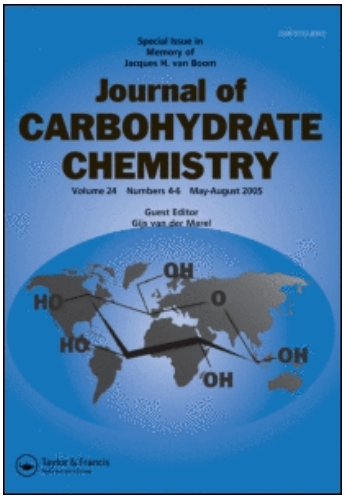

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LARGE SCALE SYNTHESIS OF A DERIVATIVE OF AN α-GALACTOSYL TRISACCHARIDE EPITOPE INVOLVED IN THE HYPERACUTE REJECTION OF XENOTRANSPLANTATION

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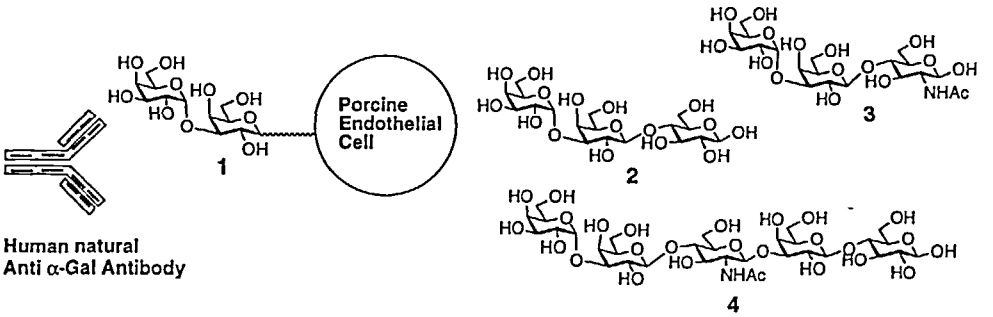
ABSTRACT

A derivative of an α-galactosyl trisaccharide xenoactive antigen, (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**5**), was synthesized on a large scale (50 gram). The synthesis involved a high yielding and highly stereoselective (α/β>20:1) glycosylation reaction utilizing a thiogalactoside as the donor and a selectively protected lactose azide as the acceptor. This derivative serves as a versatile intermediate that can be transformed into a variety of α-Gal containing glycoconjugates highly desired in xenotransplantation research and pharmaceutical development.

INTRODUCTION

Hyperacute rejection is the major obstacle in xenotransplantation,[1] a procedure to transplant animal organs or cells to human. The xenoactive antigens on porcine endothelial cells have been identified as oligosaccharide structures terminated with the Galα1-3Galβ sequence (**1, Scheme 1**).[2] Trisaccharides Galα1-3Galβ1-4Glcβ (**2**) and Galα1-3Galβ1-4GlcNAcβ (**3**), and pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ (**4**) are the major α-galactosyl epitopes (or α-Gal) responsible for initiating human

natural anti-α-Gal antibodies (or anti-Gal) mediated immune defense to reject the transplanted organ or tissue, similar to the destruction of red blood cells in the ABO-mismatched allotransplantation. The binding of anti-Gal antibodies with these α-Gal epitopes expressed on glycolipids and glycoproteins of xenograft cells induces antibody-dependent cytotoxicity by human blood monocytes and macrophages, and complement-mediated lysis resulting in the destruction of the xenograft cells.



