

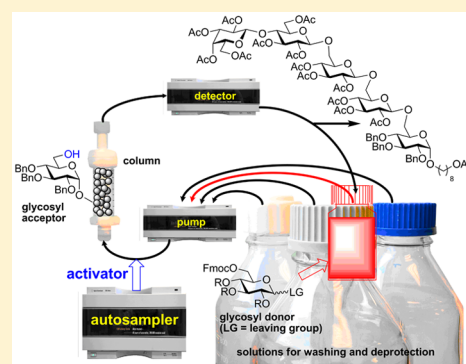
HPLC-Assisted Automated Oligosaccharide Synthesis: Implementation of the Autosampler as a Mode of the Reagent Delivery

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Supporting Information

ABSTRACT: The development of a useful methodology for simple, scalable, and transformative automation of oligosaccharide synthesis that easily interfaces with existing methods is reported. The automated synthesis can now be performed using accessible equipment where the reactants and reagents are delivered by the pump or the autosampler and the reactions can be monitored by the UV detector. The HPLC-based platform for automation is easy to setup and adapt to different systems and targets.



INTRODUCTION

Glycans are oligomeric carbohydrates wherein monomers are connected via the glycosidic linkage. This linkage is obtained by a glycosylation reaction, which remains challenging to synthetic chemists due to the requirement to achieve high stereo-control^{1,2} and yields by suppressing side reactions.³ Beyond that, glycan synthesis may require further manipulations between each glycosylation step. Due to significant advances, the chemical synthesis of many glycans can now be streamlined by using expeditious strategies.⁴ Solid-phase synthesis,^{5,6} which eliminates the need for purifying intermediates and simplifies the removal of excess reagents, has been widely used in the preparation of peptides^{7,8} and oligonucleotides.⁹ Since 1971, solid-phase synthesis has been used for the preparation of oligosaccharides,^{10–14} and in 2001 Seeberger et al. reported the first automated oligosaccharide synthesis using a modified peptide synthesizer.^{15–18} In 2012, Seeberger et al. reported “the first fully automated solid-phase oligosaccharide synthesizer,” initially in its experimental form;¹⁹ and in 2013 it was marketed as Glycoconer 2.1. Approaches by Wong,^{20,21} Takahashi,^{22,23} Chen,^{24–26} Pohl,^{27–30} Wang,^{31,32} and Nokami^{33,34} are based on the automation of chemical, enzymatic, or chemoenzymatic syntheses in solution with or without using tags.³⁵

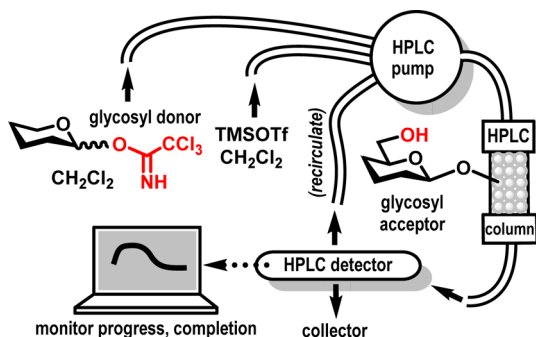
In light of recent progress made in the areas of glycobiology^{36–39} and glycomics⁴⁰ “widely applicable methods to generate both large and small quantities of glycans are needed.”⁴¹ Oligosaccharides can be obtained by isolation/release from natural sources, or prepared enzymatically and/or chemically. All three approaches are viable, each offering certain advantages, but none can significantly outperform the others.

Oligosaccharide synthesis in solution requires a significant deal of know-how. The automated platform for solid-phase synthesis developed by Seeberger introduces an idea of operational simplicity and highlights that the development of accessible methods for glycan production is essential for further innovations and practical applications in all areas of glycosciences.

The development of the automated synthesizer in our laboratories began with the introduction of the surface-tethered iterative carbohydrate synthesis (STICS).⁴³ The basis for this concept is a surface-functionalized stack of nanoporous gold plates that simplifies the transfer of the gold surface-bound molecules between reaction vessels. At the end of the synthesis, the resulting glycan can be either cleaved-off for further processing or deprotected directly on the gold surface to be used for recognition studies or immunoassay development.⁴⁴ The STICS concept was developed with robotic arm automation in mind. However, we discovered that standard HPLC equipment would offer a more accessible platform for automation. This approach was discovered with nanoporous gold,⁴⁵ but we have also investigated more traditional polymer supports. Using the acceptor-bound approach,⁴⁶ preloaded Tentagel resin was packed in the Omnifit column and integrated into the HPLC system (Scheme 1).⁴² All steps were automated using a three-headed HPLC pump and the reagent consumption was monitored using a standard UV detector. Reagents were recirculated, but still 10 equiv of

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Scheme 1. Original Set-up for HPLC-Assisted Synthesis.⁴²

trichloroacetimidate donors were used for each glycosylation.⁴² More recently, Pentelute and co-workers investigated the HPLC-assisted synthesis of peptides.⁴⁷ Other exciting developments in the area of high throughput and automated syntheses have been particularly inspiring to our own research endeavors.^{48,49}

RESULTS AND DISCUSSION

Presented herein is the development of a broadly useful technology for simple, scalable, and transformative automation of solid-phase synthesis that does not rely on specialized equipment. Broadly available and used in most laboratories, the setup of the HPLC equipment requires no investment. This platform allows for real-time UV detector monitoring of all steps including glycosylation, which, in turn, helps reduce the reaction time and the amount of reagents and solvents needed. The use of a computer interface and standard HPLC liquid handling equipment and software will allow recording a successful automated sequence as a computer program that can then be reproduced by both specialists and nonspecialists with a “press of a button”. While this approach has a potential to revolutionize the way the automation is conducted, solid-phase synthesis suffers from many inherent limitations. Practically every aspect of solid-phase synthesis needs to be refined. Along with the introduction of the autosampler for the reagent delivery, this article is also dedicated to the refinement of some basic aspects of this methodology. Our new basic setup is using standard Agilent 1260 Infinity series HPLC system equipped with the quad pump, a UV detector, and autosampler.

