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Synthesis of a divalent glycoside of an α-galactosyl disaccharide epitope involved in the hyperacute rejection of xenotransplantation☆

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Abstract

3,6-Dioxaoct-1,8-diyl di-(3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside) was synthesized for use in research on hyperacute rejection of xenotransplantation. The trichloroacetate method was successfully applied to form stereoselectively the α-D-galactosyl linkage under mild reaction conditions and a simple procedure. The divalent *O*-glycoside was formed from the corresponding trichloroacetimidate in one step with reasonable yield. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Divalent glycoside; Hyperacute rejection; Synthesis; Trichloroacetate method

1. Introduction

The shortage of donor organs and tissues is the most urgent problem in transplantation today. This has increased the interest in the possible use of xenogeneic organs for transplantation in humans.[1,2] Considered from the aspects of availability, a suitable size of the organ, genetical manipulation, ethics and virology, the pig is the most favored species at present.[3,4] However, if a pig organ is transplanted into an untreated human, it would almost certainly be destroyed over a period of minutes to hours by hyperacute rejection (HAR), an important immunological barrier to xenotransplantation. HAR has been found to be triggered by the binding of xenoreactive natural antibodies in humans to the endothelial lining of the blood vessels within the graft. These natural occurring antibodies, termed anti-Gal, specifically recognize α-Gal-(1→3)-Gal, which is presented on the surface of the endothelial cells of most mammalian species except for humans, apes, and Old World monkeys.[5,6]

One strategy to prevent anti-Gal binding to the endothial cells is the use of synthetic antigens either as immunoabsorption agents to remove anti-Gal from the recipient's circulation or as soluble substances to inhibit the binding. However, it is known that most carbohydrate–protein interactions are rather weak.[7] The multivalent effect has been recognized as an effective way to increase binding interactions between carbohydrates and proteins.[8] Accordingly, we designed and syn-

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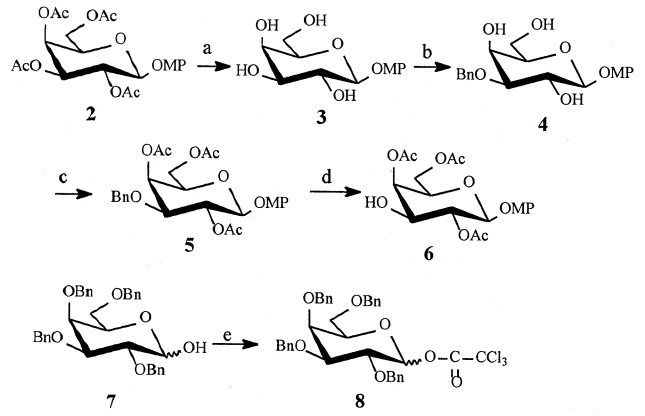
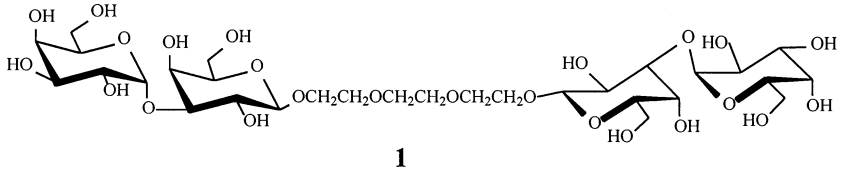


Scheme 1. (a) NaOMe, MeOH; (b) (1) Bu_2SnO, toluene, reflux, 17 h; (2) BnBr, Bu_4NI, toluene, reflux, 2 h; (c) Ac_2O, Py, rt, 18 h; (d) H_2, Pd–C (10% Pd), MeOH, rt, 10 h; (e) CCl_3COONa, $(CCl_3CO)_2O$, CH_2Cl_2, reflux, 1.5 h.

thesized the novel divalent glycoside **1**, which could be useful in the research of hyperacute rejection in xenotransplantation.



