The Synthesis of ^{13}C-Labeled Oligosaccharides Corresponding to the Phenolic Glycolipid of *Mycobacterium leprae*

by

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ABSTRACT

The development of superior immunodiagnostic reagents for the early detection of leprosy requires a detailed understanding of the interaction between antigen and antibody. This thesis describes the synthesis of oligosaccharide haptens corresponding to the phenolic glycolipid of *Mycobacterium leprae* that can be used for the detailed conformational analysis of the haptens and for the subsequent study of antibody-hapten interaction. The disaccharide, propyl 4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside, and trisaccharide, propyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-3-O-methyl-α-**L**-rhamnopyranoside have been synthesized by a new procedure, and further characterized using various NMR techniques. The use of a protected glucosyl trichloroacetimidate in the synthesis of the disaccharide and trisaccharide was efficient and convenient. The synthetic pathway leading to the disaccharide and trisaccharide was improved upon by the use of allyl 4-O-benzyl-α-**L**-rhamnopyranoside as an intermediate. In order to simplify the spectral analysis of the oligosaccharides both in the free state and bound to antibody, the following disaccharides with ^{13}C-labeled methyl groups have been synthesized: propyl 4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside, propyl 4-O-(3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside, and propyl

4-O-(3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside. Subsequent NMR results have shown that $^{1}J_{^{13}C,^{1}H}$ of the ^{13}C-labeled methyl group was 141~142Hz, and $^{3}J_{^{13}C,^{1}H}$ was 3~6Hz. The ^{13}C-labeled disaccharides will be used in subsequent isotope-filtered NMR experiments to extract conformational information.

DEDICATION

To my family

ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. Mario Pinto, for giving me the opportunity to work in a stimulating environment and to be involved in this project. I also thank him for his valuable supervision in the work and help in the preparation of this thesis. I thank Marcey Tracey for her efficient service in obtaining all the NMR spectra and M. Yang for providing all the microanalysis. I thank John Andrews, Seema Metha, and the rest of our group for their help during the work and preparation of this thesis. Finally, I thank the members of my examining committee for their time in evaluating this thesis.

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LIST OF ABBREVIATIONS

T.l.c.	Thin layer chromatography
NMR	Nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
$^{13}C\{^{1}H\}$ NMR	^{1}H-decoupled carbon-13 NMR
J coupling	Mutual interaction between two spins, which is transmitted through chemical bonds
NOE	Nuclear Overhauser enhancement
^{1}H-^{1}H COSY 2D-NMR	Two-dimensional ^{1}H homonuclear chemical shift correlated spectroscopy
^{13}C-^{1}H COSY 2D-NMR	Two-dimensional ^{13}C-^{1}H heteronuclear chemical shift correlated spectroscopy
TfOH	Trifluoromethanesulfonic acid
All-OH	Allyl alcohol
2,2,DMP	2,2-Dimethoxypropane
PTSA	p-Toluenesulfonic acid
DMF	N,N-Dimethylformamide
BOMCl	Benzyl chloromethyl ether
Pyr.	Pyridine
BOM-	Benzoxymethyl group
TESTfl	Triethylsilyl trifluoromethanesulfonate
M. leprae	*Mycobacterium leprae*

Chapter 1 Introduction

I. Background

Leprosy is a serious skin disease that forms silvery scales on the skin, causes local insensitivity to pain and may result in severe deformities such as the loss of fingers and toes. For centuries, the lives of lepers have been tragic and hopeless. *Mycobacterium leprae* (*M. leprae*) was discovered in freshly biopsied tissue of leprosy patients and was believed to be the cause of the disease[1]. For many years, species-specific antigens of *M. leprae* have been sought to be used as diagnostic reagents. Research into the mechanism of pathogenesis of *M. leprae* and its antigenic composition has been impeded by the lack of adequate supplies of the causative agent, *M. leprae*, due to the fact that the organism is extremely sensitive and incapable of growth in *vitro*[1,2]. Since 1980, the availability of large quantities of *M. leprae*-infected armadillo tissue has provided an opportunity for expanded research in this area[1,2].

In 1980, the structure of the antigenic determinant of the species-specific phenolic glycolipid of *M. leprae* was reported by Hunter *et al.*[2] and has the repeating unit shown in Fig. 1-1.

This structure is closely related to "*Mycoside A*" from *Mycobacterium Kansasii* (*M. Kansasii*). The crucial difference between the two is the composition of the attached oligosaccharides. Gas-liquid chromatography and mass

spectrometry has shown that the hydrolysis product from *M. Kansasii* is composed of 2,4-di-O-methyl rhamnose, 2-O-methyl rhamnose, and 2-O-methyl fucose, whereas that from *M. leprae* contains 2,3-di-O-methyl rhamnose, 3-O-methyl rhamnose, and 3,6-di-O-methyl glucose. The distinct composition of the oligosaccharide segment of the phenolic glycolipid in *M. leprae* makes it useful for the chemical and serological differentiation of this organism from other *Mycobacteria*, and offers the promise of a chemically defined antigen that could become an important diagnostic tool for leprosy[1].

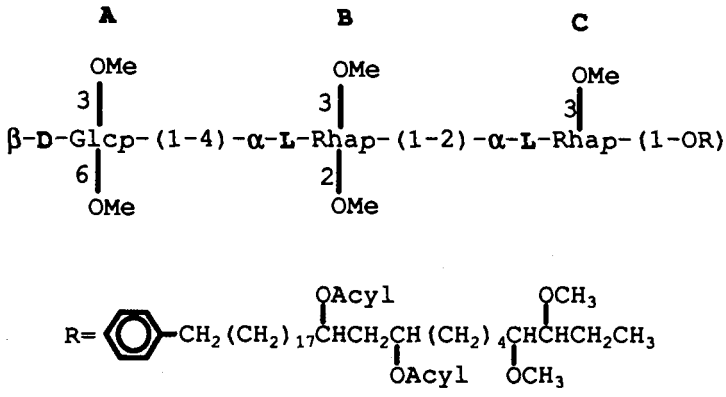


Fig. 1-1. The structure of the phenolic glycolipid of *M. leprae*

The development of carbohydrate chemistry and the application of high field NMR in carbohydrate analysis has enabled the study of complicated oligosaccharides and their analogs. The chemical synthesis of the oligosaccharides of the antigenic determinant of the phenolic glycolipid of *M. leprae* has been studied by some groups worldwide[3,4,5,6] since, its structure was reported by

Hunter *et al.*[2]. Previous studies[7] with a synthetic conjugate showed that a disaccharide is the minimal structure required for effective binding with antibodies. It has also been reported[3] that in many cases, the terminal monosaccharide (glucose residue) displayed the same binding capacity.

Although there have been notable advances in the laboratory investigation and field management of leprosy in recent years, progress continues to be hindered by the lack of an efficient method for early diagnosis and field implementation of control and treatment measures[8]. Diagnoses to date are still made using the same principles as a century ago (clinical and histopathologic findings), and only approximately one in three registered leprosy patients worldwide receives optimal chemotherapy[8]. Furthermore, as recently as 1988, nearly 10% of all newly diagnosed patients already had debilitating deformities. Thus, it can hardly be said that leprosy is "in spectacular retreat"[8]. In order to limit transmission and prevent deformity, early diagnosis of leprosy (i.e. before clinical lesions appear) by serologic test techniques has long been a desired goal. Clearly, there is a need for a reliable diagnostic reagent for the early detection of this disease.

II. Conformational Analysis

Upon injection of foreign antigens, proteins or other macromolecules, into a species (vertebrates and sharks), specific antibodies are produced in the blood serum and tissues

in response to the presence of the foreign molecules. This reaction is called the immune response, and it is the basis of the whole field of immunology[9]. An antibody may combine with an antigen to form an antigen-antibody complex through its binding sites which are believed to be complementary to specific structural features of the antigen. Antibodies are highly specific for the three-dimensional structure of antigens which evoke their formation. Therefore, if an antigen is heated or denatured to change its conformation, or, it is chemically modified, the antibody will likely not bind it. The study on the *Shigella flexneri* Y antigen and its corresponding antibody by Bundle[10], showed that the particular sugar rings and the different substituents of the antigen had significant effects on the antibody-antigen affinity. Clearly, a knowledge of the interactions of antibodies and antigens is important in understanding the mechanism of this highly specific immune response.

A specific portion of a ligand (an antigenic determinant) may interact with its complementary antibody by entering the combining site and causing the two to bind together. The intermolecular forces which contribute to the stabilization of a protein-ligand complex are mainly hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions. The interactions between the molecules vary in strength depending upon how well they complement each other and thus, the strength of the forces that are developed between them. This type of

association process has been described[11] in thermodynamic terms as the following two steps: the first is the mutual penetration of hydration layers caused by disorder of the solvent and the second involves further short-range interactions. The negative enthalpy and entropy changes of protein association reactions primarily arise from hydrogen bonds and van der Waals interactions, and the positive entropy and enthalpy changes arise from ionic and hydrophobic interactions.

For many years, the interaction of a protein-ligand system has been described as a lock-and-key model, which means the binding sites of protein and ligand are conformationally well-defined complementarily while binding[12]. Studies on the conformations of free ligand and bound ligand[13,14,15] recently have shown that the model does not apply to all protein-ligand systems. In some instances, binding leads to substantial conformational change of ligand, sometimes even the conformational change of protein. For example, in the study of protein-carbohydrate interaction by highly refined X-ray diffraction, Quiocho[15] pointed out that sugar binding induced protein conformational change. Glaudemans *et al.*[14a] reported another example, using transferred NOE experiments, they found that methyl O-β-**D**-galactopyranosyl-(1-6)-4-deoxy-4-fluoro-β-**D**-galactopyranoside underwent significant conformational changes upon binding to its antibody (mouse IgA X24). Clearly, the description of binding in thermodynamic terms can not provide us with a detailed protein-ligand interaction profile at the

molecular and atomic levels because we can not know exactly which portions of the molecules were interacting or the forces involved in binding. Since the structures of protein-ligand complexes were determined in the mid-1970s, knowledge at the atomic-level of the three-dimensional structure of protein-ligand complexes has been accumulating. X-ray diffraction is a very good method for structure analysis and has been used for many years in protein-ligand studies; it is still one of the most useful tools for obtaining conformational information. The method has several disadvantages, for example, the crystals required for X-ray diffraction are sometimes very difficult to obtain for protein-ligand systems.

Due to the above limitations, various NMR methods have been utilized to obtain the desired conformational information [14,16,17,18]. The parameters[19] of NMR which can provide conformational information include the chemical shift, spin-spin coupling (J-coupling), nuclear Overhauser effect (NOE), and spin-lattice relaxation times (T_1). J coupling is a sensitive measurement of torsion angle, it often yields valuable information about spatial structure. The relationship between the magnitude of J-coupling with torsion angle is described by the Karplus equation[20]. In the conformational analysis of carbohydrates, $^3J_{COCH}$ is of particular interest since the important conformational factors, glycosidic torsion angles, are defined by a C-O-C-H torsion, whereas $^3J_{H,H}$ does not tell much about conformation other than the individual ring

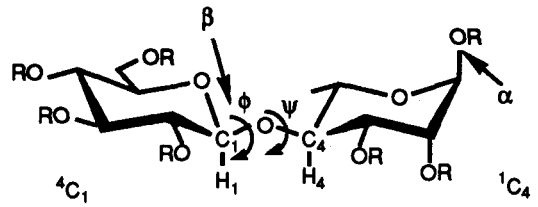
conformations[21,22,23]. Thus the glycosidic torsion angle can be deduced from long-range coupling constants by the application of a modified Karplus equation. NOE and T_1 values reflect the mutual relaxation between spatially close nuclei and therefore, provide the most important parameters for the determination of three-dimensional structures[24]. NOE and J coupling provide complementary information for structural determination.

Based on experimental data obtained from X-ray diffraction, NMR spectroscopy and theoretical calculations, such as force-field calculations, the structure of the ligand or protein may be characterized[10,18].

Information about the conformation of a protein-ligand complex can be used to develop new molecules designed to orient the functional groups of the ligand in space for maximum interaction with the protein. Knowledge of the binding site's structure will suggest ways to modify the ligand to provide a better fit in the binding site in order to enhance the binding affinity, or ways to change undesirable physical properties of the ligand (such as where to attach hydrophilic groups to improve water solubility or where to attach hydrophobic groups to improve hydrophobic properties) without altering the binding affinity of ligand. Clearly, conformational analysis can provide us with a model allowing design of specific drugs[10,18].

Carbohydrate portions of glycoconjugates (glycolipids. glycoproteins, and glycophospholipids) are biologically important molecules involved in many important intercellular and

intermolecular interactions. These interactions include cell-cell recognition, hormone interactions, cell-differentiation and immune recognition[25,26]. In contrast to nucleotides and peptides[27,28], in which the informational content is determined solely by the number and sequence of different monomer units, carbohydrate structure[28,29,30] is determined by the number of sugar units, ring size (i.e. five, six or higher membered rings), the conformation of the carbohydrate ring (i.e. $^4C_1$, $^1C_4$), the configuration (i.e. α, β linkage), and the occurrence of branching (Fig. 1-2). Biopolymers containing carbohydrates, therefore, carry considerably more information per building unit[28].



φ- Torsion angle H_1-C_1-O-C_4
ψ- Torsion angle C_1-O-C_4-H_4

Fig. 1-2. A structure of a disaccharide

III. Useful NMR Methods in Protein-Ligand Conformational Analysis

For a system containing large molecules possibly undergoing chemical exchange, such as a protein-ligand system, the NMR signals are frequently so complex and overlapped that

conformational information is difficult to obtain. The use of suitable methods to investigate such systems, therefore, becomes very important. The most common NMR methods to date for probing protein-ligand systems involve the use of transferred NOE experiments and isotopically labeled compounds.

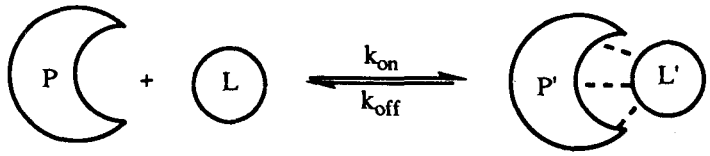
A. Transferred NOE[14,16]

Transferred NOE is a method that employs the very different relaxation properties of components in a chemical exchange system and is a suitable method for studying protein-ligand systems undergoing chemical exchange.

It is well known that small molecules (such as ligands) relax slowly and their NOE develops slowly. However, polymers or the molecules of high molecular weight (such as proteins) are not as free as small molecules, their relaxation is fast and their NOE develops quickly.

In the equilibrium system shown in the equation, NOE is built up slowly in the free ligand L and rapidly in the bound ligand L' which has the same relaxation property as that of the protein P'. When L' dissociates from the P'-L' complex, L' takes with it the NOE character of the bound property. The small amount of NOE built up in L will rapidly disappear through exchange into the protein and will be edited during the experiment. The overall NOE obtained by the transferred NOE experiment is predominantly of the bound form L'. The equilibrium of a system containing

both free ligand and bound ligand is shown in the following equation:



P- Protein
P'- Bound protein
L- Free ligand
L'- Bound ligand

K is the binding constant, $K= k_{on}/k_{off}$, k_{on} is the association rate constant and k_{off} is the dissociation rate constant. It is the dissociation rate constant, k_{off}, and the large difference in relaxation properties of components that determines the feasibility of the transferred NOE experiment. Because the transferred NOE experiment requires a relatively rapid chemical exchange of bound ligand and free ligand, and a large difference in relaxation properties, it is important to obtain suitable experimental parameters.

B. NMR Experiments Involving the Use of Isotopically-Labeled Compounds

The use of isotopically-labeled compounds in NMR experiments has recently become a very important tool in protein-ligand studies. Compared to ^{1}H NMR, routine ^{13}C NMR experiments are of low sensitivity and are time consuming. This is due to the low natural abundance of and low gyromagnetic ratio (γ) of carbon-13[31]. ^{13}C-labeled compounds, therefore, not only provide precise

NMR information, but can also simplify spectral assignment by selectively detecting only those protons which are attached to isotopically labeled nuclei. The use of ^{13}C-labeled compounds in kinetic binding experiments[17], $J_{C,H}$ measurment[23,32], and ^{13}C-isotope-filtered experiments[12,34] greatly increase the efficiency of research in protein-ligand systems.

1. Kinetic Binding Experiments[17]

The use of ^{13}C-labeled carbohydrates for probing protein-ligand (carbohydrate) systems was first reported by Neurohr *et al.* in 1981[17]. The binding kinetics of the methyl β-D-lactoside (ligand), which had a ^{13}C-label at the C-1 position of the D-galactose residue, to peanut agglutinin was studied by ^{13}C NMR spectroscopy. The labeled carbon peak of the ligand showed line broadening upon binding to the protein in the ^{13}C NMR spectrum. The residence times, dissociation rate constants, and association equilibrium constants were obtained from the study of the ^{13}C-1 line width of the disaccharide in the presence of the protein.

2. $J_{C,H}$ Measurements[23,32]

J coupling constants are important NMR parameters in conformational studies. In carbohydrate conformational analysis, long-range carbon-proton coupling constants are especially useful[21,22,23]. Since the torsion angles ϕ, ψ about the glycosidic linkage are important carbohydrate structure

parameters and sufficient NOE values are not always available for detailed linkage conformational analysis, $^{3}J_{C,H}$ coupling constants provide a valuable means of deducing values for the torsion angles ϕ, ψ.

Recently, NMR experiments for measurement of long-range carbon-proton coupling constants, based on the pulse sequence introduced by Bax and Summers *et al.*[35] for ^{1}H-detected heteronuclear multiple-bond correlation (HMBC), have been applied. This method is most sensitive for tracing long-range heteronuclear correlations and is now widely used in carbohydrate conformational studies[23]. ^{13}C-labeled compounds provide an alternative to obtaining precise $J_{C,H}$ values in direct ^{13}C-detected NMR experiments.

3. Isotope-Filtered Experiments[12,34]

The assignments of the completely unbound conformation and bound conformation of cyclosporin A (CsA) with the protein cyclophilin (CYP), and those of FK506 with the protein FKBP in solution have been obtained by the use of uniformly isotopically ^{13}C- or ^{15}N-labeled compounds and isotope-filtered techniques, allowing astonishing progress in the understanding of protein-ligand systems, and providing a new technique to investigate protein-ligand interaction[12,13].

These techniques are based on the following ideas. In a unlabeled protein-ligand system, the large number of protons in the receptor protein would complicate the ligand assignment.

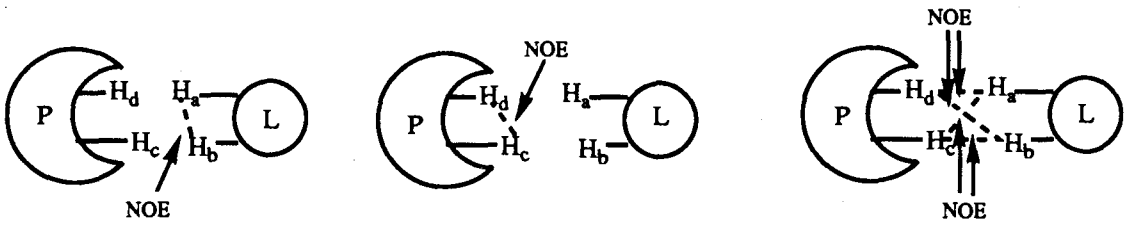
However, for proteins of intermediate size, such a system is ideally suited for isotope-filtered experiments since both components can be separately labeled with ^{13}C or ^{15}N prior to complexation. A labeled component of the protein-ligand system can be complexed with its unlabeled partner. The experiment is described in Fig. 1-3.

With ^{13}C-labeled ligand, a double-half-filter technique [13,34,36] is particularly helpful in that it produces different subspectra that contain either exclusively intramolecular NOE cross peaks between the protons of ligand (Such as the NOE between H_a and H_b) or protons of protein (Such as the NOE between H_c and H_d), or exclusively intermolecular NOE cross peaks between the protons of ligand and the protons of protein (Such as the NOE between H_a and H_c or H_d, or H_b and H_c or H_d).

These techniques are generally applicable to binary or multicomponent protein-ligand complex systems which are very stable. Complexes that exhibit large dissociation rates could, in principle, be studied by ^{13}C-filtered transfered NOE experiments (see section IIIA).

IV. Chemical Synthesis

Chemical synthesis provides a wide variety of complex compounds for studies of their biological interactions and conformational properties[37,38]. In drug design and protein-ligand study, chemical synthesis can not only provide enough material which is not easily obtained from nature but can also make it



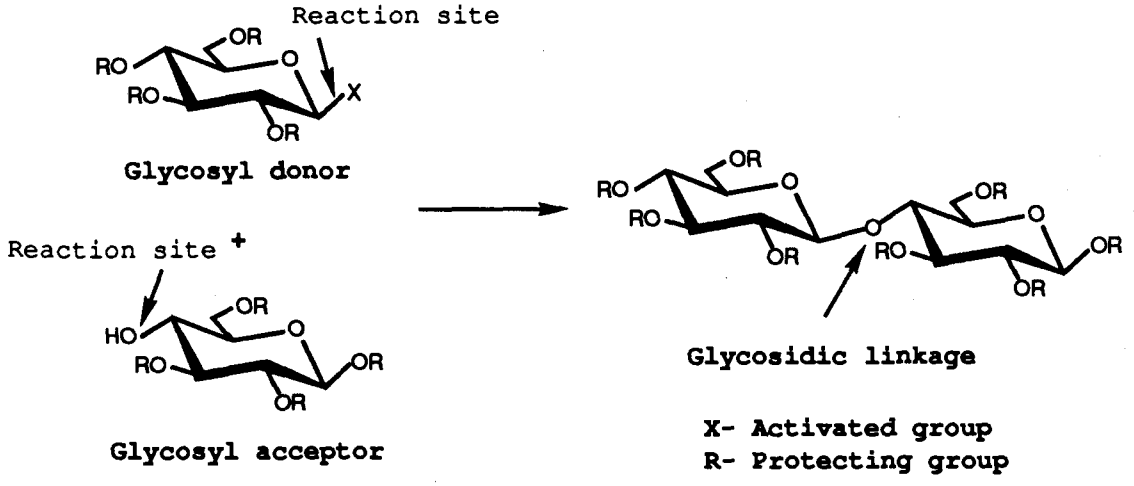
A B C

Intramolecular NOEs (A, B) **Intermolecular NOEs (C)**

(P- Protein, L- Ligand)

(Either protein or ligand is isotopically-labeled)

Fig. 1-3. Schematic illustration of the NOEs observed in isotope-filtered experiments



X- Activated group
R- Protecting group

Fig. 1-4. A generalized scheme for a glycoside synthesis

possible to obtain analogous compounds which might not exist in nature[10].

A. The General Synthesis of Oligosaccharides

The interest in carbohydrate synthesis has increased in recent years since the role of glycoconjugates in biological systems has become better understood. The synthesis of oligosaccharides involves the selective linkage by a glycosylation reaction between two selectively protected polyhydroxyl sugar units (i.e. glycosyl donor and glycosyl acceptor)[25] (Fig. 1-4).

There are two general types of glycosidic linkage that may be formed in a glycosylation reaction, 1,2-*cis* and 1,2-*trans*[39]. The hydroxyl group at the 2-position of a glycosyl donor unit and the exocyclic oxygen atom at the 1-position are *cis* in a 1,2-*cis* glycosidic linkage, and *trans* in a 1,2-*trans* glycosidic linkage (Fig. 1-5). The difference in glycosidic linkage gives rise to different compounds with different physical, chemical and biological properties.

Different strategies are used for the preparation of these two types of linkages. In general, the 1,2-*trans* linkages are easier to form than the 1,2-*cis* linkages. The usual method for the formation of 1,2-*trans* linkages is to have an activated glycosyl donor which has at the 2-position, a participating group such as an acetate or a benzoate group. Under Lewis acid catalysis the leaving group at the anomeric

position departs resulting in the formation of an oxocarbenium ion. This oxocarbenium ion is stabilized by the participation of the carbonyl oxygen of the substituent at 2-position to give a dioxocarbenium ion. The alcohol component of the acceptor substrate then opens the dioxocarbenium ring in an S_N2 type reaction, with the neighboring group protecting the *cis* face of the ring from nucleophilic attack[38,40] (Fig. 1-6).

Formation of 1,2-*cis* glycosidic linkages can be achieved by using blocking groups such as benzyl ethers at the 2-position which are inactive in neighboring group participation. The glycosylation reaction is then carried out such that the hydroxyl group of the glycosyl acceptor displaces the leaving group (which is *trans* to the group at the 2-position) of the glycosyl donor in an S_N2 type reaction resulting in inversion of configuration at the anomeric center[38,41].

The requirment for a 1,2-*cis* oriented glycosyl donor has necessitated the development of special procedures for their preparation. One such method is the halide-ion catalyzed glycosylation method of Lemieux *et al.*[38,42]. Other methods for 1,2-*cis* glycosylation take advantage of the thermodynamic stability of axially-substituted glycosides[38,42].

