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SYNTHESIS OF POLYSACCHARIDES WITH A REGULAR STRUCTURE

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1. INTRODUCTION

The synthetic studies of biopolymers have played a prominent role in the rapid development of many branches of life science As is well known from the studies of nucleic acids and proteins, the synthesis of their fragments and analogues and, finally, of the natural biopolymers themselves was of great and sometimes crucial importance in establishing some fundamental concepts like the genetic code

The brilliant advances in chemistry of proteins and nucleic acids for a long time kept in the shadow the studies of biopolymers containing the carbohydrate chains However, in the last 10–20 years a significant and specific role of the carbohydrate-containing biopolymers in the vital activity of the cell has been ascertained Together with their function as an energy source (e g starch, glycogen) and as structural material (e g. cellulose, chitin), which has been considered traditionally for many years, they play also a highly specific role, namely, they participate in the formation of structures, which determine a specificity of the cell surface (or some regions of this surface) in the

real medium of the cell's vital activity Such biological acts as cell interactions, serological reactions of the bacterial cell and primary stage of its interaction with a virus, and other related processes proceed with the participation of the specific polysaccharide structures [1-3] Such investigations revealed for the first time the significance of the specificity of structure of the carbohydrate-containing biopolymers Eventually, such a specificity is determined by the spatial structure of the polysaccharide chain (i e by its secondary and tertiary structures), nevertheless, as in the case of proteins, higher structures of the chain are determined by its primary structure, i e by the nature and the sequence of monomeric units, in this case by monosaccharides and the type of the intermonomeric (glycosidic) linkage Unlike proteins and nucleic acids, a determining role is played by the stereochemistry of both the monomer unit itself and the glycosidic linkage

As could be anticipated, the new step in the investigation of carbohydrates has stimulated a large number of synthetic studies in this complicated area The synthesis of fragments of carbohydrate chains, e g the fragments of oligosaccharide chains of glycoproteins and of *O*-specific and capsular bacterial polysaccharides, is now being developed at an increasing pace These synthetic compounds serve as important models for physicochemical and biological studies, directed eventually to the elucidation of a role played by the carbohydrate structures in the biological processes and to understanding the principles of specificity in the classical problem of structure–function relationship

A next step in investigations of this kind certainly is the synthesis of the polymeric structures themselves, the natural polysaccharides or their close analogues It should be kept in mind that at present the structure of many polysaccharides, in particular those of plant origin, is known, in essence merely in the averaged form, thus hampering the gain of sufficiently accurate information for tackling numerous problems at the molecular level Synthetic polymers, having quite unambiguous structures, are thus indispensable models for the solution of many problems, including those of a purely structural character It is known that the natural polysaccharides are distinguished by an exceptional diversity of structures, resulting from the polyfunctional nature and the stereochemical variety of the monomer, i e of the monosaccharide residue Whereas proteins and nucleic acids are linear, unbranched polymers, the overwhelming majority of natural polysaccharides turn out to be the branched polymers, which involve several different principles of construction Among these are the homopolysaccharides, containing monosaccharides of a single type and connected by glycosidic linkages of a single type (e g cellulose or amylose), or involving glycosidic linkages of different types distributed regularly or irregularly along the polymeric chain Known also are the heteropolysaccharides, the polymeric chain of which consists of irregularly repeated short and various homopolysaccharide blocks, giving rise, on the whole, to an irregular structure (e g alginic acid) Of particular biological interest are the polysaccharides, consisting of the repeating oligosaccharide fragments and having a strictly regular heteropolysaccharide chain (e g *O*-antigenic and capsular polysaccharides of bacteria) Finally, there are the polysaccharides containing irregular sequences of monosaccharide residues (e g some plant polysaccharides)

The first attempts in the synthesis of polysaccharides aimed, quite naturally, at the solution of the simplest problem, that of the synthesis of the polysaccharides having a regular structure, and the present review is devoted to the discussion of this particular aspect The synthesis of polymers of irregular structure, for which the only feasible route so far, i e a step-by-step elongation of a carbohydrate chain, is evidently a matter of future, taking into account the labour of handling each polyfunctional monosaccharide component, although at present the oligomeric structures of this type can be effectively obtained

In essence, the synthesis of the polysaccharides with a regular structure* can be accomplished via the three pathways (*cf* [4]) (i) by the step-by-step attachment of units of either the monosaccharide (for homopolysaccharides) or the oligosaccharide (for heteropolysaccharides with repeating units), (ii) by the polycondensation or polymerization of the corresponding monosaccharide or oligosaccharide derivatives by chemical methods, (iii) by the enzymatic polymerization of the corresponding monosaccharide or oligosaccharide precursors, the structures of which are known from the studies of pathways of biosynthesis of particular polysaccharides

* The present review considers the synthesis of only real polysaccharides, i e the polymers, in which the monosaccharide residues are bonded by the glycosidic linkages The synthesis of polymers, in which the monosaccharide residues are bonded by another type of linkage, e g by the C–C or ether bond (the so-called neopolysaccharides or quasipolysaccharides), and which differ in many respects from the real polysaccharides, falls beyond the scope of this review

In the present review only the second approach, the chemical polymerization or polycondensation of mono- and oligosaccharide derivatives, will be discussed The stepwise approach, representing a highly laborious procedure, has also been investigated (see, e g [5]) Some information available on the enzymatic polycondensation of biosynthetic precursors[6] appears to be quite promising

The most important requirement for the chemical polymerization or polycondensation of mono- and oligosaccharide fragments, aiming at the preparation of polysaccharides with a strictly regular structure, is its absolute regio- and stereospecificity, ensuring, in addition, a proper molecular weight of the polymer It is obvious that even a single variation in the structural and stereochemical regularity of the intermonomeric linkage within the polymeric chain immediately changes sharply the macromolecular conformation, altering essentially its physicochemical properties, and, therefore, its biological specificity This is the main reason for the serious difficulties in carrying out the specific polymerization or polycondensation, and in consequence of which the first syntheses of the regular polysaccharides have been performed only quite recently

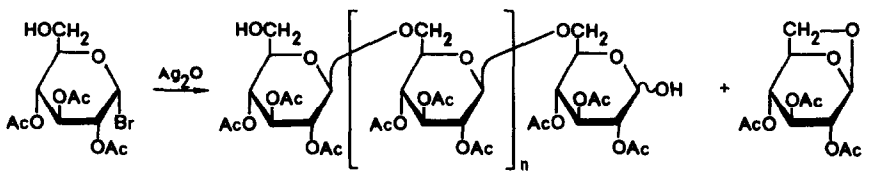
Regiospecificity of the polymerization/polycondensation can be ensured by temporary protection of the OH groups of a monomer, which take no part in the formation of a new glycosidic linkage This is achieved without particular trouble in the simplest monosaccharide monomers For more complex oligosaccharide fragments the task becomes more complicated and sometimes becomes very laborious However the present chemistry of sugars makes this task rather technical in character

The problem of the stereospecificity of the polymerization/polycondensation has proved to be much more intricate Since the reaction of polymerization and polycondensation is based on the formation of an intermonomeric glycosidic linkage, the stereospecificity of the whole process of formation of the polymeric chain is determined by the stereospecificity of the glycosylation reaction involved Meanwhile the most important glycosylation reaction leading to the formation of a 1,2-*trans* glycosidic linkage suitable for use in the polycondensation process, the Koenigs–Knorr reaction[7] and its numerous modifications do not ensure absolute stereospecificity The orthoester method for the synthesis of glycosides,[8] which is general enough in many cases, also does not provide an unambiguous stereochemical result Still more poor results are obtained in the synthesis of a 1,2-*cis* glycosidic linkage, where the classical glycosylation reactions, based on the nucleophilic substitution at C-1 atom of the pyranose or furanose ring, are far from being absolutely stereospecific

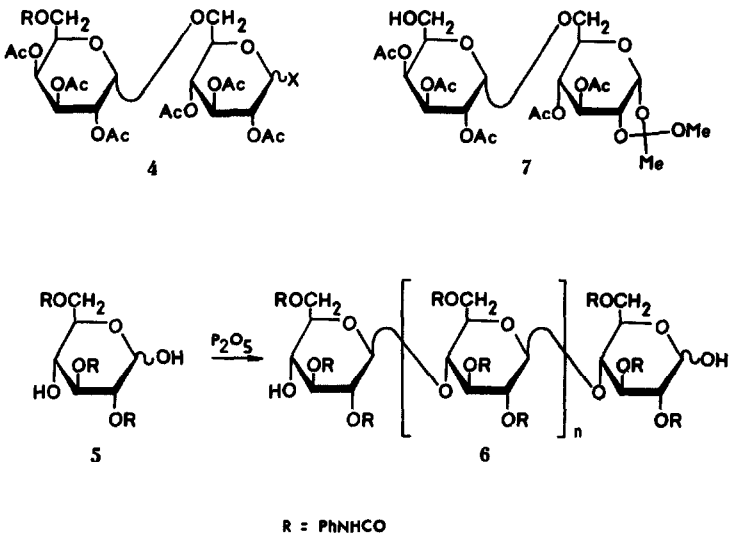
Thus, the progress in the synthesis of the stereoregular polysaccharides has become possible due to the development of sufficiently general methods for the formation of glycosidic linkages ensuring absolute stereospecificity, and which could be used as a basis for the polymerization process Two reactions of this kind, the polymerization of anhydro sugars and the polycondensation of trityl ethers of cyanoethylidene derivatives of mono- and oligosaccharides, have enabled the realization of the first syntheses of regular polysaccharides

2 EARLY ATTEMPTS TO SYNTHESIZE REGULAR POLYSACCHARIDES

The first attempt in the synthesis of a polysaccharide with a regular structure appears to be that of Haq and Whelan,[9] who studied the polycondensation of 2,3,4,-tri-*O*-acetyl-α-D-glucosyl bromide (**1**) in the presence of Ag_2O and $CaSO_4$ with a view to obtaining (1-6)-β-D-glucan However, the intermolecular polycondensation was limited mainly by the formation of the disaccharide gentiobiose (**2**, $n = 0$) and lower oligosaccharides, and the question of the stereospecificity of the reaction remained unanswered The yield of the polycondensation products was also very poor The main product formed was that of the intramolecular condensation, that is a derivative of 1,6-anhydro-β-D-glucose (levoglucosan **3**)



1 **2** **3**



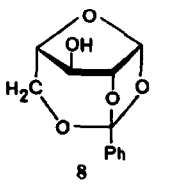
More recent attempts to apply this reaction to polycondensation did not prove successful The polycondensation of a derivative of the disaccharide,[10] 2,3,6,2′,3′,4′-hexa-*O*-acetyl-α-maltosyl bromide, in which an intramolecular glycosylation is hardly expected, in the presence of $Hg(CN)_2$ (the Helferich reaction) afforded a polysaccharide, containing 15–20 glucose units in the chain (corresponding to a degree of polymerization of 8–10) The regio- and stereospecificity of the polymer were violated, since along with the (1-6)-β-glycosidic linkages, the formation of approximately 10% of (1-4)-α-linkages was also observed Recent attempts to employ the activation of the OH group to be glycosylated by its conversion into a *t*-butyl ether also proved unsuccessful Thus, the polycondensation of **4** (R = *t*-Bu, X = Cl) also afforded products devoid of stereoregularity [11]

Some other approaches to the polysaccharide synthesis were based on the use of a monosaccharide, containing free anomeric OH, or the corresponding 1-*O*-acetate under the action of an acid catalyst Thus, Huseman and Muller[12] carried out the polycondensation of 2,3,6-tri-*O*-phenylcarbamoyl-D-glucose (**5**) in the presence of P_4O_{10} to yield a polysaccharide (**6**) with a degree of polymerization of approximately 60 The regiospecificity and the stereospecificity of the reaction has not definitely been established Later attempts[13] to obtain (1-6)-β-D-glucan by this method were unsuccessful and this approach to the polysaccharide synthesis was abandoned The desired results were also not obtained in an attempted polycondensation of the hexopyranose acetates containing the unsubstituted primary OH, 1,2,3,4-tetra-*O*-acetyl-D-glucose and 1,2,3,4-tetra-*O*-acetyl-D-mannose, in the presence of protic or Lewis acids [14–16] Some success was achieved only in the polycondensation with $ZnCl_2$, the reaction afforded (1-6)-D-glucan and (1-6)-D-mannan, respectively, in very poor yield (5–10%) and a low degree of polymerization (approximately 10) The polymer contained together with the expected 1,2-*trans*-glycosidic linkages some amount of the 1,2-*cis*-linkages A related reaction the polycondensation of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose under the action of allyl perchlorate,[17] only afforded traces of a polymeric material

Numerous attempts have been made to employ the orthoester method of glycosylation for the synthesis of polysaccharides (see review[8]) This new effective and stereospecific reaction for the formation of a 1,2-*trans*-glycosidic linkage is based on an interaction of sugar 1,2-orthoesters with hydroxyl containing compounds in the presence of acidic catalysts, namely, $HgBr_2$,[18] or lutidinium perchlorate [19] Thus, the polycondensation of melibiose orthoester acetate (**7**),[20] containing a free OH group, in the presence of $HgBr_2$, led to a low yield of a product with a low degree of polymerization, whose stereoregularity was not rigorously studied The polymerization of arabinofuranose internal tricyclic orthoesters was more successful The polymerization of a tricyclic orthoester with a free OH group at C-3 (**8**) in the presence of $HgBr_2$[21] afforded arabinofuranan with a degree of polymerization of about 60 and having an irregular structure with the branching points at O-3 and O-2 of the furanose ring the stereochemistry of the glycosidic linkages was not studied The polymerization of the monomer with a protected OH group at the C-3 (**9**) in the presence of $HgBr_2$ and using 1,2,3,4-tetra-*O*-acetyl-β-D-glucose as an alcoholic initiator of polymerization, afforded[21,22] arabinofuranan with a degree of polymerization of about 25 and glucose as the non-

reducing unit The stereochemically homogeneous polymer (only α-L-arabonifuranosidic linkages) turned out to be not fully regular and contained, together with the 1,5-glycosidic linkages, approximately 10% of the 1,2-linkages The polymerization of a unique macrocyclic orthoester **10** in the presence of *p*-toluenesulfonic acid and pyridinium perchlorate afforded[23] a regio- and stereo-regular (1-3)-β-D-glucan **11** with a degree of polymerization of about 30, which was similar in properties to the G-chains of natural laminaran The polycondensation of a xylopyranose internal orthoester **12**[24] afforded a polymer with a degree of polymerization of approximately 15–16, which possessed neither regio- nor stereo-regularity Ambiguous results obtained with orthoesters were explained later, when some side reactions in the glycosylation with orthoesters were found [25] The pathways for the formation of by-products were also studied [26]

Finally, quite recently a highly stereospecific reaction for the glycosylation of sugars[27] containing *O*-trityl groups by sugar 1,2-thioorthoesters, which has been employed successfully for oligosaccharide synthesis (see, e g [28]), was attempted for a stereospecific polycondensation However, the growth of the polymeric chain on polycondensation turned out to be inhibited by the competing reaction of the formation of thioglycosides As a consequence, the reaction yields merely short oligosaccharide chains (a degree of polymerization of approximately 3–4) [29]



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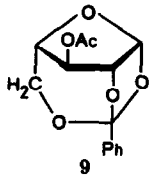

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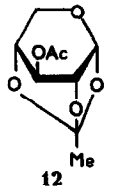

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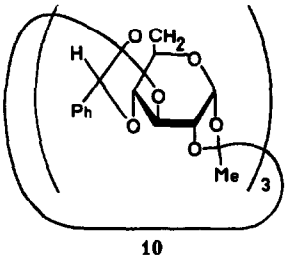

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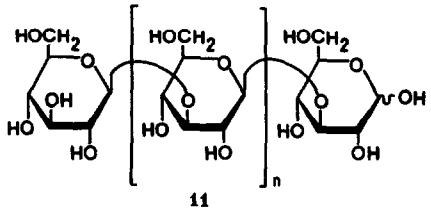

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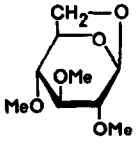

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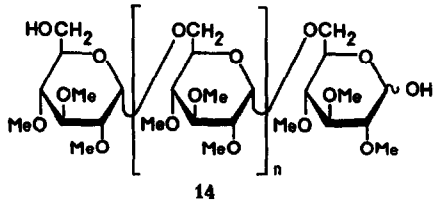

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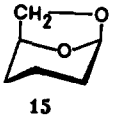

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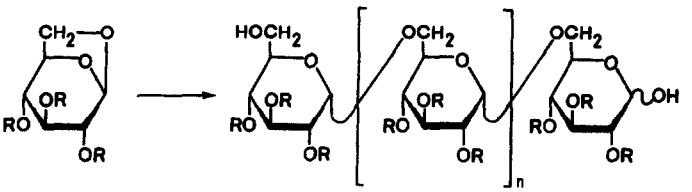
Thus, the syntheses of the regular polysaccharides by the attempted polycondensation of derivatives containing a glycosidic OH group, halogen or an acetate group, as well as the polycondensation and polymerization of various derivatives of orthoesters or their thio-analogues, failed to give the desired results However, these studies were useful because they laid the basis for other more successful approaches

3. POLYMERIZATION OF ANHYDRO SUGARS

This method for the synthesis of polysaccharides of regular structure provided some α-linked homopolysaccharides of high molecular weight and high stereoregularity Until recently the method



Scheme 1

was used mainly for the synthesis of the homopolysaccharides with a (1-6)-α-glycosidic linkage Nevertheless, the most recent investigations show that it can be used also for the synthesis of some homopolysaccharides with other types of the glycosidic linkages However, the majority of studies are devoted to the polymerization of 1,6-anhydro-monosaccharides leading to highly stereospecific syntheses of (1-6)-glycans

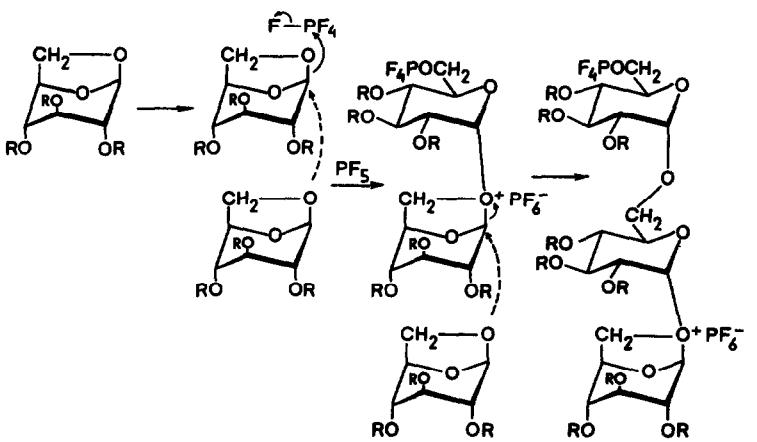
3 1 *Polymerization of* 1,6-*anhydro-hexoses*

In the early work by Pictet,[30] it was shown that the treatment of 1,6-anhydro-β-D-glucose (levoglucosan) with $ZnCl_2$ or with some acid catalysts yielded a polymeric material, which was a mixture of the highly branched polysaccharides involving both pyranose and fyranose rings The use of this reaction for the synthesis of regular polysaccharides, having an unambiguous structure, demanded the protection of the free OH groups of the sugar in order to preclude their participation in the cleavage of the anhydro ring After some unsuccessful attempts in this direction (see, e g [31,32]) the first success has been achieved by Korshak *et al* [33,34] in the polymerization of 2,3,4-tri-*O*-methyl-levoglucosan **13** in the presence of Lewis acids, of which $BF_3 \cdot Et_2O$ proved to be the best A polymer **14** was obtained in crystalline form thus demonstrating its regular structure The stereochemistry of the glycosidic linkages has not been specially studied, however, all subsequent investigations showed it to be practically unambiguous Some information is also available[35] on the polymerization of 2,3,4-tri-*O*-benzyl-levoglucosan and 2,3,4-tri-*O*-acetyl-levoglucosan in the presence of carbocations The detailed results have remained unpublished but it is known that after removal of the protecting groups a non-stereoregular polymer was obtained

The systematic investigation of the Lewis acid-catalysed polymerization of 1,6-anhydro-hexopyranoses was performed subsequently by Schuerch *et al*, and a method was developed for the synthesis of high-molecular-weight stereoregular (1-6)-α-glycans, e g (1-6)-α-D-glucan (Scheme 1)

The available reviews[36,37] on this method, including the comprehensive one by Schuerch,[38] enables us to confine our discussion primarily to the essential general and preparative aspects and to new data

3 1 1 *The reaction mechanism* The cationic polymerization of 1,6-anhydro-pyranoses in solution was established to proceed by a chain growth mechanism [34,38] Similarly to the cationic polymerization of some other oxygen-containing heterocycles (see, e g [39]), the polymerization of 1,6-anhydro-hexoses depends on the nature of substituents in the monomer Its mechanism can be represented as follows (Scheme 2)



Scheme 2

An O atom of the anhydro ring of 1,6-anhydro-β-D-glucose increases the electrophilicity of the C-1 atom due to the protonation or complex-formation with a catalyst, e g PF_5, thus initiating the polymerization process The electrophilic centre C-1 of the complex formed is subjected to the attack by an O atom of the 1,6-anhydro ring of the next molecule of the monomer As a result the anhydro ring of the initiating molecule of the monomer undergoes cleavage to give a glycosidic linkage and the oxonium ion occurs at the reducing end of the molecule At the C_6 atom of the non-reducing monomer unit an OPF_4 group appears The oxonium ion is then attacked at C_1 by the next monomer molecule to give, with formation of a glycosidic linkage, a new oxonium ion in the chain, and the polysaccharide chain grows further at the reducing end Since the electrophilic anomeric centre C-1 in the bicyclic system of the oxonium ion can be attacked, for steric reasons, only from the rear with respect to 1,6-anhydro ring, the polymerization process leads to a stereochemically unambiguous result There occurs only an α-linkage in the case of glucose derivatives and other 1,6-anhydro sugars belonging to the D-series As all OH groups of the monomer are protected, the polymerization proceeds with total regiospecificity to give only a (1-6)-glycosidic linkage

This reaction mechanism has been confirmed experimentally by the study of the polymerization of ethers of levoglucosan in the presence of PF_5 using ^{19}F- and ^{31}P-NMR spectroscopy [40] It has been possible to observe the formation of the complex of 2,3,4-tri-*O*-benzyl-levoglucosan and PF_5 in CH_2Cl_2 at $-80°C$ As the temperature is increased the complex disappears to give an anion PF_6^-, the formation of a CH_2OPF_4 group was also observed, which was assumed to be present at the non-reducing end of the growing polysaccharide chain A detailed study of the process, and, in particular, of its stereochemistry was carried out for the polymerization of 6,8-dioxabicyclo[3 2 1]octane **15**, which is a simplified unsubstituted model for 1,6-anhydro-hexose The results of these model studies are in agreement with proposed mechanism [41]

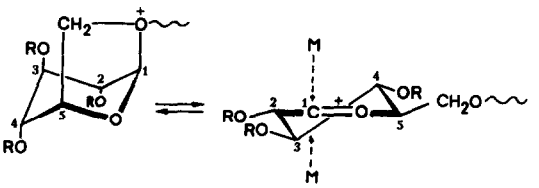
3 1 2 *Reaction conditions and general features of the process* The reaction is usually carried out at low temperatures ranging from $-60°$ to $-70°C$ At temperatures of about 0°C the stereoregularity of the synthetic polysaccharide is violated

The proposed mechanism shows that the process is catalysed by the Lewis acids of the type $BF_3 \cdot Et_2O$, $SbCl_5$ and SbF_5, $TiCl_4$ and PF_5,[42] the latter proving to be most effective and widely used The cationic initiators, such as $Ph_3C^+SbCl_6^-$, $MeCO^+PF_6^-$, triethyloxonium salts, as well as some other complex catalysts were also tested [42] However, their efficiency with regard to stereoregularity and molecular weight of the polymer formed was rather low and they found only limited use in synthetic practice Addition of PhCOF to PF_5, which gives the corresponding complex, reduced the stereospecificity and decreased the degree of polymerization evidently due to enhancement of the process of chain transfer and termination [42] At the same time, the complex $PhCOF \cdot PF_5$ proved useful in the polymerization of the monomer containing an azido group [43] It was suggested that some catalysts of the Lewis acid type act as the complexes with OH-containing compounds of the type $BF_3OH^-H^+$ or $PF_5OH^-H^+$,[33,38] and this was demonstrated by the acceleration of the polymerization upon addition of trace amounts of water or alcohol However, the best preparative results are achieved when carrying out the polymerization under anhydrous conditions

The influence of a solvent on the polymerization process has not been studied systematically Experiments were carried out with benzene, CH_2Cl_2, $MeNO_2$, and liquid SO_2 [42] The most satisfactory result was obtained with CH_2Cl_2 which was employed in almost all preparative syntheses

The most essential feature of the polymerization process is its stereospecificity because only absolute stereospecificity can lead to a fully regular polysaccharide Although in the case of (1-6)-glycans the absolute stereospecificity is generally achieved, there are examples of its violation, producing, together with the α-glycosidic linkages in the polymeric chain, also β-linkages This question has been studied very thoroughly because of its crucial importance The formation of the β-glycosidic linkage in the polymerization of 1,6-anhydro-hexoses is attributed to a side reaction of the oxonium ion, which controls the polymerization process According to Schuerch this side reaction involves the cleavage of the oxonium cycle to give a monocyclic flattened glycosyl-cation, which can be subject to a non-stereospecific attack by the next molecule of the monomer This results in formation of both types of glycosidic linkages, and the stereospecificity of the polymerization is thus violated[38,42] (Scheme 3)

This side reaction occurs when the attack of the monomer molecule proceeds at a lower rate than the intramolecular cleavage of the oxonium cycle In other words, the formation of the glycosidic linkage and the cleavage of the anhydro cycle do not occur simultaneously



