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The reaction between epichlorohydrin and polysaccharides †: Part 1, syntheses of some model substances with non-cyclic substituents

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Abstract

Five derivatives of methyl α-D-glucopyranoside, in which the substituents form noncyclic structures, have been prepared as model substances for possible structural elements formed on reaction of polysaccharides with epichlorohydrin. The substances were converted into the permethylated alditol-*1-d* derivatives and characterised by CIMS and EIMS.

1. Introduction

Polysaccharides form insoluble gels on cross-linking with epichlorohydrin, and those prepared from dextran (Sephadex®) and starch (Spherex®) are commercial products. The aim of the present investigation is to characterise the different structural elements present in such cross-linked polysaccharides, in particular in Sephadex®.

When epichlorohydrin reacts with a polysaccharide under alkaline conditions, 2,3-epoxypropyl ethers are first formed by reaction with hydroxyl groups in the glycosyl residues and their substituents. When racemic epichlorohydrin is used the substituents are mixtures of diastereoisomers. The epoxides are then opened by reaction with water or an adjacent hydroxyl group. The latter may belong to the

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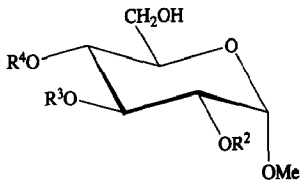
same glycosyl residue or to a substituent in such a residue, giving glycosyl residues with different types of substitution. The hydroxyl group could also belong to an interchain glycosyl residue or a substituent in such a residue, giving a cross-linkage. Reaction with intrachain residues or substituents in these is not excluded. It is thus obvious that a great number of different structural elements are formed, and that access to representative model substances would facilitate the structural studies. As we are mainly interested in cross-linked dextran, we have prepared derivatives of methyl α-D-glucopyranoside, nonsubstituted at the 6-position. The substituents may form noncyclic or cyclic structures. As a first part of our studies, we now report the syntheses of some derivatives with noncyclic substituents.

2. Results and discussion

Methyl 2-*O*-, 3-*O*-, and 4-*O*-(2,3-dihydroxypropyl)-α-D-glucopyranoside (**1**, **2**, and **3**) were prepared by reaction of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside, and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside, respectively, with 2,3-epoxypropyl trifluoromethanesulfonate (triflate), opening of the epoxide ring by hydrolysis with acid under mild conditions, and deblocking.

1-*O*-Allyl-2-*O*-benzyl-3-*O*-trifluoromethanesulfonylglycerol (**4**) was prepared from 1-allyloxy-2,3-epoxypropane (**5**) by hydrolysis with acid, tritylation of the primary hydroxyl group, benzylation, detritylation and reaction with triflic anhydride. In part of the product (**6**) obtained on etherification of methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside by reaction with **4**, the 3-*O*-allyl group was transformed into a 2,3-dihydroxypropyl group via the epoxide. Catalytic hydrogenolysis of this intermediate yielded methyl 2-*O*-(2,6,7-trihydroxy-4-oxaheptyl)-α-D-glucopyranoside (**7**).

Selective deallylation of **6** by treatment with palladium-on-charcoal in methanol–acetic acid yielded the primary alcohol **8**, which was converted into its

