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Studies of model compounds for the analysis of ester-containing polysaccharides by the reductive-cleavage method

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ABSTRACT

The four *O*-propionyl regioisomers of methyl tri-*O*-methyl-α-D-glucopyranoside and the 2- and 3-*O*-propionyl regioisomers of methyl tri-*O*-methyl-β-D-glucopyranoside were subjected to reductive cleavage in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$, $BF_3 \cdot Et_2O$, or $Me_3SiOSO_2Me–BF_3 \cdot Et_2O$. The *O*-propionyl group was stable when either $Me_3SiOSO_2CF_3$ or $BF_3 \cdot Et_2O$ was the catalyst, but was slowly reduced to the (1-propyl) ether when $Me_3SiOSO_2Me–BF_3 \cdot Et_2O$ was the catalyst. Reductive cleavages catalyzed by $Me_3SiOSO_2CF_3$ were complete in 6 h, those catalyzed by $BF_3 \cdot Et_2O$ required at least 24 h, and those catalyzed by $Me_3SiOSO_2Me–BF_3 \cdot Et_2O$ required 30 min or less. In the α-series, the rate of reductive cleavage decreased in the order 6-*O*-propionyl > 4-*O*-propionyl > 3-*O*-propionyl ≫ 2-*O*-propionyl. The reductive cleavage of β anomers was faster than that of the corresponding α anomers. This effect was particularly striking for the α and β anomers of the 2-*O*-propionyl regioisomer, as would be expected on the basis of a participation reaction.

INTRODUCTION

The reductive-cleavage method[1], which involves reductive cleavage of glycosidic linkages in fully methylated polysaccharides, has been used to determine the ring forms and positions of the linkage in various glycans[2–8] and to establish the positions of *O*-methyl[9], *O*-ethyl[9], *O*-benzyl[10], *O*-carboxymethyl[11], and *O*-(1-carboxyethylidene)[12,13] substituents. Moreover, in combination with ^{1}H-NMR spectroscopy, this technique can be used to establish the identity, sequence, and anomeric configuration of the monosaccharide residues frequently encountered in polysaccharides[14]. In seeking to extend the applicability of this method, we have examined a series of *O*-propionyl derivatives of methyl α- and β-D-glucopyranoside as model compounds for application of the method to acylated polysaccharides, and we now report our findings.

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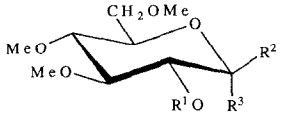
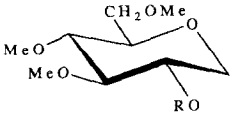
RESULTS

Since we envisioned that structural analysis of polysaccharides containing ester substituents might potentially be accomplished by sequential permethylation under neutral conditions and reductive cleavage, our initial experiments involved a series of model compounds bearing ester and *O*-methyl substituents. The model compounds were methyl tri-*O*-methyl-α- and β-D-glucopyranosides with a propionate substituent at positions 2, 3, 4, and 6. Reductive cleavage of each compound was carried out with triethylsilane and each of the catalysts previously reported[1,15,16], namely boron trifluoride etherate ($BF_3 \cdot Et_2O$), trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$), and a mixture of 5 equiv of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) and 1 equiv of $BF_3 \cdot Et_2O$ per equiv of glucoside. The products were identified by comparison of their GLC retention times and chemical ionization (CI)-mass spectra with those of authentic compounds, or by isolation and characterization by ^{1}H-NMR spectroscopy, and the appropriate details are given in the Experimental.

*Methyl 3,4,6-tri-*O*-methyl-2-*O*-propionyl-α-* (**1a**) *and -β-*D*-glucopyranosides* (**1b**). —Reductive cleavage of **1a** and **1b** gave the 1,5-anhydro derivative **2** with each of the three catalysts, as a result of reductive cleavage of the glycoside. The 1,5-anhydro-2-*O*-(1-propyl) derivative **3** was obtained also when Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ was the catalyst. Loss of the 2-*O*-propionyl group from **2** (to give **4**) occurred in some $Me_3SiOSO_2CF_3$-catalyzed cleavages when rigorously anhydrous conditions were not maintained.



1a R^1 = EtCO, R^2 = H, R^3 = OMe
1b R^1 = EtCO, R^2 = OMe, R^3 = H



2 R = EtCO
3 R = Pr
4 R = H

When $Me_3SiOSO_2CF_3$ was the catalyst, reductive cleavage of the glycosidic linkage of **1b** was complete in 1 h (see Fig. 1A), and the product (**2**) was stable in the reaction mixture for 24 h. Compound **1a** reacted much more slowly (Fig. 2A), but **2** was the only product.

Cleavage of **1a** and **2b** in the presence of $BF_3 \cdot Et_2O$ (Figs. 2B and 1B, respectively) also gave **2** as the sole product. The β anomer (**1b**) reacted more rapidly, and some anomerization to the α anomer (**1a**) was noted (Fig. 1B). Reductive cleavage of **1b** in the presence of $BF_3 \cdot Et_2O$ was also considerably slower than in the presence of $Me_3SiOSO_2CF_3$, but that of **1a** was only slightly slower.

Reductive cleavages catalyzed by Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ proceeded much faster than those catalyzed by $Me_3SiOSO_2CF_3$ or $BF_3 \cdot Et_2O$. After reaction for 10

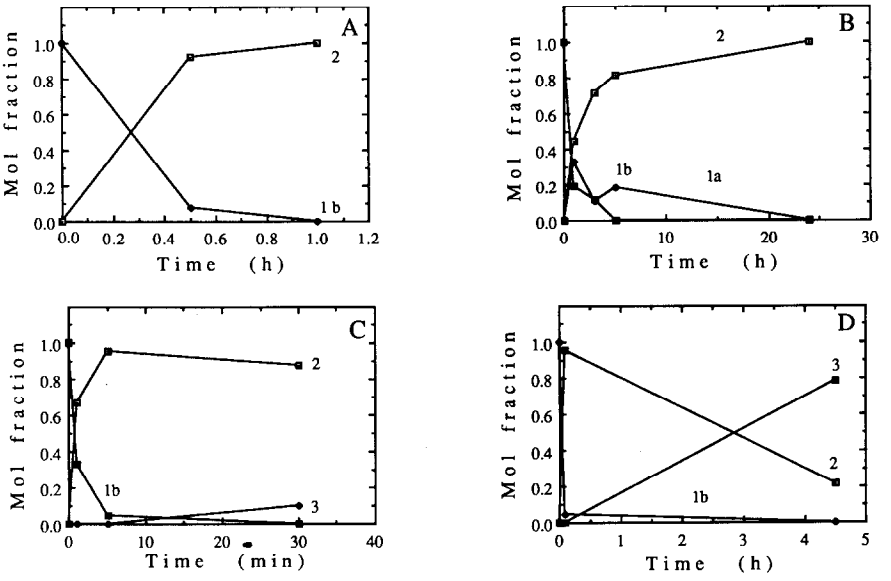


Fig. 1. Reductive cleavage of methyl 3,4,6-tri-*O*-methyl-2-*O*-propionyl-β-D-glucopyranoside (**1b**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$.

