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A Synthetic Carbohydrate–Protein Conjugate Vaccine Candidate against *Klebsiella pneumoniae* Serotype K2

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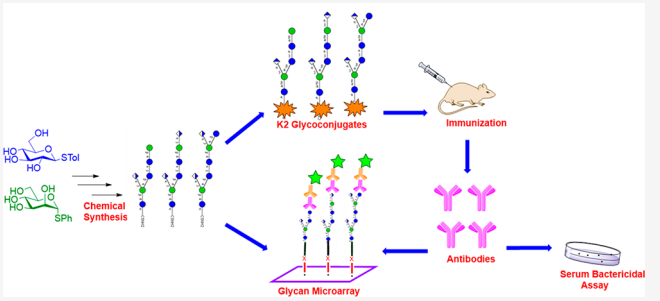
ABSTRACT: *Klebsiella pneumoniae* causes pneumonia and liver abscesses in humans worldwide and contains virulence factor capsular polysaccharides and lipopolysaccharides linked to the cell wall. Although capsular polysaccharides are good antigens for vaccine production and capsular oligosaccharides conjugate vaccines are proven effective against infections caused by encapsulated pathogens, there is still no *Klebsiella pneumoniae* vaccine available. One obstacle is that the capsular polysaccharide of a dominated *Klebsiella pneumoniae* serotype K2 is difficult to synthesize chemically due to the three 1,2-*cis* linkages in its structure. In this study, we successfully synthesized K2 capsular polysaccharides from tetra- to octasaccharides in highly a stereoselective manner. Subsequently, three synthesized glycans were conjugated to DT protein to provide glycoconjugate vaccine candidates (DT-Hexa, DT-Hepta, and DT-Octa) that were used in *in vivo* immunization experiments in mice. The results of immunized studies showed all three glycoconjugates elicited antibodies that recognized all of the synthetic glycans at 1:200-fold dilution. Particularly, the DT-Hepta conjugate elicited a higher level of antibodies that can recognize longer glycan (octasaccharide) even at 1:12800-fold dilution and exhibited good bactericidal activity. Our results concluded that heptasaccharide is the minimal epitope and a potential candidate for the vaccine against the K2 sero group of *Klebsiella pneumoniae*.



INTRODUCTION

Klebsiella pneumoniae (KP) is an opportunistic, rod-shaped, Gram-negative bacterium, belonging to the *Enterobacteriaceae* family,[1] and causes both hospital-acquired and community-acquired infections such as urinary tract infections, wound infections, and bloodstream infections in addition to pneumonia and meningitis, particularly in young children and immunodeficient adults.[2,3] More than 50% of reported pneumonia deaths were caused by KP.[4] Moreover, KP was ranked second among all pathogens that cause a pyogenic liver abscess (PLA) in humans.[5,6] Initially, KP was geographically restricted to some Asian countries, and the first liver abscess case caused by KP was reported in the 1980s in Taiwan.[7,8] In recent days, KP-associated PLA cases have been rapidly increasing globally and is now considered as an endemic in North America, Europe, and some Asian countries and regions including Taiwan, Singapore, Korea, Hong Kong, China, and Vietnam.[9−11] Of the 78 serotypes of KP identified so far, K1 and K2 serotypes are reported as the most virulent and major contributors for PLA disease.[12,13] In general, members of the genus *Klebsiella* express capsular polysaccharide (CPS, K-antigen) and lipopolysaccharide (LPS, O-antigen) components on their cell surface, and both CPS and LPS have virulent factors that contribute to the pathogenicity in humans.[14,15]

The structure of the K2 polysaccharide repeating unit was characterized by Corsaro et al. in 2005 to be →3)-*β*-D-Glc*p*-(1→4)-*β*-D-Man*p*-(1→4)-*α*-D-Glc*p*-(1→ as the backbone with a chain carrying *α*-D-Glc*p*A-(1→ branches at the 3 position of the mannoses[16] (Figure 1). Notably, the K2 serotype does not contain repeating sequences of D-mannose-*α*-2/3-D-mannose or L-rhamnose-*α*-2/3-rhamnose units that are commonly found in CPS structures of *Klebsiella* strains (K7, K14, K21, K24,

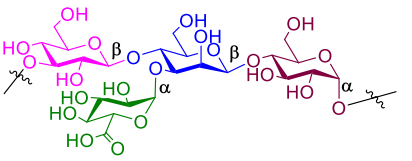


Figure 1. Structure of the tetrasaccharide repeating unit (K2 polysaccharide).

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K28, K74, K80, and others) and easily recognized by surface lectin of macrophages to enable lectinophagocytosis. The absence of these two sequences may be the cause for the more virulent nature of K1 and K2 serotypes.[1,17]

In developing countries, clinical diagnosis and treatment of *Klebsiella* remain challenging and cause an economic burden. Generally, various combinations of drugs with a prolonged duration are needed to treat the bacterial infections caused by *Klebsiella*.[18] Due to the emergence of hypervirulent and multidrug-resistant KP strains,[19,20] a safer and more effective alternate approach is urgently needed to treat or prevent the infection. Over the last three decades, numerous efforts have focused on *Klebsiella* vaccine development by conducting active and passive immunization trials; however, none has successfully brought the vaccine into the global market so far.[21−23] Most of the clinical trials were executed on LPS-based vaccines, which exhibited lots of issues during the active immunization trials due to the presence of the endotoxic Lipid A component in LPS.[21,24]

In recent days, CPS-based glycoconjugate vaccines have become very attractive alternatives due to a better immune response, superior safety, and more success in preventing infectious diseases caused by *Haemophilus influenzae*, *Streptococcus pneumonia*, and *Neisseria meningitides*.[25−28] In order to develop an effective glycoconjugate vaccine against *Klebsiella*, it is essential to identify the minimal, suitable length of the saccharide that exhibits immunogenic nature. Although oligosaccharide fragments can be isolated from natural sources, the isolated products are often heterogeneous and may include bacterial contaminants. Thus, chemical synthesis, which provides pure polysaccharides with a defined length and preinstalled linker required for conjugation, is a preferable approach to obtain oligosaccharide fragments for vaccine development.[29] To our knowledge, only one report exists on the synthetic CPS repeating unit of the K2 serotype, and the study was limited to the synthesis of the tetrasaccharide unit in the form of methyl ester methyl glycoside,[30] which may not be suitable for further vaccination studies. Therefore, an alternative synthetic strategy to prepare the desired compound is required. In our continuing effort toward the development of synthetic glycoconjugate-based vaccines,[31] here we described the chemical synthesis of the K2 antigen repeating unit and its derivatives. We, then, coupled the synthetic products to carrier proteins to study their immunological properties.

All of the target antigens, **1**−**5**, were designed to have a spacer aminopentyl group at the reducing end for further conjugation to the carrier protein for immunological studies (Figure 2). First, we constructed tetrasaccharide **1** and, then, elongated the sugar chain via the [1+4], [2+4], [3+4], and [4+4] glycosylation strategy to get **2**−**5** compounds, respectively.

RESULTS AND DISCUSSION

Part 1: Chemical Synthesis. *Retrosynthetic Analysis.* The chemical synthesis of the repeating CPS core tetrasaccharide of KP is very challenging because the tetrasaccharide unit contains three 1,2-*cis* glycosylation bonds in their structure. The installation of glycosides in a stereoselective fashion at the desired position is a difficult task, particularly at β-mannosylation and α-glucoronic acid attachments, often resulting in the formation of an inseparable mixture of anomers. We designed the retrosynthetic plan (Scheme 1) to obtain the desired molecules **2**−**5** by

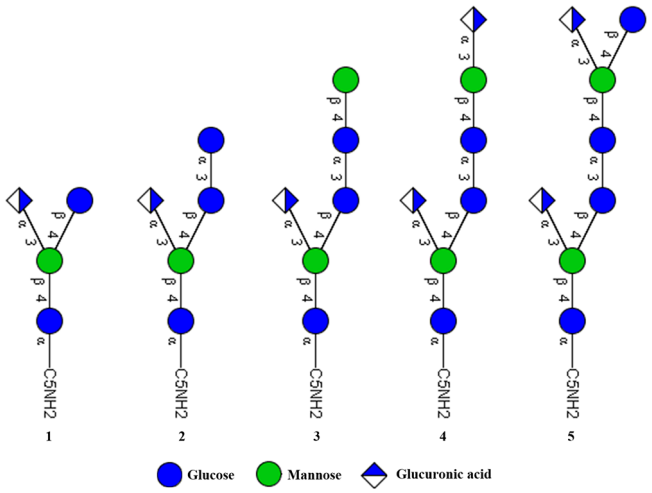

Figure 2. Targeted K2 antigens for the synthesis and immunological study.

glycosylation of common tetrasaccharide acceptor **7** with corresponding glycosyl fluorides **13**, **14**, **15**, and **16** via the [1+4], [2 + 4], [3+4], and [4+4] glycosylation strategy, respectively, and, then, global deprotection. On the other hand, target compound **1** was obtained by complete deprotection of **6**. The tetrasaccharides **6** was obtained by stereoselective β-glycosylation of donor **9** with acceptor **8**, and trisaccharide **8** is assembled via β-mannosylation followed by selective α-glucosylation on mannose **11** with corresponding glucose building blocks **12** and **10**, respectively. Our synthetic design is to protect the 6-*O*-position in **10** with the TBDPS group, which is bulky and can be selectively removable in the presence of other functional groups, to accomplish stereoselective 1,2-*cis* glycosylation. Moreover, the PMB group is used as a temporary protection group at the 3-*O*-position in **9** and **11**.

Synthesis of the Trisaccharide Acceptor. The synthesis of target K2 antigens began with the preparation of required monosaccharide building blocks **9**−**12**. Among them, **9**−**11** were prepared based on the reported procedures with modifications[32,33] (Schemes S3−S5), whereas building block **12** was prepared using a new synthetic strategy (Scheme S6). Then, we focused on the synthesis of the trisaccharide linker compound **8** by either β-mannosylation at C-1, followed by α-glucosylation at C-3 of mannose, or vice versa. We chose the first strategy because the β-mannosylation is favored when the aromatic groups (Bn, PMB) are at the C-3 position of mannose; then, bulky groups such as sugar moieties at C-3 will give poor selectivity.[34] Donor **11** was glycosylated with acceptor **12** using Prof. Crich's reaction conditions,[35] in which BSP and TTBP were used as promoters to provide desired disaccharide **17a** in high anomeric selectivity (**17a**/**17b**, β/α = 12:1) with a 62% yield. The compounds were readily separable by silica gel chromatography. The stereochemistry of the newly formed glycosidic bond was confirmed by their anomeric C−H coupling constants (β-anomer $^1J_{C-H}$ = 156.5 Hz; α-anomer $^1J_{C-H}$ = 172 Hz).

Next, the PMB ether of **17a** was removed by DDQ treatment, and the resulted alcohol **18** was treated with donor **10** in the presence of the NIS−TfOH system. Amazingly, this reaction afforded the desired trisaccharide **19** in only the α-anomer with a good yield of 84%. The glycosidic linkage was confirmed to be α-linkage by its coupling constant values ($^3J_{HH}$ = 3.5 Hz; $^1J_{C-H}$ = 172.4 Hz). At this juncture, it became

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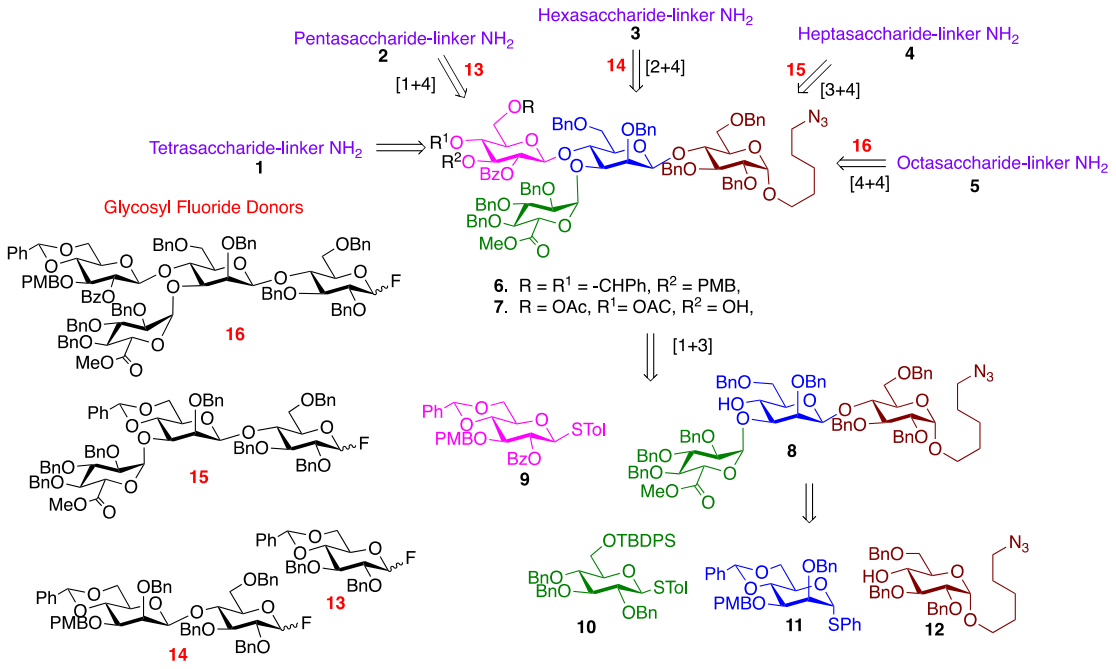
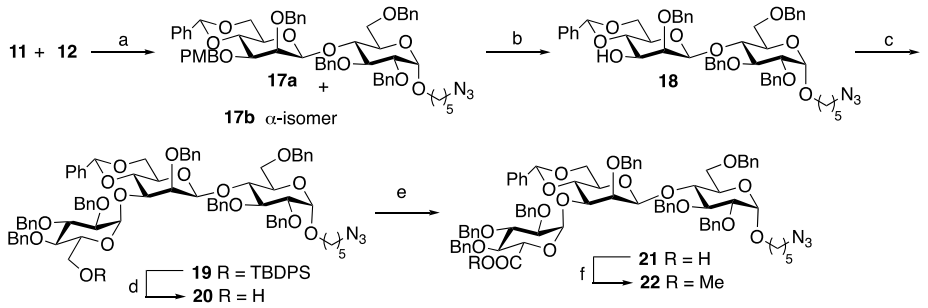
Scheme 1. Retrosynthetic Analysis of CPS Core Oligosaccharides 1−5

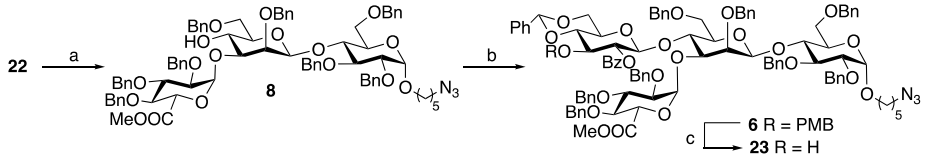


Scheme 2. Synthesis of Trisaccharide 22[a]



[a]Reagents and conditions: (a) BSP, TTBP, Tf_2O, CH_2Cl_2, 4 Å MS, −60 °C, 1.5 h, 62% (α/β = 1:12); (b) DDQ, CH_2Cl_2/phosphate buffer pH 7 (9:1), 0 °C to rt, 2 h, 81%; (c) **10**, NIS, TfOH (0.5 M), 4 Å MS, CH_2Cl_2, −40 °C, 2 h, 84%; (d) HF·py, THF/pyridine (9:1), 0 °C to rt, overnight, 91%; (e) BAIB, TEMPO, CH_2Cl_2/H_2O, rt, 2.5 h, 92%; (f) MeI, K_2CO_3, CH_2Cl_2, rt, overnight, 92%.

Scheme 3. Synthesis of Tetrasaccharide Acceptor 23[a]



[a]Reagents and conditions: (a) Et_3SiH, TfOH, CH_2Cl_2, 4 Å MS, −78 °C, 1 h, 94%; (b) **9**, NIS, TfOH, CH_2Cl_2, 4 Å MS, −20 °C, 1 h, 92%; (c) DDQ, CH_2Cl_2/phosphate buffer, pH 7 (9:1), 0 °C, 1.5 h. 72%.

necessary to free compound **19** from the TBDPS group for further manipulations. Hence, **19** was treated with HF·py to afford alcohol **20** in a 91% yield. The primary alcohol functionality of **20** was converted into the corresponding carboxylic acid moiety through 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) free radical-mediated oxidation using bis(acetoxy)iodo benzene (BAIB) as a co-oxidant to obtain **21**, which was methylated with MeI and K_2CO_3 to afford the trisaccharide ester **22** in a good yield (Scheme 2).

Synthesis of the Tetrasaccharide Acceptor. After the successful synthesis of trisaccharide **22**, we focused on the preparation of tetrasaccharide **6** (Scheme 3) by regioselectively opening the ring of the benzylidene group (**22**) in the Et_3SiH−TfOH system to provide the secondary alcohol **8** that serves as an acceptor for further reaction. Next, we examined the coupling of acceptor **8** and donor **9** in the NIS−TfOH system. Delightfully, we achieved the desired compound **6** in only the β-anomer with a good yield of 92%. The glycosidic linkage was confirmed to be β-bonds based on its anomeric C−H coupling

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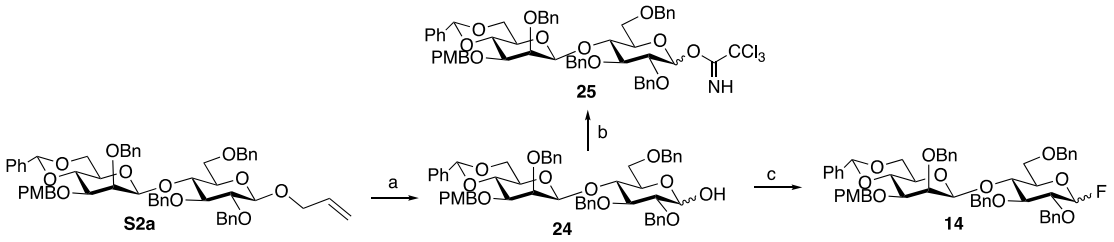
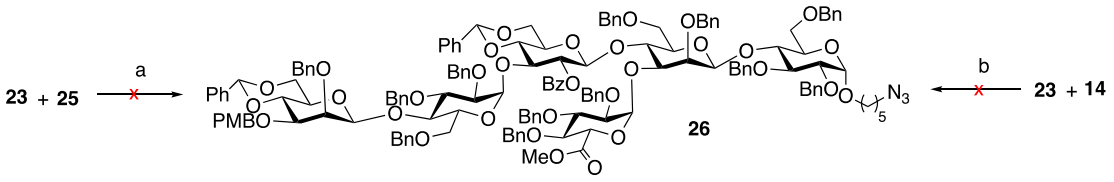
Scheme 4. Synthesis of Disaccharide Donors 14 and 25[a]



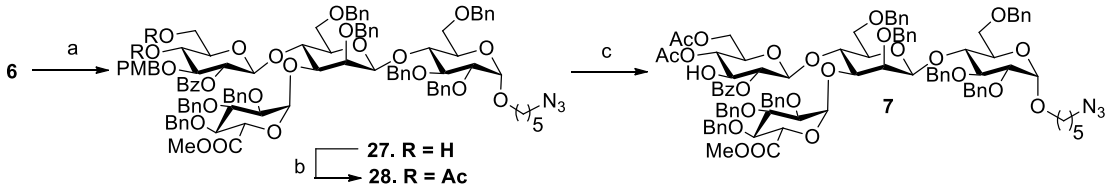
[a]Reagents and conditions: (a) $PdCl_2$, CH_3COONa, acetic acid (moist), rt, 24 h, 70%; (b) CCl_3CN, DBU, CH_2Cl_2, 0 °C to rt, 2 h, 65%; (c) DAST, CH_2Cl_2, −20 °C, 1.5 h, 92%.

Scheme 5. Attempted Synthesis of Hexasaccharide 26[a]



[a]Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å MS, −30 to −20 to −10 to 0 °C to rt; (b) Cp_2HfCl_2, AgOTf, Et_2O, 4 Å MS, −20 to 0 °C.

Scheme 6. Manipulation and Synthesis of Tetrasaccharide Acceptor 7[a]



[a]Reagents and conditions: (a) PTSA, MeOH/CH_2Cl_2 (1:1), rt, overnight, 87%; (b) Ac_2O, pyridine, 0 °C to rt, 4 h, 99%; (c) DDQ, CH_2Cl_2/phosphate buffer pH 7 (9:1), 0 °C to rt, 2 h, 48%; or $SnCl_4$, PhSH, −78 to −50 °C, 1 h, 93%.

constant ($^1J_{CH}$ = 165 Hz). Finally, compound **6** was converted into corresponding acceptor **23** by DDQ oxidation to remove 3-*O*-PMB ether. The same approach and reaction conditions were used to prepare other structural units (**S2a**, **S7**, and **S9**) that have an *O*-allyl group at the reducing end instead of the 5-azidopentyl linker, and these compounds were used as a donor source for chain extension (Scheme S1). During its synthesis, we observed that all of the reactions gave good yields with high anomeric selectivity at each glycosylation step.

Synthesis of Disaccharide Donor. With the tetrasaccharide acceptor in hand, we turned our attention to chain elongation, which is a challenging task because it requires selective α-glycosidic bond linkage. In general, an increase in the length of the donor or acceptor chain affects its reactivity and anomeric selectivity. Therefore, a study of optimization to access higher α-selectivity is highly beneficial. In this regard, we conducted a model study on hexasaccharide construction through [2+4] glycosylation. Disaccharide imidate donor **25** was prepared from **S2a** via hydrolysis of the anomeric *O*-allyl group by the treatment of $PdCl_2$ and AcONa in AcOH,[36] followed by CCl_3CN and DBU (Scheme 4). Then, donor **25** and acceptor **23** were subjected to [2+4] glycosylation using TMSOTf at various temperatures (—30/−10/0 °C and rt). Unfortunately, none has offered satisfactory results due to the decomposition of the donor (Scheme 5). Alternatively, we prepared glycosyl fluoride donor **14** from hemiacetal **24** by the treatment of DAST (Scheme 4). The resulted glycosyl fluoride, **14**, was treated with the same acceptor, **23**, using the Cp_2HfCl_2−AgOTf system[37] at various temperatures. Disappointingly, this reaction also did not proceed, and a slight decomposition of the glycosyl donor was observed (Scheme 5).

e. Synthesis of a New Tetrasaccharide Acceptor. After additional unsuccessful experiments, we suspected the problem came from the structure of acceptor **23**, in which the presence of the benzylidene ring might cause steric hindrance to the hydroxyl group. To increase reactivity and decrease the steric hindrance, we modified acceptor **23** to remove the benzylidene group and install acetyl groups at 4- and 6-OH by treating **6** with *p*-toluenesulfonic acid (PTSA) to produce diol **27**, which was later masked with acetyl groups by employing standard conditions to give **28**. The PMB ether was removed by oxidation with DDQ in a mixture of CH_2Cl_2 and neutral phosphate buffer solution, and this reaction condition afforded compound **7** in a 48% yield; however, a better yield (93%) was obtained when compound **28** was treated with $SnCl_4$ and thiophenol[38] (Scheme 6).

Synthesis of K2 Penta to Octa Oligosaccharides. With acceptor **7** in hand, we focused on screening for suitable glycosylation conditions for the synthesis of hexasaccharide via the [2+4] strategy. Glycosylation of imidate donor **25** with acceptor **7** using catalytic TMSOTf resulted in the formation of several products, which were not separable by column chromatography. Alternatively, we used the Cp_2HfCl_2−AgOTf system in toluene for glycosylation of glycosyl fluoride **14** with

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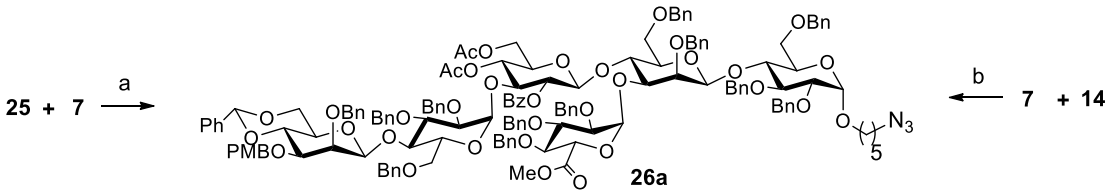
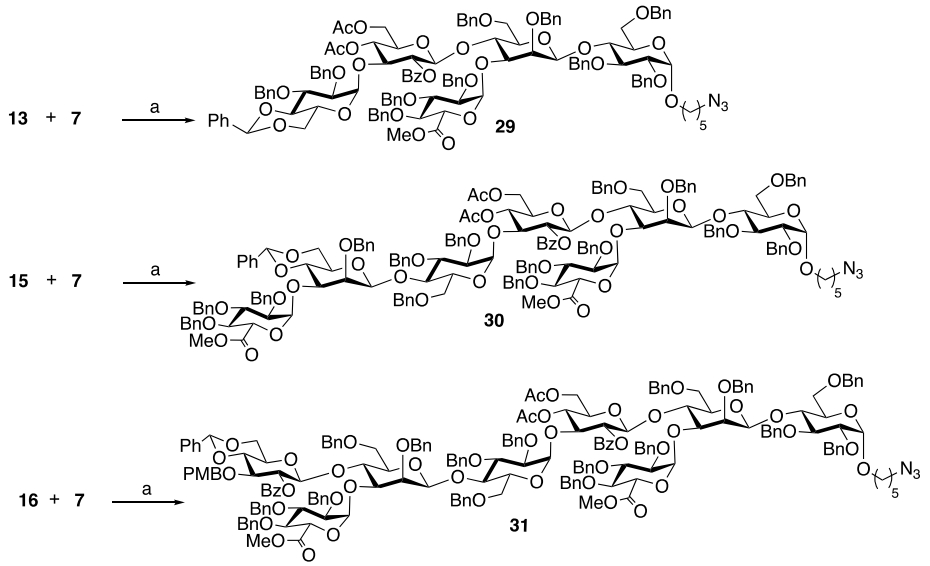
Scheme 7. Synthesis of Hexasaccharide 26a[a]



[a]Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å MS, −40 °C, 30 min; (b) Cp_2HfCl_2 (4 equiv), AgOTf (8 equiv), toluene, 4 Å MS, −30 °C, 1.5 h, 64%.

Scheme 8. Synthesis of Penta-, Hepta-, and Octasaccharides 29−31[a]



[a]Reagents and conditions: (a) Cp_2HfCl_2, AgOTf, toluene, 4 Å MS, −30 °C, 90 min, **29** (70%), **30** (68%), and **31** (70%).

acceptor **7** and found this reaction afforded fully protected hexasaccharide **26** in only the α-isomer in a modest yield (23%), which upon optimization was increased to 64% yield (Scheme 7). The glycosidic bond in **26a** was determined to be α-linkage based on its anomeric C−H coupling constant value (α-anomer $^1J_{C-H}$ = 172.6 Hz). After several attempts, we found that 4 equiv of Cp_2HfCl_2 and 8 equiv of AgOTf in toluene solvent at −30 °C are the optimal conditions for glycosylation of **14** with acceptor **7**. Meanwhile, we have also screened this glycosylation reaction using several promotors such as TfOH, Tf_2O, $BF_3(OEt)_2$, $SnCl_2$−$AgClO_4$, and Cp_2HfCl_2_AgOTf in various mole ratios and solvent systems. All conditions either led to a low yield of the desired compound or decomposition of the donor (Table ST 1).

In order to identify the optimal length of the K2 antigen for vaccine design, we prepared the remaining targeted penta-, hepta-, and octasaccharides to complete the sequence for the study. The required glycosyl fluorides, **13**, **15**, and **16**, were prepared (Scheme S2) and coupled with acceptor **7** individually through the [1+4], [3+4], and [4+4] glycosylation strategy using the optimized glycosylation conditions to afford **29**−**31** in 68−70% yields (Scheme 8). The glycosidic bonds in **29**−**31** were determined to be α-linkage based on anomeric C−H coupling constant values through 2D NMR analysis.

Finally, the synthesized oligosaccharides **6**, **26a**, and **29**−**31** were subjected to global deprotection through a two-step procedure (Scheme 9). The first saponification of all esters was carried out by the treatment of LiOH in a mixture of *p*-dioxane and water. Then, Pd/C-catalyzed hydrogenolysis was carried out in a mixture of MeOH, THF, and water with a catalytic amount of formic acid; thus, all protecting groups were removed, and the azido group was reduced to the amine group in one pot to afford antigens **1**−**5** in 39−60% yields. All synthetic antigens, **1**−**5**, were well characterized by 1D and 2D NMR and HRMS spectroscopic analysis (Supporting Information). Additionally, we compared the ^{1}H NMR spectrum of the synthetic tetrasaccharide **1** with the reported ^{1}H NMR spectrum of the isolated tetrasaccharide fragment and found both spectra are identical at all chemical shifts except in the linker region.[16]

Part 2: Preparation and Characterization of Glycoconjugates. In order to study the immunological properties of the synthesized oligosaccharides **1**−**5**, the synthetic compounds need to be coupled with the carrier protein at the spacer arm because oligosaccharides are nonimmunogenic by themselves. It should be noted that molecular weight is an important factor for CPS-based vaccine preparation. Glycoconjugates of long-chain oligosaccharides are known to be excellent immunogens in lower doses in clinical studies.[39] Short-length oligosaccharides generally do not induce antibodies, so they usually are excluded from the preparation of glycoconjugates.[40] The repeating unit of K2 polysaccharide is tetrasaccharide, which contains GlcA (Figure 1), a critical component, because the carboxyl group of this GlcA provides a

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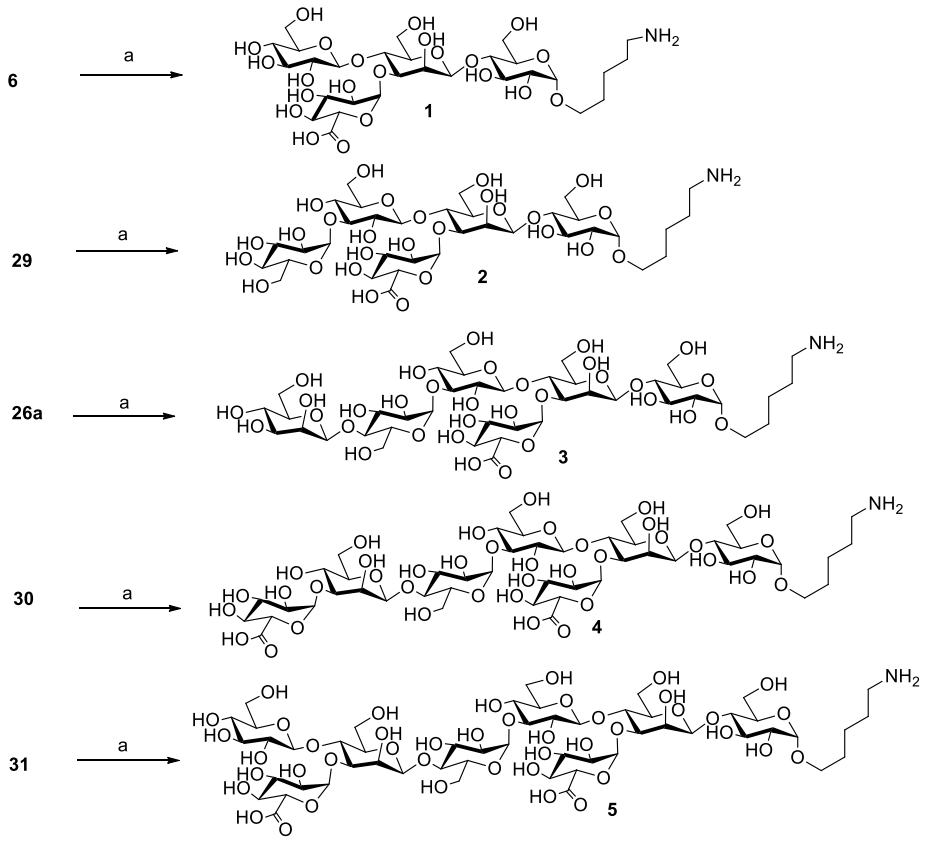
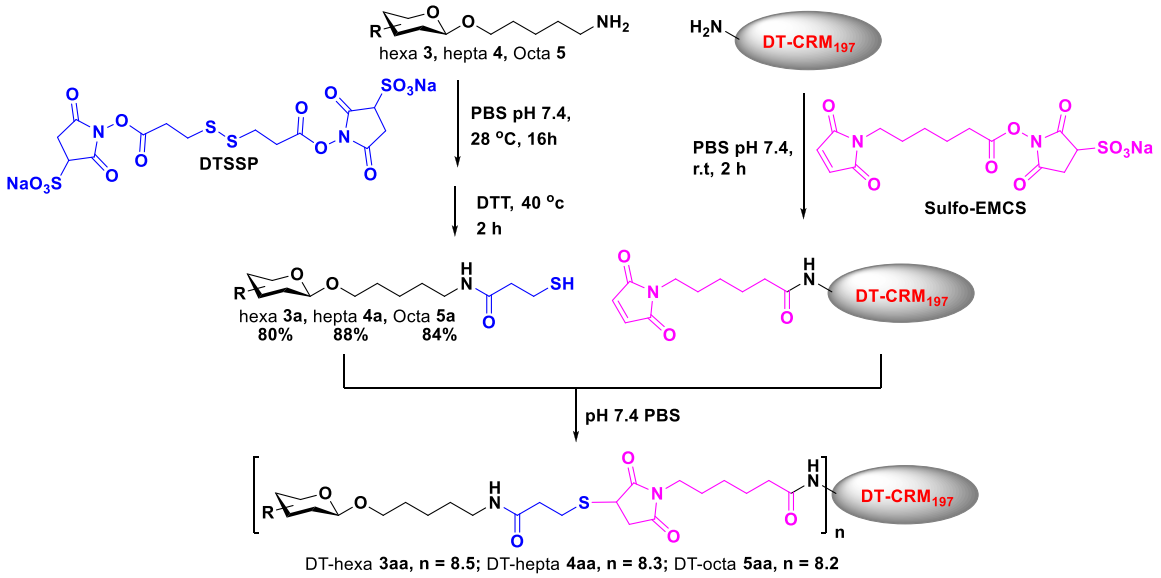
Scheme 9. Global Deprotection of Synthesized Oligosaccharides 6, 26a, and 29−31[a]



[a]Reagents and conditions: (a) (i) LiOH, *p*-dioxane/H_2O (3:1), 75 °C, overnight; (ii) $Pd(OH)_2/H_2$ (balloons), MeOH/THF/H_2O (2:1:1), HCOOH (cat.), rt, 36 h, **1** (58%), **2** (60%), **3** (39%), **4** (42%), and **5** (48%) over two steps.

Scheme 10. Oligosaccharides Conjugation to the Carrier Protein



negative charge that may lead to the change in glycan conformation. However, one GlcA in the K2 oligosaccharide does not demonstrate the charge effect. Therefore, we chose to use the hepta- and octamer with two GlcAs in order to provide a preliminary charge effect. Moreover, the glycan conformation of the hepta- or octamer is closer to the real K2 polysaccharide.

