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Base-catalyzed degradation of permethylated 3-*O*-glycosyl-glycopyranosid-2-uloses*

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ABSTRACT

In a modification of the Svensson degradation, otherwise permethylated glycopyranosid-2-uloses bearing 4-*O*-glycosyl substituents are formed by the Swern oxidation. Base-catalyzed elimination on treatment with triethylamine then gives 4-deoxy-3-*O*-methylglyc-3-enopyranosid-2-ulose-terminated oligosaccharides with liberation of glycosyl substituents as reducing sugars but without further degradation. Mild acid hydrolysis results in removal of the unsaturated sugar residues so that the overall depolymerization occurs with net loss only of the initially oxidized sugar residue.

INTRODUCTION

Svensson and collaborators[1–3] developed a specific degradation requiring selective exposure of hydroxyl groups in otherwise permethylated polysaccharides, as by controlled hydrolysis of acid-sensitive linkages or base-catalyzed removal of terminal hexuronic acid residues[4,5], followed by oxidation of exposed hydroxyl groups and base-catalyzed β-elimination from the resulting glycosiduloses. The nature of the products is dependent on the relative placing of carbonyl groups generated and glycosyl substitutents. In some instances chain scission occurs during the base treatment, but in other instances an additional mild hydrolysis of acid-sensitive enolic linkages is required before depolymerization takes place. There are well-documented examples of degradations initiated at glycopyranosid-3-uloses and glycopyranosid-4-uloses. The behaviour in the reaction sequence of glycopyranosid-2-uloses bearing 4-*O*-glycosyl substituents is less well understood. Studies by Rosell and Svensson[6] on the distribution of 4-*O*-methyl-D-glucuronic acid side-chains in birch xylan involved the degradation of such glycopyranosiduloses. Treatment of the permethylated xylan with dimsyl base resulted in the removal of uronic acid residues with exposure of aglyconic hydroxyl groups at C-2 of xylose units at branch points in the xylan backbone. Specific cleavage was effected by successive oxidation with chlorine–dimethyl sulfoxide, base-catalyzed elimination, and mild acid hydrolysis to give a mixture of xylo-oligosaccharides of varying size. The

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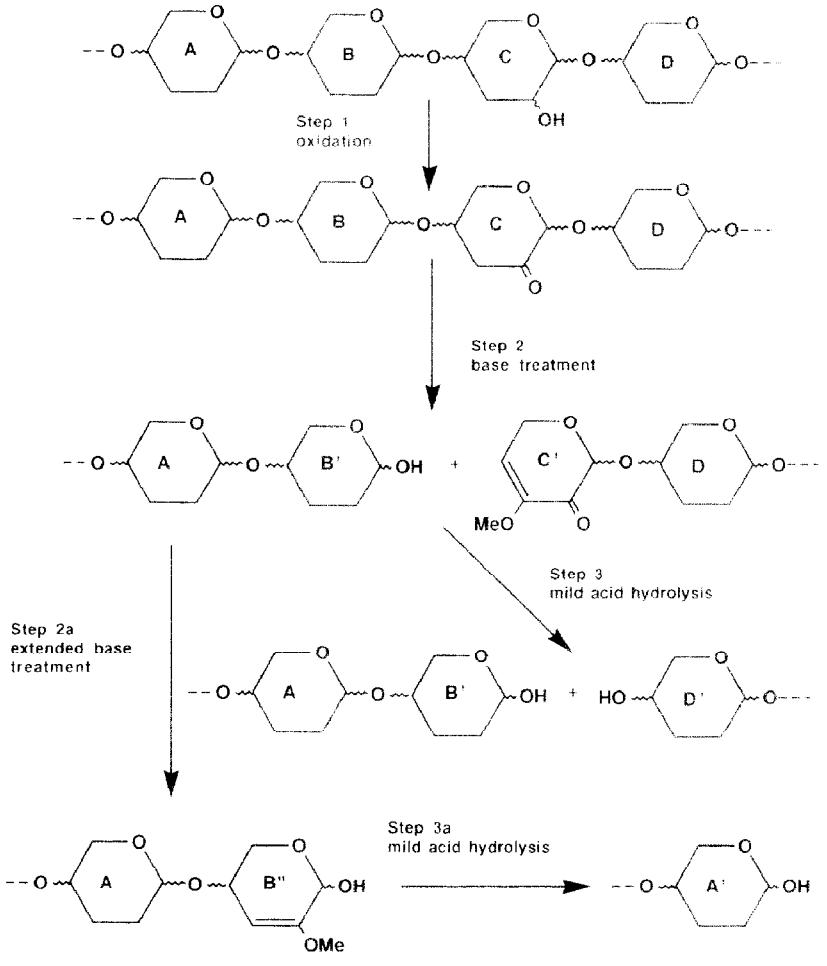
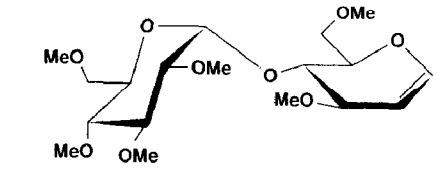
authors postulated, as shown in the generalized Scheme 1, that degradation proceeded with net loss of residue C at which oxidation (step 1) had taken place and of the initially liberated reducing residue B′. It was supposed that such a reducing sugar unit B′ would be further degraded under the conditions of the base treatment (step 2a) and then lost on mild acid treatment of the resulting pent-2-enopyranose residue (step 3a). As the degradation products were polymer-homologous oligosaccharides, it was not possible to assess the extent of degradation in the β-elimination and subsequent mild acid hydrolysis. In connection with structural studies on a highly branched fuco-4-*O*-methylglucuronoxylan[7] it was desirable to develop a modified procedure that minimizes the potential loss of structural information in the base-catalyzed β-elimination. The procedure reported here has been developed with permethylated oligosaccharides