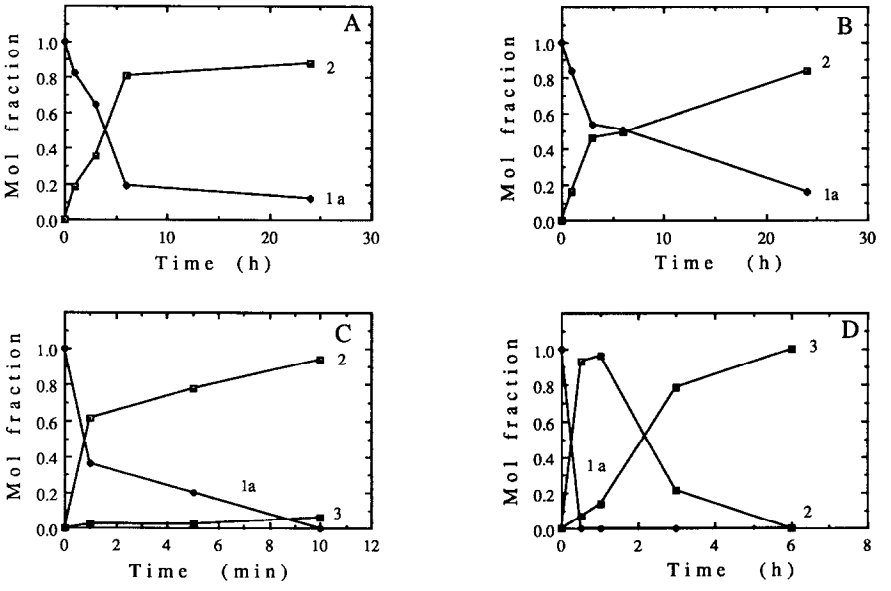


Fig. 2. Reductive cleavage of methyl 3,4,6-tri-*O*-methyl-2-*O*-propionyl-α-D-glucopyranoside (**1a**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$.

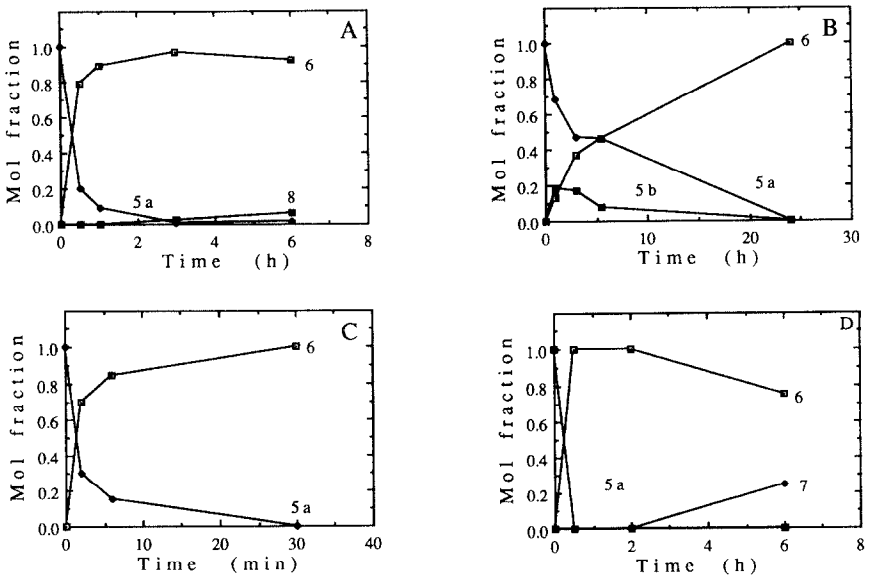


Fig. 3. Reductive cleavage of methyl 2,4,6-tri-*O*-methyl-3-*O*-propionyl-α-D-glucopyranoside (**5a**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$.

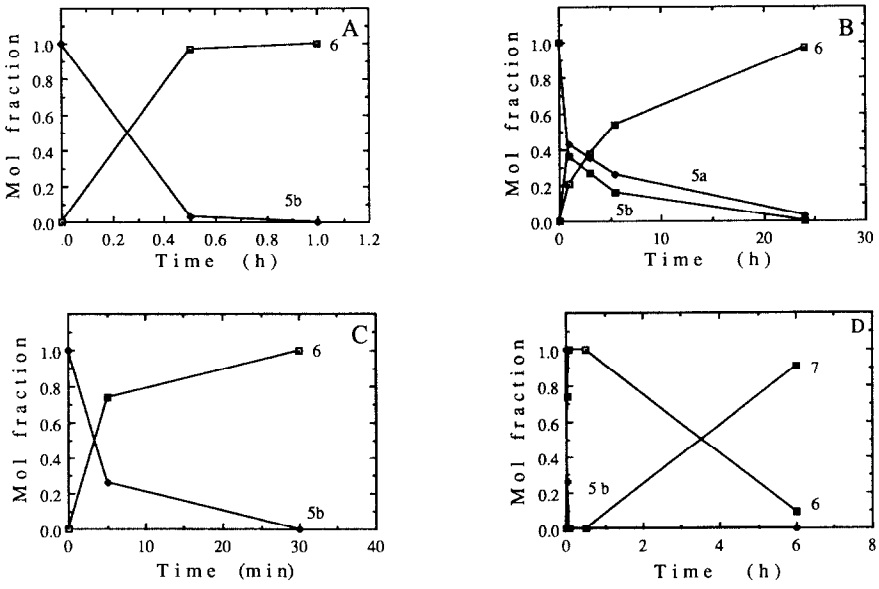
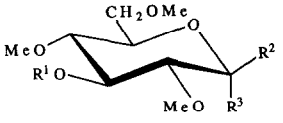


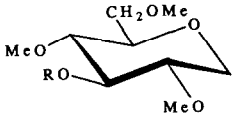
Fig. 4. Reductive cleavage of methyl 2,4,6-tri-*O*-methyl-3-*O*-propionyl-β-D-glucopyranoside (**5b**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$.

min, **2** was the major product in the reductive cleavage of both **1a** (Fig. 2C) and **1b** (Fig. 1C), and there was only a trace of **3**; after several hours, **3** was the sole or major product (Figs. 1D and 2D).

