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STEREOSPECIFIC BLOCK-POLYCONDENSATION OF OLIGOSACCHARIDES. SYNTHESIS OF A REGULAR GLUCAN WITH ALTERNATING α-1-4 AND β-1-6-LINKAGES.

N.K.Kochetkov * and I.V.Obruchnikov

N.D.Zelinsky Institute of Organic Chemistry, Academy of Sciences of USSR, Moscow, USSR

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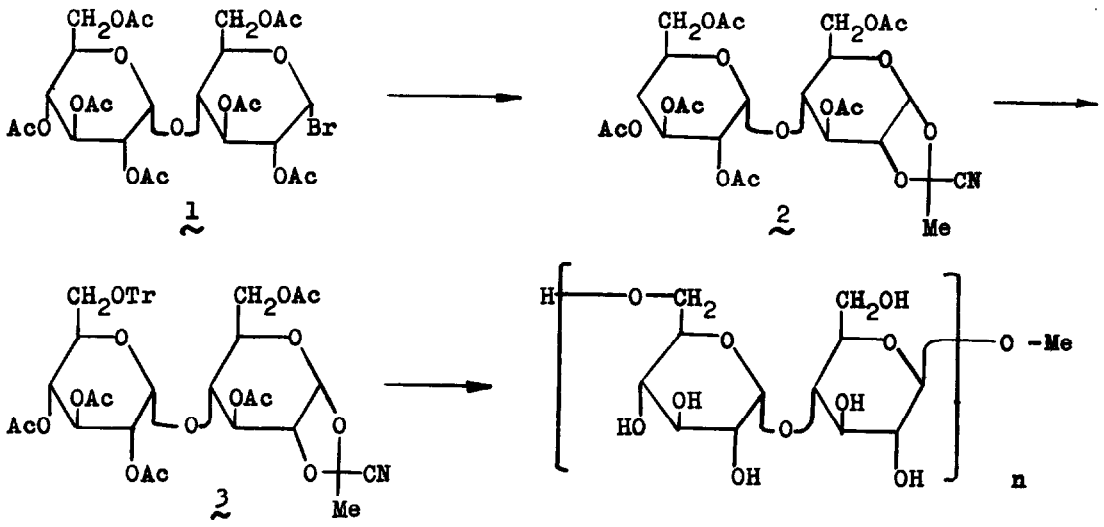
A new method of glycosylation [1] based on condensation of pyruvonitrile ketals of sugars with trityl ethers has been recently used in this laboratory[2,3] for stereo- and regio-specific synthesis of β-1-6-D-glucan. Such a polycondensation opens up possibilities for synthesis of regular polysaccharides built up of repeating units; polysaccharides of this type are widespread in Nature. Here we describe the synthesis of a regular glucan by polycondensation of the maltose derivative 3 .



Hepta-O-acetyl-α-maltosyl bromide 1 [4] was converted into pyruvonitrile ketal 2 , m.p. 172°, $[\alpha]_D$ + 84° ($CHCl_3$), by treatment with silver cyanide in dry boiling xylene [3]. Saponification of 2 (methanolic triethylamine, 20°, 48 h) followed by tritylation (1.2 mole of trityl chloride in pyridine) and acetylation

with acetic anhydride gave rise to ketal 3, $[\alpha]_D$ +70° ($CHCl_3$), in 12.3% yield based on acetobromomaltose. Analytical, PMR spectral data, and chemical properties of ketals 2 and 3 are in accord with structures proposed.

Polycondensation of 3 was carried out as described earlier [2,3], i.e. using high-vacuum technique, dry methylene chloride as a solvent and trityl perchlorate [5] (0.1 mole) as catalyst. Reaction was terminated after 50 h at 23° by addition of methanol; this also caused removal of trityl group from non-reducing terminus. Treatment of the reaction mixture with methanolic sodium methoxide followed by gel-filtration on Sephadex G-25 afforded high-molecular-weight product, glucan S-6 (for synthetic glucans S-1 - S-5 see [2,6-8]) in 33% yield. Glucan S-6, $[\alpha]_D$ +115° (H_2O), is a white powder readily soluble in water and insoluble in alcohol. Its structure was established as follows.

