Hz, 1H), 4.98 – 4.78 (m, 2H), 4.31 – 4.16 (m, 2H), 4.13 – 4.05 (m, 1H), 4.04 – 3.94 (m, 2H), 3.94 – 3.82 (m, 1H), 2.70 – 2.51 (m, 2H), 2.48 (d, $J = 7.9$ Hz, 1H), 1.23 (t, $J = 7.4$ Hz, 3H) ppm.

$^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 137.4, 134.9, 133.3, 133.2, 129.3, 128.7, 128.4, 128.1, 127.9, 127.1, 126.4, 126.4, 126.3, 125.9, 102.3, 82.6, 80.2, 79.9, 73.4, 69.2, 68.7, 64.0, 25.4, 15.0 ppm.

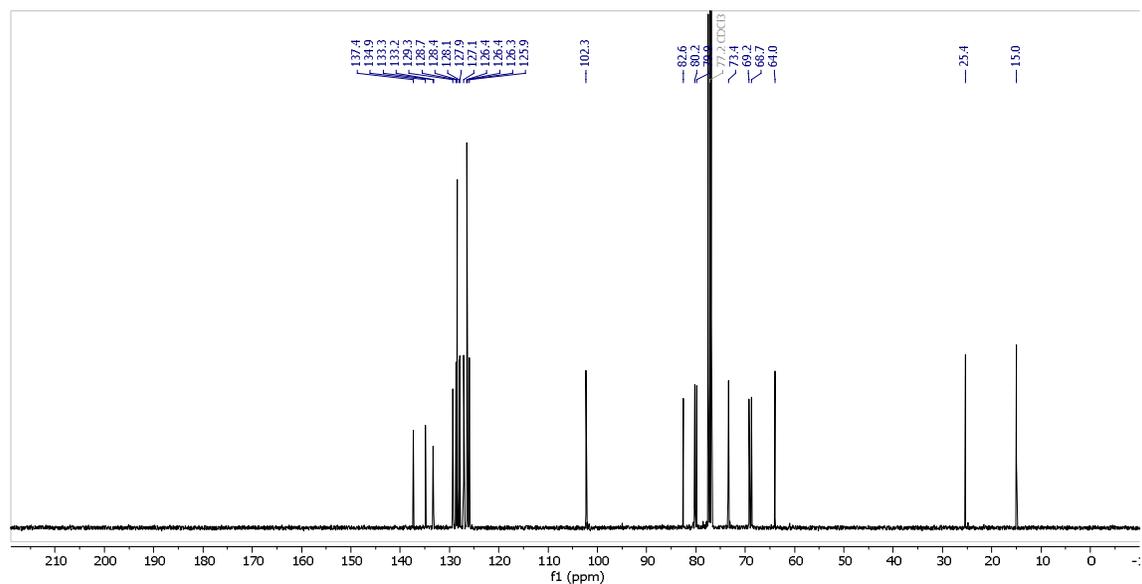
$[\alpha]_D^{25} 31.11 \text{ cm}^{-1}$ (c 1, CHCl_3).

IR (film): 3470, 3385, 3318, 2931, 2906, 2876, 1702, 1600, 1510, 1455, 1422, 1383, 1271, 1244, 1209, 1168, 1088, 1057, 1048, 1035, 972, 749 cm^{-1} . HRMS (QToF): Calcd for $\text{C}_{26}\text{H}_{28}\text{O}_5\text{SNa}$ $[\text{M} + \text{Na}]^+$ 475.1550; found 475.1552.

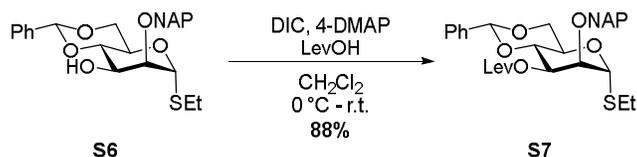
^1H NMR (400 MHz, CDCl_3) of **S6**:



^{13}C NMR (101 MHz, CDCl_3) of **S6**:



Ethyl 4,6-O-benzylidene-2-O-levulinyl-2-O-(2-naphthalenylmethyl)-1-thio- α -D-mannopyranoside (S7)



To a solution of **S6** (15.8 g, 34.91 mmol, 1.0 equiv.) and levulinic acid (7.1 mL, 69.82 mmol, 2.0 equiv.) in anhydrous CH_2Cl_2 (50 mL) was added dropwise a premixed solution of DIC (16.1 mL, 104.7 mmol, 3.0 equiv.) and DMAP (0.9 g, 6.98 mmol, 0.2 equiv.) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The reaction was stirred at room temperature overnight. The reaction mixture was filtered through Celite, the filtrate was washed with aqueous NaHCO_3 and the aqueous phase was extracted with CH_2Cl_2 . The combined organic phase was dried over Na_2SO_4 , filtered and concentrated. The title compound **S7** (17.0 g, 30.87 mmol, 88%) was obtained as a yellow syrup after purification by column chromatography (SiO_2 , Hex/EtOAc 3:1).

$R_f = 0.25$ (Hex/EtOAc 3:1).

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.88 – 7.81 (m, 4H), 7.55 (dd, $J = 8.5, 1.8$ Hz, 1H), 7.52 – 7.44 (m, 4H), 7.39 – 7.32 (m, 3H), 5.58 (s, 1H), 5.33 (d, $J = 1.5$ Hz, 1H), 5.23 (dd, $J = 9.9, 3.5$ Hz, 1H), 4.83 (q, $J = 12.0$ Hz, 2H), 4.34 – 4.21 (m, 3H), 4.14 (dd, $J = 3.5, 1.4$ Hz, 1H), 3.90 (t, $J = 10.0$ Hz, 1H), 2.66 – 2.48 (m, 6H), 2.08 (s, 3H), 1.21 (t, $J = 7.5$ Hz, 3H) ppm.

