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Practical synthesis of fully protected globotriaose and its glycopolymers

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Abstract

Convenient and useful construction of a trisaccharide moiety of globotriaosyl ceramide was performed by means of modified Ogawa's protocol. In order to evaluate an efficiency of new class of glycopolymers, further chemical transformations of the trisaccharide were accomplished to afford a globotriaosyl carbohydrate monomer, and homopolymerization of the monomer by a general radical polymerization protocol gave a high-density glycopolymer in 84.3% yield after usual work-up procedures. In addition to the homopolymer, a copolymer composed of carbohydrate units and acrylamide units was also synthesized by the radical polymerization in 96.7% yield.

Keywords: Globotriaose; Glycosides; Polymerization; Glycoclusters; Glycopolymers

1. Introduction

Globotriaose (Galα1 → 4Galβ1 → 4Glcβ1→) is known not only as a trisaccharide moiety of globotriaosyl ceramide (Gb_3) but also an epitope of Shiga toxins (Stxs) (Lingwood, 1993; Sandvig, 2001), which was produced by Shiga toxin-producing *Escherichia coli* (Karmali, 1989), including O157:H7. We have recently reported a series of carbosilane dendrimers uniformly functionalized with globotriaosyl moieties (Matsuoka, Terabatake, Esumi, Terunuma, & Kuzuhara, 1999, 2006a, 2006b) and glycopolymers having globotriaosyl moieties as pendant-type epitopes (Miyagawa et al., 2004) for therapeutic use. Although the glycoclusters have different types of carbohydrate carriers, such as carbosilane dendrimers as core scaffolds (Nishikawa et al., 2002, 2005) and linear C–C backbones (Watanabe et al., 2004, 2006), some of the glycoclusters showed excellent neutralization potency against Stxs. In order to use the trisaccharide as the epitope for either biochemical or medical use, a convenient and a versatile synthetic route for construction of globotriaose is obviously needed. The synthetic plan for construction of the trisaccharidic determinant should include reduction of laborious efforts and chromatographic purifications. Large-scale preparations of globotriaosyl derivatives by chemical and chemo-enzymatic procedures have been reported (Koike et al., 1987; Kamath et al., 2004). In this paper, we also report practical preparation of fully protected globotriaose by the modified Ogawa's protocol (Koike et al., 1987). Furthermore, an application of fully protected globotriaose is described. The application includes introduction of a polymerizable spacer as the aglycon into the trisaccharide residue and polymerization, since we have reported syntheses and biological activities of glycopolymers having globotriaosyl moieties as pendant-type epitopes. From the results of our previous biological assay, pendant-type globotriaosyl residues in the glycopolymer were effectively recognized by both Stx 1 and Stx 2 *in vitro* experiments as well as *in vivo* experiments.

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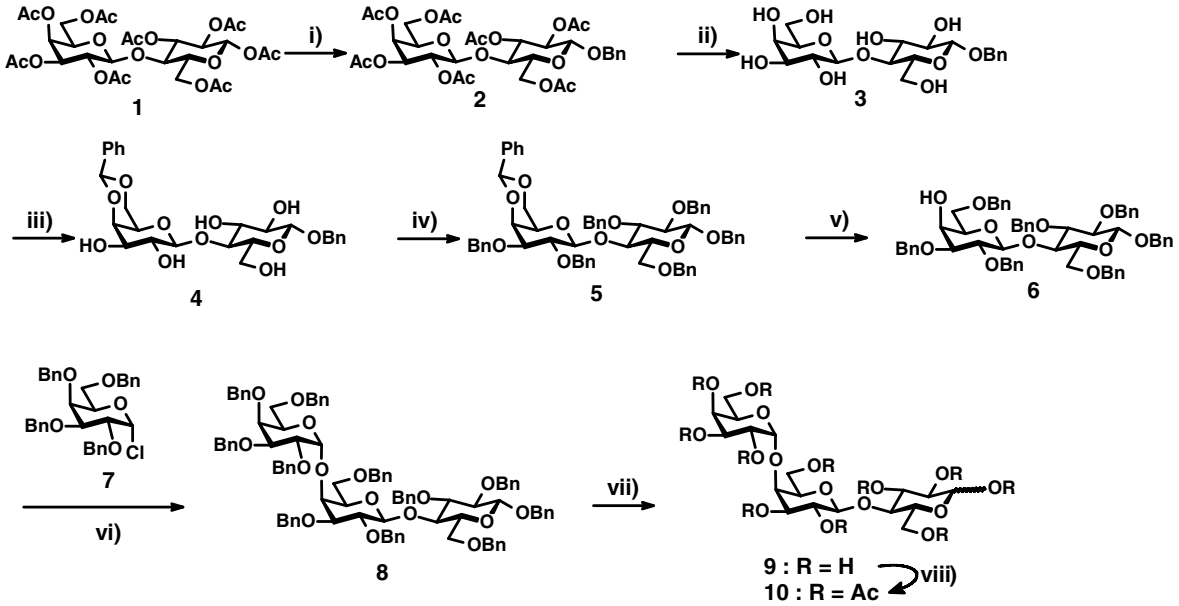
2. Results and discussion

2.1. Preparation of fully protected globotriaose

Large-scale preparations of a trisaccharide moiety of globotriaosyl ceramide have been reported by two groups (Koike et al., 1987; Kamath et al., 2004). We have selected to use the Ogawa's protocol with slight modifications, because it offers a simple and feasible strategy for our objective. The synthetic scheme for fully protected globotriaose **10** is summarized in Scheme 1. β-Selective glycosidation for β-acetate of lactose **1** with benzyl alcohol by using BF_3–OEt_2 as the promoter (Takano, Nakatsubo, & Murakami, 1990) gave β-benzyl glycoside **2** as white crystals in 46.1% yield without chromatographic purification. The ester function in **2** was removed by sodium methoxide-catalyzed transesterification to quantitatively afford crystalline **3**. Benzylidenation for **3** was performed by transacetalization of benzaldehyde dimethylacetal in the presence of TsOH as an acid catalyst. The reaction proceeded smoothly to yield amorphous **4**, in which remaining OH groups were all benzylated by usual Williamson's ether synthesis to provide crystalline **5** in high yield. The filtrate after the removal of the crystals afforded further **5** after a silica gel chromatographic purification. Reductive ring-opening of benzylidene acetal in **5** was performed according to the literature (Matsuoka, Nishimura, & Lee, 1994) to give crystalline **6** in 77.4% yield, which was used for a glycosidation as a glycosyl acceptor. Silver triflate-mediated glycosidation of acceptor **6** and a donor **7** (Austin, Hardy, Buchanan, & Baddiley, 1965) was performed in the presence of MS4A in absolute diethyl ether at low temperature, which are usual glycosidation conditions for such glycosidation, but no product was obtained due to poor solubility of acceptor **6** in diethyl ether. Various solvents for the glycosidation were tested, and tetrahydrofuran was selected for the glycosidation solvents, which had good solubility for the acceptor **6**. Under the same conditions except the use of a solvent, the reaction proceeded smoothly to afford trisaccharide **8** (Kim, Hosono, Sasai, & Shibasaki, 1995) in 85.2% yield having a newly formed α-linkage judged by the results of ^{13}C NMR. In this reaction, a chromatographic purification was needed. Because the crystallization of **8** from various solvent systems was examined, but all trials were unsuccessful. In addition, prolonged glycosidation reaction gave polymeric tetrahydrofuran and a glycosidation product was not isolated from the reaction mixture. Further 2-steps chemical modification of fully benzylated **8** was carried out. Thus, removal of all benzyl groups by hydrogenolysis in the presence of catalytic Pd on activated carbon under H_2 atmosphere gave polyol **9**, which was totally acetylated in the usual manner to give peracetate **10** in 90.5% (2 steps) yield.

