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Synthesis of Biantennary Complex-Type Nonasaccharyl Asn Building Blocks for Solid-Phase Glycopeptide Synthesis

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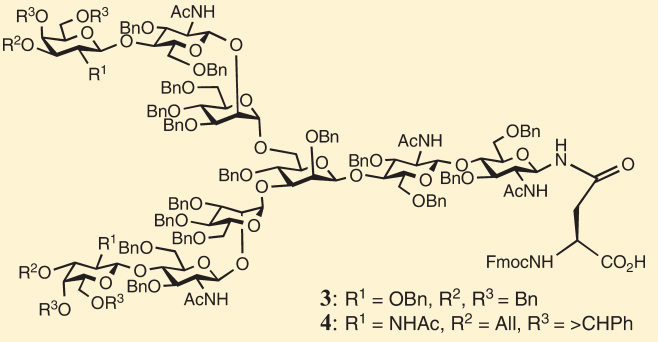
Supporting Information

ABSTRACT: The biantennary complex-type *N*-glycans bearing LacNAc and LacdiNAc as the nonreducing end motif were synthesized in a protected form suitable to use in the Fmoc solid-phase peptide synthesis studies. Two approaches for the nonasaccharide synthesis were examined by taking advantage of the highly β-selective glycosylation with GlcNTCA (*N*-phenyl)trifluoroacetimidate. An earlier approach, which involved the reaction of the trisaccharide donor (Gal-GlcNTCA-Man) and trisaccharide acceptor (Man-GlcNPhth$_2$-N$_3$), produced a mixture of nonasaccharide isomers. On the other hand, mannosylation of the trisaccharide acceptor (Man-GlcNPhth$_2$-N$_3$) stereoselectively afforded the known pentasaccharide (Man$_3$-GlcNPhth$_2$-N$_3$), which was reacted with the disaccharyl glycosyl donor (Gal-GlcNTCA or GalNTCA-GlcNTCA) to produce the desired nonasaccharide as a single stereoisomer. Selective dephthaloylation followed by N-acetylation furnished the GlcNAc$_2$ functionality. The resulting nonasaccharyl azides were condensed with Fmoc-Asp(OPfp)-OBu^t or Fmoc-Asp(OPfp)-OPac in the presence of Ph(CH$_3$)$_2$P and HOOBt. Finally, the Zn reduction and cleavage of the *tert*-butyl ester or Zn reduction alone produced the targeted nonasaccharyl Asn building blocks.



1. INTRODUCTION

Insights into the molecular mechanisms of such significant glycoprotein-mediated biological events including cancer metastasis, apoptosis, and microbe infection are expected to produce many improvements in diagnostic or therapeutic approaches. However, only a limited amount of glycoprotein samples is available from natural sources, and the attached oligosaccharides are frequently heterogeneous. Thus, a large number of studies have been directed toward establishing a reliable synthetic method for glycopeptides including those as large as glycoproteins since chemically synthesized homogeneous samples are useful to study the biological functions of not only the attached oligosaccharides but also the protein portions.[1,2]

Among these studies, we have synthesized both the *N*- and *O*-glycan-linked glycopeptides using the benzyl-protected oligosaccharyl amino acids as a building block for the Fmoc solid-phase peptide synthesis (SPPS). The benzyl groups were ultimately removed under the conditions of low-acidity TfOH with minimum rupture of the acid-labile glycosidic linkages.[3]

Recently, we accomplished the highly stereoselective synthesis of a series of LacNAc-containing *O*-glycans[3−5] based on the use of the benzyl- and/or benzylidene-protected *N*-trichloroacetyllactosaminyl fluoride as a glycosyl donor in the key glycosylation reaction (Scheme 1). In addition, the adoption of the *N*-trichloroacetyl group, which could be easily converted to an *N*-acetyl group by Zn reduction, made it possible to achieve the highly convergent synthesis of the *O*-glycoamino acid. The powerful stereocontrolling ability of the *N*-trichloroacetyl group used in the construction of the β-LacNAc glycoside prompted us to apply this method to the synthesis of the complex type *N*-glycoasparagines **1** and **2** (Figure 1).

In the last two decades, many synthetic investigations have been directed to the *N*-glycan derivatives. Chemical and chemoenzymatic syntheses of the biantennary complex-type *N*-glycan framework carrying an α-(2→6)- or α-(2→3)-disialo motif have been reported.[6] However, only a limited number of research groups have realized the further transformation of the synthetic full-size *N*-glycan into a glycosylated oligopeptide[7,8] because considerable processes are required to produce a sufficient quantity of such an *N*-glycan sample. Alternative approaches utilizing the *N*-glycans derived from natural sources have also been demonstrated,[9−11] but the natural *N*-glycans obtainable in quantity are so far structurally restricted.[12] Therefore, the

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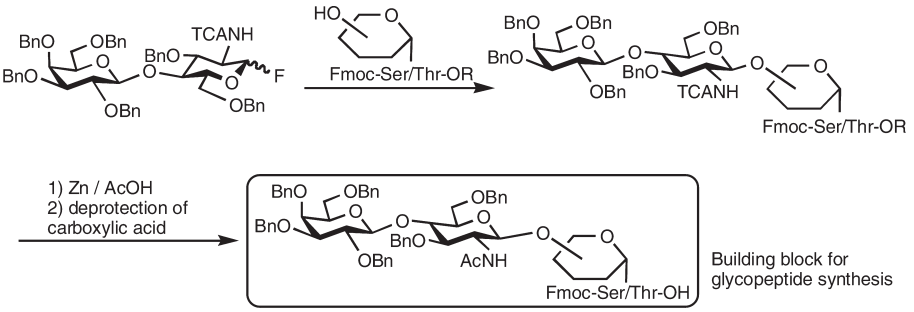
Scheme 1. Synthetic Route for *O*-Glycoamino Acid Using the Key LacNAc Donor



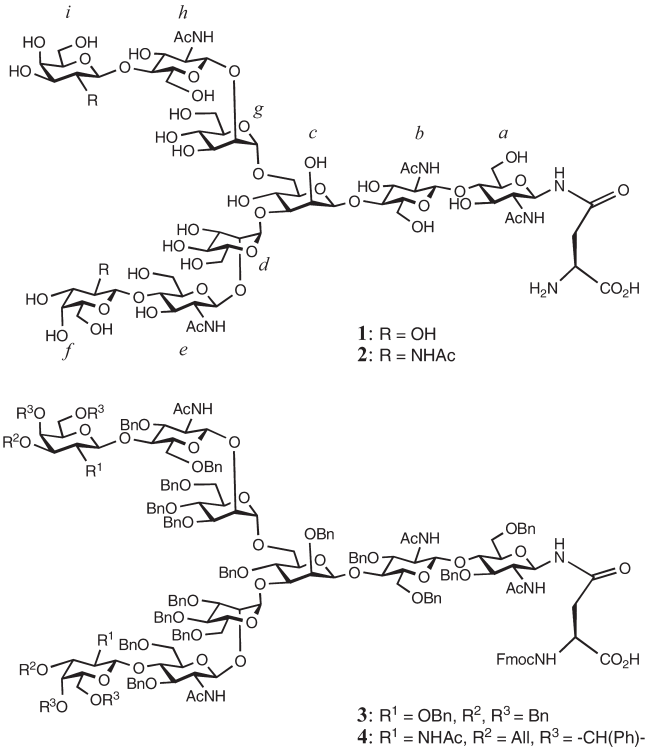


Figure 1. Structures of Asn-linked nonasaccharides **1** and **2** and synthetic targets **3** and **4**.

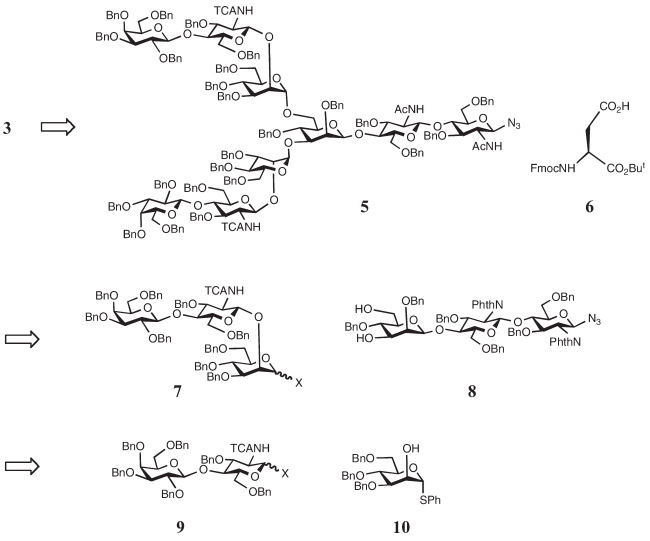
development of an efficient method to synthesize the glycoasparagine units with diverse *N*-glycan structures is still requested.

In this paper, we describe our synthetic approach to the complex type *N*-glycans **3** and **4** (Figure 1) suitably protected for SPPS. Although the latter glycoform (**4**: LacdiNAc substitution) appears only as a minor modification of the complex-type *N*-glycan, a unique sex-dependent biological function of the glycoprotein has been reported.[13] The LacdiNAc *N*-glycan identified as a female-specific glycoform of glycodelin (GdA: a glycoprotein secreted in human amniotic fluid) potentially inhibits sperm−oocyte binding, whereas the male-specific glycodelin (GdS: secreted in seminal plasma) carrying the high-mannnose type and standard complex type *N*-glycans has no such inhibiting effect.

