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Trityl Derivatives of Cellobiose. V.[1] Selective Acetylation of 6- and 6′-Mono-O-tritylcellobiose and Their Methyl β-Glycosides[2]

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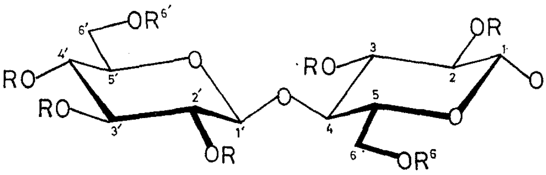
The selective acetylation of 6′-O-tritylcellobiose (**1**), 6-O-tritylcellobiose (**3**), and their methyl β-glycosides (**2** and **4**) with 6 molar equivalents of acetyl chloride in pyridine–toluene at 1—4°C has been studied. Acetylation of **1** gave the 1,2,6,2′,3′,4′-hexaacetate (**1A**) and 1,2,6,2′,3′-pentaacetate (**1B**), and that of **3** gave the peracetate, 1,2,2′,3′,4′,6′-hexaacetate (**3A**), and 1,2,3,3′,4′,6′-hexaacetate (**3B**). On the basis of the yields of partially acetylated products from **2**, *i.e.*, 2,6,2′,3′,4′-pentaacetate (**2A**, 15.5%), 2,6,2′,3′-tetraacetate (**2B**, 16.2%), 2,6,3′,4′-tetraacetate (**2C**, 10.8%), 6,2′,3′,4′-tetraacetate (**2D**, *ca.* 10.6%), 6,2′,3′-triacetate (**2E**, 7.9%), 2,6,3′-triacetate (**2F**, *ca.* 10.5%), and 3,6,2′,3′-tetraacetate (**2F′**, *ca.* 6.9%), it was deduced that the order of reactivities of the secondary hydroxyl groups in **2** is 3′>2′≃2>4′≫3. The partially acetylated products from **4** were separated by column chromatography and semipreparative HPLC to afford the peracetate (17.7%), 2,2′,3′,4′,6′-pentaacetate (**4A**, 11.1%), 2,3,3′,4′,6′-pentaacetate (**4B**, 26.0%), and 2,3,2′,3′,6′-pentaacetate (**4C**, 6.5%). It seems likely that the order of reactivities of the secondary hydroxyl groups in **4** toward acetyl chloride is 2,3′>4′>3>2′. These results suggested that TrO-6′ interacts sterically with HO-3 and HO-4′, while TrO-6 affects only HO-2′.

Keywords——selective acetylation; partially acetylated 6-O-tritylcellobioses; partially acetylated 6′-O-tritylcellobioses; partially acetylated methyl 6-O-trityl-β-cellobiosides; partially acetylated methyl 6′-O-trityl-β-cellobiosides; column chromatography; semipreparative HPLC; recycle chromatography; ^{1}H-NMR; *Rf* on TLC

There have been many studies on selective acylations of (1→4)-D-linked oligosaccharides and their derivatives.[3] It is known that although primary hydroxyl groups are normally the most reactive toward acylating agents, secondary hydroxyl groups often show significant differences in reactivity: in particular, the secondary hydroxyl group at C-3 in maltose, lactose and their methyl β-glycosides shows a remarkable inertness toward acylation. Benzoyl chloride is frequently used as the acylating agent, because acyl chlorides seem to be more regioselective than acyl anhydrides and bulkier acyl chlorides result in greater regioselectivity. However, the secondary hydroxyl groups in the cellobiose molecule do not show significant differences in reactivity[3a]: under conditions where maltose gave the 1,2,6,2′,3′,4′,6′-heptaacetate on treatment with acetyl chloride,[3b] cellobiose was converted into its octabenzoate in 98% yield by benzoylation.



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

Chart 1

Previously we attempted the selective acetylations of 6,6′-di-O-tritylcellobiose and its methyl β-glycoside with acetyl chloride in pyridine-toluene and obtained several kinds of pentaacetates, tetraacetates, triacetates, and diacetates.[1] The results show that the triphenylmethoxyl groups at C-6 and C-6′ altered the relative reactivities of the secondary hydroxyl groups in cellobiose and its methyl β-glycoside.

