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SYNTHESIS OF OLIGOSACCHARIDE STRUCTURES RELATED TO THE ANTI-I MA COMBINING SITE STUDIES

by

© Ting Chi Wong

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT

Monoclonal antibodies with anti-I specificity from patients with chronic cold agglutinin disease have been shown to recognize specific domains on the oligosaccharide structure βDGal(1→4)βDGlcNAc(1→6)[βDGal(1→4)βDGlcNAc(1→3)]βDGal(1→4)-βDGlcNAc(1→3)βDGal. In the case of the monoclonal anti-I Ma antibody, the binding had been found to be restricted to certain portions of the three sugar units, βDGal(1→4)βDGlcNAc-(1→6)DGal (or (1→6)DGlcNAc) (I).

In order to delineate the exact nature of the binding with the anti-I Ma antibody, a series of 'fraudulent' oligosaccharides designed to probe the various structural demands of the combining site were synthesized for use in inhibition studies. These included (A) the 2-amino-2-deoxy, 2-acetamido-2-deoxy and 2-deoxy-2-trifluoroacetamido derivatives of βDGal-(1→4)βDGlcNAc(1→6)1,2;3,4-di-O-isopropylidene-αDGal; (B) several homologous alkyl glycosides of βDGal(1→4)βDGlcNAc; (C) the diastereomeric βDGal(1→4)βDGlcNAc(1→6)6-C-CH_3-DGal; (D) the βDGlc(1→4)βDGlcNAc(1→6)1,2;3,4-di-O-isopropylidene-αDGal; (E) the 6"-deoxy derivatives of βDGal(1→4)βDGlcNAc-(1→6)DGal and of βDGlc(1→4)βDGlcNAc(1→6)1,2;3,4-di-O-isopropylidene-αDGal and (F) βDGlcNAc(1→6)βDGal(1→4)βDGlcNAcOCH_3.

The inhibition results obtained with these compounds showed that the C-6 methylene group of the reducing βDGal unit, the acetamido group of the βDGlcNAc, and part of the terminal βDGal including OH-4" and OH-6" of I are intimately bound by the anti-I Ma antibody. The most notable result is that the D-isomer of the βDGal(1→4)βDGlcNAc(1→6)6 - C-CH_3-DGal was found to be a superior inhibitor to I while the L-isomer was less potent. It can therefore be concluded that the I Ma trisaccharide (I) enters the combining site of the antibody in a conformation that is close to that of the D-isomer and this conformation was established by ^{1}H-nmr spectroscopy.

Our interpretation of these results is that the trisaccharide (I) is bound in a conformation that possesses a region amenable to hydrophobic bonding with a combining site that extends between the C-6 grouping of the two DGal units and over the acetamido group of the central βDGlcNAc residue with the 3-OH of this unit hydrogen-bonded to O-5" of the terminal βDGal unit. No insight was gained on the nature of the involvement of the C-4" and C-6" groupings of the terminal βDGal unit except that the interactions are between the hydroxyl groups at these positions with polar groups at the periphery of the combining site.

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INTRODUCTION

Part I Immunochemical Aspects

A. The Ii Antigens

The human Ii blood group was discovered by Wiener in 1956[1]. The I antigens are well expressed on red blood cells of 99.9% of adults, and the related i antigens are found on the red cells of the umbilical cords of newborn infants and the red cells of a rare group of adults who lack the I antigens[1,2]. These Ii antigens have also been detected on human lymphocytes, macrophages[3], various cultured cells[4,5], and in certain secretions[6].

A group of monoclonal IgM cold agglutinins, which are autoantibodies from patients with chronic cold agglutinin disease[7] that agglutinate, in vitro, red cells only in cold (up to 32°C), are directed against the Ii antigens. The studies of the reactions of the various monoclonal antibodies[8-10] with certain blood group substances from milk[11] and human ovarian cyst fluid[12,13] revealed that the Ii determinants reside in the core structure of the ABH and Lewis blood group antigens. A glycoprotein OG[11] which was isolated from ovarian cyst fluid of a Nigerian and lacked ABH and Lewis activities was found to have both I and i determinants[8-10]. Characterization of the oligosaccharides isolated after alkaline borodeuteride degradation of purified OG substance provided evidence for the composite structure shown in Fig. 1[12]. This precursor OG substance contains a branched oligosaccharide

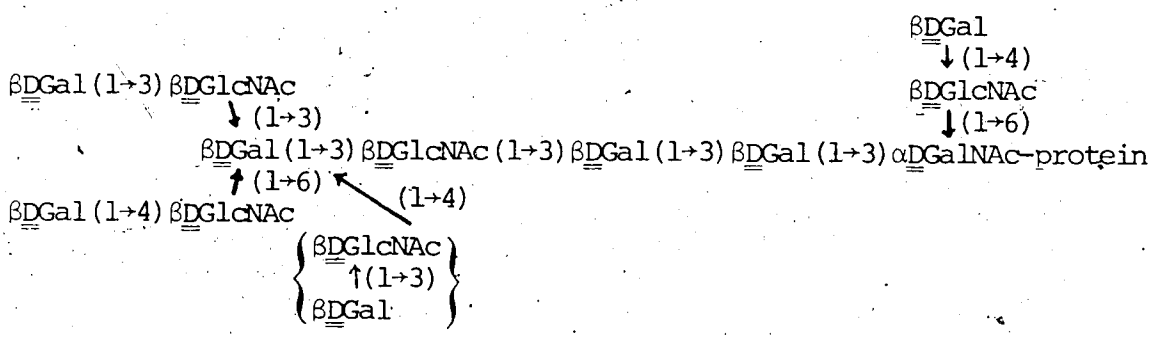


Fig. 1 A composite structure proposed by Vicari and Kabat[12], for the OG oligosaccharide moiety which was based on the earlier composite structure proposed for the A, B, H, Le^a, Le^b substances by Lloyd and Kabat[14].

portion made up of βDGal(1→3)βDGlcNAc(1→3)— (Type 1 chain) and βDGal(1→4)βDGlcNAc(1→3)— (Type 2 chain) units. It does not contain fucosyl residues. By comparison to the structure proposed for A, B, H, Le^a, Le^b substances[14], OG can reasonably be considered as a precursor to these latter blood group substances. Indeed, A and B blood group substances can be converted by Smith degradation to precursor-like substances similar to the OG glycoprotein presumably by removing the outer LFuc and DGalNAc or DGal residues[12,14].

Recent studies by Hakomori, Feizi et al[15-17] with monoclonal antibodies and glycosphingolipids further defined the I determinants to be residing on the branched oligosaccharide structure βDGal(1→4)βDGlcNAc(1→3)[βDGal(1→4)βDGlcNAc(1→6)]-βDGal(1→4)βDGlcNAc(1→3)DGal—, whereas the i determinant to be residing on the straight chain oligosaccharide structure βDGal(1→4)βDGlcNAc(1→3)βDGal(1→4)βDGlcNAc(1→3)DGal—. It was also shown by means of inhibition assays that, although no two monoclonal antibodies are identical in their reaction pattern with various haptens, three main types of anti-I can be identified[18]: type 1, the anti-I sera Ma and Woj react with the βDGal(1→4)βDGlcNAc(1→6)DGal—sequence, type 2, the anti-I sera Step, Gra, Ver and Ful react with the βDGal(1→4)-βDGlcNAc(1→3)DGal—sequence, and type 3, the anti-I sera Phi, Da, Sch and Low react only when both sequences are available. The addition of a βDGlcNAc(1→6)—branch to the reducing DGal residue of the βDGal(1→4)βDGlcNAc(1→3)DGal sequence confers an enhancement in the inhibitory activity with some

of the anti-I antibodies but results in the loss of the i activity as recognized by anti-i Tho, Den, Ho and McC[19].

B. The Immune Response, Polyclonal and Monoclonal Antibodies, and Antigenic Specificity

Antibodies in man and animals are associated with a group of plasma proteins called immunoglobulins[20]. The monomeric forms of immunoglobulin, for example, IgG, share a similar basic structure which is made up of four peptide structures consisting of two identical heavy chains and two identical light chains connected by interchain disulfide bonds. Each of these monomers has two identical ligand-binding sites capable of binding the antigenic determinants corresponding to their specificity. In general, the immunoglobulins isolated from a normal serum are a complex mixture of different antibodies with different physicochemical and biological activities. With respect to antigen-binding specificities, antibodies which have been obtained by deliberate immunization are considered to have combining sites complementary to specific structural units of the antigen employed. The immune response appears to involve an antigen-induced selection (after the clonal selection theory proposed by Burnet[21] which is widely accepted as a working hypothesis for antibody biosynthesis) and proliferation of antibody-producing lymphocytes each of which produce a clone of cells which manufacture antibodies with specificities for the inducing antigen. An antigen, however, usually possesses many immunogenic sites and the immune response to even one such site may result in antibodies

of the same specificity, but with subpopulations that recognize the structural features of a given antigenic determinant in different ways. Therefore, in inhibition studies, a topographical feature present in a given inhibitor may be recognized by that population of antibodies which is directed against that particular portion of the complete determinant but not by other antibodies of the same antigenic specificity. Recently, Zopf et al[22] examined the pattern of the binding of various complex sugars with antibodies induced by glycoconjugates of the following oligosaccharides, lacto-N-tetraose (βDGal(1→3)βDGlcNAc(1→3)βDGal(1→4)DGlc) and lacto-N-difucohexaose I (αLFuc(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc(1→3)βDGal-(1→4)DGlc). It was suggested that the polyclonal population of antibodies resemble antibodies of the same specificity but which bind different parts of the same antigenic determinant. The anti-lacto-N-difucohexaose antibody recognized the difucosyl determinant but the inhibition activity decreased significantly when either of the fucose residues was absent and was completely lost when both were missing. On the other hand, the anti-lacto-N-tetraose recognized the tetrasaccharide alone and this binding was affected only slightly by the addition of one or both of the fucose units. Thus, both of the oligosaccharide-induced antibodies could bind the lacto-N-difucohexaose but the anti-lacto-N-difucohexaose antibody recognized the surface of the oligosaccharide which possessed the fucose residues whereas the anti-lacto-N-tetraose antibody recognized another surface of the molecule. A good illustration

of the polyclonal nature of antibodies obtained by immunization is provided by results in a paper by Lemieux et al[23]. Antibodies raised in rabbits against an artificial Le^b antigen (αLFuc(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc—) were found to be extensively adsorbed by synthetic immunoadsorbents specific for anti-Le^a (βDGal(1→3)[αLFuc(1→4)]βDGlcNAc—) and anti-Le^d (αLFuc(1→2)βDGal(1→3)βDGlcNAc—) antibodies. This is attributed to different populations of antibodies in the anti-serum. Certain populations view topographical regions on the Le^b determinant which are common to the Le^a but not the Le^d determinant and vice-versa. Therefore, polyclonal antibody population in a given anti-serum can have an assortment of combining sites for a given determinant. Unless the combining site of a given monoclonal antibody has been completely defined, there is always the possibility that the best known inhibitor is a structure which is only a portion of the complete determinant.

In order to study the immunogenicity of a given antigenic determinant, either monoclonal or highly refined polyclonal antibody populations are required. Studies aimed at discerning how an antigenic determinant is accepted into the combining site of an antibody would, of course, be best based on studies involving pure monoclonal antibodies. Until recently, the main source of monoclonal antibodies was the blood of humans and animals suffering from diseases called multiple myeloma[24]. These are neoplastic diseases involving immunoglobulin-producing cells. The neoplastic cells which produce a given myeloma protein are considered to be derived from a

single clone and, therefore, produce monoclonal antibodies. Myeloma proteins are obtainable in substantial amounts from a given patient and are readily purified and characterized[25,26]. These proteins have played important roles in the elucidation of immunoglobulin structure and antigen-binding specificity.

C. The Specificity of the Anti-I Ma Antibody

The monoclonal anti-I Ma antibody, which is the concern of this thesis, reacts strongly with the glycoprotein OG (Fig. 1) and belongs to the type 1 anti-I antibodies mentioned on p. 3. The inhibition activities of different carbohydrate structures with anti-I Ma are summarized in Table I and II. It was obvious at the beginning of this investigation, from Table I, that the disaccharide residue βDGal(1→4)DGlcNAc is involved in binding which extends to the β(1→6) linkage that joins the DGlcNAc residue to the reducing DGal residue since structures 2, 3, 4 and 10 were inactive whereas 5 was fully active. However, by comparing the inhibition activities of 5 and 8, it seemed that the intact reducing DGal unit is not required. The fact that changing the β(1→4) linkage (as in 5) to β(1→3) linkage (as in 9) provided a structure that was still active and more active than the βDGal(1→4)DGlcNAc structure (less amount of 9 required for 20% inhibition than that of 1) suggested that the binding is more directed to the internal structure which is boxed in the conformational formula (Fig. 2) presented for compounds 5 and 9 than to that portion of the lactosamine residue which involves the terminal βDGal

Table I Inhibitory activities of oligosaccharides with anti-I Ma as determined by quantitative precipitin assays[10, 27, 29]

Oligosaccharides	Inhibitory Activities	References
βDGal(1→4)DGlcNAc(1)	3:1, 50%* ~0.90, 20%	29
βDGal(1→3)DGlcNAc(2)	Inactive†	10
βDGal(1→6)DGlcNAc(3)	Inactive	10
βDGlcNAc(1→6)αDGalNAcO$(CH_2)_8COOCH_3$(4)	Inactive	29
βDGal(1→4)βDGlcNAc(1→6)DGal(5)	0.45, 50%	27
βDGal(1→4)βDGlcNAc(1→6)βDGalO$(CH_2)_8COOCH_3$(6)	0.45, 50%	27
βDGal(1→4)βDGlcNAc(1→6)-3-hexenetetrols(7)	0.45, 50%	10
βDGal(1→4)βDGlcNAc(1→6)αDGalNAcO$(CH_2)_8COOCH_3$(8)	0.45, 50%	29
βDGal(1→3)βDGlcNAc(1→6)DGal(9)	~0.60, 20%§	27
βDGal(1→4)βDGlcNAc(1→3)DGal(10)	Inactive	27
βDGal(1→4)βDGlcNAc(1→6) βDGalO$(CH_2)_8COOCH_3$(11) βDGal(1→4)βDGlcNAc(1→3)	0.45, 50%	29
βDGal(1→4)βDGlcNAc(1→6) βDGalactitol (12) βDGal(1→3)βDGlcNAc(1→3)	0.45, 50%	10
αLFuc(1→3) βDGal(1→4)βDGlcNAc(1→6)3,4-dideoxy-D-hex-3-enitol(13)	Inactive	10

*μmoles of inhibitors added providing 20% or 50% (as indicated) inhibition of the precipitation of the anti-I Ma antibody from 15μL of the serum by 14.5μg of the blood group substance OG 20% from 10%[23] in a total volume of 400μL.

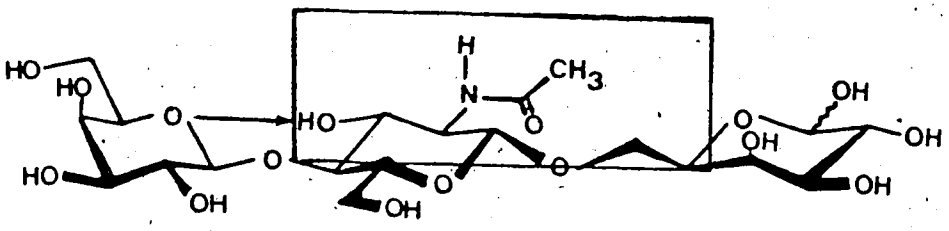
†Inactive in inhibition at the highest amount of the inhibitors tested.

§The highest inhibition attained was not more than 20% at the highest amount of inhibitor tested.

Table II Inhibitory activities of glycosphingolipids with anti-I Ma as determined by radioimmunoassay[18]

Glycosphingolipids	Inhibitory Activities
βDGal(1→4)βDGlcNAc ↘ (1→6) βDGal(1→4)βDGlcNAc(1→3)βDGal(1→4)βDGlc-Cer(14) ↗ (1→3) βDGal(1→4)βDGlcNAc	30[§]
βDGal(1→4)βDGlcNAc(1→6)βDGal(1→4)βDGlcNAc(1→3)βDGal(1→4)βDGlc-Cer(15)	9
αDGal(1→3)βDGal(1→4)βDGlcNAc ↓(1→6) βDGal(1→4)βDGlcNAc(1→3)βDGal(1→4)βDGlc-Cer(16) ↑(1→3) βDGal(1→4)βDGlcNAc	17

[§]Concentration in μg/mL required to give 50% inhibition of binding of ^{125}I-labelled I-active glycoprotein.



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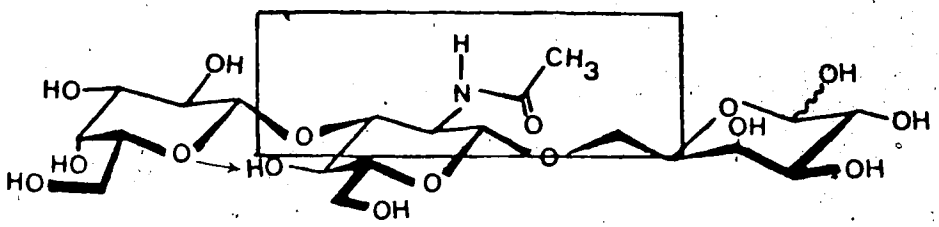

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Fig. 2 Conformational formulae of 5(βDGal(1→4)βDGlcNAc(1→6)-DGal) and 9(βDGal(1→3)βDGlcNAc(1→6)DGal) showing the common part of the two trisaccharides (boxed) against which the anti-I Ma is directed.

residue[27]. At the same time, in view of the high activities of compound 7, it was considered[27] possible that the structural unit, βDGal(1→4)βDGlcNAc(1→6)[βDGal(1→3)]αDGalNAc of the OG glycoprotein (Fig. 1) would be bound by the anti-I Ma antibody. To test this possibility, the haptens βDGal(1→4)βDGlcNAc-(1→6)αDGalNAcO$(CH_2)_8$ $COOCH_3$ (8) and βDGlcNAc(1→6)αDGalNAcO$(CH_2)_8$-$COOCH_3$ (4) were synthesized[28] and tested[29]. The trisaccharide hapten (8) was as active as were 5 and 6 but the disaccharide 4 was inactive. These results required an involvement of the βDGal(1→4) unit of the trisaccharide 5 in binding with anti-I Ma antibody. Also, it could be noted that the presence of a DGal unit α-linked to the 3-position of the terminal DGal (as in 16 in Table II) had little effect on binding.[17] Actually, it is a better inhibitor than 14 and not much less potent than 15 (Table II). In contrast, a LFuc residue α-linked to the 3-position of the central GlcNAc (as in 13 in Table I) rendered the structure inactive.[10] This suggested that the anti-I Ma antibody recognizes a certain topographical region on the trisaccharide structure 5 which is masked by the fucose in 13 but not the galactose in 16. It was thus the concern of this thesis to provide synthetic structures specially designed to shed light on the exact topographical features of the I Ma determinant which is recognized by the anti-I Ma antibody.

D. Antibody-Hapten Interaction

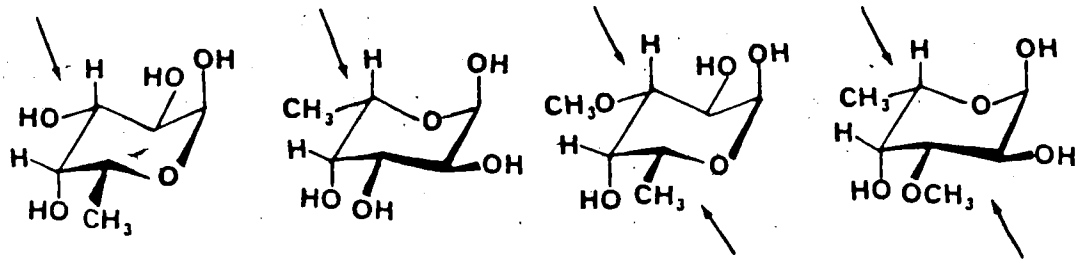
The molecular basis of the complementarity in antibody-antigen interactions has been of major theoretical interest since the classical studies by Landsteiner on serological reactions[30]. The specific binding of a ligand by an antibody is generally regarded as a competitive partitioning of a ligand between water and antibody combining site[31]. Thermodynamically, the stability of the antibody-ligand complex depends upon the free energy change due to the resultant non-covalent interactions (ionic bonds, hydrogen bonds, Van der Waals interactions or charge-transfer bonds) between atomic groups on the ligand and those on the antibody combining site as well as the transfer of the water molecules originally interacting with these regions to the bulk solvent. The summation of all the relatively weak forces over the area of interaction is responsible for the binding of a highly compatible structure and discriminates between structures which present different topographical surfaces. In favor of hydrophobic interaction as the main driving force for immunological reaction which is highly stereospecific is the fact that, as pointed out by Salem[32], London-Van der Waals interactions (dispersion forces) can cause large attractive forces between large molecules if the complex involves a substantial number of appropriate distances between atoms in both molecules. These attractive forces have an inverse sixth power dependence on the distance between the interacting groups, and therefore, for best binding, the antigenic determinant must provide a highly complementary

and conformationally well defined surface (topography of the molecule) to the antibody combining site. However, as noted in some cases, a ligand-induced conformational change or conformative response of the combining site[33] of an antibody or the active site[34] of an enzyme allows for some flexibility in the acceptance of the ligand. Cohn et al. suggested[33] that a number of amino-acid residues in the vicinity of the binding site might not contribute to the specificity of the reaction, but only to the stabilization of the antigen-antibody complex once it was formed and thus to the overall free energy change of the reaction.

Carbohydrate structures are generally strongly hydrophilic and their adsorption from solution in water by the combining site of an antibody is truly a remarkable event. Evidence has accumulated that anti-oligosaccharide antibody combining sites are highly hydrophobic[35]. In some cases, as was pointed out by Kabat[31], in which unexpected cross-reactivities were observed, studies of molecular models showed complementarity involving hydrophobic groups in the binding of carbohydrates to a protein. Springer and Williamson[36] had found that a lectin which is present in eel serum, reacted with an H blood group specific protein and also agglutinated human O red cells which have the type 2 H structure (αLFuc-(1→2)βDGal(1→4)βDGlcNAc) at their surface. The monosaccharide L-fucose was found to inhibit the agglutination at low concentrations. The enantiomeric D-fucose was found to be inactive. However, 3-O-methyl-D-fucose was found to be

considerably more active than the L-fucose, while 3-O-methyl-L-fucose was just as active as the D-isomer. These results can be rationalized in terms of the hydrophobicity of the part of the molecules which is expected to be accepted into the combining site of the lectin (Fig. 3). α-D-Fucose, which is a poor inhibitor, has an hydroxyl group in this region and is less compatible with a hydrophobic combining site on the lectin. It can be deduced, then, that the carbohydrate molecule, as a conformationally defined structure, can exhibit a considerable area of high hydrophobicity which allows the molecule to interact favorably with the hydrophobic groups in the antibody or lectin combining site while the hydrophilic part of the molecule may remain interacting either with solvent water or with polar groups at the periphery of the combining site.

Amongst the monoclonal IgM antibodies, the combining sites of the anti-I Ma and anti-I Woj antibodies (p.3) were confirmed by Lemieux and Kabat *et al*[27] to be complementary to certain portions of the three sugar units in βDGal(1→4)-βDGlcNAc(1→6)DGal (or (1→6)DGlcNAc) (5), although their inhibitory activities with different oligosaccharides are different. In order to delineate the exact nature of the binding, synthetic 'fraudulent' oligosaccharides with structural variations designed to probe the various structural demands of the combining site are required for inhibition studies. The syntheses of inhibitors related to the anti-I Ma combining site thus constitute the synthetic program of this thesis.



α-D-Fucose | α-L-Fucose | 3-O-Methyl-α-D-fucose | 3-O-Methyl-α-L-fucose

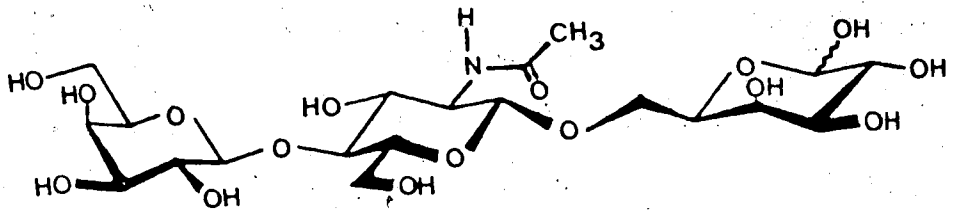
Fig. 3 The inhibition by simple sugars of the agglutination of human O red cells by an eel lectin. The arrows indicate the possible hydrophobic regions in the case of the inhibitors that are bound. In the case of αDFuc, a more hydrophilic environment is presented (an adaptation from Ref. 35).

The conformational properties of oligosaccharide structures, especially those of the blood group substances, have been of major concern in Dr. R. U. Lemieux's laboratory for the past ten years. The so-called HSEA (hard-sphere, exo-anomeric effect) method[37] had been adopted for molecular modelling and the results arrived at by this method were verified by ^{1}H-nmr experiments[38]. In the case of the I Ma determinant, Dr. H. Thøgersen deduced, by using the HSEA method, that the disaccharide βDGlcNAc(1→6)βDGal exists in a very large number of energically near-equivalent conformers, thus no conclusion could be drawn as to which conformer was most likely to be accepted by the anti-I Ma combining site. However, the range of low energy conformers was expected to be narrowed by the substitution of a methyl group for a hydrogen at the C-6 atom of the reducing βDGal unit. In view of the predicted conformational properties, the two diastereoisomers 25 and 26 were synthesized (p.77) and their conformations were verified by ^{1}H-nmr experiments which were performed by Dr. T. Nakashima.

The compounds achieved by chemical synthesis were sent to Dr. E. A. Kabat (College of Physicians and Surgeons, Columbia University, New York) for inhibition studies which were made by J. Liao. The results of these studies will be presented and discussed in a later part of this thesis. The synthetic program of this thesis which forms part of the multi-disciplinary project will be described in Part II.

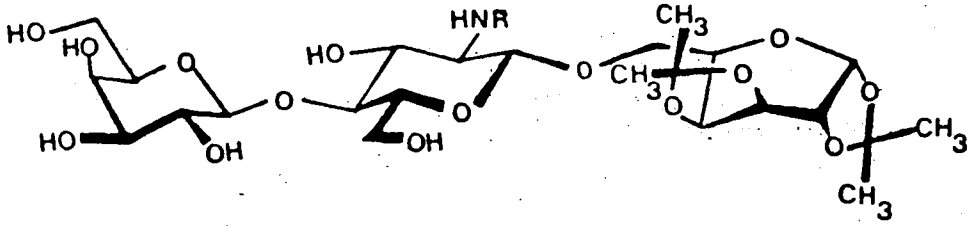
Part II Synthetic Aspects

The purpose of this section is to briefly introduce the synthetic program which forms the base of this thesis and which was designed to provide molecular probes for the examination of the binding of the trisaccharide 5 by the anti-I Ma antibody. It is expected that this overview will assist the reader to follow the presentation of the experimental results that follow.

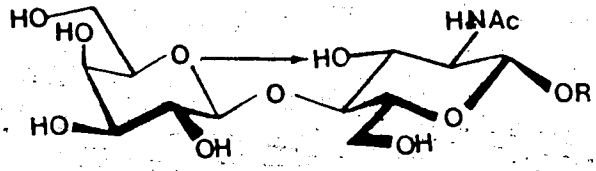


5

As already mentioned (p. 7), it had already been established that the reducing DGal unit is only marginally involved. In order to establish which atomic groups were involved, the following compounds were synthesized and tested as inhibitors of the precipitin reactions of anti-I Ma and the OG protein.

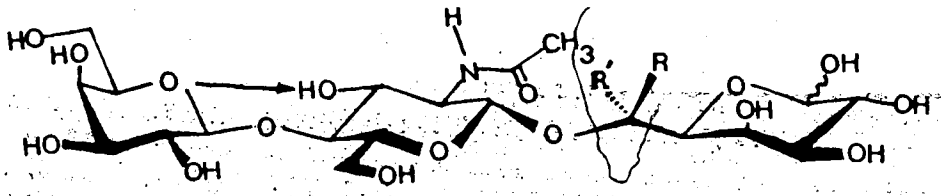


17, R = Ac ; 18, R = H ; 19, R = $COCF_3$



20, R = CH_3 ; 21, R = CH_2CH_3 ; 22, R = $CH_2CH_2CH_3$;
23, R = $CH(CH_3)_2$; 24, R = $CH_2CH(CH_3)_2$

In order to establish the orientation of the reducing βDGal unit of 5 when the trisaccharide is accepted by anti-I Ma, the conformationally well-defined homologues of 5 were synthesized; namely, 25 (the L-isomer) and 26 (the D-isomer).



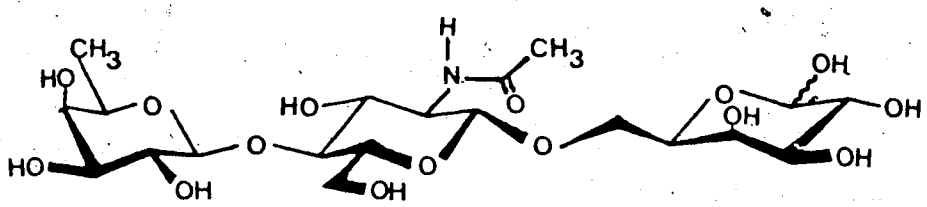
25, R = CH_3 , R' = H ; 26, R = H , R' = CH_3

All of these syntheses proceeded from 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride (27) or bromide (28), prepared from hexa-O-acetyl-lactal via the azidonitration route following the procedure reported by Lemieux et al[39]. Conden-

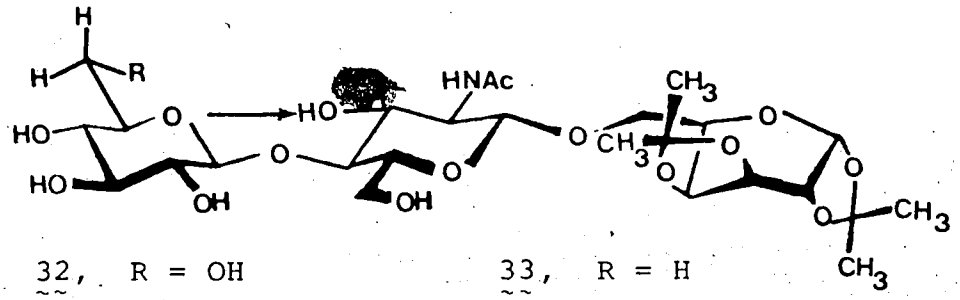
sation of the bromide (28) under standard conditions[40] with the appropriate alkanols provided the blocked derivatives of compounds 20 to 24. Compound 17 was prepared by condensation of the chloride (27) with the well-known 1,2;3,4-di-O-isopropylidene-α-D-galactose[41] followed by deblocking and N-acetylation. In order to prepare the homologues 25 and 26, a procedure modelled on that employed by Lemieux et al[42] was used to prepare 1,2;3,4-di-O-isopropylidene-7-deoxy-β-L-glycero-D-galacto-heptopyranose(29)and 1,2;3,4-di-O-isopropylidene-7-deoxy-α-D-glycero-D-galacto-heptopyranose(30).These alcohols were then condensed with the chloride 27 in the usual manner. Deblocking and N-acetylation then provided compounds 25 and 26.

In order to examine the role of the βDGlcNAc unit in the binding of 5 by the anti-I Ma antibody, the free amine(18) and its trifluoroacetyl derivative (19) were prepared. These were submitted for testing without removal of the isopropylidene groups since the N-acetyl compound (17) was highly active.

As was mentioned above, the terminal βDGal unit of 5 was implicated in the binding with anti-I Ma antibody. Syntheses were therefore designed to examine the extent of its involvement in the binding reaction. For this purpose, the 6"-deoxy derivative 31 of 5 was synthesized. The 4"-epimer (32) of 17 as well as the 6"-deoxy derivative (33) were also prepared in a manner similar to the preparation of 5[42] but starting with hexa-O-acetyl-cellobial.

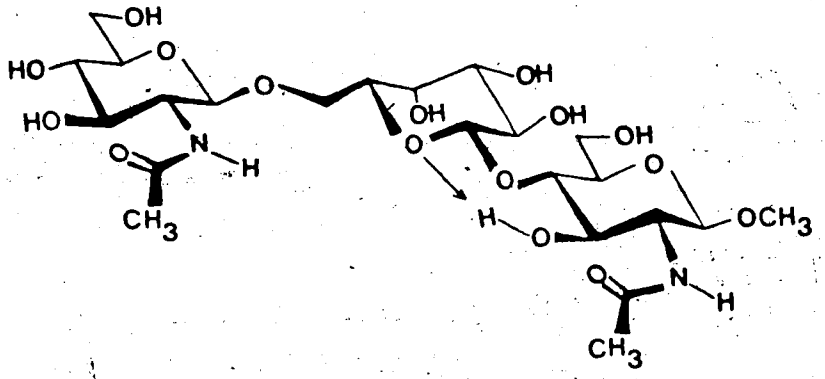


31



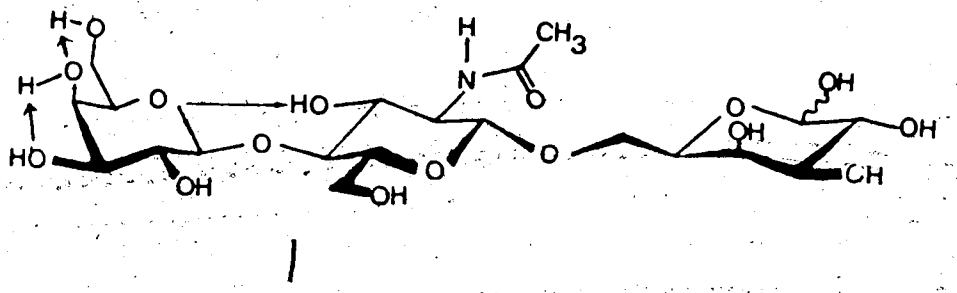
32, R = OH 33, R = H

It proved, in the inhibition studies carried out by Kabat and Liao that the simple methyl glycoside of N-acetyl-D-lactosamine was even a better inhibitor of the anti-I Ma than was the trisaccharide 5. Therefore, further syntheses aimed at elucidation of the participation of the non-reducing βDGal unit utilized the methyl glycoside as a starting material. Two possibilities were envisaged; (1) the binding that was highly specific terminated with binding of the non-reducing βDGal unit or, (2) the binding continued beyond this unit to include a fourth sugar unit. As seen above, the presence of a sugar unit at the 3"-position of 5 is unlikely. Should the binding include a fourth sugar unit, in view of the composite structure presented in Fig. 1, this would most likely be at the 6"-position and involve a βDGlcNAc residue. Even though there exists no precedent for a βDGlcNAc(1→6)βDGal(1→4)βDGlcNAc(1→6)DGal structural unit in I antigenic determinants, the methyl glycoside 34 was synthesized.



34

The other possibility is that the binding involves the 6"-hydroxymethyl group in an intramolecularly hydrogen-bonded form[43] as depicted in 35. This matter will be discussed in detail in Part IV of the Discussion of Results.



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EXPERIMENTAL

General Methods

All solvents and reagents used were reagent grade and, in the cases where further purification was required, standard procedures[44] were followed. All solid reagents for glycosylation were dried overnight over phosphorus pentoxide in high vacuum prior to use. Solution transfers where dry conditions were required were done under nitrogen using standard syringe techniques[45].

Thin layer chromatograms (tlc) were performed on precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and visualized by quenching of fluorescense and/or by charring after spraying with 5% sulfuric acid in ethanol. For column chromotography, silica gel 60(0.063-0.200mm) (Fluka A. G., Chemische Fabrik, CH-9470, Buchs S G) was used for elution under atmospheric pressure, and for medium pressure (flash) chromotography silica gel 60(0.040-0.063mm) (E. Merck, Darmstadt) was used. For reverse phase chromotography on hplc, C18 column (5μ, 8mm I.D.) (Waters Associates, Milford, MA01757) was used. For gel filtration, Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and Bio-Gel P-2 (200-400 mesh) (Bio-Rad Laboratories, Richmond, California, U.S.A.) were used. Membrane dialysis was performed under medium pressure using UM05 Diaflo membrane (Amicon, Lexington, MA02173). Dowex 1-X8(OH^-) resin was obtained from J. T. Baker

Chemical Co., Phillipsburg, N. J., and Amberlite IR-120(H^+) resin was obtained from Mallinckrodt, Inc., Paris, Kentucky 4036.

Spectral and elemental analyses were performed by the Spectral Service Laboratory under the supervision of Dr. T. Nakashima and the Analytical Service Laboratory under the supervision of Mr. R. Swindlehurst. FTIR spectra were recorded using a Nicolet 7199. Proton magnetic resonance (^{1}H-nmr) spectra were recorded on a Varian HA-100 or Brüker WH-400 at ambient temperatures. Carbon-13 nuclear magnetic resonance (^{13}C-nmr) spectra were recorded on a Brücker WP-60(15.08MHz) or HFX-90 (22.6 MHz) at ambient temperature. ^{1}H and ^{13}C chemical shifts are in ppm relative to an internal reference tetramethylsilane (TMS, δ=0.00) or chloroform ($CHCl_3$, δ=7.27) in organic solvents (chloroform-d was usually used as solvent), and in the case when deuterium oxide was the solvent, HOD=4.80. Melting points were determined using a Gallenkamp melting point apparatus in open-glass capillary tubes and are uncorrected. Optical rotations were determined by a Perkin-Elmer 241 polarimeter at 589mm in a 1-dm cell.

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-galactopyranose (37)

3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (27) (0.38 g, 0.51 mmol)[39] in dry nitromethane (2 mL) was added to a solution of 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (36) (0.142 g, 0.55 mmol), sym-collidine (62 mg, 0.51 mmol) and silver trifluoromethanesulfonate (silver triflate) (0.131 g, 0.51 mmol) in dry nitromethane (5 mL) cooled to -15°C. The mixture was stirred at -15°C for 2 h and warmed up to room temperature for 2.5 h. The reaction mixture was then diluted with chloroform (10 mL) and the solids were removed by filtration. The filtrate was washed with sodium thiosulfate solution (10 mL), cold water (10 mL) and saturated sodium bicarbonate solution (3 x 10 mL). Solvent removal after drying over sodium sulfate left a foam which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the second fraction left a white solid (0.364 g, 72%), mp 194-198°C, $[\alpha]_D^{24}$ -17.5° (c 0.7, chloroform); ^{1}H-nmr (Fig.4) δ: 7.86-7.66 (m, 4H, phthalimido), 5.80 (dd, 1H, H-3', $J_{2',3'}$= 10.5Hz, $J_{3',4'}$= 7.8Hz), 5.42 (d, 1H, H-1', $J_{1',2'}$= 8.5Hz), 5.32 (dd, 1H, H-4", $J_{3",4"}$= 3.0Hz, $J_{4",5"}$= 0.6Hz), 5.10 (m, 2H, H-1 overlapping with H-2", $J_{1,2}$= 4.8Hz), 5.00 (dd, 1H, H-3", $J_{2",3"}$= 10.5Hz), 4.51 (m, 2H, H-1" overlapping with possibly a H-6', $J_{1",2"}$= 8.0Hz), 4.36 (dd, 1H, H-3, $J_{2,3}$= 2.2Hz, $J_{3,4}$= 7.8Hz) 4.20 (m, 2H, H-2' overlapping with one other proton), 4.10 (m, 3H, H-2 overlapping with 2 other protons), 4.00 (dd, 1H,

H-4, $J_{4,5}$ = 1.5Hz), 3.82 (m, 4H, H-4' overlapping with 3 other protons, one of which is possibly H-5"), 3.66 (m, 2H, possibly H-5' and H-5), 2.15-1.91 (6S, 18H, acetyl methyl), 1.38, 1.21, 1.03 (3S, 12H, O-C(=O)$(CH_3)_2$). Anal. calcd. for $C_{44}H_{55}NO_{23}$: C 54.71, H 5.74, N 1.45; found: C 55.70, H 5.97, N 1.37.

1,2;3,4-Di-O-isopropylidene-6-O-[2-amino-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranose (18)

A solution of compound 37 (0.357g, 0.37mmol) and 85% hydrazine hydrate (0.12g, 2.39mmol) in ethanol (3mL) was refluxed for 2 h. The white solid obtained after solvent removal was applied directly to a Sephadex LH-20 column and eluted with ethanol-water (1:1). Solvent removal from the main fraction gave a white solid (0.17g, 0.29mmol, 78%); ^{1}H-nmr (Fig. 5) δ : 5.73 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.61 (dd, 1H, H-2, $J_{2,3}$ = 2.0Hz), 4.48 (2d, 2H, H-1' and H-1" overlapping, J = 7.6Hz and 7.6Hz), 4.16 (m, 2H, H-4 and H-5), 3.05 (dd, 1H, H-2'), 1.60, 1.51, 1.43, 1.42 (4s, 12H, O-C(=O)$(CH_3)_2$), 4.75, 4.05-3.53 (dd and m, 14H, remaining protons).

1,2;3,4-Di-O-isopropylidene-6-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranose (17)

Compound 18 (30mg, 0.05mmol) was taken up in methanol-water (1:1, 2 mL) containing acetic anhydride (0.4 mL) and left at room temperature for about 5 h. The oil obtained after solvent removal was applied to Sephadex LH-20 column and eluted with ethanol-water (1:1). Solvent removal and freeze-drying of

the main fraction gave a white solid (26 mg, 0.042 mmol, 82%); ^{1}H-nmr (Fig. 6) δ : 5.66 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.58 (d, 1H, H-1', $J_{1',2'}$ = 7.6Hz), 4.56 (dd, 1H, H-2, $J_{2,3}$ = 2.0Hz) 4.50 (d, 1H, H-1", $J_{1",2"}$ = 7.6Hz), 4.42 (bd, 1H, H-4), 2.05 (s, 3H, NHAc methyl), 1.60, 1.51, 1.43, 1.42 (4s, 12H, O-C(=O)-$(CH_3)_2$), 4.11-3.53 (m, 16H, remaining protons).

1,2;3,4-Di-O-isopropylidene-6-O-[2-deoxy-2-trifluoroacetamido-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranose (19)

A solution of the compound 18 (30 mg, 0.051 mmol) and S-ethyl trifluorothioacetate (90 mg, 0.63 mmol) in methanol (2 mL) was kept at room temperature for 2 days. The oil obtained after solvent removal was applied to a silica gel column and eluted with methanol. The white solid obtained after solvent removal of the first fraction was filtered through a Sephadex LH-20 column in ethanol-water (1:1). Solvent removal and freeze-drying of the main fraction gave a white solid (20 mg, 0.029 mmol, 57%). ^{1}H-nmr (Fig. 7) δ : 5.66 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.72 (d, 1H, H-1', $J_{1',2'}$ = 7.6Hz), 4.55 (dd, 1H, H-2, $J_{2,3}$ = 2.0Hz), 4.50 (d, 1H, H-1", $J_{1",2"}$ = 7.6Hz), 4.41 (dd, 1H, H-4), 1.60, 1.50, 1.42 (3s, 12H, O-C(=O)-$(CH_3)_2$), 4.08-3.54 (m, 16H, remaining protons).

Methyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (38)

3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl bromide (28) (mp 104-111°C; $[\alpha]_D^{25}$ +35.7° (c 1.0, chloroform), 0.293 g, 0.37 mmol) in dry nitromethane (2 mL) was added to a solution of dry methanol (1 mL), sym-collidine (50 µl), silver triflate (0.114 g, 0.44 mmol) in dry nitromethane (5 mL) cooled to -15°C. The mixture was stirred at -15°C for 1 h. The reaction mixture was diluted with chloroform (10 mL) and the solid was removed by filtration. The filtrate was washed with saturated sodium thiosulfate solution (10 mL), cold water (10 mL) and saturated sodium bicarbonate solution (3 x 10 mL). Solvent removal after drying with sodium sulfate left a white solid (0.287 g) which was recrystallized from ethyl acetate (0.198 g, 0.27 mmol, 73%); mp 227.5-229.5°C; $[\alpha]_D^{24}$ +19.8° (c 0.42, chloroform); ^{1}H-nmr (Fig. 8) δ: 7.90-7.70 (m, 4H, phthalimido) 5.75 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.28 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 3.42 (s, 3H, OCH_3), 2.15-1.90 (6s, 18H, acetyl methyl), 5.38-3.76 (m, 11H, remaining protons). Anal. calcd. for $C_{33}H_{39}O_{18}N$: C 53.74, H 5.33, N 1.90; found: C 53.23, H 5.34, N 1.94.

Ethyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (39)

The bromide (28) (0.3 g, 0.38 mmol) was condensed with dry ethanol (1 mL) following the same procedures as has been described for the preparation of 38 to give, after recrystallization from ethyl acetate-hexane, the glycoside (39) (0.21 g, 0.28 mmol, 74%); mp 249-250°C; $[\alpha]_D^{24}$ + 11.4° (c 0.4, chloroform); ^{1}H-nmr (Fig. 9) δ : 7.90-7.70 (m, 4H, phthalimido), 5.74 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.36 (dd, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 3.52 (m, 1H, OCH_2A), 2.15-1.91 (6H, 18H, acetyl methyl), 1.05 (t, 3H, OCH_2CH_3 methyl), 5.32-3.75 (m, 12H, remaining protons). Anal. calcd. for $C_{34}H_{41}O_{18}N$: C 54.33, H 5.30, N 1.86; found: C 54.14, H 5.58, N 1.83.

n-Propyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galacto-pyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (40)

The bromide (28) (0.3 g, 0.38 mmol) was condensed with dry n-propyl alcohol (1 mL) following the same procedures as has been described for the preparation of 38 to give, after recrystallization from ethyl acetate-hexane, the glycoside (40) (0.219 g, 0.29 mmol, 77%); mp 227-229°C; $[\alpha]_D^{24}$ + 13.5° (c 0.4, chloroform); ^{1}H-nmr (Fig.10) δ : 7.96-7.68 (m, 4H, phthalimido), 5.73 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.32 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 3.32 (m, 1H, OCH_2A), 2.14-1.89 (6s, 18H, acetyl

methyl), 1.41 (m, 2H, $(OCH_2)CH_2$), 0.66 (t, 3H, $OCH_2CH_2CH_3$ methyl), 5.30-3.68 (m, 12H, remaining protons). Anal. calcd. for $C_{35}H_{43}O_{18}N$: C 54.90, H 5.66, N 1.83; found: C 55.02, H 5.65, N 1.85.

Isopropyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glycopyranoside (41)

The bromide (28) (0.3 g, 0.38 mmol) was condensed with isopropyl alcohol (1 mL) following the same procedures described for the preparation of 38 to give, after recrystallization from ethyl acetate-hexane the glycoside (41) (0.236 g, 0.31 mmol, 81%); mp 255.5-258.5°C; $[\alpha]_D^{24}$ +3.6° (c 0.4, chloroform); ^{1}H-nmr (Fig.11) δ : 7.90-7.70 (m, 4H, phthalimido), 5.72 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.42 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 2.12-1.88 (5s, 18H, acetyl methyl), 1.12 (d, 3H, $OCH(CH_3)_2$ methyl A), 0.91 (d, 1H, $OCH(CH_3)_2$ methyl B), 5.34-3.78 (m, 12H, remaining protons). Anal. calcd. for $C_{35}H_{43}O_{18}N$: C 54.90, H 5.66, N 1.83; found: C 54.56, H 5.65, N 1.81.

Isobutyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (42)

The bromide (28) (0.3 g, 0.38 mmol) was condensed with dry isobutyl alcohol (1 mL) following the same procedures described for the preparation of 38 to give, after recrystallization from ethanol, the glycoside (42) (0.2 g, 0.26 mmol,

68%); mp 199-203°C; $[\alpha]_D^{24}$ +15.0°(c 0.4, chloroform); ^{1}H-nmr (Fig.12) δ : 7.90-7.67 (m, 4H; phthalimido), 5.75 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.32 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 3.60 (dd, 1H, $OCH_2CH(CH_3)_2$ OCH_2A), 3.12 (dd, 1H, $OCH_2CH(CH_3)_2$ OCH_2B), 2.13-0.88 (6s, 18H, acetyl methyl), 1.68 (m, 1H, $OCH_2CH(CH_3)_2$ methine), 0.63 (2d, 6H, $OCH_2CH(CH_3)_2$ methyls). Anal. calcd. for $C_{36}H_{45}O_{18}N$: C 55.46, H 5.82, N 1.80; found: C 56.19, H 5.91, N 1.83.

Methyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (20)

Method 1. A solution of the methyl glycoside (38) (52.9 mg, 0.072 mmol) in 97% hydrazine hydrate-ethanol (3:20, 1 mL) was refluxed for 2 h. The solid which deposited on cooling was removed by filtration before solvent removal. The residue was taken up in methanol-water (1:1, 2 mL) containing acetic anhydride (0.4 mL) and left at room temperature for 1.5 h. Solvent removal left a solid which was extracted with water and the solution was applied to a column of Dowex 1-X8 (OH^-) resin. Solvent removal left a solid (54 mg). This material (19 mg) was applied to a reverse phase C18 column (hplc) and eluted with methanol-water (3:7). Solvent removal of the main fraction gave a solid which was recrystallized from methanol (6 mg, 0.015 mmol, 60%); mp 283-285.5°C; $[\alpha]_D^{24}$ -26.7°(c 0.25, water); ^{1}H-nmr (Fig.13) δ : 4.52 (2d, 2H, H-1,

H-1' overlapping, J = 8.0Hz and 8.0Hz), 3.55 (s, 3H, OCH_3), 2.08 (s, 3H, NHAc methyl), 4.08-3.50 (m, 12H, remaining protons).

Method 2. The methyl glycoside (38) (60 mg, 0.081 mmol) was treated with 0.125M sodium methoxide-methanol solution (2 mL) at room temperature for 15 min before IR-120 (H^+) resin was added to bring the pH to ~6.0. Solvent removal left a white solid which was dissolved in ethanol (2 mL) containing 97% hydrazine hydrate (0.05 mL) and the solution was refluxed for 5 h. The solvent was evaporated and the residue was dried under high vacuum to remove excess hydrazine. The solid was taken up in methanol-water (2 mL) containing acetic anhydride (0.4 mL) and left at room temperature for 2 h. Solvent removal left a solid which was dissolved in small amount of water and applied to a Biogel P-2 column and eluted with water-ethanol (9:1). Solvent removal from the main fractions left a solid (26 mg, 0.065 mmol, 80%) which was recrystallized and characterized as described in Method 1.

Ethyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (21)

The ethyl glycoside (39) (0.120 g, 0.16 mmol) was dissolved in 0.025M sodium methoxide-methanol (2 mL) and left at room temperature for 1 h before Amberlite IR-120(H^+) resin was added to bring the pH down to about 5. Solvent removal left a solid which was redissolved in pyridine (2 mL) and heated

at 115°C for 10 h. Solvent removal gave an oil which was redissolved in methanol and decolorized with charcoal. The resultant solution was evaporated down to about 1 mL. Diethyl ether was added dropwise until the solution turned cloudy. On storage at -4°C for a few hours, the solution deposited a crystal (55.2 mg). A solution of this material (52 mg), 97% hydrazine hydrate (20 mg) in ethanol (1.5 mL) was refluxed at 80°C for 2 h. Solvent removal left a solid which was taken up in methanol-water (1:1, 4 mL) containing acetic anhydride (0.8 mL) and left at room temperature for 2 h. Solvent removal left a white solid which was redissolved in water and applied to a Dowex 1-X8(OH) resin column. Solvent removal left a white solid which was recrystallized from methanol (38 mg, 0.092 mmol, 61%); mp 274-275°C; $[\alpha]_D^{24}$ - 24.0° (c 0.9, water); ^{1}H-nmr (Fig.14) δ : 4.59 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.50 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 2.05 (s, 3H, NHAc methyl), 1.19 (t, OCH_2CH_3 methyl), 4.02-3.52 (m, 14H, remaining protons).

n-Propyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (22)

Treatment of n-propyl glycoside (40) (71 mg, 0.093 mmol) with hydrazine hydrate, work-up and N-acetylation as in Method 1 for the preparation of (20) resulted in a white solid (80 mg). 30 mg of this material was applied to a reverse phase C18 column (hplc) and eluted with methanol-water (3:7). Solvent removal of the main fraction left a solid which was recrystallized from methanol (10 mg, 0.024 mmol, 67%).

mp 276.5-278°C; $[\alpha]_D^{24}$ -20.7°(c 0.35, water); ^{1}H-nmr (Fig.15) δ : 4.55 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.50 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 2.04 (s, 3H, NHAc methyl), 1.54 (m, 2H, $(OCH_2)CH_2(CH_3)$ methylene), 0.90 (t, 3H, $OCH_2CH_2CH_3$ methyl), 4.05-3.50 (m, 14H, remaining protons).

Isopropyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (23)

Treatment of the isopropyl glycoside (41) (89 mg, 0.12 mmol) with hydrazine hydrate, work-up and N-acetylation as described in Method 1 for the preparation of 20, resulted in a white solid (85 mg). 30 mg of this material was applied to a reverse phase C18 column (hplc) and eluted with methanol-water (3:7). Solvent removal of the main fractions left a solid which was recrystallized from methanol (7 mg, 0.016 mmol, 40%); mp 264-266°C; $[\alpha]_D^{24}$ -16.7°(c 0.25, water); ^{1}H-nmr (Fig.16) δ : 4.68 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.54 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 1.08 (s, 3H, NHAc methyl), 1.24 and 1.18 (2d, 6H, $OCH(CH_3)_2$ methyl), 4.10-3.55 (m, 13H, remaining protons).

Isobutyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (24)

Treatment of the isobutyl glycoside (42) (50 mg, 0.064 mmol) with hydrazine hydrate, work-up and N-acetylation as described in Method 1 for the preparation of 20, resulted in

a white solid (54 mg). This material (20 mg) was applied to a reverse phase C18 column (hplc) and eluted with methanol-water (3:7). Solvent removal of the main fractions left a solid which was recrystallized from methanol (6 mg, 0.014 mmol, 58%), mp 282.5-284.5°C; $[\alpha]_D^{24}$ -23.5°(c 0.25, water); ^{1}H-nmr (Fig.17) δ : 4.58 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 4.52 (d, 1H, H-1', $J_{1,2}$ = 8.0Hz), 3.40 (dd, 1H, $OCH_2CH(CH_3)_2$ methylene A), 2.08 (s, 3H, NHAc methyl), 1.84 (m, 1H, $OCH_2CH(CH_3)_2$ methine), 0.92 (2d, 6H, $OCH_2CH(CH_3)_2$ methyl), 4.08-3.54 (m, 13H, remaining protons).

1,2;3,4-Di-O-isopropylidene-α-D-galacto-1,6-dialdohexopyranose (43)

1,2;3,4-Di-O-isopropylidene-α-D-galactose was oxidized using the chromium trioxide-pyridine complex in dichloromethane as was described by Arrick et al[53]. Chromium trioxide (47 g, 0.47 mol) was added to a stirred solution of dichloromethane (1200 mL) and anhydrous pyridine (73 g, 0.93 mol) over a period of 30 min. 1,2;3,4-di-O-isopropylidene-α-D-galactose (10 g, 0.0384 mol) in dichloromethane (40 mL) was then added and the mixture was stirred for 20 min. The supernatant solution was decanted and the tarry precipitate was extracted with diethyl ether (100 mL). The combined solutions were washed successively with cold sodium bicarbonate solution (500 mL) and ice-cold water (2 x 300 mL). Solvent removal, after drying over magnesium sulfate, left a dark green syrup (9.47 g, 0.0367 mol, 95%). Examination of the

dark green syrupy crude product (95% yield) showed it to be of as good a quality as that obtained by distillation. The ^{1}H-nmr spectrum was in accord with that published by Horton *et al*[46].

1,2;3,4-Di-O-isopropylidene-7-deoxy-β-L-glycero-D-galacto-heptopyranose (29)

Methylmagnesium iodide was prepared under standard conditions[47] using magnesium (1.42 g, 58 mmol) and methyl iodide (8.3 g, 58 mmol) in diethyl ether (60 mL). A solution of the aldehyde (43) (8.0 g, 31 mmol) in diethyl ether (45 mL) was added dropwise with stirring and the mixture was kept at room temperature for 15 h. The reaction mixture was then cooled in ice-water and a 10% solution of ammonium chloride in water (50 mL) was added. Work-up in the usual manner using diethyl ether as extractant, drying over magnesium sulfate, and solvent removal, left a syrup which was decolorized by passing a solution in ethyl acetate through a short column of silica gel. Removal of the solvent left a syrup (7.3 g) which was dissolved in hexane (15 mL). On storage, the solution deposited 3.17 g of crystals which, after three recrystallizations from hexane, provided 2.2 g (26%) of 29, mp 90-91°C; $[\alpha]_D^{23}$ -58.5° (c 1.8, chloroform); ^{1}H-nmr (Fig.19) δ : 5.58 (d, 1H, H-1, $J_{1,2}$ = 4.6Hz), 4.59 (dd, 1H, H-3, $J_{2,3}$ = 2.1Hz, $J_{3,4}$ = 8.0Hz), 4.32 (dd, 1H, H-2), 4.26 (dd, 1H, H-4, $J_{4,5}$ = 2.0Hz), 3.99 (m, 1H, H-6), 3.48 (dd, 1H, H-5, $J_{5,6}$ = 6.8Hz), 1.50, 1.44,

1.32 (3s, 12H, O-C(=O)$(CH_3)_2$), 1.26 (d, 3H, (C-7)H_3, J_{6,CH_3} ≈ 6Hz). Anal. calcd. for $C_{13}H_{22}O_6$: C 56.92, H 8.08; found: C 56.87, H 8.05.

1,2;3,4-Di-O-isopropylidene-7-deoxy-α-D-glycero-D-galacto-heptopyranose (30)

The mother liquor from the first crystallization of the isomer (29) was taken to a syrup (3.86 g) which was dissolved in dichloromethane (10 mL), pyridine (4 mL), and benzoyl chloride (2 mL). The solution was stirred at room temperature for 3 h and the product was isolated in the usual manner. Solvent removal left a syrup which was taken up in hexane (3 mL) and the solution was kept at -4°C for 2 days. Crystals (0.290 g) were deposited which were debenzoylated in methanol-sodium methoxide without further purification. The product was applied to a column of silica gel and eluted with ethyl acetate-hexane (6:4). The main fractions were combined and dissolved in a small amount of hexane and the solution deposited a white solid (0.188 g, 2%) when stored at -4°C overnight. This product, mp 57-58°C, $[\alpha]_D^{23}$ -34.8°(c 0.3, chloroform), was not further purified since the ^{1}H-nmr spectrum revealed it to be a nearly pure substance; ^{1}H-nmr (Fig.20) δ: 5.55 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.62 (dd, 1H, H-3, $J_{2,3}$ = 2.2Hz, $J_{3,4}$ = 8.0Hz), 4.46 (dd, 1H, H-4, $J_{4,5}$ = 2.0Hz), 4.30 (dd, 1H, H-2), 3.96 (m, 1H, H-6), 3.50 (dd, 1H, H-5, $J_{5,6}$ = 7.2Hz), 1.51, 1.45, 1.36, 1.32, 1.29 (5 peaks, 15H, O-C(=O)$(CH_3)_2$ and (C-7)H_3 overlapping). Anal. calcd. for $C_{13}H_{22}O_6$: C 56.92, H 8.08; found: C 56.42, H 7.77.

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-7-deoxy-β-L-glycero-D-galacto-heptopyranose(44)

3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride(27)[39] (1.3 g, 1.8 mmol) in nitromethane (5 mL) was added to a solution of the alcohol (29) (0.5 g, 1.8 mmol), sym-collidine (0.22 g, 1.8 mmol) and silver triflate (0.49 g, 1.9 mmol) in dry nitromethane (15 mL) cooled to -25°C. The mixture was stirred at -25°C for 2 h. The reaction mixture was then stirred at room temperature for 8 h. The solids that were deposited after the addition of chloroform (100 mL) were removed by filtration and the filtrate was washed with sodium thiosulfate solution, cold water, dilute aqueous HCl, and saturated sodium bicarbonate solution. Solvent removal, after drying over sodium sulfate, left a foam which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the second fraction left a white solid (0.63 g, 36%), mp 119-123°C; $[\alpha]_D^{23}$ -21.6°(c 1.1, chloroform); ^{1}H-nmr (Fig.21) δ: 7.80-7.52 (broad m, 4H, phthalimido), 5.70 (dd, 1H, H-3', $J_{3',4'}$ = 8.0Hz, $J_{2',3'}$ = 10.5Hz), 5.39 (d, 1H, H-1', $J_{1',2'}$ = 8.5Hz), 5.23 (broad d, 1H, H-4", $J_{3",4"}$= 3.1Hz), 5.04 (dd, 1H, H-2", $J_{1",2"}$ = 7.5Hz, $J_{2",3"}$ = 10.5Hz), 4.87 (dd, 1H, H-3"), 4.73 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.44 (d, 1H, H-1"), 3.18 (broad dd, 1H, H-5, $J_{5,6}$ = 8.2Hz), 2.06, 2.05, 1.99, 1.97, 1.88, 1.80 (6s, 18H, acetyl CH_3), 1.32,

1.18, 1.12, 0.80, 0.75 (5 peaks, 15H, O-C(=O)-$(CH_3)_2$ and (C-7)H_3). Anal. calcd. for $C_{45}H_{57}O_{23}N$: C 55.16, H 5.86, N 1.43; found: C 55.02, H 5.83, N 1.31.

1,2;3,4-Di-O-isopropylidene-6-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-7-deoxy-β-L-glycero-D-galacto-heptopyranose (45)

A solution of compound 44 (0.56 g, 0.57 mmol) and 85% hydrazine hydrate (0.22 g, 4.39 mmol) in ethanol (5 mL) was refluxed for 3 h. Solvent removal left a syrup which was redissolved in water (5 mL). The solution was neutralized with glacial acetic acid and the precipitate formed was removed by filtration. Dialysis of the filtrate through a UM05 Diaflo membrane afforded after freeze-drying of the dialyzed solution, a solid (0.25 g). This substance was taken up in methanol-water (1:1, 20 mL) containing acetic anhydride (4 mL) and left at room temperature for 2 H. Solvent removal and crystallization from methanol-ether gave a white solid (0.152 g, 42%), mp 165-169°C; ^{1}H-nmr (D_2O) δ: 5.62 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.44 (2d, 2H, H-1' and H-1" overlapping, J = 7.5Hz and 8.0Hz), 2.07 (s, 3H, NHAc methyl), 1.52, 1.48, 1.38 (3s, 12H, O-C(=O)$(CH_3)_2$), 1.30 (d, 3H, (C-7)H_3), 4.02-3.48 (m, 17H, remaining protons).

6-O-[2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-7-deoxy-L-glycero-D-galacto-heptopyranose (25)

A solution of compound (45) (50 mg, 0.078 mmol) in 95% trifluoroacetic acid (2 mL) was stirred for 3 min. at room temperature and rapidly evaporated in vacuo to a syrup which was applied to a Sephadex LH-20 column and eluted with ethanol-water (1:1). Solvent removal and freeze-drying of the main fraction gave a white solid (13.5 mg, 0.024 mmol, 31%); ^{1}H-nmr (Fig.23) δ: 5.28 (d, H-1α, $J_{1,2}$ = 2.5Hz, H-1β not observed, probably obscured by the HOD peak at 4.80), 4.54 (2d, 2H, H-1' and H-1" overlapping, J = 7.5Hz and 7.5Hz), 2.14, 2.13 (2s, NHAc-α and NHAc-β methyl), 1.33 (2d, 3H, (C-7)H_3α and β), 4.08-3.44 (m, 17H, remaining protons).

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-7-deoxy-α-D-glycero-D-galacto-heptopyranose (46)

3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (0.213 g, 0.29 mmol) in dry nitromethane (2 mL) was added to a solution of the alcohol (30) (60 mg, 0.22 mmol), sym-collidine (33 mg, 0.27 mmol), and silver triflate (60 mg, 0.24 mmol), in dry nitromethane (5 mL) cooled to -25°C. The mixture was stirred at -25°C for 2 h and then warmed to room temperature for 16 h. The reaction mixture was diluted with chloroform (20 mL) and the solids were removed by filtration. The filtrate was washed with cold water, aqueous sodium bicarbonate

solution, and dilute hydrochloric acid. Solvent removal after drying over sodium sulfate left a foam which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the second fraction left a white solid (0.14 g, 0.14 mmol, 65%), mp 114-119°C, $[\alpha]_D^{23}$ -20°(c 0.5, chloroform); ^{1}H-nmr (Fig.22) δ: 7.80-7.62 (broad m, 4H, phthalimido), 5.66 (dd, 1H, H-3', $J_{3',4'}$ = 8.0Hz, $J_{2',3'}$ = 10.5Hz), 5.41 (d, 1H, H-1', $J_{1',2'}$ = 8.5Hz), 5.34 (d, 1H, H-1, $J_{1,2}$ = 4.5Hz), 5.25 (broad d, 1H, H-4", $J_{3",4"}$ = 3.1Hz), 5.04 (dd, 1H, H-2", $J_{1",2"}$ = 7.5Hz, $J_{2",3"}$ = 10.5Hz), 4.86 (dd, 1H, H-3"), 4.46 (d, 1H, H-1"), 2.07, 1.99, 1.98, 1.89, 1.84 (5s, 15H, acetyl CH_3), 1.32, 1.22, 1.20 (3s, 12H, O-C(=O)$(CH_3)_2$), 0.88 (d, 3H, (C-7)H_3). Anal. calcd. for $C_{45}H_{57}O_{23}N$: C 55.16, H 5.86, N 1.43; found: C 54.51, H 5.76, N 1.34.

1,2;3,4-Di-O-isopropylidene-6-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-7-deoxy-α-D-glycero-D-galacto-heptopyranose (47)

Treatment of compound 46 (0.119 g, 0.12 mmol) with 85% hydrazine hydrate, work-up and N-acetylation as described above for the preparation of 45, resulted in the isolation of a white solid (72 mg) which was applied to a Sephadex LH-20 column and eluted with ethanol-water (1:1). Solvent removal from the main fraction gave a white solid (34 mg, 0.053 mmol, 44%), mp 155-158°C, ^{1}H-nmr (D_2O) δ: 5.66 (d, 1H, H-1,

$J_{1,2}$ = 5.0Hz), 4.50 (2d, 2H, H-1' and H-1" overlapping, J = 7.5Hz and 7.5Hz), 2.10 (s, 3H, NHAc methyl), 1.65, 1.48, 1.45, 1.43 (4s, 12H, O-C(=O)$(CH_3)_2$), 1.25 (d, 3H, (C-7)H_3), 4.20-3.50 (m, 17H, remaining protons).

6-O-[2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-7-deoxy-D-glycero-D-galacto-heptopyranose (26)

A solution of compound 47 (21 mg, 0.033 mmol) in 70% trifluoroacetic acid (2 mL) was stirred at room temperature for 4 min and rapidly evaporated in vacuo to about 0.5 mL, and 3 drops of triethylamine was added. Filtration through a column of Sephadex LH-20, as described above for the preparation of 25, provided a main fraction which was freeze-dried to a white solid (14.5 mgs, 79%); ^{1}H-nmr (Fig.24) δ : 5.24, 4.66 (2d, 1H, H-1α and H-1β, J = 3.0Hz and 7.5Hz), 4.58, 4.49 (2d, 2H, H-1' and H-1" which shows up at this field position, J = 7.5Hz and 7.5Hz), 2.08 (s, 3H, NHAc methyl), 1.26, 1.24 (2d, 3H, (C-7)H_3α and (C-7)H_3β), 4.26-3.42 (m, 17H; remaining protons).

Crude 3,6-Di-O-acetyl-2-azido-2-deoxy-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-α,β-D-glucopyranosyl nitrate (48)

Hexa-O-acetyl-cellobial (mp 129.5-133°C, $[\alpha]_D^{24}$ = -24.5° (c, 1.1, chloroform))[48] (45 g, 0.08 mol) in acetonitrile (315 mL) was added to a mixture of sodium azide (8.1 g, 0.124 mol) and ceric ammonium nitrate (135 g, 0.3 mol) cooled to

-15°C[49]. The resulting suspension was vigorously stirred with cooling for 8 h. The solid was removed by filtration and washed with toluene (700 mL) and the combined filtrate and washings were poured on ice (∿500 g). The organic layer was separated and washed with cold water (3 x 500 mL) before drying over sodium sulfate. Solvent removal by evaporation in vacuo at 45°C left a foam (45 g).

3,6-Di-O-acetyl-2-azido-2-deoxy-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl bromide (49)

N,N-dimethylbromoforminium bromide (13.3 g, 0.061 mol) was added to a solution of the crude product 48 (40 g) in dichloromethane (60 mL). The mixture was stirred at room temperature for 3 h to result in a clear red solution before lithium bromide (50 g, 0.57 mol) was added. The suspension was stirred for 7 h. The mixture was then diluted with dichloromethane (60 mL) and the supernatant was decanted onto ice (500 g). The organic layer was separated and washed with saturated sodium bicarbonate solution (4 x 200 mL) and water (200 mL) before drying with anhydrous sodium sulfate. Solvent removal by evaporation left a foam which was redissolved in the minimum amount of ethyl acetate and kept in an ether bath for 12 h. The solution deposited a solid (15.6 g, 0.0228 mole, 32% from hexa-O-acetyl-cellobial); mp 136.5-138°C; FTIR ($CHCl_3$ Cast) νmax: 2114 (N_3); ^{1}H-nmr (Fig.25) δ: 6.35 (d, 1H, H-1', $J_{1,2}$ = 3.8Hz), 5.45 (dd, 1H, H-3, $J_{2,3}$ = 10.1Hz, $J_{3,4}$ = 8.4Hz), 5.14 (dd, 1H, H-3', $J_{2',3'}$ = 9.0Hz, $J_{3',4'}$ = 9.0Hz),

5.06 (dd, 1H, H-4', $J_{4',5'}$ = 9.0Hz), 4.91 (dd, 1H, H-2', $J_{1',2'}$ = 7.8Hz), 4.51 (d, 1H, H-1'), 4.49 (dd, 1H, H-6A), 4.38 (dd, 1H, H-6'A, $J_{6'A,6'B}$ = 12.5Hz, $J_{6'A,5'}$ = 4.5Hz), 4.20 (m, 1H, H-5), 4.15 (dd, 1H, H-6B, $J_{6B,5}$ = 4.0Hz), 4.03 (dd, 1H, H-6'B, $J_{6'B,5'}$ = 2.5Hz), 3.81 (dd, 1H, H-4, $J_{4,5}$ = 9.0Hz), 3.66 (dd and m, 2H, H-2 and H-5' overlapping), 2.10-1.94 (6s, 18H, acetyl methyl). Anal. calcd. for $C_{24}H_{32}O_{15}N_3Br$: C 42.24, H 4.73, N 6.16, Br 11.76; found: C 41.93, H 4.64, N 6.01, Br 11.89.

A portion of this product was redissolved in ethyl acetate with warming and petroleum ether (hexane) was added until the solution turned cloudy. The solution, on storage at -4°C overnight, deposited a crystalline solid; mp 140-141°C; $[\alpha]_D^{24}$ +79.7°(c 1.3, chloroform).

Benzyl 3,6-Di-O-acetyl-2-azido-2-deoxy-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (50)

A solution of the azidobromide (49) (9.75 g, 14.3 mmol) in nitromethane (20 mL) was added in small portions to a stirred mixture of benzyl alcohol (6 mL), silver carbonate (15 g, 54.5 mmol), and Drierite (15 g) which was cooled to -25°C. After stirring at -25°C for 20 h, the solids were removed by filtration and washed with dichloromethane (20 mL). Solvent removal left a syrup which was applied to a silica gel column and eluted with ethyl acetate-hexane (5:6). Solvent removal of the second fraction gave an oil which crystallized on addition of diethyl ether (6.68 g, 9.4 mmol, 66%);

mp 155-158°C; $[\alpha]_D^{24}$ -16.4°(c 2.0 chloroform) ^{1}H-nmr ($CDCl_3$) δ: 7.32 (s, 5H, benzyl aromatic protons), 4.88 and 4.66 (2d, 2H, benzyl CH_2), 4.47 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 4.38 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 3.44 (dd, 1H, H-2, $J_{2,3}$ = 10.5Hz), 2.14-1.96 (4s, 12H, acetyl methyl). Anal. calcd. for $C_{31}H_{39}O_{16}N_3$: C 52.47, H 5.54, N 5.92; found: C 52.27, H 5.61, N 6.02.

Benzyl 2-azido-2-deoxy-4-O-(β-D-glucopyranosyl)-β-D-glucopyranoside (51)

The acetate (50) (4.0 g, 5.64 mmol) was dissolved in 0.01N sodium methoxide-methanol (75 mL), and left at room temperature for 1 h. The solution was treated with IR-resin 120(H^+) to bring the pH to about 6.0. The resultant solution was evaporated *in vacuo* down to about 10 mL when crystallization occurred. The crystals (2.37 g, 5.19 mmol, 92%) were collected and washed with cold methanol; mp 195-198°C, $[\alpha]_D^{24}$ -7.4°(c 0.4, methanol). Anal. calcd. for $C_{19}H_{27}O_{10}N_3$: C 49.89, H 5.95, N 9.19; found: C 49.69, H 5.99, N 9.24.

Benzyl 2-amino-2-deoxy-4-O-(β-D-glucopyranosyl)-β-D-glucopyranoside (52)

A slow stream of hydrogen sulfide was bubbled through an ice-cold solution of the azide (51) (2.0 g, 4.38 mmol) in pyridine (25 mL) and triethylamine (15 mL) for 2.5 h. The solution was evaporated to dryness and the residue was extracted with methanol-water (1:1). The extract was filtered through a bed

of diatomaceous earth and solvent removal of the filtrate resulted in a dark-colored solid (1.96 g). The solid was again taken up in water and the suspension was centrifuged. Freeze-drying of the supernatant gave a yellowish white solid (1.58 g, 3.67 mmol, 84%); $[\alpha]_D^{25}$ -28.2°(c 0.6, water); ^{1}H-nmr (D_2O) δ: 7.46 (s, 5H, benzyl aromatic) 4.54-4.50 (2d, 2H, H-1 and H-1' overlapping, J = 8.0Hz and 7.5Hz), 2.72 (dd, 1H, H-2, $J_{1,2}$ = 8.0Hz, $J_{2,3}$ = 9.5Hz).

Benzyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (53)

Phthalic anhydride (97 mg, 0.65 mmol) was added to a solution of the amine (52) (0.489 g, 1.13 mmol) in pyridine (4 mL). The solution was heated to 60°C for 20 min. Triethylamine (0.1 g, 0.99 mmol) and more phthalic anhydride (97 mg, 0.65 mmol) were added and the heating was continued for 1 h. Methanol (2 mL) was added and the solution was evaporated to dryness. The residue was taken up in pyridine-acetic anhydride (2:1, 3 mL) and heated at 80°C for 3 h. Solvent removal left a dark foam which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the main fraction left an oil which was redissolved in diethyl ether and decolorized with charcoal. The resultant solution, on storage in open-flask for 2 days, deposited crystals (0.55 g, 0.68 mmol, 60%); mp 138-140°C; $[\alpha]_D^{24}$ -22.6°(c 0.95, chloroform); ^{1}H-nmr ($CDCl_3$) δ: 7.80-7.68 (m, 4H, phthalimido), 7.12-7.04 (m, 5H, benzyl aromatic),

5.72 (dd, 1H, H-3, $J_{2,3}$ = 10.0Hz, $J_{3,4}$ = 8.0Hz), 5.35 (d, 1H, H-1, $J_{1,2}$ = 8.4Hz), 5.14 (dd, 1H, H-3', $J_{2',3'}$ = 9.0Hz, $J_{3',4'}$ = 9.0Hz), 4.92 (dd, 1H, H-2', $J_{1',2'}$ = 7.6Hz), 4.81 (d, 1H, benzyl CH_2 H-A, J_{AB} = 12.0Hz), 4.58 (dd, 1H, H-6A), 4.56 (d, 1H, H-1'), 4.51 (d, 1H, benzyl CH_2 H-B), 4.33 (dd, 1H, H-6'A, $J_{6'A,6'B}$ = 12.5Hz, $J_{6'A,5'}$ = 5.0Hz), 4.26 (dd, 1H, H-2), 4.16 (dd, 1H, H-6B, $J_{6A,6B}$ = 12Hz, $J_{6B,5}$ = 5.0Hz), 4.02 (dd, 1H, H-6'B, $J_{6'B,5'}$ = 2.0Hz), 3.87 (dd, 1H, H-4 $J_{4,5}$ = 8.0Hz), 3.78 (m, 1H, H-5), 3.64 (m, 1H, H-5').
Anal. calcd. for $C_{32}H_{37}NO_{18}$: C 57.56, H 5.33, N 1.73; found: C 57.03, H 5.33, N 1.69.

3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranose (54)

The compound 53 was hydrogenated in ethanol-ethyl acetate (1:1) at 50 psi and room temperature using 5% palladium on charcoal as catalyst for 20 h. The yield was quantitative. ^{1}H-nmr ($CDCl_3$) δ : 7.90-7.70 (m, 4H, phthalimido), 6.00 (dd, 0.2H, H-3α), 5.76 (dd, 0.8H, H-3β), 5.64 (d, 0.8H, H-1β), 5.34 (d, 0.2H, H-1α), 2.21-1.82 (m, 18H, acetyl methyl).

3,6-Di-O-acetyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-α,β-glucopyranosyl chloride (55)

N,N-Dimethylchloroforminium chloride (95 mg, 0.74 mmol), the alcohol (54) (0.13 g, 0.18 mmol), and sym-collidine (0.1 g, 0.82 mmol) in dichloromethane (2 mL) was stirred at

room temperature for 1 h. The reaction mixture was diluted with chloroform (10 mL) and washed successively with cold water (10 mL) and saturated sodium bicarbonate solution (3 x 10 mL). Drying with sodium sulfate and solvent removal left a solid (0.122 g, 0.16 mmol, 89%); ^{1}H-nmr (Fig.26) δ : 7.82-7.66 (m, 4H, phthalimido), 6.48 (dd, 0.2H, H-3α), 6.12 (d, 0.8H, H-1β, $J_{1\beta,2\beta}$ = 9.0Hz), 6.08 (d, 0.2H, H-1α, $J_{1\alpha,2\alpha}$ = 3.6Hz), 5.66 (dd, 0.8H, H-3β), 2.12-1.82 (m, 18H, acetyl methyl).

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-galactopyranose (56)

The chloride (55) (0.1 g, 0.13 mmol) in nitromethane (0.5 mL) was added to a solution of the alcohol 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (33 mg, 0.13 mmol), sym-collidine (16 mg, 0.13 mmol), and silver triflate (33 mg, 0.13 mmol) in dry nitromethane (1.5 mL) cooled to -20°C. The mixture was stirred at -20°C for 3 h and then worked-up the same way as has been described for the compound (37). The product was obtained in 65% yield after recrystallization from chloroform-diethyl ether; mp 235-237°C; $[\alpha]_D^{23}$ 20.7°(c 0.4, chloroform); ^{1}H-nmr (Fig.27) δ : 7.90-7.66 (m, 4H, phthalimido), 5.78 (dd, 1H, H-3', $J_{2',3'}$ = 10.8Hz, $J_{3,4}$ = 8.0Hz), 5.45 (d, 1H, H-1', $J_{1',2'}$ = 9.0Hz), 5.12 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz, this proton is overlapping with other protons), 4.58 (d, 1H, H-1", $J_{1",2"}$ = 8.0Hz, overlapping with other protons),

2.17-1.88 (6s, 18H, acetyl methyl), 1.40, 1.24, 1.04 (3s, 12H, O-C(=O)$(CH_3)_2$). Anal. calcd. for $C_{44}H_{55}NO_{23}$: C 54.71, H 5.74, N 1.45; found: C 55.67, H 5.96, N 1.45.

1,2;3,4-Di-O-isopropylidene-6-O-[2-acetamido-2-deoxy-4-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranose (32)

A solution of compound 56 (23.5 mg, 0.024 mmol) and 85% hydrazine hydrate (0.15 mL) in ethanol (1 mL) was refluxed for 2 h. Solvent removal left a solid which was dissolved in methanol-water (1:1, 1 mL) containing acetic anhydride (0.25 mL) and the solution was kept at room temperature for 2 h. Solvent removal left a solid which was applied to a Sephadex LH-20 column and eluted with ethanol-water (1:1). Solvent removal of a main fraction left a white solid (10.6 mg, 0.017 mmol, 71%). ^{1}H-nmr (Fig.28) δ: 5.64 (d, 1H, H-1, $J_{1,2}$ = 5 Hz); 4.55 (m, 3H, H-1', H-1", H-2 overlapping), 4.40 (dd, 1H, H-4), 2.10 (s, 3H, NHAc methyl), 1.61-1.42 (4s, 12H, O-C(=O)$(CH_3)_2$).

Benzyl 4-O-(β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (57)

(i) Sodium hydroxide solution (4N, 25 mL) was added to a suspension of benzyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside[39] (0.5 g, 0.62 mmol) in methanol (25 mL) and the mixture was stirred for 1 h. The resultant clear solution was then treated with small amount of IR-resin

120(H^+) to bring the pH down to ~5.0. Solvent removal by evaporation gave a white solid (0.313 g) which had the same R_f on tlc(7 ethyl acetate:2 ethanol:1 water) as the more polar side product observed when the starting material was treated with $NaOCH_3$-CH_3OH as described in method ii (below). The solid was taken up in dry pyridine (10 mL) and heated at 115°C for 10 h. It was observed on tlc the complete disappearance of the starting material and a new product which has the same R_f as the main product obtained in the following preparation (ii). Solvent removal left a yellowish solid which was redissolved in methanol (40 mL) and the solution was decolorized by charcoal. Solvent removal left a white solid (258 mg, 0.46 mmol, 75%); mp 255-257°C; $[\alpha]_D^{23}$ -44.0°(c 0.10, methanol; FTIR ($CHCl_3$ cast) νmax: 1701.9 cm^{-1} (phthalimido carbonyl); ^{13}C-nmr (pyridine) δ : there are 13 peaks for the ring carbon atom and the benzyl methylene carbon 105.9 (C-1'), 98.5 (C-1), 83.4, 77.2, 76.8, 75.2, 72.6, 70.9, 70.6, 70.1, 62.2 and 62.1 (C-6 and C-6'), and 57.6.

(ii) The compound benzyl 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (0.5 g, 0.62 mmol) was treated with 0.2 N sodium methoxide-methanol (150 mL) for 5 min at room temperature. The resulting mixture (the solid was not completely soluble) was treated quickly with IR-120(H^+) resin to bring the pH down to ~6. Solvent removal left

a white solid (0.39 g) which showed on tlc (7 ethyl acetate:2 ethanol:1 water) as a major faster-moving spot and a minor slow-moving spot. The mixture, on refluxing in dry pyridine, resulted in the disappearance of the more polar contaminant and after the same work-up as in method i afforded a solid (0.280 g, 0.50 mmol, 82%); mp 254-257°C; $[\alpha]_D^{23}$ -44.8°(c 0.17, methanol).
anal. calcd. for $C_{27}H_{31}O_{12}N$: C 57.75, H 5.56, N 2.49; found: C 57.62, H 5.49, N 2.30.

Benzyl 4-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (58)

The compound (57) (0.80 g, 1.42 mmol),α,α-dimethoxytoluene (0.43 g, 2.84 mmol) and p-toluenesulfonic acid monohydrate (11 mg) in dry acetonitrile (15 mL) was stirred at room temperature for 7 h. Triethylamine was added dropwise to bring the pH to about 7. Solvent removal left an oil which was applied to a silica gel column and eluted with ethyl acetate-methanol (9:1). Solvent removal of the main fraction left a solid (0.78 g). The solid was redissolved in methanol (15 mL). The solution was filtered and concentrated by evaporation to about 5 mL. Diethyl ether was then added dropwise until the solution turned cloudy. On storage at room temperature overnight and then at -4°C for 5 h, this solution deposited crystals (0.54 g, 0.83 mmol, 58%); mp 206-207°C; $[\alpha]_D^{24}$ -72.4°(c 1.1, chloroform); ^{1}H-nmr (CD_3OD) δ : 7.81 (s, 4H, phthalimido), 7.61-7.25 (m, 5H, benzylidene aromatic),

7.10 (s, 5H, benzyl aromatic), 5.60 (s, 1H, benzylidene methine), 5.20 (d, 1H, H-1); ^{13}C-nmr (Fig.31) δ : there are 14 peaks for the ring carbon, the benzyl methylene carbon and the benzylidene methine carbon: 105.5 (C-1'), 101.5 (benzylidene methine), 98.6, 82.0, 77.3, 76.9, 73.8, 71.9, 80.0, 70.3, 69.5, 67.8 (C-6'), 61.7 (C-6), 57.9. Anal. calcd. for $C_{34}H_{35}O_{12}N$: C 62.86, H 5.43, N 2.16; found: C 62.22, H 5.28, N 2.14.

Benzyl 3,6-di-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (59)

The compound 58 (0.62 g, 0.96 mmol) was dissolved in acetic-anhydride-pyridine (1:2) and heated at 80°C for 3 h. Solvent removal left a yellowish foam which was decolorized by passing through a short column of silica gel and eluted with ethyl acetate. Removal of the solvent gave a solid which was recrystallized from ethanol. The yield of this pure product was 81%; mp 210-212°C. The ^{1}H-nmr spectrum (Fig.32) is consistent with the structure assigned. Anal. calcd. for $C_{42}H_{43}O_{16}N$: C 61.69, H 5.30, N 1.71; found: C 61.35, H 5.32, N 1.24.

Benzyl 3,6-di-O-acetyl-4-O-(2,3-di-O-acetyl-β-D-galacto-pyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (60)

The benzylidene compound (59) (0.47 g, 0.58 mmol) was treated with 80% aqueous acetic acid at 80°C for 3 h. Solvent removal left a foam which was applied to silica gel column and eluted with ethyl acetate-hexane (4:1). Solvent removal of the main fraction gave a solid in 82% yield. ^{1}H-nmr spectrum (Fig.33) of this solid was consistent with the structure assigned.

Benzyl 3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (61)

Methanesulfonyl chloride (60 mg, 0.52 mmol) was added in two batches (15 min interval) to a solution of the diol (60) (0.3 g, 0.41 mmol) in dry pyridine (2 mL) cooled to -20°C. The reaction mixture was kept at -20°C for 12 h and then allowed to warm up to room temperature after a few drops of water was added. Solvent removal left an oil which was applied to a silica gel column and eluted with ethyl acetate-hexane (4:1). Solvent removal of the main fraction gave an oil (0.18 g) which was directly treated with acetic anhydride-pyridine (1:2) for 3 h. Removal of solvent left a solid (0.19 g). This material and potassium iodide (0.5 g) in N,N-dimethylformamide (2 mL) were heated at 80°C for 72 h. The reaction mixture was diluted with chloroform (10 mL) and

washed with cold water (3 x 10 mL) before drying with sodium sulfate. The residue, after solvent removal, was applied to a silica gel column and eluted with ethyl acetate-hexane (3:2). Solvent removal of the main fraction gave a solid (0.12 g, 0.13 mmol, 32%); ^{1}H-nmr (Fig.34) δ: 7.83-7.69 (m, 4H, phthalimido), 7.12 (s, 5H, benzyl aromatic), 5.80 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.2Hz), 5.55 (dd, 1H, H-4', $J_{3',4'}$ = 3.4Hz, $J_{4',5'}$ = 1.0Hz), 5.36 (d, 1H, H-1, $J_{1,2}$ = 8.5Hz), 5.11 (dd, 1H, H-2', $J_{1',2'}$ = 7.5Hz, $J_{2',3'}$ = 10.5Hz), 4.97 (dd, 1H, H-3'), 4.82 (d, 1H, benzyl methylene A, $J_{A,B}$ = 12.0Hz), 4.58 (dd, 1H, H-6A, $J_{6A,5}$ = 2.0Hz), 4.55 (d, 1H, H-1'), 4.53 (d, 1H, benzyl methylene B), 4.28 (dd, 1H, H-2), 4.17 (dd, 1H, H-6B, $J_{6B,5}$ = 4.5Hz), 3.94 (dd, 1H, H-4, $J_{4,5}$ = 10.0Hz), 3.86 (m, 1H, H-5), 3.76 (m, 1H, H-5'), 3.10 (m, 2H, H-6'A and B), 2.16-1.91 (5s, 15H, acetyl methyl). Anal. calcd. for $C_{37}H_{41}O_{16}NI$: I 14.42; found: I 14.53.

Benzyl 3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside(62)

The iodo compound (61) (0.11 g, 0.12 mmol), tri-n-butyl tin hydride (0.14 g, 0.48 mmol) 2,2'-azobisisobutyronitrile (∿5 mg) in toluene was heated at 55°C for 20 h. Solvent removal left an oil which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Removal of solvent from the second fraction gave an oil (48 mg, 0.06 mmol, 50%); ^{1}H-nmr requires this to be a near pure substance and conforms to the structure assigned. ^{1}H-nmr (Fig.35) δ:

7.86-7.70 (m, 4H, phthalimido), 7.16 (s, 5H, benzyl aromatic), 5.76 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 7.0Hz), 5.41 (d, 1H, H-1, $J_{1,2}$ = 7.2Hz), 5.22 (dd, 1H, H-4', $J_{3',4'}$ = 3.5Hz, $J_{4',5'}$ = 1.0Hz), 5.14 (dd, 1H, H-2', $J_{1',2'}$ = 7.5Hz, $J_{2',3'}$ = 10.0Hz), 4.98 (dd, 1H, H-3'), 4.84 (d, 1H, benzyl methylene A, J_{AB} = 12.0Hz), 4.58 (dd, 1H, H-6A, $J_{6A,5}$ = 1.5Hz), 4.56 (d, 1H, benzyl methylene B), 4.55 (d, 1H, H-1'), 4.26 (dd, 1H, H-2), 4.17 (dd, 1H, H-6B, $J_{6B,5}$ = 3.5Hz), 3.88 (dd, 1H, H-4), 3.80 (dd, 1H, H-5), 3.74 (dd, 1H, H-5', $J_{5',(C'_6)H_3}$ = 7.0Hz), 2.15-1.89 (5s, 15H, acetyl methyl), 1.26 (d, 3H, $(C_{6'})H_3$).

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-galactopyranose (63)

3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (64) (27 mg, 0.037 mmol) was prepared from 62 by the same procedure as was described for the preparation of the chloride (27), except N,N-dimethylbromoforminium bromide[51,52] was used as the halogenating reagent. The bromide (64) was condensed with 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (73) in the same manner as was described for the preparation of the trisaccharide 56. The yield was 18 mg (54%) of an oil after chromotography. ^{1}H-nmr (Fig.36) δ: 7.92-7.68 (m, 4H, phthalimido), 5.81 (dd, 1H, H-3', $J_{2',3'}$ = 10.5Hz, $J_{3',4'}$ = 8.0Hz), 5.46 (d, 1H, H-1', $J_{1',2'}$ = 8.5Hz), 5.20 (dd, 1H, H-4", $J_{3",4"}$ = 3.5Hz, $J_{4",5"}$ = 1.0Hz), 5.13 (d, 1H, H-1,

$J_{1,2}$ = 5.2Hz), 5.09 (dd, 1H, H-2", $J_{1",2"}$ = 7.5Hz, $J_{2",3"}$ = 10.5Hz), 4.96 (dd, 1H, H-3"), 4.53 (dd, 1H, H-6'A), 4.51 (d, 1H, H-1"), 4.40 (dd, 1H, H-3, $J_{2,3}$ = 2.5Hz, $J_{3,4}$ = 8.0Hz), 4.22 (dd, 1H, H-2'), 4.16 (dd, 1H, H-6'B), 4.11 (dd, 1H, H-2), 4.00 (dd, 1H, H-4, $J_{4,5}$ = 1.5Hz), 3.88 (dd, 1H, H-4'), 3.83 (m, 1H, H-5'), 3.74 (m, 1H, H-5"), 3.66 (m, 1H, H-5), 2.16-1.90 (5s, 15H, acetyl methyl), 1.40, 1.22, 1.03, 1.02 (4s, 12H, O-C(=O)$(CH_3)_2$), 1.16 (d, 3H, $(C_{6"})H_3$, $J_{5",(C_{6"})H_3}$ = 7.0Hz).

6-O-[2-Acetamido-2-deoxy-4-O-(6-deoxy-β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-galactopyranose (31)

Hydrazinolysis and N-acetylation was carried out by procedures as was described for the preparation of compound 32. Removal of isopropylidene groups by acid hydrolysis following the procedures described for the preparation of compound 26 afforded a white solid (4 mg). The yield was high for hydrazinolysis and N-acetylation but the yield of acid hydrolysis step was only 40% for the required structure as determined by ^{1}H-nmr. This material was separated from other products by the Sephadex LH-20 column. The other products were not characterized. ^{1}H-nmr (Fig.37) δ : 5.28 (d, 0.35H, H-1α, $J_{1\alpha,2\alpha}$ = 3.5Hz), 4.63 (d, 0.65H, H-1β, $J_{1\beta,2\beta}$ = 8.0Hz), 4.58 (d, 1H, H-1', $J_{1',2'}$ = 7.8Hz), 4.46 (d, 1H, H-1", $J_{1",2"}$ = 7.5Hz), 2.08 (s, 3H, NHAc methyl), 1.27 (d, 3H, $(C_{6"})H_3$, $J_{(C_{6"})H_3,5"}$ = 7.0Hz).