*Methyl 2,4,6-tri-*O*-methyl-3-*O*-propionyl-α-* (**5a**) *and -β-*D*-glucopyranosides* (**5b**). —The major products obtained upon reductive cleavage of **5a** and **5b** were the 1,5-anhydro derivative **6**, the 1,5-anhydro-3-*O*-(1-propyl) derivative **7**, and 1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol (**8**).



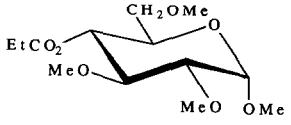
5a R^1 = EtCO, R^2 = H, R^3 = OMe
5b R^1 = EtCO, R^2 = OMe, R^3 = H



6 R = EtCO
7 R = Pr
8 R = H

When $Me_3SiOSO_2CF_3$ was the catalyst, **5a** and **5b** reacted completely in ~ 0.5 and ~ 3 h, respectively, to give **6** as the major product (Figs. 3A and 4A). After 6 h, 6% of **8** was present during the reductive cleavage of **5a** (Fig. 3A). Likewise, reductive cleavage of **5a** and **5b** in the presence of $BF_3 \cdot Et_2O$ (Figs. 3B and 4B) also gave **6** as the sole product, but anomerization occurred in each reaction and complete reduction required at least 24 h. In contrast, reductive cleavage of **5a** and **5b** in the presence of Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ was complete within 30 min (Figs. 3C and 4C), but the product (**6**) was slowly reduced to **7** (Figs. 3D and 4D).

*Methyl 2,3,6-tri-*O*-methyl-4-*O*-propionyl-α-*D*-glucopyranoside* (**9**).—Reductive cleavage of **9** gave, as expected, the 1,5-anhydro derivative **10** or the 1,5-anhydro-4-*O*-(1-propyl) derivative **11** as the major product depending upon the catalyst employed.



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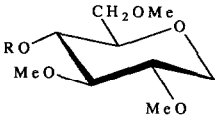


10 R = EtCO
11 R = Pr
12 R = H

With $Me_3SiOSO_2CF_3$ as the catalyst, reductive cleavage was complete in 3 h and **10** was the only product (Fig. 5A). With $BF_3 \cdot Et_2O$ as the catalyst, reaction was complete in 24 h and **10** was the only product (Fig. 5B). With Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ as the catalyst, **10** was formed within 10 min (Fig. 5C) but, after 6 h, complete conversion into **11** had occurred (Fig. 5D).

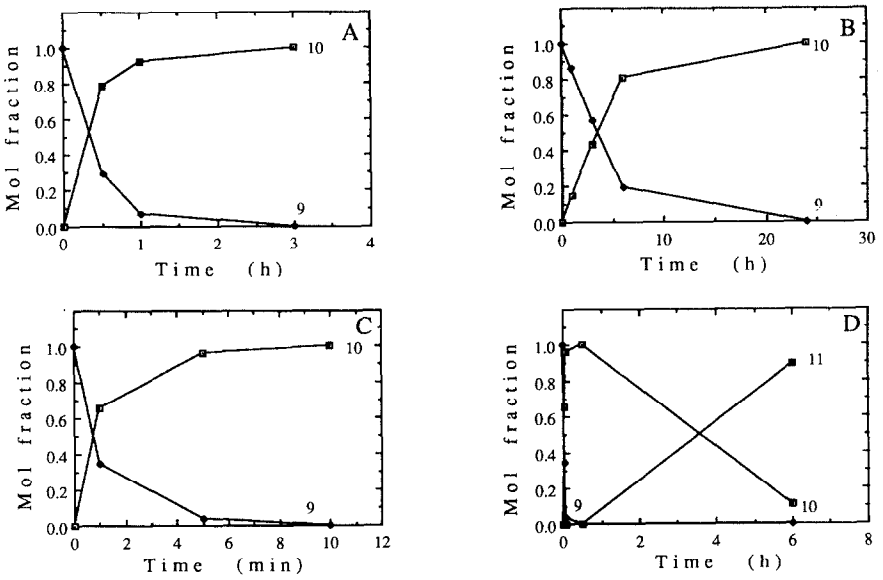
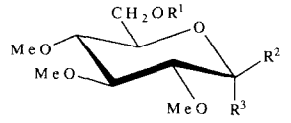
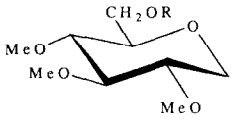


Fig. 5. Reductive cleavage of methyl 2,3,6-tri-*O*-methyl-4-*O*-propionyl-α-D-glucopyranoside (**9**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$.

Methyl 2,3,4-tri-O-methyl-6-O-propionyl-α-D-glucopyranoside (**13a**).—Reductive cleavage of **13a** gave the 1,5-anhydro derivative (**14**), the 1,5-anhydro-6-*O*-(1-propyl) derivative **15**, and **16**, formed by loss of the propionyl group from **14**.



13a R^1 = EtCO, R^2 = H, R^3 = OMe
13b R^1 = EtCO, R^2 = OMe, R^3 = H



14 R = EtCO
15 R = Pr
16 R = H

In the reaction catalyzed by $Me_3SiOSO_2CF_3$ (Fig. 6A), reductive cleavage of the glycosidic linkage of **13a** was essentially complete (96%) in 1 h to give **14** which was stable under the reaction conditions for 24 h (data not shown), although 1–3% of **16** was then present. With $BF_3 \cdot Et_2O$ as the catalyst (Fig. 6B), reaction was complete in 24 h and **14** was the only product, although some anomerization to **13b** was noted during the reaction. With Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ as the catalyst, reaction was virtually complete in 10 min to give **14** which, after 1 h, had been converted into **15** (Fig. 6D).

Synthesis.—Methyl 3,4,6-tri-*O*-methyl-2-*O*-propionyl-α-D-glucopyranoside (**1a**) was prepared by esterification of known[17] methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside. The corresponding β anomer (**1b**) was synthesized by the same method[17] starting with methyl β-D-glucopyranoside.

