JOC The Journal of Organic Chemistry

Article

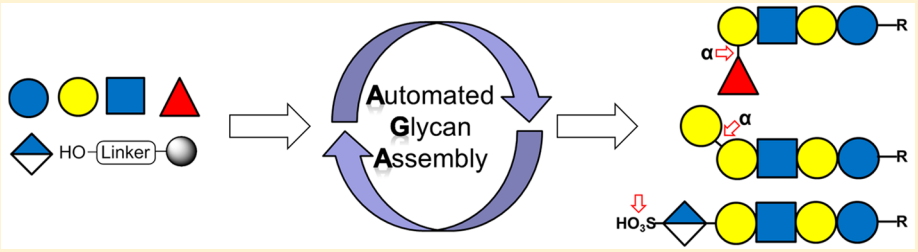
pubs.acs.org/joc

Automated Glycan Assembly of Complex Oligosaccharides Related to Blood Group Determinants

Heung Sik Hahm,[†,#] Chien-Fu Liang,[‡] Chian-Hui Lai,[§] Richard J. Fair, Frank Schuhmacher,[†] and Peter H. Seeberger*[,†]

Department of Biomolecular Systems, Max Planck Institute for Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany

Supporting Information



ABSTRACT: Lactotetraosyl (Lc4) and neo-lactotetraosyl (nLc4) are backbones that are common to many glycans. Using automated glycan assembly, these common core structures were constructed and elaborated to access synthetically challenging glycans of biological relevance. The incorporation of α-fucoses is demonstrated for H-type I and II; α(1,3)-galactose epitopes were prepared, and the pentasaccharide HNK-1 required incorporation of a 3-*O*-sulfate. In addition to preparing the target structures, essential insights were gained regarding the relationships of glycosylating agents and nucleophiles as well as the linker stability.

INTRODUCTION

Ten monosaccharides are the basis of complex mammalian glycans.[1] Nature did not make all possible combinations of glycans, but rather, based on common biosynthetic pathways, some scaffolds are present in diverse glycans that serve very different biological functions. Two isomeric tetrasaccharides, Lc4 (**1**) and nLc4 (**2**), are the core structures of the lacto- and neo-lacto subfamilies of glycosphingolipids (GSLs). GSLs are glycolipids that have been linked to many diseases.[2] Many GSLs are tumor-associated carbohydrate antigens (TACAs) that play critical roles in cancer development and survival.[3] Fucosylation of the Lc4 and nLc4 cores gives rise to the Lewis blood group antigens (**5**−**8**) as well as H-type I (**3**) and II (**4**) glycans (Figure 1).

Some of these glycans serve as receptors for bacteria and viruses.[4,5] Sialylated Lewis blood group antigens are prevalent TACAs and cancer vaccine candidates[6] but are also associated with immunodeficiency disorders and atherosclerosis.[7] Sialyl-nLc4 (SPG), for example, is connected to certain cancers,[3,7] the neurological disorder Guillain−Barré syndrome,[8] and pathogen-cell recognition.[9] Lc4, nLc4, Lewis, and sialyl Lewis antigens can be sulfated directly or may contain additional sulfated saccharide units as is the case in HNK-1, an nLc4-based pentasaccharide containing a terminal 3-*O*-SO_3-glucuronic acid that is found in glycoproteins of the central nervous system.[8] The addition of a terminal α-galactose onto nLc4 gives rise to antigens such as the P^k blood group antigen that contains a terminal α(1,4)-galactose and has been associated with several forms of bacterial and viral pathogenesis.[10] Addition of an α(1,3)-galactose onto nLC4 is associated with hyperacute rejection of xenotransplanted organs.[11]

Pure, structurally defined glycans are critical to the study of these biologically significant molecules. Different methods are used to synthesize glycans: Enzymatic methods possess extraordinary regio- and stereoselectivity without the need for protecting group manipulations. However, accessing glycosyltransferases is laborious and mainly makes sense for large scale preparations of unmodified glycans.[12] Traditional solution-phase oligosaccharide synthesis is reliable and allows for the incorporation of unnatural glycans. However, it is a time-consuming process that typically requires many purification steps and protecting-group manipulations.[13] Sequential one-pot strategies that take advantage of various building block reactivities obviate the need for intermediate purifications and greatly accelerate syntheses.[14a] Implementation of this method can be challenging as it requires multiple building blocks to install a particular linkage.[14b] Automated solid-phase strategies similarly accelerate syntheses and avoid intermediate purifications. High yields are ensured by mass action as multiple equivalents of building blocks are added during glycosylation reactions. Automated glycan assembly (AGA)[15] on a solid support has progressed in recent years to provide access to increasingly complex structures.[16,17] Still, effective building block design, linker stability, and the development of improved glycosylation and deprotection conditions is ongoing.

Received: March 14, 2016
Published: June 6, 2016

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

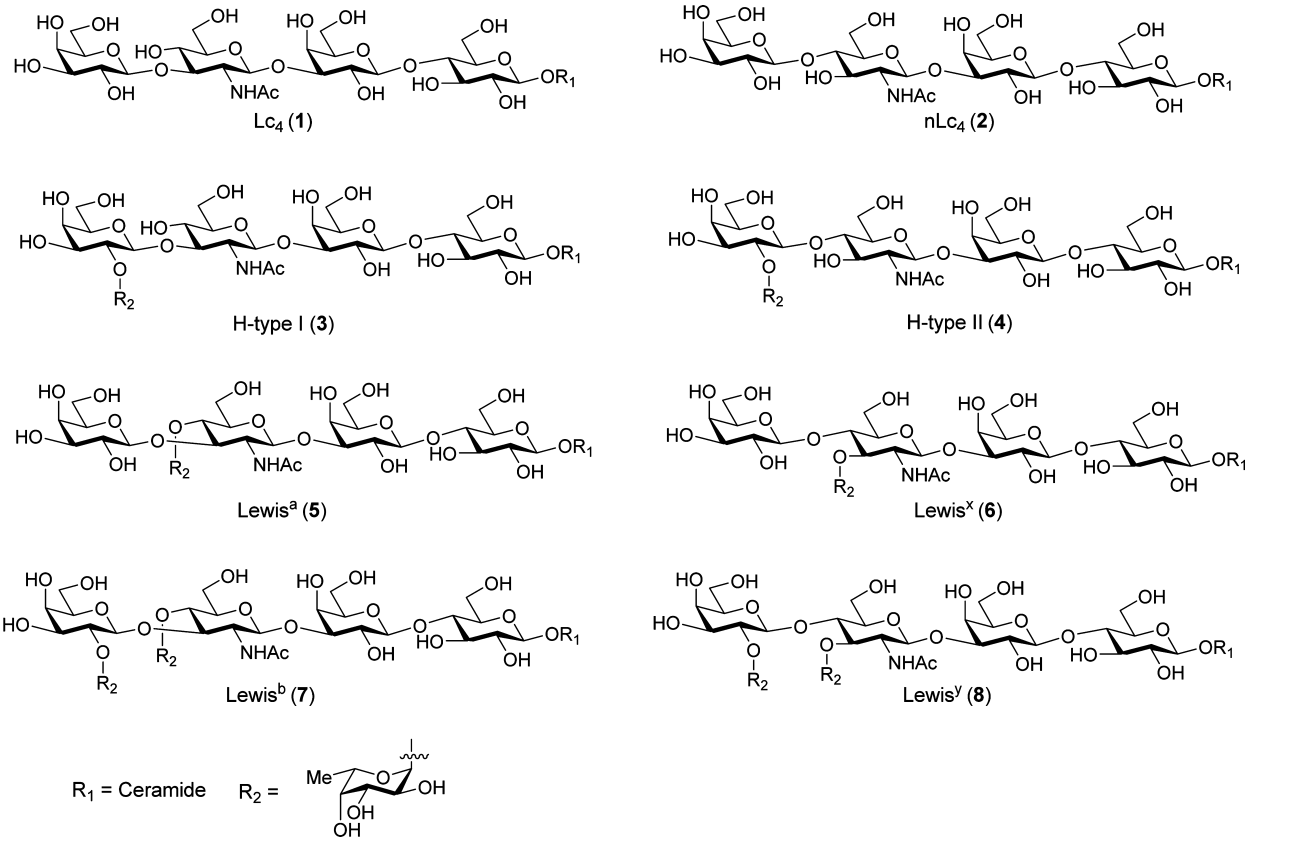


Figure 1. Oligosaccharides based on Lc4 and nLc4 core tetrasaccharides.

A set of structurally diverse, biologically relevant glycans that contain the Lc4 and nLc4 cores serve as a challenge to further develop AGA methods. Previously, Lewis X, Lewis Y, and Le^y-Le^x antigens were successfully synthesized by AGA,[16c] but H-type I and II pentasaccharides proved very challenging[18a] and automated syntheses were low yielding due to a lack of reliable methods to install α(1,2)-cis linkages.[18b] The synthesis of HNK-1 requires sulfation at the C-3 hydroxyl group of glucuronic acid and draws from our insights on the synthesis of chondroitin sulfate[17a] and dermatan sulfate[17b] fragments.

RESULTS AND DISCUSSION

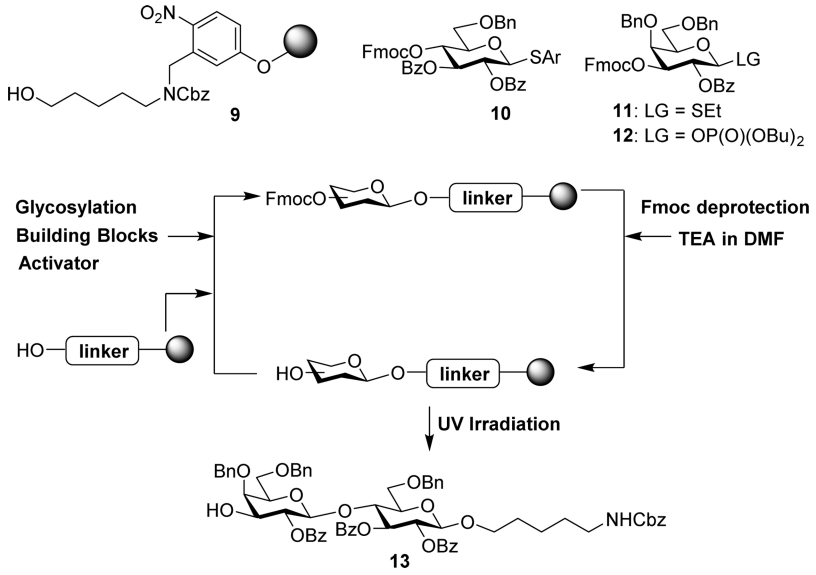
Assessment of Linker Stability. Merrifield resin equipped with photolabile linker **9** has been the basis for AGA of many complex glycans.[17] However, the stability of **9** toward commonly utilized strong acid activators, trimethylsilyl triflate (TMSOTf), or triflic acid (TfOH) at room temperature had not been explored. Activation at ambient temperature is required sometimes when deactivated building blocks are incorporated. Lactose disaccharide **13** is present in all target molecules and was selected as a model for methodological studies (Table 1). Thioglucoside **10**,[17b] thiogalactoside **11**,[17b] and galactosyl phosphate **12**[17c] building blocks were prepared. Two different anomeric leaving groups were chosen that require either catalytic (thioglycoside) or stoichiometric (glycosyl phosphate) amounts of acid for activation.

All reactions were performed using an automated synthesizer[17a] that executes reaction modules. The acidic wash module adds a TMSOTf solution at −20 °C to neutralize basic residues and remove residual water from the resin. The glycosylation module introduces five equivalents of building block and an activator solution (TMSOTf for glycosyl phosphates, TfOH and *N*-iodosuccinimide (NIS) for thioglycosides) at an activation temperature (T_a) to remain on the resin for the activation time (t_1). The temperature is then increased to the incubation temperature (T_i) for incubation time (t_2) (Table 1). Each glycosylation cycle is repeated twice. During the Fmoc deprotection module, the 9-fluorenemethylcarbonate (Fmoc) protecting group is removed using a solution of triethylamine (TEA). Following AGA, protected disaccharide **13** was cleaved from the resin by UV irradiation using a continuous flow photoreactor.[17a] Product **13** was quantitated by normal phase high-performance liquid chromatography (NP-HPLC) with an internal standard.

The result of the automated assembly of a simple disaccharide such as **13** shows a clear dependence of the overall yield on the acid concentration and the reaction temperature. The linker stability to four sets of conditions was assessed (Table 1). The conditions did not change for glucose building block **10** or T_a for galactose building blocks **11** or **12**. The overall yield of **13** did not change when thioglycoside **11** was used at T_i = −20 or 20 °C (42 vs 40% overall yield). Apparently, linker **9** is stable to catalytic quantities of TfOH at room temperature. Exposure of **19** to stoichiometric TMSOTf at room temperature may lead to some cleavage as evidenced by a slight drop in yield (36%) when glycosyl phosphate **12** was coupled at 20 °C. For testing whether the exposure to strong acid at room temperature really results in linker cleavage, the disaccharide was prepared by coupling building block **12** at T_i = −20 °C, but during a subsequent cycle, five equivalents of TMSOTf were added at room temperature without building block. The drastically decreased yield (14%) confirmed the incompatibility of linker **9** with stoichiometric amounts of strong acid at room temperature.

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

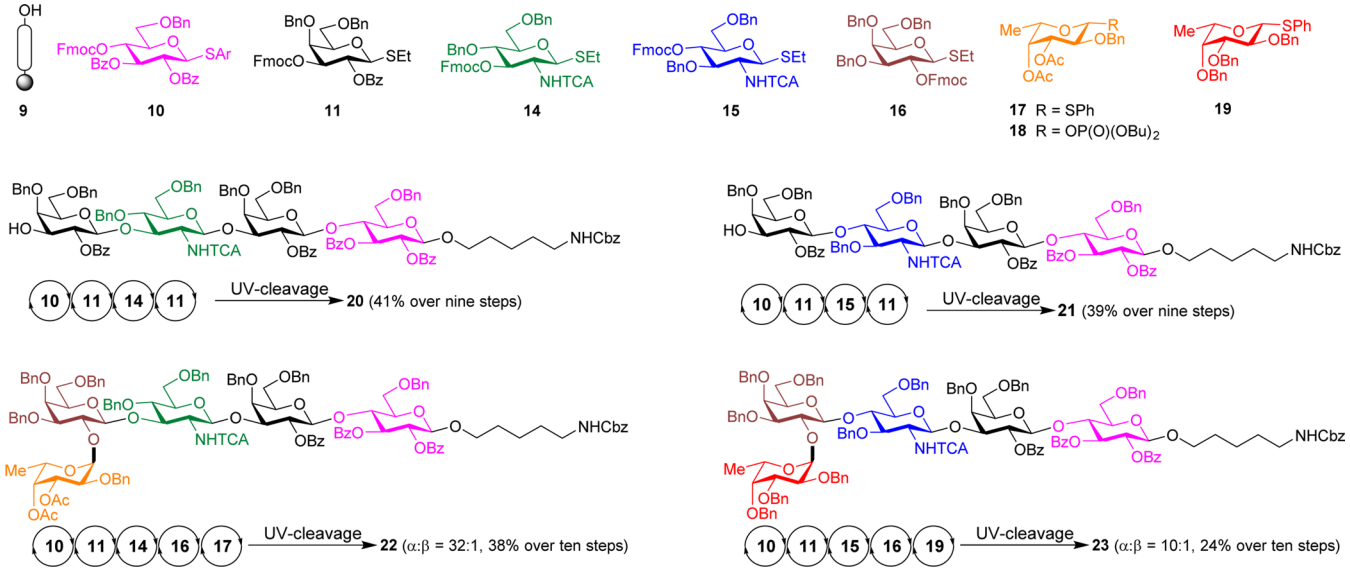
Table 1. Automated Glycan Assembly of Lactose 5 to Assess Linker Stability[a]



entry	building block	T_a (°C)	T_i (°C)	acidic wash[e]	yield (%)[f]
1	**11**[b]	−40	−20	no	42
2	**11**[c]	−40	−20 → 20	no	40
3	**12**[d]	−40	20 → 20	no	36
4	**11**[b]	−40	−20	yes	14

[a]Reaction conditions: Resin **9** (63.8 mg, 0.025 mmol, loading of resin: 0.392 mmol/g) was utilized. Building blocks **2**–**4** (0.25 mmol, 10 equiv based on the resin) were dissolved in DCM (2 mL). Glycosylation: 2 × 5 equiv of **2**, NIS, TfOH, DCM/dioxane, −30 °C (5 min) → −10 °C (25 min). [b]Reaction conditions: 2 × 5 equiv of **3**, NIS, TfOH, DCM/dioxane, −40 °C (5 min) → −20 °C (25 min). [c]Reaction conditions: 2 × 5 equiv of **3**, NIS, TfOH, DCM/dioxane, −40 °C (5 min) → −20 °C (25 min) → 20 °C (25 min). [d]Reaction conditions: 2 × 5 equiv of **4**, TMSOTf, DCM, −40 °C (5 min) → −20 °C (25 min) → 20 °C (25 min). [e]Reaction conditions: 2 × 5 equiv of TMSOTf, DCM, 20 °C (25 min). [f]Yields by preparative normal-phase HPLC.

