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Transglycosylation reactions of permethylated methyl D-glucopyranosides with partially methylated 1,5-anhydroalditols

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Abstract

Transglycosylation of methyl 2,3,4,6-tetra-*O*-methyl-α- and β-D-glucopyranosides with 1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol and 1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol took place in the presence of trimethylsilyl trifluoromethanesulfonate or boron trifluoride etherate, resulting in the formation of disaccharide derivatives. A disaccharide which could have been formed by the transglycosylation reaction of the intermediates was observed during the reductive-cleavage reaction of permethylated pullulan.

Keywords: Transglycosylation; Disaccharide derivatives; Anomerization

1. Introduction

The determination of the structure of a polysaccharide is often a complex task which requires a wide range of information, such as number and nature of residues, linkage position, configuration, and so on [1]. The reductive-cleavage method is one of the most useful methods for determining the structure of polysaccharides [2]. Total cleavage of a permethylated polysaccharide and subsequent acylation results in acylated 1,5- (or 1,4-) anhydroalditols, provided that no isomerization of the pyranose or furanose ring-skeleton takes place. Identification of the latter compounds reveals the structures of the monosaccharide residues and their linkage positions. Partial cleavage of the permethylated polysaccharide produces oligosaccharide-anhydroalditols whose structures reveal infor-

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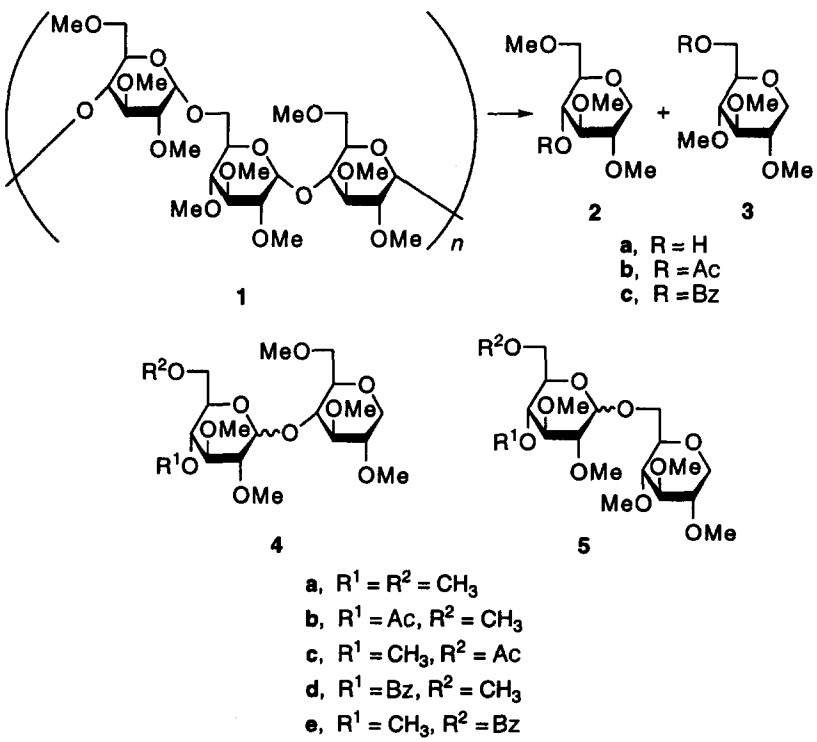
mation on the sequence of the polysaccharide as well as its anomeric configurations. This approach to the structural determination of polysaccharides has been extensively examined [3] and is a useful method for structure determination.

In the course of our investigation of the mechanism of the reductive-cleavage reaction, we noted that both anomerization of glycosidic linkages and transglycosylation took place [4]. Both phenomena are very critical in deducing the total structure of a polysaccharide if one is going to relate the identity of derived oligosaccharide-anhydroalditols to the monosaccharide sequence and the anomeric configurations present in the parent polysaccharide. For example, permethylated pullulan (**1**), a polysaccharide which has a trisaccharide repeating-unit, will upon total cleavage give its monosaccharide components as anhydroalditols **2a** and **3a**, which are usually converted to their acetates **2b** and **3b**, respectively, for identification by GLC. Benzoates such as **2c** and **3c** may be prepared for separation by HPLC. Partial cleavage of **1** and subsequent acetylation is expected to give **4b**α, **4c**α, and **5b**α if neither transglycosylation nor anomerization takes place. On the other hand, transglycosylation among the partially methylated monosaccharide may produce **5c** in addition to **4b**, **4c**, and **5b**, and their anomeric configurations could be both α and β depending on the reaction conditions.



