

PATHWAYS FOR THE SYNTHESIS OF SPECIFIC POLYSACCHARIDES

N. K. KOCHETKOV

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

ABSTRACT

Many specific polysaccharides, such as microbial ones, are block-polymers consisting of repeating oligosaccharide units. The search for approaches to the synthesis of these biopolymers requires, first, the development of the synthesis of oligosaccharides and, secondly, new methods for the polymerization of oligosaccharide blocks.

The first problem is related to the synthetic chemistry of oligosaccharides, and is illustrated here by the synthesis of trisaccharides, one of which is a repeating unit of *O*-antigenic polysaccharide from *Salmonella anatum*.

The second problem has not been solved yet, and therefore only some possible approaches to its solution have been considered: (1) polycondensation of oligosaccharide blocks; (2) stepwise extension of the polymeric chain; and (3) biochemical polymerization.

The data on a new glycosylation reaction based on generation of the 1,2-acyloxonium cation upon interaction of Fletcher's nitrile with tritylium salts are presented here, and a suggestion is made for a new method of polycondensation of carbohydrate derivatives. Other possible ways for conversion of oligosaccharide blocks into block-polymer systems are also considered.

INTRODUCTION

It is generally accepted now that the polysaccharides or a polysaccharide component of conjugated biopolymers play an important role in many biological processes, such as highly specific interaction between the cell surface and its outer surroundings.

So far, our knowledge about the carbohydrate structures responsible for this high specificity is rather limited. But it is already clear that this specificity is due to polysaccharides of various types: branched homopolysaccharides, such as yeast mannans; heteropolysaccharides with nonregular chains, such as the carbohydrate chains of blood-group substances; and finally, regular heteropolysaccharides consisting of repeating oligosaccharide units, which are in fact typical block-polymers.

The last-mentioned type of specific polysaccharides, which appear to be most common, have been studied in greater detail. Related to this type are the polysaccharides of sea-weeds and connective tissues, and, especially,

capsular and *O*-antigenic polysaccharides of microorganisms.

A large amount of data now available on the structure of microbial polysaccharides made it possible to undertake the study of the relationships between the structure of a polymer and its biological specificity which is fundamental for the chemistry of biopolymers.

It is well known from the history of protein and nucleic acid research that the development of synthesis contributes much to the general success in this field. But unlike the chemistry of polypeptides and polynucleotides, where the general strategy of the synthesis has been developed, the synthesis of polysaccharides of block-polymer type has not been subjected to any detailed study and discussion.

This lecture makes an attempt to discuss in general the problems arising in this connection, and some possible ways of solving them. By way of illustration, use is made of the results obtained during the last one or two years in this laboratory.

Since the polysaccharides are block-polymers, it seems reasonable to use the block-polymerization principle for their synthesis. Therefore, in general, the synthesis of regular heteropolysaccharides involves the solution of two independent and quite different problems: first, the synthesis of the oligomeric block which is the 'repeating unit' of the polysaccharide and, secondly, polymerization—to be more exact, polycondensation of these oligomers into polysaccharides of the appropriate molecular weight. No doubt, the second problem is the more difficult to solve.

It should be emphasized that at the second stage of the synthesis, in the oligomeric block which plays the role of monomer, the hydroxyls and other functional groups which are not involved in polycondensation must be protected, and it must contain the corresponding groups responsible for the polycondensation to form a monomeric linkage of the required type. The nature of these protecting and activating groups depends on the polycondensation method used, and this should be taken into account when planning the synthesis in order to obtain the required combination of substituents in a polyfunctional molecule in the course of the synthesis.

The first problem, the synthesis of the oligosaccharide block which is the starting monomer for polycondensation, belongs to the synthetic chemistry of oligosaccharides (for review, see reference 1). Recently, much attention has been given to the synthesis of oligosaccharides, and it seems necessary to discuss this problem in general; but I should just like to point out that its success depends on the solution of two principal problems: (1) to elaborate stereospecific methods for the formation of the glycosidic linkage and (2) to enrich the choice of stereospecific protection for the hydroxyls and other functional groups.

The choice of protection presents even greater difficulties in the synthesis of complex oligosaccharide fragments, such as trisaccharides which contain various monosaccharide residues.

The problems arising during the synthesis of oligosaccharides can vary, depending on the class of the oligosaccharides (monosaccharide composition, type of glycosidic linkages) and the ultimate object. Therefore it seems more reasonable to discuss some particular oligosaccharide syntheses rather than to speak about them in general.

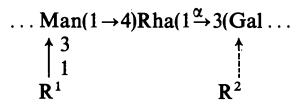
SYNTHESIS OF OLIGOSACCHARIDES

Recently, this laboratory has begun studying the approaches to the synthesis of *O*-antigenic polysaccharides from some *Salmonella* species. This work is in its initial stage but some results will be presented here by way of illustration. As is known from the literature, no synthesis of *O*-antigenic polysaccharides has been undertaken so far, although reports by Garregg and co-workers² are well known on the synthesis of disaccharides which are polysaccharide fragments from *Salmonella*, corresponding to certain immunological factors of these polysaccharides.

It has so far been established with certainty that *O*-antigenic polysaccharides from *Salmonella* are block-polymers which consist of repeating oligosaccharide blocks containing from three to six monosaccharide units³. The main backbone of these oligosaccharides is different for different *Salmonella* groups. The oligosaccharide blocks from many groups of *Salmonella* have the same sequence of mannopyranosyl 1→4 rhamnopyranosyl 1→3-galactopyranose, the mannose linkage having α- or β-configuration (*Table 1*). The polysaccharides from *Salmonella* with β-mannosyl linkages are listed in *Table 2*. The simplest linear trisaccharide with β-mannosyl linkage is a repeating unit in *S. anatum*, whereas in *O*-polysaccharides of other *Salmonella* the repeating unit is the branched oligosaccharide.

Recently we have completed the synthesis of two trisaccharides of this type. One of these is the repeating unit of the polysaccharide from *S. anatum*, β-D-mannopyranosyl = 1 → 4-α-L-rhamnopyranosyl-1 → 3-D-galactose(I)⁴. This is the first synthesis of the complete repeating unit of *O*-antigenic polysaccharide. The second is its corresponding isomer with α-D-mannopyranosidic linkage: α-D-mannopyranosyl-1 → 4-α-L-rhamnopyranosyl = 1 → 3-D-galactose(II)⁵, the common backbone of the oligosaccharide blocks of *O*-antigenic polysaccharides from some *Salmonella* of groups A, B and D. Also completed is the synthesis of the corresponding glucosyl-analogue,

Table 1. *O*-specific chains of *Salmonella* lipopolysaccharides



Serological group	Linkages		R ¹	R ²
	Man → Rha	Gal → Man		
A	α	1 ^α →2	paratose	Glc(1 ^α →4)
B	α	1 ^α →2	acetylabequose	Glc(1 ^α →4)
D ₁	α	1 ^α →2	tyvelose	Glc(1 ^α →4)
D ₂	β	1 ^α →6	tyvelose	Glc(1 ^α →4)
E ₁	β	1 ^α →6	—	Ac
E ₂	β	1 ^α →6	—	Glc(1 ^α →4)
E ₃	β	1 ^α →6	—	Glc(1 ^α →4)
E ₄	β	1 ^α →6	—	Glc(1 ^α →6)

