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Chemical Modification of Lactose. VIII.[1] Studies on the Reactivities of the Secondary Hydroxyl Groups in 1,6-Anhydro-4′,6′-O-benzylidene-β-lactose by Selective *p*-Toluenesulfonylation

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Selective tosylation of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose (**1**), using 2.2 molar equivalents of tosyl chloride in pyridine at 0°, yielded four tosylates which were designated **2** to **5** in the order of decreasing *Rf* value on thin-layer chromatography. After column chromatography on silica gel, compounds **2**—**5** were separated as the 2,3,3′-tritosylate (**2**, 1.3%), 2,3′-ditosylate (**3**, 21.9%), 3′-tosylate (**4**, 15%), and 2-tosylate (**5**, 25.2%), respectively.

Selective tosylation of **3**, using 8 molar equivalents of tosyl chloride afforded **2** (29%), **15**, and **16**, together with **3** (15%). Compounds **15** and **16** were identified as 2,2′,3,3′-tetratosylate (**15**, 38.9%) and 2,2′,3′-tritosylate (**16**, 17.1%), respectively. Thus, the order of reactivities of the secondary hydroxyl groups in **1** is 2>3′>3>2′. Compounds **2**—**5** and **16** have potential value in the chemical modification of lactose or the syntheses of lactose-containing oligosaccharides in human milk.

Keywords——synthesis of bifidus factor; lactosan; selective tosylation; methylation; acetylation; NMR

The lactose-containing oligosaccharides of human milk have been investigated as a result of their association with the "bifidus factor", a growth factor for *Lactobacillus bifidus*. Although many oligosaccharides of great complexity have been reported to occur in human milk,[3] trisaccharides and tetrasaccharides composed of lactose plus one or two L-fucose residues, of lactose plus N-acetyl-lactosamine residue, and of lactose plus one or two N-acetylneuraminic acid residues were found to occur in human milk. However, chemical syntheses of those sugars have not yet been accomplished.

1,6-Anhydro-4′,6′-O-benzylidene-β-lactose (**1**)[4] contains only unblocked secondary hydroxyl groups, those in the reducing moiety of which are *trans*-diaxial and those in the non-reducing moiety are *trans*-diequatorial. Therefore, the reactions of **1** are of potential interest in relation to obtaining useful intermediates for chemical syntheses of trisaccharides or tetrasaccharides in human milk. In the previous paper,[5] selective benzoylation of **1** was investigated and we reported that the order of reactivities of the secondary hydroxyl groups in **1** was 3′>2>3>2′.

Generally, partially acylated or sulfonylated derivatives of disaccharides have potential value in the syntheses of higher oligosaccharides. Therefore, in order to obtain useful intermediates for chemical syntheses of lactose-containing higher oligosaccharides in human milk and to elucidate further reactivities of the secondary hydroxyl groups in **1**, we investigated partial *p*-toluenesulfonylation (tosylation) of **1**. In this paper, the results are now reported in full detail.

Tosylation of **1** with 2.2 molar equivalents of *p*-toluenesulfonyl chloride (tosyl chloride) in pyridine at 0° gave four products. The reaction was monitored by thin-layer chromato-

1) Part VII: T. Chiba and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **25**, 1049 (1977).
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) K. Yamashita, Y. Tachibana, and A. Kobata, *Biochemistry*, **15**, 3950 (1976), and the references cited.
4) T. Chiba, M. Haga, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1283 (1975).
5) T. Chiba, M. Haga, and S. Tejima, *Carbohyd. Res.*, **45**, 11 (1975).

raphy (TLC), and these products were designated **2** to **5** in the order of decreasing *Rf* value. Products **2**—**5** were isolated by column chromatography on silica gel.

Compound **2**, isolated in 1.3% yield, gave a crystalline acetate (**6**, 94.5%), in the nuclear magnetic resonance (NMR) spectrum of which the signal for acetyl appeared at δ 1.86 as a singlet. From the ratio of acetyl to total protons, **6** was identified as a mono-O-acetyl-tri-O-tosyl derivative of **1**.

