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Chemical Modification of Lactose. IX.[1] Synthesis of O-β-D-Galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6′-Galactosyllactose)

Tai Gi Chung, Hideko Ishihara, and Setsuzo Tejima

Faculty of Pharmaceutical Sciences, Nagoya City University[2]

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The title trisaccharide was synthesized from 1,6-anhydro-β-lactose.

Condensation of 2,2′,3,3′-tetra-O-acetyl-1,6-anhydro-β-lactose and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide by a modified Koenigs-Knorr reaction followed by acetylation gave acetylated 1,6-anhydro-β-trisaccharide (3) in 62% yield. The β-configuration for the new galactosidic linkage was obtained from the value of the molecular rotation of 3. Deacetylation of 3 afforded 1,6-anhydro-β-trisaccharide (4). The structure was determined by a gas chromatographic (GC) analysis and a gas chromatography–mass spectrometry of acid hydrolysate of permethylated 4.

Acetolysis of 3 under mild condition proceeded cleavage of the 1,6-anhydro-β-ring and afforded acetylated trisaccharide contaminated with acetylated galactose and lactose. From the acetolysis mixture, the title trisaccharide was isolated *via* acetylated β-trisaccharide followed by deacetylation. The structure was determined by GC analysis of the partial methylated alditol acetates obtained from the hydrolysate of permethylated, borohydride reduced trisaccharide.

Keywords——trisaccharide in human milk; modified Koenigs-Knorr reaction; 1,6-anhydro-β-lactose; molecular rotation; methylation analysis; GC-MS; 6′-galactosyllactose

The title trisaccharide is the main oligosaccharide synthesized enzymatically from lactose by the transgalactosylase of *Penicillium chrysogenum* Thom.[3] More recently, Yamashita and Kobata[4] isolated the trisaccharide from human milk of "nonsecretor". They showed that it was synthesized by a specific galactosyltransferase from UDP-galactose and lactose, and not by the action of β-galactosidase on lactose.

Among many oligosaccharides in human milk, 6′-galactosyllactose has the simplest structure except lactose. Beith-Halahmi *et al.*[5] synthesized 3′-galactosyllactose from benzyl β-lactoside during the course of a study of complex glycosphingolipids. But 6′-galactosyllactose has not been chemically synthesized. Thus, as the first approach of chemical syntheses of higher oligosaccharides in human milk, we synthesized the title trisaccharide from 1,6-anhydro-β-lactose. In this paper, we report the results in full detail.

Debenzylidenation of 2,2′,3,3′-tetra-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-β-lactose[6] by hydrogenolysis on palladium black afforded crystalline 2,2′,3,3′-tetra-O-acetyl-1,6-anhydro-β-lactose (**1**) in 86% yield. The nuclear magnetic resonance (NMR) spectrum and elemental analysis were in good agreement with the structure.

Condensation of **1** and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (**2**) was carried out by a modified Koenigs-Knorr reaction, and the product was isolated as crystalline acetate (**3**) in 62% overall yield from **1**. Namely, a mixture of **1** (1 mol) and **2** (1.5 mol) in nitromethane was stirred in the presence of mercuric cyanide and Drierite at room temperature for 24 hours.

1) Part VIII: T. Takamura and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **26**, 1117 (1978).
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) *a*) A. Ballio and S. Russi, *Tetrahedron*, **9**, 125 (1960); *b*) *Idem, J. Chromatogr.*, **4**, 117 (1960).
4) K. Yamashita and A. Kobata, *Arch. Biochem. Biophys.*, **161**, 164 (1974).
5) D. Beith-Halahmi, H.M. Flowers, and D. Shapiro, *Carbohyd. Res.*, **5**, 25 (1967).
6) T. Chiba, M. Haga, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1283 (1975).

After removal of the inorganic salts, the mixture was evaporated to dryness and, without further purification, acetylated in the usual way. The crude acetate was purified through a column of silica gel. Removal of the solvent from the combined fraction containing a single spot on thin-layer chromatography (TLC) gave an amorphous powder, which was crystallized on treatment with 2-propanol. The NMR spectrum and elemental analysis suggested the composition of acetylated monoanhydro-trisaccharide. Final proof of the structure was obtained by determining the nature of the compound isolated by acid hydrolysis of its fully methylether.

