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Trityl Derivatives of Cellobiose. VI.[1] Unambiguous Assignments of Acetoxyl Group Resonances in the ^{1}H-NMR Spectra of 6,6′-Di-O-trityl-, 6-O-Trityl-, and 6′-O-Tritylcellobiose Peracetates[2]

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The ^{1}H-NMR spectra of 6,6′-di-O-trityl-, 6-O-trityl-, and 6′-O-tritylcellobiose peracetates (I-1, II-1, and III-1) showed some of the acetoxyl group resonances at abnormally high fields in $CDCl_3$ and all their acetoxyl group signals were resolved in the region of δ 1.56—2.16. Each individual acetoxyl group signal in the spectra was unambiguously assigned. For this purpose, analogs having trideuterioacetoxyl groups at specific positions were synthesized. The 2′-acetoxyl group exposed to the benzene nuclei of the triphenylmethoxyl group (TrO) at C-6 resonates at the highest field. The large upfield shifts of the 4′- and 3-acetoxyl group signals of the derivatives having TrO at C-6′ suggest that there are strong interactions between those acetoxyl groups and TrO. In the spectra of the derivatives which have TrO at C-6, a downfield shift of the 1-acetoxyl group signal was observed.

Keywords——6,6′-di-O-tritylcellobiose peracetate; 6′-O-tritylcellobiose peracetate; 6-O-tritylcellobiose peracetate; methyl 6,6′-di-O-trityl-β-cellobioside peracetate; methyl 6′-O-trityl-β-cellobioside peracetate; methyl 6-O-trityl-β-cellobioside peracetate; trideuterioacetyl analog; ^{1}H-NMR spectrum; acetoxyl group signal

The aceyoxyls group signals in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of octa-O-acetylcellobiose are concentrated in the regio of δ 1.98—2.11, whereas the ^{1}H-NMR spectra of 6,6′-di-O-trityl-, 6-O-trityl-, and 6′-O-tritylcellobiose peracetates (I-1, II-1, and III-1) show some of the acetoxyl group resonances at abnormally high fields in $CDCl_3$ and all their acetoxyl group signals are well resolved in the region of δ 1.56—2.16.[3]

In the present work, I-1, II-1, and III-1, and their methyl glycosides, having trideuterioacetoxyl groups at specific positions, were prepared to permit unambiguous assignment of each individual acetoxyl group signal in the ^{1}H-NMR spectra of I-1, II-1, and III-1.

The method of specific spectral assignments for acetoxyl group resonances by the use of trideuterioacetyl analogs was pioneered by Horton *et al.*,[4] and similar studies on partial or complete assignments of acetoxyl group signals of monosaccharide[5] and disaccharide[6] derivatives have been reported, together with related studies in which methoxyl group resonances in permethylated sugars have been assigned through syntheses of specifically trideuteriomethylated derivatives.[7]

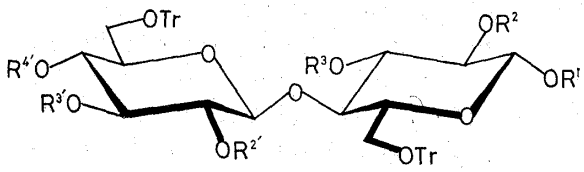
Results and Discussion

Comparison of acetoxyl group resonances of I-1, II-1, and III-1 with those of their methyl glycosides (I-2, II-2, and III-2), in each case, showed that the methoxyl group at C-1 of methyl cellobioside peracetate essentially did not affect the chemical shift of any acetoxyl group in the same cellobioside molecule. Therefore, not only trideuterioacetyl analogs of I-1, II-1, and III-1 but also those of I-2, II-2, and III-2 were used for the purpose of this study.

Preparation of Specifically Trideuterioacetylated Derivatives

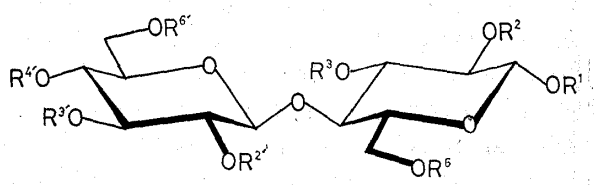
The di-O-tritylcellobiose hexaacetate (I-1) and the two isomeric mono-O-tritylcellobiose heptaacetates (II-1 and III-1) were synthesized from cellobiose by direct tritylation, separation

