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# An efficient synthesis of a biantennary sialooligosaccharide analog using a 1,6-anhydro-β-lactose derivative as a key synthetic block

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**Abstract**—An efficient and versatile method for the synthesis of a biantennary octasaccharide derivative was established by combined chemical and enzymatic manipulations of 1,6-anhydro-β-lactose as a key starting material. A key 1,6-anhydro-β-lactose derivative having two unprotected hydroxyl groups at C-3′ and C-6′ positions was prepared and employed for the chemical coupling reaction with a known 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl imidate to afford a tetrasaccharide derivative with two GlcNAc branches in 69% yield. Enzymatic galactosylation using UDP-Gal with a bovine milk β1,4-galactosyltransferase and subsequent sialylation with a recombinant α2,3-sialyltransferase in the presence of CMP-Neu5Ac proceeded smoothly and gave a desired model compound, a bivalent sialooctasaccharide (**1**), in 73% overall yield from the tetrasaccharide intermediate. 

## 1. Introduction

Lactosaminoglycans are high molecular weight carbohydrates bound to proteins or lipids and composed of unique *N*-acetyllactosamine repeating units [Galβ(1→4)GlcNAcβ(1→3)]$_n$.[1] It was also reported that the linear lactosaminoglycan chains sometimes involve branching structures at C-6 position of the D-galactose residues of this repeating disaccharide unit. In addition, it has also known that introduction of sialic acid and/or fucose residues to the lactosaminoglycans may lead to further complicated structures showing significant biological functions.[2,3] In fact, a variety of lactosaminoglycans carrying modified oligosaccharide branches play important roles as cell surface antigens and as specific ligands (markers) for the carbohydrate-binding proteins in cellular recognition processes. For example, it has been suggested that the structure of each lactosaminoglycan isolated from teratocarcinoma, Ehrlich's ascites carcinoma or human granulocyte remarkably differs in terms of the average molecular weight and the degree of branching.[4–6] The branching of the linear lactosaminoglycans has also been known as a crucial on/off switch for regulating the functional roles in Ii-antigenic structures. Thus, I-antigens produced from the linear lactosaminoglycans (i-antigens) are key antigenic structures as cell surface signals to control cell differentiation, aging, and malignant transformation of cells.[7–14]

The synthetic study of lactosaminoglycans and related compounds will apparently accelerate the investigation for the biological significance of these attractive carbohydrates and this might contribute to the development of the effective therapeutic reagents to inhibit cancer metastasis or prevent cells from malignant alterations and viral infections. Moreover, multivalency of carbohydrate chains is of growing interest from medicinal chemistry for the synthesis of carbohydrate-based therapeutic reagents as well as biological importance of naturally occurring glycoproteins with multiple sugar branches such as biantennary, triantennary, and tetraantennary structures.[15] Our interest has been, therefore, focused on the efficient and systematic synthesis of branched-type lactosaminoglycans as a key and specific carbohydrate signals in biomolecular recognitions. Although chemical syntheses of some lactosaminoglycan-related compounds were carried out by means of a conventional strategy, these syntheses often involve complicated synthetic scheme with tedious multistep protection/deprotection processes and purification of anomeric mixture

*Keywords*: lactosaminoglycan; biantennary oligosaccharide; 1,6-anhydro-β-lactose; β1,4-galactosyltransferase; α2,3-sialyltransferase; glycoprotein; glycolipid.

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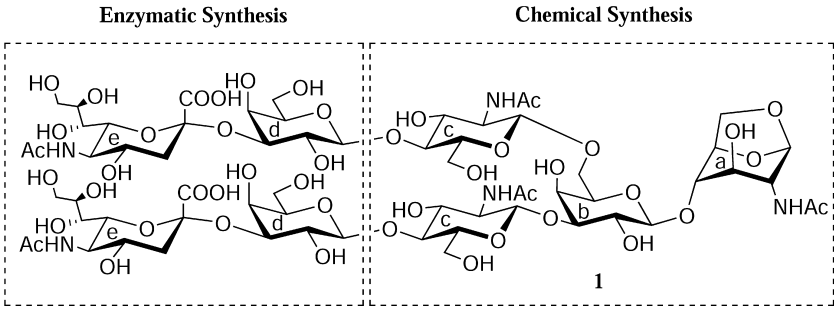



**Figure 1.** A strategy for chemo-enzymatic synthesis of a biantennary sialyllactosaminoglycan model **1**.

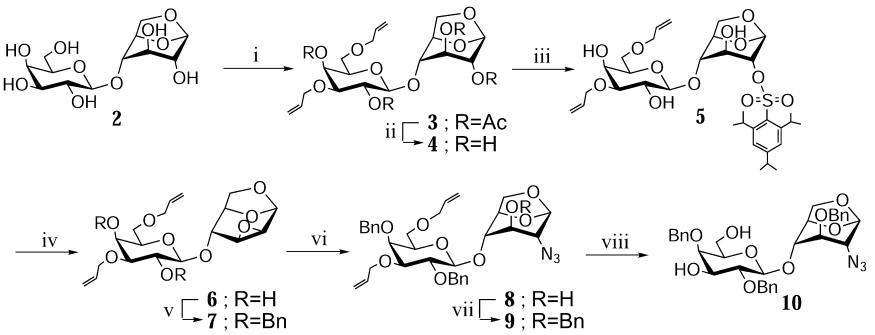
in glycosylation reactions.[16–23] Advent of an efficient and versatile strategy for rapid synthesis of the lactosaminoglycans and related compounds is strongly required. In the present study, we would like to report an efficient synthesis for these unique oligosaccharide analogs based on the combined chemical and enzymatic strategy. The method described herein involves the use of a novel key disaccharide intermediate derived from naturally abundant lactose and recombinant glycosyltransferases as biocatalysts for the sugar elongation reactions. Merits of the novel synthetic concept will be demonstrated by constructing a bivalent octasaccharide derivative **1** having two sialic acid residues as a potential candidate of the sugar-based therapeutic reagent. Some synthetic intermediates prepared herein will become versatile materials for the construction of a variety of synthetic multivalent glycoligands as stable and standard compounds. These standard glycoligands prepared in a large scale manner will become valuable tools for the investigation of the functional roles of 'multivalency' in carbohydrate–protein interactions. The synthesis of **1** was basically achieved by (i) preparation of a key disaccharide intermediate **10** from lactose, (ii) introduction of two glucosamine residues to C-3′ and C-6′ positions of compound **10**, and (iii) enzymatic galactosylation and sialylation of the tetrasaccharide intermediate as outlined in Figure 1.