This led us to investigate the selective acetylation of 6′- or 6-mono-O-tritylcellobiose

(**1** and **3**) and their methyl β-glycosides (**2** and **4**) in order to establish which secondary hydroxyl group is affected by which triphenylmethoxyl group (TrO-6 or TrO-6′). In addition, partially acetylated derivatives are useful as starting materials for the introduction of a trideuterioacetyl group into a specific position of **1**—**4** with a view to making specific ^{1}H-NMR spectral assignments of acetyl groups in their (**1**—**4**) peracetates.[4]

Results and Discussion

The Selective Acetylation of 6′-O-Tritylcellobiose (1)

Compound **1** was treated with 6 molar equivalents of acetyl chloride in pyridine and toluene at 1—4°C. Thin-layer chromatography (TLC) indicated a complex mixture comprised of two major components (**1A** and **1B**) and several minor components having lower *Rf* values. Each component consisted of a pair of α- and β-anomers. Column chromatography (CC) afforded **1A** and **1B** in the ratio of approximately 2: 1. By ^{1}H-NMR, **1A** and **1B** were shown to be the hexaacetate and the pentaacetate, respectively. The location of the unacetylated hydroxyl group(s) in the partially acetylated compound was determined as follows. First the unacetylated hydroxyl group was labeled by methylation with methyl trifluoromethanesulfonate–2,6-di-*tert*-butyl-4-methylpyridine[5] overnight at 80°C. 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, and traces of by-products. 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 a methoxyl group at the carbon which originally carried the free hydroxyl group. Methanolyzates of the 4-O-β-D-glucopyranosyl-D-glucitol derivative were identified by gas chromatography–mass spectrometry (GC–MS)[1] as the trimethylsilyl (TMS) ethers. The methanolyzates derived from **1A** consisted of methyl D-glucoside and 3-O-methyl-D-glucitol, and that from **1B** consisted of methyl 4-O-methyl-D-glucoside and 3-O-methyl-D-glucitol. Therefore **1A** was 1,2,6,2′,3′,4′-hexa-O-acetyl-6′-O-tritylcellobiose and **1B** was 1,2,6,2′,3′-penta-O-acetyl-6′-O-tritylcellobiose.

The Selective Acetylation of Methyl 6′-O-Trityl-β-cellobioside (2)

Since treatment of **2** with 5 molar equivalents of acetyl chloride in pyridine and toluene at 1—4°C resulted in an insufficient extent of reaction, the amount of acetyl chloride was increased to 6 molar equivalents. A complex mixture was obtained which contained six major

TABLE I. The partially Acetylated Methyl 6′-O-Trityl-β-cellobiosides

Compd.	*Rf*[a] on TLC	Yield[b] (%)	$[\alpha]_D^{25}$ in $CHCl_3$		Unacetylated hydroxyl group at
			(°)	*c*(%)	
2A	0.69	15.5	+20.0	2.0	C-3
2B	0.60	16.2	−10.0	3.5	C-3 and C-4′
2C	0.44	10.8	+24.1	2.2	C-3 and C-2′
2D	0.38	13.3[c]	+15.7	1.3	C-2 and C-3
2E	0.31	7.9	−5.2	1.2	C-2, C-3, and C-4′
2F[d]	0.26	17.4			
Minor products	>0.15	18.9			

a) Plates: TLC plates silica gel 60 (0.25 mm) (E. Merck).
Solvent composition: C_6H_6–$CH_3COOC_2H_5$=1: 1.
b) By TLC spectrophotometry at 260 nm.
c) Contaminated with approximately 20% of an isomer having free hydroxyl groups at C-3 and C-3′.
d) Consisted of two unresolved components, one of which has free hydroxyl groups at C-3, C-2′, and C-4′, and the other at C-2 and C-4′. The ratio is *ca.* 3: 2 by ^{1}H-NMR.

