Russian Chemical Reviews **69** (9) 795 – 820 (2000) © 2000 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2000v069n09ABEH000568

Solid-phase synthesis of oligosaccharides and glycoconjugates

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Abstract. The use of polymeric supports for directed regiospecific assembly of oligosaccharides, glycopeptides and phosphoglycans is considered. In some cases, synthesis of oligosaccharides on supports is more efficient than the corresponding synthesis in solution, although this cannot still compare favourably with the latter. Examples of successful solid-phase syntheses of complex oligosaccharides and glycoconjugates are given. Special emphasis is laid on the choice of optimum supports, linkers and glycosylation methods. The bibliography includes 105 references.

I. Introduction

The explosive development of molecular biology is closely connected with recent advances in the chemistry and biochemistry of biopolymers including the development of efficient techniques for their targeted chemical synthesis. The recently developed approaches to the synthesis of biopolymers are based on essentially new principles for elongation of the polymeric chains including their assembly on polymeric supports.

This method was first employed by Merrifield for the synthesis of peptides.[1] After solution of numerous methodological problems, this protocol, which is sometimes improperly referred to as 'solid-phase synthesis', was successfully automated and became the main routine procedure in peptide synthesis.

An enormous body of experimental evidence published in the past two-three decades point to the prominent role of biopolymers, such as polysaccharides and glycoconjugates (glycoproteins, glycolipids) in vital processes. These polymers are indispensable participants in many biological processes (*e.g.*, antigen – antibody, enzyme – substrate and ligand – receptor interactions) as well as in some other interactions of carbohydrates with proteins.[2, 3]

The first attempts aimed at synthesising oligosaccharide chains on solid supports were undertaken in the early 1970's;[4, 5] however, it became clear that rapid progress in this area was hardly possible, since chemical synthesis of oligosaccharides with a strictly determined sequence of monomeric units presented a serious problem due to the polyfunctionality of monosaccharides and the stereochemical ambiguity of intermonomeric glycosidic bond formation.

At present, synthesis of oligosaccharides on polymeric supports still cannot compete with the classical synthesis in solution, but its development is in progress and the methodology is improved. This is associated with the fact that in many cases syntheses on supports are more advantageous. Numerous examples of successful application of the solid-phase approach in the synthesis of complex oligosaccharides and glycoconjugates are currently known.

A vast body of experimental material accumulated thus far highlights the major problems and advances in solid-phase synthesis of carbohydrate-containing compounds and allows estimation of its possible application for realistic syntheses. It seems therefore expedient to sum up these data in order to inform all those who are concerned with this subject about the potential of the method and the problems it may involve.

The review covers the publications appearing up to 1999 inclusive.

II. The methodology of synthesis of oligosaccharide chains on solid supports

The synthesis of oligosaccharides on solid supports includes immobilisation of the first unit from which elongation of the oligosaccharide chain begins on a specially selected optimum support followed by elongation of the oligosaccharide chain using specific methods for the formation of interglycosidic linkages (*viz.*, by glycosylation) and removal of the oligosaccharide molecules thus synthesised from the polymeric support without any sacrifice of their structures. The peculiarity of carbohydrate chemistry is manifested in all steps of the solid-phase oligosaccharide synthesis, which makes it different from peptide and nucleotide syntheses.

1. Supports

Oligosaccharide syntheses are carried out both on insoluble solid supports and on soluble polymers. In the former case, the reaction proceeds in heterogeneous media, in the latter case, in homogeneous media.

The obvious advantage of insoluble solid supports is that the isolation and purification of the reaction products after each step is achieved by simple filtration and washing with a solvent.

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Received 28 December 1999
Uspekhi Khimii **69** (9) 869 – 896 (2000); translated by R L Birnova

Furthermore, the reaction rate can be increased if one of the reagents, which can be regenerated following the reaction, is taken in a large excess. The main disadvantage of insoluble supports is that the reaction occurs on a solid surface, which hampers the access of the reagent to the reaction centre and poses additional difficulties in regulation of regio- and stereoselectivity of the reaction.

Syntheses of oligosaccharide chains anchored to soluble polymeric supports are partly devoid of this drawback but purification of the reaction product becomes more difficult. In this case, the polymer-bound reaction product has to be precipitated by addition of another solvent and purified by repeated precipitations. The use of an excess of a reagent for acceleration of the reaction is also undesirable, since in this case its complete removal, which is necessary to ensure the desired sequence of the monomeric units in the 'growing' chain, faces difficulties. On the other hand, glycosylation in a homogeneous medium offers better opportunities for regulation of regio- and stereospecificity of the reaction.

Currently, both types of supports for the synthesis of oligosaccharides are employed; however, synthesis on soluble polymers (especially, synthesis of glycoconjugates) has acquired ever increasing importance in recent years.

a. Insoluble solid supports

The efficiency of solid-phase synthesis strongly depends on the physicochemical properties of solid supports, such as porosity and swelling capacity in different solvents. These characteristics are very important, since they determine the accessibility of immobilised molecules to the reagent present in the solution. The number of moles of the first monomeric unit from which elongation of the chain begins, which can be immobilised per g of the polymeric support (the so-called 'loading'), is yet another factor of considerable practical importance.

In the early period of solid-phase oligosaccharide synthesis, polystyrene-based resins prepared by copolymerisation of styrene with *p*-divinylbenzene were predominantly employed. Commercial resins of this type are now available as powders with particles having spherical shapes ('beads') and differing in the degree of cross-linking, porosity, swelling capacity and particle size.† Modified polystyrenes containing reactive chloromethyl (Merrifield's polymer), phenolic hydroxy groups,[7] mercaptomethyl,[8] sulfonylmethyl,[9] diphenyl- and diisopropylchlorosilyl,[10] (3-mercaptopropyl)oxy(thio)methyl [11, 12] and some other groups are most commonly used as supports. The choice of a particular polystyrene resin as a solid support is determined by the strategy of a realistic synthesis, the mode of attachment of the first monomer to the solid phase and the structure of the linker. Sometimes, the role of a linker is played by the functional group of the polystyrene resin.

A main disadvantage of polystyrene resins used in solid-phase oligosaccharide synthesis is their poor compatibility with biomolecules. For example, the yields of oligosaccharides in enzymic solid-phase synthesis catalysed by glycosyl transferases were low to such an extent that this method had to be abandoned as being impractical.

Attempts were undertaken aimed at using polyacrylamide gels as supports. Prior to the use, the gels were modified, *e.g.*, by incorporation of aminoethyl groups.[13]

Controlled-pore glasses (CPG) with a modified surface, *e.g.*, with grafted 3-aminopropyl groups serving to attach the first monomer (see, *e.g.*, Refs 14 and 15), are currently employed with increasing frequency. The surface of CPG-based supports can be modified by silanisation with prior zirconation.[16] The use of CPG has a number of advantages, since they offer broad opportunities in the selection of supports of desired porosity and allow appropriate modification of the surface.

† A brief survey of polymeric substrates for solid-phase syntheses is given in Ref. 6.

b. Soluble supports

These types of supports have currently acquired wide popularity. The simplest soluble support is polyethylene glycol (PEG) (M_r ~5.000 Da) in which one of the terminal hydroxy groups is methylated and the other one is free and serves to immobilise the first monomer (either directly or through a linker).[17] Commercially available PEG is easily soluble in many solvents.

Polymers of a hybrid type, containing both hydrophobic and hydrophilic fragments, have received considerable attention in the last few years. The synthesis on such materials is carried out in a 'micromedium', *i.e.*, under conditions mimicking those of a homogenous reaction, and at the same time allows easy isolation of the reaction products after each step. These polymers include a copolymer of acrylamide or its N-substituted derivatives with polyethylene glycol (PEGA) possessing terminal amino groups [18] and a copolymer of polystyrene with polyethylene glycol (Tentagel).[19] Other more sophisticated copolymers are also used and will be discussed below.

2. Linkers

In solid-phase syntheses of oligosaccharides, the first monomer is usually attached to the support through a bifunctional group (the so-called linker) rather than directly. The linker serves both to bind the monomer to the polymer and to make the monomer and the growing chain remote from the support in order to minimise the influence of the solid surface on the course of the reaction. The latter circumstance is especially important, since heterogeneity of the medium has a specific, sometimes rather obscure effect on the stereochemistry of the newly formed glycosidic linkage.

In deciding upon a linker, allowance should be made for the possibility of selective cleavage of one of its linkages in the release of the oligosaccharide synthesised from the solid support without affecting labile glycosidic bonds.

In early oligosaccharide syntheses, the first monosaccharide was immobilised on chloromethyl–polystyrene without linkers. However, later the use of linkers differing in structures and chemical properties became common practice.

It should be noted that in contrast with the conventional solid-phase peptide and oligonucleotide syntheses, diverse linkers are utilised in solid-phase syntheses of carbohydrates. The choice of a suitable linker for a realistic purpose is an indispensable step in the design of a synthesis. In the synthesis of glycopeptides, the peptide chain is often used as a linker which also removes the growing carbohydrate chain from the surface of the support. A more detailed description of linkers will be given further below in relation to particular syntheses.

3. Elongation of the oligosaccharide chain

This step is crucial in oligosaccharide synthesis, therefore the choice of a method for the formation of glycosidic bonds between the monosaccharides (*i.e.*, method of glycosylation) determines the overall strategy of the synthesis. The predetermined sequence of monosaccharides necessitates that the formation of glycosidic linkages is strictly regio- and stereospecific. A prerequisite for the preparation of homogeneous oligosaccharides with a predetermined structure, which should not require any additional purification (it is this that makes solid-phase synthesis advantageous in comparison with the classical synthesis in solution), is that the yields were close to quantitative in all steps. The lack of a versatile glycosylation procedure (optimal for all monosaccharides) makes the implementation of these requirements extremely difficult, which is the main reason for the relatively slow progress in the solid-phase synthesis of oligosaccharides.

Two problems seem to be especially difficult to overcome. The first of them is that successful synthesis of any biopolymer consisting of 40–50 monomeric units with a predetermined sequence (without deletions or 'erroneous' units) requires that the yield in each elongation step is no less than 97%–98%. Meanwhile, even the most advanced glycosylation methods do not ensure such high yields, which in addition vary strongly

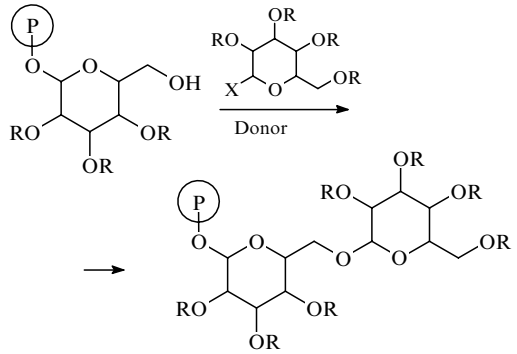
depending on the nature of the monosaccharide and the type of the newly formed glycosidic linkage. The second problem is related to stereochemical ambiguity of the glycosylation reaction,‡ which makes the oligosaccharide synthesis different in principle from the peptide and oligonucleotide syntheses, although it is known, for example, that the synthesis of the 1,2-*trans*-glycosidic bond is usually stereospecific. So far, the use of solid-phase synthesis is effective only for the preparation of relatively short oligosaccharides.

Yet another problem inherent in the solid-phase synthesis of oligosaccharides is related to the polyfunctionality of monosaccharides. The formation of (1-2), (1-3), *etc*., intermonomeric glycosidic linkages requires temporary protection of hydroxy groups which are not involved in the glycosylation reaction. In the general case, synthesis of irregular oligosaccharides (*viz*., those in which the monosaccharide components are either different or identical but differ in the type of bonding) requires specific protection depending on the type of the glycosidic bond for each monosaccharide. After the glycosylation step, the type of protection of hydroxy groups must be changed up to complete removal of the existing and introduction of new protecting groups. This sophisticated procedure makes oligosaccharide synthesis technically cumbersome in comparison with peptide and oligonucleotide syntheses. Sometimes, as in the case of homopolysaccharide synthesis, elongation of the oligosaccharide chain involves the same set of procedures, but even in these cases the passage from attachment of one monomeric fragment to the other is complicated and automation of the synthetic protocol presents a problem.

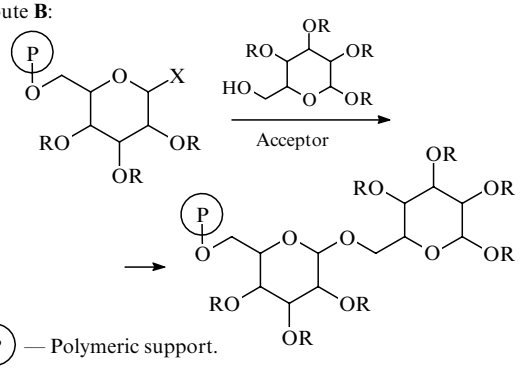
In designing a general strategy for oligosaccharide synthesis with any combination of protecting groups, it is necessary, first of all, to define the order in which the oligosaccharide chain has to be elongated. The elongation is typically carried out in two directions, *viz*., from the reducing end of the chain to the non-reducing one (route **A**) or *vice versa* (route **B**) (Scheme 1). In the former case (route **A**), the monosaccharide immobilised on a solid support through a glycosidic hydroxyl is used as a glycosyl acceptor and the second monosaccharide present in the solution plays the role of a glycosyl donor. In the latter case (route **B**), the first monosaccharide is attached to the support through one of the hydroxy (non-glycosidic) groups and serves as a glycosyl donor, whereas the second monosaccharide remains in the solution and fulfils the function of a glycosyl acceptor. Naturally, the protecting groups and manipulations with them in each stage of the solid-phase synthesis are different in these two routes. If the synthesis is to be performed *via* the route **A**, it is in the terminal monosaccharide of the glycosyl acceptor that the protection of the hydroxy groups should be changed or selectively removed, whereas in the case of the route **B** it is necessary to introduce or establish a group activating the glycosidic centre in the terminal monosaccharide of the glycosyl donor. Obviously, the reagents present in a solution, *e.g*., a glycosyl acceptor (route **B**) or a glycosyl donor (route **A**), should also contain appropriate protecting groups.

Scheme 1

Route **A**:



Route **B**:



P — Polymeric support.

Glycosylation can be carried out by chemical methods or using specific enzymes (most often, glycosyl transferases), which changes the whole strategy of the solid-phase synthesis and eliminates the problem of temporary protection of the monosaccharide units.

a. Chemical glycosylation methods

The pioneering studies of solid-phase oligosaccharide synthesis utilised classical glycosylation with glycosyl halides (predominantly, per-*O*-acetylglycosyl bromides)§ under conditions of the Koenigs–Knorr or the Helferich reaction[4] as well as the orthoester glycosylation method.[22] However, insufficiently high yields and the lack of complete stereospecificity in the formation of glycosidic bonds devaluate these methods for use in solid-phase synthesis. Other glycosylation methods based on the use of thioglycosides (in the presence of activators), glycosyl tosylates,[23] glycosyl sulfoxides,[24] *etc*., as glycosyl donors, have also been explored.

Glycosylation with sugar *O*-trichloracetimidates proved to be especially efficient.[25] This procedure provides sufficiently high yields for a wide variety of monosaccharides. Unfortunately, the stereospecificity of this reaction and the effect of the configuration of the trichloroacetimidate on the steric outcome of the reaction still present a problem; therefore, the practical utility of this reaction in solid-phase synthesis of relatively high-molecular-weight oligosaccharides still remains to be elucidated.

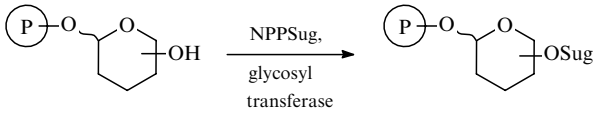
Recently, Danishefsky *et al*.[26] proposed to use the so-called glycal approach[25] for the synthesis of relatively short oligosaccharides and glycoconjugates with more complex structures where 1,2-anhydrosugars were used as donors. This procedure has not received wide application in conventional oligosaccharide syntheses yet, but has been used to develop a convenient scheme for a solid-phase synthesis (see below).

b. Enzymic glycosylation

The elongation of the oligosaccharide chain immobilised on a support can also be carried out by biochemical methods,¶ *i.e*., using enzymes which transfer a monosaccharide residue from the corresponding nucleoside diphosphate sugar (NPPSug) to the end of the growing chain (Scheme 2).

The main advantages of the enzymic approach are good yields and, which is more important, high regio- and stereospecificity of

Scheme 2



N is a nucleoside.

‡ A critical review of the stereospecificity of the main glycosylation reactions is given in Ref. 20.

§ An overview of various glycosylation methods is given in Ref. 21.

¶ The biosynthesis of oligosaccharides is described in a review.[27]

glycosylation. Moreover, in this case no protection of the monosaccharide hydroxy groups is necessary, which significantly simplifies the synthetic procedure. However, sometimes the high specificity of enzymic glycosylation may have a negative effect, *viz*., where different monosaccharides are to be introduced or different types of glycosidic bonds are to be formed. The reason is that in these cases each elongation step would require its own, specific enzyme because of the normally narrow specificities of glycosyl transferases. Moreover, the specificities of enzymes can depend both on the nature of the monosaccharide donor and the monosaccharide acceptor and on the nature of adjacent monosaccharides in the fragment of the oligosaccharide chain synthesised.

Yet another characteristic feature of enzymic glycosylation which should be taken into consideration in conducting solid-phase synthesis is a noticeable dependence of the outcome of the reaction on the nature of the support and the structure of the linker. This can be explained by the fact that the accessibility of the reaction centre of the oligosaccharide chain, which is immobilised on a solid support, for the enzyme, which itself represents a macromolecule, and the formation of an enzyme–support complex are impeded in comparison with enzymic reactions carried out in a solution. Therefore, the requirements for supports and linkers, which enable contact between the reacting molecules, are more stringent. This is the main reason why soluble and conformationally flexible polymers and structurally complex linkers are employed where enzymic methods are used for oligosaccharide synthesis on polymeric supports. (As noted above, glycosyl transferases cannot be used for enzymic oligosaccharide syntheses on polystyrene-based resins due to incompatibility of these structurally different macromolecules).

Although the first attempts to use enzymes in solid-phase syntheses of oligosaccharide date back to the 1980's, it was not until recently that this method received wide application.

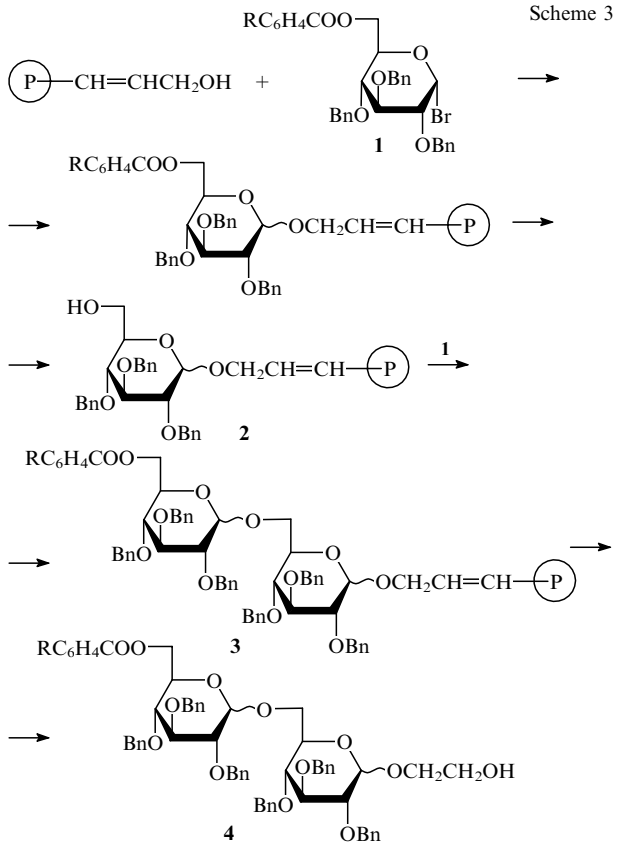
III. Model syntheses of di- and trisaccharides

The pioneering studies on solid-phase oligosaccharide synthesis were aimed at elucidating the feasibility of this technique for preparing this class of compounds. The simplest di- and trisaccharides were the first to be synthesised.