## 2. Results and discussion

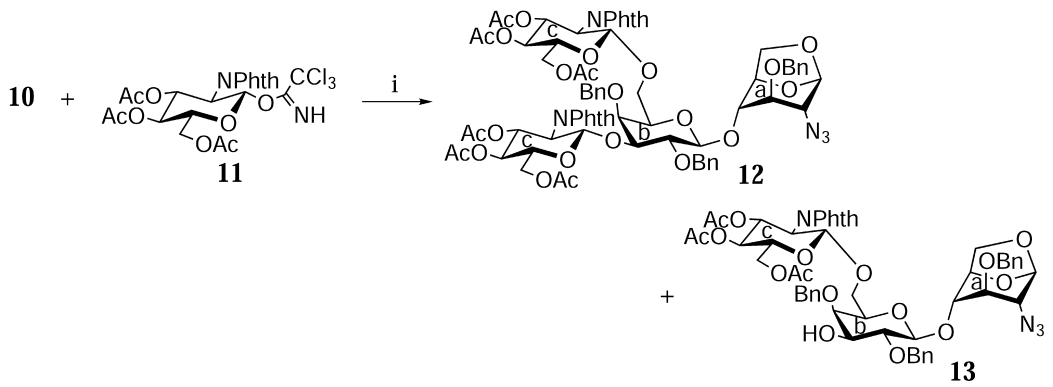
### 2.1. Synthesis of a key intermediate 10 from 1,6-anhydro-β-lactose as a starting material

Lactose, Galβ(1→4)Glc, is a naturally abundant disaccharide resource produced from bovine milk and one of the most important starting materials that can be used for the large scale syntheses of bioactive glycoconjugates. It has been widely accepted that 1,6-anhydro-β-lactose obtained readily from lactose monohydrate can be efficiently converted to some potential intermediates for the synthesis of *N*-acetyllactosamine and its derivatives as reported previously by Kuzuhara et al.,[24] Tejima et al.,[25,26] and our group.[27–29] Feasibility of an intermediate derived from 1,6-anhydro-β-lactose in the synthetic study of a complicated oligosaccharide derivative has been preliminarily demonstrated by an efficient synthesis of biologically important Lewis X ($Le^X$) trisaccharide derivative and the preparation of a macromolecular ligand carrying this $Le^X$ antigenic trisaccharide structure.[28]

In the present synthesis, we selected a 1,6-anhydro-β-lactose derivative **10** having two unprotected hydroxyl groups at C-3′ and C-6′ positions as a key intermediate for the efficient syntheses of a bivalent sialooligosaccharide derivative. Although compound **10** could be converted from a known *O*-(3-*O*-allyl-2,4-di-*O*-benzyl-6-*O*-triphenylmethyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose,[29] we prepared herein a new intermediate **9** in order to achieve an efficient and direct synthesis of the compound **10** as indicated in Scheme 1. First, 1,6-anhydro-β-lactose **2** was subjected to stannylation[30–32] by using bis(tri-*n*-butyltin)oxide, followed by the introduction of two *O*-allyl groups with 3-bromo-1-propene and acetylation to afford 3′,6′-di-*O*-allylated derivative **3** in 68% yield. After de-O-acetylation of **3**, the tetraol **4** was converted into 2-*O*-(2,4,6-triisopropylbenzenesulfonyl) (TIPBS) derivative **5** in 78% yield. Intramolecular nucleophilic substitution of compound **5** with an aqueous solution




**Scheme 1.** *Reagents and conditions*: (i) (a) $(n\text{-}Bu_3Sn)_2O$/toluene, azeotrope; (b) allyl bromide, 80°C; (c) $Ac_2O$/pyridine, room temperature, 68%. (ii) NaOMe/MeOH–THF, room temperature, q.y. (iii) TIPBS-Cl, DMAP/pyridine, room temperature, 78%. (iv) 1N NaOHaq./MeOH–THF, room temperature, 78%. (v) BnBr, NaH/DMF, 92%. (vi) $NaN_3$, CsF/DMF, 110°C, 65%. (vii) BnBr, NaH/DMF, 82%. (viii) $PdCl_2$, NaOAc/95%AcOHaq., ultrasonication, room temperature, 81%.



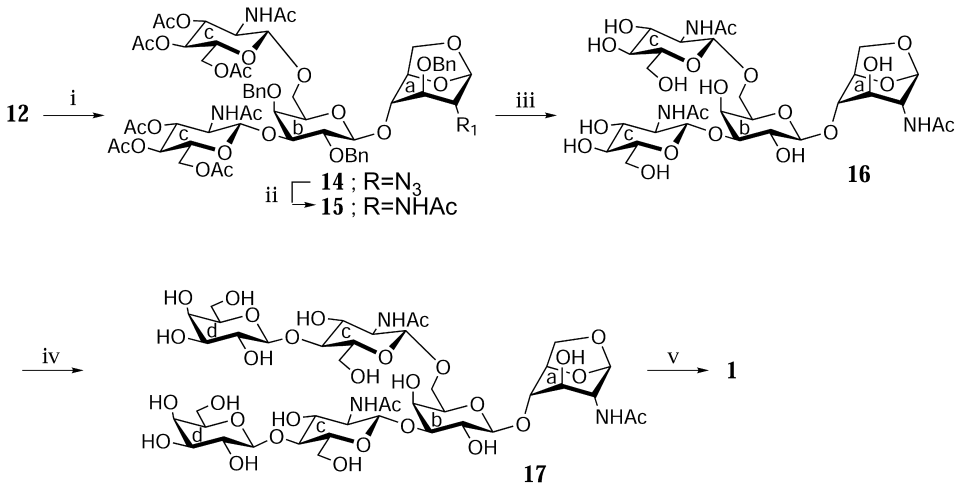
**Scheme 2.** *Reagents and conditions*: (i) TMSOTf, MS4Å/$CH_2Cl_2$, −15°C, 69% for **12**, 26% for **13**.

of 1N sodium hydroxide efficiently gave an epoxide **6** in 78% yield. After O-benzylation at C-2′ and C-4′ positions of **6**, nucleophilic attack by an azide ion in the presence of cesium salt as accelerator to the epoxide **7** gave 2-azido derivative **8** in 65% yield. O-Benzylation at C-3 position of **8** and subsequent de-O-allylation of **9** under standard conditions[30,31] afforded a desired diol **10** in 81% yield. The standardized intermediate **10** will become a key synthetic block to construct longer and more complicated branched-type lactosaminoglycan chains.

### 2.2. Chemical and enzymatic synthesis of an octasaccharide 1 from compound 10

A disaccharide synthetic block **10** was allowed to react with a known glycosyl donor **11**[33,34] readily obtained from the peracetate of 2-phthalimido-2-deoxy-D-glucopyranose (Scheme 2). Coupling reaction of **10** with **11** proceeded smoothly in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter and gave tetrasaccharide **12** in 69% yield together with trisaccharide **13** (26%) as a by-product. De-N-phthaloylation with hydradine and subsequent N-acetylation afforded compound **14** in 77% yield. Next, azide group at C-2 position of **14** was converted into acetamido group by treating with thioacetic acid–pyridine to yield **15** quantitatively. After de-O-acetylation of tetrasaccharide **15**, hydrogenation over 5% palladium on charcoal removed benzyl protections at C-3, 2′, and 4′ positions to give nonaol **16** in 81% yield (2 steps).