RESULTS AND DISCUSSION

On the basis of the merits of convergent synthesis, our first synthetic plan for **3** was the condensation between the known

Scheme 2. Retrosynthetic Pathway to 3 by the First Plan



trisaccharide **8**[14] and 2 equiv of the LacNAc-Man donor **7** (see Scheme 2). The β-mannoside synthesis of the trisaccharide **8** was facilitated using Crich's method (Scheme 3).[15,16] The mannosyl thioglycoside **11**[17] was activated with 1-benzenesulfinyl piperidine (BSP), 2,4,6-tri-*tert*-butylpyrimidine (TTBP), and Tf_2O at −60 °C and reacted with the disaccharide **12** for 2 days. The product **13** was obtained as a mixture of stereoisomers (β/α = 7/1). The major isomer was separated from the minor one by recycling HPLC, which enables the fraction containing the desired product to return to the same column and repeat the cycles until the separation with the minor component becomes perfect. The highly pure compound **13** was obtained in 73% yield. Then it was reduced with Et_3SiH and $PhBCl_2$[18] to regioselectively cleave the benzylidene acetal. The MPM group was then removed to afford **8** in a 69% overall yield. The trisaccharyl donor (**7s**) was synthesized by the $Cp_2Zr(ClO_4)_2$-mediated glycosidation[19] of the known LacNTCA fluoride **9f**[5] and **10**[20] with a high yield and stereoselectivity (85%; β/α = 98/2). The β-selective synthesis of **7s** was also achieved by the reaction of **10** and (*N*-phenyl)trifluoroacetimidate **9i** derived from the corresponding hemiacetal[5] (79%; β/α = 99/1).

Glycosylation of **8** with **7s** (2 equiv) was promoted by NIS and TfOH in CH_2Cl_2 to afford the nonasaccharide **17**, which was isolated by gel permeation chromatography in 66% yield. However, the ^1H and ^{13}C NMR spectra of the nonasaccharide showed

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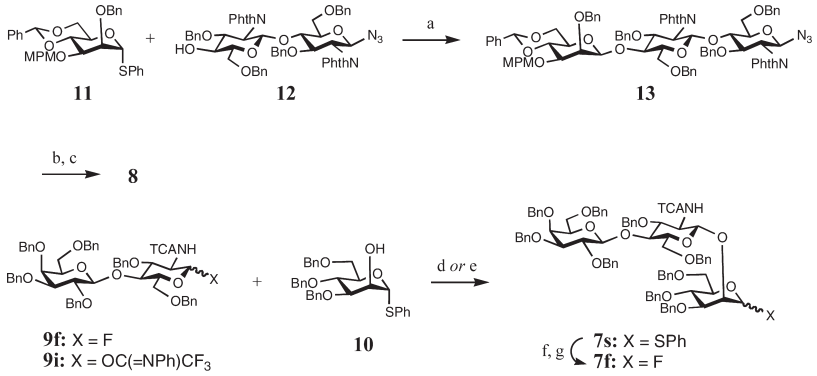
Scheme 3. Syntheses of the Trisaccharyl Intermediates 7 and 8[a]



[a] Reagents and conditions: (a) BSP, TTBP, Tf_2O, CH_2Cl_2, −60 °C, 2 days, 73%; (b) Et_3SiH, $PhBCl_2$, MS4A, CH_2Cl_2, −78 °C, 0.5 h; (c) 90% TFA aq, CH_2Cl_2, −10 °C, 0.5 h, 95% (2 steps); (d) Cp_2ZrCl_2, $AgClO_4$, MS4A, CH_2Cl_2, −60 to −50 °C, 3 h, 85% (β/α = 98/2); (e) TMSOTf, MS AW300, CH_2Cl_2, −78 to −40 °C, 2 h, 79% (β/α = 99/1); (f) NBS, acetone aq, rt, 3.5 h, 85%; (g) DAST, CH_2Cl_2, rt, 0.5 h, quant.

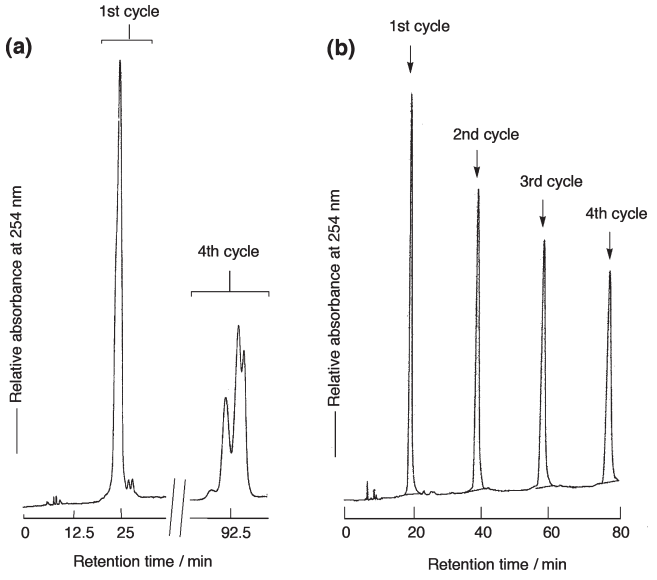
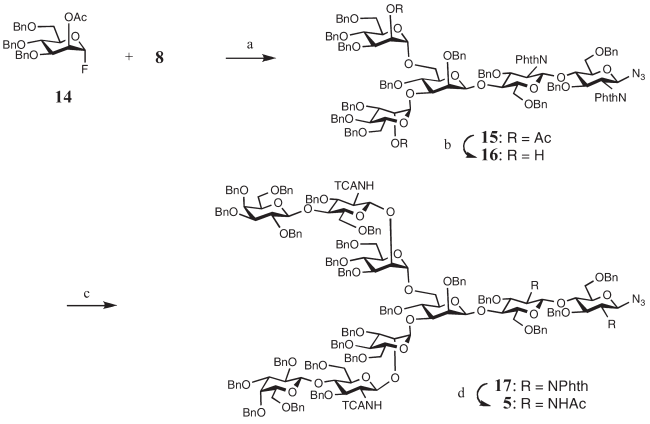


Figure 2. Analysis of nonasaccharide samples of **17** prepared from (a) **7s** and **8** and (b) **9i** and **16** by the recycling HPLC system. The major peak shown as 1st cycle was collected and returned to the same column. The sample flowed through the same column repeatedly, which was shown by the cycle number. Conditions: column: Inertsil SIL-100A (20 mm × 250 mm) at a flow rate of 9.5 mL/min using (a) 30% EtOAc/hexane and (b) 35% EtOAc/hexane as eluents.

the heterogeneous property of the product. Through careful examination of the product by recycling HPLC, it turned out that the product consisted of at least three major components as shown in Figure 2a. A similar result was obtained when acceptor **8** was glycosylated with the fluoride donor **7f**. By these glycosylation methods, we could not attain a high α-selectivity, although several examples have previously been reported for the related α-mannnoside synthesis of an *N*-glycan using the nonparticipating 2-*O*-glycosylated mannnose donors of trichloroacetimidate[6a−c] and thioglycoside.[21] Therefore, we altered the synthetic route to the nonasaccharide (Scheme 4).

Mannosylation of **8** with 2-*O*-acetyl-3,4,6-tri-*O*- benzyl-α-D-mannopyranosyl fluoride **14**[22] stereoselectively gave the α,α-dimannosylated pentasaccharide **15**,[14] which was readily deacetylated to the diol **16**. The glycosylation of **16** with **9i** (3.4 equiv) was promoted using a

Scheme 4. Synthesis of Nonasaccharide 5[a]



[a] Reagents and conditions: (a) Cp_2ZrCl_2, $AgClO_4$, MS4A, CH_2Cl_2, −15 °C, 2 h, 83%; (b) 30% H_2O_2, LiOH, THF, 0 °C, 3 d, 75%; (c) **9i**, TMSOTf, MS AW300, CH_2Cl_2, −40 °C, 2 h, 85%; (d) 1. $(CH_2NH_2)_2$, BuOH, 2. Ac_2O, CH_2Cl_2, MeOH, 75%.

catalytic amount of TMSOTf at −40 °C for 2.5 h to give the desired **17** as a single isomer in 85% yield. The homogeneity of the sample was determined by analysis using recycling HPLC (Figure 2b) as well as by the clearly split nine signals of anomeric carbon in the ^{13}C NMR spectrum. The newly generated GlcNTCA linkages were determined as β on the basis of the $^1J_{CH}$ values (166.3 and 165.5 Hz at 97.6 and 97.8 ppm, respectively).[23]

Having obtained the desired nonasaccharide with high stereoselectivity, the *N*-phthaloyl protecting groups were selectively converted to *N*-acetyl groups before condensing with the amino acid moiety. Compound **17** was heated with a large excess of 1,2-diaminoethane in BuOH for 2 h, and the extracted intermediate was heated in toluene overnight to complete cleavage of the phthalimido groups. The prolonged reaction in the presence of 1,2-diaminoethane caused a considerable decrease in the yield of the desired diamino compound. Conversion to the diacetamide was then performed by acetylation with Ac_2O to give **5** in 75% yield.

Since a stereocontrolled route to the nonasaccharide framework has been established, we next directed our attention to the synthesis of the LacdiNAc-containing congener as shown in

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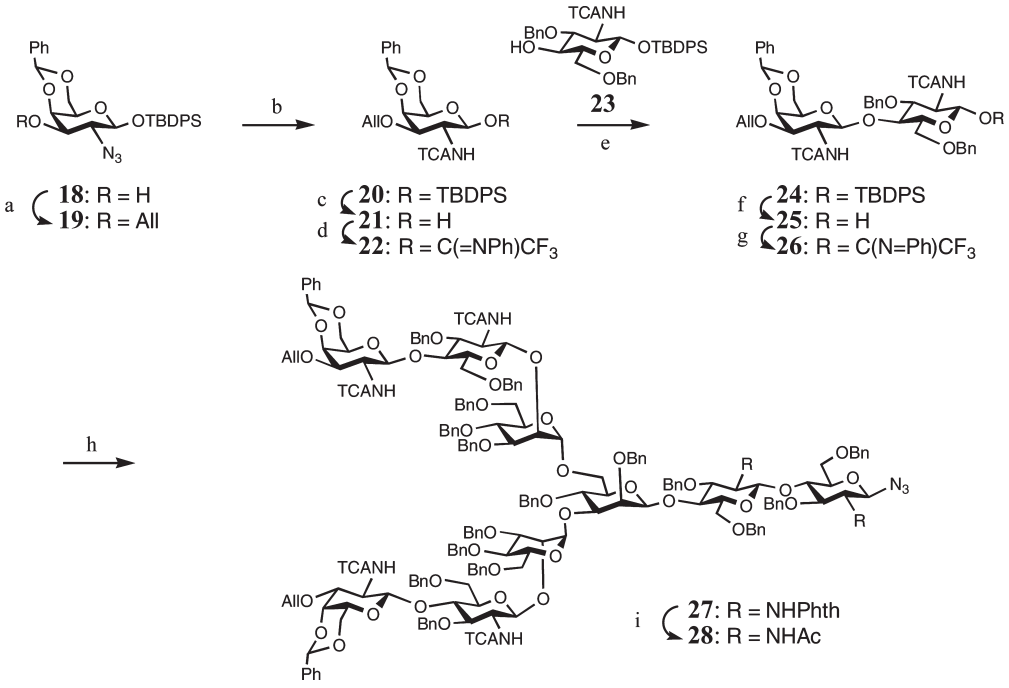
Scheme 5. Synthesis of Nonasaccharide 28[a]