Methylation of **2** with methyl iodide and silver oxide yielded a crystalline mono-O-methyl-tri-O-tosyl derivative (**7**, 94.1%) of **1**, as indicated by NMR-spectral and elemental analytical data. The location of the methyl group in **7** was determined as follows. Detosylation[6] of **7** with 2% sodium amalgam and, *via* acetylation and sequential deacetylation for purification of the detosylated product, debenzylidenation of the resulting sirup by palladium-catalyzed hydrogenolysis afforded a sirupy product which, when hydrolyzed with dilute sulfuric acid, gave glucose and 2-O-methylgalactose, identified by paper partition chromatography (PPC). Thus **2**, **6**, and **7** were assigned the structures 1,6-anhydro-4′,6′-O-benzylidene-2,3,3′-tri-O-tosyl-β-lactose (**2**), 2′-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,3,3′-tri-O-tosyl-β-lactose (**6**), and 1,6-anhydro-4′,6′-O-benzylidene-2′-O-methyl-2,3,3′-tri-O-tosyl-β-lactose (**7**), respectively.

Further structural assignment of **7** was obtained as follows. Detosylation of **7** with sodium amalgam and benzoylation of the resulting sirup afforded 1,6-anhydro-2,3,3′-tri-O-benzoyl-4′,6′-O-benzylidene-2′-O-methyl-β-lactose (**8**) in 70.4% yield. Compound **8** was characterized by a comparison with the authentic sample previously synthesized[5] by another route.

Compound **3**, isolated in 21.9% yield, gave a crystalline acetate (**9**, 73.9%). In the NMR spectrum of **9**, the signals for acetyls appeared at δ 1.87 and 2.01 as singlets. From the ratio of acetyls to total protons, **9** was identified as a di-O-acetyl-di-O-tosyl derivative of **1**. On methylation as described above, compound **3** gave a crystalline di-O-methyl-di-O-tosyl derivative (**10**, 73.1%) of **1**, as indicated by NMR-spectral and elemental analytical data. After detosylation, debenzylidenation, and acid hydrolysis, the methyl groups in **10** were shown to be located at C-2′ and C-3. Therefore, the following structures were assigned: 1,6-anhydro-4′,6′-O-benzylidene-2,3′-di-O-tosyl-β-lactose (**3**), 2′,3-di-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,3′-di-O-tosyl-β-lactose (**9**), and 1,6-anhydro-4′,6′-O-benzylidene-2′,3-di-O-methyl-2,3′-di-O-tosyl-β-lactose (**10**).

Compound **4**, isolated in 15% yield, gave a crystalline triacetate (**11**, 90%) and a crystalline trimethyl ether (**12**, 88.4%). By using procedures similar to those described for **7** and **10**, the following structures were assigned: 1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**4**), 2,2′,3-tri-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**11**), and 1,6-anhydro-4′,6′-O-benzylidene-2,2′,3-tri-O-methyl-3′-O-tosyl-β-lactose (**12**).

Compound **5**, the major product (25.2%) in the selective tosylation, yielded a crystalline triacetate (**13**, 89.4%) and an amorphous trimethyl ether (**14**, 92%). Using the procedures described above, the structures 1,6-anhydro-4′,6′-O-benzylidene-2-O-tosyl-β-lactose (**5**), 2′,3,3′-tri-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2-O-tosyl-β-lactose (**13**), and 1,6-anhydro-4′,6′-O-benzylidene-2′,3,3′-tri-O-methyl-2-O-tosyl-β-lactose (**14**) were assigned.

The yields of **2**—**5** suggest that the reactivity of the hydroxyl groups in **1** is HO-2>HO-3′>HO-3 and HO-2′. The order of reactivity of HO-3 and HO-2′ was indicated by the results of selective tosylation of **3** with 8 molar equivalents of tosyl chloride. Column chromatography of the product mixture afforded **2** and two new components (**15** and **16**) in yields of 29, 38.9, and 17.1%, respectively, together with 15% of unreacted **3**. Compound **15** was identified as 1,6-anhydro-4′,6′-O-benzylidene-2,2′,3,3′-tetra-O-tosyl-β-lactose (**15**) by compari-

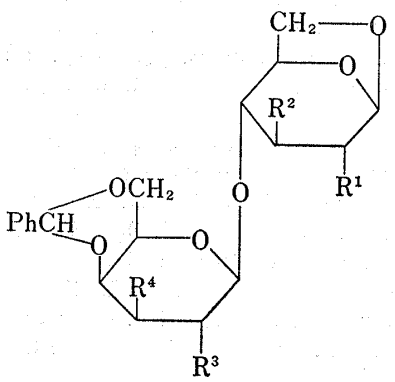
6) C.S. Hudson and M.L. Wolfrom (eds.), "Advances in Carbohydrate Chemistry," Vol. 8, Academic Press Inc., New York, 1953, p. 161.

son with an authentic sample. Compound **16** gave a crystalline monoacetate (**17**, 90.7%) and a crystalline monomethyl ether (**18**, 94.1%). By using the procedures similar to those described for **7**, **10**, and **12**, the following structures were assigned: 1,6-anhydro-4′,6′-O-benzylidene-2,2′,3′-tri-O-tosyl-β-lactose (**16**), 3-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,2′,3′-tri-O-tosyl-β-lactose (**17**), and 1,6-anhydro-4′,6′-O-benzylidene-3-O-methyl-2,2′,3′-tri-O-tosyl-β-lactose (**18**).

