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Synthetic Studies of the Repeating Unit of the C-Polysaccharide of *Streptococcus pneumoniae* and Studies of Alkylation of Carbohydrates Using Dialkylstannylene Acetals

by

Huiping Qin

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
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FACULTY OF GRADUATE STUDIES

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by Huiping Qin

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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To my parents, my husband Baoquan and my daughter Annie

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Abstract

The C-polysaccharide (**PnC**) is a common antigen to all known *Streptococcus pneumoniae* bacteria. Its repeating unit, a pentasaccharide, is comprised of D-glucopyranose, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose, 2-acetamido-2-deoxy-D-galactopyranose, ribitol phosphate and phosphorylcholine. There was uncertainty about the configuration of the ribitol unit in **PnC**. An effort was made here to determine the absolute configuration of this ribitol unit. Firstly, D-ribitol and L-ribitol derivatives (**38** and **39**) were designed and synthesized. For the selective protection of D-ribose diethyldithioacetal (**41**) at position-5, dibutylstannylene acetal methodology proved to be successful. This is the first time that the dibutylstannylene acetal method has been applied to a dialkyldithioacetal compound. Then disaccharide phosphates (**47** and **48**) and trisaccharide phosphates (**59** and **60**) containing D-ribitol and L-ribitol residues respectively were designed and synthesized. 2D NMR techniques were used for the complete assignment of their NMR spectra. Comparison of the ^{13}C NMR data of **47** and **48**, and the ^{1}H and ^{13}C NMR data of **59** and **60** with that of the corresponding part of **PnC** showed that the data for the D-ribitol derivative **47** is closer to that of **PnC** than the L-ribitol derivative (**48**); the data of **59** being closer than **60**. The data of **59** fit almost perfectly with that of **PnC**, with an average difference of 0.02 ppm of the chemical shifts in the ^{1}H NMR spectrum and 0.034 ppm in the ^{13}C NMR spectrum, respectively. On this evidence it was concluded that the ribitol unit in **PnC** has the D-configuration.

Further investigations were performed on the application of dialkylstannylene acetals. Benzylation and methylation reactions were performed under a variety of conditions on the stannylene acetals derived from methyl 4,6-*O*-benzylidene-D-glycopyranosides. Efforts were also made to synthesize heterocyclic rings on carbohydrate molecules via dibutylstannylene acetal intermediates. A new compound (**118**) was obtained.

List of Abbreviations and Symbols

A	-glycuronic acid (abbreviation used in naming a carbohydrate)
Å	-angstrom
AATG*p*	-2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose
Ac	-acetyl
ADP	-adenosine diphosphate
All	-allyl
anal.	-analysis
ATP	-adenosine triphosphate
br s	-broad singlet
br d	-broad doublet
Bn	-benzyl
bu	-*n*-butyl
Calcd	-calculated
CAN	-cerium ammonium nitrate
Cbz	-benzyloxycarbonyl
CDP	-cytidine diphosphate
CE	-cyanoethyl (substitution on phosphorus)
COSY	-proton correlated spectroscopy
CTP	-cytidine triphosphate
d	-doublet

DBU	-1,8-diazabicyclo [5,4,0] undec-7-ene
DCE	-1,2-dichloroethane
dd	-doublet of doublets
DDQ	-dichlorodicyanoquinone
DIPEA	-diisopropylethylamine
DMF	-*N*,*N*-dimethylformamide
DP	-degree of polymerization
dt	-doublet of triplets
equiv	-equivalent
ESI	-electrospray ionization
Et	-ethyl
FAB	-fast atom bombardment
Fuc*p*NAc	-2-acetamido-2-deoxy-fucopyranose
Gal*f*	-galactofuranose
Gal*p*	-galactopyranose
Gal*p*A	-galactopyranosuronic acid
Gal*p*NAc	-2-acetamido-2-deoxy-galactopyranose
Glc*p*	-glucopyranose
Glc*p*A	-glucopyranosuronic acid
Glc*p*NAc	-2-acetamido-2-deoxy-glucopyranose
GlyOH	-glycerol
h	-hour(s)

hex	-*n*-hexyl
HMBC	-heteronuclear multiple bond correlation
HMQC	-heteronuclear multiple quantum correlation
Hz	-hertz
iPr	-isopropyl
ipropyl	-isopropyl
isobu	-isobutyl
J	-coupling constant
lit.	-literature
m	-multiplet
M	-concentration unit: mole per liter
Man*p*NAc	-2-acetamido-2-deoxy-mannopyranose
MCPBA	-*m*-chloroperbenzoic acid
Me	-methyl
MHz	-megahertz
min	-minutes
mL	-milliliter
µL	-microlitter
mmol	-millimole
mp	-melting point
MW	-molecular weight
NAD^+	-nicotinamide adenine dinucleotide (oxidized form)

NADH	-nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	-nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	-nicotinamide adenine dinucleotide phosphate (reduced form)
NBS	-*N*-bromosuccinimide
neohex	-neohexyl
NMR	-nuclear magnetic resonance
Ph	-phenyl
Piv	-pivaloyl = 2,2,2-trimethylacetyl
pMBn	-*p*-methoxybenzyl
pMBnCl	-*p*-methoxybenzyl chloride
pMPh	-*p*-methoxyphenyl
pMPhCHO	-*p*-methoxybenzaldehyde
PnC	-C-polysaccharide of *Streptococcus pneumoniae*
Pp_i	-inorganic pyrophosphate
ppm	-parts per million
Py	-pyridine
q	-quartet
Rha*p*	-rhamnopyranose
RibOH	-ribitol
s	-singlet
t	-triplet
TBAB	-tetrabutylammonium bromide

TBAF	-tetrabutylammonium fluoride
TBDMS	-*t*-butyldimethylsilyl
TBDPS	-*t*-butyldiphenylsilyl
TCE	-1,1,2,2-tetrachloroethane
TEA	-triethylamine
TEAB	-tetraethylammonium bromide
THF	-tetrahydrofuran
TIPS	-*t*-butyldiisopropylsilyl
TLC	-thin layer chromatography
TMS	-tetramethylsilane
TMS-OTf	-trimethylsilyl trifluoromethanesulfonate (triflate)
trityl	-triphenylmethyl
Ts	-toluenesulfonyl group
V	-volt
δ	-chemical shifts in parts per million downfield from TMS
$[\alpha]^{t}_{D}$	-specific rotation measured at temperature t °C using the 589 nanometer sodium D line

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Chapter 1 Introduction

1.1 General

Carbohydrates are polyhydroxy aldehydes, ketones, alcohols, acids and their derivatives. They can be subdivided into monosaccharides, oligosaccharides, and polysaccharides based on molecular size. Monosaccharides are carbohydrates that cannot be converted into smaller molecules by hydrolysis. When monosaccharide units are joined by acetal linkages, oligosaccharides and polysaccharides result. Conventionally, oligosaccharides contain small numbers of monosaccharides (DP 2-10), while polysaccharides are formed with a larger number of monosaccharides (DP >10). However, the development of synthetic procedures that allow synthesis of molecules containing up to the current record of 26 monosaccharides has blurred the distinction. Unlike proteins and DNA that are long linear chains of smaller molecules, oligosaccharides or polysaccharides are not necessarily linear. The multifunctional sugar residues can be attached to each other in all sorts of ways to form a variety of branched structures. The glycosidic linkage can be either α or β. Carbohydrates exist either as free oligo- and polysaccharides or are covalently linked to other substances as in glycolipids and glycoproteins. They are present throughout nature on the surface of cells or as part of cell walls. They exist in all living organisms, from viruses and bacteria to plants and animals. Being energy storage and structural support materials, they also play fundamental roles in normal biological processes and in disease.[1] Nature expresses its individuality with these complex carbohydrates. They are essential in molecular recognition for the transmission of biological information. They can serve as receptors to bind toxins, viruses and hormones to cells. Most pathogens are selective in choosing tissues to attack; carbohydrates direct the choice. Thus, a carefully designed carbohydrate molecule could block the carbohydrate acceptor, and reduce the effects of the pathogens. Development of this type of carbohydrate-drug that stops pathogens sticking to cells could offer new weapons against the growing catalogue of diseases resistant to traditional drugs.

The growing realization of the importance of oligosaccharides has stimulated the development of synthetic organic methodology to attach one carbohydrate molecule to another carbohydrate molecule or to other non-carbohydrate molecules. It has also stimulated the development of improved methodology for determining the structures of oligosaccharide moieties or polysaccharides. Particularly significant advances have been made in the use of NMR spectroscopy. The elucidation of the structures of such fragments is very important, as it provides a great deal of information on the structures of the more complicated materials and aids in the evaluation of their biological functions. The collaboration of structural carbohydrate chemistry with biological disciplines has resulted in the identification of a great number of oligosaccharide fragments involved in biological interactions. To be able to exploit this knowledge for the understanding of health and the prevention, diagnosis, and therapy of disease, it is however, necessary to be able to obtain these fragments. Sometimes, this is possible using the natural sources, or the desired oligosaccharide fragments may be obtainable by specific enzymatic degradation techniques. However, in other cases, the desired oligosaccharides must be obtained by chemical synthesis, due to insufficiency of natural sources or the difficulty of obtaining pure compounds because of contamination. In addition, the synthetic route usually opens the way to obtaining a number of substances analogous to the one identified as having a certain biological activity. This may be of fundamental importance for the elucidation of structure-function relationships.

1.2 Current methodology of oligosaccharide synthesis

With the support of biology, synthetic carbohydrate chemistry has grown enormously. Since the seminal introduction of the Koenigs-Knorr method in 1901,[2] considerable effort has been made to try to synthesize oligosaccharides stereoselectively, regioselectively, and in high yields. As seen in Figure 1.1, for a typical glycoside synthesis, the coupling of two carbohydrate residues is involved. The anomeric carbon C-1 of one otherwise completely protected carbohydrate (glycosyl donor **1**) is coupled with a free hydroxyl group at C-4 from another appropriately protected carbohydrate (glycosyl acceptor **2**). To get the desired coupling to occur in a high yield, a well-designed procedure has to be planned or a complex mixture could be obtained. Figure 1.1 describes the synthesis of a fully protected β-D-glucopyranosyl-α-D-glucopyranoside (**3**). L in **1** indicates the leaving group, which usually needs to be

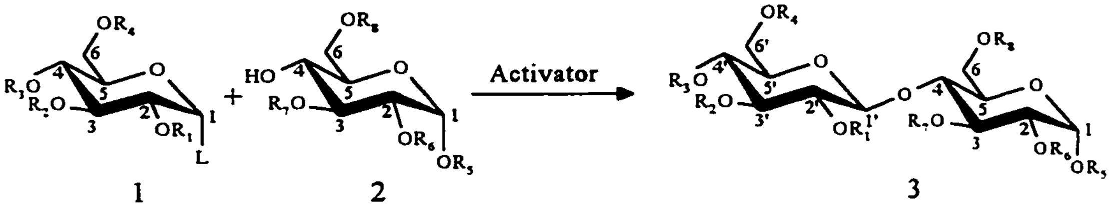


Figure 1.1 A schematic description of a disaccharide synthesis

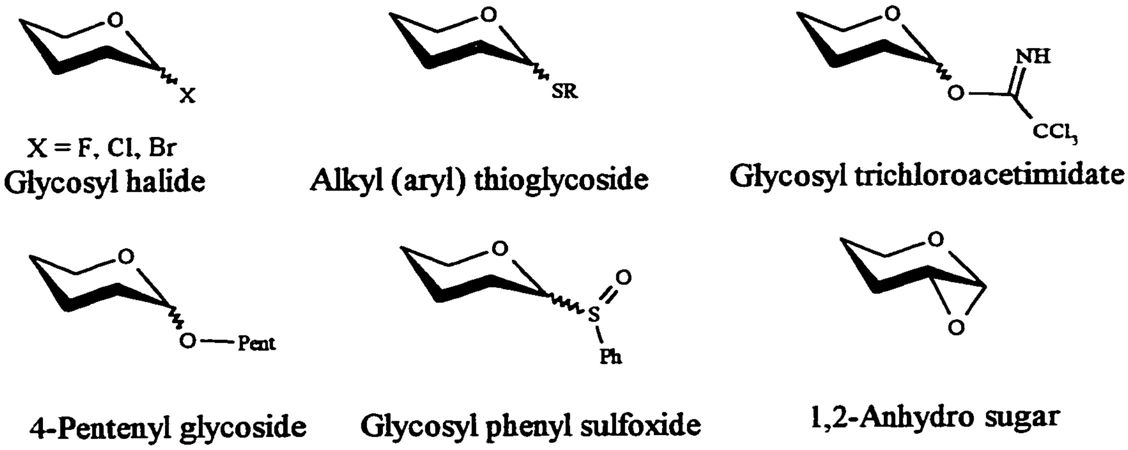
activated during the coupling of the glycosyl donor with the acceptor. This coupling will be discussed in detail in section 1.2.2. As shown in Figure 1.1, the numbering of each sugar residue in a carbohydrate molecule starts at the anomeric carbon and continues clockwise. The sugar residue shown furthest to the right is numbered 1, 2, 3 ... *etc.* On proceeding to residues to the left, numbering in this thesis will be 1', 2', 3' ... and 1", 2", 3" ... and so on.

1.2.1 Protection and deprotection in oligosaccharide synthesis

Carbohydrates are multifunctional compounds. To accomplish an unambiguous coupling of a desired hydroxyl group, all other potentially competing functional groups of the same reactivity have to be protected before the coupling reaction is performed. This includes groups on both the glycosyl donor and the glycosyl acceptor. In addition, if further glycosylations have to be performed subsequently on other hydroxyl groups, these positions have to be protected differently and in the manner that will allow facile deprotection without influencing the other protecting groups. A detailed discussion of protection and deprotection can be found in an up-to-date review of protecting groups in oligosaccharide synthesis.[3] Chapter 3 of this thesis will discuss one approach to this problem, the selective alkylation of carbohydrate molecules containing *trans*-diols using dialkylstannylene acetals as intermediates.

1.2.2 Strategies for glycosidic coupling reactions

Over the past several years, methods for the synthesis of oligosaccharides have



X = F, Cl, Br
Glycosyl halide

Alkyl (aryl) thioglycoside

Glycosyl trichloroacetimidate

4-Pentenyl glycoside

Glycosyl phenyl sulfoxide

1,2-Anhydro sugar

Figure 1.2 Major types of glycosyl donors currently in use

advanced tremendously. A variety of glycosyl donors and methods for their activation can be found in literature.[4-14] There are at least six major types among them (Figure 1.2), which are discussed below.

For over 90 years, the Koenigs-Knorr method[2] has been the most popular method and modifications of it are still being developed. This classical method uses a glycosyl halide as the glycosyl donor and silver salts as activating reagents. Tetrabutylammonium bromide was introduced by Lemieux and coworkers as a catalyst to produce the α-D-linked disaccharides.[15] Many other types of activators were also introduced and these are described in the literature.[13] The selectivity of a coupling reaction can be modulated by the choice of activators. Compared with glycosyl bromides or chlorides, glycosyl fluorides are more stable and thus easier to handle. A successful synthesis using a glycosyl fluoride as a glycosyl donor gave a 95% yield of stereoselectively coupled oligosaccharide product.[16]

Thioglycosides are another useful type of glycosyl donor. They are stable in many chemical environments and are widely used in oligosaccharide synthesis. They can be activated by various heavy metals,[13] NBS,[17] or with sulfonium triflates.[18]

Trichloroacetimidate-mediated glycosylation was introduced by Schmidt and his coworkers in 1980,[19] following the earlier use of acetimidate-mediated glycosylation by Sinaÿ.[20] The thermally and chemically stable trichloroacetimidates are easily synthesized from the corresponding 1-hydroxy sugars by treatment with trichloroacetonitrile in the presence of a base. The configurations of the trichloroacetimidates can be controlled by monitoring the basicity of the reaction solution.[21] An α-trichloroacetimidate is obtained in the presence of sodium hydride or 1,8-diazabicyclo [5,4,0] undec-7-ene (DBU), while a β trichloroacetimidate is obtained in the presence of sodium carbonate. Trichloroacetimidates can be activated under mild conditions[7,22] and the method has wide applications in the synthesis of natural products.[23-26] Further information on the application of this method can be found in a recent review.[27]

In 1988, the 4-pentenyl group was introduced by Fraser-Reid and his coworkers as a new and effective leaving group at the anomeric centre of the glycosyl donor.[28] Glycosylation reactions can be activated by Br^+ or I^+ sources.[29-31] During these studies, it was found that a 4-pentenyl glycosyl donor with an electron-withdrawing group at position-2 is less reactive than the corresponding glycosyl donor having an electron-donating group at the same position. The two different types of glycosyl donors were termed "disarmed" and "armed" sugars, respectively.[29] This method allowed for the attachment of the armed sugar to the disarmed sugar, even though both have the same leaving group at the anomeric position. Thus the newly formed disarmed oligosaccharide can be converted into an armed oligosaccharide by transformation of the electron-withdrawing group to an electron-donating group at position-2.

In 1989, glycosylation of hindered alcohols and derivatives of phenol was studied.[32] As shown in Figure 1.3, glycosyl phenyl sulfoxides were used as the glycosyl

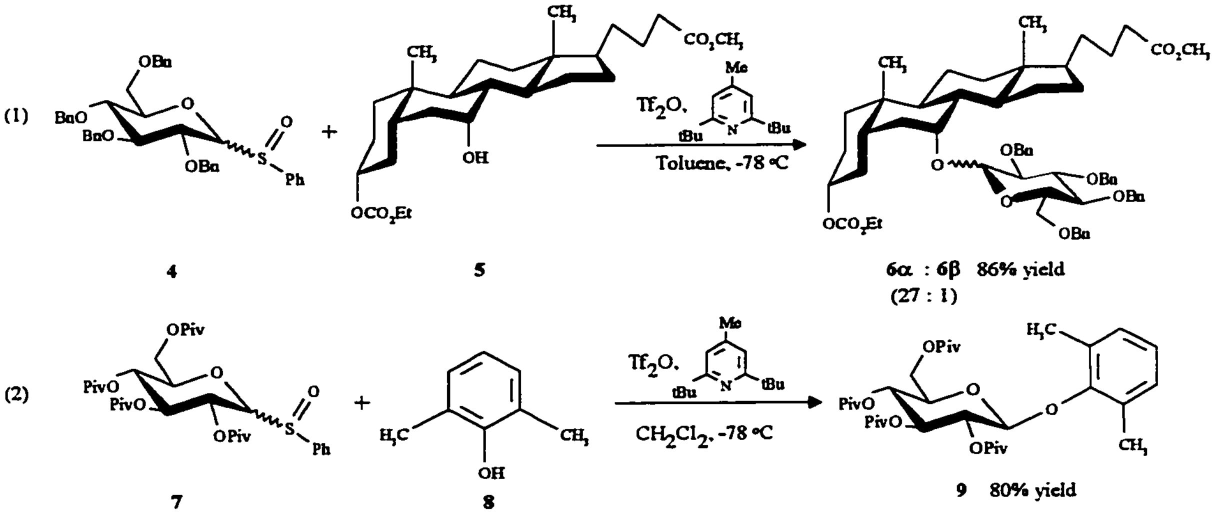


Figure 1.3 Glycosylations with glycosyl sulfoxides

donors. These glycosylation reactions can be carried out at low temperatures, and it is also worth noting that the reactions work well regardless of the electron-releasing or electron-withdrawing properties of the sugar protecting groups. However protecting groups do influence the stereochemistry of glycosidation. For instance, when a pivaloyl group is at position-2, a β-linkage is formed selectively; but with a benzyl group at position-2, an α-linkage predominates. Recently, with this methodology, blood group antigens Le^a, Le^b, and Le^x were successfully synthesized, with the yield of the glycosylation step ranging from 65% to 86%.[33] Due to its high reactivity, a glycosyl phenyl sulfoxide was successfully used for the glycosylation reactions on solid phase supported oligosaccharide, which will be described in detail in section 1.2.4.

In 1989, Danishefsky and his coworkers developed a convenient method for the direct preparation of 1,2-anhydro sugars from glycals using dimethyldioxirane as an effective epoxidation reagent.[34] Since then the usage of 1,2-anhydro sugars in oligosaccharide synthesis has been widely investigated; and in particular considerable progress having been made recently in the application of this method in solid phase oligosaccharide synthesis,[35-39] which will be described in Section 1.2.4. Furthermore, there are a number of mild, efficient methods for the activation of glycals to serve as glycosyl donors, thus providing access to various kinds of glycosidic linkages.[40,41]

1.2.3 Strategies for stereoselective coupling of carbohydrate residues

The major stereoselective coupling methods of carbohydrate residues are summarized in Figure 1.4. When there is a participating group on position-2 of a glycosyl donor, 1,2-*trans* glycosylation is obtained predominantly (Figure 1.4 (1)); when there is a non-participating group on position-2 of a glycosyl donor, the anomeric effect is most important in determining the anomeric configuration (Figure 1.4 (2)). Stereochemistry can also be influenced by solvent participation. As shown in Figure 1.4 (3), changing the solvent to acetonitrile or mixtures containing substantial amounts of acetonitrile alters the coupling stereochemistry from α to β.[9] The bromide-catalysed method (Figure 1.4 (4)) makes use of the equilibrium between α-glycosyl bromides and β-glycosyl bromides.

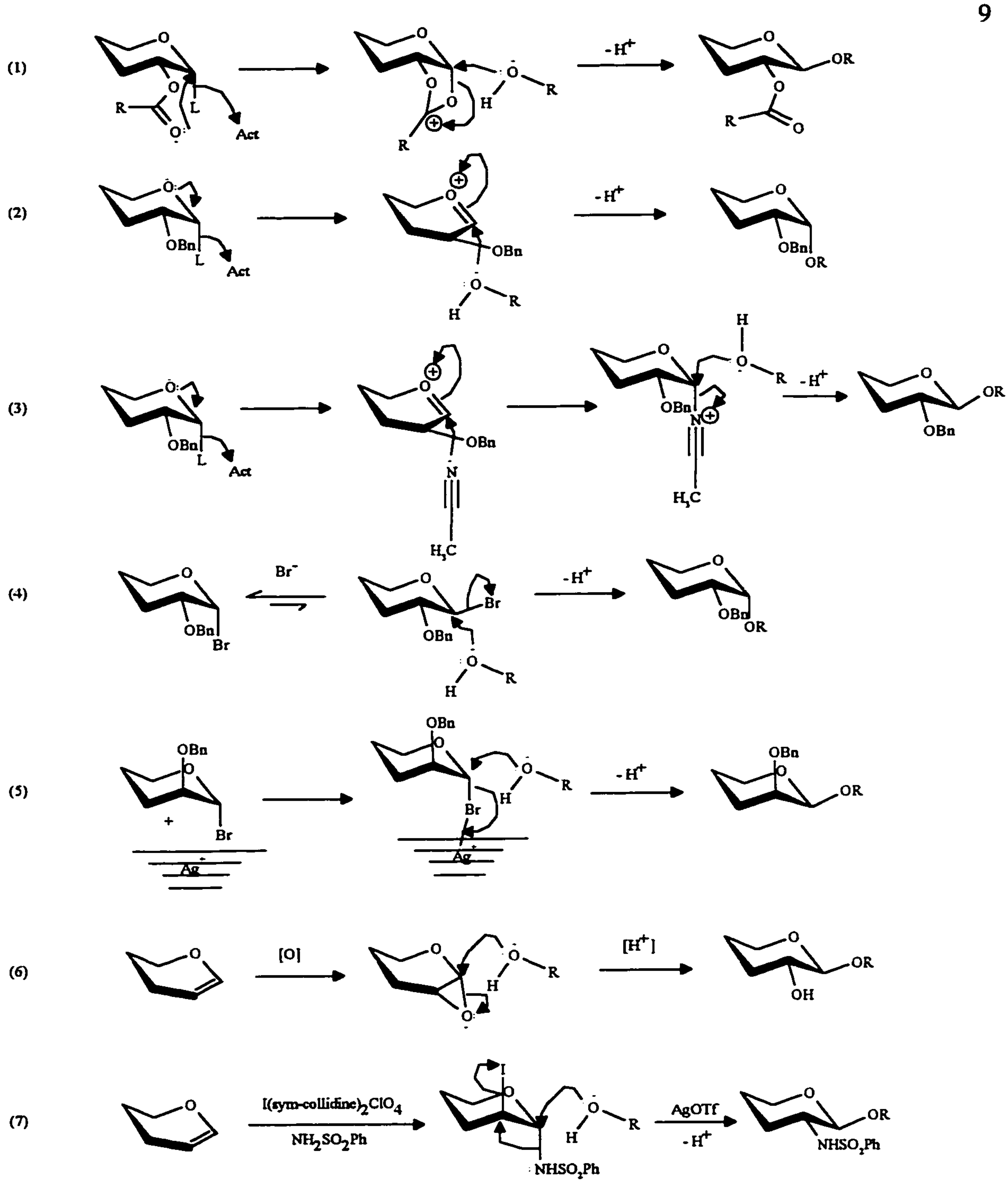


Figure 1.4 The major stereoselective coupling methods of carbohydrate residues (Act is the electrophilic activator.)

Although the equilibrium is in favour of the α-glycosyl bromide, due to the higher reactivity of the β-glycosyl bromide, the α-configuration product can be obtained selectively.[15]

Insoluble silver salts as catalysts are used to selectively synthesize the β-D-mannopyranoside linkage, which is present in all N-linked glycoproteins and is particularly difficult to form. As shown in Figure 1.4 (5), the α-side is sterically blocked; therefore β attack is favoured.[42] Methods involved intramolecular aglycon delivery through tethers have also proven to be useful for the synthesis of β-D-mannopyranoside.[43] A similar method using prearranged glycosides to synthesize β-L-rhamnopyranoside has also been reported recently.[44,45] Other methods for the synthesis of the β-D-mannopyranoside include the inversion at C-2 position by oxidation/reduction[46] or by an S_N2 reaction.[47]

Glycals are versatile synthetic intermediates. Through suitable chemical modifications, stereoselective *trans*-couplings can be obtained as shown in Figure 1.4 (6 and 7).[34,48] Entry 7 in this figure shows that a β-D-2-amino-2-deoxy sugar derivative is obtained.

In the synthesis of the 2-amino-2-deoxy carbohydrate derivatives, the most common participating group is phthalate and the most common non-participating group is the azide group (N_3). Azides can be reduced to amino groups easily with hydrogen sulfide,[49,50] zinc borohydride,[51] or borohydride exchange resin-nickel acetate,[52] or with thiolacetic acid to get acetamido groups.[53] The synthesis of oligosaccharides of 2-amino-2-deoxy sugars has been well reviewed by Banoub *et al.*[54]

1.2.4 Polymer supported oligosaccharide synthesis

Oligosaccharide synthesis is usually a difficult and time-consuming operation, requiring regio- and stereo- control in each step and tedious workup. In order to simplify the procedure, solid-phase supported glycosylation methods were introduced.[55] However, efficient and stereospecific glycosylation of secondary hydroxyl groups on insoluble resins remained a problem until 1994, when anomeric sulfoxides were employed in glycosylation reactions on the Merrifield resin.[56] As shown in Figure 1.5, the glycosyl acceptor 10 was

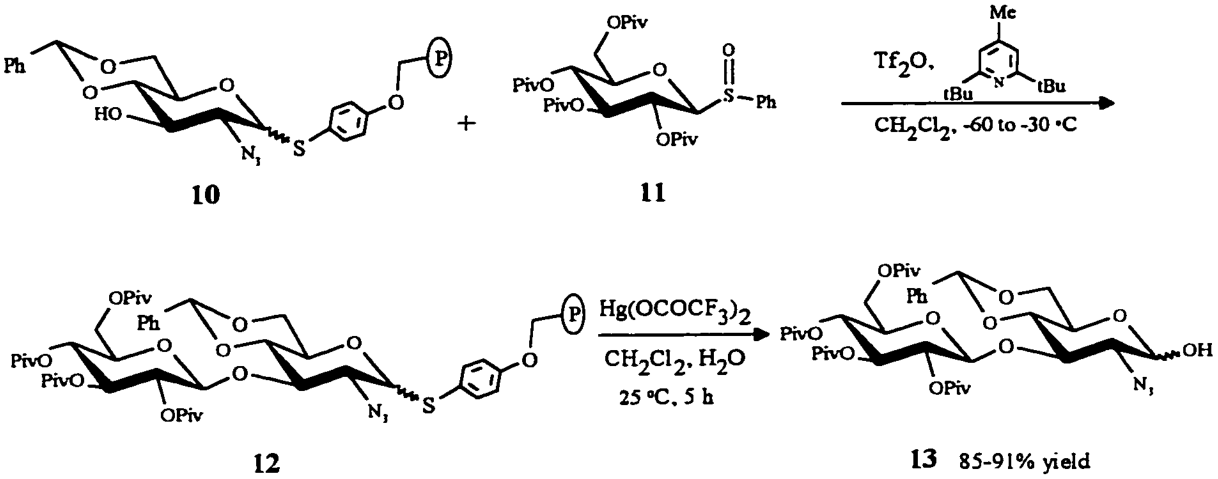


Figure 1.5 Solid phase synthesis of 13 using anomeric sulfoxide methodology

attached to a solid phase P (usually a resin polymer) via a thiophenyl ether linkage, while the glycosyl donor 11 was in the eluent. The coupling reaction occurred when the solid phase column was washed with glycosyl donor eluent. After glycosylation, disaccharide 13 was obtained by cleavage from the resin polymer by treatment with mercuric trifluoroacetate. Solid-phase supported oligosaccharide synthesis eliminates the necessity for purification of the intermediates. Instead, simple phase separations and solvent washes become the only workup procedures. The other advantages of this method are that the extent of coupling for each glycosylation reaction and the reaction rate can be increased by using an excess of reagents. It is possible in the future that this procedure could be automated, which would be useful for the industrial production of oligosaccharides. However, it should be noted that this particular approach does not give as good stereocontrol in the formation of the more difficult *cis*-1,2-glycosidic linkages as it does in the formation of the *trans*-1,2-glycosidic linkage shown.

Another earlier strategy for the solid-phase supported synthesis of oligosaccharides was presented by Danishefsky and coworkers.[35] The synthesis of **21** is shown as an example in Figure 1.6. Glycal **14** was attached to a solid phase with a silyl ether bond.

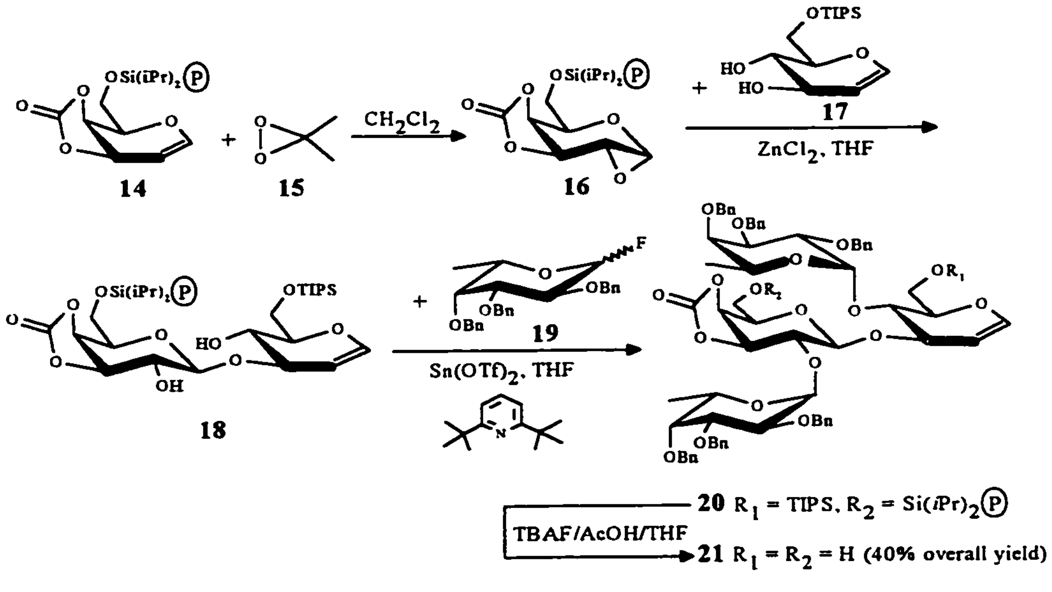


Figure 1.6 Solid phase synthesis of 21 using glycal assemble methodology

A solid-phase attached glycosyl donor **16** was obtained as a 1,2-anhydro-sugar by oxidation with 3,3-dimethyldioxirane (**15**). Glycosylation was performed by reaction with eluent-based glycal acceptor **17** in the presence of a Lewis acid, $ZnCl_2$, to give solid phase-attached disaccharide **18**. Further glycosylations can be performed by reiteration of the above procedure without worrying about protection of the 2-OH just created, thus leading to linear oligosaccharides. This method was self-corrective in that failed donors in a coupling step did not reemerge in the next cycle. This method was also useful for creating branched sugars at C-2, a common branching site. As shown in Figure 1.6, solid phase supported disaccharide **18** can also serve as a glycosyl acceptor. Reaction with solution based glycosyl donor **19** afforded the polymer supported tetrasaccharide **20**. The tetrasaccharide **21** was detached from the polymer by treatment of **20** with tetrabutylammonium fluoride (TBAF). A 40% overall yield was obtained based on **14**. It was noted that protecting group manipulations had been sharply minimized. Application of this method also proved to be successful in the synthesis of a branched oligosaccharide, the first synthesis of a complex saponin,[36] and also of some bioactive molecules such as Lewis^y and Lewis^b determinants.[37,38] Using the same strategy, the synthesis of one of the largest N-linked glycopeptides ever accessed by either solution- or solid- phase synthesis was reported recently.[39] In all cases, a high degree of β-selectivity has been observed. But of course, this method can only be used in the formation of *trans*-1,2-glycosidic linkages.

It has also been reported that precursors of 6-deoxy di- and tri-saccharides were easily synthesized using a sulfonyl chloride resin as an insoluble solid support.[57] Both glycosyl trichloroacetimidates and glycosyl acetates were used as glycosyl donors in the glycosylation steps. The synthesized oligosaccharides were removed from the solid support by treatment with various nucleophiles such as NaI, NaOAc, and NaN_3.

Despite the considerable progress that has been made in the development of solid phase oligosaccharide synthesis, problems remain. First is the lack of stereocontrol in formation of the glycosidic linkage, particularly when *cis*-1,2-glycosidic linkages are required. Secondly, glycosylation reactions go more slowly in the heterogeneous environment of a resin matrix than they do in solution. The slow reaction rates sometimes permit undesirable side reactions to predominate over glycosylation. Polymer-supported solution synthesis of oligosaccharides may provide a solution to these problems.[58] Using a soluble resin, glycosylation reactions were carried out in a more solution-like environment. In this case, the anomeric control of solution chemistry can be combined with the ease and speed of polymer-supported workup. After the glycosylation reaction, the oligosaccharide bonded polymer was precipitated with diethyl ether or *t*-butyl methyl ether and the oligosaccharide was then detached from the polymer. With this method using the trichloroacetimidate procedure, a D-mannopentose, a structural unit of D-mannans of pathogenic yeasts, was synthesized.[59] A serious drawback about this method is that the linkage of the carbohydrate to the polymer is a succinic ester bond, which may not be stable under basic chemical conditions.

1.2.5 Strategies for construction of larger oligosaccharides

The most effective methods for the construction of larger oligosaccharides is block synthesis, in which blocks of smaller oligosaccharides are coupled. For glycosylation to occur, the anomeric centre at the reducing end of one block must be reactivated.

Activation of the anomeric centre should not require many steps or, better yet, should be direct.

The coupling of donors activated by benzyl substituents with acceptors deactivated by acetyl groups had been suggested.[29,60] Glycal-type structures can also be activated by epoxidation, making renewed coupling possible.[35]

In 1994, Ogawa *et al.*[61] developed an orthogonal glycosylation strategy. As shown in Figure 1.7, X and Y were two chemically distinct (orthogonal) leaving groups, which can be activated under different conditions, i.e. (a) and (b) respectively. Group X remains unaffected under condition (b) required to activate the group Y, and *vice versa*. In addition, both X and Y should be compatible with subsequent manipulations of temporary

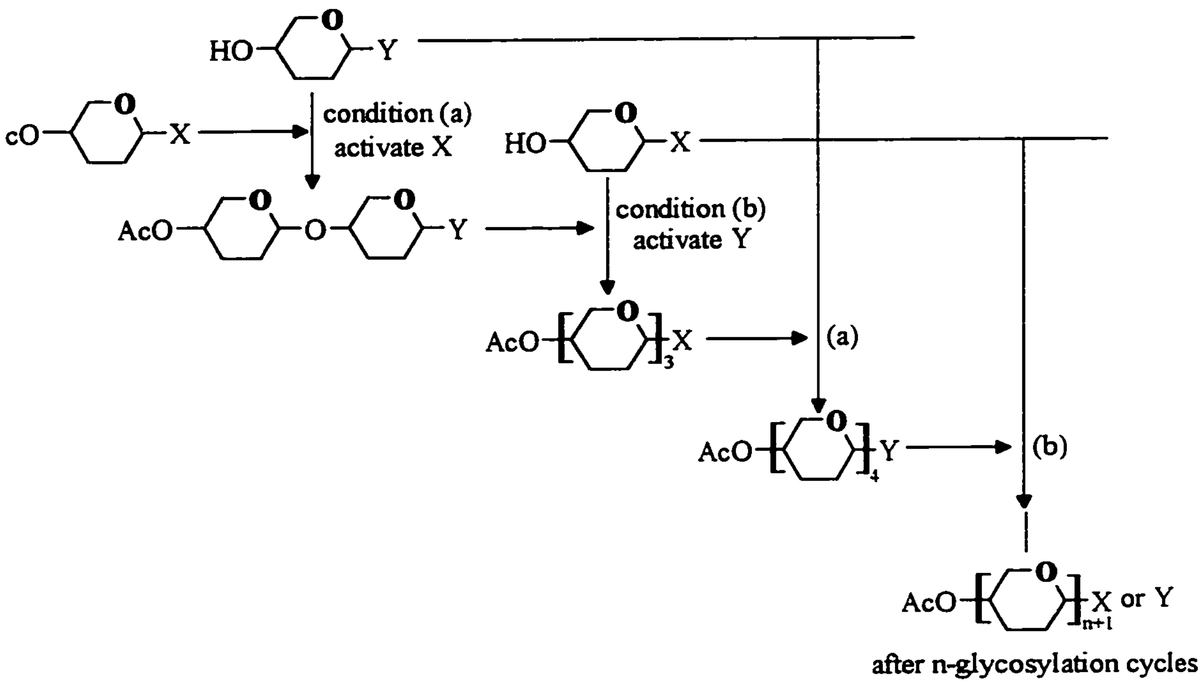


Figure 1.7 A schematic description of orthogonal glycosylation strategy

protecting groups. In their case, phenylthio group and fluoride were selected as X and Y, respectively and NIS-TfOH (or AgOTf) (condition a) and Cp_2HfCl_2-$AgClO_4$ (condition b) as promoters. A heptasaccharide was synthesized within five steps in a 27% yield from monosaccharides. In this procedure, extra steps, such as temporary protection of the anomeric position and subsequent conversion into donor were eliminated.

With a similar strategy, Kahne *et al.*[62] performed another very elegant synthesis of a trisaccharide in practically one step (Figure 1.8). The building blocks 22, 23, 25 had different reactivities at the anomeric centre. Phenylthioglycoside 23 is the least reactive, while phenylsulfoxide 22 is considerably more reactive. A solution of building blocks 22,

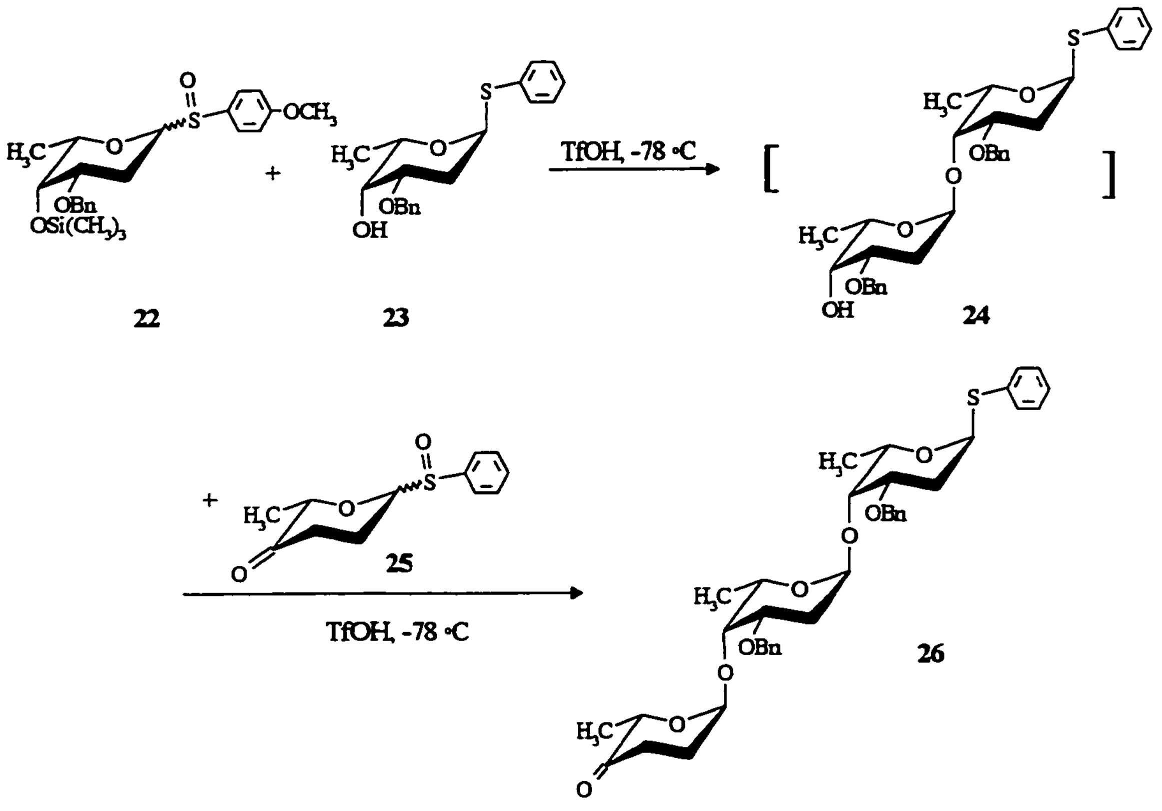


Figure 1.8 A one pot synthesis of trisaccharide 26

23, and **25** in Et_2O/CH_2Cl_2 was simply mixed in the presence of TfOH at -78 ~ -70 °C, and trisaccharide **26** was isolated in a 25% yield after 45 min. It was assumed that the modestly reactive **23** reacted first with the highly reactive **22** to give disaccharide **24**; the trimethylsilyl group was removed simultaneously, and **24** reacted with the moderately reactive **25** to provide **26**.

1.2.6 Summary

The above mentioned methods work well in some cases, but not always. Sometimes, high selectivity can be obtained, but at other times, very poor selectivity is the result.[32] There is still no universal method to selectively synthesize oligosaccharides. Frequently, mixtures are obtained. Chromatography is still a necessary procedure to obtain pure diastereomers.

The polymer supported glycosylation methods eliminate time-consuming work-up procedures and purifications steps and have been developed considerably recently. However, only relatively simple oligosaccharides have been synthesized by these methods and the glycosidic bonds of these oligosaccharides were mainly 1,2-*trans* linked. In addition, with polymer-supported oligosaccharide synthesis, it is difficult to follow the reaction. Simple techniques such as TLC cannot be used here.

Clean stereoselective control of glycosylation can be obtained by enzyme catalysis.[63] Recent progress in cloning techniques has made several glycosyltransferases available in sufficient quality and quantity,[64] and enzymatic oligosaccharide synthesis has become more practical. Glycosyltransferases and glycosidases are increasingly being used in the preparation of target molecules.[65] The most efficient use of glycosyltransferases in the combined chemical-enzymatic synthesis of oligosaccharides is to add terminal sugar residues enzymatically on to presynthesized "primers". With this technique, a multivalent sialoglycopeptide[66] and an octadecameric saccharide of branched blood group type-1[67] were successfully synthesized.

For the synthesis of a specific oligosaccharide, one needs to be very familiar with all the techniques available and work out a feasible procedure. A well-planned synthesis is more likely to produce the desired target molecule.

Chapter 2 Synthetic studies of the repeating unit of the C-polysaccharide of *Streptococcus pneumoniae*

2.1 Introduction

2.1.1 General

Streptococcus pneumoniae are Gram-positive bacteria, which can induce infections like pneumonia, otitis media, and meningitis in human beings.[68] About 90 serologically defined types of this microorganism have been described to date.[69] For more than 100 years, this family of bacteria has persisted in being a major causative agent of the above mentioned serious human diseases, especially community-acquired pneumonia. The introduction of sulfonamides in the late 1930s and of penicillin in 1945 dramatically changed the prognosis of pneumococcal infections. However, during the course of time, antibiotic-resistant strains of pneumococcus have emerged throughout the world,[70] making this family of bacteria a very severe health problem. This alarming development underscores the need for more effective preventive strategies, of which vaccination is a leading contender.