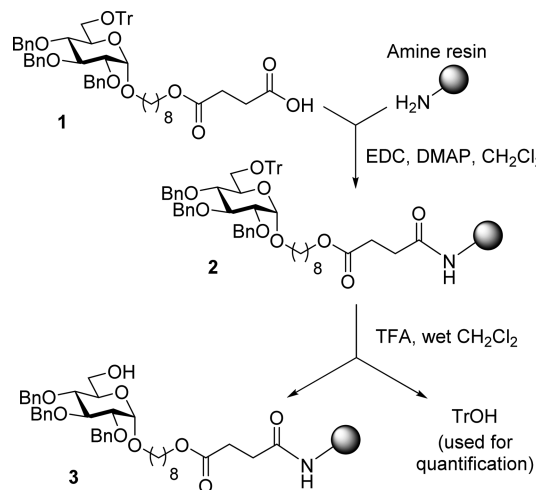
Selection of Resins, Spacers, and Linkers. Our preliminary work on the HPLC-assisted synthesis was solely based on Tentagel resin.⁴² Previously, we compared Tentagel vs Merrifield resins using the manual approach, but saw no significant difference in efficiency and yields.⁴⁶ A recent comparative study by Seeberger et al. determined that the Merrifield resin gives the best efficiency in application to their automation platform.⁵⁰ To gain a better understanding of how loading, swelling, mechanical robustness, size, and other factors may affect the HPLC-assisted synthesis we performed a side-by-side comparison study of Merrifield, Wang,⁵¹ and JandaJel⁵² resins, all of which have been found to be excellent supports for oligosaccharide synthesis and have loading capacities up to 1.0 mmol g⁻¹. Although identifying the best support for universal application might be simply impossible, in a series of comparative experiments we identified JandaJel as the most suitable resin for HPLC-mediated synthesis in terms of loading, reaction times, and yields.

It has become common knowledge that the type of the spacer and/or linker between the acceptor and the polymer

support may be of critical importance.^{53–55} Factors to consider are the chemical composition, stability toward various experimental conditions, and selective (mild) conditions for its cleavage. In our preliminary study, we were using a C4 spacer in combination with succinoyl linker that worked well, and the cleavage was reliably achieved using a small amount (~2 mL) of the recirculating 0.1 M solution of NaOMe in MeOH–CH₂Cl₂. With the general anticipation that extension of the spacer length could move the glycosyl acceptor further out into solution and enhance the efficiency of the reaction with the solution-based glycosyl donor we performed a comparative study. In our study of glycosylations using nanoporous gold, we obtained better yields with the acceptor equipped with longer C8–O–C8 spacer than those of acceptors with shorter C4 or C8 spacers.⁴⁵ With the use of polymer beads we report that while the C8–O–C8 spacer helps to enhance the yields obtained with the C4 spacer, it practically offers no advantage over the more synthetically accessible C8 spacer. Hence, all syntheses described in the article used the C8 spacer.

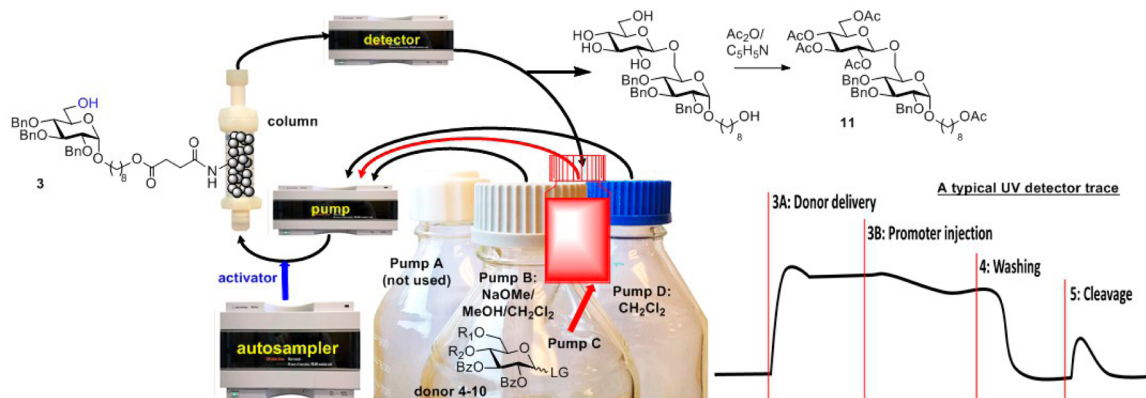
Loading Practices and Quantification. The resin loading capacity is important, but overcrowding of the reactive sites may prevent further elongation, particularly in case of sterically demanding and branched oligosaccharides. During our exploratory study with JandaGel and Tentagel resins, it was observed that the desired loading capacities could be achieved much faster using HPLC-based reagent delivery rather than the manual loading in a flask. Nevertheless, large-scale resin preloading (2–10 g) for this study was performed by the manual approach using the flask and the shaker as depicted in Scheme 2. Building block 1 was coupled with amine JandaJel

Scheme 2. Synthesis of the Solid-Phase-Bound Acceptor 3



resin in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). The loading can be confirmed by weighing the unloaded versus loaded resin, as well as cleaving and quantifying of the loaded acceptor if so desired. The preloaded JandaJel resin 2 was then subjected to detritylation with 10% trifluoroacetic acid in wet CH₂Cl₂. The detritylation results in the formation of glycosyl acceptor 3, but it also releases triphenylcarbinol (TrOH), which could be used for the initial quantification of the loading by its isolation by evaporation and weighing. Quantification of TrOH

Scheme 3. Refinement of the Glycosylation-Cleavage Sequence for the Synthesis of Disaccharide 11



Step	Operation	Mode	Flow rate	Total volume	Time
1	Washing of the resin with CH ₂ Cl ₂ (50 mg, 0.022 mmol)	Pump D	1.0 mL/min	5.0 mL	5 min
2	Swelling resin with CH ₂ Cl ₂	Pump D	1.0 mL/min	2.0 mL (recirculation)	30 min
3A	Glycosylation, delivery of the donor (4-10, 4.4 equiv, 0.10 mmol or 10 equiv, 0.22 mmol, see the table below) in CH ₂ Cl ₂	Pump C	0.5 mL/min	2.0 mL (recirculation)	10 min
3B	Glycosylation, injection of the promoter (see the table below) at 10, 12, 14 min time points	Autosampler		3 x 100 μ L	60-90 min
4	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
5	Cleavage with 0.1 N NaOMe/MeOH/CH ₂ Cl ₂ to release the disaccharide	Pump B	1.0 mL/min	10 mL (recirculation)	20 min

Entry	Donor	Promoter	Yield of 11
1	 4 (0.10 mmol)	AgOTf	50%
2	 5 (0.22 mmol)	NIS/TfOH	57%
3	 6 (0.10 mmol)	TMSOTf	73%
4	 7 (0.10 mmol)	TMSOTf	75%

Entry	Donor	Promoter	Yield of 11
5	 8 (0.10 mmol)	TMSOTf	85%
6	 9 (0.10 mmol)	TMSOTf	89%
7	 10 (0.10 mmol) (0.22 mmol)	TMSOTf	76% 95%

