[Chem. Pharm. Bull. 25(3) 464—470 (1977)]

UDC 547.458.02.04.543.422.25

Synthesis of O-α- and O-β-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranoses

CHUNG TAI GI and SETSUZO TEJIMA

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

(Received June 5, 1976)

The title new reducing trisaccharides were synthesized starting from 1,6-anhydro-β-maltose.

Reflux of 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-β-maltose with 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride in benzene in the presence of mercuric cyanide and Drierite stereospecifically afforded the corresponding trisaccharide derivative having α-D-(1→6) galactosidic linkage (yield 63%). After debenzylation with subsequent acetylation, acetolysis of the β-1,6-anhydro linkage, and deacetylation, the former title trisaccharide was obtained.

Stirring of 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-6′-O-trityl-β-maltose with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide in nitromethane in the presence of silver perchlorate and Drierite, followed by acetylation stereospecifically yielded the corresponding trisaccharide derivative having β-D-(1→6) galactosidic linkage (yield 56%). Acetolysis of the β-1,6-anhydro linkage, followed by deacetylation gave the latter title trisaccharide.

Keywords——new reducing trisaccharide; 1,6-anhydro-β-maltose; molecular rotation; methylation analysis; NMR; periodate consumption

Synthesis of reducing trisaccharides having well-defined structures may be still fascinating fundamental research subjects. Because, trisaccharides and their glycosides are not only simplified model compounds of higher oligosaccharides or polysaccharides of biological interest, but also reference compounds of sugar moiety in saponins or glycolipids. Because of the presence of many barriers have to overcome in the synthetic pathway, reducing trisaccharides so far chemically synthesized are not so numerous. Our laboratory has reported the usefulness of 1,6-anhydro-β-derivatives of lactose, maltose, and cellobiose as starting materials for chemical modification of the parent reducing disaccharides.[2] In this paper, we wish to report synthesis of the title new reducing trisaccharides starting from 1,6-anhydro-β-maltose (1).[3]

Selective tritylation with subsequent acetylation of **1** afforded 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-6′-O-trityl-β-maltose (**2**), mp 150—151°, $[\alpha]_D^{24}$ +56.4°, in 47% yield. The optical rotation of our preparation was in agreement with that obtained by Dutton and Slessor,[4] and the elemental analysis and the nuclear magnetic resonance (NMR) spectra confirm the assigned composition. But our melting point was considerably higher. Accordingly, in order to determine the assigned structure, compound (**2**) was introduced to a known 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-6′-O-*p*-toluenesulfonyl-β-maltose[4] and the corresponding 6′-deoxy-6′-iodo derivative.[4] Presumably, the deviation observed in melting point may be attributed to polymorphism.

Brederek, *et al.*[5] have reported that when 1,2,3,4-tetra-O-acetyl-6-O-trityl-β-D-glucopyranose and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide are stirred in nitromethane in the presence of silver perchlorate, the β-D-(1→6) glucosidic linkage is stereospecifically formed to

1) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
2) T. Chiba, M. Haga, and S. Tejima, *Carbohyd. Res.*, **45**, 11 (1975), and Ref. 1 to 7 cited in it.
3) M. Mori, M. Haga, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1480 (1975).
4) G.G.S. Dutton and K.N. Slessor, *Can. J. Chem.*, **44**, 1069 (1966).
5) H. Brederek, A. Wagner, G. Faber, H. Ott, and J. Rauther, *Chem. Ber.*, **92**, 1135 (1959).

afford acetylated gentiobiose in 60% yield. Later, Wolfrom and Koizumi[6] extended the method to the trisaccharide synthesis. The authors also applied the Brederek's method to the trisaccharide synthesis having β-D-(1→6) galactosidic linkage.

When a mixture of an equimolecular amount of **2** with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide[7] in dry nitromethane was stirred in the presence of silver perchlorate and Drierite, the reaction proceeded very fast as monitored with thin-layer chromatography (TLC) on silica gel. After 30 min, the mixture was chromatographed on a silica gel column to isolate four single products. The yield and structural assignment of the isolated products suggested that the major component was the acetylated trisaccharide (**3**) contaminating with fully acetylated **1** and two partly deacetylated saccharides with unknown structures which, by acetylation, yielded **3** and fully acetylated **1**. Therefore, after the glycosidation, subsequent acetylation increased the yield of **3** from 42% to 56%. Compound **3**, isolated by column chromatography and recrystallization from ethanol, had mp 217—218° and $[\alpha]_D^{22}$ +19.7°. The β-D configuration of the non-reducing terminal linkage was strongly suggested by the comparison of the molecular rotation of **3** with those of the parent monosaccharide and disaccharide derivatives (see Table I).