From the above, it is clear that stereoselectivity and regioselectivity are very important factors in carbohydrate synthesis, especially in glycosylation reactions. The hydroxyl groups in sugar residues (glycosyl donor or glycosyl acceptor) usually need to be protected with one position remaining free

1,2-trans linkage 1,2-cis linkage

R- Another sugar residue

Fig. 1-5. Two major types of glycosidic linkage

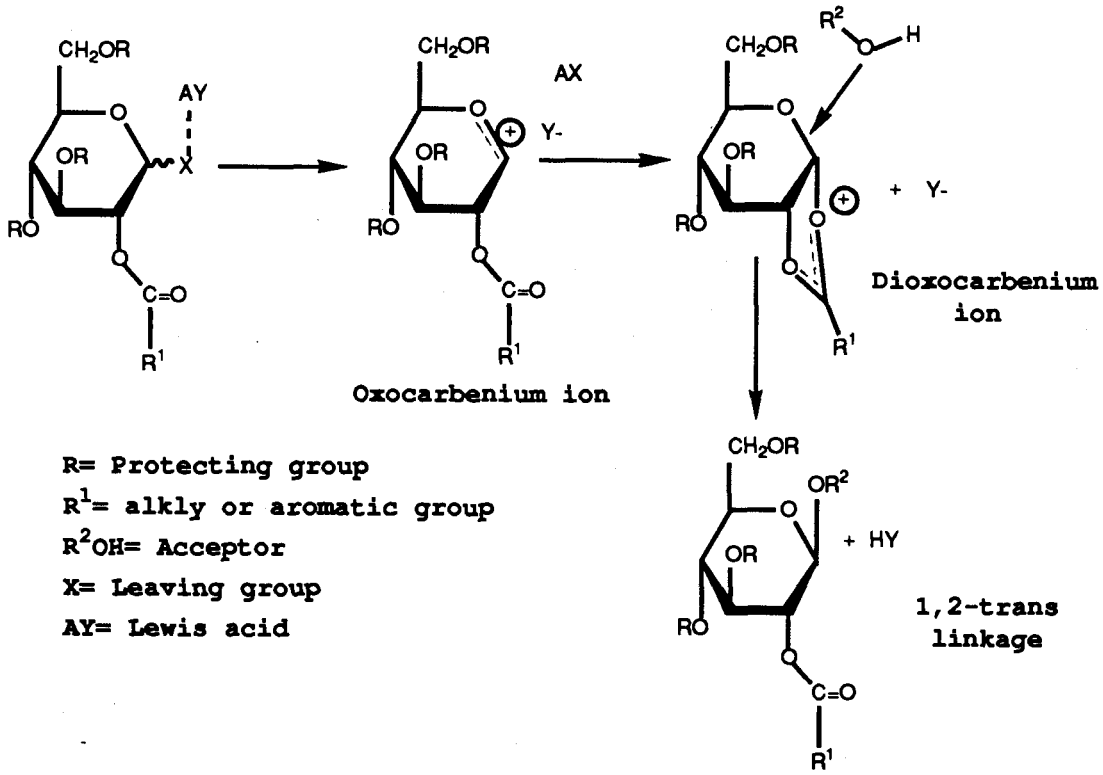


Fig. 1-6. Formation of a 1,2-trans glycosidic linkage

(for glycosyl acceptor) or one position being activated (for glycosyl donor) to meet stereochemical and regiochemical requirements (Fig. 1-4).

Several different classes of hydroxyl protecting groups are commonly used, including various acetals and ketals, ethers, and esters[25]. Different protecting groups are stable to different reaction conditions and may be removed by under a variety of conditions. For example, selective deprotection can be achieved by performing acidic or basic hydrolysis, or by hydrogenolysis. By careful selection of the combination of protecting groups, specific hydroxyl groups within a molecule may be selectively deprotected during a synthetic sequence.

Many strategies have been developed for the activation of the anomeric center of the glycosyl donor before the glycosylation reaction[39]. The most common methods involve the use of glycosyl halides[39,43], glycosyl trichloroacetimidates[39,44], thioglcosides [39,45], and selenoglycosides[46].

The other important factors in a glycosidation reaction are the polarity of solvents, the activity of catalysts, and the reactivity of the glycosyl acceptors[38].

B. The Synthesis of Isotopically-Labeled Compounds

The use of isotopically-labeled compounds in NMR experiments makes conformational analysis much more efficient. The synthetic availability of such compounds can be achieved both by chemical and biological synthesis[47].

Chemically, the carbohydrates with ^{13}C-labeled ring carbons can be synthesized by the classical cyanohydrin synthesis method of Kiliani-Fisher[48,49], which has frequently been used in the preparation of aldoses, especially those containing radioactive isotopes. The synthesis in high yield was presented by Serianni *et al.*[49]. In this synthesis, sodium cyanide was condensed with a parent aldose to produce two epimeric nitriles. The nitriles were reduced by hydrogen using palladium-barium sulfate (5% Pd-$BaSO_4$) as catalyst to give the imines with one extra carbon, which hydrolyzed rapidly to the corresponding aldose. The carbohydrates with a series of ^{13}C-labeled ring carbons can be achieved by multiple use of this method. The synthesis of ^{13}C-labeled carbohydrate side chains such as those present as methyl ethers can be achieved by conventional synthetic methods using the ^{13}C-labeled alkyl halides.

V. Research Plan

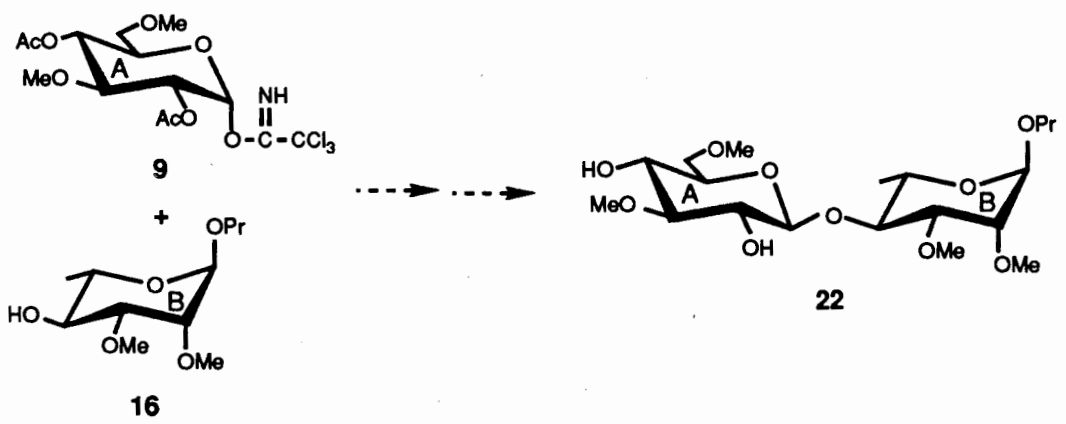
The long term goal of this project is to develop a reliable diagnostic reagent for the early detection of leprosy. Since composition of the oligosaccharide segment of the phenolic glycolipid of *M. leprae* is species-specific, the oligosaccharides or their analogs will be used as reagents to detect the antibodies present in patients' sera. Due to the high degree of stereospecificity in immunological reactions, it is necessary to better define the requirements for an effective immunodiagnostic reagent. Conformational analysis of

oligosaccharides or analogs with their complementary monoclonal antibodies may furnish details of the immunological reaction profile which may improve, on a molecular basis, our understanding, and further the exploration of the immune response. From this information, we can synthesize modified compounds which may improve the action of the forces governing protein-ligand interaction and eventually enhance the specificity of the interaction. Although the synthetic procedure has been studied and improved by different groups in the world[3,4,5,6] and the conformations of the disaccharide and trisaccharide in water, methanol, acetone have been reported by Bock *et al.*[50], the conformations of the oligosaccharides bound to complementary monoclonal antibodies have not been reported yet. In order to obtain information of bound oligosaccharides, we propose to carry out kinetic binding experiments, $J_{C,H}$ measurements, transferred NOE experiments and isotope-filtered NMR experiments with ^{13}C-labeled oligosaccharides. The development of a general synthetic route together with the synthesis of isotopically labeled oligosaccharides constitute the first part of the project. This thesis describes the efficient synthesis of di- and trisaccharides and the synthesis of ^{13}C-isotopically labeled disaccharides corresponding to the phenolic glycolipid of *M. leprae*.

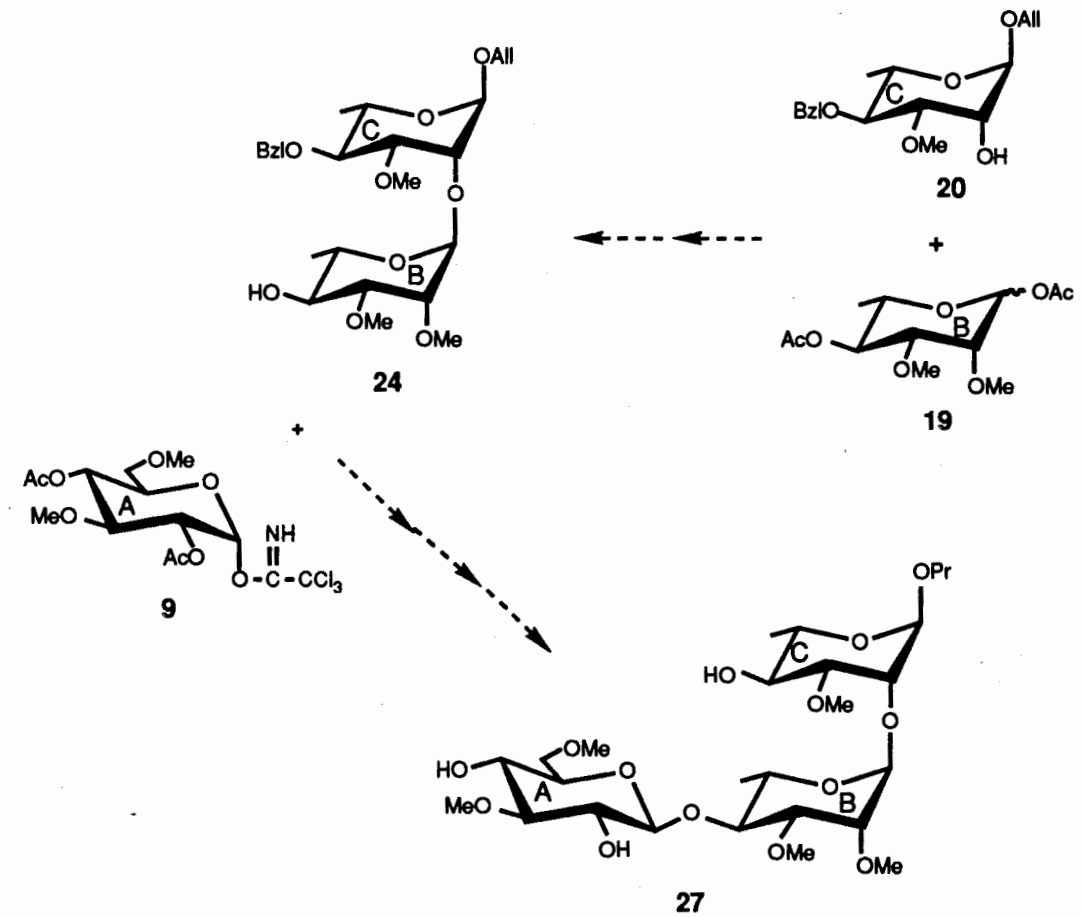
The synthetic strategy for synthesizing the disaccharide, propyl 4-O-(3,6-di-O-methyl-**β-D**-glucopyranosyl)-2,3-di-O-methyl-**α-L**-rhamnopyranoside (**22**)(Scheme 1), and the trisaccharide,

propyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-3-O-methyl-α-**L**-rhamnopyranoside (**27**) (Scheme 2) relies upon the synthesis of the monosaccharides, 2,4-di-O-acetyl-3,6-di-O-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**9**), propyl 2,3-di-O-methyl-α-**L**-rhamnopyranoside (**16**), 1,4-di-O-acetyl-2,3-di-O-methyl-α,β-**L**-rhamnopyranoside (**19**), and allyl 4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside (**20**). By utilizing the glycosyl trichloroacetimidate reaction[44], the glycosyl donor (**9**) may be coupled with glycosyl acceptor (**16**) to give disaccharide (**21**), followed by deprotection to give disaccharide (**22**) (Scheme 1). In order to synthesize trisaccharide (**27**), disaccharide (**24**) may be chosen as a glycosyl acceptor and reacted with glycosyl donor (**9**). The disaccharide (**24**) may be obtained, in turn, from the coupling of glycosyl donor (**19**) and glycosyl acceptor (**20**), followed by selective deprotection. Glycosylation of glycosyl donor (**9**) with glycosyl acceptor (**24**) would then give trisaccharide (**25**) which could be deprotected by conventional means to give trisaccharide (**27**) (Scheme 2).

With regard to the isotopically labeled compounds, we have chosen to selectively label the methyl groups with ^{13}C. These compounds will serve as initial substrates with which to test the NMR methods of analysis. The labeling of the methyl group is also of inherent interest since its orientation could have a profound influence on the nature of the hydrophobic interactions present in the antibody-oligosaccharide complex.



Scheme 1. Strategy for the synthesis of disaccharide AB



Scheme 2. Strategy for the synthesis of trisaccharide ABC

The synthesis of ^{13}C-labeled methyl groups of the disaccharide may be achieved by the use of ^{13}C-labeled methyl iodide in the methylation steps shown in Scheme 3, Scheme 4, and Scheme 5 (see later).

Chapter 2 Results and Discussion

I. Synthesis

A. Synthesis of Unlabeled Compounds

The synthesis of ring (**A**) (glucose ring) is shown in Scheme 3. The two hydroxyl groups at the 1 and 2 positions of **D**-glucurono-3,6-lactone (**1**) were protected by reaction with acetone and concentrated sulfuric acid to give compound (**2**) as crystals in 81% yield. Compound (**2**) was further protected by reflux with benzyl chloromethyl ether in dichloromethane and collidine solution to give compound (**3**) as white crystals in 68% yield. Compound (**3**) was reduced under reflux by lithium aluminum hydride in diethyl ether to give compound (**4**) as a solid in 95% yield. White crystals were obtained after recrystallization. Compound (**4**) was methylated with methyl iodide in N,N-dimethylformamide containing sodium hydride to give compound (**5**) quantitatively as a light syrup. After deprotection and mutarotation, compound (**5**) was converted to compound (**6**) which was acetylated by acetic anhydride in pyridine to give compound (**7**) as a syrup in 59% yield. The above synthesis followed the procedure of Marino-Albernas *et al.*[3]. Hydrazinolysis[51] (**7**) with hydrazine acetate in N,N-dimethylformamide gave hemiacetal (**8**). 2,4-Di-O-acetyl-3,6-di-O-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**9**) was obtained from compound (**8**) by reaction with trichloroacetonitrile and potassium carbonate according to the method of Schmidt *et al.*[28,44]. The coupling

constant ($^3J_{1,2}$=4Hz) and those ($^3J_{1,2}$=4Hz and $^1J_{C,H}$=180Hz) of compound (**32**) (see later) showed that the α-anomer was present[59].

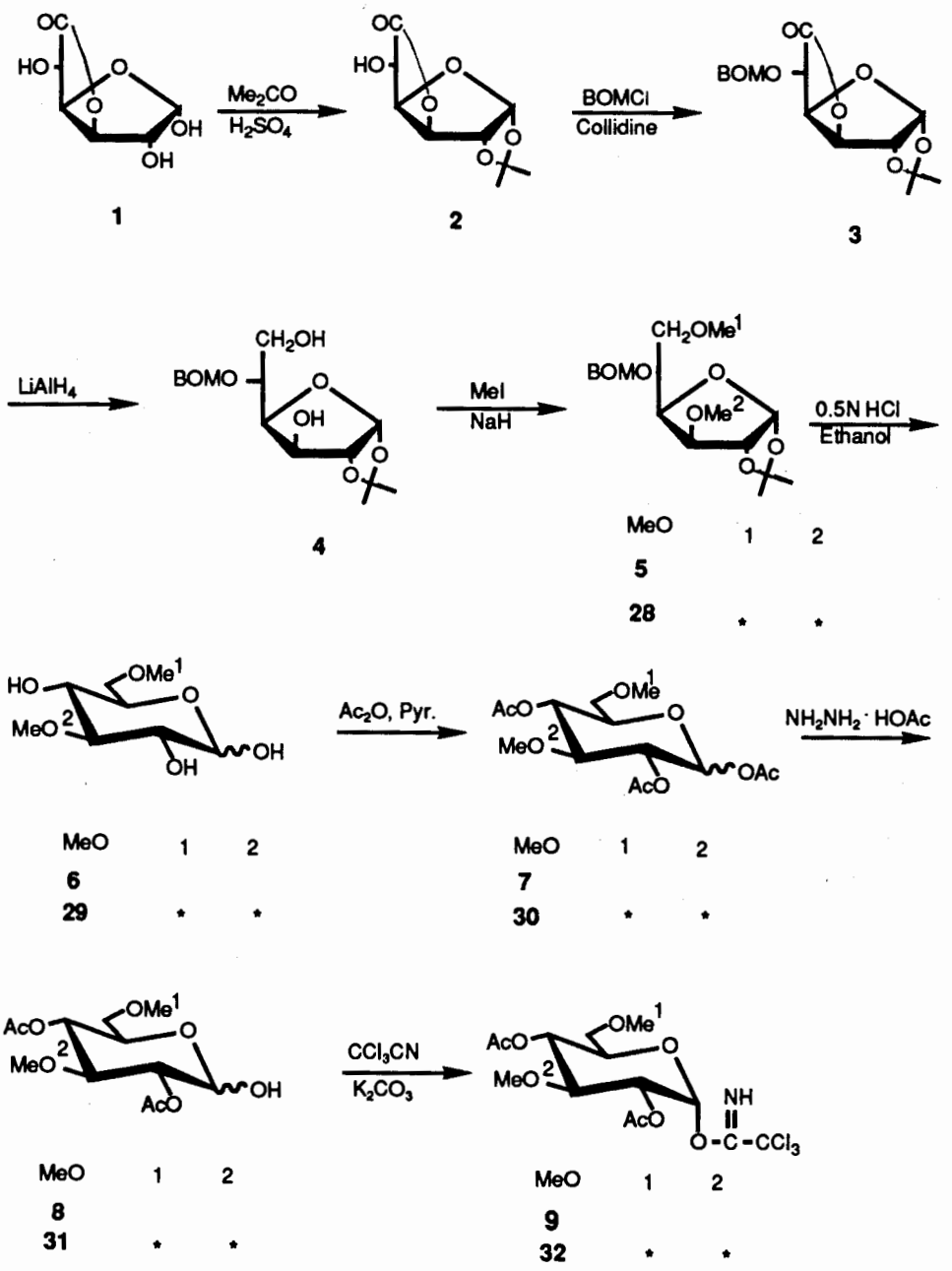
The efficient synthesis of both disaccharide (**22**) and trisaccharide (**27**) required a common rhamnopyranosyl precursor. We chose allyl 4-O-benzyl-α-**L**-rhamnopyranoside (**14**) as a starting material to synthesize these residues. The reason for using compound (**14**) was that the hydroxyl groups at 2 or 3 could be di-methylated or mono-methylated. In addition, the allyl group and benzyl group as protecting groups at the 1 and 4 position, respectively, could be selectively manipulated. Compound (**14**) was synthesized according to the procedure of Pinto *et al.*[52]. α-**L**-Rhamnose monohydrate (**10**) was refluxed with allyl alcohol and trifluoromethanesulfonic acid to give compound (**11**) which was refluxed with 2,2-dimethoxypropane and p-toluenesulfonic acid to give compound (**12**). Benzylation of compound (**12**) gave compound (**13**) which was deprotected under reflux in 0.5N hydrochloric acid-ethanol solution to give diol (**14**) as white crystals in 50% yield.

Methylation of diol (**14**) with methyl iodide in N,N-dimethylformamide solution containing sodium hydride gave dimethylated compound (**15**) quantitatively as a light syrup (Scheme 4). Hydrogenolysis of the benzyl group and hydrogenation of the allyl group with hydrogen (52psi) and using 10% palladium on charcoal as a catalyst gave compound (**16**) quantitatively. Compound (**15**) was also treated with palladium (II) chloride in

95% aqueous acetic acid solution, to remove the allyl group and give the hemiacetal (**17**) in 99% yield[53]. Compound (**17**) could be used for the attachment of other pendant groups at C-1, for example, in the synthesis of a glycoconjugate[54]. Hemiacetal (**17**) was debenzylated under hydrogen (52psi) and palladium-charcoal as a catalyst to give compound (**18**) in 90% yield. Compound (**18**) was acetylated with acetic anhydride in pyridine to give compound (**19**) in 76% yield. Compound (**19**) will form the (**B**) ring of the disaccharide (**24**) or trisaccharide (**27**).

The rhamnopyranosyl precursor (**20**) that could form the (**C**) ring was obtained in two steps from diol (**14**). Thus, diol (**14**) was refluxed with dibutyltin oxide in benzene solution[55]. The solvent was removed by coevaporation with toluene and the residue was mono-methylated with methyl iodide in N,N-dimethylformamide to give compound (**20**) in 72% yield. The regiochemistry of the mono-methylated compound (**20**) was confirmed by the ^{1}H, ^{13}C NMR, and long-range inverse ^{13}C-^{1}H COSY NMR spectroscopic data (see later).

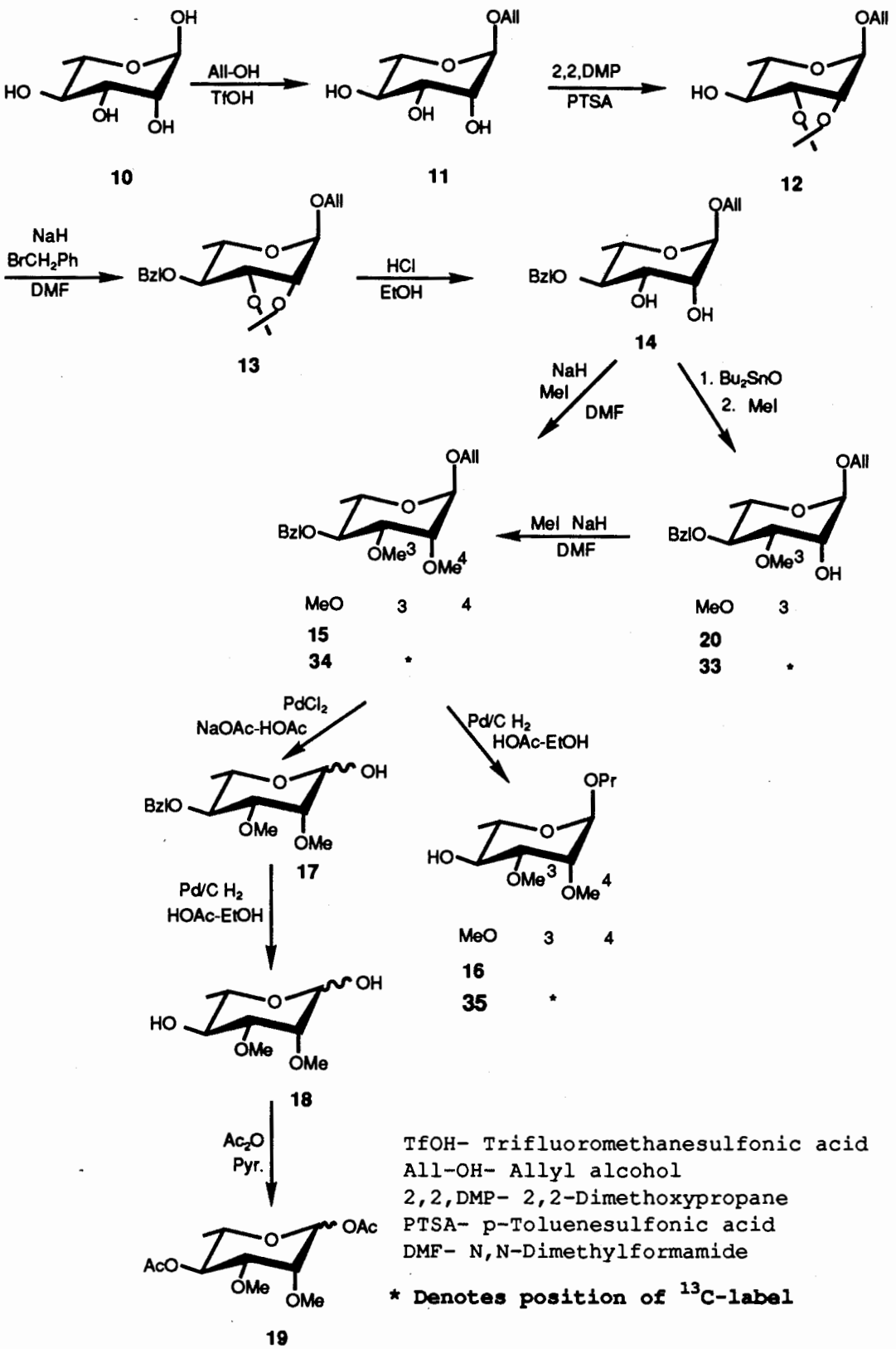
The glycosyl donor (**9**) was reacted with glycosyl acceptor (**16**) in the presence of triethylsilyl trifuloromethanesulfonate to give disaccharide (**21**) quantitatively (Scheme 5). The acetate esters were then removed with sodium methoxide in methanol to give disaccharide (**22**) in 85% yield. The glycosylation reactions were convenient and efficient. Like most glycosyl donors, the glycosyl trichloroacetimidate (**9**) with a neighbouring participating group (acetyl group) at the 2-position produced



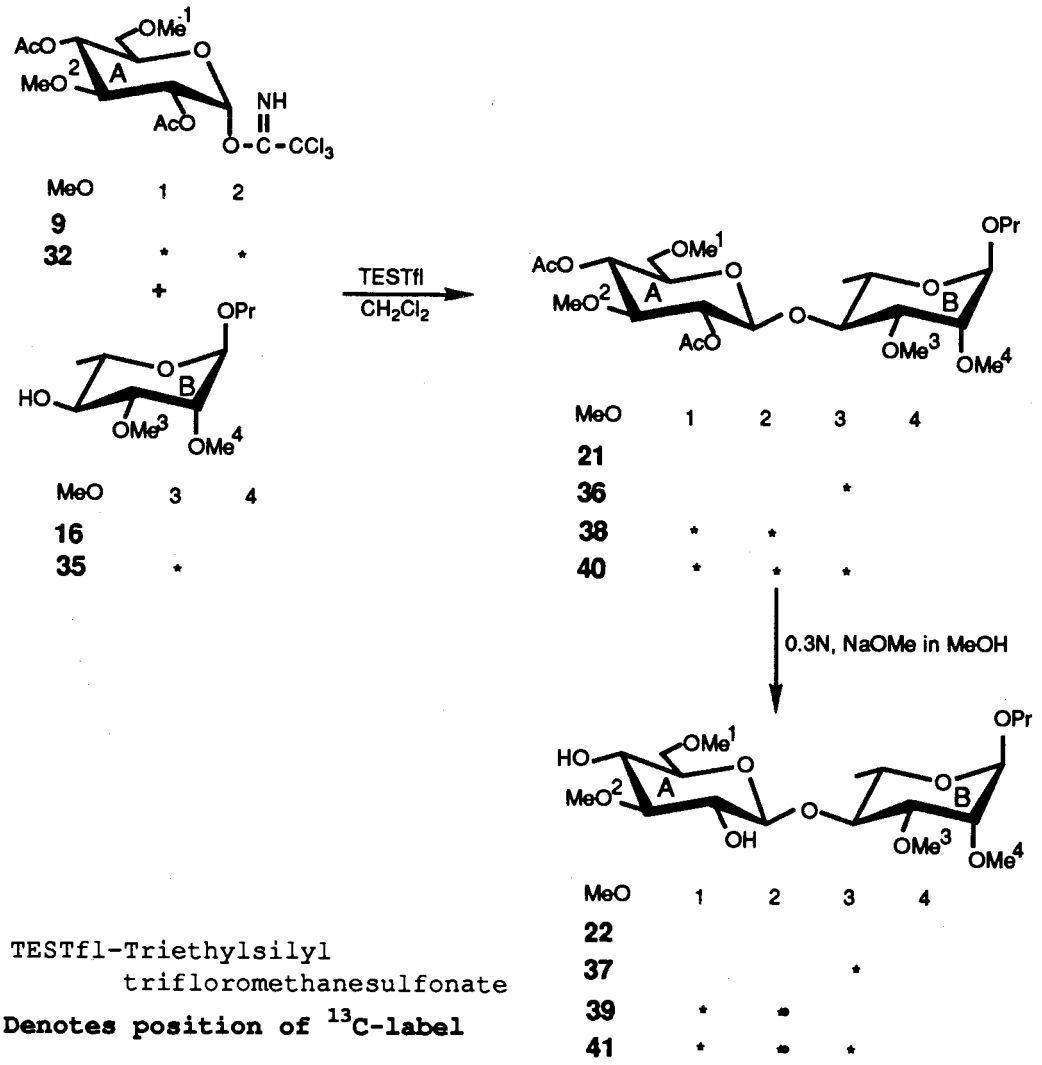
BOMCl- Benzyl chloromethyl ether
Pyr.- Pyridine
BOM- Benzoxymethyl group

