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Chemoenzymatic Synthesis of a Library of Human Milk Oligosaccharides

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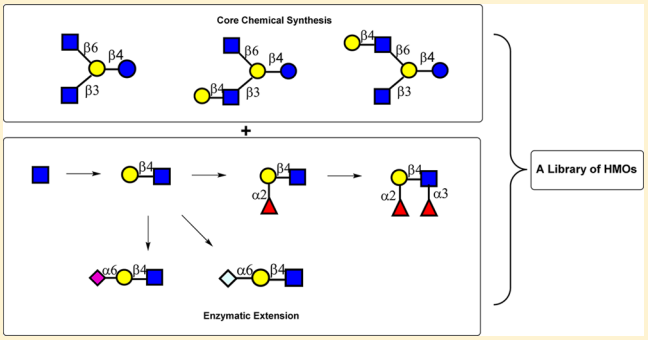
Supporting Information

ABSTRACT: Human milk oligosaccharides (HMOs) are a family of diverse unconjugated glycans that exist in human milk as one of the major components. Characterization, quantification, and biofunctional studies of HMOs remain a great challenge due to their diversity and complexity. The accessibility of a homogeneous HMO library is essential to solve these issues which have beset academia for several decades. In this study, an efficient chemoenzymatic strategy, namely core synthesis/enzymatic extension (CSEE), for rapid production of diverse HMOs was reported. On the basis of 3 versatile building blocks, 3 core structures were chemically synthesized via consistent use of oligosaccharyl thioether and oligosaccharyl bromide as glycosylation donors in a convergent fragment coupling strategy. Each of these core structures was then extended to up to 11 HMOs by 4 robust glycosyltransferases. A library of 31 HMOs were chemoenzymatically synthesized and characterized by MS and NMR. CSEE indeed provides a practical approach to harvest structurally defined HMOs for various applications.



INTRODUCTION

Human milk, as the sole dietary source, contains all the necessary nutrients for an infant to thrive in the first few months. More importantly, human milk can also provide ingredients with health benefits which traditional food cannot do. Human milk oligosaccharides (HMOs) are the third major component in human milk, only after lactose and lipid.[1] Concentrations and components of HMOs vary depending on the stages of lactation.[2] Particularly, 1 L of mature human milk contains about 12−14 g of HMOs.[3] The structures of about 130 discovered HMOs have been elucidated.[4] The major building blocks of HMOs are 5 monosaccharides, including D-glucose (Glc), D-galactose (Gal), *N*-acetyl-D-glucosamine (GlcNAc), L-fucose (Fuc), and *N*-acetylneuraminic acid (Neu5Ac). Even though HMOs were first discovered and confirmed in the 1950s, a comprehensive understanding of their functions is still out of reach, due to their inherit diversity and complexity. Increasing evidence shows that HMOs can provide significant beneficial effects to the health of breast-fed infants through several mechanisms. For instance, HMOs could serve as prebiotics to promote the growth of desired bacteria in an infant's intestine.[1c,2a,5] In addition, HMOs are antiadhesive antimicrobials by serving as receptors to prevent pathogen attachment to infant mucosal surfaces.[6] In addition, evidence has demonstrated that HMOs can modulate epithelial and immune cell responses and reduce excessive mucosal leukocyte infiltration and activation, which in turn decreases the risk of necrotizing enterocolitis (NEC), one of the most common fatal disorders in preterm infants.[7] Furthermore, sialylated HMOs may also provide necessary nutrients for the development of the brain and cognition of infants.[8]

Even though the general functions of HMOs have been explored and discovered, the functional roles of individual HMOs are far less clear because of very limited access to sufficient amounts of structurally defined HMOs. To date, only a handful of short-chain HMOs can be produced on a large scale and the supply of more complicated and branched HMOs is highly demanded.

Until now, only a few approaches have been developed for the synthesis of a small number of well-defined HMOs.[2a,9] For example, Schmidt developed strategies to synthesize some highly branched HMOs by solution-phase and solid-phase synthesis.[9b,d,e] Enzymatic methods have also been employed to achieve relatively simple structures.[9a] One of the biggest roadblocks in previous syntheses remains the small quantity and limited variety of HMOs needed for biofunctional studies. Recently, we have developed an efficient core synthesis/enzymatic extension (CSEE) strategy for the rapid preparation of *N*-glycan libraries.[10] In this study, a similar strategy was successfully applied for HMO synthesis. Briefly, 3 core oligosaccharides with 1 or 2 GlcNAc residue(s) at the nonreducing end were first synthesized by the

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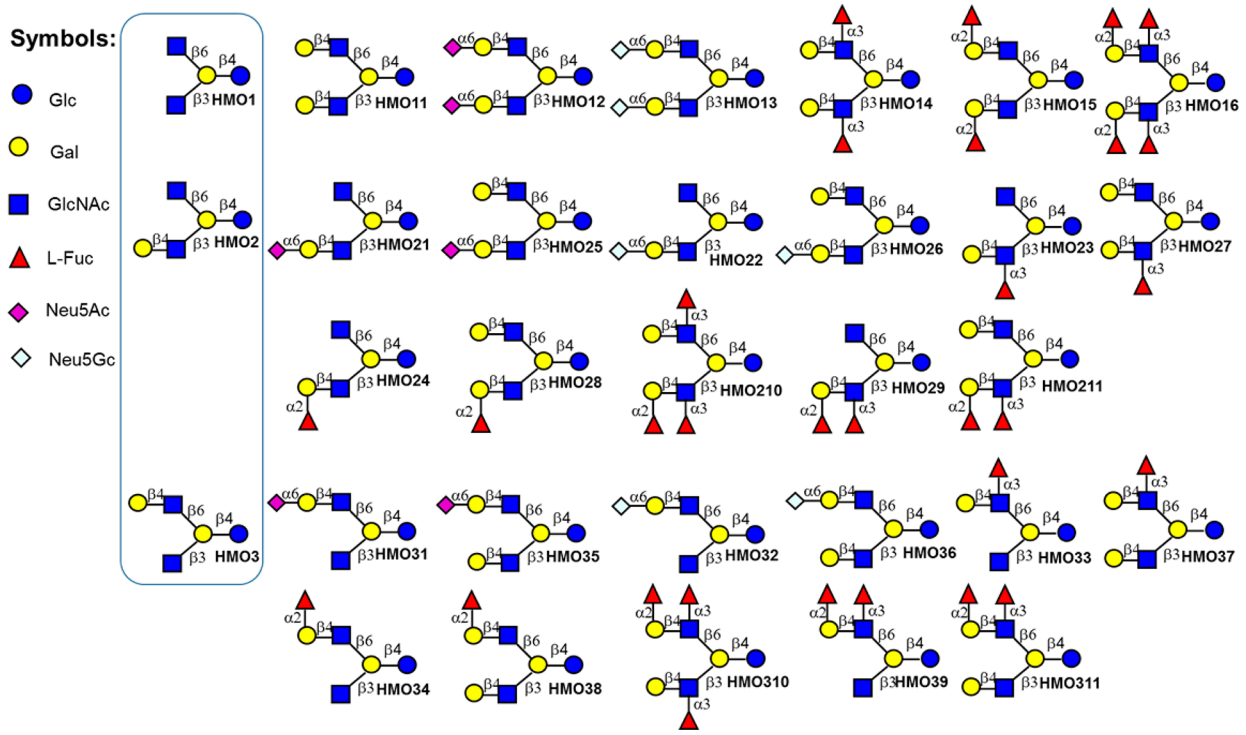


Figure 1. HMOs synthesized by the core synthesis/enzymatic extension (CSEE) strategy starting with 3 chemically prepared core structures (boxed).

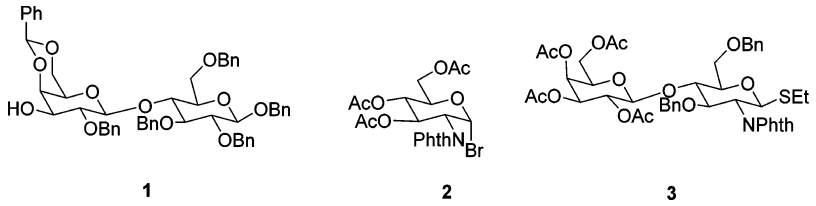
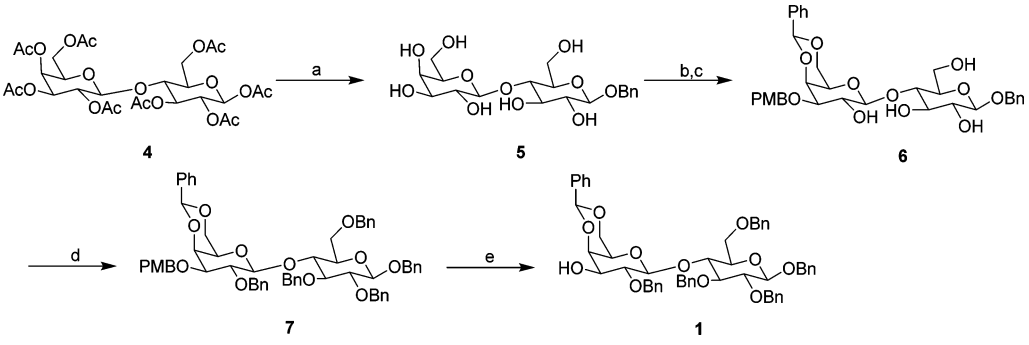


Figure 2. Three versatile building blocks for the assembly of the three core structures.

Scheme 1. Synthesis of Building Block 1[a]



[a]Reagents and conditions: (a) (i) $BF_3{\cdot}Et_2O$, BnOH, DCM, 4 Å MS, (ii) NaOMe, MeOH, 50% over two steps; (b) (i) n-Bu_2SnO, MeOH, reflux, (ii) PMBCl, TBAI, toluene, 4 Å MS reflux, 40%; (c) $PhCH(OMe)_2$, DMF, CSA, 75%; (d) BnBr, NaH, DMF, 95%; (e) DDQ, DCM/PBS Buffer 9/1, 90%.

convergent assembly of 3 simple building blocks followed by extension of the cores by 4 robust glycosyltransferases to produce a library of 31 HMOs (Figure 1).

RESULTS AND DISCUSSION

Convergent Core Synthesis. Previous studies highlighted the complexity and challenges associated with synthesizing HMOs via a block coupling strategy. Schmidt developed the sequential synthesis of lactose-containing oligosaccharides, including HMO lacto-*N*-tetraoside based on the solid-phase synthesis concept.[9d] Madsen used one-pot glycosylations to achieve several human milk oligosaccharides.[9c] Both methods can synthesize linear and simple oligosaccharides with obvious limitations in achieving more complex HMOs, especially highly branched ones. In this study, we developed an efficient and versatile methodology that utilized oligosaccharyl thioethers and oligosaccharyl bromides as convergent donors for glycosylation,

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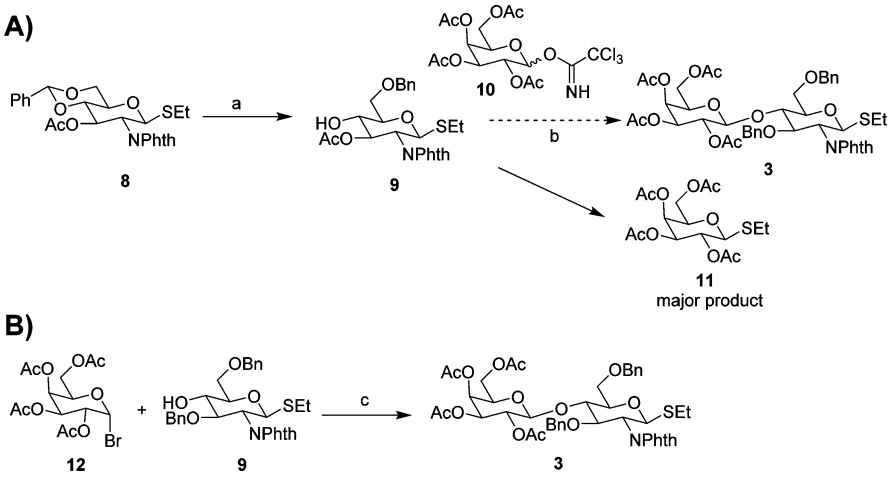
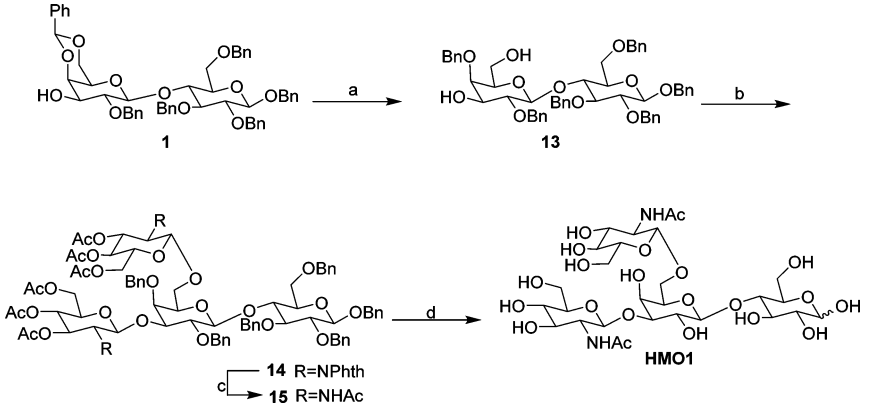
Scheme 2. Synthesis of Building Block 3: (A) Failed Reaction; (B) Successful Reaction[a]



[a]Reagents and conditions: (a) Et_3SiH, TfOH, 4 Å MS, −78 °C, 85%; (b) TMSOTf, DCM, 4 Å MS, −20 °C; (c) AgOTf, DCM/toluene, 70%.