Accordingly, we selected hexa-, hepta-, and octasaccharides **3**−**5** for glycoconjugate vaccine preparation. We adopted a well-known thiol-maleimide coupling method for carbohydrate−proteins conjugation.[41] First, the amino-linker spacer was converted into an amidothiol linker by treating the selected oligosaccharides **3**−**5** with the commercially available reagent

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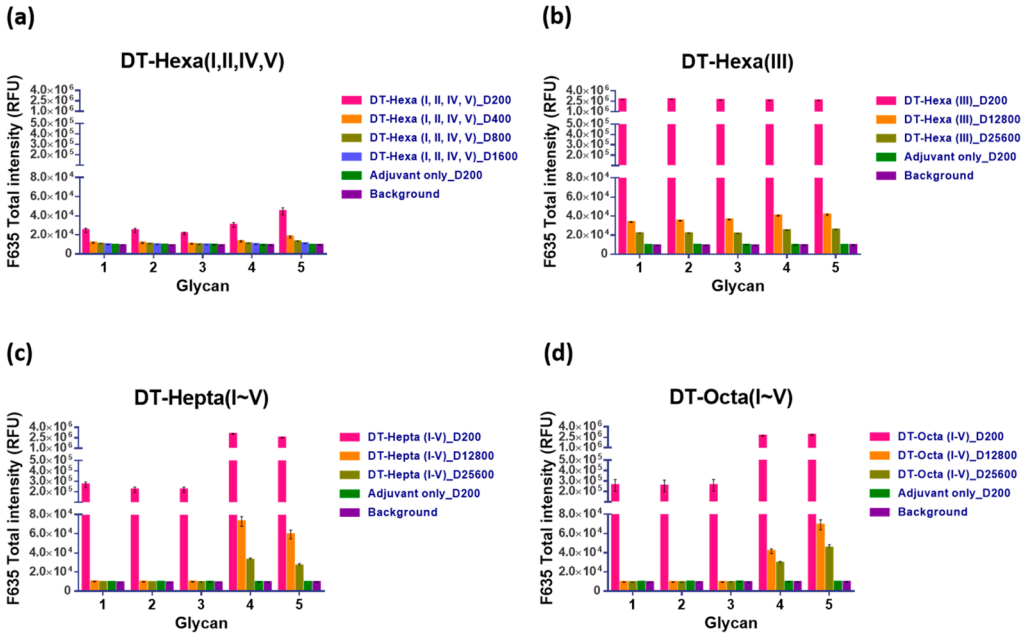

Figure 3. F635 total intensity of the synthesized oligosaccharides **1**−**5** with antibodies elicited in mice by glycoconjugates DT-Hexa **3aa**, DT-Hepta **4aa**, and DT-Octa **5aa**. (a) Binding response of serum antibodies (4 mice, no. I, II, IV, and V) elicited by DT-Hexa **3aa** to glycans **1**−**5**. (b) Binding response of serum antibodies (1 mouse, no. III) elicited by DT-Hexa **3aa** to glycans **1**−**5**. (c) Binding response of serum antibodies (5 mice) elicited by DT-Hepta **4aa** to glycans **1**−**5**. (d) Binding response of serum antibodies (5 mice) elicited by DT-Octa **5aa** to glycans **1**−**5**. RFU, relative fluorescence units. Error bars represent mean ± SD.

3,3-dithiobis(sulfosuccinimidylpropionate) (DTSSP) in PBS buffer (pH 7.4) at 28 °C overnight. The disulfide bond was then cleaved with dithiothreitol (DTT) by stirring at 40 °C for 2 h to obtain the free thiol products **3a**−**5a** in 80−88% yields after purification on a size exclusion column, LH-20 (Scheme 10).

Moreover, the maleimide group was incorporated onto the carrier protein DT (detoxified diphtheria toxin), and the number of maleimide linkers on the protein was determined by MALDI-TOF MS analysis, which revealed that 16 maleimide linkers were coupled to one DT molecule on average. Finally, the thiol-modified oligosaccharides **3a**−**5a** were conjugated to the maleimide-linked DT protein in PBS buffer (pH 7.4) individually to obtain the glycoconjugate vaccine candidates DT-Hexa **3aa**, DT-Hepta **4aa**, and DT-Octa **5aa** with various carbohydrate epitopes on the protein (Scheme 10). The resulted oligosaccharide to DT molar ratio was determined by MALDI-TOF MS spectrometry analysis (Figure SF1−3 and Table ST2).

Part 3: Immunological Studies. *Immunization of Mice.* To test whether synthetic vaccine candidates DT-Hexa **3aa**, DT-Hepta **4aa**, and DT-Octa **5aa** are sufficient epitopes for antibody recognition, we chose 6−8 week-old female BALB/c mice (n = 5) and grouped them into four groups of five mice. Mice were immunized three times with 2 μg of glycan and 2 μg of adjuvant C34[42,43] in 100 μL of PBS buffer at two week intervals. PBS buffer alone was injected into the control group. Antisera were collected one week after the third immunization, the antibody response study, and bactericidal assay.

Identification of an Oligosaccharide Vaccine Candidate by Glycan Microarray Studies. Carbohydrate antigens (glycans) **1**−**5** were immobilized through their amine linker on NHS-coated glass slides.[44] The collected mice sera were diluted to 1:200-fold, followed by 1:12800-fold and 1:25600-fold and, then, added to antigen-bonded NHS glass slides. The bound antibodies, after washing, were detected with a Cy5-labeled goat antimouse IgG secondary antibody by scanning at a 635 nm wavelength with a microarray fluorescence chip reader. The microarray results were compiled in the form of line graphs plotted by taking the mean value of the intensity on the x-axis against glycans **1**−**5** on the y-axis (Figure 3, SF4−10).

The screening results of glycans **1**−**5** with sera from mice immunized with DT-Hexa indicated (Figure 3a) very weak binding signals from four mice sera out of five at 1:200-fold dilution with respect to glycans **1**−**4** and a weak signal in glycan **5**. For the purpose of the detection study, the concentration further reduced to 1:400-, 1:800-, and 1:1600-fold, but the binding titers matches with the background at these dilutions. The most surprising and significant result was that the serum of one mouse in this group showed a high antibody titer against all glycans, **1**−**5**, at 1:200-fold dilution, and the mean intensity of all signals is around 2.5 × 10^6 (Figure 3b). Upon dilution to 1:12800- and 1:25600-fold, no significant signals were observed. Interestingly, the DT-Hexa vaccine candidate is immunogenic in one mouse but failed to induce antibodies in four mice.

The microarray results of glycans **1**−**5** against the sera collected from the group of mice immunized with DT-Hepta **4aa** (Figure 3c) revealed that all five mice responded with high antibody binding titers against glycans **1**−**3** (mean intensity around 2.5 × 10^5) and binding titers against glycans **4** and **5** were all very high (mean intensity around 3 × 10^6) at 1:200-fold dilution. Significantly, at 1:12800-fold dilution, a near-identical antibody titer was observed against glycans **4** and **5**, but no binding signal against the glycans **1**−**3** was observed. The minimal antibody titer was observed against glycans **4** and **5** even when the concentration was further reduced to 1:25600-fold. The results clearly indicated that the DT-Hepta **4aa** vaccine candidate that could induce antibodies in all

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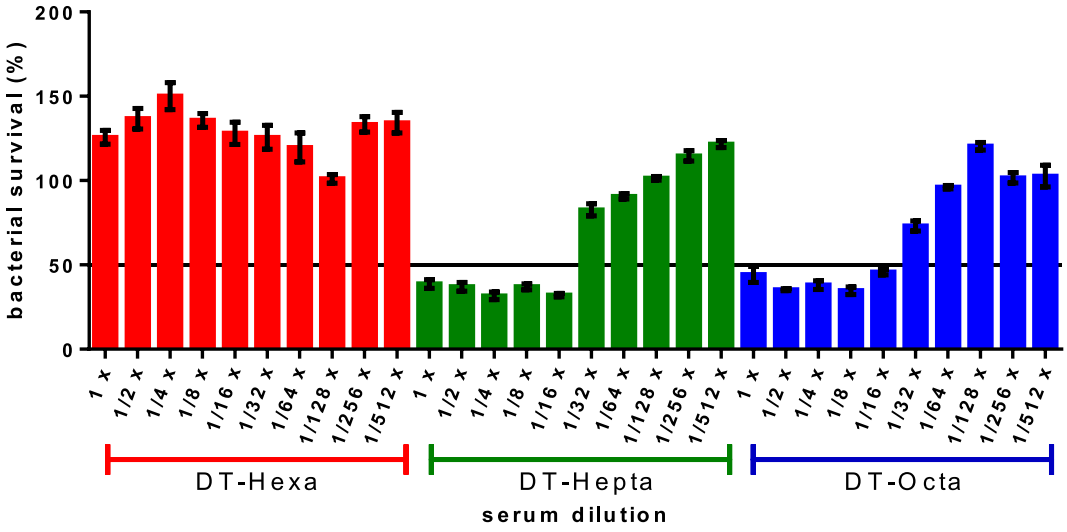

Figure 4. Serum bactericidal assay of DT-Hexa **3aa**, DT-Hepta **4aa**, and DT-Octa **5aa** glycoconjugates. Error bars represent mean ± SD.

five mice is able to recognize all glycans with different chain lengths at 1:200-fold dilutions.

We then used a microarray to study glycans **1**−**5** with sera from mice immunized with the DT-Octa **5aa** vaccine (Figure 3d). Significant antibody titers were observed against glycans **1**−**3**, and identical high-intensity antibody titers were observed in glycans **4**−**5** at 1:200-fold dilution. The observed antibody titer against glycan **5** was higher than it against glycan **4**, and no signals were observed for glycans **1**−**3** at 1:12800 dilution. When the concentration further reduced to 1:25600-fold, significant binding titers were observed for glycans **4** and **5**, and no binding signals were observed for glycans **1**−**3**. The microarray data demonstrated that the antibodies induced by DT-Hepta **4aa** and DT-Octa **5aa** vaccine candidates were able to recognize both short and long glycans. Because the antibody responded to glycans **4** and **5** but not to glycan **3**, which does not have a glucuronic acid (GlcA) moiety at the nonreducing end, we suspected that GlcA present at the nonreducing end plays an important role in binding the antigen to the IgG antibody. For economic considerations, we concluded that the DT-Hepta **4aa** glycoconjugate is a sufficient chain length for the development of vaccine candidates against the *K. pneumonia* K2 serotype.

Identification of a Suitable Glycoconjugate Vaccine Candidate by a Serum Bactericidal Assay. We further looked into the serum bactericidal assay (SBA), which represents the bactericidal ability of antibodies.[45] The principle of serum bactericidal assays is based on complement-mediated bacterial killing by bactericidal antibodies. A detail of SBA was described in the Experimental Section. SBA titers were defined as the reciprocal serum dilution at which 50% of cells are lysed, compared to the number of cells prior to incubation.[46] Interestingly, our SBA results can be correlated to the microarray results. Sera from mice immunized with DT-Hepta **4aa** showed a good bactericidal activity titer in 1/16 dilution compared to the results of DT-Octa **5aa** immunized sera, which showed a bactericidal activity titer in 1/8 dilution (Figure 4). Therefore, we concluded that DT-Hepta **4aa** is the minimal length of a glycoconjugate vaccine candidate required to kill the bacteria and a suitable epitope for the development of a vaccine candidate to prevent the diseases caused by KP.

CONCLUSION

We have described the first chemical syntheses of the K2 capsular polysaccharide of KP in various chain lengths in good yields. All target molecules, **1**−**5**, were furnished with an aminopentyl linker at the reducing end for protein conjugation. During the synthetic investigation, we attempted various approaches and glycosylation methods to formulate an optimal procedure to obtain the target molecules in a highly stereoselective manner. The α-selectivity that is the key step in chain elongation was successfully achieved using glycosyl fluorides with a tetrasaccharide acceptor by performing Cp_2HfCl_2/AgOTf-mediated glycosylation in toluene. All synthesized glycans were well characterized using NMR and mass spectroscopic analysis.

We conjugated the synthesized glycans, **3**−**5**, to carrier protein DT to provide glycoconjugate vaccine candidates (DT-Hexa **3aa**, DT-Hepta **4aa**, and DT-Octa **5aa**) and immunized mice with these vaccine candidates. Antigenicity and immunogenicity of synthetic oligosaccharides and their glycoconjugates were evaluated using glycan arrays and serum bactericidal assay studies. The immunological results demonstrated that two (**4aa** and **5aa**) of the three glycoconjugates are immunogenic and elicited antibodies that recognized all synthetic glycans at 1:200-fold dilution. The minimal immunogenic epitope of complex oligosaccharide antigens is the DT-Hepta **4aa** glycoconjugate that induced a high level of antibodies in mice, and those antibodies were able to recognize higher glycan (octasaccharide) and exhibited good bactericidal activity. Thus, heptasaccharide **4** is a promising vaccine candidate to be taken into the challenges of live animal studies. We believe that the studies reported herein will serve as a guideline for the future development of the vaccine against the K2 sero group of KP.

Experimental Section. *General Remarks.* All chemical reagents were obtained from commercial sources and used as purchased. Anhydrous dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), tetrahydrofuran (THF), *N*,*N*-dimethylformamide

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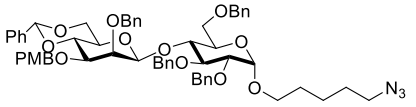
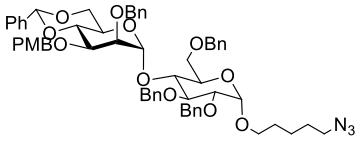
(DMF), toluene, and methanol (MeOH) were purchased from a commercial source and used without further purification. All reactions were carried out under an argon atmosphere unless mentioned otherwise, and standard syringe-septa techniques were followed. Pulverized molecular sieves (4 Å MS, Aldrich) for glycosylation were activated by heating at 350 °C for 3−4 h. Reactions were monitored by thin-layer chromatography (TLC) analysis, which was performed on glass plates precoated with Silica Gel 60 F254 (0.25 mm, Merck). The TLC was stained with either acidic *p*-anisaldehyde or ceric ammonium molybdate to be detected by UV light (254 nm). All products prior to final compounds were purified by flash chromatography with silica gel (Silicycle, 40−63 μm size, 230−400 mesh), whereas final compounds were purified by gel chromatography (LH-20 or RP-18). ^{1}H NMR, ^{13}C NMR, and 2D spectra were recorded on a Bruker AVANCE 600 (600 MHz) spectrometer at 298 K unless otherwise stated. Chemical shifts on ^{1}H NMR were assigned according to TMS (δ = 0 ppm, in $CDCl_3$) and D_2O (δ = 4.8 ppm). Chemical shift measurements are reported in δ units, and splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). Coupling constants (J) are reported in hertz (Hz). High-resolution ESI mass spectra were recorded on a Bruker Daltonics or Bruker Bio-TOF III spectrometer. MALDI-TOF spectra were recorded on a Bruker Ultraflex II spectrometer. Alexa Fluor 647-conjugated goat antimouse IgG antibody was purchased from JacksonImmunoresearch. Diphtheria toxoid cross-reactive protein material DT was purchased from Merck. NHS-coated glass slides were purchased from SCHOTT (Nexterion H). The microarray slides were scanned at a 635, 594, 532, or 488 nm wavelength with a microarray fluorescence chip reader (ArrayWorx microarray reader). The fluorescence data were analyzed by GenePix Pro software (Axon Instruments).

Chemistry Experimental Procedures.



*5-Azidopentyl-2-O-benzyl-3-O-p-methoxybenzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**17a**).* The solution of thioglycoside **11** (4.2 g, 7.36 mmol, 1 equiv), BSP (1.66 g, 8.1 mmol, 1.1 equiv), TTBP (3.66 g, 14.73 mmol, 2 equiv), and well-dried 4 Å molecular sieves (10 g) in anhydrous CH_2Cl_2 (175 mL) was stirred at rt for 10−15 min under an argon atmosphere and cooled to −60 °C. Then, Tf_2O (1.5 mL, 8.8 mmol, 1.2 equiv) was added into the mixture. After being stirred for 10−15 min at the same temperature, a solution of glycosyl acceptor **12** (3.1 g, 5.53 mmol, 0.75 equiv) in CH_2Cl_2 (30 mL) was added slowly at −60 °C. The reaction was further stirred for 90 min at the same temperature and, then, allowed to reach room temperature. After dilution with CH_2Cl_2, the mixture was filtered through a pad of Celite by repeatedly washing with CH_2Cl_2. The organic layer was then washed with satd aq $NaHCO_3$ and later by brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:4) to afford the corresponding α-anomer **17b** (272 mg) and β-anomer **17a** (3.23 g) compounds (α/β = 1:12) in viscous colorless oil compounds with a 62% yield (combined yield). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.52 (d, J = 7.3 Hz, 2H, Ar—H), 7.44−7.25 (m, 25H, Ar—H), 6.89 (d, J = 8.5 Hz, 2H, Ar—H), 5.55 (s, 1H, Ar—CH), 5.09 (d, J = 10.6 Hz, 1H, Ar—CH_aH$_b$), 4.87−4.80 (m, 4H, 2 × Ar—CH_2), 4.76 (d, J = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.71 (d, J = 11.8 Hz, 1H, Ar—CHaH_b), 4.66 (d, J = 12.4 Hz, 1H, Ar—CHaH$_b$), 4.64 (d, J = 12.4 Hz, 1H, Ar—CHaH_b), 4.57 (d, J = 11.8 Hz, 1H, Ar—CHaH$_b$), 4.45 (s, 1H, C1−H$_{\beta\text{-man}}$), 4.34 (d, J = 11.8 Hz, 1H, Ar—CHaH_b), 4.11−4.05 (m, 2H, —CH_aH$_b$, —CH_{ring}), 3.94−3.89 (m, 2H, 2 × —CH_{ring}), 3.81 (s, 3H, —OCH_3), 3.71−3.66 (m, 3H, 2 × —CH_{ring}, —CH$_a$H$_b$), 3.60−3.45 (m, 5H, —CH_{ring}, 2 × -CH$_2$), 3.38 (dd, J = 10.2, 3.1 Hz, 1H, —CH_{ring}), 3.30 (t, J = 6.7 Hz, 2H,-CH$_{2linker}$), 3.11−3.07 (m, 1H, —CH_{ring}), 1.74−1.70 (m, 2H, —CH$_{2linker}$), 1.68−1.65 (m, 2H, —CH$_{2linker}$), 1.53−1.49 (m, 2H, —CH$_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.3 (Ar—C), 139.6 (Ar—C), 138.8 (Ar—C), 138.6 (Ar—C), 137.8 (Ar—C), 137.7 (Ar—C), 130.8 (Ar—C), 129.1 (Ar—C), 128.9 (Ar—C), 128.7 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.2 (Ar—C), 128.1 × 2(Ar—C), 127.9 (Ar—C), 127.7 (Ar—C), 127.6 (Ar—C), 127.3 (Ar—C), 126.2 (Ar—C), 113.8 (Ar—C), 101.8 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.5 Hz), 101.4 (Ar—CH), 97.2 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 171.1 Hz), 80.3 (—CH_{ring}), 79.5 (—CH_{ring}), 78.8 (—CH_{ring}), 78.1 (—CH_{ring}), 77.9 (—CH_{ring}), 77.2 (—CH_{ring}), 75.2 (—CH_2), 75.0 (—CH_2), 73.7 (—CH_2), 73.6 (—CH_2), 72.4 (—CH_2), 69.9 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 68.1 (—CH_2), 67.5 (—CH_{ring}), 55.4 (—OCH_3), 51.4 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{60}H_{67}N_3O_{12}Na$, 1044.4617; found, 1044.4624.



*5-Azidopentyl 2-O-Benzyl-3-O-p-methoxybenzyl-4,6-O-benzylidene-α-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**17b**).* ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.46 (dd, J = 8.2, 1.7 Hz, 2H, Ar—H), 7.30−7.17 (m, 23H, Ar—H), 7.10 (dd, J = 7.2, 3.5 Hz, 2H, Ar—H), 6.80 (d, J = 8.7 Hz, 2H, Ar—H), 5.56 (s, 1H, Ar—CH), 5.26 (s, 1H, C1—$H_{\alpha\text{-man}}$), 5.07 (d, J = 11.2 Hz, 1H, Ar—CH_aH$_b$), 4.72 (d, J = 3.3 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.69 (d, J = 11.7 Hz, 1H, Ar—CH$_a$H$_b$), 4.63 (d, J = 12.0 Hz, 1H, Ar—CH$_a$H$_b$), 4.57−4.46 (m, 5H, 5 × Ar—CH_aH$_b$), 4.38 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.18 (d, J = 9.2 Hz, 1H, —CH_{ring}), 4.17 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.07 (dd, J = 9.9, 4.3 Hz, 1H, —CH$_a$H$_b$), 3.90 (dd, J = 9.9, 3.0 Hz, 1H, —CH_{ring}), 3.84−3.79 (m, 2H, 2 × —CH_{ring}), 3.77−3.74 (m, 6H, —OCH_3 (overlapped), 2 × —CH_{ring}, —CH$_a$H$_b$), 3.70−3.60 (m, 4H, —CH_{ring}, 3 × —CH$_a$H$_b$), 3.53 (dd, J = 9.4, 3.5 Hz, 1H, —CH_{ring}), 3.39−3.58 (m, 1H, —CH$_a$H$_b$), 3.23 (t, J = 6.8 Hz, 2H, —C$H_{2linker}$), 1.66−1.62 (m, 2H, —C$H_{2linker}$), 1.61−1.57 (m, 2H, —C$H_{2linker}$), 1.45−1.41 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.2 (Ar—C), 138.9 (Ar—C), 138.5 (Ar—C), 138.2 (Ar—C), 138.1 (Ar—C), 137.9 (Ar—C), 131.0 (Ar—C), 129.9 (Ar—C), 128.9 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.3 (Ar—C), 128.2 (Ar—C), 128.1 × 2(Ar—C), 127.8 (Ar—C), 127.7 × 2 (Ar—C), 127.6, (Ar—C), 127.5 (Ar—C), (Ar—C), 126.9 (Ar—C), 126.2 (Ar—C), 113.86 (Ar—C), 100.57 (Ar—CH), 100.53 ($C1_{\alpha\text{-man}}$, $^1J_{C-H}$ = 172.0 Hz), 96.7 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 169.0

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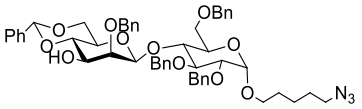
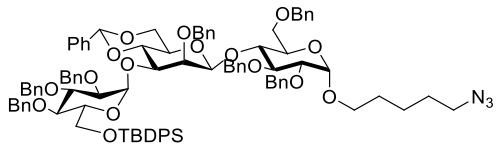
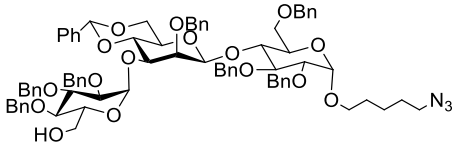
Hz), 81.6 (—CH_{ring}), 80.4 (—CH_{ring}), 79.1 (—CH_{ring}), 77.9 (—CH_{ring}), 77.8 (—CH_{ring}), 76.1 (—CH_{ring}), 75.0 (—CH_2), 73.7 (—CH_2), 73.4 (—CH_2), 73.1 (—CH_2), 72.8 (—CH_2), 69.9 (—CH_{ring}), 69.2 (—CH_2), 68.8 (—CH_2), 68.1 (—CH_2), 65.3 (—CH_{ring}), 55.4 (—OCH_3), 51.4 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (CH_2). HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{60}H_{67}N_3O_{12}Na$, 1044.4617; found, 1044.4624.



*5-Azidopentyl 2-O-Benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**18**).* DDQ (533 mg, 2.35 mmol, 1.5 equiv) was added to stirred solution of starting material **17a** (1.6 g, 1.56 mmol, 1 equiv) in a mixture of DCM/phosphate buffer pH 7 (50 mL, 9:1 = v/v) at 0 °C. The reaction mixture was vigorously stirred in the absence of light until TLC analysis indicated disappearance of the starting material (180 min). Upon completion, the reaction mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ and brine. The organic phase was washed with water until the solution became colorless; then, the organic solution was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:3) to afford pure compound **18** (1.14 g, 81%) as a viscus colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.50 (dd, J = 7.6, 1.9 Hz, 1H, Ar—*H*), 7.42−7.28 (m, 24H, Ar—*H*), 5.46 (s, 1H, Ar—C*H*), 5.07 (d, J = 10.7 Hz, 1H, Ar—CH_aH$_b$), 4.99 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.82 (s, 1H, Ar—CH_aH$_b$), 4.80 (s, 1H, Ar—CH$_a$H$_b$), 4.78 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.76 (d, J = 11.6 Hz, 1H, Ar—CH_aH$_b$), 4.66 (d, J = 12.0 Hz, 1H, Ar—CH$_a$H$_b$), 4.62 (d, J = 11.4 Hz, 1H, Ar—CH_aH$_b$), 4.51 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.42 (d, J = 12.0 Hz, 1H, Ar—CH$_a$H$_b$), 4.10 (dd, J = 10.4, 5.0 Hz, 1H, —CH_aH$_b$), 3.98 (t, J = 9.1 Hz, 1H, —CH_{ring}), 3.90 (t, J = 9.1 Hz, 1H, —CH_{ring}), 3.76−3.73 (m, 1H, —CH_{ring}), 3.71−3.66 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.65−3.59 (m, 3H, —CH_{ring}, 2 × —CH_aH$_b$), 3.58−3.56 (dd, J = 9.8, 3.1 Hz, 1H, —CH_{ring}), 3.54−3.45 (m, 3H, —CH_{ring}, 2 × -CH$_a$H$_b$), 3.28 (m, 2H, —$CH_{2linker}$), 3.07 (m, 1H, —CH_{ring}), 1.74−1.63 (m, 4H, 2 × —$CH_{2linker}$), 1.53−1.48 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.6 (Ar—*C*), 138.6 (Ar—*C*), 138.3 (Ar—*C*), 137.6 (Ar—*C*), 137.4 (Ar—*C*), 129.3 (Ar—*C*), 128.8 (Ar—*C*), 128.6 (Ar—*C*), 128.5 (Ar—*C*), 128.4 × 3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 2(Ar—*C*), 127.9 (Ar—*C*), 127.7 (Ar—*C*), 127.4 (Ar—*C*), 126.5 (Ar—*C*), 102.1 (Ar—CH), 101.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.6 Hz), 97.3 ($C1_{\alpha\text{-glc-linker}}$ $^1J_{C-H}$ = 169.5 Hz), 80.4 (—CH_{ring}), 79.4 (—CH_{ring}), 79.3 (—CH_{ring}), 79.1 (—CH_{ring}), 78.0 (—CH_{ring}), 75.9 (—CH_2), 75.3 (—CH_2), 73.9 (—CH_2), 73.6 (—CH_2), 71.0 (—CH_{ring}), 70.0 (—CH_{ring}), 68.7 (—CH_2), 68.4 (—CH_2), 68.2 (—CH_2), 67.0 (—CH_{ring}), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2). HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{52}H_{59}N_3O_{11}Na$, 924.4042; found, 924.4051.



*5-Azidopentyl 2,3,4-Tri-O-benzyl-6-O-tert-butyldiphenylsilyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**19**).* A mixture of acceptor **18** (1.1 g, 1.22 mmol, 1 equiv), donor **10** (1.455 g, 1.83 mmol, 1.5 equiv),and dried 4 Å molecular sieves (3 g) in anhydrous CH_2CL_2 (75 mL) was stirred under an argon atmosphere for 1 h and cooled to −40 °C. Then, NIS (535 mg, 2.38 mmol, 1.3 equiv with respect to the donor) and TfOH (0.5 M in Et_2O, 730 μL, 0.2 equiv with respect to the donor) were added, and the stirring was continued until TLC analysis indicated the disappearance of the starting materials (90 min). Upon completion, the reaction was quenched by the addition of NEt_3 (200 μL), and the reaction mixture was slowly warmed to room temperature and filtered through a pad of Celite. The filtrate was washed with satd aq $Na_2S_2O_3$, satd aq $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc/hexanes, 1:4) to afford pure compound **19** (1.61 g, 84%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.77 (d, J = 6.6 Hz, 2H, Ar—*H*), 7.72 (d, J = 6.6 Hz, 2H, Ar—*H*), 7.53 (d, J = 7.2 Hz, 2H, Ar—*H*), 7.38−7.15 (m, 44H, Ar—*H*), 5.53 (d, J = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.35 (s, 1H, Ar—C*H*), 5.08 (d, J = 11.2 Hz, 1H, Ar—CH_aH$_b$), 5.01 (d, J = 11.2 Hz, 1H, Ar—CH$_a$H$_b$), 4.98 (d, J = 11.2 Hz, 1H, Ar—CH_aH$_b$), 4.88 (d, J = 11.2 Hz, 2H, Ar—CH_2), 4.83−4.75 (m, 4H, 3 × Ar—C*H*aH$_b$, C1—$H_{\alpha\text{-glc-linker}}$ (overlapped), 4.70−4.63 (m, 3H, 3 × Ar—CH$_a$H$_b$), 4.55−4.53 (m, 2H, C1—$H_{\beta\text{-man}}$ (overlapped), Ar—CHaH$_b$), 4.43 (d, J = 12.3 Hz, 1H, Ar—CHaH$_b$), 4.31 (d, J = 11.7 Hz, 1H, Ar—C*H*aH$_b$), 4.22 (t, J = 9.3 Hz, 1H, —CH_{ring}), 4.08 (t, J = 9.3 Hz, 1H, —CH_{ring}), 4.03−3.93 (m, 4H, 2 × —CH_{ring}, 2 × —CH_aH$_b$), 3.90 (d, J = 11.2 Hz, 1H, —CH$_a$H$_b$), 3.84 (dd, J = 9.9, 3.3 Hz, 1H, —CH_{ring}), 3.81−3.74 (m, 3H, 3 × —CH_{ring}), 3.71−3.67 (m, 2H, —CH$_a$H$_b$, —CH_{ring}), 3.58−3.45 (m, 6H, 2 × —CH_2, 2 × —CH_{ring}), 3.31 (t, J = 7.1 Hz, 2H, —$CH_{2linker}$), 3.06−3.02 (m, 1H, —CH_{ring}), 1.76−1.70 (m, 2H, —$CH_{2linker}$), 1.69−1.65 (m, 2H, —$CH_{2linker}$), 1.55−1.50 (m, 2H, —$CH_{2linker}$), 1.17 (s, 9H, 3 × —CH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.7 (Ar—*C*), 138.8 (Ar—*C*), 138.7 (Ar—*C*), 138.6 × 2 (Ar—*C*), 38.5 (Ar—*C*), 137.7 (Ar—*C*), 137.6 (Ar—*C*), 136.0 (Ar—*C*), 135.8 (Ar—*C*), 133.5 (Ar—*C*), 133.4 (Ar—*C*), 129.8 × 2 (Ar—*C*), 129.3 (Ar—*C*), 128.6 (Ar—*C*), 128.5 (Ar—*C*), 128.4 × 3 (Ar—*C*), 128.3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 127.9 × 3 (Ar—*C*), 127.8 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 × 2 (Ar—*C*), 127.4 × 2 (Ar—*C*), 127.2 (Ar—*C*), 126.5 (Ar—*C*), 102.2 (Ph—CH), 101.2 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.7 Hz), 97.1 ($C1_{\alpha\text{-glc-linker}}$ $^1J_{C-H}$ = 171.4 Hz), 96.6 ($C1_{\alpha\text{-glc}}$ $^1J_{C-H}$ = 172.1 Hz), 81.5 (—CH_{ring}), 80.3 (—CH_{ring}), 79.7 × 2 (—CH_{ring}), 79.6 (—CH_{ring}), 79.2 (—CH_{ring}), 77.3 (—CH_{ring}), 77.2 (—CH_{ring}), 75.9 (—CH_2), 75.8 (—CH_2), 75.2 (—CH_2), 74.9 × 2 (—CH_{ring}, —CH_2), 73.6 (—CH_2), 73.5 (—CH_2), 72.5 (—CH_{ring}), 71.0 (—CH_2), 69.8 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 68.1 (—CH_2), 67.0 (—CH_{ring}), 63.1 (—CH_2), 51.4 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 27.1 (3 × —CH_3), 23.6 (—CH_2), 19.5 (*C*). HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{95}H_{105}N_3O_{16}SiNa$, 1594.7156; found, 1594.7185.



https://dx.doi.org/10.1021/acs.joc.0c01404
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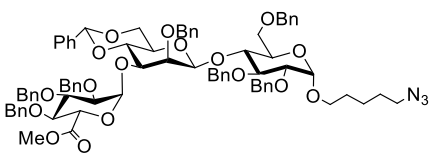
*5-Azidopentyl 2,3,4-Tri-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**20**).* To a Nalgene bottle containing a solution of silylether compound **19** (2.5 g, 1.59 mmol, 1 equiv) in a mixture of THF/pyridine (30 mL, 9:1 = v/v), the pyridinium hydorfluoride (HF·py) stock solution (25 mL, 15 equiv; [stock solution was prepared from commercially available Aldrich HF·py (70%) by dissolving 15 mL in 30 mL of pyridine, and 75 mL of THF]) was added with the help of a plastic syringe over 5 min at 0 °C. The reaction was allowed to warm to room temperature and stirred for overnight, at which time all starting material disappeared confirmed by TLC. Upon completion, the reaction mixture was poured into cold satd aq $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over $MgsO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:3) to get pure compound **20** in 1.929 g (91%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.53 (d, J = 7.3 Hz, 2H, Ar—H), 7.41−7.18 (m, 36H, Ar—H), 7.04 (d, J = 7.3 Hz, 2H, Ar—H), 5.43 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.36 (s, 1H, Ar—CH), 5.09 (d, J = 10.4 Hz, 1H, Ar—CH_aH_b), 4.99 (d, J = 10.9 Hz, 1H, Ar—CH_aH_b), 4.93 (d, J = 8.8 Hz, 1H, Ar—CH_aH_b), 4.91 (d, J = 8.8 Hz, 1H, Ar—CH_aH_b), 4.87 (d, J = 11.4 Hz, 1H, Ar—CH_aH_b), 4.84 (s, 1H, Ar—CH_aH_b), 4.82 (s, 1H, Ar—CH_aH_b), 4.78 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.76 (d, J = 11.0 Hz, 1H, Ar—CH_aH_b), 4.72 (d, J = 12.1 Hz, 1H, Ar—CH_aH_b), 4.67 (d, J = 12.1 Hz, 1H, Ar—CH_aH_b), 4.63 (d, J = 11.0 Hz, 1H, Ar—CH_aH_b), 4.59 (d, J = 12.1 Hz, 1H, Ar—CH_aH_b), 4.58 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.43 (d, J = 12.4 Hz, 1H, Ar—CH_aH_b), 4.35 (d, J = 12.4 Hz, 1H, Ar—CH_aH_b), 4.22 (t, J = 9.7 Hz, 1H, —CH_{ring}), 4.08 (dd, J = 10.3, 4.7 Hz, 1H, —CH_aH_b), 4.02 (m, 2H, 2 × —CH_{ring}), 3.96 (t, J = 9.3 Hz, 1H, —CH_{ring}), 3.80−3.78 (m, 2H, 2 × —CH_{ring}), 3.75−3.71 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.69−3.63 (m, 2H, —C$H_{2linker}$), 3.60−3.55 (m, 4H, —CH_{ring}, 3 × —CH_aH_b), 3.49−3.44 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.30 (t, 2H, —C$H_{2linker}$), 3.16−3.12 (m, 1H, —CH_{ring}), 1.76−1.70 (m, 2H, —C$H_{2linker}$), 1.69−1.65 (m, 2H, —C$H_{2linker}$), 1.54−1.48 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.6 (Ar—C), 138.8 (Ar—C), 138.6 × 2 (Ar—C), 138.4 (Ar—C), 138.3 (Ar—C), 138.0 (Ar—C), 137.5 (Ar—C), 129.4 (Ar—C), 128.8 (Ar—C), 128.6 (Ar—C), 128.5 × 3 (Ar—C), 128.4 (Ar—C), 128.3 × 2 (Ar—C), 128.2 × 2 (Ar—C), 128.1 × 2 (Ar—C), 128.0 (Ar—C), 127.9 (Ar—C), 127.7 × 2 (Ar—C), 127.4 (Ar—C), 127.3 × 2 (Ar—C), 126.5 (Ar—C), 102.4 (Ar—CH), 101.4 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 160.3 Hz), 97.2 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 171.0 Hz), 96.8 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 171.0 Hz), 81.3 (—CH_{ring}), 80.4 (—CH_{ring}), 79.5 (—CH_{ring}), 79.3 (—CH_{ring}), 79.1 (—CH_{ring}), 78.9 (—CH_{ring}), 77.5 (—CH_{ring}), 77.4 (—CH_{ring}), 75.7 × 2 (CH_2), 75.3 (—CH_{ring}), 75.2 (—CH_2), 75.1 (—CH_2), 73.8 (—CH_2), 73.6 (—CH_2), 71.6 (—CH_{ring}), 70.8 (—CH_2), 70.0 (—CH_{ring}), 68.8 (—CH_2), 68.6 (—CH_2), 68.2 (—CH_2), 67.2 (—CH_{ring}), 62.3 (—CH_2), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{79}H_{87}N_3O_{16}Na$, 1356.5979; found, 1356.5994.