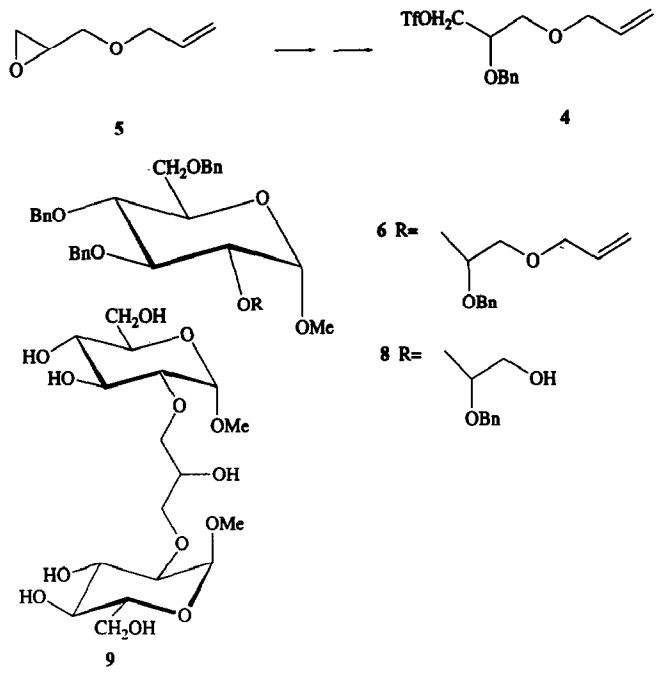


1 $R^2 = CH_2\text{-}CH(OH)\text{-}CH_2OH$ $R^3 = R^4 = H$

2 $R^3 = CH_2\text{-}CH(OH)\text{-}CH_2OH$ $R^2 = R^4 = H$

3 $R^4 = CH_2\text{-}CH(OH)\text{-}CH_2OH$ $R^2 = R^3 = H$

7 $R^2 = CH_2\text{-}CH(OH)\text{-}CH_2OCH_2\text{-}CH(OH)\text{-}CH_2OH$ $R^3 = R^4 = H$



triflate and reacted with methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside. Catalytic hydrogenolysis of the product yielded 2-hydroxy-1,3-bis(methyl α-D-glucopyranosid-2-*O*-yl)propane (**9**).

The ^{13}C NMR spectra of the model substances are given in Table 1. Substances **1**, **2**, **3**, and **7** are mixtures of diastereomers, and some signals given by the different forms are resolved. Substance **q** is a single compound, but C′-1 and C′-3 in the glycerol moiety are nonidentical.

Mass spectrometry of the permethylated alditol-1-d *derivatives obtained from the model substances.*—The model substances (**1**, **2**, **3**, **7**, and **9**) were subjected to

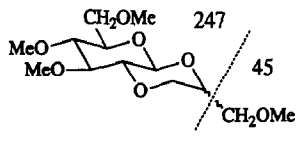
Table 1
^{13}C NMR chemical shifts for the model substances [a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C′-1	C′-2	C′-3	C′-5	C′-6	C′-7	OMe
1	97.1	79.9	72.5	69.6	71.5	60.7	71.7	70.9 70.7	62.6				54.9
2	99.5	71.8	82.8	69.4	71.1	60.7	73.9	71.1	62.8				55.2
3	99.3	71.4	73.1	78.6	70.7	60.5	73.5	71.0	62.6				55.2
7	97.1	80.0	72.5	69.6	71.5	60.6	71.7	69.4 69.2	72.1 72.0	72.2	70.5	62.7	54.9
9	97.2	80.1 80.0	72.6	69.7	71.6	60.7	71.8 71.7	69.7	71.7 71.8				54.9

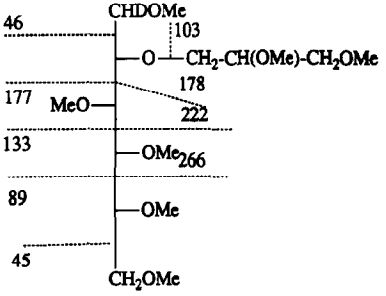
[a] Some assignments could be reversed.

hydrolysis with acid, reduction with sodium borodeuteride, and permethylation. The products were characterised by chemical ionisation GLC–MS, using ammonia as the ionisation gas, which gave $[M+H]^+$ and $[M+NH_4]^+$, and by electron impact GLC–MS, which should give fragmentation patterns in agreement with established principles [1].

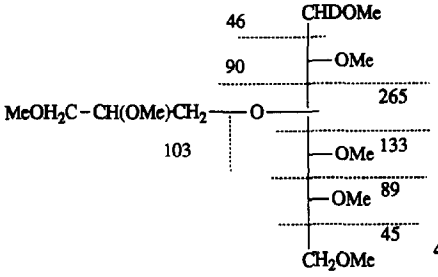
The product from **1** gave four peaks on GLC–MS. The components in the first two large peaks both had the molecular mass 292, as expected for internal glycosides formed on hydrolysis of **1**. They gave a strong ion of m/z 88 on EIMS, typical for methylated glycosides [1]. Internal glycosides, both furanoid and pyranoid, are also formed on acid hydrolysis of 2-*O*-(2-hydroxyethyl)- and 2-*O*-(2-hydroxypropyl)-glucosides [2,3]. No attempts were made to identify the individual components, exemplified by a β-pyranoside (**1a**). The two minor components, **1b**, had the molecular mass 355, and gave EI mass spectra typical for a permethylated 2-*O*-(2,3-dihydroxypropyl)-D-glucitol-*1-d*. The origins of some typical fragments are indicated in the formulas.