Benzyl 3,6-di-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside(65)

Phthalic anhydride (0.123 g, 0.83 mmol) was added to a solution of the amine (52) (0.62 g, 1.44 mmol) in pyridine (4 mL). The solution was heated to 60°C for 20 min. Triethylamine (∿0.1 g, 0.99 mmol) and more phthalic anhydride (0.123 g, 0.83 mmol) were added and the heating was continued for 1 h. Methanol (3 mL) was added before solvent removal. The residue was again taken up in dry pyridine (4 mL) and heated at 115°C for 6 h. Solvent removal left a dark oil. This material, α,α-dimethoxytoluene (0.438 g, 2.88 mmol), p-toluenesulfonic acid monohydrate (∿5 mg) in acetonitrile (3 mL) were stirred together at room temperature for 24 h before neutralization by adding triethylamine. The residue left after solvent removal was taken up in acetic anhydride-pyridine (1:2, 3 mL) and heated to 80°C for 3 h. The oil obtained after solvent removal was redissolved in ether (150 mL) and decolorized by charcoal. Removal of solvent from the resultant solution left an oil which was applied to a silica gel column and eluted with ethyl acetate-hexane (3:2). Solvent removal of the first fraction gave a solid (0.35 g, 0.43 mmol, 30%); mp 112-116°C; $[\alpha]_D^{24}$ -34.3°(c 0.7, chloroform); ^{1}H-nmr (Fig.38) δ: 7.81-7.68 (m, 4H, phthalimido), 7.38-7.31 (m, 5H, benzylidene aromatic), 7.12-7.08 (m, 5H, benzyl aromatic, 5.71 (dd, 1H, H-3, $J_{2,3}$ = 10.0Hz, $J_{3,4}$ = 8.0Hz), 5.44 (s, 1H, benzylidene methine), 5.36 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 5.25 (dd, 1H, H-3', $J_{2',3'}$ = 8.0Hz, $J_{3',4'}$ = 8.0Hz),

4.92 (dd, 1H, H-2', $J_{1',2'}$ = 7.4Hz), 4.81 (d, 1H, benzyl methylene A, $J_{A,B}$ = 12.0Hz), 4.65 (d, 1H, H-1'), 4.53 (dd, 1H, H-6A, $J_{6A,6B}$ = 12.0Hz, $J_{5,6A}$ = 2.0Hz), 4.50 (d, 1H, benzyl methylene B), 4.26 (dd, 1H, H-6'A, $J_{6'A,5'}$ = 5.0Hz, $J_{6'A,6'B}$ = 10.0Hz), 4.24 (dd, 1H, H-2), 4.13 (dd, 1H, H-6B, $J_{6B,5}$ = 4.6Hz), 3.86 (dd, 1H, H-4, $J_{4,5}$ = 10.0Hz), 3.74 (m, 1H, H-5), 3.66 (m, 2H, H-4' and H-6' overlapping), 3.44 (m, 1H, H-5'). Anal. calcd. for $C_{42}H_{43}O_{16}N$: C 61.69, H 5.30, N 1.71; found: C 61.68, H 5.29, N 1.68.

Benzyl 3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (66)

Compound 65 was debenzylidenated by the same procedure as in the case of preparation of the diol (60). This was followed by methanesulfonylation of the 6'-OH (65% yield) and acetylation of the 4'-OH (89%) in the same way as in the case of 60. The resultant mesylate (0.1 g, 0.12 mmol), sodium iodide (70 mg, 0.46 mmol) in N,N-dimethylformamide (1.5 mL) was heated at 75°C for 20 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with 10% sodium thiosulfate solution (10 mL), and cold water (2 x 10 mL) before drying with sodium sulfate. Solvent removal left a solid (0.1 g, 0.11 mmol, 92%); ^{1}H-nmr ($CDCl_3$) δ: 7.83-7.70 (m, 4H, phthalimido), 7.14-7.08 (m, 5H, benzyl aromatic), 5.78 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.8Hz), 5.36 (d, 1H, H-1, $J_{1,2}$ = 7.8Hz), 5.15 (dd, 1H, H-3', $J_{2',3'}$ = 10.0Hz, $J_{3',4'}$ =

10.0Hz), 4.88 (m, 2H, H-2' and H-4' overlapping), 4.82 (d, 1H, benzyl methylene A, $J_{A,B}$ = 12.0Hz), 4.62 (dd, 1H, H-6A, $J_{6A,6B}$ = 12.0Hz, $J_{6A,5}$ = 2.0Hz), 4.60 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 4.51 (d, 1H, benzyl methylene B), 4.30 (dd, 1H, H-2), 4.18 (dd, 1H, H-6B, $J_{6B,5}$ = 5.0Hz), 4.00 (dd, 1H, H-4, $J_{4,5}$ = 10.0Hz), 3.84 (m, 1H, H-5), 3.46 (m, 1H, H-5'), 3.27 (dd, 1H, H-6'A, $J_{6'A,6'B}$ = 11.2Hz, $J_{6'A,5'}$ = 3.8Hz), 3.06 (dd, 1H, H-6'B, $J_{6'B,5'}$ = 8.8Hz). Anal. calcd. for $C_{37}H_{41}O_{16}NI$: I 14.42; found: I 14.86.

Benzyl 3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (67)

The iodo compound (66) (67 mg, 0.076 mmol), tri-n-butyl tin hydride (90 mg, 0.31 mmol), 2,2'-azobisisobutyronitrile (~2 mg) in toluene was heated at 60°C for 4 h. The oil obtained after solvent removal was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the first fraction left an oil (21 mg, 0.028 mmol, 37%); ^{1}H-nmr required this substance to be near pure and conformed to the structure assigned. ^{1}H-nmr (Fig.39) δ: 7.83-7.66 (m, 4H, phthalimido), 7.12-7.06 (m, 5H, benzyl aromatic), 5.72 (dd, 1H, H-3, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 8.0Hz), 5.38 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 5.12 (dd, 1H, H-3', $J_{2',3'}$ = 10.0Hz, $J_{3',4'}$ = 10.0Hz), 4.90 (dd, 1H, H-2', $J_{1',2'}$ = 8.0Hz), 4.81 (m, 2H, H-4' and benzyl methylene A), 4.57 (dd, 1H, H-6A, $J_{6A,6B}$ = 2.0Hz, $J_{6A,5}$ = 2.4Hz), 4.51 (m, 2H, H-1' and benzyl

methylene B), 4.26 (dd, 1H, H-2), 4.16 (dd, 1H, H-6B, $J_{6B,5}$ = 5.0Hz), 3.86 (dd, 1H, H-4, $J_{4,5}$ = 10.0Hz), 3.78 (m, 1H, H-5), 3.50 (m, 1H, H-5'), 2.21-1.88 (5s, 15H, acetyl methyl), 1.22 (d, 3H, $(C_{6'})H_3$, $J_{(C_{6'})H_3,5'}$ = 6.5Hz).

1,2;3,4-Di-O-isopropylidene-6-O-[3,6-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-glucopyranosyl]-α-D-galactopyranose (68)

The compound 67 (20 mg, 0.026 mmol) was hydrogenated and halogenated using Vilsmeier reagent (bromide) following the same procedures as described for the preparation of 55. The resultant bromide was condensed with 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose by the method described before for the preparation of 56. However, in this case a large excess (50 equivalent) of the alcohol was used. The resultant trisaccharide, however, had the same R_f on tlc (1 ethyl acetate: 1 hexane) as that of the alcohol. The reaction mixture, after work-up, was treated with acetic anhydride-pyridine. The 6-O-acetylated alcohol was readily separated from the trisaccharide by silica gel column chromotography. The yield was 12.6 mg (54%) of an oil; ^{1}H-nmr (Fig.40) δ: 7.88-7.65 (m, 5H, phthalimido), 5.77 (dd, 1H, H-3', $J_{2',3'}$ = 10.5Hz, $J_{3',4'}$ = 8.0Hz), 5.43 (d, 1H, H-1', $J_{1',2'}$ = 8.5Hz), 5.12 (m, 2H, H-1 and H-3" overlapping, $J_{1,2}$ = 5.0Hz, $J_{2",3"}$ = 10.0Hz, $J_{3";4"}$ = 10.0Hz), 4.90 (dd, 1H, H-2"), 4.80 (dd, 1H, H-4"), 4.51 (m, 2H, H-1", $J_{1",2"}$ = 8.0Hz overlapping possibly with

H-6'A), 4.40 (dd, 1H, H-3, $J_{2,3}$ = 2.5Hz, $J_{3,4}$ = 8.0Hz), 4.21 (dd, 1H, H-2'), 4.14 (dd, 1H, H-6'B), 4.10 (dd, 1H, H-2), 4.00 (dd, 1H, H-4, $J_{4,5}$ = 1.5Hz), 3.90 (dd, 1H, H-4'), 3.84-3.50 (remaining protons consisting of H-5, H-5' and H-5"), 2.16-1.88 (5s, 15H, acetyl methyl), 1.40, 1.23, 1.03, 1.02 (4s, 12H, O-C(=O)$(CH_3)_2$), 1.22 (d, 3H, $(C_{6"})H_3$, $J_{(C_{6"})H_3,5"}$ = 6.2Hz).

1,2;3,4-Di-O-isopropylidene-6-O-[2-acetamido-2-deoxy-4-O-(6-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranose (33)

Hydrazinolysis of the compound 8 was carried out with the same method as described for the preparation of 32 to yield a white solid (4.5 mg, 0.007 mmol, 60%); ^{1}H-nmr (Fig.41) δ: 5.62 (d, 1H, H-1, $J_{1,2}$ = 5.0Hz), 4.53 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 4.52 (dd, 1H, H-2, $J_{2,3}$ = 3.0Hz), 4.39 (dd, 1H, H-4, $J_{4,5}$ = 2.0Hz), 2.03 (s, 3H, acetamido methyl), 1.55-1.37 (4s, 12H, O-C(=O)$(CH_3)_2$), 1.28 (d, 3H, $(C_{6"})H_3$).

Methyl 4-O-(β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (69)

A solution of sodium methoxide in methanol (0.12N, 5 mL) was added to the blocked methyl glycoside (38) (155 mg, 0.21 mmol) and the mixture was stirred for 15 min. IR-120 (H^+) resin was then added quickly to the resulting clear solution to bring the pH to ~6.0. Removal of the resin by filtration

and solvent removal left a solid (103 mg, 0.21 mmol, 100%). ^{1}H-nmr spectrum of this substance (Fig.42) is consistent with the structure assigned.

Methyl 3,6-di-O-acetyl-4-O-(2,3-di-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (70)

The deblocked methyl glycoside (69) (92 mg, 0.19 mmol), α,α-dimethoxytoluene (58 mg, 0.38 mmol) and p-toluenesulfonic acid monohydrate (∿2 mg) in dry acetonitrile (2 mL) were stirred together at room temperature for 24 h. An additional amount (58 mg, 0.38 mmol) of α,α-dimethoxytoluene was added and the mixture was again stirred for 48 h. Trimethylamine was added dropwise to bring the pH to about 7. Solvent removal left an oil which was directly taken up in acetic anhydride-pyridine (1:2, 2 mL) and kept at room temperature for 12 h. Solvent removal from the main fraction left a solid. This material was treated directly with 80% aqueous acetic acid at 80°C for 0.5 h. Solvent removal left an oil which was applied to a silica gel column and eluted with ethyl acetate-hexane (4:1). Solvent removal from the main fraction left a solid (58 mg, 0.089 mmol, 48%). ^{1}H-nmr (Fig.43) δ: 7.90-7.75 (m, 4H, phthalimido), 5.35 (d, 1H, H-1, $J_{1,2}$ = 8.0Hz), 5.34 (dd, 1H, H-2', $J_{1',2'}$ = 8.0Hz, $J_{2',3'}$ = 10.0Hz), 4.95 (dd, 1H, H-3', $J_{3',4'}$ = 2.4Hz), 4.60 (bd, 2H, H-1' overlapping possibly with H-6A), 4.25-4.15 (m, 3H, tentatively assigned to H-2, H-6B and H-4'), 4.00-3.80 (m, 4H, tentatively assigned to H-4, H-5 and two H-6'), 3.74 (bm, 1H, H-5'), 3.50 (s, 3H, OCH_3), 2.22, 2.16, 2.15, 2.02 (4s, 12H, acetyl methyl).

Methyl 3,6-di-O-acetyl-4-O-[2,3-di-O-acetyl-6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galacto-pyranosyl]-2-deoxy-2-phthalimido-β-D-glucopyranoside (71)

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl bromide (72) (74 mg, 0.15 mmol) in nitromethane (2 mL) was added to a solution of the diol (70) (48 mg, 0.074 mmol), sym-collidine (9.0 mg, 0.074 mmol) and silver triflate (19 mg, 0.074 mmol) in dry nitromethane (0.5 mL) cooled to -15°C. The reaction mixture was stirred for 3 h (no reaction was observed to occur). The mixture was allowed to warm to room temperature and stirred for overnight. The solids were removed by filtration and the filtrate was diluted with chloroform (5 mL) before washing with sodium thiosulfate solution (5 mL), cold water (5 mL) and sodium bicarbonate solution (2 x 5 mL). Solvent removal, after drying over anhydrous sodium sulfate, left a foam which was applied to a silica gel column and eluted with ethyl acetate-hexane (1:1). Solvent removal of the second fraction left a white solid (40 mg, 0.037 mmol, 50%). ^{1}H-nmr (Fig.44) δ: 7.90-7.70 (m, 4H, phthalimido), 5.82-5.65 (2dd, 2H, H-3 and H-3"), 5.50 and 5.30 (dd, 2H, H-1 and H-1"), 5.25-3.72 (m, 17H, remaining protons), 3.46 (s, 3H, OCH_3), 2.20, 2.12, 2.10, 1.92, 1.86 (5s, 21H, acetyl methyl).

Methyl 4-O-[6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (34)

A solution of compound 71 (35 mg, 0.033 mmol) and 97% hydrazine hydrate (0.3 g, 9.4 mmol) in ethanol (2 mL) was refluxed for 5 h. Solvent removal left a solid which was directly taken up in methanol-water (1:1, 2 mL) containing acetic anhydride (0.4 mL) and left at room temperature for 2 h. Solvent removal left a solid which was dissolved in water and applied to a Biogel P-2 column and eluted with water-ethanol (9:1). Solvent removal from the main fractions gave a solid (16 mg, 0.027 mmol, 81%). ^{1}H-nmr (Fig.46) δ: 4.65 (d, 1H, H-1', $J_{1',2'}$ = 8.0Hz), 4.50 (2d, 2H, H-1 and H-1", J = 7.5Hz and 7.5Hz), 3.55 (s, 3H, OCH_3), 2.12, 2.11 (2s, 6H, N-acetyl methyl), 4.05-3.42 (m, 18H, remaining protons); ^{13}C-nmr (Fig.47) δ: 174.7, 174.6 (NHAc carbonyl carbon), 103.2, 102.0 and 101.2 (anomeric carbons), 79.3, 76.0, 74.9, 73.9, 72.7, 72.6, 71.0, 70.1, 68.7, 68.4, 60.9 and 60.2 (two C-6s), 57.2 (OCH_3), 55.7, 55.1, 22.5, 22.4 (NHAc methyl carbon).

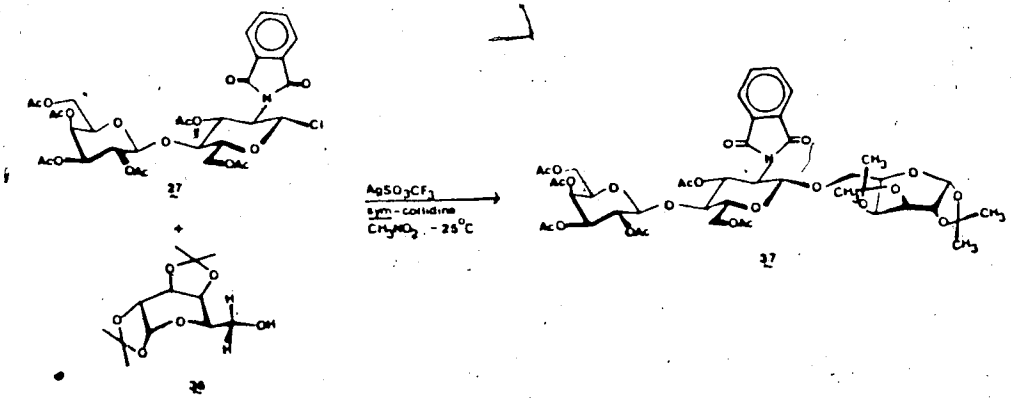
DISCUSSION OF RESULTS

This portion of the thesis is presented in four parts. Part I consists of a description of the syntheses and characterizations of the various components tested as inhibitors of the anti-I Ma antibodies. It is divided into six sections. The products prepared in each section are obtained following similar synthetic routes. Since the thesis relates to the binding of the structures to an antibody, it was desirable to investigate the conformational preferences of the various inhibitors and this is presented in Part II. Part III presents without discussion the inhibition data provided by Drs. Kaba and Liao. Part IV is concerned with the interpretation of t inhibition data in terms of the structural and conformational properties of the compounds tested.

PART I Chemical Syntheses

A. Syntheses of the 2-amino-2-deoxy- (18), the 2-acetamido-2-deoxy-(17) and the 2-deoxy-2-trifluoroacetamido- (19) derivatives of the βDGal(1→4)βDGlc(1→6)1,2;3,4-di-O-isopropylidene-αDGal

In order to prepare the blocked trisaccharide (37), a key starting material for the syntheses described in this thesis, the phthalimido chloride (27) which was prepared following published procedures[39] was condensed with 1,2;3,4-di-O-isopropylidene-α-D-galactose (36) under the condition described by Lemieux et al.[42] Compound 37 was obtained in 72% yield and characterized by the ^{1}H-nmr spectrum reproduced in Fig. 4. The spectrum is in accord with the structural assignment and indicates sufficient state of purity for this product to be used for the preparation of the amine 18.



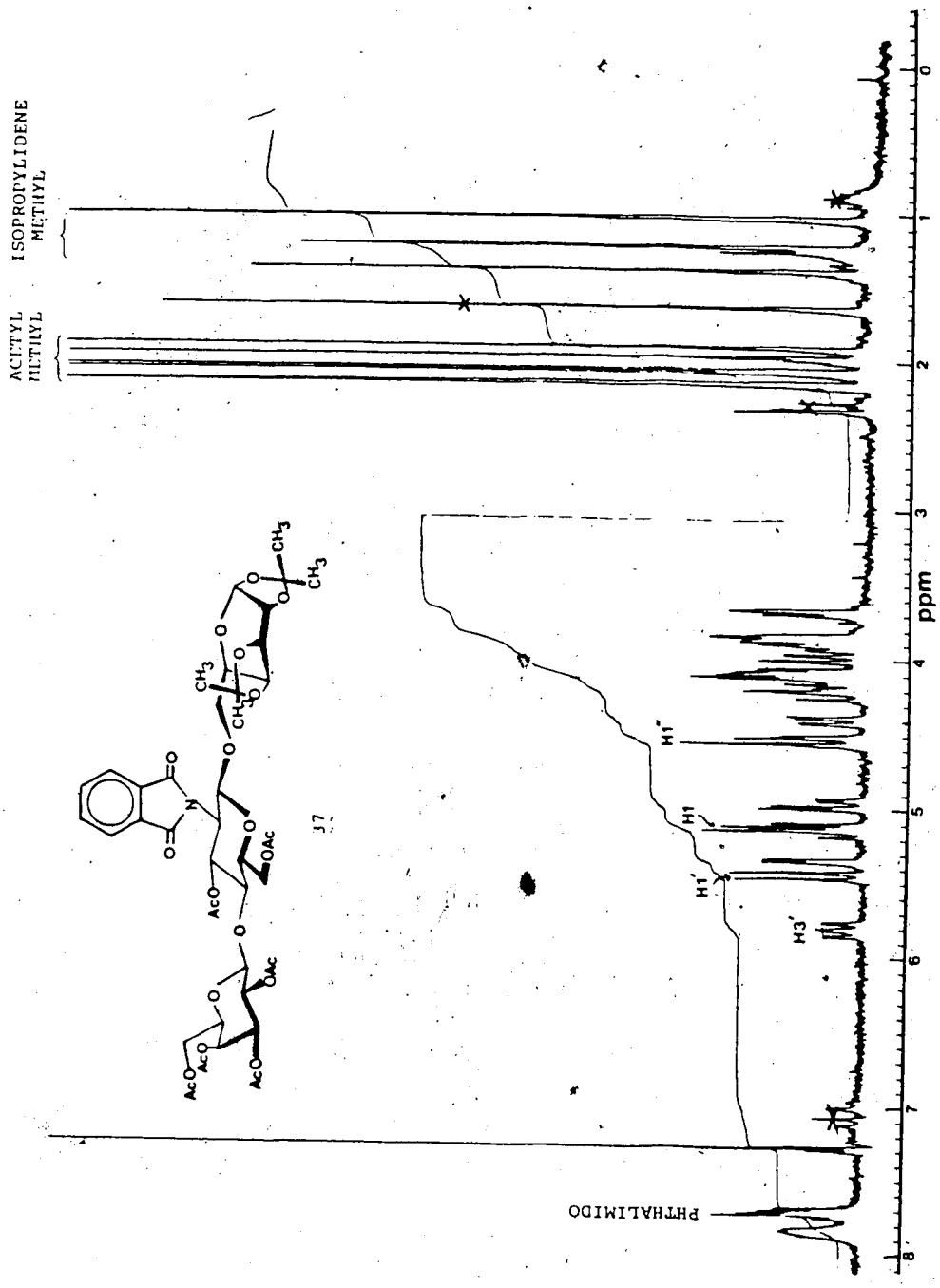


Fig. 4 The 200 MHz ^{1}H-nmr spectrum of compound 37 in $CDCl_3$.

In order to prepare the amine 18, compound 37 was treated with hydrazine in the usual manner.[42] The product was an amorphous solid obtained in 78% yield after purification by filtration through a Sephadex LH-20 column. Its characterization will be discussed in connection with the structurally related compounds 17 and 19.

The free amine (18) was converted to the acetamido- compound (17) in 82% yield by treatment with acetic anhydride in methanol-water (1:1) and to the trifluoroacetamido- compound (19) in 57% yield by treatment with S-ethyl trifluorothioacetate in methanol.[50]

The structures and ^{1}H-nmr spectra of compounds 17, 18 and 19 are presented in Fig. 5 - Fig. 7. The free amine is well characterized by the low-field triplet at δ3.05 which can be assigned to H-2', this signal is not present in the spectra for the acetamido- (17) and trifluoroacetamido- (19) compounds. It is expected that the acylation of the amine will cause a deshielding of H-2'. It seems that the signals for H-1' in 17 and 19 are also shifted downfield from that of the amine by 0.10 ppm and 0.24 ppm, respectively. This observation is as expected and confirms the structural assignment.

B. Syntheses of the glycosides βDGal(1→4)βDGlcNAcOR (20, R=CH_3; 21, R=CH_2CH_3; 22, R=$CH_2CH_2CH_3$; 23, R=$CH(CH_3)_2$; 24, R=$CH_2CH(CH_3)_2$)

As expected, 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl

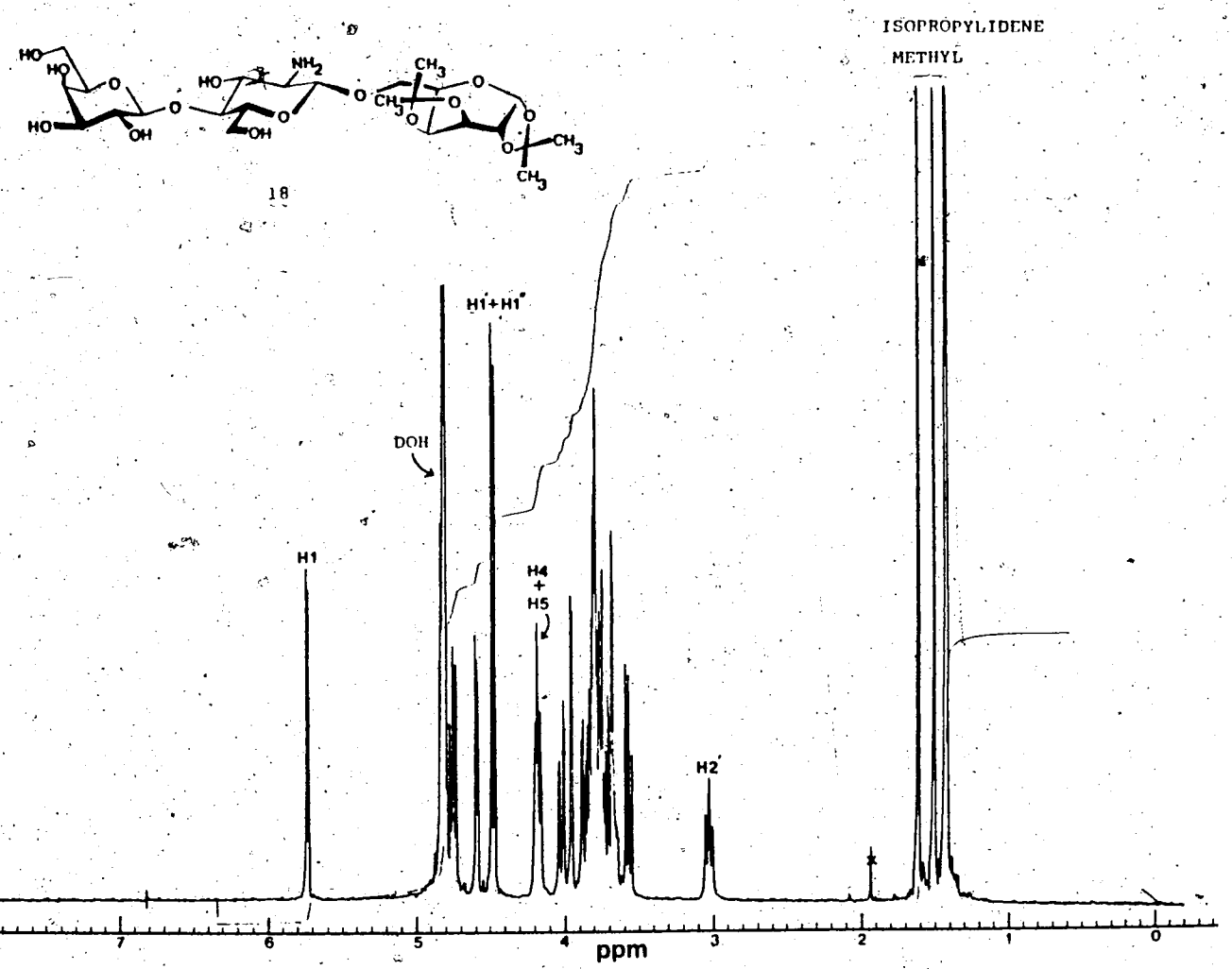


Fig. 5 The 400 MHz ^{1}H-nmr spectrum of compound 18 in D_2O.

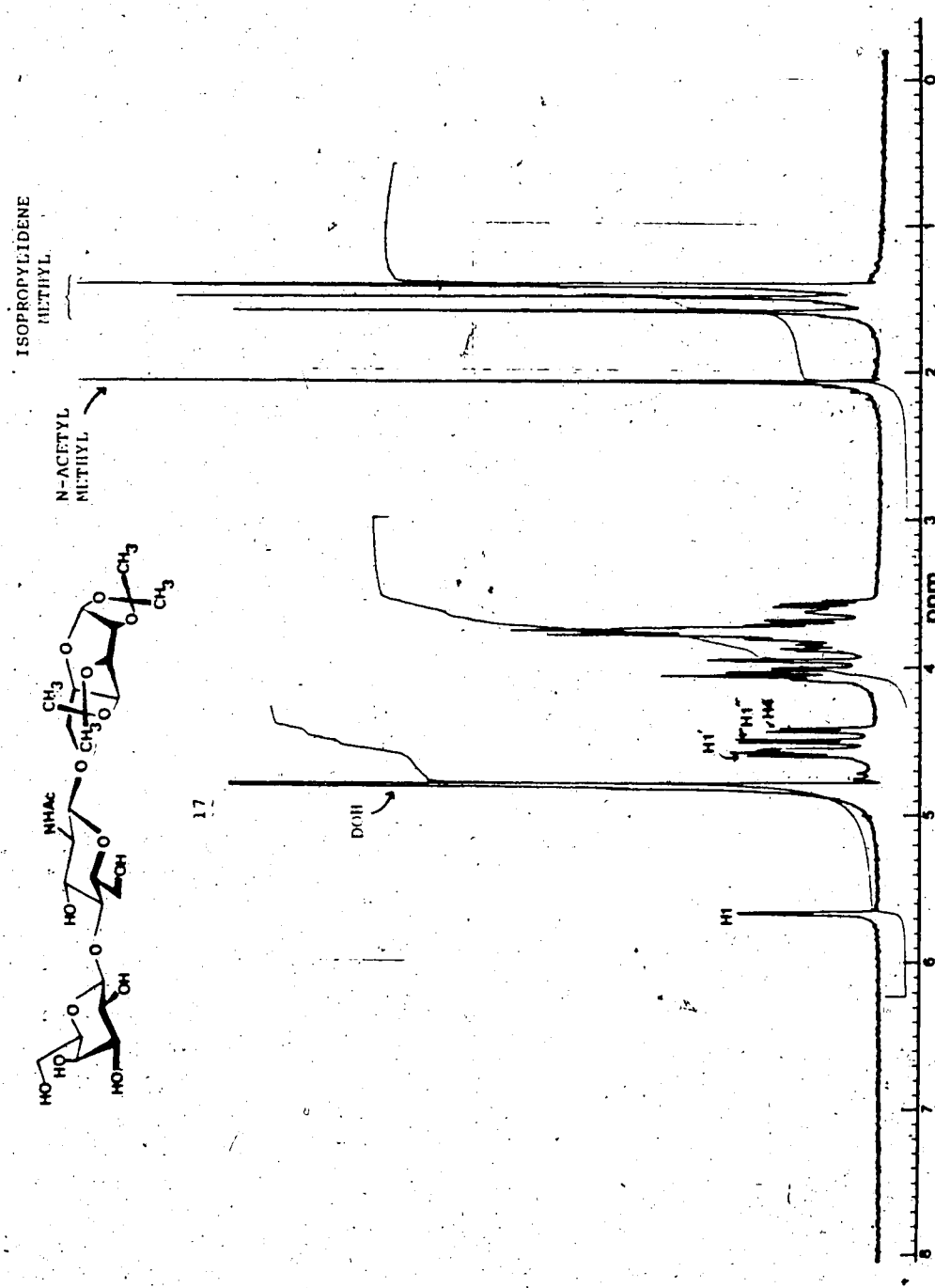


Fig. 6 The 400 MHz ^{1}H-nmr spectrum of compound 17 in D_2O.

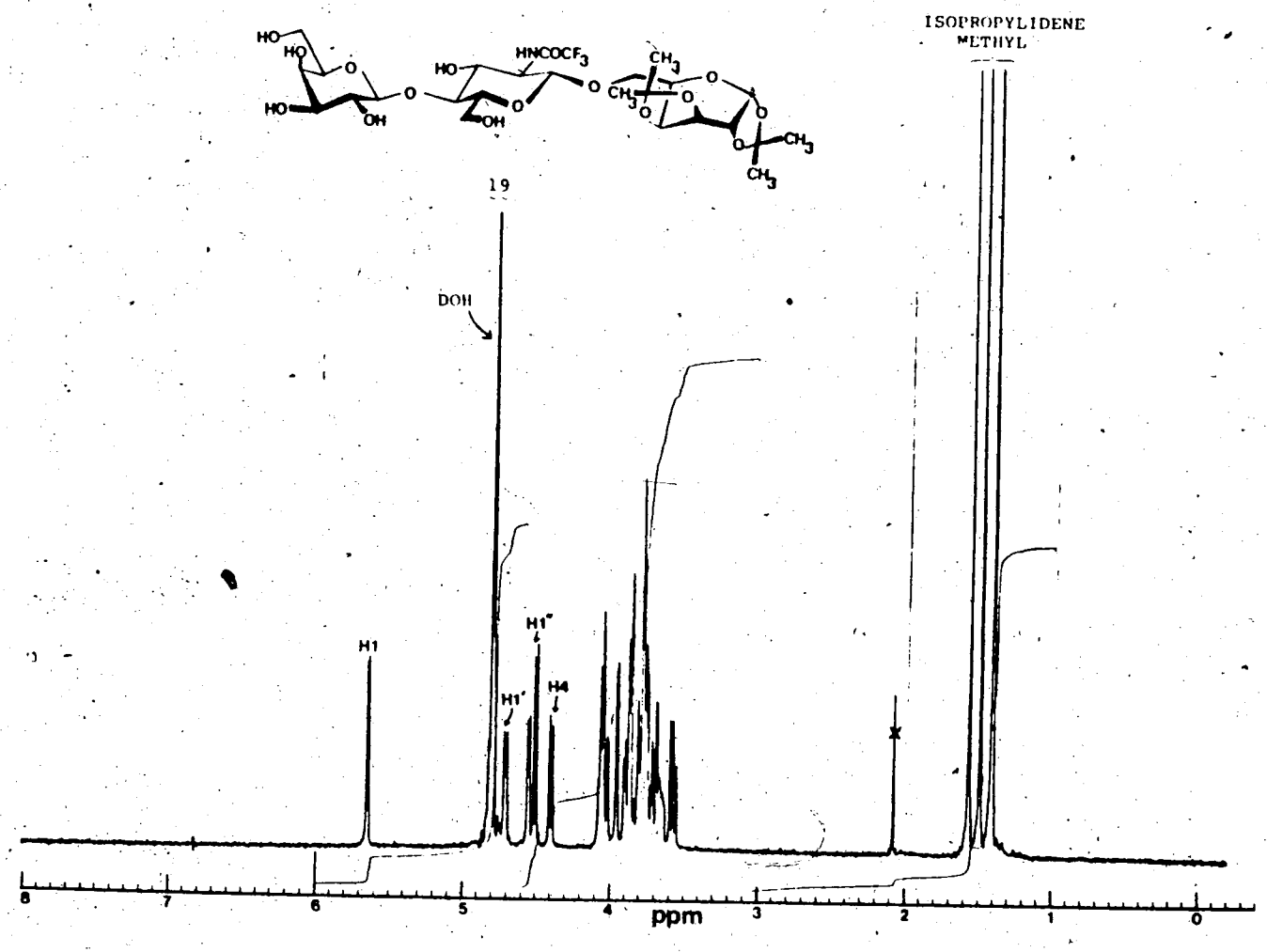


Fig. 7 The 400 MHz ^{1}H-nmr spectrum of compound 19 in D_2O.

bromide (28) could be prepared in high yield (85%) from 3,6-di-O-acetyl-4-O-(tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranose (38) by treatment with the Vilsmeier reagent (N,N-dimethylbromoforminium bromide)[51,52] under the same conditions reported for the preparation of the chloride (27).[39] This semi-crystalline product proved quite stable and could be kept for six months at 4°C in an atmosphere free of moisture. The glycosylation reactions of the bromide (28) with the various alkanols proceeded rapidly under the condition described by Lemieux et al.[42] The glycosides 38 - 42 were obtained in crystalline form with yields ranging from 70 - 80%. These products were shown to be pure and consistent with the assigned structures by the ^{1}H-nmr spectra presented in Fig. 8 - Fig. 12.

All of the glycosides except the ethyl glycoside (39) were completely deblocked by hydrazinolysis before N-acetylation.[42] The final products were then purified by hplc using reverse phase C18 column before recrystallization. The overall yields range from 40% in the case of the isopropyl glycoside (23) to 67% in the case of the n-propyl glycoside (22). The poor yield in the former case was a result of the isolation procedure. It is expected that the procedure described below for the preparation of the ethyl glycoside (21) which employs de-O-acetylation prior to removal of the phthalimido group, would have provided a higher yield.

The ethyl glycoside (39) was first de-O-acetylated by sodium methoxide in methanol before the removal of the

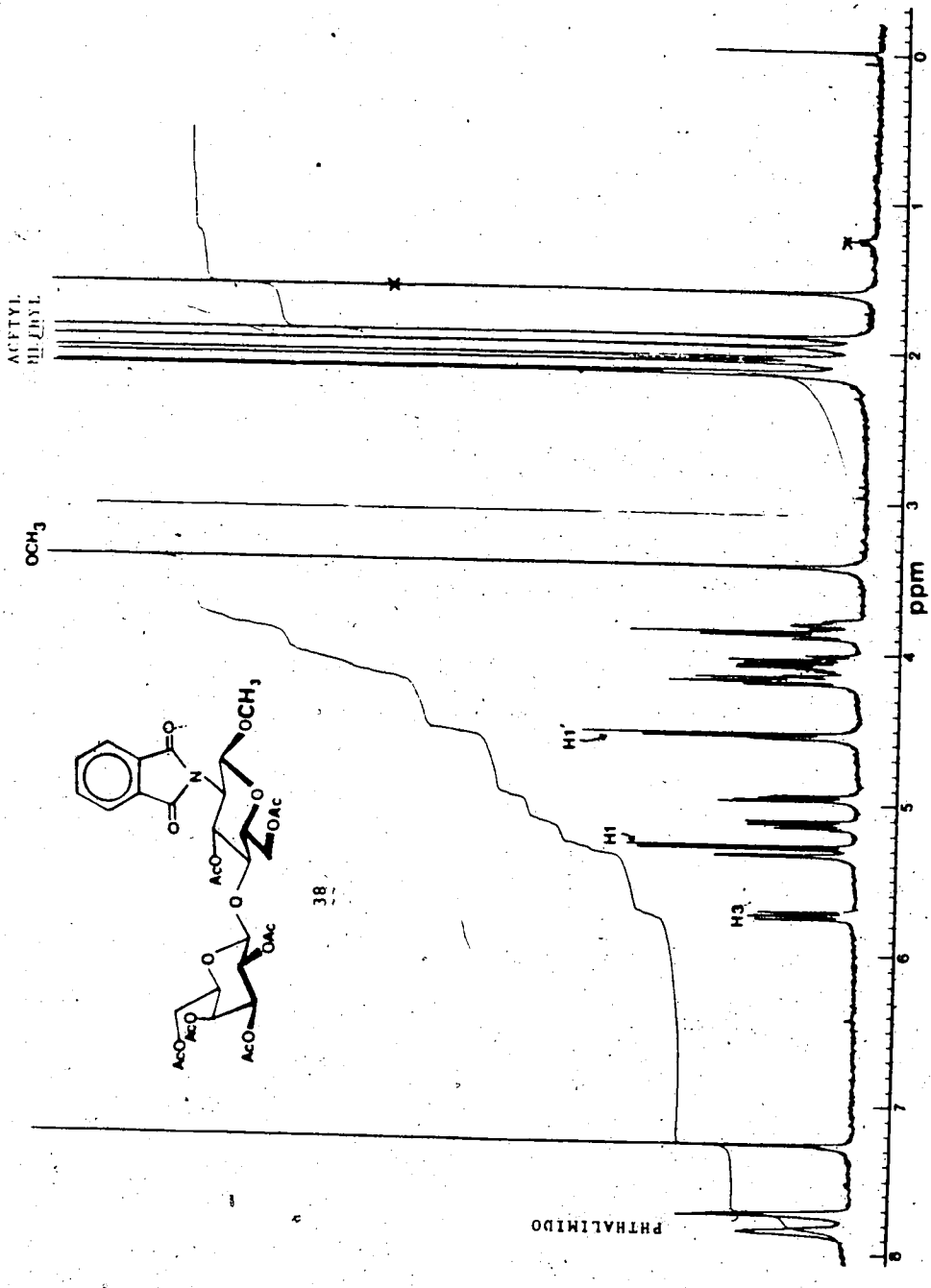


Fig. 8 The 400 MHz ^{1}H-nmr spectrum of compound 38 in $CDCl_3$.

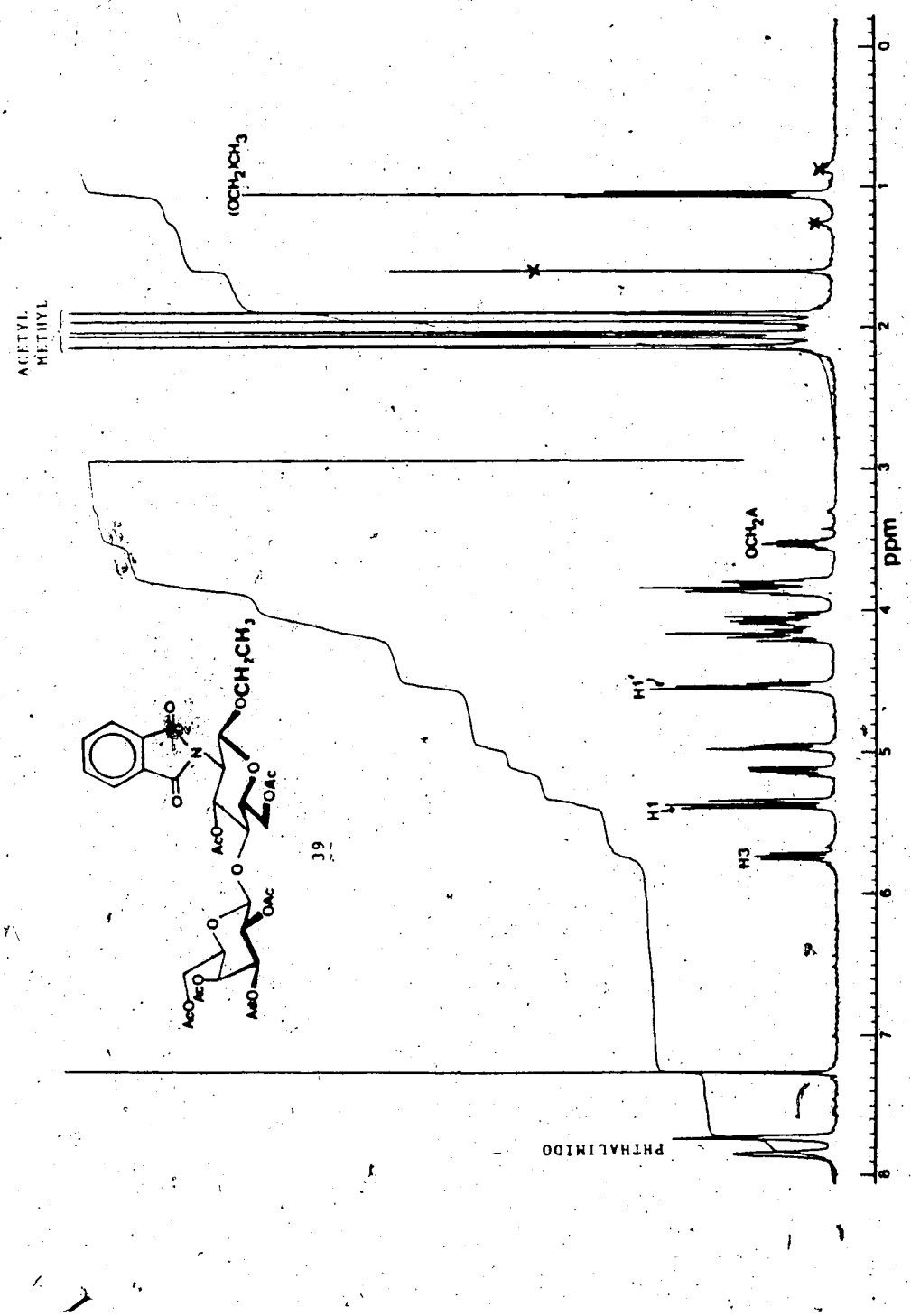
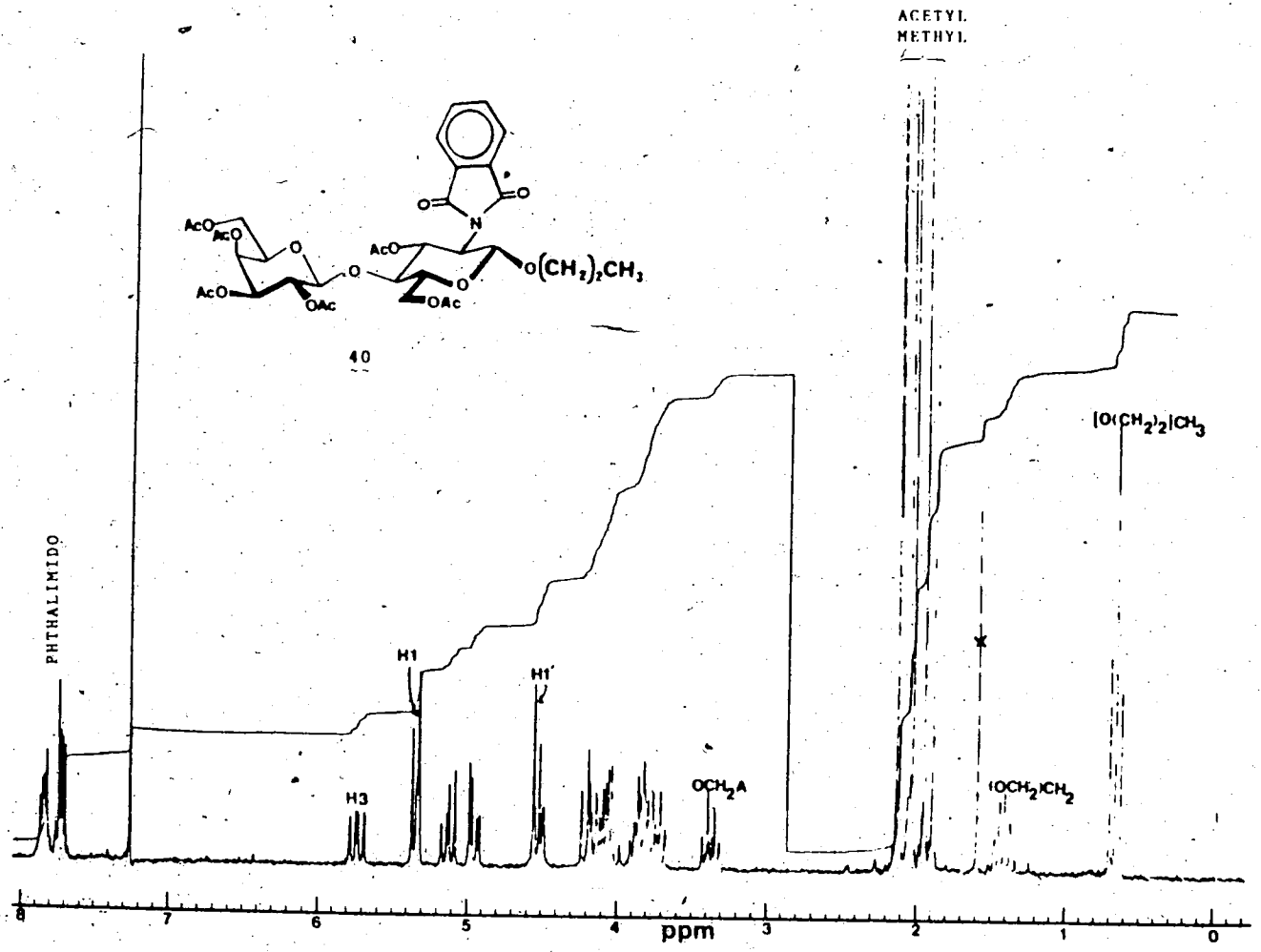


Fig. 9 The 400 MHz ^{1}H-nmr spectrum of compound 39 in $CDCl_3$.



40

Fig. 10 The 200 MHz ^{1}H-nmr spectrum of compound 40 in $CDCl_3$.