Table 2. Repeating units of *O*-antigenic polysaccharides from *Salmonella*

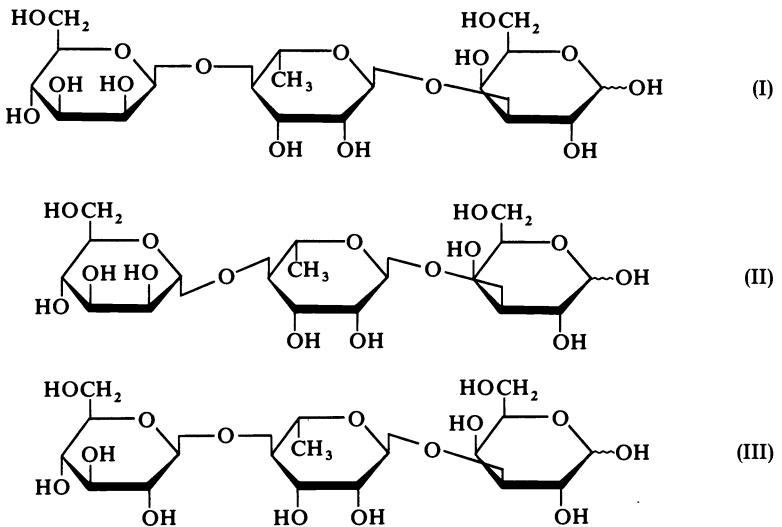
<i>S. anatum</i>	Man 1 β →4 Rha 1 α →3	Gal Glc 1 ↓ [?] 4
<i>S. muenster</i>	Man 1 β →4 Rha 1 α →3	Gal Glc 1 ↓ α 6
<i>S. seftenberg</i>	Man 1 β →4 Rha 1 α →3 Tyv 1 ↓ α 3	Gal Glc 1 ↓ α 4
<i>S. strassbourg</i>	Man 1 β →4 Rha 1→3	Gal

β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnopyranosyl-1 \rightarrow 3-D-galactose(III)⁶, whose accessibility makes it a convenient model for many experiments.

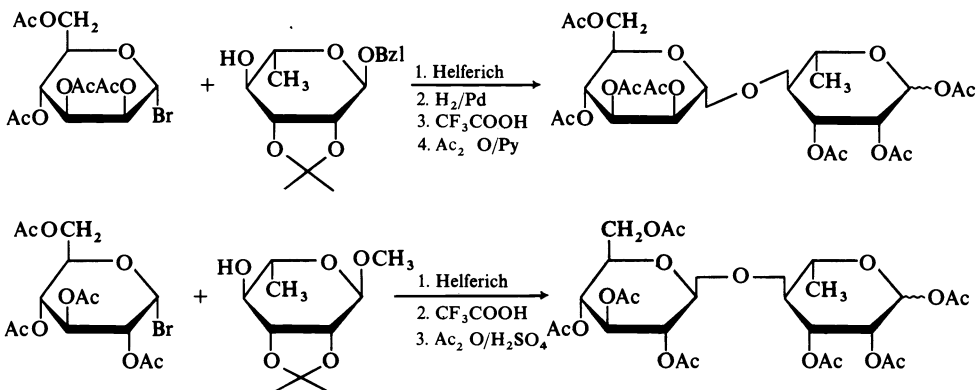
The synthesis of the three trisaccharides was carried out according to the same general scheme, and involved the extension of the monosaccharide sequence from the non-reducing to the reducing terminal.

It was not difficult to obtain the reducing disaccharide sequence for oligosaccharides II and III: the derivatives of β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnose and α -D-mannopyranosyl-1 \rightarrow 4- α -L-rhamnose were obtained in high yields upon Helferich condensation of the corresponding aceto-

SCHEME 1



SCHEME 2



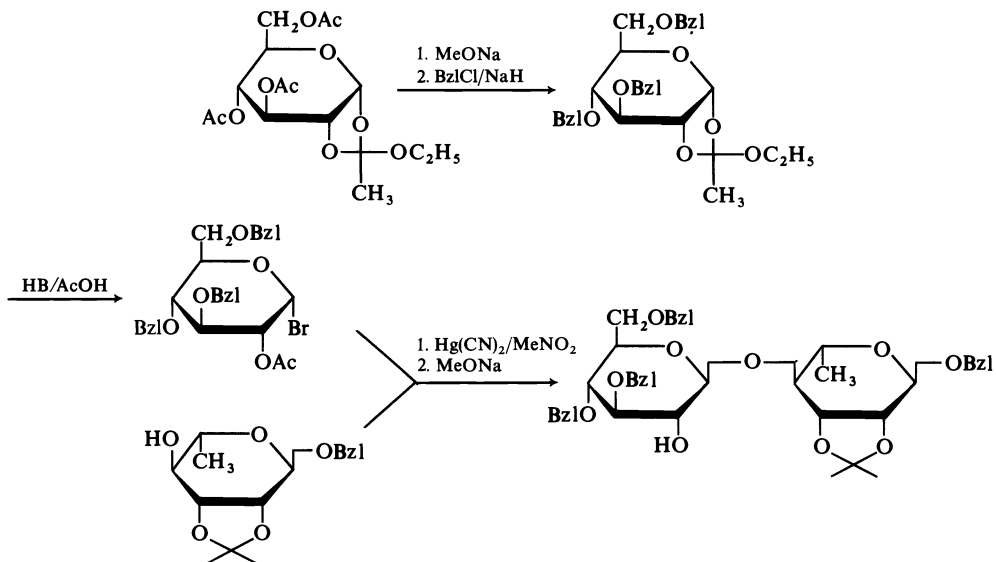
halogenoses with 2,3-isopropylidene- α -L-benzyl-(or methyl)-rhamnopyranoside. After removal of the isopropylidene group by treatment with trifluoroacetic acid and hydrogenolysis or acetolysis of the glycosyl moiety, the disaccharide was treated with acetic anhydride in pyridine. The peracetate obtained, which according to n.m.r. spectra was largely β -acetate, was used as the starting compound for the subsequent synthesis.

It was much more difficult to obtain the β -D-mannopyranosyl-1 \rightarrow 4- α -L-rhamnose required for the synthesis of the repeating unit of the polysaccharide *S. anatum*.

It is well known that a direct synthesis of the β -D-mannosidic linkage presents considerable difficulties that have not been fully overcome as yet (see, for example, reference 7). Therefore, in this case an indirect synthesis has proved to be more practicable⁴. It is based on the data by Theander on the reduction of hexos-2-ulosides⁸. For the synthesis of β -D-mannosyl-1 \rightarrow 4-L-rhamnose, first, β -D-glucopyranosyl-1 \rightarrow 4-L-rhamnose was obtained, and its glucose residue was converted into that of mannose by oxidation and subsequent stereospecific reduction of the glucose residue at C_2 . Independently, Lindberg and co-workers have recently reported the synthesis of β -D-mannosyl-1 \rightarrow 6-galactose based on the same principle⁹.

For this purpose, a mobile protection of the hydroxyl group at C_2 of the glucose residue and a more stable protection of other hydroxyls had to be found. The ortho-ester group proved to be most convenient as a protecting group. The completely acetylated D-glucopyranosyl-1,2-orthoacetate was saponified and benzylated with benzyl chloride in the presence of sodium hydride. The 3,4,6-tri-O-benzyl-L-glucopyranosyl-1,2-orthoacetate obtained was treated with a solution of hydrogen bromide in acetic acid to give the corresponding halogenose, which, upon condensation with 2,3-isopropylidene-benzyl-L-rhamnopyranoside, gave a disaccharide derivative. It should be noted that direct glycosylation by benzylated ortho-ester produced poor results¹⁰: the disaccharide was obtained with a rather low yield, the corresponding derivative of laevoglucosane (1,6-anhydroglucopyranose) being largely formed. This intramolecular glycosylation of benzyl ethers which is

