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Trityl Derivatives of Cellobiose. IV.[1] Studies on the Relative Reactivities of the Secondary Hydroxyl Groups in 6,6′-Di-O-tritylcellobiose and Methyl 6,6′-Di-O-trityl-β-cellobioside by Selective Acetylation[2]

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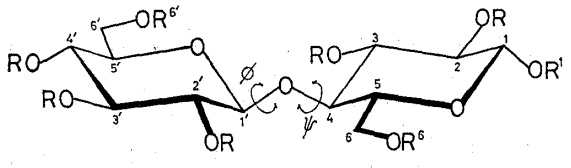
The selective acetylation of 6,6′-di-O-tritylcellobiose (**1**) and its methyl β-glycoside (**5**) with 5 molar equivalents of acetyl chloride in pyridine–toluene at 1—4°C has been studied. Acetylation of **1** gave a complex mixture from which α- and β-anomers of three main products, the 1,2,2′,3′,4′-pentaacetate (**A**), the 1,2,2′,3′-tetraacetate (**B**), and the 1,2,3′,4′-tetraacetate (**C**) were isolated by column chromatography. From **5**, the 2,2′,3′,4′-tetraacetate (**D**, 22.9%), the 2,2′,3′-triacetate (**E**, 15.7%), the 2,3′,4′-triacetate (**F**, 15.7%), the 2,3′-diacetate (**G**, 10.8%), the 2′,3′,4′-triacetate (**H**, 14.2%), the 2′,3′-diacetate (**I**, 10.8%), the 3,3′,4′-triacetate (**J**, 3.4%), and the 3,3′-diacetate (**K**, 5.6%) were obtained. The locations of the unacetylated hydroxyl groups in the partially acetylated compounds were determined by gas chromatography–mass spectrometry after the sequence of methylation (for labeling free hydroxyl groups) with methyl trifluoromethanesulfonate, detritylation, deacetylation, (reduction), methanolysis, and trimethylsilylation and by comparison of acetoxyl signals in ^{1}H-NMR spectra after trideuterioacetylation of free hydroxyl groups in the partially acetylated compounds. From the yields of partially acetylated methyl 6,6′-di-O-trityl-β-cellobiosides, it was deduced that the order of reactivity of the five secondary hydroxyl groups in **5** is HO-3′>HO-2>HO-2′>HO-4′≫HO-3.

Keywords——selective acetylation; partially acetylated 6,6′-di-O-tritylcellobioses; partially acetylated methyl 6,6′-di-O-trityl-β-cellobiosides; partially methylated D-glucoses; mono-O-methyl-D-glucitols; trimethylsilyl ether; column chromatography; semipreparative HPLC; GC–MS; ^{1}H-NMR

Previously the conformations of 6- and 6′-trityl substituted cellobioses and their methyl β-glycosides in solution were studied by the use of ^{1}H- and ^{13}C-NMR: the acetoxyl signals in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of octa-O-acetylcellobiose are concentrated in the region of δ 2.11—1.98, whereas the ^{1}H-NMR spectra of 6- and 6′-O-tritylcellobiose peracetates (**2**, **3**, and **4**) showed some of the acetoxyl resonances at abnormally high fields in $CDCl_3$ (**2**; δ 1.77, 1.67, 1.61. **3**; δ 1.82, 1.71. **4**; δ 1.56) and therefore it appeared that the trityl group at 6-O affected an acetyl substituent on a secondary hydroxyl group and the effect of that at 6′-O extended to acetyl substituents on two secondary hydroxyl groups in the cellobiose molecule.[3] On the other hand, the ^{13}C-NMR spectra of methyl 6- and 6′-O-tritylcellobiosides (**5**, **6**, and **7**) suggested that 6′-O-tritylation has no effect upon the conformation around the interglycoside linkage of cellobioside, but 6-O-tritylation alters the torsion angle φ.[4]



1: $R^6=R^{6'}=$Tr, $R^1=R=$H
2: $R^6=R^{6'}=$Tr, $R^1=R=$Ac
3: $R^{6'}=$Tr, $R^1=R^6=R=$Ac
4: $R^6=$Tr, $R^1=R^{6'}=R=$Ac
5: $R^1=$Me, $R^6=R^{6'}=$Tr, R=H
6: $R^1=$Me, $R^{6'}=$Tr, $R^6=R=$H
7: $R^1=$Me, $R^6=$Tr, $R^{6'}=R=$H
Tr=trityl, Ac=acetyl, Me=methyl

Chart 1

In the present paper, the relative reactivity of the secondary hydroxyl groups in the molecules of 6- and 6′-trityl substituted cellobiose (**1**) and its methyl β-glycoside (**5**) was

investigated by the use of selective acetylation as a tool for estimating the conformation of those molecules.