components [(**2A**)—(**2F**) in order of decreasing TLC mobility](Table I). CC afforded **2A**, **2B**, and **2C** in chromatographically pure states. **2D**, **2E**, and **2F** were separated by repeated semipreparative high-performance liquid chromatography (semiprep. HPLC) each as a syrup contaminated with a little of the preceding component. **2A** was the only pentaacetate, **2B**, **2C**, and **2D** were tetraacetates, **2E** was a triacetate, and **2F** consisted of two unresolved components, one of which was a tetraacetate and the other a triacetate. The structures of **2A**—**2E** were assigned on the basis of methylation studies as described above. In this case, labeling of the reducing residue of methyl cellobioside by reduction was impossible. Therefore, ^{1}H-NMR spectroscopy was also used for elucidation of the structures. The free hydroxyl group(s) in a partially acetylated derivative of **2** was trideuterioacetylated with acetic anhydride-d_6 and the ^{1}H-NMR spectrum of the trideuterioacetylated product was compared with that of the peracetate of **2**. Some of the well-resolved acetoxyl signals in the latter have already been assigned unambiguously[4]: δ 2.09 was assigned to AcO-6, δ 1.83 to AcO-3, δ 1.72 to AcO-4′. By this method of specific spectral assignment for acetoxyl group resonances using trideuterioacetyl analogs,[6] it was proved that every one of **2A**—**2E** had an unacetylated hydroxyl group at C-3 and consequently each methanolysis product derived from their reducing residues necessarily had a methoxyl group at C-3. Table I shows the position of the carbon bearing the unacetylated hydroxyl group in the molecules of **2A**—**2E**. It turned out by careful CC separation that the spot of **2D** on TLC contained *ca.* 20% of an isomer having free hydroxyl groups at C-3 and C-3′. The ^{1}H-NMR analysis showed that the two components of **2F** had free hydroxyl groups at C-3, -2′, and -4′, and at C-2 and -4′, respectively, and their ratio was approximately 3 : 2.

The partially acetylated derivatives having a free hydroxyl group at C-4′ are levorotatory and the other are all dextrorotatory, as was the case with the partially acetylated derivatives of 6,6′-di-O-tritylcellobiose and its methyl β-glycoside.[1]

The Selective Acetylation of 6-O-Tritylcellobiose (3)

Acetylation of **3** with 6 molar equivalents of acetyl chloride under the same conditions as described above gave a surprisingly complex mixture which was carefully and repeatedly separated by CC and semiprep. HPLC. Four compounds, the α- and β-anomers of the heptaacetate (peracetate of **3**),[7] and two kinds of hexaacetates were obtained. The methylation analyses analyses and the ^{1}H-NMR analyses indicated that one of the hexaacetates was 1,2,2′,3′,4′,6′-hexa-O-acetyl-6-O-trityl-β-cellobiose (**3A**) and the other was 1,2,3,3′,4′,6′-hexa-O-acetyl-6-O-trityl-α-cellobiose (**3B**). The ratio of the heptaacetates and the hexaacetates was approximately 1 : 8. It is noteworthy that the peracetates were obtained for the first time in this series of studies on the selective acetylation of 6 and/or 6′-tritylcellobioses.

The Selective Acetylation of Methyl 6-O-Trityl-β-cellobioside (4)

In order to minimize the amounts of lower reaction products, 6 molar equivalents (equivalent to all of the free hydroxyl groups in **4**) of acetyl chloride were used for the selective acetylation of **4**. The other conditions were the same as described above. The TLC data, obtained using a silica gel plate developed with benzene–ethyl acetate (1 : 1), indicated that there were more than ten species of partially acetylated products in the crude reaction mixture. There were six major spots and it was proved by ^{1}H-NMR analyses that the first spot, in order of decreasing *Rf* value, was the same as that of methyl 6-O-trityl-β-cellobioside hexaacetate (the peracetate of **4**)[8] and the second spot involved two kinds of pentaacetate (**4A** and **4B**); the ratio of **4A** and **4B** was 1 : 2.4. The approximate yields of major products, calculated on the basis of the UV peak areas, are shown in Table II. CC afforded the peracetate and **4C** in chromatographically pure states. The structure of **4C** was confirmed by direct comparison with an authentic sample[4] as methyl 2,3,2′,3′,6-penta-O-acetyl-6-O-trityl-β-cellobioside. The chromatographic retention behavior of **4A** and **4B** was very similar. Consequently, it was very difficult to separate the two products. This separation problem was solved by the

Table II. The partially Acetylated Methyl 6-O-Trityl-β-cellobiosides

Compd.	Rf[a] on TLC	Yield[b] (%)	$[\alpha]_D^{22}$ in $CHCl_3$ (°)	c(%)	Unacetylated hydroxyl group at
Hexaacetate	0.69	17.7	−26.1	3.1	—
4A	0.57	37.1: 11.1[c]	−20.0	1.0	C-3
4B		26.0[c]	−22.3	2.2	C-2′
4C	0.45	6.5	−41.9	1.6	C-4′
4D	0.38	26.6[d]			
4E	0.35				
4F	0.32				
Minor products	>0.20	12.1			

a), *b*) The same as in Table I.
c) Divided by ^{1}H-NMR analysis.
d) Could not be separated.