The greater yield of **2** than that of **16** indicates the reactivity sequence HO-3>HO-2′. Therefore, the order of reactivity of the secondary hydroxyl groups in **1** is 2>3′>3>2′.

Thus in **1**, HO-2′ has the lowest reactivity and HO-3 has reactivity subsequent to HO-2′. In addition, HO-2 and HO-3′ are more reactive than HO-3 and HO-2′.
These results were similar to those obtained by selective benzoylation of **1**.[5]

Compounds **2**—**5** and **16** are of potential value in the chemical modification of lactose and the syntheses of lactose-containing oligosaccharides in human milk.



1: $R^1=R^2=R^3=R^4=OH$
2: $R^1=R^2=R^4=OTs$, $R^3=OH$
3: $R^1=R^4=OTs$, $R^2=R^3=OH$
4: $R^1=R^2=R^3=OH$, $R^4=OTs$
5: $R^1=OTs$, $R^2=R^3=R^4=OH$
6: $R^1=R^2=R^4=OTs$, $R^3=OAc$
7: $R^1=R^2=R^4=OTs$, $R^3=OMe$
8: $R^1=R^2=R^4=OBz$, $R^3=OMe$
9: $R^1=R^4=OTs$, $R^2=R^3=OAc$
10: $R^1=R^4=OTs$, $R^2=R^3=OMe$
11: $R^1=R^2=R^3=OAc$, $R^4=OTs$
12: $R^1=R^2=R^3=OMe$, $R^4=OTs$
13: $R^1=OTs$, $R^2=R^3=R^4=OAc$
14: $R^1=OTs$, $R^2=R^3=R^4=OMe$
15: $R^1=R^2=R^3=R^4=OTs$
16: $R^1=R^3=R^4=OTs$, $R^2=OH$
17: $R^1=R^3=R^4=OTs$, $R^2=OAc$
18: $R^1=R^3=R^4=OTs$, $R^2=OMe$

Ac=acetyl, Bz=benzoyl, Me=methyl, Ph=phenyl, Ts=tosyl

Chart 1

Experimental

Melting points were determined by a Yanagimoto micro melting point apparatus and uncorrected. Solutions were concentrated *in vacuo* in a rotary evaporator below 40°. Optical rotations were measured in a 0.5 dm cell with a Yanagimoto OR-10 automatic polarimeter or a Union PM-201 automatic digital polarimeter. Infrared (IR) spectra were recorded for Nujol mulls or a chloroform solution with a Jasco IRA-2 spectrometer. NMR spectra were recorded at 100 MHz with a Jeol JNM-MH-100 spectrometer. Tetramethylsilane was used as the internal standard. Chemical shifts were given on the δ scale. TLC was performed on 0.25 mm precoated silica gel plates (Silica Gel 60 F_{254}, E. Merck, Darmstadt), using (A), $CHCl_3$–acetone (3: 1, v/v); (B), $CHCl_3$–acetone (1: 1); (C), benzene–ether (1: 2); and (D), CH_2Cl_2–acetone (9: 1). Detection was effected with H_2SO_4 or UV light (short wave ength). Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka). PPC was performed on Toyo Filter Paper No. 50 (Toyo Roshi Kaisha, Ltd., Tokyo) by the ascending method[7] using BuOH–pyridine–H_2O (6: 4: 3, v/v) and detection was effected with (A), aniline hydrogen phthalate,[8] and (B), alkaline silver nitrate.[9]

7) M. Ueda, *Yakugaku Zasshi*, **90**, 1322 (1970).
8) S.M. Partridge, *Nature* (London), **164**, 443 (1949).
9) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).