by column chromatography (CC), and individual acetylation.[3] Their methyl glycosides (I-2, II-2, and III-2) were synthesized from the methyl β-cellobiosides in the same manner as described above.[8] Trideuterioacetyl analogs (I-3—I-13) of I-1 and I-2 were prepared by acetylation of the selective acetylation products of 6,6′-di-O-tritylcellobiose and its methyl β-glycoside[9] with acetic anhydride-d_6 and pyridine. The structures of the selective acetylation products were unambiguously established by the following procedure; first of all, unacetylated hydroxyl groups were labelled by methylation. As reagents for the methylation, dimethylsulfinylsodium in dimethyl sulfate, which was prepared according to the method of Hakomori,[10] and diazomethane-boron trifluoride etherate in dichloromethane[11] were tested, using model compounds such as 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-β-cellobiose (**A**)[3] and methyl 6,6′-di-O-trityl-β-cellobioside (**B**).[12] On methylation by Hakomori's method, **B** was safely and completely methylated, and this proved that the O-trityl group was stable to the treatment. However, the methylation of **A** by this method resulted in deacetylation. Thin-layer chromatography (TLC) showed that the methylation of **A** and **B** with CH_2N_2–BF_3·Et_2O did not affect O-acetyl groups, but caused partial detritylation. Recently, Arnarp *et al.*[13] proposed methyl trifluoromethanesulfonate–2,6-di-*tert*-butyl-4-methylpyridine as a relatively mild methylation reagent. After it had been confirmed that both O-acetyl and O-trityl groups were entirely stable during methylation by this reagent at 80°C, unacetylated hydroxyl groups of the selective acetylation products were carefully methylated according to this method. Although a reaction time of 5 h was generally sufficient, a longer time was required for the complete methylation of HO-3 (overnight or a little longer). The methylation product was purified by CC to remove excess reagent, a small amount of the starting material, and traces of by-products. The site of the methoxyl group was determined by gas chromatography–mass spectrometry (GC–MS) after the sequence of detritylation, deacetylation, (reduction), methanolysis, and trimethylsilylation in the usual manner. In the cases of methyl cellobioside



I- 1: $R^1=R^2=R^3=R^{2'}=R^{3'}=R^{4'}=Ac$
I- 2: $R^1=Me$, $R^2=R^3=R^{2'}=R^{3'}=R^{4'}=Ac$
I- 3: $R^3=Ac\text{-}d_3$, $R^1=R^2=R^{2'}=R^{3'}=R^{4'}=Ac$
I- 4: $R^3=R^{4'}=Ac\text{-}d_3$, $R^1=R^2=R^{2'}=R^{3'}=Ac$
I- 5: $R^3=R^{2'}=Ac\text{-}d_3$, $R^1=R^2=R^{3'}=R^{4'}=Ac$
I- 6: $R^1=Me$, $R^3=Ac\text{-}d_3$, $R^2=R^{2'}=R^{3'}=R^{4'}=Ac$
I- 7: $R^1=Me$, $R^3=R^{4'}=Ac\text{-}d_3$, $R^2=R^{2'}=R^{3'}=Ac$
I- 8: $R^1=Me$, $R^3=R^{2'}=Ac\text{-}d_3$, $R^2=R^{3'}=R^{4'}=Ac$
I- 9: $R^1=Me$, $R^2=R^3=Ac\text{-}d_3$, $R^{2'}=R^{3'}=R^{4'}=Ac$
I-10: $R^1=Me$, $R^2=R^{2'}=Ac\text{-}d_3$, $R^3=R^{3'}=R^{4'}=Ac$
I-11: $R^1=Me$, $R^3=R^{2'}=R^{4'}=Ac\text{-}d_3$, $R^2=R^{3'}=Ac$
I-12: $R^1=Me$, $R^2=R^3=R^{4'}=Ac\text{-}d_3$, $R^{2'}=R^{3'}=Ac$
I-13: $R^1=Me$, $R^2=R^{2'}=R^{4'}=Ac\text{-}d_3$, $R^3=R^{3'}=Ac$
Tr=trityl, Ac=acetyl, Me=methyl

Chart 1. Derivatives of 6,6′-Di-O-trityl-β-cellobiose (I)