Scheme 1. Generalized outline for formation (step 1) and base-catalyzed degradation (step 2) of 4-*O*-glycosylglycopyranosid-2-uloses, followed by mild acid hydrolysis (step 3) [for simplicity non-involved substituents are omitted].



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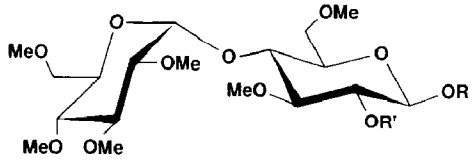
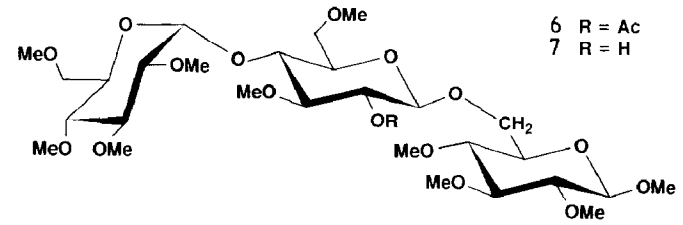
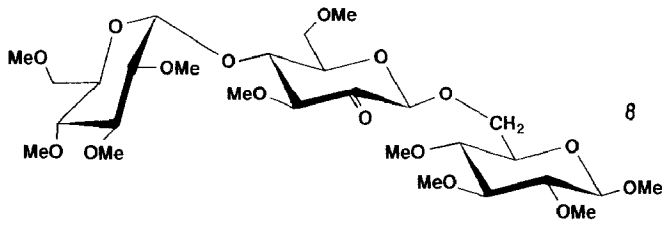


2 R = Me; R' = H
3 R = Me; R' = Me
4 R = R' = Ac
5 R = H; R' = Ac



6 R = Ac
7 R = H



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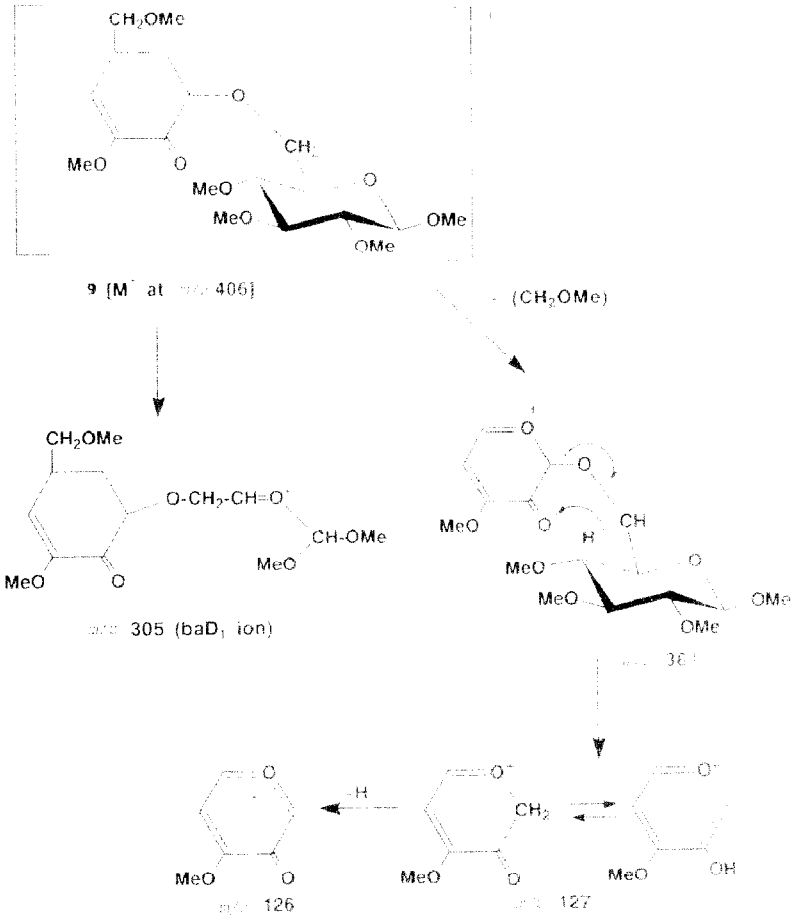
containing 4-*O*-glycosylglycosid-2-ulose units and involves the Swern oxidation[8] followed by weak-base treatment with triethylamine.

