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METHYL 4-*O*-β-D-*gluco*-HEXODIALDO-1,5-PYRANOSYL-β-D-*gluco*-HEXODIALDO-1,5-PYRANOSIDE (METHYL 6,6′-DIALDEHYDO-β-CELLOBIOSIDE) SYNTHESIS AND DEGRADATION STUDIES

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ABSTRACT

Methyl 4-*O*-β-D-*gluco*-hexodialdo-1,5-pyranosyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (methyl 6,6′-dialdehydo-β-cellobioside, **11**) was synthesized by an unambiguous route, the key intermediate (methyl 2,3,2′,3′,4′-penta-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy-β-cellobioside, **3**) being prepared by chlorination of methyl β-cellobioside (**2**) with methanesulphonyl chloride in *N*,*N*-dimethylformamide or by chlorination of cellobiose (**1**) followed by glycosidation Azide displacement of chloride from **3** gave methyl 2,3,2′,3′,4′-penta-*O*-acetyl-6,6′-diazido-6,6′-dideoxy-β-cellobioside (**9**) Photolysis of **9** and mild acid hydrolysis of the resulting imine gave **11** The β-elimination of **11** at different pH values was studied by u v spectroscopy at 30 and 80°, and pseudo-first-order rate constants were calculated 1,2,3,2′,3′,4′-Hexa-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy-α-cellobiose (**4**), 2,3,2′,3′,4′-penta-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy-α-cellobiosyl bromide (**5**), 6,6′-dichloro-6,6′-dideoxy-cellobiose (**6**), and methyl 6,6′-diazido-6,6′-dideoxy-β-cellobioside (**10**) were prepared as intermediates

INTRODUCTION

The first ω-aldehydo-glycoside to be described, methyl β-D-*gluco*-hexodialdo-1,5-pyranoside was prepared, together with the isomeric keto derivatives, by oxidizing methyl β-D-glucopyranoside with aqueous dichromate[1], chlorine and hypochlorite[2], chromium trioxide in acetone[3], nitrogen dioxide[4], and hydrogen peroxide[5]. As no protecting groups were used and the oxidants were non-specific, chromatographic methods were needed to isolate the products and the yields were low The main purpose of these studies, however, was to study the introduction and distribution of carbonyl groups in glycoside units under various conditions Preparation of methyl

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6-aldehydo-glycosides by periodate oxidation of the corresponding heptopyranosides was later used in this laboratory, and gave much improved yields[6]. It was recently shown by BeMiller *et al*[7] that potassium ferrate is a useful oxidant for primary hydroxyl groups of glycosides

The azide photolysis method of Horton *et al*[8] for the selective oxidation of primary hydroxyl groups of protected and unprotected sugars affords 6-aldehydo-glycosides in good yields[9] and has permitted for the first time the preparation of α- and β-(1→4)-linked copolymers containing D-*gluco*-hexodialdo-1,5-pyranose and D-glucose residues[10–12].