*** Denotes position of ^{13}C-label**

Scheme 3. Synthesis of residue A



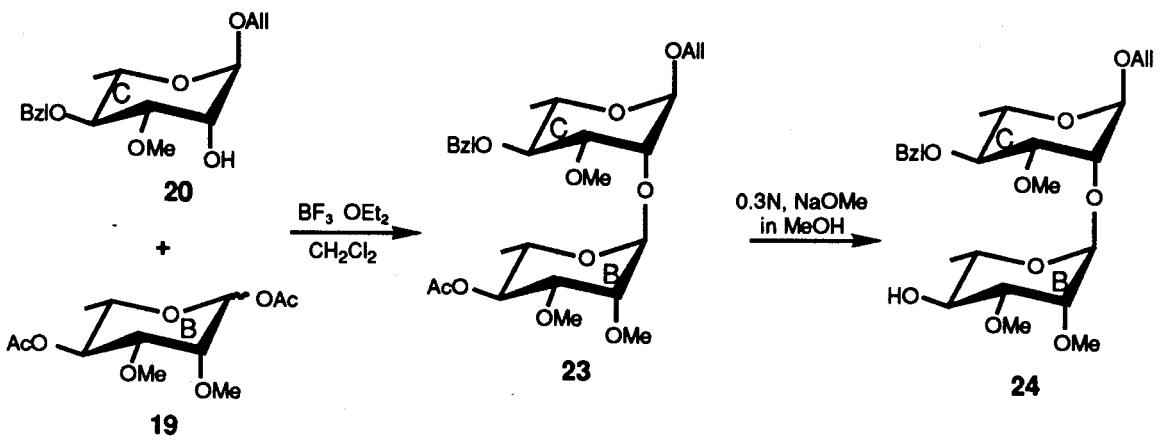
Scheme 4. Synthesis of residues B, C



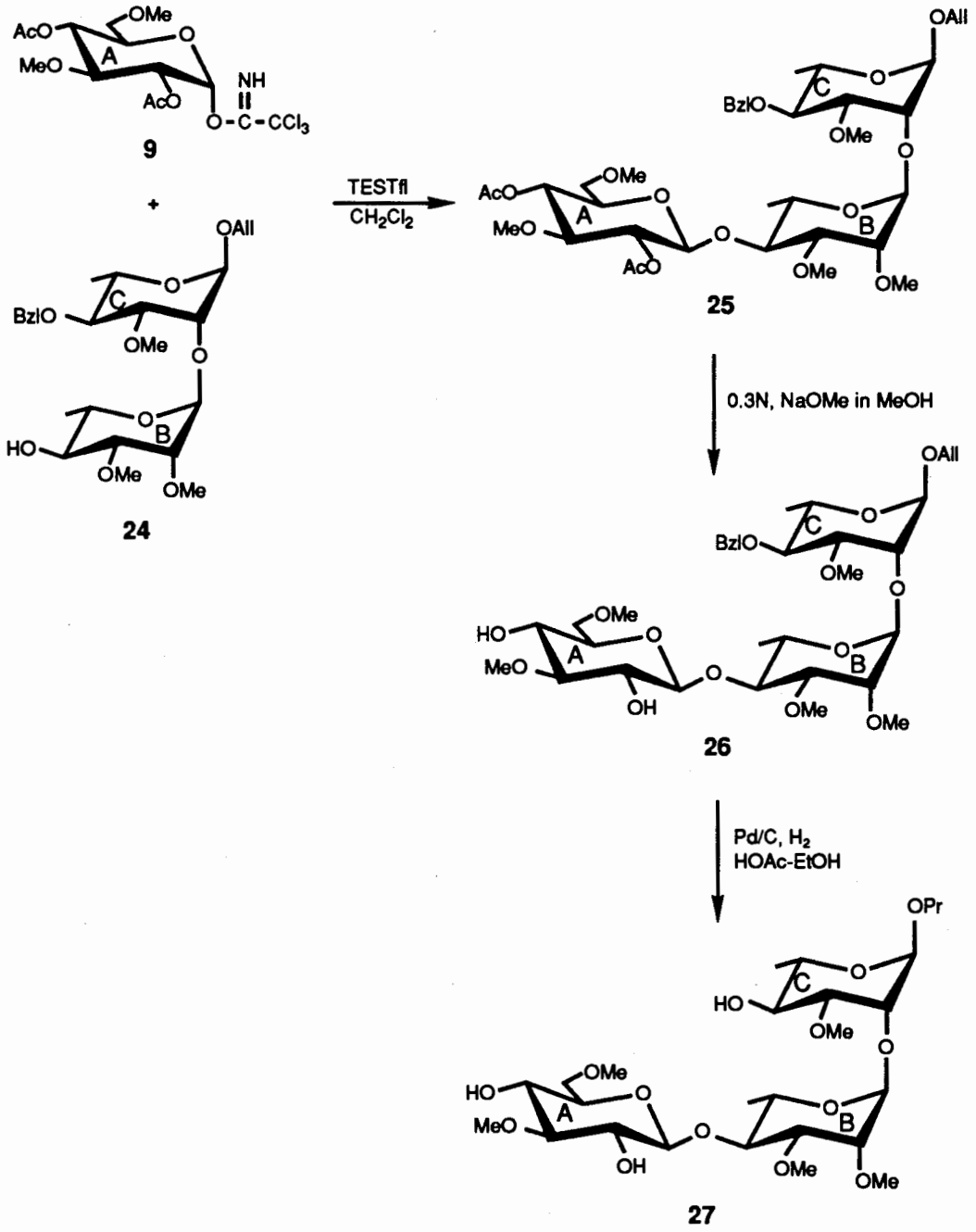
TESTfl-Triethylsilyl
trifloromethanesulfonate

* Denotes position of ^{13}C-label

Scheme 5. Synthesis of disaccharide AB



Scheme 6. Synthesis of disaccharide BC



Scheme 7. Synthesis of trisaccharide ABC

the desired 1,2-*trans* glycosyl linkage, as confirmed by the J coupling constants ($^3J_{H,H}$=8Hz and $^1J_{C,H}$=159Hz in (**22**))[59].

The synthesis of the disaccharide (**23**) was investigated next. An attempt to use 4-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranosyl trichloroacetimidate as a glycosyl donor in the synthesis of disaccharide (**23**) was not successful because this glycosyl trichloroacetimidate was difficult to obtain in pure form, therefore the direct glycosylation reaction of compound (**19**) with (**20**) in the presence of boron trifluoride etherate as described by Marino-Albernas *et al.*[3] was investigated. The disaccharide (**23**) was obtained in 70% yield. The configuration at the anomeric center was confirmed by the coupling constants ($^3J_{H,H}$=2Hz and $^1J_{C,H}$=170Hz)[59]. Disaccharide (**23**) was deacetylated to give disaccharide (**24**).

Trisaccharide (**25**) was synthesized quantitatively by coupling glycosyl donor (**9**) with acceptor (**24**) in the presence of triethylsilyl trifluoromethanesulfonate in dichloromethane solution. Deacetylation of compound (**25**) with sodium methoxide in methanol gave compound (**26**) in 72% yield. Hydrogenolysis and hydrogenation of compound (**26**) gave the final compound, trisaccharide (**27**) in 90% yield. The β-configuration of the glucosidic linkage at the anomeric center of compound (**27**) was confirmed by the coupling constants ($^3J_{H,H}$=2Hz and $^1J_{C,H}$=158Hz)[59].

B. Synthesis of ^{13}C-Labeled Compounds

The incorporation of a ^{13}C-labeled methyl group into ring (**A**) was based on the previous synthetic procedure (Scheme 3). 5-O-Benzoxymethyl-1,2-di-O-isopropylidene-α-**D**-glucofuranose (**4**) was reacted with methyl-^{13}C iodide (99%-^{13}C) in N,N-dimethylformamide containing sodium hydride to give compound (**28**) in 93% yield. ^{13}C labeling of the methyl groups at the 3 and 6 positions of compound (**28**) was confirmed by ^{1}H and ^{13}C NMR spectroscopic data (see later). In the ^{1}H NMR spectrum, the methyl groups were split into a doublet because of the $^{1}J_{^{13}C,H}$ coupling and the intensity of the methyl groups was greatly enhanced in the proton-decoupled ^{13}C NMR spectrum. In addition, $^{3}J_{^{13}C,H\text{-}3_A}$=4.5Hz was observed in the ^{1}H NMR spectrum and $^{3}J_{^{13}C,H\text{-}6_A}$=3Hz was observed in the proton coupled ^{13}C NMR spectrum. The ^{13}C-labeled compound (**28**) was further reacted with 0.5N hydrochloric acid to give compound (**29**) in 100% yield (Scheme 3). Compound (**29**) was acetylated in acetic anhydride and pyridine to give compound (**30**) in 74% yield which was, in turn, reacted with hydrazine acetate in N,N-dimethylformamide to give the hemiacetal (**31**). Compound (**31**), reacted with trichloroacetonitrile as before, afforded glycosyl trichloroacetimidate (**32**) in 64% yield. The presence of the α-anomer was confirmed by the coupling constants ($^{3}J_{1,2}$=4Hz, and $^{1}J_{C,H}$=180Hz)[59].

The incorporation of a ^{13}C-label into the methyl group at the 3-position of the rhamnose residue was carried out as shown in Scheme 4. Allyl 4-O-benzyl-α-**L**-rhamnopyranoside (**14**) was

refluxed with dibutyltin oxide in benzene solution. Removal of benzene by coevaporation with toluene gave a white residue that was mono-methylated with methyl-^{13}C iodide in N,N-dimethylformamide to give compound (**33**) in 55% yield. The regiochemistry of the methylated product was confirmed by the NMR study of compound (**35**) (see later). Compound (**33**) was methylated further with methyl iodide to give the di-methylated compound (**34**) in 100% yield. Debenzylation and hydrogenation with hydrogen and palladium-charcoal then gave glycosyl acceptor (**35**) in 80% yield. ^{1}H NMR, ^{13}C NMR and long-range inverse ^{13}C-^{1}H COSY NMR (see later) confirmed the fact that the methyl group at the 3 position of compound (**35**) was ^{13}C labeled.

Disaccharides (**36**), (**38**), and (**40**) were obtained through glycosylation with different combination of donors (**9**), (**32**) and acceptors (**16**), (**35**) in 93%, 71%, 100% yield, respectively (see Scheme 5). Deprotection of compounds (**36**), (**38**), and (**40**) with sodium methoxide in methanol gave the isotopically-labeled disaccharides (**37**), (**39**), (**41**) in 100%, 89%, 100% yield, respectively.

II. NMR Spectroscopic Results

A. NMR Spectroscopic Results of Unlabeled Compounds

The assignment of the regiochemistry of the methylation of the rhamnopyranosyl diol (**14**), by ^{13}C NMR spectroscopy was performed as follows. In the diol (**14**), the chemical shift of C-2 (67.9ppm) occurred at higher field (upfield, 3.6ppm) than that

of C-3 (71.5ppm) due to the sheilding of the axial hydroxyl group at C-2[31,58]. The chemical shifts of both C-2 (77.4ppm) and C-3 (80.9ppm) were downfield in the dimethylated compound (**15**) compared to the carbons in compound (**14**). The downfield shift observed upon methylation is due to the β substituent effect[31,56]. The chemical shift of C-2 in compound (**15**) was still at higher field (upfield, 3.5ppm) than that of C-3 because of the shielding of the axial substituent as mentioned above. In the monomethylated compound (**20**), the peak at 67.3ppm was assigned to C-2 by comparison with the assignment in the diol (**14**) (67.9ppm). The new peak appearing at 80.0ppm was assigned to C-3, indicating that the regioselective methylation had occurred at C-3. These assignments and the chemical shifts of the methyl groups attached to C-2 and C-3 in compounds (**15**) and (**16**) were also confirmed by use of a long range inverse ^{13}C-^{1}H COSY experiment on the ^{13}C-labeled compound (**35**) (see later). The assignment of the regiochemistry of methylation of the derived disaccharides and trisaccharides was based on similar arguments.

The assignments of NMR spectra of the disaccharides (**22**) and (**24**), and the trisaccharide (**27**) were obtained by the application of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY NMR experiments (Fig. 2-1 to Fig. 2-10). The ^{1}H and ^{13}C NMR data are listed in Table 1. The H-1_A, H-1_B, and H-6_B signals of disaccharide (**22**) are readily identified because of their characteristic shifts. The

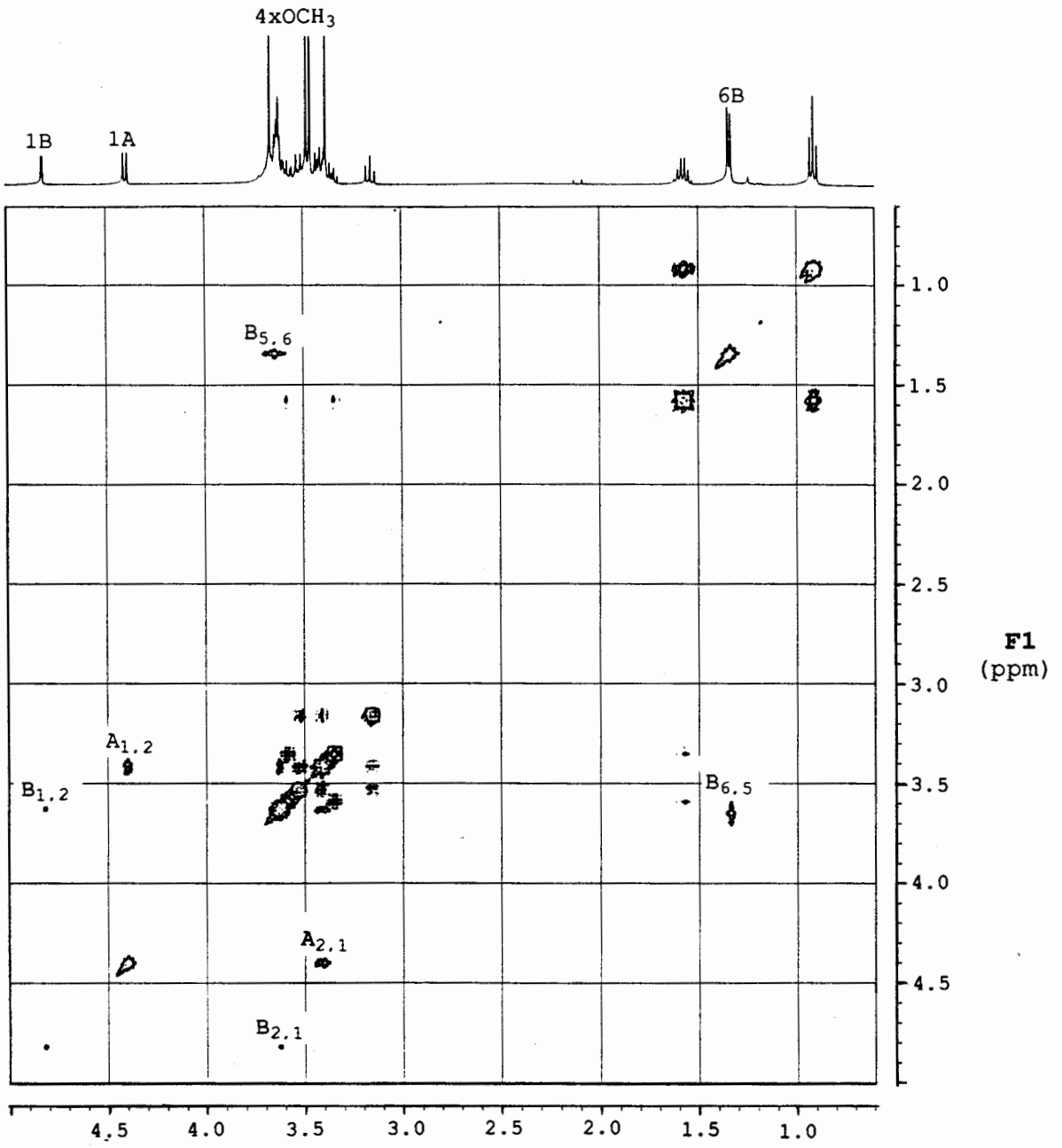


Fig. 2-1. 400 MHz ^{1}H-^{1}H COSY 2D-NMR spectrum of disaccharide (22)

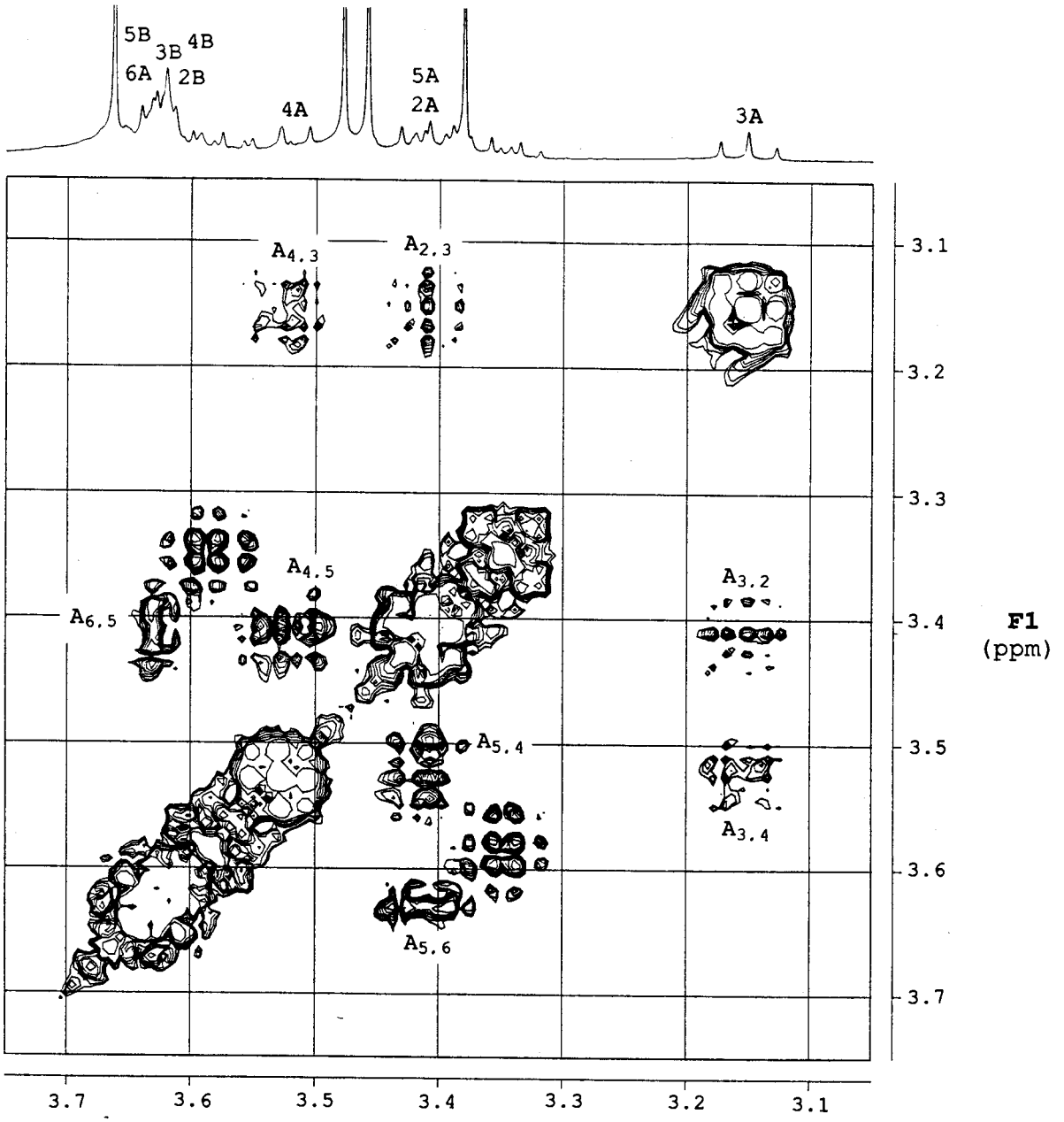


Fig. 2-2. Partial 400 MHz ^{1}H-^{1}H COSY 2D-NMR spectrum of disaccharide (22)

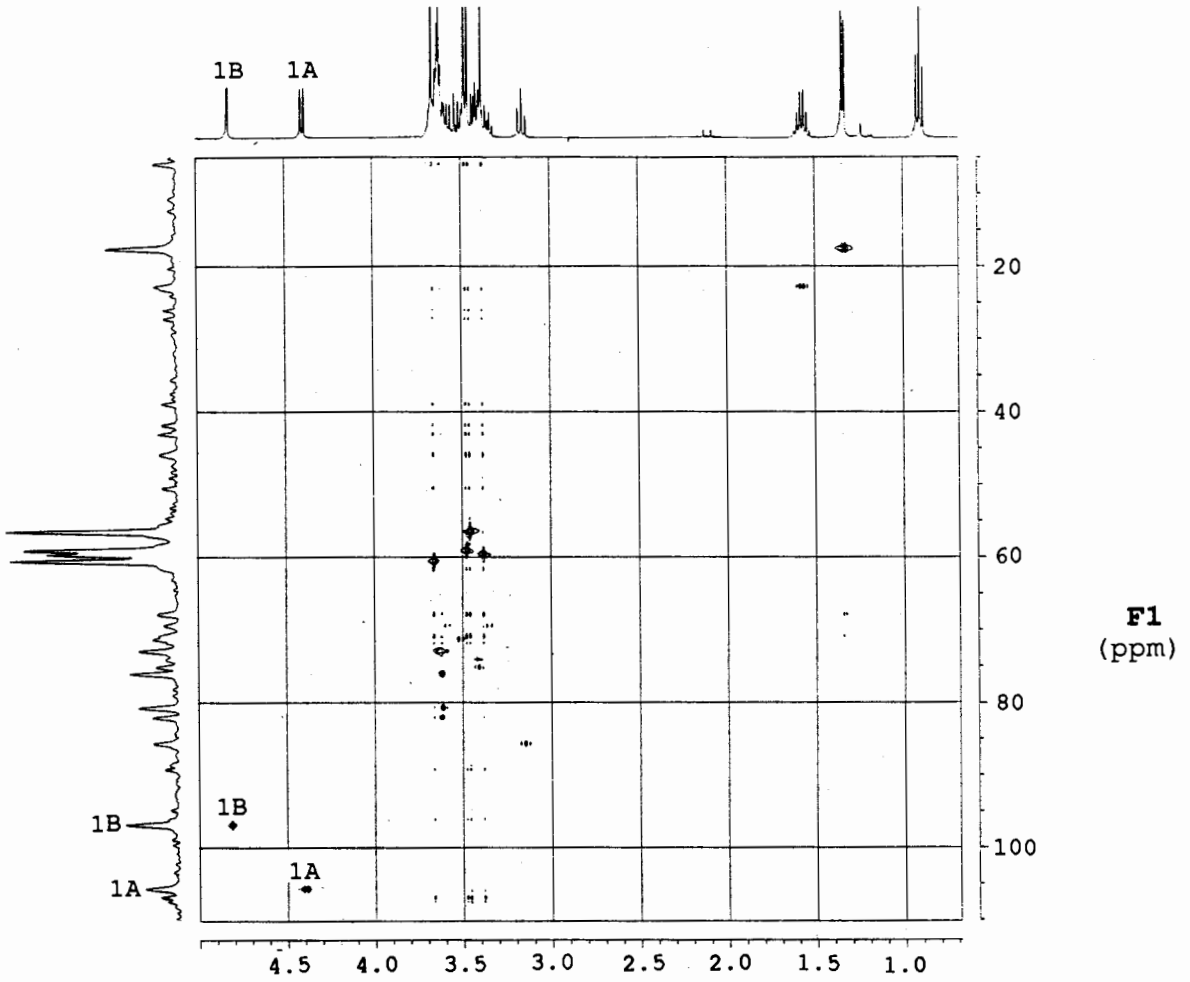


Fig. 2-3. 400 MHz inverse ^{13}C-^{1}H COSY 2D-NMR spectrum of disaccharide (22)

