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Note

A concise and practical synthesis of antigenic globotriose, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc

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Abstract—A concise and practical synthesis of the antigenic globotriose, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc (**13**), was achieved by coupling of a monosaccharide donor, 3-*O*-allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate (**4**) with a disaccharide acceptor, *p*-methoxyphenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**8**), followed by deprotection. In spite of the existence of a C-2-ester substituent capable of neighboring-group participation in the donor, the coupling gave exclusively the α-linkage in satisfactory yield. The acceptor **8** was readily obtained from selective 3-*O*-benzoylation of the galactosyl ring of *p*-methoxyphenyl 2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**7**), which was prepared from *p*-methoxyphenyl β-D-lactoside (**5**) via isopropylidenation, benzoylation, and deisopropylidenation. Donor **4** was obtained from *p*-methoxylphenyl 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (**1**) via selective 4,6-di-O-debenzoylation, oxidative removal of 1-*O*-MP, benzylidenation, and trichloroacetimidate formation.

Keywords: Globotriose; Oligosaccharide; Neighboring-group participation

Although great effort has been expended in glycochemistry over the last several decades, one focus of extensive investigation in glycochemistry still remains: the development of a rational methodology to highly stereo- and regioselectively synthesize biologically relevant oligosaccharides and their complexes that are being discovered at a markedly increased rate.[1] In principle, the stereoselectivity of glycoside synthesis relies on many factors such as the anomeric effect,[1a] neighboring-group participation,[2] and remote participation.[3] Generally, it is rather difficult to obtain pure 1,2-*cis*-linked glycosylic linkages with the donors having the gluco- and galacto configurations.

In our ongoing interest in synthetic strategies, some oligosaccharides containing 1,2-*cis* α-linked galactopyranosyl linkages have attracted our attention.[4] It is been well known that α-linked galactopyranosyl oligosaccharides play important roles in biological processes. For example, the glycosyl phosphatidylinositol (GPI) anchor of *Trypanosoma brucei* is involved in the signal transduction of insulin, IL-2, and nerve growth factor (NGF).[5] Many of the globoseries glycolipids containing the globotriose fragment, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc, have been isolated and characterized.[6] Globotriosides are the major glycosphingolipids present in the membrane of human erythrocytes from all individuals,[6c] and as antigens these are recognized by antibodies of the P blood-group system and by various bacterial proteins.[7] For example, globotrioside present in the membrane of urinary tract epithelial cells acts as a receptor for pathogenic strains of *Escherichia coli* responsible for pyelonephritis.[6c] Furthermore, there are tumor-associated antigens on human teratocarcinoma cells and other tumor cells.[8] Similarly, glycosphingolipid antigen from the breast cancer cell line MCF-7 might be clinically useful in promoting active immunity in the case of sufficient synthesis.[1b] These oligosaccharides are also apparently related to Fabry's disease, which is due to a