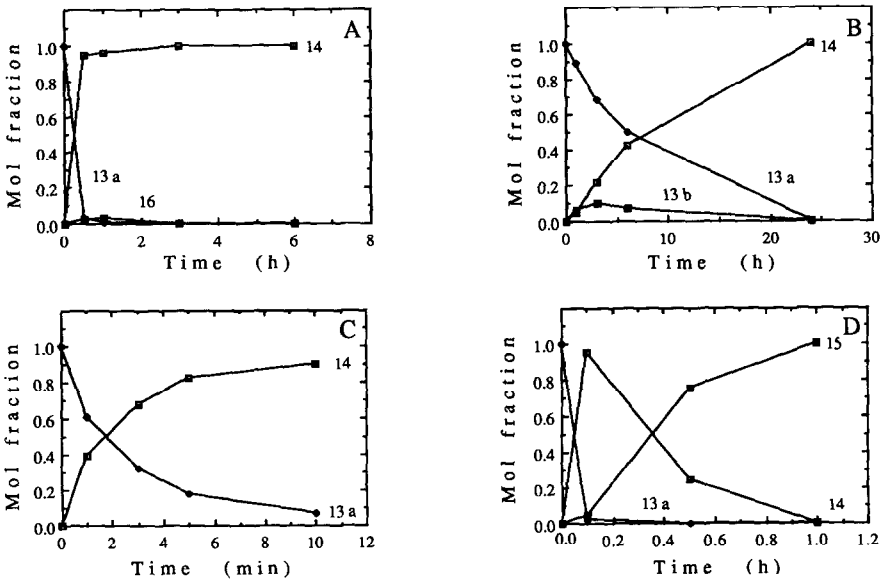


Fig. 6. Reductive cleavage of methyl 2,3,4-tri-*O*-methyl-6-*O*-propionyl-α-D-glucopyranoside (**13a**) in the presence of A, $Me_3SiOSO_2CF_3$; B, $BF_3 \cdot Et_2O$; and C and D, $Me_3SiOSO_2Me–BF_3 \cdot Et_2O$.

Methyl 2,4,6-tri-*O*-methyl-3-*O*-propionyl-α- (**5a**) and -β-D-glucopyranoside (**5b**) were synthesized from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Benzylation[18] followed by hydrolysis with aqueous acid gave 3-*O*-benzyl-D-glucose, which was converted into the methyl α- and β-glycosides by Fisher glycosidation. Methylation[19] followed by hydrogenolysis of the benzyl group then gave methyl 2,4,6-tri-*O*-methyl-α,β-D-glucopyranoside, which was esterified to yield **5a** and **5b**. The latter were obtained in pure form by chromatography on silica gel.

Methyl 2,3,6-tri-*O*-methyl-4-*O*-propionyl-α-D-glucopyranoside (**9**) was prepared from methylated cyclomaltoheptaose by treatment with methanolic hydrogen chloride to yield methyl 2,3,6-tri-*O*-methyl-α,β-D-glucopyranoside which was esterified with propionic anhydride; the α anomer (**9**) was isolated by chromatography.

Methyl 2,3,4-tri-*O*-methyl-6-*O*-propionyl-α-D-glucopyranoside (**13a**) was prepared by esterification of known[20] methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside.

The positional isomers (**3**, **7**, **11**, and **15**) of 1,5-anhydro-tri-*O*-methyl-*O*-(1-propyl)-D-glucitol were prepared from the above corresponding methyl glycosides by sequential propylation and reductive cleavage[16] with Et_3SiH in the presence of Me_3SiOSO_2Me and $BF_3 \cdot Et_2O$.

The positional isomers (**4**, **8**, **12**, and **16**) of 1,5-anhydro-tri-*O*-methyl-D-glucitol were prepared from the above corresponding glycosides, either by reductive cleavage in the presence of $Me_3SiOSO_2Me–BF_3 \cdot Et_2O$ (for **8**, **12**, and **16**) or by treatment with bis(trimethylsilyl)trifluoroacetamide in acetonitrile and then reductive cleavage in situ[21] with $Me_3SiOSO_2CF_3$ as the catalyst (for **4**).

The products isolated from the reductive cleavage reactions of the propionyl esters were identified, where appropriate, by comparison with the above authentic compounds.

DISCUSSION

Determination of the structures of polysaccharides bearing ester substituents can be accomplished in principle by reductive cleavage, provided that (*a*) methylation can be achieved under conditions that do not cleave the ester groups or cause their migration, and (*b*) the esters are stable under the conditions of reductive cleavage. The foregoing results with trimethylsilyl trifluoromethanesulfonate or boron trifluoride etherate as the catalyst indicate that the latter requirement can be met. The ester-bearing products of reductive cleavage were stable in the reaction mixtures if strictly anhydrous conditions were maintained. However, when the combination of trimethylsilyl methanesulfonate and boron trifluoride etherate was the catalyst, gradual reduction of the *O*-propionyl group to the (1-propyl) ether was observed.

The rate of reductive cleavage and the composition of the product mixture depended on the catalyst used. Cleavages catalyzed by trimethylsilyl trifluoromethanesulfonate were generally complete in 6 h or less, those catalyzed by boron trifluoride etherate generally required 24 h for completion, and those catalyzed by the combination of trimethylsilyl methanesulfonate and boron trifluoride etherate were usually complete within 30 min. With the last-named catalyst, reduction of the propionyl ester was negligible in the time required to achieve total reductive cleavage of the glycoside.

In order to determine how the relative rates of reductive cleavage were affected by the position of the ester substituent, the anomeric configuration, and the catalyst employed, pseudo-first-order rate constants and half-life times ($T_{1/2}$) were obtained. The rates of reductive cleavage varied with the catalyst in the order $Me_3SiOSO_2Me–BF_3 \cdot Et_2O > Me_3SiOSO_2CF_3 > BF_3 \cdot Et_2O$ and the corresponding $T_{1/2}$ values for **5a**, for example, were 1.7 min, 19.8 min, and 2.2 h. The position of the ester substituent also affected the rate of reductive cleavage, particularly when trimethylsilyl trifluoromethanesulfonate was the catalyst. For the series of α anomers **1a**, **5a**, **9**, and **13a**, the $T_{1/2}$ values associated with the positions of the propionyl group were as follows: 6, 7.8 min; 4, 13.8 min; 3, 20 min; and 2, 3 h. These observations are consistent with the mechanism proposed[1] for reductive cleavage, which postulates the formation of an oxonium ion at the anomeric position. Such an ion would be destabilized by an ester substituent at C-2 and the rate of cleavage would thereby be decreased. As expected, the rate of cleavage increased with the distance of the ester substituent from the anomeric position. Similar results were obtained with the other catalysts employed for reductive cleavage.