1

2 **3**

a, R = H
b, R = Ac
c, R = Bz

4 **5**

a, $R^1 = R^2 = CH_3$
b, R^1 = Ac, $R^2 = CH_3$
c, $R^1 = CH_3$, R^2 = Ac
d, R^1 = Bz, $R^2 = CH_3$
e, $R^1 = CH_3$, R^2 = Bz

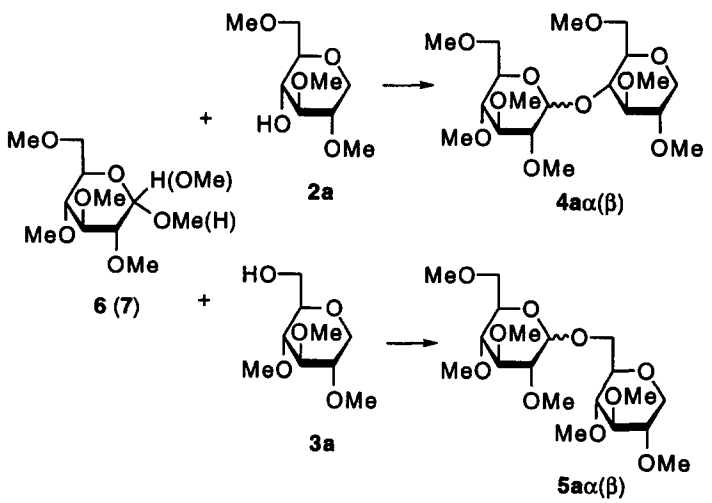
This study was undertaken to determine whether such products were formed under reductive-cleavage conditions and, if so, whether the results could be duplicated in model studies employing monosaccharide derivatives.

2. Results and discussion

When permethylated pullulan (**1**) was treated with triethylsilane in the presence of $BF_3 \cdot OEt_2$ for 3 h, a partial reductive cleavage took place. Benzoylation of the products with benzoic anhydride in pyridine and separation by HPLC gave **2c**, **3c**, **5d**α, and **5e**α in the relative molar ratios of 12.4:2.5:2.1:1, respectively. The formation of **5e**α could be explained by a transglycosylation among the monomeric units. This observation led us to investigate whether transglycosylation occurred when monosaccharide derivatives were subjected to reductive-cleavage conditions.

Transglycosylations among monosaccharides and oligosaccharides catalyzed by enzymes have been reported in the literature [5], but similar reactions catalyzed by acid are less common and the yields of oligosaccharides are generally extremely low [6,7] when dilute solutions are employed.

To explore whether transglycosylation occurred in the course of reductive cleavage, we have examined the formation of oligosaccharides from the reaction of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (**6**) with 1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**2a**) or 1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**3a**).



When a solution of **6**, **2a**, and $Me_3SiOSO_2CF_3$ (1:3.5:25 by mole) in dichloromethane was stirred for 12 h at room temperature and the reaction mixture was examined by GLC–MS there were present two major components whose GLC retention times and mass spectra were consistent with oligosaccharides **4a**α and **4a**β ($\sim$ 1:4). Based on

integration of the GLC profile, ~70% of **6** was converted into **4a**α and **4a**β. The transglycosylation reaction also took place when **3a** was used, giving **5a**α and **5a**β, but the ratio of the anomers was reversed ($\alpha:\beta = 4:1$).

The oligosaccharides were separated by HPLC using a Supelco-NH_2 column with 3:2 (v/v) acetonitrile–water. The CI (NH_3) mass spectra of all the oligosaccharides showed base peaks at 442 ($M + NH_4^+$) and peaks at 425 ($M + H^+$), indicating that they possessed the identical molecular weights.