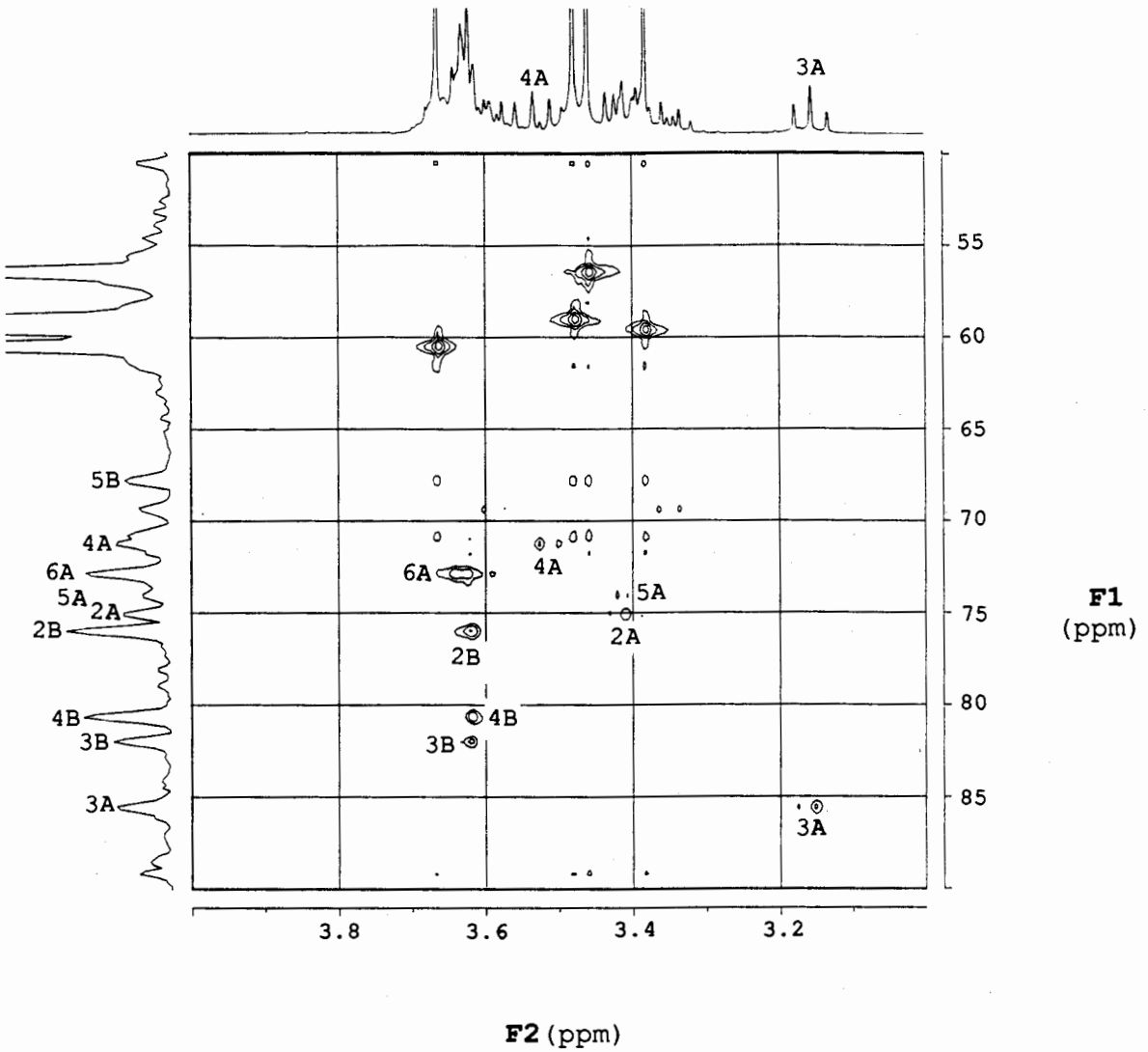


Fig. 2-4. Partial 400 MHz inverse ^{13}C-^{1}H COSY 2D-NMR spectrum of disaccharide (22)

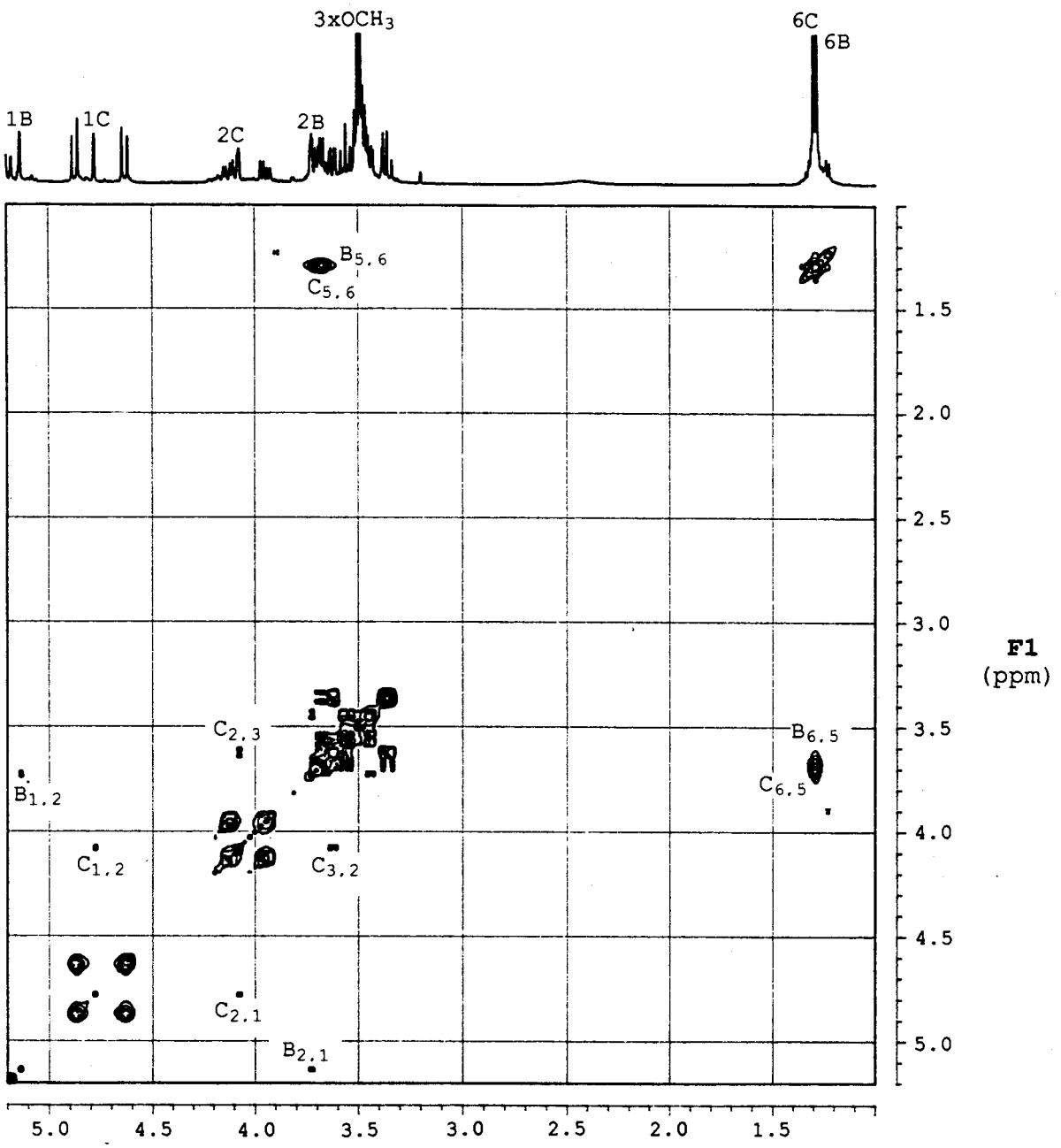


Fig. 2-5. 400 MHz ^{1}H-^{1}H COSY 2D-NMR spectrum of disaccharide (24)

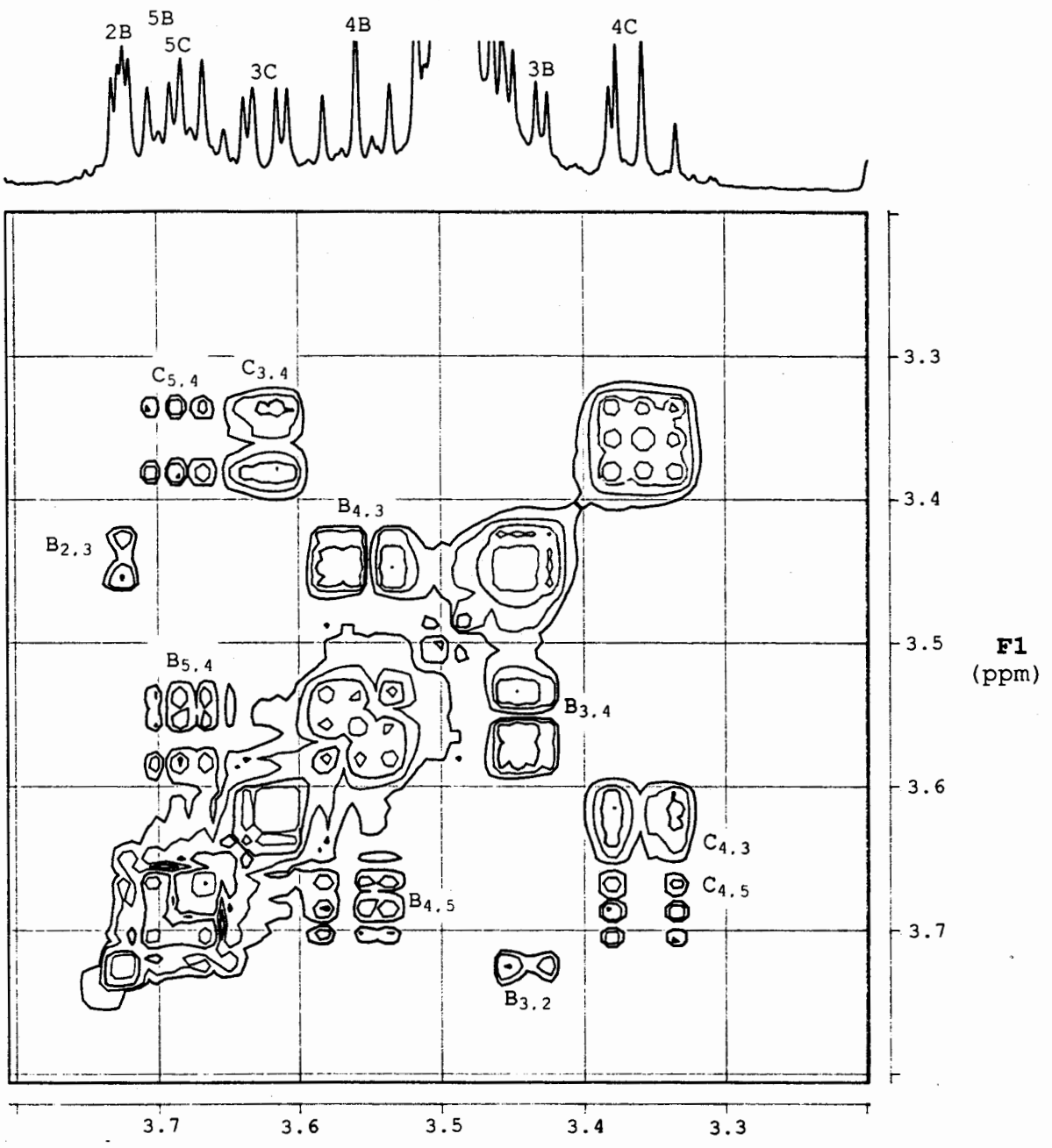


Fig. 2-6. Partial 400 MHz ^1H-^1H COSY 2D-NMR spectrum of disaccharide (24)

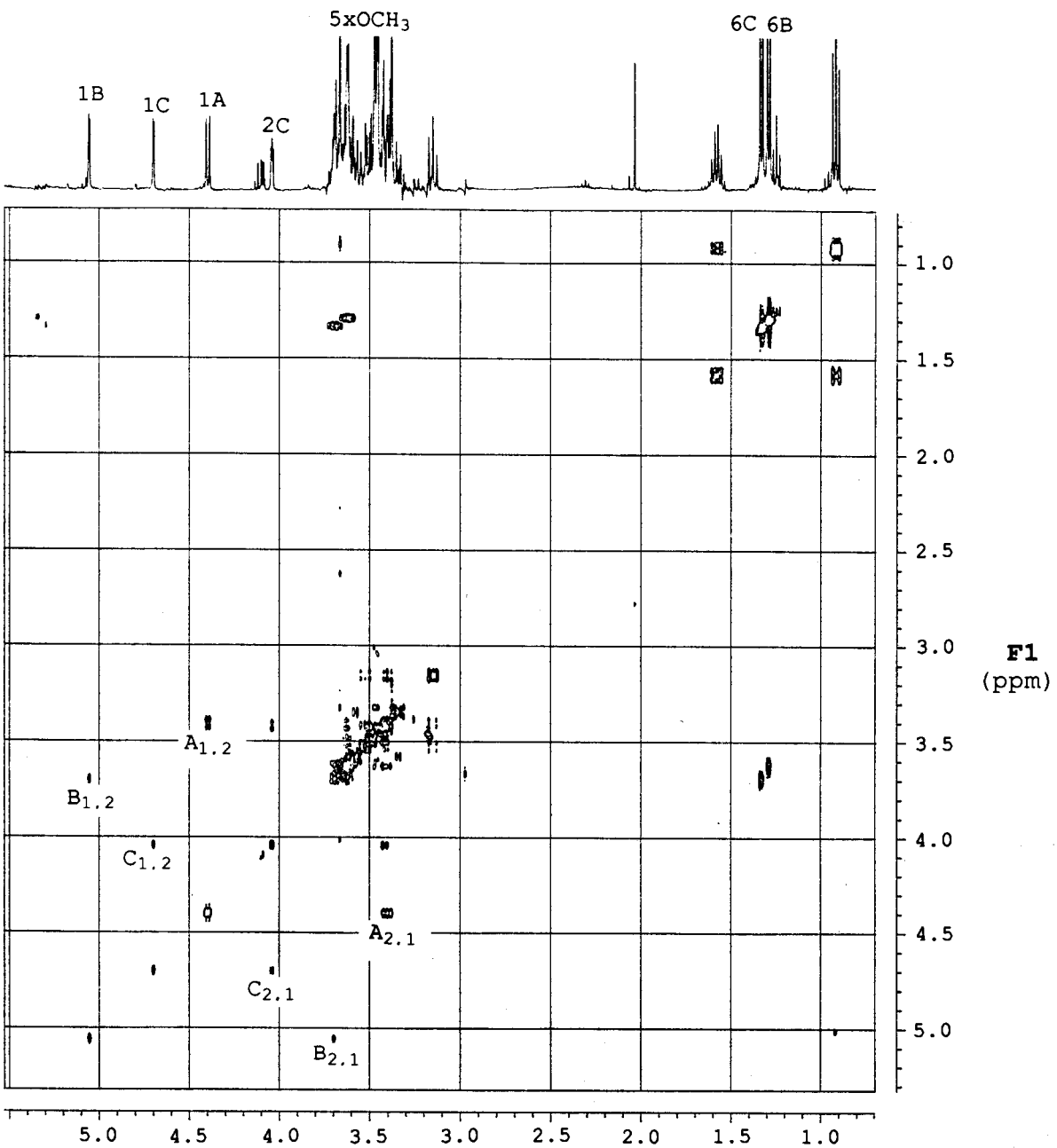


Fig. 2-7. 400 MHz ^{1}H-^{1}H COSY 2D-NMR spectrum of trisaccharide (27)

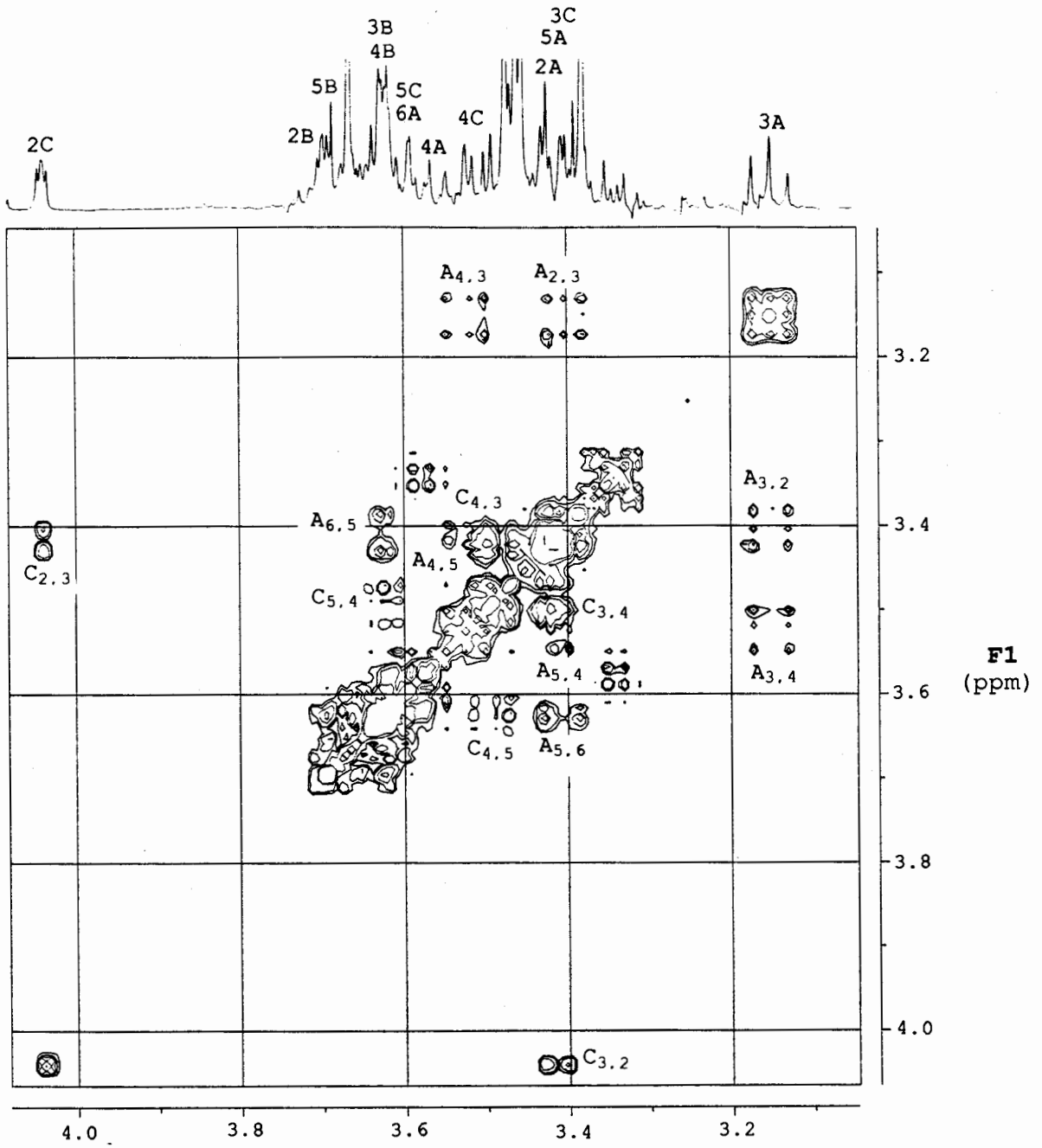


Fig. 2-8. Partial 400 MHz ^{1}H-^{1}H COSY 2D-NMR spectrum of trisaccharide (27)

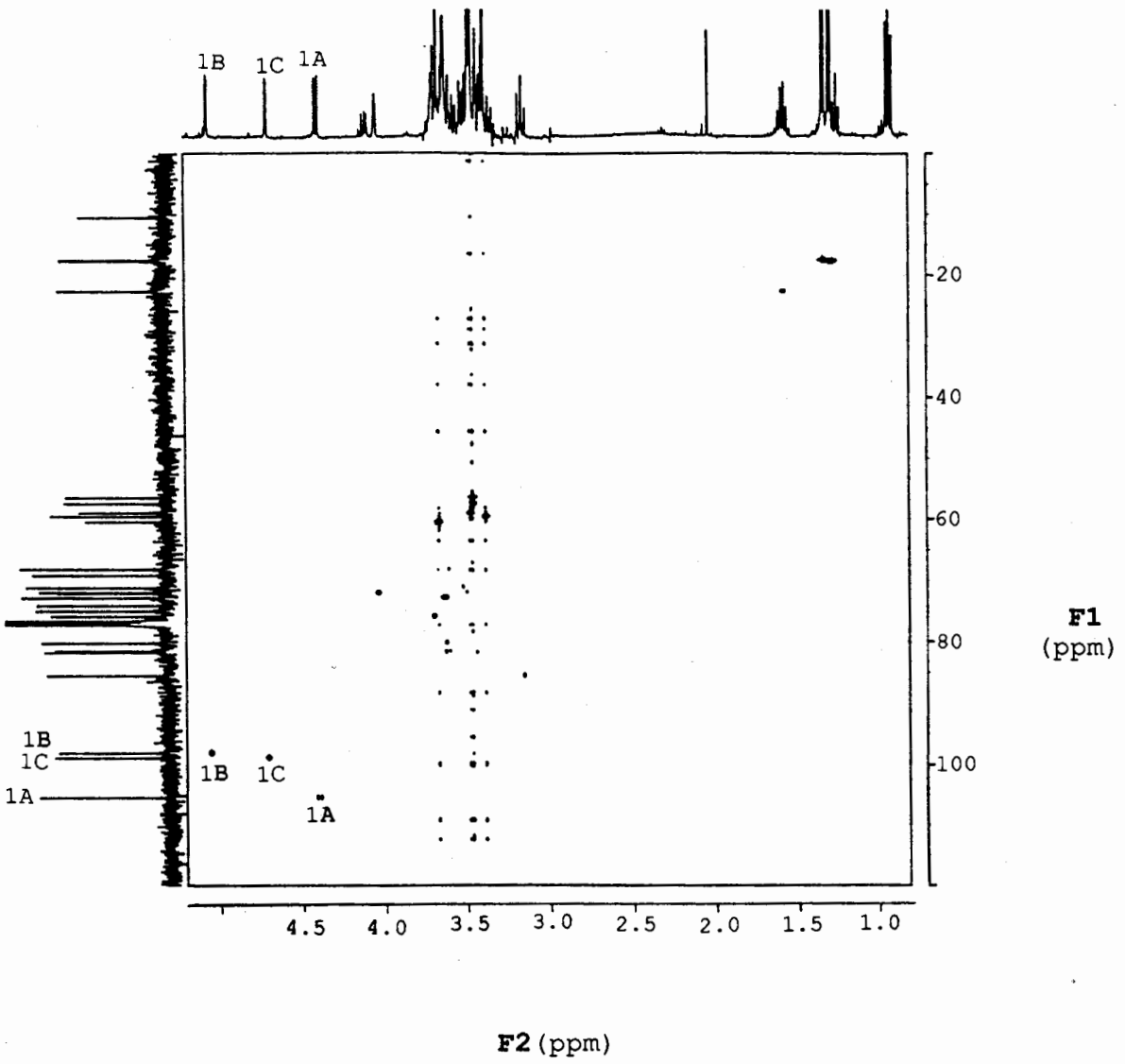


Fig. 2-9. 400 MHz inverse ^{13}C-^{1}H COSY 2D-NMR spectrum of trisaccharide (27)

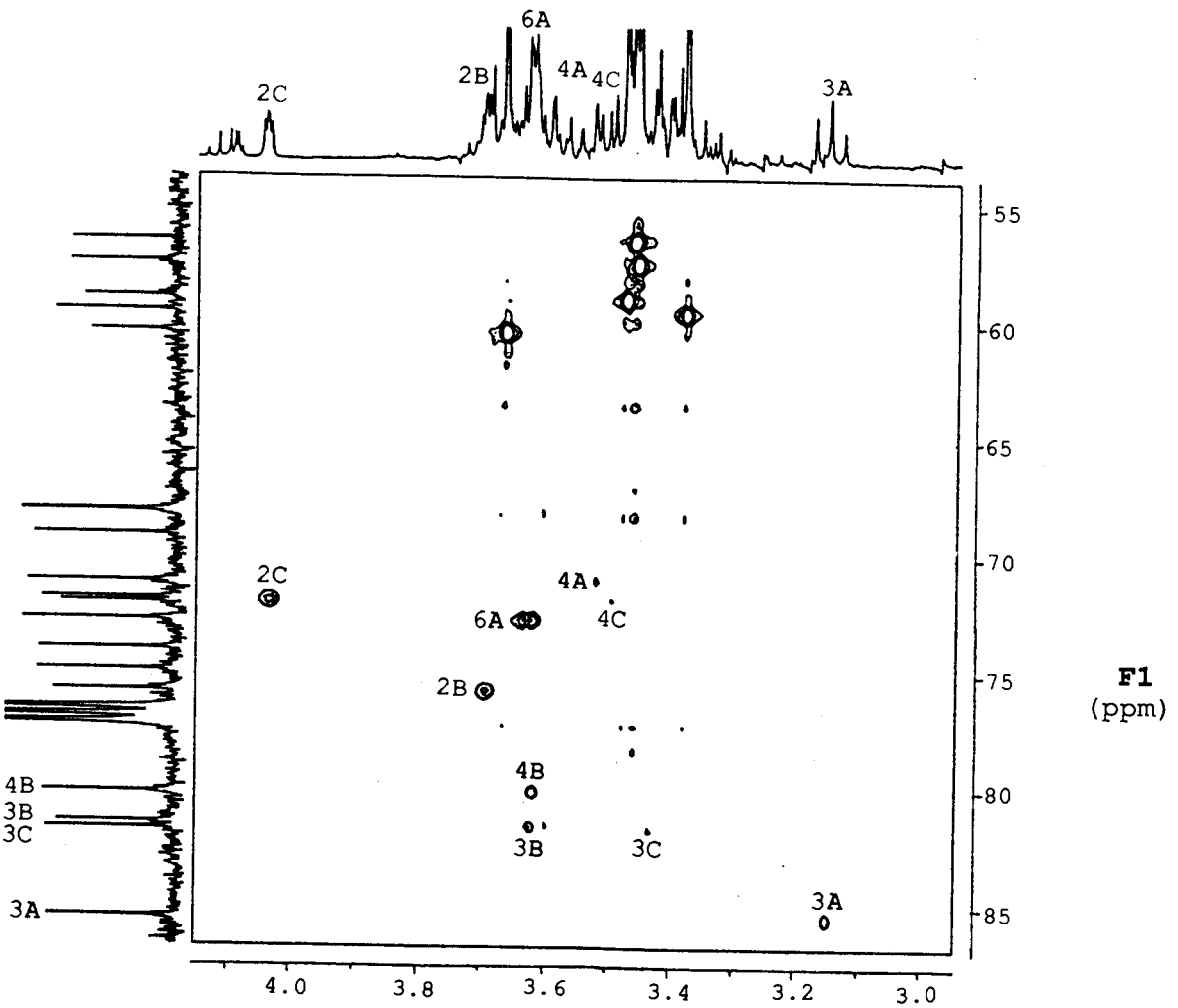


Fig. 2-10. Partial 400 MHz inverse ^{13}C-^{1}H COSY 2D-NMR spectrum of trisaccharide (27)

Table 1. ^{1}H and ^{13}C NMR data[a] of compounds (**22**), (**24**) and (**27**)

	^{1}H NMR data			^{13}C NMR data		
Ring	(**22**)	(**24**)	(**27**)	(**22**)	(**24**)	(**27**)
1A	4.40		4.40	105.6 (159Hz)[b]		105.6 (158Hz)[b]
2A	3.41		3.40	75.1		75.1
3A	3.15		3.15	85.6		85.6
4A	3.53		3.53	71.2		71.2
5A	3.41		3.40	74.2		74.2
6A	3.63		3.61	72.8		72.9
OCH_3-3_A	3.67		3.67	60.4		60.4
OCH_3-6_A	3.38		3.38	59.5		59.6
1B	4.81	5.13	5.06	96.8 (168Hz)[b]	98.9 (163Hz)[b]	98.3 (170Hz)[b]
2B	3.62	3.73	3.70	76.0	76.0	75.9
3B	3.62	3.45	3.61	81.8	80.9	81.6
4B	3.62	3.56	3.62	80.7	71.7	80.3
5B	3.64	3.68	3.70	67.5	67.9	68.2
6B	1.34	1.29	1.35	17.5	17.5	17.6
OCH_3-2_B	3.48	3.50	3.48	59.0	58.8	59.0
OCH_3-3_B	3.46	3.48[c]	3.46	56.4	57.0	56.5
1C		4.78	4.70		98.1 (165Hz)[b]	99.1 (169Hz)[b]
2C		4.07	4.05		73.7	72.2
3C		3.62	3.43		82.0	81.9
4C		3.36	3.50		80.2	72.0
5C		3.68	3.64		68.1	68.2
6C		1.30	1.29		17.7	17.7
OCH_3-3_C		3.48[c]	3.47		57.9	57.5

[a] In $CDCl_3$. [b] $^{1}J_{C-H}$. [c] The ^{1}H NMR signals of OCH_3-3_B and OCH_3-3_C of (**24**) were overlapped.