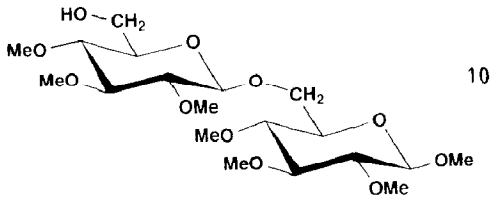
DISCUSSION

Suitable 4-*O*-glycosylglycopyranosid-2-uloses were synthesized from hexa-*O*-methylmaltal (**1**). Treatment of **1** with *m*-chloroperoxybenzoic acid in methanol proceeded with high stereoselectivity to form methyl 2',3,3',4',6,6'-hexa-*O*-methyl-β-maltoside (**2**) as the sole detectable product whose identity was confirmed by conversion into methyl hepta-*O*-methyl-β-maltoside[9] (**3**). In preliminary studies[10], oxidation of **2** with pyridinium chlorochromate[11] gave the corresponding 2-ulose and base degradation afforded 2,3,4,6-tetra-*O*-methylglucose as the sole detectable product. In order to ascertain the fate of both the residue undergoing oxidation and adjacent residues, higher oligosaccharides containing a central 4-*O*-substituted glycopyranosid-2-ulose residue were prepared. Glycal **1**, again used as starting material, reacted with *m*-chloroperoxy-

benzoic acid in acetic acid and the whole reaction mixture was acetylated before product isolation. As before, the reaction was highly stereoselective and resulted in the formation of the *β*-D-*gluco* isomer (**4**) as the major product together with two minor components, one of which was probably the α-D-*manno* isomer resulting from epoxidation from the opposite face. *O*-Deacetylation of **4** with hydrazine acetate[17] afforded glycose **5**, which was converted as required into the corresponding α-trichloroacetimidate[7] to be used directly for glycosylation. Reaction of the trichloroacetimidate with methyl 2,3,4-tri-*O*-methyl-*β*-D-glucopyranoside with boron trifluoride etherate as catalyst gave trisaccharide **6** which was *O*-deacetylated to **7**. Oxidation of **7** by the Swern method[8] with dimethyl sulfoxide–oxalyl chloride (or trifluoroacetic anhydride) furnished three products, unsaturated disaccharide **9**, tetra-*O*-methylglucose, and a substance whose ^{1}H-n.m.r. spectrum indicated that it was probably the expected trisaccharide glycosidulose **8**. On further treatment with triethylamine, the third product disappeared. We conclude that the initially formed glycosidulose **8** undergoes facile *β*-elimination when



Scheme 2. Some mass-spectral fragmentations of compound **9**.



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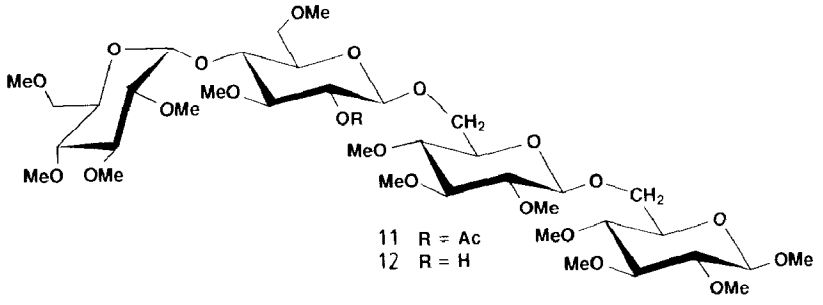
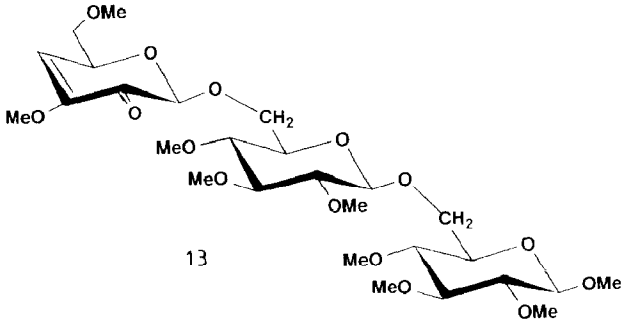


11 R = Ac
12 R = H



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triethylamine is added during work-up of the Swern oxidation and that the more-strongly basic conditions used by Svensson *et al.*[1-3] are not required to effect β-elimination. The structure of **9** as methyl 4-deoxy-3,6-di-*O*-methyl-β-D-*glycero*-hex-3-enopyranosylulose-(1→6)-2,3,4-tri-*O*-methyl-β-D-glucopyranoside was assigned on the basis of (*a*) resonances in the ^{1}H-n.m.r. spectrum for a vinylic proton (H-4′) at δ 5.90 and for an anomeric singlet at δ 5.05, and (*b*) salient ions in the mass spectrum which are shown here (Scheme 2).

