SOME NEW TRENDS IN THE CHEMISTRY OF POLYSACCHARIDES

N. K. KOCHETKOV

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

ABSTRACT

The data on the fine structure of polysaccharides should form the basis for understanding on a molecular level the biological role played by carbohydrate systems, but the methods of structural chemistry are not always sufficient. The most general approach for determination of the primary structure of polysaccharides might be the development of standard methods, which involve specific cleavage to oligosaccharidic blocks and their subsequent analysis.

The possibilities of application of some reaction for selective modification of monosaccharide units in a polymer chain with subsequent hydrolysis of glycosidic linkages in the modified unit will be discussed.

The application of mass spectrometry for the determination of monosaccharide units in oligosaccharides provides possibilities for effective structural analysis of oligomeric blocks using very small amounts of material. Finally, the development of methods for the synthesis of polysaccharides is discussed.

INTRODUCTION

The progress made in biochemistry, molecular biology, immunochemistry, and related branches of the life sciences, in the last two decades has demonstrated that specificity of biological processes is primarily due to the effect of macromolecular systems. Up to now, the role of nucleic acids and proteins has been understood in general, and during the last decade polysaccharides were added to the group of specific macromolecules. There is no need to give many examples illustrating the specific role of polysaccharide systems in biological processes. It is sufficient to mention the work of several laboratories on lipopolysaccharides of gram-negative bacteria, with the polysaccharide chain as an *O*-antigene determinant, the work of blood-group substances which has shown that the polysaccharide fragments are also immunological determinants. Recent work has demonstrated that the phage receptors localized on the surface of a bacterial cell are also carbohydrate systems.

It is most likely that the polysaccharide systems localized on the surface of a cell are responsible for the specificity of intercellular interaction and for that between a cell and its media. On the other hand, due to their high hydrophilicity and conformational mobility polysaccharides can form ordered structures in aqueous solutions and gels. And it is the polysaccharides,

glycoproteins, and other biopolymers containing carbohydrates that may be responsible for the structural order of intra- and intercellular media.

There is no doubt that the data on the structure of polysaccharides should form the basis for understanding on the molecular level the biological role played by carbohydrate systems, and it is the chemists who should provide such data for their colleagues working in biochemistry and other life sciences. But we have to admit that the chemistry of polysaccharides often falls behind and the methods available are not always adequate. One of the reasons for this, and maybe a principal one, is the difficulty of establishing the structure of polysaccharides due to the polyfunctional monomeric unit and stereochemical problems complicated by high conformational mobility of the system.

As a result of the efforts by many outstanding schools there have been developed a set of methods now conventionally used in the chemistry of polysaccharides to establish the sequence in the polysaccharide chain. But new problems arise which require new approaches. The facts that carbohydrate chains of very complicated primary structures without repeated units have been found in some natural objects, that the dogma about the notorious repeated unit is not always acceptable, that the biological consequences of establishing microheterogeneity of carbohydrate chains are still vague—all these require new approaches for establishing the fine structure of polysaccharides. On the other hand, there should be developed synthetic methods which would make it possible to produce model polysaccharides of predetermined and definite structure, and this is another aspect of the same problem.

The most general approach in establishing the primary structure of any polysaccharide or of a polysaccharide fragment of glycoproteins or lipopolysaccharides might be the development of standard methods similar to those which brought such brilliant success to the chemistry of nucleic acids and proteins. These methods involve specific cleavage of a biopolymer into oligomeric blocks and their subsequent analysis, using minimum amounts of substances.

But these approaches face serious difficulties in the chemistry of polysaccharides as there is no required set of endo- polysaccharidases with a wide range of substrate specificity. As is well known the conventional methods for the analysis of the primary structure of polynucleotides and polysaccharides make use of such endo-enzymes.

On the other hand, the standard methods for the structural analysis of small oligosaccharide fragments have not been sufficiently developed so far, and in each case the set of exo-glycosidases specific for a given monosaccharide unit, and sometimes for the type of an intermonomeric bond, should be different.

The first part of the lecture is concerned with the search for new approaches to developing standard 'block methods' for establishing the primary structure of polysaccharide chains.

Since the principle of one enzyme–one substrate seems to be most frequently valid for polysaccharides there are practically no sufficiently universal endopolysaccharidases which would cleave the chain at a certain unit of different

complicated polysaccharides. Therefore, the principle of selective fermentolysis into blocks efficient for other biopolymers would not work more or less universally in the case of polysaccharides, and chemical approaches to a specific cleavage of a polysaccharide chain might prove interesting.

It is well known that stability of glycosidic linkages to solvolysis varies insignificantly for common monosaccharides. The only exception is furanoside linkages, and selective acidic solvolysis of furanoside units is conventionally used in the chemistry of polysaccharides for splitting the side-chains. The presence of some functional groups in a monosaccharide unit may also affect the stability of glycosidic linkages. An example of this is an increased stability of glycuronic and glycosaminidic linkages. These differences can be used sometimes for selective solvolysis and isolation of disaccharide fragments from polysaccharides.

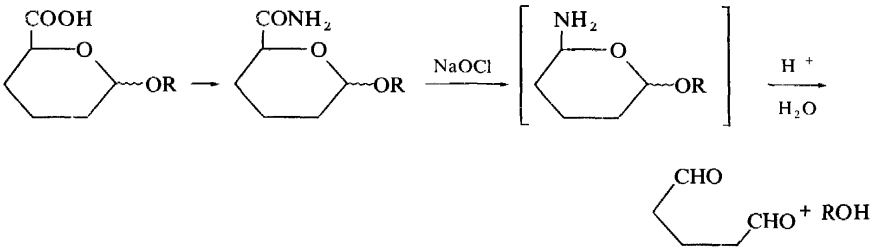
DEVELOPMENT OF 'BLOCK METHODS'

Since the rates of solvolysis of glycosidic linkages vary insignificantly for most of the monosaccharides comprising polysaccharide systems there is so far no real hope of attaining selective cleavage of polysaccharide chains using direct solvolysis, and new approaches to the problem may prove interesting.

One of the most attractive possibilities is the search for a reaction of selective modification of the monosaccharide unit in a polymer chain which would weaken the glycosidic linkages at certain units. Then, subsequent solvolysis would result in their selective cleavage. It should be remembered that similar approaches have been used successfully in the chemistry of polypeptides and polynucleotides.

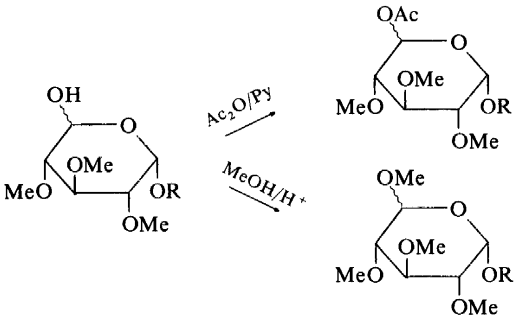
The search for selective reactions which would modify the functional groups other than hydroxyl in a monosaccharide unit appears to be most reasonable to begin with.

A successful method for degradation of uronides using the Hofmann rearrangement can illustrate this approach[1]. The general idea of this method is represented as follows.



