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Pre-activation Based One-pot Synthesis of an α-(2,3)-Sialylated Core-Fucosylated Complex Type Bi-antennary N-Glycan Dodecasaccharide

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Abstract

Synthesis of N-glycans is of high current interests due to their important biological properties. A highly efficient convergent strategy based on the pre-activation method for assembly of the complex type core fucosylated bi-antennary N-glycan dodecasaccharide has been developed. Retrosynthetically, this extremely challenging target is broken down to three modules: a sialyl disaccharide, a glucosamine building block and a hexasaccharide diol acceptor. The sialyl disaccharide was easily obtained by selective activation of a new 5-*N*-trichloroacetyl protected sialyl donor in the presence of a thiogalactoside acceptor. The hexasaccharide diol module was produced by double mannosylation of a fucosylated tetrasaccharide acceptor, which in turn was generated by glycosylation of a α-fucosylated disaccharide with a β-mannose containing disaccharide donor. The union of the three modules was performed in one pot giving the fully protected dodecasaccharide in high yield. This synthesis is characterized by minimum protective group and aglycon adjustment on oligosaccharide intermediates, thus greatly enhancing the overall synthetic efficiency. The modular feature of this strategy suggests that this method can be readily adapted to the synthesis of a wide variety of N-glycan structures.

Introduction

Glycosylation is one of the major types of postsynthetic modification of mammalian proteins, which include the attachment of oligosaccharides to asparagine (N-glycan) and to serine or threorine (O-glycan).[1] Unlike protein synthesis, the addition of carbohydrates to proteins is not under direct genetic control, which often leads to heterogeneous glycosylation patterns on the same protein backbone. The identities of carbohydrate moieties can have a profound effect on biological functions of glycoproteins such as their immunogenecities, stabilities, and affinities to receptors.[1–4] However, despite intensive studies, detailed structure-function relationships have not been established in many cases, mainly due to the difficulties in accessing sufficient quantities of these glycan structures.

We became interested in the synthesis of α-(2,3)-sialylated core-fucosylated complex type bi-antennary N-Glycan dodecasaccharide **1**, which is one of the prototypical structures of mammalian N-glycans.[5] The dodecasaccharide **1** has been found on alpha fetoprotein (AFP) isolated from patients having hepatocellular carcinoma, a form of liver cancer. It is proposed

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Dedicated to Prof. Chi-Huey Wong on the Occasion of his 60th Birthday

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that the structures of N-glycans can be a much more reliable marker to differentiate benign and malignant liver diseases.[6,7] Similarly, studies on N-glycans linked to prostate specific antigen (PSA) suggest that α-(2,3) linked sialylated N-glycan on PSA can be potentially used to identify prostate cancer.[8] Dodecasaccharide **1** has also been found on the surface of erythropoietin.[9] The presence of fucose and sialic acid moieties in attached carbohydrates is believed to be important for the *in vivo* activity of erythropoietin.[10] There are intense interests in remodeling glycoproteins such as erythropoietin with homogeneous carbohydrate structures.[11] Thus, the ready availability of dodecasaccharide **1** can greatly facilitate the exploration of its glyco-biological functions and biomedical applications.

Although N-glycan structures can be assembled via several approaches,[12–23] total synthesis of dodecasaccharide **1** via chemical methods still presents a daunting task, which requires the construction of many difficult glycosyl linkages such as β-mannose, α-sialic acid, α-fucose as well as branching sequences. Moreover, the acid lability of the fucosyl linkage limits the scope of reagents that can be used. To date the only chemical total synthesis of the dodesaccharide in its fully protected form was accomplished by the Danishefsky group using a variety of glycosyl donors including glycals, thioglycosides, glycosyl sulfoxide, glycosyl fluoride and glycosyl phosphite. The tour-de-force total synthesis took 20 steps starting from protected monosaccharide building blocks with 13 steps and 3.1% overall yield for the longest linear sequence.[11,24] Herein, we report our studies on an efficient chemical synthesis of dodecasaccharide **1** via the pre-activation based one pot method predominantly using *p*-tolyl thioglycosides to simplify building block design.

Results and Discussion

In order to enhance the overall efficiency, our synthetic sequence was designed to minimize protective group manipulations and aglycon adjustments on intermediate oligosaccharides while achieving high stereoselectivities and yields. Previously, we discovered that the reaction of a mannosyl diol acceptor with a thiomannosyl donor in the absence of a participating protective group on its 2-*O* position led to products as an anomeric mixture.[26] Therefore, the strategic disconnections between glycan rings C/D and C'/D' in dodecasaccharide **2** were chosen, leading to ABC trisaccharide fragment **3** and the core DD'EFGH hexasaccharide **4** (Scheme 1).

For the installation of DD' units in **4**, to minimize the steric hindrance to the 3-*O* position of the E mannosyl unit, a popular strategy is to introduce the D' unit first followed by protective group adjustment and glycosylation on the 6-*O* position.[12,27] For higher efficiency, we decided to explore the possibility of double mannosylation[17,28] of tetrasaccharide **6** by the 2-*O* acetylated thiomannoside **5**. In tetrasaccharide **6**, the formation of β-mannosyl linkage between ring E and F is crucial. β-Mannosides can be accessed via a variety of methods, including SN_2 inversion of β glucosides[15,18,29] intramolecular aglycon delivery,[27,30, 31] insoluble silver salt promoted mannosyl halide reaction[32] and glycosylation by benzylidene bearing mannosyl donors.[33–35] The ground breaking work by Crich and coworkers has demonstrated high β selectivity can be obtained using benzylidene bearing mannosyl donors,[33–36] although the aglycon of the product typically needs to be further modified to convert it into a suitable donor for subsequent glycosylation to extend the chain.[20]

Our journey commenced from the preparation of the reducing end disaccharide **9** through fucosylation of diol acceptor **8**[37] by donor **7** (Scheme 2a).[38] Although exclusive α selectivity was obtained when donor **7** reacted with acceptors containing secondary hydroxyl groups using *N*-iodosuccinimide/triflic acid or *p*-TolSCl/AgOTf promoter systems,[39–41] α:β mixtures were generated with acceptors bearing primary hydroxyl groups under these

conditions. In order to enhance the α selectivity, we applied the *in situ* anomerization protocol ($CuBr_2$, tetrabutylammonium bromide)[27,28,42] first developed by Lemieux and coworkers.[43] Satisfactory yield (75 %) was obtained for the desired disaccharide **9**, with its regio- and stereo-chemistry confirmed by NMR analysis.

The formation of the key β mannosyl linkage using the benzylidene bearing thiomannosyl donor **10** was examined next. Activation of thiomannosyl donor **10α** in the absence of any acceptor (pre-activation)[44] by *p*-TolSOTf, formed *in situ* through the reaction of *p*-TolSCl and AgOTf,[44,45] was followed by addition of thioglycosyl acceptor **11**,[46] which formed disaccharide **12** in 57% yield (82% based on the amount of donor consumed) with an β:α ratio of 6:1 (Scheme 2b). The aglycon stereochemistry of the donor did not affect the reaction as donor **10β** gave identical result as **10α**. The newly formed glycosyl linkage in **12β** was confirmed by the one bond coupling constant between the anomeric carbon and proton of the mannose moiety (^1J (C_1,H_1) = 157 Hz).[47] Disaccharide **12β** was then directly used as a donor without any aglycon manipulations.

The reaction of disaccharide **12β** and **9** promoted by *p*-TolSCl/AgOTf produced the desired tetrasaccharide **13** despite the presence of the bulky fucosyl group on the 6-*O* position of acceptor **9**. However, treatment of **13** with $PhBCl_2$ and Et_3SiH for opening the benzylidene group[48] cleaved off the fucose unit. After several alternative reaction routes including postponing the introduction of fucose were explored, the most efficient way was determined to be adjusting the protective groups on disaccharide **12β**. The *p*-methoxybenzyl (PMB) group in **12β** can be oxidatively removed first followed by benzylidene opening.[19,49] We discovered that mixing $PhBCl_2$ and Et_3SiH with **12β** not only regioselectively converted the benzylidene group to 4-*O* benzyl but also removed the 3-*O*-PMB group simultaneously, leading to a diol which was subsequently protected as levulinoyl ester **14** (Scheme 2c, 77%). Reaction of the levulinoyl disaccharide donor **14** with fucosylated acceptor **9** proceeded smoothly in 86% yield. The two Lev moieties in the tetrasaccharide product **15** were selectively deprotected using hydrazine acetate giving diol **6** (Scheme 2c). Double mannosylation of **6** by donor **5** was explored next with our *p*-TolSCl/AgOTf promoter. A product with the same molecular weight as the desired hexasaccharide was isolated, which was found to be extremely labile to acid. NMR studies suggested that it was an orthoester. To overcome this problem, TMSOTf[50] was added at the end of the glycosylation reaction at 0 °C to rearrange the orthoester *in situ*. Gratifyingly, the desired hexasaccharide **16** was obtained in 77% yield following TMSOTf promoted rearrangement (Scheme 2d). Hexasaccharide **16** was then de-acetylated resulting in the core hexasaccharide diol acceptor **4**.

Next we focused our attention on the assembly of the branching sequence. Sialylation is known to be notoriously difficult due to the low reactivity of the sialyl donor and the challenge in stereochemical control.[51] The presence of the carboxylic acid moiety in sialic acid also necessitates additional consideration of protective group compatibility in synthetic design.[22] Recently, it was discovered that the replacement of the 5-*N*-acetyl group on a sialyl donor with an electron withdrawing protective group[52–61] significantly enhanced the reaction yield and stereoselectivity. Thus, we designed sialyl donor **17** bearing the 5-*N* trichloroacetyl (TCA) moiety, as *NH*-TCA can be converted to acetamide under a variety of reaction conditions including hydrogenolysis, radical reduction and basic cleavage followed by acetylation.[62–64] The trifluoroacetimidate aglycon leaving group[58,65,66] was chosen due to the possibility for its selective activation over a thioglycoside. This was realized by treating a mixture of donor **17** and thiogalactoside acceptor **18**[62–64] with a catalytic amount of TMSOTf leading to disaccharide **24** in 68% yield (Table 1, entry 1), which was benzoylated to give disaccharide **30**. The α sialyl linkage was confirmed by the three bond coupling constant between C_1 and H_{3ax} (^3J (C_1,H_{3ax}) = 5.8 Hz) of the sialic acid.[67] The scope of this sialylation reaction was examined next. A variety of acceptors including primary alkyl alcohol, carbohydrate hydroxyl

groups of galactose, galactosamine and glucosamine were sialylated in good yields and stereoselectivities, which represented some of the common natural sialyl linkages (Table 1). Acid labile benzylidene and isopropylidene groups were stable under the reaction condition. In addition to acceptor **18**, thioglycoside **23** also served as an excellent substrate for the reaction. This selective activation protocol is attractive as the resulting sialylated thioglycoside product can be used as a donor for further glycosylation without additional aglycon leaving group adjustment.