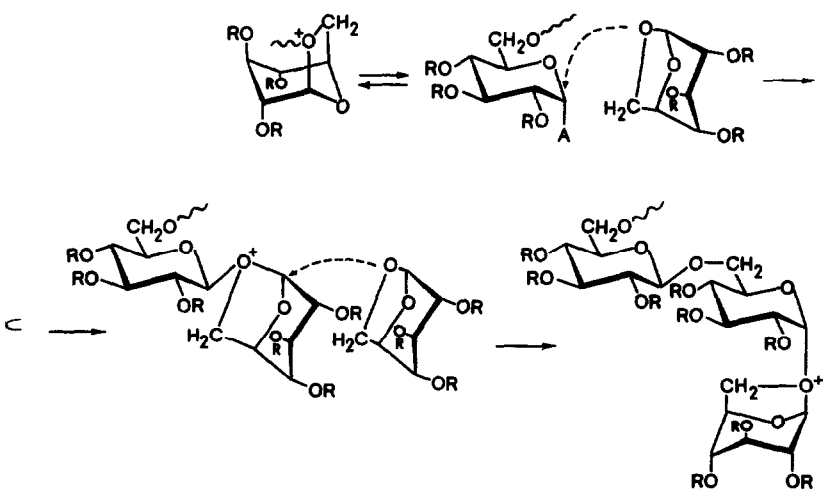
Scheme 3

Another viewpoint concerning the loss of stereospecificity has been suggested by Ponomarenko *et al* [44] The study of the kinetic isotopic effect of the reaction with the derivatives of levoglucosan has led the authors to reject the carbenium mechanism for the formation of β-glycosidic linkage, and its formation is attributed to the competing attack by a sufficiently nucleophilic anion (e g ClO_4^-) at the active site of the growing polymer to give a covalent ester The subsequent attack of the ester by the monomer leads, due to double inversion at the glycosidic centre, to the production of a β-linkage (Scheme 4) The stereospecificity of the polymerization of *O*-substituted levoglucosan depends on the catalyst's counter-ion and decreases in the series

$$PF_6^-, SbF_6^-, BF_4^+ > SbCl_6^- > CF_3SO_3^- > ClO_4^-$$

The violation of the reaction stereospecificity increases with increase in temperature, as well as with increase in the catalyst/monomer ratio Thus, for example, the polymerization of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucose in CH_2Cl_2 under the action of PF_5 at $-78°C$ proceeds fully stereospecifically However, with increasing temperature, β-linkages are formed and at room temperature the reaction is stereo-random [44] Zachoval and Schuerch estimated the critical temperature, when the violation becomes apparent, as $-40°C$ [42] A similar effect was also observed in the polymerization of model 6,8-dioxabicyclo[3 2 1]octane [41] This model reaction also showed that the reaction stereospecificity within the temperature interval from $-60°$ to 0°C drops with the increase in polarity of the solvent toluene > dichloromethane > 1-nitropropane [41] This confirms that the polymerization proceeds via an S_N2 mechanism, but that this is violated with increase in temperature and with increasing solvent polarity

An important preparative aspect of the method is the molecular weight of the resulting polymer This is essential for the synthesis of the simplest homopolysaccharides, since natural polysaccharides of this type, e g dextrans, are of high molecular weight The polymerization of 1,6-anhydrohexoses in most cases allows the preparation of polysaccharides of high molecular weight with a degree of polymerization of hundreds In accordance with the general regularities of the polymerization processes, the low temperature decelerates the process and the yield of the polymer decreases, but the molecular weight of the polymer thus obtained is higher than that achieved at elevated temperatures The molecular weight of the polymer increases with the decrease in the catalyst/monomer



Scheme 4

ratio, with decrease in the monomer concentration, and with increase in the reaction time [42] Since the Lewis acids, serving as catalyst, also cause the destruction of the polysaccharide[46] by cleaving the glycosidic linkages, each reaction requires its own optimal reaction time Thus for the polymerization of 2,3,4-tri-*O*-benzyl-levoglucosan, the optimal reaction time is 40 min [46]

The structure of the starting monomer is naturally the most important factor affecting the course of the polymerization process The main driving force of the process for the polymerization of the bicyclic system of 1,6-anhydro-hexoses is the decrease in steric strain Therefore the conformational and electronic factors which are determined both by stereochemistry of the anhydro sugar and by the substituents involved make the most significant contribution to the reactivity of the monomer

The nature of the *O*-substituents in levoglucosan slightly affects both the stereospecificity and the degree of polymerization Thus, tri-*O*-methyl-, tri-*O*-ethyl-,[45] tri-*O*-benzyl-,[42,45-49] tri-*O*-(*p*-methylbenzyl)-, and tri-*O*-(*p*-bromobenzyl)-[52] ethers of levoglucosan all yield the polymer with high degree of stereoregularity and high molecular weight on polymerization at $-60°$ to $-78°$C under the action of PF_5, although some distinctions are nevertheless observed The monomer with an alkenyl substituent, 2,4-di-*O*-benzyl-3-*O*-crotyl-levoglucosan,[53] was readily polymerized In contrast it has not been possible to polymerize the tri-*O*-trimethylsilyl ether [45]

Difficulties in the polymerization of the levoglucosan esters are connected with additional complexation of the catalyst with the carbonyls of the acyl groups [42] Thus, the complex of 2,3,4-tri-*O*-acetyl-levoglucosan and PF_5 at $-78°$C is precipitated and this precludes the polymerization [45] A reaction does occur at 0°C but this yields a non-stereoregular polymer of low molecular weight [42] Tris(monofluoroacetate) does not polymerize at all [45] Levoglucosan trinitrate does polymerize but the polymer was not characterized [42] Introduction of an azido group instead of the *O*-benzyl one decreases the rate of polymerization, 2-, 3- and 4-azidodeoxy-1,6-anhydro-hexoses polymerize at lower rates than the levoglucosan derivatives, and the rate decreases in the series 3-N_3 > 4-N_3 > 2-N_3 [43]

The activity of different 1,6-anhydro-hexopyranoses in the polymerization reaction varies considerably Their relative reactivity, which was established on the basis of the results of copolymerization of benzylated monosaccharide monomers, decreases in the series manno > gluco > galacto > allo > altro [38] The reactivity of the first three anhydro-pyranoses is similar but the allo-isomer polymerizes slowly and 1,6-anhydro-β-D-altropyranose under usual reaction conditions does not polymerize Although some quantitative data on their reactivity are available,[38] this is calculated on the basis of copolymerization data and is rather tentative The main driving force for the polymerization reaction is considered to be the decrease in the steric strain due to the elimination of the 1,3-interactions in the starting 1,6-anhydro-hexose, which exists in the $^1C_4$ conformation and possesses axial substituents Schuerch indicates, however, that if one takes into account only this factor, then the order in the reactivity series should be gluco > manno > galacto It has therefore been suggested[54] that an additional factor, which determines the reactivity of the anhydro-hexose, is the conformational changes ($^1C_4 \rightarrow B_{3,0}$) which occurs in the monomer upon interaction with the oxonium ion

3 1 3 *Synthesis of* (1-6)-α-D-*glycopyranans* The polymerization of 1,6-anhydro-hexoses* represented a very successful method for the synthesis of several linear homopolysaccharides and homopolysaccharides with short branchings (the comb-like polysaccharides) The copolymerization of two different 1,6-anhydro-hexoses yielded a series of heteropolysaccharides with irregular (statistical) distribution of the monosaccharide units along their chains

Polymerization affords high-molecular-weight glycans as a mixture of polymer-homologs with different molecular weight distribution and different average degrees of polymerization For the preparative synthesis of unprotected linear homopolysaccharides, use was made almost exclusively of the polymerization of *O*-benzyl ethers of 1,6-anhydro-hexoses the benzyl groups can easily be removed from the polymerization product In most of preparative syntheses of linear (1-6)-α-D-glycans the polymerization was carried out in the presence of PF_5 in CH_2Cl_2 at temperatures from $-40°$ to $-78°$C As the reactivity of monomers differs quite considerably, the amount of the catalyst (the catalyst/monomer ratio), the monomer concentration, and the reaction time vary within rather wide ranges Optimal conditions for the preparative synthesis of each of the glycans were selected after a large series of experiments High purity of reagents is necessary for a successful synthesis

* For a review on the synthesis of 1,6-anhydro sugars see Ref 38

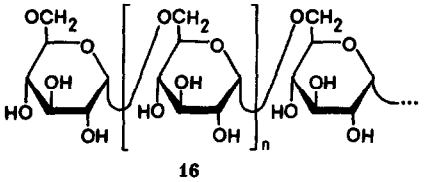
The experiments are carried out using a high-vacuum technique usually employed in polymer chemistry When the reaction comes to an end the polymerization process is terminated by adding methanol Then the *O*-benzyl ether of the polysaccharide was isolated and purified by conventional methods

The polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-D-glucose, -D-mannose and -D-galactose afforded the corresponding high-molecular-weight (1-6)-α-D-glycans with high yields The synthesis of (1-6)-α-D-glucopyranan **16**[42,45-49 55-59] which is similar in structure to natural dextran, as well as of some of its *O*-substituted derivatives, has been studied most thoroughly The polymerization at sufficiently low temperatures (−60°, −70°C) yields the fully stereoregular glucan, which does not contain β-linkages With increasing temperature the stereoregularity of the polymer is violated The molecular weight of the polymer varies considerably with the polymerization conditions the use of 1 mol % of the catalyst and a short reaction time (about 40 min) gives a degree of polymerization of 1400, which corresponds to a molecular weight of the polymer of 500,000 [46] By decreasing the monomer concentration, there is a decrease in the degree of conversion but an even higher molecular weight product is obtained

The polymerization of 1,6-anhydro-2,3,4-tetra-*O*-benzyl-β-D-mannose proceeds readily giving a stereoregular mannopyranan **17** with a degree of polymerization of 1050 and molecular weight of about 450,000 [48 49] The polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactose occurs much less readily [47,48 60] the reaction carried out at −78°C for 100 h even using up to 20 mol % of the catalyst afforded (1-6)-α-D-galactopyranan **18** in only 60% yield, the increase in the temperature up to −60°C reduces the molecular weight of the polymer Polymerization for 24 h, in the presence of 0 8 mol % of the catalyst gives a degree of polymerization of about 500 (a molecular weight of 200,000) [47]

Recently, it has been possible to carry out the polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-allose,[61] which possesses rather low reactivity The best results were obtained with a high concentration of the monomer and a short reaction time, which gave (1-6)-α-D-allopyranan **19** in 67% yield Depending on the reaction temperature (−60° or −78°C) its molecular weight was 37,000 and 62,000, respectively

Until now, it was not possible to carry out the polymerization of an altrose derivative [62] This is consistent with the low reactivity of this monomer under ionic polymerization conditions



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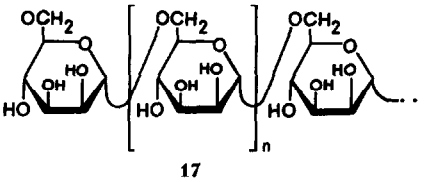

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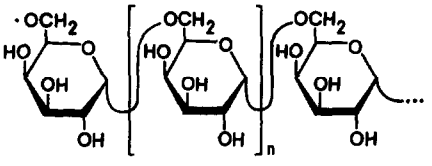

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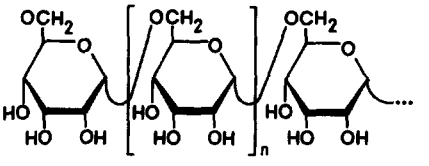

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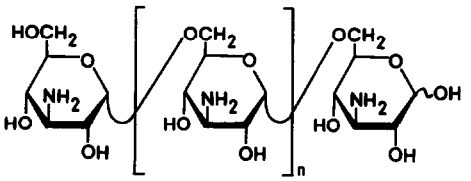
Debenzylation of the products of polymerization in order to obtain the unprotected polysaccharide is achieved by treatment with sodium in liquid ammonia at −78°C During the treatment the polymeric chain of the synthetic polysaccharide is partially degraded to result, after the usual work-up and purification, in a polysaccharide of lower molecular weight Uryu[46] has estimated that the molecular weight is reduced by a factor of 2 5–3

The structure of the resultant high molecular weight polysaccharides, containing from several hundred to 1500 monosaccharide units, was studied by chemical and physicochemical methods, including the occasional use of NMR-spectroscopy for the unprotected polysaccharides In these cases both ^{1}H- and ^{13}C-NMR data demonstrated complete stereoregularity (absence of the signals for the β-glycosidic linkages) and regioregularity (simplicity of the spectra and absence of excessive

signals for C-atoms) However, in many cases the stereoregularity of synthetic samples was estimated only by the measurement of optical rotations Occasionally, the regioregularity was confirmed by the methylation analysis and oxidation with periodate [59]

Enzymatic hydrolysis of synthetic α-D-glucan **16** and α-D-mannan **17** with the specific enzymes (dextranase,[63] α-D-mannanase[64]) also confirms their very high regularity The absence of branchings in the glucan was also supported by its lack of precipitation with concanavalin A [65] The synthetic mannan **17** and glucan **16** were also successfully used in immunochemical studies The distinction has been confirmed between the synthetic linear mannan and the branched natural mannan from yeast [38] The synthetic glucan which is close in structure to natural dextran was employed to establish the size of the antigenic site of the latter [66]



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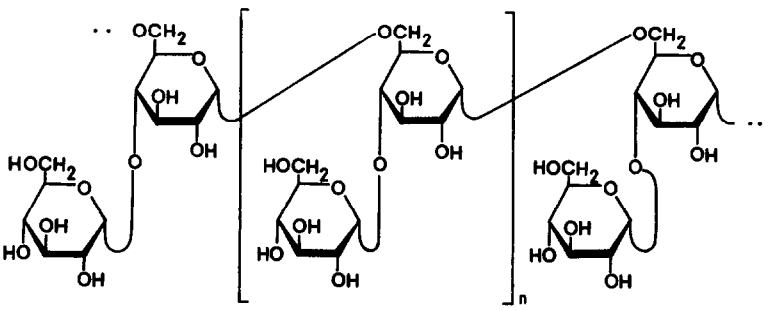

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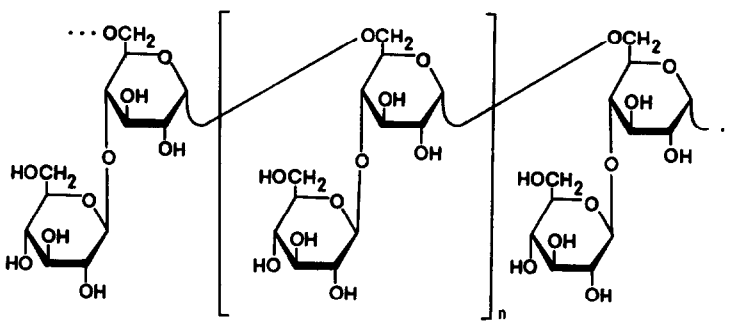

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The polymerization of 1,6-anhydro sugars was recently used also for the synthesis of the polysaccharides containing an amino group The polymerization of a derivative of 1,6-anhydro-β-D-glucopyranose, containing a protected amino group, was unsuccessful, and, hence, the polymerization of derivatives of 1,6-anhydro-glucose containing the azido group, namely the di-*O*-benzyl-ethers of 1,6-anhydro-2-azido-2-deoxy -, -3-azido-3-deoxy -, and-4-azido-4-deoxy-β-D-glucopyranose[43] was studied The polymerization of these compounds proceeded less readily than that of the corresponding glucose derivatives The polymerization of the 3-azido-3-deoxy derivative was carried out successfully using the complex PF_5–PhCOF as a catalyst at −60°C for 20 h A highly stereospecific (according to the NMR data) polymer with a degree of polymerization of 130–150 (a molecular weight of 47,000–55,000) was obtained The use of pure PF_5 affected the azido group in the course of the reaction and the spectrum of the polymer showed the presence of carbonyl groups The polymerization of 1,6-anhydro-2-azido-2-deoxy-β-D-glucose did not yield a polymer Only lower

oligosaccharides, from tri- to hexasaccharides were formed In the case of the 4-azido-isomer the reactivity of the monomer was somewhat higher, however, the yield of the polymer (with a degree of polymerization of 13) was but 19% The polysaccharide obtained in the polymerization of the 3-azido derivative was converted into the respective 3-amino-3-deoxy-α-D-glucan by reduction of the azido group with $LiAlH_4$, followed by debenzylation Such a modification caused certain destruction and the final 3-amino-glucan **20** had a somewhat lower molecular weight

The polymerization of 1,6-anhydro-sugars also yielded two comb-like polysaccharides with a regular structure The polymerization of hexa-*O*-benzyl ethers of 1,6-anhydro-β-maltose[67] and -cellobiose[68] yielded the comb-like regular glycans **21** and **22**, containing as the substituents at C-4 of each α-linked glucose unit of the backbone chain the α- and β- linked glucose units, respectively The polymerization of the disaccharide monomers occurs less readily The molecular weight and the stereoregularity of the polymeric structure is much more dependent upon the reaction conditions than in the case of monosaccharide derivatives The polymers with a relatively high molecular weight (5000–11,000) were obtained only under the action of PF_5 together with 20% of PhCOF and with longer reaction times Otherwise the conversion of the monomer was very poor, and the molecular weight of the polymer was low Debenzylation of the polymers obtained with sodium in liquid ammonia yields the unprotected comb-like polysaccharides The stereoregularity of the polymer was determined only by measurement of its optical rotation This, of course, cannot be considered rigorous, although, taking into account general regularities in the polymerization of 1,6-anhydro-hexoses, the stereospecificity should be high

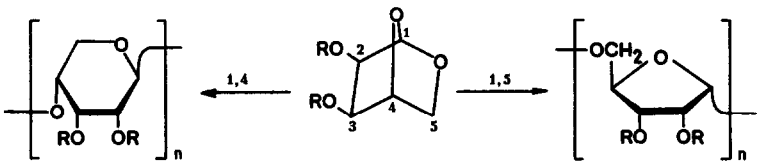
The copolymerization of some 1,6-anhydro-hexose monomers has been studied in greater detail This establishes the order of reactivity of these derivatives by using the known regularities in the copolymerization processes [38] The copolymerization of the respective derivatives, followed by debenzylation, afforded D-gluco-D-mannan,[69] D-gluco-D-galactan[70] and D-manno-D-galactan [71] Synthesis of a polysaccharide, which is close in structure to natural dextran, was accomplished by the copolymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucose and 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-crotyl-β-D-glucose, followed by the subsequent removal of the 3-*O*-crotyl protection and the glucosylation of the recovered OH groups at C-3 of the glucose units [53] Another, similar polysaccharide was obtained by the copolymerization of the 1,6-anhydro derivatives of glucose and maltose [72] The copolymerization of two different monosaccharide monomers yields the polysaccharides with a different ratio of the monosaccharide units, depending upon the ratio of the monomers and their relative reactivity The polymers obtained are claimed to have a high stereoregularity, which was established, unfortunately, only on the basis of optical rotation measurements, and irregular distribution of the monosaccharide units along the chain Hence, these polysaccharides do not belong to the class of polymers with a regular structure, and detailed discussion of their syntheses falls beyond the scope of this review Details can be found in a recent review by Schuerch [38]

Along with the synthesis of 1,6-α-D-glycopyranans attempts were made to obtain a polysaccharide containing furanose units by polymerization of 1,6-anhydro-2,3,5-tri-*O*-benzyl-α-D-galactofuranose [73] The polymerization in the presence of PF_5 afforded a polymer, whose structure was not studied in detail It appears that the polymer contains both furanose and septanose units and that it is stereochemically irregular

3 2 *Polymerization of* 1,4-*anhydro sugars**

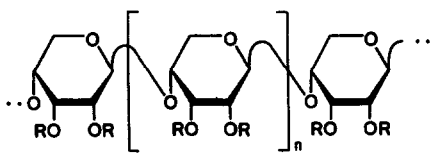
The polymerization of the derivatives of monosaccharides containing an 1,4-anhydro ring has come across much more serious difficulties, although the results obtained quite recently seem to provide the first opportunities for the synthesis of polysaccharides with a regular structure The most abundant natural polysaccharides are 1,4-glycans (cellulose, amylose, xylan), and this explains why the first syntheses of such polysaccharides by the polymerization of 1,4-anhydrides were attempted at an early stage in the development of the method Kops and Schuerch, who studied the polymerization of 1,4-anhydro-2,3,6-tri-*O*-methyl-β-D-galactose and 1,4-anhydro-2,3-di-*O*-methyl-α-L-arabinose under the action of PF_5 or $BF_3 \cdot Et_2O$,[74] obtained polymers possessing neither regio- nor stereoregularity The polymers obtained contained both pyranose and furanose units, the ratio of which depended upon the polymerization conditions Stereochemically ambiguous results were

* For a review on the synthesis of 1,4-anhydro sugars see Ref 38



Scheme 5

also obtained in the polymerization of 1,4-anhydro-2,3,6-tri-*O*-benzyl-α-D-glucopyranose [75,76] This was explained by the fact that these monomers may be considered as derivatives of both 1,4-anhydro-pyranose and 1,5-anhydro-furanose systems They are the hydroxy-derivatives of 2,7-dioxabicyclo[2 2 1]heptane, and, thus, the formation of the furanose or pyranose units is determined by the relative rate of scission of the 1,4- or 1,5-carbon-oxygen bonds (Scheme 5) These relative rates are determined by complexation at the respective O atoms that is by their relative basicity Stereochemical ambiguity of the polymerization as a result of an opening of the anhydro-ring, and, hence, of a glycosidic linkage formed was rationalized by the authors by assuming that the growth of the polymeric chain involves not only the oxonium ion but also the carbenium ion arising upon isomerization of the former A thorough study of the influence of the catalyst's nature and the type of substitution in the starting monomer on the direction of an opening of the bicyclic system of the 1,4-anhydro sugar appears to have succeeded in finding the way to a more specific polymerization of the 1,4-anhydrides The Japanese group has studied in detail the polymerization of 2,3-*O*-substituted derivatives of 1,4-anhydro-α-D-ribopyranose under the action of different Lewis acids [77,78] The polymerization of 2,3-*O*-benzylidene- and 2,3-*O*-isopropylidene derivatives under the action of $SbCl_5$, and of the 2,3-di-*O*-methyl derivative under the action of PF_5 and $SbCl_5$ at $-40°$ to $-60°C$ yields 2,3-substituted (1-4)-β-D-ribopyranans **23** On changing the reaction temperature and using other catalysts (e g $BF_3 \cdot Et_2O$), the structural regularity of the polymer is violated, and pyranose units appear with furanose units Moreover, the polymerization of the 2,3-di-*O*-methyl or 2,3-di-*O*-benzyl derivatives of 1,4-anhydro-α-D-ribose in the presence of NbF_5, $SnCl_4$, or $BF_3 \cdot Et_2O$ affords a polymer with a high molecular weight, built of ribofuranose units, 2,3-di-*O*-benzyl-and 2,3-di-*O*-methyl-(1-5)-α-D-ribofuranans **24** The molecular weight of the polymers varies considerably depending upon polymerization conditions Other combinations of catalyst and temperature lead to irregular polymers containing both furanose and pyranose units Treatment of the 2,3-*O*-benzylidene and 2,3-di-*O*-benzyl derivatives with sodium in liquid ammonia yielded unsubstituted polysaccharides built up of pyranose and furanose units Their structures were established by NMR spectroscopy with the assignment of signals being made on the basis of the monosaccharide models This is questionable because the "monosaccharide approximation" is known[79] to be insufficient for the analysis of the structure of polysaccharides because the adjacent unit in the chain affects the chemical shift The proper interpretation of this type of NMR data requires at least a disaccharide model



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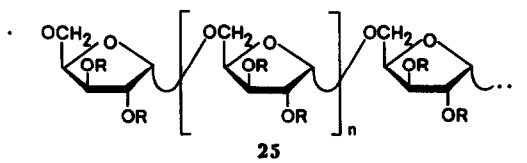

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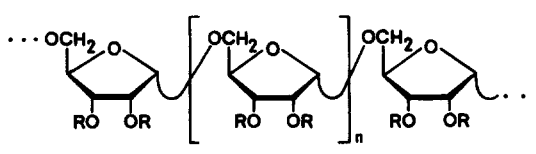

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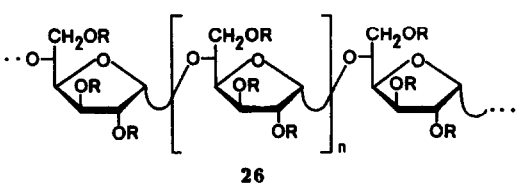

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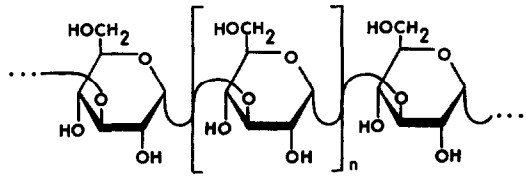
Nevertheless, the results obtained so far show that the proper choice of the catalyst and substituents in the starting monomer provides some control over the opening of the 1,4-anhydro system and directs the polymerization in a less ambiguous way However, a very thorough structural and

stereochemical analysis of the polysaccharides is required in order to prove reliably the results of polymerization of 1,4-anhydro sugars

The polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-α-D-xylopyranose[80] was also studied The reaction carried out in the presence of $BF_3 \cdot Et_2O$ or $SnCl_4$ at $-20°$ to $-60°C$ afforded a polymer with a high molecular weight (of 14,000), to which the authors on the basis of the ^1H- and ^{13}C-NMR spectra ascribe a strictly regular structure 2,3-di-*O*-benzyl-(1-5)-α-D-xylofuranan **25** Treatment of this product with sodium in liquid ammonia yielded the unprotected (1-5)-α-D-xylofuranan It should be pointed out that the presence of the furanose ring was claimed only from the difference with the spectrum of natural xylan, which is known to be (1-4)-β-D-xylopyranan The use of other catalysts, SiF_4, PF_5, NbF_5, or SbF_5, gives an irregular polymer, which contains xylopyranose units both α- and β-linked In order to explain the steric ambiguity of an opening of the anhydro system, the authors consider the mechanism, in which the β-linkage is formed on polymerization via the oxonium mechanism, whereas the "anomalous" α-linkage is the result of the polymerization proceeding via the carbenium ion The reason for the distinction between the polymerization of the 1,4-anhydroribose and 1,4-anhydroxylose systems was not discussed