The study of *Streptococcus pneumoniae* has led to many insights into the pathogenesis of bacterial infections, however, many questions about its virulence are still unanswered.[71] Like other encapsulated bacteria, the pneumococcus relies on its polysaccharide surface capsule to evade the host's phagocytic defenses. Induction of anti-capsular antibodies by active or passive immunization has long been known to protect against disease by enhancing phagocytosis of the bacteria.[72] However, the development of a vaccine with adequate coverage of pneumococci is complicated by the existence of various distinct pneumococcal capsular polysaccharide serotypes.[69] This obstacle was partially overcome with the introduction of a polyvalent pneumococcal vaccine. In 1977, a vaccine containing the purified capsular polysaccharides of 14 of the most prevalent types was licensed; this vaccine provided coverage against about 80% of invasive pneumococcal isolates in the United States. With the introduction of the 23-valent pneumococcal polysaccharide vaccine in 1983, coverage was increased to above 90% of isolates.[68] Preparation of these vaccines poses formidable challenges, because a certain number of separate vaccines must be manufactured, controlled, and combined in appropriate concentrations. A major problem with the current polysaccharide vaccine is that the purified capsular polysaccharides of pneumococcus do not reliably induce protective antibody responses in children younger than 2 years - the age group that shows the highest incidence of invasive pneumococcal infections. In addition, polysaccharides did not induce the T cell memory required for booster responses. Considering these limitations of this type of vaccine, efforts have been made to prepare better alternatives based on polysaccharide or oligosaccharide conjugates, having proteins as the carrier.[73]

Alternative approaches to pneumococcal vaccines are also being considered. The C-polysaccharide, a common antigen to all the known types of *Streptococcus pneumoniae*,[74] is one of the candidates. Immunization with noncapsular antigens that might induce protection against all serotypes would be advantageous either instead of or as a complement to, capsular polysaccharide-based vaccines.[75] Such a vaccine could enhance protective efficacy of type-specific conjugate vaccines and extend protection to strains not included in the vaccine. In addition, though concealed by capsular polysaccharides in the log phase pneumococci, the C-polysaccharide is found to be exposed on decaying cells. It is therefore possible that a host infected by pneumococci can benefit from antibodies to these antigens, because they may facilitate the clearance of potentially harmful disintegrated bacterial residues such as the peptidoglycan. Studies showed that anti-C-polysaccharide antibody may contribute to the removal of pneumococci with no or partially disintegrated capsules during the course of pneumococcal infections, thereby diminishing inflammatory reactions.[76] It has been reported that non-type-specific antibodies could protect mice against pneumococcal infections.[77,78] On the other hand, it was also reported that mice were not protected against pneumococcal infections by passive immunization with polyclonal rabbit antiserum to C-polysaccharide.[79] More research needs to be conducted to clarify this situation.

C-Polysaccharide was found to be a contaminant in most preparations of capsular polysaccharides and it could not be removed.[80] Capsular polysaccharides were found to be strongly attached to the cell walls of different types of pneumococci and they persisted even after enzymatic disintegration of cell walls.[81] It is usually very difficult to isolate large polar molecules like those from biological specimens in very high purity, which makes it difficult to accurately evaluate the immunoefficacy of different parts of the cell. To understand the structure-activity of the natural antigen, the availability of the pure polysaccharide or the oligosaccharide of the repeating unit or analogues would be helpful. Chemical synthesis in this regards is a necessary alternative to provide pure research substances. Development of a reasonable way to synthesize the compound could be the most economic way to obtain it.

In order to clarify the confusion about the immunoefficacy of the C-polysaccharide, an effort was made here to synthesize the repeating unit of the C-polysaccharide. However, the time constraints associated with the Ph.D degree necessitated synthesis of only part of the C-polysaccharide. There was also uncertainty with respect to one element of the structure of this biomolecule and the part of the C-polysaccharide synthesized was selected to answer this uncertainty (see section 2.3.2 and section 2.3.3).

2.1.2 The polysaccharides on the cell surface of *Streptococcus pneumoniae*

As shown in Figure 2.1, three major distinguishable layers exist on the cell surface: plasma membrane, cell wall and capsule. The pneumococcus bacterial cell is surrounded by a capsule that contains a polysaccharide (capsular polysaccharide).[82] The capsule is the

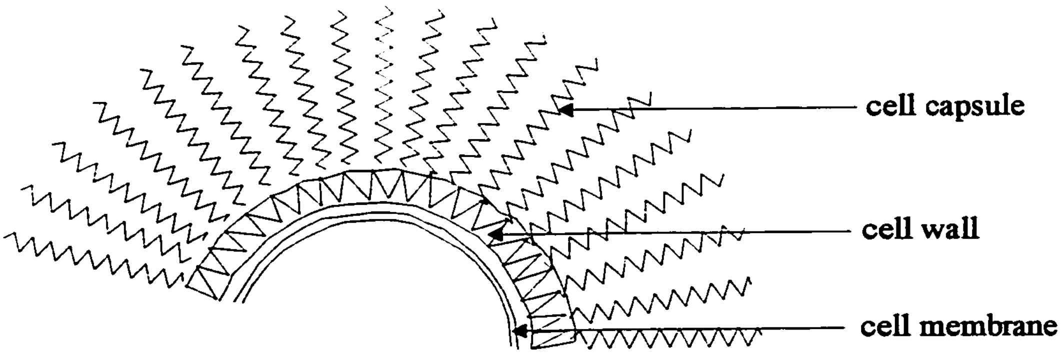


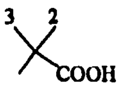
Figure 2.1 A graphic description of the cell surface anatomy of pneumococcus

thickest layer, completely concealing the inner structures in pneumococci.[83] The cell wall consists of a triple-layered peptidoglycan backbone that anchors the capsular polysaccharide, the cell wall polysaccharide, and possibly also proteins.[84] The chemical compositions of the polysaccharides on the cell surface of *Streptococcus pneumoniae* will be described in detail in the following sections.

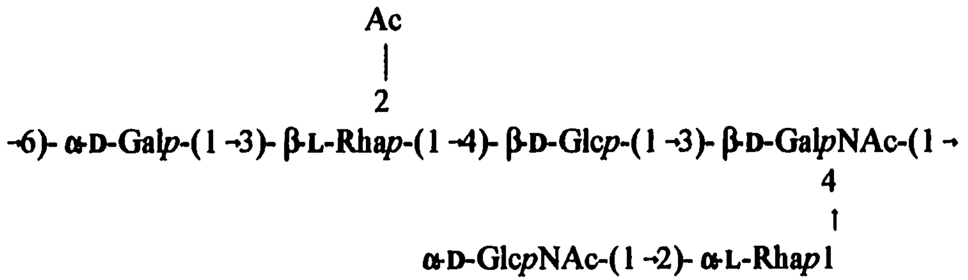
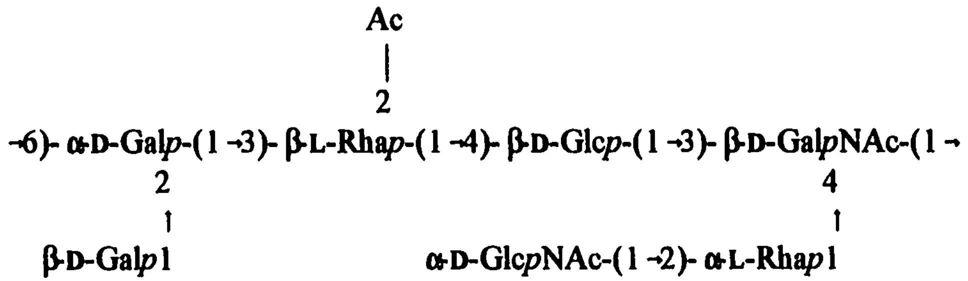
2.1.2.1 The capsular polysaccharides

The chemical composition of the capsular polysaccharide determines its specific serological type, of which there are now 90 types known.[69] The capsular polysaccharides of *Streptococcus pneumoniae* bacteria are usually regular, periodic polymers with diverse structures that contain, in several instances, comparatively rare monosaccharides. Extensive studies on capsular polysaccharides have been carried out, particularly starting from the time of the development of the pneumococcal vaccine.[72] The oligosaccharides that constitute the repeating units of these polysaccharides provide a demanding challenge in terms of oligosaccharide synthesis. Advances in glycoside synthesis with ancillary techniques of chromatographic separation and high resolution NMR spectroscopy have allowed rational synthesis of such oligosaccharides to be planned and successfully completed. Much work has been done on pneumococcal capsular polysaccharides, both on structural elucidation and on oligosaccharide synthesis, which is summarized in Table 2.1. A previous summary of the structures of pneumococcal capsular polysaccharides had been presented by Kenne and Lindberg.[85] Some of the structures has been revised and some new types have been elucidated since then.

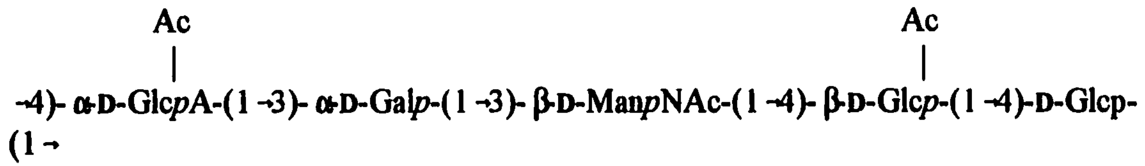
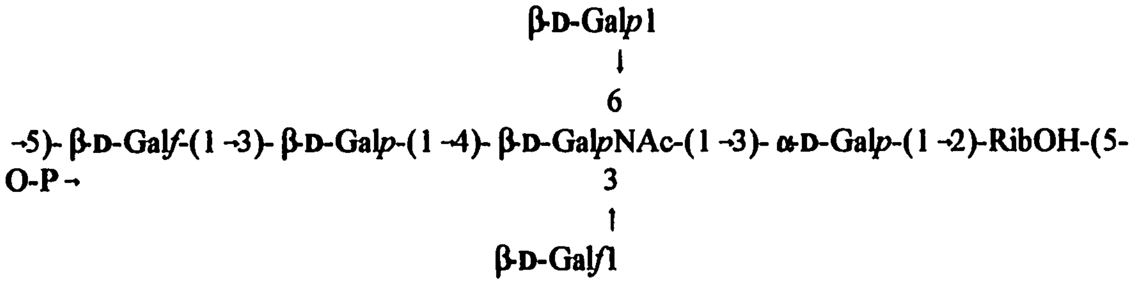
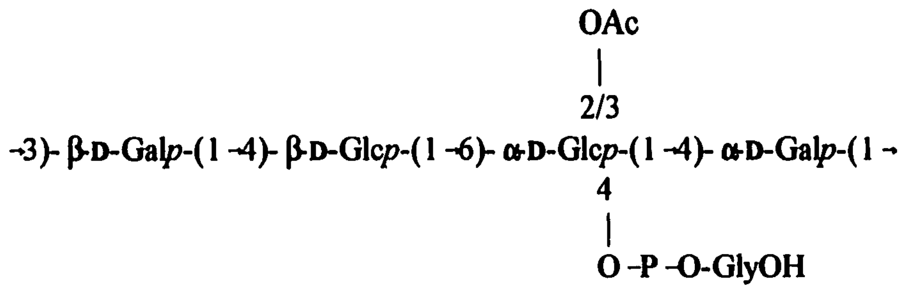
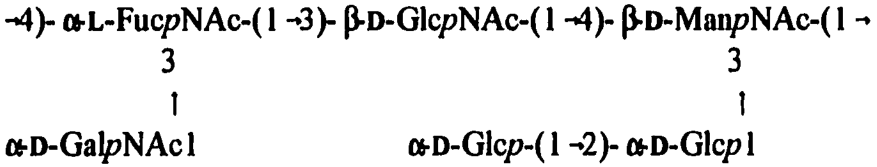
Table 2.1. The capsular polysaccharides in *Streptococcus pneumoniae*

Type	Structural Repeating Unit	Structural Studies (ref)	Synthetic Work (ref)
1	→3)-α-D-AATG*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A(1→	86	87
2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*(1→ 2 ↑ α-D-Glc*p*A-(1→6)-α-D-Glc*p*1	88	
3	→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*A-(1→	89	
4	→3)-β-D-Man*p*NAc-(1→3)-α-D-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-(1→  3 2 COOH	90	
5	→4)-β-D-Glc*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Sug*p*-(1→ 4 ↑ α-L-Pne*p*NAc-(1→4)-β-D-Glc*p*A1	91	
6A	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-RibOH-(5-O-P→	92	93,94
6B	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5-O-P→	92,95	93,94

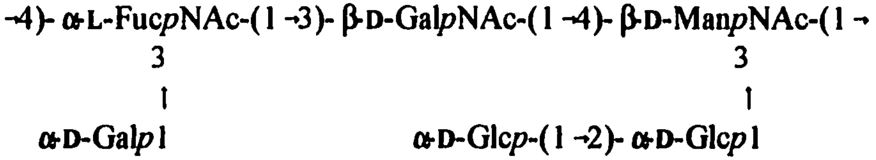
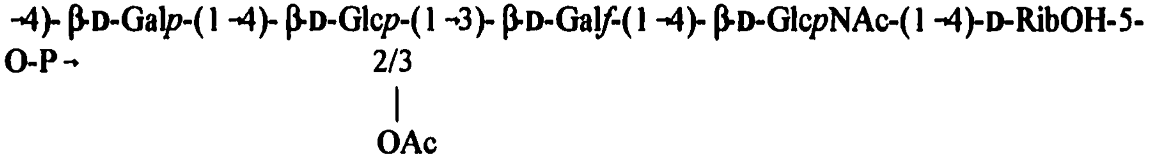
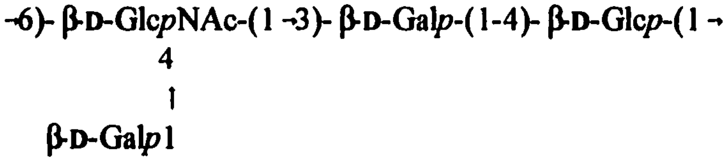
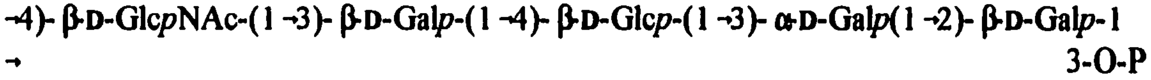
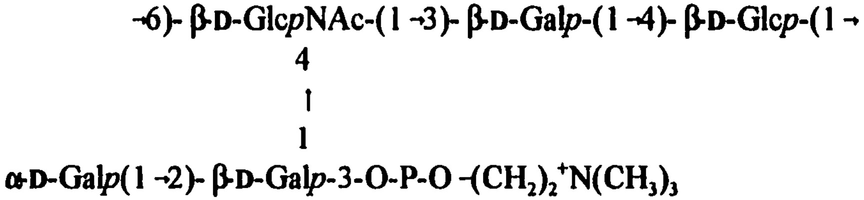
continued

7A	 $\rightarrow 6)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\overset{\substack{\text{Ac}\\ \vert\\ 2}}{\beta\text{-L-Rha}p}\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\underset{\substack{4\\ \uparrow\\ \alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p1}}{\beta\text{-D-Gal}p\text{NAc}}\text{-}(1\rightarrow$	96	
7F	 $\rightarrow 6)\text{-}\underset{\substack{2\\ \uparrow\\ \beta\text{-D-Gal}p1}}{\alpha\text{-D-Gal}p}\text{-}(1\rightarrow 3)\text{-}\overset{\substack{\text{Ac}\\ \vert\\ 2}}{\beta\text{-L-Rha}p}\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\underset{\substack{4\\ \uparrow\\ \alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p1}}{\beta\text{-D-Gal}p\text{NAc}}\text{-}(1\rightarrow$	97	
8	→4)- β-D-Glc*p*A-(1 →4)- β-D-Glc*p*-(1 →4)- α-D-Glc*p*-(1 →4)- α-D-Gal*p*-(1 →	98	
9A	→3)- α-D-Gal*p*-(1 →3)- β-D-Man*p*NAc-(1 →4)- α-D-Glc*p*-(1 →4)- β-D-Glc*p*-(1 →4)- α-D-Glc*p*A-(1 →	99,100	101
9L	→3)- α-D-Gal*p*-(1 →3)- β-D-Man*p*NAc-(1 →4)- β-D-Glc*p*-(1 →4)- α-D-Glc*p*NAc-(1 →4)- α-D-Glc*p*A-(1 →	100,102	
9N	→3)- α-D-Glc*p*-(1 →3)- β-D-Man*p*NAc-(1 →4)- β-D-Glc*p*-(1 →4)- α-D-Glc*p*NAc-(1 →4)- α-D-Glc*p*A-(1 →	100,103,104	

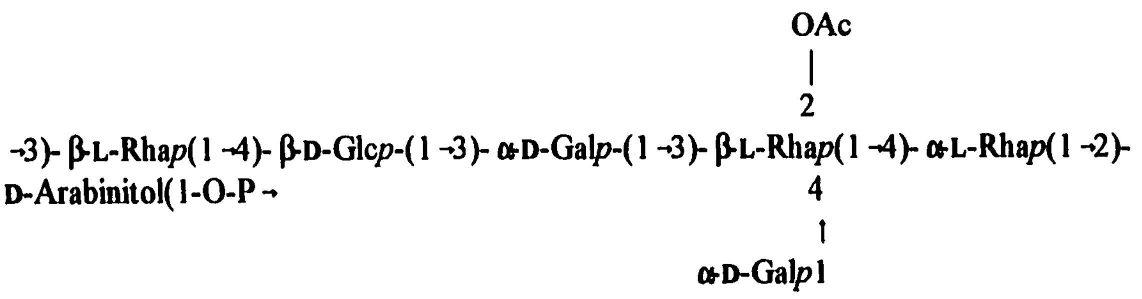
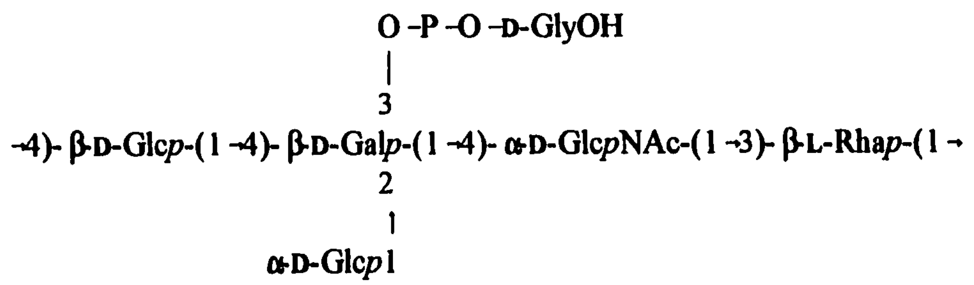
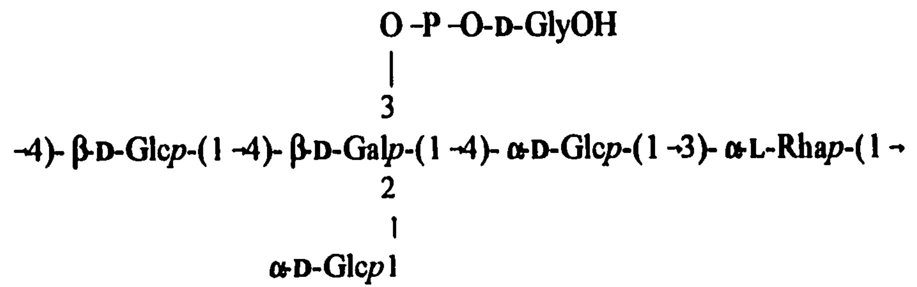
continued

9V	 $\rightarrow 4)\text{-}\overset{\text{Ac}}{\overset{\vert}{\alpha\text{-D-Glc}p\text{A}}}\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Man}p\text{NAc-}(1\rightarrow 4)\text{-}\overset{\text{Ac}}{\overset{\vert}{\beta\text{-D-Glc}p}}\text{-}(1\rightarrow 4)\text{-D-Glc}p\text{-}(1\rightarrow$	100,105	101,108
10A	 $\rightarrow 5)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\overset{\beta\text{-D-Gal}p1}{\overset{\downarrow}{\overset{6}{\underset{\underset{\underset{\beta\text{-D-Gal}f1}{\uparrow}}{3}}{\beta\text{-D-Gal}p\text{NAc}}}}}\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 2)\text{-RibOH-}(5\text{-O-P}\rightarrow$	107	
11A	 $\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 6)\text{-}\overset{\text{OAc}}{\overset{\vert}{\overset{2/3}{\underset{\underset{\underset{\text{O-P-O-GlyOH}}{\vert}}{4}}{\alpha\text{-D-Glc}p}}}}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow$	108	
12A	 $\rightarrow 4)\text{-}\underset{\underset{\underset{\alpha\text{-D-Gal}p\text{NAc}1}{\uparrow}}{3}}{\alpha\text{-L-Fuc}p\text{NAc}}\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-}\underset{\underset{\underset{\alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Glc}p1}{\uparrow}}{3}}{\beta\text{-D-Man}p\text{NAc}}\text{-}(1\rightarrow$	109	

continued

12F	 →4)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Man*p*NAc-(1→ 3 ↑ α-D-Gal*p*1 (on FucpNAc); 3 ↑ α-D-Glc*p*-(1→2)-α-D-Glc*p*1 (on ManpNAc)	110	
13	 →4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*f*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-RibOH-5-O-P→ 2/3 | OAc	111	
14	 →6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1-4)-β-D-Glc*p*-(1→ 4 ↑ β-D-Gal*p*1	112	113,114
15F	 →4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-α-D-Gal*p*(1→2)-β-D-Gal*p*-1→ 3-O-P	115	
15B	 →6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ 1 α-D-Gal*p*(1→2)-β-D-Gal*p*-3-O-P-O-(CH$_2$)$_2$$^+$N(CH$_3$)$_3$	116	

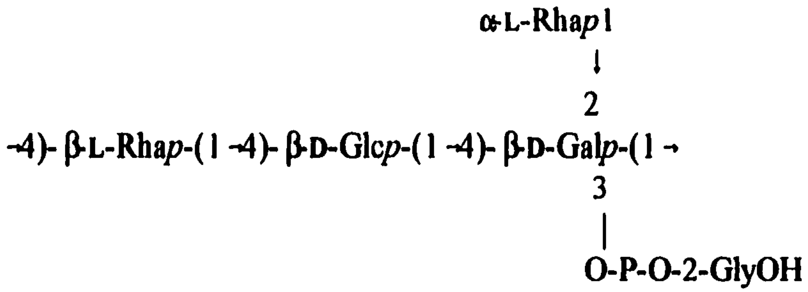
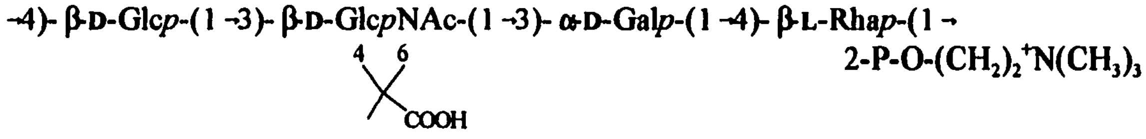
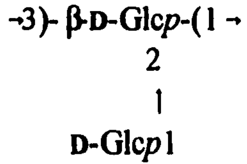
continued

17F	 $\rightarrow 3)\text{-}\beta\text{-L-Rha}p(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\underset{\substack{4\\ \uparrow\\ \alpha\text{-D-Gal}p1}}{\overset{\substack{\text{OAc}\\ \vert\\ 2}}{\beta\text{-L-Rha}p}}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p(1\rightarrow 2)\text{-D-Arabinitol}(1\text{-O-P}\rightarrow$	117	
18A	 $\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\underset{\substack{2\\ \uparrow\\ \alpha\text{-D-Glc}p1}}{\overset{\substack{\text{O-P-O-D-GlyOH}\\ \vert\\ 3}}{\beta\text{-D-Gal}p}}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow 3)\text{-}\beta\text{-L-Rha}p\text{-}(1\rightarrow$	118	
18C	 $\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\underset{\substack{2\\ \uparrow\\ \alpha\text{-D-Glc}p1}}{\overset{\substack{\text{O-P-O-D-GlyOH}\\ \vert\\ 3}}{\beta\text{-D-Gal}p}}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow$	119	120,121

continued

18F	O-P-O→D-GlyOH | 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→ 2 2 ↑ | Ac-6-α-D-Glc*p*1 Ac	122	
19A	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1-O-P→	123,124	101,125
19F	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1-O-P→	126-128	101,129,130
20	→6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Glc*p*NAc(1-P- 4 ↑ β-D-Gal*f*1	131	
22F	Ac 1-α-D-Glc*p* | ↑ 2 3 →4)-β-D-Glc*p*A-(1→4)-β-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→3)-α-D-Gal*f*-(1→2)-α-L-Rha*p*-(1→	132	

continued

23F	 $\rightarrow 4)\text{-}\beta\text{-L-Rha}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\underset{\substack{3\\ \vert \\ \text{O-P-O-2-GlyOH}}}{\overset{\substack{\alpha\text{-L-Rha}p1\\ \downarrow \\ 2}}{\beta\text{-D-Gal}p}}\text{-}(1\rightarrow$	133	134
27	 $\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\underset{\substack{4\;\;6\\ \times\,\mathrm{COOH}}}{\beta\text{-D-Glc}p\text{NAc}}\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\underset{\text{2-P-O-}(\mathrm{CH_2})_2{}^{+}\mathrm{N(CH_3)_3}}{\beta\text{-L-Rha}p}\text{-}(1\rightarrow$	135	136,137
29	→4)- β-D-Gal*p*NAc-(1→6)- β-D-Gal*f*-(1→3)- β-D-Gal*p*-(1→6)- β-D-Gal*f*-(1→1)-D-RibOH-(5-O-P→	138	
33	→3)- α-D-Gal*p*-(1→3)- β-D-Man*p*NAc-(1→4)- α-D-Glc*p*-(1→4)- β-D-Glc*p*-(1→4)- α-D-Glc*p*A-(1→	139	
35B	→4)- β-D-Gal*p*NAc-(1→6)- β-D-Gal*f*-(1→3)- β-D-Glc*p*-(1→6)- β-D-Gal*f*-(1→1)-D-RibOH-(5-O-P→	140	
37	 $\rightarrow 3)\text{-}\underset{\substack{2\\ \uparrow \\ \text{D-Glc}p1}}{\beta\text{-D-Glc}p}\text{-}(1\rightarrow$	141	

2.1.2.2 Cell wall and cell membrane polysaccharides

The pneumococcal cell wall is typical of a Gram-positive microorganism, which is relatively thick and homogeneous. It is constructed from three layers of cross-linked peptidoglycan chains with a C-polysaccharide covalently attached.[142] The peptidoglycan is an enormous polymer composed of many identical subunits. The polymer contains two carbohydrate derivatives, N-acetylglucosamine and N-acetylmuramic acid, and several different amino acids. Chains of linked peptidoglycan subunits are joined by cross-links between the peptides.

The C-polysaccharide is uniformly distributed on both the inside and outside of the cell walls.[74,83] It is also covalently bonded to a lipid moiety in the plasmic membrane through a phosphodiester linkage,[143,144] which is called the F antigen. The C-polysaccharide was first described as a somatic antigen (fraction "C") of the pneumococcus in 1930.[145] Structural studies[146-150] showed that the polysaccharide was

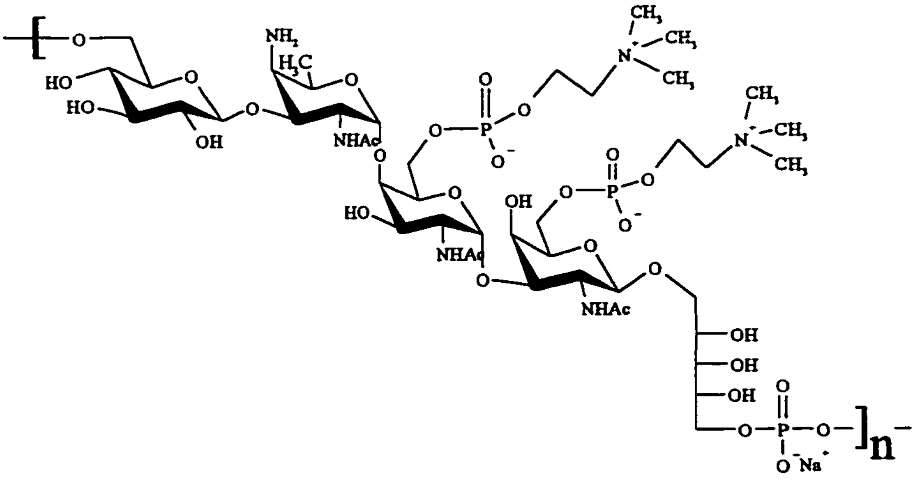


Figure 2.2 The structure of the C-polysaccharide of *Streptococcus pneumoniae*

composed of a repeating unit (27) containing residues of β-D-glucopyranose, 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose, 2-acetamido-2-deoxy-β-D-galactopyranose, ribitol phosphate, and phosphorylcholine. The phosphodiester linkage joins *O*-5 of ribitol with *O*-6 of a β-D-glucopyranose in the next repeating unit. With the development of modern NMR techniques, the structure of the C-polysaccharide was almost completely elucidated.[151] The sequence of the carbohydrate residues, most of their absolute configurations, the positions of the *N*-acetyl groups, the locations of phosphorylcholine substituents and other phosphorus linkage sites were clearly identified. Figure 2.2 shows the structure of the C-polysaccharide.[151]

2.1.3 The configuration of the ribitol unit in the C-polysaccharide

There was still one uncertainty remaining in the structure of the C-polysaccharide: the configuration of the ribitol unit. Careful examination of the literature showed that no direct experimental evidence had ever been provided to assign this configuration. Because the C-polysaccharide contains ribitol phosphate and sugar residues and occurs in the cell wall, it was classified as a teichoic acid.[148] The ribitol unit in the C-polysaccharide had been assumed to have the D-configuration based on the biosynthesis of teichoic acids.[148,149]

The teichoic acids are a group of phosphate-containing polymers found in many Gram-positive bacterial cell walls and membranes. The name was originally applied to

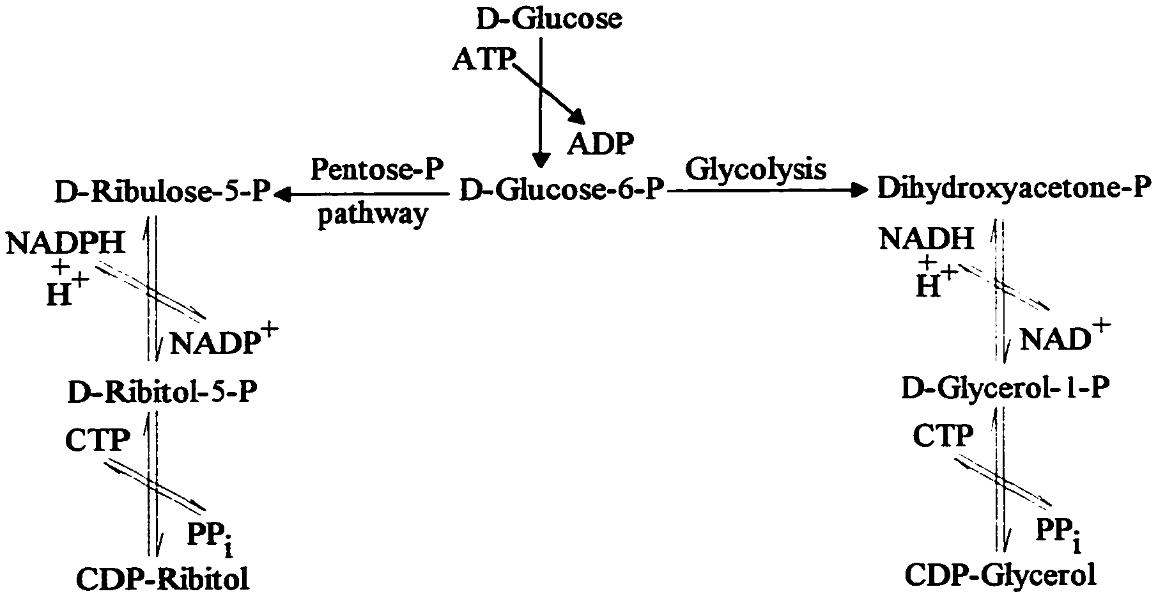


Figure 2.3 Biosynthesis of CDP-glycerol and CDP-ribitol

polymers of ribitol and glycerol phosphate but is now more generally applied to include related polymers which, in addition to ribitol and glycerol phosphate, also contain sugar residues as an integral part of the polymer chain. In the biosynthesis of teichoic acids,[152,153] the donors of glycerol and ribitol phosphate residues are CDP-glycerol and CDP-ribitol, respectively. As shown in Figure 2.3, these nucleotide precursors are synthesized from CTP and D-glycerol-1-phosphate or D-ribitol-5-phosphate and eventually from D-glucose. Therefore, the configuration of ribitol unit in teichoic acids is D, with position-5 linked to phosphate, and the configuration of glycerol unit is also D, with position-1 linked to phosphate.

It is very easy to assume that the CDP-glycerol and CDP-ribitol are the precursors of all the glycerol- or ribitol-containing polysaccharides, respectively. However, it is very difficult to rule out different biosynthetic routes for specific teichoic acid-like molecules. There have been reports about the absolute configuration of glycerol unit in teichoic acid-like molecules.[154-159] The configurations of the glycerol units were determined either by comparing the chromatographic mobilities of the partially hydrolyzed glycerol-containing derivatives with that of authentic compound,[154] by NMR techniques[157] or by GLC-MS by comparison with synthesized standard compounds.[159] Most of the time, the glycerol unit was found to have the D-configuration with position-1 linked to phosphate, in agreement with the biosynthesis assumption. However, it was also reported that an L-glycerol unit with position-1 linked to a phosphate was found in the membrane bound teichoic acid from *Streptococcus lactis*.[155] From biosynthetic studies of various compounds, it had been established that D-glycerol-1-phosphate was derived from CDP-glycerol,[160] whereas the L-glycerol-1-phosphate was transferred from phosphatidylglycerol.[161] It would be interesting to find out the situation about the ribitol-containing teichoic acids.

The ribitol unit has two more stereogenic centres than the glycerol unit does, which makes the determination of the configuration a more challenging issue. Several of the capsular polysaccharides of *Streptococcus pneumoniae* also contain this ribitol unit [92,95,107,138,140] as shown in Table 2.1. It is important to determine the configurations of the ribitol units in the C-polysaccharide as well as in the capsular polysaccharides. Based on these results, it would be possible to provide evidence that would confirm the biosynthetic pathway of D-ribitol-5-phosphate, or to discover other biosynthetic pathways. However, no work has been published to determine the absolute configurations of the ribitol units in these biomolecules. An effort was made here to determine the configuration of the ribitol unit in the C-polysaccharide of *Streptococcus pneumoniae*. A retrosynthetic analysis of the repeating unit of the C-polysaccharide was performed in the following section. However, due to time limitations, only part of the synthesis was carried out. Fragments of the repeating unit of the C-polysaccharide containing both D- and L-ribitol units were designed and synthesized. With 2D NMR techniques, the ^{1}H and ^{13}C NMR spectra of these diastereomerically different products were fully assigned. Comparison of these data with that of the corresponding part of the natural product would allow the assignment of the configuration of the ribitol unit in the C-polysaccharide. This process would remove any ambiguity in the structure of the C-polysaccharide as well as provide potential vaccine candidates.

2.2 Retrosynthetic analysis of the repeating unit of the C-polysaccharide

Retrosynthetic analysis for the synthesis of the repeating unit (**27**) of C-polysaccharide of *Streptococcus pneumoniae* was carried out by first designing a completely protected pentasaccharide **28**. As seen in Figure 2.4, **28** had two dioxaphospholane rings. As the precursors of the phosphocholine chains, they also served as temporary protecting groups. The dioxaphospholane ring can be opened easily with trimethylamine.[162] As in other organic synthesis, block synthesis usually gives better yields

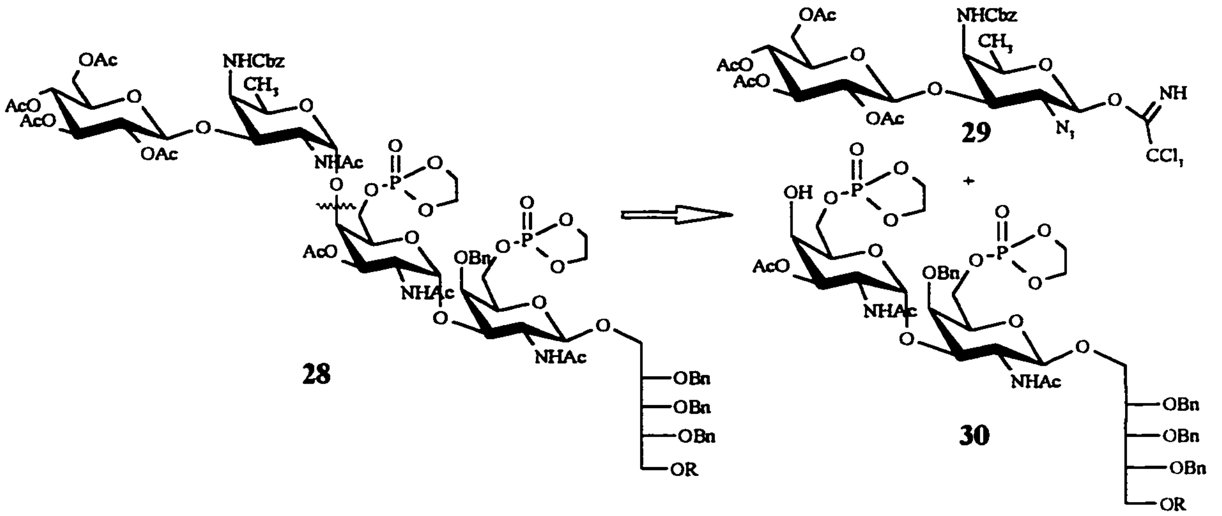


Figure 2.4 Retrosynthetic analysis (scheme 1)

than stepwise synthesis do. Disconnection of **28** led to two oligosaccharide blocks, a disaccharide glycosyl donor **29** and a trisaccharide glycosyl acceptor **30**. The easily activated trichloroacetimidate group was chosen as the glycosyl donor group here. Because the coupling between **29** and **30** must give an α-configuration, a nonparticipating group (N_3), which could be easily transformed to AcNH by treatment with thiolacetic acid, was put at position-2 beside the leaving group of **29**. As seen in Figure 2.5, These two groups could be introduced through several steps of chemical modifications of **31**, a glycal derivative.[21,163] Further disconnection of **31** afforded glycosyl donor **32**, which could easily be synthesized from D-glucose,[164] and glycosyl acceptor **33**, which is a rare sugar derivative also to be synthesized from D-glucose.

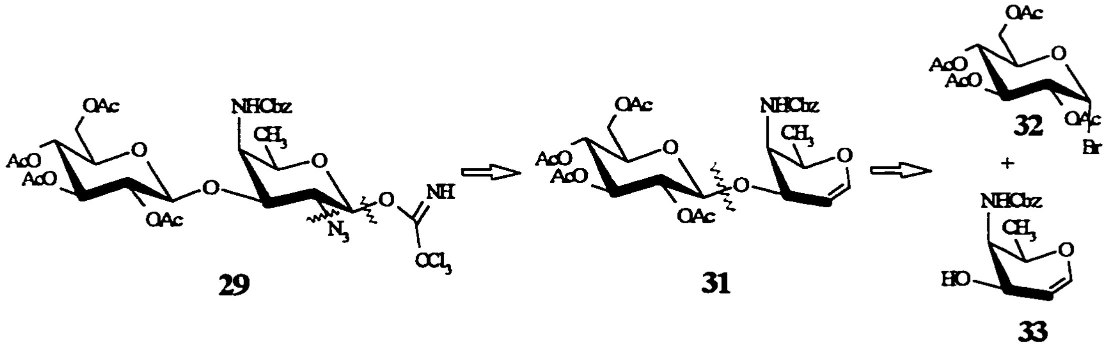


Figure 2.5 Retrosynthetic analysis (scheme 2)

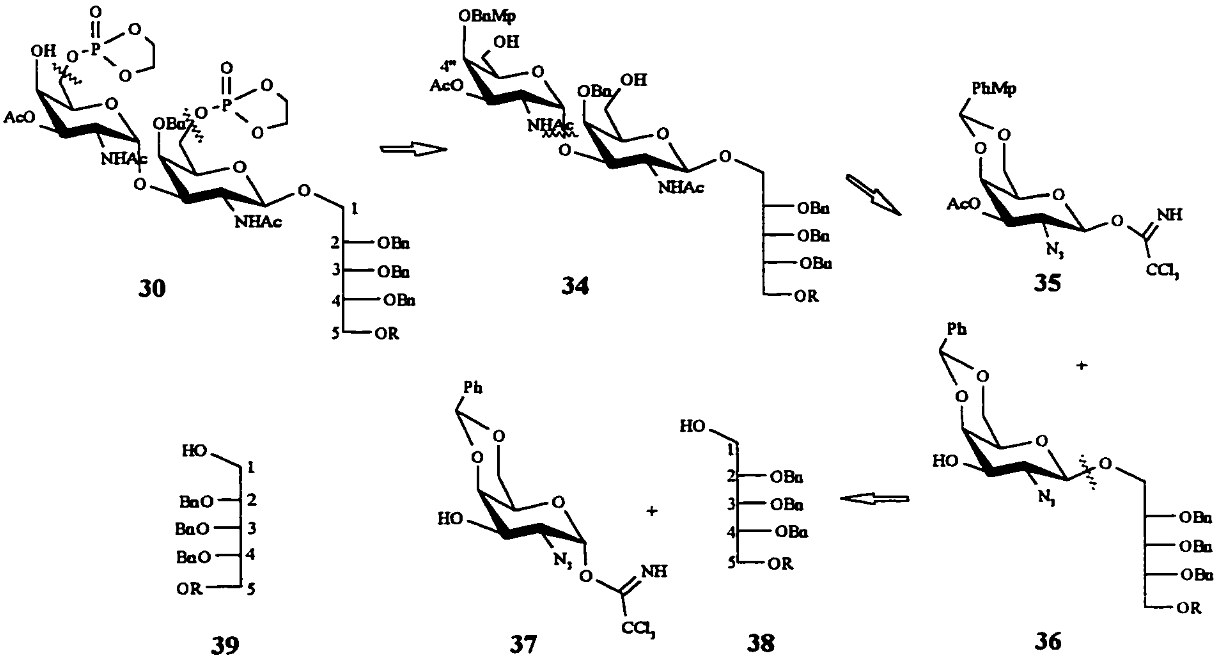


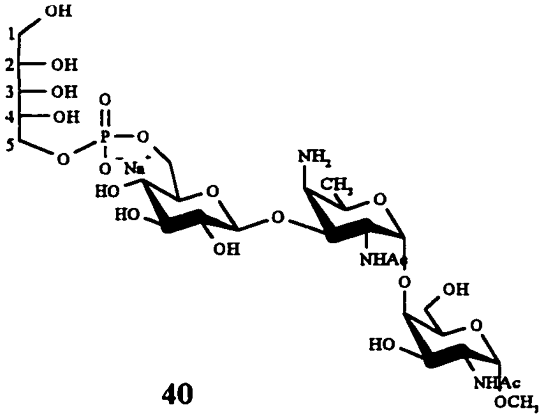
Figure 2.6 Retrosynthetic analysis (scheme 3)

As shown in Figure 2.6, disconnection of **30** removes the two dioxaphospholane rings first to give **34**. The 4"-hydroxyl group is protected with a *p*-methoxybenzyl group in order to be selectively deprotected later for further glycosylation. Further disconnection of the sugar residues one by one from **34** gave two glycosyl donors (**35** and **37**) and two glycosyl acceptors (**36** and **38**). The coupling between **35** and **36** is another α-linkage. The same strategy was proposed as before for the design of the glycosyl donor **35**. For the β-linkage between **37** and **38**, it was planned that the solvent participation strategy[9] would be used. The two primary hydroxyl groups were protected by *p*-methoxybenzylidene and benzylidene groups, respectively. Both rings would be opened selectively, leaving the remaining groups still protected. Benzyl was selected as the major protecting group, because it can be removed easily by hydrogenation. The C-5 of the ribitol residue was protected differently so that it could be deprotected selectively later to be used in the phosphorus linkage. The synthesis of the analogue of **27** containing the L-ribitol unit would follow the same procedure. Instead of D-ribitol derivative **38**, an L-ribitol derivative **39** could also be incorporated.

2.3 Results and discussion

The only uncertainty about the structure of the repeating unit of the C-polysaccharide of *Streptococcus pneumoniae* is the configuration of the ribitol unit. Unsubstituted ribitol is a *meso* compound with an internal plane of symmetry. When different substituents are put on the two ends, two enantiomers are obtained or two diastereomers if the substituents are chiral. The ribitol unit in the C-polysaccharide, a teichoic acid, is assumed to have the D-configuration based on the biosynthesis mechanism as shown in section 2.1.3.[152,153] Neither of the methods used for the structural determination of the C-polysaccharide, hydrolysis or NMR spectroscopy, gave any experimental evidence about this configuration.

It was reported that a tetrameric fragment (40) of the C-polysaccharide had been synthesized.[165] As shown in Figure 2.7, tetramer 40 was composed of residues of ribitol, D-glucopyranose, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose and 2-acetamido-2-deoxy-D-galactopyranose. The ribitol and the glucose were connected by a



40

Figure 2.7 The structure of the reported tetramer 40

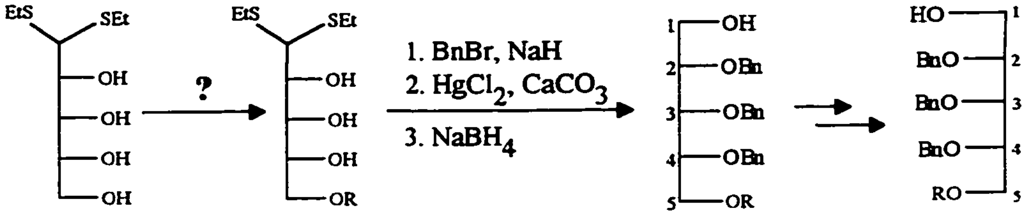
phosphodiester linkage. The other end of the ribitol unit was a free hydroxyl group. If the free end was defined as position-1, the ribitol unit was assumed to have the D-configuration in 40. However, the environment of the ribitol unit here was quite different from that of the repeating unit of the C-polysaccharide. No information could be retrieved from this study to prove the configuration of the ribitol unit in the natural product.

It was decided to determine the configuration of the ribitol unit in the C-polysaccharide through synthesis of oligosaccharide fragments of the repeating unit. These oligosaccharide fragments would incorporate ribitol units with both D- and L-configurations. It was expected that they would have different ^{1}H and ^{13}C NMR spectral data. Comparison of their ^{1}H and ^{13}C NMR data with that of the C-polysaccharide would allow the assignment of the configuration of the ribitol unit in the C-polysaccharide. The product that gave data that fit better would have the same configuration as that of the ribitol unit in the C-polysaccharide. Disaccharide and trisaccharide phosphates containing the ribitol units with both D- and L- configurations have been synthesized here using trichloroacetimidate methodology[6,7] as described below.