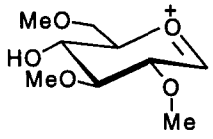
The anomeric configurations of the four oligosaccharides could be readily established by the chemical-shift values and the coupling constants of the protons on the anomeric carbons. Thus, H-1′ of **4a**α appeared at δ 5.68 with $J = 3.5$ Hz while that of **4a**β appeared at δ 4.29 with $J = 8.0$ Hz. Similarly, H-1′ of **5a**α appeared at δ 5.05 ($J = 3.0$ Hz) and that of **5a**β appeared at δ 4.24 ($J = 8.0$ Hz). One of the methylene protons in the 1,5-anhydroalditol ring, H-1*e*, of the both **4a**α and **4a**β appeared at δ 4.07 (dd, $J = 5.0$ and 11.0 Hz), but those of **5a**α and **5a**β appeared at δ 4.06 (dd, $J = 5.5$ and 10.5 Hz) and 3.95 (dd, $J = 5.0$ and 11.5 Hz), respectively.

In our previous report [4] we observed that transglycosylation of **6** in the presence of 10 equiv of ethanol and 10 equiv of $Me_3SiOSO_2CF_3$ for 24 h resulted in an $\alpha:\beta$ ratio of 3.68:1. In the present study, transglycosylation of **6** in the presence of **3a** gave **5a**α and **5a**β in a ratio of 4:1, in agreement with the results using ethanol as the glycosyl acceptor. However, transglycosylation of **6** in the presence of **2a** gave the α and β anomers (**4a**α and **4a**β, respectively) in the ratio of 1:4. This observation is particularly striking because glycopyranosides readily undergo anomerization in the presence of $Me_3SiOSO_2CF_3$ leading to the α anomer as the major component in an equilibrium state [4]. The 4:1 preference of β to α in the presence of an excess amount of $Me_3SiOSO_2CF_3$ in the reaction mixture of **6** and **2a** may be due to an unfavorable α approach of **2a** to the cyclic oxonium-ion intermediate due to steric interference between the 2′-methoxyl of **6** and the 3-methoxyl of **2a**. On the other hand, a β approach would likely be favorable because of the reduced steric interaction between methoxyl groups. Steric interference is not likely when **3a** approaches from the α direction because this glycosyl acceptor contains a primary hydroxyl group. A similar $\alpha:\beta$ ratio could be expected in the reaction of the β anomer **7** and **2a** because **7** will be readily isomerized to **6** under the reaction conditions as previously reported [4]. Indeed, this was the case as **4a**α and **4a**β were formed in a ratio of 1:3.5 from **7** and **2a**.

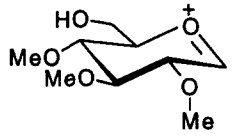
Although the yields of the oligosaccharides were moderate (50–80%) the present procedure does not seem to be suitable for preparation of oligosaccharides because of the difficulty in transforming OCH_3 to OH groups.

Rolf et al. [8] reported the isolation of **5b**α upon conducting the reductive-cleavage reaction of permethylated pullulan (**1**) with Et_3SiH in the presence of $BF_3 \cdot OEt_2$ for 20 h. Compounds **2b** and **3b** were also present in the reaction mixture and the molar ratio of **2b**:**3b**:**5b**α was 0.42:0.07:0.37. However, as mentioned in the Introduction, only two disaccharide derivatives (**5b**α and **5c**α) were detected by GLC–CIMS analysis of the reductive-cleavage reaction mixture of **1** after acetylation even though there were eight possible disaccharides (α and β anomers of **4b**, **4c**, **5b**, and **5c**) which could be formed from the monomeric components. Benzoylation of the mixture and separation using a C_{18} column with acetonitrile–water provided **5d**α and **5e**α. Apparently, the (1 → 4)-α-

glycoside linkage is cleaved much faster than the $(1 \rightarrow 6)$-α-linkage when $BF_3 \cdot OEt_2$ was used as catalyst, although the cause of this preference is not yet clear. The former cleavage would produce cyclic oxonium ion **8** while the latter would form **9**. Formation of **5c** α (also **5e** α) may be the result of the nucleophilic attack of the primary hydroxyl group of **3a** to **9**. However, **5b** α (also **5d** α) may be the product either of a similar reaction between **3a** and **8** or partial reductive cleavage of the repeating unit.



