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Synthesis of Modular Building Blocks using Glycosyl Phosphate Donors for the Construction of Asymmetric N-Glycans

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Abstract

Glycosyl phosphates are known as versatile donors for the synthesis of complex oligosaccharides both chemically and enzymatically. Herein, we report the stereoselective construction of modular building blocks for the synthesis of N-glycan using glycosyl phosphates as donors. We have synthesized four trisaccharide building blocks with orthogonal protecting groups, namely, Man β 2GlcNAc(OAc)₃ β 6GlcNAc (**9**), Man β 2GlcNAc- β 6GlcNAc(OAc)₃ (**15**), Man β 2GlcNAc(OAc)₃ β 4GlcNAc (**18**) and Man β 2GlcNAc β 4GlcNAc(OAc) (**22**) for further selective elongation using glycosyltransferases. The glycosylation reaction using glycosyl phosphate was found to be high yielding with shorter reaction time. Initially, The phthalimide protected glucosamine donor was exploited to ensure the formation of β -glycosidic linkage and later converted to the N-acetyl group before the enzymatic synthesis. The selective deprotection of O-benzyl group was performed prior to enzymatic synthesis to avoid its negative interference.

Graphical Abstract

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Supplementary Material

Supplementary material is included for the ¹H and ¹³C NMR spectra of all intermediates.

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Keywords

N-Glycans; Glycosyl phosphate donors; Chemo-enzymatic; Oligosaccharides; Orthogonal protecting groups; Modular building blocks

1. Introduction

Glycans are composed of a number of monosaccharides linked together *via* glycosidic bond(s) and constitute the carbohydrate portion of glycoproteins, glycolipids or other glycoconjugates. In glycoproteins, glycans are broadly divided into O-glycans and N-glycans. They affect several biological processes in the cell such as protein folding, cell signaling, embryogenesis etc.¹ N-Glycans are assembled during protein biosynthesis through attachment to the amide nitrogen of asparagine in a defined peptide chain. The synthesis and processing of N-glycans generally takes place in the endoplasmic reticulum and Golgi complex involving different stages of glycosylation and deglycosylation.² The glycan precursor $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ is first transferred from a lipid pyrophosphate derivative to a growing polypeptide in the endoplasmic reticulum (ER) and further trimmed by ER glucosidases before travelling to the Golgi complex for further processing³ down to $\text{Man}_5\text{GlcNAc}_2$, which is further glycosylated under the catalysis of N-acetylglucosamine transferase (GnT) to convert it to a complex or hybrid-type glycan. Next, a series of enzymes of the GnT family modify the glycoform with the addition of GlcNAc moieties to the D1 arm of the glycan.⁴ Then, the Golgi-generated glycoforms is converted to highly diverse glycan species through a series of sequential galactosylation, sialylation and fucosylation. The naturally occurring glycans can only be isolated in minor quantities and as complex mixtures, difficult to separate, and cannot provide a reliable source for detailed biological studies. More than twenty thousand structures of N-glycans exist depending on the linkages of monosaccharides, and many of which are difficult to sequence due to their isomeric nature. In order, to study the effect of individual N-glycans on the function of a particular glycoprotein would ideally require a structurally diverse collection of synthetic N-glycans with systematic variations in compositions and branching, terminal sugars, and core modifications. The chemical synthesis to access diverse and complex N-glycan structures is challenging due to step-wise synthesis and multiple purification processes. However, over the last two decades, chemical and chemo-enzymatic synthesis methods have been reported for the synthesis of complex and hybrid type N-glycans.^{5,6} In 2013, we reported a

convergent synthesis of multi-antennary N-glycans and their application to the study of HIV-antigenicity.^{7a} Recently, we also developed a modular chemo-enzymatic approach for the synthesis of high-mannose, hybrid- and complex-type N-glycan structures for the development of glycan arrays for the rapid screening and identification of epitopes on HIV-1 recognized by the broadly neutralizing antibodies isolated from HIV-1 patients.^{7b}

In the last 100 years, synthetic chemists have been developing efficient glycosyl donors for the synthesis of complex oligosaccharides. Numerous versatile glycosyl donors have been developed, among which glycosyl trichloroacetimidates⁸ and thioglycosides⁹ are more commonly utilized. However, the application of glycosyl donors, such as glycosyl sulfoxides,¹⁰ *n*-pentenyl glycosides,¹¹ glycosyl phosphites,¹² glycosyl halides,¹³ anhydrosugars¹⁴, glucals¹⁵, has been also widely documented for the synthesis of complex oligosaccharide. Since mid-1990's, glycosyl phosphates have become an attractive class of glycosylating donors due to their stability, ease of activation and high reactivity at low temperature. The use of glycosyl phosphate donors for enzymatic syntheses is also well known. It is known that glycosyltransferases use nucleotide 5'-phosphosugars (NDPs) for the biosynthesis of complex oligosaccharides.¹⁶ Seeberger and co-workers reported the synthesis of glycosyl phosphates for the synthesis of Oglycosides,¹⁷ C-glycosides¹⁸, and complex oligosaccharides.¹⁹ Previously, we have reported the synthesis of glycosyl phosphates and their application in the chemical and chemoenzymatic synthesis of oligosaccharides.¹² In the current context, we extend our chemo-enzymatic modular approach using glycosyl phosphates and glycosyltransferases to construct several modular building blocks which are useful for the assembly of symmetric and asymmetric N-glycans.

2. Result and Discussion

The synthesis of the targeted trisaccharide **9** was initiated from orthogonally protected glycosyl donor **2** and acceptor **1**, which were synthesized from commercially available D-mannose and Dglucosamine, respectively, using known procedures.^{7b} Initially, glucosamine donor **2** and acceptor **1** were coupled using NIS/TMSOTf to furnish disaccharide **3** with 1,2- β -glycosidic linkage in 86% yield. Selective opening of the 4,6-*O*-benzylidene ring in the presence of BH₃-THF/*n*-Bu₂BOTf led to 6-hydroxy mannosyl acceptor **4** in 82%. On the other hand, the glycosyl phosphate donor **6** was prepared from thioglycoside donor **2**, which upon deacetylation under Zemplén condition followed by benzyl protection using BnBr/NaH provided *O*-benzyl protected thioglycoside **5**. The anomeric phosphate group was installed using dibutyl phosphate in the presence of NIS/TfOH to generate **6** with excellent yield. Next, disaccharide **4** and phosphate donor **6** were coupled using TMSOTf at -50 °C to obtain trisaccharide **7** in 80% yield. Later, removal of phthalimide protecting groups using ethylene diamine/*n*-BuOH followed by N-acetylation using Ac₂O/Py generated trisaccharide **8**. In order to avoid any negative interference of the bulky -OBn groups during the enzymatic reaction, we planned to remove the benzyl groups using Pd(OH)₂/C-H₂ to furnish **9** (Scheme 1). The C4 hydroxyl group at the GlcNAc (β 6 arm) of trisaccharide **9** can be elongated by enzymatic glycosylation to form the desired N-glycan structure.

To further investigate the application of the phosphate donor towards the synthesis of diverse branching units, we installed tri *O*-acetyl glucosamine entity on the β 6 arm of the

trisaccharide motif. Glycosyl phosphate **6** was coupled with mannosyl acceptor **1** using TMSOTf to furnish disaccharide **10** in good yield. The presence of doublet at 5.27 ppm with a coupling constant ($J = 8.4$ Hz) in ^1H NMR spectrum along with the signal at 96.9 ppm in ^{13}C NMR indicated the formation of 1,2- β -linked disaccharide **10**. Selective opening of the 4,6-*O*-benzylidene ring in the presence of $\text{BH}_3\text{-THF}/n\text{-Bu}_2\text{BOTf}$ led to the 6-hydroxy disaccharide acceptor **11** in 78% yield. The absence of signal at 5.42 ppm and 101.5 ppm in ^1H and ^{13}C NMR spectrum, respectively, indicated the cleavage of 4,6-*O*-benzylidene ring. Next, the disaccharide acceptor **11** was coupled with phosphate donor **12** using TMSOTf to furnish the β 2- β 6 linked trisaccharide **13**. The presence of two doublet signals at 5.20 (d, $J = 8.5$ Hz, 1H) and 5.10 (d, $J = 8.4$ Hz, 1H) in ^1H NMR confirmed that the two glucosamine units are β 2- β 6 linked with mannose in **13**. Removal of the phthalimide protecting group using ethylene diamine at 90 °C, followed by N-acetylation with $\text{Ac}_2\text{O}/\text{Py}$ generated **14**. Finally, $\text{Pd}(\text{OH})_2/\text{C-H}_2$ mediated hydrogenation furnished the desired trisaccharide **15** in 87% (Scheme 2).

