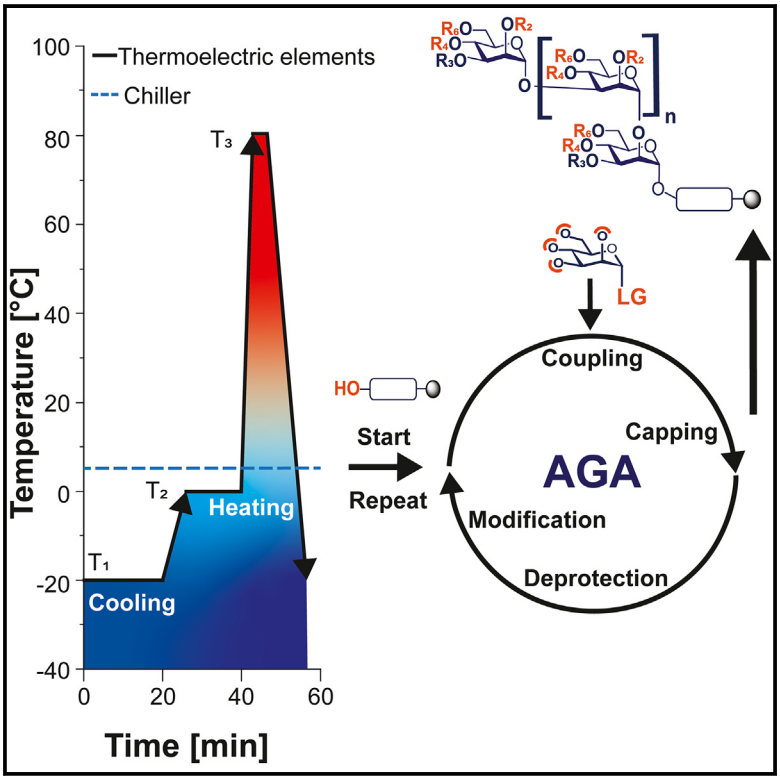
Device

Article

Optimized platform for automated glycan assembly

Graphical abstract



Highlights

- Automated solid-phase reactor for efficient glycan synthesis
- Reaction temperatures were adjusted from −40°C to 90°C
- Thermal and microwave effects promoted reaction equally
- Device size and energy demand were optimized while the capabilities expanded

Authors

José Danglad-Flores, Eric T. Sletten, Emelie E. Reuber, Klaus Bienert, Hans Riegler, Peter H. Seeberger

Correspondence

peter.seeberger@mpikg.mpg.de

In brief

Despite being the most abundant biopolymers in nature, the synthesis of tailor-made glycans has lagged behind that of peptides and oligonucleotides. We present a new cost- and energy-efficient automated instrument based on a local-temperature-controlled (LTC) reactor that enables automated assembly of complex oligosaccharides. The system has a modular design for future expansion.

3 Develop
Prototype with demonstrated applications in relevant environment

Danglad-Flores et al., 2024, Device 2, 100499
October 18, 2024 Published by Elsevier Inc.
https://doi.org/10.1016/j.device.2024.100499

CellPress

Device

CellPress
OPEN ACCESS

Article

Optimized platform for automated glycan assembly

José Danglad-Flores,[1,5] Eric T. Sletten,[1,5] Emelie E. Reuber,[1,2] Klaus Bienert,[3] Hans Riegler,[4] and Peter H. Seeberger[1,2,6,*]
[1]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, 14476 Potsdam, Germany
[2]Freie Universität Berlin, Institute of Chemistry and Biochemistry, 14195 Berlin, Germany
[3]Max Planck Institute of Colloids and Interfaces, 14476 Potsdam, Germany
[4]Riegler & Kirstein GmbH, Berliner Street 133, 14476 Potsdam, Germany
[5]These authors contributed equally
[6]Lead contact
*Correspondence: peter.seeberger@mpikg.mpg.de
https://doi.org/10.1016/j.device.2024.100499

THE BIGGER PICTURE Carbohydrates are the most abundant biopolymer in nature, serving a multitude of functions that range from materials to nutrients and even as mediators of biological signaling processes. Access to tailor-made glycans has traditionally lagged behind that of peptides and oligonucleotides, impeding advancements in molecular glycobiology and drug discovery. The first generation of commercial instruments for automated glycan assembly was large and costly. Herein, we describe a complete overhaul of the original configuration with a cost- and energy-efficient local-temperature-controlled (LTC) reactor. The resulting new instrument enables the automated assembly of complex oligosaccharides and can be expanded in a modular fashion.

SUMMARY

Fast, affordable, and reliable access to complex glycans is essential to meet the demand for custom-made research tools for glycosciences. Automated glycan assembly (AGA)' based on the solid-phase synthesis paradigm allows quickly producing a range of biologically relevant oligosaccharides. The instrument sets the limits as to the types of chemistries that can be executed. The new, smaller, and more economical synthesis device allows quick temperature adjustments between −40°C and +90°C based on an energy-efficient local-temperature-controlled (LTC) reactor. The power of the new instrument is illustrated in the context of several AGA syntheses of complex oligosaccharides.

INTRODUCTION

Automated synthesis platforms are indispensable tools to meet the demand for more and more complex synthetic molecules. Iterative operations used for the synthesis of biooligomers[1–5] and small molecules[6] are executed in automated devices that precisely adjust reaction conditions in a reproducible manner while safely handling toxic reagents and freeing the practitioner from the tedious manipulation and purification of intermediates.

While automated solid-phase synthesis of peptides and oligonucleotides is well established, automated glycan assembly (AGA) to prepare complex oligosaccharides[7] relies on costly instrumentation.[8] Apart from the solid-phase strategy,[9] alternative platforms have been proposed for enzyme-mediated,[10] solution-phase,[11–13] and high-performance liquid chromatography (HPLC)-based[12] oligosaccharide synthesis. AGA[14] has to offer solutions to the general challenges of carbohydrate synthesis, including (1) incorporating a variety of building blocks (glycosylating agents) of unknown and widespread reactivity,[15] (2) reaction temperatures ranging from subzero[15,16] to above room temperature,[2] (3) strictly anhydrous conditions, and (4) consecutive interchange of incompatible reagents (see Figure 1). Initial efforts to address these challenges resulted in operational instruments[1] that left room for significant improvements.

