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THE SYNTHESIS OF OLIGOSACCHARIDES CORRESPONDING TO THE CELL-WALL POLYSACCHARIDES OF THE β-HEMOLYTIC *STREPTOCOCCI* GROUP A

by

Kerry Bruce Reimer

B.Sc. University of British Columbia, 1985

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The Synthesis of Oligosaccharides Corresponding to the Cell-Wall Lipopolysaccharides of the Gram-Negative Streptococcus Group A

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ABSTRACT

The overall strategy for the preparation of higher-order oligosaccharides corresponding to the repeating unit of cell-wall polysaccharides of the β-hemolytic *Streptococci* Group A is described. A linear trisaccharide β-D-Glc*p*NAc-(1-3)-α-L-Rha*p*-(1-3)-α-L-Rha*p*, has been prepared by a series of Königs-Knorr reactions. Glycosylation of the selectively protected rhamnose monosaccharide, allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside, with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide as a glycosyl donor, gave the fully blocked disaccharide. Removal of the allyl group was accomplished by isomerisation of the allyl group to the prop-1-enyl group, followed by hydrolysis of the vinyl ether to give the hemiacetals. The hemiacetals were then treated with the Vilsmeier-Haack reagent, *N*,*N*-dimethyl(chloromethylene)ammonium chloride, affording the disaccharide as a glycosyl chloride. Addition of this glycosyl donor to the same selectively protected rhamnose monosaccharide unit used in the preparation of the disaccharide, gave the trisaccharide as its allyl glycoside. Deprotection was accomplished by transesterification, followed by hydrazinolysis, selective N-acetylation, and hydrogenolysis to give the pure trisaccharide as its propyl glycoside for use as a hapten in binding studies with monoclonal antibodies, and in n.m.r. studies. The fully blocked disaccharide was similarly deprotected, affording the hapten, β-D-Glc*p*NAc-(1-3)-α-L-Rha*p*, as its propyl glycoside.

The trisaccharide incorporating a linking arm was prepared by the addition of the disaccharide chloride to the rhamnose monosaccharide unit, 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside. Deprotection, as before, gave the trisaccharide in a form suitable for the preparation of glycoconjugates.

DEDICATION

To my wife Carol, for her support and understanding.

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I would like to thank my supervisor Dr. Mario Pinto, for giving me the opportunity to work in a stimulating environment, and for his close and instructive supervision, and also for his valuable assistance in the preparation of this manuscript. I thank also Marcey Tracey for her efficient and friendly service in the course of obtaining several n.m.r. spectra. I am grateful to Dr. D. R. Bundle of the National Research Council of Canada, Ottawa, for recording the two-dimensional n.m.r. spectra. Microanalyses were kindly provided by M. Yang. I also thank the members of my examining committee for the time they have taken in appraisal of this thesis.

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LIST OF ABBREVIATIONS

Ac	Acetyl
All	Allyl
Bz	Benzoyl
Bzl	Benzyl
DMF	*N,N*-Dimethylformamide
α-D-Gal*p*NAc	2-Acetamido-2-deoxy-α-D-galactopyranosyl
β-D-Glc*p*NAc	2-Acetamido-2-deoxy-β-D-glucopyranosyl
α-L-Rha*p*	α-L-Rhamnopyranosyl
NHAc	Acetamido
Phth	Phthalimido
silver triflate	silver trifluoromethanesulphonate
UDP	Uridine diphosphate
n.m.r.	Nuclear magnetic resonance
$^{13}C\{^{1}H\}$ n.m.r.	Proton-decoupled carbon-13 n.m.r.
CHORTLE	*c*arbon *h*ydrogen correlations from *o*ne-dimensional pola*r*isation-*t*ransfer spectra by *l*east squares analysis
2D-^{1}H n.m.r. COSY	Two-dimensional proton *c*oupling *c*orrelated *s*pectroscop*y*
n.O.e.	Nuclear Overhauser enhancement

I. INTRODUCTION

Background

The Gram positive β-hemolytic *Streptococci* Group A is a commonly occurring organism and is one of the primary infective agents in humans. Infection by *Streptococci* Group A may lead to the commonly known condition of streptococcal pharyngitis, or strep throat[1]. Although strep throat is not itself a serious condition it can, in a small number of cases develop into the more serious disorder of rheumatic fever. In addition, there appears to be a connection between the initial *Streptococcus* infection and other disorders such as acute glomerulonepheritis, rheumatoid arthritis, and other rheumatoid diseases[2].

Each *Streptococcus* bacterium is surrounded by a slime capsule, below which lies the cell wall (Figure 1). The cell-wall is composed of protein, carbohydrate, and mucopeptide.

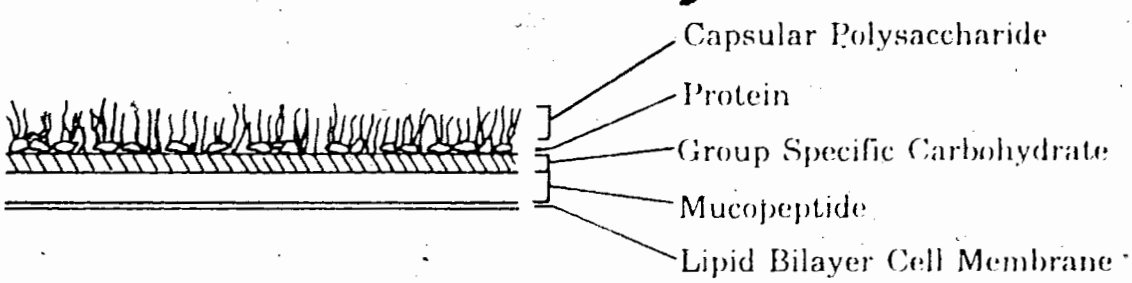


Figure 1

Schematic representation of the cell-wall of Gram-positive bacteria

Below the cell-wall is the protoplast, or cell-membrane. The cell-wall carbohydrates are the structures responsible for the

serological classification of *Streptococci* into main groups, Groups A, A-variant, and C. The group-specific carbohydrates of β-hemolytic *Streptococci* (Figure 2) have been found to contain a

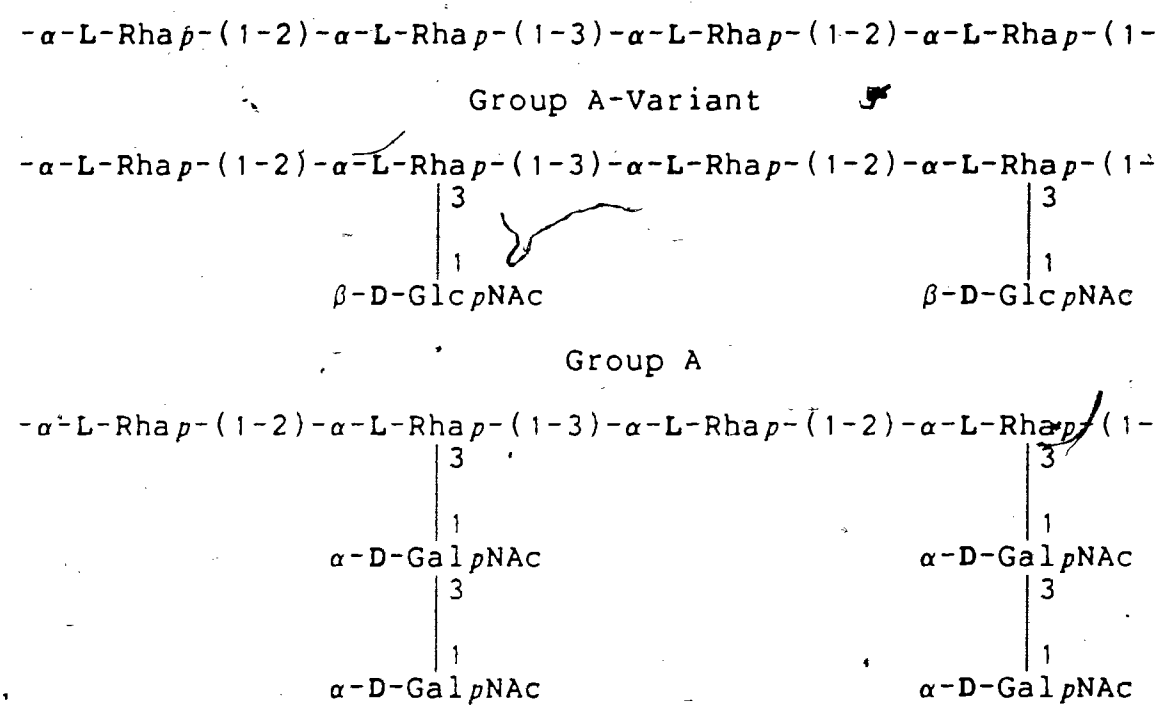


Figure 2
Group-specific carbohydrates of β-hemolytic *Streptococci*

common poly-rhamnopyranosyl backbone of alternating α-L-(1-2) and α-L-(1-3) linkages[3]. The A-variant structure is a polymer made up of only this sequence of sugars. The Group A structure has branching β-D-N-acetylglucosamine residues attached to the 3-position of the rhamnose backbone. The Group C structure has branching disaccharide units, 3-*O*-α-D-N-acetylgalactosaminosyl-α-D-N-acetylgalactosamine, linked to the 3-position of the rhamnose backbone. Of the β-hemolytic *Streptococci* the group of most importance clinically is that of Group A; however the Group C N-acetylgalactosaminosyl disaccharide has been shown to be

cross reactive with the tumor-associated Forssman antigen[4]. The cell-wall polysaccharides of *Streptococci* Group A have been shown to be immunologically cross-reactive with structural glycoproteins of heart valves[5]. This cross-reactivity means that, in some cases, an immune response to a streptococcal infection, and directed against streptococcal antigens, may actually act against the host's own tissue antigens; this is known as an autoimmune disorder. It is this cross-reactivity of streptococcal antigens with heart valve glycoproteins that is thought to be important in the development of valvular disease in patients with rheumatic fever. Cross-reactivity with other connective tissues and structural proteins may be involved in many other rheumatic disorders.

Traditionally, streptococcal infections have been diagnosed by growing bacterial cultures from patients' throat swabs, and then using standard microbiological and immunological techniques to identify the resulting colonies. The time required for conventional culture techniques can be overnight or longer; consequently, treatment is often delayed or initiated without proper confirmation of infection. A rapid diagnostic reagent is therefore desirable. Recently, several rapid detection methods have been developed[6] which can detect streptococcal antigen directly from a throat swab. For the most part, these tests rely on the extraction of bacterial antigen from a patient's throat swab, using enzymatic or nitrous acid extraction, and detection of the extracted polysaccharide antigen by macroscopic agglutination of antibody-coated latex particles (Figure 3).

Clinical studies[7-9] have compared some of these methods[10] to the traditional culture method and have found that they showed good specificity; however, in some cases[8,9] these tests have been found to suffer from moderate sensitivity requiring the

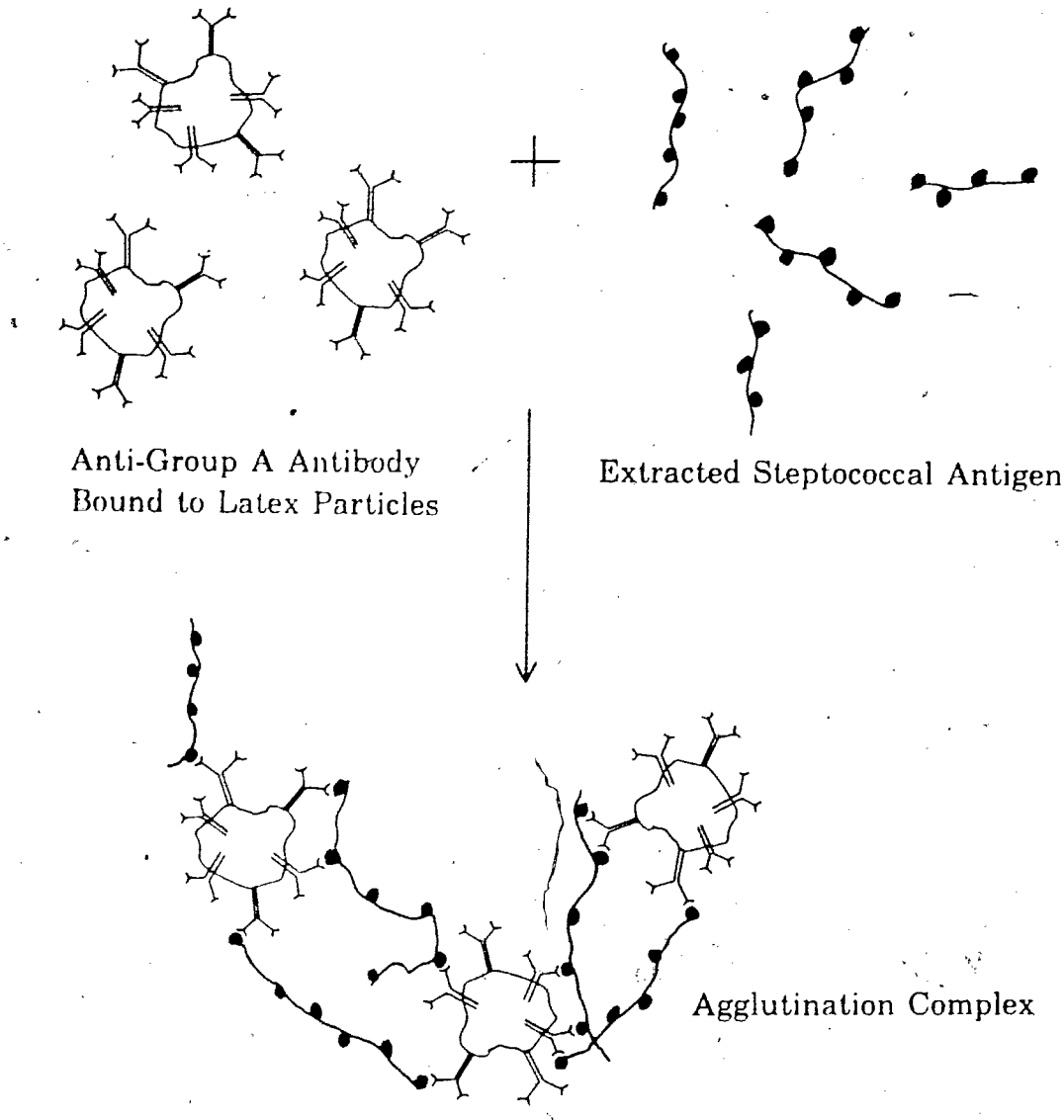


Figure 3

Detection of extracted bacterial antigen by the latex-agglutination method

confirmation of negative test results by some other method.

This thesis describes the chemical synthesis of portions of

the antigenic determinant of the β-hemolytic *Streptococci* Group A. Glycoconjugates of these oligosaccharide structures may be prepared by coupling them to a carrier protein, enzyme, or immunoadsorbent *via* the 8-methoxycarbonyloctyl linking arm[11], using the acyl azide methodology[12] (Figure 4). The artificial

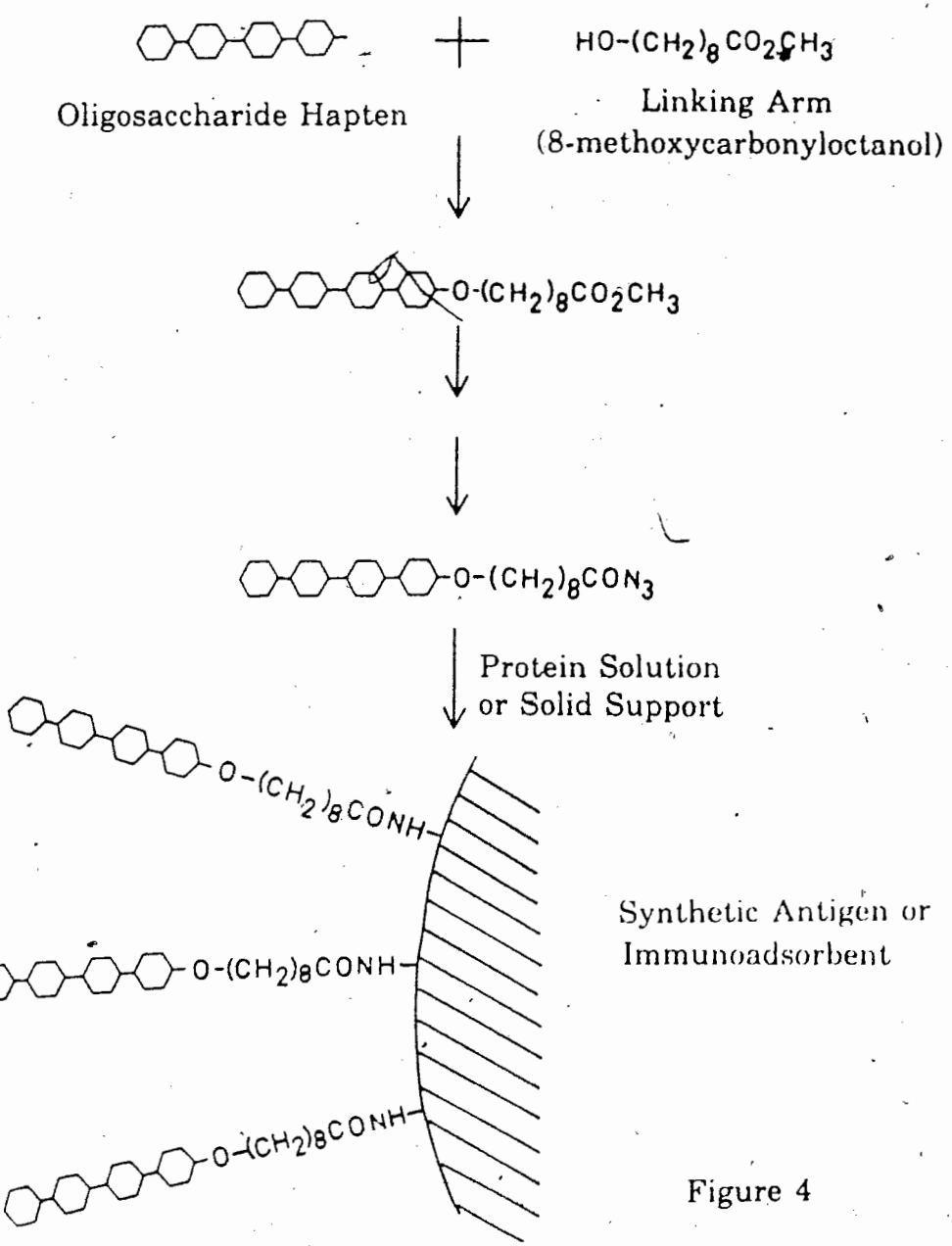


Figure 4

Attachment of oligosaccharide hapten to a protein or solid support

antigens thus obtained could then be used to test for anti-

Streptococci Group A antibody in patients' sera (Figure 5). Alternatively, the hybrid-myeloma technique[13] could be used to produce monoclonal antibodies of highly defined specificity