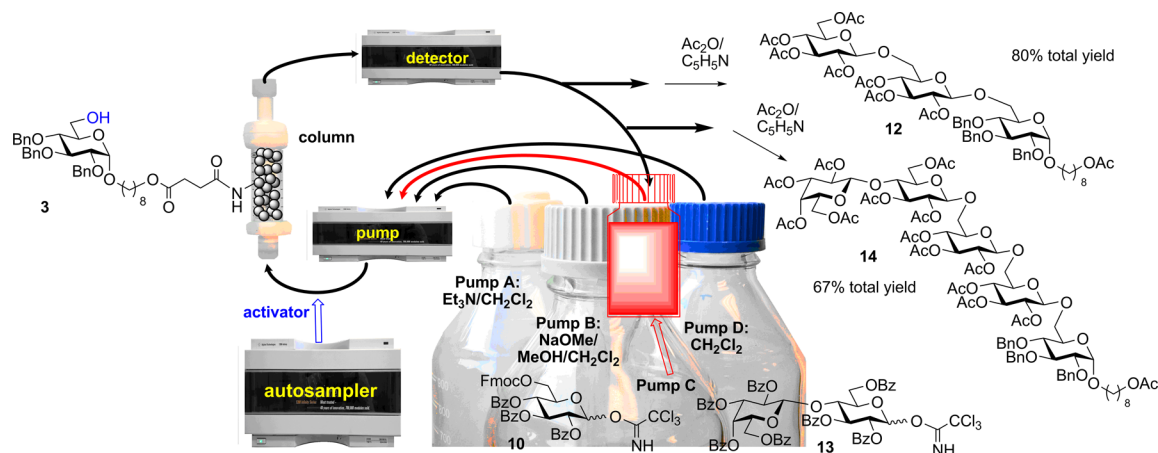
is the key step for determining of the loading capacity of the resin.

Glycosylation: Reagent Delivery, Recirculation, Monitoring, and Synthetic Methods. Glycosylation is a complex multistep process, and reactions on solid supports bring additional hurdles related to the mismatch between highly reactive solution-based vs unreactive solid-phase-based reactants. This mismatch is typically addressed by using a large excess (5–10 equiv) of the solution-based reactant, most commonly the donor, and repeating the reaction 2–3 times to ensure that all solid-supported acceptor is consumed.¹⁴ Automation offers some operational simplicity to oligosaccharide synthesis, but the entire concept may suffer from the inherited drawbacks of conventional methods.

Our experience with HPLC-assisted reactions is still limited, but we already established the protocol for separate delivery of

solutions of glycosyl donor and promoter using HPLC pumps.⁴² The primary focus of the earlier study was to determine ranges of the variables, beginning with reagent ratios, concentration, velocity, and pressure. The reaction efficiency is likely to improve with increased speed of the reagent delivery. However, this may have potential downsides if not properly addressed. If the reagents are delivered too fast, the internal column pressure may increase to a point where the resin beads collapse or fracture.⁶ We have not observed this at our operating velocity of 0.5–2 mL/min (1–12 bar).

The initial reagent delivery via HPLC pump offered a notable limitation of our platform in comparison to Seeberger's automated synthesizer that has 32 intake lines.¹⁹ In principle, essentially the same capability can be achieved with the HPLC setup by splitting of the pump intake lines with eight-way split valves. However, as further steps toward complete automation,

Scheme 4. Automation of Glycosylation-Deprotection-Cleavage Sequences for the Synthesis of Oligosaccharides **12** and **14**

Step	Operation	Mode	Flow rate	Total volume	Time
1	Washing of the resin with CH ₂ Cl ₂ (50 mg, 0.022 mmol)	Pump D	1.0 mL/min	5.0 mL	5 min
2	Swelling resin with CH ₂ Cl ₂	Pump D	1.0 mL/min	2.0 mL (recirculation)	30 min
3	Glycosylation, delivery of 10 (10 equiv, 0.22 mmol) in CH ₂ Cl ₂	Pump C	0.5 mL/min	2.0 mL (recirculation)	60-90 min
	Injection of TMSOTf at 10, 12, 14 min time points	Autosampler		3 x 100 μL	
4	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
5	Fmoc deprotection, delivery of Et ₃ N/CH ₂ Cl ₂	Pump A	1.0 mL/min	20 mL	20 min
6	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
7	Glycosylation, delivery of 10 (10 equiv, 0.22 mmol) in CH ₂ Cl ₂	Pump C	0.5 mL/min	2.0 mL (recirculation)	60-90 min
	Injection of TMSOTf at 10, 12, 14 min time points	Autosampler		3 x 100 μL	
8	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
	<i>Synthesis of 12 go to step 13, synthesis of 14 continue to step 9</i>				
9	Fmoc deprotection, delivery of Et ₃ N/CH ₂ Cl ₂	Pump A	1.0 mL/min	20 mL	20 min
10	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
11	Glycosylation, delivery of 13 (10 equiv 0.22 mmol) in CH ₂ Cl ₂	Pump C	0.5 mL/min	2.0 mL (recirculation)	60-90 min
	Injection of TMSOTf at 10, 12, 14 min time points	Autosampler		3 x 100 μL	
12	Washing with CH ₂ Cl ₂	Pump D	1.0 mL/min	10 mL	10 min
13	Cleavage with 0.1 N NaOMe/MeOH/CH ₂ Cl ₂ to release the oligosaccharide	Pump B	1.0 mL/min	10 mL (recirculation)	20 min

we envisaged the use of a standard HPLC autosampler. Autosamplers are abundant, cheap, easily fit into the HPLC-automation concept, and this approach opens access to hundreds of intake/delivery lines. This approach allows us to liberate other pump intake lines for the delivery of solvent for reactions, washing, and deprotection because only one line is now used for the donor delivery and recirculation. It should be mentioned that the recirculation has already been previously optimized with the purpose of addressing the main drawback of all solid-phase syntheses: the requirement for a large excess of solution-based reagents.

The outline of the automation setup, program sequence, and the key results for basic glycosylation reactions are depicted in Scheme 3. JandaJel resin (50 mg) functionalized with glycosyl acceptor **3** (0.022 mmol) was packed in Omnifit glass chromatography column. The column was connected to the standard Agilent Infinity 1260 HPLC system and the automation sequence was programmed as follows. Pump D was programmed to deliver CH₂Cl₂ at a flow rate of 1.0 mL/min. After discarding the first ~5 mL of the eluate (washing, step 1, Scheme 3) the system was switched to the recirculation mode and 2 mL of CH₂Cl₂ was recirculated for 30 min at a flow rate of 1.0 mL/min (swelling, step 2). After that, pump C was

programmed to deliver a solution of the glycosyl donor (0.10 mmol) in CH₂Cl₂ (2 mL) at 0.5 mL/min and the system was left recirculating for 10 min (step 3A). Beginning from this stage the synthesis was monitored using the integrated UV detector set at 254 nm. A typical trace is shown in Scheme 3.