2.2. Synthetic conversion of fully acetylated globotriaose into glycomonomer

Given the success of the preparation of fully acetylated globotriaose **10**, we examined the further transformation of **10** to a glycomonomer that can be used as a precursor of glycopolymers. Thus, the reactivity of the acetate **10** was first demonstrated against functional alcohols by BF_3–OEt_2-mediated reaction (Takano et al., 1990). Thus, **10** was treated with a 5 molar excess of ω-alkenyl alcohols in the presence of a 10 molar excess of BF_3–OEt_2 in dichloromethane to give corresponding β-glycoside **11** and **12** in moderate yields. Both known glycosides were able to be

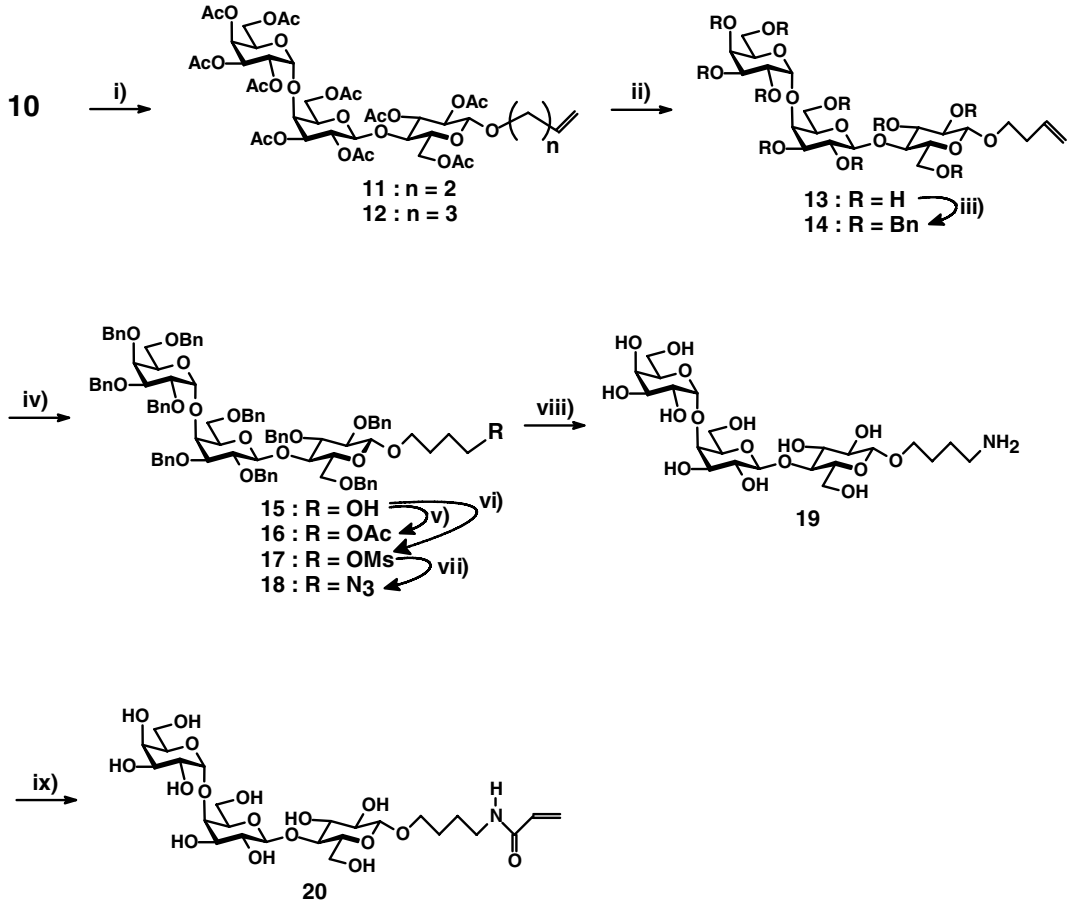


Scheme 1. Reagents and conditions: (i) BnOH, BF_3–OEt_2, CH_2Cl_2, −5 °C → rt, 2 h, (ii) NaOMe, MeOH, rt, 1 h, (iii) α,α-dimethoxytoluene, TsOH, DMF, 60 °C, 17 h, under reduced pressure, (iv) BnBr, NaH, DMF, 0 °C, 2 h, (v) BH_3–NMe_3, $AlCl_3$, MS4A, THF, rt, (vi) **7** (ref. Matsuoka et al., 2006a), AgOTf, MS4A, THF, −20 °C, 1 h, (vii) H_2, Pd/C, DMF–MeOH, rt, then, NaOAc, Ac_2O, 110 °C.

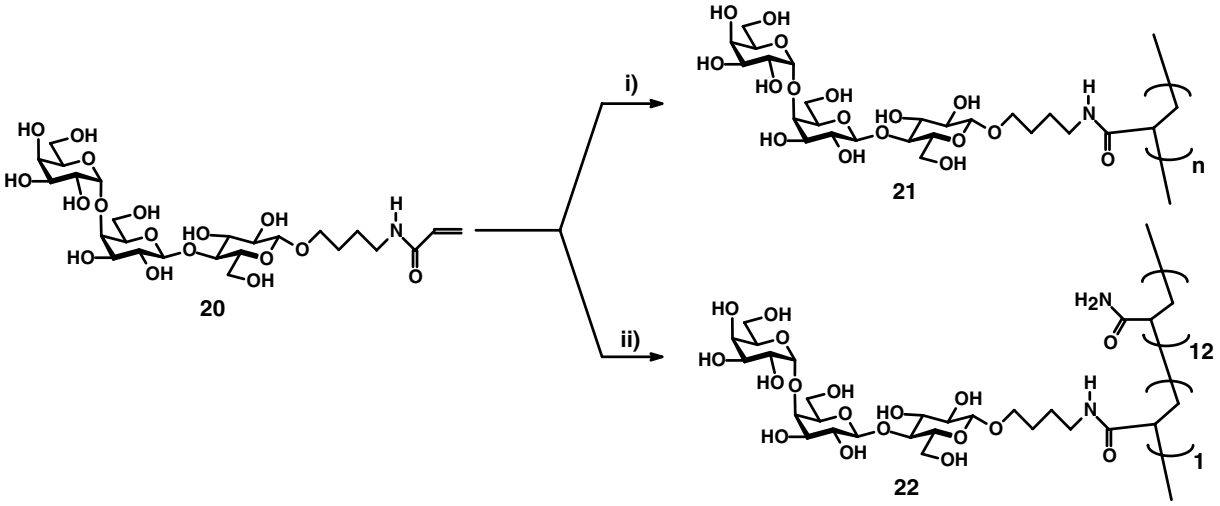
converted into their functional derivative by methods previously reported (Matsuoka et al., 2006a; Miyagawa et al., 2004). 3-Butenyl glycoside **11** was used for this study as a model compound. Transesterification of **11** by the usual procedure gave polyol **13**, which was then benzylated to afford fully benzylated glycoside **14** in high yield. Hydroboration to the terminal C=C double bond in aglycon of **14** was examined by a couple of conditions. Whereas the use of 9-BBN (Dubber & Lindhorst, 1998) as method A gave alcohol **15** in 91.8% yield, the use of cyclohexylborane as method B gave **15** in 96.2% yield. The results suggest that cyclohexylborane is a better reagent for the hydroboration for the C=C double bond in **14**. Regioselectivity of a newly formed hydroxyl group in **15** was determined by ^{1}H NMR study using acetylated product **16**. Mesylation of **15** yielded mesylate **17** in high yield, which was then treated with a large amount of sodium azide to provide azide derivative **18** having strong infrared absorption at 2096 cm^{-1}. Removal of all benzyl groups and reduction of the azide group in **18** were accomplished in the presence of $Pd(OH)_2/C$ under H_2 atmosphere to give amino alcohol **19**. The amino group of **19** was condensed with acryloyl chloride by Schotten-Baumann conditions to give acrylamide derivative **20** in moderate yield (Scheme 2).