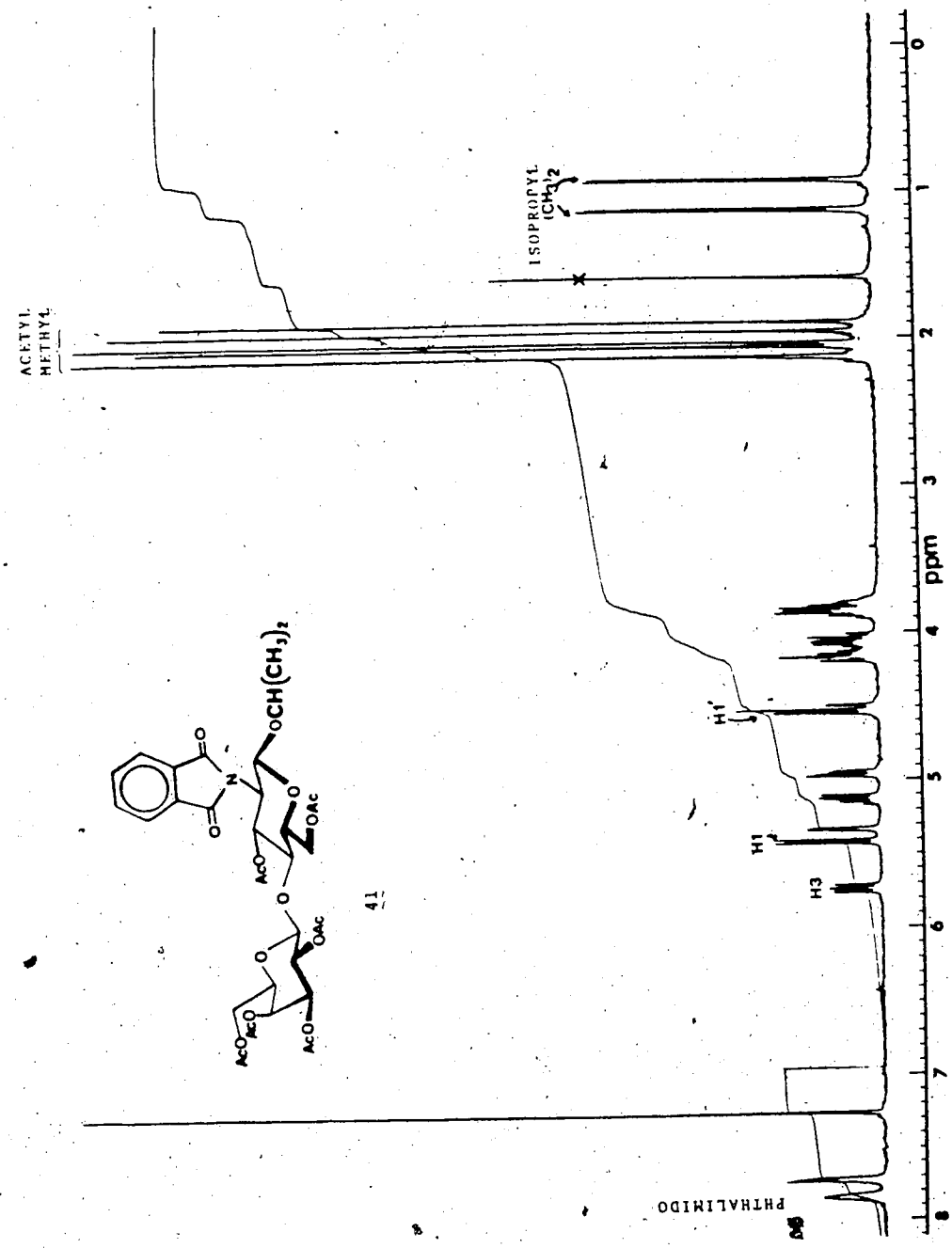


Fig. 11 The 400 MHz ^{1}H-nmr spectrum of compound 41 in $CDCl_3$.

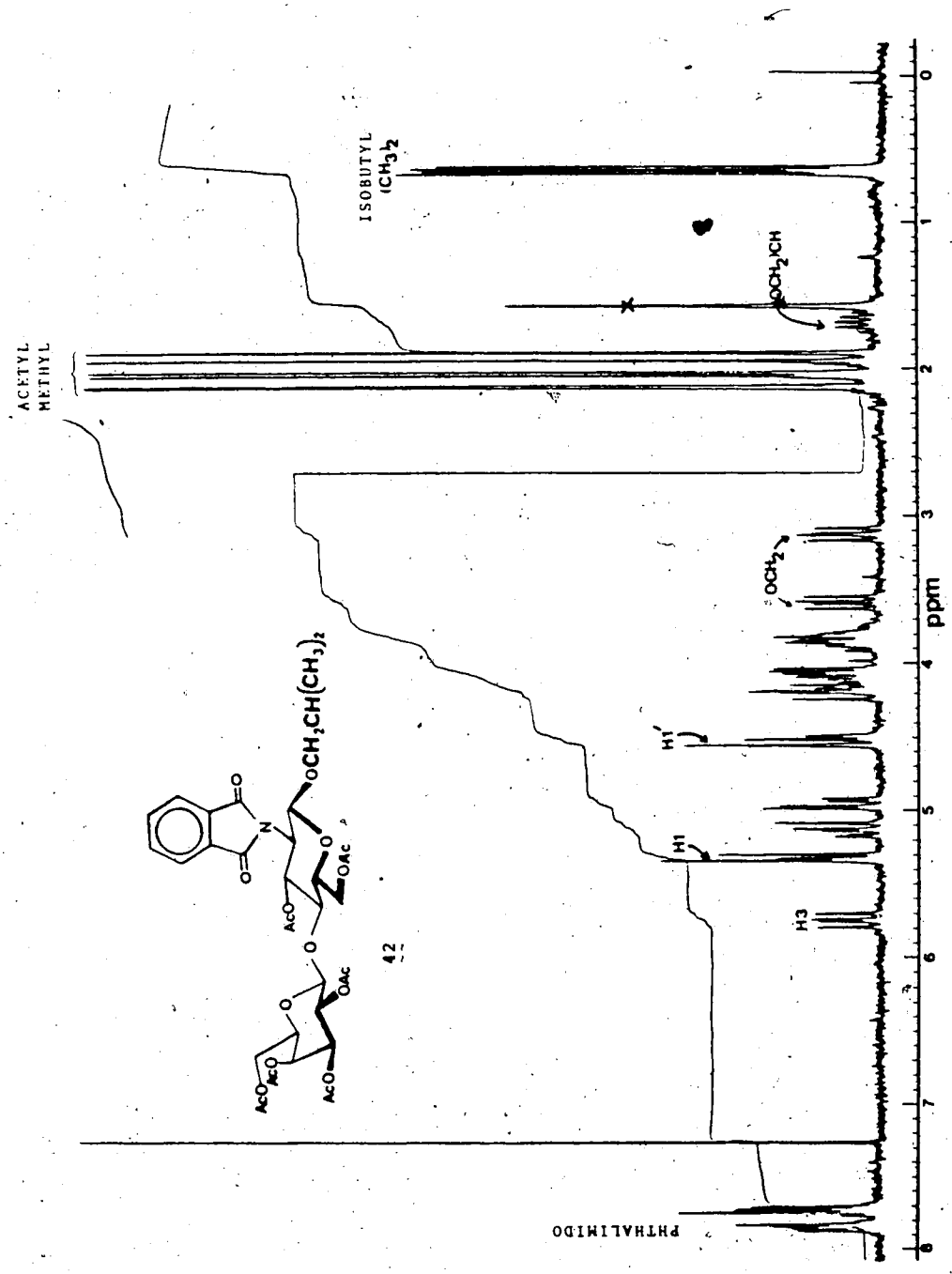


Fig. 12 The 200 MHz ^{1}H-nmr spectrum of compound 42 in $CDCl_3$.

phthalimido group by hydrazinolysis. The material obtained after N-acetylation was purified directly by recrystallization from methanol and the overall yield of 21 was 61%. The methyl glycoside (20) was prepared again following this procedure except that the final product was purified by Biogel P-2 column. In this way, the yield after purification was improved to 80%.

The ^{1}H-nmr spectra of the alkyl glycosides 20-24 are presented in Fig. 13 - Fig. 17. The signals which could be assigned (Table III) on the basis of splitting patterns confirmed the assignments for βDGal(1→4)βDGlcNAcO$(CH_2)_8COOCH_3$ published by Lemieux et al.[38] It can be seen (Table III) that the chemical shifts of the ring protons are essentially the same for all the glycosides except for certain anomeric H-1 atoms which are attached to the different alkoxy groups. As could be expected, the signals for H-1 are essentially the same in the case of the ethyl, propyl and the isobutyl glycosides. The chemical shift for H-1 of the methyl glycoside is 0.06 ppm upfield and that of the isopropyl glycoside is 0.12 ppm downfield.

C. Syntheses of the diastereoisomeric βDGal(1→4)βDGlcNAc-(1→6)6-C-CH_3-DGal trisaccharides (25 and 26)

It was anticipated that the preparation of the trisaccharide 25 and 26 would restrict the conformational preferences as compared to the related trisaccharide (5) which, to date, has been the natural structure most active in the

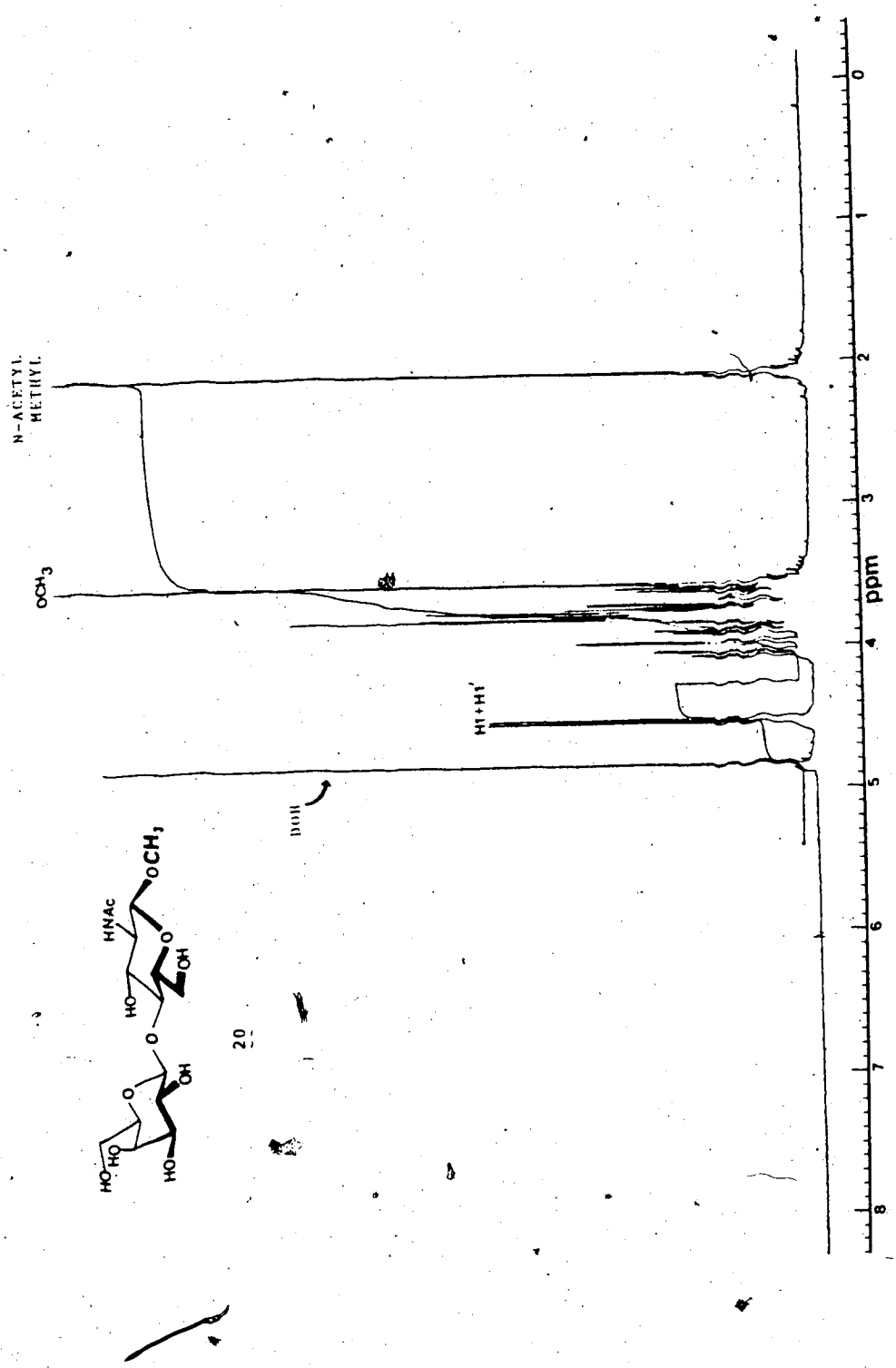


Fig. 13 The 400 MHz ^{1}H-nmr spectrum of compound 20 in D_2O.

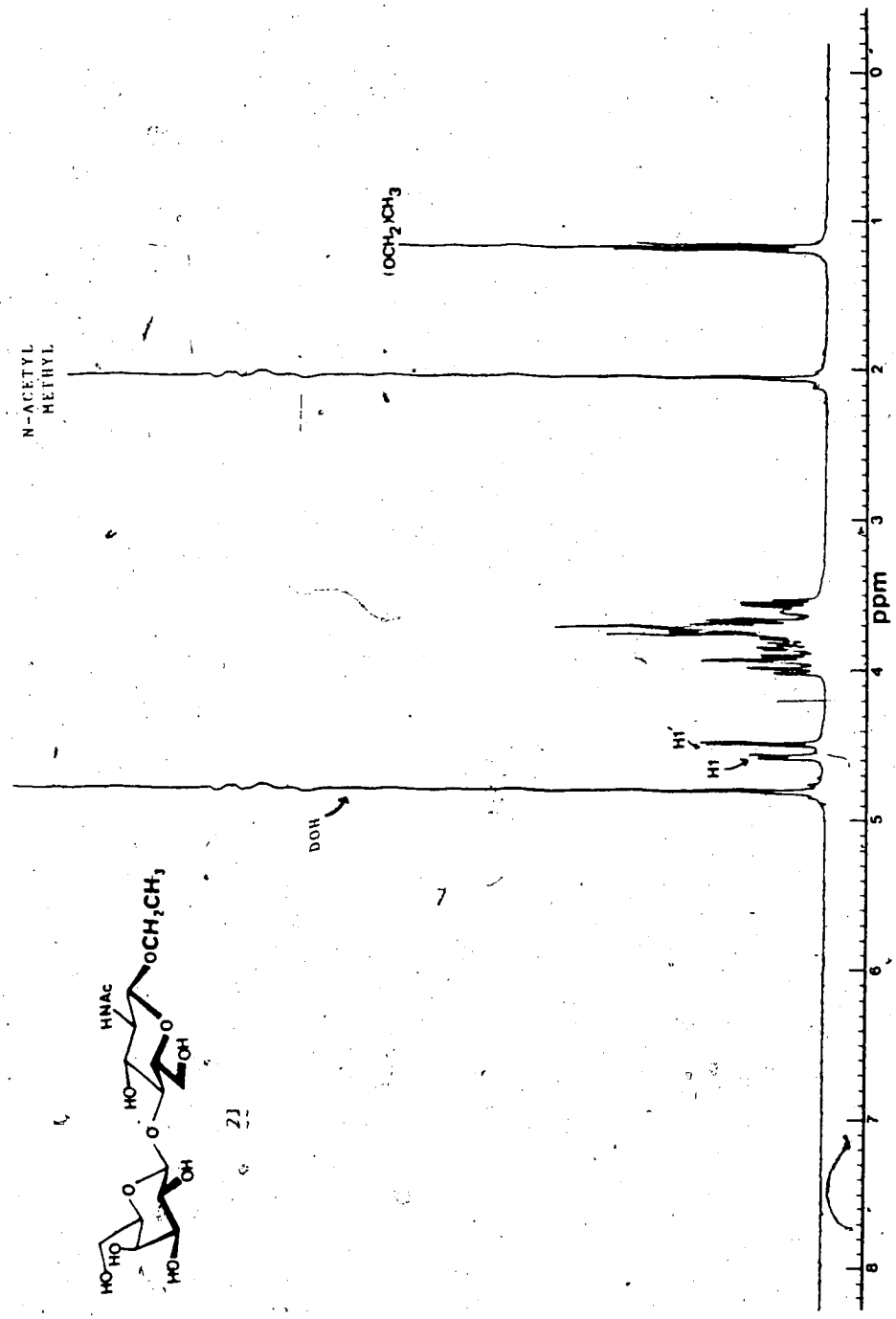


Fig. 14 The 400 MHz ^{1}H-nmr spectrum of compound 21 in D_2O.

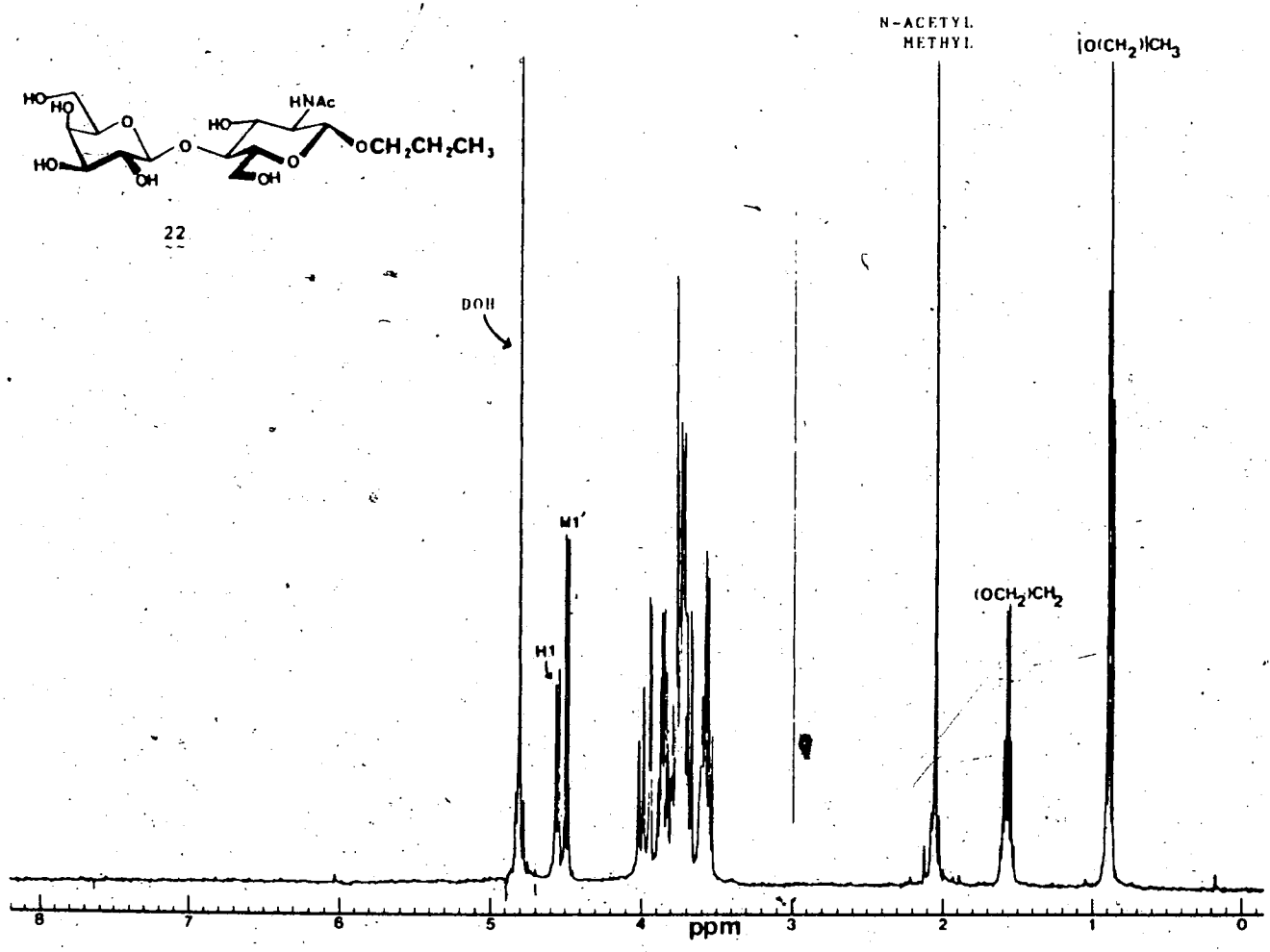


Fig. 15 The 400 MHz ^{1}H-nmr spectrum of compound 22 in D_2O.

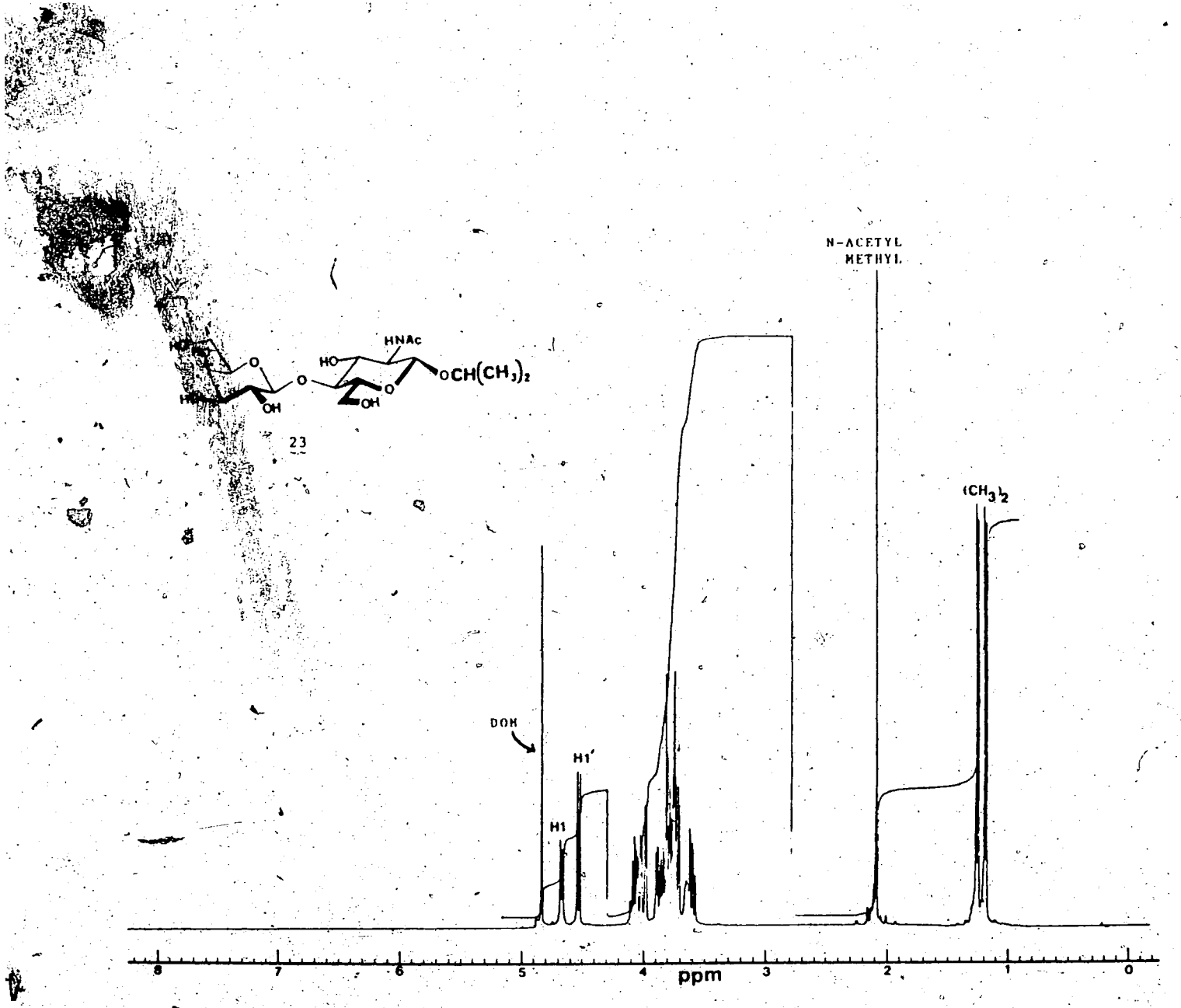


Fig. 16 The 400 MHz ^{1}H-nmr spectrum of compound 23 in D_2O.

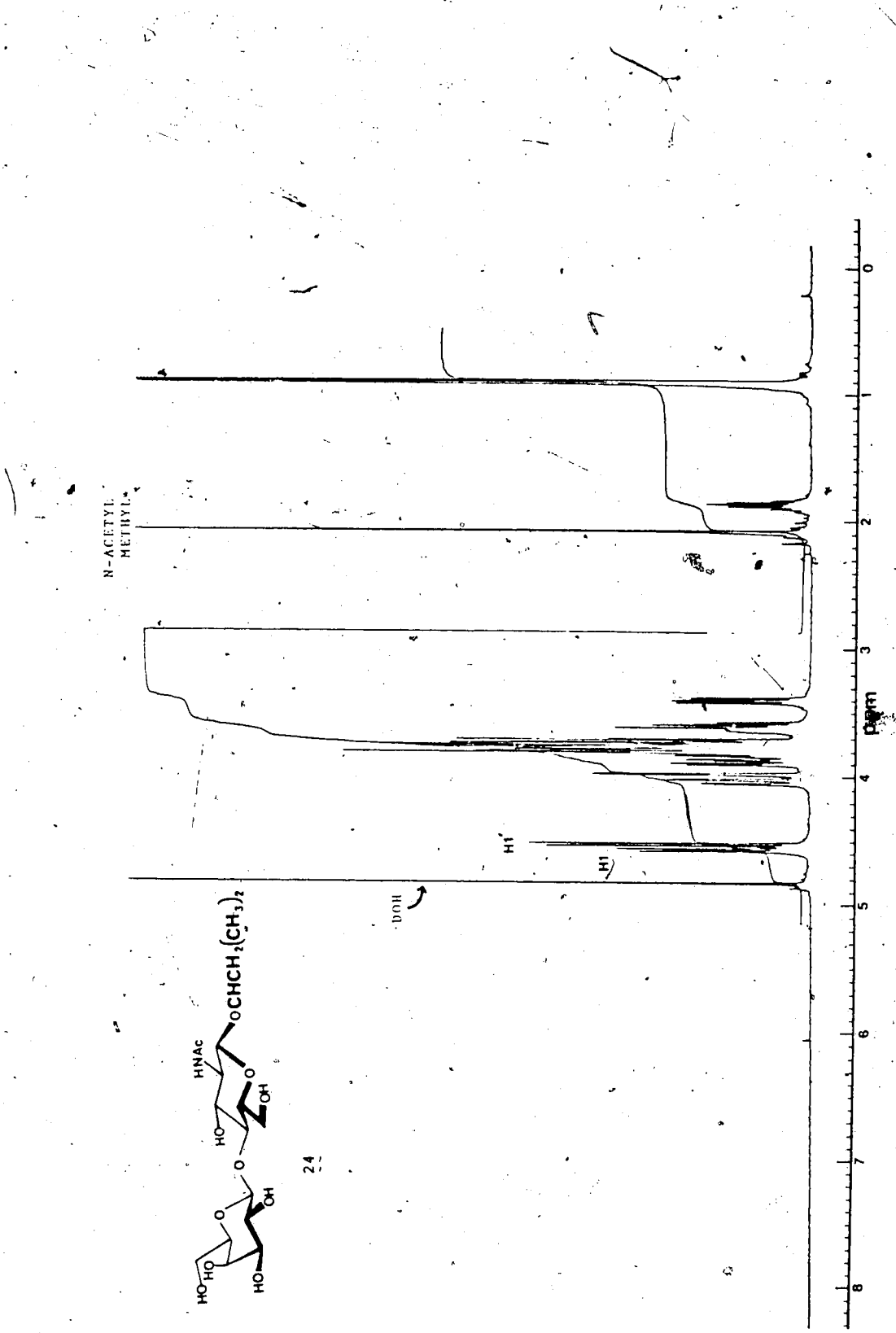


Fig. 17 The 400 MHz ^{1}H-nmr spectrum of compound 24 in D_2O.

Table III ^{1}H-nmr Chemical Shifts for βDGal(1→4)βDGlcNAcOR Glycosides

	βDGal							βDGlcNAc							
βDGal(1→4)βDGlcNAcOR	1	2	3	4	5	6A	6B	1	2	3	4	5	6A	6B	Aglyconic Protons
R=$(CH_2)_8COOCH_3$*	4.50	3.58	3.70	3.96	3.75	3.82-3.76		4.55	3.74	3.74	3.72	3.61	4.00	3.86	— §
R=CH_3	4.50	3.60	3.72	4.00	—†	—	—	4.50	—	—	—	3.65	4.06	3.90	3.55 (CH_3)
R=CH_2CH_3	4.50	3.54	3.67	3.95	—	—	—	4.56	—	—	—	3.60	4.00	3.84	3.90, 3.69 (CH_2), 1.20 (CH_3)
R=$CH_2CH_2CH_3$	4.50	3.55	3.68	3.95	—	—	—	4.56	—	—	—	3.60	4.00	3.82	3.86 (CH_2), 1.59 (CH_2), 0.84 (CH_3)
R=$CH(CH_3)_2$	4.50	3.60	3.72	4.00	—	—	—	4.68	—	—	—	3.65	4.04	3.86	4.09 (CH), 1.36 (CH_3), 1.20 (CH_3)
R=$CHCH_2(CH_3)_2$	4.50	3.58	3.71	4.00	—	—	—	4.56	—	—	—	3.62	4.03	3.90	3.40 (CH_2), 1.86 (CH), 0.92 (CH_3), 0.92 (CH_3)

* ^{1}H-nmr chemical shifts (270MHz) were reported by Lemieux et al[38] in D_2O relative to 1% acetone, δ=2.48 as internal reference. The ^{1}H-nmr chemical shifts for the other glycosides obtained in the present work were recorded at 400 MHz with internal reference HOD=4.80. For convenience of comparison in this table, the values reported by Lemieux et al for βDGal(1→4)βDGlcNAcO$(CH_2)_8COOCH_3$ are adjusted by assuming all signals for H-1'(βDGal) are the same at δ=4.50.

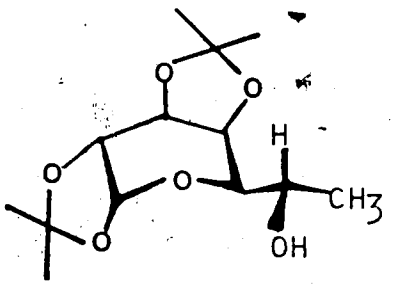
§ These values were not reported by Lemieux et al.[38]

† Signals for these protons are not assigned.

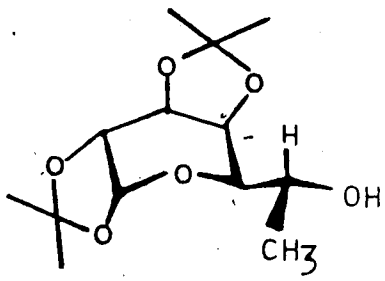
binding of the anti-I Ma antibody. These considerations will be discussed later on in Part IV of this discussion.

The compound 1,2;3,4-di-O-isopropylidene-α-D-galacto-1,6-dialdo-hexopyranose (43) was obtained in 95% yield by oxidation of 1,2;3,4-di-O-isopropylidene-α-D-galactose using chromium trioxide-pyridine complex in dichloromethane as was described by Arrick et al.[53] This syrupy product was used in the preparation of the compound 29 and 30 since the ^{1}H-nmr spectrum showed this product to be of as good a quality as that obtained by distillation[53] which resulted in a lower yield (62%).

In a study of the binding of antibodies specific for the β-D-galactopyranosyl group, Lemieux et al[43] observed that the addition of methylmagnesium bromide to 1,2;3,4-di-O-isopropylidene-α-D-galacto-1,6-dialdohexopyranose (43)[46,53] provided the L- and D-isomer 29 and 30, respectively, in a ratio of about 3:1. The two isomers were not separated, but were carried on to the next reaction and separated only as the methyl β-glycosides. In the present work, two products were obtained



29

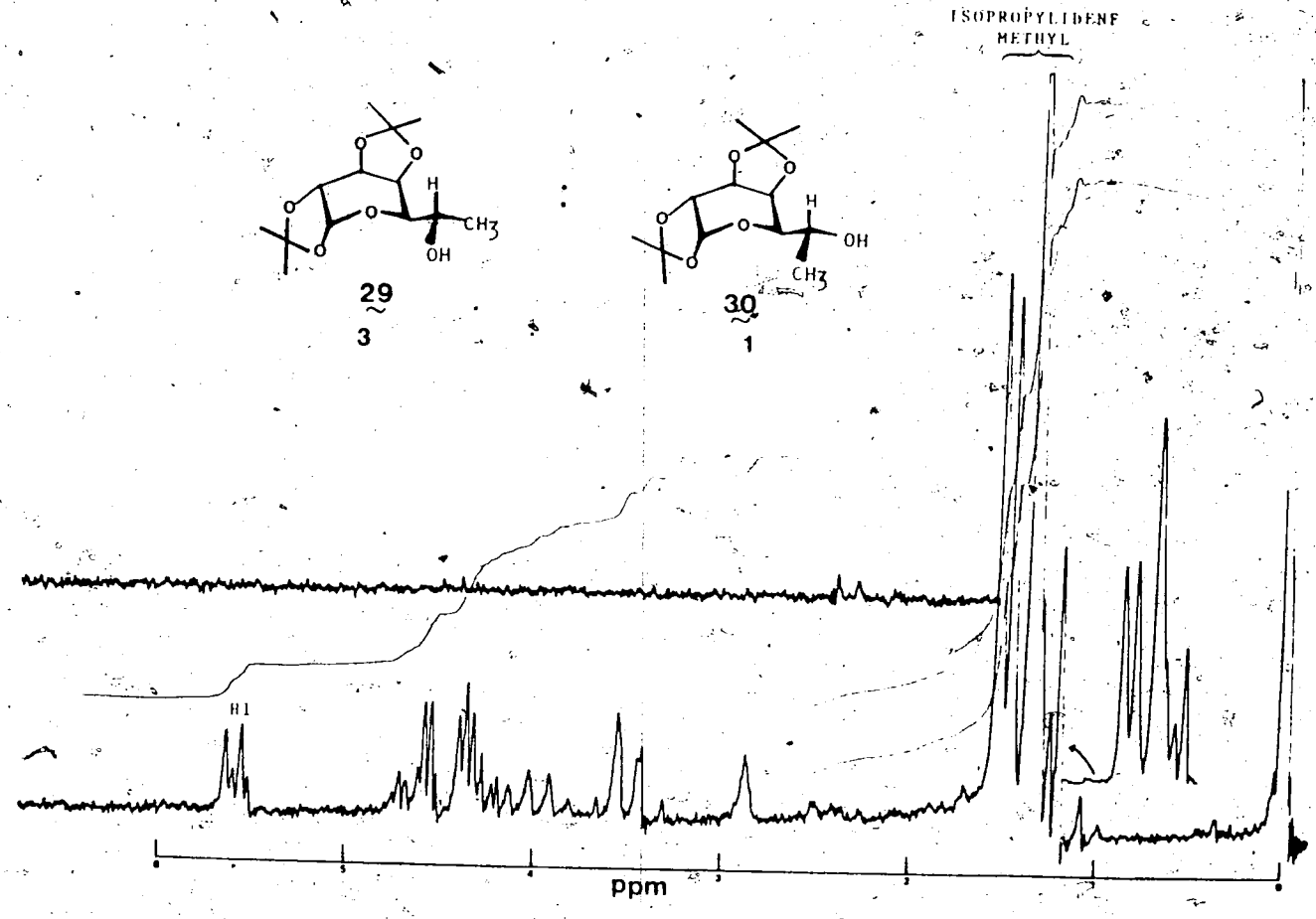


30

in a similar addition of methylmagnesium iodide to the aldehyde (43).[47,54] Two anomeric proton signals (doublets at δ5.60 and δ5.55, respectively) could be identified in the 60 MHz ^{1}H-nmr spectrum of the reaction mixture (Fig. 18). The relative intensities of the two signals indicated a 3:1 ratio. This is consistent with the result reported by Lemieux et al.[43]

The mixture of isomers was obtained as a syrup in 90% yield. Since a solvent system that provided an acceptable separation on tlc was not found, no attempt was made to separate the compounds by column chromotography. However, the major product 29 crystallized from a solution of the syrupy mixture in hot hexane. The yield was 26% after three recrystallizations from hexane. The ^{1}H-nmr spectrum (Fig.19) confirmed the purity. In order to isolate the other isomer, the benzoate derivatives were prepared of the mixture present in the mother liquors. Crystals deposited from a solution of the benzoates in hexane. De-O-benzoylation provided a material, mp 57-58°C, which produced the ^{1}H-nmr spectrum (presented in Fig. 20). The spectrum is in accordance with that expected for the isomer (30) of 29. The yield was 2%.

Lemieux et al,[43] as has been mentioned above, prepared the methyl β-glycoside (72) of the minor product which had a melting point of 143-145°C and $[\alpha]_D^{20}$ -17.9° (water). Since methyl 7-deoxy-β-L-glycero-L-galacto-heptopyranoside had been reported by Jackson and Hudson[55] to have melting point 143.5-144.0°C and $[\alpha]_D^{20}$ +18.5° (water), the D-glycero-D-galacto-



29
3

30
1

Fig. 18 The 60 MHz ^{1}H-nmr spectrum showing the 3:1 ratio of compounds 29 and 30 in the mixture of addition products resulted by treating the aldehyde 43 with methylmagnesium iodide.

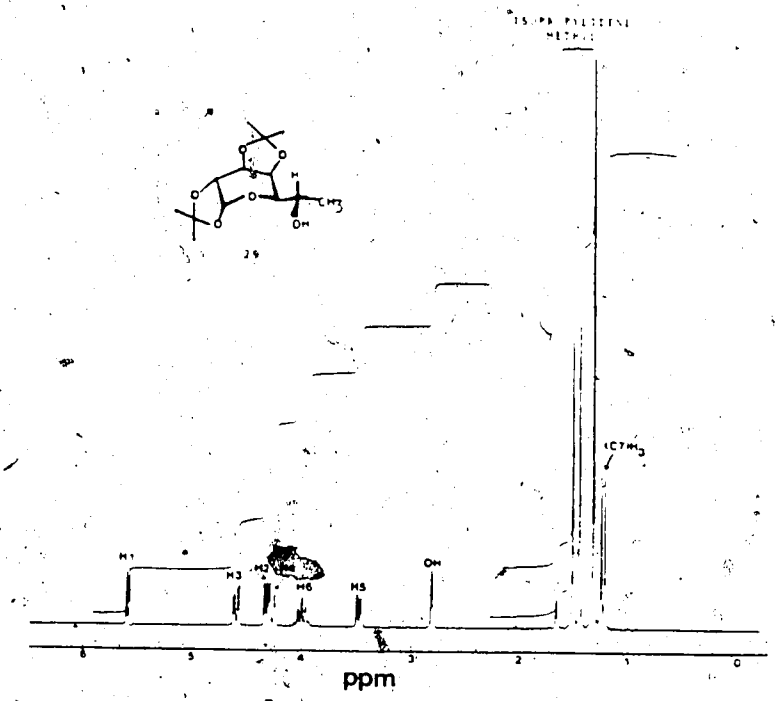


Fig. 19 The 200 MHz ^{1}H-nmr spectrum of compound 29 in $CDCl_3$.

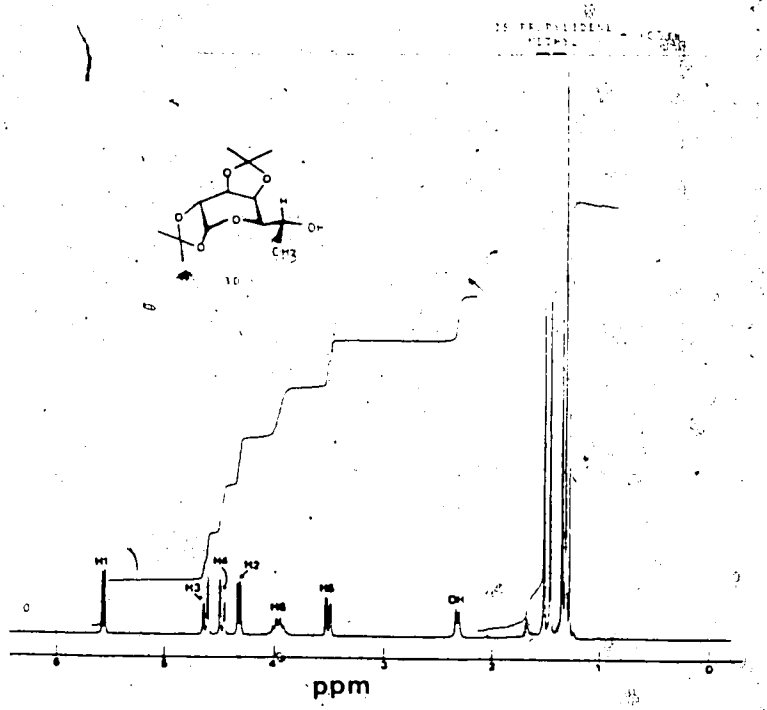
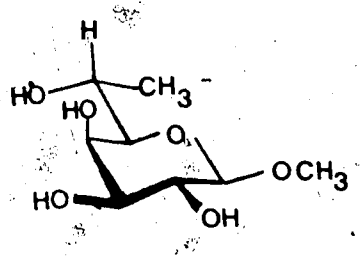


Fig. 20 The 200 MHz ^{1}H-nmr spectrum of compound 30 in $CDCl_3$.

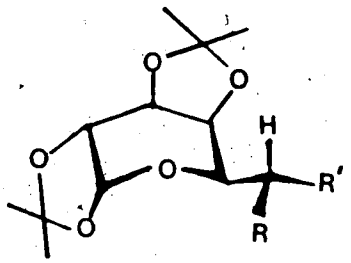


72

configuration was assigned to 72. Thus, the new assymmetric center in the minor product 30 was assigned the D-configuration. This stereochemical outcome conforms to Cram's rule.[56]

The ^{1}H-nmr chemical shifts and coupling constants of 29 and 30 are listed in Table IV where it is seen that spacings for the signals for the protons of the pyranose rings in compounds 29 and 30 are virtually the same as those published by Cone and Hough[57] for 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (73). The chemical shifts of H-1, H-2 and H-3 were essentially the same for the three compounds (73, 29, 30). The shift of H-4 was 4.26 ppm for both the galactose compound and the L-isomer (29), but the signal for H-4 of the D-isomer (30) was 0.2 ppm to lower field probably due to the deshielding by O-6 atom . In view of the magnitudes of the coupling

TABLE IV. ^{1}H-nmr Chemical Shifts and Coupling Constants of Compounds 29, 30, 73



29 R=OH, R'=CH_3

30 R=CH_3, R'=OH

73 R=H, R'=OH

Compound	H-1	H-2	H-3	H-4	H-5	H-6
29*	5.58	4.32	4.59	4.26	3.48	3.99
30*	5.55	4.30	4.62	4.46	3.50	3.96
73†	5.60	4.36	4.66	4.29	—	—

* In $CDCl_3$ at 300°K with $CHCl_3$, δ = 7.27 ppm as internal reference and measured at 200.13 MHz.

† Values taken from Cone and Hough[57] measured at 60 MHz

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
29	4.6	2.1	8.0	2.0	6.8
30	5.0	2.2	8.0	2.0	7.2
73	5.0	2.4	8.0	1.4	—

constants for H-5 and H-6, the compounds are expected to exist extensively in $CDCl_3$ in the conformers which maintain these two hydrogens in near anti-periplanar relationship. The coupling constants of the protons on the pyranose ring indicate that the ring in these compounds is strongly distorted from the $^4C_1$ conformation into the conformation inferred by the conformational formula drawn for 29 and 30.

It was planned to confirm the configurations of the products 29 and 30 by nuclear Overhauser enhancement studies[38,58] since the C6-methyl group may reside closer to H-4 in the L-isomer (29) than in the D-isomer (30). However, although saturation of the signals assigned to these methyl groups did, in fact, cause a greater enhancement of H-4 in the case of the L-isomer, the significance of this result was obscured by the fact that the experiment could not avoid simultaneous saturation of the isopropylidene methyl groups. This matter was dealt with later when the trisaccharides 21 and 24 were synthesized.

Reaction of the glycosyl chloride with 29 and with 30 gave the blocked trisaccharides 44 and 46 in 36% and 65% yields, respectively. The ^{1}H-nmr spectra for these products are presented in Figs. 21 and 22. Treatments with hydrazine hydrate completely deblocked these compounds to produce amines that were not isolated. N-acetylation provided 45 and 47. Removal of the isopropylidene groups by hydrolysis using aqueous trifluoroacetic acid then afforded the trisaccharides 25 and 26 whose ^{1}H-nmr spectra are presented in Figs. 23 and 24. The

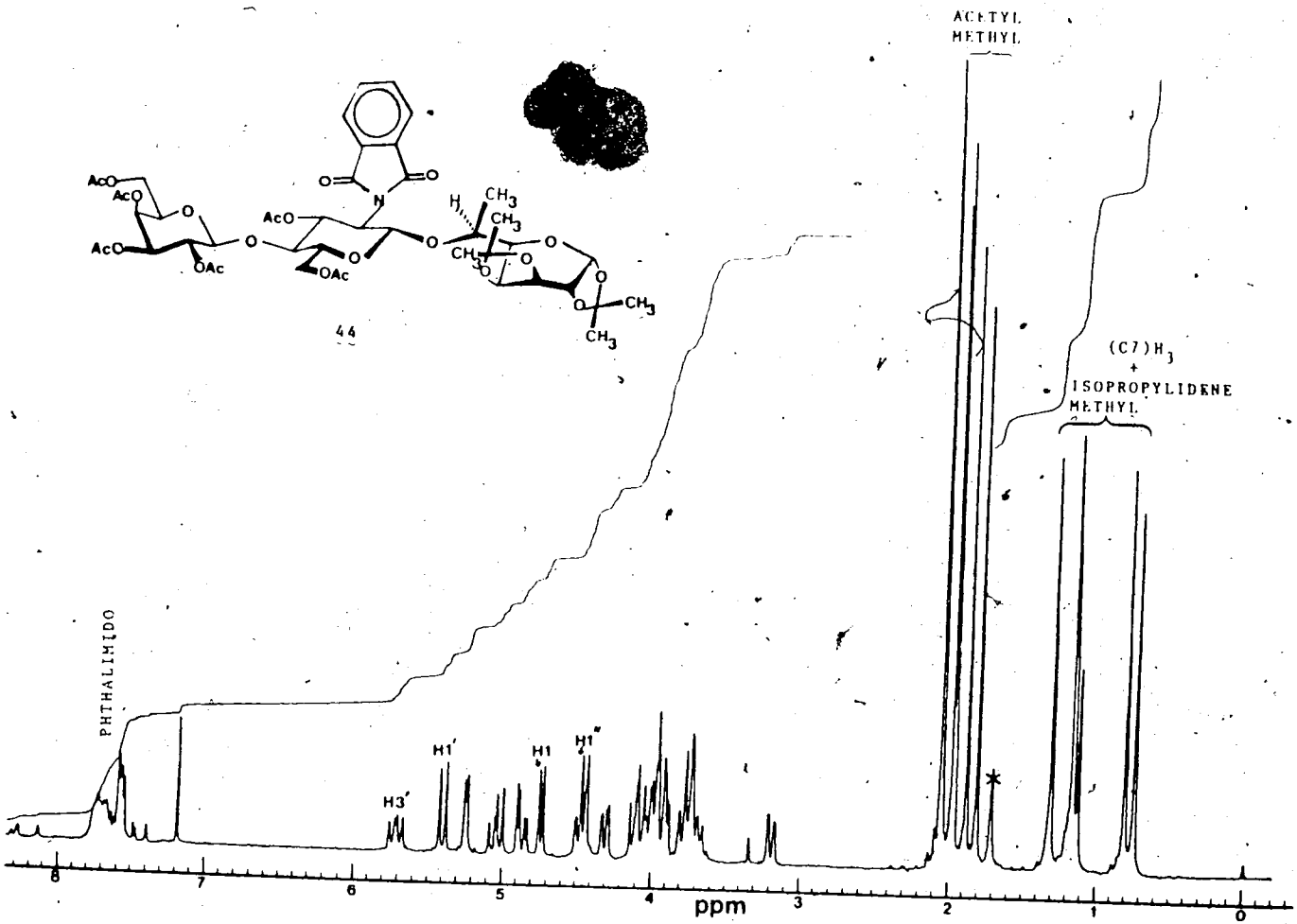


Fig. 21 The 200 MHz ^{1}H-nmr spectrum of compound 44 in $CDCl_3$.