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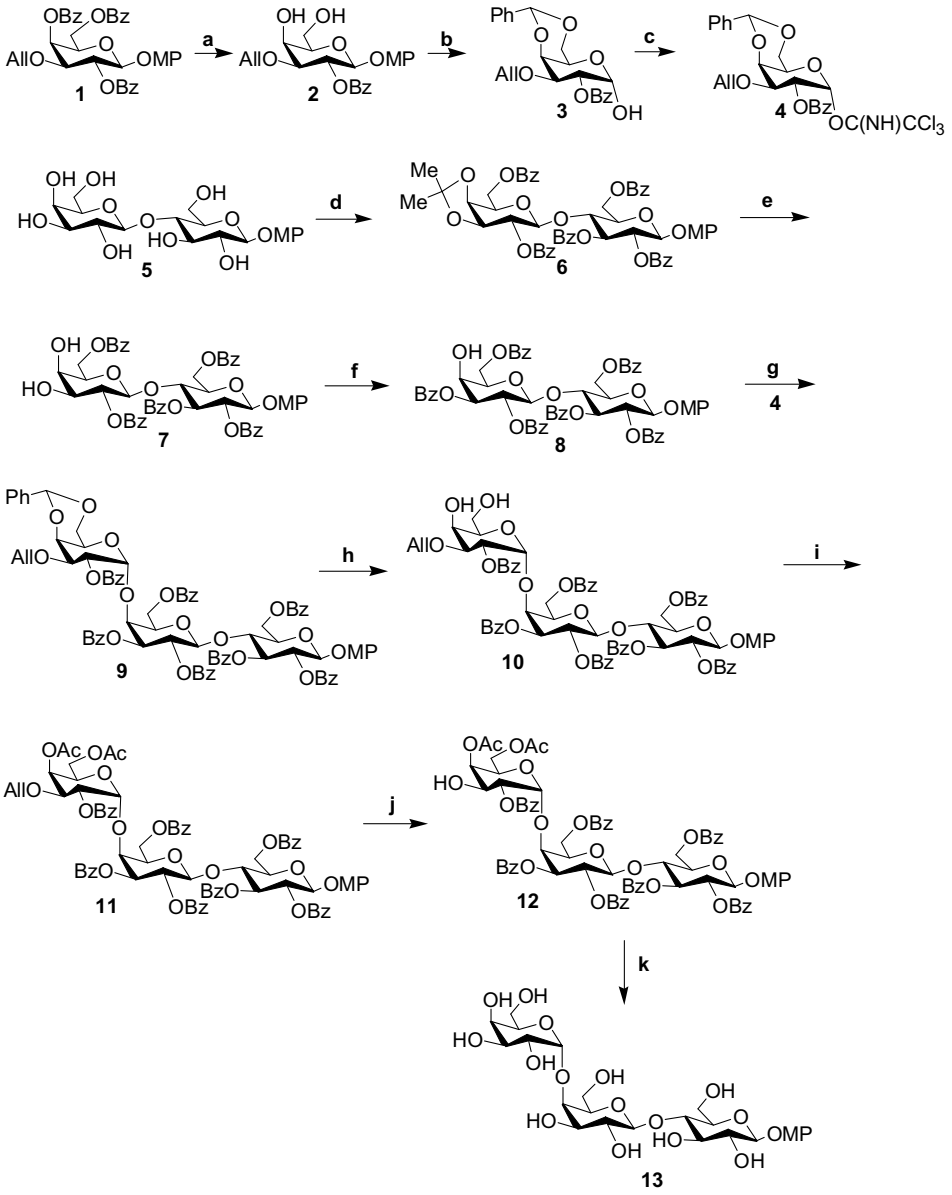
deficiency of α-galactosidase activity.[9] Globotriosyl ceramide is located on the surface of the kidney glomerular endothelial cell and is known as the host receptor for verotoxins. The glycosphingolipid is extremely selective, and its potent affinity is mainly attributable to its globotriose component. Artificially created materials, including its dendrimers, might be applicable as potential glycomaterials for medicinal uses.[10]

Globotriose, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc, and its derivatives and analogs have been previously synthesized.[6a,b,11] In the crucial α-D-galactosylation step, applicable donors are mainly D-galactosyl donors with an ether-type, non-participating group at the their C-2 position, such as a perbenzylated galactosyl halide,[10,12] a sulfoxide,[11d] a tetramethylphosphoroamidate,[6a] a dibenzyl phosphite,[13] a trichloroacetimidate,[1c] a thioglycoside,[14] 2,4,6-tri-*O*-benzyl-3-*O*-*p*-methoxyphenyl-β-D-galactopyranosyl fluoride,[15] and 3-*O*-allyl-2,4,6-tri-*O*-benzyl-D-galactopyranosyl chloride.[6b] However, it was difficult to get high stereoselectivity for these methods such as the coupling with galactosyl chloride as the donor.[6b] On the other hand, Yang et al.[16] found an unexpected α-stereochemical 1,2-*cis* outcome for a galactosyl heptasaccharide with a galactosyl tetrasaccharide donor with acetyl group in its C-2 position. Meanwhile, Boons and co-workers[17] proposed through-bond remote neighboring-group participation arising from the C-4 position of the galactosyl donor, as well as solvent effects, based on their α-anomeric selective studies of the coupling of various donors such as ethyl 4-*O*-acyl-2,3,6-tri-*O*-benzyl-1-thio-D-galactoside and acceptors like 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside. Earlier, we[4a] found an intriguing strategy for synthesis of α-linked galactopyranosides by using the thio donors with a C-2 ester capable of neighboring-group participation. These results prompted us to put more effort into researching the issue. As a part of our ongoing synthetic studies concerning structure–bioactivity relationships of the immunological specificity of the globoseries of glycolipids,[6b] we describe herein a very facile synthesis of the target compound, globotriose, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc.