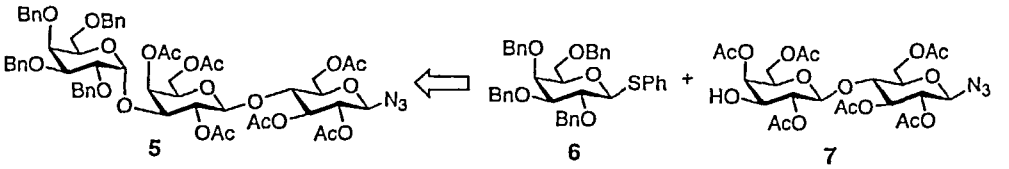
Scheme 1

The understanding of the interaction of anti-Gal with α-Gal epitopes in xenotransplantation has led to experimental attempts to overcome hyperacute rejection by either depleting the recipient's anti-Gal through α-Gal immobilized affinity columns or antagonizing anti-Gal by infusing soluble synthetic α-Gal oligosaccharides.[3] These procedures would require access to a substantial amount of α-Gal oligosaccharides as well as their derivatives with high-affinity to anti-Gal antibodies. We have previously reported synthesis of several α-Gal oligosaccharides using a recombinant α1-3 galactosyltransferase.[4] The chemical syntheses of derivatives of trisaccharide **2** have been reported previously.[5] However, these methods were limited to milligram scales and may not be suitable to prepare large quantity of the trisaccharide. Besides, they suffer either low yields or poor stereoselectivity. Here, we describe an efficient chemical route for the synthesis of a versatile α-Gal trisaccharide derivative (**5**) on a 50 gram scale.

RESULTS AND DISCUSSION

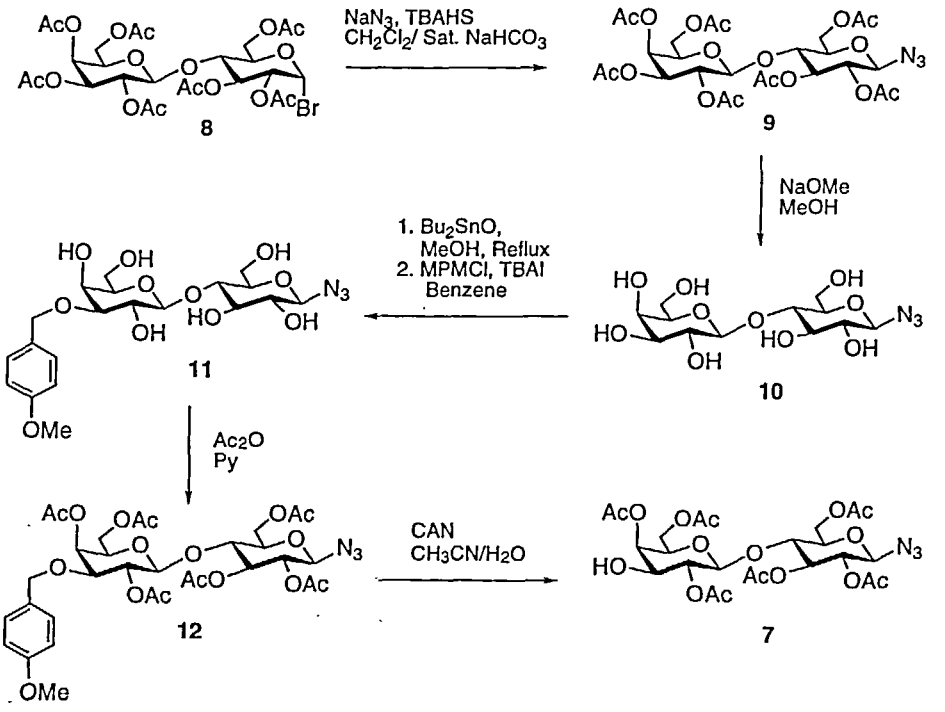
To construct the trisaccharide **5**, we chose to use a stereoselective and high yielding glycosylation method with perbenzylated phenyl thiogalactoside **6** as the donor and selectively protected lactosyl azide **7** as the acceptor. The anomeric azido group was

introduced to the structure for its convenient transformations to other derivatives and glycoconjugates.[6]