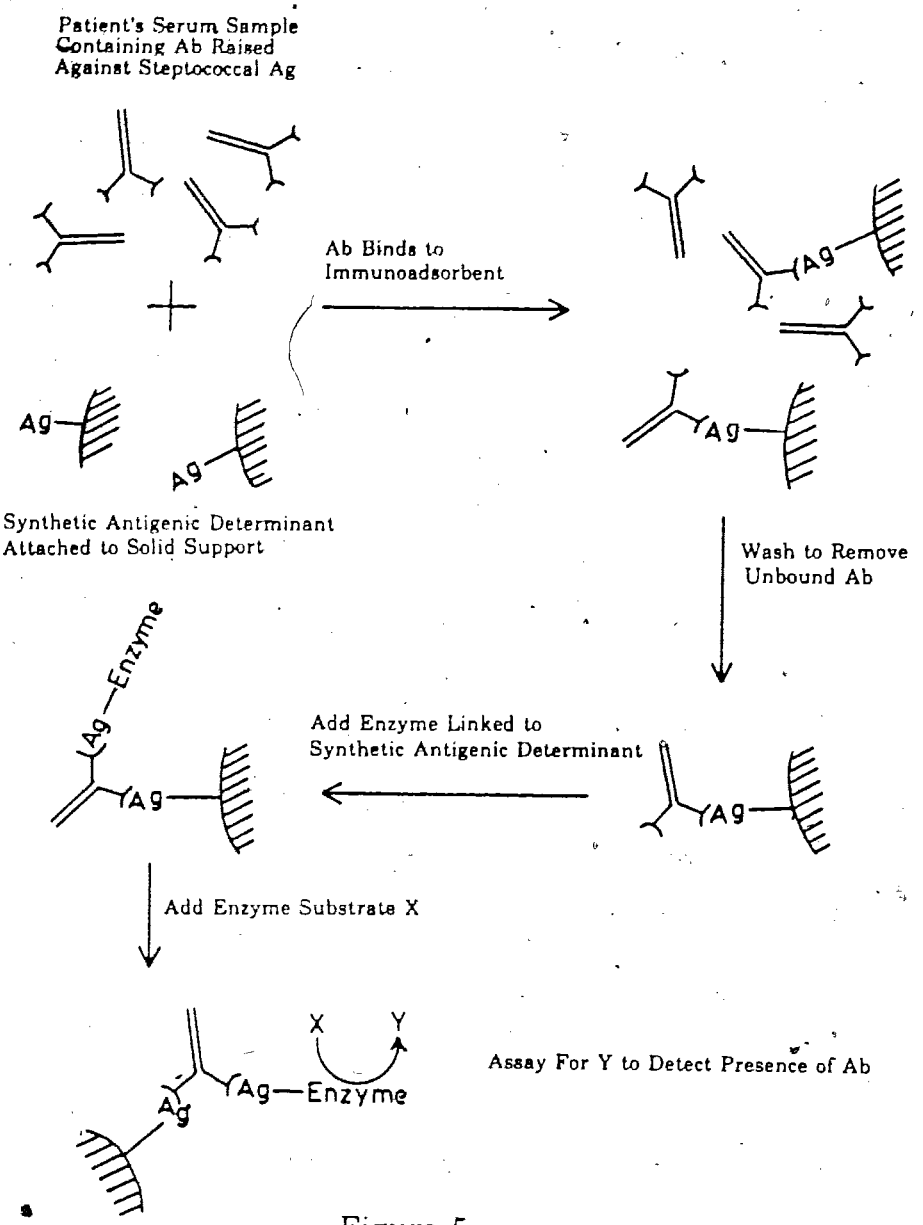


Figure 5

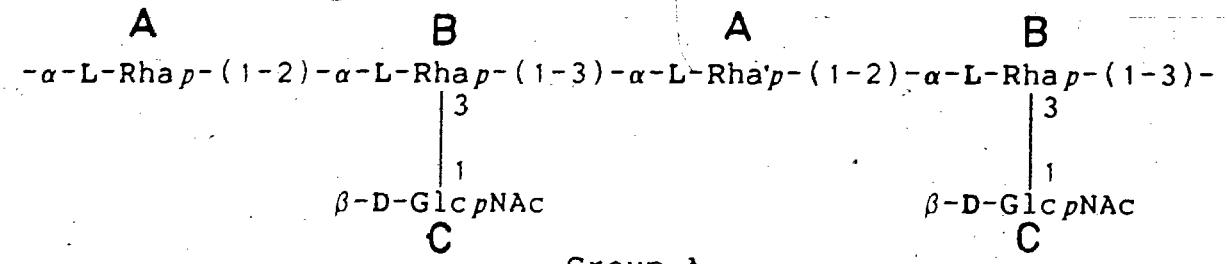
Detection of antibody by enzyme-linked immunoadsorbent assay

against the artificial antigens. The monoclonal antibodies thus obtained could be used to directly detect *Streptococci* Group A

antigen from a throat swab. In this fashion they could be incorporated into existing test kits or used to develop new diagnostic test reagents. Presumably, the detection of streptococcal infections utilizing glycoconjugates derived from highly defined chemical structures (or monoclonal antibodies to these glycoconjugates) would be both more sensitive and more specific than methods currently available.

The synthetic structures will be used as haptens in binding studies to determine the optimium binding requirements for a highly defined *Streptococci* A antigen. Oligosaccharides of varying chain length and degree of branching may be used in competitive binding studies with natural antigen to determine the structure which gives the greatest inhibition and hence the greatest specificity. Computer modelling[14] of the Group A polysaccharide has indicated a three-dimensional structure in which the poly-rhamnopyranosyl backbone takes up a helical arrangement with the *N*-acetylglucosamine residues protruding outward (Figure 6). The binding studies would help elucidate the topographical features of this macroscopic structure of importance in antigen-antibody recognition.

A review of the literature revealed the synthesis of a terminal disaccharide of the Group A determinant, the trisaccharide portion of the C determinant, and a tetrasaccharide portion of the Group A-variant structure[15]. The Group A and Group A-variant structures have also been coupled to protein[12] and the semi-synthetic antigens thus obtained have been used to investigate the fine binding specificities of two



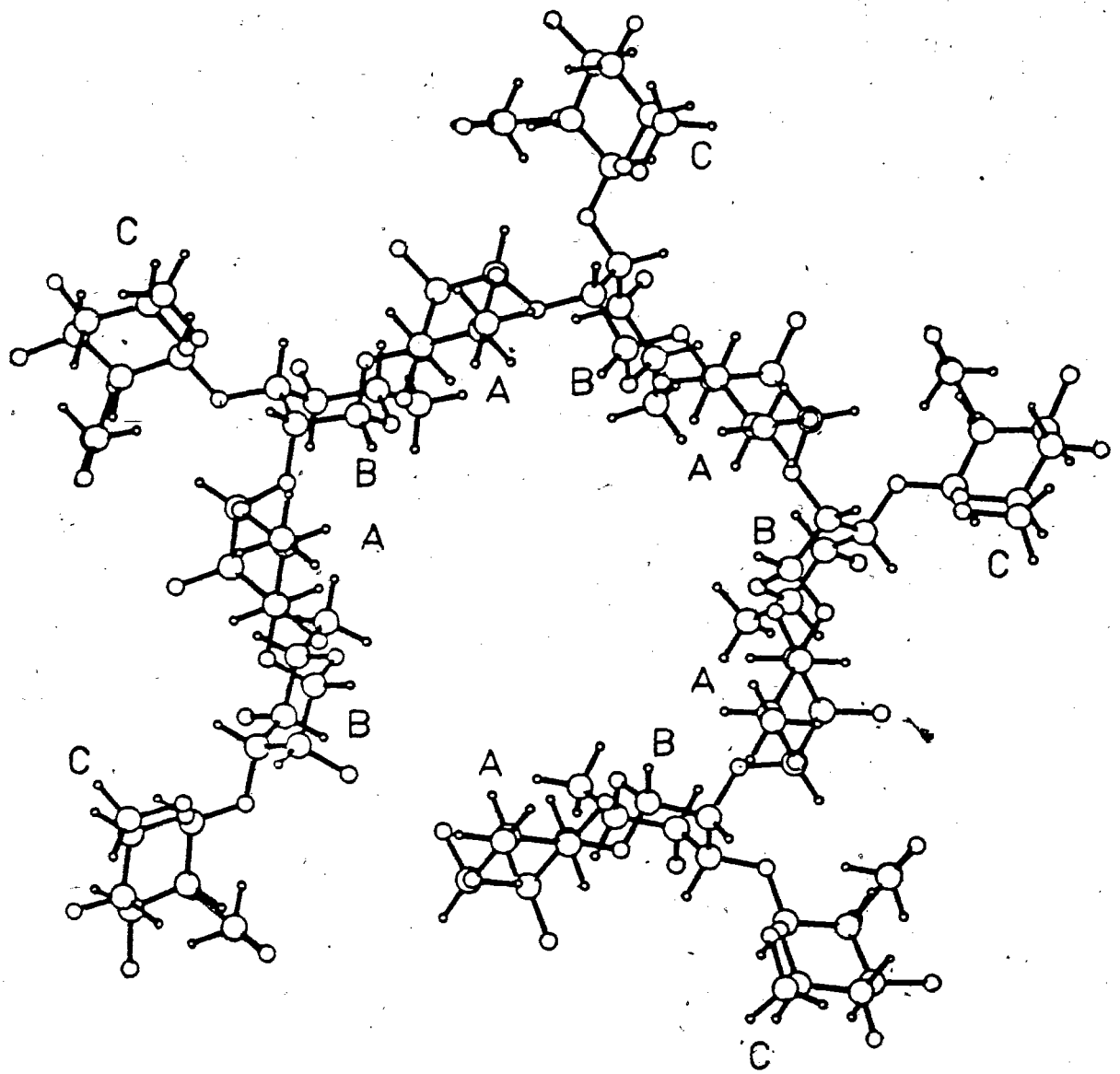


Figure 6

Computer-generated model of the three-dimensional structure of the *Streptococci* Group A cell-wall polysaccharide

IgM myeloma proteins directed against streptococcal cell-wall carbohydrate antigens[16]. **Glycoside Synthesis**

The interest in oligosaccharide synthesis has increased in recent years since the role of glycoproteins and glycolipids in biological systems has become better understood[17]. The oligosaccharide portions of glycoconjugates are often the biologically important structures involved in cell-cell recognition, hormone receptors, immune recognition, and several other biologically important interactions.

The chemical synthesis of oligosaccharides has been reviewed in the literature[18] and what are presented in this section are only the salient features. The synthesis of oligosaccharides involves the selective linkage between two polyhydroxyl sugar units. These units may be individual monosaccharides or more complex oligosaccharides. The strategy involves the selective protection of all functional groups intended not to take part in the reaction and their selective deprotection when necessary. In a glycosylation reaction the reaction partners are a fully protected glycosyl derivative, functionalised at the anomeric position with a good leaving group, often a halide, (a glycosyl donor), and an acceptor molecule with, in general, one free hydroxyl group intended for coupling (Figure 7).

Since the biological function of oligosaccharides is dependent upon the three-dimensional shape of the molecule in solution, and the conformation of oligosaccharides is affected by the stereochemistry about the glycosidic linkages, it is necessary that the synthesis of oligosaccharides be carried out

such that the stereochemical outcome of the newly formed glycosidic linkages is predictable. There are two general types

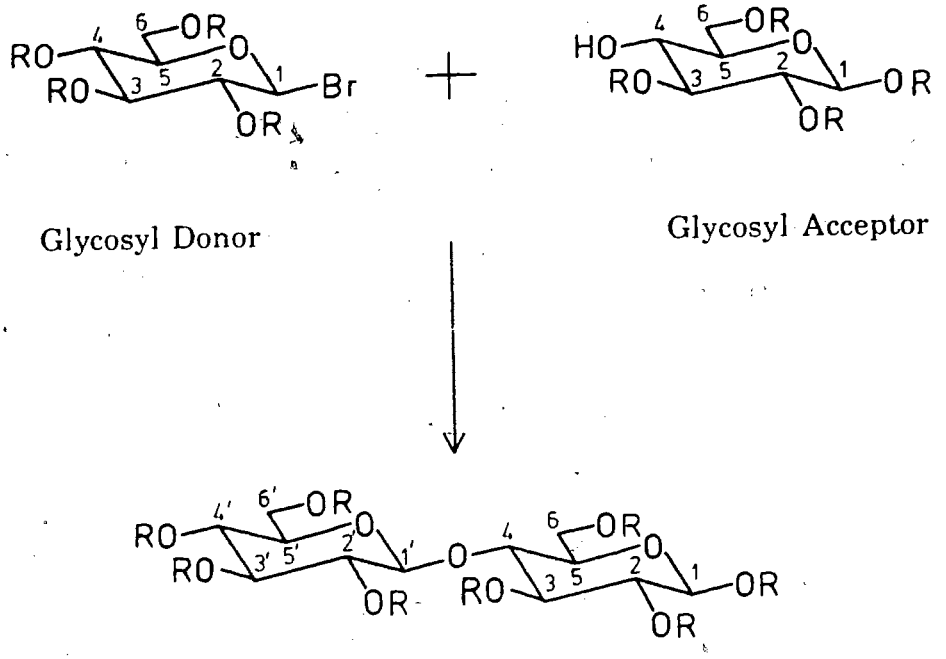
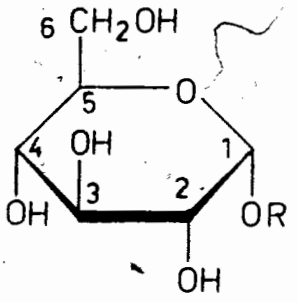


Figure 7

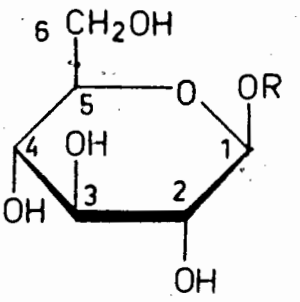
General glycosylation reaction

of glycosidic linkages; 1,2-*cis* and 1,2-*trans*, in which the hydroxyl group at the 2-position of the glycosyl unit and the exocyclic oxygen atom at the 1-position (the anomeric centre) are *cis* and *trans* to each other, respectively (Figure 8). The 1,2-*cis* linkage applies to the synthesis of an α-glycosidic linkage in the *gluco* and *galacto* series and a β-glycosidic linkage in the *manno* and *rhamno* series. The 1,2-*trans* linkage is seen in the β-glycosidic linkage of the *gluco* and *galacto* series and in the α-glycosidic link of the *manno* and *rhamno*

series. Strategies for the formation of 1,2-cis and 1,2-trans glycosidic linkages differ.