Access to complex carbohydrates by AGA commences at subzero temperatures (Figure 1A) with an acidic wash of the reaction vessel to ensure that any residual basic reagents are neutralized/removed to avoid quenching of the ensuing glycosylation reaction (see the supplemental information). The glycosylating agent (donor) coupling to a solid-support-bound nucleophile (acceptor) typically occurs between −40°C and 0°C under acidic and strictly anhydrous conditions. "Capping" of unreacted nucleophiles via acetylation at room temperature eases purification by preventing them from engaging in subsequent coupling reactions.[17] Temporary protecting group removal on the support-bound saccharide reveals the position for elongation, closing the cycle. This last step typically requires a drastic change in solvent, basicity, and reactions above room

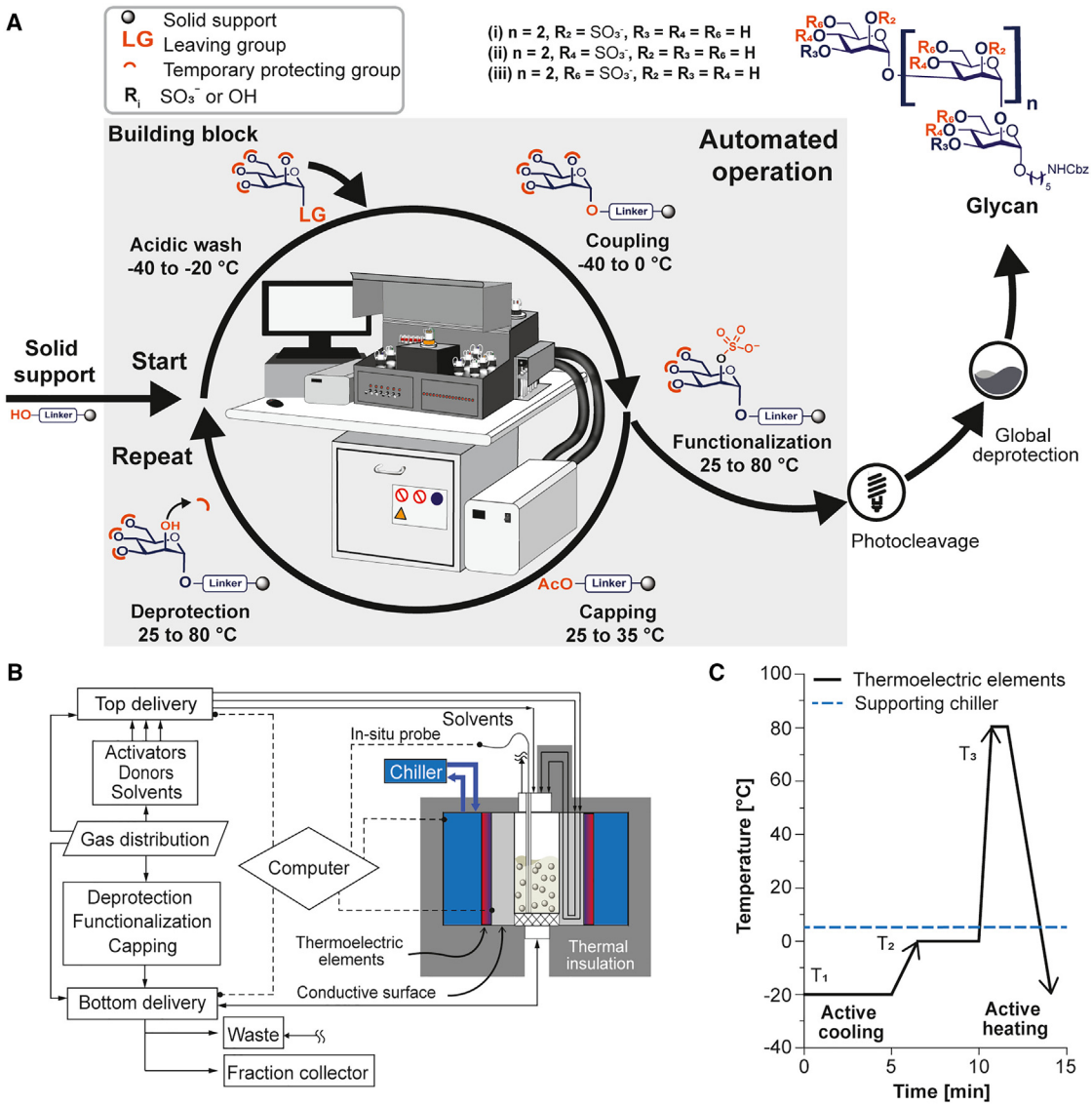


Figure 1. Schematic representation and operational principles of the new AGA platform

(A) Glycan assembly. Highlighted in gray are standard operations performed automatically by the Glyconeer 3.1. Cleavage from the solid support and final deprotection are performed outside the synthesizer.

(B) Detailed schematic depiction of the local temperature control system.

(C) Illustration of the time-resolved temperature graph at the reaction vessel wall adjusted by the action of thermoelectric elements and a cooling support unit (operating at constant temperature).

temperature. Sulfates[2,18] and phosphates[19,20] are introduced into fully constructed backbones. Washing steps with the appropriate solvent remove reagents between each reaction step. Upon completion, the tailor-made compound is released from the resin by photocleavage.[21] The remaining protecting groups are removed by global deprotection.

The growing demand for tailor-made carbohydrates motivated the design of a more efficient automated platform. Here, we present a more cost- and energy-efficient Glyconeer 3.1 synthesizer and demonstrate its capabilities in the context of the synthesis of several complex glycans.