There are also the data on the polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-α-D-lyxose Under the action of $SbCl_5$ at $-60°C$ this reaction yielded a product with a low molecular weight containing only α-furanose units [82]

Quite recently the polymerization of the 1,4-anhydro derivatives of glucose and galactose has been reinvestigated [81] The polymerization of 1,4-anhydro-2,3,6-tri-*O*-benzyl-α-D-glucopyranose under the action of PF_5 at $-20°C$ afforded 2,3,6-tri-*O*-benzyl-(1-5)-α-D-glucofuranan **26** with a low degree of polymerization (about 20) The fully stereoregular structure was ascribed to the polymer Unfortunately the proposed structure was based only on differences between the ^{13}C-NMR spectra for **26** and for the *O*-benzyl ethers of natural amylose and cellulose The polymerization of 1,4-anhydro-2,3,6-tri-*O*-benzyl-β-D-galactopyranose in the presence of PF_5 or $SbCl_5$ afforded a product with a low molecular weight, which possessed neither regio- nor stereoregularity, thus confirming the former result [74]



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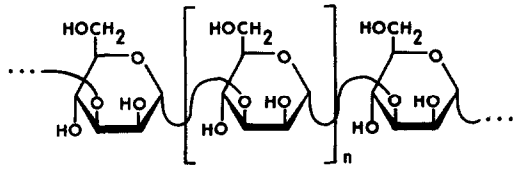

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The available data on the polymerization of 1,4-anhydro sugars do not permit the assessment of general preparative possibilities It is not yet possible to predict the course of the polymerization reaction and the influence of the catalyst's nature on the direction of anhydro-ring opening

3 3 *Polymerization of* 1,3-*anhydro sugars*

There are only a few publications concerning the polymerization of the 1,3-anhydro sugars because the starting anhydro derivatives containing pyranose and oxetane rings became available only recently [83] The polymerization of 1,3-anhydro-2,4,6-tri-*O*-benzyl-β-D-glucopyranose in the presence of PF_5 at $-60°C$ yielded a linear polymer[84] with a low molecular weight, which showed no stereoregularity (the content of the α-linkages was approximately 75%) Even more unsuccessful were the experiments with $SbCl_5$, triethyloxonium hexafluorophosphate, $BF_3 \cdot Et_2O$ and SiF_4 This was explained by involvement not of the oxonium but of the carbenium intermediate Much higher stereospecificity was obtained by using catalysts such as tris(4-bromophenyl)aminium hexafluoroantimonate, triphenylmethylium perchlorate (tritylium perchlorate) and trifluoro-

methanesulfonic anhydride (triflic anhydride) [85] It is proposed that there is a stronger interaction between the counterions of these catalysts and the oxonium ion of the growing chain This leads to the stabilization of the oxonium cation and directs the polymerization towards the oxonium-ionic route Higher stereospecificity of the reaction was also achieved with the *p*-bromobenzyl protection of the OH groups of the monomer It was observed that the stereoselectivity of the process drops in the series *p*-bromobenzyl > benzyl > *p*-methylbenzyl The authors suggest this distinction to be caused by the fact that the electron-donating groups (*p*-methylbenzyl) increase the basicity of the ether O atom, whereas the electron-withdrawing groups (*p*-bromobenzyl) reduce it As a consequence, the position of the complexation equilibrium between the initiator and ether or ring oxygens is shifted towards the formers for electron-donating groups and towards the latter for electron-withdrawing groups

Using these data Good and Schuerch succeeded in obtaining linear (1-3)-α-D-glucopyranan **27** by the polymerization of 1,3-anhydro-2,4,6-tri-*O*-(*p*-bromobenzyl)-β-D-glucose in the presence of triflic anhydride in toluene at −40°C or silver triflate in benzene, followed by the standard procedure for removal of the protective groups According to the ^{13}C-NMR data, the polymer is stereoregular, has only the α-linkages and, under optimum conditions, its molecular weight is 16,000–31,000

The polymerization of 1,3-anhydro-2,4,6-tri-*O*-(*p*-bromobenzyl)mannose in the presence of triflic anhydride afforded the corresponding polysaccharide[86] with a degree of polymerization of approximately 60–90 After the standard procedure for the removal of the protecting groups, it was converted into the unsubstituted (1-3)-α-D-mannopyranan **28** associated with a high stereospecificity as determined by its NMR spectrum In this case again, the *p*-bromobenzyl derivatives offer better results

3 4 *Polymerization of* 1,2-*anhydro sugars*

Thermal polymerization of 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose ("Brigl's anhydride"), which produced a mixture of polymers of unknown structure, has been studied by Haq and Whelan [87] The cationic polymerization of this monomer under the action of various Lewis acids afforded a polymer containing both α- and β-linkages, as well as orthoester cross-linkings It is evident that the reaction proceeded via the carbenium mechanism and the protective acetyl groups of the monomer are also involved

Somewhat more successful results were obtained in the polymerization of the *O*-benzyl derivatives of 1,2-anhydrides of α-D-glucose and β-D-mannose The former on polymerization (PF_5, −60° to −70°C) gave a polymer with a molecular weight of 10,000 which after debenzylation yielded a free (1-2)-glucan, which contained, according to the ^{13}C-NMR data, approximately 90% of β- and 10% of α-linkages [88] The use of the other cationic catalysts gave a polymer with less stereoregularity The polymerization of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannose under these conditions afforded, in a poor yield, the polymers with a low molecular weight and unsatisfactory stereoregularity [89]

Concluding this discussion of methods for the synthesis of polysaccharides by polymerization of anhydro sugars, it is worth noting that undoubtedly this method is the best to obtain the simplest high-molecular weight (1-6)-α-glycopyranans, as well as (1-3)-α-glucopyranans and (1-3)-α-mannopyranans

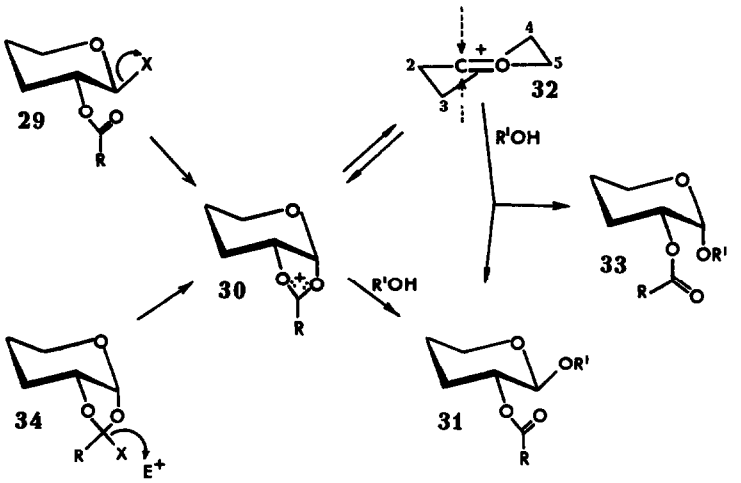
4 POLYCONDENSATION OF TRITYL ETHERS OF CYANOETHYLIDENE DERIVATIVES OF SUGARS

This approach to the synthesis of the regular polysaccharides is based on an entirely new glycosylation reaction and is of a general character It yields 1,2-*trans*-linked homopolysaccharides and polymers built of regularly repeating oligosaccharide units It is for the synthesis of complex heteropolysaccharides with these repeating units that is the attractive feature of this method It is a flexible method and has provided for the first time biologically active natural complex heteropolysaccharides

Since this method is based upon a new glycosylation reaction, the essence and limitation of this reaction will be discussed first

4 1 *Glycosylation of trityl ethers with cyanoethylidene derivatives of sugars*

Analysis of the mechanism of the glycosidic linkage formation upon glycosylation with acylglycosyl halides **29** (Koenigs–Knorr reaction and its modifications) leads to the conclusion that the



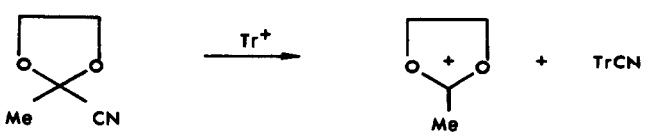
Scheme 6

formation of a 1,2-*trans* glycosidic linkage is connected with the participation of an acyl substituent at C-2 of the aldose it proceeds via the formation of a bicyclic dioxolenium intermediate **30**, the so-called acyloxonium ion The nucleophilic attack of this intermediate towards its rear side by an alcohol leads to the 1,2-*trans* glycoside **31** The stereospecificity of glycosylation depends on the ratio of the rates of this nucleophilic attack and of the competing reaction of isomerization of the acyloxonium ion into the monocyclic glycosyl cation **32**, which is further subject to the non-stereospecific attack to give besides the 1,2-*trans* glycoside **31** also the 1,2-*cis* isomer **33** (Scheme 6)

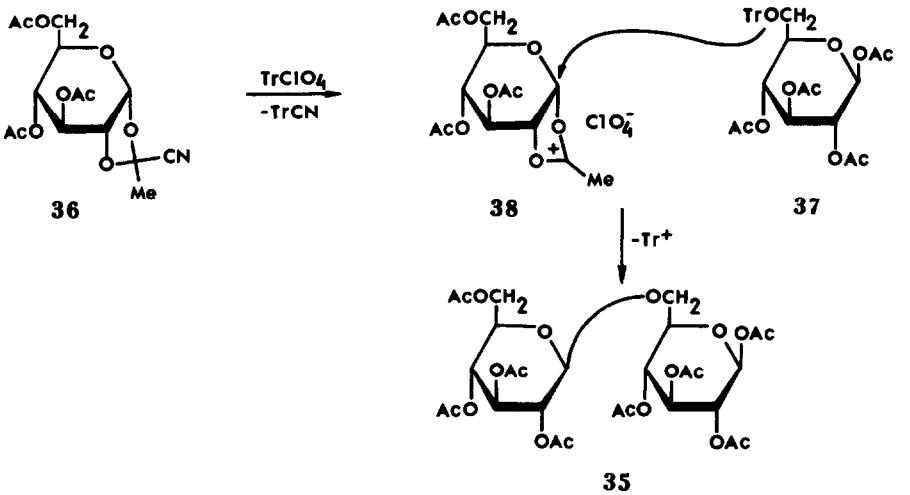
These concepts led to the examination of a more effective way to generate the dioxolenium intermediate **30**, which could provide higher stereospecificity for the formation of the 1,2-*trans* glycosidic linkages Generation of such an intermediate from a sugar derivative of the type **34**, in which the bicyclic trioxahydrindane system already pre-exists, appeared most favourable The new method of glycosylation with the 1,2-orthoesters and 1,2-thioorthoesters of sugars (Section 2) were the realizations of this idea However, due to different reasons these methods proved unsuitable for the development of stereospecific methods of polycondensation Meerwein *et al* [90] have suggested an interesting approach to generate the simplest cyclic ion of the dioxolenium(acyloxonium) type from 2-cyano-2-methyl-1,3-dioxolane (Scheme 7) Upon formation of the dioxolenium ion, the triphenylacetonitrile (tritylcyanide) leaves the reaction site thus making this reaction irreversible

Starting from this finding a new method of glycosylation was developed[91] in the laboratory of Zelinsky Institute of Organic Chemistry Its essence can be exemplified by the synthesis of gentiobiose octa-acetate **35** by the interaction of 3,4,6-tri-*O*-acetyl-1,2-*O*-cyanoethylidene-α-D-glucopyranose **36** and 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose **37** under the action of tritylium perchlorate (Scheme 8) The trityl-cation abstracts a cyano group from the cyanoethylidene moiety, and generates, as in the Meerwein reaction, the acyloxonium cation **38** The latter is subject to nucleophilic attack by the O atom of the trityl ether giving rise to a glycosidic linkage formation This regenerates the trityl-cation which then continues the process Thus tritylium perchlorate serves as a catalyst It is of particular importance that the nucleophilic attack of the intermediate **38**, having the bicyclic system of *cis*-hydrindane is possibly only from the rear side, and hence, only a 1,2-*trans* linkage is formed, while the 1,2-*cis* was not found

This glycosylation reaction is general in nature and it has found the application for the synthesis of oligosaccharides, and, what is most important, it became the basis for a new polycondensation reaction which allowed for the first time the rational synthesis of a wide class of polysaccharides [92,93]



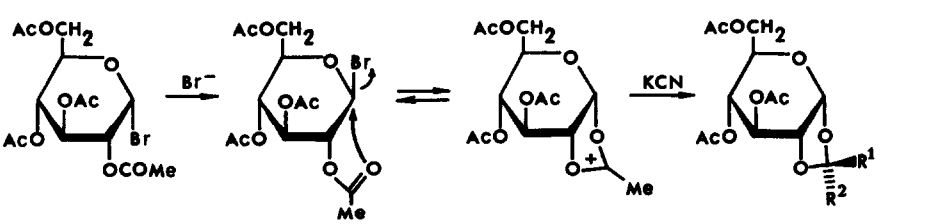
Scheme 7



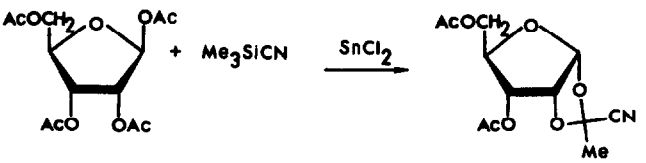
Scheme 8

The synthetic approach based on this reaction required a reliable method for the synthesis of the cyanoethylidene derivatives and of trityl ethers For the synthesis of polysaccharides this requires the synthesis derivatives carrying both these functions in the same molecule

4 1 1 *Synthesis of cyanoethylidene derivatives of mono- and oligosaccharides* In the majority of studies, acylated (acetylated and, in some cases, benzoylated) cyanoethylidene derivatives were used as glycosylating agents A first compound of this type, namely, **36** was described by Coxon and Fletcher[94] and was obtained by the interaction of acetobromglucose and AgCN in boiling xylene A cyanoethylidene derivative of maltose was obtained in a similar way [95] This reaction frequently affords low yields and is accompanied by the side-formation of glycosylisocyanides [96] A much more advantageous and general method for the synthesis of the acylated cyanoalkylidene, in particular cyanoethylidene derivatives, consists in the interaction of per-*O*-acetyl-glycosyl bromides and KCN or NaCN in dry acetonitrile [97] In the case of the less reactive 1,2-*cis*-glycosyl bromides, the reaction is carried out in the presence of some source of bromide ions (tetrabutylammonium bromide) which promotes the conversion of the 1,2-*cis*-bromo-derivative into the more reactive 1,2-*trans*-glycosyl bromide The reaction proceeds smoothly within 12–24 h and affords peracetates of the cyanoethylidene derivatives **39** in high yields The reaction mechanism can be represented by the following scheme (Scheme 9) This general method has been applied for the synthesis of the cyanoalkylidene derivatives of hexopyranoses,[97 98] pentopyranoses,[99,100] pentofuranoses,[97] as well as for the synthesis of the cyanoethylidene derivatives of disaccharides[97] and of a large number of more complex oligosaccharides [101,102] Numerous examples of its application will be given below (Section 4 2) Unsatisfactory results were obtained for the cyanoethylidene derivatives of the uronic acids the reaction was complicated and the yields were very low It has been possible to obtain the latter derivatives only by treatment of methyl (2,3,4-tri-*O*-acetylglycosylbromide)uronates with an excess of AgCN in boiling xylene, although the yields were low as well [103] Recently, it has been found that the acetates of the cyanoethylidene derivatives of furanoses can be obtained from the corresponding peracetates and trimethylsilyl cyanide in the presence of stannous chloride[104,105] (Scheme 10)



Scheme 9



Scheme 10

This reaction has not yet been studied in detail, though it appears to be very promising, and has already found synthetic application [106] The interaction of the glycosyl bromides, containing *O*-benzoyl groups instead of *O*-acetyl groups with KCN or NaCN gives *O*-benzoates of the 1,2-cyanobenzylidene derivatives of some monosaccharides,[97,99] but these have not been widely employed in synthesis

The formation of the cyanoethylidene derivative is accompanied by the appearance of a new chiral centre at C-2′ of the dioxolane ring so the reaction affords a mixture of *exo*-(**39a**) and *endo*-cyano-(**39b**) derivatives Their ratio, which can easily be determined from ^{1}H-NMR data, varies within a broad range No clear-cut dependence of this ratio on the structure of the starting sugar has been observed, although there is a tendency towards the formation of the *exo*-cyano isomer in greater amounts This seems to relate to a greater spatial accessibility of the *exo*- side in the dioxolenium cation to attack by the CN anion The reactivity of both diastereomers in the glycosylation reaction was practically identical (see 4 1 4) so the synthesis of glycosides can be performed using a mixture The individual isomers can easily be obtained by crystallization or chromatographic separation

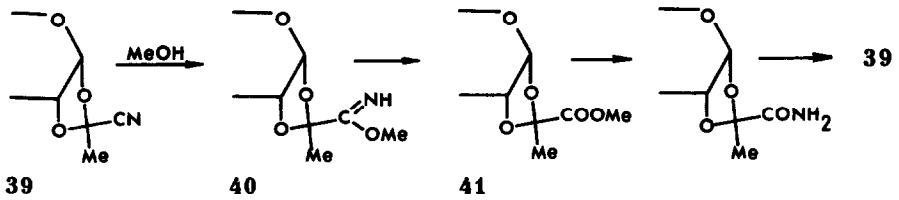
The cyanoethylidene derivatives are stable compounds which in most cases can be isolated after the chromatographic purification and kept without any changes for a long time They can be characterized reliably by spectral data The presence of a cyano group is demonstrated by an intensive peak at 2230–2240 cm^{-1} in their Raman spectra The ^{1}H-NMR spectrum of 1,2-*O*-cyanoethylidene derivatives is characterized by the presence of the three-proton singlets for the methyl groups at C-2′ of the dioxolane ring located at high field (δ 1 77–1 97), as compared to the chemical shifts of acetyl groups Low-field doublets (δ 5 47–6 14) for H-1 of sugar moiety with coupling constants of about 5 Hz (for the derivatives of gluco-, galacto-, and xylopyranoses) and about 2 Hz (for manno- and rhamnopyranoses), and *ca* 4 Hz (for galacto-, gluco-, arabinofuranoses and arabinopyranose) are observed

The diagnostic signals in the ^{13}C-NMR spectra are those of a methyl group at C-2′ (δ 24–27), cyano-group (δ 116–117), quaternary carbon C-2′ (δ 96–104), as well as the signals for C-1 of pyranoses (δ 96–99) and furanoses (δ 104–107), and C-2 of pyranoses (δ 72–79) and furanoses (δ 84–88)

The configuration at C-2′ of the diastereoisomeric cyanoethylidene derivatives is determined by the deshielding effect of the carbohydrate ring The signals in the ^{1}H-NMR spectra for the methyl groups of an *endo*-methyl isomer are shifted downfield as compared to those of an *exo*-isomer The X-ray studies of a series of 1,2-cyanoethylidene derivatives have confirmed the validity of this assignment [107]

The cyanoethylidene group is rather stable towards acids In contrast treatment with bases sometimes affects this group so deacetylation of these derivatives has to be performed under especially mild conditions The main reason for this instability is their conversion into the corresponding imidates **40** and methoxycarbonyl derivatives **41** [108] This side reaction has caused some difficulties in the synthesis of complex heteropolysaccharides At the same time, it can be used for the "temporary protection" of the cyano group For this purpose the methoxycarbonyl group, after an appropriate modification of the monomer, is converted into the carboxamide group by ammonolysis, and then the cyano group of the cyanoethylidene moiety is recovered by treatment with benzoyl chloride in pyridine[109] (Scheme 11)

The carbohydrate part of the cyanoethylidene derivative can be transformed by a series of reactions without affecting the cyanoethylidene group Of these transformations the most important are de-*O*-acetylation and introduction of a trityl group These transformations are the basis of the synthesis of monomers for polycondensation (Section 4 2) Also important is the ability of the cyanoethylidene derivatives to participate in the glycosylation reaction as the aglycones without involving the cyanoethylidene group [110] This rather unexpected reaction proceeds smoothly upon



Scheme 11

glycosylation of the cyanoethylidene derivatives, possessing a free OH, with glycosyl bromides under the conditions of the Helferich reaction (Scheme 12) This reaction is important because it provides new opportunities for the synthesis of cyanoethylidene derivatives of complex oligosaccharides which can then serve as the monomers for the synthesis of heteropolysaccharides (Section 4 2 5)

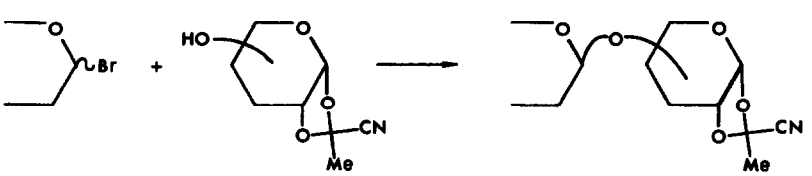
4 1 2 *Synthesis of trityl ethers of sugars* Trityl ethers of sugars are well known and are widely used in carbohydrate synthesis (see review[111]) Until recently only primary trityl ethers of mono- and oligosaccharides were available These derivatives, which are frequently used for the temporary protection of the primary OH groups sugars, are obtained smoothly by treatment of mono- and oligosaccharides with triphenylchloromethane (trityl chloride) in pyridine [111] The reaction proceeds selectively and the substitution affects only primary OHs Substitution of secondary OHs of sugars can be achieved as well under more drastic conditions

A mild and general method for the synthesis of secondary trityl ethers consists in the treatment of mono- and oligosaccharides, containing one or several OHs, with tritylium perchlorate in the presence of a hindered pyridine base,[112] 2,4,6-tri-*t*-butyl-pyridine or the more easily accessible 2,4,6-collidine[114] in CH_2Cl_2 at room temperature In this way it has been possible to obtain secondary trityl ethers of both simplest monosaccharides in the pyranose or furanose forms, and complex oligosaccharides in good to excellent yields The reaction is not always regioselective and hence, for the synthesis of the *O*-trityl derivatives of definite structure so the remaining OHs should preferably be protected In most syntheses acyl protective groups (*O*-acetates or *O*-benzoates) are used However, the derivatives containing *O*-benzyl, benzylidene[113] or isopropylidene groups,[114] can also be employed Tritylation reactions do not usually affect the cyanoethylidene group and this is of particular importance in the synthesis of monomers to be used further in polysaccharide synthesis

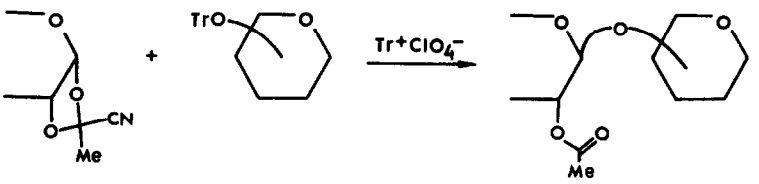
Trityl ethers of sugars are stable and, as a rule, crystalline substances Each has its typical NMR spectrum but no regularities were found which would allow the determination of the position of the *O*-trityl group from spectral data In order to determine or confirm the location of the *O*-trityl group in the acetylated derivative use is made of methylation analysis (sequential alkaline hydrolysis, *O*-methylation, de-*O*-tritylation, and GLC-MS analysis of the partially substituted sugar obtained)

4 1 3 *Trityl-cyanoethylidene condensation Synthesis of oligosaccharides* The interaction between cyanoethylidene derivatives and trityl ethers, the so-called "trityl-cyanoethylidene condensation", can be generally employed for the synthesis of oligosaccharides containing the 1,2-*trans*-glycosidic linkage (Scheme 13) The discussion of the oligosaccharide synthesis falls beyond the scope of this review However, the limitations of this reaction and, in particular, its stereoselectivity are relevant and the oligosaccharides obtained by this method often serve as models for the determination of the structure using the NMR technique of the corresponding synthetic polysaccharide

The condensation of trityl ethers and cyanoethylidene derivatives is usually performed by reaction of equivalent amounts of the reagents at room temperature although some excess of trityl ether is sometimes helpful The result of the reaction depends on the solvent used The most convenient solvent is CH_2Cl_2, in MeCN the reaction does not take place [91] Various tritylium salts,



Scheme 12



Scheme 13

such as perchlorate, tetrafluoroborate, hexafluorophosphate, were tested to serve as initiators but only the perchlorate salt (usually in 5–10 mol % amount) has found application in the synthesis Strictly anhydrous conditions are essential which is expected for reactions proceeding via carbenium mechanisms Along with the usual precautions, it is most convenient to carry out the reaction using a high vacuum technique [91] Solutions of the reagents and the initiator are placed in separate limbs of a tuning-fork-shaped tube (⅄) The reaction components are dried by a 2–3-fold distillation of a dry solvent, and then the solutions are mixed The reaction proceeds in a sealed evacuated vessel Large-scale experiments can also be performed in normal glassware with the usual precautions [114] The reaction takes several hours and when it is complete the trityl perchlorate is destroyed by the addition of a methanol–pyridine mixture Addition of methanol prior to pyridine results in detritylation of the aglyconic component