$H\text{-}3_A$ signal is also readily identified by its unique coupling pattern and chemical shift. However, the rest of the peaks which appeared in the 3.4~3.7 ppm region were highly overlapped. The assignments of $H\text{-}2_A$, $H\text{-}4_A$, $H\text{-}2_B$, and $H\text{-}5_B$ were obtained by $^1H\text{-}^1H$ COSY experiments through their chemical-shift correlated relationship with $H\text{-}1_A$, $H\text{-}3_A$, $H\text{-}1_B$, and $H\text{-}6_B$, respectively. The NMR data of $H\text{-}5_A$, $H\text{-}6_A$, $H\text{-}3_B$, $H\text{-}4_B$ were difficult to obtain from the $^1H\text{-}^1H$ COSY spectrum. The assignment of $H\text{-}5_A$, $H\text{-}6_A$, $H\text{-}3_B$, $H\text{-}4_B$ was completed, therefore, by comparison of spectral integration and by a $^{13}C\text{-}^1H$ COSY experiment. The assignment of the signals in the spectra of the disaccharide (**24**) was easily obtained from ^1H, $^1H\text{-}^1H$ COSY, and $^{13}C\text{-}^1H$ COSY NMR experiments. Overlap also occurred in the $^1H\text{-}^1H$ COSY NMR spectrum of the trisaccharide (**27**) and made it difficult to assign $H\text{-}5_A$, $H\text{-}6_A$, $H\text{-}3_B$, $H\text{-}4_B$, $H\text{-}3_C$, and $H\text{-}4_C$, but the assigment was completed by comparison with $^{13}C\text{-}^1H$ COSY NMR spectra. Most of the data for disaccharides (**22**) and (**24**), and trisaccharide (**27**) were similar to those reported by Bock *et al.*[50] in their study of allyl 4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhammopyranoside (**AB**), allyl 2-O-(2,3-di-O-methyl-α-**L**-rhamnopyranosyl)-3-O-methyl-α-**L**-rhamnopyranoside (**BC**), and allyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-3-O-methyl-α-**L**-rhamnopyranoside (**ABC**).

B. NMR Spectroscopic Results of ^{13}C-Labeled Compounds

The ^{13}C-labeled mono-methylated hydroxyl group at C-3 of the rhamnopyranosyl ring was confirmed by performing a long-range inverse ^{13}C-^{1}H COSY NMR experiment on propyl 2-O-methyl-3-O-^{13}C-methyl-**α-L**-rhamnopyranoside (**35**). As shown in Fig. 2-11 and Fig. 2-12, there is an intense long-range correlated peak between the ^{13}C-labeled methyl group (56.9ppm) and H-3 (3.43ppm) [$^{3}J_{(^{13}C\text{-}O\text{-}C(3)\text{-}H(3))}$]. Clearly, the methylation had occurred at the 3-position. Another long-range correlated peak was also observed between the ^{13}C-labeled methyl group and H-4 (3.54ppm) [$^{4}J_{(^{13}C\text{-}O\text{-}C(3)\text{-}C(4)\text{-}H(4))}$]. This observation may provide important information for the investigation of the glycosidic linkage of disaccharide (**22**), and trisaccharide (**27**) since the coupling information of H-4 of the (**B**) ring is an important factor for the glycosidic linkage analysis. It also provides a possibility to study further the rhamnopyranosidic linkage (**B**-(1,2)-**C**) since H-1 of (**B**) and H-2 of (**C**) are important factors for the glycosidic linkage analysis. The assignments of the NMR spectra of the ^{13}C-labeled disaccharides (**37**), (**39**) and (**41**) were obtained by comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of the disaccharide (**22**). The ^{1}H and ^{13}C NMR data are shown in Table 2. The ^{1}H NMR spectrum of ^{13}C-labeled disaccharide (**37**) was similar to that of unlabeled disaccharide (**22**) in chemical shift and peak pattern in $CDCl_3$ solution. As the methyl group at the 3 position of ring (**B**) in (**37**) was ^{13}C labeled, the singlet of the methyl group in (**22**) became a

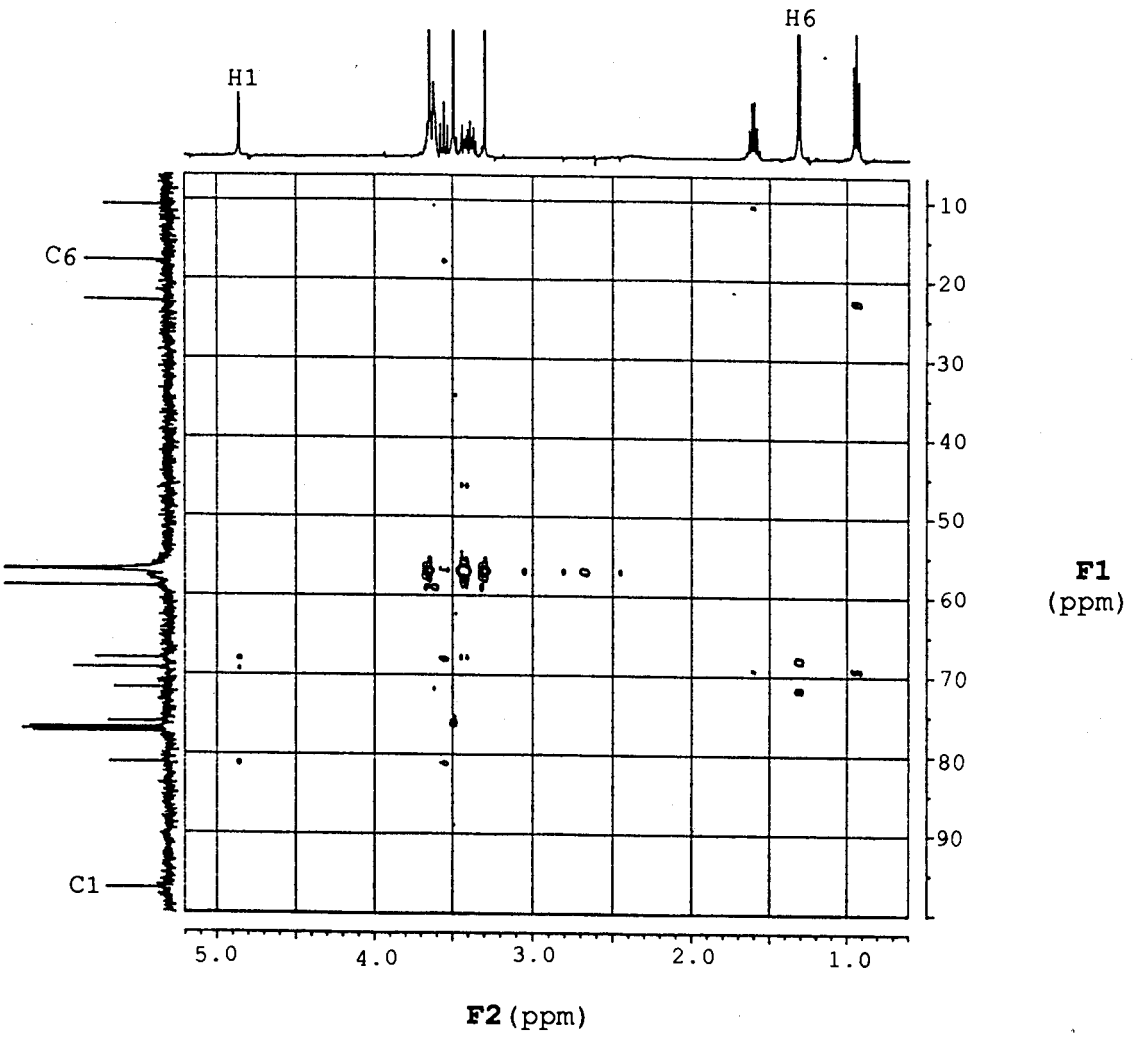


Fig. 2-11. 400 MHz inverse ^{13}C-^{1}H COSY (for long-range correlation) 2D-NMR spectrum of ^{13}C-labeled compound (35)

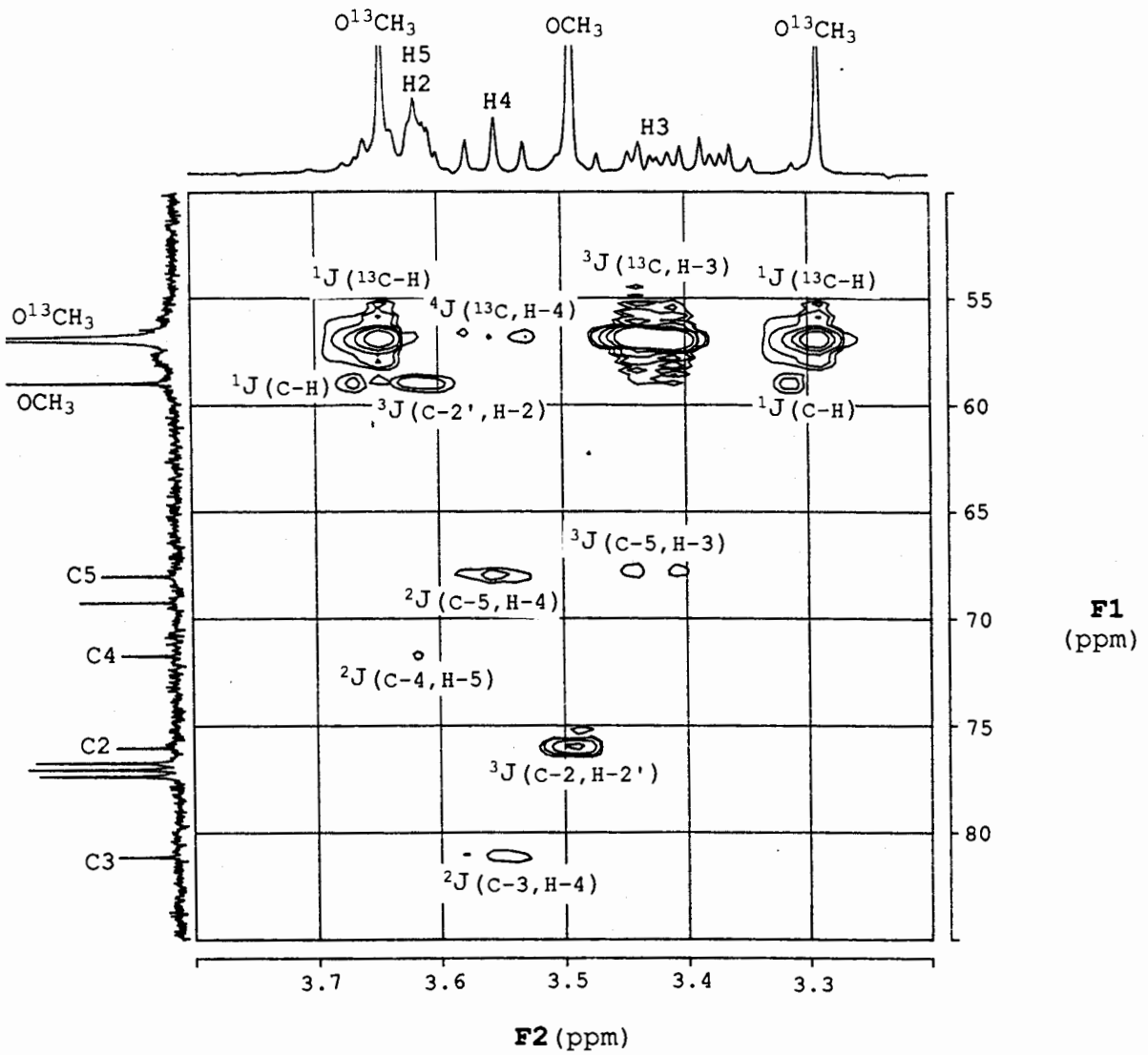


Fig. 2-12. Partial 400 MHz inverse ^{13}C-^{1}H COSY (for long-range correlation) 2D-NMR spectrum of ^{13}C-labeled compound (35)

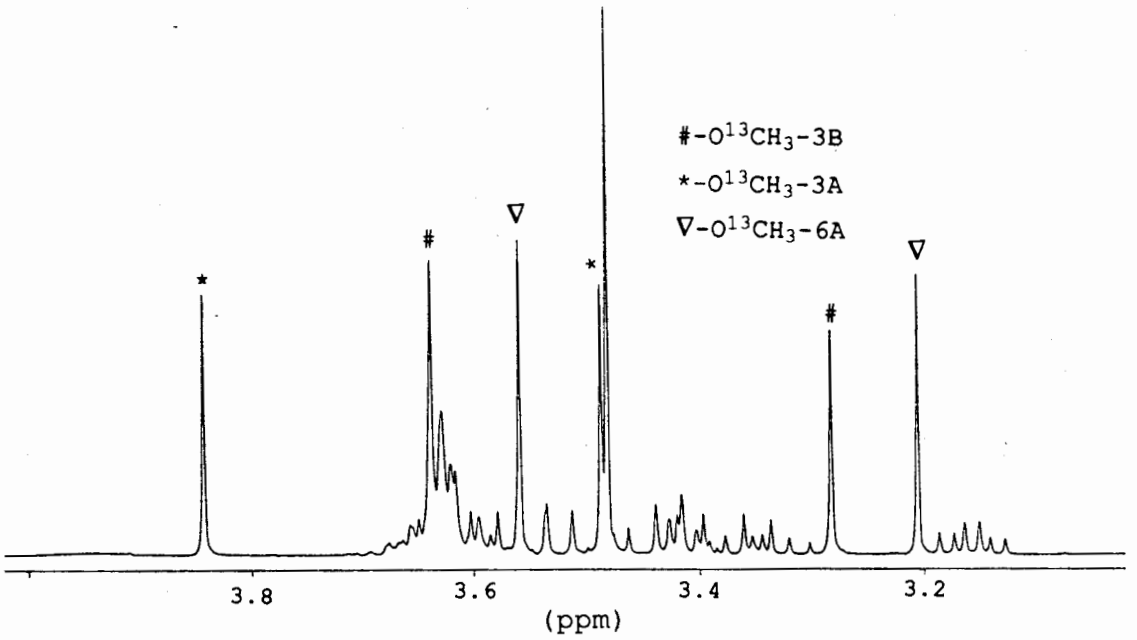


Fig. 2-13. Partial 400 MHz ^{1}H-NMR spectrum of ^{13}C-labeled disaccharide (41)

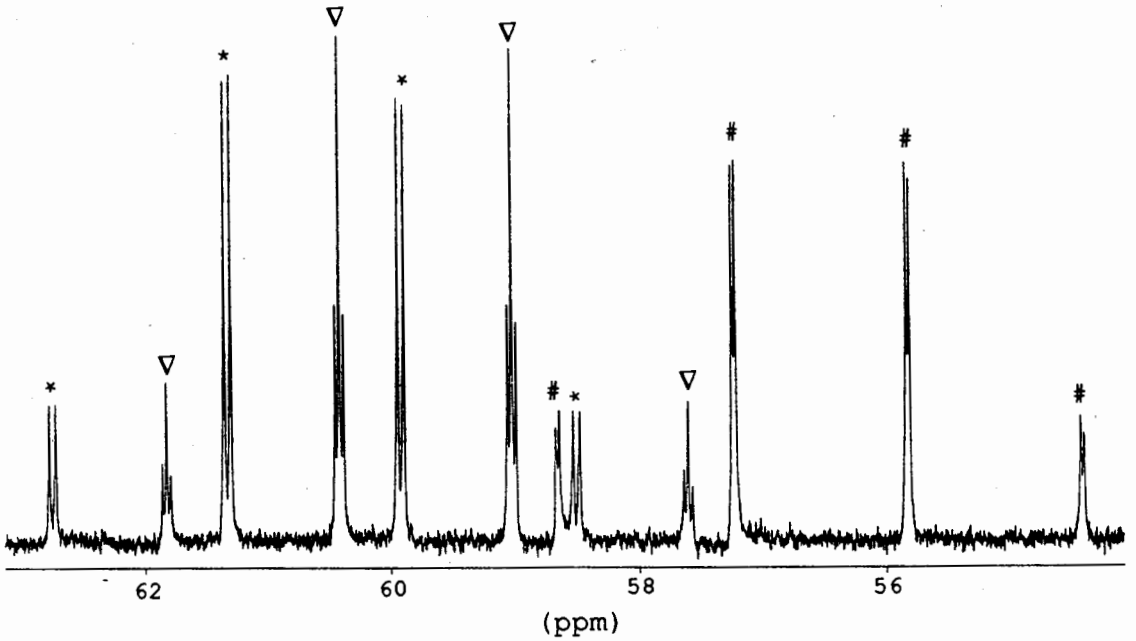


Fig. 2-14. Partial 400 MHz ^{13}C-NMR spectrum of ^{13}C-labeled disaccharide (41)

Table 2. ^{1}H and ^{13}C NMR data[a] of compounds (**37**), (**39**) and (**41**)

	^{1}H NMR data			^{13}C NMR data		
Ring	(**37**)	(**39**)	(**41**)	(**37**)	(**39**)	(**41**)
1A	4.40	4.40	4.40	105.6	105.7	105.7
				(161Hz)[b]	(160Hz)[b]	(158Hz)[b]
2A	3.41	3.41	3.41	75.1	75.1	75.1
3A	3.15	3.16	3.16	85.6	85.6	85.6
4A	3.53	3.53	3.53	71.2	71.3	71.3
5A	3.41	3.41	3.41	74.2	74.1	74.1
6A	3.63	3.63	3.63	72.9	72.9	72.9
OCH_3-3_A	3.66	3.66	3.66	60.4	60.4	60.4
		(~142Hz)[e]	(~142Hz)[e]		(142Hz)[c]	(142Hz)[c]
		(5Hz)[f]	(5Hz)[f]		(5Hz)[d]	(5Hz)[d]
OCH_3-6_A	3.37	3.38	3.38	59.6	59.6	59.6
		(~141Hz)[e]	(~141Hz)[e]		(142Hz)[c]	(142Hz)[c]
		N/A[g]	N/A[g]		(4Hz)[d]	(4Hz)[d]
1B	4.81	4.82	4.82	96.9	96.9	96.8
				(168Hz)[b]	(167Hz)[b]	(168Hz)[b]
2B	3.62	3.62	3.62	76.1	76.0	76.0
3B	3.62	3.62	3.62	81.9	81.9	81.9
4B	3.62	3.62	3.62	80.7	80.7	80.7
5B	3.64	3.64	3.64	67.6	67.5	67.5
6B	1.34	1.34	1.34	17.5	17.5	17.5
OCH_3-2_B	3.47	3.48	3.48	59.0	59.0	59.0
OCH_3-3_B	3.45	3.46	3.46	56.4	56.4	56.4
	(~142Hz)[e]		(~142Hz)[e]	(142Hz)[c]		(142Hz)[c]
	N/A[g]		N/A[g]	(3Hz)[d]		(3Hz)[d]

[a] In $CDCl_3$. [b] $^{1}J_{C-H}$. [c] $^{1}J_{13C-H}$ from ^{13}C NMR. [d] $^{3}J_{13C,H}$ from ^{13}C NMR. [e] $^{1}J_{13C-H}$ from ^{1}H NMR. [f] $^{3}J_{13C,H}$ from ^{1}H NMR. [g] $^{3}J_{13C,H}$ could not be obtained from the ^{1}H NMR spectrum.

doublet in (**37**) because of the $^{1}J_{^{13}C,H}$ coupling. The $^{3}J_{^{13}C,H\text{-}3_B}$ coupling was not observed in the ^{1}H NMR spectrum because of spectral overlap around 3.5~3.6ppm where H-3_B appeared. In the proton-coupled ^{13}C NMR spectrum of (**37**), $^{1}J_{^{13}C,H}$=142 and $^{3}J_{^{13}C,H\text{-}3_B}$=3Hz were clearly observed. Similar results were found for (**39**) and (**41**) (see Fig. 2-13 and Fig. 2-14). The methyl groups at the 3 and 6-positions of ring (**A**) in (**39**) and (**41**) and the 3-position of ring (**B**) in (**41**) were ^{13}C-labeled; the labeled methyl groups were doublets in the ^{1}H NMR spectra because of the $^{1}J_{^{13}C,H}$ coupling. The $^{3}J_{^{13}C,H\text{-}3_A}$ coupling of H-3_A was identified, but that of H-6_A, and H-3_B was difficult to assign because of spectral overlap, but that data could be obtained from the proton-coupled ^{13}C spectra.

The coupling constants obtained in the ^{1}H NMR spectra, and proton-coupled ^{13}C NMR spectra were similar and the values were $^{1}J_{^{13}C,H}$=142Hz in (**37**), $^{1}J_{^{13}C,H}$=142Hz in (**39**), and $^{1}J_{^{13}C,H}$=142Hz in (**41**) while $^{3}J_{^{13}C,H\text{-}3_B}$=3Hz in (**37**), $^{3}J_{^{13}C,H\text{-}3_A}$=5Hz, $^{3}J_{^{13}C,H\text{-}6_A}$=4Hz in (**39**), and $^{3}J_{^{13}C,H\text{-}3_A}$=5HZ, $^{3}J_{^{13}C,H\text{-}6_A}$=4Hz, $^{3}J_{^{13}C,H\text{-}3_B}$=3Hz in (**41**).

No isotope shift was observed in the ^{13}C NMR spectra of the above ^{13}C-labeled compounds (see Table 2.). The reason is probably that the effect of the ^{13}C-labeled methyl groups was decreased by the number of bonds between the ^{13}C-label and the proton since the atom directly connected to the ^{13}C-labeled methyl groups is oxygen.

III. Conclusion

Disaccharide (**22**), and trisaccharide (**27**) corresponding to the antigenic determinant of the phenolic glycolipid of *Mycobacterium leprae* have been synthesized, and characterized by 2D-NMR techniques. The use of a glucosyl trichloroacetimidate as a glycosyl donor in the glycosylation reaction was efficient and convenient. Compound (**14**) used as an intermediate to synthesize the rhamnose rings of the disaccharide and trisaccharide provided an efficient synthetic pathway. The synthetic route could be extended to synthesize artificial antigens by coupling the trisaccharide to protein.

^{13}C-isotopically labeled disaccharides (**37**), (**39**), and (**41**) have been synthesized. These disaccharides will be available for future detailed study of $J_{C,H}$ coupling constants ($J_{C,H}$ measurment), kinetic binding experiments, and ^{13}C isotope-edited experiments. Through these NMR experiments, better conformational information may be obtained. In particular, information on the conformation of the disaccharides when bound to antibody may be obtained.

Chapter 3 Experimental

I. General

^{1}H-NMR(400.13MHz), ^{13}C-NMR(100.6MHz) spectra were recorded on a Bruker AMX-400 NMR spectrometer. All spectra were measured in deuteriochloroform ($CDCl_3$) unless otherwise stated. ^{13}C-NMR and ^{1}H-NMR spectra were referenced to solvent (residual $CHCl_3$, δ_H 7.24ppm, δ_C 77.0ppm, respectively) as an internal standard. Chemical shifts and coupling constants were obtained from a first order analysis of spectra.

^{1}H-^{1}H COSY and inverse ^{13}C-^{1}H COSY spectra were acquired with data sets of 2K(F2)x512(F1), the FIDs were zero filled to a 1K(F2)x1K(F1) data set, and processed using a sine squared apodization function with a shift of 2 in F1 and F2. The spectra were displayed in the absolute value mode. The digital resolution in the inverse ^{13}C-^{1}H spectra was ± 1Hz/pt. The inverse ^{13}C-^{1}H COSY spectra for detecting long-range correlation were acquired with data sets of 1K(F2)x250(F1). The FIDs were processed using a sine squared apodization function. The mixing time was 90ms and the digital resolution was ± 2Hz/pt.

Microanalyses were measured with an Elemental Analyzer-MDD 1106. The percentage of carbon in a particular compound was calculated by using the weight of carbon divided by the molecular weight of the compound. For the labeled compounds, the molecular weight was calculated using 13.01 as atomic weight of

the labeled carbon. However, for the calculation of the weight of carbon, the atomic weight was taken as 12.01. This is because during the actual experimental determination of the percentage of carbon, the quantity being detected is the number of moles of CO_2 produced as opposed to the weight of CO_2. Therefore, for the calculation of the percentage of carbon in a compound, the weight of carbon in the compound is not sensitive to the presence of ^{13}C unlike the molecular weight of the compound.