Scheme 1. Automated Glycan Assembly of the Protected Lc4 13, H-type I 15, nLc4 14, and H-type II 16 Oligosaccharides[a]

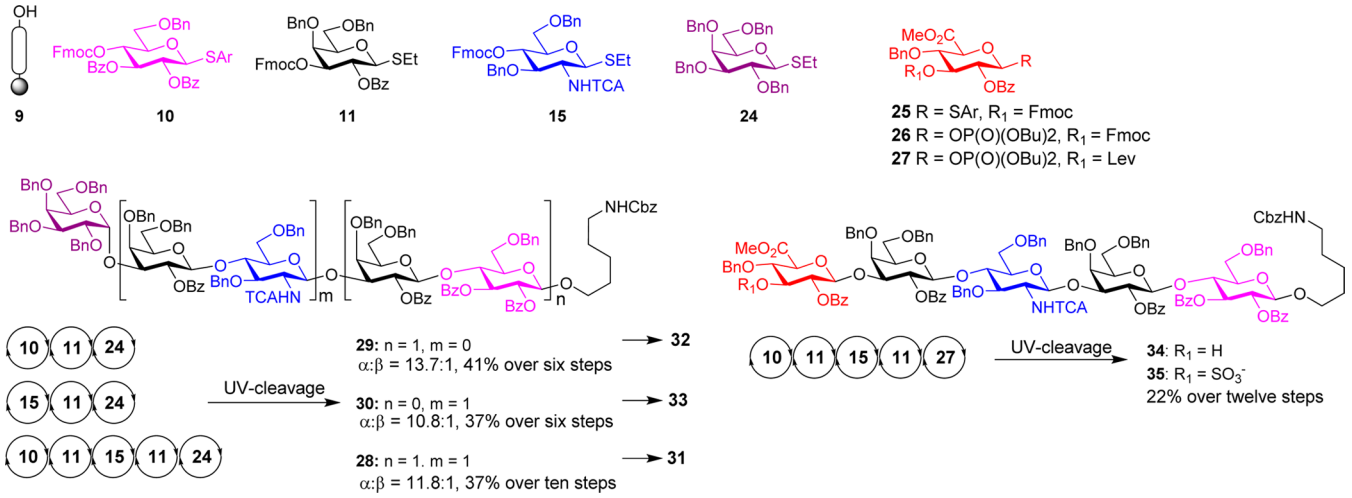


[a]Reaction conditions: Building blocks (0.025 mmol) were dissolved in DCM (2 mL). Glycosylation: 2 × 5 equiv of **10**, **14**, or **15**, NIS, TfOH, DCM/dioxane, −30 °C (5 min) → −10 °C (25 min); 2 × 5 equiv of **11**, **16**, or **19**, NIS, TfOH, DCM/dioxane, −40 °C (5 min) → −20 °C (25 min); 2 × 5 equiv of **17**, TMSOTf, DCM, −40 °C (5 min) → −20 °C (25 min). Deprotection: three cycles of 20% NEt_3 in DMF, 25 °C for **10**, **11**, **14**, **15**, **17**–**19**, or 30 °C for **16**. UV cleavage: hv (305 nm). Yields are based on resin loading and obtained by preparative normal-phase HPLC (ELSD detection).

Synthesis of H-type I and II. H-type I,[19a,b] H-type II,[19b,c] HNK-1,[20] and α-Gal[21] epitope oligosaccharides were prepared using similar strategies but using mono- and disaccharide building blocks that differed in anomeric leaving and protecting groups (Schemes 1 and 2). The lack of a commonly agreed-upon set of "approved" building blocks that can be prepared in large

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

Scheme 2. Automated Glycan Assembly of the Protected Three α-Gal Epitopes 28−30 and Nonsulfated and Sulfated HNK-1 31 and 32 Oligosaccharides[a]



[a]Reaction conditions: Glycosylation: 2 × 5 equiv of **10** or **15**, NIS, TfOH, DCM/dioxane, −30 °C (5 min) → −10 °C (25 min); 2 × 5 equiv of **11** or **24**, NIS, TfOH, DCM/dioxane, −40 °C (5 min) → −20 °C (25 min); 2 × 7.5 equiv of **27**, TMSOTf, DCM, −10 °C (5 min) → 0 °C (50 min). Deprotection: three cycles of 20% NEt_3 in DMF, 25 °C; three cycles of hydrazine hydrate (0.56 M in pyridine/acetic acid (v/v, 3:2)). Sulfation: three cycles of 0.5 M sulfur trioxide pyridine complex in DMF/TEA (v/v, 1:1) at 50 °C. UV cleavage: hv (305 nm). Yields are based on resin loading and obtained by preparative HPLC (ELSD detection).

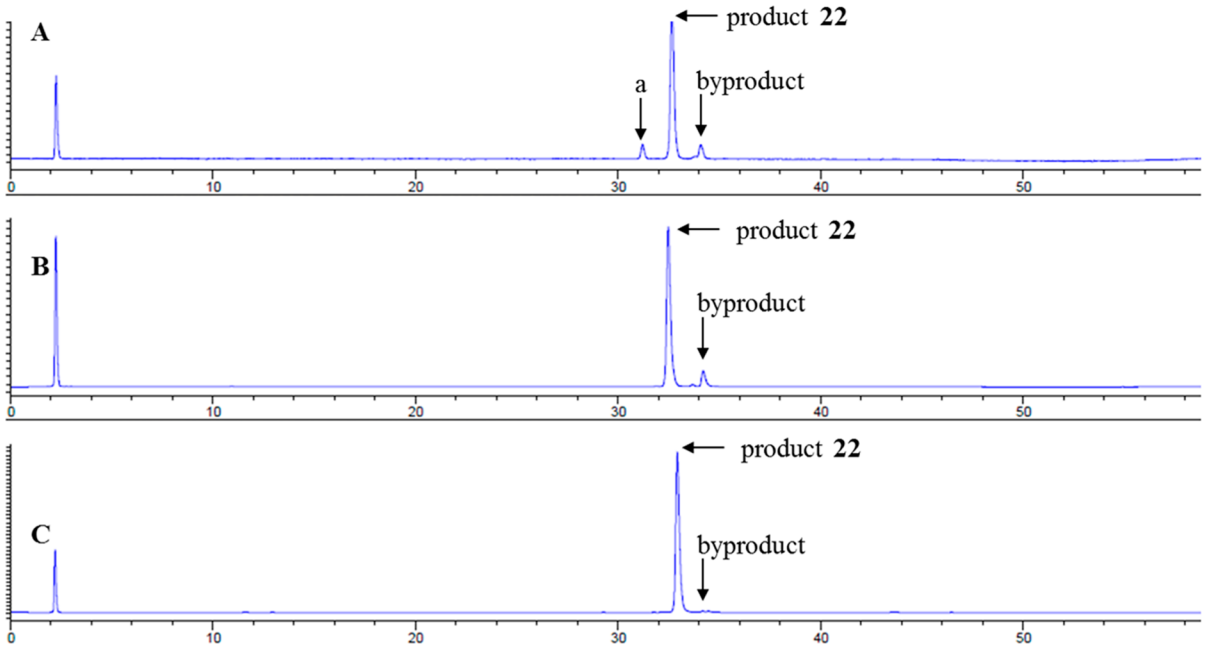


Figure 2. NP-HPLC of protected pentasaccharide **22**. HPLC was performed using Luna 5 μm Silica. A: The Fmoc deprotection module was performed at 25 °C. **17** was dissolved in DCM/Et_2O (1:3, v/v). B: The Fmoc deprotection module was performed at 30 °C. **17** was dissolved in DCM/Et_2O (1:3, v/v). C: The Fmoc deprotection module was performed at 30 °C. **17** was dissolved in DCM.

quantities, are stable for months, and can be activated at a specific temperature to reliably and selectively form the desired glycosidic linkage is a major impediment to carbohydrate chemistry. In the context of the oligosaccharide syntheses, several reliable building blocks were identified.

Fully protected oligosaccharides were prepared by AGA. Following photocleavage from the resin and normal phase-HPLC purification, the two core oligosaccharides Lc4 **20** (41% over nine steps based on resin loading) and nLc4 **21** (39% over nine steps) were obtained.

Fully protected H-type I pentasaccharide **22** (38% over ten steps) was assembled by sequential incorporation of building blocks **10**, **11**, **14**,[22] **16**, and **17**[23] (Scheme 1). NP-HPLC analysis of the initial attempt indicated an Fmoc-protected tetrasaccharide deletion sequence (a, Figure 2A) as well as desired product **22** (Figure 2) with the final fucose attached with good stereoselectivity (α:β = 11:1, Figure 2). Increasing the temperature of the Fmoc deprotection module from 25 to 30 °C eliminated the Fmoc-protected tetrasaccharide (a, Figure 2A). Changing the glycosylation solvent for building block **17** from a cosolvent

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

(DCM/Et_2O = 1:3) to DCM also significantly improved the stereoselectivity of the fucosylation (α:β = 32:1, Figure 2C).

H-type II pentasaccharide **23** was assembled using building blocks **10**, **11**, **15**[16g], **16**, and **19**[23] (Scheme 1). Prior automated synthetic efforts toward this target were unsuccessful.[18a] Our initial approach utilized fucose building block **17** in line with the synthesis of pentasaccharide **22**. However, this building block worked poorly due to the low reactivity as well as poor stereoselectivity (α:β = 1:1.8) (see Supporting Information). Glycosyl phosphate **18**, a variant of building block **17**, resolved the reactivity issues but resulted in even worse stereoselectivity (α:β = 1:2.8). Perbenzylated fucose building block **19** was used to overcome these problems providing H-type II pentasaccharide **23** in good yield (24% over ten steps) with good stereoselectivity (α:β = 10:1). Perbenzylated fucose building block **19** gave drastically improved stereoselectivity on AGA, although 3,4-di-*O*-acetyl fucoside as well as perbenzyl fucoside are known to provide excellent α-stereoselectivity in solution-phase chemistry.[24]

The automated syntheses of H-type I and II pentasaccharides **22** and **23** illustrated that the formation of α(1,2)-fucosidic linkages is dependent upon oligosaccharide connectivity (Galβ1-3GlcNTCA or Galβ1-4GlcNTCA) even with the same sequence (Gal-GluNTCA-Gal-Glu). The differences in stereoselectivity may be a result of mismatch between the donors and the acceptor on the solid support.[25] By varying the reaction conditions, some challenges are overcome, whereas in other cases, building blocks have to be changed. By employing "approved" building blocks regarding target oligosaccharides, protected H-type I (**22**) and II (**23**) pentasaccharides were synthesized in good yields with good to excellent stereoselectivities. The conjugation-ready unprotected glycans **1**[17b,19a] (48% over two steps), **2**[17d] (39%), and **3**[19a] (46%) were obtained by using reverse-phase HPLC following deprotection of oligosaccharides **20**–**22** by methanolysis and hydrogenolysis.

Synthesis of α-Gal Epitopes. α-Gal pentasaccharide **28** and two α-Gal trisaccharide epitopes (**29**, **30**) were synthesized to investigate the structural influence on 1,2-cis glycosylation (Scheme 2). Some acceptor sequence dependence on stereoselectivity was observed, though the results were not as pronounced as during the synthesis of the H-type oligosaccharides. The three target epitopes were prepared by AGA employing building block **24**[16g] at lower temperature (T_a = −40 °C, t_1 = 5 min, T_i = −20 °C, t_2 = 25 min). The oligosaccharide targets were prepared in high yield with good stereoselectivity: pentasaccharide **28** (37% over ten steps on resin, α:β = 11.8:1), trisaccharide **29** (41% over six steps on resin, α:β = 13.7:1), and **30** (38% over six steps on resin, α:β = 10.8:1). Coupling using **24** at higher temperature (T_a = −10 °C, t_1 = 5 min, T_i = −10 °C, t_2 = 25 min) led to incomplete glycosylation and the formation of deletion sequence nLc4 **21** in addition to desired product α-Gal epitope **28**. Under these conditions, the decomposition of activated building block **24** was faster than glycosylation of the resin-bound acceptor. A change of solvents from DCM to Et_2O/DCM (3:1) mixture during glycosylations with **24**[16d] had no effect on the stereochemical outcome of the coupling. The automated syntheses of the α-Gal epitopes demonstrated that the installation of a *cis*-galactosidic linkage is sequence dependent. Fully protected α-Gal epitopes **28**–**30** were deprotected to furnish the conjugation-ready glycans **31** (51% over two steps), **32** (44%), and **33** (37%).

Synthesis of HNK-1. Addition of 3-*O*-SO_3-glucuronic acid on the nonreducing end of nLc4 tetrasaccharide backbone **21** gives rise to HNK-1 **35** (Scheme 2). An initial synthetic attempt employed the deactivated glucuronic acid thioglycoside building block **25**[26] (T_a = −20 °C, t_1 = 5 min, T_i = −10 °C, t_2 = 50 min). Under these conditions, little nonsulfated pentasaccharide **34** and mostly nLc4 **21** (**21**:**34** = 90:10) were obtained. The use of glycosyl phosphate **26** using the same coupling cycle based on time and temperature resulted in little improvement (**21**:**34** = 84:16). Phosphate building block **27** bearing a C-3 levulinoyl ester instead of an Fmoc carbonate improved conversion to **34** only incrementally (**21**:**34** = 64:36). By raising the coupling temperature for building block **27** (T_a = −10 °C, t_1 = 5 min, T_i = 0 °C, t_2 = 50 min), the conversion of **21** to **34** (**21**:**34** = 39:61) improved. Finally, using these conditions with 15 equiv of building block added over three cycles transformed tetrasaccharide backbone **21** to **34** in good yield (26% over 11 steps). With the glycosylation conditions of **27** optimized, the automated glycan assembly of HNK-1 3-*O*-sulfated pentasaccharide **35** was performed using a modified, sulfation module with the more reactive SO_3Py in TEA/DMF rather than the standard pyridine/DMF solution. The crude product was purified by RP-HPLC to afford **35** (22% over 12 steps).