Reductive cleavages of the β anomers (**1b** and **5b**) were more rapid than those of the respective α anomers (**1a** and **5a**). For example, when $Me_3SiOSO_2CF_3$ was the catalyst, the $T_{1/2}$ values for the 2-esters **1a** and **1b** were 3 h and ~8 min, respectively, and those for the 3-esters **5a** and **5b** were ~20 min and ~5 min, respectively. The relatively rapid reaction of the β anomer of the 2-ester (**1b**) probably reflects participation by the propionate group in the cleavage of the glycosidic bond in a rate-determining step.

Extension of these studies to acylated polysaccharides is in progress.

EXPERIMENTAL

General.—^{1}H-NMR spectra were recorded with an IBM NR-200 NMR spectrometer on solutions in $CDCl_3$ and were referenced to internal Me_4Si. GLC–MS was performed with either a Finnegan 4000 mass spectrometer equipped with a VG Multispec data system or a VG Analytical LTD Model VG 7070E-HF high-resolution, double-focusing mass spectrometer. Column effluents were analyzed by CIMS with either ammonia or isobutane as the reagent gas. Analytical GLC was performed using He as the carrier gas and a Hewlett–Packard Model 5890A chromatograph equipped with a Hewlett–Packard 3392A integrator, a flame-ionization detector, and a J.&W. Scientific DB-5 fused-silica capillary column (0.25 mm × 30 m) with the temperature program 110° for 2 min, then to 300° at 6°/min. Medium pressure liquid chromatography (MPLC) involved a Rheodyne 7125 injector, an Eldex model B-100-S4 pump, a Scientific Systems Model LP-21 pulse dampener, and glass columns (2.1 × 30 cm) packed with 60 Å silica gel (Merck, 60–200 mesh). Elemental analyses were performed by M-H-W Laboratories, Inc. (Phoenix, AZ) on samples purified by MPLC, using hexane–ethyl acetate mixtures.

Triethylsilane, trimethylsilyl trifluoromethanesulfonate, boron trifluoride etherate, methanesulfonic acid, chlorotrimethylsilane, and dichlorodimethylsilane were obtained from the Aldrich Chemical Company. Trimethylsilyl methanesulfonate was prepared as described[16]. Acetylation and methylation were carried out as described[9].

Reductive cleavages were carried out in Wheaton V-vials equipped with Teflon-lined screw caps. The glassware was silylated inside by treatment with 20% Me_2SiCl_2 in toluene for 2 h, then rinsed successively with MeOH, CH_2Cl_2, and acetone, and dried in an oven overnight. The sample (1–10 mg) and a small stirring bar were added to each vial, and the contents were dried under N_2 and then kept under vacuum overnight. To each vial was added a sufficient quantity of a stock solution of reducing agent and catalyst in CH_2Cl_2 to yield a 0.1 M solution of the substrate. The vials were then sealed, the contents were stirred at room temperature for the times indicated, the reactions were quenched as described[6,9,21], and the products were analyzed. The stock solutions of reducing agent and catalyst consisted of M Et_3SiH and M $Me_3SiOSO_2CF_3$, M $BF_3 \cdot Et_2O$, or M

Me_3SiOSO_2Me, and 0.2 M $BF_3 \cdot Et_2O$[16] in dry CH_2Cl_2, which were prepared immediately before use in a vial that had been silylated.

For time-course studies, a solution of each dry glucoside (~ 10 mg) in dry CH_2Cl_2 (1.0 mL) was divided amongst 5 silylated V-vials. A stock solution consisting of dry CH_2Cl_2 (1 mL), Et_3SiH (161 μL), and either $Me_3SiOSO_2CF_3$ (192 μL), Me_3SiOSO_2Me (139 μL) plus $BF_3 \cdot Et_2O$ (25 μL), or $BF_3 \cdot Et_2O$ (123 μL) was prepared immediately prior to use and an aliquot (0.2 mL) was added to each vial. The vials were then sealed and, at the appropriate times, the reactions were quenched. The organic layer was used directly for GLC (retention times are designated by *T*); filtration or concentration were avoided in order to minimize loss of any product by evaporation.

Reactions in which Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$ was the catalyst were quenched after 1, 5, 10, and 30 min, and 4–6 h. Trimethylsilyl trifluoromethanesulfonate-catalyzed reactions were quenched after 30 min, and 1, 3, 6, and 24 h. Boron trifluoride etherate-catalyzed reactions were quenched at 1, 3, 6, and 24 h.

Methyl 3,4,6-tri-O-methyl-2-O-propionyl-α-D-glucopyranoside (**1a**).—Methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside[17] (120 mg) was esterified with propionic anhydride (0.5 mL, 8 equiv) in pyridine (2 mL) overnight at room temperature, and the mixture was processed as reported[9] for acetylation. The product (113 mg, 77%) was purified by MPLC (9:1 hexane–EtOAc) to give **1a**, *T* 13.6 min. ^{1}H-NMR data: δ 1.16 (t, 3 H, *J* 7.6 Hz, CH_3CH_2), 2.40 (q, 2 H, CH_3CH_2), 3.36, 3.41, 3.53, 3.55 (4 s, 12 H, 4 MeO), 3.25–3.63 (complex, 4 H, H-3,4,6a,6b), 4.74 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.0 Hz, H-2), and 4.86 (d, 1 H, H-1). CI(NH_3)-mass spectrum: *m/z* 261 [100%, $(M - MeOH + H)^+$], 278 [28%, $(M - MeOH + NH_4)^+$], 293 [17%, $(M + H)^+$], and 310 [22%, $(M + NH_4)^+$].

Anal. Calcd for $C_{13}H_{24}O_7$: C, 53.41; H, 8.28. Found: C, 53.66; H, 8.21.

Methyl 3,4,6-tri-O-methyl-2-O-propionyl-β-D-glucopyranoside (**1b**).—Methyl 3,4,6-tri-*O*-methyl-β-D-glucopyranoside[17] (112 mg) was propionylated and the product (135 mg, 97%) was purified as for **1a** to give **1b**, *T* 13.4 min. ^{1}H-NMR data: δ 1.21 (t, 3 H, *J* 7.6 Hz, CH_3CH_2), 2.36 (q, 2 H, CH_3CH_2), 3.35, 3.50, 3.55, 3.57 (4 s, 12 H, 4 MeO), 3.0–3.6 (complex, 4 H, H-3,4,6a,6b), 4.14 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), and 4.85 (dd, $J_{2,3}$ 10.0 Hz, H-2). CI(NH_3)-mass spectrum: *m/z* 261 [100%, $(M - MeOH + H)^+$], 278 [76%, $(M - MeOH + NH_4)^+$], 293 [12%, $(M + H)^+$], and 310 [5%, $(M + NH_4)^+$].