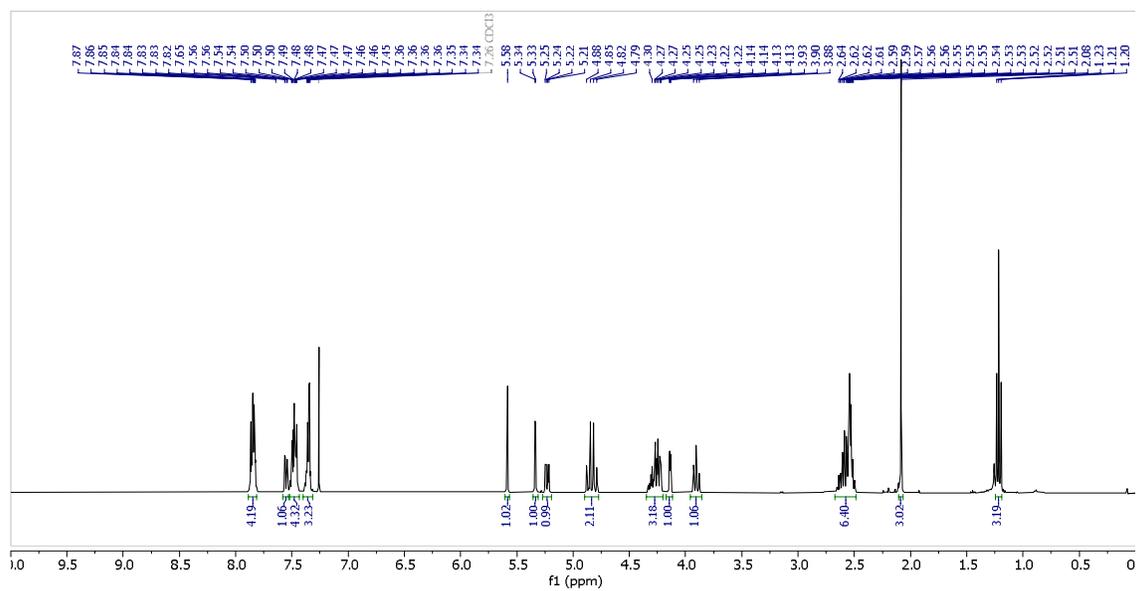
$^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 206.5, 172.2, 137.4, 135.2, 133.3, 133.2, 129.2, 128.5, 128.4, 128.1, 127.8, 127.3, 126.4, 126.4, 126.3, 126.2, 101.3, 83.3, 79.2, 76.5, 73.7, 71.2, 68.8, 64.6, 37.8, 29.9, 28.0, 25.5, 14.9 ppm.

$[\alpha]_D^{25} 32.53 \text{ cm}^{-1}$ (c 1, CHCl_3).

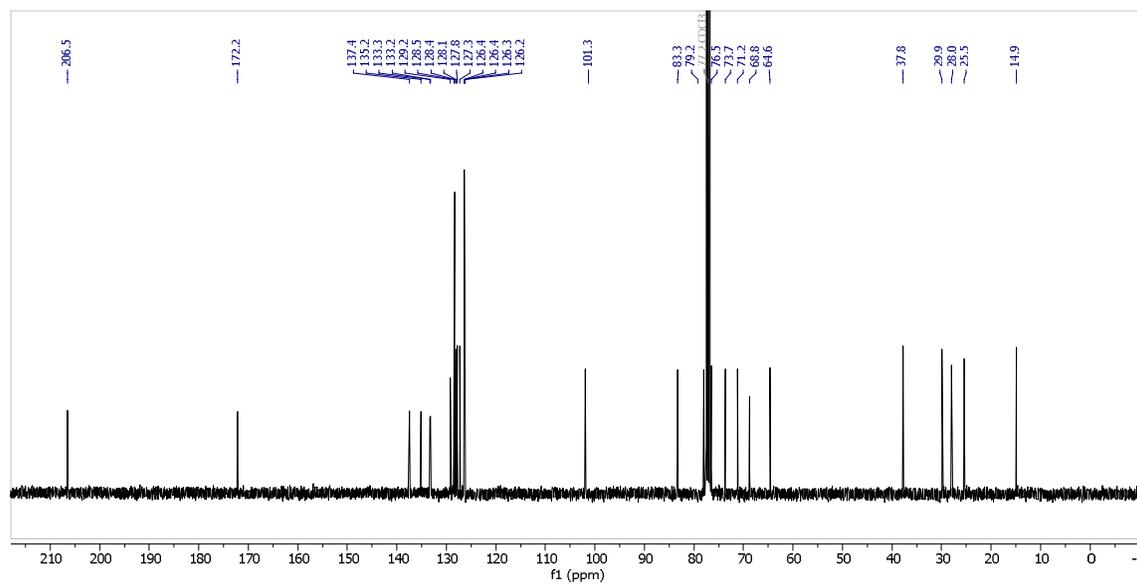
IR (film): 2930, 1741, 1719, 1365, 1206, 1180, 1157, 1097, 1014, 753, 700 cm^{-1} .

HRMS (QToF): Calcd for $\text{C}_{31}\text{H}_{34}\text{O}_7\text{SNa}$ $[\text{M} + \text{Na}]^+$ 573.1917; found 573.1921.

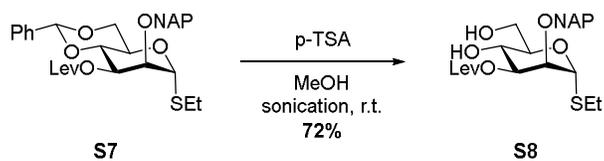
^1H NMR (400 MHz, CDCl_3) of **S7**:



^{13}C NMR (101 MHz, CDCl_3) of **S7**:



Ethyl 2-O-levulinyl-2-O-(2-naphthalenylmethyl)-1-thio- α -D-mannopyranoside (**S8**)



To a suspension of **S7** (16.5 g, 36.46 mmol, 1.0 equiv.) in MeOH (100 mL) was added *p*-TSA (3.1 g, 18.23 mmol, 0.5 equiv.). The reaction mixture was sonicated for one hour at room temperature before pyridine (5 mL) was added. The solvent was removed and the residue was redissolved in ethyl acetate, washed with water, dried over Na₂SO₄, filtered and concentrated. The title compound **S8** (12.2 g, 26.37 mmol, 72%) was obtained as a colorless oil after purification by column chromatography (SiO₂, Hex/EtOAc = 1:1 to 2:3).

R_f = 0.25 (Hex/EtOAc 2:3).

¹H NMR (400 MHz, CDCl₃): δ 7.87 – 7.77 (m, 4H), 7.54 – 7.44 (m, 3H), 5.35 (d, J = 1.5 Hz, 1H), 5.08 (dd, J = 9.8, 3.3 Hz, 1H), 4.88 – 4.70 (m, 2H), 4.17 (t, J = 9.7 Hz, 1H), 4.11 – 4.02 (m, 1H), 3.98 (dd, J = 3.4, 1.5 Hz, 1H), 3.90 (s, 2H), 2.74 – 2.37 (m, 6H), 2.12 (s, 3H), 1.23 (t, J = 7.4 Hz, 3H) ppm.