Melting points (m.p.) were measured on a Fisher-Johns melting point apparatus and uncorrected. Optical rotations were measured on a Rudolph Research Autopol II automatic polarimeter.

Analytical thin-layer chromatography (T.l.c.) was performed on precoated aluminum plates with Kieselgel silica gel 60 F_{254} (E. Merck) and detected with u.v. light and/or charred with 5% sulfuric acid in ethanol solution. All compounds were purified by medium-pressure column chromatography or flash column chromatography with Kieselgel silica gel 60 (230-400mesh) according to published procedures[57].

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

Methyl-^{13}C iodide (99%-^{13}C) was purchased from Sigma company.

II. Specific procedures

A. General synthesis

1. Residue A (Glucose ring)

*1,2-O-isopropylidene-α-**D**-3,6-glucofuranolactone* (**2**).--- A mixture of **D**-glucurono-3,6-lactone (**1**) (12g, 69mmol) and concentrated sulfuric acid (5ml) in dried acetone (300ml) was stirred for 12h at room temperature. Anhydrous potassium carbonate was added to neutralize the acid. The excess potassium carbonate was removed by filtration and the filtrate was concentrated to give a yellowish syrup (14.8g, 99%). Recrystallization of the syrup from diethyl ether-petroleum ether yielded the *title compound* (**2**) as white needle crystals (11.9g, 81%). m.p. 118-119°C, $[\alpha]_D^{22}$ +82° (c 1.1, CH_2Cl_2) [lit[3]. m.p. 118-120°C, $[\alpha]_D$ +70° (c 1, acetone:methanol 1:1)]. ^{1}H-NMR: δ5.88(d, 1H, $J_{1,2}$=3.5Hz, H-1), 4..94(dd, 1H, $J_{4,5}$=3.0Hz, $J_{4,3}$=4.5Hz, H-4), 4.83(d, 1H, $J_{5,4}$=3.0Hz, H-5), 4.82(d, 1H, $J_{2,1}$=3.5Hz, H-2), 4.52(d, 1H, $J_{3,4}$=4.5Hz, H-3), 1.53(s, 3H, $(CH_3)_2C$), 1.36 (s, 3H, $(CH_3)_2C$). ^{13}C-NMR: δ173.7(C=O), 113.6$(CH_3)_2C$), 106.7(C-1), 82.9(C-2), 81.3(C-3), 78.1(C-4), 70.6(C-5), 26.9($(CH_3)_2C$), 26.5($(CH_3)_2C$).

*5-O-Benzoxymethyl-1,2-O-isopropylidene-α-**D**-3,6-glucofuranolactone* (**3**).--- Benzyl chloromethyl ether (9.5ml, 68 mmol) was added dropwise under nitrogen into dried dichloromethane (60ml) which contained 1,2-O-isopropylidine-α-**D**-

3,6-glucofuranolactone (**2**) (6.0g, 28mmol) and collidine (40ml, 300mmol). After refluxing for 12h, the mixture was cooled, diluted with dichloromethane, and washed successively with water, 10% hydrochloric acid aqueous solution, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated to give a reddish syrup. Recrystallization from petroleum ether-diethyl ether, yielded the *title compound* (**3**) as white crystals (6.4g, 68%). m.p. 96-97°C [lit[3]. m.p. 96-97°C], $[\alpha]_D^{22}$ +33.6° (c 0.7, CH_2Cl_2). ^{1}H-NMR: δ7.34(m, 5H, Ar), 6.04(d, 1H, $J_{1,2}$=4Hz, H-1), 5.03(dd, 2H, J=7.5Hz, OCH_2O), 4.90(dd, 1H, $J_{4,3+4,5}$=7.5Hz, H-4), 4.81(d, 1H, $J_{2,1}$=4Hz, H-2), 4.80(d, 1H, J=11Hz, OC*H*HPh), 4.76(d, 1H, $J_{5,4}$=3Hz, H-5), 4.69(d, 1H, J=11Hz, OCH*H*Ph), 4.52(d, 1H, $J_{3,4}$=4Hz, H-3), 1.50(s, 3H, $(CH_3)_2C$), 1.35(s, 3H, $(CH_3)_2C$). ^{13}C-NMR: δ171.6(C=O), 137.1, 128.4, 128.3, 127.9(Ar), 113.1($(CH_3)_2C$), 106.9(C-1), 94.9(OCH_2O), 82.5(C-2), 81.7(C-3), 77.8(C-4), 73.6(C-5), 70.5(OCH_2Ph), 26.8($(CH_3)_2C$), 26.4($(CH_3)_2C$).

5-O-Benzoxymethyl-1,2-O-isopropylidene-α-D-glucofuranose (**4**).--- A solution of 5-O-benzoxymethyl-1,2-O-isopropylidene-α-**D**-3,6-glucofuranolactone (**3**) (3.2g, 9.4mmol) in diethyl ether (150ml) was transferred by means of a cannula under nitrogen to a flask containing fresh lithium aluminum hydride ($LiAlH_4$) (1.1g, 30mmol). The mixture was refluxed under nitrogen for 1h, then cooled in an ice-water bath. Water (1ml) was added dropwise to the mixture to destroy excess $LiAlH_4$. When evolution of

bubbles had ceased, the inorganic residue was filtered and washed with diethyl ether. The filtrate was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave white solid (3.0g, 95%). Recrystallization from ethyl acetate-hexane yielded the *title compound* (**4**) as white needles. m.p. 48-50°C, $[\alpha]_D^{22}$ +10° (c 1, CH_2Cl_2). ^{1}H-NMR: δ7.34(m, 5H, Ar), 5.89(d, 1H, $J_{1,2}$=4Hz, H-1), 4.86(dd, 2H, J=7Hz, OCH_2O), 4.65(AB pattern, 2H, J=11Hz, OCH_2Ph), 4.49(d, 1H, $J_{2,1}$=4Hz, H-2), 4.27(d, 1H, $J_{3,4}$=2Hz, H-3), 4.14(dd, 1H, $J_{4,3}$=2Hz, $J_{4,5}$=8Hz, H-4), 3.95(m, 1H, H-5), 3.87(dd, 1H, $J_{6a,6b}$=12Hz, $J_{6a,5}$=5Hz, H-6a), 3.72(dd, 1H, $J_{6b,6a}$=12Hz, $J_{6b,5}$=3Hz, H-6b), 2.15-1.80(br., 2H, 2xO*H*), 1.25(s, 3H, $(CH_3)_2C$), 1.15(s, 3H, $(CH_3)_2C$). ^{13}C-NMR: δ136.6, 128.6, 128.2, 127.9(Ar), 111.7($(CH_3)_2C$), 104.9(C-1), 95.7(OCH_2O), 84.9(C-2), 79.6(C-4), 78.0(C-5), 74.9(C-3), 70.5(OCH_2Ph), 63.5(C-6), 26.8($(CH_3)_2C$), 26.1($(CH_3)_2C$).

5-O-Benzoxymethyl-1,2-O-isopropylidene-3,6-di-O-methyl-α-D-glucofuranose (**5**).--- 5-O-Benzoxymethyl-1,2-O-isopropylidene-α-D-glucofuranose (**4**) (6.6g, 19mmol) in N,N-dimethyformamide (50ml) was transferred by means of a cannula under nitrogen to a cooled solution of sodium hydride (3.4g, 85mmol, 60% in mineral oil) in N,N-dimethylformamide (10ml). After stirring for 30min in an ice bath, methyl iodide (3ml, 48mmol) was added to the mixture, and the reaction was left at room temperature for 2h. Methanol (5ml) was added to remove excess sodium hydride. The

solution was poured into ice-water and extracted with dichloromethane. The extracts were dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:3 gave the *title compound* (**5**) as a syrup (8.9g, 100%). $[\alpha]_D^{22}$ +0.8° (c 1.3, CH_2Cl_2). ^{1}H-NMR: δ7.36(m, 5H, Ar), 5.88(d, 1H, $J_{1,2}$=4Hz, H-1), 4.88(dd, 2H, J=7Hz, OCH_2O), 4.73(d, 1H, J=12Hz, OC*H*HPh), 4.61(d, 1H, J=12Hz, OCH*H*Ph), 4.58(d, 1H, $J_{2,1}$=4Hz, H-2), 4.30(dd, 1H, $J_{4,3}$=3Hz, $J_{4,5}$=9.5Hz, H-4), 4.05(m, 1H, $J_{5,4}$=9.5Hz, $J_{5,6a}$=2Hz, $J_{5,6b}$=4Hz, H-5), 3.79(dd, 1H, $J_{3,4}$=3Hz, H-3), 3.76(dd, 1H, $J_{6a,6b}$=11Hz, $J_{6a,5}$=2Hz, H-6a), 3.60(dd, 1H, $J_{6b,6a}$=11Hz, $J_{6b,5}$=4Hz, H-6b), 3.39(s, 3H, OCH_3), 3.37(s, 3H, OCH_3), 1.48(s, 3H, $(CH_3)_2C$), 1.32(s, 3H, $(CH_3)_2C$). ^{13}C-NMR: δ138.0, 128.3, 127.8, 127.6(Ar), 111.7($(CH_3)_2C$), 105.0(C-1), 94.7(OCH_2O), 83.5(C-3), 81.0(C-2), 78.6(C-4), 73.0(C-6), 73.7(C-5), 69.6(OCH_2Ph), 59.3(OCH_3), 57.2(OCH_3), 26.7($(CH_3)_2C$), 26.3($(CH_3)_2C$).

3,6-Di-O-methyl-α,β-D-glucopyranose (**6**).--- 5-O-Benzoxymethyl-1,2-O-isopropylidene-3,6-di-O-methyl-α-**D**-glucofuranose (**5**) (8.9g, 25mmol) was refluxed for 1h with 0.5N hydrochloric acid (60ml) in dioxane (40ml). Toluene was added to coevaporate the solvents. Column chromatography with ethyl acetate-hexane-methanol 4:4:1.5 yielded the *title compound* (**6**) as white crystals (10.9g, 100%). m.p. 125-130°C (α,β mixture) [lit[3]. m.p. 112-115°C]. ^{13}C-NMR(acetone-d_6) (α-anomer): δ93.7(C-

1), 85.2(C-3), 73.8, 73.4, 71.7, 71.5(C-2,4,5,6), 60.8(OCH_3), 59.1(OCH_3).

1,2,4-Tri-O-acetyl-3,6-di-O-methyl-α,β-D-glucopyranose (**7**).--- 3,6-Di-O-methyl-α,β-D-glucopyranose (**6**) (5.6g, 27mmol) was stirred with acetic anhydride (25ml) and pyridine (50ml) for 24h at room temperature. The mixture was poured onto ice-water and extracted with dichloromethane. The extracts were washed successively with 10% hydrochloric acid, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:2 as eluant gave a syrup (5.3g, 59%). Recrystallization from ethyl acetate-hexane solution yielded the *title compound* (**7**) as white crystals. m.p. 63-65°C (α,β mixture), 108.5-109.5°C (β form), $[\alpha]_D^{22}$ +8.6° (c 1, CH_2Cl_2, β form)[lit[4]. m.p. 64-66°C, $[\alpha]_D^{25}$ +49.7° (c 1, $CHCl_3$)]. ^{1}H-NMR(β form): δ5.64(d, 1H, $J_{1,2}$=8Hz, H-1), 5.08(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2), 5.06(t, 1H, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4), 3.66(m, 1H, $J_{5,4}$=10Hz, $J_{5,6a}$=3Hz, $J_{5,6b}$=5Hz, H-5), 3.53(t, 1H, $J_{3,2}$=10Hz, $J_{3,4}$=10Hz, H-3), 3.49(dd, 1H, $J_{6a,6b}$=11Hz, $J_{6a,5}$=3Hz, H-6a), 3.41(dd, 1H, $J_{6b,6a}$=11Hz, $J_{6b,5}$=5Hz, H-6b), 3.41(s, 3H, OCH_3), 3.32(s, 3H, OCH_3), 2.55(s, 3H, CH_3CO), 2.41(s, 3H, CH_3CO), 2.43(s, 3H, CH_3CO). ^{13}C-NMR(β form): δ169.3(C=O), 169.1(C=O), 169.1(C=O), 92.1(C-1), 81.3(C-3), 74.2(C-2), 71.2(C-5), 71.2(C-4), 69.5(C-6), 59.4(OCH_3), 58.8(OCH_3), 20.8(CH_3CO), 20.7(CH_3CO).

2,4-Di-O-acetyl-3,6-di-O-methyl-α-D-glucopyranosyl trichloroacetimidate (**9**).--- 1,2,4-Tri-O-acetyl-3,6-di-O-methyl-α,β-D-glucopyranose (**7**) (0.76g, 2.2mmol) and hydrazine acetate (0.25g, 2.8mmol) were stirred for 24h in N,N-dimethylformamide (12ml) under nitrogen. The mixture was washed with water, and extracted with dichloromethane. The extracts were dried over sodium sulfate and concentrated to give a syrup, 2,4-di-O-acetyl-3,6-di-O-methyl-α,β-D-glucose (**8**) (crude, 0.91g). After drying overnight under high vacuum, (**8**) was dissolved in dichloromethane (50ml) and stirred with anhydrous potassium carbonate (3.1g, 23mmol), and trichloroacetonitrile (3.2ml, 32mmol) for 48h. The remaining potassium carbonate was removed by filtration and the filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:3 as eluant gave the *title compound* (**9**) as a colorless syrup (0.97g, 46%). ^{1}H-NMR: δ8.60(s, C=NH), 6.48(d, 1H, $J_{1,2}$=4Hz, H-1), 5.08(dd, 1H, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4), 4.98(dd, 1H, $J_{2,1}$=4Hz, $J_{2,3}$=10Hz, H-2), 4.01(m, 1H, H-5), 3.80(t, 1H, $J_{3,2}$=10Hz, $J_{3,4}$=10Hz, H-3), 3.46(dd, 1H, $J_{6a,5}$=3Hz, $J_{6a,6b}$=11Hz, H-6a), 3.43(dd, 1H, $J_{6',5}$=5Hz, $J_{6b,6a}$=11Hz, H-6b), 3.46(s, 3H, OCH_3), 3.30(s, 3H, OCH_3), 2.11(s, 3H, CH_3CO), 2.02(s, 3H, CH_3CO). ^{13}C-NMR: δ169.7(C=O), 169.3(C=O), 160.8(*C*=NH), 93.5(C-1), 91.0(CCl_3), 78.2(C-3), 71.7(C-2), 71.6(C-5), 71.2(C-6), 69.8(C-4), 59.9(OCH_3), 59.3(OCH_3), 20.7(*C*H_3CO), 20.5(*C*H_3CO).

2. Residue B, C (Rhamnose rings)

Allyl 4-O-benzyl-α-L-rhamnopyranoside (**14**).--- A mixture of α-**L**-rhamnose monohydrate (**10**) (15g, 84mmol), allyl alcohol (120ml), and trifluoromethanesulfonic acid (1.2ml) was refluxed for 4h. After the mixture was cooled, triethylamine (3ml) was added to neutralize the acid. Coevaporation with toluene gave a yellowish residue, allyl α-**L**-rhamnopyranoside (**11**). Compound (**11**) was dried overnight under high vacuum and dissolved in dry acetone (140ml). 2,2-Dimethoxypropane (35ml) and p-toluenesulfonic acid (0.23g) was added to the solution and the mixture was stirred at room temperature. After 3h, triethylamine (1.5ml) was added to terminate the reaction. The mixture was concentrated to give a yellowish syrup, allyl 2,3-O-isopropylidene-α-**L**-rhamnopyranoside (**12**). The dried syrup (**12**) was dissolved in freshly distilled N,N-dimethylformamide (95ml) and was transfered slowly by means of cannula under nitrogen to a suspension of sodium hydride (7.4g, 0.19mol, 60% in mineral oil) in N,N-dimethylformamide (30ml). After cooling in an ice-bath for 30min, benzyl bromide (14ml, 118mmol) was added dropwise to the reaction mixture. After 22h at room temperature, methanol (30ml) was added to destroy excess sodium hydride. When evolution of bubbles had ceased, the mixture was poured into ice water (450ml). The mixture was extracted with ethyl acetate, the extracts were dried over sodium sulfate and concentrated to give a syrup, allyl 4-O-benzyl-2,3-O-isopropylidene-α-**L**-rhamnopyranoside (**13**). Compound (**13**) was refluxed for 1h with

hydrochloric acid (0.5N, 140ml) in ethanol (150ml), after which t.l.c. showed that hydrolysis was complete. The cooled mixture was neutralized with solid potassium carbonate and extracted with ethyl acetate. The extracts were dried with sodium sulfate and concentrated to give a brown syrup. Separation by preparative h.p.l.c. with ethyl acetate-hexane 1:3 as eluant gave a colorless syrup. Recrystallization from ethyl acetate-hexane yielded the *title compound* (**14**) as white crystals (12g, 50%). m.p. 66-67°C, $[\alpha]_D^{22}$ -72.5° (c 1.1, CH_2Cl_2) [lit[52]. m.p. 66.0-67.5°C, $[\alpha]_D$ -75.2° (c 1.0, CH_2Cl_2)]. ^{1}H-NMR: δ7.35(m, 5H, Ar), 5.87(m, 1H, $OCH_2CH{=}CH_2$), 5.22(m, 2H, $OCH_2CH{=}CH_2$), 4.81(s, 1H, $J_{1,2}$=2Hz, H-1), 4.74(AB pattern, 2H, J=12Hz, OCH_2Ph), 4.06(m, 2H, $OCH_2CH{=}CH_2$), 3.94-3.92(m, 2H, overlapped, H-2 and H-3), 3.75(m, 1H, $J_{5,4}$=9.5Hz, H-5), 3.34(t, 1H, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4), 2.5-1.5(br., 2H, 2xOH), 1.35(d, 3H, H-6). ^{13}C-NMR: δ133.7($OCH_2CH{=}CH_2$), 128.6, 127.9(Ar), 117.3($OCH_2CH{=}CH_2$), 98.5(C-1), 81.8(C-4), 75.0(OCH_2Ph), 71.5(C-3), 71.2($OCH_2CH{=}CH_2$), 67.9(C-2), 67.2(C-5), 18.0(C-6).

Allyl 4-O-benzyl-2,3-di-O-methyl-α-L-rhamnopyranoside (**15**).--- A solution of allyl 4-O-benzyl-α-L-rhamnopyranoside (**14**) (3.2g, 11mmol) in N,N-dimethylformamide (20ml) was transferred by means of a cannula under nitrogen to a cooled suspension of sodium hydride (2.0g, 82mmol) in N,N-dimethylformamide (10ml). After stirring for 15min in an ice bath, methyl iodide (2ml, 32mmol) was added slowly to the mixture. The mixture was stirred

for 2h at room temperature, and methanol (5ml) was added to destroy the excess sodium hydride. The mixture was poured into ice-water and extracted with dichloromethane. The extracts were dried with sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:3 yielded the *title compound* (**15**) as a syrup (3.5g, 100%). $[\alpha]_D^{22}$ -67° (c 1.5, CH_2Cl_2). ^{1}H-NMR: δ7.35(m, 5H, Ar), 5.89(m, 1H, OCH_2CH=CH_2), 5.23(m, 2H, OCH_2CH=CH_2), 4.91(d, 1H, J=11Hz, OC*H*HPh), 4.89(d, 1H, $J_{1,2}$=2Hz, H-1), 4.60(d, 1H, J=11Hz, OCH*H*Ph), 4.05(m, 2H, OCH_2CH=CH_2), 3.68(m, 1H, $J_{5,4}$=9Hz, H-5), 3.62-3.60(m, 2H, overlapped, H-2 and H-3), 3.51(s, 6H, overlapped, 2xOCH_3), 3.43(t, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4), 1.32(d, 3H, H-6). ^{13}C-NMR: δ133.8(OCH_2CH=CH_2), 128.3, 127.9, 127.5(Ar), 117.3(OCH_2CH=CH_2), 96.0($J_{C,H}$=167Hz, C-1), 81.5(C-4), 80.4(C-3), 77.4(C-2), 75.2(OCH_2Ph), 67.8(overlapped, C-5 and OCH_2CH=CH_2) , 59.0(OCH_3), 57.7(OCH_3), 17.8(C-6).

Anal. Calc. for $C_{18}H_{26}O_5$: C, 67.05; H, 8.14. Found: C, 66.98; H, 8.24.

Propyl 2,3-di-O-methyl-α-L-rhamnopyranoside (**16**).--- Allyl 4-O-benzyl-2,3-di-O-methyl-α-L-rhamnopyranoside (**15**) (1.1g, 3.5mmol) in ethanol (15ml) and 80% aqueous acetic acid (30ml) was hydrogenolyzed overnight under hydrogen (52psi pressure) and Pd/C (0.10g) as catalyst. The black solid was removed by filtration and the filtrate was concentrated by coevaporation with toluene. Column chromatography with ethyl acetate-hexane

1:1 yielded the *title compound* (**16**) as a syrup (0.81g, 100%). $[\alpha]_D^{22}$ -32.4° (c 0.7, CH_2Cl_2). ^{1}H-NMR: δ4.81(d, 1H, $J_{1,2}$=2Hz, H-1), 3.61(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.58-3.57(m, 2H, overlapped, H-2 and H-5), 3.51(t, 1H, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4), 3.45(s, 3H, OCH_3), 3.43(s, 3H, OCH_3), 3.39(dd, 1H, $J_{3,4}$=9.5Hz, $J_{3,2}$=3Hz, H-3), 3.34(dt, 1H, $OCH_aH_bCH_2CH_3$), 1.57(m, 2H, $OCH_2CH_2CH_3$), 1.27(d, 3H, $J_{6,5}$=6Hz, H-6), 0.9(t, 3H, $OCH_2CH_2CH_3$). ^{13}C-NMR: δ97.2($^1J_{C,H}$=167Hz, C-1), 81.3(C-3), 76.1(C-2), 71.8(C-4), 69.2($OCH_2CH_2CH_3$), 68.1(C-5), 58.9(OCH_3), 56.9(OCH_3), 22.8(OCH_2CH_2CH3), 17.7(C-6), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{11}H_{22}O_5$: C, 56.38; H, 9.48. Found: C, 56.43; H, 9.52.

4-O-Benzyl-2,3-di-O-methyl-α,β-L-rhamnose (**17**).--- A mixture of allyl 4-O-benzyl-2,3-di-O-methyl-α-L-rhamnpyranoside (**15**) (2.1g, 6.7mmol), palladium(II) chloride (1.4g, 8.0mmol), and sodium acetate (1.6g, 20mmol) in 95% aqueous acetic acid solution (120ml) was stirred for 48h. After filtration, the filtrate was extracted with dichloromethane and the extracts were washed successively with water, saturated sodium bicarbonate solution, saturated sodium chloride solution, then dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant yielded a syrup (1.7g, 99%). Recrystallization from ethyl acetate-hexane 1:8 gave white crystals. ^{1}H-NMR showed the crystals were a mixture of anomers, (α:β=2:1). m.p. 57.5-58.5°C, $[\alpha]_D^{22}$ -34° (c

0.9, CH_2Cl_2). ^{1}H-NMR: δ7.32 (Ar), 5.26(H-1α), 4.65(H-1β), 3.43(H-4α), 3.36(H-4β), 1.32(H-6β), 1.28(H-6α). ^{13}C-NMR: δ138.8, 128.4, 128.3, 127.9, 127.8, 127.6(Ar), 93.6(C-1β), 92.0(C-1α), 61.8(OCH_3-β), 59.2(OCH_3-α), 58.1(OCH_3-β), 57.8(OCH_3-α), 18.0(C-6α), 17.9(C-6β).

Anal. Calc. for $C_{15}H_{22}O_5$: C, 63.80; H, 7.87. Found: C, 63.70; H, 7.74.

1,4-Di-O-acetyl-2,3-di-O-methyl-α,β-L-rhamnopyranoside (**19**).--- 4-O-Benzyl-2,3-di-O-methyl-α,β-L-rhamnose (**17**) (1.6g, 6.0mmol) in ethanol (45ml), and 80% acetic acid (45ml) was stirred for 7h under hydrogen (52psi, pressure) and Pd/C (0.18g) as a catalyst. The black solid was removed by filtration and the filtrate was concentrated by coevaporation with toluene. Column chromatography with ethyl acetate-hexane-methanol 4:4:1 as eluant gave 2,3-di-O-methyl-α,β-L-rhamnose (**18**) as a syrup (1.1g, 90%). Compound (**18**) (0.88g, 4.6mmol) was stirred with acetic anhydride (12ml) and pyridine (25ml) for 24h at room temperature. The mixture was poured into ice water and extracted with dichloromethane. The extracts were washed successively with 10% hydrochloric acid, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave the *title compound* (**19**) as a syrup (0.97g, 76%), α:β=7:1. ^{1}H-NMR(α form): δ6.17(d, 1H, $J_{1,2}$=2Hz, H-1), 5.08(t, 1H, $J_{4,3}$=10Hz,

$J_{4,5}$=10Hz, H-4), 3.79(m, 1H, H-5), 3.61(dd, 1H, $J_{2,1}$=2Hz, $J_{2,3}$=3Hz, H-2), 3.54(dd, $J_{3,2}$=3Hz, $J_{3,4}$=10Hz, H-3), 3.52(s, 3H, OCH_3), 3.43(s, 3H, OCH_3), 2.65(s, 3H, CH_3CO), 2.42(s, 3H, CH_3CO), 1.91(d, 3H, $J_{6,5}$=4Hz, H-6). ^{1}H-NMR(β form): δ5.60(d, 1H, $J_{1,2}$=1Hz, H-1), 4.99(t, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4), 3.75(dd, 1H, $J_{2,1}$=1Hz, $J_{2,3}$=3Hz, H-2), 3.59(s, 3H, OCH_3), 3.39(s, 3H, OCH_3), 3.47(m, 1H, H-5), 3.28(dd, 1H, $J_{3,2}$=3Hz, $J_{3,4}$=10Hz, H-3), 2.13(s, CH_3CO), 2.05(s, CH_3CO), 1.20(d, 3H, H-6). ^{13}C-NMR(α form): δ169.7(C=O), 168.9(C=O), 91.3(C-1), 78.5(C-3), 76.0(C-2), 72.0(C-4), 69.2(C-5), 59.2(OCH_3), 57.8(OCH_3), 21.0(CH_3CO), 20.9(CH_3CO), 17.5(C-6). ^{13}C-NMR(β form): δ169.7(C=O), 169.1(C=O), 93.0(C-1), 81.3(C-3), 76.2(C-2), 72.1(C-4), 71.5(C-5), 61.4(OCH_3), 57.7(OCH_3), 29.6(CH_3CO), 20.9(CH_3CO), 17.4(C-6).