Table I. Molecular Rotations of Compounds (**3** and **12**) Compared to the Sum of the Molecular Rotations of the 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′,4′-Penta-O-acetyl-1,6-anhydro-β-maltose (**9**)	+43.4°	534	+231.7
Compound (**3**)	+19.7°	864	+170
Methyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside+ Compound (**9**)			+179
Compound (**12**)	+83.1°	864	+718
Methyl 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranoside+ Compound (**9**)			+714

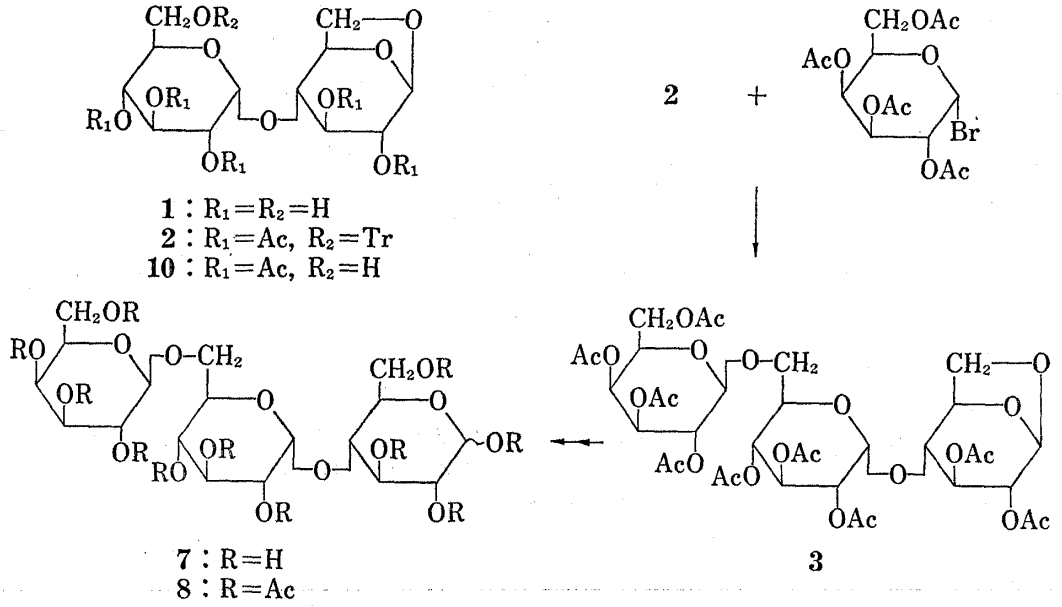
a) optical rotations determined in chloroform
b) J. Świderski and A. Temeriusz, *Carbohyd. Res.*, **3**, 225 (1966)

Further structural assignments of **3** were as follows. 1) Deacetylation of **3** afforded 1,6-anhydro-β-trisaccharide (**4**) as an amorphous powder. Compound (**4**) consumed 4.5 moles of sodium metaperiodate with concomitant formation of 1.73 moles of formic acid for 48 hr, in agreement with the structure posturated. 2) Methylation of **4** with the Hakomori's method[8] gave nona-O-methyl ether (**5**) as an amorphous powder. Acid hydrolysis of **5** yielded 2,3-di-O-methylglucose, 2,3,4-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylgalactose which were identified with the authentic specimens by paper partition chromatography (PPC). This methylation analysis eliminated the possibility of acetylmigration encountered in the synthesis of **3**.