Selective Tosylation of 1,6-Anhydro-4′,6′-O-benzylidene-β-lactose (1)——To a chilled solution of **1**[4] (4 g, 9.7 mmol) in dry pyridine (20 ml), a solution of tosyl chloride (4.07 g, 21.34 mmol) in dry pyridine (20 ml) was added dropwise with stirring at 0°, and the stirring was continued, with exclusion of moisture, for a further 1 hr. The mixture was stored for 4 days at room temperature, then poured into ice-water (300 ml) with the aid of CH_2Cl_2 (80 ml), and stirred for 1 hr. After separation of the organic layer, the H_2O-layer was extracted with CH_2Cl_2 (100 ml×2). The combined organic layer and extracts were successively washed with H_2O (200 ml), 10% H_2SO_4 (150 ml×2), H_2O (200 ml), aq. $NaHCO_3$ (200 ml×2), and H_2O (200 ml×3), dried (Na_2SO_4), and concentrated to dryness. The resulting white solid contained four components having *Rf* 0.74, 0.57, 0.21, and 0.11 (major) (TLC, solvent B). Successive elution of the mixture from silica gel column with $CHCl_3$–acetone (20: 1, v/v) and CH_2Cl_2–acetone (3: 2) gave the following products.

The component having *Rf* 0.74 crystallized from MeOH, and recrystallization from MeOH gave 1,6-anhydro-4′,6′-O-benzylidene-2,3,3′-tri-O-tosyl-β-lactose (**2**; 110 mg, 1.3%) as white needles, mp 157—159°, $[\alpha]_D^{28}$ +71° (*c*=1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660, 3570 (OH). NMR ($CDCl_3$) δ: 2.22 (3H, singlet, $C_6H_4C\underline{H}_3$), 2.40 (6H, singlet, $C_6H_4C\underline{H}_3$×2). TLC: *Rf* 0.53 (solvent A), 0.74 (B), 0.27 (C), 0.53 (D). *Anal.* Calcd. for $C_{40}H_{42}O_{16}S_3$: C, 54.91; H, 4.84. Found: C, 54.82; H, 4.78.

The component having *Rf* 0.57 spontaneously crystallized from eluting solvent of silica gel column, and recrystallization from CH_2Cl_2–acetone (3: 2, v/v) gave 1,6-anhydro-4′,6′-O-benzylidene-2,3′-di-O-tosyl-β-lactose (**3**; 1.53 g, 21.9%) as white prisms, mp 198—200° (dec.), $[\alpha]_D^{26}$ −3.3° (*c*=1, pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3470 (OH). NMR (pyridine-d_5) δ: 2.40 (3H, singlet, $C_6H_4C\underline{H}_3$), 2.21 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.19 (solvent A), 0.57 (B), 0.03 (C), 0.08 (D). *Anal.* Calcd. for $C_{33}H_{36}O_{14}S_2$: C, 54.99; H, 5.03. Found: C, 54.71; H, 5.03.

The component having *Rf* 0.21 crystallized from CH_2Cl_2, and recrystallization from CH_2Cl_2 gave 1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**4**; 820 mg, 15%) as white needles, mp 184—186° (dec.), $[\alpha]_D^{26}$ +5.5° (*c*=1, acetone). IR ν_{max}^{Nujol} cm^{-1}: 3460 (OH). NMR (acetone-d_6) δ: 2.40 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.04 (solvent A), 0.21 (B). *Anal.* Calcd. for $C_{26}H_{30}O_{12}S$: C, 55.12; H, 5.34. Found: C, 54.95; H, 5.30.

The component having *Rf* 0.11 crystallized from EtOH, and recrystallization from AcOEt gave 1,6-anhydro-4′,6′-O-benzylidene-2-O-tosyl-β-lactose (**5**; 1.38 g, 25.2%), as white needles, mp 159—161°, $[\alpha]_D^{27}$ −22.4° (*c*=1, acetone). IR ν_{max}^{Nujol} cm^{-1}: 3470 (OH). NMR (acetone-d_6) δ: 2.39 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.02 (solvent A), 0.11 (B). *Anal.* Calcd. for $C_{26}H_{30}O_{12}S$: C, 55.12; H, 5.34. Found: C, 55.17; H, 5.48.