Allyl 4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**20**).--- Allyl 4-O-benzyl-α-L-rhamnopyranoside (**14**) (1.8g, 6.1mmol) was refluxed for 3h with dibutyltin oxide (1.8g, 7.2mmol) in benzene (80ml). After the mixture was cooled, toluene was added and the solvents were coevaporated to give a white syrup. The dried syrup was dissovled in N,N-dimethylformamide (8ml) and methyl iodide (0.42ml, 6.7mmol) was added dropwise. The mixture was stirred overnight at 35-40°C. Water was added and the mixture was extracted with dichloromethane. The organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave the *title compound* (**20**) as a syrup (1.3g, 71%). $[\alpha]_D^{22}$ -89° (c 0.9, CH_2Cl_2). ^{1}H-NMR:

δ7.36(m, 5H, Ar), 5.88(m, 1H, $OCH_2CH=CH_2$), 5.24(m, 2H, $OCH_2CH=CH_2$), 4.85(d, 1H, $J_{1,2}$=2Hz, H-1), 4.84(d, 1H, J=11Hz OCHHPh), 4.62(d, 1H, J=11Hz OCHHPh), 4.07(dd, 1H, $J_{2,1}$=2Hz, $J_{2,3}$=3.5Hz, H-2), 4.06(m, 2H, $OCH_2CH=CH_2$), 3.72(m, 1H, H-5), 3.59(dd, 1H, $J_{3,2}$=3.5Hz, $J_{3,4}$=9.5Hz, H-3), 3.50(s, 3H, OCH_3), 3.37(t, 1H, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4), 2.50-2.36(br., 1H, OH), 1.30(d, 3H, H-6). ^{13}C-NMR: δ133.9($OCH_2CH=CH_2$), 138.6, 128.4, 127.9, 127.7(Ar), 117.4($OCH_2CH=CH_2$), 98.3(C-1), 81.8, 80.0(C-3, C-4), 75.2(OCH_2Ph), 68.0(C-5), 67.9, 67.3(C-2, $OCH_2CH=CH_2$), 57.4(OCH_3), 17.9(C-6).

Anal. Calc. for $C_{17}H_{24}O_5$: C, 66.20; H, 7.86. Found: C, 66.42; H, 8.05.

3. Disaccharides

*Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside* (**21**).--- A mixture of dry propyl 2,3-di-O-methyl-α-**L**-rhamnopyranoside (**16**) (0.19g, 0.82mmol) and dry 2,4-di-O-acetyl-3,6-di-O-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**9**) (0.41g, 0.95mmol) in dichloromethane (20ml) was stirred with molecular sieves (4Å) for 1h under nitrogen. The mixture was cooled for 1h in a dry ice-acetone bath before triethylsilyl trifluoromethanesulfonate (0.03ml, 0.13mmol) was added. After stirring for 15min at -78°C, the mixture was left at room temperature for 15min. T.l.c. (dichloromethane-acetone 9:1) showed that the reaction was complete. Triethylamine (1 drop) was added to stop the reaction.

The molecular sieves were removed by filtration and the filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant yielded the *title compound* (**21**) as a syrup (0.43g, 100%). $[\alpha]_D^{22}$ -51° (c 0.7, CH_2Cl_2). ^{1}H-NMR: δ4.98(t, 1H, $J_{4,3}$=9Hz, $J_{4,5}$=9Hz, H-4_A), 4.90(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2_A), 4.82(d, 1H, $J_{1,2}$=2Hz, H-1_B), 4.74(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.61-3.51(m, 4H, H-$6a_A$, H-$6b_A$, H-5_B, $OCH_aH_bCH_2CH_3$), 3.56(dd, 1H, $J_{2,1}$=2Hz, $J_{2,3}$=3Hz, H-2_B), 3.48-3.30(m, 5H, H-3_B, H-4_B, H-3_A, H-5_A, $OCH_aH_bCH_2CH_3$), 3.48(s, 3H, OCH_3), 3.42(s, 3H, OCH_3), 3.38(s, 3H, OCH_3), 3.30(s, 3H, OCH_3), 3.34(dd, 1H, $OCH_aH_bCH_2CH_3$), 2.30(CH_3CO), 2.50(CH_3CO), 1.32(m, 2H, $OCH_2CH_2CH_3$), 1.13(d, 3H, H-6_B), 0.96($OCH_2CH_2CH_3$). ^{13}C-NMR: δ169.3(C=O), 168.9(C=O), 100.9($^1J_{C,H}$=167Hz, C-1_A), 96.6($^1J_{C,H}$=167Hz, C-1_B), 81.5, 81.3(C-3_B, C-4_B), 77.6, 76.7(C-2_B, C-6_A), 73.0, 72.4, 72.0(C-2_A, C-3_A, C-5_A), 70.2(C-4_A), 69.3($OCH_2CH_2CH_3$), 67.0(C-5_B), 59.6(OCH_3), 58.9(OCH_3), 58.3(OCH_3), 57.0(OCH_3), 22.6($OCH_2CH_2CH_3$), 20.9(CH_3CO), 20.8(CH_3CO), 17.7(C-6_B), 10.5($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{23}H_{40}O_{12}$: C, 54.33; H, 7.87. Found: C, 54.42; H, 8.03.

*Propyl 4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside* (**22**).--- Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside (**21**) (0.41g, 0.81mmol) was stirred for 2h in sodium methoxide-methanol (0.3N, 4ml). Aqueous hydrochloric acid (1ml, 1N) was added to neutralize the base. The aqueous solution

was extracted with dichloromethane and the extracts were washed with saturated sodium bicarbonate solution and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane-methanol 6:6:1 as eluant gave the *title compound* (**22**) as a syrup (0.29g, 85%). m.p. 52-54°C, $[\alpha]_D^{22}$ -53° (c 0.6, CH_2Cl_2) [lit[4]. $[\alpha]_D^{26}$ -46.1° (c 1.2, $CHCl_3$)]. ^{1}H-NMR: δ4.81(d, 1H, $J_{1,2}$=2Hz, H-1_B), 4.40(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.67(s, 3H, OCH_3), 3.64(H-5_B), 3.63(m, 2H, 2xH-6_A), 3.62(m, 3H, H-2_B, H-3_B, H-4_B), 3.59(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.52(t, 1H, $J_{4,3+4,5}$=18Hz, H-4_A), 3.48(s, 3H, OCH_3), 3.46(s, 3H, OCH_3), 3.41(m, 1H, H-5_A), 3.38(s, 3H, OCH_3), 3.15(t, 1H, $J_{3,2}$=10Hz, $J_{3,4}$=10Hz, H-3_A), 3.35(dt, 1H, $OCH_aH_bCH_2CH_3$), 1.57($OCH_2CH_2CH_3$), 1.34(d, 3H, H-6_B), 0.90($OCH_2CH_2CH_3$). ^{13}C-NMR: δ105.6($^1J_{C,H}$ =159Hz, C-1_A), 96.8($^1J_{C,H}$=168Hz, C-1_B), 85.6(C-3_A), 81.8(C-3_B), 80.7(C-4_B), 76.0(C-2_B), 75.0(C-2_A), 74.2(C-5_A), 72.8(C-6_A), 71.2(C-4_A), 69.3($OCH_2CH_2CH_3$), 67.5(C-5_B), 60..4(OCH_3), 59.5(OCH_3), 59.0(OCH_3), 56.4(OCH_3), 22.7($OCH_2CH_2CH_3$)), 17.5(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{19}H_{36}O_{10}$: C, 53.75; H, 8.56. Found: C, 53.64; H, 8.62.

Allyl 2-O-(4-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranosyl)-4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**23**).--- The mixture of dry allyl 4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**20**) (0.33g, 1.1mmol) and dry 1,4-di-O-acetyl-2,3-di-O-methyl-α,β-L-rhamnopyranoside (**19**) (0.33g, 1.2mmol) was stirred for 1h in

dichloromethane (50ml) containing molecular sieves (4Å), under nitrogen. Boron trifluoride (BF_3) in diethyl ether (0.06ml, 45%, 0.22mmol) was added and the mixture was stirred. After 16h at room temperature, triethylamine (1 drop) was added to terminate the reaction, the molecular sieves were removed by filtration and the filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave a syrup (0.54g, 97%). Recrystallization from ethanol-hexane gave the *title compound* (**23**) as white crystals. m.p. 109-110°C, $[\alpha]_D^{22}$ -65.6° (c 0.7, CH_2Cl_2). ^{1}H-NMR: δ7.36(m, 5H, Ar), 5.87(m, 1H, OCH_2CH=CH_2), 5.23(m, 2H, OCH_2CH=CH_2), 5.10(d, 1H, $J_{1,2}$=2Hz, H-1_B), 5.03(t, 1H, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4_B), 4.88(d, 1H, J=11Hz OCHHPh), 4.77(d, 1H, $J_{1,2}$=2Hz, H-1_C), 4.63(d, 1H, J=11Hz OCHHPh), 4.06(dd, 1H, $J_{2,3}$=3Hz, $J_{2,1}$=2Hz, H-2_C), 4.04(m, 2H, OCH_2CH=CH_2), 3.72(dd, 1H, $J_{2,3}$=3Hz, $J_{2,1}$=2Hz, H-2_B), 3.76(m, H-5_B), 3.69(m, H-5_C), 3.62(dd, 1H, $J_{3,2}$=3Hz, $J_{3,4}$=10Hz, H-3_C), 3.56(dd, 1H, $J_{3,2}$=3Hz, $J_{3,4}$=10Hz, H-3_B), 3.52(s, 3H, OCH_3), 3.48(s, 3H, OCH_3), 3.44(s, 3H, OCH_3), 3.37(t, 1H, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4_C), 2.05(s, 3H, CH_3CO), 1.30(d, 3H, H-6_C), 1.15(d, 3H, H-6_B). ^{13}C-NMR: δ169.9(C=O), 133.8(OCH_2CH=CH_2), 138.6, 128.4, 128.0, 127.7(Ar), 117.4(OCH_2CH=CH_2), 99.2($^1J_{C,H}$=171Hz, C-1_B), 98.1($^1J_{C,H}$=170Hz, C-1_C), 81.9(C-3_C), 80.2(C-4_C), 78.7(C-3_B), 75.0(OCH_2Ph), 77.1(C-2_B), 74.1(C-2_C), 73.1(C-4_B), 67.9, 67.8, 67.1(C-5_B, C-5_C, OCH_2CH=CH_2), 59.1(OCH_3), 57.9(OCH_3), 57.8(OCH_3), 21.0(CH_3CO), 17.9(C-6_C), 17.5(C-6_B).

Anal. Calc. for $C_{27}H_{40}O_{10}$: C, 61.80; H, 7.70. Found: C, 62.01; H, 7.85.

*Allyl 2-O-(2,3-di-O-methyl-α-**L**-rhamnopyranosyl)-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside* (**24**).--- Allyl 2-O-(4-O-acetyl-2,3-di-O-methyl-α-**L**-rhamnopyranosyl)-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside (**23**) (0.40g, 0.76mmol) was stirred for 1h in sodium methoxide-methanol (0.3N, 10ml). Aqueous hydrochloric acid (3ml, 1N) was added to neutralize the base and the aqueous solution was extracted with dichloromethane. The extracts were washed with saturated sodium bicarbonate and saturated sodium chloride after which the organic layer was dried over sodium sulfate and concentrated. Chromatography with ethyl acetate-hexane-methanol 4:8:1 gave the *title compound* (**24**) as a syrup (0.24g, 66%). $[\alpha]_D^{22}$ -62.8° (c 0.8, CH_2Cl_2). ^{1}H-NMR: δ7.32(m, 5H, Ar), 5.87(m, 1H, $OCH_2CH{=}CH_2$), 5.22(m, 2H, $OCH_2CH{=}CH_2$), 5.13(d, 1H, $J_{1,2}$=1.5Hz, H-1_B), 4.87(d, 1H, J=11Hz, OC*H*HPh), 4.78(d, 1H, $J_{1,2}$=1.5Hz, H-1_C), 4.63(d, 1H, J=11Hz, OCH*H*Ph), 4.07(dd, 1H, $J_{2,3}$=3Hz, $J_{2,1}$=1.5Hz, H-2_C), 4.04(m, 2H, $OCH_2CH{=}CH_2$), 3.73(dd, 1H, $J_{2,3}$=3Hz, $J_{2,1}$=1.5Hz, H-2_B), 3.68(m, 2H, overlapped, H-5_B, H-5_C), 3.62(dd, $J_{3,4}$=9.5Hz, $J_{3,2}$=3Hz, H-3_C), 3.56(t, 1H, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4_B), 3.50(s, 3H, OCH_3), 3.49-3.48(2xs, 6H, overlapped, 2xOCH_3), 3.45(dd, $J_{3,2}$=3Hz, $J_{3,4}$=9.5Hz, H-3_B), 3.36(t, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4_C), 1.30(d, 3H, H-6_C), 1.29(d, 3H, H-6_B). ^{13}C-NMR: δ133.8($OCH_2CH{=}CH_2$), 138.7, 128.3, 128, 127.7(Ar), 117.4($OCH_2CH{=}CH_2$), 98.9($^1J_{C,H}$=163Hz, C-1_B),

98.1($^1J_{C,H}$=165Hz, C-1_C), 82.0(C-3_C), 80.9(C-3_B), 80.2(C-4_C), 76.0(C-2_B), 75.0(OCH_2Ph), 73.7(C-2_C), 71.7(C-4_B), 68.7, 67.9, 67.8(C-5_B, C-5_C, $OCH_2CH=CH_2$), 58.8(OCH_3-2_B), 57.9(OCH_3-3_C), 57.0(OCH_3-3_B), 18.0, 17.7(C-6_B, C-6_C).

Anal. Calc. for $C_{25}H_{38}O_9$: C, 62.21; H, 7.95. Found: C, 62.00; H, 8.03.

4. Trisaccharides

*Allyl 2-O-[4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside* (**25**).--- A mixture of dry allyl 2-O-(2,3-di-O-methyl-α-**L**-rhamnopyranosyl)-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside (**24**) (0.21g, 0.44mmol) and dry 2,4-di-O-acetyl-3,6-di-O-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**9**) (0.24g, 0.54mmol) in dichloromethane (20ml) containing molecular sieves (4Å) was stirred for 5h under nitrogen. The solution was cooled in a dry ice-acetone bath for 1h and triethylsilyl trifluoromethanesulfonate (0.02ml, 0.08mmol) was added. After stirring for 20min at -78°C, the reaction was left for 30min at room temperature. Triethylamine (1 drop) was added to terminate the reaction. The molecular sieves were removed by filtration and the filtrate was concentrated. Column chromatography with ethyl acetate-hexane-methanol 4:8:1 gave the *title compound* (**25**) as a syrup (0.37g, 100%). $[\alpha]_D^{22}$ -63.7° (c 0.3, CH_2Cl_2). ^{1}H-NMR: δ7.35(m, 5H, Ar), 5.87(m, 1H, $OCH_2CH=CH_2$), 5.23(m, 2H, $OCH_2CH=CH_2$), 5.17(d, $J_{1,2}$=2Hz, H-1_B), 4.98(t, 1H,

$J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4_A), 4.89(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2_A), 4.86(d, 1H, J=11Hz, OC*H*HPh), 4.76(d, 1H, $J_{1,2}$=8Hz, H-1_A), 4.74(d, 1H, $J_{1,2}$=2Hz, H-1_C), 4.64(d, 1H, J=11Hz, OCH*H*Ph), 4.11(m, 2H, OC*H*$_2$CH=CH$_2$), 4.10(dd, 1H, $J_{2,1+2,3}$=5Hz, H-2_C), 3.73-3.56(m, 6H, overlapped, 2xH-6_A, H-2_B, H-5_B, H-3_C, H-5_C), 3.50-3.47(m, 2H, overlapped, H-3_B, H-4_B), 3.50(s, 3H, OCH$_3$), 3.48(s, 3H, OCH$_3$), 3.47(s, 3H, OCH$_3$), 3.38(s, 3H, OCH$_3$), 3.32(s, 3H, OCH$_3$), 3.40-3.38(m, 2H, overlapped, H-3_A, H-5_A), 3.37(t, 1H, $J_{4,3+4,5}$=18Hz, H-4_C), 2.70(s, 3H, CH$_3$CO), 2.40(s, 3H, CH$_3$CO), 1.32, 1.25(H-6_B, H-6_C). ^{13}C-NMR: δ169.6(C=O), 169.1(C=O), 133.8(OCH$_2$*C*H=CH$_2$), 138.7, 128.3, 128.0, 127.7(Ar), 117.5(OCH$_2$CH=*C*H$_2$), 100.9(C-1_A), 98.2, 98.1(C-1_B, C-1_C), 82.3(C-3_C), 81.5, 80.9(C-3_B, C-4_B), 80.2(C-4_C), 77.5, 76.9(C-2_B, C-6_A), 75.0(O*C*H$_2$Ph), 73.1, 72.8, 72.3, 72.2(C-2_A, C-3_A, C-5_A, C-2_C), 70.2(C-4_A), 67.9, 67.8, 67.7(C-5_B, C-5_C, O*C*H$_2$CH=CH$_2$), 59.6(OCH$_3$), 58.87(OCH$_3$), 58.1(OCH$_3$), 57.9(OCH$_3$), 57.3(OCH$_3$), 21.0(*C*H$_3$CO), 20.9(*C*H$_3$CO), 18.0, 17.8(C-6_B, C-6_C).

Anal. Calc. for $C_{37}H_{56}O_{16}$: C, 58.71; H, 7.47. Found: C, 58.49; H, 7.37.

*Allyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside* (**26**).--- Allyl 2-O-[4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside (**25**) (0.37g, 0.44mmol) was stirred for 2h with sodium methoxide-

methanol (6ml, 3N). Aqueous hydrochloric acid (2ml, 1N) was added to neutralize the base and the aqueous solution was extracted with dichloromethane. The extracts were washed with saturated sodium bicarbonate solution and saturated sodium chloride solution after which the organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane-methanol 4:4:1 as eluant gave the *title compound* (**26**) as a syrup (0.21g, 72%). $[\alpha]_D^{22}$ -62° (c 0.4, CH_2Cl_2). 7.43(m, 5H, Ar), 5.85(m, 1H, $OCH_2CH{=}CH_2$), 5.21(m, 2H, $OCH_2CH{=}CH_2$), 5.11(d, $J_{1,2}$=2Hz, H-1_B), 4.86(d, 1H, J=11Hz, OC*H*HPh), 4.72(d, 1H, $J_{1,2}$=2Hz, H-1_C), 4.62(d, 1H, J=11Hz, OCH*H*Ph), 4.40(d, 1H, $J_{1,2}$=8Hz, H-1_A), 4.00(m, 2H, $OCH_2CH{=}CH_2$), 4.04(dd, 1H, $J_{2,1+2,3}$=5Hz, H-2_C), 3.74(d, 1H, $J_{2,1+2,3}$=5Hz, H-2_B), 3.67-3.50(m, overlapped, 6H, 2xH-6_A, H-3_B, H-4_B, H-5_B, H-5_C), 3.67(H-3_C), 3.55(H-4_A), 3.44(t, 1H, H-2_A), 3.42(1H, H-5_A), 3.67(s, 3H, OCH_3), 3.50(s, 3H, OCH_3), 3.49(s, 3H, OCH_3), 3.48(s, 3H, OCH_3), 3.39(s, 3H, OCH_3), 3.32(t, 1H, $J_{4,3+4,5}$=18Hz, H-4_C), 3.19(t, 1H, $J_{3,4+3,2}$=18Hz, H-3_A), 1.32, 1.25(H-6_B, H-6_C). ^{13}C-NMR: δ133.8($OCH_2CH{=}CH_2$), 138.6, 128.3, 127.9, 127.7(Ar), 117.4($OCH_2CH{=}CH_2$), 105.5($^1J_{C,H}$=161Hz, C-1_A), 98.5($^1J_{C,H}$=171Hz, C-1_B), 98.0($^1J_{C,H}$=170Hz, C-1_C), 85.5(C-3_A), 82.0, 81.5, 80.3, 80.2(C-3_B, C-4_B, C-3_C, C-4_C), 75.9, 75.0(C-2_B, C-5_A), 74.0(OCH_2Ph), 73.8(C-2_A), 73.4(C-2_C), 72.9(C-6_A), 71.2(C-4_A), 68.1, 67.9, 67.8(C-5_B, C-5_C, $OCH_2CH{=}CH_2$), 60.3(OCH_3), 59.6(OCH_3), 58.8(OCH_3), 57.9(OCH_3), 56.4(OCH_3), 18.0, 17.5(C-6_B, C-6_C).

Anal. Calc. for $C_{33}H_{52}O_{14}$: C, 58.90; H, 7.81. Found: C, 58.60; H, 8.08.

*Propyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-3-O-methyl-α-**L**-rhamnopyranoside* (**27**).--- Allyl 2-O-[4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranosyl]-4-O-benzyl-3-O-methyl-α-**L**-rhamnopyranoside (**26**) (0.24g, 0.35mmol) in acetic acid (8ml, 80%) and ethanol (10ml) was stirred overnight under hydrogen (52 psi) and Pd/C (78mg) as a catalyst. The black solid was removed by filtration and the filtrate was concentrated by coevaporation with toluene. Column chromatography with ethyl acetate-hexane-methanol 4:4:1 as eluant gave the *title compound* (**27**) as a syrup (0.19g, 90%). $[\alpha]_D^{22}$ -46° (c 1, CH_2Cl_2). ^{1}H-NMR: 5.06(d, $J_{1,2}$=2Hz, H-1_B), 4.70(d, $J_{1,2}$=2Hz, H-1_C), 4.40(d, $J_{1,2}$=8Hz, H-1_A), 4.05(dd, $J_{2,1}$=2Hz, $J_{2,3}$=2.5Hz, H-2_C), 3.70(m, 2H, H-2_B, H-5_B), 3.67(s, 3H, OCH_3-3_A), 3.64(H-5_C), 3.62(H-4_B), 3.61(H-3_B), 3.53(t, 1H, H-4_A), 3.50(t, 1H, H-4_C), 3.48(s, 3H, OCH_3-2_B), 3.47(s, 3H, OCH_3-3_C), 3.46(s, 3H, OCH_3-3_B), 3..43(H-3_C), 3.40(m, H-2_A, H-5_A), 3.38(s, 3H, OCH_3-6_A), 3.15(t, J=9.5Hz, H-3_A), 3.59(dd, 1H, $OCH_aH_bCH_2CH_3$), 3.34(dd, 1H, $OCH_aH_bCH_2CH_3$), 3.58(m, 2H, 2xH-6_A), 1.59(m, 2H, $OCH_2CH_2CH_3$), 1.32, 1.25(H-6_B, H-6_C), 0.92(t, 3H, $OCH_2CH_2CH_3$). ^{13}C-NMR: δ105.6($^1J_{C,H}$=158Hz, C-1_A), 99.1($^1J_{C,H}$=169Hz, C-1_C), 98.3($^1J_{C,H}$=170Hz, C-1_B), 85.6(C-3_A), 81.9(C-3_C), 81.6(C-3_B), 80.3(C-4_B), 75.9(C-2_B), 75.1(C-2_A), 74.2(C-5_A), 72.9(C-6_A), 72.2(C-2_C), 72.0(C-4_C), 71.2(C-4_A), 69.2($OCH_2CH_2CH_3$),

68.2(overlapped, C-5_B, C-5_C), 60.4(OCH_3-3_A), 59.6(OCH_3-6_A), 59.0(OCH_3-2_B), 57.5(OCH_3-3_C), 56.5(OCH_3-3_B), 22.7($OCH_2CH_2CH_3$), 17.7(C-6_C), 17.6(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{26}H_{48}O_{14}$: C, 53.40; H, 8.29. Found: C, 53.20; H, 8.28.

B. ^{13}C-Labeled Compounds

5-O-Benzoxymethyl-1,2-O-isopropylidene-3,6-di-O-^{13}C-methyl-α-D-glucofuranose (**28**).--- 5-O-Benzoxymethyl-1,2-O-isopropylidene-α-D-glucofuranose (**4**) (2.4g, 6.9mmol) in N,N-dimethylformamide (10ml) was transferred by means of a cannula under nitrogen to a cooled suspension of sodium hydride (60% in mineral oil, 1.4g, 34mmol) in N,N-dimethylformamide (3ml). After stirring for 30min in an ice bath, methyl-^{13}C iodide (2g, 99%-^{13}C, 14mmol) was added dropwise. T.l.c. showed that the reaction was complete after stirring for 6h at room temperature. Methanol (4ml) was added to destroy excess sodium hydride. The mixture was poured into ice-water (50ml), extracted with dichloromethane, and the extracts were washed with water. The oganic layer was dried with sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:3 as eluant gave the *title compound* (**28**) as a syrup (2.4g, 93%). ^{1}H-NMR: δ7.36(m, 5H, Ar), 5.86(d, 1H, $J_{1,2}$=4Hz, H-1), 4.88(dd, 2H, J=7Hz, OCH_2O), 4.73(d, 1H, J=12Hz, OC*H*HPh), 4.61(d, 1H, J=12Hz, OCH*H*Ph), 4.58(d, 1H, $J_{2,1}$=4Hz, H-2), 4.30(dd, 1H, $J_{4,3}$=3Hz, $J_{4,5}$=9.5Hz, H-4), 4.05(m, 1H, $J_{5,6a}$=2Hz, $J_{5,6b}$=3Hz, H-5), 3.78(dd, 1H, $J_{3,4}$=3Hz, $^3J_{^{13}C,H-3}$=4.5Hz,

H-3), 3.76(dt, 1H, $J_{6a,6b}$=11Hz, $J_{6a,5+6a,^{13}C}$=5Hz, H-6a), 3.60(dt, 1H, $J_{6a,6b}$=11Hz, $J_{6b,5+6b,^{13}C}$=7Hz, H-6b), 3.39(d, 3H, $^{1}J_{^{13}C,H}$=141Hz, $O^{13}CH_3$), 3.37(d, 3H, $^{1}J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 1.50(s, 3H, $(CH_3)_2C$), 1.32(s, 3H, $(CH_3)_2C$). ^{13}C-NMR: δ138.0, 128.4, 127.8, 127.6(Ar), 111.8($(CH_3)_2C$), 105.1(C-1), 94.7(OCH_2O), 83.5(C-3), 81.0(C-2), 78.7(C-4), 73.8(C-5), 73.0(C-6), 69.6(OCH_2Ph), 59.3(qt, $^{1}J_{^{13}C,H}$=141Hz, $^{3}J_{^{13}C,H-3}$=3Hz, $O^{13}CH_3$-6), 57.2(qd, $^{1}J_{^{13}C,H}$ =142Hz, $^{3}J_{^{13}C,H-3}$=5Hz, $O^{13}CH_3$-3), 26.8($(CH_3)_2C$), 26.4($(CH_3)_2C$).