On selective acylations of (1→4)-linked disaccharides (maltose, lactose, and cellobiose) and some of their derivatives with acid chlorides, it has been reported that the secondary hydroxyl group at C-3 is the least reactive.[5] Among those reports, comparatively little information is available concerning the relative reactivity of hydroxyl groups in cellobiose and its derivatives.[5b,6]

We first attempted the selective acetylation of 6,6′-di-O-trityl cellobiose (**1**), but the resulting products included many α- and β-anomers and the complexity of composition made it impossible to estimate the yield of each component. This led us to investigate the selective acetylation of methyl 6,6′-di-O-trityl-β-cellobioside (**5**) in order to establish the order of reactivity of the secondary hydroxyl groups toward acetyl chloride. The locations of the unacetylated hydroxyl groups in the partially acetylated compounds were determined by gas chromatography-mass spectrometry (GC–MS) after converting to the trimethylsilyl (TMS) ethers, labeled the free hydroxyl groups with methyl groups and by comparison of acetoxyl signals in the ^{1}H-NMR spectra after trideuterioacetylation of unacetylated hydroxyl groups.[7]

Results and Discussion

The Selective Acetylation of 6,6′-Di-O-tritylcellobiose (1)

Acetylation of **1** with 5 molar equivalents of acetyl chloride in pyridine and toluene at 1—4°C for 6 h gave a complex mixture containing three pairs of main products which was fractionated by column chromatography (CC). Each pair consisted of α- and β-anomers of the same compound and usually the faster moving β-anomer was obtained in a pure state more easily. Only one pair had one unacetylated hydroxyl group in the molecule and the other two pairs had two unacetylated hydroxyl groups each (Table I). The peracetylated α- and β-derivatives of **1** could not be detected on thin-layer chromatography (TLC).

Table I. The Partially Acetylated 6,6′-Di-O-tritylcellobiose

Compd.	*Rf*[a] on TLC	$[\alpha]_D^{24}$ in $CHCl_3$ (°)	*c* (%)	Ratio[b]	Unacetylated hydroxyl group at
A(β)	0.75	+31.7	2.5	3	C-3
A(α)	0.71	+58.0	2.7		
B(β)	0.64	−4.0	0.5	2	C-3 and C-4′
B(α)	0.59				
C(β)	0.49	+34.6	1.6	4	C-3 and C-2′
C(α)	0.45				

a) Plates: TLC plates silica gel 60 (0.25 mm) (E. Merck). Solvent composition: C_6H_6–$CH_3COOC_2H_5$=2:1.
b) By TLC spectrophotometry at 260 nm (approximately).

The Selective Acetylation of Methyl 6,6′-Di-O-trityl-β-cellobioside (5)

In order to minimize the amounts of lower reaction products, 5 molar equivalents (equivalent to all of the free hydroxyl groups in **5**) of acetyl chloride were used for the selective acetylation of **5**. The other conditions were the same as described above. Eight partially acetylated derivatives, designated **D** to **K** in order of decreasing *Rf* value on Silica gel TLC, were separated by CC. **D**, **E**, and **F** were oislated by the use of a Lobar prepacked column (E. Merck) with benzene–ethyl acetate (5:1) as the eluting solvent, and **G**, **H**, **I**, **J**, and **K** were isolated in chromatographically pure states by repeated semipreparative high-performance liquid chromatography (semiprep. HPLC), using the solvent system: benzene–