[a] Reagents and conditions: (a) NaH, CH_2=$CHCH_2Br$, DMF, 0 °C, 2 h, 87%; (b) 1. Zn, AcOH, CH_2Cl_2, rt, 0.5 h, 2. Cl_3CCOCl, pyridine, CH_2Cl_2, 0 °C, 1 h, 77%; (c) TBAF, AcOH, THF, rt, 20 h, 96%; (d) ClC(=NPh)CF_3, K_2CO_3, acetone, rt, 1 h, 88%; (e) TMSOTf, MS AW300, CH_2Cl_2, −78 to −40 °C, 2.5 h, 94%; (f) TBAF, AcOH, THF, rt, 20 h, 96%; (g) ClC(=NPh)CF_3, K_2CO_3, acetone, rt, 1 h, 96%; (h) **16**, TMSOTf, MS AW300, CH_2Cl_2, −78 to −40 °C, overnight, 69%; (i) 1. $(CH_2NH_2)_2$, BuOH, 2. Ac_2O, CH_2Cl_2, MeOH, 52%.

Scheme 5, before conjugation with an amino acid and dechlorination of the trichloroacetyl groups. The disaccharide intermediate for LacdiNAc was synthesized using the GalNTCA and GlcNTCA derivatives. The hydroxyl group at the 3-position of *tert*-butyldiphenylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside **18**[24] was protected as an allyl ether by taking into account the potential glycan elongation. Recently, we found that the allyl group was concomitantly removed when a synthetic glycopeptide was deprotected under the low-acidity TfOH conditions.[25] The rather unstable compound **19** bearing azide and allyl groups in a molecule was immediately reduced by Zn/AcOH and then trichloroacetylated to give **20** (77%), which was converted to **22** (84%) through desilylation and imidate formation. The glycosylation of **23**[26] with **22** stereoselectively produced the disaccharide **24** (94%), which was converted into the glycosyl donor **26** (92% in 2 steps). Crucial glycosylation of **16** required increased equivalences of the donor **26** to be 5 and TMSOTf to be 0.25, in addition to the longer reaction time (overnight) in contrast to the smooth coupling between **9i** and **16**. On the basis of monitoring the reaction by TLC, the oxazoline intermediate derived from **26** seemed more stable than that from **9i**. The product **27** was obtained in 69% yield, and the $^1J_{CH}$ value (both 166.3 Hz at 97.7 and 97.9 ppm for C-1e and C-1 h) supported the β-configuration of the newly formed glycosidic linkages. Conversion of the diphthalimide **27** to the diacetamide **28** (52%) was less efficient than the preparation of **5**, when similar conditions were used.

Conjugation of the synthetic nonasaccharides, **5** and **28**, with a properly protected aspartic acid derivative was performed by a modified "Staudinger Ligation".[27] The nonasaccharyl azide **5** was treated with dimethylphenylphosphine [$Ph(CH_3)_2P$], O^1-*tert*-butyl O^4-pentafluorophenyl aspartate [Fmoc-Asp(OPfp)-OBu^t] and 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HOBt) in 98% aq. THF.

Among the tested phosphines ($Ph(CH_3)_2P$, [$(Et)_3P$], [$(n\text{-}Bu)_3P$], Ph_3P) using a monosaccharide model, the use of $Ph(CH_3)_2P$ effectively minimized the side products derived from the dechlorination of the trichloroacetyl group. The desired glycoamino acid **29** was obtained in 84% yield. In addition, two fractions were separated by HPLC as the more hydrophilic side products (9 and 2%, respectively, based on the HPLC analysis). Their structures were determined by HRMS to be monodechlorinated and didechlorinated compounds, respectively. Similarly, the glycoamino acid derivative **30** was obtained by the condensation of **28** and O^1-phenacyl O^4-pentafluorophenyl aspartate [Fmoc-Asp(OPfp)-OPac] in 78% yield (Scheme 6). The trichloroacetamide groups were then converted into the acetamide groups by the reported procedure using microwave irradiation with Zn and AcOH in EtOAc, and the dechlorinated product **31** was obtained from **29** in 86% yield.[5] After the cleavage of the *tert*-butyl ester in **31** by treatment with aq. TFA, the title compound **3** (71%) was successfully obtained. In the case of compound **30**, the reduction of the trichloroacetamide group and the cleavage of Pac ester were simultaneously achieved by treatment with Zn and AcOH in EtOAc, and the LacdiNAc congener **4** was successfully obtained in 45% yield.

In conclusion, we have synthesized two nonasaccharyl asparagines of the biantennary *N*-glycan, each containing LacNAc and LacdiNAc as the nonreducing end motifs. The synthesis was achieved by combination of the N-phthaloylated and N-trichloroacetylated glucosamine derivatives, both promoting the exclusive 1,2-trans-glycoside formation. Selective conversion of phthalimide

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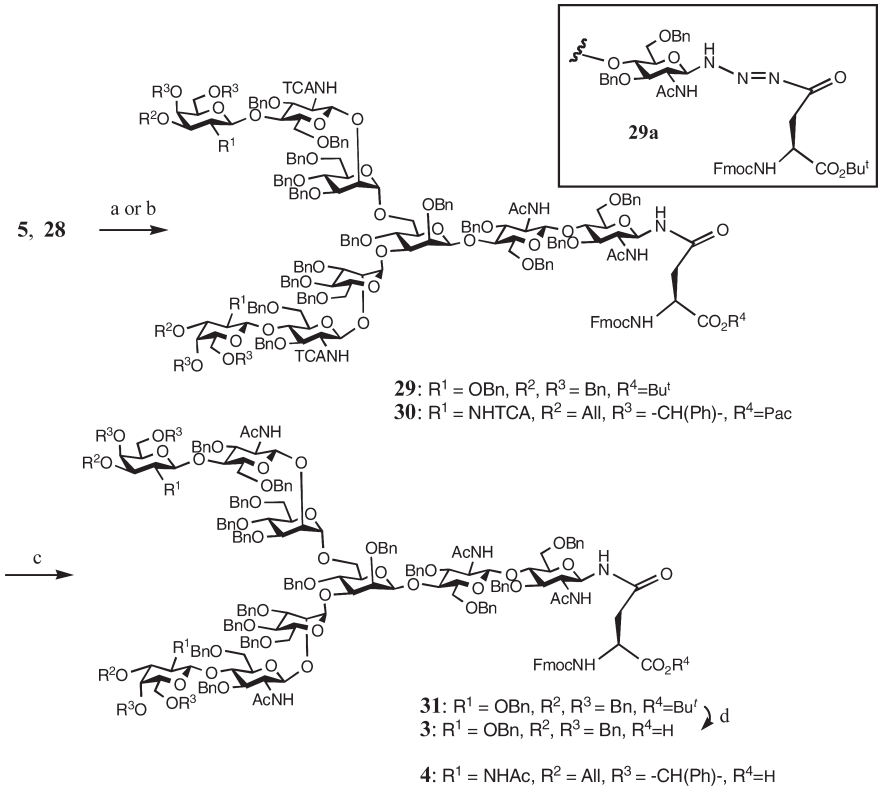
Scheme 6. Synthesis of Nonasaccharyl Asn Building Blocks[a]



[a] Reagents and conditions: (a) $Ph(CH_3)_2P$, Fmoc-Asp(OPfp)-OBu^t, HOOBt, 98% THF aq, rt, 2 h, **29**: 84%; (b) $Ph(CH_3)_2P$, Fmoc-Asp(OPfp)-OPac, HOOBt, 98% THF aq, rt, 2 h, **30**: 83%; (c) Zn, AcOH, EtOAc, microwave (150 W), 1 h, **31**: 86%, **4**: 45%; (d) 80% TFA aq, **3**: 71%.

to acetamide was necessary before coupling with the base-labile *N*-Fmoc aspartic acid derivative, in which the dephthaloylation conditions were carefully explored to avoid any side reactions on the susceptible trichloroacetyl groups. Coupling of the nonasaccharides with aspartic acid derivatives was successfully promoted under the modified Staudinger reaction by selecting $Ph(CH_3)_2P$. The benzyl-protected **3** and **4** would be used for the solid-phase synthesis of glycopeptides carrying *N*-glycan, which would contribute to the biological studies to discriminate the functions between the LacNAc and LacdiNAc *N*-glycans. Syntheses of these glycopeptides are currently being investigated.

EXPERIMENTAL SECTION

General. Specific rotation values were at 20 ± 2 °C for solutions in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded on a 400 or 600 MHz spectrometer. H−H and C−H COSY spectra were measured to confirm the NMR peak assignments. Chemical shifts are expressed in parts per million downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. For description of the NMR data, each sugar residue in oligosaccharide is indicated by an alphabetical mark as shown in Figure 1. Recycling HPLC was performed with a recycling preparative HPLC. MALDI TOF mass spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix. High-resolution mass spectra were obtained with a Fourier transform ion cyclotron resonance mass spectrometer.