Deacetylation of **3** afforded 1,6-anhydro-β-trisaccharide (**4**) as an amorphous powder. It was dissolved in dimethylsulfoxide (DMSO) and methylated by Hakomori's procedure.[7] The permethylated 1,6-anhydro-β-trisaccharide (**5**) was purified through a column of silica gel to afford a sirup in 96% yield. The NMR spectrum showed five separate singlets due to nine methoxyl protons.

The purified permethylate was hydrolyzed in 5% sulfuric acid at 100° for five hours. The hydrolysate was neutralized and evaporated to dryness, from which the partially methylated alditol acetates were prepared by borohydride reduction followed by acetylation. The product was dissolved in chloroform and subjected to a gas chromatographic (GC) analysis and a gas chromatography–mass spectrometry (GC–MS).

The gas chromatogram of the methylated alditol acetates obtained from the hydrolysate of **5** showed three peaks, (a), (b), and (c), of about equal amount. Peak (a), (b), and (c) were assigned to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol (**6**), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**7**), and 1,4,5,6-tetra-O-acetyl-2,3-di-O-methylglucitol (**8**), respectively, by comparing their retention times and mass spectra with the products of 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-O-methyl-β-lactose (**9**) and 1,6-anhydro-2,3,4-tri-O-methyl-β-D-galactopyranose.[8] The mass spectra of peak (a), (b), and (c) were in good agreement with the reported spectra of partially methylated alditol acetates.[4,9]

The negative value of the optical rotation of **3** and the synthetic route suggested a β-configuration for the new galactosidic linkage in **3**. Further evidence of the β-configuration was obtained from the value of the molecular rotation of **3** (−35,850). The value differed markedly from the value which was calculated for α-1,6-linked galactosyllactose (+25,870) by the summation of the molecular rotations of **1** and methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (see Table I).

Table I. Molecular Rotation of Compound **3** to the Sum of the Molecular Rotations of Constituents

Compound	$[\alpha]_D$ [a]	Mol. wt.	$[M]_D$ (degree) $\times 10^{-2}$
Methyl 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranoside[b]	+133.3°	362	+482.5
Methyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside[b]	−14.5°	362	−52.4
2,2′,3,3′-Tetra-O-acetyl-1,6-anhydro-β-lactose (**1**)	−45.5°	492	−223.8
Compound 3[c]	−41.5°	864	−358.5
1+Methyl 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranoside			+258.7
1+Methyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside			−276.2

a) Optical rotations determined in chloroform.

b) J. Swiderski and A. Temeriusz, *Carbohyd. Res.*, **3**, 225 (1966).

c) O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose

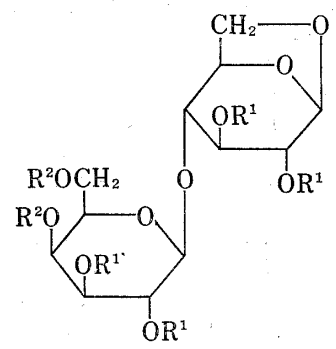
7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
8) D. McCreath, F. Smith, E.G. Cox, and A.I. Wagstaff, *J. Chem. Soc.*, **1939**, 387.
9) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. Res.*, **5**, 433 (1967).

Therefore, the structures of **3**, **4**, and **5** were assigned to O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (**3**), O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (**4**), and O-(2,3,4,6-tetra-O-methyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-methyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (**5**), respectively.

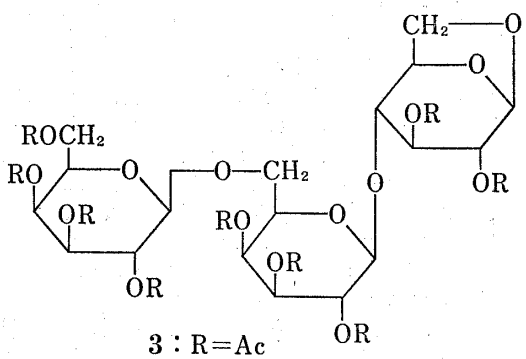
In the preparation of **3**, although **1** contains two unblocked hydroxyl groups at C-4′ and C-6′ positions, condensation of galactopyranosyl group occurred predominantly on the C-6′ position. The result is attributed to differential reactivity between primary and secondary hydroxyl groups and the low reactivity of the C-4 hydroxyl group in hexopyranose.[10]