With the success towards the β 2- β 6 linked trisaccharide unit, we were further intrigued to investigate the reactivity of phosphate donor towards the systematic synthesis of β 2- β 4 linked modules. In this regard, the benzylidene protected disaccharide **3** was treated $\text{Et}_3\text{SiH-TFA}$ to provide C-4 hydroxy derivative **16**, selectively. Next, glycosylation of the phosphate donor **6** with acceptor **16** in the presence of TMSOTf led to the trisaccharide with required β 2- β 4 linkage. Further, the phthalimide protecting groups of trisaccharide were deprotected followed by Nacetylation using $\text{Ac}_2\text{O}/\text{Py}$ generated **17** in 77%. Finally, removal of the *O*-benzyl group using $\text{Pd}(\text{OH})_2/\text{C-H}_2$ led to the formation of trisaccharide **18** in 90% yield (Scheme 3). To show the diversity of phosphate donors towards various trisaccharide modules, we also focused on the synthesis of the trisaccharide building block containing C-4-OAc N-acetyl glucosamine unit attached with β 4 glycosidic linkages to the -OPMP-mannose unit. Selective opening of the 4,6-*O*-benzylidene ring of **10** in the presence of $\text{Et}_3\text{SiH/TFA}$ led to 4-hydroxy disaccharide acceptor **20** in 74% yield. Next, the phosphate donor **19** was coupled with disaccharide acceptor **20** using TMSOTf to generate trisaccharide **21**. The removal of phthalimide protecting groups, followed by N-acetylation and hydrogenolysis provided trisaccharide **22** with GlcNAc(OAc) at the β 4 arm in 86 % yield (Scheme 4).

The structural patterns of all these trisaccharides **9**, **15**, **18** and **22** provide a platform for the selective elongation at the GlcNAc unit using enzymes to create libraries of asymmetric branching motifs. We utilized one of the trisaccharide, **18** to demonstrate the applicability of enzymatic elongation. Initially, trisaccharide **18** was treated with β -1,4-galactosyltransferase (GalT) using UDPGal in the presence of MnCl_2 to furnish tetrasaccharide **23** with complete stereospecificity in 75% yield. To further extend the chain length and complexity, tetrasaccharide **23** was treated with α -2,3-sialyltransferase (ST) along with ATP (0.05 eq.), CTP (0.05 eq.), phosphoenolpyruvate (2.4 eq.), cytidine monophosphate kinase (CMK, 5 units) and CMP-sialic acid synthetase (CSS, 10U) in Tris buffer (50mM, pH 7.5) to generate pentasaccharide **24** in 83% yield (Scheme 5).

In conclusion, we have developed a new and highly efficient chemo-enzymatic strategy for the synthesis of modular building blocks using glycosyl phosphate as donors for the synthesis of complex/hybrid N-glycans. The phosphate donors were synthesized from the corresponding thioglycosides. Overall, the glycosylation reactions using phosphate donors were found to be high yielding and highly stereoselective. One of the trisaccharide building block **18** was further exploited for the enzymatic synthesis. With the aid of purified enzymes β -1,4-GalT and α 2,3-ST, we were able to elongate the β 4 arm of the trisaccharide even in the presence of three O-acetyl moieties at the β 2 arm. This result indicates that the presence of multiple OAc protecting groups can be tolerated by the enzymes during the enzymatic reactions. This work emphasizes the use of glycosyl phosphate donors and enzymes to access complex and diverse N-glycan structures.

Experimental Section:

General procedures.

All chemicals were purchased as reagent grade and used without further purification. All anhydrous solvents were purchased from commercial source and further dried in accordance with standard procedures. Molecular sieves (MS 4 Å) were purchased from Aldrich, which were ground into powdered form and activated before use. Reactions were performed under an inert atmosphere and strictly anhydrous conditions. Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel 60 F254 plates and visualized under UV (254 nm) and/or by spraying with 20% anisaldehyde in ethanol or with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ 25 g/L. ^1H and ^{13}C NMR were recorded on 600/500 MHz and 151 MHz spectrometers, respectively, with the residual solvent signal acting as the internal standard. Chemical shift (in ppm) was determined relative to tetramethylsilane in deuterated chloroform (δ 0 ppm). Coupling constant(s) in hertz (Hz) were measured from one-dimensional spectra. High-resolution mass spectra were obtained from a Q-TOF instrument by the electrospray ionization (ESI) technique.

General procedure for glycosyl phosphate coupling:

To a solution of glycosyl phosphate donor (1.2 equiv.) and acceptor (1.0 eq) in CH_2Cl_2 was mixed with molecular sieves (4 Å). After stirring for 1 h under N_2 -atmosphere at room temperature, the mixture was cooled to -50°C and was added TMSOTf (1.0 equiv.). After the reaction mixture was stirred for additional 1 h at the same temperature, triethylamine (2 equiv.) was added and the mixture was filtered through a pad of Celite and concentrated in *vacuo*. The crude residue was purified through a silica gel column chromatography to afford the corresponding glycosylated product.

General procedure for the synthesis of glycosyl phosphates:

A solution of thioglycoside donor (1.2 equiv.) and molecular sieves 4 Å (1.0 equiv.) in CH_2Cl_2 was stirred for 1 h under N_2 -atmosphere at room temperature and then cooled to -40°C . Dibutyl phosphate (1.0 equiv.), NIS (1.2 equiv.) and TfOH (0.3 equiv.) were added to the mixture, and stirring was continued until the complete conversion of the donor. The reaction mixture was quenched with Et_3N (2 equiv.) and filtered through a Celite pad. The mixture was diluted with CH_2Cl_2 (2×30 mL), washed with sat. NaHCO_3 (25 mL),

Na₂S₂O₃ (25 mL) and brine (20 mL). The crude product was dried over MgSO₄ and concentrated in *vacuo*, and the residue was purified through a silica gel column chromatography to afford the corresponding glycosylated product.

General procedure for 4,6-*O*-benzylidene ring opening using BH₃-THF:

A solution of compound (0.50 mmol) in anhydrous THF (2mL) was mixed with BH₃-THF complex (1M solution in THF), followed by *n*-Bu₂BOTf (1M solution in CH₂Cl₂) at 0 °C under N₂-atmosphere. After the complete conversion of the starting material to product, Et₃N (2 equiv.) was added. The reaction mixture was further quenched with dropwise addition of MeOH. When no more hydrogen was evolved, the reaction mixture was concentrated in *vacuo* and the residue was purified by flash column chromatography on silica gel to afford the corresponding 6-hydroxy product.

General procedure for 4,6-*O*-benzylidene ring opening using Et₃SiH/TFA:

To a solution of 4,6-*O*-benzylidene protected compound (1.0 mmol) in anhydrous CH₂Cl₂ (20 mL) was added triethyl silane (10 equiv.) followed by trifluoroacetic acid (10 equiv.) at 0 °C. After 2 h, the reaction mixture was slowly quenched with sodium bicarbonate solution (50 mL). The aqueous layer was further extracted with CH₂Cl₂ (3 × 50 mL), and the combined organic layer was washed with brine solution (50 mL), dried over MgSO₄, filtered and concentrated in *vacuo*. The crude reaction mixture was purified by flash column chromatography on silica gel to afford the corresponding 4hydroxy product.

General procedure for phthalimide deprotection and Nacetylation:

A protected trisaccharide (1 mmol) was dissolved in a mixture of ethylene diamine: ⁿBuOH (1:4) and stirred at 90 °C for 12 h. Solvents were evaporated in *vacuo* and the reaction mixture was dried for 2 h on high vacuum. The crude product was treated with a mixture of Ac₂O/pyridine (1: 2) at 0 °C. Then, the reaction mixture was stirred at room temperature for 12 h. After complete conversion of the starting material, solvents were evaporated in *vacuo*. The mixture was diluted with EtOAc (2 × 30 mL), washed with sat. NaHCO₃ (25 mL), and brine (20 mL). It was dried over MgSO₄ and concentrated in *vacuo*. The crude residue was purified through a silica gel column chromatography to afford the corresponding N-acetyl amine derivative.

General procedure for hydrogenolysis:

A protected trisaccharide derivative (0.5 mmol) was dissolved in 10 mL MeOH : HCOOH (9 : 1), and 20%-Pd(OH)₂/C was added. The mixture was placed under an atmosphere of hydrogen gas bubbling through a needle. After 12h, the reaction mixture was filtered through a pad of Celite and washed with a mixture of CH₃OH and H₂O (1/1, v/v, 6 mL). Solvents were evaporated in *vacuo* and the crude mixture was purified using silica gel column chromatography to furnish the protecting group free trisaccharide.