Acetolysis of **3** for 2 hr at room temperature proceeded cleavage of the 1,6-anhydro-β-ring to give crude acetylated trisaccharide (**6**). In this stage, the authors encountered the difficulties in purifying **6**. Therefore, without further purification, **6** was deacetylated to yield trisaccharide (**7**) contaminated with a small amount of galactose and maltose, identified with PPC. According to the literature, disaccharides or polysaccharides containing (1→6) linkage are

6) M.L. Wolfrom and K. Koizumi, *J. Org. Chem.*, **32**, 656 (1967).
7) H. Ohle, W. Marecek, and W. Bourjau, *Chem. Ber.*, **62**, 833 (1929).
8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

extremely labile to acetolysis.[9] Therefore, it is reasonable to notice that only the terminal (1→6) linkage was hydrolyzed to isolate galactose and maltose. After column chromatography on charcoal using aqueous alcohol as the eluant,[10] pure **7** was isolated as an amorphous powder, $[\alpha]_D^{22}$ +85° (in water). The product showed the elemental analysis for such a formulation with 2 moles of water and was cleaved by acid to glucose and galactose, identified by PPC.



Ac=acetyl
Tr=triphenylmethyl

Chart 1

In the next step, synthesis of the trisaccharide having α-D-(1→6) galactosidic linkage is described.

In general, oligosaccharide synthesis having α-D glycosidic linkage is more difficult than that of β. However, according to the recent publication reported by Suami, *et al.*[11] when 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride (**9**)[12] and 1′,2,3,3′,4,4′,6′-hepta-O-acetylsucrose were refluxed in dry benzene in the presence of mercuric cyanide, α-D-(1→6) galactosidic linkage is stereospecifically formed to afford raffinose derivative in 53% yield. The authors extended the method to the α-D-linked trisaccharide synthesis.

2,2′,3,3′,4′-Penta-O-acetyl-1,6-anhydro-β-maltose (**10**), mp 161—162°, $[\alpha]_D^{23}$ +48.5°, was prepared from **2** by the method of Dutton and Slessor.[4] Similarly as pointed out in **2**, the optical rotation of our preparation was almost in agreement with that obtained by the previous authors, but our melting point was considerably higher. Previously, the authors noticed that when crystals having approximately same melting point as reported by Dutton and Slessor were analyzed by NMR spectroscopy, protons assigned to isopropanol, a solvent of recrystallization of **10**, were identified. Therefore, we assume that, presumably, low melting point of the previous authors may be attributed to the presence of the solvent of crystallization.

Reflux of a mixture of **9** (1.3 moles) with **10** (1 mole) in dry benzene in the presence of mercuric cyanide and Drierite for 24 hr, followed by purification on a column chromatography, afforded trisaccharide derivative (**11**), mp 59—60°, $[\alpha]_D^{22}$ +48.2°, in 63% yield. In this glyco-

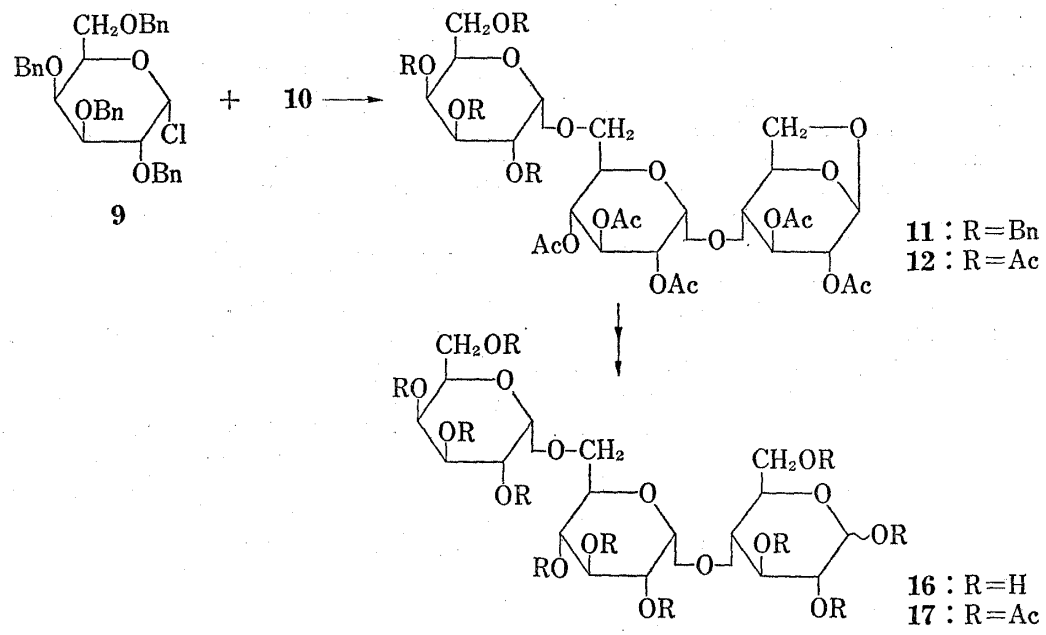
9) V.I. Govorchenko, V.I. Gorbatch, and Yu. S. Ovodov, *Carbohyd. Res.*, **29**, 421 (1973); L. Rosenfeld and C.E. Ballou, *ibid.*, **32**, 287 (1974).
10) R.L. Whistler and D.F. Durso, *J. Am. Chem. Soc.*, **72**, 677 (1950).
11) T. Suami, T. Otake, T. Nishimura, and T. Ikeda, *Carbohyd. Res.*, **26**, 234 (1973).
12) P.W. Austin, F.E. Hardy, J.G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, **1965**, 1419.