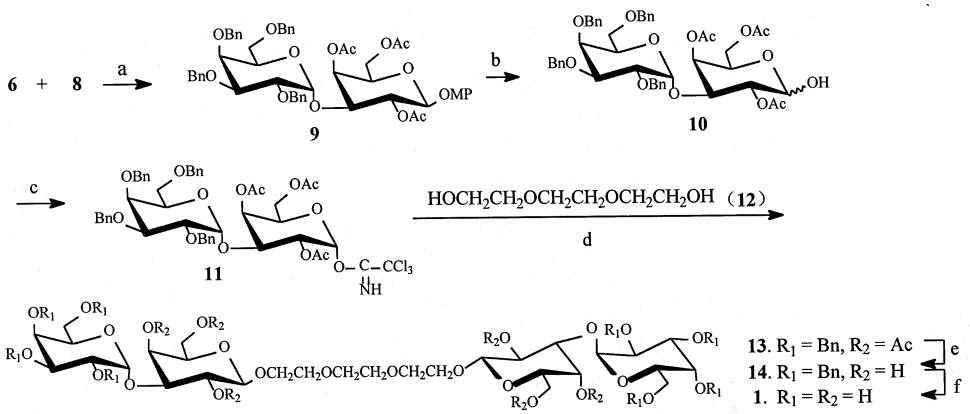
1

2. Results and discussion

The synthetic route for dimer **1** is outlined in Schemes 1 and 2. A fully protected glycotetraose **13** is designed as a direct precursor for compound **1**.

The galactopyranosyl acceptor **6**, unsubstituted at O-3, was conveniently synthesized from *p*-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**2**) by the 'dibutyltin oxide alkylation' procedure[9] in four steps and 67% overall yield. Compound **2**[10] was deacetylated and then regioselectively benzylated via a dibutyl tin oxide-mediated reaction with benzyl bromide as the reagent and Bu_4NI as the catalyst to afford compound **4** as a pale yellow solid. TLC indicated **4** to be contaminated with 10–15% of unknown compound, which was difficult to separate by chromatography. Conventional direct acetylation of crude **4** afforded the fully protected compound **5**, which could be readily crystallized pure from ice-water. The unknown contaminant was readily separated in this step as it still stayed in the aqueous layer. Catalytic hydrogenolysis under standard conditions gave the nucleophile **6**, isolated as an amorphous white solid.

Trichloroacetoxy, a novel glycosyl anomeric leaving group, was reported by our laboratory in successful preparations of many glycosides and oligosaccharides under mild reaction conditions and simple procedures.[11–17] Here we used this method to prepare disaccharide **9**. 2,3,4,6 - Tetra - *O* - benzyl - D - galactopyranose (**7**)[19] was converted into trichloroacetyl



Scheme 2. (a) Me_3SiOTf, CH_2Cl_2–Et_2O, 4 Å MS, −20 °C, 30 min; (b) CAN, CH_3COCH_3–H_2O, rt, 5 h; (c) CCl_3CN, DBU, 0 °C, 4 h; (d) Me_3SiOTf, CH_2Cl_2, 4 Å MS, 0 °C, 20 min; (e) NaOMe, MeOH–CH_2Cl_2 (1:1), rt, 24 h; (f) Pd–C, H_2, MeOH, 20 h.

2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (**8**), an activated glycosyl donor, by treatment with trichloroacetic anhydride in the presence of sodium trichloroacetate.[16] Compound **8** is stable and can be stored at room temperature for a long time. The yield is nearly quantitative and the product pure enough for use in the next step without further purification. The trichloroacetate **8** reacted with **6** at room temperature in 1:3 CH_2Cl_2–Et_2O with Me_3SiOTf as promoter to give the α-disaccharide **9** in good yield (two crops, 69%) with high stereoselectivity (α:β = 11:1). The configuration of the disaccharide was indicated by the characteristic coupling constant ($J_{1',2'}$ 3.3 Hz) and confirmed in ^{13}C NMR by the C-1′ chemical shift (δ 94.9 ppm). When the condensation was carried out in dichloromethane at 0 °C, the ratio of α:β is only 16:5.

Removal of the *p*-methoxyphenyl group in **9** using ammonium cerium(IV) nitrate (CAN) afforded **10** as an anomeric mixture (67%, α:β = 4:1), which on treatment with DBU and trichloroacetonitrile in dichloromethane gave the α-imidate **11** in 81% yield. Condensation of **11** with triethylene glycol (**12**) in dry dichloromethane, using 0.25 equiv (based on the donor) of Me_3SiOTf as promotor, gave the desired dimer **13** (57%) in one step. The symmetric β configuration was confirmed by ^1H NMR ($J_{1,2}$ 8.0 Hz), ^{13}C NMR (δ 101.6 ppm), and TOF-MS ($[M + Na]^+$: 1793.4; $[M + K]^+$: 1809.8).