Galactosylation of tetrasaccharide **16** was carried out by using β1,4-galactosyltransferase with UDP-Gal in 50 mM HEPES buffer (pH 6.0) for 24 h according to the condition reported previously (Scheme 3).[35–37] Hexasaccharide **17** was isolated by subjecting a reaction mixture to the simple gel filtration by means of a column of Sephadex G-15 with water as an eluent in 87% yield. No pentasaccharide derivative (by-product) was detected and obtained by this procedure, suggesting that the terminal GlcNAc residues in lactosaminoglycans could be galactosylated equally by this enzyme. Finally, compound **17** was subjected to the following sialylation by means of rat liver α2,3-sialyltransferase in the presence of CMP-Neu5Ac in 50 mM sodium cacodylate buffer (pH 7.3) to give octasaccharide **1** in 84% yield. Chemical structure of the final product was confirmed by a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), and electrospray ionization high resolution (ESI-HRMS) mass spectrometry, and $^1H$ and $^{13}C$ NMR spectroscopy. The MALDI-TOF mass spectra of hexasaccharide **17** and octasaccharide **1** are shown in Figure 2. It was suggested that the molecular weight observed at 1700.8 was found to be in good agreement with



**Scheme 3.** *Reagents and conditions*: (i) (a) $NH_2NH_2{\cdot}H_2O$/EtOH, reflux; (b) $Ac_2O$/Pyr, room temperature, 77%. (ii) AcSH/Pyr, room temperature, 96%. (iii) (a) NaOMe/MeOH, room temperature; (b) $H_2$, 5% Pd–C/MeOH–$H_2O$, room temperature, 81%. (iv) UDP-galactose, β-1,4-galactosyltransferase, α-lactalbumin, $MnCl_2{\cdot}4H_2O$/50 mM HEPES buffer (pH6.0), 37°C, 87%. (v) CMP-NANA, α-2,3-sialyltransferase, BSA, $MnCl_2{\cdot}4H_2O$, Triton CF-54/50 mM sodium cacodylate buffer (pH 7.4), 37°C, 84%.

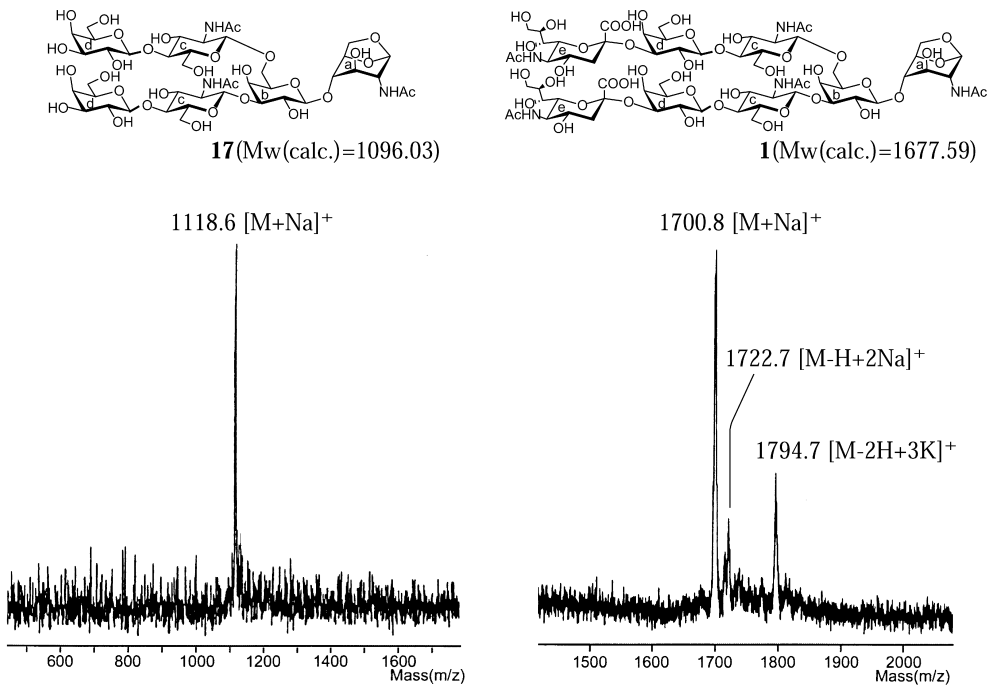



**Figure 2.** MALDI-TOF mass spectra of hexasaccharide **17** and octasaccharide **1**.

its $[M+Na]^+$ adduct of compound **1**. Fully assigned $^{13}C$ NMR spectrum of octasaccharide **1** is also shown in Figure 3. The data obtained by $^1H$ and $^{13}C$ NMR measurements were described in Section 3. These results clearly demonstrate that both galactosyl and sialyltransferases recognize non-reducing two GlcNAc moieties bound to the 1,6-anhydro-β-lactose and an unusual 1C type conformation at the reducing end of **16** does not reduce the efficiency of the sugar elongation reaction catalyzed by this enzyme at all. These findings mean obviously the wide applicability of 1,6-anhydro-β-lactose derivatives as versatile scaffolds in the combined chemical and enzymatic synthesis of a variety of complicated glycoconjugates involving non-natural oligosaccharides. A series of oligosaccharide derivatives might become valuable tools for the preparation of carbohydrate-based therapeutic reagents, since octasaccharide derivative **1** seems to be one of the bivalent sialooligosaccharide ligands with potent biological