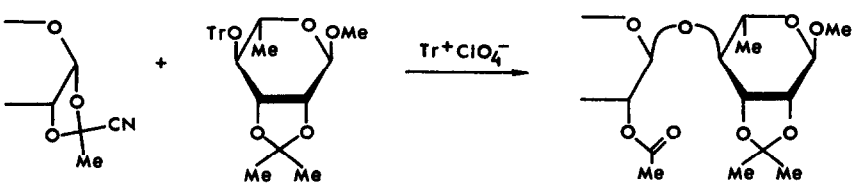
The reactivity of the cyanoethylidene derivative in this reaction is not significantly influenced by the structure of the starting sugar Thus the reaction between methyl 2,3-*O*-isopropylidene-4-*O*-trityl-α-L-rhamnopyranoside **42** and 1,2-*O*-cyanoethylidene derivatives of neutral sugars under identical conditions afforded in all cases the corresponding disaccharides **43** (Scheme 14) in almost equally high yields [114] It is worth noting that the reactivity of the cyanoethylidene derivatives of rhamnose seems to be somewhat higher than that of other neutral sugars whereas the cyanoethylidene derivatives of uronic acids esters seem to be less reactive [103,115] The experience in the use of cyanoethylidene derivatives of oligosaccharides (Section 4 2 5) shows that the additional monosaccharide substituents in the cyanoethylidene derivative do not obviously affect its reactivity

The 1,2-*O*-cyanoethylidene derivatives of furanoses have found useful application in synthesis [106,116] The preparation of furanosides by the classical methods often presents difficulties Their reactivity seems to be higher than that of the respective pyranose derivatives [106,117]

A large number of trityl ethers of sugars has been employed in the trityl-cyanoethylidene condensation, including primary and secondary trityl ethers of hexopyranoses, pentofuranoses, and oligosaccharides [118] Along with neutral sugars trityl ethers of 2-acylamino sugars[119] and deoxyoctulosonic acid[120] can also be used

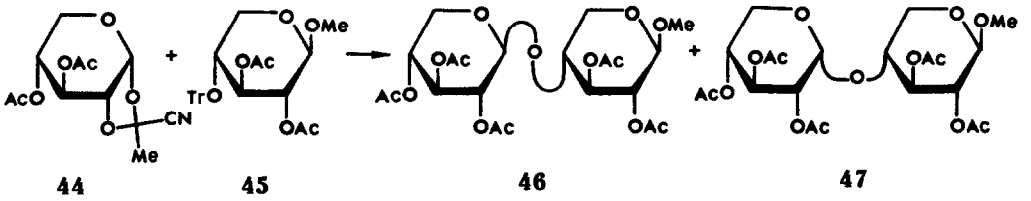
No special studies on the comparative reactivity of trityl ethers have been performed Primary trityl ethers of hexopyranoses and pentofuranoses are close in their reactivity and give oligosaccharides in high yields The reactivity and, in particular, the stereoselectivity of the reaction of the secondary trityl ethers, unlike their primary analogues, depend on the structure of the ether, the stereochemistry of the sugar and the position of the *O*-trityl group The yields of oligosaccharides from secondary trityl ethers are sometimes lower

The stereospecificity of glycosylation of trityl ethers by cyanoethylidene derivatives is crucial because only absolute stereospecificity of the glycosidic linkage formation would allow the synthesis of regular polysaccharides (Section 4 2) The data available show that the glycosylation of primary trityl ethers by the cyanoethylidene derivatives of different structure yields only 1,2-*trans* glycoside



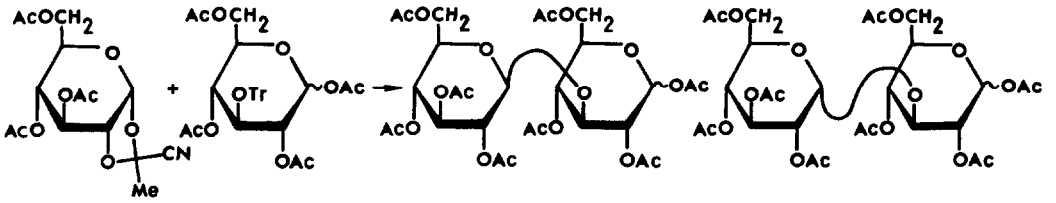
42 **43**

Scheme 14



44 45 46 47

Scheme 15



48 49 50a 50b

Scheme 16

derivatives Absolute stereospecificity for the reaction has been achieved * Judging from polycondensation data (see below), the same result is obtained in the reaction of 3-*O*- and 4-*O*-trityl ethers in the D-mannose and L-rhamnose series The absolute stereospecificity of glycosylation is supported most conclusively by the total stereoregularity of the polysaccharides obtained from the monomers of similar structure (Section 4 2)

At the same time, recently it was found that the stereospecificity of the trityl-cyanoethylidene polycondensation in its standard tritylium–perchlorate initiated version is violated in the glycosylation of 3-*O*- and 4-*O*-trityl ethers of xylo-,[121] arabino-,[122] gluco-,[123] and galactopyranosides [124]† In these cases the glycosylation by different cyanoethylidene derivatives yields the 1,2-*trans*-linked product along with 1,2-*cis* glycosidic product The proportion of the *cis*-product varies from 5 to 50% depending upon the structure of trityl ether Violation of stereospecificity in the synthesis of disaccharides conforms with the violation of polycondensation stereospecificity in the synthesis of polysaccharides (See 4 2 4 2) These violations seem to depend primarily on the structure of the trityl ether, since the glycosylation of a given trityl ether by different cyanoethylidene derivatives affords very similar amounts of the 1,2-*cis* derivatives [121]‡

It should be pointed out that random stereochemistry of glycosylation appears to be characteristic only of trityl ethers of pyranoses The glycosylation of the 3-trityl ether of L-arabinofuranose, in contrast to the corresponding pyranose analogue, is stereochemically unambiguous [106]

The stereospecificity of glycosylation depends strongly on the nature of the initiator, or, more exactly, on the tritylium salt anion Thus the reaction of 3,4-di-*O*-acetyl-1,2-*O*-cyanoethylidene-α-D-xylopyranose **44** with methyl 2,3-di-*O*-acetyl-4-*O*-trityl-β-D-xylopyranoside **45** in the presence of tritylium perchlorate gives a mixture of 1,2-*trans*- (**46**) and 1,2-*cis*- (**47**) isomeric disaccharides in the 2 1 ratio, whereas in the presence of tritylium tetrafluoroborate this ratio becomes 25 1, that is the reaction proceeds almost stereospecifically[125] (Scheme 15) A similar effect is also observed for the glycosylation of 1,2,4,6-tetra-*O*-acetyl-3-*O*-trityl-D-glucopyranose[123] (Scheme 16) Unfortunately, the reaction in the presence of tritylium tetrafluoroborate proceeds very slowly and the yields of disaccharides sharply decrease From a practical standpoint the use of this initiator is hardly reasonable

Recently, it has been established that high pressure affects very strongly the stereochemistry of products of glycosylation by cyanoethylidene derivatives The reaction carried out under a pressure

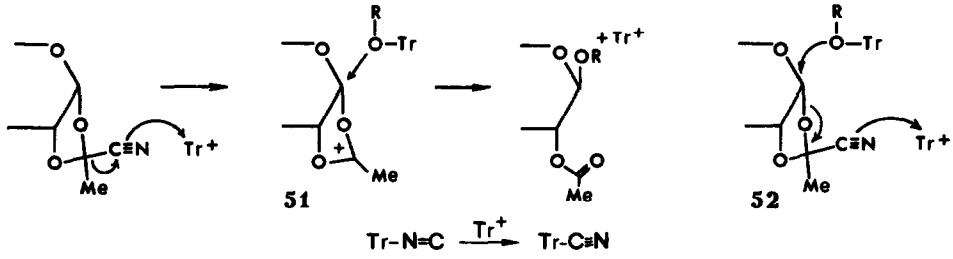
* It was proved by analysis of the ^{1}H- and ^{13}C-NMR spectra of total disaccharide fractions, obtained directly from the reaction mixture The modern NMR technique allows an admixture of an anomer with 1–2% accuracy to be determined

† The only known exception so far is the reaction of 3-trityl ethers of *N*-acyl-D-glucosamine with the cyanoethylidene derivatives of rhamnose This reaction proceeds with complete stereospecificity

‡ Moreover, the glycosylation of trityl ethers by thioorthoesters of sugars, which is distinguished by very high specificity, is also violated only in case of the trityl ethers of this type [121]

of 14 kbar leads to a striking result Only the 1,2-*trans* glycosidic linkage is formed and the reaction proceeds in a totally stereospecific way even in those cases when under the normal conditions it proceeds with a strong violation of stereospecificity Thus the reaction between 3,4,6-tri-*O*-acetyl-1,2-*O*-cyanoethylidene-α-D-glucose **48** and 1,2,4,6-tetra-*O*-acetyl-3-*O*-trityl-α,β-D-glucose **49** under the usual conditions of trityl-cyanoethylidene condensation afforded disaccharides **50a,b** with the 1,2-*trans*/1,2-*cis* ratio of 59 41 In dramatic contrast the same reaction carried out at a pressure of 14 kbar proceeds with absolute stereospecificity to give only the 1,2-*trans* disaccharide **50a**[126] (Scheme 16) The striking effect of high pressure is also manifested in the synthesis of polysaccharides (see 4 2 4 2) Thus, the glycosylation reaction carried out at high pressure proceeds with absolute stereospecificity even in those cases when at normal pressure the stereospecificity is violated

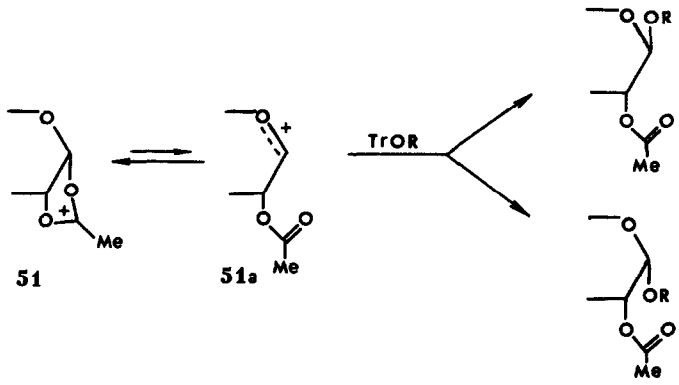
4 1 4 *On the mechanism of the trityl-cyanoethylidene condensation* Since no special studies on the mechanism of this new reaction have been carried out we can only discuss here some general considerations based upon common ideas on glycosylation reactions leading to 1,2-*trans*-glycosidic linkages A mechanism of the trityl-cyanoethylidene condensation can be represented schematically in the following way[102] (Scheme 17)



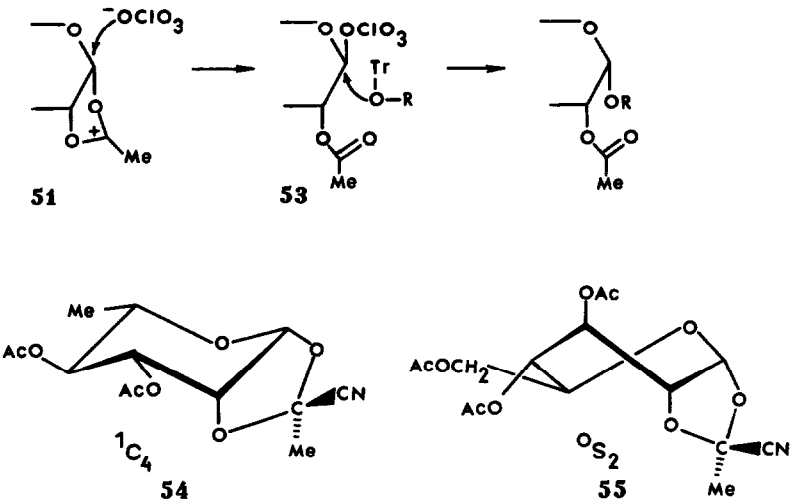
Scheme 17

The electrophilic attack of the tritylium cation, which initiates the reaction, directed on nitrogen of the cyano group, results in its abstraction, thus giving rise to the bicyclic dioxolenium cation **51**, the latter is subject to the nucleophilic attack by an O atom of the *O*-trityl group of the aglycone to give a glycosidic linkage In the presence of the tritylium ion, trityl isocyanide formed undergoes rearrangement into trityl cyanide, which leaves the reaction site As the bicyclic system of the dioxolenium ion is open to attack only from its rearside, the process leads to the formation of a 1,2-*trans* glycosidic linkage The whole process seems to be close to a concerted one of the type **52** with a push–pull attack of the tritylium cation and oxygen of the *O*-trityl group this explains the absolute stereospecificity of the reaction in most cases On the other hand, when the process is not concerted, the intermediate **51** then fails to enter the reaction immediately and may therefore undergo conversions leading to side processes and the formation of a 1,2-*cis* glycosidic product

There are two possible pathways which could give rise to a 1,2-*cis* glycosidic linkage (see 4 1 3) Partial isomerization of the dioxolenium ion **51** into the monocyclic glycosyl cation **51a** could take place which, because of its flattened structure, is open to a non-stereospecific nucleophilic attack towards both faces This explains the formation of a 1,2-*cis* isomer (Scheme 18) This suggestion is



Scheme 18



Scheme 19

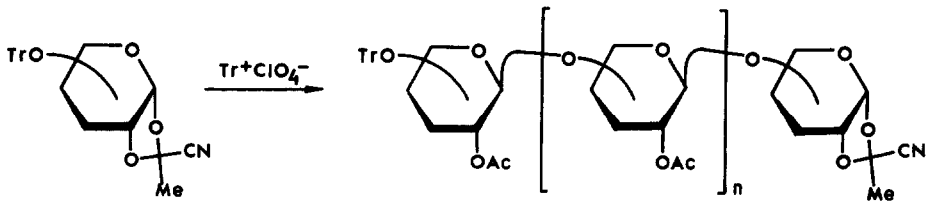
confirmed by the fact that the reaction, carried out under high pressure, affords mainly 1,2-*trans* glycosides because the pressure shifts the equilibrium **51** ⇌ **51a** to the left side This also accounts for the rather high volume effect of the reaction amounting to about 9 cm^3/mol [126]

Alternatively, the 1,2-*cis* glycosidic linkage may be produced because the intermediate **51** is subject to a competing substitution by the nucleophilic perchlorate ion to give the perchlorate **53** with the 1,2-*trans* configuration * **53** is then attacked by an oxygen of the *O*-trityl group from the other side (Scheme 19) This double inversion gives rise to the 1,2-*cis* glycosidic linkage It should be pointed out that the nucleophilicity of the perchlorate ion,[128] as well as the existence of carbohydrate 1-*O*-perchlorates,[129] are well documented This explanation is strongly supported by decrease in the amount of the 1,2-*cis* isomer[125] when tritylium perchlorate is replaced by the other tritylium salts with less nucleophilic anion[127] such as tritylium triflate [125]

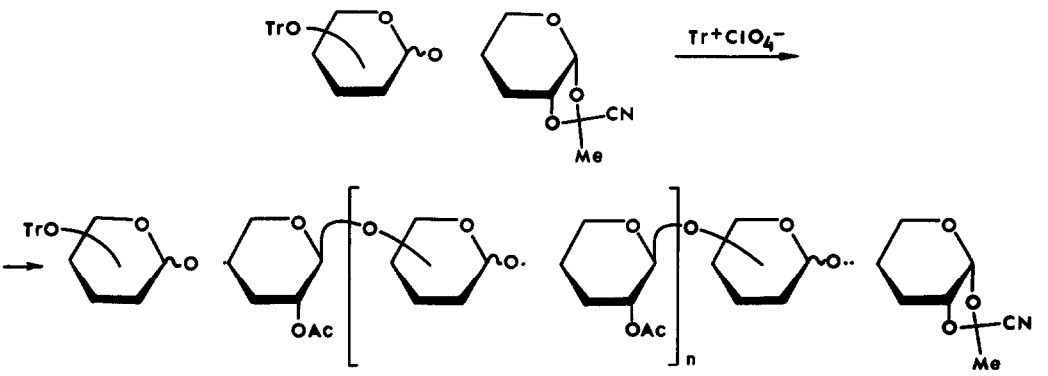
Whatever suggestion is correct, it appears quite clear that the anomalous 1,2-*cis* glycosylation occurs when the normal concerted process is disturbed due to a delay in the attack of the intermediate **51** This intermediate then has time to participate in side reactions The formation of the anomalous 1,2-*cis* glycosidic linkage depends upon both the nature of the cyanoethylidene component, and more distinctly on the structural features of the trityl ether It was observed only in the cases of the less reactive 3- and 4-trityl ethers of D-glucose, D-galactose, D-xylose, and L-arabinose At the same time, derivatives of D-mannose and L-rhamnose interact with the cyanoethylidene derivatives with high stereospecificity to give only 1,2-*trans* glycosidic linkages This is also demonstrated by the polycondensation reaction (see below)

The reasons of the decreased reactivity of the above-mentioned trityl ethers are still rather obscure It appears that they are determined by the steric and electron factors and X-ray analysis of some trityl ethers shows different accessibilities of the O atom of the trityl groups to nucleophilic attack in ethers of different structure In those derivatives giving on glycosylation stereochemically ambiguous results, the O atom is sterically shielded to a greater extent [130] It is also evident that the higher reactivity of the cyanoethylidene derivatives of L-rhamnose and D-mannose compensates for the hindrance arising in the attack by less reactive trityl ethers Therefore the intermediate **51** is subjected to the attack more effectively and the anomalous 1,2-*cis* glycosylation does not take place A higher reactivity of these cyanoethylidene derivatives can be explained by the absence in, say, a derivative of L-rhamnose (which according to the X-ray data[107] exists in the $^1C_4$ conformation **54**) of 1,3-interactions which hinder the attack of the C_1-centre of the cyanoethylidene derivative In contrast, in a derivative of glucose, which exists[131] in a skew-boat conformation $^OS_2$ (**55**), there arise 1,3-interactions hindering the attack at the C_1-centre, thus slowing down the reaction and, hence, leading to the anomalous 1,2-*cis* glycosylation X-ray data may serve only as a guide because in

* It appears that the perchlorate can exist as a compound containing a covalent bond or a tight ion pair



Scheme 20



Scheme 21

solution the molecules may have a different conformation, so steric and electronic factors could be displayed in some other way

4 2 *Synthesis of polysaccharides*

The formation of the glycosidic linkage via the trityl-cyanoethylidene condensation has revealed a completely new pathway for the synthesis of the 1,2-*trans*-linked polysaccharide chains with a regular structure In fact, when both the *O*-trityl and the cyanoethylidene groups are in the same molecule then under glycosylation conditions the polycondensation takes place giving a polysaccharide chain When both these groups belong to a monosaccharide unit, the reaction gives a homopolysaccharide (Scheme 20) If these groups are present in an oligosaccharide then the polycondensation gives a chain consisting of repeating units, the structure of which corresponds to the oligosaccharide monomer (Scheme 21) It is quite obvious that the polymer formed upon polycondensation is actually a mixture of polymer-homologues The regioselectivity of polycondensation is determined by the position of the *O*-trityl group in the molecule of a mono- or oligosaccharide monomer The trityl-cyanoethylidene condensation proceeds most often with absolute stereospecificity to give a stereochemically regular polysaccharide containing only 1,2-*trans* glycosidic linkages However, it was pointed out above (see 4 1 3) that in some cases the stereochemistry of the resulting polysaccharide may be irregular

The most serious disadvantage of the method is a low degree of polymerization of the polysaccharide formed which is averaged for a mixture * Depending on the nature of the monomer it varies within wide range and sometimes is close to that typical of the natural polymers However, in some cases (e g in the polymerization of uronic acids' monomers and some simplest neutral monosaccharides) the degree of polymerization is very low and the reaction yields in fact oligosaccharides

Nevertheless, the method of synthesis of the 1,2-*trans*-linked polysaccharide chains based on the trityl-cyanoethylidene polycondensation provides wide possibilities It yields homo- and heteropolysaccharide structures containing both neutral sugars, amino sugars or uronic acids The monosaccharide units may be in the pyranose or furanose form, and can be linked by various types of intermonomeric linkages, excluding the 1,2-glycosidic linkage It should be emphasized that the method for the first time opens the possibility for the synthesis of regular polysaccharides to which type belong numerous natural polysaccharide possessing high biological specificity

* Naturally, the average degree of polymerization depends on a "width" of a fraction obtained upon fractionation Higher molecular weight product can be isolated at the expense of yield if a narrow fraction is cut off

4 2 1 *Synthesis of monomers* The OH groups in all the monomers used were properly protected generally as acetates The *O*-benzoyl protection appears to give polymers with a higher degree of polymerization (see, e g [132]), but removal of this protection presents some difficulties The acetal and *O*-benzyl protections have been examined in much lesser detail, and they have found practically no application The preparation of monomers required for the synthesis of homopolysaccharides is currently a routine procedure On the contrary, the synthesis of the oligosaccharide monomers used in the preparation of heteropolysaccharides with repeating units is still a problem and requires specific strategy in each particular case

4 2 2 *The polycondensation reaction* An elementary act of the polycondensation is the formation of a 1,2-*trans* glycosidic linkage as a result of an interaction between *O*-trityl and cyanoethylidene groups The conditions for polycondensation are practically the same as those described in the synthesis of disaccharides (Section 4 1 3) The polycondensation is usually carried out by treatment of a monomer with an initiator in dichloromethane Tritylium perchlorate was practically the only initiator used Other tritylium salts have been used so far very rarely, despite a higher stereoselectivity of polycondensation (Section 4 1 3) Although the amount of initiator has some influence on the reaction, it has been found that the best results are obtained with the use of 5–10 mol % of tritylium perchlorate An increase in the amount of initiator accelerates the polycondensation process, while at low concentration of the initiator the reaction is slow and this may lead to undesirable side processes Thus the polycondensation of 3,4-di-*O*-acetyl-1,2-*O*-cyanoethylidene-6-*O*-trityl-α-D-glucopyranose in the presence of 1 mol % of tritylium perchlorate has not reached completion even after 40 h in the presence of 20 mol % of the initiator it was completed in 14 h [133,134] On the other hand, a decrease in the initiator concentration does not produce the polymer with a higher molecular weight The degree of polymerization of (1-6)-β-D-galactan was not changed when either 1 mol % or 10 mol % of the initiator were used, although in the former case the reaction was not completed even after several days [135] The polycondensation is usually carried out at room temperature An increase in temperature up to 50°C has only insignificant effect on the result although the reaction is slightly accelerated A decrease in temperature down to 0°C and below highly decelerates the process and in this case the reaction is not completed even after several days

The complete exclusion of traces of moisture and other nucleophiles is very important for the success of polycondensation. The best results are therefore obtained when the reaction is carried out with the use of high-vacuum technique[91] (Section 4 1 3) A large-scale experiment may also be carried out in ordinary equipment with usual precautions This leads to some decrease in the yield and sometimes in the molecular weight of the polymer

The reaction time varied within a wide range Although at room temperature the reaction is initiated practically at once upon addition of catalyst, its rate strongly depends on the structure of the monomer used, and the optimum reaction time, naturally, differs with the monomer For example, the reaction of the arabinofuranose derivatives is completed in 15 h [136] In contrast, the polycondensation of the oligosaccharide monomers requires longer time, usually 50–70 h [102] Numerous attempts to increase the molecular weight of the polymer or its yield by increasing the reaction time (up to 250–300 h) were unsuccessful This may find a tentative explanation in that the reactive species are exhausted rather rapidly The reactive intermediate at the reducing end of the chain or the *O*-trityl groups at the non-reducing end could disappear because of some still unknown side reactions A precise control of the reaction termination presents some difficulty, although it can be roughly followed by disappearance of the monomer and *O*-trityl groups using TLC

4 2 3 *Isolation and characterization of polysaccharides* In order to decompose the remaining initiator and to stop the polycondensation process the reaction mixture is treated with methanol or trifluoroacetic acid, then pyridine is added for neutralization The protected polysaccharide is then isolated by column chromatography on silica gel It is then subjected to conventional deacylation by treatment with bases, and the free polysaccharide is isolated, purified, and fractionated by gel filtration The deacylation is easily followed by the disappearance of the band for the acyl group (1735–1750 cm^{-1}) in the IR spectrum of the isolated polysaccharide, or by their NMR spectra The polysaccharides containing the 1,3-glycosidic linkages are known to undergo partial decomposition under the action of bases ("peeling reaction"), therefore in this case the polysaccharide is treated first with sodium borohydride to reduce the terminal monosaccharide unit to the respective alditol and the product is then deacetylated

The structure of all the polysaccharides synthesized by this method has been checked carefully

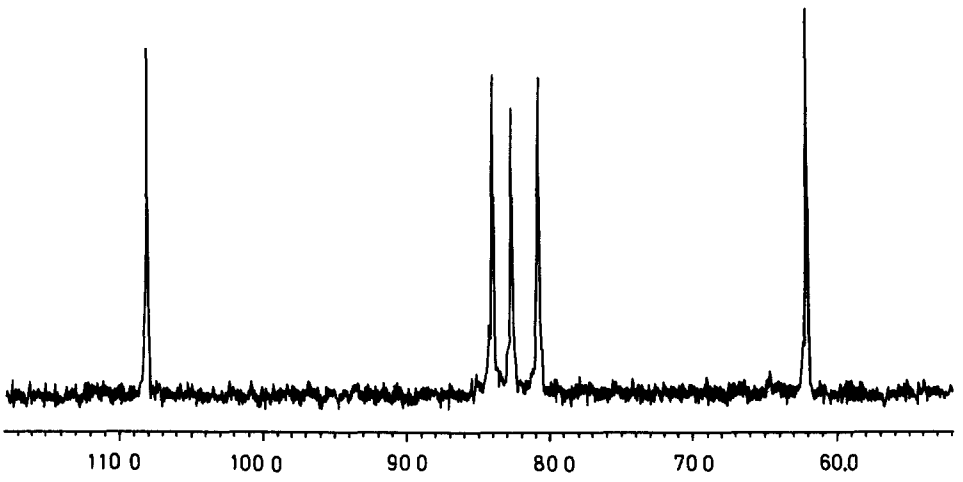


Fig 1 ^{13}C-NMR spectrum of (1-3)-α-L-arabinofuranan

by chemical and spectroscopic methods The regularity of their structure and therefore the regiospecificity of the polycondensation reaction have been checked by the classical methylation analysis,[137] including the analysis (GLC-MS) of acetates of partially methylated alditols In the cases of polysaccharides with a low molecular weight, this method estimated the degree of polymerization from the ratio of the alditols corresponding to the non-terminal units and those corresponding to the terminal monosaccharide unit In some cases the molecular weight of the polymer has been determined by estimating its reducing power [102] For some polysaccharides, especially those with the repeating units and having the molecular weight within the range 4000–6000, their molecular weight has been roughly estimated by gel filtration with reference to conventional standards

