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Note

A new approach to synthesis of polysaccharides: synthesis of a (1→6)-glucan

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The few successful syntheses of regular polysaccharides are based on polymerization of internal sugar orthoesters[1] or 1,6-anhydro sugars[2]. Polycondensation of a monomer containing an active glycosylating moiety and a free hydroxyl group (or its equivalent) could provide a more-general method. For the synthesis of regular heteropolysaccharides, the oligosaccharide corresponding to the repeating unit of the polymer would serve as the monomer for polycondensation. Attempts to use the orthoester of a disaccharide with a free hydroxyl group for this purpose have been rather disappointing[3], probably because an alcohol is formed during the reaction[4] and also because of the moderate glycosylating activity of the orthoester group. The polycondensation of acetohaloglucoses bearing a free hydroxyl group[5] gave very poor yields of polymer, possibly due to the instability of this type of monomer.

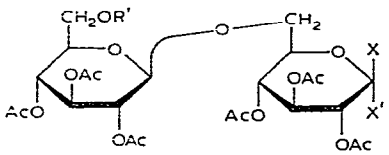
We have shown that glycosylation of *tert*-butyl ethers of simple alcohols[6] or monosaccharides[7] under Helferich conditions (nitromethane–mercuric cyanide) gave alkyl glycosides or disaccharides, respectively, in good yield. The nucleophilic attack at the oxygen atom of the *tert*-butoxy group could be facilitated because of the +I effect. This view is supported by the higher yields obtained on glycosylation of *tert*-butyl ethers as compared to the corresponding alcohols.

Glycosylation of *tert*-butyl ethers may therefore be of use in the synthesis of polysaccharides and we now report the results of preliminary experiments.

2,3,4-Tri-*O*-acetyl-6-*O*-*tert*-butyl-β-D-glucopyranosyl chloride (**1**) was first investigated as a monomer for polycondensation. Treatment of 1,2,3,4-tetra-*O*-acetyl-6-*O*-*tert*-butyl-β-D-glucopyranose[7] with titanium tetrachloride gave **1**. When a solution of **1** in nitromethane was heated in the presence of $Hg(CN)_2$, only a small amount of polymeric material was found and 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose was the main product. Thus, glycosylation occurred easily, but the sterically more-favourable intramolecular reaction prevailed.

The use of the disaccharide derivative 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6′-*O*-*tert*-butylgentiobiosyl bromide (**2**), which cannot undergo intramolecular glycosylation, was more successful. Gentiobiose (**3**) was converted conventionally into 6′-*O*-trityl-gentiobiose (**4**), which was acetylated without isolation. Removal of the trityl group

from the hepta-acetate **5** with hydrogen bromide–acetic acid gave crystalline 1,2,2′,3,3′,4,4′-hepta-*O*-acetyl-β-gentiobiose (**6**), which was converted into the 6′-*O*-*tert*-butyl derivative **7** with liquid isobutylene in the presence of a small amount of sulphuric acid. Compound **7** was obtained crystalline in good yield, after chromatography on silica gel, and its structure was supported by i.r., p.m.r., and m.s. data.



2 R′ = ^{t}Bu, X = H, X′ = Br
5 R′ = Tr, X = OAc, X′ = H
6 R′ = H, X = OAc, X′ = H
7 R′ = ^{t}Bu, X = OAc, X′ = H

Application to **7**, in sequence, of Zemplén deacetylation, Hakomori methylation hydrolysis, and standard conversion into the partially methylated alditol gave 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol as the only product. This result proves the location of the *tert*-butyl group in **7** to be at position 6′ and shows that there is no acetyl migration during the conversion **4** → **7**. Treatment of **7** with hydrogen bromide–acetic acid in dry benzene at 0° gave the corresponding glycosyl bromide **2**, which was used without isolation for polycondensation. The presence of the *tert*-butyl group in **2** was proved when a small portion, isolated by p.l.c., was heated with aqueous acetone–silver nitrate, and the product was deacetylated with methanolic sodium methoxide to give 6′-*O*-*tert*-butylgentiobiose. The configuration of **2** was not proved, but would be expected to be α (*cf.* ref. 9).

Under the Helferich conditions of glycosylation (boiling nitromethane in the presence of mercuric cyanide for 30 min), **2** readily gave a polysaccharide. The product was acetylated with acetic anhydride in pyridine, and a polymeric fraction was isolated (15–18% from **7**) by p.l.c. The synthetic polysaccharide was then deacetylated, and treated with KU-2 (H^+) resin in aqueous methanol to remove the *tert*-butyl groups; 6′-*O*-*tert*-butylgentiobiose gave gentiobiose in quantitative yield when treated with KU-2 (H^+) resin in aqueous methanol at 20° for 15 h.

The deacetylated polymer fraction gave a diffuse spot with $R_{\text{GENTIOBIOSE}}$ 0.05 on a paper chromatogram (1-butanol–acetone–water, 2:7:1), and was eluted with 0.1M acetic acid from Biogel P-2 in the void volume.

Hydrolysis of the polysaccharide (0.5M HCl, 100°, 8 h) afforded glucose (p.c., and g.l.c. of the hexitol hexa-acetate).

The polysaccharide was subjected in sequence to methylation (Hakomori), acid hydrolysis, borohydride reduction, and acetylation. G.l.c. of the resulting mixture revealed only 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol. These data indicate the presence of (1→6)-linkages only in the polysaccharide. The ratio of tetra- to tri-methylglucitol was 1:12.7 (quantitative g.l.c. data), indicating an average chain-length of 14 and a molecular weight of ~2200.