Table II. The Partially Acetylated Methyl 6,6′-Di-O-trityl-β-cellobiosides

Compd.	Rf[a] on TLC	Yield[b] (%)	$[\alpha]_D^t$ in $CHCl_3$ (°)	t(°)	c(%)	Unacetylated hydroxyl group at
D	0.74	22.9	+31.1	27	3.6	C-3
E	0.63	15.7	−10.0	27	2.0	C-3 and C-4′
F	0.46	15.7	+20.5	27	2.5	C-3 and C-2′
G	0.35	10.8	−8.7	24	2.3	C-3, C-2′, and C-4′
H	0.31	14.2	+18.0	20	1.5	C-2 and C-3
I	0.26	10.8	−10.0	23	1.4	C-2, C-3, and C-4′
J	0.14	3.4	+18.0	21	1.0	C-2 and C-2′
K	0.12	5.6	−6.0	20	1.0	C-2, C-2′, and C-4′
Minor products	>0.06	0.9				

a) Plates: TLC plates silica gel 60 (0.25 mm) (E. Merck). Solvent composition: C_6H_6–$CH_3COOC_2H_5$=2 : 1.
b) By TLC spectrophotometry at 260 nm.

ethyl acetate=3: 1, 2: 1 or 1: 1. The sum of the yields of the eight partially acetylated products was 99.1% and the peracetylated derivative could not be detected. It was shown by ^{1}H-NMR that **D** was the only tetraacetate, **E**, **F**, **H**, and **J** were triacetates, and **G**, **I**, and **K** were diacetates. At present only **E** has been crystallized from ethanol (mp 138—139°C).

The *Rf* value of **D**, having the 3-hydroxyl group free, is higher than that of the methyl 6,6′-di-O-trityl-β-cellobioside (**5**) peracetate (*Rf* 0.71). If HO-3 in **D** is hydrogen-bonded to O-5′, the polarity of the compound will be decreased and its mobility on hydrated silica gel will be increased. In fact, all the derivatives containing a 3-O-acetyl group in the series of esters of the compound **5** (**J** and **K**) have much lower mobilities than those of the corresponding isomers (**E**—**I**) (Table II). In the case of monosaccharides, a correlation has been found between intramolecular hydrogen-bonding and enhanced reactivity toward acetic anhydride.[8] However, a strong HO-3....O-5′ intramolecular hydrogen-bonding could cause the molecule to fold in such a way that access to HO-3 would be severely hindered and a molecular model of the favored conformation of **5** indeed shows that HO-3 is subject to considerable steric interaction. Some degree of intramolecular hydrogen-bonding also may occur between HO-4′ ...O-6′ and HO-2′...O-6. This interpretation is supported by the relative polarity of the triacetates (**E**, **F**, and **H**), as inferred from their mobilities on TLC.

As regards the optical rotations of partially acetylated derivatives of **5**, it was found that compounds having the free hydroxyl group at C-4′ are levorotatory and the others are all dextrorotatory.

A consideration of the yields of all products led to a reactivity order for the secondary hydroxyl groups of 3′>2>2′>4′≫3.

Determination of the Site of the Unacetylated Hydroxyl Group

The location of the free hydroxyl group(s) in the partially acetylated compound was determined as follows: the partially acetylated compound was methylated with methyl trifluoromethanesulfonate[9]-2,6-di-*tert*-butyl-4-methylpyridine[9a] at 80°C for 5 h. During this methylation, both O-acetyl and O-trityl groups were stable. The methylation product was purified by CC to remove excess reagent, small amounts of the starting material remaining, and traces of by-products. In the cases of partially acetylated derivatives from **1**, detritylation of the resulting syrup with 80% acetic acid, followed by deacetylation with methanolic sodium methoxide, and by reduction with sodium borohydride in the usual manner afforded a syrupy product, the 4-O-β-D-glucopyranosyl-D-glucitol derivative having an -OCH_3 group at the carbon which originally carried the free -OH group. Methanolysis of the 4-O-β-D-glucopyranosyl-D-glucitiol derivative gave partially methylated methyl D-glucopyranoside and D-glucitol deriva-

tives which were identified by GC–MS as their TMS ethers. Treatment of partially acetylated derivatives from the methyl glycoside (**5**) in the same manner with the exception of reduction usually gave two kinds of partially methylated methyl glucosides. Which of the two methyl glucosides arose from the reducing residue was determined from the ^{1}H-NMR spectrum of the trideuterioacetyl ester of the original partially acetylated compound: there are marked differences in the chemical shifts of acetoxyl signals between AcO-2 and AcO-2′ (δ 2.04 and 1.63) or AcO-3 and AcO-3′ (δ 1,78 and 1.92). Details of assignments for acetoxyl signals in the ^{1}H-NMR spectra of 6,6′-di-O-tritylcellobiose peracetate and its methyl glycoside will be reported in the near future.[7] The methanolyzate from **J** contained only one kind of compound, methyl 2-O-methylglucoside (2 mol).