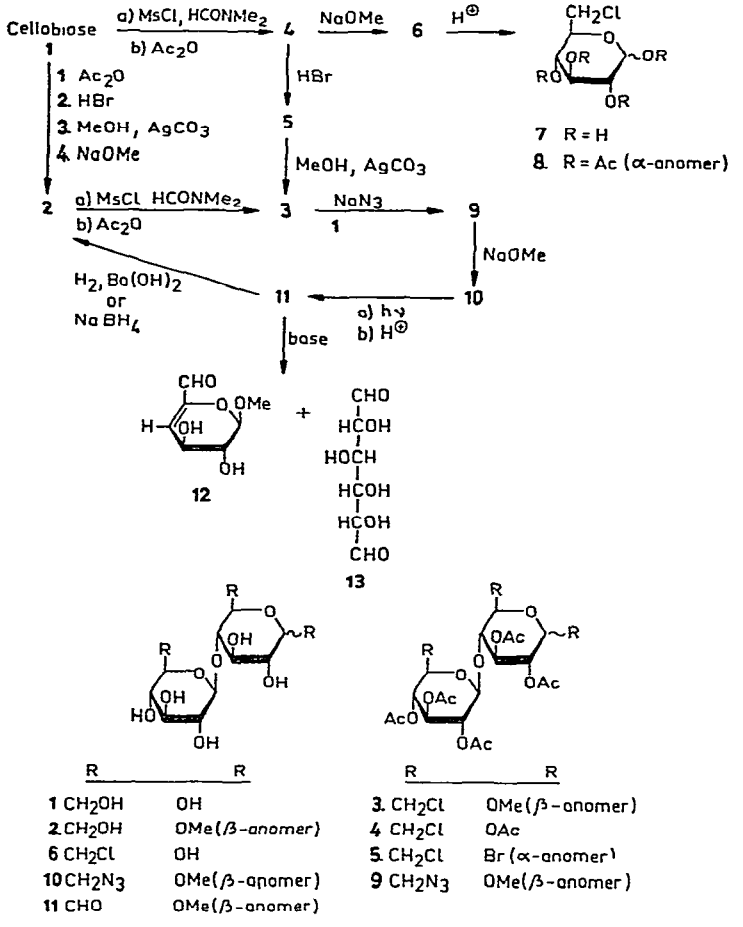
Degradative studies of the low-molecular weight 6-aldehydo-glycosides and the 2- and 3-keto-glycosides used as model compounds afforded useful information relating to the degradation of oxidized polysaccharides under various technical conditions[13–15] Degradative studies on 6-aldehydo-celluloses[16] (partially oxidized) over a broad pH range were consistent with the conclusions drawn from corresponding studies on the 6-aldehydo-glycosides The introduction of 6-aldehydo groups into a polysaccharide destabilizes the molecule towards both acid-catalyzed hydrolysis and alkaline decomposition via β-elimination β-Eliminative degradation of carbohydrates containing uronic acid residues has been reviewed by Kiss[17]

We now describe an extension of the photolytic method to permit the selective oxidation of the primary hydroxyl groups of a disaccharide derivative, methyl β-cellobioside The degradation by elimination of the oxidized methyl β-cellobioside (methyl 6,6′-dialdehydo-β-cellobioside, **11**), used as a model for cellulose fully oxidized in the 6-position, will also be discussed

RESULTS AND DISCUSSION

The approach used for the synthesis of methyl 6,6′-dialdehydo-β-cellobioside (**11**) is shown in Scheme 1 The key intermediate, methyl 2,3,2′,3′,4′-penta-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy-β-cellobioside (**3**), was prepared by two pathways In the first route, cellobiose (**1**) was converted into methyl β-cellobioside (**2**) by the four-step synthesis of Wolfrom and Haq[18] Treatment of **2** with methanesulphonyl chloride in *N*,*N*-dimethylformamide at 65°, followed by removal of the formic ester groups with sodium methoxide and subsequent acetylation, gave crystalline **3** in 58% yield

In previous work on the preparation of 6-chloro-6-deoxy-amyloses[11], some indication was found that a reducing sugar could be directly chlorinated. Treatment of cellobiose (**1**) under standard conditions for the reaction with methanesulphonyl chloride gave a dark mixture The formic ester groups were removed by acid-catalyzed hydrolysis[19], and the product was isolated as the hexa-acetate (**4**) by acetylating the mixture, with the hydrochloric acid present acting as catalyst The hexa-acetate (**4**) was isolated in 70% yield as an α,β-anomeric mixture, m p 223–228° (dec), $[\alpha]_D$ +1 8°. Treatment with acetic anhydride–zinc chloride raised the melting point and optical rotation to 263–269° (dec) and +38 8°, respectively Deacetylation of **4** with sodium methoxide gave crystalline 6,6′-dichloro-6,6′-dideoxycellobiose (**6**)