2-O-Benzyl-4,6-O-benzylidene-3-O-(4-methoxyphenyl)methyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***13***. A stirred mixture of **11** (1.02 g, 1.79 mmol), BSP (0.56 g, 2.67 mmol), TTBP (0.89 g, 3.58 mmol), and dried MS 4A (6.42 g) in anhydrous CH_2Cl_2 (35 mL) was cooled at −60 °C for 30 min. The mixture was stirred for a further 20 min after addition of Tf_2O (0.33 mL, 1.98 mmol). A solution of **12** (0.88 g, 0.89 mmol) in anhydrous CH_2Cl_2 (10 mL) was slowly added to the mixture through a cannula. Then the mixture was allowed to stir for 2 days at the temperature, before the reaction was quenched by addition of satd $NaHCO_3$ aq. The mixture was diluted with $CHCl_3$ and filtered through Celite. The organic layer of the combined filtrate and $CHCl_3$ was successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene−EtOAc (9:1) to give trisaccharides as a mixture (1.30 g). Separation of the isomers was performed by recycling HPLC to give **13** (0.94 g, 73%). The α-isomer was not obtained as a homogeneous sample even by the recycling HPLC. Compound **13**: $[\alpha]_D$ +0.3 (*c* 1.3). R_f 0.38 (9:1 toluene−EtOAc). ^1H NMR: δ 7.87−7.21 (m, 20H, Ar), 6.95−6.75 (m, 12H, Ar), 5.49 [s, 1H, PhC*H*(O)$_2$<], 5.29 (d, 1H, *J* = 8.0 Hz, H-1b), 5.16 (d, 1H, *J* = 9.3 Hz, H-1a), 4.90−4.82 (m, 4H, −C*H*$_2$Ar × 4), 4.66 (d, 1H, *J* = 12.0 Hz, −C*H*$_2$Ar), 4.56 (s, 1H, H-1c), 4.67−4.48 (m, 5H, −C*H*$_2$Ar × 5), 4.40 (d, 1H, *J* = 12.4 Hz, −C*H*$_2$Ar), 4.37 (d, 1H, *J* = 12.2 Hz, −C*H*$_2$Ar), 4.28−4.02 (m, 8H), 3.78 (s, 3H, −OC*H*$_3$), 3.72 (d, 1H, *J* = 3.2 Hz, H-2c), 3.62−3.49 (m, 3H), 3.45−3.37 (m, 4H), 3.20 (m, 1H), 3.12 (m, 1H, H-5c). ^{13}C NMR: δ 85.8 (C-1a, $^1J_{CH}$ = 165.1 Hz), 97.3 (C-1b, $^1J_{CH}$ = 167.2 Hz), 101.5 (C-1c, $^1J_{CH}$ = 168.5 Hz), 102.0 [PhC*H*<]. Anal. Calcd for $C_{84}H_{79}N_5O_{18}$: C, 69.75; H, 5.50; N, 4.84. Found: C, 69.85; H, 5.54; N, 4.80.

2,4-Di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***8***. To a stirred mixture of **13** (338 mg, 0.23 mmol) and dried MS 4A (1.7 g) in anhydrous CH_2Cl_2 (20 mL) were successively added Et_3SiH (0.11 mL, 0.70 mmol)

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and $PhBCl_2$ (0.11 mL, 0.82 mmol) at −78 °C. The mixture was stirred for 1 h at that temperature. The reaction was quenched by adding Et_3N (1.7 mL) and MeOH (3 mL). The resulting mixture was stirred for 30 min at room temperature, diluted with $CHCl_3$, and filtered through Celite. The combined filtrate and washings were successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (8 mL) and stirred with 90% aq. TFA (8 mL) at −10 °C for 30 min. Then the acidic mixture was carefully neutralized with satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene−EtOAc (4:1) to afford **8** (294 mg, 95%), NMR data of which were superimposable on those of the authentic sample prepared previously.[14] $[\alpha]_D$ +0.3 (*c* 1.3). R_f 0.32 (3:1 toluene−EtOAc). ^{1}H NMR: δ 5.30 (d, 1H, *J* = 7.8 Hz, H-1b), 5.17 (d, 1H, *J* = 9.3 Hz, H-1a), 5.17 (s, 1H, H-1c). ^{13}C NMR: d 101.1 (C-1c), 96.9 (C-1b), 85.4 (C-1a). MALDI TOF MS: calcd for $C_{76}H_{73}N_5O_{17}$ $(M+Na)^+$, *m*/*z* 1350.50. Found: 1350.60.

2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-D-glucopyranosyl (N-Phenyl)trifluoroacetimidate ***9i***. A mixture of known hemiacetal[5] (87 mg, 85 μmol), (*N*-phenyl)trifluoroacetimidoyl chloride (35 mg, 170 μmol), and K_2CO_3 (24 mg, 170 μmol) in anhydrous acetone (1 mL) was stirred at room temperature for 1 h. Then the insoluble material was filtered off through Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel with toluene−EtOAc (49:1) to quantitatively afford **9i** (102 mg) as a mixture of stereoisomers. R_f 0.28 (49:1 toluene−EtOAc). ^{1}H NMR: δ 7.36−7.16 (m, 32H, Ar), 7.07 (t, 1H, *J* = 7.5 Hz, Ar), 6.76 (d, 2H, *J* = 7.8 Hz, Ar), 6.61 (d, 1H, *J* = 7.6 Hz, −N*H*), 4.14 (brt, 1H, *J* = 9.2 Hz, H-4a), 3.91 (d, 1H, *J* = 2.7 Hz, H-4b). Anal. Calcd for $C_{64}H_{62}Cl_3F_3N_2O_{11}$: C, 64.14; H, 5.21; N, 2.34. Found: C, 64.24; H, 5.27; N, 2.36.

Thiophenyl 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside ***7s***. *Method A (Reaction of* ***9f*** *and* ***10****)*. A mixture of Cp_2ZrCl_2 (121 mg, 0.41 mmol), $AgClO_4$ (171 mg, 0.82 mmol), and dried MS 4A (380 mg) in anhydrous CH_2Cl_2 (4.6 mL) was stirred under Ar at room temperature for 30 min and then cooled at −60 °C. To the stirred mixture was added a mixture of **9f** (215 mg, 0.21 mmol) and **10** (124 mg, 0.23 mmol) in anhydrous CH_2Cl_2 (5 mL) though a cannula. The resulting mixture was stirred at −60 °C for 1 h and then at −50 °C for 2.5 h before the reaction was quenched by addition of sat. $NaHCO_3$ aq. The mixture was diluted with EtOAc and filtered through Celite. The organic layer was successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on Bio-Beads S-X1 with toluene and then on silica gel with hexane−EtOAc (4:1−3:1) to afford **7s** (276 mg, 85%, α/β = 1/49). The minor α-isomer was separated by HPLC and identified by the mass spectrum. **Compound 7s**: $[\alpha]_D$ +38.6 (*c* 1.1). R_f 0.35 (8:3 hexane−EtOAc). ^{1}H NMR: δ 7.45−7.10 (m, 51H, Ar, −N*H*), 5.54 (d, 1H, *J* = 1.5 Hz, H-1a), 5.23 (d, 1H, *J* = 8.3 Hz, H-1b), 5.06 (d, 1H, *J* = 10.2 Hz, −CH_2Ph), 4.98 (d, 1H, *J* = 11.2 Hz, −CH_2Ph), 4.85−4.72 (m, 6H, −CH_2Ph), 4.56−4.37 (m, 11H, H-1c, H-2a, H-3b, −CH_2Ph), 4.27 (d, 1H, *J* = 10.2 Hz, −CH_2Ph), 4.25 (m, 1H, H-5a), 4.24 (d, 1H, *J* = 11.7 Hz, −CH_2Ph), 4.03 (brt, 1H, *J* = 8.8, 9.3 Hz, H-4b), 3.92−3.63 (m, 8H, H-3a, H-4a, H-5a, H-6a, H-6a, H-2c, H-4c, H-6c, H-6c), 3.54−3.51 (m, 2H, H-5b, H-6b), 3.44−3.34 (m, 4H, H-2b, H-6b, H-3c, H-5c). ^{13}C NMR δ: 102.8 (C-1c), 97.0 (C-1b), 92.2 (-CCl_3), 86.2 (C-1a). MALDI TOF MS: calcd for $C_{89}H_{92}Cl_3NO_{15}S$ $[M+Na]^+$, *m*/*z* 1574.51 (100%). Found: 1573.12. Anal. Calcd for $C_{89}H_{92}Cl_3NO_{15}S$: C, 68.87; H, 5.84; N, 0.90. Found: C, 68.82; H, 5.90; N, 0.96.

α-isomer: R_f 0.35 (8:3 hexane−EtOAc). MALDI TOF MS: Found: 1573.09.

Method B (Reaction of ***9i*** *and* ***10****)*. A stirred mixture of **9i** (102 mg, 85 μmol), **10** (48 mg, 89 μmol), and dried MS AW300 (300 mg) in anhydrous CH_2Cl_2 (3 mL) was cooled at −78 °C. Then 2% TMSOTf/CH_2Cl_2 (39 μL, 4.3 μmol) was added to the mixture. The stirring was continued for 1.5 h at that temperature and for 0.5 h at −40 °C. The reaction was quenched by addition of Et_3N, and the insoluble material was filtered off. The filtrate was concentrated in vacuo. The residue was chromatographed on Bio-Beads and then on silica gel as described above to give **7s** (105 mg, 79%, α/β = 1/99). The minor isomer was separated by HPLC and identified by the mass spectrum.