We also examined various other reaction conditions. When the condensation was promoted by 0.12 equiv Me_3SiOTf, most of **11** was converted into a self-condensation product. Attempted glycosylations with boron trifluoride etherate or silver trifluoromethanesulfonate as promotor were unsuccessful and most of the donor was converted into the hemiacetal **10**. The result showed that the condensation required a strong catalyst. In order to decrease the decomposition of the donor, we also tried the 'inverse procedure' (addition of the donor to an acceptor–catalyst solution).[18] Unfortunately, no obvious reaction occurred after 45 min and prolongation of the reaction time only led to the decomposition of the donor. Addition of a second portion of the catalyst before decomposition of the donor occurred can lead to glycosylation. Possibly, the catalyst had already been decomposed by the triethylene glycol before the addition of the donor.

Deacetylation of **13** followed by debenzylation furnished the target divalent glycoside **1** in 94% yield.

Immunological inhibition experiments with this novel dimer are now in progress.

3. Experimental

General methods.—Solvents were purified conventionally. Optical rotations were measured using an optical activity AA-10R type polarimeter. Melting points were uncorrected. NMR spectra were recorded with Bruker ARX-400 or Varian VXR-300 spectrometers. Mass spectra were recorded with a ZAB-HS spectrometer (FAB) and a LDI-1700 spectrometer (MALDI-TOF). Column chromatography was performed on silica gel H (10–40 μm) (Hai Yang Chemical Factory, Qingdao, Shandong, China). The purity of the product was determined by TLC on silica gel GF254 (Hai Yang Chemical Factory, Qingdao, Shandong, China). Elemental analyses were performed on Perkin–Elmer 240C instrument.

p-*Methoxyphenyl 3*-O-*benzyl*-β-D-*galactopyranoside* (**4**).—To a solution of *p*-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**2**)[10] (9.0 g, 20 mmol) in dry MeOH (100 ml) was added NaOMe (0.15 g, 2.8 mmol). After 2 h, the mixture was neutralized with H^+ cation-exchange resin, filtered, and concentrated to afford **3**. The crude product **3** (5.0 g, 17 mmol) was dissolved in dry toluene, and dibutyltin oxide (4.4 g, 17 mmol) was added. The mixture was refluxed with azeotropic removal of the generated water for 17 h. Tetrabutylammonium iodide (6.5 g, 17 mmol) and BnBr (3.1 mL, 26 mmol) were then added, and the mixture was stirred at reflux temperature for additional 2 h. Examination by TLC (4:1 CH_2Cl_2–CH_3COCH_3) showed only traces of **3** remaining and the predominant presence of **4** (R_f 0.42). The dark-orange solution was concentrated to dryness and the resulting residue was chromatographed (25:1 CH_2Cl_2–MeOH) to give **4**

as an amorphous solid (5.7 g, 87%), contaminated by 10–15% of a co-eluting compound. A portion of **4** was recrystallized from MeOH–Et_2O to give a pure sample: mp 155–156 °C, $[\alpha]_D^{26}$ −8.0° (*c* 1.0, CH_3OH); ^1H NMR (Me_2SO-d_6): δ 7.46–7.27 (m, 5 H, $PhCH_2$), 6.99 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 6.85 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 5.35 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1), 4.75–4.65 (m, 5 H, $PhCH_2$, 3 × OH), 4.58 (d, 1 H, *J* 12 Hz, $PhCH_2$), 3.99 (dd, 1 H, *J* 3.3, *J* 4.8 Hz, H-4), 3.75 (m, 1 H, H-2), 3.70 (s, 3 H, OCH_3), 3.60–3.33 (m, 4 H, H-5, H-6a, H-6b, H-3); ^{13}C NMR (Me_2SO-d_6): δ 154.3, 151.5, 139.0, 128.0, 127.5, 127.1, 117.8 and 114.4 (Ph), 102.1 (C-1), 81.2 (C-3), 75.2 (C-5), 70.2 ($PhCH_2$), 69.4 (C-2), 64.6 (C-4), 60.3 (C-6), 55.3 (OCH_3); FAB-MS: $[M+K]^+$ 414.8. Anal. Calcd for $C_{20}H_{24}O_7$: C, 63.83; H, 6.38. Found: C, 64.08; H, 6.46.