Scheme 1

In an alternative route, compound **6** was also isolated from the foregoing, acid-hydrolyzed mixture by column chromatography on Dowex-1 (acetate) resin with water as the eluent The resin retarded the elution of **6** and salts were eluted from the column first However, this method is less efficient than the acetylation route Hydrolysis of **6** in sulphuric acid, followed by zinc chloride-catalyzed acetylation afforded 1,2,3,4-tetra-*O*-acetyl-6-chloro-6-deoxy-α-D-glucopyranose (**8**), identical with an authentic sample

Treatment of **4** with hydrogen bromide in acetic acid gave the crystalline 2,3,2',3',4'-penta-*O*-acetyl-6,6'-dichloro-6,6'-dideoxy-α-cellobiosyl bromide (**5**) Treatment of **5** with methanol in the presence of silver carbonate gave **3** Displacement of chloride from **3** by azide was performed in *N*,*N*-dimethylformamide for 3 days at 80° The diazido derivative (**9**) was isolated in good yield as analytically pure crystals The compound showed the azido group absorption at 2100 cm^{-1}, on t l c plates it

decomposed to a yellowish-brown spot in the presence of sulphuric acid, a characteristic colour reaction of 6-azido-substituted glycosides[9].

To prevent exposure of the dialdehydo-cellobioside (**11**) to alkali, the acetyl groups were removed from **9** before photolysis Methyl 6,6'-diazido-6,6'-dideoxy-β-cellobioside (**10**) was obtained as a syrup The aldimine presumed to be formed initially by the irradiation of **10** was hydrolyzed by means of Dowex-50 (H^+) resin

The methyl 6,6'-dialdehydo-β-cellobioside (**11**) was isolated as a syrup containing no detectable amount of azido derivatives The structure of compound **11** is evident from its mode of formation from the 6,6'-diazide **10**, by analogy with similar reactions of the corresponding derivatives of methyl α- and β-glucopyranosides[20,21] The i r spectrum of **11** showed a weak carbonyl absorption at 1720 cm^{-1} attributable to the aldehydo group It appears that most of the aldehydo groups are present either as hydrates or as hemiacetals. Similar i r observations were recorded for methyl α-D-*gluco*-hexodialdo-1,5-pyranoside[9], 6-aldehydo-amyloses[11], and 6-aldehydo-celluloses[12] With Schiff's reagent, **11** slowly developed a colour The D-*gluco*-hexodialdo-1,5-pyranoside derivatives behaved similarly with Schiff's reagent By electrophoresis in hydrogen sulphite[22] (at 40°), compound **11** gave two spots (M_V values of 0 80 and 1 17, respectively) with weak streaking between, indicating an equilibrium between two forms of **11**, having one and two available aldehyde groups, respectively. This is analogous to the behaviour of periodate-oxidized methyl β-D-glucopyranoside, which shows M_V values of 1 02 and 1 48, respectively (at room temperature)[22] The equilibrium between the various forms of periodate-oxidized hexopyranosides has been further discussed by Guthrie[23]

Attempts to prepare the 2,4-dinitrophenylhydrazone and oxime of **11** failed, possible reasons are the stability of the hemiacetal bonds, sensitivity towards β-elimination, and steric factors

Reduction of **11** by hydrogen with Adams' catalyst or by sodium borhydride gave methyl β-cellobioside (**2**) as the only detectable reaction product The reduced product was identified as **2** by comparing the mass spectra of the permethylated derivatives To suppress β-elimination during the reduction with sodium borohydride, a reaction temperature of 4° was used, and a pH of less than 8 was maintained by the addition of acetic acid

Previous experiments on the degradation of 6-aldehydo-celluloses indicated that β-elimination operated at pH values as low as 3 5 (at 80°)[16]. The degradation of **11** by β-elimination was monitored by u v spectroscopy (Figs 1 and 2) The 4,5-unsaturated, 6-aldehydo system has a u v maximum at 253 nm[24] Experiments were performed at 30 and 80° in buffer solutions of pH 1–10. At 30°, no reaction was observed at pH 1 2 and 4 5, some β-elimination was indicated at pH 7, and rapid reaction occurred at pH 9 and above At 80°, some reaction was indicated at pH 2, significant β-elimination was observed at pH 4 5, and, at pH values above 6 0, the reaction was rapid The absorbance curves for the β-elimination of **11** resembled those reported in β-elimination studies on 6-aldehydo-celluloses[16].