In II series, $R^{6'}=Tr$
II- 1: $R^1=R^2=R^3=R^6=R^{2'}=R^{3'}=R^{4'}=Ac$
II- 2: $R^1=Me$, $R^2=R^3=R^6=R^{2'}=R^{3'}=R^{4'}=Ac$
II- 3*: $R^3=Ac\text{-}d_3$, $R^1=R^2=R^6=R^{2'}=R^{3'}=R^{4'}=Ac$
II- 4*: $R^3=R^{4'}=Ac\text{-}d_3$, $R^1=R^2=R^6=R^{2'}=R^{3'}=Ac$
II- 5: $R^1=Me$, $R^{4'}=Ac\text{-}d_3$, $R^2=R^3=R^6=R^{2'}=R^{3'}=Ac$
II- 6: $R^1=Me$, $R^6=Ac\text{-}d_3$, $R^2=R^3=R^{2'}=R^{3'}=R^{4'}=Ac$
II- 7: $R^1=Me$, $R^3=Ac\text{-}d_3$, $R^2=R^6=R^{2'}=R^{3'}=R^{4'}=Ac$
II- 8: $R^1=Me$, $R^3=R^{4'}=Ac\text{-}d_3$, $R^2=R^6=R^{2'}=R^{3'}=Ac$
II- 9: $R^1=Me$, $R^3=R^{2'}=Ac\text{-}d_3$, $R^2=R^6=R^{3'}=R^{4'}=Ac$
II-10: $R^1=Me$, $R^2=R^3=Ac\text{-}d_3$, $R^6=R^{2'}=R^{3'}=R^{4'}=Ac$
II-11: $R^1=Me$, $R^2=R^3=R^{4'}=Ac\text{-}d_3$, $R^6=R^{2'}=R^{3'}=Ac$
In III series, $R^6\triangleq Tr$
III-1: $R^1=R^2=R^3=R^{2'}=R^{3'}=R^{4'}=R^{6'}=Ac$
III-2: $R^1=Me$, $R^2=R^3=R^{2'}=R^{3'}=R^{4'}=R^{6'}=Ac$
III-3: $R^3=Ac\text{-}d_3$, $R^1=R^2=R^{2'}=R^{3'}=R^{4'}=R^{6'}=Ac$
III-4*: $R^{2'}=Ac\text{-}d_3$, $R^1=R^2=R^3=R^{3'}=R^{4'}=R^{6'}=Ac$
III-5: $R^1=Me$, $R^{6'}=Ac\text{-}d_3$, $R^2=R^3=R^{2'}=R^{3'}=R^{4'}=Ac$
III-6: $R^1=Me$, $R^{4'}=Ac\text{-}d_3$, $R^2=R^3=R^{2'}=R^{3'}=R^{6'}=Ac$
III-7: $R^1=Me$, $R^3=Ac\text{-}d_3$, $R^2=R^{2'}=R^{3'}=R^{4'}=R^{6'}=Ac$
III-8: $R^1=Me$, $R^{2'}=Ac\text{-}d_3$, $R^2=R^3=R^{3'}=R^{4'}=R^{6'}=Ac$
Tr=trityl, Ac=acetyl, Me=methyl, * α-anomer

Chart 2. Derivatives of 6′-O-Trityl-β-cellobiose (II) and 6-O-Trityl-β-cellobiose (III)

derivatives, the labelling of the reducing glucose unit by reduction before methanolysis was impossible. Hence, which of two methyl glucosides in methanolyzates arose from the reducing residue was judged by ^{1}H-NMR of the trideuterioacetyl ester of the original partially acetylated compound on the basis of the acetoxyl group resonances in the ^{1}H-NMR spectra of I-3, I-4, and I-5, the structures of which had already been estabilshed by the method mentioned above. For the GC–MS, methyl glucoside, four kinds of monomethylglucoses, four kinds of methyl monomethylglucosides, five kinds of methyl dimethyl glucosides, methyl 2,3,4-tri-O-methyl-glucoside, and four kinds of monomethylglucitols were prepared by unambiguous routes.[9] Each component of the methanolyzates was identified by comparison with those reference compounds, and thus the structures of the selective acetylation products were unambiguously established.

II-5, the structure of which is given in Chart 2, was synthesized from methyl 4′,6′-O-benzylidene-β-cellobioside peracetate by sequential debenzylidenation, tritylation of HO-6′, and acetylation of HO-4′ with acetic anhydride-d_6.

II-6, III-5, and III-6 were prepared as follows: detritylation of methyl 6,6′-di-O-trityl-β-cellobioside peracetate (I-2) with 80% acetic acid afforded a mixture of methyl 2,3,2′,3′,4′-penta-O-acetyl-β-cellobioside and methyl 2,3,2′,3′,6′-penta-O-acetyl-β-cellobioside (on TLC). The latter was produced by acetyl migration from O-4′ to O-6′. The mixture was retritylated with 1.5 molar equivalents of trityl chloride. The resulting methyl 6′-O-trityl-β-cellobioside pentaacetate, having a free hydroxyl group at C-6 (II-6′), and two isomeric methyl 6-O-trityl-β-cellobioside pentaacetates, one of which had a free hydroxyl group at C-6′ (III-5′) and the other at C-4′ (III-6′), were separated by CC and then acetylated with acetic anhydride-d_6 to afford II-6, III-5, and III-6. The structures of these compounds were elucidated in the following way: II-6 is an analog of II-2, and III-5 and III-6 are analogs of III-2. Tritylation of III-5′ gave the 6,6′-di-O-trityl derivative I-2, whereas III-6′ could not be tritylated with trityl chloride.