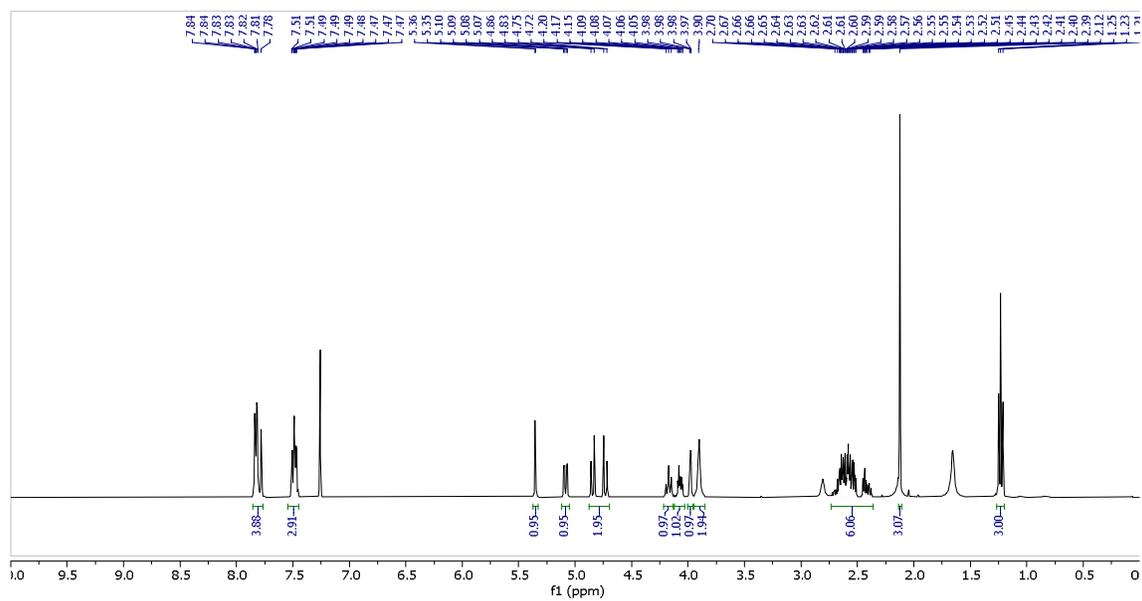
¹³C NMR (101 MHz, CDCl₃): δ 207.4, 172.8, 135.2, 133.2, 133.1, 128.3, 127.9, 127.7, 126.8, 126.3, 126.1, 125.9, 82.1, 77.5, 74.7, 73.0, 72.4, 66.9, 62.7, 38.1, 29.8, 28.1, 25.2, 14.7 ppm.

$[\alpha]_D^{25}$ 5.33 cm⁻¹ (c 1, CHCl₃).

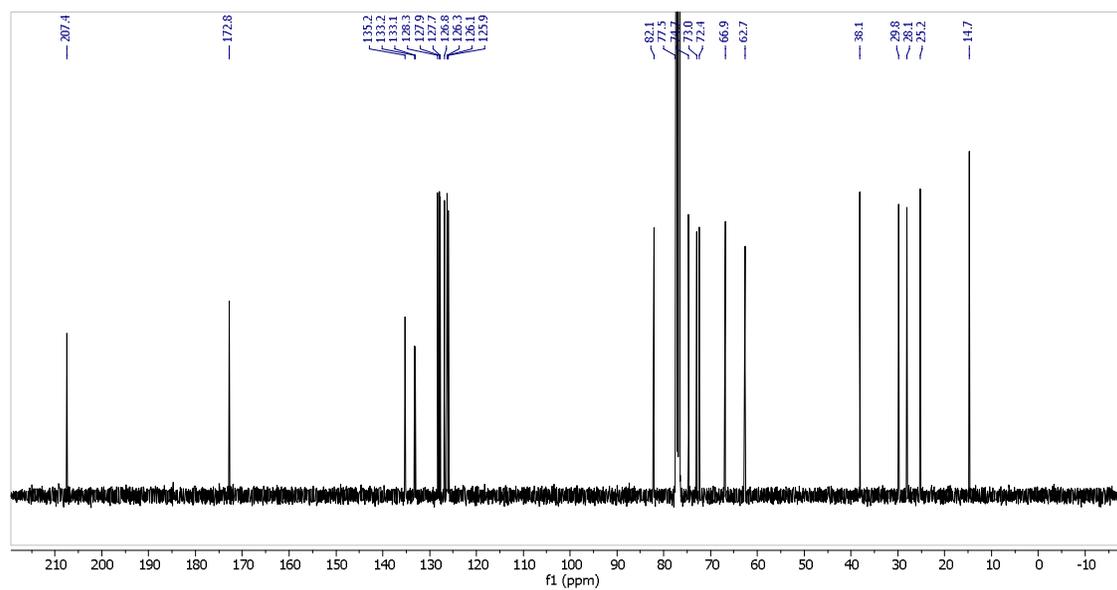
IR (film): 3456, 2928, 1720, 1365, 1159, 1083, 774 cm⁻¹.

HRMS (QToF): Calcd for C₂₄H₃₀O₇SNa [M + Na]⁺ 485.1604; found 485.1605.

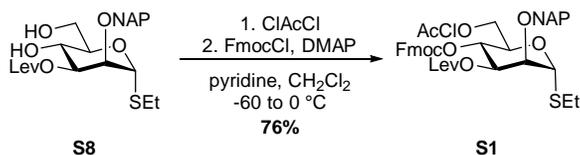
^1H NMR (400 MHz, CDCl_3) of **S8**:



^{13}C NMR (101 MHz, CDCl_3) of **S8**:



Ethyl 6-O-(2-chloroacetyl)-4-O-fluorenylmethoxycarbonyl-2-O-levulinyl-2-O-(2-naphthalenylmethyl)-1-thio- α -D-mannopyranoside (S1)



To a solution of **S8** (2.0 g, 4.32 mmol, 1.0 equiv.) and anhydrous pyridine (1.75 mL, 21.62 mmol, 5.0 equiv.) in anhydrous CH_2Cl_2 (20 mL) at $-60 \text{ }^\circ\text{C}$ was added chloroacetyl chloride (0.38 mL, 4.76 mmol, 1.1 equiv.). The reaction was stirred for 10 min, aqueous citric acid solution (2 mL) was added and the mixture was allowed to warm up to room temperature. Water was added and the aqueous phase was extracted with CH_2Cl_2 . The combined organic phase was dried over Na_2SO_4 , filtered and concentrated. The residue ($R_f = 0.53$ (Hex/AcOEt 2:3)) was dissolved in anhydrous CH_2Cl_2 (30 mL) and anhydrous pyridine (1.75 mL, 21.62 mmol, 5.0 equiv.) was added followed by a solution of FmocCl (1.7 g, 6.47 mmol, 1.5 equiv.) in anhydrous CH_2Cl_2 (20 mL) at $0 \text{ }^\circ\text{C}$. DMAP (53 mg, 0.43 mmol, 0.1 equiv.) was added and the reaction mixture was stirred for one hour at $0 \text{ }^\circ\text{C}$. Aqueous citric acid solution (10 mL) was added and the mixture was allowed to warm up to room temperature. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phase was dried over Na_2SO_4 , filtered and concentrated. The title compound **S1** (2.5 g, 3.28 mmol, 76%) was obtained as a white solid after purification by column chromatography (SiO_2 , Hex/EtOAc = 9:1 to 3:1).