The β-elimination product, methyl 4-deoxy-β-L-*threo*-hex-4-enodialdo-1,5-

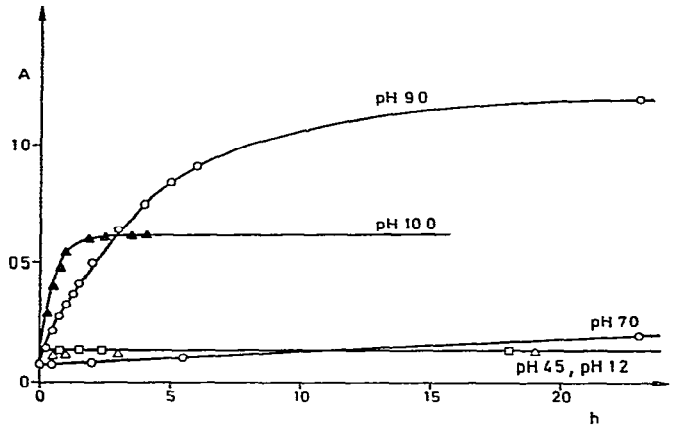


Fig 1. Monitoring the β-elimination of methyl 6,6'-dialdehydo-β-cellobioside (**11**) by u v. spectroscopy at 30° in buffer solutions of pH 1 2, 4 5, 7 0, 9 0, and 10 0

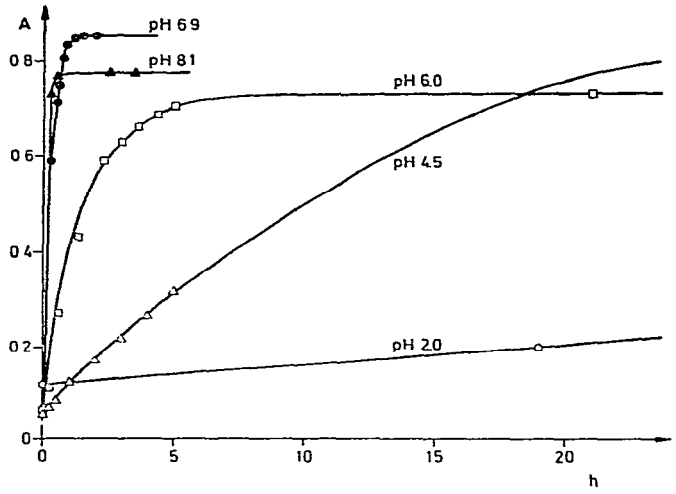


Fig 2 Monitoring the β-elimination of methyl 6,6'-dialdehydo-β-cellobioside (**11**) by u v spectroscopy at 80° in buffer solutions of pH 2 0, 4 5, 6 0, 6 9, and 8 1

pyranoside (**12**), could be detected by paper electrophoresis in the buffer solutions from pH 4 5 upwards[14] On the other hand, no starting material was detected when constant u v absorbance had been reached In other experiments, the corresponding unsaturated 6-aldehydo-glycoside was shown to be the main low-molecular reaction product by treatment[14] of methyl α- and β-D-*gluco*-hexodialdo-1,5-pyranoside at pH 6 It was also found that the rate of the further degradation of **12** is much lower than that of its saturated precursors[21]. The kinetic data for the β-elimination gave

straight lines when analyzed according to pseudo-first-order kinetics Rate constants calculated by this method are listed in Table I It is noteworthy that these rates are of the same order as those found for the 6-aldehydo-celluloses[16] (having a low degree of substitution) and the monomeric 6-aldehydo-glycosides[14] The effect of acid hydrolysis of the glycosidic linkage is not reported here