In similar manner, an homologous tetrasaccharide **11** was synthesized by condensation, with boron trifluoride as catalyst, of the trichloroacetimidate from **5** with methyl 2,2′,3,3′,4,4′-hexa-*O*-methyl-β-gentiobioside (**10**) (prepared by standard methods from methyl β-gentiobioside), followed by *O*-deacetylation to **12**. Swern oxidation of **12** followed by further treatment with triethylamine without isolation of the initial oxidation product gave only two compounds, namely, tetra-*O*-methylglucose and the unsaturated trisaccharide **13** whose structure was evident from the ^{1}H-n.m.r. data. Although the unsaturated oligosaccharides **9** and **13** were insufficiently stable to obtain satis-

factory elemental analyses, both compounds were further characterized by exact mass determinations. No degradation of tetra-*O*-methylglucose was detected in these reactions and a branched methylated reducing trisaccharide, 2,6-di-*O*-methyl-3,4-bis-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-D-galactose [J. Szafranek, unpublished results], was also stable when treated with triethylamine.

As degradations of glycans containing 4-linked glycosid-2-ulose units would result in the formation of oligosaccharides with 4-deoxy-3,6-di-*O*-methyl-β-D-*glycero*-hex-3-enopyranosylulose or similar residues at the non-reducing termini, it was necessary to ascertain the mildest conditions for their selective removal with minimum hydrolysis of normal glycosidic linkages (step 3). Trial experiments on **9** and **13** and the corresponding 4-deoxyhex-3-enopyranosides formed on reduction with sodium borohydride showed that the latter compounds were hydrolyzed more readily than the former when heated at 80° with Amberlite IR-120(H^+) resin. With erythritol tetraacetate as internal standard, reduction of **9** followed by hydrolysis afforded methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside in 71% yield. A similar preparative-scale treatment of **13** led to the isolation of **10** in 72% yield with no other detectable products. Reduction with sodium borohydride (or borodeuteride) also serves to identify reducing glycose residues exposed during the β-elimination.

This modified method offers advantages over the original procedure of Svensson *et al.*[13] in that the Swern oxidation and β-elimination (steps 1 and 2) are compressed into a single operation without intermediate isolation of the oxidation product, and that the triethylamine used in the β-elimination step causes no degradation at exposed reducing sugar residues. The accompanying paper[7] gives an example of the method applied to a polysaccharide substrate.

EXPERIMENTAL

General methods. — Solvents and reagents were purified and dried according to standard procedures[14]. Molecular sieves were activated and K_2CO_3 was dried by heating to 400°. Evaporations were conducted under diminished pressure at <40°. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at ~20°. N.m.r. spectra were recorded with a Bruker AM 300 spectrometer for solutions in $CDCl_3$ unless otherwise stated. G.l.c. was performed with a Perkin–Elmer Sigma 3B chromatograph, using fused-silica columns (Chromatographic Specialties Limited): A, a 15-m wide-bore capillary of DB-225, or B, a 5-m narrow-bore capillary of DB-5. For g.l.c.–m.s., columns were attached by a jet separator to a VG Micromass 16F mass spectrometer, which was operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°. Accurate mass determinations by fast-atom bombardment mass spectrometry in a thioglycerol matrix were performed by Dr. Henrianna Pang and her associates at the University of Toronto Carbohydrate Research Centre. Microanalyses were carried out by Guelph Chemical Laboratories, Ltd.

*Hexa-*O*-methylmaltal* **1**. — Methanolic M NaOMe (1 mL) was added to hexa-*O*-acetylmaltal[15] (1.12 g, 2 mmol) in dry MeOH (100 mL) and the solution was kept for 18

h. Sodium ions were removed by treatment with Amberlite IR-120 (H^+) resin and the filtrate was concentrated to a syrupy residue which was dried to constant weight, dissolved in *N,N*-dimethylformamide (25 mL) under an argon atmosphere and NaH (900 mg, 60% suspension in Nujol) was added[16]. The mixture was stirred at room temperature for 3 h, cooled to 0° and MeI (3 mL) was added slowly with stirring. The solution was allowed to reach room temperature and was left overnight. Methanol was added dropwise to ensure that all NaH had reacted, solvents were evaporated, the residue was dissolved in $CHCl_3$ and the solution was washed 3 times with water and dried. The solution was concentrated and the residue was chromatographed on silica gel (10:1, $CHCl_3$–acetone) to give **1** as a syrup (703 mg, 89%), $[\alpha]_D$ +112.5° (*c*, 1.0); ^{1}H-n.m.r.: δ 3.32–3.63 (6 s, each 3 H, 6 OMe), 4.81 (dd, 1 H, $J_{1,2}$ 6.71, $J_{2,3}$ 2.51 Hz, H-2), 5.63 (d, 1 H, $J_{1',2'}$ 3.75 Hz, H-1'), and 6.44 (dd, 1 H, $J_{1,3}$ 1.11 Hz, H-1).