8



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The structure of **5d** α was readily confirmed by converting it into **2c** and **3c** ($\sim$ 1:1) by reductive cleavage with Et_3SiD in the presence of a mixture of trimethylsilyl methanesulfonate ($Me_3SiOSO_2CH_3$) and $BF_3 \cdot OEt_2$ (5:1 molar ratio) and subsequent benzoylation of the mixture. The deuterium atom was found to be present in **2c** by GLC–CIMS analysis. On the other hand, **5e** α gave **3c** as a sole product upon sequential treatment with Et_3SiH–$Me_3SiOSO_2CH_3$–$BF_3 \cdot OEt_2$ (5:5:1 molar ratio) and benzoic anhydride and pyridine.

Although the amount of the unexpected oligosaccharide-anhydroalditol (**5c** α) formed from permethylated pullulan was quite small, our findings suggest that structural information derived from the analysis of such products should not be used as the sole evidence for determining the sequence and anomeric configurations of polysaccharides. One should be absolutely certain that no anomerization or transglycosylation takes place when the partial reductive cleavage method is employed.

3. Experimental

Starting materials.—Methyl α- and β-D-glucopyranosides, pullulan, and β-cyclodextrin were all commercial products. Commercial trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$), trimethylsilyl methanesulfonate ($Me_3SiOSO_2CH_3$), and boron trifluoride etherate ($BF_3 \cdot OEt_2$) were distilled under mild vacuum prior to use. Dichloromethane was dried over CaH_2 and distilled prior to use. Methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (**6**) and methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (**7**) were prepared as described by Fügedi et al. [9] and of Ciucanu et al. [10], respectively. 1,5-Anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**2a**) was prepared by the reductive cleavage reaction of permethylated β-cyclodextrin with 5 equiv of Et_3SiH, 5 equiv of $Me_3SiOSO_2CH_3$, and 1 equiv of $BF_3 \cdot OEt_2$ [11]. 1,5-Anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**3a**) was prepared as described by Rolf et al. [8].

Analytical methods.—^{1}H-NMR spectra were recorded on a Varian 500 VXR-FT NMR spectrometer in $CDCl_3$ as the solvent and were referenced to internal $CHCl_3$ as δ

7.24. Infrared (IR) spectra were recorded on a Perkin–Elmer Model 1410 IR spectrophotometer. GC–CI-mass spectra were obtained using Finnigan MAT-95 mass spectrometer (DB-5 capillary column; 6 min at 120 °C and 6 °C/min) by the Mass Spectroscopy Laboratory at the University of Minnesota. GLC analyses were performed using a Hewlett–Packard 5890A gas–liquid chromatograph equipped with an on-column injector connected to a DB-5 capillary column (J & W Scientific, 30 m, film thickness 0.25 μm, ID 0.25 mm), a flame-ionization detector, and a Hewlett–Packard 3392A integrator; helium was used as the carrier gas. On column analysis conditions were as follows: initial temperature, 120 °C; initial hold, 2 min; temperature increase, 6 °C/min; final temperature 280 °C; final hold, 30 min. The retention times of each disaccharides were as follows (min): **4a**α, 23.80; **4a**β, 22.98; **5a**α, 23.46; **5a**β, 24.12; **5d**α, 31.20; **5e**α, 31.70. HPLC analyses were performed using a Beckman System Gold instrument equipped with either a Beckman 156 refractive index detector and a Supelco LC-NH_2 column (25 cm × 4.6 mm; 5-μm particle size) or a Beckman UV detector and a C_{18} reverse phase column (25 cm × 4.6 mm; 5-μm particle size). An isocratic combination of MeCN and water (3:2 by volume) was used as the eluent at a flow rate of 1 mL/min.