RESULTS

System description

AGA, unlike oligonucleotide and oligopeptide syntheses, requires subzero temperature control. Previous instruments used an expensive circulation thermostat, employing 5 L of siloxane-based thermal fluid to serve 5 mL reaction volume[22] (energy consumption = 3.0 kW h/cycle). The Glyconeer 3.1 employs a precise local temperature control system based on Peltier elements around the reaction chamber (Figure 1B), maintaining ±1°C from −40°C to +90°C.[23–26] Previously, the reported glass jacketed

reactors[1] demanded periodic hydrophobization and an entire unit replacement once the frit reached the end of the operational life. A modular fluoropolymer of tetrafluoroethylene (PTFE) reactor affords individual component replacement and ensures chemical stability, preventing adhesion of the solid support, while a thermal conductive layer distributes homogeneously the heat transfer area. In comparison to glass, the new PTFE reactor has a slight reduction in thermal conductivity, and the opaqueness of PTFE prevents the direct observation of the reaction. The thermoelectric elements operate at a reference temperature of 5°C, which is sustained by a circulating water-based coolant via a small chiller (4× smaller) in the outer layer of the reactor assembly (Figure 1B).[26] This allows the dissipation of energy during cooling processes (Figure 1C). The full assembly for local temperate control consumes 0.7 kWh per cycle (estimated as average power consumption by the reference cycle time), 75% less energy than the circulating thermostat. The optimization of components and reagent positions resulted in a device that fits on a regular laboratory bench (0.3 m^2, represented in Figure 1A and depicted in the supplemental information).

The local temperature control has an integrated precooling circuit for building block and activator solutions (Figure 1B) and two extra inlets for optional delivery of different chemicals. The new system standardizes the use of syringe pumps for the delivery of monomers and activators, allowing for dropwise addition for volumes $\leq$1 mL with greater accuracy. The increased accuracy comes at the cost of a marginal increase in operating time. The installed delivery system has six building block stock solution positions (9 mL each, or approximately 54 couplings), with the possibility of a 30% future expansion. A significant improvement over the previous commercial version is the splitting of reagent delivery, such that solvents, building blocks, and activator solutions are added from the top of the reaction vessel from independent pathways, while deprotection, capping, and post-modification solutions are supplied from the bottom. Isolating the atmosphere (through the gas distribution system) and the reagent transport path limits the possibility of cross-contamination and the mixing of incompatible reagents. The gas flow for bubbling and the reagent drainage are connected to the bottom; the exhausted solution could be disposed to the waste or collected for recovering glycosylating agents (Figure 1C).

Temperature control

Previously, a dual temperature regulation system for rapid temperature changes across a broad range was established by combining a circulation thermostat with a microwave generator. In this costly system, microwave irradiation elevates the temperature above the reference temperature (typically −30°C). For benchmarking, the Peltier-based system was compared to the microwave-supported AGA.

Differences between the two temperature adjustment systems were evaluated in the context of Lewis X tetramer **5** synthesis (Figure 2A), and the temperature profiles (continuous line) of instruments with local temperature control (Peltier, Figure 2B) and dual temperature regulation (microwave supported, Figure 2C) were compared. In this way, events such as reagent delivery and drainage can be correlated with thermal perturbations. The microwave-supported system facilitates rapid adjustments across a wide temperature range to allow for diverse reactions, including deprotection methods and on-resin modifications.[2] However, the heating operation depends on the microwave radiation susceptibility of the reagents. Dichloromethane, with a dielectric constant (ε) of 9, requires around 150 W of microwave power to increase the temperature by 6°C/min. Deprotection by fluorenylmethoxycarbonyl (Fmoc) cleavage in dimethylformamide (DMF, ε = 36) requires just 50 W for the temperature to rise as fast as 85°C/min. The Peltier platform adjusts the temperature by a rate of 16°C/min (below 0°C) and 26°C/min (above 0°C), irrespective of the reagents and solvent. Both systems completed every reaction within comparable reaction times (Figures 2A and 2B), with the overall synthesis time of the tetramer differing by only 1 h. The crude product was recovered with the same quality (Figure 2D) and comparable amounts for the Peltier reactor (15 mg, 69% yield) and the microwave-supported platform (14 mg, 64%). In both cases, the levulinoyl ester protecting group (Lev) was cleaved within 30 min (see supplemental information).

Cooling relies on a classical conduction-convention mechanism in these systems. When comparing the cooling rate as a function of the temperature (Figure 2E), the conventional system using a circulating thermostat is less efficient, with a constant cooling rate of 3°C/min, while cooling the reactor by Peltier increases the rate linearly from 6°C to 15°C/min. When a microwave elevates the temperature, the cooling rate shifts from a constant behavior (3°C/min, controlled by the coolant circulation) at subzero temperatures to a linear function of the temperature when operating above zero degrees, reaching up to 30-fold of the constant value. The constant thermal rate obeys a linear increase in the temperature differential with respect to the chiller reference temperature (−30°C). With the size, cost, and energy consumption of the cooling system optimized, the time required to reach the desired temperature may be further shortened.

Completing one coupling-deprotection cycle on the new platform requires 1 h, while challenging couplings, such as those involving glucosamine donors, may demand up to 2 h. Coupling cycles will be drastically shortened when the current trial-and-error approach to reaction optimization will be replaced by a more rational strategy to assess glycosylation kinetics.

AGA of oligosaccharides

PI-88, a hyper-sulfated mannan mimic of heparan sulfate that is isolated or synthesized as a randomly sulfated mixture of molecules with an average sulfation of three sulfates per mannose residue,[27–32] is undergoing evaluation as an anticancer[33,34] and antiviral[35,36] therapeutic. The highly charged, highly hydrophilic molecules are poorly bioavailable, with unwanted side effects.[37] Structure-activity relationships have remained elusive to date, but a collection of synthetic glycans resembling PI-88 with defined sulfation patterns should help reduce its off-target biological activity.[38] AGA of an oligomannoside carrying temporary protecting groups was achieved using mannoside building block **12**. Selective cleavage of (2-Naphthylmethyl) NAP ether, Lev ester, Fmoc carbonate, and ClAc ester protecting groups, capping, and, finally, on-resin sulfation were all achieved under conditions (reagents, time, and temperature) similar to those utilized in the microwave-supported synthesizer (see the supplemental information for details).[2] Tetrasaccharides **6–8**