Anal. Calc. for $C_{18}H_{32}O_9$: C, 55.09; H, 8.22. Found: C, 54.83; H, 8.05.

*Methyl 2',3,3',4',6,6'-hexa-*O*-methyl-β-maltoside* **2** *and hepta-*O*-methyl derivative* **3**. — *m*-Chloroperoxybenzoic acid (345 mg, 2.0 mmol) in CH_2Cl_2 (6 mL) was added with stirring to **1** (0.39 g, 0.98 mmol) in MeOH (6 mL) at room temperature. T.l.c. showed that reaction was complete after 1 h and the excess of peroxyacid was reduced by addition of aq. 10% Na_2SO_3. The separated organic layer was washed with aq. $NaHCO_3$, dried, and concentrated. The residue was chromatographed on silica gel (6:1 $CHCl_3$–acetone) to yield syrupy **2** (0.37 g, 83%); ^{1}H-n.m.r.: δ 3.33–3.64 (7 s, each 3 H, 7 OMe), 4.15 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), and 5.63 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1'). Hydrolysis of a sample of **2** followed by reduction with $NaBD_4$ and acetylation gave 3,6-di- and 2,3,4,6-tetra-*O*-methylglucitol acetates, which were identified by g.l.c.–mass spectrometry on column A.

Anal. Calc. for $C_{19}H_{36}O_{11}$: C, 51.82; H, 8.18. Found: C, 51.97; H, 8.34.

Methylation of **2** (60 mg) in oxolane (4 mL) using NaH (70 mg) and MeI (0.9 mL) afforded methyl hepta-*O*-methyl-β-maltoside (**3**) (47 mg, 74%), $[\alpha]_D$ +81° (*c* 1.0, $CHCl_3$) [lit.[9] $[\alpha]_D$ +81°]. Characterization of hydrolysis products as before afforded 2,3,6-tri- and 2,3,4,6-tetra-*O*-methylglucitol acetates.

*1,2-Di-*O*-acetyl-2',3,3',4',6,6'-hexa-*O*-methyl-β-maltose* **4**. — Hexa-*O*-methylmaltal (0.7 g, 1.8 mmol) in AcOH (12 mL) at 0° was treated with *m*-chloroperoxybenzoic acid (0.587 g, 3.4 mmol) in CH_2Cl_2 (15 mL). The solution was kept for 3 h at room temperature, at which time t.l.c. indicated complete disappearance of the starting material. Excess of peroxy acid was decomposed by the addition of aq. 10% $NaHSO_3$, CH_2Cl_2 (50 mL) was added, and the organic layer was washed with aq. 5% $NaHCO_3$ and then water, and dried. Concentration gave a syrup (0.697 g, 83%) which was immediately dissolved in anhydrous pyridine and treated with Ac_2O at 0°, and the solution was kept overnight. Concentration of the solution and normal work-up gave a syrupy mixture which was chromatographed on silica gel (5:2 EtOAc–light petroleum) to give a major fraction (563 mg, 75%) containing **4** with only traces of the anomeric α-maltose derivative, $[\alpha]_D$ +104° (*c* 1.2); ^{1}H-n.m.r.: δ 2.08, 2.12 (2 s, each 3 H, 2 OAc), 3.34–3.62 (6 s, each 3 H, 6 OMe), 4.02 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 8.94 Hz, H-2'), 5.07 (dd, H, $J_{1,2}$ 8.16, $J_{2,3}$ 8.97 Hz, H-2), 5.54 (d, 1 H, $J_{1',2'}$ 3.76 Hz, H-1'), and 5.58 (d, 1 H, $J_{1,2}$ 7.98 Hz, H-1);

^{13}C-n.m.r.: δ_C 20.3 ($OCOCH_3$), 58.0, 58.5, 58.7, 58.8, 58.9, 60.1 (6 OCH_3), 69.9, 70.3 (C-6, C-6'), 70.4 (C-2'), 71.2 (C-2), 91.7 (C-1), 96.0 (C-1'), and 168.6 ($OCOCH_3$).

Anal. Calc. for $C_{33}H_{58}O_{17}$: C, 51.75; H, 7.50. Found: C, 51.78; H, 7.69.