Scheme 2

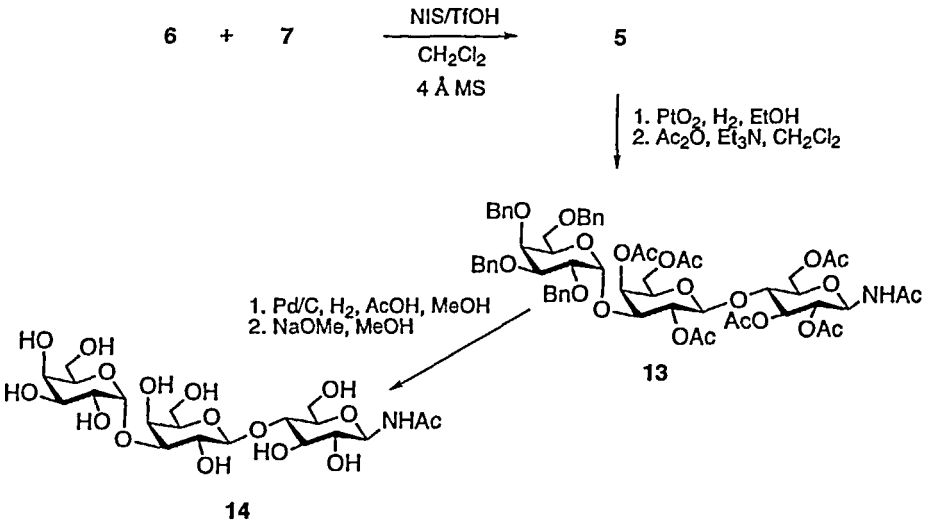
In preparation of the acceptor **7**, lactosyl bromide **8** was converted to azide **9**[7] and then deacetylated to generate lactosyl azide **10**. The regioselective monoalkylation of the C3 hydroxy group of the galactose unit with *p*-methoxybenzyl chloride (MPMCl) was achieved through a one-pot reaction sequence involving a dibutylstannylene acetal intermediate.[8] The selectively protected azide **11** was peracetylated to give compound



Scheme 3

12, which was selectively deprotected at C3-OH through oxidative cleavage of the MPM ether with cerium(IV) ammonium nitrate (CAN)[9] to yield acceptor building block 7.

Large scale (50 gram) glycosylation between perbenzylated phenyl thiogalactoside donor 6[10] and acceptor 7 was carried out at -40 °C under activation of *N*-iodosuccinimide/triflic acid[11] to afford the protected trisaccharide 5 in 94% yield and with a high level of stereoselectivity (α:β>20:1). We have examined several other glycosylation donors (including methyl thiogalactopyranoside, imidate, and bromide) using different activating systems, and it was found that this glycosylation protocol provided the highest yield and best α/β stereoselectivity.



Scheme 4

In our ongoing α-Gal research program, the trisaccharide 5 serves as the most important intermediate for the synthesis of α-Gal containing *N*- and *O*- linked glycopeptides, glycolipids, multivalent α-Gal epitope clusters and polymers, and other glycoconjugates. For example, trisaccharide 5 was converted to *N*-acetyl derivative 14 using the following sequence: selective hydrogenolysis of the azido group with Adam's catalyst (PtO_2)[12] followed by *N*-acetylation gave compound 13, which was deacetylated and debenzylated to form *N*-linked α-Gal trisacchride 14.[13] The overall yield of this synthesis (from 8 to 14) is 44%. Using a similar approach, this azido trisaccharide derivative can be transformed into a variety of glycoconjugates conveniently.

EXPERIMENTAL

General Methods. ^1H and ^{13}C spectra were recorded on a 500 MHz Varian Unity spectrometer. Mass spectra (FAB or ESI) were run on the mass spectrometry facility at the University of California, Riverside. Thin-layer chromatography was conducted on Baker Si_{250F} silica gel TLC plates with a fluorescent indicator. Column chromatography was conducted with silica gel, grade 62, 60-200 mesh, 150 Å.

2,3,4,6-Tetra-*O*-acetyl -β -D-galactopyranosyl-(1→4) - 2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (9). A biphasic mixture of heptaacetobromo-α-D-lactose **8** (122.4 g, 0.175 mol), NaN_3 (34.1 g, 0.525 mol), Bu_4NHSO_4 (59.4 g, 0.175 mol), CH_2Cl_2 (400 mL) and saturated aqueous Na_2CO_3 (400 mL) was vigorously stirred for 8 h. EtOAc (800 mL) was then added to the mixture and the layers were separated. The aqueous layer was washed with EtOAc (2 × 100 mL) and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was chromatographed (EtOAc:Hexane = 1:1) to afford azide **9** as a white foamy solid (109.5 g, 95%). Recrystallization from ether/hexane yielded a white crystalline solid. mp 71-73 °C. (Lit.[14b] 72-74 °C). $[\alpha]_D^{25}$ -19.8° (*c* 0.98, $CHCl_3$. Lit. -18°,[14a] -22°,[14b] -20.4°.[7]). ^1H NMR (500 MHz, $CDCl_3$) δ 5.32 (d, *J* = 3.2 Hz, 1 H, H-4'), 5.18 (t, *J* = 9.6 Hz, 1 H, H-3), 5.08 (dd, *J* = 10.4, 7.6 Hz, 1 H, H-2'), 4.93 (dd, *J* = 10.4, 7.6 Hz, 1 H, H-3'), 4.83 (t, *J* = 9.6 Hz, 1 H, H-2), 4.60 (d, *J* = 8.4 Hz, 1 H, H-1), 4.50 (m, 1 H), 4.46 (d, *J* = 7.6 Hz, 1 H, H-1'), 4.08 (m, 3 H), 3.85 (t, *J* = 7.0 Hz, 1 H), 3.79 (t, *J* = 9.0 Hz, 1 H), 3.68 (ddd, *J* = 9.6, 4.8, 1.6 Hz, 1 H, H-5), 2.13 (s, 3 H), 2.11 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 6 H), 1.94 (s, 3 H); MS *m/z* 684 ($M+Na^+$).