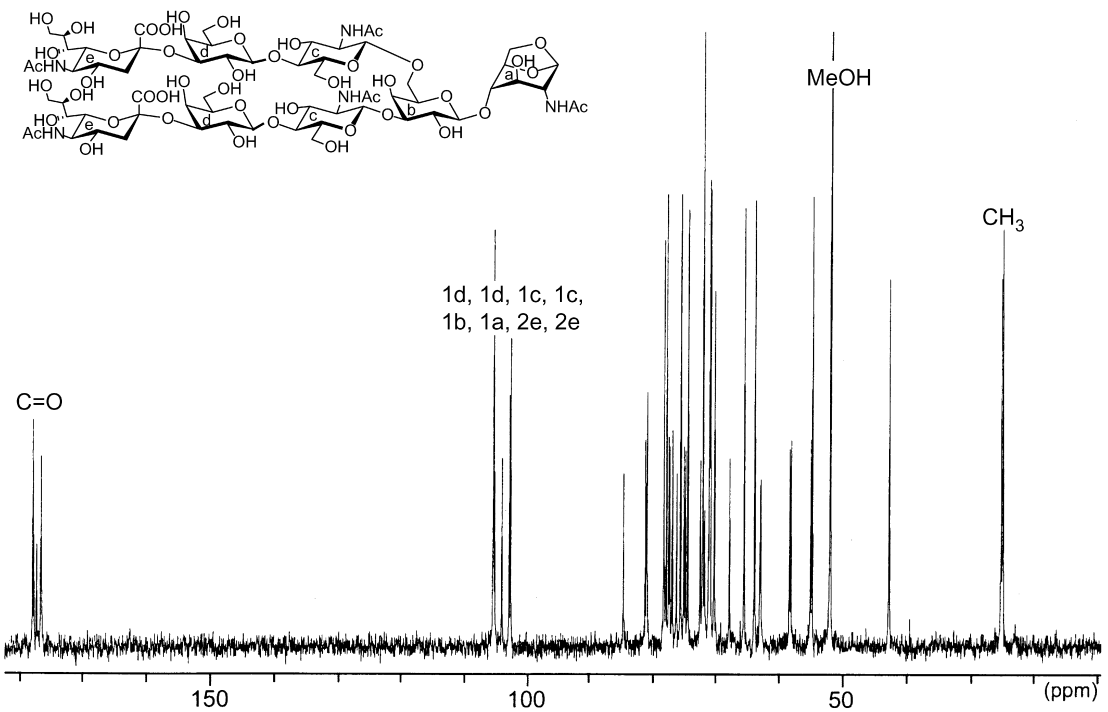



**Figure 3.** $^{13}C$ NMR spectrum of octasaccharide **1**.

activity such as antiinfluenza and antitumor activities. Application of these synthetic multivalent glycoligands in medical field is under investigation and the result will be reported in the nearest future.

In conclusion, 1,6-anhydro-β-lactose was efficiently converted into a key lactosamine intermediate having unprotected hydroxyl groups at C-3′ and C-6′ positions. It was clearly demonstrated that combined chemical and enzymatic modifications of this key compound greatly accelerated the large-scale synthesis of a bivalent sialooctasaccharide derivative as a potent glycoligand.

## 3. Experimental

### 3.1. General methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Optical rotations were determined with a Horiba SEPA-200 digital polarimeter at 25°C. $^1$H and proton decoupled carbon NMR spectra were recorded at 400 and 100.4 MHz, respectively, on a JEOL ALPHA-400 spectrometer. Ring-proton assignments in NMR were made by first-order analysis of the spectra and supported by HH-COSY experiments. Elemental analyses were performed with a Sartorius 4503 Micro, 7079 printer. Bovine milk β1,4-galactosyltransferase (EC 2.4.1.22) was purchased from Sigma Co. Ltd.. Rat liver α2,3-sialyltransferase was purchased from Calbiochem Co. Ltd. Reactions were monitored by thin-layer chromatography (t.l.c.) on a precoated plate of silica gel 60$F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co. Ltd. Japan). MALDI-TOF mass spectrometry was carried out on a Finnigan LASERMAT 2000 instrumental using 2,5-dihydroxybenzoic acid. The instrument was operated in the positive ion reflection mode with an accelerating potential of 20 kV. Solvent extracts were dried with anhydrous magnesium sulfate and concentrated below 40°C under diminished pressure.

**3.1.1. 2,4-Di-*O*-acetyl-3,6-di-*O*-allyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (3).** To a solution of **2** (2.5 g, 6.18 mmol) in toluene (300 mL) was added bis(tri-*n*-butyltin)oxide (4.27 mL, 8.03 mmol), and the mixture was stirred for 4 h at 140°C with continuous azeotropic removal of water. After evaporation to the half volume, 3-bromo-1-propene (20 mL) was added to the solution and the reaction mixture was stirred for 12 h at 80°C. After concentration, the residual syrup was dissolved in pyridine (50 mL) and to the mixture were added DMAP (0.76 g, 6.18 mmol) and acetic anhydride (50 mL). After being stirred for 15 h at room temperature, the mixture was evaporated and diluted with chloroform. The solution was washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica gel with toluene–ethyl acetate (5:1, v/v) as an eluent to afford compound **3** (2.4 g, 68%): mp 116°C; $[\alpha]_D$25=−37.4° (*c* 0.149, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 5.90–5.70 (m, 2H, $CH_2$=C*H*), 5.47 (d, 1H, $J_{3',4'}$=3.3 Hz, H-4′), 5.44 (s, 1H, H-1), 5.21–5.11 (m, 5H, C$H_2$=CH and H-2′), 5.10 (s, 1H, H-2), 4.71 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1′), 4.61 (d, 1H, $J_{5,6a}$=5.0 Hz, H-5), 4.53 (s, 1H, H-3), 4.17–4.10 (br, 2H, H-6′a and H-6′b), 4.19–3.86 (m, 6H, $CH_2$=CH–C$H_2$, H-6a, and H-6b), 3.59 (s, 1H, H-4), 3.55–3.45 (br, 2H, H-3′ and H-5′), 2.13–2.08 (all s, 12H, 4$COCH_3$). Anal. calcd for $C_{26}H_{36}O_{14}$·0.5$H_2O$: C, 53.70; H, 6.41%. Found: C, 53.77; H, 6.26%.

**3.1.2. 3,6-Di-*O*-allyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-*O*-triisopropylbenzenesulfonyl-β-D-glucopyranose (5).** A solution of compound **3** (1.10 g, 1.92 mmol) and sodium methoxide (60 mg) in 1:1 (v/v) methanol–THF (20 mL) were stirred for 2 h at room temperature. The mixture was made neutral with the Dowex 50W-X8($H^+$) resin, and the suspension was filtered. The filtrate was evaporated to give a quantitative yield of **4** (0.77 g). To a solution of **4** in pyridine (10 mL) were added 4-dimethylaminopyridine (DMAP) (560 mg, 4.61 mmol) and TIPBS-Cl (1.56 g, 4.61 mmol) at 0°C, and the mixture was stirred for 72 h at room temperature under nitrogen atmosphere. After concentration, the obtained residue was dissolved in chloroform and washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica gel with chloroform–methanol (100:1, v/v) to give **5** (1.0 g, 78%) as a crystalline mass: mp 80°C; $[\alpha]_D^{25}$=−7.6° (*c* 0.240, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ7.17 (s, 2H, aromatic), 5.88 (m, 2H, $CH_2$=C*H*), 5.43 (br s, 1H, H-1), 5.35–5.15 (m, 4H, C$H_2$=CH), 4.60 (d, 1H, $J_{5,6a}$=6.8 Hz, H-5), 4.38 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.36 (d, 1H, $J_{1,2}$=5.6 Hz, H-2), 4.20 (m, 2H, $CH_2$=CH–C$H_2$), 4.14 [m, 3H, $(CH_3)_2$–C*H*], 4.02–3.93 (m, 2H, H-3, and H-4′), 3.81 (t, 2H, H-2′), 3.70–3.63 (m, 5H, H-4, H-6a, H-6b, H-6′a, and H-6′b), 3.55 (d, 1H, H-5′), 3.37 (dd, 1H, $J_{3',4'}$=3.2 Hz, $J_{2',3'}$=10.4 Hz, H-3′), 2.90 (br, 2H, 2′-OH and 4′-OH), 2.50 (br, 1H, 3-OH), 1.27–1.25 [each s, each 6H, $(CH_3)_2$CH]. Anal. calcd for $C_{33}H_{50}O_{12}S$: C, 59.08; H, 7.51; S, 4.78%. Found: C, 59.05; H, 7.57; S, 5.07%.