Table III shows the relative retention times of glucose, fifteen partially methylated glucoses, glucitol, and four monomethylglucitols as their TMS ethers on GC using the 3% SE-30 column at 180°C.

Table III. Relative Retention Times of Glucose, Partially Methylated Glucoses, Glucitol, and Monomethyl glucitols as the Pertrimethylsilyl Ethers at 180°C

Compound	Abbreviation	Relative t_R
Glucose		1.11(α),[a] 1.66(β)
Methyl glucoside	1-OCH_3	1.00(α),[b] 1.12(β)
2-O-Methylglucose	2-OCH_3	1.08
3-O-Methylglucose	3-OCH_3	0.65
4-O-Methylglucose	4-OCH_3	0.71,[a] 1.10
6-O-Methylglucose	6-OCH_3	0.93
Methyl 2-O-methylglucoside	1,2-OCH_3	0.67
Methyl 3-O-methylglucoside	1,3-OCH_3	0.55(α),[b] 0.57(β)
Methyl 4-O-methhlglucoside	1,4-OCH_3	0.60(α),[b] 0.67(β)
Methyl 6-O-methylglucoside	1,6-OCH_3	0.82(α)
Methyl 2,3-di-O-methylglucoside	1,2,3-OCH_3	0.37(β), 0.41(α)[b]
Methyl 2,4-di-O-methylglucoside	1,2,4-OCH_3	0.41
Methyl 2,6-di-O-methylglucoside	1,2,6-OCH_3	0.52
Methyl 3,4-di-O-methylglucoside	1,3,4-OCH_3	0.37
Methyl 3,6-di-O-methylglucoside	1,3,6-OCH_3	0.43
Methyl 2,3,4-tri-O-methylglucoside	1,2,3,4-OCH_3	0.27(α)
Glucitol		1.42
2-O-Methylglucitol	2-OCH_3	1.07
3-O-Methylglucitol	3-OCH_3	1.05
4-O-Methylglucitol	4-OCH_3	1.01
6-O-Methylglucitol	6-OCH_3	0.94

a) The main product on trimethylsilylation.
b) The main product on methanolysis.

The mass spectra of pertrimethylsilylated glucose and its methyl glucoside,[10] and permethylated glucoside[11] were investigated in great detail. Petersson *et al.* measured the mass spectra of several kinds of mono- and di-O-methylaldohexoses as O-TMS derivatives[12] and also measured the mass spectra of pertrimethylsilylated alditols.[13] The separation and structural determination of four possible methyl tri-O-methylglucosides and six possible methyl di-O-methylglucosides by GC–MS were described by Hayashi *et al.*[14] However, we cannot find any report concerning the analysis of TMS ethers of methyl mono-O-methylglucosides and partially methylated glucitols by GC–MS in the literature.

Partial mass spectra of TMS ethers of methyl mono-O-methylglucosides are presented in Table IV. For comparison, partial mass spectra of TMS ethers of glucose, mono-O-methylglucoses, and methyl di- and tri-O-methylglucosides, measured under the same conditions are also given in Table IV. These are consistent with the data described in the literature.

Essentially no difference could be detected between the mass spectral patterns of the α- and β-anomers.

Partial mass spectra of the TMS ethers of glucitol and mono-O-methylglucitols are shown in Table V.