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (10). To a solution of azide **9** (92.5 g, 0.140 mol) in anhydrous methanol (1.2 L) was added NaOMe portionwise until pH 9. Stirring was continued for 2 h followed by addition of Dowex resin (H form) to neutralize the solution. The resin was filtered off and the filtrate was concentrated and dried to afford azide **10** as a white solid (51.3 g, 100%). mp 165-167 °C. $[\alpha]_D^{25}$ -9.1° (*c* 1.02, H_2O). ^1H NMR (500 MHz; D_2O) δ 4.59 (d, *J* = 8.4 Hz, 1 H, H-1), 4.28 (d, *J* = 7.5 Hz, 1 H, H-1'), 3.78 (dd, *J* = 12.0, 8.0 Hz, 1 H), 3.73 (m, 1 H), 3.69 (m, 1 H), 3.62 (dd, *J* = 12.0, 2.8 Hz, 1 H), 3.61 (m, 1 H), 3.38-3.55 (m, 6 H), 3.13 (t, *J* = 8.8 Hz, 1 H); ^{13}C (125.69 MHz; D_2O) δ 102.8 (C-1'), 89.8 (C-1), 77.5, 76.6, 75.3, 74.2, 72.4 (2 C), 70.8, 68.4, 60.9 (C-6'), 59.7 (C-6); MS *m/z* 390($M+Na^+$); HRMS for $C_{12}H_{21}N_3O_{10}Na$ calcd 390.1125, found 390.1107.

[3-*O*-(4-Methoxybenzyl)-β-D-galactopyranosyl]-(1→4)-β-D-glucopyranosyl azide (11). A suspension of azide **10** (48.2 g, 0.131 mol) and dibutyltin oxide (39.1 g, 0.157 mol) in anhydrous methanol (400 mL)) was heated to reflux for 6 h. The reaction

mixture was then cooled to rt followed by removal of methanol *in vacuo*. To the residue were added dry benzene (500 mL), *p*-methoxybenzyl chloride (24.6 g, 0.157 mmol), tetrabutylammonium iodide (19.3 g, 0.0522 mol), and 4 Å molecular sieve (15 g). The mixture was heated to reflux for 6 h and then cooled to room temperature. The suspension was filtered through a celite pad and the filtrate was concentrated and chromatographed (chloroform:methanol = 6:1) to afford **11** as a white solid (47.2 g, 74%). $[\alpha]_D^{25}$ 2.9° (*c* 1.03, CH_3OH). ^1H NMR (500 MHz, D_2O) δ 7.26 (d, *J* = 8.5 Hz, 2 H, Ar), 6.87 (d, *J* = 8.5 Hz, 2 H, Ar), 4.61 (d, *J* = 9.0 Hz, 1 H, H-1), 4.53 (d, *J* = 11.5 Hz, 1 H, CH_2Ph), 4.43 (d, *J* = 11.5 Hz, 1 H, CH_2Ph), 4.28 (d, *J* = 7.5 Hz, 1 H, H-1'), 3.92 (d, *J* = 3.0 Hz, 1 H), 3.82 (dd, *J* = 13.0, 1.5 Hz, 1 H), 3.69 (s, 3 H, OCH_3), 3.66-3.43 (m, 8 H), 3.37 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.15 (t, *J* = 9.0 Hz, 1 H); ^{13}C (125.69 MHz; D_2O) δ 159.5, 131.0, 130.4, 114.7, 103.4 (C-1'), 90.6 (C-1), 80.1, 78.2, 77.3, 75.9, 75.0, 73.2 (CH_2Ph), 71.5, 70.7, 65.8, 61.7 (C-6'), 60.4 (C-6), 56.0 (OCH_3); MS (*m/z*) 510 ($M+Na^+$); HRMS calcd for $C_{20}H_{29}N_3O_{11}Na$ 510.1700, found 510.1701.