Scheme 3. Synthesis of HMO1[a]



[a]Reagents and conditions: (a) Et_3SiH, $PhBCl_2$, 4 Å MS, DCM, −78 °C, 85%; (b) **2**, AgOTf, 2,4,6-collidine, −20 °C, 85%; (c) (i) ethanediamine, *n*-BuOH, (ii) Ac_2O, pyridine, DMAP; (d) (i) NaOMe/MeOH 75% over two steps, (ii) H_2, $Pd(OH)_2/C$, 80% over two steps.

enabling branching assembly in one or two steps of glycosylations with excellent stereoselectivity and yields.

We envisaged that protected lactose **1** (Figure 2) would be a versatile precursor for the synthesis of core structures, including symmetric and asymmetric ones, as C4,C6-hydroxy groups (OH) on Gal are protected by benzylidene and the C3-OH is unprotected for chemical glycosylation. In order to achieve the selective protection of building block **1**, the C3-OH group was selectively protected by 4-methoxybenzyl ether (PMB) using standard conditions, followed by C4,C6-OH protection with a benzylidene group. In order to furnish the target cores, two oligosaccharyl thioethers and oligosaccharyl bromide were prepared (Figure 2).

Synthesis of Building Blocks. The synthesis of precursor **1** began with lactose peracetate, which was converted to the *β*-lactoside **4** by reaction with benzyl alcohol in the presence of $BF_3 \cdot Et_2O$, followed by deacetylation with NaOMe/MeOH to furnish compound **5**. Then **5** was treated with dibutyltin oxide, followed by a reaction with 4-methoxybenzyl chloride to provide a selectively 3′-*O*-PMB protected lactoside in fair yield, which has been extensively studied.[11] The following benzylidene protection on 4′,6′-OH was conducted with benzaldehyde dimethyl acetal, catalyzed by camphorsulfonic acid (CSA), to give compound **6**. The perbenzylation of the remaining hydroxyls of **6** was performed by using sodium hydride and benzyl bromide in anhydrous DMF to give compound **7**. After the PMB protecting group of **7** was removed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), the synthesis of building block **1** was achieved (Scheme 1).

The lactosamine building block **3** was initially envisioned to be synthesized using a straightforward fashion by coupling monosaccharides **9** and **10** (Scheme 2A). Unfortunately, the desired product was isolated in minor amounts by silica gel chromatography, and a substantial amount of byproduct **11** was generated through a thioether migration reaction.[12] Therefore, a Koenigs−Knorr reaction coupling approach was carried out by installing the C4 hydroxy group of glucosamine with high yield (Scheme 2B). Building block **2** can be conveniently used in the synthesis of two asymmetric core oligosaccharides.

Synthesis of Core Oligosaccharides. With all of the building blocks in hand, we began to assemble the three core oligosaccharides using our convergent strategy. The synthesis of **HMO1** was depicted in Scheme 3. Disaccharide **13** was obtained in good yield (85%) by selective opening of the benzylidene ring at C6 of building block **1** using $Et_3SiH/PhBCl_2$. Oligosaccharyl bromide **2** was freshly prepared using HBr/AcOH. Silver triflate (AgOTf) promoted glycosylation of **13** with donor **2** in dichloromethane resulted in the formation of tetrasaccharide

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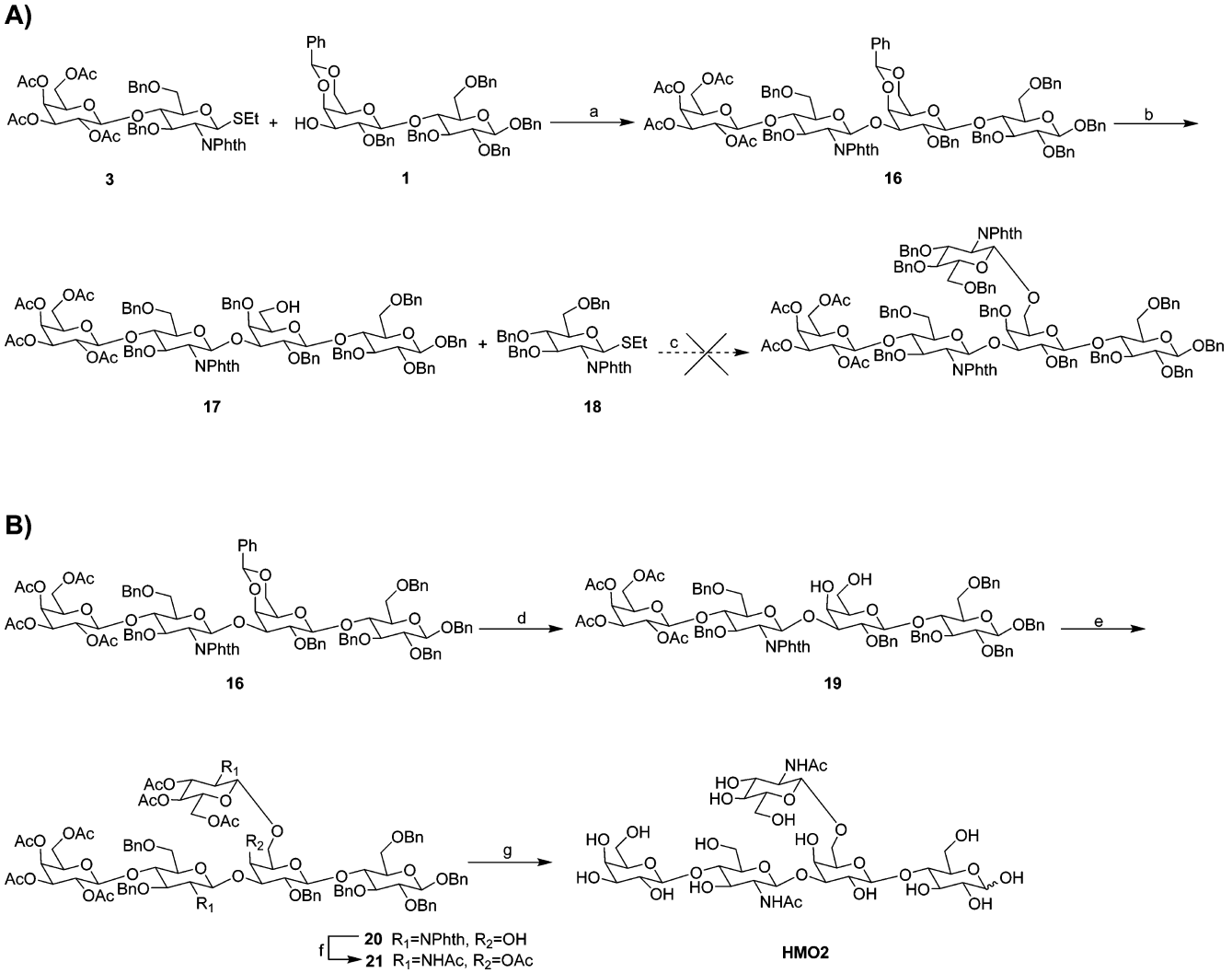
Scheme 4. Synthesis of HMO2: (A) Failed Reaction; (B) Successful Reaction[a]



[a]Reagents and conditions: (a) NIS, TfOH, DCM, 4 Å MS, −20 °C, 85%; (b) Et_3SiH, $PhBCl_2$, DCM, −78 °C, 80%; (c) NIS, AgOTf, DCM, 4 Å MS, −20 °C; (d) TsOH, EtSH, DCM, 90%; (e) **2**, AgOTf, 2,4,6-collidine, DCM, 4 Å MS, −20 °C, 85%; (f) (i) ethanediamine, *n*-buOH, (ii) Ac_2O, pyridine, DMAP 62% over two steps; (g) (i) NaOMe/MeOH, (ii) H_2, $Pd(OH)_2/C$, 80% over two steps.

14 in an excellent yield of 85%. Deprotection of **14** with ethylenediamine, followed by treatment with acetic anhydride, furnished the peracetylated tetrasaccharide **15** in 75% yield. *O*-Deacetylation of compound **15** was performed under Zemplén conditions, followed by the global deprotection of the benzyl group (Bn) by catalytic hydrogenolysis with $Pd(OH)_2/H_2$ in $MeOH/H_2O$ (10/1). The core structure **HMO1** was produced in a total yield of 80% over the two steps.

Glycosylation of 3′-*O*-unprotected acceptor **1** with donor **3** proceeded at −20 °C under AgOTf/NIS conditions to furnish the desired tetrasaccharide **16** in 85% yield. Then selective opening of the benzylidene ring at C6 of **16** using $Et_3SiH/PhBCl_2$ provided 6′-*O*-unprotected acceptor **17** in 80% yield. The fully protected pentasaccharide was initially attempted to be synthesized by a convergent glycosylation of acceptor **17** and thiol donor **18**. Unfortunately, the desired product was not detected by TLC and ESI mass spectrometry analysis (Scheme 4A). Several other donors, including oligosaccharyl trichloroacetimidate and thioether donors, were tried to install the pentasaccharide, but no product was detected. The main challenge should be due to the bulky benzyl group at the 4′-OH position, which has very large steric hindrance and stops the glycosylation on the 6′-OH position, even though the primary alcohol is very nucleophilic. Therefore, 4′,6′-*O*-unprotected lacto-*N*-tetraose **19** was proposed as an acceptor for the glycosylation. Removal of the 4′,6′-*O*-benzylidene of **16** by treatment with ethanethiol in the presence of TsOH afforded acceptor **19**. Glycosylation of acceptor **19** with glycosyl bromide **2** to give the protected target pentasaccharide **20** proceeded smoothly and regioselectively by use of AgOTf as a Lewis acid at −20 °C in an excellent yield (85%). The two phthalimides of **20** were then converted into acetamides **21**, followed by the global deprotection of Ac and Bn groups. The core oligosaccharide **HMO2** was produced in a total yield of 53% over four steps (Scheme 4B).

AgOTf promoted glycosylation of 3′-*O*-unprotected acceptor **1** with donor **2** proceeded at −20 °C to furnish the trisaccharide **22** in a good yield of 85%. Then deprotection of the 4′,6′-*O*-benzylidene of **22** by treatment with EtSH/TsOH provided the dialcohol **23**. Glycosylation of acceptor **23** with thiol donor **3** by treatment with AgOTf/NIS at −20 °C gave the protected target pentasaccharide **24** in 70% yield. The two phthalimides of **24**

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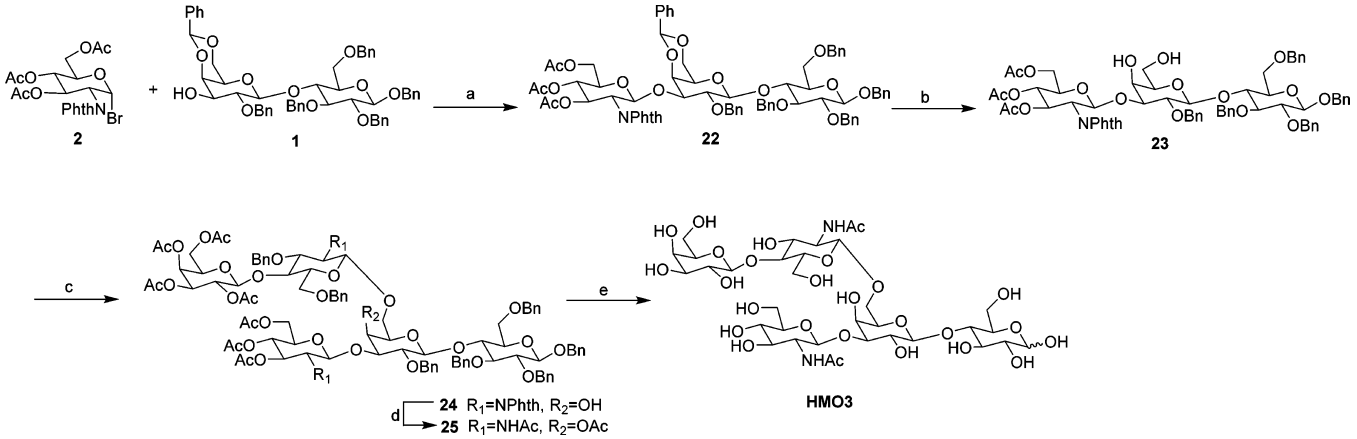
Scheme 5. Synthesis of HMO3[a]



[a]Reagents and conditions: (a) AgOTf, 2,4,6-collidine, DCM, 4 Å MS, 85%; (b) TsOH, EtSH, DCM, 80%; (c) **3**, NIS, AgOTf, DCM, 4 Å MS, −20 °C, 70%; (d) (i) ethanediamine, *n*-BuOH, (ii) Ac_2O, pyridine, DMAP, 70% over two steps; (e) (i) NaOMe, MeOH; (ii) $Pd(OH)_2$, H_2, 95% over two steps.

were then converted into acetamides **25**. Complete deprotection of **25** was achieved by hydrogenolytic debenzylation ($Pd(OH)_2$/C, H_2) and complete de-*O*-acetylation using sodium methoxide in methanol, resulting in core oligosaccharide **HMO3** in a total yield of 67% over four steps (Scheme 5).