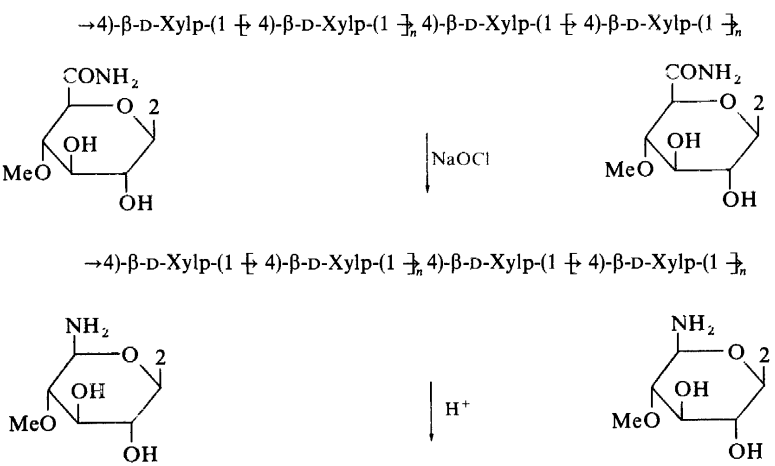
Uronic acid is converted into a carboxamide. The latter is treated with sodium hypochlorite, and quantitatively converted into a derivative of pentadialdose. In the resulting derivative, the glycosidic linkage is very labile and cleaves quantitatively at pH 5, whereas the other glycosidic linkages are completely unaffected.

The experiments with the monosaccharide models[2,3] provide support for this scheme of a degradation reaction. The pentadialdoses resulting from the degradation are isolated as acetal and acylal derivatives.

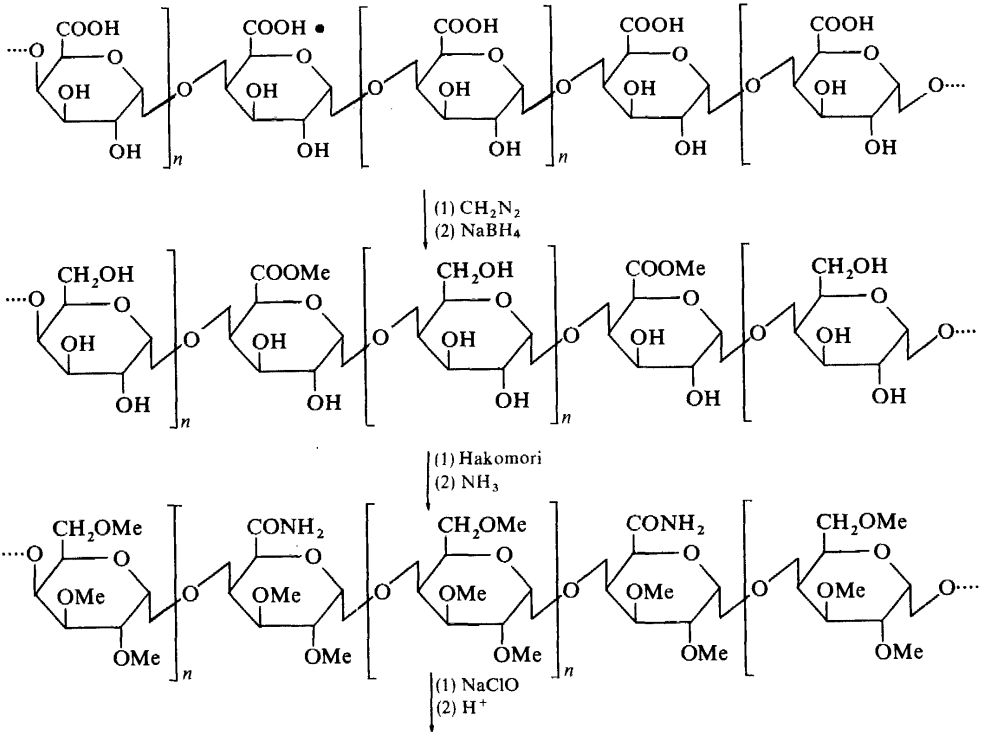


These structures have been proved by mass spectrometry[3]. It is most practical to identify the degraded moiety by reducing a dialdose derivative to its respective alditol which can easily be identified as acetate by g.l.c. and m.s.

The application of the method to polysaccharide degradation can be demonstrated by selective cleavage of white birch xylan[2]. The latter is known to have a β-1,4-xylopyranose backbone with residues of 4-methyl-glucuronic acid attached to C_2 of the xylose units of the backbone; the content of uronic acid is nine to ten per cent. The xylan was converted into carboxamide under the action of liquid ammonia, and subjected to degradation with hypochlorite. The resulting polysaccharide was free from uronic acid, and it practically retained its molecular weight. Thus, as a result of the degradation the residues of uronic acids were split off quantitatively, whereas the glycosidic linkages of the xylose residues were completely retained.



Some preliminary experiments with partially reduced pectins show that this method can be applied to selective degradation of the main chain of polysaccharides[4]. Pectin has been reduced by sodium borohydride so that about 80 per cent of galacturonic acid residues are converted into galactose and the backbone of partially reduced polyuronide in it consists of galactose and galacturonic acid units, on average every fourth and fifth unit being uronic acid. This modified biopolymer after Hakomori methylation has been converted into carboxamide and degraded as above. From the reaction mixture the oligosaccharides fraction has been isolated by conventional procedures.

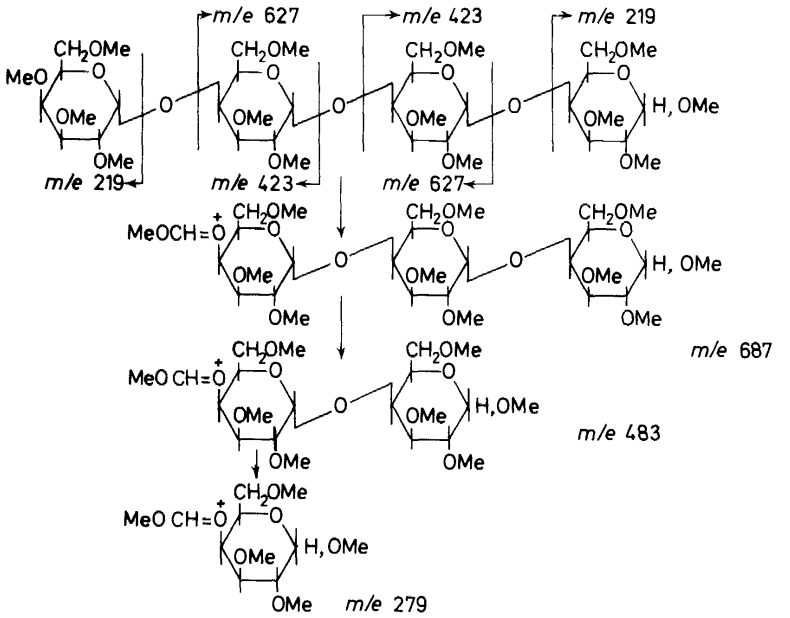


Oligosaccharides

The investigation of this fraction by g.l.c. and m.s. shows that it consists of oligosaccharides, containing only galactose units. It proves again the complete and selective degradation of uronic acid links in the polysaccharide. The mass spectra of one such oligosaccharide can be demonstrated as an example.

These experiments which are now in progress present possibilities for practical use of the method in the chemistry of polysaccharides.

A more important and much more difficult problem is the development of methods for selective modification and subsequent splitting of neutral monosaccharide units with differences only in the stereochemistry and the type of glycosidic linkage.