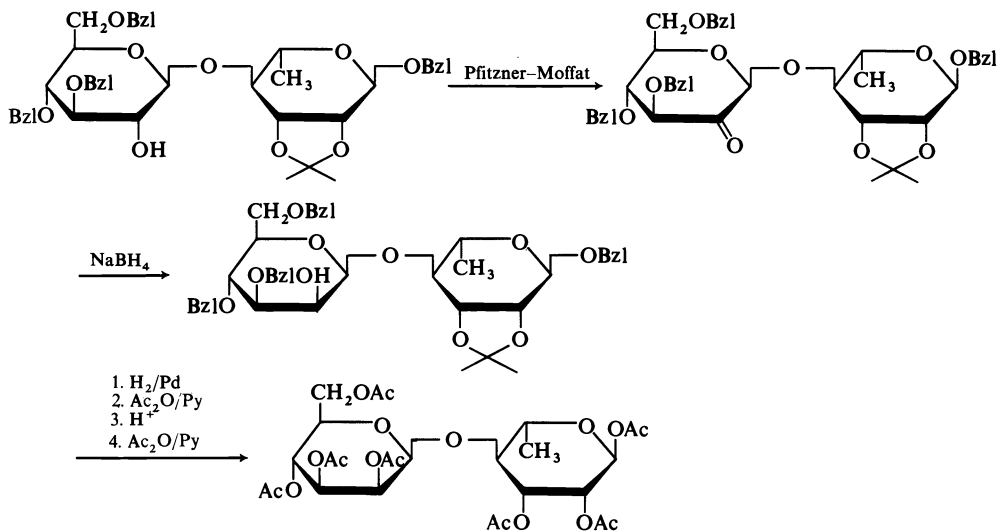
SCHEME 3



reminiscent of the Brederick reaction has been encountered for the first time, and should be studied separately.

After removal of the acetyl group at C_2 by sodium methylate, the disaccharide derivative with a free hydroxyl group was oxidized with Pfitzner–Moffat reagent, which produced more satisfactory results than any other method. After crystallization from alcohol, ketone was isolated with a 70 per cent yield as a semi-acetal derivative, as evidenced from i.r. and n.m.r. spectra.

SCHEME 4



Theander⁸ studied the reduction of simple glycosides of *arabino*-hexos-2-ulose and established that the stereospecificity of the reduction depends on the nature of the reducing agent. He recommended the use of Adams platinum catalyst, over which the ratio of mannose/glucose in the reduction product is 19:1. As was found, the reduction of disaccharide also proceeded stereospecifically to give largely a mannose derivative. Unfortunately, the reduction of the carbonyl group is apparently accompanied by a side reaction of hydrogenation of the aromatic rings of benzyl residues, which was to be expected with the Adams catalyst. This side reaction causes some difficulties on account of the subsequent removal of the protecting groups which makes the yields of β -D-mannopyranosyl-1 \rightarrow 4-L-rhamnose unstable and sometimes low.

Theander also found that on reduction of hexosulose with sodium borohydride the mannose/glucose ratio is 2:1, which is not satisfactory for a multi-stage synthesis. A detailed study of the problem¹¹ has shown that this ratio is also temperature-dependent. It increases in favour of mannose as the temperature increases. It was found that reduction of hexosulosyl-rhamnose derivative with sodium borohydride at 60° in aqueous ethanol is practically stereospecific and gives β -D-mannopyranosyl-1 \rightarrow 4-L-rhamnose with a high yield and without noticeable admixture of glucose derivatives. The disaccharide derivative thus obtained was subjected to hydrogenolysis over palladium, treated with trifluoroacetic acid to eliminate the isopropylidene group, and acetylated with acetic anhydride in pyridine to give a peracetate of β -D-mannosyl-1 \rightarrow 4-L-rhamnose.

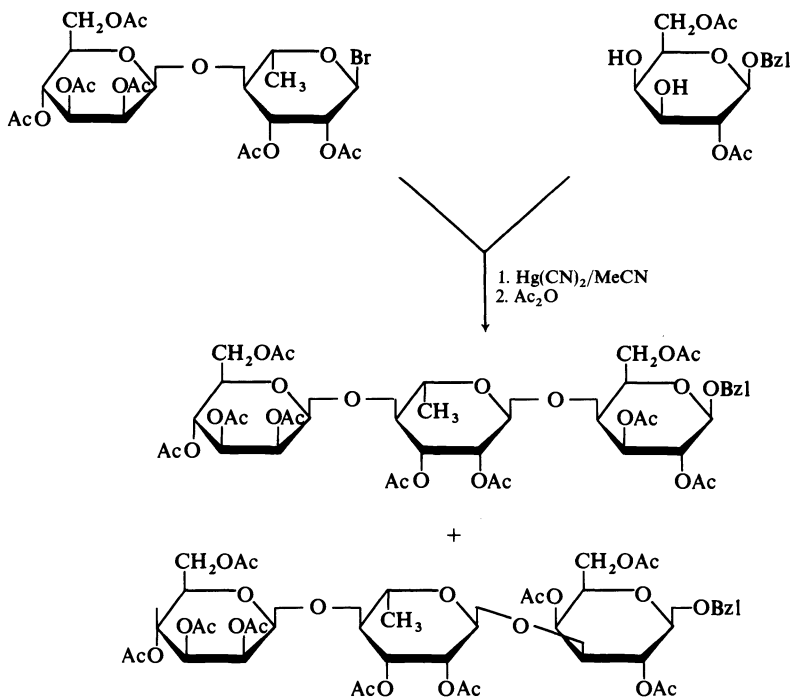
The structure of the resulting disaccharide was proved by standard methods, including n.m.r. and m.s. techniques, and the analysis of partially methylated polyol acetates. Much attention was given to identification of the mannosidic linkage configuration. As we had the samples of mannopyranosyl-1 \rightarrow 4-rhamnose with both β - and α -mannosidic linkages, the most convincing evidence was obtained from oxidation with chromium anhydride, following Angyal and James¹² and a recent suggestion by Lindberg to identify the configuration of some polysaccharides. The disaccharide obtained undergoes oxidation to decompose qualitatively the mannose residue, whereas the reference experiment with α -mannosyl derivative showed that it was stable to oxidation.

Thus, fortunately it turned out that the glycosidic linkage in the hexosulose derivative did not undergo epimerization in spite of the presence of the carbonyl group in the α -position.

The second stage of the trisaccharide synthesis involved attachment of the third monosaccharide residue of galactose. Many repeating oligosaccharide units of *O*-antigenic polysaccharides from *Salmonella* contain the 1 \rightarrow 3 rhamnosylgalactose linkage (Tables 1 and 2). Therefore the synthesis of the corresponding oligosaccharides required a rational method to make this linkage. The synthesis of galactose derivatives with a substituent at position 3 is known to be a very unpleasant problem that has not been satisfactorily solved so far. This is due to the inaccessibility of the galactose derivative with a single free hydroxyl at C₃ in the pyranose form. Therefore, Flowers and co-workers suggested¹³ that benzyl-2,6-di-*O*-acetylgalactopyranoside with two free hydroxyl groups should be used for the synthesis of 3-sub-

stituted galactoses. These authors claimed that in this compound glycosylation proceeds selectively at equatorial hydroxyl C₃ only. We used this approach for the synthesis of trisaccharides, but failed to support Flowers's data. The glycosylation of benzyl-2,6-di-*O*-acetyl-galactopyranoside with acetobromohalogenose obtained from β -D-mannopyranosyl-1 \rightarrow 4-L-rhamnopyranose proceeded at the two free hydroxyls of galactose to give a mixture of two trisaccharides containing both 1 \rightarrow 3 and 1 \rightarrow 4 rhamnopyranosyl-galactose linkages⁴. A similar result was obtained¹⁴ (SCHEME 5) from

SCHEME 5



glycosylation of the same galactose derivative with acetobromohalogenose produced from β -D-glucopyranosyl = 1 \rightarrow 4-L-rhamnopyranose. Fortunately in both cases, with glucose as well as with the mannose derivative, both the trisaccharides could be separated by chromatography on silica gel, the yield of the desired compounds with the 1 \rightarrow 3 linkage exceeding 15 per cent. After removal of the isopropylidene group and benzyl residue, the trisaccharides obtained were converted into acetates by treatment with acetic anhydride in pyridine. The latter appeared to be mainly β -acetates with a small amount of α -anomer.