TABLE IV. Relative Intensities of Significant Peaks[a] in the Mass Spectra of TMS Ethers of Glucose and Partially Methylated Glucoses

m/e	75	88	101	133	146	159	191	204	205	217
Glucose							50.0	100.0	26.6	
1-OCH_3				29.4				100.0		26.1
2-OCH_3					100.0		36.3			
3-OCH_3				100.0	97.5					85.6
4-OCH_3							49.2	100.0		
6-OCH_3							28.3	100.0		
1,2-OCH_3				36.3	100.0					
1,3-OCH_3					100.0					84.3
1,4-OCH_3				42.2				100.0	27.2	
1,6-OCH_3								100.0		28.5
1,2,3-OCH_3	58.3	100.0				51.3				
1,2,4-OCH_3				27.6	100.0					
1,2,6-OCH_3				37.9	100.0	28.8				
1,3,4-OCH_3	74.3				100.0	88.4				
1,3,6-OCH_3					100.0					86.5
1,2,3,4-OCH_3	89.9	100.0	47.1							

a) Greater than 25% of the base peak.

TABLE V. Relative Intensities of Significant Peaks[a] in the Mass Spectra of TMS Ethers of Glucitol and Monomethylglucitols

m/e	103	147	205	217	261	307	319
Glucitol	42.0		97.4	45.9		40.6	100.0
2-OCH_3	58.9	35.7	53.5	46.0			100.0
3-OCH_3	38.1		100.0	38.3	37.5		
4-OCH_3	43.3		100.0	39.8	40.7		
6-OCH_3	62.4	100.0	71.3	39.9	57.7		55.3

a) Greater than 35% of the base peak.

TABLE VI. Presumed Structures of Main Fragment Ions

Source compound	*m/e*	Presumed structure
Glucose derivatives	75	$CH_3O-\overset{+}{C}H-OCH_3$
	88	$CH_3O-\dot{C}H-\overset{+}{C}H-OCH_3$
	101	$CH_3O-CH=CH-\overset{+}{C}H-OCH_3$
	133	$CH_3O-\overset{+}{C}H-OTMS$
	146	$CH_3O-\overset{+}{C}H-\dot{C}H-OTMS$
	159	$TMSO-\overset{+}{C}H-CH=CH-OCH_3$
		$TMSO-CH=CH-\overset{+}{C}H-OCH_3$
	191	$TMSO-\overset{+}{C}H-OTMS$
	204	$TMSO-\dot{C}H-\overset{+}{C}H-OTMS$
	205	$TMSO-CH_2-\overset{+}{C}H-OTMS$
	217	$TMSO-CH=CH-\overset{+}{C}H-OTMS$

Source compound	*m/e*	Presumed structure
Glucitol derivatives	103	$\overset{+}{C}H_2$–OTMS
	147	TMSO–CH_2–$\overset{+}{C}$H–OCH_3
		TMSO–$\overset{+}{C}$H–CH_2–OCH_3
	205	TMSO–CH_2–$\overset{+}{C}$H–OTMS
	217	TMSO–CH=CH–$\overset{+}{C}$H–OTMS
	261	TMSO–CH=CH–CH–$\overset{+}{C}$H–OTMS OCH_3
		CH_3O–CH=CH–CH–$\overset{+}{C}$H–OTMS OTMS
	307	TMSO–CH_2–CH–$\overset{+}{C}$H–OTMS OTMS
	319	TMSO–CH_2–CH=C–$\overset{+}{C}$H–OTMS OTMS

The differentiation of all methyl mono-, di-, and tri-O-methylglucosides and mono-O-methylglucitols could be easily achieved on the basis of the characteristic mass spectral patterns of their TMS ethers. (The mass spectral patterns of 3-O- and 4-O-methylgulcitols are similar, but in this study the possibility of 4-O-methylglucitol can be ruled out since the hydroxyl group at C-4 in the reducing residue is involved in the glucoside linkage of cellobiose.) Presumed structures of main fragment ions obtained from TMS ethers of glucose, partially methylated glucoses, glucitol, and monomethylglucitols are given in Table VI.

From the above results, each component of the methanolyzates was identified by comparison with a reference compound (Table VII) and thus the structures of partially acetylated **1** and **5** were unambiguously established as shown in Tables I and II.