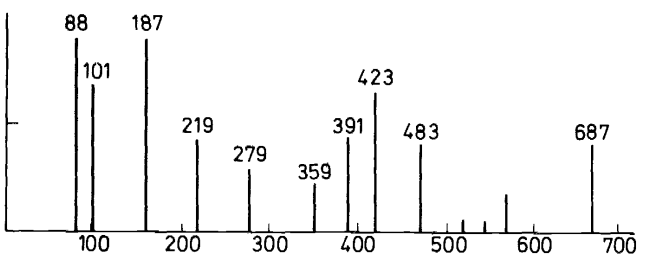
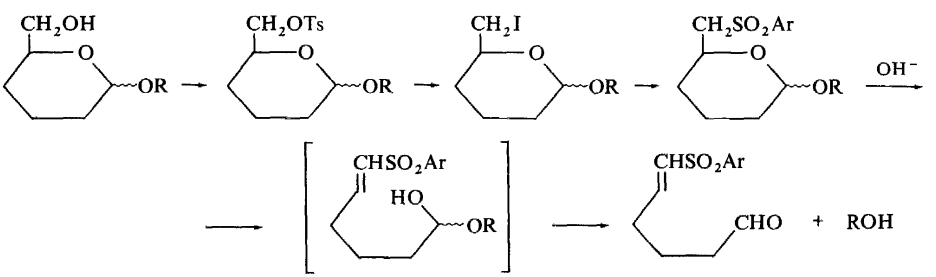


Figure 1

A reaction can be selective only if the reactivity of the hydroxyl groups varies significantly. Therefore, so far this problem presents considerable difficulties. Only one successful attempt has been undertaken by Lindberg and Lundström, who tried to solve the problem of selective modification of the hexopyranose link with a free CH_2OH group[5]. The idea of the method can be represented as follows:



The CH_2OH group is converted via toluene sulphonate into CH_2I in which the iodine atom is substituted by an arylsulphonyl group. Due to the electron-accepting properties of this grouping, the β-elimination at C_5 is facilitated. As a result, the glycosidic linkage in the hemiacetal system arising under the action of the base can be readily and selectively cleaved.

Recently[6] this method has been used by Lindberg and his colleagues for partial degradation of dextrane to determine the number and the length of its sidechains. Dextrane is known to be $1 \rightarrow 6$ glucane with several branchings at C_3 of the main chain. After selective methylation of the secondary hydroxyls, dextrane was converted into C_6-sulphone, and the terminal residues of the glucose located in the branchings were split off by treatment with a base.

When repeated, this procedure allowed a conclusion about the number and length of the sidechains linked to the main 1–6 glucone backbone with 1–3 linkages.

A weak point of the practical application of this method is that tosylation would not proceed quite quantitatively. Therefore, the secondary hydroxyls should be first selectively protected, which requires a long and multistep process.

But despite some weak points of these first two methods of selective degradation they seem to open up new ways for studying the primary structure of complex polysaccharides. The search for further such ways seems to be most desirable for future progress.

In order to be efficient in practical work such methods should be based on quantitative reactions of high selectivity, and involve the fewest possible stages. However, it should be noted that even those methods which would not involve complete quantitative degradation but guarantee selectivity might be of considerable interest for studying complex polysaccharide systems.

Now, let's consider the second problem the solution of which would facilitate progress in establishing the structure of polysaccharides and polysaccharide fragments of glycoproteins, lipopolysaccharides and other conjugated biopolymers. This implies the development of efficient and, if possible, universal methods for establishing the structure of oligosaccharide fragments arising from the degradation of the polysaccharide chains.

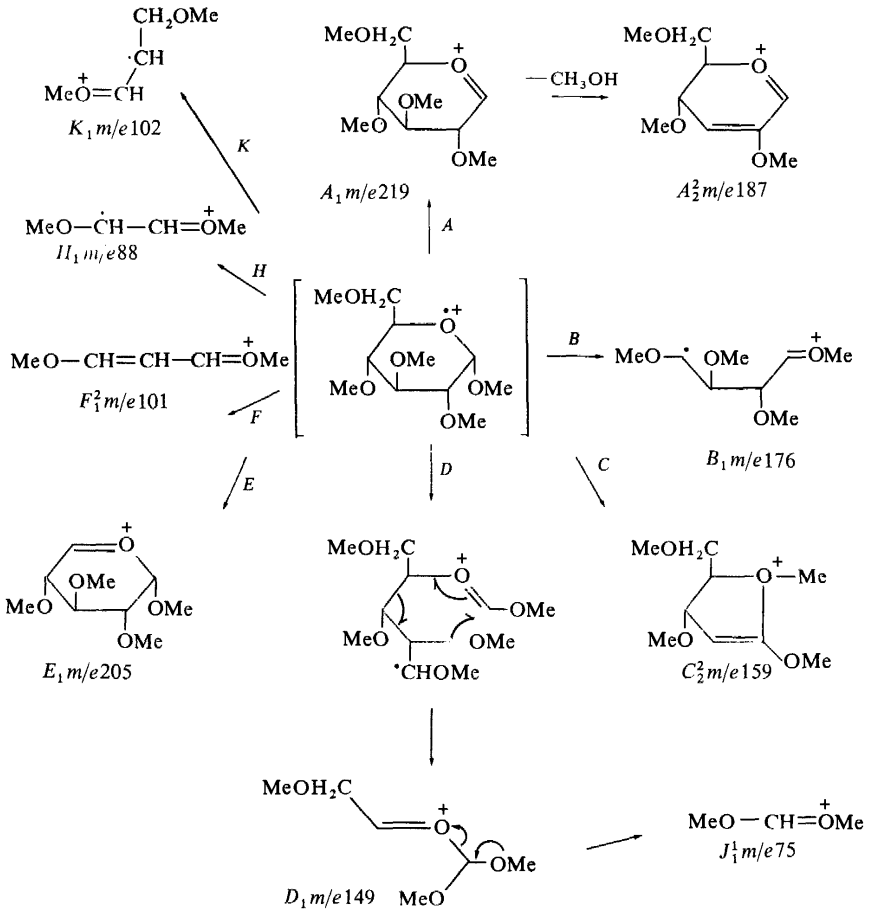
As a rule, this problem is resolved by using methylation and the Smith degradation which show the type of linkages, the general architectonics of a fragment and, if you have luck, short monosaccharide sequences. This analysis does not always provide elucidation of the structure and frequently requires much material, which is seldom available because there is still no efficient and reliable technique for the separation of oligosaccharides.

This problem can be solved more satisfactorily by using sets of glycosidases of high specificity. By selecting various enzymes for successive action it is possible to establish the sequence of monosaccharide units, and, with highly specific glycosidases, the type of a linkage.

Along with the availability of appropriate enzymes, another necessary prerequisite of success is the information about their specificity. Therefore, this way is rather laborious and time-consuming as it requires the search, isolation and study of the enzymes necessary for the solution of each particular problem.

In this connection, the use of mass spectrometry seems to be attractive for

solving the problem. Mass spectrometry was first used in the chemistry of carbohydrates ten years ago[7]. Nowadays, it is quite indispensable for the structural chemistry of sugars. The general scheme of fragmentation of a monosaccharide under electron impact is presented below.



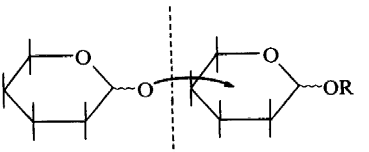
Without entering into details, we should note two features characteristic of these patterns. First there is no molecular ion, which may be accounted for by the lack of electron-accepting groupings stabilizing it. Secondly, there are many different ways of fragmentation which produce complicated patterns. This can also be accounted for by the absence of a stable molecular ion. On the other hand, such diversified degradation makes possible the mass-spectrometric analysis of various derivatives of monosaccharides and related compounds, and mass spectrometry has become a routine analytical technique for establishing the structure of many different monosaccharide derivatives[8]. As for the modern application of mass spectrometry for the structural analysis of polysaccharides, it should be pointed out that,

in combination with g.l.c., it is the best route to identification and structural determination of partially methylated monosaccharides resulting from the solvolysis of methylated polysaccharides.