1,2-cis Glycoside



1,2-trans Glycoside

Figure 8

1,2-*cis* and 1,2-*trans* Glycosides

1,2-trans Glycosylations

The most common method for the formation of a 1,2-*trans* type linkage is to use a glycosyl halide with a participating group, such as an ester, as substituent at the 2-position. Under Lewis acid catalysis an oxocarbocation may be formed which can then be stabilised by the substituent at the 2-position to give a dioxocarbenium ion (Figure 9). The alcohol component in the reaction mixture then opens the dioxocarbenium ring in an S_N2 type fashion with the neighbouring-group protecting the *cis* face of the ring from nucleophilic attack[19]. A useful method for the preparation of 1,2-*trans* linkages in 2-amino sugars utilises the phthalimido group as substituent at the 2-position[20]. In this case a dioxocarbenium ion is not formed; however, the steric bulk of the phthalimido group blocks nucleophilic attack

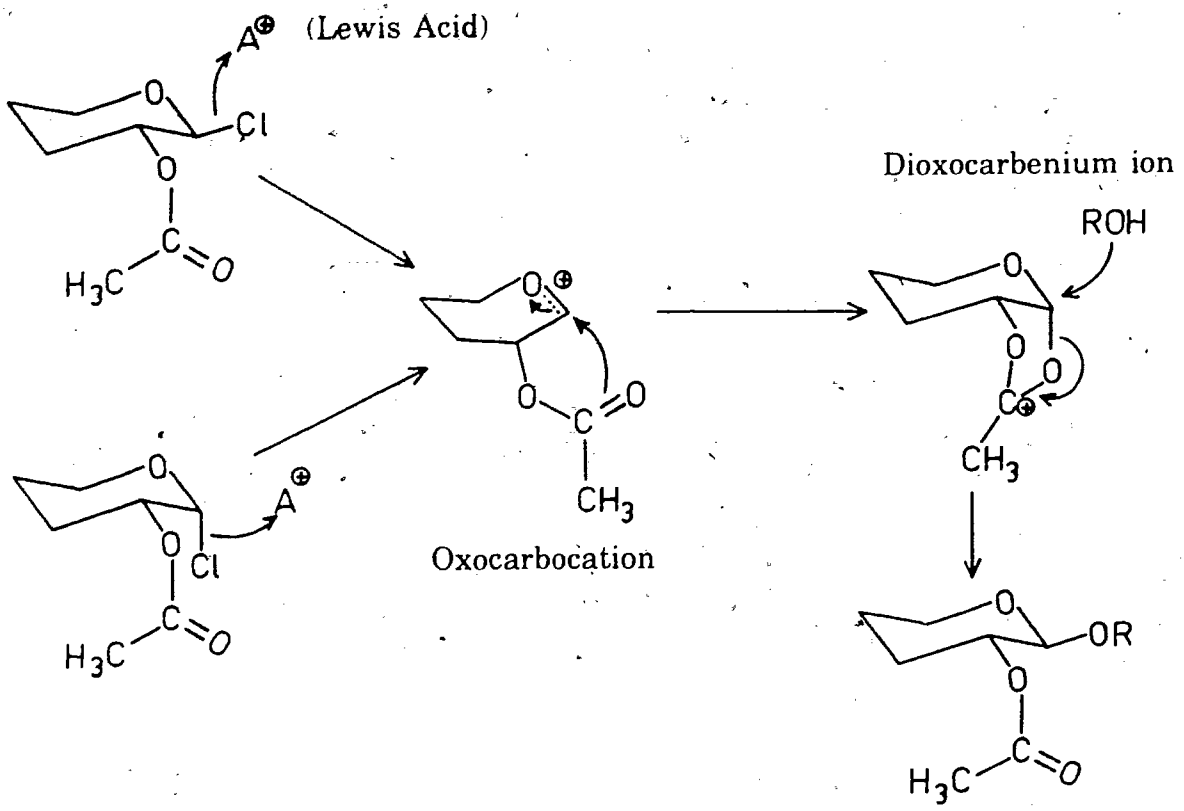


Figure 9 1,2-trans Gylcoside

Preparation of a 1,2-*trans* glycosidic linkage, in the *gluco* and *galacto* series, with neighbouring-group participation

by the alcohol component on the *cis* face of the oxocarbocation (Figure 10).

1,2-cis Glycosylations

The formation of a 1,2-*cis* glycosidic linkage requires a different strategy. One method is to have a non-participating group such as Bzl, $OCOCCl_3$, NO_2, or N_3 at the 2-position of the glycosyl donor, and in conjunction with a *trans* halide at the anomeric position, use reaction conditions such that

nucleophilic attack by the hydroxyl component (the glycosyl acceptor) proceeds with inversion of configuration in an S_N2 sense. Use of this method to form an α-glycosidic linkage in

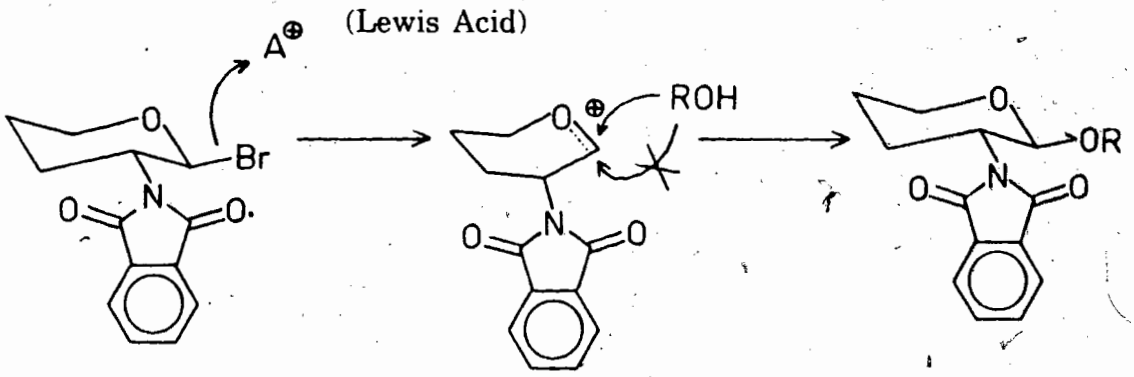


Figure 10

Preparation of a 1,2-*trans* glycosidic linkage, in the *gluco* and *galacto* series, with amino sugars

the *gluco* and *galacto* series requires the use of the highly reactive β-halide. However, reaction of the β-halide is often complicated by anomerisation to the α-anomer, leading to an undesirable anomeric mixture of glycosides.

One method that circumvents this problem is the use of the "halide-ion catalysed glycosylation" reaction of Lemieux *et al* [21]. Using this approach one can form an α-glycosidic linkage starting from the more stable α-halide. The α-halides are stabilised by the anomeric effect[22]. The isomerisation of the α-halide to the more reactive β-halide is catalysed, *in situ*, by halide ions and silver salts (Figure 11). The alcohol component then reacts with the more reactive β-halide, and proceeds with inversion to give an α-glycoside. This difference in reactivities of glycosyl halides (Figure 12) can be used to

prepare α-glycosides from the more stable α-halides in the *gluco* and *galacto* series.

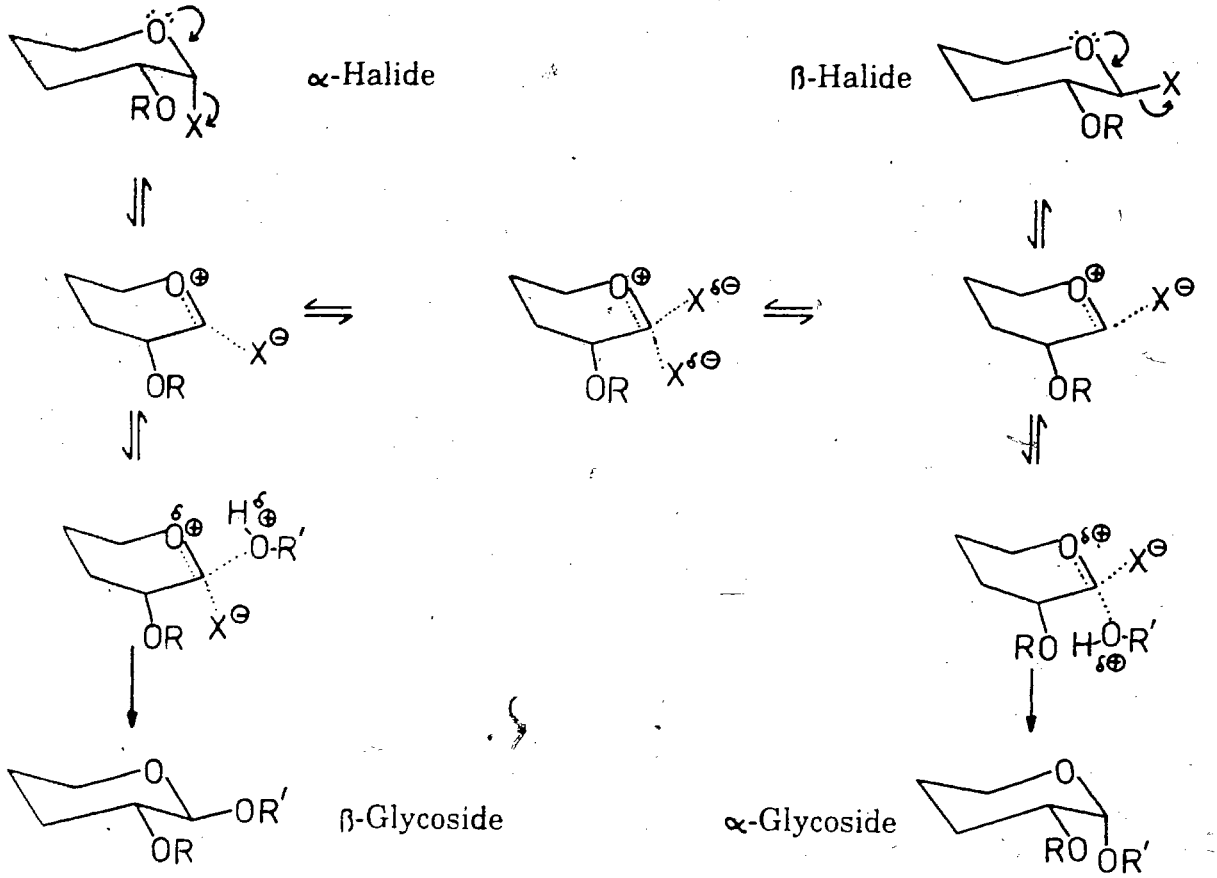


Figure 11

Preparation of a 1,2-*cis* glycosidic linkage, in the *gluco* and *galacto* series, using the "halide-ion catalysed glycosylation" method

Factors important in a glycosylation reaction are the polarity of the solvent, the reactivity of the glycosylation promotor, and the reactivities of the glycosyl donor and alcohol acceptor. The reactions are generally carried out in solvents of low polarity such as dichloromethane. More polar solvents may lead to a decrease in the stereoselectivity of the reaction. The reactivities of glycosyl promotors range from the mild tetraethylammonium halide, through the more reactive mercury

salts, mercuric cyanide and mercuric bromide, to the highly reactive silver salts, silver perchlorate and silver triflate. The choice of promotor is determined by the reactivities of the glycosyl donor and alcohol acceptor; more reactive species require a less reactive promotor. An increase in the reactivity of the promotor may lead to a decrease in the stereoselectivity

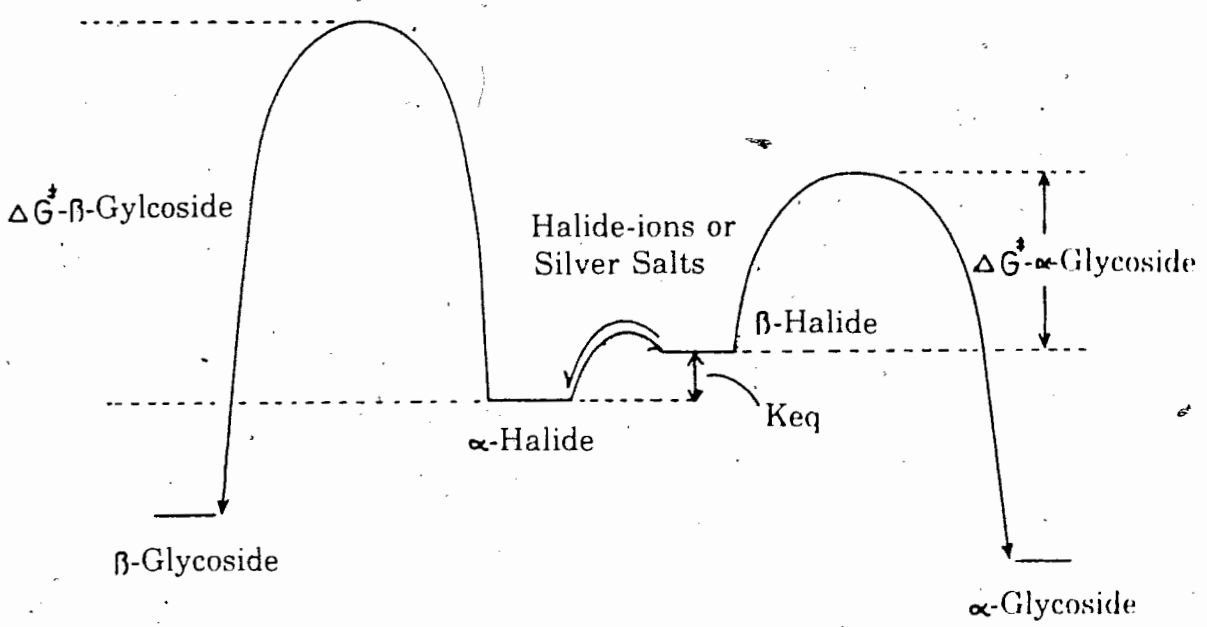


Figure 12

Energy profile for the "halide-ion catalysed glycosylation" reaction

of the glycosylation reaction; however, this can be minimised by performing the reactions at lower temperatures. The reactivity of the glycosyl donor is affected by the protecting groups present. In general, electron donating substituents on the glycosyl donor groups increase its reactivity, whereas substitution with electron withdrawing groups decreases the reactivity. When glycosyl halides are used, the order of reactivity of the donor is found to be I>Br>Cl. The reactivity

of the alcohol acceptor molecule also depends on the nature of the protecting groups; in general, electron donating groups increase the reactivity of the glycosyl acceptor whereas electron withdrawing groups decrease the reactivity. In addition, glycosyl acceptor molecules with primary alcohols have been found to be considerably more reactive than those with secondary alcohols.

The most common leaving groups utilised in glycosyl donors are the halides, chloride and bromide; however, many other glycosyl donors have been used successfully. Nicolaou *et al*[23] have used glycosyl fluorides (readily obtainable from the corresponding phenylthioglycosides) successfully in the preparation of oligosaccharides. Many glycosyl donors which have an activating substituent other than a halide have also been developed. Thioglycosides are versatile reagents, being stable to the conditions encountered in many protecting group manipulations and being readily converted to the corresponding glycosyl halides. However, thioglycosides, may be used directly in glycosylation reactions as glycosyl donors, and have been used successfully in the preparation of both 1,2-*cis* and 1,2-*trans* glycosides[24]. Other glycosyl donors include 1,2-orthoesters (and modifications thereof)[25], anomeric xanthates[26], and trichloroacetimidates[27].

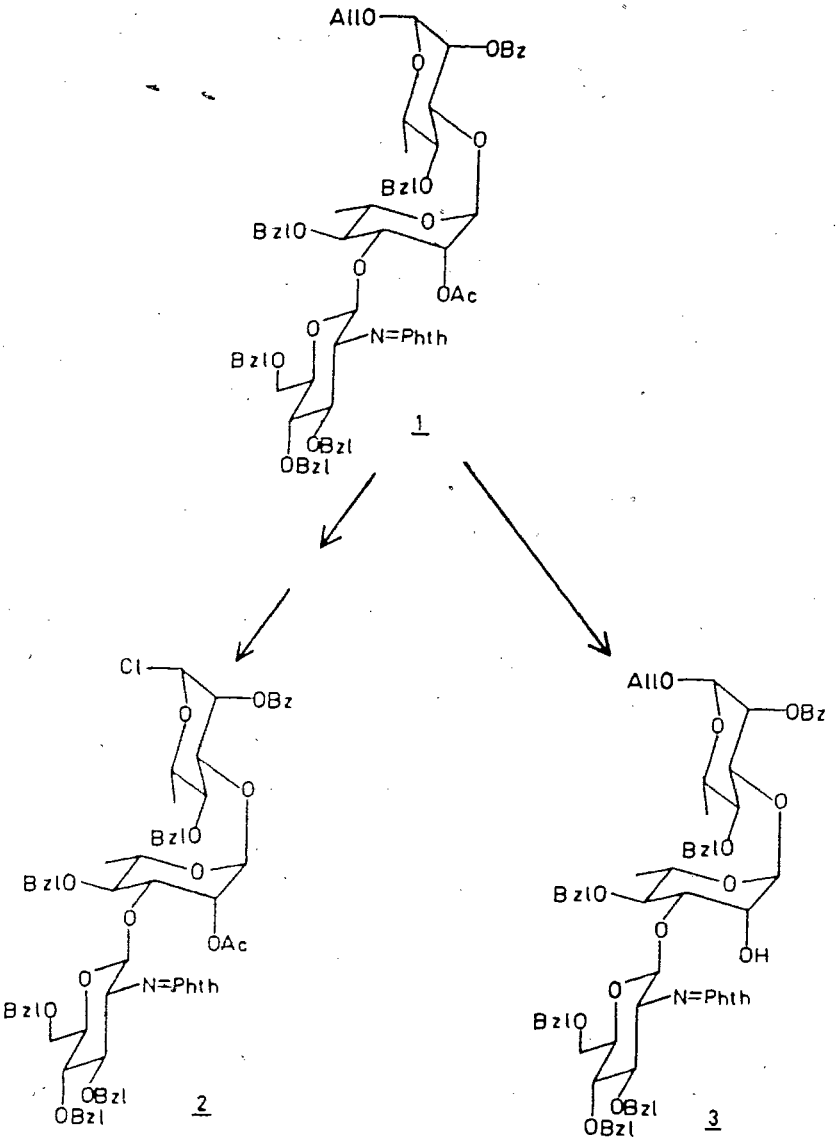
Recently, there has been much interest in the use of enzymes as catalysts in organic synthesis[28]. Enzymes have also been used successfully in the synthesis of some oligosaccharides. Glycosidases, a class of enzymes that normally catalyse the

degradation of complex carbohydrates, may be used in the reverse sense to catalyse the synthesis of glycosidic linkages. One enzyme which has been used in this fashion is the enzyme β-galactosidase[29]. Glycosyltransferases, enzymes that catalyse the transfer of a nucleotide activated sugar onto an alcohol acceptor, have also been used in glycoside synthesis. Thus, for example galactosyltransferases have been used to transfer β-D-galactopyranosyl units from UDP-galactose to alcohol acceptors[30], and N-acetylglucosaminosyltransferase V, has been used to transfer β-D-N-acetylglucosamine units (β-D-Glc*p*NAc) from UDP-Glc*p*NAc to oligosaccharide acceptor molecules[31]. Whitesides *et al*[32] have used sialyltransferases to prepare sialyloligosaccharides, and Sabesan *et al*[33] have utilised a combination of chemical and enzymatic synthesis (galactosyltransferase as well as three mammalian sialyltransferases) to prepare similar compounds. The enzymatic methods have made use of both immobilised[29], and membrane enclosed[32] enzymes.