TABLE VII. The Compositions of the Methanolyzates

Starting compound	From non-reducing residue	From reducing residue
A	1-OCH_3-glucose	3-OCH_3-glucitol
B	1,4-OCH_3-glucose	3-OCH_3-glucitol
C	1,2-OCH_3-glucose	3-OCH_3-glucitol
D	1-OCH_3-glucose	1,3-OCH_3-glucose
E	1,4-OCH_3-glucose	1,3-OCH_3-glucose
F	1,2-OCH_3-glucose	1,3-OCH_3-glucose
G	1,2,4-OCH_3-glucose	1,3-OCH_3-glucose
H	1-OCH_3-glucose	1,2,3-OCH_3-glucose
I	1,4-OCH_3-glucose	1,2,3-OCH_3-glucose
J	1,2-OCH_3-glucose	1,2-OCH_3-glucose
K	1,2,4-OCH_3-glucose	1,2-OCH_3-glucose

Experimental

Column chromatography was performed on a Lobar prepacked column, LiChroprep Si 60 (40—63 μm), size B or C (E. Merck). Semiprep. HPLC was conducted with a high pressure micro pump KHD-W-104, a high pressure universal injector KHP-UI-130, both from Kyowa Seimitsu Co. Ltd., a differential refractometer R-401 (Waters Assoc.), and a recorder VP-653B (National). For HPLC, four stainless steel columns (30 cm × 4 mm I.D. each, Waters Assoc.), packed with LiChroprep Si 60 (15—25 μm, E. Merck) were connected in series. The following solvent systems (v/v) were used: (a) 5: 1, (b) 4: 1, (c) 3: 1, (d) 2: 1, (e) 1: 1 benzene–

ethyl acetate. TLC was performed on TLC plates, silica gel 60 (0.25 mm, E. Merck) with solvent systems (d) and (e). Spots were detected by spraying with anthrone-sulfuric acid. A Carl Zeiss chromatogram spectrodensitometer, set at 260 nm, was used for quantitative analyses of the partially acetylated products on TLC. Optical rotations were determined with a Jasco DIP-4 automatic polarimeter. GC–MS was carried out using a Hitachi RMU-6MG gas chromatograph-mass spectrometer. The ionization potential was 20 eV, and the accelerating voltage 3.27 kV. The separator temperature was maintained at 235°C and the ion source temperature at 170°C. A glass column (2 m×3 mm I.D.) filled with 3% SE-30 on Chromosorb W (AW-DMCS, 80—100 mesh) was used isothermally at 180°C. The carrier gas was helium (0.68 kg/cm²). NMR spectra were recorded with solutions in chloroform-*d* on a Varian A-60A spectrometer, with tetramethylsilane as an internal standard. Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected.

Selective Acetylation of 6,6′-Di-O-tritylcellobiose (1)——All solvents used were anhydrous. Compound **1**[3] (1 g) was finely powdered in a mortar and then dissolved in pyridine (3 ml) and toluene (15 ml). The solution was kept below 5°C and acetyl chloride (0.43 ml, 5 mol. equiv.) in toluene (3 ml) was added to the solution dropwise during 15 min. The resulting slurry was stirred at 1—4°C for 6 h and then the white precipitate was filtered off and rinsed twice with fresh toluene. The combined filtrates were washed with water, dried with $MgSO_4$, and evaporated to a syrup (0.94 g). TLC [solvent (d)] showed the presence of three pairs of major components (**A**, **B**, and **C**) and several minor components.

Separation of the Partially Acetylated Derivatives of 1——The mixture of reaction products was fractionated on a Lobar column, size C. First of all, a pair of **A** (α, β) was eluted with solvent (a) and the second fraction, containing a pair of **B** (α, β), followed. The third fraction, eluted with solvent (b), included α- and β-anomers of **C**. Each fraction was rechromatographed several times by semiprep. HPLC with solvent (c) and **A** (α), **A**(β), **B**(β), and **C**(β) were obtained in chromatographically pure states.

Selective Acetylation of Methyl 6,6′-Di-O-trityl-β-cellobioside (5) and Separation of the Partially Acetylated Products——Compound **5** (2 g) was partially acetylated with 5 mol. equiv. (0.85 ml) of acetyl chloride (*d* 1.104) as described above and a mixture of syrupy products (2.11 g) was obtained. Elution of the mixture from a Lobar column (size C) with solvents (a), (b), and (c) gave the following products: **D**, **E**, and **F** in chromatographically pure states (the 1st—3rd fractions), **G** containing a small amount of **F** (the 4th fraction), a mixture of **G** and **H** (the 5th fraction), **I** accompanied by **H** (the 6th fraction), and a mixture of **J** and **K** (the 7th fraction). **G** was isolated from the 4th fraction with solvent (c) and from the 5th fraction with solvent (d) by repeated semiprep. HPLC. Similarly **H** was isolated from the 5th fraction with solvent (d), **I** was isolated from the 6th fraction with solvent (d), and **J** and **K** were isolated from the 7th fraction with solvent (e).