The minor fractions when examined by ^{1}H-n.m.r. showed the presence of (*i*) 2-*O*-acetyl-1-*O*-*m*-chlorobenzoyl-hexa-*O*-methylmaltose (21 mg, 3%) and (*ii*) 1,2-di-*O*-acetyl-3,6-di-*O*-methyl-4-*O*-[2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl]-α-D-mannopyranose (21 mg, 3%).

2-O-Acetyl-2',3,3',4',6,6'-hexa-O-methyl-α,β-maltose **5**. — Hydrazine acetate (50 mg) was added with stirring to compound **4** (230 mg, 0.457 mmol) in *N,N*-dimethylformamide (2 mL) and the mixture was stirred for 1 h, whereupon t.l.c. showed that reaction was complete. Chloroform (15 mL) was added and the organic layer was washed twice with saturated aq. NaCl, and then water, dried, and concentrated. The resulting syrup was chromatographed on silica gel (10:3 $CHCl_3$–acetone) to give the title compound (209 mg) in virtually quantitative yield and for which t.l.c. showed the presence of both anomers. $[\alpha]_D$ +141° (*c* 0.55); ^{1}H-n.m.r.: δ 2.13 (s, 3 H, OAc), 3.33–3.64 (5 s, 4 × 3 H, 1 × 6 H, 6 OMe), 5.38 (d, 1 H, $J_{1,2}$ 3.55 Hz, H-1), and 5.57 (d, 1 H, $J_{1,2}$ 3.72 Hz, H-1'); *exact mass* calc. for ($C_{30}H_{56}O_{17}$ + Na): 491.2104; found: 491.2113.

Anal. Calc. for $C_{30}H_{56}O_{17}$: C, 51.27; H, 7.74. Found: C, 51.66; H, 8.12.

Methyl 2-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-(1→4)-2-O-(2-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-methyl-β-D-glucopyranoside **6**. — Trichloroacetonitrile (0.4 μL) was added with a syringe through a septum to the foregoing hemiacetal **5** (86 mg, 0.1183 mmol) in dry CH_2Cl_2 (2 mL) under an argon atmosphere. Later, anhydrous K_2CO_3 (200 mg) was added and the suspension was stirred for 3 days, whereupon t.l.c. showed formation of a single product and disappearance of starting material. The mixture was diluted with CH_2Cl_2 (10 mL), filtered through a pad of Celite, and the filtrate was concentrated to dryness to give the trichloroacetimidate (120 mg), $[\alpha]_D$ +96° (*c* 0.18); ^{1}H-n.m.r.: δ 2.03 (s, 3 H, OAc), 3.50–3.64 (5 s, 4 × 3 H, 1 × 6 H, 6 OMe), 5.61 (d, 1 H, $J_{1,2}$ 3.54 Hz, H-1'), 6.51 (d, 1 H, $J_{1,2}$ 3.12 Hz, H-1), and 8.58 (s, 1 H, NH).

Freshly activated molecular sieves (4 Å, 0.5–5 μm, 100 mg) were added to the aforementioned imidate (150 mg, 0.245 mmol, without further purification) and methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside (58 mg, 0.245 mmol) in dry CH_2Cl_2 (2 mL). The mixture was stirred for 1 h, BF_3–OEt_2 (10 μL) was added and the mixture was kept for 2 days at room temperature with stirring. The reaction was terminated by the addition of solid $NaHCO_3$ (100 mg), the mixture was diluted with CH_2Cl_2 (10 mL), and the filtrate was washed with water, dried and concentrated to a syrup in which t.l.c. showed a major product at R_f 0.87 (2:1 $CHCl_3$–acetone). Separation of the mixture on the "Chromatotron" on 1-mm silica gel plates using gradient elution with $CHCl_3$–$CHCl_3$ containing 10% of acetone furnished **6** (138 mg, 72%), m.p. 123–124° (after recrystallization from acetone–hexane). $[\alpha]_D$ +52° (*c* 1.32); ^{1}H-n.m.r.: δ 2.07 (s, 3 H, OAc), 3.34 (s, 3 H, glycosidic OMe), 3.41–3.64 (9 s, each 3 H, 9 × OMe), 4.11 (d, 1 H, $J_{1,2}$ 7.83 Hz, H-1), 4.87 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1'), 4.99 (dd, 1 H, $J_{2,3} = J_{2,1}$, 8.08 Hz, H-2'), and 5.58 (d, 1 H, $J_{1,2}$ 3.76 Hz, H-1''); ^{13}C-n.m.r.: δ_C 68.12 (C-6), 70.77, 70.84 (C-6' and

C-6′), 96.23 (C-1″), 100.86 (C-1), and 103.97 (C-1′). G.l.c.–mass spectrometric examination (DB-5 column) showed a single peak with the fragment ions discussed elsewhere.

Anal. Calc. for $C_{30}H_{54}O_{17}$: C, 52.46; H, 7.92. Found: C, 52.26; H, 8.13.