With all building blocks in hand, one pot glycosylation was performed using the pre-activation based protocol (Scheme 3).[39,44,46,68,69] Pre-activation of disaccharide **30** by *p*-TolSCl/AgOTf,[44,45] was rapidly achieved at −78 °C. Addition of the thioglycosyl acceptor **31**[39] to the reaction mixture produced trisaccharide **3**,[38] which underwent double glycosylation of hexasaccharide **4** in the same reaction flask leading to dodecasaccharide **2**. It took only four hours for this three-component one pot assembly process and the desired dodecasaccharide **2** was easily isolated from the reaction by flash column chromatography in an excellent 65% yield from disaccharide **30**.

Deprotection in complex oligosaccharide synthesis can be very challenging. Previously, Ito and coworkers reported that the removal of dichlorophthalimide (DCPhth) group in the presence of protected sialic acids did not lead to the desired product presumably due to cross reactivities of the methyl ester of sialic acids with reagents used to remove DCPhth and vice versa.[22] They designed an elegant approach by first converting all DCPhth groups into azide prior to the attachment of sialic acid, thus bypassing the cross reactivity problem. As an alternative, we explored the possibility of deprotecting sialic esters in the presence of phthalimide (Phth) groups. Treatment of the fully protected dodecasaccharide **2** with LiI in pyridine[54,70] at 110 °C cleanly cleaved the two methyl esters without affecting the Phth groups (Scheme 4). The resulting dicarboxylic acid was then added to hydrazine in refluxing ethanol, which was followed by selective acetylation in methanol to yield compound **32**. The usage of ethylene diamine instead of hydrazine to remove the Phth groups led to a lower yield. Finally, the fully deprotected dodecasaccharide **1** was produced by catalytic hydrogenolysis of **32** with $Pd(OH)_2/C$ (49% overall yield from **2**). The NMR and MS data of **1** are consistent with its structure.

Conclusion

We have achieved a stereocontrolled synthesis of the fucosylated complex type N glycan dodecasaccharide **2** via the pre-activation based chemoselective glycosylation method. This is the most complex oligosaccharide assembled by any one pot methods to date, which contains twelve monosaccharide units and several synthetically challenging linkages such as α-sialic acids, β-mannose, α-fucose as well as branching. Starting from monosaccharide building blocks, dodecasaccharide **2** was produced in a total of 10 steps with 7 steps and 11% overall yield for the longest linear sequence. The successful completion and high efficiency of this synthesis highlight the power of the pre-activation based approach. Due to the convergent modular approach taken, this strategy can be readily adapted towards the assembly of a library of N glycan sequences including bisecting GlcNAc and multi-antennary structures as well as un-natural N glycan analogs. We are currently synthesizing other N-glycan sequences and investigating the usage of these molecules as disease markers for early diagnosis.

Experimental Section

General Experimental Procedures

All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. All glycosylation reactions were performed in the presence of

molecular sieves, which were flame-dried right before the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. Chemicals used were reagent grade as supplied except where noted. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates; Compound spots were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}$ $4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230–400 Mesh). NMR spectra were referenced using Me_4Si (0 ppm), residual $CHCl_3$ (δ ^{1}H-NMR 7.26 ppm, ^{13}C-NMR 77.0 ppm). Peak and coupling constant assignments are based on ^{1}H-NMR, ^{1}H-^{1}H gCOSY and (or) ^{1}H-^{13}C gHMQC and ^{1}H-^{13}C gHMBC experiments. All optical rotations were measured using the sodium D line. ESI mass spectra were recorded using ESQUIRE Ion Trap LC/MS. High-resolution mass spectra were recorded on a Micromass electrospray mass spectrometer equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode.

Characterization of anomeric stereochemistry

The stereochemistries of the newly formed glycosidic linkages in oligosaccharides (except sialyl and mannosyl linkages) are determined by ^3J (H_1, H_2) through ^{1}H-NMR and/or ^1J (C_1, H_1) through gHMQC 2-D NMR (without ^{1}H decoupling). Smaller coupling constants of $^3J_{H1.H2}$ (around 3 Hz) indicate 1,2-cis α linkages and larger coupling constants ^3J (H_1,H_2) (7.2 Hz or larger) indicate 1,2-trans β linkages. This can be further confirmed by $^1J_{C1,H1}$ (~170 Hz) for α linkages and ^1J (C_1,H_1) (~160 Hz) for β linkages.[47] For the mannosyl linkages, one bond coupling constants between C_1 and H_1 were measured by gHMQC 2-D NMR. ^1J (C_1, H_1) ~170 Hz indicates α-mannosyl linkages and ^1J (C_1, H_1) ~160 Hz suggests β linkages. For the sialyl linkages, three bond coupling constants between C_1 and H_{3ax} of the sialic acid was measured by gHMBC 2-D NMR. ^1J (C_1, H_{3ax}) ~ 5.8 Hz indicates α-sialyl linkage and ^1J (C_1, H_{3ax}) ~ 1Hz suggests β linkage.

Benzyl 2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→6)-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)—A mixture of $CuBr_2$ (0.9 g, 6.27 mmol), Bu_4NBr (1.31 g, 4.06 mmol), and molecular sieves 4A (1.2 g) in CH_2Cl_2/DMF (2:1, 18 mL) was stirred and cooled over an ice-water bath. A solution of compound **7** (1.07 g, 1.98 mmol) and **8** (0.9 g, 1.84 mmol) in CH_2Cl_2 (12 mL) was added dropwise and the mixture was stirred for 17 h from 0°C to room temperature. The resulting mixture was quenched with aq. $NaHCO_3$, diluted with EtOAc, and filtered through Celite. The filtrate was washed successively with aq. $NaHCO_3$ and brine, and dried over Na_2SO_4. The organic layer was then evaporated in vacuo. The residue was purified by flush column chromatography (hexanes: EtOAc = 2:1) to afford **9** (1.25 g, 75%). $[\alpha]_D^{20}$ = −78.1, (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 7.79 (bs, 1 H), 7.67 (bs, 2 H), 7.52 (bs, 1 H), 7.42-7.40 (m, 2 H), 7.38-7.22 (m, 14 H), 7.08-6.98 (m, 6 H), 6.89-6.86 (m, 3 H), 5.12-5.08 (m, 1 H), 4.98 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.87 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.84 (d, ^3J (H, H)= 12 Hz, 1 H), 4.81 (d, ^3J(H, H) = 3.6 Hz, 1 H), 4.78 (d, ^3J = 12 Hz, 1 H), 4.74-4.70 (m, 2 H), 4.68 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.65 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.46 (d, ^3J (H, H) = 12 Hz, 1 H), 4.41 (d, ^3J (H, H) = 12.6 Hz, 1 H), 4.16-4.14 (m, 2 H), 4.08-4.06 (dd, ^3J (H, H) = 3.6, 10.2 Hz, 1 H), 4.00-3.97 (m, 2 H), 3.94-3.90 (m, 2 H), 3.88-3.84 (dd, ^3J (H, H) = 4.2, 10.8 Hz, 1 H), 3.70-3.67 (m, 2 H), 3.57-3.53 (m, 1 H), 1.12 (d, ^3J ()H, H = 6.6 Hz, 3 H). ^{13}C NMR (150 Hz, $CDCl_3$): δ = 138.80, 138.79, 138.7, 138.3, 137.3, 133.8, 131.9, 128.72, 128.66, 128.6, 128.52, 128.47, 128.34, 128.25, 128.24, 128.18, 128.1, 127.9, 127.83, 127.76, 127.5, 123.3, 98.9 (C_1 fucose, ^1J (C_1, H_1) = 172 Hz), 97.6 (C_1 glucosamine, ^1J (C_1, H_1) = 161 Hz), 79.5, 77.8, 77,7, 76.6, 75.1, 74.4, 74.4, 74.1, 73.2, 73.2, 60.6, 55.8, 16.7. Correlations between C_1 of fucose and H_6 and H_6, of the glucosamine unit were observed in gHMBC 2-D NMR spectrum confirming the fucose is linked to 6-*O* of the glucosamine. HRMS: m/z: calcd for $C_{55}H_{55}NO_{11}Na$: $[M+Na]^+$, 928.3673, found: 928.3654.

p-Tolyl 2-O-benzyl-4,6-O-benzylidene-3-O-p-methoxybenzyl-1-thio-α-D-mannopyranoside (10α)—n-Bu_2SnO (3.72 g, 14.6 mmol) was added to a solution of *p*-tolyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[71] (4.9 g, 13.6 mmol) in toluene (65 mL) and the resulting mixture was refluxed using a Dean-Stark apparatus for 3 h. The reaction mixture was cooled to room temperature and *p*-methoxybenzyl chloride (2.8 mL, 20.4 mmol) and n-Bu_4NI (1.01 g, 2.7 mmol) were added. The reaction mixture was refluxed again for 2 h followed by addition of H_2O (2 mL) to quench the reaction. After removing the solvents, the residue was purified by column chromatography (silica gel, hexanes: EtOAc 7 : 3) to give *p*-tolyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (5.96 g, 92%). This product was dissolved in anhydrous DMF (50 mL) and NaH (95%, 457 mg, 18 mmol) was added to the mixture. After 1 h at room temperature, benzyl bromide (2.47g 18 mmol) was added to the mixture and stirred overnight. The reaction mixture was diluted with EtOAc (300 mL), washed with water, 10% of NH_4Cl and brine and dried over anhydrous Na_2SO_4. The organic phase was evaporated and the residue was purified by flash column chromatography (silica gel, hexanes: EtOAc = 4 : 1) to give compound **10α** (6.3 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.58-7.53 (m, 2 H), 7.44-7.27 (m, 12 H), 7.15-7.11 (m, 2 H), 6.93-6.87 (m, 2 H), 5.67 (s, 1 H), 5.46 (d, ^3J (H, H) = 1.8 Hz, 1 H), 4.76 (d, ^3J (H, H) = 12 Hz, 1 H), 4.73 (s, 2 H), 4.62 (d, ^3J (H, H) = 12 Hz, 1 H), 4.36-4.30 (m, 2 H), 4.27-4.22 (dd, ^3J (H, H) = 10.8, 4.2 Hz, 1 H), 4.40-4.10 (m, 1 H), 4.10-3.97 (m, 1 H), 3.94- 3.88 (m, 1 H), 3.82 (s, 3 H), 2.35 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$): δ =159.5, 138.2, 138.1, 137.9, 132.6, 130.7, 130.2, 130.16, 129.6, 129.1, 128.7, 128.5, 128.4, 128.1, 126.4, 114.0, 101.7, 87.7, 79.3, 78.2, 76.0, 73.2, 73.0, 68.8, 65.8, 21.4. HRMS: m/z calcd for $C_{35}H_{36}O_6SNa$, $[M+Na]^+$, 607.2130, found 607.2089.