Enzymatic Extension of Core Structures. A total of 31 HMOs were enzymatically synthesized starting from the 3 core structures (**HMO1**, **HMO2**, **HMO3**) via an enzymatic extension approach using 4 robust GTs: β-1,4-galactosyltransferase from *Neisseria meningitidis* (LgtB),[13] α-2,6-sialyltransferase from *Photobacterium damselae* (Pd2,6ST),[14] C-terminal 66 amino acid truncated α-1,3-fucosyltransferase from *Helicobacter pylori* (Hpα1,3FT),[15] and α-1,2-fucosyltransferase from *Helicobacter mustelae* (Hmα1,2FT).[16] All GTs were obtained from bacteria and had high expression levels in *Escherichia coli*, high activity, and relatively relaxed substrate tolerance. As shown in Figure 3A, glycans **HMO11**−**HMO16** were prepared by starting with the chemically prepared core **HMO1**. Briefly, in a 2 mL reaction system, 30 mg of **HMO1** (20 mM) was incubated with Gal (20 mM), $MgCl_2$ (20 mM), ATP (20 mM), UTP (20 mM), and varying amounts of BiGalK, BiUSP, and LgtB[17] (Figure 4). After overnight reaction, the reaction was terminated by boiling for 10 min and analyzed by MALDI-MS, which shows a single peak at m/z 1095.748, corresponding to **HMO11** $[M + Na]^+$. Meanwhile, on the HPLC-ELSD (evaporative light scattering detector) profile, a new peak (TR = 11.946 min) was observed. The reaction mixture was purified by HPLC using a water/acetonitrile gradient elution, giving 40 mg of **HMO11** (93% yield). The purified **HMO11** (99% pure) was then utilized for the syntheses of **HMO12**−**HMO16** (Figure 3A) catalyzed by Pd2,6ST, and α1,2FT, α1,2FT respectively (see the Supporting Information for details). Interestingly α1,3FT can specifically distinguish the GlcNAc from terminal Galβ1,4GlcNAc and α1,2FT preferentially attaches to terminal Gal. We basically use this feature to biosynthesize Lewis X (Le^x) and Lewis Y (Le^y). In addition, the difucosylated LacNAc motif (Fucα1, 2-Gal-β1, 4-(Fucα1,3-)GlcNAc) was also generated while using Hmα1,2FT. The syntheses of asymmetric biantennary **HMO2x** and **HMO3x** (Figure 3B,C) were carried out by enzymatic extension of core **HMO2** and **HMO3**. Asymmetric core **HMO2** and **HMO3** can more efficiently take advantage of different substrate specificities of GTs over symmetric **HMO1** via coupling of Gal to the terminal GlcNAc of one antennary. For example, to obtain **HMO311**, Gal from the β6GlcNAc branch was sequentially extended by Hmα1,2FT, Hpα1,3FT, and LgtB (Figure 3C). In contrast, **HMO310** was sequentially synthesized by Hmα1,2FT, LgtB, and Hpα1,3FT (Figure 3C). Such synthetic routes were designed according to the substrate specificities of GTs to avoid undesirable glycosylation. Each glycosylation step is further described in detail in Figure 4.

To avoid tedious purification steps of producing much more complex HMO structures, one-pot multienzyme systems (OPME) were used for LgtB and Pd2,6ST catalyzed glycosylation steps (Figure 4a−c).[18] LgtB, which can specifically recognize terminal GlcNAc, was combined with enzymes (BiGalK and AtUSP) involved in the biosynthesis of their corresponding sugar nucleotides (UDP-Gal) to produce the target glycan with the desired β1,4 linkage (Figure 4a). Similarly, Pd2,6ST was combined with NmCSS, a CMP-sialic acid synthetase, to form the α-2,6 configuration (Figure 4b,c). Both Hmα1,2FT and Hpα1,3FT simply used pure GDP-Fuc to form α-1,2 and α-1,3 linkages, respectively (Figure 4d,e).

CONCLUSION

In summary, we have utilized our well-developed CSEE strategy for the efficient synthesis of a library of structure-defined HMOs, which was assisted by rapid HPLC purification. The combination of CSEE and HPLC purification allowed us to deliver 31 diverse, high-purity homogeneous HMOs. These HMOs are valuable materials for bioactivity evaluations as well as glycan analyses. In this work, oligosaccharyl thioethers and oligosaccharyl bromide were consistently utilized as chemical glycosylation donors for the convergent installation of branched lactose-terminated antennae. This general and efficient method furnished 3 core oligosaccharides with high stereoselectivity and excellent yields. This work further confirmed that any GlcNAc-terminated glycan could be extended to 5 or more glycans, including Le^X and SLe^X, which are very important epitopes in glycobiology. The CSEE strategy demonstrated a practical way to harvest diverse and complex HMOs with defined structures for various applications. The "mass" production of more homogeneous HMOs and bioactivity evaluations are underway.

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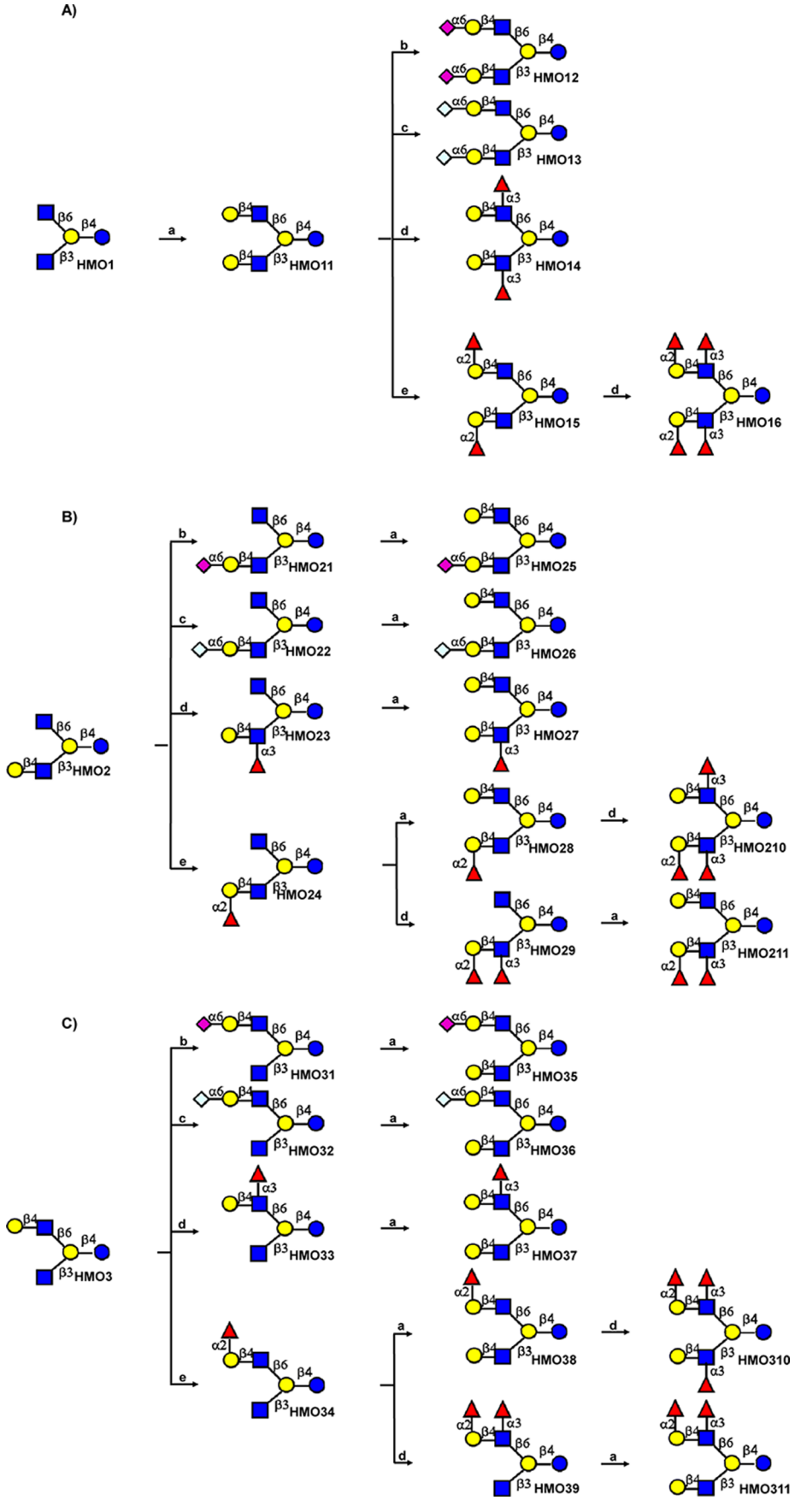

Figure 3. . Enzymatic extension of human milk oligosaccharides.

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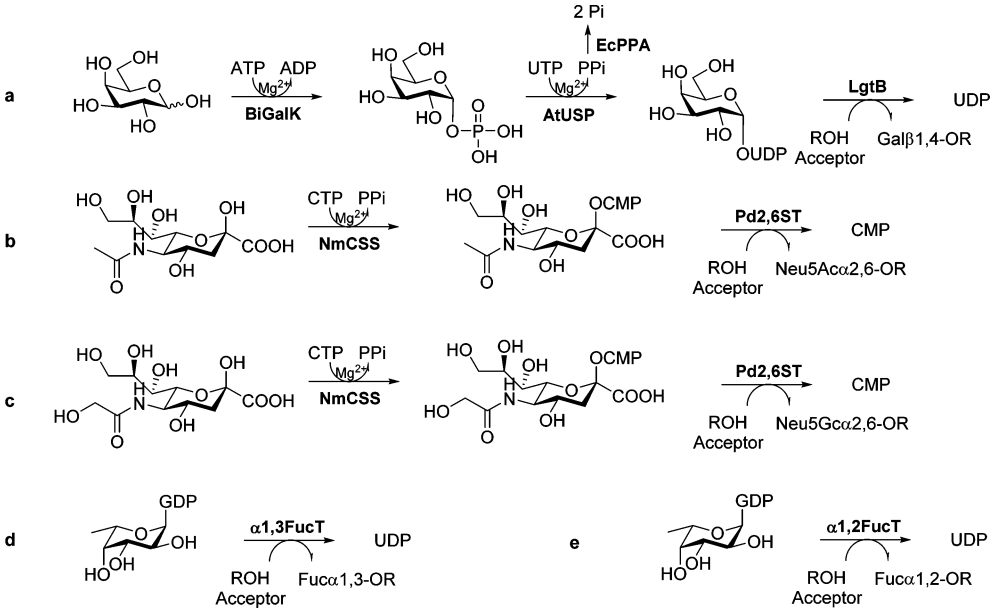

Figure 4. (a) LgtB, BiGalK, AtUSP, Gal, ATP, UTP, Mg^{2+}. (b) Pd2,6ST, NmCSS, Neu5Ac, CTP, Mg^{2+}. (c) Pd2,6ST, NmCSS, Neu5Gc, CTP, Mg^{2+}. (d) Hpα1,3FT, GDP-Fuc, Mn^{2+}. (e) Hmα1,2FT, GDP-Fuc, Mn^{2+}. Abbreviations: LgtB, *Neisseria meningitidis* β-1,4-galactosyltransferase; BiGalk, *Bifidobacterium infantis* galactokinase; AtUSP, *Arabidopsis thaliana* pyrophosphorylase; Pd2,6ST, *Photobacterium damselae* α-2,6-sialyltransferase; NmCSS, *Neisseria meningitidis* CMP-sialic acid synthetase; Hpα1,3FT, C-terminal 66 amino acids truncated *Helicobacter pylori* α-1,3-fucosyltransferase; Hmα1,2FT, *Helicobacter mustelae* α-1,2-fucosyltransferase.

EXPERIMENTAL SECTION

General Methods. All chemicals were purchased as reagent grade and used without further purification. Anhydrous dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), toluene, and methanol (MeOH) were purchased from a commercial source without further distillation. Pulverized molecular sieves MS-4 Å (Aldrich) for glycosylation was activated by heating at 350 °C for 3 h. All reactions were performed with dry solvents and under nitrogen unless otherwise stated. Thin-layer chromatography (TLC) with 60 F_{254} silica gel plastic plates was visualized under UV (254 nm) and/or by staining with a solution of 10 mL of anisaldehyde and 10 mL of 95% H_2SO_4 in 400 mL of ethanol, followed by heating on a hot plate. Column chromatography was carried out on silica gel (EMD 230−400 mesh ASTM) and P2 gel. Optical rotation values were measured using a polarimeter at ambient temperature in the specified solvents. ^{1}H NMR spectra were recorded on a 400 or 500 MHz NMR spectrometer at 25 °C. All ^{1}H chemical shifts (in ppm) were assigned according to $CDCl_3$ (δ 7.24 ppm) or D_2O (δ 4.79 ppm). ^{13}C NMR spectra were obtained with a 400 MHz NMR spectrometer and calibrated with $CDCl_3$ (δ 77.00 ppm). Coupling constants (J) are reported in hertz (Hz). Splitting patterns are described using the following abbreviations: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet. ^{1}H NMR spectra are reported in the following order: chemical shift, multiplicity, coupling constant(s), and number(s) of protons. All NMR signals were assigned on the basis of ^{1}H NMR and ^{13}C NMR experiments. High-resolution MALDI mass spectra were recorded on an ESI-Orbitrap or MALDI-TOF spectrometer.

Neu5Ac and Neu5Gc were purchased from Carbosynth Limited. ATP and CTP were purchased from Sigma. Thermosensitive alkaline phosphatase from shrimp (FastAP) was purchased from Thermo Scientific. Other enzymes, including *Neisseria meninngitidis* β1,4-galactosyltransferase (NmLgtB), α-2,6-sialyltransferase from *Photobacterium damselae* (Pd2,6ST), C-terminal 66 amino acid truncated *Helicobacter pylori* α-1,3-fucosyltransferase (Hpα1,3FT), *Helicobacter mustelae* α-1,2-fucosyltransferase (Hmα1,2FT), and CMP-sialic acid synthetase from *N. meningitides* (NmCSS), were expressed and purified as previously described. Enzymes were then desalted against 50 mM Tris-HCl, 100 mM NaCl, and 50% glycerol and stored at −20 °C for long-term use. Sugar nucleotide guanoside 5′-diphospho-L-fucose (GDP-Fuc) was prepared as described in our previous paper.[19]

General Procedures. *A. Transformation of N-Phth to NHAc.* A mixture of N-Phth protected oligosaccharide was dissolved in *n*-BuOH at room temperature, followed by addition of ethylenediamine (*n*-BuOH/ethylenediamine 2/1). After it was stirred at 90 °C for 12 h, the mixture was evaporated in vacuo to give a residue for the next step without further purification. To a solution of the residue in pyridine was added Ac_2O. After it was stirred at room temperature for 12 h, the solution was diluted with EtOAc and washed with aqueous 1 M HCl, saturated aqueous $NaHCO_3$, and brine solution. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to give a residue, which was purified by silica gel column chromatography to give the NHAc compound.