sidation, other product could not be identified. According to the paper entitled synthesis of isomaltose and isomaltotriose by benzyl brocking method, reported by Takiura, *et al.*,[13] when 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose and 2-O-benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl chloride are reacted in nitromethane in the presence of mercuric cyanide and Drierite, α-D-(1→6) glucosidic linkage formed predominantly (yield 32%) with concurrent formation of the β-isomer (yield 6%).

Debenzylation and successive acetylation of **11** afforded acetylated 1,6-anhydro-β-trisaccharide (**12**). The comparison of the molecular rotation of **12** with those of the parent monosaccharide and disaccharide derivatives strongly suggested the α-D configuration of the nonreducing terminal linkage of **11** and **12** (see Table I).

Deacetylation of **12** gave 1,6-anhydro-β-trisaccharide (**13**) which consumed 5 moles of sodium metaperiodate with concomitant formation of 1.7 moles of formic acid for 24 hr. Methylation of **13** afforded a sirupy nona-O-methyl ether (**14**) in which, after acid hydrolysis, 2,3-di-O-methylglucose, 2,3,4-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylgalactose were identified by PPC. The result eliminated, similarly to synthesis of the β-D-isomer, the possibility of acetyl migration encountered.

Acetolysis of **12** with subsequent deacetylation gave, after purification of a column chromatography, an amorphous trisaccharide (**16**), $[\alpha]_D^{23}$ +159.2° (in water).



Bn=benzyl

Chart 2

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and uncorrected. Solutions were evaporated in a rotary evaporator below 40° under vacuum. Optical rotations were measured with a Yanagimoto OR-10 automatic polarimeter in a 0.5 dm tube. 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 are given on the δ scale. TLC on Kieselgel GF_{254} (E. Merck, Darmstadt, Germany) activated at 110° was performed with solvent system (A) 9: 1 (v/v) CH_2Cl_2–acetone, (B) 5: 2 CH_2Cl_2–acetone, (C) 5: 1 benzene–AcOEt. Detection was effected with H_2SO_4 or UV light (short wave length). Column chromatography was performed on a column of Wakogel C-200 as the adsorbent, with 1 g of the mixture to

13) K. Takiura, K. Kakehi, and S. Honda, *Chem. Pharm. Bull.* (Tokyo), **21**, 523 (1973).

be separated per 20 g of adsorbent. PPC was performed on a Toyo Filter Paper No. 50 by the ascending method with (A) 6: 4: 3 (v/v) BuOH–pyridine–H_2O, (B) 5: 1: 4 (upper phase) BuOH–EtOH–H_2O, (C) 40: 11: 19 BuOH–EtOH–H_2O, (D) 3: 3: 1 AcOEt–AcOH–H_2O by the procedure of Ueda[14] and detection was effected with (A) alkaline silver nitrate,[15] or aniline hydrogen phthalate.[16]