The other trideuterioacetyl analogs of II-1 and II-2 given in Chart 2 were prepared by acetylation of the selective acetylation products of 6′-O-tritylcellobiose and its methyl β-glycoside[1] with acetic anhydride-d_6. Trideuterioacetyl analogs of III-1 (III-3 and III-4) and those of III-2 (III-7 and III-8), together with III-6, were prepared by trideuterioacetylation of the selective acetylation products of 6-O-tritylcellobiose and its methyl β-glycoside,[1] respectively. The structures of the selective acetylation products from 6- or 6′-mono-O-tritylcellobioses and their methyl β-glycosides were also unambiguously established as mentioned previously. It should be noted that complete acetylation of HO-3 in the molecule having TrO-6′ with acetic anhydride-d_6 and pyridine at room temperature is relatively difficult that is, the complete acetylation of HO-3 in partially acetylated 6,6′-di-O-trityl and 6′-mono-O-trityl derivatives required 40—45 h at 40°C and approximate 40 molar equivalents of the reagent, acetic anhydride-d_6. On the other hand, HO-3 in partially acetylated 6-mono-O-trityl derivatives was completely acetylated with half the above amount of the reagent, overnight at room temperature.

^{1}H-NMR Spectra of 6,6′-Di-O-trityl-β-cellobiose Derivatives (I-1—I-13)

The chemical shifts of the acetoxyl group signals in the 100 MHz spectra of the series of 6,6′-di-O-trityl-β-cellobiose derivatives selectively labelled with trideuterioacetoxyl groups are given in Table I. Chloroform-*d* which gives the best separation of all signals in most cases,[5c] was used as the solvent. For example, the pentaacetate having a trideuterioacetoxyl group at C-3, I-3, gave an ^{1}H-NMR spectrum identical with that of I-1 except that one of the acetoxyl group signals was absent, and this missing signal could thus be assigned to the 3-acetoxyl group. In the same manner, the six individual acetoxyl group signals in the ^{1}H-NMR spectrum of 1,2,3,2′,3′,4′-hexa-O-acetyl-6,6′-di-O-trityl-β-cellobiose (I-1) could be unequivocally assigned as shown in Table I.

Table I. Chemical Shifts of Acetoxyl Group Signals in the ^{1}H-NMR Spectra of 6,6′-Di-O-trityl-β-cellobiose Derivatives (I-1—I-13) in $CDCl_3$ and Their Assignments

Compds.	OAc-d_3 at	Acetoxyl group signals δ (ppm)					
I- 1		2.16	2.02	1.91	1.77	1.67	1.61
I- 2			2.04	1.92	1.78	1.69	1.63
I- 3	C-3	2.16	2.02	1.91		1.67	1.61
I- 4	C-3, C-4′	2.16	2.02	1.91			1.61
I- 5	C-3, C-2′	2.16	2.02	1.91		1.67	
I- 6	C-3		2.04	1.92		1.69	1.63
I- 7	C-3, C-4′		2.04	1.92			1.63
I- 8	C-3, C-2′		2.04	1.92		1.69	
I- 9	C-2, C-3			1.92		1.69	1.63
I-10	C-2, C-2′			1.92	1.78	1.69	
I-11	C-3, C-2′, C-4′		2.04	1.92			
I-12	C-2, C-3, C-4′			1.92			1.63
I-13	C-2, C-2′, C-4′			1.92	1.78		
Assigned to		AcO-1	AcO-2	AcO-3′	AcO-3	AcO-4′	AcO-2′

Table II. Chemical Shifts of Acetoxyl Group Signals in the ^{1}H-NMR Spectra of 6′-O-Tritylcellobiose Derivatives (II-1—II-11) in $CDCl_3$ and Their Assignments