Thus Frechet and Scheurch[4] carried out the synthesis of D-glucopyranosyl-(1-6)-D-glucose and the corresponding trisaccharide with 1-6-bonds using elongation of the oligosaccharide chain immobilised on an insoluble support from the reducing end (Scheme 3). Treatment of a polystyrene resin modified by introduction of allyl alcohol residues[28] with 2,3,4-tri-*O*-benzyl-6-*O*-(*p*-nitrobenzoyl)-D-glucopyranosyl bromide (**1**, R = NO_2) or its *p*-methoxybenzoyl analogue (**1**, R = MeO) resulted in immobilisation of a protected glucose residue after which the acyl protection of the hydroxy group at position 6 was removed. The glucose residues in the polymer **2** were glycosylated with the same bromides. The immobilised disaccharide residues in the polymer **3** were subjected to repeated deacylation and glycosylation to obtain a trisaccharide. The polymer **3** containing disaccharide residues (or its analogue containing trisaccharide residues) was subjected to ozonolysis in order to detach the reaction product, *viz*., the corresponding di- or trisaccharide. After reduction, the reaction products were isolated as the corresponding 2-hydroxyethyl glycosides. The disaccharide **4** represented an anomeric mixture of derivatives of α- and β-D-glucopyranosyl-(1-6)-D-glucose (*i.e*., isomaltose and gentiobiose). Thus, the solid-phase synthesis was not stereospecific and did not differ in this respect from the corresponding reaction in solution.

An alternative scheme for the solid-phase synthesis of β-D-glucopyranosyl-(1-6)-D-glucose (gentiobiose) was proposed by Guthrie *et al*.[5] who used an orthoester glycosylation method†

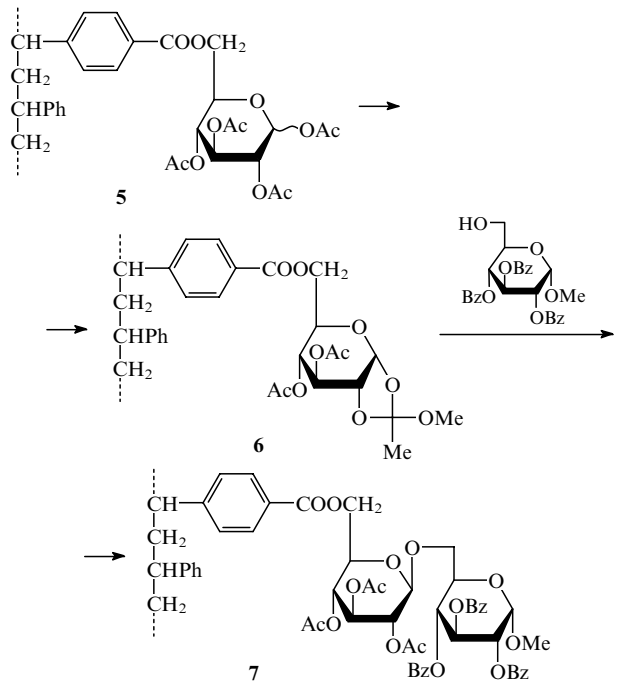
Scheme 3



R = NO_2, MeO.

for the elongation of the chain (Scheme 4). The linear polymer **5**, which contained glucose residues linked through a 6-hydroxy group, was prepared by copolymerisation of styrene with 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-D-glucopyranose or its 6-*O*-(*p*-vinylphenyl)sulfonyl analogue. The glucose residues immobilised on the polymer were converted into the corresponding 1,2-orthoester residues. The resulting polymer **6** was used as a glycosyl donor. Its reaction with methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyr-

Scheme 4



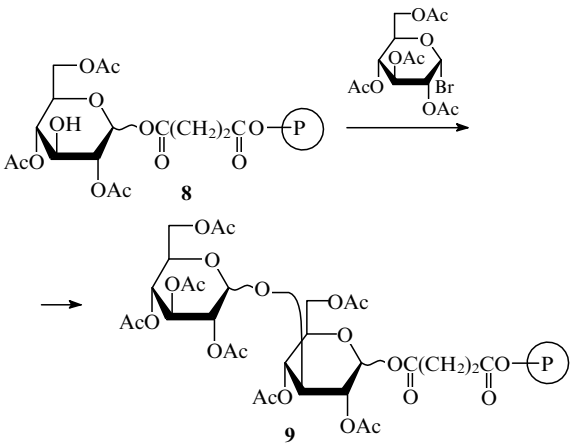
† For a review on the orthoester glycosylation method, see Ref. 22.

anoside under standard conditions of orthoester glycosylation afforded the gentiobiose derivative **7** (yield 64%) which was detached from the polymer by treatment with sodium methoxide. Since glycosylation of primary alcohols with orthoesters is stereospecific, this solid-phase synthesis was also stereospecific and yielded exclusively gentiobiose.

These two studies demonstrated that glycosidic bonds can be formed in heterogeneous reactions and that the value of solid-phase oligosaccharide syntheses does not exceed that of conventional syntheses in solution. Further developments in this field were aimed at overcoming some of the methodological problems, such as the choice of supports, construction of linkers suitable for support immobilisation, intermittent treatment of the polymer, *etc*. The applicability of the solid-phase approach to the synthesis of other simplest oligosaccharides was studied in parallel.

Thus the solid-phase synthesis of D-glucopyranosyl-(1-3)-D-glucose [29] was carried out with the aim of examining the possibility of glycosylation of a less reactive secondary hydroxy group (Scheme 5). To this end, 2,4,6-tri-*O*-acetyl-D-glucopyranose was immobilised on a polystyrene-based resin through a linker (succinic acid residue). The glycosyl acceptor **8** thus immobilised was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide under conditions of the Helferich reaction (48 h, 30 °C). The polymer **9** was separated and washed, after which glycosylation was repeated two more times. The acylglycosidic linkage in the polymer **9** was cleaved with hydrazinium acetate. The yield of the disaccharide, which represented a mixture of an α- (nigerose) and a β-isomer (laminaribiose) (~ 3 : 2), was 81%.

Scheme 5



Glycosylation with 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide was stereospecific; the yield of the α-linked disaccharide was 55%. Thus, the feasibility of glycosylation of immobilised monosaccharides at a less reactive secondary hydroxy group under conditions of a solid-phase synthesis has been demonstrated.

In more recent studies, a succinic acid-based linker has been applied for chemical and enzymic syntheses of oligosaccharides on soluble supports. Krepinsky *et al*.[30] were the first to use this linker for immobilisation of the first monosaccharide on the monomethyl ether of PEG. Glycosylation of the thus immobilised monosaccharide derivatives (*e.g*., methyl galactopyranoside, methyl 2-deoxy-2-phthalimido-β-D-glucopyranoside or 1,6-anhydro-β-D-glucopyranose) was carried out with glycosyl bromides or *O*-glycosyltrichloroacetimidates in the presence of the corresponding promoters. The resulting disaccharides ‡ were easily split off the support after completion of the synthesis. At the same time, there were some difficulties associated with the separation of the immobilised product from an excess of the glycosylating reagent present in the solution which, as was mentioned above, is a disadvantage of the use of soluble polymers as supports.

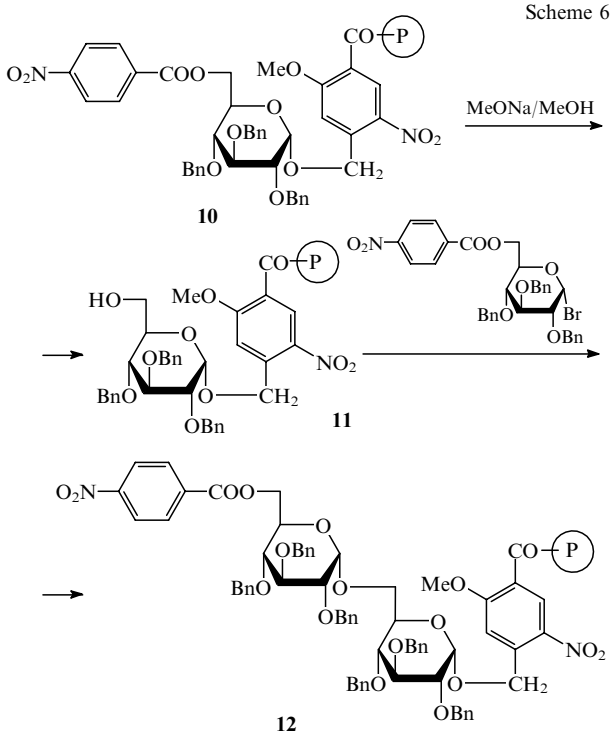
A similar approach was used in the synthesis of β-D-glucopyranosyl-(1-6)-D-galactose. In this case, the glycosyl acceptor (*viz*., selectively protected methyl α-D-galactopyranoside with a free primary hydroxy group) was immobilised on a soluble support (Tentagel) through an OH group at position 2 or 3 using succinic acid as the linker. Glycosylation of the immobilised monosaccharide with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl) trichloroacetimidate was stereospecific; the yield of the disaccharide liberated from the polymer by treatment with aqueous ammonia varied from 50% to 70% depending on the promoter.[19]

In order to facilitate detachment of oligosaccharides from the supports, it was proposed [8] to use the thioglycoside linkage for the binding of the first monosaccharide. This linkage was formed either in a reaction of 1-thio sugars with chloromethylated polystyrene or in a reaction of acylglycosyl halides with polystyrene containing $HSCH_2$ groups. A model synthesis was that of gentiobiose. The resulting disaccharide was split off the polymer as benzyl glycoside by boiling in benzene containing methyl iodide and benzyl alcohol.

Zehavi *et al*.[32–34] proposed to use 4-hydroxymethyl-3-nitrobenzoic and 4-hydroxymethyl-6-methoxy-3-nitrobenzoic acid derivatives as photosensitive linkers. The hydroxy groups of these linkers are glycosylated with monosaccharides, while the carboxy group is used for the binding to the polymeric support. The presence of an *o*-nitrobenzyl alcohol fragment enables smooth decomposition of these linkers upon photolysis in neutral media, which results in the liberation of the oligosaccharides.

These linkers were used for the first time in the synthesis of α-D-glucopyranosyl-(1-6)-D-glucose (isomaltose) (Scheme 6).[32] The 2,3,4-tri-*O*-benzyl-6-*O*-(*p*-nitrobenzoyl)-D-glucopyranose residue was immobilised as an *O*-glycoside on a polystyrene-based resin (**10**) through the linker. The 6-OH group was deprotected by treatment with MeONa after which the polymer **11** containing partially substituted glucose residues was glycosylated with 2,3,4-tri-*O*-benzyl- 6-*O*-(*p*-nitrobenzoyl)-α-D-glycopyranosyl bromide. In order to liberate the target product, which represents an isomaltose derivative, the polymer **12** was irradiated in dioxane for 32 h (yield 80%).

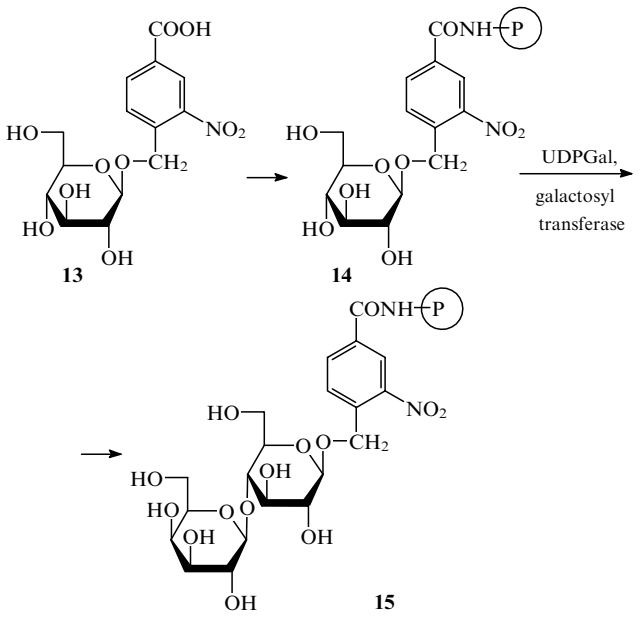
Scheme 6



‡ For the 'methathesis' of oligosaccharides occurring in this glycosylation technique, see Ref. 31.

In more recent studies, the photosensitive linker based on 4-hydroxymethyl-3-nitrobenzoic acid was used for the enzymic synthesis of oligosaccharides on other supports. The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with alkyl 4-hydroxymethyl-3-nitrobenzoate followed by *O*-deacetylation and saponification gave the glycoside **13** (Scheme 7), which was then immobilised on modified polyacrylamide containing 2-aminoethyl groups [13] or on modified polyvinyl alcohol bearing amino groups.[33] The glucose residues in the resulting polymers **14** were linked by glycosidic bonds and served as acceptors in enzymic syntheses. These polymers were incubated with galactosyl transferase in the presence of uridine diphosphate galactose (UDPGal) as a galactosyl donor. β-D-Galactopyranosyl-(1-4)-D-glucose (lactose) formed was split off by irradiation of the polymer **15** in aqueous solution for 20 h. This synthesis was the first to demonstrate the usefulness of glycosyl transferase in regio- and stereospecific synthesis of a disaccharide using glycosylation of an immobilised monosaccharide residue devoid of protecting groups.

Scheme 7



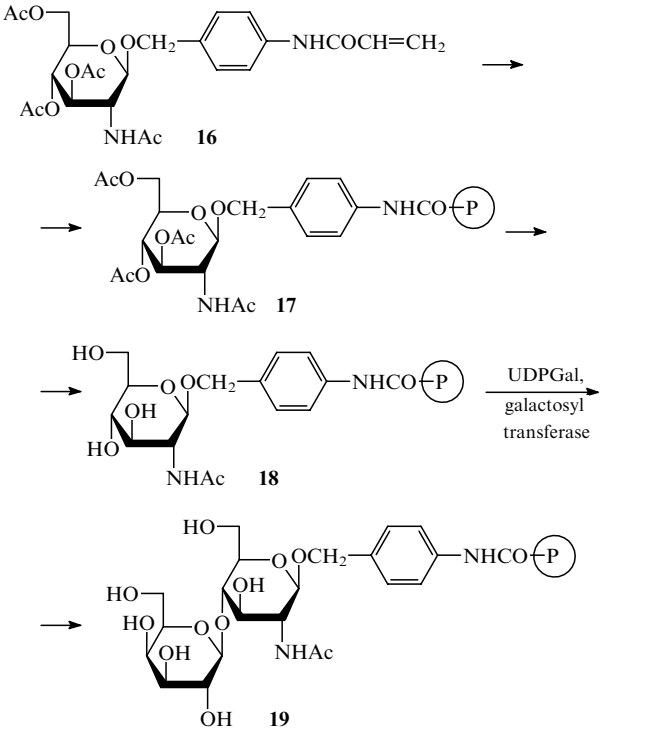
Similarly, the glycosylation of 4-hydroxymethyl-3-nitrobenzoic acid immobilised on polyacrylamide with cellobiose 1,2-orthoester and subsequent deacetylation afforded a polymer containing cellobiose residues with free hydroxy groups. These played the role of acceptors in the enzymic reaction with galactosyl transferase and UDPGal which gave the trisaccharide β-Gal*p*(1-4)-β-Glc*p*(1-4)Glc. Cellobiose immobilised on polyacrylamide was also used in the synthesis of oligosaccharides containing *N*-acetyl-β-D-glucosamine residues which were transferred from chitin in the presence of lysozyme.[13]

Maltose [α-Glc*p*(1-4)Glc], maltotriose [α-Glc*p*(1-4)-α-Glc*p*(1-4)Glc] and panose [αGlc*p*(1-6)-α-Glc*p*(1-4)Glc] were immobilised on *N*-(2-aminoethyl)polyacrylamide through the same photosensitive linker in order to investigate the specificity of glycogen synthase, which transfers glucose residues from uridine diphosphate glucose (UDPG) to glucooligosaccharides, and to elucidate the role of primers in the biosynthesis of glycogen.[34] Oligosaccharide derivatives were attached to 4-hydroxymethyl-3-nitrobenzoic acid; the resulting glycosides were deacylated and immobilised on a polymer through the carboxy group of the linker by treatment with water-soluble carbodiimide. Reactions of these immobilised acceptors with UDPG in the presence of glycogen synthase shed additional light on some essential details of the mechanism of glycogen biosynthesis, *e.g.*, the role of primers, the appearance of branching, *etc.*

In the enzymic synthesis of *N*-acetyllactosamine [2-acetamido-4-*O*-(β-D-galactopyranosyl)-2-deoxy-D-glucose],[35] the *N*-acetylglucosamine residue was immobilised on a water-soluble copolymer of acrylamide and *N*-acryloyloxysuccinimide modified by incorporation of 2-aminoethyl groups through its glycosidic centre and a photosensitive linker (*viz.*, 4-hydroxymethyl-3-nitrobenzoic acid). After *O*-deacetylation of the glucosamine residue, the polymer was incubated with UDPG in the presence of the UDPG epimerase – galactosyl transferase system which provided epimerisation of the glucose residue and transfer of the galactose residue to the acceptor. The *N*-acetyllactosamine thus formed was liberated from the polymer by photolysis.

Copolymerisation of acrylamide with its derivative **16** (Scheme 8) gave rise to polyacrylamide **17** bearing glucosamine residues glycosidically linked to a benzyl-type linker. After *O*-deacetylation, the polymer **18** was incubated with UDPGal and galactosyl transferase. The resulting *N*-acetyllactosamine **19** was split off the polymeric carrier by hydrogenolysis in the presence of Pd/C.[36]

Scheme 8



These examples illustrate that the use of enzymes in solid-phase synthesis of oligosaccharides is promising. The efficacy of enzymic elongation of oligosaccharide chains depends on the distance between the monosaccharide acceptor and the surface of the support. This distance and, as a consequence, the optimum size of the linker, are determined by the molecular mass of the enzyme. If the galactose residue is transferred by galactosyl transferase, it can be attached to the polymer by a chain containing only two methylene units, whereas successful glucose transfer effected by glycogen synthase with a higher molecular mass requires that the chain contained at least six methylene units.

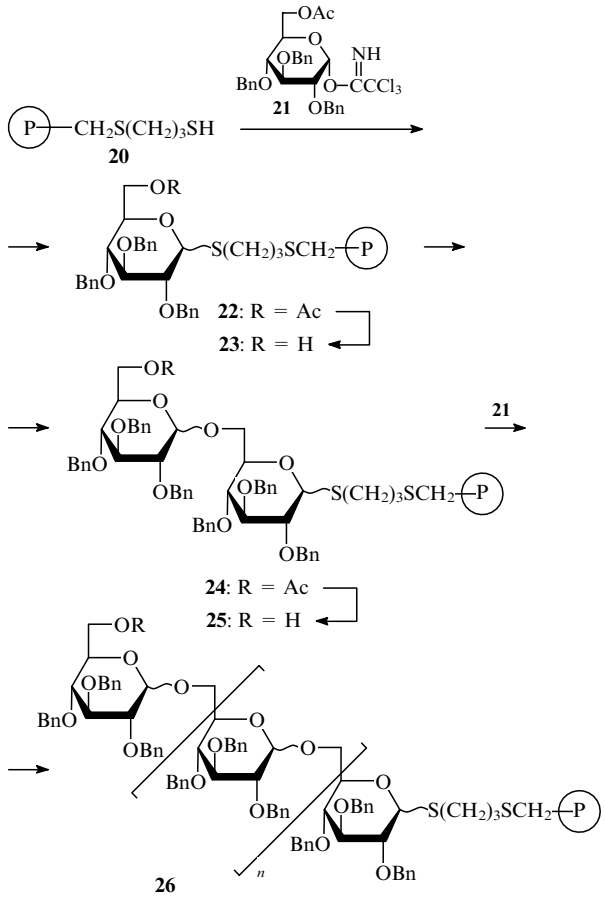
The solid-phase syntheses of di- and trisaccharides described above do not have any obvious advantages over the corresponding syntheses in solution. However, they provided a clue to the solution of some urgent methodological problems in order to pass to targeted synthesis of fragments of homo- and heteropolysaccharides and glycopeptides and contributed also to progress in the combinatorial chemistry of carbohydrates.

IV. Synthesis of higher oligosaccharides

Several solid-phase syntheses of regular homooligosaccharides containing up to 10 monosaccharide units have been carried out to date. Although some of compounds of this type can be prepared by polymerisation of monosaccharide derivatives,[37,38] their synthesis on solid supports made it possible to develop a standard procedure for stepwise elongation of an oligosaccharide chain suitable for concrete method of glycosylation.