Acetolysis of **3** at room temperature for two hours proceeded cleavage of the 1,6-anhydro-β-ring to give acetylated α-trisaccharide, which was contaminated with a small amount of acetylated galactose and lactose as indicated by paper partition chromatography (PPC) of deacetylated product. Isolation of the acetylated α-trisaccharide through a column of silica gel failed. After deacetylation, the free trisaccharide was reacetylated with sodium acetate and acetic anhydride at 100° for three hours. Purification through a column of silica gel gave acetylated β-trisaccharide (**10**) as an amorphous powder in 56% overall yield from **3**. In the NMR spectrum of **10**, was observed the signal due to C-1 proton of the reducing terminal (δ 5.68, 1H, d, $J_{1,2}$=8 Hz).

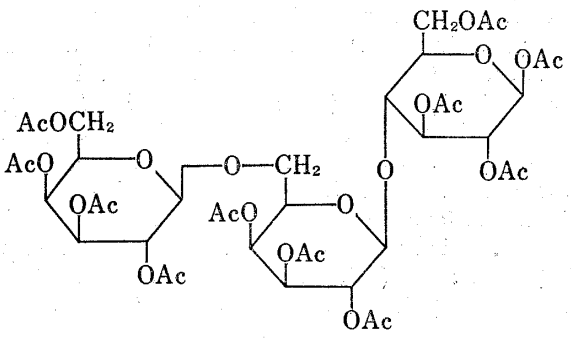
Deacetylation of **10** yielded the title trisaccharide (**11**) as a white powder in 87% yield. The value of specific rotation, $[\alpha]_D^{22}$ +36°, was in good agreement with the reported value,[3a] $[\alpha]_D^{20}$ +34°. Complete acid hydrolysis resulted in the liberation of galactose and glucose as indicated by PPC. PPC of the product of partial acid hydrolysis showed, besides galactose and glucose, two disaccharides, lactose and 6-O-β-D-galactopyranosyl-D-galactopyranose.



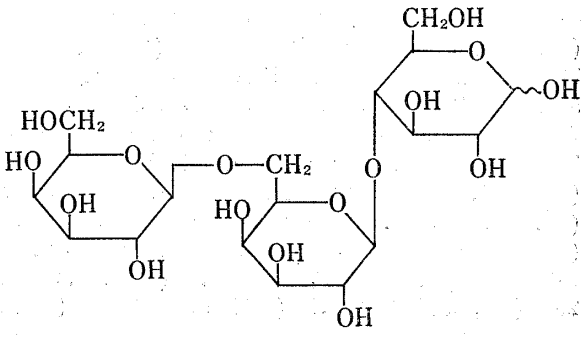
1 : R^1=Ac, R^2=H
9 : R^1=R^2=Me



3 : R=Ac
4 : R=H
5 : R=Me



10



11

Chart 1

10) A.H. Haines, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 33, Academic Press, New York, San Francisco, and London, 1976, p. 11.

Final proof of the structure of **11** was obtained by GC analysis of the partially methylated alditol acetates obtained from the hydrolysate of permethylated, borohydride reduced **11**. The gas chromatogram showed three peaks, (a), (b), and (d), of about equal amount. Peak (a) and (b) were assigned to **6** and **7**, respectively, by comparing their retention times with the methylated alditol acetates obtained from acid hydrolysate of **5**. Peak (d) was assigned to 4-O-acetyl-1,2,3,5,6-penta-O-methylglucitol (**12**) by comparing its retention time with the products of lactose.

Our synthetic trisaccharide (**11**) gave crystalline phenylosazone, mp 230—232° (dec.). The value was in good agreement with the reported datum,[3a] mp 229—231°.