B. Deacetylation. An Ac-protected oligosaccharide was dissolved in MeOH, and NaOMe in MeOH was added until the pH was about 10. After it was stirred at room temperature for 12 h, the solution was neutralized with ion exchange resin (H^+) and then filtered. The residue was concentrated in vacuo to afford the desired deacetylated product.

C. Deprotection of Benzyl Group. $Pd(OH)_2$ on carbon was added to a solution of protected oligosaccharide in MeOH/H_2O (10/1). The mixture was stirred under 1 atm of hydrogen. After it was stirred for 24 h, the mixture was filtered through a PTFE syringe filter and concentrated in vacuo. The residue was purified by Bio-Gel P-2 (BIO-RAD) column chromatography using water as eluent. The product was then lyophilized to give the target compound as a white powder.

D. Production of Oligosaccharyl Bromide. Peracetylated oligosaccharide was added portionwise to a stirred solution of HBr (33%) in glacial acetic acid (20.0 mL) at 0 °C. After all the sugar had been added, the reaction mixture was stirred at room temperature for 45 min. TLC analysis (hexanes/ethyl acetate 1/1) indicated formation of the product and consumption of starting material. Then the reaction was quenched with ice water (200 mL) and the product extracted with DCM (2 × 200 mL). The combined organic extracts were washed with a solution of $NaHCO_3$ (aqueous saturated, 2 × 200 mL), dried with Na_2SO_4, filtered, and then concentrated in vacuo. The crude product was used without further purification.

E. Enzyme Treatment and HPLC Purification. In general, 31 HMOs were enzymatically synthesized by 4 glycosyltransferases (NmLgtB, Pd2,6ST, Hpα 1,3FT, Hmα 1,2FT) under the nearly same reaction

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conditions. Reaction mixtures contain 50 mM Tris-HCl (pH 8.0), 10 mM acceptor HMOs, 12 mM sugar nucleotide (or its corresponding synthetase), 10 mM $MnCl_2$, and varying amounts of glycotransferases. FastAP (1 U/200 μL) was also added to digest the reaction byproduct UDP to drive the reaction forward. Reaction mixtures were incubated at 37 °C overnight and monitored by HILIC-ELSD (Waters XBridge BEH amide column, 130 Å, 4.6 mm × 250 mm under a gradient running condition (solvent A, 100 mM ammonium formate, pH 3.4; solvent B, acetonitrile; flow rate 1 mL/min; B% 65−50% within 25 min)). The desired products were detected by a highly efficient ELSD (evaporative light scattering detector), which increases the sample concentration to minimize the noise and deliver higher sensitivity. After over 90% of the acceptor was converted, the reaction was quenched by boiling for 10 min, followed by concentration with a rotary evaporator. HPLC-A210 nm was then used to purify target HMOs using a semipreparative column (Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm) under the following gradient running conditions: solvent A, 100 mM ammonium formate, pH 3.4; solvent B, acetonitrile; flow rate 4 mL/min; B%: 65−50% within 25 min.[10] MS data for purified HMOs were obtained by ESI-MS and MALDI-MS.

Benzyl *O*-(4,6-*O*-Benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (6). A suspension of benzyllactose **5** (12.0 g, 27.78 mmol) and Bu_2SnO (7.6 g, 30.54 mmol) in anhydrous MeOH (100 mL) was heated to reflux and stirred for 8 h. The solvent was removed in vacuo. Then the residue was dissolved in dry toluene (100 mL). *p*-Methoxybenzyl chloride (3.76 mL, 20.37 mmol), tetrabutylammonium iodide (2.05 g, 11.10 mmol), and 4 Å molecular sieves (5 g) were added. The resulting mixture was heated to reflux for another 8 h and then cooled to room temperature. The suspension was filtered through a Celite pad, and the filtrate was concentrated and chromatographied (dichloromethane/methanol 6/1) to afford 9.2 g of crude product (60% yield).

Benzaldehyde dimethyl acetal (2.75 mL, 18.33 mmol) was added to a solution of the above crude product (7.8 g, 14.10 mmol) in anhydrous dimethylformamide (100 mL), and then camphorsulfonic acid was added to adjust the pH to about 2.0−3.0. The reaction mixture was stirred overnight and then quenched with triethylamine. The mixture was concentrated under vacuum. The residue was purified by flash column chromatography (dichloromethane/methanol 10/1) to give **6** as a white solid (8.47 g, 87.0%). $[\alpha]_D^{20}$ = +6.7 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48−7.51 (dd, 2H), 7.28−7.38 (m, 10 H), 6.85−6.87 (m, 2 H), 5.34 (s, 1 H), 4.87 (d, *J* = 11.95 Hz, 1 H), 4.59−4.63 (m, 4 H), 4.45 (d, *J* = 7.8 Hz, 1 H), 4.36 (d, *J* = 8.1 Hz, 1 H), 4.21 (d, *J* = 14.0 Hz, 1 H), 4.10 (s, 1 H), 3.92−4.00 (m, 3 H), 3.79−3.89 (m, 2 H), 3.78 (s, 3 H), 3.60−3.69 (m, 3 H), 3.40−3.50 (m, 2 H), 3.27−3.40 (m, 4 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.6,137.2, 130.0,129.5, 128.5, 128.3, 128.2, 127.9, 126.3, 113.9, 103.6, 101.8, 101.1, 78.8, 74.9,74.7, 73.5, 72.7, 71.3, 71.2, 69.1, 69.0, 66.9, 61.9, 55.3. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{34}H_{40}NaO_{12}$ 663.2417, found 663.2420.

Benzyl *O*-(2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranos-yl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). NaH (60%; 2.25 g, 56.25 mmol) and BnBr (6.66 mL, 56.25 mmol) were added to a stirred solution of **6** (6.0 g, 9.38 mmol) in DMF (60 mL) cooled to 0 °C. The solution turned light yellow. The reaction mixture was maintained at room temperature for 4 h. Then the solution was quenched with MeOH. The mixture was diluted with EtOAc and washed with water. The organic layer was dried with Na_2SO_4 and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 6/1) to afford the product 7 (1.85 g, 92.5%) as a white powder. $[\alpha]_D^{20}$ = +7.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57−7.59 (m, 2 H), 7.51−7.54 (m, 2 H), 7.42−7.45 (m, 2 H), 7.31−7.38 (m,23 H), 7.23−7.24 (m, 2 H), 6.90−6.92 (m, 2 H), 5.51 (s, 1H), 5.25 (d, *J* = 11.0 Hz, 1 H), 4.98−5.03 (m, 2 H), 4.90 (d, *J* = 11.1 Hz, 1 H), 4.79−4.85 (m, 3 H),4.71−4.74 (m, 3 H), 4.63 (d, *J* = 12.0 Hz, 1 H), 4.52−4.55 (m, 2 H), 4.43 (d, *J* = 12.0 Hz, 1 H), 4.26 (dd, *J* = 1.4 Hz, 12.4 Hz, 1 H), 4.04−4.08 (m, 2 H), 3.96 (dd, *J* = 4.2, 11.3 Hz, 1 H), 3.90 (dd, *J* = 1.8, 12.5 Hz, 1 H), 3.85 (s, 3 H), 3.79−3.83 (m, 2 H), 3.69 (t, *J* = 8.8 Hz, 1 H), 3.56−3.60 (m, 1 H), 3.40−3.46 (m, 2 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.3, 139.0, 139.0, 138.7, 138.6, 138.2, 137.6, 130.5, 129.4, 128.9, 128.7, 128.4, 128.3, 128.1, 128.0, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 126.6, 113.8, 103.0, 102.6, 101.4, 83.1, 81.9, 79.4, 78.9, 77.7, 75.9, 75.3, 75.2, 75.1, 73.8, 73.0, 71.4, 71.1, 69.0, 68.3,66.4, 55.3. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{62}H_{64}NaO_{12}$ 1023.4295, found 1023.4285.

Benzyl *O*-(2-*O*-Benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (1). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (2.75 g, 12.10 mmol) was added to a solution of **7** (6.0 g, 6.05 mmol) in 9/1 CH_2Cl_2/phosphate-buffered saline (200 mL). The solution was stirred for 1.5 h at room temperature and diluted with CH_2Cl_2. The solution was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **1** (5.02 g, 95%) as a white powder. $[\alpha]_D^{20}$ = +15.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52−7.55 (m, 4 H), 7.20−7.45 (m, 26 H), 5.56 (s, 1 H), 5.21 (d, *J* = 8.0 Hz 1 H), 4.97−5.02 (m, 2 H), 4.78−4.83 (m, 4 H), 4.65−4.73 (m, 2 H), 4.51−4.54 (m, 2 H), 4.46 (d, *J* = 12.3 Hz, 1 H), 4.30 (d, *J* = 12.3 Hz, 1 H), 4.06−4.13 (m, 2 H), 3.93−3.97 (m, 2 H), 3.79 (dd, *J* = 1.3, 11.0 Hz, 1 H), 3.68 (m, 1 H), 3.55−3.60 (m, 3 H), 3.38−3.41 (m, 1 H), 3.14 (s, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.9, 138.7, 138.6, 138.5, 137.9, 137.5, 129.2, 128.8, 128.4, 128.2, 128.0, 127.8, 127.6, 127.4, 126.5, 102.8, 102.6, 101.5, 83.1, 81.9, 80.2, 77.6, 75.9, 75.7, 75.2, 75.1, 73.1, 72.9, 71.0, 68.9, 68.2, 66.5. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{54}H_{56}NaO_{11}$ 903.3720, found 903.3725.

Ethyl 3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (9). A mixture of compound **8** (2 g, 4.09 mmol) and 4 Å molecular sieves (2 g) in dry CH_2Cl_2 was stirred at room temperature under nitrogen for 2 h. Triethylsilane (2.1 mL, 13.1 mmol) and TfOH (1.05 mL, 11.9 mmol) were sequentially added at −78 °C. The reaction mixture was stirred at −78 °C for 2 h and then quenched with MeOH (2 mL) and Et_3N (2 mL). The resulting mixture was filtered. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **9** (1.85 g, 92.5%) as a white powder. $[\alpha]_D^{20}$ = +91.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82 (d, *J* = 5.9 Hz, 1 H), 7.66−7.72 (m, 3 H), 7.28−7.42 (m, 5 H), 7.03−7.10 (m, 2 H), 6.92−7.00 (m, 3 H), 5.32 (d, *J* = 9.9 Hz, 1 H), 4.80 (d, *J* = 12.0 Hz, 1 H), 4.54−4.70 (m, 3 H), 4.25−4.34 (m, 2 H), 3.80−3.89 (m, 3 H), 3.70−3.73 (m, 1 H), 2.59−2.72 (m, 2 H), 1.19 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.1, 167.6, 138.2, 137.7, 134.0, 133.9, 131.6, 128.5, 127.9, 127.9, 127.8, 127.5, 123.5, 123.3, 81.2, 79.7, 78.0, 74.5, 74.2, 73.8, 70.7, 54.5, 24.0, 15.0. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{30}H_{31}NNaO_6S$ 556.1770, found 556.1760.

Ethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3). 2,3,4,6-Tetra-*O*-acetyl-β-D-galactosyl bromide (3.35 g, 8.17 mmol) was prepared by following general procedure D. Then the bromide donor (3.35 g, 8.17 mmol) and 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **9** (2.64 g. 5.43 mmol) were dissolved in a mixture of dry toluene and CH_2Cl_2 (1/1, 30 mL). Powdered molecular sieves (4 Å) were added, and the mixture was stirred under nitrogen for 1 h. The flask was wrapped in aluminum foil and cooled to −45 °C. AgOTf (2.79 g, 10.86 mmol) dissolved in dry toluene (20 mL) was added during 1 h with the exclusion of light. After additional stirring for 30 min at −45 °C, the reaction mixture was quenched by aqueous $Na_2S_2O_3$. The mixture was transferred to a separatory funnel via a Celite-packed glass filter funnel. The organic phase was separated, dried with Na_2SO_4, filtered, and concentrated. Purification of the residue by silica gel column chromatography (hexanes/EtOAc 4/1) gave compound **3** (5.81 g, 80%). $[\alpha]_D^{20}$ = +31.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (d, *J* = 6.3 Hz, 1 H), 7.62−7.66 (m, 3 H), 7.29−743(m, 5 H), 7.01(d, *J* = 7.0 H), 6.82−6.92 (m, 3 H), 5.13−5.28 (m, 3 H), 4.78−4.87 (m, 3 H), 4.62 (d, *J* = 7.9 Hz, 1 H), 4.43−4.51 (m, 2 H), 4.20−4.28 (m, 2 H), 4.09 (t, *J* = 9.5 Hz, 1 H), 3.89−4.02 (m, 2 H), 3.79 (s, 2 H), 3.64 (t, *J* = 6.8 Hz, 1 H), 3.56 (d, *J* = 10.0 Hz, 1 H), 2.55−2.75 (m, 2 H), 2.06 (s, 3 H), 2.02 (s, 6 H), 1.97 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 170.2, 170.0, 169.2, 167.9, 167.4, 138.5, 137.9, 133.9, 133.7, 131.6, 128.6, 128.0, 127.9, 127.9, 127.1, 123.4, 123.3, 100.3, 81.1, 79.1, 77.8, 77.6, 74.5, 73.6,

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70.4,69.5, 67.7, 66.9, 60.7, 54.7, 23.9, 20.8, 20.7, 20.6, 20.6, 14.9. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{44}H_{49}NNaO_{15}S$ 886.2721, found 886.2729.