For this purpose the following procedures are recommended: (1) mass spectrometry of partially methylated glycosides permethylated with deuteromethyl iodide[9]; (2) mass spectrometry of the acetates of partially methylated polyols resulting from reduction of partially methylated aldoses[10]; (3) mass spectrometry of the acetates of partially methylated nitriles of aldonic acids resulting from the conversion of partially methylated aldoses into aldononitriles upon heating with hydroxylamine in pyridine[11].

These methods made methylation a much more convenient procedure.

But at present it has become possible to apply mass spectrometry to the direct analysis of oligosaccharides, and to establish the sequence of monosaccharide units and, in many instances, the type of linkage between them. This analysis is based on the fragmentation pattern of glycosides of a disaccharide under electron impact[12]. In this case both of the monosaccharide units can be considered as independently degraded moieties.



unsubstituted glycoside degradation

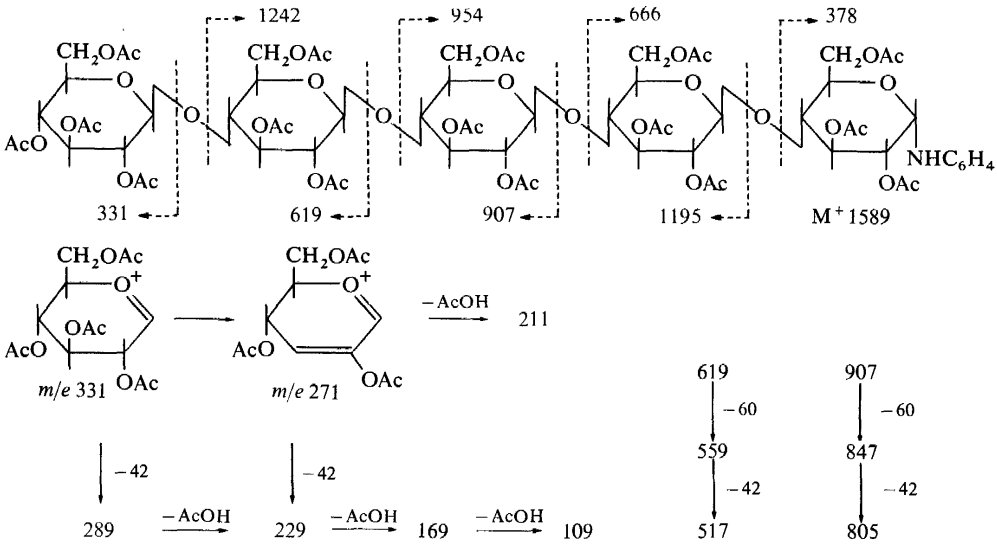
substituted (at C_2, C_3, etc.) glycoside degradation

The non-reducing monosaccharide unit undergoes degradation as does the glycoside of a monosaccharide, and its fragmentation pattern would indicate its location on the non-reducing end. The reducing monosaccharide undergoes degradation as does the monosaccharide substituted either at C_2 or C_3 and so on depending on the type of intermonosaccharidic linkage. The fragmentation pattern of the reducing monosaccharide would indicate its location and the type of a glycosidic linkage.

However, mass spectrometry of high oligosaccharides presented considerable difficulty. Due to a complicated and diversified fragmentation of the monosaccharide units the mass spectra are very complicated and their unambiguous interpretation becomes difficult even for trisaccharide.

The principal difficulty is that all of the oligosaccharide derivatives so far studied—simple glycosides and glycosides of alditols—undergo degradation under electron impact without forming a stable molecular ion. This results in an undirected fragmentation and complicated spectra. After a careful search there was found a derivative which gives a molecular ion and further undergoes well regulated fragmentation of the oligosaccharide chain. It turned out that there should be an aromatic system at the reducing end of the oligosaccharide chain, which stabilizes the molecular ion[13]. Anilides proved to be the most practical derivatives[14]. Oligosaccharides in a micro scale can be readily converted into the acetates of the respective anilides or substituted anilides merely upon heating with an aromatic amine in acetic

acid. The next scheme shows the fragmentation pattern of one such anilide acetate.



The molecular ion of the oligosaccharide undergoes regular fragmentation successfully splitting off the monosaccharide units and breaking the glycosidic linkage either at the carbon of the glycosidic centre, or at the carbon of aglycone. The *m/e* value from the resulting primary fragments suggests the character of the splitting units and hence their sequence. The subsequent degradation of the primary fragments depends on the nature of the glycosidic linkage. Thus, the secondary fragments provide information about the type of intermonomeric glycosidic linkage. It makes it possible to establish the sequence of monosaccharides in an oligosaccharide if these monosaccharides can be differentiated by mass spectrometry. The mass spectra of β-D-Glpl → 3 β-D-Arpl → 6-β-D-Gl can be demonstrated as example (p 63).

There is no doubt that mass spectrometry should make an important contribution to establishing the structure of oligosaccharides. But so far, it cannot distinguish the stereochemistry of systems with non-rigid conformation and hence, the epimeric monosaccharides. However, this may become possible in the near future. In combination with biochemical and immunochemical techniques, mass spectrometry makes structural determination possible with very small amounts of oligosaccharide material. Most likely, this would allow a new technique for establishing the structure of polysaccharides.

In conclusion, it should be added that quite recently it has been demonstrated that mass spectrometry in combination with a computer technique can facilitate simultaneous structural elucidation of several polypeptides without their preliminary separation into individual substances[15]. The extension of this principle to the analysis of oligosaccharide mixtures, where the problem of separation is most intractable would expand the application of mass spectrometry in the chemistry of polysaccharides.