2,2′,3,3′,4′-Penta-O-acetyl-1,6-anhydro-6′-O-trityl-β-maltose (2)——To a solution of **1** (11.5 g, 35.5 mmole) in dry pyridine (100 ml) was added trityl chloride (14.9 g, 55.2 mmole) with exclusion of moisture. The mixture was stirred for 4 hr at room temperature and then left for 15 hr. After cooling at 0°, Ac_2O (100 ml) was added under stirring, the mixture was left overnight, and then poured into ice-water (2 liters). The resulting precipitate was collected by filtration, dried, and dissolved in CH_2Cl_2. The solution was washed with H_2O, satd. $NaHCO_3$, and H_2O, dried ($CaCl_2$), and evaporated to dryness. The resulting sirup was chromatographed on a silica gel column using CH_2Cl_2–acetone (20: 1, v/v) as eluant. Removal of the solvent from the fractions having *Rf* 0.72 on TLC (solvent A), and recrystallization of the resulting residue from EtOH–H_2O (6: 1, v/v) afforded **2** (12.9 g, 47%), mp 150—151°, $[\alpha]_D^{24}$ +56.4° (c=2.26, $CHCl_3$) [lit.[4] mp 101—102°, $[\alpha]_D$ +55° (c=1.55, $CHCl_3$)]; NMR ($CDCl_3$) δ ppm: 1.73, 1.97, 2.07, 2.17 (15H, each s, 5 OAc), 7.15—7.47 (15H, m, aromatic protons). *Anal.* Calcd. for $C_{41}H_{44}O_{15}$: C, 63.40; H, 5.70. Found: C, 63.35; H, 5.76.

Detritylation of **2** with 80% aq. AcOH[4] afforded 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-β-maltose (yield 95%), mp 161—162°, $[\alpha]_D^{23}$ +48.5° (c=0.91, $CHCl_3$). *p*-Toluenesulfonylation[4] of the pentaacetate gave 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-6′-O-*p*-toluenesulfonyl-β-maltose, mp 170—171°, $[\alpha]_D^{22}$ +51.5° (c=0.96, $CHCl_3$) [lit.[4] mp 170—171°, $[\alpha]_D$ +55.7°, (c=1.00, $CHCl_3$)]. Treatment of the tosylate with NaI in boiling acetonitrile for 2 hr yielded 2,2′,3,3′,4′-penta-O-acetyl-1,6-anhydro-6′-deoxy-6′-iodo-β-maltose, mp 194—195°, $[\alpha]_D^{24}$ +39° (c=1.17, $CHCl_3$) [lit,[4] mp 194—195°, $[\alpha]_D$ +42° (c=1.03, $CHCl_3$)].

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (3)——To a solution of silver perchlorate (320 mg, 1.54 mmole) in dry nitromethane (10 ml) were added Drierite (200 mg) and **2** (980 mg, 1.26 mmole) under stirring, and the mixture stirred for 30 min. After cooling at 0°, 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide[7] (520 mg, 1.26 mmole) was added, and the mixture was stirred vigorously with exclusion of moisture, during which the solution turned to orange and then AgBr and trityl perchlorate began to precipitate. Stirring was continued for further 30 min, filtered, and the solid was washed with CH_2Cl_2. The combined filtrate and washings were washed with H_2O, satd. $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was acetylated with Ac_2O (15 ml) and pyridine (15 ml), the mixture was left overnight, poured into ice-water (300 ml), and extracted with CH_2Cl_2 (3×30 ml). The combined extracts were washed with H_2O, dil. H_2SO_4, and satd. $NaHCO_3$, respectively, dried (Na_2SO_4), and removed the solvent to afford a sirup which was chromatographed on a column of silica gel using CH_2Cl_2–acetone (15: 1, v/v) as the eluant. Removal of the solvent from the fractions having *Rf* 0.26 (TLC, solvent A) gave an amorphous powder which crystallized from EtOH. Recrystallization from EtOH gave pure **3** (620 mg, 56%), mp 217—218°, $[\alpha]_D^{22}$ +19.7° (c=0.89, $CHCl_3$); NMR ($CDCl_3$) δ ppm: 1.97, 1.99, 2.04, 2.09, 2.14, 2.17 (27H, each s, 9 OAc); TLC: *Rf* 0.26 (solvent A). *Anal.* Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59. Found: C, 49.76; H, 5.72.