As outlined in Scheme 1, 3-*O*-allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate (**4**) was chosen as the donor since our previous study[4a] indicated that either the sole 3-O-allylation or the sole 4,6-O-benzylidenation of benzoylated galactopyranosyl donors gave α- and β-mixed products, while the galactopyranosyl donors with both 3-*O*-allyl and 4,6-*O*-benzylidene groups afforded very high α-stereoselectivity in spite of the existence of the C-2 ester group. The rationale for the high α-selectivity in this case is still not clear. For the synthesis of **4**, selective 4- and 6-O-debenzoylation of 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside **1**[18] with MeONa in MeOH at room temperature was carried out within a short time, giving *p*-methoxylphenyl 3-*O*-allyl-2-*O*-benzoyl-β-D-galactopyranoside (**2**) in acceptable yield (69%). Subsequent removal of C-1-*O*-MP by oxidative cleavage of **2** with ammonium cerium(IV) nitrate (CAN) and benzylidenation yielded **3** (46% for two steps). Subsequent trichloroacetimidation afforded the donor **4** in satisfactory yield (86%). The disaccharide acceptor, 2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**8**) was also readily prepared. Thus, *p*-methoxyphenyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**5**)[19] was chosen as the starting material. Isopropylidenation of **5** with dimethoxypropane and benzoylation with benzoyl chloride in pyridine gave *p*-methoxyphenyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (**6**) (67% for two steps). O-Deisopropylidenation of **6** with 80% HOAc–H_2O at reflux was carried out smoothly giving *p*-methoxyphenyl 2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**7**) in high yield (95%). Subsequent selective benzoylation of **7** with benzoyl chloride in pyridine afforded **8** in 87% yield. The ^{1}H NMR spectrum of **8** showed the salient H′-4 upfield (δ 4.15 ppm) with a small coupling constant ($J_{4,5}$ 0.3 Hz), indicating selective 3-O-benzoylation. The coupling of donor **4** and acceptor **8** gave trisaccharide **9** in 89% yield, and its ^{1}H and ^{13}C NMR spectra showed that the new glycosidic bond is α-linked (δ 5.33 ppm with $J_{1,2}$ 3.2 Hz for H-1, and δ 100.0 ppm with J_{C1-H1} 170.0 Hz for C-1). Debenzylidenation of **9** in 80% HOAc–H_2O at 80 °C give **10** (91%), followed by acetylation of **10** with Ac_2O in pyridine, yielded *p*-methoxyphenyl 4,6-di-*O*-acetyl-3-*O*-allyl-2-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**11**) (93%). Subsequent deallylation of **11** with $PdCl_2$ in MeOH gave **12** (74%), and finally deacylation of **12** with saturated ammonia–methanol furnished the unprotected globotriose glycoside, *p*-methoxyphenyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**13**) (86%). The ^{1}H NMR spectrum of **13** was in agreement with the structure assigned. There are some salient characteristic signals such as a doublet at δ 4.42 ppm with $J_{1,2}$ 7.6 Hz for H-1 of β-Gal residue, a doublet at δ 4.84 ppm with $J_{1,2}$ 3.6 Hz for the H-1 of the α-Gal residue, a doublet at δ 4.89 ppm with $J_{1,2}$ 7.6 Hz for the H-1 of the β-Glc residue. (See Supplementary data for details.)

In summary, we present herein a facile synthesis of globotriose, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc, with a galactosyl donor containing a C-2 ester capable of neighboring-group participation. Furthermore, the intermediates **10**, **11**, and **12** are useful synthons that can be used to further elongate sugar chains to obtain higher oligosaccharides with similar structures such as lipopolysaccharides.



Scheme 1. Reagents and conditions: (a) MeONa–MeOH, rt; (b) CAN, CH_3CN–H_2O, rt; PhCHO, $HC(OEt)_3$, *p*-TsOH, rt; (c) CCl_3CN, K_2CO_3, rt; (d) $Me_2C(OMe)_2$, *p*-TsOH, rt; PhCOCl, Pyr. rt; (e) 80% HOAc–H_2O, heat; (f) PhCOCl, Pyr. 0 °C; (g) TMSOTf, −25 °C; (h) 80% HOAc–H_2O, heat; (i) Ac_2O, Pyr., rt; (j) $PdCl_2$, MeOH, 40 °C; (k) NH_3–MeOH, rt.

1. Experimental

1.1. General methods

Melting points were determined with a 'Mel-Temp' apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1-dm, jacketed cell. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AV 400 spectrometer at 400 and 100.6 MHz, respectively. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Mass spectra were recorded with a VG PLATFORM mass spectrometer using the electrospray-ionization (ESI) mode. The progress of all reactions was followed by thin-layer chromatography (TLC) that was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in MeOH or by UV detection. Purification of the crude product by chromatography was conducted by elution of a column (8×100 mm, 16×240 mm, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether as the eluent. HPLC was performed with a Gilson instrument consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO_2, 10×300 mm or 4.6×250 mm), differential refractometer (132-RI detector) and a UV/vis detector

(model 118). EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature of 60 °C under diminished pressure.