General procedure for enzyme expression and purifications:

Recombinant enzymes β-1,4 galactosyltransferase (β-1,4-GalT), α-2,3 sialyltransferase, pyruvate kinase (PK), pyrophosphatase (PPA), cytidine monophosphate kinase (CMK) and

CMP-sialic acid synthetases (CSS) were over expressed and purified from *E. coli* BL21(DE3) using Ni-NTA column as our previous protocol.^{7b}

General procedure for enzymatic β -1, 4-galactosylation:

A trisaccharide (1 equiv.) and UDP-Gal (2 equiv.) were dissolved in Tris buffer (50 mM, pH 7.5) containing MnCl_2 (5 mM) in a small capped glass tube with β -1,4-GalT (15 units). The resulting reaction mixture was incubated at 37 °C for 12 h. The protein was removed by heating and centrifugation followed by purification using C18 column chromatography. The pure fractions were pooled and concentrated to give the corresponding product.

General procedure for enzymatic α -2,3-sialylation:

Trisaccharide (1 eq.), Neu5Ac (2 equiv.), ATP (0.05 equiv.), CTP (0.05 equiv.), phosphoenolpyruvate (2.4 equiv.), cytidine monophosphate kinase (CMK, 5U), CMP-sialic acid synthetases (CSS, 10U), pyruvate kinase (PK, 5 units), pyrophosphatase (PPA, 2.5U) and α -2,3 sialyltransferase (15 units) were dissolved in Tris buffer (50 mM, pH 7.5) and transferred to a small glass tube. The reaction was incubated at 37 °C and monitored by TLC until completion. Heating and centrifugation was performed to remove the protein and followed by purification using C18 column chromatography. The pure fractions were pooled and concentrated to give the respective product.

p-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-4,6-O-benzylidene-3-Obenzyl- α -D-mannopyranoside (3)

Glycosyl donor **2** (0.5 g, 0.924 mmol, 1.2 equiv. based on acceptor) and acceptor **1** (0.36 g, 0.775 mmol) in dry CH_2Cl_2 was added 4 Å molecular sieves and the mixture was stirred under N_2 atmosphere for 1h, then cooled to -50 °C. NIS (0.249 g, 1.10 mmol) and TMSOTf (25 μL , 0.272 mmol) were added, and the reaction mixture was stirred for another 1 h. After complete conversion of the starting materials, the reaction mixture was quenched with Et_3N (2 equiv.) and filtered through a Celite pad. The mixture was diluted with CH_2Cl_2 (2 $\times$ 30 mL), washed with sat. NaHCO_3 (25 mL), $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL) and brine (50 mL). It was then dried over MgSO_4 and concentrated for column chromatography purification on silica gel (PhCH_3 : EtOAc , 9:1) to furnish **3** (0.7 g, 86 %) as yellow oil. R_f 0.52 ($\text{PhCH}_3/\text{EtOAc}$ 9:1); ^1H NMR (600 MHz, CDCl_3) δ 7.89 – 7.85 (m, 2H), 7.78 – 7.73 (m, 2H), 7.41 (td, J = 7.1, 6.5, 3.8 Hz, 4H), 7.34 – 7.31 (m, 4H), 7.31 – 7.28 (m, 2H), 6.76 – 6.72 (m, 2H), 6.67 – 6.63 (m, 2H), 5.89 (dd, J = 10.9, 9.1 Hz, 1H), 5.52 (d, J = 8.5 Hz, 1H), 5.46 (s, 1H), 5.22 (dd, J = 10.1, 9.1 Hz, 1H), 5.13 (d, J = 2.0 Hz, 1H), 4.79 – 4.71 (m, 2H), 4.52 (dd, J = 11.0, 8.5 Hz, 1H), 4.34 – 4.31 (m, 1H), 4.27 (dd, J = 3.2, 1.9 Hz, 1H), 4.23 (dd, J = 12.2, 2.4 Hz, 1H), 4.04 (dd, J = 10.0, 3.1 Hz, 1H), 3.97 (t, J = 9.5 Hz, 1H), 3.90 (ddt, J = 9.4, 4.6, 2.3 Hz, 1H), 3.76 (s, 3H), 3.68 – 3.60 (m, 2H), 3.18 – 3.11 (m, 1H), 2.06 (s, 3H), 2.05 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 170.8, 170.3, 169.5, 155.1, 149.5, 138.4, 137.5, 134.4, 133.9, 131.7, 130.1, 128.9, 128.3, 128.2, 127.7, 127.6, 126.1, 123.5, 117.0, 114.6, 101.6, 97.0, 96.24, 78.1, 77.3, 77.1, 76.9, 75.6, 73.7, 72.3, 71.9, 70.4, 69.1, 68.2, 64.4, 62.2, 55.7, 54.6, 20.8, 20.7, 20.6; m/z (HRMS) calcd for $\text{C}_{47}\text{H}_{47}\text{NO}_{16}$ $[\text{M}+\text{Na}]^+$: 904.2793, found: 904.2789.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-3,4-di-O-benzyl- α -D-mannopyranoside (4)**

Disaccharide **4** was prepared from **3** (0.7 g, 0.774 mmol) using the general procedure for 4,6-*O*-benzylidene ring opening using BH_3 -THF, followed by purification using silica gel column chromatography (PhCH_3 : EtOAc = 8:2). Yield (0.575 g, 82 %). ^1H NMR (600 MHz, CDCl_3) δ 7.88 (dt, J = 7.2, 3.6 Hz, 2H), 7.79 (dd, J = 5.5, 3.0 Hz, 2H), 7.45 – 7.41 (m, 2H), 7.34 – 7.28 (m, 5H), 7.28 – 7.23 (m, 3H), 6.70 – 6.64 (m, 2H), 6.52 – 6.48 (m, 2H), 5.97 (dd, J = 10.8, 9.0 Hz, 1H), 5.44 (d, J = 8.5 Hz, 1H), 5.22 (dd, J = 10.2, 9.0 Hz, 1H), 5.01 (d, J = 2.2 Hz, 1H), 4.87 (d, J = 10.8 Hz, 1H), 4.81 (d, J = 11.1 Hz, 1H), 4.62 (d, J = 11.2 Hz, 1H), 4.58 – 4.50 (m, 2H), 4.35 (dd, J = 12.2, 5.3 Hz, 1H), 4.26 – 4.22 (m, 2H), 4.03 (dd, J = 9.1, 3.1 Hz, 1H), 3.93 (ddd, J = 10.2, 5.3, 2.4 Hz, 1H), 3.81 (t, J = 9.4 Hz, 1H), 3.73 (s, 3H), 3.47 (ddd, J = 10.0, 4.6, 2.6 Hz, 1H), 3.38 (ddd, J = 12.1, 5.1, 2.6 Hz, 1H), 3.29 (ddd, J = 12.3, 8.2, 4.6 Hz, 1H), 2.05 (app d, J = 3.4 Hz, 6H), 1.92 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.6, 170.2, 169.5, 154.8, 149.5, 138.3, 138.0, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 116.8, 114.5, 97.5, 95.6, 77.5, 75.2, 74.5, 73.9, 72.5, 72.1, 71.4, 70.2, 69.0, 62.4, 62.1, 55.6, 54.8, 20.7, 20.6, 20.5; m/z (HRMS) calcd for $\text{C}_{47}\text{H}_{49}\text{NO}_{16}$ $[\text{M}+\text{Na}]^+$: 906.2649, found: 906.2655.

***p*-Tolyl-3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside (5)**

Compound **2** (0.6 g, 1.09 mmol) was dissolved in anhydrous methanol (8 mL) and sodium methoxide (0.15 g) was added. The reaction mixture was stirred at room temperature for 3 h, and was neutralized using acid resin (IR-120), filtered and concentrated in *vacuo*, and later co-evaporated with toluene to remove traces of waters. A solution of the tri hydroxy intermediate (0.5 g, 1.20 mmol) in DMF (10 mL) was added tetrabutylammonium iodide (TBAI, 40 mg, 0.12 mmol) and benzyl bromide (0.49 mL, 4.21 mmol) under N_2 atmosphere. The solution was cooled to 0 $^\circ\text{C}$ and then NaH (60% dispersion in mineral oil, 0.24 g, 6.0 mmol) was added slowly. The reaction mixture was stirred at 0 $^\circ\text{C}$ for 1 h and then stirred at room temperature for additional 7 h. The reaction mixture was quenched with aqueous NH_4Cl and was diluted with CH_2Cl_2 (2×30 mL), washed with sat. NH_4Cl (25 mL), and brine (50 mL). It was dried over MgSO_4 and concentrated in *vacuo*. The crude mixture was purified using silica gel column chromatography (hexane: EtOAc, 9:1) to furnish **5** (0.57 g, 69 %) as colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.74 (d, J = 7.3 Hz, 1H), 7.65 – 7.53 (m, 3H), 7.34 – 7.32 (m, 4H), 7.32 – 7.29 (m, 6H), 7.25 – 7.21 (m, 5H), 6.96 – 6.92 (m, 2H), 6.85 – 6.82 (m, 1H), 6.82 – 6.78 (m, 1H), 5.46 (dd, J = 10.0, 2.7 Hz, 1H), 4.78 (d, J = 10.9 Hz, 1H), 4.73 (d, J = 12.1 Hz, 1H), 4.64 – 4.57 (m, 2H), 4.53 – 4.49 (m, 1H), 4.39 (d, J = 12.1 Hz, 1H), 4.32 (dd, J = 10.4, 8.5 Hz, 1H), 4.21 (td, J = 10.3, 3.4 Hz, 1H), 3.77 (td, J = 12.2, 11.6, 3.2 Hz, 2H), 3.74 – 3.67 (m, 1H), 3.62 (ddd, J = 9.9, 4.4, 2.1 Hz, 1H), 2.22 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 168.2, 167.5, 141.1, 138.3, 138.2, 138.0, 137.8, 134.0, 133.9, 133.5, 131.7, 131.5, 129.7, 129.7, 128.7, 128.7 (2), 128.5, 128.5, 128.4, 128.2, 128.1, 128.1 (2), 127.9, 127.8, 127.7, 127.7(2), 127.6, 127.6(2), 127.5, 127.0, 123.5(2), 123.4, 83.5, 80.3, 79.5, 79.4, 75.1, 75.0, 73.5, 68.9, 55.1, 21.2.; m/z (HRMS) calcd for $\text{C}_{42}\text{H}_{39}\text{NSO}_6$ $[\text{M}+\text{Na}]^+$: 708.2396, found: 708.2399.