The integrated autosampler was then programmed to inject a solution of promoter (40 μL) in CH₂Cl₂ (3 injections of 100 μL) at 10, 12, and 14 min after the initial delivery of the donor (step 3B). The system was left recirculating for 60–90 min, and the reaction was monitored by the UV detector in real-time. When the detector trace reaches the plateau, no change in the absorbance of the recirculating solution is observed, the reaction is stopped. In principle, low-efficiency reactions can be supplemented with fresh reagents/reactants at this time. After a typical reaction time of 60–90 min, the system was switched to pump D and washed with CH₂Cl₂ (1.0 mL/min flow rate) to remove excess reagents (step 4). The eluate from the washing step (~10 mL) is discarded. Again, this step was monitored by the UV detector, and the washing was typically stopped after ~10 min when the detector trace reached the baseline corresponding to pure CH₂Cl₂.

To affect the product cleavage from the solid support, pump B was then programmed to deliver a solution of NaOMe/

CH₂Cl₂/MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min (step 5). This step was also monitored by the UV detector. Typically, the cleavage is completed at this stage and the use of the detector monitoring is discontinued. The resulting mixture was recirculated for an additional ~10 min. The eluate was collected, neutralized, concentrated under the reduced pressure and the residue was acetylated with Ac₂O in pyridine to afford disaccharide **11**. The purification of **11** was achieved by conventional column chromatography and its identity was proven by traditional spectral methods.

Our initial study of the HPLC-assisted synthesis⁴² was exclusively based on trichloroacetimidates^{56–58} as glycosyl donors. In an attempt to broaden the scope of this methodology, we performed a comparative study of other common and novel leaving groups. Thioglycosides are generally much less reactive than O-imidates and hence considered less desirable for polymer-supported synthesis. With some prior success of using thioglycosides in glycosylations using polymer⁴⁶ and nanoporous supports⁴³ we investigated S-benzoxazolyl (SBox) donor **4**⁵⁹ and S-phenyl glycosyl donor **5**⁶⁰ in the HPLC-automated reactions. Glycosylation of SBox donor **4** with resin-bound acceptor **3** was performed in the presence of AgOTf. Following the general programming described above, disaccharide **11** was obtained in a good yield of 50% (Scheme 3, entry 1). A similar result was achieved with SPh donor **5**, wherein NIS/TfOH promoted reaction afforded disaccharide **11** in 57% yield (entry 2). While the outcome of these reactions could be improved by injecting additional quantities of reagents, we chose to explore other classes of glycosyl donors.

Recently, we developed a new class of glycosyl donors, O-benzoxazolyl (OBox) imidates, which were also tested in the HPLC-based applications, but could not outperform traditional trichloroacetimidates.⁶¹ We also introduced 3,3-difluoro-3*H*-indol-2-yl (OFox) imidates,⁶² which showed a very high reactivity and allowed us to obtain impressive results in the HPLC-based application. Thus, glycosylation of OFox donor **6** with resin-bound acceptor **3** was performed in the presence of TMSOTf. Following the general programming, disaccharide **11** was obtained in a good yield of 73% (entry 3). A very similar outcome was obtained with phosphate donor **7**, a glycosylation approach frequently used in Seeberger's automation method.⁶³ The phosphate donor **7** also provided a very impressive result in our HPLC-based platform wherein TMSOTf-promoted activation led to disaccharide **11** in 75% yield (entry 4). Nevertheless, the most consistent result and the highest yield was obtained with trichloroacetimidate **8**.⁶⁴ TMSOTf-promoted activation led to disaccharide **11** in an excellent yield of 85% using only 4.4 equiv of the donor (entry 5). In order to expand this procedure to selectively protected imidates we investigated donors **9** and **10**⁴² containing a selectively removable Fmoc protecting group at C-4 and C-6, respectively. TMSOTf promoted glycosylations afforded disaccharide **11** in 89 and 76% yields, respectively. The latter yield could be increased to 95% by using 10 equiv of donor **10**.

Fmoc Deprotection and Reiteration for the Synthesis of Oligosaccharides. Having optimized conditions for glycosylation we decided to undertake the synthesis of two linear oligosaccharides **12** and **14**. General programming outline is presented in Scheme 4. For the synthesis of trisaccharide **12** we selected glycosyl donor **10** equipped with the selectively removable Fmoc group at C-6. Previously, we have shown that Fmoc can be removed using mild reagents

(piperidine/DMF, 2–5 min or TEA/CH₂Cl₂, 10–20 min using HPLC setup) and also provides a very straightforward and informative mode for monitoring the deprotection step and quantification of the glycosylation.⁴² To gain better yields and minimize side reactions we decided to use a larger excess of donor **10** (10 equiv). After washing and swelling of the resin containing acceptor **3** (0.022 mmol, pump D, steps 1 and 2, Scheme 4), pump C was programmed to deliver donor **10** (0.22 mmol) in CH₂Cl₂ (2 mL total volume) at 0.5 mL/min, which was then recirculated for 10 min. Again, all automated sequence steps have been monitored with the UV detector. The autosampler was programmed to deliver a solution of promoter in CH₂Cl₂ (3 injections of 100 μL each) and the resulting reaction mixture was recirculated for 60–90 min. When the UV-monitoring showed no change in absorbance of the eluate passing through the detector, the system was washed with CH₂Cl₂ (pump D, 1.0 mL/min rate flow for 10 min).

A capping step in the synthetic cycle is important because it prevents the accumulation of shorter oligosaccharides due to incomplete reactions. Capping can be as simple as acetylation with Ac₂O in pyridine,⁶⁵ or by using benzoyl isocyanate in CH₂Cl₂, a procedure developed by Schmidt.⁶⁶ It should be mentioned that due to high yields achieved in glycosylations of reactive primary hydroxyls with trichloroacetimidates capping was found unnecessary. To affect the deprotection of the Fmoc group, pump A was programmed to deliver a solution of triethylamine/CH₂Cl₂ (1/1, v/v 1.0 mL/min flow rate).

The release of the dibenzofulvene-triethylamine adduct was monitored by using the UV detector set at 312 nm. Upon reaching the baseline indicating that dibenzofulvene-triethylamine is no longer produced (20 min/20 mL total volume for step 5), the pump D was engaged for washing (step 6, 10 min). The resulting solid-phase bound disaccharide acceptor was glycosylated with donor **10** following essentially the same programming sequence as that for the first cycle. Upon completion of the glycosylation and washing (steps 7 and 8) pump B was engaged to deliver a solution of NaOMe/CH₂Cl₂/MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min to remove the resulting trisaccharide (step 13). The eluate was collected, neutralized, concentrated, and the residue was acetylated with Ac₂O in pyridine to afford trisaccharide **12** in 80% yield.

To investigate whether the trisaccharide sequence achieved during the synthesis of **12** could be extended further we explored a possibility for the chain elongation. For this purpose, we repeated the same steps 1–8 as those described for the synthesis of **12**. It should be noted that in this case a completely automated sequence was reproduced simply by using the same program as previously. The solid phase bound trisaccharide intermediated was subjected to Fmoc deprotection (step 9) and washing (step 10). The subsequent glycosylation step was performed using lactosyl donor **13**⁶⁷ with the main aim of determining the scope of using larger building blocks (step 11). The glycosylation with disaccharide donor **13** was performed following essentially the same programming sequence as that for other glycosylations described in this article. Upon completion of the glycosylation and washing (steps 11 and 12) pump B was engaged to deliver a solution of NaOMe/CH₂Cl₂/MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min followed by recirculation for additional 10 min to remove the resulting pentasaccharide (step 13). The eluate was collected, neutralized, concentrated, and the residue was

acetylated with Ac₂O in pyridine to afford pentasaccharide **14** in 67% yield overall.