p-Tolyl 2-O-benzyl-4,6-O-benzylidene-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12β)—A mixture of donor **10α** (226 mg, 387 μmol) and TTBP (180 mg, 726 μmol) in dry CH_2Cl_2 (20 mL) was stirred with activated molecular sieves 4Å (800 mg) for 30 minutes at room temperature and then cooled to −75°C. AgOTf (118 mg, 0.46 mmol) in acetonitrile (300 μL) was added directly to the solution without touching the flask wall. After 10 minutes, orange colored *p*-TolSCl (55 μl, 387 μmol) was added via a microsyringe. Since the reaction temperature was lower than the freezing point of *p*-TolSCl, *p*-TolSCl was added directly into the reaction mixture to prevent it from freezing on the flask wall. The characteristic yellow color of *p*-TolSCl in the reaction solution dissipated rapidly within a few seconds indicating depletion of *p*-TolSCl. After the donor was completely consumed according to TLC analysis (about 5 minutes at −78 °C), a solution of acceptor **11** (345 mg, 579 μmol) in CH_2Cl_2 (6 mL) was added slowly along the flask wall. The reaction mixture was allowed to warm up to −20°C and quenched by using triethylamine. The reaction mixture was diluted with CH_2Cl_2 and filterer through Celite. The Celite was washed extensively with CH_2Cl_2 until TLC indicated no more organic compounds in the filtrate. The organic layer was then extracted with satd. $NaHCO_3$ solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. After flash column chromatography (hexanes : EtOAc = 7 : 3), the α and β mixture (231 mg, 82% based on consumption donor, recover donor 70 mg) was obtained. The pure **12α** (33 mg) and **12β** (198 mg) (α:β = 1:6) were isolated by column chromatography using 3% acetone in toluene. $[\alpha]_D^{20}$ = +19.4, (c = 1.0 in $CHCl_3$). Data for **12β**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83-7.80 (m, 1H), 7.71-7.64 (m, 2H), 7.63-7.60 (m, 1H), 7.48-7.42 (m, 4H), 7.38-7.30 (m, 7H), 7.29-7.21 (m, 8H), 7.01-6.98 (m, 2H), 6.92-6.89 (m, 2H), 6.86-6.80 (m, 5H), 5.51 (s, 1H), 5.44 (d, ^3J (H, H) = 10.2 Hz, 1 H), 4.90-4.82 (m, 3H), 4.65 (d, ^3J (H, H) = 12 Hz, 1 H), 4.58 (d, ^3J (H, H) = 12 Hz, 1 H), 4.55 (s, 1H), 4.51 (d, ^3J (H, H) = 12 Hz, 1 H), 4.40 (t, 2H, *J* = 12.6 Hz), 4.25-4.22 (m, 1H), 4.19-4.15 (m, 2H), 4.05 (t, 1H, *J* = 9.6 Hz), 3.96 (t, 1H, *J* = 9.6 Hz), 3.78 (s, 3H), 3.75 (d, ^3J (H, H)= 3 Hz, 1 H), 3.68 (d, ^3J (H, H) = 12 Hz, 1 H), 3.59-3.51 (m, 3H), 3.43 (dd, 1H, *J* = 3.0, 10.2 Hz), 3.16-3.12 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.4, 167.5, 159.4, 138.9, 138.8, 138.5, 138.2, 137.9, 134.1,

134.1, 133.9, 133.7, 131.9, 131.8, 130.8, 129.8, 129.4, 129.3, 129.1, 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, 127.2, 126.3, 123.7, 123.5, 113.9, 102.3 (C_1 mannose, ^1J (C_1, H_1) = 154 Hz), 101.6, 83.4 (C_1 glucosamine, ^1J (C_1, H_1) = 157 Hz), 79.5, 79.4, 78.9, 78.2, 77.3, 75.2, 75.1, 73.8, 72.6, 68.9, 68.8, 67.6, 55.5, 55.0, 21.4; HRMS: m/z: calcd for $C_{63}H_{61}NNaO_{12}S$ $[M+Na]^+$: 1078.3812, found: 1078.3807.

p-Tolyl 2,4-di-O-benzyl-4,6-di-O-levulinoyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (14)—A solution of compound **12β** (210 mg, 0.20 mmol) in CH_2Cl_2 (6 mL) was stirred with activated molecular sieves (MS AW 300) (600 mg) and cooled to −78°C. Triethylsilane (130 μL, 0.20 mmol) was added followed by dichlorophenyl borane (105 μL, 0.20 mmol). The reaction was stirred for 6 h and quenched by adding triethyl amine. The reaction mass was diluted with CH_2Cl_2 and extracted with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Diol *p*-tolyl 2,4-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (167 mg) was obtained from flash column chromatography (EtOAc: hexanes, 1: 1) in 89% yield. $[\alpha]_D^{20}$ = + 40.6, (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83-7.80 (m, 1H), 7.71-7.61 (m, 3H), 7.40-7.25 (m, 16H), 7.04-7.01 (m, 2H), 6.92-6.88 (m, 5H), 5.43 (d, ^3J (H, H) = 10.2 Hz, 1 H), 4.98 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.86 (d, ^3J (H, H) = 12.6 Hz, 1 H), 4.81-4.79 (d, ^3J (H, H) = 10.8 Hz, 1 H), 4.66-4.58 (m, 3H), 4.53 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.52-4.48 (d, ^3J (H, H) = 12 Hz, 1 H), 4.25-4.17 (m, 2H), 3.99-3.96 (t, ^3J (H, H) = 8.4 Hz, 1 H), 3.80-3.69 (m, 3H), 3.64-3.61 (m, 2H), 3.51-3.47 (m, 1H), 3.44-3.39 (m, 2H), 3.14-3.11 (m, 1H), 2.28 (s, 3H), 1.86-1.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5, 167.9, 138.8, 138.6, 137.8, 137.5, 136.6, 136.2, 134.3, 134.2, 134.1, 133.9, 133.2, 132.1, 131.9, 131.6, 129.9, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 123.9, 123.5, 123.3, 96.8, 96.7, 78.776.3, 75.5, 74.7, 74.6, 74.4, 74.0, 73.6, 70.9, 69.9, 62.5, 60.7, 56.5, 56.1, 27.2; ESI-MS: m/z: calcd for $C_{55}H_{55}NO_{11}SNa$: $[M+Na]^+$, 960.35, found: 960.36. To a solution of *p*-tolyl 2,4-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (167 mg, 0.178 mmol) in CH_2Cl_2 (5 mL), levulinic acid (73 μL, 0.712 mmol), *N*-ethyl *N*,*N*-dimethylaminopropyl carbodiimide hydrochloride (EDC) (136.5 mg, 0.712 mmol) and *N*,*N*-dimethylamino pyridine (4.4 mg, 0.036 mmol) were added and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and extracted with satd. $NaHCO_3$ solution. The organic layer was evaporated to dryness and compound **14** (173 mg) was obtained in 86 % yield after flash column chromatographic purification (hexanes : EtOAc = 2:1). $[\alpha]_D^{20}$ = +17.8, (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 7.78-7.74 (m, 1 H), 7.68-7.62 (m, 2H), 7.64-7.61 (m, 1 H), 7.42-7.24 (m, 17 H), 6.99-6.97 (m, 1 H), 6.83-6.81 (m, 2 H), 6.73-6.69 (m, 3 H), 5.39 (d, ^3J (H, H) = 10.2 Hz, 1 H), 4.85 (d, ^3J (H, H) = 12 Hz, 1H), 4.80 (d, ^3J (H, H) = 12 Hz, 1 H), 4.77 (dd, ^3J (H, H) = 3.0, 9.6 Hz, 1 H), 4.70 (d, ^3J (H, H) = 12 Hz, 1 H), 4.67 (s, 2 H), 4.54 (d, ^3J (H, H) = 12 Hz, 1 H), 4.53 (d, ^3J (H, H) = 12 Hz, 1 H), 4.42 (d, ^3J (H, H) = 12.6 Hz, 1 H), 4.32 (dd, ^3J (H, H) = 4.8, 12 Hz, 1 H), 4.24-4.10 (m, 4 H), 4.02 (t, ^3J (H, H)= 9.6 Hz, 1 H), 3.92 (d, ^3J (H, H) = 3.0 Hz, 1 H), 3.87 (t, ^3J (H, H) = 9.6 Hz, 1 H), 3.77 (d, ^3J (H, H) = 10.8 Hz, 1 H), 3.68 (dd, ^3J (H, H) = 3.0, 12 Hz, 1 H), 3.55 (d, ^3J (H, H) =.6 Hz, 1 H), 3.38-3.33 (m, 1 H), 2.67-2.33 (m, 8 H), 2.25 (s, 3 H), 2.12 (s, 3 H), 2.01 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.7, 206.2, 172.3, 172.0, 167.9, 167.2, 138.7, 138.6, 138.2, 137.89, 137.85, 133.8, 133.6, 129.5, 128.5, 128.4, 128.2, 127.94, 127.89, 127.78, 127.75, 127.71, 127.66, 127.51, 126.8, 123.3, 123.2, 100.7, 83.18, 79.0, 78.9, 77.9, 76.2, 76.1, 74.8, 74.7, 74.6, 73.4, 73.07, 73.05, 68.5, 63.1, 54.7, 37.74, 37.69, 29.8, 29.7, 27.88, 27.85, 21.1. MALDI-MS: m/z: calcd for $C_{65}H_{67}NO_{15}SNa$: $[M+Na]^+$, 1156.42, found: 1156.61.