**3.1.3. 3,6-Di-*O*-allyl-β-D-galactopyranosyl-(1→4)-1,6:2,3-dianhydro-β-D-mannopyranose (6).** To a solution of **5** (650 mg, 0.969 mmol) in 1:1 (v/v) methanol–THF (10 mL) was added an aqueous solution of 1N sodium hydroxide (2.90 mL) and the mixture was stirred for 24 h at room temperature. After concentration, the residual oil was dissolved in chloroform, washed with brine, dried and evaporated in vacuo. The residue was purified by chromatography on silica gel with chloroform–methanol (80:1, v/v) as an eluent to give a syrup **6** (290 mg, 78%): $[\alpha]_D^{25}$=−16.4° (*c* 0.251, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 6.00–5.84 (m, 2H, $CH_2$=C*H*) 5.71 (d, 1H, $J_{1,2}$=3.0 Hz, H-1), 5.36–5.19 (m, 4H, C$H_2$=CH), 4.54 (t, 1H, H-5), 4.45 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.21 (m, 2H, $CH_2$=CH–C$H_2$), 4.06–4.04 (m, 3H, H-4′ and $CH_2$=CH–C$H_2$), 3.98 (br s, 1H, H-3), 3.84–3.79 (m, 2H, H-2′ and H-6b), 3.78–3.68 (m, 1H, H-6a), 3.63 (dd, 1H, $J_{5',6'a}$=5.7, $J_{6'a,6'b}$=11.6 Hz, H-6′b), 3.47–3.42 (m, 4H, H-2, H-4, H-5′ and H-6′a), 3.38 (dd, 1H, $J_{3',4'}$=3.3, $J_{2',3'}$=9.4 Hz, H-3′), 2.77 (br, 2H, 2′-OH and 4′-OH). Anal. calcd for $C_{18}H_{26}O_9$·0.5$H_2O$: C, 54.67; H, 6.88%. Found: C, 54.50; H, 6.58%.

**3.1.4. 3,6-Di-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyrano-**

**syl-(1→4)-1,6:2,3-dianhydro-β-D-mannopyranose (7).** To a solution of **6** (200 mg, 0.518 mmol) dissolved in DMF (10 mL) were added sodium hydride (60% oil suspension; 62 mg, 1.55 mmol) and benzyl bromide (0.32 mL, 2.59 mmol), and the mixture was stirred at room temperature. After 24 h, excess of sodium hydride was carefully quenched by addition of methanol. The resulting solution was dissolved in ethyl acetate, washed with water, brine and dried. After evaporation, the residual oil was purified by silica gel column chromatography with toluene–ethyl acetate (5:1, v/v) as an eluent, and recrystallization from ethanol gave **7** (270 mg, 92 %): mp 138°C; $[\alpha]_D^{25}=-23.3°$ (*c* 0.227, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 7.42–7.25 (m, 10H, aromatic), 5.90–5.78 (m, 2H, $CH_2$=C*H*), 5.72 (d, 1H, $J_{1,2}$=2.8 Hz, H-1), 5.37–5.15 (m, 4H, $CH_2$=CH), 4.97–4.64 (m, 4H, PhC$H_2$), 4.50 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.46 (m, 1H, H-5), 4.22–4.20 (m, 2H, $CH_2$=CH–C$H_2$), 3.91–3.86 (m, 4H, H-3, H-6b and $CH_2$=CH–C$H_2$), 3.89 (d, 1H, H-4′), 3.82 (dd, 1H, $J_{1',2'}$=7.6 Hz, $J_{2',3'}$=9.7 Hz, H-2′), 3.67 (d, 1H, H-6a), 3.52 (m, 2H, H-6′a and H-6′b), 3.45–3.41 (m, 4H, H-2, H-4, H-3′, and H-5′). Anal. calcd for $C_{32}H_{38}O_9$: C, 67.83; H, 6.76%. Found: C, 67.71; H, 6.89%.

**3.1.5. 3,6-Di-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (8).** A mixture of **7** (100 mg, 0.176 mmol), cesium fluoride (270 mg, 1.76 mmol) and sodium azide (114 mg, 1.76 mmol) dissolved in DMF (2.0 mL) were stirred for 24 h at 110°C under nitrogen atmosphere. After cooling to room temperature, the mixture was diluted with water, extracted with ethyl acetate and washed with brine. The mixture was dried, evaporated and subjected to chromatography on silica gel column with *n*-hexane–ethyl acetate (3:1, v/v) as an eluent to give **8** (70 mg, 65%) as a crystalline powder: mp 87°C; $[\alpha]_D^{25}=-40.7°$ (*c* 0.229, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 7.35–7.30 (m, 10H, aromatic), 6.00–5.80 (m, 2H, $CH_2$=C*H*), 5.38–5.16 (m, 4H, $CH_2$=CH), 5.20 (d, 1H, H-1), 4.97–4.51 (m, 4H, PhC$H_2$), 4.38 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.34 (br t, 1H, H-5), 4.23 (m, 2H, $CH_2$=CH–C$H_2$), 3.91 (m, 2H, $CH_2$=CH–C$H_2$), 3.84 (d, 1H, $J_{1',2'}$=7.7 Hz, H-2′), 3.88–3.79 (m, 1H, H-3), 3.75 (d, 1H, $J_{3',4'}$=3.3 Hz, H-4′), 3.64–3.53 (m, 4H, H-6a, H-6b, H-6′a and H-6′b), 3.43 (dd, 1H, $J_{3'4'}$=2.9 Hz and $J_{2',3'}$=9.8 Hz, H-3′), 3.36 (d, 1H, $J_{3,4}$=6.3 Hz, H-4), 3.26 (d, 1H, $J_{5',6'a}$=6.3 Hz, H-5′), 3.22 (d, 1H, $J_{2,3}$=8.6 Hz, H-2), 1.64 (br s, 1H, 3-OH). Anal. calcd for $C_{32}H_{39}N_3O_9$: C, 63.04; H, 6.45; N, 6.89%. Found: C, 63.08; H, 6.45; N, 6.74%.