O-β-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (4)——To a solution of **3** (105 mg) in dry MeOH (5 ml) was added methanolic 0.1N sodium methoxide (0.5 ml) at 0°, and the mixture was stirred for 3 hr under cooling; complete deacetylation was monitored by TLC. Dry Amberlite IR-120 (H^+) resin was added for neutralization, filtered, and the filtrate evaporated to dryness. To a solution of the residue in MeOH was added 2 vol. of AcOEt, and the resulting precipitate was collected by filtration. Compound (**4**) (56 mg, 95%), amorphous powder, $[\alpha]_D^{27}$ +40° (c=0.63, H_2O), was negative the Fehling's test.

The $NaIO_4$-consumption (mole)[17] of **4** (122 mg) at room temperature was as follows: 2.40 (30 min), 2.80 (1 hr), 3.30 (2 hr), 3.70 (4 hr), 4.00 (8 hr), 4.30 (24 hr), 4.50 (48 hr, constant) with concomitant formation of 1.73 moles of formic acid for 48 hr.

O-(2,3,4,6-Tetra-O-methyl-β-D-galactopyranosyl-(1→6)-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (5)——A suspension of NaH (1.28 g, defatted with *n*-hexane beforehand) in dry dimethylsulfoxide (DMSO) (15 ml) was heated at 40—50° for 3 hr under N_2 atmosphere to furnish the solution of dimsyl carbanion. To a solution of **4** (completely dried over P_2O_5 under vacuum for 24 hr, 400 mg, 82.3 mmole) in dry DMSO (15 ml) was added the above prepared dimsyl carbanion, and the mixture was kept stirring under N_2 atmosphere for 2 hr, treated with CH_3I (10 ml), and stirred for further 15 hr in the dark. After removal of DMSO under vacuum, the residue was dissolved in CH_2Cl_2, washed with H_2O, aq. $Na_2S_2O_3$, and H_2O, dried (Na_2SO_4), and evaporated to dryness to afford an amorphous powder (470 mg, 93%), $[\alpha]_D^{23}$ +45.2° (c=1.02, $CHCl_3$); TLC: *Rf* 0.37 (solvent B). *Anal.* Calcd. for $C_{27}H_{48}O_{15}$: C, 52.93; H, 7.89. Found: C, 52.92; H, 7.72.

14) M. Ueda, *Yakugaku Zasshi*, **90**, 1322 (1970).
15) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).
16) S.M. Partridge, *Nature*, **164**, 443 (1949).
17) S. Okui, *Yakugaku Zasshi*, **75**, 1216 (1955).

PPC of Acid Hydrolyzate of 5——Authentic 2,3-di-O-methyl-D-glucose[18] (PPC: *Rf* 0.58, solvent B), 2,3,4-tri-O-methyl-D-glucose[19] (*Rf* 0.75, solvent B), and 2,3,4,6-tetra-O-methyl-D-galactose[20] (*Rf* 0.85, solvent B) were prepared according to methods described in the literature.

A mixture of **5** (35 mg) and 5% H_2SO_4 (6 ml) was heated at 95—100° for 6 hr. After neutralization with $BaCO_3$, the filtrate was concentrated to a thin sirup in which 2,3-di-O-methylglucose, 2,3,4-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylgalactose were identified by PPC with solvent B.

O-β-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose (7)——Compound 3 (430 mg, 4.9 mmole) was dissolved in acetolysis mixture (10 ml, 1: 70: 30, v/v, H_2SO_4–Ac_2O–AcOH). After stirring for 2 hr at room temperature, the solution was poured into ice-water (300 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with H_2O, satd. $NaHCO_3$, and H_2O, dried (Na_2SO_4), and removed the solvent to afford an amorphous powder. The crude material was purified by dissolving it in EtOH, followed by addition of H_2O to yield O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-α-D-glucopyranose (**6**) (350 mg), $[\alpha]_D^{25}$ +76.5° (*c*=1.32, $CHCl_3$); NMR ($CDCl_3$) δ ppm: 6.24 (1H, d, $J_{1,2}$=4 Hz, H-1).