Experimental

Melting points were determined by a Yanagimoto micro melting point apparatus and uncorrected. Solutions were evaporated in a rotary evaporator below 40° under vacuum unless otherwise indicated. Optical rotations were measured with a Unikon Giken Co., Ltd., automatic digital polarimeter, Model PM-201, in a 0.5 dm tube. Infrared (IR) spectra were measured with a Jasco Model IRA-2 spectrometer. NMR spectra were recorded at 100 MHz with a Jeol Model JNM-MH-100 spectrometer. Tetramethylsilane was used as the internal standard in $CDCl_3$. Chemical shifts were given in the δ scale.

A Shimadzu gas chromatograph, Model GC-4BPTF, equipped with a hydrogen flame ionization detector, was employed for GC analysis. The conditions were as follows: column: a glass column (2m×4 mm) packed with Gas-Chrom Q (100—120 mesh), coated with 3% ECNSS-M; column temp., 190°; carrier gas; N_2, flow rate 60 ml/min. Retention times (t_R)were given in min. scale.

Mass spectra of partially methylated alditol acetates were measured with a Hitachi M-52 GC-MS spectrometer with a glass column (2 m×3 mm) packed with Gas-Chrom Q (80—100 mesh), coated with 2% OV-17. Conditions for recording mass spectra were as follows: ionization potential, 20 eV; ion source temp., 170°; separation temp., 160°.

TLC on Kieselgel GF_{254} (5×20 cm) (E. Merck, Darmstadt, Germany) was performed with solvent combination (v/v): (A), CH_2Cl_2-acetone (6: 1); (B), CH_2Cl_2–acetone (5: 2), (C), 70% 2-PrOH–AcOEt (2: 1). Detection was effected with H_2SO_4 or UV light (short wave length). Column chromatography was performed on a column of Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka) as the adsorbent, with 1 g of a sample to be separated per 20 g of adsorbent. Solvent combination of eluent was indicated by v/v. PPC was performed on Toyo Filter Paper No. 50 (Toyo Roshi Kaisha, Ltd., Tokyo) by the ascending method[11] with BuOH–pyridine–H_2O (6: 4: 3, v/v) and detection was effected with alkaline silver nitrate.[12]

2,2′,3,3′-Tetra-O-acetyl-1,6-anhydro-β-lactose (**1**)—A suspension of 2,2′,3,3′-tetra-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-β-lactose[6] (10 g, 17.2 mmol) in MeOH (150 ml) was hydrogenated over Pd catalyst at room temperature under atmospheric pressure until absorption of H_2 ceased: the Pd catalyst was freshly prepared from $PdCl_2$ (2.5 g) according to the method of Schmidt and Staab.[13] After removal of the catalyst, the filtrate was evaporated to dryness. Recrystallization from 2-PrOH gave white needles (7.3 g. 86%), mp 166—168°, $[\alpha]_D^{22}$ −45.5° (c=1.12, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3460 (OH); NMR ($CDCl_3$): 2.05, 2.09, 2.11, 2.15 (12H, all s, 4 OAc), 3.18 (2H, s, disappeared with D_2O); TLC: Rf 0.27 (solvent B). *Anal.* Calcd. for $C_{20}H_{28}O_{14}$: C, 48.78; H, 5.73. Found: C, 48.61; H, 5.76.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (**3**)——To a solution of **1** (2 g, 4.1 mmol) and **2**[14] (2.5 g, 6.1 mmol) in dry nitromethane (40 ml), mercuric cyanide (4 g, 15.8 mmol) and Drierite (4 g,) were added. The mixture was stirred, with exclusion of light and moisture, at room temperature for 24 hr. It was filtered, and the filtrate was evaporated to a sirup, which was acetylated with Ac_2O (20 ml) and pyridine (20 ml). After storage at room temperature overnight, the mixture was poured into ice-H_2O, and extracted with CH_2Cl_2. The combined extract was successively washed with H_2O, dil. H_2SO_4, H_2O, satd. $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated to dryness. It was purified through a column of silica gel with CH_2Cl_2–acetone (10: 1) as eluent. Removal of the solvent from the combined fractions containing a single spot on TLC (Rf 0.32, solvent A) gave an amorphous powder (2.16 g, 62%), which was crystallized on treatment with 2-PrOH, mp 105—107°, $[\alpha]_D^{21}$ −41.5° (c=2.31, $CHCl_3$); NMR ($CDCl_3$): 1.98 (6H, s, 2 OAc), 2.04 (3H, s, OAc), 2.07 (6H, s, 2 OAc), 2.13 (3H, s, OAc,) 2.16 (9H, s, 3 OAc); TLC: Rf 0.32 (solvent A). *Anal.* Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59. Found: C, 49.86; H, 5.88.