2.3. Polymerization of globotriaosyl glycomonomer

Water-soluble *N*-acryloylated derivative **20** was initially polymerized itself in water by means of a general polymerization protocol, APS-TEMED as the radical initiator system (Matsuoka & Nishimura, 1995). The polymerization reaction proceeded smoothly at 50 °C and the viscosity of the reaction mixture gradually increased with progress of the reaction. Chromatographic purification by a gel filtration followed by lyophilization gave a white powdery glycopolymer **21** in 84.3% yield. The reaction conditions are summarized in Table 1. ^{1}H NMR spectra of the glycomonomer and the glycopolymers are shown in Fig. 1. Protons attached to C=C in the monomer **20** (a) at around 5–6 ppm completely vanished after radical polymerizations (b) and broadening of the peaks was observed. The ^{1}H NMR spectrum of copolymer **22** is shown in Fig. 1(c). The difference between homopolymer **21** and copolymer **22** is the relative area around 1.5 and 2.2 ppm due to methylene and methine



Scheme 2. Reagents and conditions: (i) 3-Buten-1-ol for **11**, 4-penten-1-ol for **12**, BF_3–OEt_2,CH_2Cl_2, −15 to 10 °C → rt, 2–3 h, (ii) NaOMe, MeOH, rt, 4.5 h, (iii) BnBr, NaH, DMF, 0 °C, (iv) 0.5 M 9-BBN–THF for method A, 1 M cyclohexylborane–THF for method B, 0 °C, then, 3 M aqueous NaOH, 30% aqueous H_2O_2, (v) Ac_2O–Pyr, rt, (vi) MsCl, Pyr, 0 °C, 1.5 h, (vii) NaN_3, DMF, 80 °C, (viii) H_2, $Pd(OH)_2/C$, DMF–MeOH, rt, (ix) CH_2=CHCOCl, Na_2CO_3, water, 0 °C.



Scheme 3. Reagents and conditions: (i) APS, TEMED,water, 50 °C, (ii) CH_2=CHCONH$_2$, APS, TEMED, water, 50 °C.

Table 1
Results of radical polymerization of **20** with or without acrylamide (AAm)

	Monomer ratio **20**:AAm	Total yield (%)	Polym. Comp.[a]	Sugar content (wt%)	$\overline{Mw}$ (kDa) [b]	$\overline{Mw}/\overline{Mn}$
Homopolymer **21**	1:0	84.3	1:0	100	330	2.0
Copolymer **22**	1:10	96.7	1:12	42.5	620	1.6

[a] Polymer compositions of sugar unit:acrylamide unit were estimated on the basis of the results of ^{1}H NMR.
[b] The weight-average molecular weights were estimated by size-exclusion chromatography in aqueous NaCl solution using tandem-bonded Shodex SB-803 and SB-804 columns. Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, 404, and 788 kDa; Shodex P-82).

protons, respectively. The weight-average molecular weight ($\overline{Mw}$) of the glycopolymer was estimated by size exclusion chromatography in 0.01 M aqueous NaCl solution. When pure water was used for the solvent to determine the molecular weight, the estimations were unsuccessful due to the aggregations of glycopolymers. We speculate that the hydrophobic region of a glycopolymer, such as a C–C backbone, may aggregate by hydrophobic interaction in pure water; however, addition of an electrolyte, such as NaCl, decreased hydrophobic interaction to give the proper molecular weight of the glycopolymers. Copolymerization of the glycosyl monomer **20** with acrylamide was also performed by same manner as that for **21** to afford water-soluble glycopolymer **22** having a molecular weight of 620 kDa in 96.7% yield. Polymer composition of the glycopolymer was estimated by the results of ^{1}H NMR, and the sugar content of the polymer was calculated according to the polymer composition on the basis of formula weights of the glycomonomer unit and the acrylamide unit Scheme 3.

In summary, we have described a practical synthesis of fully protected globotriaosyl derivative and its chemical modification to provide a water-soluble glycomonomer. Homopolymerization and copolymerization with acrylamide using the glycomonomer were performed to give water-soluble glycopolymer having high $\overline{Mw}$ in high yield. The biological activity of the glycopolymer is now under investigation, and the results will be reported in the near future.

3. Experimental section

3.1. General procedures

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Pyridine (Pyr) and *N*,*N*-dimethylformamide (DMF) were stored over molecular sieves (MS4Å), and methanol (MeOH) was stored over MS3Å before use. Dichloromethane was stored over MS4 Å after distillation. Tetrahydrofuran (THF) was distilled from the sodium benzophenone ketyl solution just before use. Removal of water from *p*-toluenesulfonic acid monohydrate was performed by azeotropic distillation with toluene just before use. Melting points were measured with a Laboratory Devices MELTEMP II apparatus and were uncorrected. The optical rotations were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a JASCO FT/IR-300E spectrophotometer. The ^{1}H NMR spectra were recorded at 400 MHz with a Bruker AM-400 or a Bruker DRX-400 spectrometer, or at 300 MHz with a Varian Mercury 300 spectrometer, or at 200 MHz with a Varian Gemini-2000 spectrometer in chloroform-*d* including tetramethylsilane (TMS) as the internal standard or D_2O including HDO (4.78 ppm) as the internal standard. The internal standards used for ^{13}C NMR spectra were $CDCl_3$ (77.0 ppm) in $CDCl_3$, MeOD (49.0 ppm) and acetone (29.8 ppm). Ring-proton assignments in NMR were made

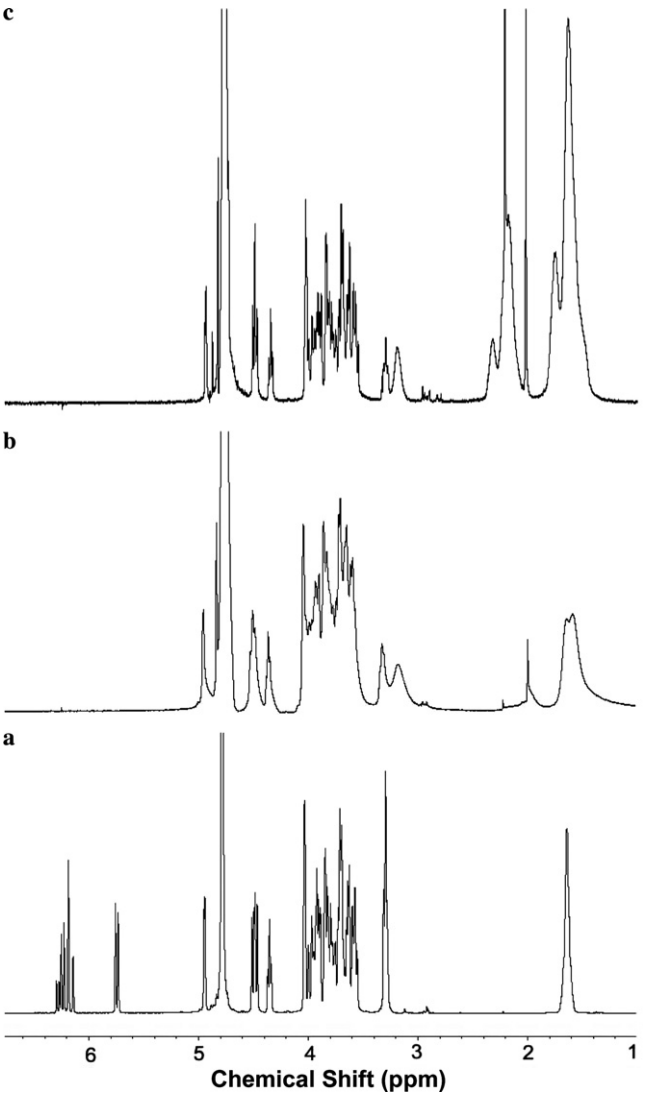


Fig. 1. ^{1}H NMR spectra of (a) glycomonomer **20**, (b) homopolymer **21**, and (c) copolymer **21** in D_2O.