Benzyl *O*-(2,4-Di-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (13). A mixture of compound **1** (2 g, 2.27 mmol) and 4 Å molecular sieves (2 g) in dry CH_2Cl_2 was stirred at room temperature under nitrogen for 2 h. Triethylsilane (0.69 mL, 4.34 mmol) and $PhBCl_2$ (0.56 mL, 4.34 mmol) were sequentially added at −78 °C. The reaction mixture was stirred at −78 °C for 2 h and then quenched by the addition of MeOH (2 mL) and Et_3N (2 mL). The resulting mixture was filtered. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **13** (1.70 g, 85%) as a white powder. $[\alpha]_D^{20}$ = +8.9 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.35−7.52 (m, 27 H), 7.26−7.34 (m, 3 H), 5.03−5.16 (m, 3 H), 4.78−4.97 (m, 6 H), 4.70−4.75 (m, 2 H), 4.59−4.65 (m, 2 H), 4.52 (d, *J* = 7.0 Hz, 1 H), 4.08 (t, *J* = 8.7 Hz, 1 H), 3.92 (d, *J* = 2.6 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 138.9, 138.7 (2 C), 138.5, 137.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 127.6, 102.8, 102.6, 82.8, 81.9, 80.5, 77.0, 75.8, 75.5, 75.3, 75.2 (2 C), 75.1, 74.4, 73.4, 71.1, 68.4,61.7. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{54}H_{58}NaO_{11}$ 905.3877, found 905.3867.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl]-(1→6)-(2,4-di-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (14). 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucosyl bromide was prepared by following the general procedure D. Powdered molecular sieves (4 Å; 3.0 g) was added to a solution of the above bromide donor (4.80 g, 5.44 mmol) and **13** (800 mg, 0.907 mmol) in anhydrous dichloromethane (20 mL). The suspension was stirred under nitrogen for 1.5 h at room temperature and then cooled to −30 °C. Then 2,4,6-collidine (0.72 mL, 5.44 mmol), and freshly dried AgOTf (1.40 g, 5.44 mmol) were sequentially added to the reaction mixture. After it was stirred for 2 h at −30 °C, the mixture was warmed to room temperature overnight, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **14** (1.33 g, 85%) as a white powder. $[\alpha]_D^{20}$ = +6.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.85 (t, *J* = 4.01 Hz, 1 H), 7.72−7.74 (m, 2 H), 7.43−7.56 (m, 2 H), 7.18−7.40 (m, 27 H), 7.08−7.16 (m, 3 H), 6.97 (m, 2 H), 5.76−5.87 (m, 2 H), 5.62 (d, *J* = 8.24 Hz, 1 H), 5.39 (d, *J* = 8.43, 1H), 5.16−5.24 (m, 2 H), 4.85−4.93 (m, 4 H), 4.75 (d, *J* = 10.6 Hz, 1 H), 4.55−4.66 (m, 3 H), 4.35−4.44 (m, 5 H), 4.19−4.33 (m, 5 H), 4.12 (d, *J* = 12.7 Hz, 1 H), 3.91 (t, *J* = 10.1 Hz, 1 H), 3.71−3.86 (m, 5 H), 3.47−3.59 (m, 5 H), 3.33−3.47 (m, 2 H), 3.21 (t, *J* = 5.9 Hz, 1 H), 3.05(dd, *J* = 3.2, 9.7 Hz, 1 H), 2.09 (s, 3 H), 2.09 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.87(d, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 170.7, 170.6, 170.1, 170.0, 169.5, 169.5, 139.1, 139.0, 138.6, 138.5, 138.4, 137.7, 134.4, 134.0, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 127.4, 127.1, 127.0, 126.9, 126.5, 102.4, 102.3, 99.0, 97.5, 83.1, 81.7, 81.7, 78.6, 76.2, 76.1, 75.6, 75.2, 75.0, 74.9, 74.1, 73.1, 72.8 (2 C), 71.7, 71.6, 70.8, 70.8 (2 C), 70.5, 68.9, 68.8, 68.0, 66.8, 61.7, 61.6, 55.1, 54.7, 20.8, 20.7, 20.7, 20.6, 20.5, 20.4. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{94}H_{96}N_2NaO_{29}$ 1739.5996, found 1739.5980.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxyacetamido-*β*-D-glucopyranosyl)-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-(2,4-di-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (15). Following the general procedure A compound **14** (1.16 g, 0.66 mmol) yielded the compound **15** (762 mg, 75% over two steps). $[\alpha]_D^{20}$ = −1.9 (*c* 0.4, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.25−7.48 (m, 27 H), 7.10−7.18 (m, 3 H), 5.78 (d, *J* = 9.5 Hz, 1 H), 5.01−5.16 (m, 6 H), 4.90−5.00 (m, 3 H), 4.75−4.91 (m, 4 H), 4.60−4.71 (m, 3 H), 4.41−4.55 (m, 4 H), 4.29 (d, *J* = 3.5 Hz, 1 H), 3.96−4.15 (m, 5 H), 3.64−3.86 (m, 8 H), 3.52−3.64 (m, 4 H), 3.45−3.51 (m, 1 H), 3.23 (d, *J* = 9.7 Hz, 1 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.51 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 170.9, 170.8, 170.6, 170.5, 170.3, 169.8, 169.3, 169.2, 139.4, 138.9, 138.8, 138.6, 137.9, 137.4, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 126.2, 102.6, 102.5, 102.4, 101.4, 84.3, 82.4, 82.1, 79.5, 76.7, 76.4, 76.2, 75.2, 74.9, 74.7, 74.5, 74.4, 73.3, 73.2, 72.7, 72.0, 70.9, 70.6, 68.8, 68.5, 67.9, 67.5, 62.0, 61.6, 54.4, 53.7, 23.6, 22.8, 20.8, 20.8, 20.7, 20.6 (3 C). HRMS: $[M + Na]^+$ calcd for $C_{82}H_{96}N_2NaO_{27}$ 1563.6098, found 1563.6126.

2-Deoxyacetamido-*β*-D-glucopyranosyl-(1→3)-[2-deoxyacetamido-*β*-D-glucopyranos-yl]-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*,*β*-D-glucopyranose (HMO1). Following the general procedures B and C, compound **15** (400 mg, 0.26 mmol) yielded the compound **HMO1** (152 mg, 80% over two steps). ^{1}H NMR (D_2O, 400 MHz): *δ* 5.15 (d, *J* = 3.6 Hz, 0.55 H, Glc-1 H-1 of *α* form), 4.53−4.62 (m, overlap with D_2O, 2.45 H, GlcNAc-1 H-1, GlcNAc-2 H-1 and Glc-1 H-1 of *β* form), 4.36 (d, *J* = 7.7 Hz, 1 H, Gal-1 H-1), 4.07(d, *J* = 2.4 Hz, 1 H), 3.58−3.95 (m, 13 H), 3.45−3.58 (m, 5 H), 3.32−3.45 (m, 4 H), 3.16−3.26 (m, 1 H), 1.99 (s, 3 H), 1.96 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): *δ* 174.9, 174.6, 102.9, 102.8 (GlcNAc-1, GlcNAc-2, C-1),101.1 (Gal-1, C-1), 95.7 (Glc-1, C-1 of *β* form), 91.8 (Glc-1, C-1 of *α* form), 81.7, 78.8, 75.8 (2 C), 75.6, 73.8, 74.7, 74.3, 73.8, 73.5, 73.4,69.9, 69.8, 69.6, 68.7, 68.4,60.7, 60.5, 55.6, 55.5, 22.4, 22.2. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{28}H_{48}N_2NaO_{21}$ 771.2647, found 771.2665.

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-(2-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (16). Powdered molecular sieves (4 Å; 3.0 g) was added to a solution of compounds **3** (871 mg, 0.96 mmol) and **1** (652 mg, 0.74 mmol) in anhydrous dichloromethane (20 mL). The suspension was stirred under nitrogen for 1.5 h at room temperature and then cooled to −30 °C. Then NIS (260 mg, 1.15 mmol) and TMSOTf (35 *μ*L, 0.19 mmol) were sequentially added to the reaction mixture. After it was stirred for 2 h at −30 °C, the mixture was warmed to room temperature overnight, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **16** (1.04 g, 86%) as a white powder. $[\alpha]_D^{20}$ = +12.9 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.53−7.55 (m, 2 H), 7.45−7.50 (m, 2 H), 7.27−7.45 (m, 27 H), 7.19 (t, *J* = 3.6 Hz, 2 H), 7.09−7.16 (m, 3 H), 7.02−7.06 (m, 2 H), 6.84−6.94 (m, 5 H), 5.49 (s, 1 H), 5.47 (d, *J* = 7.4 Hz, 1 H), 5.35 (d, *J* = 3.2 Hz, 1 H), 5.25 (dd, *J* = 7.9, 10.4 Hz, 1 H), 5.09 (d, *J* = 10.7 Hz, 1 H), 4.88−4.99 (m, 3 H), 4.85 (d, *J* = 12.0 Hz, 1 H), 4.65−4.78 (m, 4 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.44−4.55 (m, 3 H), 4.30−4.39 (m, 4 H), 4.18−4.28 (m, 5 H), 4.09 (t, *J* = 9.3 Hz, 1 H), 3.98−4.04 (m, 2 H), 3.89−3.96 (m, 1 H), 3.80−3.89 (m 2 H), 3.66−3.79 (m, 3 H), 3.53−3.64 (m, 2 H), 3.44−3.51 (m, 3 H), 3.38 (d, *J* = 10.1 Hz, 1 H), 2.93−3.02 (m, 2 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 170.3, 170.2, 170.1, 169.2, 139.0, 138.6 (2 C), 138.5, 138.3, 137.8, 137.6, 133.5, 131.3, 128.7, 128.6 (2 C), 128.4, 128.3 (2 C), 128.2, 128.1 (2 C), 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 127.1, 126.7, 126.5, 126.3, 123.1, 102.4 (2 C), 100.7, 100.6, 99.6, 83.1, 81.8, 80.9, 78.4, 77.7, 76.0, 75.7, 75.0, 74.8, 74.7, 74.5, 74.3, 73.8 (2 C), 73.0 (2 C), 71.1, 70.9 (2 C), 70.6, 69.7, 68.8, 68.7, 67.9, 67.0, 66.4,60.8, 55.8, 20.9, 20.7 (2 C), 20.6. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{96}H_{99}NNaO_{26}$ 1704.6353, found 1704.6383.

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-(2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (19). To a solution of compound **16** (800 mg, 0.49 mmol) in anhydrous MeOH (10 mL) were added TsOH (8.4 mg) and EtSH (0.21 mL, 2.93 mmol). The reaction mixture was stirred at room temperature for 6 h and then quenched with triethylamine and evaporated under reduced pressure. The mixture was purified with silica column (hexanes/acetone 5/1) to afford compound **19** as a white powder (702 mg, 90%). $[\alpha]_D^{20}$ = +18.4 (*c* 0.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.38−7.52 (m, 11 H), 7.25−7.36 (m, 16 H), 7.22−7.26 (m, 2 H), 7.03−7.14 (m, 3 H), 6.99−7.03 (m, 2 H), 6.85−6.92 (m, 2 H), 6.78 (d, *J* = 7.1 Hz, 2 H), 5.29−5.31 (m, 2 H), 5.19−5.25 (m, 1 H), 4.86−4.98 (m, 4 H), 4.83 (d, *J* = 12.2 Hz, 1 H), 4.68−4.77 (m, 3 H), 4.65 (d, *J* = 8.3 Hz, 1 H), 4.57−4.62 (m, 2 H), 4.46

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(t, J = 12.4 Hz, 2 H), 4.34−4.40 (m, 1 H), 4.26−4.34 (m, 3 H), 4.17−4.26 (m, 3 H), 3.95−4.08 (m, 3 H), 3.81−3.88 (m, 1 H), 3.72−3.79 (m, 3 H), 3.64−3.72 (m, 2 H), 3.47−3.55 (m, 2 H), 3.34−3.46 (m, 5 H), 3.17−3.23 (m, 1 H), 3.00−3.06 (m, 1 H), 2.87 (s, 1 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 170.2, 170.1, 169.2, 138.9, 138.4, 138.3 (2 C), 137.6, 137.5, 133.6, 131.1, 128.8, 128.4, 128.3 (3 C), 128.2 (2 C), 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 126.7, 126.4, 123.2, 102.4, 102.1, 100.6, 99.0, 83.8, 82.8, 81.5, 78.3, 77.9, 76.6, 76.4, 75.6, 74.9, 74.6, 74.3, 73.8, 73.7, 73.1, 71.0, 70.9, 70.7, 69.6, 68.3,67.9, 67.7, 66.9, 62.2, 60.8, 55.6, 20.8, 20.68, 20.6, 20.6. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{89}H_{95}NNaO_{26}$ 1616.6040, found 1616.6065.