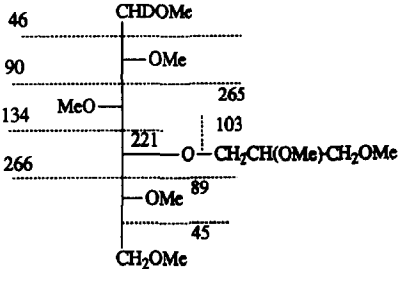
1a M=292



1b M=355



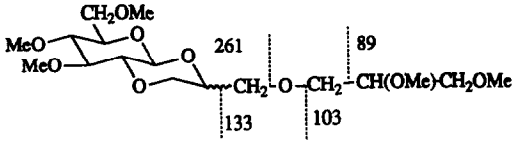
2a M=355



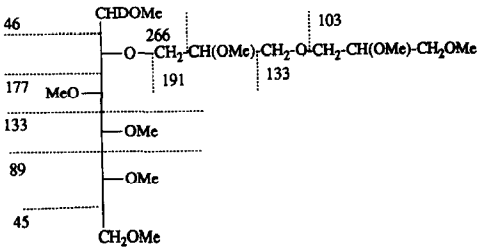
3a M=355

The derivatives prepared from **2** gave a large and a small peak on GLC, both with a molecular mass of 355, and the EI mass spectra were in agreement with the postulated structures (**2a**). The derivatives prepared from **3** gave a single peak on GLC, with a molecular mass of 355 and an EI mass spectrum in agreement with the postulated structure (**3a**).

The product from **7** gave five peaks on GLC. The components in the three minor peaks all had a molecular mass of 380, as expected for internal glycosides, e.g., **7a**. The components in the two major peaks both had a molecular mass of 433, as expected for the glucitol derivatives **7b**.

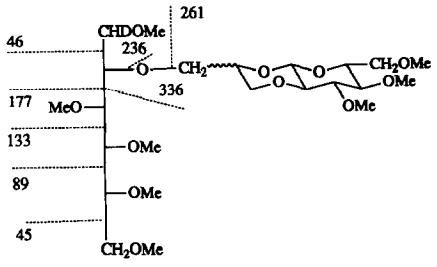


7a M=380



7b M=443

The product from **9** gave three peaks on GLC. They all had the molecular mass 513, indicating that they were internal glucosides. Again the formation of both furanosides and pyranosides is expected, and the identification of the individual components was not attempted. In the El mass spectra, the fragments obtained on fissions in the alditol moiety should, however, have the same mass numbers for the different isomers, and were in agreement with the postulated structures, as exemplified for the β-pyranoside, **9a**.



9a M=513

3. Experimental

General methods.—Reactions were followed by TLC on precoated Merck Silica Gel 60 F_{254}, using toluene–EtOAc mixtures as irrigants. Spots were detected by exposure to UV light and/or by spraying with 3 M H_2SO_4 and heating. Lipophilic substances were purified by chromatography on Merck Silica Gel 60, using the same solvent systems. Hydrophilic substances were purified by gel filtration on Sephadex® G15. Solutions were concentrated to dryness under reduced pressure. ^{13}C NMR spectra were recorded on a Jeol FX 200 instrument. Chemical shifts are reported in ppm, using internal ($CDCl_3$) or external (D_2O) Me_4Si as reference.

For GLC–MS, a Finnigan 4021 instrument equipped with a Finnigan 9610 gas chromatograph and Incos data system was used. Separations were performed on an OV-1 fused-silica capillary column, using a temperature program from 200 to 300°C at 1.3°C min^{-1}. Electron impact spectra were recorded at 70 eV. For chemical ionisation spectra, ammonia was used as ionisation gas.