**3.1.6. 3,6-Di-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (9).** To a solution of **8** (180 mg, 0.295 mmol) in DMF (10 mL) were added sodium hydride (60% oil suspension; 24 mg, 0.590 mmol) and benzyl bromide (0.11 mL, 0.885 mmol), and stirred for 14 h at room temperature. The same manner as described for **7** was carried out, and the obtained syrup was purified on silica gel chromatography with *n*-hexane–ethyl acetate (5:1, v/v) as an eluent to give **9** (170 mg, 82%): $[\alpha]_D^{25}=-6.6°$ (*c* 0.290, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 7.38–7.18 (m, 15H, aromatic), 5.97–5.65 (m, 2H, $CH_2$=C*H*), 5.42 (s, 1H, H-1), 5.30–5.02 (m, 4H, $CH_2$=CH), 4.95–4.50 (m, 6H, PhC$H_2$), 4.55 (d, 1H, H-5), 4.38 (d, 1H, $J_{1',2'}$=7.6 Hz, H-1′), 4.14 (m, 2H, $CH_2$=CH–C$H_2$), 3.94 (d, 1H, $J_{6a,6b}$=7.1 Hz, H-6b), 3.85 (s, 1H, H-3), 3.82–3.70 (m, 5H, H-4, H-2′, H-4′ and $CH_2$=CH–C$H_2$), 3.63 (t, 1H, $J_{5,6a}$=6.1 Hz and $J_{6a,6b}$=7.2 Hz, H-6a), 3.43–3.32 (m, 4H, H-3′, H-5′ H-6′a and H-6′b), 3.13 (s, 1H, H-2). Anal. calcd for $C_{39}H_{45}N_3O_9{\cdot}0.5H_2O$: C, 66.09; H, 6.54; N, 5.92%. Found: C, 65.80; H, 6.53; N, 5.70%.

**3.1.7. 2,4-Di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (10).** To a solution of **9** (80 mg, 0.114 mmol) in acetic acid (1.9 mL) and $H_2O$ (0.1 mL) were added sodium acetate (93 mg, 1.14 mmol) and palladium (II) chloride (81 mg, 0.456 mmol), and the mixture was kept for 2 h under ultrasonication. After filtration off through a celite bed, the filtrate was extracted with chloroform. The organic layer was washed with saturated sodium hydrogen carbonate, brine, dried and evaporated in vacuo. The residual oil was chromatographed on a silica gel column with chloroform: methanol (60:1, v/v) as an eluent to afford **10** (57 mg, 81%) as a syrup foam: $[\alpha]_D^{25}=+2.0°$ (*c* 0.19, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$7.40–7.30 (m, 15H, aromatic), 5.51 (brs, 1H, H-1), 5.10–4.64 (m, 6H, PhC$H_2$), 4.61 (d, 1H, $J_{5,6a}$=6.1 Hz, H-5), 4.44 (d, 1H, $J_{1',2'}$=7.0 Hz, H-1′), 4.07 (d, 1H, $J_{6a,6b}$=7.4 Hz, H-6b), 3.91 (br s, 1H, H-3), 3.78 (m, 2H, H-2′ and H-4′), 3.75 (br s, 1H, H-4), 3.72 (d, 1H, $J_{5,6a}$=6.1 Hz, $J_{6a,6b}$=7.4 Hz, H-6a), 3.68 (m, 1H, H-6′b), 3.68 (dd, 1H, $J_{3',4'}$=3.2 Hz, $J_{2',3'}$=9.9 Hz, H-3′), 3.56 (dd, 1H, $J_{5',6'a}$=5.6 Hz, $J_{6'a,6'b}$=10.1 Hz, H-6′a), 3.41 (t, 1H, $J_{5',6'a}$=5.6 Hz, $J_{5',6'b}$=6.5 Hz, H-5′), 3.27 (br s, 1H, H-2), 3.09 (br, 2H, 3′-OH and 6′-OH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 138.3, 138.0, 137.3, 128.7–127.5 (Bn), 103.1 (C-1′), 100.5 (C-1), 79.0 (C-2′), 77.4 (C-3), 76.1 (C-4), 75.0, 74.8, 74.6 (Ph*C*$H_2$×3), 74.4 (C-5′), 74.1 (C-5,4′), 72.3 (C-3′), 64.9 (C-6), 61.7 (C-2), 59.6 (C-6′). Anal. calcd for $C_{33}H_{37}N_3O_9$: C, 63.96; H, 6.02; N, 6.78%. Found: C, 63.78; H, 6.20; N, 6.80%.

**3.1.8. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (12) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (13).** To a mixture of 1,6-anhydrolactose derivative **10** (160 mg, 0.259 mmol), glucosaminyl imidate **11** (360 mg, 0.622 mmol) and MS4Å (300 mg) in dry dichloromethane (4.0 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf) (40 μL, 0.207 mmol) at −15°C under nitrogen atmosphere. After stirring for 1 h at room temperature, the mixture was neutralized with triethylamine and concentrated. The resulting residue was purified by chromatography on a silica gel first with 3:1 and then *n*-hexane–ethyl acetate (1:1, v/v) to give tetrasaccharide **12** (260 mg, 69%) and trisaccharide **13** (70 mg, 26%).

*Compound* **12**. $[\alpha]_D^{25}=+13.0°$ (*c* 0.258, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 7.81–7.57 [4m, 8H, aromatic(Phth)], 7.31–7.20 [m, 15H, aromatic(Bn)], 5.70 (dd, 1H, *J*=10.6, 9.1 Hz, H-3c), 5.70 (d, 1H, *J*=8.3 Hz, H-1c), 5.62 (dd, 1H, *J*=10.6, 9.2 Hz, H-3c), 5.48 (d, 1H, *J*=8.4 Hz, H-1c), 5.32 (s, 1H, H-1a), 5.18 (t, 1H, *J*=9.2, 9.5 Hz, H-4c), 5.11 (t, 1H, *J*=9.4,

9.6 Hz, H-4c), 4.85–4.30 (6d, 6H, PhC$H_2$), 4.36 (d, 1H, $J$=7.3 Hz, H-1b), 4.35 (dd, 1H, $J$=8.4, 10.9 Hz, H-2c), 4.22 (d, 1H, $J$=8.6 Hz, H-2c), 3.96 (d, 1H, $J$=7.4 Hz, H-2b), 3.73 (d, 1H, H-4a), 3.62 (s, 1H, H-3a), 3.36 (br t, 1H, H-5b), 3.15 (d, 1H, H-2a), 2.04–1.82 (all s, 18H, 6COC$H_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.6–169.5 (*C*OCH$_3$), 138.4, 138.3, 137.5, (Bn), 134.4, 134.1, (Phth 4/5), 131.2, 131.1 (Phth 1/2), 128.4–127.3 (Bn), 123.6, 123.5 (Phth 3/6), 102.2 (C-1b), 100.7 (C-1a), 98.5, 97.5 (C-1c×2), 80.3 (C-3b), 78.1, 77.7 (C-2b, C-3a), 76.1 (C-4a), 74.7, 74.5, 74.2 (Ph*C*H$_2$×3, C-4b, C-5a), 72.8 (C-5b), 71.8, 71.7 (C-5c×2), 70.8, 70.4 (C-4c×2), 68.8, 68.5, 68.0 (C-3c×2, C-6b), 65.5 (C-6a), 61.6 (C-2a, C-6c×2), 55.1, 54.4 (C-2c×2), 20.7–20.4 (CO*C*H$_3$). Anal. calcd for $C_{73}H_{75}N_5O_{27}\cdot H_2O$: C, 59.55; H, 5.27; N, 4.76%. Found: C, 59.64; H, 5.07; N, 4.78%.

