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Note

Practical Synthesis of the Disaccharide Epitope, D-Galactopyranosyl-α-1,3-D-galactopyranose, by using 1,2;5,6-Di-*O*-cyclohexylidene-α-D-galactofuranose as the Glycosyl Acceptor

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D-Galactosyl-α-1,3-D-galactopyranose (1) was chemically prepared in a good yield by coupling phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (5) or 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide (8) with 1,2:5,6-di-*O*-cyclohexylidene-α-D-galactofuranose (3) with subsequent de-*O*-benzylation and de-*O*-cyclohexylidenation of the resulting protected α-1,3-disaccharide.

Key words: 1,2:5,6-di-*O*-cyclohexylidene-α-D-galactofuranose; D-galactopyranosyl-α-1,3-D-galactopyranose; xenotransplantation; α-glycosydation

D-Galactopyranosyl-α-1,3-D-galactopyranose (**1**) is the well-known epitope found on the surface of pig endothelial cells,[1,2] and has been shown to be effective in removing the cytotoxic activity of human serum to pig cells and pig-to-primate xeno graft rejection during xenotransplantation.[3] Although **1** has been synthesized by both chemical[4] and enzymatic methods,[5,6] these methods have some drawbacks. The chemical method by Lemieux employed 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**2**) as a glycosyl acceptor for α-glycosidation, but it is very difficult to obtain **2** on large scale; for example, Lemiuex prepared **2** from D-glucose by four steps in a 13% yield,[4] and direct isopropylidenation of D-galactose always gave a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (predominant) and **2**, their separation by chromatography being very difficult.[7] On the other hand, the α-D-galactosidase-catalyzed self-condensation of *p*-nitrophenyl α-D-galactopyranoside to give the desired α-1,3 disaccharide was low yielding and gave undesired α-1,2 and α-1,6 disaccharides which must be separated by column chromatography.[5] In this paper, we describe a chemical method that can be used for the large-scale synthesis of **1**.

In order to develop a chemical method which can be used for the large scale preparation of **1**, we first studied the conditions for a facile synthesis of **2**, but did not achieve a successful result.

We then directed our attention to 1,2:5,6-di-*O*-cyclohexylidene-α-D-galactofuranose (**3**), which was expected to have chemical properties similar to those of **2**, and studied the reaction of D-galactose with cyclohexanone under various acidic conditions. The results are summarized in Table 1. The reaction of D-galactose with cyclohexanone under acidic conditions gave a mixture of **3** and 1,2:3,4-di-*O*-cyclohexylidene-α-D-galactopyranose (**4**), which is similar to the reaction of D-galactose with acetone, but **3** and **4** could be easily separated by silica gel column chromatography. This is in striking contrast to the cyclohexylidene and isopropylidene derivatives of D-galactose. The PPTS-catalyzed reaction of D-galactose with cyclohexanone in the presence of DMF selectively gave **3** in a 66% yield (Table 1). We thus developed a facile synthetic method for properly protected D-galactofuranose **3** which can be used for 3-*O*-glycosilation of D-galactose.

Having obtained a sufficient quantity of **3**, the α-glycosidation of **3** with galactopyranosyl donors was next studied. The NIS-catalyzed glycosidation[8] of **3** with phenyl 2,3,4,5,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**5**)[9] in dichloromethane was first studied. The reaction always gave α,β-mixture of 1,3-disaccharides in different ratios. The best α-selectivity (α:β = 10:1, total yield = 82%) was achieved at −45°C. These were separated by medium-pressure silica gel column chromatography. Slower-eluting 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-1,2:5,6-di-*O*-cyclohexylidene-α-D-galactofuranose (**6**) was obtained in a 69% yield, and the minor product, 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galac-

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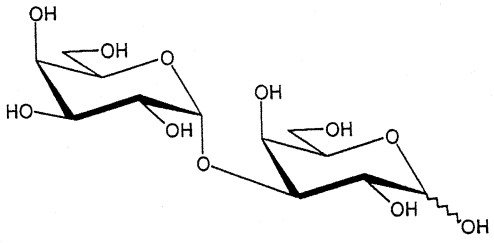
Abbreveations: CSA, *d*-camphor-10-sulfonic acid; DMF, *N*,*N*-dimethylformamide; NIS, *N*-iodosuccinimide; PPTS, pyridinium *p*-toluenesulfonate; TBAB, tetrabutylammonium bromide; TFA, trifluoroacetic acid; TfOH, trifluoromethanesulfonic acid; TsOH, *p*-toluenesulfonic acid monohydrate