by first-order analysis of the spectra and were supported by the results of homonuclear decoupling experiments and H–H or C–H cosy experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried at 50–60 °C over phosphorus pentoxide for 4–5 h. Fast atom bombardment mass (FAB-MS) spectra were recorded with a JEOL JMS-HX110 spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60F_{254} (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the intermediates, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) MeOH–*p*-anisaldehyde-concentrated sulfuric acid and heated for a few minutes (for carbohydrate) or (b) an aqueous solution of 5 wt% potassium permanganate and heated similarly (for C=C double bond). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm, E. Merck) or octadesyl (C_{18})-bonded silica gel (BAKERBOND® Octadecyl (C18) Prep LC Packing; 40 μm, J.T.Baker, Phillipsburg, NJ). Flush column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck). Preparative GPC was performed by a recycling system GPC apparatus [HLC-50G system (Shimamura, Instruments, Works, Co., Tokyo Japan)] using JAIGEL W252 with MilliQ water as the eluent (Kimura, Xue, Kobayashi, Onoda, & Yamamoto, 2004). All extractions were concentrated below 45 °C under diminished pressure.

3.2. Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl- β-D-glucopyranoside (2)

To a stirred solution of lactose peracetate **1** (61.8 g, 91.1 mmol) and benzyl alcohol (47.1 mL, 0.456 mol) in dichloromethane (370 mL) was dropwise added boron trifluoride diethyl etherate (115 mL, 0.911 mol) at −5 °C under N_2 atmosphere. After stirring at room temperature for 2 h, the solution was poured into ice-cold water and extracted with chloroform. The organic layer was successively washed with ice-cold aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The residual syrup was washed with hexane to remove an unreacted benzyl alcohol several times and dried *in vacuo*. The residue was crystallized from methanol to give pure **2** (30.4 g, 46.1%): mp 145–146 °C [145–146 °C (Richtmyer, 1946), 148–150 °C (Koike et al., 1987)]; R_F 0.51 [1:1 (v/v) toluene–ethyl acetate]; IR (KBr) 1751 ($\nu_{C=O}$), 1227 (ν_{C-O}), 1057 (ν_{C-O-C}) cm^{-1}; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.96, 2.01, 2.04, 2.05, 2.05, 2.14, and 2.15 (each s, 21 H, 7 $COCH_3$), 3.58 (ddd, 1 H, $J_{5,6a}$ 4.9 Hz and $J_{5,6b}$ = 1.9 Hz, H-5), 3.82 (t, 1 H, $J_{4,5}$ = 9.8 Hz, H-4), 3.87 (dd, 1 H, $J_{5',6'a}$ = 7.3 Hz and $J_{5',6'b}$ = 6.6 Hz, H-5′), 4.07 (dd, 1 H, $J_{6'a,6'b}$ = 11.2 Hz, H-6′a), 4.10 (dd, 1 H, $J_{6a,6b}$ = 12.7 Hz, H-6a), 4.12 (dd, 1 H, H-6′b), 4.48 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.51 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 4.53 (dd, 1 H, H-6b), 4.60 (d, 1 H, J_{gem} = 12.3 Hz, CH_aPh), 4.86 (d, 1 H, J_{gem} = 12.3 Hz, CH_bPh), 4.95 (dd, 1 H, $J_{3',4'}$ = 3.4 Hz, H-3′), 4.97 (dd, 1 H, $J_{2,3}$ = 9.2 Hz, H-2), 5.10 (dd, 1 H, $J_{2',3'}$ = 10.4 Hz, H-2′), 5.16 (t, 1 H, $J_{3,4}$ = 9.4 Hz, H-3), 5.34 (d, 1 H, $J_{4',5'}$ =∼ 0 Hz, H-4′), 7.13 (m, 5 H, Ph).

3.3. Benzyl O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (3)

To a solution of acetate **2** (24.2 g, 33.4 mmol) in methanol (180 mL) was added sodium methoxide (1.26 g, 23.4 mmol) with stirring at room temperature under nitrogen atmosphere. The solution became gel after 1 h, and a tlc of the reaction mixture indicated complete conversion of **2**. The mixture was treated with Dowex 50W-X8 (H^+) resin and the suspension was filtered. The filtrate was concentrated *in vacuo* to give crystalline **3** (14.4 g, quant.): mp 174–175 °C [180 °C (Beith-Halahmi, Flowers, & Shapiro,

1967)] R_F 0.63 [3:2 (v/v) chloroform–methanol]; IR (KBr) 3422 (ν_{O-H}), 1053 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ (400 MHz, D_2O) 3.34 (t, 1 H, $J_{5',6'a} = J_{5',6'b}$ 8.6 Hz, H-5′), 3.90 (d, 1 H, $J_{3',4'} = 3.2$ Hz, H-4′), 3.97 (dd, 1 H, $J_{5,6b} = 2.14$ Hz and $J_{6a,6b} = 12.3$ Hz, H-6b), 4.42 (d, 1 H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.53 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.74 (d, 1 H, $J_{gem} = 11.8$ Hz, CH_aPh), 4.92 (d, 1 H, $J_{gem} = 11.8$ Hz, CH_bPh), 7.43 (m, 5 H, Ph).

3.4. Benzyl O-(4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (4)

A solution of alcohol **3** (5.00 g, 11.6 mmol) in *N*,*N*-dimethylformamide (25 mL) was treated with α,α-dimethoxytoluene (2.61 mL, 17.4 mmol) and *p*-toluenesulfonic acid (600 mg, 3.48 mmol) at room temperature for 17 h. After disappearance of **3** judged by tlc, triethylamine (0.65 mL, 4.64 mmol) was added to the mixture. The mixture was evaporated, followed by repeated evaporation with toluene. Ethanolic solution of the residue gave amorphous **4** (5.25 g, 86.8%): R_F 0.46 [5:1 (v/v) chloroform—methanol]; IR (KBr) 3422 (ν_{O-H}), 2886 (ν_{C-H}), 1651 ($\nu_{C=C}$), 1068 (ν_{C-O-C}), 1034 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ (400 MHz, DMSO-d_6) 5.03 (s, 1 H, PhCH).