Anal. Calcd for $C_{20}H_{29}N_3O_{11}$: C, 49.28; H, 6.00; N, 8.62. Found: C, 49.01; H, 6.16; N, 8.81.

[3-*O*-(4-Methoxybenzyl) 2,4,6-tri-*O*- acetyl-β-D-galactopyranosyl]-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (12). To a solution of selectively protected azide **11** (42.3 g, 0.0869 mol) in pyridine (500 mL) were added acetic anhydride (300 mL, 3.18 mol) and DMAP (300 mg). The reaction mixture was stirred for 6 h and then concentrated under reduced pressure. The residue was dissolved in chloroform (800 mL) and the solution was washed with 0.1 N HCl, water, aqueous $NaHCO_3$, and brine and dried over Na_2SO_4. Solvent removal followed by drying *in vacuo* afforded **12** as a white foamy solid (59.1 g, 92%). $[\alpha]_D^{25}$ 21.3° (*c* 0.68, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 2 H, Ar), 6.85 (d, *J* = 9.0 Hz, 2 H, Ar), 5.44 (d, *J* = 3.0 Hz, 1 H), 5.18 (t, *J* = 9.0 Hz, 1 H), 4.98 (dd, *J* = 10.0, 8.0 Hz, 1 H, H-2'), 4.84 (t, *J* = 9.0 Hz, 1 H), 4.62 (d, *J* = 8.5 Hz, 1 H, H-1), 4.56 (d, *J* = 12.0 Hz, 1 H, CH_2Ph), 4.45 (dd, *J* = 12.5, 2.0 Hz, 1 H, CH_2Ph), 4.35 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.28 (d, *J* = 12.0 Hz, 1 H), 4.14-4.09 (m, 3 H), 3.80 (s, 3 H), 3.77-3.73 (m, 2 H), 3.70-3.67 (m, 1H), 3.45 (dd, *J* = 10.0, 3.5 Hz, 1 H), 2.14 (s, 3 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 2.00 (s, 3 H); ^{13}C (125.69 MHz; $CDCl_3$) δ 171.2, 171.1, 171.0, 170.3, 170.2, 169.8, 160.1, 130.1, 130.0, 114.5, 102.0 (C-1'), 88.4 (C-1), 77.0, 76.4, 75.5, 73.3, 71.8, 71.7 (2 C), 71.3, 66.1, 62.7 (C-6'), 62.2 (C-6), 56.0 (OCH_3), 21.5 (3 C), 21.4 (2 C), 21.3. MS (*m/z*): 762 ($M+Na^+$); HRMS calcd for $C_{32}H_{41}N_3O_{17}Na$, 762.2334, found 762.2360.

(2,4,6-Tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (7). To the azide **12** (43.6 g, 0.059 mol) in a mixture of CH_3CN and water (9/1, 800 mL) at 0 °C was added $(NH_4)_2Ce(NO_3)_6$ (80.3 g, 0.148 mol) in small