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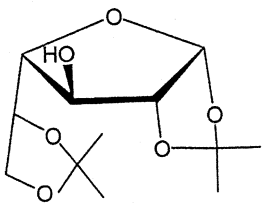

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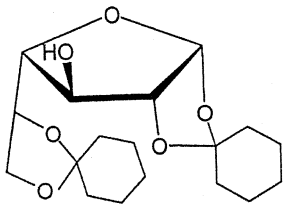

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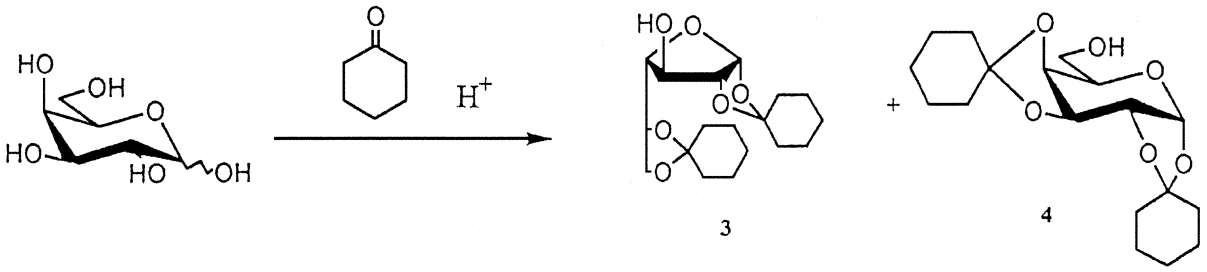
Fig. 1. Structures of **1**, **2** and **3**.

Table 1. Yields of Furanose **3** and Pyranose **4**



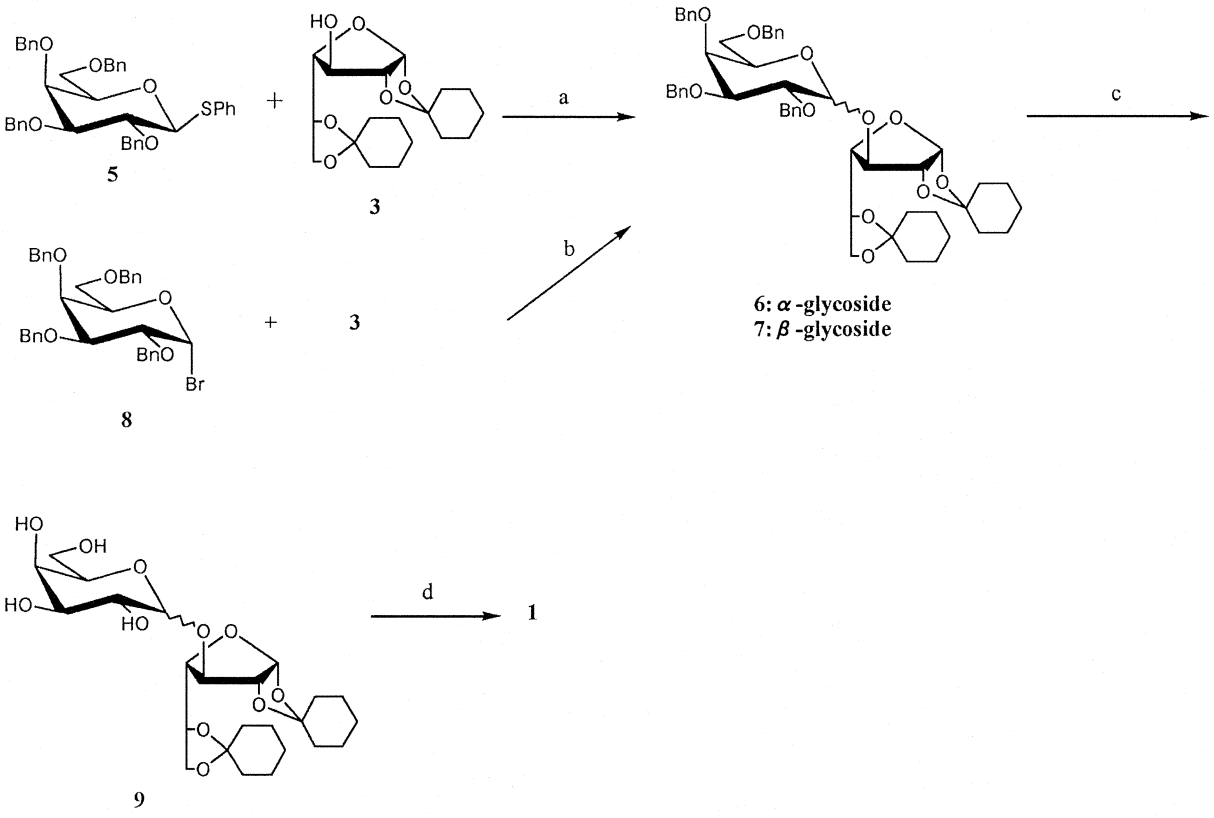
Entry	H^+	Temp. (°C)	Time (h)	Yield (%)	
				Furanose	Pyranose
1	H_2SO_4	rt	overnight	30	27
2	H_2SO_4	80	8	16	35
3	CSA	rt	overnight	N.R	N.R
4	CSA	120 (then rt)	1.5 (then 16)	37	20
5	PPTS	120	8	40	16
6	TsOH	rt	overnight	16	15
7	$CuSO_4$/DMF	reflux	overnight	18	1
8	TsOH/DMF	rt	overnight	N.R	N.R
9	TsOH/DMF	reflux	overnight	44	3
10	H_2SO_4/DMF	rt	overnight	32	30
11	CSA/DMF	reflux	overnight	32	16
12	PPTS/DMF	reflux	8	66	trace