The HNK-1 synthesis showed that building block design and optimal glycosylation conditions are crucial for achieving high glycosylation efficiencies. Modification of protecting groups (Fmoc to Lev) and leaving groups (thioglycoside to glycosyl phosphate), and fine-tuning of glycosylation conditions all played a central role in the automated synthesis of the HNK-1 pentasaccharide (**35**).

CONCLUSIONS

We describe the automated glycan assembly of fully protected H-type I and II, α-Gal epitopes, and the HNK-1 pentasaccharide using sequential combinations of monosaccharide building blocks. During these syntheses, we discovered that the stereoselectivity of 1,2-*cis*-glycosidic bond formation depends on the sequence of the resin-bound nucleophile. Fucoside **17** allowed for automated synthesis of H-type I **22**, but fucoside **18** was required to assemble H-type II **23**. Glycosylation efficiencies were affected by leaving groups as well as protecting groups in the synthesis of HNK-1. The identification of "approved" building blocks that are used in defined coupling cycles under conditions adjusted to the building block enables access to challenging oligosaccharides.

EXPERIMENTAL SECTION

General Experimental Methods. All chemicals used were reagent grade and used without further purification as commercially available unless otherwise noted. All reactions were performed in oven-dried glassware under an argon atmosphere. *N*,*N*-Dimethylformamide (DMF), dichloromethane (DCM), toluene, and tetrahydrofuran (THF) were purified in a Cycle-Tainer Solvent Delivery System. Analytical thin layer chromatography (TLC) was performed on Merch silica gel 60 F254 plates (0.25 mm) using UV light (254 and 365 nm). Flash chromatography was conducted using forced flow of the indicated solvent on Fluka silica gel 60 (230–400 mesh). All automated glycosylations were performed on an automated oligosaccharide synthesizer demonstrator unit using anhydrous solvents of the Cycle-Tainer Solvent Delivery System. LCMS chromatograms were recorded on an Agilent 1100 Series spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. ^1H and ^{13}C spectra were recorded on a Varian Mercury 400 (400 MHz), 600 (600 MHz), or Bruker AVIII 700 (700 MHz) spectrometer in $CDCl_3$ or CD_3OD with chemical shifts (δ)

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866–5877

referenced to internal standards ($CDCl_3$: 7.26 ppm, ^1H; 77.16 ppm, ^{13}C; CD_3OD: 4.87 or 3.31 ppm, ^1H; 49.0 ppm, ^{13}C) unless stated otherwise. High resolution mass spectrometry (HRMS) analyses were performed by the MS service in the Department of Organic Chemistry at Free University Berlin using an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA).

Synthesis of Building Blocks. *(2-Methyl-5-tert-butylphenyl) 2,3-Di-O-benzoyl-6-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside* ***10***.[17c] To a solution of (2-methyl-5-*tert*-butylphenyl) 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (4.45 g, 6.94 mmol, 1.0 equiv) were added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol, 2.0 equiv) and pyridine (1.68 mL, 20.82 mmol, 3.0 equiv) successively at 0 °C. The reaction was stirred overnight at room temperature. Then, the mixture was quenched with 1 M aqueous HCl and diluted with DCM. The organic layer was dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **10** (5.74 g, 6.65 mmol, 96%). R_f = 0.18 (hexane/ethyl acetate/DCM: 9:1:0.5). $[\alpha]_D^{25}$ +3.1 (*c* 3.05, $CHCl_3$). IR (thin film): υ 2960, 1755, 1732, 1278, 1249 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.0 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.71 (dd, *J* = 7.6, 3.3 Hz, 2H), 7.61 (d, *J* = 1.7 Hz, 1H), 7.56−7.50 (m, 1H), 7.46−7.20 (m, 16H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 5.80 (t, *J* = 9.5 Hz, 1H, H-3), 5.54 (t, *J* = 9.8 Hz, 1H, H-2), 5.25 (t, *J* = 9.8 Hz, 1H, H-4), 4.94 (d, *J* = 10.1 Hz, 1H, H-1), 4.58 (q, *J* = 12.2 Hz, 2H, CH_2Ph), 4.22 (dd, *J* = 10.4, 7.3 Hz, 1H, C*H*HPh of Fmoc), 4.08 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6, C*H*HPh of Fmoc), 4.00−3.85 (m, 2H, H-5, C*H* of Fmoc), 3.74 (d, *J* = 3.9 Hz, 2H, H-6), 2.25 (s, 3H, Me), 1.26 (d, *J* = 0.8 Hz, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8 (OBz), 165.2 (OBz), 154.2, 149.9, 143.3, 143.0, 141.3, 141.2, 137.8, 137.3, 133.4, 132.1, 130.3, 130.1, 130.00, 129.98, 129.4, 128.9, 128.49, 128.46, 128.4, 127.9, 127.8, 127.2, 125.5, 125.3, 125.1, 120.0 (Ar), 87.6 (C-1), 77.3 (C-5), 74.6 (C-3), 73.8 (CH_2Ph), 73.4 (C-4), 70.8 (C-2), 70.4 (CH_2 of Fmoc), 69.0 (C-6), 46.6 (CH of Fmoc), 34.5 (Cq, *t*-Bu), 31.4 (Me, *t*-Bu), 20.5 (Me). HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{53}H_{50}O_9SNa$ 885.3068, found 885.3094.

Ethyl 2-O-Benzoyl-4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside ***11***.[17c] To a solution of ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (3.8 g, 6.1 mmol) in anhydrous acetonitrile (76.0 mL, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$, 0.92 mL, 7.3 mmol) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C. Then, the mixture was diluted with DCM and quenched with saturated aqueous $NaHCO_3$ (30.0 mL). The organic layer was dried over $MgSO_4$, and the solvent was evaporated in vacuo. To a solution of this crude product in anhydrous DCM was added 9-fluorenylmethylchloroformate (3.9 g, 15.1 mmol) and pyridine (1.5 mL, 18.1 mmol) at 0 °C. The reaction was stirred overnight at room temperature. Then, the mixture was diluted with DCM and then quenched with 1 M aqueous HCl. The organic layer was dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 8:1:1 to 8:2:1) to afford **11** (4.21 g, 5.76 mmol, 95%). R_f (hexane/ethyl acetate/DCM = 8:2:1) = 0.49; $[\alpha]_D^{20}$ +34.08 (*c* 2.69, $CHCl_3$). IR (thin film): υ 2870, 1730, 1602, 1496, 1451 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (dt, *J* = 8.5, 1.6 Hz, 2H), 7.74−7.66 (m, 2H), 7.62−7.27 (m, 9H), 7.13 (dtd, *J* = 8.6, 7.5, 1.1 Hz, 17H), 5.76 (t, *J* = 9.9 Hz, 1H, H-2), 5.09 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.80 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.58−4.42 (m, 3H, Bn, CH*H*Ph), 4.31 (dd, *J* = 10.4, 7.1 Hz, 1H, H-6a), 4.23 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6b), 4.18−4.14 (m, 1H, H-4), 4.07 (t, *J* = 7.4 Hz, 1H, H-5), 3.84 (t, *J* = 6.9 Hz, 1H, CH Fmoc), 3.68 (d, *J* = 6.7 Hz, 2H, CH_2 Fmoc), 2.76 (qq, *J* = 12.4, 7.5 Hz, 2H, CH_2), 1.25 (t, *J* = 7.5 Hz, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (Bz), 154.6 (Fmoc), 143.4, 143.0, 141.3, 141.2, 138.0, 137.8, 133.3, 130.1, 129.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.98, 127.9, 127.8, 127.22, 127.19, 125.3, 125.1, 120.07, 120.06 (Ar), 83.8 (C-1), 79.1 (C-3), 77.4 (CH, Fmoc), 75.2 (Bn), 74.1 (C-4), 73.7 (Bn), 70.2 (C-6), 68.7 (C-2), 68.2 (CH_2, Fmoc), 46.6 (C-5), 24.0 (CH_2), 14.9(CH_3). HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{44}H_{42}O_8SNa$ 753.2493, found 753.2488.

Dibutyl 2-O-Benzoyl-4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-β-D-glucopyranosyl Phosphate ***12***. Thioglycoside **11** (3.7 g, 5.1 mmol) was coevaporated twice with toluene. The remainder and NIS (1.6 g, 5.6 mmol) were dissolved in DCM (25.0 mL) under an Ar atmosphere, and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (2.5 mL, 12.7 mmol) and triflic acid (22 μL, 0.253 mmol) were added into reaction flask, the reaction was stirred at 0 °C for 1 h. After complete conversion of the starting material, it was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and then saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 3:1 to 1:1) to afford title compound **12** (4.1 g, 92%). R_f = 0.61 (hexane/ethyl acetate, 1:1). $[\alpha]_D^{25}$ +29.4 (*c* 3.00, $CHCl_3$). IR (thin film): υ 2960, 1735, 1451, 1270, 1096, 1027 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.10−8.04 (m, 2H), 7.67 (t, *J* = 7.1 Hz, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.50−7.39 (m, 5H), 7.39−7.27 (m, 11H), 7.18−7.05 (m, 2H), 5.80 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2), 5.43 (t, *J* = 7.7 Hz, 1H, H-1), 5.03 (dd, *J* = 10.5, 3.0 Hz, 1H, H-3), 4.76 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.56−4.42 (m, 3H, CH_2Ph, C*H*HPh), 4.30 (ddd, *J* = 27.4, 10.4, 7.4 Hz, 2H, CH_2, Fmoc), 4.15 (d, *J* = 2.7 Hz, 1H, H-4), 4.11−3.90 (m, 4H, H-5, CH of Fmoc, OCH_2), 3.72 (qdd, *J* = 14.5, 9.4, 6.2 Hz, 4H, H-6, OCH_2), 1.63−1.53 (m, 2H, $-CH_2-$), 1.39−1.25 (m, 4H, 2 x $-CH_2-$), 1.03 (dq, *J* = 14.6, 7.4 Hz, 2H, $-CH_2-$), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3), 0.69 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1 (Bz), 154.5 (Fmoc), 143.3, 142.9, 141.3, 141.2, 137.8, 137.7, 133.5, 130.1, 129.4, 128.6, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.20, 127.17, 125.2, 125.0, 120.1 (Ar), 97.0 (d, *J* = 4.7 Hz, C-1), 77.5 (d, *J* = 1.7 Hz, C-3), 75.4 (CH_2Ph), 74.0 (C-5), 73.6 (CH_2Ph), 73.5 (C-4), 70.2 (CH_2, Fmoc), 70.1 (d, *J* = 9.1 Hz, C-2), 68.1 (d, *J* = 6.4 Hz, OCH_2), 67.9 (d, *J* = 6.4 Hz, OCH_2), 67.5 (C-6), 46.6 (CH, Fmoc), 32.1 (d, *J* = 7.4 Hz, $-CH_2-$), 31.9 (d, *J* = 7.3 Hz, $-CH_2-$), 18.7 ($-CH_2-$), 18.3 ($-CH_2-$), 13.7, 13.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ −2.84. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{50}H_{55}O_{12}NaP$ 901.3323, found 901.3336.

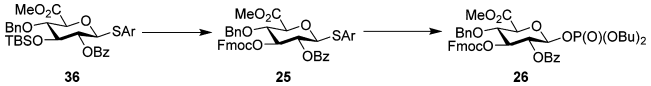
Ethyl 4,6-Di-O-benzyl-3-O-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside ***14***.[22] To a solution of ethyl 4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside (3.3 g, 5.0 mmol) in anhydrous acetonitrile (62 mL, 0.08 M) was added BF_3OEt_2 (0.82 mL, 5.5 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and quenched with saturated aqueous $NaHCO_3$. The precipitated solid was filtered and dried in vacuo. The crude product was used for the next reaction. To a solution of the crude product and FmocCl (2.6 g, 9.9 mmol) in DCM (28 mL, 0.18 M) was added anhydrous pyridine (1.2 mL, 4.6 mmol) at 0 °C. The reaction mixture was stirred at rt for 15 h. The reaction mixture was diluted with DCM, and 1 M aqueous HCl was added for quenching the reaction. The combined organic layer was dried over Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography (hexane/EtOAc/DCM = 7:2:1 to 7:3:1) to give **14** (3.50 g, 92%) over two steps. R_f = 0.29 (hexane/ethyl acetate/DCM = 8:1:2). $[\alpha]_D$ −27.6 (*c* 2.67, $CHCl_3$). IR (thin film): υ 3361, 3032, 2921, 2868, 1720, 1526, 1451, 1390, 1265 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.6, 3.0 Hz, 2H), 7.55 (ddd, *J* = 10.3, 7.5, 0.6 Hz, 2H), 7.45−7.17 (m, 12H), 7.14−7.07 (m, 2H), 6.99 (d, *J* = 9.4 Hz, 1H, N*H*), 5.14 (dd, *J* = 10.4, 9.3 Hz, 1H, H-3), 4.70−4.50 (m, 4H, CH_2 x 2), 4.48 (d, *J* = 10.2 Hz, 1H, H-1), 4.30 (m, 2H, CH_2 of Fmoc), 4.22−4.09 (m, 2H, C*H* of Fmoc, H-2), 3.85 (t, *J* = 9.5 Hz, 1H, H-3), 3.67 (dd, *J* = 11.1, 3.9 Hz, 1H, H-6a), 3.60 (dd, *J* = 11.1, 1.7 Hz, 1H, H-6b), 3.52 (ddd, *J* = 9.8, 3.8, 1.8 Hz, 1H, H-5), 2.67 (qd, *J* = 7.4, 2.5 Hz, 2H), 1.21 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 155.5, 143.0, 142.8, 141.2, 141.2, 137.9, 137.4, 128.4, 128.4, 128.0, 128.0, 127.9, 127.8, 127.7, 127.3, 127.2, 125.2, 125.1, 120.08, 120.06 (Ar), 92.3 (CCl_3), 83.8 (C-1), 80.2 (C-3), 79.1 (C-5), 75.7 (C-4), 75.1 (CH_2), 73.5 (CH_2), 70.8 (CH_2 of Fmoc), 68.3 (C-6), 54.9 (C-2), 46.4 (CH of Fmoc), 24.1, 14.9. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{39}H_{38}NO_7Cl_3SNa^+$ 792.1327, found 792.1325.