11) M. Ueda, *Yakugaku Zasshi*, **90**, 1322 (1970).
12) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).
13) O.Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 388 (1954).
14) H. Ohle, W. Marecek, and W. Bourjau, *Chem. Ber.*, **62**, 833 (1929).

O-β-D-Galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (4)——To a solution of 3 (1.22 g, 1.4 mmol) in dry MeOH (20 ml) was added methanolic 0.1 N sodium methoxide (0.5 ml). The mixture was stirred at room temperature for 4 hr. Deacetylation was complete as indicated by TLC (solvent C). The solution was neutralized with dry Amberlite IR-120 (H^+) resin, and filtered. Evaporation of the filtrate gave **4** (630 mg, 92%) as an amorphous powder, $[\alpha]_D^{19}$ −26.2° (c=1.19, H_2O); TLC: *Rf* 0.27 (solvent C). Compound **4** was negative to the Fehling's test. *Anal.* Calcd. for $C_{18}H_{30}O_{15}\cdot 3/2\ H_2O$: C, 42.10; H, 6.47. Found: C, 42.00; H, 6.26.

O-(2,3,4,6-Tetra-O-methyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-methyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (5)——A suspension of NaH (500 mg, defatted with *n*-hexane beforehand) in dry DMSO (4 ml) was stirred at 50° for 2 hr under N_2 to furnish the solution of dimsyl carbanion. To a solution of **4** (250 mg, 0.51 mmol) in dry DMSO (3 ml) was added above prepared carbanion. The mixture was kept stirring under N_2 for 2 hr, treated with CH_3I (5 ml), and stirred for further 15 hr in the dark. After removal of DMSO under vacuum, the residue was purified through a column of silica gel with CH_2Cl_2–acetone (9:1) as eluent to afford a sirup (302 mg, 96%). $[\alpha]_D^{21}$ −59.9° (c=1.14, $CHCl_3$); NMR ($CDCl_3$): 3.32, 3.36, 3.47, 3.50, 3.57 (27H, all s, 9 OMe), 5.32 (1H, s, H-1); TLC: *Rf* 0.46 (solvent B). *Anal.* Calcd. for $C_{27}H_{48}O_{15}$: C, 52.93; H, 7.89. Found: C, 52,74; H, 8.05.

GC Analysis of Acid Hydrolysate of 5——A mixture of **5** (9 mg) and 5% H_2SO_4 (3 ml) was heated at 100° for 5 hr under stirring. The hydrolysate was neutralized with $BaCO_3$, filtered on Ceilite, and evaporated to dryness. The residue in H_2O (2 ml) was hydrogenated with $NaBH_4$ (20 mg) in H_2O (2 ml) at room temperature for 3 hr. Excess $NaBH_4$ was destroyed with Amberlite IR-120 (H^+) resin and, after removal of the resin, boric acid was removed by repeated evaporation with MeOH. The residue was acetylated with Ac_2O (2 ml) and pyridine (2 ml), and the mixture was left overnight. It was evaporated by repeated co-distillation with EtOH and toluene to afford a sirup, which was dissolved in $CHCl_3$ and subjected to GC analysis. The product gave three peaks of methylated alditol acetates (a), (b), and (c). They were assigned to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol (**6**), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**7**), and 1,4,5,6-tetra-O-acetyl-2,3,-di-O-methylglucitol (**8**), respectively, by comparing their retention times and mass spectra with the products of **9** and 1,6-anhydro-2,3,4-tri-O-methyl-β-D-galactopyranose.[8] t_R: (a)=7.76; (b)=20.00; (c)=31.16; **6**=7.74; **7**=20.05; **8**=30.52.