Anal. Calc. for $C_{17}{}^{13}C_2H_{28}O_7$: C, 61.59; H, 7.63. Found: C, 61.84; H, 7.68.

3,6-Di-O-^{13}C-methyl-α,β-D-glucopyranose (**29**).--- 5-O-Benzoxymethyl-1,2-O-isopropylidene-3,6-di-O-^{13}C-methyl-α-D-glucofuranose (**28**) (2.3g, 6.3mmol) was refluxed for 1h with 0.5N hydrochloric acid (16ml) in dioxane (13ml). Toluene was added and the solvents were codistilled. Column chromatography with ethyl acetate-hexane-methanol 4:4:1.5 gave the *title compound* (**29**) as a white solid (2.1g, 100%). ^{1}H-NMR(D_2O): 5.14(d, $J_{1,2}$=4Hz, H-1α), 4.58(d, $J_{1,2}$=8Hz, H-1β), 3.56(d, $^{1}J_{^{13}C,H}$=143Hz, $O^{13}CH_3$-α), 3.33(d, $^{1}J_{^{13}C,H}$=143Hz, $O^{13}CH_3$-α). ^{13}C-NMR(D_2O): δ98.7(C-1β), 94.8(C-1α), 88.0, 85.4, 77.1($^{3}J_{^{13}C,H}$=3Hz, C-3 or C-6), 76.2, 73.9, 73.8, 73.6, 72.8($^{3}J_{^{13}C,H}$=3Hz, C-6 or C-3), 72.0, 71.9, 62.7($O^{13}CH_3$), 62.4($O^{13}CH_3$), 61.5(2x$O^{13}CH_3$).

Anal. Calc. for $C_6{}^{13}C_2H_{16}O_6$: C, 45.70; H, 7.69. Found: C, 46.82; H, 7.86.

1,2,4-Tri-O-acetyl-3,6-di-O-^{13}C-methyl-α,β-D-glucopyranoside (**30**).--- 3,6-Di-O-^{13}C-methyl-α,β-D-glucopyranose (**29**) (2.1g, 6.3mmol) was stirred for 24h with acetic anhydride (10ml) and pyridine (20ml). The mixture was poured into ice-water and extracted with dichloromethane. The extracts were washed successively with 10% hydrochloric acid, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried with sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:2 as eluant gave the *title compound* (**30**) as a white solid (α,β mixture, 1.5g, 74%). The mixture was recrystallized from diethyl ether-petroleum ether. m.p. 65-66°C.

Anal. Calc. for $C_{12}{}^{13}C_2H_{22}O_9$: C, 49.99; H, 6.61. Found: C, 50.68; H, 6.70.

2,4-Di-O-acetyl-3,6-di-O-^{13}C-methyl-α-D-glucopyranosyl trichloroacetimidate (**32**).--- 1,2,4-Tri-O-acetyl-3,6-di-O-^{13}C-methyl-α,β-D-glucopyranoside (**30**) (0.55g, 1.6mmol) was stirred for 24h under nitrogen with hydrazine acetate (0.23g, 2.5mmol) in N,N-dimethylformamide (10ml). The mixture was washed with water and extracted with ethyl acetate. The extracts were dried over sodium sulfate and concentrated to give a syrup, 2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-α,β-D-glucopyranose (**31**) (crude, 0.57g). The dried compound (**31**) was stirred for 24h with anhydrous potassium carbonate (2.3g, 16mmol), and trichloroacetonitrile (2.5ml, 25mmol) in dichloromethane (40ml).

Excess potassium carbonate was removed by filtration through Celite 545, and the filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:3 as eluant gave the *title compound* (**32**) as a colorless syrup (0.41g, 64%). $[\alpha]_D^{22}$ +96.5° (c 0.48, CH_2Cl_2). ^{1}H-NMR: δ8.6(s, C=NH), 6.48(d, 1H, $J_{1,2}$=4Hz, H-1), 5.08(dd, 1H, $J_{4,3}$=10Hz, $J_{4,5}$=10Hz, H-4), 4.98(dd, 1H, $J_{2,1}$=4Hz, $J_{2,3}$=10Hz, H-2), 4.01(m, 1H, H-5), 3.80(tt, 1H, $J_{3,2}$=10Hz, $J_{3,4}$=10Hz, $^3J_{^{13}C,H-3}$=6Hz, H-3), 3.43(m, 2H, overlapped, 2xH-6), 3.46(d, 3H, $^1J_{^{13}C,H}$ =142Hz, O^{13}CH$_3$), 3.31(d, 3H, $^1J_{^{13}C-H}$=142Hz, O^{13}CH$_3$), 2.11(s, 3H, CH_3CO), 2.02(s, 3H, CH_3CO). ^{13}C-NMR: δ169.7(C=O), 169.4(C=O), 160.7(*C*=NH), 93.4($J_{C,H}$=179.9Hz, C-1), 90.9(CCl_3), 78.2(C-3), 71.6(C-2), 71.5(C-5), 71.2(C-6), 69.7(C-4), 59.9(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3}$=6Hz, O^{13}CH$_3$-3), 59.3(qt, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-6}$=3Hz, O^{13}CH$_3$-6), 20.8(*C*H$_3$CO), 20.5(*C*H$_3$CO).

Propyl 2-O-methyl-3-O-^{13}C*-methyl-*α*-**L**-rhamnopyranoside* (**35**).--- Allyl 4-O-benzyl-α-**L**-rhamnopyranoside (**14**) (1.6g, 5.6mmol) was refluxed for 3.5h with dibutyltin oxide (1.7g, 6.7mmol) in benzene (80ml). Toluene was added and the solvents were coevaporated to give a syrup. The dried syrup was dissolved in N,N-dimethylformamide (9ml) and methyl-^{13}C iodide (1g, 7.0mmol) was added dropwise to the mixture. The mixture was stirred overnight at 34-37°C, and then concentrated by removing N,N-dimethylformamide under high vacuum. Column chromatography with ethyl acetate-hexane 1:1 as eluant afforded allyl 4-O-benzyl-3-

O-^{13}C-methyl-α-**L**-rhamnopyranoside (**33**) as a syrup (0.94g, 54%). A solution of (**33**) (0.94g, 3.0mmol) in N,N-dimethylformamide (4.5ml) was transferred by means of a cannula under nitrogen to a cooled suspension of sodium hydride (0.31g, 7.9mmol) in N,N-dimethylformamide (2ml). After stirring for 30min in an ice bath, methyl iodide (0.41g, 6.1mmol) was added dropwise to the mixture. T.l.c. showed the reaction was complete after stirring for 2h under nitrogen. Methanol (2ml) was added to destroy excess sodium hydride. The mixture was poured into ice-water (10ml) and extracted with ethyl acetate. The extracts were washed with water, dried over sodium sulfate and concentrated to give allyl 4-O-benzyl-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside (**34**) as a syrup (1.1g, 100%). Compound (**34**) (1.1g, 3.0mmol) in ethanol (15ml) and 80% acetic acid (30ml) containing Pd/C (0.13g) was stirred overnight under hydrogen (52psi pressure). The black solid was filtered through Celite 545, the filtrate was diluted with dichloromethane, and washed successively with water, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant yielded the *title compound* (**35**) as a syrup (0.71g, 80%). $[\alpha]_D^{22}$ -33.6° (c 0.7, CH_2Cl_2). ^{1}H-NMR: δ4.85(d, 1H, $H_{1,2}$=2Hz, H-1), 3.61(m, 3H, overlapped, H-2, H-5, $OCH_aH_bCH_2CH_3$), 3.54(t, 1H, $J_{4,3}$=9.5Hz, $J_{4,5}$=9.5Hz, H-4), 3.48(s, 3H, OCH_3-2), 3.46(d, 3H, $J_{^{13}C,H}$=141Hz, $O^{13}CH_3$-3), 3.41(dt, 1H, $J_{3,4}$=9.5Hz, $J_{3,2}$=3.5Hz, $^3J_{^{13}C,H\text{-}3}$=3.5Hz, H-

3), 3.36(dt, 1H, $OCH_aH_bCH_2CH_3$), 2.4-2.3(br., 1H, O*H*), 1.60(m, 2H, $OCH_2CH_2CH_3$), 1.30(d, 3H, $J_{6,5}$=6Hz, H-6), 0.92(t, 3H, $OCH_2CH_2CH_3$). ^{13}C-NMR: δ97.2($^1J_{C,H}$=167Hz, C-1), 81.2(C-3), 76.1(C-2), 71.8(C-4), 69.2($OCH_2CH_2CH_3$), 68.1(C-5), 58.9($^1J_{C,H}$=142Hz, $^3J_{C,H-2}$=5Hz, OCH_3-2), 56.9(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3}$=4Hz, $O^{13}CH_3$-3), 22.8($OCH_2CH_2CH_3$), 17.7(C-6), 10.6($OCH_2CH_2CH_3$).

CI-MS. Calc. for $C_{10}{}^{13}C_1H_{22}O_5$: $M^{+\cdot}$, 235. Found: $M+1^{+\cdot}$, 236

Anal. Calc. for $C_{10}{}^{13}C_1H_{22}O_5$: C, 56.13; H, 9.44. Found: C, 56.30; H, 9.47.

Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-L-rhamnopyranoside (**36**).--- The mixture of dry propyl 2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside (**35**) (0.16g, 0.69mmol) and dry 2,4-di-O-acetyl-3,6-di-O-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**9**) (0.3g, 0.68mmol) in dichloromethane (12ml) containing molecular sieves (4Å) was stirred for 1h under nitrogen. The solution was cooled for 1h in a dry ice-acetone bath before triethylsilyl trifluoromethanesulfonate (0.014ml, 0.11mmol) was added. After stirring for 20min at -78°C, the solution was left for 20min at room temperature. T.l.c. showed that the reaction was complete. Triethylamine (2 drops) was added, the molecular sieves were filtered and washed with dichloromethane several times. The filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:1 gave the *title compound* (**36**) as a syrup (0.32g, 93%). ^{1}H-NMR: δ4.98(t, 1H, $J_{4,3}$=9Hz, $J_{4,5}$=9Hz, H-4_A),

4.90(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2_A), 4.82(d, 1H, $J_{1,2}$=2Hz, H-1_B), 4.74(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.59(m, 1H, H-5_B), 3.61(dd, 1H, $OCH_aH_bCH_2CH_3$), 3.56(dd, $J_{2,1}$=2Hz, $J_{2,3}$=3Hz, H-2_B), 3.56-3.51(m, overlapped, 2xH-6_A), 3.48-3.42(m, 3H, overlapped, H-3_B, H-4_B, H-5_A), 3.43(d, 3H, $^1J_{^{13}C,H}$=141Hz, $O^{13}CH_3$-3_B), 3.48(s, 3H, OCH_3), 3.38(s, 3H, OCH_3), 3.32(s, 3H, OCH_3), 3.44(t, $J_{3,2}$=10Hz, $J_{3,4}$=10Hz, H-3_A), 3.34(dd, 1H, $OCH_aH_bCH_2CH_3$), 2.30(CH_3CO), 2.50(CH_3CO), 1.32($OCH_2CH_2CH_3$), 1.13(d, 3H, H-6_B), 0.96($OCH_2CH_2CH_3$). ^{13}C-NMR: δ101.0(C-1_A), 96.7(C-1_B), 81.5, 81.4(C-3_B, C-4_B), 77.7, 76.8(C-2_B, C-6_A), 73.0, 72.5, 72.1(C-2_A, C-3_A, C-5_A), 70.3(C-4_A), 69.4($OCH_2CH_2CH_3$), 67.0(C-5_B), 59.6(OCH_3-6_A), 58.9(OCH_3-2_B), 58.2(OCH_3-3_A), 57.1($^1J_{^{13}C,H}$=141Hz, $^3J_{^{13}C,H}$-$_3$=4Hz, $O^{13}CH_3$-3_B), 22.7($OCH_2CH_2CH_3$), 21.0(CH_3CO), 20.8(CH_3CO), 17.8(C-6_B), 10.6($OCH_2CH_2CH_3$).

*Propyl 4-O-(3,6-di-O-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside* (**37**).--- Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside (**36**) (0.19g, 0.38mmol) was stirred for 1h in sodium methoxide-methanol solution (5ml, 0.3N) after which t.l.c. showed that deacetylation was complete. Aqueous hydrochloric acid (2ml, 1N) was added to neutralize the base and the aqueous solution was extracted with dichloromethane. The extracts were washed with saturated sodium bicarbonate solution and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Chromatography with

ethyl acetate-hexane-methanol 4:4:1 gave the *title compound* (**37**) as a syrup (0.16g, 100%). $[\alpha]_D^{22}$ -51.6° (c 1.2, CH_2Cl_2). ^{1}H-NMR: δ4.81(d, 1H, $J_{1,2}$=1.5Hz, H-1_B), 4.40(d, 1H, $J_{1,2}$=7.5Hz, H-1_A), 3.45(d, $^1J_{^{13}C,H}$ =142Hz, O^{13}CH$_3$-3_B), 3.66(s, 3H, OCH_3), 3.62(m, 7H, overlapped, 2xH-6_A, H-2_B, H-3_B, H-4_B, H-5_B, $OCH_aH_bCH_2CH_3$), 3.53(t, $J_{4,3+4,5}$=20Hz, H-4_A), 3.47(s, 3H, OCH_3), 3.46-3.37(m, 2H, overlapped, H-2_A, H-5_A), 3.37(s, 3H, OCH_3), 3.35(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.15(t, 1H, $J_{2,3}$=9.0HZ, $J_{3,4}$=9.0Hz, H-3_A), 1.57(m, 2H, $OCH_2CH_2CH_3$), 1.34(d, 3H, H-6_B), 0.90(t, 3H, $OCH_2CH_2CH_3$). ^{13}C-NMR: δ105.6($^1J_{C,H}$=161Hz, C-1_A), 96.9($^1J_{C,H}$=168Hz, C-1_B), 85.6(C-3_A), 81.9(C-3_B), 80.7(C-4_B), 76.1(C-2_B), 75.1(C-2_A), 74.2(C-5_A), 72.9(C-6_A), 71.2(C-4_A), 69.3($OCH_2CH_2CH_3$), 67.6(C-5_B), 60.4(OCH_3), 59.6(OCH_3), 59.0(OCH_3), 56.4(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3B}$=3Hz, O^{13}CH$_3$-3_B), 22.8($OCH_2CH_2CH_3$), 17.5(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{18}{}^{13}C_1H_{36}O_{10}$: C, 53.62; H, 8.54. Found: C, 53.49; H, 8.67.

*Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside* (**38**).--- The mixture of dry propyl 2,3-di-O-methyl-α-**L**-rhamnopyranoside (**16**) (55mg, 0.24mmol) and dry 2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**32**) (94mg, 0.22mmol) in dichloromethane (4ml) containing molecular sieves (4Å) was stirred for 1h under nitrogen. The solution was cooled for 1h in dry ice-acetone before triethylsilyl trifluoromethanesulfonate (0.008ml, 0.035mmol) was added. After

stirring for 20min at -78°C, the solution was left for 20min at room temperature. T.l.c. (dichloromethane-acetone 9:1) showed that the reaction was complete. Triethylamine (1 drop) was added, the molecular sieves were filtered and washed with dichloromethane several times. The filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave the *title compound* (**38**) as a syrup (78mg, 71%). $[\alpha]_D$ -50.3° (c 2.3, CH_2Cl_2). ^{1}H-NMR: δ4.98(t, 1H, $J_{4,3}$=9Hz, $J_{4,5}$=9Hz, H-4_A), 4.90(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2_A), 4.82(d, 1H, $J_{1,2}$=2Hz, H-1_B), 4.76(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.48(s, 3H, OCH_3), 3.38(d, 3H, $^1J_{^{13}C,H}$=141Hz, $O^{13}CH_3$), 3.32(d, 3H, $^1J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 3.49(s, 3H, OCH_3-2_B), 3.34(dt, 1H, $OCH_aH_bCH_2CH_3$), 2.10(s, 3H, CH_3CO), 2.08(s, 3H, CH_3CO), 1.59(m, 3H, $OCH_2CH_2CH_3$), 1.24(d, 3H, H-6_B), 0.92(t, 3H, $OCH_2CH_2CH_3$). ^{13}C-NMR: δ169.6(C=O), 169.3(C=O), 101(C-1_A), 96.6(C-1_B), 81.5, 81.4(C-3_B, C-4_B), 77.7, 76.8(C-2_B, C-6_A), 73.0, 72.4, 72.1(C-2_A, C-3_A, C-5_A), 70.7(C-4_A), 69.4, 67.0(C-5_B), 59.6(qt, $^1J_{^{13}C,H}$=141Hz, $^3J_{^{13}C,H\text{-}6A}$=4Hz, $O^{13}CH_3$-6_A), 58.9(OCH_3-2_B), 58.2(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H\text{-}3A}$=6Hz, $O^{13}CH_3$-3_A), 58.0(OCH_3-3_B), 22.7($OCH_2CH_2CH_3$), 21.0(CH_3CO), 20.9(CH_3CO), 17.8(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{21}{}^{13}C_2H_{40}O_{12}$: C, 54.10; H, 7.91. Found: C, 54.21; H, 8.09.

*Propyl 4-O-(3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-rhamnopyranoside* (**39**).---Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2,3-di-O-methyl-α-**L**-

rhamnopyranoside (**38**) (78mg, 0.15mmol) was stirred for 2h in sodium methoxide-methanol (3ml, 0.3N) after which t.l.c. showed that deacetylation was complete. Aqueous hydrochloric acid (1ml, 1N) was added to neutralize the base. The aqueous solution was extracted with dichloromethane and the extracts were washed with saturated sodium bicarbonate solution and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Chromatography with ethyl acetate-hexane-methanol 6:6:1 gave the *title compound* (**39**) as a syrup (58mg, 89%). ^{1}H-NMR: δ4.82(d, 1H, $J_{1,2}$=1.5Hz, H-1_B), 4.40(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.68~3.48(m, 6H, overlapped, 2xH-6_A, H-2_B, H-3_B, H-4_B, H-5_B), 3.59(dt, 1H, OCH_aH$_b$CH$_2$CH$_3$), 3.53(H-4_A), 3.41(m, 2H, overlapped, H-2_A, H-5_A), 3.67(d, 3H, $^1J_{^{13}C,H}$=142Hz, O^{13}CH$_3$), 3.38(d, 3H, $^1J_{^{13}C,H}$=142Hz, O^{13}CH$_3$), 3.48(s, 3H, OCH$_3$), 3.46(s, 3H, OCH$_3$), 3.35(dt, 1H, OCH$_a$H$_b$CH$_2$CH$_3$), 3.16(tt, 1H, $J_{3,2}$=9.0Hz, $J_{3,4}$=9.0Hz, $^3J_{^{13}C,H}$=5Hz, H-3_A), 1.57(OCH$_2$CH$_2$CH$_3$), 1.34(d, 3H, H-6_B), 0.91(OCH$_2$CH$_2$CH$_3$). ^{13}C-NMR: δ105.7($^1J_{C,H}$=160Hz, C-1_A), 96.9($^1J_{C,H}$=167Hz, C-1_B), 85.6(C-3_A), 81.9(C-3_B), 80.7(C-4_B), 76.0(C-2_B), 75.1(C-2_A), 74.1(C-5_A), 72.9(C-6_A), 71.3(C-4_A), 69.3(OCH$_2$CH$_2$CH$_3$), 67.5(C-5_B), 60.4(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3A}$=5Hz, O^{13}CH$_3$-3_A), 59.6(qt, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-6A}$=4Hz, O^{13}CH$_3$-6_A), 59.0(OCH$_3$-2_B), 56.4(OCH$_3$-3_B), 22.7(OCH$_2$CH$_2$CH$_3$), 17.5(C-6_B), 10.6(OCH$_2$CH$_2$CH$_3$).

Anal. Calc. for C$_{17}$^{13}C$_2$H$_{36}$O$_{10}$: C, 53.50; H, 8.52. Found: C, 53.70; H, 8.40.

*Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside* (**40**).--- The mixture of dry propyl 2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside (**35**) (58mg, 0.25mmol) and dry 2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-α-**D**-glucopyranosyl trichloroacetimidate (**32**) (0.11g, 0.25mmol) in dichloromethane (4.5ml) containing molecular sieves (4Å) was stirred for 1h under nitrogen. The solution was cooled for 1h in dry ice-acetone before triethylsilyl trifluoromethanesulfonate (0.0085 ml, 0.037mmol) was added. After stirring for 20min at -78°C, the solution was left for 20min at room temperature. T.l.c. (dichloromethane-acetone 9:1) showed that the reaction was complete. Triethylamine (1 drop) was added, the molecular sieves were filtered and washed with dichloromethane several times. The filtrate was concentrated. Column chromatography with ethyl acetate-hexane 1:1 as eluant gave the *title compound* (**40**) as a syrup (0.12g, 100%). $[\alpha]_D$ -51.5° (c 2.3, CH_2Cl_2). ^{1}H-NMR: δ4.98(t, 1H, $J_{4,3}$=9Hz, $J_{4,5}$=9Hz, H-4_A), 4.90(dd, 1H, $J_{2,1}$=8Hz, $J_{2,3}$=10Hz, H-2_A), 4.82(d, 1H, $J_{1,2}$=2Hz, H-1_B), 4.76(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.61(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.44(d, 3H, $^1J_{^{13}C-H}$=141Hz, $O^{13}CH_3$), 3.38(d, 3H, $^1J_{^{13}C,H}$=141Hz, $O^{13}CH_3$), 3.32(d, 3H, $^1J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 3.49(s, 3H, OCH_3-2_B), 3.34(dt, 1H, $OCH_aH_bCH_2CH_3$), 2.10(CH_3CO), 2.08(CH_3CO), 1.59($OCH_2CH_2CH_3$), 1.24(d, H-6_B), 0.92($OCH_2CH_2CH_3$). ^{13}C-NMR: δ169.6(C=O), 169.3(C=O), 101.0(C-1_A), 96.6(C-1_B), 81.5, 81.4(C-3_B, C-4_B), 77.7, 76.8(C-2_B, C-6_A), 73.0, 72.4, 72.1(C-2_A, C-3_A, C-5_A),

70.3(C-4_A), 69.4($OCH_2CH_2CH_3$), 67.0(C-5_B), 59.6(qt, $^1J_{^{13}C,H}$=141Hz, $^3J_{^{13}C,H\text{-}6A}$=4Hz, $O^{13}CH_3$-6_A), 58.9(OCH_3-2_B), 58.2($^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H\text{-}3A}$=6Hz, $O^{13}CH_3$-3_A), 57.1(qd, $^1J_{^{13}C,H}$=141Hz, $^3J_{^{13}C,H\text{-}3B}$=5Hz, $O^{13}CH_3$-3_B), 22.7($OCH_2CH_2CH_3$), 23.0(CH_3CO), 20.9(CH_3CO), 17.8(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{20}{}^{13}C_3H_{40}O_{12}$: C, 54.10; H, 7.90. Found C, 54.35; H 7.85.

*Propyl 4-O-(3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside* (**41**).--- Propyl 4-O-(2,4-di-O-acetyl-3,6-di-O-^{13}C-methyl-β-**D**-glucopyranosyl)-2-O-methyl-3-O-^{13}C-methyl-α-**L**-rhamnopyranoside (**40**) (66mg, 0.13mmol) was stirred for 2h in sodium methoxide-methanol (3ml, 0.3N). Aqueous hydrochloric acid (2ml, 1N) was added to neutralize the base and the solution was extracted with dichloromethane. The extracts were washed with saturated sodium bicarbonate solution and saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated. Chromatography with ethyl acetate-hexane-methanol 6:6:1 gave the *title compond* (**41**) as a syrup (58mg, 100%). ^{1}H-NMR: δ4.82(d, 1H, $J_{1,2}$=1.5Hz, H-1_B), 4.40(d, 1H, $J_{1,2}$=8Hz, H-1_A), 3.68-3.48(m, 6H, overlapped, 2xH-6_A, H-2_B, H-3_B, H-4_B, H-5_B), 3.59(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.53(H-4_A), 3.41(H-2_A, H-5_A), 3.67(d, 3H, $^1J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 3.46(d, 3H, $^1J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 3.38(d, 3H, $^1J_{^{13}C,H}$=142Hz, $O^{13}CH_3$), 3.48(s, 3H, OCH_3), 3.35(dt, 1H, $OCH_aH_bCH_2CH_3$), 3.16(tt, 1H, $J_{3,2}$=9.0Hz, $J_{3,4}$=9.0Hz, $^3J_{^{13}C,H}$=5Hz, H-3_A), 1.57($OCH_2CH_2CH_3$), 1.34(d, 3H, H-

6_B), 0.91($OCH_2CH_2CH_3$). ^{13}C-NMR: δ105.7($^1J_{C,H}$=158Hz, C-1_A), 96.8($^1J_{C,H}$=168Hz, C-1_B), 85.6(C-3_A), 81.9(C-3_B), 80.7(C-4_B), 76.(C-2_B), 75.1(C-2_A), 74.1(C-5_A), 72.9(C-6_A), 71.3(C-4_A), 69.3($OCH_2CH_2CH_3$) 67.5(C-5_B), 60.4(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3A}$=5Hz, $O^{13}CH_3$-3_A), 59.6(qt, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-6A}$=4Hz, $O^{13}CH_3$-6_A), 59.0(OCH_3-2_B), 56.4(qd, $^1J_{^{13}C,H}$=142Hz, $^3J_{^{13}C,H-3B}$=3Hz, $O^{13}CH_3$-3_B), 22.8($OCH_2CH_2CH_3$), 17.5(C-6_B), 10.6($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{16}{}^{13}C_3H_{36}O_{10}$: C, 53.37%; H, 8.50%. Found: C, 53.55%; H, 8.54%.

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