TABLE I

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE β-ELIMINATION OF METHYL 6,6′-DIALDEHYDO-β-CELLOBIOSIDE (11) AND METHYL β-D-*gluco*-HEXODIALDO-1,5-PYRANOSIDE[14]

	Temp (*degrees*)	*pH*	*k* $min^{-1} \times 10^6$
Methyl 6,6′-dialdehydo-β-cellobioside (11)	30	9 0	63 7
11	30	10 0	488
11	80	4 5	21 5
11	80	6 0	182
11	80	6 9	1310
Methyl β-D-*gluco*-hexodialdo-1,5-pyranoside	90	6 0	1820

EXPERIMENTAL

General methods — Evaporations were performed under vacuum at temperatures below 50° Melting points were measured with a Mettler FP-2 automatic melting-point apparatus Elemental analyses were performed by A. Bernhardt Microanalytical Laboratory, Elbach uber Engelskirchen, Germany

T l c analysis was performed on silica gel HF_{254} (Merck), solvent systems were (*a*) 3 1 ethyl acetate–petroleum ether (b p 60–71°), (*b*) 1 1 benzene–ether, (*c*) 4 1 ethyl acetate–methanol, (*d*) 1 1 ethyl acetate–petroleum ether (b p 60–71°), (*e*) 17 3 propanol–water, and (*f*) butanone saturated with water Paper chromatography was performed on Whatman No 1 paper, solvent systems were (*a*) 3 1 1 ethyl acetate–acetic acid–water and (*b*) 8·2 1 ethyl acetate–pyridine–water Paper electrophoresis was effected in (*a*) 0 1M borate buffer[25] pH 10 (20°) at 1500 V for 2 h and in (*b*) sodium hydrogen sulphite[22] pH 4 7 (at 40°) at 1200 V for 1 5 h Components on chromatograms were detected with *p*-anisidine hydrochloride, Schiff's reagent, sodium hydroxide–silver nitrate, or 50% aqueous sulphuric acid G l c separations were effected on a glass column (3% ECNSS-M on Gas-Chrom Q, 100–120 mesh) at an oven temperature of 190° with a Perkin–Elmer 801 apparatus.

I r spectra were recorded with a Perkin–Elmer Model 337 spectrophotometer U.v measurements were made with Beckman DK 2 and Carl Zeiss PMQ II spectrophotometers Mass spectra were recorded with a Perkin–Elmer 270 spectrometer Optical rotations were measured in a 1-dm tube with a Perkin–Elmer Model 141 polarimeter Irradiations were conducted in a 1-liter, Hanovia photochemical reactor as previously described[12]

Methyl 2,3,2',3',4'-penta-O-*acetyl-6,6'-dichloro-6,6'-dideoxy-β-cellobioside* (**3**) — *Method 1. From methyl β-cellobioside*[18] (**2**) Compound **2** (3 0 g) was dissolved in *N,N*-dimethylformamide (30 ml) and methanesulphonyl chloride (6 5 ml) was added dropwise to the stirred solution at 65° by the general procedure of Evans *et al* [26] After 18 h, the mixture was cooled, methanol (60 ml) was added, and the pH was adjusted to 10 with solid sodium methoxide The precipitated salts were filtered off, washed with methanol, and the combined filtrate and washings were evaporated Toluene was evaporated several times from the residual syrup Pyridine (10 ml) and acetic anhydride (5 ml) were added and the solution was kept for 16 h at room temperature Excess reagent was decomposed by the addition of water and the solution was evaporated. Treatment of the syrup with activated charcoal in hot chloroform, and recrystallization from ethanol yielded **3** as colourless crystals (3 0 g, 58%), m p 225–227°, $[\alpha]_D^{25}$ −31° (*c* 0 5, chloroform) T l c analysis gave one spot, R_F 0 49 (solvent *a*)

Anal Calc for $C_{23}H_{32}Cl_2O_{14}$ C, 45 8, H, 5 35, Cl, 11 8, O, 37 1 Found C, 45 8, H, 5 33, Cl, 11 7; O, 37 4