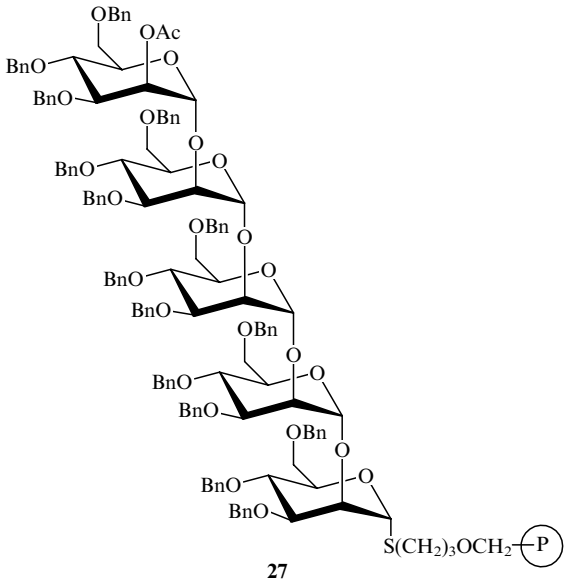
Gluco- and mannooligosaccharides were prepared by the Schmidt trichloroacetimidate method,[39] which is the most popular. The synthesis of (1-6)-D-glucooligosaccharides is shown in Scheme 9. The polymer **20** modified with 3-mercaptopropylthiomethyl groups was prepared by the reaction of the Merrifield polymer with propane-1,3-dithiol. The substituted glucose residue was attached to the free thiol group of the polymer to form the thioglycosidic bond using *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl) trichloroacetimidate (**21**). The 6-*O*-acetyl group in the resulting polymer **22** was removed and the polymer **23** formed was treated with a threefold excess of the imidate **21** in the presence of trimethylsilyl triflate. The *O*-acetyl group in the polymer **24** was removed again, the polymer **25** was glycosylated by treatment with the glycosyl donor **21**, and the whole procedure was repeated as many times as required. After termination of the synthesis, the oligosaccharide was split off from the polymer **26** with a thiophilic reagent [*e.g.*, dimethyl(methylthio)sulfonium triflate] in the presence of a base. This approach was used in the synthesis of di-, tri-, tetra- and pentasaccharides. The yields of the products in each step of the chain elongation were determined by the increase in the polymer mass. In addition, this reaction was monitored by a mass-spectrometric procedure which had been specially developed for this purpose. An aliquot of the polymer was treated with dimethyl(methylthio)sulfonium tetrafluoroborate in a dichloromethane – methanol mixture in the presence of the Hünig base and the oligosaccharides liberated were analysed by mass spectrometry, which made it possible to judge the completeness of glycosylation in each step. According to mass spectrometric data (MALDI – TOF), the yield was 95%.

Scheme 9



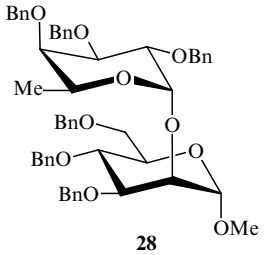
Each glycosylation step afforded a mixture of α- and β-anomers in an α : β ratio of 1 : 1; thus, the pentamer represented a mixture of 32 diastereomers. Hence, this methodology is well-suited for combinatorial syntheses.

A similar approach was used in the synthesis of α-1,2-linked oligomannosides.[11] 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranose residues were incorporated into the polystyrene resin Ⓟ—$CH_2OCH_2CH_2CH_2SH$ prepared in an analogous way. After deacetylation and glycosylation of the deacetylated product with *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl) trichloroacetimidate and reiteration of the reaction cycle, the immobilised oligomannosides **27** (up to the hexasaccharide) were split off the support by treatment with *N*-bromosuccinimide in THF – MeOH in the presence of 2,6-di-*tert*-butylpyridine. The elongation of the chain was monitored by mass spectrometry. The yields of di-, tri- and tetramannosides were 75%, 54% and 34%, respectively.



27

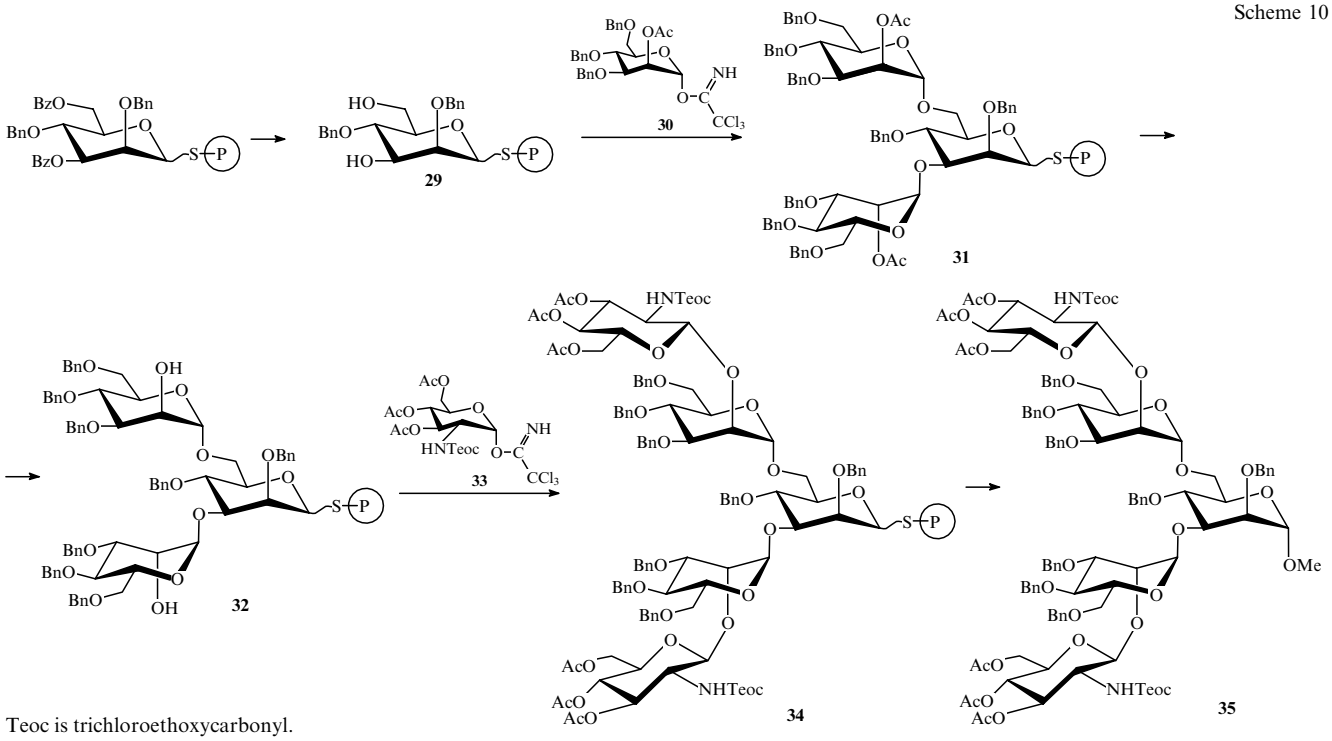
Glycosylation of the first mannose residue with an L-fucose analogue instead of *O*-mannosyl trichloroacetimidate yields the disaccharide **28** containing an α-L-fucosidic (1,2-*cis*-glycosidic) bond.



28

This approach was also used in the synthesis of a well-known core of *N*-glycoproteins, which represents a branched pentasaccharide (Scheme 10).[12] Mannose residues were immobilised on the same polymer, *viz.*, Ⓟ—$CH_2OCH_2CH_2CH_2SH$, by treatment with *O*-(2,4-di-*O*-benzyl-3,6-di-*O*-benzoyl-α-D-mannopyranosyl) trichloroacetimidate. Both benzoyl groups in the resulting polymer were removed, and the diol **29** formed was glycosylated with an excess of *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl) trichloroacetimidate (**30**). The acyl groups in the polymer **31** were removed again, and the immobilised trisaccharide **32** was glycosylated with *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroethoxycarbonylamino-α-D-glucopyranosyl) trichloroacetimidate

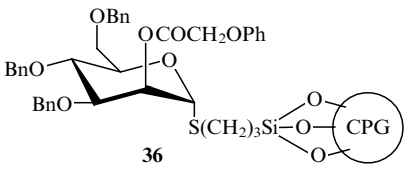
Scheme 10



Teoc is trichloroethoxycarbonyl.

(**33**), which afforded a polymer with an immobilised pentasaccharide (**34**). The thioglycosidic linkage was cleaved with *N*-bromosuccinimide in the presence of methanol and 2,6-di-*tert*-butylpyridine resulting in the pentasaccharide **35** in 20% yield; hence, the yield of glycosylation was 85% in each step. The yields of the reaction products were monitored by mass spectrometry, while the stereochemistry of the glycosidic bonds was established by NMR spectroscopy.

Analogous syntheses of α-(1-2)-linked mannooligosaccharides were performed on other supports as well, *e.g.*, on porous glasses with grafted mercaptopropyl groups.[40] The 3,4,6-tri-*O*-benzyl-2-*O*-phenoxyacetyl-D-mannose residues were attached to the support modified by treatment with 3-mercaptopropyltrimethoxysilane. The phenoxyacetyl group in the immobilised saccharide **36** was removed with guanidine and the next mannose residue was attached to the liberated hydroxy group after which the whole reaction cycle was reiterated. The oligomannoside synthesised was split off the support by treatment with *N*-bromosuccinimide in the presence of methanol and 2,6-di-*tert*-butylpyridine. Mass-spectrometric analysis revealed that the resulting trisaccharide contained only trace amounts of the mono- and the disaccharide, which suggests that glycosylation occurred with high yields in each step.



36

Synthesis of this type of oligomannosides have also been carried out on a soluble support, *viz.*, monomethyl ether of polyethyleneglycol.[17] 1,4-Bis(hydroxymethyl)benzene§ has been used as a linker and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranose residue was attached through this linker. The elongation of the oligosaccharide chain was carried out as described above. A scandium(III) triflate – acetic anhydride system was recommended for the splitting of the reaction product from the polymer.[41]

In all these oligomannoside syntheses, the chain was elongated from the reducing end to the non-reducing one. However, an alternative route is documented, which utilises an opposite strategy, *i.e.*, the elongation is performed from the non-reducing end of the chain to its reducing end. The synthesis on the soluble support (PEG) was carried out according to the following scheme.[42] The linker used was a 6-(4-hydroxymethylphenoxy)-hexanoic acid derivative, which alkylated methyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside at position 2 and esterified PEG (Scheme 11). The substituted methyl 1-thiomannopyranoside residue in the polymer **37** served as the glycosyl donor, reacted with 3,4,6-tri-*O*-benzyl-D-mannopyranosyl fluoride in the presence of methyl triflate to give the disaccharide derivative **38** which also played the role of a glycosyl donor. The Suzuki glycosylation[43] of 2-(trimethylsilyl)ethyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside resulted in the immobilised trisaccharide **39**; the free trisaccharide was split off the polymer by treatment with a base and deprotected by hydrogenolysis.

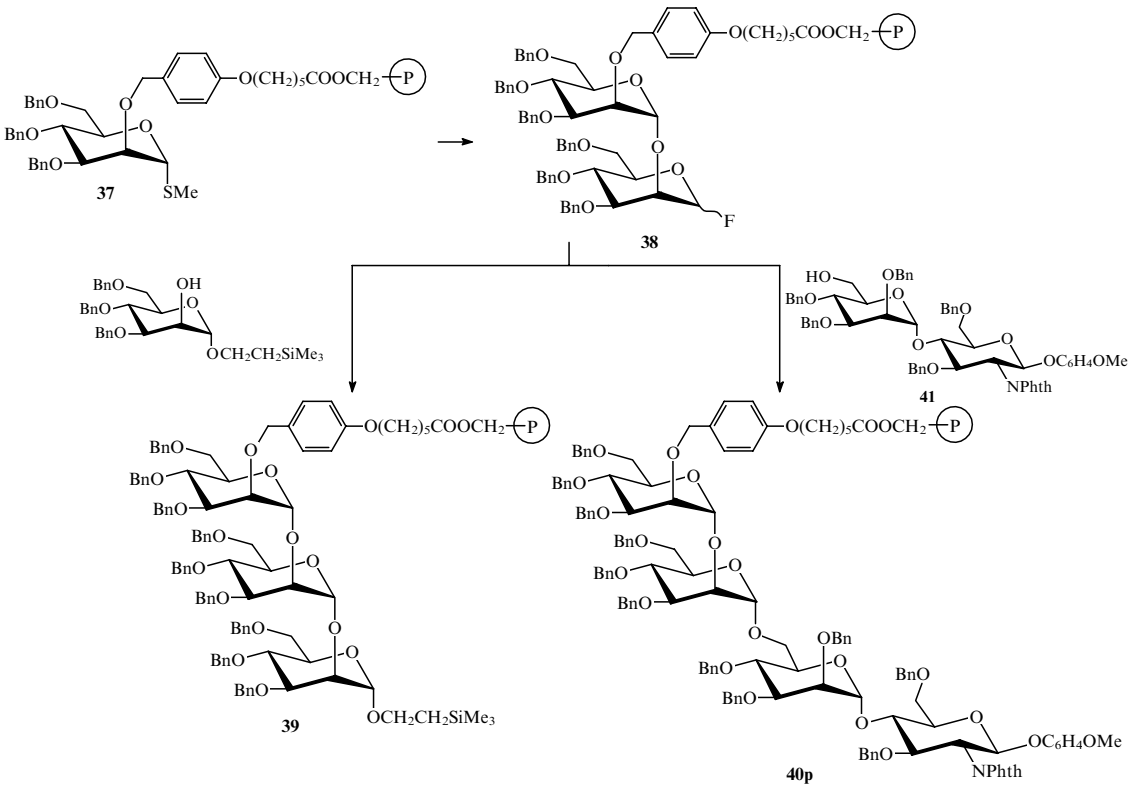
The target product (yield 40%) was easily separated from by-products owing to the presence of a hydrophobic trimethylsilylethyl group.

Incorporation of hydrophobic groups to facilitate isolation of the target product was also used[42] in the synthesis of a structurally more complex heterooligosaccharide **40**, which is a fragment of glycophosphatidylinositol. The latter was prepared by a reaction of the disaccharide **41** with the glycosyl donor **38**. The tetrasaccharide **40**, which was cleaved from the polymer **40p** by hydrogenolysis, could easily be purified by reversed-phase chromatography owing to the presence of hydrophobic phthalimido- and methoxyphenyl groups. This methodology is an interesting example of modification of carbohydrate synthesis on solid phase supports.

From the oligomannoside syntheses described above one cannot draw any final conclusion in favour of one or another synthetic procedure, but the flexibility and high potential of the solid-phase method have been demonstrated.

§ It should be noted that this type of linker has also been used in some other syntheses on soluble supports, *e.g.*, in the synthesis of model disaccharides.

Scheme 11



The solid-phase synthesis of the natural immunologically active heptasaccharide **42**

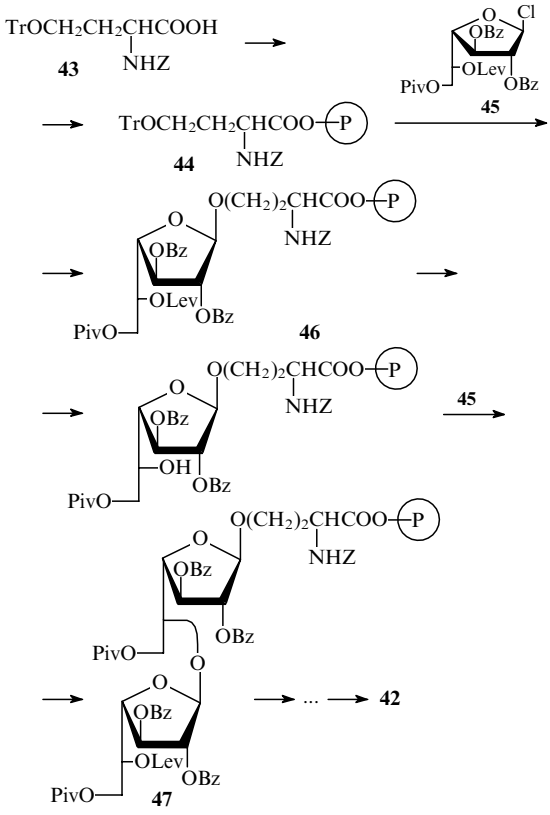
β-Gal*f*(1-5)[β-Gal*f*(1-5)]$_5$β-Gal*f*OCH$_2$CH$_2$CHCOOH
42 NH$_2$

containing β-(1-5)-linked galactofuranose units and an L-homoserine residue at the reducing end (Scheme 12) [44] deserves special mention. L-Homoserine **43** with protected OH and NH_2 groups was immobilised on Merrifield's resin. The polymer **44** was detritylated and the resulting hydroxyl-containing derivative was glycosylated with 2,3-di-*O*-benzoyl-5-*O*-levulinoyl-6-*O*-pivaloyl-β-D-galactofuranosyl chloride (**45**) under conditions of the Helferich reaction. The resulting polymer **46**, which contained immobilised galactofuranose residues (the support loading was 0.5 mmol g^{-1}), was used as a starting material in the synthesis of the heptasaccharide **42**. Selective removal of the levulinic acid residue from the polymer **46** and glycosylation of the liberated hydroxy group with the chloride **45** under identical conditions resulted in the elongation of the chain with one more galactofuranose unit and afforded the polymer **47**. Reiteration of this reaction sequence resulted in the heptasaccharide derivative **42**, which was split off the polymer with simultaneous removal of all *O*-benzoyl groups by treatment with a base. The benzyloxycarbonyl group was removed by hydrogenolysis. The overall yield of the heptasaccharide **42** was 23%.

The resulting oligosaccharide contained exclusively β-glycosidic linkages and was virtually free from shorter oligomers, which suggested high yields in each glycosylation step and stability of the protecting groups. The choice of protecting groups was of particular importance in this synthesis. Thus upon substitution of a selectively removable chloroacetyl group for a levulinoyl group a large amount of admixtures was formed. The synthetic oligosaccharide **42** can be easily converted into the corresponding glycoconjugate owing to the presence of the homoserine residue. This is the first example of the synthesis of an oligosaccharide chain attached to an amino acid residue, which opens a route to the solid-phase glycopeptide syntheses.