CONCLUSIONS

In conclusion, we optimized the synthetic and operational strategies for HPLC-based automation, and have created a generally useful tool for accelerating glycan synthesis. This automated technology offers a transformative, semiautomated approach to synthesis. Automated HPLC-based synthesis introduces rather sophisticated yet affordable *in situ* monitoring and reagent recirculation concepts. This basic approach provided a solid basis for the implementation of a standard autosampler system for the fully automated delivery of reagents. Further optimization of HPLC technology and its application using different resin, spacers, linkers is currently underway. Our efforts are also focusing on developing efficient protocols for the synthesis of branched hetero-oligosaccharides as well as using the autosampler for delivering all sugar building blocks and deprotecting reagents necessary for the synthesis.

EXPERIMENTAL SECTION

General Methods. The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70–230 mesh), reactions were monitored by TLC on Kieselgel 60 F₂₅₄. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH₂Cl₂ was distilled from CaH₂ directly prior to application. Pyridine and acetonitrile were dried by refluxing with CaH₂ and then distilled and stored over molecular sieves (3 Å). Molecular sieves (4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2–3 h at 390 °C directly prior to application. Dowex Monosphere 650C (H⁺) was washed three times with MeOH and stored under MeOH. Optical rotations were measured using a polarimeter. ¹H NMR spectra were recorded at 300 or 600 MHz, ¹³C NMR spectra were recorded at 75 or 150 MHz. The ¹H chemical shifts are referenced to the signal of the residual CHCl₃ (δ_H = 7.24 ppm). The ¹³C chemical shifts are referenced to the central signal of CDCl₃ (δ_C = 77.23 ppm). HRMS determinations were made with the use of a mass spectrometer with FAB ionization and ion-trap detection. Agilent 1260 infinity HPLC System and Agilent 1260 Variable Wavelength UV–vis Detector were used to assemble the automated synthesizer.

Synthesis of Glycosyl Acceptor 3. 8-(*tert*-Butyldiphenylsilyloxy)oct-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl- α -D-glucopyranoside (**17**). A mixture of ethyl 2,3,4-tri-*O*-benzyl-1-thio-6-*O*-triphenylmethyl- α -D-glucopyranoside (**15**,⁶⁸ 3.0 g, 4 mmol), 8-(*tert*-butyldiphenylsilyloxy)octan-1-ol (**16**,⁶⁹ 1.3 g, 3.3 mmol), and freshly activated molecular sieves (4 Å, 3.0 g) in diethyl ether (100 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 1.8 g, 8.0 mmol) and TfOH (71 μ L, 0.8 mmol) were added, and the resulting mixture was stirred for 20 min at rt. After that, the solids were filtered off and washed successively with CH₂Cl₂. The combined filtrate (~200 mL) was washed with sat. aq. Na₂SO₄ (10 mL) and water (3 \times 10 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate–hexane gradient elution) to afford compound **17** (2.27 g, 65%) as a colorless foam. Analytical data for **17**: R_f = 0.62 (ethyl acetate/hexanes, 1/4, v/v); [α]_D²⁵ + 25.3 (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 0.89 (s, 9H, C(CH₃)₃), 1.12–1.20 (m, 8H, 4 \times CH₂), 1.38, 1.50 (2 m, 4H, 2 \times CH₂), 3.03 (dd, 1H, J_{5,6b} = 4.8 Hz, J_{6a,6b} = 9.9 Hz, H-6a), 3.31–3.32 (m, 2H, H-6b, OCH₂^a), 3.42–3.50 (m, 4H, H-2, 4, CH₂), 3.56 (m, 1H, OCH₂^b), 3.68 (m, 1H, H-5), 3.82 (dd, 1H, J_{3,4} = 9.2 Hz, H-3), 4.13 (d, 1H, ²J = 10.4 Hz, 1/2 CH₂Ph), 4.52–4.72 (m, 5H, H-1, 2 \times CH₂Ph), 4.81 (d, 1H, ²J = 10.6 Hz, 1/2 CH₂Ph), 6.69–7.52 (m, 40H,

aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 19.4, 25.9 (\times 3), 26.4, 29.5 (\times 2), 29.6 (\times 4), 30.2, 32.7 (\times 2), 63.8, 64.2, 70.5, 73.3, 75.2, 76.1, 78.5, 80.6, 82.5, 86.4, 96.7, 126.9 (\times 2), 127.1, 127.7 (\times 6), 127.8 (\times 4), 127.9 (\times 3), 128.1, 128.3 (\times 2), 128.4, 128.6 (\times 3), 128.9 (\times 4), 129.0 (\times 3), 129.6 (\times 3), 134.3, 135.7 (\times 6), 138.1, 138.7, 139.0, 144.1, 144.7 ppm; HR-FAB MS [M+Na]⁺ calcd for C₇₀H₇₈O₇SiNa 1081.5415, found 1081.5435.

8-Hydroxyoct-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl- α -D-glucopyranoside (18**).** Tetrabutylammonium fluoride (TBAF, 1.72 mL, 1.995 mmol) was added to a solution of **17** (2.0 g, 1.995 mmol) in THF (14 mL) and the resulting mixture was stirred under argon for 3 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~150 mL), washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) to afford compound **18** (1.68 g, 95%) as a colorless foam. Analytical data for **18**: R_f = 0.45 (ethyl acetate/hexanes, 1/2, v/v); [α]_D²⁶ + 19.7 (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.21–1.29 (m, 8H, 4 \times CH₂), 1.43, 1.61 (2 m, 4H, 2 \times CH₂), 3.17 (dd, 1H, J_{5,6a} = 3.0 Hz, J_{6a,6b} = 9.8 Hz, H-6a), 3.45–3.61 (m, 6H, H-2, 4, 6b, OCH₂^a, OCH₂^b), 3.66 (m, 1H, OCH₂^b), 3.83 (m, 1H, H-5), 3.96 (dd, 1H, J_{3,4} = 10.2 Hz, H-3), 4.27 (d, 1H, ²J = 10.4 Hz, 1/2 CH₂Ph), 4.67 (d, 1H, ²J = 10.3 Hz, 1/2 CH₂Ph), 4.69 (d, 1H, ²J = 12.1 Hz, 1/2 CH₂Ph), 4.76–4.86 (m, 3H, H-1, CH₂Ph), 4.95 (d, 1H, ²J = 10.6 Hz, 1/2 CH₂Ph), 6.83–7.46 (m, 30H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 25.8, 26.3, 29.4, 29.5, 29.6, 32.9, 62.8, 63.1, 68.1, 70.5, 73.2, 75.2, 76.0, 78.4, 80.6, 82.4, 86.4, 96.7, 127.0 (\times 3), 127.7, 127.8, 127.9 (\times 6), 128.0 (\times 3), 128.2 (\times 2), 128.3 (\times 5), 128.4 (\times 4), 128.9 (\times 6), 138.0, 138.6, 138.9, 144.1 (\times 2) ppm; HR-FAB MS [M+Na]⁺ calcd for C₅₄H₆₀O₇Na 843.4237, found 843.4257.