Compds.	OAc-d_3 at	Acetoxyl group signals δ (ppm)						
II- 1		2.09	2.06	2.02	2.01	1.95	1.82	1.71
II- 1′[a]		2.10	2.07	2.02	1.99	1.95	1.81	1.71
II- 2		2.09		2.03	2.02	1.95	1.83	1.72
II- 3[a]	C-3	2.10	2.07	2.02	1.99	1.95		1.71
II- 4[a]	C-3, C-4′	2.10	2.07	2.02	1.99	1.95		
II- 5	C-4′	2.09		2.03	2.02	1.95	1.83	
II- 6	C-6			2.03	2.02	1.95	1.83	1.72
II- 7	C-3	2.09		2.03	2.02	1.95		1.72
II- 8	C-3, C-4′	2.09		2.03	2.02	1.95		
II- 9	C-3, C-2′	2.09			2.02	1.95		1.72
II-10	C-2, C-3	2.09		2.03		1.95		1.72
II-11	C-2, C-3, C-4′	2.09		2.03		1.95		
Assigned to		AcO-6	AcO-1	AcO-2′	AcO-2	AcO-3′	AcO-3	AcO-4′

a) The α-anomer. The others are all the β-anomers.

^{1}H-NMR Spectra of 6′-O-Tritylcellobiose Derivatives (II-1—II-11)

By comparison of the acetoxyl group resonances in the spectra of 6′-O-tritylcellobiose peracetate (II-1) and its methyl glycoside (II-2) with those of their trideuterioacetyl analogs (II-3—II-11) as given in Table II, each individual acetoxyl group signal could be unambiguously assigned. The large upfield shift of the 4′-acetoxyl group signal suggested that there is strong interaction between the acetoxyl group and the benzene nuclei of the trityl group at C-6′. The previous study on the selective acetylation of 6′-O-tritylcellobiose and its methyl glycoside[1] proved that HO-3 is more sterically hindered by TrO-6′ than HO-4′ is, but nevertheless, the upfield shift of the 3-acetoxyl group signal was smaller than that of the 4′-acetoxyl group signal. This result suggests that the orientation of the benzene ring of TrO-6′ is such that the protons on the 4′-acetoxyl group are in the shielding region of the benzene ring. The 2- and 2′-acetoxyl group signals in the spectra of β-anomers overlap considerably and hence it is difficult to distinguish them. On the other hand, those in the spectra of α-anomers are clearly resolved. The assignment of the signal at δ 1.99 to the 2-acetoxyl group was based on analysis of the acetoxyl group signals in the spectrum of 1,6,2′,3′,4′-penta-O-acetyl-2,3-di-O-trideuterioacetyl-6′-O-trityl-α-cellobiose, which was prepared from an unusual di-O-trityl

derivative of cellobiose, 2,6′-di-O-tritylcellobiose[14] by the following sequential treatments; acetylation under the usual conditions to give 1,6,2′,3′,4′-penta-O-acetyl-2,6′-di-O-trityl-α-cellobiose, partial detritylation in $CHCl_3$ at 80 °C to remove TrO-2, and trideuterioacetylation of HO-2 and HO-3 to afford the title compound.

Table III. Chemical Shifts of Acetoxyl Group Signals in the ^{1}H-NMR Spectra of 6-O-Tritylcellobiose Derivatives (III-1—III-8) in $CDCl_3$ and Their Assignments

Compds	OAc-d_3 at	Acetoxyl group signals δ(ppm)						
III-1		2.14	2.09	2.04	2.04	1.97	1.92	1.56
III-1′[a]		2.11	2.09	2.06	2.02	1.98	1.93	1.60
III-2			2.10	2.06	2.04	1.98	1.94	1.57
III-3	C-3	2.14	2.09	2.04		1.97	1.92	1.56
III-4[a]	C-2′	2.11	2.09	2.06	2.02	1.98	1.93	
III-5	C-6′			2.06	2.04	1.98	1.94	1.57
III-6	C-4′		2.10	2.06	2.04		1.94	1.57
III-7	C-3		2.10	2.06		1.98	1.94	1.57
III-8	C-2′		2.10	2.06	2.04	1.98	1.94	
Assigned to		AcO-1	AcO-6′		AcO-3	AcO-4′		AcO-2′

a) The α-anomer. The others are all the β-anomers.