2′-O-Acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,3,3′-tri-O-tosyl-β-lactose (6)——To an ice-cold solution of **2** (100 mg) in dry pyridine (3 ml), Ac_2O (3 ml) was added dropwise with stirring. The mixture was stored overnight at 5°, and concentrated to dryness by repeated co-distillation with toluene. A solution of the residue in CH_2Cl_2 was successively washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($CaCl_2$), and concentrated to dryness to give an amorphous powder, which crystallized from MeOH. Recrystallization from MeOH gave white prisms (99 mg, 94.5%), mp 179—181°, $[\alpha]_D^{26}$ +65.2° (*c*=1.1, $CHCl_3$). NMR ($CDCl_3$) δ: 1.86 (3H, singlet, OAc), 2.21 (3H, singlet, $C_6H_4C\underline{H}_3$), 2.41 (6H, singlet, $C_6H_4C\underline{H}_3$×2). TLC: *Rf* 0.65 (solvent A), 0.75 (B), 0.29 (C), 0.76 (D). *Anal.* Calcd. for $C_{42}H_{44}O_{17}S_3$: C, 55.01; H, 4.84. Found: C, 54.80; H, 5.04.

1,6-Anhydro-4′,6′-O-benzylidene-2′-O-methyl-2,3,3′-tri-O-tosyl-β-lactose (7)——To a solution of **2** (920 mg) in freshly distilled CH_3I (60 ml), freshly prepared Ag_2O (1.2 g) was added. The mixture was refluxed for 10 hr with stirring in the dark, filtered, and concentrated to dryness. A solution of the residue in CH_2Cl_2 was successively washed with 10% aq. $Na_2S_2O_3$ and H_2O, dried (Na_2SO_4), and concentrated to afford an amorphous powder, which crystallized from EtOH. Recrystallization from EtOH gave white needles (880 mg, 94.1%), mp 200—203°, $[\alpha]_D^{27}$ +75.7° (*c*=1.1, $CHCl_3$). NMR ($CDCl_3$) δ: 2.20 (3H, singlet, $C_6H_4C\underline{H}_3$), 2.40 (6H, singlet, $C_6H_4C\underline{H}_3$×2), 3.24 (3H, singlet, OMe). TLC: *Rf* 0.67 (solvent A), 0.68 (B), 0.42 (C), 0.79 (D). *Anal.* Calcd. for $C_{41}H_{44}O_{16}S_3$: C, 55.39; H, 4.99. Found: C, 55.29; H, 4.94.

Identification of the Component Monosaccharides in 7——To a suspension of **7** (500 mg) in dry MeOH (60 ml), 2% Na–Hg (7 g) was added, and the mixture was stirred for 40 hr at room temperature; detosylation was monitored by TLC (solvent A). After detosylation was completed, the mixture was filtered, and concentrated to dryness. The residue was acetylated with Ac_2O (5 ml) and pyridine (5 ml) for 24 hr at 5°. The mixture was similarly treated as described above for **6** to afford a sirup which was dissolved in CH_2Cl_2. The CH_2Cl_2-solution was chromatographed on a column of silica gel with $CHCl_3$–acetone (20: 1, v/v) as eluent. Removal of the solvent afforded an amorphous powder. To a solution of the residue (230 mg) in dry MeOH (8 ml), methanolic 0.5 N sodium methoxide (0.3 ml) was added at room temperature. The mixture was stirred, with exclusion of moisture, for 3 hr; complete deacetylation was monitored by TLC (solvent B). Dry Amberlite IR-120 (H^+) resin (200 mg) was added to the mixture, stirred for 30 min, and then filtered. The filtrate was concentrated to dryness to give a sirup which was dissolved in MeOH (15 ml). To the solution, Pd catalyst,[10] freshly prepared from $PdCl_2$ (120 mg), was added, and the mixture was hydrogenated with stirring at room temperature under atmospheric pressure; theoretical amounts of hydrogen

10) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 393 (1954).

were absorbed within 1 hr. The mixture was filtered and concentrated, and a solution of the sirupy residue in 0.5 M H_2SO_4 (8 ml) was kept at 95° for 3 hr. The hydrolyzate was neutralized with $BaCO_3$, filtered, and concentrated to a thin sirup, in which glucose and 2-methylgalactose[11] (R_{GLC} 1.35) were identified by PPC.

1,6-Anhydro-2,3,3′-tri-O-benzoyl-4′,6′-O-benzylidene-2′-O-methyl-β-lactose (8)——Starting from **7**, sirupy 1,6-anhydro-4′,6′-O-benzylidene-2′-O-methyl-β-lactose was prepared by detosylation followed by acetylation and deacetylation as described above for identification of the component monosaccharides in **7**. The sirup (50 mg) was dissolved in pyridine (3 ml) and benzoyl chloride (1 ml) was added with stirring at room temperature. The mixture was left to stand overnight, treated with ice to decompose excess benzoyl chloride, and extracted with CH_2Cl_2 (20 ml × 2). The combined extracts were successively washed with H_2O, 10% H_2SO_4, aq. $NaHCO_3$, and H_2O, dried ($CaCl_2$), and concentrated to dryness. The resulting sirup was dissolved in CH_2Cl_2, and the CH_2Cl_2-solution was chromatographed on a column of silica gel with $CHCl_3$–acetone (25: 1, v/v) as eluent. Removal of the solvent afforded an amorphous powder which crystallized from EtOH. Recrystallization from EtOH gave white needles (61 mg, 70.4%), mp 221—223.5°, $[\alpha]_D^{23}$ +75.2° (c=0.8, $CHCl_3$). It was indistinguishable from the authentic sample.[5]