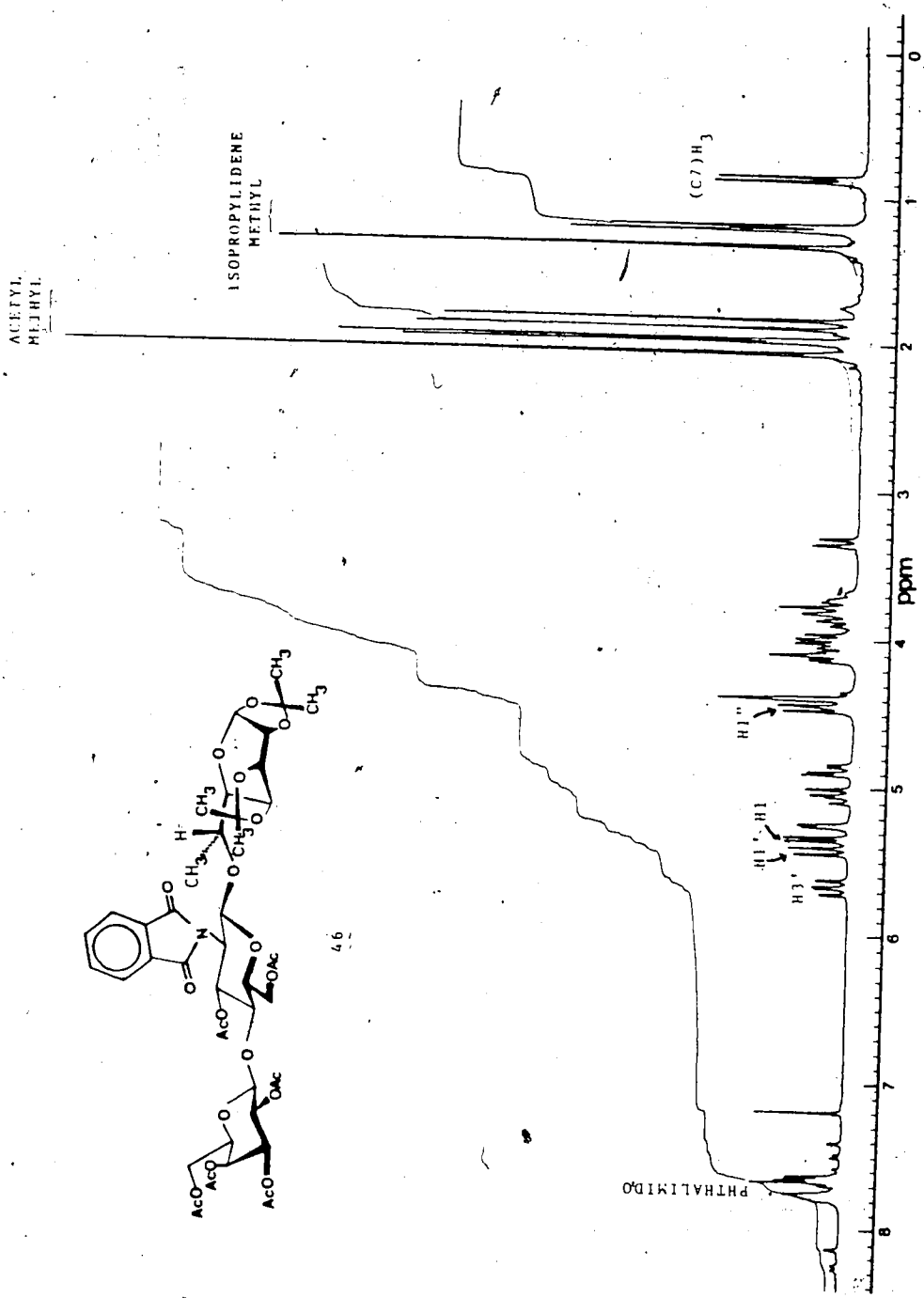
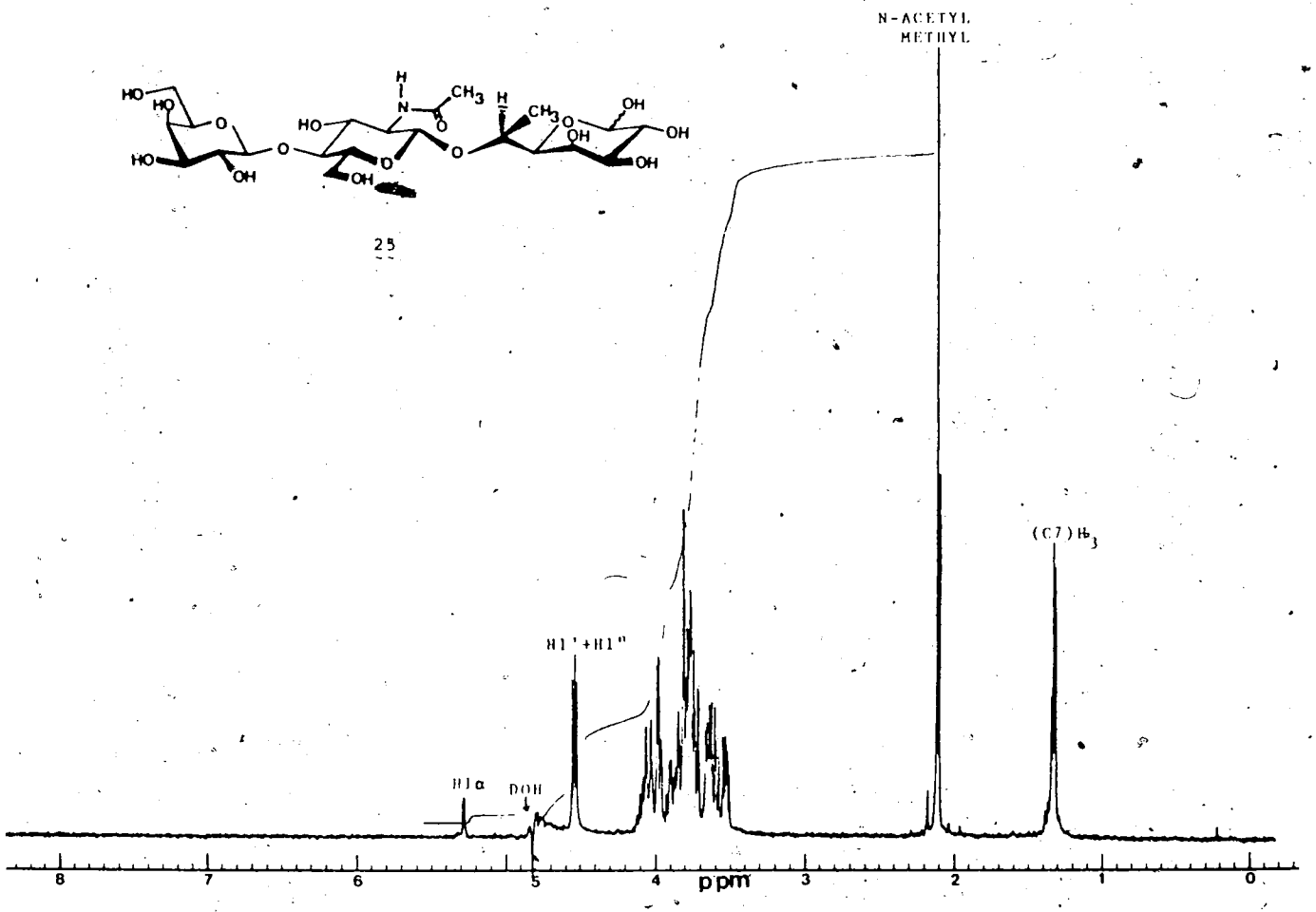
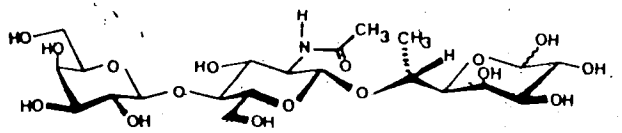


Fig. 22 The 200 MHz ^{1}H-nmr spectrum of compound 46 in $CDCl_3$.



25

Fig. 23 The 400 MHz ^{1}H-nmr spectrum of compound 25 in D_2O.



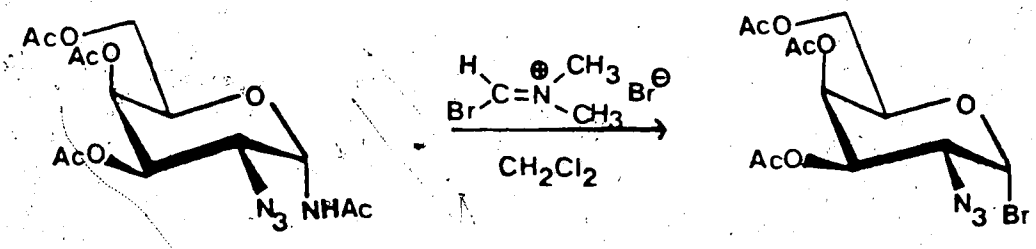
26

Fig. 24 The 400 MHz ^{1}H-nmr spectrum of compound 26 in D_2O.

overall yields (4.7% and 22.6%) were poor mainly because the preparations were made only once. It is expected that these could be substantially improved by an effort to optimize the reaction and isolation procedures.

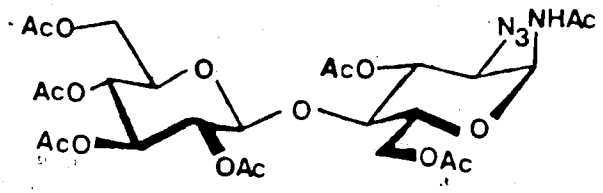
D. Synthesis of the βDGlc(1→4)βDGlcNAc(1→6)1,2;3,4-di-O-isopropylidene-αDGal trisaccharide (4"-epimer) (32)

The hexa-O-acetyl-2-deoxy-2-phthalimido-β-D-cellobiosyl chloride (55) was prepared starting from the hexa-O-acetyl-cellobial following the azidonitration route as in the case of the lactosyl analogue.[39] During the time of this preparation, S. Sabesan, a fellow graduate student in the group, observed that the reaction[59] of the N-acetyl 2-azido-2-deoxy-α-D-glycosylamine (74a), one of the by-products in the azidonitration reaction,[49] with N,N-dimethylbromoforminium bromide[51,52] in dichloromethane smoothly replaced the acetamido group to provide the α-bromide (74b). As usual,[39] the main products of



74a 74b

the reaction were the α and β azidoglycosyl nitrates (about 75%) but the reaction product also contained about 10% of the 1-acetamide compound 75. Since the separation of 75 from the mixture of nitrates requires chromatography, the crude product was first treated with Vilsmeier bromide in dichloromethane. The disappearance of the N-acetyl compound (75) could be confirmed



75

by tlc. Lithium bromide was then added to replace the nitrate groups. Reversal of the order of addition did not cause the disappearance of the N-acetyl glycosylamine (75). The reason for this behaviour was not investigated. An overall yield of 32% of the α-bromide (49) after crystallization was obtained in contrast to the 24% yield obtained by previous procedures.[39,49] The ^{1}H-nmr spectrum of the crystalline α-bromide (49) is presented in Fig. 25. The sequence of reactions used to prepare the phthalimido chloride 55 from 49 were those described by Lemieux et al[42] with very little modification. It may be noted that in the reduction of the azido compound (51) by hydrogen sulfide, sulfur is generated and it was found helpful to remove the sulfur by centrifugation. The ^{1}H-nmr spectra of

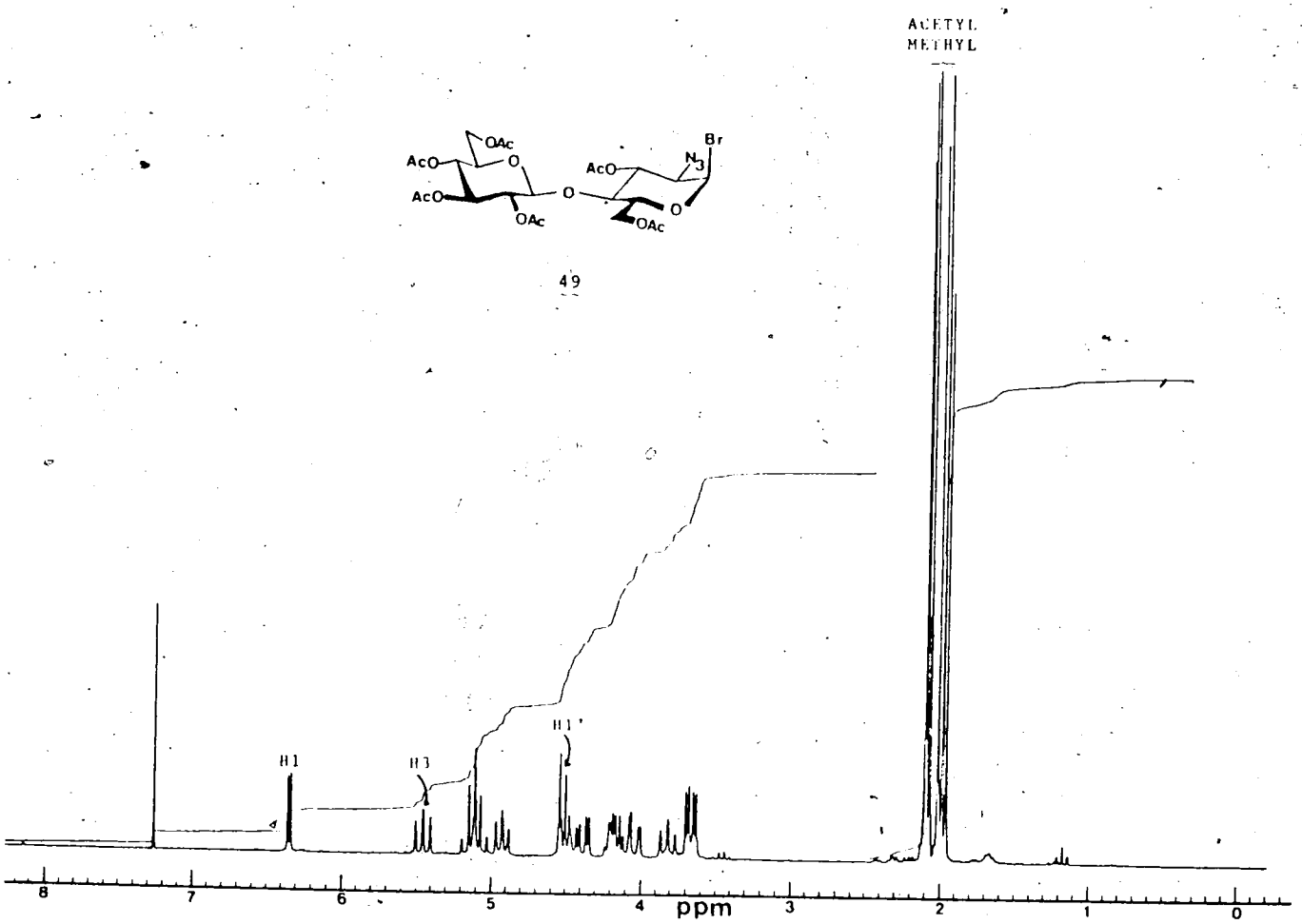
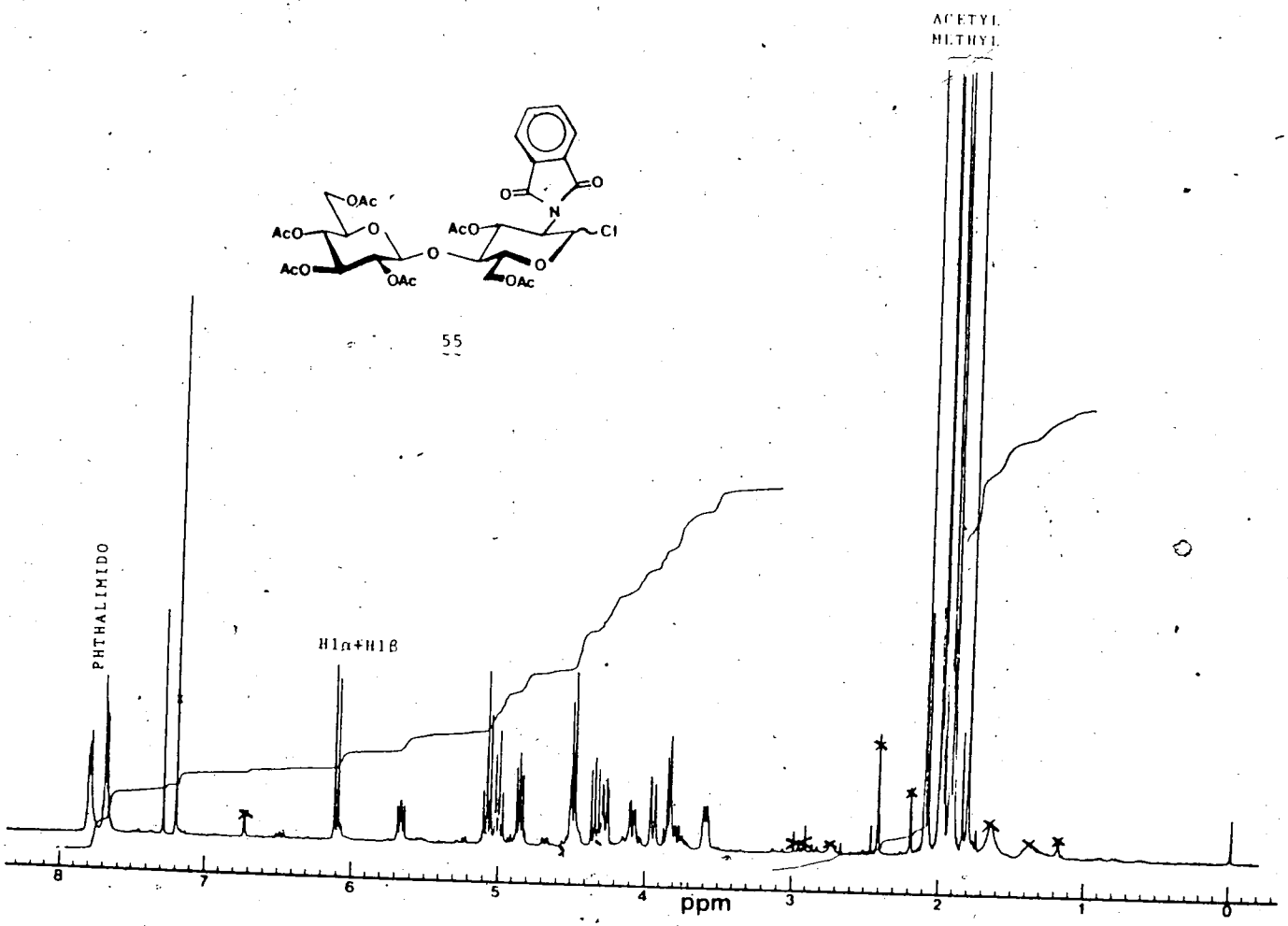


Fig. 25 The 200 MHz ^{1}H-nmr spectrum of compound 49 in $CDCl_3$.

the chloride (55) is presented in Fig. 26. The signals for the anomeric protons of the α- and β- forms are present at δ6.12 and δ6.08 and show the product to be a mixture of these anomers (α:β≈1:4).[40] The mixture was used for the glycosylation reaction with 1,2;3,4-di-O-isopropylidene-α-D-galactose to give the blocked trisaccharide (56) in 65% yield. The quality of this product can be judged from its ^{1}H-nmr spectrum presented in Fig. 27. Removal of acetyl and phthalimido groups on 56 followed by N-acetylation afforded the 4"-epimer (32) in 71% yield. The ^{1}H-nmr spectrum of 32 is presented in Fig. 28.

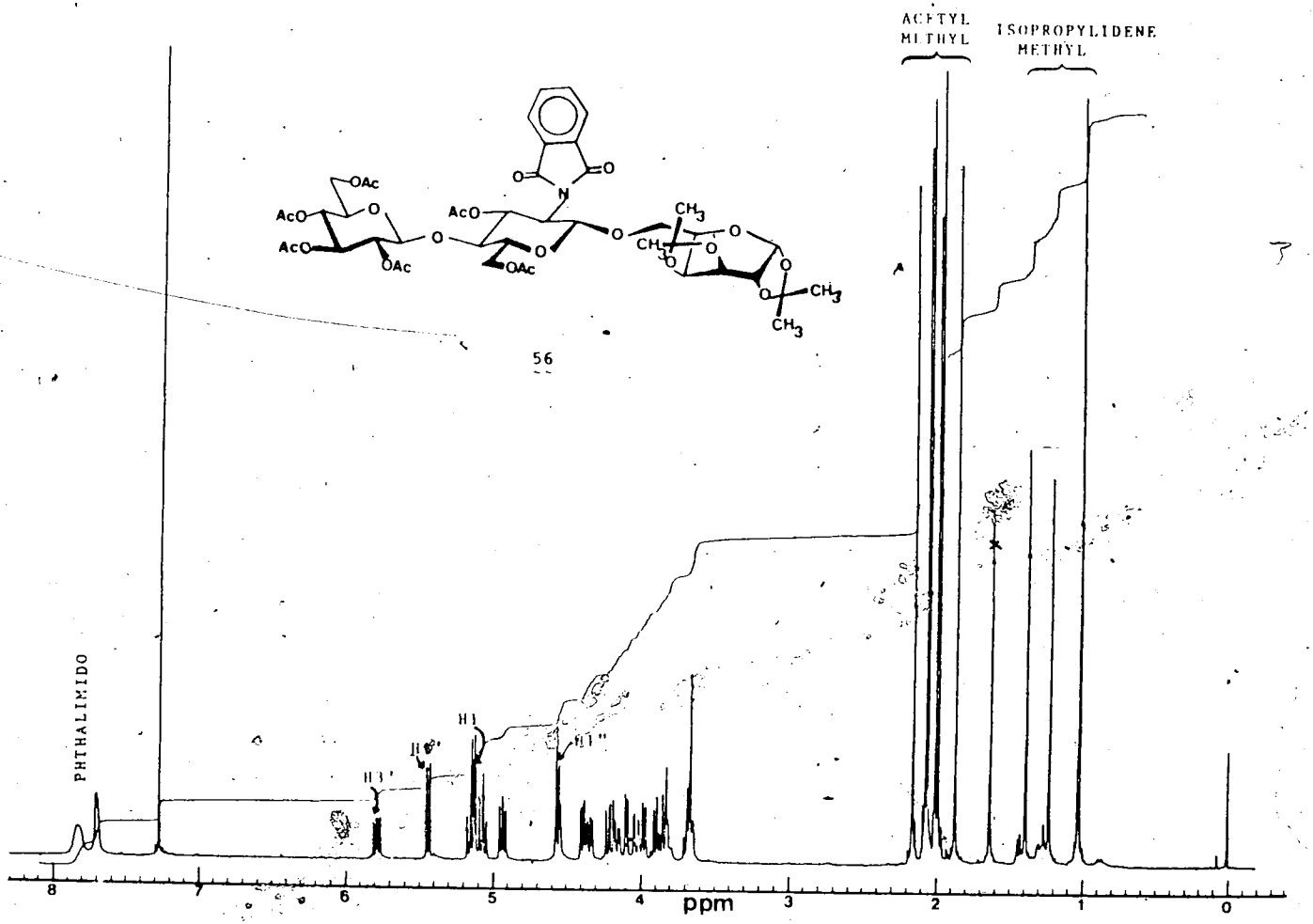
E. Syntheses of the 6"-deoxy derivatives of βDGal(1→4)-βDGlcNAc(1→6)DGal (31) and of βDGlc(1→4)βDGlcNAc(1→6)-1,2;3,4-di-O-isopropylidene-αDGal (33)

Lemieux and Burzynska[28] as well as Bundle and Josephson[60] had observed that acetyl protecting groups can be selectively removed by strictly anhydrous sodium methoxide-methanol in the presence of phthalimido group. Since the deacetylation is very rapid, short reaction times can limit the extent of hydrolytic cleavage of the phthalimido group by the invariably present traces of water. It was anticipated, therefore, that benzyl 3,6-di-O-acetyl-4-O-2-deoxy-(tetra-O-acetyl-β-D-galactopyranosyl)-2-phthalimido-glucopyranoside (76)[39] should be a convenient precursor to the corresponding 6"-deoxy compound (31). However, it appeared that part of the phthalimido compound had hydrolysed during the deacetylation reaction to the o-carboxy-



55

Fig. 26 The 400 MHz ^{1}H-nmr spectrum of compound 55 in $CDCl_3$.



56

Fig. 27 The 400 MHz ^{1}H-nmr spectrum of compound 56 in $CDCl_3$.

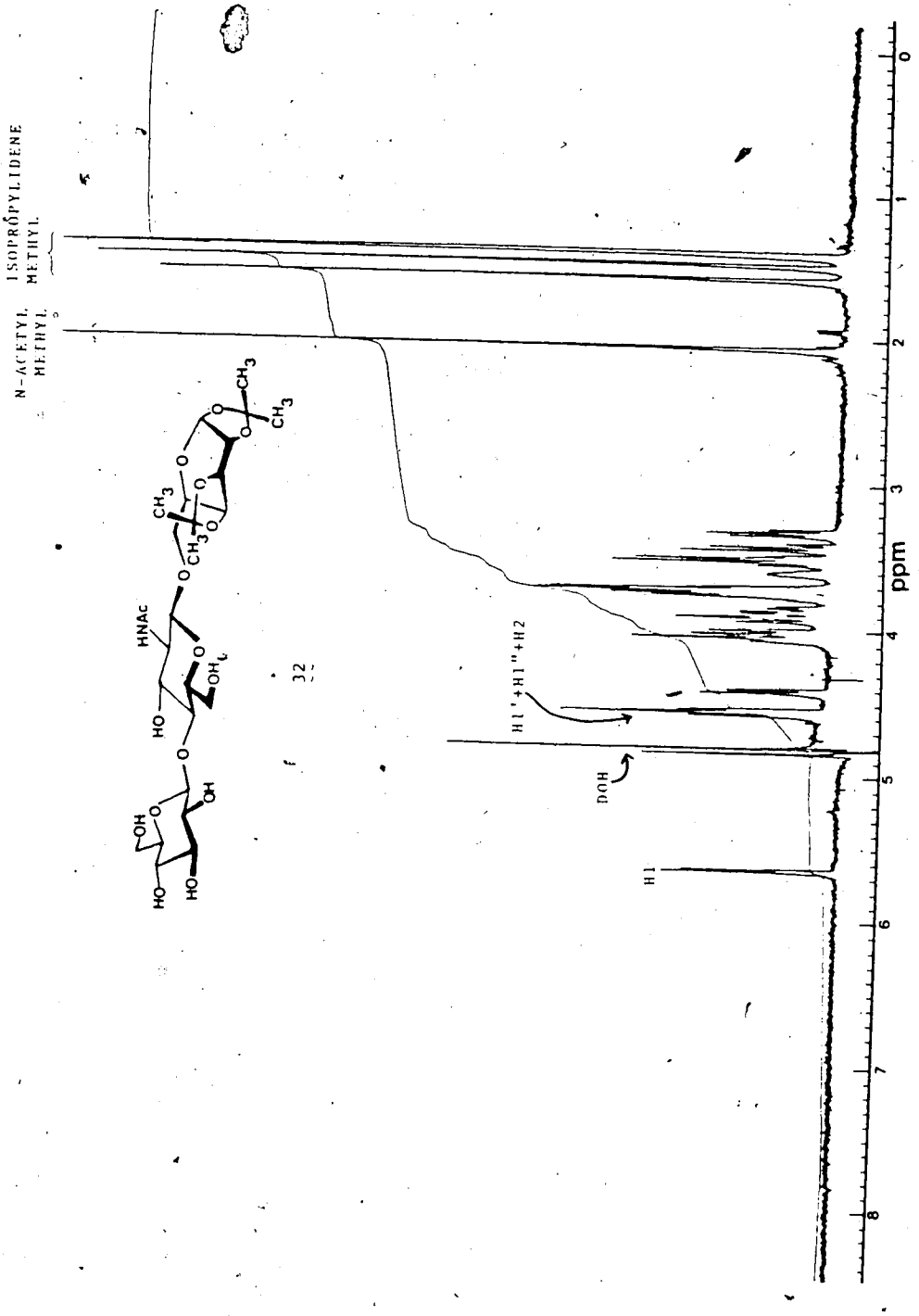
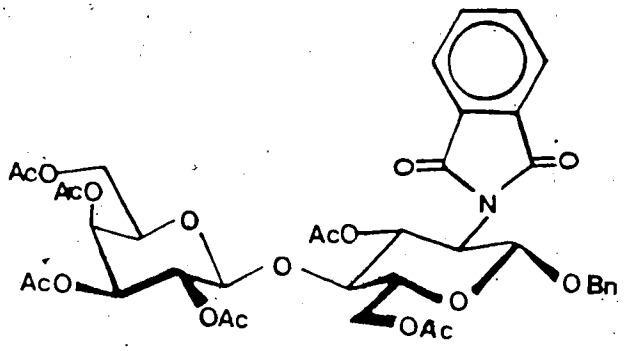
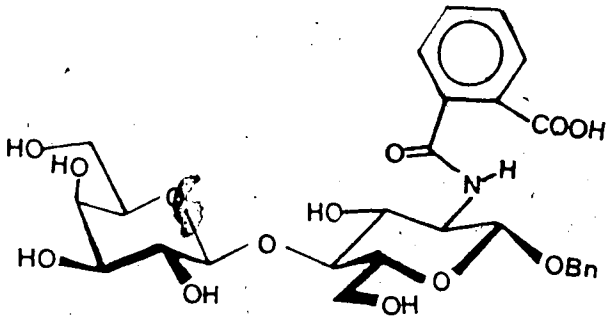


Fig. 28 The 400 MHz ^{1}H-nmr spectrum of compound 32 in D_2O.



76

benzamido derivative 77. The formulation of 77 was apparent from the observation of a highly polar component on tlc and



77

the absence of a signal for a methoxy group in the ^{1}H-nmr spectrum (Fig.29b). The removal of this side product proved to be quite difficult as well as wasteful. It was observed, however, that treatment of the crude product in refluxing dry pyridine for about 10 h caused the ring closure to form the desired phthalimido compound (57). In order to check on this

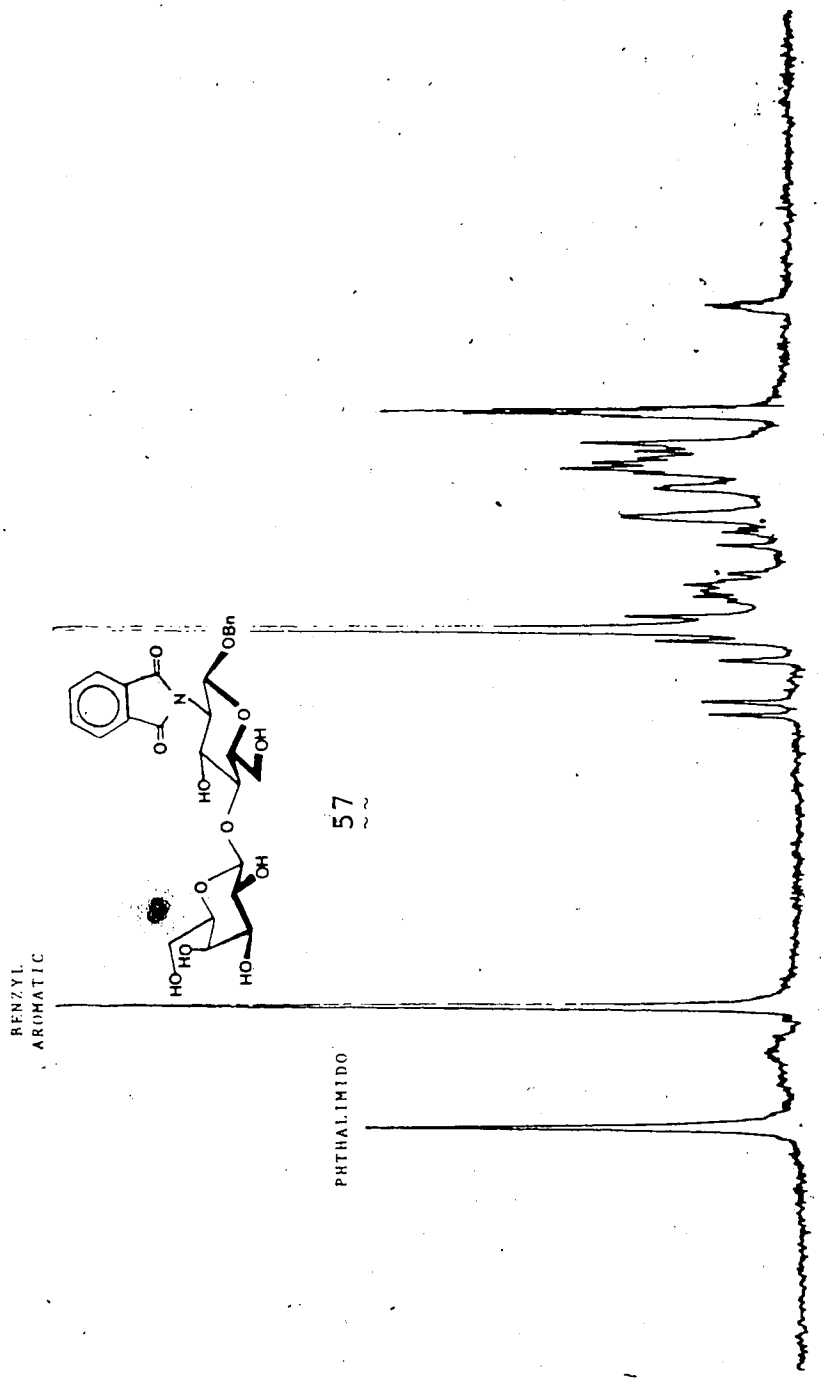


Fig. 29a The 100 MHz ^{1}H-nmr spectrum of compound 57 in DMSO-d_6-CD_3OD obtained by deacetylation of 76 using anhydrous sodium methoxide-methanol.

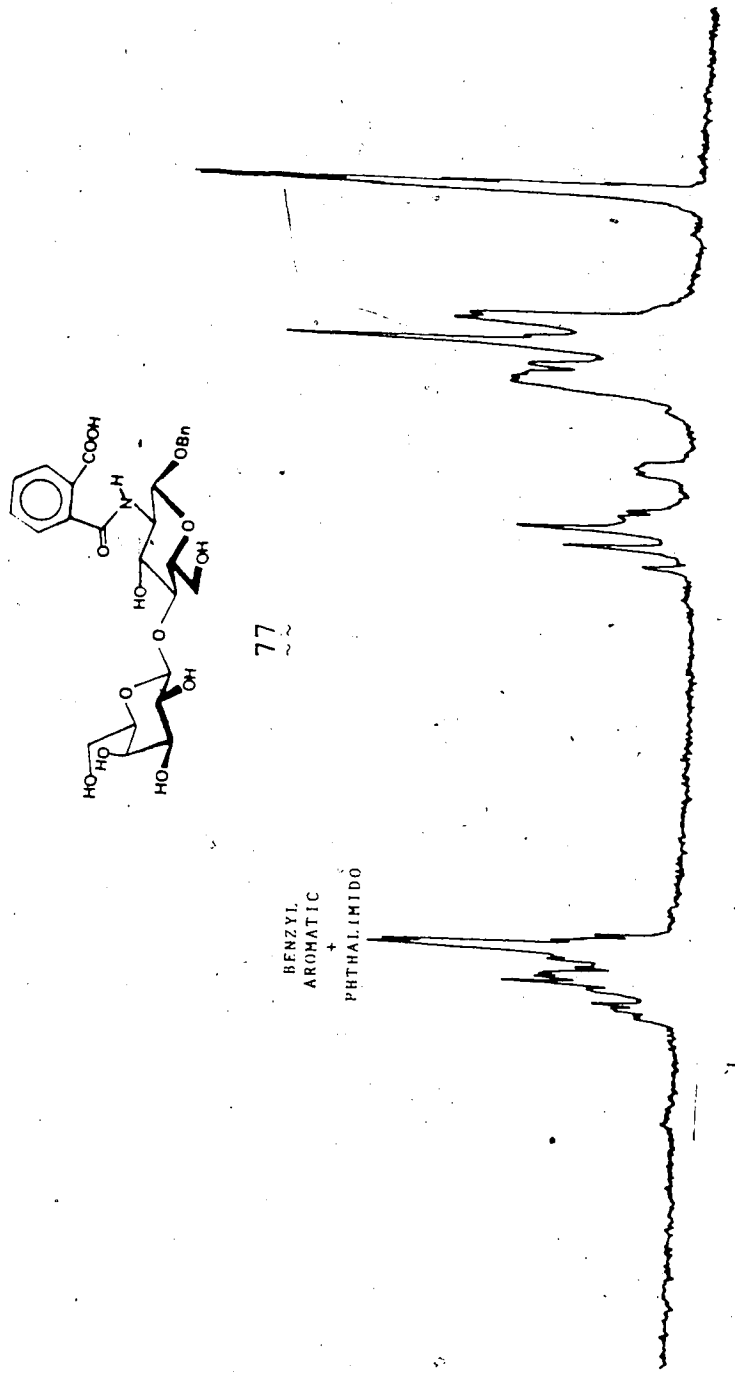
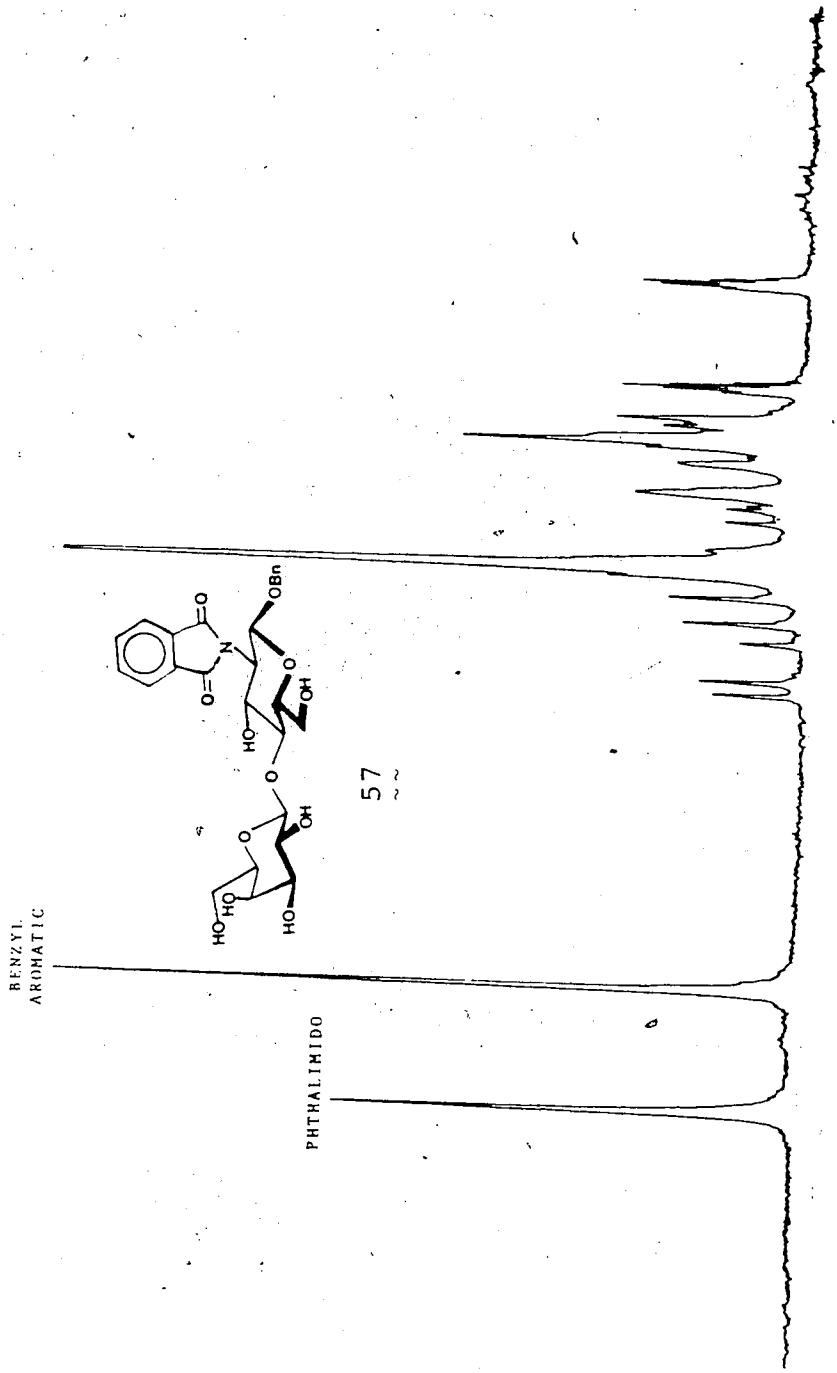


Fig. 29b The 100 MHz ^{1}H-nmr spectrum of compound 77 in DMSO-d_6.



57
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Fig. 29c The 100 MHz ^{1}H-nmr spectrum of compound 57 ~~ obtained by refluxing 77 ~~ in pyridine.

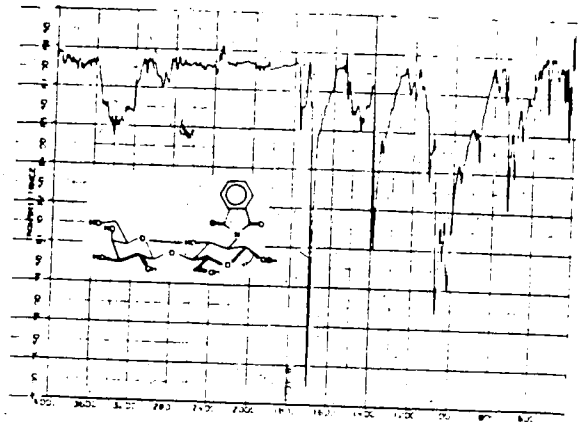


Fig. 30a The FTIR spectrum ($CHCl_3$ cast) of the compound 57 obtained by deacetylation of 76 using anhydrous sodium methoxide-methanol.

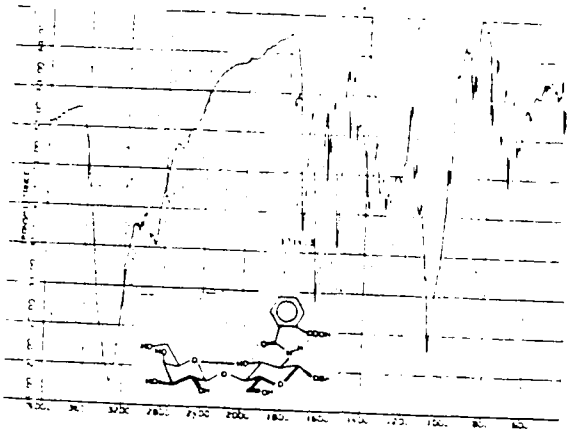


Fig. 30b The FTIR spectrum (CH_3OH cast) of the compound 77.

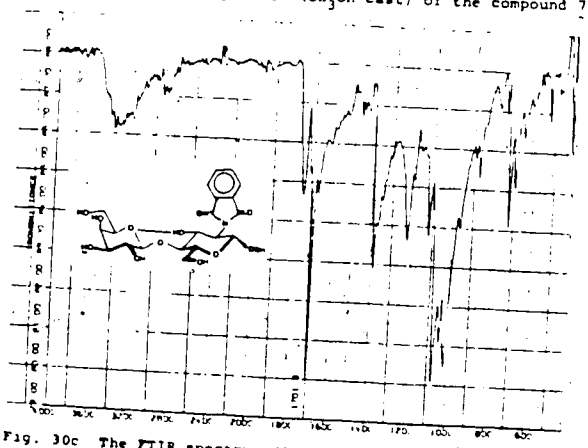
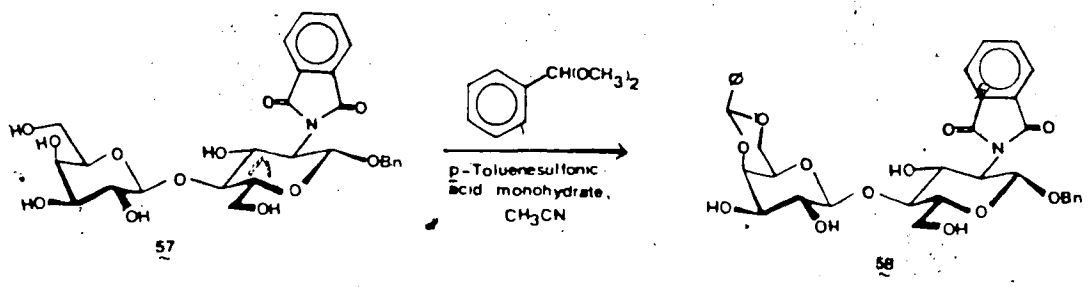


Fig. 30c The FTIR spectrum ($CHCl_3$ cast) of the compound 57 obtained by refluxing 77 in pyridine.

matter, an authentic sample of the O-carboxybenzamide (77) was prepared by alkaline hydrolysis. This acid was characterized by its ^{1}H-nmr (Fig.29b) and FTIR (Fig.30b) spectra. In fact, on refluxing this product in pyridine, compound 57 was obtained in quantitative yield as characterized by ^{1}H-nmr (Fig.29c) and FTIR (Fig.30c) spectra. It is seen that these spectra are identical to those (Figs.29a & 30a) of the sample prepared by deacetylation using anhydrous sodium methoxide-methanol.