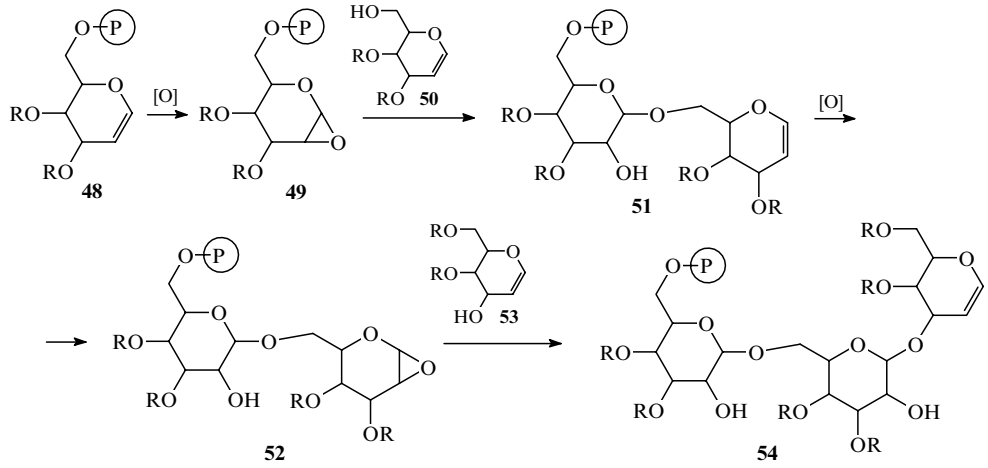
Further developments in solid-phase oligosaccharide synthesis were aimed at a search for novel methods of glycosylation. The so-called glycal method (Scheme 13) [10] offers the most interesting opportunities for the synthesis of complex (including branched) oligosaccharides. After treatment with a mild epoxidising reagent (*viz*., 3,3-dimethyldioxirane), the glycal **48** immobilised on a support through a hydroxy group is converted into the 1,2-anhydrosugar **49**. The latter reacted with the glycal **50** with a free

Scheme 12

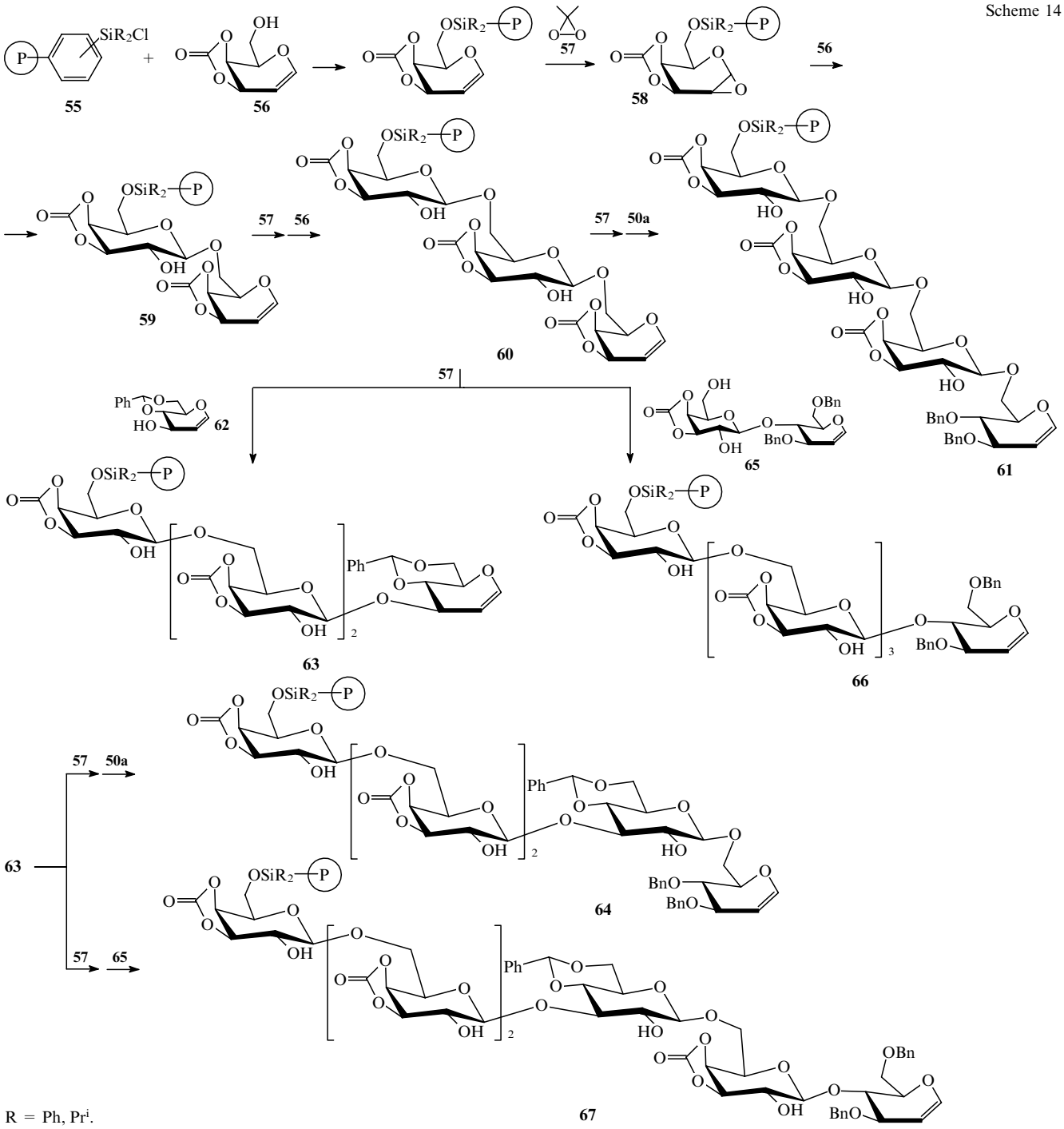


Tr = Ph_3C; Z = $PhCH_2OCO$; Piv = Me_3CCO; Lev = $MeCO(CH_2)_2CO$.

Scheme 13



Scheme 14



R = Ph, Pr^{i}.

hydroxy group in the presence of $ZnCl_2$ to yield the disaccharide glycal **51** which is further converted into the 1,2-anhydrosugar **52**. Its reaction with the monosaccharide glycal **53** afforded the immobilised trisaccharide glycal **54**, and so on. The yields in each glycosylation step amounted to 90%–95%. High stereospecificity of glycosidic linkage formation is governed by well-established regularities in the epoxide ring opening. As can be seen, the main characteristic feature of this approach is the reconstruction of a reactive epoxide group (*i.e.*, a glycosyl donor group) at the reducing end of the growing chain under standard mild conditions. By varying the structure of the glycal acceptor, one can obtain both regular and irregular homo- and heterooligosaccharides. Immobilised glycals with free hydroxy groups resulting either from the reaction or after selective removal of protecting groups can also play the role of glycosyl acceptors which permits their glycosylation leading to branched oligosaccharides.

It should be emphasised that the use of the glycal method simplifies the purification of the reaction product, since possible admixtures formed as a result of incomplete glycosylation in the intermediate steps of the synthesis become more polar after final treatment and can easily be separated from the target oligosaccharides by chromatography.

The high potentials and flexibility of the glycal method can be exemplified in the convergent synthesis of several oligosaccharides on an insoluble support (Scheme 14).[10] Here, R_2SiCl groups (R = Ph, Pr^i), which are used as linkers for binding the first monomer to the polymeric support, were incorporated into a polystyrene resin by sequential treatment with butyllithium and diphenyl- or diisopropyldichlorosilane. The use of such linkers allows easy cleavage of the O–Si bond upon treatment with Bu_4NF, which enables detachment of the synthesised product from the support under mild conditions.

Treatment of the thus modified polystyrene **55** with a D-galactal derivative **56** in the presence of a base followed by oxidation with dimethyldioxirane (**57**) affords the polymer **58**, which contains the immobilised anhydrosugar residue, which is employed as the glycosyl donor for the elongation of the chain. Glycosylidation of the polymer **58** with D-galactal **56** in the presence of anhydrous $ZnCl_2$ yields the disaccharide glycal derivative **59**. Reiteration of the reaction cycle results in the trisaccharide glycal **60**; its reaction with 3,4-di-*O*-benzyl-D-glucal (**50a**) yields the tetrasaccharide glycal **61** with (1-6) glycosidic linkages. The introduction of a structurally different glycal acceptor (*e.g.*, 4,6-*O*-benzylidene-D-glucal **62**) into the reaction with an immobilised glycosyl donor prepared from the trisaccharide glycal **60** results in the formation of the tetrasaccharide glycal **63** of irregular structure. After conversion into the glycosyl donor, the glycal **63** reacts with 3,4-di-*O*-benzyl-D-glucal as described above to yield the irregular pentasaccharide derivative **64**. Finally, oxidation of the trisaccharide glycal **60** and subsequent reaction with the disaccharide glycal **65** afforded the pentasaccharide glycal **66**. Conversion of the tetrasaccharide glycal **63** into a glycosyl donor using the conventional scheme and reaction of the latter with the disaccharide glycal **65** result in the hexasaccharide derivative **67**.

In each step of this convergent synthesis, the oligosaccharides can be split off the polymer by treatment with Bu_4NF and protecting groups can be removed by standard methods. The yields of the oligosaccharides are 80%–90% in each step, which gives the final yields of the order of 20% to 40%. The stereochemistry of glycosidic linkages in all steps of this synthesis was established by NMR spectroscopy.

The usefulness of two other, recently proposed glycosylation methods for solid-phase synthesis, *viz*., those utilising glycosyl sulfoxides[24] and pent-4-enyl glycosides[45] as glycosyl donors, is more difficult to evaluate.

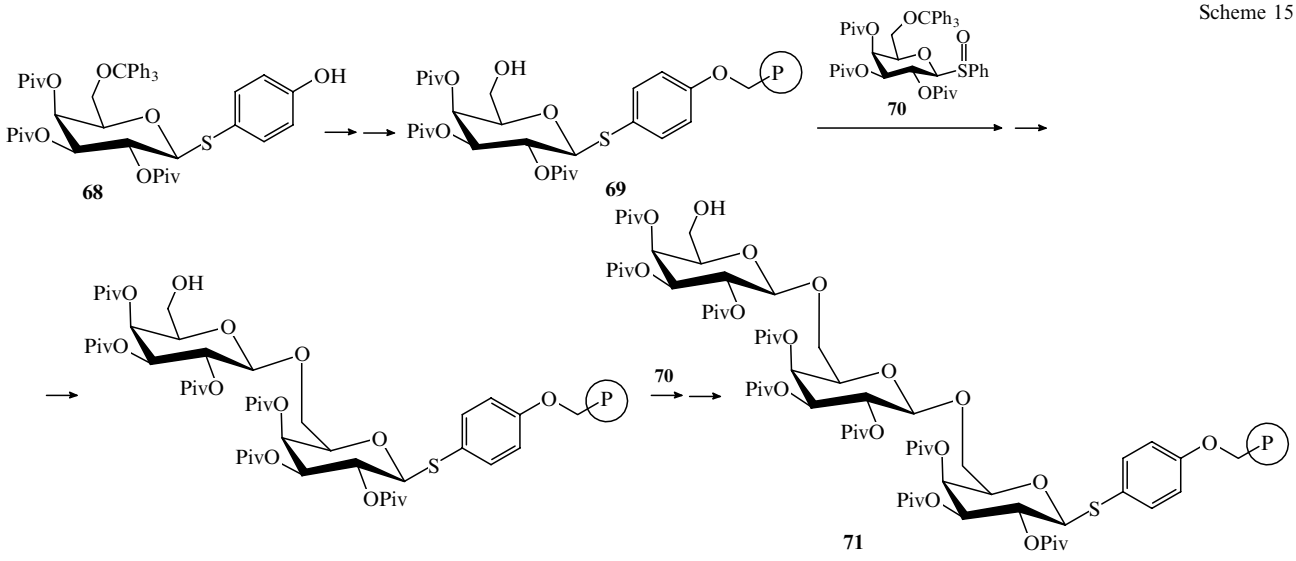
Several examples of solid-phase syntheses with the use of these glycosylating reagents are considered below.

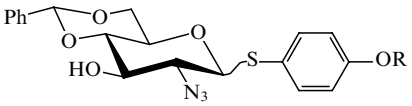
p-Hydroxyphenyl 2,3,4-tri-*O*-pivaloyl-6-*O*-trityl-1-thio-β-D-galactopyranoside (**68**) was immobilised on Merrifield's resin (Scheme 15).[24] After removal of the trityl group from the immobilised monosaccharide, the hydroxy group in the polymer **69** was glycosylated with the sulfoxide **70** in the presence of trifluoromethanesulfonic acid anhydride and 2,4,6-tri-*tert*-butylpyridine. After removal of the trityl group from the immobilised disaccharide, the reaction sequence was repeated. The resulting derivative of the corresponding trisaccharide **71** was split off the support by treatment with mercury trifluoroacetate in a dichloromethane–water mixture. The glycosylation occurred stereospecifically with formation of a β-galactosidic bond. The yield of the trisaccharide was 52%. Taking into account the fact that only 70% to 75% of the oligosaccharide is liberated from the polymer, the yields of di- and trisaccharides in the glycosylation step were no less than 94%–95%.

Glycosyl sulfoxides can be used as glycosyl donors in the glycosylation of secondary hydroxy groups as is exemplified in the synthesis of two synthetic precursors of Lewis antigens of group-specific blood glycoproteins.

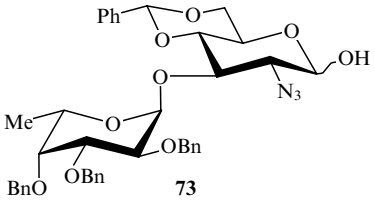
Glycosylation of 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-D-glucoside immobilised in an analogous way (**72**) with sulfoxides based on 2,3,4-tri-*O*-benzyl-L-fucose and 2,3,4,6-tetra-*O*-pivaloyl-D-galactose afforded the disaccharides **73** and **74** (yields 67% and 64%, respectively); these reactions were stereospecific.

Scheme 15

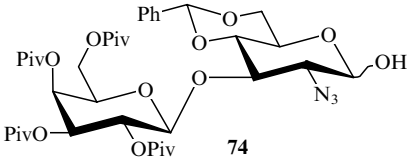




72



73



74

The stereospecificity of these reactions, which is particularly emphasised by the authors,[24] is quite obviously governed by the nature of a substituent at the O(2) of the glycosyl donor. The same regularity is observed when glycosylation is carried out in solution.

Further utilisation of glycosyl sulfoxides in solid-phase syntheses of oligosaccharides is still not documented.

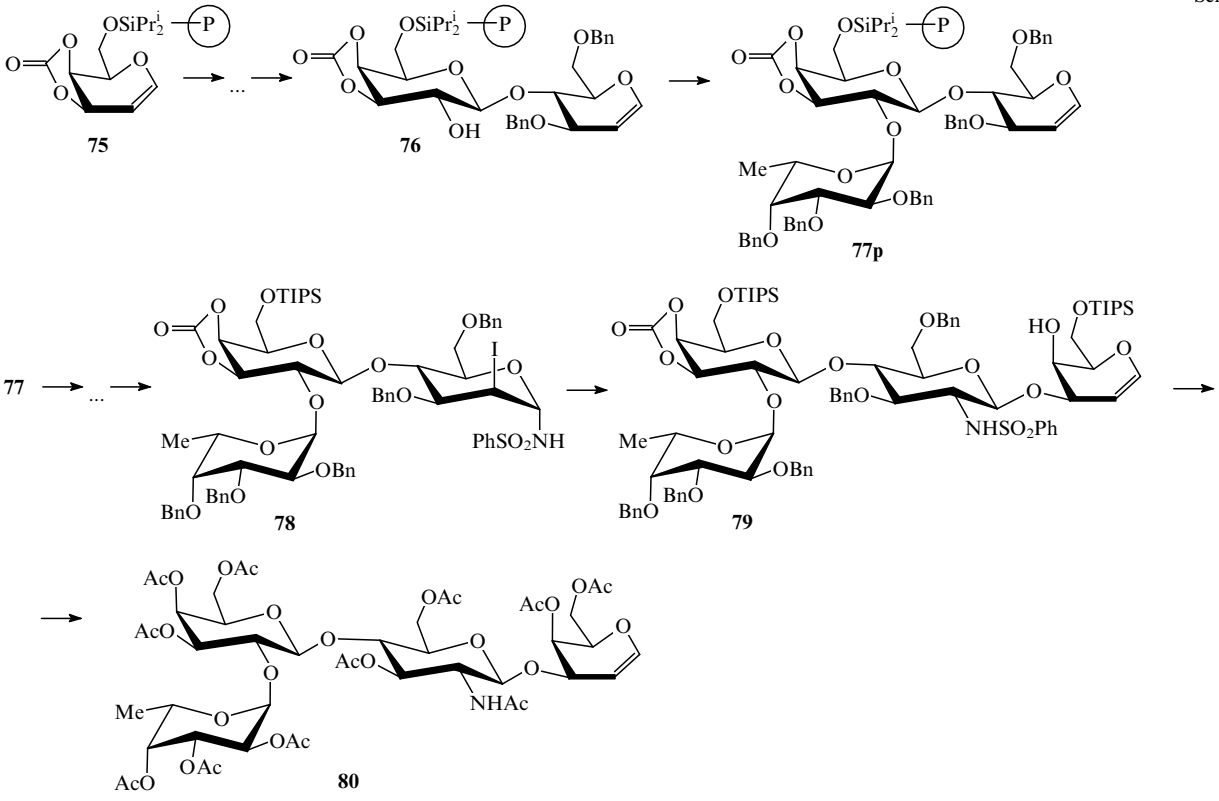
Rather specific experimental conditions and uncertainty of the problem of stereospecificity in the case of other monosaccharides seem to preclude the widespread application of glycosylation based on the use of glycosyl sulfoxides.

Pent-4-enyl glycosides act as glycosylating agents following generation of a carbocation at the glycosidic centre of the sugar as a result of treatment with *N*-iodosuccinimide and triethylsilyl triflate. The glycosylation with n-pent-4-enyl glycosides has been described in detail;[45] some general considerations concerning the applicability of this reaction to solid-phase syntheses on different supports, with different linkers and monosaccharides were briefly surveyed in Ref. 46. According to Fraser-Reid *et al.*,[46] solid-phase glycosylation gives satisfactory yields. Since the stereoselectivity of this reaction is not very high, it is recommended for use in combinatorial chemistry for the acquisition of 'libraries' of immobilised oligosaccharides designed for direct activity assays. The principles common to combinatorial chemistry have been applied for the acquisition of a library of deprotected oligosaccharides concomitant with the synthesis of the trisaccharide β-Gal*p*(1-2)-α-Glc*p*(1-6)GlcNAc (no experimental details were given).

The advantages of solid-phase oligosaccharide synthesis are most conspicuous in the directed synthesis of complex fragments of natural biopolymers. Thus, the glycal method was used in the synthesis of a series of biologically important oligosaccharides which play an essential role in carbohydrate – protein interactions and represent antigenic determinants. Some steps of this synthesis were carried out on a polystyrene support.

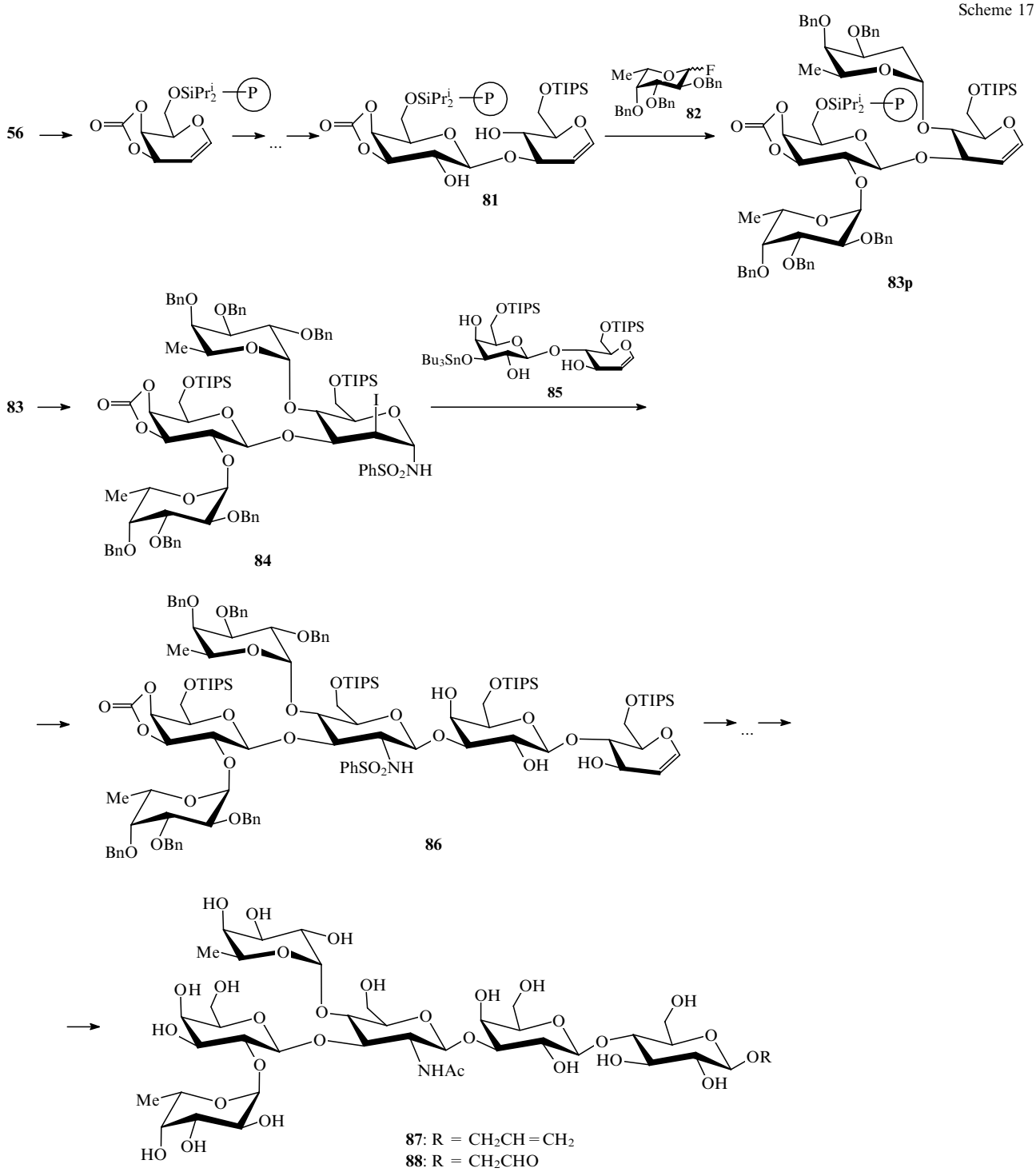
The synthesis of the blood-group H-determinant (type 2) is shown in Scheme 16.[47] 3,4-*O*-Carbonyl-D-galactal **75** immobilised on a polystyrene resin through a diisopropylsilyl linker was oxidised with dimethyldioxirane into the corresponding 1,2-anhydrosugar which was then introduced into a reaction with 3,6-di-*O*-benzyl-D-glucal in the presence of $ZnCl_2$. The resulting immobilised disaccharide glycal **76** was glycosylated at the free OH group at position 2′ with 2,3,4-tri-*O*-benzyl-L-fucopyranosyl fluoride in the presence of tin triflate and 2,6-di-*tert*-butylpyridine. The immobilised trisaccharide **77p** was treated with Bu_4NF to liberate the trisaccharide **77** (overall yield was 50%) after which the synthesis was continued in the solution. The hydroxy group at C(6) of the galactose residue in the trisaccharide **77** was protected with a triisopropylsilyl group and treated with benzenesulfamide in the presence of iodonium(dicollidine) perchlorate. The resulting 2-deoxy-2-iodo-*N*-phenylsulfonylglycosylamine **78** (*cf.* Ref. 48) underwent rearrangement in a reaction with 3-*O*-tributylstannyl-6-*O*-triisopropylsilyl-D-galactal in the presence of silver tetrafluoroborate.[49] The resulting tetrasaccharide **79** was subjected to

Scheme 16



TIPS = $SiPr^i_3$.