Method 2. From 2,3,2',3',4'-penta-O-*acetyl-6,6'-dichloro-6,6'-dideoxy-β-cellobiosyl bromide* (**5**) Compound **5** (0 15 g) was dissolved in ethanol-free chloroform (3 ml) and abs methanol (3 ml), and anhydrous calcium sulphate (1 g), dry silver carbonate (0 2 g), and a small crystal of iodine were added The mixture was shaken in the dark for 48 h and then filtered through Celite 545. After removal of the solvent, the residue was dissolved in chloroform and washed successively with 10% aqueous sodium thiosulphate and twice with water The solution was dried with magnesium sulphate and evaporated Crystallisation of the syrup from methanol gave **3** (49 mg), m p 218–221°, $[\alpha]_D^{25}$ −16° (*c* 0 72, chloroform) A second crystallisation from methanol gave pure **3** having m p 225–227°, $[\alpha]_D^{25}$ −31° (*c* 0 44, chloroform)

1,2,3,2',3',4'-Hexa-O-*acetyl-6,6'-dichloro-6,6'-dideoxy-α-cellobiose* (**4**) — Cellobiose (**1**, 4 g) was treated at 60° with methanesulphonyl chloride (25 ml) in *N,N*-dimethylformamide (300 ml) by the procedure already described After 16 h, the dark mixture was cooled and then diluted with water (300 ml) After 3 h, the solution was evaporated to a syrup, ensuring that most of the *N,N*-dimethylformamide had been evaporated An excess of acetic anhydride (100 ml) was added and the solution was warmed for 1 h on a steam bath and then kept for 16 h at room temperature The mixture was poured into ice–water, from which the product crystallized The brown solid was recrystallized from a charcoal-treated ethanol solution, yield 6 0 g (70%) (two crops), m p 223–228° (dec), $[\alpha]_D^{25}$ +1 8° (*c* 0 5, chloroform) T l c analysis gave one spot, R_F 0 64 (solvent *b*) The product was an anomeric mixture

A portion of the product (200 mg) was treated with acetic anhydride (10 ml) and anhydrous zinc chloride (50 mg) for 8 min on a steam bath Isolation as before gave **4** having m p 263–269° (dec), $[\alpha]_D^{25}$ +38 8° (*c* 0 4, chloroform)

Anal Calc for $C_{24}H_{32}Cl_2O_{15}$ C, 45 7, H, 5 10, Cl, 11 2 Found C, 45 5, H, 5 11, Cl, 11 4

6,6'-Dichloro-6,6'-dideoxy-D-cellobiose (**6**) — *Method 1. From 1,2,3,2',3',4'-hexa-O-acetyl-6,6'-dichloro-6,6'-dideoxy-α-cellobiose* (**4**) A catalytic amount of sodium methoxide was added to a stirred suspension of **4** (200 mg) in abs methanol (50 ml) After 5 min, a clear solution resulted which was kept for an additional 2 h at room temperature Neutralization was effected with Dowex-50 (H^+) resin The product crystallized from ethanol–ether, yielding 100 mg (80%) of **6**, m p 193–199° (dec), $[\alpha]_D^{25}$ +58 2°→+29 4° (10 h, *c* 0 6, water); t l c R_F 0 64 (solvent *c*), M_G 0 18 (buffer *a*)

Anal Calc for $C_{12}H_{20}Cl_2O_9$ C, 38 0, H, 5 31, Cl, 18 7; O, 38 0 Found C, 38 2, H, 5 40, Cl, 18 7, O, 37 8

Method 2 From cellobiose (**1**) Cellobiose (**1**, 1 g) and methanesulphonyl chloride (10 ml) in *N,N*-dimethylformamide (100 ml) were treated as before After dilution with water and evaporation, the remaining syrup was chromatographed on column (3 × 100 cm) of Dowex-1 (acetate) that was eluted with water The first fractions contained salts The next fractions contained product Evaporation and crystallization as before gave pure **6**