GC-MS of Acid Hydrolysate of 5——Compound **5** was treated as for GC analysis to prepare a sample of GC–MS, which was subjected to GC–MS. MS *m/e* (%) of methylated alditol acetates were as follows; (a): 43 (100), 45 (97.3), 71 (86.6), 87 (98), 101 (98), 117 (98.6), 129 (98.6), 145 (98), 161 (97.3), 205 (97.3); (b): 43 (100), 87 (96.6), 99 (98.6), 101 (98.6), 117 (98.6), 129 (98), 161 (94.6), 189 (94.6); (c): 43 (100), 101 (34.9), 117 (100), 261 (44.3).

GC-MS of 1,5,6-Tri-O-acetyl-2,3,4-tri-O-methylgalactitol (7)——Acid hydrolysis, borohydride reduction and acetylation of 1,6-anhydro-2,3,4-tri-O-methyl-β-D-galactopyranose[8] were performed as for **5** to prepare **7**. MS *m/e* (%): 43 (100), 87 (78.6), 99 (98.6), 101 (98.6), 101 (98.6), 117 (100), 129 (98), 161 (69.3), 189 (66.6).

1,6-Anhydro-2,2′,3,3′,4′,6′,-hexa-O-methyl-β-lactose (9)——Methylation of 1,6-anhydro-β-lactose (500 mg, 1.54 mmol) as for **5** afforded **9** (518 mg, 82%) as a colorless powder, $[\alpha]_D^{22}$ −69.5° (c=1.59, $CHCl_3$); NMR ($CDCl_3$): 3.32, 3.38, 3.48, 3.51, 3.57 (18H, all s, 6 OMe), 5.32 (1H, s, H-1); TLC: *Rf* 0.26 (solvent B). *Anal.* Calcd. for $C_{18}H_{32}O_{10}$: C, 52.93; H, 7.89. Found: C, 53.10; H, 8.08.

GC Analysis of Acid Hydrolysate of 9——Acid hydrolysis of **9**, borohydride reduction, and acetylation were carried out as for **5** to give methylated alditol acetates, which were subjected to GC analysis under the same conditions as for acid hydrolysate of **5**. The product gave two peaks, (a) and (c), of about equal amount. Peak (a) and (c) were assigned to **6** and **8**, respectively, by comparing the recorded retention times[15] and mass spectra. t_R: (a)=7.74, (c)=30.52.

GC-MS of Acid Hydrolysate of 9——MS *m/e* (%) of methylated alditiol acetates were as follows; (a): 43 (100), 45 (70.4), 71 (60.1), 87 (89.4), 101 (98.6), 117 (98.7), 129 (97.4), 145 (97.4), 161 (97.4), 205 (96.1); (c): 43 (100), 101 (96.7), 117 (98), 261 (84.8).

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranose (10)——Compound **3** (2.75 g, 3.2 mmol) was dissolved in acetolysis mixture (20 ml, 1:70:30, v/v, H_2SO_4–Ac_2O–AcOH). After keeping at room temperature for 2 hr, it was poured into ice-H_2O and extracted with CH_2Cl_2. The combined extract was washed with H_2O, satd. $NaHCO_3$, H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was deacetylated with 0.1 N sodium methoxide (0.5 ml) in MeOH (30 ml) as for **3** to afford an amorphous powder (1.5 g). It was reacetylated with anhyd. AcONa (1.5 g) and Ac_2O (15 ml) at 100° under stirring for 3 hr. The mixture was poured into ice-H_2O and treated as the acetolysis mixture mentioned above to give an amorphous powder (2.5 g). The product was purified through a column of silica gel with CH_2Cl_2–acetone (15:1) as eluent. Removal of the solvent from the fraction containing a single spot on TLC (*Rf* 0.40, solvent A) gave an amorphous powder (1.72 g, 56%), $[\alpha]_D^{20}$ −9.7° (c=1.17, $CHCl_3$); NMR ($CDCl_3$): 1.94, 1.97, 2.00 (9H, all s, 3 OAc), 2.05 (9H, s, 3 OAc), 2.08 (6H, s, 2 OAc), 2.13 (6H, s, 2 OAc), 2.15 (3H, s, OAc), 5.68 (1H, d, $J_{1,2}$=8 Hz, H-1); TLC: *Rf* 0.40 (solvent A). *Anal.* Calcd. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.62. Found: C, 49.45; H, 5.41.