*5-Azidopentyl Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**22**).* Compound **20** (1.925 g, 1.443 mmol, 1 equiv) was dissolved in a mixture of CH_2Cl_2/H_2O (20 mL, 1:1 = v/v), and the reaction mixture was cooled to 0 °C. BAIB (1.395 g, 4.33 mmol, 3 equiv) and TEMPO (68 mg, 0.433 mmol, 0.3 equiv) were added, and the reaction mixture was allowed to warm to rt and stirred for 3 h. Upon completion, the reaction mixture was quenched by the addition of satd aq $Na_2S_2O_3$ solution (10 mL) and diluted with CH_2Cl_2 (20 mL). The organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (15 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH, 98:2) to obtain acid compound **21** in 1.80 g (92%) as a white foam.

The above purified compound was dissolved in dry DMF (20 mL). Then, MeI (416 μL, 6.68 mmol, 5 equiv) and oven-dried K_2CO_3 (553 mg, 4 mmol, 3 equiv) were added at rt under a nitrogen atmosphere, and the reaction mixture was stirred overnight. Upon completion, the solvent was removed under a high vacuum, and the remaining mixture was diluted with CH_2Cl_2 (30 mL) and washed with water (15 mL). The aqueous layer was extracted with CH_2Cl_2 (15 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:3) to get pure compound **22** (1.68 g, 92%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.53 (d, J = 7.2 Hz, 2H, Ar—H), 7.33−7.18 (m, 36H, Ar—H), 7.03 (d, J = 7.2 Hz, 2H, Ar—H), 5.56 (d, J = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc-COOMe}}$), 5.30 (s, 1H, Ar—C$H$), 5.08 (d, J = 10.8 Hz, 1H, Ar—CH_aH_b), 4.94 (d, J = 11.0 Hz, 1H, Ar—CH_aH_b), 4.88 (d, J = 11.5 Hz, 1H, Ar—CH_aH_b), 4.84−4.82 (m, 4H, 2 × Ar—CH_2), 4.78 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.73 (d, J = 11.6 Hz, 1H, Ar—CH_aH_b), 4.71 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.67 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.59 (d, J = 11.0 Hz, 1H, Ar—CH_aH_b), 4.55 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.52 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.42 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.33 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.29 (d, J = 10.0 Hz, 1H, —CH_{ring}), 4.19 (t, J = 9.3 Hz, 1H, —CH_{ring}), 4.02−3.96 (m, 3H, 2 × —CH_{ring}), —CH_aH_b), 3.95 (t, J = 8.9 Hz, 1H, —CH_{ring}), 3.82−3.74 (m, 4H, 4 × —CH_{ring}), 3.72 (m, 1H, —CH_aH_b), 3.69 (s, 3H, —COOCH_3), 3.68−3.62 (m, 2H, —C$H_{2linker}$), 3.58−3.54 (m, 2H, 2 × —CH_{ring}), 3.53−3.45 (m, 2H, 2 × -CH_aH_b), 3.31 (t, J = 6.6 Hz, 2H, —C$H_{2linker}$), 3.05−3.06 (m, 1H, —CH_{ring}), 1.75−1.70 (m, 2H, —C$H_{2linker}$), 1.69−1.66 (m, 2H, —C$H_{2linker}$), 1.56−1.49 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 170.06 (CO), 139.6 (Ar—C), 138.6 × 3 (Ar—C), 138.2 (Ar—C), 138.1 (Ar—C), 137.8 (Ar—C), 137.4 (Ar—C), 129.4 (Ar—C), 128.8 (Ar—C), 128.5 × 2 (Ar—C), 128.4 × 2 (Ar—C), 128.3 (Ar—C), 128.2 × 2 (Ar—C), 128.1(Ar—C), 128.0 (Ar—C), 127.9 × 2 (Ar—C), 127.7 × 3 (Ar—C), 127.5 (Ar—C), 127.3 × 2 (Ar—C), 126.5 (Ar—C), 102.3 (Ar—CH), 101.3 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 155.4 Hz), 97.4 ($C1_{\alpha\text{-glc-coome}}$, $^1J_{C-H}$ = 172.2 Hz), 97.3 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 171.4 Hz), 80.7 (—CH_{ring}), 80.3 (—CH_{ring}), 79.5 (—CH_{ring}), 79.3 (—CH_{ring}), 79.2 (—CH_{ring}), 79.1 (—CH_{ring}), 78.6 (—CH_{ring}), 77.5 (—CH_{ring}), 75.9 (—CH_2), 75.8 (—CH_2), 75.3 (—CH_{ring}), 75.2 × 2 (—CH_2), 73.8 (—CH_2), 73.6 (—CH_2), 71.2 (—CH_{ring}), 71.0 (—CH_2), 69.9 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 68.2 (—CH_2), 67.0

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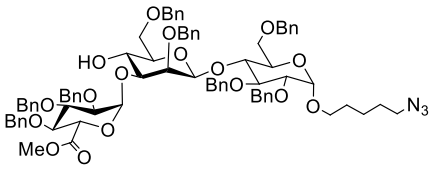
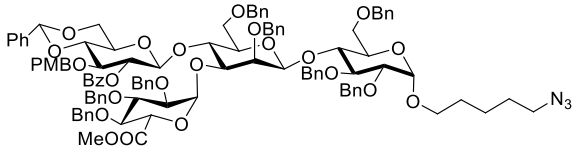
(—CH_{ring}), 52.6 (—$COOCH_3$), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2). HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{80}H_{87}N_3O_{17}Na$, 1384.5928; found, 1384.5940.



*5-Azidopentyl Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**8**).* A mixture of trisaccharide **22** (1.55 g, 1.14 mmol, 1 equiv) and activated 4 Å molecular sieves (3 g) in anhydrous CH_2Cl_2 (50 mL) was stirred under argon for 1 h. The reaction mixture was cooled to −78 °C, Et_3SiH (545 μL, 3.41 mmol, 3 equiv) and TfOH (354 μL, 3.5 equiv) were added, and the stirring was continued at −78 °C until TLC analysis (EtOAc/hexanes) indicated the disappearance of the starting material (1 h). Upon completion, the reaction mixture was quenched by the addition of satd aq $NaHCO_3$ (1 mL). The reaction mixture was slowly warmed to room temperature. Then, the mixture was filtered through a pad of Celite, and the filtrate was washed with satd aq $NaHCO_3$ (25 mL) and brine (25 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes) to obtain pure compound **8** (1.45 g, 94%) in a viscous colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K):*δ* 7.45 (d, *J* = 7.2 Hz, 2H, Ar—*H*), 7.37−7.19 (m, 38H, Ar—*H*), 5.15 (d, *J* = 11.2 Hz, 1H, Ar—CH_aH_b), 5.12 (d, *J* = 3.5 Hz, 1H, C1—$H_{α\text{-glc-COOMe}}$), 4.98 (d, *J* = 11.4 Hz, 1H, Ar—CH_aH_b), 4.93 (d, *J* = 10.9 Hz, 1H, Ar—CH_aH_b), 4.84−4.79 (m, 4H, 2 × Ar—CH_2), 4.78−4.73 (m, 4H, C1—$H_{α\text{-glc-linker}}$), 3 × Ar—CH_aH_b), 4.69 (d, *J* = 12.2 Hz, 1H, Ar—CH_aH_b), 4.63 (d, *J* = 12.2 Hz, 1H, Ar—CH_aH_b), 4.60 (d, *J* = 11.2 Hz, 1H, Ar—CH_aH_b), 4.50 (d, *J* = 11.8 Hz, 1H, Ar—CH_aH_b), 4.49 (s, 1H, C1—$H_{β\text{-man}}$), 4.45 (d, *J* = 11.8 Hz, 1H, Ar—CH_aH_b), 4.41 (d, *J* = 9.9 Hz, 1H, —CH_{ring}), 4.33 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.06 (t, *J* = 9.2 Hz, 1H, —CH_{ring}), 4.02 (d, *J* = 9.5 Hz, 1H, —CH_{ring}), 3.97 (d, *J* = 10.2 Hz, 1H, —CH_{ring}), 3.92 (t, *J* = 9.2 Hz, 1H, —CH_{ring}), 3.79−3.74 (m, 3H, 3 × —CH_{ring}), 3.69−3.61 (m, 8H, —$CH_{2linker}$, —CH_{ring}, 2 × —C*H*aH_b, —$COOCH_3$), 3.51−3.45 (m, 3H, —CH_{ring}, 2 × —CH_aH_b), 3.28−3.25 (m, 4H, 2 × —CH_{ring}, 2 × —CH_aH_b), 1.73−1.67 (m, 2H, —$CH_{2linker}$), 1.66−1.63 (m, 2H, —$CH_{2linker}$), 1.51−1.47 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): *δ* 170.2 (CO), 139.8 (Ar—*C*), 138.8 (Ar—*C*), 138.6 × 2 (Ar—*C*), 138.5 (Ar—*C*), 138.3 (Ar—*C*), 137.8 (Ar—*C*), 137.7 (Ar—*C*), 128.7 (Ar—*C*), 128.6 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 × 2 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 127.9 (Ar—*C*), 127.8 (Ar—*C*), 127.7 (Ar—*C*), 127.5 (Ar—*C*), 127.4 (Ar—*C*), 127.1 (Ar—*C*), 100.8 ($C1_{β\text{-man}}$, $^1J_{C-H}$ = 156.8 Hz), 100.5 ($C1_{α\text{-glc-COOMe}}$, $^1J_{C-H}$ = 171.0 Hz), 97.3 ($C1_{α\text{-glc-linker}}$, $^1J_{C-H}$ = 170.7 Hz), 83.8 (—CH_{ring}), 81.5 (—CH_{ring}), 80.4 (—CH_{ring}), 79.7 (—CH_{ring}), 79.4 (—CH_{ring}), 79.1 (—CH_{ring}), 78.7 (—CH_{ring}), 77.0 (—CH_{ring}), 75.9 (—CH_2), 75.2 (—CH_2), 75.1 (—CH_2), 74.9 (—CH_2), 73.8 × 2 (—CH_2), 73.7 (—CH_2), 73.6 (—CH_2), 71.2 (—CH_{ring}), 71.0 (—CH_2), 70.1 (—CH_{ring}), 69.0 (—CH_{ring}), 68.6 (—CH_2), 68.1 (—CH_2), 52.6 (—$COOCH_3$), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2). HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{80}H_{89}N_3O_{17}Na$, 1386.6084; found, 1386.6084.



*5-Azidopnetyl 2-O-Benzoyl-3-O-p-methoxybenzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**6**).* A mixture of acceptor **8** (1.05 g, 0.77 mmol), donor **9** (690 mg, 1.15 mmol, 1.5 equiv), and activated 4 Å molecular sieves (2 g) in anhydrous CH_2Cl_2 (55 mL) was stirred under an argon atmosphere for 30 min. Then, the reaction mixture was cooled to −30 °C. NIS (310 mg, 1.38 mmol, 1.2 equiv with respect to the donor) and TfOH (0.5 M in Et_2O, 690 μL, 0.34 mmol, 0.3 equiv with respect to the donor) were added, and the mixture was stirred at −20 °C until TLC analysis (EtOAc/toluene, 1:5) indicated the complete disappearance of the starting materials (2 h). Upon completion, the reaction mixture was quenched by the addition of NEt_3 (75 μL) and slowly warmed to room temperature. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 3:7) to afford pure product **6** (1.3 g, 92%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): *δ* 7.86 (d, *J* = 7.2 Hz, 2H, Ar—*H*), 7.60−7.00 (m, 48H, Ar—*H*), 6.96 (d, *J* = 8.6 Hz, 2H, Ar—*H*), 6.56 (d, *J* = 8.6 Hz, 2H, Ar—*H*), 5.19 (t, *J* = 8.3 Hz, 1H, —CH_{ring}), 5.14 (d, *J* = 3.1 Hz, 1H, C1—$H_{α\text{-glc-COOMe}}$), 5.05 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.97 (m, 2H, Ar—CH_aH_b, PhC*H*), 4.87−4.70 (m, 6H, 3 × Ar—CH_2), 4.69−4.67(m, 2H, C1—$H_{α\text{-glc-linker}}$ (overlapped), Ar—CH_aH_b), 4.65 (s, 1H, Ar—CH_aH_b), 4.63−4.60 (m, 2H, C1—$H_{β\text{-glc}}$ (overlapped) Ar—CH_aH_b), 4.56−4.42 (m, 6H, 2 × Ar—CH_2, Ar—CH_aH_b, —CH_{ring}), 4.33 (t, *J* = 9.1 Hz, 1H, —CH_{ring}), 4.28−4.26 (m, 2H, C1—$H_{β\text{-man}}$ (overlapped), Ar—CH_aH_b), 4.14−4.09 (m, 2H, —CH_{ring}, CH_aH_b), 4.02 (d, *J* = 12 Hz, 1H, Ar—CH_aH_b), 3.81−3.72 (m, 4H, 4 × CH_{ring}), 3.68 (m, 4H, —OCH_3, —CH_{ring}), 3.61−3.57 (m, 5H, —$COOCH_3$, 2 × —CH_aH_b), 3.56−3.48 (m, 5H, 2 × —CH_{ring}, 3 × —CH_aH_b), 3.45−3.40 (m, 2H, 2 × —CH_{ring}), 3.38−3.34 (m, 1H, −CH_aH_b), 3.29 (d, *J* = 10.8 Hz, 1H, —CH_aH_b), 3.22−3.18 (m, 3H, —CH_{ring}, —$CH_{2linker}$), 3.12−3.07 (m, 1H, —CH_{ring}), 2.75 (d, 1H, *J* = 9.3 Hz, —CH_{ring}), 1.64−1.60 (m, 2H, —$CH_{2linker}$), 1.59−1.54 (m, 2H, —$CH_{2linker}$), 1.42−1.38 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): *δ* 170.4 (CO), 164.8 (CO), 159.2 (Ar—*C*), 139.9 (Ar—*C*), 139.3 (Ar—*C*), 138.9 (Ar—*C*), 138.8 (Ar—*C*), 138.6 (Ar—*C*), 138.5 (Ar—*C*), 138.4 (Ar—*C*), 137.8 (Ar—*C*), 137.7 (Ar—*C*), 133.3 (Ar—*C*), 130.4(Ar—*C*), 130.0 (Ar—*C*), 129.9 (Ar—*C*), 129.6 (Ar—*C*), 129.0 (Ar—*C*), 128.7 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 (Ar—*C*), 128.3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 (Ar—*C*), 127.5 (Ar—*C*), 127.2 (Ar—*C*), 127.1 (Ar—*C*), 126.9 (Ar—*C*), 126.2 (Ar—*C*), 113.6 (Ar—*C*), 101.3 ($C1_{β\text{-man}}$, $^1J_{C-H}$ = 155.6 Hz), 100.9 (Ph−CH), 100.3 ($C1_{β\text{-glc}}$, $^1J_{C-H}$ = 165.1 Hz), 97.6 ($C1_{α\text{-glc-COOMe}}$, $^1J_{C-H}$ = 171.4 Hz), 97.2 ($C1_{α\text{-glc-linker}}$, $^1J_{C-H}$ = 171.3 Hz), 81.63 (—CH_{ring}), 80.7 (—CH_{ring}), 80.6 (—CH_{ring}), 80.0 (—CH_{ring}), 79.5 (—

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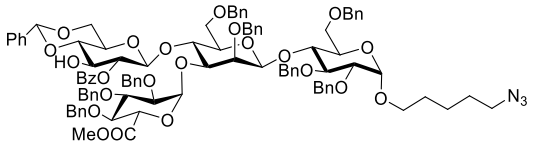
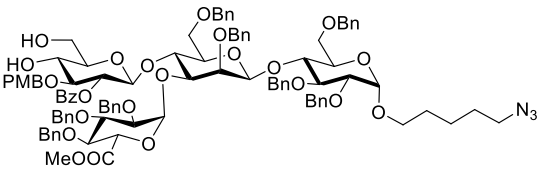
CH_{ring}), 79.3 (—CH_{ring}), 79.0 (—CH_{ring}), 77.9 (—CH_{ring}), 77.7 (—CH_{ring}), 75.9 (—CH_{ring}), 75.6 (—CH_2), 75.0 (—CH_2), 74.9 (—CH_2), 74.2 (—CH_2), 73.8 (—CH_{ring}), 73.6 (CH_2), 73.5 (—CH_2), 73.4 (—CH_2), 73.3 (—CH_2), 72.6 (—CH_2), 72.1 (—CH_{ring}), 71.6 (—CH_{ring}), 70.0 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—$CH_{2linker}$), 68.3 (—CH_2), 68.1 (—CH_2), 66.2 (—CH_{ring}), 55.3 (—OCH_3), 52.5 (—$COOCH_3$), 51.5 (—$CH_{2linker}$), 29.1 (—$CH_{2linker}$), 28.8 (—$CH_{2linker}$), 23.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{108}H_{115}N_3O_{24}Na$, 1860.7763; found, 1860.7758.



*5-Azidopnetyl 2-O-Benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**23**).* DDQ (91 mg, 4.00 mmol, 1.75 equiv) was added to a stirred solution of tetrasaccharide **6** (420 mg, 0.229 mmol) in DCM/phosphate buffer pH 7 (10 mL, 9:1 = v/v) at 0 °C. The reaction mixture was vigorously stirred in the absence of light until TLC analysis indicated the disappearance of the starting material (180 min). Upon completion, the reaction mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ and brine. The organic phase was washed with water until the solution becomes colorless; then, the organic solution was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 3:7) to afford pure compound **23** (281 mg, 72%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.94 (d, J = 7.3 Hz, 2H, Ar—H), 7.35−7.00 (m, 48H, Ar—H), 5.12 (d, J = 3.0 Hz, 1H, C1—$H_{\alpha\text{-glc-COOMe}}$), 5.05−5.02 (m, 2H, Ar—CH_aH$_b$, —CH_{ring}), 4.94 (d, J = 11.0 Hz, 1H, Ar—CH$_a$H$_b$), 4.89 (s, 1H, PhCH—), 4.87 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.82 (d, J = 11.8 Hz, 2H, Ar—CH_2), 4.77 (d, J = 10.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.73−4.62 (m, 6H, 2 × Ar—CH_2, C1—$H_{\alpha\text{-glc-linker}}$ (overlapped), C1—$H_{\beta\text{-glc}}$, (overlapped)), 4.55−4.47 (m, 4H, 2 × Ar—CH_2), 4.43 (d, J = 9.1 Hz, 1H, —CH_{ring}), 4.34 (t, 1H, J = 9.1 Hz, —CH_{ring}), 4.28 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.26 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.10−4.06 (m, 3H, —CH_{ring}, Ar—CH$_a$H$_b$, —CH_aH$_b$OBn), 3.80−3.73 (m, 4H, 4 × —CH_{ring}), 3.67−3.52 (m, 10H, —$COOCH_3$, 1 × —CH_2OBn, —CH$_a$H$_b$OBn 1 × —CH_aH$_{blinker}$ 3 × —CH_{ring}), 3.48−3.32 (m, 5H, 1 × —CH_2OBn, 2 × —CH_{ring}, 1 × —CH$_a$H$_{blinker}$), 3.18 (t, J = 6.8 Hz, 2H, —$CH_{2linker}$), 3.08−3.04 (m, 1H, —CH_{ring}), 2.88 (t, 1H, J = 9.3 Hz, —CH_{ring}), 2.79 (d, 1H, J = 8.5 Hz, —CH_{ring}), 1.62−1.52 (m, 4H, 2 × —$CH_{2linker}$), 1.40−1.36 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 170.4 (CO), 165.6 (CO), 139.9 (Ar—C), 139.3 (Ar—C), 139.0 (Ar—C), 138.8 (Ar—C), 138.6 (Ar—C), 138.5 (Ar—C), 138.3 (Ar—C), 137.8 (Ar—C), 137.3 (Ar—C), 133.6 (Ar—C), 130.0 (Ar—C), 129.6 (Ar—C), 129.4 (Ar—C), 128.7 × 2 (2 × Ar—C), 128.6 × 2 (2 × Ar—C), 128.5 × 3 3 × (Ar—C), 128.4 (Ar—C), 128.2 (Ar—C), 128.1 × 3 (Ar—C), 128.0 (Ar—C), 127.9 × 5 (Ar—C), 127.8 (Ar—C), 127.7 (Ar—C), 127.4 (Ar—C), 127.3 (Ar—C), 127.1 (Ar—C), 126.9 (Ar—C), 126.5 (Ar—C), 101.6 ($C1_{\beta\text{-man}}$), 101.4 (Ph—CH), 99.9 ($C1_{\beta\text{-glc}}$), 97.8 ($C1_{\alpha\text{-glc-COOMe}}$), 97.2 ($C1_{\alpha\text{-glc-linker}}$), 80.7 × 2 (2 × —$CH_{ring}$), 80.6 (—$CH_{ring}$), 80.2 (—$CH_{ring}$), 79.5 (—$CH_{ring}$), 79.3 (—$CH_{ring}$), 79.2 (—$CH_{ring}$), 77.9 (—$CH_{ring}$), 77.7 (—$CH_{ring}$), 75.9 (—$CH_{ring}$), 75.6 (—$CH_2$), 75.0 × 2 (—$CH_{ring}$, —$CH_2$), 74.9 (—$CH_2$), 74.2 (—$CH_2$), 73.6 (—$CH_2$), 73.5 × 2 (2 × —$CH_2$), 72.6 × 2 (—$CH_{ring}$, —$CH_2$), 72.0 (—$CH_{ring}$), 71.6 (—$CH_{ring}$), 70.0 (—$CH_{ring}$), 68.7 (—$CH_2$), 68.5 × 2 (—$CH_2$, —$CH_{2linker}$), 68.1 (—$CH_2$), 66.1 (—$CH_{ring}$), 52.5 (—$COOCH_3$), 51.5 (—$CH_2$ $_{linker}$), 29.1 (—$CH_{2linker}$), 28.8 (—$CH_{2linker}$), 23.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{100}H_{107}N_3O_{23}Na$, 1740.7188; found, 1740.7182.



*5-Azidopnetyl 2-O-Benzoyl-3-O-p-methoxybenzyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**27**).* To a stirred solution of tetrasaccharide **6** (1.175 g, 0.639 mmol) in CH_2Cl_2/MeOH (14 mL 1:1 = v/v), p-TsOH (120 mg, 0.639 mmol, 1 equiv) was added at rt. The reaction mixture was allowed to stir overnight. Upon completion, the reaction mixture was quenched with Et_3N (100 μL) and concentrated by rotary evaporation. The obtained residue was purified by flash column chromatography (EtOAc/hexanes, 2:3) to give pure compound **27** (110 mg, 87%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.96 (d, J = 7.1 Hz, 2H, Ar—H), 7.63−7.03 (m, 45H, Ar—H), 6.69 (d, J = 8.1 Hz, 2H, Ar—H), 5.24 (t, J = 8.4, —CH_{ring}), 5.10 (dd, J = 11.8, 4.0 Hz, 2H, 2 × Ar—CH_aH$_b$), 5.02 (d, J = 10.7 Hz, 1H, Ar—CH$_a$H$_b$), 4.97 (d, J = 3.4 Hz, 1H, C1−H$_{\alpha\text{-glc-COOMe}}$), 4.86 (d, J = 12.9 Hz, 1H, Ar—CH$_a$H$_b$), 4.78−4.74 (m, 3H, 3 × Ar—CH$_a$H$_b$), 4.72−4.69 (m, 3H, Ar—CH_2, C1−H$_{\alpha\text{-glc-linker}}$), 4.66 (s, 1H, C1—H$_{\beta\text{-glc}}$), 4.65−4.54 (m, 5H, 2 × Ar—CH_2, Ar—CH$_a$H$_b$), 4.52 (s, 1H, Ar—CH$_a$H$_b$), 4.47 (d, J = 11.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.37−4.33 (m, 2H, Ar—CH$_a$H$_b$, —CH_{ring}), 4.29−4.27 (m, 2H, C1—H$_{\beta\text{-man}}$, Ar—CH$_a$H$_b$), 4.02−3.95 (m, 2H, —CH$_a$H$_b$ −CH_{ring}), 3.88−3.84 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.82−3.79 (m, 3H, 3 × —CH_{ring}), 3.72−3.66 (m, 6H, —OCH_3, 3 × —CH_{ring}), 3.63−3.59 (m, 4H, —CH_{ring}, 3 × —CH$_a$H$_b$), 3.57 (s, 3H, —$COOCH_3$), 3.56−3.51 (m, 2H, —CH_{ring}, CH$_a$H$_b$), 3.48 (dd, J = 9.1, 4.0 Hz, 1H, —CH_{ring}), 3.43−3.35 (m, 3H, —CH_{ring}, 2 × —CH$_a$H$_b$), 3.22 (t, J = 7.0 Hz, 2H, —$CH_{2linker}$), 3.11−3.06 (m, 2H, —CH_{ring}, —OH), 2.80 (d, J = 9.2 Hz, 1H, —CH_{ring}), 2.25 (d, J = 36 Hz, 1H, —OH), 1.65−1.61 (m, 2H, —$CH_{2linker}$), 1.60−1.56 (m, 2H, —$CH_{2linker}$), 1.45−1.34 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 170.0 (COOMe), 165.0 (COBz), 159.3 (Ar—C), 139.9 (Ar—C), 139.3 (Ar—C), 138.8 (Ar—C), 138.6 (Ar—C), 138.5 (Ar—C), 138.4 (Ar—C), 137.7 (Ar—C), 137.6 (Ar—C), 133.5 (Ar—C), 130.4 (Ar—C), 129.8 × 2 (Ar—C), 129.5 (Ar—C), 128.8 × 2 (Ar—C), 128.7 (Ar—C), 128.5 × 2 (Ar—C), 128.3 × 2 (Ar—C), 128.2 (Ar—C), 128.1 × 2 (Ar—C), 128.0 × 2 (Ar—C), 127.9 × 2 (Ar—C), 127.7 (Ar—C), 127.6 (Ar—C), 127.5 (Ar—C), 127.4 (Ar—C), 127.2 (Ar—C), 127.1 (Ar—C), 126.9 (Ar—C), 113.9 (Ar—C), 101.8 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.0 Hz), 100.2 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 170.1 Hz), 99.9 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 163.6 Hz), 97.2 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 171.6 Hz), 82.6 (—CH_{ring}), 81.5 (—CH_{ring}), 80.6 × 2 (—CH_{ring}), 79.8 (—CH_{ring}), 79.3

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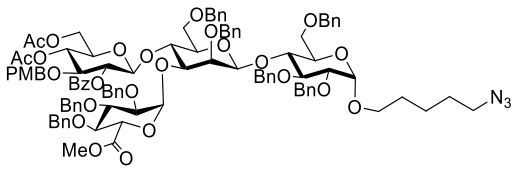
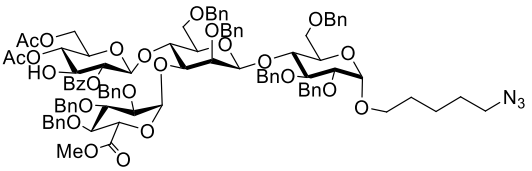
(—CH_{ring}), 78.3 × 2 (—CH_{ring}), 76.0 (—CH_{ring}), 75.7 (—CH_2), 75.3 (—CH_{ring}), 75.03 (—CH_2), 74.6 (—CH_2), 74.2 (—CH_2), 73.9 (—CH_2), 73.8 (—CH_{ring}), 73.6 (—CH_2), 73.5 × 2 (—CH_2), 72.4 (—CH_2), 71.4 (—CH_{ring}), 70.9 (—CH_{ring}), 70.1 (—CH_{ring}), 69.4 (—CH_{ring}), 68.6 (CH_2), 68.1 (—CH_2), 68.0 (—CH_2), 60.7 (—CH_2), 55.3 (—OCH_3), 52.5 (—$COOCH_3$), 51.4 (—CH_2), 29.0 (—CH_2), 28.8 (—CH_2), 23.5 (—CH_2). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{101}H_{111}N_3O_{24}Na$, 1772.7450; found, 1772.7460.



*5-Azidopnetyl 4,6-Di-O-acetyl-2-O-benzoyl-3-O-p-methoxybenzyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**28**).* To a stirred solution of diol **27** (920 mg, 0.525 mmol) in a pyridine (10 mL) were added DMAP (7 mg, 0.1 mol %) and acetic anhydride (200 μL, 2.10 mmol) at 0 °C under an argon atmosphere. The reaction mixture was stirred at rt until TLC analysis indicated the disappearance of the starting material (3 h). Upon completion, pyridine was removed by rotary evaporation under a high vacuum. The obtained residue was diluted with CH_2Cl_2 (25 mL) and washed with 1 N HCl (2 × 10 mL), satd aq $NaHCO_3$ (10 mL), and water (10 mL). The organic solvent was dried over anhydrous $MgSO_4$, and the residue was concentrated by rotary evaporation. The concentrate was purified by flash column chromatography (EtOAc/hexanes, 2:3) to yield pure compound **28** (953 mg, 99%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.92 (d, *J* = 7.3 Hz, 2H, Ar—*H*), 7.59−7.05 (m, 43H, Ar—*H*), 6.94 (d, *J* = 8.4 Hz, 2H, Ar—*H*), 6.64 (d, *J* = 8.7 Hz, 2H, Ar—*H*), 5.22 (t, *J* = 8.7 Hz, 1H, —CH_{ring}), 5.12 (d, *J* = 3.1 Hz, 1H, C1−$H_{α\text{-glc-COOMe}}$), 5.07 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.92 (s, 1H, Ar—CH_aH_b), 4.90 (s, 1H, Ar—CH_aH_b), 4.86 (d, *J* = 12.4 Hz, 1H, Ar—CH_aH_b), 4.80−4.71 (m, 4H, —CH_{ring}, 3 × Ar—CH_aH_b), 4.69−4.68 (m, 4H, C1−$H_{α\text{-glc-linker}}$, C1−$H_{β\text{-glc}}$, Ar—CH_2), 4.64 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.58 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.54−4.50 (m, 3H, 3 × Ar—CH_aH_b), 4.44 (d, *J* = 9.4 Hz, 1H, —CH_{ring}), 4.36−4.29 (m, 4H, C1−$H_{β\text{-man}}$, Ar—CH_2, —CH_{ring}), 4.28 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.07−4.02 (m, 3H, —CH_{ring}, Ar—CH_aH_b, —CH_aH_b), 3.98 (dd, *J* = 12.1, 2.5 Hz, 1H, —CH_aH_b), 3.82−3.72 (m, 4H, 4 × —CH_{ring}), 3.68−3.63 (m, 5H, —OCH_3, —CH_aH_b, —CH_{ring}), 3.62−3.51 (m, 8H, 2 × —CH_{ring}, —$COOCH_3$, 3 × —CH_aH_b), 3.46 (dd, *J* = 9.1, 3.5 Hz, 1H, —CH_{ring}), 3.43 (d, *J* = 11.1, 1H, —CH_aH_b), 3.38−3.34 (m, 2H, —CH_{ring}, —CH_aH_b), 3.32 (dd, *J* = 9.3, 2.3 Hz, 1H, —CH_{ring}), 3.19 (t, *J* = 7.1 Hz, 2H, —$CH_{2linker}$), 2.88 (d, 1H, *J* = 8.0 Hz, 1H, —CH_{ring}), 1.93 (s, 3H, —$COCH_3$), 1.69 (s, 3H, —$COCH_3$), 1.62−1.58 (m, 2H, —$CH_{2linker}$), 1.57−1.53 (m, 2H, —$CH_{2linker}$), 1.41−1.37 (m, 2H, —$CH_{2linker}$). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 171.0 (*CO*), 170.4 (*CO*), 169.4 (*CO*), 164.6 (*CO*), 159.2 (Ar—*C*), 139.9 (Ar—*C*), 139.2 (Ar—*C*), 138.7 × 2 (Ar—*C*), 138.6 (Ar—*C*), 138.4 (Ar—*C*), 137.8 (Ar—*C*), 133.4 (Ar—*C*), 130.0 (Ar—*C*), 129.8 × 2 (Ar—*C*), 129.4 (Ar—*C*), 128.7 × 2 (Ar—*C*), 128.6 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 (Ar—*C*), 128.2 (Ar—*C*), 128.1 × 3 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 (Ar—*C*), 127.1 (Ar—*C*), 126.9 (Ar—CH), 113.7 (Ar—*C*), 101.4 ($C1_{β\text{-man}}$, $^1J_{C-H}$ = 155.8 Hz), 99.8 ($C1_{β\text{-glc}}$, $^1J_{C-H}$ = 163.2 Hz), 98.6 ($C1_{α\text{-glc-COOMe}}$, $^1J_{C-H}$ = 171.0 Hz), 97.1 ($C1_{α\text{-glc-linker}}$, $^1J_{C-H}$ = 172.5 Hz), 80.7 (—CH_{ring}), 80.6 (—CH_{ring}), 79.9 (—CH_{ring}), 79.5 (—CH_{ring}), 79.4 (—CH_{ring}), 79.1 (—CH_{ring}), 78.9 (—CH_{ring}), 78.0 (—CH_{ring}), 77.4 (—CH_{ring}), 75.9 (—CH_{ring}), 75.3 (—CH_2), 75.0 (—CH_2), 74.7 (—CH_2), 73.8 (—CH_2), 73.6 (—CH_2), 73.5 (—CH_2), 73.4 (—CH_2), 73.3 (—CH_2), 72.3 (—CH_2), 72.1 (—CH_{ring}), 71.5 (—CH_{ring}), 70.5 (—CH_{ring}), 70.0 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 68.1 (CH_2), 62.8 (—CH_2), 55.3 (—OCH_3), 52.3 (—$COOCH_3$), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2), 20.9 (—$COCH_3$), 20.6 (—$COCH_3$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{105}H_{115}N_3O_{26}Na$, 1856.7661; found, 1856.7662.