For a more convenient and general solution for the synthesis of 3-substituted galactoses, we attempted the synthesis of galactofuranose derivatives. In one of his mass-spectrometric studies Biemann demonstrated¹⁵ that treatment of galactose with acetone at elevated temperatures gave

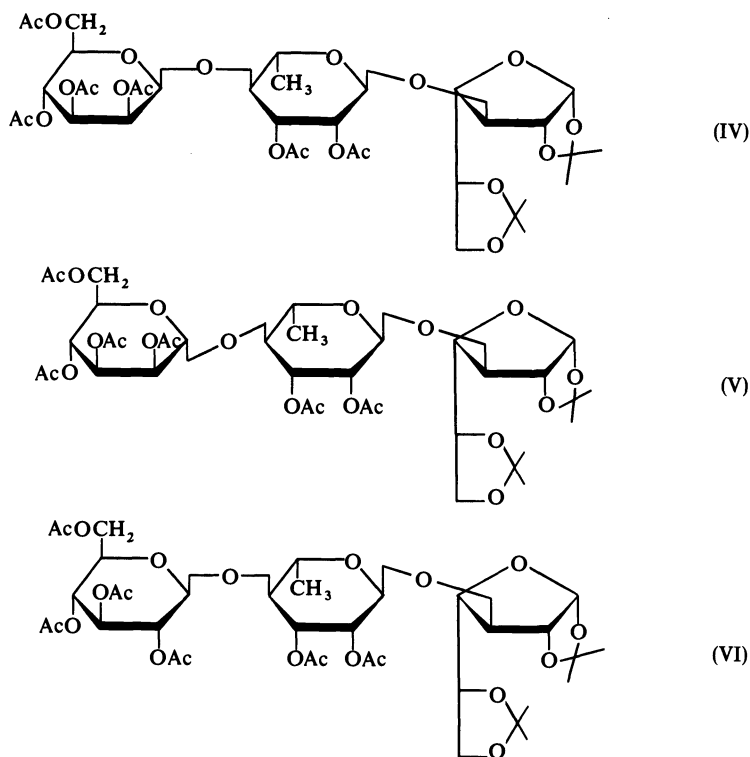
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traces of diisopropylidengalactofuranose. A more detailed study⁶ showed that upon heating a galactose solution in acetone in the presence of copper sulphate a mixture of 1,2;3,4-diisopropylidengalactopyranose and 1,2;5,6-galactofuranose was formed; their ratio was temperature-dependent. almost equal amounts of these derivatives are formed upon heating for ten hours at 100–120°. They can be separated readily by chromatography on silica gel and diisopropylidengalactofuranose obtained with 30 per cent yield: for the formation of the furanose derivative it is necessary that acetone should be free of any trace of moisture.

Upon glycosylation of diisopropylidengalactofuranose with halogenoses obtained from β -D-mannopyranosyl-, α -D-mannopyranosyl- and β -D-glucopyranosyl-1 \rightarrow 4-L-rhamnopyranose by heating in acetonitrile in the presence of mercury cyanide, the corresponding trisaccharide derivatives IV–VI were formed. Of prime importance in this case is the purity of the acetonitrile: the presence of impurities, most likely traces of amine, results in a sharp decrease of the yield.

At the final stage of the synthesis to obtain trisaccharides with a completely pyranose unit structure, it was necessary to remove the protecting groups from the galactofuranose derivative. Here we are confronted rather

SCHEME 6



unexpectedly with some difficulties. Direct removal of isopropylidene groups in the presence of acids caused considerable degradation of the trisaccharide to give several reaction products with no galactose found after hydrolysis. Apparently, this is due to elimination of 1,3-bound galactose (probably according to the elimination mechanism), which results in degradation of the galactofuranose residue. The mechanism of this decomposition has not been clarified as yet, and requires special study. The most surprising thing is the effect of the nature of the non-reducing monosaccharide and the type of its glycosidic linkage on the degree of degradation: the trisaccharide with a β -mannosyl linkage decomposes most strongly, whereas that with a β -glucosidic residue undergoes but little destruction. From these data we have to assume that the stability of the 1,3-rhamnagalatosidic linkage is affected by the nature of the neighbouring glycosidic linkage. So far no explanation can be given for this fact.

Increased lability of the 1,3-linkage to acids demanded special conditions for removal of the isopropylidene groups. The best results were produced by heating the trisaccharide derivative in chloroform-trifluoroacetic acid mixture. Under these conditions the trisaccharide, containing a glucose unit and also the trisaccharide with the α -mannosyl linkage, was obtained in sufficient yield, but the treatment of a β -mannosyl derivative was also accompanied by the formation of several side-products which produced no galactose after acidic hydrolysis. To isolate the β -mannosyl trisaccharide, we had to use a more complicated procedure which included saponification of acetoxy groups first and then mild acid hydrolysis.

It should be mentioned that despite the accessibility of galactofuranose derivative with only C_3 -hydroxyl free, the practical use of this derivative for general synthesis of 3-substituted galactoses still demands a good procedure for removing isopropylidene groups without destruction of the galactose residue.

The three trisaccharides I-III were obtained as peracetates, and contained galactose in its pyranose form. Their structure was confirmed by standard techniques—analysis of methylated polyols, m.s. and n.m.r. spectra. *N*-Tolylglycosylamine derivatives obtained directly from acetates were used for mass spectrometry¹⁶; in the mass spectra of these derivatives one could easily follow the monosaccharide sequence in the trisaccharide. A more detailed analysis of the spectra partially confirmed additionally the type of glycosidic linkages.

The data reported on the synthesis of oligosaccharides illustrate the ideas put forward at the beginning of this paper. In the course of the synthesis we had to solve some particular problems arising during the synthesis of these oligosaccharides; that is, the practical synthesis of the β -mannosidic linkage and 3-substituted galactose. It is related to general problems of stereospecific synthesis of the 1,2-*cis*-glycosidic linkage and of the introduction and removal of selective protection.

ROUTES TO POLYSACCHARIDES

As was indicated, a more difficult task is the second stage of the synthesis of block-polymer polysaccharides; that is, conversion of oligosaccharide

blocks into a polymeric structure. This problem has not been solved yet and it is quite clear that its solution would be very important for the synthetic chemistry of carbohydrates.

Generally speaking, there may be three different approaches to convert the oligomeric block into the corresponding block-polymeric polysaccharide: (1) Polycondensation of the oligomeric blocks by chemical methods; (2) stepwise chemical synthesis to extend the polymeric chain; and (3) biochemical polymerization of the oligomeric blocks.

Polycondensation

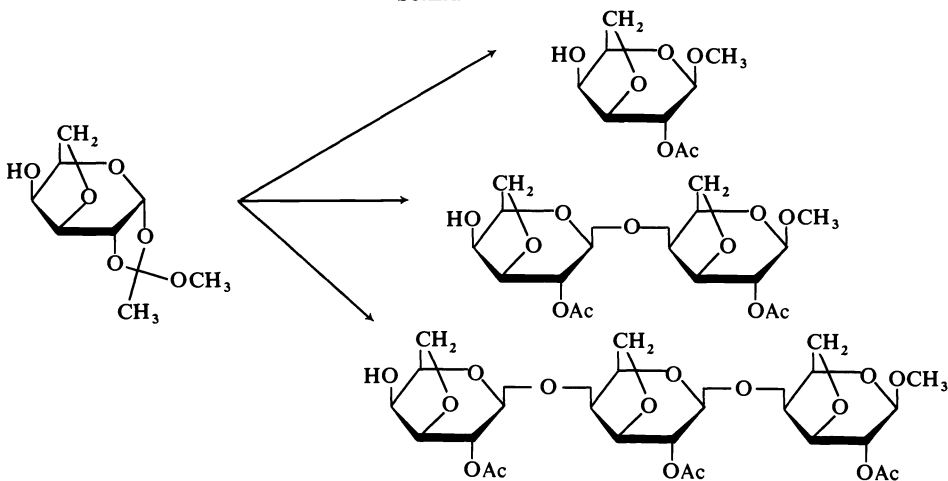
No doubt, the polycondensation of oligomeric blocks would provide the direct and fast route to polysaccharides of the block-polymer type.