Benzyl 2,4-di-O-benzyl-3,6-di-O-levulinoyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-(2,3,4-tri-O-benzyl-α-

L-fucopyranosyl-(1→6))-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (15)—The mixture of donor **14** (100 mg, 88 μmol), acceptor **9** (64 mg, 70.4 μmol) and freshly activated MS 4Å (600 mg) in dry CH_2Cl_2 (6 mL) was stirred for 30 min at room temperature, and cooled down to −70°C followed by the addition of AgOTf (68 mg, 264 μmol) in anhydrous acetonitrile (0.1 mL) directly to the solution without touching the wall of reaction flask. After 5 min, *p*-TolSCl (12.8 μL, 88 μmol) was added via a microsyringe. The reaction mixture was stirred for 1.5 h until the temperature reached −20°C and triethylamine (30 μL) was added. The mixture was diluted with CH_2Cl_2 (50 mL) filtered through Celite. The filtrate was concentrated and purified by flash column chromatography (hexanes : EtOAc = 3 : 2) to give compound **15** (116 mg, 86%). $[\alpha]_D^{20}$ = −28.0 (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 7.85-7.81 (m, 1 H), 7.80-7.76 (m, 1 H), 7.72-7.60 (m, 4H), 7.59-7.56 (m, 1H), 7.52-7.40 (m, 6H), 7.36-7.20 (m, 18H), 7.17-7.12 (m, 3H), 7.05-7.02 (m, 3 H), 6.98-6.94 (m, 4 H), 6.92-6.88 (m, 2 H), 6.80-6.71 (m, 8 H), 5.52 (d, 1 H, *J* = 8.4 Hz), 4.92-4.70 (m, 10H), 4.68 (s, 1 H), 4.66-4.47 (m, 8 H), 4.39 (d, ^3J (H, H) = 12.6 Hz, 1 H), 4.33 (d, ^3J (H, H) = 12 Hz, 1 H), 4.30-4.10 (m, 8 H), 4.09-4.04 (m, 1 H), 3.95-3.92 (m, 3 H), 3.84-3.76 (m, 4 H), 3.68 (d, ^3J (H, H) = 9.0 Hz, 1 H), 3.60-3.56 (m, 2 H), 3.37-3.30 (m, 2 H), 3.22 (d, ^3J (H, H) = 9.6 Hz, 1 H), 2.62-2.27 (m, 8 H), 2.10 (s, 3 H), 1.93 (s, 3 H), 0.95 (d, ^3J (H, H) = 5.4 Hz, 3 H). ^{13}C NMR (150 Hz, $CDCl_3$): δ = 206.9, 206.4, 172.5, 172.3, 168.4, 168.0, 167.94, 167.85, 139.3, 139.2, 139.1, 139.00, 138.86, 138.85, 138.2, 138.1, 137.3, 134.1, 133.9, 132.1, 131.9, 131.8, 128.9, 128.8, 128.68, 128.67, 128.6, 128.40, 128.35, 128.3, 128.13, 128.11, 128.08, 128.04, 128.00, 127.99, 127.9, 127.81, 127.77, 127.74, 127.71, 127.6, 127.50, 127.48, 127.2, 127.1, 123.7, 123.5, 100.8, 97.1, 96.92, 96.85, 79.8, 79.2, 77.9, 77.1, 76.9, 76.5, 76.2, 75.4, 75.3, 75.0, 74.96, 74.7, 74.6, 74.6, 73.9, 73.5, 73.46, 73.3, 73.28, 72.7, 70.1, 68.2, 66.2, 64.0, 63.3, 60.6, 56.8, 56.1, 38.0, 37.99, 30.0, 29.9, 28.2, 28.1, 16.6. HRMS: m/z: calcd for $C_{113}H_{114}N_2O_{26}Na$, $[M+Na]^+$, 1938.7592, found: 1938.7534.

Benzyl 2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→6))-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6)—To a solution of **15** (300 mg, 0.157 mmol) in CH_2Cl_2/MeOH (1:1, 8 mL), hydrazine acetate (60 mg, 0.65 mmol) was added and stirred at room temperature for 90 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and extracted with satd. NH_4Cl solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The compound **6** (217 mg, 81%) was isolated by flash column chromatography (hexanes: EtOAc = 2 : 3). $[\alpha]_D^{20}$ = −16.5 (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.92-7.88 (m, 1 H), 7.84-7.78 (m, 1 H), 7.76-7.62 (m, 5 H), 7.58-7.55 (m, 1H), 7.52-7.42 (m, 1H), 7.40-7.22 (m, 22H), 7.08-7.00 (m, 7H), 6.98-6.90 (m 7H), 6.81-6.76 (m, 3H), 5.64 (d, ^3J (H, H) = 8.4 Hz, 1 H), 5.00-4.90 (m, 7 H), 4.86-4.76 (m, 4 H), 4.67-4.60 (m, 5 H), 4.57-4.51 (m, 2 H), 4.46-4.26 (m, 6 H), 4.24-4.16 (m, 3 H), 4.10-4.00 (m, 3 H), 3.93 (d, ^3J (H, H) = 10.2 Hz, 1 H), 3.86 (d, ^3J (H, H) = 9.6 Hz, 1 H), 3.77 (d, ^3J (H, H) = 10.2 Hz, 1 H), 3.75 (d, ^3J (H, H) = 11.4 Hz, 1 H), 3.69-3.64 (m, 2 H), 3.60 (d, ^3J (H, H) = 3.6 Hz, 1 H), 3.55-3.49 (m, 1 H), 3.45-3.40 (m, 3 H), 3.29 (d, ^3J (H, H)= 9.6 Hz, 1 H), 3.18-3.13 (m, 1 H), 2.34 (d, ^3J (H, H) = 9.0 Hz, 1 H), 1.02 (d, ^3J (H, H) = 6.6 Hz, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.7, 168.1, 168.0, 167.9, 139.2, 139.1, 139.0, 138.9, 138.8, 138.5, 138.3, 1378.0, 137.3, 134.3, 134.1, 133.9, 132.1, 132.0, 131.7, 128.9, 128.74, 128.69, 128.66, 128.64, 128.61, 128.4, 128.38, 128.3, 128.26, 128.2, 128.1, 128.08, 128.0, 127.94, 127.81, 127.8, 127.77, 127.7, 127.64, 127.6, 127.4, 127.2, 123.9, 123.5, 101.4, 97.0, 96.99, 96.9, 79.8, 79.2, 79.0, 77.8, 77.4, 76.8, 76.2, 75.4, 75.37, 75.36, 75.3, 75.0, 74.9, 74.7, 74.67, 74.4, 73.7, 73.69, 73.4, 72.7, 70.2, 68.1, 66.3, 64.0, 62.6, 60.7, 56.7, 56.1, 16.6. HRMS: m/z: calcd for $[M+Na]^+$, $C_{103}H_{102}N_2O_{22}Na$, 1742.6855, found: 1742.6771.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3))-2,4-di-O-benzyl-β-D-

mannopyranosyl-(1→4)-O-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→6))-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16)—The mixture of donor **5** (26 mg, 45 μmol), acceptor **6** (17 mg, 15.7 μmol) and freshly activated MS 4Å (600 mg) in anhydrous CH_2Cl_2 (6 mL) was stirred for 30 minutes at room temperature and cooled down to −78°C followed by the addition of AgOTf (34 mg, 133 μmol) in anhydrous acetonitrile (0.1 mL) directly to the solution without touching the wall of reaction flask. After 5 min, *p*-TolSCl (6.8 μL, 45 μmol) was added via a microsyringe. The reaction mixture was stirred for 1.5 h until the temperature reached 0°C, which was followed by addition of TMSOTf (2 μL). The reaction was stirred further for 1.5 h from 0°C to room temperature and triethylamine (25 μL) was added. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through Celite. The Celite was washed with CH_2Cl_2 until no organic product was present in the filtrate by TLC. The filtrate was combined, concentrated and purified by flash column chromatography (hexanes : EtOAc = 3 : 2) to give compound **16** (32 mg, 76%). $[\alpha]_D^{20}$ = + 4.5 (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 7.76-7.71 (m, 2 H), 7.69-7.58 (m, 4 H), 7.50-7.45 (m, 4 H), 7.41-7.16 (m, 42 H), 7.14-7.01 (m, 11 H), 6.99-6.93 (m, 4 H), 6.93-6.88 (m, 2 H), 6.78-6.68 (m, 6 H), 6.62-6.58 (m, 2 H), 5.49-5.46 (m, 2 H), 5.30-5.28 (m, 1 H), 5.14 (s, 1 H), 4.93 (d, ^3J (H, H) = 8.4 Hz, 1 H), 4.92-4.80 (m, 10 H), 4.77 (d, ^3J (H, H)= 10.8 Hz, 1 H), 4.71 (d, ^3J (H, H)= 11.4 Hz, 1 H), 4.67 (d, ^3J (H, H)= 11.4 Hz, 1 H), 4.64-4.52 (m, 9 H), 4.50-4.43 (m, 4 H), 4.42-4.33 (m, 5 H), 4.29 (d, ^3J (H, H)= 12.6 Hz, 1 H), 4.28-4.12 (m, 6 H), 4.10-4.04 (m, 2 H), 3.99-3.87 (m, 6 H), 3.85-3.70 (m, 7 H), 3.67-3.56 (m, 7 H), 3.5-3.48 (m, 2 H), 3.32-3.28 (dd, ^3J (H, H) = 3.0, 10.2 Hz, 1 H), 3.21 (d, ^3J (H, H) = 9.6 Hz, 1 H), 3.17-3.13 (m, 1 H), 2.10 (s, 3 H), 1.80 (s, 3 H), 0.96 (d, ^3J (H, H)= 6.6 Hz, 3 H). ^{13}C NMR (150 Hz, $CDCl_3$): δ = 170.4, 169.9, 168.8, 167.78, 139.2, 139.0, 138.9, 138.85, 138.6, 138.3, 138.2, 138.15, 137.8, 137.7, 137.3, 133.9, 133.7, 132.1, 131.9, 131.7, 128.9, 128.7, 128.61, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 127.3, 127.1, 127.0, 123.7, 123.4, 102.0, 99.7, 99.6, 98.5, 97.1, 96.8, 81.5, 79.8, 79.5, 78.9, 78.4, 77.98, 77.9, 76.7, 76.4, 75.7, 75.3, 75.2, 75.19, 75.0, 74.9, 74.85, 74.8, 74.7, 74.69, 74.67, 74.6, 74.5, 74.4, 74.2, 74.0, 73.7, 73.6, 73.5, 73.46, 72.6, 72.5, 72.0, 71.6, 71.4, 70.1, 70.0, 69.0, 68.9, 68.34, 68.129, 66.7, 66.2, 65.3, 64.0, 56.8, 56.0, 24.9, 21.3, 21.2, 16.7. MALDI-MS: m/z: calcd for $C_{161}H_{162}N_2O_{34}Na$, $[M+Na]^+$, 2690.10, found: 2690.49.

Benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3))-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→6))-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4)—The solution of compound **16** (242 mg, 90.6 μmol) in methanol (5 mL) and CH_2Cl_2 (2 mL) was stirred with NaOMe (12 mg, 0.5 mmol) at room temperature for 3 h, which was then neutralized to pH 5-6 with acetic acid. After evaporating the solvent, the residue was purified by flash column chromatography (hexanes: EtOAc = 1 : 1) to give **4** (184 mg, 79%). $[\alpha]_D^{20}$ = +4.0 (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 7.77-7.83 (m, 2 H), 7.72-7.61 (m, 4 H), 7.56-7.47 (m, 4 H), 7.45-7.39 (m, 4 H), 7.36-7.10 (m, 53 H), 7.09-7.03 (m, 2 H), 7.02-6.97 (m, 4 H), 6.94-6.91 (m, 2 H), 6.80-6.73 (m, 6 H), 6.70-6.66 (m, 2 H), 5.52 (d, 1 H, *J* = 8.4 Hz), 5.19 (s, 1 H), 5.00-4.82 (m, 11 H), 4.79-4.73 (m, 2 H), 4.67-4.45 (m, 15 H), 4.45-4.37 (m, 3 H), 4.36-4.23 (m, 6 H), 4.21-4.10 (m, 4 H), 4.04-3.96 (m, 4 H), 3.92-3.50 (m, 20 H), 3.35 (dd, ^3J (H, H) = 2.4, 10.8 Hz, 1 H), 3.28 (d, ^3J (H, H)= 9.6 Hz, 1 H), 3.21 (d, ^3J (H, H)= 9.6 Hz, 1 H), 2.38 (bs, 1 H), 2.07 (bs, 1 H), 0.99 (d, ^3J (H, H) = 6.6 Hz, 3 H). ^{13}C NMR (150 Hz, $CDCl_3$): δ = 168.3, 168.0, 139.2, 139.2, 139.15, 139.1, 138.9, 138.8, 138.75, 138.6, 138.4, 138.3, 138.2, 138.16, 138.1, 137.3, 134.1, 133.8, 132.1, 131.7, 128.9, 128.73, 128.70, 128.69, 128.67, 128.6, 128.5, 128.48, 128.46, 128.4, 128.3, 128.2, 128.16, 128.1, 128.09, 128.07, 128.04, 128.02, 127.98, 127.95, 127.93, 127.89, 127.85, 127.8, 127.76, 127.74, 127.72, 127.67, 127.6, 127.57, 127.3, 127.1, 127.06, 123.7, 123.4, 101.8, 101.5, 100.1,

97.2, 97.1, 96.8, 81.9, 80.3, 80.0, 79.56, 79.3, 78.8, 77.9, 76.5, 75.8, 75.5, 75.3, 75.1, 75.07, 75.0, 74.8, 74.75, 74.7, 74.6, 74.5, 74.4, 74.3, 74.0, 73.7, 73.5, 73.47, 72.7, 72.3, 72.2, 71.8, 71.4, 70.1, 69.0, 68.9, 68.2, 67.9, 66.7, 66.2, 64.0, 56.8, 56.1, 16.7. HRMS: m/z: calcd for $C_{157}H_{158}N_2O_{32}Na$, $[M+Na]^+$, 2607.0730, found: 2607.0669.

Methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-D-glycero-α-D-galacto-2-nonulopyranosonate-2-(N-phenyl)-trifluoroacetimidate (17)—To a solution of methyl (*p*-tolyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-2-nonulopyranosid)onate (1.8 g, 3 mmol)[67] in methanol (30 mL) was added methanesulfonic acid (1.95 mL, 30 mmol) at room temperature. After being stirred at 60°C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated *in vacuo*. The residue was used for next reaction without further purification. Methyl trichloroacetate (3.57 mL, 30 mmol) and triethylamine (0.84 mL, 6 mmol) were added to a solution of the residue in methanol (30 mL) at 0°C. The reaction mixture was stirred at room temperature for 6 h and all volatile solvents were removed. The remaining residue was dissolved in pyridine (10 mL), to which acetic anhydride (2 mL) and a catalytic amount of DMAP were added at 0°C. After being stirred at room temperature overnight, the reaction mixture was diluted with ethyl acetate, washed with water, 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc : hexanes = 2 : 3) to give methyl (*p*-tolyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-trichloroacetamido-D-glycero-D-galacto-2-nonulopyranosid) onate (1.40 g, 2 mmol, 67%). To a solution of this thioglycoside (1.4 g, 2 mmol) in acetone (20 mL) and water (1 mL) was added NBS (930 mg, 5.23 mmol).[72] After being stirred for 0.5 h at room temperature, the solution was concentrated. The residue was diluted with CH_2Cl_2 and washed with saturated aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated leading to methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trichloroacetamido-β-D-glycero-D-galacto-2-nonulopyranosonate. To a solution of methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trichloroacetamido-D-glycero-D-galacto-2-nonulopyranosonate in acetone was added K_2CO_3 (3 eq) and *N*-phenyl trifluoroacetimidoyl chloride[66] (10 eq). After stirred at room temperature for 3 h, the mixture was filtered and then concentrated. The residue was subject to chromatography on silica gel column (hexanes: EtOAc = 3 : 2, containing 0.5% of triethylamine) leading to imidate **17** (α : β= 1 : 2, 79%). α : β mixture ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32-7.25 (m, 1.5 H), 7.15-7.08 (m, 1.5 H), 6.83-6.77 (m, 1.5 H), 6.76-6.61 (m, 3 H), 5.50-5.47 (m, 1 H, β), 5.46-5.40 (m, 1 H, β), 5.36-5.33 (m, 0.5 H, α), 5.33-5.28 (m, 0.5 H, α), 5.18-5.13 (m, 1 H, β), 4.91-4.84 (m, 0.5 H, α), 4.55-4.49 (m, 1 H, β), 4.46-4.41 (m, 1 H, β), 4.38-4.32 (m, 0.5 H, α), 4.29-4.23 (m, 0.5 H, α), 4.18-4.12 (m, 2 H, β), 4.10-3.97 (m, 1 H, α), 2.94-2.87 (dd, ^3J (H, H) = 13.6, 4.8 Hz, β, H-3eq, 1 H), 2.83-2.77 (dd, ^3J (H, H) = 4.8, 13.6 Hz, 3-Heq,α, 0.5 H), 2.38-2.30 (dd, ^3J (H, H)= 10.8, 13.6 Hz, 3-H_{ax},α, 0.5 H), 2.26-2.18 (dd, ^3J (H, H) = 11.6, 13.6 Hz, 3-Hax, β 1 H), 2.20 (s, 1.5 H, α), 2.19 (s, 3 H, β), 2.09, 2.05, 1.84, 1.62 (each 3 H, each s, OAc from β isomer), 2.04 (s, 3 H, from 2 OAc of α isomer), 1.98 (s, 1.5 H, α), 1.96 (1.5 H, α). HRMS: m/z calcd for $C_{28}H_{30}Cl_3F_3N_2O_{13}Na$, $[M+Na]^+$, 787.0663, found 787.0626.

p-Tolyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-D-glycero-α-D-galacto-2-nonulopyranosonate-(2→3))-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (24)—A mixture of the donor **17** (514 mg, 0.67 mmol) and acceptor **18** (411 mg, 1.1 mmol) and MS 3Å in anhydrous CH_2Cl_2/CH_3CN (1 : 1, 30 mL) was stirred at room temperature under N_2 for 30 min and then cooled to −65°C. TMSOTf (0.2 eq) was added. After stirred at −65°C for 2 h, the mixture was warmed up to room temperature and quenched with triethylamine (50 μL). The resulting mixture was filtered and concentrated. The residue was chromatographed on a silica gel column to afford the desired coupling product **24** (346 mg, 68% based on acceptor consumed, recover acceptor 210 mg). $[\alpha]_D^{20}$ = −11.2, (c

= 1.0 in $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.61-7.57 (m, 2 H), 7.38-7.30 (m, 5 H), 7.07-7.04 (m, 2 H), 6.52 (d, ^3J (H, H) = 9.6 Hz, 1 H), 5.43-5.39 (m, 1 H), 5.32 (s, 1 H), 5.27-5.24 (dd, 1 H, J = 1.8, 9.6 Hz), 5.11-5.05 (m, 1 H), 4.63 (d, ^3J (H, H) = 9.6 Hz, 1 H), 4.32 (dd, ^3J (H, H) = 1.2, 12 Hz, 1 H), 4.29-4.24 (m, 2 H), 4.22 (dd, ^3J (H, H) = 1.8, 10.8 Hz, 1 H), 4.10-4.05 (m, 2H), 3.97 (d, ^3J (H, H)= 3.0 Hz, 1 H), 3.82 (q, ^3J (H, H) = 10.2 Hz, 1 H), 3.76 (dt, ^3J (H, H) = 0.6, 9.6 Hz, 1 H), 3.58 (s, 3 H), 3.54 (s, 1 H), 2.72 (dd, ^3J (H, H) = 4.2, 12.6 Hz, 1 H), 2.58 (d, ^3J (H, H) = 1.8 Hz, 1 H), 2.32 (s, 3 H), 2.19 (s, 3 H), 2.18 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.94 (dd, ^3J (H, H) = 6.0, 12.6 Hz, 1 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.9, 170.7, 170.5, 170.4, 168.4, 162.3, 138.2, 138.15, 134.42, 134.41, 129.81, 129.77, 129.2, 128.3, 127.4, 126.8, 101.1, 97.4, 92.3, 86.8, 76.4, 74.0, 71.9, 69.7, 69.5, 68.1, 67.6, 67.3, 66.0, 62.5, 53.3, 51.8, 38.7, 21.6, 21.5, 21.04, 21.01, 20.97. HRMS: m/z, calcd for $C_{40}H_{46}Cl_3NO_{17}SNa$, $[M+Na]^+$, 972.1444, found: 972.1448. The sialyl α2→3 galactose linkage was confirmed by a HMBC correlation between C_2 of the sialic acid and H_3 of the galactose unit. Stereochemistry of the linkage was established using compound **30**.

p-Tolyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-ᴅ-glycero-α-ᴅ-galacto-2-nonulopyranosonate-(2→3))-2-O-benzoyl-4,6-O-benzylidene-1-thio-β-ᴅ-galactopyranoside (30)—The mixture of compound **24** (475 mg, 0.5 mmol), pyridine (2 mL) and benzoyl chloride (1 mL) was stirred overnight at room temperature. The mixture was then diluted with CH_2Cl_2 (100 mL) and washed with HCl (1 M), water and satd. sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. After flash column chromatography (EtOAc:CH_2Cl_2:hexanes = 3 : 3 : 4), compound **30** was obtained (501 mg, 95%). $[\alpha]_D^{20}$= −15.0, (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.16-8.12 (m, 2 H), 7.60-7.56 (m, 1H), 7.52-7.46 (m, 2 H), 7.45-7.42 (m, 2 H), 7.39-7.35 (m, 2 H), 7.35-7.30 (m, 3 H), 7.02-7.00 (m, 2 H), 6.58 (d, ^3J (H, H) = 10.2 Hz, 1 H), 5.49-5.44 (m, 1 H), 5.33 (t, ^3J (H, H) = 9.6 Hz, 1 H), 5.32 (s, 1 H), 5.18 (dd, ^3J (H, H) = 1.8, 9.6 Hz, 1 H), 4.92-4.86 (m, 2 H), 4.56 (dd, ^3J (H, H) = 3.6, 9.6 Hz, 1 H), 4.34 (d, ^3J (H, H) = 11.4 Hz, 1 H), 4.30 (dd, ^3J (H, H) = 2.4, 6.6 Hz, 1 H), 4.10-4.03 (m, 2 H), 4.02-3.96 (m, 2 H), 3.72 (q, ^3J (H, H) = 10.2 Hz, 1 H), 3.62 (s, 1 H), 3.54 (s, 3 H), 2.58 (dd, ^3J (H, H) = 4.8, 13.2 Hz, 1 H), 2.29 (s, 3 H), 2.18 (s, 3 H), 2.00 (s, 3 H), 1.89 (s, 3 H), 1.76 (s, 3 H), 1.64 (t, ^3J (H, H) = 13.2 Hz, 1 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.9, 170.6, 170.1, 168.8, 165.1, 162.3, 138.4, 138.0, 134.6, 133.3, 130.8, 130.7, 130.2, 129.7, 129.3, 129.2, 128.7, 128.3, 127.7, 126.8, 101.3, 96.9, 92.2, 85.5, 73.73, 73.68, 71.9, 69.7, 69.5, 68.5, 68.0, 67.9, 67.2, 62.7, 53.2, 51.5, 38.5, 21.7, 21.5, 21.01, 20.93, 20.6. HRMS: m/z: calcd for $C_{47}H_{50}Cl_3NO_{18}SNa$, $[M+Na]^+$, 1076.1712, found: 1076.1676. Three-bond coupling between CO_2Me and H_{3ax} of sialic acid was determined to be 5.8 Hz indicating α sialyl linkage.

Benzyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-ᴅ-glycero-α-ᴅ-galacto-2-nonulopyranosonate-(2→3))-2-O-benzoyl-4,6-O-benzylidene-β-ᴅ-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-ᴅ-glucopyranoside-(1→2)-3,4,6-tri-O-benzyl-α-ᴅ-mannopyranosyl-(1→6)-((methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-ᴅ-glycero-α-ᴅ-galacto-2-nonulopyranosonate-(2→3))-2-O-benzoyl-4,6-O-benzylidene-β-ᴅ-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-ᴅ-glucopyranoside-(1→2)-3,4,6-tri-O-benzyl-α-ᴅ-mannopyranosyl-(1→3))-2,4-di-O-benzyl-β-ᴅ-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-ᴅ-glucopyranosyl-(1→4)-(2,3,4-tri-O-benzyl-α-ʟ-fucopyranosyl-(1→6))-3-O-benzyl-2-deoxy-2-phthalimido-β-ᴅ-glucopyranoside 2—A mixture of donor **30** (53 mg, 0.05 mmol) and freshly activated molecular sieves 4Å (800 mg) in CH_2Cl_2 (6 mL) was stirred at room temperature for 30 minutes and then cooled to −78 °C, which was followed by the addition of AgOTf (38 mg, 0.15 mmol)

dissolved in acetonitrile (0.1 mL) without touching the wall of the flask. After 5 minutes, orange colored *p*-TolSCl (7.6 μL, 0.05 mmol) was added through a microsyringe. Since the reaction temperature was lower than the freezing point of *p*-TolSCl, *p*-TolSCl was added directly into the reaction mixture to prevent it from freezing on the flask wall. The characteristic yellow color of *p*-TolSCl in the reaction solution dissipated rapidly within a few seconds indicating its depletion. After the donor was completely consumed according to TLC analysis (about 5 minutes at −78 °C), a solution of acceptor **31** (21 mg, 0.04 mmol) and TTBP (20 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) was slowly added dropwise via a syringe. The reaction mixture was stirred for 1.5 h until the temperature reached 0 °C, then cooled to −78°C again. A solution of diol acceptor **4** (32 mg, 0.0125 mmol) in CH_2Cl_2 (1 mL) was added, followed by AgOTf (26 mg, 0.1 mmol) in acetonitrile (0.1 mL). After 5 minutes, *p*-TolSCl (6.1 μL, 0.04 mmol) was added and the reaction mixture was stirred for 1.5 h until the temperature reached 0 °C, at which point triethylamine (30 μL) was added to quench the reaction. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered through Celite. The Celite was washed extensively with CH_2Cl_2 until TLC showed no products in the filtrate. The filtrate was combined, concentrated and purified by flash column chromatography (hexanes : EtOAc = 2 : 3) to give compound **2** (42 mg, 64.5%). $[\alpha]_D^{20} = -2.5$ (c = 1.0 in $CHCl_3$). ^{1}H NMR (600 Hz, $CDCl_3$): δ = 8.20-8.12 (m, 4 H), 7.82-7.76 (m, 3 H), 7.72-6.84 (m, 103 H), 6.84-6.79 (m, 4 H), 6.78-6.74 (m, 3 H), 6.70-6.66 (m, 1 H), 6.49-6.45 (m, 1 H), 6.44-6.34 (m, 4 H), 5.56-5.40 (m, 5 H), 5.34 (s, 1 H), 5.32 (s, 1 H), 5.34-5.31 (m, 1 H), 5.24-5.19 (m, 2 H), 4.96- 4.85 (m, 6 H), 4.83-4.65 (m, 10 H), 4.63-4.41 (m, 12 H), 4.37-4.29 (m, 5 H), 4.28-4.19 (m, 8 H), 4.17-3.98 (m, 23 H), 3.94-3.83 (m, 7 H), 3.81-3.72 (m, 5 H), 3.68 (s, 2 H), 3.66-3.57 (m, 7 H), 3.56 (s, 3 H), 3.54-3.48 (m, 5 H), 3.47 (s, 3 H), 3.45-3.37 (m, 4 H), 3.33-3.15 (m, 8 H), 3.13-3.05 (m, 2 H), 2.90 (d, ^3J (H, H) = 10.2 Hz, 1 H), 2.86 (d, ^3J (H, H) = 10.8 Hz, 1 H), 2.77 (dd, ^3J (H, H) = 5.4, 10.8 Hz, 1 H), 2.68 (dd, ^3J (H, H) = 4.2, 12.6 Hz, 1 H), 2.64-2.56 (m, 2 H), 2.20 (s, 3 H), 2.19 (s, 3 H), 1.97 (s, 6 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.91 (s, 3 H), 1.90 (s, 3 H), 1.65-1.56 (m, 2 H), 0.93 (d, ^3J (H, H) = 6.6 Hz, 3 H). ^{13}C NMR (150 Hz, $CDCl_3$): δ = 170.9, 170.6, 170.5, 170.4, 170.3, 168.68, 168.65, 168.5, 168.11, 168.05, 168.00, 164.98, 164.90, 162.3, 139.4, 139.3, 139.1, 139.04, 138.99, 138.90, 138.82, 138.6, 138.5, 138.3, 138.2, 137.9, 137.8, 137.3, 133.7, 133.6, 132.1, 132.0, 131.1, 130.3, 130.2, 130.0, 129.2, 129.1, 129.0, 128.9, 128.6, 128.5, 128.42, 128.41, 128.36, 128.33, 128.27, 128.24, 128.22, 128.17, 128.14, 128.07, 128.02, 127.90, 127.82, 127.79, 127.76, 127.66, 127.57, 127.54, 127.45, 127.34, 127.23, 127.17, 127.00, 126.97, 126.91, 126.61, 126.59, 126.52, 125.41, 123.31, 101.9, 101.5, 101.5, 101.0, 98.5, 97.7, 97.10, 97.08, 96.98, 96.96, 96.85, 95.7, 92.4, 92.1, 81.6, 81.4, 80.3, 79.3, 78.3, 78.0, 77.0, 76.2, 76.1, 75.5, 75.0, 74.9, 74.7, 74.4, 74.3, 74.1, 74.0, 73.8, 73.40, 73.35, 73.33, 73.24, 73.1, 72.8, 72.7, 72.54, 72.45, 72.1, 72.0, 70.9, 70.6, 70.3, 70.1, 69.7, 69.4, 68.8, 68.4, 68.3, 67.8, 67.6, 67.3, 66.7, 66.6, 66.1, 62.7, 62.6, 56.8, 56.2, 56.0, 53.3, 53.2, 51.6, 38.9, 38.9, 21.6, 20.9, 20.9, 20.82, 20.76, 16.7. MALDI-MS: Calcd for $C_{279}H_{280}Cl_6N_6NaO_{80}$, $[M+Na]^+$, 5232.61 (one of the highest isotope peaks), found: 5232.24.

p-Tolyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroacetamido-D-glycero-α-D-galacto-2-nonulopyranosonate-(2→3))-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosonyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3)—See procedure for compound **2**. The one pot reaction for synthesis of compound **2** was terminated prior to addition of acceptor **4**. Compound **3** was purified from the reaction mixture by flash column chromatography (hexanes : EtOAc : CH_2Cl_2 = 4 : 3 : 3) in 82% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.15-8.12 (m, 2 H), 7.87-7.84 (m, 1 H), 7.81-7.77 (m, 1 H), 7.70-7.66 (m, 2 H), 7.63-7.59 (m, 1 H), 7.51-7.47 (m, 2 H), 7.46-7.42 (m, 2 H), 7.36-7.28 (m, 6 H), 7.28-7.24 (m, 2 H), 7.20-7.16 (m, 2 H), 6.93-6.90 (m, 2 H), 6.58 (d, ^3J (H, H) = 9.6 Hz, 1 H), 5.56-5.46 (m, 3 H), 5.30 (s, 1 H), 5.21 (dd, ^3J (H, H) = 1.8, 9.6 Hz, 1 H), 4.92-4.85 (m, 1 H), 4.76 (d, ^3J (H, H) = 7.8 Hz, 1 H), 4.53 (dd, ^3J (H, H) = 3.6, 10.2 Hz, 1 H), 4.43-4.38 (m, 2 H), 4.30 (dd, ^3J (H, H) = 2.4, 12.6

Hz, 1 H), 4.22-4.06 (m, 7H), 4.05-4.00 (m, 1 H), 3.81-3.74 (m, 1 H), 3.68-3.64 (m, 1 H), 3.63 (s, 1 H), 3.61-3.57 (m, 1 H), 3.52-3.45 (m, 5 H), 2.63 (dd, ^3J (H, H) = 4.2, 12.6 Hz, 1 H), 2.22 (s, 3 H), 2.20 (s, 3 H), 2.00 (s, 3 H), 1.95 (s, 3 H), 1.91 (s, 3 H), 1.62 (t, ^3J (H, H) = 12.6 Hz, 1 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 171.0, 170.59, 170.55, 170.1, 168.7, 168.2, 168.0, 165.1, 162.3, 138.8, 138.2, 137.7, 134.24, 134.18, 133.6, 133.4, 132.1, 132.0, 130.2, 130.1, 129.7, 129.2, 128.8, 128.5, 128.41, 128.35, 127.68, 127.66, 126.6, 123.9, 123.5, 101.7, 101.0, 96.9, 92.1, 83.6, 82.1, 78.2, 73.1, 72.8, 72.3, 72.0, 71.2, 70.0, 68.7, 68.6, 67.7, 67.5, 67.3, 66.7, 62.9, 60.7, 55.4, 53.2, 51.6, 38.8, 21.7, 21.31, 21.30, 20.9, 20.8. HRMS: calcd for $C_{68}H_{69}Cl_3N_2O_{24}SNa$, $[M+Na]^+$,1457.2924, found: 1457.2908. The Gal β1→4 GlcN linkage was confirmed by a HMBC correlation between C_1 of the galactose and H_4 of the glucosamine unit.