3.5. Benzyl O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)- 2,3,6-tri-O-benzyl-β-D-glucopyranoside (5)

To a suspension of NaH (50%, 9.40 g, 196 mmol, washed with hexane) in *N*,*N*-dimethylformamide (100 mL) was dropwise added alcohol **4** (10.2 g, 19.6 mmol) in *N*,*N*-dimethylformamide (100 mL) at 0 °C, and the mixture was stirred for 30 min under reduced pressure. Benzyl bromide (23.4 mL, 196 mmol) was dropwise added to the mixture at 0 °C, and the whole mixture was stirred for 2 h. To the mixture was added methanol (20.0 mL) at 0 °C, and the mixture was evaporated and poured into ice-cold water. The mixture was extracted with diethylether and the etherate solution was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. Crystallization of the residual syrup from ethanol gave white crystal **5** (11.2 g, 58.9%) after filtration. The filtrate was concentrated and the residue was applied to a column of silica gel with 4:1 (v/v) *n*-hexane–ethyl acetate as the eluent to yield further **5** (2.40 g, 12.6%). The total yield was 71.5% : mp 120–123 °C [122–123 °C (Lipták, Jodál, & Nánási, 1976), 118–120 °C (Sarkar & Roy, 1987), 120.2 °C (Qiu & Schmidt, 1992)]; R_F 0.61 [4:1 (v/v) toluene–ethyl acetate]; $[\alpha]_D^{31}$ +1.3° (*c* 1.53, $CHCl_3$) $[\alpha]_D^{25}$ −19° (*c* 1.35, $CHCl_3$) (Lipták et al., 1976), $[\alpha]_D^{25}$ −9° (*c* 1.5, $CHCl_3$) (Sarkar & Roy, 1987), $[\alpha]_D^{20}$ +11.0° (*c* 1, $CHCl_3$) (Qiu & Schmidt, 1992)]; IR (KBr) 3032 ($\nu_{C-H;aromatic}$), 2866 (ν_{C-H}),1497 (δ_{C-H}), 1454 (δ_{C-H}), 1096 (ν_{C-O-C}), 733 (δ_{C-H}), 698 (δ_{C-H}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 2.94 (br s, 1 H, H-5′), 3.35 (ddd, 1 H, $J_{5,6a} = 2.4$ Hz and $J_{5,6b} = 4.0$ Hz, H-5), 3.38 (dd, 1 H, $J_{3',4'} = 3.6$Hz, H-3′), 3.51 (t, 1 H, $J_{2,3} = 9.0$ Hz, H-2), 3.62 (t, 1 H, $J_{3,4} = 9.0$ Hz, H-3), 3.72 (dd, 1 H, H-6a), 3.76 (dd, 1 H, $J_{2',3'} = 9.6$Hz, H-2′), 3.83 (dd, 1 H, $J_{5',6'a} = 1.3$ Hz, H-6′a), 3.89 (dd, 1 H, $J_{6a,6b} = 10.9$ Hz, H-6b), 4.00 (t, 1 H, $J_{4,5} = 9.8$ Hz, H-4), 4.02 (d, 1 H, $J_{4',5'} = \sim 0$Hz, H-4′), 4.21 (br d, 1 H, $J_{6'a,6'b} = 12.3$ Hz, H-6′b), 4.34 (d, 1 H, $J_{gem} = 12.1$ Hz, C*H*Ph), 4.46 (d, 1 H, $J_{1,2} = 7.9$ Hz, H-1), 4.50 (d, 1 H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.57 (d, 1 H, J_{gem} 12.1 = Hz, C*H*Ph), 4.66 (d, 1 H, $J_{gem} = 12.0$ Hz, C*H*Ph), 4.74 (d, 1 H, $J_{gem} = 11.2$ Hz, C*H*Ph), 4.75 (m, 4 H, 4 C*H*Ph), 4.84 (d, 1 H, $J_{gem} = 11.2$ Hz, C*H*Ph), 4.92 (d, 1 H, $J_{gem} = 10.5$ Hz, C*H*Ph), 4.95 (d, 1 H, $J_{gem} = 11.7$ Hz, C*H*Ph), 5.18 (d, 1 H, $J_{gem} = 10.6$ Hz, C*H*Ph), 5.45 [s, 1 H, C*H*Ph (benzylidene)], 7.34 (m, 35 H, 7 Ph); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 66.1 (C-4′), 68.0 (C-6), 68.7 (C-6′), 70.8, 71.4, 72.7, 73.4, 74.8, 74.9, 75.0, 75.5, 77.4, 78.6, 79.4, 81.6, 82.8, 101.1, *C*HPh, benzylidene, 102.3, 102.6.

Anal. Calcd for $C_{61}H_{62}O_{11}$: C, 75.44; H, 6.43. Found: C, 75.49; H, 6.42.

3.6. Benzyl O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)- 2,3,6-tri-O-benzyl-β-D-glucopyranoside (6)

A suspension of acetal **5** (5.00 g, 5.15 mmol) and powdered MS4Å (5.00 g) in tetrahydrofuran (50 mL) was stirred for 30 min at room temperature under N_2 atmosphere. To the suspension was added BH_3–Me_3N complex (2.63 g, 36.1 mmol) at room temperature, and the mixture was cooled to 0 °C. Aluminum chloride (4.81 g, 36.1 mmol) was portionwise added to the mixture and the mixture was stirred overnight at room temperature. Chloroform was added to the mixture and the whole mixture was filtered on a pad of Celite to remove insoluble mass. The filtrate was successively washed with ice-cold water, 1 M aqueous hydrochloric acid, aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was crystallized from methanol to give pure **6** (3.88 g, 77.4%): mp 114–115°C [106–108 °C (Sarkar & Roy, 1987), 113–114 °C (Jung, Hoch, & Schmidt, 1989)]; R_F 0.65 [4:1 (v/v) toluene–ethyl acetate]; $[\alpha]_D^{29}$ +11° (*c* 1.2, $CHCl_3$) $[[\alpha]_D^{25}$ +4° (*c* 0.55, $CHCl_3$) (Lipták et al., 1976), $[\alpha]_D^{25}$ +22.1° (*c* 0.53, $CHCl_3$) (Koike et al., 1987), $[\alpha]_D^{25}$ +4° (*c* 1.6, $CHCl_3$) (Sarkar & Roy, 1987), $[\alpha]_D^{22}$ +8.9° (*c* 1, $CHCl_3$) (Jung et al., 1989)]; IR (KBr) 3464 (ν_{O-H}), 3032 ($\nu_{C-H;aromatic}$), 2878 (ν_{C-H}), 1651 ($\nu_{C=C}$), 1072 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 3.32 (brt, 1 H, H-5′), 3.36 (m, 1 H, H-5), 3.37 (dd, 1 H, $J_{3',4'} = 3.2$ Hz, H-3′), 3.47 (m, 1 H, H-6′a), 3.48 (t, 1 H, $J_{2,3} = 9.1$ Hz, H-2), 3.54 (t, 1 H, $J_{3,4} = 9.1$ Hz, H-3), 3.58 (t, 1 H, $J_{2',3'} = 9.1$ Hz, H-2′), 3.65 (dd, 1 H, $J_{5',6'b} = 7.5$ Hz and $J_{6'\,a,6'b} = 9.6$ Hz, H-6′b), 3.73 (dd, 1 H, $J_{5,6a} = 1.1$ Hz, H-6a), 3.82 (dd, 1 H, $J_{5,6b} = 4.3$ Hz and $J_{6a,6b} = 10.7$ Hz, H-6b), 3.99 (t, 1 H, $J_{4,5} = 9.6$ Hz, H-4), 4.01 (d, 1 H, $J_{4',5'} = 0$ Hz, H-4′), 4.39 (d, 1 H, $J_{gem} = 11.8$ Hz, C*H*Ph), 4.40 (d, 1 H, $J_{gem} = 12.9$ Hz, C*H*Ph), 4.43 (d, 1 H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.45 (d, 1 H, J_{gem} 11.8 = Hz, C*H*Ph),