Overall Synthetic Plan

The long term goal of the work is to synthesize tri- and higher-order oligosaccharide portions of the cell-wall polysaccharides of *Streptococci* Group A. This panel of synthetic structures could then be used as haptens in binding studies to help elucidate the minimum number of sugar residues, and the terminal chain-end sequence, required for optimum binding to complementary monoclonal antibodies. The procedure

would help define the requirements for a highly defined *Streptococci* Group A antigen. Such an antigen could then be used as a diagnostic reagent, as described previously. The overall synthetic plan is described below.



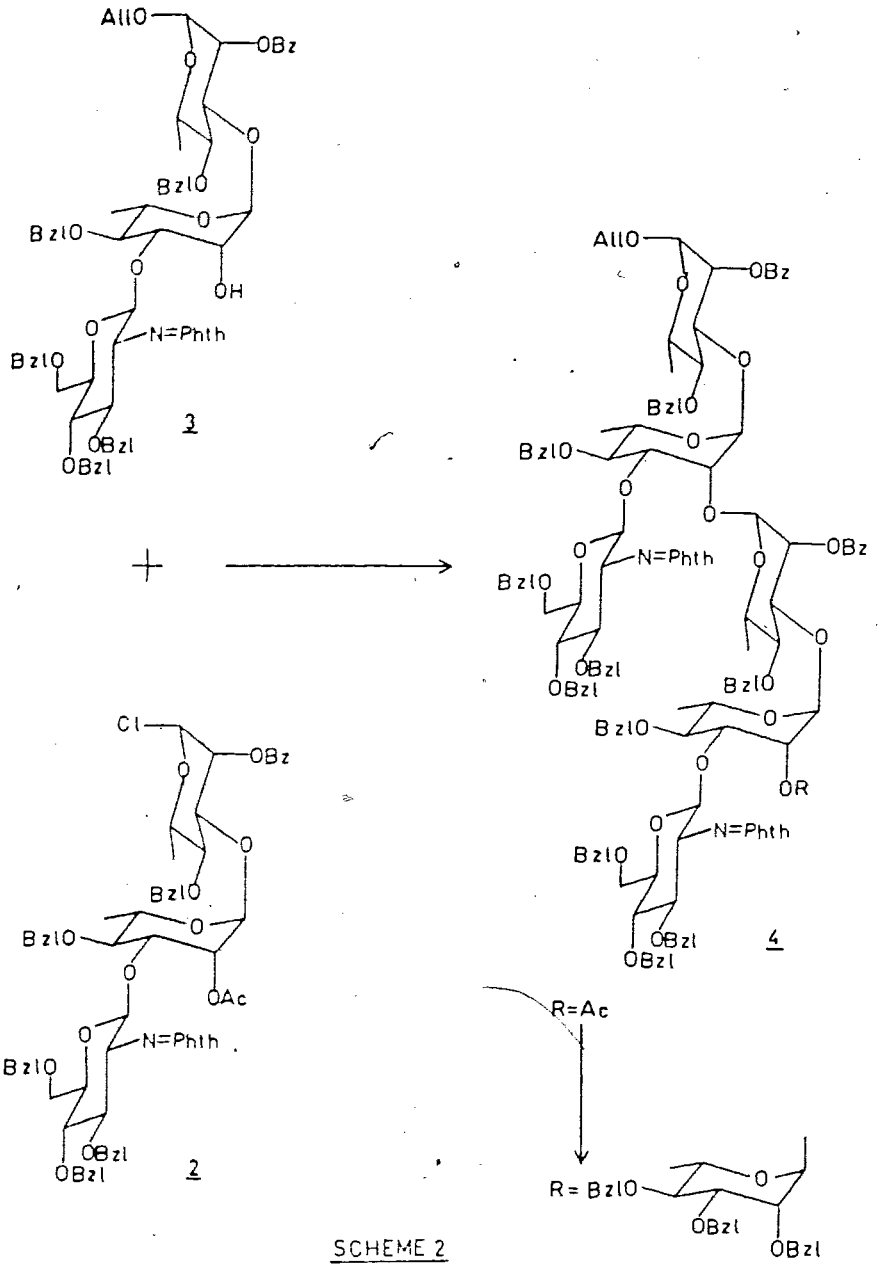
SCHEME 1

Retrosynthetic analysis of the natural structure [3] indicated that the trisaccharide repeating unit, β-D-Glc*p*NAc-(1-3)-α-L-Rha*p*-(1-3)-α-L-Rha*p*, could be prepared as a fully protected unit, and used in the block synthesis of all the higher-order sequences. The synthesis of this key trisaccharide unit (1) would involve conventional blocking group chemistry and was

based on recent work done in our laboratory on the synthesis of penta- up to octa-saccharides corresponding to the biological repeating unit of *Shigella flexneri* variant Y *O*-antigen[34,35]. The main feature of the key trisaccharide intermediate is its ability to function as a glycosyl donor or glycosyl acceptor in future glycosylation reactions. It was envisaged that selective removal of the 1-*O*-allyl group by isomerisation to the prop-1-enyl group[36], followed by hydrolysis to the hemiacetals[37], and conversion of the hemiacetals to the glycosyl halide (2) using Vilsmeier-Haack reagents[34,38], would allow the trisaccharide to function as a glycosyl donor in glycosylation reactions. Alternatively, selective transesterification of the 2'-*O*-acetyl group[39] to give a free hydroxyl at the 2'-position would allow the trisaccharide to function as a glycosyl acceptor (3) (Scheme 1). Glycosylation of the trisaccharide donor (2) with the trisaccharide acceptor (3) would give a hexasaccharide (4) of two repeating units which could be manipulated in the same fashion as the key trisaccharide intermediate (Scheme 2) (the 2-position of the penultimate residue could be selectively deprotected to give a glycosyl acceptor, or the 1-*O*-allyl group could be removed to allow synthesis of a glycosyl halide). Using this methodology, oligosaccharides of increasing size could be prepared.

Since the structure of the "non-reducing" terminus has not yet been firmly established, the synthesis of branched as well as linear chain-end sequences is desirable. The branched sequences could be prepared by capping the hydroxyl at the 2-

position of the penultimate residue with a rhamnopyranosyl halide, thus affording branched tetra-,hepta-, and deca-saccharide sequences (Scheme 2).



SCHEME 2

II. RESULTS AND DISCUSSION

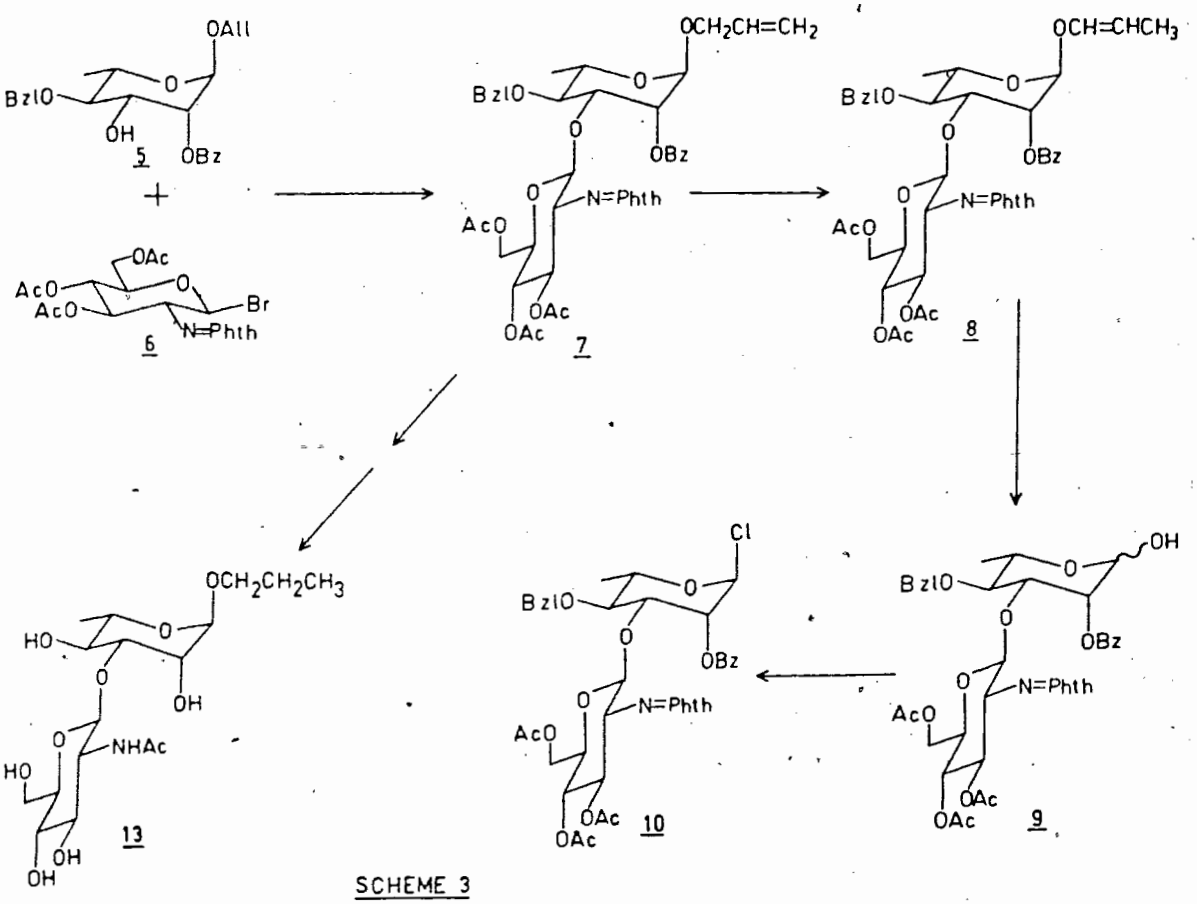
Synthesis

This thesis describes the synthesis of a disaccharide and a trisaccharide portion of the bacterial cell-wall polysaccharide of the β-hemolytic *Streptococci* Group A, as propyl glycoside haptens to be used in inhibition studies with the natural antigen, and also for use in n.m.r. studies. The trisaccharide was also prepared as its 8-methoxycarbonyloctyl glycoside. In this form, the trisaccharide may be linked to a protein[12] to serve as an artificial antigen, or to a solid support to function as an immunoadsorbent.

The first step in the synthesis was a Königs-Knorr reaction of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-**L**-rhamnopyranoside[34] (**5**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-**D**-glucopyranosyl bromide[20] (**6**), using silver trifluoromethanesulphonate as promotor, and 2,4,6-trimethylpyridine as proton acceptor, to give the blocked disaccharide (**7**) in 91% yield. The phthalimido group at the 2-position of (**6**) sterically shields the α-face of the ring from nucleophilic attack, thus directing the glycosylation to give exclusively the β-glycoside.

The anomeric centre of the disaccharide (**7**) was accessed by removal of the 1-*O*-allyl group, using rhodium (I) catalysed-isomerisation[36] to give the prop-1-enyl glycosides (**8**) (80%). Mercuric chloride-mercuric oxide hydrolysis[37] of the vinyl ether

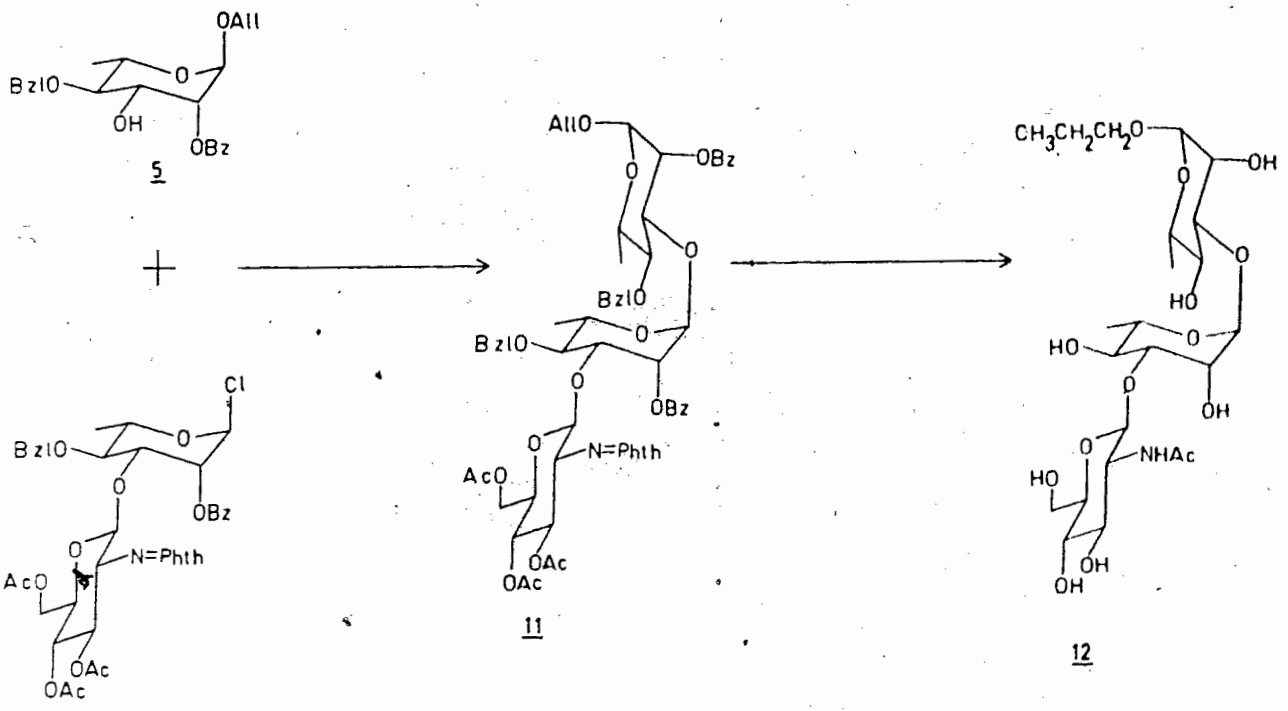
then gave the hemiacetals (9) (90%). The hemiacetals were converted into the glycosyl donor, specifically the glycosyl chloride (10), by reaction of (9) with the Vilsmeier-Haack reagent *NN*-dimethyl(chloromethylene)ammonium chloride[34,38] (Scheme 3). Reaction of the glycosyl donor (10) with the glycosyl accceptor (5), using silver trifluoromethanesulphonate promotion in the presence of 1,1,3,3-



SCHEME 3

tetramethylurea[40], gave the fully protected trisaccharide (11) in 75% yield (Scheme 4). This trisaccharide (11) differs from the key trisaccharide intermediate (1) outlined in the overall synthetic plan in that (11) cannot be selectively deprotected at the 2'-position. Deprotection at the 2'-position of

trisaccharide (11) is not possible since this structure has benzoyl groups at the 2-, and the 2'-positions and there is no method for their independent removal; furthermore, the acetate groups present at the 3''-,4''- , and 6''-positions would be labile under the conditions necessary to deprotect the 2'-position. These factors dictate that the trisaccharide (11) may



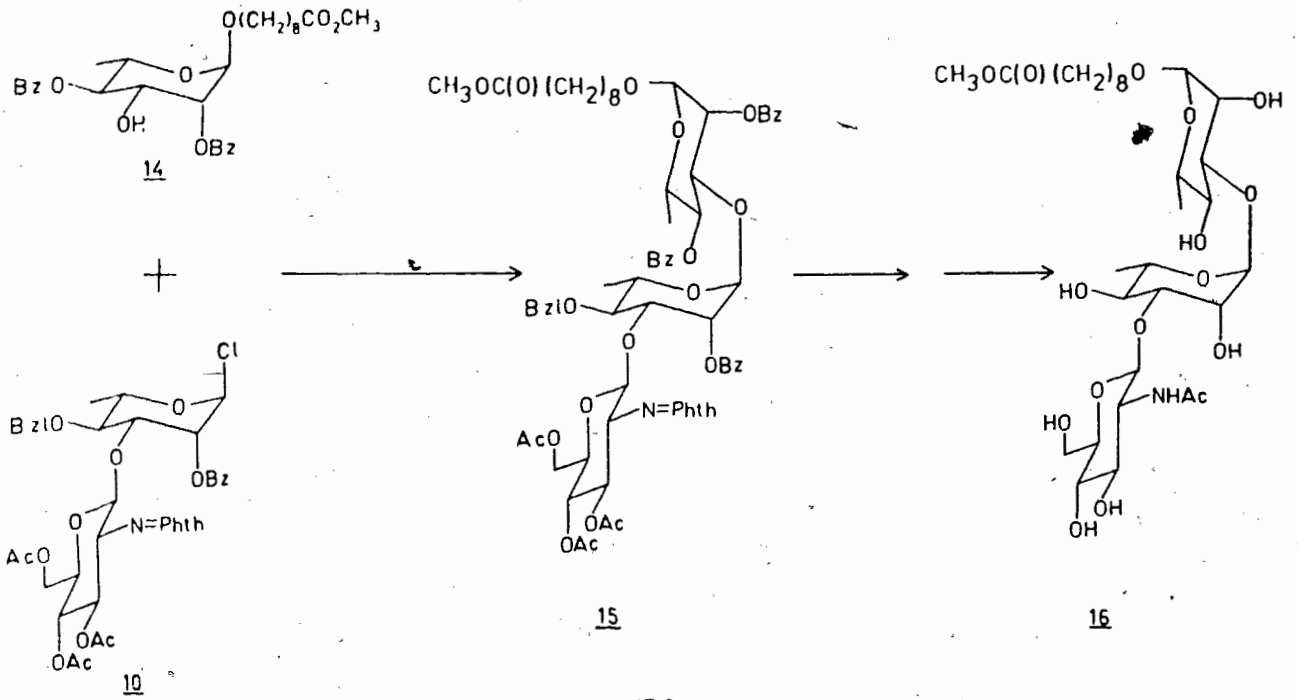
SCHEME 4

not be used in the block synthesis of higher-order oligosaccharides. The trisaccharide (11) gives, however, the first of the series of sequences for use in inhibition studies required to establish the structure which affords the greatest specificity in antibody recognition.