Ethyl 3,4,6-Tri-O-benzyl-2-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside ***16***. To a solution of compound ethyl 3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[26] in anhydrous DCM (15 mL, 0.3 M) were added 9-fluorenylmethylchloroformate (2.3 g, 8.8 mmol) and

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

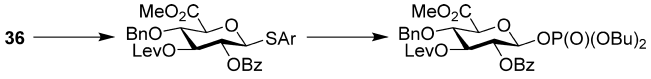
pyridine (1.1 mL, 13.2 mmol) at 0 °C. The reaction was stirred overnight at rt. After the reaction completed, the mixture was diluted with DCM and quenched with 1 M aqueous HCl. The combined organic layer was dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 8:1:1 to 8:2:1) to afford **16** (2.7 g, 3.77 mmol, 86%). R_f = 0.17 (hexane/ethyl acetate/DCM = 9:1:0.5). $[\alpha]_D^{20}$ −12.3 (*c* 1.30, $CHCl_3$). IR (thin film): υ 2869, 1752, 1452, 1251 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 8.03 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.7 Hz, 3H), 7.49−7.39 (m, 4H), 7.38−7.26 (m, 7H), 7.23−7.14 (m, 5H), 5.71 (t, *J* = 9.7 Hz, 1H, H-2), 5.04 (d, *J* = 11.7 Hz, 1H, CHHPh), 4.68 (d, *J* = 12.2 Hz, 1H, CHHPh), 4.67 (d, *J* = 11.7 Hz, 1H, CHHPh), 4.56 (d, *J* = 12.2 Hz, 1H, CHHPh), 4.52 (d, *J* = 9.9 Hz, 1H, 4.44−4.34 (m, 3H, H-6, CH2 of Fmoc), 4.25 (t, *J* = 7.4 Hz, 1H, CH of Fmoc), 4.18 (dd, *J* = 11.1, 5.8 Hz, 1H, H-6), 3.97 (d, *J* = 1.9 Hz, 1H, H-4), 3.72 (dd, *J* = 13.2, 4.1 Hz, 2H, H-3, H-5), 2.82−2.64 (m, 2H), 1.22 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 154.66 (Fmoc), 143.7, 143.5, 141.42, 141.40, 138.7, 138.0, 137.9, 128.6, 128.5, 128.3, 128.1, 128.04, 127.96, 127.9, 127.9, 127.8, 127.63, 127.57, 127.29, 127.26, 125.4, 125.3, 120.1 (Ar), 83.7 (C-1), 81.6 (C-3), 77.7 (C-5), 74.6 (CH2Ph), 74.4 (C-2), 73.7 (CH2Ph), 73.4 (C-4), 72.5 (CH2Ph), 70.2 (CH2, Fmoc), 68.6 (C-6), 46.9 (CH, Fmoc), 24.0, 15.0. HRMS (ESI-TOF): *m*/*z* [M + Na]+ calcd for $C_{44}H_{44}O_7SNa$ 739.2700, found 739.2699.

Di-O-butyl 3,4-Di-O-acetyl-2-O-benzyl-α/β-L-fucopyranosylphosphate ***18***. Thioglycoside **17** (2.24 g, 2.84 mmol) was coevaporated twice with toluene. The remainder and NIS (0.71 g, 3.13 mmol) were dissolved in DCM (14 mL) under an Ar atmosphere, and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (1.41 mL, 7.11 mmol) and triflic acid (13 μL, 0.142 mmol) were added, and the reaction was stirred at 0 °C. After complete conversion of the starting material, it was diluted with DCM and extracted with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate, 3:1 to 1:1) to afford the title compound **18** (2.20 g, 95%). Rf = 0.61 (hexane/ethyl acetate = 1:1). IR (thin film): υ 2963, 1750, 1372, 1241, 1223 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.42−7.14 (m, 5H, Bn), 5.92 (br s, 1H, H-1), 5.30 (br s, 2H, H-3, H-4), 4.75 (d, *J* = 11.3 Hz, 1H, CH*H* of Bn), 4.60 (d, *J* = 10.2 Hz, 1H, CH*H* of Bn), 4.32 (d, *J* = 5.1 Hz, 1H, H-5), 4.13−3.93 (m, 4H, 2 CH_2O of Bu), 3.88 (d, *J* = 8.4 Hz, 1H, H-2), 2.13 (s, 3H, CH_3 of Ac), 2.00 (s, 3H, CH_3 of Ac), 1.71−1.59 (m, 2H, CH_2 of Bu), 1.59−1.47 (m, 2H, CH_2 of Bu), 1.47−1.33 (m, 2H, CH_2 of Bu), 1.33−1.19 (m, 2H, CH_2 of Bu), 1.13 (d, *J* = 5.0 Hz, 3H, CH_3-6), 0.92 (t, *J* = 6.4 Hz, 3H, CH_3 of Bu), 0.84 (t, *J* = 6.4 Hz, 3H, CH_3 of Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (Ac), 170.2 (Ac), 137.7, 128.4, 127.9, 127.8 (Bn), 95.5 (C-1), 72.9 (CH_2 of Bn), 72.9 (C-2), 71.1 (C-4), 69.7 (C-3), 68.0 (CH_2O of Bu), 67.6 (CH_2O of Bu), 66.6 (C-5), 32.3 (CH_2 of Bu), 32.2 (CH_2 of Bu), 20.9 (CH_3 of Ac), 20.8 (CH_3 of Ac), 18.8 (CH_2 of Bu), 18.6 (CH_2 of Bu), 16.0 (C-6), 13.71 (CH_3 of Bu), 13.66 (CH_3 of Bu). ^{31}P NMR (162 MHz, $CDCl_3$) δ −2.15. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{39}O_{10}PNa$ 553.2173, found 553.2177.



Methyl (2-Methyl-5-tert-butyl-phenyl)-2-O-benzoyl-4-O-benzyl-3-O-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranosyluronate ***25***. To a solution of **36**[27] (1.88 g, 2.77 mmol) in anhydrous acetonitrile (35 mL, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (0.42 mL, 3.32 mmol) at 0 °C, and the solution was stirred for 20 min. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$. The combined organic layer was evaporated in vacuo. To a solution of this crude product in anhydrous DCM (15 mL) was added 9-fluorenylmethyl chloroformate (1.91 g, 7.35 mmol) and pyridine (0.90 mL, 11.1 mmol) successively at 0 °C. The reaction was stirred overnight at room temperature. Then, the mixture was quenched with 1 M aqueous HCl, diluted with DCM, and dried over $MgSO_4$. The organic layer was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 8.5:1.5:0.5) to afford **25** (2.08 g, 2.64 mmol, 93%) over two steps. R_f = 0.33 (hexane/ethyl acetate/DCM: = 8/1/1). $[\alpha]_D^{25}$ +41.2 (*c* 2.96, $CHCl_3$). IR (thin film): υ 2954, 1751, 1451, 1267 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08−7.99 (m, 2H), 7.73 (dd, *J* = 7.6, 3.6 Hz, 2H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.56−7.33 (m, 7H), 7.24 (dddd, *J* = 11.5, 8.6, 5.5, 1.4 Hz, 8H), 7.12 (d, *J* = 8.0 Hz, 1H), 5.42 (t, *J* = 9.6 Hz, 1H, H-2), 5.34 (t, *J* = 9.2 Hz, 1H, H-3), 4.90 (d, *J* = 9.9 Hz, 1H, H-1), 4.70 (s, 2H, CH_2Ph), 4.28 (dd, *J* = 10.4, 7.1 Hz, 1H, CH*H* of Fmoc), 4.21−4.06 (m, 3H, H-4, H-5, CH*H* of Fmoc), 4.00 (t, *J* = 7.5 Hz, 1H, C*H* of Fmoc), 3.80 (s, 3H, Me), 2.23 (s, 3H, Me), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6 (CO_2Me), 165.1 (Bz), 154.5 (Fmoc), 149.7, 143.3, 142.9, 141.1, 141.0, 137.2, 137.0, 133.4, 131.7, 130.1, 130.0, 129.9, 129.1, 128.4, 128.3, 127.9, 127.85, 127.78, 127.1, 125.5, 125.2, 124.9, 119.9 (Ar), 87.7 (C-1), 80.0 (C-3), 77.9 (C-5), 76.9 (C-4), 75.0 (CH_2Ph), 70.4 (CH_2 of Fmoc), 70.4 (C-2), 52.7 (Me), 46.4 (CH of Fmoc), 34.4 (Cq, *t*-Bu), 31.2 (*t*-Bu), 20.2 (Me). HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{47}H_{46}O_9SNa$ 809.2780, found 809.2805.

Methyl-2-O-benzoyl-4-O-benzyl-3-O-fluorenylmethoxycarbonyl-1-di-O-butylphosphatidyl-β-D-glucopyranosyluronate ***26***. Compound **25** (2.238 g, 2.84 mmol, 1.0 equiv) was coevaporated twice with toluene. The remainder and NIS (0.71 g, 3.13 mmol) were dissolved in DCM (14 mL) under an Ar atmosphere, and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (1.41 mL, 7.11, 2.5 equiv) and triflic acid (13 μL, 0.142 mmol) were added to the mixture. The mixture was stirred for 2 h at 0 °C, diluted with DCM, quenched with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. The organic layer was evaporated in vacuo. The crude product was purified by silica gel flash column chromatography (hexane/ethyl acetate = 3:1 to 1:1) to afford compound **26** (2.20 g, 95%). R_f = 0.61 (hexane/ethyl acetate = 1:1). $[\alpha]_D^{25}$ +18.8 (*c* 3.11, $CHCl_3$). IR (thin film): υ 2960, 1751, 1452, 1270, 1096, 1029 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.3, 1.1 Hz, 2H), 7.70 (dd, *J* = 7.5, 4.5 Hz, 2H), 7.53−7.29 (m, 7H), 7.26−7.14 (m, 7H), 5.50 (t, *J* = 7.5 Hz, 1H, H-1), 5.47−5.40 (m, 1H, H-2), 5.28 (t, *J* = 9.2 Hz, 1H, H-3), 4.70−4.57 (m, 2H, CH_2Ph), 4.29 (dd, *J* = 10.4, 7.0 Hz, 1H, CH*H* of Fmoc), 4.18 (t, *J* = 9.0 Hz, 1H, H-5), 4.15−3.96 (m, 5H, H-4, CH*H* of Fmoc, C*H* of Fmoc, CH_2O of Bu), 3.75 (s, 3H, Me), 3.84−3.66 (m, 2H, CH_2O of Bu), 1.68−1.58 (m, 2H,, CH_2 of Bu), 1.42−1.22 (m, 4H, 2 x CH_2 of Bu), 1.04 (dq, *J* = 14.6, 7.4 Hz, 2H, CH_2 of Bu), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3 of Bu), 0.69 (t, *J* = 7.4 Hz, 3H, CH_3 of Bu).8.08−7.99 (m, 2H), 7.73 (dd, *J* = 7.6, 3.6 Hz, 2H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.56−7.33 (m, 7H), 7.24 (dddd, *J* = 11.5, 8.6, 5.5, 1.4 Hz, 8H), 7.12 (d, *J* = 8.0 Hz, 1H), 5.42 (t, *J* = 9.6 Hz, 1H, H-2), 5.34 (t, *J* = 9.2 Hz, 1H, H-3), 4.90 (d, *J* = 9.9 Hz, 1H, H-1), 4.70 (s, 2H, CH_2Ph), 4.28 (dd, *J* = 10.4, 7.1 Hz, 1H, CH*H* of Fmoc), 4.21−4.06 (m, 3H, H-4, H-5, CH*H* of Fmoc), 4.00 (t, *J* = 7.5 Hz, 1H, C*H* of Fmoc), 3.80 (s, 3H, Me), 2.23 (s, 3H, Me), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7 (CO_2Me), 165.0 (Bz), 154.4 (Fmoc), 143.4, 143.0, 141.3, 141.2, 137.1, 133.7, 130.1, 128.8, 128.6, 128.5, 128.1, 128.0, 127.9, 127.2, 125.3, 125.0, 120.0, 96.4 (d, *J* = 4.8 Hz, C-1), 78.3 (d, *J* = 1.6 Hz, C-3), 77.0 (C-4), 75.1 (CH_2Ph), 74.6 (C-5), 71.7 (d, *J* = 9.2 Hz, C-2), 70.5 (CH_2 of Fmoc), 68.3 (d, *J* = 6.3 Hz, CH_2O, Bu), 68.1 (d, *J* = 6.3 Hz, CH_2O, Bu), 52.8 (Me), 46.6 (CH of Fmoc), 32.1 (d, *J* = 7.4 Hz, CH_2, Bu), 31.9 (d, *J* = 7.3 Hz, CH_2, Bu), 18.6 (CH_2, Bu), 18.3 (CH_2, Bu), 13.6 (CH_3, Bu), 13.5 (CH_3, Bu). ^{31}P NMR (162 MHz, $CDCl_3$) δ −3.05. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{44}H_{49}O_{13}PNa$ 839.2803, found 839.2820.



Methyl (2-Methyl-5-tert-butyl-phenyl)-2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-thio-β-D-glucopyranosyluronate ***37***.[27] To a solution of compound **36**[27] (1.88 g, 2.77 mmol) in anhydrous acetonitrile (35 mL, 0.08 M) was added boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (0.42 mL, 3.32 mmol) for 20 min at 0 °C. Then, the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$. The combined organic layer was evaporated in vacuo. To a solution of the crude product in anhydrous DCM (14 mL, 0.2 M) was added levulinic acid (1.29 g, 11.1 mmol), DIC (1.73 mL, 11.08 mmol), and a

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

catalytic amount of DMAP (0.068 g, 0.554 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$. The combined organic layer was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/EtOAc/DCM = 9:0.5:0.5 to 8:2:0.5) to afford **37** (1.76 g, 2.66 mmol, 96%) over two steps. R_f = 0.24 (hexane/EtOAc/DCM = 8:2:0.5). $[\alpha]_D^{25}$ +17.5 (*c* 2.60, $CHCl_3$). IR (thin film): υ 2958, 1748, 1264, 1093, 1071 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.6 Hz, 2H), 7.63−7.53 (m, 2H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.37−7.16 (m, 6H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.45 (t, *J* = 8.3 Hz, 1H, H-3), 5.26 (t, *J* = 9.7 Hz, 1H, H-2), 4.83 (d, *J* = 10.1 Hz, 1H, H-1), 4.72−4.55 (m, 2H, CH_2Ph), 4.16−3.99 (m, 2H, H-4, H-5), 3.76 (s, 3H, Me), 2.44−2.30 (m, 2H, Lev), 2.18 (s, 3H, Me), 2.01 (s, 3H, Me), 1.27 (s, 9H, *i*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.8 (CO, Lev), 171.9 (Lev), 168.0 (CO_2Me), 165.3 (Bz), 149.8, 137.6, 137.1, 133.5, 131.9, 130.12, 130.06, 129.4, 128.6, 128.5, 128.2, 128.1, 125.5 (Ar), 87.7 (C-1), 78.1 (C-5), 77.1 (C-4), 75.6 (C-3), 74.9 (CH_2Ph), 70.6 (C-2), 52.8 (Me), 37.8 (CH_2, Lev), 34.6 (Cq, *t*-Bu), 31.3 (*t*-Bu), 29.7 (Me, Lev), 28.1 (CH_2, Lev), 20.3 (Me). HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{37}H_{42}O_9SNa$ 685.2442, found 685.2448.