*5-Azidopnetyl 4,6-Di-O-acetyl-2-O-benzoyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**7**).* To a stirred solution of tetrasaccharide **28** (953 mg, 0.520 mmol) and thiophenol (75 μL, 0.676 mmol, 1.3 equiv) in dry CH_2Cl_2 (10 mL) was added $SnCl_4$ (162 mg, 0.624 mmol, 1.2 equiv) at −78 °C under an argon atmosphere. After 10 min, the resulting mixture was stirred at −50 °C until TLC analysis indicated the disappearance of the starting material (30−40 min). Upon completion, the reaction mixture was quenched with satd aq $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated by rotary evaporation. The resulting residue was purified by flash column chromatography (EtOAc/Hexanes, 2:3) to give title compound **7** (833 mg, 93%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.95 (d, *J* = 7.3 Hz, 2H, Ar—*H*), 7.60−7.03 (m, 43H, Ar—*H*), 5.14 (d, *J* = 3.0 Hz, 1H, C1−$H_{α\text{-glc-coome}}$), 5.07 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.94 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.89−4.83 (m, 3H, Ar—CH_2, —CH_{ring}), 4.76 (d, *J* = 11.4 Hz, 1H, Ar—CH_aH_b), 4.72 (d, *J* = 12.8 Hz, 1H, Ar—CH_aH_b), 4.71−4.62 (m, 6H, C1−$H_{α\text{-glc-link}}$, C1−$H_{β\text{-glc}}$), 2 × Ar—CH_2), 4.58 (d, *J* = 11.6 Hz, 1H, Ar—CH_aH_b), 4.54−4.51 (m, 3H, Ar—CH_2, —CH_{ring}), 4.47 (d, *J* = 9.4 Hz, 1H, —CH_{ring}), 4.37−4.33 (m, 3H, C1−$H_{β\text{-man}}$, 2 × —CH_{ring}), 4.28 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.08−4.00 (m, 3H, —CH_{ring}, Ar—CH_aH_b, —CH_aH_b), 3.94 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 3.80−3.74 (m, 4H, 4 × —CH_{ring}), 3.67−3.65 (m, 2H, —CH_{ring}, —CH_aH_b), 3.64 (dd, 1H, *J* = 8.9, 3.1 Hz, —CH_{ring}), 3.60−3.54 (m, 3H, —CH_2, 1 × —$CHaH_b$), 3.53 (s, 3H, —$COOCH_3$), 3.47−3.44 (m, 3H, —CH_{ring}, —CH_2), 3.37−3.28 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.19 (t, *J* = 7.1 Hz, 2H, —$CH_{2linker}$), 2.91 (d, *J* = 8.0 Hz, 1H, —CH_{ring}), 2.07 (d 1H, *J* = 6.7, OH), 2.00 (s, 3H, —$COCH_3$), 1.67 (s, 3H, —$COCH_3$), 1.62−1.58 (m, 2H, —$CH_{2linker}$), 1.56−1.53 (m, 2H, —$CH_{2linker}$), 1.41−1.36 (m, 2H,−$CH_{2linker}$). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 171.0 (*CO*), 170.7 (*CO*), 170.5 (*CO*), 165.8 (*CO*), 139.8 (Ar—*C*), 139.1 (Ar—*C*), 138.8 (Ar—*C*), 138.6 (Ar—*C*), 138.4 (Ar—*C*), 138.3 (Ar—*C*),

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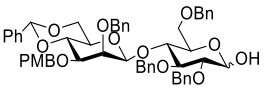
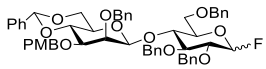
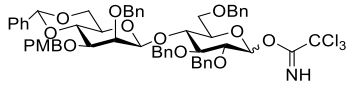
137.6 (Ar—*C*), 133.7 (Ar—*C*), 129.9 (Ar—*C*), 129.4 (Ar—*C*), 128.8 (Ar—*C*), 128.7 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 (Ar—*C*), 128.2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.7 (Ar—*C*), 127.5 (Ar—*C*), 127.3 (Ar—CH), 127.2 (Ar—CH), 127.0 (Ar—CH), 126.9 (Ar—CH), 101.5 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 155.1 Hz), 99.3 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.7 Hz), 98.6 ($C1_{\alpha\text{-glc-coome}}$, $^1J_{C-H}$ = 170.2 Hz), 97.1 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 170.5 Hz), 80.7 (—CH_{ring}), 80.5 (—CH_{ring}), 79.8 (—CH_{ring}), 79.2 (—CH_{ring}), 78.9 (—CH_{ring}), 78.8 (—CH_{ring}), 77.9 × 2 (—CH_{ring}), 75.8 (—CH_{ring}), 75.3 (—CH_2), 75.0 (—CH_2), 74.8 (—CH_{ring}), 74.6 (—CH_2), 74.12 (—CH_{ring}), 73.7 (—CH_2), 73.5 (—CH_2), 73.4 × 2 (—CH_2), 72.1 (—CH_2), 71.9 (—CH_{ring}), 71.6 × 2 (—CH_{ring}), 71.5 (—CH_{ring}), 69.8 (—CH_{ring}), 68.5 (—CH_2), 68.4 (—CH_2), 68.0 (—CH_2), 62.7 (—CH_2), 52.5 (—$COOCH_3$), 51.4 (—CH_2), 29.0 (—CH_2), 28.8 (—CH_2), 23.5 (—CH_2), 21.0 (—$COCH_3$), 20.5 (—$COCH_3$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{97}H_{107}N_3O_{25}Na$, 1736.7086; found, 1736.7130.



*2-O-Benzyl-3-O-p-methoxybezyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**24**).* To a stirred solution of disaccharide **S2a** (650 mg, 0.683 mmol) in moist acetic acid (10 mL, acetic acid/water, 10:1 = v/v) were added CH_3COONa (280 mg, 3.42 mmol, 5 equiv) and $PdCl_2$ (242 mg, 1.36 mmol, 2 equiv) at rt. The reaction mixture continued to stir until TLC analysis indicated the disappearance of the starting material (16−20 h). Upon completion, the reaction mixture was diluted with ethyl acetate (20 mL) and poured into saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with ethyl acetate (2 × 25 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography over silica gel (EtOAc/hexanes, 1:2) afforded hemiacetal **24** (431 mg, 70%, white foam) in an *α,β*-mixture. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): (major anomer) *δ* 7.53 (d, *J* = 8.2 Hz, 2H, Ar—*H*), 7.45−7.26 (m, 25H, Ar—*H*), 6.89 (d, *J* = 8.6 Hz, 2H, Ar—*H*), 5.56 (s, 1H, Ph−C*H*), 5.24 (d, *J* = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.14 (d, *J* = 10.7 Hz, 1H, Ar—CH_aH_b), 4.84−4.80 (m, 4H, 2 × Ar—CH_2), 4.75 (d, *J* = 10.6 Hz, 1H, Ar—CH_aH_b), 4.71−4.68 (m, 2H, Ar—CH_2), 4.65 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.57 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.46 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.36 (d, *J* = 11.8 Hz, 1H, —CH_aH_b), 4.12−4.09 (m, 2H, —CH_{ring}, —CH_aH_b), 3.96−3.93 (m, 2H, 2 × —CH_{ring}), 3.81 (s, 3H, —OCH_3), 3.70−3.69 (m, 1H, —CH_{ring}), 3.63−3.52 (m, 4H, 2 × —CH_{ring}, —CH_2), 3.39−3.37 (m, 1H, —CH_{ring}), 3.13−3.10 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): (major anomer) *δ* 159.3 (Ar—*C*), 139.4 (Ar—*C*), 138.7 (Ar—*C*), 138.0 (Ar—*C*), 137.8 (Ar—*C*), 137.7 (Ar—*C*), 130.7 (Ar—*C*), 129.1 (Ar—*C*), 128.7 (Ar—*C*), 128.6 (Ar—*C*), 128.4 (Ar—*C*), 128.3 (Ar—*C*), 128.2 (Ar—*C*), 128.1 (Ar—*C*), 127.8 (Ar—*C*), 127.6 (Ar—*C*), 127.4 (Ar—*C*), 126.2 (Ar—*C*), 113.9 (Ar—*C*), 101.8 (*C*-$1_{\beta\text{-man}}$), 101.5 (PhCH), 91.5 (*C*-1_{glc}), 80.1 (—CH_{ring}), 79.3 (—CH_{ring}), 78.8 (—CH_{ring}), 78.0 (—CH_{ring}), 77.7 (—CH_{ring}), 77.1 (—CH_{ring}), 75.3 (—CH_2), 75.1 (—CH_2), 73.7 × 2 (—CH_2), 72.3 (—CH_2), 70.1 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 67.5 (—CH_{ring}), 55.4 (—OCH_3). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{55}H_{58}O_{12}Na$, 933.3820; found, 933.3828.



*2-O-Benzyl-3-O-p-methoxy bezyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl Fluoride (**14**).* To a stirred solution of hemiacetal **24** (330 mg, 0.362 mmol) in anhydrous CH_2Cl_2 (5 mL) was added DAST (133 *μ*L, 1.08 mmol) at −20 °C under an argon atmosphere. The reaction mixture continued to stir until TLC analysis (EtOAc/hexanes) indicated the disappearance of the starting material (1.5 h). Upon completion, the mixture was diluted with CH_2Cl_2 (10 mL), washed with satd aq $NaHCO_3$ (3 mL) and brine (5 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:5) to give pure compound **14** (302 mg, 92%, white foam) in an *α,β*-mixture (*α*/*β* = 1:2). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): (major anomer) *δ* 7.52 (d, *J* = 6.6 Hz, 2H, 2 × Ar—*H*), 7.43−7.26 (m, 25H, 25 × Ar—*H*), 6.89 (d, *J* = 8.5 Hz, 2H, 2 × Ar—*H*), 5.55 (s, 1H, Ph−C*H*), 5.35 (dd, J_{HF} = 53.1, 6.9 Hz, 1H, C1—$H_{glc\text{-}F}$), 5.06 (d, *J* = 10.9 Hz, 1H, Ar—CH_aH_b), 4.88−4.79 (m, 3H, 3 × Ar—CH_aH_b), 4.75−4.70 (m, 3H, 3 × Ar—CH_aH_b), 4.66 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.58 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.53 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.43 (m 1H, Ar—CH_aH_b), 4.12−4.06 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.81 (s, 3H, —OCH_3), 3.71 (d, *J* = 3.6 Hz, 1H, —CH_{ring}), 3.68−3.48 (m, 6H, 3 × —CH_{ring}, 3 × —CH_aH_b), 3.44 (dd, *J* = 9.8, 3.1 Hz, 1H, —CH_{ring}), 3.14−3.08 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): (major anomer) *δ* 159.3 (Ar—*C*), 138.9 (Ar—*C*), 138.7 (Ar—*C*), 137.8 × 2 (Ar—*C*), 137.7 (Ar—*C*), 130.7 (Ar—*C*), 129.2 (Ar—*C*), 129.0 (Ar—*C*), 128.7 (Ar—*C*), 128.6 (Ar—*C*), 128.5 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 × 3 (Ar—*C*), 128.0 (Ar—*C*), 127.9 (Ar—*C*), 127.8 (Ar—*C*), 126.2 (Ar—*C*), 113.9 (Ar—*C*), 110.4 (d, *C1*-F, J_{C-F} = 216 Hz), 101.7 (C-1_{man}, $^1J_{C-H}$ = 159.7 Hz), 101.5 (Ph−CH), 80.8 (—CH_{ring}), 79.8 (—CH_{ring}), 78.8 (—CH_{ring}), 78.1 (—CH_{ring}), 77.2 (—CH_{ring}), 77.1 (—CH_{ring}), 75.1 (—CH_2), 75.0 (—CH_2), 74.6 (—CH_2), 73.8 (—CH_2), 72.5 (—CH_2), 72.4 (—CH_{ring}), 68.7 (—CH_2), 68.3 (—CH_2), 67.5 (—CH_{ring}), 55.4 (—OCH_3). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{55}H_{57}FO_{11}Na$, 935.3777; found, 935.3783.



*2-O-Benzyl-3-O-p-methoxy Bezyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl Trichloroacetimidate (**25**).* To a stirred solution of hemiacetal **24** (455 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (5 mL) were added trichloroacetonitrile (250 *μ*L, 2.5 mmol, 5 equiv) and DBU (28 *μ*L, 0.2 mmol, 0.4 equiv) at 0 °C under an argon atmosphere. The reaction mixture was stirred at rt for 2 h. Upon completion, the solvent was removed under reduced pressure, and the residue was purified by column chromatography over neutral alumina (EtOAc/hexanes, 2:8) without applying any pressure to afford pure compound **25** (342 mg, 65%, white foam) in an *α,β*-mixture (*α*/*β* = 13:1). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): (*α*-anomer) *δ* 8.58 (s, 1H, —N*H*), 7.47−7.19 (d, m, 27H, Ar—*H*), 6.84 (d, *J* = 8.7 Hz, 2H, Ar—*H*), 6.44 (d, *J* = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.51 (s, 1H, Ph−C*H*), 5.00 (d, *J* = 10.5 Hz, 1H, Ar—CH_aH_b—), 4.85 (d, *J* = 11.8 H, 1H, Ar—CH_aH_b—), 4.77 (m, 2H, Ar—CH_2—), 4.68 (s, 2H, Ar—CH_2—), 4.67 (d, *J* = 11.8, 1H, Ar—CH_aH_b—),

https://dx.doi.org/10.1021/acs.joc.0c01404
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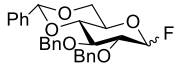
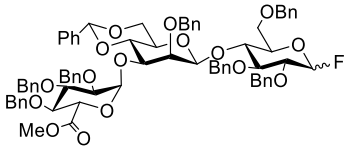
4.56 (d, J = 12.3, 1H, Ar—CH_aH_b—), 4.52 (d, J = 11.6 H, 1H, Ar—CH_aH_b—), 4.43 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.28 (d, J = 11.6 H, 1H, Ar—CH_aH_b—), 4.08 (t, J = 9.1 Hz, 1H, —CH_{ring}), 4.02–3.92(m, 2H, —CH_{ring}, —CH_aH_b—), 3.96 (t, J = 9.1 Hz, 1H, —CH_{ring}), 3.89 (m, 1H, —CH_{ring}), 3.76 (s, 3H, —OCH_3), 3.71 (dd, J = 9.3, 3.5 Hz, 1H, —CH_{ring}), 3.64 (d, J = 3.0 Hz, 1H, —CH_{ring}), 3.58 (t, J = 10.5 Hz, 1H, —CH_aH_b—), 3.54 (dd, J = 11.2, 2.0 Hz, 1H, —CH_aH_b—), 3.45 (dd, J = 11.2, 2.7 Hz, 1H, —CH_aH_b—), 3.35 (dd, J = 10.1, 3.0 Hz, 1H, —CH_{ring}), 3.06–3.02 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): (α-anomer) δ 161.4 (C=NH), 159.3 (Ar—C), 139.3 (Ar—C), 138.7 (Ar—C), 138.2 (Ar—C), 137.8 × 2 (Ar—C), 130.7 (Ar—C), 129.2 (Ar—C), 129.0 (Ar—C), 128.7 (Ar—C), 128.5 × 2 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.2 × 2 (Ar—C), 128.8 × 3 (Ar—C), 127.7 (Ar—C), 127.5 (Ar—C), 126.3 (Ar—C), 113.9 (Ar—C), 101.9 (C-$1_{\beta\text{-man}}$), 101.5 (PhCH—), 94.6 (C-$1_{\alpha\text{-glc}}$), 91.5 (—CCl_3), 79.6 (—CH_{ring}), 78.9 (—CH_{ring}), 78.8 (—CH_{ring}), 78.3 (—CH_{ring}), 77.2 (—CH_{ring}), 77.0 (—CH_{ring}), 75.2 (—CH_2), 75.0 (—CH_2), 73.7 (—CH_2), 73.3 (—CH_2), 72.8 (—CH_{ring}), 72.5 (—CH_2), 68.7 (—CH_2), 68.2 (—CH_2), 67.6 (—CH_{ring}), 5.4 (—OCH_3).



*2,3-Di-O-benzyl-4,6-O-benzylidene-D-glucopyranosyl Fluoride (**13**).* To a stirred solution of tetrasaccharide **S10** (600 mg, 1.23 mmol) in moist acetic acid (11 mL, acetic acid/water, 10:1 = v/v) were added CH_3COONa (500 mg, 6.14 mmol) and $PdCl_2$ (435 mg, 2.45 mmol) at rt. The reaction mixture was continued to stir until TLC analysis indicated the disappearance of the starting material (12 h). Upon completion, the mixture was diluted with ethyl acetate (20 mL) and poured into saturated $NaHCO_3$ (30 mL). The aqueous layer was extracted with ethyl acetate (2 × 25 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography over silica gel (EtOAc/hexanes, 1:4) afforded hemiacetal **S11** (420 mg, 76%, white foam) in an α,β-mixture.

A portion of the above dried hemiacetal **S11** (300 mg, 0.669 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL), and DAST (245 μL, 2 mmol, 3 equiv) was added at −20 °C. The reaction mixture was stirred until TLC analysis indicated the disappearance of the starting material (75 min). Upon completion, the mixture was diluted with CH_2Cl_2 (10 mL), washed with satd aq $NaHCO_3$ (5 mL) and brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:4) to afford pure product **13** (283 mg, 94%, white foam) in an α,β-mixture (α/β = 1:4). NMR is in accordance with the literature.[47] ^1H NMR (600 MHz, $CDCl_3$, 298 K): (major β-anomer) δ 7.52–7.50 (m, 2H, 2 × Ar—H), 7.42–7.29 (m, 13H, 2 × Ar—H), 5.59 (s, 1H, PhCH−), 5.41 (d, J_{HF} = 53.2, 6.1 Hz, 1H, C1—$H_{glc\text{-}F}$), 4.97 (d, J = 11.1 Hz, 1H, Ar—CH_aH_b), 4.80–4.73 (m, 3 H, Ar—CH_aH_b, Ar—CH_2), 4.42 (dd, 1H J = 5.0, 10.5 Hz, —CH_aH_b), 3.88–3.80 (m, 3 H, —CH_aH_b, 2 × —CH_{ring}), 3.67–3.58 (m, 2 H, 2 × —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): (major β-anomer) δ 138.5 (Ar—C), 137.8 (Ar—C), 137.2 (Ar—C), 129.2 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.2 (Ar—C), 128.1(Ar—C), 127.9(Ar—C), 126.1 (Ar—C), 110.3 (d, $C1_{glc\text{-}F}$, J_{C-F} = 217 Hz), 101.5 (Ph−CH), 81.1 (—CH_{ring}), 80.8 (—CH_{ring}), 79.7 (d, J_{C3-F} = 8.8 Hz, —CH_{ring}−CH_{ring}), 74.7 (Ph−CH_2), 74.6 (Ph−CH_2), 68.7 (C−6CH_2), 65.6 (d, J_{C5-F} = 3.6 Hz, —CH_{ring}). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{27}H_{28}FO_5$, 451.1915; found, 451.1917.



*Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-D-glucopyranosyl Fluoride (**15**).* To a stirred solution of trisaccharide **S7** (400 mg, 0.31 mmol, 1 equiv) in moist acetic acid (4.4 mL, acetic acid/water, 10:1 = v/v), CH_3COONa (127 mg, 1.55 mmol, 5 equiv) and $PdCl_2$ (109 mg, 0.62 mmol, 2 equiv) were added at rt. The reaction mixture was stirred until TLC analysis indicated disappearance of starting material (16–20 h). Upon completion, the mixture was diluted with ethyl acetate (15 mL) and poured into saturated $NaHCO_3$ (30 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography over silica gel (EtOAc/hexanes, 1:2) afforded hemiacetal **S12** (285 mg, 74%, white foam) in an α,β-mixture.

A portion of the above dried hemiacetal **S12** (260 mg, 0.21 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL), and DAST (77 μL, 0.62 mmol, 3 equiv) was added at −20 °C. The reaction mixture was stirred until TLC analysis (EtOAc/hexanes) indicated the disappearance of the starting material (1.5 h). Upon completion, the mixture was diluted with CH_2Cl_2 (10 mL), washed with satd aq $NaHCO_3$ (10 mL) and brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:3) to afford pure product **15** (249 mg, 96%, white foam) in an α,β-mixture (α,β = 1:2). ^1H NMR (600 MHz, $CDCl_3$, 298 K): (major anomer) δ 7.52 (d, J = 7.2 Hz, 2H, 2 × Ar—H), 7.40–7.18 (m, 36H, 36 × Ar—H), 7.03 (d, J = 7.3 Hz, 2H, 2 × Ar—H), 5.56 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc-coome}}$), 5.37 (dd, J_{HF} = 53.3, 6.4 Hz, 1H, C1—$H_{glc\text{-}F}$), 5.30 (s, 1H, PhCH—), 5.04 (d, J = 11.0 Hz, 1H, Ar—CH_aH_b), 4.95–4.89 (m, 2H, Ar—CH_2), 4.87- 4.81 (m, 3H, 3 × Ar—CH_aH_b), 4.77–4.68 (m, 5H, 5 × Ar—CH_aH_b), 4.62 (s, 1H, C1—H_{β}-man), 4.58–4.53 (m, 2H, Ar—CH_2), 4.34 (d, J = 12.0 Hz, 1H, Ar—CH_aH_b), 4.28 (d, J = 10.0 Hz, 1H, —CH_{ring}), 4.21 (t, J = 9.5 Hz, 1H, —CH_{ring}), 4.15 (t, J = 9.2 Hz, 1H, —CH_{ring}), 4.08–3.97 (m, 2H, 2 × —CH_{ring}), 3.95–3.91 (m, 1H, —CH_{ring}), 3.90–3.87 (m, 1H, —CH_{ring}), 3.84–3.73 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.69–3.65 (m, 4H, $COOCH_3$, —CH_aH_b), 3.64–3.47 (m, 4H, 2 × —CH_{ring}, 2 × —$CHaH_b$), 3.10–3.05 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): (major anomer) δ 170.0 (Ar—C), 138.9 (Ar—C), 138.6 (Ar—C), 138.5 (Ar—C), 138.2 (Ar—C), 138.1 (Ar—C), 137.9 (Ar—C), 137.8 (Ar—C), 137.4 (Ar—C), 129.5 (Ar—C), 128.8 (Ar—C), 128.7 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.3 × 3 (Ar—C), 128.2 × 2 (Ar—C), 128.1 (Ar—C), 127.9 (Ar—C), 127.8 (Ar—C), 127.7 (Ar—C), 127.5 (Ar—C), 127.3 (Ar—C), 126.5 (Ar—C), 110.4 (d, $C1_{glc\text{-}F}$, J_{C-F} = 218 Hz), 102.4 (Ph−CH), 101.3 $C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 157.5 Hz), 97.4 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 172.2 Hz), 81.8 × 2 (—CH_{ring}), 80.8

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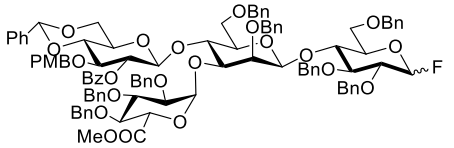
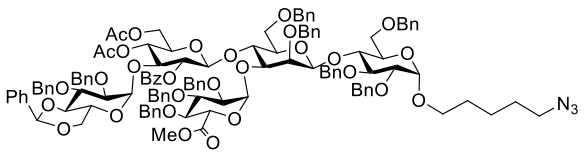
(—CH_{ring}), 80.7 (—CH_{ring}), 79.8 (—CH_{ring}), 79.3 (—CH_{ring}), 78.2 (—CH_{ring}), 78.9 (—CH_{ring}), 78.6 (—CH_{ring}), 76.0 (CH_2), 75.8 (CH_2), 75.3 (—CH_{ring}), 75.3 (CH_2), 74.9 (CH_2), 74.6 (CH_2), 73.8 (CH_2), 71.3 (—CH_{ring}), 71.1 (CH_2), 68.7 (CH_2), 68.2 (CH_2), 67.1 (—CH_{ring}), 52.6 (—$COOCH_3$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{75}H_{77}FO_{16}Na$, 1275.5088; found, 1275.5094.



*2-O-Benzoyl-3-O-p-methoxy-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-D-glucopyranosyl Fluoride (**16**).* To a stirred solution of tetrasaccharide **S9** (300 mg, 0.17 mmol) in moist acetic acid (4.4 mL, acetic acid/water, 10:1 = v/v) were added CH_3COONa (70 mg, 0.85 mmol) and $PdCl_2$ (60 mg, 0.34 mmol) at rt. The reaction mixture was stirred until TLC analysis indicated the disappearance of the starting material (20 h). Upon completion, the mixture was diluted with ethyl acetate (20 mL) and poured into saturated $NaHCO_3$ (30 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography over silica gel (EtOAc/hexanes, 2:3) afforded hemiacetal **S13** (207 mg, 71%, white foam) in an α,β-mixture.

A portion of the above dried hemiacetal **S13** (115 mg, 0.066 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL), and DAST (25 μL, 0.2 mmol, 3 equiv) was added at −20 °C. The reaction mixture was stirred until TLC analysis indicated the disappearance of the starting material (1.5 h). Upon completion, the mixture was diluted with CH_2Cl_2 (10 mL), washed with satd aq $NaHCO_3$ (10 mL) and brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:2) to afford pure product **16** (108 mg, 94%, white foam) in an α,β-mixture (α/β = 1:2). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): (major anomer) δ 7.91−7.89 (m, 2H, Ar—H), 7.65−7.10 (m, 48H, 48 × Ar—H), 7.02 (d, J = 8.6 Hz, 2H, 2 × Ar—H), 6.61 (d, J = 8.6 Hz, 2H, 2 × Ar—H), 5.27−5.17 (m, 3H, C1—$H_{\alpha\text{-glc-COOMe}}$, —$CH_{ring}$, C1—$H_{glc\text{-}F}$, overlapped), 5.03−5.00 (m, 3H, PhCH, Ar—CH_2), 4.90−4.83 (m, 4H, 2 × Ar—CH_2), 4.80−4.68 (m, 4H, 3 × Ar—CH_aH_b, C1—$H_{\beta\text{-glc}}$ (overlapped), 4.66−4.59 (m, 4H, 2 × Ar—CH_2), 4.56−4.46 (m, 4H, 2 × Ar—CH_2), 4.40 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.38−4.34 (m, 1H, —CH_{ring}), 4.32 (m, 1H, —CH_{ring}), 4.20−4.13 (m, 2H, —CH_{ring}, —CH_aH_b), 4.08 (d, J = 11.8 Hz, 1H, Ar—CH_aH_b), 3.97 (t, J = 9.1 Hz, 1H, —CH_{ring}), 3.90−3.81 (m, 3H, 3 × —CH_{ring}), 3.73 (s, 3H, OCH_3), 3.70−3.45 (m, 12H, $COOCH_3$, 5 × CH_{ring}, 2 × —CH_2), 3.39 (t, 1H, J = 12.2 Hz, —CH_aH_b), 3.31−3.27 (m, 1H, —CH_{ring}), 3.19−3.15 (m, 1H, —CH_{ring}), 2.85−2.84(m, 1H, —CH_{ring}). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 170.3 (CO), 164.8 (CO), 159.2 Ar—C), 139.1 × 2 (Ar—C), 138.9 (Ar—C), 138.8 (Ar—C), 138.4 (Ar—C), 138.3 (Ar—C), 137.8 × 2 (Ar—C), 137.6 (Ar—C), 133.4 (Ar—C), 130.4 (Ar—C), 129.9 × 2 (Ar—C), 129.6 × 2 (Ar—C), 128.7 (Ar—C), 128.6 × 3 (Ar—C), 128.5 × 2 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.2 × 3 (Ar—C), 128.1 × 3 (Ar—C), 128.0 × 2 (Ar—C), 127.9 (Ar—C), 127.7 (Ar—C), 127.6 (Ar—C), 127.4 (Ar—C), 126.2 (Ar—C), 113.6 (Ar—C), 110.2 (d, C1-$F_{glc\text{-}F}$, J_{C-F} = 216 Hz), 100.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 160.9 Hz), 100.8 (Ph−CH), 100.4 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 166.2 Hz), 97.6 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 172.6 Hz), 81.6 (—CH_{ring}), 80.8 (—CH_{ring}), 80.1 (—CH_{ring}), 79.3 (—CH_{ring}), 78.8 (—CH_{ring}), 77.7 (—CH_{ring}), 77.4 (—CH_{ring}), 77.0 (—CH_{ring}), 76.7 (—CH_{ring}), 75.7 (—CH_{ring}), 75.6 (CH_2), 75.0 (CH_2), 74.5 (CH_2), 74.4 (CH_2), 74.2 (CH_2), 73.8 (—CH_{ring}), 73.6 (CH_2), 73.5 × 2 (CH_2), 73.4 (CH_2), 72.7 (CH_2), 72.3 (—CH_{ring}), 71.5 (—CH_{ring}), 71.6 (—CH_{ring}), 68.6 (CH_2), 68.4 (CH_2), 66.3 × 2 (—CH_{ring}), 55.3 (—OCH_3), 52.5 (—$COOCH_3$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{103}H_{105}FO_{23}Na$, 1751.6923; found, 1751.6929.



*5-Azidopnetyl 4,6-O-Benzylidine-2,3-di-O-benzyl-α-D-glucopyranosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**29**).* A mixture of silver triflate (AgOTf) (114 mg, 0.44 mmol, 8 equiv with respect to the donor), bis (cyclopentadienyl) hafnium dichloride (CP_2HfCl_2) (85 mg, 0.22 mmol, 4 equiv with respect to the donor), and freshly activated 4 Å molecular sieves (200 mg) in dry toluene (2 mL) was stirred at room temperature under an argon atmosphere for 30 min. Then the reaction mixture was cooled to −30 °C, and a solution of donor **13** (25 mg, 0.055 mmol, 1 equiv) and acceptor **7** (47.5 mg, 0.027 mmol, 0.5 equiv) in dry toluene (1.5 mL) was added. Stirring was continued at the same temperature for 2 h. Then, the reaction mixture was quenched with Et_3N (100 μL), diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed twice with satd aq $NaHCO_3$ and brine solution. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The obtained residue was purified by open column chromatography over silica gel (EtOAc/hexanes, 1:2) to afford pure pentasaccharide **29** (42 mg, 70%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_{3\beta}$ 298 K): δ 7.89 (d, J = 7.2 Hz, 2H, Ar—H), 7.62−7.05 (m, 58H, Ar—H), 5.38 (s, 1H, Ph−CH), 5.15 (d, J = 3.3 Hz, 1H, C1—$H_{\alpha\text{-glc-COOMe}}$12 (d, 1H, J = 11.8 Hz, −ArCH_aH_b), 5.03−4.98 (m, 4H, Ar—CH_2, —CH_aH_b, —CH_{ring}), 4.83 (d, J = 8.9 Hz, 1H, Ar—CH_aH_b), 4.82 (d, J = 8.9 Hz, 1H, Ar—CH_aH_b), 4.79−4.70 (m, 7H, 2 × Ar—CH_2, C1—$H_{\alpha\text{-glc-linker}}$, —$CH_aH_b$, —$CH_{ring}$), 4.69−4.61 (m, 5H, 2 × Ar—CH_2, C1—$H_{\beta\text{-glc}}$), 4.59−4.57 (m, 3H, Ar—CH_2, Ar—CH_aH_b), 4.52 (d, J = 3.4 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 4.50 (d, J = 9.6 Hz, 1H, Ar—CH_aH_b), 4.39 (t, J = 9.3 Hz, 1H, —CH_{ring}), 4.32 (d, J = 11.6 Hz, 1H, Ar—CH_aH_b), 4.29 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.17−4.13 (m, 2H, —CH_2), 4.11−4.06 (m, 2H, —CH_aH_b, —CH_{ring}), 4.05 (d, J = 11.6 Hz, 1H, Ar—CH_aH_b), 3.86−3.79 (m, 5H, 5 × CH_{ring}), 3.77−3.73 (m, 1H, —CH_{ring}), 3.72−3.67 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.66−3.22 (m, 2H, —CH_2), 3.60−3.57 (m, 4H, COOMe, —CH_aH_b), 3.56 (t, J = 8.9 Hz, 1H, —CH_{ring}), 3.51−3.45 (m, 2H, 2 × —CH_{ring}), 3.43−3.39 (m, 2H, —CH_2), 3.35 (t, J = 9.3 Hz, 1H, —CH_{ring}), 3.30 (dd, J = 3.1, 9.2 Hz, 1H, —CH_{ring}), 3.25−3.22 (m, 3H, —CH_2, —CH_aH_b), 3.19 (dd, J = 2.4, 9.2 Hz, 1H, —CH_{ring}), 2.86 (d, J = 7.9 Hz, 1H, —CH_{ring}), 1.78 (s, 3H, —$COCH_3$), 1.72

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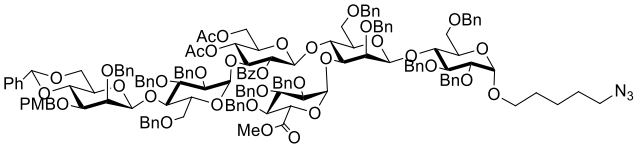
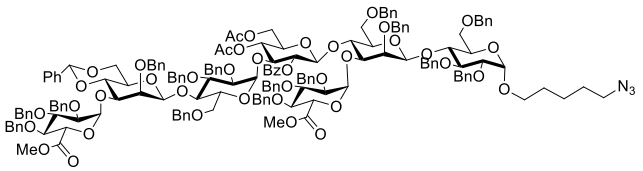
(s, 3H, —COCH_3), 1.67−1.60 (m, 4H, 2 × —C$H_{2linker}$), 1.47−1.41 (m, 2H,−C$H_{2linker}$). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 171.1 (*C*O), 170.6 (*C*O), 169.5 (*C*O), 164.5 (*C*O), 140.0 (Ar—*C*), 139.2 (Ar—*C*), 138.9 (Ar—*C*), 138.8 (Ar—*C*), 138.7 × 2 (Ar—*C*), 138.6 (Ar—*C*), 138.5 × 2 (Ar—*C*), 137.8 (Ar—*C*), 137.7 (Ar—*C*), 133.3 (Ar—*C*), 130.1 (Ar—*C*), 129.5 (Ar—*C*), 128.9 (Ar—*C*), 128.8 × 2 (Ar—*C*), 128.7 × 2 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 3 (Ar—*C*), 128.4 × 4 (Ar—*C*), 128.3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 128.0 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 (Ar—*C*), 127.2 (Ar—*C*), 127.0 (Ar—*C*), 126.9 (Ar—*C*), 126.4 (Ar—*C*), 101.7 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.6 Hz), 101.5 (Ph−*C*H), 100.6 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 171.0 Hz), 99.7 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 160.2 Hz), 99.3 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 169.8 Hz), 97.2 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 168.8 Hz), 82.6 (—CH_{ring}), 82.2 (—CH_{ring}), 80.8 (—CH_{ring}), 80.7 (—CH_{ring}), 80.6 (—CH_{ring}), 80.5 (—CH_{ring}), 79.3 × 2 (—CH_{ring}), 79.1 (—CH_{ring}), 78.2 (—CH_{ring}), 78.1 (—CH_{ring}), 77.9 (—CH_{ring}), 76.1 (—CH_{ring}), 75.4 (—CH_2), 75.3 (—CH_2), 75.0 (—CH_2), 74.7 (—CH_2), 74.2 (—CH_2), 73.7 (—CH_2), 73.6 (—CH_2), 73.5 × 3 (2 × —CH_2, —CH_{ring}), 71.8 × 2 (—CH_{ring}), 71.7 (—CH_2), 71.4 (—CH_{ring}), 70.0 (—CH_{ring}), 69.1 (—CH_{ring}), 68.6 (—CH_2), 68.4 (—CH_2), 68.2 (—CH_2), 63.7 (—CH_{ring}), 62.9 (—CH_2), 52.4 (—COOCH_3), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2), 20.8 (—COCH_3), 20.6 (—COCH_3). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{124}H_{133}N_3O_{30}Na$, 2166.8866; found, 2166.8917.