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3))-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl]-(1→6)-(2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (20). 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucosyl bromide was prepared by following the general procedure D. Powdered molecular sieves (4 Å; 3.0 g) was added to a solution of **19** (460 mg, 0.29 mmol), 2,4,6-collidine (76 μL, 0.58 mmol), and freshly dried AgOTf (150 mg, 0.58 mmol) in anhydrous dichloromethane (20 mL). The suspension was stirred under nitrogen for 1.5 h at room temperature and then cooled to −30 °C. Then a solution of the above bromide donor (460 mg, 0.29 mmol) in dichloromethane (5.0 mL) was added dropwise during 30 min to the reaction mixture. After it was stirred for 2 h at −30 °C, the mixture was warmed to room temperature overnight, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/EtOAc 5/1) to afford the product **20** (495 mg, 85%) as a white powder. $[\alpha]_D^{20}$ = +11.7 (c 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76−7.91 (m, 4 H), 7.49−7.56 (m, 2 H), 7.41−7.49 (m, 8 H), 7.14−7.20 (m, 2 H), 7.05−7.11 (m, 1 H), 6.97−7.04 (m, 4 H), 6.85−6.94 (m, 3 H), 6.75 (d, J = 7.4 Hz, 2 H), 5.73 (dd, J = 9.1, 10.61 Hz, 1 H), 5.43 (d, J = 8.3 Hz, 1 H), 5.33 (d, J = 3.5 Hz, 1 H), 5.12−5.25 (m, 2 H), 4.99 (d, J = 8.9 Hz, 1 H), 4.77−4.95 (m, 3 H), 4.72−4.84 (m, 3 H), 4.61−4.71 (m, 2 H), 4.48−4.58 (m, 2 H), 4.33−4.47 (m, 3 H), 4.09−4.31 (m, 8 H), 3.80−4.06 (m, 6 H), 3.65−3.80 (m, 5 H), 3.62 (d, J = 10.0 Hz, 1 H), 3.50−3.56 (m, 2 H), 3.36−3.45 (m, 3 H), 3.20−3.27 (m, 2 H), 3.09−3.17 (m, 2 H), 2.87 (s, 1 H), 2.14 (s, 3 H), 2.11 (s, 3 H), 2.09 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.87 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 170.3, 170.2, 170.0 (2 C), 169.5, 169.5, 139.1, 138.7, 138.4, 138.3 (2 C), 137.7, 137.6, 131.2, 128.8, 128.4, 128.3 (3 C), 128.2, 128.1, 127.9 (2 C), 127.8, 127.7, 127.6 (2 C), 127.4, 127.2, 126.5, 123.2, 102.4, 101.9, 100.7, 98.5, 97.6, 83.8, 83.1, 81.9, 78.4, 77.8, 76.5, 76.3, 75.7, 75.1, 74.9, 74.5, 74.4, 74.4, 73.9, 72.9 (2 C), 72.0, 71.7, 71.1, 71.0, 70.8, 70.6, 69.5, 68.8, 68.1, 67.6, 66.9, 66.6, 61.7, 60.8, 55.4, 54.7, 20.9, 20.8, 20.67, 20.6 (3 C), 20.5. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{109}H_{114}N_2NaO_{35}$ 2033.7100, found 2033.7089.

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxyacetamido-*β*-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-(4-*O*-acetyl-2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (21). Following the general procedure A, compound **20** (400 mg, 0.20 mmol) yielded the compound **21** (232 mg, 62% over two steps). $[\alpha]_D^{20}$ = −1.0 (c 1.2, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51 (m, 2H), 7.21−7.43 (m, 33 H), 5.89 (d, J = 9.5 Hz, 1 H), 5.43 (d, J = 8.4 Hz, 1 H), 5.33 (d, J = 2.8 Hz, 1 H), 4.33−5.24 (m, 23 H), 4.24 (d, J = 8.2 Hz, 1 H), 3.94−4.10 (m, 6 H), 3.45−3.86 (m, 15 H), 3.23 (d, J = 9.7 Hz, 1 H), 2.12 (s, 3 H), 2.11 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.97 (s, 3 H), 1.94 (s, 3 H), 1.54 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.8, 170.7, 170.4, 170.3, 170.2, 170.0, 170.0 (2 C), 169.7, 169.2, 139.4, 138.7, 138.6 (2 C), 138.0, 137.7, 137.4, 128.7, 128.6, 128.4, 128.4 (2 C), 128.3, 128.2, 128.1, 128.0, 127.9 (2 C), 127.8, 127.7, 127.6, 127.0, 102.6, 102.3, 101.1, 100.7, 100.0, 83.8, 82.4, 79.5, 78.3, 77.9, 76.2 (2 C), 75.6, 75.0, 74.9, 74.6, 74.4, 73.9, 73.5, 73.3, 73.2 (2 C), 73.2, 70.9, 70.8, 70.7, 70.6, 70.0, 69.4,68.3,68.0, 67.9, 67.9, 67.3,66.9, 61.5, 60.8, 54.5, 53.6, 20.9, 20.8, 20.7 (3 C), 20.6 (3 C), 20.5 (2 C). HRMS: $[M + Na]^+$ calcd for $C_{99}H_{116}N_2NaO_{34}$ 1899.7307, found 1899.7287.

***β*-D-Galactopyranosyl-(1→4)-2-deoxyacetamido-*β*-D-glucopyranosyl-(1→3)-[2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*,*β*-D-glucopyranose (HMO2).** Following the general procedures B and C, compound **21** (200 mg, 0.11 mmol) yielded the compound **HMO2** (87 mg, 90%). ^{1}H NMR (D_2O, 400 MHz): δ 5.11 (d, J = 3.6 Hz, 0.42 H, Glc-1 H-1 of α form), 4.53−4.62 (m, 1.58 H, GlcNAc-1 H-1, Glc-1 H-1 of β form), 4.50 (d, J = 8.4 Hz, 1 H, GlcNHAC-2 H-1), 4.36 (d, J = 7.9 Hz, 1 H, Gal-1 H-1), 4.31 (d, J = 8.0 Hz, 1 H, Gal-2 H-1), 4.03 (d, J = 3.0 Hz, 1 H), 3.77−3.92 (m, 5 H), 3.40−3.77 (m, 21 H), 3.30−3.38 (m, 2 H), 3.17 (t, J = 8.9 Hz, 1 H), 1.95 (s, 3 H), 1.92 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): δ 174.9, 174.5, 103.0, 102.8, 102.7 (GlcNAc-1, GlcNAc-2, Gal-2, C-1), 101.0 (Gal-1, C-1), 95.7 (Glc-1, C-1 of β form), 91.8 (Glc-1, C-1 of α form), 81.8, 78.9, 78.8, 78.1, 75.8, 75.3, 74.7, 74.5, 74.3, 73.8, 73.4, 72.5, 72.1, 71.4, 71.2, 70.9, 69.6, 68.5, 68.4,61.0, 60.1, 60.0, 59.8, 55.5, 55.2, 22.4, 22.2. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{34}H_{58}N_2NaO_{26}$ 933.3175, found 933.3195.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→3)-(2-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (22). 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucosyl bromide was prepared by following the general procedure D. Powdered molecular sieves (4 Å; 2.0 g) was added to a solution of the above bromide donor **2** (1.2 g, 2.43 mmol) and **1** (500 mg, 0.61 mmol) in anhydrous dichloromethane (20 mL). The suspension was stirred under nitrogen for 1.5 h at room temperature and then cooled to −30 °C. Then 2,4,6-collidine (0.32 mL, 2.43 mmol), and freshly dried AgOTf (624 mg, 2.43 mmol) were sequentially added to the reaction mixture. After it was stirred for 2 h at −30 °C, the mixture was warmed to room temperature overnight, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/acetone 6/1) to afford the product **22** (626 mg, 85%) as a white powder. $[\alpha]_D^{20}$ = +12.6 (c 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30−7.63 (m, 26 H), 7.22−7.27 (m, 3 H), 7.16−7.21 (m, 3 H), 6.89−6.96 (m, 2 H), 5.90 (dd, J = 9.4, 10.65 Hz, 1 H), 5.76 (d, J = 8.3 Hz, 1 H), 5.59 (s, 1 H), 5.29 (t, J = 9.7 Hz, 1 H), 5.17 (d, J = 10.6 Hz, 1 H), 4.92−4.99 (m, 2 H), 4.72−4.82 (m, 2 H), 4.63 (t, J = 11.6 Hz, 1 H), 4.54 (dd, J = 8.3, 10.8 Hz, 1 H), 4.25−4.47 (m, 9 H), 3.93−4.05 (m, 3 H), 3.61−3.73 (m, 2 H), 3.49−3.60 (m, 3 H), 3.44 (d, J = 11.3 Hz, 1 H), 3.14 (s, 1 H), 2.98−3.04 (m, 1 H), 2.13 (s, 3 H), 2.12 (s, 3 H), 1.90 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): δ 170.5, 170.2, 169.6, 139.0, 138.7, 138.6, 138.5, 138.3, 137.6, 134.2, 128.7, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.3, 126.9, 126.4, 123.4, 102.4 (2 C), 100.9, 99.3, 83.1, 81.8, 81.3, 76.9, 75.9, 75.8, 75.1, 74.8, 74.3, 73.1, 71.9, 71.0, 70.8, 69.0, 68.9, 67.9, 66.4,62.2, 54.8, 53.7, 20.9, 20.8, 20.5. HRMS (ESI-Orbitrap): $[M + Na]^+$ calcd for $C_{74}H_{75}NNaO_{20}$ 1320.4780, found 1320.4806.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→3)-(2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (23). To a solution of compound **22** (602 mg, 0.46 mmol) in anhydrous MeOH (10 mL) were added TsOH (16.0 mg) and EtSH (0.21 mL, 2.93 mmol). The reaction mixture was stirred at room temperature for 6 h and then quenched with triethylamine and evaporated under reduced pressure. The mixture was purified with a silica column (hexanes/acetone 5/1) to give compound **23** as a white powder (445 mg, 80%). $[\alpha]_D^{20}$ = +17.7 (c 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51−7.66 (m, 4 H), 7.41−7.45 (m, 2 H), 7.25−7.40 (m, 18 H), 7.06−7.17 (m, 3 H), 6.83 (d, J = 6.7 Hz, 2 H), 5.86 (dd, J = 9.1, 10.6 Hz, 1 H), 5.65 (d, J = 8.5 Hz, 1 H), 5.18 (t, J = 9.4 Hz, 1 H), 4.88−4.96 (m, 3 H), 4.69−4.76 (m, 2 H), 4.62 (d, J = 12.0 Hz, 1 H), 4.43−4.56 (m, 2 H), 4.37−4.41 (m, 1 H), 4.23−4.32 (m, 9 H), 3.95−4.03 (m, 3 H), 3.87−3.93 (m, 1 H), 3.68−3.75 (m, 1 H), 3.52−3.66 (m, 2 H), 3.38−3.50 (m, 5 H), 3.23−3.29 (m, 1 H), 3.03−3.08 (m, 1 H), 2.83 (d, J = 1.5 Hz, 1 H), 2.15 (s, 3 H), 2.09 (s, 3 H), 1.88 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): δ 170.8, 170.1, 169.5, 138.8, 138.6, 138.4, 138.3, 134.3, 128.4 (2 C), 128.3, 128.2, 128.2, 128.0, 127.9, 127.8, 127.7 (2 C), 127.6, 127.5, 126.8, 126.3, 123.5, 102.4, 102.1, 98.7, 84.3, 82.8, 81.7, 77.8, 76.3, 75.7, 75.0, 74.7, 74.3, 73.9, 73.1, 72.1, 70.9, 70.5, 69.0,

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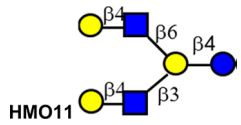
68.0, 67.7, 62.2, 61.9, 54.6, 20.8, 20.7, 20.4. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{67}H_{71}NNaO_{20}$ 1232.4467, found 1232.4490.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-(2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (24). Powdered molecular sieves (4 Å; 1.0 g) was added to a solution of compound **3** (230 mg, 0.27 mmol) and **23** (261 mg, 0.21 mmol) in anhydrous dichloromethane (10 mL). The suspension was stirred under nitrogen for 1.5 h at room temperature and then cooled to −30 °C. Then NIS (61 mg, 0.27 mmol), and AgOTf (35 mg, 0.105 mmol) were sequentially added to the reaction mixture. After it was stirred for 2 h at −30 °C, the mixture was warmed to room temperature overnight, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was diluted with CH_2Cl, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on a silica gel column (hexanes/acetone 5/1) to afford the product **24** (295 mg, 70%) as a white powder. $[\alpha]_D^{20}$ = +13.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.76−8.08 (m, 5 H), 7.49−7.72 (m, 3 H), 7.17−7.48 (m, 25 H), 6.96−7.11 (m, 5 H), 6.87−6.95 (m, 3 H), 6.74 (d, *J* = 7.3 Hz, 2 H), 5.55−5.61 (dd, *J* = 9.3, 10.37 Hz, 1 H), 5.30 (d, *J* = 3.3 Hz, 1 H), 5.11−5.20 (m, 2 H), 5.04 (d, *J* = 8.5 Hz, 1 H), 4.69−4.95 (m, 9 H), 4.42−4.66 (m, 6 H), 4.21−4.39 (m, 6 H), 4.03−4.15 (m, 5 H), 3.91−4.02 (m, 3 H), 3.70−3.88 (m, 4 H), 3.50−3.68 (m, 4 H), 3.23−3.46 (m, 8 H), 3.00−3.07 (m, 1 H), 2.60−2.69 (m, 1 H), 2.11 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.03 (s, 3 H), 2.00 (s, 3 H), 1.85 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): *δ* 171.2, 170.4, 170.3, 170.1, 170.0, 169.2, 169.2, 138.8, 138.7, 138.5, 138.3, 138.2, 137.7, 137.6, 128.7, 128.5, 128.4, 128.3, 128.2 (2 C), 128.0 (2 C), 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 126.6, 126.2, 102.5, 101.8, 100.3, 97.8, 97.4, 84.6, 82.9, 81.8, 77.9 (2 C), 77.3, 76.5, 76.1, 75.7, 75.0, 74.8, 74.7, 74.5, 74.4, 73.7, 73.1, 71.4, 71.0, 70.9, 70.4, 70.1, 70.0, 69.5, 69.4,69.2, 67.8, 67.2, 66.9, 65.5, 64.5, 62.2, 60.7, 55.6, 54.1, 20.9, 20.7, 20.7, 20.6 (3 C), 20.4. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{109}H_{114}N_2NaO_{35}$ 2033.7100, found 2033.7129.

Benzyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxyacetamido-*β*-D-glucopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-(4-*O*-acetyl-2-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (25). Following the general procedure A, compound **24** (400 mg, 0.20 mmol) yielded compound **25** (183 mg, 70% over two steps). $[\alpha]_D^{20}$ = −2.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.16−7.51 (m, 35 H), 5.20−5.36 (m, 2 H), 4.97−5.20 (m, 6 H), 4.89−4.97 (m, 2 H), 4.69−4.89 (m, 8 H), 4.56−4.69 (m, 4 H), 4.29−4.56 (m, 7 H), 4.08−4.26 (m, 2 H), 3.41−4.04 (m, 20 H), 3.32−3.40 (m, 1 H), 3.23−3.31 (m, 1 H), 3.08−3.19 (d, *J* = 8.6 Hz, 1 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 1.98 (s, 9 H), 1.92 (s, 3 H), 1.57 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): *δ* 171.0, 170.9, 170.6 (2 C), 170.2, 170.0, 169.8, 169.7, 169.3 (2 C), 139.1, 139.0, 138.7, 138.5, 138.2, 137.9, 137.4, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7 (2 C), 127.6, 127.5, 126.6, 102.4, 102.0, 101.1, 100.0, 99.8, 82.9, 82.0, 79.9, 78.4, 78.2, 77.4, 76.4, 75.5, 75.0, 74.8, 74.1, 73.7, 73.6, 73.3, 72.7, 72.3, 71.7, 71.0, 70.9, 70.4,69.5, 68.3,67.9, 66.9, 66.7, 61.5, 60.8, 55.2, 54.3, 53.9, 20.9, 20.8 (2 C), 20.7 (2 C), 20.6 (3 C), 20.50 (2 C). HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{99}H_{116}N_2NaO_{34}$ 1899.7307, found 1899.7357.

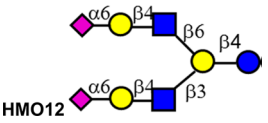
2-Deoxyacetamido-*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→4)-2-deoxyacetamido-*β*-D-glucopyranosyl]-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*,*β*-D-glucopyranose (HMO3). Following the general procedures B and C, compound **25** (183 mg, 0.098 mmol) yielded the compound **HMO3** (85 mg, 95%). ^{1}H NMR (D_2O, 400 MHz): *δ* 5.14 (d, *J* = 3.8 Hz, 0.54 H, Glc-1 H-1 of *α* form), 4.54−4.63 (m, overlap with D_2O, 2.46 H, GlcNAc-1, GlcNAc-2, H-1, Glc-1 H-1 of *β* form), 4.39 (d, *J* = 7.8 Hz, Gal-1, 1 H), 4.35 (d, *J* = 7.8 Hz, Gal-1, 1 H), 4.06 (d, *J* = 3.1 Hz, 1 H), 3.82−3.95 (m, 4 H), 3.42−3.82(m, 22 H), 3.33−3.42 (m, 2 H), 3.21 (t, *J* = 8.39 Hz, 1 H), 1.98 (s, 3 H), 1.96 (s, 3 H). ^{13}C NMR (D_2O, 100 MHz): *δ* 174.9, 174.5, 103.0, 102.9, 102.8 (GlcNAc-1, GlcNAc-2, Gal-2, C-1), 101.0 (Gal-1, C-1), 95.7 (Glc-1, C-1 of *β* form), 91.8 (Glc-1, C-1 of *α* form), 81.7, 79.0, 78.9, 78.4, 75.7, 75.3, 74.7, 74.7, 74.4, 73.9, 73.6, 73.5, 72.5, 72.4, 71.0, 69.9, 69.7, 68.7, 68.6, 68.4,61.0, 60.5, 60.0, 55.7, 55.0, 22.4, 22.2. HRMS (ESI-Orbitrap): [M + Na]$^+$ calcd for $C_{34}H_{58}N_2NaO_{26}$ 933.3175, found 933.3161.

HMO11. Yield: 40.0 mg, 92%. ^{1}H NMR (D_2O, 500 MHz): *δ* 5.10 (d, *J* = 3.5 Hz, 0.61 H, Glc-1 H-1 of *α* form), 4.50−4.60 (m, 2.39 H,



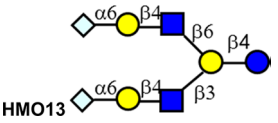
GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of *β* form), 4.33−4.37 (m, 2 H, Gal-2 H-1, Gal-3 H-1), 4.31 (d, *J* = 7.9 Hz, 1 H, Gal-1 H-1), 4.02 (d, *J* = 3.1 Hz, 1 H), 3.78−3.79 (m, 6 H), 3.57−3.76 (m, 18 H), 3.53−3.57 (m, 2 H), 3.39−3.51 (m, 7 H), 3.17 (t, *J* = 8.3 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 3 H). MS (MALDI-TOF): [M + Na]$^+$ calcd for $C_{40}H_{68}N_2NaO_{31}$ 1095.370, found 1095.368.

HMO12.



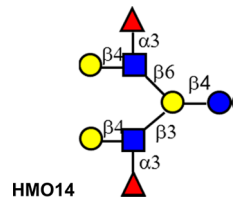
Yield: 2.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): *δ* 5.10 (d, *J* = 3.5 Hz, 0.51 H, Glc-1 H-1 of *α* form), 4.50−4.58 (m, 2.49 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of *β* form), 4.28−4.36 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.03 (d, *J* = 3.0 Hz, 1 H), 3.38−3.91 (m, 47H), 3.17 (t, *J* = 8.3 Hz, 1 H), 2.52−2.57 (m, 2 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.91 (s, 6 H), 1.55−1.63 (m, 2 H). HRMS (ESI-Orbitrap): [M − 2H]$^{2-}$ calcd for $C_{62}H_{100}N_4O_{47}$ 826.2784, found 826.2760.

HMO13.



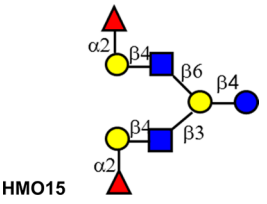
Yield: 2.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): *δ* 5.09 (d, *J* = 3.7 Hz, 0.29 H, Glc-1 H-1 of *α* form), 4.50−4.63 (m, 2.71 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of *β* form), 4.28−4.36 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.02 (d, *J* = 3.0 Hz, 1 H), 3.99 (s, 4 H), 3.38−3.91 (m, 48 H), 3.18 (t, *J* = 8.3 Hz, 1 H), 2.52−2.60 (m, 2 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.55−1.65 (m, 2 H). HRMS (ESI-Orbitrap): [M − 2H]$^{2-}$ calcd for $C_{62}H_{100}N_4O_{49}$ 842.2734, found 842.2760.

HMO14.



Yield: 2.3 mg, 95%. ^{1}H NMR (D_2O, 500 MHz): *δ* 5.10 (d, *J* = 3.5 Hz, 0.33 H, Glc-1 H-1 of *α* form), 4.96−5.02 (m, 2 H, Fuc-1 H-1, Fuc-2 H-1), 4.50−4.61 (m, 2.67 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of *β* form), 4.28−4.36 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.01 (d, *J* = 3.1 Hz, 1 H), 3.63−3.91 (m, 25 H), 3.40−3.63 (m, 21 H), 3.35−3.39 (m, 2 H), 3.17 (t, *J* = 8.4 Hz, 1 H), 1.90−1.93 (d, *J* = 6.5 Hz, 6 H). MS (MALDI-TOF): [M + Na]$^+$ calcd for $C_{52}H_{88}N_2NaO_{39}$ 1387.487, found 1387.486.

HMO15.



Yield: 2.0 mg, 93%. ^{1}H NMR (D_2O, 500 MHz): *δ* 5.15−5.22 (m, 2 H, Fuc-1 H-1, Fuc-2 H-1), 5.10(d, *J* = 3.5 Hz, 0.33 H, Glc-1 H-1 of *α* form),

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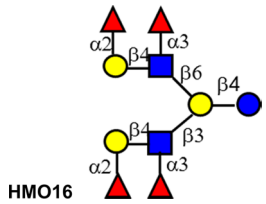
4.45−4.61 (m, 2.67 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.39−4.45 (m, 2 H, Gal-2 H-1, Gal-3 H-1), 4.30 (d, J = 7.9 Hz, 1 H, Gal-1 H-1), 4.06−4.13 (m, 2 H), 4.01 (d, J = 3.1 Hz, 1 H), 3.41−3.91 (m, 44 H), 3.30−3.39 (m, 2 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.93 (s, 3 H), 1.91 (s, 3 H), 1.09−1.11 (d, J = 6.7 Hz, 6 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{52}H_{88}N_2NaO_{39}$ 1387.487, found 1387.490.

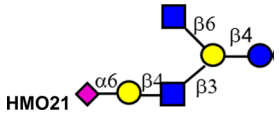
HMO16.



HMO16

Yield: 1.0 mg, 95%. ^{1}H NMR (D_2O, 500 MHz): δ 5.12−5.18(m, 2 H, Fuc-2 H-1, Fuc-4 H-1), 5.10 (d, J = 3.5 Hz, 0.40 H, Glc-1 H-1 of α form), 4.93−5.01 (m, 2 H, Fuc-1 H-1, Fuc-3 H-1), 4.70−4.78 (m, 2 H), 4.45−4.61 (m, 2.60 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.35−4.41 (m, 2 H, Gal-2 H-1, Gal-3 H-1), 4.31 (d, J = 7.9 Hz, 1 H, Gal-1 H-1), 4.09−4.16 (m, 2 H), 4.00 (d, J = 3.1 Hz, 1 H), 3.41−3.91 (m, 44 H), 3.28−3.38 (m, 2 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.93 (s, 3 H), 1.90 (s, 3 H), 1.10−1.15 (m, 12 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{64}H_{108}N_2NaO_{47}$ 1679.602, found 1679.607.

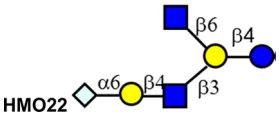
HMO21.



HMO21

Yield: 2 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.6 Hz, 0.22 H, Glc-1 H-1 of α form), 4.45−4.61 (m, 2.78 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.26−4.36 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.17−4.22 (m, 1 H), 4.09−4.16 (m, 2 H), 4.00 (d, J = 3.1 Hz, 1 H), 3.36−3.90 (m, 32 H), 3.26−3.36 (m, 2 H), 3.16 (t, J = 8.4 Hz, 1 H), 2.50−2.56 (m, 1 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.58 (t, J = 12.3 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{45}H_{74}N_3O_{34}$ 1200.4159, found 1200.4190.

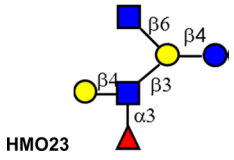
HMO22.



HMO22

Yield: 2.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.6 Hz, 0.27 H, Glc-1 H-1 of α form), 4.47−4.62 (m, 2.73 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.35 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.06−4.16 (m, 1 H), 4.01 (d, J = 3.1 Hz, 1 H), 3.99 (s, 2 H), 3.38−3.90 (m, 33 H), 3.29−3.37 (m, 2 H), 3.16 (t, J = 8.4 Hz, 1 H), 2.53−2.58 (m, 1 H), 1.93 (s, 3 H), 1.92 (s, 3 H), 1.60 (t, J = 12.3 Hz, 1 H). HRMS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{45}H_{74}N_3O_{35}$ 1216.4108, found 1216.4129.

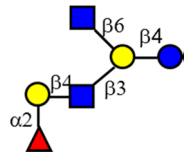
HMO23.



HMO23

Yield: 2.0 mg, 95%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.6 Hz, 0.36 H, Glc-1 H-1 of α form), 5.00 (d, J = 3.9 Hz, 1 H, Fuc-1 H-1), 4.46−4.60 (m, 2.64 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.36 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.02 (d, J = 3.1 Hz, 1 H), 3.40−3.90 (m, 31 H), 3.29−3.40 (m, 2 H), 3.16 (t, J = 8.4 Hz, 1 H), 2.50−2.56 (m, 1 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 1.05 (d, J = 6.7 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{40}H_{68}N_2NaO_{30}$ 1079.376, found 1079.371.

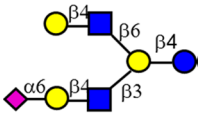
HMO24.



HMO24

Yield: 2.0 mg, 92%. ^{1}H NMR (D_2O, 500 MHz): δ 5.19 (d, J = 2.6 Hz, 1 H, Fuc-1 H-1), 5.09 (d, J = 3.6 Hz, 0.50 H, Glc-1 H-1 of α form), 4.46−4.60 (m, 2.50 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.42 (d, J = 7.8 Hz, 1 H, Gal-2 H-1), 4.31 (d, J = 8.0 Hz, 1 H, Gal-1 H-1), 4.07−4.12 (m, 1 H), 4.01 (d, J = 3.1 Hz, 1 H), 3.41−3.90 (m, 28 H), 3.29−3.38 (m, 3 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 3 H), 1.10 (d, J = 6.5 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{40}H_{68}N_2NaO_{30}$ 1079.376, found 1079.379.

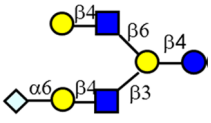
HMO25.



HMO25

Yield: 1.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09(d, J = 3.9 Hz, 0.28 H, Glc-1 H-1 of α form), 4.48−4.62 (m, 2.72 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.37 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.01 (d, J = 3.1 Hz, 1 H), 3.38−3.90 (m, 41 H), 3.17 (t, J = 8.4 Hz, 1 H), 2.50−2.57 (m, 1 H), 1.93 (s, 3 H), 1.92 (s, 3 H), 1.90 (s, 3 H), 1.59 (t, J = 11.9 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{51}H_{84}N_3O_{39}$ 1362.4687, found 1362.4759.

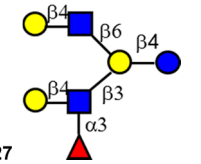
HMO26.



HMO26

Yield: 1.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.10 (d, J = 3.7 Hz, 0.51 H, Glc-1 H-1 of α form), 4.50−4.63 (m, 2.49 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.29−4.37 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.02 (d, J = 2.9 Hz, 1 H), 3.99 (s, 2 H), 3.39−3.90 (m, 41 H), 3.17 (t, J = 8.3 Hz, 1 H), 2.54−2.59 (m, 1 H), 1.94 (s, 3 H), 1.93 (s, 3 H), 1.61 (t, J = 12.2 Hz, 1 H). HRMS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{51}H_{84}N_3O_{40}$ 1378.4637, found 1378.4660.