Deprotection[15,34] of the resulting trisaccharide (11) was accomplished by sodium methoxide-catalysed transesterification of the acyl groups, followed by hydrazinolysis of the

phthalimido group, selective N-acetylation of the resulting free amino group, and palladium-catalysed hydrogenation of the benzyl ethers to give the fully deprotected trisaccharide as its propyl glycoside (12) in 54% yield. A similar deprotection sequence was carried out on the disaccharide (7) to give the fully deprotected disaccharide as its propyl glycoside (13) in 55% yield.

To enable the trisaccharide to be used in the preparation of glycoconjugates, a linking arm at the 1-*O*-position was required.



SCHEME 5

This was accomplished by addition of the disaccharide chloride (10) to the monosaccharide, 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[41,42] (14), using silver trifluouromethanesulphonate as promotor, and 1,1,3,3-tetramethylurea as proton acceptor[40]. Chromatography of the

resulting products afforded a pure sample of the trisaccharide as its 8-methoxycarbonyloctyl glycoside (15)in 53% yield. Deprotection ,as before with compounds (7) and (11), gave the trisaccharide (16) in 71% yield, suitable for use in the preparation of glycoconjugates (Scheme 5).

The above compounds were obtained as analytically pure samples.

NMR Spectroscopic Results

Various nuclear magnetic resonance (n.m.r.) spectroscopic techniques were utilised to characterise the prepared compounds. These include the use of routine ^{1}H, ^{13}C, and $^{13}C\{^{1}H\}$ spectra, spin decoupling experiments, nuclear Overhauser enhancement (n.O.e.) experiments[43], as well as ^{1}H homonuclear chemical-shift correlated experiments (COSY)[44], and ^{13}C-^{1}H correlated (CHORTLE) experiments[45].

The vicinal coupling constants of the ring-protons in the monosaccharide units within the oligosaccharides were found to be consistent with a $^{4}C_{1}$(D) conformation for the *N*-acetylglucosamine ring and a $^{1}C_{4}$(L) conformation for the rhamnopyranosyl residues.

The stereochemical integrity of the various oligosaccharides was established by examination of the one-bond ^{13}C-^{1}H coupling constants, $^{1}J_{13C-1H}$, for the anomeric carbons. These values were consistent with an α-L-configuration about both rhamnosyl residues and the β-D-configuration about the *N*-acetylglucosamine residue.[46]

The analysis of the ^1H n.m.r spectrum of disaccharide (7) was made by comparison with spectra of the starting monosaccharide units and by comparison with spectra of related compounds[47]. Assignments were confirmed by means of homo-decoupling experiments.

The one-dimensional 400MHz ^1H n.m.r. spectrum of trisaccharide (11) revealed considerable overlap of the ring-proton signals in the region 3.5-5.5p.p.m. Therefore, a 500MHz COSY spectrum (Figures 13 and 14) of trisaccharide (11) was obtained in order to establish the intra-ring connectivities. For example, the signal at 5.18p.p.m., assigned to an anomeric proton from a rhamnosyl unit, shows a cross-peak in the COSY spectrum at 5.39p.p.m. indicating the position of the 2-H to which it is coupled. The 2-H in turn shows a cross-peak to a signal at 4.06p.p.m., indicating the position of the 3-H of the same ring. The ring protons 4,5 and 6 of the same rhamnosyl ring were assigned in a similar fashion (Figures 13 and 14). By following the path of the two- and three-bond couplings in the COSY spectrum all of the intra-ring connectivities were assigned. In order to distinguish one set of rhamnosyl ring protons from the other, an n.O.e. experiment was performed in which irradiation of one of the rhamnosyl anomeric protons was used to identify the proton across the inter-glycosidic linkage[48]. Thus, for example, irradiation of the anomeric proton at 4.90p.p.m. showed a significant n.O.e. of the signal at 3.98p.p.m., this signal being attributed to one of the diastereotopic protons of the allyl aglycone $-CH_2CH=CH_2$

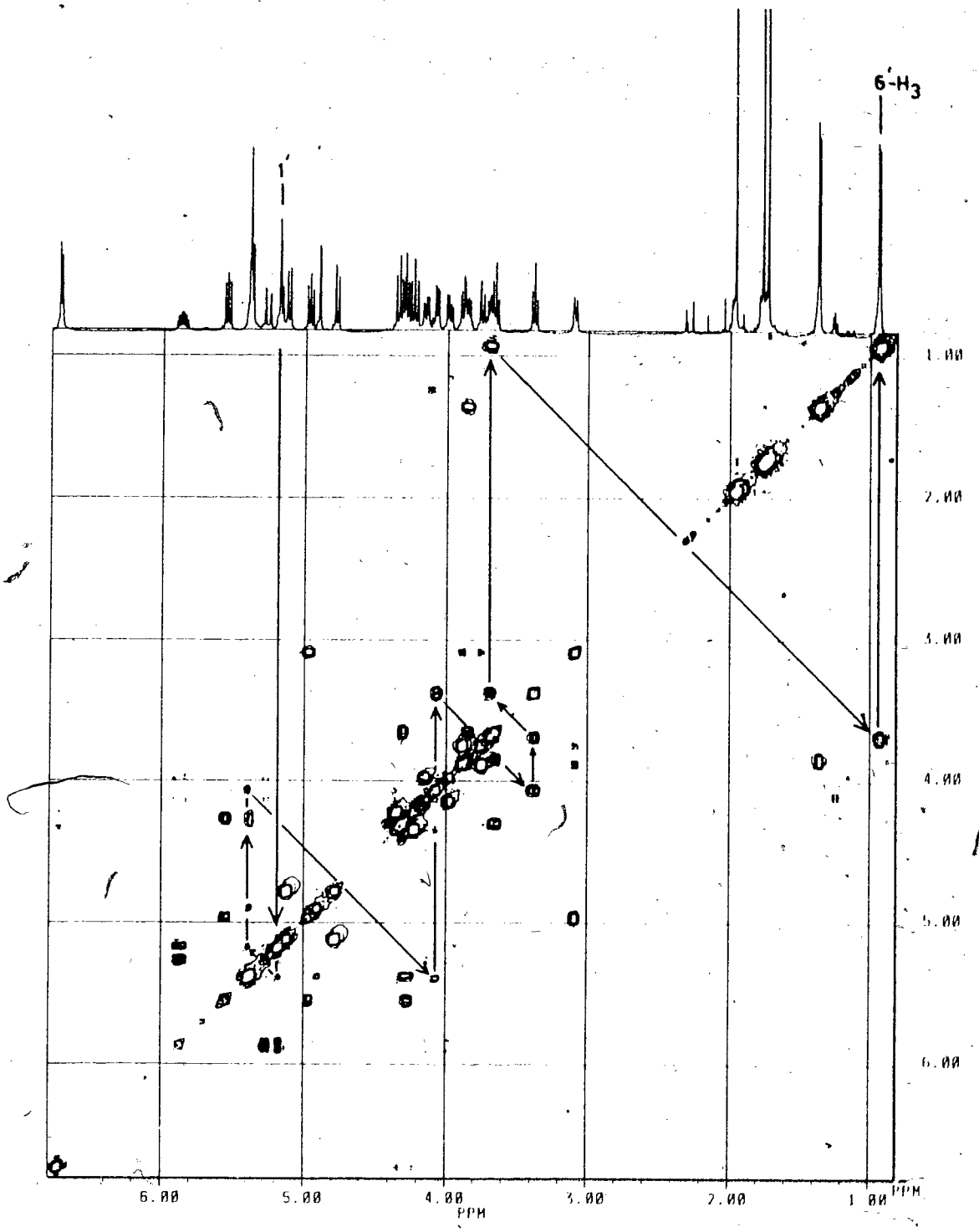


Figure 13

Partial 500MHz 2D-^{1}H n.m.r. COSY spectrum of trisaccharide (11)

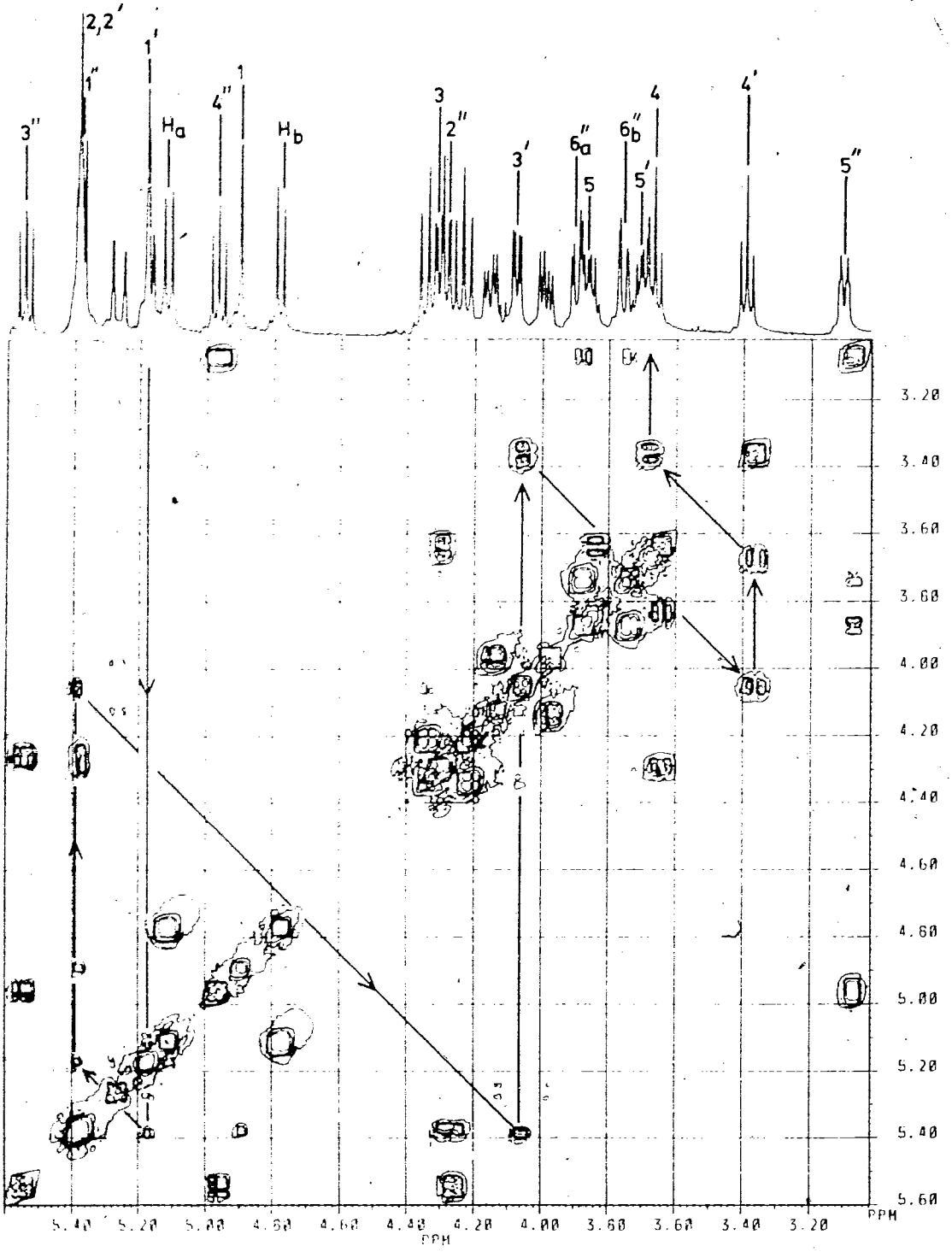


Figure 14

Expanded region of the 2D-^{1}H n.m.r. COSY spectrum of trisaccharide (11)

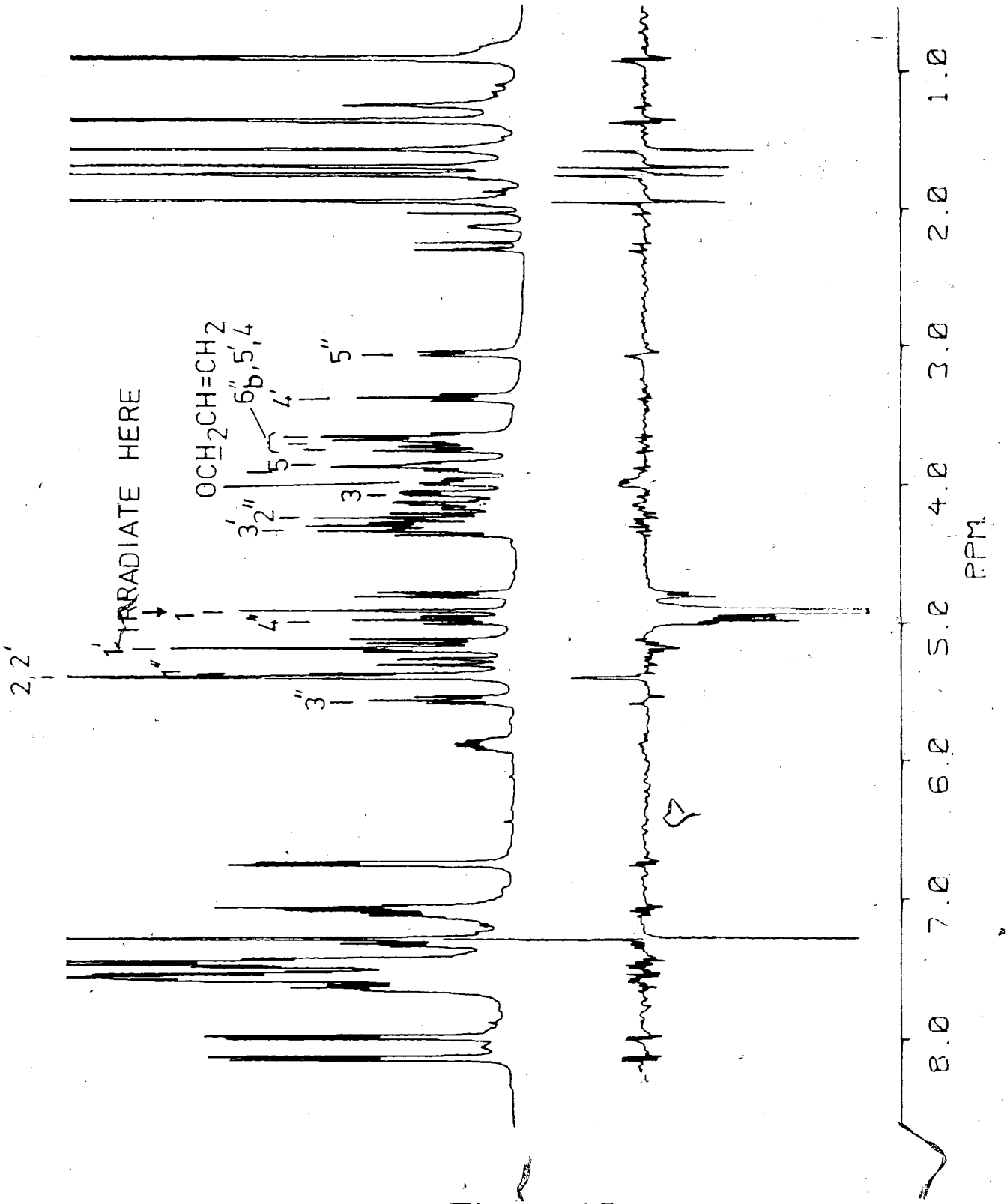


Figure 15

400MHz Difference n.O.e. ^{1}H n.m.r. spectrum of trisaccharide (11)

(Figure 15). This enhancement indicated that the irradiated signal was in fact the 1-H proton; the signal at 5.18ppm was therefore assigned to 1'-H. The ring-proton signals of the *N*-acetylglucosamine ring were readily distinguished from the rhamnosyl rings on the basis of vicinal coupling constants and hence assigned to the terminal ring of trisaccharide (11). The assignment of the ^{1}H n.m.r.spectrum of trisaccharide (15) was made by comparison with assignments made in the corresponding spectrum of trisaccharide (11). The assignments were confirmed by means of spin-decoupling experiments, and similar n.O.e. experiments to those described above (see Appendix).

Analysis of the ^{13}C-^{1}H correlation (CHORTLE) spectral data of (11) (Table 1), following the assignment of the ^{1}H n.m.r. spectrum from the COSY experiment, permitted the assignment of the $^{13}C\{^{1}H\}$ n.m.r. spectrum of trisaccharide (11). Chemical-shift assignments in the $^{13}C\{^{1}H\}$ n.m.r. spectra of compounds (7) and (15) were then made by comparison those in the n.m.r. spectrum of compound (11).

Owing to the complex overlap of signals in the ^{1}H n.m.r.spectra of the deprotected compounds (12), (13),and (16) in the region 3.5-4.0p.p.m. a 500MHz COSY spectrum of one of these compounds, namely, trisaccharide (12) was obtained; this proved to be invaluable in the assignment of some of the signals in that region (Figures 16-18). This spectrum, together with the ^{13}C-^{1}H correlation (CHORTLE) spectral data of the trisaccharide (12) (Table 2), and comparison with ^{1}H n.m.r data of the natural polymer[47], permitted the assignment of most of

Table 1

^{13}C-^{1}H correlation (CHORTLE) spectral data of trisaccharide (11)

CHORTLE can find a CH2 group instead of a CH group. Hence for the geminal pair, H(1) and H(2), its center, H-1, is also given.

Table of C-H chemical shifts (ppm).