The configuration of the glycosidic bonds in the synthetic (1→6)-glucan was not established, but the $[\alpha]_D^{20}$ value (+1.5°, water) suggests the presence of both α- and

β-linkages. This inference is not unexpected, because the lack of complete stereospecificity of Helferich glycosylation is well known.

EXPERIMENTAL

Solutions were concentrated under diminished pressure. Melting points were determined on a Kofler microscope hot-stage and are uncorrected. Rotations were determined (1-dm path-length) at 20° with a Perkin–Elmer 141 polarimeter. P.m.r. spectra were recorded with a Varian DA-60-JL spectrometer. Tetramethylsilane was used as internal standard. Mass spectrometry was carried out on a Varian MAT CH-6 spectrometer. G.l.c. was performed on an LKhM 8 MD chromatograph at 212° (3-m column, 5% of PNPGS with chromatone N-AW). Chromatography was performed on silica gel with *A*, chloroform–acetone (80:20); *B*, chloroform–acetone 90:10); *C*, 1-butanol–pyridine–water (6:4:3); *D*, 1-butanol–acetone–water (2:7:1).

6′-O-tert-*Butyl-β-gentiobiose hepta-acetate* (**7**). — A mixture of conc. H_2SO_4 (0.1 ml), liquid isobutylene (35 ml), and a solution of 1.5 g of β-gentiobiose hepta-acetate[11] in 15 ml of dichloromethane was stored in a sealed ampoule for 3 days at 20°. The excess of isobutylene was then evaporated, the residue was dissolved in 100 ml of chloroform, and the solution was washed with water, aqueous sodium hydrogen carbonate, water, and dried (Na_2SO_4). Elution of the product from silica gel with a chloroform–acetone gradient gave 1.4 g of material which was recrystallised from ethanol to give **7**, m.p. 184–185°, $[\alpha]_D^{20}$ +20.5° (*c* 2, chloroform); ν_{max} 1050, 1080 (ether), and 1750 cm^{-1} (ester) (Found: C, 52.42; H, 6.33. $C_{30}H_{44}O_{18}$ calc.: C, 52.02; H, 6.40%). P.m.r. data: δ 7.9–8.1 (21 H, 7 AcO), 8.88 (9 H, *tert*-butyl).

Synthesis of the (1→6)-glucan. — To a solution of **7** (110 mg) in 2.5 ml of benzene, 0.4 ml of a 32% solution of hydrogen bromide in acetic acid was added. After 3 h at 0°, benzene (15 ml) was added, and the mixture was washed with ice-cold water, aqueous sodium hydrogen carbonate, and water. T.l.c. (solvent *B*) revealed mainly 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6′-*O*-*tert*-butyl-α-gentiobiosyl bromide (**2**) (R_F 0.7) and its hydrolysis products (R_F 0.2). After treatment of the mixture with $AgNO_3$ in aqueous acetone, the component with R_F 0.7 disappeared and an intense spot with R_F 0.2 was the sole product. Deacetylation of the $AgNO_3$-treated glycosyl halide gave a product with mobility (R_{GLC} 1.4) p.c. (solvent *C*) identical to that of 6′-*O*-*tert*-butylgentiobiose obtained by deacetylation of **7**.

The benzene solution of **2** described above was concentrated to dryness and the residue was used without purification for the polycondensation reaction. To a solution of **2** in 0.5 ml of nitromethane, $Hg(CN)_2$ (40 mg) was added, and the mixture was boiled for 30 min. T.l.c. (solvent *B*) was used to monitor the reaction. The mixture was diluted with 20 ml of nitromethane and washed with water (3 × 10 ml), and the organic layer was dried (Na_2SO_4) and concentrated. The residue was treated with 10 ml of pyridine and 6 ml of acetic anhydride (15 h, 20°). Methanol (20 ml) was then added and the mixture was concentrated *in vacuo*. Methanol was repeatedly distilled from the residue, and pyridine was removed by co-evaporation with heptane. The

product was fractionated by p.l.c. (solvent *B*), and the zone with $R_F \leqslant 0.1$ was eluted with acetone to give 16.5–19.5 mg of material of high molecular weight. Deacetylation (Zemplén), followed by treatment with KU-2 (H^+) resin in aqueous methanol (15 h) and concentration of the solution, gave 8–9 mg (15–18% from 7) of a polysaccharide, $[\alpha]_D^{20}$ +1.5° (*c* 0.8, water). This material was eluted from Biogel P-2 within the void volume of the column, with 0.1M acetic acid; the elution was monitored by the phenol–sulphuric acid reaction.

Acid hydrolysis of the glucan. — A mixture of the glucan (1 mg) and 1 ml of 0.5M hydrochloric acid was heated in a sealed tube for 12 h at 100°. The hydrolysate contained (p.c., solvent *C*) glucose only. After deionisation with Dowex-1 x8 (CO_3^{2-}) resin, the hydrolysate was treated with $NaBH_4$ (20°, 3 h), and the resulting alditols were acetylated. G.l.c. revealed only glucitol hexa-acetate.

Methylation of the glucan. — Hakomori methylation of the polysaccharide, followed by formolysis, hydrolysis, reduction with $NaBH_4$, and acetylation, gave only 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol (ratio 1:12.7), which were identified by g.l.c.

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