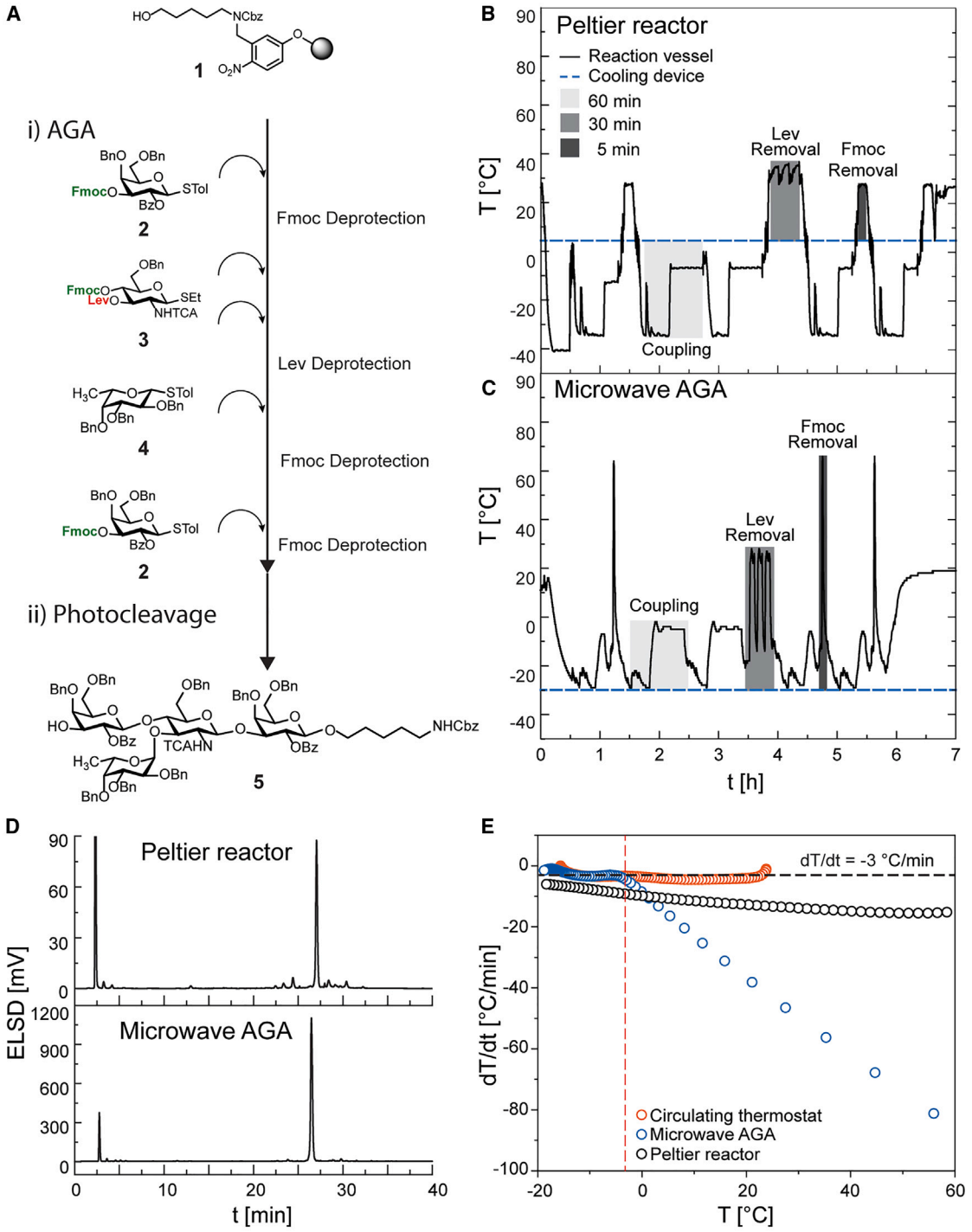


Figure 2. Comparison of different heating/cooling approaches

(A) Schematic overview of AGA of tetrasaccharide **5**.

(B and C) The temperature inside the reaction vessel (black continuous line, measured with a thermal probe) during Lewis X tetrasaccharide **5** synthesis by (B) Peltier reactor and (C) microwave-supported AGA. The dashed blue lines mark the cooling fluid temperature for each platform. The reference timescales (60, 30, and 5 min) are highlighted on a gray background for coupling and deprotections.

(D) HPLC of the crude tetrasaccharide **5** (evaporative light scattering detector, ELSD trace, t_R = 26.5 min).

(E) Cooling rate (°C/min) as a function of the reagent (3 mL) temperature (°C) for circulating thermostat, microwave AGA, and Peltier systems.

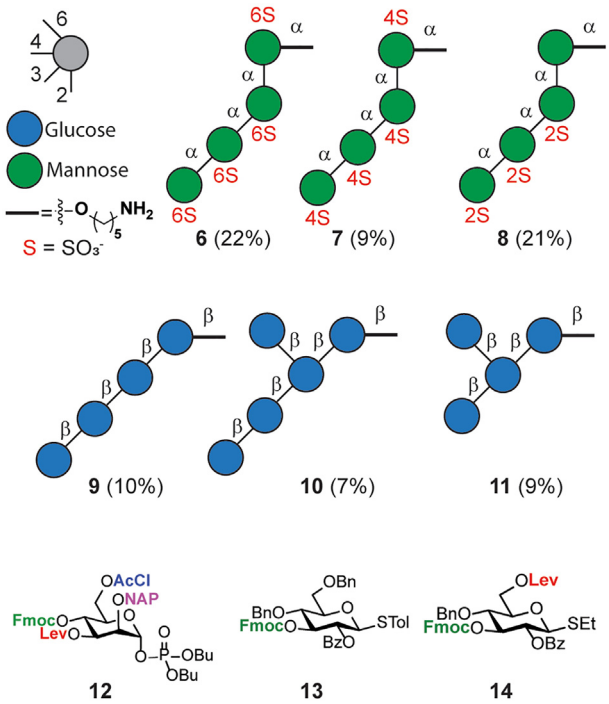


Figure 3. Oligosaccharides 6–11 (isolated yields) were prepared to illustrate the capabilities of the Glyconeer 3.1 synthesizer

with regiospecific sulfation on C-2, C-4, or C-6 (Figure 3) are the basis for detailed structure-activity studies of PI-88.