$R_f = 0.26$ (Hex/EtOAc 3:1).

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.87 – 7.72 (m, 6H), 7.64 – 7.57 (m, 2H), 7.54 – 7.45 (m, 3H), 7.44 – 7.38 (m, 2H), 7.35 – 7.28 (m, 2H), 5.38 – 5.30 (m, 2H), 5.22 (dd, $J = 10.1, 3.2$ Hz, 1H), 4.89 – 4.74 (m, 2H), 4.50 – 4.24 (m, 6H), 4.10 (d, $J = 2.1$ Hz, 2H), 4.06 (dd, $J = 3.2, 1.6$ Hz, 1H), 2.69 – 2.52 (m, 2H), 2.49 – 2.34 (m, 4H), 2.00 (s, 3H), 1.24 (t, $J = 7.4$ Hz, 3H) ppm.

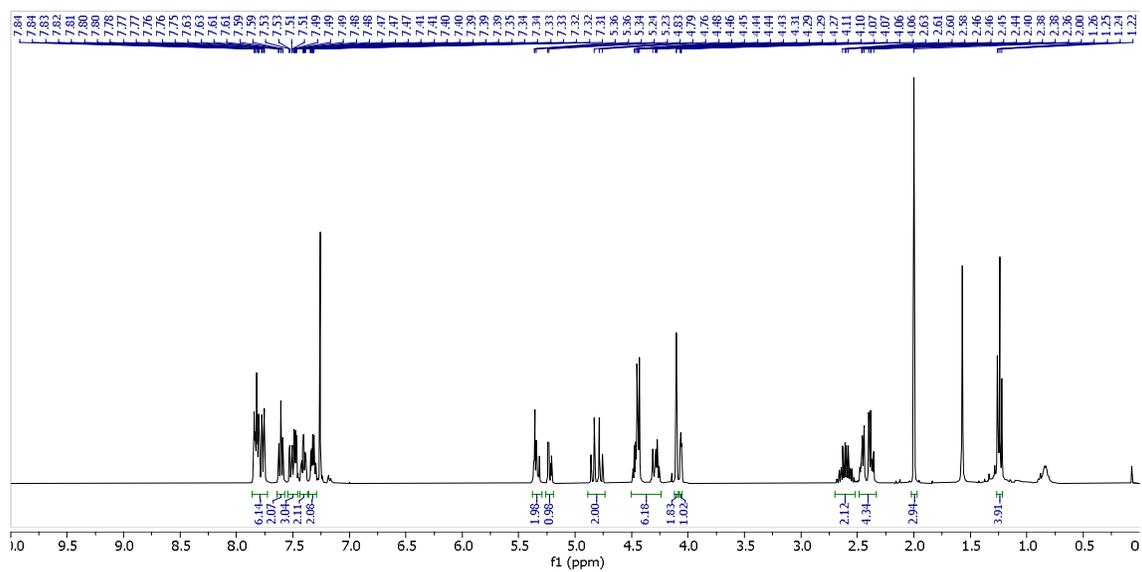
$^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 206.2, 171.9, 167.3, 154.5, 143.4, 143.3, 141.4, 141.4, 135.1, 133.3, 133.2, 129.2, 128.4, 128.4, 128.1, 128.0, 127.8, 127.4, 127.3, 127.0, 126.4, 126.2, 126.1, 125.4, 125.4, 125.2, 120.2, 120.2, 82.4, 73.2, 71.8, 70.8, 70.5, 68.6, 64.2, 46.7, 40.9, 37.6, 29.8, 29.8, 27.9, 25.5, 14.9 ppm.

$[\alpha]_D$ 8.93 cm^{-1} (c 1, CHCl_3).

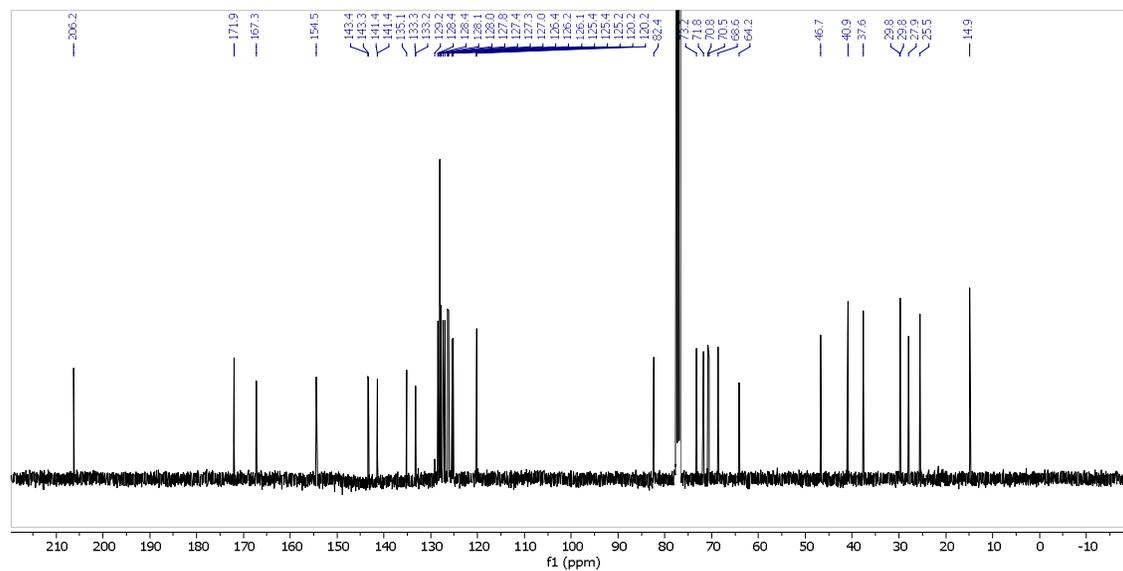
IR (film): 2958, 1754, 1720, 1451, 1364, 1261, 1156, 1101, 989, 760, 744 cm^{-1} .

HRMS (QToF): Calcd for $\text{C}_{41}\text{H}_{41}\text{SO}_{10}\text{ClNa}$ $[\text{M} + \text{Na}]^+$ 783.2001; found 783.2043.