To a solution of **6** (900 mg) in dry MeOH (20 ml) was added methanolic 0.1 N sodium methoxide (0.5 ml) at 0°. The mixture was similarly treated as for **4** to afford an amorphous powder (440 mg) in which small amounts of galactose and maltose contaminated with **7**, identified by PPC with solvent A. The crude product was chromatographed on a charcoal column (2×30 cm) with H_2O, 2% 5%, and 10% aq. EtOH as eluants. Compound (**7**) was eluted with 10% aq. EtOH. Removal of the solvent from the fractions having a single spot afforded pure **7** (270 mg, 42%), amorphous powder, $[\alpha]_D^{22}$ +85° (*c*=0.76, H_2O); PPC: *Rf* 0.12 (solvent A), 0.08 (solvent C), 0.05 (solvent D). *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot 2H_2O$: C, 40.00; H, 6.71. Found: C, 39.91; H, 6.99.

PPC of Acid Hydrolyzate of 7——A mixture of **7** (10 mg) and 5% H_2SO_4 (5 ml) was treated as described for **5** in which glucose (*Rf* 0.38 with solvent A, 0.29 with solvent C, and 0.27 with solvent D) and galactose (*Rf* 0.33 with solvent A, 0.27 with solvent C, and 0.26 with solvent D) were identified by PPC.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (8)——After acetylation of **7** (200 mg) in pyridine (10 ml) with Ac_2O (10 ml), the solvent was removed by repeated co-distillation with EtOH and toluene to give a sirupy residue which was purified on silica gel column chromatography with CH_2Cl_2–acetone (20: 1, v/v) as eluant to afford an amorphous powder (376 mg, 98%), $[\alpha]_D^{21}$ +67° (*c*=0.80, $CHCl_3$); TLC: *Rf* 0.29 (solvent A). *Anal.* Calcd. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.62. Found: C, 49.40; H, 5.80.

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl Chloride (9)——The product, $[\alpha]_D^{23}$ +131° (*c*=1.85, benzene), was prepared by the method of Austin, *et al.*[12] NMR ($CDCl_3$) δ ppm: 6.13 (1H, d, $J_{1,2}$=3.8 Hz,H-1),7.24—7.31 (20H, m, aromatic protons); lit.[12] $[\alpha]_D^{20}$ +135° (*c*=4.27, benzene); lit.[13] $[\alpha]_D$ +147° (*c*=2.00, benzene).

2,2′,3,3′,4′-Penta-O-acetyl-1,6-anhydro-β-maltose (10)——The product (yield 95%), mp 161—162°, $[\alpha]_D^{23}$ +48.5° (*c*=0.91, $CHCl_3$), was prepared from **2** by the method of Dutton and Slessor.[4] NMR ($CDCl_3$) δ ppm: 2.04, 2.08, 2.12, 2.20 (15H, each s, 5 OAc); lit.[4] mp 82—83°, $[\alpha]_D^{20}$ +43.4° (*c*=2.43, $CHCl_3$).

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (11)——To a solution of **9** (1.59 g, 2.84 mmole) and **10** (1.16 g, 2.18 mmole) in dry benzene (20 ml) were added mercuric cyanide (1.80 g, 7.18 mmole) and Drierite (1.80 g). The mixture was heated under reflux for 24 hr with mechanical stirring and exclusion of moisture, filtered, the filtrate was washed with H_2O, and evaporated to give an amorphous powder which was chromatographed on a silica gel column with benzene–AcOEt (10: 1, v/v) as eluant. Removal of the solvent from the combined fractions containing a single spot on TLC (solvent C) gave a sirup which solidified from petr. ether. The product was collected by filtration to give a white powder (1.45 g, 63%), mp 59—60°, $[\alpha]_D^{22}$ +48.2° (*c*=1.17, $CHCl_3$); NMR ($CDCl_3$) δ ppm: 1.94, 2.00, 2.06, 2.16 (15H, each s, 5 OAc), 7.21—7.30 (20H, m, aromatic protons); TLC: *Rf* 0.11 (solvent C). *Anal.* Calcd. for $C_{56}H_{64}O_{20}$: C, 63.62; H, 6.10. Found: C, 63.46; H, 6.06.