Hydrolysis of **6** — A solution of **6** (50 mg) in 72% sulphuric acid (6 drops) was kept for 3 h at room temperature The solution was diluted with water (5 ml) and then heated for 4 h on a steam bath After neutralisation (pH 5–6) with saturated barium hydroxide and removal of the barium sulphate, the solution was evaporated The product (M_G 0 91) contained no starting material (M_G 0 18) according to paper electrophoresis in buffer *a* Acetylation of the syrup with acetic anhydride–zinc chloride and crystallization from ethanol gave 1,2,3,4-tetra-*O*-acetyl-6-chloro-6-deoxy-α-D-glucopyranose (**8**), m p 163–164°, $[\alpha]_D^{25}$ +119° (*c* 0 34, chloroform), lit [19] m p 164°, $[\alpha]_D^{25}$ +120° (*c* 1, chloroform)

2,3,2',3',4'-Penta-O-acetyl-6,6'-dichloro-6,6'-dideoxy-α-cellobiosyl bromide (**5**) — Compound **4** (0 5 g) was dissolved in acetic acid (2 5 ml) The solution was saturated at 0° with hydrogen bromide and then shaken at room temperature for 20 min The yellow solution was poured into ice–water, and the resultant precipitate was dissolved in 1 ml of chloroform The chloroform layer was separated, washed with water, and dried with calcium chloride Petroleum ether was added until the solution became turbid The product crystallized readily and was recrystallized from 1 1 ethyl acetate–petroleum ether, yield 0 25 g (48%), m p 221–222°, $[\alpha]_D^{25}$ +98 3° (*c* 1 13, chloroform)

Anal Calc for $C_{24}H_{32}BrCl_2O_{14}$ C, 41 5, H, 4 61, Br, 11 6, Cl, 10 1, O, 32 3 Found C, 41 3, H, 4 67, Br, 11.4, Cl, 10 2, O, 32 0.

Methyl 2,3,2',3',4'-penta-O-acetyl-6,6'-diazido-6,6'-dideoxy-β-cellobioside (**9**) — A solution of **3** (2 g) and sodium azide (2 g) in *N,N*-dimethylformamide (50 ml) was stirred for 72 h at 80° The mixture was cooled in an ice bath, filtered, and the filtrate evaporated Treatment of the resultant syrup in hot chloroform with activated charcoal and then crystallization from ethanol gave pure crystals of **9**, yield 1 9 g (88%), m p 149–151°, $[\alpha]_D^{25}$ −3 0° (*c* 0 5, chloroform) T l c analysis gave one spot, R_F 0 50 (solvent *d*), ν_{max}^{KBr} 2100 cm^{-1} (azide)

Anal Calc for $C_{23}H_{32}N_6O_{14}$: C, 44 8, H, 5 23, N, 13 6, O, 36 3 Found C, 44 8, H, 5 24, N, 13 9, O, 35 7.

Methyl 6,6'-diazido-6,6'-dideoxy-β-cellobioside (**10**) — A catalytic amount of sodium methoxide was added to a stirred suspension of **9** (0 39 g) in abs methanol (60 ml) After 3 h, a clear solution resulted and only one component was detected by t l c Dowex-50 (H^+) resin (5 ml) and water (5 ml) were then added, and the suspension was stirred for 30 min After filtration, and evaporation of toluene from the filtrate, a colourless syrup remained, yield 0 25 g, $[\alpha]_D^{25}$ −4 2° (*c* 1 3, methanol), t l c R_T 0 40 (solvent *c*); ν_{max}^{KBr} 2100 cm^{-1} (azide) After acid hydrolysis of a sample of the syrup, paper electrophoresis in borate buffer showed only 6-azido-6-deoxy-D-glucose (M_G 0 53, buffer *a*)