The treatment of 57 with two equivalents of α,α-dimethoxytoluene and catalytic amount of p-toluenesulfonic acid monohydrate[61] in acetonitrile provided the 4',6'-O-benzylidene derivative in 55% yield. The solubility of 57 in acetonitrile was low, but the material disappeared in solution as reaction proceeded. The structure assigned to 58 was established by



^{13}C-nmr spectrum (Fig.31) which shows only one CH_2OH carbon signal at δ 61.7 which is assigned to C-6 and the signal for C-6' which is expected[62] to be shifted downfield due to substitution of benzylidene group is found at δ67.8. Compound 58 was then acetylated to provide the tetraacetate (59). The

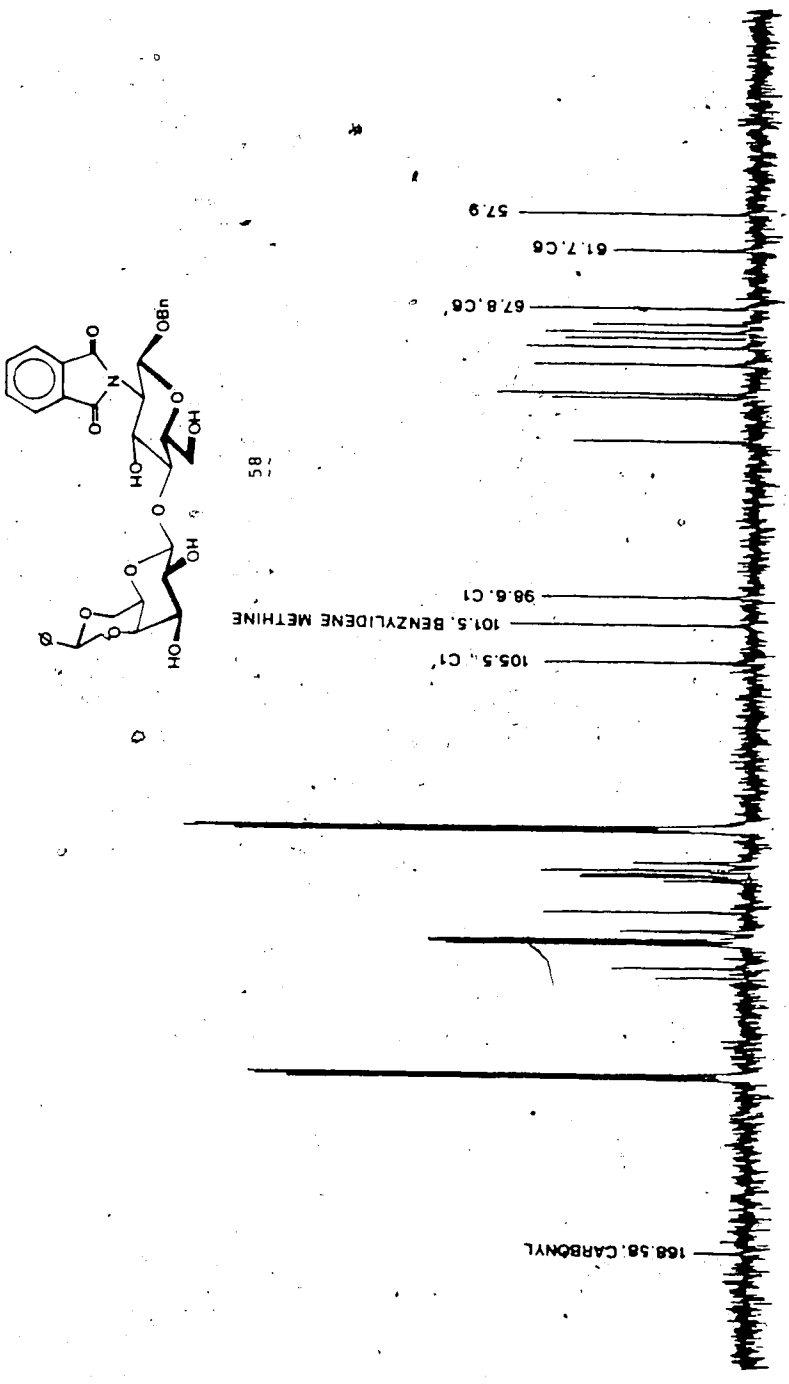
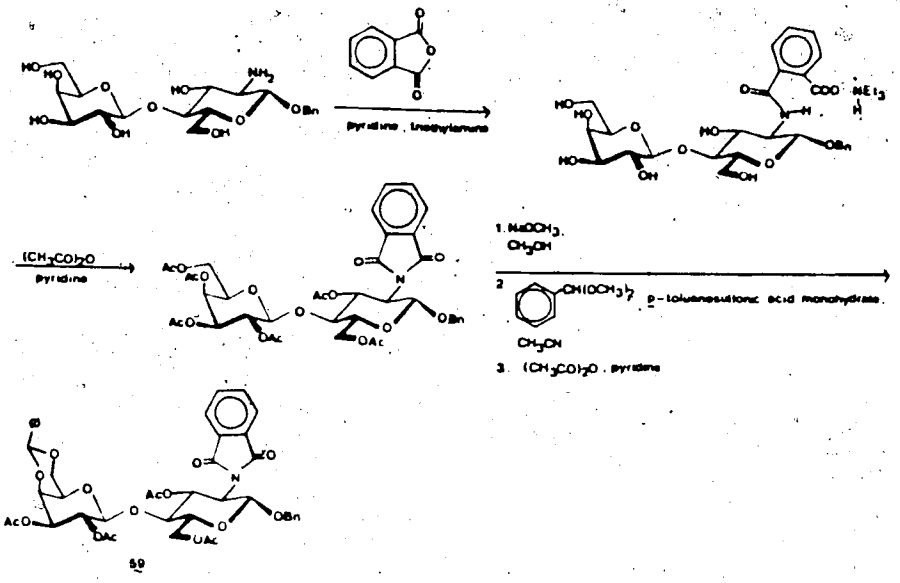
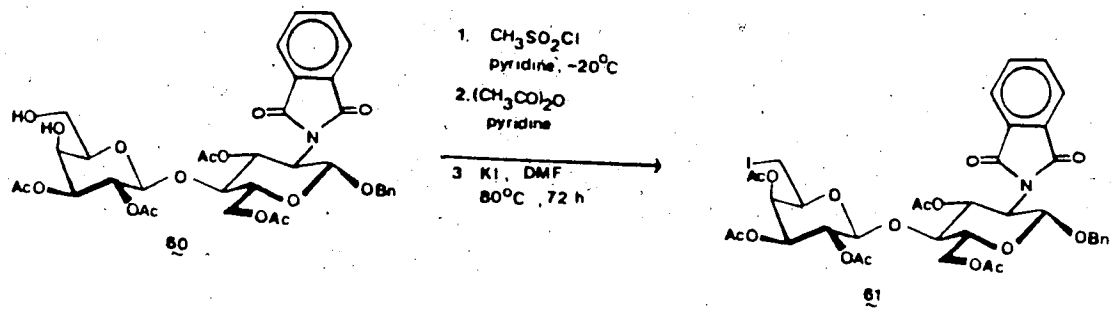


Fig. 31 The 100 MHz ^{13}C-nmr spectrum of compound 58 in pyridine-d_5.



overall yield of compound 59 from benzyl 2-amino-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside was 35%.[39] ^{1}H-nmr spectrum of this compound (59) is presented in Fig. 32. Removal of the benzylidene group was effected by hydrolysis in 80% aqueous acetic acid and provided the 4',6'-diol (60) whose ^{1}H-nmr spectrum is presented in Fig. 33 which shows the absence of the benzylidene aromatic and the methine signals that are present in the spectrum (Fig.32) of 59.

In order to prepare the desired 6"-deoxy compound (31), it was decided to attempt a preferential mesylation of the 6'-hydroxy group. Reaction of the diol(60) with one equivalent



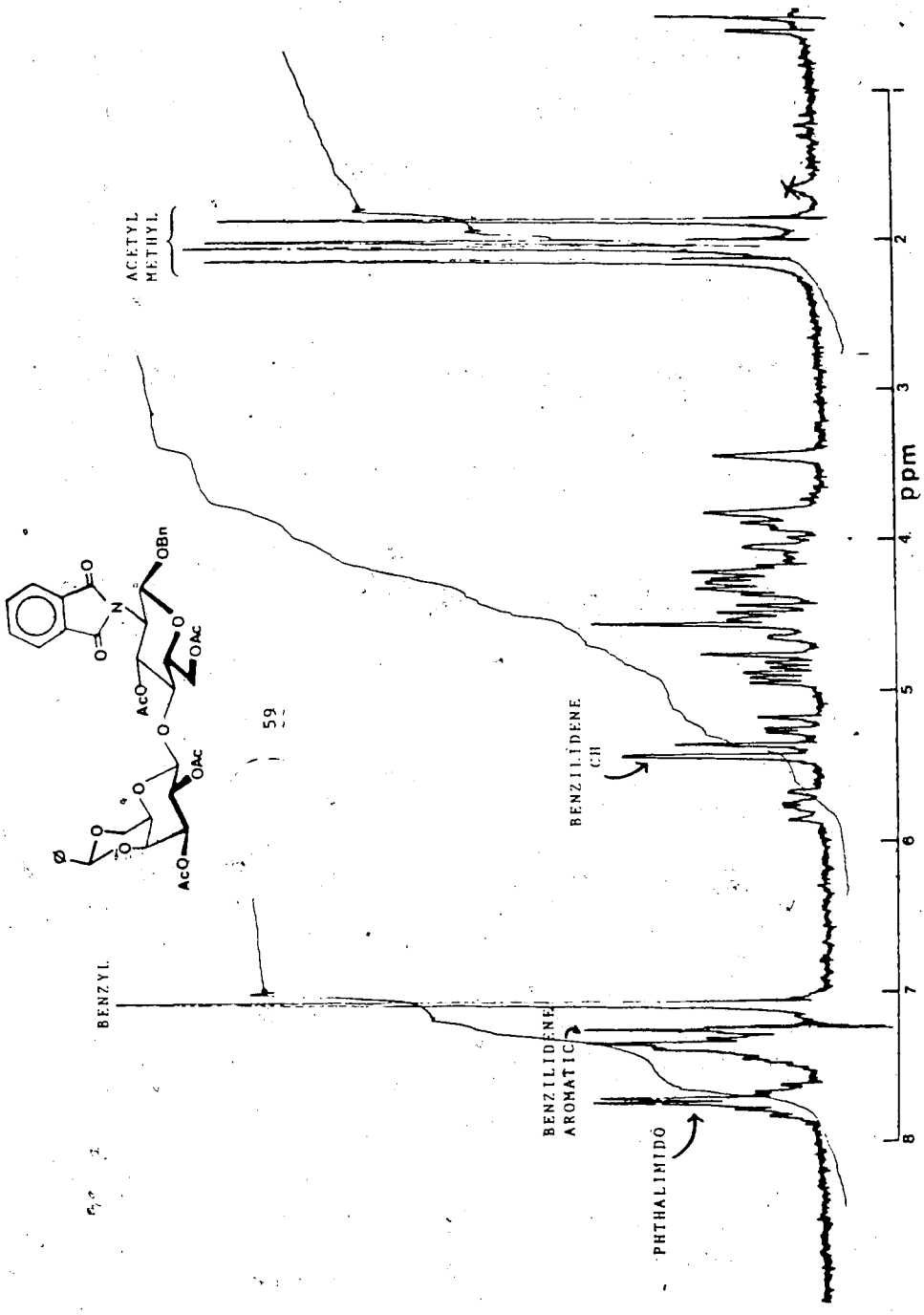


Fig. 32 The 100 MHz ^{1}H-nmr spectrum of compound 59 in $CDCl_3$.

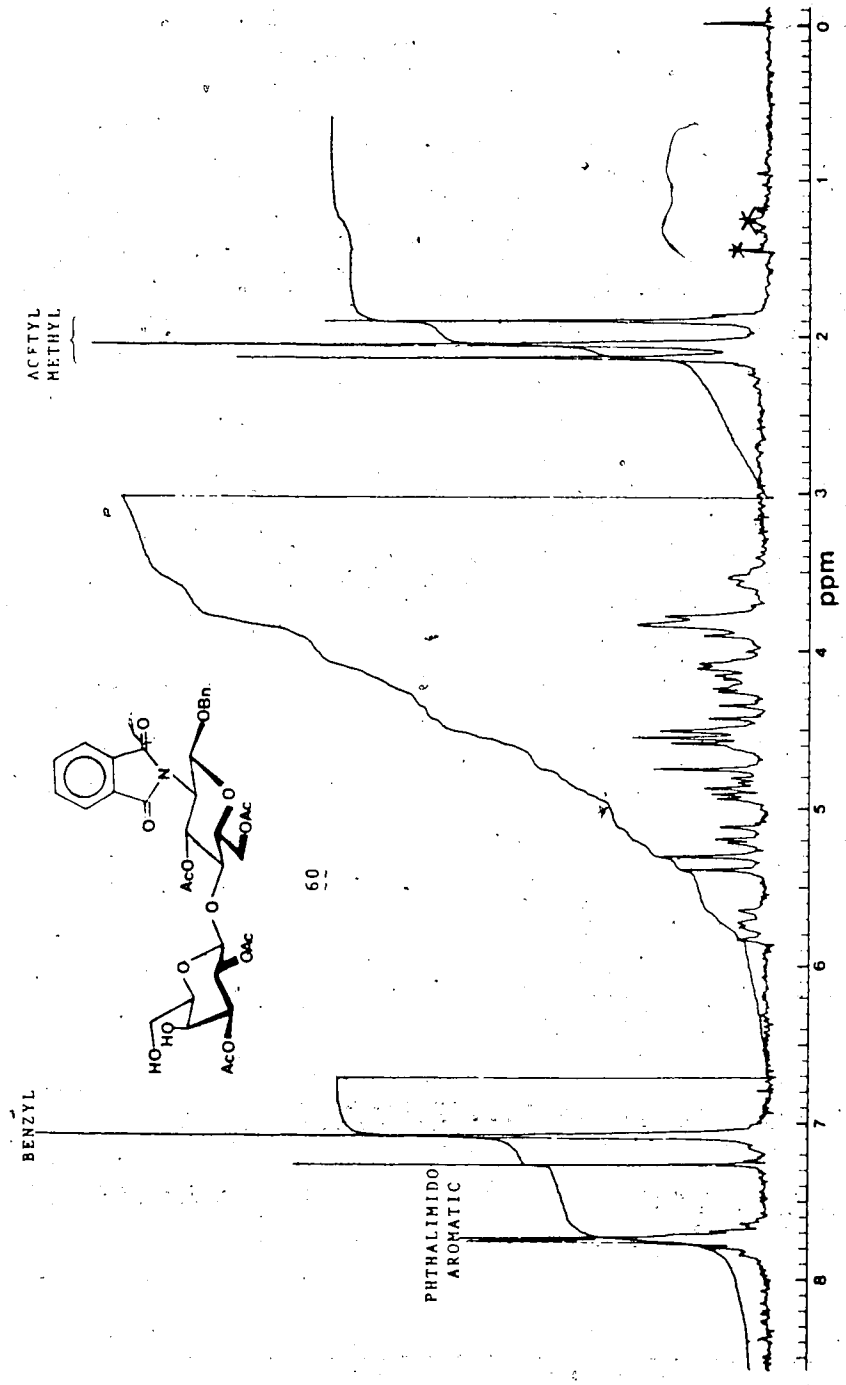


Fig. 33 The 100 MHz ^{1}H-nmr spectrum of compound 60 in $CDCl_3$.

of methanesulfonyl chloride provided a major product (54%) which was acetylated. The product of acetylation was not further purified and was treated directly with potassium iodide in N,N-dimethylformamide at 80°C. It took 72 h for the reaction to go to near completion. In the case of a similar displacement reaction in the preparation of compound 66, the reaction was complete in 20 h. This result is not surprising since the greater resistance of the 6-sulfonate of galactose derivatives to displacement reaction as compared to the glucose derivatives had been pointed out by Richardson.[63] The yield of the iodide 61 was 32% and this product was characterized by its ^{1}H-nmr spectrum presented in Fig. 34 which shows a multiplet signal for the two H-6' protons at δ 3.10. Tri-n-butyltin hydride reduction[64] of 61 in the presence of catalytic amount of 2,2'-azobisisobutyronitrile as radical initiator at 55°C resulted in a mixture of products as can be seen on tlc. The desired deoxy compound (62) was isolated by silica gel chromotography and characterized by ^{1}H-nmr spectrum reproduced in Fig. 35 which shows the product to be in accord with the structure assigned and is of sufficient purity to be carried on to the next reaction. This product was obtained in 50% yield and the other products of the reduction reaction were not characterized. Compound 62 was then converted to the corresponding bromide 64 following similar procedure as was described for the preparation of the chloride 27 except N,N-dimethylbromoforminium bromide was used as the halogenating

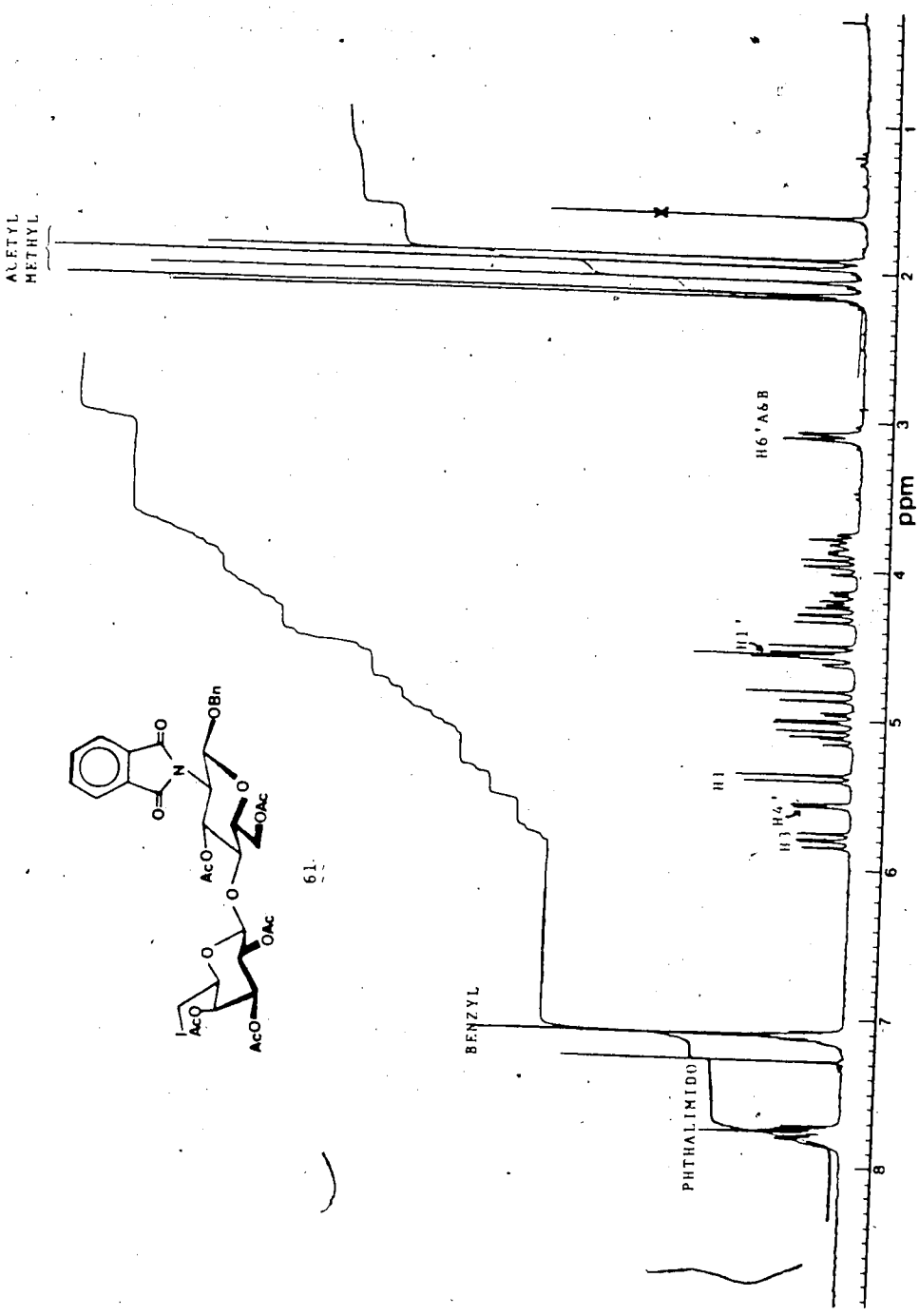


Fig. 34 The 200 MHz ^{1}H-nmr spectrum of compound 61 in $CDCl_3$.

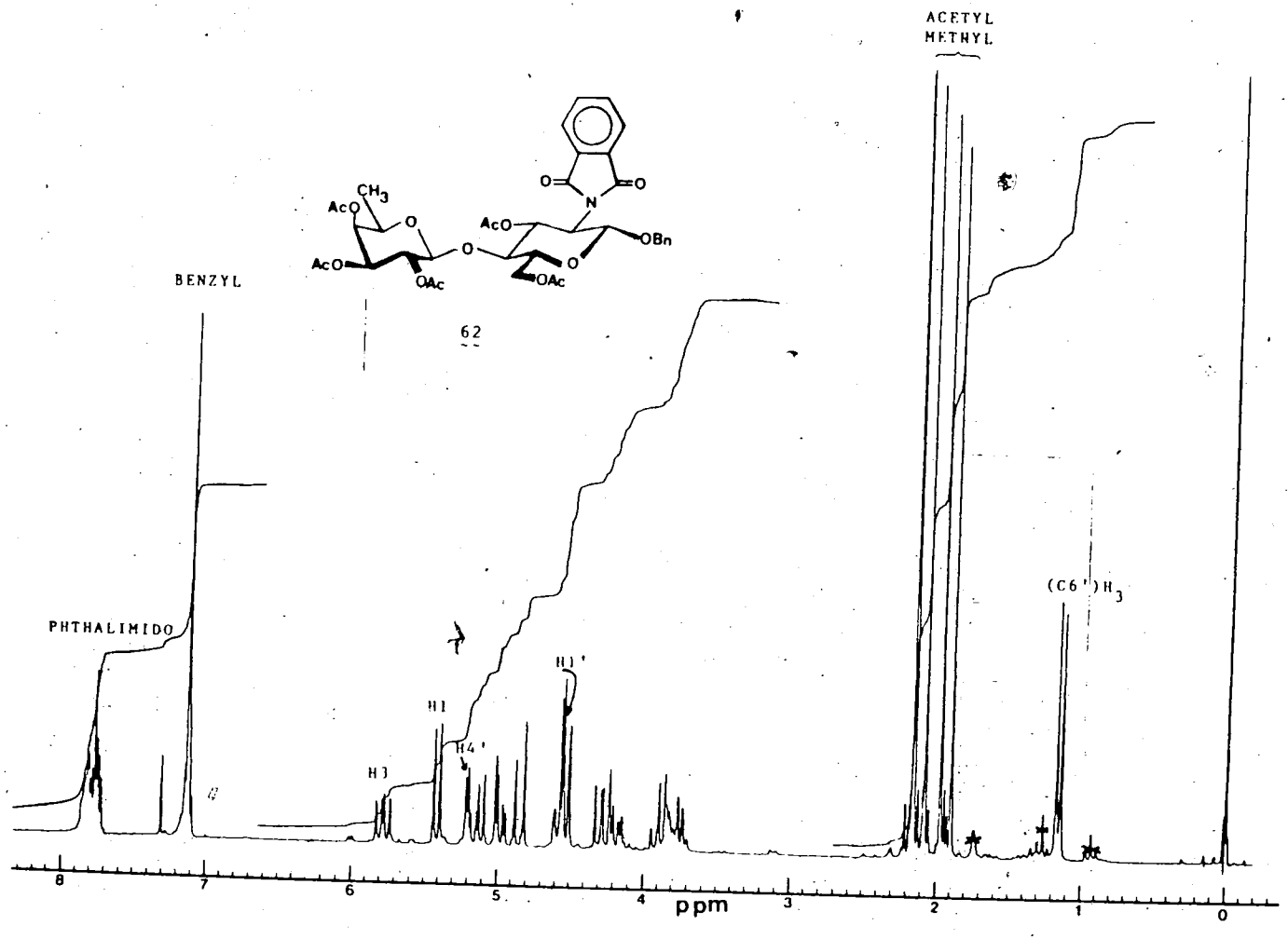
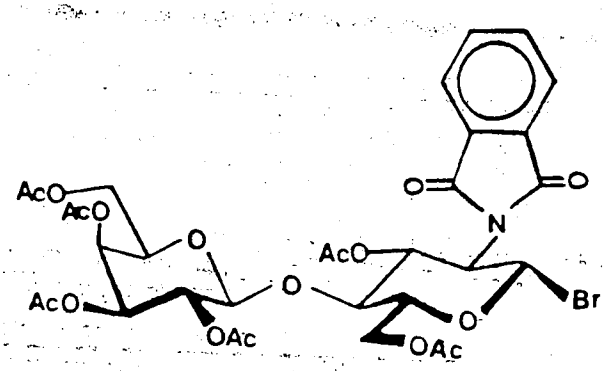


Fig. 35 The 200 MHz ^{1}H-nmr spectrum of compound 62 in $CDCl_3$.



64

agent. The bromide 64 was used directly in the glycosylation reaction with 1,2;3,4-di-O-isopropylidene-α-D-galactose by the method described by Lemieux et al.[42] The ^{1}H-nmr spectrum (Fig.36) of the product obtained in 54% yield after purification by silica gel column chromotography is in accord with the structure of 63 and is in a sufficiently pure state. Removal of the acetyl and phthalimido group by hydrazinolysis and N-acetylation was effected smoothly following the usual procedures.[42] The removal of the isopropylidene groups by acid hydrolysis, however, resulted in a mixture of products. The product 31 was isolated by column chromotography (Sephadex LH -20) and characterized by ^{1}H-nmr spectrum.shown in Fig. 37. This product, obtained in 40% yield, is in accord with the structure of 31 and is of acceptable purity to be used as an inhibitor.

Synthesis of the 6"-deoxy derivative of the 4"-epimer (33) followed a scheme similar to that described for the preparation of 31, except the isopropylidene groups were retained. In this case, however, with the result for the closing

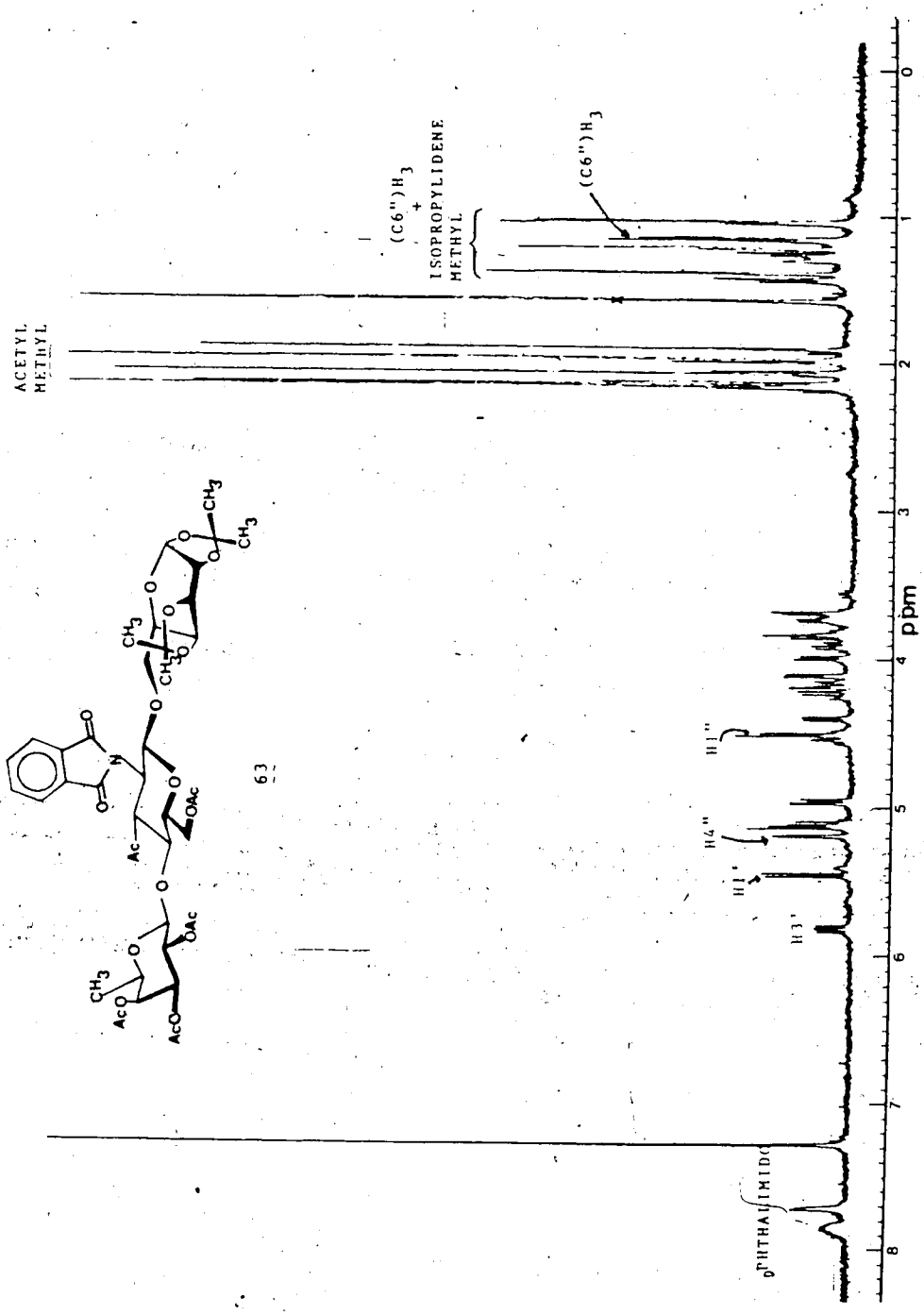


Fig. 36 The 400 MHz ^{1}H-nmr spectrum of compound 63 in $CDCl_3$.

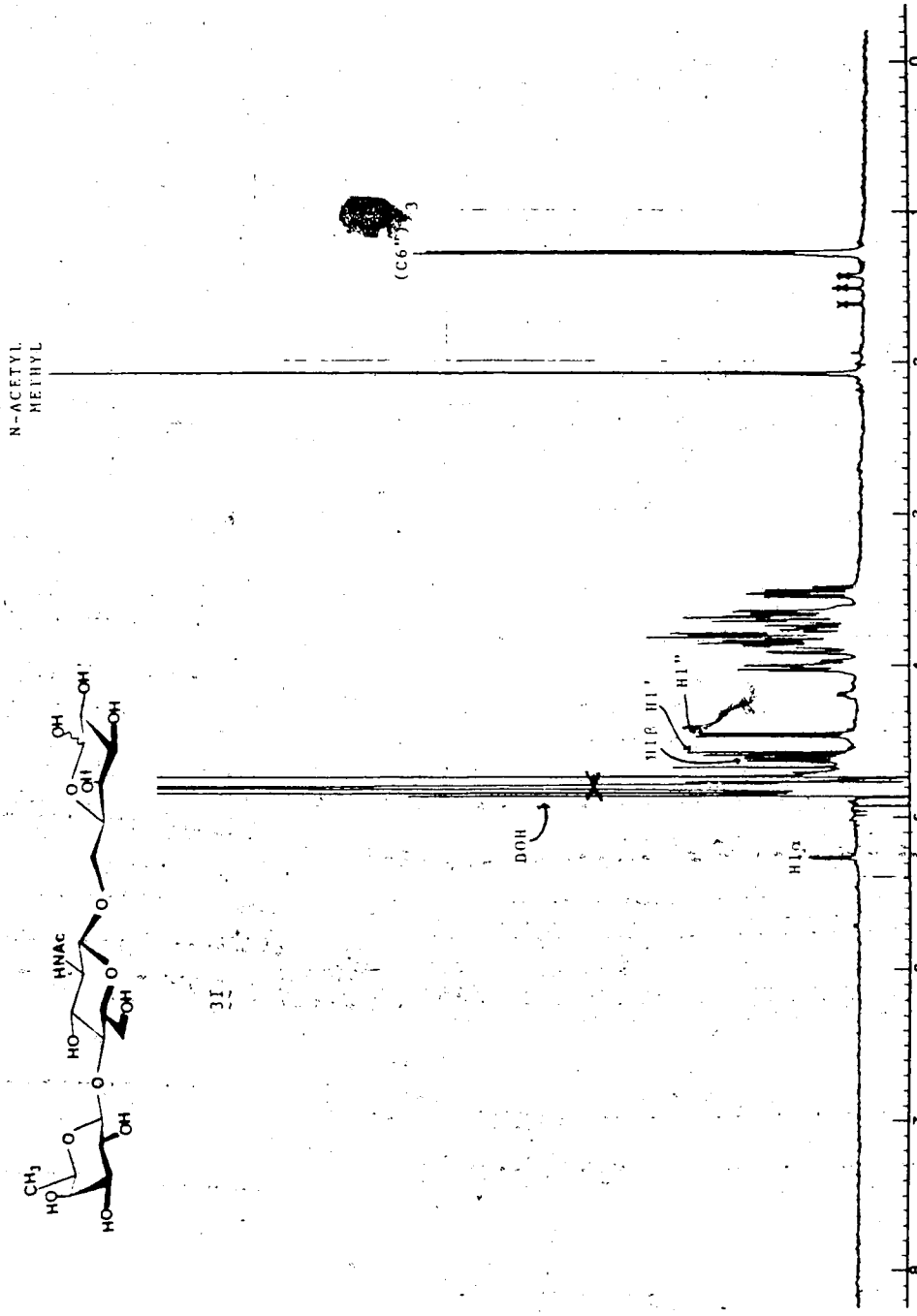
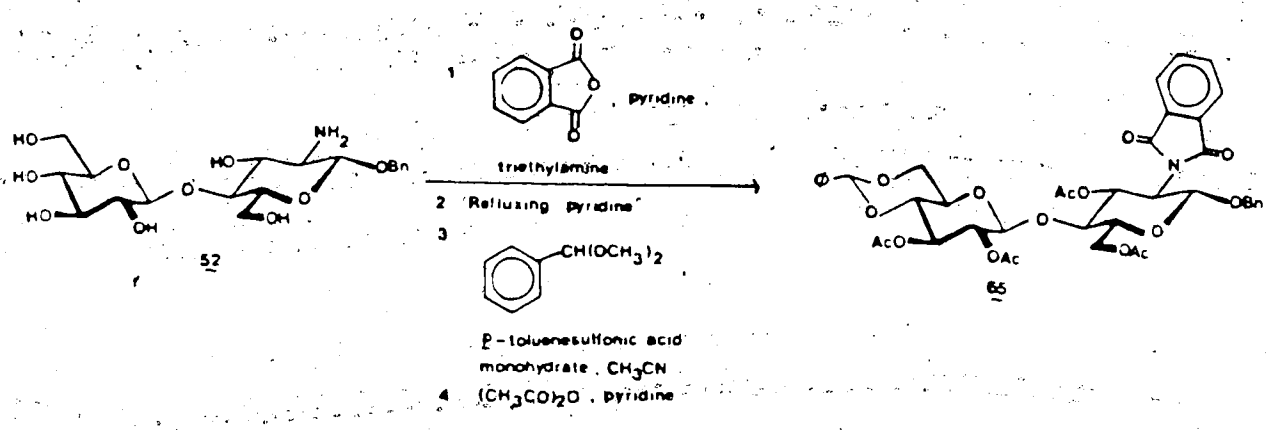
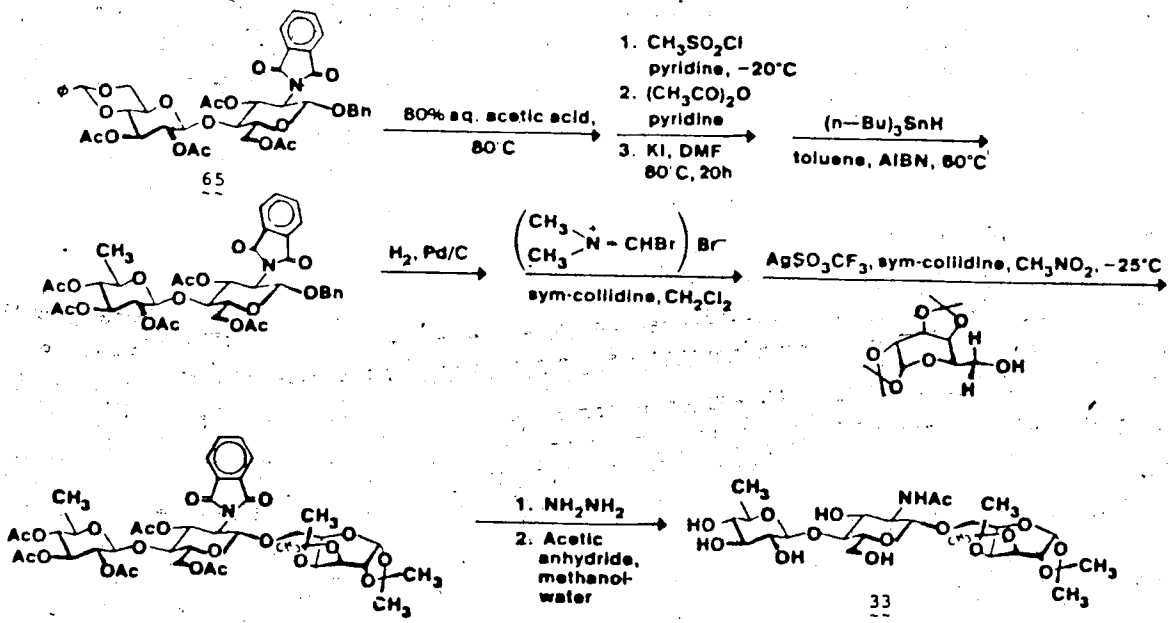


Fig. 37 The 400 MHz ^{1}H-nmr spectrum of compound 31 in D_2O.

of the phthalimido group discussed above (i.e., from the O-carboxybenzamide to the phthalimido derivative), it was decided to make the benzylidene compound 65 directly from the amine (52) without isolation of intermediates as was done in the preparation of 59. The sequence of the reactions used are described below. The product obtained by careful column chromotography was characterized by ^{1}H-nmr spectrum reproduced in Fig. 38. The spectrum is in



accord with the structural assignment and indicates a high state of purity. The reaction sequence from 65 to 33 was similar to that used for the preparation of 31, only short



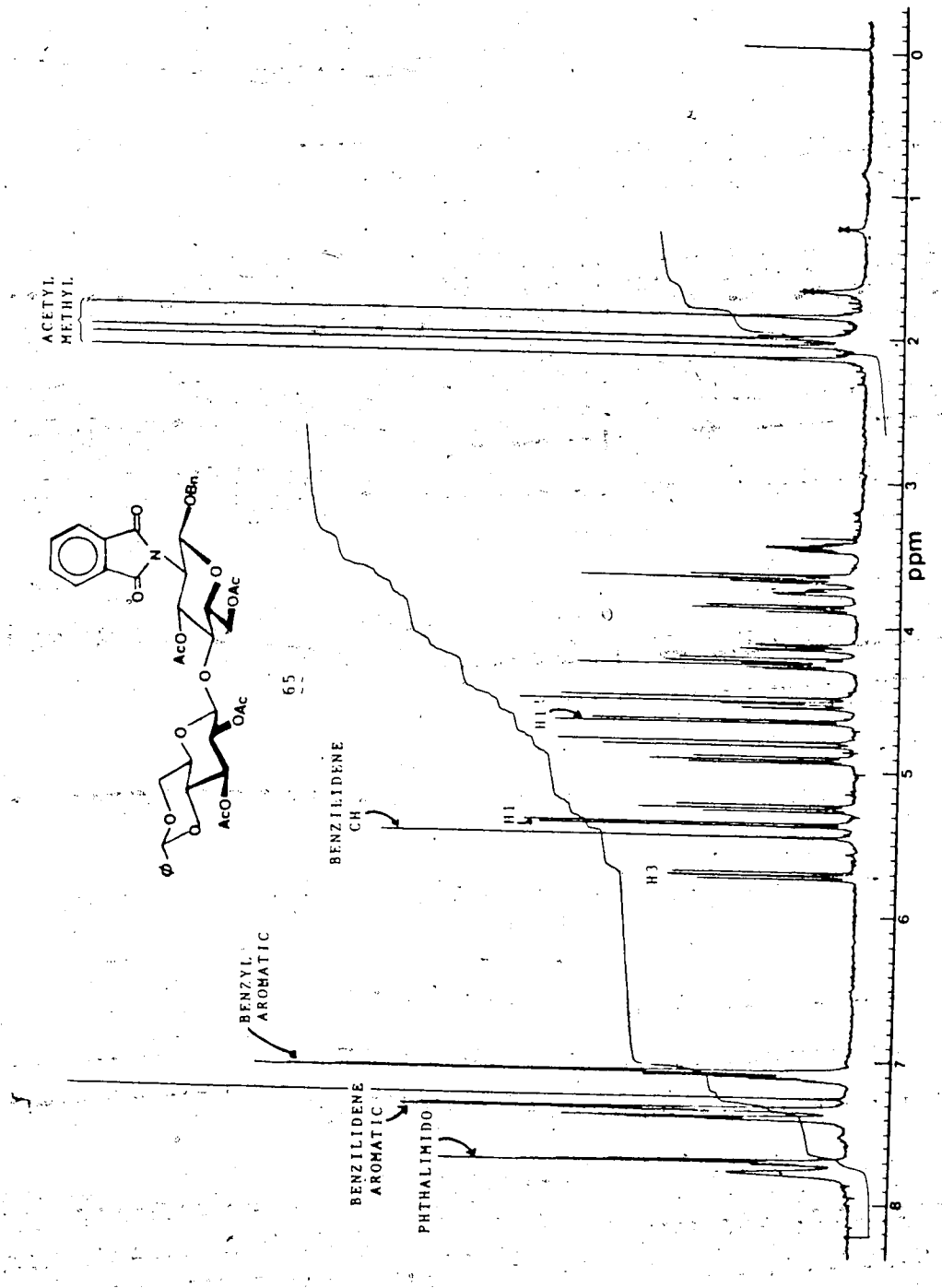


Fig. 38 The 400 MHz ^{1}H-nmr spectrum of compound 65 in $CDCl_3$.

of the last acid hydrolysis step for the removal of the isopropylidene groups. The results were essentially the same except for a smoother reaction in the preparation of the iodo compound 66 which has been commented on before. The ^{1}H-nmr spectra for 67 and 68 are presented in Fig. 39 and Fig. 40 which show the two intermediates to be in essentially pure form to be used in subsequent reactions. It can be seen in Table V that the chemical shifts of the ring protons in 67 is essentially the same as those in the compound 62 (the 4'-epimer which is described on p.112) except the H-4" in 62 is shifted downfield by 0.41 ppm from that in 67, which is expected as the configuration is inverted in that position. The compound 63 and 68 can be compared likewise in the same table. The ^{1}H-nmr spectrum of the compound 33 is presented in Fig. 41 which shows this product to be in highly pure state. This product with the blocked reducing DGal unit (cf. the completely deblocked trisaccharide 31) was used as inhibitor since the compound 17 was found to be highly active in inhibition as has already been mentioned in the introduction of the thesis.

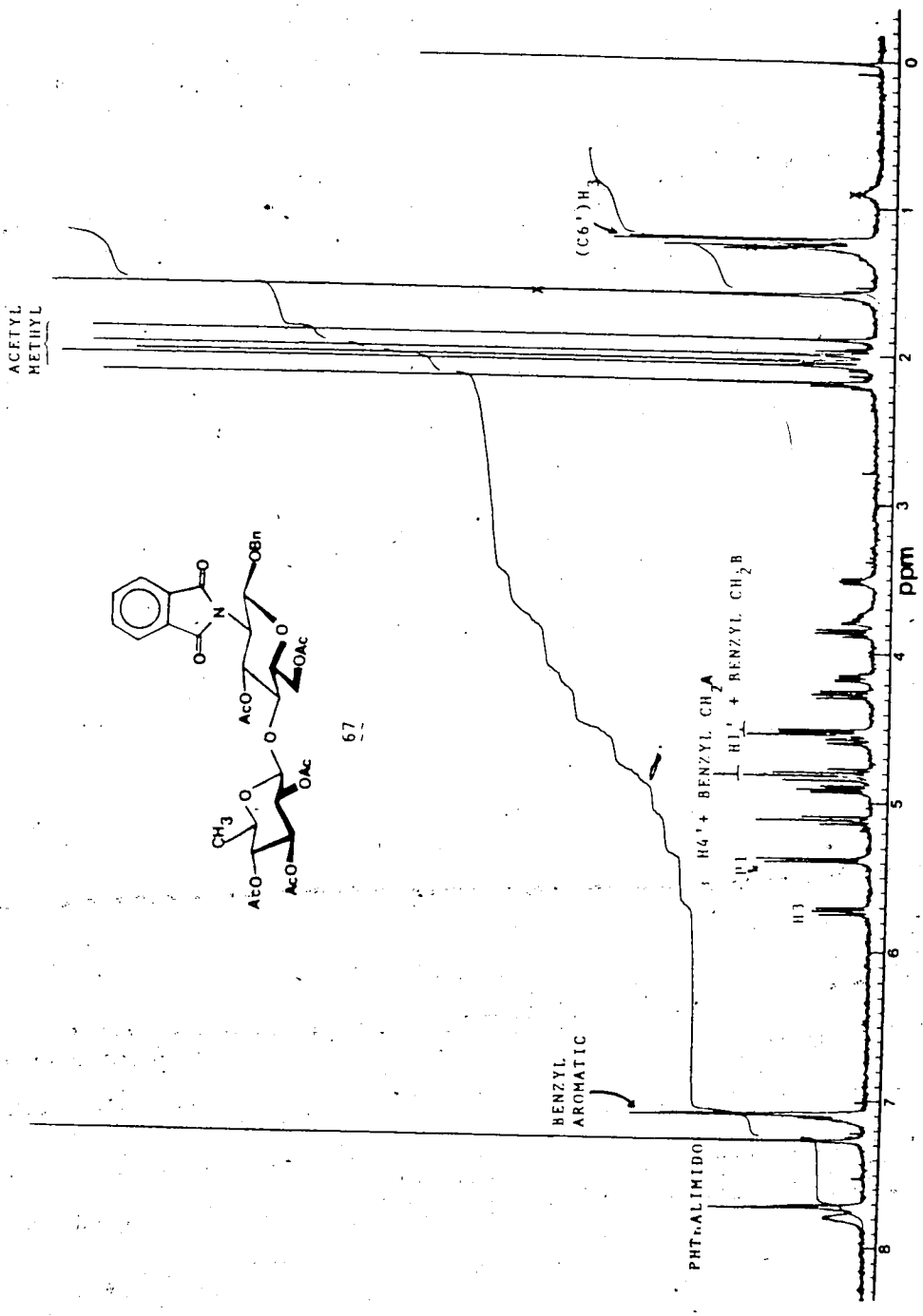


Fig. 39 The 400 MHz ^{1}H-nmr spectrum of compound 67 in $CDCl_3$.