Determination of the Site of the Unacetylated Hydroxyl Group——(i) Methylation: The partially acetylated compound (0.2 mmol), contained in a sealed tube, was dissolved in dichloromethane (5 ml), then 2,6-di-*tert*-butyl-4-methylpyridine (4.0 mmol) was added and the tube was flushed with nitrogen. Next, methyl trifluoromethanesulfonate (2.0 mmol) was added to the tube. The tube was sealed and heated at 80°C overnight. The reaction mixture was concentrated to dryness. The product was then isolated by CC, using a Lobar column (size B).

(ii) Detritylation and Deacetylation:[15] The methylated compound (0.1 mmol) was dissolved in 10 ml of 80% aqueous acetic acid and heated for 30 min at 100°C. The solvent was removed under reduced pressure, and traces of acetic acid were removed from the residue by repeated codistillation with methanol. The residue was dissolved in 0.05 M methanolic sodium methoxide (2—3 ml) and the solution was stirred for 30 min at room temperature. After complete deacetylation had been confirmed by TLC, Amberlite IR-120B (H^+) resin was added and the suspension was stirred for 30 min, then filtered. Water was added to the filtrate, the precipitated triphenylmethanol was filtered off, and the filtrate was evaporated to dryness.

(iii) Reduction:[16] The sugar derivative (0.1 mmol) solution in 2 ml of water was cooled in ice and mechanically stirred. To this solution, sodium borohydride (0.1 mmol) in 0.5 ml of water was added gradually. Reduction is complete when an aliquot of the solution acidified with acetic acid fails to give a positive reducing test with aniline hydrogen phthalate reagent. At this point, 6 N acetic acid was slowly added to the stirred solution until the evolution of hydrogen ceased and solution was just acidified (pH 6). Amberlite IR-120B (H^+) resin was added and the suspension was stirred for 30 min, then filtered. The filtrate was concentrated to dryness, the syrupy residue was taken up repeatedly in methanol, and the solvent was distilled under reduced pressure to remove methyl borate.

(iv) Methanolysis:[17] Methanolic 0.5% HCl (0.5 ml) was added to the sugar derivative (0.05 mmol) in a glass tube and N_2 was bubbled through for 30 sec, after which the tube was sealed and heated overnight at 90°C. The acid was then neutralized with Amberlite IRA-410 (OH^-) resin and the mixture was filtered. The filtrate was concentrated to dryness.

(v) Trimethylsilylation:[18] A sample sugar (1—2 mg) in dry pyridine (500 μl) was shaken vigorously with N-trimethylsilylimidazole (100 μl) and trimethylchlorosilane (50 μl), and the mixture was allowed to stand for 30 min at room temperature.

Syntheses of Partially Methylated Glucoses and Glucitols——Methyl α-D-glucoside was prepared by condensation of D-glucose with methanol, using Dowex 50 (H^+) as a catalyst.[19] mp 167—169°C [lit. mp 167—169°C].

Methyl β-D-glucoside was prepared by methylation and deacetylation of tetra-O-acetyl-α-D-glucosyl bromide. mp 111°C, $[\alpha]_D^{22}$ −32.7° (c=2, H_2O) [lit.[20] mp 108—100°C, $[\alpha]_D^{20}$ −32° (c=8, H_2O)].

2-O-Methyl-D-glucose was prepared by methylation and deacetylation of 1,3,4,6-tetra-O-acetyl-α-D-glucose, mp 98°C [lit.[21] mp 98—99°C]. mp 157—158°C [lit.[22] mp 157—159°C].

3-O-Methyl-D-glucose: 3-O-Methyl-1,2: 5,6-diisopropylidene-D-glucofuranose (prepared by methylation according to Hakomori's method[23] from 1,2: 5,6-diisopropylidene-D-glucofuranose) was deisopropyridenated with Amberlite IR-120B (H^+)[24] to give 3-O-methyl-D-glucose. mp 166.5—168°C [lit.[22] mp 168°C].