portions over a period of 2 h. The reaction mixture was allowed to warm slowly to 10 °C, and then the solvent was partially evaporated *in vacuo*. Water (500 mL) was added to this concentrated suspension and the resulting mixture was extracted with $CHCl_3$ (3 × 500 mL). The combined organic phase was washed with aqueous $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was chromatographed (EtOAc:Hexane = 1:1) to afford **7** as a clear oil (32.9 g, 90%). $[\alpha]_D^{25}$ -16.3° (*c* 1.34, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 5.28 (d, *J* = 3.6 Hz, 1 H, H-4'), 5.18 (t, *J* = 9.2 Hz, 1 H), 4.84 (m, 2 H), 4.60 (d, *J* = 8.8 Hz, 1 H, H-1), 4.50 (d, *J* = 8.8 Hz, 1 H), 4.39 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.16 (dd, *J* = 12.0, 4.2 Hz, 1 H), 4.08 (m, 1 H), 3.77 (m, 1 H), 3.70 (m, 1 H), 2.15 (s, 3 H), 2.14 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H); ^{13}C (125.69 MHz; $CDCl_3$) δ 170.9, 170.7, 170.5, 169.8, 169.5, 100.9 (C-1'), 87.7 (C-1), 75.8, 74.9, 72.8, 72.4, 71.4, 71.0, 70.8, 69.2, 61.9, 61.5, 20.8 (2 C), 20.7, 20.6. MS (*m/z*) 642 ($M+Na^+$); HRMS calcd for $C_{24}H_{33}N_3O_{16}Na$ 642.1759, found 642.1774.

Anal. Calcd for $C_{24}H_{33}N_3O_{16}$: C, 46.53; H, 5.37; N, 6.78. Found: C, 46.34; H, 5.46; N, 6.61.

(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (5). A suspension of acceptor **7** (30.1 g, 0.049 mol), donor **6** (32.2 g, 0.051 mol), 4 Å MS (30 g), and anhydrous CH_2Cl_2 (400 mL) was stirred for 30 min at room temperature. It was cooled to –30 °C and *N*-iodosuccinimide (12.0 g, 0.054 mol) was added to the mixture. After stirring for 10 min, triflic acid (0.71 mL, 8.0 mmol) was added dropwise over a period of 30 min. Stirring was continued for 2 h at the same temperature, and then the reaction was allowed to warm slowly to 10 °C. The reaction mixture was diluted with 400 mL of CH_2Cl_2 and filtered through a celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ solution (300 mL) and dried over Na_2SO_4. Solvent removal followed by chromatographic purification (EtOAc:hexane = 1:1) afforded trisaccharide **5** as a foamy solid (50.2 g, 94%). $[\alpha]_D^{25}$ 42.3° (*c* 0.78, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.22-7.34 (m, 20 H, Ar), 5.40 (d, *J* = 2.8 Hz, 1 H, H-4"), 5.16 (t, *J* = 8 Hz, 2 H), 5.01-5.08 (m, 2 H), 4.77-4.89 (m, 3 H), 4.57-4.69 (m, 4 H), 4.48 (m, 3 H), 4.38 (t, *J* = 8.0 Hz, 1 H), 4.28 (d, *J* = 8.0 Hz, 1 H, H-1'), 3.94-4.09 (m, 3 H), 3.61-3.79 (m, 6 H), 3.47 (d, *J* = 6.4 Hz, 2 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.98 (s, 3 H), 1.90 (s, 3 H), 1.78 (s, 3 H); ^{13}C (125.69 MHz; $CDCl_3$) δ 170.3, 170.2, 170.1, 169.5, 169.4, 168.8, 138.6, 138.0, 128.3, 128.2, 128.1(2 C), 127.9, 127.7, 127.6, 127.5, 127.4, 101.1 (C-1'), 95.1 (C-1"), 87.6 (C-1), 78.4, 77.5, 77.0, 76.6, 75.7, 75.3 (2 C), 74.9, 74.8, 73.6, 73.3, 73.2, 73.0, 72.5, 71.1, 71.0, 70.5, 69.9, 68.5, 64.8, 61.9, 61.3, 20.6 (2 C), 20.4. MS (*m/z*) 1164 ($M+Na^+$); HRMS calcd for $C_{58}H_{67}N_3O_{21}Na$ 1164.4165, found 1164.4117.

Anal. Calcd for $C_{58}H_{67}N_3O_{21}$: C, 60.99; H, 5.91; N, 3.68. Found: C, 60.89; H, 5.88; N, 3.68.