Unfortunately, there are no polycondensation methods which could give satisfactory results. Therefore it seems desirable to consider the possibilities of this approach, although the experiments have been performed only with models which are far from the natural polysaccharides of block-polymer type.

The ortho-ester method of glycosylation is known to open up some possibilities for obtaining polysaccharide structures by means of polymerization of mono- or oligosaccharide derivatives¹⁷. About five years ago we succeeded in carrying out polycondensation of a melibiose derivative into a regular galactoglucane using the ortho-ester group as a glycosylating moiety¹⁸.

It was found that the yield of the polycondensation product and the degree of its polymerization were very low. Actually, oligomerization stopped after addition of three or four monomeric units. Numerous attempts to perform polycondensation of other model compounds produced, in fact, the same result: the polycondensation process stopped at the very beginning and the yield of polymer was very low. Polycondensation of 1,2-ortho-ester of 3,6-anhydrogalactose¹⁹ can be taken as an illustration. This reaction, when carried out under the conditions of ortho-ester glycosylation, stopped at the stage of dimer or trimer formation, the yield being very low.

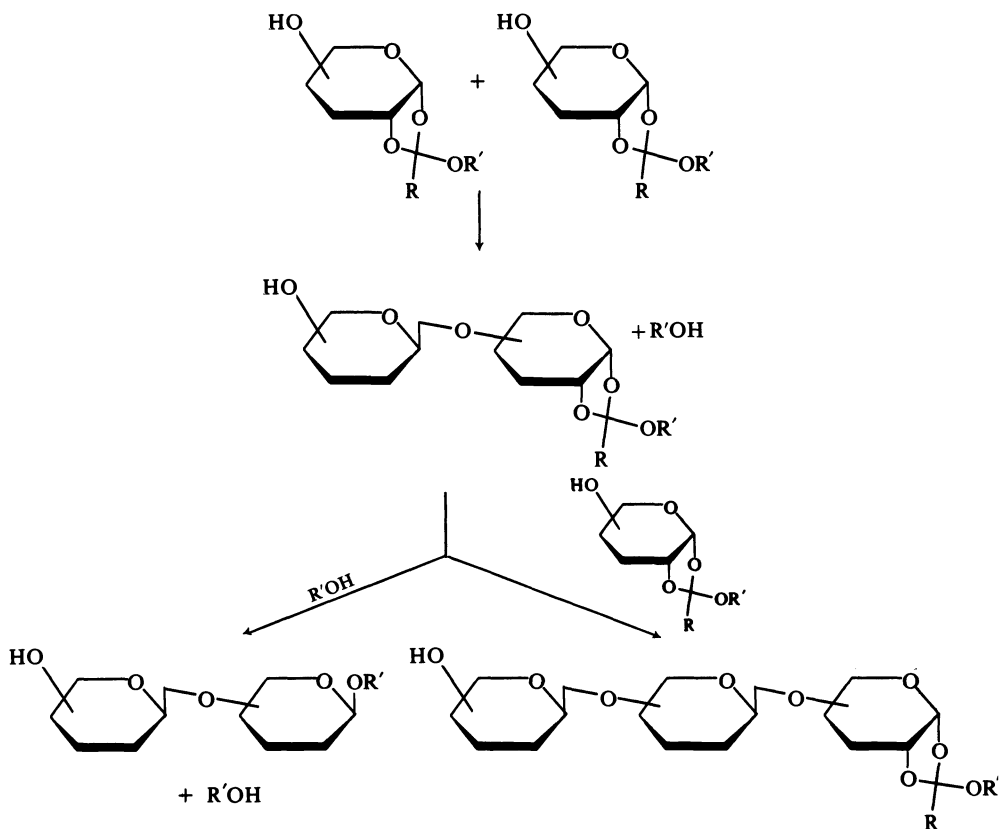
SCHEME 7



It is necessary to emphasize that during the synthesis of galactoglucane one could suggest that the insufficient extent of the process might be due to the low reactivity of the free hydroxyl group in 2,3,4-tri-*O*-acetylgalactose residue. But in the latter case this supposition was not valid, because it is known that the hydroxyl group at C₄ of the anhydrogalactose unit shows high reactivity.

A more detailed study of the problem led to the conclusion that the competing glycosylation of the low alcohol liberated during the reaction may be responsible for interruption of polycondensation chain. This conclusion was prompted by the fact that in the resulting oligomers the glycosidic groups proved to be terminal ends. It is quite likely that the end ortho-ester group of the growing chain can react either with another molecule of the monosaccharide (monomer) or with a molecule of the alcohol formed during the previous steps of glycosylation. As previously found, the reaction of the ortho-ester group with alcohol proceeds more readily, and therefore this side reaction may interrupt the polycondensation chain at the early stage of polycondensation in spite of a low concentration of methanol or ethanol.

SCHEME 8



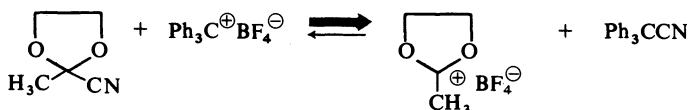
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Attempts have been made to remove the liberated alcohol, for example, by using molecular sieves of the zeolite type which entrap the low molecular weight alcohol. But in spite of some improvement in the ortho-ester glycosylation reaction, this did not in practice affect the result of the polycondensation.

All this makes doubtful the application of the ortho-ester glycosylation for the reaction of polycondensation. Therefore we shall have to look for some other approaches to polycondensation. One of the possible ways may be the use of a glycosylating compound capable of generating the acyloxonium cation without the formation of alcohol or any other compound active in the glycosylation process.

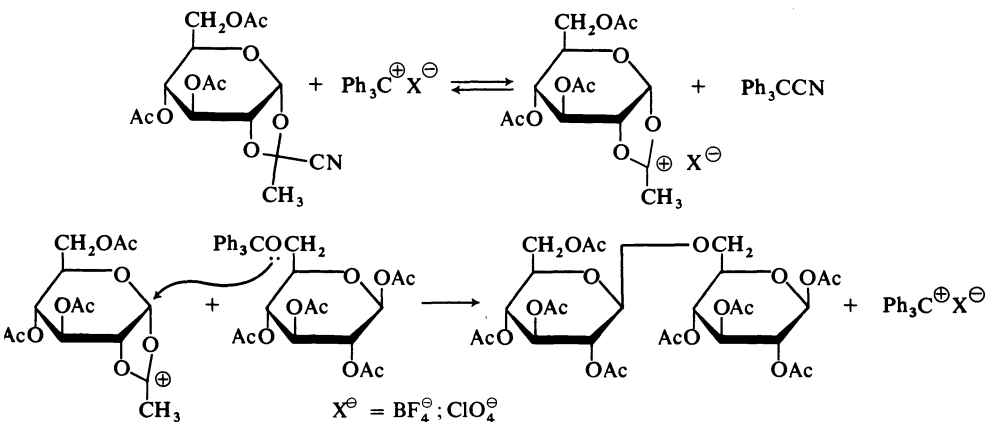
About fifteen years ago Meerwein demonstrated²⁰ that pyruvinnitrile cyclic ketal could readily regenerate a five-membered acyloxonium ion upon interaction with triphenylcarbonium salts. The equilibrium is shifted towards the formation of acyloxonium ion owing to formation of the insoluble tritylnitrile.

SCHEME 9



This idea of acyloxonium ion generation was used for the development of a new glycosylation method with the nitriles, which have been obtained by Fletcher as side products of Helferich glycosylation. I should like to call it Fletcher's nitrile. Later a good method of synthesis of these nitriles was developed²¹ based on the interaction of acetohalogenoses with silver cyanide. Using the synthesis of gentiobiose²² as an illustration, the reaction can be presented as follows.