1.2. *p*-Methoxylphenyl 3-*O*-allyl-2-*O*-benzoyl-β-D-galactopyranoside (2)

To a solution of **1**[18] (19.3 g, 30.2 mmol) in CH_2Cl_2 (20 mL)–MeOH (200 mL) was added 4.0 M NaOMe–MeOH solution dropwise to pH 10. After stirring the mixture at rt within 0.5 h, TLC (1:1 EtOAc–MeOH) indicated no remaining starting material and the formation of a major product. The reaction mixture was neutralized with acidic ion-exchange resin, the resin was removed by filtration, and the filtrate was concentrated. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **2** as a foamy solid (8.97 g, 69%): $[\alpha]_D$ +15.6 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 2.99 (2H, br, OH), 3.71 (3H, s, C*H*$_3$O), 3.71–3.76 (2H, m, H-3, H-5), 3.93 (1H, dd, $J_{5,6}$ 4.8 Hz, $J_{6,6}$ 11.9 Hz, H-6), 4.02–4.21 (4H, m, H-4, H-6′, CH_2=CHC*H*$_2$O), 5.01 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 5.10–5.23 (2H, m, C*H*$_2$=CHCH$_2$O), 5.68 (1H, dd, $J_{2,3}$ 9.5 Hz, H-2), 5.68–5.81 (2H, m, CH_2=C*H*CH$_2$O), 6.72–6.75 (2H, m, $CH_3OC_6H_4$–), 6.89–6.91 (2H, m, $CH_3OC_6H_4$–), 7.43–7.47 (2H, m, Ar–H), 7.58 (1H, m, Ar–H), 8.05–8.07 (2H, m, Ar–H). Anal. Calcd for $C_{23}H_{26}O_8$: C, 64.17; H, 6.10; Found: C, 63.89; H, 6.07.

1.3. 3-*O*-Allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranose (3)

To a solution of **2** (4.42 g, 10.3 mmol) in 8:1 CH_3CN–H_2O (90 mL) was added CAN ($(NH_4)_2Ce(NO_3)_6$) (25.3 g, 46.1 mmol), and the mixture was stirred for 30 min at room temperature, at the end of which time TLC (EtOAc) indicated that the reaction was complete. The mixture was extracted with EtOAc and washed with satd aq $NaHCO_3$. The organic layer was concentrated under reduced pressure and purified by column chromatography (EtOAc) to afford a syrup that was used directly for the next step.

The syrup was dissolved in DMF (50 mL), and PhCHO (2.4 mL, 24.0 mmol), $(EtO)_3CH$ (3.18 mL, 19.1 mmol), and *p*-TsOH·H_2O (0.03 g) were added. The reaction mixture was stirred at room temperature overnight, after which time TLC showed the reaction was complete. Et_3N (0.3 mL) was added, the reaction mixture was concentrated, and the residue was purified by column chromatography (2:1 petroleum–EtOAc) to give **3** as a foamy solid (1.96 g, 46%): $[\alpha]_D$ +105.9 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 2.84 (1H, s, OH), 3.96 (1H, m, H-5), 4.06–4.08 (1H, dd, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 1.8 Hz, H-3), 4.16–4.19 (1H, dd, $J_{5,6}$ 3.3 Hz, $J_{6,6}$ 10.4 Hz, H-6), 4.19–4.21 (2H, m, CH_2=CHC*H*$_2$O), 4.25 (1H, dd, $J_{5,6}$ 1.2 Hz, H-6′), 4.35 (1H, dd, $J_{4,5}$ 1.5 Hz, H-4), 5.14–5.31 (2H, m, C*H*$_2$=CHCH$_2$O), 5.50 (1H, d, H-2), 5.57 (1H, s, PhC*H*=), 5.69 (1H, d, $J_{1,2}$ 3.5 Hz, H-1), 5.80–5.94 (2H, m, CH_2=C*H*CH$_2$O), 7.33–7.35 (3H, m, Ar–H), 7.41–7.45 (3H, m, Ar–H), 7.54–7.57 (2H, m, Ar–H), 8.06–8.08 (2H, m, Ar–H). Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.97; H, 5.88; Found: C, 66.72; H, 5.85.

1.4. 3-*O*-Allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate (4)

Compound **3** (1.40 g, 3.39 mmol) was dissolved in CH_2Cl_2 (20 mL), and CCl_3CN (1.5 mL) and anhyd K_2CO_3 (1.4 g) were added. The reaction mixture was stirred overnight at room temperature, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was then filtered. Concentration of the filtrate, followed by purification of the crude product on a silica-gel column with 2.5:1 petroleum ether–EtOAc as the eluant, gave donor **4** as foamy solid (1.62 g, 86%): $[\alpha]_D$ +93.0 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 4.00 (1H, m, H-5), 4.12 (1H, dd, $J_{5,6}$ 1.6 Hz, $J_{6,6}$ 12.8 Hz, H-6), 4.22–4.25 (2H, m, CH_2=CHC*H*$_2$O), 4.28 (1H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.37 (1H, dd, $J_{5,6}$ 1.5 Hz, H-6′), 4.49 (1H, dd, $J_{4,5}$ 0.9 Hz, H-4), 5.16–5.32 (2H, m, C*H*$_2$=CHCH$_2$O), 5.63 (1H, s, PhCH=), 5.81 (1H, dd, H-2), 5.84–5.94 (2H, m, CH_2=C*H*CH$_2$O), 6.80 (1H, dd, $J_{1,2}$ 3.4 Hz, H-1), 7.37–7.45 (5H, m, Ar–H), 7.57–7.59 (3H, m, Ar–H), 8.03–8.05 (2H, m, Ar–H), 8.53 (1H, s, NH). Anal. Calcd for $C_{25}H_{24}O_7NCl_3$: C, 53.92; H, 4.35; Found: C, 54.14; H, 4.37.