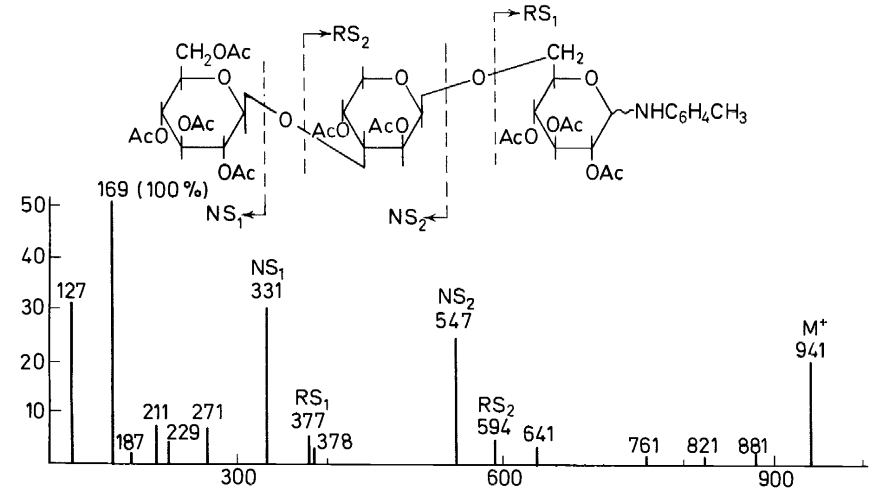


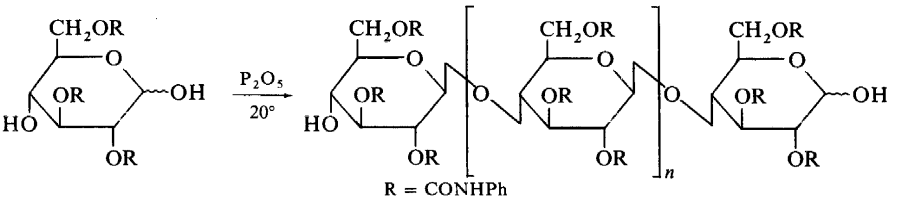
Figure 2

SYNTHESIS OF POLYSACCHARIDES

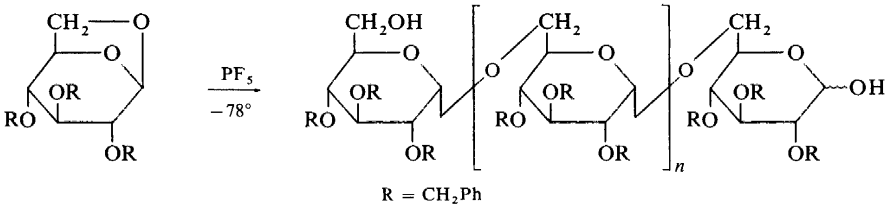
Another aspect in the study of any group of natural compounds is the development of synthetic approaches. Compared with advances in the synthetic chemistry of polypeptides and polynucleotides the progress made in the synthesis of polysaccharides is very modest, and evidently the synthetic chemistry of polysaccharides is still in its infancy. Meanwhile, with no distinct characteristics of individual natural polysaccharides, and also often with the microheterogeneity of carbohydrate chains, any study, no matter how thorough, of a complicated natural polysaccharide would usually give only a very general idea of its structure.

Therefore, the synthesis of polysaccharides with predetermined structure would give models important for physicochemical and biochemical experiments. This would be of particular importance for the study of specificity and the mechanism of carbohydrate metabolism of enzymes, for example, polysaccharidases. It should be remembered that synthetic polypeptides and polynucleotides have played and are still playing a very important role in the solution of many problems of biochemistry and molecular biology.

The directed synthesis of polysaccharides only attracted attention during the last few years. The first process of this kind was probably the re-synthesis of cellulose by polymerization of 2,3,6-tri-*O*-phenylcarbamoyl glucose in dimethylsulphoxide in the presence of phosphorus pentoxide[16]. The starting monomer was prepared from carbamoylcellulose. The stereochemistry



of the synthetic polysaccharide has not been proved in its entirety. More recently Scheuer, Schuerch and collaborators succeeded in the stereospecific synthesis of 1,6-glycans by polymerization of 1,6-anhydromonosaccharides under the action of phosphorus pentafluoride in dichloromethane under mild conditions[17]. The synthetic polysaccharide has only α-glycosidic linkages.

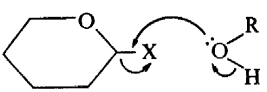


R = CH_2Ph

The procedure required strong protection of secondary hydroxyls at C_2, C_3 and C_4 by benzylation, which is rather disadvantageous as the full removal of benzyl groups from the polysaccharide is still a problem. This important synthesis is of interest but it unfortunately deals only with 1,6-glycans.

One of the most general problems which arises in finding a more general approach to the synthesis of polysaccharide systems with predetermined structures is to develop a suitable method or set of methods for making up a glycosidic linkage. In this connection it should be stressed that the development of efficient methods for the synthesis of peptide and phosphodiester linkages was of prime importance in the synthetic chemistry of peptides and polynucleotides.

The glycosidic linkage is formed from a reaction of the general type shown below.



This means electrophilic substitution at the oxygen atom of the hydroxyl group or, respectively, nucleophilic substitution at the carbon atom of the glycosidic centre. The success of the reaction depends on the activation of both reaction centres, that is the increase of electron accepting properties of the glycosidic centre and electron donating properties of the oxygen of the hydroxyl group in the aglycone under glycosylation (p. 65).

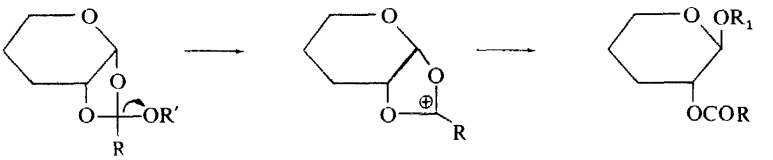
First, I would like to discuss the activation of the glycosidic centre. Until recently the only method for such activation was the use of acetohalogenoses on which the famous Koenigs–Knorr reaction is based. For 70 years this reaction has been used as the only practical way to glycosylation.

Well-known weak points of this reaction are insufficiently high stereospecificity and frequently variable yields. I would like to mention also that this method in its modern modifications is unsuitable for the synthesis of polysaccharides by polymerization of monosaccharides, since acetohalo-

genoses with a free hydroxyl group are not stable, and cannot be used as suitable monomers.

During the last few years we succeeded in finding a new method of glycosylation, which is based on a different principle of glycosidic centre activation. This method has a good promise for the synthesis of polysaccharides.

According to this method sugar 1,2-ortho-esters are used as glycosylating agents, which react with the hydroxyl groups of aglycon in the presence of an acid catalyst[18]. The mechanism of the reaction can be represented as follows:



The splitting of the alkoxyl gives rise to an ambident cation which is subjected to an attack by the hydroxyl group of aglycon from the rear leading to strongly stereospecific substitution at the glycosidic centre.

The strong stereospecificity of the reaction is a particular advantage of this method. There are some modifications of the reaction which include the use of mercury bromide as a catalyst in boiling nitromethane[18] or lutidinium perchlorate in boiling dichloroethane or chlorobenzene[19]. In all cases the reaction proceeds in homogeneous media, under rather mild conditions and gives consistent and relatively high yields.

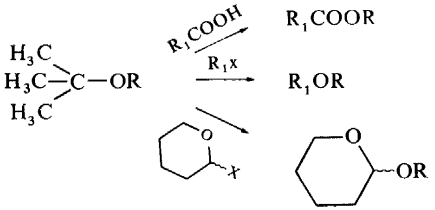
Up to now this so-called 'ortho-esters method' has been used for the synthesis of glycosides and lower oligosaccharides of various types[20].

As will be seen this method can be used as a basis for the synthesis of the polysaccharide systems.

And now a few words about the activation of the other reaction centre involved in the glycosylation—the oxygen atom of the hydroxyl group in the aglycone under glycosylation. As has been mentioned already, this means increasing the electron donating property of the oxygen atom in the electrophilic substitution. It may seem strange that this problem, common for organic chemistry, concerned with such reactions as acetylation and alkylation of the hydroxyl groups, has not been investigated and was solved sometimes only by using alcoholate of various metals. But alcoholates were not used in the reaction of glycosylation because of some difficult technical problems, and a problem of solubility in particular.

Although no really practical method for activating a hydroxyl group has been developed so far there is one observation which may have some promise. It has been found that tertiary-butyl ethers of alcohol can enter into electrophilic substitution at the oxygen (alkylation[21], acylation[22] and glycosylation[23]) in the presence of acidic catalysts, with elimination of isobutylene, the yields being slightly higher than those usually obtained by acetylation,

acylation or glycosylation of alcohols. It has been shown experimentally that there is no preliminary degradation of tert-butyl ether, and that tert-butyl ether undergoes direct substitution.



The principle seems to be of a general character, since other ethers of tertiary alcohols produce the same effect and there is hope that this would be a real approach to activation of the hydroxyl group.

Preliminary experiments show that this method is suitable for making up glycosidic linkages in oligosaccharide synthesis[24]. For example, we were able to prepare β-D-glucopyranosyl-1 → 6-D-galactose and β-D-glucopyranosyl 1 → 6 D-glucose by glycosylation of 6-*O*-tert-butyl-1,2; 3,4-diisopropylidene galactose and -1,2,3,4-tetra-*O*-acetyl glucose.