The configuration of the glycosidic linkages formed on polycondensation has been checked most carefully It presents evidence on the stereoregularity of the polymer and the stereospecificity of the polycondensation process Total stereoregularity of the polysaccharide obtained provides the most fundamental criterion for the absolute stereospecificity of the trityl-cyanoethylidene condensation (*cf* Section 4 1 3)

^{1}H- and ^{13}C-NMR spectroscopy is the most convenient and accurate method for the determination of glycosidic linkage stereochemistry the latter being more helpful * Frequently, even the number of signals in the anomeric region of the high-resolution ^{13}C-NMR spectrum indicated quite decisively the stereospecificity of the polysaccharide obtained because the signals for 1,2-*trans*- and 1,2-*cis*-glycosidic linkages are usually resolved However, since the chemical shifts for the anomeric carbons in the ^{13}C-NMR spectrum, as is known,[79] are affected strongly by the structure of the neighbouring units, the complete interpretation of the ^{13}C-NMR spectrum of the synthetic polymer was accomplished in many cases by using the data on the corresponding model disaccharides for the correct assignment of the signals for C atoms † For the synthetic homopolysaccharides, which, as a rule, have a relatively simple spectrum, it has been usually possible to assign the signals of all the ring carbons [see, for example, the ^{13}C-NMR spectrum of (1-3)-α-L-arabinofuranan,[117] Fig 1] Furthermore, a simple and distinct picture of the ^{13}C-NMR spectrum and the absence of unidentified signals are very strong evidence in favour of the regularity of the polysaccharide chain When the stereospecificity was violated and the polycondensation yielded both 1,2-*trans* and 1,2-*cis* glycosidic linkage, the ^{13}C-NMR spectrum accurately determined the ratio of these two types of linkages from the integrated intensity of corresponding signals

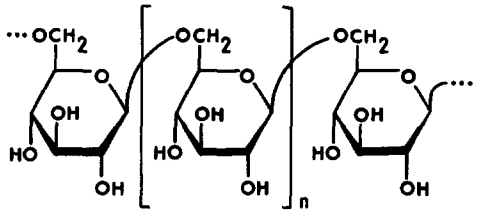
The measurement of the optical rotation of the synthetic polymer often proved helpful, although it must be emphasized that reliable data on the natural polysaccharide of the corresponding structure to serve as a reference are scarce

* The ^{13}C-NMR spectroscopy was not used in the examination of the first syntheses of polysaccharides by this method Its application for the investigation of polysaccharide structure was developed later

† Usually, the synthesis of such model disaccharide was also very helpful in determining the optimum conditions for the polycondensation of the corresponding monomers in the polysaccharide synthesis

Finally, in some cases quite exact information on the configuration of the glycosidic linkage may be determined in a purely chemical way Selective degradation of the polysaccharide, followed by the isolation and analysis of the unit identifies the newly formed glycosidic linkage [102]

4 2 4 *Synthesis of homopolysaccharides* Of the polysaccharides of this class there were synthesized (1-6)-, (1-4)-, and (1-3)-glycopyranans, containing both the neutral monosaccharides and uronic acids, as well as the first representatives of glycofuranans Most of the polysaccharides obtained possessed a low degree of polycondensation and sometimes they were rather oligosaccharides The results are given in Table 1



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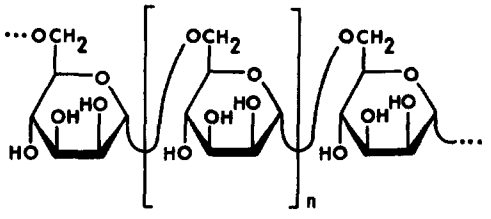

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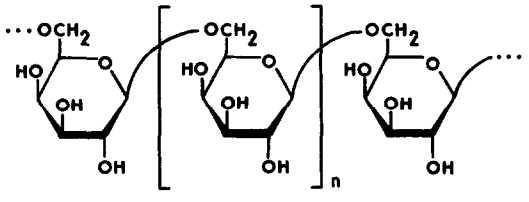

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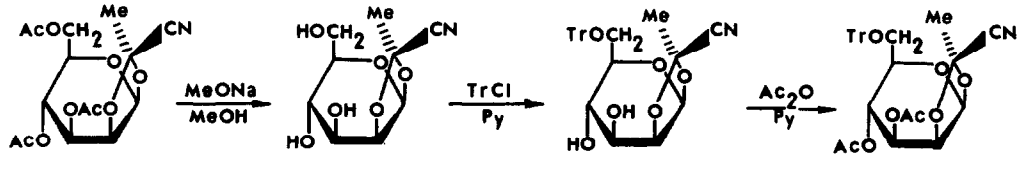
4 2 4 1 (1-6)-*Glycopyranans* The monomers for the synthesis of (1-6)-β-D-glucan **56**,[133,134] (1-6)-α-D-mannan **57**,[138] and (1-6)-β-D-galactan **58**[135] were obtained by standard procedures based upon initial formation of the cyanoethylidene group in the monosaccharide molecule followed by the selective introduction of a primary *O*-trityl group This can be exemplified by the synthesis of 3,4-di-*O*-acetyl-1,2-*O*-cyanoethylidene-6-*O*-trityl-β-D-mannopyranose **59**,[138] which is a monomer for the synthesis of (1-6)-α-D-mannan (Scheme 22) 2,3,4,6-Tetra-*O*-acetyl-D-mannosyl bromide was converted by the general method[97] into 3,4,6-tri-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-D-mannose **60**, which was then deacetylated with dilute methanolic sodium methoxide The unprotected cyanoethylidene derivative **61** was then treated with trityl chloride in pyridine, and acetylated with acetic anhydride in pyridine 3,4-Di-*O*-acetyl-1,2-*O*-(1-cyanoethylidene)-6-*O*-trityl-α-D-glucose **62**,[133,134] and 3,4-di-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-6-*O*-trityl-α-D-galactose **63**[135] were obtained in a similar way In order to obtain the galactose analogue with *O*-benzoyl protection, the isolated unprotected trityl derivative was treated with PhCOCl in pyridine Deacetylation of the cyanoethylidene derivative **62** under more severe conditions caused a noticeable conversion of the cyano group into the imidate (*cf* Section 4 1 1), which then required additional purification of the monomer

The monomers obtained may be used in the polycondensation directly as a mixture of *endo*- and *exo*-derivatives (*cf* 4 1 1), because the reactivity of both isomers is practically identical However, the use of the individual isomers sometimes facilitated spectroscopic control in the synthesis of the



60 **61** **59**

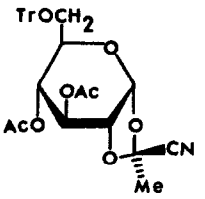
Scheme 22

Table 1 Synthesis of homopolysaccharides*

Polysaccharide	Monomer	Amount of initiator (mol %)	Reaction time (h)	Yield (%)	Degree of polymerization	Contents of 1,2-*cis* glycosidic linkages	Ref	Notes
(1-6)-β-D-glucopyranan **56**	**62**	20	15	13 5	16	0	134	
(1-6)-α-D-mannopyranan **57**	**59**	10	70	40	10	0	138	
(1-6)-β-D-galactopyranan **58**	**63**	10	100	51	6	0	135	
(1-6)-β-D-galactopyranan **58**	**63**	10		90	11	0		Synthesis at 14 kbar pressure
(1-4)-α-L-arabinopyranan **71**	**79**	10	110	54	11	25	100	
(1-3)-α-L-arabinopyranan **70**	**78**	10	110	43	7	25	100	
(1-4)-β-D-xylopyranan **73**	**101**	11	17	70	17	18	144	
(1-3)-β-D-xylopyranan **72**	**100**	10	17	60	11	11	144	
(1-3)-β-D-glucopyranan **64**	**116**	10	110	48	14	50	140	
(1-3)-β-D-galactopyranan **65**	**88**	10	110	50	8	30	124	
(1-3)-β-D-galactopyranan **65**	**88**	10		55	22	0		Synthesis at 14 kbar pressure
(1-4)-α-D-mannopyranan **66**	**104**	10	110	72	15	0	141	
(1-4)-α-L-rhamnopyranan **68**	**107** (R_1 = Ac)	10	70	71	22	0	133	
(1-4)-α-L-rhamnopyranan **68**	**107** (R_1 = Bz)	10	70	82	40	0	133	
(1-3)-α-L-rhamnopyranan **67**	**83**	10	60	59	30	0	142	
(1-3)-β-D-(6-deoxy)-glucopyranan **69**	**92**	10	100	63	15	59		
(1-3)-β-D-(6-deoxy)-glucopyranan **69**	**92**	10	20	77	25	0		Synthesis at 14 kbar pressure
(1-3)-β-D-glucuronopyranan (methyl ester) **74**	**96**	10	17	55	7	0	145	Protection groups were not removed
(1-4)-β-D-glucuronopyranan (methyl ester) **75**	**110**	10	17	70	6	0		
(1-5)-α-L-arabinofuranan	**120**	6	15		20	0		
(1-3)-α-L-arabinofuranan	**122**	10	16	93	45	0		

* All syntheses were carried out in CH_2Cl_2 at room temperature

monomers In this case, the isomers can be separated by crystallization or chromatography The structure of the synthesized monomers can be confirmed unambiguously by the NMR spectroscopy (see 4 1 1)



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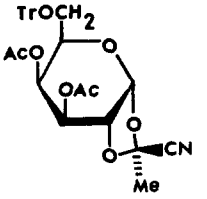

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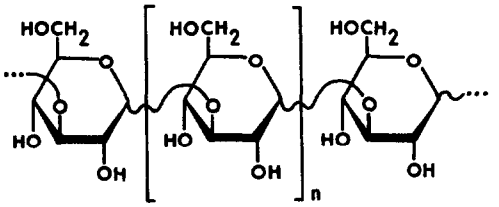

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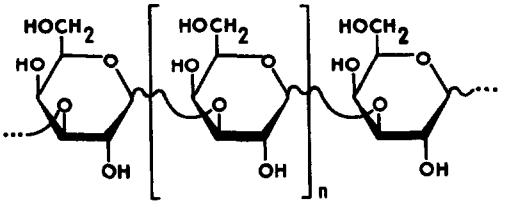

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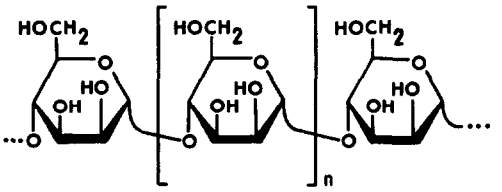

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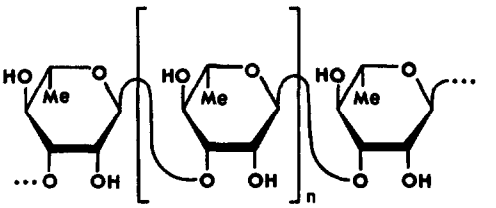

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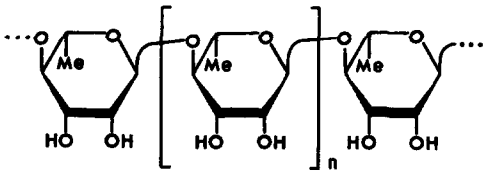

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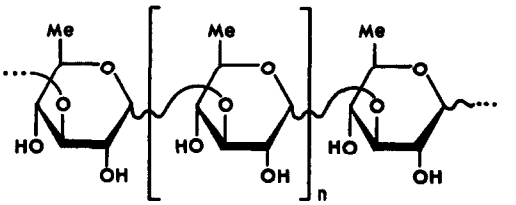

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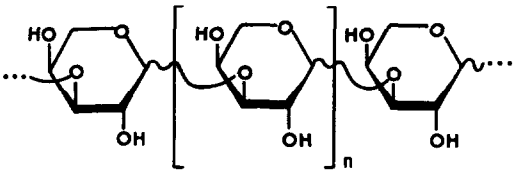

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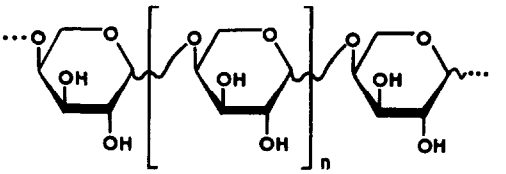

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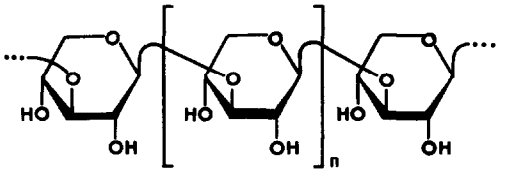

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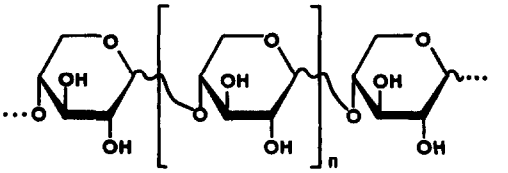


73

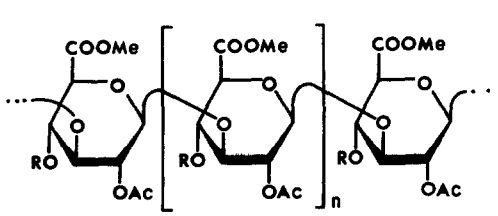
The polycondensation of the monomers was carried out under conventional conditions (10 mol % of tritylium perchlorate, room temperature, 40–70 h) yielding (1-6)-glycans which possessed structural and stereochemical regularity, and contained only the 1,2-*trans* glycosidic linkages They had, however, a low degree of polycondensation particularly in the case of mannan and galactan The attempts to increase the degree of polymerization of the galactan by decreasing the initiator quantity (to 1%) were unsuccessful [135] However, recent experiments showed that the polycondensation under high pressure offers a promising way for the increase of both the degree of polymerization and the yield of polysaccharides Thus, the polymerization carried out under standard conditions at a pressure of 14 kbar gave a totally stereospecific (1-6)-β-D-galactan with a degree of polymerization of 10–12 and 90% yield [139]

4 2 4 2 1,3- *and* 1,4-*Glycopyranans* The synthesis has been performed for glycopyranans containing the residues of hexoses [(1-3)-D-glucopyranan **64**,[140] (1-3)-D-galactopyranan **65**,[124] (1-4)-α-D-mannopyranan **66**[141]], 6-deoxy hexoses [(1-3)-α-L-rhamnopyranan **67**,[142] (1-4)-α-L-rhamnopyranan **68**,[133] (1-3)-6-deoxy-D-glucopyranan **69**[139,143]], pentose [(1-3)- and (1-4)-L-arabinopyranans **70** and **71**,[100] (1-3)- and (1-4)-D-xylopyranans **72** and **73**[144]], and uronic acids (methyl esters of (1-3)- and (1-4)-β-D-glucuronopyranans **74** and **75** [145]

The synthesis of the monomers was accomplished via the standard introduction of the cyanoethylidene group in the monosaccharide molecule by the treatment of acylglycosyl halide with KCN or NaCN, or in the case of uronates, by AgCN[103] (see 4 1 1) Peracetates of the cyanoethylidene derivative were then deacetylated with methanolic sodium methoxide The introduction of an *O*-trityl group at one of the secondary OHs was performed by the reaction of tritylium perchlorate in the presence of a sterically hindered pyridine base (see 4 1 2) As tritylation is not selective there were two ways to achieve the desired result (i) a direct tritylation of the cyanoethylidene derivative containing several OHs, followed by the subsequent separation of the resulting mixture of mono-*O*-trityl derivatives, one of which is usually produced preferentially or (ii) a preliminary protection of OHs of a polyhydroxyl derivative, followed by tritylation of a free OH The first route is demonstrated by the synthesis of the monomers used for the synthesis of (1-3)- and (1-4)-arabinopyranans [100] 3,4-Di-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-L-arabinopyranose **76**, prepared by the standard procedure, was deacetylated with 0 01 M methanolic sodium methoxide The diol **77** was then tritylated with an equimolar amount of tritylium perchlorate, giving the resulting mixture of 3-*O*-(**78**) and 4-*O*-trityl derivatives (**79**), formed in a 3 1 ratio This mixture was acetylated and fractionated by crystallization (Scheme 23)

In a similar way, the sequence **80** → **81** → **82** → **83** has led to 4-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano) ethylidene]-3-*O*-trityl-β-L-rhamnopyranose **83**, the monomer for the synthesis of (1-3)-α-L-rhamnan,[142] the sequence **84** → **85** → **86** → **87** → **88** gave 4-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-3-*O*-trityl-6-*O*-benzoyl-α-D-galactopyranose **88**, the monomer for the synthesis of (1-3)-D-galactan,[124] the sequence **89** → **90** → **91** → **92** gave 4-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-6-deoxy-3-*O*-trityl-α-D-glucopyranose **92**, the monomer for the synthesis of (1-3)-6-deoxy-D-glucan,[139] the sequence **93** → **94** → **95** → **96** gave the monomers **96** for the synthesis of (1-3)-polyuronates [146]

The second route is exemplified by the synthesis of monomers for (1-3)- and (1-4)-D-xylopyranans [144] 1,2-*O*-Cyanoethylidene-α-D-xylopyranose **97** (a mixture of *exo*- and *endo*-isomers), obtained by the deacetylation of the corresponding acetate, was subjected to benzoylation with 1 mol of PhCOCl or PhCOCN in pyridine The resulting 3-*O*- and 4-*O*-monobenzoyl derivatives **98** and **99** were then separated by chromatography and tritylated giving the corresponding monomers **100** and **101**



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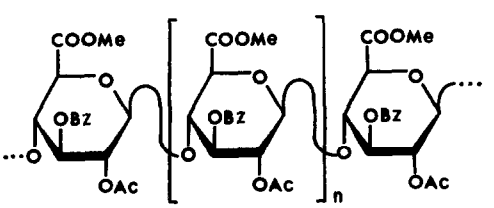

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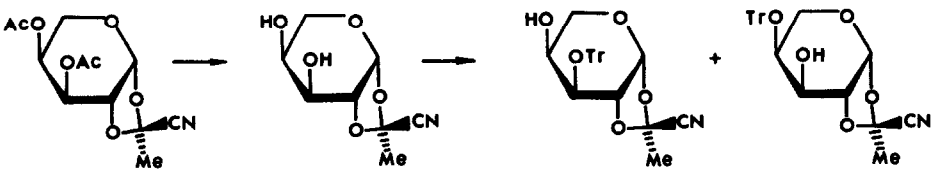
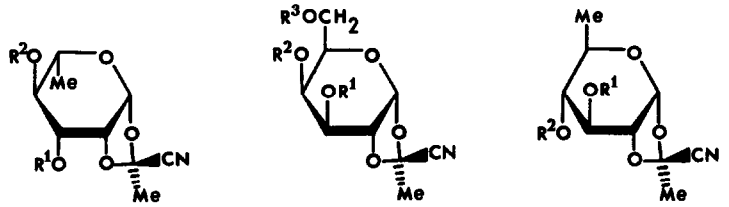


76 **77** **78** **79**

Scheme 23

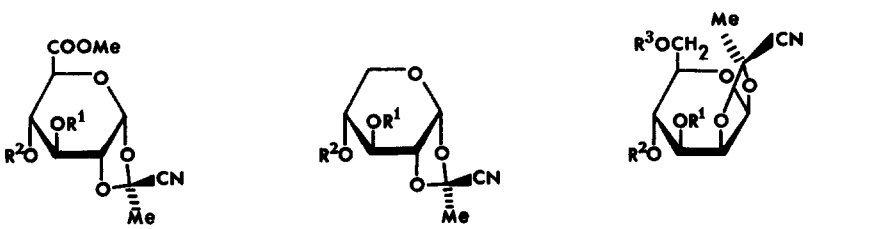


80 $R^1 = R^2 = Ac$
81 $R^1 = R^2 = H$
82 $R^1 = Tr$, $R^2 = H$
83 $R^1 = Tr$, $R^2 = Ac$

84 $R^1 = R^2 = R^3 = Ac$
85 $R^1 = R^2 = R^3 = H$
86 $R^1 = R^2 = H$, $R^3 = Bz$
87 $R^1 = Tr$, $R^2 = H$, $R^3 = Bz$
88 $R^1 = Tr$, $R^2 = Ac$, $R^3 = Bz$

89 $R^1 = R^2 = Ac$
90 $R^1 = R^2 = H$
91 $R^1 = Tr$, $R^2 = H$
92 $R^1 = Tr$, $R^2 = Ac$

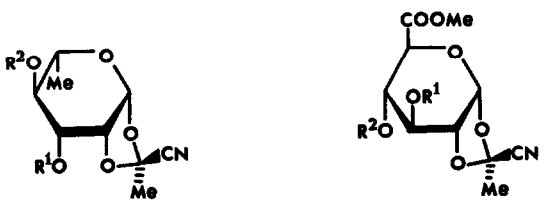
In a similar way, the sequence **102** → **103** → **104** led to 3,6-di-*O*-benzoyl-1,2-*O*-[1-(*exo*-cyano) ethylidene]-4-*O*-trityl-β-D-mannopyranose **104**, the monomer for the synthesis of (1-4)-α-D-mannan,[141] the sequence **105** → **106** → **107** gave 3-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-4-*O*-trityl-β-L-rhamnopyranose (and its 3-*O*-benzoyl analogue), the monomer for the synthesis of (1-4)-α-L-rhamnan,[133] the sequence **108** → **109** → **110** gave the monomer **110** for the synthesis of (1-4)-polyuronate [146]



93 $R^1 = R^2 = Ac$
94 $R^1 = R^2 = H$
95 $R^1 = Tr$, $R^2 = H$
96 $R^1 = Tr$, $R^2 = Ac(Bz)$

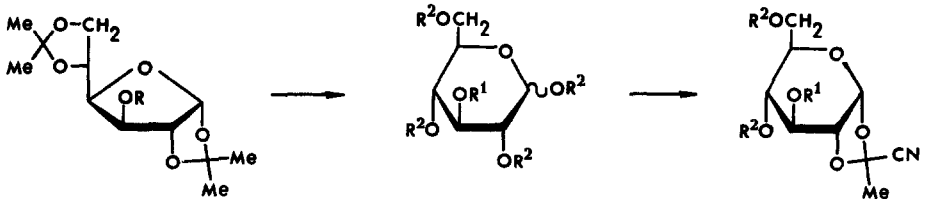
97 $R^1 = R^2 = H$
98 $R^1 = Bz$, $R^2 = H$
99 $R^1 = H$, $R^2 = Bz$
100 $R^1 = Tr$, $R^2 = Bz$
101 $R^1 = Bz$, $R^2 = Tr$

102 $R^1 = R^2 = R^3 = H$
103 $R^1 = R^3 = Bz$, $R^2 = H$
104 $R^1 = R^3 = Bz$, $R^2 = Tr$



105 $R^1 = R^2 = H$
106 $R^1 = Ac(Bz)$, $R^2 = H$
107 $R^1 = Ac(Bz)$, $R^2 = Tr$

108 $R^1 = R^2 = H$
109 $R^1 = Bz$, $R^2 = H$
110 $R^1 = Bz$, $R^2 = Tr$



111 R = $PhOCH_2CO$

112 R^1 = $PhOCH_2CO$, R^2 = Ac

113 R^1 = $PhOCH_2CO$, R^2 = Ac
114 R^1 = H, R^2 = Ac
115 R^1 = Tr, R^2 = Ac

Scheme 24

In some cases the synthesis of the monomers required a temporary protection of the OH to be tritylated, which later was selectively removed directly prior to tritylation This scheme is exemplified by the synthesis of the monomer for (1-3)-D-glucan[140] (Scheme 24) 1,2 5,6-Di-*O*-isopropylidene-α-D-glucofuranose was acylated with phenoxyacetic acid in pyridine in the presence of *N,N'*-dicyclohexylcarbodiimide to give **111**, after removal of isopropylidene groups and acetylation, **112** was converted into the acetylated cyanoethylidene derivative **113**, the phenoxyacetyl group was removed selectively by treatment with methanolic NH_3, and the monohydroxyl derivative **114** was tritylated with tritylium perchlorate giving the monomer **115** This approach is much more laborious and is used primarily for the synthesis of oligosaccharide monomers (see 4 2 5 2)

All the monomers thus obtained are crystalline substances and their structure has been firmly established by NMR spectroscopy and in some cases by chemical methods

The polycondensation of monomers was carried out under the standard conditions (10 mol % of the initiator, room temperature) The reactivity of the monomers obviously varies but the reaction is usually completed in 50–70 h The synthesis of rhamnans showed[133 142] that neither the degree of polymerization nor the yield of the polysaccharide is changed by increasing the reaction time up to 120–150 h The reaction was terminated (see 4 2 3), the protected polysaccharide was deacetylated and isolated by the usual methods, usually by chromatography The results are presented in Table 1 All the polysaccharides obtained possessed a regular structure, confirmed by standard methylation analysis,[137] which also showed that the degree of polymerization of these polysaccharides varied significantly

The synthetic (1-4)- and (1-3)-L-rhamnans were shown to possess a degree of polymerization of 30–40 and molecular weights of 4000–6000 It is noteworthy that the polycondensation of the respective monomer with the *O*-benzoyl protection gave (1-4)-α-L-rhamnan with a higher degree of polymerization than the corresponding *O*-acetate [132] The synthesis of (1-3)-α-L-rhamnan is accompanied by a precipitate, which is characteristic of the polysaccharides with a highly regular spatial structure The polycondensation of the derivatives of uronic acids yields the polymers with a very low degree of polymerization (about 4–7), so the reaction in this case leads to homo-oligosaccharides This is evidently a result of a rapid detritylation of the monomer due to a side reaction of unknown character [145] It is worth noting that in this case again the *O*-benzoylated monomer leads to a higher degree of polymerization than does the *O*-acetate