*Compound* **13**. $[\alpha]_D^{25}$=+14.0° (*c* 0.253, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.82–7.69 [2m, 4H, aromatic(Phth)], 7.36–7.32 [m, 15H, aromatic(Bn)], 5.70 (dd, 1H, $J$=10.5, 9.1 Hz, H-3c), 5.47 (d, 1H, $J$=8.5 Hz, H-1c), 5.46 (s, 1H, H-1a), 5.12 (t, 1H, $J$=9.1, 9.9 Hz, H-4c), 5.03–4.57 (6d, 6H, PhC$H_2$), 4.62 (m, 1H, H-5a), 4.39 (d, 1H, $J$=7.4 Hz, H-1b), 4.27 (dd, 1H, $J$=8.5, 10.4 Hz, H-2c), 4.22 (dd, 1H, $J$=4.4, 12.5 Hz, H-6c), 4.08 (dd, 1H, $J$=7.4 Hz, H-6a), 3.97 (dd, 1H, $J$=12.5 Hz, H-6c), 3.86 (dd, 1H, $J$=10.2 Hz, H-5c), 3.82 (s, 1H, H-3a), 3.79 (s, 1H, H-4a), 3.44 (dd, 1H, $J$=7.5, 9.7 Hz, H-2b), 3.39 (m, 1H, H-3b), 3.24 (s, 1H, H-2a), 2.16 (br s, 1H, 3b-OH), 2.00, 1.99, 1.84 (all s, 9H, 3COC$H_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.5, 170.0, 169.3 (*C*OCH$_3$), 138.3, 138.2, 137.5 (Bn), 134.4, (Phth 4/5), 131.0 (Phth 1/2), 128.4–127.7 (Bn), 123.6 (Phth 3/6), 102.2 (C-1b), 100.6 (C-1a), 97.7 (C-1c), 79.0 (C-2b), 77.4 (C-3a), 76.1 (C-4a), 75.1, 74.9, 74.8 (Ph*C*H$_2$×3), 74.1, 73.8 (C-5a, C-5b, C-4b), 72.4 (C-3b), 71.9 (C-5c), 70.7 (C-4c), 68.7 (C-3c), 67.6 (C-6b), 65.2 (C-6a), 61.7 (C-2a), 60.4 (C-6c), 54.5 (C-2c), 20.7–20.4 (CO*C*H$_3$). Anal. calcd for $C_{53}H_{56}N_4O_{18}\cdot H_2O$: C,60.34; H, 5.54; N, 5.31%. Found: C, 60.34; H, 5.39; N, 5.34%.

**3.1.9. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-[(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (14).** To a solution of tetrasaccharide **12** (150 mg, 0.103 mmol) in ethanol (5.0 mL) was added hydrazine monohydrate (0.50 mL). After refluxing for 5 h, the mixture was evaporated in vacuo, and the residual syrup was dissolved in pyridine (10 mL). DMAP (13 mg) and acetic anhydride (10 mL) were added to the solution at 0°C and the mixture was stirred for 14 h at room temperature. After evaporation, the residue was purified by silica gel column chromatography with chloroform–methanol (40:1, v/v) as an eluent to give **14** (102 mg, 77%): $[\alpha]_D^{25}$=−17.7° (*c* 0.579, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.45–7.22 [m, 15H, aromatic(Bn)], 5.47 (t, 1H, H-3c), 5.44 (s, 1H, H-1a), 5.25 (dd, 1H, $J$=9.5, 10.4 Hz, H-3c), 5.13 (d, 1H, N*H*), 5.09 (t, 1H, $J$=8.3, 9.7 Hz, H-4c), 5.00 (d, 1H, N*H*), 4.96 (t, 1H, $J$=9.5, 10.7 Hz, H-4c), 4.92–4.56 (6d, 6H, PhC$H_2$), 4.67 (d, 1H, $J$=8.2 Hz, H-1c), 4.62 (d, 1H, $J$=7.2 Hz, H-1c), 4.49 (d, 1H, $J$=7.6 Hz, H-1b), 3.27 (br, 1H, H-2a), 2.08–1.62 (all s, 24H, 8COC$H_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 170.7, 170.6, 170.6, 170.2, 169.8, 169.4, 169.3, (*C*OCH$_3$×8), 139.0, 138.6, 137.7 (Bn×3), 128.8–126.8 (Bn), 102.2 (C-1b), 101.5 (C-1c), 100.7 (C-1a), 100.3 (C-1c), 80.1, 79.2, 78.1, 76.2, 75.5, 74.6, 74.6, 74.2, 73.8, 72.7, 72.6, 71.9, 71.9, 71.7, 68.5, 68.4, 68.3, 65.4, 61.9, 61.7, 60.8, 55.1, 54.2, 22.8, 23.2 (NHCO*C*H$_3$×2), 20.8–20.6 (OCO*C*H$_3$). Anal. calcd for $C_{61}H_{75}N_5O_{25}\cdot CH_3OH$: C, 56.84; H, 6.08; N, 5.34%. Found: C, 56.74; H, 6.04; N, 5.21%.

**3.1.10. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-*O*-2,4-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-1,6-anhydro-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (15).** To a solution of tetrasaccharide **14** (100 mg, 78.2 μmol) in pyridine (1.5 mL) was added thioacetic acid (3.0 mL) and the mixture was stirred for 24 h at room temperature. After concentration, the residual oil was purified by silica gel column chromatography with chloroform–methanol (40:1, v/v) as an eluent to give **15** (97 mg, 96%): $[\alpha]_D^{25}$=−47.8° (*c* 0.524, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.30–7.40 [m, 15H, aromatic(Bn)], 6.72 (d, 1H, N*H*), 6.05 (d, 1H, N*H*), 5.53 (t, 1H, $J$=9.6, 10.0 Hz, H-3c), 5.34 (s, 1H, H-1a), 5.10 (dd, 1H, $J$=9.4, 9.8 Hz, H-3c), 5.01 (t, 1H, H-4c), 5.00 (t, 1H, H-4c), 4.98 (d, 1H, $J$=7.7 Hz, H-1c), 4.97–4.41 (6d, 6H, PhC$H_2$), 4.82 (d, 1H, $J$=8.7 Hz, H-1c), 4.28 (d, 1H, $J$=8.7 Hz, H-1b), 2.07–1.59 (all s, 27H, 9COC$H_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.9, 170.9, 170.6, 170.6, 170.5, 170.2, 169.8, 169.5, 169.3 (*C*OCH$_3$×9), 138.8, 138.4, 137.9 (Bn×3), 128.8–127.7 (Bn), 103.6 (C-1b), 101.9 (C-1c), 100.6 (C-1a), 100.2 (C-1c), 81.0, 79.6, 78.1, 75.8, 75.7, 74.7, 74.6, 74.4, 73.7, 72.6, 72.3, 72.0, 71.9, 71.8, 68.8, 68.6, 68.4, 64.9, 61.9, 61.8, 55.9, 54.3, 48.2, 23.2, 22.9, 22.6 (NHCO*C*H$_3$×3), 20.8–20.6 (OCO*C*H$_3$). Anal. calcd for $C_{63}H_{79}N_3O_{26}\cdot 2H_2O$: C, 56.88; H, 6.28; N, 3.15%. Found: C, 56.91; H, 6.04; N, 3.18%.