*1,5-Anhydro-4-*O*-(2,3,4,6-tetra-*O*-methyl-*α*(and *β*)-*D*-glucopyranosyl)-2,3,6-tri-*O*-methyl-*D*-glucitol* (**4a**α and **4a**β).—A solution of **6** (72 mg, 0.29 mmol), **2a** (270 mg, 1.31 mmol), and $Me_3SiOSO_2CF_3$ (1.60 g, 7.21 mmol) in CH_2Cl_2 (10 mL) was capped and stirred at room temperature for 12 h. Saturated $NaHCO_3$ solution (~ 4 mL) was carefully added and the mixture was stirred for 30 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (15 mL). The combined organic layers was concentrated and the components were separated by HPLC using MeCN and water (3:2) as the eluent. Fractions at 16.1 and 16.9 min were pooled and concentrated and dried under vacuum. The first fraction was the α anomer **4a**α (16%): ^{1}H-NMR ($CDCl_3$) δ 3.11 (t, 1 H, *J* 9.0 and 11.5 Hz, H-6 (or H-6′)), 3.10–3.79 (m, total 33 H, overlapping singlets of 3 H each at 3.32, 3.40, 3.44, 3.55, 3.56, 3.58, 3.64), 4.07 (dd, 1 H, *J* 5.0 and 15.0 Hz, H-1*e*), 5.68 (d, 1 H, *J* 3.5 Hz, H-1′); CIMS (NH_3), *m/z* (%) 442 (100, $M + NH_4^+$), 425 (6, $M + H^+$); EIMS (70 eV), *m/z* (%) 249 (27), 189 (24), 157 (10), 131 (12), 101 (32), 89 (12), 88 (100), 75 (12), 71 (13), 45 (19).

The second fraction was the β anomer **4a**β (65%): ^{1}H-NMR ($CDCl_3$) δ 2.94 (dd, 1 H, *J* 7.5 and 9.0 Hz, H-6 (or H-6′)), 3.08–3.75 (m, total 33 H, overlapping singlets of 3 H each at 3.38, 3.40, 3.47, 3.53, 3.55, 3.60, 3.63), 4.07 (dd, 1 H, *J* 5.0 and 11.5 Hz, H-1*e*), 4.29 (d, 1 H, *J* 8.0 Hz, H-1′); CIMS (NH_3), *m/z* (%) 442 (100, $M + NH_4^+$), 425 (33, $M + H^+$); EIMS (70 eV), *m/z* (%) 249 (53), 189 (47), 157 (19), 131 (21), 115 (12), 101 (63), 89 (19), 88 (100), 75 (18), 71 (23), 45 (40).

*1,5-Anhydro-6-*O*-(2,3,4,6-tetra-*O*-methyl-*α*(and *β*)-*D*-glucopyranosyl)-2,3,4-tri-*O*-methyl-*D*-glucitol* (**5a**α and **5a**β).—A solution of **6** (81 mg, 0.32 mmol), **3a** (261 mg, 1.27 mmol), and $Me_3SiOSO_2CF_3$ (1.55 g, 6.98 mmol) in CH_2Cl_2 (10 mL) was capped and stirred at room temperature for 12 h. Similar work-up as the preparation of **4a**α and **4a**β gave the disaccharides. The first fraction at 15.0 min was the α anomer **5a**α (40%): ^{1}H-NMR ($CDCl_3$) δ 3.04 (dd, 1 H, *J* 7.5 and 11.0 Hz, H-6 (or H-6′)), 3.13–3.80 (m, total 33 H, overlapping singlets of 3 H each at 3.39, 3.45, 3.46, 3.52, 3.53, 3.61, 3.62), 3.95 (dd, 1 H, *J* 5.0 and 11.5 Hz, H-1*e*), 5.05 (d, 1 H, *J* 3.5 Hz, H-1′); CIMS (NH_3), *m/z* (%) 442 (100, $M + NH_4^+$), 425 (3, $M + H^+$); EIMS (70 eV),

m/z (%) 249 (56), 189 (16), 157 (6), 101 (34), 99 (8), 89 (8), 88 (100), 75 (18), 71 (12), 45 (10).