4.46 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.57 (d, 1 H, $J_{gem} = 11.8$ Hz, C*H*Ph), 4.65 (d, 1 H, $J_{gem} = 12.3$ Hz, C*H*Ph), 4.68 (d, 1 H, $J_{gem} = 11.8$ Hz, C*H*Ph), 4.70 (m, 3 H, 3 C*H*Ph), 4.72 (d, 1 H, $J_{gem} = 10.7$ Hz, C*H*Ph), 4.75 (d, 1 H, $J_{gem} = 11.2$ Hz, C*H*Ph), 4.90 (d, 1 H, $J_{gem} = 11.2$ Hz, C*H*Ph), 4.94 (d, 1 H, $J_{gem} = 12.3$ Hz, C*H*Ph), 4.97 (d, 1 H, $J_{gem} = 10.7$ Hz, C*H*Ph), 7.30 (m, 35 H, 7 Ph); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 66.1 (C-4′), 68.2 (C-6), 68.4 (C-6′), 70.9 (OCH_2), 72.0 (OCH_2), 72.7 (C-5′), 73.1 (OCH_2), 73.5 (OCH_2), 75.0 (OCH_2), 75.1 (C-5), 75.2 (OCH_2), 75.3 (OCH_2), 76.6 (C-4), 79.4 (C-2′), 81.1 (C-3′), 81.8 (C-2), 82.9 (C-3), 102.5 (C-1 & C-1′).

3.7. Benzyl O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (8)

To a suspension of known glycosyl donor **7** (Austin et al., 1965) (10.1 g, 18.0 mmol) and powdered MS4Å (10.0 g) in tetrahydrofuran (50 mL) was added a solution of glycosyl acceptor **6** (8.75 g, 8.99 mmol) in tetrahydrofuran (100 mL) at room temperature under N_2 atmosphere. After cooling to −20 °C, silver triflate (6.93 g, 27.0 mmol) was portionwise added to the mixture with vigorous stirring. The reaction mixture was continuously stirred for 1 h at the same temperature. When the tlc indicated complete conversion of the acceptor, the whole mixture was filtered through a pad of Celite. The filtrate was diluted with chloroform and the organic solution was successively washed with ice-cold water, aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. Chromatographic purification of the residue using a silica gel column with 7:1 (v/v) *n*-hexane–ethyl acetate as the eluent afforded known trisaccharide (Koike et al., 1987; Sarkar & Matta, 1992) **8** (11.4 g, 85.2%): R_F 0.31 [4:1 (v/v) *n*-hexane–ethyl acetate]; ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 100.7 (C-1′), 102.5(C-1), 102.8 (C-1′).

3.8. O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (10)

A solution of **8** (11.4 g, 7.62 mmol) in 1:1 (v/v) *N*,*N*-dimethylformamide–methanol (60 mL) was added to a mixture of 20 wt% $Pd(OH)_2$/C (8.0 g) in 1:1 (v/v) *N*,*N*-dimethylformamide–methanol (60 mL) at room temperature. The reaction mixture was vigorously stirred at ambient temperature until complete removal of benzyl groups on the tlc. The reaction mixture was filtered and evaporated *in vacuo* to afford polyol **9**, which was used for the next step without purification: R_F 0.29 [3:3:1 (v/v/v) chloroform–methanol–water]. A solution of the alcohol **9** (~7.62 mmol) in *N*,*N*-dimethylformamide (20 mL) was dropwise added to a mixture of sodium acetate (813 mg, 9.91 mmol) in acetic anhydride (39.6 mL, 419 mmol) at 110 °C. When the tlc showed conversion of alcohols into corresponding acetate, the reaction mixture was poured into ice-water and extracted with chloroform. The organic layer was successively washed with aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel with 1:1 (v/v) toluene–ethyl acetate as the eluent to give pure acetate **10** (6.94 g, 90.5%) as a 1:2 (α:β) anomeric mixture: R_F 0.32 [1:2 (v/v) toluene–ethyl acetate]; ^{1}H NMR δ (200 MHz, $CDCl_3$) 5.68 (d, 2/3 H, $J_{1,2} = 8.1$ Hz, H-1β), 6.22 (d, 1/3 H, $J_{1,2} = 4.1$ Hz, H-1α).

3.9. 3-Butenyl O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-acetyl-D-glucopyranoside (11)

To a cooled solution of acetate **10** (4.30 g, 4.46 mmol) and 3-buten-1-ol (1.92 mL, 22.3 mmol) in dichloromethane (40 mL) was added dropwise boron trifluoride—diethyl etherate (5.65 mL, 44.6 mmol) at −10 °C under N_2 atmosphere. The solution was stirred for 2.5 h at 0 °C and the reaction mixture was poured into ice-water. Chloroform was added to the mixture, and then the mixture was partitioned. The organic layer was successively washed with aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The residual syrup was purified by silica gel chromatography with 5:4 (v/v) toluene–ethyl acetate as the eluent to yield known glycoside **11** (1.43 g, 32.9%): R_F 0.48 [1:2 (v/v) toluene–ethyl acetate]. Structural confirmation was performed using spectroscopic analysis, and the results were in good agreement with previously reported results (Matsuoka et al., 2006a).

3.10. 4-Pentenyl O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-acetyl-D-glucopyranoside (12)

Boron trifluoride–diethyl etherate (1.5 mL, 11.8 mmol) was added dropwise to a stirred solution of acetate **10** (1.15 g, 1.19 mmol) and 4-penten-1-ol (0.614 mL, 5.95 mmol) in dichloromethane (10 mL) at −15 °C under N_2 atmosphere. The solution was stirred at 0 °C for 0.5 h and then at room temperature for another 1.5 h. When the tlc showed vanishment of **10**, the reaction mixture was poured into ice-water, followed by addition of chloroform. The mixture was shaken and partitioned. The chloroform extraction was successively washed with aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. Chromatographic purification of the residue using a column of silica gel with 2:1 (v/v) toluene–ethyl acetate as the eluent gave known glycoside **13** (573 mg, 48.6%) as an amorphous solid: R_F 0.59 [1:2 (v/v) toluene–ethyl acetate]. Structural

elucidation was accomplished by a direct comparison to previously reported **13**, and the results of the spectral analyses were in good agreement (Miyagawa et al., 2004).

3.11. 3-Butenyl O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-benzyl-D-glucopyranoside (14)

To a stirred solution of acetate **11** (1.43 g, 1.46 mmol) in methanol (15 mL) was added sodium methoxide (55.2 mg, 1.02 mmol) at room temperature under N_2 atmosphere and the solution was further stirred for 4.5 h. The solution was neutralized with IRA-120B (H^+) resin and the suspension was filtered. The filtrate was concentrated *in vacuo* to give pure **13** (790 mg, 96.5%), which was used for the next step without further purification: R_F 0.59 [65:25:4 (v/v/v) chloroform–ethyl acetate–methanol]. A solution of alcohol **13** in *N*,*N*-dimethylformamide (8 mL) was dropwise added to the stirred suspension of NaH (50%, 1.05 g, 21.9 mmol, washed with hexane) in *N*,*N*-dimethylformamide (10 mL) at 0°C under N_2 atmosphere. To the suspension was dropwise added benzyl bromide (3.47 mL, 29.2 mmol) at 0°C. The work-up procedure was the same as that described for **5** to afford perbenzylated product **14** (1.84 g, 89.3%), for which structural elucidation was performed by a direct comparison to previously reported **14**, and the results of the spectral analyses were in good agreement (Matsuoka et al., 2006a): R_F 0.44 [:4:1 (v/v) *n*-hexane–ethyl acetate].