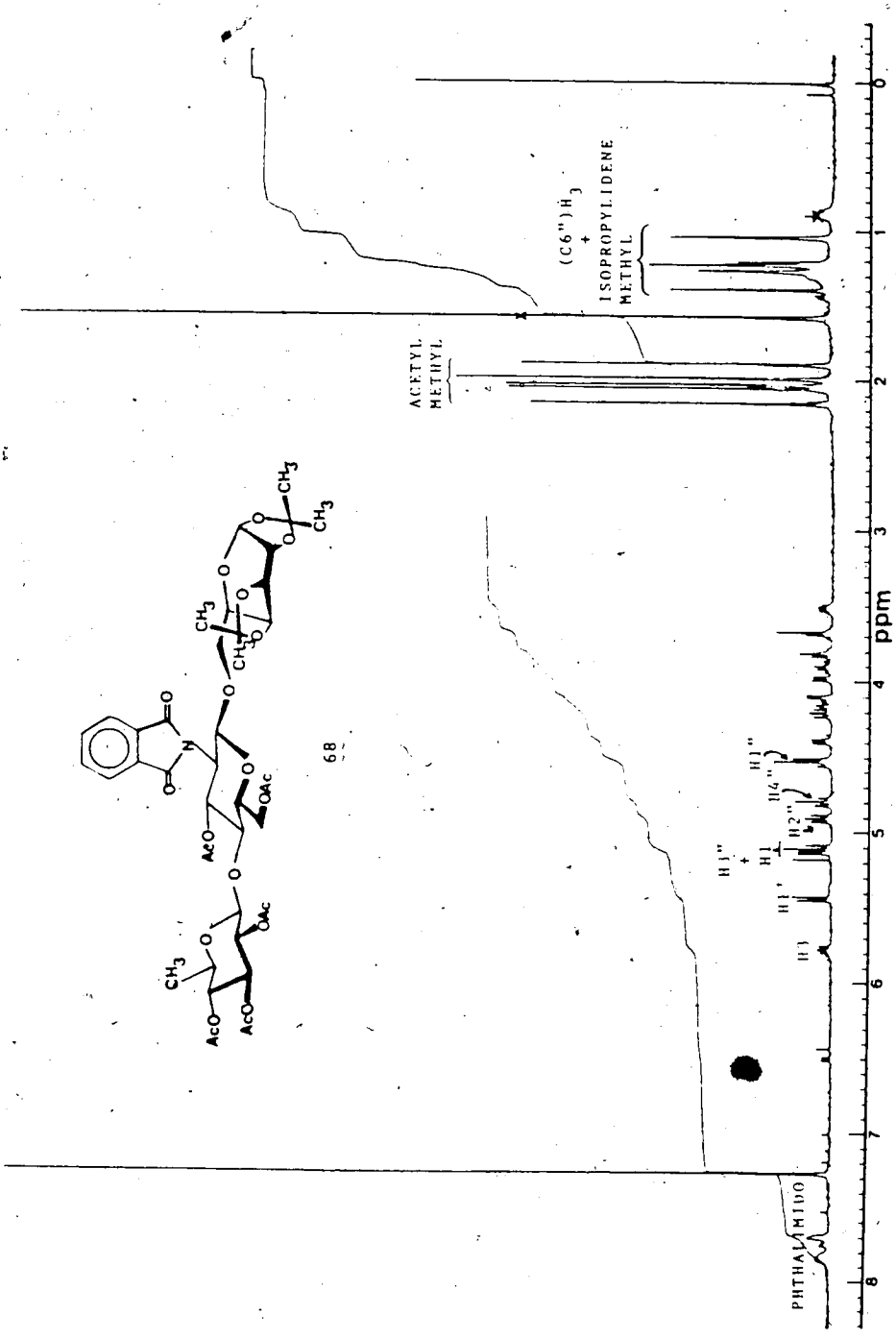


Fig. 40 The 200 MHz ^{1}H-nmr spectrum of compound 68 in $CDCl_3$.

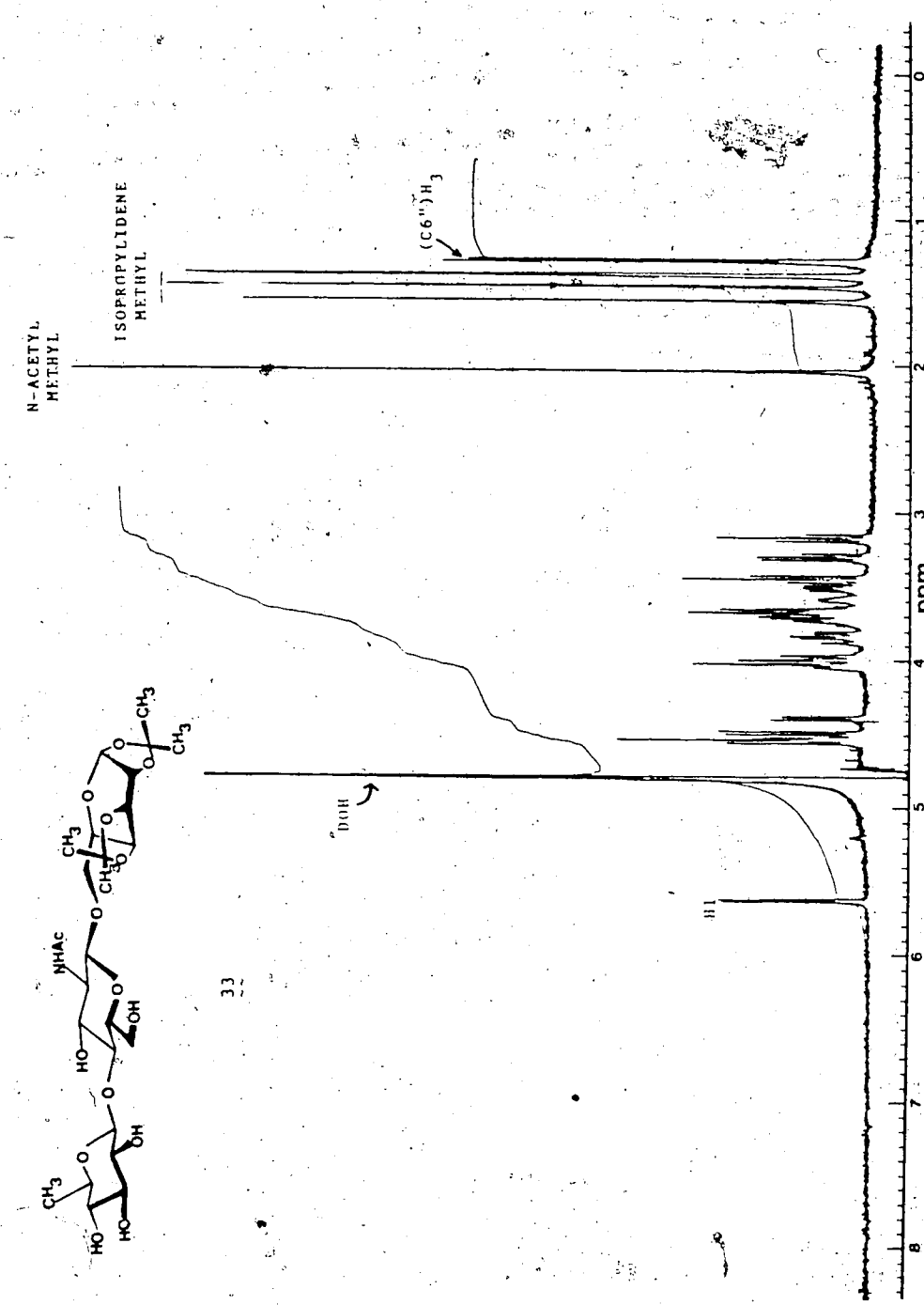
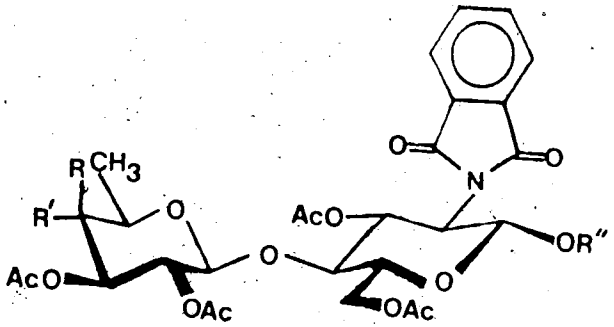


Fig. 41 The 400 MHz ^{1}H-nmr spectrum of compound 33 in D_2O.

TABLE V. ^{1}Hnmr Chemical Shifts* for the Compounds 62, 63, 67 and 68



62 R = OAc, R' = H, R" = Bn

63 R = OAc, R' = H, R" = 1,2;3,4-di-O-isopropylidene-αDGal

67 R = H, R' = OAc, R" = Bn

68 R = H, R' = OAc, R" = 1,2;3,4-di-O-isopropylidene-αDGal

Compound	1	2	3	4	5	6A	6B	1'	2'	3'	4'	5'	6A'	6B'	1"	2"	3"	4"	5"	(C6)"H_3
62								5.41	4.26	5.76	3.88	3.80	4.58	4.17	4.55	5.14	4.98	5.22	3.74	1.26
63	5.13	4.11	4.40	4.00	3.66	—†	—	5.46	4.22	5.81	3.88	3.83	4.53	4.16	4.51	5.09	4.96	5.20	3.74	1.16§
67								5.38	4.26	5.72	3.86	3.78	4.57	4.16	4.51	4.90	5.12	4.81	3.50	1.22
68	5.12	4.10	4.40	4.00	—	—	—	5.43	4.21	5.77	3.90	—	—	4.14	4.51	4.90	5.12	4.80	—	1.22

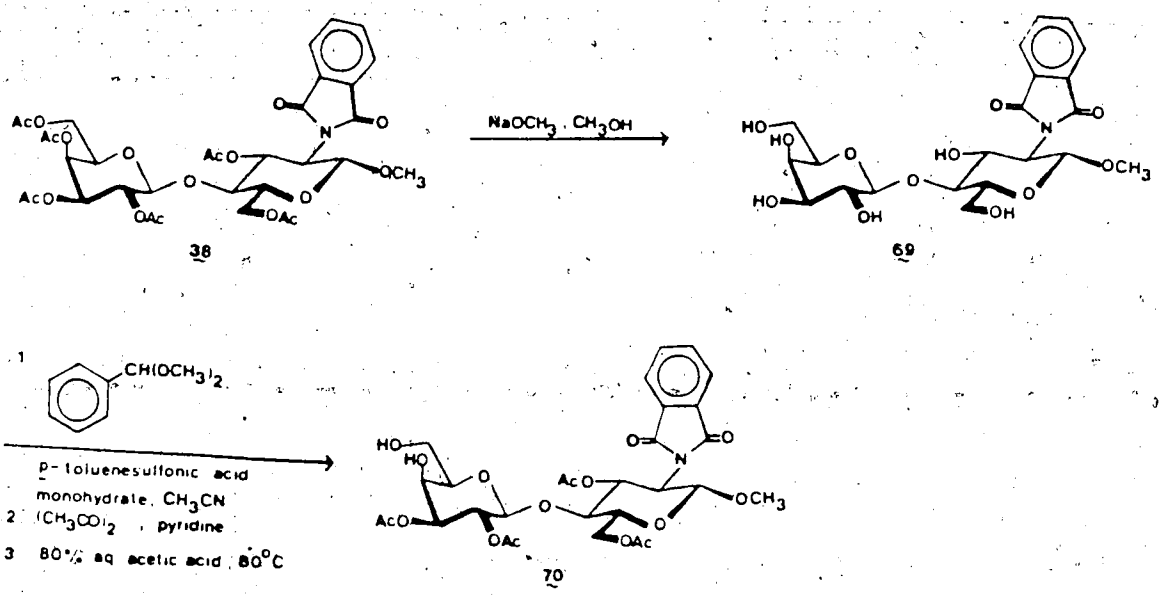
* In $CDCl_3$ at 294°K with $CHCl_3$, δ = 7.27 ppm as internal reference measured at 200 MHz for 62 and 68 and 400 MHz for 63 and 67.

† Signals not assigned

§ This signal is assigned tentatively

F. Synthesis of βDGlcNAc(1→6)βDGal(1→4)βDGlcNAcOCH$_3$ (34)

In order to synthesize the trisaccharide 34, it was decided to attempt a preferential glycosylation on the 6'-OH of the diol (70). This diol was synthesized starting with the methyl glycoside 38 according to the scheme shown below.



Deacetylation of 38 using 0.12M anhydrous sodium methoxide solution[28] proceeded smoothly to provide 69 in quantitative yield. The compound 69 could be characterized by ^{1}H-nmr spectrum presented in Fig. 42. The benzylidenation reaction of 69 proceeded considerably slower than in the case of the benzyl glycoside (76) described before on p.102 presumably due to the lower solubility of the methyl glycoside (69) in acetonitrile. The diol (70) was obtained in 48% overall yield after acetylation and debenzylidenation. The ^{1}H-nmr

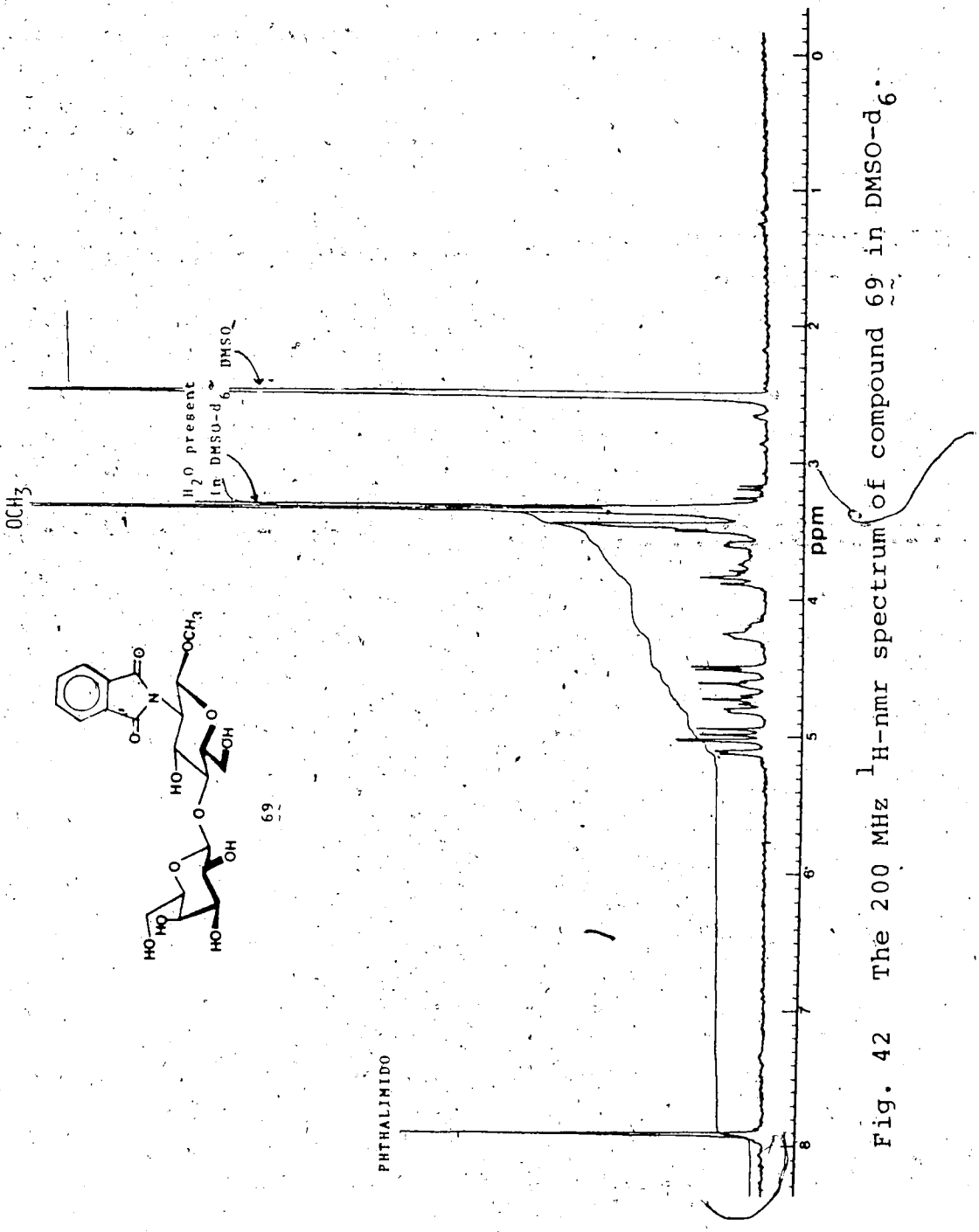
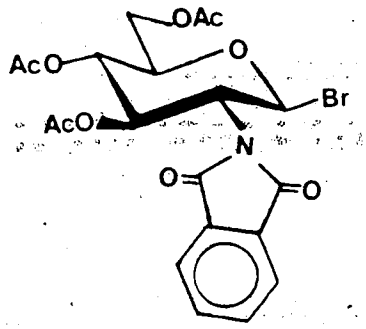


Fig. 42 The 200 MHz ^{1}H-nmr spectrum of compound 69 in DMSO-d_6.

spectrum of this compound is presented in Fig. 43. The fact that the signal for H-4' at δ4.18 is 0.77 ppm to the high field from the signal for H-3' at δ4.95 requires a substitution of an acetyl group on 3'-OH and not on the 4'-OH. This indicates that the product is in accord with the structural assignment and is not the other possible product; namely, the 3',4'-diol.

The glycosylation reaction between the 4',6'-diol (70) and the bromide (78) (kindly provided by S. Sabesan) was per-



78

formed at room temperature as no reaction was observed to occur at low temperature (-10°C) to provide the blocked trisaccharide 71 in 50% yield. The ^{1}H-nmr of this product (71) is presented in Fig. 44. In order to prove that the glycosidic bond was formed with the 6'-OH and not the 4'-OH in this product, the ^{1}H-nmr spectrum (Fig.45) was obtained after 71 was treated with trichloroacetyl isocyanate in deuterated chloroform. This spectrum indicates a one-proton signal appearing at δ5.25 which is not found in the spectrum of 71. This is assigned to H-4' and is a result of the substitution of the

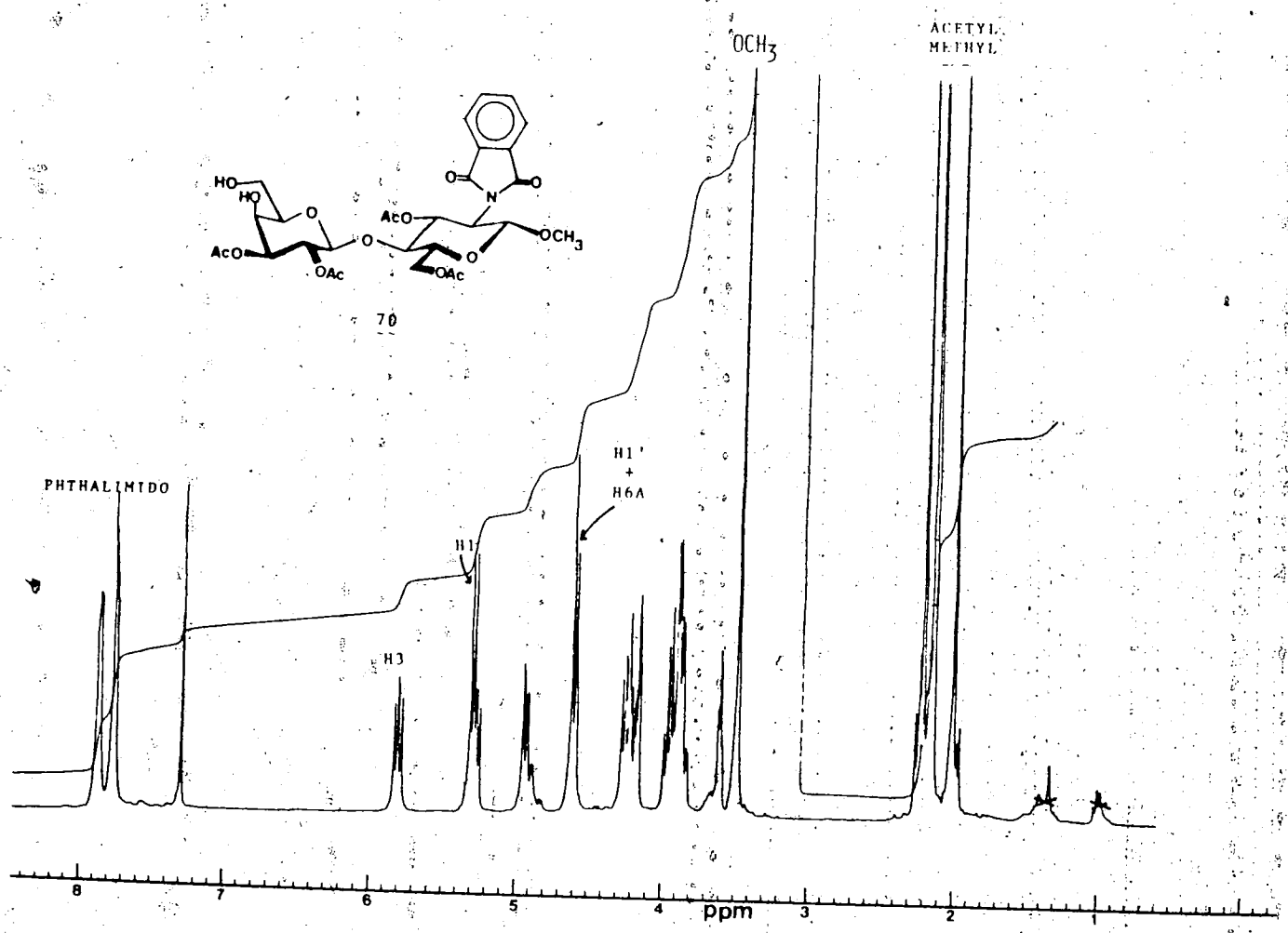


Fig. 43 The 360 MHz ^{1}H-nmr spectrum of compound 70 in $CDCl_3$ containing deuterium oxide.

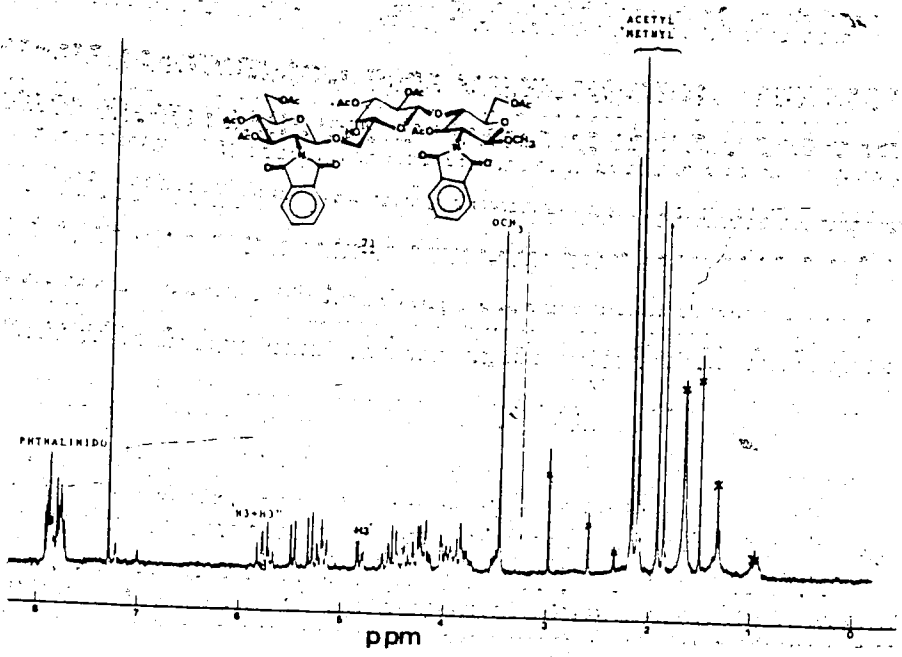


Fig. 44. The 200 MHz ^{1}H-nmr spectrum of compound 71 in $CDCl_3$.

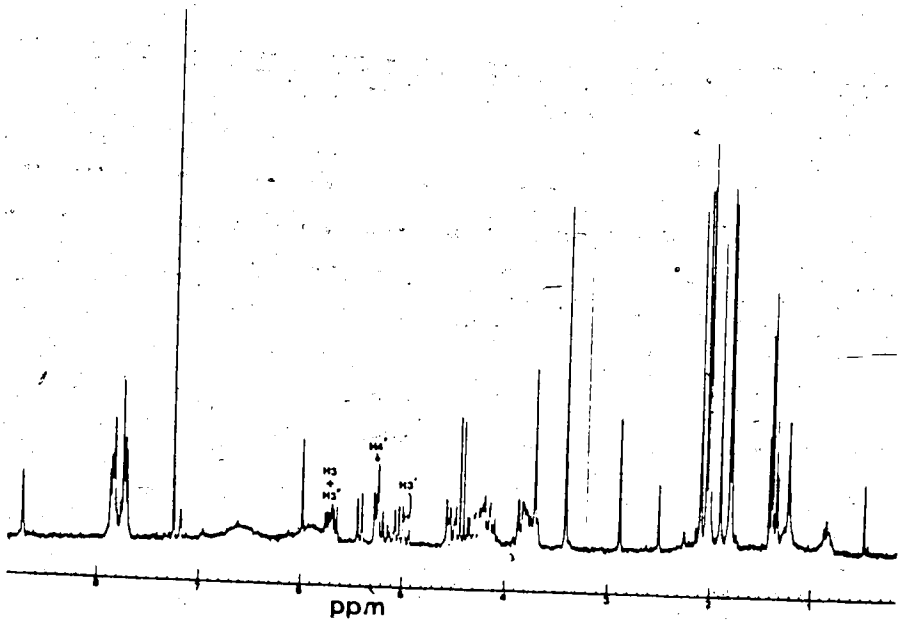
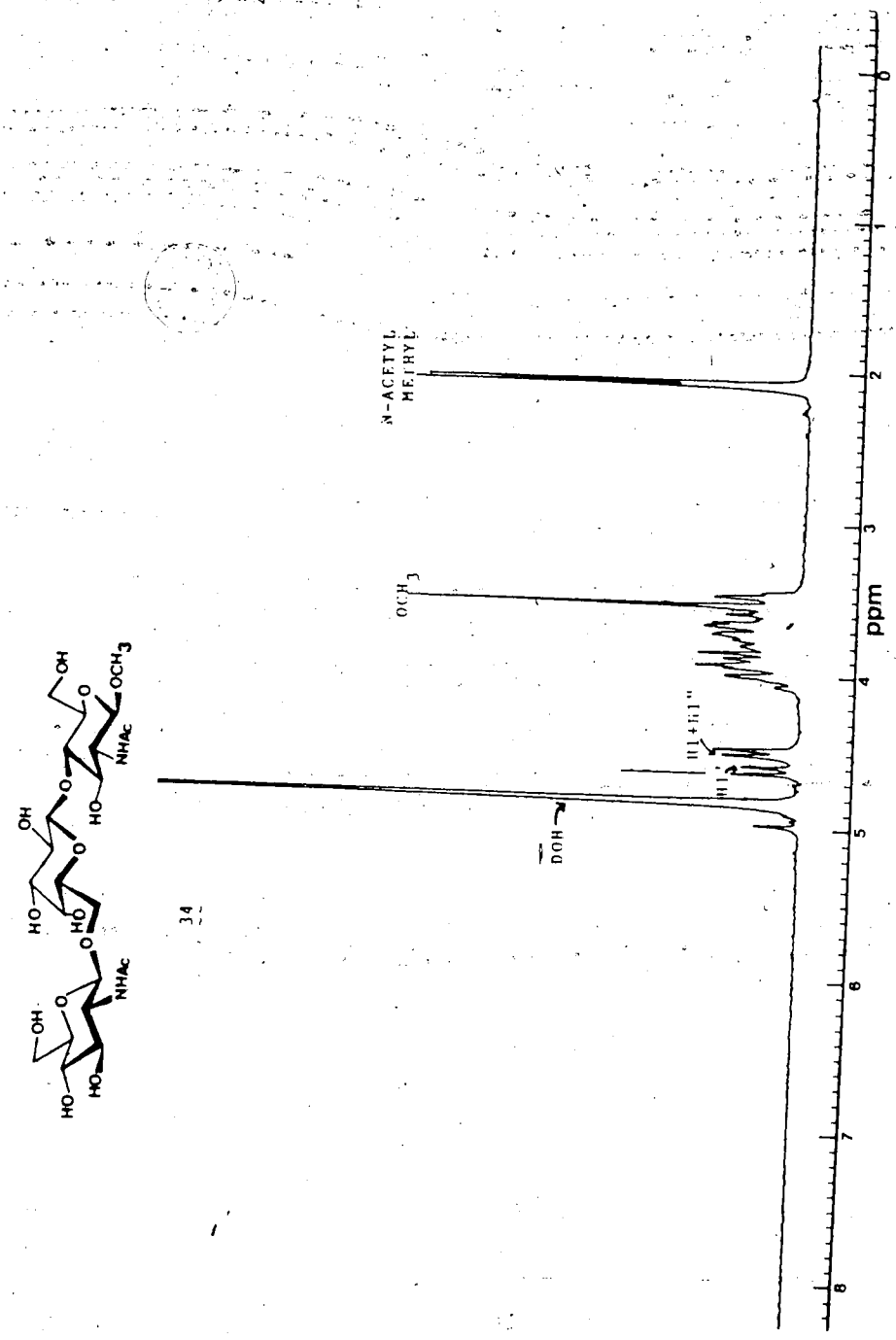


Fig. 45 The 200 MHz ^{1}H-nmr spectrum of the N-trichloacetyl urethane derivative of 71.

N-trichloroacetyl urethane group on the 4'-OH and not the 6'-OH since in the latter case the substitution is expected to cause a much smaller shift of the signals for the two H-6'.

The removal of protecting groups on 71 was achieved by hydrazinolysis and N-acetylation of the product to give the compound 34 in 81% overall yield. This product is characterized by ^{1}H-nmr and ^{13}C-nmr spectra presented in Fig. 46 and Fig. 47, respectively. The spectra are in accord with the structural assignment and indicate a high state of purity. It is to be noted that two signals at δ 60.9 and δ 60.2 on the ^{13}C-nmr spectrum are assigned to C-6 and C-6" while the signal of C-6' is expected to be shifted downfield. This further confirms the 1→6 linkage between βDGlcNAc unit and the central βDGal unit in compound 34.



34

Fig. 46 The 200 MHz ^{1}H-nmr spectrum of compound 34 in D_2O.

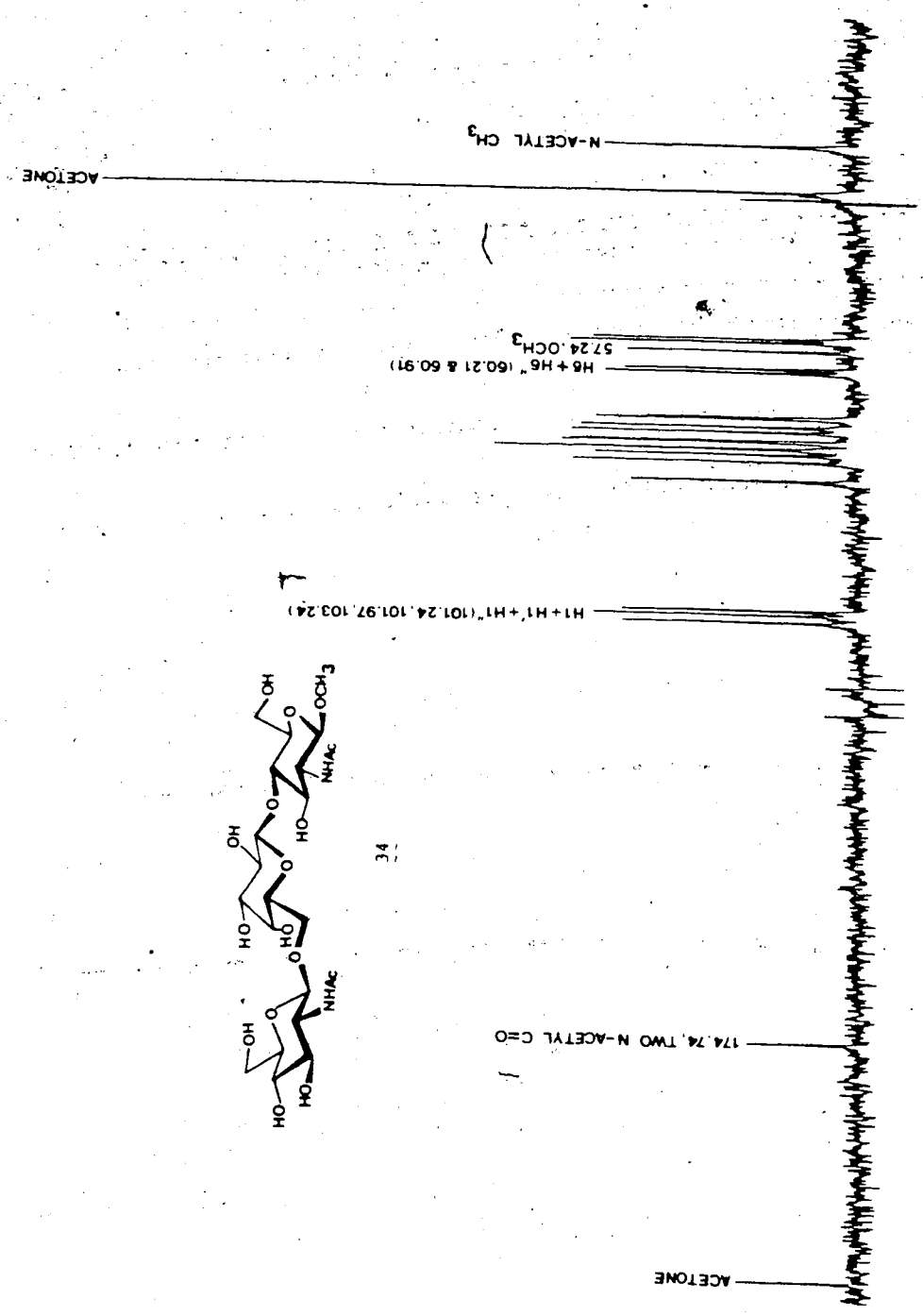
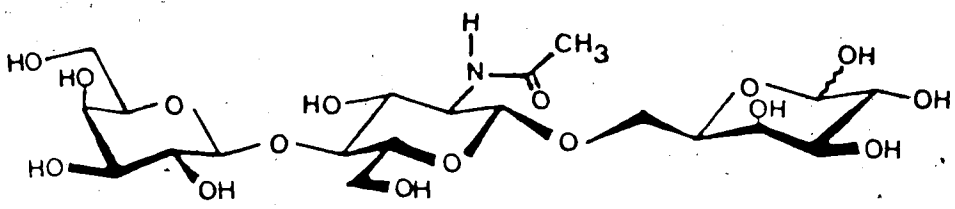


Fig. 47 The 50 MHz ^{13}C-nmr spectrum of compound 34 in D_2O with acetone CH_3 = 30.4 as internal reference.

PART II Conformational Analysis

As discussed in the Introduction, the monoclonal anti-I Ma antibody combining site is at least in part directed towards a certain portion of the surface of the trisaccharide (5).



5

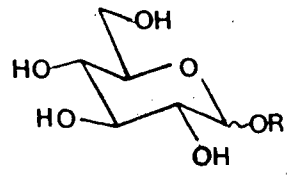
The specificity in the binding of carbohydrate to an antibody results from the presentation of a highly complementary surface by the oligosaccharide to the antibody combining site.[30,31,35] The topographical feature is necessarily related to the stereochemistry and the three-dimensional structure of the sugar molecule. When the chemical structure of the antigenic determinant[65,66] is known; for example, as may be the case for I Ma (5), it is possible to anticipate the various conformations which the molecule can adopt by rotation about carbon-carbon and carbon-oxygen bonds. However, even if the structure is completely imbedded into the combining site, each

conformation can offer a wide range of topographical features. Should the binding occur at the surface of the protein, even though the oligosaccharide were a rigid body of known conformation, there would still remain to establish which topographical feature is involved.

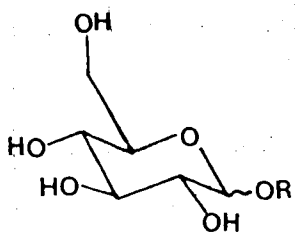
However, oligosaccharides are not rigid structures. The conformational properties of oligosaccharide, which is reviewed by Stoddart[67] in his treatise concerning the stereochemistry of carbohydrates, can be seen to be determined by two factors: (i) the conformations of the individual monosaccharide residues, and (ii) the relative conformations of respective pairs of monosaccharide residues linked glycosidically to each other.

As seen in structure 5, the I Ma determinant consists of an N-acetyl-D-glucosamine residue β-linked to the 6-position of a D-galactose residue and substituted at its 4-position by another D-galactose in the β-configuration. In general, the conformations of the pyranose rings[67] in the sugar unit can be expected to be those preferred for the sugar as a simple glycoside and this can usually be readily ascertained by nuclear magnetic resonance spectroscopy.[68] The three sugar units in the trisaccharide 5 are known to exist virtually entirely in the $^4C_1$ conformers[67] as is indicated by the conformational formula 5. As for the orientation of the hydroxymethyl groups for sugars in aqueous solution, it has been established[69-72] that the conformational preference is importantly affected by the configuration at the C-4 position.

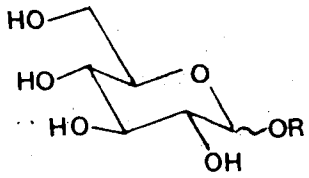
In the case of D-glucose, Lemieux and Brewer[72] had established the orientation of the hydroxymethyl group to be mainly the structure 79a. In the case of D-galactose, Lemieux et al[37] concluded, on the basis of ^{1}H-nmr data, that the conformer 80a



79a

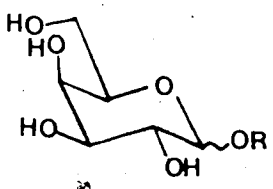


79b

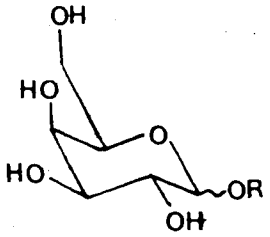


79c

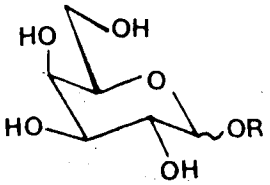
is even more populated than is 79a for D-glucose.



80a

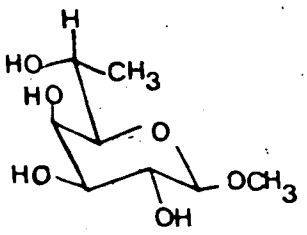


80b

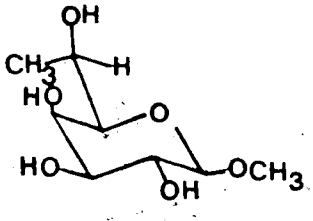


80c

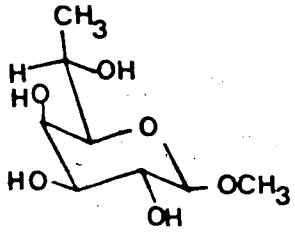
No experimental evidence exists for the existence of appreciable amounts of the conformers 79c and 80b wherein the 6-OH is in syn-axial like orientation with the 4-OH. However, Lemieux et al[73] predicted by hard-sphere calculations and proved by ^{1}H-nmr data that the synthetic diastereoisomeric 6-C-methyl derivatives of D-galactose (81 and 82)[43] exist mainly in the conformation shown as 81a (D-isomer) and 82a (L-isomer). Such conformational preference was found to hold true in the diastereoisomeric βDGal(1→4)βDGlcNAc(1→6)6-C-CH_3-D-Gal trisaccharides (25 and 26) which will be discussed in detail later on.



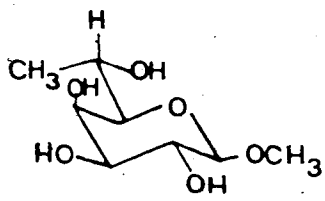
81a



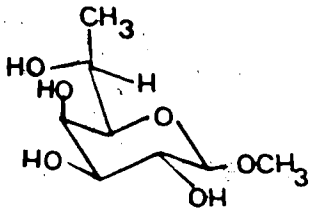
81b



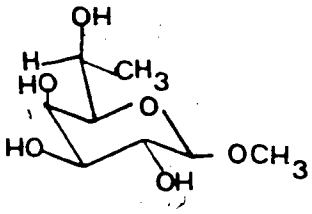
81c



82a

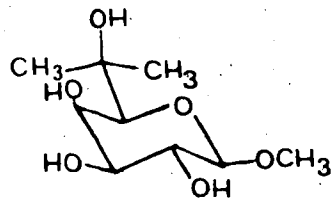


82b

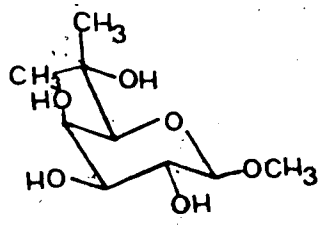


82c

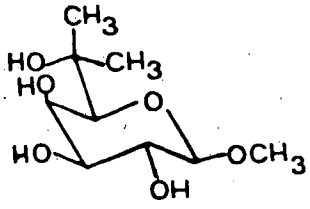
Conformational analyses of the synthetic 6,6-C-dimethyl derivative of D-galactose (83), predicted[73] that the compound should reside mainly in the conformation shown in 83a. This conclusion was supported by ^{1}H-nmr and ^{13}C-nmr data.[73]



83a



83b

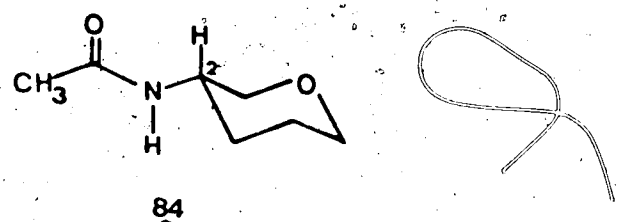


83c

Although the 6-C-methyl and 6,6-C-dimethyl derivatives of D-galactose are not found in nature, the conformational preferences exhibited by these derivatives, which was discussed

above, render them useful for the investigation of the conformation in which the D-galactose was accepted into the combining site of antibody. This matter will be discussed in more detail later in Part IV.

The orientation of the acetamido group is found by X-ray analysis[74] to be near that displayed in 84 where the



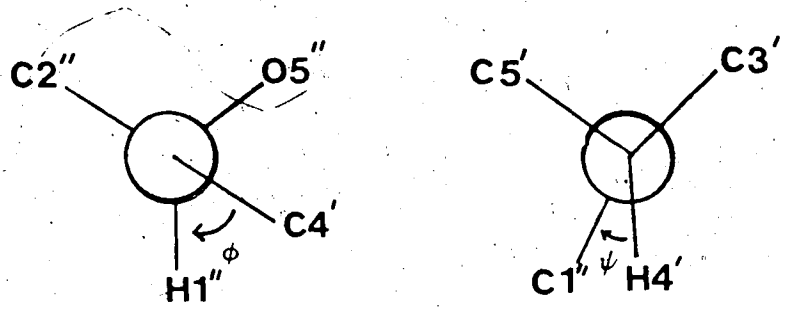
84

C-O and C-N bonds are virtually eclipsed and the N-H bond is nearly trans to the C2-H bond. That this orientation is strongly preferred for the acetamido group of N-acetyl aminosugars is supported by the deshielding of H-2 by the carbonyl group[38] and the large coupling constant between the N-H and the C-H bonds which is normally observed to be 9.0 Hz.[75]

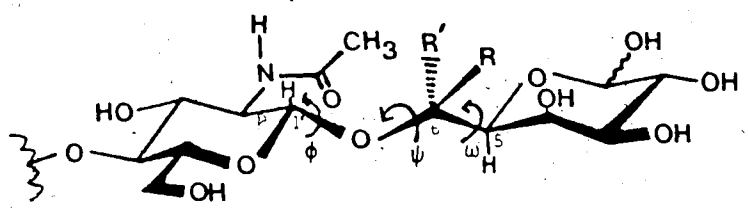
The rotation of the glycosidic and aglyconic bonds gives rise to numerous conformations, each of which provides a different topography for the molecule. Thus, in order to study the binding behaviour of oligosaccharides to proteins, it is essential to appreciate the conformational preferences. In this regard, high-resolution nuclear magnetic resonance spectroscopy[37,38,68] has been found to be the most useful physical method presently available for conformational assignments. However, it must also

be realized that, in the absence of molecular models that define the spatial arrangement of the protons in molecules, it would be extremely difficult to design nmr experiments (nOe, T_1 measurements and other nmr parameters) and to assess the significance of the data obtained by these experiments. In this regard, Lemieux et al[37,38] have been looking into a simple and relatively unbiased empirical method called the HSEA (hard-sphere, exo-anomeric effect) calculations, which, to date, had provided conformational preferences that are in good accord with the nmr data.[37,38] This method is composed of two parts. The first part is the so-called hard-sphere calculations[76] which estimate the non-bonded interactions (based on the Kitaigorodsky expression[77,78] for the ranges of conformational change as one rotates the bonds concerned). Superimposed on this consideration (which usually gives a wide range of conformations of similar energy) is the restriction of the exo-anomeric effect.[79] In the procedure,[37] the potential energy surfaces published by Jeffrey and Pople et al[80] for the internal rotation barrier present in dimethoxymethane, which was derived by ab initio molecular orbital calculations, were used to introduce the influence of the exo-anomeric effect. The assessment of the exo-anomeric effect in different anomers (α- and β-anomers) was also carried out by Lemieux et al.[37]

By means of HSEA molecular modelling method, the conformational preference for the glycosidic linkage at the 4-position of the βDGlcNAc residue of (63) was predicted by Lemieux et al[38] to be near $\phi^{C4'/H1''} = 50°$, $\psi^{H4'/C1''} = 10°$. It is expected that the same conformational preference will be retained



when the molecule complexes with the antibody. Lemieux et al[29] also estimated the glycosyl bond of the βDGlcNAc residue to the 6-position of the reducing βDGal (or αDGalNAc) unit to have the $\phi^{C6/H1'}$ torsion angle close to +60°. However, rotation about either the O6-C6 ($\psi^{C5/C1'}$ torsion angle) or C5-C6



5 R = R' = H

25 R = CH_3, R' = H

26 R = H, R' = CH_3

($\omega^{H5/O6}$ torsion angle) bonds was expected to lead to a number of conformers which differ very little in energy. HSEA calculations[54] showed that the $\psi^{C5/C1'}$ torsion angle could vary within the following ranges (Table VI) without encountering energy barriers greater than ±0.5 kcal/mole.

TABLE VI Conformers of Near Equal Energy for βDGlcNAc(1→6)βDGal by HSEA Calculations[54]

$\phi^{C6/H1'}$	$\omega^{H5/O6}$	$\psi^{C5/C1'}$
60°	60°	120-290° (-70°)
60°	180°	110-240° (-120°)
60°	-60°	100-260° (-100°)

Under this circumstance, no conclusion could be drawn as to which conformer was most likely to be accepted by the I Ma combining site. However, it was expected that the range of low energy conformers could be narrowed by the substitution of a methyl group for a hydrogen at the C-6 atom of the reducing βDGal residue in 5. This would lead to the two diastereoisomers 25 (the L-isomer) and 26 (the D-isomer). Indeed, HSEA calculations[54] showed the most favorable conformer in the case of 26 (the D-isomer) to be near that with $\phi^{C6/H1'}$ = 60°, $\omega^{H5/O6}$ = 60° and $\psi^{C5/C1'}$ = 130°. The $\omega^{H5/O6}$ = 60° torsion angle requires that the torsion angle defined by H-5 and H-6 be near 180°. The ^{1}H-nmr spectrum of 26 (Fig.48a) indicates a coupling constant of 8.5 Hz between H5 and H6 which is of the magnitude that requires the vicinal H5 and H6 atoms to be anti-periplanar. Examination of a molecular model in this conformation shows the C-6 methyl group to be close to H-5 and H-6 only (Fig.49). In fact, saturation of the methyl group led to nuclear Overhauser enhancement[81,82] of only these two hydrogen (Fig.50). The signals for H-4 at 4.17 and 4.24 ppm (α and β anomers) was not affected.

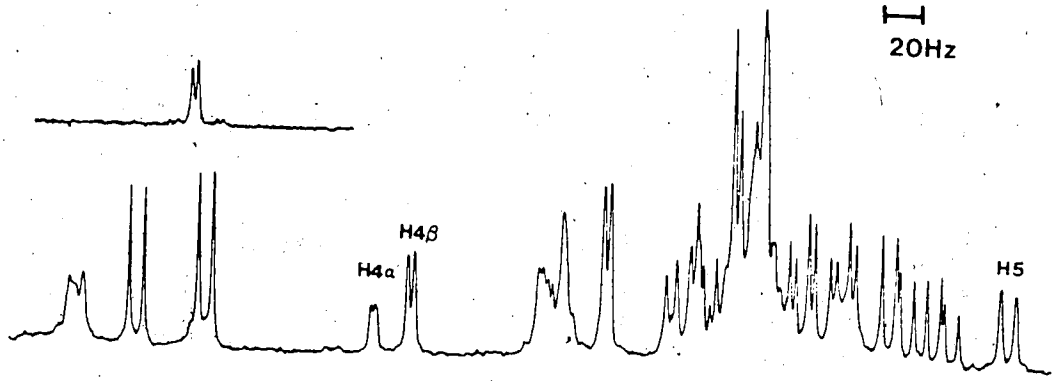


Fig. 48a The 400 MHz ^{1}H-nmr spectrum of compound 26 showing the signals for the ring protons and indicating the coupling constant of 8.5 Hz between H5 and H6.