2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl Fluoride ***7f***. Compound **7s** (210 mg, 0.14 mmol) was dissolved in 85% aq. acetone (2.7 mL). *N*-Bromosuccimide (NBS, 133 mg, 0.75 mmol) was added to the stirred solution, which was stirred at room temperature for 2.5 h. The reaction mixture was diluted with EtOAc, washed with 5% aq. $Na_2S_2O_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with hexane−EtOAc (7:3−3:2) to afford trisaccharyl hemiacetal (168 mg, 85%). R_f 0.41 (3:2 hexane−EtOAc). MALDI TOF MS: calcd for $C_{83}H_{86}Cl_3NO_{16}$ $[M + Na]^+$, *m*/*z* 1480.58. Found: 1480.50. To a stirred solution of the hemiacetal in anhydrous CH_2Cl_2 (5.2 mL) was added (diethylamino)sulfur trifluoride (DAST, 28 μL, 0.21 mmol) at 0 °C. The mixture was stirred for 0.5 h, and the reaction was quenched by adding satd $NaHCO_3$ aq. The mixture was diluted with EtOAc, successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene−EtOAc (9:1) to give **7f** [α-fluoride (151 mg, 90%) and β-fluoride (13 mg, 8%)]. **α-Fluoride**: R_f 0.41 (3:2 toluene−EtOAc). ^{1}H NMR: δ 5.58 (brd, 1H, *J* = 50.7 Hz, H-1a), 5.20 (d, 1H, *J* = 8.3 Hz, H-1b), 4.48 (d, 1H, *J* = 7.3 Hz, H-1c). MALDI TOF MS: calcd for $C_{83}H_{86}Cl_3NO_{16}$ $[M + Na]^+$, *m*/*z* 1482.50. Found: 1482.54. **β-Fluoride**: R_f 0.27 (3:2 toluene−EtOAc). ^{1}H NMR: δ 5.31 (brd, 1H, *J* = 51.7 Hz, H-1a), 5.09 (d, 1H, *J* = 8.3 Hz, H-1b), 4.43 (d, 1H, *J* = 7.3 Hz, H-1c). MALDI TOF MS: calcd for $C_{83}H_{86}Cl_3NO_{16}$ $[M + Na]^+$, *m*/*z* 1482.50 (96%). Found: 1482.61.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***15***. A mixture of Cp_2ZrCl_2 (497 mg, 1.70 mmol), $AgClO_4$ (500 mg, 2.41 mmol), and dried MS 4A (1.2 g) in anhydrous CH_2Cl_2 (4 mL) was stirred under Ar at room temperature for 30 min and then cooled at −60 °C. To the stirred mixture was added a mixture of **8** (263 mg, 0.20 mmol) and **14** (293 mg, 0.59 mmol) in anhydrous CH_2Cl_2 (8 mL) through a cannula. Then the temperature was raised to −15 °C. The mixture was stirred for 2 h at the temperature before the reaction was quenched by addition of satd $NaHCO_3$ aq. The mixture was diluted with EtOAc and filtered through Celite. The organic layer was successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on Bio-Beads S-X1 with toluene to afford **15** (373 mg, 83%). The NMR data of **15** were identical with those of the sample reported previously.[14] R_f 0.46 (4:1 toluene−EtOAc). ^{1}H NMR: δ 5.49 (brs, 1H, H-2e), 5.31 (brs, 1H, H-2d), 5.21 (d, 1H, *J* = 7.8 Hz, H-1b), 5.14 (s, 1H, H-1e), 5.13 (d, 1H, *J* = 7.8 Hz, H-1a), 4.84 (s, 1H, H-1d), 2.09 (s, 3H, −COCH_3), 1.79 (s, 3H, −COCH_3). ^{13}C NMR: δ 102.2 (C-1c), 99.9 (C-1d), 98.5 (C-1e), 97.2 (C-1b), 85.8 (C-1a), 21.3 and 21.0 (−$COCH_3$). MALDI TOF MS: calcd for $C_{134}H_{133}N_5O_{29}$ $[M + Na]^+$, *m*/*z* 2298.91. Found: 2297.93.

3,4,6-Tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-O-benno-pyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***16***. To a solution of **15** (130 mg, 57 μmol) in THF (32 mL)

were added 30% H_2O_2 (6.5 mL, 80.3 mmol) and 1 N LiOH aq (6.5 mL, 6.5 mmol) at 0 °C. The mixture was stirred for 18 h at 0 °C. Then the mixture was diluted with EtOAc, successively washed with aq $Na_2S_2O_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by two chromatographies on silica gel with toluene−EtOAc (9:1−7:3) and then with hexane−EtOAc (3:2−1:1) to afford **16** (91 mg, 72%). $[\alpha]_D$ +19.9 (*c* 0.5). R_f 0.36 (7:3 toluene−EtOAc). ^{1}H NMR: δ 5.24 (m, 1H, H-1b), 5.15 (s, 1H, H-1e), 5.14 (d, 1H, J = 7.8 Hz, H-1a), 4.81 (s, 1H, H-1d), 4.59 (s, 1H, H-1c). ^{13}C NMR: δ 101.7, 101.5, and 99.8 (C-1c, C-1d, C-1e), 97.1 (C-1b), 85.5 (C-1a). MALDI TOF MS: calcd for $C_{130}H_{129}N_5O_{27}$ $[M + Na]^+$, m/z 2215.88 (100%). Found: 2215.99. Anal. Calcd for $C_{130}H_{129}N_5O_{27}$: C, 71.18; H, 5.93; N, 3.19. Found: C, 70.92; H, 6.00; N, 3.16.

2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-D-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***17***. *Method A (Reaction of* ***7s*** *and* ***8****).* A mixture of **7s** (97 mg, 62 μmol), **8** (42 mg, 32 μmol), *N*-iodosuccimide (NIS, 22 mg, 98 μmol), and dried MS 3A (130 mg) in anhydrous CH_2Cl_2 (1 mL) was stirred at −20 °C under Ar for 30 min. To the mixture was added a solution of TfOH (0.83 μL, 9 μmol) in anhydrous CH_2Cl_2 (0.1 mL). The reaction mixture was stirred at −20 °C for 1 h before quenching with satd $NaHCO_3$ aq. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with aq NaS_2O_3, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on Bio-Beads S X1 in toluene to afford a fraction composed of nonasaccharides (88 mg). The separated fraction was concentrated in vacuo, and the residue was analyzed by recycling HPLC with a column of Inertsil SIL-100A (20 mm × 250 mm) in hexane−EtOAc (13:7). After five times recycling elution, the chromatogram showed three major components.

Method B (Reaction of ***7f*** *and* ***8****).* A mixture of Cp_2ZrCl_2 (55 mg, 187 μmol), $AgClO_4$ (75 mg, 362 μmol), and dried MS 4A (190 mg) in anhydrous CH_2Cl_2 (0.4 mL) was stirred under Ar at room temperature for 0.5 h and then cooled at −40 °C. To the stirred mixture was slowly added a mixture of **7f** (132 mg, 90 μmol) and **8** (55 mg, 41 μmol) in anhydrous CH_2Cl_2 (1.1 mL). The mixture was stirred at the temperature for 0.5 h, and then the temperature was raised to −20 °C during 1.5 h. Finally, the mixture was stirred at 0 °C for 1.5 h before quenching with satd $NaHCO_3$ aq. The mixture was diluted with EtOAc and filtered through Celite. The organic layer was successively washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on Bio-Beads S-X1 in toluene to afford a nonasaccharide fraction (73 mg). Recycling HPLC demonstrated a pattern of the chromatogram for this sample similar to that obtained by method A.

Method C (Reaction of ***9i*** *and* ***16****).* A stirred mixture of **9i** (1.40 g, 1.17 mmol), **16** (0.75 g, 0.34 mmol), and dried MS AW300 (10 g) in anhydrous CH_2Cl_2 (30 mL) was cooled at −40 °C. Then TMSOTf (30 μL, μmol) was added to the mixture. The stirring was continued for 2 h at that temperature. The reaction was quenched by addition of satd $NaHCO_3$ aq, and the insoluble material was filtered off. The filtrate was concentrated in vacuo. The residue was chromatographed on Bio-Beads S-X1 in toluene and then on silica gel with hexane−EtOAc (3:2) to give **17** (1.23 g, 85%) as a single stereoisomer. Homogeneity of the product was evidenced by recycling HPLC. $[\alpha]_D$ +17.8 (*c* 0.8). R_f 0.36 (7:5 toluene−EtOAc). ^{1}H NMR: δ 5.18 (d, 1H, J = 7.8 Hz, H-1b), 5.12 (d, 1H, J = 8.8 Hz, H-1a). ^{13}C NMR: δ 102.7 and 102.5 ($^1J_{CH}$ = 160.6 Hz, C-1f and C-1i), 101.8 ($^1J_{CH}$ = 158.9 Hz, C-1c), 100.0 ($^1J_{CH}$ = 173.0 Hz) and 98.8 ($^1J_{CH}$ = 168.3 Hz) (C-1d and C-1 g), 97.8 ($^1J_{CH}$ = 165.5 Hz) and 97.6 ($^1J_{CH}$ = 166.3 Hz) (C-1 h and C-1e), 96.7 ($^1J_{CH}$ = 168.0 Hz, C-1b), 92.4 and 92.2 ($-CCl_3$), 85.3 ($^1J_{CH}$ = 170.5 Hz, C-1a). Anal. Calcd for $C_{242}H_{241}Cl_6N_7O_{47}$: C, 69.00; H, 5.77; N, 2.33. Found: C, 68.99; H, 5.86; N, 2.38.