**3.1.11. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (16).** To a solution of tetrasaccharide **15** (60 mg, 46.4 μmol) in 1:1 (v/v) methanol–THF (2.0 mL) was added sodium methoxide (1 mg). After being stirred for 2 h at room temperature, the mixture was neutralized with the Dowex 50W-X8($H^+$) resin, filtered, and concentrated. 5% Pd/C (30 mg) was added to a solution of the residual syrup dissolved in methanol (2.0 mL) and the reaction mixture was stirred for 48 h at room temperature under hydrogen atmosphere. After filtration, the filtrate was evaporated to give **16** (29 mg, 81%): $[\alpha]_D^{25}$=−28.7° (*c* 0.483, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 5.37 (s, 1H, H-1a), 4.61 (d, 1H, $J$=8.7 Hz, H-1c), 4.46 (d, 2H, $J$=8.3 Hz, H-1b and H-1c), and 1.95, 1.95, 1.93 (3s, 9H, 3COC$H_3$); $^{13}$C NMR ($D_2O$) $\delta$ 175.8, 175.3, 174.5 (*C*OCH$_3$×3), 103.6 (C-1c), 103.2 (C-1b), 102.1 (C-1c), 100.8 (C-1a), 82.6, 78.9, 76.6, 76.4, 75.0, 74.6, 74.3, 74.2, 70.7, 70.6, 70.5, 70.1, 69.7, 69.2, 65.9, 61.5, 61.3, 56.5, 56.3, 52.7, 23.1, 23.1, 22.7 (NHCO*C*H$_3$×3); MALDI-TOF MS calcd for $C_{30}H_{49}N_3O_{20}$: 771.7. Found: *m*/*z* 793.7 $[M+Na]^+$. Anal. calcd for $C_{30}H_{49}N_3O_{20}\cdot 3H_2O$: C, 43.63; H, 6.71; N, 5.09%. Found: C, 43.27; H, 6.38; N, 4.87%.

**3.1.12. β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-**

**(1→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (17).** Tetrasaccharide **16** (10.5 mg, 13.6 μmol), UDP-galactose (25 mg, 41 μmol), α-lactalbumin (241.2 μg), $MnCl_2{\cdot}4H_2O$ (990 μg) and β-1,4-galactosyltransferase (3 U) in a HEPES buffer (50 mM, pH 6.0, 0.50 mL) were incubated for 48 h at 37°C. The mixture was purified by a column of Sephadex G-15 with water as an eluent and afford hexasaccharide **17** (13 mg, 87%); $[\alpha]_D^{25}=-21.2°$ (*c* 0.156, $H_2O$); $^1H$ NMR ($D_2O$, 50°C) δ 5.48 (s, 1H, H-1a), 4.71 (d, 1H, *J*=8.0 Hz, H-1c), 4.56 (d, 1H, *J*=7.8 Hz, H-1b), 4.54 (d, 1H, *J*=7.8 Hz, H-1c), 4.49 (d, 1H, *J*=7.5 Hz, H-1d), 4.47 (d, 1H, *J*=7.6 Hz, H-1d), 2.04, 2.04, 2.03 (3s, 9H, $3COCH_3$); $^{13}C$ NMR ($D_2O$, 50°C) δ177.7, 177.2, 176.4 ($COCH_3$×3), 105.7 (C-1d), 105.7 (C-1d), 105.4 (C-1c), 105.3 (C-1c), 104.0 (C-1b), 102.9 (C-1a), 84.7, 81.5, 81.3, 81.2, 78.1, 78.1, 77.6, 77.4, 77.1, 76.3, 75.4, 75.4, 75.2, 75.0, 73.8, 73.8, 72.6, 72.2, 71.6, 71.4, 71.4, 71.2, 67.9, 63.8, 63.8, 63.0, 62.9, 58.1, 57.9, 54.9, 25.0, 25.0, 24.7 ($NHCOCH_3$×3); MALDI-TOF MS calcd for $C_{42}H_{69}N_3O_{30}$ $[M+Na]^+$: 1096.0. Found: 1118.6. Anal. calcd for $C_{42}H_{69}N_3O_{30}{\cdot}3H_2O$: C, 43.86; H, 6.57; N, 3.65%. Found: C, 43.67; H, 6.38; N, 3.92%.

**3.1.13. 5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (1).** Hexasaccharide **17** (8.0 mg, 7.30 μmol), CMP-NANA (13.0 mg, 14.6 μmol), BSA (400 μg), $MnCl_2{\cdot}4H_2O$ (400 μg) and α-2,3-sialyltransferase (50 mU) in a sodium cacodylate buffer (50 mM, pH 7.3, 0.40 mL) were incubated for 48 h at 37°C. The mixture was purified by DEAE-Sephacel chromatography using 0.01–0.07 M $NH_4HCO_3$ solution as eluent. Ion-exchange chromatography (Dowex50W-X8($Na^+$)) and subsequent desalting on Sephadex G-25 column with deionized water as an eluent afforded the desired octasaccharide **1** (10.5 mg, 84%): $[\alpha]_D^{25}=-22.4°$ (*c* 0.324, $H_2O$); $^1H$ NMR ($D_2O$) δ 5.47 (s, 1H, H-1a), 4.70 (d, 1H, *J*=8.3 Hz, H-1c), 4.58–4.51 (m, 4H, H-1b, H-1c, H-1d, H-1d), 2.75 (dd, 1H, *J*=4.1, 12.1 Hz, H-3eq(e)), 2.03 (brs, 15H, $5COCH_3$), 1.80 (t, 1H, H-3ax(e)); $^{13}C$ NMR ($D_2O$, 40°C) δ 177.8, 177.8, 177.6, 177.2, 176.5, 176.5, 176.4 ($C$=O×7), 105.4, 105.4, 105.4, 105.2, 104.0, 102.8, 102.6, 102.6, 84.7, 81.2, 81.0, 80.9, 78.3, 78.3, 77.9, 77.9, 77.5, 77.4, 77.0, 76.3, 75.7, 75.7, 75.1, 74.9, 74.5, 74.5, 72.5, 72.1, 72.1, 72.1, 71.8, 71.2, 71.0, 71.0, 70.9, 70.9, 70.3, 70.3, 67.8, 65.4, 65.4, 63.7, 63.7, 62.9, 62.7, 58.0, 57.8, 54.7, 54.5, 54.5, 42.4, 42.4, 25.0, 25.0, 24.8, 24.8, 24.7 ($NHCOCH_3$×5); MALDI-TOF MS calcd for $C_{64}H_{103}N_5O_{46}$ $[M+Na]^+$, 1700.58 Found: 1700.8; ESI-HRMS calcd for $C_{64}H_{101}N_5O_{46}Na$ $[M-Na]^-$,1698.5616 Found: 1698.5657.

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