5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-β-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-β-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3))-β-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-(α-L-fucopyranosyl-(1→6))-2-amino-2-deoxy-D-glucopyranoside (1)—Lithium iodide (200 mg) was added to a solution of compound **2** (80 mg) in dry pyridine (7 mL). The reaction mixture was refluxed at 120°C for 8 h under nitrogen atmosphere. The dark yellow solution was then evaporated to dryness and co-evaporated with toluene to yield a dark yellow amorphous solid which was directly used for next reaction. A solution of the above solid in ethanol (5 mL) was treated with NH_2-NH_2·H_2O (1 mL) at 85°C for 48 h. The reaction mixture was concentrated and co-evaporated with toluene then selective acetylated with Ac_2O (0.3 mL), Et_3N (0.3 mL) in methanol (3 mL) at room temperature overnight. The acetylated mixture was concentrated and passed through a short column of silica gel and eluted with CH_2Cl_2/MeOH to give compound **22**. A mixture of **22**, 10% $Pd(OH)_2$/C (30 mg) in methanol (3 mL) and water (1 mL) was stirred for 24 h at room temperature under H_2 atmosphere. The solid was filtered off and the solution was concentrated to give compound **1** (18 mg, 49%). $[\alpha]_D^{20} = -4.2$ (c = 1.0 in H_2O). ^{1}H NMR (600 Hz, $CDCl_3$): α:β mixture at the reducing end fucose δ 4.97 (d, ^3J (H, H) = 2.4 Hz, 0.5 H), 4.91 (s, 1 H), 4.72 (s, 1H), 4.70-4.68 (m, 1 H), 4.57-4.54 (m, 1 H), 4.47-4.44 (m, 1 H), 4.38-4.33 (m, 4 H), 4.08 (bs, 1 H), 4.02 (bs, 1 H), 3.94-3.88 (m, 4H), 3.80-3.28 (m, 68H), 2.58 (dd, ^3J (H, H) = 3.6, 12.0 Hz, 2 H), 1.88-1.82 (m, 12 H), 1.62 (t, ^3J (H, H) = 12.0 Hz, 2 H), 1.06-1.02 (m, 3 H). ESI-MS: (neg. mode) Calcd for $C_{90}H_{148}N_6O_{66}$ $(M-2H)^{2-}$: 1183.42, found: 1183.83.

Supplementary Material

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Acknowledgements

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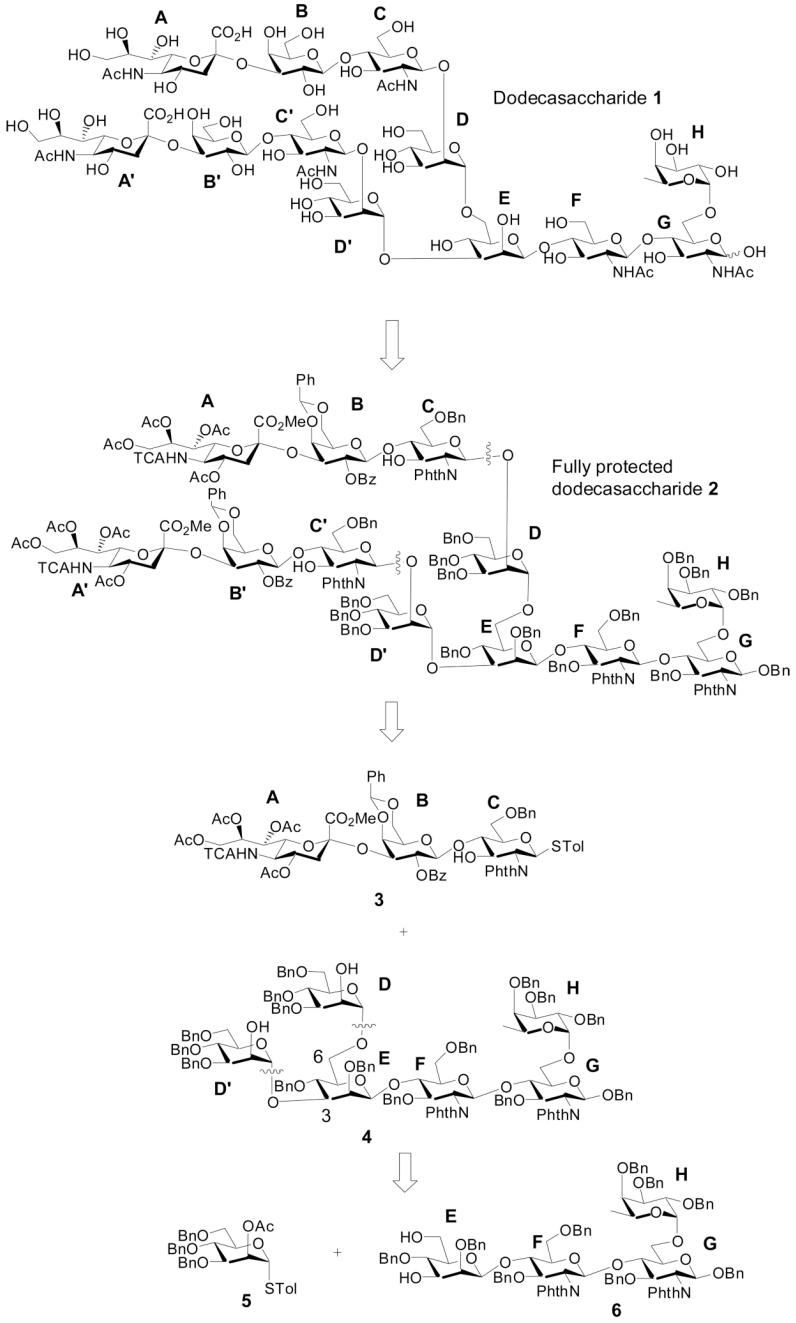
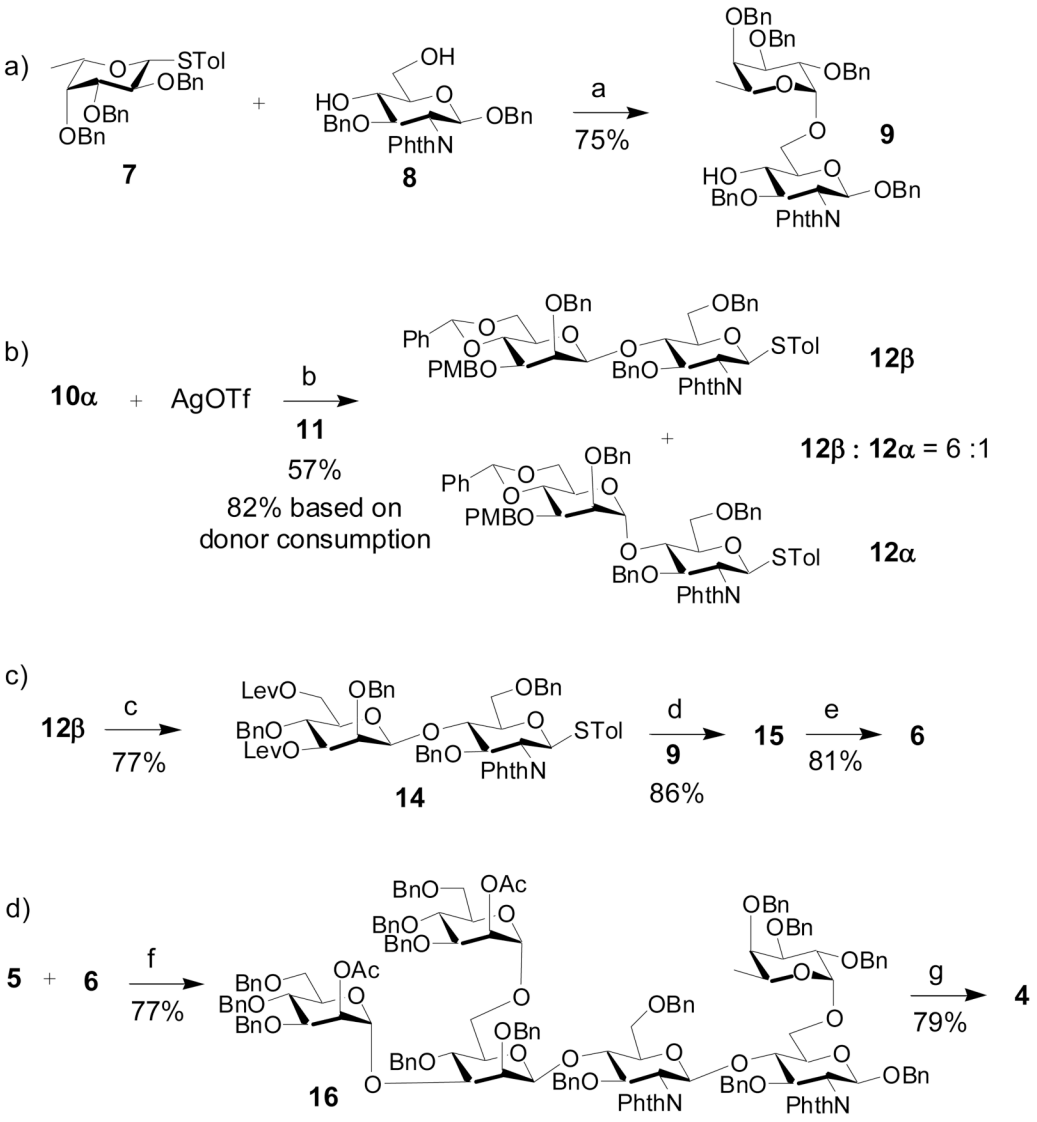
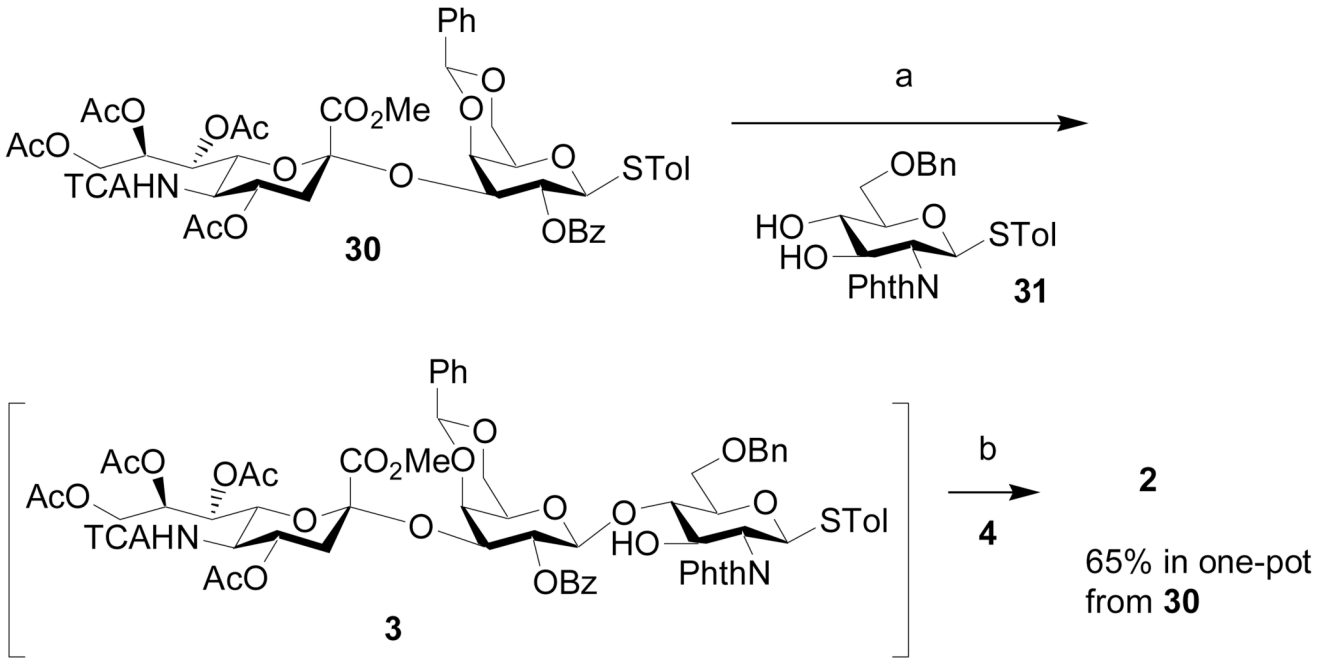


Scheme 1.
Retrosynthetic analysis of dodecasaccharide **1**.