2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl Azide ***5***. A mixture of **17** (245 mg, 58 μmol) and 1,2-diaminoethane (785 μL, 11.7 mmol) in *n*-BuOH (5 mL) was heated at 90 °C for 2 h. After cooling, the mixture was diluted with EtOAc and washed with water until the washings became neutral pH. The organic extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in toluene (10 mL), heated at 90 °C overnight, and then concentrated in vacuo. The crude diamino product was stirred with Ac_2O (1 mL) in a mixture of CH_2Cl_2 (2 mL) and MeOH (4 mL) at room temperature for 0.5 h. The reaction mixture was concentrated in vacuo, and the volatile materials were removed by coevaporation with toluene. The crude product was chromatographed on silica gel with hexane−EtOAc (2:3) to give **5** (75%). $[\alpha]_D$ +1.9 (*c* 2.3). R_f 0.28 (2:3 hexane−EtOAc). ^{1}H NMR: δ 1.64 (s, 3H, $-COCH_3$), 1.40 (s, 3H, $-COCH_3$). ^{13}C NMR: δ 102.8 and 102.6 (C-1f and C-1i), 101.0, 100.0, 99.6, 99.0, 97.9, and 97.2 (C-1b, C-1c, C-1d, C-1e, C-1 g, C-1 h), 92.6 and 92.3 (-CCl_3), 88.0 (C-1a), 23.2 ($-COCH_3$), 22.7 ($-COCH_3$). MALDI TOF MS: calcd for $C_{230}H_{241}Cl_6N_7O_{45}$ $[M + Na]^+$, m/z 4059.1. Found: 4060.9. Anal. Calcd for $C_{230}H_{241}Cl_6N_7O_{45}$: C, 68.44; H, 6.02; N, 2.43. Found: C, 68.09; H, 6.05; N, 2.42.

tert-Butyldiphenylsilyl 3-O-Allyl-2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside ***19***. To a stirred mixture of **18** (1.46 g, 2.7 mmol) and 60% NaH/mineral oil (0.16 g, 4.1 mmol) in anhydrous DMF (25 mL) was added allyl bromide (0.36 mL, 4.1 mmol). The mixture was stirred at room temperature for 1.5 h. Then the reaction was quenched carefully by adding a few pieces of ice, and the mixture was concentrated in vacuo. The residue was extracted with EtOAc, successively washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene−EtOAc (39:1−19:1) to give **19** (1.36 g, 87%). $[\alpha]_D$ +27.0 (*c* 0.5). R_f 0.44 (19:1 toluene−EtOAc). ^{1}H NMR: δ 7.80 (m, 2H, Ar), 7.73 (m, 2H, Ar), 7.53 (m, 2H, Ar), 7.43−7.31 (m, 9H, Ar), 5.92 (m, 1H, $-CH{=}CH_2$), 5.47 [s, 1H, PhC*H*<], 5.30 (dq, 1H, J = 1.5, 17.1 Hz, $-CH{=}CH_2$), 5.17 (dd, 1H, J = 1.5, 10.2 Hz, $-CH{=}CH_2$), 4.40 (d, 1H, J = 7.8 Hz, H-1), 4.20−4.10 (m, 2H, $-CH_2CH{=}CH_2$), 4.07 (d, 1H, J = 2.9 Hz, H-4), 3.95 (dd, 1H, J = 1.5, 12.2 Hz, H-6), 3.89−3.83 (m, 2H, H-2, H-6), 3.21 (dd, 1H, J = 3.4, 10.2 Hz, H-3), 2.94 (d, 1H, J = 1.0 Hz, H-5), 1.13 (s, 9H, Bu^t). Anal. Calcd for $C_{32}H_{37}N_3O_5Si$: C, 67.22; H, 6.52; N, 7.35. Found: C, 67.45; H, 6.57; N, 7.33.

tert-Butyldiphenylsilyl 3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside ***20***. A mixture of **19** (200 mg, 0.35 mmol), powdered Zn (875 mg, 13.4 mmol), and AcOH (0.4 mL, 7.0 mmol) in CH_2Cl_2 (8.7 mL) was stirred at room temperature for 40 min. The mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was successively washed with satd $NaHCO_3$ aq and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in pyridine (3.5 mL) and stirred with trichloroacetyl chloride (59 μL) at 0 °C for 30 min. Most of the pyridine was evaporated in vacuo, and the residue was extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel with toluene−EtOAc (29:1) to afford **20** (187 mg, 77%). $[\alpha]_D$ +16.7 (*c* 0.5). R_f 0.32 (19:1 toluene−EtOAc). ^{1}H

NMR: δ 7.76 (m, 2H, Ar), 7.65 (m, 2H, Ar), 7.53 (m, 2H, Ar), 7.40−7.30 (m, 9H, Ar), 6.92 (d, 1H, *J* = 6.8 Hz, −N*H*), 5.86 (m, 1H, −C*H*═CH_2), 5.50 [s, 1H, PhC*H*<], 5.24 (brd, 1H, *J* = 17.4 Hz, −CH═CH_2), 5.16 (d, 1H, *J* = 7.8 Hz, H-1), 5.14 (m, 1H, CH═CH_2), 4.17 (d, 1H, *J* = 3.4 Hz, H-4), 4.13−4.08 (m, 2H, H-3, −CH_2CH═CH_2), 4.02 (dd, 1H, *J* = 5.8, 12.7 HZ, −CH_2CH═CH_2), 3.97 (dd, 1H, *J* = 1.0, 12.2 Hz, H-6), 3.93−3.86 (m, 2H, H-2, H-6), 3.09 (s, 1H, H-5), 1.09 (s, 9H, Bu^t). ^{13}C NMR: δ 100.9 [PhCH(O)$_2$<], 94.2 (C-1)1, 92.5 (−CCl_3). Anal. Calcd for $C_{34}H_{38}Cl_3NO_6Si$: C, 59.09; H, 5.54; N, 2.03. Found: C, 59.16; H, 5.55; N, 2.07.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-D-galactopyranose ***21***. To a stirred mixture of **20** (216 mg, 0.18 mmol) and AcOH (105 μL, 1.8 mmol) in freshly distilled THF (2 mL) was added 1 M tetra-*n*-butylammonium fluoride/THF (TBAF, 3.5 mL, 3.5 mmol) at 0 °C. Then the mixture was stirred at room temperature for 20 h. THF was evaporated in vacuo, and the product was extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene−EtOAc (1:1) to give **21** (166 mg, 96%). R_f 0.35 (1:1 toluene−-EtOAc). ^{1}H NMR: δ 7.53−7.51 (m, 2H, Ar), 7.38−7.30 (m, 3H, Ar), 6.90 (d, 1H, *J* = 8.3 Hz, −N*H*), 5.88 (m, 1H, −C *H*═CH_2), 5.53 [s, 1H, PhC*H*<], 5.45 (brt, 1H, *J* = 3.4 Hz, H-1), 5.29 (dd, 1H, *J* = 1.5, 17.1 Hz, −CH═CH_2), 5.18 (dd, 1H, *J* = 1.5, 10.2 Hz, −CH═CH_2), 4.47 (m, 1H, H-2), 4.26−4.14 (m, 3H, H-4, H-6, −CH_2CH═CH_2), 4.05−3.97 (m, 3H, H-6, −CH_2CH═CH_2, −O*H*), 3.82 (dd, 1H, *J* = 2.9, 10.7 Hz, H-3), 3.81 (brs, 1H, H-5). Anal. Calcd for $C_{18}H_{20}Cl_3NO_6$: C, 47.75; H, 4.45; N, 3.09. Found: C, 47.85; H, 4.51; N, 3.12.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-D-galactopyranosyl (N-Phenyl)trifluoroacetimidate ***22***. A mixture of **21** (373 mg, 0.83 mmol), (*N*-phenyl)trifluoroacetimidoyl chloride (343 mg, 1.65 mmol), and K_2CO_3 (570 mg, 4.13 mmol) in anhydrous acetone (8 mL) was stirred at room temperature for 1 h. Then the mixture was diluted with EtOAc, and the insoluble material was filtered off through Celite. The filtrate was concentrated in vacuo to the residue, which was chromatographed on silica gel with toluene−EtOAc (29:1) to quantitatively afford **22** (455 mg, 88%). R_f 0.44 (9:1 toluene−EtOAc). ^{1}H NMR: δ 7.52 (m, 2H, Ar), 7.38−7.23 (m, 5H, Ar), 7.11 (t, 1H, *J* = 7.8 Hz, Ar), 6.80 (d, 2H, *J* = 7.8 Hz, Ar), 6.66 (d, 1H, *J* = 6.8 Hz, −N*H*), 5.93 (m, 1H, −C*H*═CH_2), 5.60 [s, 1H, PhC*H*<], 5.34 (dd, 1H, *J* = 1.5, 17.4 Hz, −CH═CH_2), 5.26 (dd, 1H, *J* = 1.0, 10.2 Hz, −CH═CH_2), 4.69 (m,1H, H-2), 4.42−4.25 (m, 3H, H-6, −CH_2CH═CH_2), 4.10−4.04 (m, 2H, H-3, H-4), 3.93 (dd, 1H, *J* = 2.4, 10.7 Hz, H-6), 3.79 (brs, 1H, H-5). Anal. Calcd for $C_{26}H_{24}Cl_3F_3N_2O_6$: C, 50.06; H, 3.88; N, 4.49. Found: C, 50.16; H, 3.95; N, 4.40.

tert-Butyldiphenylsilyl 3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside ***24***. A stirred mixture of **22** (153 mg, 0.25 mmol), **23** (151 mg, 0.20 mmol), and dried MS AW300 (610 mg) in anhydrous CH_2Cl_2 (6 mL) was cooled at −78 °C. Then 10% TMSOTf/CH_2Cl_2 (20 μL, 0.01 mmol) was added to the mixture. The stirring was continued for 1 h at that temperature and for 1.5 h at −40 °C. The reaction was quenched by addition of satd $NaHCO_3$ aq; the mixture was diluted with EtOAc; and the insoluble material was filtered off. The combined filtrate and washings were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with hexane−EtOAc (4:1−2:1) to give **24** (224 mg, 94%). $[\alpha]_D$ +4.0 (*c* 1.0). R_f 0.40 (3:2 hexane−EtOAc). ^{1}H NMR: δ 7.68 (d, 2H, *J* = 6.8 Hz, Ar), 7.61 (d, 2H, *J* = 6.8 Hz, Ar), 7.60−7.09 (m, 21H, Ar), 6.83 (d, 1H, *J* = 8.3 Hz, −N*H*a), 6.79 (d, 1H, *J* = 7.3 Hz, −N*H*b), 5.86 (m, 1H, −C*H*═CH_2), 5.51 [s, 1H, PhC*H*<], 5.27 (dd, 1H, *J* = 1.5, 17.1 Hz, −CH═CH_2), 5.17 (brd, 1H, *J* = 10.7 Hz, −CH═CH_2), 5.07 (d, 1H, *J* = 10.7 Hz, −CH_2Ph), 5.03 (d, 1H, *J* = 8.3 Hz, H-1b), 4.80 (d, 1H, *J* = 7.3 Hz, H-1a), 4.56 (d, 1H, *J* = 10.7 Hz, −CH_2Ph), 4.49 (d, 1H, *J* = 11.7 Hz, −CH_2Ph), 4.36 (d, 1H, *J* = 11.7 Hz, −CH_2Ph), 4.28−4.19 (m, 3H, H-4a, H-4b, H-6b), 4.14−4.00 (m, 3H, H-3b, −CH_2CH═CH_2), 3.92−3.71 (m, 4H, H-6b, H-3a, H-2a, H-2b), 3.52 (dd, 1H, *J* = 2.9, 11.2 Hz, H-6a), 3.30 (brd, 1H, *J* = 10.3 Hz, H-6a), 3.31 (s, 1H, H-5b), 3.00 (brd, 1H, *J* = 8.8 Hz, H-5a), 1.04 (s, 9H, Bu^t). ^{13}C NMR: δ 100.9 [PhC*H*<], 97.8 (C-1b), 95.1 (C-1a), 92.5 (−CCl_3), 92.3 (−CCl_3). MALDI TOF MS: calcd for $C_{56}H_{60}Cl_6N_2O_{11}Si$ [M + Na]$^+$, *m*/*z* 1199.20 (100%). Found: 1199.28. Anal. Calcd for $C_{56}H_{60}Cl_6N_2O_{11}Si$: C, 57.10; H, 5.13; N, 2.38. Found: C, 57.08; H, 5.12; N, 2.36.