No	C-13	H-1	+/-	H(1)	H(2)	+/-
1	98.8	5.154	0.005			
2	98.4	5.354	0.003			
3	96.2	4.878	0.003			
4	80.0	3.630	0.003			
5	79.7	4.050	0.003			
6	78.6	3.357	0.003			
7	78.3	4.279	0.004			
8	75.2	4.929	0.005	4.760	5.098	0.005
* 9	73.7	4.263	0.005			
9	73.7	4.262	0.002	4.203	4.320	0.004
10	72.6	5.359	0.003			
11	72.0	5.371	0.003			
* 11	72.0	5.371	0.003	5.347	5.394	0.009
12	71.2	3.068	0.003			
13	70.7	5.530	0.003			
14	68.2	3.667	0.008			
15	68.2	4.039	0.007	3.931	4.147	0.009
16	68.0	4.945	0.004			
17	67.7	3.827	0.003			
** 18	61.0	3.793	0.006			
19	54.6	4.250	0.003			
20	20.5	1.918	0.004			
21	20.3	1.723	0.003			
22	20.3	1.710	0.003			
23	18.0	1.334	0.003			
24	17.5	0.903	0.003			

* These peaks are attributed to artifacts.

** The expected geminal pair was not detected, only its centre is given.

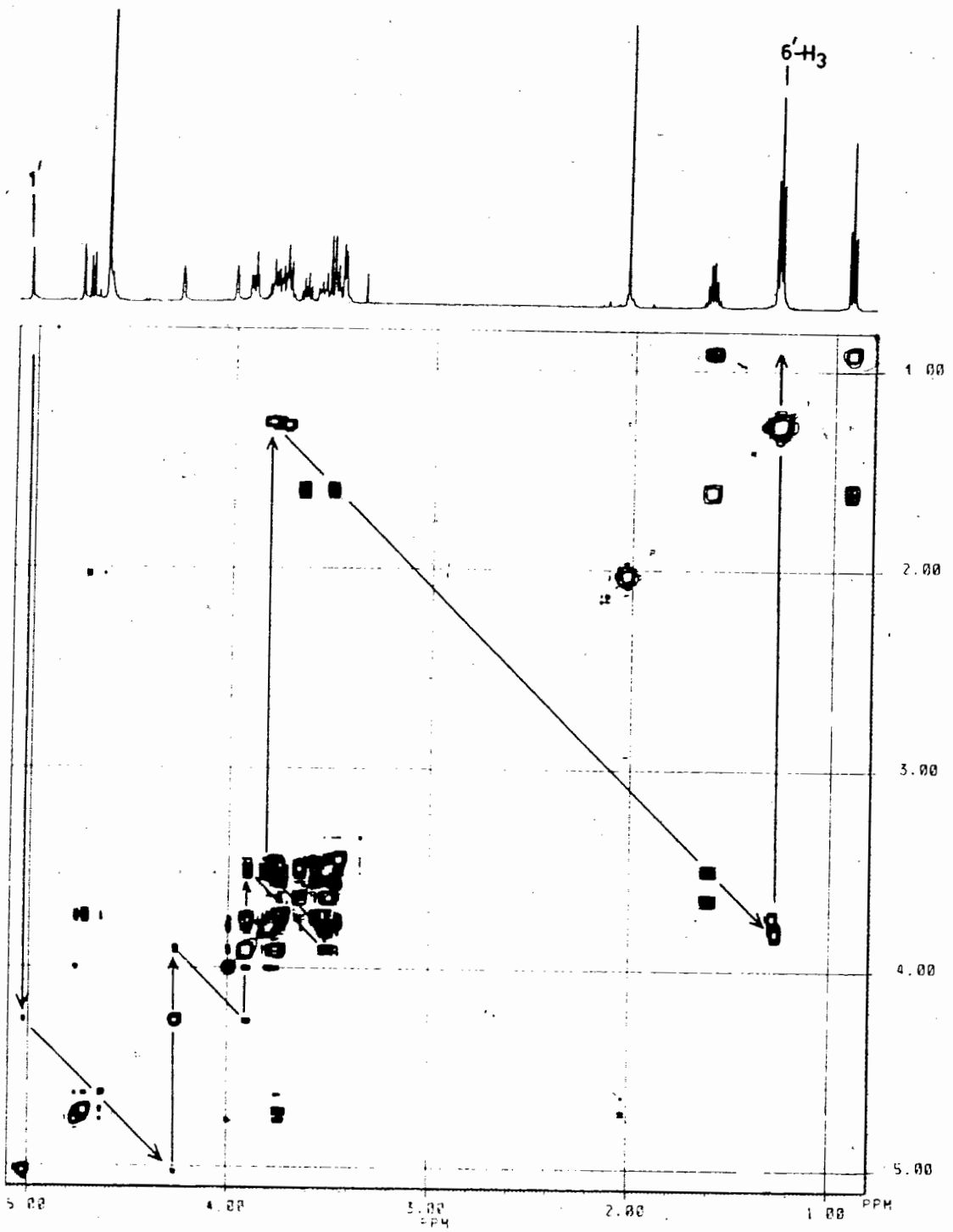


Figure 16

500MHz 2D-^{1}H n.m.r. COSY spectrum of deprotected trisaccharide (12)

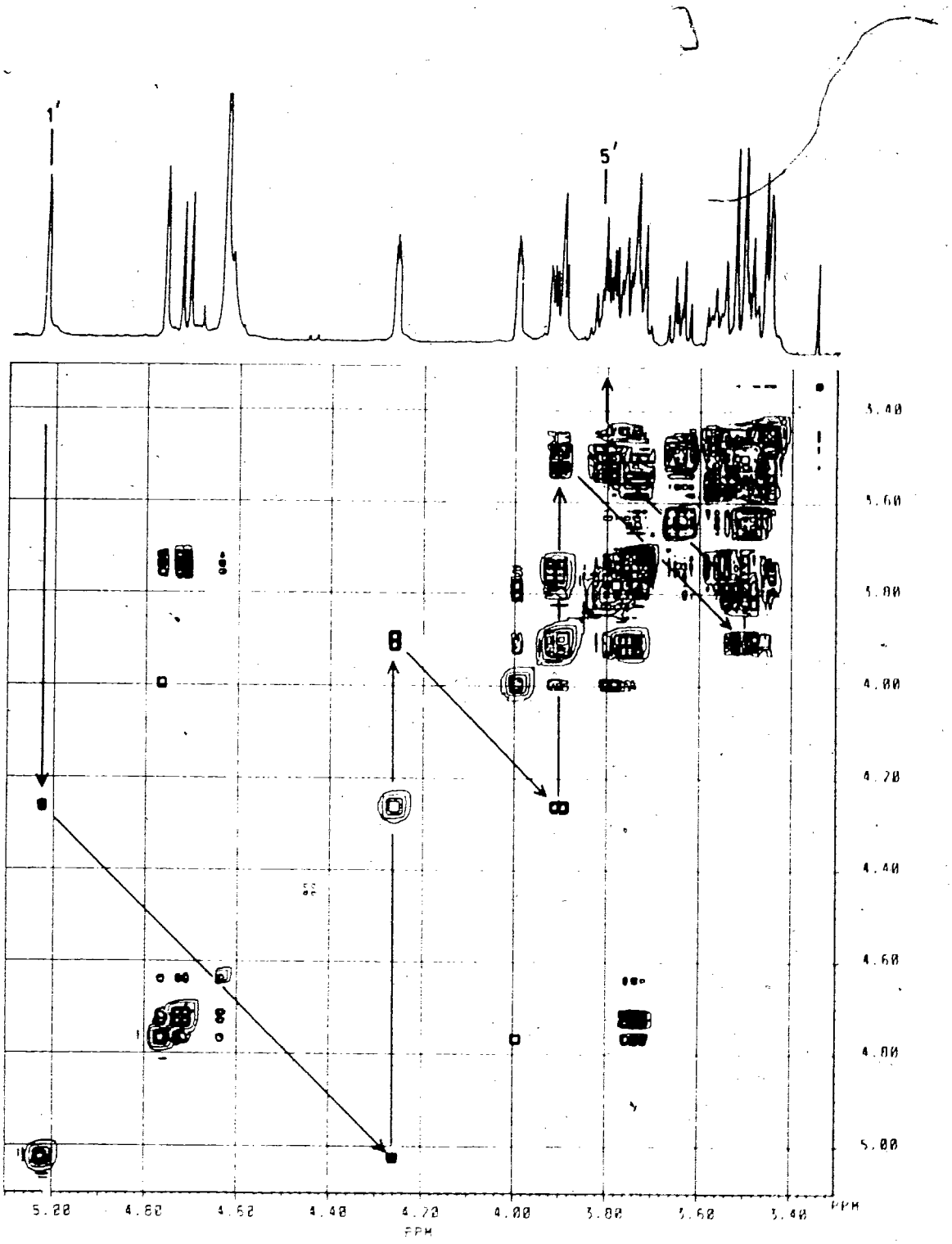


Figure 17

Expanded region of the 2D-^{1}H n.m.r. COSY spectrum of deprotected trisaccharide (12)

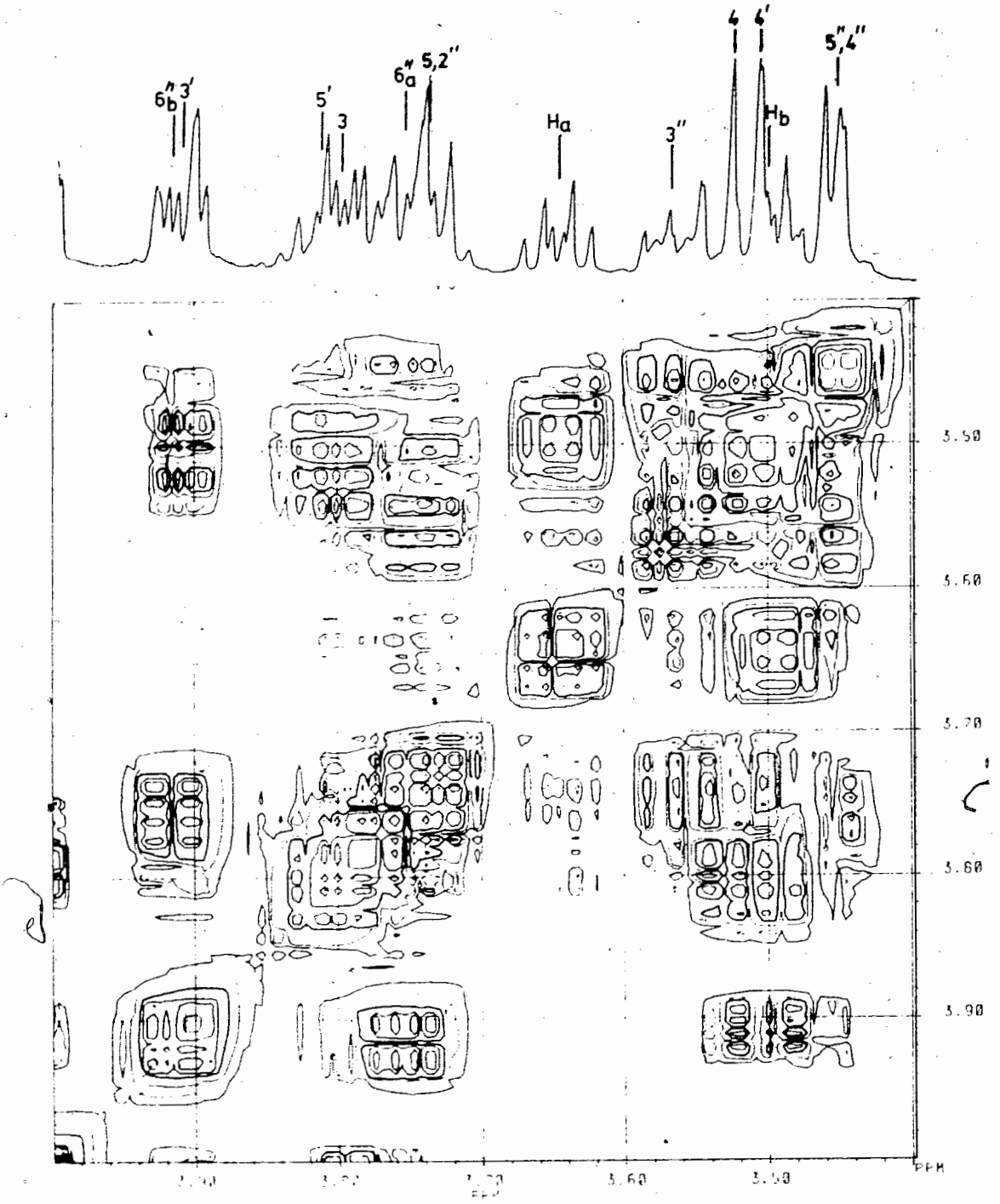


Figure 18

Expanded region of the 2D-^{1}H n.m.r. COSY spectrum of deprotected trisaccharide (12)

Table 2

^{13}C-^{1}H correlation (CHORTLE) spectral data of trisaccharide (12)

CHORTLE can find a CH2 group instead of a CH group. Hence for the geminal pair, H(1) and H(2), its center, H-1, is also given.

Table of C-H chemical shifts (ppm).

No	C-13	H-1	+/-	H(1)	H(2)	+/-
1	103.4	4.732	0.002			
2	102.7	5.033	0.002			
3	100.4	4.775	0.002			
4	80.8	3.917	0.003			
5	79.0	3.806	0.002			
6	76.5	3.461	0.002			
7	74.6	3.580	0.002			
*7	74.6	3.580	0.002	3.559	3.601	0.008
8	72.3	3.538	0.002			
9	71.8	3.519	0.002			
10	70.9	4.011	0.003			
11	70.7	3.465	0.180	3.559	3.372	0.192
12	70.5	3.581	0.009			
13	70.1	3.822	0.002			
14	69.5	3.747	0.002			
*14	69.5	3.747	0.002	3.726	3.768	0.003
*15	61.5	3.848	0.008			
15	61.5	3.847	0.003	3.771	3.923	0.004
16	56.6	3.752	0.002			
17	23.1	2.040	0.003			
18	22.8	1.621	0.003			
19	17.5	1.291	0.002			

* These peaks are attributed to artifacts.

the carbon and proton signals in the spectra of (12). Most of the signals in the $^{13}C\{^{1}H\}$ and ^{1}H n.m.r. spectra of the deprotected structures (13) and (16) were then assigned by comparison with the data for (12).

Conclusion

The disaccharide (13) and the trisaccharide (12) may be used as haptens in inhibition studies with the natural antigen to help establish the requirements for a highly defined *Streptococci* Group A antigen. The trisaccharide (16) may be coupled, *via* the 8-methoxycarbonyloctyl linking arm,[11] to carrier proteins, enzymes or immunoadsorbent support materials, as required, using the simplified acyl azide methodology.[12] The ability of the immunoadsorbents incorporating the oligosaccharide to detect anti-*Streptococci* Group A antibodies in sera can then be tested. Alternatively, the glycoconjugates can be used in the hybrid-myeloma protocol as immunizing antigens and screening aids to produce mouse monoclonal antibodies of defined specificities which can themselves be used, in a latex agglutination test, for example, to detect *Streptococci* Group A antigen. Thus, in the long term, both antibody and antigen may be incorporated into diagnostic reagents for the detection of streptococcal Group A pharyngitis.

III. EXPERIMENTAL

General Procedures

Routine ^{1}H n.m.r. (400.13MHz) and ^{13}C n.m.r. (100.6MHz) were recorded on a Bruker WM-400 spectrometer. The ^{1}H homonuclear chemical-shift-correlated (COSY) spectra and the ^{13}C-^{1}H correlated (CHORTLE) spectra were recorded by Dr. D. R. Bundle of the National Research Council of Canada, Ottawa, on a Bruker AM-500 spectrometer operating at 500.13MHz for ^{1}H and 125.7MHz for ^{13}C. Spectra were recorded in deuteriochloroform solution unless otherwise stated. Chemical shifts are given in p.p.m. downfield from $SiMe_4$. For spectra recorded in deuterium oxide, chemical shifts are given in p.p.m. downfield from $MeSi[CH_2]_3SO_3Na$. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. Nuclear Overhauser enhancement (n.O.e.) experiments were performed in the difference mode. Optical rotations were performed on a Perkin-Elmer P22 spectropolarimeter.

Analytical t.l.c. was performed on pre-coated aluminum foil plates with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to u.v. light and/or sprayed with 5% sulphuric acid in ethanol, and heated at 150°C. Medium pressure chromatography was performed according to a published procedure[49].

Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated

under reduced pressure and below 40°C.