5-Azidopnetyl 2-O-Benzyl-3-O-p-methoxy-4,6-O-benzylidine-β-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (***26a***). A mixture of silver triflate (AgOTf) (170 mg, 0.66 mmol, 8 equiv with respect to the donor), bis (cyclopentadienyl) hafnium dichloride (CP_2HfCl_2) (125 mg, 0.33 mmol, 4 equiv with respect to the donor), and freshly activated 4 Å molecular sieves (300 mg) in dry toluene (4 mL) was stirred at room temperature under an argon atmosphere for 30 min. Then the reaction mixture was cooled to −30 °C, and a solution of donor **14** (75 mg, 0.082 mmol, 1 equiv) and acceptor **7** (70 mg, 0.041 mmol, 0.5 equiv) in dry toluene (3 mL) was added. Stirring was continued at the same temperature for 2 h, quenched with Et_3N (150 μL), diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed twice with satd aq $NaHCO_3$ and brine solution. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The obtained residue was purified by open column chromatography over silica gel (EtOAc/hexanes, 1:2) to afford pure hexasaccharide **26a** (69 mg, 64%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.89 (d, *J* = 7.2 Hz, 2H, Ar—*H*), 7.43−7.39 (m, 5H, Ar—*H*), 7.35−7.14 (m, 56H, Ar—*H*), 7.09−7.07 (m, 5H, Ar—*H*), 7.04−7.00 (m, 4H, Ar—*H*), 6.82 (d, *J* = 8.8 Hz, 2H, Ar—*H*), 5.43 (s, 1H, Ph−C*H*), 5.11−5.10 (m, 2H, C1—$H_{\alpha\text{-glc-COOMe}}$, —C$H_{ring}$), 5.05 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.94−4.91 (m, 2H, Ar—CH_2), 4.90 (d, *J* = 12.2 Hz, 1H, Ar—CH_aH_b), 4.77−4.70 (m, 5H, 2 × Ar—CH_2, —CH_{ring}), 4.68−4.63 (m, 4H, C1—$H_{\alpha\text{-glc-linker}}$, Ar—C$H_2$, Ar—C$H_aH_b$), 4.62−4.58 (m, 5H, C1—$H_{\alpha\text{-glc}}$, C1—$H_{\beta\text{-glc}}$ Ar—CH_2, Ar—CH_aH_b), 4.56−4.49 (m, 5H, 2 × Ar—CH_2, Ar—CH_aH_b), 4.47−4.42 (m, 3H, 3 × Ar—CH_aH_b), 4.34−4.31 (m, 1H, —CH_{ring}), 4.27−4.25 (m, 2H, C1−$H_{\beta\text{-man}}$, Ar—CH_aH_b), 4.23 (s, 1H, C1−$H_{\beta\text{-man}}$), 4.16 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.07−3.94 (m, 7H, 3 × —CH_{ring}, Ar—CH_aH_b 3 × —CH_aH_b), 3.78−3.74 (m, 10H, 6 × CH_{ring}, —OCH_3, Ar—CH_aH_b), 3.66−3.54 (m, 9H, 5 × CH_{ring}, 2 × —CH_2), 3.53−3.51 (m 4H, —CH_{ring}, —COOCH_3), 3.44 (dd, 1H, *J* = 9.3, 3.8 Hz, —CH_{ring}), 3.41−3.58 (m, 4H, 2 × —CH_2), 3.26−3.19 (m, 4H, 3 × —CH_{ring}, —CH_aH_b), 3.18 (t, *J* = 6.9 Hz, 2H, —C$H_{2linker}$), 2.98−2.94 (m, 1H, —CH_{ring}), 2.84 (d, 1H, *J* = 9.0, —CH_{ring}), 1.69 (s, 3H, —COCH_3), 1.65 (s, 3H, —COCH_3), 1.60−1.53 (m, 4H, 2 × —C$H_{2linker}$), 1.40−1.36 (m, 2H,−C$H_{2linker}$). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 171.0 (*C*O), 170.5 (*C*O), 169.5 (*C*O), 164.8 (*C*O), 159.3 (Ar—*C*), 139.9 (Ar—*C*), 139.7 (Ar—*C*), 139.1 (Ar—*C*), 138.8 × 2 (Ar—*C*), 138.7 (Ar—*C*), 138.6 (Ar—*C*), 138.5 (Ar—*C*), 138.4 (Ar—*C*), 137.9 × 2 (Ar—*C*), 137.7 (Ar—*C*), 133.5 (Ar—*C*), 130.9 (Ar—*C*), 130.2 (Ar—*C*), 129.8 × 2 (Ar—*C*), 129.1 (Ar—*C*), 128.8 (Ar—*C*), 128.7 × 2 (Ar—*C*), 128.6 (Ar—*C*), 128.5 × 3 (Ar—*C*), 128.4 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 4 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 (Ar—*C*), 127.5 (Ar—*C*), 127.4 (Ar—*C*), 127.2 (Ar—*C*), 127.1 (Ar—*C*), 126.9 (Ar—*C*), 126.3 (Ar—*C*), 113.9 (Ar—*C*), 101.5 (Ph−*C*H), 101.4 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.5 Hz, $C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.1 Hz), 100.1 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 163.5 Hz), 99.8 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 172.6 Hz), 99.0 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 172.3 Hz), 97.2 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 171.1 Hz), 82.0 (—CH_{ring}), 80.7 (—CH_{ring}), 80.2 (—CH_{ring}), 79.9 (—CH_{ring}), 79.5 (—CH_{ring}), 79.3 (—CH_{ring}), 79.2 × 2 (—CH_{ring}), 78.8 (—CH_{ring}), 78.0 (—CH_{ring}), 77.6 (—CH_{ring}), 77.4 (—CH_{ring}), 76.9 (—CH_{ring}), 76.0 (—CH_{ring}), 75.4 × 2 (—CH_2), 75.3 (—CH_2), 75.0 (—CH_2), 74.7 (—CH_2), 74.1 (—CH_2), 73.7 × 2 (—CH_2, —CH_{ring}), 73.6 × 2 (—CH_2), 73.5 × 2 (—CH_2), 72.1 (—CH_2), 72.0 (—CH_2), 71.6 (—CH_{ring}), 71.4 (—CH_{ring}), 70.0 (—CH_{ring}), 69.2 (—CH_{ring}), 68.7 (—CH_2), 68.5 × 2 (—CH_2), 68.1 (CH_2), 67.9 (—CH_2), 67.4 (—CH_{ring}), 62.8 (—CH_2), 55.4 (—OCH_3), 52.4 (—COOCH_3), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2), 20.9 (—COCH_3), 20.6 (—COCH_3). HRMS (ESI) *m*/*z*: [M + 2Na/2]$^+$ calcd for $C_{152}H_{163}N_3O_{36}Na_2$, 1326.0400; found, 1326.0439.



5-Azidopnetyl Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2-O-benzyl-4,6-O-benzylidine-β-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (***30***). A mixture of silver triflate (262 mg, 1.02 mmol, 8 equiv with respect to the donor), bis (cyclopentadienyl) hafnium dichloride (CP_2HfCl_2) (193 mg, 0.51 mmol, 4 equiv with respect to the donor), and freshly activated 4 Å molecular sieves (500 mg) in dry toluene

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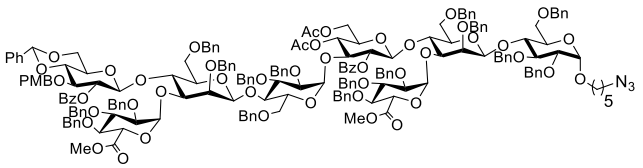
(7 mL) was stirred at room temperature under an argon atmosphere for 30 min. Then the reaction mixture was cooled to −30 °C, and a solution of donor **15** (160 mg, 0.127 mmol, 1 equiv) and acceptor **7** (109 mg, 0.064 mmol, 0.5 equiv) in dry toluene (3 mL) was added. Stirring was continued at the same temperature for 2 h, and the reaction mixture was quenched with Et_3N (160 μL), diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed twice with satd aq $NaHCO_3$ and brine solution. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The obtained residue was purified by open column chromatography over silica gel (EtOAc/hexanes, 1:2) to afford pure heptasaccharide **30** (127 mg, 68%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.91 (d, *J* = 7.1 Hz, 2H, Ar—*H*), 7.47−7.41 (m, 6H, Ar—*H*), 7.32−7.08 (m, 73H, Ar—*H*), 7.05 (t, 2H, *J* = 7.3 Hz, Ar—*H*), 6.98 (d, 2H, *J* = 7.5 Hz, Ar—*H*), 5.49 (d, *J* = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glcCOOMe}}$), 5.19 (s, 1H, Ph−C*H*), 5.18 (t, *J* = 8.9 Hz, 1H, —CH_{ring}), 5.11 (d, *J* = 3.3 Hz, 1H, C1—$H_{\alpha\text{-glcCOOMe}}$), 5.08 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.96−4.86 (m, 5H, 5 × Ar—CH_aH_b), 4.80−4.72 (m, 6H, 3 × Ar—CH_2), 4.70−4.61 (m, 11H, C1—$H_{\alpha\text{-glc-linker}}$, C1—$H_{\alpha\text{-glc}}$, C1—$H_{\beta\text{-glc}}$, 4 × Ar—C$H_2$), 4.56−4.43 (m, 8H, 3 × Ar—CH_2, 2 × —CH_{ring}), 4.37 (t, *J* = 9.0 Hz, 1H, —CH_{ring}), 4.28−4.24 (m, 4H, 2 × C1—$H_{\beta\text{-man}}$, Ar—CH_2), 4.22 (d, *J* = 9.8 Hz, 1H, —CH_{ring}), 4.10−4.00 (m, 5H, 2 × —CH_{ring}, Ar—CH_aH_b −CH_2), 3.96−3.88 (m, 3H, —CH_{ring}, Ar—CH_aH_b −CH_aH_b), 3.81−3.73 (m, 5H, 5 × —CH_{ring}), 3.70−3.62 (m, 11H, 7 × —CH_{ring}, —COOCH_3, —CH_aH_b), 3.60−3.52 (m, 7H, —CH_{ring}, —COOCH_3, 3 × —CH_aH_b), 3.50 (dd, 1H, *J* = 9.7, 3.5 Hz, —CH_{ring}), 3.47 (dd, 1H, *J* = 9.3, 4.1 Hz, —CH_{ring}), 3.44−3.29 (m, 6H, —CH_{ring}, 5 × —CH_aH_b), 3.27 (dd, 1H, *J* = 9.5, 3.1 Hz, —CH_{ring}), 3.20−3.17 (m, 3H, —CH_{ring}, —C$H_{2linker}$), 2.94−2.90 (m, 1H, —CH_{ring}), 2.85 (d, 1H, *J* = 8.8, —CH_{ring}), 1.72 (s, 3H, —COCH_3), 1.67 (s, 3H, —COCH_3), 1.63−1.54 (m, 4H, 2 × —C$H_{2linker}$), 1.42−1.38 (m, 2H,−C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 171.0 (*C*O), 170.5 (*C*O), 170.2 (*C*O), 169.5 (*C*O), 164.8 (*C*O), 140.0 (Ar—*C*), 139.7 (Ar—*C*), 139.2 (Ar—*C*), 138.8 × 3 (Ar—*C*), 138.7 × 3 (Ar—*C*), 138.6 (Ar—*C*), 138.5 (Ar—*C*), 138.3 (Ar—*C*), 138.2 (Ar—*C*), 138.0 (Ar—*C*), 137.8 (Ar—*C*), 137.5 (Ar—*C*), 133.7 (Ar—*C*), 130.1 (Ar—*C*), 129.8 (Ar—*C*), 129.4 (Ar—*C*), 128.9 (Ar—*C*), 128.8 × 2 (Ar—*C*), 128.7 × 2 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 × 3 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 × 3 (Ar—*C*), 128.1 (Ar—*C*), 128.0 (Ar—*C*), 127.9 × 3 (Ar—*C*), 127.8 × 3 (Ar—*C*), 127.7 × 2 (Ar—*C*), 127.6 (Ar—*C*), 127.5 (Ar—*C*), 127.4 (Ar—*C*), 127.3 (Ar—*C*), 127.2 × 2 (Ar—*C*), 127.1 (Ar—*C*), 126.9 (Ar—*C*), 126.5 (Ar—*C*), 102.2 (Ph−*C*H), 101.6 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 157.1 Hz), 100.8 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 158.8 Hz), 99.8 × 2 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 169.1 Hz, $C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 158.4 Hz), 99.1 ($C1_{\alpha\text{-glcCOOMe}}$, $^1J_{C-H}$ = 171.9 Hz), 97.3 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 171.0 Hz), 97.2 ($C1_{\alpha\text{-glclinker}}$, $^1J_{C-H}$ = 174.0 Hz), 81.3 (—CH_{ring}), 80.8 × 2 (—CH_{ring}), 80.2 (—CH_{ring}), 79.6 (—CH_{ring}), 79.5 (—CH_{ring}), 79.4 × 2 (—CH_{ring}), 79.3 × 2 (—CH_{ring}), 79.2 (—CH_{ring}), 79.1 (—CH_{ring}), 78.7 (—CH_{ring}), 78.1 × 2 (—CH_{ring}), 77.4 (—CH_{ring}), 76.6 (—CH_{ring}), 76.4 (—CH_2), 76.1 (—CH_{ring}), 75.9 (—CH_2), 75.4 (—CH_2), 75.3 (—CH_2), 75.2 (—CH_2), 75.1 (—CH_2), 74.7 (—CH_2), 74.1 (—CH_2), 73.7 × 2 (—CH_2), 73.6 × 2 (—CH_2), 73.5 (—CH_2), 72.1 (—CH_2), 72.0 (—CH_{ring}), 71.9 (—CH_{ring}), 71.6 (—CH_{ring}), 71.4 (—CH_{ring}), 71.3 (—CH_{ring}), 71.0 (—CH_2), 70.1 (—CH_{ring}), 69.7 (—CH_{ring}), 68.7 (—CH_2), 68.6 (—CH_2), 68.5 (—CH_2), 68.2 (CH_2), 67.9 (—CH_2), 66.8 (—CH_{ring}), 62.9 (—CH_2), 52.7 (—COOCH_3), 52.4 (—COOCH_3), 51.5 (—CH_2), 29.1 (—CH_2), 28.8 (—CH_2), 23.6 (—CH_2), 20.9 (—COCH_3), 20.6 (—COCH_3). HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{172}H_{183}N_3O_{41}Na$, 2969.2219; found, 2969.2276.



5-Azidopnetyl 2-O-Benzoyl-3-O-p-methoxybenzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (***31***). A mixture of silver triflate (118 mg, 0.462 mmol, 8 equiv with respect to the donor), bis (cyclopentadienyl) hafnium dichloride ($C_{p2}HfCl_2$) (87.7 mg, 0.231 mmol, 4 equiv with respect to the donor), and freshly activated 4 Å molecular sieves (300 mg) in dry toluene (4 mL) was stirred at room temperature under an argon atmosphere for 30 min. Then the reaction mixture was cooled to −30 °C, and a solution of donor **16** (100 mg, 0.058 mmol, 1 equiv) and acceptor **7** (49.5 mg, 0.029 mmol, 0.5 equiv) in dry toluene (3 mL) was added. Stirring was continued at the same temperature for 2 h, and the reaction mixture was quenched with Et_3N (125 μL), diluted with EtOAc, and filtered through a pad of Celite. The filtrate was washed twice with satd aq $NaHCO_3$ and brine solution. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by open column chromatography over silica gel (EtOAc/hexanes, 1:2) to afford pure heptasaccharide **31** (70 mg, 70%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.88 (d, *J* = 7.3 Hz, 2H, Ar—*H*), 7.85 (d, *J* = 7.5 Hz, 2H, Ar—*H*), 7.61−7.55 (m, 2H, Ar—*H*), 7.44−7.15 (m, 97H, Ar—*H*), 6.55 (d, 2H, *J* = 8.4 Hz, Ar—*H*), 5.18 (t, 1H, *J* = 8.7 Hz, —CH_{ring}), 5.10 (d, *J* = 3.4 Hz, 1H, C1—$H_{\alpha\text{-glcCOOMe}}$), 5.09 (d, *J* = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glcCOOMe}}$), 5.07−5.00 (m, 3H, —C$H_{ring}$, Ar—C$H_2$), 4.97.4.89 (m, 6H, Ph−C*H*, 2 × Ar—CH_2, —CH_{ring}), 4.86−4.71 (m, 7H, 3 × Ar—CH_2, —CH_{ring}), 4.69−4.66 (m, 4H, C1—$H_{\alpha\text{-glclinker}}$, —C$H_{ring}$, Ar—C$H_2$), 4.64−4.59 (m, 5H, C1—$H_{\beta\text{-glc}}$, 2 × Ar—C$H_2$), 4.59−4.50 (m, 7H, C1—$H_{\alpha\text{-glc}}$ (overlapped), C1—$H_{\beta\text{-glc}}$ (overlapped), 2 × Ar—CH_2, Ar—CH_aH_b), 4.49−4.36 (m, 6H, 2 × Ar—CH_2, 2 × —CH_{ring}), 4.33−4.25 (m, 4H, C1—$H_{\beta\text{-man}}$ (overlapped), —CH_2, —CH_aH_b), 4.14−4.06 (m, 4H, C1—$H_{\beta\text{-man}}$ (overlapped), 2 × —CH_{ring}, —CH_aH_b), 4.05−3.97 (m, 5H, 2 × Ar—CH_2, —CH_{ring}), 3.80−3.73 (m, 5H, 5 × —CH_{ring}), 3.72−3.66 (m, 6H, —COOCH_3, 2 × —CH_{ring}, Ar—CH_aH_b), 3.64−3.45 (m, 20H, 3 × —CH_2, 2 × —COCH_3, 8 × —CH_{ring}), 3.41−3.29 (m, 5H, 2 × —CH_2, —CH_{ring}), 3.22−3.15 (m, 6H, 2 × —CH_2, 2 × —CH_{ring}), 3.13−3.06 (m, 2H, 2 × —CH_{ring}), 2.84 (d, 1H, *J* = 8.8, —CH_{ring}), 2.71 (d, 1H, *J* = 8.5, —CH_{ring}), 1.65 (s, 3H, —COCH_3), 1.64 (s, 3H, —COCH_3), 1.61−1.54 (m, 4H, 2 × —C$H_{2linker}$), 1.42−1.37 (m, 2H,−C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 171.0 (*C*O), 170.5 (*C*O), 170.4 (*C*O), 169.5 (*C*O), 164.9 (*C*O), 164.8 (*C*O), 159.1 (Ar—*C*), 140.0 (Ar—*C*), 139.9 (Ar—*C*), 139.2 (Ar—*C*), 139.1 (Ar—*C*), 139.0

https://dx.doi.org/10.1021/acs.joc.0c01404
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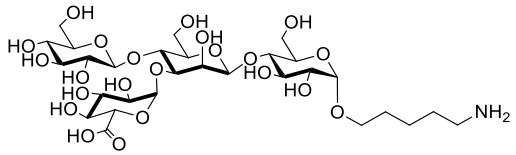
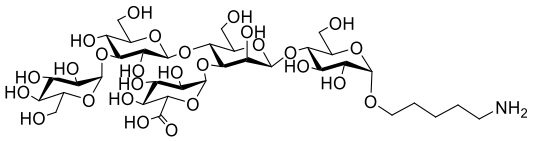
(Ar—*C*), 138.9 (Ar—*C*), 138.8 × 2 (Ar—*C*), 138.7 (Ar—*C*), 138.6 × 3 (Ar—*C*), 138.5 × 2 (Ar—*C*), 137.8 (Ar—*C*), 137.7 × 2 (Ar—*C*), 133.5 (Ar—*C*), 133.3 (Ar—*C*), 130.5 (Ar—*C*), 130.1 (Ar—*C*), 130.0 (Ar—*C*), 129.8 (Ar—*C*), 129.7 (Ar—*C*), 129.6 (Ar—*C*), 129.0 (Ar—*C*), 128.9 (Ar—*C*), 128.8 (Ar—*C*), 128.7 × 2 (Ar—*C*), 128.6 (Ar—*C*), 128.5 × 4 (Ar—*C*), 128.4 × 3 (Ar—*C*), 128.3 × 3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 × 2 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 × 4 (Ar—*C*), 127.8 (Ar—*C*), 127.7 × 3 (Ar—*C*), 127.6 (Ar—*C*), 127.5 (Ar—*C*), 127.4 × 2 (Ar—*C*), 127.3 × 2 (Ar—*C*), 127.2 × 2 (Ar—*C*), 127.1 × 2 (Ar—*C*), 127.1 (Ar—*C*), 127.0 (Ar—*C*), 126.9 (Ar—*C*), 126.8 (Ar—*C*), 126.7 (Ar—*C*), 126.2 (Ar—*C*), 113.6 (Ar—*C*), 101.6 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 159.5 Hz), 101.3 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 158.6 Hz), 100.9 (Ph−*C*H), 100.2 × 2 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.1 Hz, $C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.1 Hz), 99.8 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 168.3 Hz), 99.2 ($C1_{\alpha\text{-glcCOOMe}}$, $^1J_{C-H}$ = 171.1 Hz), 98.1 ($C1_{\alpha\text{-glcCOOMe}}$, $^1J_{C-H}$ = 173.6 Hz), 97.2 ($C1_{\alpha\text{-glclinker}}$, $^1J_{C-H}$ = 171.1 Hz), 82.2 (—CH$_{ring}$), 81.5 (—CH$_{ring}$), 80.7 × 3 (—CH$_{ring}$), 80.2 (—CH$_{ring}$), 80.1 (—CH$_{ring}$), 80.0 (—CH$_{ring}$), 79.9 (—CH$_{ring}$), 79.5 × 2 (—CH$_{ring}$), 79.3 × 2 (—CH$_{ring}$), 79.2 (—CH$_{ring}$), 78.3 (—CH$_{ring}$), 78.1 (—CH$_{ring}$), 77.7 (—CH$_{ring}$), 77.4 × 2 (—CH$_{ring}$), 76.2 (—CH$_{ring}$), 76.1 (—CH$_{ring}$), 75.6 (—CH$_2$), 75.4 (—CH$_2$), 75.3 (—CH$_2$), 75.0 (—CH$_2$), 74.9 (—CH$_2$), 74.7 (—CH$_2$), 74.4 (—CH$_2$), 74.1 (—CH$_2$), 73.8 (—CH$_{ring}$), 73.7 (—CH$_2$), 73.6 (—CH$_{ring}$), 73.5 × 2 (—CH$_2$), 73.4 (—CH$_2$), 73.3 (—CH$_2$), 72.4 (—CH$_2$), 72.1 (—CH$_{ring}$), 72.0 (—CH$_{ring}$), 71.9 (—CH$_{ring}$), 71.8 (—CH$_2$), 71.7 (—CH$_{ring}$), 71.4 (—CH$_{ring}$), 71.3 (—CH$_{ring}$), 70.1 (—CH$_{ring}$), 69.3 (—CH$_{ring}$), 68.6 (—CH$_2$), 68.5 (—CH$_2$), 68.4 (—CH$_2$), 68.2 × 2 (—CH$_2$), 67.8 (—CH$_2$), 66.1 (—CH$_{ring}$), 62.9 (—CH$_2$), 55.3 (—OCH$_3$), 52.5 (—COOCH$_3$), 52.4 (—COOCH$_3$), 51.5 (—CH$_2$), 29.9 (—CH$_2$), 29.5 (—CH$_2$), 29.1 (—CH$_2$), 28.8 (—CH$_2$), 23.6 (—CH$_2$), 20.7 (—COCH$_3$), 20.5 (—COCH$_3$). HRMS (ESI) *m/z*: $[M + 2Na/2]^+$ calcd for $C_{200}H_{211}N_3O_{48}Na_2$, 1734.1973; found, 1734.2006.



5-Aminopnetyl β-D-Glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyra-nosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (1). To a well-stirred solution of tetrasaccharide **6** (90 mg, 0.049 mmol) in a mixture of *p*-dioxane and H_2O (4 mL, *p*-dioxane/H_2O, 3:1 = v/v) was added LiOH·H_2O (123 mg, 0.29 mmol, 6 equiv). The reaction mixture was heated to 75 °C overnight. Upon completion, the reaction mixture was neutralized with IR-120, filtered, and concentrated. The obtained residue was dissolved in 4 mL of MeOH/THF/H_2O (2:1:1). Then, a drop of HCOOH and 20% $Pd(OH)_2$/C (90 mg, 100 wt %) were added. The reaction mixture was stirred under a H_2 atmosphere for 36 h at rt. Upon completion, it was filtered over a Whatman filter paper and concentrated under reduced pressure. The obtained residue was purified by reverse-phase column chromatography (Bond Eluct-C18) by elution with H_2O. Fractions containing the product were pooled and lyophilized to give the free tetrasaccharide **1** (21.6 mg, 58% over two steps) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.30 (d, *J* = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glcA}}$), 5.05 (d, *J* = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.90 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.65 (d, *J* = 7.9 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.37 (s, 1H, —CH_{ring}), 4.26 (d, *J* = 10.0 Hz, 1H, —CH_{ring}), 4.20 (t, *J* = 9.8 Hz, 1H, —CH_{ring}), 4.17−4.12 (m, 2H, 2 × —CH_aH$_b$), 4.00−3.94 (m, 4H, 2 × —CH_aH$_b$, 2 × —CH_{ring}), 3.93−3.85 (m, 5H, 3 × —CH_aH$_b$, 2 × —CH_{ring}), 3.81 (t, *J* = 9.0 Hz, 1H, —CH_{ring}), 3.76−3.63 (m, 7H, 6 × —CH_{ring}, 1 × —CH_aH$_b$), 3.46 (d, *J* = 10.0 Hz, 1H, —CH_{ring}), 3.43 (d, *J* = 9.0 Hz, 1H, —CH_{ring}), 3.16 (t, *J* = 7.8 Hz, 2H, —CH_2), 1.86−1.78 (m, 4H, 2 × —CH_2), 1.63−1.59 (m, 2H, —CH_2). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.4 (CO), 102.4 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 163.5 Hz), 101.7 ($C1_{\alpha\text{-glcA}}$, $^1J_{C-H}$ = 172.0 Hz), 99.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 163.7 Hz), 98.0 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 172.1 Hz), 81.1 (—CH$_{ring}$), 79.0 (—CH$_{ring}$), 76.8 (—CH$_{ring}$), 75.5 (—CH$_{ring}$), 73.7 (—CH$_{ring}$), 73.5 (—CH$_{ring}$), 73.0 (—CH$_{ring}$), 72.3 (—CH$_{ring}$), 72.0 (—CH$_{ring}$), 71.9 (—CH$_{ring}$), 71.8 (—CH$_{ring}$), 71.0 (—CH$_{ring}$), 70.9 (—CH$_{ring}$), 70.5 (—CH$_{ring}$), 70.1 (—CH$_{ring}$), 68.1 (—CH$_{2linker}$), 61.3 (—CH$_2$), 60.2 (—CH$_2$), 60.0 (—CH$_2$), 39.5 (—CH$_{2linker}$), 28.2 (—CH$_{2linker}$), 26.6 (—CH$_{2linker}$), 22.6 (—CH$_{2linker}$). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{52}NO_{22}$, 766.2975; found, 766.2984.



5-Aminopnetyl α-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (2). To a stirred solution of pentasaccharide **29** (29 mg, 0.0135 mmol) in *p*-dioxane and H_2O (2 mL, *p*-dioxane/H_2O, 3:1 = v/v) was added LiOH·H_2O (68 mg, 0.162 mmol, 12 equiv). The reaction mixture was heated to 75 °C overnight. Upon completion, the mixture was neutralized with IR-120, filtered, and concentrated. The obtained residue was dissolved in 4 mL of a MeOH/THF/H_2O mixture (2:1:1), and a drop of HCOOH and 20% $Pd(OH)_2$/C (29 mg) were added. The reaction mixture was stirred under a H_2 atmosphere for 36 h at rt, filtered over a Whatman filter paper, and concentrated under reduced pressure. The residue was purified by reverse-phase column chromatography (Bond Eluct-C18) by elution with H_2O. Fractions containing the product were pooled and lyophilized to give free pentasaccharide **2** (7.5 mg, 60% over two steps) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.46 (d, *J* = 3.9 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.30 (d, *J* = 3.9 Hz, 1H, C1—$H_{\alpha\text{-glc-A}}$), 5.05 (d, *J* = 3.7 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.91 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.69 (d, *J* = 7.9 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.37 (d, *J* = 2.9 Hz, 1H, —CH_{ring}), 4.26 (d, *J* = 10.1 Hz, 1H, —CH_{ring}), 4.20 (t, *J* = 9.9 Hz, 1H, —CH_{ring}), 4.17−4.12 (m, 3H, —CH_{ring}, 2 × —CH_aH$_b$), 4.00−3.87 (m, 12H, 3 × —CH_2, 1 × CH_aH$_b$, 5 ×CH_{ring}), 3.81−3.59 (m, 10H, 9 × CH_{ring}, 1 × CH_aH$_b$), 3.63 (t, *J* = 9.6 Hz, 1H, —CH_{ring}), 3.55 (t, *J* = 8.6 Hz, 1H, —CH_{ring}), 3.17 (t, *J* = 7.5 Hz, 2H, —C$H_{2linker}$), 1.87−1.79 (m, 4H, 2 × —C$H_{2linker}$), 1.63−1.59 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.5 (CO), 102.5 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 165.1 Hz), 101.7 ($C1_{\alpha\text{-glcA}}$, $^1J_{C-H}$ = 172.8 Hz), 99.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 163.4 Hz), 99.4 ($C1_{-\ \alpha\text{-glc}}$, $^1J_{C-H}$ = 172.0 Hz), 98.0 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 172.1 Hz), 82.5 (—CH$_{ring}$), 81.2 (—CH$_{ring}$), 79.0 (—CH$_{ring}$), 76.4 (—CH$_{ring}$), 75.6 (—CH$_{ring}$), 73.7 (—CH$_{ring}$), 73.0 × 2 (—CH$_{ring}$), 72.4 (—CH$_{ring}$), 72.3 (—CH$_{ring}$), 72.0 (—CH$_{ring}$), 71.9 × 2 (2 × —CH$_{ring}$), 71.8 × 2 (2 × —CH$_{ring}$), 71.1 (—CH$_{ring}$), 70.9 (—CH$_{ring}$), 70.5 (—CH$_{ring}$), 70.4 (—CH$_{ring}$), 69.5 (—CH$_{ring}$), 68.1 (—CH$_{2linker}$), 61.1 (—CH$_2$), 60.4 (—CH$_2$), 60.3 (—

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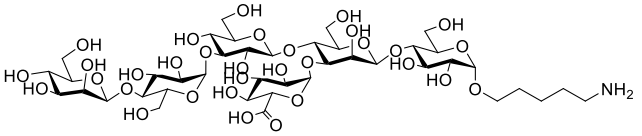
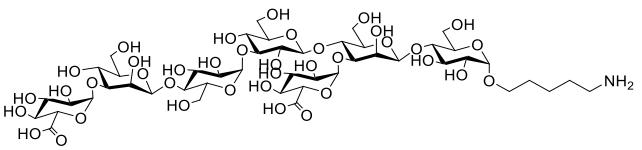
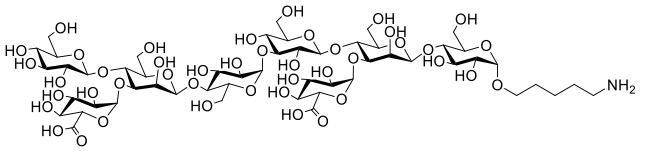
CH_2), 60.1 (—CH_2), 39.5 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 26.6 (—$CH_{2linker}$), 22.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{35}H_{62}NO_{27}$, 928.3504; found, 928.3537.



*5-Aminopnetyl β-D-Mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**3**).* To a stirred solution of hexasaccharide **26** (37 mg, 0.0142 mmol) in a mixture of *p*-dioxane and H_2O (2 mL, *p*-dioxane/H_2O, 3:1 = v/v) was added LiOH·H_2O (71.5 mg, 0.17 mmol, 12 equiv). The reaction mixture was heated to 75 °C overnight. Upon completion, the mixture was neutralized with IR-120, filtered, and concentrated. The residue was dissolved in 4 mL of MeOH/THF/H_2O (2:1:1), and a drop of HCOOH and 20% $Pd(OH)_2/C$ (37 mg) were added. The reaction mixture was stirred under a H_2 atmosphere for 36 h at rt, filtered over a Whatman filter paper, and concentrated under reduced pressure. The obtained residue was purified by reverse-phase column chromatography (Bond Eluct-C18) by elution with H_2O. Fractions containing the product were pooled and lyophilized to give free pentasaccharide **3** (6 mg, 39% over two steps) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.45 (d, J = 3.9 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.30 (d, J = 4.0 Hz, 1H, C1—$H_{\alpha\text{-glcA}}$, 5.05 (d, J = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.90 (s, 2H, 2 × C1-$H_{\beta\text{-man}}$), 4.68 (d, J = 7.9 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.37 (d, J = 3.1 Hz, 1H, —CH_{ring}), 4.26−4.18 (m, 4H, 4 × CH_{ring}), 4.17−4.11 (m, 2H, —CH_2), 4.10 (dd, J = 12.2, 2.0 Hz, 1H, —CH_aH_b), 4.06 (t, J = 9.3 Hz, 1H, —CH_{ring}), 4.01−3.95 (m, 5H, 2 × —CH_{ring}, —CH_aH_b, —CH_2), 3.93−3.87 (m, 7H, —CH_aH_b, 2 × —CH_{ring}, 2 × —CH_2), 3.85−3.77 (m, 4H, 4 × CH_{ring}), 3.76−3.71 (m, 4H, 4 × CH_{ring}), 3.70−3.63 (m, 5H, 4 × CH_{ring}, —CH_aH_b), 3.58−3.55 (m, 1H, —CH_{ring}), 3.55 (t, J = 8.3 Hz, 1H, —CH_{ring}), 3.17 (t, J = 7.6 Hz, 2H, —$CH_{2linker}$), 1.87−1.77 (m, 4H, 2 × —$CH_{2linker}$), 1.63−1.58 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.5 (CO), 102.5 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.9 Hz), 101.7 ($C1_{\alpha\text{-glc-A}}$, $^1J_{C-H}$ = 173.6 Hz), 100.13 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.5 Hz), 99.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.5 Hz), 99.2 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 173.6 Hz), 98.0 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 173.6 Hz), 82.7 (—CH_{ring}), 81.1 (—CH_{ring}), 79.0 (—CH_{ring}), 78.6 (—CH_{ring}), 76.5 (—CH_{ring}), 76.4 (—CH_{ring}), 75.6 (—CH_{ring}), 73.7 (—CH_{ring}), 73.0 × 2 (—CH_{ring}), 72.3 × 2 (—CH_{ring}), 72.0 (—CH_{ring}), 71.9 (—CH_{ring}), 71.8 (—CH_{ring}), 71.6 (—CH_{ring}), 71.5 (—CH_{ring}), 71.0 (—CH_{ring}), 70.9 (—CH_{ring}), 70.7 (—CH_{ring}), 70.5 (—CH_{ring}), 70.4 (—CH_{ring}), 68.1 (—$CH_{2linker}$), 66.8 (—CH_{ring}), 61.1 × 2 (—CH_2), 60.2 (—CH_2), 60.0 (—CH_2), 59.9 (—CH_2), 39.5 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 26.6 (—$CH_{2linker}$), 22.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{41}H_{72}NO_{32}$, 1090.4032; found, 1090.4084.



*5-Aminopnetyl α-D-Glucopyranosyl Uronate-(1→3)-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**4**).* To a stirred solution of heptasaccharide **30** (96 mg, 0.0325 mmol) in a mixture of *p*-dioxane and H_2O (4 mL, *p*-dioxane/H_2O, 3:1 = v/v) was added LiOH·H_2O (20.5 mg, 0.487 mmol, 15 equiv). The reaction mixture was heated to 75 °C overnight. Upon completion, the mixture was neutralized with IR-120, filtered, and concentrated. The residue was dissolved in 4 mL of MeOH/THF/H_2O (2:1:1), and a drop of HCOOH and 20% $Pd(OH)_2/C$ (96 mg) were added. The reaction mixture was stirred under a H_2 atmosphere for 36 h at rt, filtered over a Whatman filter paper, and concentrated under reduced pressure. The obtained residue was purified by reverse-phase column chromatography (Bond Eluct-C18) by elution with H_2O. Fractions containing the product were pooled and lyophilized to give free hexasaccharide **4** (17.5 mg, 42% over two steps) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.44 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.41 (d, J = 4.0 Hz, 1H, C1—$H_{\alpha\text{-glcA}}$), 5.32 (d, J = 4.0 Hz, 1H, C1—$H_{\alpha\text{-glc-A}}$), 5.05 (d, J = 4.1 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.92 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.91 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.68 (d, J = 8.1 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.36−4.32 (m, 4H, 4 × CH_{ring}), 4.23−4.20 (m, 2H, 2 × —CH_{ring}), 4.19−4.07 (m, 4H, 2 × —CH_2), 4.05−3.93 (m, 9H, 2 × —CH_2, 5 × CH_{ring}), 3.92−3.81 (m, 9H, 3 × —CH_aH_b, 6 × CH_{ring}), 3.79−3.72 (m, 5H, 5 × CH_{ring}), 3.71−3.67 (m, 5H, —CH_aH_b, 4 × CH_{ring}), 3.61 (t, J = 7.1 Hz, 1H, —CH_{ring}), 3.54 (t, J = 8.6 Hz, 1H, —CH_{ring}), 3.17 (t, J = 7.6 Hz, 2H, —$CH_{2linker}$), 1.87−1.79 (m, 4H, 2 × —$CH_{2linker}$), 1.64−1.59 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 175.4 (CO), 175.1 (CO), 102.5 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.8 Hz), 101.7 ($C1_{\alpha\text{-glcA}}$, $^1J_{C-H}$ = 172.7 Hz), 100.8 ($C1_{\alpha\text{-glc-A}}$, $^1J_{C-H}$ = 173.1 Hz), 100.0 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.2 Hz), 99.8 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.2 Hz), 99.2 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 172.7 Hz), 98.1 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 168.7 Hz), 82.6 (—CH_{ring}), 81.2 (—CH_{ring}), 78.9 (—CH_{ring}), 78.7 (—CH_{ring}), 76.4 (—CH_{ring}), 76.3 (—CH_{ring}), 75.6 (—CH_{ring}), 73.6 (—CH_{ring}), 72.7 (—CH_{ring}), 72.3 (—CH_{ring}), 72.1 (—CH_{ring}), 71.9 (—CH_{ring}), 71.8 × 2 (—CH_{ring}), 71.7 (—CH_{ring}), 71.6 (—CH_{ring}), 71.5 (—CH_{ring}), 71.1 (—CH_{ring}), 70.9 (—CH_{ring}), 70.7 (—CH_{ring}), 70.5 (—CH_{ring}), 70.4 (—CH_{ring}), 68.1 (—$CH_{2linker}$), 66.0 (—CH_{ring}), 61.1 (—CH_2), 61.0 (—CH_2), 60.3 (—CH_2), 60.0 (—CH_2), 59.9 (—CH_2), 39.5 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 26.6 (—$CH_{2linker}$), 22.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{47}H_{80}NO_{38}$, 1266.4353; found, 1266.4379.