topyranosyl) - 1,2:5,6 - di - *O* - cyclohexylidene - α - D-galactofuranose (**7**), was obtained in a 7% yield.

Next, Lemiex's halide ion catalyzed α-glycosylation[4] of **3** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide (**8**) was studied. The reaction gave desired product **6** alone in a 65% yield. The catalytic hydrogenation of **6** over 10% Pd–C gave crystalline 3-*O*-α-D-galactopyranosyl-1,2:5,6-di-*O*-cyclohexylidene-α-D-galactofuranose (**9**) in an 80% yield. Hydrolysis of the *O*-cyclohexylidene group of **9** in 10% TFA gave **1** in a 70% yield. When these two glycosylation methods were compared, Lemieux's protocol was superior to the other in that it could be carried out at room temperature and gave only the desired α-glycoside. We thus developed an efficient synthetic method, which could be used for a large-scale preparation of **1**.

Experimental

General methods. Melting point (mp) data were recorded with Shibata melting point apparatus and



Scheme 1. Synthesis of D-Galactopyranosyl-α-1,3-D-galactopyranose (**1**).
a) NIS-TfOH, CH_2Cl_2; b) TBAB, $^iPr_2NEt$, CH_2Cl_2, 2 days, 65%; c) H_2, 10% Pd-C, 80%; d) 10% TFA, 30 min, 70%.

are uncorrected. ^{1}H-NMR spectra were recorded with a Varian UNITY plus-500 spectrometer at 21–23°C in $CDCl_3$, using Me_4Si as an internal standard, and mass spectra were recorded with a Hitachi M-80B spectrometer at 70 eV. Specific rotation values were measured with a JASCO DIP-360 instrument at 589 nm. Merck silica gel Art. 9385 was used for column chromatography, and Merck silica gel Art. 5554 for analytical thin-layer chromatography.