Reactions performed under nitrogen were also carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

Specific Procedures

Allyl 3-*O*-(3',4',6'-tri-*O*-Acetyl-2'-deoxy-2'-phthalimido-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (7).-- A mixture of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (5)[34] (1.30g, 3.26mmol), silver trifluoromethanesulphonate (1.23g, 4.77mmol), and 4A molecular sieves in anhydrous dichloromethane (20cm^3) was stirred for 0.5 h under nitrogen in a flask fitted with a dropping funnel, equipped with a cooling jacket. A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (6)[20] (2.40g, 4.82mmol) and collidine (0.63cm^3, 4.8mmol) in anhydrous dichloromethane (5.0cm^3), previously stirred under an atmosphere of nitrogen for 0.5 h in the presence of 4A molecular sieves was transferred under nitrogen to the dropping funnel by means of a cannula. The flask was rinsed with additional portions of dichloromethane (2x2cm^3). The solution of (6) was cooled to -78°C and then added dropwise, over a period of 10 min, to the mixture containing the allyl glycoside (5). The mixture was allowed to warm gradually to room temperature and was stirred for 12 h in the dark under nitrogen. The solids were removed by filtration and the filtrate was washed successively with 1*M*

hydrochloric acid solution, sodium hydrogen carbonate solution, and sodium chloride solution. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup which was purified by column chromatography using hexane-ethyl acetate (1:1) as eluant; R_f0.43. The *title compound* (7) was obtained as a clear syrup (2.43g, 91%); $[\alpha]^{25}_D$ -15.5° (*c* 0.9 in CH_2Cl_2); δ_C(100.6 MHz) 17.7 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 54.8 (C-2'), 61.7 (C-6'), 67.3 (C-5), 68.3, and 68.4 ($OCH_2CH=CH_2$, and C-4'), 70.9 (C-3'), 71.6 (C-5'), 72.1 (C-2), 74.6 ($-CH_2Ph$), 79.2 (C-3), 80.2 (C-4), 96.2 (C-1), 98.5 (C-1'), 117.9 ($O-CH_2CH=CH_2$), 133.7 ($O-CH_2CH=CH_2$), and 166.1, 169.5, 170.2, and 170.8 (C=O); δ_H(400.13MHz) 1.13 (3H, d, $J_{5,6}$=6.3Hz, 6-H_3), 1.78, 1.80, and 1.97 (3x3H, s, $OCOCH_3$), 3.48 (1H, t, $J_{3,4}+J_{4,5}$=19Hz, 4-H), 3.69 (1H, m, 5-H), 3.85 (1H, ddd, $J_{4',5'}$=10.1, $J_{5',6a'}$=4.0, $J_{5',6b'}$=2.3Hz, 5'-H), 4.04 (1H, dd, $J_{5',6b'}$=2.3, $J_{6a',6b'}$=12.0Hz, 6'-H_b), 4.13 (1H, dd, $J_{5',6a'}$=4.0, $J_{6a',6b'}$=12.0Hz, 6'-H_a), 4.18 (1H, dd, $J_{2,3}$=3.5Hz, $J_{3,4}$=9.5, 3-H), 4.37 (1H, dd, $J_{2',3'}$=10.8, $J_{1',2'}$=8.5Hz, 2'-H), 4.89 (1H, d, $J_{1,2}$=1.8Hz, 1-H), 5.05 (1H, dd, $J_{3',4'}$=9.2, $J_{4',5'}$=10.1Hz, 4'-H), 5.44 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.5Hz, 2-H), 5.67 (1H, d, $J_{1',2'}$=8.5Hz, 1'-H), and 5.70 (1H, dd, $J_{3',4'}$=9.2Hz, $J_{2',3'}$=10.8, 3'-H) (Found: C, 63.3; H, 5.5; N, 1.7. $C_{43}H_{45}NO_{15}$ requires C, 63.3; H, 5.56; N, 1.72%).

Prop-1-enyl 3-*O*-(3',4',6'-tri-*O*-Acetyl-2'-deoxy-2'-phthalimido-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (8).---Tris(triphenylphosphine)rhodium (I) chloride (85.7mg,

0.093mmol) was added to a solution of the allyl glycoside (7) (1.36g, 1.66mmol) in ethanol-water (9:1) (60cm^3) and the mixture was heated at reflux for 16 h under nitrogen. The solvent was removed by evaporation to give a dark brown residue which was taken up in ethyl acetate and filtered through a short column of silica gel.

Removal of the solvent gave a light brown foam which was further purified by column chromatography with hexane-ethyl acetate (1:1) as eluant R_f0.47. The *title compound* (8) was obtained as a clear light brown syrup as a mixture of **E** and **Z** isomers (1.17g, 86%); δ_C(100.6MHz) 9.2, and 12.1 (CH=CH*C*H$_3$ **E**, **Z**) 17.7 (C-6), 20.1, 20.2, and 20.4 (OCO*C*H$_3$), 96.4, and 96.5 (C-1 **E**, **Z**), 98.7, and 98.8 (C-1' **E**, **Z**), 104.5, and 105.0 (CH=*C*HCH$_3$ **E**, **Z**), 166.0 (O*C*OC$_6$H$_5$), 167.8 (phthaloyl C=O), and 169.4, 170.1, and 170.7 (O*C*OCH$_3$); δ_H(400.13MHz) 1.12 (3H, m, 6-H$_3$, **E**, **Z**), 1.55, and 1.65 (3H, 2 dd, *J*=1.9, 7.0Hz, CH=CHC*H*$_3$ **E**, **Z**), 1.79, 1.81, and 1.98 (3x3H, s, OCOC*H*$_3$), 4.59, and 5.14 (1H, 2 m, *J*=6.4, 7.0Hz, CH=C*H*CH$_3$ **Z**, and *J*=7.0, 12.1Hz, CH=C*H*CH$_3$ **E**) , 5.45, and 5.53 (1H, 2dd, *J*=1.8, 3.5Hz, 2-H **E**, **Z**), and 6.06, and 6.13 (1H, 2 m, *J*=1.9, 6.4Hz, C*H*=CHCH$_3$ **Z**, and *J*=1.9, 12.1 Hz, C*H*=CHCH$_3$ **E**).

3-*O*-(3',4',6'-tri-*O*-Acetyl-2'-deoxy-2'-phthalimido-β-D-gluco-pyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-L-rhamnopyranose (9).---The prop-1-enyl glycosides (8) (1.01g, 1.23mmol) were dissolved in 90% aqueous acetone (40cm^3) and the solution was stirred.

Yellow mercury (II) oxide (0.279g, 1.29mmol) was added followed by the dropwise addition, over 2 min, of a solution of mercury (II) chloride (0.336g, 1.24mmol) in 90% aqueous acetone (25cm^3). The mixture was stirred for 5 h, the solvent was evaporated, and the resulting residue dissolved in ethyl acetate and filtered through celite. The filtrate was washed successively with saturated aqueous potassium iodide (2x), aqueous sodium thiosulphate (2x), and water (2x). The organic layer was dried (Na_2SO_4), the solvent was evaporated and the resulting yellow syrup was chromatographed with hexane-ethyl acetate (3:2) as eluant. The *title compound* (**9**) was obtained as a clear light yellow syrup (1.04g, 93%); δ_C(100.6MHz) (α-anomer) 17.8 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 91.9 ($^1J_{13C-H}$=172Hz, C-1), 98.6 ($^1J_{13C-H}$=167Hz, C-1'), and 166.1, 169.5, 170.1, and 170.9 (C=O).

3-*O*-(3',4',6'-tri-*O*-Acetyl-2'-deoxy-2'-phthalimido-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl chloride (**10**).---Oxalyl chloride (0.57cm^3, 6.5mmol) was added to a stirred solution of DMF (0.5cm^3, 6.5mmol) in anhydrous dichloromethane (4.0cm^3) and the mixture was stirred under nitrogen for 5 min. The solvent was evaporated under reduced pressure and the white salt was dried *in vacuo* for 50 min. The *NN*-dimethyl(chloromethylene)ammonium chloride was then dissolved in anhydrous dichloromethane (4cm^3) and a solution of the hemiacetals (**9**) (1.04g, 1.33mmol) in anhydrous dichloromethane (2cm^3) was transferred to the flask under nitrogen by means of a

cannula. The flask was rinsed with additional portions of solvent and transferred as before. The mixture was stirred under nitrogen for 2 h, and then the reaction was quenched by the addition of cold aqueous sodium hydrogen carbonate ($20cm^3$). The organic layer was diluted with dichloromethane and washed successively with aqueous sodium hydrogen carbonate, and aqueous sodium chloride. The organic layer was dried over anhydrous potassium carbonate, and the solvent was evaporated to give a clear light yellow syrup (10) (1.07g, 99%) which was dried *in vacuo* and used directly in the subsequent glycosylation reaction; δ_C(100.6MHz) (α-anomer) 17.4 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 54.8 (C-2'), 61.9 (C-6'), 68.6 (C-5), 74.7 ($-OCH_2Ph$), 78.4 (C-3), 78.8 (C-4), 89.8 ($^1J_{13C-H}$=184Hz, C-1), 98.9 ($^1J_{13C-H}$=158Hz, C-1'), and 165.8, 169.5, 170.1, and 170.8 (C=O).

Allyl 3-*O*-[3'-*O*-(3'',4'',6''-tri-*O*-Acetyl-2''-deoxy-2''-phthalimido-β-D-glucopyranosyl)-2'-*O*-benzoyl-4'-*O*-benzyl-α-L-rhamnopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (11).---A mixture of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (5)[34] (0.381g, 0.957mmol), silver trifluoromethanesulphonate (0.382g, 1.49mmol), and 4A molecular sieves in anhydrous dichloromethane ($8cm^3$) was stirred under an atmosphere of nitrogen for 0.5 h in a flask fitted with a dropping funnel which was equipped with a cooling jacket. A solution of the glycosyl chloride (10) (1.07g, 1.34mmol), and 1,1,3,3-tetramethylurea ($0.18cm^3$, 1.5mmol) in anhydrous

dichloromethane ($4cm^3$), previously stirred with 4A molecular sieves for 0.5 h under nitrogen, was transferred under nitrogen to the dropping funnel by means of a cannula. The flask was rinsed with additional portions of anhydrous dichloromethane ($2x2cm^3$) and transferred as before. The solution of the glycosyl chloride was cooled to -78°C and added dropwise, during 20 min, to the cooled (-50°C) solution of (5). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane ($2x2cm^3$). The mixture was allowed to warm to room temperature was stirred in the dark under an atmosphere of nitrogen for 36 h. The solids were removed by filtration and the filtrate was diluted with dichloromethane ($30cm^3$) and washed successively with aqueous sodium hydrogen carbonate, and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and the solvent was evaporated to give a syrup which was chromatographed using hexane-ethyl acetate (1:1) as eluant; R_f 0.51. The *title compound* (11) was obtained as a clear colourless syrup (0.835g, 76%); $[\alpha]^{25}_D$ -4.5° (*c* 2.4 in CH_2Cl_2); δ_C(125.7MHz) 17.5 (C-6'), 18.0 (C-6), 20.3 (2x $OCOCH_3$), 20.5 ($OCOCH_3$), 54.6 (C-2''), 61.0 (C-6''), 67.7 (C-5), 68.0 (C-4''), 68.2 ($O-CH_2CH=CH_2$), 68.3 (C-5'), 70.8 (C-3''), 71.2 (C-5''), 72.0 (C-2'), 72.6 (C-2), 73.7, and 75.2 ($O-CH_2Ph$), 78.3 (C-3), 78.6 (C-4'), 79.7 (C-3'), 80.1 (C-4), 96.3 (C-1), 98.4 (C-1''), 98.8 (C-1'), 117.5 ($O-CH_2CH=CH_2$), and 165.67, 165.73, 169.1, 170.0 and 170.6 (C=O); δ_H(400.13MHz) 0.92 (3H, d, $J_{5',6'}$=6.2Hz, 6'-H_3), 1.36 (3H, d, $J_{5,6}$=6.2Hz, 6-H_3), 1.71, 1.76, and 1.96 (3x3H, s, $OCOCH_3$), 3.06 (1H, ddd, $J_{5'',6b''}$=2.2, $J_{5'',6a''}$=3.2,

$J_{4'',5''}$=10.2Hz, 5''-H), 3.38 (1H, t, $J_{3',4'}+J_{4',5'}$=18.8Hz, 4'-H), 3.65 (1H, t, $J_{3,4}+J_{4,5}$=18.8Hz, 4-H), 3.68 (1H, m, 5'-H), 3.73 (1H, dd, $J_{5'',6b''}$=2.2, $J_{6a'',6b''}$=12.6Hz, 6''-H_b), 3.85 (1H, m, 5-H), 3.88 (1H, dd, $J_{5'',6a''}$=3.2, $J_{6a'',6b''}$=12.6Hz, 6''-H_a), 4.06 (1H, dd, $J_{2',3'}$=3.5, $J_{3',4'}$=9.2Hz, 3'-H), 4.27 (1H, dd, $J_{1'',2''}$=8.5, $J_{2'',3''}$=10.5Hz, 2''-H), 4.32 (1H, dd, $J_{2,3}$=3.5, $J_{3,4}$=9.2Hz, 3-H), 4.90 (1H, d, $J_{1,2}$=1.8Hz, 1-H), 4.97 (1H, dd, $J_{3'',4''}$=9.3, $J_{4'',5''}$=10.2Hz, 4''-H), 5.18 (1H, d, $J_{1',2'}$=1.8Hz, 1'-H), 5.37 (1H, d, $J_{1'',2''}$=8.5Hz, 1''-H), 5.39 (2x1H, dd, J=1.8, 3.5Hz, 2-H, and 2'-H), and 5.55 (1H, dd, $J_{3'',4''}$=9.3, $J_{2'',3''}$=10.5Hz, 3''-H) (Found: C, 65.4; H, 5.7; N, 1.4. $C_{63}H_{65}NO_{20}$ requires C, 65.45; H, 5.67; N, 1.21%).

Propyl 3-*O*-[3'-*O*-(2''-Acetamido-2''-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (12).---A sample of the fully protected trisaccharide (11) (0.755g, 0.653mmol) was dissolved in a solution of 1*M* sodium methoxide in methanol (10cm^3). The mixture was let stand under nitrogen for 20 h. The solution was then neutralised by addition of Rexyn 101 (H^+) resin. The resin was removed by filtration and the filtrate was concentrated to give a clear syrup. The syrup was dissolved in ethanol (10cm^3), containing hydrazine hydrate 100% (2cm^3), and the mixture was refluxed for 16 h. Removal of a white rubbery material by filtration, and solvent evaporation , gave a clear colourless syrup which was chromatographed using ethyl acetate-methanol-water (85:10:5) as eluant; R_f0.4. The resulting clear

colourless syrup was dissolved in methanol ($25cm^3$), containing acetic anhydride ($2.5cm^3$), and stirred overnight. Solvent evaporation gave a clear colourless syrup which was taken up in 80% aqueous acetic acid ($20cm^3$) and hydrogenolysed over 10% palladium-carbon (0.20g) at a hydrogen pressure of 52 p.s.i. for 48 h. The solids were removed by filtration through celite and the solvent removed by evaporation to give a clear light brown syrup which was chromatographed using ethyl acetate-methanol-water (7:2:1) as eluant; R_f 0.41. The *title compound* (12) was obtained as a white amorphous solid (0.195g, 53.9%); $[\alpha]^{29}_D$ - 59.2° (*c* 1.0 in H_2O); δ_C(D_2O, 100.6 MHz) 12.8 ($OCH_2CH_2CH_3$), 19.6 (C-6, C-6'), 24.9 ($OCH_2CH_2CH_3$), 25.2 ($NHCOCH_3$), 58.8 (C-2''), 63.7 (C-6''), 71.7 (C-5), 72.2 (C-5'), 72.6, 72.8, 72.9, and 73.1 (C-2, C-2', C-4'', and $-OCH_2CH_2CH_3$), 74.0 (C-4'), 74.5 (C-4), 76.8 (C-3''), 78.7 (C-5''), 81.1 (C-3), 83.0 (C-3'), 102.6 ($^1J_{13C-1H}$=170Hz, C-1), 104.8 ($^1J_{13C-1H}$=167Hz, C-1'), 105.6 ($^1J_{13C-1H}$=162Hz, C-1''), and 178.0 ($NHCOCH_3$); δ_H(D_2O, 500.13MHz) 1.27, and 1.29 (2x3H, d, *J*=6.4Hz, 6-H_3, and 6'-H_3), 2.04 (3H, s, $NHCOCH_3$), 3.45 (2H, m, 4''-H, 5''-H), 3.46-3.60 (4H, m, $OCH_2CH_2CH_3$, 4'-H, 4-H, and 3''-H), 3.64 (1H, m, $OCH_2CH_2CH_3$), 3.70-3.77 (3H, m, 2''-H, 5-H, and 6''-H_a), 3.79 (1H, dd, $J_{2,3}$=3.2, $J_{3,4}$=9.7Hz, 3-H), 3.91 (1H, br d, $J_{6a'',6b''}$=12.3Hz, 6''-H_b), 3.99 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.2Hz, 2-H), 4.26 (1H, dd, $J_{1',2'}$=1.8, $J_{2',3'}$=3.3Hz, 2'-H), 4.71 (1H, d, $J_{1'',2''}$=8.6Hz, 1''-H), 4.76 (1H, d, $J_{1,2}$=1.8Hz, 1-H), and 5.01 (1H, d, $J_{1',2'}$=1.8Hz, 1'-H) (Found: C, 49.5; H, 7.5; N, 2.3. $C_{23}H_{41}O_{14}N$ requires C, 49.7; H, 7.5; N, 2.5%).

Propyl 3-*O*-(2'-Acetamido-2'-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (13).---A sample of the fully protected disaccharide (7) (0.784g, 0.960mmol) was dissolved in a solution of 1.0*M* sodium methoxide in methanol (10cm^3). The solution was let stand at room temperature, under an atmosphere of nitrogen. After 40 h the mixture was neutralised by stirring with Rexyn 101(H^+) resin. The resin was removed by filtration and the solvent evaporated to give a clear syrup which was then dissolved in ethanol (30cm^3) containing hydrazine hydrate 100% (0.08cm^3, 1.65mmol). The mixture was refluxed for 24 h under an atmosphere of nitrogen. Following filtration and solvent evaporation the resulting clear colourless syrup was dried *in vacuo* to remove traces of hydrazine. The white amorphous solid was dissolved in methanol (25cm^3) containing acetic anhydride (2.5cm^3) and let stand under nitrogen. After 16 h the solvent was removed by evaporation and the resulting syrup was chromatographed using ethyl acetate-methanol-water as eluant (85:10:5); R_f0.36. The resulting clear colourless glass was then dissolved in 80% aqueous acetic acid (30cm^3) and hydrogenolysed over 10% palladium-carbon (0.098g) at a hydrogen pressure of 55 p.s.i. for 3 days. The solids were removed by filtration through a pad of celite and the solvent removed by evaporation. Following chromatography, using ethyl acetate-methanol-water (7:2:1) as eluant; R_f 0.47, the *title compound* (13) was obtained as a white amorphous solid (0.216g, 55%); $[\alpha]^{29}_D$ -47.2° (*c*0.9 in H_2O); δ_C(D_2O, 100.6MHz) 12.6

($OCH_2CH_2CH_3$), 19.4 (C-6), 24.8 ($CH_2CH_2CH_3$), 25.0 ($NHCOCH_3$), 58.7 (C-2'), 63.6 (C-6'), 71.7 (C-5), 72.6, 72.8, and 72.9 (C-2, C-4', and $-OCH_2CH_2CH_3$), 73.9 (C-4), 76.6 (C-3'), 78.6 (C-5'), 83.2 (C-3), 102.2 ($^1J_{13C-1H}$=170Hz, C-1), 105.7 ($^1J_{13C-1H}$=163Hz, C-1'), and 178.0 ($NHCOCH_3$); δ_H(D_2O, 400.13MHz) 0.88 (3H, t, J=7.5Hz, $OCH_2CH_2CH_3$), 1.24 (3H, d, $J_{5,6}$=6.1Hz, 6-H_3), 1.58 (2H, m, $OCH_2CH_2CH_3$), 2.00 (3H, s, $NHCOCH_3$), 3.40-3.82 (10H, complex multiplet, ring hydrogens, and $OCH_2CH_2CH_3$), 3.87 (1H, br d, $J_{6a',6b'}$=12.0Hz, 6'-H_b), 4.11 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.2Hz, 2-H), and 4.64 (1H, d, $J_{1',2'}$=8.3Hz, 1'-H) (Found: C, 49.7; H,7.5; N, 3.3. $C_{17}H_{31}NO_{10}$ requires C, 49.87; H, 7.63; N, 3.42%).