Dibutyl-3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside phosphate (6)

Dibutyl phosphate derivative **6** was prepared from **5** (0.57 g, 0.774 mmol) using the general procedure for the synthesis of glycosyl phosphates, followed by purification using silica gel column chromatography (hexane: EtOAc = 7:3). Yield (0.564 g, 88 %). ¹H NMR (600 MHz, CDCl₃) δ 7.70 – 7.64 (m, 4H), 7.37 – 7.33 (m, 3H), 7.33 – 7.27 (m, 3H), 7.26 – 7.22 (m, 3H), 6.99 (dt, *J* = 6.2, 1.4 Hz, 2H), 6.90 – 6.86 (m, 2H), 6.86 – 6.82 (m, 2H), 5.82 (dd, *J* = 8.5, 7.2 Hz, 1H), 4.84 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 12.2 Hz, 1H), 4.66 (dd, *J* = 11.5, 9.2 Hz, 2H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.50 – 4.42 (m, 2H), 4.27 (dd, *J* = 10.8, 8.5 Hz, 1H), 3.99 – 3.95 (m, 1H), 3.95 – 3.90 (m, 1H), 3.89 – 3.85 (m, 1H), 3.85 – 3.81 (m, 1H), 3.78 (d, *J* = 1.9 Hz, 1H), 3.77 – 3.75 (m, 1H), 3.75 – 3.72 (m, 1H), 3.72 – 3.69 (m, 1H), 3.69 – 3.66 (m, 1H), 1.54 – 1.48 (m, 2H), 1.27 (ddt, *J* = 14.5, 11.9, 6.3 Hz, 4H), 1.07 – 1.00 (m, 2H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.69 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 167.7, 138.0, 138.0(2), 137.9, 133.8, 131.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.8(2), 127.7, 127.4, 123.3, 94.2, 94.2(2), 79.0, 78.7, 75.5, 75.1, 74.1, 73.6, 68.3, 68.0, 68.0 (2), 67.8, 67.8, 56.3, 56.3, 32.1, 32.1, 31.9, 31.8, 18.6, 18.4, 13.6, 13.5.; *m/z* (HRMS) calcd for C₄₃H₅₀NPO₁₀ [M+Na]⁺: 794.3070, found: 794.3078.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranoside (7)**

Trisaccharide **7** was prepared from glycosyl phosphate donor **6** (0.104 g, 0.135 mmol) and disaccharide acceptor **4** (0.1 g, 0.113 mmol) using the general procedure for glycosyl phosphate coupling and followed by purification using silica gel column chromatography (PhCH₃: EtOAc = 8:2). Yield (0.130 g, 80 %). ¹H NMR (600 MHz, CDCl₃) δ 7.84 – 7.54 (m, 4H), 7.52 (dd, *J* = 5.5, 3.0 Hz, 4H), 7.33 – 7.21 (m, 16H), 7.10 – 7.07 (m, 3H), 6.98 – 6.95 (m, 2H), 6.91 – 6.81 (m, 4H), 6.65 – 6.58 (m, 4H), 5.83 (dd, *J* = 10.8, 9.0 Hz, 1H), 5.45 (d, *J* = 8.5 Hz, 1H), 5.16 (dd, *J* = 10.2, 9.0 Hz, 1H), 4.96 (dd, *J* = 5.5, 2.9 Hz, 2H), 4.80 (d, *J* = 10.9 Hz, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.62 (dd, *J* = 11.2, 5.9 Hz, 2H), 4.50 – 4.45 (m, 3H), 4.43 – 4.42 (m, 1H), 4.42 – 4.37 (m, 2H), 4.27 (dd, *J* = 12.2, 5.0 Hz, 1H), 4.22 (dd, *J* = 10.7, 8.6 Hz, 1H), 4.19 (d, *J* = 2.4 Hz, 1H), 4.18 – 4.14 (m, 1H), 4.12 (t, *J* = 2.9 Hz, 1H), 3.99 (dd, *J* = 10.7, 8.4 Hz, 1H), 3.85 (dd, *J* = 8.2, 3.2 Hz, 1H), 3.82 (ddd, *J* = 10.2, 5.1, 2.4 Hz, 1H), 3.74 (s, 3H), 3.73 – 3.71 (m, 1H), 3.65 (dd, *J* = 11.4, 2.3 Hz, 2H), 3.63 – 3.58 (m, 1H), 3.58 – 3.54 (m, 1H), 3.48 (ddd, *J* = 9.9, 4.6, 1.9 Hz, 1H), 3.38 (dd, *J* = 9.5, 8.2 Hz, 1H), 3.10 (dd, *J* = 11.1, 6.2 Hz, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.88 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.8, 170.3, 169.6, 154.9, 150.3, 138.3, 138.3(2), 138.1, 138.1, 138.0, 134.1, 131.5, 128.5, 128.5(2), 128.4, 128.3, 128.3, 128.2, 128.1, 128.1(2), 128.0, 128.0(2), 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 117.9, 114.4, 98.7, 97.3, 96.6, 79.8, 79.3, 77.9, 75.1, 74.9, 74.9, 73.5, 72.2, 71.7, 71.7, 70.6, 69.4, 69.2, 68.8, 62.4, 55.8, 55.7, 54.6, 20.8, 20.8, 20.6; *m/z* (HRMS) calcd for C₈₂H₈₀N₂O₂₂ [M+Na]: 1467.5100, found: 1467.5116.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)-6-O-(3,4,6-tri-O-benzyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)-3,4-di-O-benzyl- α -D-mannopyranoside (8)**

Trisaccharide **8** was prepared from trisaccharide **7** (0.130 g, 0.09 mmol) using the general procedure for phthalimide deprotection and N-acetylation. The crude mixture was purified using silica gel column chromatography (PhCH₃: EtOAc = 6:4). Yield (0.09 g, 79 %). ¹H NMR (600 MHz, CDCl₃) δ 7.82 – 7.72 (m, 2H), 7.45 – 7.42 (m, 2H), 7.39 – 7.35 (m, 2H), 7.35 – 7.31 (m, 4H), 7.31 – 7.25 (m, 9H), 7.25 – 7.23 (m, 3H), 7.23 – 7.16 (m, 3H), 6.90 – 6.87 (m, 2H), 6.81 – 6.78 (m, 2H), 5.83 (t, *J* = 10.0 Hz, 1H), 5.50 (d, *J* = 1.9 Hz, 1H), 5.46 – 5.36 (m, 1H), 5.09 – 5.04 (m, 1H), 4.89 (dd, *J* = 16.7, 11.5 Hz, 2H), 4.80 (dd, *J* = 11.3, 7.8 Hz, 2H), 4.67–4.63 (m, 2H), 4.59 – 4.55 (m, 1H), 4.55 – 4.49 (m, 3H), 4.37 (dd, *J* = 3.5, 2.0 Hz, 1H), 4.25 (dd, *J* = 12.2, 5.0 Hz, 1H), 4.14 (dd, *J* = 9.1, 3.5 Hz, 1H), 4.10 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.92 – 3.86 (m, 2H), 3.84 (t, *J* = 9.4 Hz, 1H), 3.72 (app s, 4H), 3.67 (d, *J* = 3.3 Hz, 2H), 3.62 – 3.54 (m, 2H), 3.53 – 3.49 (m, 1H), 3.42–3.39 (m, 2H), 3.33 (dt, *J* = 10.0, 3.3 Hz, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.82 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 173.1, 171.2, 170.7, 170.5, 169.7, 154.8, 149.8, 139.5, 138.5(2), 138.4, 138.1, 137.96, 128.5127.6, 116.8, 114.7, 101.6, 97.8, 95.1, 80.6, 79.1, 78.1, 75.0, 74.9, 74.6, 73.4, 73.4, 72.5, 71.7, 71.6, 71.1, 70.9, 70.1, 69.8, 68.7, 67.3, 62.5, 55.7, 23.9, 23.8, 20.7, 20.7(2), 20.6; *m/z* (HRMS) calcd for C₇₀H₈₀N₂O₂₀ [M+Na]⁺: 1291.5202, found: 1291.5212.