Now let me return to the problem of the synthesis of polysaccharide systems.

Provided there are efficient methods for the formation glycosidic linkages there should be two ways for the synthesis of polysaccharide systems. To synthesize heteropolysaccharides with any sequence of monomeric units there is only one way—step-by-step synthesis with successive 'seaming' of the blocks. With polyfunctional monomers, stereochemical problems, and the required flexible protection of the extra hydroxyl groups, this problem presents considerable difficulties and in general is far from solution for the synthesis of higher oligosaccharides or polysaccharides.

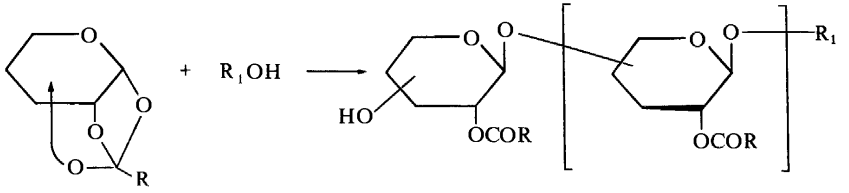
Fortunately, most polysaccharide systems, including many biologically important polysaccharides, are block-polymers, consisting of repeated units, or homopolysaccharides. The synthesis of such polysaccharides seems to be more real, and the importance of this task cannot be refuted.

To synthesize such systems it is reasonable to use polymerization of the respective monomers. For the synthesis of a homopolymer with one type of linkage the respective monosaccharide derivative should be used as a monomer, and for the synthesis of heteropolysaccharides with a repeated unit a derivative of an oligosaccharide corresponding to the repeated unit should be used.

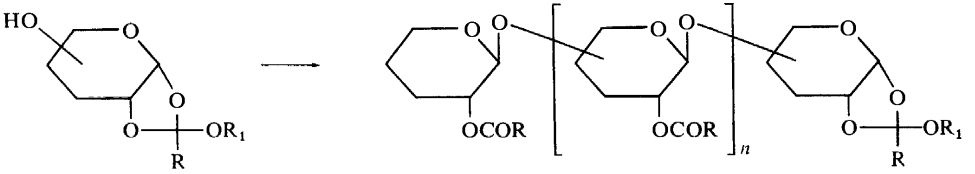
Polymerization and polycondensation of suitable halogenose derivatives according to the Koenigs–Knorr reaction failed due to instability of the monomers of this type.

On the contrary, the orthoester method of glycosylation holds promise as it allows us to carry out the synthesis of several polysaccharides. The polymeric systems can be obtained on the basis of ortho-esters in two ways. One is the polymerization of the internal ortho-esters which proves to be a stepwise polymerization in the presence of a hydroxyl containing initiator,

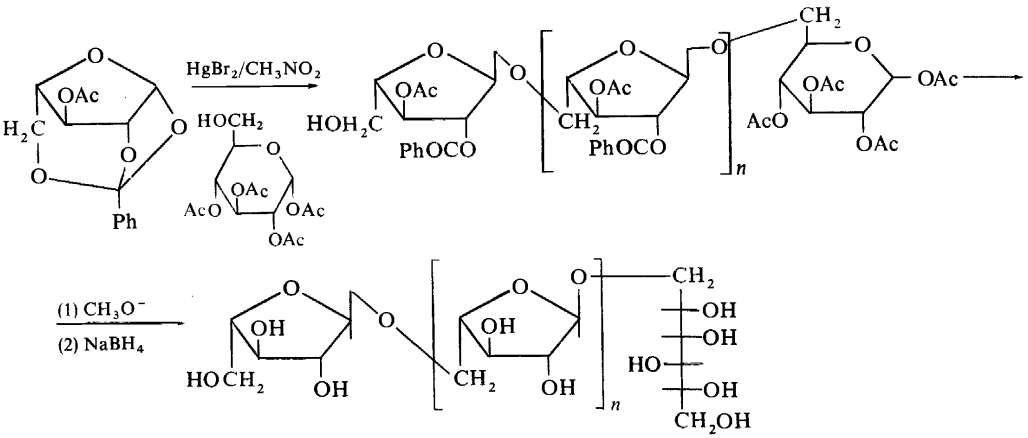
its molecule occupies the reducing end of the growing polysaccharide chain.



The other way, a more general and more important one, is the polycondensation of monomers with an ortho-ester group and a free hydroxyl group capable of glycosylation. These monomers can be the derivatives of both monosaccharides and oligosaccharides.

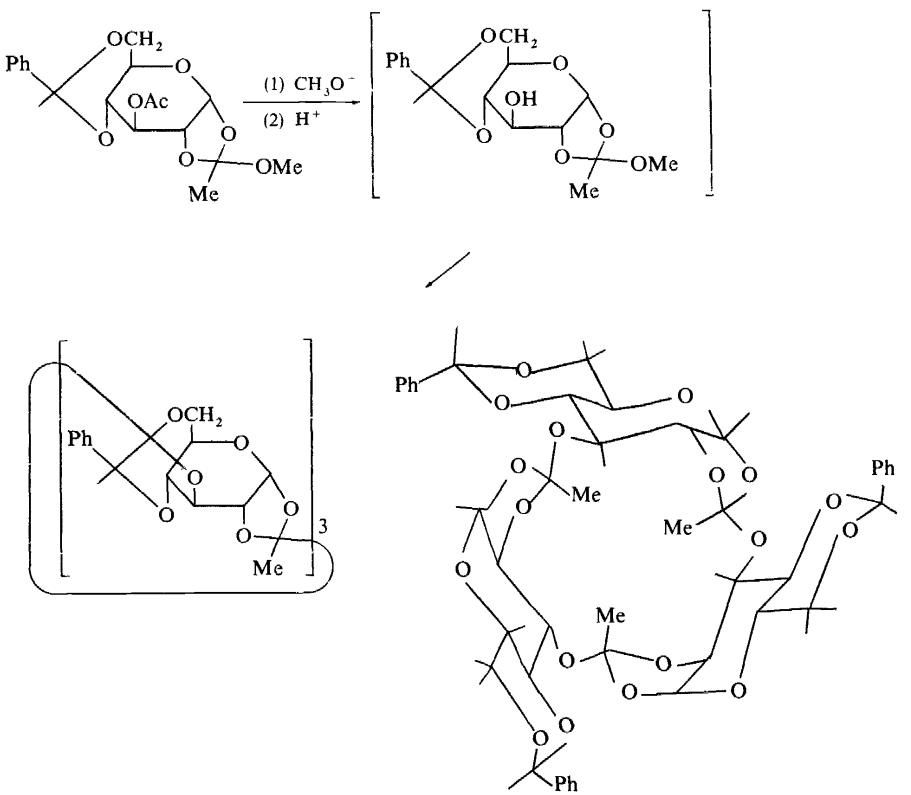


Polymerization of internal ortho-esters can be illustrated by the synthesis of arabinan containing arabofuranose units bound by 1,5-linkages whose structure is close to that of some natural arabinans[25]. 1,2,5-Orthobenzoate of arabofuranose was used as a monomer. Polymerization of this ortho-ester proceeds in boiling nitromethane in the presence of mercury bromide, using 1,2,3,4-tetraacetate of glucose as an initiator. After isolation and deacetylation of the polysaccharide according to the standard procedure, the terminal glucose residue was reduced with $NaBH_4$. The degree of polymerization of the fraction with a higher molecular weight isolated with a yield of 20 per cent was about 20 to 25 calculated from the arabinose:sorbitol ratio. It should be emphasized that as would be expected the degree of polymerization depends on a monomer:initiator ratio.