Methyl-2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-di-O-butylphosphatidyl-β-D-glucopyranosyluronate ***27***.[27] Compound **37** (2.238 g, 2.84 mmol) was coevaporated twice with toluene. The remainder and NIS (0.71 g, 3.13 mmol) were dissolved in DCM (14 mL) under an Ar atmosphere, and the solution was cooled 0 °C. Dibutyl hydrogen phosphate (1.41 mL, 7.11 mmol) and triflic acid (13 μL, 0.142 mmol) were added, and the reaction was stirred for 2 h at 0 °C. After the mixture was quenched with 10% aqueous $Na_2S_2O_3$ and with saturated aqueous $NaHCO_3$, it was diluted with DCM and dried over $MgSO_4$. The organic layer was removed in vacuo. The crude product was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 3:1 to 1:1) to afford title compound **27** (2.20 g, 95%). R_f = 0.61 (hexane/EtOAc = 1:1). $[\alpha]_D^{25}$ +18.8 (*c* 3.11, $CHCl_3$). IR (thin film): υ 2960, 1751, 1452, 1270, 1096, 1029 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.4 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.35−7.21 (m, 5H), 5.45 (dd, *J* = 16.0, 8.5 Hz, 2H, H-1, H-3), 5.35−5.27 (m, 1H, H-2), 4.62 (q, *J* = 11.3 Hz, 2H, CH_2Ph), 4.17 (d, *J* = 9.6 Hz, 1H, H-5), 4.09−3.92 (m, 3H, H-4, CH_2 of Fmoc), 3.74 (s, 3H, Me), 3.81−3.62 (m, 2H, CH_2O of Bu), 2.59−2.28 (m, 4H, Lev), 2.00 (s, 3H, Lev), 1.66−1.52 (m, 2H, CH_2 of Bu), 1.39−1.21 (m, 4H, 2 x CH_2 of Bu), 1.10−0.95 (m, 2H, CH_2 of Bu), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3 of Bu), 0.67 (t, *J* = 7.4 Hz, 3H, CH_3 of Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.7 (CO, Lev), 171.6 (Lev), 167.7 (CO_2Me), 165.0 (Bz), 137.2, 133.5, 129.9, 128.8, 128.5, 128.4, 128.1, 128.0 (Ar), 96.3 (d, *J* = 4.9 Hz, C-1), 76.9 (C-4), 74.8 (CH_2Ph), 74.6 (C-5), 73.7 (d, *J* = 1.6 Hz, C-3), 71.7 (d, *J* = 9.1 Hz, C-2), 68.1 (d, *J* = 6.3 Hz, CH_2O, Bu), 67.9 (d, *J* = 6.3 Hz, CH_2O, Bu), 52.7 (Me), 37.6 (Lev), 31.9 (d, *J* = 7.4 Hz, CH_2, Bu), 31.7 (d, *J* = 7.3 Hz, CH_2, Bu), 29.5 (Lev), 27.8 (Lev), 18.5 (CH_3, Bu), 18.2 (CH_3, Bu), 13.5 (CH_3, Bu), 13.3 (CH_3, Bu). ^{31}P NMR (162 MHz, $CDCl_3$) δ −3.04. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{34}H_{45}O_{13}PNa$ 715.2490, found 715.2512.

Automated Glycan Assembly. *Building Block Solution.* For the glycosylation using 5 equiv twice, 0.25 mmol building block was dissolved in 2.0 mL of DCM.

Acidic TMSOTf Wash Solution. For the acidic TMSOTf wash, 480 μL of TMSOTf was dissolved in 20 mL of DCM.

Activator Solution. For the thioglycoside monomer, *N*-iodosuccinimide (1.35 g) was dissolved in a 9:1 mixture of anhydrous DCM and dioxane (40.0 mL), and then TfOH (60 μL) was added in an ice bath. For the phosphate monomer, 480 μL of TMSOTf was dissolved in 20 mL of DCM.

Fmoc Deprotection Solution. A solution of 20% triethylamine (TEA) in DMF (v/v) was prepared.

Lev Deprotection Solution. For Lev deprotection, hydrazine hydrate (0.68 mL) was dissolved in pyridine/acetic acid (25 mL, v/v, 3:2) to give a 0.56 M solution.

Sulfation Solution. A 0.5 M sulfur trioxide pyridine complex in DMF/TEA. For sulfation, sulfur trioxide pyridine complex (1.6 g) was dissolved in DMF/TEA (20 mL, v/v, 1:1).

Preparation of the Resin and the Synthesizer for Automated Synthesis. The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL of DCM. To start the synthesis sequence, the resin was washed using Module 1. The building blocks were coevaporated with toluene three times, dissolved in DCM under an argon atmosphere, and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer. Activation temperature (T_a) and time (t_1) and incubation temperature (T_i) and time (t_2) were used (Table S1, see the Supporting Information).

Module 1: Acidic TMSOTf Wash. The resin was washed with DMF, THF, DCM (three times each with 2 mL for 25 s), and 0.350 mL of TMSOTf solution in DCM once at −20 °C. The resin was swollen in 2 mL of CH_2Cl_2, and the temperature of the reaction vessel was adjusted to T_a.

Module 2: Glycosylation Using Thioglycoside. For glycosylation, the DCM was drained, and a solution of thioglycoside building block (5 equiv in 1.0 mL of DCM) was delivered to the reaction vessel. After the set temperature was reached (T_a), the reaction started with the addition of 1 mL of NIS (5 equiv in 1.0 mL of DCM) and TfOH (0.1 equiv in 1.0 mL of DCM) solution. The glycosylation was performed for t_1 at T_a and for t_2 at T_i. After the reaction, the solution was drained, and the resin was washed with DCM (six times with 2 mL for 15 s). This procedure was repeated twice.

Module 3: Fmoc Deprotection. The resin was washed with DMF (six times with 2 mL for 15 s) and swollen in 2 mL of DMF, and the temperature of the reaction vessel was adjusted to 25 °C. For Fmoc deprotection, the DMF was drained, and 2 mL of a solution of 20% TEA in DMF was delivered to the reaction vessel. After 5 min, the reaction solution was collected in the fraction collector of the oligosaccharide synthesizer, and 2 mL of a solution of 20% TEA in DMF was delivered to the resin. This procedure was repeated three times.

Module 4-1: Glycosylation Using Phosphate. For glycosylation, DCM was drained, and a solution of phosphate building block (5 equiv in 1.0 mL of DCM) was delivered to the reaction vessel. After the set temperature was reached (T_a), the reaction started with the addition of 1 mL of TMSOTf solution. The glycosylation was performed for t_1 at T_a and for t_2 at T_i. After the reaction, the solution was drained, and the resin was washed with DCM (six times with 2 mL for 15 s). This procedure was repeated twice.

Module 4-2: Glycosylation Using Phosphate. For glycosylation, DCM was drained, and a solution of phosphate building block (5 equiv in 1.0 mL of DCM) was delivered to the reaction vessel. After the set temperature was reached (T_a), the reaction started with the addition of 1 mL of TMSOTf solution. The glycosylation was performed for t_1 at T_a and for t_2 at T_i. After the reaction, the solution was drained, and the resin was washed with DCM (six times with 2 mL for 15 s). This procedure was repeated three times.

Module 5: Lev Deprotection. The resin was washed with DCM (six times with 2 mL for 25 s) and swollen in 1.3 mL of DCM, and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection, 0.8 mL of the hydrazine hydrate solution was delivered into the reaction vessel. After 30 min, the reaction solution was drained, and the resin was washed with 0.2 M acetic acid in DCM and DCM alone (six times each with 2 mL for 25 s). The entire procedure was performed three times.

Module 6: Sulfation. The resin was washed with DMF and TEA (three times each with 2 mL for 15 s) and swollen in 2 mL of TEA, and the temperature of the reaction vessel was adjusted to 50 °C. For sulfation, 2 mL of a 0.5 M solution of sulfur trioxide pyridine complex in DMF/TEA (v/v, 1:1) was added. After 1 h, the reaction solution was drained, and the resin was washed with DMF and TEA (three times each with 2 mL for 15 s). The entire procedure was performed three times.

Post-Automation: Linker Cleavage and Purification. *Resin Cleavage.* To prepare the photoreactor, the FEP tubing was washed with 20 mL of DCM using a flow rate of 5 mL/min. For the cleavage, the resin was slowly injected from the disposable syringe (20 mL) into the reactor and pushed through the tubing with 18 mL of DCM (flow rate: 600 μL/min). The tubing was washed with 20 mL of DCM (flow rate: 2

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

mL/min) to remove any remaining resin. The suspension leaving the reactor was directed into a filter where the resin was filtered off. The tubing was re-equilibrated with 20 mL of DCM using a flow rate of 5 mL/min. The entire procedure was performed twice. The resulting solution was evaporated, and the crude material was analyzed by NMR and HPLC.

Analytical HPLC. The crude material was analyzed by HPLC (column: Luna 5 μm Silica 100A, (260 × 4.60 mm); flow rate: 1 mL/min; eluents: hexane/ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: ELSD).

Preparative HPLC. The crude mixture was carefully dissolved in a minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was injected for purification using semipreparative HPLC (column: Luna 5 μm Silica (260 × 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: ELSD) to afford the fully protected target oligosaccharide.

Post-Automation: Global Deprotection and Purification. To a solution of the fully protected oligosaccharides in MeOH (5 mL) was added 0.5 M NaOMe solution (0.25 equiv per acetyl or benzoyl group) in MeOH at 40 °C. The mixture was stirred and then neutralized by 200 mg of Amberite IR120 hydrogen form (400 mg per 100 μL of NaOMe solution) after completion of the reaction. This crude mixture was dissolved in MeOH, ethyl acetate, and AcOH (v/v/v = 5:0.5:0.2) with 5% Pd/C (V/V) purged first with argon and then with hydrogen and left to stir overnight at room temperature using a balloon. The reaction mixture was filtered through a modified cellulose filter and washed with 20 mL of water/MeOH (9:1), and the combined solution was evaporated to provide the crude product.

Analytical HPLC. The crude material was analyzed by HPLC (column: Hypercarb, (150 × 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in acetonitrile/0.1% formic acid (FA) in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD).

Preparative HPLC. The crude solution was purified by preparative HPLC (column: Hypercarb, (150 × 10.00 mm); flow rate: 3.6 mL/min; eluents: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.

N-Benzyloxycarbonyl-5-amino-pentyl (2-O-Benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***13***. Yield: 12.0 mg, 0.0105 mmol, 42% over five steps based on resin loading, 0.025 mmol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97−7.89 (m, 6H), 7.58−7.53 (m, 1H), 7.49−7.38 (m, 4H), 7.37−7.27 (m, 18H), 7.23 (dt, J = 8.3, 5.3 Hz, 6H), 5.59 (t, J = 9.4 Hz, 1H, H-3), 5.35 (dd, J = 9.8, 8.0 Hz, 1H, H-2), 5.11 (dd, J = 10.0, 7.9 Hz, 1H, H′-2), 5.06 (s, 2H, Cbz), 4.59 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.57−4.49 (m, 5H, H-1, H′-1, CH_2Ph, NH), 4.36 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.17−4.08 (m, 3H, H-4, 2 x C*H*HPh), 3.86−3.80 (m, 1H, OC*H*H, linker), 3.75 (d, J = 3.4 Hz, 1H, H′-4), 3.71 (dd, J = 10.9, 3.9 Hz, 1H, H-6), 3.61 (dd, J = 10.9, 1.6 Hz, 1H, H-6), 3.56−3.49 (m, 2H, H-5, H′-3), 3.40 (dd, J = 15.5, 6.8 Hz, 1H, OC*H*H, linker), 3.31 (dd, J = 9.3, 5.2 Hz, 1H, H′-5), 2.96 (dd, J = 9.0, 4.9 Hz, 1H, H′-6), 2.91 (td, J = 12.7, 6.3 Hz, 2H, CH_2NHCbz), 2.85 (t, J = 9.2 Hz, 1H, H′-6), 2.24 (d, J = 10.4 Hz, 1H, OH), 1.48 (ddd, J = 20.5, 12.9, 6.5 Hz, 2H), 1.34−1.24 (m, 2H), 1.23−1.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (Bz), 165.3 (Bz), 165.3 (Bz), 156.3 (Cbz), 138.2, 138.2, 137.7, 136.8, 133.3, 133.2, 132.8, 130.4, 123.0, 129.9, 129.84, 129.77, 129.6, 128.6, 128.58, 128.57, 128.51, 128.47, 128.22, 128.18, 128.0, 127.94, 127.87, 127.8, 127.7 (Ar), 101.1 (C-1), 100.6 (C′-1), 76.0 (C′-4), 75.6 (C-4), 75.1 (CH_2Ph), 74.7 (C′-3), 74.3 (C′-2), 73.6 (C-3), 73.5 (CH_2Ph), 73.2 (CH_2Ph), 72.9 (C-5), 72.7 (C′-5), 72.0 (C-2), 69.9 (OCH_2, linker), 67.8 (C-6), 66.9 (C′-6), 66.6 (Cbz), 40.9 (CH_2NHCbz), 29.5, 29.0, 23.1. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{67}H_{69}O_{16}$NNa 1166.4509, found 1166.4505.

N-Benzyloxycarbonyl-5-amino-pentyl (2-O-Benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***20***. Yield: 21.3 mg, 0.0103 mmol, 41% over nine steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, J = 7.2 Hz, 2H), 7.91 (d, J = 7.3 Hz, 2H), 7.85 (dd, J = 8.2, 7.3 Hz, 4H), 7.50 (td, J = 7.1, 1.1 Hz, 2H), 7.46 (t, J = 7.4 Hz, 1H), 7.40−7.18 (m, 37H), 7.18−7.08 (m, 12H), 6.60 (t, J = 6.8 Hz, 1H, NHTCA), 5.53 (t, J = 9.4 Hz, 1H, H-3), 5.31 (dt, J = 17.6, 8.9 Hz, 2H, 2 x H-2), 5.23 (dd, J = 10.0, 8.0 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.90 (d, J = 10.4 Hz, 1H, C*H*HPh), 4.76 (d, J = 7.9 Hz, 1H, H-1), 4.74−4.59 (m, 4H, H-1, 3 x C*H*HPh), 4.57−4.53 (m, 2H, C*H*HPh, NHCbz), 4.48 (d, J = 7.9 Hz, 1H, H-1), 4.46−4.28 (m, 8H, H-1, 6 x C*H*HPh), 4.23 (d, J = 12.3 Hz, 1H), 4.05 (dt, J = 9.3, 8.5 Hz, 3H), 3.88 (dd, J = 8.0, 3.0 Hz, 2H), 3.82−3.74 (m, 2H), 3.70 (dd, J = 10.2, 1.7 Hz, 1H), 3.65−3.59 (m, 2H), 3.57−3.49 (m, 3H), 3.47−3.41 (m, 3H), 3.36 (d, J = 9.4 Hz, 3H), 3.31 (dd, J = 8.4, 5.1 Hz, 1H), 3.09 (d, J = 7.5 Hz, 1H, H-2), 2.90 (dt, J = 14.3, 7.3 Hz, 3H, H-6, CH_2NHCbz), 2.76 (t, J = 8.7 Hz, 1H, H-6), 2.41 (d, J = 7.9 Hz, 1H, OH), 1.52−1.36 (m, 2H, CH_2, pentane), 1.34−1.21 (m, 2H, CH_2, pentane), 1.20−1.05 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1 (Bz), 165.4 (Bz), 165.3 (Bz), 164.7 (Bz), 161.3 (TCA), 156.4 (Cbz), 139.3, 138.2, 138.10, 138.06, 137.8, 136.8, 133.6, 133.3, 133.2, 132.5, 130.6, 130.03, 130.00, 129.97, 129.9, 129.8, 129.7, 129.6, 128.7, 128.61, 128.59, 128.57, 128.55, 128.51, 128.50, 128.47, 128.4, 128.24, 128.20, 128.17, 128.1, 127.98, 127.96, 127.93, 127.88, 127.79, 127.77, 127.7, 127.2 (Ar), 101.0 (C-1), 100.8 (C-1), 99.7 (C-1), 98.9 (C-1), 92.4 (CCl_3), 77.8, 76.7, 76.4, 76.3, 75.7, 75.1, 74.9, 74.8, 74.7, 74.5, 73.6, 73.5, 73.4, 73.3, 73.2, 73.0, 72.7, 72.1, 69.7, 69.3, 67.8, 67.5, 67.3, 66.6, 59.4, 40.9, 29.8, 29.5, 29.0, 23.1. HRMS (ESI-TOF): m/z [M + Na]$^+$ $C_{116}H_{117}Cl_3N_2O_{27}$Na 2097.6802, found 2097.6765.