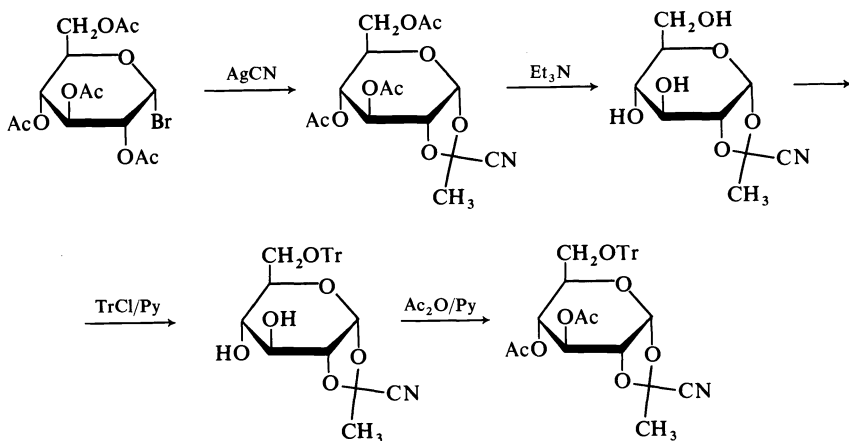
SCHEME 10



Upon interaction of Fletcher's nitrile, obtained from acetobromoglucose, with triphenylcarbonium salt (tetrafluoroborate or perchlorate), acyloxonium and insoluble tritylcyanide are formed. Upon addition of 6-*O*-trityl-1,2,3,4-tetra-*O*-acetylglucose to the reaction mixture, the cation attacks the oxygen of the trityloxy group to give a gentiobiose derivative, thus achieving the equilibrium shift; triphenylcarbonium ion is regenerated to continue the reaction. Catalytic amounts of triphenylcarbonium salt are sufficient for the reaction to proceed. Upon glycosylation, only neutral tritylcyanide is formed, without any alcohol or strong acid. This is quite a new type of real catalytic glycosylation. The best results are obtained when the reaction is performed in methylene chloride for 1–1.5 h at 45° and the catalyst/reagent ratio is 1:20. A high-vacuum technique was used, which is common practice for reactions with carbocations of this type. The yield of gentiobiose was as high as 65 per cent with tritylperchlorate, and 45 per cent with trityl borofluoride.

This new reaction of glycosylation may prove to be the basis of a new polycondensation method, when Fletcher's nitrile containing a trityl group is used as a monomer^{2,3}. This idea has been verified in the case of polycondensation of 6-*O*-trityl glucose derivative which was obtained from acetobromoglucose. This substance undergoes polymerization at room temperature in

SCHEME 11

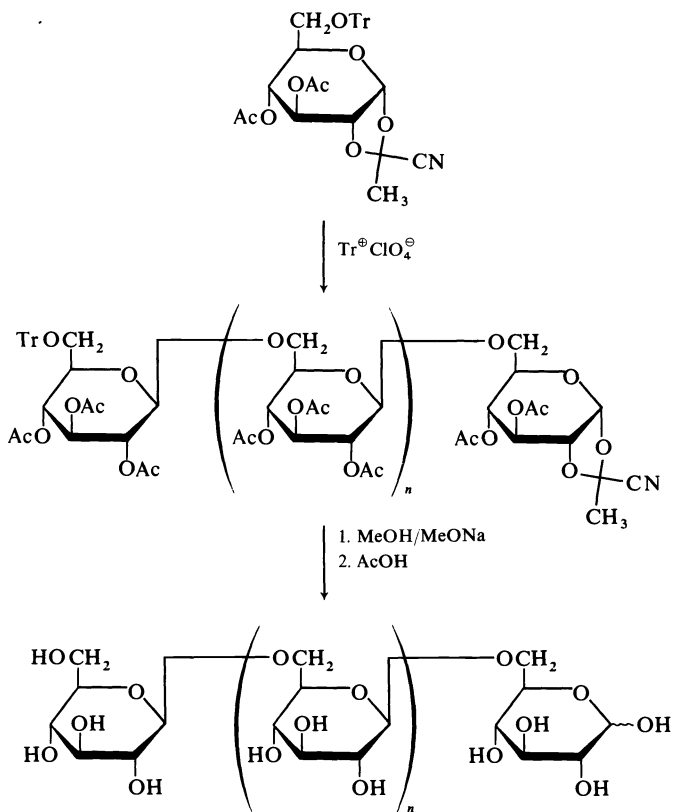


the presence of catalytic amounts of triphenylcarbonium perchlorate in methylene chloride solution.

Along with the polymeric fraction some laevoglucosane was formed due to the nitrile intramolecular glycosylation. The preparative experiment on nitrile polymerization showed that the polymer is formed with a yield of 40 per cent; its degree of polymerization is 40–50, according to the data of gel-filtration on biogel P-4.

It could be suggested that the resulting polymer might be formed as a result of laevoglucosane polymerization rather than from the polycondensa-

SCHEME 12



tion of the tritylated Fletcher's nitrile. This supposition has been refuted, since upon treatment of laevoglucosane acetate under the reaction conditions no polymer was formed.

Another supposition was that the formation of the polymer is the result of copolymerization of Fletcher's nitrile with laevoglucosane, which might be initiated by the original formation of acyloxonium ion from the starting nitrile. In order to refute it, a series of experiments was undertaken to effect polycondensation of tritylated nitrile in the presence of laevoglucosane acetate labelled with ^{14}C . No incorporation of the label into the polymeric fraction was observed. This provides evidence that nitrile polycondensation did indeed take place in the course of the reaction.

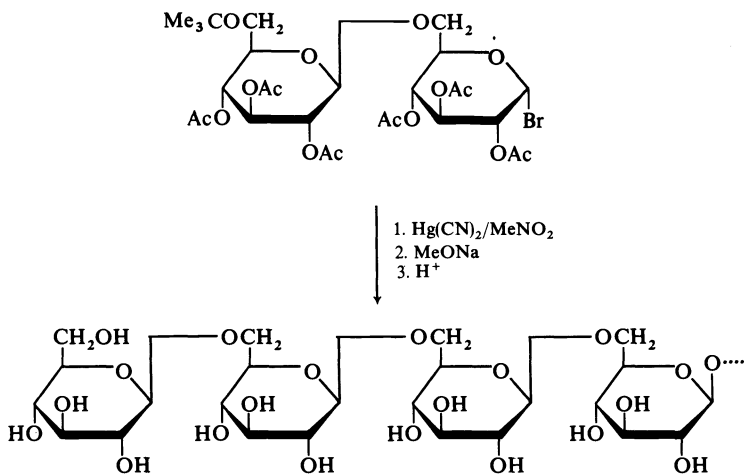
Hence, we have a new polycondensation process based on a new principle. It is very important that during the process there should appear only one neutral molecule — tritylcyanide, which does not react with starting or intermediate substances. It is a challenging problem to try to use this process for obtaining regular heteropolysaccharides. This would require answers to several questions. First, it is necessary to develop synthetic schemes to obtain the corresponding tritylated oligosaccharide nitriles. Next, it is important to

study in detail all the possible side reactions which may occur under the action of so active an agent as triphenylcarbonium ion. The most dangerous reaction would be the cleavage of glycosidic linkages and transglycosylation reactions. And finally, in order to extend the possibilities of glycosylation of both primary and secondary hydroxyl groups, it is necessary to select new convenient 'leaving groups' such as the diphenylcyclopropenyl group suggested by Khorlin²⁴.

Another possible approach to a rational procedure of polycondensation could be based on a modified Koenigs-Knorr reaction. In this case a higher reactivity of halogenoses as compared with that of ortho-esters should provide a higher degree of polymerization. It is known that polymerization of acetohalogenoses with a free hydroxyl group is connected with some difficulties due to the instability of these derivatives; very low yields were obtained in the experiments reported by Whelan²⁵. Therefore this approach did not find application for the synthesis of polysaccharides.