Scheme 17

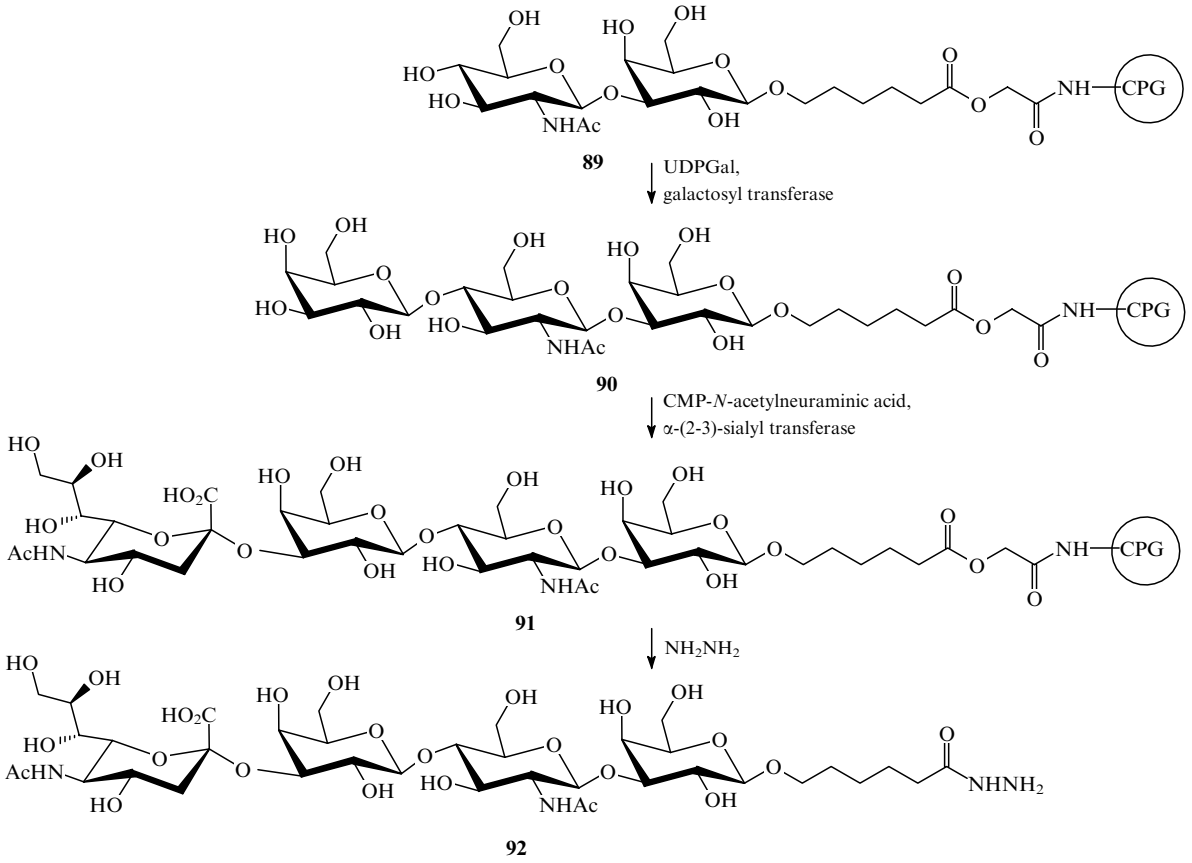


deprotection and *N*-acetylation to give the tetrasaccharide **80**, which is a derivative of the H-antigen (type 2) of a blood group-specific glycoprotein.

A similar approach was used in the synthesis of a Le^b-specific hexasaccharide, which was prepared in a form suitable for conjugation with proteins (Scheme 17).[47] The starting galactal **56** was immobilised on a polystyrene resin; the reaction product was converted into an 1,2-anhydrosugar, which further glycosylated 6-*O*-triisopropylsilyl-D-glucal [its most reactive hydroxy group at C(3) reacts selectively]. The (1-3)-linked disaccharide **81** with two free hydroxy groups was glycosylated with an excess of 2,3,4-tri-*O*-benzyl-L-fucopyranosyl fluoride (**82**). The resulting tetrasaccharide **83p** bearing two L-fucose residues was split off the support, the primary alcohol groups in the tetrasaccharide liberated were silylated after which the synthesis was continued in solution. The tetrasaccharide **83** was converted into tetrasaccharide 2-deoxy-2-iodo-*N*-phenylsulfonylglycosylamine **84**. The latter reacted with 3-*O*-tributylstannyllactal derivative **85** to give the hexasaccharide glycal **86**, which was converted into an 1,2-anhydro derivative and then made to react with allyl alcohol to obtain the allyl glycoside of the corresponding hexasaccharide **87**. To prepare antibodies against Le^b antigen, the hexasaccharide **87** was converted into the aldehyde **88** suitable for conjugation with proteins.

Halcomb *et al.*[15] performed an enzymic synthesis in solution of the pentasaccharide NeuAc-α-(2-3)-Gal-β-(1-4)-[Fuc-α-(1-3)]-GlcNAc-β-(1-3)-Gal and an enzymic solid-phase synthesis of the tetrasaccharide NeuAc-α-(2-3)-Gal-β-(1-4)-GlcNAc-β-(1-3)-Gal,

Scheme 18

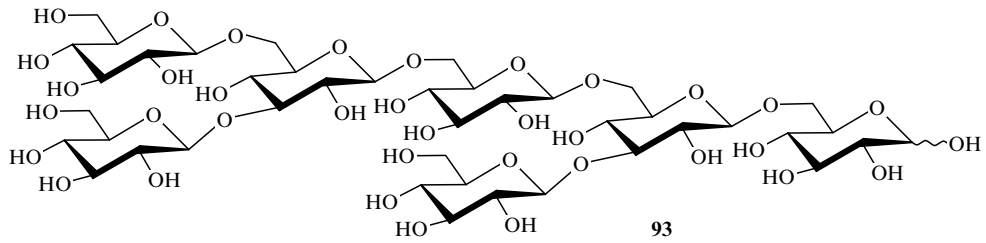


which are known as inhibitors of E-selectin and *Helicobacter pylori*, respectively. A fragment of the pentasaccharide, *viz*., the tetrasaccharide NeuAc-α-(2-3)-Gal-β-(1-4)-[Fuc-α-(1-3)]-GlcNAc (sialyl-Lewis X, SLe^X), is a tumour-associated antigen; it is involved in processes of cell adhesion, metastasis, inflammation and thrombosis. This antigen contains neuraminic acid; its stereospecific introduction into oligosaccharides by chemical methods presents a problem, therefore the use of enzymic glycosylation is more preferable in this case.

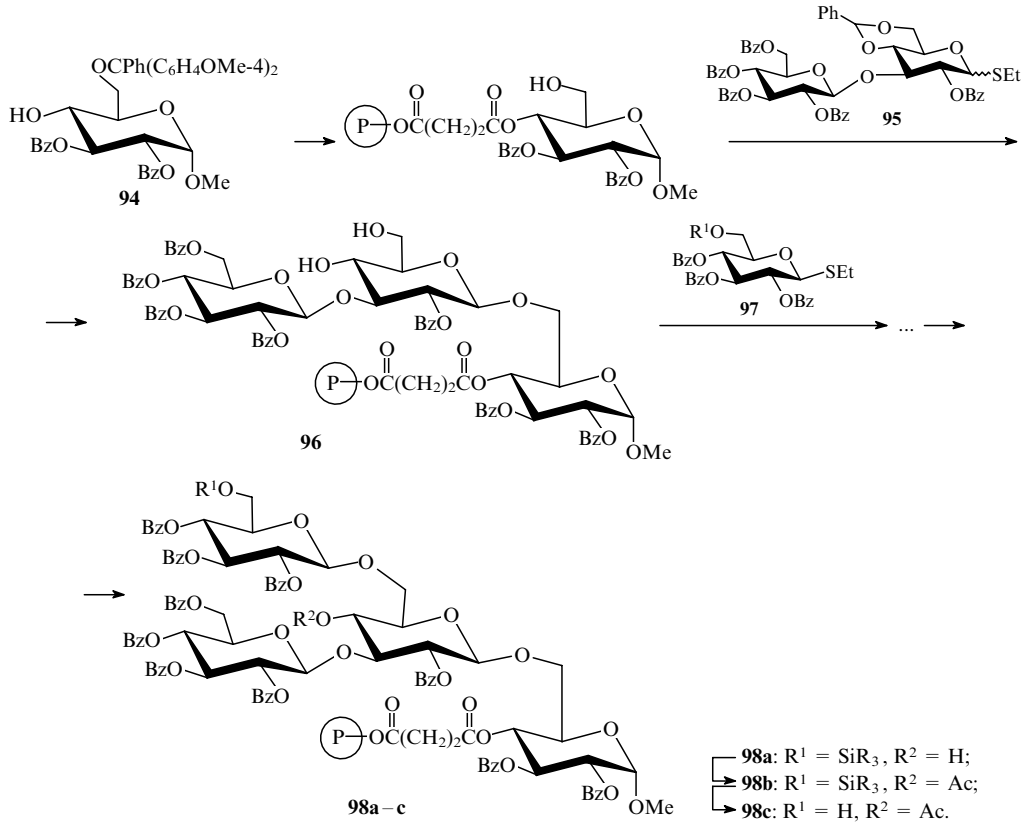
The solid-phase synthesis of the tetrasaccharide NeuAc-α-(2-3)-Gal-β-(1-4)-GlcNAc-β-(1-3)-Gal was performed on CPG modified with iodoacetylamino groups, which made it possible to conduct the synthetic operations in both aqueous solution and organic solvents as required (Scheme 18).[15] The starting disaccharide, *viz*., 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1-3)-D-galactopyranose, prepared by chemical synthesis as a glycoside of ω-hydroxyhexanoic acid, was immobilised on the modified CPG. After incubation with UDPGal in the presence of β-(1-4)-galactosyl transferase, the immobilised disaccharide **89** yielded the trisaccharide **90**, which was made to react with cytidine 5′-phospho-*N*-acetylneuraminic acid (CMP-*N*-acetylneuraminic acid) in the presence of α-(2-3)-sialyl transferase. The resulting tetrasaccharide **91** was split off the support with hydrazine and isolated as ω-hydroxyhexanohydrazide glycoside **92** suitable for conjugation with proteins. This synthesis demonstrates again the advantages of the enzymic approach in solid-phase oligosaccharide syntheses where the regio- and stereospecificity of the glycosidic bonds formed are determined by the specificities of glycosyl transferases.

The synthesis of the phytoalexin elicitor (PAE), which prevents viral and bacterial infections in plants, is yet another example of successful application of the solid-phase methodology in directed synthesis of biologically active and structurally complex oligosaccharides. Synthetic PAE (a branched heptasaccharide) presents substantial practical interest for agriculture, since the natural product is difficultly accessible due to its low concentration in plants. The synthesis of PAE **93** was carried out using two different approaches.

In the first approach (Scheme 19),[50] the oligosaccharide chain was elongated by sequential addition of blocks on a soluble polymeric support (PEG) using a thioglycoside glycosylation method. The starting monosaccharide, *viz*., methyl 2,3-di-*O*-benzoyl-6-*O*-dimethoxytrityl-α-D-glucopyranoside (**94**), was attached to the polymer modified with succinic acid through the hydroxy group at C(4) (the support loading was 148 mmol g^{-1}). After mild acidolysis of the dimethoxytrityl group, the polymer was introduced into the reaction with the disaccharide thioglycoside **95** in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid. Benzylidene protection was removed by acid hydrolysis after which the immobilised trisaccharide **96** was subjected to regioselective glycosylation with the thioglycoside **97** at O(6′). Subsequent acylation of the hydroxy group at C(4′)



Scheme 19



(**98a** → **98b**) and removal of silyl protection resulted in the tetrasaccharide **98c**.

Reiteration of this reaction sequence, *viz*., the reaction with the disaccharide **95**, debenzylidation and the reaction with the thioglycoside **97**, afforded the protected immobilised heptasaccharide. Purification of the reaction products formed in each step from excess of the soluble reagents was achieved by precipitation of the polymeric material and subsequent washing with diethyl ether. Deprotection by treatment with sodium methoxide was accompanied by splitting of the heptasaccharide from the polymer eventually resulting in the biologically active PAE methyl glycoside (yield 18%).

According to the second scheme, PAE and its lower analogues were synthesised on a hydroxylated polystyrene resin using a photosensitive linker **99** and thioglycoside glycosylation.[7] Nitrobenzyl alcohol **99** was glycosylated with phenyl-2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside (**100**), and the resulting glycoside was immobilised. This glucose residue served as the origin of the oligosaccharide chain which was elongated by successive treatment with the thioglucosides **100**, **101** and **102** (Scheme 20). The thioglucoside **100** contained a selectively removable *tert*-butyldiphenylsilyl protecting group at O(6), while the thioglucoside **101** contained a 9-fluorenylmethoxycarbonyl (Fmoc) group at O(4). These groups were selectively removed when required, and the hydroxy groups liberated were glycosylated with the corresponding thioglucoside in the presence of dimethyl(methylthio)sulfonium triflate. The resulting heptasaccharide (overall yield 20%) was split off the support by photolysis.

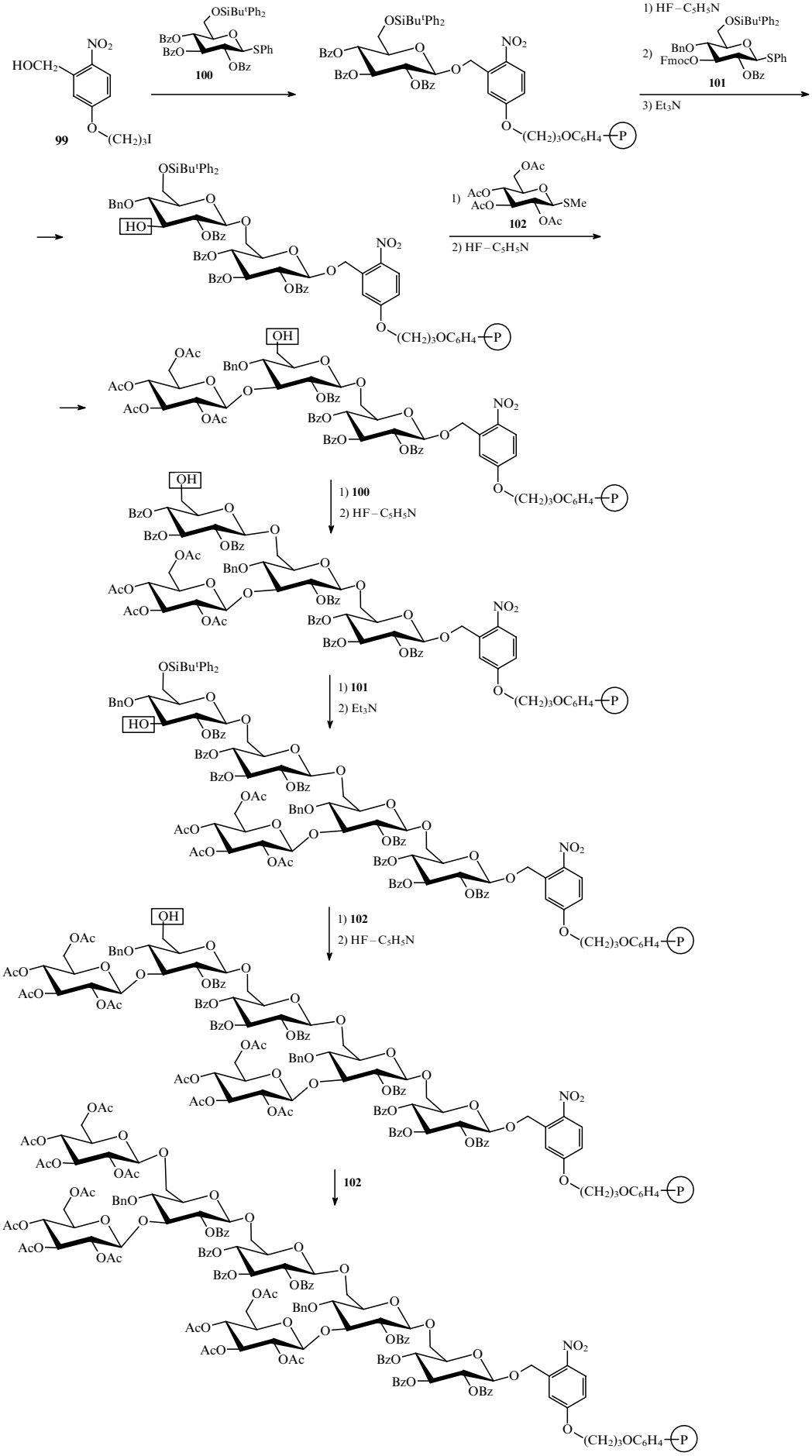
Solid-phase synthesis was also used for the preparation of oligosaccharides containing 6-deoxy- and/or 2,6-dideoxymonosaccharide units which represent carbohydrate chains of some antibiotics.[9] This synthesis combines ingeniously detachment of the oligosaccharide from the support with nucleophilic substitution of the hydroxy group involved in immobilisation of the sugar on the polymer. The Merrifield's resin modified by introduction of ethanesulfonic acid was used as the solid support. Methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- and methyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-glucopyranoside **103a**(**b**) were immobilised on this polymer (Scheme 21) after which *O*-acetyl groups were removed by treatment with guanidine. The immobilised monosaccharides **104a**(**b**) reacted with the glycosyl donors **105** and **106** in the presence of trimethylsilyl triflate to afford the corresponding immobilised disaccharides **107**–**110**. These disaccharides were split off the support by treatment with NaI in ethyl methyl ketone with simultaneous substitution of iodine for the sulfonyloxy group. The yields of iododeoxysaccharides (**111**–**114**) varied from 85% to 91%. Their treatment with Bu_3SnH gave the corresponding deoxy sugars **115**–**118** in 92% yield.

This method was used in the targeted synthesis of the carbohydrate fragment of the antibiotic olivomycin A (Scheme 22).[9] Glucal **119** was immobilised on a sulfonylethylated Merrifield's polymer after which it was desilylated and treated with trichloroacetimidate **122**. The immobilised disaccharide **123** formed was desilylated (**123** → **124**) and glycosylated with glycosyl acetate **106** in the presence of trimethylsilyl triflate. The trisaccharide **125** was detached from the polymer with simultaneous substitution of iodine for bromine (yield 67%). The glycal **125** was used for direct incorporation of the carbohydrate fragment into the aglycon of olivomycin A.