Anal. Calcd for $C_{13}H_{24}O_7$: C, 53.41; H, 8.28. Found: C, 53.61; H, 8.12.

1,5-Anhydro-3,4,6-tri-O-methyl-2-O propionyl-D-glucitol (**2**).—Compound **1a** (13.7 mg) was treated with 5 equiv of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$ for 30 min. MPLC (9:1 hexane EtOAc) of the product gave **2** (7.4 mg, 54%), *T* 12.2 min. ^{1}H-NMR data: δ 1.14 (t, 3 H, *J* 7.6 Hz, CH_3CH_2), 2.36 (q, 2 H, CH_3CH_2), 3.22, 3.54, 3.56 (3 s, 9 H, 3 MeO), 3.06–3.60 (complex, 5 H, H-1*ax*,3,4,6a,6b), 4.05 (dd, 1 H, $J_{1eq,2}$ 5.6, $J_{1eq,1ax}$ 11.0 Hz, H-1*eq*), and 4.81 (ddd, 1 H, $J_{1ax,2}$ 8.9, $J_{2,3}$ 10.1 Hz, H-2). CI(NH_3)-mass spectrum: *m/z* 263 [100%, $(M + H)^+$] and 280 [12%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 55.12; H, 8.37.

1,5-Anhydro-3,4,6-tri-O-methyl-2-O-(1-propyl)-D-glucitol (**3**).—Methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside[17] (66 mg) was propylated by a variation of the procedure of Hakomori[19], namely, deprotonation in Me_2SO with 1.5 equiv of lithium dimsyl for 1 h followed by alkylation with 2.5 equiv of propyl iodide for 48 h. The product was extracted into CH_2Cl_2, the organic layer was washed with cold water (6 × 50 mL), then dried (Na_2SO_4), and the solvent was evaporated. MPLC (10:1 hexane–EtOAc) of the product (77 mg, 98%) gave methyl 3,4,6-tri-*O*-methyl-2-*O*-(1-propyl)-α-D-glucopyranoside, *T* 12.2 min.

A portion (23.5 mg) of the above product was treated with 10 equiv of Et_3SiH, 10 equiv of Me_3SiOSO_2Me, and 2 equiv of $BF_3 \cdot Et_2O$ for 1 h, and the product was subjected to MPLC (10:1 hexane–EtOAc) to give **3** (12.4 mg, 53%), *T* 10.4 min. ^{1}H-NMR data: δ 0.91 (t, 3 H, *J* 7.4 Hz, $CH_3CH_2CH_2O$), 1.56 (sextet, 2 H, $CH_3CH_2CH_2O$), 3.40, 3.54, 3.65 (3 s, 9 H, 3 MeO), 3.02–3.58 (complex, 9 H, $CH_3CH_2CH_2O$ and H-1*ax*,2,3,4,5,6a,6b), and 4.01 (dd, 1 H, $J_{1eq,2}$ 5.0, $J_{1eq,1ax}$ 10.9 Hz, H-1*eq*). CI(isobutane)-mass spectrum: *m/z* 271 [100%, $(M - MeOH + H)^+$] and 249 [63%, $(M + H)^+$].

Anal. Calcd for $C_{12}H_{24}O_5$: C, 58.04; H, 9.74. Found: C, 57.93; H, 9.71.

1,5-Anhydro-3,4,6-tri-O-methyl-D-glucitol (**4**).—Methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside[17] (24 mg) was subjected in sequence to silylation and reductive cleavage as described by Bennek and Gray[21]. GLC of the product revealed peaks with *T* 10.1 min (starting material, 26%) and 7.7 min (**4**, 75%). MPLC (3:1 hexane–EtOAc) of the mixture gave pure **4** (6 mg, 25%). ^{1}H-NMR data: δ 1.25 (s, 1 H, OH), 3.05–3.57 (complex, 6 H, H-1*ax*,2,3,4,6a,6b), 3.40, 3.52, 3.67 (3 s, 9 H, 3 MeO), and 4.00 (dd, 1 H, $J_{1eq,2}$ 5.3, $J_{1eq,1ax}$ 11.0 Hz, H-1*eq*). CI(isobutane)-mass spectrum: *m/z* 175 [100%, $(M - MeOH + H)^+$] and 207 [4%, $(M + H^+)$].

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.32; H, 8.91.

Methyl 2,4,6-tri-O-methyl-3-O-propionyl-α- (**5a**) *and -β-D-glucopyranoside* (**5b**).—3-*O*-Benzyl-D-glucose was prepared by a variation of the procedure of Srivastava and Srivastava[18]. A solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1.16 g) in Me_2SO was stirred for 2 h with 1.8 equiv of lithium dimsyl. Benzyl bromide (1.2 equiv) was added, the solution was stirred overnight, the reaction was quenched by the addition of ice, the product was extracted with CH_2Cl_2, the extract was dried (Na_2SO_4), and the solvent was evaporated. Column chromatography (3:1 hexane–EtOAc) of the syrupy product gave the 3-*O*-benzyl derivative (1.25 g, 80%), a solution of which in 1:1 acetic acid–water was boiled under reflux overnight. A methanolic solution of the product was boiled under reflux in the presence of Dowex 50 (H^+) resin for 24 h. The product, methyl 3-*O*-benzyl-α,β-D-glucopyranoside (1.00 g), was methylated by the procedure of Hakomori[19], a portion (352 mg) was then hydrogenolyzed (Pd–C) to give methyl 2,4,6-tri-*O*-methyl-α,β-D-glucopyranoside (230 mg) (*T* values: α, 10.10 min; β, 9.55 min; α,β-ratio 85:15), and a portion (174 mg) of the latter was propionylated as described. GLC of the product revealed components with *T* 15.0 (85%, **5a**) and

14.1 min (15%, **5b**). MPLC (9:1 hexane–EtOAc) of the mixture gave **5a** (158 mg) and **5b** (28 mg). ^{1}H-NMR data (**5a**): δ 1.37 (t, 3 H, J 7.6 Hz, CH_3CH$_2$), 2.37 (q, 2 H, CH$_3$CH_2), 3.39, 3.41 (2 s, 6 H, 2 MeO), 3.40 (s, 6 H, 2 MeO), 3.29–3.68 (complex, 5 H, H-2,4,5,6a,6b), 4.86 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), and 5.37 (t, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3). CI(NH$_3$)-mass spectrum: m/z 293 [7%, $(M + H)^+$] and 310 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_{13}H_{24}O_7$: C, 53.41; H, 8.28. Found: C, 53.20; H, 8.09.