N-Benzyloxycarbonyl-5-amino-pentyl (2-O-Benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***21***. Yield: 20.3 mg, 0.0098 mmol, 39% over 9 steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 7.8 Hz, 2H), 7.90 (t, J = 6.7 Hz, 4H), 7.84 (d, J = 7.9 Hz, 2H), 7.56 (t, J = 7.4 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.47−7.02 (m, 50H), 6.54 (d, J = 8.0 Hz, 1H, NHTCA), 5.55 (t, J = 9.3 Hz, 1H, H-3), 5.38 (dd, J = 9.9, 8.0 Hz, 1H, H-2), 5.29 (t, J = 8.7 Hz, 1H, H-2), 5.18 (dd, J = 9.8, 8.1 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.87 (d, J = 10.7, 1H, C*H*HPh), 4.86 (d, J = 11.9 Hz, 1H, C*H*HPh), 4.75 (d, J = 7.6 Hz, 1H, H-1), 4.65 (s, 2H, CH_2Ph), 4.58 (d, J = 7.9 Hz, 1H, H-1), 4.53−4.41 (m, 6H, 2 x H-1, 3 x C*H*HPh), 4.39−4.31 (m, 2H, 2 x C*H*HPh), 4.26 (dd, J = 11.9, 5.0 Hz, 3H, 3 x C*H*HPh), 4.07−3.97 (m, 3H, 2 x C*H*HPh), 3.95 (d, J = 8.0 Hz, 1H), 3.89 (s, 2H), 3.82−3.58 (m, 5H), 3.56−3.24 (m, 11H), 2.89 (dd, J = 8.6, 4.7 Hz, 3H, CH_2NHCbz, H-6), 2.82 (t, J = 8.7 Hz, 1H, H-6), 1.53−1.35 (m, 2H, CH_2, pentane), 1.35−1.23 (m, 2H, CH_2, pentane), 1.22−1.08 (m, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (Bz), 165.35 (Bz), 165.30 (Bz), 164.6 (Bz), 161.7 (TCA), 156.4 (Cbz), 139.3, 138.5, 138.5, 138.20, 138.17, 138.0, 137.8, 136.9, 133.4, 133.3, 133.1, 132.5, 130.6, 130.0, 129.9, 129.9, 129.8, 129.75, 128.70, 128.66, 128.61, 128.58, 128.4, 128.2, 128.12, 128.10, 128.04, 128.01, 127.9, 127.83, 127.80, 127.74, 127.67, 127.4, 127.2 (Ar), 101.1 (C-1), 101.0 (C-1), 100.4 (C-1), 100.2 (C-1), 92.2 (CCl_3), 79.2, 77.8, 77.4, 76.6, 76.5, 76.0, 75.6, 75.2, 75.0, 74.8, 74.6, 74.3, 73.6, 73.5, 73.4, 73.2, 73.0, 72.9, 72.5, 72.2, 69.7, 68.3, 67.8, 67.7, 67.4, 66.6, 58.0, 40.9, 29.5, 29.0, 23.2. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{116}H_{117}Cl_3N_2O_{27}$Na 2097.6802, found 2097.6811.

N-Benzyloxycarbonyl-5-amino-pentyl (3,4-Di-O-acetyl-2-O-benzoyl-α-L-fucopyranosyl)-(1→2)-(4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***22***. Yield: 22.6 mg, 0.0095 mmol, 38% over ten steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, J = 7.6 Hz, 2H), 7.87 (d, J = 4.4 Hz, 2H), 7.81 (d, J = 7.3 Hz, 2H), 7.66 (s, 1H), 7.50 (t, J = 6.7 Hz, 1H), 7.45 (t, J = 7.3 Hz, 1H), 7.42−7.08 (m, 52H), 7.02 (dd, J = 9.0, 6.0 Hz, 4H), 5.64 (d, J = 3.0 Hz, 1H, H_{Fuc}-1), 5.56−5.43 (m, 3H, H-3, H_{Fuc}-3, H′-2), 5.30 (dd, J = 11.5, 5.9 Hz, 1H, H-2), 5.20 (d, J = 1.3 Hz, 1H, H_{Fuc}-4), 5.15 (d, J = 12.0 Hz, 1H, C*H*HPh), 5.06 (s, 2H, CH_2, Cbz), 4.93 (d, J = 9.6 Hz, 1H, C*H*HPh), 4.88 (d, J = 11.3 Hz, 1H, C*H*HPh), 4.78 (dd, J = 12.4, 6.0 Hz, 2H, H_{Fuc}-5), 4.66 (d, J = 11.9 Hz, 1H, C*H*HPh), 4.55−4.44 (m, 6H, H-1, 4 x C*H*HPh), 4.41−4.28 (m, 8H, 2 x H-1, 6 x C*H*HPh), 4.22 (dd, J = 29.7, 11.8 Hz, 2H, 2 x C*H*HPh), 4.15−4.10 (m, 1H), 4.04 (dt, J = 9.0, 8.1 Hz, 3H, 2 x C*H*HPh), 3.95 (d, J = 6.4 Hz, 3H, 2 x H-4), 3.81−3.71 (m, 4H), 3.54 (dd, J = 19.4, 10.9 Hz, 2H), 3.46−3.30 (m, 10H), 3.12 (s, 1H),

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

2.93−2.85 (m, 3H, H-6, CH_2NHCbz), 2.82 (t, J = 8.7 Hz, 1H, H-6), 2.10 (s, 3H, Ac), 1.57 (s, 3H, Ac), 1.51−1.38 (m, 2H, CH_2, pentane), 1.33−1.21 (m, 2H, CH_2, pentane), 1.19−1.10 (m, 2H, CH_2, pentane), 1.08 (d, J = 6.5 Hz, 3H, H_{Fuc}-6). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4 (Ac), 170.5 (Ac), 165.4 (Bz), 165.3 (Bz), 164.6 (Bz), 162.2 (TCA), 156.4 (Cbz), 139.9, 139.0, 138.2, 138.1, 138.0, 133.1, 133.0, 132.4, 130.7, 130.2, 130.0, 129.8, 129.8, 128.9, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.24, 128.20, 128.17, 128.05, 128.02, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 126.9, 126.8 (Ar), 101.4 (C-1), 101.3 (C-1), 101.1 (C-1), 98.5 (C-1), 96.5 (C_{Fuc}-1), 92.3 (CCl_3), 84.1, 77.8, 77.6, 76.6, 75.4, 75.2, 75.0, 74.9, 74.7, 73.6, 73.5, 73.3, 73.2, 73.1, 72.9, 72.6, 72.4, 72.1, 72.0, 71.6, 69.7, 69.1, 67.7, 67.5, 66.6, 65.0, 60.8, 40.9, 29.5, 29.0, 23.2, 20.8, 20.4, 15.8 (C_{Fuc}-6). HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{133}H_{139}N_2O_{32}Cl_3Na$ 2403.8269, found 2403.8303.

N-Benzyloxycarbonyl-5-amino-pentyl (2,3,4-Tri-O-benzoyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***23***. Yield: 14.9 mg, 0.006 mmol, 24% over ten steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.3 Hz, 2H), 7.85−7.80 (m, 2H), 7.52−7.48 (m, 2H), 7.45 (dd, J = 13.1, 5.7 Hz, 2H), 7.39−7.07 (m, 61H), 7.05 (d, J = 7.2 Hz, 2H), 7.00 (t, J = 7.6 Hz, 2H), 6.73 (d, J = 7.5 Hz, 1H), 5.68 (d, J = 3.7 Hz, 1H), 5.56 (t, J = 9.4 Hz, 1H), 5.43 (dd, J = 10.0, 8.0 Hz, 1H), 5.29 (dd, J = 9.6, 8.0 Hz, 1H), 5.05 (s, 2H), 4.94 (t, J = 10.1 Hz, 2H), 4.90−4.82 (m, 2H), 4.79−4.69 (m, 3H), 4.64−4.60 (m, 2H), 4.53−4.41 (m, 8H), 4.38 (d, J = 11.5 Hz, 2H), 4.34 (dd, J = 13.2, 4.8 Hz, 2H), 4.29 (d, J = 2.0 Hz, 1H), 4.27 (d, J = 8.6 Hz, 3H), 4.19 (d, J = 11.9 Hz, 1H), 4.15 (dd, J = 9.4, 7.9 Hz, 1H), 4.06−4.00 (m, 4H), 3.96 (d, J = 2.3 Hz, 1H), 3.95−3.91 (m, 1H), 3.90 (d, J = 2.0 Hz, 1H), 3.86−3.76 (m, 4H), 3.72−3.64 (m, 3H), 3.55 (dd, J = 9.6, 2.7 Hz, 1H), 3.51 (dd, J = 11.0, 3.9 Hz, 1H), 3.43 (dd, J = 19.0, 10.4 Hz, 3H), 3.38−3.30 (m, 5H), 3.26 (dd, J = 8.7, 5.0 Hz, 1H), 2.93−2.87 (m, 3H), 2.83 (t, J = 8.7 Hz, 1H), 1.51−1.38 (m, 2H), 1.31−1.24 (m, 4H), 1.18 (d, J = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.4 (Bz), 165.3 (Bz), 164.7 (Bz), 161.7 (NHTCA), 156.4 (Cbz), 139.2, 139.0, 138.9, 138.7, 138.6, 138.4, 138.1, 138.0, 133.3, 133.2, 132.5, 130.5, 130.1, 130.0, 129.8, 129.7, 128.63, 128.58, 128.51, 128.48, 128.45, 128.43, 128.35, 128.28, 128.15, 128.13, 128.09, 128.0, 127.9, 127.81, 127.78, 127.6, 127.5, 127.4, 127.35, 127.30, 127.2, 126.3, 101.3 (C-1), 101.1 (C-1), 99.8 (C-1), 97.5 (C-1), 92.1 (C_{Fuc}-1), 84.1, 79.4, 79.3, 78.2, 76.5, 76.0, 75.9, 75.5, 75.2, 75.0, 74.9, 74.8, 74.5, 73.6, 73.5, 73.4, 73.2, 73.0, 72.8, 72.5, 72.3, 72.2, 71.1, 69.8, 68.7, 68.1, 67.8, 67.3, 66.6, 66.5, 58.1, 40.9, 29.9, 29.5, 29.0, 23.2, 16.9. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{143}H_{147}N_2O_{30}Na$ 2499.8996, found 2499.9001.

5-Amino-pentyl β-D-Galactopyranosyl-(1→3)-2-deoxy-2-acetoamido-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside ***1***. Yield: 3.9 mg, 4.92 μmol, 48% over two steps. ^{1}H NMR (600 MHz, D_2O) δ 4.75 (d, J = 8.5 Hz, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.48−4.43 (m, 2H), 4.17 (d, J = 3.1 Hz, 1H), 4.03−3.87 (m, 5H), 3.87−3.46 (m, 19H), 3.32 (t, J = 8.5 Hz, 1H), 3.02 (t, J = 7.8 Hz, 2H), 2.04 (s, 3H), 1.76−1.64 (m, 4H), 1.52−1.43 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 172.1, 104.9, 104.3, 103.9, 103.4, 83.5, 83.4, 79.8, 76.7, 76.6, 76.3, 76.2, 75.9, 74.2, 73.9, 72.1, 71.5, 71.4, 69.9, 69.9, 69.7, 62.4, 62.3, 61.9, 61.5, 56.1, 40.7, 29.6, 27.8, 23.6, 23.5. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{31}H_{56}N_2O_{21}Na$ 815.3273, found 815.3285.

5-Amino-pentyl β-D-Galactopyranosyl-(1→4)-2-deoxy-2-acetoamido-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside ***2***. Yield: 3.0 mg, 3.80 μmol, 39% over two steps. ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, J = 8.3 Hz, 1H), 4.55−4.49 (m, 2H), 4.46 (d, J = 7.8 Hz, 1H), 4.18 (br s, 1H), 4.04−3.92 (m, 4H), 3.91−3.53 (m, 20H), 3.37−3.29 (m, 1H), 3.03 (t, J = 7.4 Hz, 2H), 2.05 (s, 3H), 1.76−1.65 (m, 4H), 1.53−1.44 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 172.3, 104.3, 104.3, 104.1, 103.4, 83.4, 79.8, 79.6, 76.7, 76.3, 76.2, 75.9, 75.9, 74.2, 73.9, 73.6, 72.3, 71.4, 71.3, 69.9, 69.7, 62.4, 62.3, 61.5, 61.3, 56.6, 40.7, 29.5, 27.8, 23.6, 23.5. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for calcd for $C_{31}H_{56}N_2O_{21}Na$ 815.3273, found 815.3297.

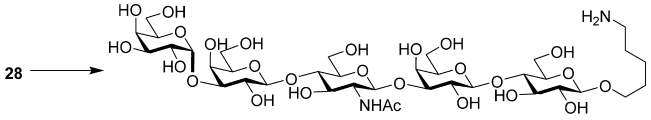
5-Amino-pentyl α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetoamido-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside ***3***. Yield: 4.1 mg, 4.37 μmol, 46% over two steps. ^{1}H NMR (600 MHz, D_2O) δ 5.06 (d, J = 4.1 Hz, 1H), 4.52 (d, J = 7.7 Hz, 1H), 4.49 (d, J = 8.5 Hz, 1H), 4.36 (d, J = 8.0 Hz, 1H), 4.29 (d, J = 7.9 Hz, 1H), 4.19−4.15 (m, 1H), 4.01 (dd, J = 3.2, 0.9 Hz, 1H), 3.88−3.75 (m, 5H), 3.72−3.41 (m, 23H), 3.40−3.38 (m, 1H), 3.36 (ddd, J = 10.0, 4.7, 2.2 Hz, 1H), 3.20−3.15 (m, 1H), 2.89−2.85 (m, 2H), 1.93 (s, 3H), 1.55 (ddt, J = 14.6, 11.6, 7.3 Hz, 4H), 1.33 (tt, J = 9.8, 6.5 Hz, 2H), 1.11 (d, J = 6.7 Hz, 3H). ^{13}C NMR (150 MHz, D_2O) δ 176.8, 105.8, 105.6, 104.6, 102.8, 102.1, 84.2, 80.9, 79.8, 79.3, 77.9, 77.7, 77.43, 77.40, 77.0, 76.1, 75.4, 74.5, 72.8, 72.7, 72.0, 71.7, 71.2, 71.1, 70.6, 69.1, 63.7, 63.6, 63.0, 62.7, 57.6, 42.0, 30.8, 29.1, 24.7, 24.7, 17.9. HRMS (ESI-TOF): m/z [M + Na]+ calcd for calcd for $C_{37}H_{66}N_2O_{25}Na$ 961.3847, found 961.3845.