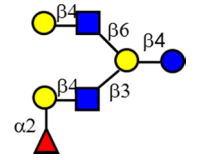
HMO27.



HMO27

Yield: 1.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.10 (d, J = 3.7 Hz, 0.55 H, Glc-1 H-1 of α form), 5.00 (d, J = 4.0 Hz, 1 H, Fuc-1 H-1), 4.69−4.74 (m, 1 H), 4.50−4.61 (m, 2.45 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.38 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.02 (d, J = 2.9 Hz, 1 H), 3.34−3.91 (m, 32 H), 3.17 (t, J = 8.3 Hz, 1 H), 2.54−2.59 (m, 1 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 1.05 (d, J = 6.6 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{35}$ 1241.428, found 1241.432.

HMO28.



HMO28

Yield: 1.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.19 (d, J = 2.4 Hz, 1H, Fuc-1 H-1), 5.10 (d, J = 3.7 Hz, 0.42 H, Glc-1 H-1 of α form), 4.48−4.60 (m, 2.58 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.45 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.06−4.15 (m, 1 H), 4.01 (d, J = 2.9 Hz, 1 H), 3.38−3.90 (m, 31 H), 3.31−3.37 (m, 1 H), 3.17(t, J = 8.8 Hz, 1 H), 1.93 (s, 3 H), 1.91 (s, 3 H), 1.10 (d, J = 6.5 Hz, 3

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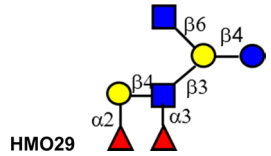
H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{35}$ 1241.428, found 1241.423.

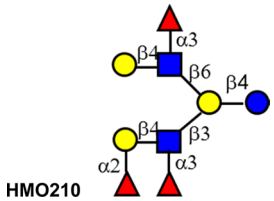
HMO29.



HMO29

Yield: 1.0 mg, 93%. ^{1}H NMR (D_2O, 500 MHz): δ 5.15 (d, J = 2.6 Hz, 1 H, Fuc-2 H-1), 5.09(d, J = 3.7 Hz, 0.36 H, Glc-1 H-1 of α form), 4.99 (d, J = 3.7 Hz, 1 H, Fuc-1 H-1), 4.72−4.78 (m, 1 H), 4.46−4.61 (m, 2.64 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.42 (d, J = 7.8 Hz, 1 H, Gal-2 H-1), 4.31 (d, J = 8.0 Hz, 1 H, Gal-1 H-1), 4.10−4.16 (m, 1 H), 4.01 (d, J = 2.9 Hz, 1 H), 3.40−3.92 (m, 33 H), 3.29−3.37 (m, 3 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 1.09−1.16 (m, 6 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{34}$ 1225.433, found 1225.438.

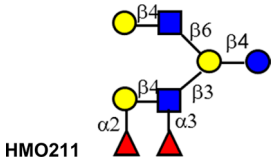
HMO210.



HMO210

Yield: 0.5 mg, 95%. ^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, J = 2.6 Hz, 1 H, Fuc-2 H-1), 5.10 (d, J = 3.5 Hz, 0.40 H, Glc-1 H-1 of α form), 4.93−5.01 (m, 2 H, Fuc-1 H-1, Fuc-3 H-1), 4.67−4.79 (m, 2 H), 4.45−4.61 (m, 2.60 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.26−4.41 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.09−4.16 (m, 1 H), 4.00 (d, J = 3.1 Hz, 1 H), 3.41−3.91 (m, 44 H), 3.28−3.40 (m, 3 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.93 (s, 3 H), 1.90 (s, 3 H), 1.02−1.17 (m, 9 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{58}H_{98}N_2NaO_{43}$ 1533.544, found 1533.538.

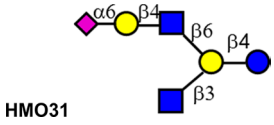
HMO211.



HMO211

Yield: 0.5 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, J = 2.7 Hz, 1 H, Fuc-2 H-1), 5.10 (d, J = 3.7 Hz, 0.47 H, Glc-1 H-1 of α form), 4.99 (d, J = 3.7 Hz, 1 H, Fuc-1 H-1), 4.72−4.78 (m, 1 H), 4.49−4.62 (m, 2.53 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.29−4.41 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.10−4.16 (m, 1 H), 4.01 (d, J = 3.2 Hz, 1 H), 3.77−3.91 (m, 8 H), 3.39−3.77 (m, 35 H), 3.30−3.36 (m, 1 H), 3.17 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 1.09−1.16 (m, 6 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{52}H_{88}N_2NaO_{39}$ 1387.486, found 1387.490.

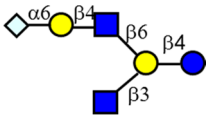
HMO31.



HMO31

Yield: 2.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09(d, J = 3.7 Hz, 0.31 H, Glc-1 H-1 of α form), 4.47−4.58 (m, 2.69 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.34 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.06−4.16 (m, 1 H), 4.02 (d, J = 3.1 Hz, 1 H), 3.28−3.91 (m, 35 H), 3.26−3.36 (m, 2 H), 3.18 (t, J = 8.4 Hz, 1 H), 2.51−2.57 (m, 1 H), 1.96 (s, 3 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.59 (t, J = 12.2 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{45}H_{74}N_3O_{34}$ 1200.4159, found 1200.4122.

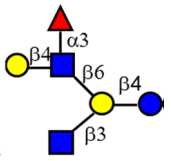
HMO32.



HMO32

Yield: 2.0 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.9 Hz, 0.28 H, Glc-1 H-1 of α form), 4.50−4.58 (m, 2.72 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.34 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.02 (d, J = 3.2 Hz, 1 H), 3.28−3.91 (m, 35 H), 3.18 (t, J = 8.3 Hz, 1 H), 2.53−2.58 (m, 1 H), 1.96 (s, 3 H), 1.91 (s, 3 H), 1.59 (t, J = 12.2 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{45}H_{74}N_3O_{35}$ 1216.4108, found 1216.4139.

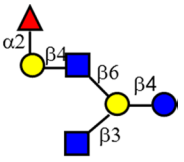
HMO33.



HMO33

Yield: 2.2 mg, 95%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.7 Hz, 0.19 H, Glc-1 H-1 of α form), 4.98 (d, J = 3.9 Hz, 1 H, Fuc-1 H-1), 4.66−4.73 (m, 1 H), 4.48−4.58 (m, 2.81 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.27−4.35 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.02 (d, J = 3.1 Hz, 1 H), 3.28−3.90 (m, 38 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.92 (s, 3 H), 1.90 (s, 3 H), 1.04 (d, J = 6.6 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{40}H_{68}N_2NaO_{30}$ 1079.376, found 1079.380.

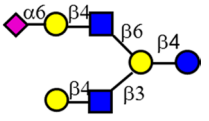
HMO34.



HMO34

Yield: 2.0 mg, 92%. ^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, J = 2.6 Hz, 1 H, Fuc-1 H-1), 5.09(d, J = 3.9 Hz, 0.41 H, Glc-1 H-1 of α form), 4.66−4.73 (m, 1 H), 4.45−4.58 (m, 2.59 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.39−4.44 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.31 (d, J = 7.5 Hz, 1 H), 4.06−4.12 (m, 2 H), 4.02 (d, J = 3.1 Hz, 1 H), 3.39−3.90 (m, 28 H), 3.28−3.38 (m, 3 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 3 H), 1.11 (d, J = 6.7 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{40}H_{68}N_2NaO_{30}$ 1079.376, found 1079.372.

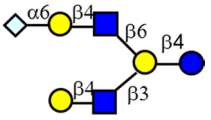
HMO35.



HMO35

Yield: 1 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.8 Hz, 0.53 H, Glc-1 H-1 of α form), 4.51−4.60 (m, 2.47 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.37 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.02 (d, J = 3.0 Hz, 1 H), 3.38−3.91 (m, 41 H), 3.18 (t, J = 8.4 Hz, 1 H), 2.50−2.57 (m, 1 H), 1.96 (s, 3 H), 1.91 (s, 6 H), 1.59 (t, J = 12.0 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{51}H_{84}N_3O_{39}$ 1362.4687, found 1362.4799.

HMO36.



HMO36

Yield: 1 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.8 Hz, 0.52 H, Glc-1 H-1 of α form), 4.50−4.60 (m, 2.48 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.38 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.02 (d, J = 3.0 Hz, 1 H), 3.99 (s, 2 H), 3.38−3.91 (m, 41 H), 3.18 (t, J = 8.4 Hz, 1 H), 2.53−2.59 (m, 1 H), 1.96 (s, 3 H), 1.91 (s, 3 H), 1.59 (t, J = 12.0 Hz, 1 H). MS (ESI-Orbitrap): $[M - H]^-$ calcd for $C_{51}H_{84}N_3O_{40}$ 1378.4637, found 1378.4609.

DOI: 10.1021/acs.joc.6b00478
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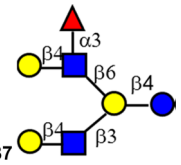
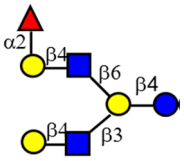
HMO37.



HMO37

Yield: 1.1 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, J = 3.8 Hz, 0.34 H, Glc-1 H-1 of α form), 4.98 (d, J = 4.0 Hz, 1 H, Fuc-1 H-1), 4.67−4.74 (m, 1 H), 4.50−4.61 (m, 2.66 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.27−4.38 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.01 (d, J = 3.1 Hz, 1 H), 3.30−3.90 (m, 37 H), 3.16 (t, J = 8.6 Hz, 1 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.05 (d, J = 6.6 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{35}$ 1241.428, found 1241.436.

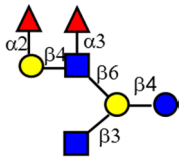
HMO38.



HMO38

Yield: 1 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, J = 2.6 Hz, 1 H, Fuc-1 H-1), 5.09 (d, J = 3.8 Hz, 0.69 H, Glc-1 H-1 of α form), 4.46−4.60 (m, 2.31 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.43 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.07−4.13 (m, 1 H), 4.02 (d, J = 3.2 Hz, 1 H), 3.39−3.89 (m, 36 H), 3.33−3.38 (m, 1 H), 3.17 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 3 H), 1.11 (d, J = 6.5 Hz, 3 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{35}$ 1241.428, found 1241.435.

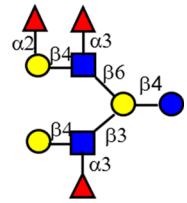
HMO39.



HMO39

Yield: 1.1 mg, 93%. ^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, J = 2.9 Hz, 1 H, Fuc-2 H-1), 5.10(d, J = 3.7 Hz, 0.39 H, Glc-1 H-1 of α form), 4.97 (d, J = 4.0 Hz, 1 H, Fuc-1 H-1), 4.72−4.78 (m, 1 H), 4.45−4.58 (m, 2.61 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.40 (m, 2 H, Gal-1 H-1, Gal-2 H-1), 4.10−4.16 (m, 1 H), 4.02 (d, J = 3.06 Hz, 1 H), 3.40−3.93 (m, 33 H), 3.29−3.38 (m, 3 H), 3.16(t, J = 8.4 Hz, 1 H), 1.93 (s, 3 H), 1.91 (s, 3 H), 1.08−1.17 (m, 6 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{46}H_{78}N_2NaO_{34}$ 1225.433, found 1225.430.

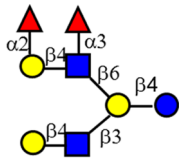
HMO310.



HMO310

Yield: 0.4 mg, 93%. ^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, J = 3.0 Hz, 1 H, Fuc-3 H-1), 5.09 (d, J = 3.7 Hz, 0.62 H, Glc-1 H-1 of α form), 4.96−5.01 (m, 2 H), 4.69−4.78 (m, overlap with D_2O, 2 H), 4.47−4.61 (m, 2.38 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.28−4.40 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.10−4.16 (m, 1 H), 4.02 (d, J = 3.2 Hz, 1 H), 3.32−3.93 (m, 43 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.93 (s, 3 H), 1.90 (s, 3 H), 1.02−1.17 (m, 9 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{58}H_{98}N_2NaO_{43}$ 1533.544, found 1533.550.

HMO311.



HMO311

Yield: 0.5 mg, 90%. ^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, J = 3.2 Hz, 1 H, Fuc-2 H-1), 5.10 (d, J = 3.6 Hz, 0.67 H, Glc-1 H-1 of α form), 4.97 (d, J = 3.9 Hz, 1 H, Fuc-1 H-1), 4.72−4.77 (m, 1 H), 4.47−4.60 (m, 2.37 H, GlcNAc-1 H-1, GlcNAc-2 H-1, Glc-1 H-1 of β form), 4.29−4.39 (m, 3 H, Gal-1 H-1, Gal-2 H-1, Gal-3 H-1), 4.10−4.16 (m, 1 H), 4.02 (d, J = 3.1 Hz, 1 H), 3.39−3.93 (m, 41 H), 3.30−3.38 (m, 1 H), 3.16 (t, J = 8.4 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 3 H), 1.09−1.17 (m, 6 H). MS (MALDI-TOF): $[M + Na]^+$ calcd for $C_{52}H_{88}N_2NaO_{39}$ 1387.486, found 1387.478.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of The Supporting Information is available free of charge on the ACS Publications Web site The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00478.

^{1}H and ^{13}C spectra for compounds **6**, **7**, **1**, **9**, **3**, **13**−**16**, and **19**−**25**, ^{1}H, ^{13}C, COSY. and HSQC spectra for **HMO1**−**HMO3**, and ^{1}H spectra and HPLC chromatograms for **HMO11**−**HMO311** (PDF)

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The authors declare no competing financial interest.

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