1.5. *p*-Methoxyphenyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (6)

To a solution of **5**[19] (4.28 g, 9.54 mmol) in dry DMF (50 mL) were added *p*-TsOH·H_2O (0.2 g) and 2,2-dimethoxypropane (3.6 mL, 29.3 mmol). After stirring the mixture for 4 h at 40 °C, TLC (5:1 EtOAc–MeOH) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N (0.5 mL), and the mixture was concentrated. Purification of the crude product by column chromatography (5:1 EtOAc–MeOH) gave a syrup. To the solution of the syrup in pyridine (15 mL) was added PhCOCl (5.0 mL, 43.1 mmol) dropwise, and the mixture was stirred overnight at rt. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification of the crude product by column chromatography (2.5:1 petroleum ether–EtOAc) gave **6** as a foamy solid (6.45 g, 67%): $[\alpha]_D$ +46.9

(c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (3H, s, $(CH_3)_2C$), 1.54 (3H, s, $(CH_3)_2C$), 3.69 (3H, s, CH_3O), 3.67–3.72 (1H, m), 3.84 (1H, m, H-5), 3.96 (1H, s, H-5′), 4.10 (1H, dd, $J_{3,4}$ 2.0 Hz, $J_{4,5}$ 5.6 Hz, H-4′), 4.21–4.28 (3H, m, H-4, H-3′, H-6′), 4.48 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{6,6}$ 12.0 Hz, H-6), 4.62 (1H, d, $J_{1,2}$ 7.9 Hz, H-1′), 4.64 (1H, dd, $J_{5,6}$ 2.4 Hz, H-6), 5.09 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 5.17 (1H, dd, $J_{2,3}$ 7.4 Hz, H-2′), 5.66 (1H, dd, H-2), 5.78 (1H, dd, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 6.63 (2H, d, J 8.8 Hz, $CH_3OC_6H_4$–), 6.85 (2H, d, J 8.8 Hz, $CH_3OC_6H_4$–), 7.31–7.37 (8H, m, Ar–H), 7.51–7.61 (7H, m, Ar–H), 7.93–8.10 (10H, m, Ar–H). Anal. Calcd for $C_{57}H_{52}O_{17}$: C, 67.84; H, 5.20; Found: C, 68.13; H, 5.23.

1.6. *p*-Methoxyphenyl 2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (7)

Compound **6** (4.84 g, 4.80 mmol) was dissolved in 80% HOAc (100 mL), and the mixture was refluxed for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was passed through a silica-gel column with 1.5:1 petroleum ether–EtOAc as the eluent to give **7** as foamy solid (4.41 g, 95%): $[\alpha]_D$ +51.3 (c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.29 (2H, br, OH), 3.54–3.60 (2H, m), 3.69 (3H, s, CH_3O), 3.73–3.76 (1H, m, H-5), 3.89–3.93 (2H, m), 4.02 (1H, dd, $J_{5,6}$ 8.9 Hz, $J_{6,6}$ 14.0 Hz, H-6′), 4.14 (1H, dd, $J_{4,5}$ 8.8 Hz, H-4), 4.52 (1H, dd, $J_{5,6}$ 5.7 Hz, $J_{6,6}$ 11.9 Hz, H-6), 4.59 (1H, dd, $J_{5,6}$ 1.9 Hz, H-6), 4.62 (1H, d, $J_{1,2}$ 8.0 Hz, H-1′), 5.04 (1H, d, $J_{1,2}$ 7.5 Hz, H-1), 5.41 (1H, dd, $J_{2,3}$ 8.8 Hz, H-2′), 5.64 (1H, dd, H-2), 5.69 (1H, dd, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 6.63 (2H, d, J 8.8 Hz, $CH_3OC_6H_4$–), 6.85 (2H, d, J 8.8 Hz, $CH_3OC_6H_4$–), 7.29–7.44 (15H, m, Ar–H), 7.91–8.05 (10H, m, Ar–H). Anal. Calcd for $C_{54}H_{48}O_{17}$: C, 66.93; H, 5.00; Found: C, 66.63; H, 4.98.