*5-Aminopnetyl-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyl uronate-(1→3)]-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**5**).* To a stirred solution of octasaccharide **31** (35 mg, 0.010 mmol) in a mixture of *p*-dioxane and H_2O (2 mL, *p*-dioxane/H_2O, 3:1 = v/v) was added LiOH·H_2O (77 mg, 0.18 mmol, 18 equiv). The reaction mixture was heated to 75 °C overnight. Upon completion, the mixture was neutralized with IR-120, filtered, and concentrated. The residue was dissolved in 4 mL of MeOH/THF/H_2O (2:1:1), and a drop of HCOOH and 20% $Pd(OH)_2/C$ (35 mg) were added. The reaction mixture was stirred under a H_2

https://dx.doi.org/10.1021/acs.joc.0c01404
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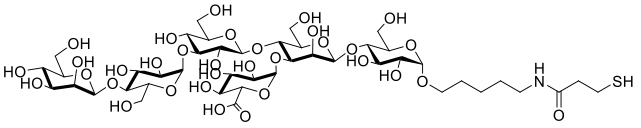
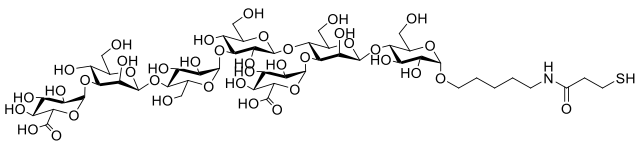
atmosphere for 36 h at rt, filtered over a Whatman filter paper, and concentrated under reduced pressure. The residue was purified by reverse-phase column chromatography (Bond Eluct-C18) by elution with H_2O. Fractions containing the product were pooled and lyophilized to give free hexasaccharide **5** (7 mg, 48% over two steps) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.45 (d, J = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 5.31 (t, J = 4.1 Hz, 2H, 2 × C1—$H_{\alpha\text{-glcA}}$, overlapped), 5.05 (d, J = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 4.93 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.92 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.68 (t, J = 8.2 Hz, 2H, 2 × C1—$H_{\beta\text{-glc}}$, overlapped), 4.38 (t, J = 3.0 Hz, 2H, 2 × —CH_{ring}), 4.27–4.12 (m, 9H, 5 × CH_{ring}, 2 × —CH_2OH), 4.06–3.84 (m, 17H, 8 × CH_{ring}, 4 × CH_2OH, —$CH_aH_{blinker}$), 3.82–3.63 (m, 15H, 14 × CH_{ring}, —$CH_aH_{blinker}$), 3.52 (t, J = 8.5 Hz, 1H, —CH_{ring}), 3.47 (d, J = 10.2 Hz, 1H, —CH_{ring}), 3.43 (d, J = 9.1 Hz, 1H, —CH_{ring}), 3.18 (t, J = 7.7 Hz, 2H, —$CH_{2linker}$), 1.86–1.77 (m, 4H, 2 × —$CH_{2linker}$), 1.67–1.58 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.5 × 2 (CO), 102.6 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 163.7 Hz), 102.4 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 163.7 Hz), 101.7 ($C1_{\alpha\text{-glcA}}$, $^1J_{C-H}$ = 171.4 Hz), 101.6 ($C1_{\alpha\text{-glc-A}}$, $^1J_{C-H}$ = 171.4 Hz), 99.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 160.2 Hz), 99.7 (C $C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 160.2 Hz), 99.2 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 173.2 Hz), 98.0 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 170.3 Hz), 82.5 (—CH_{ring}), 81.3 (—CH_{ring}), 81.0 (—CH_{ring} 78.9 (—CH_{ring}), 78.8 (—CH_{ring}), 76.8 (—CH_{ring}), 76.4 (—CH_{ring}), 75.6 × 3 (—CH_{ring}), 73.7 (—CH_{ring}), 73.6 (—CH_{ring}), 73.0 × 2 (—CH_{ring}), 72.4 (—CH_{ring}), 72.3 (—CH_{ring}), 72.1 (—CH_{ring}), 72.0 (—CH_{ring}), 71.9 × 2 (—CH_{ring}), 71.7 (—CH_{ring}), 71.6 (—CH_{ring}), 71.5 (—CH_{ring}), 71.1 (—CH_{ring}), 70.9 × 2 (—CH_{ring}), 70.5 × 2 (—CH_{ring}), 70.4 (—CH_{ring}), 70.1 (—CH_{ring}), 68.1 (—$CH_{2linker}$), 61.3 (—CH_2), 61.2 (—CH_2), 60.3 (—CH_2), 60.1 × 2 (—CH_2), 59.9 (—CH_2), 39.5 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 26.6 (—$CH_{2linker}$), 22.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{53}H_{90}NO_{43}$, 1428.4881; found, 1428.4924.



*3-Mercapto-N-propanamidopentyl-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**3a**).* To a solution of free amine **3** (3.4 mg, 0.0031 mmol, 1 equiv) in PBS buffer (pH 7.4, 1 mL) was added 3,3′-dithiobis-(sulfosuccinimidylpropionate (DTSSP) (7.6 mg, 0.0124 mmol, 4 equiv) at room temperature. The pH value of the reaction mixture was adjusted to 7 by adding 1 N NaOH (aq) dropwise every 20 min up to 1 h, and the mixture was stirred overnight. Then, dithiothreitol (DTT) (7.2 mg, 0.0465 mmol, 15 equiv) was added to the reaction mixture, which was stirred at 40 °C for another 2 h. The solvent was evaporated under reduced pressure, and the residue was purified by Sephadex LH-20 column chromatography using H_2O as an eluent. Fractions containing the product were pooled and lyophilized to give the desired thiolate compound **3a** (2.94 mg, 80%) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.48 (d, J = 3.1 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.34 (d, J = 2.9 Hz, 1H, C1—$H_{\alpha\text{-glcA}}$, 5.08 (d, J = 3.0 Hz, 1H, C1—$H_{\alpha\text{-glc-linker}}$), 4.94 (s, 2H, 2 × C1—$H_{\beta\text{-man}}$), 4.72 (d, J = 7.7 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.41 (s, 1H, —CH_{ring}), 4.28–4.23 (m, 4H, 4 × CH_{ring}), 4.19–4.06 (m, 4H), 4.05–3.99 (m, 5H), 3.97–3.87 (m, 7H, —CH_aH_b, 2 × —CH_{ring}, 2 × —CH_2), 3.84–3.76 (m, 6H, 4 × CH_{ring}), 3.72–3.66 (m, 5H, 4 × CH_{ring}), 3.61–3.55 (m, 1H, —CH_{ring}), 3.41 (t, J = 6.5 Hz, 1H, —CH_{ring}), 2.97 (t, J = 6.6 Hz, 2H, —CH_2), 2.73 (t, J = 6.6 Hz, 2H, —CH_2), 1.85–1.80 (m, 2H, —$CH_{2linker}$), 1.75–1.71 (m, 2H, —$CH_{2linker}$)1.62–1.52 (m, 2H, —$CH_{2linker}$), ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.4 (CO), 174.2 ($CONH$), 102.4 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 162.9 Hz), 101.6 ($C1_{\alpha\text{-glc-A}}$, $^1J_{C-H}$ = 173.6 Hz), 99.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.5 Hz), 99.7 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 162.5 Hz), 99.1 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 173.6 Hz), 97.8 ($C1_{\alpha\text{-glc-linker}}$, $^1J_{C-H}$ = 173.6 Hz), 82.4 (—CH_{ring}), 80.9 (—CH_{ring}), 78.8 (—CH_{ring}), 78.4 (—CH_{ring}), 76.4 (—CH_{ring}), 76.3 (—CH_{ring}), 75.4 (—CH_{ring}), 73.5 (—CH_{ring}), 72.9 (—CH_{ring}), 72.8 (—CH_{ring}), 72.2 (—CH_{ring}), 71.9 (—CH_{ring}), 71.8 (—CH_{ring}), 71.6 (—CH_{ring}), 71.4 (—CH_{ring}), 71.3 (—CH_{ring}), 70.9 (—CH_{ring}), 70.8 (—CH_{ring}), 70.5 (—CH_{ring}), 70.3 (—CH_{ring}), 70.2 (—CH_{ring}), 68.2 (—$CH_{2linker}$), 66.6 (—CH_{ring}), 60.9 × 2 (—CH_2), 60.0 (—CH_2), 59.9 (—CH_2), 59.7 (—$CH_{2linker}$), 39.4 (—$CH_{2linker}$), 39.2 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 28.05 (—$CH_{2linker}$), 22.7 (—$CH_{2linker}$), 20.0 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{44}H_{75}NO_{33}SNa$, 1200.3834; found, 1200.3860.



*3-Mercapto-N-propanamidopentyl-α-D-glucopyranosyluronate-(1→3)-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**4a**).* To a solution of free amine **4** (15 mg, 0.0118 mmol, 1 equiv) in PBS buffer (pH 7.4, 1 mL) was added 3,3′-dithiobis(sulfosuccinimidylpropionate (DTSSP) (29 mg, 0.0475 mmol, 4 equiv) at room temperature. The pH value of the reaction mixture was adjusted to 7 by adding 1 N NaOH (aq) dropwise every 20 min up to 1 h, and the mixture was stirred overnight. Then, dithiothreitol (DTT) (2.7 mg, 0.177 mmol, 15 equiv) was added to the reaction mixture, which was stirred at 40 °C for another 2 h. The solvent was evaporated under reduced pressure, and the residue was purified by Sephadex LH-20 column chromatography using H_2O as an eluent. Fractions containing the product were pooled and lyophilized to give the desired thiolate compound **4a** (14 mg, 88%) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.44 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.40 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glcA}}$), 5.30 (d, J = 3.8 Hz, 1H, C1—$H_{\alpha\text{-glc-A}}$), 5.04 (d, J = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glclinker}}$), 4.92 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.91 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.68 (d, J = 8.2 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.37 (s, 1H, —CH_{ring}), 4.33 (s, 1H, —CH_{ring}), 4.25–4.20 (m, 4H, 4 × CH_{ring}), 4.18–4.13 (m, 2H), 4.11–4.02 (m, 3H), 4.01–3.82 (m, 17H), 3.82–3.72 (m, 7H), 3.68–3.65 (m, 6H), 3.63 (t, J = 9.4 Hz, 1H, —CH_{ring}), 3.54 (t, J = 8.2 Hz, 1H, —CH_{ring}), 3.37 (t, J = 6.9 Hz, 2H, —$CH_{2linker}$), 2.94 (t, J = 6.6 Hz, 2H, —$CH_{2linker}$), 2.69 (t, J = 6.6 Hz, 2H, —$CH_{2linker}$), 1.83–1.75 (m, 2H, —$CH_{2linker}$), 1.72–1.67 (m, 2H, —$CH_{2linker}$), 1.60–1.52 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.4 (CO), 176.3 (CO), 174.1 ($CONH$), 102.4 ($C1_{\beta\text{-glc}}$), 101.6 ($C1_{\alpha\text{-glcA}}$), 100.6 ($C1_{\alpha\text{-glc-A}}$), 99.8 ($C1_{\beta\text{-man}}$), 99.6 ($C1_{\beta\text{-man}}$), 99.1 ($C1_{\alpha\text{-glc}}$), 97.7 ($C1_{\alpha\text{-glc-linker}}$), 82.3 (—$CH_{ring}$), 81.1 (—$CH_{ring}$), 8.06 (—$CH_{ring}$), 78.7 (—$CH_{ring}$), 78.4 (—$CH_{ring}$), 76.3 (—$CH_{ring}$), 76.1 (—$CH_{ring}$), 75.4 (—$CH_{ring}$), 73.5 (—$CH_{ring}$), 72.9 (—

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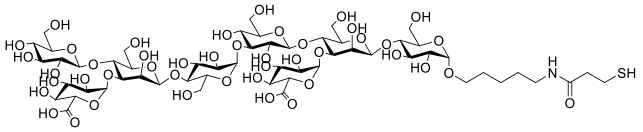
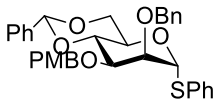
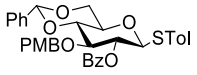
CH_{ring}), 72.6 × 2 (—CH_{ring}), 72.2 (—CH_{ring}), 72.2 (—CH_{ring}), 71.9 (—CH_{ring}), 71.9 (—CH_{ring}), 71.8 (—CH_{ring}), 71.6 (—CH_{ring}), 71.5 (—CH_{ring}), 71.4 (—CH_{ring}), 71.2 (—CH_{ring}), 70.9 (—CH_{ring}), 70.7 (—CH_{ring}), 70.5 (—CH_{ring}), 70.3 (—CH_{ring}), 70.2 (—CH_{ring}), 70.1 (—CH_{ring}), 68.2 (—$CH_{2linker}$), 65.9 (—CH_{ring}), 60.9 (—CH_2), 60.8 (—CH_2), 59.9 (—CH_2), 59.8 (—CH_2), 59.6 (—CH_2), 39.4 (—$CH_{2linker}$), 39.1 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 28.0 (—$CH_{2linker}$), 22.7 (—$CH_{2linker}$), 20.03 (—$CH_{2linker}$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{50}H_{83}NO_{39}SNa$, 1376.4155; found, 1376.4184.



*3-Mercapto-N-propanamidopentyl-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyl uronate-(1→3)]-(1→4)-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-mannopyranosyl-[α-D-glucopyranosyluronate-(1→3)]-(1→4)-α-D-glucopyranoside (**5a**).* To a solution of free amine **5** (9 mg, 0.0063 mmol, 1 equiv) in PBS buffer (pH 7.4, 1 mL) was added 3,3′-dithiobis-(sulfosuccinimidylpropionate (DTSSP) (15.3 mg, 0.025 mmol, 4 equiv) at room temperature. The pH value of the reaction mixture was adjusted to 7 by adding 1 N NaOH (aq) dropwise every 20 min up to 1 h, and the mixture was stirred overnight. Then, dithiothreitol (DTT) (14.5 mg, 0.094 mmol, 15 equiv) was added to the reaction mixture, which was stirred at 40 °C for another 2 h. The solvent was evaporated under reduced pressure, and the residue was purified by Sephadex LH-20 column chromatography using H_2O as an eluent. Fractions containing the product were pooled and lyophilized to give the desired thiolate compound **5a** (7 mg, 84%) in a white powder. ^{1}H NMR (600 MHz, D_2O, 313 K): δ 5.45 (d, *J* = 3.3 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.31 (br s, *J* = 3.5 Hz, 2H, 2 × C1—$H_{\alpha\text{-glcA}}$, overlapped), 5.05 (d, *J* = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 4.93 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.92 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.68 (like t, *J* = 8.3 Hz, 2H, 2 × C1—$H_{\beta\text{-glc}}$, overlapped), 4.38 (s, 2H, 2 × —CH_{ring}), 4.26–4.12 (m, 9H, 5 × CH_{ring}, 2 × —CH_2OH), 4.07–3.85 (m, 17H, 8 × CH_{ring}, 4 × CH_2OH, —$CH_aH_{blinker}$), 3.83–3.74 (m, 6H, —$CH_aH_{blinker}$, 5 × CH_{ring}), 3.78–3.64 (m, 8H, 8 × CH_{ring}), 3.54 (t, *J* = 8.8 Hz, 1H, —CH_{ring}), 3.47–3.42 (m, 2H, 2 × —CH_{ring}), 3.38 (t, *J* = 6.9 Hz, 2H, —$CH_{2linker}$), 2.95 (t, *J* = 6.6 Hz, 2H, —$CH_{2linker}$), 2.71 (t, *J* = 6.6 Hz, 2H, —$CH_{2linker}$), 1.83–1.77 (m, 2H, —$CH_{2linker}$), 1.73–1.68 (m, 2H, —$CH_{2linker}$), 1.607–1.52 (m, 2H, —$CH_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, D_2O, 313 K): δ 176.5 × 2 (*C*O), 174.3 (*C*ONH), 102.5 ($C1_{\beta\text{-glc}}$), 102.4 ($C1_{\beta\text{-glc}}$), 101.7 ($C1_{\alpha\text{-glcA}}$), 101.6 ($C1_{\alpha\text{-glc-A}}$), 99.9 ($C1_{\beta\text{-man}}$), 99.8 ($C1_{\beta\text{-man}}$), 99.2 ($C1_{\alpha\text{-glc}}$), 98.0 ($C1_{\alpha\text{-glc-linker}}$), 82.6 (—$CH_{ring}$), 81.1 (—$CH_{ring}$), 80.9 (—$CH_{ring}$ 79.0 (—CH_{ring}), 78.7 (—CH_{ring}), 76.8 (—CH_{ring}), 76.4 (—CH_{ring}), 75.6 × 3 (—CH_{ring}), 73.7 (—CH_{ring}), 73.6 (—CH_{ring}), 73.5 (—CH_{ring}), 73.0 × 2 (—CH_{ring}), 72.4 (—CH_{ring}), 72.3 × 2 (—CH_{ring}), 72.1 × 3 (—CH_{ring}), 71.9 × 3 (—CH_{ring}), 71.8 (—CH_{ring}), 71.6 (—CH_{ring}), 71.5 (—CH_{ring}), 70.9 (—CH_{ring}), 70.5 (—CH_{ring}), 70.4 (—CH_{ring}), 70.1 (—CH_{ring}), 68.4 (—$CH_{2linker}$), 61.3 (—CH_2), 61.2 (—CH_2), 60.3 (—CH_2), 60.1 × 2 (—CH_2), 59.9 (—CH_2), 39.4 (—$CH_{2linker}$), 39.6 (—$CH_{2linker}$), 28.3 (—$CH_{2linker}$), 28.2 (—$CH_{2linker}$), 22.9 (—$CH_{2linker}$), 20.2 (—$CH_{2linker}$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{56}H_{93}NO_{44}SNa$, 1538.4683; found, 1538.4717.



*Phenyl 2-O-Benzyl-4,6-O-benzylidene-3-p-methoxybenzyl-1-thio-α-D-mannopyranoside (**11**).* To a suspension of NaH (0.9 g, 22.5 mmol, 1.75 equiv) in dry DMF (60 mL) was added alcohol compound **S19** (6 g, 12.86 mmol) dissolved in dry DMF (35 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 10 min; then, benzyl bromide (2.3 mL, 19.2 mmol, 1.5 equiv) was added dropwise. The mixture was stirred for another 2 h at room temperature. Upon completion, the reaction was carefully quenched with MeOH at 0 °C, and the mixture was concentrated *in vacuo*. The residue was diluted with ice-cold water, and the organic material was extracted with Et_2O (3 × 60 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAC/hexanes, 100% hexane followed by1:5) to yield compound **11** (7.25 g, 99%) in a viscus oily compound. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.55 (dd, *J* = 7.9, 1.4 Hz, 2H, Ar—*H*), 7.43–7.28 (m, 15H, Ar—*H*), 6.90 (d, *J* = 8.7 Hz, 2H, Ar—*H*), 5.67 (s, 1H), 5.52 (brd, *J* = 1.3 Hz, 1H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.78 (s, 2H), 4.63 (d, *J* = 11.7 Hz, 1H), 4.33–4.28 (m, 2H), 4.25–4.23 (m, 1H), 4.03 (dd, *J* = 3.2, 1.2 Hz, 1H), 3.98 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.92 (m, 1H), 3.84 (s, 3H, —OCH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.4 (Ar—*C*), 137.9 (Ar—*C*), 137.8 (Ar—*C*), 133.9 (Ar—*C*), 131.8 (Ar—*C*), 130.6 (Ar—*C*), 129.6 (Ar—*C*), 129.3 (Ar—*C*), 129.1 (Ar—*C*), 128.6 (Ar—*C*), 128.4 (Ar—*C*), 128.3 (Ar—*C*), 128.1 (Ar—*C*), 127.8 (Ar—*C*), 126.3 (Ar—*C*), 113.9 (Ar—*C*), 101.7 (PhCH—), 87.3 ($C1_{\alpha\text{-man-SPh}}$), 79.2 (—$CH_{ring}$), 78.3 (—$CH_{ring}$), 76.0 (—$CH_{ring}$), 73.2 (—$CH_2$), 72.9 (—$CH_2$), 68.7 (—$CH_2$), 65.7 (—$CH_{ring}$), 55.5 (—$OCH_3$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{34}H_{34}O_6SNa$, 593.1968; found, 593.2000.



*p-Methylphenyl 2-Benzoyl-4,6-O-benzylidene-3-p-methoxybenzyl-1-thio-β-D-glucopyranoside (**9**).* Compound **S21** (6.74 g, 13.63 mmol) was dissolved in pyridine (60 mL), and DMAP (167 mg, 0.1 equiv) was added. Then, benzoyl chloride (3.17 mL, 27.25 mmol, 2 equiv) was added dropwise over 5 min at 0 °C. The reaction mixture was warmed to rt and stirred for 1 h. Upon completion, methanol was added at 0 °C to decompose the excess BzCl, and the reaction mixture was concentrated *in vacuo*. The obtained white solid was dissolved in CH_2Cl_2 (100 mL) and washed with 1 N HCl (2 × 30 mL), satd aq $NaHCO_3$ (2 × 25 mL), and water (25 mL). The organic layer was dried over $MgSO_4$ and concentrated. The obtained white solid was suspended in CH_2Cl_2/hexane (1:3), and the title compound **9** was obtained after filtration in a white solid (7.7 g, 94%). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.99 (d, *J* = 7.5 Hz, 2H, Ar—*H*), 7.65–7.29 (m, 10H, Ar—*H*), 7.06 (d, *J* = 7.8 Hz, 2H, Ar—*H*), 7.00 (d, *J* = 8.5 Hz, 2H, Ar—*H*), 6.55 (d, *J* = 8.5 Hz, 2H, Ar—*H*), 5.57 (s, 1H), 5.21 (t, *J* = 9.1 Hz, 1H), 4.75 (d, *J* = 10.1 Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.40 (dd, *J* = 10.6, 5.0 Hz, 1H), 3.84–3.79 (m, 2H), 3.77 (t, *J* = 9.3 Hz, 1H), 3.66 (s, 3H, —OCH_3), 3.53–3.49 (m, 1H), 2.30 (s, 3H, —CH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ

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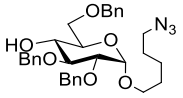
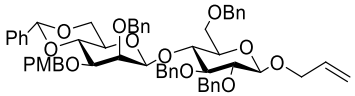
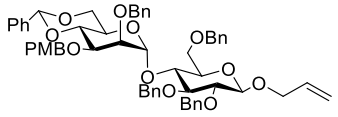
165.1 (—CO), 159.3 (Ar—C), 138.7 (Ar—C), 137.4 (Ar—C), 133.8 (Ar—C), 133.3 (Ar—C), 130.1 × 2 (Ar—C), 130.0 (Ar—C), 129.9 (Ar—C), 129.8 (Ar—C), 129.2 (Ar—C), 128.5 × 2 (Ar—C), 128.4 (Ar—C), 126.2 (Ar—C), 113.7 (Ar—C), 101.4 (PhCH—), 87.3 ($C1_{\beta\text{-glc}}$), 81.6 (—CH_{ring}), 78.9 (—CH_{ring}), 74.0 (—CH_2), 72.2 (—CH_{ring}), 70.8 (—CH_{ring}), 68.8 (—CH_2), 55.3 (—OCH_3), 21.3 (—CH_3). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{35}H_{34}O_7SNa$, 621.1917; found, 621.1937.



*5-Azidopentyl 2,3,6-Tri-O-benzyl-α-D-glucopyranoside (**12**).* A mixture of starting material **S24** (6.5 g, 11.62 mmol, 1 equiv) and 4 Å molecular sieves (7 g) in CH_2Cl_2 (150 mL) was stirred under argon for 1 h. The reaction was cooled to −78 °C, and triethylsilane (5.6 mL, 34.86 mmol, 3 equiv) and trifluoroacetic acid (3.6 mL, 40.67 mmol, 3.5 equiv) were added. The mixture was stirred at −78 °C until TLC analysis indicated the disappearance of the starting material (1 h). The reaction mixture was quenched by the addition of satd aq $NaHCO_3$ (10 mL) and, then, slowly warmed to rt. The mixture was filtered, and the filtrate was washed with saturated $NaHCO_3$ (50 mL) and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:4) to give pure product **12** (5.07 g, 78%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.36−7.23 (m, 15H, Ar—*H*), 5.00 (d, *J* = 11.4 Hz, 1H, Ar—CH_aH_b), 4.74−4.71 (m, 3H, 2 × Ar—CH_aH_b, C1—*H* (overlapped), 4.62 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.57 (d, *J* = 12.2 Hz, 1H, Ar—CH_aH_b), 4.53 (d, *J* = 12.2 Hz, 1H, Ar—CH_aH_b), 3.79 (t, *J* = 9.2 Hz, 1H, —CH_{ring}), 3.72−3.70 (m, 1H, —CH_{ring}), 3.65−3.61 (m, 3H, —C$H_aH_{blinker}$, —CH_2OBn), 3.60 (t, *J* = 9.2 Hz, 1H, —CH_{ring}), 3.52 (dd, *J* = 9.6, 3.6 Hz, 1H, —CH_{ring}), 3.41 (dt, *J* = 9.7, 6.2 Hz, 1H, —C$H_aH_{blinker}$), 3.23 (t, *J* = 6.9 Hz, 2H, —C$H_{2linker}$), 2.36 (brs, 1H,-*OH*), 1.67−1.57 (m, 4H, 2 × —C$H_{2linker}$), 1.46−1.41 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.1 (Ar—C), 138.4 (Ar—C), 138.2 (Ar—C), 128.7 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.2 (Ar—C), 128.1 × 2 (Ar—C), 128.0 (Ar—C), 127.8 × 2 (Ar—C), 97.2 ($C1_{\alpha\text{-glc-linker}}$), 81.6 (—$CH_{ring}$), 79.9 (—$CH_{ring}$), 75.5 (Ar—$CH_2$), 73.7(Ar—$CH_2$), 73.1 (Ar—$CH_2$), 71.0 (—$CH_{ring}$), 70.2 (—$CH_{ring}$), 69.7 (—$CH_2$OBn), 68.1 (—$CH_{2linker}$), 51.5 (—$CH_{2linker}$), 29.1 (—$CH_{2linker}$), 28.8 (—$CH_{2linker}$), 23.6 (—$CH_{2linker}$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{32}H_{39}N_3O_6Na$, 584.2764; found, 584.2731.



*Allyl 2-O-Benzyl-3-O-p-methoxybezyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S2a**).* To a stirred solution of thioglycoside **11** (4.2 g, 7.36 mmol, 1 equiv) were added BSP (1.69 g, 8.1 mmol, 1.1 equiv), TTBP (3.66 g, 14.73 mmol, 2 equiv), and activated 4 Å molecular sieves (9 g) in CH_2Cl_2 (120 mL). The reaction mixture was stirred at rt for 10−15 min under an argon atmosphere and, then, cooled to −60 °C before Tf_2O (1.49 mL, 8.84 mmol, 1.2 equiv) was added. After stirring for another 10−15 min at −60 °C, a solution of glucosyl acceptor **S1** (2.71 g, 5.53 mmol, 0.75 equiv) in CH_2Cl_2 (25 mL) was added slowly at the same temperature. The reaction was stirred for another 3 h at the same temperature and, then, allowed to reach room temperature before it was diluted with CH_2Cl_2, filtered, and washed with excess CH_2Cl_2. The organic layer was washed with saturated sodium bicarbonate solution and brine, then dried over sodium sulfate, evaporated to dryness, and purified by flash chromatography over silica gel (EtOAc/hexanes, 1:5) to afforded the corresponding α-anomer **S2b** (365 mg, viscous oil) and β-anomer **S2a** (3.653 g, viscous oil) compounds in 77% yield (yields based on the acceptor used). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.51 (d, *J* = 7.2 Hz, 2H, Ar—H), 7.43−7.25 (m, 25H, Ar—*H*), 6.88 (d, *J* = 7.7 Hz, 2H, Ar—H), 6.02−5.97 (m, 1H, —C*H*=CH_2), 5.55 (s, 1H, PhC*H*), 5.39 (d, *J* = 17.1 Hz, 1H, =CH_aH_b), 5.25 (d, *J* = 10.5 Hz, 1H, =CH_aH_b), 5.07 (d, *J* = 10.8 Hz, 1H, Ar—CH_aH_b), 4.94 (d, *J* = 10.8 Hz, 1H, Ar—CH_aH_b), 4.88 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.83 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.76 (d, *J* = 10.6 Hz, 2H, Ar—CH_2), 4.69 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.65 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.55−4.53 (m, 2H, C1−$H_{\beta\text{-man}}$ (overlapped), Ar—CH_aH_b), 4.48−4.42 (m, 3H, C1−$H_{\beta\text{-glc}}$, Ar−CH_aH_b, —CH_aH_b), 4.20 (dd, *J* = 12.7, 5.6 Hz, 1H, —CH_aH_b), 4.12−4.07 (m, 2H, —CH_{ring}, —CH_aH_b), 3.97 (t, *J* = 9.2 Hz, 1H, —CH_{ring}), 3.81 (s, 3H, —OCH_3), 3.73 (s, 1H, —CH_{ring}), 3.68 (d, *J* = 10.7 Hz, 1H, —CH_aH_b), 3.62−3.56 (m, 3H, —CH_{ring}, —CH_2), 3.50 (t, *J* = 8.3 Hz, 1H, —CH_{ring}), 3.43 (d, *J* = 9.7 Hz, 1H, —CH_{ring}), 3.38 (d, *J* = 9.7 Hz, 1H, —CH_{ring}), 3.14 (m, 1H, —CH_{ring}). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.3 (Ar—C), 139.3 (Ar—C), 138.8 (Ar—C), 138.6 (Ar—C), 138.0 (Ar—C), 137.8 (Ar—C), 134.2 (CH=), 130.7 (Ar—C), 129.2 (Ar—C), 129.0 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.2 (Ar—C), 128.1 (Ar—C), 129.0 (Ar—C), 127.9 (Ar—C), 127.8 × 2 (2 × Ar—C), 127.6 (Ar—C), 127.4 (Ar—C), 126.3 (Ar—C), 117.4 (=CH_2), 113.9 (Ar—C), 102.9 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 160.1 Hz), 101.9 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 157.8 Hz), 101.5 (PhCH), 83.1 (—CH_{ring}), 81.9 (—CH_{ring}), 78.8 (—CH_{ring}), 78.2 (—CH_{ring}), 78.1 (—CH_{ring}), 77.2 (—CH_{ring}), 75.4 (—CH_2), 75.2 (—CH_2), 75.1 (—CH_2), 74.8 (—CH_{ring}), 73.7 (—CH_2), 72.4 (—CH_2), 70.5 (—CH_2), 68.9 (—CH_2), 68.7 (—CH_2), 67.5 (—CH_{ring}), 55.4 (—OCH_3). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{58}H_{62}O_{12}Na$, 973.4133; found, 973.4139.



*Allyl 2-O-Benzyl-3-O-p-methoxybezyl-4,6-O-benzylidene-α-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S2b**).* ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.53 (d, *J* = 6.6 Hz, 2H, Ar—*H*), 7.40−7.13 (m, 25H, Ar—*H*), 6.86 (d, *J* = 8.3 Hz, 2H, Ar—*H*), 6.02−5.96 (m, 1H, —C*H*=CH_2), 5.63 (s, 1H, PhC*H*—), 5.39 (d, *J* = 15.9 Hz, 1H, =C*H*aH_b), 5.31 (s, 1H, C1—$H_{\alpha\text{-man}}$), 5.25 (d, *J* = 9.26 Hz, 1H, =CH_aH_b), 5.07 (d, *J* = 11.4 Hz, 1H, Ar—CH_aH_b), 4.96 (d, *J* = 10.8 Hz, 1H, Ar—CH_aH_b), 4.75 (d, *J* = 11.8 Hz, 1H, Ar—CH_aH_b), 4.67 (dd, *J* = 12.6, 8.6 Hz, 2H, Ar—CH_2), 4.60 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.58 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.55 (d, *J* = 11.7 Hz, 1H, Ar—CH_aH_b), 4.49 (d, *J* = 7.5 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.46 (dd, *J* = 12.6, 7.7 Hz, 1H, Ar—CH_aH_b), 4.41 (d, *J* = 11.9 Hz, 1H, Ar—CH_aH_b), 4.24−4.13 (m, 4H, 3 × —CH_aH_b, —CH_{ring}), 3.92 (dd, *J* = 9.8, 3.5 Hz,

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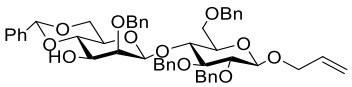
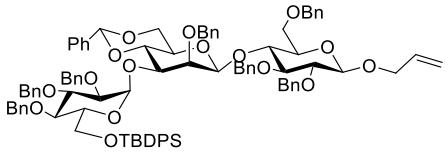
1H, —CH_{ring}), 3.88−3.85 (m, 1H, —CH_{ring}), 3.83−3.78 (m, 6H, —OCH_3, —CH_{ring}, —OCH_2), 3.74−3.71 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.58 (t, J = 9.0 Hz, 1H, —CH_{ring}), 3.53 (t, 1H, —CH_{ring}), 3.47 (m, 1H, —CH_{ring}). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.3 (Ar—C), 138.6 (Ar—C), 138.5 (Ar—C), 138.4 (Ar—C), 138.3 (Ar—C), 137.9 (Ar—C), 134.2 (CH═), 131.0 (Ar—C), 129.3 (Ar—C), 128.9 (Ar—C), 128.6 (Ar—C), 128.5 × 3 (Ar—C), 128.3 × 2 (Ar—C), 127.9 (Ar—C), 127.8 (Ar—C), 127.7 × 2 (Ar—C), 127.5 (Ar—C), 126.9 (Ar—C), 126.3 (Ar—C), 117.4 (=CH_2), 113.8 (Ar—C), 102.7 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 160.1 Hz), 101.6 (PhCH), 101.2 ($C1_{\alpha\text{-man}}$, $^1J_{C-H}$ = 173.6 Hz), 84.5 (—CH_{ring}), 82.3 (—CH_{ring}), 79.1 (—CH_{ring}), 77.9 (—CH_{ring}), 76.7 (—CH_{ring}), 76.1 (—CH_{ring}), 74.9 (—CH_2), 74.8 (—CH_2), 74.6 (—CH_{ring}), 73.7 (—CH_2), 73.4 (—CH_2), 72.8 (—CH_2), 70.5 (—CH_2), 69.5 (—CH_2), 68.8 (—CH_2), 65.4 (—CH_{ring}), 55.4 (—OCH_3). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{58}H_{62}O_{12}Na$, 973.4133; found, 973.4141.