use of recycle chromatography. The resulting chromatographically homogeneous **4A** and **4B** were both pentaacetates and after trideuterioacetylation, the chemical shifts of acetoxyl signals in their ^{1}H-NMR spectra coincided with those of trideuterioacetylated **3A** and **3B**, respectively, except for the lack of the AcO-1 signal in the spectra of **4A** and **4B** trideuterioacetates. Thus, **4A** and **4B** were identified as methyl 2,2′,3′,4′,6′-penta-O-acetyl-6-O-trityl-β-cellobioside and methyl 2,3,3′,4′,6′-penta-O-acetyl-6-O-trityl-β-cellobioside, respectively. On the basis of the *Rf* values, **4D**—**4F** were presumed to be tetraacetates, but could not be separated, because the actual components of the three spots, designated **4D**—**4F** for convenience, amounted to more than three and on CC they gave a complicated overlapping pattern.

The derivatives of **4** were all levorotatory. Among them, the specific rotation of **4C**, having a free hydroxyl group at C-4′, had a larger negative value than the others. The reason why the derivatives containing a HO-4′ group in this series of partially acetylated compounds rotate the plane of polarization to the left is unclear.

The Influence of 6 and 6′-Trityl Substituents (TrO-6 and TrO-6′) on the Reactivities of Secondary Hydroxyl Groups in the Cellobiose Molecule

From the results mentioned above, it was deduced that the order of reactivity of the five secondary hydroxyl groups in methyl 6′-O-trityl-β-cellobioside (**2**) is $3'>2'\simeq 2>4'\gg 3$ and that in methyl 6-O-trityl-β-cellobioside (**4**) is $2,3'>4'>3>2'$. In this connection, the order of reactivity of the secondary hydroxyl groups in methyl 6,6′-di-O-trityl-β-cellobioside was $3'>2\gg 2'>4'\gg 3$.[1] These results suggest that the influence of TrO-6 on the secondary hydroxyl groups is much smaller than that of TrO-6′. The low reactivity of HO-3 in the cellobiose molecule, which was recognized in the reaction with benzyl chloride[3c] is further decreased to a great extent by the influence of TrO-6′. Indeed, the complete trideuterioacetylation of HO-3 in the partially acetylated compound having TrO-6′ was extremely difficult. However, in the case of derivatives which do not have TrO-6′, the reactivity of HO-3 toward acetyl chloride is not particularly low, in spite of the observation of the short O-3....O-5′ nonbonding contact (3.34 Å) between the two glucopyranosyl residues of 6-O-trityl-α-cellobiose heptaacetate in an X-ray crystallographic study.[9] In addition, the polarity of 6′-O-trityl-substituted derivatives having an unacetylated hydroxyl group at C-3 (*e.g.* **2A**) is decreased by the influence on the free HO-3 of TrO-6′ and this effect results in an increase of mobility on silica gel. This effect is much smaller in the case of **6**-O-trityl-substituted derivatives having an unacetylated hydroxyl group at C-3 (*e.g.* **4A**). This interpretation is supported by their *Rf* values on TLC: the *Rf* value of **2A** is the same as that of the peracetate of **2** (0.69), while on the other hand the *Rf* value of **4A** (0.57) is smaller than that of the peracetate of **4** (0.69). Although HO-2′ is adjacent to the junction of the two glucopyranose rings, the effect of the reducing glucose unit in cellobiose on HO-2′ is relatively small. It seems that the reactivity of HO-2′ is decreased only by the presence of TrO-6. The X-ray crystallographic study of 6-O-trityl-α-cellobiose heptaacetate[9] showed that the C-O nonbonding distance between one carbon of TrO-6 and O-2′ was the shortest (3.70 Å) in the molecule. While the low reactivity of HO-4′ in the compound containing TrO-6′ can be explained by steric hindrance, the relatively low reactivity of HO-4′ in the compound which does not have TrO-6′ cannot be accounted for. However, similar low reactivity of HO-4′ in glucobioses was observed in the benzoylation of maltose (1→4-α-linked),[3c] gentiobiose (1→6-β-linked),[10] and sophorose (1→2-β-linked)[11] with benzoyl chloride. Although it is impossible to determine the reactivity order of HO-2 and HO-3′ in **4** from this selective acetylation, a reactivity order of HO-3′>HO-2 is inferred by analogy with the reactivities of HO-2 and HO-3′ in the 6′-O-trityl- and 6,6′-di-O-trityl derivatives, since it is likely that HO-2 and HO-3′ are little affected by either TrO-6 or TrO-6′.