2′,3-Di-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,3′-di-O-tosyl-β-lactose (9)——Acetylation of **3** (80 mg) with Ac_2O (3 ml) and pyridine (3 ml) as described above for **6** gave an amorphous powder, which crystallized from EtOH. Recrystallization from EtOH gave white needles (66 mg, 73.9%), mp 130—135°, $[\alpha]_D^{27}$+29.1° (c=1.1, $CHCl_3$). NMR ($CDCl_3$) δ: 1.87, 2.01 (6H, singlet, OAc × 2), 2.29, 2.40 (6H, singlets, $C_6H_4C\underline{H}_3 \times 2$). TLC: *Rf* 0.54 (solvent A), 0.71 (B), 0.10 (C), 0.65 (D). *Anal.* Calcd. for $C_{37}H_{40}O_{16}S_2$: C, 55.22; H, 5.01. Found: C, 55.13; H, 5.05.

1,6-Anhydro-4′,6′-O-benzylidene-2′,3-di-O-methyl-2,3′-di-O-tosyl-β-lactose (10)——Methylation of **3** (1 g) with CH_3I (100 ml) and Ag_2O (3.4 g) as described above for **7** afforded an amorphous powder which was dissolved in CH_2Cl_2. The CH_2Cl_2-solution was chromatographed on a column of silica gel with $CHCl_3$–acetone (20: 1, v/v) as eluent. Removal of the solvent afforded an amorphous powder. Twice recrystallizations from EtOH gave white powder (799 mg, 73.1%), mp 95—97°, $[\alpha]_D^{26}$ −10° (c=1.1, $CHCl_3$). NMR ($CDCl_3$) δ: 2.35, 2.43 (6H, singlets, $C_6H_4C\underline{H}_3 \times 2$), 3.25, 3.28 (6H, singlets, OMe × 2). TLC: *Rf* 0.50 (solvent A), 0.73 (B), 0.21 (C), 0.68 (D). *Anal.* Calcd. for $C_{35}H_{40}O_{14}S_2$: C, 56.14; H, 5.38. Found: C, 56.27; H, 5.47.

Identification of the Component Monosaccharides in 10——Compound **10** (300 mg) was successively detosylated, acetylated, deacetylated, debenzylidenated, and hydrolyzed as described above for **7**. The product contained 3-O-methylglucose[12] (R_{GLC} 1.49) and 2-O-methylgalactose (R_{GLC} 1.35) which were identified by PPC.

2,2′,3-Tri-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (11)——Acetylation of **4** (150 mg) with Ac_2O (3 ml) and pyridine (3 ml) as described above for **6** gave an amorphous powder which crystallized from acetone. Recrystallization from acetone gave white plates (165 mg, 90%), mp 224—227° (dec.), $[\alpha]_D^{26}$ +7.1° (c=1.2, CH_2Cl_2). NMR (pyridine-d_5) δ: 1.96, 1.98, 2.01 (9H, singlets, OAc × 3), 2.21 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.52 (solvent A), 0.67 (B), 0.10 (C), 0.54 (D). *Anal.* Calcd. for $C_{32}H_{36}O_{15}S$: C, 55.49; H, 5.24. Found: C, 55.42; H, 5.31.

1,6-Anhydro-4′,6′-O-benzylidene-2,2′,3-tri-O-methyl-3′-O-tosyl-β-lactose (12)——Methylation of **4** (300 mg) with CH_3I (30 ml) and Ag_2O (2.5 g) as described above for **7** afforded an amorphous powder, which was dissolved in CH_2Cl_2. The CH_2Cl_2-solution was chromatographed on a column of silica gel with $CHCl_3$–acetone (25: 1, v/v) as eluent. Removal of the solvent afforded an amorphous powder. Twice recrystallizations from MeOH–H_2O gave white powder (285 mg, 88.4%), mp 76—79°, $[\alpha]_D^{22}$ −14.4° (c=1, $CHCl_3$). NMR ($CDCl_3$) δ: 2.37 (3H, singlet, $C_6H_4C\underline{H}_3$), 3.29, 3.37, 3.38 (9H, singlets, OMe × 3). TLC: *Rf* 0.50 (solvent A), 0.72 (B), 0.17 (C), 0.53 (D). *Anal.* Calcd. for $C_{29}H_{36}O_{12}S$: C, 57.23; H, 5.96. Found: C, 57.22; H, 6.03.