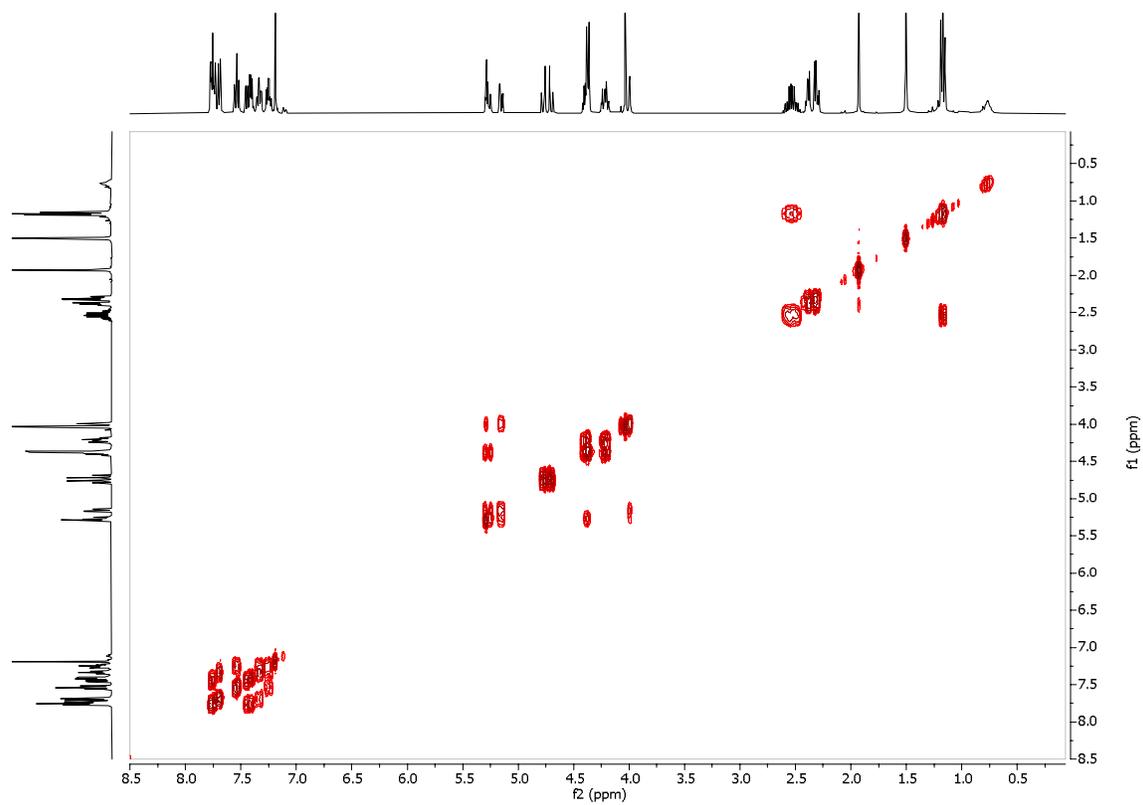
^1H NMR (400 MHz, CDCl_3) of **S1**:



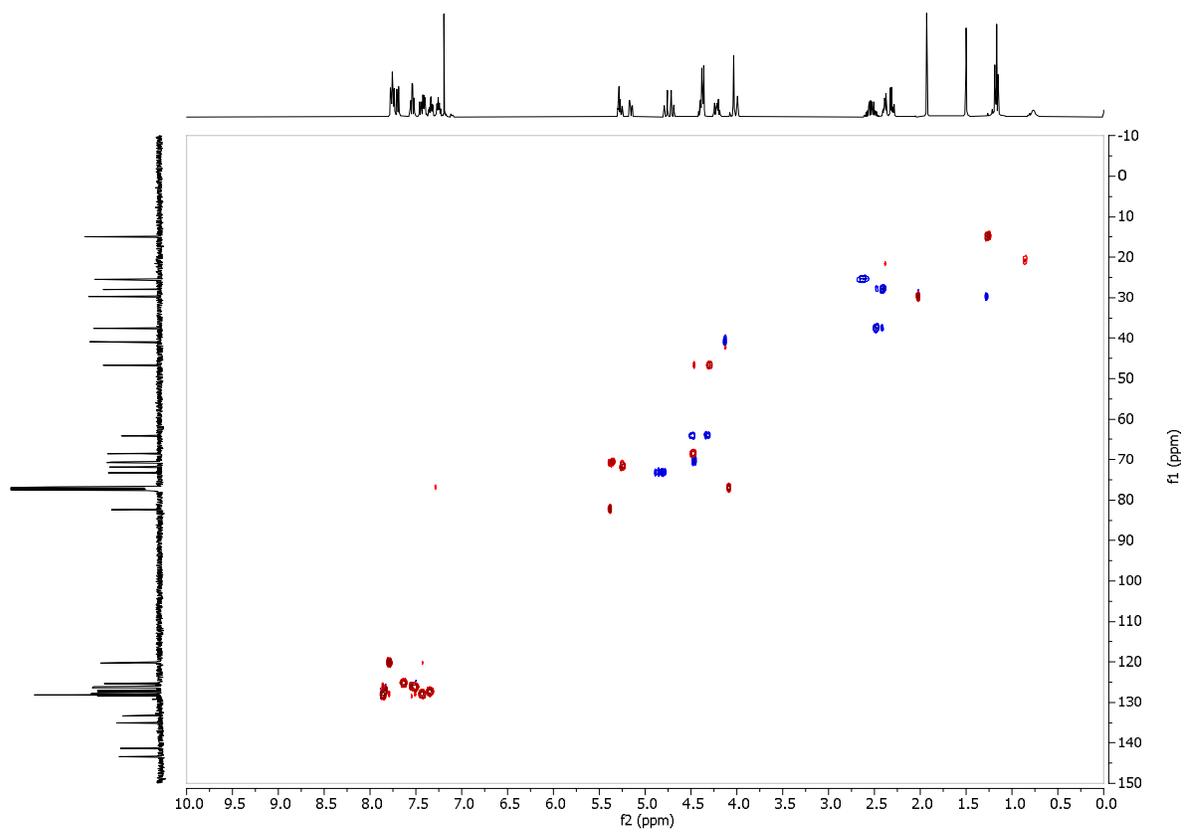
^{13}C NMR (101 MHz, CDCl_3) of **S1**:



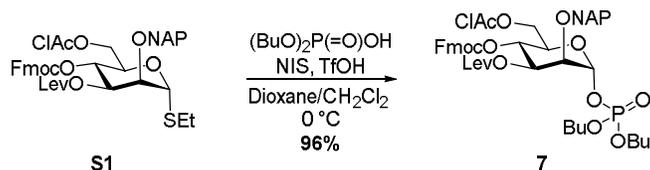
$^1\text{H}, ^1\text{H}$ COSY of **S1**:



$^{13}\text{C}, ^1\text{H}$ HSQC of **S1**:



Dibutoxyphosphoryloxy 6-O-(2-chloroacetyl)-4-O-fluorenylmethoxycarbonyl-2-O-levulinyl-2-O-(2-naphthalenylmethyl)- α -D-mannopyranoside (7)



To a solution of thioglycoside **S1** (1.0 g, 1.31 mmol, 1.0 equiv.) and dibutyl hydrogen phosphate (0.52 mL, 2.63 mmol, 2.0 equiv.) in anhydrous CH₂Cl₂ (6 mL) a solution of NIS (530 mg, 2.36 mmol, 1.8 equiv.) and TfOH (35 μ L, 0.39 mmol, 0.3 equiv.) in anhydrous CH₂Cl₂/dioxane (1:1, 4 mL) was added dropwise at 0 °C. The reaction was stirred at the same temperature for 2 h. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and 10% sodium thiosulfate solution was added. The bilayer mixture was extracted with CH₂Cl₂, dried over Na₂SO₄, filtered and concentrated. The title compound **7** (1.2 g, 1.26 mmol, 96%) was obtained as a white solid after purification by column chromatography (SiO₂, Hex/EtOAc = 2:1).

R_f = 0.23 (Hex/EtOAc 3:1).

¹H NMR (400 MHz, CDCl₃): δ 7.86 – 7.74 (m, 6H), 7.60 (t, J = 7.3 Hz, 2H), 7.54 – 7.46 (m, 3H), 7.45 – 7.36 (m, 2H), 7.33 (dd, J = 8.7, 3.7 Hz, 2H), 5.68 (dd, J = 6.4, 2.0 Hz, 1H), 5.43 – 5.28 (m, 2H), 4.86 – 4.82 (m, 2H), 4.48 – 4.25 (m, 5H), 4.26 – 4.20 (m, 1H), 4.10 (d, J = 3.1 Hz, 2H), 4.07 – 3.95 (m, 5H), 2.50 – 2.36 (m, 4H), 2.01 (s, 3H), 1.65 – 1.54 (m, 4H), 1.42 – 1.30 (m, 4H), 0.92 (q, J = 7.5 Hz, 6H) ppm.

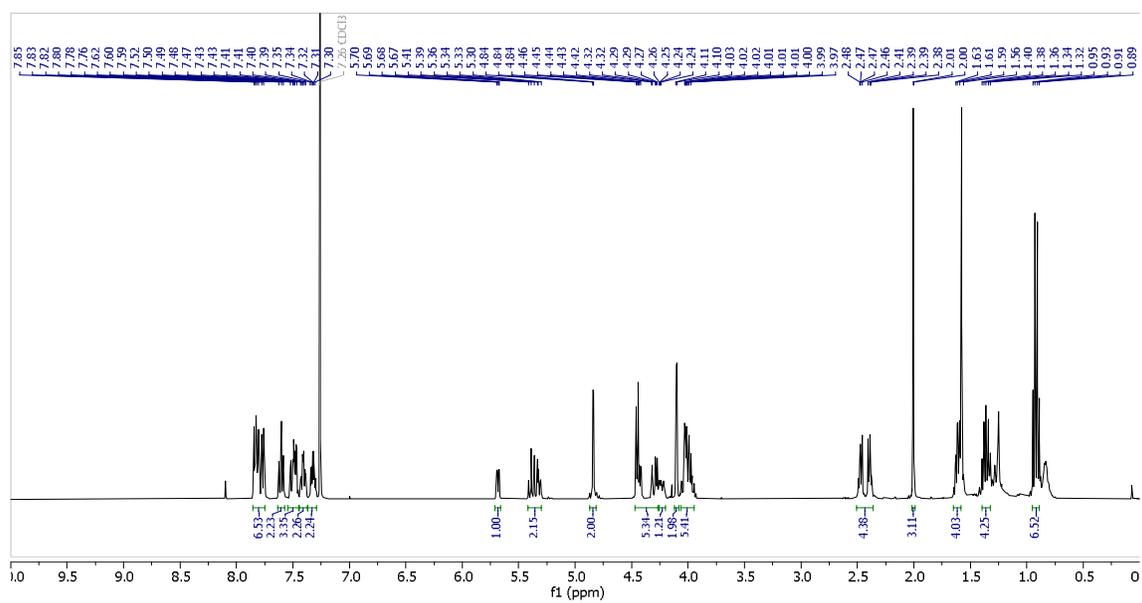
¹³C NMR (101 MHz, CDCl₃): δ 205.2, 172.3, 167.3, 155.1, 143.4, 142.2, 134.8, 133.8, 128.5, 128.1, 127.8, 127.4, 127.3, 127.2, 126.4, 126.3, 126.1, 125.4, 125.2, 119.8, 95.9, 75.1, 73.8, 70.6, 70.5, 70.0, 69.9, 68.2, 63.6, 46.7, 40.9, 37.6, 32.4, 29.7, 27.9, 18.7, 13.7 ppm.

$[\alpha]_D$ -6.32 cm⁻¹ (c 1, CHCl₃).

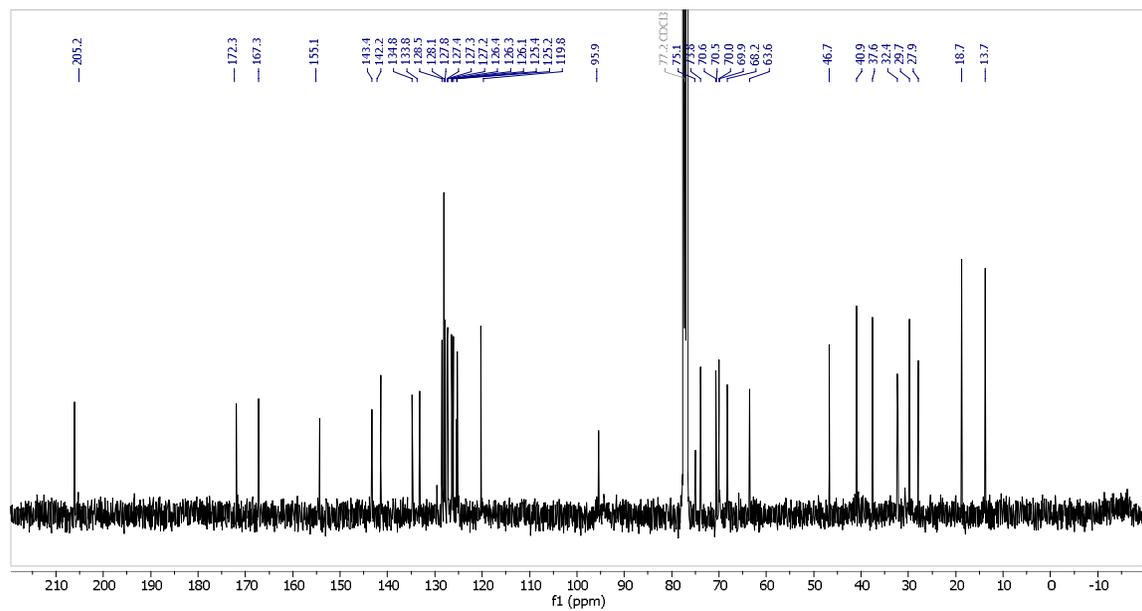
IR (film): 2964, 1754, 1720, 1451, 1365, 1261, 1153, 1029, 957, 744 cm⁻¹.