In a more mobile fraction, the α-isomer (8 mg, 3%) was also obtained. R_f 0.48 (3:2 hexane−EtOAc). ^{1}H NMR: δ 5.68 (d, 1H, *J* = 3.9 Hz, H-1b), 5.02 (d, 1H, *J* = 7.3 Hz, H-1a). MALDI TOF MS: *m*/*z* 1200.16.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-D-glucopyranose ***25***. To a stirred mixture of **24** (102 mg, 86 μmol) and AcOH (49 μL, 860 μmol) in freshly distilled THF (1 mL) was added 1 M tetra-*n*-butylammonium fluoride/THF (0.34 mL, 340 μmol) at 0 °C. Then the mixture was stirred at room temperature for 20 h. THF was evaporated in vacuo, and the product was extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene−EtOAc (7:1−2:1) to give **25** (77 mg, 96%) as an α-anomer-rich mixture (α/β = 4.4/1). R_f 0.38 (1:1 hexane−EtOAc). ^{1}H NMR (α-anomer): δ 7.41−7.14 (m, 15H, Ar), 6.83 (d, 1H, *J* = 7.3 Hz, −N*H*), 6.77 (d, 1H, *J* = 7.8 Hz, −N*H*), 5.89 (m, 1H, −C*H*═CH_2), 5.52 [s, 1H, PhC*H*<], 5.39 (d, 1H, *J* = 3,4 Hz, H-1a), 5.32 (dq, 1H, *J* = 1.5, 17.1 Hz, −CH═CH_2), 5.17 (dd, 1H, *J* = 1.5, 10.3 Hz, −CH═CH_2), 5.01 (d, 1H. *J* = 8.3 Hz, H-1b). Anal. Calcd for $C_{40}H_{42}Cl_6N_2O_{11}$: C, 51.14; H, 4.51; N, 2.98. Found: C, 51.15; H, 4.47; N, 2.92.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-D-glucopyranosyl (N-phenyl)trifluoroacetimidate ***26***. A mixture of **25** (74 mg, 80 μmol), (*N*-phenyl)trifluoroacetimidoyl chloride (33 mg, 158 μmol), and K_2CO_3 (55 mg, 398 μmol) in anhydrous acetone (1 mL) was stirred at room temperature for 1 h. Then the mixture was diluted with EtOAc, and the insoluble material was filtered off through Celite. The filtrate was concentrated in vacuo to the residue, which was chromatographed on silica gel with toluene−EtOAc (9:1−7:1) to give **26** (85 mg, 96%) as a mixture of isomers. R_f 0.41 and 0.34 (4:1 toluene−EtOAc). ^{1}H NMR (major isomer): δ 7.41−7.18 (m, 17H, Ar), 7.09 (t, 1H, *J* = 7.3 Hz, Ar), 6.84 (d, 1H, *J* = 7.8 Hz, −N*H*), 6.74 (d, 2H, *J* = 7.8 Hz, Ar), 6.51 (d, 1H, *J* = 7.3 Hz, −N*H*), 5.90 (m, 1H, −C*H*═CH_2), 5.54 [s, 1H, PhC*H*<], 5.32 (dd, 1H, *J* = 1.5, 17.1 Hz, −CH═CH_2), 5.22 (dd, 1H, *J* = 1.0, 10.5 Hz, −CH═CH_2), 5.08 (d, 1H, *J* = 8.3 Hz, H-1b), 3.15 (s, 1H, H-5b). Anal. Calcd for $C_{48}H_{46}Cl_6F_3N_3O_{11}$: C, 51.91; H, 4.17; N, 3.78. Found: C, 51.95; H, 4.28; N, 3.71.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3-O-allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide ***27***. A stirred mixture of **26** (243 mg, 219 μmol), **16** (92 mg, 42 μmol), and dried MS AW300 (180 mg) in anhydrous CH_2Cl_2 (2 mL) was cooled at −78 °C. Then 10% TMSOTf/CH_2Cl_2 (38 μL, 21 μmol) was added to the mixture, which was stirred for 2 days at at −40 °C. The reaction was quenched by addition of satd $NaHCO_3$ aq, and the insoluble material was filtered off. The filtrate was concentrated in vacuo. The residue was chromatographed on Bio-Beads S-X1 in toluene to give a nonasaccharide fraction, which was further purified by chromatography on silica with hexane−$CHCl_3$−EtOAc (2:1:1) to give **27** (117 mg, 69%). $[\alpha]_D$ +5.1 (*c* 0.5). R_f 0.22 (1:1 hexane−EtOAc). ^{1}H NMR: δ

7.64−7.44 (m, 9H, Ar), 7.34−6.76 (m, 86H, Ar, −N*H* × 3), 6.67−6.55 (m, 7H, Ar, −N*H*), 5.86−5.74 (m, 2H, −C*H*═CH_2 × 2), 5.43 [s, 1H, PhC*H*(O)$_2$<], 5.40 [s, 1H, PhC*H*(O)$_2$<], 5.22 (brd, 1H, *J* = 17.0 Hz, −CH═C*H*$_2$), 5.20 (brd, 1H, *J* = 17.1 Hz, −CH═C*H*$_2$), 5.18−4.91 (m, 9H, −CH═C*H*$_2$ × 2, H-1b, H-1a, H-1d, H-1e, H-1h, −C*H*$_2$Ph × 2), 4.49 (s, 1H, H-1 g), 4.46 (s, 1H, H-1c), 2.88 and 2.74 (2s, 2H, H-5f and H-5i). ^{13}C NMR: δ 102.1 ($^1J_{CH}$ = 159.7 Hz, C-1c), 101.1 [PhC*H*<], 100.6 ($^1J_{CH}$ = 172.9 Hz) and 99.0 ($^1J_{CH}$ = 170.5 Hz) (C-1d and C-1g), 98.3 and 98.2 ($^1J_{CH}$ = 163.8 Hz, C-1f and C-1i), 97.9 and 97.7 ($^1J_{CH}$ = 166.3 Hz, C-1h and C-1e), 97.1 ($^1J_{CH}$ = 168.0 Hz, C-1b), 92.8 and 92.7 (−*C*Cl$_3$), 85.7 ($^1J_{CH}$ = 165.5 Hz, C-1a). Anal. Calcd for $C_{210}H_{209}Cl_{12}N_9O_{47}$: C, 62.49; H, 5.22; N, 3.12. Found: C, 62.20; H, 5.22; N, 3.05.

The crude heptasaccharides (33 mg) derived by the incomplete glycosylation were also obtained by the Biobeads column separation.

3-O-Allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3-O-allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy -β-D-glucopyranosyl Azide ***28***. Compound **27** (34 mg, 8.4 μmol) was converted to **28** (18 mg, 52%) by dephthaloylation and acetylation, in a similar manner as described for **5**.