Methyl O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-(1→4)-O-(3,6-di-O-methyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-methyl-β-D-glucopyranoside **7**. — Trisaccharide methyl glycoside (**6**, 26 mg, 0.38 mmol) in dry MeOH (10 mL) was treated with a catalytic quantity of NaOMe for 10 h. Sodium ions were removed on treatment with Amberlite IR-120(H^+) resin and concentration of the filtrate afforded **7** (18 mg, 75%), $[\alpha]_D$ +34.8° (*c* 0.16); ^{1}H-n.m.r.: δ 4.15 (d, 1 H, $J_{1,2}$ 7.67 Hz), 4.33 (d, 1 H, $J_{1',2'}$ 7.42 Hz, H-1′), and 5.64 (d, 1 H, $J_{1'',2''}$ 3.81 Hz, H-1″).

Anal. Calc. for $C_{28}H_{52}O_{16}$: C, 52.16; H, 8.13. Found: C, 51.96; H, 8.10.

Oxidation of methylated trisaccharide glycoside **7** *with oxalyl chloride–dimethyl sulfoxide, followed by treatment with triethylamine.* — Dimethyl sulfoxide (175 μL) was added to freshly distilled oxalyl chloride (100 μL) in dry CH_2Cl_2 (2.5 mL) at −50°. The mixture was stirred for 2 min and **7** (13 mg) in CH_2Cl_2 (0.5 mL) was added slowly and stirring was continued for 15 min. Triethylamine (0.7 mL) was added and the mixture was stirred for 15 min and allowed to warm to room temperature. The mixture was concentrated to dryness under vacuum, and the non-volatile residue in CH_2Cl_2 was washed three times with water, dried and concentrated to a syrup from which trisaccharide **7** was absent. Chromatography on silica gel with gradient elution with $CHCl_3$ containing 20–50% of acetone furnished three fractions (each 2–3 mg). Fraction (*i*) contained a single component with ^{1}H-n.m.r. data and electron-impact mass-spectral data (Scheme 2) consistent with the structure methyl 4-deoxy-3,6-di-*O*-methyl-β-D-*glycero*-hex-3-enopyranosylulose-(1→6)-2,3,4-tri-*O*-methyl-β-D-glucopyranoside (**9**); ^{1}H-n.m.r.: δ 3.42–3.66 (5 s, 4 × 3 H, 1 × 6 H, 6 OMe), 4.14 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1′), 5.05 (s, 1 H, H-1′), and 5.90 ($J_{4',5'}$ 3.6 Hz, H-4′); *exact mass* calc. for fragment ion [M − (CH_3OMe), − (H_2O), $C_{16}H_{23}O_8$]: 343.1393; found: 343.1383. Fraction (*ii*) was chromatographically indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-glucopyranose; ^{1}H-n.m.r.: δ 3.40–3.62 (4 s, each 3 H, 4 OMe), 4.57 and 5.33 (2 d, each 1 H, $J_{1,2}$ 7.6 and 3.5 Hz, H-1β and H-1α). Fraction (*iii*) contained the two aforementioned compounds and a third component whose ^{1}H-n.m.r. spectrum showed anomeric protons consistent with those of oxidized trisaccharide, methyl *O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-(1→4)-*O*-(3,6-di-*O*-methyl-β-D-*arabino*-hexopyranosyl-2-ulose)-(1→6)-2,3,4-tri-*O*-methyl-β-D-glucopyranoside (**8**); δ 4.14 (d, $J_{1,2}$ 7.7 Hz, H-1), 4.92 (s, H-1′), and 5.50 (d, $J_{1'',2''}$ H-1″). G.l.c.–mass-spectrometric examination of the mixture using column B showed no peaks in the trisaccharide region but a single peak was observed in the disaccharide region whose molecular ion and fragment ions were consistent with those arising from the proposed unsaturated disaccharide (**9**). A portion of the mixture of reaction products was treated with Ac_2O and pyridine, and the resulting compounds were examined by g.l.c.–m.s. Peaks were observed which corresponded to the aforementioned unsaturated disaccharide (**9**) and 2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl acetate (α,β-anomers not resolved) [M^+ at *m/z* 278, and fragment ions at *m/z* 219, 187, and 155], but no trace of trisaccharide glycoside **6** could be detected, indicating

completeness of oxidation of **7**. A portion of the mixture from the Swern oxidation was treated with Et_3N 10% in CH_2Cl_2 for 7 h, and t.l.c. examination of the products [10:3 $CHCl_3$–acetone] showed complete disappearance of the trisaccharide oxidation-product (**8**).

Methyl 2,2',3,3',4,4'-hexa-O-methyl-β-gentiobioside **10**. — Methyl β-gentiobioside[17] was successively converted into the 6-*O*-trityl derivative[18], methylated by the Brimacombe procedure[16], and *O*-detritylated with HBr in AcOH[18] to give **10**, $[\alpha]_D$ −16° (*c* 0.5); ^{1}H-n.m.r.: δ 3.16–3.64 (7 s, each 3 H, 7 OMe), 4.14 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), and 4.36 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1').