The second fraction at 15.4 min was the β anomer **5a**β (10%): ^{1}H-NMR ($CDCl_3$) δ 3.02–3.80 (m, total 34 H, overlapping singlets of 3 H each at 3.39, 3.46, 3.51, 3.56, 3.59, 3.62, 3.64), 4.06 (dd, 1 H, *J* 5.5 and 10.5 Hz, H-1*e*), 4.24 (d, 1 H, *J* 8.0 Hz, H-1′); CIMS (NH_3), *m/z* (%) 442 (100, M + NH_4^+), 425 (3, M + H^+); EIMS (70 eV), *m/z* (%) 249 (91), 189 (26), 101 (39), 88 (100), 75 (11), 71 (17), 45 (13).

Reductive cleavage of permethylated pullulan (**1**).—Et_3SiH (0.40 mL) and $BF_3 \cdot OEt_2$ (0.06 mL) were added to a solution of **1** (106 mg, vacuum-dried) in CH_2Cl_2 (5 mL). The resulting solution was stirred for 3 h at room temperature then quenched by addition of MeOH (0.5 mL) and stirring was continued for 2 h. The solution was evaporated to dryness and the resulting residue was dissolved in pyridine (2 mL) and cooled in an ice bath. Benzoic anhydride (340 mg) was added and the mixture was stirred in an oil-bath for 24 h at 50 °C. The mixture was poured into ice–water (30 mL) and the aqueous mixture was extracted with CH_2Cl_2 (5 × 20 mL). The organic layer was washed with 2 M HCl (2 × 30 mL), satd $NaHCO_3$ (30 mL), and water (30 mL). After drying, the solvent was evaporated giving a residue of a mixture of **2c** [3], **3c** [3], **5d**α, and **5e**α (12.4:2.5:2.1:1 by UV absorption in HPLC chromatography) which were separated and identified by ^{1}H-NMR and GC–CIMS spectrometry.

*1,5-Anhydro-6-*O*-(4-*O*-benzoyl-2,3,6-tri-*O*-methyl-α-*D*-glucopyranosyl)-2,3,4-tri-*O*-methyl-*D*-glucitol* (**5d**α).—^{1}H-NMR ($CDCl_3$) δ 3.00–4.20 (m, total 32 H, overlapping singlets of 3 H each at 3.30, 3.46, 3.47, 3.50, 3.58, 3.65), 5.09 (d, 1 H, *J* 3.5 Hz, H-1′), 5.17 (apparent t, 1 H, *J* 9.5 Hz, H-4′); CIMS (NH_3), *m/z* (%) 532 (100, M + NH_4^+), 515 (1, M + H^+), 7.45 (m, 2 H, *m*-Ar), 7.60 (m, 1 H, *p*-Ar), 8.05 (m, 2 H, *o*-Ar).

*1,5-Anhydro-6-*O*-(6-*O*-benzoyl-2,3,4-tri-*O*-methyl-α-*D*-glucopyranosyl)-2,3,4-tri-*O*-methyl-*D*-glucitol* (**5e**α).—^{1}H-NMR ($CDCl_3$) δ 3.00–3.90 (m, total 32 H, overlapping singlets of 3 H each at 3.44, 3.49, 3.50, 3.55, 3.63, 3.64), 3.98 (dd, 1 H, *J* 4.0 and 9.0 Hz, H-1*e*), 4.42 (dd, 1 H, *J* 5.0 and 11.7 Hz, H-6′), 4.57 (dd, 1 H, *J* 2.0 and 11.7 Hz, H-6′), 4.98 (d, 1 H, *J* 3.5 Hz, H-1′), 7.45 (m, 2 H, *m*-Ar), 7.60 (m, 1 H, *p*-Ar), 8.05 (m, 2 H, *o*-Ar); CIMS (NH_3), *m/z* (%) 532 (100, M + NH_4^+), 515 (3, M + H^+).

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