A collection of linear and branched beta-glucan oligosaccharides was constructed next from glycosyl donors **13–14**. Beta-glucans **9–11**[39] play a role in biofilm resistance to antimicrobial therapy.[40] In order to explore the resistance mechanism at a molecular level and identify new strategies to treat biofilm infections,[41] defined oligosaccharides are helpful. The target oligosaccharides were prepared by AGA before global deprotection, and HPLC yielded 1–2 mg (7%–10% yield, depending on the structure) of each fully deprotected glycan (see the supplemental information).

DISCUSSION

A benchtop, solid-support AGA platform based on a thermoelectric control system was described. The system ensures precise temperature control for efficient reactions while reducing the cost, size, and energy consumption of the instrument. A Peltier-based reactor unit offers a balance between operational range, process time, component cost, and energy usage. The resulting device has a small footprint and is less costly to construct and operate while still facilitating access to well-defined glycans for structure-function studies. The reduction in platform size by over 50% did not affect any of the operations. Two types of glycosyl activations were demonstrated: the removal of four different protecting groups, one post-functionalization, and a capping reaction.

Previous versions of the automated glycan synthesizer had a circulating thermostat with a limited temperature range of −60°C to 40°C.[1,4,42,43] With such a device, the temperature oscillations required within a 1-h span are energy intensive and compromised the operational lifespan of the dynamic thermostat. Consequently, alternatives for an energy-efficient and extended thermal range were explored utilizing microwave radiation, though the overall cost was increased.[2] Thus, we devised a thermoelectric-based temperature reactor to demonstrate the following technical advantages: (1) economical construction, (2) compact assembly, (3) higher energy efficiency, and (4) suitability for iterative temperature oscillation on a short timescale.

In regards to process time, the previous thermostat synthesizers reported cycles of at least 100 min.[2] The latest technologies (Figure 2) shorten the cycle to 60 min for the microwave system and 72 min for the Peltier-based reactor due to inherent slower temperature adjustments (Figure 2E).

In addition to the increase in efficiency resulting from the hardware modification, a more comprehensive understanding of the washing process, including the specific steps involved, also contributed to a reduction in the size of the platform and the minimization of waste generated. The number of washing solutions required in the earlier custom-built devices[42,44] and Glyconeer 2.1[1] (13 L total) was minimized from six solvents to just DCM and DMF (2 L each) in the new system. Additionally, highly carcinogenic 1,2-dichloroethane, previously used[2] as buffer liquid between the reagents and the syringe pump, was replaced with a mixture of 1:3 DCM/toluene (1.5 L bottle) with equivalent physicochemical properties.

A return to a more modular design (for details, please refer to the supplemental information) was undertaken with the intention of creating a closer resemblance to the home-built

synthesizer.[43] This approach was designed to facilitate maintenance and troubleshooting, with a preference for individual solenoid valve arrangements over block constructions. The software and hardware architectures were developed to incorporate extra components such as valves and syringe pumps, allowing for the system build-up. Additionally, the reaction vessel was designed with two extra inlets to support independent reagent delivery, paving the way for future chemistry advancements. Overall, process intensification strategies and the systematic identification of optimal reaction conditions will help reduce synthesis time and enhance reaction efficiency.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Peter H. Seeberger (peter.seeberger@mpikg.mpg.de).

Materials availability

This study did not generate new unique reagents. There are no restrictions to the availability of reagents. For a detailed description of the reagents, solutions, and materials, refer to the supplemental information.

Data and code availability

The codes to execute process modules used during all synthesis are accessible in the following public repository[45]: https://edmond.mpg.de/dataset.xhtml?persistentId=doi:10.17617/3.Z8Y7OM.

Glycan assembly

Automated syntheses were performed on a synthesizer developed at the Max Planck Institute of Colloids and Interfaces with the technical support of Riegler & Kirstein GmbH and GlycoUniverse. The supplemental information provides all details concerning the device, preautomation steps, building blocks, reagents, and modules used for the automated synthesis and post-assembly manipulations.

Reactor unit

The reaction vessel (Figure 1B) includes an arrangement of thermoelectric elements for temperature adjustment supported by an auxiliary cooling unit (maintaining a water base coolant at 5°C in a close circuit surrounding the Peltier elements). The Peltier system can control temperatures from −40°C to 90°C. The heat transfer area is homogeneously distributed around the reactor surface by a highly conductive block between the reactor and the thermoelectric elements. The Teflon reaction vessel was built in three parts: an upper Teflon cap for top delivery and an outlet that vents the exhausted gases. From the top of the reaction vessel, a type K thermocouple extends to the bottom through a closed-end channel, enabling *in situ* temperature monitoring. This process allows for the accurate correlation between the set and experimental temperatures in the reaction environment. The bottom fritted stopper holds the resin inside the reactor.

Delivery systems

Building block and activator solutions are delivered via independent lines at the top of the reaction vessel by an arrangement of syringe pumps and rotary valves (∼3 min of operational time per aliquot). Both reagents are precooled in heat exchangers built in the reactor heat transfer section. Washing solvent and draining Ar are dispensed from a spread nozzle in the top stopper. The top stopper possesses a controlled vent for exhausted gases and two additional inlets that can be used as delivery points when needed.

Up to six vials could be loaded per operation, hosting up to 9 mL of solution each. The activator, deprotection, and capping solutions are stored in 100 mL bottles, while solvents demand 2 L containers of all pressure-resistant glass. NIS (N-Iodosuccinimide)-containing solution is kept between 4°C and 10°C hosted by a cooling holder.

Deprotection, capping, and post-modification reagents are accommodated in six containers and delivered pneumatically to the bottom of the reaction vessel controlled by rotary and solenoid valves (operational time: ∼10 s or less per aliquot). The bottom of the reaction vessel provides an Ar inlet for bubbling and an outlet for draining them toward the waste container or the fraction collector with six collecting positions.