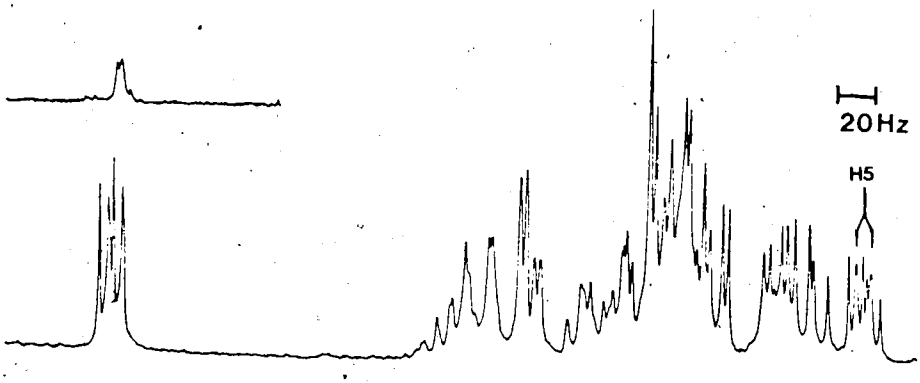
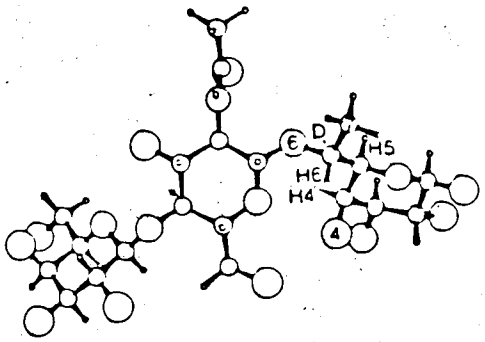
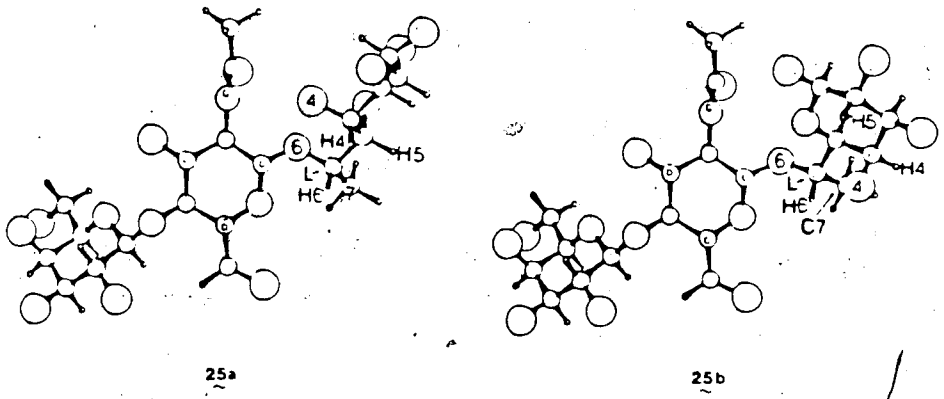


Fig. 48b The 400 MHz ^{1}H-nmr spectrum of compound 25 showing the signals for the ring protons and indicating the coupling constant of 8.0 Hz between H5 and H6.



26

Fig. 49a Computer drawing of the D-trisaccharide (26) in the conformation anticipated by HSEA calculation in order to display the anti-periplanar orientation of H5 and H6 and the orientation of the C7-methyl group relative to H4.



25a 25b

Fig. 49b Computer drawing of the L-trisaccharide (25) in the conformation (25a) anticipated to be most favorable by HSEA calculation and in the conformation (25b) best indicated by the ^{1}H-nmr spectrum. In the case of 25a, note the syn-axial like arrangement of O4 and O6, the syn-clinal orientation of H5 and H6, and that the C7-methyl group is distant from H4. In the case of 25b, note H5 and H6 are in anti-periplanar orientation and that the C7-methyl group is in syn-axial like orientation with H4.

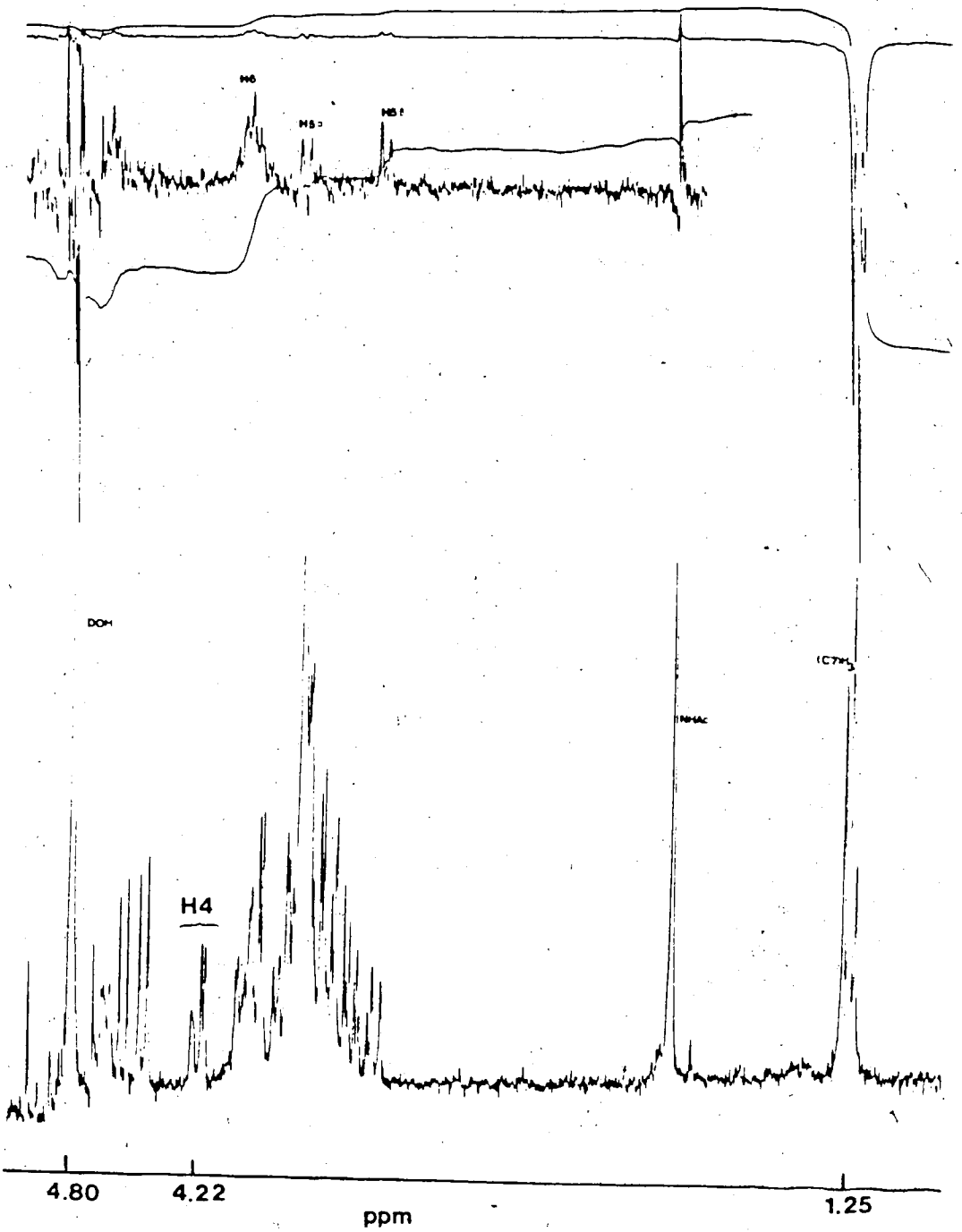


Fig. 50 The 200 MHz ^{1}H-nmr spectrum of compound 26 and the nuclear Overhauser enhancement of the signals for H6, H5α and H5β observed on irradiation of $(C7)H_3$.

The HSEA calculations for the structure 25 (the L-isomer), however, indicated a conformation 25a (Fig.49) which has the torsion angles $\phi^{C6/H1'} = 50°$, $\psi^{C5/C1'} = 230°$ and $\omega^{H5/O6} = 190°$ to be 0.6 Kcal/mole lower in energy than the conformation 25b which has the torsion angles $\phi^{C6/H1'} = 45°$, $\psi^{C5/C1'} = 250°$ $(-110°)$ and $\omega^{H5/O6} = 290°$ $(-70°)$. It appeared that the conformer 25a should be more abundant than 25b. However, a coupling constant of 8.0 Hz between H5 and H6 was observed on ^{1}H-nmr (Fig.48b) which implies an anti-periplanar orientation of the vicinal protons. This would be expected of 25b and not 25a conformer where these atoms are in syn-clinal orientations. Saturation of the signal for the C-methyl group resulted in strong enhancement of the signal for H-4 (see Fig.51) as well as those for H5 and H6 in keeping with conformation 25b and not 25a. The discrepancy between the ^{1}H-nmr observations and the HSEA prediction in this case might be due to the fact that the syn-axial like non-bonded interactions between O4 and O6 in 25a was underestimated by the hard-sphere calculation. In fact, Lemieux and Pavia[69] had noted that the effective radius for oxygen may vary depending on its substitution and in HSEA calculations this was assumed to be 1.5 Å which is the Van der Waals radius. Moreover, the calculations do not take into account the electrostatic effect[83] and the effect on the apparent volume of hydroxyl groups when these are hydrogen-bonded to solvent water.[84]

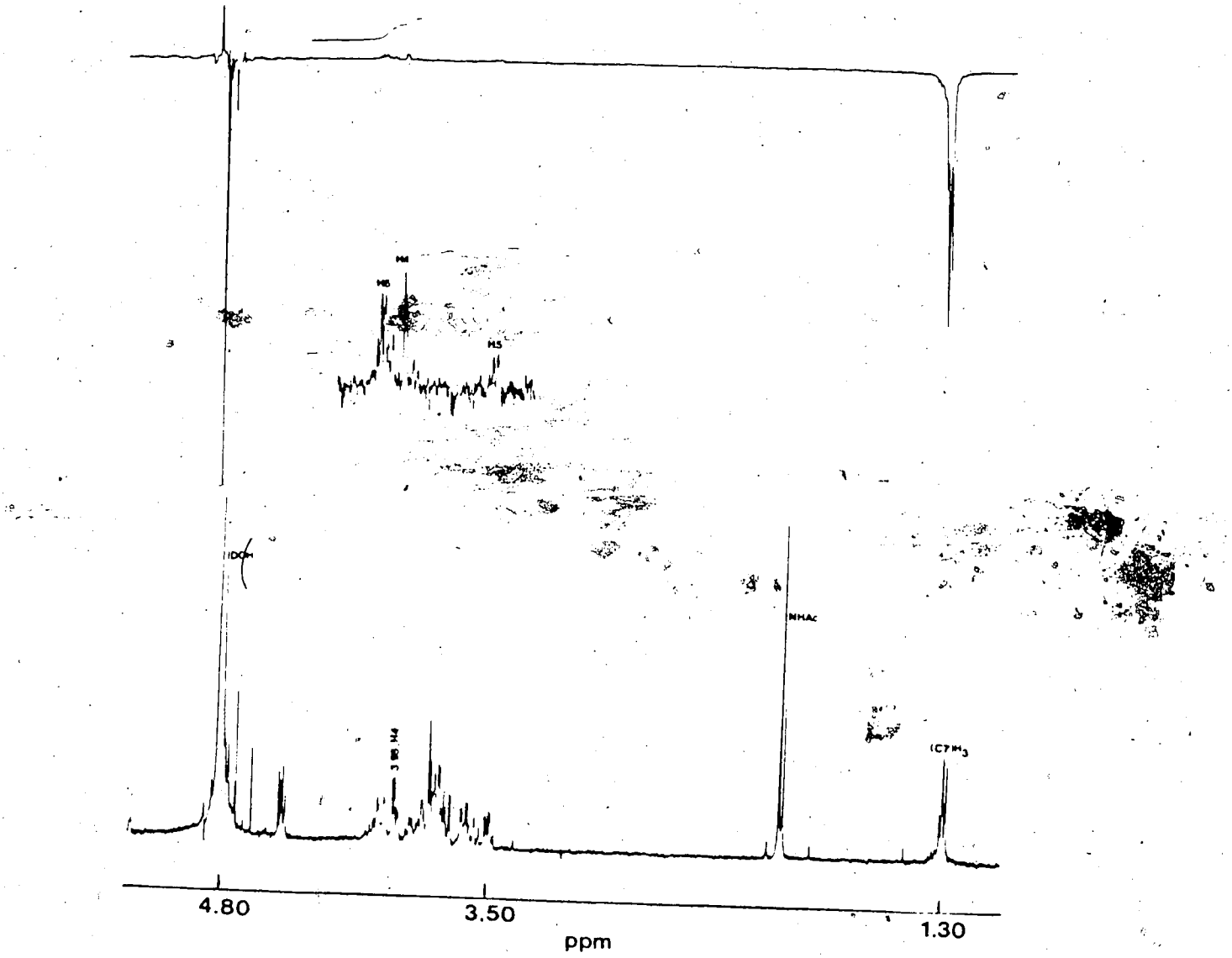


Fig. 51 The 400 MHz ^{1}H-nmr spectrum of compound 25 and the nuclear Overhauser enhancement of the signals for H6, H4 and H5 observed on irradiation of (C7)H .

The nOe results mentioned above also confirms the configurations of the two isomers which has not been accomplished in the precursor 29 and 30 due to the presence of the isopropylidene groups.

The compounds 25 and 26 are thus considered to be conformationally more rigid structure than 5 about the C5-C6 and the O6-C6 bonds. This aspect of their conformational properties were taken advantage of in binding studies which were intended to determine as to which conformation the I Ma determinant (5) is accepted into the combining site of the antibody. Although it was predicted by HSEA calculations that 5 is distributed among a wide range of conformers described in Table VI, an observation of the ^{1}H-nmr chemical shifts of 17, 18 and 19 suggests that the compound 18, which is the 1,2;3,4-di-O-isopropylidene derivative of 5, might be restricted to certain conformations. The signals of H-4 in the acylated derivatives (18 and 19) of 17 are shifted by 0.24 ppm from that of 17 (Figs.5-7) which indicates that 18 and 19 might reside in a conformation that requires the acetamido- or trifluoroacetamido group to be on the same side of the molecule as O-4. This means that the compound 18 are highly populated in conformers which are close to that preferred by the D-isomer (26) which has the acetamido group close to H-4 (Fig.49a). Since the conformation preferred by the L-isomer has the acetamido group away from H-4 (Fig.49b), the above-mentioned deshielding effect is unlikely to occur if 18 occupies such a conformation. Although it is difficult to assess

the effect of the isopropylidene groups on the conformation of the partially blocked trisaccharide (18) as compared to 5, a good guess can be made that the conformational preference of the two compounds are not appreciably different since the compound 18 was found to be a good inhibitor and is expected to resemble 5. This matter will be discussed in Part IV after the inhibition results are presented in Part III.

PART III Inhibition Assays

All of the inhibition tests were conducted by Jerry Liao in the laboratory of Professor E. A. Kabat. The method used by J. Liao will be discussed below followed by the results of such tests.

A. Method

The Anti-I Ma (group 1) serum was obtained from a patient (Ma) of group O and had a titer of 16,000 at 4°C.[8] The precursor substance, OG-20%-from-10%, was used as an antigen.[12] This is a fraction obtained from human ovarian cyst fluid by digestion with pepsin, precipitation with ethanol, extraction of the dried precipitate with 90% phenol and then isolating that fraction which is not precipitated from phenol by 10% ethanol but is precipitated by 20% ethanol.

The quantitative precipitin inhibition assays were carried out as follows.

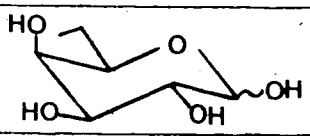
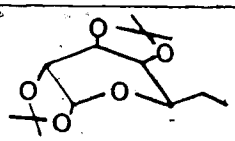
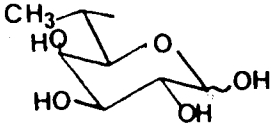
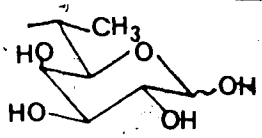
Varying quantities of oligosaccharides (reported below in μmoles) were added at 0°C to 30 μL of a 1:2 dilution of anti-I Ma.[11,27] Total volumes were adjusted with saline to 400 μL. After 30 min at 0°C, 14.5 μg of OG-20%-from-10%, a quantity which had been shown previously to precipitate all of the antibody in the absence of inhibitor, was added. The contents of the tubes were mixed, kept at 0°C for 7 days, stirred twice daily. The tubes were then centrifuged in the cold to

collect the precipitates, which were washed twice with 0.5 mL cold saline. The washed precipitates were analysed for nitrogen by the ninhydrin method.[85] The per cent inhibition was calculated from the decrease in specifically precipitated nitrogen in the presence of inhibitor as compared with a control to which no inhibitor had been added.

B. Results of Inhibition Assays

TABLE VII

Inhibition of the precipitin reactions of the anti-I, Ma antibody and the OG glycoprotein. Effects of changes in the aglycon (R)[§] (βDGal(1→4)βDGlcNHAcOR)

Compound	R	μmoles added for 50% inhibition	ΔΔG° * (kcal/mole)	Relative potencies
(5)		0.45	0.00	1.00
(17)		0.59	0.16	0.76
(20)	CH_3-	0.16	-0.61	2.81
(21)	CH_3CH_2-	0.16	-0.61	2.81
(22)	$CH_3CH_2CH_2$-	0.29	-0.26	1.55
(23)	$(CH_3)_2CH$-	0.22	-0.42	2.04
(24)	$(CH_3)_2CH\ CH_2$-	0.83	0.36	0.54
(25)		>1.25	>0.60	<0.36
(26)		0.16	-0.61	2.81

§ The experimental plots are presented in Fig. 52.

* The ratio of the molar amounts of two inhibitors which reduce the amount of precipitin formation by 50% (μmole of reference compound/μmoles of other inhibitors) is taken as the relative equilibrium constant Krel and this value is used[35] to calculate the difference of the binding free energies ($\Delta\Delta G^{\circ}$) for the two inhibitors with the antibody using the usual expression.

$$-\Delta\Delta G^{\circ} = RT \ln K_{rel}$$

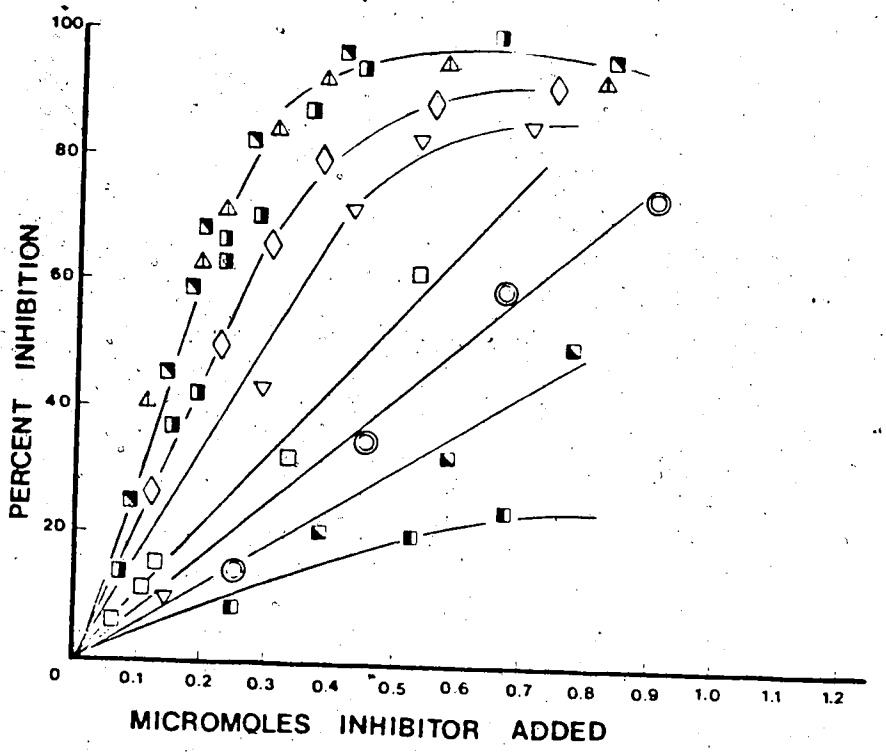
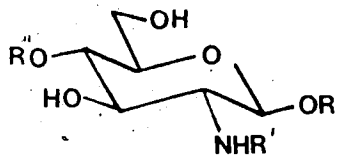
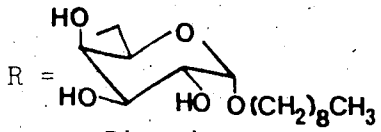
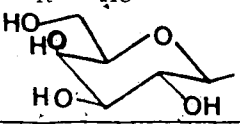
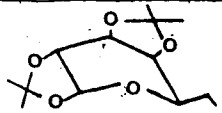
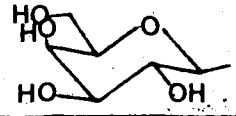
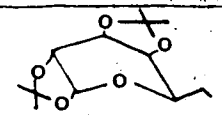
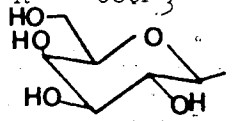


Fig. 52 Plots of inhibition of precipitation on mixing 15 µL of anti-I Ma (30 µL of a 1:2 dilution) with 14.5 µg of OG 20% from 10% in a total volume of 400 µL and using compounds 5 (□), 17 (◎), 20 (◪), 21 (△), 22, (▽), 23 (◇), 24 (◪), 25 (◧) and 26 (◨) as inhibitor.

TABLE VIII

Effects of changes in the β-D-GlcNAc unit[§]



Compound	Structure	μmoles added for 50% inhibition	$\Delta\Delta G^{o}$ * (kcal/mole)	Relative potencies
(4)	 R' = Ac, R" = H	Inactive	Inactive	Inactive
(1)	R = H, R' = Ac, R" = 	3.1	1.14	0.15†
(18)	R =  R' = H, R" = 	Inactive	Inactive	Inactive
(19)	R =  R' = $COCF_3$, R" = 	Inactive	Inactive	Inactive

§ The experimental plots are presented in Fig. 53.

* See Table VII (p.152), compound (5) is also used as the reference.

† Compound (5) is used as the reference.

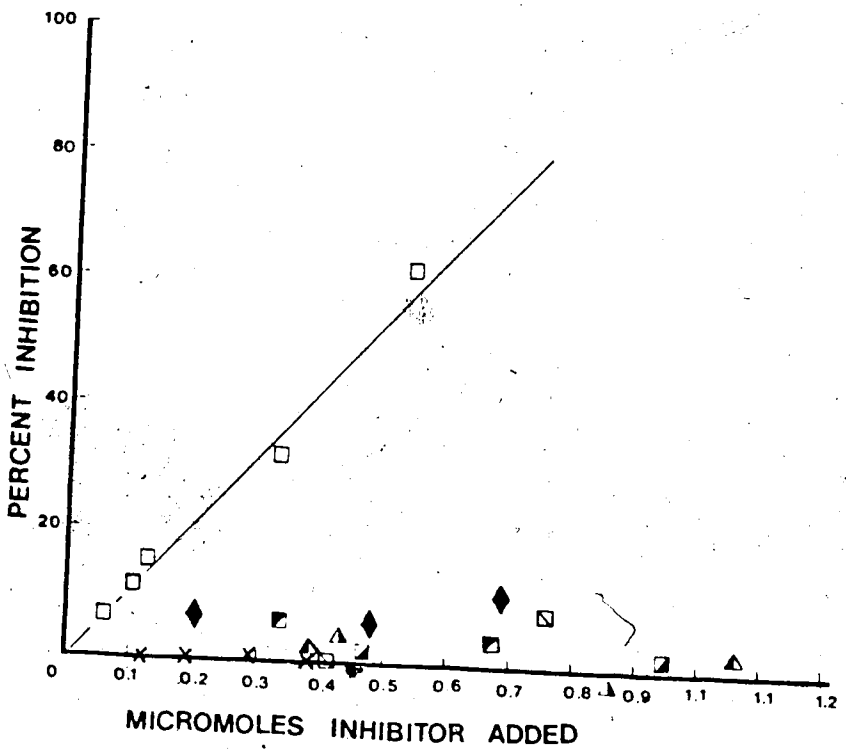
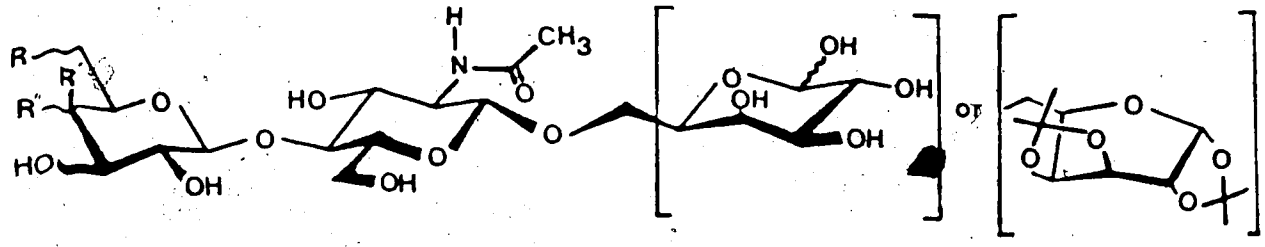
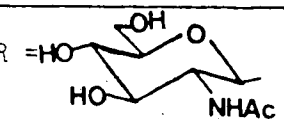


Fig. 53 Plots of inhibition of precipitation on mixing 15 µL of anti-I Ma (30 µL of a 1:2 dilution) with 14.5 µg of OG 20% from 10% in a total volume of 400 µL and using compounds 5 (□), 4 (◆), 18 (◭), 19 (◭), 31 (◩), 32 (◪), 33 (◪) and 34 (×) as inhibitors. The compound 1 (LacNAc) provided 50% inhibition at the level of 3.1 µmoles.

TABLE IX

Effects of changes in the terminal βDGal unit[§]



Compound	Structure	Potency as an Inhibitor
(31)	R = H, R' = OH, R" = H	Inactive at 0.68 μmole
(32)	R = OH, R' = H, R" = OH	Inactive at 0.95 μmole
(33)	R = H, R' = H, R" = OH	Inactive at 0.75 μmole
(34)	 R' = OH, R" = H	Inactive at 0.4 μmole

[§] The experimental plots are presented in Fig. 53.

PART IV. The binding of βDGal(1→4)βDGlcNAc(1→6)DGal by Monoclonal Anti-I Ma Antibody

The evidence was presented in the introduction which indicates that the anti-I Ma antibody combining site is directed towards a certain part on the trisaccharide βDGal(1→4)-βDGlcNAc(1→6)DGal(or (1→6)DGlcNAc) (5). The hypothesis that the complementarity arises due to the steric requirement (with some flexibility arising from conformative response) of the combining site as well as hydrophobic bonding between carbohydrate molecule and the atomic groupings of the combining site was also discussed. In the present work, the binding of the trisaccharide (5) with the anti-I Ma antibody was examined in terms of this hypothesis by inhibition assays using synthetic 'fraudulent' structure as inhibitors.

The size of the combining site of the monoclonal anti-I Ma antibody can be defined by the relative potencies in inhibition of the compounds 4 - 6, 17, 18 and 31 - 34. Compound 5, which is the natural structure most active in the binding of the anti-I Ma antibody, is designated the value of 1.0 in its relative potency in inhibition as compared to various synthetic inhibitors whose values are also reported in Table VII in Part III. It has been mentioned in the Introduction that the intact reducing DGal unit is not required in the binding. This is confirmed by the fact that compound 17 was found to be 0.76 times as good an inhibitor as 5 (Table VII). The

was to be expected,[10,27] the isopropylidene groups present in 17 do not cause much hindrance to the binding. Of major interest is the observation that the simple methyl glycoside (20) is a 2.8 times more potent inhibitor than the I Ma trisaccharide 5. Consequently, the major portion of the reducing DGal unit is not involved. The addition of a methyl group to form the ethyl glycoside (21) had no appreciable influence on the potency. Thus, it can be concluded that the binding of the reducing βDGal unit of the I Ma trisaccharide (5) is largely restricted to the C-6 methylene group. The addition of a second methyl group to the methyl group of 20 to form the isopropyl glycoside (23) lowered the inhibition potency but to such a small amount that it can be concluded that neither of the methyl groups are importantly involved. Thus, the indication is that the role of the methyl, ethyl and isopropyl groups is to render the topography about the aglyconic carbon hydrophobic in nature. The significance of the higher activities of 20 and 21 than that of 5 and the decreasing activities of 22 - 24 will be discussed later on in conjunction to the binding activities of the compounds 25 and 26.

The involvement of the acetamido group of the central βDGlcNAc unit in the binding is supported by the binding activities of compounds 17 - 19 (Tables VII and VIII). The replacements of the more hydrophobic acetamido group in 18 by an amino group as well as the polar (but of about the same size) trifluoroacetamido group in 19 render both structures completely inactive in binding. Since the trifluoroacetamido compound (19)

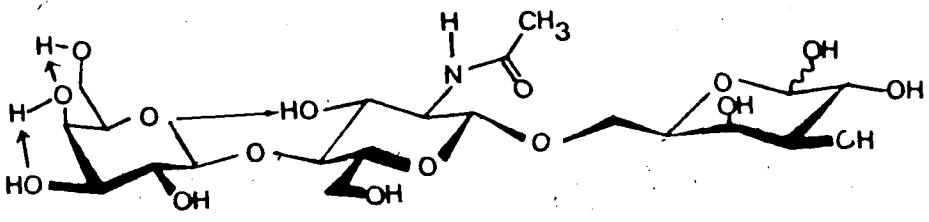
is inactive, it is expected that the acetamido group in the I Ma determinant is orientated in a way that the methyl group is directed towards the combining site in the antibody. This point will become more obvious when the conformation of 5 accepted into the combining site is discussed later.

As for the terminal βDGal unit, it has been mentioned in the Introduction that this unit is importantly involved in binding since the compound 4 is completely inactive (Table I) and that the OH-3" is not involved in the binding. The fact that the compounds 31 - 33 (Table IX) were found to be inactive suggested that the OH-4" and OH-6" are involved in binding. Since compound 34 (Table IX) was found to be inactive, the possibility that the I Ma determinant consists of an additional sugar unit, βDGlcNAc, linked to the 6-position of the terminal DGal residue can be ruled out. Further investigations are thus required in order to elucidate the way that the two hydroxyl groups enter the combining site.

The possibility exists that, in fact, the anti-I Ma combining site is designed to accept the terminal βDGal unit with the favored orientation for the hydroxylmethyl group as is shown in 5 (p.133). In fact, it has already been demonstrated [43] that the inhibition of the binding of methyl βDGal by anti-βDGal antibodies is strongly diminished (relative to the inhibition by methyl βDGal) for methyl 6-deoxy-βDGal, methyl 4-deoxy-βDGal and methyl βDGlc. Thus, the results presented in Table IX are compatible with the binding of 5 about the C-4" to C-6" region of a simple unsubstituted βDGal

residue. Furthermore, the inhibition provided by the D-6-C-methyl derivative of methyl βDGal which resides in the conformation 81a, although weaker ($\Delta\Delta G°$ = 1.3 kcal/mol) than that provided by methyl βDGal, was substantially ($\Delta\Delta G°$ = 0.8 kcal/mol) greater than that provided by the L-isomer (82a). As discussed in a recently submitted paper,[73] the lower activity of 81a as compared to methyl βDGal may simply arise because the binding of 81a requires the intrusion of the 6-C-methyl group into a rigidly held hydrophilic region of the combining site. This would be an interesting contrast to the results to be presented below in connection with Fig. 54 where the introduction of methyl group at the C-6 position of 5 actually enhanced the binding. In this case, it seems evident that the binding of the C-6 methylene group occurs in an hydrophobic region of the combining site.

The possibility also exists that the binding involves the 6"-hydroxymethyl group in an intramolecularly hydrogen-bonded form as depicted in 35. The binding of a βDGal unit in this manner was proposed by Lemieux et al.[43]



35

What the present results prove beyond doubt is that the combining site of the monoclonal anti-I Ma antibody extends from the C-6-methyl group, over the acetamido group and continues to include OH-4" and OH-6".

The trisaccharide 5 is expected to exist in solution in a large number of conformations of near equal energy (Table VI). However, as discussed in Part II, compounds 25 (L-isomer) and 26 (D-isomer) reside in the conformations that are indicated by the formula in Fig. 54. The fact that the D-isomer is a 2.8 times better inhibitor than the trisaccharide (5) and that the L-isomer is a poor inhibitor (Fig.54) indicates that the combining site recognizes the trisaccharide (5) in a conformation that is close to that of the D-isomer (26). The higher binding activity of 26 than 5 might arise because 5 is distributed among several conformations and therefore the binding of 5 must involve an important entropy of mixing as compared to 26. Since the di-O-isopropylidene derivative of 5; namely 18, is almost as potent an inhibitor as 5, the conformation of that portion of 18 which is bound is probably not appreciably different from that of 5. As has been mentioned in Part II, the conformation of 18 is in fact close to that preferred by the D-isomer (26). It is likely, therefore, that compound 5 is highly populated in those conformations which are close to that shown as 5b in Fig. 54, with minor subpopulations of conformers close to that shown as 5a. It is also seen in Fig. 54 that the disaccharide 1 is 19.4 times less potent than that of its methyl glycoside (20). This

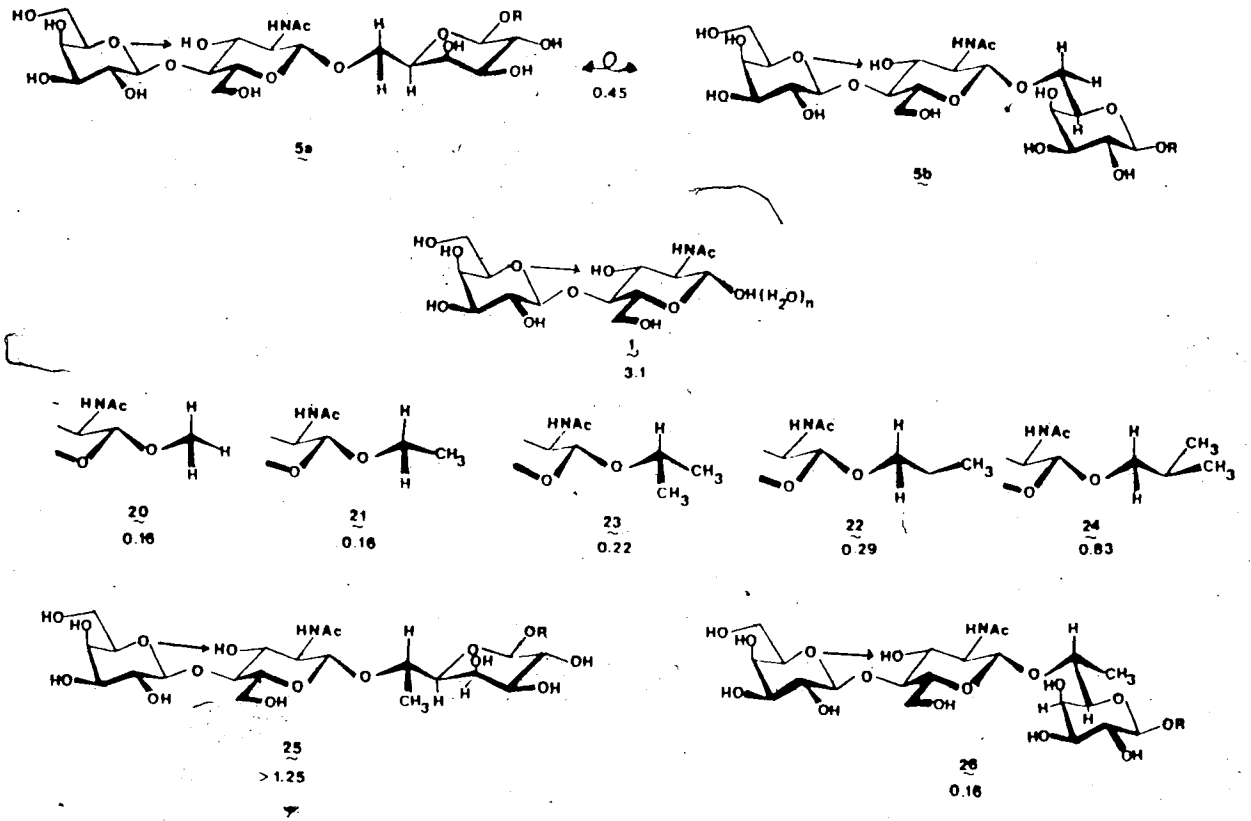


Fig. 54 The inhibition data of compounds 5, 1, 20 - 24, 25 and 26 are presented to show the steric requirement of the anti-I Ma antibody combining site in the C1'-O6-C6-C5 region of the trisaccharide 5.

indicates that the hydroxyl group in 1 is occupying the position of the methoxy group in 20 and is less compatible with an hydrophobic region of the combining site. Since the trisaccharide 5 can occupy a large range of energically nearly equivalent orientations, it can be anticipated that C-1 to C-5 region (L-arabino-portion) can occupy the orientation of one of the two methyl groups of the isopropyl glycoside (23). An indication as to which one is provided by the inhibition data for the n-propyl and isobutyl glycosides (22 and 24). It is expected that the conformational preference for the n-propyl group would be the so-called zig-zag conformers depicted in Fig. 54. Since the inhibition provided by this compound was only 1.8 times less than the methyl glycoside (20), it can be concluded that the methyl group of the n-propyl glycoside (22) does not appreciably influence the binding activity. However, the addition of a methyl group to form the isobutyl glycoside (24) provided a distinctly poorer inhibitor (5.2 times less potent than 20 and 2.9 times less potent than 22). This additional methyl group can be seen to occupy the same position (Fig. 54) as the O-5 grouping in 5a. Therefore, it is deduced that the L-arabino grouping of the reducing unit of 5 would occupy the pro-R and not the pro-S methyl group of the isopropyl glycoside (23), as in 5b, when it is accepted into the combining site. This interpretation is consistent with the results of the D- and L-isomers (25 and 26) as the conformation shown as 5b is deduced from the highly potent inhibitor 26.

On examination of a molecular model, the trisaccharide 5 which resides in a conformation that is inferred from that of the D-isomer (26) displays a large surface (Fig.55) which can be expected to be energically quite readily freed from water for descent into a hydrophobic cleft. This surface is wedge-like, and, for reasons discussed above concerning the inhibition results, is expected to extend from C-6 of the reducing DGal unit along the edge which includes the acetamido group to the region around O5"-C5"-C6"-C4" of the terminal DGal unit. The preferred conformation for the βDGal(1→4)-βDGlcNAc unit has O-3' in close proximity to O-5" (3.17Å) and therefore is disposed for the intramolecular hydrogen bonding that is indicated in Fig. 56. The presence of this bond is expected[43] to render the structure more favorable to binding with an hydrophobic surface.

The evidence presented above concerning the elucidation of the binding in the region of the O5"-C5"-C6"-C4" indicates that both the OH-4" and OH-6" are involved in the binding since the compounds 31 - 34 are all inactive. It remains, however, that the exact manner as to how these two hydroxyl groups are involved in the binding is not understood. Nevertheless, it is remarkable, on the basis of the conformation for the I Ma determinant (Fig.55) and how it appears to bind with the antibody (Fig.56), that the large surface that is expected to be 'buried' in the combining site is essentially hydrophobic in nature and at least seven hydroxyl groups on 5 are readily available for hydrogen bonding with the aqueous phase. As

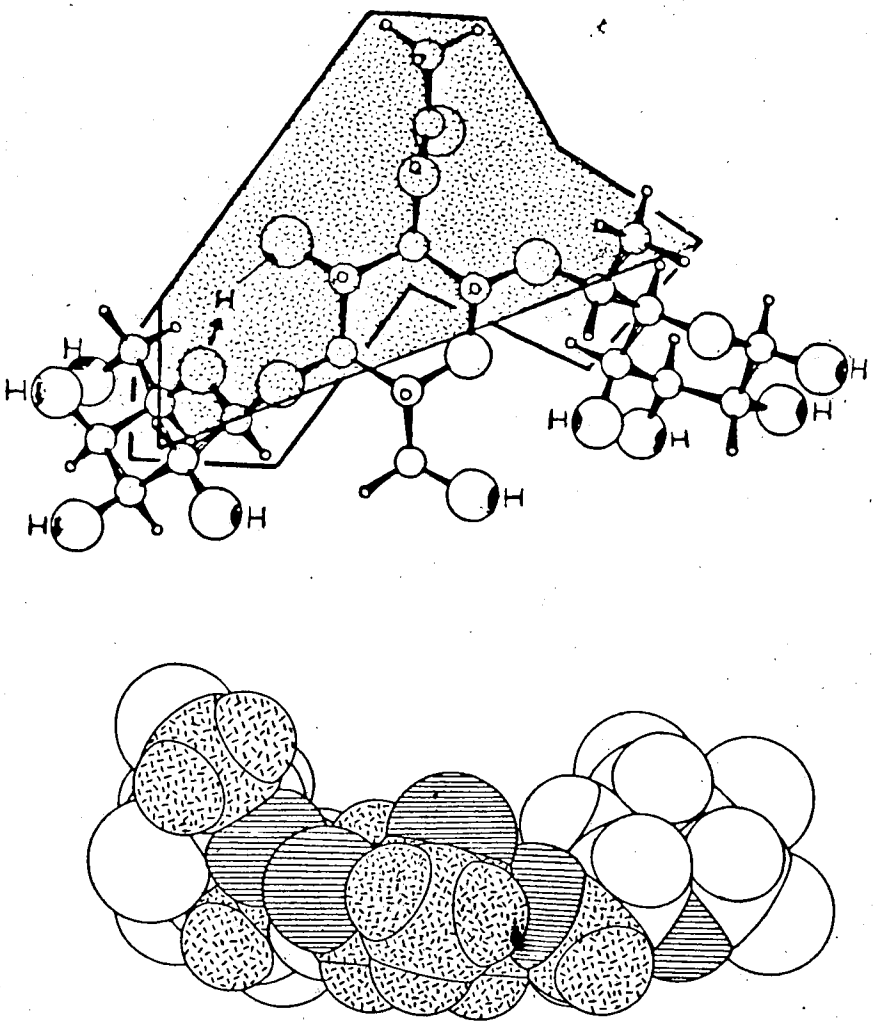
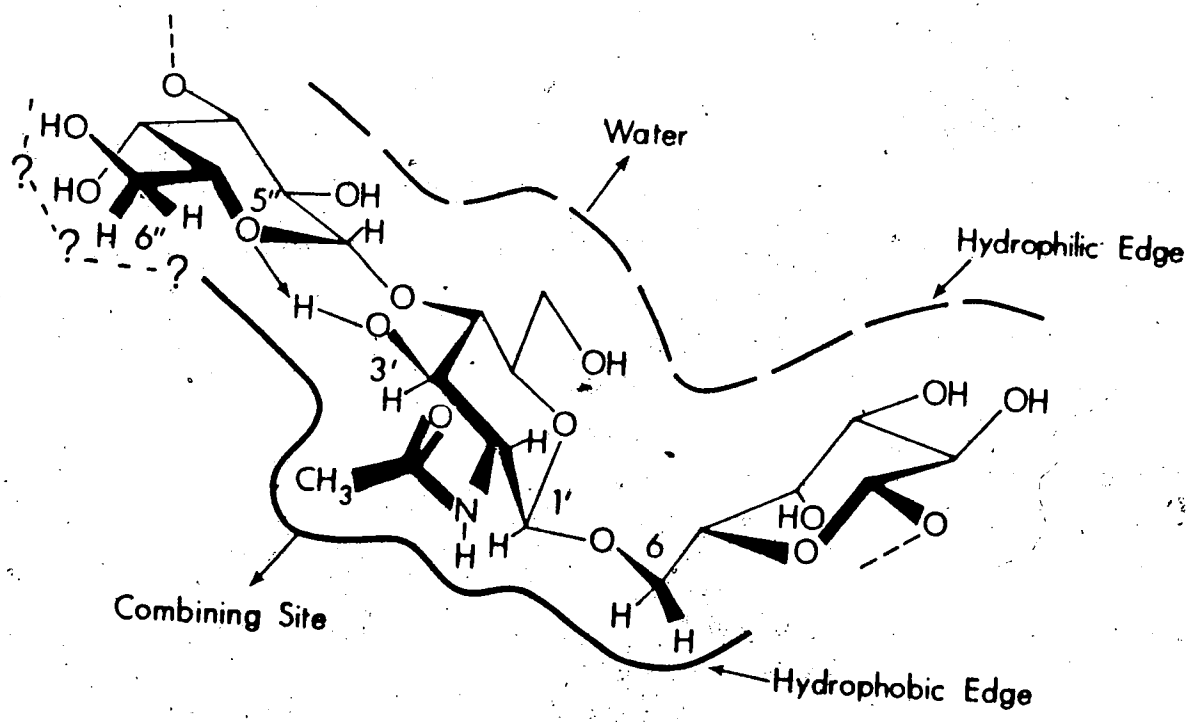


Fig. 55 Computer drawn projection formula of the trisaccharide (26) (upper) as derived by HSEA calculation in order to display how it is expected to interact with a hydrophobic combining site. The projection of the trisaccharide (lower) is to display the lipophilic region of this antigenic determinant in this conformation.



ANTI I(Ma)

Fig. 56 Display showing that the conformation of the I Ma antigenic determinant possesses a region along C6-C1'-C3'-O5"-C6" which can be expected to be compatible with a hydrophobic region for the combining site. The area marked with question marks indicates the region where the exact manner of binding is not known.

recently discussed,[73] some of these hydroxyl groups may be in contact with rigidly held hydrophilic groups at the periphery of the combining site.

Since the anti-I Ma antibody possesses an unique combining site expressed by a single clone, no general conclusions can be made in the present findings. However, it is noteworthy that the hydrophobic nature of the major portion of the anti-I Ma antibody combining site is in keeping with the expectation[43] that the main driving force for antibody-carbohydrate binding should tend toward hydrophobic bonding and, when necessary and stereochemically possible, the carbohydrate may assume intramolecular hydrogen bonds in order to become more compatible for such binding.

Finally, of major interest are the compounds 26 and 20 - 23 which provide the first examples of synthetic structures which are closely related to a natural antigenic determinant and which bind the antibody more strongly than does the natural antigen. This occurs likely because of the conformational preferences of these molecules which more readily present the topography which is complementary to the combining site. The modern technique developed for chemical synthesis and conformational analysis together with the now greater availability of monoclonal antibodies[87] and lectins[88] augurs well for studies concerning the exact nature of binding of carbohydrate structures with receptor sites—a matter which is now of fundamental importance to the understanding of a wide variety of biological communications.[89]

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