p-*Methoxyphenyl 2,4,6-tri*-O-*acetyl-3*-O-*benzyl-β*-D-*galactopyranoside* (**5**).—A solution of crude **4** (4.0 g) in pyridine (60 mL) was treated with Ac_2O (30 mL) at 0 °C and the mixture was stirred for 18 h at rt. The mixture was then poured into ice-water and stirred, whereupon the product crystallized. Recrystallization from EtOH gave pure **5** (6.0 g, 69% in two crops) as white needles: mp 91–92 °C, $[\alpha]_D^{26}$ +55.4° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.34–7.25 (m, 5 H, $PhCH_2$), 6.93 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 6.79 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 5.55 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.37 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.9 Hz, H-2), 4.81 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.72 (d, 1 H, *J* 12.0 Hz, $PhCH_2$), 4.43 (d, 1 H, *J* 12.6 Hz, $PhCH_2$), 4.21 (d, 2 H, H-6a, H-6b), 3.90 (m, 1 H, H-5), 3.76 (s, 3 H, OCH_3), 3.61 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 2.18, 2.08 and 2.05 (3s, 9 H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.4 and 169.2 (3 C, 3 × CH_3CO), 155.7, 151.3, 137.4, 128.4 (2 C), 127.9, 127.8, 118.6 (2 C), and 114.5 (2 C) (Ph), 100.9 (C-1), 71.4, 71.2, and 70.4 (C-3, C-2, C-5), 65.8 (C-4), 62.0 (C-6), 55.7 (OCH_3), 20.8 (2 C) and 20.6 (3 × CH_3CO); FAB-MS: $[M+K]^+$ 541.6. Anal. Calcd for $C_{26}H_{30}O_{10}$: C, 62.15; H, 5.98. Found: C, 61.95; H, 6.02.

p-*Methoxyphenyl 2,4,6-tri*-O-*acetyl-β*-D-*galactopyranoside* (**6**).—A solution of **5** (3.1 g, 6.2 mmol) in MeOH was treated with a catalytic amount of Pd–C (10% Pd) and reduced with hydrogen. After 10 h TLC (1:1 petroleum ether–EtOAc) indicated the reaction to be complete and the product was isolated conventionally and collected as a white solid, mp 130–132 °C, $[\alpha]_D^{26}$ +8.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.96 (d, 2 H, *J* 8.7 Hz, $C_6H_4OCH_3$), 6.82 (d, 2 H, *J* 8.7 Hz, $C_6H_4OCH_3$), 5.38 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.22 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.9 Hz, H-2), 4.88 (d, 1 H, H-1), 4.19 (d, 2 H, H-6a, H-6b), 3.97–3.89 (m, 2 H, H-5, H-3), 3.78 (s, 3 H, OCH_3), 2.20, 2.16 and 2.06 (3s, 3 × 3 H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 171.1, 170.8, 170.4 (3 C, CH_3CO), 155.8, 151.2, 118.7 (2 C) and 114.6 (2 C) (Ph), 100.6 (C-1), 72.7 (C-2), 71.5, 71.3 (C-5, C-3), 69.6 (C-4), 61.9 (C-6), 55.7 (OCH_3), 20.9, 20.7 and 20.6 (3 C, 3 × CH_3CO); FAB-MS: $[M+K]^+$ 450.8. Anal. Calcd for $C_{19}H_{24}O_{10}$: C, 55.34; H, 5.82. Found: C, 55.09; H, 5.79.

p-*Methoxyphenyl 2,4,6-tri*-O-*acetyl-3*-O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-β*-D-*galactopyranoside* (**9**).—To a solution of **7**[19] (1.00 g, 1.85 mmol) in dry CH_2Cl_2 (20 mL) was added Cl_3CCO_2Na (0.35 g, 1.89 mmol) and trichloroacetic anhydride (0.50 mL, 2.74 mmol). The mixture was refluxed with stirring for 1.5 h, and then 15 mL CH_2Cl_2 was added to the cooled flask. The mixture was washed with aq $NaHCO_3$, ice-water, dried (Na_2SO_4) and concentrated to give **8** as a syrup (1.36 g, 99%).