Identification of the Component Monosaccharides in 12——Compound **12** (180 mg), when treated as described above for **7**, gave 2,3-di-O-methylglucose[13] (R_{GLC} 1.80) and 2-O-methylgalactose (R_{GLC} 1.35) which were identified by PPC.

2′,3,3′-Tri-O-acetyl-1,6-anhydro-4′,6′-O-benzylidene-2-O-tosyl-β-lactose (13)——Acetylation of **5** (150 mg) with Ac_2O (3 ml) and pyridine (3 ml) as described above for **6** gave an amorphous powder. Twice recrystallizations from 2-propanol gave white powder (164 mg, 89.4%), mp 109—110°, $[\alpha]_D^{27}$ +25.4° (c=1, $CHCl_3$). NMR ($CDCl_3$) δ: 1.95, 1.99, 2.01 (9H, singlets, OAc × 3), 2.23 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.50 (solvent A), 0.70 (B), 0.10 (C), 0.56 (D). *Anal.* Calcd. for $C_{32}H_{36}O_{15}S$: C, 55.49; H, 5.24. Found: C, 55.24; H, 5.16.

1,6-Anhydro-4′,6′-O-benzylidene-2′,3,3′-tri-O-methyl-2-O-tosyl-β-lactose (14)——Methylation of **5** (250 mg) with CH_3I (20 ml) and Ag_2O (1.8 g) as described above for **7** afforded an amorphous powder, which was dissolved in CH_2Cl_2. The CH_2Cl_2-solution was chromatographed on a column of silica gel with $CHCl_3$–acetone (20: 1, v/v) as eluent. Evaporation of the solvent gave an amorphous powder (247 mg, 92%),

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13) C.M. McCloskey and G.H. Coleman, *J. Org. Chem.*, **10**, 184 (1945).

$[\alpha]_D^{20}$ −19.6° (c=0.8, $CHCl_3$). All attempts to crystallize the compound failed. NMR ($CDCl_3$) δ: 2.34 (3H, singlet, $C_6H_4C\underline{H}_3$), 3.27, 3.54, 3.59 (9H, singlets, OMe×3). TLC: *Rf* 0.38 (solvent A), 0.59 (B), 0.06 (C), 0.41 (D). *Anal.* Calcd. for $C_{29}H_{36}O_{12}S$: C, 57.23; H, 5.96. Found: C, 56.98; H, 6.08.

Identification of the Component Monosaccharides in 14——Treatment of **14** (600 mg), as described above for **7**, gave 3-O-methylglucose (R_{GLC} 1.49) and 2.3-di-O-methylgalactose[14] (R_{GLC} 1.63), which were identified by PPC.

Selective Tosylation of 3——To a solution of **3** (400 mg, 0.56 mmol) in dry pyridine (5 ml), a solution of tosyl chloride (850 mg, 4.45 mmol) in dry pyridine (5 ml) was added dropwise under stirring at room temperature. Stirring was continued, with exclusion of moisture, for a further 10 min. The mixture was stored for 4 days at room temperature and treated as described above for the selective tosylation of **1**. Elution of the products from silica gel column with $CHCl_3$–acetone (25: 1, v/v) gave **2** (140 mg, 29%) and new components having *Rf* 0.71 and 0.40 (solvent A) together with unreacted **3** (60 mg, 15%).

The component having *Rf* 0.71 crystallized from MeOH, and recrystallization from MeOH gave white plates (222 mg, 38.9%), mp 127—130°, $[\alpha]_D^{22}$ +119.8° (c=1.1, $CHCl_3$), which were indistinguishable (mixed mp, IR, and TLC) from 1,6-anhydro-4′,6′-O-benzylidene-2,2′,3,3′-tetra-O-tosyl-β-lactose (**15**) mentioned below.