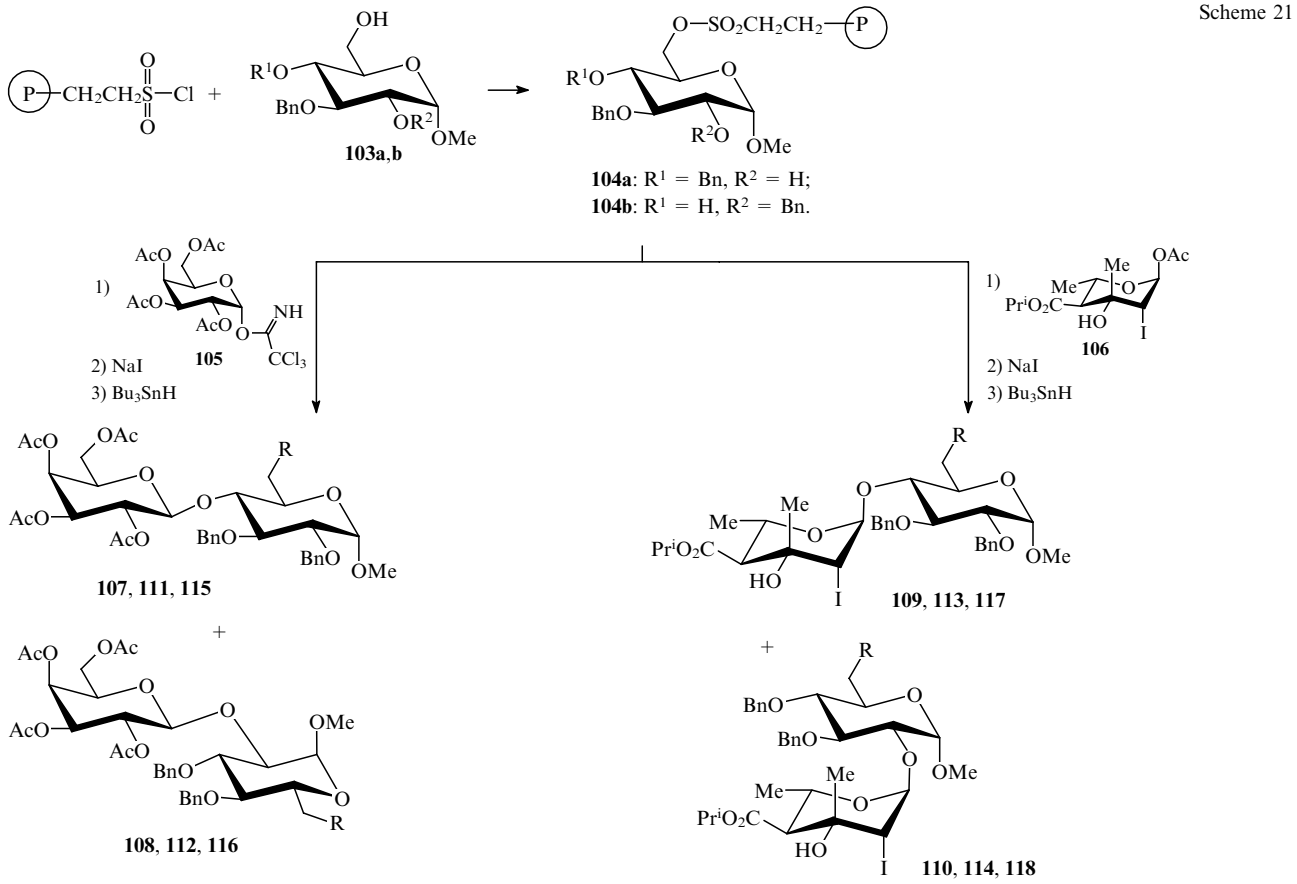
An important observation which awaits rationalisation is the fact that the glycosylation of the immobilised acceptor with trichloroacetimidate **122** was stereospecific leading exclusively to β-linkages, whereas the product synthesised in solution contained some proportion of α-linked oligosaccharide.

This example is also interesting in that it illustrates that the solid-phase synthesis of carbohydrates can be combined with simultaneous transformation of the monosaccharide residues. This significantly extends the range of reactions in synthetic chemistry of carbohydrates performed on solid supports. The same approach can be used for the introduction of amino sugar units into oligosaccharides if sodium azide is employed instead of NaI for splitting off the reaction product.[9]

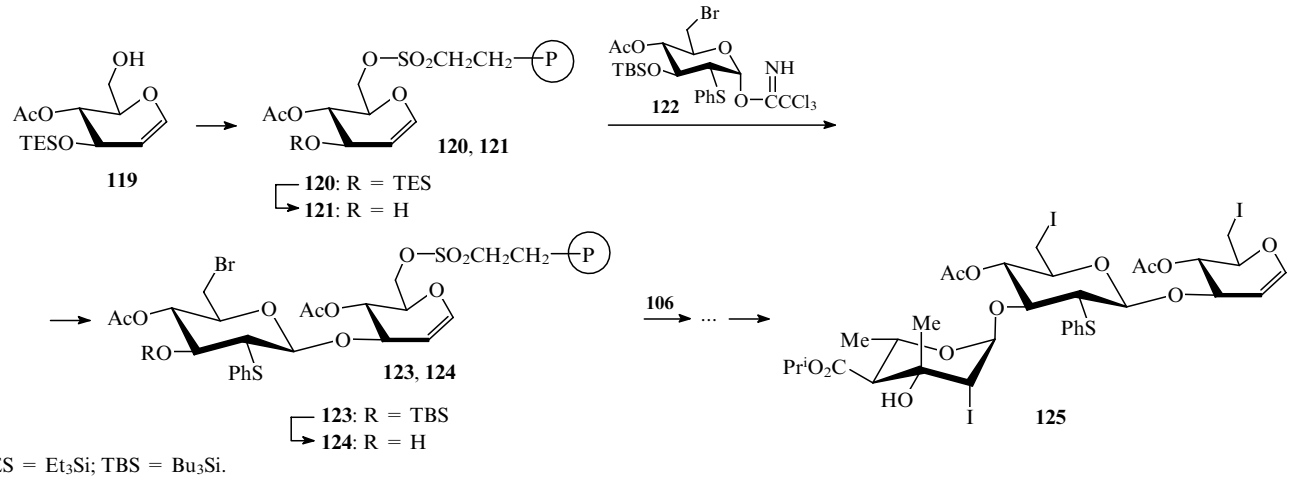
Scheme 20



Scheme 21



Scheme 22



V. Synthesis of glycopeptides

Glycopeptides are compounds in which oligosaccharides are covalently linked to amino acids or peptides by *O*- or *N*-glycosidic bonds. They are structural components of glycoproteins, the important naturally occurring glycoconjugates responsible for a great diversity of vital cell functions and are used as the simplest models in studies of biological activities of glycoproteins. In recent years, glycopeptides have acquired considerable practical importance, since many of them served as the basis for the development of new generations of highly efficient drugs.

Glycopeptides are most commonly prepared by solid-phase synthesis, since their synthesis in solution faces serious problems. Synthesis of glycopeptides can be performed by elongation of a peptide or an oligosaccharide chain. Those peptides in which an amino acid is glycosylated with a monosaccharide are the simplest representatives of the glycopeptide family.¶ These can be prepared by direct glycosylation of the corresponding amino acid residue in a presynthesised immobilised peptide chain.†

¶ It is interesting to note that the mode of incorporation of the first monosaccharide residue is a crucial factor which determines the physicochemical characteristics of these peptides.

† For direct *O*-glycosylation of peptides containing serine and threonine residues in solution, see Refs 51 and 52.

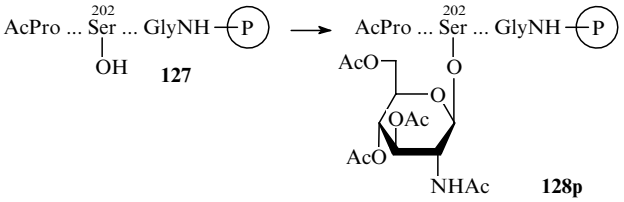
This method has been briefly described by Hollósi *et al.*[53] in the example of the synthesis of a glycosylated fragment 189-207 of tau-protein (**126**), responsible for a certain stage of Alzheimer's disease, and of its simplified analogue.

AcPro-Lys-Ser-Gly-Asp-Arg-Ser-Gly-Tyr-Ser-Ser-Pro-Gly-$\overset{202}{\text{Ser}}$-Pro-Gly-Thr-Pro-GlyNH$_2$

126

The immobilised 19-membered peptide **127** possessing the necessary protecting groups in its amino acid residues (with the exception of the hydroxy group of serine-202 which was subject to glycosylation) was prepared by solid-phase peptide synthesis. This peptide was treated with 3,4,6-tri-*O*-acetyl-2-methyl-D-*gluco*-oxazoline‡ prepared by treatment of per-*O*-acetyl-β-D-glucosamine with trimethylsilyl triflate as described in Ref. 54 (Scheme 23).

Scheme 23

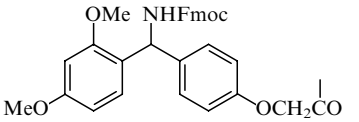


The resulting *O*-glycopeptide **128p** (yield 23%) was split off the polymer by treatment with a trifluoroacetic acid – anisole mixture and purified by chromatography. The structure of the glycopeptide **128** was confirmed by mass spectrometry. The glycosylation of the serine residue in its simple peptide analogue AcGly-Ser-Pro-Val-Glu-Lys was carried out in a similar way. The corresponding *O*-glycopeptide was obtained in 33% yield. This synthesis demonstrated the possibility of glycosylation of the serine residue adjacent to the proline residue of immobilised peptides, which normally hardly undergoes glycosylation.

The synthesis of *N*-glycopeptides in which mono- and oligosaccharide residues (including hexasaccharides) are linked to the asparagine residue of the hexapeptide Asn-Tyr-Gly-Gly-Phe-Leu by an *N*-glycosidic bond is yet another example of direct glycosylation. In this synthesis, the hexapeptide with *N*-terminal aspartic acid was immobilised on a support and the aspartic acid was converted into the pentafluorophenyl ester. The activated ester was brought into reaction with the corresponding glycosylamines in the presence of hydroxybenzotriazole and amine to afford *N*-glycosylasparagines.[55]

It is necessary to note that direct glycosylation of immobilised peptide chains has not received wide recognition in synthetic practice. It is the classical solid-phase peptide synthesis (see, *e.g.*, Refs 56 – 90) that is mainly employed for the preparation of glycopeptides bearing a monosaccharide residue or a short oligosaccharide chain, which makes use of the corresponding glycosylated amino acids.

However, implementation of solid-phase glycopeptide synthesis in an automated regime of conventional peptide synthesis required that a number of problems was solved, such as a search for supports and linkers providing both the optimum conditions for the condensation in the elongation of the peptide chain and smooth non-destructive liberation of the target glycopeptide from the support. The most common support is Merrifield's peptide resin[56] modified by incorporation of benzyl alcohol fragments ($HOCH_2C_6H_4OCH_2$–(P)),[58] the role of linker is played by a compound with benzhydrylamine and phenoxyacetic acid fragment (the so-called Rink linker),[61] a variety of compounds containing β-hydroxyallylic groups (Hycron and Hycram linkers)[62,63] and nitrobenzophenone oxime.[65]



Rink linker (RINK)

$HOCH_2CH=CHCONHCH_2-$

Hycram linker

$HOCH_2CH=CHCH_2O(CH_2CH_2O)_3CH_2CH_2CONHCH_2-$

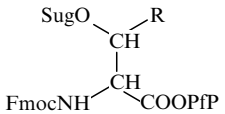
Hycron linker

In parallel with a search for linkers, the general strategy of glycopeptide synthesis required unification of procedures used for incorporation of glycosylated amino acids into the peptide chain.

To this end, efficient methods for incorporation of glycosylated amino acid residues into the peptide chain have been developed and synthesis of such fragments has been proposed.§

And, finally, particular attention has been paid to the protection of hydroxy groups of carbohydrates and functional groups of amino acids as well as to protecting group introduction, removal and manipulations as required in the synthesis.

The Fmoc/PfP strategy was found to be the most efficient in the synthesis of *O*-glycopeptides. In this case, the amino group of serine or threonine (**129**) derivatives is protected with a Fmoc group which is easily removed upon treatment with mild bases, while the carboxy group is activated by conversion into the pentafluorophenyl ester (PfP).



129

R = H, Me.

The use of these derivatives allows incorporation of glycosylated amino acids into the peptide chain in an automated mode of a conventional peptide synthesis.

An alternative approach to the synthesis of glycopeptides with a complex carbohydrate chain consists in the elongation of the oligosaccharide chain starting from the monosaccharide residue that has been incorporated into the immobilised peptide chain. In this case, as in the oligosaccharide synthesis in solution, the choice of protecting groups in the monosaccharide residues and the method of glycosylation which provide the highest regio- and stereoselectivity of glycosylation are of great importance. A factor which largely determines the successful outcome of the glycopeptide synthesis is the proper choice of a support which ensures effective synthesis of both peptide and oligosaccharide fragments within a single system '(support + linker) – solvent'. The conditions for the peptide and oligosaccharide syntheses can differ (*e.g.*, the synthesis of an oligosaccharide fragment should preclude the presence of water or any other hydroxyl-containing components), which may require additional technical manipulations connected with a change of solvents in the course of the synthesis.

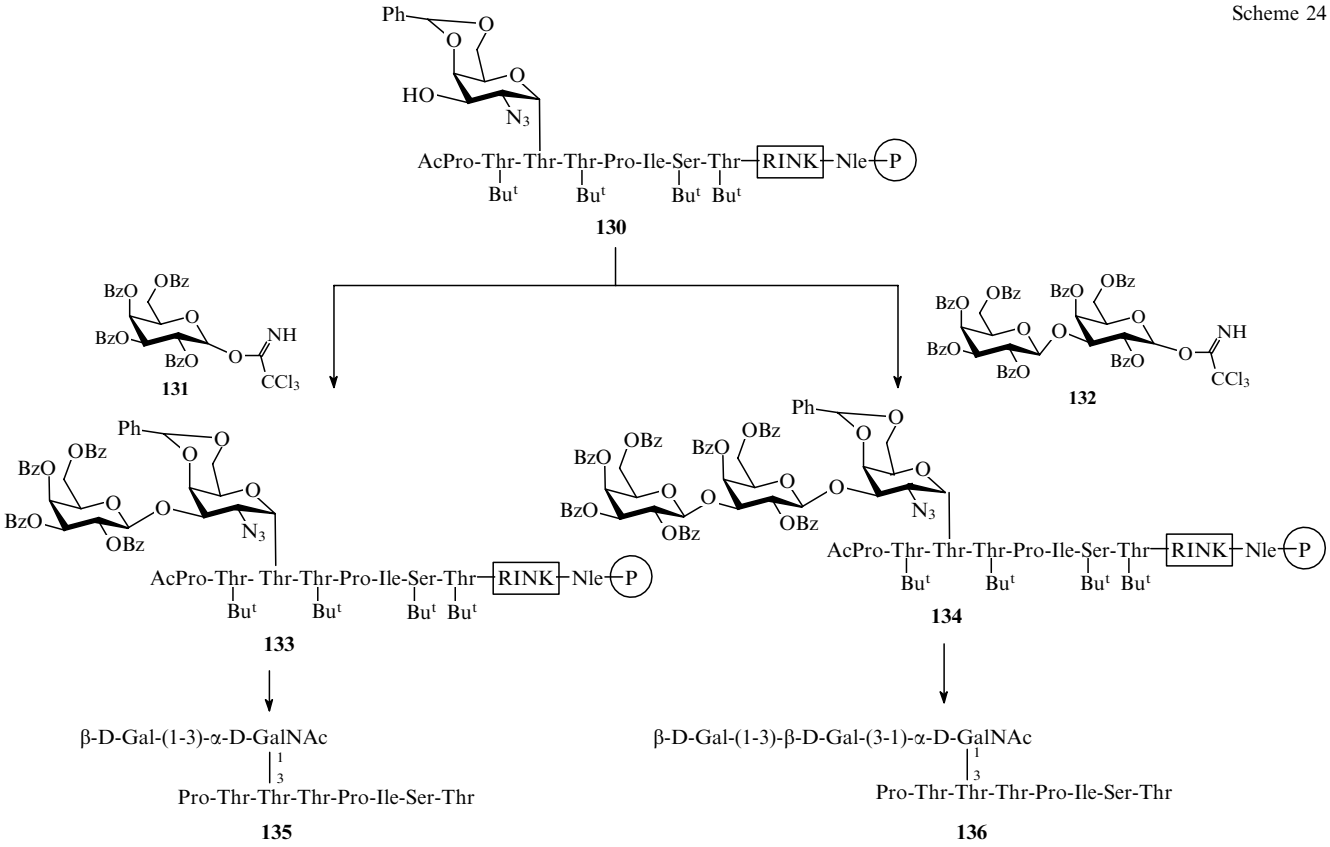
Glycopeptide syntheses by chemical elongation of the oligosaccharide chain was described in detail in a fundamental study by Paulsen *et al.*[91]

The Rink linker and norleucine (Nle) as the internal standard were used in the synthesis of mucin core fragments, *viz.*, glycopeptides with the peptide chain comprising eight amino acids and a di- or a trisaccharide residue (Scheme 24). One of the threonine residues incorporated into the peptide chain immobilised on a

‡ An attempt to incorporate the first monosaccharide into an immobilised peptide by direct glycosylation with sugar trichloroacetimidates was unsuccessful.

§ These residues include *O*-glycosylated threonine and serine residues in the case of *O*-glycopeptide synthesis and *N*-glycosylated asparagine in the case of *N*-glycopeptide synthesis.

Scheme 24



PEGA support contained a substituted 2-azido-2-deoxy-α-D-galactose residue linked by an *O*-glycosidic bond. The only free hydroxy group at the C(3) atom of the sugar residue in this immobilised glycopeptide (**130**) was glycosylated with the corresponding *O*-glycosyl trichloroacetimidates. As was noted above, this method is used for the formation of β-glycosidic linkages in good yields and with high stereoselectivity. Thus, the reaction of the glycopeptide **130** with *O*-(tetra-*O*-benzoyl-D-galactopyranosyl)- (**131**) and *O*-(perbenzoyl-β-D-galactopyranosyl-(1-3)-D-galactopyranosyl) trichloroacetimidates (**132**) in the presence of trimethylsilyl triflate afforded the glycopeptide derivatives **133** and **134**, respectively. The latter were split off the support with trifluoroacetic acid (yields 67% and 33%, respectively), this was accompanied by removal of *O*-*tert*-butyl protecting groups. The azido groups in the resulting compounds were transformed into acetamido groups with subsequent removal of *O*-benzoyl protection. This procedure afforded the glycopeptides **135** and **136** with the *N*-acetylgalactosamine residue in the carbohydrate–peptide linkage site.

An attempt to perform this synthesis on other supports and substitution of acetyl groups in the trichloroacetimidates **131** and **132** for *O*-benzoyl groups decreased the yields of the resulting glycopeptides **133** and **134**.

The same linker and the support were used in the synthesis of a series of isomeric glycopeptides with (1-3) and (1-6) glycosidic linkages. The starting monoglycosylated peptide comprised a threonine residue glycosylated with 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranose (Scheme 25). The glycosylation of the peptide **137** with the trichloroacetimidates **131** and **132** gave the glycopeptide derivatives **138** and **139** (yields 69% and 53%, respectively) which were further converted into the corresponding *O*-glycopeptides after transformation of azido groups into acetamido groups with subsequent liberation from the support and deprotection.