A mixture of **8** (1.24 g, 1.81 mmol), **6** (0.90 g, 2.18 mmol) and powered 4 Å molecular sieves in 80 mL of dry 1:3 CH_2Cl_2–Et_2O was stirred at rt. After 30 min, 0.76 mL Me_3SiOTf was added dropwise and the mixture was stirred at rt for 30 min. The mixture was neutralized with Et_3N, diluted with CH_2Cl_2, and filtered over Celite. The filtrate was washed with satd aq $NaHCO_3$, water, then dried (Na_2SO_4) and concentrated. The residue was chromatographed 20:1 toluene–acetone to afford **9** (1.19 g, two steps 69%) as a foam, $[\alpha]_D^{26}$ +74.2° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.24 (m, 20 H, $PhCH_2$), 6.92 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 6.80 (d, 2 H, *J* 9.3 Hz, $C_6H_4OCH_3$), 5.49 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.43 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.2 Hz, H-2), 5.10 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 4.95–4.36 (m,

9 H, 4 × $PhCH_2$, H-1), 4.13 (t, 2 H, H-6a, H-6b), 4.02 (dd, 1 H, $J_{1',2'}$ 3.3, $J_{2',3'}$ 11.1 Hz, H-2′), 3.92–3.90 (m, 2 H, H-5, H-5′), 3.86–3.80 (m, 3 H, H-3, H-3′, H-4′), 3.78 (s, 3 H, OCH_3), 3.51 (t, 2 H, H-6′a, H-6′b), 2.06, 1.97 and 1.84 (3s, 3 × H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.3 and 169.0 (3 C, 3 × CH_3CO), 155.7, 151.4, 138.8, 138.7, 138.2, 128.4, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 118.6, 114.6 (Ph), 101.1 (C-1), 94.9 (C-1′), 78.6 (C-4′), 75.9 (C-2′), 75.5 (C-3′), 74.8, 73.5 and 73.3 (4 C, 4 × $PhCH_2$), 72.8 (C-3), 71.4 (C-5), 70.2 and 70.1 (C-2, C-5′), 68.9 (C-6′), 65.1 (C-4), 62.0 (C-6), 55.7 (OCH_3), 20.8, 20.7 and 20.4 (3 × CH_3CO); TOF-MS: $[M + Na]^+$ 956.3, $[M + K]^+$ 972.0. Anal. Calcd for $C_{53}H_{58}O_{15}$: C, 68.09; H, 6.21. Found: C, 68.23; H, 6.39.

*2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-*D*-galactopyranose* (**10**).—Compound **9** (1.34 g, 1.41 mmol) was dissolved in acetone (70 mL) and water (23 mL). The mixture was cooled (ice-water bath) and a solution of ceric ammonium nitrate (CAN, 4.58 g, 8.35 mmol) in 3:1 acetone–water was added. After stirring at rt for 5 h, the mixture was concentrated to 40 mL, diluted with EtOAc (150 mL), washed with water. The aqueous layer was extracted twice with EtOAc. The organic extracts were washed with aq satd NaCl solution, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue (1.8:1 petroleum ether (60–90 °C)–EtOAc) afforded **10** (0.78 g, 67%, α:β = 4:1) as a foam, $[\alpha]_D^{26}$ + 93.7° (*c* 1.7, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 170.5 and 170.2 (3 C, 3 × CH_3CO), 138.7, 138.6, 138.1, 128.3, 128.1, 128.0, 127.6, 127.5 (Ph), 96.2 (C-1β), 94.6 (C-1′), 90.6 (C-1α), 20.8 and 20.4 (3 C, 3 × CH_3CO); TOF-MS: $[M + Na]^+$ 851.0, $[M + K]^+$ 866.7.