^{1}H-NMR Spectra of 6-O-Tritylcellobiose Derivatives (III-1—III-8)

The chemical shifts of the acetoxyl group signals in the ^{1}H-NMR spectra of the series of 6-O-trityl cellobiose derivatives in $CDCl_3$ and their assignments are given in Table III. The 2′-acetoxyl group signal shows an extremely large upfield shift and therefore, the 2′-acetoxyl group must lie within the shielding cone(s) of one or more of the benzene rings of the TrO-6. It is known that all glucopyranose derivatives which have an acetoxyl group at C-6 show the resonance of this group at the lowest field of the set of acetoxyl group signals.[5c] The 6-acetoxyl group in the series of II and the 6′-acetoxyl group in the series of III resonated at about the same low field, δ 2.09—2.10. In the ^{1}H-NMR spectra of the series of III having TrO-6, a downfield shift of the 1-acetoxyl group signal across the chemical shift of the 6′-acetoxyl group signal was observed. A similar downfield shift of the 1-acetoxyl group signal was seen in the spectra of the series of I which also have TrO-6. Although it is impossible to assign the signals at δ 2.06 and δ 1.94 in the spectrum of III-2 from the present study, the former may be that of the 2-acetoxyl group and the latter, that of the 3′-acetoxyl group by analogy with the resonances of the 2- and 3′-acetoxyl groups in the spectra of the series of I and II, as it is likely that the 2- and 3′-acetoxyl groups are little affected by either TrO-6 or TrO-6′.

Experimental

The ^{1}H-NMR spectra were recorded on a Varian HA-100 (100 MHz) spectrometer, using chloroform-*d* solutions and with tetramethylsilane as an internal reference. The other general methods were the same as described previously.[9] The solvent systems (v/v) for CC and TLC were (a) 1:1, (b) 2:1, (c) 3:1, (d) 4:1, (e) 5:1 benzene–ethyl acetate.

Methyl 2,3,6,2′,3′-Penta-O-acetyl-4′-O-trideuterioacetyl-6′-O-trityl-β-cellobioside (II-5)——A mixture of well-dried methyl β-cellobioside (5 g), freshly fused and powdered zinc chloride (5 g), and freshly distilled benzaldehyde (25 ml) was shaken for 22 h at room temperature. The mixture was poured into ice-water (60 ml) containing methanol (6 ml). Excess benzaldehyde was removed by repeated extraction with petroleum ether (bp 40—50°C, 50 ml × 6). Aqueous sodium carbonate was added to the aqueous layer for neutralization. The mixture was filtered and the residue was washed with hot methanol (30 ml × 2). The combined filtrate and washings were evaporated to dryness and the residue was crystallized from 85% ethanol. Recrystallization from 95% ethanol gave pure methyl 4′,6′-O-benzylidene-β-cellobioside, yield 2.9 g (46.8%), mp 154—155°C. A solution of methyl 4′,6′-O-benzylidene-β-cellobioside (1.2 g) in dry pyridine (7 ml) was treated with acetic anhydride (7 ml) at 0°C under stirring, and the mixture was kept overnight at room temperature, then poured into ice-water. The resulting precipitate was filtered off, air-dried, and recrystallized twice from ethanol to give methyl 2,3,6,2′,3′-penta-O-acetyl-4′,6′-O-benzylidene-β-cellobioside, yield

1.4 g (79.1%), mp 241°C, $[\alpha]_D^{21}$ −40.0° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{18}O_{16}$: C, 55.04; H, 5.85. Found: C, 54.87; H, 5.73. This compound (1.4 g) was dissolved in 100 ml of 50% aqueous acetic acid and heated for 10 min at 100°C. The solvents were evaporated off under reduced pressure and the last trace of acetic acid was removed from the residue by repeated codistillation with ethanol to give chromatographically pure syrup, 1.2 g (96%). Trityl chloride (370 mg, 1.5 mol. equiv.) was added to a solution of this syrup (methyl 2,3,6,2′,3′-penta-O-acetyl-β-cellobioside) (0.5 g) in dry pyridine (10 ml), and the mixture was stirred overnight at 45°C. The mixture was cooled to 20°C, fresh pyridine (2 ml) and acetic anhydride-d_6 (1 ml) were added, and the whole was kept overnight at room temperature, then concentrated to dryness. The residue was purified by CC on a Lobar column with solvent (d) to give an amorphous powder which was crystallized from ethanol, yield 0.45 g (58.6%), mp 153—154°C, $[\alpha]_D^{25}$ +4.5° (c=2.0, $CHCl_3$). *Anal.* Calcd for $C_{44}H_{47}O_{17}D_3$: C, 61.89; H, 5.55. Found: C, 61.85; H, 5.95.