The component having *Rf* 0.40 solidified from MeOH. Twice recrystallizations from MeOH gave white powder (83 mg, 17.1%), mp 109—110°, $[\alpha]_D^{18}$ +35.8° (c=0.8, $CHCl_3$). The product was identified to 1,6-anhydro-4′,6′-O-benzylidene-2,2′,3′-tri-O-tosyl-β-lactose (**16**) as mentioned later. IR ν_{max}^{Nujol} cm^{-1}: 3600—3320 (OH). NMR ($CDCl_3$) δ: 2.38 (6H, singlet, $C_6H_4C\underline{H}_3\times2$), 2.42 (3H, singlet, $C_6H_4C\underline{H}_3$). TLC: *Rf* 0.40 (solvent A), 0.71 (B), 0.09 (C), 0.35 (D). *Anal.* Calcd. for $C_{40}H_{42}O_{16}S_3$: C, 54.91; H, 4.84. Found: C, 54.79; H, 4.83.

1,6-Anhydro-4′,6′-O-benzylidene-2,2′,3,3′-tetra-O-tosyl-β-lactose (**15**)——To a chilled solution of **1** (450 mg, 1.09 mmol) in dry pyridine (5 ml), a solution of tosyl chloride (2.08 g, 10.9 mmol) in dry pyridine (5 ml) was added dropwise under stirring at 0°, and stirring was continued, with exclusion of moisture, for a further 1 hr. The mixture was kept for two weeks at room temperature and then similarly treated as described above for selective tosylation of **1** to afford an amorphous powder. The product was dissolved in CH_2Cl_2, and chromatographed on a column of silica gel with $CHCl_3$–acetone (20: 1, v/v) as eluent. Removal of the solvent afforded an amorphous powder, which crystallized from MeOH. Recrystallization from MeOH gave white plates (784 mg, 69.9%), mp 127—130°, $[\alpha]_D^{22}$ +119.8° (c=1.1, $CHCl_3$). NMR ($CDCl_3$) δ: 2.17, 2.35 (6H, singlets, $C_6H_4C\underline{H}_3\times2$), 2.42 (6H, singlet, $C_6H_4C\underline{H}_3\times2$). TLC: *Rf* 0.71 (solvent A), 0.81 (B), 0.51 (C), 0.83 (D). *Anal.* Calcd. for $C_{47}H_{48}O_{18}S_4$: C, 54.85; H, 4.70. Found: C, 54.86; H, 4.69.

3-O-Acetyl-1,6-anhydro-4′,6′-O-benzylidene-2,2′,3′-tri-O-tosyl-β-lactose (**17**)——Acetylation of **16** (120 mg) with Ac_2O (2 ml) and pyridine (2 ml) as described above for **6** gave an amorphous powder which crystallized from EtOH. Recrystallization from EtOH gave white needles (114 mg, 90.7%), mp 211—214°, $[\alpha]_D^{16}$ 93.3° (c=0.6, $CHCl_3$). NMR ($CDCl_3$) δ: 2.05 (3H, singlet, OAc), 2.20, 2.34, 2.43 (9H, singlets, $C_6H_4C\underline{H}_3\times3$). TLC: *Rf* 0.62 (solvent A), 0.76 (B), 0.22 (C), 0.72 (D). *Anal.* Calcd. for $C_{42}H_{44}O_{17}S_3$: C, 55.01; H, 4.84. Found: C, 54.87; H, 4.82.

1,6-Anhydro-4′,6′-O-benzylidene-3-O-methyl-2,2′,3′-tri-O-tosyl-β-lactose (**18**)——Methylation of **16** (230 mg) with CH_3I (16 ml) and Ag_2O (400 mg) as described above for **7** gave an amorphous powder which crystallized from 2-propanol. Recrystallization from 2-propanol gave white grains (220 mg, 94.1%), mp 96—98°, $[\alpha]_D^{15}$ +44.1° (c=0.8, $CHCl_3$). NMR ($CDCl_3$) δ: 2.33, 2.36, 2.42 (9H, singlets, $C_6H_4C\underline{H}_3\times3$), 3.22 (3H, singlet, OMe). TLC: *Rf* 0.59 (solvent A), 0.76 (B), 0.19 (C), 0.69 (D). *Anal.* Calcd. for $C_{41}H_{44}O_{16}S_3$: C, 55.39; H, 4.99. Found: C, 55.28; H, 5.19.

Identification of the Component Monosaccharides in 18——Treatment of **18** (140 mg), as described above for **7**, gave 3-O-methylglucose (R_{GLC} 1.49) and galactose (R_{GLC} 0.88) which were identified by PPC.

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