The (1-3)- and (1-4)-glycopyranans, containing the residues of mannose, glucose, galactose, arabinose, and xylose, possessed a degree of polymerization of 8–17 the (1-4)-linked polysaccharides had a somewhat higher molecular weight It can be expected that the polycondensation, carried out under high pressure, will afford the products with higher molecular weights [139]

Unlike the total structural regularity of the synthesized (1-3)- and (1-4)-glycans, their stereochemical regularity varied with the monomer used The polysaccharides containing the mannose and rhamnose residues were completely stereoregular and contained only 1,2-*trans* glycosidic linkages This has been confirmed unambiguously by the in ^{13}C-NMR spectra, which contained a single signal in the anomeric region The NMR spectra of these glycans were very simple and distinct and contained only one signal for each of the ring carbons The products of polymerization of the uronic acid derivatives, although with gluco-configuration, also were stereoregular and contained only 1,2-*trans* glycosidic linkages

In contrast, the (1-3)- and (1-4)- glycopyranans containing glucose, galactose, arabinose, and xylose residues were stereochemically irregular and contained both 1,2-*trans* and 1,2-*cis* glycosidic

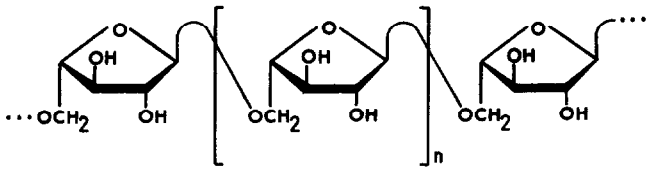
linkages Their ^{13}C-NMR spectra contained two signals for the anomeric carbons, whose chemical shifts corresponded to the 1,2-*trans* and 1,2-*cis* glycosidic linkages These linkages were irregular distributed along the polysaccharide chain This is clearly demonstrated by their complex ^{13}C-NMR spectra, which contained in the region of ring carbons a large number of signals of varying intensity, which could not be assigned and which originate from a stereochemically different neighbouring units in the polymeric chain The content of the anomalous 1,2-*cis* glycosidic linkage in the synthetic (1-3)- and (1-4)-glycopyranans varied very considerably (Table 1) and ranged from 5 to 50–60% The reasons for these violations of stereochemistry during the trityl-cyanoethylidene polycondensation which have been found so far for 1,3- and 1,4-linkages in the arabino-, xylo, gluco-, and galactopyranose systems, remain unclear These violations are consistent with those found in the synthesis of the corresponding disaccharides (Section 4 1 3), and seem to relate to the violation of stereochemistry of an elementary act of the process (*cf* 4 1 4) It is evident that a decisive role is played by the position of the *O*-trityl group, which displays probably lower reactivity in positions 3 and 4 of pyranoses with the xylo- and arabino configurations At the same time, when the reactivity of the cyanoethylidene group, or, more exactly, of the bicyclic dioxolenium intermediate derived from it, is high, then the reaction proceeds with high stereospecificity Thus in the synthesis of the natural heteropolysaccharide *Shigella flexneri* (Section 4 2 5 2) the polycondensation of a complex oligosaccharide monomer, having an *O*-trityl group at C-3 of glucosamine and a highly reactive cyanoethylidene group at the rhamnose residue, was completely stereospecific although the sugar carrying the *O*-trityl group had the gluco configuration

Thus, the reason for stereochemical violations in polycondensation seems to be linked with the influence of the spatial structure of the monomer on the reactivity of both of its functional groups The investigations of the conformation of monomers and intermediates in solution as well as of their influence on reactivity would be very helpful for the elucidation of this phenomenon

Some attempts have been undertaken to improve the stereochemistry of polycondensation As it was already pointed out[125] (*cf* 4 1 3), the content of the anomalous 1,2-*cis* glycosidic linkages was sharply reduced by replacing tritylium perchlorate by tritylium tetrafluoroborate Thus, the polycondensation of the cyanoethylidene derivative of xylopyranose **100** in the presence of tritylium tetrafluoroborate was totally stereospecific, and the (1-3)-xylan obtained contained only 1,2-*trans* glycosidic linkages [144] However, the degree of polymerization obtained in the latter case was only 4–5, so this initiator cannot be used in the preparative synthesis of polysaccharides

A more promising result has been obtained in the polycondensation under high pressure (Section 4 1 3) In this connection, one of the most unfavourable examples has been studied, i e the synthesis of (1-3)-6-deoxy-D-glucopyranan, the polycondensation under usual conditions gave 1,2-*trans* and 1,2-*cis* glycosidic linkages in the 1 1 ratio This process when carried out under high pressure (14 kbar) was absolutely stereospecific and gave a regular (1-3)-6-deoxy-β-D-glucopyranan, containing only the 1,2-*trans* glycosidic linkages and the degree of polymerization (of 25) being twice as that achieved without pressure [139,143] The structural and stereochemical regularity of the polymer obtained has been demonstrated most clearly by its ^{13}C-NMR spectrum, very distinct and different from that of the polysaccharide obtained under normal pressure (Fig 2) Similarly, the polycondensation of the monomer **88** under usual conditions gave (1-3)-D-galactan in 25% yield, which contained, according to the ^{13}C-NMR data, about 30% of 1,2-*cis* (α-galactosidic) linkages The polycondensation under a pressure of 14 kbar stereospecifically afforded (1-3)-β-D-galactan containing no 1,2-*cis* linkages (55% yield) These examples show that the stereochemical limitations of the trityl-cyanoethylidene polycondensation can be overcome thus providing real opportunities for the synthesis of polysaccharides by this method

4 2 4 3 *Glycofuranans* So far only two examples of syntheses of polysaccharides containing furanose units are known, the recently accomplished syntheses of (1-5)- (**116**)[136] and (1-3)-α-L-arabinofuranans (**117**) [147] The starting 3,5-di-*O*-acetyl-1,2-*O*-[1-(*endo*-cyano)ethylidene]-β-L-arabinofuranose **118**, which was obtained by treatment of 1,2,3,5-tetra-*O*-acetyl-L-arabinofuranose with trimethylsilyl cyanide,[106] was deacetylated, the resulting diol on treatment with trityl chloride in pyridine was converted regioselectively into the 5-trityl ether **119**, which was then benzoylated giving the monomer **120** for the synthesis of (1-5)-α-L-arabinofuranan Treatment of **118** with a dilute methanolic sodium methoxide leads to selective deacetylation from position 3 Tritylation of the monoacetate **121** with tritylium perchlorate gives the monomer **122** for the synthesis of (1-3)-α-L-arabinofuranan



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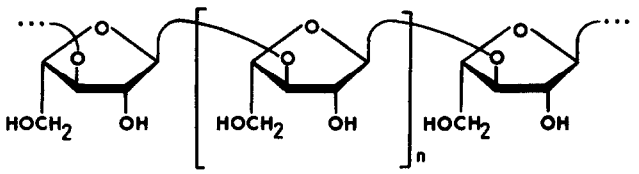


117

The polycondensation of the monomers **120** and **122** was carried out in the presence of 6% or 10% trityhum perchlorate, respectively After the usual work-up the acylated polysaccharides were isolated by chromatography Their deacylation then gave the unprotected arabinofuranans in very high yields

The ^{13}C-NMR data for both the acylated and the unprotected synthetic arabinofuranans showed their structural and stereochemical regularity, as well as the absence of 1,2-*cis* glycosidic linkages (Fig 1) Their simple and distinct spectra contained a single signal in the region of anomeric carbons and four signals for the ring carbons were easily assigned using the data for model oligosaccharides

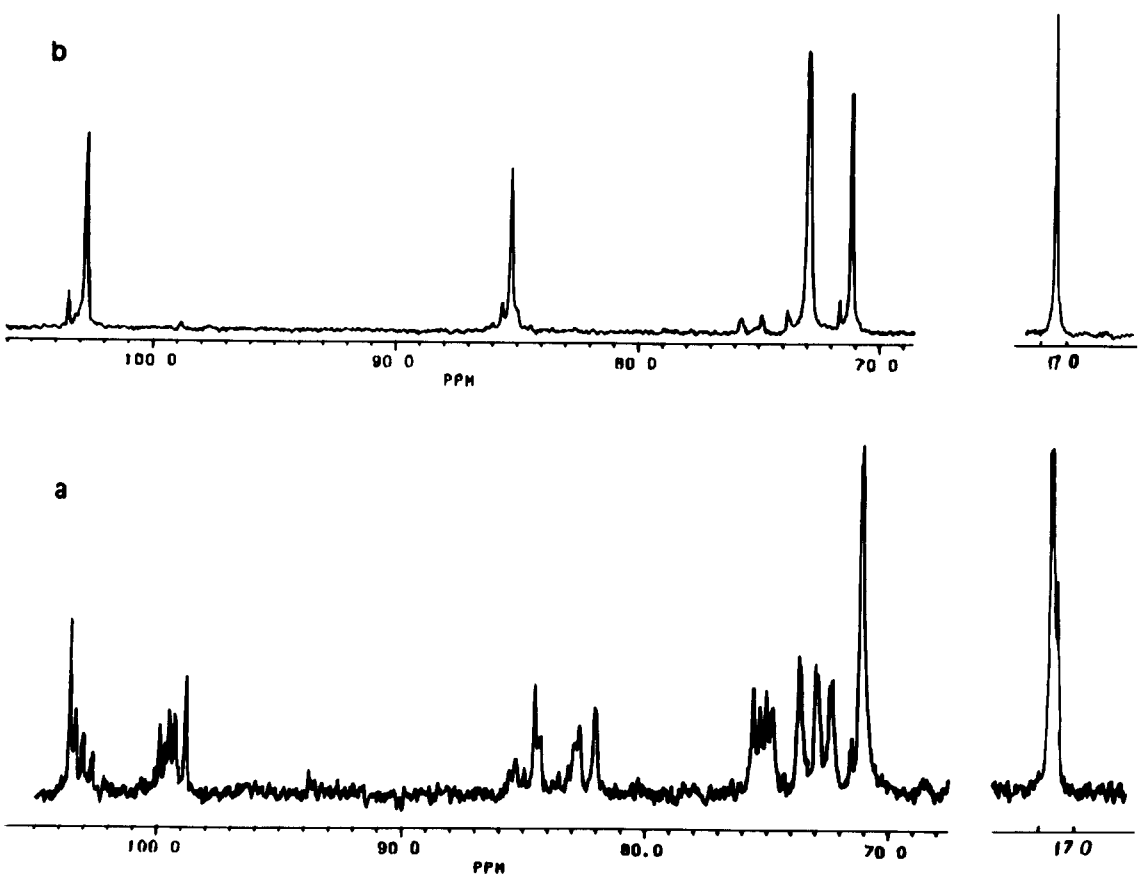
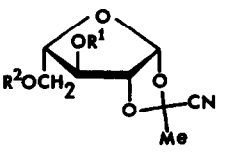
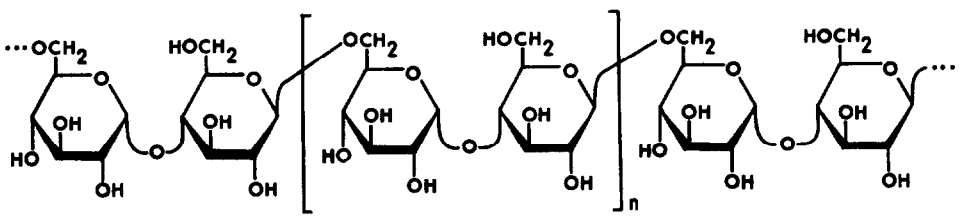


Fig 2 ^{13}C-NMR spectra of (1-3)-6-deoxy-D-glucan obtained at (a) normal pressure, (b) 14 kbar



118 $R^1 = R^2 = Ac$
119 $R^1 = H$ $R^2 = Tr$
120 $R^1 = Bz$ $R^2 = Tr$
121 $R^1 = H$ $R^2 = Ac$
122 $R^1 = Tr$ $R^2 = Ac$



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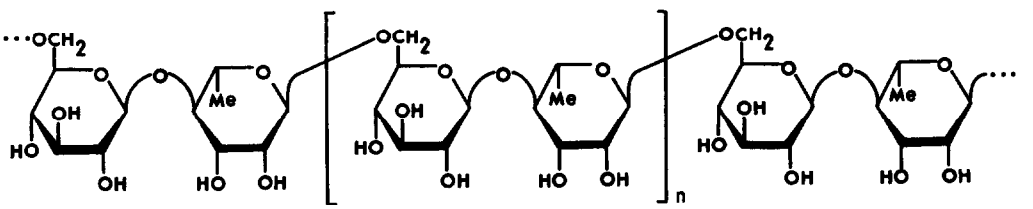

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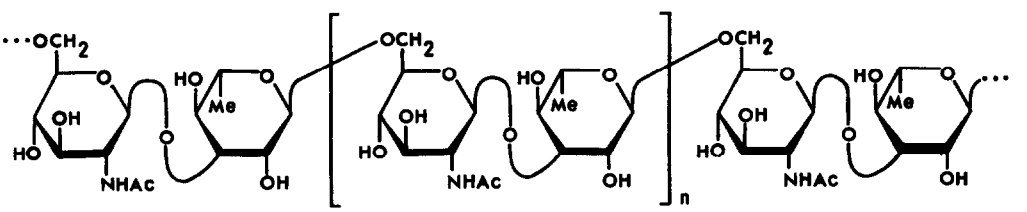

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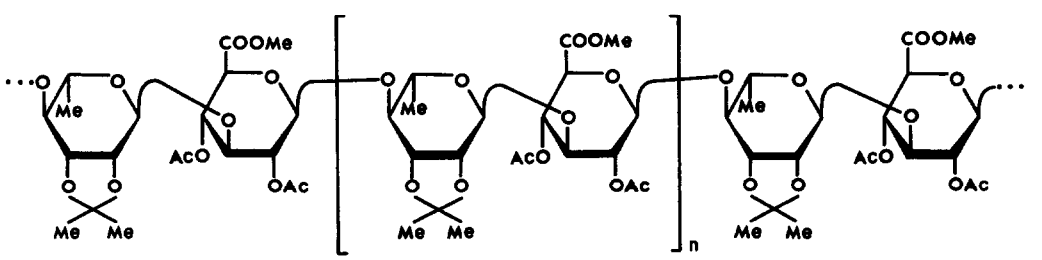


126

High negative value of the optical rotation of the both synthetic arabinofuranans was also consistent with a strictly regular structure The degree of polymerization was 15–23 for **116** (molecular weight of 2000–3000) and 42–45 for **117** (molecular weight of about 6000)

It is noteworthy that unlike the monomers having the arabinopyranose structure (see 4.2 4 2),

the polymerization of furanose monomers proceeds with absolute stereospecificity These results open the way for stereospecific synthesis of other glycofuranans

4 2 5 *Synthesis of heteropolysaccharides* The trityl-cyanoethylidene condensation is of particular interest for the synthesis of complex heteropolysaccharides built of repeating oligosaccharide units, including the natural heteropolysaccharides possessing high biological specificity and activity This approach to the synthesis of such complex polysaccharides is so far the only real possibility, since a stepwise attachment of complex oligosaccharide units is so laborious[148,149] that the preparation of a polymer with a proper degree of polymerization by this method is hardly practical

Several regular polysaccharides built up of disaccharide units have already been synthesized by polycondensation of the oligosaccharide monomers The first syntheses of natural polysaccharides of microbial origin have been accomplished

The synthesis of the necessary monomer whose structure corresponds to that of the oligosaccharide repeating unit of the heteropolysaccharide and which contains a cyanoethylidene group at the reducing terminus and an *O*-trityl group at the OH, which is to form a glycosidic linkage in the future polysaccharide, was often a complex problem The main difficulty was the selective tritylation of polyhydroxyl cyanoethylidene derivatives In relatively simple cases it was possible to perform direct tritylation followed by the separation of the mixture of trityl derivatives However, this became unreasonable in more complex cases Thus, for the synthesis of complex monomers another route has been used involving temporary selective protection of the OH to be tritylated, protection of the other OHs with a "permanent" group, removal of the former protective group, and tritylation (*cf* 4 2 4 2) The most important point in the realization of this strategy was the differentiation of the two simplest and most useful protective groups in carbohydrate chemistry, namely, *O*-acetyl- and *O*-benzoyl groups This was achieved by selective removal of the former in the presence of the latter by mild acid methanolysis [150] Next, it has been established that the OH-containing cyanoethylidene derivatives of mono- and oligosaccharides can be glycosylated without affecting the cyanoethylidene group [110] This was the second important point for the development of a general strategy for the synthesis of complex monomers Thus, the oligosaccharide monomers can be assembled from the two fragments, one of which already has the cyanoethylidene group, while the other contains a potential site for the introduction of the trityl group The assembled oligosaccharide derivative is then subjected to tritylation This strategy will be exemplified in the discussion of concrete syntheses

4 2 5 1 *Heteropolysaccharides consisting of disaccharide units* Polysaccharides have been synthesized containing neutral sugars, namely, glucan **123** and glucorhamnan **124**, as well as polysaccharides containing amino sugars and uronic acids, namely, hexosaminoglucan **125** and heteropolyuronide **126**

Glucan with the alternating (1-6)-*β- and* (1-4)-*α-glycosidic linkages* [151] The synthesis of this polysaccharide* built up of repeating units, was the first example of the trityl-cyanoethylidene polycondensation of the oligosaccharide blocks with *O*-trityl and cyanoethylidene groups in different monosaccharide units

The monomer was obtained[95] from the 4-*O*-(α-D-glucopyranosyl)-D-glucose (maltose) octaacetate, which was converted via its bromide into the acetate of the cyanoethylidene derivative **127** The latter was then deacetylated giving the unprotected cyanoethylidene derivative **128**, which was then treated with 1 mole of trityl chloride in pyridine giving a mixture of the trityl derivatives After acetylation the required monomer 3,6,2′,3′,4′-penta-*O*-acetyl-1,2-*O*-cyanoethylidene-6′-*O*-trityl-4-*O*-α-D-glucopyranosyl-α-D-glucopyranose **129**, was isolated by chromatography Its structure was supported by chemical and spectral methods Fortunately, of the two primary OHs present the tritylation affected predominantly that in the unsubstituted monosaccharide unit This is obviously related to the conformational features of the maltose molecule

The polycondensation of **129** was carried out in the presence of 10 mol % of the initiator at room temperature The optimum reaction time (50 h) was determined by preliminary experiments The protected polymer was deacetylated without purification, and the unsubstituted polysaccharide was isolated by gel chromatography Its structural regularity was confirmed by methylation analysis, which also showed that the degree of polycondensation of the polymer amounted to 10, referring

* According to the strict nomenclature this polysaccharide, containing only the glucose units, is not a heteropolysaccharide, however, as it has a block structure it is more reasonable to discuss it in this section

to the disaccharide monomer Its molecular weight was of about 3200 The stereochemical regularity of the polymer was established by comparing the values of its specific rotation (+115°) with those for the higher oligomers of maltose and gentiobiose This confirmed the 1,2-*trans* configuration for the newly formed (1-6)-glycosidic linkages in the polymer

Glucorhamnan **124** [152] In order to obtain the monomer, the *O*-(β-D-glucopyranosyl)-(1-4)-1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-L-rhamnopyranose peracetate **130** was deacetylated, the only primary OH of the resulting compound was selectively tritylated with trityl chloride in pyridine and then acetylation gave the monomer **131**

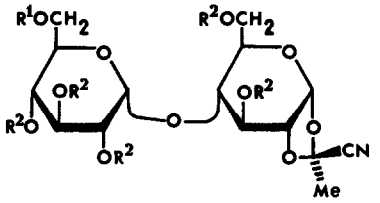
The polycondensation of **131** under the standard conditions (10 mol % of the initiator, 50 h, room temperature), followed by deacetylation of the resulting polymer and purification by chromatography gave the polysaccharide, which was fractionated into two fractions, both of them proved to be structurally identical and differed only in the degree of polymerization Methylation analysis showed the total structural regularity of both fractions and the degrees of polymerization (referring to the disaccharide units) of 30 and 23, respectively, which correspond to the molecular weights of about 9000 and 7000 The stereoregularity of the polysaccharide was unambiguously confirmed by its ^{13}C-NMR spectrum, which contained in the anomeric region along with the signal for the β-glucosidic linkage (104 7 ppm) the only signal at 101 45 ppm, corresponding to the α-rhamnosidic linkage The total stereochemical unambiguity of the polymer was confirmed also by the other spectral features, in particular, a very distinct picture of the spectrum, which enabled the assignment of all the signals

Hexosaminoglycan **125** The synthesis of this heteropolysaccharide was of particular interest, as it contained an amino sugar residue It is well known that many natural polysaccharides are hexosaminoglycans so this synthesis is an essential extension of the trityl-cyanoethylidene polycondensation method The choice of a reasonable protection for the amino group was of considerable importance Almost all natural hexosaminoglycans are the *N*-acetyl derivatives However, preliminary model experiments, including polycondensation, have shown that the glycosylation of *N*-acetylamino sugar derivatives with the cyanoethylidene derivatives proceeds at a very low rate,[153] evidently, because of the complexation between the initiator (tritylium cation) and the *N*-acetyl group (*cf* 3 1 3) For that reason the synthesis of glucosaminorhamnan was carried out starting from the monomer with the phthaloyl-protected amino group which, after the polycondensation, was replaced by an *N*-acetyl group

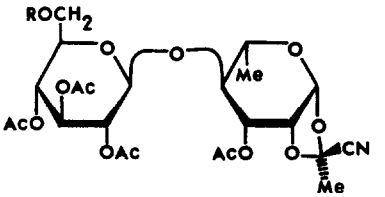
The key step in the synthesis of the monomer was the glycosylation[110] of the preformed L-rhamnose cyanoethylidene derivative (Scheme 25) (*cf* 4 1 1) [98] The unprotected cyanoethylidene derivative **132** was selectively acetylated, the 3-*O*-acetate **133** was benzylated with benzyl trichloroacetimidate, and then deacetylated The 4-*O*-benzyl derivative **134** was glycosylated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide **135** in the presence of $Hg(CN)_2$ The disaccharide derivative **136** was then deacetylated giving, following tritylation, the crystalline monomer **137**, whose structure was confirmed by the ^{1}H- and ^{13}C-NMR data

The condensation of **137** proceeded smoothly and at a high rate (16 h) under the usual conditions (10 mol % of the initiator, room temperature) The presence of the phthalimido group, as opposed to the acetamido group, did not complicate the polycondensation process The reaction was terminated by addition of aqueous pyridine, and the polymer isolated was dephthaloylated and deacetylated by treatment with hydrazine, followed by *N*-acetylation with acetic anhydride in methanol After hydrogenolysis of the *O*-benzyl group, the *N*-acetylated hexosaminoglycan **125** was isolated by chromatography

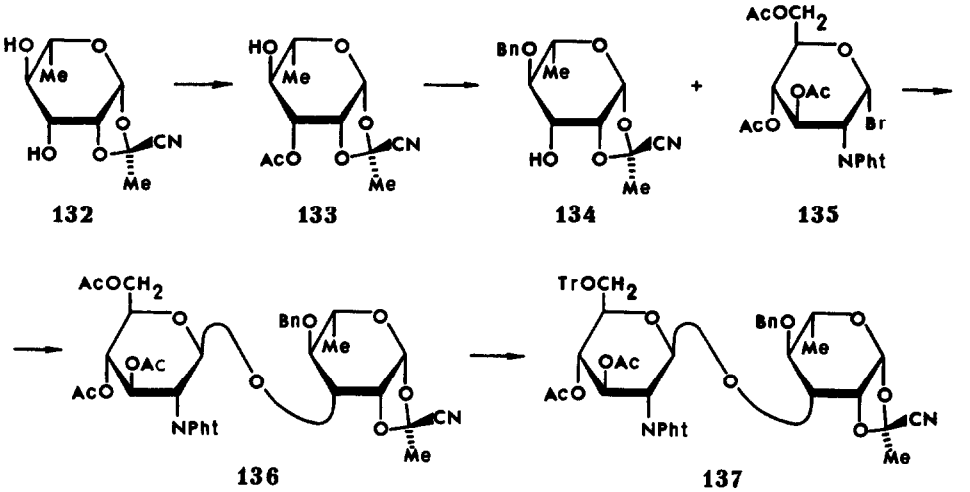
Total structural regularity of the polysaccharide was proved by methylation analysis This also showed a rather high of polymerization of about 45 which corresponds to a molecular weight of about 14000 This was confirmed by gel filtration data The ^{13}C-NMR spectrum demonstrated stereochemical and structural regularity of the synthetic hexosaminoglycan The presence of the low-field signal for C-6 of *N*-acetylglucosamine (67 3 ppm) and the absence of signals in the region of 62–63 ppm, characteristic of the unsubstituted hydroxymethyl group, proved that the rhamnose-to-glucosamine linkage is only (1–6) The chemical shift for C-5 of the rhamnose unit (70 6 ppm) and the total absence of signals in the regions of 73–74 ppm and 83–84 ppm, which are typical for C-5 and C-3, respectively, of β-rhamnosidic linkage, was evidence in favour of the α-rhamnosidic linkage Some spectral features, such as the absence of signals at 94–95 ppm, characteristic of the reducing rhamnose unit, also supported a high molecular weight of the polymer