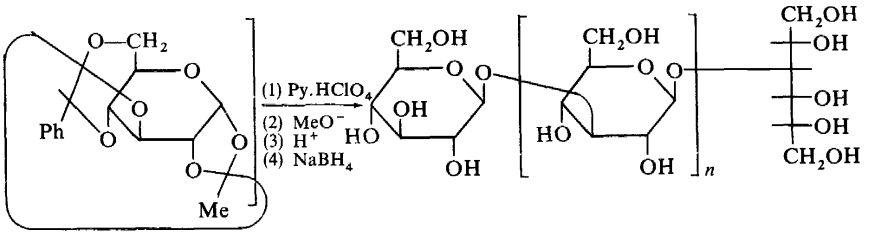
The polymerization proved to be highly stereospecific since the synthetic arabinane can be completely cleaved by highly specific α-arabinase. By using methylation and periodate oxidation it has been shown that about 95 per cent of the glycosidic linkages are 1,5. But there are some 1,2-linkages, which is indicated by the fact that a small amount of 3,5-*O*-dimethylarabinose was isolated from the hydrolysate of the methylated polymer. There is no final explanation for these anomalous linkages, but most likely they arise as a result of the migration of the acetyl groups during polymerization at the moment of opening the ortho-ester cycle.

Another example of a more successful preparation of synthetic polysaccharide by polymerization of internal ortho-esters is the synthesis of β-1,3-glucane. Its properties completely correspond to those of the so-called G-chain of natural laminarine isolated from *Laminaria japonica*. The starting monomer for this synthesis is the trimeric macrocyclic ortho-ester which forms rather unexpectedly as a result of spontaneous cyclization[26]:



The structure of the macrocyclic ortho-ester was supported by chemical and physicochemical methods, including n.m.r. spectroscopy. Upon heating in dichloroethane in the presence of lutidinium perchlorate, the macroester gives a polymer with a yield of about 50 per cent. After removal of the benzylidene groups by a mild acidic hydrolysis and reduction of the terminal

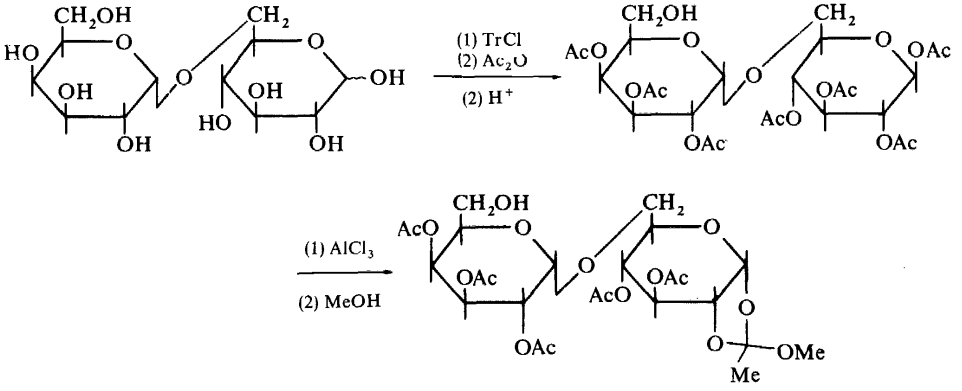
glucose residue with $NaBH_4$ there was obtained a polysaccharide[27], whose physicochemical properties completely correspond to those of a natural laminarine.



As I've mentioned before it would be more promising for the preparation of synthetic polysaccharides to use polycondensation of monomers with an ortho-ester grouping and a hydroxyl group capable of glycosylation. Particularly it would be more thrilling to use it for the preparation of heteropolysaccharides with an alternating repeated unit. Unfortunately this proved to be difficult because suitable and universal conditions for polycondensation are still to be found. But the first experiments on polycondensation give hope that this method would open up a way to the synthesis of regular heteropolysaccharides.

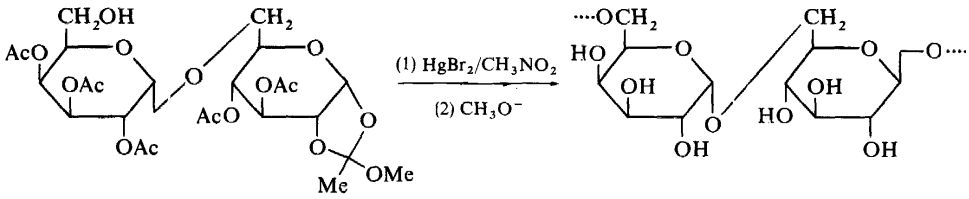
The experiments on the preparation of a galactoglucane with a repeated sequence[28] can be used as an illustration.

A scheme for the synthesis of a derivative of melibiose which was used as a monomer is outlined below.



The scheme involves tritylation of melibiose in order to protect the primary hydroxyl, acetylation of secondary hydroxyls, and introduction of an ortho-ester grouping by the well-known method at the last stage of the synthesis. Thus, in the resulting monomer there is a glycosylating ortho-ester grouping in the reducing unit of glucose and a free primary hydroxyl group at the galactose end.

This monomer undergoes polycondensation in boiling nitromethane, in the presence of mercury bromide, and results in a galactoglucane:

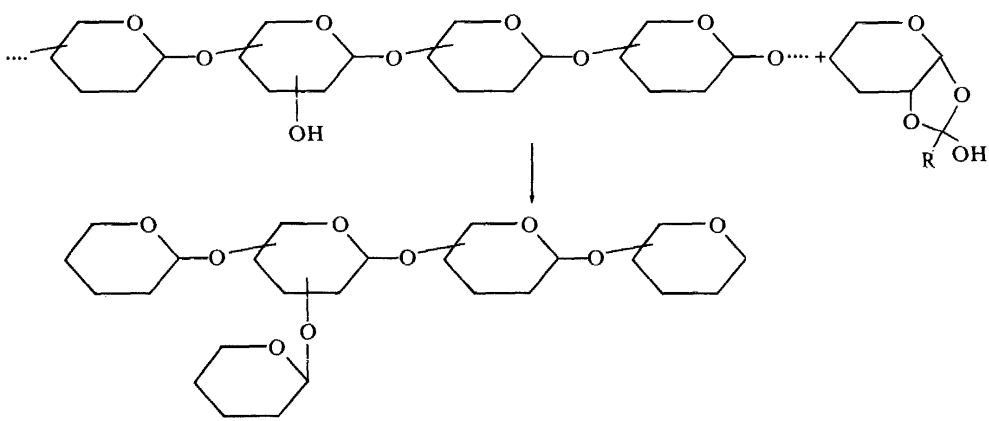


The product of the polycondensation was obtained in low yield (about five per cent) and a low degree of polymerization (about four or five), and was an octa- or deca-saccharide.

The reason for this insufficiently intense process of polycondensation is connected with a low reactivity of the primary hydroxyl group in the residue of 2,3,4-triacetate galactose. It has been found that this hydroxyl group is rather inert in such reactions as methylation, acylation or glycosylation. The reason for this is not clear.

This experiment has demonstrated the first synthesis of a polysaccharide fragment with a regularly alternating sequence of monosaccharide units. And despite a very modest yield and molecular weight it gives hope that the polycondensation method would open up a way to heteropolysaccharides of regular structure.