*3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-*D*-galactopyranosyl)-2,4,6-tri-*O*-acetyl-α-*D*-galactopyranosyl trichloroacetimidate* (**11**).—To a solution of **10** (700 mg, 0.845 mmol) and trichloroacetonitrile (1.96 mL, 19.4 mmol) in dry CH_2Cl_2 (35 mL) was added DBU (0.035 mL, 0.23 mmol) at 0 °C. The mixture was stirred in an ice-water bath. After 4 h, TLC (3:1 petroleum ether–EtOAc) indicated the reaction to be complete. The mixture was concentrated and the residue was chromatographed (6:1 petroleum ether (60–90 °C)–EtOAc + 1% Et_3N) to give **11** (665 mg, 81%) as a colorless foam; ^{1}H NMR ($CDCl_3$): δ 8.61 (s, 1 H, NH), 7.38–7.20 (m, 20 H, 4 × $PhCH_2$), 6.60 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.62 (d, 1 H, $J_{3,4}$ 2.4 Hz, H-4), 5.30 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.15 (d, 1 H, $J_{1',2''}$ 3.0 Hz, H-1′), 4.93–4.49 (m, 6 H, 3 × $PhCH_2$), 4.43–4.30 and 4.06–3.93 (2m, 2 × 4 H, $PhCH_2$, H-4′, H-3′, H-6′, H-3, H-5, H-6), 4.15 (dd, 1 H, *J* 6.0, *J* 11.4 Hz, H-6), 3.85 (dd, 1 H, *J* 2.7, *J* 10.2 Hz, H-2′), 3.58 (t, 1 H, *J* 8.4 Hz, H-5′), 3.40 (dd, 1 H, $J_{1',2'}$ 5.4, $J_{2',3'}$ 8.7 Hz, H-2′), 3.58 (t, 1 H, *J* 6.0 Hz, H-5′), 3.40 (dd, 1 H, *J* 5.4, *J* 8.7 Hz, H-6′), 2.03, 1.90 and 1.86 (3s, 3 × 3 H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.1, 169.9, 163.3 and 160.9 (4 C, 3 × CH_3CO, $HNCCCl_3$), 138.7, 138.6, 137.9, 128.3, 128.1, 128.0, 127.7, 127.5, 127.4 (24 C, Ph), 94.4 (C-1′), 93.8 and 91.0 (C-1, CCl_3), 78.5 (C-4′), 75.9 (C-2′), 75.0, 74.8, 73.6, 73.4, 73.0 (5 C, C-3′, 4 × $PhCH_2$), 69.7, 69.5, 68.6, 68.3 and 68.1 (C-5, C-2, C-5′, C-6′, C-3), 65.7 (C-4), 61.8 (C-6), 20.6, 20.4, and 20.3 (3 C, 3 × CH_3CO).

*3,6-Dioxaoct-1,8-diyl-di-[2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside]* (**13**).—A mixture of **11** (300 mg, 0.308 mmol), triethylene glycol (**12**, 21 mg, 0.140 mmol) and powered 4 Å molecular sieves in 10 mL of dry CH_2Cl_2 was stirred at rt for 45 min. Then the mixture was cooled to 0 °C in an ice-water bath and 0.03 M Me_3SiOTf (2.37 mL) was added dropwise. The reaction was complete after 20 min, as indicated by TLC. After addition of solid $NaHCO_3$ and filtration, the solution was concentrated and the residue was chromatographed (1:1 petroleum ether (60–90 °C)–EtOAc) to give **13** (141 mg, 57%) as a foam, $[\alpha]_D^{26}$ + 68.4° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.24 (m, 20 H, Ph), 5.45 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 5.18 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2), 5.08 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.93–4.38 (m, 9 H, 4 × $PhCH_2$, H-1), 4.08 (d, 2 H, *J* 6.5 Hz, H-6a, H-6b), 3.99 (dd, $J_{1'',2'}$ 3.4, $J_{2',3'}$ 11.2 Hz, H-2′), 3.94–3.90 (m, 2 H, H-5, OCH_2CHOCH_2), 3.87–3.80 (m, 3 H, H-3, H-3′, H-4′), 3.69–3.59 (m, 6 H, OCH_2CHOCH_2, H-5′), 3.52–3.51 (d, 2 H, H-

6′a, H-6′b), 2.06, 1.95 and 1.81 (3s, 3 × 3 H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.4, 170.3 and 169.2 (3 × CH_3CO), 138.8, 138.7, 138.1, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5 and 127.4 (Ph), 101.6 (C-1), 94.7 (C-1′), 78.5 (C-4′), 75.8 (C-2′), 75.4 (C-3′), 74.8, 73.6 (2 C) and 73.3 (4 × $PhCH_2$), 72.7 (C-3), 71.1 (C-5), 70.8, 70.2 and 68.9 ($OCH_2CH_2OCH_2$), 70.1 (C-2), 69.9 (C-5′), 68.8 (C-6′), 65.2 (C-4), 61.9 (C-6), 20.8, 20.7, and 20.5 (3 × CH_3CO); TOF-MS: $[M + Na]^+$ 1793.4, $[M + K]^+$ 1809.8. Anal. Calcd for $C_{98}H_{114}O_{30}$: C, 66.44; H, 6.44. Found: C, 66.16; H, 6.50.