2.3.1 Synthesis of ribitol derivatives having both D- and L- configurations (38 and 39)

The synthesis of **38** and **39** started from D-ribose diethyl dithioacetal (**41**), for which the configuration was absolutely known. Compound **41** was prepared according to a literature procedure.[166] The workup procedure was made more environmentally friendly. Instead of using the lead carbonate, hydrogen sulfide, and silver carbonate procedure to remove the remaining acid, the reaction solution was neutralized by sodium bicarbonate first and then concentrated to dryness under vacuum. The product was taken up in ethyl acetate and filtered to remove the inorganic salts. These changes also improved the yield from 62%[166] to 75%.

As shown in Figure 2.8, for a short efficient synthetic procedure, the crucial step was the selective protection of position-5 of **41**. It had been reported that this position



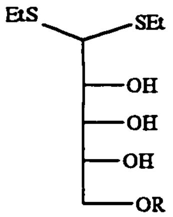
41 **38** **39**

Figure 2.8 Synthetic plan of ribitol derivatives 38 and 39

had been selectively protected with trityl[167,168] or acyl groups.[169] Neither approach was compatible with the following steps in the synthetic sequence adopted here. An effort was made to find a suitable protecting group at position-5 of **41**. Dibutylstannylene acetals and tributylstannyl ethers have been shown to work well in selective acylation and alkylation reactions. Both methods were tried here. Benzylation of **41** via a dibutylstannylene acetal was carried out in the presence of tetrabutylammonium iodide. However, decomposition of the diethyl dithioacetal was faster than benzylation. Cesium fluoride has been shown to be a very promising reagent that enhances the rates of reactions of dibutylstannylene acetals.[170,171] Therefore, it was used here instead of tetrabutylammonium iodide. Several reactions were carried out. Alkylation did occur and the reaction was fast. However, the yields were low. Besides the major compound, there were always some other unidentified compounds in the product mixture. Tributylstannyl ethers are well known to be effective in directing electrophiles to primary centres. However, this ether did not show any advantage over the dibutylstannylene acetal method for this particular molecule.

Silyl ethers have been shown to be useful protecting groups in many organic syntheses.[172,173] Regioselective silylation via stannylene acetal intermediates had been successfully performed on a secondary *cis*-diol[174] and several diols and a triol with primary hydroxyl groups.[175] Silylation occurred selectively on the equatorial and primary hydroxyl groups, respectively. It was interesting to notice that when the substrate was butane-1,2,4-triol, only the 4-*O*-silyl product was obtained. In all the cases, *t*-butyldimethylsilyl chloride was used as the silylating reagent. Silylation of **41** via its dibutylstannylene acetal intermediate was attempted here. Compound **41** was reacted with *t*-butyldiphenylsilyl chloride via its dibutylstannylene acetal intermediate in DMF. The reaction was complete within 1 h and only one product was obtained. The NMR spectra showed that it was the desired compound. It was surprising to observe that **41** did not react with *t*-butyldiphenylsilyl chloride in the presence of imidazole, a normal procedure for the preparation of silyl ethers. Next **41** was reacted with *t*-butyldimethylsilyl chloride, a cheaper silylating reagent, via the same procedure. Compound **46** was obtained in a 97% yield. The substitution results of **41** are summarized in Table 2.2. Obviously, **46** was the

Table 2.2 The selective substitution results of 41

Structure	R	Products	Yields (%)
	All	**42**	68
	Bn	**43**	15
	pMBn	**44**	48
	TBDPS	**45**	96
	TBDMS	**46**	97

best choice.

Next was benzylation of the remaining hydroxyl groups. It had been thought that the benzylation of **46** would be a straightforward reaction, but it turned out to be dominated by side reactions. No desired benzylation product was obtained. The *t*-butyldimethylsilyl group was unfortunately not stable in the presence of sodium hydride, which was in agreement with a report that silyl groups were removed by sodium hydride.[176] It had been reported that for the synthesis of the tribenzyl derivative of **45**, four steps were involved using the same starting material **41**, which included tritylation, benzylation, detritylation and silylation.[167] It was hoped to have a shorter and efficient synthetic procedure. The *t*-butyldiphenylsilyl group could have been tried, because it is more stable than the *t*-butyldimethylsilyl group.[172] However, the *t*-butyldiphenylsilyl group is more expensive and it is not wise to start with costly materials for a long synthesis. It was decided to use the allyl group, which proved compatible in the subsequent procedures. In this case, only two steps were required for the synthesis of the fully and selectively protected D-ribose diethyl dithioacetal derivative. An alternative synthesis of

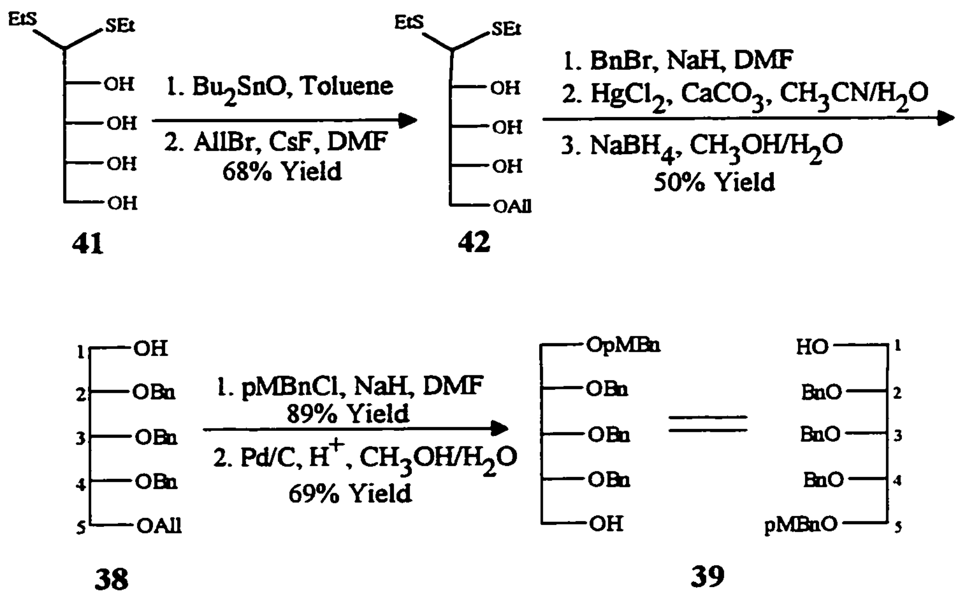


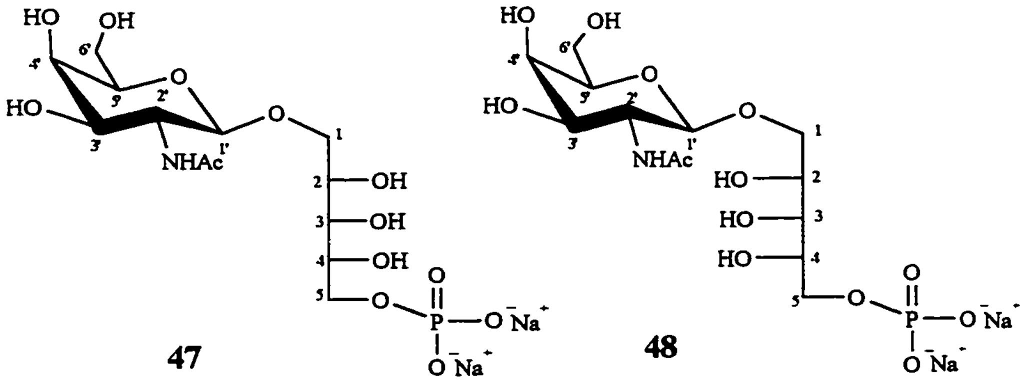
Figure 2.9 Synthesis of the ribitol derivatives 38 and 39

42 had been reported, which involved three chemical transformations, using methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside as the starting material,[177] which itself is obtained in two steps in the same pot from D-ribose.[178] By comparison, the synthetic sequence adopted here is shorter and more efficient. In addition, this is the first time that dialkyldithioacetal derivatives have been selectively protected with an alkyl group at the primary position via a dibutylstannylene acetal intermediate in a single step.

As shown in Figure 2.9, following benzylation of the selectively allylated 42, the diethyl dithioacetal group was removed with mercury salts. After reduction with sodium borohydride, the desired ribitol derivative 38 was obtained. If the free hydroxymethyl group of the ribitol derivative is defined as position-1, 38 is a D-ribitol derivative. Following *p*-methoxybenzylation of the free hydroxy group and deallylation of the other end of **38**, L-ribitol derivative **39** was obtained, following the same definition. In the deallylation step, control of the temperature was found to be crucial. At room temperature, the reaction was very slow, as followed by TLC. Above 70 °C, the *p*-methoxybenzyl (pMBn) group was also removed. It was found that at 50-60 °C, deallylation was complete in 10 h with 69% isolated yield and only a trace amount of the product due to the removal of the pMBn group was formed.

2.3.2 Synthetic studies of disaccharide phosphates containing ribitol units having both D- and L- configurations

In order to see the effect of the configuration of the ribitol unit on the ^{13}C NMR chemical shifts, two initial target disaccharide phosphates (**47** and **48**) were designed and synthesized. As shown in Figure 2.10, the two compounds are only different in the configuration of their ribitol units; compound **47** is a D-ribitol derivative and **48** an L-ribitol derivative. The synthesis is described in the following sections.



47

48

Figure 2.10 The structures of the target disaccharide phosphates 47 and 48

2.3.2.1 Retrosynthetic analysis of disaccharide phosphate (47)

As shown in Figure 2.11, retrosynthetic analysis of 47 led to fully protected disaccharide phosphate 49. Only one type of protecting group was present, benzyl ether, and all of these would be removed in a single step. Further cleavage would afford the glycosyl donor 50, glycosyl acceptor 38, and phosphitylating reagent 51. There were

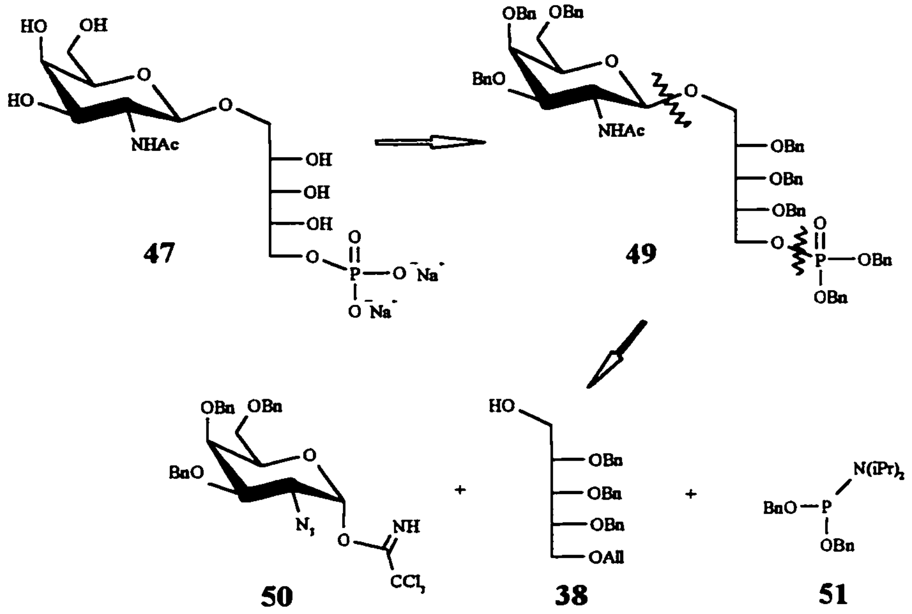


Figure 2.11 Retrosynthetic analysis of 47

two reasons for the selection of 50 as the glycosyl donor. Firstly, α-trichloroacetimidates usually give high stereoselectivity in glycosylation reactions in acetonitrile due to solvent participation as discussed in section 1.2.3. Secondly, galactosamine was considered to be prohibitively costly. 2-Azido-2-deoxy-D-galactopyranose derivative can be obtained from D-galactal in reasonable yield.[179] Compound 51 could easily be prepared from phosphorus trichloride.[180,181] Synthesis of **48** would follow the same procedure, except that **39** would be incorporated as the glycosyl acceptor.

2.3.2.2 Synthesis of disaccharide phosphates containing ribitol units having both D- and L- configurations (47 and 48)

Starting from D-galactose, glycosyl donor **50** was synthesized in eight steps following a literature procedure.[179] Besides **50**, its β-isomer **50β** was also obtained. It was noticed that the β-isomer was more stable than the α-isomer. The β-isomer was exposed to air for one week at room temperature and did not change as shown by TLC and NMR spectra. However, **50** decomposed overnight under the same conditions as shown by TLC.

As shown in Figure 2.12, coupling of **50** with **38** afforded **52**. Due to the solvent participation of acetonitrile as discussed previously, the β-linkage was obtained selectively. The large coupling constant of $J_{1',2'}$ (7.87 Hz) unambiguously confirmed this β-linkage. No α-disaccharide was observed. This glycosylation reaction of **38** was first attempted using silver triflate as activator, following the method reported by Krepinsky and coworkers.[182] The reaction was performed at room temperature for 30 h and was still not complete. Not surprisingly, a relatively low yield was obtained (47%). A more efficient activator was needed. TMS triflate was the next choice.

The glycosylation reaction using TMS triflate as catalyst was tried first using

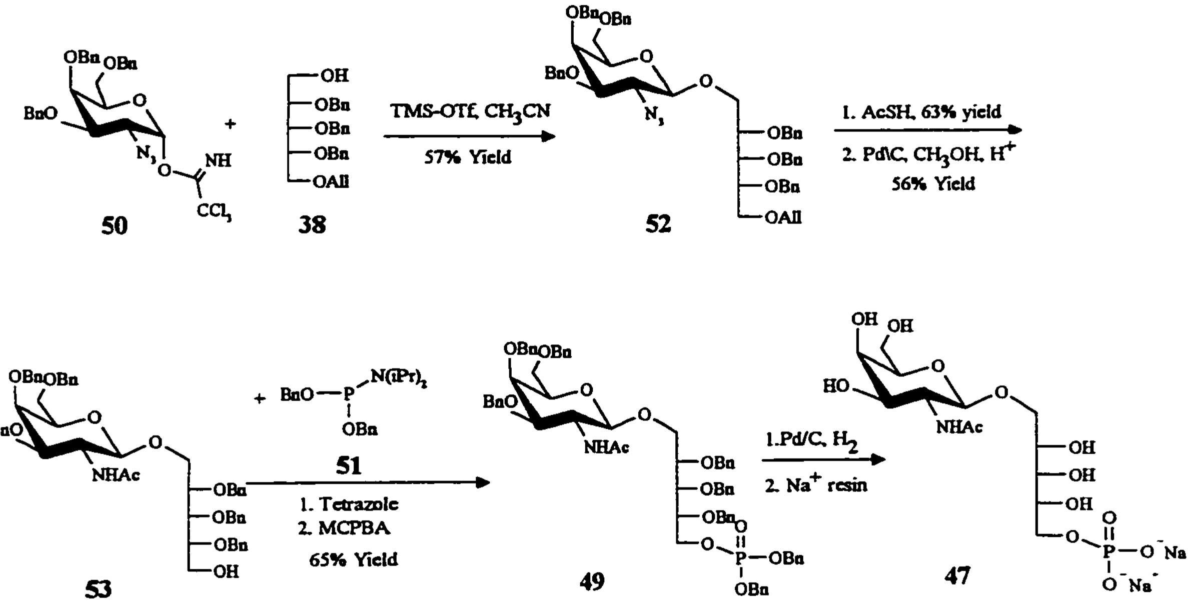


Figure 2.12 Synthesis of the target disaccharide phosphate 47

ethanol as a test glycosyl acceptor and the ethyl β-D-glycoside (54) was formed in 55% yield. When 38 was used instead of ethanol as the glycosyl acceptor, disaccharide 52 was obtained in 57% yield as shown in Figure 2.12. It was also found that coupling of the anomeric mixture (50 and 50β) with 38 in acetonitrile also gave the β-linkage with high stereoselectivity in 58% yield. Products containing an α-linkage were not observed. It was concluded that it was not necessary to separate the anomeric mixture of the trichloroacetimidates, which could just be used directly. The reaction with TMS triflate as catalyst was completed within 1 h at -40 °C and it gave higher yield (57% vs. 43%) in comparison with the procedure using silver triflate as catalyst. Thus, reaction under the second conditions was more efficient.

Higher yields than that obtained here have been reported for glycosylations using trichloroacetimidate.[183] It was thought that the relative low yield here might be due to possible moisture in the system, although caution was taken to exclude moisture.

As planned in section 2.2, the azido group of **52** was changed to an acetamido group in one step by treatment with thiolacetic acid at room temperature. The appearance of NH (δ 5.32) and CH_3CO (δ 2.18) signals in the ^1H NMR spectrum confirmed the success of the reaction. The chemical shift of C-2' changed from δ 63.44 to δ 54.70.

The allyl group was removed by stirring in methanol in the presence of Pd/C and toluenesulfonic acid at 60 °C. This was confirmed by the disappearance of the allyl group signals at δ 72.15 (CH_2), 116.7 (CH_2=), and 134.9 (CH=), respectively in the ^{13}C NMR spectrum. The chemical shift of the C-5 changed from δ 70.27 to δ 61.58 due to the removal of the substituent effect of the allyl group. In the ^1H NMR spectrum, a triplet appeared at δ 2.51, assigned to OH-5, confirming that a primary *O* had been deprotected.

Next, dibenzyl phosphate ester **49** was synthesized. The phosphitylating reagent **51** was prepared from phosphorus trichloride in two steps following literature procedures.[180,181] The reaction of **53** with **51** was performed at room temperature in the presence of tetrazole, followed by oxidation of the phosphite to a phosphate with *m*-chloroperbenzoic acid (MCPBA) at 0 °C. The two steps afforded a 65% yield. The choice of benzyl as the protecting group for the phosphitylating reagent allowed removal of all the protecting groups in a single step. The success of the phosphorylation reaction was indicated by the one single peak at δ -0.09 in the ^{31}P NMR spectrum of **49** and splitting of the C-5 ($J_{5,P}$ = 8.6 Hz) signal in its ^{13}C NMR spectrum. In addition, in comparison with the ^{13}C NMR spectrum of **53**, two more CH_2 signals (δ 68.9) and two more phenyl quaternary carbon signals (δ 135.4, 135.6) appeared in the ^{13}C NMR spectrum of 49 due to the presence of the two benzyl groups on phosphorus.

The fully benzylated disaccharide phosphate ester 49 was deprotected in one step by hydrogenation. The disappearance of the aromatic proton and carbon signals in the ^{1}H and ^{13}C NMR spectra, respectively, of 47a, the hydrogenation product, confirmed the success of the reaction. Following treatment with Amberlite IR-120 (Na^{+}) resin, the target disaccharide phosphate 47 was obtained quantitatively. The ESI-MS of 47 showed signals for [M-H]$^{-}$, [M-Na]$^{-}$, and [M-2Na+H]$^{-}$, respectively.

As shown in Figure 2.13, L-ribitol derivative 48 was synthesized in a similar way. Firstly, reaction of 50 and 39 in the presence of TMS triflate from a newly opened bottle

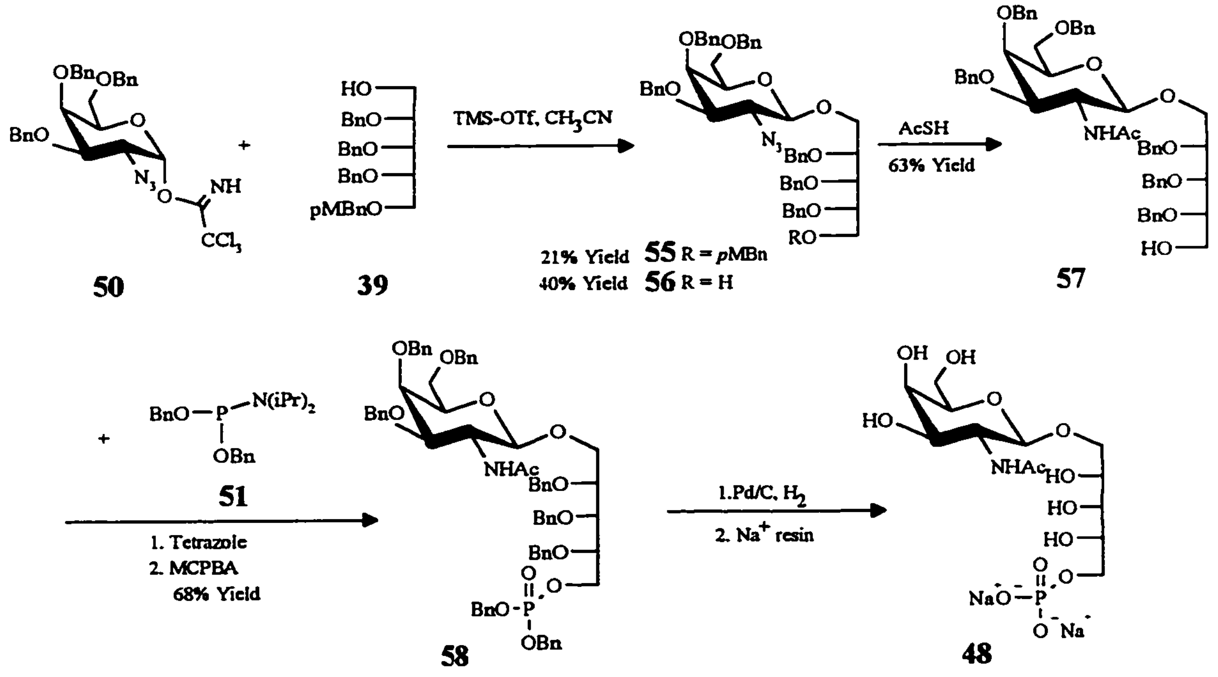


Figure 2.13 Synthesis of the target disaccharide phosphate 48

afforded the desired disaccharide **55** in a 65% yield. However, **55** was not stable. Another spot was observed on TLC after **55** had been kept at 5 °C for three weeks. The same glycosylation reaction using TMS triflate that had been kept at 5 °C for eight months afforded two products **55** and **56**. The yields were shown in Figure 2.13. The *p*-methoxybenzyl (pMBn) group was partially lost during the glycosylation. Luckily, it seemed that the glycosylation occurred faster than the removal of the pMBn group, because the ^{13}C NMR of **56** only had one set of signals, showing that it was not a mixture of diasteromers. It has been reported that both primary and secondary pMBn group can be removed by 10% of trifluoroacetic acid in dichloromethane at room temperature within 5 min.[184] It was thought that acidic species like triflic acid formed after the glycosylation step may be responsible for the removal of the pMBn group.

It was interesting to note that treatment of **56** with AcSH successfully afforded the acetamido product **57**. The primary hydroxyl group was left untouched. The subsequent procedures were the same as that of the formation of the disaccharide phosphate **47**. The target disaccharide **48** was synthesized successfully.

2.3.2.3 NMR analysis

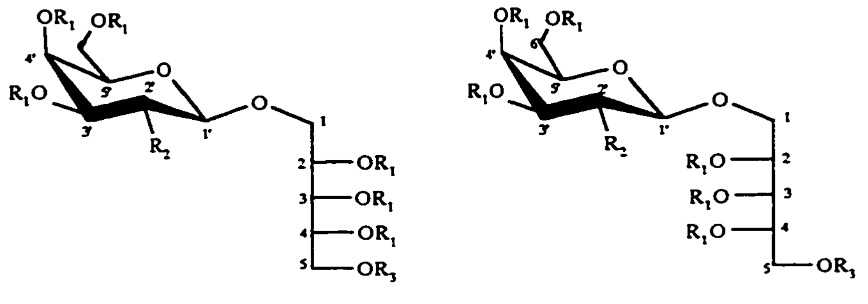
For the convenience of comparison, the structures of the disaccharides synthesized are shown in Figure 2.14. Their ^{13}C NMR spectra were analysed by means of JMOD, COSY, HMQC, HMBC, and TOCSY experiments. As shown in Table 2.3, there were significant differences between the two sets of ^{13}C NMR data for the two series of compounds. For **52, 55** and **53, 57**, the largest chemical shift differences ($\Delta\delta$) were for

Table 2.3 ^{13}C NMR Data of Disaccharides synthesized and PnC[137]

Cpd	C1	C2*	C3*	C4*	C5	C1'	C2'	C3'	C4'	C5'	C6'
52	**68.71**	*78.20*	*78.39*	78.25	70.02	102.0	63.44	**81.43**	72.02	73.21	68.11
55	**70.18**	*78.24*	*78.69*	*78.82*	69.43	102.4	63.44	**80.66**	72.03	73.21	68.12
53	**67.64**	77.52	79.05	78.77	61.58	100.2	54.77	77.94	72.28	73.15	68.45
57	**69.09**	*78.27*	*78.99*	*78.65*	60.99	99.97	54.32	78.02	72.24	73.01	68.24
49	68.93	*77.24*	*77.71*	**77.79**	67.33	100.1	54.18	**78.03**	72.22	72.91	68.30
58	68.92	*77.50*	*77.80*	**78.89**	66.69	99.99	54.24	**78.65**	72.21	72.95	68.19
47a	**71.27**	71.31	71.84	**71.55**	66.95	102.6	53.30	75.94	68.66	72.24	61.84
48a	**71.74**	71.33	71.74	**72.93**	67.26	103.0	53.28	75.92	68.62	72.37	61.84
47	**71.31**	71.27	71.77	**71.55**	66.75	**102.6**	53.24	75.91	68.61	72.24	61.80
Δδ	-0.15	-0.08	-0.46	+0.09	-0.71	+0.5					
48	**71.72**	71.34	71.72	**72.92**	66.93	**103.0**	53.27	75.92	68.63	72.44	61.84
Δδ	+0.26	-0.01	-0.51	+1.46	-0.53	+0.9					
PnC	**71.46**	71.35	72.23	**71.46**	67.46	**102.1**	51.46	75.49	64.07	74.30	65.33

* The data in italic face are assigned tentatively. The data in bold face are the places where the significant difference can be found.

the C-1s of the ribitol units, with 1.47 ppm for **52**, **55** and 1.45 ppm for **53**, **57**, respectively. When the C-5 was phosphorylated, the Δδ for C-1s became smaller, with 0.01 and 0.41 ppm for **49**, **58** and **47**, **48**, respectively, while the Δδ for C-4s became larger, with 1.10 and 1.37 ppm for **49**, **58** and **47**, **48**, respectively. The chemical transformation from the phosphoric acid derivative to the disodium phosphate did not influence the ^{13}C NMR chemical shifts of the carbohydrate parts of the compounds much, which was obvious by comparing the ^{13}C NMR spectral data of **47**, **47a** and **48** and **48a**,



Cpd	R_1	R_2	R_3	Cpd
52	Bn	N_3	All	**55** (R_3 = *p*MBn)
53	Bn	NHAc	H	**57**
49	Bn	NHAc	$PO(OBn)_2$	**58**
47a	H	NHAc	$PO(OH)_2$	**48a**
47	H	NHAc	$PO(ONa)_2$	**48**

Figure 2.14 The structures of the synthesized disaccharides 47-49, 52, 53, 55, 57, 58

respectively. All the chemical shifts except that for the C-5 remained the same. The slight change at C-5 was due to the change in the electron density on phosphorus on changing from phosphoric acid to its sodium salts.

2.3.2.4 Preliminary conclusion

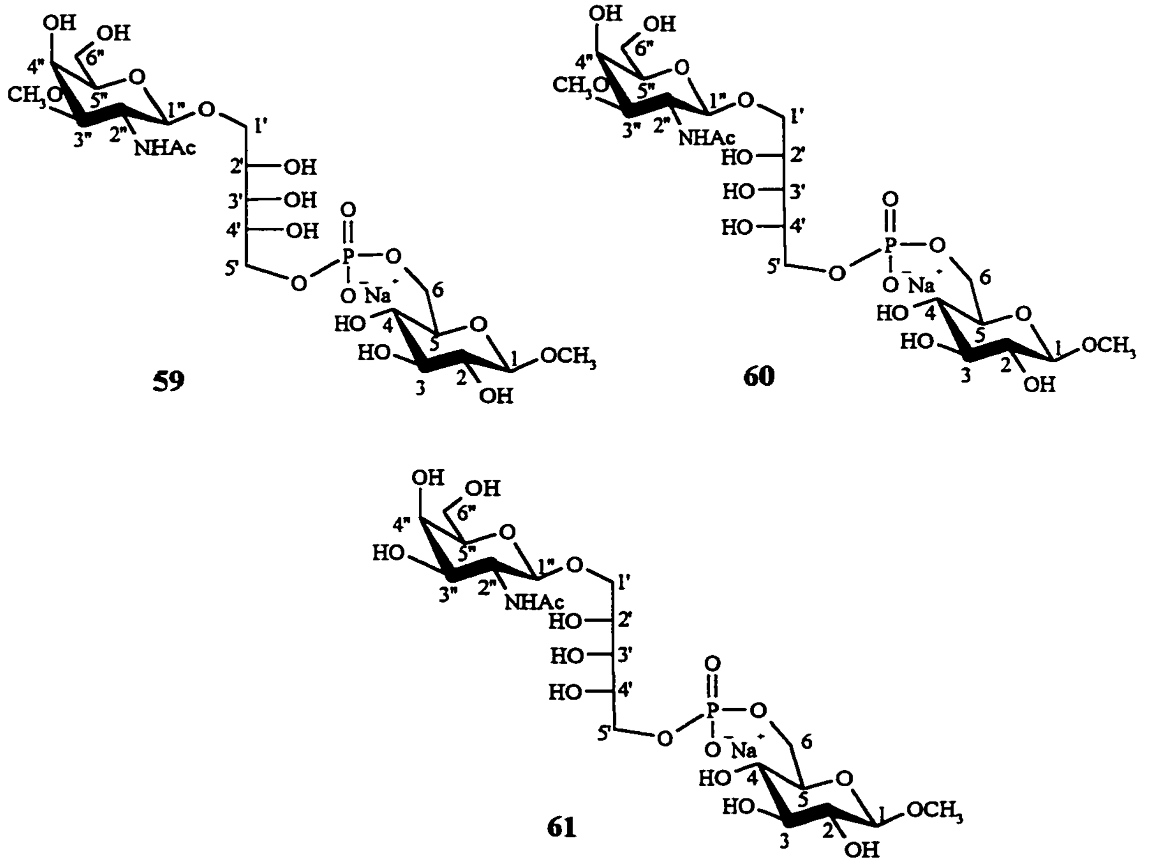
Using trichloroacetimidate methodology, disaccharide phosphates containing both D- and L-ribitol units were synthesized in reasonable yields. The synthetic procedure developed here laid a basis for the more extended synthesis of more complicated oligosaccharides that will be described in the next sections.

Significant differences of the chemical shifts in the ribitol unit between the D- and L-series of disaccharides synthesized were observed. The chemical shifts in the galactose unit were almost the same except C-1', which had a Δδ of 0.4 ppm. Comparison of the ^{13}C NMR data of **47**, **48**, the target disaccharide phosphates, with that of the corresponding part of the C-polysaccharide (**PnC**) showed that **48**, the L-ribitol derivative, had a Δδ of 0.9 ppm in C-1', 0.26 ppm in C-1, and 1.46 ppm in C-4, respectively. The average absolute deviation of the chemical shifts of ribitol unit part from the corresponding part of **PnC** was 0.55 ppm. The D-ribitol derivative, **47**, had a Δδ of 0.5 ppm in C-1', -0.15 ppm in C-1, and 0.09 ppm in C-4, respectively and the average deviation was 0.30 ppm. Although the differences were not large, it was obvious that the ^{13}C NMR spectral data of **47** was closer to that of the **PnC**. Therefore, the configuration of the ribitol unit in the **PnC** was tentatively assigned as D. By comparison of the ^{13}C NMR spectral data with that of the **PnC**, it was also observed that there were some significant differences in the galactose part and in the C-5s of ribitol. To better simulate the environment of the ribitol unit in the C-polysaccharide, it was decided to synthesize trisaccharide fragment of the repeating unit of the **PnC** containing ribitol unit having both D- and L- configurations (**59-61**) in the next step. It was hoped that the above observed differences would persist, leading to a more certain assignment. The synthesis of **59-61** and their NMR data will be presented in the following sections.

2.3.3 Synthetic studies of trisaccharide phosphates containing ribitol units having both D- and L- configurations

2.3.3.1 Synthetic analysis

To better simulate the environment of the ribitol unit in the repeating unit of the C-polysaccharide, three trisaccharide phosphate target molecules were designed. Their structures were shown in Figure 2.15. In 59 and 60, instead of linking to another-2-acetamido-2-deoxy galactopyranose residue as in the C-polysaccharide, the 3" position was substituted with a methyl group, which should partially make up for the substitution



59

60

61

Figure 2.15 The structures of the target trisaccharide phosphates 59-61 effect at the 3" position. Compound **61** was also targeted to be synthesized in order to see if a substituent at the 3" position caused differences. In order to observe the substitution effects of the phosphate on the chemical shifts of C-5', a phosphodiester was designed. As in the natural product, where the *O*-5 of the ribitol unit was connected to *O*-6 of a β-glucopyranose residue through a phosphodiester linkage, methyl β-D-glucopyranoside was similarly linked at *O*-5 of the ribitol unit.

The synthesis of the three trisaccharide phosphates could have followed the same procedure as in the synthesis of the two disaccharide phosphates **47** and **48**. However, due to the specific substitution at the 3"-position, the protecting groups at the other

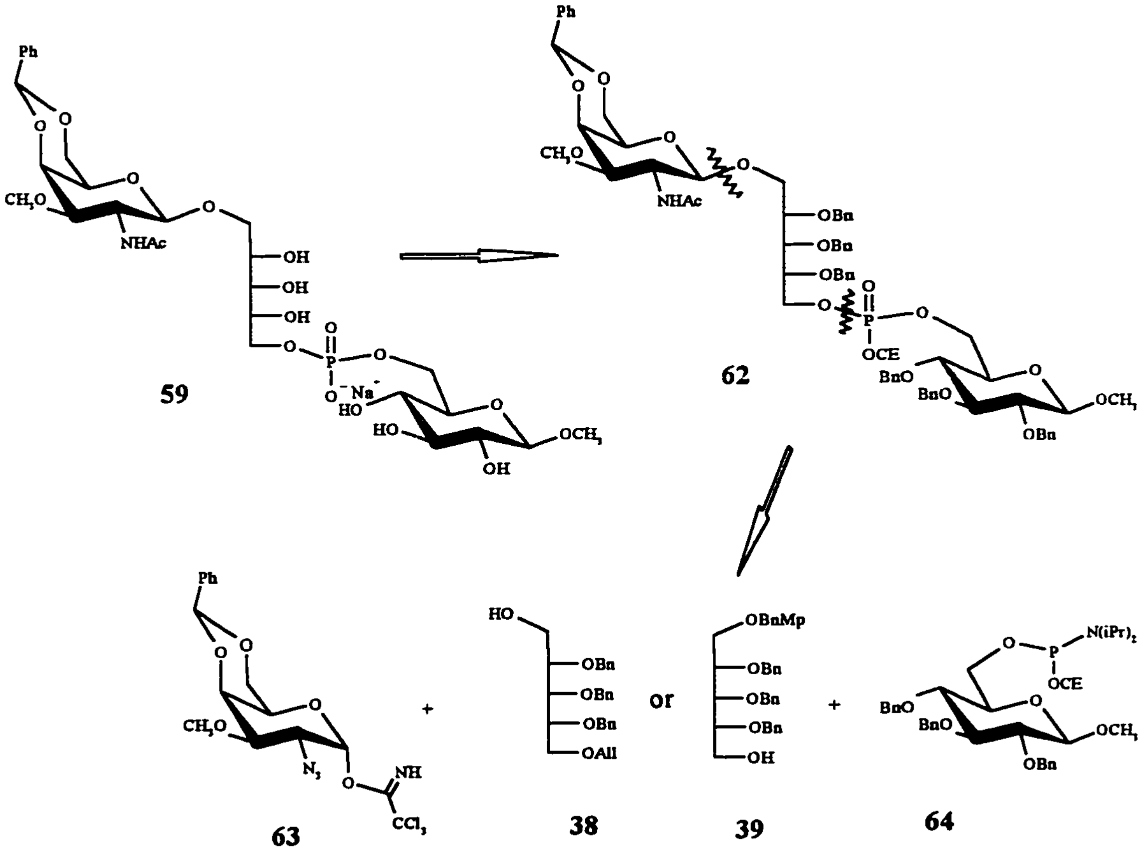


Figure 2.16 Retrosynthetic analysis of the target trisaccharide phosphate 59

positions of the glycosyl donor had to be different. As planned in section 2.2, a similar glycosyl donor was chosen. As shown in Figure 2.16, retrosynthetic analysis of **59** led to the glycosyl donor **63**, D-ribitol derivative **38** or L-ribitol derivative **39**, and methyl β-D-glucopyranoside phosphitylating reagent **64**. The benzyl group was again chosen as the protecting group for **64**. Thus, all protecting groups could be removed in one step.

If **59** was synthesized in the same way as **47** by linking **63** and **38** first, then with **64**, it was uncertain whether the benzylidene ring in the glycosyl donor **63** would survive the following reaction conditions: AcSH, or acidic conditions at elevated temperature needed for the removal of allyl group. If it did not, the whole synthetic procedure would be invalidated. It was hoped that this dilemma could be avoided by reversing the order of glycoside and phosphate formation, that is by linking the ribitol unit **39** and **64** with a phosphodiester linkage first and then coupling the just formed disaccharide phosphate with **63**. For the synthesis of **60**, similarly there were two choices: glycosylation of **39** with **63** first, then linking with **64**, or linking of **38** and **64** first, then performing glycosylation with **63**. However, for the latter strategy, besides the formation of the phosphate triester linkage between **38** and **64**, the allyl double bond in **38** would also be oxidized by *m*-chloroperbenzoic acid (MCPBA), which would make the removal of the allyl group impossible. It seemed that **38** was not a reasonable choice for the synthesis of both **59** and **60**. For the synthesis of **59**, the removal of the allyl group would endanger the benzylidene ring. For the synthesis of **60**, the allyl group could not be removed under normal conditions once it had been oxidized. It was decided that **39** could be used as middle combinant in the synthesis of both **59** and **60**. As shown in Figure 2.17,

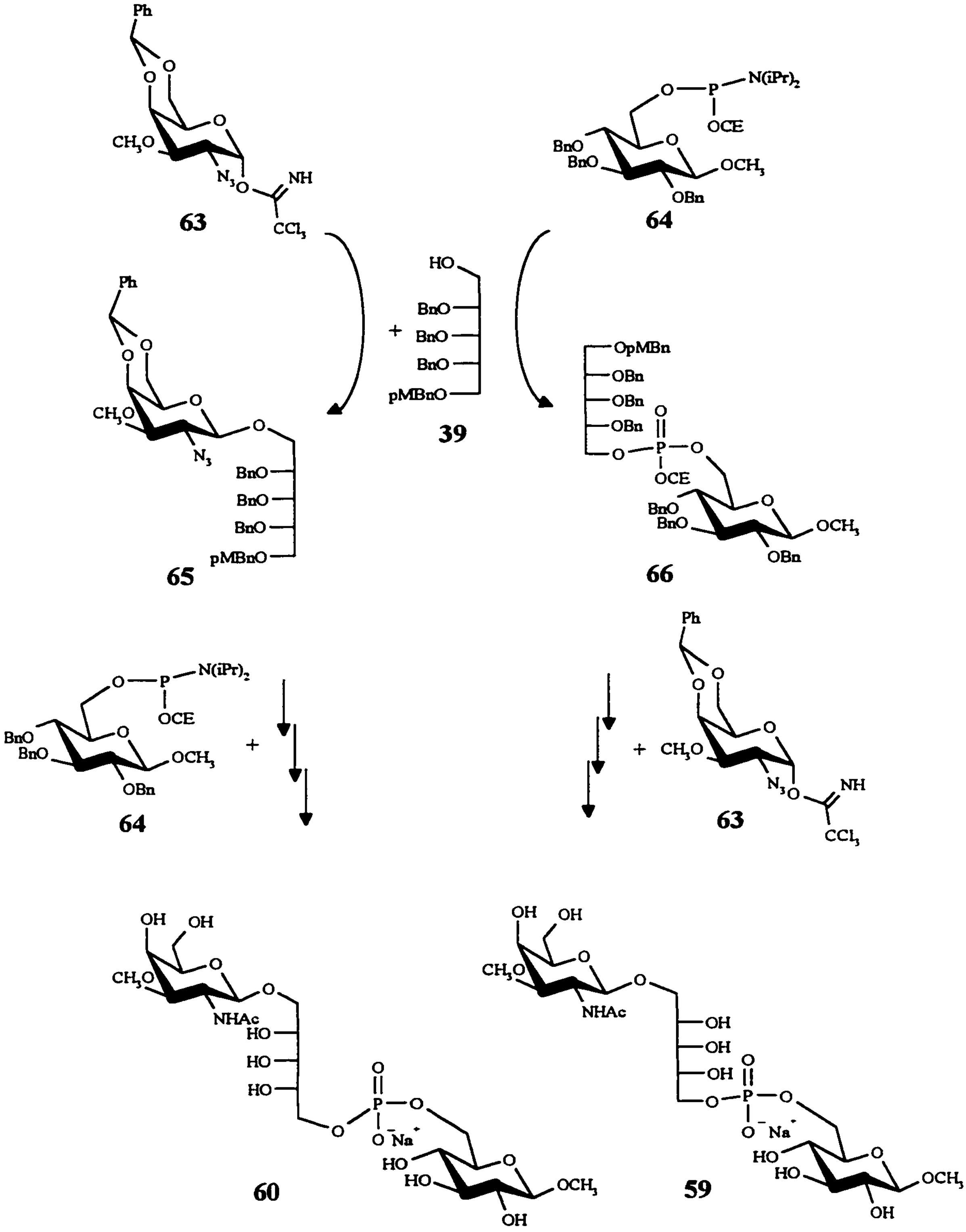


Figure 2.17 Synthetic analysis of the target trisaccharide phosphates 59 and 60

in the approach shown on the left, a trisaccharide phosphate containing a ribitol unit with an L-configuration would be obtained; while in the approach shown on the right, a trisaccharide phosphate containing a ribitol unit with a D-configuration would be obtained.

Target trisaccharide phosphate **61** was planned to be synthesized by coupling **57** with **64** first, then following the same procedure as that of the synthesis of **60**.

2.3.3.2 Preparation of glycosyl donor 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl trichloroacetimidate (63)

(*t*-Butyldimethyl)silyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**67**) was an important intermediate in the synthetic sequence. As a precursor of **37** in the retrosynthetic analysis in section 2.2, it would act as a glycosyl acceptor as in the preparation of disaccharide **68** shown in Figure 2.18. The anomeric effect caused the reaction to proceed selectively to give an α : β ratio of about 150 : 1 based on the ^{1}H NMR spectrum of **68**. Compound **68** could further be transformed to a glycosyl donor in the same procedure as the formation of **63**, thus, be used as a disaccharide glycosyl donor in further block oligosaccharide synthesis.

Compound **67** could also be transformed easily into a glycosyl donor **63**. The anomeric (*t*-butyldimethyl)silyl group turned out to be stable in the presence of sodium hydride. The position-3 of **67** was alkylated successfully with both benzyl bromide and methyl iodide, a temporary and a permanent protecting group to give **69** and **70**, respectively.

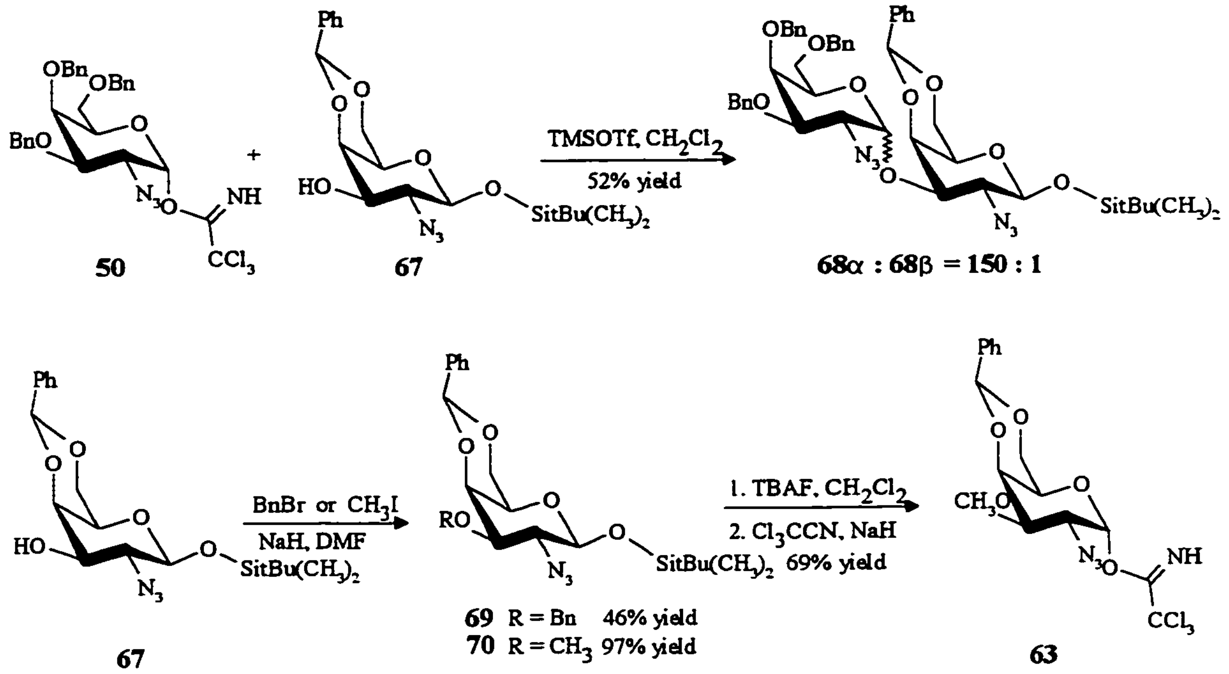


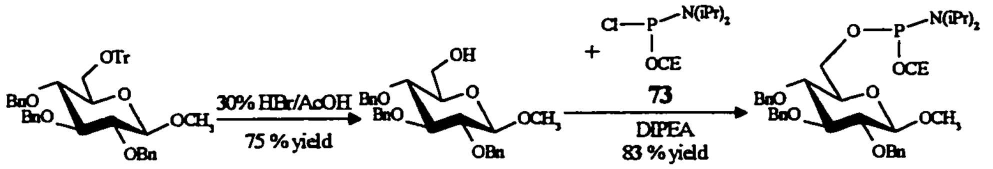
Figure 2.18 Reactions with 67 and synthesis of 63

The choice of solvent played a crucial role in desilylation reaction. The reaction was faster in THF. For a slow reaction, THF was a better choice, for example for the removal of the *t*-butyldimethylsilyl group of the methyl 2,3,4-tri-O-benzoyl-6-*O*-*t*-butyldimethylsilyl-β-D-glucopyranoside. But when the substrate was active as in the case here, THF was not appropriate. When 70 was treated with TBAF in THF, not only the silyl group but also the methyl group at position-3 was removed, as indicated by the disappearance of the methoxy carbon signal at δ 57 in the ^{13}C NMR spectrum of the product. No desired product was obtained. It had been reported by Schmidt[179] that the anomeric silyl group had been removed with TBAF at room temperature in dichloromethane. Following the same procedure, as shown in Figure 2.18, treatment of

70 with TBAF in dichloromethane successfully afforded the desired desilylation product. Subsequent reaction with trichloroacetonitrile in the presence of sodium hydride, a strong base, afforded the α-trichloroacetimidate **63** selectively in a 69% yield for the two steps.