Methyl 4-O-β-D-gluco-hexodialdo-1,5-pyranosyl-β-D-gluco-hexodialdo-1,5-pyranoside (**11**) — Syrupy **10** (0 2 g) was dissolved in ethanol (100 ml) Photolysis of the solution for 3 h caused complete decomposition of the azide groups Ethanol was chosen as solvent because of its ease of removal T l c analysis (solvents *c* and *e*) showed one spot, R_F 0. Dowex-50 (H^+) resin (5 ml) was added and the suspension was stirred for 2 h. T l c analysis (solvent *c*) showed two reducing spots, R_F 0 21 and R_F 0 14 (traces), and no azido compounds (no yellow colour with 50% sulphuric acid) A part of the solution was evaporated The yellow syrup (50 mg) was dissolved in water (5 ml) and treated with charcoal for 1 h at room temperature Evaporation yielded a colourless syrup (35 mg), $[\alpha]_D^{25}$ −17 5° (*c* 1 0, water) On paper chromatograms, one spot was obtained, R_F 0 53 (solvent *a*), positive to *p*-anisidine and developing colour slowly with Schiff's reagent Paper electrophoresis in hydrogen sulphite buffer revealed two spots having $M_{Vanillin}$ 0 80 and 1 17, respectively The syrup showed ν_{max}^{KBr} 1720 cm^{-1} (weak, C=O)

Preparation of methyl β-cellobioside (**2**) *by reduction of* **11** — *Method 1* Hydrogen was bubbled for 4 h through a 1% solution of **11** in water (2 ml) containing Adams' catalyst (20 mg) After removal of the catalyst, t l c analysis (solvent *e*) and paper chromatography (solvent *b*) showed that the product was identical with **2** Methylation[27] of the product (by methyl sulphoxide–sodium hydride–methyl iodide), followed by g l c–m s analysis, gave a mass spectrum identical with that of authentic per-*O*-methylated methyl β-cellobioside

Method 2 A solution of **11** (35 mg) in water (2 ml) was cooled in an ice bath An ice-cold solution of sodium borohydride (35 mg) in water (2 ml) was added slowly. The pH of the solution was kept below 8 0 by adding aqueous acetic acid (9 1 water–acetic acid) Stirring was continued for 1 h and the solution was stored for 16 h at 4° Water (10 ml) and Dowex-50 (H^+) resin in excess were added The suspension was stirred for 15 min and filtered The filtrate was evaporated and several portions of methanol were evaporated from the syrup, yield 26 mg of **2** as a syrup The identity with **2** was established by t l c, R_F 0 29 (solvent *e*) The product was methylated[27] (methyl sulphoxide–sodium hydride–methyl iodide), hydrolysed, reduced (sodium borohydride), and then acetylated G l c analysis gave two peaks, and mass spectrometry indicated the structure to be 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-

methyl-D-glucitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol, by comparison with authentic samples

Experiments to detect β-elimination of **11** — Samples of **11** (10 mg) were dissolved in buffer solutions (50 ml) The solutions were kept in stoppered glass flasks placed in a constants-temperature bath at 30 or 80° At various time-intervals, aliquots (4 ml) were removed, cooled, and the absorbance at 260 nm was measured Plots of absorbancy as a function of time are shown in Figs 1 and 2 Pseudo-first-order rate constants (Table I) were calculated by using the equation

$$k = \frac{2\,303}{t_2 - t_1}\left[\log\frac{A_\infty - A_0}{A_\infty - A_{t_2}} - \log\frac{A_\infty - A_0}{A_\infty - A_{t_1}}\right].$$

Experiments were conducted in 0 1 mol per litre aqueous buffers pH 1 2, hydrochloric acid; pH 4 5, potassium acetate; pH 6 0 and 7 0, potassium phosphate, pH 8 0 and 9 0, potassium borate, and pH 10.0, potassium hydroxide The solutions also contained 0 1 mol per litre of potassium chloride

Evaporation of the buffer solutions and analysis by t l c showed the presence of **12**, identical with an authentic sample of methyl 4-deoxy-α-L-*threo*-hex-4-enodialdo-1,5-pyranoside, R_F 0 65 (solvent *f*) and R_F 0 62 (solvent *c*). An authentic sample of **12** was prepared by deacetylation of the 2,3-diacetate[14]

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