*3,6-Dioxaoct-1,8-diyl-di-(3-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranoside)* (**1**).—To a solution of **13** (200 mg, 0.113 mmol) in dry 1:1 MeOH–CH_2Cl_2 was added a catalytic amount of NaOMe (pH 12). After stirring at rt for 24 h, the mixture was neutralized with H^+ cation-exchange resin, filtered, and concentrated. The residue was chromatographed to afford **14** (161 mg, 94%). A mixture of **14** and Pd–C (10% Pd) in MeOH was stirred for 20 h at 25 °C under H_2. After filtration, the filtrate was evaporated to afford **1** as a white foamy solid in quantitative yield, $[\alpha]_D^{26}$ +95.3° (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD): δ 5.05 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.35 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1); ^{13}C NMR (CD_3OD): δ 104.9 (C-1), 97.7 (C-1′), 80.0 (C-3), 76.3 (C-5), 72.2 (C-5′), 71.3, 70.9, 70.2, and 69.7 (7 C, $OCH_2CH_2OCH_2$, C-2, C-2′, C-4′, C-3′), 66.8 (C-4), 62.9 and 62.5 (C-6, C-6′); TOF-MS: $[M + Na]^+$ 821.2, $[M + K]^+$ 837.4.

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References

1. Auchincloss, Jr, H. *Transplantation* **1998**, *46*, 1–20.
2. Platt, J. L.; Vercellotti, G. M.; Dalmasso, A. P.; Matas, A. J.; Bolman, R. M.; Najarian, J. S.; Bach, F. H. *Immunol. Today* **1990**, *11*, 450–456.
3. Ye, Y.; Niekrasz, M.; Kosanke, S.; Welsh, R.; Jordan, H. E.; Fox, J. C.; Edwards, W. C.; Maxwell, C.; Cooper, D. K. C. *Transplantation* **1994**, *57*, 694–703.
4. Hammer, C.; Linke, R.; Wanger, F.; Diefenbeck, M. *Int. Arch. Allergy Immunol.* **1998**, *116*, 5–21.
5. Good, A. H.; Cooper, D. K.; Malcolm, A. J.; Ippolito, R. M.; Koren, E.; Neethling, F. A.; Ye, Y.; Zuhdi, N.; Lamontagne, L. R. *Transpl. Proc.* **1992**, *24*, 559–562.
6. Rother, R. P.; Squinto, S. P. *Cell* **1996**, *86*, 185–188.
7. Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728.
8. (a) Roy, R.; Zanini, D. *J. Org. Chem.* **1996**, *61*, 7348–7354;
   (b) Roy, R. *Trends Glycosci. Glyctotechnol.* **1996**, *8*, 79–99;
   (c) Heerze, L. D.; Kelm, M. A.; Talbot, J. A.; Armstrong, G. D. *J. Infect. Dis.* **1994**, *169*, 1291–1296.
9. David, S.; Thieffry, A.; Veyrières, A. *J. Chem. Soc. Perkin Trans. 1* **1981**, 1796–1801.
10. Zhang, Z. Y.; G(ran, M. *Carbohydr. Res.* **1996**, *295*, 41–55.
11. Li, Z. J.; Huang, H. Q.; Cai, M. S. *Carbohydr. Res.* **1994**, *265*, 227–236.
12. Li, Z. J.; Huang, H. Q.; Cai, M. S. *J. Carbohydr. Chem.* **1996**, *15*, 501–506.
13. Zhu, X. X.; Ding, P. Y.; Cai, M. S. *Tetrahedron: Asymmetry* **1996**, *7*, 2833–2838.
14. Zhu, X. X.; Ding, P. Y.; Cai, M. S. *Tetrahedron Lett.* **1996**, *37*, 8549–8552.
15. Zhang, S. Q.; Li, Z. J.; Wang, A. B.; Cai, M. S. *Carbohydr. Res.* **1998**, *308*, 281–285.
16. Mao, J. M.; Chen, H. M.; Zhang, J.; Cai, M. S. *Synth. Commun.* **1995**, *25*, 1563–1565.
17. Li, Q.; Li, H.; Cai, M. S.; Li, Z. J.; Zou, R. L. *Tetrahedron: Asymmetry* **1999**, *10*, 2675–2683.
18. Greilich, U.; Zimmermann, P.; Jung, K. H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1993**, 859–864.
19. Koto, S.; Morishima, N.; Miyata, Y.; Zen, S. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2639–2640.