Experimental

The general experimental conditions were the same as described previously.[1] The measurements of optical rotations and melting points, CC, semiprep. HPLC, TLC, GC–MS, ^{1}H-NMR, and chromatogram spectrodensitometry were performed under the same conditions as described previously.[1] The solvent systems (v/v) on CC and TLC were (a) 4: 1, (b) 3: 1, (c) 2: 1, (d) 1: 1, (e) 2: 3 benzene–ethyl acetate. Recycle chromatography was conducted with a 6000A solvent delivery system, a U6K injector, and an R-401 differential refractometer, all from Waters Assoc.

Selective Acetylation of 6′-O-Tritylcellobiose (1) and Separation of the Products——All solvents used were anhydrous. Compound **1**[7] (0.7 g) was dissolved in pyridine (3 ml) and toluene (15 ml). The solution was kept below 5°C and treated dropwise with acetyl chloride (0.49 ml, 6 mol. equiv.) in toluene (5 ml). When the addition was complete, the resulting slurry was stirred at 1—4°C for 20 h and then a white precipitate was filtered off and rinsed twice with toluene. The combined filtrates were washed with water, dried with magnesium sulfate, and evaporated to dryness. The resulting syrup was fractionated on a Lobar column (size C) with solvent (a) to give α-anomers of **1A** (118 mg) and **1B** (60 mg) contaminated with a little of the corresponding β-anomer.

Selective Acetylation of Methyl 6′-O-Trityl-β-cellobioside (2) and Separation of the Products——Compound **2**[12] (2.0 g) was partially acetylated with 6 mol. equiv. (1.43 ml) of acetyl chloride as described above. The reaction was followed by TLC [solvent (d)] and was discontinued after 3 h. The mixture was then processed in the way described above and the resulting syrup (2.5 g) was fractionated by CC on a Lobar column with solvent (b) and (c), and by repeating semiprep. HPLC with solvents (c) and (d). Yield: **2A** 292 mg, **2B** 286 mg, **2C** 266 mg, **2D** 250 mg, **2E** 110 mg, **2F** 230 mg.

Selective Acetylation of 6-O-Tritylcellobiose (3) and Separation of the Products——Selective acetylation of **3**[7] (1.1 g) with acetyl chloride (0.8 ml, 6 mol. equiv.) under the same conditions as for **1**, followed by fractionation on a Lobar column with solvent (b), gave the β-anomer (26 mg) and the α-anomer (42 mg) of hepta-O-acetyl-6-O-tritylcellobiose, and a mixture of the hexaacetates (**3A** and **3B**, 540 mg). Hepta-O-acetyl-6-O-trityl-α-cellobiose: mp 201—202°C (from isopropanol), $[\alpha]_D^{25}$ +24.0° (c=2.0, $CHCl_3$). Hepta-O-acetyl-6-O-trityl-β-cellobiose: mp 113°C (from ethanol), $[\alpha]_D^{21}$ −28.0° (c=2.0, $CHCl_3$). The mixture of hexaacetates was separated by repeating semiprep. HPLC with solvent (c). The fastest moving β-anomer of **3A** was isolated first as a chromatographically pure syrup, and from the last fraction, the α-anomer of **3B** was obtained as a syrup contaminated with a little of the β-anomer.

Selective Acetylation of Methyl 6-O-Trityl-β-cellobioside (4) and Separation of the Products——Treatment of **4**[12] (1.7 g) with acetyl chloride (1.22 ml, 6 mol. equiv.) under the same conditions as for **2** gave a complex mixture (2.3 g) which was chromatographed on a Lobar column with solvent (c). The initial fraction from the column gave methyl 6-O-trityl-β-cellobioside (**4**) hexaacetate (270 mg). The second fraction afforded a mixture of **4A** and **4B** (746 mg). From the third fraction, an additional amount of **4A**, **B** mixture (22 mg) and **4C** (101 mg) were separated by semiprep. HPLC with solvent (d). Continued elution of the Lobar column gave a very complex mixture of tetraacetates (489 mg), which could not be further separated. The separation of **4A** and **4B** by the use of recycle chromatography was performed on four stainless steel columns (30 cm × 4 mm I.D. each, Waters Assoc.), packed with LiChroprep Si 60 (15—25 μm, E. Merck) and connected in series, with solvent (d). The purity of the collected fractions was checked by TLC using solvent (e).

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