Compound **5b** was isolated as described for **5a**. ^{1}H-NMR data: δ 1.38 (t, 3 H, J 7.6 Hz, CH_3CH$_2$), 2.53 (q, 2 H, CH$_3$CH_2), 3.5–3.8 (complex, 5 H, H-2,4,5,6a,6b), 4.43 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), and 5.26 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3).

Anal. Found: *C, 53.18; H, 8.20.*

*1,5-Anhydro-2,4,6-tri-*O*-methyl-3-*O*-propionyl-*D*-glucitol* (**6**).—Reductive cleavage of **5a** for 30 min in the presence of 5 equiv of Et$_3$SiH, 5 equiv of Me$_3$SiOSO$_2$Me, and 1 equiv of BF$_3$ · Et$_2$O, with MPLC (9:1 hexane–EtOAc) of the product, gave **6** (7.5 mg), T 12.7 min. ^{1}H-NMR data: δ 1.20 (t, 3 H, J 7.6 Hz, CH_3CH$_2$), 2.38 (q, 2 H, CH$_3$CH_2), 3.36, 3.40, 3.41 (3 s, 9 H, 3 MeO), 3.19–3.59 (complex, 6 H, H-1*ax*,2,4,5,6a,6b), 4.12 (dd, 1 H, $J_{1eq,2}$ 4.4, $J_{1eq,1ax}$ 10.4 Hz, H-1*eq*), and 5.05 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3). CI(NH$_3$)-mass spectrum: m/z 263 [100%, $(M + H)^+$] and 280 [5%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 55.26; H, 8.56.

*1,5-Anhydro-2,4,6-tri-*O*-methyl-3-*O*-(1-propyl)-*D*-glucitol* (**7**).—Methyl 2,4,6-tri-*O*-methyl-α-D-glucopyranoside was propylated as described for **3** and a portion (10 mg) of the product was subjected to reductive cleavage for 1 h in the presence of 5 equiv of Et$_3$SiH, 5 equiv of Me$_3$SiOSO$_2$Me, and 1 equiv of BF$_3$ · Et$_2$O. The mixture was processed as described[16], and MPLC (10:1 hexane–EtOAc) of the product gave **7** (8 mg), T 10.4 min. ^{1}H-NMR data: δ 0.95 (t, 3 H, J 7.4 Hz, CH_3CH$_2$CH$_2$O), 1.54 (m, 2 H, CH$_3$CH_2CH$_2$O), 3.39, 3.46, 3.53 (3 s, 9 H, 3 MeO), 3.20–3.70 (complex, 8 H, CH$_3$CH$_2$CH_2O and H-2,3,4,5,6a,6b), and 4.10 (dd, 1 H, $J_{1eq,2}$ 4.4, $J_{1eq,1ax}$ 10.4 Hz, H-1*eq*). CI(NH$_3$)-mass spectrum: m/z 249 [29%, $(M + H)^+$] and 266 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{24}O_5$: C, 58.04; H, 9.74. Found: C, 57.93; H, 9.43.

*1,5-Anhydro-2,4,6-tri-*O*-methyl-D-glucitol* (**8**).—Reductive cleavage of methyl 2,4,6-tri-*O*-methyl-α-D-glucopyranoside (29 mg) in the presence of 10 equiv of Et$_3$SiH, 10 equiv of Me$_3$SiOSO$_2$Me, and 2 equiv of BF$_3$ · Et$_2$O, work-up as described[16], and MPLC (3:1 hexane–EtOAc) of the product gave **8** (4 mg), T 8.0 min. ^{1}H-NMR data: δ 2.63 (s, 1 H, OH), 3.40, 3.46, 3.50 (3 s, 9 H, 3 MeO), 3.10–3.59 (complex, 7 H, H-1*ax*,2,3,4,5,6a,6b), and 4.13 (dd, 1 H, $J_{1eq,2}$ 4.4, $J_{1eq,1ax}$ 10.5 Hz, H-1*eq*). CI(NH$_3$)-mass spectrum: m/z 207 [20%, $(M + H)^+$] and 224 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.56; H, 8.81.

*Methyl 2,3,6-tri-*O*-methyl-4-*O*propionyl-α-D-glucopyranoside* (**9**).—A solution of methylated cyclomaltoheptaose (1.58 g) in 1:20 acetyl chloride–MeOH was boiled under reflux for 72 h to give methyl 2,3,6-tri-*O*-methyl-α,β-D-glucopyranoside, a

portion (790 mg) of which was propionylated to give **9** (920 mg). GLC of the product revealed components with T 13.6 (β-**9**) and 14.1 min (**9**). MPLC (9:1 hexane–EtOAc) gave pure **9**. ^{1}H-NMR data: δ 1.17 (t, 3 H, J 7.5 Hz, CH_3CH_2), 2.36 (q, 2 H, CH_3CH_2), 3.35, 3.44, 3.50, 3.51 (4 s, 12 H, 4 MeO), 3.30–3.77 (complex, 4 H, H-2,3,6a,6b), 4.85 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), and 4.89 (t, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4). CI(NH_3)-mass spectrum: m/z 310 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_{13}H_{24}O_7$: C, 53.41; H, 8.28. Found: C, 53.40; H, 8.10.

1,5-Anhydro-2,3,6-tri-O-methyl-4-O-propionyl-D-glucitol (**10**).—Reductive cleavage of **9** (25 mg) in the presence of 5 equiv of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$, with work-up as described[16], followed by MPLC (9:1 hexane–EtOAc) of the product, gave **10** (13 mg), T 12.1 min. ^{1}H-NMR data: δ 1.17 (t, 3 H, J 7.5 Hz, CH_3CH_2), 2.30 (q, 2 H, CH_3CH_2), 3.34, 3.47, 3.51 (3 s, 9 H, 3 MeO), 3.0–3.64 (complex, 6 H, H-1*ax*,2,3,5,6a,6b), 4.09 (dd, 1 H, $J_{1eq,2}$ 4.7, $J_{1eq,1ax}$ 11.0 Hz, H-1*eq*), and 4.81 (t, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4). CI(NH_3)-mass spectrum: m/z 263 [100%, $(M + H)^+$] and 280 [3%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.85; H, 8.61.