***p*-Methoxyphenyl-2-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-6-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)- α -D-mannopyranoside (9)**

Trisaccharide **9** was prepared from trisaccharide **8** (0.09 g, 0.07 mmol) using the general procedure for hydrogenolysis and followed by purification using silica gel column chromatography (MeOH: CHCl₃ = 1:20). Yield (0.05 g, 91 %). ¹H NMR (600 MHz, MeOD) δ 8.10 (s, 2H), 7.06 – 6.96 (m, 2H), 6.90 – 6.84 (m, 2H), 5.55 (td, *J* = 10.3, 3.7 Hz, 1H), 5.38 (s, 1H), 4.47 – 4.41 (m, 1H), 4.45–4.40 (m, 1H), 4.32 (ddd, *J* = 12.3, 4.4, 1.8 Hz, 1H), 4.17 – 4.11 (m, 1H), 4.10 (dd, *J* = 12.4, 2.4 Hz, 1H), 4.06 (d, *J* = 10.7 Hz, 1H), 3.94 – 3.87 (m, 1H), 3.86–3.84 (m, 1H), 3.76 (s, 3H), 3.73 – 3.68 (m, 2H), 3.68 – 3.59 (m, 2H), 3.66–3.60 (m, 2H), 3.48 (ddd, *J* = 10.3, 8.4, 1.7 Hz, 1H), 3.35 – 3.33 (m, 1H), 3.30 – 3.22 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H), 1.99 (s, 6H), 1.87 (s, 3H); ¹³C NMR (151 MHz, MeOD) δ 174.6, 173.9, 172.3, 171.9, 171.2, 164.7, 156.6, 151.9, 119.1, 119.1, 115.7, 103.1, 99.5, 97.8, 78.5, 77.8, 75.9, 73.9, 72.9, 72.8, 72.1, 71.3, 70.3, 69.7, 68.5, 63.1, 62.7, 57.3, 56.3, 56.1, 23.7, 23.7(2), 23.4, 23.4(2), 20.6, 20.5; *m/z* (HRMS) calcd for C₃₅H₅₀N₂O₁₀ [M+Na]⁺: 841.2855, found: 841.2860.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3-O-benzyl-4,6-Obenzylidene- α -D-mannopyranoside (10)**

Disaccharide **10** was prepared from glycosyl phosphate donor **6** (0.6 g, 0.775 mmol) and acceptor **1** (0.4 g, 0.646 mmol) using the general procedure for glycosyl phosphates coupling and followed by purification using silica gel column chromatography (PhCH₃: EtOAc = 20:1). Yield (0.530 g, 80 %). ¹H NMR (600 MHz, CDCl₃) δ 7.72 – 7.62 (m, 2H), 7.43 – 7.35 (m, 5H), 7.35–7.28 (m, 9H), 7.27 – 7.20 (m, 5H), 7.07 – 7.02 (m, 4H), 6.93–

6.87 (m, 2H), 6.87 – 6.82 (m, 2H), 6.74 – 6.67 (m, 2H), 6.65 – 6.58 (m, 2H), 5.42 (s, 1H), 5.27 (d, $J = 8.4$ Hz, 1H), 5.06 (d, $J = 1.9$ Hz, 1H), 4.87 (d, $J = 10.8$ Hz, 1H), 4.83 (d, $J = 12.2$ Hz, 1H), 4.78 (d, $J = 12.3$ Hz, 1H), 4.67 (dd, $J = 16.5, 11.6$ Hz, 2H), 4.60 (d, $J = 12.1$ Hz, 1H), 4.55 (d, $J = 12.2$ Hz, 1H), 4.48 (d, $J = 12.2$ Hz, 1H), 4.41 (dd, $J = 10.8, 8.3$ Hz, 1H), 4.35 (dd, $J = 10.8, 8.3$ Hz, 1H), 4.24 (dd, $J = 3.1, 1.9$ Hz, 1H), 4.12 (q, $J = 7.2$ Hz, 1H), 3.98 (dd, $J = 10.0, 3.1$ Hz, 1H), 3.96 – 3.91 (m, 1H), 3.82 – 3.76 (m, 2H), 3.74 (s, 3H), 3.72 – 3.68 (m, 1H), 3.59 – 3.53 (m, 2H), 3.21 (s, 1H), 3.06 – 2.99 (m, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 176.4, 171.2, 155.0, 149.7, 138.5–137.6, 133.8, 132.0, 128.8–127.4, 126.1, 123.1, 117.1, 114.6, 101.5, 96.9, 96.3, 79.8, 79.2, 78.1, 75.6, 75.2, 74.9, 74.9(2), 73.7, 73.4, 71.5, 69.3, 68.3, 64.3, 60.5, 59.9, 56.0, 55.7; m/z (HRMS) calcd for $\text{C}_{62}\text{H}_{59}\text{N}_2\text{O}_{13}$ [$\text{M}+\text{Na}$] $^+$: 1048.3884, found: 1048.3889.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- α -D-mannopyranoside (11)**

Disaccharide **11** was prepared from **10** (0.53 g, 0.687 mmol) using the general procedure for 4,6-*O*-benzylidene ring opening with BH_3 -THF, followed by purification using silica gel column chromatography (PhCH_3 : EtOAc = 8:2). Yield (0.413 g, 78 %). ^1H NMR (600 MHz, CDCl_3) δ 7.78 – 7.51 (m, 5H), 7.35 – 7.32 (m, 2H), 7.28 – 7.21 (m, 8H), 7.21 – 7.12 (m, 9H), 7.00 – 6.97 (m, 2H), 6.83 (dd, $J = 8.2, 6.8$ Hz, 2H), 6.78 – 6.74 (m, 1H), 6.58 – 6.54 (m, 2H), 6.40 – 6.35 (m, 2H), 5.10 (d, $J = 8.5$ Hz, 1H), 4.83 (d, $J = 2.2$ Hz, 1H), 4.80 (s, 1H), 4.79 – 4.78 (m, 2H), 4.76 (dd, $J = 11.8, 1.9$ Hz, 2H), 4.58 (d, $J = 10.9$ Hz, 1H), 4.53 – 4.48 (m, 2H), 4.48 – 4.43 (m, 2H), 4.43 – 4.38 (m, 2H), 4.27 (dd, $J = 10.8, 8.5$ Hz, 1H), 4.11 (t, $J = 6.0$ Hz, 1H), 3.89 (dd, $J = 9.2, 3.1$ Hz, 1H), 3.72 – 3.68 (m, 1H), 3.68 (d, $J = 4.0$ Hz, 1H), 3.65 (app s, 4H), 3.36 – 3.31 (m, 2H), 3.24 (dd, $J = 12.7, 2.8$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 168.8, 168.0, 154.8, 149.7, 138.5, 138.3, 138.3(2), 138.2, 138.1, 137.8, 134.2, 133.7, 128.6 – 127.4, 116.9, 114.5, 97.6, 95.8, 79.7, 79.1, 75.3, 75.2, 74.8, 74.1, 73.7, 72.4, 70.9, 70.7, 69.5, 62.5, 56.3, 55.7; m/z (HRMS) calcd for $\text{C}_{62}\text{H}_{59}\text{N}_2\text{O}_{13}$ [$\text{M}+\text{Na}$] $^+$: 1048.3884, found: 1048.3889.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)3,4-di-O-benzyl- α -D-mannopyranoside (13)**