8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl- α -D-glucopyranoside (1**).** Succinic anhydride (0.440 g, 4.39 mmol) and 4-dimethylaminopyridine (DMAP, 0.053 g, 0.438 mmol) were added to a solution of compound **18** (1.3 g, 1.464 mmol) in pyridine (5.0 mL) and the resulting mixture was stirred under argon for 16 h at 65 °C. After that, the volatiles were removed under the reduced pressure and the residue was coevaporated with toluene (3 \times 10 mL) and purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) to afford the title compound (1.30 g, 97%) as a colorless foam. Analytical data for **1**: R_f = 0.25 (ethyl acetate/hexanes, 1/1, v/v); [α]_D²⁷ + 21.8 (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.28–1.37 (m, 8H, CH₂), 1.54–1.66 (m, 4H, 2 \times CH₂), 2.55–2.63 (m, 4H, 2 \times CH₂), 3.17 (dd, 1H, J_{5,6a} = 4.9 Hz, J_{6a,6b} = 9.8 Hz, H-6a), 3.44–3.47 (m, 2H, H-6b, OCH₂^a), 3.53–3.61 (m, 2H, H-2, 4), 3.69–3.72 (m, 1H, OCH₂^b), 3.82 (m, 1H, H-5), 3.95 (dd, 1H, J_{3,4} = 9.2 Hz, H-3), 4.03 (t, 2H, J = 6.6 Hz, CH₂), 4.25 (d, 1H, ²J = 10.3 Hz, 1/2 CH₂Ph), 4.66 (d, 1H, ²J = 10.4 Hz, 1/2 CH₂Ph), 4.68 (d, 1H, ²J = 12.1 Hz, 1/2 CH₂Ph), 4.75–4.84 (m, 4H, H-1, 1/2 CH₂Ph) 4.93 (d, 1H, ²J = 10.7 Hz, 1/2 CH₂Ph), 6.83–7.44 (m, 30H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 25.1, 26.1, 28.5, 28.7, 28.9, 29.1, 29.3, 20.4, 62.7, 65.0, 67.9, 70.3, 73.1, 75.1, 75.9, 78.3, 80.4, 82.2, 86.3, 95.5, 126.9 (\times 3), 127.7 (\times 2), 127.8 (\times 5), 127.9 (\times 2), 128.1 (\times 3), 128.2 (\times 3), 128.3 (\times 5), 128.4, 128.8 (\times 5), 137.8, 138.4, 138.6, 138.8, 144.0 (\times 3), 144.5, 172.2 ppm; HR-FAB [M+Na]⁺ calcd for C₅₈H₆₄NaO₁₀ 943.4397, found 943.4371.

Resin-Bound Acceptor 3. JandaJel amine resin (1% cross-linked polystyrene, 500 mg, 0.25 mmol) was added to a solution of **1** (253 mg, 0.275 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 105.4 mg, 0.55 mmol), and DMAP (30 mg, 0.25 mmol) in CH₂Cl₂ (5.0 mL) and the resulting suspension was agitated under argon for 18 h at rt. When the Kaiser test⁷⁰ showed the negative result, the resin was filtered off; washed with CH₂Cl₂ (3 \times 20 mL), methanol (3 \times 20 mL), and CH₂Cl₂ (3 \times 20 mL); and dried *in vacuo* for 4 h. The resulting resin **2** was swelled in CH₂Cl₂ (10 mL) for 60 min at rt. A 10% solution of TFA in wet CH₂Cl₂ (5.0 mL) was added dropwise and the resulting suspension was agitated for 3 h at rt. The resin was filtered off; washed with CH₂Cl₂ (3 \times 20 mL), methanol (3 \times 20 mL), and CH₂Cl₂ (3 \times 20 mL); and dried *in vacuo* for 6 h to afford the title compound. The loading (0.44 mmol/g) was

determined by the quantification of TrOH formed as a result of the treatment with TFA.

Synthesis of Glycosyl Donors. Benzoxazolyl 2,3,4,6-Tetra-O-benzoyl-1-thio- β -D-glucopyranoside (4). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁵⁹

Phenyl 2,3,4,6-Tetra-O-benzoyl-1-thio- β -D-glucopyranoside (5). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁷¹

3,3-Difluoro-3H-indol-2-yl 2,3,4,6-Tetra-O-benzoyl- α -D-glucopyranoside (6). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁶²

Bis(buthyl)phosphoryl 2,3,4,6-Tetra-O-benzoyl- β -D-glucopyranoside (7). A mixture of 5 (700 mg, 1.0 mmol), dibutyl phosphate (0.58 mL, 3.0 mmol), and freshly activated molecular sieves (4 Å, 1.5 g) in CH_2Cl_2 (15 mL) was stirred under argon for 1 h at rt. After that, NIS (265 mg, 1.2 mmol) and TFOH (10 μL , 0.12 mmol) were added and the resulting mixture was stirred for 18 h at rt. The solid was then filtered off and rinsed successively with CH_2Cl_2 . The combined filtrate (~40 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 \times 10 mL). The organic phase was separated, dried with MgSO_4 , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate–hexane gradient elution) to afford the title compound (1.30 g, 97%) as a clear syrup. Analytical data for 7: R_f = 0.24 (ethyl acetate/hexanes, 1/1, v/v); $[\alpha]_{\text{D}}^{26} + 33.9$ (c = 1.0, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 0.64, 0.79 (2 t, 6H, 2 \times CH_3), 0.90 (2 m, 4H, 2 \times CH_2), 1.18–1.69 (m, 4H, 2 \times CH_2), 3.73 (m, 2H, OCH_2^a), 3.99 (m, 2H, OCH_2^b), 4.28 (m, 1H, H-5), 4.46 (dd, 1H, $J_{5,6a} = 5.0$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.64 (dd, 1H, $J_{5,6b} = 2.5$ Hz, H-6b), 5.60–5.75 (m, 3H, H-1, 2, 4), 5.91 (dd, 1H, $J_{3,4} = 10.5$ Hz, H-3), 7.15–7.80 (m, 20H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 13.4, 13.5, 18.2, 18.5, 31.7, 31.8, 32.0, 62.6, 67.0, 68.0, 68.1, 68.2, 69.0, 71.7, 71.8, 72.5, 73.0, 96.6, 128.3 ($\times 4$), 128.5 ($\times 4$), 128.6, 128.7, 129.4, 129.8 ($\times 5$), 129.9 ($\times 4$), 133.3, 133.4, 133.5, 133.6 ppm. HR-FAB $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{45}\text{O}_{13}\text{PNa}$ 811.2495, found 811.2505.