2.3.3.3 Synthesis of (methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidite (64)

Following a literature procedure,[185] methyl 2, 3, 4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside (**71**) was prepared from methyl β-D-glucopyranoside[186] in two steps. Detritylation of **71** can be done either with 80% acetic acid at reflux[185] or with HBr in acetic acid at 0 °C.[187] The second method proceeded instantaneously and the first took 1 h. For the first method, it was very tedious to remove the trityl alcohol formed, which crystallized readily but not completely. It took many repetitions of the crystallization-filtration cycle to remove the trityl alcohol completely. In this regard, performing detritylation with HBr/AcOH was advantageous. The trityl group was detached as a trityl bromide precipitate that was removed conveniently and completely by immediate filtration. It had been reported that detritylation with 40% HBr/AcOH afforded a 61.2% yield.[187] As shown in Figure 2.19, with this same method using 30% HBr/AcOH, the yield was



71 **72** **64**

Figure 2.19 Synthesis of the phosphitylating reagent 64

improved to 75% here.

Compound **73** was prepared from phosphorus trichloride following a literature procedure.[188] Reaction of **72** with **73** in the presence of diisopropylethylamine (DIPEA) afforded **64** in 83% yield.

2.3.3.4 Synthesis of trisaccharide phosphate containing ribitol unit with D-configuration (59)

As explained in the section 2.3.3.1, trisaccharide phosphate **59** was synthesized by glycosylation of a phosphorus linked disaccharide **74** with trichloroacetimidate **63**. As shown in Figure 2.20, the synthesis of the disaccharide glycosyl acceptor (**74**) was fulfilled by phosphitylation of L-ribitol derivative **39** with **64**, followed by oxidation with *m*-chloroperbenzoic acid (MCPBA) in the same pot. The *p*-methoxybenzyl group survived both acidic conditions provided by tetrazole and MCPBA, and was removed easily with 2,3-dichloro-4,5-dicyano-quinone (DDQ). The two steps afforded a 57% yield. The formation of the phosphorous linkage was clearly evidenced by the coupling of the phosphorus to the C-4 (doublet) and C-5 (doublet) of the ribitol unit and the disappearance of isopropyl group signals (δ 24, 43) in the ^{13}C NMR spectrum. Compound **74** was a mixture of epimers at phosphorus as indicated by the two signals (δ -0.72, -0.77) in the ^{31}P NMR spectrum.

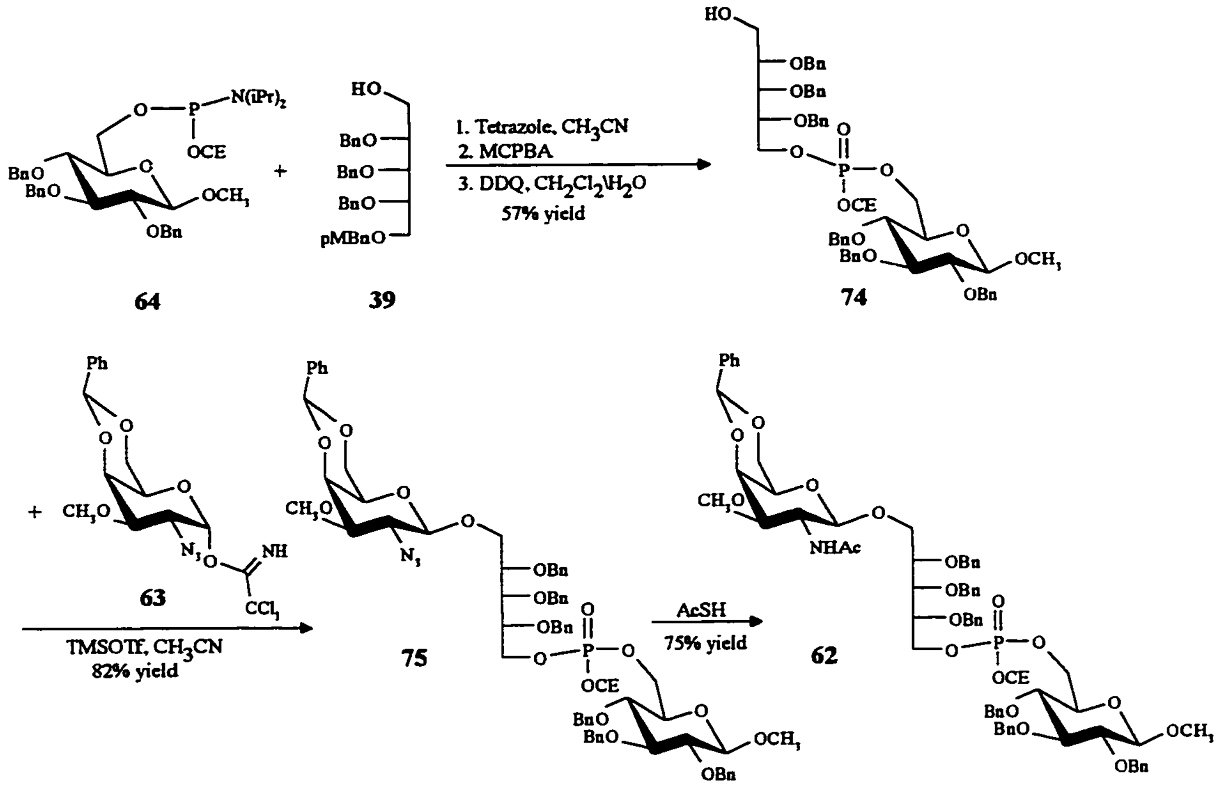


Figure 2.20 Synthesis of the fully protected trisaccharide 62

Glycosylation of the disaccharide glycosyl acceptor **74** by **63** was successfully performed in an 82% yield. Again, solvent participation by acetonitrile caused the β-linkage to be formed selectively, which was clearly shown by the observation of a large vicinal coupling constant between H-1 and H-2 of 7.8 Hz. No products having an α-linkage were observed. This observation showed promise that this disaccharide glycosyl acceptor could be successfully linked with disaccharide or higher oligosaccharide glycosyl donor in a block oligosaccharide synthesis in high yield. Extra caution was taken to avoid moisture in this reaction system: the TMS triflate used was newly distilled and was introduced into the reaction system through rubber septa with a syringe; the acetonitrile was refluxed with calcium hydride for 24 h; the glassware used was kept in an oven for a week and then cooled in a desiccator. Comparison with the previous glycosylation reaction using a similar trichloroacetimidate showed that the extra care was worthwhile; the yield was improved greatly from about 60% to 82%.

The next step was the transformation of the azido group to the acetamido group. As shown in Figure 2.20, the benzylidene ring surprisingly survived overnight treatment with thiolacetic acid at room temperature and gave the reduction product in 75% yield.

Due to the stereogenic centre at phosphorus, **64**, **74** and **75** are diastereomeric mixtures, as shown by their ^{31}P NMR spectra, each of which contained two peaks. Surprisingly, treatment of **75** with thiolacetic acid brought another pair of peaks. In the ^{31}P NMR spectrum of **62**, there are two large peaks (δ -0.68, -0.96) and two small peaks (δ 0.17, -0.73). A possible explanation might be hydrogen bond formation between the acetamido proton and the oxygen on phosphorus. This hydrogen bond restricts the inversion of the acetamido group, which may result in the appearance of the other pair of peaks in the ^{31}P NMR spectrum. The same phenomenon was observed for the L-derivatives **79** and **81**; their synthesis will be described in the following sections.

As shown in Figure 2.21, the cyanoethyl group on phosphorus was removed by treatment with triethylamine, pyridine and water mixture in 2 h at room temperature. The product had only a single peak in its ^{31}P NMR spectrum at δ 1.41. The disappearance of the CN (δ 116) and two CH_2 (δ 19, 61) signals from the ^{13}C NMR spectrum confirmed the removal of cyanoethyl (CE) group. Hydrogenation removed the 4,6-*O*-benzylidene ring and all of the *O*-benzyl groups, as confirmed by disappearance of all of the signals

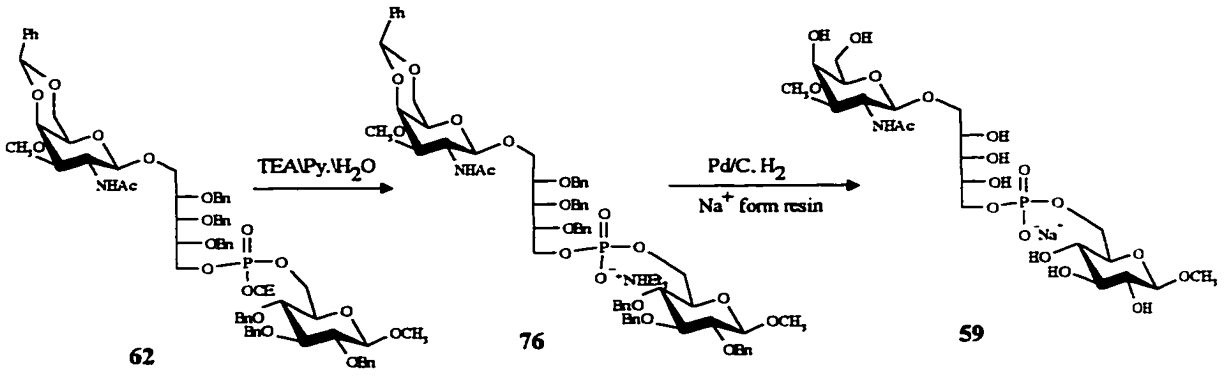


Figure 2.21 Synthesis of the target trisaccharide phosphate 59

assigned to aromatic proton and carbon signals in the ^1H and ^{13}C NMR spectra respectively. Treatment with Amberlite IR-120 (Na^+) resin afforded the target trisaccharide phosphate 59 in a quantitative yield. The disappearance of the signals for the ethyl group in the NMR spectra indicated the removal of the triethylammonium ion and the completion of the ion exchange reaction.

2.3.3.5 Synthesis of trisaccharide phosphates containing ribitol unit with L-configuration (60 and 61)

The synthesis of the target trisaccharide phosphate 60 followed a different procedure. As outlined previously in Figure 2.17, the synthesis of disaccharide 65 had to be performed first. Though extra caution was exercised to keep the reaction flask as dry as possible and the reagent TMS triflate was redistilled before use, coupling of 63 and 39 still gave a low yield. The reaction gave a complicated product mixture, which was separated with a flash column. As shown in Figure 2.22, besides the desired disaccharide

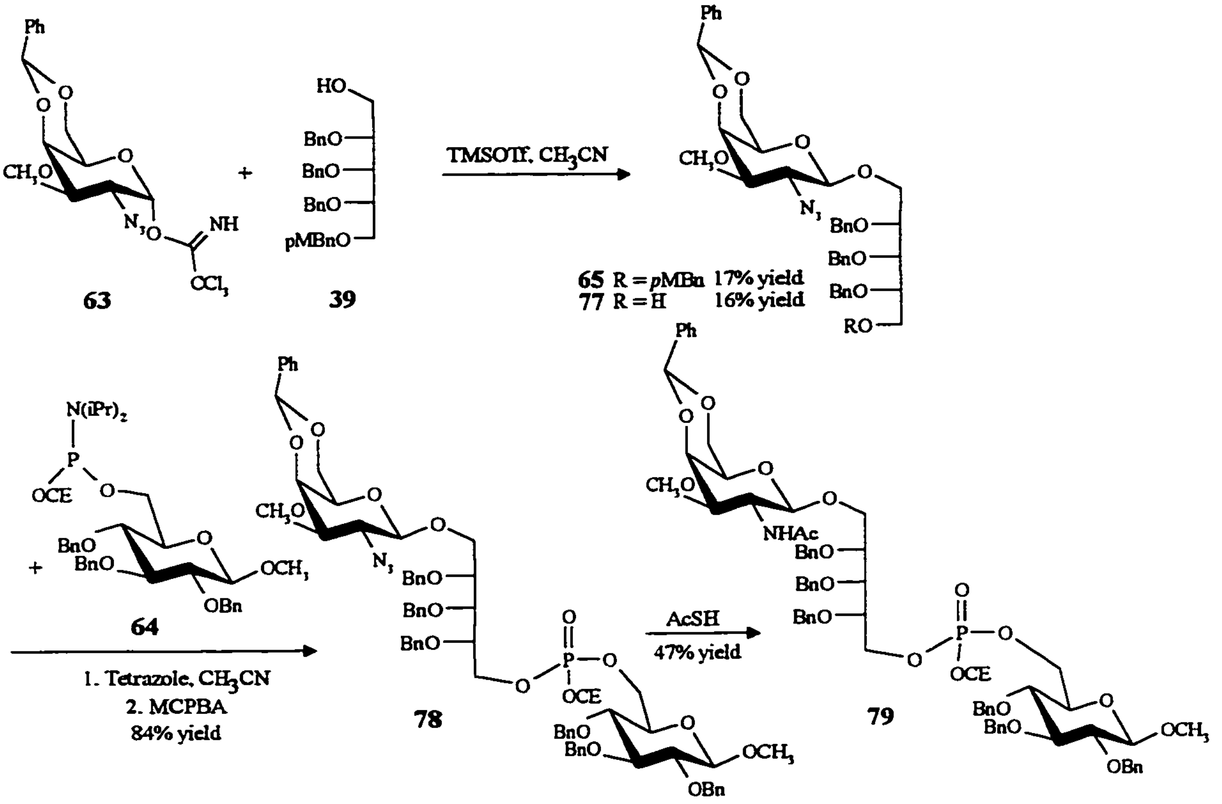


Figure 2.22 Synthesis of the fully protected trisaccharide 79

65, another product was 77, with the *p*-methoxybenzyl group lost. The other minor products were not identified. The two compounds 65 and 77 were obtained with a combined yield of 33%. 65 was easily transformed to 77 by treatment with DDQ (63% yield) at room temperature. The results shown here once again demonstrated that the primary *p*-methoxybenzyl group was not stable in the presence of TMSOTf. Apparently, the formation of the glycosidic linkage was faster than the removal of the primary *p*-methoxybenzyl group. Disaccharide 77 contained only one set of signals in its ^{13}C NMR spectrum. If the *p*-methoxybenzyl group had been cleaved more rapidly, it would be expected that both 77 and its diastereomer with the ribitol unit having the D-configuration

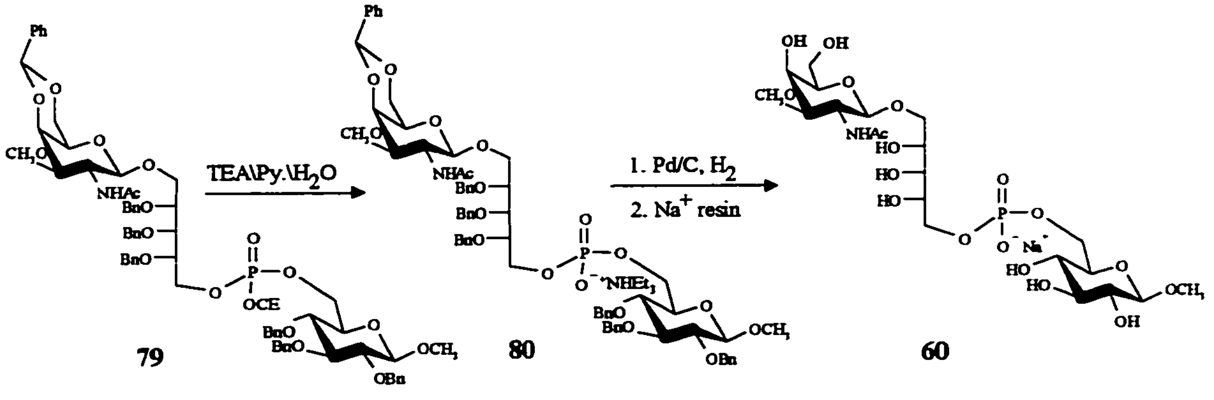


Figure 2.23 Synthesis of the target trisaccharide phosphate 60

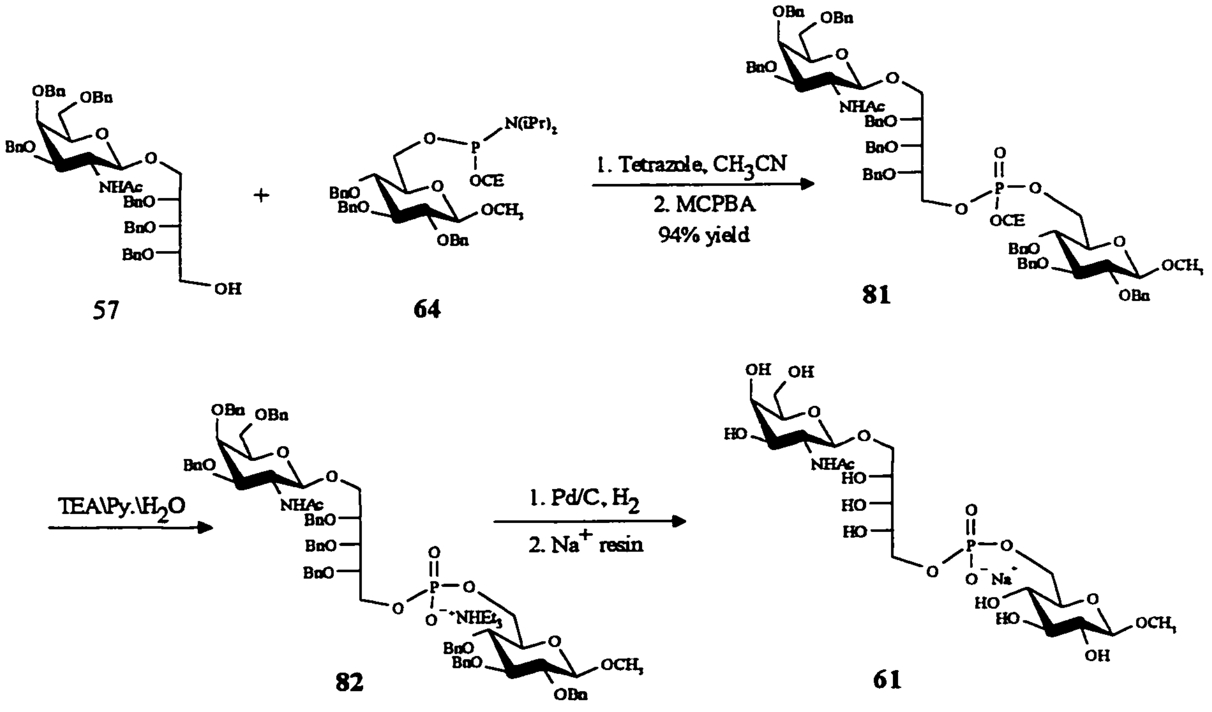


Figure 2.24 Synthesis of the target trisaccharide phosphate 61

would have been formed. This compound 77D, would have different chromatographic properties, so it may have been one of the minor products that were discarded. The phosphotriester linkage between the disaccharide 77 and 64 was formed as previously in an 84% yield. A diastereomeric mixture 74 having both configurations at phosphorus was obtained. The transformation of the azido group in 78 to an acetamido group and the subsequent transformation from 79 to 60, as shown in Figure 2.23, followed the same procedure as before. The low yield (47%) for the reaction with AcSH was due to some loss on the column.

Synthesis of the trisaccharide phosphate 61 followed a similar procedure as that of 60. As shown in Figure 2.24, coupling of disaccharide 57 with 64 afforded trisaccharide 81 in a 94% yield, which was modified in the same way as that of 62 to afford the intermediate 82 and then the target trisaccharide phosphate 61 in a quantitative fashion.

2.3.3.6 NMR analysis of 59 and 60

Complete assignment of all proton and carbon signals in the NMR spectra was conducted using many 2D NMR techniques. In accordance with the literature report for the C-polysaccharide, acetone was used as an internal reference, using chemical shifts of the methyl group at δ 2.225 and δ 31.07 in the ^{1}H and ^{13}C NMR spectra, respectively, as references. All the 1D and 2D NMR spectra of 59 and 60 are attached in the Appendix.

The ^{1}H NMR spectrum of 59 was assigned by using JMOD, COSY, HMBC and HMQC experiments. As shown in Figure A.8 (2), at δ 4.39 and δ 4.49, there were two doublets for the two anomeric protons H-1 and H-1". In the HMBC spectrum (Figure

A.1), the signal at δ 4.49 in the ^{1}H projection has a cross peak with the signal at δ 71.38 in the ^{13}C projection, the only CH_2 signal in the down field region in the JMOD spectrum of 59 (Figure A.9) that was due to C-1'. The other signal at δ 4.39 in the ^{1}H projection has a cross peak with the signal at δ 58.11 in the ^{13}C projection that was due to a methoxy carbon. Therefore, the two signals at δ 4.39 and δ 4.49 were assigned to H-1 and H-1", respectively. The large coupling constants of 8.0 Hz and 8.6 Hz, respectively, confirmed the two β-linkages. Starting from the signal for H-1, all other protons in the glucose residue can be assigned by finding cross peaks in the COSY spectrum (Figure A.2). In the ^{13}C NMR spectrum, the signal for C-5 was a doublet due to coupling to phosphorus. With HMQC (Figure A.4), the assignment of H-5 in the ^{1}H projection can be confirmed by the cross peak with C-5. The assignment for the protons of the galactosamine residue was done in a similar way. Starting from the signal for H-1", the signals for H-2", H-3" and H-4" were assigned by finding cross peaks in the COSY (Figure A.2) spectrum. There was no cross peak between H-4" and H-5" due to the weak coupling. In the JMOD spectrum (Figure A.9(1)), the signal at δ 61.88 of CH_2 was assigned to C-6", thus the signal for H-6" was assigned from the cross peak in the HMQC spectrum (Figure A.4). Then the signal for H-5" was assigned by the correlation with H-6" in the COSY spectrum (Figure A.2).

The most difficult part was the assignment of signals for the ribitol residue. In the JMOD spectrum (Figure A.9(1)), at δ 64.97 and δ 67.41, there were two signals for CH_2. The one at δ 64.97 was assigned to C-6 because it had a cross peak with H-6 in the HMQC spectrum (Figure A.4). The other one at δ 67.41 was thus assigned to C-5'.

From the cross peak in the HMQC spectrum, the signals at δ 3.99 and δ 4.06 in the ^{1}H NMR spectrum were assigned to H-5'. Starting from here, the signals for H-4', H-3', H-2' were assigned from their cross peaks in the COSY spectrum (Figure A.3). There was a strong cross peak between signals at δ 3.83 and δ 3.95 (H-2'), thus the signal at δ 3.83 was assigned to one of the H-1's (H-1'a). In the ^{13}C NMR spectrum (Figure A.9(2), at δ 71.33, δ 71.54, there were a large peak and a small side peak, respectively. According to the cross peaks in the HMQC spectrum (Figure A.4) to H-1'a, H-2' and H-4' in the ^{1}H projection, C-2', C-4' and C-1' were assigned to this region. The small side peak at δ 71.54 was due to half of the doublet of C-4', which was coupled to a phosphorus. In the JMOD spectrum (Figure A.9(1)), there were three peaks at δ 71.34, δ 71.42 and δ 71.54 in the same phase. Thus, C-2' was assigned at δ 71.34 and C-4' was assigned at the middle of δ 71.42 and δ 71.54 (δ 71.48). The signal of C-1', which is primary, should have had the other phase, but was cancelled out. Under this circumstance, the assignment of the chemical shift of C-1' was difficult.

From the expansion of the part at δ 71.33-δ 71.54 of the HMQC spectrum (Figure A.4), the cross peak from the down field side in the ^{13}C projection to H-4' in the ^{1}H projection confirmed the assignment for both C-4' and H-4'. In addition, the cross peak from the shielded side in the ^{13}C projection to H-2' in the ^{1}H projection confirmed the assignment for both C-2' and H-2'. It was seen from the ^{13}C projection that the signal of C-1' was in the middle of C-2' and C-4'. Thus, C-1' was assigned a chemical shift of δ 71.41. It was also noted that the other cross peak correlated to C-1' was overlapped with H-2' in the ^{1}H projection (H-1'b). Taken this into account, the strong cross peak between signals at δ 3.83 and δ 3.95 (H-2') in the COSY spectrum (Figure A.2) was assumed to be due to an overlap of the two cross peaks between H-1'b and H-2' and between H-1'a and H-1'b. Due to the poor resolution of the COSY spectrum, it was hard to determine if there was cross peak between H-1'b and H-2'. The H-1'b was assigned the same chemical shift at δ 3.95 as that of H-2'.

The ^{1}H-^{13}C chemical shift correlated experiment, HMQC (Figure A.4), permitted assignment of all the remaining carbon atoms of **59** that were directly bonded to protons. The major ^{1}H and ^{13}C NMR chemical shifts of **59** are given in Tables 2.4 and Table 2.5, respectively.

The ^{1}H NMR spectrum of **60** was assigned by using COSY, TOCSY and HMQC experiments. At δ 4.40 and δ 4.48, similarly, there were two doublets for the two anomeric protons H-1 and H-1". At δ 103.15 and δ 104.15 in the ^{13}C NMR spectrum, there were two peaks that were assigned for C-1" and C-1, respectively, by comparison with that of **59**. The chemical shift of C-1 of the glucose residue was not expected to change much. Then from the cross peaks in the HMQC spectrum (Figure A.7), the two signals at δ 4.40 and δ 4.48 in the ^{1}H projection were assigned to H-1 and H-1", respectively. In the COSY spectrum (Figure A.5), starting from H-1, all the remaining protons in the glucose residue were assigned from the cross peaks. However, for assignment of the remaining protons of the galactosamine residue, the correlation stopped at H-4", due to the weak coupling between H-4" and H-5". More information was required from other experiments. In the TOCSY spectrum (Figure A.6), there were three distinctive spin systems. All the correlations in glucose residue were confirmed by the strong cross peaks. For the galactosamine residue, it was different. The continuous cross peak correlation stopped at H-4". For the remaining part of the galactosamine residue, it was like a COSY spectrum. H-4" correlated to H-3" and H-5", and H-5" correlated to H-6"a and H-6"b *etc.* The important information was the cross peak between H-4" and H-5", which allowed the assignment of H-5" and H-6".

Before the assignment of ^1H signals for the ribitol residue, the ^{13}C signals for the glucose and galactosamine residues were clearly assigned from the cross peaks in the HMQC spectrum (Figure A.7). The remaining five signals belonged to the ribitol residue. Two of them were doublets. From the chemical shift, the one at δ 67.36 was assigned to C-5' and the one at δ 71.34 was assigned to C-4'. The chemical shift was taken as the middles of the doublets. The remaining three peaks at δ 71.34, 71.70 and 72.41 were due to C-1', C-2' and C-3' that were more difficult to assign. From the expansion of the HMQC spectrum, the middle signal at δ 71.70 was correlated to two protons that were assigned to C-1', a CH_2 carbon. The two correlated proton signals at δ 3.74 and δ 4.07 were assigned to H-1'a and H-1'b, respectively. The signal for H-2' or H-3' was sitting on top of H-1'a at δ 3.74, the other signal was at δ 3.95, close to H-4' and H-5'a. From the COSY spectrum (Figure A.5) at δ 3.74, close to the diagonal, there was no obvious cross peak, indicating that the signal sitting on top of H-1'a could not be assigned to H-2', thus, H-3' was assigned to this position. The strong cross peak between the signals at δ 3.74 and δ 3.95 were assumed to be due to the overlap of the couplings between H-1'b~H-2', H-5'b~H-5'a and H-5'b~H-4'. From the cross peaks in the HMQC spectrum (Figure A.7) correlated to H-2' and H-3', the C-2' and C-3' signals were assigned chemical shifts of δ

71.43 and δ 72.41, respectively. The ^{1}H and ^{13}C NMR chemical shifts of **59**-**61**, the corresponding parts of the tetramer **40** synthesized by van Booms' group,[165] and the C-polysaccharide (**PnC**) are given in Tables 2.4 and Table 2.5, respectively.

Table 2.4 ^{1}H NMR data of PnC,[151] 59 and 60

Cpd	Unit	H1a, H1b	H2	H3	H4	H5a, H5b	H6a, H6b
PnC	GalNAc	4.640	4.10	3.85	4.17	3.83	4.06, 4.06
	Rib	3.86, 3.96	3.99	3.76	3.88	3.98, 4.04	
	Glc	4.619	3.31	3.50	3.54	3.55	4.11, 4.11
59	GalNAc	4.49	3.93	3.44	4.22	3.66	3.77, 3.84
	Rib	3.83, 3.95	3.95	3.77	3.89	3.99, 4.06	
	Glc	4.39	3.28	3.50	3.50	3.58	4.07, 4.17
	ΔδRib	-0.03, -0.01	-0.04	+0.01	+0.01	+0.01, +0.02	
60	GalNAc	4.48	3.94	3.45	4.22	3.66	3.78, 3.83
	Rib	3.740, 4.07	3.95	3.744	3.93	3.98, 4.08	
	Glc	4.40	3.28	3.50	3.50	3.58	4.09, 4.18
	ΔδRib	-0.12, +0.11	-0.04	-0.016	+0.05	0.00, +0.04	

ΔδRib refer to the chemical shift difference of the ribitol unit between 59, 60 and PnC. Digital resolution: 0.22 Hz/point for 59, and 0.43 Hz/point for 60

Table 2.5 ^{13}C NMR data of PnC,[151] 40,[165] and 59-61

Cpd	Unit	C1	C2	C3	C4	C5	C6
PnC	GalNAc	102.08	51.46	75.49	64.07	74.30	65.33
	Rib	71.46	71.35	72.23	71.46	67.46	
	Glc	104.81	73.79	76.37	69.68	75.22	64.92
40	Rib	63.1	72.8	72.4	71.68	67.35	
	Glc	104.8	73.5	76.1	69.6	75.15	65.03
59	GalNAc	102.67	51.99	80.70	64.35	75.78	61.88
	Rib	71.41	71.34	72.19	71.48	67.41	
	Glc	104.12	73.86	76.36	69.87	75.38	64.97
	ΔδRib	-0.05	-0.01	-0.04	+0.02	-0.05	
60	GalNAc	103.15	51.98	80.63	64.30	75.77	61.88
	Rib	71.70	71.34	72.41	71.67	67.36	
	Glc	104.15	73.89	76.39	69.87	75.38	64.96
	Δδ Rib	+0.24	-0.01	+0.18	+0.21	-0.10	
61	GalNAc	103.02	53.27	71.75	68.64	75.89	61.82
	Rib	71.69	71.36	72.43	71.65	67.38	
	Glc	104.14	73.89	76.39	69.89	75.38	64.98

ΔδRib refer to the chemical shift difference of the ribitol unit between 59, 60 and PnC. Digital resolution: 0.015 ppm/point for 59 and 61, 0.0076 ppm/point for 60.

2.3.3.7 The determination of the configuration of the ribitol unit in the C-polysaccharide (PnC)

As shown in Figure A.8, the ^{1}H NMR spectra of the final products **59** and **60** were similar, but significant differences were observed in the part due to the ribitol unit, indicating that the configuration of the ribitol did have significant effect on the chemical shifts of the protons of the corresponding part. As shown in Table 2.4, the chemical shifts for the galactosamine (GalNAc) and glucose (Glc) parts of **59** and **60** were almost the same. This set up the basis for comparison. The largest differences were observed for the signals of H-1a and H-1b of the ribitol part. Comparing with the ^{1}H NMR data of the ribitol unit in the C-polysaccharide (**PnC**), for **59**, the D-ribitol derivative, the chemical shifts of the two H-1' had Δδs of -0.03 ppm and -0.01 ppm, respectively; for **60**, the L-ribitol derivative, the two H-1s had Δδs of -0.12 ppm and +0.11 ppm, respectively. At H-4 and H-5b, **60** also had larger Δδs than **59**, with 0.05 ppm *vs* 0.01 ppm and 0.04 ppm *vs* 0.02 ppm, respectively. This clearly demonstrated that the data for the D-ribitol derivative **59** was closer to that of **PnC** than that of the L-ribitol derivative **60**. In addition, from the Δδ of every proton of the ribitol part of **59**, it was shown that the fit was almost exact with the largest Δδ of -0.04 ppm at H-2'. These observations led to the assignment of the ribitol unit in **PnC** as having the D-configuration.

As shown in Figure A.9, the ^{13}C NMR spectrum of **59** and that of **60** are quite similar. The largest differences lie in C-1' and C-4' of the ribitol unit. From Table 2.5, it was interesting to note that the glucopyranose units of **59**, **60**, and **61** have almost the same chemical shifts, which indicated that there were not any systematic shift differences.

This set up the basis of comparison. In **59-61**, the β-D-glucopyranose residue has an anomeric methyl group instead of a sugar residue, which resulted in the chemical shift differences with respect to the spectra of **PnC**. By comparison with **PnC**, there were -0.69 ppm and -0.66 ppm differences at C-1 of glucose residue for **59** and **60**, respectively. In **40**, the anomeric centre of the glucose residue was linked to the same sugar residue as in **PnC** and the C-1 of the glucose residue had the same chemical shift as that of the corresponding carbon in the **PnC**. However, in **40**, the ribitol unit was terminal. Thus, there was no basis for comparison and no further information could be inferred with respect to the configuration of the ribitol unit in **PnC**.

As shown for **60** and **61**, the methyl substitution at C-3" of the GalNAc part caused the chemical shift of C-3" to change from δ 71.75 to δ 80.63 (Δδ 8.88). For **59**, the chemical shift at C-3" was δ 80.70 and the corresponding C-3" for the **PnC** was at δ 75.49. Glycosylation at C-3" caused a smaller β-effect as would be expected, because a sugar residue causes shielding γ-effects on C-3" as well as the deshielding β-effects. By comparing **60** and **61**, it was found that the methyl group at C-3" in **60** had a γ-effect of -1.29 ppm and -4.34 ppm at C-2" and C-4", respectively and a δ-effect of +0.13 ppm and -0.12 ppm at C-1" and C-5", respectively. For the anomeric centre of the galactosamine part (C-1"), **59** had a 0.59 ppm difference and **60** had a 1.07 ppm difference. The difference of the δ-effects of glycosylation and methylation should be taken into account. The deviation from the **PnC** at C-1" could be smaller.

By comparison with the disaccharide phosphate **47**, trisaccharide phosphate **59** better simulated the environment of the ribitol unit in the **PnC**. At C-1' end, it had a 2-

acetamido-β-D-galactose residue (GalNAc), and at the C-5' end, a β-D-glucose residue (Glc). In addition, at C-3" and C-1, two methyl groups were substituted for two sugar residues as in the **PnC**. All these changes brought a much closer fit of the NMR data with that of the **PnC**, in fact, an almost perfect fit. The average deviation of the data for the ribitol segment of the D-disaccharide phosphate **47** from the corresponding segment of the **PnC** was 0.30 ppm whereas that the corresponding deviation for **59** had decreased to an almost insignificant 0.034 ppm. It is unlikely that data for the complete repeating unit of the C-polysaccharide would have agreed better.

Comparison of the spectra for the ribitol unit will be considered next. It was found that the chemical shifts of the ribitol unit part of the D-trisaccharide phosphate **59** had an exact fit with that of the corresponding part of the **PnC**. The average absolute deviation of the chemical shifts of the ribitol unit part from the corresponding part of the **PnC** was 0.034 ppm. The chemical shifts for the same part of the L-trisaccharide phosphate **60** had an average absolute deviation of 0.15 ppm with the largest differences at C-1 (+0.24 ppm), C-3 (+0.18 ppm), and C-4 (+0.21 ppm), respectively. Therefore, it was concluded that the ribitol unit in the C-polysaccharide of *Streptococcus pneumoniae* had a D-configuration.

2.3.4 Preliminary results directed towards the synthesis of glycosyl acceptor 33

There had been reports[87,189,190] about the synthesis of derivatives of the 2-acetamido-4-amino-2,4,6-trideoxy-galactopyranose residue. However, they were not

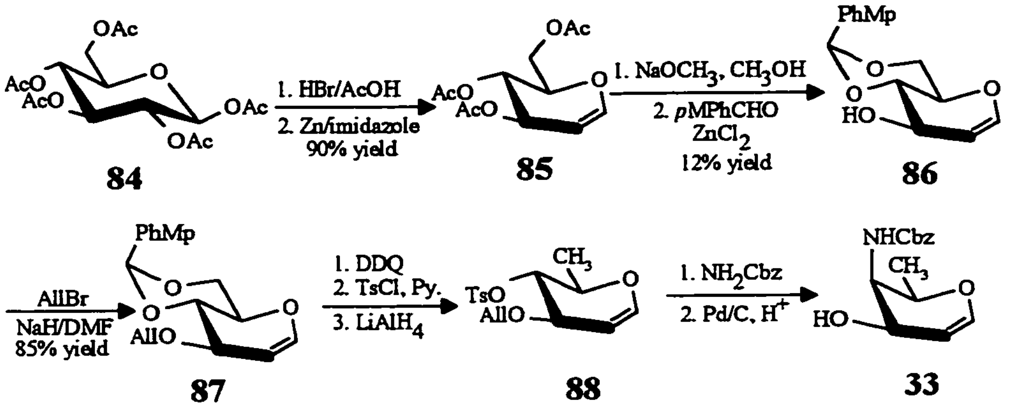


Figure 2.25 The synthetic scheme of 33

compatible with the current synthetic procedure, due to the presence of participating NHAc group at C-2. As seen in Figure 2.4 in section 2.2, it was required that an α-linkage be formed between **29** and **30**. It was planned to put a non-participating group (N_3) at C-2 via azidonitration reaction of **31**, as Schmidt and coworkers did,[179] using the procedure of Ratcliffe and Lemieux.[191] For the synthesis of **31**, **33** was the key compound. The synthesis of the 3-*O*-Ac derivative of **33** (**83**) had been reported.[192] Starting from ethyl 1-thio-α-D-mannopyranoside, in an eleven step chemical transformation, **83** was obtained in a 20% overall yield. The 4-amino group was introduced by an oxidation and imine reduction procedure and the 1,2-double bond was introduced in the last step by reduction with zinc powder. It was decided to design a shorter, more efficient alternative synthetic procedure for the preparation of **33**.

As shown in Figure 2.25, the synthetic procedure started from readily available β-D-glucopyranose pentaacetate **84**. Following a literature procedure,[193] **85** was prepared in a 90% yield. Deacetylation of **85** with sodium methoxide afforded D-glucal quantitatively, which was used without further purification. Reaction with *p*-methoxybenzaldehyde in the presence of zinc dichloride, yielded **86**. The reaction was not complete and the yield was only 12%. It was believed that the low yield was due to the insufficient stirring, because the magnetic bar ceased moving when the reaction mixture become gelatinous. It was thought that this yield could be improved by vigorous mechanical stirring over a longer reaction time.

Subsequent allylation of **86** afforded **87** in a 85% yield. The allyl group is a stable protecting group, which should survive the synthetic procedure shown in Figure 2.25. Due to time limitations, this synthetic procedure was halted at this point.

The removal of the *p*-methoxybenzylidene ring by treatment with DDQ would have been followed by di-*O*-toluenesulfonylation. It was hoped that treatment of the di-*O*-toluenesulfonyled product with lithium aluminum hydride would allow the primary *O*-toluenesulfonyl group to be removed selectively. This reaction might have required a prior selective S_N2 reaction with sodium iodide. Then an S_N2 reaction was designed to replace the 4-*O*-toluenesulfonyl group by a benzyloxycarbonylamide group (Cbz) via reaction with the commercially available benzyloxycarbonylamide or its anion. Amino groups are normally introduced by S_N2 reactions with azide,[189] followed by reduction and then protection of the resulting amino group with Cbz group. If the reaction with benzyloxycarbonylamide worked, this sequence would save two steps. Subsequent removal of the allyl group of **88** with Pd/C would afford the desired glycosyl acceptor **33**.

In comparison with the literature report,[192] the synthetic procedure designed here would use readily available starting material (**85**) and take two less steps. It is hoped that the yield of reaction with *p*-methoxybenzaldehyde can be improved and the remaining steps work well. If these improvements could be made, this sequence would be an short and efficient synthetic procedure.

2.3.5 Conclusion

For the determination of the configuration of the ribitol residue in the repeating unit of the C-polysaccharide of *Streptococcus pneumoniae*, disaccharide and trisaccharide fragments of the repeating unit containing ribitol unit with both D- and L-configurations were designed and synthesized. NMR analysis of these disaccharide and trisaccharide phosphate derivatives with 2D NMR techniques allowed the full assignment of both the ^{1}H and ^{13}C NMR signals.

From the differences of the NMR data of **47** and **48**, **59** and **60**, it was demonstrated that the configuration of the ribitol unit in these molecules had significant influences on the chemical shifts of the corresponding ^{1}H and ^{13}C NMR signals. Of the two configurations, only one can be present in the natural polysaccharide. The configuration for which the NMR data was in closer agreement to that of the corresponding part of the C-polysaccharide (**PnC**) should be the right one present in the natural material as long as there is reasonable agreement. Based on the comparison of the ^{1}H and ^{13}C NMR data of the synthesized disaccharide and trisaccharide phosphates containing ribitol unit of both D- and L-configurations with the corresponding part of the **PnC**, the derivatives with the D-configuration consistently had a better fit with the data of **PnC** than those of the L- configuration. From the ^{1}H and ^{13}C NMR spectra, **47** fit better than **48** and **59** fit better than **60**. In fact, both the ^1H and ^{13}C NMR data of **59** agreed almost perfectly with that of the **PnC**; the agreement was a remarkable one with an average $\Delta\delta$ of 0.02 ppm and 0.034 ppm, respectively. Thus, the ribitol unit in the C-polysaccharide of *Streptococcus pneumoniae* can now be reliably assigned the D-configuration.

A synthetic procedure was also proposed for the synthesis of the 2-acetamido-4-amino-2,4,6-tri-deoxy-D-galactose derivative, an unusual sugar residue in the repeating unit of the C-polysaccharide. Due to the time limitations, only part of it was carried out. It is hoped that this procedure can be completed and proven to be an efficient method in the future.

2.4 Experimental

2.4.1 General methods

Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer model 141 polarimeter. TLC was performed on 0.20 mm thick Merck silica gel 60F-254 aluminum sheets cut to be approximately 7 cm long. Components were located by spraying with 2% ceric sulfate in 1M sulfuric acid and heating on a hot plate until coloration occurred. Dry flash column chromatography was performed on silica gel 60 PF-254 for TLC. Low pressure flash column chromatography was performed on silica gel Merck grade 9385, 230-400 mesh, 60 Å. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AC-250 NMR spectrometer, unless otherwise noted. Some spectra were recorded on Bruker AMX-400 or AMX-500 NMR spectrometers. The ^{1}H NMR spectrum of **94** was recorded on a Bruker AMX-600 NMR spectrometer. Solutions for NMR spectra were prepared in chloroform-*d* unless otherwise stated. Chemical shifts are reported in ppm downfield from internal TMS for ^{1}H and ^{13}C NMR spectra. The central peak for chloroform-*d*, at δ 77.0, was used as a secondary reference for ^{13}C NMR spectra. Phosphoric acid (85%) was used as an external chemical shift reference for ^{31}P NMR (δ 0.00). For compounds **76**, **80**, and **82**, the methyl group of methanol was used as an internal chemical shift reference for ^{1}H NMR (δ 3.31) and for ^{13}C NMR (δ 49.15). For compounds **47**, **48**, **59-61**, acetone was used as an internal chemical shift reference for ^{1}H NMR (δ 2.225) and for ^{13}C NMR (δ 31.07). Exact masses for **47**, **60**, **61**, **64**, **68**, **70**, and **94** were measured on a Kratos IIH mass spectrometer using fast atom bombardment (FAB). The fast atom beam was provided by a cesium gun. Solvents for the matrix were glycerol and/or 3-nitrobenzyl alcohol and combinations there of. Exact masses for **59**, **62**, **75**, **76**, **78**, **80** were measured on a MS50 mass spectrometer using electrospray ionization (ESI). Electrospray ionization mass spectra for **39**, **47**, **48**, **74**, and **77** were run on a VG Micromass Quattro spectrometer in acetonitrile-water (1:1) using a loop injector. The solution concentration was approximately 5.21 x 10^{-4} M and injection was 10 μL. The sample capillary was at 3500 V, the sample cone was at 20 V and the source temperature was at 100 °C. For **39** and **74**, the intensity of the isotopic peaks of the molecular ions were measured.

2.4.2 Synthesis of ribitol derivatives having both D- and L- configurations

2.4.2.1 D-Ribose diethyl dithioacetal (41)

To a solution of D-ribose (30.00 g, 0.20 mol) and concentrated hydrochloric acid (36 mL) was added ethanethiol (36 mL) dropwise at 0 °C during 15 min and the reaction mixture was stirred for another 30 min. Then a saturated sodium bicarbonate solution was added dropwise until the solution was neutral. The resulting solution was concentrated to a residue that was extracted with ethyl acetate. The insoluble inorganic salts were removed by filtration. The ethyl acetate filtrate was concentrated to solid **41**, recrystallized from ethanol as needles (38.61 g, 75% yield): mp 82-83 °C; lit.[166] mp 82-83 °C.