Trisaccharide **13** was prepared from glycosyl phosphate donor **12** (0.302 g, 0.483 mmol) and disaccharide acceptor **11** (0.413 g, 0.402 mmol) using the general procedure for glycosyl phosphates coupling and followed by column chromatography on silica gel (PhCH_3 : EtOAc = 8:2). Yield (0.471 g, 81 %). ^1H NMR (600 MHz, CDCl_3) δ 7.61 (s, 2H), 7.49 (s, 3H), 7.31 – 7.12 (m, 21H), 7.06 – 7.02 (m, 2H), 6.98 – 6.94 (m, 2H), 6.85 – 6.81 (m, 2H), 6.80 – 6.76 (m, 1H), 6.61 – 6.53 (m, 2H), 6.52 – 6.41 (m, 2H), 5.59 (dd, $J = 10.7, 9.0$ Hz, 1H), 5.20 (d, $J = 8.5$ Hz, 1H), 5.10 (d, $J = 8.4$ Hz, 1H), 4.90 – 4.86 (m, 2H), 4.85 (d, $J = 10.9$ Hz, 1H), 4.79 (d, $J = 12.1$ Hz, 1H), 4.74 (d, $J = 11.7$ Hz, 1H), 4.63–4.58 (m, 2H), 4.56 (d, $J = 12.1$ Hz, 1H), 4.51 (s, 1H), 4.49 (d, $J = 3.5$ Hz, 1H), 4.47 – 4.44 (m, 2H), 4.40 (dd, $J = 10.8, 8.3$ Hz, 1H), 4.28 (dd, $J = 10.8, 8.5$ Hz, 1H), 4.19 (d, $J = 11.0$ Hz, 1H), 4.14 – 4.12 (m, 1H), 4.05 (dd, $J = 10.7, 8.4$ Hz, 1H), 3.85 – 3.82 (m, 2H), 3.77 (s, 3H), 3.75 – 3.72 (m, 2H), 3.72 – 3.69 (m, 1H), 3.67 (dq, $J = 9.9, 2.3$ Hz, 1H), 3.56–3.51 (m, 2H), 3.30 (dd, $J = 9.7, 8.3$ Hz, 1H), 2.02 – 1.96 (m, 3H), 1.91 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.7, 170.2,

169.6, 154.8, 150.4, 138.4, 138.3, 138.2, 138.0, 134.3, 133.6, 128.6–127.4, 123.6, 117.8, 114.3, 98.6, 97.5, 97.0, 79.7, 79.3, 75.2, 75.1, 74.8, 73.6, 71.5, 71.4, 70.9, 70.6, 69.4, 69.2, 62.3, 56.0, 55.7, 54.6, 31.1, 20.7, 20.5. m/z (HRMS) calcd for C₈₂H₈₀N₂O₂₂ [M+Na]: 1467.5100, found: 1467.5109.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-Dmannopyranoside (14)**

Trisaccharide **14** was prepared from trisaccharide **13** (0.471 g, 0.326 mmol) using the general procedure for phthalimide deprotection and N-acetylation and followed by column chromatography on silica gel (PhCH₃: EtOAc = 6:4). Yield (0.302 g, 73 %). ¹H NMR (600 MHz, CDCl₃) δ 7.68 (s, 2H), 7.56 (s, 2H), 7.33 – 7.29 (m, 4H), 7.29 – 7.26 (m, 5H), 7.25 – 7.23 (m, 2H), 7.22 (dt, *J* = 6.4, 1.7 Hz, 3H), 7.13 – 7.10 (m, 2H), 7.04 – 7.01 (m, 2H), 6.92 – 6.88 (m, 2H), 6.87 – 6.84 (m, 1H), 6.65 – 6.63 (m, 2H), 6.57 – 6.54 (m, 2H), 5.59 (dd, *J* = 10.7, 9.0 Hz, 1H), 5.20 (d, *J* = 8.5 Hz, 1H), 5.10 (d, *J* = 8.4 Hz, 1H), 4.91 – 4.86 (m, 2H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.79 (d, *J* = 12.1 Hz, 1H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 10.9 Hz, 1H), 4.59 (d, *J* = 10.9 Hz, 1H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.51 (s, 1H), 4.49 (d, *J* = 3.5 Hz, 1H), 4.48 – 4.43 (m, 2H), 4.40 (dd, *J* = 10.8, 8.3 Hz, 1H), 4.28 (dd, *J* = 10.8, 8.5 Hz, 1H), 4.19 (d, *J* = 11.0 Hz, 1H), 4.13 (t, *J* = 6.0 Hz, 1H), 4.05 (dd, *J* = 10.7, 8.4 Hz, 1H), 3.85 – 3.82 (m, 3H), 3.77 (s, 3H), 3.75 – 3.71 (m, 2H), 3.71 – 3.67 (m, 2H), 3.56 – 3.52 (m, 2H), 3.30 (dd, *J* = 9.7, 8.3 Hz, 1H), 3.10 – 3.04 (m, 1H), 1.99 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.84 (s, 3H), 1.82 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.7, 170.2, 169.6, 154.8, 150.4, 138.4, 138.3, 138.2, 138.0, 134.3, 133.6, 128.6–127.4, 123.6, 117.8, 114.3, 98.6, 97.5, 97.0, 79.7, 79.3, 75.2, 75.1, 74.8, 73.6, 71.5, 71.4, 70.9, 70.6, 69.4, 69.2, 62.3, 56.0, 55.7, 54.6, 31.1, 20.7, 20.7, 20.5; m/z (HRMS) calcd for C₇₀H₈₀N₂O₂₀ [M+Na]⁺: 1291.5202, found: 1291.5208

***p*-Methoxyphenyl-2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-(2-acetamido-2-deoxy-β-Dglucopyranosyl)-α-D-mannopyranoside (15)**

Trisaccharide **15** was prepared from trisaccharide **14** (0.3 g, 0.236 mmol) using the general procedure for hydrogenolysis and followed by column chromatography on silica gel (MeOH: CHCl₃ = 1:20). Yield (0.168 g, 87 %). ¹H NMR (600 MHz, MeOD) δ 7.88 – 7.80 (m, 1H), 7.11 – 7.00 (m, 2H), 6.93 – 6.81 (m, 2H), 5.34 – 5.30 (m, 1H), 5.24 (ddd, *J* = 10.5, 9.3, 1.1 Hz, 1H), 4.96 – 4.90 (m, 1H), 4.85–4.82 (m, 4H), 4.71 – 4.67 (m, 1H), 4.63 (dd, *J* = 8.2, 1.3 Hz, 1H), 4.16 (dd, *J* = 12.2, 4.9 Hz, 1H), 4.11 (dd, *J* = 3.4, 1.9 Hz, 1H), 4.05 (ddd, *J* = 12.4, 10.0, 2.2 Hz, 2H), 3.90 – 3.84 (m, 3H), 3.84 – 3.79 (m, 1H), 3.77 (s, 3H), 3.76 – 3.72 (m, 1H), 3.69 (dt, *J* = 11.8, 5.7 Hz, 1H), 3.61 – 3.56 (m, 1H), 3.55–3.48 (m, 2H), 3.20 (q, *J* = 7.3 Hz, 1H), 2.02 (app d, 6H), 2.01 (s, 3H), 1.98 (s, 3H), 1.89 (s, 3H); ¹³C NMR (151 MHz, MeOD) δ 174.3, 173.4, 172.3, 171.7, 171.2, 156.6, 152.0, 119.5, 115.6, 102.5, 100.9, 98.5, 79.3, 79.1, 78.9, 78.4, 77.9, 74.7, 73.9, 73.8, 72.6, 72.0, 71.4, 70.8, 70.2, 68.7, 63.2, 62.4, 57.7, 56.1, 55.6, 23.5, 23.1, 23.0, 20.7, 20.6; m/z (HRMS) calcd for C₃₅H₅₀N₂O₁₀ [M+Na]⁺: 841.2855, found: 841.2860.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (16)**

Disaccharide **16** was prepared from **3** (0.3 g, 0.34 mmol) using the general procedure for 4,6-*O*-benzylidene ring opening using Et₃SiH/TFA, followed by purification using silica gel column chromatography (PhCH₃: EtOAc = 8:2). Yield (0.210 g, 70 %). ¹H NMR (600 MHz, CDCl₃) δ 7.86 – 7.75 (m, 1H), 7.73 – 7.64 (m, 1H), 7.44 – 7.40 (m, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.32 – 7.28 (m, 2H), 7.28 – 7.25 (m, 2H), 7.25 – 7.22 (m, 2H), 7.09 – 7.03 (m, 2H), 6.79 – 6.75 (m, 2H), 6.75 – 6.69 (m, 2H), 5.83 (dd, *J* = 10.8, 9.1 Hz, 1H), 5.54 (d, *J* = 8.5 Hz, 1H), 5.26 – 5.14 (m, 2H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.53 (d, *J* = 11.0 Hz, 1H), 4.47 (dd, *J* = 10.8, 8.5 Hz, 1H), 4.31 – 4.28 (m, 1H), 4.27 (d, *J* = 4.9 Hz, 1H), 4.21 (dd, *J* = 12.2, 2.4 Hz, 1H), 4.07 (s, 2H), 3.89 (ddd, *J* = 10.3, 5.0, 2.4 Hz, 1H), 3.84 (dd, *J* = 9.1, 3.1 Hz, 1H), 3.76 – 3.72 (m, 4H), 3.70 – 3.65 (m, 1H), 3.41 (dd, *J* = 10.7, 3.2 Hz, 1H), 3.04 (dd, *J* = 10.7, 6.4 Hz, 1H), 2.61 (dt, *J* = 7.3, 2.3 Hz, 1H), 2.03 (s, 3H), 2.03 – 2.00 (s, 3H), 1.87 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.7, 170.2, 169.5, 155.0, 149.9, 138.2, 137.9, 137.8, 134.2, 129.1–127.3, 125.3, 123.5, 117.5, 114.5, 96.8, 95.9, 77.0, 73.2, 72.9, 72.1, 71.7, 70.9, 70.5, 70.5(2), 69.0, 67.5, 62.2, 55.6, 54.5, 21.5, 20.7, 20.7, 20.5; *m/z* (HRMS) calcd for C₄₇H₄₉NO₁₆ [M+Na]⁺: 906.2649, found: 906.2653.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-O-(3,4,6-tri-O-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranoside (17)**