Preparation of Methyl 2,3,2′,3′,4′-Penta-O-acetyl-6′-O-trityl-β-cellobioside (II-6′), Methyl 2,3,2′,3′,4′-Penta-O-acetyl-6-O-trityl-β-cellobioside (III-5′), and Methyl 2,3,2′,3′,6′-Penta-O-acetyl-6-O-trityl-β-cellobioside (III-6′)——Methyl 6,6′-di-O-trityl-β-cellobioside peracetate (I-2)[8] (4.2 g) was dissolved in 250 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 dry pyridine (30 ml), trityl chloride (2.67 g) was added, and the mixture was stirred at 40°C for 45 h, and then poured into ice-water. The precipitate was collected by filtration. The yield of amorphous powder was 6.56 g. TLC with solvent (a) showed the presence of four compounds, *Rf* 0.80, 0.49, 0.45, and 0.39. This mixture (6.56 g) was divided into two parts and fractionated on a Lobar column (size C) with solvent (c). Triphenylmethanol (mp 164—165°C from) methanol, yield 787 mg) was obtained from the 1st fraction, I-2 (*Rf* 0.80, mp 135—137°C from ethanol, $[\alpha]_D^{22}$ +15.6° (c=2.0, $CHCl_3$), yield 1.170 g) from the 2nd fraction, and III-5′ (*Rf* 0.49, mp 124—125°C from acetone and ethanol, $[\alpha]_D^{20}$ −20.0° (c=3.3, $CHCl_3$), yield 712 mg) from the 3rd fraction. The 4th fraction, containing III-6′ accompanied by III-5′, was rechromatographed twice on a Lobar column (size B) with solvent (b) to afford chromatographically pure III-6′, *Rf* 0.45, $[\alpha]_D^{22}$ −41.5° (c=1.8, $CHCl_3$), yield 246 mg. The 5th fraction containing traces of III-5′, III-6′, and II-6′ was discarded. II-6′ from the 6th fraction was crystallized from acetone and ethanol, *Rf* 0.39, mp 241—242°C, $[\alpha]_D^{20}$ +2.5° (c=2.0, $CHCl_3$), yield 443 mg. *Anal.* Calcd for $C_{42}H_{48}O_{16}$: C, 62.37; H, 5.98. Found: C, 62.21; H, 6.11.

General Method of Trideuterioacetylation——a) I and II Series: A sugar derivative having free hydroxyl group(s) at specific position(s) (30—100 mg) was dissolved in 1—3 ml of dry pyridine and then acetylated with acetic anhydride-d_6 (0.5—1.5 ml) for 40—45 h at 40°C. The purity of the product was tested by TLC. If necessary, it was purified by CC using solvent (d) or (e) for the ditrityl derivative and solvent (c) or (d) for the monotrityl derivative.

TABLE IV. Trideuterioacetyl Analogs of 6,6′-Di-O-trityl-β-cellobiose hexaacetate (I-1) and Its Methyl β-Glycoside (I-2)

Compound	$[\alpha]_D$ (in $CHCl_3$) (°)	temp (°C)	c(%)	Yield (%) on trideuterioacetylation
1,2,2′,3′,4′-Penta-O-acetyl-3-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobiose (I-3)	+20.9	24	1.6	99.7
1,2,2′,3′-Tetra-O-acetyl-3,4′-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobiose (I-4)	+19.2	24	1.3	99.0
1,2,3′,4′-Tetra-O-acetyl-3,2′-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobiose (I-5)	+18.5	22	1.0	95.4
Methyl 2,2′,3′,4′-tetra-O-acetyl-3-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-6)	+15.2	24	2.5	71.9
Methyl 2,2′,3′-tri-O-acetyl-3,4′-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-7)	+15.6	24	1.8	82.6
Methyl 2,3′,4′-tri-O-acetyl-3,2′-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-8)	+15.3	24	1.8	68.7
Methyl 2′,3′,4′-tri-O-acetyl-2,3-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-9)	+15.4	21	1.3	67.2
Methyl 3,3′,4′-tri-O-acetyl-2,2′-di-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-10)	+15.0	21	1.4	94.8
Methyl 2,3′-di-O-acetyl-3,2′,4′-tri-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-11)	+15.6	24	1.8	68.5
Methyl 2′,3′-di-O-acetyl-2,3,4′-tri-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-12)	+15.7	24	2.1	69.0
Methyl 3,3′-di-O-acetyl-2,2′,4′-tri-O-trideuterioacetyl-6,6′-di-O-trityl-β-cellobioside (I-13)	+14.2	20	1.2	93.4

Table V. Trideuterioacetyl Analogs of 6′-O-Trityl-α-cellobiose hexaacetate (II-1′) and Its Methyl β-Glycoside (II-2)