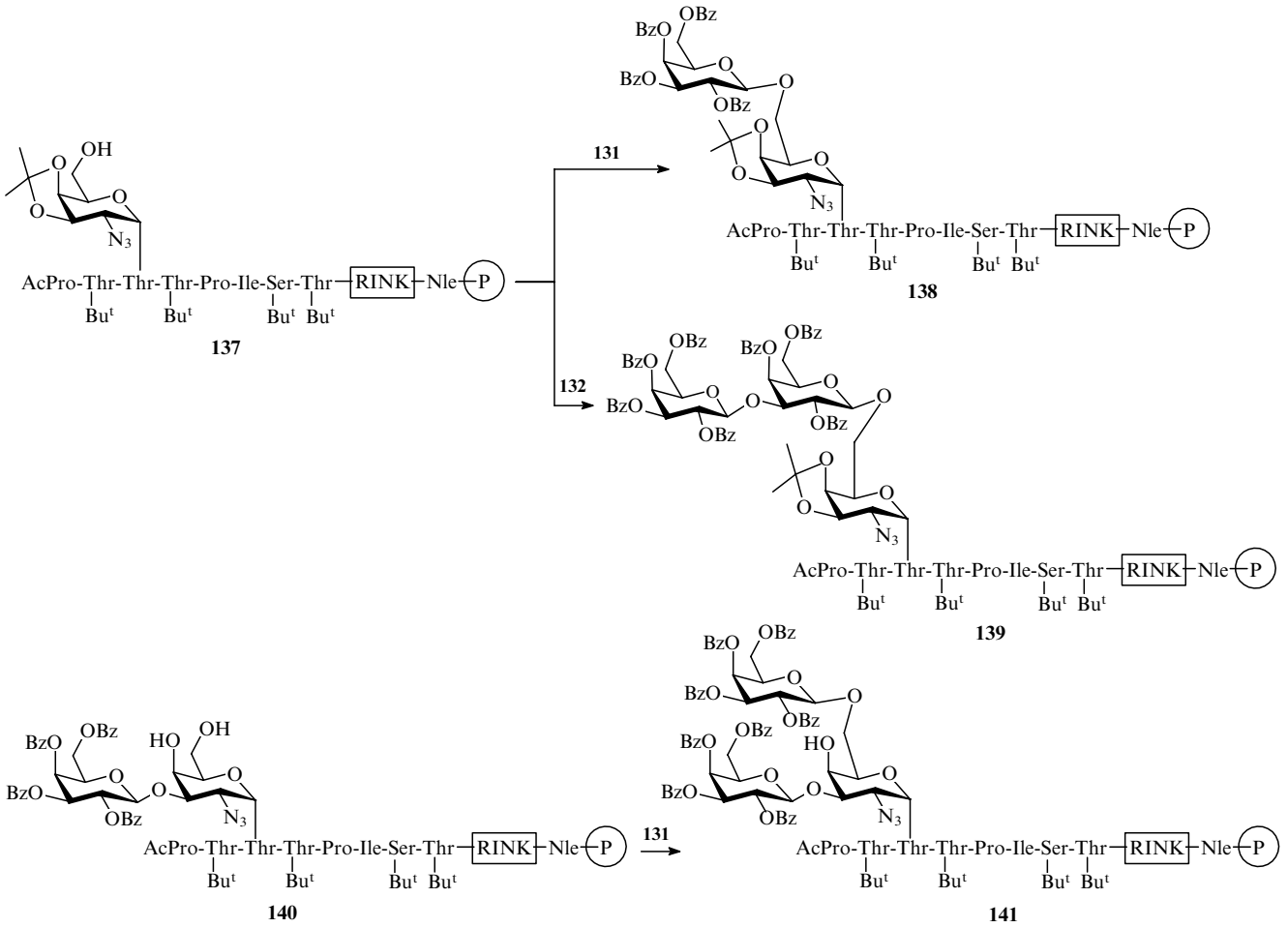
This scheme was also used in the synthesis of *O*-glycopeptides having branched oligosaccharide chains. Here, the protecting benzylidene group in the immobilised glycopeptide **133** was removed by mild acid hydrolysis, and the glycopeptide **140** was selectively glycosylated with the trichloroacetimidate **131** at the primary hydroxy group of the partially protected disaccharide fragment. This procedure afforded the glycopeptide **141** with a branched oligosaccharide chain.

This approach was also used in the preparation of glycopeptides with carbohydrate chains built of amino sugars. The immobilised glycononapeptides **142** and **143** structurally related to compounds **130** and **137** with an additional N-terminal glutamic acid residue were glycosylated with an excess of *O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-trichloroethoxycarbonylamino-α-D-glucopyranosyl) trichloroacetimidate **144** (Scheme 26). The *N*-trichloroethoxycarbonylamino- and azido groups in the resulting glycopeptide derivatives **145** and **146** were transformed into *N*-acetylamino groups, the protecting groups in amino acid and sugar residues were removed after which the reaction products were split off the support. The corresponding glycopeptides, which represent mucin core fragments, were obtained in 62% yield.

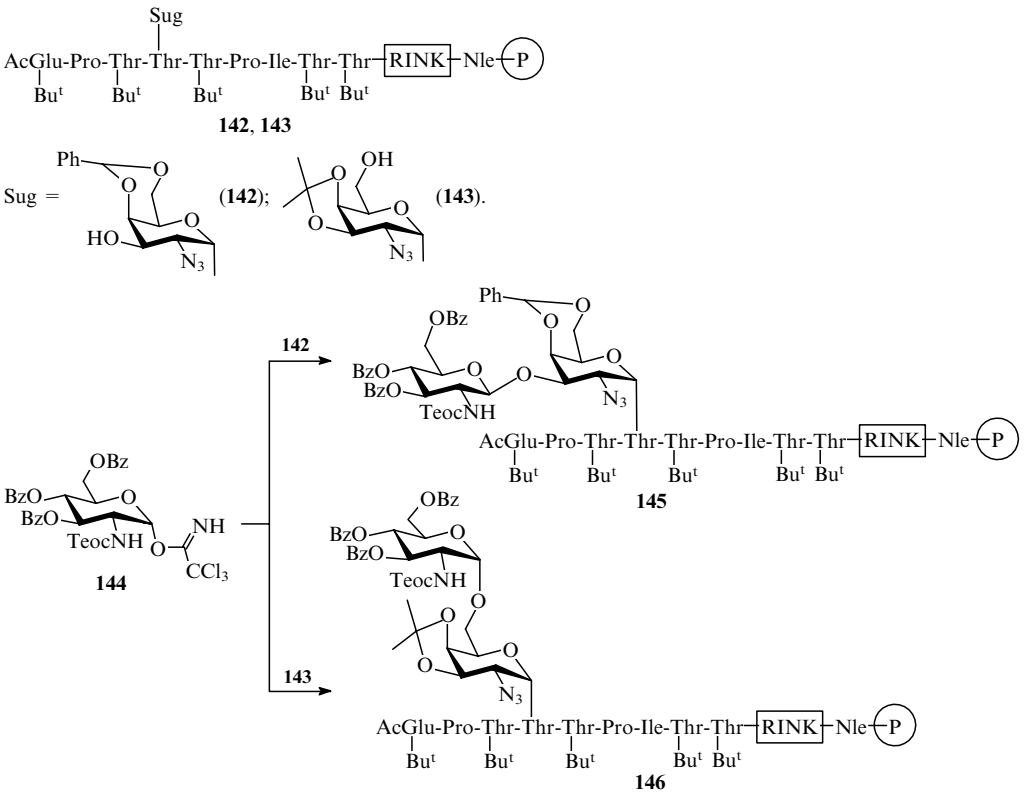
Obviously, the elongation of an oligosaccharide chain in an immobilised glycopeptide bearing a monosaccharide residue is a flexible and reliable, albeit labour-consuming, method for the synthesis of complex glycopeptides. This procedure can be considerably simplified if glycosylation is performed with the use of glycosyl transferases. The use of enzymes makes monosaccharide protection unnecessary and ensures high regio- and stereoselectivity in the formation of glycosidic bonds. The supports employed in enzymic glycopeptide synthesis involving consecutive elongation of peptide and oligosaccharide chains must provide adequate conditions both for the synthesis of the peptide chain and for the transfer of monosaccharide residues under the action of glycosyl transferases.¶ This problem was successfully overcome

¶ In choosing supports for such syntheses, one should take into account the fact that peptide syntheses are performed in organic solvents, while the elongation of the oligosaccharide chain is carried out in aqueous media.

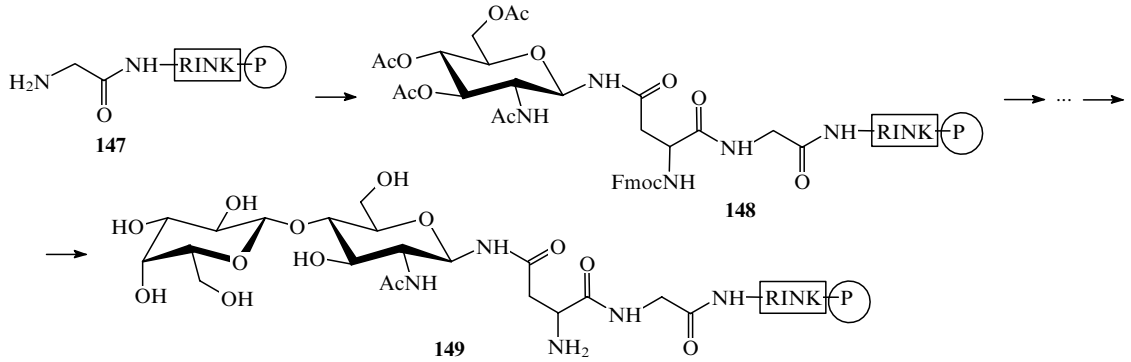
Scheme 25



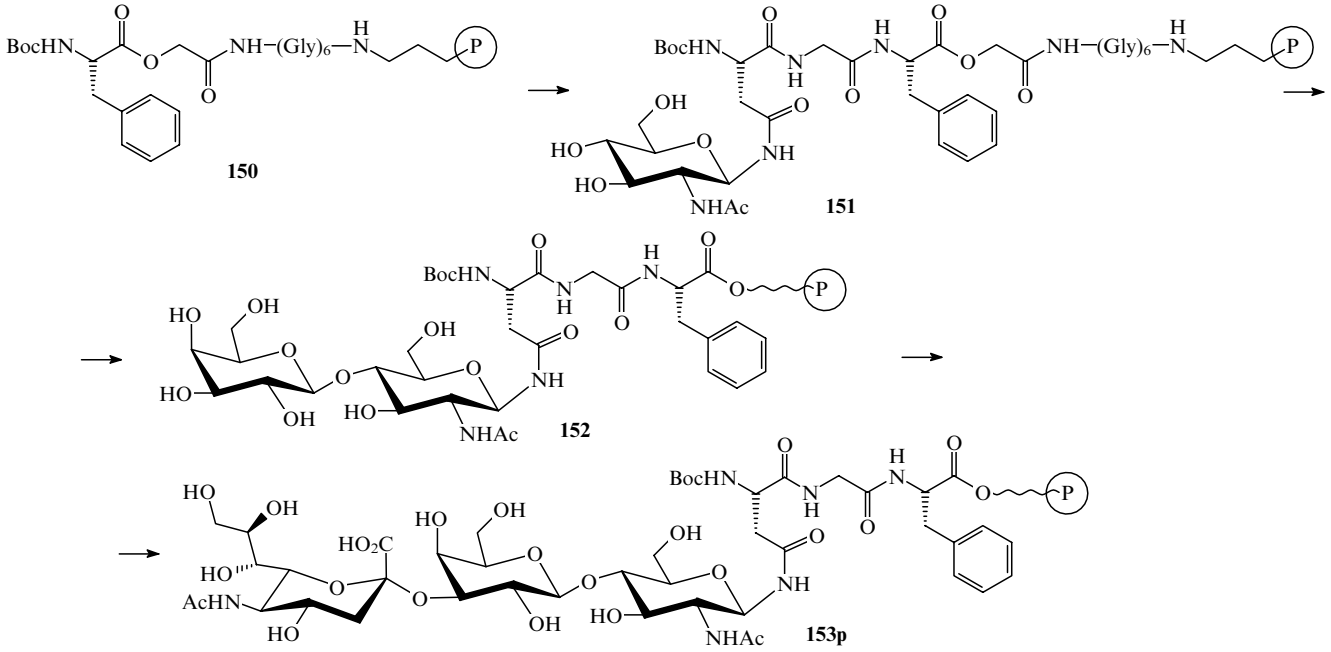
Scheme 26



Scheme 27



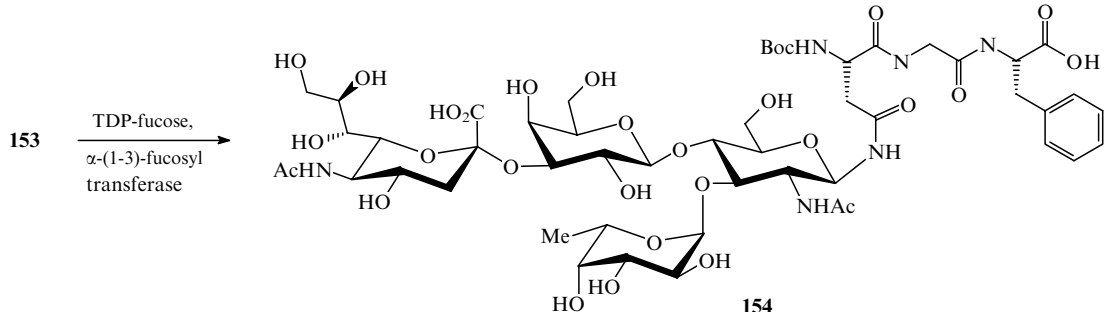
Scheme 28



through the use of complex polymeric supports (PEGA) capable of swelling both in water and in organic solvents, or of mineral supports (*e.g.*, CPG modified by introduction of amino groups).

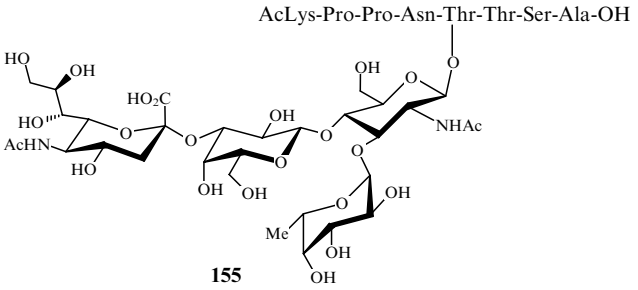
The synthesis of a model *N*-glycopeptide, *viz.*, {β-D-Gal*p*-(1-4)-β-D-Glc*p*NAc-[1-N(4)]}-Asn-Gly, on a PEGA-type support modified by attachment of the Rink linker is shown in Scheme 27.[18] A C-terminal amino acid, *viz.*, *N*-Fmoc-glycine, was immobilised on a modified support. After removal of the Fmoc-group, the immobilised glycine **147** was made to react with *N*(4)-glycosylated *N*(2)-Fmoc-asparagine to give the glycopeptide **148**. Incubation with UDPGal and β-(1-4)galactosyl transferase was carried out after removal of Fmoc- and *O*-acetyl protecting groups. The resulting glycopeptide **149** was split off the polymer with CF_3COOH or used further in the immobilised form. The glycosylation reaction was monitored by ^1H NMR spectroscopy.

The use of CPG modified with 3-aminopropyl groups for solid-phase chemo-enzymic synthesis of glycopeptides was exemplified in the preparation of sialyl-Lewis-X glycopeptide (Scheme 28).[14] In this synthesis, the hexaglycin fragment was attached to the amino groups of the modified CPG; the unreacted amino groups of the polymer were 'capped' by acetylation to prevent their involvement in further reactions after which the terminal amino group of the hexapeptide was acylated with *O*-(*N*-Boc-phenylalanyl)glycolic acid. The ester bond formed is susceptible to hydrolysis with chymotrypsin and is well-suited for splitting the reaction product off the support. Following liberation of the amino group in the ester **150**, glycine and *N*(4)-glycosylated asparagine residues were introduced by conventional methods of solid-phase peptide synthesis. The immobilised glycopeptide **151** was incubated with UDPGal and β-(1-4)-galactosyl transferase to afford the immobilised glycopeptide **152**. The latter was made to react with cytidine 5′-phospho-*N*-acetylneuraminic acid (CMP-*N*-acetylneuraminic acid, the activated form of *N*-acetylneuraminic acid) and α-(2-3)-sialyl transferase to yield the



glycopeptide **153p** containing a trisaccharide chain. The latter is easily split off the support with α-chymotrypsin. After liberation, the *N*-glycopeptide **153** was further glycosylated in solution with thymidine 5′-diphospho-L-fucose (TDP-fucose) in the presence of α-(1-3)-fucosyl transferase eventually resulting in the branched *N*-glycopeptide **154**.

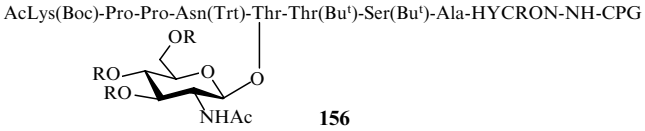
The synthesis of a more complex biologically important *O*-glycopeptide **155** with the same tetrasaccharide moiety, which represents a fragment of the mucin-type glycoprotein MAdCAM-1 (a specific ligand for selectins),[92] was carried out in a similar way. The problems which arose in its synthesis were connected with the high lability of the *O*-glycosidic bond between *N*-acetylglucosamine and threonine.



155

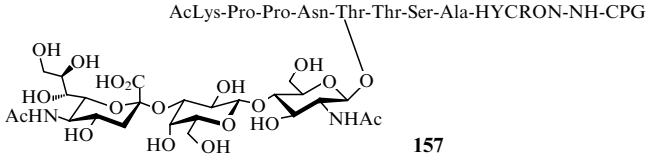
The synthesis of the glycopeptide **155** was carried out on PEGA and CPG modified with aminopropyl groups. CPG appeared to be more convenient than organic supports of the PEGA type. The Hycron linker containing an allylic hydroxy group with an attached *N*-Fmoc-alanine as a terminal amino acid was used for the immobilisation of the peptide chain.

After removal of the Fmoc-group in the polymer Fmoc-Ala-HYCRON-NH-CPG, the peptide chain was elongated from the alanine residue using a standard *N*-Boc/OBu^t procedure. *N*-Acetylglucosamine linked to threonine served as a carbohydrate–peptide junction. After removal of *N*- and *O*-protecting acid-labile groups (treatment with trifluoroacetic acid in the presence of ethanedithiol) from the immobilised monoglycosyloctapeptide **156**, this



156

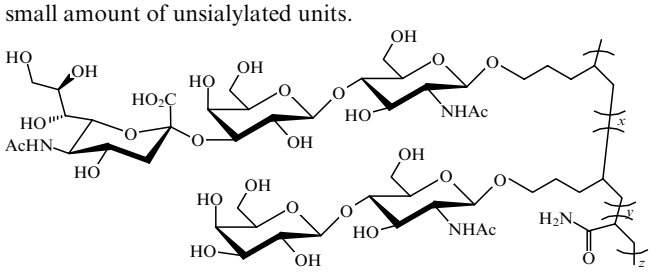
was incubated first with UDPGal in the presence of galactosyl transferase and then with CMP-*N*-acetylneuraminic acid in the presence of α-(2-3)-sialyl transferase. The resulting triglycosyl peptide derivative **157** was split off the support in the presence of Pd(0) as a catalyst. The yield of the glycopeptide **157** was 9% with respect to the immobilised alanine. The glycopeptide **157** was converted into the glycopeptide **155** by enzymic fucosylation as described above.



157

The glycopeptide **155** has also been synthesised in a solution. The yield of the target product was higher in this case, but the reaction time increased significantly (up to 9 days). In the authors' opinion,[92] the shorter duration of solid-phase synthesis may fully compensate for the lower yields of the target product.

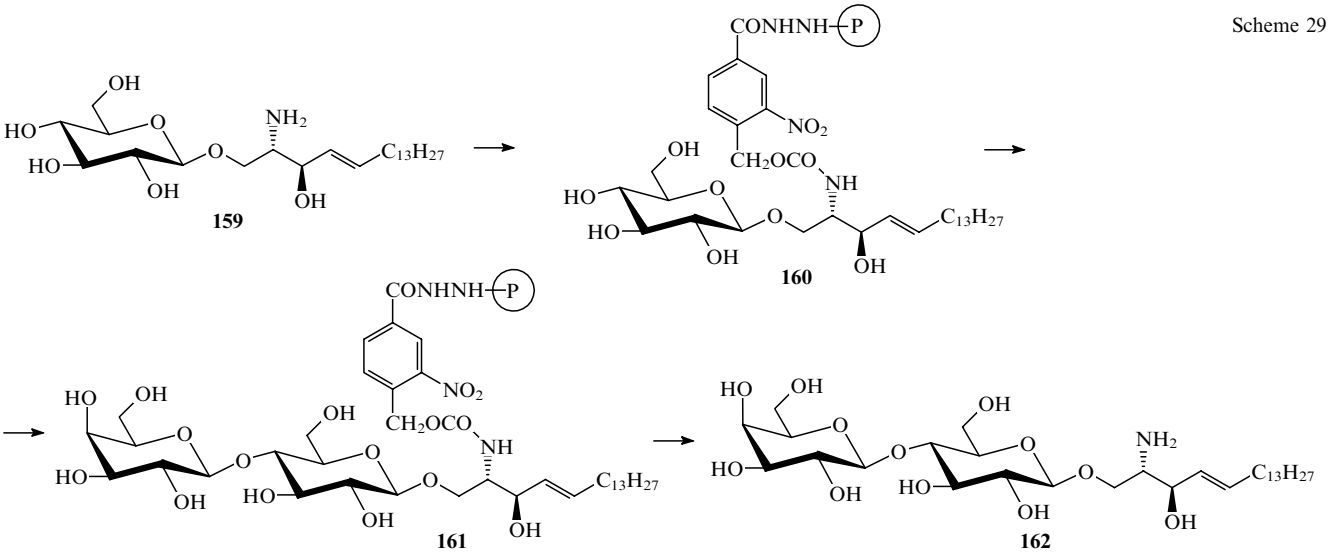
The enzymic elongation of oligosaccharide chains was also employed in the synthesis of glycoconjugates which are used in the manufacture of diagnostic preparations (artificial antigens), *e.g.*, in the synthesis of a polymer containing the α-NeuNAc(2-3)-β-D-Gal*p*(1-4)-β-D-GlcNAc chains attached to a polyacrylamide carrier.[93] The polyacrylamide carrying monosaccharide residues was obtained by copolymerisation of n-pent-4-enyl 2-acetamido-2-deoxy-β-D-glucopyranoside and acrylamide.[94] This was incubated consecutively with UDPGal in the presence of β-(1-4)galactosyl transferase and *p*-nitrophenyl *N*-acetylneuraminate in the presence of Tc-Ts-transsialidase to afford the synthetic glycoconjugate **158**, which contained trisaccharide units together with a small amount of unsialylated units.