15) H. Björndal, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, **21**, 1801 (1967).

O-β-D-Galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (11)——To a solution of **10** (260 mg, 2.69 mmol) in dry MeOH (4 ml) was added methanolic 0.1 N sodium methoxide (0.1 ml). After the mixture was stirred at room temperature for 4 hr, it was neutralized with dry Amberlite IR-120 (H^+) resin. Removal of the resin and solvent gave **11** (118 mg, 87%) as a white powder. It was purified by precipitation from MeOH–acetone, $[\alpha]_D^{22}+36°$ ($c=1.15$, H_2O) (lit.[3a] $[\alpha]_D^{20}+34°$ ($c=1$, H_2O)); TLC: *Rf* 0.19 (solvent C); PPC: *Rf* 0.13. *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot 1/2H_2O$: C, 42.10; H, 6.47. Found: C, 41.85; H, 6.52.

PPC of Acid Hydrolysate of 11——1) Complete Hydrolysis: Compound **11** (5 mg) was heated with 1 N HCl (2 ml) in a sealed tube at 100° for 3 hr. Removal of HCl by repeated co-distillation with EtOH gave hydrolysate, in which glucose (*Rf* 0.39) and galactose (*Rf* 0.35) were identified by PPC.

2) Partial Hydrolysis: A mixture of **11** (5 mg) and 0.1 N HCl (2 ml) was treated as 1) to afford hydrolysate, in which glucose, galactose, lactose (*Rf* 0.24), O-β-D-galactopyranosyl-(1→6)-D-galactopyranose (*Rf* 0.18), and **11** were identified by PPC.

GC Analysis of Acid Hydrolysate of Permethylated, Borohydride Reduced 11——Compound **11** (15 mg) in H_2O (2 ml) was hydrogenated with $NaBH_4$ (45 mg) in H_2O (2 ml) at room temperature for 3 hr. Excess $NaBH_4$ was destroyed with Amberlite IR-120 (H^+) resin and, after removal of the resin, boric acid was removed by co-distillation with MeOH. The residue was dissolved in dry DMSO (1 ml) and methylated by the same procedure as for **5**. The permethylated product was purified by preparative TLC on a silica gel plate with solvent C (*Rf* 0.59).

The purified product was hydrolyzed in 5% H_2SO_4 (6 ml) at 100° for 5 hr. It was neutralized with $BaCO_3$, filtered, and the filtrate was evaporated to dryness. The residue was dissolved in H_2O (2 ml), and hydrogenated with $NaBH_4$ (25 mg) in H_2O (2 ml) at room temperature for 3 hr. After destruction of excess $NaBH_4$ and removal of boric acid, the residue was acetylated with Ac_2O (2 ml) and pyridine (2 ml). After evaporation to dryness, the residue was dissolved in $CHCl_3$ and subjected to GC analysis.

The gas chromatogram gave three peaks, (a), (b), and (d), of about equal amount. Peak (a) and (b) were identified to **6** and **7**, respectively, by comparing their retention times with authentic sample. Peak (d) was assigned to 4-O-acetyl-1,2,3,5,6-penta-O-methylglucitol (**12**) by comparing its retention time with the product of lactose. t_R: (a)=7.45; (b)=19.46; (d)=2.59; **6**=7.49; **7**=19.44; **12**=2.59.

O-β-D-Galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose Phenylosazone——A mixture of **11** (150 mg, 0.29 mmol), phenylhydrazine hydrochloride (300 mg, 2.1 mmol), and AcONa (600 mg, 7.3 mmol) in H_2O (3 ml) was heated at 100° under stirring for 30 min. After cooling, yellow needles appeared within few min. The product was collected by filtration, washed repeatedly with EtOH, and air-dried. Yellow needles (65 mg, 32%), mp 230—232° (dec.) (lit.[3a] mp 229—231°), TLC: *Rf* 0.74 (solvent C). *Anal.* Calcd. for $C_{30}H_{42}O_{14}N_4\cdot 2H_2O$: C, 50.13; H, 6.45; N, 7.79. Found: C, 50.33; H, 6.36; N, 7.77.

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