***N*-Acetyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (13).** In the presence of PtO_2 (10 mg), trisaccharide **5** (100 mg, 0.088 mmol) in ethanol (10 mL) was charged with hydrogen (40 lb/in^2) for 1 h. The solid was then filtered off, and the filtrate was concentrated to give a residue. To this residue in dry dichloromethane (10 mL) was added TEA (1 mL) and acetyl chloride (0.2 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The solution was washed with 0.2 N HCl, $NaHCO_3$ and brine and dried over Na_2SO_4. Solvent removal followed by chromatographic purification (EtOAc:hexane = 1:1) afforded **13** as a foamy solid (86 mg, 88%). $[\alpha]_D^{25}$ 40.5° (*c* 0.58, $CHCl_3$). ^{1}H NMR (550 MHz, $CDCl_3$) δ 7.36-7.25 (m, 20 H, Ar), 6.18 (d, *J* = 9.5 Hz, 1 H, NH), 5.43 (d, *J* = 3.0 Hz, 1 H), 5.28 (t, *J* = 9.0 Hz, 1H), 5.19 (t, *J* = 9.0 Hz, 1 H), 5.10 (dd, *J* = 10.5, 8.5 Hz, 1 H), 5.04 (d, *J* = 3.5 Hz, 1 H), 4.90 (d, *J* = 11.5 Hz, 1 H), 4.83-4.80 (m, 2 H), 4.72-4.63 (m, 3 H), 4.49 (d, *J* = 11.5 Hz, 2 H), 4.39 (d, *J* = 12 Hz, 2 H), 4.30 (d, *J* = 7.5 Hz, 1 H), 4.13-3.97 (m, 4 H), 3.84-3.78 (m, 4 H), 3.73-3.65 (m, 3H), 3.50 (d, *J* = 7.0 Hz, 2 H), 2.09 (s, 3H), 2.08 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.93 (s, 3 H), 1.82 (s, 3 H); ^{13}C (125.69 MHz; $CDCl_3$) δ 172.1, 171.1 (2 C), 170.9 (2 C), 170.0, 169.5, 139.4, 139.3, 138.7, 129.1 (2 C), 128.9 (3 C), 128.7, 128.4 (3 C), 128.3, 128.2, 128.1, 101.5 (C-1'), 95.7 (C-1"), 79.1 (C-1), 78.8, 76.4, 76.1, 76.0, 75.5, 75.2, 74.3, 74.0, 73.9, 73.7, 73.0, 71.8, 71.7, 71.2, 70.5, 69.1, 65.5, 62.8, 62.2 (C-6), 24.1, 21.5, 21.4 (2 C), 21.1; MS (*m/z*) 1180 (M+Na$^+$).

***N*-Acetyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (14).** A mixture of **13** (70 mg, 0.061 mmol), $Pd(OH)_2$/C (10%wt, 10 mg), acetic acid (0.5 mL), and methanol (10 mL) was charged with compressed hydrogen (50 lb/in^2) with shaking for 12 h. The mixture was filtered and the filtrate was concentrated to give a residue. To this residue in anhydrous methanol (20 mL) was added NaOMe portionwise until pH = 9. After 1 h, Dowex (H form) was added to neutralize the solution. The resin was filtered off and the filtrate was concentrated to afford **14** as a white foamy solid in quantitative yield. $[\alpha]_D^{25}$ 59.7° (*c* 1.16, $CHCl_3$). ^{1}H NMR (500 MHz, D_2O) δ 5.03 (d, *J* = 3.6 Hz, 1 H, H-1"), 4.86 (d, *J* = 8.8 Hz, 1 H, H-1), 4.42 (d, *J* = 8.0 Hz, 1H, H-1'), 4.07 (m, 2 H, H-6), 3.10-3.91 (m, 16 H), 1.96 (s, 3 H, $COCH_3$);. ^{13}C (125.69 MHz; D_2O) δ 175.5 (C=O), 103.7 (C-1'), 96.4 (C-1'), 80.0 (C-1), 79.1 (C-4), 78.2 (C-3'), 77.2 (C-5), 76.0 (C-3), 75.9 (C-5'), 72.4 (C-2'), 72.3 (C-2), 71.7 (C-5"), 70.2 (C-3"), 70.0 (C-4"), , 69.1 (C-2"), 65.8 (C-4'), 61.8 (C-6', C-6"). 60.9 (C-6), 21.8 (CH_3CO). HRMS calcd for $C_{20}H_{35}NO_{16}Na^+$ 568.1854, found 568.1829.

Anal. Calcd for $C_{20}H_{35}NO_{16}$: C, 44.04; H, 6.47; N, 2.57. Found: C, 44.28; H, 6.29; N, 2.39.

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