Anal. Calc. for $C_{19}H_{36}O_{11}$: C, 51.80; H, 8.20. Found: C, 51.87; H, 8.30.

Methyl O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-(1→4)-O-(2-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-methyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-methyl-β-D-glucopyranoside **11**. — A mixture of molecular sieves (4Å, 0.5–5 μm, 170 mg), **10** (144 mg, 0.36 mmol) and α-trichloroacetimidate (220 mg, 0.36 μm, prepared from **5** as already described) in dry CH_2Cl_2 (4 mL) was stirred for 1 h, $BF_3 \cdot OEt_2$ (17 μL) was added and the mixture was stirred for 2 days at room temperature. The mixture was worked up as for **6** and chromatography on silica gel (20:1 EtOAc–hexane) gave **11** (170 mg, 53%), $[\alpha]_D$ +29° (*c* 1.0); ^{1}H-n.m.r.: δ 2.08 (s, 3 H, OAc), 3.34–3.63 (12 s, 11 × 3 H, 1 × 6 H, 13 OMe), 4.13 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.26 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 4.46 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1''), and 5.55 (d, 1 H, $J_{1''',2'''}$ 3.7 Hz, H-1'''); *exact mass* calc. for ($C_{39}H_{70}O_{22}$ + Na): 913.4256; found: 913.4272.

Methyl O-2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl-(1→4)-O-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-methyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-methyl-β-D-glucopyranoside **12**. — Catalytic *O*-deacetylation of **11** (170 mg) with NaOMe as for **4** afforded **12** (140 mg), $[\alpha]_D$ +26° (*c* 5.0); ^{1}H-n.m.r.: δ 3.32–3.63 (11 s, 9 × 3 H, 2 × 6 H, 13 OMe), 4.14 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.26 (d, 1 H, $J_{1',2'}$ 7.2 Hz, H-1'), 4.31 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1''), and 5.67 (d, 1 H, $J_{1''',2'''}$ 3.7 Hz, H-1'''); *exact mass* calc. for ($C_{37}H_{68}O_{21}$ + Na): 871.4151; found: 871.4129.

Anal. Calc. for $C_{37}H_{68}O_{21}$: C, 52.30; H, 8.07. Found: C, 51.90; H, 7.58.

Oxidation of methylated tetrasaccharide **12** *with trifluoroacetic anhydride–dimethyl sulfoxide, followed by treatment with triethylamine.* — Trifluoroacetic anhydride (800 μL, 5.2 mmol) in CH_2Cl_2 (1 mL) was added to Me_2SO (500 μL, 7 mmol) in CH_2Cl_2 (3 mL) at −65–70°, the solution was stirred for 15 min, and **12** (60 mg, 0.07 mmol) in CH_2Cl_2 (0.5 mL) was added with maintenance of temperature below −65°. After 4 days Et_3N (1.5 mL, 10.9 mmol) was added dropwise, the mixture was allowed to warm to room temperature and the CH_2Cl_2 solution was washed with saturated NH_4Cl solution, and then water, and dried and concentrated. The residue was kept overnight in CH_2Cl_2 containing ~ 10% of Et_3N. Concentration of the solution followed by chromatography on silica gel (10:3 $CHCl_3$–acetone) gave (*i*) 2,3,4,6-tetra-*O*-methyl-D-glucose (9 mg) and (*ii*) unsaturated trisaccharide **13** (32 mg), $[\alpha]_D$ −64° (*c* 1.44); ^{1}H-n.m.r.: δ 3.44–3.66 (9 s, each 3 H, 9 OMe), 4.11 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.29 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 5.06 (s, 1 H, H-1''), and 5.87 (d, $J_{1''',2'''}$ 3.5 Hz, H-4''); ^{13}C-n.m.r.: δ_C 99.28 (C-1''), 103.70, 104.25 (C-1, 1'), 114.07 (C-4''), 147.60 (C-3''), and 183.83 (C-3''); *exact mass* calc. for ($C_{25}H_{46}O_{15}$ + Na): 633.2734; Found: 633.2737.

Reduction and selective hydrolysis of unsaturated trisaccharide **13**. — Sodium borohydride (10 mg) was added to **13** (25 mg) in water (1 mL). The solution was kept for 2 h, AcOH was added to decompose excess hydride, Na ions were removed by treatment with Amberlite IR-120 (H^+) resin, and the solution was concentrated with additions of MeOH to remove boric acid. The residue in water was heated with stirring with Amberlite IR-120 (H^+) resin at 80° for 5 h. Filtration of the mixture followed by concentration of the filtrate and chromatography on silica gel (10:3 $CHCl_3$–acetone) afforded as the sole detectable product **10** (13.2 mg) which was chromatographically and spectroscopically indistinguishable from the previous sample.

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