2,3,4,6-Tetra-O-benzoyl- β -D-glucopyranosyl Trichloroacetimidate (8). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.^{64,72}

2,3,6-Tri-O-benzoyl-4-O-(9-fluorenylmethoxycarbonyl)- α - β -D-glucopyranosyl Trichloroacetimidate (9). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁴²

2,3,4-Tri-O-benzoyl-6-O-(9-fluorenylmethoxycarbonyl)- α - β -D-glucopyranosyl Trichloroacetimidate (10). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁴²

O-(2,3,4,6-Tetra-O-benzoyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-O-benzoyl- β -D-glucopyranosyl Trichloroacetimidate (13). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.⁶⁷

HPLC-Mediated Synthesis of Oligosaccharides. General Procedure for Glycosylation and Cleavage. Functionalized JandaJel resin 3 (50 mg, 0.022 mmol) was packed in an Omnifit glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of glycosyl donor (4–10, 0.10 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector ($\lambda_{\text{max}} = 254$ nm). The integrated autosampler was programmed to inject a solution of the promoter in CH_2Cl_2 (3 \times 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL)

was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 5). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH , and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation.

General Procedure for Acetylation of Released Disaccharide. A crude residue was redissolved in pyridine (2.0 mL), Ac_2O (73 μL , 0.771 mmol) was added dropwise, and the resulting mixture was stirred for 16 h at rt. The reaction mixture was quenched with CH_3OH (~1.0 mL) and the volatiles were removed under the reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (2 \times 10 mL), water (20 mL), sat. aq. NaHCO_3 (20 mL), and water (2 \times 20 mL). The organic phase was separated, dried with MgSO_4 , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) to afford disaccharide 11.

8-Acetyloxyoctan-1-yl O-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-O-benzyl- α -D-glucopyranoside (11). The title compound was synthesized from glycosyl donors 4–10 and glycosyl acceptor 3 in 50–89% yield. Analytical data for 11: R_f = 0.57 (ethyl acetate/hexanes, 1/1, v/v); $[\alpha]_{\text{D}}^{27} + 8.90$ (c = 1.0, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.22–1.29 (m, 8H, 4 \times CH_2), 1.50–1.58 (m, 4H, 2 \times CH_2), 1.91–2.07 (s s, 15H, 5 \times COCH_3), 3.32–3.50 (m, 3H, H-2, 4, OCH_2^a), 3.55–3.75 (m, 4H, 1/2 OCH_2^a , H-5, H-6b, H-5'), 3.95 (dd, 1H, $J_{3,4} = 9.3$ Hz, H-3), 3.98–4.23 (m, 5H, H-6a, 6'a, 6'b, OCH_2^b), 4.47 (d, 1H, $J_{1,2'} = 7.5$ Hz, H-1'), 4.50 (dd, 1H, $^2J = 9.8$ Hz, 1/2 CH_2Ph), 4.60 (d, 1H, $^2J = 12.0$ Hz, 1/2 CH_2Ph), 4.68 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.74 (d, 1H, $^2J = 12.0$ Hz, 1/2 CH_2Ph), 4.76 (d, 1H, $^2J = 10.9$ Hz, 1/2 CH_2Ph), 4.83 (d, 1H, $^2J = 10.7$ Hz, 1/2 CH_2Ph), 4.96 (d, 1H, $^2J = 11.0$ Hz, 1/2 CH_2Ph), 5.02 (dd, 1H, $J_{2,3'} = 9.2$ Hz, H-2'), 5.05 (dd, 1H, $J_{4,5'} = 9.6$ Hz, H-4'), 5.15 (dd, 1H, $J_{3,4'} = 9.4$ Hz, H-3'), 7.22–7.31 (m, 15H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 20.8 ($\times 2$), 20.9 ($\times 2$), 21.3, 26.1, 26.3, 28.7, 29.4, 29.5, 29.6, 57.2, 62.1, 64.8, 68.2, 68.3, 68.5, 68.6, 69.6, 71.4, 71.9, 73.0, 73.2, 73.3, 75.1, 75.8, 80.2, 96.8, 100.8, 101.8, 127.7, 128.0 ($\times 2$), 128.1, 128.2, 128.3 ($\times 2$), 128.5 ($\times 2$), 128.6 ($\times 2$), 128.7 ($\times 2$), 138.3, 138.4, 139.0, 169.2, 169.5, 170.5, 170.8, 171.4 ppm; HR-FAB $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{51}\text{H}_{66}\text{O}_{17}\text{Na}$ 973.4198, found 973.4175.

8-Acetyloxyoct-1-yl O-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-O-acetyl- α -D-glucopyranoside (12). Functionalized JandaJel resin 3 (50 mg, 0.022 mmol) was packed in an Omnifit glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor 10 (188 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector ($\lambda_{\text{max}} = 254$ nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL , 0.44 mmol) in CH_2Cl_2 (3 \times 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/ CH_2Cl_2 (1/1, v/v) for 20 min at 1.0 mL/min (step 5). This step was monitored by the integrated UV detector ($\lambda_{\text{max}} = 312$ nm). After that, pump A was stopped and pump D was programmed to deliver CH_2Cl_2