1,2:5,6-di-O-cyclohexylidene-α-D-galactofuranose (3). A mixture of D-galactose (20 g, 111 mmol) in a mixture of DMF (150 ml) and cyclohexanone (150 ml) was heated to 120°C while stirring. After the galactose had dissolved, toluene (300 ml) was added, and the mixture was refluxed for 1 h. PPTS (100 mg) was then added, and the mixture was refluxed for 24 h. After the toluene had been removed under reduced pressure, the mixture was partitioned between $CHCl_3$ (700 ml) and sat. aq. $NaHCO_3$ (300 ml). The aqueous layer was extracted with $CHCl_3$ (200 ml × 2). The combined $CHCl_3$ extracts were washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. After filtering off the $MgSO_4$, the filtrate was concentrated under reduced pressure to give a syrup. This syrup was chromatograhed in a column of silica gel (hexane:ethyl acetate = 4:1, v/v) to give an analytically sample of **3**, mp 109–110°C, $[\alpha]_D$ −15.0° (c = 1.15, $CHCl_3$); ^{1}H-NMR (ppm) δ: 5.85 (1H, d, J = 4.12 Hz, H-1), 4.44 (1H, m, H-2), 3.34 (1H, m, H-6), 4.16 (1H, m, H-3), 4.06 (1H, m, H-5), 3.89–3.83 (2H, m, H-4, H-6′), 1.98 (1H, OH), 1.87–1.38 (20H, m, cyclohexylidene). *Anal.* Found: C, 63.51; H, 8.30%. Calcd. for $C_{18}H_{28}O_6$: C, 63.50; H, 8.30%.

3-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl) -1,2:5,6-di-O-cyclohexylidene-α-D-galactofuranose (6). (a) To a solution of **3** (7.56 g, 22.2 mmol) and phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**5**; 13.3 g, 21.1 mmol) and 4A molecular sieves (6.20 g) in dichloromethane (100 ml) were added NIS (11.2 g, 49.8 mmol) and a few drops of TfOH [1.56 g in CH_2Cl_2 (50 ml)] at −45°C under Ar. The mixture was stirred for 10 min at −45°C, $CHCl_3$ (100 ml) was added, and the final mixture successively washed with aq. 10% $Na_2S_2O_3$ (20 ml × 2) and sat. aq. $NaHCO_3$ (20 ml). The organic layer was concentrated *in vacuo* to give a syrup. This syrup was chromatographed over silicic acid (hexane:ethyl acetate = 10:1, v/v) to give white powder (α:β = 10:1) of mixed disaccharides (14.8 g, 82%). This mixture was separated by medium-pressure silica gel chromatography (toluene:ether = 20:1, v/v) to give the β-isomer (7:1, 19 g, 7%) as the first eluting compound and then the α-isomer (**6**; 12.41 g, 69%). **6**: mp 112–114°C, $[\alpha]_D$ +37.1° (c = 1.06, $CHCl_3$); ^{1}H-NMR