2.4.2.2 Synthesis of 5-*O*-substituted D-ribose diethyl dithioacetals

2.4.2.2.1 5-*O*-Allyl D-ribose diethyl dithioacetal (42)

Dibutyltin oxide (0.248 g, 1.0 equiv) and **41** (0.256 g, 1.0 mmol) were refluxed in toluene (20 mL) for 4 h in a Dean-Stark apparatus for the azeotropic removal of water. Then the solvent was evaporated and cesium fluoride (0.222 g, 1.45 equiv) added. After the mixture had been kept under vacuum for 1 h, a solution of allyl bromide (0.12 mL, 1.3 equiv) in DMF (6 mL) solution was added. The reaction mixture was stirred at room temperature for 1 h when TLC showed that all starting material had been consumed. The reaction solution was concentrated to a residue that was taken up in ethyl acetate (50 mL). The solution was dried (Na_2SO_4) and concentrated to a liquid residue that was purified by dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **42** (0.202 g, 68% yield); the ^{1}H NMR spectrum was in full agreement with literature data;[177] ^{13}C NMR δ 14.4, 14.5 (2 CH_3), 25.6 (2 CH_2S), 54.4 (C-1), 71.1 (C-4), 71.5 (C-5), 72.4 (CH_2-All), 72.8 (C-3), 75.0 (C-2), 117.6 (CH_2=), 133.9 (-CH=).

2.4.2.2.2 5-*O*-Benzyl D-ribose diethyl dithioacetal (43)

Dibutyltin oxide (0.249 g, 1.0 equiv) and **41** (0.253 g, 1.0 mmol) were refluxed in toluene (22 mL) for 5 h in a Dean-Stark apparatus for the azeotropic removal of water. Then the solvent was evaporated and cesium fluoride (0.214 g, 1.4 equiv) added. After the mixture had been kept under vacuum for 1 h, a solution of benzyl bromide (0.26 mL, 2.2 equiv) in DMF (6 mL) was added. The reaction mixture was stirred at room temperature for 3 h then concentrated to a residue that was taken up in ethyl acetate (50 mL). The solution was dried (Na_2SO_4) and concentrated to a liquid residue that was purified by dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **43** (0.050 g, 15% yield); ^{1}H NMR δ 1.28 (t, 6H, 2 CH_3), 2.67 (m, 4H, 2 CH_2S), 3.16 (d, 1H, 2-OH), 3.21 (d, 1H, 3-OH), 3.28 (br, 1H, 4-OH), 3.74 (d, 2H, H-5, $J_{4,5}$ = 4.6 Hz), 3.87 (m, 1H, H-2), 3.97 (m, 2H, H-3,4), 4.25 (d, 1H, H-1, $J_{1,2}$ = 3.1 Hz), 4.56 (q, 2H, $J_{A,B}$=-11.9 Hz, CH_2Ph), 7.34 (m, 5H, Ph-H); ^{13}C NMR δ 14.4, 14.6 (2 CH_3), 25.7 (2 CH_2S), 54.5 (C-1), 71.1 (C-4), 71.7 (C-5), 73.0 (C-3), 73.7 (CH_2Ph), 75.0 (C-2), 127.8, 127.9,128.5, 137.3 (C-Ph).

2.4.2.2.3 5-*O*-*p*-Methoxybenzyl D-ribose diethyl dithioacetal (44)

Dibutyltin oxide (0.250 g, 1.0 equiv) and **41** (0.259 g, 1.0 mmol) were refluxed in toluene (22 mL) for 5 h in a Dean-Stark apparatus for the azeotropic removal of water. Then the solvent was evaporated and cesium fluoride (0.228 g, 1.5 equiv) added. After the reaction mixture had been kept under vacuum for 1 h, a solution of *p*-methoxybenzyl chloride[194] (0.19 mL, 1.4 equiv) in DMF (6 mL) was added. The reaction mixture was stirred at room temperature for 16 h then concentrated to a residue that was taken up in ethyl acetate (50 mL). The solution was dried (Na_2SO_4) and concentrated to a liquid residue that was purified by dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **44** (0.181 g, 48% yield); ^{1}H NMR δ 1.27 (t, 6H, 2 CH_3), 2.67 (m, 4H, 2 CH_2S), 3.3 (br, 3H, 2,3,4-OH), 3.70 (d, 2H, H-5 $J_{4,5}$ = 4.3 Hz), 3.79 (s, 3H, CH_3O), 3.86 (br, 1H, H-2), 3.97 (m, 2H, H-3,4), 4.24 (d, 1H, H-1, $J_{1,2}$ = 2.7 Hz), 4.48 (q, 2H, CH_2PhOCH_3-*p*, $J_{A,B}$=-11.3 Hz), 6.87, 7.24 (2 d, 4H, AA'BB' pattern, Ph-H, J = 7.0 Hz); ^{13}C NMR δ 14.4, 14.5 (2 CH_3), 25.6 (2 CH_2S), 54.4 (C-1), 55.1 (CH_3O), 71.1 (C-4), 71.3 (C-5), 72.9 (C-3), 73.2 (CH_2PhOCH_3-*p*), 75.1 (C-2), 113.8, 128.5, 129.5, 159.3 (C-$PhOCH_3$-*p*).

2.4.2.2.4 5-*O*-*t*-Butyldiphenylsilyl-D-ribose diethyl dithioacetal (45)

2.4.2.2.4.1 In the presence of imidazole

Imidazole (0.150 g, 2.2 mmol) and **41** (0.253 g, 1.0 mmol) were dissolved in DMF (5 mL). Then *t*-butyldiphenylsilyl chloride (0.29 mL, 1.1 equiv) was added. The reaction mixture was stirred at room temperature for 6 d. TLC showed that only starting material was present.

2.4.2.2.4.2 Via dibutylstannylene acetal intermediate

Dibutyltin oxide (0.250 g, 1.0 equiv) and **41** (0.252 g, 1.0 mmol) were refluxed in toluene (22 mL) for 4 h in a Dean-Stark apparatus for the azeotropic removal of water. Then the solvent was evaporated and the reaction mixture was taken up in DMF (5 mL). *t*-Butyldiphenylsilyl chloride (0.32 mL, 1.2 equiv) was added. The reaction mixture was stirred at room temperature for 1 h when TLC showed that all starting material had been consumed. The reaction solution was concentrated to a residue that was taken up in ethyl acetate (50 mL). The solution was dried (Na_2SO_4) and concentrated to a liquid residue that was purified by dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **45** (0.464 g, 96% yield); ^{1}H NMR δ 1.08 (s, 9H, *t*-Bu), 1.28 (m, 6H, 2 CH_3), 2.68 (m, 4H, 2 CH_2S), 3.2 (br, 3H, 2,3,4-OH), 3.95 (br, 5H, H-2,3,4,5), 4.28 (d, 1H, H-1, $J_{1,2}$ = 3.2 Hz), 7.5 (m, 10H, Ph-H); ^{13}C NMR δ 14.5, 14.6 (2 CH_3), 19.1 (*q*-C, *t*-Bu), 25.6 (2 CH_2S), 26.8 (3 CH_3, *t*-Bu), 54.4 (C-1), 65.6 (C-5), 72.2, 72.7 (C-3,4), 75.2 (C-2), 127.8, 130.0, 132.4, 135.5 (C-Ph).

2.4.2.2.5 5-*O*-*t*-Butyldimethylsilyl D-ribose diethyl dithioacetal (46)

Dibutyltin oxide (0.253 g, 1.0 equiv) and **41** (0.256 g, 1.0 mmol) were refluxed in toluene (30 mL) for 6 h as in a Dean-Stark apparatus for the azeotropic removal of water. Then the solvent was evaporated and the residue was dissolved in DMF (5 mL). *t*-Butyldimethylsilyl chloride (0.190 g, 1.2 equiv) was added and the reaction mixture was stirred at room temperature for 1.5 h until TLC showed that all starting material had been consumed. The reaction solution was concentrated to a residue that was taken up in ethyl acetate (50 mL). The solution was dried (Na_2SO_4) and concentrated to a liquid residue that was purified by dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **46** (0.364 g, 97% yield); $[\alpha]_D^{25}$ -10.8° (*c* 7.1, $CHCl_3$); ^{1}H NMR δ 0.11 (s, 6H, 2 CH_3-Si), 0.91 (s, 9H, *t*-Bu), 1.29 (t, 6H, 2 CH_3), 2.70 (m, 4H, 2 CH_2S), 3.37, 3.42, 3.47 (br, d, d, 3H, 2,3,4-OH), 3.9 (br-m, 5H, H-2,3,4,5), 4.29 (d, 1H, H-1, $J_{1,2}$ = 2.4 Hz); ^{13}C NMR δ -5.6 (2 CH_3-Si), 14.4, 14.5 (2 CH_3), 18.0 (q-C, *t*-Bu), 25.5 (2 CH_2S), 25.7 (3 CH_3, *t*-Bu), 54.3 (C-1), 65.0 (C-5), 71.2, 73.0, 75.3 (C-2,3,4).

2.4.2.3 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribose diethyl dithioacetal (89)

A solution of **42** (0.425 g, 1.44 mmol) in DMF (5 mL) was treated with excess NaH (0.42 g, 60% suspension in mineral oil, washed with hexanes 3 times before use) at 0°C. The resulting mixture was stirred for 30 min. Then a solution of benzyl bromide (1.36 mL) in DMF (2 mL) was added dropwise. The reaction solution was stirred at 0°C for 3.5 h when TLC showed that all the starting material had been consumed. Cold water was added until the total volume was about 70 mL. The water layer was extracted with pentane (5 x 40 mL). The pentane solution was dried (Na_2SO_4) and concentrated to a liquid residue that was separated with dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. The title compound **89** was obtained as a syrup (0.514 g, 63% yield); $[\alpha]_D^{23}$ 24° (*c* 6.76, $CHCl_3$); the ^1H NMR spectrum was in full agreement with literature data;[177] ^{13}C NMR δ 14.6 (2 CH_3 SEt), 24.8, 26.1 (2 CH_2 SEt), 53.8 (C-1), 69.3 (C-5), 72.0, 72.4, 73.4, 74.5 (3 CH_2Ph, 1 CH_2 All), 78.9, 79.8, 82.1 (C-2, C-3, C-4), 116.4 (CH_2=), 134.7 (-CH=), 127.2-128.7, 138.2, 138.3, 138.7 (C-Ph).

2.4.2.4 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribose (90)

A solution of **89** (0.310 g, 0.55 mmol) in aqueous acetonitrile (7.25 mL CH_3CN + 0.72 mL H_2O) was treated with mercury (II) chloride (0.604 g, 2.21 mmol) and calcium carbonate (0.229 g, 2.29 mmol). The resulting mixture was stirred at room temperature for 1 h when TLC showed that all starting material had been consumed. The reaction mixture was filtered through celite and the filtrate was concentrated to a residue that was taken up in dichloromethane (100 mL). The solution was washed with potassium iodide solution (1M, 3 x 100 mL) and sodium thiosulfate solution (30%, 2 x 100 mL) subsequently and then dried (Na_2SO_4) and concentrated. The title compound **90** was obtained as a colourless liquid (0.216 g, 86% yield); ^{1}H NMR δ 3.53 (dd, 1H, H-5$_a$, $J_{5a,5b}$ = -10.7 Hz, $J_{5a,4}$ = 4.6 Hz), 3.67 (dd, 1H, H-5$_b$, $J_{5b,4}$ = 2.1 Hz), 3.87-4.01 (m, 4H, H-3, H-4, CH_2 All), 4.09 (br d, 1H, H-2), 4.47-4.82 (m, 6H, 3 CH_2Ph), 5.11-5.26 (m, 2H, CH_2=), 5.78-5.93 (m, 1H, -CH=), 7.22-7.33 (m, 15H, H-Ph), 9.47 (br s, 1H, -CH=O); ^{13}C NMR δ 69.0 (C-5), 72.1 (CH_2 All), 72.5, 72.7, 79.9 (3 CH_2Ph), 76.5, 80.3 (2C C-3, C-4), 82.2 (C-2), 116.8 (CH_2=), 134.6 (-CH=), 127.4-128.3, 137.3, 137.5, 137.9 (C-Ph), 200.9 (C-1 -HC=O).

2.4.2.5 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribitol (38)

A solution of **90** (0.215 g, 0.47 mmol) in aqueous methanol (methanol/water : 1/1, 3 mL) was stirred with sodium borohydride (0.032 g, 0.81 mmol) at room temperature for 10 h. Then water (10 mL) was added and the resulting mixture was extracted with chloroform (3 x 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated to a liquid residue that was purified with dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **38** (0.194 g, 90% yield); $[\alpha]_D^{23}$ -17° (*c* 2.52, $CHCl_3$); lit.[177] $[\alpha]_D^{23}$ -23.15°; the ^{1}H NMR spectrum was in full agreement with literature data;[177] ^{13}C NMR δ 61.2 (C-1), 69.6 (C-5), 71.8, 72.1, 72.3, 73.9 (3 CH_2Ph, 1 CH_2 All), 78.1, 78.7, 78.9 (C-2, C-3, C-4), 116.8 (CH_2=), 134.7 (-CH=), 127.5-128.3, 138.1, 138.2 (C-Ph).

2.4.2.6 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-*p*-methoxybenzyl-D-ribitol (91)

A solution of **38** (0.150 g, 0.325 mmol) in DMF (3 mL) was stirred with sodium hydride (0.06 g, 60% suspension in mineral oil, washed with hexanes before use) at 0°C for 20 min and a solution of *p*-methoxybenzyl chloride (300 µL, 2.21 mmol) in DMF (2 mL) was added dropwise in 6 min. The resulting mixture was stirred at room temperature for 4 h when TLC showed that all starting material had been consumed. Water (15 mL) was added and the resulting mixture was extracted with pentane (6 x 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated to a liquid residue that was purified with dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **91** was obtained as a syrup (0.169 g, 89% yield); $[\alpha]_D^{24}$ -0.11° (*c* 2.48, $CHCl_3$); ^1H NMR δ 3.63-3.70 (m, 4H, H-1, H-5), 3.75 (s, 3H, OCH_3), 3.85-3.95 (m, 5H, H-2, H-3, H-4, CH_2 All), 4.42 (s, 2H, CH_2PhOCH_3-*p*), 4.57-4.72 (m, 6H, 3 CH_AH_BPh, $J_{A,B}$ = -11.6 Hz, -11.9 Hz), 5.10-5.28 (m, 2H, CH_2=), 5.80-5.93 (m, 1H, -CH=), 6.82 (d, 2H, 1/2 AA'BB' pattern, pMPh, J = 8.5 Hz), 7.18-7.25 (m, 17H, H-Ph); ^{13}C NMR δ 55.1 (OCH_3), 69.6, 70.0 (C-1, C-5), 72.1, 72.2, 72.3, 73.7 (3 CH_2Ph, 1 CH_2 All), 72.8 (CH_2PhOCH_3-*p*), 78.4, 78.6 (C-2, C-3, C-4), 113.6 (2C *o*-CH *p*MBn), 116.6 (CH_2=), 134.8 (-CH=), 127.3-129.2, 130.4, 138.6, 159.0 (C-Ph).

2.4.2.7 2,3,4-Tri-*O*-benzyl-5-*O*-*p*-methoxybenzyl-L-ribitol (39)

A solution of 91 (6.052 g, 10.4 mmol) in aqueous methanol (methanol/water : 5/1 55 mL) was stirred with Pd/C (10%, 0.60 g) at 60 °C for 10 h. The reaction mixture was filtered through celite and diluted with water (30 mL). The resulting mixture was extracted with dichloromethane (4 x 50 mL). The combined extracts were dried (Na_2SO_4), and concentrated to a liquid residue that was purified with dry flash column chromatography using a gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5% to yield **39** (3.88 g, 69% yield); $[\alpha]_D^{24}$ 11° (*c* 2.29, $CHCl_3$); ^1H NMR δ 3.5-4.0 (m, 7H, H-1~5), 3.8 (s, 3H, OCH_3), 4.4 (s, 2H, CH_2PhOCH_3-*p*), 4.5-4.8 (m, 6H, 3 CH_2Ph), 6.82 (1/2 AA'BB' pattern, 2H, *o*-CH *p*MBn, J = 8.5 Hz), 7.25 (m, 17H, H-Ph); ^{13}C NMR δ 55.3 (OCH_3), 61.4 (C-1), 69.3 (C-5), 71.9, 72.4, 73.0, 74.0 (3 CH_2Ph, 1 CH_2PhOCH_3-*p*), 78.2, 78.9, 79.1 (3C, C-2, C-3, C-4), 113.8 (2C *o*-CH *p*MBn), 127.7-129.4, 130.3, 138.2, 138.3, 159.2 (C-Ph); ESI-MS (g/mol): 565 for $[C_{34}H_{38}O_6Na]^+$ $[M+Na]^+$ (100), isotopic peaks of $[M+Na]^+$: 566 (calc 38.6, measured 38.4), 567 (calc 8.5, measured 8.5), 568 (calc 1.3, measured 1.2); 581 for $[C_{34}H_{38}O_6K]^+$ $[M+K]^+$; calculated mass 542.3 (M); ESI-MS (g/mol) (in the presence of trace amount of formic acid): 543 for $C_{34}H_{39}O_6$ $[M+H]^+$, 565 for $C_{34}H_{38}O_6Na$ $[M+Na]^+$, 581 for $C_{34}H_{38}O_6K$ $[M+K]^+$.

2.4.3 Synthesis of disaccharide phosphates containing ribitol units having both D- and L- configurations

2.4.3.1 Dibenzyl (*N*,*N*-diisopropylamino)phosphine (51)

To a solution of benzyl alcohol (6.5 mL, 2.0 equiv) and triethylamine (18.0 mL, 4.0 equiv) in diethyl ether (40 mL) was added dichloro(*N*,*N*-diisopropylamino) phosphine[180] (^{31}P NMR δ 170.2; lit.[180] 169.8) [6.35 g (31.4 mmol) in diethyl ether (10 mL)] dropwise at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h and then filtered. The filtrate was diluted with acid-free ethyl acetate (500 mL) to give a solution that was extracted with sodium phosphate buffer (pH 7, 3 x 80 mL). The organic layer was then dried (Na_2SO_4) and concentrated to a colourless syrup (9.36 g, 86% yield) of mainly **51** that was used without further purification; ^{31}P NMR δ 148.3, 13.86; lit.[181] δ 149-150 for similar compounds.

2.4.3.2 Synthesis of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (50)

2.4.3.2.1 3,4,6-Tri-*O*-benzyl-D-galactal (92)

Deacetylation of 3,4,6-tri-*O*-acetyl-D-galactal[193] with sodium in dry methanol[195] gave a white solid, crude D-galactal. Benzylation of D-galactal with benzyl bromide in the presence of sodium hydride gave **92** as a colourless solid: mp 48-49°C; $[\alpha]_D^{24}$ -42° (*c* 16.0, $CHCl_3$); lit.[196] mp 51°; $[\alpha]_D^{20}$ -45°.

2.4.3.2.2 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (50)

A solution of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-galactopyranose[179] (**93**, 5.30 g, 11.2 mmol) in trichloroacetonitrile (7.0 mL, 69.8 mmol) was stirred with sodium hydride (0.69 g, 60% suspension in mineral oil) under argon at 0 °C for 2 h. Then the reaction mixture was filtered through celite. The filtrate was concentrated to a liquid residue that was separated through dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound 50 was obtained as a colourless syrup (2.81 g, 41% yield): IR (neat, cm^{-1}) 3340 (N-H), 2109 (N_3), 1627 (C=N); lit.[179] 3340 (N-H), 2115 (N_3), 1675 (C=N); the ^{1}H NMR data were in agreement with literature report;[179] ^{13}C NMR δ 59.1 (C-2), 67.9 (C-6), 72.1 (CH_2Ph), 72.2 (C-4), 72.6 (C-5), 73.5, 74.9 (2 CH_2Ph), 77.1 (C-3), 95.4 (C-1), 127.9-128.5, 137.2-138.1 (C-Ph), 160.7 (C=N).

Another slower moving compound was also isolated as a colourless syrup that was identified as the 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl trichloroacetimidate (50β, 1.55 g, 22% yield); ^{1}H NMR δ 3.45 (dd, 1H, H-3, $J_{3,4}$ = 2.8 Hz, $J_{2,3}$ = 10.4 Hz), 3.61 (m, 2H, H-6), 3.69 (m, 1H, H-5), 3.95 (br d, 1H, H-4), 4.06 (dd, 1H, H-2), 4.45 (dd, 2H, CH_2Ph, J = -11.6 Hz), 4.59 (d, 1H, CH_2Ph, J = -11.3 Hz), 4.71 (dd, 2H, CH_2Ph, J = -11.6 Hz), 4.32-4.90 (d, 1H, CH_2Ph, J = -11.3 Hz), 5.54 (d, 1H, H-1, $J_{1,2}$ = 8.6 Hz), 7.33 (m, 15H, H-Ph), 8.66 (s, 1H, NH); ^{13}C NMR δ 62.6 (C-2), 67.9 (C-6), 71.9 (C-4), 72.6, 73.5 (2 CH_2Ph), 74.5 (C-5), 77.5 (CH_2Ph), 80.5 (C-3), 97.1 (C-1), 127.8-128.5, 137.3-138.0 (C-Ph), 161.3 (C=N).

2.4.3.3 Synthesis of sodium 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D- ribityl-5-*O*-phosphate (47)

2.4.3.3.1 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (52) (with silver triflate as catalyst)

A solution of **50** (0.806 g, 1.3 mmol) and **38** (0.462 g, 1.0 mmol) in acetonitrile (6.0 mL) was stirred with silver triflate (0.338 g, 1.3 mmol) at room temperature under argon for 67 h when TLC showed no further changes in the product mixture. The silver salts were removed by a column filtration of the reaction mixture. Then a separation was conducted with dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **52** was obtained as a colourless syrup (0.397 g, 43% yield); $[\alpha]_D^{22}$ -15.3° (*c* 1.06, $CHCl_3$); ^{1}H NMR δ 3.33 (dd, 1H, H-3', $J_{2',3'}$ = 7.3 Hz, $J_{3',4'}$ = 2.4 Hz), 3.45 (br t, 1H, H-5'), 3.56 (m, 2H, H-6'), 3.69 (m, 2H, H-5), 4.00 (m, 8H, H-2',4', 1_A, 2, 3, 4, CH_2 All), 4.16 (dd, 1H, H-1_B, $J_{1A,1B}$ = -11.0 Hz, $J_{1B,2}$ = 4.3 Hz), 4.27 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.43-5.02 (m, 6 CH_2Ph), 5.26 (2 br d, 2H, CH_AH_B= All, $J_{A,C}$ = 10.3 Hz, $J_{B,C}$ = 17.1 Hz), 5.86 (m, 1H, -CH_C= All), 7.65 (m, 30H, H-Ph); ^{13}C NMR δ 63.44 (C-2'), 68.11 (C-6'), 68.71 (C-1), 70.02 (C-5), 72.02 (C-4', CH_2 All), 72.12, 72.16, 72.24 (3 CH_2Ph), 73.21 (C-5'), 73.37, 73.63, 74.48 (3 CH_2Ph), 78.20, 78.25, 78.39 (C-2, C-3, C-4), 80.43 (C-3'), 102.0 (C-1'), 116.50 (CH_2=), 134.8 (-CH=), 127.2-128.3, 137.5-138.5 (C-Ph).

Anal. calcd for $C_{56}H_{61}N_3O_9$ (920.11): C 73.09, H 6.68, N 4.57; found: C 72.92, H 6.53, N 4.58.

2.4.3.3.2 Ethyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranoside (54)

A solution of **50** (0.187 g, 0.30 mmol) and ethanol (20 μL, 0.34 mmol) in acetonitrile (10 mL) was stirred with 4Å molecular sieves (0.5 g) at room temperature for 1 h. Then the solution was cooled to -36°C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 14 μL, 0.072 mmol) was added. The reaction solution was stirred at -30°C~-36°C for 1 h when TLC showed no further change in the reaction. Pyridine (1 mL) was added. The solution was diluted with dichloromethane (50 mL), filtered through celite and washed with water (3 x 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to a brownish syrup. Dry flash column chromatography with gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **54** was obtained as a solid (0.083 g, 55% yield); mp 44-45°C; $[\alpha]_D^{22}$ -20.7° (*c* 2.54, $CHCl_3$); ^{1}H NMR δ 1.22 (t, 3H, C**H**$_3$CH$_2$, X$_3$ part of ABX$_3$, $J_{AX} = J_{BX} = 7.0$ Hz), 3.30 (dd, 1H, H-3), 3.49 (m, 1H, H-5), 3.59 (m, 3H, H-6, CH$_3$C**H**H, A part of ABX$_3$), 3.81 (dd, 1H, H-2), 3.87 (br d, 1H, H-4), 3.91 (dq, 1H, CH$_3$CH**H**, B part of ABX$_3$, $J_{A,B} = -9.5$ Hz), 4.21 (d, 1H, H-1, $J_{1,2} = 7.9$ Hz), 4.42, 4.67, 4.73 (3 dd, $J_{A',B'} = -11.9$ Hz, -11.9 Hz, -11.6 Hz, 3 C**H**$_2$Ph), 7.31 (m, 15H, H-Ph); ^{13}C NMR δ 15.0 (CH$_3$CH$_2$), 63.1 (C-2), 65.5 (CH$_2$CH$_3$), 68.6 (C-6), 72.0 (C-4), 72.5 (CH$_2$Ph), 73.5 (C-5), 73.6, 74.5 (2 CH$_2$Ph), 80.6 (C-3), 102.1 (C-1), 127.6, 127.8, 127.9, 128.3, 128.4, 137.6, 137.7, 138.3 (C-Ph).

Anal. calcd for $C_{29}H_{33}N_3O_5$ (503.60): C 69.17, H 6.60, N 8.34; found: C 68.78, H 6.45, N 8.30.

2.4.3.3.3 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (52)

A solution of **50** (0.373 g, 0.60 mmol) and **38** (0.232 g, 0.50 mmol) in acetonitrile (15 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at room temperature. Then the temperature of the solution was lowered to -36°C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 50 μL, 0.26 mmol) was added. The reaction solution was stirred at -34°C~ -38°C for 2 h when TLC showed no further change. Pyridine (1 mL) was added. The solution was diluted with dichloromethane (50 mL), filtered through celite and washed with water (3 x 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to a residue. Dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **52** was obtained as a colourless syrup (0.264 g, 57% yield).

Reaction of a mixture of **50** and **50β** (1 : 1 mixture, 1.55 g, 2.50 mmol) with **38** (1.29 g, 2.80 mmol) followed the same procedure and afforded **52** as a colourless syrup (1.34 g, 58% yield).

2.4.3.3.4 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (94)

A solution of **52** (1.34 g, 1.46 mmol) in thiolacetic acid (1.6 mL) was stirred under argon at room temperature for 20 h. The reaction solution was concentrated to a residue that was separated by dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%.

Compound 94 was obtained as a colourless solid (0.85 g, 63% yield); mp 108-109°C; $[\alpha]_D^{24}$ 7.5° (*c* 0.92, $CHCl_3$); ^1H NMR (600 MHz) δ 2.16 (s, 3H, CH_3CO), 3.50-3.57 (m, 2H, H-6'), 3.60-3.64 (m, 3H, H-2', H-5a, H-5'), 3.69 (dd, 1H, H-5b, $J_{5b,5a}$ = -10.6 Hz, $J_{5b,4}$ = 3.0 Hz), 3.79-3.83 (m, 3H, H-1a, H-2, H-3), 3.92-3.93 (m, 4H, H-4, H-4', CH_2 All), 4.03 (br d, 1H, H-1b, $J_{1b,2}$ = 7.1 Hz), 4.11 (br d, 1H, H-3', $J_{3',2'}$ = 10.8 Hz), 4.38-4.70 (m, 11H, 5.5 x CH_2Ph), 4.81 (d, 1H, H-1', $J_{1',2'}$ = 8.3 Hz), 4.87 (d, 1H, 0.5 x CH_2Ph, $J_{A,B}$ = -11.5 Hz), 5.12-5.24 (m, 2H, CH_2=), 5.32 (d, 1H, NH, $J_{NH,2'}$ = 7.4 Hz), 5.83-5.90 (m, 1H, -CH=), 7.22-7.33 (m, 30H, H-Ph); ^{13}C NMR δ 23.55 (CH_3CO), 54.70 (C-2'), 68.02 (C-1), 68.45 (C-6'), 70.27 (C-5), 71.88, 72.03, (2 CH_2Ph), 72.15 (CH_2 All), 72.35 (CH_2Ph, C-4'), 73.10 (C-5'), 73.39, 73.57, 74.45 (3 CH_2Ph), 77.82 (C-2), 78.12 (C-3'), 78.45, 78.49 (C-3, C-4), 100.2 (C-1'), 116.7 (CH_2=, All), 134.9 (CH=, All), 127.4-128.4, 138.1-138.7 (C-Ph), 170.5 (C=O); Exact mass (FAB) calcd for $C_{58}H_{64}NO_{10}$ $[M-H]^+$: 934.4530; found: 934.440.

Anal. calcd for $C_{58}H_{65}NO_{10}.H_2O$ (954.17): C 73.01, H 7.08, N 1.47; found: C 72.52, H 6.88, N 1.57.

2.4.3.3.5 2,3,4-Tri-*O*-benzyl-1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (53)

To a solution of 94 (0.852 g, 0.91 mmol) in aqueous methanol (methanol/water : 10/1 60 mL) was added *p*-toluenesulfonic acid (0.16 g, 1.0 equiv) and Pd/C (10%, 0.35 g). The suspension was stirred at 60°C for 5 h when TLC showed that all the starting material had been consumed. The reaction mixture was filtered and diluted with water (50 mL). The resulting mixture was extracted with dichloromethane (3 x 60 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to a residue that was purified using dry flash column chromatography to yield **53** as a colourless solid (0.455 g, 56% yield); mp 94-95 °C; $[\alpha]_D^{24}$ 13.5° (*c* 1.79, $CHCl_3$); ^{1}H NMR δ 1.73 (s, 3H, CH_3CO), 2.51 (t, 1H, 5-OH, $J_{OH,5}$ = 6.1 Hz), 3.51 (m, 4H, H-6', H-5', H-2'), 3.73-3.88 (m, 6H, H-5, H-1a, H-2, H-3, H-4), 3.94 (br s, 1H, H-4'), 4.02-4.13 (m, 2H, H-1b, H-3'), 4.36-4.90 (m, 12H, 6 CH_2Ph), 4.84 (d, 1H, H-1', $J_{1',2'}$ = 6.7 Hz), 5.39 (d, 1H, NH, $J_{NH,2'}$ = 7.3 Hz) 7.30-7.45 (m, 30H, H-Ph); ^{13}C NMR δ 23.56 (CH_3CO), 54.77 (C-2'), 61.58 (C-5), 67.64 (C-1), 68.45 (C-6'), 71.92, 71.98, 72.06 (3 CH_2Ph), 72.28 (C-4'), 73.15 (C-5'), 73.42, 73.69, 74.48 (3 CH_2Ph), 77.52 (C-2), 77.94 (C-3'), 78.77, 79.05 (C-3, C-4), 100.2 (C-1'), 127.8-128.4, 137.8-138.5 (C-Ph), 170.6 (C=O).

Anal. calcd for $C_{55}H_{61}NO_{10}$ (896.09): C 73.72, H 6.86, N 1.56; found: C 73.20, H 6.68, N 1.56.

2.4.3.3.6 Dibenzyl [2,3,4-Tri-*O*-benzyl-1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribityl-] phosphate (49)

To a solution of **53** (0.415 g, 0.463 mmol) in dichloromethane (15 mL) was added **51** (0.962, 2.8 equiv) and tetrazole (0.198 g, 2.8 equiv). The resulting solution was stirred at room temperature for 1.5 h when TLC showed that all starting material had been consumed. The reaction mixture was cooled to 0 °C and a solution of *m*-chloroperbenzoic acid (MCPBA, 0.405 g, 2.5 equiv) in dichloromethane (5 mL) was added dropwise. The reaction was complete in 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and the resulting solution was washed with $Na_2S_2O_5$ (10%, 2 x 30 mL) and Na_2CO_3 (10%, 2 x 30 mL), then was dried ($MgSO_4$) and concentrated. Dry flash column chromatography with gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **49** was obtained as a colourless syrup (0.345 g, 65% yield); $[\alpha]_D^{24}$ 9.2° (*c* 3.54, $CHCl_3$); ^{31}P NMR δ -0.09; ^1H NMR δ 1.70 (s, 3H, CH_3CO), 3.50-4.20 (m, 11H, H-6', H-5', H-2', H-2, H-3, H-4, H-4', H-3', H-5), 4.30-4.70 (m, 15H, 7.5 x CH_2Ph), 4.80-5.00 (m, 4H, H-1, 2H-1', 0.5 x CH_2Ph), 6.05 (d, 1H, NH, $J_{NH,2'}$ = 7.7 Hz) 7.10-7.45 (m, 40H, H-Ph); ^{13}C NMR δ 23.21 (CH_3CO), 54.18 (C-2'), 67.33 (C-5, $J_{5,P}$ = 8.6 Hz), 68.30 (C-6'), 68.93 (C-1), 68.86, 68.93, 71.65, 71.86, 72.22 (5 CH_2Ph), 72.22 (C-4'), 72.91 (C-5'), 73.10 (2C), 74.15 (3 CH_2Ph), 77.24 (C-2), 77.71 (C-3), 77.79 (C-4, $J_{4,P}$ = 10.0 Hz), 78.03 (C-3'), 100.1 (C-1'), 127.2-128.2, 135.4-138.4 (C-Ph), 170.4 (C=O).

Anal. calcd for $C_{69}H_{74}NO_{13}P.H_2O$ (1174.33): C 70.57, H 6.52, N 1.19, P 2.64; found: C 70.13, H 6.45, N 1.36, P 2.40.

2.4.3.3.7 Sodium 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-ribityl-5-*O*-phosphate (47)

A solution of 49 (0.250 g, 0.217 mmol) in a mixture of ethyl acetate, methanol, and acetic acid (16/24/1.5, 41.5 mL) was stirred under hydrogen (1 atm) in the presence of Pd/C (10%, 0.417 g) for 21 h at room temperature. The mixture was filtered through celite and the celite was washed with methanol (250 mL). The combined filtrate and washings were concentrated to a colourless syrup **47a**; ^{31}P NMR (D_2O) δ -0.29; ^1H NMR

(D_2O) δ 2.06 (s, 3H, CH_3CO), 3.75-3.98 (m, 13H, H-1~H-5, H-2'~H-6'), 4.05 (d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz); ^{13}C NMR (D_2O) δ 24.99 (CH_3CO), 53.29 (C-2'), 61.84 (C-6'), 66.95 (d, C-5, $J_{5,P}$ = 4.8 Hz), 68.64 (C-4'), 71.27 (C-1), 71.31 (C-2), 71.55 (d, C-4, $J_{4,P}$ = 7.6 Hz), 71.83 (C-3), 72.75 (C-5'), 75.94 (C-3'), 102.6 (C-1'), 175.8 (C=O).

Compound **47a** was dissolved in water (3 mL) and stirred with Amberlite IR-120 (Na^+) resin for 12 h. Then the suspension was filtered and the filtrate was concentrated to yield **47** (0.103 g, 99% yield); $[\alpha]_D^{24}$ 5.7° (*c* 2.06, H_2O); ^{31}P NMR (D_2O) δ 1.92; ^1H NMR (D_2O) δ 1.96 (s, 3H, CH_3CO), 3.67-4.04 (m, 13H, H-1~H-5, H-2'~H-6'), 4.50 (d, 1H, H-1', $J_{1',2'}$ = 8.5 Hz); ^{13}C NMR δ 23.09 (CH_3CO), 53.24 (C-2'), 61.80 (C-6'), 66.74 (d, C-5, $J_{5C,P}$ = 4.8 Hz), 68.61 (C-4'), 71.27 (C-2), 71.31 (C-1), 71.55 (d, C-4, $J_{4C,P}$ = 7.6 Hz), 71.77 (C-3), 72.24 (C-5'), 75.91 (C-3'), 102.6 (C-1'), 175.8 (C=O); ESI-MS (g/mol) $[M-H]^-$ 478, $[M-Na]^-$ 456, $[M-2Na+H]^-$ 434; calculated mass 479.3; exact mass (FAB) calcd for $C_{13}H_{25}NO_{13}PNa_2$ $[M+H]^+$: 480.086; found 480.097.

2.4.3.4 Synthesis of sodium 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-ribityl-5-*O*-phosphate (48)

2.4.3.4.1 2,3,4-Tri-*O*-benzyl-5-*O*-*p*-methoxybenzyl-1-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-L-ribitol (55)

A solution of **50** (0.360 g, 0.58 mmol) and **39** (0.255 g, 0.47 mmol) in acetonitrile (5 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at room temperature. Then the temperature of the solution was lowered to -37°C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 50 μL, 0.26 mmol) was added. The reaction solution was stirred at -34 °C~ -38 °C for 2 h when TLC showed no further change. Pyridine (1 mL) was added. The solution was diluted with dichloromethane (50 mL), filtered through celite and washed with water (3 x 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to a residue that was purified with dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **55** was obtained as a pale yellow syrup (0.305 g, 65% yield); $[\alpha]_D^{22}$ -13.2° (*c* 2.01, $CHCl_3$); ^{1}H NMR δ 3.28 (dd, 1H, H-3', $J_{2,3'}$ = 10.4 Hz, $J_{3',4'}$ = 2.9 Hz), 3.41 (br dd, 1H, H-5'), 3.52 (m, 2H, H-6'), 3.63 (m, 2H, H-5), 3.73 (m, 1H, H-1_A), 3.77 (s, 3H, OMe), 3.81 (br s, 1H, H-2'), 3.86 (m, 2H, H-4, H-4'), 3.95 (m, 2H, H-2, H-3), 4.15 (dd, 1H, H-1_B, $J_{A,B}$ = -11.0 Hz, $J_{1,2}$ = 2.1 Hz), 4.19 (d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz), 4.38-4.91 (m, 14H, 7 CH_2Ph), 6.81-7.41 (m, 34H, H-Ph); ^{13}C NMR δ 55.18 (OCH_3), 63.44 (C-2'), 68.12 (C-6'), 69.43 (C-5), 70.18 (C-1), 72.03 (C-4'), 72.35, 72.51, 72.85 (4 CH_2Ph), 73.21 (C-5'), 73.47, 73.49, 74.61 (3 CH_2Ph), 78.24, 78.69, 78.82 (C-2, C-3, C-4), 80.66 (C-3'), 102.4 (C-1'), 113.6, 127.4-130.4, 137.7-138.5, 159.0 (C-Ph).

2.4.3.4.2 2,3,4-Tri-*O*-benzyl-1-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-L-ribitol (56)

A solution of **50** and **50β** (2.69 g, 4.34 mmol) and **39** (2.16 g, 3.98 mmol) in acetonitrile (15 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at room temperature. Then the reaction mixture was cooled to -42 °C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 0.35 mL, 1.18 mmol) was added. The resulting mixture was stirred at ~-40°C for 1 h when TLC showed no further change. Pyridine (1 mL) was added. The solution was diluted with dichloromethane (50 mL), filtered through celite and washed with water (3 x 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to a brownish liquid residue that was separated with dry flash column chromatography using gradient elution from hexane to ethyl acetate to give both **55** (0.836 g, 21% yield) and **56** (1.384 g, 40% yield) as pale yellow syrups. **56**: $[\alpha]_D^{24}$ -2.9° (*c* 5.85, $CHCl_3$); ^1H NMR δ 3.28 (dd, 1H, H-3', $J_{2,3'}$ = 10.1 Hz, $J_{3',4'}$ = 1.7 Hz), 3.43 (m, 1H, H-5'), 3.52 (m, 2H, H-6'), 3.63-4.00 (m, 8H, H-5, H-1a, H-2', H-4', H-2, H-3, H-4), 4.16 (br d, 1H, H-1b), 4.20 (d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz), 4.31-4.92 (m, 12H, 6 CH_2Ph), 7.10-7.45 (m, 30H, H-Ph); ^{13}C NMR δ 60.11 (C-5), 63.26 (C-2'), 68.01 (C-6'), 69.48 (C-1), 71.80 (CH_2Ph), 71.88 (C-4'), 72.13, 72.36, (2 CH_2Ph), 73.12 (C-5'), 73.25, 73.50, 74.44 (3 CH_2Ph), 78.33, 78.64 (3C, C-2, C-3, C-4), 80.40 (C-3'), 102.1 (C-1'), 127.3-127.7, 137.7-138.5 (C-Ph).

2.4.3.4.3 2,3,4-Tri-*O*-benzyl-1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-L-ribitol (57)

A solution of **56** (1.316 g, 1.50 mmol) in thiolacetic acid (2 mL) was stirred under argon at room temperature for 10 h. The reaction solution was concentrated to a residue that was purified by dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **57** was obtained as a colourless solid (0.839 g, 63% yield); mp 84-85°; $[\alpha]_D^{22}$ -2.9° (*c*

5.85, $CHCl_3$); ^1H NMR δ 2.00 (s, 3H, CH_3CO), 3.48-3.94 (m, 9H, H-6', H-5', H-2', H-5, H-2, H-3, H-4), 3.96 (d, 1H, H-4', $J_{3',4'}$ = 2.4 Hz), 4.10-4.20 (m, 3H, H-1, H-3', $J_{3',2'}$ = 11.0 Hz), 4.35-4.93 (m, 12H, 6 CH_2Ph), 4.90 (d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz), 5.55 (d, 1H, NH, $J_{2',NH}$ = 7.6 Hz), 7.10-7.40 (m, 30H, H-Ph); ^{13}C NMR δ 23.34 (CH_3CO), 54.32 (C-2'), 60.99 (C-5), 68.24 (C-6'), 69.09 (C-1), 71.74, 71.86 (2 CH_2Ph), 72.24 (2C C-4', CH_2Ph), 73.01 (C-5'), 73.21, 73.50, 74.36 (3 CH_2Ph), 78.02 (C-3'), 78.27, 78.65, 78.99 (C-2, C-3, C-4), 99.97 (C-1'), 127.3-128.2, 137.6-138.4 (C-Ph), 170.4 (C=O).

Anal. calcd for $C_{55}H_{61}NO_{10}$ (896.09): C 73.72, H 6.86, N 1.56; found: C 73.31, H 6.86, N 1.58.

2.4.3.4.4 Dibenzyl [2,3,4-Tri-*O*-benzyl-1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-L-ribityl] phosphate (58)

To a solution of **57** (0.265 g, 0.296 mmol) and **51** (0.982 g, 9.5 equiv) in dichloromethane (10 mL) was added tetrazole (0.127 g, 1.81 mmol). The resulting solution was stirred under argon for 2 h when TLC showed that all starting material had been consumed. The reaction mixture was cooled to 0 °C and a solution of *m*-chloroperbenzoic acid (MCPBA, 0.430 g, 2.49 mmol) in dichloromethane (5 mL) was added dropwise. The reaction was complete in 2 h. The reaction mixture was diluted with dichloromethane (20 mL) and the resulting mixture was washed with $Na_2S_2O_5$ (10%, 2 x 30 mL) and Na_2CO_3 (10%, 2 x 30 mL), then dried ($MgSO_4$) and concentrated. Dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **58** was obtained as a colourless syrup (0.183 g, 68% yield); $[\alpha]_D^{24}$ -0.3° (*c* 3.83, $CHCl_3$); ^{31}P NMR δ -2.54; ^1H NMR 1.70 (s, 3H, CH_3CO), 3.45-4.25 (m, 11H, H-6', H-5', H-2', H-2, H-3, H-4, H-4', H-3', H-5), 4.30-4.75 (m, 15H, 7.5 x CH_2Ph), 4.80-5.00 (m, 4H, H-1, H-1', 0.5 x CH_2Ph), 5.45 (d, 1H, NH, $J_{NH,2'}$ = 7.1 Hz), 7.10-7.50 (m, 40H, H-Ph); ^{13}C NMR δ 23.29 (CH_3CO), 54.24 (C-2'), 66.69 (br s, C-5), 68.19 (C-6'), 68.92 (C-1), 68.92, 71.81, 72.20 (5 CH_2Ph), 72.21 (C-4'), 72.95 (C-5'), 73.15, 73.45, 74.30 (3 CH_2Ph), 77.50 77.80 (C-2, C-3), 78.89 (d, C-4, $J_{4C,P}$ = 11.4 Hz), 78.65 (C-3'), 99.99 (C-1'), 127.4-128.6, 135.4-138.4 (C-Ph), 170.4 (C=O).

Anal. calcd for $C_{69}H_{74}NO_{13}P$ (1156.32): C 71.67, H 6.45, N 1.21; found: C 71.06, H 6.23, N 1.21.

2.4.3.4.5 Sodium 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-ribityl-5-*O*-phosphate (48)

A solution of **58** (0.106 g, 0.092 mmol) in a mixture of ethyl acetate, methanol, and acetic acid (4/5.8/0.5, 10.3 mL) was stirred under hydrogen (1 atm) in the presence of Pd/C (10%, 0.137 g) for 18 h at room temperature. The mixture was filtered though celite and the celite was washed with methanol (250 mL). The combined filtrate and washings were concentrated to a colourless syrup (**48a**); ^{31}P NMR (D_2O) δ 3.48; ^1H NMR (D_2O) δ 2.03 (s, 3H, CH_3CO), 3.62-3.83 (m, 6H, H-3', H-5', H-2, H-6', H-4), 3.86 (m, 4H, H-2', H-4', H-3, H-5a), 4.03-4.15 (m, 3H, H-5b, H-1), 4.48 (br d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz); ^{13}C NMR (D_2O) δ 23.09 (CH_3CO), 53.28 (C-2'), 61.84 (C-6'), 67.26 (d, C-5, $J_{5C,P}$ = 5.7 Hz), 68.63 (C-4'), 71.33 (C-3), 71.74 (C-1, C-2), 72.37 (C-5'), 72.88 (d, C-4, $J_{4C,P}$ =

7.6 Hz), 76.92 (C-3'), 103.0 (C-1'), 175.9 (C=O).

Compound **48a** was dissolved in water (3 mL) and stirred with Amberlite resin IR-120 (Na^+) resin for 1 h. Then the mixture was filtered and the filtrate was concentrated to yield the title compound **48** (0.044 g, 100% yield); $[\alpha]_D^{24}$ 3.4° (*c* 2.2, H_2O); ^{31}P NMR (D_2O) δ 1.47; ^1H NMR (D_2O) δ 2.05 (s, 3H, CH_3CO), 3.60-4.15 (m, 13H, H-1~H-5, H-2'~H-6'), 4.48 (br d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz); ^{13}C NMR (D_2O) δ 23.11 (CH_3CO), 53.27 (C-2'), 61.84 (C-6'), 66.93 (br s, C-5), 68.63 (C-4'), 71.33 (C-3), 71.72 (C-1, C-2), 72.43 (C-5'), 72.92 (br s, C-4), 75.92 (C-3'), 103.0 (C-1'), 175.9 (C=O); ESI-MS (g/mol) $[M-H]^-$ 478, $[M-Na]^-$ 456, $[M-2Na+H]^-$ 434; calculated mass 479.3.