1.7. *p*-Methoxyphenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (8)

To the solution of **7** (1.97 g, 2.0 mmol) in pyridine (5 mL) at 0 °C was added dropwise PhCOCl (0.24 mL, 2.1 mmol), and the mixture was stirred overnight. TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification of the crude product by column chromatography (2:1 petroleum ether–EtOAc) gave **8** as a foamy solid (1.87 g, 87%): $[\alpha]_D$ +54.5 (c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (1H, s and br, OH), 3.64–3.73 (2H, m), 3.68 (3H, s, CH_3O), 3.99 (1H, m, H-5), 4.10 (1H, dd, $J_{5,6}$ 5.9 Hz, $J_{6,6}$ 12.7 Hz, H-6′), 4.15 (1H, dd, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 0.3 Hz, H-4′), 4.45 (1H, dd, $J_{4,5}$ 9.2 Hz, H-4), 4.49 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{6,6}$ 11.9 Hz, H-6), 4.61 (1H, dd, $J_{5,6}$ 0.8 Hz, H-6), 4.80 (1H, d, $J_{1,2}$ 7.9 Hz, H-1′), 5.12 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 5.17 (1H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.2 Hz, H-3′), 5.67 (1H, dd, H-2), 5.75 (1H, dd, H-2′), 5.82 (1H, dd, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 6.62 (2H, d, J 8.9 Hz, $CH_3OC_6H_4$–), 6.86 (2H, d, J 8.9 Hz, $CH_3OC_6H_4$–), 7.25–7.48 (18H, m, Ar–H), 7.91–8.01 (12H, m, Ar–H). Anal. Calcd for $C_{61}H_{52}O_{18}$: C, 68.27; H, 4.89; Found: C, 68.57; H, 4.87.

1.8. *p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (9)

Donor **4** (0.25 g, 0.45 mmol) and acceptor **8** (0.38 g, 0.35 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (10 mL). TMSOTf (6.8 μL, 0.035 mmol) was added at −25 °C with N_2 protection. The reaction mixture was stirred for 2 h, at the end of which time TLC indicated that the reaction was complete. The mixture was then neutralized with Et_3N and concentrated under reduced pressure to dryness. Purification of the crude product by column chromatography (2.5:1 petroleum ether–EtOAc) gave **9** as a foamy solid (0.46 g, 89%): $[\alpha]_D$ +61.7 (c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.55 (1H, m), 3.68 (3H, s, CH_3O), 3.63–3.72 (2H, m), 3.96–4.12 (4H, m), 4.18–4.31 (5H, m), 4.44 (2H, m), 4.73 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6}$ 11.8 Hz, H-6), 4.96 (1H, d, $J_{1,2}$ 7.7 Hz, H-1′), 5.08 (1H, dd, $J_{3,4}$ 2.6 Hz, H-3′), 5.10 (1H, d, $J_{1,2}$ 7.7 Hz, H-1), 5.13–5.32 (2H, m, CH_2=CHCH$_2$O), 5.33 (1H, d, $J_{1,2}$ 3.2 Hz, H-1″), 5.46 (1H, s, PhCH=), 5.55 (1H, dd, $J_{1,2}$ 3.2 Hz, $J_{2,3}$ 10.5 Hz, H-2″), 5.59 (1H, dd, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.0 Hz, H-2′), 5.72 (1H, dd, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 10.8 Hz, H-2), 5.87 (1H, dd, $J_{2,3} = J_{3,4}$ 7.8 Hz, H-3), 5.80–5.93 (1H, m, CH_2=CHCH$_2$O), 6.61 (2H, d, J 9.0 Hz, $CH_3OC_6H_4$–), 6.84 (2H, d, J 9.0 Hz, $CH_3OC_6H_4$–), 7.19–7.46 (24H, m, Ar–H), 7.71–8.00 (16H, m, Ar–H); ^{13}C NMR ($CDCl_3$): δ 55.4 (CH_3O), 60.3, 62.6, 63.7, 69.0, 69.7, 71.0, 71.0, 72.1, 72.4, 72.8, 72.9, 73.0, 73.2, 73.7, 74.5, 75.9, 100.0 (J_{C1-H1} 170.0 Hz, C-1″, α), 100.3 (J_{PhC-H} 162.4 Hz, PhCH=), 100.8 (J_{C1-H1} 160.7 Hz, C-1′, β), 100.8 (J_{C1-H1} 160.7 Hz, C-1, β), 114.3 ($CH_3OC_6H_4$–), 116.9 (CH_2=CHCH$_2$O), 118.9 ($CH_3OC_6H_4$–), 128.0, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 129.5, 129.5, 129.6, 129.6, 129.7, 129.7, 133.0, 133.0, 133.1, 133.2, 133.2, 133.3, 133.6, 134.7, 137.7, 150.8 ($CH_3OC_6H_4$–), 155.6 ($CH_3OC_6H_4$–), 165.0, 165.1, 165.1, 165.4, 165.6, 165.9, 166.0 (7 PhCO). Anal. Calcd for $C_{84}H_{74}O_{24}$: C, 68.74; H, 5.09; Found: C, 68.97; H, 5.11.