8-Methoxycarbonyloctyl 3-*O*-[3'-*O*-(3'',4'',6''-tri-*O*-Acetyl-2''-deoxy-2''-phthalimido-β-D-glucopyranosyl)-2'-*O*-benzoyl-4'-*O*-benzyl-α-L-rhamnopyranosyl)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (15).---A mixture of 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (14)[41,42] (0.146g, 0.270mmol), silver trifluoromethanesulphonate (0.12g, 0.46mmol) and 4A molecular sieves in anhydrous dichloromethane (2.0cm^3) was stirred for 0.5 h under nitrogen in a Schlenk tube fitted with a dropping funnel, equipped with a cooling jacket. A solution of the disaccharide chloride (10) (0.368g, 0.464mmol), and 1,1,3,3-tetramethylurea (0.05cm^3, 0.42mmol) in anhydrous dichloromethane (2.0cm^3), previously stirred with 4A molecular sieves for 0.5 h under nitrogen, was transferred *via* cannula to the dropping funnel. The flask was rinsed with additional

portions of anhydrous dichloromethane (2x1.0cm^3) and transferred to the dropping funnel as before. The cooled (-78°C) solution of the glycosyl chloride was added dropwise, over 30 min, to the cooled (-35°C) mixture containing the alcohol (**14**). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane (2x1.0cm^3). The reaction mixture was stirred in the dark under nitrogen and allowed to warm to room temperature. After 72 h the solids were removed by filtration and the filtrate diluted with dichloromethane (20cm^3) and washed successively with aqueous sodium hydrogen carbonate, and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and the solvent evaporated to give a syrup which was chromatographed using hexane-ethyl acetate (1:1) as eluant; R_f0.46. The *title compound* (**15**) was obtained as a clear light yellow syrup (0.186g, 53%); $[\alpha]^{29}_D$ -71.6° (*c* 0.9 in CH_2Cl_2); δ_C(100.6MHz) 17.2 (C-6'), 17.5 (C-6), 19.9, 20.0, and 20.3 ($OCOCH_3$), 24.7, 25.8, 28.86, 28.90, 28.94, 29.2, and 33.8 ($CH_3OC(O)(CH_2)_7CH_2O$-), 51.1 ($CH_3OC(O)(CH_2)_8O$-), 54.4 (C-2''), 60.7 (C-6''), 66.3 ($CH_3OC(O)(CH_2)_7CH_2O$-), 67.9, 68.1, 68.2, 70.6, 70.7, 71.8, 72.0, 72.9, 73.5, 76.7, 78.4, and 79.7 (other CH and $-CH_2Ph$), 97.2 (C-1), 98.35, 98.42 (C-1'', C-1'), and 165.59, 165.62, 165.9, 167.5, 169.0, 169.9, 170.5, and 174.1 (C=O); δ_H(400.13MHz) 0.77 (3H, d, $J_{5',6'}$=6.3Hz, 6'-H_3), 1.32 (3H, d, $J_{5,6}$ =6.3Hz, 6'-H_3), 1.59, 1.76, and 1.97 (3x3H, s, $OCOCH_3$), 2.57 (1H, m, 5''-H), 3.28 (1H, t, $J_{3',4'}+J_{4',5'}$=19Hz, 4'-H), 3.41 (1H, dd, $J_{5'',6b''}$=1.8, $J_{6a'',6b''}$=12.3Hz, 6''-H_b), 3.63 (1H, m, 5'-H), 3.65 (3H, s, CH_3O-), 3.74 (1H, dd, $J_{5'',6a''}$=3.0,

$J_{6a'',6b''}$=12.3Hz, 6''-H_a), 3.95 (1H, dd, $J_{2',3'}$=3.4, $J_{3',4'}$=9.5Hz, 3'-H), 4.04 (1H, m, 5-H), 4.16, and 4.30 (2x1H, d, $J_{Ha,Hb}$=12.4Hz, -CH_2Ph), 4.21 (1H, dd, $J_{1'',2''}$=8.4, $J_{2'',3''}$=10.5Hz, 2''-H), 4.33 (1H, dd, $J_{2,3}$=3.5, $J_{3,4}$=9.8Hz, 3-H), 4.87 (1H, dd, $J_{3'',4''}$=9.1, $J_{4'',5''}$=10.0Hz, 4''-H), 4.92 (1H, d, $J_{1,2}$=1.8Hz, 1-H), 4.97 (1H, dd, $J_{1',2'}$=2.0, $J_{2',3'}$=3.4Hz, 2'-H), 5.01 (1H, d, $J_{1',2'}$=2.0Hz, 1'-H), 5.10 (1H, d, $J_{1'',2''}$=8.4Hz, 1''-H), 5.40 (1H, dd, $J_{1,2}$ =1.8, $J_{2,3}$=3.5Hz, 2-H), 5.52 (1H, dd, $J_{3'',4''}$=9.1, $J_{2'',3''}$=10.5Hz, 3''-H), and 5.53 (1H, t, $J_{3,4}+J_{4,5}$=19.5Hz, 4-H) (Found: C, 64.5; H, 5.9; N, 1.2. $C_{70}H_{77}NO_{23}$ requires C, 64.66; H, 5.97; N,1.08%).

8-Methoxycarbonyloctyl 3-*O*-[3'-*O*-(2''-Acetamido-2''-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (16). ---A sample of the fully protected trisaccharide (15) (0.250g, 0.192mmol) was dissolved in a solution of 1*M* sodium methoxide in methanol (14cm^3) and the solution was let stand for 24 h. The reaction mixture was neutralised by stirring with Rexyn 101 (H^+) resin, the resin was removed by filtration, and the solvent evaporated to give a clear light brown syrup. The syrup was dissolved in ethanol (25cm^3) containing hydrazine hydrate 100% (0.05cm^3, 1.03mmol) and the mixture was refluxed under nitrogen for 18 h. A fine white precipitate was removed by filtration and the solvent was dried *in vacuo* to remove traces of hydrazine. The syrup was then dissolved in methanol (20cm^3) containing acetic anhydride (2.0cm^3), and let stand at room

temperature for 18 h. The solvent was evaporated to give a clear light brown syrup which was dissolved in 80% aqueous acetic acid ($20cm^3$) and hydrogenolysed over 10% palladium-carbon (0.10g) at a hydrogen pressure of 52 p.s.i. for 20 h. The mixture was filtered through celite and the filtrate was evaporated to dryness. Chromatography with ethyl acetate-methanol-water (7:2:1) as eluant; R_f 0.49 gave the *title compound* (16) as a clear light brown syrup (0.099g, 75%); $[\alpha]^{29}_D$ -48.3° (*c* 1.0 in H_2O); δ_C(D_2O, 100.6MHz) 19.4 (C-6, C-6'), 25.0 ($NHCOCH_3$), 27.1, 28.1, 31.0, 31.1, 31.3, and 36.4 ($CH_3OC(O)(CH_2)_7CH_2O$-), 54.7, ($CH_3OC(O)(CH_2)_8O$-), 58.6 (C-2''), 63.5 (C-6''), 70.7 ($CH_3OC(O)(CH_2)_7CH_2O$-),71.6 (C-5), 72.0 (C-5'), 72.6, 72.7, and 73.0 (C-2, C-2', and C-4''), 73.7 (C-4'), 74.3 (C-4), 76.5 (C-3''), 78.5 (C-5''), 80.8 (C-3), 82.8 (C-3'), 102.6 ($^1J_{13C-1H}$=169Hz, C-1), 104.7 ($^1J_{13C-H}$=171Hz, C-1'), 105.4 ($^1J_{13C-1H}$=163Hz, C-1''), 177.8 ($NHCOCH_3$), and 180.0 ($CH_3OC(O)(CH_2)_8O$); δ_H(D_2O, 400.13MHz) 1.20-1.35 (14H, m), 1.51-1.61 (4H, m), 1.99 (3H, s, $NHCOCH_3$), 2.34 (2H, t, $CH_3OC(O)CH_2(CH_2)_7O$-), 3.37-3.57 (6H, complex m, ring H's), 3.61-3.81 (9h, complex m, ring H's); 3.87 (1H, dd, $J_{2',3'}$=3.2, $J_{3',4'}$=9.8Hz, 3'-H), 3.88 (1H, d, $J_{6a'',6b''}$=12.0Hz, 6''-H_b), 3.95 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.2Hz, 2-H), 4.24 (1H, dd, $J_{1',2'}$=1.8, $J_{2',3'}$=3.2Hz, 2'-H), 4.67 (1H, d, $J_{1'',2''}$=8.2Hz, 1''-H), 4.71 (1H, d, $J_{1,2}$=1.8Hz, 1-H), and 4.98 (1H, d, $J_{1',2'}$=1.8Hz, 1'-H) (Found C, 52.8; H, 8.1; N, 1.8. $C_{30}H_{53}O_{16}N$ requires C, 52.7; H, 7.8; N, 2.0%).

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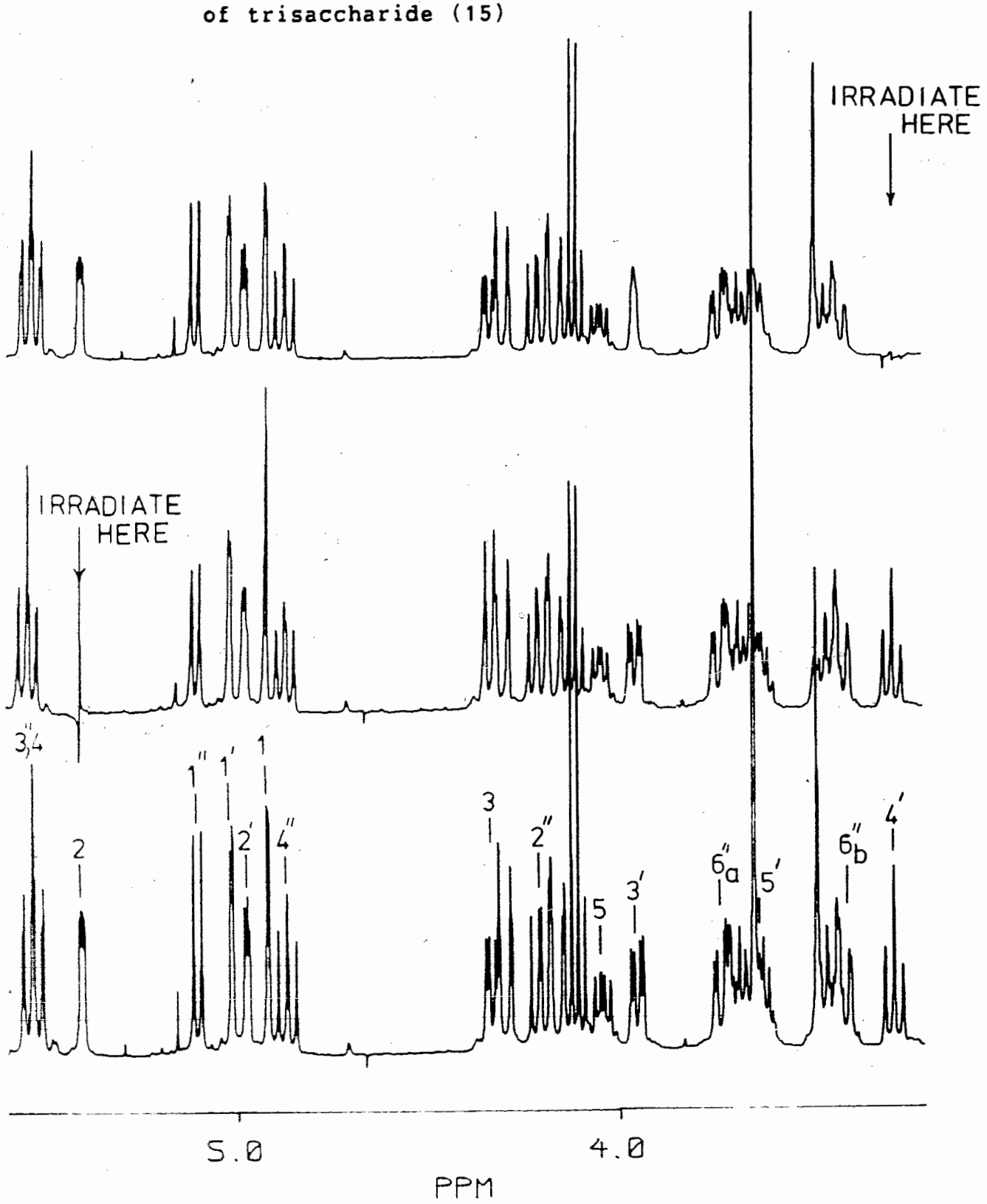
V. APPENDIX

Figure 19

Homo-decoupled 400MHz ^{1}H n.m.r. spectra of trisaccharide (15)



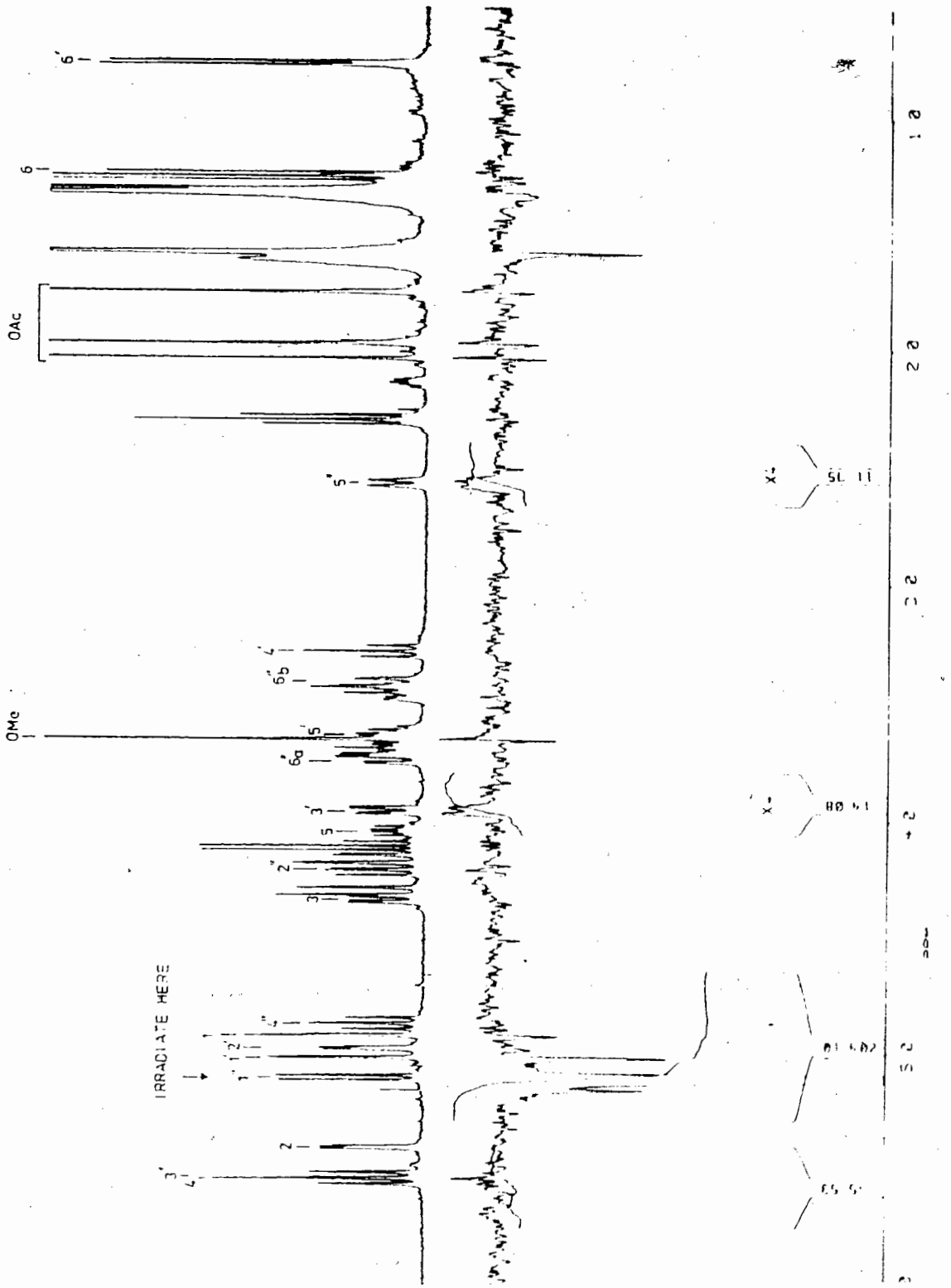


Figure 20

400MHz Difference n.O.e. ^{1}H n.m.r. spectrum of trisaccharide (15)