Automated control

An electronics compartment separated from the reactor unit serves as an interface between the computer and the actuators in the unit. The status of the valve, temperature in the reactor, and pressure through the machine are computer readable. A LabVIEW-based software controls the handling of the reagents and temperature regulation. Developing and storing modular operations is possible through the software interface, which lists ground-level commands (basic operations). The modules execute standard processes (https://edmond.mpg.de/dataset.xhtml?persistentId=doi:10.17617/3.Z8Y7OM) to any synthesis strategy,[42] such as initialization, couplings, deprotections, capping, functionalization, and standby operation. The user sets the process parameters and compiles the modules to design synthetic programs.

AGA cycle modules

The modules utilized during AGA are described below; refer to the supplemental information for further details.

Resin preparation for synthesis

Based on loading, an appropriate amount of resin is placed in the reaction vessel. Once the synthesis starts, the system initializes all components, washes with DMF and CH_2Cl_2 (3 × 3 mL for 10 s each), and swells in 2 mL CH_2Cl_2 for 20 min before the synthesis commences at the temperature selected for the first coupling. The required reagent lines are primed during this time.

Acidic wash with TMSOTf solution

The reactor is drained, and the temperature is adjusted to T_1 (starting glycosylation temperature typically from −40°C to −20°C). The resin is washed with CH_2Cl_2 (5 × 3 mL for 30 s each). Upon reaching the set temperature T_1, 2 mL CH_2Cl_2 is delivered to dilute the trimethylsilyl trifluoromethanesulfonate (TMSOTf) solution (1 mL) and added to the reaction vessel. After incubation for 3 min, the acidic solution is drained and washed with 3 mL CH_2Cl_2 for 30 s.

Glycosylation

The building block and activator solution delivery lines to the reaction vessel are primed. The building block solution (6.5 or 5 equiv of donor in 1 mL CH_2Cl_2 per glycosylation) is delivered to the reaction vessel after reaching the set temperature (T_1). The reaction is started by slowly adding the NIS/triflic acid (TfOH) activator solution (1.0 mL) in the case of thioglycosylation or 1.0 mL TMSOTf solution in the case of a phosphate coupling. The reaction is performed in two thermal steps. Starting at T_1, the reagents incubate for a time t_1, then the temperature is linearly increased by a rate of 4°C/min to a T_2, incubating for a t_2 (times and temperatures are building block dependent). The solution is removed, and the solid support is washed with CH_2Cl_2:dioxane (2:1, 3 mL for 6 s) or CH_2Cl_2 (in the case of phosphate activation). If required, a double cycle is performed by repeating the operation. The resin is washed with CH_2Cl_2 (2 × 3 mL for 15 s).

Capping

The temperature is adjusted to 30°C while the solid support is washed with DMF (3 × 3 mL for 10 s). Pyridine solution (10% in DMF) is delivered (2 mL) into the reaction vessel. After 1 min, the reaction solution is removed, and the solid support is washed with CH_2Cl_2 (3 × 3 mL for 10 s). A capping solution (2 mL) is delivered into the reaction vessel. After 10 min, the reaction solution is drained, and the capping reaction is repeated once more. Finally, the resin is washed with CH_2Cl_2 (3 × 3 mL for 25 s) and once with DMF for 1 min.

Fmoc deprotection

The resin rested in 3 mL DMF until the reaction vessel adjusts to 25°C, and then it is washed with DMF (three times with 2 mL for 15 s). Fmoc

deprotection solution is selected in accordance with which building block is being used. Piperidine or triethylamine (TEA) in DMF (2 mL) is delivered to the reactor. After 5 min, the reaction solution is removed. In the case of using a TEA solution, the reaction is repeated twice more. Then, the resin is washed with DMF (1 × 3 mL for 15 s each) and DCM (3 × 3 mL for 30 s each). The temperature is adjusted accordingly for the next module.

Lev deprotection

The resin is washed with CH_2Cl_2 (three times with 3 mL for 15 s), and the temperature is adjusted to 35°C. CH_2Cl_2 (2 mL) is delivered to dilute the Lev deprotection solution (1 mL). The reagents are kept under pulsed Ar bubbling for 10 min and washed three times with DCM (three times with 3 mL for 15 s). This procedure is repeated three times. The reaction solution is drained, and the resin is washed with DMF (three times with 3 mL for 30 s) and CH_2Cl_2 (three times each with 2 mL for 30 s). The temperature is adjusted accordingly for the next module.

NAP deprotection

The solid support is washed with CH_2Cl_2 (3 × 3 mL for 15 s) at 20°C, and then NAP deprotection solution (2 mL) is delivered to the reaction vessel. The temperature of the reagents is then adjusted to and maintained at 60°C. After 30 min, the reaction solution is removed from the reactor vessel. Then, fresh NAP deprotection solution (2 mL) is delivered, and the process is repeated twice more. The temperature is adjusted to 20°C. The resin is washed with DMF and CH_2Cl_2 (3 mL for 60 s each). The temperature is adjusted accordingly for the next module.

ClAc deprotection

The temperature of the reactor vessel is adjusted to 80°C while the resin is washed with DMF (3 × 3 mL for 15 s). Then, ClAc deprotection solution (2 mL) is delivered to the reaction vessel. After 22.5 min, the reaction solution is drained from the reactor vessel. The resin is washed with DMF (1 × 2 mL for 15 s). Then, a fresh ClAc deprotection solution (2 mL) is delivered and the process repeated. Then, the solid support is washed with DMF (3 × 3 mL for 30 s) while the temperature is adjusted for the next glycosylation cycle.

Sulfation

The temperature is then adjusted to 90°C while being washed with DMF (3 × 2 mL for 15 s); then, sulfation solution (2 mL) is delivered to the reaction vessel. After 20 min, the reaction solution is drained from the reactor vessel. The sulfation cycle is repeated twice more. Upon completion, the resin is washed with DMF (3 × 3 mL for 60 s), and the temperature is adjusted to 25°C.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.device.2024.100499.

ACKNOWLEDGMENTS

We gratefully acknowledge the Max Planck Society for its generous financial support.

AUTHOR CONTRIBUTIONS

J.D.-F., E.T.S., and E.E.R. conducted the research, performed the experiments, and wrote the original draft. K.B. developed the software. H.R., in collaboration with J.D.-F., designed and built the device. P.H.S. supervised the project and edited the manuscript. J.D.-F. and E.T.S. contributed equally to the project from the engineering and chemistry points of view, respectively.