Disaccharide acceptor **16** upon glycosylation with glycosyl donor **6** using general procedure for glycosyl phosphates coupling provided trisaccharide. The phthalimide groups of trisaccharide were converted to NHAc using general procedure for phthalimide deprotection and N-acetylation and followed by column chromatography on silica gel (PhCH₃: EtOAc = 6:4) to provide trisaccharide **17**. Yield (0.141 g, 77 %). ¹H NMR (600 MHz, CDCl₃) δ 7.41 – 7.36 (m, 3H), 7.33 – 7.21 (m, 20H), 7.19 – 7.15 (m, 2H), 6.98 – 6.94 (m, 2H), 6.81 – 6.74 (m, 2H), 5.91 (s, 1H), 5.54 (s, 1H), 5.36 (d, *J* = 2.8 Hz, 1H), 5.30 (d, *J* = 8.8 Hz, 1H), 5.11 (d, *J* = 8.4 Hz, 1H), 5.01 (t, *J* = 9.7 Hz, 1H), 4.77 (d, *J* = 6.9 Hz, 1H), 4.76 – 4.74 (m, 2H), 4.74 – 4.67 (m, 3H), 4.60 – 4.55 (m, 2H), 4.54 (d, *J* = 10.9 Hz, 1H), 4.48 (q, *J* = 12.2 Hz, 2H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.26 (d, *J* = 2.8 Hz, 1H), 4.15 (dd, *J* = 12.2, 4.6 Hz, 1H), 4.13 – 4.08 (m, 3H), 3.89 – 3.85 (m, 1H), 3.84 – 3.81 (m, 1H), 3.75 (s, 3H), 3.73 – 3.70 (m, 1H), 3.68 – 3.64 (m, 1H), 3.64 – 3.59 (m, 1H), 3.58 (dd, *J* = 10.5, 4.6 Hz, 1H), 3.52 – 3.44 (m, 1H), 3.37 (ddd, *J* = 9.3, 4.7, 2.2 Hz, 1H), 1.98 (s, 3H), 1.92 (s, 3H), 1.89 (s, 3H), 1.70 (app s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 171.2, 170.7, 170.4, 170.2, 169.5, 155.0, 150.3, 138.6, 138.4, 138.4(2), 138.2, 138.0, 128.5–127.6, 117.6, 114.7, 100.5, 98.3, 96.7, 81.9, 78.4, 77.3, 74.9, 74.7, 74.6, 73.4, 73.1, 72.3, 71.7, 71.5, 69.3, 69.0, 68.9, 62.1, 56.3, 55.7, 55.4, 23.5, 23.2, 20.7, 20.6, 20.6(2); *m/z* (HRMS) calcd for C₇₀H₈₀N₂O₂₀ [M+Na]⁺: 1291.5202, found: 1291.5208.

***p*-Methoxyphenyl-2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (18)**

Trisaccharide **18** was prepared from trisaccharide **17** (0.14 g, 0.110 mmol) using the general procedure for hydrogenolysis and followed by column chromatography on silica gel

(MeOH: CHCl₃ = 1:20). Yield (0.081 g, 90 %). ¹H NMR (600 MHz, MeOD) δ 7.02 (d, *J* = 9.0 Hz, 2H), 6.90 – 6.77 (m, 2H), 5.47 – 5.36 (m, 1H), 5.31 – 5.20 (m, 1H), 4.87 – 4.77 (m, 1H), 4.67 – 4.51 (m, 1H), 4.50 (d, *J* = 8.4 Hz, 1H), 4.33 (dp, *J* = 11.9, 6.8 Hz, 2H), 4.23 – 4.10 (m, 2H), 4.07 (tt, *J* = 6.4, 3.9 Hz, 1H), 3.99 – 3.86 (m, 2H), 3.87 – 3.75 (m, 2H), 3.74 (app s, 3H), 3.73 – 3.67 (m, 2H), 3.68 – 3.55 (m, 2H), 3.55 – 3.44 (m, 1H), 3.42 – 3.32 (m, 2H), 2.04 (app s, 3H), 1.99 (app s, 6H), 1.96 (app s, 6H); ¹³C NMR (151 MHz, MeOD) δ 174.2, 173.8, 172.5, 171.9, 171.3, 164.7, 156.7, 151.7(2), 119.1, 119.1, 119.0, 115.6, 103.1, 101.2, 97.9, 78.5, 78.4, 78.0, 75.8, 73.9, 73.5, 72.9, 71.8, 71.8(2), 70.0, 69.7, 63.1, 62.5, 62.1, 62.0, 57.3, 56.1, 55.4, 23.3, 23.0, 20.7, 20.5; *m/z* (HRMS) calcd for C₃₅H₅₀N₂O₁₀ [M + Na]⁺: 841.2855, found: 841.2859.

***p*-Methoxyphenyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-α-D-mannopyranoside (20)**

Disaccharide **20** was obtained from the disaccharide **10** (0.4 g, 0.39 mmol) using the general procedure for 4,6-*O*-benzylidene ring opening using Et₃SiH/TFA and followed by column chromatography on silica gel (PhCH₃: EtOAc = 9:1). Yield (0.294 g, 74 %). ¹H NMR (500 MHz, CDCl₃) δ 7.51 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.42 – 7.37 (m, 2H), 7.33 – 7.21 (m, 18H), 7.05 – 6.98 (m, 4H), 6.90 – 6.85 (m, 2H), 6.85 – 6.81 (m, 1H), 6.77 – 6.72 (m, 2H), 6.71 – 6.67 (m, 2H), 5.29 (d, *J* = 8.3 Hz, 1H), 5.08 (d, *J* = 1.9 Hz, 1H), 4.86 (dd, *J* = 11.0, 8.2 Hz, 2H), 4.80 (d, *J* = 12.1 Hz, 1H), 4.64 (d, *J* = 10.9 Hz, 1H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.48 (s, 1H), 4.47 – 4.43 (m, 2H), 4.36 (dd, *J* = 10.9, 8.0 Hz, 1H), 4.32 – 4.26 (m, 2H), 3.80 – 3.74 (m, 4H), 3.73 (app s, 4H), 3.71 – 3.59 (m, 3H), 3.38 (dd, *J* = 10.7, 3.1 Hz, 1H), 2.92 (dd, *J* = 10.7, 6.4 Hz, 1H); *m/z* (HRMS) calcd for C₆₂H₆₁NO₁₃ [M+Na]⁺: 1050.4041, found: 1050.4043.

***p*-Methoxyphenyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-*O*-(4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-*O*-benzyl-α-D-mannopyranoside (21)**

Trisaccharide **21** was prepared from glycosyl phosphate donor **19** (0.225 g, 0.291 mmol) and disaccharide acceptor **20** (0.250 g, 0.243 mmol) using the general procedure for glycosyl phosphate coupling and followed by column chromatography on silica gel (PhCH₃: EtOAc = 8:2). Yield (0.292 g, 78 %). ¹H NMR (600 MHz, CDCl₃) δ 7.63–7.56 (m, 6H), 7.43 – 7.33 (m, 1H), 7.29 (qd, *J* = 5.9, 2.8 Hz, 12H), 7.25 – 7.14 (m, 10H), 7.08 – 6.99 (m, 5H), 6.99 – 6.95 (m, 2H), 6.95 – 6.81 (m, 7H), 6.63 – 6.58 (m, 2H), 6.58 – 6.51 (m, 2H), 5.21 (d, *J* = 8.4 Hz, 1H), 4.93 – 4.86 (m, 3H), 4.84 (d, *J* = 10.9 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 10.9 Hz, 1H), 4.55 (dd, *J* = 12.0, 9.5 Hz, 2H), 4.50 (d, *J* = 12.0 Hz, 2H), 4.45 (dd, *J* = 11.7, 2.9 Hz, 2H), 4.38 (dd, *J* = 10.8, 8.4 Hz, 1H), 4.33 – 4.28 (m, 3H), 4.28 – 4.22 (m, 2H), 4.13 (t, *J* = 2.8 Hz, 1H), 4.10 (d, *J* = 11.0 Hz, 1H), 3.92 (dd, *J* = 10.7, 8.4 Hz, 1H), 3.82 (dd, *J* = 8.4, 3.2 Hz, 1H), 3.74 (app s, 4H), 3.72 – 3.68 (m, 1H), 3.66 (td, *J* = 7.2, 3.6 Hz, 2H), 3.55 (dd, *J* = 11.3, 2.3 Hz, 1H), 3.51 (ddd, *J* = 9.8, 5.7, 4.0 Hz, 1H), 3.33 – 3.27 (m, 2H), 3.18 (dd, *J* = 10.6, 5.6 Hz, 1H), 3.06 (dd, *J* = 11.2, 6.7 Hz, 1H), 2.36 (s, 1H), 1.89 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 169.8, 154.8, 150.5, 138.4, 138.2, 138.2, 138.1, 138.0, 137.7, 133.8, 131.6, 129.1–127.4, 125.4, 117.8, 114.4, 98.7, 97.3, 96.4, 79.7, 79.4, 77.6, 75.2, 75.1, 74.9, 74.8, 73.9, 73.6, 73.5, 73.1, 72.9, 71.3, 71.1, 70.1, 69.9, 69.4,

55.9, 55.7, 55.4, 21.0; m/z (HRMS) calcd for $C_{92}H_{88}N_2O_{22}$ $[M+Na]^+$: 1563.5822, found: 1563.5859.