Acid hydrolysis (2N H_2SO_4, 100°, 6 h) gave D-glucose as the only reducing component. Glucan S-6 was methylated according to Hakomori procedure [9]. Formolysis of the permethylated product (92% HCOOH, 100°, 2 h) followed by acid hydrolysis (0.05 N H_2SO_4, 100°, 8 h) and sodium borohydride reduction afforded the mixture of partially methylated alditols. Its acetylation by ordinary procedure and GLC-analysis (SE-30) revealed the presence of 2,3,4,6-tetra-O-methyl-glucitol acetate and a mixture of 2,3,4- and 2,3,6-tri-O-methyl-glucitol acetates in the ratio 1:18.6. Due to poor resolution of this pair of methylated glucitol acetates (cf. [10]), the mixture of partially methylated alditols was subjected to periodate oxidation, borohydride reduction and acetylation (acetic anhydride in pyridine, 100°, 15 min, evaporation <u>in vacuo</u> at room temperature). GLC-analysis indicated the presence of 2,3-di-O-methyl-threitol and 2,3,4-tri-O-methyl-xylitol acetates in a ratio 1:1, identified by comparison with authentic samples. Thus glucan S-6 is a linear polysaccharide with alternating 1-4 and 1-6 linkages. Its degree of polymerisation, based on D-glucose units, is 20.

The anomeric configurations of glucan S-6 were determined from the following data. The high positive $[\alpha]_D$ value (+115°) is in qualitative accordance with values for a stereochemically related pair of methyl biosides, viz., methyl β-maltoside (+78.8°, +84.6°)[11,12] and methyl α-gentiobioside (+65.5°)[13]. More definite data were obtained from CrO_3-oxidation [14,15] of glucan S-6

acetate. It was obtained by acetylation with acetic anhydride and pyridine in formamide at room temperature followed by coevaporation of volatile components with toluene under reducing pressure and precipitation with water from solution in formamide. The glucan acetate thus obtained was treated with CrO_3 in acetic anhydride - glacial acetic acid (1:10) mixture at 50° for 2 h and the product obtained after the usual work-up was reacted with excess of sodium borohydride in 98% aqueous methanol for 12 h. Glucosyl-hexitol was identified as a major component of the reaction product by means of anion-exchange chromatography (Technicon sugar analyzer SC-2) with maltitol as a reference compound. The oxidation-reduction product was methylated according to Hakomori [9], permethylated glucosyl-hexitol being identified by GLC in comparison with the product of β-maltose octaacetate CrO_3-oxidation with subsequent borohydride reduction and permethylation. A mixture of partially methylated alditol acetates derived from the aforementioned glucosyl-hexitol was shown by GLC to contain 1,2,3,5,6-penta-O-methyl-hexitol and 2,3,4,6-tetra-O-methyl-glucitol acetates as main components besides ca. 18% of tri-O-methyl-glucitol acetate(s).

The data obtained seem to be in accord with expected regular structure of synthetic glucan. The presence of tri-O-methyl-glucose derivatives may be obviously rationalised by incomplete CrO_3-oxidation of glucan acetate since even disaccharide derivatives are known not to undergo quantitative oxidation [15]. Another possibility explaining the presence of tri-O-methyl-glucose is the existence of a single, "anomalous" α-1-6-linkage per macromolecule. Bearing in mind the complete stereoregularity of β-1-6-D-glucan obtained by analogous polycondensation of a monosaccharide derivative [2,3], a regular structure,with alternating α-1-4- and β-1-6-linkages should be ascribed to glucan S-6.

This work seems to open wide perspectives for chemical synthesis of regular biologically significant polysaccharides.

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