2.4.4 Synthesis of trisaccharides containing ribitol units having both D- and L- configurations

2.4.4.1 Synthesis of (methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidite (64)

2.4.4.1.1 Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (72)

A solution of methyl 2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside[171] (**71**, 19.52 g, 27.6 mmol) in glacial acetic acid (35 mL) was treated with a solution of HBr in acetic acid (30% wt., 7.5 mL, 1.0 equiv) at 0 °C. The resulting precipitate was removed by filtration and washed with glacial acetic acid. The combined filtrate and washings were poured onto a mixture of ice and solid sodium carbonate. The resulting mixture was extracted with dichloromethane (3 x 100 mL). The combined extracts were washed with saturated sodium hydrogen carbonate solutions (2 x 100 mL) and distilled water (3 x 100). The organic layer was dried (Na_2SO_4) and concentrated to a solid that was applied to a low pressure flash chromatography column. Elution with hexane and ethyl acetate (2 : 1) gave **72** as a colourless solid (9.61 g, 75% yield), which was recrystallized from ether and hexane to give fine needles: mp 90-91 °C; $[\alpha]_D^{20}$ 8.8° (*c* 0.34, $CHCl_3$); lit.[187] mp 91-92 °C; $[\alpha]_D$ 9.9°.

A faster moving compound was also obtained, identified as 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**72a**, 0.40 g, 3% yield); mp 62-63 °C; lit.[187] mp 60-62 °C.

2.4.4.1.2 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidite (64)

To a solution of **72** (3.25 g, 7.00 mmol) in dichloromethane (70 mL), stirred under argon at room temperature, were added diisopropylethylamine (DIPEA, 3.4 mL, 2.8 equiv) and chloro 2-cyanethyl (*N*,*N*-diisopropyl)phosphoramidite[188] (**73**, 2.22 g, 1.3 equiv). The reaction was complete in 30 min. The reaction solution was diluted with dichloromethane (30 mL), washed with saturated sodium chloride containing 5% triethylamine (3 x 30 mL), and tetraethylammonium bromide (TEAB, 2M, 2 x 30 mL). The organic solution was dried ($MgSO_4$) and concentrated to a syrup that was purified with dry flash column chromatography using hexane/ethyl acetate (5/1) containing 4% triethylamine as eluant. The title compound (**64**) was obtained as a colourless syrup (3.86 g, 83% yield); $[\alpha]_D^{20}$ 4.4° (*c* 3.03, $CHCl_3$); ^{31}P NMR δ 150.0, 149.4; ^1H NMR δ 1.14 (m, 12H, CH_3 *i*Pr), 2.48-2.60 (m, 2H, OCH_2CH_2CN), 3.36-4.05 (m, 13H, H-2~H-6, 2 CH *i*Pr, OCH_2CH_2CN, OCH_3), 3.53 (s, OCH_3), 4.31 (d, 1H, H-1, $J_{1,2}$ = 7.6 Hz), 4.64-4.97 (m, 6H, 3 CH_2Ph), 7.15-7.37 (m, 15H, H-Ph); ^{13}C NMR δ 19.9, 20.0 (OCH_2CH_2CN), 24.3, 24.4 (CH_3 *i*Pr), 42.7, 42.9, 43.1 (CH *i*Pr), 56.5, 56.6 (OCH_3), 58.1, 58.4 (OCH_2CH_2CN), 62.2, 62.4, 62.7 (C-6), 74.3, 74.6, 75.3 (3 CH_2Ph, C-5), 77.5 (C-3), 82.1 (C-2), 84.26, 84.34 (C-4), 104.3 (C-1), 117.3, 117.5 (CN), 127.3-128.1, 137.9, 138.3 (C-Ph); exact mass (FAB) calcd for $C_{37}H_{50}N_2O_7P$ $[M+H]^+$: 665.3356, $C_{37}H_{48}N_2O_7P$ $[M-H]^+$: 663.3199; found 665.3323 $[M+H]^+$, 663.3210 $[M-H]^+$.

2.4.4.2 Synthesis of 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl trichloroacetimidate (63)

2.4.4.2.1 (*t*-Butyldimethyl)silyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (67)

Starting from 3,4,6-tri-*O*-acetyl-D-galactal,[179] 67, a syrup, was prepared in five steps using the method of Schmidt[179]; $[\alpha]_D^{20}$ -8.1° (*c* 3.58, $CHCl_3$); lit.[179] $[\alpha]_D^{25}$ -10°; the ^1H NMR spectrum was in full agreement with literature data;[179] ^{13}C NMR δ -4.92, -4.15 (2 CH_3Si), 17.98 $[C(CH_3)_3]$, 25.64 $[C(CH_3)_3]$, 66.39, 66.46 (C-2,3), 69.07 (C-6), 71.01(C-4), 74.54 (C-5), 97.14 (C-1), 101.3 (CHPh), 126.4, 128.2, 129.2, 137.5 (C-Ph).

2.4.4.2.2 (*t*-Butyldimethyl)silyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranoside (70)

A solution of 67 (3.14g 7.71 mmol) in DMF (50 mL) was stirred with NaH (0.78 g, 60% suspension in mineral oil, washed with pentane 3 times before use) at 0 °C for 0.5 h before methyl iodide (1.2 mL) was added. The reaction mixture was stirred at 0 °C for 15 min when TLC showed that all starting materials had been consumed. Cold water was added until the evolution of bubbles ceased. The resulting mixture was extracted with dichloromethane (3 x 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated to a syrup that was purified by low pressure flash column chromatography using hexane/ethyl acetate (4/1) as eluent to yield **70** as a syrup (3.14 g, 97% yield); $[\alpha]_D^{20}$ 8.5° (*c* 2.31, $CHCl_3$); ^1H NMR (400 MHz) δ 0.10, 0.12 (2 s, 6H, CH_3Si), 0.88 [s, 9H, $SiC(CH_3)_3$], 3.03 (dd, 1H, H-3, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.7 Hz), 3.25 (br d, 1H, H-4,), 3.41 (s, 3H, OCH_3), 3.62 (dd, 1H, H-2, $J_{1,2}$ = 7.8 Hz), 3.97 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 2.0 Hz), 4.13-4.20 (m, 2H, H-5, H-6b), 4.47 (d, 1H, H-1), 5.50 (s, 1H, CHPh), 7.25-7.45 (m, 5H, H-Ph); ^{13}C NMR δ -4.87, -4.14 (2 CH_3Si), 18.0 [$SiC(CH_3)_3$], 25.6 [$SiC(CH_3)_3$], 57.2 (OCH_3), 64.3 (C-2), 66.5 (C-4), 69.3 (C-6), 71.7 (C-5), 79.6 (C-3), 97.3 (C-1), 101.2 (CHPh), 126.4, 128.7, 129.0, 137.7 (C-Ph); exact mass (FAB) calcd for $C_{20}H_{30}N_3O_5Si$ $[M-H]^+$: 420.1955; found 420.196.

2.4.4.2.3 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl trichloroacetimidate (63)

A solution of 70 (1.53 g, 3.64 mmol) in dichloromethane (100 mL) was cooled to -

25 °C under argon and a solution of tetrabutylammonium fluoride (TBAF) in THF (1M, 18.2 mL, 5 equiv) was added dropwise. TLC analysis showed that all the starting material had been consumed in 10 min. The reaction mixture was poured into ice-water and the resulting mixture was extracted with dichloromethane (3 x 100 mL). The combined extracts were dried (Na_2SO_4) and concentrated to a residue, a mixture of anomers of 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-D-galactopyranose. To a solution of the anomeric mixture in dichloromethane (50 mL) was added trichloroacetonitrile (4.5 mL, 45 mmol). The solution was cooled to 0 °C and sodium hydride (0.19 g, 60% suspension, washed with pentane three times before use) was added. The reaction was complete in 1 h. The reaction mixture was filtered through celite and concentrated to a foam that was purified with low pressure flash column chromatography using as eluent, hexane/ethyl acetate, 5/1 to yield **63** as a foam (1.14 g, 69% yield); $[\alpha]_D^{24}$ 128° (*c* 0.84, $CHCl_3$); ^1H NMR (400 MHz) δ 3.56 (s, 3H, OCH_3), 3.87 (m, 2H, H-3, H-5), 4.08 (dd, 1H, H-6a, $J_{6a,6b}$ = -12.7 Hz, $J_{6a,5}$ = 1.5 Hz), 4.15 (dd, 1H, H-2, $J_{2,3}$ = 10.3 Hz), 4.32 (dd, 1H, H-6b, $J_{5,6b}$ = 1.5 Hz), 4.48 (br d, 1H, H-4, $J_{4,3}$ = 2.9 Hz), 5.59 (s, 1H, CHPh), 6.51 (d, 1H, H-1, $J_{1,2}$ = 3.3 Hz), 7.34-7.53 (m, 5H, H-Ph), 8.52 (s, 1H, NH); ^{13}C NMR δ 56.60 (OCH_3), 58.20 (C-2), 65.23(C-4), 69.04 (C-6), 71.78 (C-5), 76.26 (C-3), 95.64 (C-1), 101.1 (CHPh), 126.2, 128.2, 129.1, 137.4 (C-Ph), 160.59 (C=NH).

A slower moving portion (0.31 g) was also obtained, which was mainly the β-isomer of **63**. However, it was difficult to purify because the unreacted intermediate 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-D-galactopyranose was present that had a very similar Rf values in all solvents tested.

2.4.4.3 Synthesis of sodium (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl)-D-ribityl-5-phosphate (59)

2.4.4.3.1 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-*p*-methoxybenzyl-2,3,4-tri-*O*-benzyl-D-ribityl-5-phosphate (95)

To a stirred solution of **64** (0.412 g, 0.62 mmole) and **39** (0.343 g, 1.02 equiv) in acetonitrile (10 mL) under argon was added tetrazole (77.5 mg, 1.8 equiv). After 1 h, TLC showed that all starting material had been consumed. The reaction mixture was cooled to 0 °C and a solution of *m*-chloroperbenzoic acid (MCPBA, 0.544 g, 2.6 equiv) in dichloromethane was added dropwise. The reaction was complete in 20 min. The reaction mixture was diluted with dichloromethane (20 mL) and the resulting solution was washed with TEAB (2 M, 1 x 20 mL) and water (1 x 30 mL). The dichloromethane solution was dried ($MgSO_4$) and concentrated to a residue that was purified by low pressure flash column chromatography using as eluent, hexane/ethyl acetate : 1/1. The title compound **95** was obtained as a colourless syrup (0.480 g, 69% yield); $[\alpha]_D^{20}$ 6.5° (*c* 3.72, $CHCl_3$); ^{31}P NMR δ -0.73, -0.81; ^1H NMR (400 MHz) δ 2.41 (m, 2H, OCH_2CH_2CN), 3.30-4.12 (m, H-1~H-5, H-1'~H-6', OCH_2CH_2CN, OCH_3), 3.49, 3.51, 3.75 (OCH_3), 4.39 (d, H-1, $J_{1,2}$ = 8.3 Hz), 4.15-4.95 (m, CH_2Ph), 6.79-6.84, 7.16-7.35 (H-Ph); ^{13}C NMR δ 19.00 (OCH_2CH_2CN), 55.14, 57.03 (2 x OCH_3), 61.64 (OCH_2CH_2CN), 66.57, 67.45, 67.76 (C-5, 6'), 69.20, 69.30 (C-1), 72.38, 72.89 (CH_2Ph), 73.35 (C-5'), 73.60, 73.69, 74.62, 74.90, 75.48 (CH_2Ph), 78.03 (C-2, C-3, C-4), 77.00, 82.11, 84.26, 84.33 (C-2', C-3', C-4'), 104.6 (C-1'), 116.3 (CN), 113.6, 127.6-129.2, 130.2, 137.8-138.4, 159.1 (C-Ph).

2.4.4.3.2 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 2,3,4-tri-*O*-benzyl-D-ribityl-5-phosphate (74)

To a solution of 95 (0.330 g, 0.294 mmol) in dichloromethane (9 mL) was added water (0.5 mL) and 2,3-dichloro-5,6-dicyano-quinone (DDQ, 0.082 g, 1.2 equiv). The reaction mixture was stirred vigorously at room temperature for 4 h until TLC showed that all starting material had been consumed. The reaction mixture was extracted with saturated sodium hydrogen carbonate (3 x 50 mL), then dried (Na_2SO_4) and concentrated to a residue that was purified with low pressure flash column chromatography to yield 74 as a colourless syrup (0.185 g, 63% yield); $[\alpha]_D^{20}$ 5.3° (*c* 1.36, $CHCl_3$); ^{31}P NMR δ -0.72, -0.77; ^1H NMR (400 MHz) δ 2.24, 2.29 (2 br t, 1-OH), 2.49 (m, OCH_2CH_2CN), 3.39 (m, H-2'), 3.45 (m, H- 5'), 3.50, 3.52 (OCH_3), 3.55 (br s, H-4'), 3.65 (m, H-3'), 3.86-3.95 (m, H-1), 3.99-4.05 (m, OCH_2CH_2CN), 4.18-4.50 (m, H-5, H-6', H-1', $J_{1',2'}$ = 7.9 Hz), 4.53-4.96 (m, CH_2Ph), 7.20-7.70 (H-Ph); ^{13}C NMR δ 19.14 (OCH_2CH_2CN), 57.03 (OCH_3), 61.00, 61.05 (C-1), 61.62, 61.77 (OCH_2CH_2CN), 66.58, 67.23, 67.37 (C-5, 6'), 72.00, 72.32, 72.43 (CH_2Ph), 73.30 (C-5'), 73.79, 73.90, 74.85, 74.91, 75.45 (CH_2Ph), 76.92 (C-4'), 77.73, 77.79, 78.03, 78.75, 78.85 (C-2, C-3, C-4), 82.06 (C-2'), 84.18, 84.25 (C-3'), 104.5 (C-1'), 116.3 (CN), 127.7-128.4, 137.6-138.3 (C-Ph); ESI-MS (g/mol) 1024 for $[C_{57}H_{64}O_{13}NPNa]^+$ $[M+Na]^+$ (100), isotopic peaks: 1025 (calc 65.2, measured 64.9), 1026 (calc 23.5, measured 23.5), 1027 (calc 5.7, measured 5.8); 1040 for $[C_{57}H_{64}O_{13}NPK]^+$ $[M+K]^+$; calculated mass 1001.4 (M).

Following the same procedure, starting from **64** (1.389 g, 2.09 mmol) and **39** (1.133 g, 1.0 equiv), **95** was obtained quantitatively and was treated with water and DDQ directly without further purification. Compound **74** was obtained in a 57% yield (1.190 g) based on **64** and **39**.

2.4.4.3.3 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-D-ribityl-5-phosphate (75)

A solution of **74** (0.845 g, 0.844 mmol) and **63** (0.568 g, 1.2 equiv) in acetonitrile (30 mL) was stirred with 4 Å molecular sieves for 2 h, then cooled to -40 °C and treated with TMS triflate (120 µL, 0.74 equiv). It was shown by TLC that the reaction was complete in 30 min. Pyridine (1 mL) was added and the reaction mixture was filtered through celite. The filtrate was washed with water (3 x 80 mL), dried (Na_2SO_4) and concentrated to a syrup. The product was purified with low pressure flash column chromatography using hexane/ethyl acetate (1/ 1.3) as eluent. The title compound **75** was obtained as a colourless syrup (0.892 g, 82% yield); $[\alpha]_D^{20}$ 8.2° (*c* 3.40, $CHCl_3$); ^{31}P NMR δ - 0.73, - 0.83; ^1H NMR δ 2.39 (t, 2H, OCH_2CH_2CN, J = 6.4 Hz), 3.13 (dd, 1H, H-3", $J_{2",3"}$ = 10.27 Hz, $J_{3",4"}$ = 3.4 Hz), 3.18 (br s, 1H, H-5"), 3.38 (t, 1H, H-2, $J_{1,2}$ = 8.3, $J_{2,3}$ = 8.8 Hz), 3.45 (m, 1H, H-5), 3.48, 3.50 (2 s, 6H, 2 OCH_3), 3.51 (m, 1H, H-4), 3.63 (t, 1H, H-3, $J_{3,4}$ = 8.8 Hz), 3.77 (dd, 1H, H-2", $J_{1",2"}$ = 7.8 Hz), 3.87 (dd, 1H, H-1'$_A$, $J_{1'A,1'B}$ = 10.8

Hz, $J_{1'A,2'}$ = 3.7 Hz), 3.98 (m, H-2, H-3, H-4, H-6', OCH_2CH_2CN), 4.16 (dd, 1H, H-1'b, $J_{1'b,2'}$ = 3.7 Hz), 4.21 (m, H-5'a, 4"), 4.26 (d, H-1"), 4.29 (d, H-1), 4.30 (m, H-6a), 4.37 (m, H-5'b), 4.48 (m, H-6b), 4.55-4.95 (m, 6 CH_2Ph), 5.53 (s, 1H, CHPh), 7.2-7.6 (m, 35H, H-Ph); ^{13}C NMR δ 19.0 (OCH_2CH_2CN), 57.1 (2 x OCH_3), 61.6 (OCH_2CH_2CN), 62.2 (C-2"), 66.4 (C-5"), 66.6 (C-5'), 67.4 (C-6), 68.3 (C-1'), 69.0 (C-6"), 71.5 (C-4"), 72.2 (2 CH_2Ph), 73.3 (C-5), 73.6, 74.6, 74.9, 75.4 (4 CH_2Ph), 77.0 (C-4), 77.8 (3C, C-2', C-3', C-4'), 79.9 (C-3"), 82.1 (C-2), 84.3 (C-3), 101.1 (CHPh), 101.9 (C-1"), 104.5 (C-1), 116.3 (CN), 126.3-128.9, 137.5-138.4 (C-Ph); exact mass (ESI) calcd for $C_{71}H_{79}N_4O_{17}PNa$ $[M+Na]^+$: 1313.5076; found 1313.5092.

2.4.4.3.4 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-D-ribityl-5-phosphate (62)

A solution of **75** (0.651 g, 0.505 mmol) in AcSH (4 mL) was stirred under argon at room temperature for 17 h and then was concentrated to a liquid residue. Low pressure flash column chromatography using ethyl acetate as eluent to yield **62** as a foam (0.493 g, 75% yield); $[\alpha]_D^{20}$ 22.3 ° (*c* 0.35, $CHCl_3$); ^{31}P NMR δ 0.17, -0.68, -0.73, -0.96; ^1H NMR (400 MHz) δ 1.80, 1.81 (2 s, CH_3CO), 2.32-2.36, 2.46-2.50 (m, OCH_2CH_2CN), 3.41, 3.48, 3.51 (3 s, OCH_3), 5.527, 5.533, 5.539 (CHPh), 6.10, 6.38 (2 d, NH, $J_{NH,2''}$ = 6.8, 7.3 Hz), 7.20-7.48 (m, H-Ph); ^{13}C NMR δ 18.99, 19.08, 19.15 (OCH_2CH_2CN), 23.49 (CH_3CO), 54.31, 54.50, 54.62 (C-2"), 56.89, 57.06 (OCH_3), 61.78 (OCH_2CH_2CN), 66.39 (C-5"), 66.67 (C-5'), 67.60 (C-6), 68.41 (C-1'), 69.35 (C-6"), 71.89 (CH_2Ph), 72.36 (C-

4"), 72.68 (CH_2Ph), 73.36 (C-5), 73.47, 74.93, 75.09, 75.49 (4 CH_2Ph), 77.0 (C-4), 77.61, 77.95, 78.20, 78.51, 78.66 (C-2', C-3', C-4', C-3"), 82.13 (C-2), 84.27, 84.35 (C-3), 99.61, 99.89 (C-1"), 101.0 (CHPh), 104.6 (C-1), 116.5 (CN), 126.2-128.9, 137.8-138.4 (C-Ph), 170.9, 171.0 (CH_3CO); exact mass (ESI) calcd for $C_{73}H_{83}N_2O_{18}PNa$ $[M+Na]^+$: 1329.5276; found 1329.5275.

2.4.4.3.5 Triethylammonium (methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-D-ribityl-5-phosphate (76)

A solution of **62** (0.312 g, 0.239 mmol) in a mixture of triethylamine, pyridine and water (3/1/1, 20 mL) was stirred at room temperature for 2 h when TLC showed that all starting material had been consumed. The reaction solution was concentrated to a foam of **76** (0.323 g, 99.6% yield); $[\alpha]_D^{25}$ -8.2° (*c* 0.48, CH_3OH); ^{31}P NMR (CD_3OD) δ 1.41; ^1H NMR (400 MHz, CD_3OD) δ 1.22 (t, 3H, CH_3CH_2), 1.84 (s, 3H, CH_3CO), 2.95 (q, 2H, CH_3CH_2), 3.15-3.55 (m, 12H, H-5", H-2, H-3, H-3", H-4, H-5, 2 OCH_3), 3.34, 3.38 (2 s, 2 x OCH_3), 3.88-4.36 (m, 14H, H-1'~H-5', H-6", H-6, H-2", H-4", H-1), 4.23 (d, H-1, $J_{1,2}$ = 7.3 Hz)), 4.05(br dd, H-2", $J_{1",2"}$ = 8.8 Hz, $J_{2",3"}$ = 10.3 Hz), 4.53 (d, 1H, H-1"), 4.54-4.83 (m, 6 CH_2Ph), 5.54 (s, 1H, CHPh), 7.10-7.48 (m, 35H, H-Ph); ^{13}C NMR (CD_3OD) δ 9.31 (CH_3CH_2), 23.56 (CH_3CO), 47.72 (CH_3CH_2), 52.77 (C-2"), 57.20, 57.52 (2 x OCH_3), 65.70, 66.32 (C-5', C-6), 67.95 (C-5"), 69.81 (C-1'), 70.45 (C-6"), 72.91 (C-4"), 73.53, 74.86, 75.58 (4 CH_2Ph), 75.77 (C-5), 75.95, 76.48 (2 CH_2Ph), 79.22 (C-4), 80.10, 80.28, 80.60 (C-2', C-3', C-4', C-3"), 83.47 (C-2), 85.73 (C-3), 102.2 (CHPh), 102.8 (C-1"), 105.9 (C-1), 127.2-129.9, 139.8-140.2 (C-Ph), 173.6 (C=O); exact mass (ESI) calcd for $C_{70}H_{79}NO_{18}PNa_2$ $[M-NHEt_3+2Na]^+$: 1298.4830; found 1298.4814.

2.4.4.3.6 Sodium (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl)-D-ribityl-5-phosphate (59)

A solution of **76** (0.323 g, 0.238 mmol) in a mixture of ethyl acetate, methanol, and acetic acid (10/14.5/0.5, 25 mL) was stirred with Pd/C (10%, 0.38 g) under hydrogen (1 atm) for 70 h when TLC showed that the reaction was complete. The reaction mixture was filtered through celite and the filtrate was concentrated. A solution of the residue in water (3 mL) was stirred with Amberlite IR-120 (Na^+) resin overnight. The filtrate was concentrated to give **59** quantitatively; $[\alpha]_D^{20}$ -9.9° (*c* 0.68, H_2O); ^{31}P NMR (D_2O) δ 1.73; ^1H NMR (500 MHz, D_2O) δ 2.04 (s, 3H, CH_3CO), 3.28 (m, 1H, H-2, $J_{2,3}$ = 9.4 Hz), 3.40 (s, 3H, 3"-OCH_3), 3.44 (dd, 1H, H-3", $J_{2'',3''}$ = 10.9 Hz), 3.50 (m, 2H, H-3,4), 3.57 (s, 3H, 1-OCH_3), 3.58 (m, 1H, H-5), 3.66 (br dd, 1H, H-5", $J_{5'',6a''}$ = 4.4 Hz $J_{5'',6b''}$ = 8.1 Hz), 3.77 (dd, 1H, H-3', $J_{2',3'}$ = 7.0 Hz, $J_{3',4'}$ = 4.8 Hz), 3.77 (dd, 1H, H-6"a, $J_{6a'',6b''}$ = 11.4 Hz), 3.83 (dd, 1H, H-2', $J_{1',2'}$ = 6.4 Hz), 3.84 (dt, H-6"b), 3.89 (m, 1H, H-4'), 3.93 (dd, 1H, H-2"), 3.95 (d, 2H, H-1'), 3.99 (t, 1H, H-5'a, $J_{5'a,4'}$ = 6.1 Hz), 4.06 (m, 1H, H-5'b, $J_{5'b,5'a}$ = 10.4 Hz, $J_{5'b,4'}$ = 5.4 Hz), 4.07 (m, 1H, H-6a), 4.17 (dddd, 1H, H-6b, $J_{6b,6a}$ = 11.6 Hz, $J_{6b,5}$ = 5.2 Hz, $J_{6b,P}$ = 2.1 Hz), 4.22 (d, 1H, H-4", $J_{4'',3''}$ = 3.3 Hz), 4.39 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.49 (d, 1H, H-1", $J_{1'',2''}$ = 8.6 Hz); ^{13}C NMR (D_2O) δ 23.94 (CH_3CO), 51.99 (C-2"), 57.18 (3"-OCH_3), 58.10 (1-OCH_3), 61.88 (C-6"), 64.35 (C-4"), 64.97 (d, C-6, $J_{6,P}$ = 4.8

Hz), 67.41 (d, C-5', $J_{5',P}$ = 4.8 Hz), 69.87 (C-4), 71.34 (C-2'), 71.41 (C-1'), 71.48 (d, C-4', $J_{4',P}$ = 7.6 Hz), 72.19, (C-3'), 73.86 (C-2), 75.38 (d, C-5, $J_{5,P}$ = 7.6 Hz), 75.78 (C-5"), 76.36 (C-3), 80.70 (C-3"), 102.67 (C-1"), 104.12 (C-1), 175.65 (C=O); exact mass (ESI) calcd for $C_{21}H_{40}NO_{18}PNa$ $[M+H]^+$: 648.1881; found 648.1900; calcd for $C_{21}H_{39}NO_{18}PNa_2$ $[M+Na]^+$: 670.1700; found 670.1697; calcd for $C_{21}H_{38}NO_{18}PNa_3$ $[M-H+2Na]^+$: 692.1519; found 692.1536.

2.4.4.4 Synthesis of (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl)-L-ribityl-5-phosphate (60)

2.4.4.4.1 1-*O*-(2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 5-*O*-*p*-methoxybenzyl-2,3,4-tri-*O*-benzyl-L-ribitol (65) and 1-*O*-(2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) tri-*O*-benzyl-L-ribitol (77)

A solution of 63 (0.460 g, 1.02 mmol) and 39 (0.554 g, 1.02 mmol) in acetonitrile (20 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at room temperature. Then the solution was cooled to -50°C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 130 µL, 0.66 equiv) was added. The reaction solution was stirred at -50°C for 40 min when TLC showed no further change. Pyridine (1 mL) was added and the reaction mixture was filtered through celite. The filtrate was washed with water (3 x 80 mL), dried (Na_2SO_4) and concentrated to a brownish syrup that was purified with low pressure flash column chromatography using hexane/ethyl acetate (2/1) as eluent. The title compound

65 was obtained as a syrup (0.145 g, 17% yield); $[\alpha]_D^{20}$ 2.2° (*c* 0.68, $CHCl_3$); ^1H NMR (400 MHz) δ 3.11 (dd, 1H, H-3', $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.4 Hz), 3.20 (br s, 1H, H-5'), 3.49 (s, 3H, OCH_3), 3.61-4.06 (m, 11H, H-2, H-3, H-4, H-6'a, H-1a, H-5, H-2', OCH_3), 3.75 (s, OCH_3), 4.18-4.34 (m, 4H, H-4', H-6'b, H-1b, H-1', $J_{1',2'}$ = 8.3 Hz), 4.42 (t, 2H, CH_2PhOMe-*p*), 4.55-4.84 (m, 6H, 3 CH_2Ph), 5.53 (s, 1H, CHPh), 6.82 (d, 2H, AA'BB' pattern, H*o*-*p*MPh, J = 8.8 Hz), 7.15-7.55 (m, 22H, H-Ph); ^{13}C NMR δ 55.12, 57.14 (2 x OCH_3), 62.16 (C-2'), 66.34 (C-5'), 69.07, 69.44, 70.31 (C-1, C-6', C-5), 71.63 (C-4'), 72.34, 72.56, 72.84, 73.53 (4 CH_2Ph), 78.22, 78.91, 79.03 (C-2, C-3, C-4), 80.07 (C-3'), 101.1 (CHPh), 102.3 (C-1'), 113.7, 127.2-129.7, 130.4, 137.6-138.7, 159.1 (C-Ph).

Another product, obtained as a syrup (0.118 g, 16% yield), was 2,3,4-tri-*O*-benzyl-1-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-L-ribitol (**77**); $[\alpha]_D^{24}$ -3.5° (*c* 0.31, $CHCl_3$); ^1H NMR (400 MHz) δ 2.29 (br t, 5-OH), 3.14 (dd, 1H, H-3', $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.4 Hz), 3.26 (br s, 1H, H-5'), 3.50 (s, 3H, OCH_3), 3.65-4.07 (m, 8H, H-2, H-3, H-4, H-6'a, H-1a, H-5, H-2'), 4.18-4.34 (m, 4H, H-4', H-6'b, H-1b, H-1'), 4.53-4.86 (m, 6H, 3 CH_2Ph), 5.58 (s, 1H, CHPh), 7.15-7.55 (m, 20H, H-Ph); ^{13}C NMR δ 57.19 (OCH_3), 61.25 (C-5), 62.16 (C-2'), 66.44 (C-5'), 69.45, 69.74 (C-1, C-6'), 71.63 (C-4'), 72.07, 72.70, 73.81 (3 CH_2Ph), 78.78, 79.09 (3C C-2, C-3, C-4), 80.07 (C-3'), 101.2 (CHPh), 102.2 (C-1'), 126.3-128.3, 137.6-138.4 (C-Ph); ESI-MS (g/mol) 706 for $[M+Na-N_2]^+$, 734 for $[M+Na]^+$, 750 for $[M+K]^+$; calculated mass 711.3 (M).

A solution of **65** (0.107 g, 0.128 mmol) in dichloromethane (5 mL) was stirred with water (0.4 mL) and 2,3-dichloro-5,6-dicyano-quinone (DDQ, 0.0327 g, 1.1 equiv) vigorously at room temperature for 4 h until TLC showed that all starting material had been consumed. The reaction mixture was extracted with saturated sodium hydrogen carbonate (3 x 20 mL), then dried (Na_2SO_4) and concentrated to a syrup that was purified with low pressure column chromatography using ethyl acetate and hexane (1 : 1.2) as eluent to yield the product as a colourless syrup (0.059 g, 63% yield). The ^{1}H and ^{13}C NMR spectra of this product were identical in all respects to that of 77 above.

2.4.4.4.2 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-L-ribityl-5-phosphate (78)

To a solution of **64** (0.105 g, 1.1 equiv) and **77** (0.102 g, 0.144 mmol) in acetonitrile (20 mL) was added tetrazole (23.2 mg, 2.3 equiv). The reaction solution was stirred under argon for 40 min when TLC (hexane : ethyl acetate = 1.5 : 1) showed that all the starting material had been consumed. The reaction mixture was cooled to 0 °C and a solution of *m*-chloroperbenzoic acid (MCPBA, 0.142 g, 2.6 equiv) in dichloromethane (5 mL) was added dropwise. The reaction was complete in 20 min. The reaction mixture was diluted with dichromethane (30 mL) and was washed with TEAB (2 M, 1 x 30 mL) and water (1 x 30 mL). Then the organic layer was dried ($MgSO_4$) and concentrated. The residue was purified with low pressure flash column chromatography using hexane/ethyl acetate : 1/2 as eluent. The title compound **78** was obtained as colourless foam (0.155 g, 84% yield); $[\alpha]_D^{25}$ 6.6° (*c* 0.33, $CHCl_3$); ^{31}P NMR δ -0.77, -1.28; ^{1}H NMR (400 MHz) δ 2.42-2.46 (m, 2H, OCH_2CH_2CN), 3.14 (m, H-3"), 3.35 (br d, 1H, H-5"), 3.37 (m, 1H, H-2), 3.49 (m, 1H, H-4), 3.50 (s, OCH_3), 3.63 (m, 1H, H-3), 3.75-3.86 (m, 2H, H-1'a, H-

2"), 3.88-4.15 (m, H-2', H-3', H-4', H-6"a, OCH_2CH_2CN), 4.16-4.53 (m, H-1'b, H-6"b, H-5', H-4", H-1", H-1, H-6), 4.54-4.96 (m, 6 CH_2Ph), 5.54 (s, 1H, CHPh), 7.2-7.6 (m, 35H, H-Ph); ^{13}C NMR δ 19.24 (OCH_2CH_2CN), 57.11, 57.24 (2 x OCH_3), 61.96 (OCH_2CH_2CN), 62.15 (C-2"), 66.45 (C-5"), 66.83 (C-5'), 67.39, 67.69 (C-6), 69.08 (C-6"), 69.59 (C-1'), 71.63 (C-4"), 72.46, 72.65 (2 CH_2Ph), 73.30 (C-5), 73.56, 74.61, 74.94, 75.49 (4 CH_2Ph), 77.0 (C-4), 77.62, 78.18, 78.45 (C-2', C-3', C-4'), 80.06 (C-3"), 82.06 (C-2), 84.26 (C-3), 101.1 (CHPh), 102.2 (C-1"), 104.5 (C-1), 116.2, 116.4 (CN), 126.2-134.4, 137.4-138.3 (C-Ph); exact mass (ESI) calcd for $C_{71}H_{79}N_4O_{17}PNa$ [M+Na]$^+$: 1313.5076; found 1313.5102.

2.4.4.4.3 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-L-ribityl-5-phosphate (79)

A solution of **78** (0.139 g, 0.108 mmol) in thiolacetic acid (2 mL) was stirred under argon at room temperature for 17 h. Then the reaction mixture was concentrated and was purified by low pressure flash column chromatography using ethyl acetate as eluent. The title compound **79** was obtained as a colourless foam (0.066 g, 47% yield); ^{31}P NMR δ -0.03, -0.58, -1.49, -1.50; ^{1}H NMR (400 MHz) δ 1.80, 1.84 (2 s, CH_3CO), 2.34-2.46 (m, OCH_2CH_2CN), 3.30-4.5 (m, H-1~H-6, H-1'~H-5', H-1"~H-6", OCH_3, OCH_2CH_2CN), 3.41 (s, OCH_3), 4.52-4.96 (m, 6 CH_2Ph), 5.54, 5.55 (2 s, CHPh), 5.9, 6.2, 6.5 (2 br, NH), 7.2-7.6 (m, 35H, H-Ph); ^{13}C NMR δ 19.13 (OCH_2CH_2CN), 23.54 (CH_3CO), 54.36 (C-2"), 56.88, 57.16 (2 x OCH_3), 61.91 (OCH_2CH_2CN), 66.46 (C-5"), 66.70, 67.30, 67.84 (C-5', C-6), 68.63, 68.99, 69.40 (C-6", C-1'), 72.23 (C-4"), 72.49 (CH_2Ph), 73.32, 73.39 (C-5), 73.53, 74.59, 74.94, 75.50 (4 CH_2Ph), 76.16, 77.00, 77.62, 78.15, 78.33, 78.49 (C-4, C-2', C-3', C-4', C-3"), 82.10 (C-2), 84.28 (C-3), 99.52, 99.60 (C-1"), 100.9 (CHPh), 104.6 (C-1), 116.5 (CN), 126.2-128.8, 137.8-138.4 (C-Ph), 171.2 (C=O).

2.4.4.4.4 Triethylammonium (methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-L-ribityl-5-phosphate (80)

A solution of 79 (0.066 g, 0.051 mmol) in a mixture of triethylamine, pyridine and water (3/1/1, 5 mL) was stirred at room temperature for 2 h. The solution was concentrated to a foam of 80 (0.068 g, 99.6% yield); $[\alpha]_D^{20}$ -7.9° (*c* 0.30, CH_3OH); ^{31}P NMR (CD_3OD) δ 1.22; ^1H NMR (400 MHz, CD_3OD) δ 1.18 (t, 3H, CH_3CH_2), 1.75 (s, 3H, CH_3CO), 2.97 (q, 2H, CH_3CH_2), 3.22 (dd, 1H, H-2, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.29 Hz), 3.35-3.45 (m, H-4,5,5"), 3.39, 3.43 (2 s, 2 x OCH_3), 3.51 (m, H-3",3), 3.75 (dd, 1H, H-1', $J_{1'A,1'B}$ = -10.8 Hz, $J_{1'A,2'}$ = 6.4 Hz), 3.92 (m, H-3',4'), 4.02 (m, H-2',6), 4.25 (d, 1H, H-1), 4.40 (br d, 1H, H-4", $J_{3",4"}$ = 3.4 Hz), 4.52 (d, 1H, H-1", $J_{1",2"}$ = 8.8 Hz), 4.57-4.83 (m, 6 CH_2Ph), 5.60 (s, 1H, CHPh), 7.1-7.5 (m, 35H, H-Ph); ^{13}C NMR (CD_3OD) δ 9.40 (CH_3CH_2), 23.34 (CH_3CO), 47.67 (CH_3CH_2), 52.83(C-2"), 57.16, 57.57 (2 x OCH_3), 65.85 (C-6), 66.39 (C-5'), 68.04 (C-5"), 70.54 (C-6"), 71.37 (C-1'), 72.93 (C-4"), 73.56, 73.77, 75.00, 75.50 (4 CH_2Ph), 75.73 (C-5), 75.96, 76.41 (2 CH_2Ph), 79.27 (C-4), 80.23

(3C C-3", 2', 3' or 4'), 80.55 (C-3' or 4'), 83.39 (C-2), 85.71 (C-3), 102.2 (CHPh), 103.1 (C-1"), 105.9 (C-1), 127.7-130.0, 138.5-140.5 (C-Ph), 173.5 (C=O); exact mass (ESI) calcd for $C_{70}H_{79}NO_{18}PNa_2$ [M-NHEt$_3$+2Na]$^+$: 1298.4830; found 1298.4808.

2.4.4.4.5 Sodium (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-galactopyranosyl)-L-ribityl-5-phosphate (60)

A solution of **80** (0.323 g, 0.238 mmol) in a mixture of ethyl acetate, methanol, and acetic acid (10/14.5/0.5, 25 mL) was stirred with Pd/C (10%, 0.38 g) under hydrogen (1 atm) for 50 h when TLC showed that the reaction was complete. The reaction mixture was filtered through celite and the filtrate was concentrated. A solution of the residue in water (3 mL) was stirred with Amberlite IR-120 (Na$^+$) resin overnight. The filtrate was concentrated to give **60** quantitatively; $[\alpha]_D^{20}$ -14.2° (*c* 0.12, H_2O); ^{31}P NMR (D_2O) δ 1.71; ^1H NMR (500 MHz) (D_2O) δ 2.04 (s, 3H, CH_3CO), 3.28 (m, 1H, H-2, $J_{2,3}$ = 9.4 Hz), 3.41 (s, 3H, 3"-OCH_3), 3.45 (dd, 1H, H-3", $J_{2'',3''}$ = 11.0 Hz), 3.50 (m, 2H, H-3,4), 3.57 (s, 3H, 1-OCH_3), 3.58 (m, 1H, H-5), 3.66 (br dd, 1H, H-5", $J_{5'',6a''}$ = 4.3 Hz $J_{5'',6b''}$ = 6.9 Hz), 3.740 (dd, 1H, H-1'a, $J_{1'a,1'b}$ = 11.6 Hz, $J_{1'a,2'}$ = 6.3 Hz), 3.744 (t, 1H, H-3', $J_{2',3'}$ = $J_{4',3'}$ = 6.4 Hz), 3.78 (dd, 1H, H-6"a, $J_{6a'',6b''}$ = 11.8 Hz, $J_{6''a,5''}$ = 4.3 Hz), 3.83 (dd, 1H, H-6"b, $J_{6''b,5''}$ = 7.9 Hz), 3.93 (m, 1H, H-4'), 3.94 (dd, H-2", $J_{2''.,3''}$ = 10.6 Hz), 3.95 (m, 1H, H-2'), 3.98 (m, 1H, H-5'a), 4.07 (dd, 1H, H-1'b, $J_{1'b,2'}$ = 5.2 Hz), 4.08 (m, H-5'b), 4.09 (dd, 1H, H-6a, $J_{6a,5}$ = 2.9 Hz), 4.18 (dddd, 1H, H-6b, $J_{6b,6a}$ = 11.6 Hz, $J_{6b,5}$ = 5.1 Hz, $J_{6b,P}$ = 2.1 Hz), 4.22 (d, 1H, H-4", $J_{4'',3''}$ = 3.2 Hz), 4.40 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.48 (d, 1H, H-1", $J_{1'',2''}$ = 8.5 Hz); ^{13}C NMR 23.10 (CH_3CO), 51.98 (C-2"), 57.13 (3"-OCH_3), 58.14 (1-

OCH$_3$), 61.88 (C-6"), 64.30 (C-4"), 64.96 (d, C-6, $J_{6,P}$ = 5.0 Hz), 67.36 (d, C-5', $J_{5',P}$ = 6.3 Hz), 69.87 (C-4), 71.34 (C-2'), 71.67 (d, C-4', $J_{4',P}$ = 7.7 Hz), 71.70 (C-1'), 72.41, (C-3'), 73.89 (C-2), 75.38 (d, C-5, $J_{5,P}$ = 7.8 Hz), 75.77 (C-5"), 76.39 (C-3), 80.63 (C-3"), 103.15 (C-1"), 104.15 (C-1), 175.72 (C=O); exact mass (FAB) calcd for $C_{21}H_{40}NO_{18}PNa$ $[M+H]^+$: 648.1881; found 648.188.

2.4.4.5 Synthesis of sodium (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-ribityl-5-phosphate (61)

2.4.4.5.1 (Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 2-cyanoethyl 1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-L-ribityl-5-phosphate (81)

To a solution of **64** (0.196 g, 1.1 equiv) and **57** (0.240 g, 0.268 mmol) in acetonitrile (20 mL) was added tetrazole (38.9 mg, 2.1 equiv). The reaction mixture was stirred under argon for 40 min when TLC showed that all starting material had been consumed. The reaction mixture was cooled to 0 °C and a solution of *m*-chloroperbenzoic acid (MCPBA, 0.270 g, 2.6 equiv) in dichloromethane was added dropwise. The reaction was complete in 20 min. The reaction mixture was diluted with dichromethane (30 mL), washed with TEAB (2 M, 1 x 25 mL) and water (1 x 30 mL), dried (Na_2SO_4) and concentrated. The residue was purified with low pressure flash column chromatography using hexane/ethyl acetate (1/1) as eluent. The title compound **81** was obtained as a colourless syrup (0.373 g, 94% yield); $[\alpha]_D^{20}$ 0.5° (*c* 2.97, $CHCl_3$);

^{31}P NMR δ -0.64, -1.18, -1.44; ^{1}H NMR (400 MHz) δ 1.71 (s, CH_3CO), 2.41 (m, OCH_2CH_2CN), 3.30-5.0 (m, H-1~H-6, H-1'~H-5', H-1"~H-6", OCH_3, OCH_2CH_2CN, CH_2Ph), 3.48, 3.49 (s, OCH_3), 5.60, 7.50 (2 d, NH, $J_{NH,2"}$ = 7.3 Hz), 7.2-7.6 (m, H-Ph); ^{13}C NMR δ 19.19 (OCH_2CH_2CN), 23.45 (CH_3CO), 54.88 (C-2"), 57.13 (OCH_3), 61.85 (OCH_2CH_2CN), 66.66, 67.27, 67.81 (C-5', C-6), 68.43, 68.69, 68.92 (C-6", C-1'), 72.19, 72.42, 72.55, 73.42, 74.66, 74.97, 75.53 (CH_2Ph), 72.21, 73.42, 77.00, 77.83 (C-4, C-5, C-5", C-4", C-2', C-3', C-4', C-3"), 82.13 (C-2), 84.31 (C-3), 99.98 (C-1"), 104.6 (C-1), 116.5 (CN), 126.2-128.4, 137.8-138.6 (C-Ph), 169.2 (C=O).