at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 6). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **10** (188 mg, 0.22 mmol) in CH₂Cl₂ (2 mL) at a flow rate of 0.5 mL/min (step 7). This step was monitored by the integrated UV detector ($\lambda_{\max} = 254$ nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μ L, 0.44 mmol) in CH₂Cl₂ (3 \times 100 μ L) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 8). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH₃OH/CH₂Cl₂ (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 9). Pump B was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H⁺) resin. The resin was filtered off, washed successively with CH₂Cl₂ and CH₃OH, and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound **11**. The crude residue was purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) to afford trisaccharide **12** in 80% yield. Analytical data for **12**: R_f = 0.44 (ethyl acetate/hexanes, 1/1, v/v); [α]_D²⁷ + 6.34 (c = 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ , 1.22–1.28 (m, 8H, 4 \times CH₂), 1.58–1.59 (m, 4H, 2 \times CH₂), 1.95–2.06 (8 s, 24H, 8 \times COCH₃), 3.31 (m, 1H, OCH₂^a), 3.45–3.49 (m, 2H, H-2, 4), 3.56–3.63 (m, 4H, H-5', 5'', 6'a, OCH₂^b), 3.74–3.70 (m, 2H, H-5, 6a), 3.81 (d, 1H, J_{6'a,6'b} = 10.5 Hz, H-6'b), 3.96 (dd, 1H, J_{3,4} = 9.1 Hz, H-3), 3.93–4.08 (m, 4H, H-6''a, 6b, CH₂), 4.22 (dd, 1H, J_{5',6'b} = 4.4 Hz, J_{6'a,6'b} = 12.3 Hz, H-6''b), 4.46–4.49 (m, 3H, H-1', 1'', 1/2 CH₂Ph), 4.60 (d, 1H, ²J = 12.1 Hz, 1/2 CH₂Ph), 4.68 (d, 1H, J_{1,2} = 2.8 Hz, H-1), 4.75 (dd, 2H, ²J = 13.7 Hz, CH₂Ph), 4.86–4.82 (m, 2H, H-4', 1/2 CH₂Ph), 4.90–5.01 (m, 4H, H-2', 2'', 4'', 1/2 CH₂Ph), 5.08–5.12 (m, 2H, H-3', 3''), 7.23–7.32 (m, 15H, aromatic) ppm; ¹³C NMR (150 MHz, CDCl₃): δ , 20.7, 20.8, 20.9 (\times 2), 20.92, 21.2, 25.9, 26.4, 28.7, 29.4, 29.5, 29.6, 61.9, 64.7, 68.0, 68.1, 63.3, 68.4, 69.2, 69.7, 71.2, 71.5, 72.1, 72.8, 73.1, 73.2, 73.4, 75.1, 75.8, 77.6, 80.2, 82.0, 96.9, 100.5, 100.9, 127.7, 128.0 (\times 3), 128.1 (\times 3), 128.2 (\times 2), 128.4, 128.5 (\times 2), 128.6 (\times 2), 128.7, 128.8 (\times 2), 138.3, 138.4, 139.0, 169.2, 169.3, 169.5, 169.7, 170.3, 170.4, 170.8, 171.4 ppm; HR-FAB [M+Na]⁺ calcd for C₆₃H₈₂O₂₅Na 1261.5025, found 1261.5071.

8-Acetyloxyoct-1-yl O-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-O-(2,3,6-tri-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-O-(2,3,4-tri-O-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-O-benzyl- α -D-glucopyranoside (14**).** Functionalized JandaJel resin **3** (50 mg, 0.022 mmol) was packed in an Omnifit glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH₂Cl₂ continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **10** (188 mg, 0.22 mmol) in CH₂Cl₂ (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector ($\lambda_{\max} = 254$ nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μ L, 0.44 mmol) in CH₂Cl₂ (3 \times 100 μ L) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/CH₂Cl₂ (1/1, v/v) for 20 min at 1.0 mL/min (step 5). This step was monitored by the integrated UV detector ($\lambda_{\max} = 312$ nm). After that, pump A was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 6). After that, pump D was stopped and pump C

was programmed to deliver a solution of donor **10** (188 mg, 0.22 mmol) in CH₂Cl₂ (2 mL) at a flow rate of 0.5 mL/min (step 7). This step was monitored by the integrated UV detector ($\lambda_{\max} = 254$ nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μ L, 0.44 mmol) in CH₂Cl₂ (3 \times 100 μ L) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 8). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/CH₂Cl₂ (1/1, v/v) for 20 min at 1.0 mL/min (step 9). This step was monitored by the integrated UV detector ($\lambda_{\max} = 312$ nm). After that, pump A was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 10). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **13** (266 mg, 0.22 mmol) in CH₂Cl₂ (2 mL) at a flow rate of 0.5 mL/min (step 11). This step was monitored by the integrated UV detector ($\lambda_{\max} = 254$ nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μ L, 0.44 mmol) in CH₂Cl₂ (3 \times 100 μ L) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60–90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 12). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH₃OH/CH₂Cl₂ (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 13). Pump B was stopped and pump D was programmed to deliver CH₂Cl₂ at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H⁺) resin. The resin was filtered off, washed successively with CH₂Cl₂ and CH₃OH, and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound **11**. The crude residue was purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) to afford pentasaccharide **14** in 67% yield. Analytical data for **14**: R_f = 0.26 (ethyl acetate/hexanes, 1/1, v/v); [α]_D²⁷ –1.94 (c = 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ , 1.26–1.30 (m, 8H, 4 \times CH₂), 1.55–1.59 (m, 4H, 2 \times CH₂), 1.90–2.12 (m, 42H, 14 \times COCH₃), 3.30 (m, 1H, OCH₂^a), 3.44–3.46 (m, 2H, H-2, 4), 3.53–3.63 (m, 6H, H-5', 5'', 5''', 6'a, 6''a, OCH₂^b), 3.68–3.73 (m, 2H, H-6a, 6''b), 3.77–3.86 (m, 4H, H-5, 5''', 6''', 6''b), 3.94 (dd, 1H, J_{3,4} = 9.2 Hz, H-3), 4.03–4.10 (m, 7H, H-2', 2''', 6b, 6''b, 6''b, CH₂), 4.42–4.52 (m, 6H, H-1', 1'', 1''', 1''', 6''b, 1/2 CH₂Ph), 4.59 (d, 1H, ²J = 12.0 Hz, 1/2 CH₂Ph), 4.69 (d, 1H, J_{1,2} = 3.1 Hz, H-1), 4.73 (dd, 2H, ²J = 11.6 Hz, CH₂Ph), 4.82–7.97 (m, 6H, H-2', 2'', 4', 4'', 1/2 CH₂Ph), 4.99 (dd, 1H, J_{3''',4''''} = 8.4 Hz, H-3'''), 5.06–5.16 (m, 4H, H-3', 3'', 3''', 1/2 CH₂Ph), 5.31 (d, 1H, J_{4''',5''''} = 2.1 Hz, H-4'''), 7.24–7.32 (m, 15H, aromatic) ppm; ¹³C NMR (150 MHz, CDCl₃): δ , 20.6 (\times 2), 20.7 (\times 3), 20.8, 20.9 (\times 2), 21.1, 26.0, 26.2, 28.7, 29.4, 29.5, 29.8 (\times 2), 62.0, 64.4 (\times 2), 66.6, 68.0, 68.1, 69.0, 69.1, 69.2 (\times 2), 69.7, 70.7 (\times 2), 71.0, 71.2, 71.4 (\times 2), 71.5, 72.8, 72.9, 73.0, 73.1 (\times 2), 73.2, 75.0, 75.7, 76.2, 77.5, 80.1, 81.9, 96.7, 100.5, 100.7, 100.9, 101.1, 127.6, 127.9 (\times 7), 128.0, 128.1 (\times 3), 128.4 (\times 3), 128.5 (\times 3), 128.6 (\times 3), 138.3, 138.4, 138.9, 169.1, 169.2, 169.3, 169.5, 169.6, 169.8, 170.2, 170.3, 170.4 (\times 3), 171.3 ppm; HR-FAB [M+Na]⁺ calcd for C₈₇H₁₁₄O₄₁ Na 1837.6716, found 1837.6703.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01439.

Spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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