General procedure for the preparation of triflates [4].—Trifluoromethanesulfonic anhydride (1.1 equiv) was dissolved in CH_2Cl_2 (5–20 mL), previously cooled to −40°C. Pyridine (1.2 equiv) was added, and the mixture stirred for 5 min before the alcohol (1 equiv) in CH_2Cl_2 (2–5 mL) was added dropwise. The mixture was allowed to reach room temperature, then filtered, and the freshly prepared solution used directly, without further purification.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3-epoxypropyl)-α-D-glucopyranoside.—Sodium hydride (0.36 g, 15 mmol) was added in portions to a stirred solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside [5] (2.0 g, 5.4 mmol) in THF (30 mL). The mixture was stirred for 30 min at room temperature, then cooled to −10°C, and 2,3-epoxpropyl triflate (1.7 g, 8.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The mixture was allowed to reach room temperature, excess of sodium hydride was destroyed by addition of MeOH (5 mL), and the solution concentrated. A solution of the residue in CH_2Cl_2 (100 mL) was washed with water (5 × 25 mL), dried over $MgSO_4$, concentrated, and subjected to silica gel chromatography to yield the title compound (1.5 g, 66%). ^{13}C NMR: δ 44.1, 44.3, 50.6, 51.1, 55.3, 62.3, 69.0, 71.7, 75.1, 78.5, 80.8, 82.1, 98.8, 99.1, 101.2, 125.9, 127.4, 127.8, 128.1, 128.7.

Methyl 3-O-benzyl-2-O-(2,3-hydroxypropyl)-α-D-glucopyranoside.—A solution of the foregoing epoxide (1.4 g, 3.3 mmol) and 0.5 M H_2SO_4 (1 mL) in 5:1 Me_2CO–water (60 mL) was refluxed for 3 h, allowed to cool, neutralised with $BaCO_3$, filtered, and concentrated. Silica gel chromatography yielded the title compound as a syrup (0.9 g, 76%). ^{13}C NMR (D_2O): δ 54.9, 60.6, 62.8, 69.5, 70.8, 70.9, 71.7, 71.8, 72.1, 75.3, 75.4, 79.9, 80.9, 81.1, 97.2, 128.8, 128.7, 128.8, 137.3.

Methyl 2-O-(2,3-hydroxypropyl)-α-D-glucopyranoside (**1**).—A solution of the foregoing 2,3-hydroxypropyl ether (0.8 g, 2.3 mmol) in EtOH (10 mL) containing AcOH (0.1 mL) was hydrogenolysed at room temperature and atmospheric pressure over Pd–C (10%, 75 mg). The solution was filtered and concentrated. Gel filtration yielded pure **1** as a syrup (0.59 g, 98%).

Methyl 3-O-(2,3-hydroxypropyl)-α-D-glucopyranoside (**2**) *and methyl 4-O-(2,3-hydroxypropyl)-α-D-glucopyranoside* (**3**).—These substances were prepared as described for **1**, starting from methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside [5] and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside [5], respectively, and in comparable yields.

1-O-Allylglycerol.—A solution of 1-allyloxy-2,3-epoxypropane (**5**; 95 g, 830 mmol) in 0.25 M CF_3CO_2H (100 mL) was refluxed until no starting material was left, then concentrated to dryness and repeatedly codistilled with water, until an aqueous solution of the product was neutral. The product (92 g, 82%) was purified by distillation; bp 75–77°C at 1 mmHg. ^{13}C NMR ($CDCl_3$): δ 63.9, 71.1, 71.5, 72.3, 117.1, 138.4.

1-O-Allyl-3-O-triphenylmethylglycerol.—A solution of chlorotriphenylmethane (143 g, 510 mmol) and 1-*O*-allylglycerol (58 g, 440 mmol) in pyridine (400 mL was refluxed for 2 h, allowed to cool, and poured into ice–water (2 L) under vigorous stirring. The nonaqueous phase was separated and dissolved in CH_2Cl_2 (500 mL), and the solution washed with water (3 × 200 mL), dried over $MgSO_4$, and concentrated to dryness, to give the crude title compound (105 g). Chromatography of part of this material yielded the pure substance as a syrup. ^{13}C NMR: δ 64.6, 69.8, 71.5, 72.2, 86.6, 116.9, 126.9, 127.7, 128.6, 134.4, 143.7.