N-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-O-benzoyl-4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***28***. Yield: 24.1 mg, 9.25 μmol, 37% over ten steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95−7.79 (m, 8H), 7.48 (ddd, J = 25.6, 16.1, 7.6 Hz, 4H), 7.39−7.06 (m, 66H), 7.05−6.98 (m, 2H), 5.55 (dt, J = 19.1, 9.4 Hz, 2H), 5.35 (dd, J = 9.8, 8.1 Hz, 1H), 5.29 (dd, J = 11.2, 5.0 Hz, 1H), 5.05 (s, 2H), 5.01 (s, 1H), 4.93 (d, J = 3.3 Hz, 1H), 4.88−4.78 (m, 4H), 4.69 (d, J = 7.6 Hz, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.56−4.52 (m, 3H), 4.50−4.46 (m, 3H), 4.43−4.38 (m, 3H), 4.32 (dd, J = 27.0, 11.8 Hz, 2H), 4.25−4.21 (m, 2H), 4.19 (d, J = 2.7 Hz, 1H), 4.14 (dd, J = 23.0, 10.1 Hz, 2H), 4.01 (ddd, J = 12.6, 8.6, 5.1 Hz, 4H), 3.95−3.87 (m, 3H), 3.83 (s, 1H), 3.78 (dd, J = 10.0, 2.4 Hz, 2H), 3.74−3.60 (m, 4H), 3.54 (dd, J = 10.6, 3.4 Hz, 1H), 3.50−3.29 (m, 9H), 3.28−3.17 (m, 4H), 2.97 (dd, J = 8.5, 5.2 Hz, 1H), 2.91−2.83 (m, 3H), 2.78 (t, J = 8.7 Hz, 1H), 1.51−1.35 (m, 2H), 1.34−1.22 (m, 2H), 1.20−1.05 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (Bz), 165.3 (Bz), 165.1 (Bz), 164.6 (Bz), 161.7 (TCA), 156.4 (Cbz), 139.2, 139.2, 138.8, 138.6, 138.5, 138.3, 138.20, 138.17, 138.1, 133.3, 133.23, 133.17, 132.5, 130.6, 130.01, 129.95, 129.8, 129.7, 128.63, 128.58, 128.48, 128.46, 128.4, 128.3, 128.2, 128.1, 128.05, 128.00, 127.96, 127.92, 127.89, 127.85, 127.78, 127.74, 127.72, 127.65, 127.60, 127.5, 127.4, 127.31, 127.26, 127.1 (Ar), 101.1 (C-1), 101.0 (C-1), 100.8 (C-1), 100.2 (C-1), 99.7 (C-1), 92.2, 81.0, 79.2, 79.0, 77.7, 76.6, 76.2, 76.0, 75.2, 75.0, 74.8, 74.8, 74.4, 74.3, 73.8, 73.5, 73.4, 73.4, 73.3, 73.1, 73.0, 72.7, 72.5, 72.4, 72.2, 69.8, 69.7, 68.2, 68.1, 67.9, 67.7, 67.4, 66.6, 57.9, 40.9, 31.1, 29.9, 29.5, 29.0, 23.2. HRMS (ESI-TOF): m/z [M + Na]+ calcd for $C_{150}H_{151}N_2O_{32}Cl_3Na$ 2621.9275, found 2621.9260.

N-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***29***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 7.7 Hz, 2H), 7.92 (d, J = 7.9 Hz, 2H), 7.61 (t, J = 7.0 Hz, 1H), 7.53 (dd, J = 13.1, 6.2 Hz, 2H), 7.47 (t, J = 7.4 Hz, 2H), 7.44−7.20 (m, 31H), 7.16 (t, J = 7.2 Hz, 2H), 7.12 (d, J = 7.2 Hz, 2H), 7.04−6.98 (m, 3H), 6.95 (t, J = 6.8 Hz, 1H), 5.48 (t, J = 8.8 Hz, 1H, H-2), 5.28−5.23 (m, 1H, H-2), 5.22 (d, J = 7.6 Hz, 1H, H″-1), 5.11 (t, J = 8.5 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.92 (d, J = 11.5 Hz, 1H, C*H*HPh), 4.72 (d, J = 11.6 Hz, 1H, C*H*HPh), 4.66 (d, J = 12.2 Hz, 1H, C*H*HPh), 4.59−4.54 (m, 3H, H-1, 2 x C*H*HPh), 4.50−4.43 (m, 4H, H′-4, CH_2Ph, C*H*HPh), 4.40 (d, J = 12.0 Hz, 1H, C*H*HPh), 4.30 (d, J = 7.9 Hz, 1H, H-1), 4.26 (d, J = 12.0 Hz, 2H, 2 x C*H*HPh), 4.16 (d, J = 10.7 Hz, 1H, C*H*HPh), 3.93 (d, J = 2.4 Hz, 1H, H″-4), 3.90 (t, J = 9.0 Hz, 1H, H-4), 3.82−3.72 (m, 2H, H″-3, OC*H*H$(CH_2)_4$), 3.68 (dd, J = 12.4, 6.4 Hz, 1H, H-6), 3.65−3.57 (m, 3H, H-5, 2 x H-6), 3.57−3.50 (m, 2H, H-3, H-6), 3.49−3.42 (m, 3H, H-3, H-5, H-6), 3.34−3.27 (m, 2H, H-6, OC*H*H$(CH_2)_4$), 3.19 (d, J = 8.1 Hz, 1H, H-5), 3.00 (br, 1H, OH), 2.89 (d, J = 6.7 Hz, 2H, CH_2NHCbz), 1.51−1.35 (m, 2H, CH_2, pentane), 1.34−1.22 (m, 2H, CH_2, pentane), 1.20−1.06 (m, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9 (Bz), 165.1 (Bz), 164.6 (Bz), 156.3 (Cbz), 138.7, 138.5, 138.4, 138.3, 137.9, 137.7, 136.8, 133.2, 133.0, 132.9, 130.7, 130.2, 130.0, 129.8, 129.7, 128.6, 128.6, 128.5, 128.5, 128.44, 128.42, 128.36, 128.1, 128.1, 128.0, 127.92, 127.87, 127.8, 127.6, 127.5, 127.0 (Ar), 101.3 (C-1), 100.8 (C-1), 99.7 (C-1), 80.7, 80.1, 77.0, 76.7, 75.9, 75.2, 74.99, 74.97, 74.2, 73.8, 73.63, 73.60, 73.5, 73.4, 72.3, 71.8, 69.4, 69.1, 68.7, 68.5, 68.0, 66.5, 40.9, 29.8, 29.4, 28.9, 23.1, 22.8, 14.2. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{101}H_{103}NO_{21}Na$ 1688.6915, found 1688.6920.

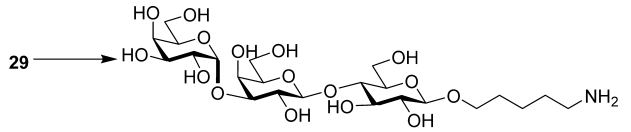
DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

N-Benzyloxycarbonyl-5-amino-pentyl (2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside ***30***. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 7.5 Hz, 2H), 7.49 (t, J = 7.3 Hz, 1H), 7.36−7.14 (m, 45H), 7.10 (t, J = 7.3 Hz, 2H), 7.01 (s, 1H), 5.64−5.58 (m, 1H, H′-2), 5.08−5.03 (m, 3H, C*H*HPh, CH_2, Cbz), 4.95 (d, J = 3.2 Hz, 1H, H″-1), 4.90 (d, J = 10.6 Hz, 1H, C*H*HPh), 4.85 (d, J = 11.5 Hz, 1H, C*H*HPh), 4.79 (d, J = 11.2 Hz, 1H, C*H*HPh), 4.74 (s, 1H, NHCbz), 4.63 (d, J = 11.4 Hz, 2H, H-1, C*H*HPh), 4.56 (dd, J = 20.0, 9.0 Hz, 4H, H′-1, 3 x C*H*HPh), 4.49 (d, J = 11.9 Hz, 1H, C*H*HPh), 4.39 (dd, J = 11.4, 6.1 Hz, 2H, CH_2Ph), 4.34 (d, J = 12.1 Hz, 2H, 2 x C*H*HPh), 4.26−4.20 (m, 2H, 2 x C*H*HPh), 4.16−4.11 (m, 1H, C*H*HPh), 4.02 (dd, J = 10.2, 3.3 Hz, 1H, H″-2), 3.98 (d, J = 1.9 Hz, 2H), 3.93−3.90 (m, 2H), 3.79 (dd, J = 10.1, 2.5 Hz, 1H), 3.70 (d, J = 10.9 Hz, 3H), 3.62−3.49 (m, 3H), 3.48−3.43 (m, 2H), 3.35 (dd, J = 16.9, 13.1 Hz, 2H), 3.26 (t, J = 8.6 Hz, 1H), 3.21 (d, J = 8.0 Hz, 1H), 3.12 (d, J = 6.0 Hz, 2H), 2.99 (dd, J = 8.6, 5.3 Hz, 1H), 1.50−1.39 (m, 4H), 1.32−1.22 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (Bz), 161.8 (Bz), 156.5 (Cbz), 139.2, 138.8, 138.7, 138.6, 138.3, 138.3, 138.3, 138.2, 136.8, 133.2, 130.0, 128.6, 128.6, 128.54, 128.49, 128.46, 128.4, 128.34, 128.27, 128.23, 128.18, 128.0, 127.9, 127.84, 127.79, 127.7, 127.59, 127.54, 127.50, 127.4, 127.3 (Ar), 100.6 (C-1), 99.63 (C-1), 99.60 (C-1), 92.7, 80.85, 79.02, 78.0, 76.6, 75.8, 75.2, 75.03, 75.01, 74.8, 74.4, 74.2, 73.8, 73.6, 73.5, 73.3, 72.7, 72.4, 69.8, 69.6, 68.4, 68.2, 67.9, 66.7, 60.5, 57.3, 41.0, 29.8, 29.7, 29.1, 23.3, 14.3. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{96}H_{101}N_2O_{19}$ Cl_3Na 1714.5990, found 1714.6020.



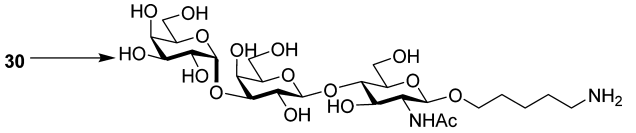
31

5-Amino-pentyl α-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetoamido-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside ***31***. Yield: 3.9 mg, 4.07 μmol, 44% over two steps. ^{1}H NMR (500 MHz, D_2O) δ 5.13 (d, J = 3.8 Hz, 1H, H-1), 4.69 (d, J = 8.3 Hz, 1H, H-1), 4.54 (d, J = 7.8 Hz, 1H, H-1), 4.47 (d, J = 8.0 Hz, 1H, H-1), 4.42 (d, J = 7.9 Hz, 1H, H-1), 4.17 (dd, J = 8.3, 4.6 Hz, 2H), 4.14 (d, J = 2.9 Hz, 1H), 4.00 (d, J = 2.8 Hz, 1H), 3.98−3.89 (m, 4H), 3.86−3.53 (m, 23H), 3.29 (dd, J = 11.3, 5.7 Hz, 1H), 3.01−2.96 (m, 2H), 2.02 (s, 3H), 1.71−1.59 (m, 4H), 1.44 (dt, J = 15.2, 7.7 Hz, 2H). ^{13}C NMR (176 MHz, D_2O) δ 177.49 (NHAc), 105.54 (C-1), 105.38 (C-1), 105.34 (C-1), 104.59 (C-1), 98.04 (C-1), 84.67, 81.03, 79.81, 77.65, 77.49, 77.38, 77.14, 77.06, 75.41, 74.85, 73.45, 72.68, 72.55, 72.20, 71.89, 71.73, 70.91, 70.79, 67.41, 63.59, 63.55, 63.53, 62.69, 62.55, 57.78, 41.97, 30.75, 29.05, 25.87, 24.79, 24.68. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{37}H_{66}N_2O_{26}Na$ 977.3796, found 977.3804.



32

5-Amino-pentyl α-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside ***32*** *(3.1 mg, 5.53 μmol, 51% over two steps).* ^{1}H NMR (600 MHz, D_2O) δ 5.17 (d, J = 4.0 Hz, 1H, H, H-1), 4.55 (d, J = 8.0 Hz, 1H, H-1), 4.52 (d, J = 8.1 Hz, 1H, H-1), 4.25−4.19 (m, 2H), 4.05 (d, J = 3.1 Hz, 1H), 4.02 (dd, J = 12.3, 1.8 Hz, 1H), 3.99−3.94 (m, 2H), 3.89 (dd, J = 10.4, 3.9 Hz, 1H), 3.86−3.65 (m, 12H), 3.64−3.61 (m, 1H), 3.36−3.32 (m, 1H), 2.98 (t, J = 7.3 Hz, 2H, OCH_2(CH_2)$_4$$NH_2$), 1.73−1.66 (m, 4H, OCH$_2CH_2CH_2CH_2CH_2NH_2$), 1.52−1.44 (m, 2H OCH$_2CH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (150 MHz, D_2O) δ 173.63 (CO_2H), 105.45 (C-1), 104.62(C-1), 98.03(C-1), 81.28, 79.81, 77.66, 77.35, 77.13, 75.41, 73.44, 72.75, 72.18, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.77, 42.09 (CH_2NH_2), 30.80, 29.63, 24.73. HRMS (ESI-TOF): m/z [M + H]$^+$ calcd for $C_{23}H_{44}NO_{16}$ 590.2655, found 590.2644.



33

5-Amino-pentyl α-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetoamido-β-D-glucopyranoside ***33***. Yield: 2.4 mg, 3.79 μmol, 37% over two steps. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, J = 3.9 Hz, 1H, H-1), 4.52 (dd, J = 9.9, 7.8 Hz, 2H), 4.53 (d, J = 7.9 Hz, 1H, H-1), 4.51 (d, J = 7.7 Hz, 1H, H-1), 4.02−3.56 (m, 21H), 2.99−2.95 (m, 2H), 1.66 (dt, J = 14.9, 7.4 Hz, 2H), 1.62−1.54 (m, 2H), 1.42−1.36 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.02 (NHAc), 105.41 (C-1), 103.72 (C-1), 98.04 (C-1), 81.36, 79.81, 77.67, 77.34, 75.09, 73.45, 72.71, 72.20, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.76, 57.68, 41.94, 30.68, 28.98, 24.76, 24.72. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{25}H_{46}N_2O_{16}Na$ 653.2740, found 653.2748.