158

Thus, in some cases the solid-phase synthesis is confined exclusively to the preparation of a glycopeptide containing only one monosaccharide residue in the carbohydrate–protein junction, which is followed by enzymic elongation of the carbohydrate chain in solution. The synthesis of a large series of *N*-glycopeptides the chains of which contained different amino acid sequences including two asparagine residues glycosylated with *N*-acetylglucosamine, *N*-acetyllactosamine and sialyl-(2-6′)-*N*-acetyllactosamine, provides an example.[95]

Scheme 29



159 **160** **161** **162**

The solid-phase synthesis of lactosylceramide on a soluble support with a photosensitive linker is yet another, thus far unique example of enzymic elongation of a carbohydrate chain in glycolipids.[96] Synthetic (2*S*,3*R*,4*E*)-2-amino-1-(β-D-glucopyranosyloxy)-3-hydroxyoctadec-4-ene (**159**, glucosylsphingosine) was converted into a urethane (4-hydroxymethyl-3-nitrobenzoic acid derivative) and immobilised on a copolymer of acrylamide with *N*-acryloyloxysuccinimide (Scheme 29). The immobilised glycolipid **160** was incubated with UDPGal and galactosyl transferase. The resulting disaccharide derivative **161** was purified from low-molecular-weight admixtures by dialysis and the reaction product **162** was split off the polymer by photolysis.

VI. Synthesis of phosphoglycans

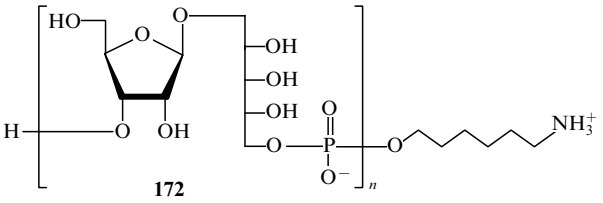
Solid-phase synthesis has successfully been used for the preparation of fragments of phosphoglycans, *i.e.*, the regular biopolymers built of repeating carbohydrate units linked through phosphodiester bonds. This class of biopolymers, *e.g.*, teichoic acids, are widespread in microorganisms where they play the role of antigens.

Synthesis of phosphoglycans in solution results exclusively in low-molecular-weight compounds which contain no more than two or three repeating units of the biopolymer. Apparently, solid-phase synthesis is more preferable in the preparation of high-molecular-weight phosphoglycan fragments. Several examples of such syntheses are currently known.

In general, the synthesis of phosphoglycans is very close to the synthesis of polynucleotides and differs in the structure of the repeating units which incorporate more or less complex carbohydrate structures.

The synthesis of a fragment of teichoic acid from *Bacillus licheniformis* is the first example of solid-phase phosphoglycan synthesis. This biopolymer is built of 1-*O*-(β-D-galactopyranosyl)glycerol repeating units linked *via* (6′-3)-phosphodiester bonds. The solid-phase synthesis of the trimer of the repeating unit of this phosphoglycan was performed on a modified CPG; the phosphodiester bond was formed using the phosphoramidite method (Scheme 30).[97] The galactopyranosylglycerol derivative **163**, which represents a fragment of the repeating unit of this biopolymer, was synthesised in solution and was treated with succinic anhydride to yield the succinic acid monoester **164**, which was then immobilised on the modified CPG. The reaction of the derivative **163** with $Pr^i_2NP(OCH_2CH_2CN)Cl$ gave (galactosylglyceryl) (2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite **165**, which was used as a phosphorylating agent. The elongation of the oligomeric chain was carried out in an automated mode using a well-established technique for the synthesis of oligonucleotides, This included reiteration of the following operations: liberation of the primary hydroxy group in the galactose residue of the polymer **164p** by removal of the 6′-dimethoxytrityl group, the reaction with phosphoramidite **165** in the presence of 1*H*-tetrazole, acetylation of unreacted free hydroxy groups in order to prevent their interaction at subsequent steps and oxidation of trivalent phosphorus into pentavalent phosphorus with iodine. The yield in each reaction cycle was 95%. The target product **166** ($n = 3$) was split off the support by ammonolysis with simultaneous removal of *O*-acetyl and cyanoethyl groups. The structure of the teichoic acid fragment thus prepared was confirmed by ^{1}H NMR spectroscopy and FAB-mass spectrometry.

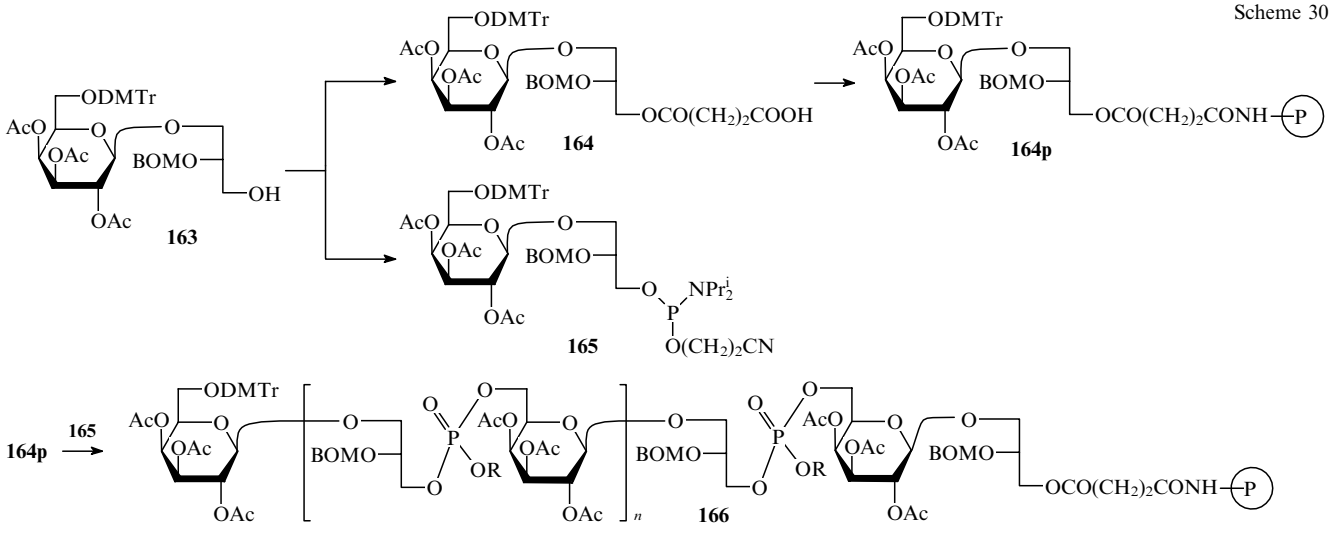
The synthesis of a fragment of the capsular polysaccharide of *Haemophilus influenza* (type b), which is known to induce meningitis in children, proved to be more difficult. This synthesis was undertaken [98] with the aim of using this phosphoglycan as an artificial antigen in the preparation of a synthetic vaccine (Scheme 31). This synthesis utilised the methodology of the oligonucleotide synthesis and was carried out on a modified CPG. The substituted ribosylribitol **167** was used as the starting compound. The disiloxanediyl group was removed by treatment with Bu_4NF, the primary hydroxy group was selectively protected by silylation and the secondary hydroxy group at C(3′) was converted into the succinic acid monoester. The resulting ester **168** was immobilised on CPG and detritylated to afford the polymer **169**. The ribosylribitol **167** was converted into 2-cyanoethyl *N*,*N*-diisopropyl phosphoramidite **170**. This was then introduced into reaction with the polymer **169**, which was carried out in an automated synthesiser under conditions of nucleotide synthesis. The reaction cycle was repeated as many times as required after which a (6-aminohexyl) phosphate spacer was attached to the oligomer **171** ($n = 6$) in order to ensure its further transformation into a synthetic antigen following binding to the protein. The reaction product was split off the support by



172

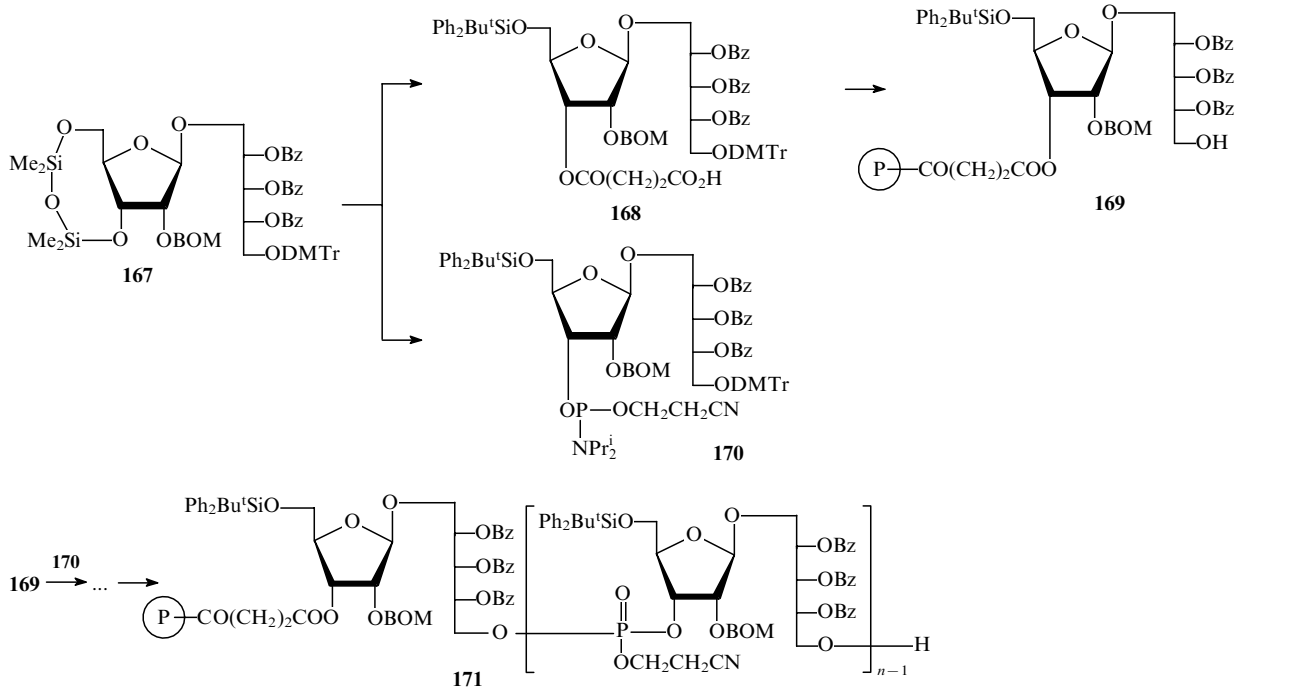
ammonolysis; the protecting groups were removed by treatment with Bu_4NF and hydrogenolysis. The overall yield of the target oligomer **172** ($n = 6$) was 24%.

Scheme 30



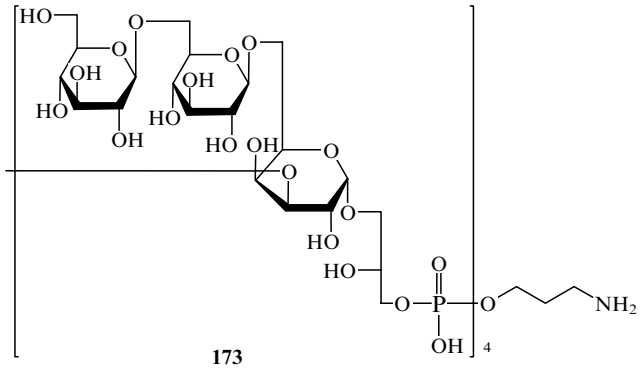
DMTr is dimethoxytrityl; BOM is benzyloxymethyl.

Scheme 31

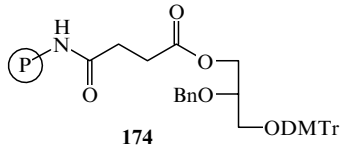


Synthesis of the phosphoglycan on a soluble support (PEGA) [99] using an analogous scheme with *O*-benzyl protection, was found to be more efficient and suitable for practical purposes. In this case, the loading of the support was larger (109 mmol g^{-1}) than in the previous synthesis (34 mmol g^{-1}); higher oligomers were synthesised in 35% yield.

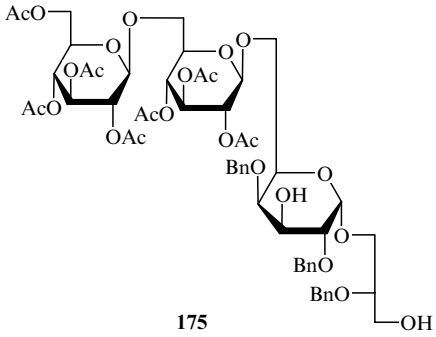
The same standard procedure was used in the synthesis [100] of a fragment **173** of the phosphoglycan of *Haemophilus pleuropneumoniae* (serotype 2) which induces swine diseases. This fragment is made up of β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl-(1-6)-α-D-galactopyranosyl-(1-3)-glycerol repeating units linked through (1-3′)-phosphodiester bonds.



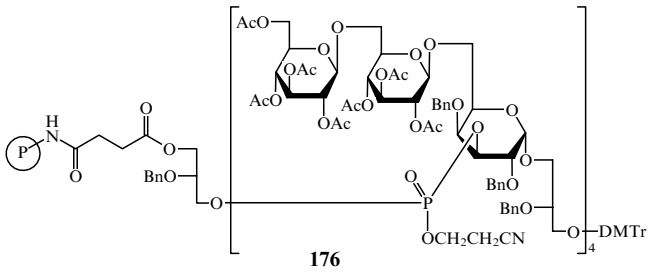
The synthesis of the tetrameric fragment of the repeating unit [100] was carried out according to a scheme similar to those used in previous syntheses. CPG modified with aminopropyl groups served as the support and the phosphoramidite method was employed for the formation of the phosphodiester bond.



2-*O*-Benzyl-3-*O*-dimethoxytritylglycerol was converted into a monoester of succinic acid and immobilised on CPG as in the previous syntheses. The resulting product **174** was detritylated and used as an initial unit for the elongation of the chain. The



primary hydroxy group in the glycerol portion of the repeating unit, *viz*., triglycosylglycerol **175**, was protected with dimethoxytrityl ether, and the hydroxy group at C(3) of the galactose residue was converted into (2-cyanoethyl) *N*,*N*-diethylphosphoramidite and used as a phosphorylating agent under conditions of automated oligonucleotide synthesis. The immobilised tetramer of the phosphoglycan repeating unit **176** obtained after four reaction cycles was detritylated with subsequent introduction of a phosphorylated spacer. The reaction product was split off the support by ammonolysis with simultaneous removal of protecting ester groups. Subsequent hydrogenolysis gave compound **173** which could be employed for the preparation of a synthetic vaccine.



These results demonstrate the high efficiency of the solid-phase synthesis of phosphoglycans of the teichoic acid type, which utilises well-established techniques of nucleotide synthesis.

VII. Conclusion

The examples of solid-phase carbohydrate syntheses described above demonstrate that advances in this area are modest yet. The problems encountered in solution synthesis of various carbohydrate structures are related to the polyfunctionality of monosaccharides and non-stereospecificity of the formation of glycosidic bonds; these problems persist and preclude automation of the synthesis.

It is quite natural that the 'technology' of solid-phase synthesis of carbohydrates demands further improvement and studies in this area are currently under way. It is desirable that the elongation of the carbohydrate chain by chemical methods should involve standard procedures for the formation of active glycosyl-donor function and for the liberation of hydroxy groups after each elongation step. The solution of this problem would open a way to the automation of the synthetic process.

An example of a successful solution of this problem is described in this review, particularly, in the section devoted to oligosaccharide synthesis by the glycal method. It should be noted that elongation of the oligosaccharide chain is more conveniently performed in the direction from the reducing end of the chain to its non-reducing end, since in this case the elongation of the chain by addition of the next fragment to the immobilised reagent should be preceded only by a selective liberation of the required hydroxy group, which is much easier to perform than to reconstruct the activated glycosidic centre.

Yet another problem which demands urgent solution is the choice of ideal polymeric supports and linkers compatible with them. Their wide availability will significantly simplify the synthesis and open up a way to the automated synthesis.

Obviously, solid-phase synthesis can successfully be employed for the preparation of lower oligosaccharides, but in this case its advantages over syntheses in solution are not so apparent. However, on going to higher-molecular-weight oligosaccharides and glycoconjugates the merits of the solid-phase technique will become more conspicuous as in the case of glycoconjugate (*viz*., glycopeptide and phosphoglycan) synthesis. It is this trend that has become particularly popular in the last few years. And yet major progress was achieved in those syntheses where the elongation of the carbohydrate chain was carried out by enzymic methods, which made it possible to overcome the problem of selective protection of hydroxy groups and its changes in each step of the synthesis.

The feasibility of modification of immobilised monosaccharides presents particular interest and can be illustrated by several examples.

The use of solid-phase techniques in combinatorial chemistry, particularly in a search for carbohydrate structures possessing the optimum biological activity, is one of the most attractive fields. The use of solid-phase synthesis for the preparation of an immense diversity of the so-called 'libraries' of structurally related polyfunctional oligosaccharide structures, excels other methods in many points.

A search for medicinal drugs and other substances possessing specific biological activities among carbohydrate-containing compounds demanded a rapid synthesis of a vast variety of analogues and fragments of biopolymers aimed at a selection of the most active compounds. This in turn required the acquisition of 'libraries' of compounds synthesised by methods of combinatorial chemistry and their further analysis.

It is known that 'combinatorial chemistry' is based on the principle of simultaneous synthesis of a series of related compounds and includes reactions of structurally similar acceptors with donors analogous to each other. This principle can be used sequentially in different stages of a multistep synthesis.[101] The most active compounds are further selected from such 'libraries'.

This strategy attracts considerable attention of specialists in the chemistry of biopolymers who widely utilise the strategy of solid-phase synthesis in the design of peptide and oligonucleotide 'libraries'. Very often, the selection of active components does not require their splitting off the support. The advantages of this approach in a search for biologically active oligosaccharides are especially apparent against the background of their immense structural diversity, which is due to the polyfunctionality of the constituent monosaccharides. The applicability of solid-phase synthesis to the construction of oligosaccharide 'libraries' has been considered.[102] Acquisition of a 'library' comprising $\sim$ 1300 compounds which represent derivatives of β-D-galactopyranosyl-(1-3)-2-deoxy-2-*N*-acylaminogalactopyranoside and include di- and trisaccharides differing in the nature and sequence of monosaccharides, type of intermonomeric glycosidic bonds and substituents at hydroxy and amino groups is a clear example.[103] A Tentagel-type polymer was used as a support and glycosyl sulfoxides served as glycosyl donors. The resulting 'library' of immobilised oligosaccharides was examined for the specificity of carbohydrate–protein interactions. The most active components were selected using a lectin test.

At present, the principles of combinatorial chemistry including carbohydrate synthesis have become widely used by large manufacturing companies.[104]

Special mention should be made in conclusion of a recent review[105] devoted to solid-phase synthesis of oligosaccharides, which suggests that interest in this method continues.

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