2.4.4.5.2 Triethylammonium (methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl) 2,3,4-tri-*O*-benzyl-L-ribityl-5-phosphate (82)

A solution of **81** (0.196 g, 0.133 mmol) in a mixture of triethylamine, pyridine and water (3/1/1, 5 mL) was stirred at room temperature for 2 h. The solution was concentrated to a foam of **82** (0.192 g, 95% yield); $[\alpha]_D^{25}$ 0.4° (*c* 1.10, CH_3OH); ^{31}P NMR (CD_3OD) δ 1.11; ^{1}H NMR (400 MHz, CD_3OD) δ 1.11 (t, 9H, CH_3CH_2, J = 7.3 Hz), 1.71 (s, 3H, CH_3CO), 2.90 (q, 6H, CH_3CH_2), 3.18 (dd, 1H, H-2, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 8.8 Hz), 3.36 (m, 2H, H-4, H-5), 3.38 (s, 3H, OCH_3), 3.45-3.63 (m, 5H, H-3, H-6", H-5", H-3"), 3.61 (dd, 1H, H-1'$_A$, $J_{1'A,1'B}$ = 10.5 Hz, $J_{1'A,2'}$ = 6.6 Hz), 3.83-3.42 (m, 6H, H-2', H-3', H-4', H-4", H-6), 4.20 (d, H-1), 4.47 (d, H-1", $J_{1",2"}$ = 8.8 Hz), 4.15-4.83 (m, 9 CH_2Ph), 7.1-7.45 (m, 45H, H-Ph); ^{13}C NMR (CD_3OD) δ 9.30 (CH_3CH_2), 23.4 (CH_3CO), 47.5 (CH_3CH_2), 53.7 (C-2"), 57.6 (OCH_3), 65.8, 66.3 (C-5', 6), 70.2 (C-6"), 71.0 (C-1'), 73.4, 73.6 (3 CH_2Ph), 74.4 (C-4"), 74.5 (CH_2Ph), 74.8 (C-5"), 75.0, 75.5 (2 CH_2Ph), 75.6 (C-5), 75.9, 76.4 (3 CH_2Ph), 79.2 (C-4), 80.0, 80.35, 80.34, (C-2', C-3', C-4'), 81.6 (C-3"), 83.4 (C-2), 85.7 (C-3), 103.1 (C-1"), 105.8 (C-1), 128.4-131.4, 139.5-140.4 (C-Ph), 173.3 (C=O).

2.4.4.5.3 Sodium (methyl β-D-glucopyranosid-6-yl) 1-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-ribityl-5-phosphate (61)

A solution of **82** (0.192 g, 0.13 mmol) in a mixture of ethyl acetate, methanol, and acetic acid (10/14.5/0.5, 25 mL) was stirred with Pd/C (10%, 0.30 g) under hydrogen (1 atm) for 48 h when TLC showed that the reaction was complete. The reaction mixture was filtered through celite and the filtrate was concentrated. A solution of the residue in water (3 mL) was stirred with Amberlite IR-120 (Na^+) resin for 12 h. The filtrate was concentrated to give **61** quantitatively; $[\alpha]_D^{20}$ -15.2° (*c* 0.11, H_2O); ^{31}P NMR (D_2O) δ 1.72; ^1H NMR (400 MHz, D_2O) δ 2.06 (s, 3H, CH_3CO), 3.28 (m, 1H, H-2), 3.47-3.53 (s, 4H, H-5, OCH_3), 3.67 (m, 1H, H-5"), 3.72-3.84 (m, 5H, H-4', H-3', H-1'a, H-6"), 3.90-4.01 (m, 5H, H-4", H-3", H-2", H-2', H-5'a), 4.05-4.12 (m, 3H, H-6a, H-5'b, H-1'b), 4.16-4.21 (m, 1H, H-6b), 4.40 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.49 (d, 1H, H-1", $J_{1",2"}$ = 8.5 Hz); ^{13}C NMR (D_2O) δ 23.11 (CH_3CO), 53.27 (C-2"), 58.12 (OCH_3), 61.82 (C-6"), 64.98 (d, C-6, $J_{6,P}$ = 4.8 Hz), 67.38 (d, C-5', $J_{5',P}$ = 4.8 Hz), 68.64 (C-4"), 69.89 (C-4), 71.36 (C-2'), 71.49 (d, C-4', $J_{4',P}$ = 4.8 Hz), 71.69 (C-1'), 71.75(C-3"), 72.43 (C-3'), 73.89 (C-2), 75.38 (d, C-5, $J_{5,P}$ = 8.6 Hz), 75.89 (C-5"), 76.39 (C-3), 103.02 (C-1"), 104.14 (C-1), 175.84 (C=O); exact mass (FAB) calcd for $C_{20}H_{38}NO_{18}PNa$ $[M+H]^+$: 634.1725; found 634.173.

2.4.4.6 Synthesis of other derivatives of (*t*-butyldimethyl)silyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside

2.4.4.6.1 (*t*-Butyldimethyl)silyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (69)

A solution of **67** (0.50 g, 1.23 mmol) in DMF (10 mL) was treated with NaH (0.13 g, 60% suspension in mineral oil, washed with pentane 3 times before use) at 0 °C. The resulting mixture was treated with benzyl bromide (0.23 mL, 1.5 equiv). The reaction mixture was stirred at 0 °C for 20 min when TLC showed that all starting material had been consumed. Cold water was added until evolution of bubbles ceased. The resulting mixture was extracted with dichloromethane (3 x 30 mL). The combined dichloromethane solutions were dried (Na_2SO_4) and concentrated to a syrup. Low pressure flash column chromatography using hexane/ethyl acetate (5/1) as eluant yielded **69** as a colourless solid (0.28 g, 46% yield); mp 82-83 °C; $[\alpha]_D^{24}$ 11.1° (*c* 7.22, $CHCl_3$); ^{1}H NMR (400 MHz) δ 0.14, 0.18 (2 s, 6H, CH_3Si), 0.94 [s, 9H, $SiC(CH_3)_3$], 3.26 (br d, H-4,), 3.30 (dd, 1H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.4 Hz), 3.76 (dd, 1H, H-2, $J_{1,2}$ = 7.6 Hz), 3.98 (dd, 1H, dd, H-6a, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 1.8 Hz), 4.04 (br d, 1H, H-5, $J_{4,5}$ = 3.7 Hz), 4.33 (dd, 1H, H-6b, $J_{6b,5}$ = 1.5 Hz), 4.52 (d, 1H, H-1), 4.72 (s, 2H, CH_2Ph), 5.45 (s, 1H, CHPh), 7.24-7.31 (m, 10H, H-Ph); ^{13}C NMR δ -4.82, -4.06 (2 CH_3Si), 18.0 [$C(CH_3)_3$], 25.7 [$C(CH_3)_3$], 64.5 (C-2), 66.5 (C-4), 69.3 (C-6), 71.5 (CH_2Ph), 72.5 (C-5), 77.4 (C-3), 97.3 (C-1), 101.1 (CHPh), 126.4-129.0, 137.77, 137.85 (C-Ph).

2.4.4.6.2 Synthesis of *t*-butyldimethylsilyl 2-azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α, β-D-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (68)

A solution of **50** (0.260 g, 0.42 mmol) and 67 (0.255 g, 0.63 mmol) in acetonitrile (5 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at room temperature. Then the temperature of the solution was lowered to -37°C and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 50 μL, 0.26 mmol) was added. The reaction mixture was stirred at ~ -38°C for 30 min when TLC showed no further change in composition. Pyridine (1 mL) was added and the reaction mixture was filtered through celite. The filtrate was washed with water (3 x 80 mL), dried (Na_2SO_4) and concentrated to a brownish syrup that was purified with dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing by 5%. Compound **68** was obtained as a colourless syrup (0.190 g, 52% yield); ^{1}H NMR (400 MHz) δ 0.16, 0.17 (2 s, CH_3Si), 0.94 [$SiC(CH_3)_3$], 3.23 (br s, H-5), 3.51-3.62 (m, H-3, H-6'), 3.77 (dd, H-2, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.3 Hz), 3.79 (m, H-2'), 4.01 (br dd, H-6), 4.05 (br d, H-5'), 4.11 (H-3'), 4.23 (m, H-4, H-4'), 4.48 (d, H-1), 4.45-4.90 (m, CH_2Ph), 5.14 (H-1', $J_{1',2'}$ = 3.9 Hz), 5.47 (s, integration 0.0061, CHPh for β-disaccharide), 5.54 (s, integration 1.0000 CHPh for α-disaccharide), 7.25-7.56 (m, H-Ph); ^{13}C NMR δ **68α** 59.1 (C-2'), 66.8 (C-2), 66.3 (C-5), 68.7 (C-6'), 69.2 (C-6), 70.0 (C-4'), 70.4 (C-4), 72.3, 73.1 (2 CH_2Ph), 73.3 (C-3), 73.6 (C-5'), 74.7 (CH_2Ph), 76.7 (C-3'), 94.2 (C-1'), 97.1 (C-1), 100.6 (CHPh); **68β** 62.9, 64.8, 66.5, 68.7, 68.9, 70.4, 72.4, 72.8, 73.4, 74.6, 75.0, 77.3, 80.6, 97.5 (C-1), 100.6 (CHPh), 103.4 (C-1').

The α-isomer crystallized from ethyl acetate and hexane: $[\alpha]_D^{20}$ -2.5° (*c* 0.20, $CHCl_3$); mp 65-67 °C; exact mass (FAB) calcd for $C_{46}H_{55}N_6O_9Si$ $[M-H]^+$: 863.3799, $C_{46}H_{57}N_4O_9Si$ $[M+H-N_2]^+$: 837.3895; found 863.3731, 837.3888.

2.4.4.7 Synthesis of monosaccharide derivatives containing 2-acetamido-4-amino-2,4,6-trideoxy-galactopyranoside unit

2.4.4.7.1 4,6-*O*-*p*-Methoxybenzylidene-D-glucal (86)

To a solution of 3,4,6-tri-*O*-acetyl-D-glucal[193] (**85**, 16.07 g, 59.1 mmol) in *p*-methoxybenzaldehyde (15 mL) was added a thick milky mixture of $ZnCl_2$ (9.62 g, 1.2 equiv) in *p*-methoxybenzaldehyde (50 mL). The resulting gelatinous mixture was kept at room temperature for 4 h and then poured into ice-water (300 mL). The mixture was extracted with chloroform (3 x 80 mL). The organic layer was dried (Na_2SO_4) and concentrated to a residue that was separated with dry flash column chromatography using gradient elution from hexane to ethyl acetate with the amount of ethyl acetate increasing gradually by 5%. Compound **86** was obtained as a colourless solid (1.811 g, 12% yield); mp 132-133 °C; $[\alpha]_D^{20}$ -18.9° (*c* 0.65, $CHCl_3$); ^1H NMR δ 3.85 (s, 3H, OCH_3), 3.90 (m, 3H, H-4, H-5, H-6a), 4.35 (m, 1H, H-6b), 4.50 (m, 1H, H-3), 4.75 (dd, 1H, H-2), 5.55 (s, 1H, CHPh), 6.40 (dd, 1H, H-1), 6.90 (d, 2H, *o*-H pMPh), 7.42 (d, 2H, *m*-H *p*MPh); ^{13}C NMR δ 55.3 (OCH_3), 66.5 (C-3), 68.3 (C-6), 68.4 (C-5), 80.7 (C-4), 101.8 (CHPh), 103.7 (C-2), 113.7, 127.6, 132.3, 160.3 (C-PhOMe), 144.1 (C-1).

2.4.4.7.2 3-*O*-Allyl-4,6-*O*-*p*-methoxybenzylidene-D-glucal (87)

To a solution of **86** (0.515 g, 2 mmol) in DMF (15 mL) was added NaH (0.20 g, 60% suspension in mineral oil, washed with pentane before use) and then allyl bromide (0.5 mL, 1.5 equiv). The reaction mixture was stirred at room temperature for 18 h when TLC showed that all the starting material had been consumed. Cold water was added until the evolution of bubbles ceased. The resulting mixture was extracted with pentane (6 x 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated to a solid. Dry flash column chromatography using hexane/ethyl acetate (3/1) as eluant afforded **87** as colourless needles (0.505 g, 85% yield); mp 91-92°C; $[\alpha]_D^{20}$ -44.5° (*c* 0.42, $CHCl_3$); ^1H NMR δ 3.80 (s, 3H, OCH_3), 3.78-3.98 (m, 3H, H-5, H-6), 4.12-4.37 (m, 4H, H-3, H-4, CH_2 All), 4.80 (dd, 1H, H-2, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 1.8 Hz), 5.23 (m, 2H, CH_2= All), 5.58 (s, 1H, CHPh), 5.85-6.00 (m, 1H, -CH= All), 6.35 (dd, 1H, H-1, $J_{1,3}$ = 1.5 Hz), 6.90 (d, 2H, 1/2 AA'BB' pattern, *o*-H pMPh, J = 8.7 Hz), 7.42 (d, 2H, *m*-H pMPh); ^{13}C NMR δ 55.3 (OCH_3), 66.5 (C-3), 68.3 (C-6), 68.4 (C-5), 80.7 (C-4), 101.8 (CHPh), 103.7 (C-2), 113.7, 127.6, 132.3, 160.3 (C-PhOMep), 144.1 (C-1).

Chapter 3 Selective alkylation of methyl 4,6-*O*-benzylidene-D-glycopyranosides via dialkylstannyleneacetal intermediates

3.1 General

Regioselective alkylations of carbohydrates are of interest to obtain synthetic intermediates and the many naturally occurring compounds that contain *O*-methyl and other *O*-alkyl groups. Several methods are available for the regioselective introduction of alkyl groups: reductive cleavage of benzylidene groups,[197,198] activation of hydroxyl groups through the formation of tributylstannyl ethers,[199-202] phase transfer catalysis,[1203] selective activation through copper chelates,[204] activation via electrochemical reduction,[205] and the selective activation of diols with dialkylstannylene acetals.[199,200] A number of different synthetic procedures have been used in the last technique. An evaluation of the regioselectivity obtained with existing procedures and the approach adopted here to obtaining improved regioselectivity is described here.

Dibutylstannylene acetals often undergo acylation and oxidation reactions with excellent regioselectivity.[199,200,206] However, alkylation reactions on the same substrate often occur with considerably less regioselectivity. For instance, benzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**96**) in benzene gave only the 2-*O*-benzoyl derivative in 93% yield;[207] benzylation with benzyl bromide in DMF gave the 2-*O*-benzyl and 3-*O*-benzyl derivatives in 70% and 20% yields, respectively,[208] while methylation with methyl iodide in DMF was even less selective, yielding 57% of the 2-*O*-methyl derivative and 30% of the 3-*O*-methyl derivative.[207] Benzoylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside in benzene gave the 3-*O*-benzoate in 95% yield;[207] benzylation in benzene containing tetrabutylammonium bromide at reflux gave a mixture of the 3- and 2-*O*-benzyl ethers in 55% and 10% yields, respectively.[209]

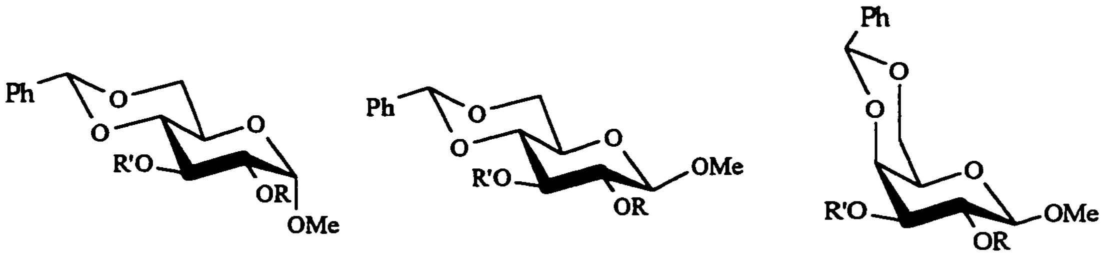
Dialkylstannylene acetals exist in solutions of non-polar solvents as mixtures of dimers and higher oligomers, of which one dimer is often predominant.[206,210,211] When equilibratio of the two most populated dimers (dimer 1 and dimer 2) is fast relative to the rate of reaction, it was shown that: regioselectivity = 2 x (rate constant from dimer 1 / rate constant from dimer 2) x ([dimer 1] / [dimer 2]). It was thought that the regioselectivity of alkylation reactions could be improved by employing reaction conditions under which dimers are the most important species present in solution, that is, in non-polar solvents without added nucleophiles. An investigation of the effects of these reaction conditions and also the effects of using different alkyl groups on tin on the regioselectivity obtained in alkylation reactions of dialkylstannylene acetals derived from three hexopyranosides containing *trans*-1,2-diol units are carried out here and the results are as follows.

3.2 Results and discussion

Benzylation and methylation reactions have been performed under a variety of conditions on stannylene acetals derived from *trans*-1,2-diols on pyranose rings in methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**96**), methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**97**), and methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**98**). Table 3.1 summarizes the results obtained for these compounds, including literature results, and also including literature results for related compounds. The structures of compounds 96-

110 are shown in Figure 3.1.

As mentioned in the introduction, reaction of the dibutylstannylene acetal of **96** with benzyl bromide in DMF gave a 2-*O*-benzyl / 3-*O*-benzyl mixture in the ratio of 70 to 20.[208] Better selectivity for benzylation of **96** was obtained by performing the reaction in neat benzyl bromide particularly if a dialkyltin derivative having a larger alkyl group on tin was employed. Dialkyltin oxides, other than butyl or *t*-butyl are not available commercially but can be prepared easily from the alkyl halide.[212] The best regioselectivity was obtained using the dihexylstannylene acetal in benzyl bromide, which gave a selectivity of 82:2. Reaction of the dibutylstannylene acetal of **96** with 2-nitrobenzyl bromide and tetrabutylammonium bromide in acetonitrile yielded 24% of 2-*O*-substitution



96	R = R' = H	**97**	R = R' = H	**98**	R = R' = H
99	R = Bn, R' = H	**101**	R = Bn, R' = H	**103**	R = Bn, R' = H
100	R = H, R' = Bn	**102**	R = H, R' = Bn	**104**	R = H, R' = Bn
105	R = Me, R' = H	**107**	R = Me, R' = H	**109**	R = Me, R' = H
106	R = H, R' = Me	**108**	R = H, R' = Me	**110**	R = H, R' = Me

Figure 3.1 The structures of 96-110

Table 3.1 The alkylations of methyl 4,6-*O*-benzylidene-D-glycopyranosides

Compound		Conditions					% yield		Ref
	alkyl group in stannylene	Solvent[a]	Temp (°C)	time (h)	reagent	added nucleophile[b]	O-2	O-3	
96	bu	DMF, BnBr	100	2	BnBr	none	70	20	208
	bu	CH_3CN	80	24	2-NO_2BnBr	none	24	12	213
	bu	BnBr	85	30	BnBr	none	74	8	
	hex	BnBr	85	96	BnBr	none	82	2	
	bu	DMF	45	20	MeI	none	57	30	207
	bu	TCE	130	22	MeI	none	84	13	
	neohex	TCE	130	22	MeI	none	89	10	
	bu	DCE	25	10	MeTf	none	64	14	
	bu	$CHCl_3$	25	1	MeTf	none	90	8	
97	bu	benzene	80	18	BnBr	TBAB	29	61	215
	bu	BnBr	85	34	BnBr	none	46	48	
	bu	DMF	45	10	MeI	none	22	66	215
	bu	TCE	130	22	MeI	none	52	46	
	neohex	TCE	130	22	MeI	none	62	36	
	isobu	TCE	130	22	MeI	none	46	53	
	bu	$CHCl_3$	25	1	MeTf	none	34	64	
	bu	benzene	80	18	AllBr	TBAB	33	60	215
98	bu	benzene	80	c	BnBr	TBAB	10	55	209
	bu	BnBr	85	45	BnBr	none	15	72	
	hex	BnBr	85	24	BnBr	none	20	73	
	hexamethylene	BnBr	85	29	BnBr	none	16	61	
	bu[d]	TCE	130	27	MeI	none	5	77	
	bu	DCE	25	1	MeTf	none	6	77	
	bu	$CHCl_3$	25	1	MeTf	none	7	72	

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[a] TCE is 1,1,2,2-tetrachloroethane, DCE is 1,2-dichloroethane. [b] TBAB is tetrabutylammonium bromide. [c] Not reported. [d] 16% yield of 2,3-di-*O*-methyl derivative also isolated.

and 12% of 3-*O*-substitution.[213] Phase transfer catalysed benzylation[213] and allylation[214] of **96** gave 54% and 52.5% of the O-2 products and 20% and 20% of the O-3 products, respectively.

The conditions developed here gave better yields for benzylation of **98** than previously reported[209] but the regioselectivity was not improved. For **97**, the regioselectivity under these conditions was altered to favour reaction at O-2 more but good regioselectivity was not achieved. O-2 is thought[206] to be dicoordinate in the most populated dimer of the dibutylstannylene acetal of **97** and hence should be favoured for the reaction more under the conditions used here. Good regioselectivity (70%) for benzylation at O-3 of **97** can be obtained by reaction of benzyl bromide and tetrabutylammonium iodide with the copper chelate in THF.[216]

The conditions most commonly used for methylation and those previously used for **96** and **97** are methyl iodide in DMF at 45 °C. Regioselectivity under these conditions was much worse than that achieved in benzylation for **96**[208] and slightly more in favour of O-3 for **97**.[215] In the ^{119}Sn NMR spectra of stannylene acetals recorded in DMF, all signals were very broad.[217] This observation indicates that an exchange process is occurring in solution, most likely between the species that are predominant in non-coordinating solvents, the dimers, with a species having a considerably different chemical shift, most likely the coordinated monomer.[217]

Two sets of methylation conditions have been developed that give better regioselectivity than that previously reported. Reaction with the dibutylstannylene acetal of **96** in 1,1,2,2-tetrachloroethane (TCE) with methyl iodide at 130 °C gave an O-2/O-3 product ratio of 84 to 13, much better than ratio of 57 to 30 obtained previously.[193] Further improvement to 89 to 10 was achieved if the dihexylstannylene acetal was employed. Application of these conditions to **97** reversed the direction of the regioselectivity obtained under normal conditions[215] of 22 to 66 of O-2/ O-3. Yield ratios for reaction at O-2 to O-3 of 52 to 46 were obtained using the dibutylstannylene acetal and 62 to 36 using the dineohexylstannylene acetal. Methylation had not been performed previously on the dibutylstannylene acetal of **98**. In TCE, excellent regioselectivity (77 to 5) for reaction at O-3 over O-2 of was obtained.

It was hoped that better regioselectivity could be obtained using a more active methylating reagent at lower reaction temperatures. Methyl triflate reacts with stannylene acetals in chloroform at room temperature more rapidly than does methyl iodide in TCE at 130 °C. The regioselectivity obtained is of the same order as in TCE but varies from compound to compound. For instance, that obtained for the dibutylstannylene acetal of **96** was better, 90 to 8 versus 84 to 13 in TCE. However, nearly identical regioselectivity was obtained for the dibutylstannylene acetal of **98** and that for the dibutylstannylene acetal of **97** was similar to the result in DMF.

3.3 Experimental

3.3.1.1 General method for benzylation. Benzylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (96) via its dibutylstannylene acetal Compound 96 (0.280 g, 1.0 mmol) and dibutyltin oxide (0.249 g, 1.0 equiv) were refluxed in toluene (40 mL) for 12 h in a Dean-Stark apparatus. The solvent was evaporated then benzyl bromide (10 mL) was added. The mixture was stirred at 80-90 °C until TLC indicated that the starting material had disappeared (30 h). The reaction mixture was cooled, diluted with chloroform (20 mL), and the solution was washed with water (50 mL). The organic layer was dried ($MgSO_4$) and concentrated. The remaining liquid residue was fractionated by flash chromatography using a solvent gradient changing from toluene to ethyl acetate to give methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (99, 0.275 g, 74% yield), which crystallized from ethyl acetate as fine colourless needles: mp 123-124 °C; $[\alpha]_D^{25}$ +33.7° (*c* 8.0, chloroform); lit.[203] 131-132 °C, $[\alpha]_D^{25}$ +35°; ^{13}C NMR δ 55.4 (MeO), 62.1 (C-5), 69.0 (C-6), 70.3 (C-3), 73.4 (CH_2Ph), 79.6 (C-4), 81.3 (C-2), 98.7 (C-1), 102.0 (CHPh), 126.4-138.0 (Ar-C); and methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (100, 0.029 g, 8% yield), which was a colourless solid, mp 162-3 °C, $[\alpha]_D^{25}$ +54.3° (*c* 0.4, chloroform); lit.[218] 183-5 °C, $[\alpha]_D^{14}$ +36.2°; lit.[219] 178-180 °C, $[\alpha]_D^{20}$ +47°; ^{13}C NMR δ 55.4 (MeO), 62.6 (C-5), 69.0 (C-6), 72.4 (C-2), 74.8 (CH_2Ph), 78.9 (C-4), 82.0 (C-3), 99.9 (C-1), 101.3 (CHPh), 126.1-138.5 (Ar-C).

3.3.1.2 Benzylation of 96 via its dihexylstannylene acetal intermediate

Compound **96** (0.284 g, 1.0 mmol) was reacted with dihexyltin oxide (0.306 g, 1.0 equiv) in toluene (25 mL), then with excess benzyl bromide (6 mL) for 96 h as in the general procedure in section 3.3.1.1. Flash column chromatography with gradient elution from toluene : ethyl acetate (15:1) to ethyl acetate yielded **99** (0.308 g, 82% yield) and

100 (6 mg, 2% yield).

3.3.1.3 Benzylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (97) via its dibutylstannylene acetal intermediate

Compound **97** (0.283 g, 1.0 mmol) was reacted with dibutyltin oxide (0.249 g, 1.0 equiv) in toluene (28 mL), then with excess benzyl bromide (6 mL) for 34 h as in the general procedure in section 3.3.1.1. Dry flash chromatography with gradient elution from toluene: ethyl acetate (15:1) to ethyl acetate yielded methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**101**, 0.170 g, 46% yield); mp 113-114 °C; $[\alpha]_D^{25}$ -24.0° (*c* 1.82, chloroform); lit.[218] 124-125 °C; $[\alpha]_D^{25}$ -26.0°; ^{13}C NMR δ 57.5, (CH_3O), 66.4 (C-5), 68.7 (C-6), 74.2 (C-2), 74.7 (CH_2Ph), 80.2 (C-4), 81.5 (C-3), 101.3 (CHPh), 104.2 (C-1), 126-137 (Ar-C); and methyl 3-O-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**102**, 0.180 g, 48% yield); mp 177-178 °C, $[\alpha]_D^{25}$ -40.0° (*c* 0.14, chloroform); lit.[218] mp 180°, $[\alpha]_D^{25}$ -45.5°.

3.3.1.4 Benzylation of 97 via its diisobutylstannylene acetal and dineohexylstannylene acetal intermediates

Reaction of **97** (1.0 mmol) with diisobutyltin oxide or dineohexyltin oxide (1.0 equiv) in toluene (28 mL), then with excess benzyl bromide (6 mL) for 42 h was performed as in the general procedure in section 3.3.1.1. TLC indicated that two products were obtained in approximately equal amounts.

3.3.1.5 Benzylation of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (98) via its dibutylstannylene acetal intermediate

Reaction of **98** (0.281 g, 1.0 mmol) with dibutyltin oxide (0.251 g, 1.0 equiv) in toluene (30 mL), then with excess benzyl bromide (6 mL) for 45 h was performed as in the general procedure in section 3.3.1.1. A dry flash column chromatography with an eluent of hexane : ethyl acetate (2:1) yielded methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**103**, 0.055 g, 15% yield), a colourless solid; $[\alpha]_D^{24}$ +21.0° (*c* 1.15, chloroform); lit.[220] mp 108-111°C; $[\alpha]_D^{25}$ +22°; and methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**104**, 0.268 g, 72% yield); needles; mp 199-200 °C, $[\alpha]_D^{25}$ +56.2° (*c* 0.48, chloroform); lit.[220] 200-201 °C, $[\alpha]_D^{25}$ +56°.

3.3.1.6 Benzylation of 98 via its dihexylstannylene acetal intermediate

Reaction of **98** (0.280 g, 1.0 mmol) with dihexyltin oxide (0.311 g, 1.0 equiv) in toluene (30 mL), then with excess benzyl bromide (6 mL) for 24 h was performed as in the general procedure in section 3.3.1.1. Compounds **103** (0.060 g, 20% yield) and **104** (0.224 g, 73% yield) were obtained along with trace amount of starting material.

3.3.1.7 Benzylation of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (98) via its hexamethylenestannylene acetal intermediate

Reaction of **98** (0.280 g, 1.0 mmol) with hexamethylenetin oxide (0.222 g, 1.0 equiv) in toluene (40 mL), then with excess benzyl bromide (6 mL) for 29 h was performed as in the general procedure in section 3.3.1.1. Compounds **103** (0.056 g, 16%

yield) and **104** (0.212 g, 61% yield) were obtained with starting material (0.016 g, 4% yield).

3.3.2.1 General method for methylation of methyl 4,6-*O*-benzylidene-D-hexopyranosides with methyl iodide. Methylation of 96 via dibutylstannylene acetal intermediate

Compound **96** (0.283 g, 1.0 mmol) and dibutyltin oxide (0.249 g, 1.0 equiv) were refluxed in toluene (30 mL) for 12 h in a Dean-Stark apparatus. The solvent was evaporated, then 1,1,2,2-tetrachloroethane (30 mL) and methyl iodide (3 mL) were added subsequently under nitrogen. The mixture was stirred at 130 °C until TLC indicated that the starting material had disappeared (22 h). The reaction mixture was cooled, diluted with chloroform (20 mL) and the solution was washed with water (50 mL). The organic layer was dried ($MgSO_4$) and concentrated. The remaining liquid residue was fractionated by dry flash chromatography using a solvent gradient changing from hexane to hexane : ethyl acetate (2:1) to give methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside (**105**, 0.039 g, 13% yield); mp 144-145°C; $[\alpha]_D^{25}$ +101.6° (*c* 0.37, chloroform); lit.[218] 153-4 °C; $[\alpha]_D^{25}$ +114.4°; ^{1}H NMR δ 3.45 (s, 3H, CH3O), 3.55 (m, 2H, H-3, H-4), 3.6-3.72 (m, 1H, H-2), 3.66 (s, 3H, CH_3O), 3.74 (m, 2H, H-6a, H-5), 4.28 (dd, 1H, $J_{5,6e}$ = 3.6 Hz, $J_{6a,6e}$ = 9.1 Hz, H-6e), 4.81 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 5.55 (s, 1H, CHPh), 7.20-7.60 (m, 5H, ArH); ^{13}C NMR δ 55.4 (OCH_3), 61.1 (OMe), 62.5 (C-5), 69.0 (C-6), 76.5 (C-2), 80.6 (C-4), 82.0 (C-3), 99.8 (C-1), 101.3 (CHPh), 126.0-137.3 (Ar-C); and methyl 4,6-O-benzylidene-2-*O*-methyl-α-D-glucopyranoside (**106**, 0.249 g, 84% yield), crystallized from ethyl acetate as fine needles: mp 170-171 °C; $[\alpha]_D^{25}$ +91.1° (*c* 0.73, chloroform); lit.[218] mp 166 °C; $[\alpha]_D^{25}$ +96.4°.

3.3.2.2 Methylation of 96 via its dineohexylstannylene acetal intermediate

Reaction of **96** (0.284 g, 1.0 mmol) with dineohexyltin oxide (0.305 g, 1.0 equiv) in toluene (30 mL), then with excess methyl iodide (3 mL) in 1,1,2,2-tetrachloroethane (30 mL) for 22 h as in the general procedure in section 3.3.2.1 yielded **105** (0.030 g) and **106** (0.264 g) in 10% and 89% yields, respectively.

3.3.2.3 Methylation of 97 via its dibutylstannylene acetal intermediate

Reaction of **97** (0.284 g, 1.0 mmol) with dibutyltin oxide (0.249 g, 1.0 equiv) in toluene (28 mL), then with excess methyl iodide (3 mL) in 1,1,2,2-tetrachloroethane (30 mL) for 22 h as in the general procedure in section 3.3.2.1 yielded methyl 4,6-*O*-benzylidene-2-*O*-methyl-β-D-glucopyranoside (**107**, 0.155 g, 52% yield), mp 176-177 °C, $[\alpha]_D^{25}$ -65.4° (*c* 1.65, chloroform); lit.[219] 175-176 °C; $[\alpha]_D^{25}$ -67.3°; and methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside (**108**, 0.138 g, 46% yield), mp 174-175 °C, $[\alpha]_D^{25}$ -50.8° (*c* 0.54, chloroform); lit.[218] 172-3 °C, $[\alpha]_D^{25}$ -40.0°.

3.3.2.4 Methylation of 97 via its dineohexylstannylene acetal intermediate

Reaction of **97** (0.283 g, 1.0 mmol) with dineohexyltin oxide (0.305 g, 1.0 equiv) in toluene (28 mL), then with excess methyl iodide (3 mL) in 1,1,2,2-tetrachloroethane (30 mL) for 22 h as in the general procedure in section 3.3.2.1 yielded **107** (0.183 g, 62%

yield) and **108** (0.108 g, 36% yield).

3.3.2.5 Methylation of 97 via its diisobutylstannylene acetal intermediate

Reaction of **97** (0.284 g, 1.0 mmol) with diisobutyltin oxide (0.249 g, 1.0 equiv) in toluene (28 mL), then with excess methyl iodide (3 mL) in 1,1,2,2-tetrachloroethane (25 mL) for 22 h as in the general procedure in section 3.3.2.1 yielded **107** (0.139 g, 46% yield) and **108** (0.157 g, 53% yield).

3.3.2.6 Methylation of 98 via its dibutylstannylene acetal intermediate

Reaction of **98** (0.368 g, 1.3 mmol) with dibutyltin oxide (0.251 g, 1.0 equiv) in toluene (25 mL), then with excess methyl iodide (3 mL) in 1,1,2,2-tetrachloroethane (20 mL) was performed for 27 h as in the general procedure in section 3.3.2.1. Dry flash column chromatography of the product mixture with chloroform : ethyl acetate (from 3:1 to 1:2) as eluent yielded methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-β-D-galactopyranoside (**111**, 0.066 g, 16% yield): mp 148-149 °C; $[\alpha]_D^{25}$ +15.8° (*c* 0.94, chloroform); lit.[221] 148 °C; $[\alpha]_D^{25}$ +18.2°; methyl 4,6-*O*-benzylidene-2-*O*-methyl-β-D-galactopyranoside (**109**, 0.019 g, 5% yield): mp 148-150 °C; $[\alpha]_D^{25}$ -3.2° (*c* 0.50, chloroform); lit.[222] 169-171 °C; $[\alpha]_D^{25}$ -29°; ^{13}C NMR δ 57.0 (OCH_3), 60.9 (OCH_3), 66.4 (C-3), 69.1 (C-6), 72.5 (C-5), 75.5 (C-4), 80.7 (C-2), 101.5 (CHPh), 104.5 (C-1), 126-137 (Ar-C); and methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-galactopyranoside (**110**, 0.296 g, 77% yield), mp 211-2 °C, $[\alpha]_D^{25}$ +31.7° (*c* 0.24, chloroform); lit.[223] 216-7 °C, $[\alpha]_D^{25}$ +25°.

3.3.3.1 General method for methylation of methyl 4,6-*O*-benzylidene-D-hexopyranosides with methyl triflate. Methylation of 96 via its dibutylstannylene acetal in 1,2-dichloroethane

Compound **96** (0.274 g, 1.0 mmol) and dibutyltin oxide (0.330 g, 1.2 equiv) were refluxed in toluene (25 mL) for 12 h in a Dean-Stark apparatus. The solvent was evaporated, then 1,2-dichloroethane (10 mL) and methyl triflate (0.8 mL) were added subsequently under nitrogen. The mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with chloroform (20 mL) and the solution was washed with water (40 mL). The organic layer was dried ($MgSO_4$) and concentrated. The remaining liquid residue was fractionated by flash column chromatography using hexane/ethyl acetate (2/1) as eluant to give **105** (0.040 g, 14%), **106** (0.177 g, 64% yield), and starting material **96** (0.011 g, 4% yield).

3.3.3.2 Methylation of 96 via its dibutylstannylene acetal in chloroform

Compound **96** (0.281 g, 1.0 mmol) and dibutyltin oxide (0.302 g, 1.2 equiv) were refluxed in toluene (40 mL) for 8 h in a Dean-Stark apparatus. The solvent was evaporated, then anhydrous chloroform (10 mL) and methyl triflate (0.6 mL) were added subsequently under nitrogen. The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with chloroform (20 mL) and the solution was washed with aqueous sodium bicarbonate solution (40 mL). The organic layer was dried ($MgSO_4$) and concentrated. The remaining liquid residue was fractionated by flash column chromatography as in the general procedure in section 3.3.3.1 to give **105** (0.024 g, 8%

yield), and **106** (0.266 g, 90% yield).

3.3.3.3 Methylation of 97 via its dibutylstannylene acetal in chloroform

Compound **97** (0.278 g, 1.0 mmol) and dibutyltin oxide (0.306 g, 1.2 equiv) were refluxed in toluene (30 mL) for 12 h in a Dean-Stark apparatus. Methylation with methyl triflate (0.6 mL) in 5 mL chloroform was complete within 1 h. Dry flash column chromatography gave **107** (0.099 g, 34% yield) and **108** (0.187 g, 64% yield).

3.3.3.4 Methylation of 98 via its dibutylstannylene acetal in 1,2-dichloroethane

Reaction of **98** (0.278 g, 1.0 mmol) with dibutyltin oxide (0.306 g, 1.2 equiv) in toluene (25 mL), then with excess methyl triflate (0.8 mL) in 1,2-dichloroethane (10 mL) was performed for 1 h as in the general procedure in section 3.3.3.1. Flash coumn chromatography of the product mixture with chloroform : ethyl acetate (2:1) as eluent yielded **109** (0.018 g, 6% yield) and **110** (0.225 g, 77% yield).

3.3.3.5 Methylation of 98 via its dibutylstannylene acetal in chloroform

Reaction of **98** (0.275 g, 1.0 mmol) with dibutyltin oxide (0.296 g, 1.2 equiv) in toluene (20 mL), then with excess methyl triflate (0.6 mL) in chloroform (5 mL) was performed for 1 h as in section 3.3.3.4, yielding **109** (0.022 g, 7% yield) and **110** (0.208 g, 72% yield).

Chapter 4 Synthesis of disaccharides and carbohydrates with heterocyclic rings via dibutylstannylene acetal intermediates

4.1 General

As seen in Chapter 1, much effort has been devoted to the development of a mild, efficient, and stereospecific procedure for the synthesis of oligosaccharides. One possible approach to an efficient synthesis of oligosaccharides is to increase the nucleophilicity of the oxygen atom of the aglycone compared with that of a free hydroxy group. Reaction with dibutyltin oxide to form a dibutylstannylene acetal has been shown to be an effective method in this regard.[199] Alkylation and acylation of primary and secondary 1,2-diols via stannylene acetal intermediates mostly occur selectively on the primary hydroxyl groups. It was deduced that dibutylstannylene acetal derivatives of primary-secondary 1,2-diol might be glycosylated in the same way as in the alkylation or acylation. Stannyl ethers had been used to synthesize simple glycosides successfully.[223] The dibutylstannylene acetal had also been used successfully in the synthesis of a trisaccharide.[224] However, quite often orthoesters were formed. Few applications of this method have been published. As part of the study, disaccharide formation via stannylene acetals was further investigated here.

An effort was also made to synthesize heterocycles via dialkylstannylene acetal intermediates. Many heterocyclic compounds function as drugs. Hopefully, forming heterocyclic rings using carbohydrate-based stannylene acetals could give novel heterocycles.

4.2 Results and discussion

For the investigation of the disaccharide synthesis via stannylene acetal intermediates, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**32**) was selected as the glycosyl donor and 3,4-di-*O*-benzyl-D-mannitol (**112**) as the glycosyl acceptor. Unfortunately, the major product obtained was not the expected disaccharide, but an orthoester (**113**) instead. Under the conditions employed here, the oxonium ion resulting from participation of the 2-*O*- acyl group reacts selectively at the orthoester centre rather than the anomeric centre. None of the expected disaccharide was detected by NMR techniques. A long range ^{13}C-^{1}H HETCOR spectrum showed the product to be an 1,2-orthoester unambiguously. To avoid the formation of an orthoester, the glycosyl donor was changed to 2,3,4,6-tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (**114**[225]). As the structure of **114** (Figure 4.1) shows, when the 2-*O*-pivalate participates, the orthoester

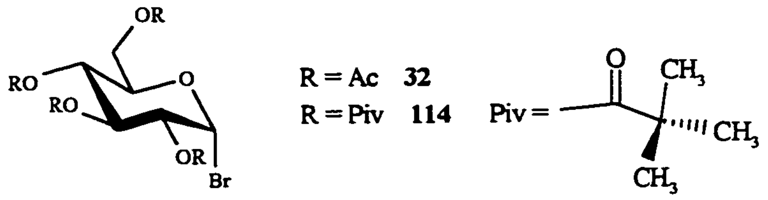
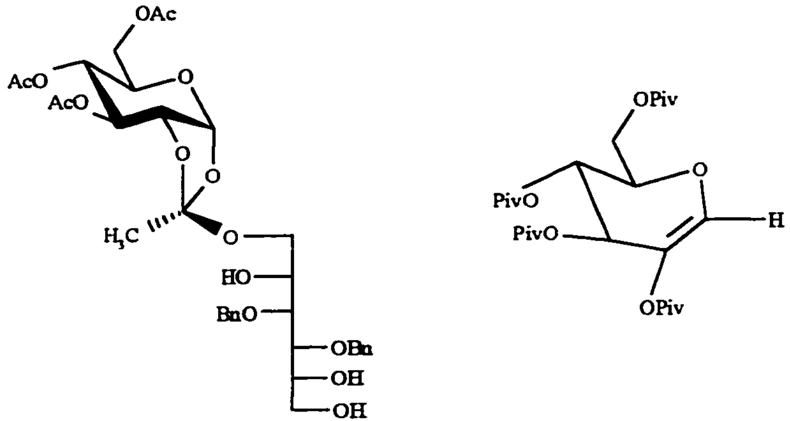


Figure 4.1 The structures of 32 and 114

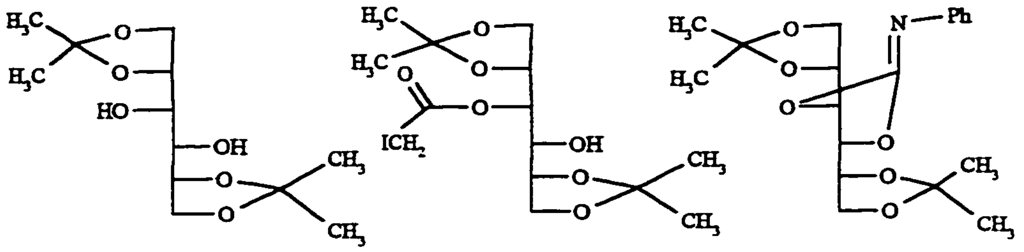
centre would be too sterically hindered to be attacked, thus disaccharide would be expected. However, the major product obtained was a glycal (**115**). The ^{1}H NMR spectrum shows that there is a sharp singlet for H-1, indicating that there are no protons at C-2. The ^{1}H and ^{13}C NMR spectra clearly showed that there were four pivaloyl groups in the product. In the HMQC NMR spectrum, the signal at δ 139.0 in the ^{13}C NMR correlated with the δ 6.6 signal for H-1 in the ^{1}H NMR spectrum. The other alkene area signal at δ 127.9 was from C-2, a quaternary carbon. This result suggested that **114** was unstable in the presence of the dibutylstannylene acetal. Instead of a nucleophilic substitution, a 1,2-elimination of HBr occurred. Figure 4.2 shows the structures of the products (**113** and **115**) from the above two reactions.



113 **115**

Figure 4.2 The structures of 113 and 115

To form a heterocyclic ring, 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**116**) was reacted with iodoacetyl chloride[226] via its dibutylstannylene acetal. The product obtained was identified as 3-*O*-iodoacetyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol (**117**). The peak at δ -6 in the ^{13}C NMR spectrum clearly indicates that the iodine is still attached. An effort was made to form the ring product by intramolecular alkylation via the tributylstannyl ether. However, deacylation of **117** resulted.



116 **117** **118**

Figure 4.3 The structures of 116-118

Some 1,3-dioxolane-2-imine derivatives had been reported to be synthesized by reactions of phenyl isothiocyanate with simple glycol- based stannylene acetals.[227] It was hoped that this kind of heterocyclic ring derivatives could also be formed via carbohydrate-based stannylene acetals. Compound **116** was reacted with phenyl isothiocyanate via the dibutylstannylene acetal intermediate in the presence of triethylamine. A heterocyclic ring was formed. The signal at δ 152.2 in the ^{13}C NMR spectrum suggested the presence of the carbonimidoyl group. Compound **118** is a new compound. The structures of **116-118** are shown in Figure 4.3.

4.3 Experimental

4.3.1 Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (32) with 3,4-di-*O*-benzyl-D-mannitol (112) via its dibutylstannylene acetal intermediate

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**32**), prepared by the procedure described by Vogel[164] as a white powdered solid, was used without purification; mp 82-83 °C; lit.[164] 88-89 °C.

Compound **112** (0.359 g, 1.0 mmol) and dibutyltin oxide (0.309 g, 1.2 equiv) were refluxed in toluene (22 mL) for 11 h in a Dean-Stark apparatus. Then the toluene was evaporated and the residue was dissolved in 1,2-dichloroethane (15 mL). Then 4 Å molecular sieves (1 g), tetrabutylammonium iodide (0.77 g, 2.1 mmol) and **32** (0.889 g, 2 mmol, in 10 mL 1,2-dichloroethane) was added. The reaction solution was stirred at room temperature for 23 h, then heated up to 85 °C for another 9 h until TLC showed no more change in the reaction. Then the reaction solution was diluted with dichloromethane. The solution was washed with water once, then dried ($MgSO_4$) and concentrated to a dark coloured residue that was purified using a dry flash column chromatography with chloroform as the eluent. The major product was obtained as a liquid residue (0.426 g, 62% yield) of the 3,4,6-tri-*O*-acetyl-α-D-glucopyranose-1,2-orthoester of 3,4-di-*O*-benzyl-D-mannitol (**113**); ^{1}H NMR δ 1.67 (s, 3H, CH_3-CO_3), 2.07 (s, 9H, 3 $CH_3C{=}O$), 3.65 (m, 2H, H-1'), 3.75 (br d, 2H, H,3', 4'), 3.95 (m, 3H, H-5, 2', 5'), 4.17 (m, 4H, H-6, 6'), 4.29 (t, 1H, H-2), 4.62 (q, 4H, 2 CH_2Ph), 4.87 (dd, 1H, $J_{3,4}$ = 3.1

Hz, $J_{4,5}$ = 9.5 Hz, H-4), 5.14 (t, 1H, $J_{2,3}$ = 3.1 Hz, H-3), 5.65 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1), 7.3 (m, 10H, H-Ph); ^{13}C NMR δ 20.7 (3 CH_3C=O), 21.2 (CH_3-CO_3), 62.9 (C-6, 6'), 64.8 (C-1'), 67.1 (C-5,5'), 67.9 (C-4), 69.6 (C-2'), 70.1 (C-3), 73.4 (C-2), 73.7 (2 CH_2Ph), 77.8 (C-3', 4'), 96.9 (C-1), 121.4 (CO_3CH_3), 121.8, 128.0, 128.4, 137.7 (C-Ph), 169.1, 169.5, 170.6 (3 CH_3C=O).