3.12. 4-Hydroxybutyl O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-benzyl-D-glucopyranoside (15)

Method A: To a stirred solution of alkene **14** (4.32 g, 2.96 mmol) in tetrahydrofuran (45 mL) was dropwise added 0.5 M 9-BBN–THF solution (17.8 mL, 8.88 mmol) at 0 °C under Ar atmosphere and the solution was further stirred overnight at room temperature. To the reaction mixture was added water (1.78 mL), followed by addition of 3 M aqueous NaOH (8.88 mL, 26.6 mmol) and 30% aqueous H_2O_2 (8.88 mL) and the mixture was stirred further overnight at room temperature. To the reaction mixture was added solid NaCl until the solution became saturated. The whole mixture was filtered to remove insoluble mass and the filtrate was partitioned. The aqueous solution was extracted with tetrahydrofuran and the organic extractions were combined, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. Chromatographic purification of the residue using a column of silica gel with 2:1 to 1:1 (v/v) *n*-hexane–ethyl acetate as the eluent gave pure alcohol **15** (4.01 g, 91.8%) as a syrup: R_F 0.44 [2:1 (v/v) *n*-hexane–ethyl acetate]; $[\alpha]_D^{30}$ +30° (*c* 1.1, $CHCl_3$); IR (KBr) 3484 (ν_{O-H}), 3030 ($\nu_{C-H;aromatic}$), 2877 (ν_{C-H}), 1497 (δ_{C-H}), 1454 (δ_{C-H}), 1096 (ν_{C-O-C}), 735 (δ_{C-H}), 697 (δ_{C-H}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 1.46 (br s, 1 H, OH), 1.67 (m, 4 H, 2 CH_2), 3.16 (dd, 1 H, $J = 4.6$ Hz and $J = 8.2$ Hz), 3.36 (dd, 1 H, $J_{2,3} = 9.1$ Hz, H-2), 3.62 (dd, 1 H, $J_{2',3'} = 10.2$ Hz, H-2′), 3.71 (dd, 1 H, $J_{5,6a} = \sim 2$ Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6b} = 4.5$ Hz and $J_{6a,6b} = 10.9$ Hz, H-6b), 3.96 (dd, 1 H, $J = 2.6$ Hz and $J = 10.0$ Hz), 4.02 (dd, 1 H, $J = 2.5$ Hz and $J = 8.6$ Hz), 4.07 (dd, 1 H, $J = 3.3$ Hz and $J = 10.3$ Hz), 4.48 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.50 (d, 1 H, $J_{1',2'} = 8.1$ Hz, H-1′), 5.04 (d, 1 H, $J_{1'',2''} = 2.8$ Hz, H-1′), 7.24 (m, 50 H, 10 Ph); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 26.1 (CH_2), 29.4 (CH_2), 62.3 (CH_2CH_2OH), 67.8, 67.8, 68.2, 69.4, 69.6, 72.0, 72.4, 72.9, 73.1, 73.3, 73.6, 74.8, 74.8, 74.8, 75.0, 75.1, 76.6, 77.1, 77.2, 79.3, 79.3, 81.5, 81.6, 82.5, 100.5 (C-1″), 102.7 (C-1), 103.4 (C-1′) 138.0 [C-1(Ph)], 138.3 [C-1(Ph)], 138.4 [C-1(Ph)], 138.5 [C-1(Ph)], 138.6 [C-1(Ph)], 138.6 [C-1(Ph)], 138.7 [C-1(Ph)], 138.7 [C-1(Ph)], 138.9 [C-1(Ph)], 139.1 [C-1(Ph)]; FAB MS Calcd for $[M+H^+]$: 1477.7. Found: *m*/*z* 1477.3; Anal. Calcd for $C_{92}H_{100}O_{17}$;· H_2O: C, 73.87; H, 6.87 Found: C, 74.01; H, 6.82.

Method B: Cyclohexene (191 μL, 1.89 mmol) was dropwise added to a 1 M solution of BH_3–THF (1.89 mL, 1.89 mmol) at 0 °C under N_2 atmosphere, to which was then added a solution of alkene **14** (1.84 g, 1.26 mmol) in tetrahydrofuran (15 mL). The reaction mixture was stirred for 1.5 h at room temperature and the tlc showed complete conversion of the alkene. Dropwise additions of methanol (3.5 mL), 3 M aqueous NaOH (12 mL), and 30% aqueous H_2O_2 (3.5 mL) to the mixture were successively performed, and the mixture was stirred overnight at 60 °C under N_2 atmosphere. Chloroform was added to the reaction mixture, and the mixture was partitioned. The organic solution was washed with water and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to dryness. The resulting mass was purified by silica gel chromatography with 3:1 (v/v) *n*-hexane–ethyl acetate as the eluent to afford pure alcohol **15** (1.79 g, 96.2%).

3.13. 4-(Acetyloxy)butyl O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)- (1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)- (1 → 4)-1,2,3,6-tetra-O-benzyl-D-glucopyranoside (16)

An acetylation of alcohol **15** (38.1 mg, 25.8 μmol) in 1:1 (v/v) acetic anhydride–pyridine (2 mL) gave monoacetate **16** (36.9 mg) in 94.1% yield: R_F 0.44 [2:1 (v/v) *n*-hexane–ethyl acetate]; IR (neat) 1737 ($\nu_{C=O}$), 1497 (δ_{C-H}), 1454 (δ_{C-H}), 1096 (ν_{C-O-C}), 736 (δ_{C-H}), 698 (δ_{C-H}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 2.01 (s, 3 H, Ac).

3.14. 4-(Methylsulfonyloxy)butyl O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-benzyl-D-glucopyranoside (17)

To a stirred solution of alcohol **15** (2.27 g, 1.54 mmol) in pyridine (23 mL) was dropwise added methanesulfonyl

chloride at 0 °C under N_2 atmosphere, and the reaction mixture was stirred for 1.5 h at the same temperature. Water was added to the mixture, and chloroform extraction was carried out. The organic layer was successively washed with 1 M aqueous hydrogen chloride, aqueous saturated sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo* to give mesylate **17** (2.35 g, 97.9%): R_F 0.57 [1:1 (v/v) *n*-hexane–ethyl acetate]; IR (neat) 3033 ($\nu_{C-H;aromatic}$), 2874 (ν_{C-H}), 1497 (δ_{C-H}), 1454 (δ_{C-H}), 1359 ($\nu_{O=S=O}$), 1174 ($\nu_{O=S=O}$), 1051 (ν_{C-O-C}), 736 (δ_{C-H}), 697 (δ_{C-H}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 2.86 (s, 3 H, CH_3SO_2); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 37.1 (CH_3SO_2), 100.6 (C-1″), 102.7 (C-1), 103.3 (C-1′).