N-Benzyloxycarbonyl-5-amino-pentyl (Methyl 2-O-Bbenzoyl-4-O-benzyl-β-D-glucopyranosyluronate)-(1→3)-(2-O-benzoyl-4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***34***. Yield: 16.0 mg, 6.5 μmol, 26% over 2 steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 (dd, J = 14.7, 7.2 Hz, 4H), 7.83 (d, J = 7.5 Hz, 2H), 7.71 (d, J = 7.5 Hz, 4H), 7.58−7.42 (m, 5H), 7.42−7.08 (m, 52H), 7.07−7.01 (m, 3H), 6.53 (d, J = 7.3 Hz, 1H), 5.55 (t, J = 9.1 Hz, 1H), 5.48−5.43 (m, 1H), 5.38−5.33 (m, 1H), 5.30 (dd, J = 11.7, 5.2 Hz, 1H), 5.06 (t, J = 8.3 Hz, 3H), 4.98 (d, J = 11.4 Hz, 1H), 4.80 (ddd, J = 39.1, 23.4, 9.7 Hz, 4H), 4.69 (d, J = 11.2 Hz, 2H), 4.56 (s, 1H), 4.52−4.44 (m, 5H), 4.38 (dd, J = 16.3, 9.1 Hz, 2H), 4.30 (d, J = 11.9 Hz, 1H), 4.28−4.21 (m, 2H), 4.15−4.08 (m, 3H), 4.05−3.96 (m, 5H), 3.87 (dd, J = 20.1, 10.8 Hz, 4H), 3.80−3.72 (m, 5H), 3.68−3.59 (m, 2H), 3.48 (dd, J = 13.5, 6.1 Hz, 4H), 3.43−3.31 (m, 6H), 3.24−3.18 (m, 2H), 3.14 (s, 1H), 2.93−2.83 (m, 3H), 2.78 (t, J = 8.7 Hz, 1H), 1.52−1.38 (m, 2H), 1.33−1.22 (m, 2H), 1.19−1.10 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3 (CO_2Me), 168.6 (Bz), 166.0 (Bz), 165.3 (Bz), 164.6 (Bz), 164.2 (Bz), 161.6 (TCA), 156.4 (Cbz), 139.2, 138.8, 138.4, 138.3, 138.2, 138.1, 138.0, 137.7, 136.8, 133.3, 133.1, 132.5, 130.5, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.1, 128.7, 128.6, 128.55, 128.51, 128.48, 128.43, 128.40, 128.29, 128.26, 128.24, 128.21, 128.18, 128.1, 128.04, 127.98, 127.91, 127.87, 127.8, 127.75, 127.69, 127.6, 127.5, 127.2, 127.1, 124.1, 123.6, 116.0, 114.2, 101.5 (C-1), 101.0 (C-1), 101.0 (C-1), 100.6 (C-1), 100.3 (C-1), 92.1 (TCA), 79.4, 79.3, 79.0, 77.6, 77.4, 77.2, 77.0, 76.1, 75.95, 75.93, 75.25, 75.23, 75.17, 75.1, 75.0, 74.7, 74.6, 74.39, 74.36, 73.7, 73.6, 73.3, 73.1, 72.9, 72.7, 72.3, 72.1, 69.7, 68.10, 68.01, 67.7, 67.3, 66.6, 60.5, 57.8, 52.8, 40.9, 33.9, 32.0, 31.7, 31.5, 30.3, 29.8, 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 23.1, 22.8, 21.2, 18.0, 14.3, 14.2, 13.5. HRMS (ESI-TOF): m/z [M + Na]$^+$ calcd for $C_{137}H_{137}N_2O_{34}Cl_3Na$ 2483.7981, found 2483.7969.

N-Benzyloxycarbonyl-5-amino-pentyl (Methyl 2-O-Bbenzoyl-4-O-benzyl-3-O-sulfato-β-D-glucopyranosyluronate)-(1→3)-(2-O-benzoyl-4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-(4,6-di-O-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside ***35***. Yield: 14 mg, 5.50 μmol, 22% over two steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (d, J = 7.4 Hz, 2H), 7.82 (dd, J = 14.1, 7.4 Hz, 4H), 7.59 (t, J = 8.4 Hz, 3H), 7.49 (t, J = 7.3 Hz, 1H), 7.46−7.41 (m, 1H), 7.37−7.17 (m, 41H), 7.17−7.07 (m, 16H), 7.02 (dd, J = 15.2, 7.7 Hz, 2H), 6.56 (d, J = 6.2 Hz, 1H), 5.95 (s, 1H), 5.52 (t, J = 9.4 Hz, 2H), 5.40−5.32 (m, 1H), 5.28 (dd, J = 9.4, 8.2 Hz, 2H), 5.23 (s, 1H), 5.05 (s, 2H), 4.96 (d, J = 11.5 Hz, 1H), 4.90 (s, 2H), 4.83−4.77 (m, 2H), 4.66 (d, J = 7.2 Hz, 1H), 4.50 (dd, J = 26.5, 15.8 Hz, 5H), 4.43−4.32 (m, 4H), 4.27 (d, J = 11.9 Hz, 1H), 4.24−4.16 (m, 3H), 4.08 (dd, J = 11.4, 7.6 Hz, 2H), 3.99 (dt, J = 27.2, 8.3 Hz, 4H), 3.82 (d, J = 10.3 Hz, 2H), 3.79−3.73 (m, 2H), 3.69−3.63 (m, 1H), 3.58 (d, J = 10.4 Hz, 3H), 3.47−3.23 (m, 9H), 3.20 (d, J = 5.1 Hz, 2H), 3.07 (d, J = 7.7 Hz, 1H), 2.88 (s, 2H), 2.82 (d, J = 4.0 Hz, 1H), 2.77−2.68 (m, 2H), 1.49−1.36 (m, 2H), 1.29 (dd, J = 19.3, 8.5 Hz, 2H), 1.13 (dd, J = 13.8, 7.2 Hz, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 170.3 (CO_2Me), 167.3 (Bz), 167.1 (Bz), 166.9 (Bz), 166.45 (Bz), 166.41 (Bz), 164.0 (NHTCA), 158.7 (Cbz), 140.3, 140.2, 139.9, 139.45, 139.41, 139.39,

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877

139.25, 139.23, 138.4, 134.6, 134.4, 134.0, 133.5, 131.5, 131.3, 131.13, 131.10, 131.04, 130.99, 130.8, 130.7, 130.6, 130.5, 129.9, 129.8, 129.7, 129.70, 129.6, 129.6, 129.5, 129.43, 129.41, 129.33, 129.29, 129.26, 129.19, 129.17, 129.1, 129.0, 128.94, 128.91, 128.89, 128.82, 128.76, 128.74, 128.72, 128.62, 128.59, 128.3, 128.1 (Ar), 103.3 (C-1), 102.5 (C-1), 102.2 (C-1), 101.9 (C-1), 101.8 (C-1), 93.6 (CCl_3), 81.2, 81.0, 80.3, 80.1, 79.2, 77.7, 77.6, 76.1, 76.0, 75.9, 75.8, 75.8, 75.5, 75.4, 75.2, 75.1, 74.5, 74.4, 74.3, 74.1, 74.0, 73.9, 73.5, 73.5, 73.0, 70.8, 69.9, 69.2, 68.9, 68.5, 67.3, 64.9, 58.7, 53.2, 47.9, 44.7, 41.5, 30.2, 30.1, 30.0, 24.0. HRMS (ESI-TOF): m/z $[M + 2Na]^{2+}$ calcd for $C_{137}H_{137}N_2O_{37}Cl_3SNa_2$ 1292.3735, found 1292.3728.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00554.

^1H, ^{13}C, and 2D NMR spectra of novel compounds, HPLC chromatograms, and tables (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: peter.seeberger@mpikg.mpg.de.

Present Addresses

†H.S.H. and P.H.S.: Institute of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany.

#H.S.H.: Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904-4319, United States.

‡C.-F.L.: Department of Chemistry, National Chung Hsing University, 145 Xingda Road, South District, Taichung City 402, Taiwan.

§C.-H.L.: Genomic Research Center, Academia Sinica, 128, Section 2, Academia Road, Taipei 115, Taiwan.

Notes

The authors declare the following competing financial interest(s): P.H.S. declares a competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the Max-Planck Society and an ERC Advanced Grant (AUTOHEPARIN to P.H.S.).

■ REFERENCES

(1) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem. Biol.* **2007**, *2*, 685−691.

(2) (a) Hakomori, S. *Biochim. Biophys. Acta, Gen. Subj.* **2008**, *1780*, 325−346. (b) Zhang, X.; Kiechle, F. L. *Ann. Clin. Lab. Sci.* **2004**, *34*, 3−13.

(3) Hakomori, S.; Zhang, Y. *Chem. Biol.* **1997**, *4*, 97−104.

(4) Berenson, C. S.; Sayles, K. B.; Huang, J.; Reinhold, V. N.; Garlipp, M. A.; Yohe, H. C. *FEMS Immunol. Med. Microbiol.* **2005**, *45*, 171−182.

(5) Fantini, J.; Hammache, D.; Pieroni, G.; Yahi, N. *Glycoconjugate J.* **2000**, *17*, 199−204.

(6) (a) Wilson, R. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 14462−14472. (b) Danishefsky, S. J.; Shue, Y.-K.; Chang, M. N.; Wong, C.-H. *Acc. Chem. Res.* **2015**, *48*, 643−652.

(7) (a) Astronomo, R. D.; Burton, D. R. *Nat. Rev. Drug Discovery* **2010**, *9*, 308−324. (b) Fuster, M. M.; Esko, J. D. *Nat. Rev. Cancer* **2005**, *5*, 526−542.

(8) Kleene, R.; Schachner, M. *Nat. Rev. Neurosci.* **2004**, *5*, 195−208.

(9) Hughes, R. A. C.; Cornblath, D. R. *Lancet* **2005**, *366*, 1653−1666.

(10) (a) Lemichez, E.; Lecuit, M.; Nassif, X.; Bourdoulous, S. *Nat. Rev. Microbiol.* **2010**, *8*, 93−104. (b) Lindberg, A. A.; Brown, J. E.; Stromberg, N.; Westling-Ryd, M.; Schultz, J. E.; Karlsson, K. A. *J. Biol. Chem.* **1987**, *262*, 1779−1785. (c) Puri, A.; Hug, P.; Jernigan, K.; Barchi, J.; Kim, H. Y.; Hamilton, J.; Wiels, J.; Murray, G. J.; Brady, R. O.; Blumenthal, R. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 14435−14440.

(11) (a) Fryer, J. P.; Leventhal, J. R.; Matas, A. *Transplant Immunol.* **1995**, *3*, 21−31. (b) Cascalho, M. J.; Platt, L. *Nat. Rev. Immunol.* **2001**, *1*, 154−160.

(12) Hancock, S. M.; Vaughan, M. D.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2006**, *10*, 509−519.

(13) Werz, D. B.; Chevolot, Y. *Methods Mol. Biol.* **2012**, *808*, 13−29.

(14) (a) Burkhart, F.; Zhang, Z.; Wacowich-Sgarbi, S.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2001**, *40*, 1274−1277. (b) Huang, C.-Y.; Thayer, D. A.; Chang, A. Y.; Best, M. D.; Hoffmann, J.; Head, S.; Wong, C.-H. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15−20.

(15) Seeberger, P. H. *Acc. Chem. Res.* **2015**, *48*, 1450−1463.

(16) For selected examples of the automated glycan assembly using the olefinic linker, see: (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523−1527. (b) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 602−605. (c) Werz, D. B.; Castagner, B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2007**, *129*, 2770−2771. For selected examples of the automated glycan assembly using the bifunctional linker, see: (d) Kröck, L.; Esposito, D.; Castagner, B.; Wang, C.-C.; Bindschadler, P.; Seeberger, P. H. *Chem. Sci.* **2012**, *3*, 1617−1622. (e) Esposito, D.; Hurevich, M.; Castagner, B.; Wang, C.-C.; Seeberger, P. H. *Beilstein J. Org. Chem.* **2012**, *8*, 1601−1609.

(17) For selected examples of the automated glycan assembly using the photolabile linker, see: (a) Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2013**, *52*, 5858−5861. (b) Hofmann, J.; Hahm, H. S.; Seeberger, P. H.; Pagel, K. *Nature* **2015**, *526*, 241−244. (c) Bartetzko, M. P.; Schuhmacher, F.; Hahm, H. S.; Seeberger, P. H.; Pfrengle, F. *Org. Lett.* **2015**, *17*, 4344−4347. (d) Fair, R. J.; Hahm, H. S.; Seeberger, P. H. *Chem. Commun.* **2015**, *52*, 6183−6185.

(18) (a) Love, K. R.; Seeberger, P. H. *J. Org. Chem.* **2005**, *70*, 3168−3177. (b) Love, K. R. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA, 2004.

(19) (a) Hsu, Y.; Lu, X.-A.; Zulueta, M. M. L.; Tsai, C.-M.; Lin, K.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **2012**, *134*, 4549−4552. (b) Jennum, C. A.; Fenger, T. H.; Bruun, L. M.; Madsen, R. *Eur. J. Org. Chem.* **2014**, *66*, 3232−3241. (c) Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 8165−8176.

(20) (a) Isogai, Y.; Kawase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1996**, *15*, 1001−1023. (b) Isogai, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1996**, *15*, 1119−1137. (c) Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43−69. (d) Kornilov, A. V.; Sherman, A. A.; Kononov, L. O.; Shashkov, A. S.; Nifant'ev, N. E. *Carbohydr. Res.* **2000**, *329*, 717−730. (e) Tsvetkov, Y. E.; Burg-Roderfeld, M.; Loers, G.; Arda, A.; Sukhova, E. V.; Khatuntseva, E. A.; Grachev, A. A.; Chizhov, A. O.; Siebert, H.-C.; Schachner, M.; Jimenez-Barbero, J.; Nifantiev, N. E. *J. Am. Chem. Soc.* **2012**, *134*, 426−435.

(21) (a) Zhu, T.; Boons, G.-J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 857−861. (b) Gege, C.; Kinzy, W.; Schmidt, R. R. *Carbohydr. Res.* **2000**, *328*, 459−466. (c) Wang, Y.; Huang, X.; Zhang, L.-H.; Ye, X.-S. *Org. Lett.* **2004**, *6*, 4415−4417.

(22) Calin, O.; Eller, S.; Hahm, H. S.; Seeberger, P. H. *Chem. - Eur. J.* **2013**, *19*, 3995−4002.

(23) Kiyoi, T.; Nakai, Y.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1167−1176.

(24) (a) Gerbst, A. G.; Ustuzhanina, N. E.; Grachev, A. A.; Khatuntseva, E. A.; Tsvetkov, D. E.; Whitfield, D.; Berces, A.; Nifantiev, N. E. *J. Carbohydr. Chem.* **2001**, *20*, 821−831. (b) Nigudkar, S. S.; Demchenko, A. V. *Chem. Sci.* **2015**, *6*, 2687−2704. (c) Lee, j.-C.; Greenberg, W. A.; Wong, C.-H. *Nat. Protoc.* **2006**, *6*, 3143−3152.

(25) Spijker, N. M.; van Boeckel, C. A. A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 180−183.

(26) Thijssen, M.-J. L.; Halkes, K. M.; Kamerling, J. P.; Vliegenthart, J. K. G. *Bioorg. Med. Chem.* **1994**, *2*, 1309−1318.

(27) Weishaupt, M. W.; Matthies, S.; Hurevich, M.; Pereira, C. L.; Hahm, H. S.; Seeberger, P. H. *Beilstein J. Org. Chem.* **2016**, *12*, accepted.

DOI: 10.1021/acs.joc.6b00554
J. Org. Chem. 2016, 81, 5866−5877