*Allyl 2-O-Benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S3**).* To a stirred solution of starting material **S2a** (1.67 g, 1.83 mmol, 1 equiv) in CH_2Cl_2/Phosphate buffer (pH = 7, 60 mL, 9:1 = v/v), DDQ (625 mg, 2.75 mmol, 1.5 equiv) was added at 0 °C. The reaction mixture was vigorously stirred until TLC analysis (EtOAc/hexanes) indicated the disappearance of starting material (2 h). Upon completion, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with satd aq $NaHCO_3$ and brine. The organic phase was washed with water until the solution colorless became colorless; then, the organic solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:3) to obtain pure compound **S3** (1.215 g, 80%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.50 (d, J = 8.0 Hz, 2H, Ar—H), 7.42−7.28 (m, 23H, Ar—H), 6.03−5.97 (m, 1H, —CH═CH_2), 5.48 (s, 1H, PhCH), 5.40 (dd, J = 17.5, 1.6 Hz, 1H, ═CH_aH$_b$), 5.26 (dd, J = 10.2, 1.0 Hz, 1H, ═CHaH_b), 5.06 (d, J = 10.7 Hz, 1H, Ar—CH_aH$_b$), 4.98 (d, J = 11.5 Hz, 1H, Ar—CH_aH$_b$), 4.95 (d, J = 10.8 Hz, 1H, Ar—CH$_a$H$_b$), 4.77 (m, 3H, 3 × Ar—CH$_a$H$_b$), 4.65−4.63 (m, 2H, C1—$H_{\beta\text{-man}}$ (overlapped), Ar—CH$_a$H$_b$), 4.51−4.45 (m, 3H, C1—$H_{\beta\text{-glc}}$ (overlapped), Ar—CH$_a$H$_b$, —OCH$_a$H$_b$), 4.21−4.17 (m, 1H, —OCH$_a$H$_b$), 4.12 (dd, J = 10.3, 4.7 Hz, 1H, —CH_{ring}), 4.03 (t, J = 9.2 Hz, 1H, —CH_{ring}), 3.75−3.72 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.70−3.67 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.60 (t, 1H, J = 9.0 Hz, —CH_{ring}), 3.58−3.50 (m, 3H, —CH_2, —CH_{ring}), 3.54−3.41(m, 1H, —CH_{ring}), 3.13−3.09 (m, 1H, —CH_{ring}), 2.38 (d, J = 8.5 Hz, 1H, OH). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.3 (Ar—C), 138.6 (Ar—C), 138.3 (Ar—C), 137.8 (Ar—C), 137.4 (Ar—C), 134.2 (CH═), 129.3 (Ar—C), 128.8 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.3 (Ar—C), 128.3 (Ar—C), 128.2 × 2 (Ar—C), 128.1 (Ar—C), 127.8 (Ar—C), 127.5 (Ar—C), 126.5 (Ar—C), 117.5 (=CH_2), 102.8 ($C1_{\beta\text{-glc}}$), 102.2 ($C1_{\beta\text{-man}}$), 101.9 (PhCH), 83.2 (—CH_{ring}), 81.8 (—CH_{ring}), 79.3 (—CH_{ring}), 79.1 (—CH_{ring}), 78.2 (—CH_{ring}), 75.9 (—CH_2), 75.5 (—CH_2), 75.2 (—CH_2), 74.9 (—CH_{ring}), 73.8 (—CH_2), 71.1 (—CH_{ring}), 70.4 (—CH_2), 68.7 (—CH_2), 68.7 (—CH_2), 67.10 (—CH_{ring}). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{50}H_{54}O_{11}Na$, 853.3558; found, 853.3563.



*Allyl 2,3,4-Tri-O-benzyl-6-O-tert-butyldiphenylsilyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S4**).* A mixture of the acceptor **S3** (1.21 g, 1.45 mmol), donor **10** (1.622 g, 2.04 mmol, 1.5 equiv), and activated 4 Å molecular sieves (3 g) in anhydrous CH_2Cl_2 (75 mL) was stirred under an argon atmosphere for 1 h. The reaction mixture was cooled to −40 °C; then, NIS (550 mg, 2.45 mmol, 1.2 equiv with respect to the donor) and TfOH (0.5 M in Et_2O, 1.25 mL, 0.61 mmol, 0.3 equiv with respect to the donor) were added into the mixture. The stirring continued until TLC analysis (EtOAc/hexanes) indicated the disappearance of the starting materials (2 h). Upon completion, the reaction was quenched by the addition of NEt_3 (100 μL), and the reaction mixture was slowly warmed to room temperature before it was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel (EtOAc/hexanes 1:5) to obtain pure product **S4** (2.05 g, 95%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.68 (d, J = 6.7 Hz, 2H, Ar—H), 7.64 (d, J = 6.7 Hz, 2H, Ar—H), 7.45 (d, J = 6.6 Hz, 2H, Ar—H), 7.37−7.11 (m, 42H, Ar—H), 7.08 (d, J = 6.9 Hz, 2H, Ar—H), 5.97−5.90 (m, 1H, CH═), 5.45 (d, J = 3.6 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.33 (m, 1H, ═CHaH$_b$), 5.27 (s, 1H, PhCH), 5.19 (m, 1H, ═CHaH_b), 4.98 (d, J = 10.9 Hz, 1H, Ar—CHaH$_b$), 4.91−4.87 (m, 3H, 3 × Ar—CH$_a$H$_b$), 4.83 (d, J = 11.2 Hz, 1H, Ar—CH_aH$_b$), 4.74 (dd, J = 11.2, 2.7 Hz, 2H, Ar—CH_2), 4.69 (dd, J = 11.2, 2.7 Hz, 2H, Ar—CH_2), 4.60 (d, J = 10.7 Hz, 1H, Ar—CH_aH$_b$), 4.57 (d, J = 11.9 Hz, 1H, Ar—CH$_a$H$_b$), 4.55 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.47 (d, J = 12.0 Hz, 1H, Ar—CH_aH$_b$), 4.41 (d, J = 7.8 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 4.39−4.37 (m, 1H, —CH_aH$_b$), 4.35 (d, J = 12.0 Hz, 1H, Ar—CH_aH$_b$), 4.31 (d, J = 12.0 Hz, 1H, Ar—CH$_a$H$_b$), 4.15−4.10 (m, 2H, —CH_{ring}, —CH_aH$_b$), 3.99 (t, J = 9.2 Hz, 1H, —CH_{ring}), 3.94 (dd, J = 10.4, 4.9 Hz, 1H, —CH$_a$H$_b$), 3.90−3.87 (m, 2H, —CH_{ring}, —CH_aH$_b$), 3.81−3.79 (m, 2H, —CH_{ring}, —CH$_a$H$_b$), 3.71−3.64 (m, 3H, 3 × —CH_{ring}), 3.59−3.55 (m, 2H, —CH_{ring}, —CH_aH$_b$), 3.48−3.41 (m, 4H, 2 × —CH_{ring}, —CH_2), 3.34−3.31 (m, 1H, —CH_{ring}), 3.03−2.99 (m, 1H, —CH_{ring}), 1.02 (s, 9H, 3 × —CH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.4 (Ar—C), 138.9 (Ar—C), 138.7 (Ar—C), 138.6 × 3 (Ar—C), 138.0 (Ar—C), 137.6 (Ar—C), 136.0 (Ar—C), 135.8 (Ar—C), 134.2 (CH═), 133.6 (Ar—C), 133.4 (Ar—C), 129.9 (Ar—C), 129.8 (Ar—C), 129.4 (Ar—C), 128.6 (Ar—C), 128.5 × 2 (Ar—C), 128.4 × 2 (Ar—C), 128.3 × 3 (Ar—C), 128.0 (Ar—C), 127.9 (Ar—C), 127.8 × 2 (Ar—C), 127.7 × 2 (Ar—C), 127.5 (Ar—C), 127.4 × 2 (Ar—C), 127.3 (Ar—C), 126.6 (Ar—C), 117.5 (=CH_2), 102.8 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 161.0 Hz), 102.3 (PhCH), 101.3 ($C1_{\beta\text{-man}}$, $^1J_{C-H}$ = 156.7 Hz), 96.6 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 173.3 Hz), 83.2 (—CH_{ring}), 81.9 (—CH_{ring}), 81.6 (—CH_{ring}), 79.7 (—CH_{ring}), 79.6 (—CH_{ring}), 79.2 (—CH_{ring}), 77.4 × 2 (2 × —CH_{ring}), 75.9 × 2 (2 × CH_2), 75.2 × 2 (2 × CH_2), 75.1 (—CH_2), 74.9 (—CH_{ring}), 74.8 (—CH_{ring}), 73.6 (—CH_2), 72.5 (—CH_{ring}), 71.1 (—CH_2), 70.5 (—CH_2), 68.8 (—CH_2), 68.7 (—CH_2),

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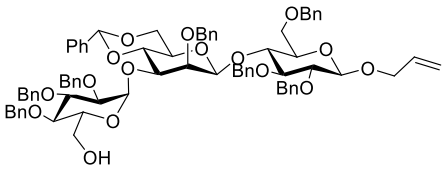
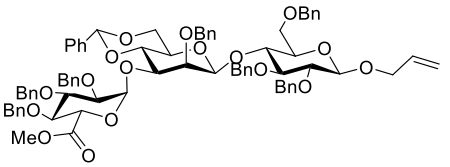
67.10 (—CH_{ring}), 63.18 (—CH_2), 27.1 (—CH_3), 19.5 (*tert-C*). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{93}H_{100}O_{16}SiNa$, 1523.6673; found, 1523.6681.



*Allyl 2,3,4-Tri-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S5**).* To a Nalgene bottle containing silylether compound **S4** (2.05 g, 1.37 mmol, 1 equiv) in THF/pyridine (30 mL, 9:1 = v/v), pyridinium hydorfluoride (HF·py) stock solution (22 mL, 15 equiv; stock solution was prepared from commercially available Aldrich HF·py (70%) by dissolving 15 mL in 30 mL of pyridine, and 75 mL of THF) was added by plastic syringe over 5 min at 0 °C. The reaction was allowed to warm to room temperature and stirred overnight. By then, TLC confirmed all starting material had disappeared. The reaction mixture was poured into cold satd aq $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over $MgsO_4$, filtered and concentrated. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 2:3) to afford pure compound **S5** (1.62 g, 94%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.43 (d, J = 7.2 Hz, 2H, Ar—H), 7.30−7.09 (m, 36H, Ar—*H*), 6.95 (d, J = 7.2 Hz, 2H, Ar—H), 5.93−5.87 (m, 1H, C*H*═), 5.36 (d, J = 3.6 Hz, 1H, C1−$H_{\alpha\text{-glc}}$), 5.30 (dd, J = 17.3, 1.6 Hz, 1H, ═C*H*aH_b), 5.26 (s, 1H, PhC*H*—), 5.16 (dd, J = 10.6, 1.4 Hz, 1H, ═CHaH_b), 4.98 (d, J = 10.7 Hz, 1H, Ar—C*H*aH_b), 4.89−4.81 (m, 4H, 2 × Ar—CH_2), 4.78 (d, J = 11.6, 1H, Hz, Ar—CH_aH_b), 4.69−4.61 (m, 4H, 2 × Ar—CH_2), 4.59 (s, 1H, C1−$H_{\beta\text{-man}}$), 4.53 (d, J = 11.0 Hz, 1H, Ar—C*H*$_a$H$_b$), 4.49 (d, J = 12.3 Hz, 1H, Ar—CH_aH_b), 4.41 (d, J = 11.7 Hz, 1H, Ar—C*H*$_a$H$_b$), 4.39 (d, J = 7.7 Hz, 1H, C1−$H_{\beta\text{-glc}}$), 4.37−4.34 (m, 1H, —C*H*$_a$H$_b$), 4.26 (d, J = 12.2 Hz, 1H, Ar—CH_aH_b), 4.14−4.08 (m, 2H, —CH_{ring}, —CH_aH_b), 3.97−3.89 (m, 3H, 2 × —CH_{ring}, —C*H*$_a$H$_b$), 3.78 (dd, J = 9.8, 3.0 Hz, 1H, —CH_{ring}), 3.73 (d, J = 2.6 Hz, 1H,−CH_{ring}), 3.65−3.61 (m, 3H, —CH_{ring}, 2 × —C*H*$_a$H$_b$), 3.56−3.51 (m, 3H, —CH_{ring}, 2 × -CH_aH_b), 3.47−3.35 (m, 4H, 3 × —CH_{ring}, —CH_aH_b), 3.31−3.29 (m, 1H, —CH_{ring}), 3.09−3.05 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 139.3 (Ar—*C*), 138.8 (Ar—*C*), 138.7 (Ar—*C*), 138.6 (Ar—*C*), 138.4 (Ar—*C*), 138.3 (Ar—*C*), 138.2 (Ar—*C*), 137.5 (Ar—*C*), 134.2 (*C*H═), 129.4 (Ar—*C*), 128.7 (Ar—*C*), 128.6 (Ar—*C*), 128.5 (Ar—*C*), 128.3 × 3 (Ar—*C*), 128.2 × 3 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.7 (Ar—*C*), 127.5 (Ar—*C*), 127.4 (Ar—*C*), 127.3 (Ar—*C*), 126.5 (Ar—*C*), 117.5 (=CH_2), 102.9 (C1$_{\beta\text{-glc}}$, $^1J_{C-H}$ = 160.8 Hz), 102.4 (C1$_{\beta\text{-man}}$, $^1J_{C-H}$ = 163.4 Hz), 101.5 (PhCH), 96.8 (C1$_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 171.6 Hz), 83.1 (—CH_{ring}), 81.9 − (*C*H$_{ring}$), 81.4 (—CH_{ring}), 79.4 (—CH_{ring}), 79.2 (—CH_{ring}), 78.9 (—CH_{ring}), 77.4 (—CH_{ring}), 77.3 (—CH_{ring}), 75.7 × 2 (2 × —CH_2), 75.3 × 2 (—CH_2, —CH_{ring}), 75.2 × 2 (2 × —CH_2), 74.9 (—CH_{ring}), 73.7 (—CH_2), 71.7 (—CH_{ring}), 70.9 (—CH_2), 70.5 (—CH_2), 68.8 (—CH_2), 68.7 (—CH_2), 67.30 (—CH_{ring}), 62.30 (—CH_2). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{77}H_{82}O_{16}Na$, 1285.5495; found, 1285.5503.



*Allyl Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S7**).* Compound **S5** (1.62 g, 1.28 mmol, 1 equiv) was dissolved in a mixture of CH_2Cl_2/H_2O (30 mL, 1:1 = v/v). The reaction mixture was cooled to 0 °C; then, BAIB (1.24 g, 3.85 mmol, 3 equiv) and TEMPO (61 mg, 0.385 mmol, 0.3 equiv) were added. The reaction mixture was allowed to warm to rt and stirred for 2h. Upon completion, the reaction was quenched by the addition of satd aq $Na_2S_2O_3$. The reaction mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (15 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (100% CH_2Cl_2 followed by CH_2Cl_2/MeOH, 98:2) to yield pure compound **S6** (1.488 g, 93%) in cream color foam.

The above purified compound was dissolved in dry DMF (20 mL), and MeI (363 μL, 5.83 mmol, 5 equiv) and K_2CO_3 (483 mg, 3.5 mmol, 3 equiv) were added at room temperature under an argon atmosphere. The reaction mixture was stirred overnight. Upon completion, the solvent was removed under high vacuum, diluted with CH_2Cl_2 (25 mL) and washed with water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (10 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The obtained residue was purified by flash column chromatography (EtOAc/hexanes, 1:3) to yield the pure compound **S7** (1.387 g, 92%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.52 (d, J = 7.2 Hz, 2H, 2 × Ar—*H*), 7.38−7.18 (m, 36H, 36 × Ar—*H*), 7.03 (d, J = 7.3 Hz, 2H, 2 × Ar—*H*), 6.04−5.97 (m, 1H, C*H*═), 5.56 (d, J = 3.5 Hz, 1H, C1—$H_{\alpha\text{-glc-COOMe}}$), 5.40 (dd, J = 17.2, 1.6 Hz, 1H, ═C*H*aH_b), 5.31 (s, 1H, PhC*H*−), 5.26 (dd, J = 10.7, 1.6 Hz, 1H, ═CHaH_b), 5.06 (d, J = 10.8 Hz, 1H, Ar—C*H*$_a$Hb), 4.96 (dd, J = 11.0, 6.5 Hz, 2H, Ar—CH_2), 4.91 (d, J = 11.5 Hz, 1H, Ar—C*H*$_a$H$_b$), 4.86 (d, J = 11.4 Hz, 1H, Ar—C*H*$_a$H$_b$), 4.84 (d, J = 11.0 Hz, 1H, Ar—C*H*$_a$H$_b$), 4.78 (dd, J = 6.8, 10.6 Hz, 2H, Ar—CH_2), 4.73 (s, 1H, Ar—C*H*$_a$H$_b$), 4.71 (s, 1H, Ar—C*H*$_a$H$_b$), 4.65 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.59 (d, J = 11.1 Hz, 1H, Ar—CH_2—), 4.55 (d, J = 12.1 Hz, 1H, Ar—CH_2—), 4.50 (s,1H, Ar—CH_2), 4.49 (s, 1H, C1—$H_{\beta\text{-glc}}$), 4.47−4.45 (m, 1H, Ar—CH_2—), 4.33 (d, J = 12.0 Hz, 1H, Ar—CH_2—), 4.29 (d, J = 9.7 Hz, 1H, —CH_{ring}), 4.21−4.18 (m, 2H, —CH_{ring}, Ar—C*H*$_a$H$_b$—), 4.04−4.01 (m, 2H, —CH_{ring}, Ar—CH_aH_b—), 3.99 (t, J = 9.3 Hz, 1H, —CH_{ring}), 3.88−3.85 (m, 2H, 2 × —CH_{ring}), 3.76−3.72 (m, 2H, —CH_{ring}, Ar—CH_2—), 3.68 (s, 3H, —COOC*H*$_3$), 3.67−3.62 (m, 2H, —CH_{ring}, Ar—CH_aH_b), 3.56−3.49 (m, 3H, 2 × —CH_{ring}, Ar—CH_aH_b—), 3.43−3.40 (m, 1H, —CH_{ring}), 3.10−3.06 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 170.0 (*C*O), 139.3 (Ar—*C*), 138.6 × 3 (Ar—*C*), 137.4 (Ar—*C*), 134.2 (*C*H═), 129.5 (Ar—*C*), 128.7 (Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 (Ar—*C*), 128.0 (Ar—*C*), 127.9 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.5 (Ar—*C*), 127.4 (Ar—*C*), 127.3 (Ar—*C*), 126.5 (Ar—*C*), 117.5 (═CH_2), 102.9 (C1β-glc, $^1J_{C-H}$ = 157.6 Hz), 102.3 (Ph−CH), 101.4 (C1$_{\beta\text{-man}}$, $^1J_{C-H}$

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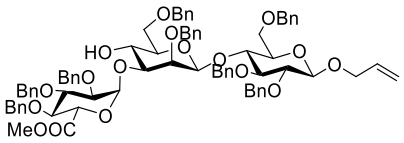
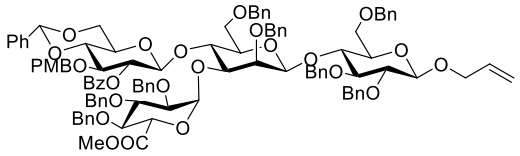
= 158.7 Hz), 97.4 ($C1_{\alpha\text{-glc-COOMe}}$, $^1J_{C-H}$ = 175.8 Hz), 83.0 (—CH_{ring}), 81.9 (—CH_{ring}), 80.7 (—CH_{ring}), 79.3 (—CH_{ring}), 79.2 (—CH_{ring}), 79.1 (—CH_{ring}), 78.6 (—CH_{ring}), 77.8 (—CH_{ring}), 75.9 (—CH_2), 75.8 (—CH_2), 75.4 (—CH_{ring}), 75.3 (—CH_2), 75.2 × 2 (—CH_2), 74.8 (—CH_{ring}), 73.7 (CH_2), 71.2 (—CH_{ring}), 71.0 (—CH_2), 70.5 (—CH_2), 68.7 × 2 (—CH_2), 67.1 (—CH_{ring}), 52.6 (—$COOCH_3$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{78}H_{82}O_{17}Na$, 1313.5444; found, 1313.5463.



*Allyl Methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)-2,6-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S8**).* A mixture of the starting material **S7** (615 mg, 0.476 mmol, 1 equiv) and 4 Å molecular sieves (3 g) in CH_2Cl_2 was stirred under an argon atmosphere for 1 h. The reaction mixture was cooled to −78 °C, and triethylsilane (230 μL, 1.43 mmol, 3 equiv) and TfOH (150 μL, 1.67 mmol, 3.5 equiv) were added. The reaction mixture continued to stir at −78 °C until the complete disappearance of the starting material (monitored by TLC, 1 h). Then, the reaction was quenched by the addition of satd aq $NaHCO_3$. The reaction mixture was warmed to room temperature and filtered, and the filtrate was washed with saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:5) to give pure compound **S8** (578 mg, 89%) as a viscous colorless oil. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.43 (d, *J* = 7.1 Hz, 2H, 2 × Ar—*H*), 7.35−7.19 (m, 38H, 38 × Ar—*H*), 6.02−5.95 (m, 1H, C*H*═), 5.38 (dd, *J* = 17.2, 1.5 Hz, 1H, ═CH_aH_b), 5.24 (dd, *J* = 10.2, 1.5 Hz, 1H, ═CH_aH_b), 5.10−5.09 (m, 2H, C1—$H_{\alpha\text{-glc-COOMe}}$ (overlapped), Ar—CH_aH_b), 4.97−4.91 (m, 3H, Ar—CH_2, Ar—CH_aH_b), 4.83−4.72 (m, 7H, 3 × Ar—CH_2, Ar—CH_aH_b), 4.68 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.60 (d, *J* = 11.2 Hz, 1H, Ar—CH_aH_b), 4.56 (s, 1H, C1—$H_{\beta\text{-man}}$), 4.53 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.46 (s, 1H, C1—$H_{\beta\text{-glc}}$), 4.45−4.42 (m, 2H, Ar—CH_aH_b, —CH_aH_b), 4.40 (d, *J* = 10.1 Hz, 1H, —CH_{ring}), 4.37 (d, *J* = 12.1 Hz, 1H Ar—CH_aH_b), 4.17 (dd, *J* = 12.8, 5.8 Hz, 1H −CH_aH_b), 4.05−3.97 (m, 3H, 3 × —CH_{ring}), 3.78−3.73 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.69−3.61 (m, 7H, 2 × —CH_{ring}, —CH_2, —$COOCH_3$), 3.51−3.46 (m, 3H, 2 × —CH_{ring}, —CH_aH_b), 3.31 (dd, 1H, *J* = 9.3, 2.9 Hz, —CH_{ring}), 3.26 (m, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 170.2 (*C*O), 139.5 (Ar—*C*), 138.9 (Ar—*C*), 138.7 (Ar—*C*), 138.6 (Ar—*C*), 138.5 (Ar—*C*), 138.3 (Ar—*C*), 138.2 (Ar—*C*), 137.4 (Ar—C), 134.3 (*C*H═), 128.7 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 3 (Ar—*C*), 128.4 × 2 (Ar—*C*), 128.3 × 2 (Ar—*C*), 128.2 (Ar—*C*), 128.1 (Ar—*C*), 128.0 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 × 2 (Ar—*C*), 127.7 (Ar—*C*), 127.6 (Ar—*C*), 127.5 (Ar—*C*), 127.2 (Ar—*C*), 117.5 (=CH_2), 102.8 ($C1_{\beta\text{-allylicglc}}$, $^1J_{C-H}$ = 162.6 Hz), 100.7 ($C1_{\beta\text{-man}}$ $^1J_{C-H}$ = 155.8 Hz), 100.6 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 171.4 Hz), 83.9 (—CH_{ring}), 82.7 (—CH_{ring}), 82.0 (—CH_{ring}), 81.5 (—CH_{ring}), 79.7 (—CH_{ring}), 79.1 (—CH_{ring}), 78.7 (—CH_{ring}), 77.2 (—CH_{ring}), 75.2 × 2 (—CH_2), 75.1 (—CH_{ring}), 74.9 (—CH_2), 73.8 (—CH_2), 73.7 (—CH_2), 73.6 (—CH_2), 71.2 (—CH_{ring}), 71.0 (—CH_2), 70.5 (—CH_2), 68.9 × 2 (—CH_2, —CH_{ring}), 52.6 (—$COOCH_3$). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{78}H_{84}O_{17}Na$, 1315.5601; found, 1315.5615.



*Allyl 2-O-Benzoyl-3-O-p-methoxy benzyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-[methyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate-(1→3)]-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**S9**).* A mixture of acceptor **S8** (692 mg, 0.53 mmol), donor **9** (480 mg, 0.8 mmol, 1.5 equiv), and dried 4 Å molecular sieves (1 g) in an anhydrous CH_2Cl_2 (30 mL) was stirred under an argon atmosphere for 30 min. The reaction mixture was cooled to −30 °C. Then, NIS (216 mg, 0.96 mmol, 1.2 equiv with respect to the donor) and TfOH (0.5 M in Et_2O, 480 μL, 0.24 mmol, 0.3 equiv with respect to the donor) were added, and the reaction mixture was stirred at −20 °C until TLC analysis (EtOAc/toluene, 1:10) indicated the disappearance of the starting materials (50 min). Upon completion, the reaction was quenched by the addition of NEt_3 (35 μL). The reaction mixture was slowly warmed to rt. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (EtOAc/toluene, 1:10) to give pure compound **S9** (836 mg, 93%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.89 (d, *J* = 8.0 Hz, 2H, 2 × Ar—*H*), 7.48−7.06 (m, 48H, 48 × Ar—*H*), 7.01 (d, *J* = 8.7 Hz, 2H, 2 × Ar—*H*), 6.60 (d, *J* = 8.6 Hz, 2H, 2 × Ar—*H*), 5.99−5.92 (m, 1H, C*H*═), 5.35 (dd, *J* = 17.1, 1.9 Hz, 1H, ═CH_aH_b), 5.22−5.19 (m, 2H, ═CH_aH_b, —CH_{ring}), 5.18 (d, *J* = 3.1 Hz, 1H, C1—$H_{\alpha\text{-glc}}$), 5.06 (d, *J* = 11.8 Hz, 1H, Ar—CH_aH_b), 5.02 (d, *J* = 11.2 Hz, 1H, Ar—CH_aH_b), 4.99 (s, 1H, PhC*H*—), 4.90−4.82 (m, 5H, 2 × Ar—CH_2, Ar—CH_aH_b), 4.80 (d, *J* = 11.6 Hz, 1H, Ar—CH_aH_b), 4.77 (d, *J* = 12.1 Hz, 1H, Ar—CH_aH_b), 4.68−4.65 (m, 4H, Ar—CH_2, Ar—CH_aH_b, C1—$H_{\beta\text{-glc}}$), 4.61 (d, *J* = 10.8 Hz, 1H, Ar—CH_aH_b), 4.57 (dd, *J* = 11.9, 3.7 Hz, 2H, Ar—CH_2), 4.51 (d, *J* = 12.0 Hz, 1H, Ar—CH_aH_b), 4.48 (d, *J* = 9.4 Hz, 1H, —CH_{ring}), 4.42−4.34 (m, 5H, C1—$H_{\beta\text{-man}}$, C1—$H_{\beta\text{-allylglc}}$, —$CH_{ring}$, Ar—$CH_2$), 4.19−4.10 (m, 3H, —$CH_{ring}$, 2 × —$CH_aH_b$), 4.05 (d, *J* = 11.8 Hz, 1H, —CH_aH_b), 3.84−3.80 (m, 3H, 3 × —CH_{ring}), 3.72 (s, 3H, OCH_3), 3.69 (d, *J* = 10.1 Hz, 1H, —CH_aH_b), 3.65 (dd, *J* = 9.5, 3.0 Hz, 1H, —CH_{ring}), 3.63 (s, 3H, $COOCH_3$), 3.62 (dd, *J* = 11.0, 4.1 Hz, 1H, —CH_aH_b), 3.57−3.51 (m, 5H, 3 × —CH_{ring}, 2 × —CH_aH_b), 3.44 (t, 1H, *J* = 8.0 Hz, —CH_{ring}), 3.39 (dd, 1H, *J* = 9.7, 2.2 Hz, —CH_{ring}), 3.33 (d, *J* = 11.2 Hz, 1H, —CH_aH_b), 3.27 (t, 1H, *J* = 9.3 Hz, —CH_{ring}), 3.16 (m, 1H, —CH_{ring}), 2.81 (d, 1H, *J* = 9.1 Hz, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): δ 170.4 (*C*O), 164.86 (*C*O), 159.1 (Ar—*C*), 139.5 (Ar—*C*), 139.2 (Ar—*C*), 138.9 (Ar—*C*), 138.8 (Ar—*C*), 138.6 (Ar—*C*), 138.5 (Ar—*C*), 138.4 (Ar—*C*), 138.1 (Ar—*C*), 137.7 (Ar—*C*), 134.2 (*C*H═), 133.3 (Ar—*C*), 130.4 (Ar—*C*), 129.9 (Ar—*C*), 129.8 (Ar—*C*), 129.6 (Ar—*C*), 129.0 (Ar—*C*), 128.7 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 3 (Ar—*C*), 128.4 × 2 (Ar—*C*), 128.3 (Ar—*C*), 128.2 × 2 (Ar—*C*), 128.1 (Ar—*C*), 128.0 × 2 (Ar—*C*), 127.9 × 2 (Ar—*C*), 127.8 (Ar—*C*), 127.7 (Ar—*C*), 127.5 (Ar—*C*), 127.3 (Ar—*C*), 127.2 (Ar—*C*), 127.0 (Ar—*C*), 126.2 (Ar—*C*), 117.4 (=CH_2), 113.6

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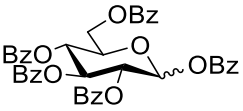
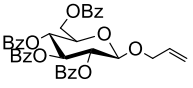
(Ar—*C*), 102.8 ($C1_{\beta\text{-allylicglc}}$, $^1J_{C-H}$ = 162.2 Hz), 101.21 ($C1_{\beta\text{-man}}$ $^1J_{C-H}$ = 158.3 Hz), 100.9 (Ph—*C*H), 100.4 ($C1_{\beta\text{-glc}}$, $^1J_{C-H}$ = 164.2 Hz), 97.6 ($C1_{\alpha\text{-glc}}$, $^1J_{C-H}$ = 171.3 Hz), 82.9 (—CH_{ring}), 81.9 (—CH_{ring}), 81.6 (—CH_{ring}), 80.7 (—CH_{ring}), 80.1 (—CH_{ring}), 79.3 (—CH_{ring}), 78.9 (—CH_{ring}), 78.1 (—CH_{ring}), 77.7 (—CH_{ring}), 77.6 (—CH_{ring}), 75.8 (—CH_{ring}), 75.6 (CH_2), 75.1 (CH_2), 75.0 (CH_2), 74.9 (CH_2), 74.8 (—CH_{ring}), 74.2 (CH_2), 73.8 (—CH_{ring}), 73.5 × 2 (CH_2), 73.4 (CH_2), 72.7 (CH_2), 72.1 (—CH_{ring}), 71.5 (—CH_{ring}), 70.4 (CH_2), 68.8 (CH_2), 68.6 (CH_2), 68.3 (CH_2), 66.2 (—CH_{ring}), 55.3 (—OCH_3), 52.5 (—$COOCH_3$). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{106}H_{110}O_{24}Na$, 1789.7279; found, 1789.7279.

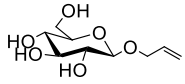


1,2,3,4,6-Penta-O-benzyl-β-D-glucopyranoside (***S14***). D-Glucose (15 g, 83.2 mmol) and a catalytic amount of DMAP (1.02 g, 8.32 mmol, 0.1 equiv) were dissolved in pyridine (175 mL). After cooling the reaction mixture to 0 °C, benzoyl chloride (72 mL, 62.44 mmol, 7.5 equiv) was added, and the mixture was left stirring overnight at rt. Upon completion, pyridine was removed under reduced pressure, and the obtained residue was dissolved in CH_2Cl_2 (175 mL). Then, H_2O (75 mL) was added carefully, and the reaction mixture was stirred vigorously to decompose excess benzyol chloride. The product was extracted with CH_2Cl_2, and the organic phase was washed with satd aq $NaHCO_3$ (75 mL) and brine (50 mL) and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from CH_2Cl_2/hexane (1:3) to give the title compound **S14** (58 g, 99%, white solid) in an *α*,*β*-mixture (*α*/*β*, 6:1). ^1H NMR (600 MHz, $CDCl_3$, 298 K): (major anomer) *δ* 8.15 (dd, *J* = 7.1, 1.3 Hz, 2H, Ar—*H*), 8.01 (dd, *J* = 7.2, 0.9 Hz, 2H, Ar—*H*), 7.94 (dd, *J* = 7.2, 0.9 Hz, 2H, Ar—*H*), 7.87−7.85 (m, 4H, Ar—*H*), 7.65−7.63 (m 1H, Ar—*H*), 7.53−7.27 (m 14H, Ar—*H*), 6.84 (d, *J* = 3.7 Hz, 1H, C1—*H*), 6.32 (q, *J* = 9.9 Hz, 1H, —CH_{ring}), 5.86 (t, *J* = 9.9 Hz, 1H, —CH_{ring}), 5.68 (dd, *J* = 10.2, 3.6 Hz, 1H, —CH_{ring}), 4.62−4.59 (m, 2H, —CH_{ring}, —CH_aH_bOBz), 4.48−4.45 (m, 1H, —CH$_a$$H_b$OBz). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): *δ* 166.3 (*C*O), 166.1 (*C*O), 165.5 (*C*O), 165.3 (*C*O), 164.6 (*C*O), 134.1 (Ar—*C*), 133.7 × 2 (Ar—*C*), 133.5 (Ar—*C*), 133.3 (Ar—*C*), 130.2 (Ar—*C*), 130.1 (Ar—*C*), 130.0 (Ar—*C*), 129.9 × 2 (Ar—*C*), 129.0 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.5 × 2 (Ar—*C*), 90.2 ($C1_{\alpha\text{-glc}}$), 70.6 × 3 (—CH_{ring}), 69.0 (—CH_{ring}), 62.6 (—CH_2).

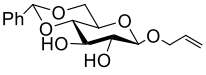


Allyl 2,3,4,6-Tetra-O-benzyl-β-D-glucopyranoside (***S15***). Glucose pentabenzoate **S14** (50 g) was dissolved in CH_2Cl_2 (250 mL), and 100 mL of HBr (33% in AcOH, 2 mL/1 g) was added at 0 °C over 20 min under argon. Then, the reaction mixture was allowed to react for 1 h at the same temperature and for another 3 h at room temperature. Upon completion, the reaction mixture was diluted with 75 mL of CH_2Cl_2 and poured over ice. The organic layer was washed with cold H_2O (2 × 100 mL), satd aq $NaHCO_3$ (2 × 150 mL), and brine (2 × 100 mL) and dried over $MgSO_4$. The sample was filtered and concentrated, and the solid was crystallized from EtOAC/hexane (1:3) to give pure glycosyl bromide (38 g, 79%) in white crystals.