DECLARATION OF INTERESTS

P.H.S. declares a significant financial interest in GlycoUniverse GmbH & Co. KGaA, the company that commercialized the previous AGA synthesis instrument, building blocks, and other reagents. H.R. declares a significant financial interest in Riegler & Kirstein GmbH, the contractor that assembled the device. The gas distribution system and the precooling of sensitive reagents are in the process of patenting under US20230026870A1 and US20220395800A1, respectively.

Received: March 26, 2024
Revised: June 9, 2024
Accepted: July 11, 2024
Published: August 7, 2024

REFERENCES

1. Hahm, H.S., Schlegel, M.K., Hurevich, M., Eller, S., Schuhmacher, F., Hofmann, J., Pagel, K., and Seeberger, P.H. (2017). Automated glycan assembly using the Glyconeer 2.1 synthesizer. Proc. Natl. Acad. Sci. USA *114*, E3385–E3389. https://doi.org/10.1073/pnas.1700141114.
2. Danglad-Flores, J., Leichnitz, S., Sletten, E.T., Abragam Joseph, A., Bienert, K., Le Mai Hoang, K., and Seeberger, P.H. (2021). Microwave-Assisted Automated Glycan Assembly. J. Am. Chem. Soc. *143*, 8893–8901. https://doi.org/10.1021/jacs.1c03851.
3. Merrifield, R. (1965). Automated Synthesis of Peptides: Solid-phase peptide synthesis, a simple and rapid synthetic method, has now been automated. Science *150*, 178–185.
4. Plante, O.J., Palmacci, E.R., and Seeberger, P.H. (2001). Automated solid-phase synthesis of oligosaccharides. Science *291*, 1523–1527.
5. Caruthers, M.H. (1985). Gene synthesis machines: DNA chemistry and its uses. Science *230*, 281–285.
6. Li, J., Ballmer, S.G., Gillis, E.P., Fujii, S., Schmidt, M.J., Palazzolo, A.M.E., Lehmann, J.W., Morehouse, G.F., and Burke, M.D. (2015). Synthesis of many different types of organic small molecules using one automated process. Science *347*, 1221–1226. https://doi.org/10.1126/science.aaa5414.
7. Gagneux, P., Panin, V., Hennet, T., Aebi, M., and Varki, A. (2022). Evolution of Glycan Diversity. Essentials of Glycobiology, 4th edition. [Internet].
8. Corolleur, F., Level, A., Matt, M., and Perez, S. (2020). Innovation potentials triggered by glycoscience research. Carbohydr. Polym. *233*, 115833.
9. Seeberger, P.H. (2015). The logic of automated glycan assembly. Acc. Chem. Res. *48*, 1450–1463.
10. Li, T., Liu, L., Wei, N., Yang, J.-Y., Chapla, D.G., Moremen, K.W., and Boons, G.-J. (2019). An automated platform for the enzyme-mediated assembly of complex oligosaccharides. Nat. Chem. *11*, 229–236.
11. Nokami, T., Hayashi, R., Saigusa, Y., Shimizu, A., Liu, C.-Y., Mong, K.-K.T., and Yoshida, J.-i. (2013). Automated solution-phase synthesis of oligosaccharides via iterative electrochemical assembly of thioglycosides. Org. Lett. *15*, 4520–4523.
12. Kashiwagi, G., Petrosilli, L., Escopy, S., Lay, L., Stine, K., De Meo, C., and Demchenko, A.V. (2024). HPLC-Based Automated Synthesis and Purification of Carbohydrates. Chem. Eur J., e202401214.
13. Tang, S.-L., and Pohl, N.L. (2015). Automated solution-phase synthesis of β-1, 4-mannuronate and β-1, 4-mannan. Org. Lett. *17*, 2642–2645.
14. Lin, M.H., Wolf, J.B., Sletten, E.T., Cambié, D., Danglad-Flores, J., and Seeberger, P.H. (2023). Enabling technologies in carbohydrate chemistry: automated glycan assembly, flow chemistry and data science. Chembiochem *24*, e202200607.
15. Tuck, O.T., Sletten, E.T., Danglad-Flores, J., and Seeberger, P.H. (2022). Towards a systematic understanding of the influence of temperature on glycosylation reactions. Angew. Chem. Int. Ed. *61*, e202115433.
16. Chatterjee, S., Moon, S., Hentschel, F., Gilmore, K., and Seeberger, P.H. (2018). An empirical understanding of the glycosylation reaction. J. Am. Chem. Soc. *140*, 11942–11953.
17. Yu, Y., Kononov, A., Delbianco, M., and Seeberger, P.H. (2018). A capping step during automated glycan assembly enables access to complex glycans in high yield. Chem. Eur J. *24*, 6075–6078.

18. Tyrikos-Ergas, T., Sletten, E.T., Huang, J.-Y., Seeberger, P.H., and Delbianco, M. (2022). On resin synthesis of sulfated oligosaccharides. Chem. Sci. *13*, 2115–2120.

19. Sletten, E.T., Danglad-Flores, J., Leichnitz, S., Joseph, A.A., and Seeberger, P.H. (2022). Expedited synthesis of mannose-6-phosphate containing oligosaccharides. Carbohydr. Res. *511*, 108489.

20. Sletten, E.T., Fittolani, G., Hribernik, N., Dal Colle, M.C., Seeberger, P.H., and Delbianco, M. (2024). Phosphates as Assisting Groups in Glycan Synthesis. ACS Cent. Sci. *10*, 138–142.

21. Teschers, C.S., and Gilmour, R. (2020). Flow Photocleavage for Automated Glycan Assembly (AGA). Org. Process Res. Dev. *24*, 2234–2239.

22. Pinzón-López, S., Kraume, M., Danglad-Flores, J., and Seeberger, P. (2023). Transport Phenomena in Solid Phase Synthesis Supported by Cross-Linked Polymer Beads. React. Chem. Eng. *8*, 2951–2962. https://doi.org/10.1039/D3RE00359K.