O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (12)——To a solution of **11** (200 mg) in MeOH (15 ml) was hydrogenated over Pd catalyst at room temperature under atmospheric pressure until absorption of hydrogen ceased; the Pd catalyst was freshly prepared from $PdCl_2$ (200 mg) according to the method of Schmidt and Staab.[21] After removal of the catalyst, the filtrate was evaporated to an amorphous powder (127 mg) which was acetylated with Ac_2O (5 ml) in pyridine (5 ml) overnight at 5°. After treatment of the mixture as for **8**, **12** was yielded as an amorphous powder (148 mg, 91%), $[\alpha]_D^{21}$ +83.1° (*c*=1.09, $CHCl_3$); NMR ($CDCl_3$) δ ppm; 1.99, 2.06, 2.08, 2.11, 2.13 (27H, each s, 9 OAc); TLC: *Rf* 0.31 (solvent A). *Anal.* Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59. Found: C, 49.80; H, 5.63.

18) C.M. McClosky and G.H. Coleman, *J. Org. Chem.*, **10**, 184 (1945).
19) F. Smith, M. Stacey, and P.I. Wilson, *J. Chem. Soc.*, **1944**, 131.
20) E. Hirst, E. G. V. Percival, and C.B. Wylam, *J. Chem. Soc.*, **1954**, 189.
21) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 393 (1954).

O-α-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (13)——To a solution of **12** (1.01 g) in dry MeOH (10 ml) was added 0.1 N sodium methoxide (0.5 ml) in MeOH. After stirring for 6 hr at room temperature, neutralization with Amberlite IR-120 (H^+) and evaporation afforded **13** as an amorphous powder (530 mg, 93%), $[\alpha]_D^{24}$ +148.4° (c=1.02, H_2O), negative the Fehling's test. Acid hydrolysis afforded glucose (*Rf* 0.40) and galactose (*Rf* 0.36), identified by PPC (solvent A).

The $NaIO_4$-consumption (mole)[17] of **13** (72.8 mg) at room temperature was as follows: 3.40 (30 min), 3.50 (1 hr), 3.80 (2 hr), 4.00 (4 hr), 4.80 (8 hr), 5.00 (24 hr, constant), with concomitant formation of 1.70 moles of formic acid for 24 hr.

O-(2,3,4,6-Tetra-O-methyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (14)——Methylation of **13** (200 mg) was performed as for **5** to afford a sirup (231 mg, 91%), $[\alpha]_D^{25}$ +116.4° (c=0.43, $CHCl_3$); TLC: *Rf* 0.37 (solvent B). *Anal.* Calcd. for $C_{27}H_{48}O_{15}$: C, 52.93; H, 7.89. Found: C, 52.75; H, 8.10.

Acid hydrolysis of **14** as for **5** afforded 2,3-di-O-methylglucose (*Rf* 0.58), 2,3,4-tri-O-methylglucose (*Rf* 0.75), and 2,3,4,6-tetra-O-methylgalactose (*Rf* 0.85), identified by PPC with solvent B.

O-α-D-Galactopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose (16)——Acetolysis of **12** (487 mg) was performed as for **3** to afford O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-α-D-glucopyranose (**15**) (470 mg, 86%), amorphous powder, $[\alpha]_D^{23}$ +124.7° (c=1.10, $CHCl_3$); NMR ($CDCl_3$) δ ppm: 6.24 (1H, d, $J_{1,2}$=4 Hz, H-1).

Deacetylation of **15** (825 mg) as for **6** yielded an amorphous powder in which small amounts of galactose (*Rf* 0.36) and maltose (*Rf* 0.29) contamination with **16**, identified by PPC with solvent A. The crude product was chromatographed on a column of charcoal as for **7** to afford pure **16** (203 mg), amorphous powder, $[\alpha]_D^{24}$ +159.2° (c=0.85, H_2O). *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot 2H_2O$: C, 40.00; H, 6.71. Found: C, 39.77; H, 6.99.

After acid hydrolysis as for **7**, glucose and galactose were identified by PPC.

O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (17)——Acetylation of **16** (185 mg) in pyridine (5 ml) with Ac_2O (5 ml) and successive treatment of the product as for **8** gave an amorphous powder (295 mg), $[\alpha]_D^{25}$ +131.9° (c=1.05, $CHCl_3$); TLC: *Rf* 0.34 (solvent A). *Anal.* Calcd. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.62. Found: C, 49.36; H, 5.73.

Acknowledgement We thank Miss M. Nakashima for the NMR spectral measurements and Misses M. Ishiguro and S. Iwauchi for the microanalyses. This work was supported by a Grant-In-Aid for Scientific Research from the Ministry of Education, Science and Culture to which the authors thanks are due.