***p*-Methoxyphenyl-2-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-(4-O-acetyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)- α -D-mannopyranoside (22)**

Trisaccharide **21** (0.2 g, 0.13 mmol) was converted to protecting group free trisaccharide **22** in a two steps sequence: (i) deprotection of phthalimide groups and converted to NHAc using general procedure for phthalimide deprotection and N-acetylation (ii) catalytic hydrogenation in the presence of $Pd(OH)_2/C$ removed all benzyl ethers. Finally, the crude trisaccharide was purified by column chromatography on silica gel (MeOH: $CHCl_3$ = 1:9) to furnish **24**. Yield (0.059 g, 86 %). 1H NMR (600 MHz, MeOD) δ 7.11 – 7.02 (m, 2H), 6.95 – 6.85 (m, 2H), 5.36 (s, 1H), 4.87 – 4.81 (m, 3H), 4.78 (t, J = 9.2 Hz, 1H), 4.68 (d, J = 8.3 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 4.17 – 4.07 (m, 2H), 3.95 – 3.86 (m, 2H), 3.78 (s, 3H), 3.77 – 3.74 (m, 1H), 3.73 – 3.68 (m, 1H), 3.66 – 3.59 (m, 3H), 3.59 – 3.54 (m, 2H), 3.52 (dd, J = 12.2, 5.8 Hz, 1H), 3.45 (ddd, J = 8.9, 6.0, 2.5 Hz, 1H), 2.11 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (151 MHz, MeOD) δ 174.5, 172.1, 156.8, 152.0, 119.5, 115.7, 102.9, 98.7, 78.9, 78.0, 75.8, 74.8, 74.0, 73.3, 73.2, 72.1, 71.5, 70.1, 68.8, 62.5, 62.4, 57.8, 57.6, 56.1, 23.6, 23.3, 20.9. m/z (HRMS) calcd for $C_{31}H_{46}N_2O_{18}$ $[M+Na]^+$: 757.2638, found: 757.2647.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)-4-O-(β -D-galactopyranosyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)- α -D-mannopyranoside (23)**

Glycan **23** was prepared from **18** using the general procedure for enzymatic β -1, 4-galactosylation. The crude compound was purified using C18 column chromatography using MeOH/ H_2O as eluent. Fractions containing the product were combined, evaporated, and lyophilized to give the tetrasaccharide **23** (9 mg, 75 %). 1H NMR (600 MHz, D_2O) δ 7.16 – 7.12 (m, 2H), 7.03 – 6.99 (m, 2H), 5.49 (d, J = 2.1 Hz, 1H), 5.28 (dd, J = 10.7, 9.2 Hz, 1H), 5.12 (dd, J = 10.1, 9.3 Hz, 1H), 4.91 (d, J = 8.4 Hz, 1H), 4.58 (d, J = 8.3 Hz, 1H), 4.50 (d, J = 7.9 Hz, 1H), 4.45 (dd, J = 12.6, 3.4 Hz, 1H), 4.35 (dd, J = 3.4, 2.1 Hz, 1H), 4.26 – 4.20 (m, 2H), 4.17 – 4.11 (m, 1H), 4.06 – 4.03 (m, 1H), 4.04 – 3.99 (m, 1H), 3.95 (d, J = 3.5 Hz, 1H), 3.90 – 3.86 (m, 1H), 3.83 (s, 3H), 3.82 – 3.79 (m, 1H), 3.81 – 3.76 (m, 1H), 3.76 (ddd, J = 5.0, 3.6, 2.0 Hz, 2H), 3.75 – 3.71 (m, 1H), 3.71 – 3.68 (m, 1H), 3.62 – 3.57 (m, 1H), 3.57 – 3.54 (m, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.06 – 2.04 (m, 3H), 1.99 (s, 3H). ^{13}C NMR (151 MHz, D_2O) δ 177.2, 177.0, 176.2, 175.7, 175.4, 157.3, 152.3, 121.2, 117.6, 105.4, 104.0, 102.1, 98.9, 80.6, 79.8, 79.2, 77.9, 77.4, 75.0, 74.9, 74.7, 73.7, 73.5, 71.1, 71.0, 70.7, 65.0, 64.3, 63.5, 63.2, 62.5, 58.3, 57.7, 56.0, 25.8, 24.7, 24.6, 22.6, 22.6(2), 22.5; m/z (HRMS) calcd for $C_{41}H_{60}N_2O_{23}$ $[M+Na]^+$: 1003.3383, found: 1003.3384.

***p*-Methoxyphenyl-2-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-O-(5-Acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl-2 $\rightarrow$ 3)- α -D-galactopyranosyl- β -(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)- α -D-mannopyranoside (24)**

Glycan **24** was prepared from **23** using the general procedure for enzymatic α -2, 3-sialylation. The crude compound was purified using C18 column chromatography using

MeOH/H₂O as eluent. Fractions containing the product were combined, evaporated, and lyophilized to give the pentasaccharide **24** (11.8 mg, 83 %). ¹H NMR (600 MHz, D₂O) δ 7.06 (d, *J* = 8.3 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 5.40 (s, 1H), 5.19 (t, *J* = 10.0 Hz, 1H), 5.04 (t, *J* = 9.7 Hz, 1H), 4.82 (d, *J* = 8.3 Hz, 1H), 4.74–4.78 (m, 1H), 4.50 (d, *J* = 8.3 Hz, 1H), 4.41 (d, *J* = 7.5 Hz, 1H), 4.37 (d, *J* = 12.7 Hz, 1H), 4.27 (s, 1H), 4.18–4.10 (m, 2H), 3.98–3.90 (m, 3H), 3.86 (s, 1H), 3.83–3.76 (m, 1H), 3.75 (d, *J* = 2.1 Hz, 3H), 3.72–3.63 (m, 12H), 3.63–3.54 (m, 2H), 3.54–3.44 (m, 2H), 2.97–2.87 (m, 1H), 2.07 (app s, 3H), 2.04–1.98 (app m, 9H), 1.97 (s, 3H), 1.93 (s, 3H), 1.29–1.18 (m, 1H); ¹³C NMR (151 MHz, D₂O) δ 174.7, 174.5, 173.6, 173.2, 172.8, 154.7, 149.7, 118.6, 115.1, 102.9, 101.5, 99.5, 96.3, 78.1, 77.3, 76.7, 75.3, 74.8, 72.5, 72.4, 72.1, 72.0, 71.1, 70.9, 68.5, 68.4, 68.2, 62.5, 61.8, 61.0, 59.9, 59.5, 55.8, 55.1, 53.5, 30.2, 23.2, 22.1, 22.1, 20.1, 20.1(2), 19.9.

Supplementary Material

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Scheme 1:
Synthesis of Man β 2GlcNAc(OAc) $_3\beta$ 6GlcNAc

Scheme 2:
Synthesis of Man β 2GlcNAc β 6GlcNAc(OAc)₃

Scheme 3:
Synthesis of Man β 2GlcNAc(OAc) $_3$ β 4GlcNAc

Scheme 4:
Synthesis of Man β 2GlcN- β 4GlcN(OAc)

Scheme 5: Stepwise Enzymatic elongation

Reaction conditions: (i) β -1,4-GalT (15 U), UDP-Gal, Tris buffer (50 mM), MnCl_2 (5mM), 37 °C, 12 h, 75%. (ii) α -2,3-ST (15U), CMK (5 U), CSS (10 U), ATP (0.05 eq.), CTP (0.05 eq.), phosphoenolpyruvate (2.4 eq.), 37 °C, 54 h, 83%.