In this laboratory it was found that *t*-butyl ethers of alcohols can undergo the reaction of electrophilic substitution at the oxygen atom with removal of the *t*-butyl group²⁶. This principle proved to be applicable also to glycosylation²⁷. Upon glycosylation of *t*-butyl ethers of certain monosaccharides with acetohalogenoses in the presence of Lewis acids as catalysts, disaccharides with satisfactory yields were obtained²⁸. It is important to stress that the substitution reaction of the *t*-butyl group can proceed as readily as that of a free hydroxyl, or even more efficiently. We tried to use this principle for developing a new polycondensation process. Bromoacetohalogenose obtained from peracetate 6-*O*-*t*-butyl-gentiobiose under the action of titanium tetrachloride was treated with mercury cyanide in nitromethane. A wide range of products from low oligomers to high molecular substances was formed. The high molecular weight fraction isolated with about ten per cent yield was treated with sodium methylate to remove

SCHEME 13



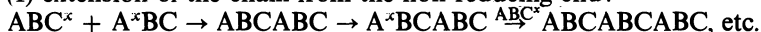
the acetyl groups, then with acids to eliminate the remaining t.-butyl groups and possible ortho-ester groups, re-acetylated with acetic anhydride in pyridine for purification, and again saponified with methylate. The resulting polymer after methylation and subsequent analysis of the methylated polyols showed the presence of a regular 1,6-glucane.

These findings demonstrate that the use of t.-butyl ethers in the Koenings-Knorr reaction probably also opens up some possibilities for obtaining polysaccharides by means of polycondensation. The next step is to try to extend this principle to the synthesis of block-polymer heteropolysaccharides. In this case again we shall have to study in detail all possible side processes which may distort the pattern of polymerization. The greatest danger again lies in the processes of transglycosylation and cleavage of the glycosidic linkage.

Stepwise synthesis

The stepwise synthesis of the polymeric chain should involve gradual glycosylation of oligo- or polysaccharide molecules according to the general scheme :

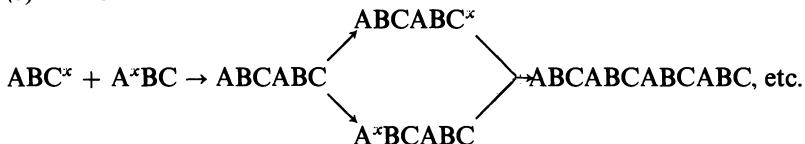
(1) extension of the chain from the non-reducing end :



(2) extension of the chain from the reducing end :



(3) condensation of dimeric blocks :



where A^x is the non-reducing monosaccharide unit with a free hydroxyl or activating group and C^x is the reducing monosaccharide unit activated at C-1.

Although this route seems to be tedious, it may prove efficient in obtaining polysaccharides of low molecular weight. Its most difficult point, in addition to the selection of the proper glycosylation method, is introduction of the corresponding groups to activate the glycosidic centre and the hydroxyl group to be glycosylated. As seen from the above scheme, these groups are introduced at successive steps, and the method of introduction should be equally good both for the simplest starting block and for the corresponding dimeric, trimeric and other blocks. Therefore, when planning the synthesis of an oligosaccharide, it is advisable to consider possible ways of introducing the appropriate groups.

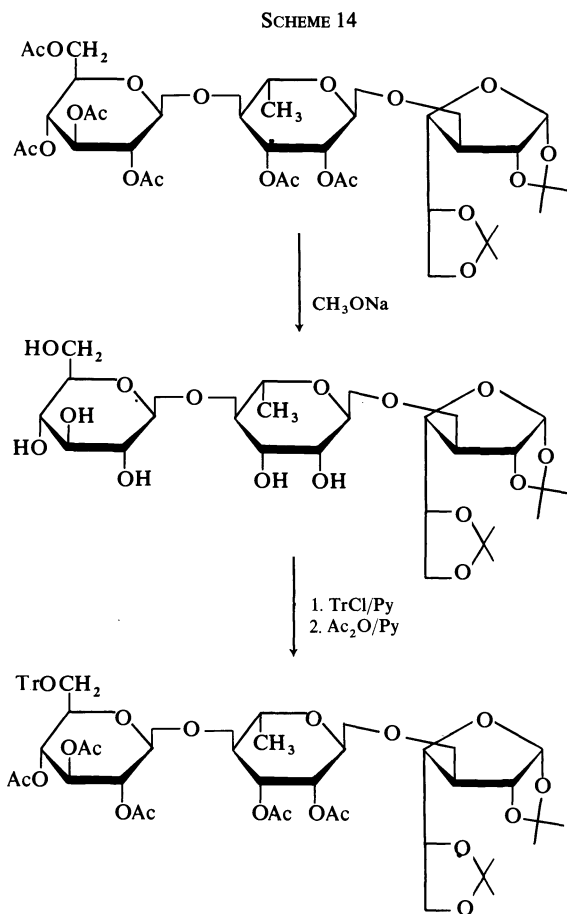
We attempted to use this method for the synthesis of hexasaccharide by condensation of two trisaccharide blocks. A more accessible glucose analogue of the repeating unit from *S. anatum*, β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnopyranosyl-1-3-D-galactopyranose, was used as a trisaccharide block. In the project two blocks have to be connected by the β -D-galactopyranosyl-1 \rightarrow 6-glucose linkage, which corresponds to a galactose-mannose linkage

in the natural polysaccharide from *S. anatum*. The Brederick reaction was chosen for glycosylation, which gives rather good yields and satisfactory stereospecificity.

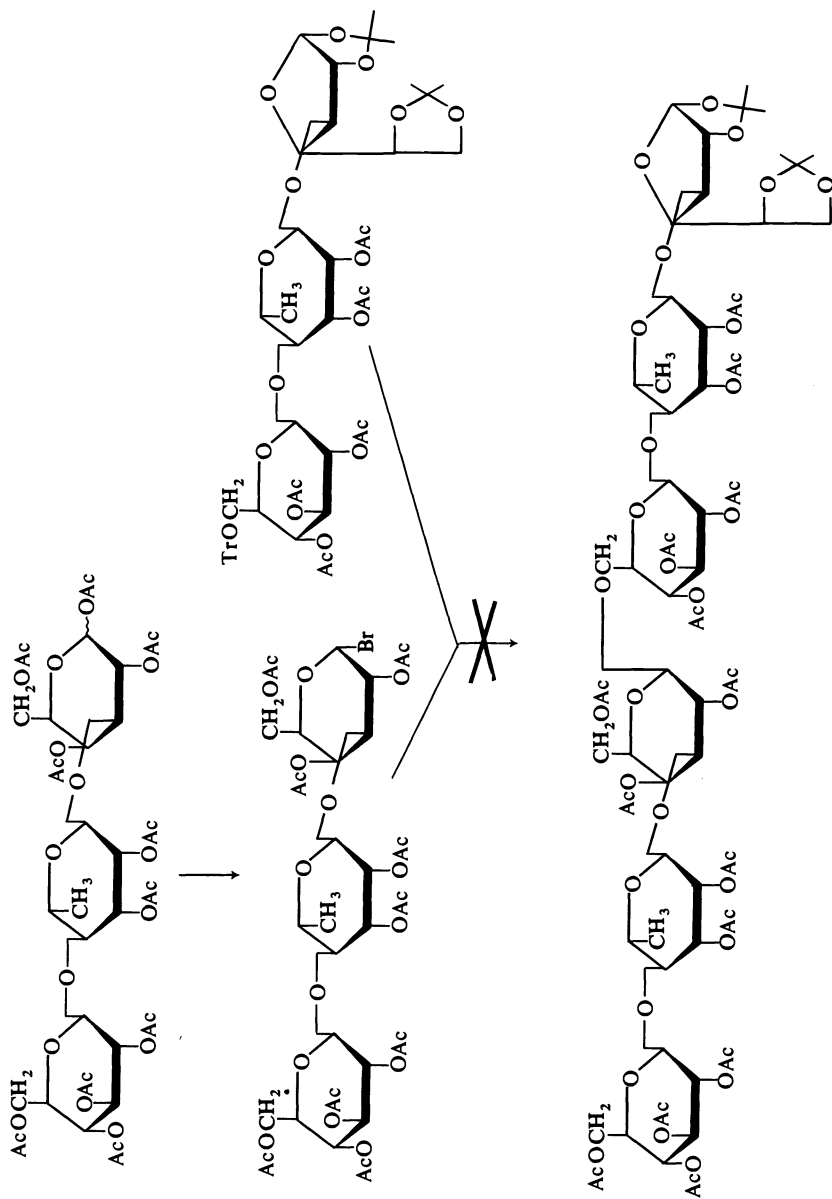
According to this plan, the glycosylating trisaccharide ABC^x had to be the corresponding halogenose, and in the trisaccharide A^xBC to be glycosylated the primary group of the glucose residue must be selectively replaced by the trityl group.