1,5-Anhydro-2,3,6-tri-O-methyl-4-O-(1-propyl)-D-glucitol (**11**).—Compound **9** was propylated as described for **3**. Reductive cleavage of a portion (12 mg) of the 4-*O*-(1-propyl) derivative for 1 h in the presence of 5 equiv of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$, then work-up as described[16], and MPLC (10:1 hexane–EtOAc) of the product gave **11** (6.8 mg), T 10.2 min. ^{1}H-NMR data: δ 0.93 (t, 3 H, J 7.5 Hz, $CH_3CH_2CH_2O$), 1.58 (m, 2 H, $CH_3CH_2CH_2O$), 3.38, 3.46, 3.63 (3 s, 9 H, 3 MeO), 3.03–3.79 (complex, 9 H, $CH_3CH_2CH_2O$ and H-1*ax*,2,3,4,5,6a,6b), and 4.05 (dd, 1 H, $J_{1eq,2}$ 4.2, $J_{1eq,1ax}$ 10.4 Hz, H-1*eq*). CI(NH_3)-mass spectrum: m/z 249 [100%, $(M + H)^+$] and 266 [4%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{24}O_5$: C, 58.04; H, 9.74. Found: C, 58.30; H, 9.74.

1,5-Anhydro-2,3,6-tri-O-methyl-D-glucitol (**12**).—Reductive cleavage of methyl 2,3,6-tri-*O*-methyl-α-D-glucopyranoside (28 mg) for 1 h in the presence of 10 equiv of Et_3SiH, 10 equiv of Me_3SiOSO_2Me, and 2 equiv of $BF_3 \cdot Et_2O$, with work-up as described[16], followed by MPLC (3:1 hexane–EtOAc) of the product, gave **12** (16 mg), T 7.8 min. ^{1}H-NMR data: δ 1.25 (s, 1 H, OH), 3.40, 3.45, 3.64 (3 s, 9 H, 3 MeO), 3.10–3.60 (complex, 7 H, H-1*ax*,2,3,4,5,6a,6b), and 4.10 (dd, 1 H, $J_{1eq,2}$ 4.6, $J_{1eq,1ax}$ 10.0 Hz, H-1*eq*). CI(NH_3)-mass spectrum: m/z 207 [100%, $(M + H)^+$] and 224 [68%, $(M + NH_4)^+$].

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.19; H, 8.88.

Methyl 2,3,4-tri-O-methyl-6-O-propionyl-α-D-glucopyranoside (**13a**).—Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside[20] (96 mg) was propionylated and the product was purified by MPLC (9:1 hexane–EtOAc), to give **13a** (100 mg), T 14.3 min. ^{1}H-NMR data: δ 1.14 (t, 3 H, J 7.6 Hz, CH_3CH_2), 2.36 (q, 2 H, CH_3CH_2), 3.39, 3.49, 3.50, 3.62 (4 s, 12 H, 4 MeO), 3.05–3.72 (complex, 4 H, H-2,3,4,5), 4.20 (dd, 1 H, $J_{5,6a}$ 4.9, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.24 (dd, 1 H, $J_{5,6b}$ 2.3, H-6b), and 4.79 (d, 1 H,

$J_{1,2}$ 3.5 Hz, H-1). CI(NH_3)-mass spectrum: m/z 293 [9%, $(M + H)^+$] and 310 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_{13}H_{24}O_7$; C, 53.41; H, 8.28. Found: C, 53.63; H, 8.30.

1,5-Anhydro-2,3,4-tri-O-methyl-6-O-propionyl-D-glucitol (**14**).—Reductive cleavage of **13a** (14 mg) for 1 h in the presence of 5 equiv of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$, then work-up as described[16], and MPLC (9:1 hexane–EtOAc) of the product gave **14** (6.1 mg), T 12.3 min. ^{1}H-NMR data: δ 1.14 (t, 3 H, J 7.5 Hz, CH_3CH_2), 2.37 (q, 2 H, CH_3CH_2), 3.47, 3.52, 3.64 (3 s, 9 H, 3 MeO), 2.97–3.40 (complex, 5 H, H-1*ax*,2,3,4,5), 4.04 (dd, 1 H, $J_{1eq,2}$ 4.3, $J_{1eq,1ax}$ 10.5 Hz, H-1*eq*), 4.18 (dd, 1 H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.0 Hz, H-6a), and 4.33 (dd, 1 H, $J_{5,6b}$ 2.1 Hz, H-6b). CI(NH_3)-mass spectrum: m/z 263 [100%, $(M + H)^+$] and 280 [13%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.82; H, 8.43.

1,5-Anhydro-2,3,4-tri-O-methyl-6-O-(1-propyl)-D-glucitol (**15**).—Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside was propylated as described above. Reductive cleavage of the product (11 mg) for 1 h in the presence of 5 equiv of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$[16], with MPLC (10:1 hexane–EtOAc) of the product, gave **15** (5.9 mg), T 10.2 min. ^{1}H-NMR data: δ 0.91 (t, 3 H, J 7.4 Hz, $CH_3CH_2CH_2O$), 1.64 (m, 2 H, $CH_3CH_2CH_2O$), 3.01–3.83 (complex, 9 H, $CH_3CH_2CH_2O$ and H-1*ax*,2,3,4,5,6a,6b), 3.46, 3.53, 3.64 (3 s, 9 H, 3 MeO), 4.05 (dd, 1 H, $J_{1eq,2}$ 4.6, $J_{1eq,1ax}$ 10.6 Hz, H-1*eq*). CI(NH_3)-mass spectrum: m/z 249 [100%, $(M + H)^+$] and 266 [62%, $(M + NH_4)^+$].

Anal. Calcd for $C_{12}H_{24}O_5$: C, 58.04; H, 9.74. Found: C, 58.29; H, 9.42.

1,5-Anhydro-2,3,4-tri-O-methyl-D-glucitol (**16**).—Reductive cleavage of methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside (12 mg) for 1 h in the presence of 10 equiv of Et_3SiH, 10 equiv of Me_3SiOSO_2Me, and 2 equiv of $BF_3 \cdot Et_2O$, with MPLC (3:1 hexane–EtOAc) of the product, gave **16** (5.4 mg), T 7.4 min. ^{1}H-NMR data: δ 1.57 (s, 1 H, OH), 3.48, 3.55, 3.64 (3 s, 9 H, 3 MeO), 3.05–3.84 (complex, 7 H, H-1*ax*,2,3,4,5,6a,6b), and 4.05 (dd, 1 H, $J_{1eq,2}$ 4.4, $J_{1eq,1ax}$ 9.7 Hz, H-1*eq*). CI(NH_3)-mass spectrum: m/z 207 [14%, $(M + H)^+$] and 224 [100%, $(M + NH_4)^+$].

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.29; H, 8.59.

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