(ppm) δ; 7.40–7.24 (20H, m, Ph), 5.83 (1H, d, J= 4.0 Hz, H-1), 4.95 (1H, d, J=11.5 Hz, benzyl-H), 4.95 (1H, d, J=3.5 Hz, H-1′), 4.82 (1H, d, b J= 12.0 Hz, benzyl-H), 4.80 (1H, d, J=12.0 Hz, benzyl-H), 4.73 (1H, d, J=11.5 Hz, benzyl-H), 4.66 (1H, m, H-2), 4.63 (1H, d, J=12.0 Hz, benzyl-H), 4.57 (1H, d, J=11.5 Hz, benzyl-H), 4.46 (1H, d, J=12.0 Hz, benzyl-H), 4.41 (1H, d, J=11.5 Hz, benzyl-H), 4.26 (1H, m, H-6), 4.04 (1H, m, H-6), 4.01 (1H, m, H-3), 3.98–3.89 (4H, m, H-4,5, H-4′,5′), 3.88 (1H, m, H-3′), 3.76 (2H, m, H-6′), 1.81–1.31 (20H, m, cyclohexylidene). *Anal.* Found: C, 72.36; H, 7.23%. Calcd. for $C_{52}H_{62}O_{11}$; C, 72.37; H, 7.24%. β-Isomer (**7**): mp 142–144°C, $[\alpha]_D$ −21.7° (c=1.04, $CHCl_3$); ^{1}H-NMR (ppm) δ; 7.36–7.26 (20H, m, Ph), 5.75 (1H, d, J=4.0 Hz, H-1), 4.92 (1H, d, J=12.0 Hz, benzyl-H), 4.79 (1H, d, J=11.0 Hz, benzyl-H), 4.77 (1H, d, J=11.0 Hz, benzyl-H), 4.73 (1H, d, J=11.0 Hz, benzyl-H), 4.71 (1H, d, J=12.0 Hz, benzyl-H), 4.62 (1H, d, J=12.0 Hz, benzyl-H), 4.55 (1H, m, H-2), 4.45 (1H, d, J=8.0 Hz, H-1′), 4.43 (1H, d, J= 13.0 Hz, benzyl-H), 4.40 (1H, d, J=13.0 Hz, benzyl-H), 4.31 (1H, m, H-6), 4.10 (1H, m, H-3), 3.98–3.89 (4H, m, H-4, H-5′), 3.89 (1H, m, H-4′), 3.84–3.76 (2H, m, H-5, H-2′), 3.53–3.50 (4H, m, H-3′, H-6, H-6′), 1.80–1.36 (2H, m, H-2′, H-5), 3.53–3.50 (4H, m, H-3′, H-6, H-6′), 1.80–1.36 (20H, m, cyclohexylidene). *Anal.* Found: C, 72.37; H, 7.24%. Calcd. for $C_{52}H_{62}O_{11}$: C, 72.37; H, 7.24%. (b) *Lemiex's α-galactosidation.* 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (**8**: 6 g, 10 mmol) was dissolved in dichloromethane (30 ml), and to this solution were added TBAB (2.1 g, 10 mmol), diisoproplethylamine (1.7 g, 10 mmol), and **2** (2.9 g, 11 mmol). The mixture was stirred until becoming homogeneous and kept for **2** days at rt. The solution was then diluted with dichloromethane (50 ml) and successively washed with water, dilute hydrochloric acid and water. After being dried over $MgSO_4$, the solution was concentrated to dryness. The resulting oil was applied to silica gel column chromatography, using a mixture of hexane and ethyl ether (3:1, v/v), to give crystalline **6** (5.7 g, 65%) as the sole product.

3-O-α-D-Galactopyranosyl-1,2:5,6-di-O-cyclohexylidene-α-D-galactofuranose (9). A mixture of **6** (5 g, 5.8 mmol) and 10% Pd-C (1 g) in methanol (20 ml) was stirred in an H_2 atmosphere for 48 hr. The catalyst was removed by filtration, and the filtrate was concentrated to give a syrup. This syrup was purified by silica gel column chromatography ($CHCl_3$:MeOH=30:1, v/v) to give crystalline **9** (2.33 g, 80%), mp 84–85°C, $[\alpha]_D$ +77.8° (c=0.74, $CHCl_3$); ^{1}H-NMR (ppm) δ: 5.80 (1H, d, J=3.0 Hz, H-1), 5.11 (1H, d, J=3.5 Hz, H-1′), 4.66 (1H, m, H-2), 4.40 (1H, m, H-5), 4.09 (1H, m, H-3′), 4.05 (1H, m, H-6), 4.04–3.98 (2H, m, H-3, H6), 2.74 (1H, OH), 2.60 (1H, OH), 2.49 (1H, OH), 1.93 (1H, OH), 1.84–1.34 (20H, m, cyclohexylidene). *Anal.* Found: C, 57.15; H, 7.76%. Calcd. for $C_{24}H_{38}O_{11}$: C, 57.36; H, 7.62%.

3-O-α-D-Galactopyranosyl-α-D-galactopyranose (1). A solution of **9** (1 g, 2.28 mmol) in 10% aq. trifluoroacetic acid (10 ml) was kept at room temperature for 30 min, before being rapidly concentrated *in vacuo* to a syrup. Water was added to this syrup, and the resulting solution was extracted with $CHCl_3$ (15 ml×3). The aqueous layer was submitted to active carbon column chromatography. Elution with 10 % EtOH gave the titled compound (432 mg, 70%) as an amorphous powder, $[\alpha]_D$ +170° (c=0.45, H_2O), ^{1}H-NMR in D_2O displayed a broad band for 12 protons in the range 4.25–3.61, a doublet signal for one proton at 5.30 (J=3.6 Hz), and doublet signals at 5.18 (J=3.6 Hz) and 4.65 (J=7.8 Hz) with a total intensity for one hydrogen. FAB-HRMS m/z (M^+): Calcd. as 342.1162; ($[M+H]^+$), 343.1240; found, 343.1242.

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