Peracetate of glucosyl-rhamnosyl-galactose was converted into the corresponding halogenose as usual by treatment with a solution of hydrogen bromide in acetic acid, and this was accompanied by only small destruction of the starting trisaccharide.

In order to perform selective tritylation of the primary group of the glucose residue to produce the glycosylated component a derivative obtained at the previous stage of the synthesis (VI) rather than the final trisaccharide (III) was taken as the starting substance. In this derivative the galactose residue was still in its furanose form and was protected by isopropylidene groups.



SCHEME 15



After removal of its acetyl groups by treatment with sodium methylate, a trisaccharide derivative was obtained which contained a single primary hydroxyl group in the glucose residue. Its tritylation with trityl bromide in pyridine and subsequent standard acetylation gave a trisaccharide trityl-derivative which served as a second component of the reaction.

The structure of this compound was supported by n.m.r. and mass spectra and did not raise any doubts. Condensation of the acetohalogenose obtained from the above trityl derivative was carried out under the standard conditions of Brederick's glycosilation.

The reaction proceeds quite readily, and in several minutes silver bromide was completely precipitated. But the result of the reaction turned out to be quite unexpected. The chromatographic study of the reaction mixture showed the presence of two major components which were isolated and proved to be glucosyl-rhamnosyl-galactopyranose peracetate and the 6-*O*-trityl derivative of glucosyl-rhamnose peracetate.

It can be suggested that the trisaccharide peracetate is formed from the starting acetohalogenose, whereas the disaccharide results from the degradation of the trityl derivative. Hence, during the reaction the cleavage of the 1,3-rhamnosylgalactose linkage took place followed by degradation of the galactofuranose residue as no galactose was found in the reaction mixture. The cause of this unusual reaction is not clear, but it suggests destruction of the trisaccharide VI containing a diisopropylidene-galactofuranose residue by the action of acids. In both cases it is apparently due to a cation (a proton or triphenylcarbonium ion) which seems to catalyse the cleavage of the glycosidic linkage of 3-substituted galactose with simultaneous destruction of galactofuranose.

Nevertheless, this unusual degradation does not seem to exclude the possibility of applying stepwise synthesis for the gradual extension of a heteropolysaccharide chain. The study of other glucosyl-rhamnosyl-galactose derivatives for this purpose is in progress in this laboratory.

Biochemical polymerization

In conclusion, the possibilities of the synthesis of heteropolysaccharides using biochemical polymerization may be briefly discussed. This principle, which can be used for the conversion of an oligomeric repeating unit into *O*-antigenic polysaccharide, involves the action of polymerases contained in the enzyme complex located in the endoplasmatic membrane of a microbial cell.

At present, the scheme of biosynthesis of *O*-antigenic polysaccharides of Gram-negative bacteria is generally clear³⁰. Moreover, Wright³¹ has demonstrated the polymerization of an oligomeric block under the action of an enzymatic preparation from the walls of Gram-negative bacteria.

It is well known that the polymerases from Gram-negative bacteria show very high specificity. This may indicate the limit of the possible application of this method; biochemical polymerization can be used only for synthesis of the natural *O*-antigenic polysaccharides and is not suitable for their analogues or modified derivatives.

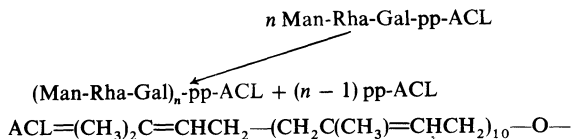
There is another important aspect in using synthetic oligosaccharide units in biochemical polymerization. Owing to high specificity of the corres-

ponding polymerase, a successful result of biopolymerization would give strong evidence that the structure of the synthetic oligosaccharide coincides with the natural one; the failure of the experiment would mean that these structures are different.

Thus, here the chemical synthesis would serve for its direct purpose: it would support conclusively or refute the structure of the natural compound assumed on the basis of the analytical data.

As is known, the substrate of biopolymerization is not the oligosaccharide itself but an asymmetric pyrophosphate substituted with an oligosaccharide and a polyprenol, a natural isoprenoid alcohol with 55 carbon atoms.

SCHEME 16



Therefore, for successful biopolymerization, it is necessary to start from the oligosaccharide corresponding to the biological repeating unit preliminarily converted into a polyprenolpyrophosphate derivative. Hence, the first question in using this route for oligosaccharide conversion into a biopolymer is its conversion into α -phosphate. This question is now solved because we find that the MacDonald method³² can be successfully applied for oligosaccharide phosphorylation³³: it was demonstrated with the phosphorylation of maltose, cellobiose and a disaccharide with a very labile rhamnosidic linkage, peracetate of α -L-rhamno-pyranosyl-1 \rightarrow 3- β -D-galactopyranose. The yields of oligosaccharide α -phosphates are not different from those obtained upon phosphorylation of monosaccharides, and the admixture of the resulting anomeric phosphates can be easily separated by ion-exchange chromatography. The structure of α -phosphates was identified by standard chemical methods and the configuration of the glycosidic centre from n.m.r. spectra exhibiting characteristic features (Table 3).

This method was also used to obtain more complex oligosaccharide-phosphate, namely α -phosphate of mannopyranosyl-rhamnopyranosyl-galactose—a biological repeating unit from *S. anatum*, which can be used for further studies. Upon phosphorylation of β -peracetate of this trisaccharide by interaction with phosphoric acid, only insignificant destruction of the trisaccharide was observed.

The next step of the synthesis—a conversion of the phosphate into polyprenolpyrophosphate derivative—can be performed probably taking into account the data of Warren and Jeanloz³⁵ on the synthesis of simpler poly-prenolpyrophosphate systems. A very important and difficult point here is obtaining the polyprenole phosphate. Phosphorylation of polyprenole, which is very labile and easily oxidized with oxygen in the air, may develop considerable complications³⁶.

We also checked the known method for isolation of a polymerase preparation from the cell walls of some *Salmonella* strains and found it suitable for making preparative amounts of enzyme complexes.

Table 3. Phosphates of oligosaccharides

Structure	Yield, %	$[M]_D$	p.m.r. data δ , p.p.m.	$(H-I)$ $J_{1,2} J_{1,P}$
	40	285°	5.34	3.0 7.0
	33	642°	5.39	3.0 7.0
	24	208°	5.39	2.5 7.5
	20	175°	—	—

It seems that synthesis of regular heteropolysaccharides by means of biopolymerization, after solution of some problems connected mainly with chemistry of polyprenol phosphates and synthesis of polyprenolpyrophosphates, might be possible and useful as well.

The data presented in this paper should be considered only as a preliminary discussion of a very complicated problem. Its actual solution would require a great deal of hard work.

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