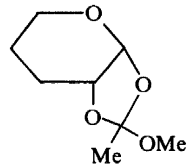
One of the considerable disadvantages of the above approach to the synthesis of polysaccharides on the basis of polymerization and polycondensation of ortho-esters is a possible migration of the protecting groups in the monomer. We were sure in some cases that acetyl and benzoyl protection is not always reliable under the conditions of ortho-ester condensation. This is understandable, since these conditions are favourable for the formation of carbonium ion which is proved to be an intermediate in the process of the migration of acetyl groups. It is possible that the acetal protection, a benzylidene grouping for example, may also prove insufficient under these conditions. To find a more reliable protection is one of the most important problems to be solved. This is also true for the synthesis of oligosaccharide systems, in general, and is one of the general problems in the synthetic chemistry of carbohydrates.



As for the problem of the synthesis of polysaccharides as a whole, it is necessary to mention one more possibility for their preparation. I mean the preparation of semi-synthetic polysaccharides by modification of natural polysaccharides, in particular the synthesis of branched polysaccharides from linear ones, according to the general scheme (p. 70).

An example of such synthesis is the preparation of a branched semi-synthetic glucane from cellulose[29]. The starting compound for this synthesis was cellulose diacetate with free primary hydroxyl groups. After its interaction with the acetylated ortho-ester of glucose in boiling chlorobenzene in the presence of lutidinium perchlorate, and subsequent removal of the acetyl groups, there was obtained a branched glucane, which unlike classical insoluble cellulose, can be dissolved in water.

Cellulose diacetate (DS 2.2) +



(1) Lut.$HClO_4$/C_6H_5Cl
(2) CH_3O^-

$\ldots Gl1 \rightarrow 4Gl1 \rightarrow 4Gl1 \rightarrow 4Gl1 \rightarrow 4Gl \ldots$

2(3) 2(3)

$Gl1$ $Gl1$

The structural studies of the semisynthetic glucane by periodate oxidation have shown that every other glucose unit of the backbone has a branch consisting of one glucosyl unit. But it was quite unexpected to find that these branches are located not at the primary hydroxyl groups of the backbone, which were free in the starting diacetate of cellulose, but at the secondary hydroxyl groups C_2 and C_3. This can be accounted for only by migration of the acetyl groups in the starting compound during the synthesis. Since from steric restrictions the direct migration of the acyl residue from C_6 to C_3 or C_6 to C_2 is impossible, it is most reasonable to suggest that such migration occurs between the neighbouring glycosyl units, so that the C_3-acyl group of one unit migrates via the formation of an intermediate acyloxonium ion to C_6 of the neighbouring unit.

It is natural to expect that this principle could be applied to the synthesis of branched polysaccharides by one-unit-branching or by engrafting of linear polysaccharides.

This paper is concerned with some general problems of the chemistry of polysaccharides which, in the author's opinion, seem to be most important and fascinating. Some possible ways to the solution of these problems have been discussed. The examples given here for illustration show, to some extent, the author's inclination.

What will be the actual way of development of the chemistry of polysaccharides is a matter of the future. But as the great significance of this branch of the chemistry of natural products for the solution of important biological problems is unquestionable, it seems reasonable and useful to discuss possible alternatives which could be developed.

REFERENCES

[1] N. K. Kochetkov, O. S. Chizhov and A. F. Sviridov, *Carbohyd. Res.* **5**, 492 (1967).
[2] N. K. Kochetkov, O. S. Chizhov and A. F. Sviridov, *Carbohyd. Res.* **14**, 277 (1970).
[3] N. K. Kochetkov, O. S. Chizhov and A. F. Sviridov, *Izvest. Akad. Nauk SSSR*, Ser. chim, 1920 (1969).
[4] N. K. Kochetkov, O. S. Chizhov and A. F. Sviridov. To be published.
[5] B. Lindberg and H. Lundström, *Acta Chem. Scand.* **20**, 2423 (1966).
[6] O. Larm, B. Lindberg and S. Svensen, *Carbohyd. Res.* **20**, 39 (1971).
[7] N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov and B. M. Zolotarev, *Dokl. Akad. Nauk SSSR*, **147**, 1369 (1962).
K. Biemann, H. K. Schnoes and I. A. McCloskey, *Chem. & Ind.* (*London*), 148 (1963).
[8] N. K. Kochetkov and O. S. Chizhov, *Advanc. Carbohydr. Chem.* **21**, 39 (1966).
[9] N. K. Kochetkov and O. S. Chizhov, *Biochim. Biophys. Acta*, **83**, 134 (1964).
[10] L. S. Golovkina, O. S. Chizhov and N. S. Wulfson, *Izvest. Akad. Nauk SSSR*, 1915 (1966).
H. Bjorndal, B. Lindberg and S. Svensen, *Carbohyd. Res.* **5**, 433 (1967).
[11] B. A. Dmitriev, L. V. Backinovsky, O. S. Chizhov, B. M. Zolotarev and N. K. Kochetkov, *Carbohyd. Res.* **19**, 432 (1971).
[12] N. K. Kochetkov, O. S. Chizhov and L. A. Polyakova, *Dokl. Akad. Nauk SSSR*, **158**, 685 (1964).
[13] G. S. Johnson, W. S. Ruliffson and R. G. Cooks, *Chem. Commun.* 587 (1970), N. K. Kochetkov, O. S. Chizhov, N. N. Malysheva and A. I. Shiyonok, *Org. Mass Spectr.* **5**, 481 (1971).
[14] O. S. Chizhov, N. K. Kochetkov, N. N. Malysheva, A. I. Shiyonok and V. L. Chashchin, *Org. Mass Spectr.* **5**, 1157 (1971).
[15] F. W. McLafferty, XXIII IUPAC Congress, Boston, 1971, Vol. 7, p 61. Butterworths: London (1971).
[16] H. O. Huseman and H. O. J. Müller, *Makromol. Chemie*, **49** 238 (1961).
[17] E. R. Rückel and C. Schuerch, *J. Org. Chem.* **31**, 2233 (1966).
[18] N. K. Kochetkov, A. Ja. Khorlin and A. F. Bochkov, *Tetrahedron Letters*, 289 (1964); *Tetrahedron*, **23**, 693 (1967).
[19] N. K. Kochetkov, A. F. Bochkov, V. A. Snjatkova and T. A. Sokolovskaya. *Carbohyd. Res.* **16**, 17 (1971).
[20] N. K. Kochetkov and A. F. Bochkov, *Recent Developments in the Chemistry of Natural Carbon Compounds*, Vol. IV, p 77. Akademiai KIADO: Budapest (1971).
[21] V. A. Derevitskaya, E. M. Klimov and N. K. Kochetkov. To be published.
[22] N. K. Kochetkov, V. A. Derevitskaya and E. M. Klimov, *Tetrahedron Letters*, 4269 (1970).
[23] N. K. Kochetkov, V. A. Derevitskaya and E. M. Klimov, *Tetrahedron Letters*, 4769 (1969); *Dokl. Akad. Nauk SSSR*, **192**, 336 (1970).
[24] N. K. Kochetkov, E. M. Klimov and V. I. Torgov. Unpublished results.
[25] N. K. Kochetkov, A. F. Bochkov and I. G. Yazlovetsky, *Carbohyd. Res.* **9**, 49 (1969).
[26] N. K. Kochetkov and A. F. Bochkov, *Tetrahedron Letters*, 4669 (1967).
[27] N. K. Kochetkov and A. F. Bochkov, *Carbohyd. Res.* **9**, 61 (1969).
[28] N. K. Kochetkov, A. F. Bochkov and I. G. Yazlovetsky, *Izvest. Akad. Nauk SSSR*, 1802 (1968).
[29] N. K. Kochetkov, A. F. Bochkov and T. A. Sokolovskaya, *Carbohyd. Res.* **19**, 1 (1971).