23. Läppchen, T., Vlaming, M.L., Custers, E., Lub, J., Sio, C.F., DeGroot, J., and Steinbach, O.C. (2012). Automated synthesis of [18F] gefitinib on a modular system. Appl. Radiat. Isot. *70*, 205–209.

24. Huiban, M., Pampols-Maso, S., and Passchier, J. (2011). Fully automated synthesis of the M_1 receptor agonist [^{11}C]GSK1034702 for clinical use on an Eckert & Ziegler Modular Lab system. Appl. Radiat. Isot. *69*, 1390–1394.

25. Frede, T.A., vom Hofe, N., Reuß, R.J., Kemmerling, N., Kock, T., Herbstritt, F., and Kockmann, N. (2023). Design and characterization of a flow reaction calorimeter based on FlowPlate® Lab and Peltier elements. React. Chem. Eng. *8*, 1051–1060.

26. Christensen, M., Yunker, L.P., Shiri, P., Zepel, T., Prieto, P.L., Grunert, S., Bork, F., and Hein, J.E. (2021). Automation isn't automatic. Chem. Sci. *12*, 15473–15490.

27. Chhabra, M., and Ferro, V. (2020). PI-88 and Related Heparan Sulfate Mimetics. In Heparanase: From Basic Research to Clinical Applications, I. Vlodavsky, R.D. Sanderson, and N. Ilan, eds. (Springer International Publishing), pp. 473–491. https://doi.org/10.1007/978-3-030-34521-1_19.

28. Handley, P.N., Carroll, A., and Ferro, V. (2017). New structural insights into the oligosaccharide phosphate fraction of Pichia (Hansenula) holstii NRRL Y2448 phosphomannan. Carbohydr. Res. *446–447*, 68–75.

29. Elli, S., Stancanelli, E., Handley, P.N., Carroll, A., Urso, E., Guerrini, M., and Ferro, V. (2018). Structural and conformational studies of the heparan sulfate mimetic PI-88. Glycobiology *28*, 731–740.

30. Namme, R., Mitsugi, T., Takahashi, H., and Ikegami, S. (2005). Synthesis of PI-88 analogue using novel O-glycosidation of exo-methylenesugars. Tetrahedron Lett. *46*, 3033–3036.

31. Fairweather, J.K., Hammond, E., Johnstone, K.D., and Ferro, V. (2008). Synthesis and heparanase inhibitory activity of sulfated mannooligosaccharides related to the antiangiogenic agent PI-88. Bioorg. Med. Chem. *16*, 699–709.

32. Ventura, J., Uriel, C., Gómez, A.M., and López, J.C. (2022). Access to n-pentenyl tetra-and pentasaccharide analogues of the antitumor drug PI-88 based on 1, 2-methyl orthoester glycosyl donors. Carbohydr. Res. *516*, 108557.

33. Kudchadkar, R., Gonzalez, R., and Lewis, K.D. (2008). PI-88: a novel inhibitor of angiogenesis. Expert Opin. Investig. Drugs *17*, 1769–1776.

34. Yang, Y., Yuan, F., Zhou, H., Quan, J., Liu, C., Wang, Y., Xiao, F., Liu, Q., Liu, J., Zhang, Y., and Yu, X. (2023). Potential roles of heparanase in cancer therapy: Current trends and future direction. J. Cell. Physiol. *238*, 896–917.

35. Lee, E., Pavy, M., Young, N., Freeman, C., and Lobigs, M. (2006). Antiviral effect of the heparan sulfate mimetic, PI-88, against dengue and encephalitic flaviviruses. Antiviral Res. *69*, 31–38.

36. Nyberg, K., Ekblad, M., Bergström, T., Freeman, C., Parish, C.R., Ferro, V., and Trybala, E. (2004). The low molecular weight heparan sulfate-mimetic, PI-88, inhibits cell-to-cell spread of herpes simplex virus. Antiviral Res. *63*, 15–24.

37. Zhang, Y., and Cui, L. (2023). Discovery and development of small-molecule heparanase inhibitors. Bioorg. Med. Chem. *90*, 117335.

38. Wakpal, J., Pathiranage, V., Walker, A.R., and Nguyen, H.M. (2023). Rational Design and Expedient Synthesis of Heparan Sulfate Mimetics from Natural Aminoglycosides for Structure and Activity Relationship Studies. Angew. Chem. Int. Ed. *62*, e202304325.

39. Weishaupt, M., Hahm, a.H., Geissner, A., and Seeberger, P.H. (2017). Automated glycan assembly of branched β-(1, 3)-glucans to identify antibody epitopes. Chem. Commun. *53*, 3591–3594.

40. Nett, J., Lincoln, L., Marchillo, K., Massey, R., Holoyda, K., Hoff, B., VanHandel, M., and Andes, D. (2007). Putative role of β-1, 3 glucans in Candida albicans biofilm resistance. Antimicrob. Agents Chemother. *51*, 510–520.

41. Träger, J., Dräger, S., Mihai, S., Cipa, F., Busse Grawitz, A., Epting, T., Meyer, R., Rappold, E., and Held, J. (2023). Detailed β-(1→3)-D-glucan and mannan antigen kinetics in patients with candidemia. J. Clin. Microbiol. *61*. e00598-e00523.

42. Walvoort, M.T., van den Elst, H., Plante, O.J., Krock, L., Seeberger, P.H., Overkleeft, H.S., van der, Marel, and Codée, J.D. (2012). Automated solid-phase synthesis of b-mannuronic acid alginates. Angew. Chem. Int. Ed *51*, 4393–4396.

43. Guberman, M., Bräutigam, M., and Seeberger, P.H. (2019). Automated glycan assembly of Lewis type I and II oligosaccharide antigens. Chemical Science *10*, 5634–5640.

44. Werz, D.B., Castagner, B., and Seeberger, P.H. (2007). Automated synthesis of the tumor-associated carbohydrate antigens Gb-3 and Globo-H: incorporation of α-galactosidic linkages. Journal of the American Chemical Society *129*, 2770–2771.

45. Danglad-Flores, J. (2024). GlycoMerge Software Operative Module Codes, V1 ed.