3.15. 4-Azidobutyl O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-benzyl-D-glucopyranoside (18)

A suspension of mesylate **17** (1.42 g, 0.914 mmol) and sodium azide (297 mg, 4.57 mmol) in *N*,*N*-dimethylformamide (15 mL) was stirred at 80 °C overnight under N_2 atmosphere. The mixture was poured into water followed by addition of chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The residue was applied to a column of silica gel with 4:1 (v/v) *n*-hexane–ethyl acetate as the eluent to yield **18** (1.34 g, 97.8%) as a syrup: R_F 0.58 [2:1 (v/v) *n*-hexane–ethyl acetate]; $[\alpha]_D^{30}$ +30° (*c* 1.1, $CHCl_3$); IR (neat) 3030($\nu_{C-H;aromatic}$), 2865 (ν_{C-H}), 2096 ($\nu_{N=N=N}$), 1497 (δ_{C-H}), 1455 (δ_{C-H}) 1096 (ν_{C-O-C}), 735 (δ_{C-H}), 698 (δ_{C-H}) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 1.68 (m, 4 H, 2 CH_2), 3.16 (dd, 1 H, $J = 4.6$ Hz and $J = 8.3$ Hz), 3.36 (dd, 1 H, $J_{2,3} = 9.1$ Hz, H-2), 3.56 (dd, 1 H, $J_{2',3'} = 9.9$ Hz, H-2′), 3.70 (dd, 1 H, $J_{5,6a} = 2$ Hz, H-6a), 3.81 (dd, 1 H, $J_{5,6b} = 4.5$ Hz and $J_{6a,6b} = 10.9$ Hz, H-6b), 3.93 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-4), 3.97 (dd, 1 H, $J = 2.5$ Hz and $J = 10.3$ Hz), 4.02 (dd, 1 H, $J = 2.5$ Hz and $J = 8.8$ Hz), 4.07 (dd, 1 H, $J = 3.3$ Hz and $J = 10.3$ Hz), 4.46 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1), 4.50 (d, 1 H, $J_{1',2'} = 7.7$ Hz, H-1′), 5.04 (d, 1 H,$J_{1'',2''} = 2.8$ Hz, H-1″), 7.25 (m, 50 H, 10 Ph); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 25.7 (CH_2), 26.9 (CH_2), 51.1 ($CH_2CH_2N_3$), 100.7 (C-1″), 102.8 (C-1), 103.5 (C-1′); FAB MS Calcd for [M+H$^+$]: 1500.69. Found: *m*/*z* 1500.61; Anal. Calcd for $C_{92}H_{99}O_{16}N_3$: C, 73.53; H, 6.64; N, 2.80. Found: C, 73.66; H, 6.76; N, 2.86.

3.16. 4-Aminobutyl O-α-D-galactopyranosyl-(1 → 4)-O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (19)

A mixture of **18** (500 mg, 0.333 mmol) in 1:1 (v/v) *N*,*N*-dimethylformamide–methanol (5 mL) was stirred in the presence of $Pd(OH)_2$/C (300 mg) at room temperature overnight under H_2 atmosphere. After consuming the starting material on the tlc, the suspension was filtered through a pad of activated carbon and the filtrate was concentrated. The analytical sample was purified by using a column of octadesyl (C_{18})-bonded silica gel with 0:1 to 1:0 (v/v) methanol–water as the eluent to provide aminoalcohol **19** as a syrup: positive ninhydrin test; R_F 0.01 [5:5:1 (v/v/v) chloroform–methanol–water]; ^1H NMR δ (300 MHz, D_2O) 4.45 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.46 (d, 1 H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.90 (d, 1 H, $J_{1'',2''} = 3.7$ Hz, H-1″); ^{13}C NMR δ (75.4 MHz, D_2O) 23.7 (CH_2), 25.9 (CH_2), 39.3 (CH_2CH_2N), 60.1, 60.5, 60.6, 68.7, 69.1, 69.2, 69.7, 70.9, 71.0, 72.3, 73.0, 74.6, 74.9, 75.5, 77.5, 78.8,100.4 (C-1″), 102.1 (C-1), 103.4 (C-1′).

3.17. 4-Acrylamidobutyl O-α-D-galactopyranosyl-(1 → 4)-O-β-D- galactopyranosyl-(1 → 4)-β-D-glucopyranoside (20)

To a solution of amine **19** (191 mg, 0.333 mmol) and Na_2CO_3 (352 mg, 3.32 mmol) in water (4 mL) was added dropwise acryloyl chloride (202 μL, 2.49 mmol) at 0 °C under N_2 atmosphere and the reaction mixture was stirred overnight. The reaction mixture was filtered and the filtrate was passed through a column of Sephadex LH-20 with methanol to give crude acrylamide **20**, which was further purified by using GPC to give pure **20** (76 mg, 36.4%): R_F 0.57 [3:3:1 (v/v/v) chloroform—ethanol–water]; $[\alpha]_D^{25}$ +50° (*c* 1.1, H_2O); IR (KBr) 3402 (ν_{O-H}), 2928 (ν_{C-H}), 2884 (ν_{C-H}), 1655 ($\nu_{C=O}$, Amide I), 1555 (δ_{N-H}, Amide II), 1076 (ν_{C-O-C})cm^{-1}; ^1H NMR δ (400 MHz, D_2O) 4.47 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1), 4.50 (d, 1 H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.94 (d, 1 H, $J_{1'',2''} = 3.3$ Hz, H-1″), 5.74 (d, 1 H, $J_{cis} = 10.0$ Hz, CO–CH=CH_{cis}), 6.16 (d, 1 H, $J_{trans} = 17.1$ Hz, CO–CH=CH_{trans}), 6.26 (dd, 1 H, CO–CH=CH$_2$); ^{13}C NMR δ (100.6 MHz, D_2O) 24.4 (CH_2), 25.7 (CH_2), 38.6 (CH_2CH_2N), 59.6, 59.9, 60.1, 68.1, 68.5, 68.7, 69.6, 70.4, 70.5, 71.3, 72.5, 74.0, 74.4, 75.0, 76.9, 78.2, 99.9 (C-1″), 101.5 (C-1), 102.8 (C-1′), 126.6 (CH_2=), 129.6 (CH=), 168.0 (C=O); FAB MS Calcd for [M+H$^+$]: 630.26. Found: *m*/*z* 630.73, [M+Na$^+$]: 652.24. Found: *m*/*z* 652.66; Anal. Calcd for $C_{25}H_{43}O_{17}N_1 \cdot 1.5\ H_2O$: C, 45.73; H, 7.06; N, 2.13. Found: C, 45.64; H, 6.94; N, 2.22.

3.18. Radical polymerization

Homopolymerization: A solution of carbohydrate monomer **20** (40.1 mg, 63.7 μmol) in deionized water (0.4 mL) was deaerated under reduced pressure for a few minutes, and then TEMED (0.96 μL, 6.4 μmol) and APS (0.58 mg, 2.5 μmol) were added. The mixture was stirred at 50 °C for 27 h and diluted with 1 M aqueous pyridine–acetic acid buffer (pH 5, 1 mL). The viscous solution was directly applied to a column of Sephadex G-50 (220 mL) with 5% aqueous acetic acid as the eluent to give white powdery homopolymer **21** (33.8 mg, 84.3%) after lyophilization: $\overline{M}$n 164 kDa, $\overline{M}$w 330 kDa, $\overline{M}$n/$\overline{M}$w 2.0; ^1H NMR δ (400 MHz, D_2O) 1.58 (br m, 4 H, 2 CH_2), 4.36

(t, 1 H), 4.50 (br t, 2 H, H-1 and H-1′), 4.95 (br s, 1 H, H-1″).

Copolymerization: A carbohydrate monomer **20** (25.3 mg, 40.2 μmol) was copolymerized with acrylamide (28.6 mg, 402 μmol) by the same method as that described for homopolymerization to yield copolymer **22** (52.1 mg, 96.7%) after lyophilization: $\overline{Mn}$ 391 kDa, $\overline{Mw}$ 620 kDa, $\overline{Mn}$ / $\overline{Mw}$ 1.6; ^{1}H NMR δ (400 MHz, D_2O) 1.70 (br m, 26 H, 13 CH_2), 2.21 (m, 13 H, 13 CH), 4.48 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1), 4.50 (d, 1 H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.94 (d, 1 H, $J_{1'',2''} = 3.8$ Hz, H-1″).

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