127 $R^1 = R^2 = Ac$
128 $R^1 = R^2 = H$
129 $R^1 = Tr$, $R^2 = Ac$



130 R = Ac
131 R = Tr

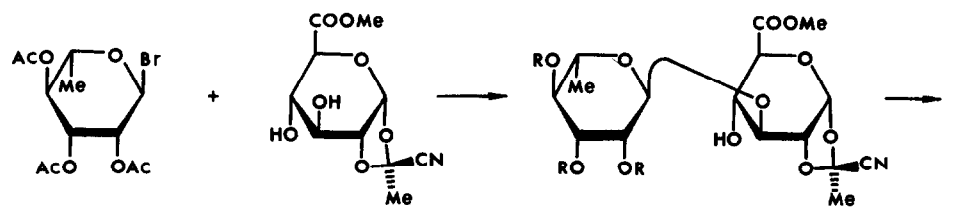


132 **133** **134** **135**

136 **137**

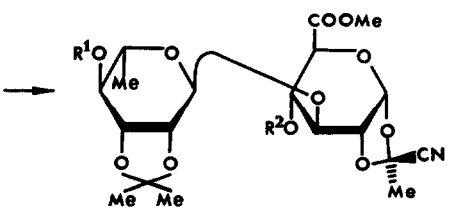
Scheme 25

Heteropolyuronide **126** [145] The synthesis of polysaccharides containing the uronic acid residues was also of considerable interest, since such heteropolyuronides are very abundant in nature Synthetic approaches to even the simplest oligosaccharides of this class are not well developed The monomer was synthesized[146] by the glycosylation of the methyl glucuronate cyanoethylidene derivative **139** with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide **138** The 3-*O*-rhamnosyl derivative **140** was isolated from the mixture of disaccharides by chromatography and was then carefully deacetylated by a brief treatment with sodium methoxide The tetraol **141** was converted



138 **139**

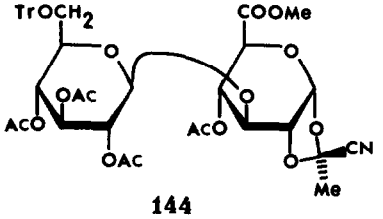
140 R = Ac
141 R = H



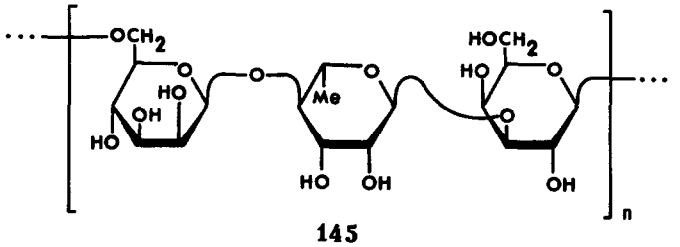
142 $R^1 = R^2 = H$
143 $R^1 = Tr$, $R^2 = Ac$

into the isopropylidene derivative **142**, which was tritylated regioselectively with trityhum perchlorate in the presence of 2,4,6-collidine giving the monomer **143**

The polycondensation[145] in the presence of 10 mol % of the initiator at room temperature was over in 17 h Aqueous pyridine was added to destroy trityhum perchlorate The protected polysaccharide, isolated by chromatography, proved to be regular, both structurally and stereochemically The ^{13}C-NMR spectrum contained in the anomeric region, along with the signal for C-1 of the rhamnose unit (98 3 ppm), the only signal for C-1 of glucuronic acid (99 7 ppm), which was consistent with the β-glycosidic linkage However, the degree of polymerization was very low, of about 5, which corresponds to a molecular weight of 1800



144



145

An attempt was undertaken to carry out the polycondensation of the derivative of a pseudo-aldobiouronic acid **144** It gave only a dimer, although the reaction proceeded stereospecifically These results show that the monomers containing uronic acids also enter the trityl-cyanoethylidene condensation without serious complications but so far, it has been possible to obtain only oligomeric products

4 2 5 2 *Microbial heteropolysaccharides* Synthesis of natural biopolymers is a serious test of modern synthetic methods, particularly when the synthetic product can be compared with authentic natural material Somatic antigens of Gram-negative bacteria are the lipopolysaccharides, in which a polysaccharide chain (the so-called *O*-polysaccharide) is linked to the lipid fragment Its structure determines the very high specificity of the antigen and of the surface of the bacterial cell Capsular polysaccharides of many bacteria also play the role of highly specific antigens It is a vast diversity of the heteropolysaccharide chains occurring on the surface or the capsule of the bacterial cell that forms the basis for fine differentiation of microorganisms

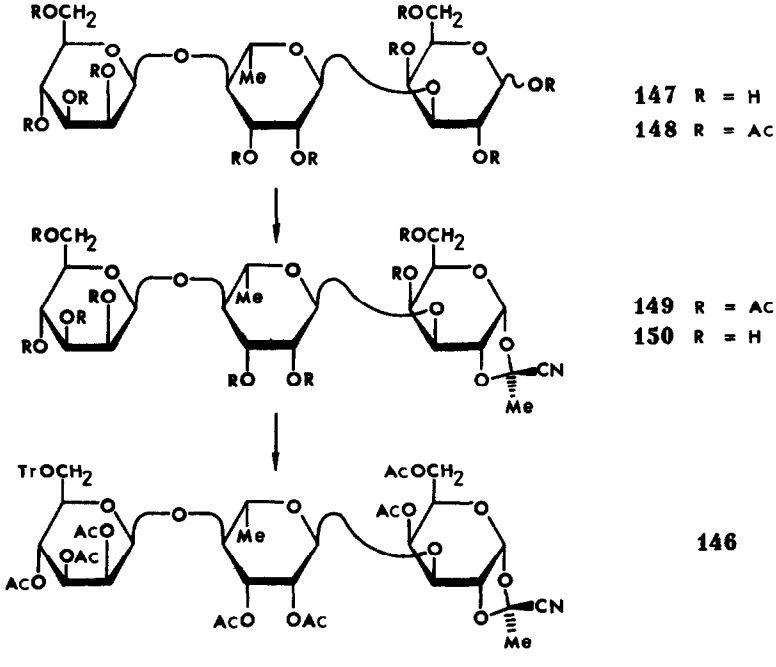
The *O*-antigenic and capsular polysaccharides have common structural features [154] They are the block-polymers built of the regularly repeating oligosaccharide units which may involve from 2 to 10 monosaccharides As a synthetic target these bacterial polysaccharides are both difficult and beneficial, since the identity of the natural and synthetic polymers may be established by chemical, spectral, and immunochemical methods In addition, the block structure of the polysaccharides offers directly the route to their synthesis by the polymerization of the monomer, whose structure corresponds to that of the oligosaccharide repeating unit To date the synthesis of two *O*-antigenic heteropolysaccharides of Gram-negative bacteria, the polysaccharides of *Salmonella newington* and *Shigella flexneri*, and the capsular polysaccharide of *Streptococcus pneumoniae* type 14 has been performed

O-*Specific polysaccharide of* Salmonella newington [101,102] The polysaccharide is built of trisaccharide repeating units containing D-mannose, L-rhamnose, and D-galactose residues It has the structure **145**[155] with the oligosaccharide unit being repeated in the polysaccharide chains 2 to 27 times [156] The most apparent route to its synthesis is the polycondensation of the block, Man β (1-4) Rha α (1-3) Gal, with the formation of the galactosyl-(1-6)-mannose linkage with the 1,2-*trans*-configuration Thus the starting monomer must have the structure **146** with an *O*-trityl group at C-

6, the mannose residue, and a cyanoethylidene group at the galactose residue The synthesis of the monomer was accomplished by a direct functionalization of the trisaccharide **147**, for which the most rational and convenient synthetic route has been developed [157] Using standard procedures the trisaccharide peracetate **148** was converted *via* the bromide into the acetate of the cyanoethylidene derivative **149** and then by careful deacetylation into the unsubstituted cyanoethylidene derivative **150** The presence of two primary OH groups posed a problem for the subsequent selective tritylation Nevertheless, treatment of **150** with 1 5 mole of trityl chloride in pyridine and acetylation, followed by chromatography, afforded the required monomer **146**, albeit in modest yield (Scheme 26) It should be pointed out that such a route for the synthesis of the structurally complex monomer is a somewhat adventurous enterprise the separation of a complex mixture of oligosaccharide derivatives is far from being an easy task

The monomer **146** was subjected to polycondensation in the presence of 10 mol % of the initiator at room temperature for 65 h The reaction product was deacetylated without purification and the unprotected polysaccharide was isolated by gel filtration Methylation analysis has confirmed its structural regularity Its molecular weight was determined by the content of the terminal galactitol after reduction of the polymer with sodium borohydride and methanolysis, by gel filtration, as well as by measurement of the reducing power Its molecular weight was 4000–6000 (a degree of polymerization of 8–12, referring to the trisaccharide) This is consistent with the average value for the natural *O*-antigenic polysaccharide of *S newington* [156]

The stereoregularity of the polysaccharide and stereochemistry of the newly formed D-galactopyranosidic linkages have been checked very thoroughly The ^{13}C-NMR spectrum contained in the anomeric region three signals of roughly equal intensity (104 3, 103 4, and 101 8 ppm), which correspond to the signals for C-1 of β-D-galactopyranose, α-L-rhamnopyranose, and β-D-mannopyranose, no signals for α-D-galactopyranose have been observed It was possible to assign the signals of all the 18 carbons in the ^{13}C-NMR spectrum, and the spectrum itself resembled very closely that of the natural *O*-antigenic polysaccharide isolated from *S newington* (Table 2) The absolute stereospecificity of polycondensation and the absence of 1,2-*cis* glycosidic linkages was confirmed also by Smith degradation of the synthetic polymer **145** (Scheme 27) Treatment with $NaIO_4$ resulted in oxidation of the mannose and rhamnose moieties, while the galactose remained unaffected, subsequent borohydride reduction and hydrolysis gave 1-*O*-galactopyranosyl-glycerol **151**, which contained the galactosidic linkage formed on polycondensation GLC analysis of galactosylglycerol isolated as peracetate with peracetates of α- and β-galactosylglycerols as authentic



Scheme 26

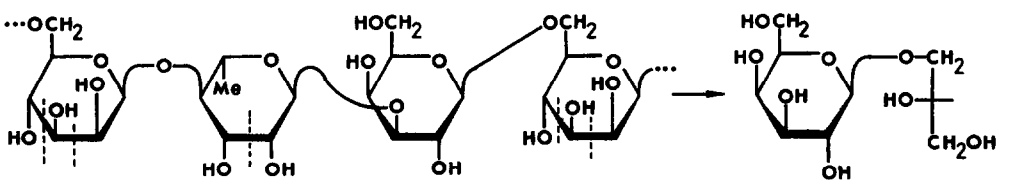
Table 2 Comparative data of ^{13}C-NMR spectra of natural and synthetic *O*-antigenic polysaccharide of *Salmonella newington*

Unit	Polysaccharide	Chemical shifts (p p m) C-1	C-2	C-3	C-4	C-5	C-6
Man	Natural	101 75	71 7	74 3	68 1	76 25	70 55
	Synthetic	101 8	71 6	74 3	68 0	76 25	70 25
Rha	Natural	103 3	71 45	71 7	80 7	69 1	18 3
	Synthetic	103 4	71 4	71 6	80 8	69 1	18 5
Gal	Natural	104 4	71 45	81 8	69 75	76 4	62 1
	Synthetic	104 3	71 4	81 8	69 7	76 45	62 1

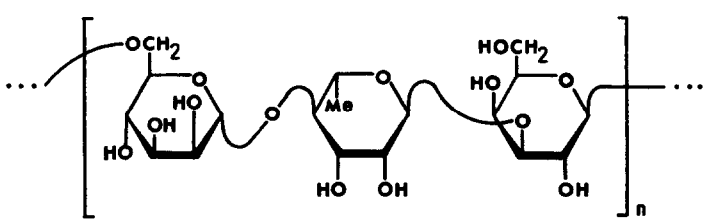
standards proved the degradation porduct to be pure β-isomer (to within 1%) Hence, the synthetic polysaccharide contained only the β-galactosidic linkages so that the polysaccharide is fully stereoregular

It is well known that the *O*-antigenic polysaccharides are characterized by a highly sensitive and specific interaction with antisera raised against the corresponding microorganism The response in this reaction varies drastically with even slight changes in the polysaccharide structure In order to examine this most important property of the synthetic *O*-antigenic polysaccharide, that is its biological specificity, the synthesis of its close analogue **152**, containing an α-mannosyl-rhamnose linkage instead of the β-mannosyl-rhamnose was carried out in parallel [102] This synthesis has been accomplished by the route identical to that proposed for the synthesis of the natural polysaccharide **145** The structure of **152** was confirmed by the spectral data, its ^{13}C-NMR spectrum, which on the whole resembled that of the natural polysaccharide, contained the signal for C-1 of the α-mannose residue instead of that for C-1 of the β-mannose in the former The configuration of the newly formed galactosidic linkages was also checked by Smith degradation, which led to only 1-*O*-β-galactosyl-glycerol **151** Comparison of **145** and **152** in passive haemagglutionation test in the *O*-factor 3–anti 3 system of *S newington* showed that the synthetic *O*-antigenic polysaccharide **145** was highly active as an inhibitor of passive agglutination, whereas its analogue **152** was practically inactive These data prove convincingly that the structure of the synthetic polysaccharide **145** is completely identical to that of the natural polysaccharide This is the first direct synthetic demonstration of the structure of the natural polysaccharide and, in particular, of its regular structure

O-*Antigenic polysaccharide of* Shigella flexneri [158] This polysaccharide is built of the tetrasaccharide repeating units with one residue of *N*-acetyl-D-glucosamine and three L-rhamnose residues It has the structure **153** It represents an *O*-antigenic polysaccharide of *Sh flexneri* serotypes



151



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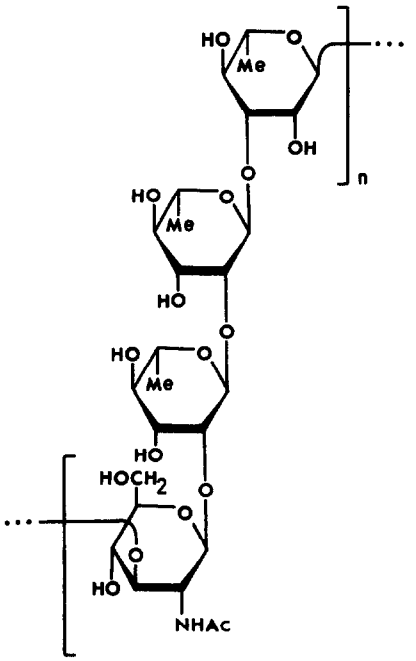
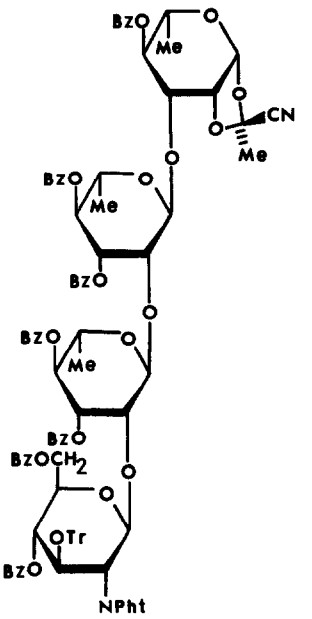
Scheme 27



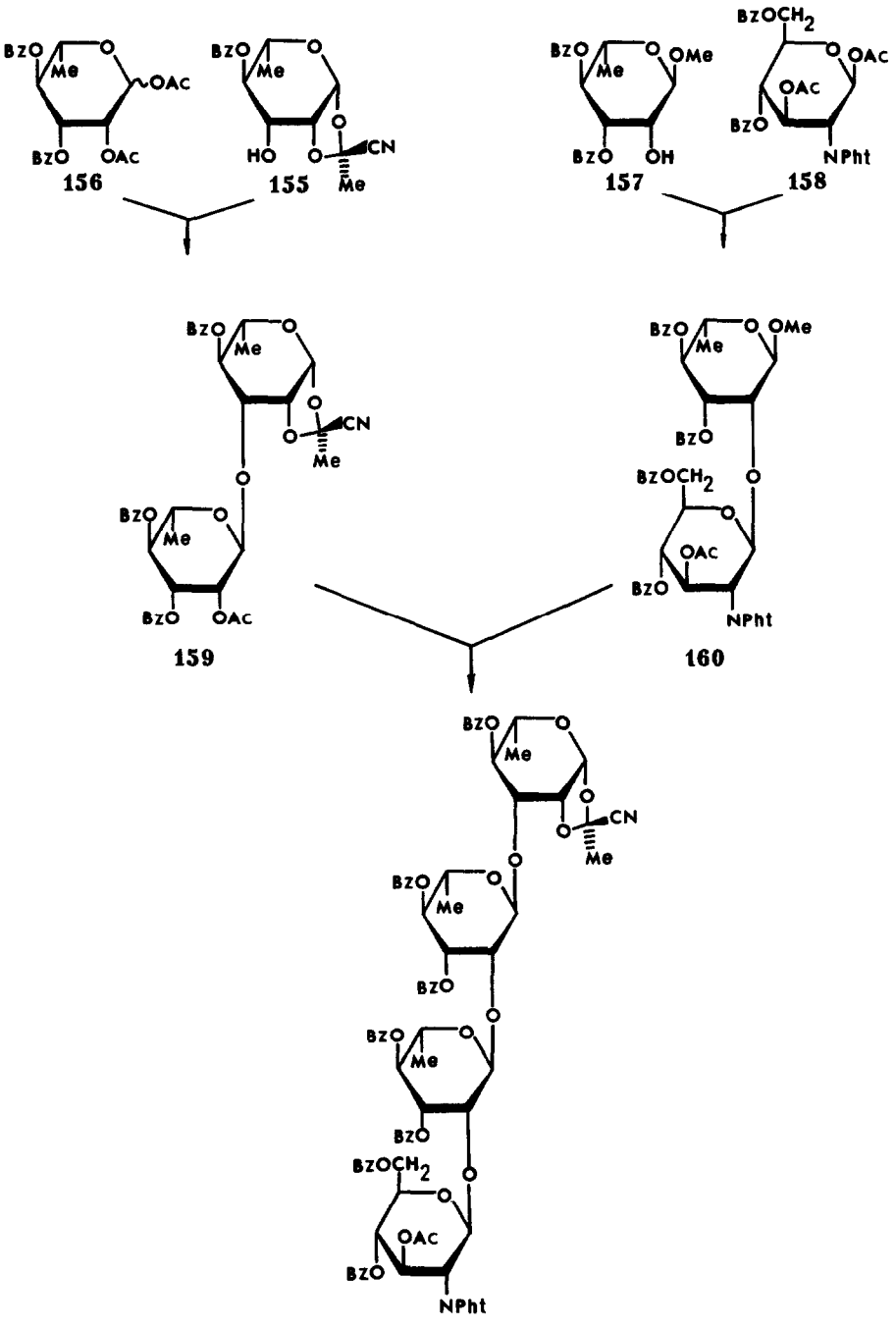
153



154

3b, 3c and variant Y, and is also the basic chain of the other serotypes of *Sh flexneri*[159] which may contain some branchings in the form of single glucose units and/or *O*-acetyl groups The synthesis of this polysaccharide has been achieved by polycondensation of the monomer **154** containing trityl group at O-3 of the non-reducing *N*-acetylglucosamine terminus and the cyanoethylidene group at the reducing rhamnose terminus It was anticipated that the polycondensation of this monomer would be successful since it was already established that the rhamnose cyanoethylidene derivatives are highly reactive and stereospecific glycosylating agents (*cf* 4 1 3) The glycosylation of the *O*-tritylated *N*-phthaloyl-glucosamine also proceeds effectively[153] (*cf* 4 2 5 1) The synthesis of the monomer **154** was based on the use of the monosaccharide synthons **155–158**, which are related to each of the monosaccharide units of the tetrasaccharide The OHs of each of these synthons which should not participate in the subsequent transformations in the course of the monomer synthesis were protected with the "permanent" protection (benzoyl groups) The OHs of the synthons participating in the formation of the glycosidic linkages of the tetrasaccharide were protected with a "temporary" protection (acetyl group) Selective removal of the *O*-acetates without affecting the *O*-benzoates, under the conditions of mild acid methanolysis[150] has made possible, at each step of the synthesis, to reveal selectively the required OH group The use of this new strategy (see 4 2 5), which proved helpful in the oligosaccharide synthesis allowed a rational synthetic scheme The synthesis of the derivatives **155**, **156**, **157**, and **158**, each containing a specific protection, was carried out by using the traditional methods of synthetic carbohydrate chemistry [160–163] The tetrasaccharide monomer **154** was assembled from these synthons in a blockwise route (synthesis of two disaccharides, followed by their connection in the tetrasaccharide, the 2+2 scheme) (Scheme 28)

The synthon **155** was obtained from 1,2,3-tri-*O*-acetyl-4-*O*-benzoyl-L-rhamnose via the corresponding glycosyl bromide, using the general method for the synthesis of the cyanoethylidene derivatives,[97] followed by deacetylation It was glycosylated, in the presence of mercuric cyanide, with a glycosyl bromide derived from the synthon **156**, giving a derivative of the disaccharide **159** This compound **159** contained the cyanoethylidene group and the only "temporary" protected (by an *O*-acetyl group) OH, while the remaining OHs were protected with the "permanent" benzoyl groups [162] For the synthesis of the second disaccharide block the rhamnose dibenzoate **157** was glycosylated with the bromide obtained by the standard procedure from the synthon **158**, giving the disaccharide **160**, containing along with the other protective groups the only OH protected as *O*-acetate,[163] which was replaced subsequently by an *O*-trityl group For the synthesis of tetrasaccharide the OH at C-2′ of the rhamnose residue of the disaccharide **159** was selectively deprotected, and the disaccharide **160** was converted into the corresponding bromide by the sequential acetolysis of methyl glycoside



Scheme 28

and treatment with HBr The condensation of the bromide with the monohydroxyl derivative of the disaccharide **159** in the presence of $Hg(CN)_2$ gave the tetrasaccharide **161**, which again contained along with the benzoyl protection the only *O*-acetyl group [164] In order to transform **161** into the monomer **154** the OH at C-3 of the glucosamine moiety was deprotected by mild acid methanolysis, and then tritylated with trityhum perchlorate in the presence of 2,4,6-collidine [165] Each step of the synthesis was followed by NMR spectroscopy, thus ensuring the absence of any isomerization or rearrangement processes The synthesis of the monomer **154** was accomplished also by the sequential chain elongation from the reducing end, using just the same synthons, although this route proved to be less efficient

Table 3 Comparative data of ^{13}C-NMR spectra of synthetic and natural *O*-antigenic polysaccharide of *Shigella flexneri*

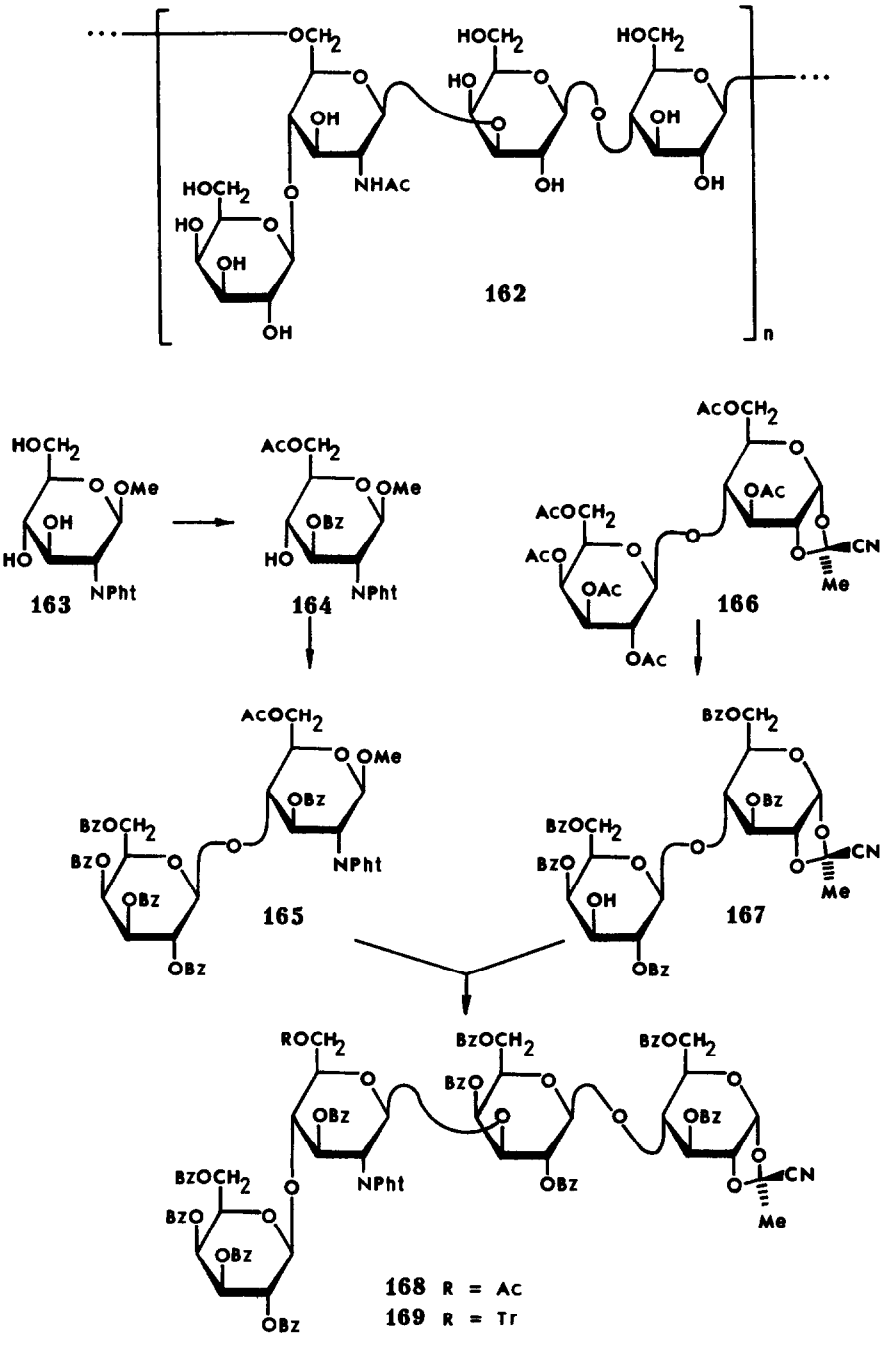
Unit	Polysaccharide	Chemical shifts (p p m) C-1	C-2	C-3	C-4	C-5	C-6	$^1J_{C1\,H1}$
Rha A	Synthetic	102 21	71 80	78 64	72 79	70 35	17 63	170 2
	Natural	102 03	71 41	78 10	72 45	69 81	17 22	169
Rha B	Synthetic	101 88	79 26	71 32	73 53	69 81	17 78	172 0
	Natural	101 62	78 91	70 82	72 96	69 14	17 47	171
Rha C	Synthetic	102 10	79 89	71 13	73 65	70 23	17 86	171 1
	Natural	101 85	79 52	70 58	73 10	69 94	17 47	172
GlcNAc	Synthetic	103 24	56 73	82 68	70 23	77 05	62 09	162 7
	Natural	102 95	56 46	82 29	69 94	76 68	61 56	162