4-O-Methyl-D-glucose was prepared by methylation and deacetylation of 1,2,3,6-tetra-O-acetyl-β-D-glucose, mp 127—127.5°C, $[\alpha]_D^{21.5}$ −33.5° [lit.[25] mp 131—133°C, $[\alpha]_D^{23}$ −32.2°]. Chromatographically pure syrup.

6-O-Methyl-D-glucose was prepared by methylation and deacetylation of 1,2,3,4-tetra-O-acetyl-β-D-glucose, mp 118—119°C, $[\alpha]_D^{21.5}$ +8.3° [lit.[26] mp 128—129°C, $[\alpha]_D^{20}$ +12.1°]. mp 146—147°C [lit.[22] mp 143—145°C].

Methyl 2-O-methyl-D-glucoside was prepared by methanolysis of 2-O-methyl-D-glucose.

Methyl 3-O-methyl-β-D-glucoside was prepared by deacetylation of methyl 3-O-methyl-2,4,6-tri-O-acetyl-β-D-glucoside, mp 91°C [lit.[22] mp 90°C]. Chromatographically pure syrup.

Methyl 4-O-methyl-β-D-glucoside was prepared by deacetylation of methyl 4-O-methyl-2,3,6-tri-*O*-acetyl-β-D-glucoside, mp 110°C, $[\alpha]_D^{20}$ −33.5° [lit.[22] mp 106—108°C, $[\alpha]_D$ −34°]. Needles, mp 101°C, $[\alpha]_D^{20}$ −15.0° (c=2.0, CH_3OH). *Anal.* Calcd for $C_8H_{16}O_6$: C, 46.15; H, 7.75. Found: C, 46.27; H, 8.00 [lit.[22] liquid].

Methyl 6-O-methyl-α-D-glucoside was prepared by methylation and deacetylation of methyl 2,3,4-tri-O-acetyl-α-D-glucoside, mp 108—108.5°C, $[\alpha]_D^{22}$ +120.0° which was synthesized from methyl 2,3,4-tri-O-acetyl-6-O-trityl-α-D-glucoside, mp 137—138°C [lit.[27] mp 136°C]. Chromatographically pure syrup.

Methyl mono-O-methyl-D-glucosides were also prepared by methanolyses of the corresponding mono-O-methyl-D-glucoses.

Methyl 2,3-di-O-methyl-α-D-glucoside was prepared by methylation and debenzylidenation of methyl 4,6-O-benzylidene-α-D-glucoside, mp 168—169.5°C, $[\alpha]_D^{20}$ +85.0° [lit.[28] mp 161—162°C, $[\alpha]_D$ +85°]. mp 84—85°C [lit.[22] 83—84°C].

Methyl 2,4-Di-O-methyl-D-glucoside and Methyl 2,6-Di-O-methyl-D-glucoside: Detritylation of 2-O-methyl-6-O-trityl-α-D-glucose triacetate (mp 85.5—87.0°C) with a saturated solution of hydrogen bromide in acetic acid[29] gave two products, which were separated by CC. One of them was 1,3,4-tri-O-acetyl-2-O-methyl-α-D-glucose, which could be converted to the starting compound by retritylation, and the other was 1,3,6-tri-O-acetyl-2-O-methyl-α-D-glucose, which was generated by acetyl migration from O-4 to O-6. Both compounds were methylated with methyl trifluoromethanesulfonate and deacetylated. Methanolyses of the resulting products afforded the title compounds.

Methyl 3,4-Di-O-methyl-D-glucoside and Methyl 3,6-Di-O-methyl-D-glucoside: 3-O-Methyl-6-O-trityl-α-D-glucose triacetate was treated in the same manner as described above.

Methyl 2,3,4-tri-O-methyl-α-D-glucoside was prepared by methylation and detritylation of methyl 6-O-trityl-α-D-glucoside, mp 152—152.5°C, $[\alpha]_D^{26}$ +56.5° [lit.[27] mp 151—152°C]. Chromatographically pure syrup.

Mono-O-methyl-D-glucitols were prepared by reduction with $NaBH_4$ of the corresponding mono-O-methyl-D-glucoses.

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