Glycosyl bromide (20 g, 30.3 mmol) and allyl alcohol (2.7 mL, 39.4 mmol, 1.3 equiv) were dissolved in anhydrous CH_2Cl_2 (200 mL) under argon. Freshly activated powdered 4 Å MS (15 g) were added, and the reaction mixture was stirred for 30 min. Then, it was cool to 0 °C, and AgOTf (10.13 g, 39.4 mmol, 1.3 equiv) was added in portions over 10 min; the reaction mixture was stirred overnight at room temperature in darkness. Upon completion, the reaction was quenched by the addition of NEt_3 (4 mL). Then the mixture was stirred for 10 min and filtered through a wet Celite pad. The filtrate was washed with saturated $NaHCO_3$ (2 × 40 mL) and brine (40 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was subjected to flash chromatography on silica gel (EtOAC/hexanes, 1:4) to give pure compound **S15** (17 g, 88%) as a white foam. ^1H NMR (600 MHz, $CDCl_3$, 298 K): *δ* 8.07 (dd, *J* = 8.6, 1.5 Hz, 2H, Ar—*H*), 8.01 (dd, *J* = 8.2, 1.2 Hz, 2H, Ar—*H*), 7.94 (dd, *J* = 8.2, 1.2 Hz, 2H, Ar—*H*), 7.87 (dd, *J* = 8.2, 1.5 Hz, 2H, Ar—*H*), 7.58−7.50 (m 3H, Ar—*H*), 7.46−7.35 (m 7H, Ar—*H*), 7.31−7.28 (m, 2H, Ar—*H*), 5.96 (t, *J* = 9.6 Hz, 1H, —CH_{ring}), 5.86−5.80 (m, 1H, —C*H*═), 5.73 (t, *J* = 9.6 Hz, 1H, —CH_{ring}), 5.62 (dd, *J* = 9.6, 7.7 Hz, 1H, —CH_{ring}), 5.27−5.23 (m, 1H, ═CH_aH_b), 5.16−5.14 (m, 1H, ═CH$_a$$H_b$), 4.95 (d, *J* = 7.8 Hz, 1H, C1—*H*), 4.69 (dd, *J* = 12.1, 3.6 Hz, 1H, —CH_aH_bOBz), 4.56 (dd, *J* = 12.1, 5.6 Hz, 1H, —CH$_a$$H_b$OBz), 4.41−4.38 (m, 1H, —CH_{ring}), 4.22−4.18 (m, 2H, —OCH_2CH═CH). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, 298 K): *δ* 166.3 (*C*O), 166.0 (*C*O), 165.4 (*C*O), 165.3 (*C*O), 133.6 (Ar—*C*), 133.5 (Ar—*C*), 133.4 × 2 (Ar—*C*), 133.3 (Ar—*C*), 130.0 (Ar—*C*), 129.9(Ar—*C*), 129.9 (Ar—*C*), 129.8 (Ar—*C*), 129.5, 128.9 (Ar—*C*), 128.6 (Ar—*C*), 128.5 × 3 (Ar—*C*), 118.1 (—CH_2), 100.0 ($C1_{\beta\text{-glc}}$), 73.1 (—CH_{ring}), 72.4 (—CH_{ring}), 72.0 (—CH_{ring}), 70.3 (—CH_2), 70.0 (—CH_{ring}), 63.4 (—CH_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{37}H_{32}O_{10}Na$ 659.1888; found, 659.1932.



Allyl β-D-Glucopyranoside (***S16***). To a stirred solution of **S15** (17 g, 26.7 mmol) in methanol was added NaOMe (30% in MeOH, 1.4 mL, 8 mmol, 0.3 equiv) under an argon atmosphere. After overnight stirring, the reaction mixture was neutralized with Amberlite IR-120 resin. The resin was removed by filtration, and the filtrate was concentrated. The residue was purified by flash chromatography over silica (MeOH/CH_2Cl_2, 1:6) to afford title compound **S16** (5.62 g, 95%) as a white solid. ^1H NMR (600 MHz, CD_3OD, 298 K): *δ* 5.94−5.88 (m, 1H, ═CH), 5.29 (dd, *J* = 17.1, 1.5 Hz, 1H, ═CH_aH_b), 5.11 (d, *J* = 10.3 Hz, 1H, ═CH$_a$$H_b$), 4.33 (dd, *J* = 12.7, 5.0 Hz, 1H, —OCH_aH_bCH═CH_2), 4.25 (d, *J* = 7.9 Hz, 1H, C1−H), 4.10 (dd, *J* = 12.7, 6.0 Hz, 1H, OCH$_a$$H_b$CH═$CH_2$), 3.81 (dd, *J* = 12.0, 2.1 Hz, 1H, —H_aH_bOH), 3.62 (dd, *J* = 11.9, 5.6 Hz, 1H, —H$_a$$H_b$OH), 3.31−3.28 (m, 1H, —$CH_{ring}$), 3.25−3.17 (m, 2H, 2 × —CH_{ring}), 3.17−3.12 (t, *J* = 7.8 Hz, 1H, —CH_{ring}). $^{13}C\{^1H\}$ NMR (150 MHz, CD_3OD, 298 K): *δ* 135.8 (*C*H═), 117.6 (—CH_2), 103.4 ($C1_{\beta\text{-glc}}$), 78.2 (—CH_{ring}), 78.0 (—CH_{ring}), 75.2 (—CH_{ring}), 71.7 (—CH_{ring}), 71.2 (—CH_2), 62.8 (—CH_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_9H_{16}O_6Na$, 243.0839; found, 243.0854.



https://dx.doi.org/10.1021/acs.joc.0c01404
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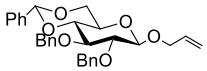
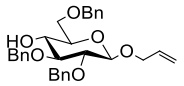
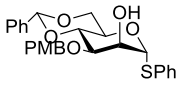
*Allyl 4,6-O-Benzylidene-β-D-glucopyranoside (**S17**).* To a suspension of the tetraol **S16** (8 g, 36.35 mmol) in anhydrous acetonitrile (120 mL) were added DL-10-camphorsulfonic acid (CSA) (2.110 g, 9.09 mmol, 0.25 equiv) and benzaldehyde dimethyl acetal (13.7 mL, 90.09 mmol, 2.5 equiv) dropwise under an argon atmosphere at room temperature, and the mixture was allowed to stir vigorously for 15 min. The reaction was quenched with Et_3N (1 mL). Then, the reaction mixture was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ (50 mL) and water. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The solid residue was recrystallized in ethyl acetate and hexane (1:3) to afford pure compound **S17** (10 g, 89%) in a white solid, which was pure enough for further reaction. ^{1}H NMR (600 MHz, CD_3OD, 298 K): δ 7.42−7.41 (m, 2H, Ar—*H*), 7.26−7.25 (m, 3H, Ar—*H*), 5.91−5.84 (m, 1H, C*H*═), 5.49 (s, 1H, Ph−C*H*), 5.27 (dd, J = 17.5, 1.0 Hz, 1H, ═CH_aH_b), 5.10 (dd, J = 10.5, 1.0 Hz, 1H, ═CH$_a$*H*$_b$), 4.37 (dd, J = 7.7, 2.1 Hz, C1—*H*), 4.26−4.23 (m, 2H, —CH_{ring}, —CH_aH_b), 4.21−4.18 (m, 1H, —CH$_a$*H*$_b$), 3.70 (ddd, J = 10.2, 2.4 Hz, 1H, —CH_{ring}), 3.57 (ddd, J = 9.0, 2.4 Hz, 1H, —CH_{ring}), 3.40 (ddd, J = 9.2, 2.2 Hz, 1H, —CH_{ring}), 3.36 (ddd, J = 4.8, 2.4 Hz, 1H, —CH_{ring}), 3.25−3.22 (m, 3H, 2 × —OH). ^{13}C{^{1}H} NMR (150 MHz, CD_3OD, 298 K): δ 139.3 (Ar—*C*), 135.7 (Ar—*C*), 130.1 (Ar—*C*), 129.2 (Ar—*C*), 127.6 (Ar—*C*), 117.7 (—CH_2), 104.3 (Ph−*C*H), 103.1 (*C1*$_{\beta\text{-glc}}$), 82.4 (—CH_{ring}), 76.1 (—CH_{ring}), 74.8 (—CH_{ring}), 71.6 (—CH_2), 69.8 (—CH_2), 67.7 (—CH_{ring}). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{16}H_{20}O_6Na$, 331.1152; found, 331.1169.



*Allyl 2,3-Di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (**S18**).* To a suspension of NaH (60%, 2.65 g, 66.2 mmol, 3 equiv) in dry DMF (70 mL) was added diol compound **S17** (6.8 g, 22.06 mmol) dissolved in dry DMF (35 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 10 min; then, benzyl bromide (10.5 mL, 88.27 mmol, 4 equiv) was added dropwise, and the reaction mixture was stirred for 5−6 h at room temperature. Upon completion, the reaction was carefully quenched with MeOH at 0 °C, and the mixture was concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (75 mL) and washed with H_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (100% hexane and EtOAC/hexanes, 1:9) to yield compound **S18** (10.5 g, 98%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.48−7.42 (m, 2H, Ar—*H*), 7.38−7.25 (m, 13H, Ar—*H*), 5.97−5.5.91 (m, 1H, C*H*═), 5.56 (s, 1H, Ph−C*H*), 5.36 (dd, J = 17.3, 1.6 Hz, 1H, ═CH_aH_b), 5.23 (dd, J = 10.5, 2.8 Hz, 1H, ═CH_aH_b), 4.91 (d, J = 11.1 Hz, 2H, Ar—CH_2), 4.81 (d, J = 11.4 Hz, 1H, Ar—CH_aH_b), 4.77 (d, J = 10.9 Hz, 1H, Ar—CH$_a$*H*$_b$), 4.56 (d, J = 7.6 Hz, 1H, C1—*H*$_{\beta\text{-glc}}$), 4.42−4.38 (m, 1H, —CH$_a$*H*$_b$), 4.35 (dd, J = 10.5, 5.0 Hz, 1H, —CH$_a$*H*$_b$), 4.17−4.14 (m, 1H, —CH$_a$*H*$_b$), 3.80 (t, J = 9.8 Hz, 1H, —CH$_a$*H*$_b$), 3.76 (t, J = 9.5 Hz, 1H, —CH_{ring}), 3.68 (t, J = 9.5 Hz, 1H, —CH_{ring}), 3.50 (dd, J = 8.6, 7.6 Hz, 1H, —CH_{ring}), 3.42−3.38 (m, 1H, —CH_{ring}). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 138.7 (Ar—*C*), 138.5 (Ar—*C*), 137.5 (Ar—*C*), 133.9 (Ar—*C*), 129.1(Ar—*C*), 128.5 × 2 (Ar—*C*), 128.4 × 2 (Ar—*C*), 128.2 (Ar—*C*), 127.9 (Ar—*C*), 127.8 (Ar—*C*), 126.2 (Ar—*C*), 117.8 (—CH_2), 103.4 (Ph−CH), 101.3 (*C1*$_{\beta\text{-glc}}$), 82.3 (—CH_{ring}), 81.7 (—CH_{ring}), 81.1 (—CH_{ring}), 75.6 (—CH_2), 75.3 (—CH_2), 70.9 (—CH_2), 68.9 (—CH_2), 66.2 (—CH_{ring}). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{30}H_{32}O_6Na$, 511.2091; found, 511.2125.



*Allyl 2,3,6-Tri-O-benzyl-β-D-glucopyranoside (**S1**).* A mixture of starting material **S18** (6 g, 12.29 mmol, 1 equiv) and 4 Å molecular sieves (9 g) in CH_2Cl_2 (150 mL) was stirred under argon for 1 h. The reaction was cooled to −78 °C, and triethylsilane (5.9 mL, 36.86 mmol, 3 equiv) and TFA (3.805 mL, 43.01 mmol, 3.5 equiv) were added. The reaction mixture was stirred at −78 °C until TLC analysis indicated the disappearance of the starting material (1 h); then, the reaction was quenched by the addition of satd aq $NaHCO_3$ (10 mL) solution. The reaction mixture was slowly warmed to room temperature and filtered. The filtrate was washed with saturated $NaHCO_3$ (50 mL) and brine. The organic phase was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:4) to give pure product **S1** (4.88 g, 81%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.36−7.26 (m, 15H, Ar—*H*), 5.98−5.92 (m, 1H, C*H*═), 5.34 (dd, J = 17.2, 1.5 Hz, 1H, ═CH_aH_b), 5.21 (dd, J = 10.2, 1.5 Hz, 1H, ═CH$_a$*H*$_b$), 4.96 (dd, J = 16.6, 10.9 Hz, 2H, Ar—CH_2), 4.73 (dd, J = 11.6, 5.3 Hz, 2H, Ar—CH_2), 4.60 (d, J = 12.2 Hz, 1H, Ar—CH_aH_b), 4.57 (d, J = 12.2 Hz, 1H, Ar—CH$_a$*H*$_b$), 4.46 (dd, J = 7.5, 2.0 Hz, 1H, C1—*H*$_{\beta\text{-glc}}$), 4.43−4.39 (m, 1H, —CH_aH_b), 4.15−4.12 (m, 1H, —CH_aH_b), 3.77 (dd, J = 10.5, 3.9 Hz, 1H, —CH_aH_b), 3.70 (dd, J = 10.5, 5.5 Hz, 1H, —CH_aH_b), 3.60−3.56 (m, 1H, —CH_{ring}), 3.46−3.41 (m, 3H, 3 × —CH_{ring}), 2.53 (d, J = 2.0 Hz, 1H, −*OH*). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 138.8 (Ar—*C*), 138.6 (Ar—*C*), 138.1 (Ar—*C*), 134.2 (Ar—*C*), 128.7 (Ar—*C*), 128.6 × 2 (Ar—*C*), 128.4 (Ar—*C*), 128.2 (Ar—*C*), 128.0 (Ar—*C*), 127.9 (Ar—*C*), 127.9 (Ar—*C*), 117.5 (—CH_2), 102.9 (*C1*$_{\beta\text{-glc}}$), 84.2 (—CH_{ring}), 81.9 (—CH_{ring}), 75.5 (—CH_2), 75.0 (—CH_2), 74.2 (—CH_{ring}), 73.8 (—CH_2), 71.7 (—CH_{ring}), 70.5 × 2 (—CH_2). HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{30}H_{34}O_6Na$, 513.2248; found, 513.2277.



*Phenyl 4,6-O-Benzylidene-3-p-methoxybenzyl-1-thio-α-D-mannopyranoside (**S19**).* To a well-stirred suspension of α-mannose thioglycoside (8.17 g, 30 mmol, 1 equiv) in dry CH_3CN (200 mL) were added CSA (70 mg, 3 mmol, 10 mol %) and benzaldehyde dimethyl acetal (BDA) (5.17 mL, 36.0 mmol, 1.2 equiv) at room temperature. The reaction mixture was stirred under argon until TLC analysis indicated the disappearance of the starting material (45 min); then, the reaction was quenched by triethyl amine (600 μL, 2 equiv to CSA). The solvent was removed under reduced pressure to give a solid compound, which was dissolved in CH_2Cl_2 (125 mL) and washed with satd aq $NaHCO_3$ (2×) and water. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting solid compound was recrystallized from CH_2Cl_2:hexanes (1:3), and the crystallized compound was separated and repeatedly

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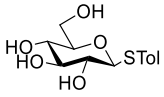
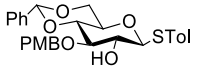
washed with hexane or diethyl ether to remove excess BDA. The obtained white solid was almost pure and can be used as is for further reactions (9.3 g, 86%).

A portion of the above dried mannose diol (6.1 g, 17 mmol) and Bu_2SnO (4.655 g, 18.7 mmol, 1.1 equiv) in toluene (120 mL) were stirred at reflux for 3 h with azeotropic removal of water using a Dean−Stark apparatus (clear solution was formed during the refluxing). The solvent was removed under reduced pressure and coevaporated twice with toluene. The residue was dissolved in DMF (50 mL), and Bu_4NBr (5.77 g, 17.9 mmol, 1.05 equiv, CsF (2.72 g, 17.9 mmol, 1.05 equiv), and PMBCl (2.5 mL, 17.9 mmol, 1.05 equiv) were added sequentially under an argon atmosphere. The reaction mixture was stirred overnight. Upon completion, the solvent was removed under reduced pressure, and the residue was diluted with EtOAc (120 mL) and washed with sat aq $NaHCO_3$ solution (75 mL) and brine. The separated organic layer was filtered through a pad of Celite to remove the inorganic salts. The residue was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained solid was recrystallized from Et_2O/hexane (1:3) to get white crystallized compound **S19**, which was pure enough for further reactions (7.8 g, 88%). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.51−7.42 (m, 4H, Ar—*H*), 7.39−7.35 (m, 3H, Ar—*H*), 7.31−7.25 (m, 5H, Ar—*H*), 6.88−6.86 (m, 2H, Ar—*H*), 5.60 (s, 1H, Ph−C*H*), 5.57 (s, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.33−4.29 (m, 1H), 4.22 (br d, *J* = 3.2 Hz, 1H), 4.20 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.16 (t, *J* = 9.4 Hz, 1H), 3.9 (dd, *J* = 9.6, 3.4 Hz, 1H), 3.85 (t, *J* = 10.3 Hz, 1H), 3.80 (s, 3H, —OCH_3), 2.89 (s, 1H, —*OH*). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.7 (Ar—*C*), 137.6 (Ar—*C*), 133.5 (Ar—*C*), 131.9 (Ar—*C*), 129.9 (Ar—*C*), 129.8 (Ar—*C*), 129.3 (Ar—*C*), 129.2 (Ar—*C*), 128.4 (Ar—*C*), 127.9 (Ar—*C*), 126.3 (Ar—*C*), 114.1 (Ar—*C*), 101.8 (Ph−*C*H), 87.9 (*C*1α-man), 79.2 (—CH_{ring}), 75.6 (—CH_{ring}), 73.1 (—CH_2), 71.6 (—CH_{ring}), 68.7 (—CH_2), 64.8 (—CH_{ring}), 55.5 (—OCH_3). HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{27}H_{28}O_6SNa$, 503.1499; found, 503.1530.



*p-Methylphenyl 1-Thio-β-D-glucopyranoside (**S20**).*[48] D-Glucose penta-acetate (45 g, 11.54 mmol) and *p*-thiocreosl (21.5 g, 17.31 mmol, 1.5 equiv) were dissolved in anhydrous CH_2Cl_2 (250 mL) under argon in a flame-dried 1 L flask. The solution was cooled to 0 °C, and $BF_3{\cdot}Et_2O$ (43 mL, 14.4 mmol, 1.25 equiv) was added. The mixture was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and neutralized with satd aq $NaHCO_3$ solution (until the evaluation of CO_2 ceased). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from the EtOAc/hexane system (1:3) by storing in a 4 °C refrigerator overnight. The crystals were filtered through a sintered funnel by repeatedly washing with hexane to afford pure tetra-acetyl β-thioglycoside (47 g, 90%) in a white crystalline solid. The compound was dissolved in methanol. Sodium methoxide in methanol (30 wt %, 9.3 mL, 51.8 mmol, 0.5 equiv) was added to the solution at rt, and the mixture was stirred for 4−5 h. Then, ion-exchange resin (IR-120) was added portionwise until the solution was neutralized. The reaction mixture was filtered and concentrated *in vacuo* to afford tetraol **S20** (28 g, 94%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD, 298 K): δ 7.37 (d, *J* = 8.0 Hz, 2H, Ar—*H*), 7.03 (d, *J* = 8.0 Hz, 2H, Ar—*H*), 4.42 (d, *J* = 9.8 Hz, 1H, C1—$H_{\beta\text{-glc}}$), 3.76 (dd, *J* = 12.1, 1.5 Hz, 1H, —CH_aH$_b$), 3.57 (dd, *J* = 12.1, 5.1 Hz, 1H, —CH$_a$$H_b$), 3.28 (t, *J* = 8.6 Hz, 1H, —CH_{ring}), 3.19−3.15 (m, 2H, −CH_{ring}), 3.09 (t, *J* = 9.3 Hz, 1H, (—CH_{ring}), 2.22 (s, 3H, PhCH_3). ^{13}C{^{1}H} NMR (150 MHz, CD_3OD, 298 K): δ 138.9 (Ar—*C*), 133.6 (Ar—*C*), 131.3 (Ar—*C*), 130.6 (Ar—*C*), 89.8 (*C*1$_{\beta\text{-glc}}$), 82.1 (—CH_{ring}), 79.8 (—CH_{ring}), 73.8 (—CH_{ring}), 71.5 (—CH_{ring}), 63.0 (—CH_2), 21.2 (—CH_3). HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{13}H_{18}O_5SNa$, 309.0767; found, 309.0786.



*p-Methylphenyl 4,6-O-Benzylidene-3-p-methoxybenzyl-1-thio-β-D-glucopyranoside (**S21**).* To a suspension of the tetraol **S20** (7.5 g, 26.2 mmol) in anhydrous acetonitrile (100 mL) were added DL-10-camphorsulfonic acid (CSA) (1.52 g, 6.55 mmol, 0.25 equiv) and benzaldehyde dimethyl acetal (7.9 mL, 52.4 mmol, 2 equiv) dropwise under an argon atmosphere at room temperature. The mixture was stirred vigorously for 45 min. Then, the reaction was quenched with Et_3N (2 mL), and the mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with satd aq $NaHCO_3$ (50 mL) and water. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The solid residue was crystallized from CH_2Cl_2/hexane (1:3) and separated by filtering. The solid mass was repeatedly washed with hexane or diethyl ether to remove excess BDA and later dried under a vacuum. The white solid was almost pure and could be used as is for further reactions (9.5 g, 88%).

A portion of the above dried thioglycoside (5.5 g, 14.69 mmol) and Bu_2SnO (4.02 g, 16.89 mmol, 1.15 equiv) in toluene (75 mL) were stirred at reflux for 3−4 h with azeotropic removal of water using a Dean−Stark apparatus (clear solution was formed during the refluxing). The solvent was removed under reduced pressure and coevaporated twice with toluene. The residue was dissolved in DMF (50 mL), and Bu_4NBr (5.21 g, 16.16 mmol, 1.1 equiv), CsF (2.46 g, 16.16 mmol, 1.1 equiv), and PMBCl (2.3 mL, 16.16 mmol, 1.1 equiv) were added sequentially under an argon atmosphere. The reaction mixture was stirred overnight at rt. Upon completion, DMF was removed under reduced pressure, and the residue was diluted with EtOAc (150 mL) and washed with sat aq $NaHCO_3$ solution (75 mL) and brine. The separated organic layer was filtered through a pad of Celite to remove the inorganic salts, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The solid residue was purified by flash chromatography over silica gel (hexanes/EtOAc/CH_2Cl_2, 2:1:1) to give pure compound **S21** as a white solid (6.4 g, 88%). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.46−7.22 (m, 9H, Ar—*H*), 7.10 (d, *J* = 7.9 Hz, 2H, Ar—*H*), 6.82 (d, *J* = 8.5 Hz, 2H, Ar—*H*), 5.52 (s, 1H), 4.85 (d, *J* = 11.2 Hz, 1H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.53 (d, *J* = 9.7 Hz, 1H), 4.35 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.78−3.73 (m, 4H, —OCH_3), 3.61 (dt, *J* = 18.4, 9.0 Hz, 2H), 3.47−3.40 (m, 2H), 2.32 (s, 3H, —CH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 159.6 (Ar—*C*), 138.9 (Ar—*C*), 137.4 (Ar—*C*), 134.1 (Ar—*C*), 130.5 (Ar—*C*), 130.0 (Ar—*C*), 129.2 (Ar—*C*), 128.5 (Ar—*C*), 127.4 (Ar—*C*), 126.2 (Ar—*C*), 114.1 (Ar—*C*),

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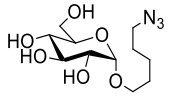
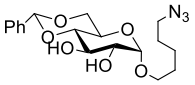
101.4 (PhCH—), 88.7 ($C1_{\beta\text{-glc}}$), 81.4 (—CH_{ring}), 81.3 (—CH_{ring}), 74.6 (—CH_{ring}), 72.2 (—CH_{ring}), 70.9 (—CH_{ring}), 68.8 (—CH_2), 55.5 (—OCH_3), 21.4 (—CH_3). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{28}H_{30}O_6SNa$, 517.1655; found, 517.1670.

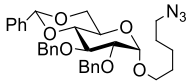


*5-Azidopentyl α-D-Glucopyranoside (**S22**).* TMSOTf (1.5 mL, 8.32 mmol, 0.1 equiv) was added to a suspension of D-glucose (15 g, 83.25 mmol) and HMDS (53 mL, 249.75 mmol) in CH_2Cl_2 (150 mL) at rt under argon. The reaction mixture was stirred for 40 min. Upon completion, the mixture was poured into ice−water and extracted with hexane (3 × 300 mL). The combined organic extracts were washed with water (2 × 100 mL), dried ($MgSO_4$), and concentrated under reduced pressure to afford per-*O*-trimethylsilylglucose in a viscous colorless oil (45 g, quantitative).

The above prepared compound (20 g, 36.6 mmol) was dissolved in CH_2Cl_2 (150 mL), and TMSI (6.25 mL, 44 mmol) was added at 0 °C. The reaction mixture was stirred under argon for 20 min before benzene (40 mL) was added. The solvent was removed under reduced pressure, and the glycosyl iodide residue was dried under a vacuum for 10 min and, then, dissolved in CH_2Cl_2 (50 mL) under argon. In a separate flask, a mixture of activated 4 Å molecular sieves (10 g), nBu$_4$NI (54 g, 146 mmol), iPr$_2$NEt (18.15 mL, 109.8 mmol), and 1-azidopentanol (4.72 mL, 36.6 mmol) in CH_2Cl_2 (175 mL) was stirred under argon at rt for 15 min. Then, the solution of glycosyl iodide in CH_2Cl_2 was added dropwise over 5 min to this mixture at rt, and the resulting mixture was stirred overnight. After removal of the solvent under reduced pressure, Et_2O (150 mL) and H_2O (75 mL) were added, and the phases were separated. The organic phase was concentrated under reduced pressure, MeOH (50 mL) and IR-120 (3 g) were added, and the reaction mixture was stirred overnight. The reaction was quenched by the addition of $NaHCO_3$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (15% MeOH in $CHCl_3$) to afford tetraol **S22** as a pale-yellow viscous compound (4.52 g, 43%). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 5.25 (s, 1H), 5.03 (s, 1H), 4.81 (d, J = 2.8 Hz, 1H), 4.10 (s, 1H), 3.83 (m, 2H), 3.72−3.62 (m, 3H), 3.56−3.50 (m, 3H), 3.44 (dd, J = 15.6, 6.6 Hz, 1H), 3.27 (t, J = 6.6 Hz, 2H, —C$H_{2linker}$), 1.65−1.56 (m, 4H, 2 × —C$H_{2linker}$), 1.44−1.36 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 98.8 ($C1_{\alpha\text{-glc-linker}}$), 74.3 (—$CH_{ring}$), 72.1 (—$CH_{ring}$), 71.7 (—$CH_{ring}$), 69.5 (—$CH_{ring}$), 68.2 (—$CH_2$OH), 61.28 (—$CH_{2linker}$), 51.4 (—$CH_{2linker}$), 29.1 (—$CH_{2linker}$), 28.7 (—$CH_{2linker}$), 23.4 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{11}H_{21}N_3O_6Na$, 314.1323; found, 314.1341.



*5-Azidopentyl 4,6-O-Benzylidene-α-D-glucopyranoside (**S23**).* To a stirred solution of **S22** (5.9 g, 20.26 mmol) in acetonitrile (60 mL) were added DL-10-camphorsulfonic acid (CSA) (1.175 g, 5.06 mmol) and benzaldehyde dimethyl acetal (7.65 mL, 50.6 mmol) dropwise under a N_2 atmosphere at room temperature. The mixture was stirred for 3.5 h. Upon completion, the reaction was quenched with Et_3N (1 mL), solvents were evaporated, and the obtained residue was chromatographed on silica gel (MeOH/CH_2Cl_2, 3:97) to afford pure compound **S23** (7.19 g, 94%) as an oily compound. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.48−7.46 (m, 2H, Ar—H), 7.36−7.32 (m, 3H, Ar—H), 5.50 (s, 1H), 4.85 (d, J = 4.0 Hz, 1H), 4.26 (dd, J = 10.1, 4.7 Hz, 1H), 3.91 (td, J = 9.2, 1.7 Hz, 1H), 3.79−3.75 (m, 1H), 3.74−3.69 (m, 2H), 3.61 (td, J = 9.6, 4.0 Hz, 1H), 3.48−3.43 (m, 2H), 3.28 (t, J = 7.0 Hz, 2H), 2.87 (d, J = 1.7 Hz, 1H), 2.31 (d, J = 10.0 Hz, 1H), 1.67−1.59 (m, 4H), 1.47−1.42 (m, 2H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 137.2 (Ar—C), 129.4 (Ar—C), 128.5 (Ar—C), 126.4 (Ar—C), 102.1 (PhCH—), 98.9 ($C1_{\alpha\text{-glc-linker}}$), 81.1 (—$CH_{ring}$), 73.1 (—$CH_{ring}$), 72.0 (—$CH_{ring}$), 69.1 (—$CH_2$O—), 68.4 (—$CH_{2linker}$), 62.7 (—$CH_{ring}$), 51.4 (—$CH_{2linker}$), 29.2 (—$CH_{2linker}$), 28.8 (—$CH_{2linker}$), 23.5 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{18}H_{25}N_3O_6Na$, 402.1636; found, 402.1648.



*5-Azidopentyl 2,3-Di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (**S24**).* To the suspension of NaH (3.03g, 75.8 mmol) in dry DMF (50 mL) was added diol compound **S23** (7.19 g, 18.95 mmol) dissolved in dry DMF (80 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 10 min. Then, benzyl bromide (7.88 mL, 66.32 mmol) was added dropwise, and the reaction mixture was stirred for 6 h at rt. Upon completion, the reaction was carefully quenched with MeOH at 0 °C. The reaction mixture concentrated *in vacuo*. The obtained residue was diluted with EtOAc (150 mL) and washed with H_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAC/hexanes, 100% hexane followed by 1:9) to yield compound **S24** (9.8 g, 93%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.48−7.23 (m, 15H, Ar—H), 5.54 (s, 1H, PhCH—), 4.91 (d, J = 11.2 Hz, 1H, Ar—CH_aH$_b$), 4.83 (d, J = 11.5 Hz, 2H, Ar—CH_2), 4.71 (d, J = 3.6 Hz, 1H, C1−H), 4.66 (d, J = 12.0 Hz, 1H, Ar—CH$_a$$H_b$), 4.25 (dd, J = 10.2, 4.9 Hz, 1H), 4.04 (t, J = 9.4 Hz, 1H), 3.83 (td, J = 10.0, 4.9 Hz, 1H), 3.70 t, J = 10.3 Hz, 1H), 3.66 (dt, J = 9.8, 6.7 Hz, 1H), 3.61 (t, J = 9.3 Hz, 1H), 3.55 (dd, J = 9.3, 3.7 Hz, 1H), 3.41 (dt, J = 9.8, 6.6 Hz, 1H), 3.25 (t, J = 6.9 Hz, 2H, —C$H_{2linker}$), 1.67−1.58 (m, 4H, 2 × —C$H_{2linker}$), 1.48−1.42 (m, 2H, —C$H_{2linker}$). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, 298 K): δ 139.0 (Ar—C), 138.5 (Ar—C), 137.6 (Ar—C), 129.1 (Ar—C), 128.6 (Ar—C), 128.5 (Ar—C), 128.4 (Ar—C), 128.2 (Ar—C), 128.1 × 2 (Ar—C), 127.7 (Ar—C), 126.2 (Ar—C), 101.4 (PhCH—), 98.3 ($C1_{\alpha\text{-glc-linker}}$), 82.4 (—$CH_{ring}$), 79.6 (—$CH_{ring}$), 78.8 (—$CH_{ring}$), 75.5 (—$CH_2$), 73.7 (—$CH_2$), 69.3 (—$CH_2$O—), 68.4 (—$CH_{2linker}$), 62.7 (—$CH_{ring}$), 51.5(—$CH_{2linker}$), 29.2 (—$CH_{2linker}$), 28.8 (—$CH_{2linker}$), 23.6 (—$CH_{2linker}$). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{32}H_{37}N_3O_6Na$, 582.2575; found, 582.2610.

General Procedure for Maleimide−DT Activation and Oligosaccharide−Protein Conjugation. Carrier protein, CRM197 (diphtheria toxin mutant, DT), 6.0 mg, was dissolved in 3.0 mL of pH 7.6 PBS buffer, and Sulfo-EMCS (2.4 mg, 60 equiv) was added to the solution. The solution was stirred at room temperature for 1 h. The mixture was then diluted with

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ddH2O and centrifuged against 4 changes of deionized water by Amicon Ultra-0.5, 10 kDa. The obtained DT−maleimide was characterized by MALDI-TOF (positive mode, sinapinic acid matrix, H_2O) analysis to determine the maleimide incorporation number (Figure SF1). Modified DT was dissolved in pH 7.2 PBS buffer by the buffer exchange method using Amicon by centrifuging two times, and the modified DT concentration was determined by the BCA protein quantified method.

Next, 1.5 mg equivalents of modified DT and 1.5 mg of modified glycan (**3a**/**4a**/**5a**) were mixed and stirred at room temperature for 2 h. Then, the mixture was diluted with ddH_2O and centrifuged against 6 changes of ddH_2O by Amicon Ultra-0.5, 10 kDa. The solution was lyophilized to a white solid. The obtained oligosaccharide−protein conjugates were characterized by MALDI-TOF (positive mode, matrix sinapinic acid, H_2O) analysis to determine the oligosaccharide incorporation number (Figure SF1-SF3 and Table ST2).

Mice Immunization. All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine (Approval Number 20140054). Animal housing and experiments were in strict accordance with the regulations of the guidelines in the Handbook of Laboratory Animal Care of the National Laboratory Animal Breeding and Research Center, National Science Council of Taiwan. All mice were housed under specific pathogen-free conditions. K2-hexamer, -heptamer, -and octamer conjugate vaccines were diluted to 100 μg/mL in PBS, and the glycolipid adjuvant C34 was dissolved to 100 μg/mL in DMSO. Twenty mice (6−8 week-old female BALB/c, LASCO) were separated to 4 groups randomly (5 mice in the same cage, marked mouse I−V), and each group was immunized subcutaneously through intramuscular (IM)[49] injections of the K2-hexamer, -heptamer, and -octamer conjugate vaccines (2 μg glycan/dose) with 2 μg of glycolipid adjuvant C34 in a PBS buffer to a total volume of 100 μL. Controlled mice were injected with phosphate buffer saline (PBS). Booster doses were given at days 14 and 28 using the same strategy. Mice sera were collected 7 days after the third immunization by puncturing the submandibular vein to study antibody detection and the bactericidal assay.

Microarray Fabrication and Detection. To fabricate the microarray, K2-tetramer **1**, -pentamer **2**, -hexamer **3**, -heptamer **4**, and -octamer **5** were dissolved in the printing buffer (300 mM phosphate buffer, 0.005% triton, pH 8.5) in 100 μM concentration. Microarrays were printed (BioDot; Cartesian Technologies) by a robotic pin (SMP3; TeleChem International) with the deposition of ∼0.6 nL of various solutions from 96-well plates onto NHS-coated glass slides (Nexterion H slide; SCHOTT North America). The microarray was designed to be 16 grids in one slide and 5 columns (5 kinds of K2-oligosaccharide) × 10 rows (10 spots for 1 saccharide) in one grid. Printed slides were allowed to react in an atmosphere of 80% humidity for an hour followed by desiccation overnight.

Antibody Specificity and Antigen Immunogenicity Comparison. Before serum antibody binding, the glycan microarrays were blocked with Superblock blocking buffer (Pierce) at 4 °C for 1 h and then washed with PBST (PBS + 0.05% triton) buffer twice. Sera from mice that were immunized with oligosaccharide−protein conjugates were diluted 200-fold with PBST and were incubated with a microarray at 4 °C for 1 h. Excess serum antibodies were washed out, and the microarrays were incubated with goat antimouse IgG antibodies labeled with fluorescence as the second antibody at 4 °C in the dark for 1 h. The slides were washed thoroughly and, then, scanned at a 635 nm wavelength with a microarray fluorescence chip reader (GenePix 4300A; Molecular Devices Corporation). The scanned images were analyzed by GenePix Pro-6.0 analysis software (Axon Instruments, Union City, CA, USA). Data are presented by GraphPad Prism version 6.01 as the means ± SD from three array-independent experiments.

Serum Bactericidal Assay (SBA). Aliquots of mouse sera were inactivated by heating at 56 °C for 30 min and then subjected to 2-fold serial dilution (1:2 to 1:512) with normal saline. Ten μL of diluted sera and 20 μL of bacteria suspension (2000 CFU) were incubated at 37 °C for 15 min. After incubation, 25 μL of new born rabbit complement (Pel-Freez, USA) and 25 μL of normal saline were added, and incubation was continued for another hour at 37 °C. Then, 2 μL of the reaction mixture was plated on the LB plate. After culture overnight, the number of surviving bacteria was counted. Serum bactericidal titers were defined as the reciprocal of the serum dilution that resulted in ≥50% killing of the bacteria that achieved the bacteria-complement-buffer controls.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01404.

Optimization of glycosylation reaction conditions for the synthesis of hexasaccharide, related molecule synthesis schemes, MALDI analysis of glycoconjugate, and glycan microarray analysis of individual mouse sera (PDF)

NMR and mass spectra (PDF)

NMR and mass spectra of additional compounds (PDF)

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[⊥]M.R. and K.-S.L. contributed equally.

Notes

The authors declare no competing financial interest.

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