The polycondensation of the monomer **154** was carried out under the standard conditions (10 mol % of the initiator, room temperature) [165] The reaction has terminated in 16 h and the protected polysaccharide was isolated by chromatography All the protective groups were removed by hydrazinolysis and after the standard *N*-acetylation the free heteropolysaccharide was isolated by gel chromatography The ^{13}C-NMR spectrum of the polysaccharide contained only four signals in the anomeric region (102 21, 101 88, 102 10, 103 24 ppm), and the values of the $^1J_{C1,H1}$ coupling constants for the rhamnose units (170 2–172 0 Hz) showed the presence of only the α-L-rhamnosidic linkages These and some other spectral features fully supported stereoregularity of the polysaccharide and consequently the absolute stereospecificity of the polycondensation process Comparison of the ^{13}C-NMR spectra of the synthetic and natural polysaccharide of *Sh flexneri* has demonstrated full agreement for all 24 carbons (Table 3) The molecular weight of the synthetic polysaccharide, as determined by gel chromatography, and by ^{13}C-NMR spectral data, amounted to about 6000 (a degree of polymerization of about 10, referring to the tetrasaccharide unit)

Capsular polysaccharide of Streptococcus pneumoniae *type* 14 [166] This polysaccharide is derived from tetrasaccharide repeating units and has the branched structure **162** [167] Its synthesis was the first example of the synthesis of a regular branched heteropolysaccharide The monomer used was a tetrasaccharide with an *O*-trityl group at C-6 of the glucosamine moiety and the cyanoethylidene group in the glucose moiety This formed the (1-6)-β-glucosyl-glucosamine linkage of the polysaccharide backbone upon polycondensation The terminal galactose would form the regular branching in the polymer formed

The synthesis of the monomer **169** (Scheme 29) was accomplished using the blockwise approach according to the 2+2 scheme based on the strategy described in the previous synthesis (see 4 2 5) The first disaccharide block, containing the glucosamine and galactose residues and the only *O*-acetyl protection, was obtained from methyl 2-deoxy-2-phthalimido-β-D-glucopyranoside **163**, which by sequential benzylidenation, 3-*O*-benzoylation, debenzylidenation, and selective 6-*O*-acetylation was converted into the synthon **164**, containing the only free OH Condensation of **164** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide in the presence of silver triflate gave the disaccharide block **165** In order to obtain the second disacharide block the acetylated cyanoethylidene derivative of lactose **166**, obtained from peracetyl lactosyl bromide by the standard procedure,[97] was converted by sequential deacetylation, isopropylidenation, benzoylation, mild hydrolysis, conversion into the 3′,4′-cyclic orthobenzoate and its selective cleavage, into the benzoylated cyanoethylidene derivative containing the only free OH at C-3 of the galactose residue **167** Condensation of the biosyl bromide, obtained from the disaccharide **165**, with the disaccharide **167** gave the tetrasaccharide **168**, corresponding to the repeating unit of the capsular polysaccharide and possessing the cyanoethylidene group The *O*-acetyl group in the resulting tetrasaccharide **168** was removed by mild acid methanolysis and the recovered OH was tritylated with tritylium perchlorate in the presence of 2,4,6-collidine giving the monomer **169**

The monomer **169** was subjected to polycondensation (10 mol % of the initiator, 18 h, room temperature) The initiator was destroyed by addition of aqueous pyridine and the polycondensation product was isolated with high yield and purified by chromatography on silica gel Hydrazinolysis removed the protective groups and the resulting compound was *N*-acetylated with acetic anhydride in methanol The full regioregularity of this complex, branched polysaccharide was established by methylation analysis Its ^{13}C-NMR spectrum confirmed the configuration of all its glycosidic



Scheme 29

linkages, including the unambiguous proof of the β-configuration of a newly formed glycosidic linkage The spectrum contained four signals in the anomeric region which correspond to two C-1s of β-D-galactose (104 1 and 103 4 ppm), β-D-glucosamine (103 8 ppm) and β-D-glucose (103 6 ppm) The signals for the other 20 carbons also were easily assigned Thus, the synthetic polysaccharide is totally stereoregular Its optical rotation value (+8 4°) is close to that of the natural polymer (+5°) Good agreement of this sensitive figure for both polysaccharides is an excellent demonstration of the identity of the synthetic and natural samples The molecular weight of the synthetic polysaccharide, as determined by gel filtration, amounted to 6000, which corresponds to a degree of polymerization of about 10 This synthesis clearly demonstrates that the trityl-cyanoethylidene polycondensation can be employed with success for the synthesis of branched heteropolysaccharides

5. CONCLUSION

The synthetic chemistry of polysaccharides makes its first steps and this progress is discussed in this review The polymerization of anhydrides yielded some simple homopolysaccharides with α-glycosidic intermonomeric linkage The extension of this limited method is determined by the search of conditions for regioselective and stereoselective ring-opening of the dioxabicyclic systems to which sugar anhydrides belong This will offer a route to the synthesis of other polysaccharides although only linear homopolysaccharide chains can be obtained by this method

The trityl-cyanoethylidene condensation is much more promising This route is also limited to the synthesis of polysaccharides with only a 1,2-*trans* glycosidic intermonomeric linkage The opportunity provided by this method appears to be a unique possibility for the synthesis of complex heteropolysaccharide systems with a regular structure, including the natural polysaccharides possessing biological specificity The main disadvantage of the method is the insufficient degree of polymerization This is especially disadvantageous in the synthesis of homopolysaccharides, as well as an occasional violation of the reaction's stereospecificity Further efforts in overcoming these drawbacks will, hopefully, broaden the scope of this method

A further fundamental development in the synthetic chemistry of polysaccharides necessitates not only the improvement of the already existing methods but also the search for new reactions of stereospecific glycosylation The development of new methods of polycondensation are required It must be emphasized that the search for a glycosylation reaction leading with absolute stereospecificity to a 1,2-*cis* glycosidic linkage is a very demanding problem

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REFERENCES

[1] R C Hughes, *Essays in Biochemistry* **11**, 1 (1975)
[2] R Ivatt (Ed), *Biology of Glycoproteins* Plenum Press, New York (1984)
[3] A G Darviel and P A A Albersheim, *Rev Physiol* **35**, 249 (1984)
[4] N K Kochetkov, *Pure Appl Chem* **42**, 327 (1975)
[5] S Koto, T Uchida and S Zen, *Bull Chem Soc Japan* **46**, 2520 (1973), B A Dmitriev, A V Nikolaev, A S Shashkov and N K Kochetkov, *Carbohydr Res* **100**, 195 (1982)
[6] V N Shibaev, L L Danilov, T N Druzhinina, L M Gogilashvili, S D Maltsev and N K Kochetkov, *FEBS Letters* **139**, 177 (1982)
[7] K Igarashi, *Adv Carbohydr Chem Biochem* **34**, 243 (1977)
[8] N K Kochetkov and A F Bochkov, in *Recent Developments in the Chemistry of Natural Compounds*, Vol 4, p 77 Akad Kiado, Budapest (1971)
[9] S Haq and W J Whelan, *J Chem Soc* 4543 (1956)
[10] N K Kochetkov, E M Klimov and M V Ovchinnikov, *Izv Akad Nauk SSSR, Ser Khim* 1864 (1977)
[11] N K Kochetkov, E M Klimov and M B Kislov, *Izv Akad Nauk SSSR, Ser Khim* 2400 (1973)
[12] E Huseman and G Muller, *Makromol Chem* **91**, 212 (1966)
[13] R Eby and C Schuerch, *Carbohydr Res* **25**, 133 (1972)
[14] D McGrath, E Lee and P O'Colla, *Carbohydr Res* **11**, 453 (1969)
[15] D McGrath, E Lee and P O'Colla, *Carbohydr Res* **11**, 461 (1969)
[16] E O'Brien, E E Lee and P O'Colla, *Carbohydr Res* **32**, 31 (1974)
[17] V A Nesmeyanov, S E Zurabyan and A Ya Khorlin, *Tetrahedron Lett* 3213 (1973)
[18] N K Kochetkov, A Ya Khorlin and A F Bochkov, *Tetrahedron* **23**, 693 (1967)
[19] N K Kochetkov, A F Bochkov, T A Sokolovskaya and V I Snyatkova, *Carbohydr Res* **16**, 17 (1971)
[20] N K Kochetkov, A F Bochkov, I G Yaslovetsky and V I Snyatkova, *Izv Akad Nauk SSSR, Ser Khim* 1802 (1968)
[21] N K Kochetkov, A F Bochkov and I G Yaslovetsky, *Carbohydr Res* **2**, 84 (1966), **9**, 49 (1969)
[22] N K Kochetkov, A F Bochkov and I G Yaslovetsky, *Izv Akad Nauk SSSR, Ser Khim* 1818 (1968)
[23] N K Kochetkov and A F Bochkov, *Carbohydr Res* **9**, 61 (1969)
[24] A F Bochkov, I V Obruchnikov and N K Kochetkov, *Zh Obshch Khim* **44**, 1197 (1974)
[25] A F Bochkov, V I Betaneli and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1379 (1974)
[26] A F Bochkov, V I Betaneli and N K Kochetkov, *Bioorg Khim (USSR)* **2**, 927 (1976)
[27] N K Kochetkov, L V Backinowsky and Yu E Tsvetkov, *Tetrahedron Lett* **41**, 3681 (1977)
[28] L V Backinowsky, Yu E Tsvetkov, N F Balan, N E Byramova and N K Kochetkov, *Carbohydr Res* **85**, 209 (1980)
[29] L V Backinowsky, Yu E Tsvetkov and N K Kochetkov, *Bioorg Khim* (USSR) **7**, 750 (1981)
[30] A Pictet, *Helv Chim Acta* **1**, 226 (1918), **4**, 788 (1921)
[31] H Pringsheim and R Schmalz, *Ber* **55**, 3001 (1922)
[32] J C Irvine and J W H Oldham, *J Chem Soc* **127**, 2903 (1925)
[33] V V Korshak, O P Golova, V A Sergeev, N M Merlis and R Ya Pernikis, *Vysokomol Soed* **3**, 477 (1961)
[34] V V Korshak, V A Sergeev, Ya A Surna and R Ya Pernikis, *Vysokomol Soed* **5**, 1593 (1963)

[35] H Bredereck and V Hutte, cited in J Klar, *Chem Ztg* **87**, 731 (1963)
[36] C Schuerch, J Zachoval and B Veruovic, *Chem Listy* **66**, 1124 (1972)
[37] C Schuerch, *Acc Chem Res* **6**, 184 (1973)
[38] C Schuerch, *Adv Carbohydr Chem Biochem* **39**, 157 (1981)
[39] K S Frish and S L Reegen, *Ring Opening Polymerization* Dekker, New York (1969)
[40] T Uryu, K Ito, K Kobayashi and K Matsuzaki, *Makromol Chem* **180**, 1509 (1979)
[41] H Sugimoto and M Okada, in *Cationic Polymerization and Related Processes* (Edited by E Goethall), p 197 Academic Press, London (1984)
[42] J Zachoval and C Schuerch, *J Am Chem Soc* **91**, 1165 (1969)
[43] T Uryu, K Hatanaka, K Matsuzaki and H Kuzuhara, *Macromolecules* **16**, 853 (1983)
[44] V A Ponomarenko, E L Berman, A M Sakharov and Z N Nysenko, in *Cationic Polymerization and Related Processes* (Edited by E Goethall), p 155 Academic Press, London (1984)
[45] E Ruckel and C Schuerch, *J Org Chem* **31**, 2233 (1966)
[46] T Uryu, H Tachikawa, K -J Ohaki, K Terui and K Matsuzaki, *Makromol Chem* **178**, 1929 (1977)
[47] T Uryu and C Schuerch, *Macromolecules* **4**, 342 (1971)
[48] J W P Lin and C Schuerch, *J Polym Sci*, Part A1, **10**, 2045 (1972)
[49] T Uryu, K Ito, K Kobayashi and K Matsuzaki, *Makromol Chem* **180**, 1509 (1977)
[50] J W P Lin and C Schuerch, *Macromolecules* **6**, 320 (1973)
[51] K Kobayashi, R Eby and C Schuerch, *Biopolymers* **16**, 415 (1977)
[52] H Ito, *J Polym Sci*, *Polym Lett Ed* **19**, 43 (1981)
[53] H Ito and C Schuerch, *J Am Chem Soc* **101**, 5797 (1979)
[54] K Kobayashi and C Schuerch, *J Polym Sci*, *Polym Chem Ed* **15**, 913 (1977)
[55] C Schuerch, T Uryu and J W P Lin, *Macromol Synth* **4**, 151 (1972)
[56] C Tu and C Schuerch, *Polymer Lett* **1**, 163 (1963)
[57] E Ruckel and C Schuerch, *J Am Chem Soc* **88**, 2605 (1966)
[58] E Ruckel and C Schuerch, *Biopolymers* **5**, 515 (1967)
[59] J Frechet and C Schuerch, *J Am Chem Soc* **91**, 1161 (1969)
[60] T Uryu, H Libert, J Zachoval and C Schuerch, *Macromolecules* **3**, 345 (1970)
[61] T Uryu, Y Sakamoto, K Hatanaka and K Matsuzaki, *Macromolecules* **17**, 1307 (1984)
[62] K Matsuzaki, US–Japan Seminar Synth Polysaccharides, Syracuse (1979) (cited in Ref 38)
[63] E T Reese and F W Parrish, *Biopolymers* **4**, 1043 (1966)
[64] J S Tkacz, J O Lampen and C Schuerch, *Carbohydr Res* **21**, 465 (1972)
[65] I J Goldstein, R D Poretz, L L So and Y Yang, *Arch Biochem Biophys* **127**, 787 (1968)
[66] W Richter, *Int Arch Allergy Appl Immunol* **46**, 438 (1974)
[67] B Veruovic and C Schuerch, *Carbohydr Res* **14**, 199 (1970)
[68] V Masura and C Schuerch, *Carbohydr Res* **15**, 65 (1970)
[69] K Kobayashi and C Schuerch, *J Polym Sci*, *Polym Chem Ed* **15**, 913 (1977)
[70] J W P Lin and C Schuerch, *Macromolecules* **6**, 320 (1973)
[71] H Ito, V Marousek and C Schuerch, *J Polym Sci*, *Polym Chem Ed* **17**, 1299 (1979)
[72] W H Linderberger and C Schuerch, *J Polym Sci*, *Polym Chem Ed* **11**, 1225 (1973)
[73] J W P Lin and C Schuerch, *Macromolecules* **5**, 656 (1972)
[74] J Kops and C Schuerch, *J Polym Sci*, Part C, **11**, 119 (1965)
[75] F Micheel, O E Brodde and K Reinking, *Lieb Ann* 124 (1974)
[76] F Micheel and O E Brodde, *Lieb Ann* 702 (1974)
[77] T Uryu, K Kitano, K Ito, J Yamanouchi and K Matsuzaki, *Macromolecules* **14**, 1 (1981)
[78] T Uryu, J Yamanouchi, Y Kato, S Higushi and K Matsuzaki, *J Am Chem Soc* **105**, 6865 (1983)
[79] N K Kochetkov, O S Chizhov and A S Shashkov, *Carbohydr Res* **133**, 173 (1984)
[80] T Uryu, J Yamanouchi, S Hayashi, H Tamaki and K Matsuzaki, *Macromolecules* **16**, 320 (1983)
[81] T Uryu, C Yamagushi, K Morikawa, K Terui, T Kanai and K Matsuzaki, *Macromolecules* **18**, 599 (1985)
[82] T Uryu and H Hatanaka, *J Synth Org Chem*, *Japan* **42**, 507 (1984)
[83] H Ito, R Eby, S Kramer and C Schuerch, *Carbohydr Res* **86**, 193 (1980)
[84] H Ito and C Schuerch, *Macromolecules* **14**, 246 (1981)
[85] F I Good and C Schuerch, *Macromolecules* **18**, 595 (1985)
[86] F Kong and C Schuerch, *Macromolecules* **17**, 983 (1984)
[87] S Haq and W Whelan, *Nature* **178**, 1224 (1956)
[88] J Zachoval and C Schuerch, *J Polym Sci*, Part C **28**, 187 (1969)
[89] H Yamagushi and C Schuerch, *Biopolymer* **19**, 297 (1980)
[90] H Meerwein, V Hederich, H Morschel and K Wunderlich, *Lieb Ann* **635**, 1 (1960)
[91] A F Bochkov and N K Kochetkov, *Carbohydr Res* **39**, 355 (1975)
[92] N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1543 (1982)
[93] N K Kochetkov, *Sov Sci Rev*, *Chem Rev* Sect B, **4**, 1 (1982)
[94] B Coxon and H G Fletcher Jr, *J Am Chem Soc* **85**, 2637 (1963)
[95] I V Obruchnikov and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 2571 (1977)
[96] M Martin-Lomas and M E Chacon-Fuertes, *Carbohydr Res* **59**, 604 (1977)
[97] V I Betaneli, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Carbohydr Res* **68**, C11 (1979) V I Betaneli, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 2751 (1979)
[98] L V Backinowsky, Yu E Tsvetkov, M V Ovchinnikov, N E Byramova and N K Kochetkov, *Bioorg Khim* (*USSR*) **11**, 66 (1985)
[99] L V Backinowsky, N E Nifant'ev, V I Betaneli, M I Struchkova and N K Kochetkov, *Bioorg Khim* (*USSR*) **9**, 74 (1983)
[100] N K Kochetkov, A Ya Ott and A S Shashkov, *Izv Akad Nauk SSSR, Ser Khim* 196 (1986)
[101] V I Betaneli, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Dokl Akad Nauk SSSR* **251**, 108 (1980)
[102] N K Kochetkov, V I Betaneli, M V Ovchinnikov and L V Backinowsky, *Tetrahedron* **37**, Suppl 9, 149 (1981)
[103] M M Litvak, V I Betaneli, L V Backinowsky and N K Kochetkov, *Bioorg Khim* (*USSR*) **8**, 1133 (1982)

[104] K Utimoto and T Horie, *Tetrahedron Lett* **23**, 237 (1982)
[105] F G de las Heras and P Fernandez-Resa, *J Chem Soc , Perkin I* 903 (1982)
[106] L V Backinowsky, S A Nepogod'ev, A S Shashkov and N K Kochetkov, *Carbohydr Res* **138**, 41 (1985)
[107] L G Vorontsova, M O Dekaprilevich, O S Chizhov, L V Backinowsky, V I Betaneli and M V Ovchinnikov, *Izv Akad Nauk SSSR, Ser Khim* 2312 (1980)
[108] N E Byramova, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* , in press
[109] N E Byramova, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* , in press
[110] V I Betaneli, L V Backinowsky, N E Byramova, M V Ovchinnikov, M M Litvak and N K Kochetkov, *Carbohydr Res* **113**, C1 (1983)
[111] B Helferich, *Adv Carbohydr Chem* **3**, 79 (1948)
[112] Ya V Wozney and N K Kochetkov, *Carbohydr Res* **54**, 300 (1977)
[113] M V Ovchinnikov, N E Byramova, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **9**, 391 (1983)
[114] V I Betaneli, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Carbohydr Res* **76**, 252 (1979)
[115] V I Betaneli, M M Litvak, L V Backinowsky and N K Kochetkov, *Carbohydr Res* **94**, C1 (1981)
[116] K Hatanaka and H Kuzuhara, *J Carbohydr Chem* **4**, 333 (1985)
[117] S A Nepogod'ev, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **12**, 1139 (1986)
[118] Yu E Tsvetkov, N E Byramova, L V Backinowsky, N K Kochetkov and N F Yankina, *Bioorg Khim (USSR)* **12**, 1213 (1986)
[119] M V Ovchinnikov, N E Byramova, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **9**, 401 (1983)
[120] P Kosma, private communication
[121] L V Backinowsky, N E Nifant'ev and N K Kochetkov, *Bioorg Khim (USSR)* **9**, 1089 (1983)
[122] N K Kochetkov, A Ya Ott and A S Shashkov, *Izv Akad Nauk SSSR, Ser Khim* 200 (1986)
[123] N K Kochetkov, N N Malysheva, M I Struchkova and E M Klimov, *Bioorg Khim (USSR)* **11**, 391 (1985)
[124] N K Kochetkov and A Ya Ott, *Izv Akad Nauk SSSR, Ser Khim* 1177 (1983)
[125] L V Backinowsky, N E Nifant'ev and N K Kochetkov, *Bioorg Khim (USSR)* **10**, 226 (1984)
[126] V M Zhulin, E M Klimov, Z G Makarova, N N Malysheva and N K Kochetkov, *Dokl Akad Nauk SSSR* **289**, 105 (1986)
[127] D N Kevill and G M L Lin, *Tetrahedron Lett* 949 (1978)
[128] N S Zefirov, A S Kozmin, V D Sorokin and V V Zhdankin, *Zhur Org Khim (USSR)* **18**, 1768 (1982)
[129] K Igarashi, T Honma and J Irisawa, *Carbohydr Res* **15**, 329 (1970)
[130] L G Vorontsova, M O Dekaprilevich and O S Chizhov, *Bioorg Khim (USSR)* **12**, 257 (1986)
[131] C Foces-Foces, F H Cano and S Garcia-Blanco, *Acta Crystallogr* **B32**, 3029 (1976)
[132] N N Malysheva and N K Kochetkov, *Carbohydr Res* **104**, 173 (1982)
[133] A F Bochkov, I V Obruchnikov, V M Kalinevich and N K Kochetkov, *Bioorg Khim (USSR)* **2**, 1085 (1976)
[134] A F Bochkov, I V Obruchnikov, V M Kalinevich and N K Kochetkov, *Tetrahedron Lett* **16**, 3403 (1975)
[135] N K Kochetkov and A Ya Ott, *Izv Akad Nauk SSSR, Ser Khim* 2358 (1984)
[136] L V Backinowsky, S A Nepogod'ev and N K Kochetkov, *Carbohydr Res* **137**, C1 (1985) S A Nepogod'ev, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **12**, 803 (1986)
[137] P-E Jansson, L Kenne, H Liedgren, B Lindberg and J Lonngren, *Chem Commun Univ Stockholm* **8**, 1 (1978)
[138] L V Backinowsky, T A Oseledchik and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1387 (1981)
[139] N K Kochetkov, V M Zhulin, Z G Makarova, E M Klimov and N N Malysheva, *Carbohydr Res* , in press
[140] N K Kochetkov, N N Malysheva and E M Klimov, *Izv Akad Nauk SSSR, Ser Khim* 1170 (1983)
[141] N K Kochetkov, N N Malysheva and E M Klimov, *Izv Akad Nauk SSSR, Ser Khim* 1884 (1986)
[142] N K Kochetkov and N N Malysheva, *Tetrahedron Lett* **21** 3093 (1980)
[143] V M Zhulin E M Klimov N N Malysheva, Z G Makarova and N K Kochetkov *Dokl Akad Nauk SSSR* in press
[144] L V Backinowsky, N E Nifant'ev, A S Shashkov and N K Kochetkov *Bioorg Khim (USSR)* **10**, 1212 (1984)
[145] V I Betaneli, M M Litvak, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1172 (1985)
[146] V I Betaneli, M M Litvak, M I Struchkova, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **9**, 87 (1983)
[147] S A Nepogod'ev, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **12**, 940 (1986)
[148] N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 243 (1984)
[149] N K Kochetkov, *Pure Appl Chem* **56**, 923 (1984)
[150] N E Byramova, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Carbohydr Res* **124**, C8 (1983)
[151] I V Obruchnikov and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 2574 (1977)
[152] N K Kochetkov and E M Klimov, *Tetrahedron Lett* **22**, 337 (1981)
[153] M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **9**, 401 (1983) Yu E Tsvetkov, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **11**, 77 (1985)
[154] K Jann and O Westphal, in *The Antigens*, Vol 3, p 1 Academic Press, New York (1975)
[155] C G Hellerqvist, B Lindberg, J Lonngren and A A Lindberg, *Acta Chem Scand* **25**, 939 (1971)
[156] J M Ryan and H E Conrad, *Arch Biochem Biophys* **162**, 530 (1974)
[157] V I Betaneli, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Carbohydr Res* **84**, 211 (1980)
[158] N K Kochetkov, N E Byramova, Yu E Tsvetkov and L V Backinowsky, *Tetrahedron* **41**, 3363 (1985)
[159] L Kenne, B Lindberg, K Petersson, E Katzenellenbogen and E Romanowska, *Eur J Biochem* **91**, 279 (1979)
[160] N E Byramova, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1122 (1985)
[161] N E Byramova, M V Ovchinnikov, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1129 (1985)
[162] N E Byramova, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1134 (1985)
[163] N E Byramova, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1140 (1985)
[164] N E Byramova, Yu E Tsvetkov, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1145 (1985)
[165] N E Byramova, Yu E Tsvetkov, L V Backinowsky and N K Kochetkov, *Izv Akad Nauk SSSR, Ser Khim* 1151 (1985)
[166] N E Nifant'ev, L V Backinowsky and N K Kochetkov, *Bioorg Khim (USSR)* **13**, 273 (1987)
[167] B Lindberg, J Lonngren and D A Powell, *Carbohydr Res* **58**, 177 (1977)