1.9. *p*-Methoxyphenyl 3-*O*-allyl-2-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (10)

Compound **9** (0.68 g, 0.46 mmol) was dissolved in 80% HOAc (16 mL), and the mixture was heated at 80 °C for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was passed through a silica-gel column with 1.5:1 petroleum ether–EtOAc as the eluent to give **10** as foamy solid (0.58 g, 91%): $[\alpha]_D$ +30.6 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 2.73 (1H, s, br, OH), 2.85 (1H, s, br, OH), 3.67 (3H, s, C*H*$_3$O), 3.66–3.68 (1H, m), 3.83–3.89 (3H, m), 3.94–4.07 (2H, m), 4.08–4.32 (6H, m), 4.35 (1H, m), 4.46 (1H, dd, H-6), 4.67 (1H, dd, H-6), 4.92 (1H, d, $J_{1,2}$ 7.9 Hz, H-1′), 5.08 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 5.12–5.27 (2H, m, C*H*$_2$=CHCH$_2$O), 5.21 (1H, dd, $J_{1,2}$ 2.3 Hz, H-2″), 5.27 (1H, d, $J_{1,2}$ 2.3 Hz, H-1″), 5.32 (1H, dd, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 3.2 Hz, H-3′), 5.60 (1H, dd, H-2′), 5.69 (1H, dd, H-2), 5.80–5.90 (1H, m, CH$_2$=C*H*CH$_2$O), 5.84 (1H, dd, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 6.60 (2H, d, *J* 8.8 Hz, $CH_3OC_6H_4$–), 6.83 (2H, d, *J* 8.8 Hz, $CH_3OC_6H_4$–), 7.21–7.41 (21H, m, Ar–H), 7.71–8.02 (14H, m, Ar–H). Anal. Calcd for $C_{77}H_{70}O_{24}$: C, 67.04; H, 5.13; Found: C, 66.75; H, 5.14.

1.10. *p*-Methoxyphenyl 4,6-di-*O*-acetyl-3-*O*-allyl-2-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (11)

To a solution of **10** (0.35 g, 0.25 mmol) in pyridine (1 mL) was added Ac_2O (0.5 mL), and the mixture was stirred overnight at rt. TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification of the crude product by column chromatography (2:1 petroleum ether–EtOAc) gave **11** as a foamy solid (0.34 g, 93%): $[\alpha]_D$ +36.0 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, C*H*$_3$CO), 2.08 (3H, s, C*H*$_3$CO), 3.67 (3H, s, C*H*$_3$O), 3.66–3.68 (1H, m), 3.93–4.04 (6H, m), 4.17–4.21 (3H, m), 4.27 (1H, dd, $J_{5,6} = J_{6,6}$ 9.3 Hz, H-6′), 4.44 (1H, dd, $J_{5,6}$ 5.9 Hz, $J_{6,6}$ 12.0 Hz, H-6), 4.52 (1H, dd, $J_{5,6} = J_{6,6}$ 6.9 Hz, H-6″), 4.69 (1H, dd, $J_{5,6}$ 1.7 Hz, $J_{6,6}$ 9.3 Hz, H-6), 4.91 (1H, d, $J_{1,2}$ 7.9 Hz, H-1′), 5.08 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 5.10–5.28 (2H, m, C*H*$_2$=CHCH$_2$O), 5.18–5.28 (3H, m, H-1″, H-2″, H-3″), 5.57–5.61 (2H, m, H-2′, H-4″), 5.69–5.73 (1H, dd, H-2), 5.76–5.85 (1H, m, CH$_2$=C*H*CH$_2$O), 5.83 (1H, dd, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 6.59 (2H, d, *J* 9.1 Hz, $CH_3OC_6H_4$–), 6.83 (2H, d, *J* 9.1 Hz, $CH_3OC_6H_4$–), 7.30–7.40 (21H, m, Ar–H), 7.85–7.94 (14H, m, Ar–H); ^{13}C NMR ($CDCl_3$): δ 20.1, 20.1 (2 *C*H$_3$CO), 55.6 (*C*H$_3$O), 61.4, 61.4, 62.7 (3 C-6), 67.5, 67.9, 69.9, 71.2, 71.2, 71.5, 71.5, 71.7, 72.3, 72.9, 73.0, 73.0, 73.3, 73.5, 75.8, 98.8 (α), 100.5 (β), 101.1 (β) (3 C-1), 114.5 ($CH_3OC_6H_4$–), 117.3 (*C*H$_2$=CHCH$_2$O), 119.0 ($CH_3OC_6H_4$–), 128.5, 128.6, 128.8, 129.2, 129.3, 129.5, 129.6, 129.8, 130.0, 133.2, 133.3, 133.4, 133.7, 134.2, 150.2, 155.6, 165.1, 165.2, 165.3, 165.6, 165.7, 166.2, 166.3 (7 Ph*C*O), 170.3, 170.6 (2 CH$_3$CO). Anal. Calcd for $C_{81}H_{74}O_{26}$: C, 66.47; H, 5.11; Found: C, 66.17; H, 5.13.