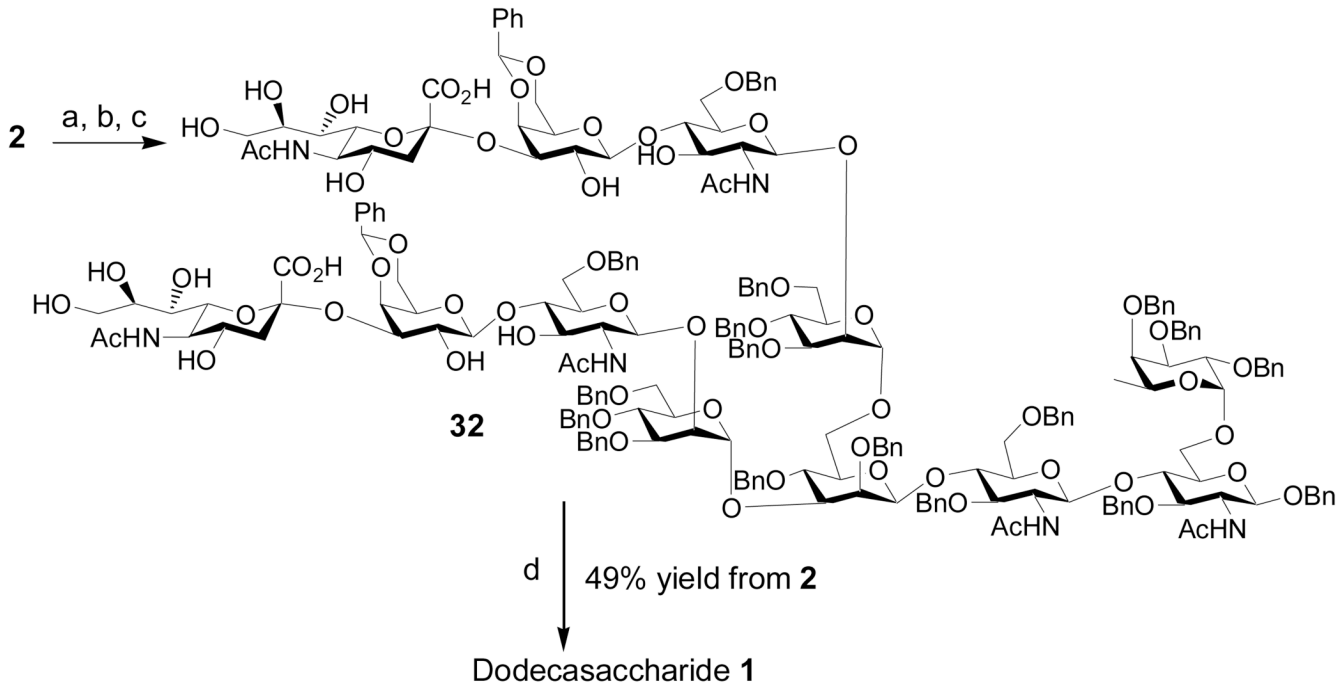
Reagents and conditions: a) $CuBr_2$, Bu_4NBr, CH_2Cl_2, DMF, 0 °C to rt; b) *p*-TolSCl, CH_2Cl_2, - 78 °C; then TTBP, **11**; c) $PhBCl_2$, Et_3SiH, CH_2Cl_2, - 78 °C; then levulinic acid, EDC, DMAP; d) AgOTf, *p*-TolSCl, TTBP, **9**, CH_2Cl_2, - 78 °C to 0 °C; e) hydrazine acetate, CH_3OH, CH_2Cl_2; f) AgOTf, *p*-TolSCl, CH_2Cl_2, - 78 °C to 0 °C then TMSOTf, 0 °C ; g) $NaOCH_3$, CH_3OH, rt.

Scheme 2.



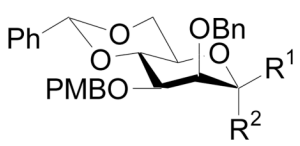
Reagents and conditions: a) *p*-TolSCl, AgOTf, CH_2Cl_2, - 78 °C; then TTBP, **21**; b) AgOTf, *p*-TolSCl, **4**, CH_2Cl_2, - 78 °C to 0 °C.

Scheme 3.

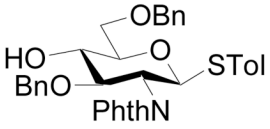


Reagents and Conditions: a) LiI, pyridine, 110 °C; b) hydrazine, ethanol, reflux; c) Ac_2O, Et_3N, MeOH; d) H_2, $Pd(OH)_2/C$, MeOH, H_2O.

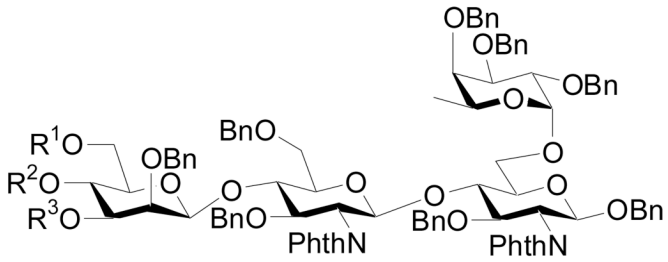
Scheme 4.



10α: R_1 = H, R_2 = STol
10β: R_1 = STol, R_2 = H



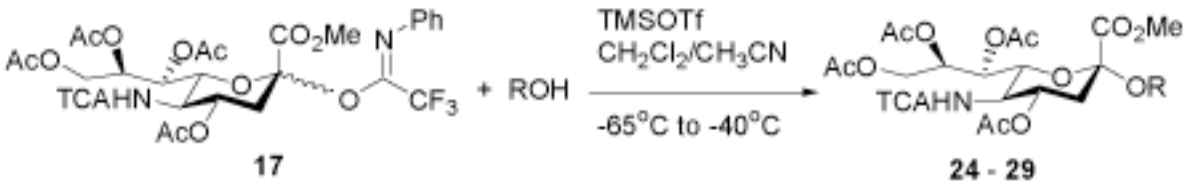
11

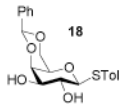
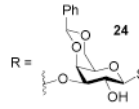
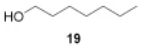
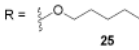
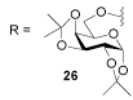
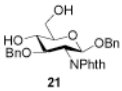
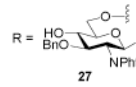
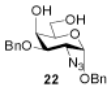
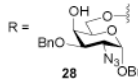
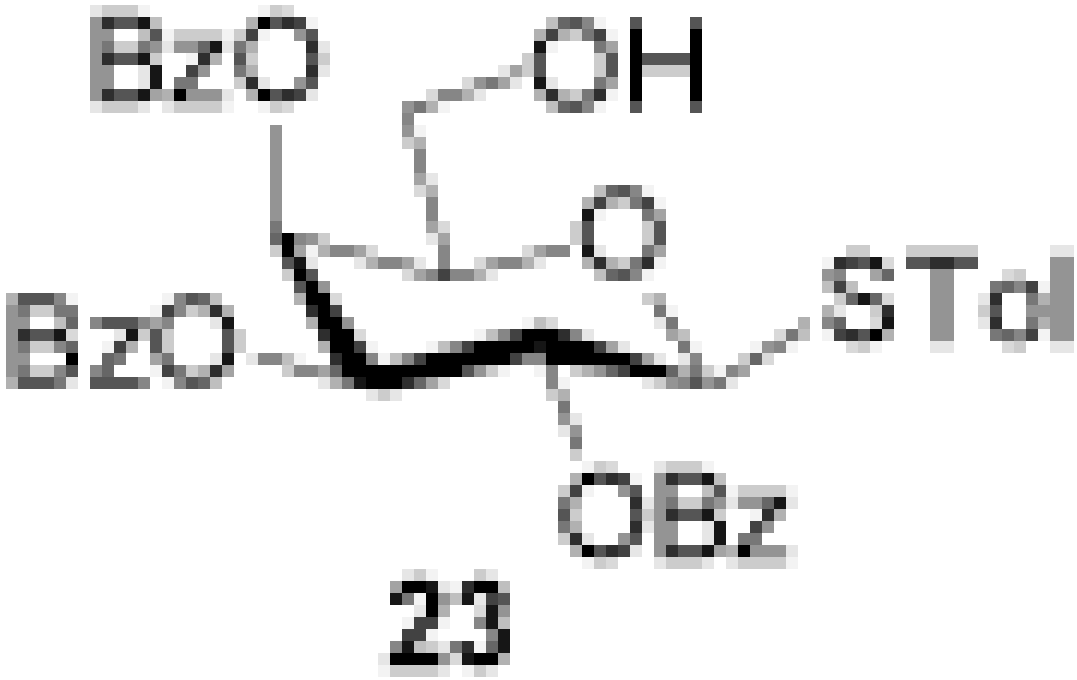
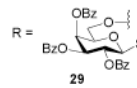


13: R^1, R^2 = PhCH, R^3 = OPMB
15: R^1, R^3 = Lev, R^2 = Bn

Table 1

Sialylation Results using Sialyl Donor **17**



Entry	Acceptor	Product
1	 18	 24
2	 19	 25
3	 20	 26
4	 21	 27
5	 22	 28
6	 23	 29

[a] Anomeric ratios were determined by ^{1}H NMR analysis