Compound	$[\alpha]_D$ (in $CHCl_3$) (°)	temp (°C)	c(%)	Yield (%) on trideuterio-acetylation
1,2,6,2′,3′,4′-Hexa-O-acetyl-3-O-trideurterio-acetyl-6′-O-trityl-α-cellobiose (II-3)	+50.5	22	2.2	96.5
1,2,6,2′,3′-Penta-O-acetyl-3,4′-di-O-tri-deuterioacetyl-6′-O-trityl-α-cellobiose (II-4)	+48.5	21	2.0	95.0
Methyl 2,3,2′,3′,4′-penta-O-acetyl-6-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-6)	+4.5	20	2.5	99.5
Methyl 2,6,2′,3′,4′-penta-O-acetyl-3-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-7)	+5.0	24	2.2	92.5
Methyl 2,6,2′,3′-tetra-O-acetyl-3,4′-di-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-8)	+5.3	22	1.9	93.5
Methyl 2,6,3′,4′-tetra-O-acetyl-3,2′-di-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-9)	+6.0	21	2.0	82.7
Methyl 6,2′,3′,4′-tetra-O-acetyl-2,3-di-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-10)	+7.7	21	1.3	89.5
Methyl 6,2′,3′-tri-O-acetyl-2,3,4′-tri-O-tri-deuterioacetyl-6′-O-trityl-β-cellobioside (II-11)	+5.5	20	1.1	95.3

Table VI. Trideuterioacetyl Analogs of 6-O-Tritylcellobiose hexaacetates (III-1 and III-1′) and the Methyl β-Glycoside (III-2)

Compound	$[\alpha]_D$ (in $CHCl_3$) (°)	temp (°C)	c(%)	Yield (%) on trideuterio-acetylation
1,2,2′,3′,4′,6′-Hexa-O-acetyl-3-O-trideuterio-acetyl-6-O-trityl-β-cellobiose (III-3)	−24.9	25	0.7	95.0
1,2,3,3′,4′,6′-Hexa-O-acetyl-2′-O-trideuterio-acetyl-6-O-trityl-α-cellobiose (III-4)	+23.5	23	1.0	98.2
Methyl 2,3,2′,3′,4′-penta-O-acetyl-6′-O-tri-deuterioacetyl-6-O-trityl-β-cellobioside (III-5)	−27.5	20	3.4	97.4
Methyl 2,3,2′,3′,6′-penta-O-acetyl-4′-O-tri-deuterioacetyl-6-O-trityl-β-cellobioside (III-6)	−25.0	22	2.3	88.3
Methyl 2,2′,3′,4′,6′-penta-O-acetyl-3-O-tri-deuterioacetyl-6-O-trityl-β-cellobioside (III-7)	−26.0	21	1.0	98.2
Methyl 2,3,3′,4′,6′-penta-O-acetyl-2′-O-tri-deuterioacetyl-6-O-trityl-β-cellobioside (III-8)	−25.2	22	2.1	98.0

(b) III Series: Acetic anhydride-d_6 (0.2—0.5 ml) was added to a solution of the sugar derivative (26—70 mg) in dry pyridine (1—2 ml), and the solution was allowed to stand overnight at room temperature. A chromatographically homogeneous product was always obtained.

Trideuterioacetyl analogs of 1,2,3,2′,3′,4′-hexa-O-acetyl-6,6′-di-O-trityl-β-cellobiose (**I-1**), mp 134—136°C, $[\alpha]_D^{22}$ +17.0° (c=2.0, $CHCl_3$), and methyl 2,3,2′,3′,4′-penta-O-acetyl-6,6′-di-O-trityl-β-cellobioside (I-2), mp 135—137°C, $[\alpha]_D^{22}$ +15.6° (c=2.0, $CHCl_3$), are listed in Table IV. Those of 1,2,3,6,2′,3′,4′-hepta-O-acetyl-6′-O-trityl-α-cellobiose (II-1′), mp 165—166°C, $[\alpha]_D^{24}$ +53.3°(c=2.3, $CHCl_3$), and methyl 2,3,6,2′,3′,4′-hexa-O-acetyl-6′-O-trityl-β-cellobioside (II-2), mp 158—160°C, $[\alpha]_D^{20}$ +5.6° (c=2.0, $CHCl_3$), are listed in Table V. Table VI shows the analogs of 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-O-trityl-β-cellobiose (III-1), mp 113°C, $[\alpha]_D^{21}$ −28.0° (c=5.76, $CHCl_3$), its α-anomer (III-1′), mp 201—202°C, $[\alpha]_D^{25}$ +24.0° (c=2.0, $CHCl_3$), and methyl 2,3,2′,3′,4′,6′-hexa-O-acetyl-6-O-trityl-β-cellobioside (III-2), $[\alpha]_D^{24}$ −26.1° (c=3.1, $CHCl_3$).

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