HRMS (QToF): Calcd for C₄₇H₅₄ClPO₁₄Na [M + Na]⁺ 931.2832; found 931.2884.

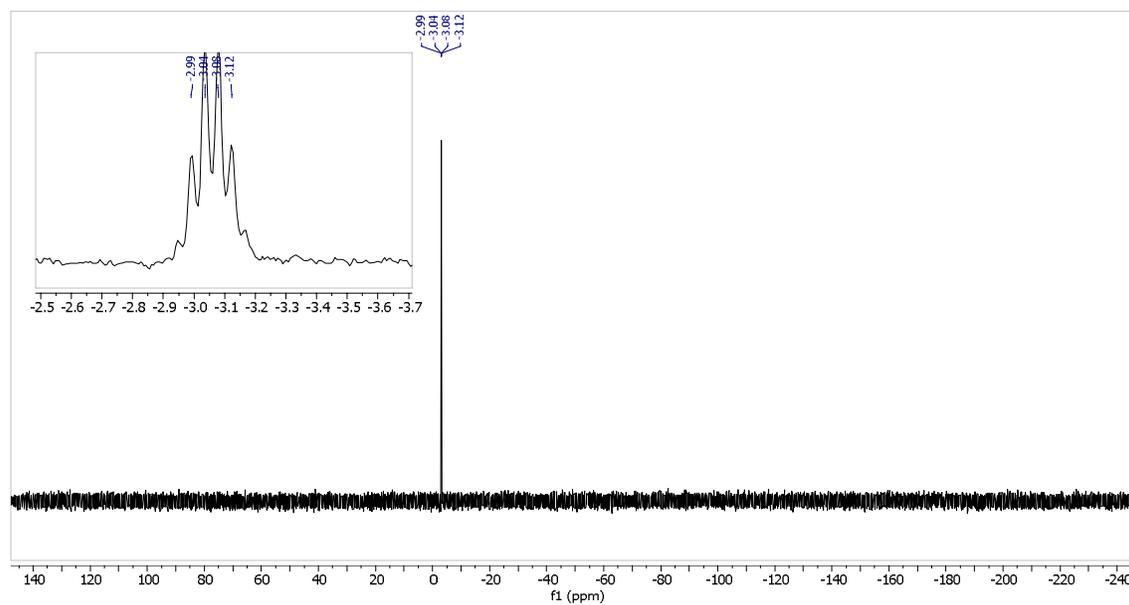
¹H NMR (400 MHz, CDCl₃) of 7:



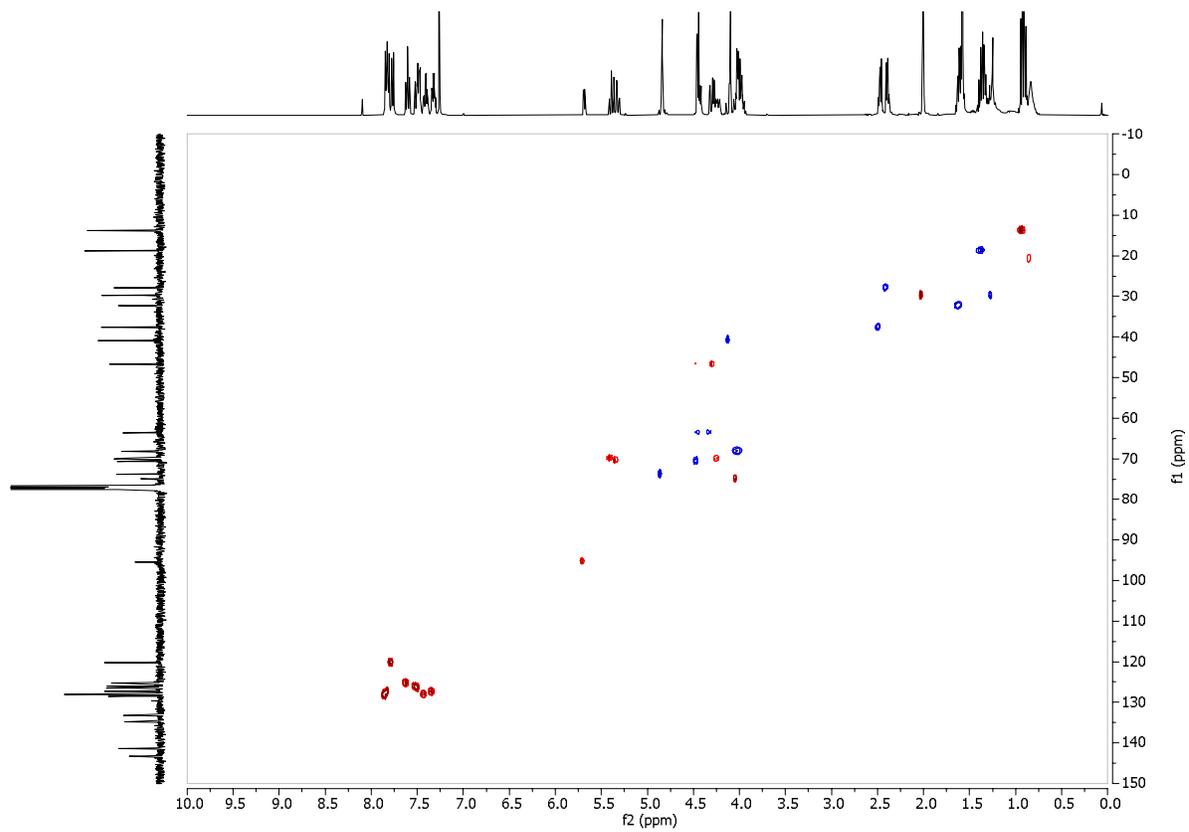
¹³C NMR (101 MHz, CDCl₃) of 7:



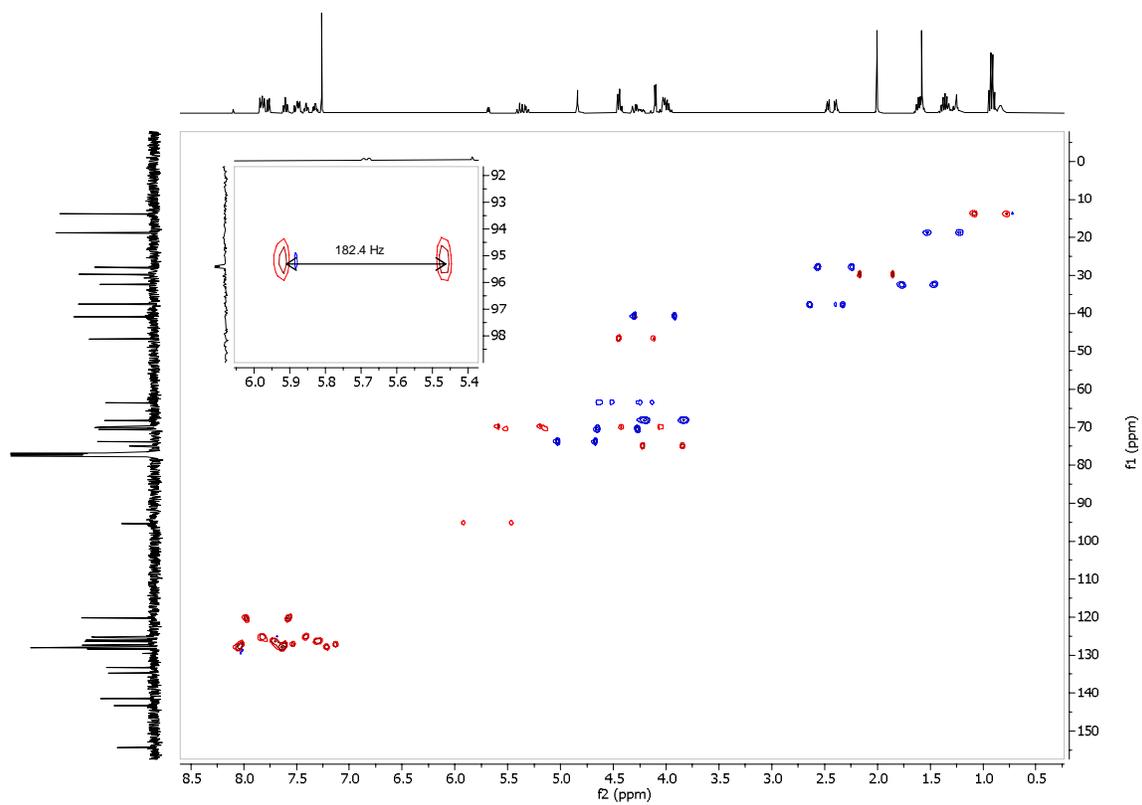
^{31}P NMR (162 MHz, CDCl_3) of 7:



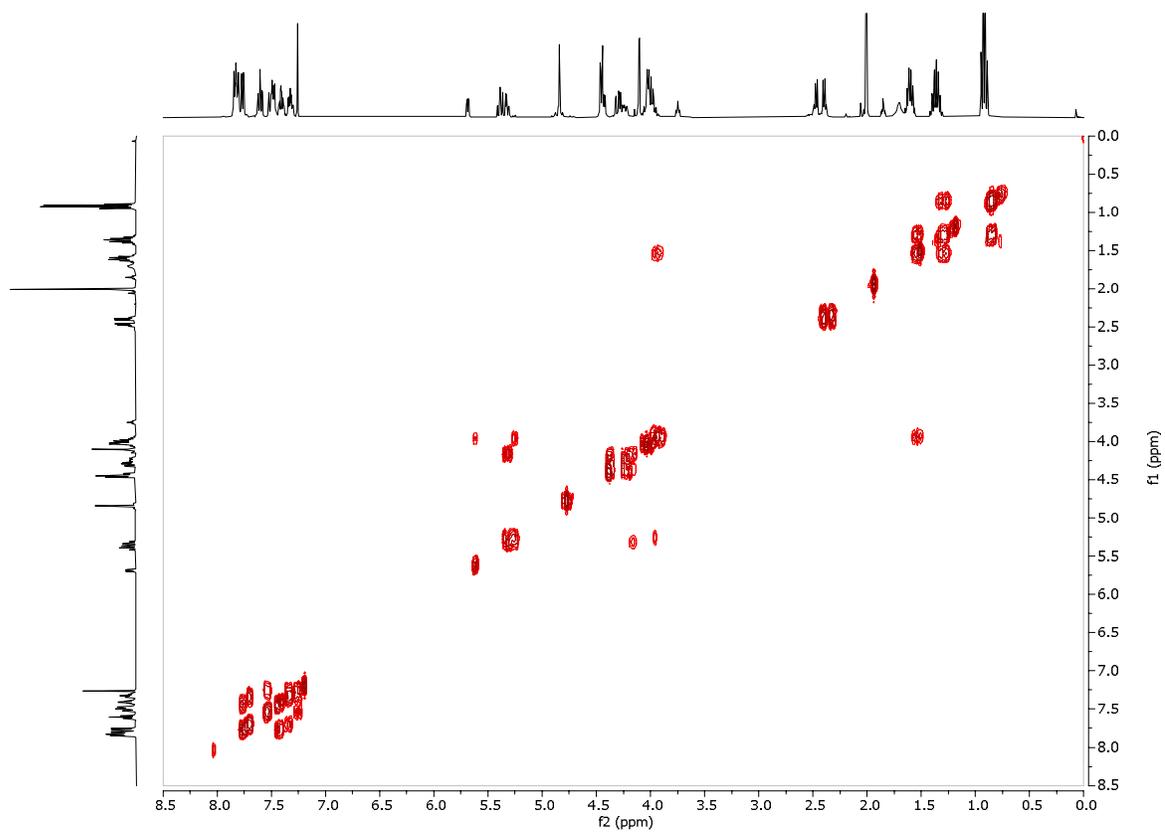
$^{13}\text{C}, ^1\text{H}$ HSQC of 7:



Coupled $^{13}\text{C}, ^1\text{H}$ HSQC of 7:



$^1\text{H}, ^1\text{H}$ COSY of 7:



References

1. Le Mai Hoang, K. et al. Traceless Photolabile Linker Expedites the Chemical Synthesis of Complex Oligosaccharides by Automated Glycan Assembly. *Journal of the American Chemical Society* **141**, 9079-9086 (2019).
2. Gude, M., Ryf, J. & White, P.D. An accurate method for the quantitation of Fmoc-derivatized solid phase supports. *Letters in Peptide Science* **9**, 203-206 (2002).

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