4.3.2 Reaction of 2,3,4,6-tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (114) with 112 via its dibutylstannylene acetal intermediate

Compound **114** was prepared by the procedure described by Kunz and Harreus[225] as colourless needle crystals, mp 140-141°C; lit.[225] mp 142-143 °C.

3,4-Di-*O*-benzyl-D-mannitol (**112**, 0.352 g, 1.0 mmol) and dibutyltin oxide (0.251 g, 1.0 equiv) were refluxed in toluene (22 mL) for 12 h in a Dean-Stark apparatus. Then the toluene was evaporated and cesium fluoride (0.198 g, 1.3 equiv) was added. The reaction flask was kept under vacuum for 2 h. Then DMF (6 mL) was added to dissolve the residue and **114** (0.695 g, 1.2 mmol) was added. The reaction solution was stirred at room temperature for 2.5 h, then evaporated under vacuum and the residue was dissolved in ethyl acetate (25 mL). The ethyl acetate solution was dried ($MgSO_4$) and concentrated to a residue that was separated using a dry flash column chromatography with gradient elution from hexane to ethyl acetate. The major product was obtained as a white solid of 2,3,4,6-tetra-*O*-pivaloyl-D-glucal (**115**, 0.210 g, 44% yield); mp 81-82 °C; ^{1}H NMR δ 1.3 (m, 36H, 12 CH_3), 4.4 (m, 3H, H-5,6), 5.2 (t, 1H, H-4), 5.6 (d, 1H, H-3), 6.6 (s, 1H, H-1); ^{13}C NMR δ 27.1 (CH_3-Piv), 38.8 (q-C-tBu), 60.9 (C-6), 66.4 (C-3), 67.4 (C-4), 74.2

(C-5), 127.9 (C-2), 139.0 (C-1), 176.6,177.1,177.2,178.0 (4 O=C-Piv).

4.3.3 Reaction of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (116) with iodoacetyl chloride via its dibutylstannylene acetal intermediate

Compound **116** (2.709 g, 10.0 mmol) and dibutyltin oxide (2.506 g, 1.0 equiv) were refluxed in toluene (170 mL) for 12 h in a Dean-Stark apparatus. Then iodoacetyl chloride (prepared from 2.947 g, 15.0 mmol of iodoacetic acid[226]) was added. It was stirred at room temperature for 15 h until TLC showed that all starting materials had been consumed. The reaction mixture was concentrated to a liquid residue that was purified with dry flash column chromatography using gradient elution from hexane to ethyl acetate. 3-*O*-Iodoacetyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol (**117**) was obtained as a yellowish gel (3.3.82 g, 76% yield); ^{13}C NMR δ -6 (**CH_2I**), 25.1, 25.2, 26.5, 26.8 (4 CH_3), 66.5 (2C C-1 and C-6), 71.1, 73.8, 74.7, 74.9 (C-2, 3, 4, 5), 109.4, 109.7 (q-C-isopropyl), 168.0 (-C=O).

4.3.4 Reaction of 116 with phenyl isothiocyanate via its dibutylstannylene acetal intermediate

Compound **73** (0.265 g, 1.0 mmol) and dibutyltin oxide (0.253 g, 1.0 equiv) were refluxed in toluene (27 mL) for 12 h in a Dean-Stark apparatus. The toluene was evaporated and the residue was dissolved in 1,2-dichloroethane (15 mL). Then phenyl isothiocyanate (120 μL, 1.0 mmol) and triethylamine (140 μL, 1.0 mmol) was added. The reaction mixture was stirred at 70 °C for 48 h until TLC showed that all starting materials had been consumed. The reaction solution was diluted with chloroform (20 mL) and extracted with water (1 x 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to a residue that was purified with dry flash column chromatography using hexane and ethyl acetate (2:1) as eluent to give 1,2:5,6-di-*O*-isopropylidene-3,4-*O*-(N-phenyl-carbonimidoyl)-D-mannitol (**118**) as a colourless solid (0.313 g, 85% yield); mp 113-114 °C; ^{13}C NMR δ 24.8, 26.5, 26.6 (4 CH_3), 65.3, 65.5 (2C C-1 and C-6), 74.4, 74.5(C-2, 5), 77.4, 80.3 (C-3, 4), 110.5, 110.6 (2 q-C-isopropyl), 123.3, 123.5, 128.7 (C-Ph), 144.8 (q-C-Ph), 152.2 (-C=N-).

Anal. calcd for $C_{19}H_{25}O_6N$ (363.4096): C 62.8, H 6.93, N 3.85; found C 62.5, H 6.85, N 3.73.

Chapter 5 Concluding remarks

As shown in previous chapters, oligosaccharide synthesis is a difficult and tedious task. Comprehensive synthetic procedures need to be carefully designed beforehand. For each glycosidic linkage, both stereochemistry and regiochemistry need to be considered. One needs to be familiar with all the glycosylation strategies as well as protection and deprotection strategies. On the other hand, it is rewarding that one can obtain many analogues during the preparation of the target molecule. These are valuable sources for drug candidates. In addition, once the synthetic procedure is completed, it can be modified to produce efficiently pure oligosaccharides on a large scale. The structure of the synthetic compounds can be assigned unambiguously, and this provides solid evidence for the structure of natural products, like the configuration of the ribitol unit in the C-polysaccharide of *Streptococcus pneumoniae*.

As shown in this thesis, NMR is an indispensable tool in oligosaccharide identification. The protons and carbons on the carbohydrate rings resonate at more or less the same region in both ^{1}H NMR and ^{13}C NMR, respectively. 1D spectra give only limited information. 2D experiments are routinely required to solve the structure problem of oligosaccharides.

As seen in Chapter 2, for the determination of the configuration of the ribitol residue in the repeating unit of the C-polysaccharide of *Streptococcus pneumoniae*, substituted ribitol derivatives with both D- and L- configurations were designed and synthesized. Disaccharide and trisaccharide phosphates containing these ribitol units were also designed and synthesized. The configuration of the ribitol unit in the C-polysaccharide of *Streptococcus pneumoniae* was determined to be D. For the total synthesis of the repeating unit of the C-polysaccharide, a retrosynthetic analysis was performed. A synthetic procedure was proposed for the synthesis of the 2-acetamido-4-amino-2,4,6-tri-deoxy-galactopyranose residue and part of it was completed.

Despite the successful synthesis of disaccharide and trisaccharide phosphates containing ribitol units having both D- and L- configurations, there were problems in the glycosylation of the L-ribitol derivative **39**. It seemed that the primary *p*-methoxybenzyl (pMBn) group was not compatible with TMS triflate, which resulted in low yields in the glycosylation step. In future, for the synthesis of the L-ribitol containing oligosaccharides, it is suggested that another protecting group be used instead of pMBn, such as *t*-butyldiphenylsilyl group.

Alkylation via dibutylstannylene acetal methods once again proved to be a good choice for regioselective protection. Regioselectivity can be increased by increasing the size of the alkyl group on the tin atom.

More and more evidence shows that carbohydrates play many important roles in living organisms. Oligosaccharide synthesis is an indispensable way to provide enough materials for research, drug screening, and other purposes. It is hoped that both the regioselective alkylation of carbohydrate diols via dialkylstannylene acetal intermediates and the methodology adopted here for oligosaccharide synthesis, in addition to the oligosaccharide phosphates synthesized can be useful to other research endeavours.

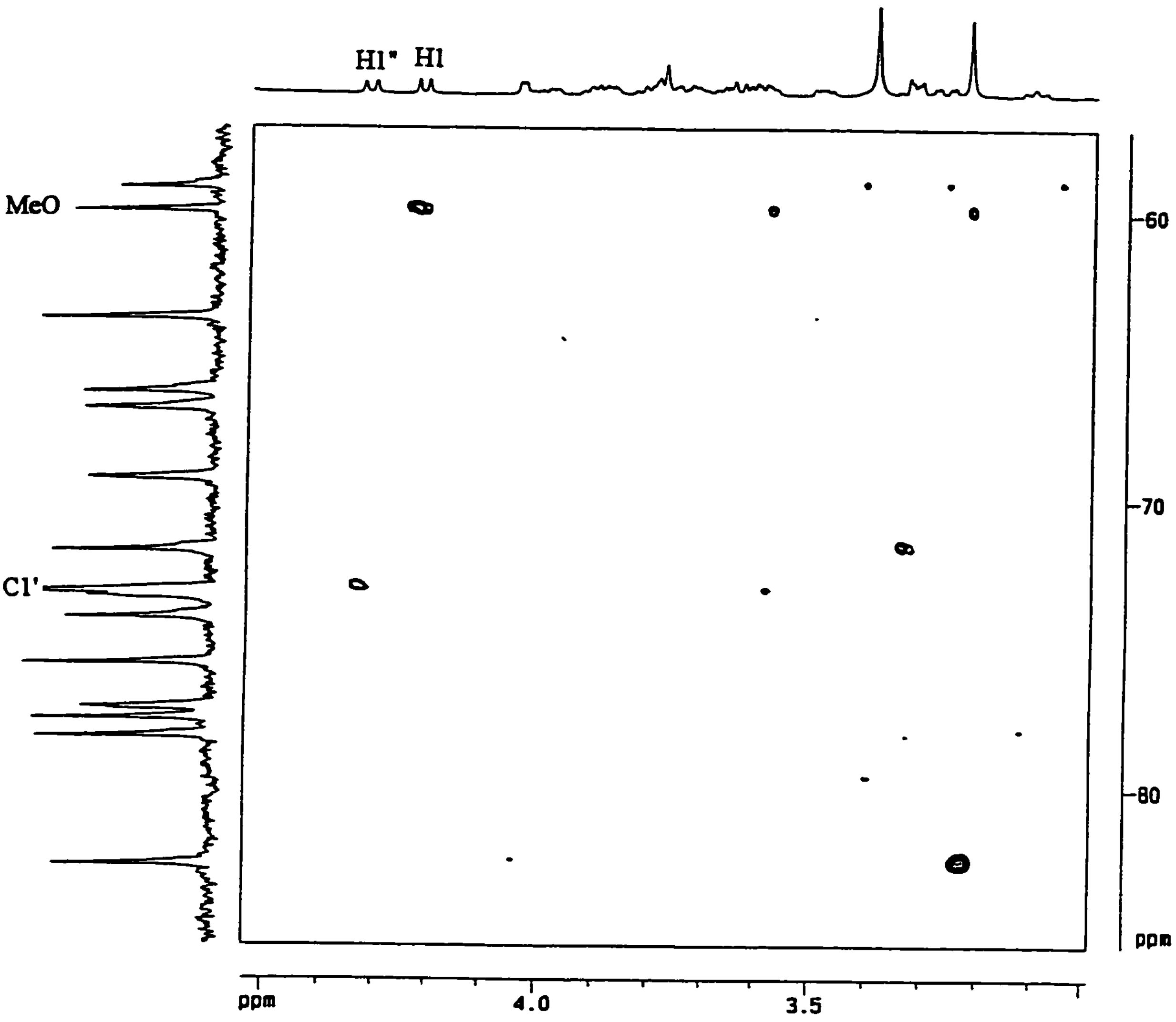


Figure A.1 The 400 MHz HMBC spectrum of 59

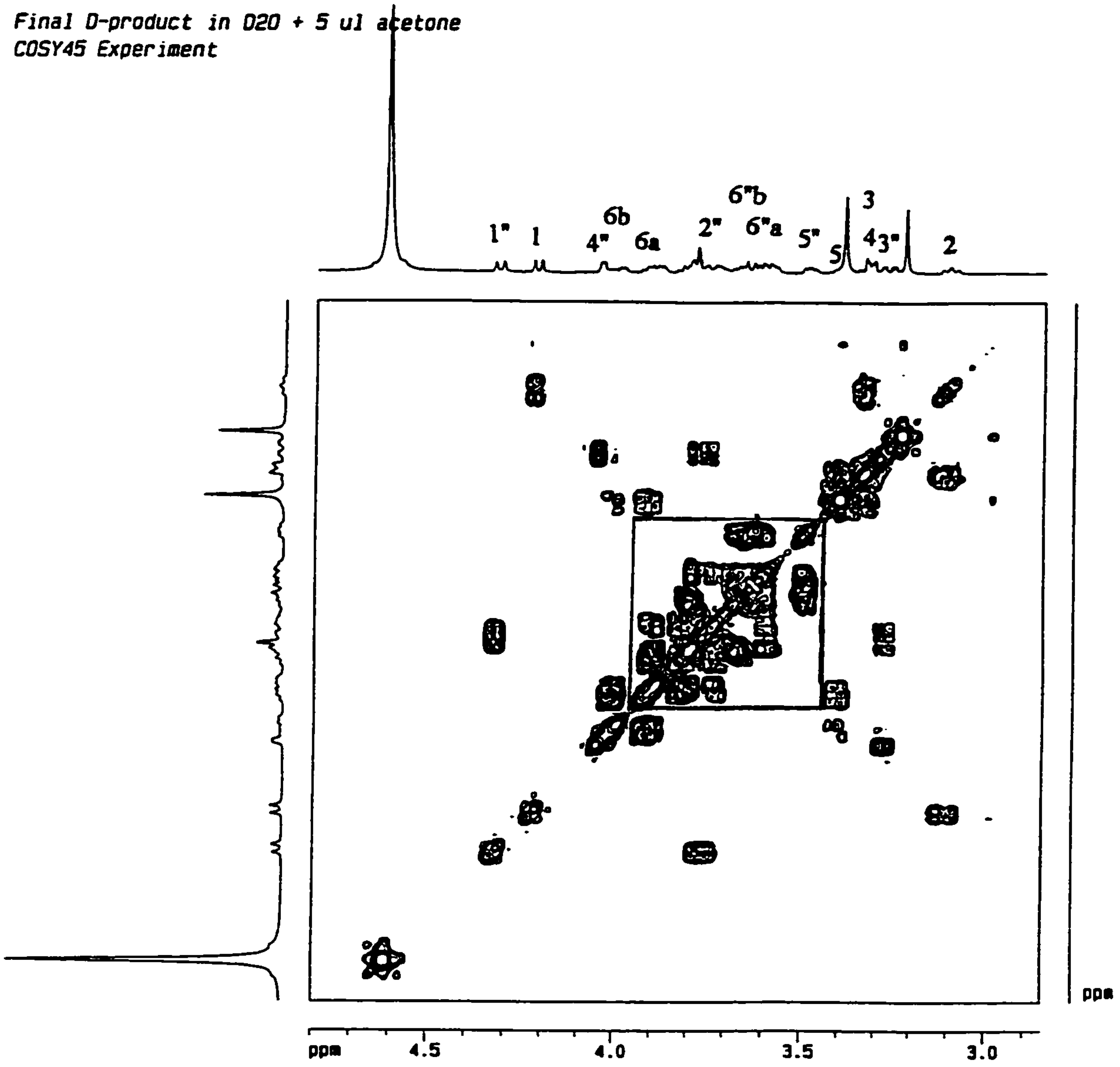


Figure A.2 The 400 MHz COSY spectrum of 59

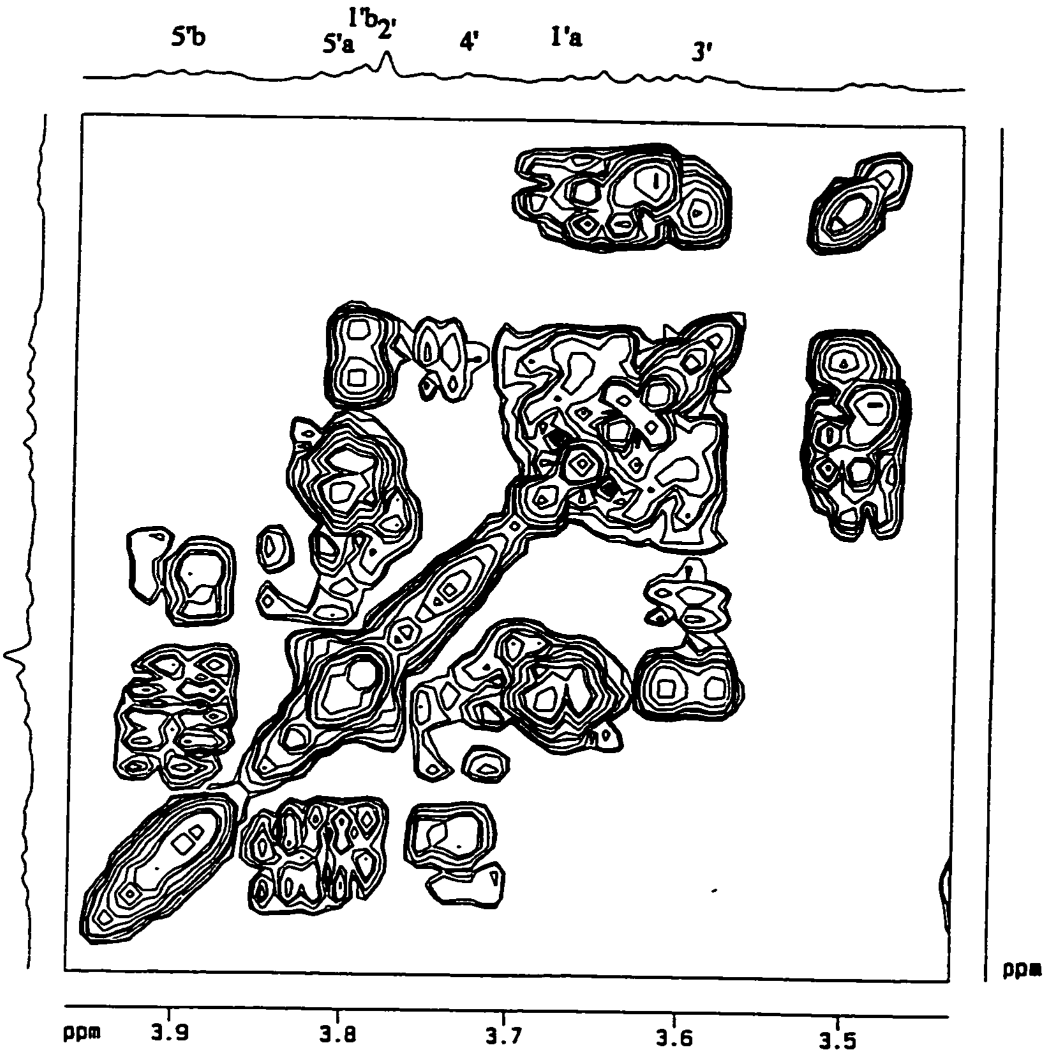
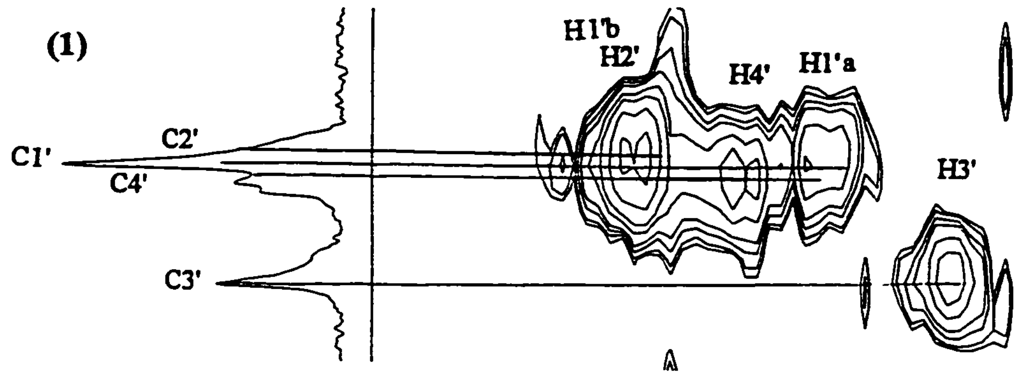


Figure A.3 An expansion of the specified part of Figure A.2



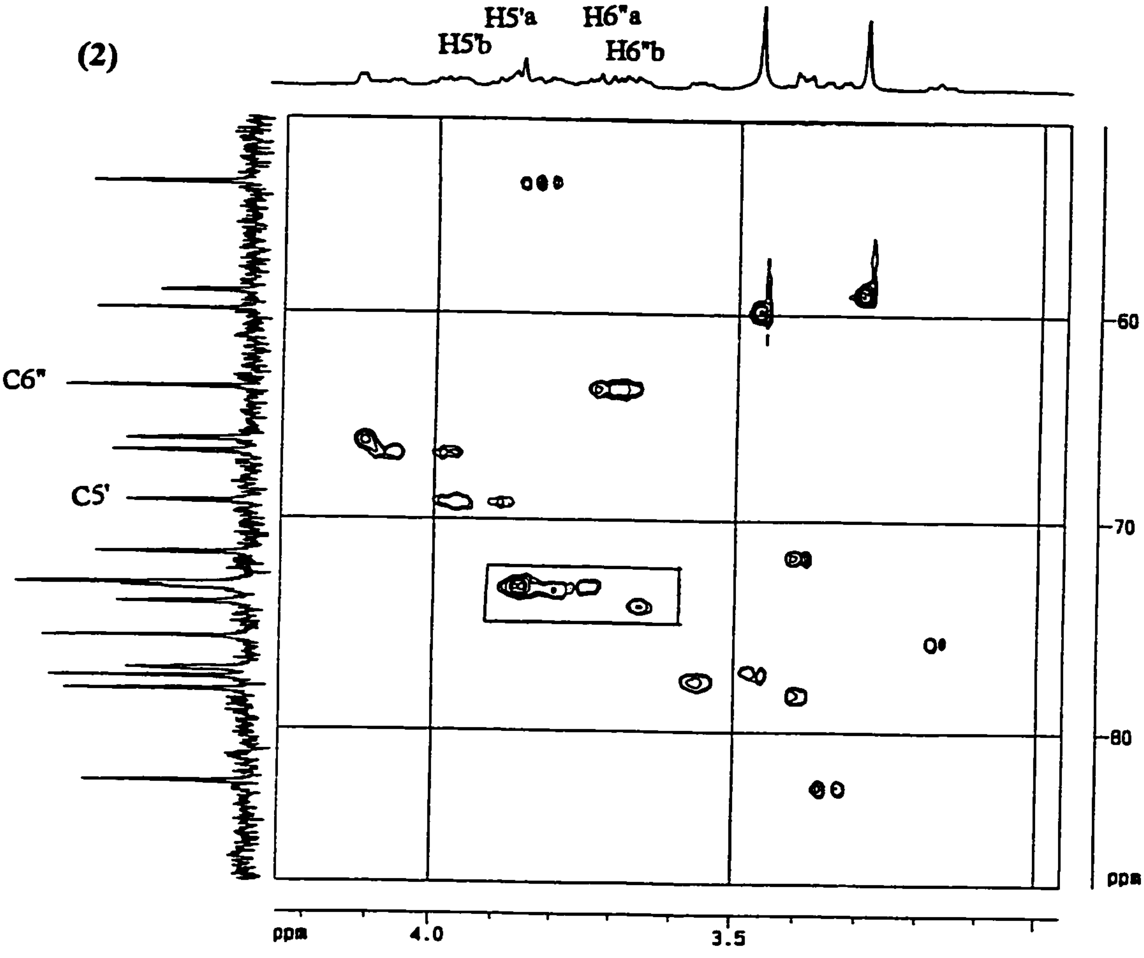


Figure A.4 (1) An expansion of the specified part of (2);
(2) The 400 MHz HMQC spectrum of 59

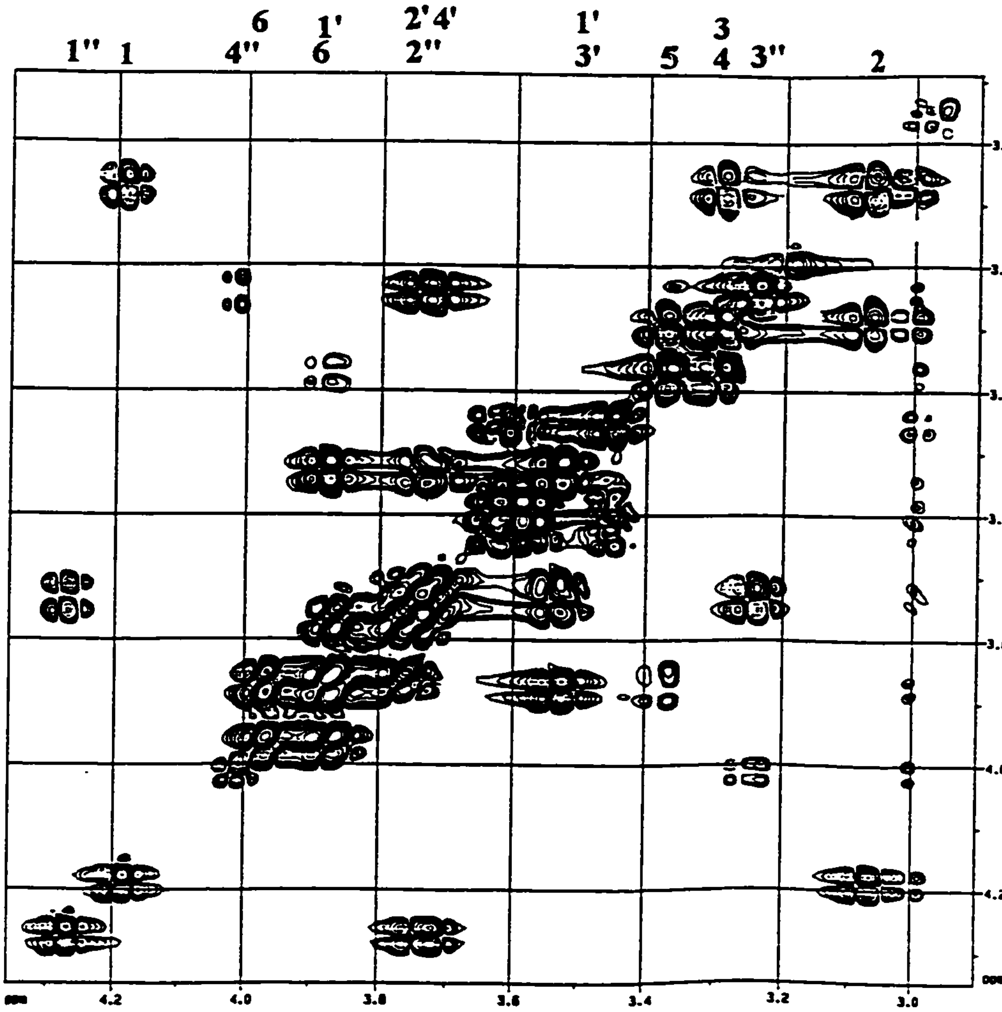


Figure A.5 The 500 MHz COSY spectrum of 60

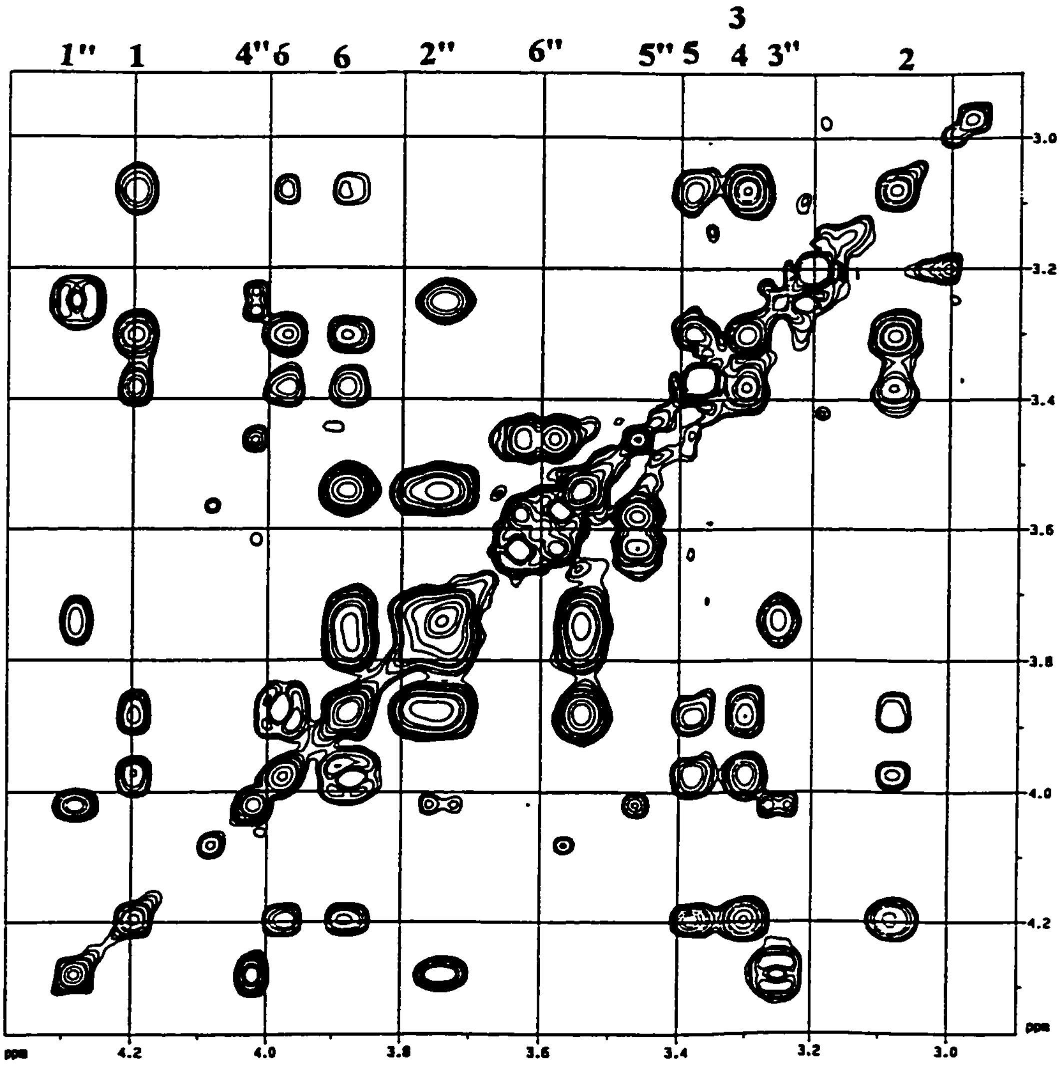


Figure A.6 The 500 MHz TOCSY spectrum of 60

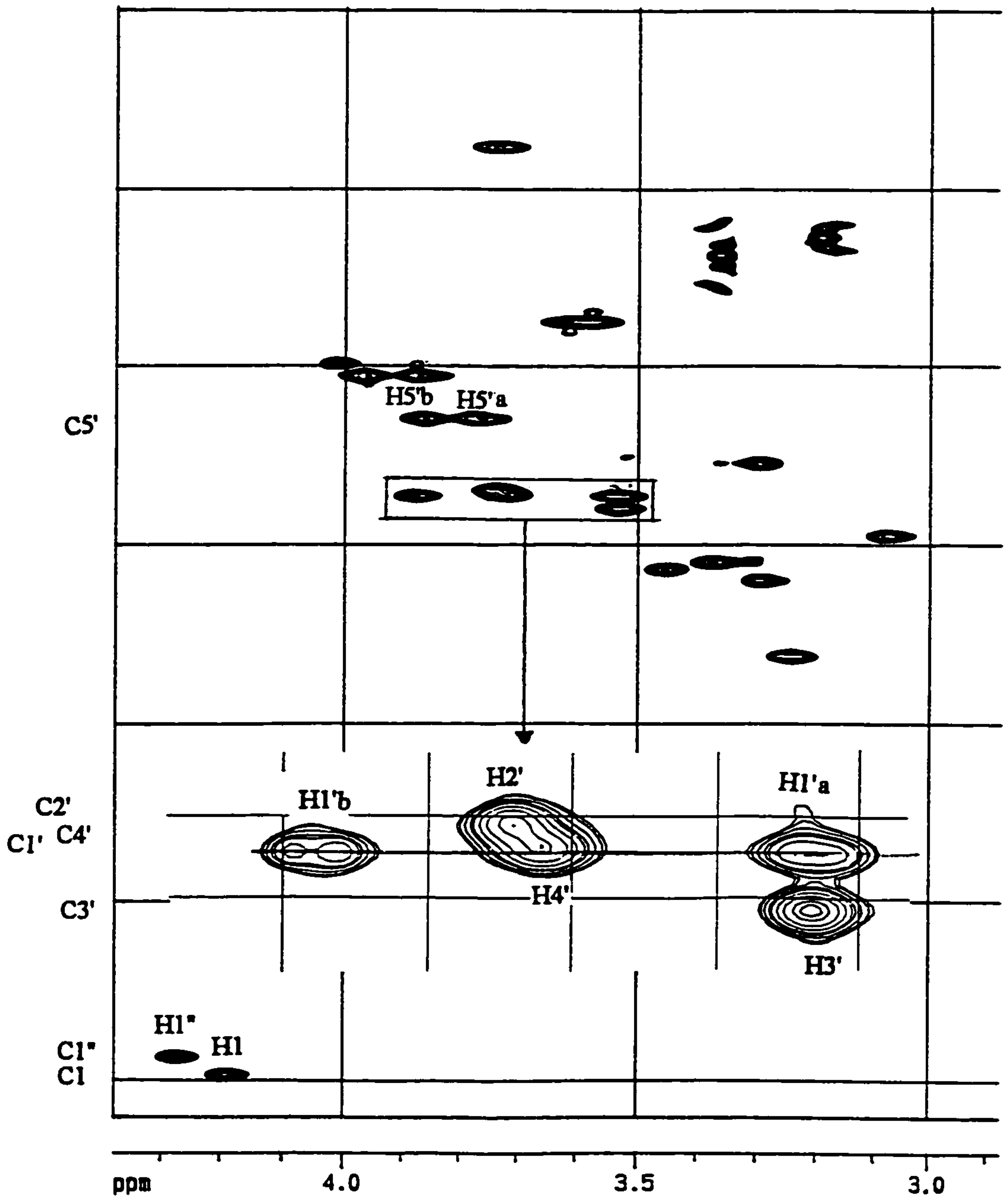


Figure A.7 The 500 MHz HMQC spectrum of 60

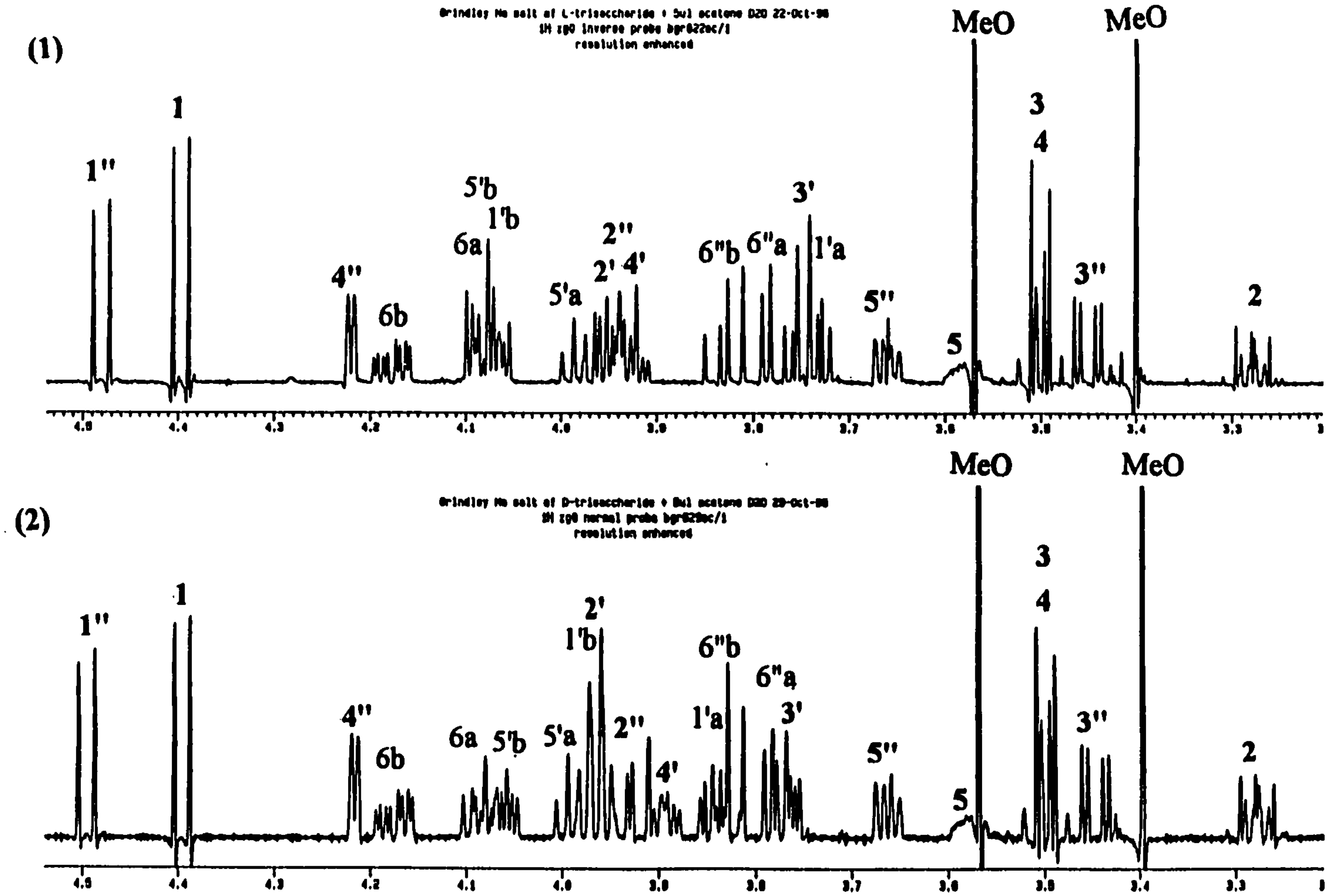


Figure A.8 (1) The 500 MHz ^{1}H NMR spectrum of 60; (2) The 500 MHz ^{1}H NMR spectrum of 59

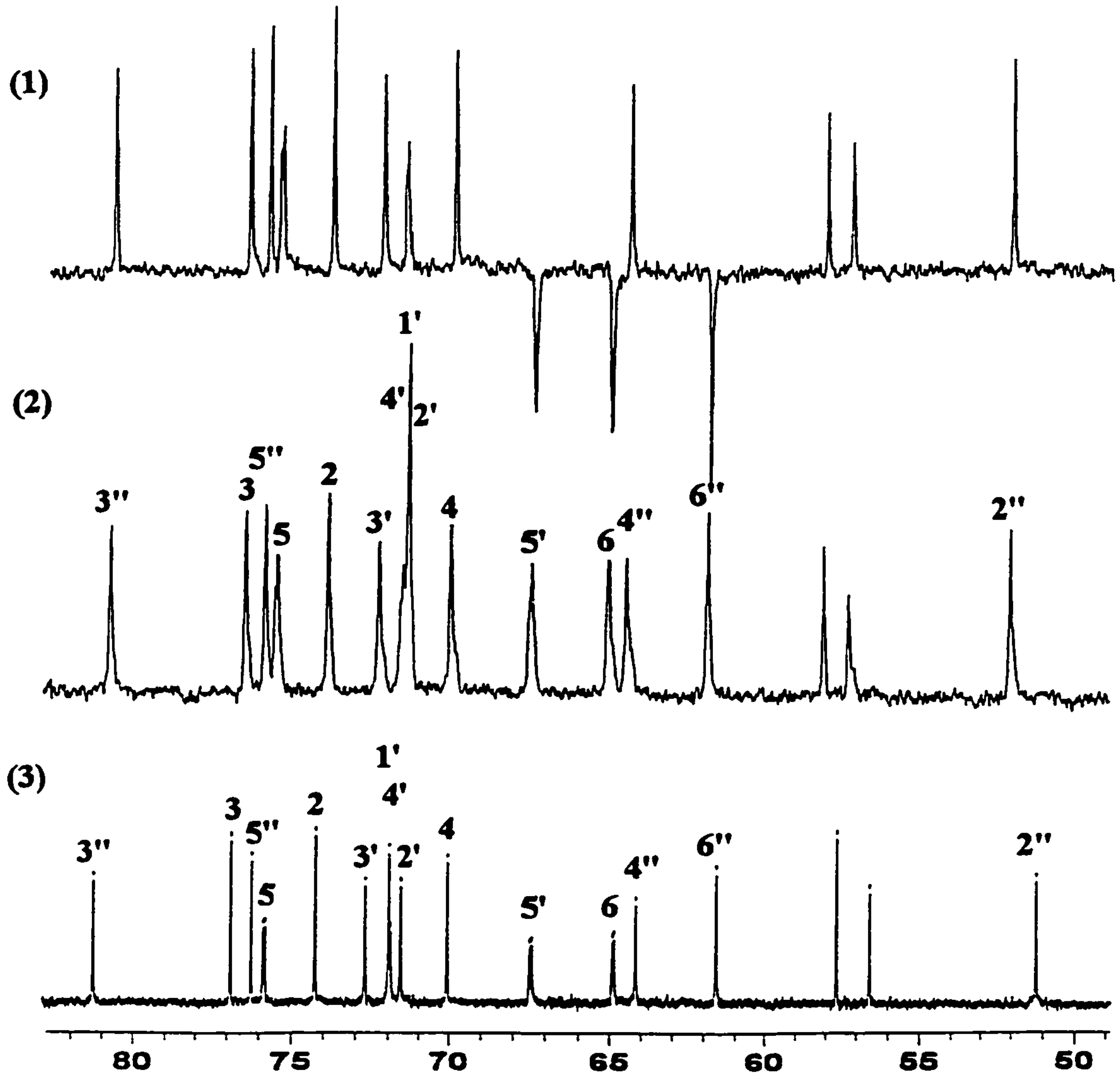


Figure A.9 (1) The 100.6 MHz JMOD spectrum of 59;
(2) The 100.6 MHz ^{13}C NMR spectrum of 59;
(3) The 125.8 MHz ^{13}C NMR spectrum of 60

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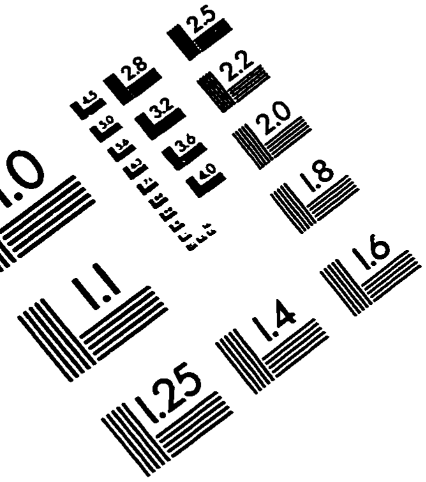

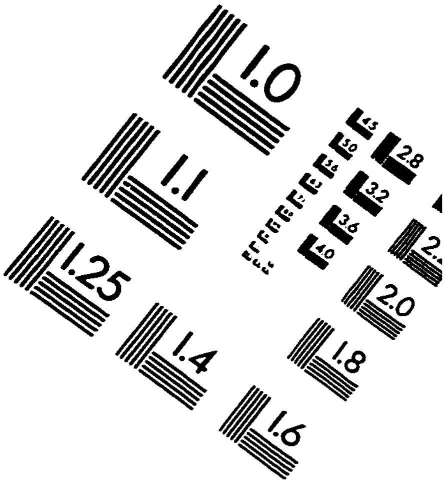

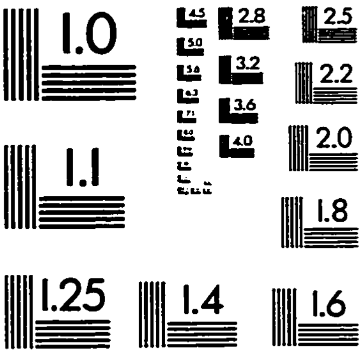

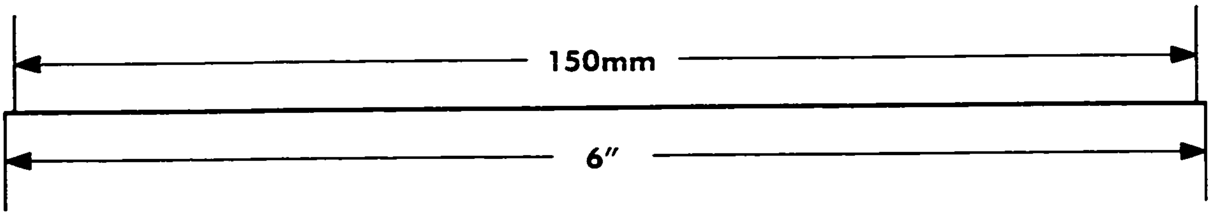

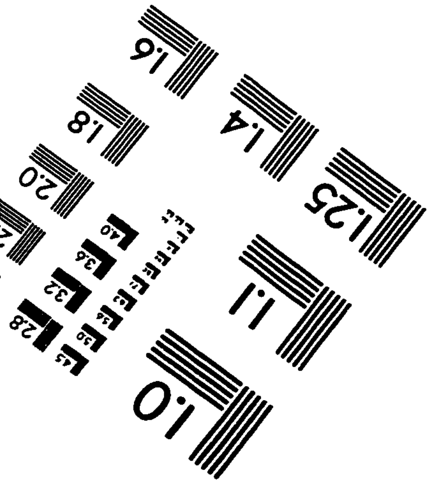

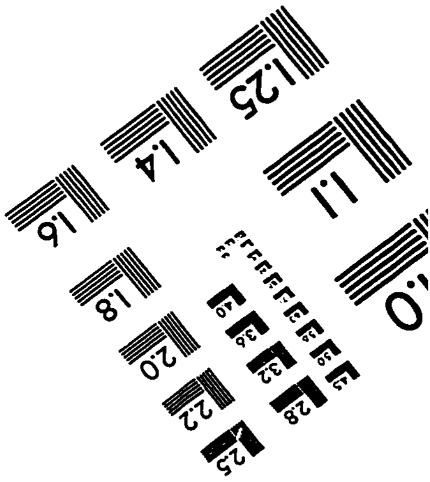

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