[α]$_D$ −9.4 (*c* 0.9). R_f 0.49 (1:4 hexane−EtOAc). ^{1}H NMR: δ 6.85 (d, 1H, *J* = 7.3 Hz, −N*H*), 6.61 (d, 1H, *J* = 7.3 Hz, −N*H*), 6.16 (d, 1H, *J* = 8.8 Hz, −N*H*), 5.94−5.83 (m, 2H, −C*H*═CH_2 × 2), 5.52 [s, 1H, PhC*H*<], 5.50 [s, 1H, PhC*H*<], 5.30 (brd, 2H, *J* = 17.1 Hz, −CH═C*H*$_2$), 5.19 (brd, 2H, *J* = 10.2 Hz, −CH═C*H*$_2$), 2.98 and 2.86 (2s, 2H, H-5f and H-5i), 1.73 (s, 3H, −COC*H*$_3$), 1.49 (s, 3H, −COC*H*$_3$). ^{13}C NMR: δ 101.1 (C-1c), 100.9 [PhC*H*(O)$_2$<], 100.2, 99.8, 98.9, 98.2, 97.5, and 97.4 (C-1d, C-1g, C-1f, C-1i, C-1h, C-1e, and C-1b), 92.6, 92.5, 92.4, and 92.3 (−*C*Cl$_3$), 88.1 (C-1a), 23.3 (−CO*C*H$_3$), 23.0 (−CO*C*H$_3$). Anal. Calcd for $C_{198}H_{209}Cl_{12}N_9O_{45}$: C, 61.61; H, 5.46; N, 3.27. Found: C, 62.00; H, 5.27; N, 3.05.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-{2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine tert-Butyl Ester ***29***. A mixture of **5** (236 mg, 59 μmol), Fmoc-Asp(OPfp)-OBu^t (53 mg, 92 μmol), HOOBt (18 mg, 110 μmol), and Ph(CH$_3$)$_2$P (15 μL, 105 μmol) in 98% aq. THF (6 mL) was stirred at room temperature for 3 h under Ar. Then the mixture was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed by HPLC using a gel-permeation column (JAIGEL 2H, 20 × 600 mm) with $CHCl_3$ (3.5 mL/min) and then a column (Inertsil SIL100A, 20 × 250 mm) with hexane−EtOAc (35:65, 5 mL/min) to give **29** (216 mg, 84%). [α]$_D$ +12.4 (*c* 2.8). R_f 0.44 (95:5 toluene−EtOH). ^{1}H and ^{13}C NMR spectra in $CDCl_3$ showed multiple signals due to the presence of conformational isomers. ^{1}H NMR: δ 7.74 [brd, 2H, *J* = 7.6 Hz, Ar (Fmoc)], 7.58 [brd, 2H, *J* = 7.3 Hz, Ar (Fmoc)], 2.80 (dd, 1H, *J* = 3.9, 16.1 Hz, Asn-β*H*), 2.62 (dd, 1H, *J* = 3.4, 16.1 Hz, Asn-β*H*), 1.71 and 1.68 (2s, 3H, −COC*H*$_3$), 1.54, 1.53, 1.50, and 1.49 (4s, 3H, −COC*H*$_3$), 1.41 (s, 9H, Bu^t). ^{13}C NMR: δ 102.8 and 102.7 (C-1f and C-1i), 101.2, 100.0, 99.6, 99.4, 99.0, 98.4, and 97.2 (C-1b, C-1c, C-1d, C-1e, C-1g, C-1h), 92.6 and 92.4 (−*C*Cl$_3$), 79.9 (C-1a), 42.6 [−C(CH$_3$)$_3$], 27.9 [−C(*C*H$_3$)$_3$], 23.2 (−CO*C*H$_3$), 22.7 (−CO*C*H$_3$). ^{1}H NMR (DMSO-d_6): δ 1.71 (s, 3H, −COC*H*$_3$), 1.69 (s, 3H, −COC*H*$_3$), 1.33 (s, 9H, Bu^t). HRMS: calcd for $C_{253}H_{266}Cl_6N_6O_{50}Na_2$ [M + 2Na]$^{2+}$, *m*/*z* 2224.3212 (100%). Found: 2224.3228.

In the two more mobile fractions separated by the HPLC, monodechlorinated and didechlorinated compounds were identified by HRMS analysis. HRMS for (−Cl + H): calcd for $C_{253}H_{267}Cl_5N_6O_{50}Na_2$ [M + 2Na]$^{2+}$, *m*/*z* 2207.3421 (100%). Found: 2207.3458. HRMS for (−2Cl + 2H): calcd for $C_{253}H_{268}Cl_4N_6O_{50}Na_2$ [M + 2Na]$^{2+}$, *m*/*z* 2189.8607 (100%). Found: 2189.8645.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-{3-O-allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[3-O-allyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine Phenacyl Ester ***30***. A mixture of **28** (45 mg, 12 μmol), Fmoc-Asp(OPfp)-OPac (8.4 mg, 13 μmol), HOOBt (2.2 mg, 13 μmol), and Ph(CH$_3$)$_2$P (2.0 μL, 14 μmol) in 98% aq THF (0.2 mL) was stirred at room temperature for 5 h under Ar. Then the mixture was diluted with EtOAc, washed successively with saturated aq NH_4Cl, saturated aq $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on Bio-Beads SX1 in toluene−EtOAc (1:1), followed by HPLC purification by InertSIL 100A (10 × 250 mm) using hexane−EtOAc (1:3) as an eluent to give **30** (43 mg, 83%). [α]$_D$ −1.7 (*c* 0.9). R_f 0.13 (3:7 hexane−EtOAc). ^{1}H NMR: δ 7.83 [d, 2H, *J* = 7.3 Hz, Ar (Fmoc)], 7.74 [d, 2H, *J* = 7.8 Hz, Ar (Fmoc)], 6.18 (d, 1H, *J* = 8.8 Hz, Asn-αN*H*), 5.94−5.84 (m, 2H, −C*H*═CH_2 × 2), 5.52 [s, 1H, PhC*H*<], 5.50 [s, 1H, PhC*H*<], 5.30 (brd, 2H, *J* = 17.0 Hz, −CH═C*H*$_2$), 2.99 and 2.84 (2s, 2H, H-5f and H-5i), 2.78 (dd, 1H, *J* = 4.1, 16.4 Hz, Asn-β*H*), 1.62 (s, 3H, −COC*H*$_3$), 1.52 (s, 3H, −COC*H*$_3$). Anal. Calcd for $C_{225}H_{232}Cl_{12}N_8O_{51}\cdot 5H_2O$: C, 61.70; H, 5.57; N, 2.56. Found: C, 61.90; H, 5.81; N, 2.43.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-{2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine tert-Butyl Ester ***31***. A mixture of **29** (170 mg, 39 μmol), powdered Zn (380 mg, 5.8 mmol), and AcOH (0.4 mL, 7.0 mmol) in EtOAc (15 mL) was stirred under microwave irradiation at 150 W for 1.5 h. The microwave machine was controlled to keep gentle refluxing during this period. After cooling, the insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residual crude product was purified by chromatography on silica gel with 1% $CH_3OH/CHCl_3$ and then by HPLC with Mightysil Si 60 (10 × 250 mm) in 1% $CH_3OH/CHCl_3$ to afford **31** (139 mg, 86%). [α]$_D$ +14.2 (*c* 2.4). R_f 0.23 (2% $CH_3OH/CHCl_3$). ^{1}H NMR (600 MHz, DMSO-d_6): δ 5.08 (brs, 1H, H-1d), 4.92 (brt, 1H, H-1a), 2.60 (br, 1H, Asn-β*H*), 2.46 (br, 1H, Asn-β*H*), 1.77 (s, 3H, −COC*H*$_3$), 1.75 (s, 3H, −COC*H*$_3$), 1.72 (s, 3H, −COC*H*$_3$), 1.71 (s, 3H, −COC*H*$_3$), 1.33 (s, 9H, Bu^t). ^{13}C NMR (150 MHz, DMSO-d_6): δ 102.2 and 102.0 (C-1f and C-1i), 100.7 (C-1c), 100.4 and 99.8 (× 2) (C-1e, C-1h, and C-1b), 99.4 (C-1d), 97.3 (C-1g), 78.4 (C-1a), 27.5 [−C(*C*H$_3$)$_3$], 23.0, 22.9 (× 2), and 22.7 (−COCH$_3$ × 4). HRMS: calcd for $C_{253}H_{272}N_6O_{50}Na_2$ [M + 2Na]$^{2+}$, *m*/*z* 2120.9388 (100%). Found: 2120.9370.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-{2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-acetamido-3-O-allyl-4,6-O-

benzylidene-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine **4**. A mixture of **30** (12 mg, 2.8 μmol), powdered Zn (280 mg, 4.3 mmol), and AcOH (0.28 mL, 4.9 mmol) in EtOAc (3.0 mL) was stirred under microwave irradiation at 150 W for 1 h. The microwave machine was controlled to keep gentle refluxing during this period. After cooling, the insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residual crude product was purified by preparative thin-layer chromatography with $CHCl_3$−CH_3OH (95:5) containing 1% AcOH to give **4** (4.8 mg, 45%). $[\alpha]_D$ −2.34 (*c* 0.75). R_f 0.34 ($CHCl_3$−CH_3OH (95:5) containing 1% AcOH). ^{1}H NMR (DMSO-d_6): δ 7.89 [d, 2H, *J* = 7.3 Hz, Ar (Fmoc)], 7.70 [d, 2H, *J* = 7.8 Hz, Ar (Fmoc)], 5.92−5.84 (m, 2H, −C*H*═CH_2 × 2), 5.63 [s, 1H, PhC*H*<], 5.61 [s, 1H, PhC*H*<], 1.91, 1.90, 1.88 (s, 6H, −COCH_3 × 6), 1.76, 1.72 (s, 12H, −COCH_3 × 4). HRMS: calcd for $C_{217}H_{237}N_8O_{50}Na_3$ $[M - H + 3Na]^{2+}$, *m*/*z* 1912.7998 (100%). Found: 1912.8007.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-{2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine **3**. A solution of **31** (40 mg, 10 μmol) in 80% aq TFA (1 mL) was stirred at room temperature for 3 h. The solvent was evaporated in vacuo, and the residue was purified by HPLC on a column of Mightysil Si 60 (10 × 250 mm) with 1% AcOH/2% $CH_3OH/CHCl_3$ to give **3** (28 mg, 71%). $[\alpha]_D$ +10.1 (*c* 1.5). R_f 0.23 (3% $CH_3OH/CHCl_3$). ^{1}H NMR (600 MHz, DMSO-d_6): δ 5.09 (brs, 1H, H-1d), 4.93 (brt, 1H, *J* = 9.0 Hz, H-1a), 2.63 (dd, 1H, *J* = 4.1, 15.8 Hz, Asn-βH), 2.49 (brd, 1H, Asn-βH), 1.76 (s, 3H, −COCH_3), 1.74 (s, 3H, −COCH_3), 1.71 (s, 6H, −COCH_3 × 2). ^{13}C NMR (150 MHz, DMSO-d_6): δ 102.0 and 101.9 (C-1f and C-1i), 100.7 (C-1c), 100.3 and 99.7 (× 2) (C-1e, C-1h, and C-1b), 99.3 (C-1d), 97.3 (C-1g), 78.4 (C-1a), 22.8, 22.7 (× 2), and 22.5 (−COCH_3 × 4). HRMS: calcd for $C_{249}H_{264}N_6O_{50}Na_2$ $[M + 2Na]^{2+}$, *m*/*z* 2092.9075 (100%). Found: 2092.9079; calcd for $C_{249}H_{263}N_6O_{50}Na_3$ $[M - H + 3Na]^{2+}$, *m*/*z* 2103.8985 (100%). Found: 2103.8991.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H and ^{13}C NMR spectra for new compounds and the result of the Low-TfOH treatment of compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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