1.11. *p*-Methoxyphenyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (12)

To a solution of **11** (0.3 g, 0.20 mmol) in anhyd CH_3OH (10 mL) was added $PdCl_2$ (0.035 g), and the mixture was stirred for 4 h at 40 °C, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filtrate was concentrated, and the residue was chromatographed on a silica-gel column with 1.5:1 petroleum ether–EtOAc as the eluant to gave **12** as a foamy solid (0.21 g, 74%): $[\alpha]_D$ +42.0 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 2.00 (3H, s, CH_3CO), 2.13 (3H, s, C*H*$_3$CO), 3.68 (3H, s, C*H*$_3$O), 3.68–3.72 (1H, m), 3.96–4.06 (3H, m), 4.09–4.17 (4H, m), 4.26–4.31 (1H, dd, $J_{5,6} = J_{6,6}$ 9.4 Hz, H-6′), 4.43 (1H, dd, $J_{5,6}$ 5.9 Hz, $J_{6,6}$ 12.0 Hz, H-6), 4.49 (1H, dd, $J_{5,6} = J_{6,6}$ 6.9 Hz, H-6″), 4.68 (1H, dd, $J_{5,6}$ 1.7 Hz, $J_{6,6}$ 12.0 Hz, H-6), 4.88 (1H, d, $J_{1,2}$ 7.8 Hz, H-1′), 5.09 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 5.14 (1H, dd, $J_{2,3}$ 10.0 Hz, H-2′), 5.20 (1H, d, $J_{1,2}$ 3.3 Hz, H-1″), 5.18–5.21 (1H, dd, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 0.9 Hz, H-3′), 5.47 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 1.0 Hz, H-4″), 5.62–5.67 (2H, m, H-2, H-2′), 5.83 (1H, dd, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 6.62 (2H, d, *J* 9.1 Hz, $CH_3OC_6H_4$–), 6.85 (2H, d, *J* 9.1 Hz, $CH_3OC_6H_4$–), 7.26–7.54 (21H, m, Ar–H), 7.87–8.12 (14H, m, Ar–H); ^{13}C NMR ($CDCl_3$): δ 20.5, 29.4 (2 CH_3CO), 55.2 (CH_3O), 61.0, 61.3, 62.3 (3 C-6), 65.9, 67.6, 69.6, 70.3, 71.6, 72.2, 72.4, 72.6, 72.9, 73.0, 75.4, 75.8, 98.1, 100.3, 100.5 (3 C-1), 114.1 ($CH_3OC_6H_4$–), 118.6 (CH_3O-C_6H_4–), 128.1, 128.3, 128.4, 128.9, 129.2, 129.3, 129.5, 129.6, 129.7, 132.9, 133.0, 150.6, 155.4, 164.6, 164.8, 164.9, 165.0, 165.4, 165.7, 166.3 (7 Ph*C*O), 170.3, 170.8 (2 CH_3CO). Anal. Calcd for $C_{78}H_{70}O_{26}$: C, 65.81; H, 4.97; Found: C, 66.10; H, 4.98.

1.12. *p*-Methoxyphenyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (13)

Compound **12** (0.12 g, 0.084 mmol) was dissolved in a satd solution of NH_3 in anhyd CH_3OH (10 mL). After

a week at room temperature, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **13** as a syrup (0.044 g, 86%): $[\alpha]_D$ −5.7 (*c* 0.9, H_2O); ^1H NMR (400 MHz, D_2O): δ 3.45–3.93 (17H, m), 3.68 (3H, s, CH_3O), 4.23–4.26 (1H, m), 4.42 (1H, d, $J_{1,2}$ 7.6 Hz, H-1′), 4.84 (1H, d, $J_{1,2}$ 3.6 Hz, H-1″), 4.89 (1H, d, $J_{1,2}$ 7.6 Hz, H-1), 6.84 (2H, d, *J* 9.0 Hz, $-OC_6H_4OCH_3$), 7.00 (2H, d, *J* 9.0 Hz, $-OC_6H_4OCH_3$); ^{13}C NMR (D_2O): δ 58.4 (CH_3O), 62.5, 63.0, 63.1, 71.2, 71.6, 71.8, 73.5, 73.5, 74.8, 75.4, 76.9, 77.5, 78.0, 80.0, 81.0, 102.9, 103.6, 105.9 (3 C-1), 117.6, 120.8, 153.5, 157.3. MALDI-TOF MS: calcd for $C_{25}H_{38}O_{17}$: *m*/*z* 610.21 [M]; found: *m*/*z* 633.21 [M+Na].

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.029.

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