1-O-Allyl-2-O-benzyl-3-O-triphenylmethylglycerol.—Sodium hydride (7.7 g, 320 mmol) was added in portions to a solution of the foregoing crude triphenylmethyl ether (85 g, 230 mmol) in DMF (500 mL). The solution was stirred for a further 30 min, then cooled to 0°C, and benzyl bromide (30 g, 240 mmol) was added. The mixture was kept at room temperature overnight, and excess of sodium hydride destroyed by MeOH (25 mL). The solution was concentrated to dryness, the residue dissolved in CH_2Cl_2 (750 mL), and the solution washed with water (4 × 200 mL), dried over $MgSO_4$, and concentrated to dryness, to give the crude title compound (95 g). Chromatography of part of this material yielded the pure substance as a syrup. ^{13}C NMR: 63.8, 70.8, 72.3, 77.8, 86.8, 116.7, 127.0, 127.5, 127.7, 128.0, 128.3, 128.8, 128.9, 129.1, 134.9, 144.2.

1-O-Allyl-2-O-benzylglycerol.—The foregoing crude product (90 g, 190 mmol) in aq 80% AcOH (500 mL) was refluxed for 30 min, and the solution was cooled to room temperature, filtered, and concentrated to dryness. Chromatography yielded the pure substance (21 g), bp 115–117°C at 1 mmHg. ^{13}C NMR: δ 62.3, 70.2, 72.2, 78.6, 116.7, 127.6, 127.7, 128.3, 134.8, 138.7.

Methyl 2-O-(3-allyloxy-2-benzyloxypropyl)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (**6**).—Sodium hydride (0.65 g, 27 mmol) was added in portions and under stirring to a solution of methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside [6] (4.0 g, 8.6 mmol) in THF (100 mL). The solution was cooled to −10°C after 30 min, a solution of 1-*O*-allyl-2-*O*-benzyl-3-*O*-trifluoromethanesulfonylglycerol (**4**; 4.0 g, 11 mmol; prepared as above) in CH_2Cl_2 (20 mL) was added dropwise, and the mixture was allowed to reach room temperature. After 1 h, excess of sodium hydride was destroyed with MeOH (15 mL), the mixture was concentrated, and a solution of the residue in CH_2Cl_2 (500 mL) was washed with water (3 × 150 mL), dried over $MgSO_4$, and concentrated. Chromatography yielded pure **6** (4.7 g, 87%). ^{13}C

NMR: δ 55.0, 68.7, 70.1, 70.2, 70.3, 70.8, 71.3, 72.0, 72.2, 73.4, 74.8, 75.3, 76.5, 77.1, 77.4, 77.6, 77.7, 81.3, 81.4, 81.8, 81.9, 97.8, 116.6, 127.3, 127.5, 127.7, 128.2, 134.7, 138.0, 138.3, 138.5, 138.6, 138.8.

*Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-benzyloxy-6,7-epoxy-4-oxaheptyl)-α-*D*-glucopyranoside.*—Bromine (260 mg, 1.6 mmol) in water (10 mL) was added dropwise to a solution of **6** (500 mg, 0.75 mmol) in a mixture of THF (20 mL) and water (5 mL). The pH was kept at 5.5 by addition of 0.5 M NaOH. When all Br_2 was consumed, the pH was raised to 12 by addition of 2 M NaOH. After 1 h at room temperature, the solution was neutralised with 1 M AcOH and concentrated, and a solution of the product in CH_2Cl_2 (100 mL) was washed with water (3 × 25 mL), dried over $MgSO_4$, and concentrated. Chromatography yielded the pure title compound (162 mg, 32%). ^{13}C NMR: δ 44.2, 50.7, 55.1, 68.7, 70.1, 70.7, 71.1, 71.2, 71.3, 71.4, 72.0, 72.1, 72.2, 72.3, 73.5, 75.0, 75.5, 77.4, 77.7, 77.8, 81.4, 81.5, 81.9, 82.0, 97.9, 127.4, 127.5, 127.6, 127.7, 127.8, 128.3, 138.0, 138.3, 138.5, 138.6, 138.8.

*Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-benzyloxy-6,7-dihydroxy-4-oxaheptyl)-α-*D*-glucopyranoside.*—A solution containing the foregoing epoxide (150 mg, 0.22 mmol), CF_3CO_2H (1 mL), Me_2CO (5 mL), and water (5 mL) was refluxed and the reaction followed by TLC. When all starting material had reacted, the solution was concentrated. Chromatography of the product yielded the pure title compound (140 mg, 91%). ^{13}C NMR: δ 55.1, 68.6, 70.3, 70.5, 71.2, 72.1, 72.3, 73.0, 73.5, 75.0, 75.5, 77.2, 77.3, 77.7, 81.4, 81.5, 81.9, 82.0, 97.7, 97.8, 127.5, 127.7, 127.8, 127.9, 128.4, 138.0, 138.2, 138.3, 138.8.

*Methyl 2-*O*-(2,6,7-trihydroxy-4-oxaheptyl)-α-*D*-glucopyranoside* (**7**).—Catalytic hydrogenolysis of the foregoing substance (103 mg, 0.15 mmol) and workup, as described for the preparation of **1**, yielded pure **7** (22 mg, 44%).

*Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-benzyloxy-3-hydroxypropyl)-α-*D*-glucopyranoside* (**8**).—Pd–C (10%, 400 mg) was added to a solution of **6** (3.9 g, 0.59 mmol) in a mixture of MeOH (50 mL), AcOH (10 mL), and water (10 mL), and the mixture was refluxed for 3 h. The catalyst was filtered off and the solution concentrated. Chromatography of the product yielded pure **8** (2.4 g, 64%). ^{13}C NMR: δ 55.8, 62.2, 68.4 70.0 70.6, 71.6, 73.2, 74.7, 75.2, 77.5, 77.8, 78.0, 81.1, 81.6, 81.7, 97.4, 127.2, 127.4, 127.5, 128.0, 128.1, 137.7, 138.0, 138.5.

*2-Benzyloxy-1,3-bis(methyl 3,4,6-tri-*O*-benzyl-α-*D*-glucopyranosid-2-*O*-yl)propane.*—Sodium hydride (200 mg, 8.3 mmol) was added in portions to a stirred solution of methyl 3,4,6-tri-*O*-benzyl-α-*D*-glucopyranoside (200 mg, 0.43 mmol) in THF (10 mL), and the solution was stirred for a further 30 min and then cooled to −10°C. The triflate prepared from methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-benzyloxy-3-hydroxypropyl)-α-*D*-glucopyranoside (365 mg, 0.58 mmol) in CH_2Cl_2 (3 mL) was added dropwise, and the solution was allowed to reach room temperature. Excess of sodium hydride was destroyed by MeOH (3 mL), the solution concentrated to dryness, the residue dissolved in CH_2Cl_2 (50 mL), and the solution washed with water (3 × 10 mL), dried over $MgSO_4$, and concentrated. Chromatography of the product yielded the pure substance (298 mg, 65%). ^{13}C NMR: δ 54.8, 68.4, 70.0, 70.7, 71.1, 71.9, 73.2, 74.7, 75.2, 77.4, 77.7, 81.2, 81.7, 97.6, 127.2, 127.4, 127.5, 127.6, 128.1, 137.8, 138.1, 138.3, 138.7.

2-Hydroxy-1,3-bis(methyl α-D-glucopyranosid-2-O-yl)propane (**9**).—The foregoing substance (290 mg, 0.27 mmol) was hydrogenolysed and worked up as described for **1**, yielding pure **9** (91 mg, 73%).

Preparation of permethylated alditol-1-d *derivatives.*—The model substance (2 mg) in 3 M aq H_2SO_4 (1 mL) was kept at 100°C for 5 h, and the solution was neutralised with $BaCO_3$, filtered, treated with Dowex 50 (H^+) resin, and then with $NaBD_4$. The product, after conventional work-up, was methylated according to Hakomori [7], and investigated by GLC–MS.

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